2007

Differential effects of mutant TAp63γ on transactivation of p53 and/or p63 responsive genes and their effects on global gene expression

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Differential effects of mutant TAp63γ on transactivation of p53 and/or p63 responsive genes and their effects on global gene expression.

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

By

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2007

WRIGHT STATE UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Date of Defense: 12-03-07

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY SHAMA KHAN KHOKHAR ENTITLED **Differential effects of mutant TAp63γ on transactivation of p53 and/or p63 responsive genes and their effects on global gene expression** BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science

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Abstract

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Differential effect of TAp63γ mutants on transactivation of p53 and/or p63 responsive genes and their effects on global gene expression.

p63, a member of the p53 gene family, known to play a role in development, has more recently also been implicated in cancer progression. Mice lacking p63 exhibit severe developmental defects such as limb truncations, abnormal skin, and absence of hair follicles, teeth, and mammary glands. Germline missense mutations of p63 have been shown to be responsible for several human developmental syndromes including SHFM, EEC and ADULT syndromes and are associated with anomalies in the development of organs of epithelial origin. The contrasting phenotypes associated with the different classes of p63 mutations might be in part due to the differential regulation of target genes. A previous report has demonstrated that heterozygous p63 mutations display high predisposition to tumor formation. Moreover, it has been shown that both p63 and p73, another member of the p53 family, are required for p53 mediated DNA damage induced apoptosis. Finally, differential splicing of p63 gene gives rise to p63 isoforms which can either act as tumor suppressors or oncogenes. The goal of this study is to determine the effects of naturally occurring TAp63γ mutants on regulation of p53/p63 and p63 specific target genes and their effects on global gene expression. Our results indicate that both TAp63γ(R227Q) and TAp63γ(R298Q) mutants mimic wildtype TAp63γ effects on its target genes. TAp63γ(K194E) and TAp63γ(R280C) significantly induced genes regulated by p63 and p53, but not those specific for p63. TAp63γ(R279H) and TAp63γ(R204W) were unable to induce any of the targets tested in this study. Co-transfection of p63 mutants along with wildtype p63 was performed to assess the effects of p63 mutants on ability of wildtype p63 to induce its target genes, while co-

transfection of TAp63γ(R279H) and TAp63γ(R204W) led to a complete inhibition of the wildtype TAp63γ mediated induction of p63 specific target genes, they had no effect on p53/p63 target genes. We demonstrated that the ability of these mutants to regulate wildtype activity was independent of their ability to either interact with wildtype TAp63γ or affect its localization. In addition, we demonstrated that the effects of these mutants on cell growth and survival were consistent with their ability to regulate the downstream targets when compared to wildtype TAp63γ. Furthermore, our analysis of the GeneChip data using GeneSpring led to the identification of several common and unique genes regulated by specific p63 mutants when compared to cells transfected with wildtype p63. Additionally, the specific genes regulated by the p63 mutants observed in EEC, SHFM and ADULT syndrome might offer unique insights in understanding the involvement of p63 in development, ectodermal-mesenchymal interactions and differentiation. In summary, we show that p63 mutants exhibit a differential effect on p63 specific and p53/p63 specific target genes and on induction of apoptosis. This, in turn might have a significant impact on p63 mutation associated abnormalities of human developmental syndromes. Taken together, our data shows that p63 mutants differentially regulate gene expression and provide an insight into the molecular biology of p63. Further, these results will aid in better understanding of role of p63 mutants in development and cancer.

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ACKNOWLEDGEMENTS

I wish to express my heartfelt gratitude to my advisor Dr Madhavi P. Kadakia for her mentoring and support during my research. I would also like to thank my committee members: Dr Steven J. Berberich and Dr Michael Leffak for their valuable time and helpful suggestions. Thanks to all the past and present members of Berberich and Kadakia lab for their friendship and helpful suggestions. I would also like to thank both the Biochemistry and Molecular Biology and the Biomedical Science Department for their patience and help during my program switches.

Last but not the least; I would like to thank my husband and my parents for their love, support and constant encouragement. I couldn't have done it without you.

I. Introduction:

1. Discovery of p63 and its structure:

p63 is a member of the p53 tumor suppressor gene family. The p63 gene is composed of 15 exons and is located on chromosome 3q27-29 (Mills et al., 1999; Yang et al., 1998). Although, p53 was considered to be different from its other family members due to absence of different isoforms, recent evidence supports that even p53 has many isoforms (Muller et al., 2006). p63 exhibits high sequence and structure parallel to p53 which led to early speculations that p63 would function as a back up tumor suppressor to p53 and could substitute p53 in promoting its tumor suppressive functions through transactivation of its target genes. Like p53, p63 also serves as a sequence specific DNA binding transcription factor that activates target genes involved in cell cycle arrest, DNA repair and apoptosis (Osada et al., 1998; Yang et al., 1998). The p63 gene with alternate promoter usage and differential C terminal splicing gives rise to six isoforms with remarkably diverse activities as transcription factors. TAp63α, TAp63β and TAp63γ contain the N terminal transactivation domain (TA), whereas the ΔNp63α, ΔNp63β and ΔNp63γ are transcribed from an internal promoter and lack the transactivation domain (Figure 1). Both TA and ΔNp63 isoforms have a DNA-binding domain, which is approximately 65% identical to the DNA-binding domain of p53, and an oligomerization domain with about 35% identity to that of p53. The TA isoforms with the transactivation domain are more closely related to p53 because apart from the above mentioned two domains, they also have partially homologous TA domain with 25% identity to p53. The TA isoforms are able to drive expression of the p53 target genes due to their ability to bind to p53 responsive elements

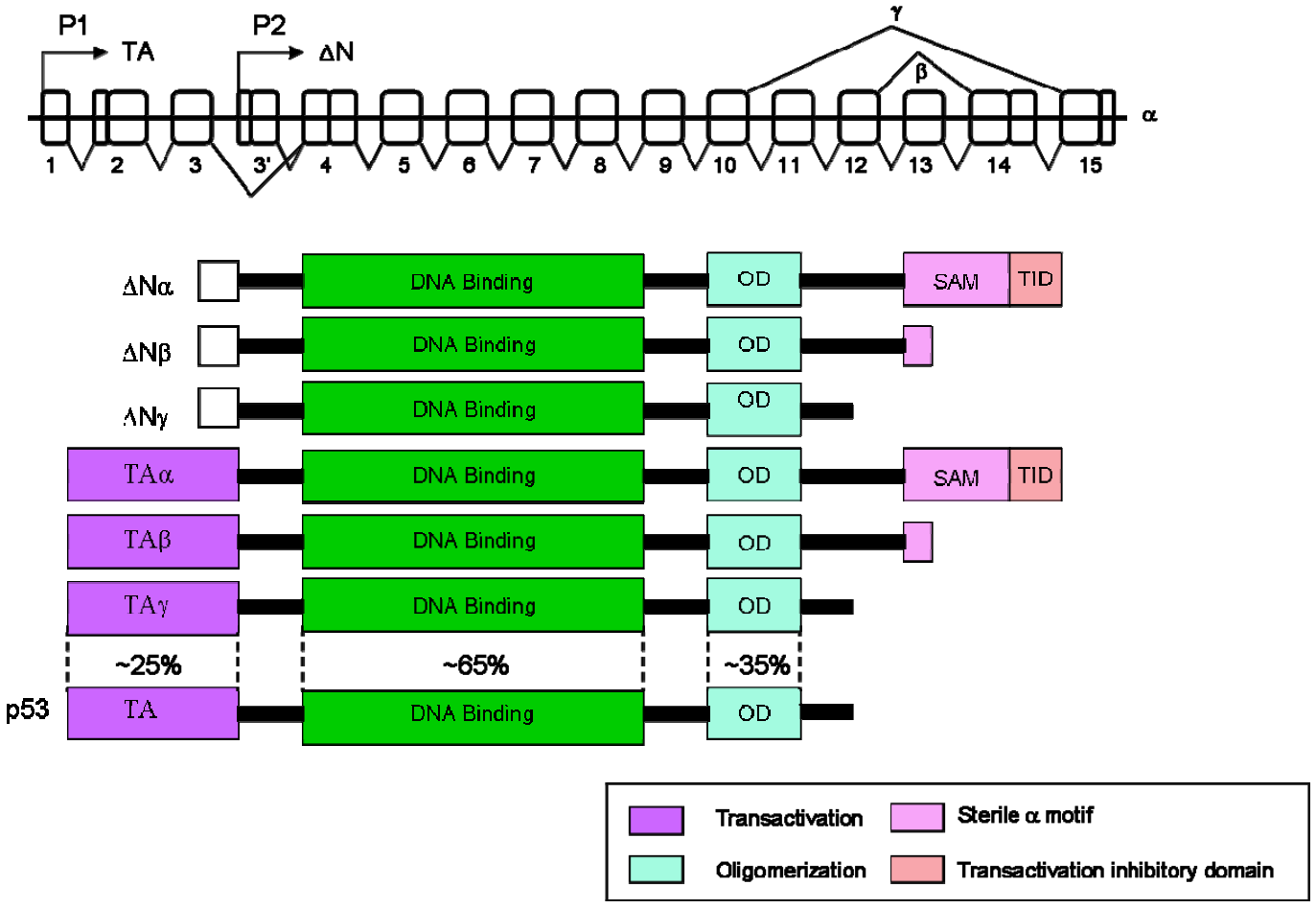

P1
TA
P2
ΔN
γ
β
α
1
2
3
3'
4
5
6
7
8
9
10
11
12
13
14
15
ΔNα
ΔNβ
ΔNγ
TAα
TAβ
TAγ
p53
TA
DNA Binding
OD
SAM
TID
~25%
~65%
~35%
Transactivation
Oligomerization
Sterile α motif
Transactivation inhibitory domain

Figure 1: *Schematic of p63 gene structure*: Representation of the high sequence and structure homology of p63 with p53 protein, in dictating the amino acid identity among the different domains. p63 has six different variants generated due to differential promoter usage at the N terminal and C terminal alternate splicing. The TA isoforms contain the transactivation domain while the ΔN isoforms lack the TA domain. The TA isoforms are more similar to p53 in structure because both of them contain the Transactivation domain (TA), DNA-binding domain and Oligomerization domain (OD). In addition to these domains, the α-isoforms contain a sterile α motif (SAM) domain and a transactivation inhibitory domain (TID) at their C-termini. The percentage represents the amino acid identity between similar domains of p53 and p63 (adapted from Bokhoven *et al*, 2002).

(Westfall et al., 2003). However, the divergent outcomes of the mice knock out models of these two genes clearly imply that they regulate distinct subsets of target genes. This can be partially explained by the existence of a distinct p63 consensus DNA binding site, to which p63 proteins bind preferentially (Osada et al., 2005; Perez et al., 2007) as shown in Figure 2. The ΔN isoforms can also bind to p53 RE and exert dominant negative effects over p53, by competing for DNA binding sites (Murray-Zmijewski et al., 2006). Reports also showed that ΔNp63 isoforms are able to activate target genes which are not induced by TA isoforms (Dohn et al., 2001; Wu et al., 2003). The carboxy terminal of the alpha isoform contains a SAM (sterile alpha motif) domain which is a protein-protein interaction domain also found in other developmentally important proteins (Ianakiev et al., 2000). SAM domain has been shown to be involved in other cellular processes such as chromatin remodeling, focal adhesion apoptosis and receptor kinase signaling (Loenen, 2006; Thanos and Bowie, 1999). Downstream from the SAM domain is a transcriptional inhibitory domain (TID) that can act either in *cis* or *trans* to regulate transcriptional activity of p63 (van Bokhoven and McKeon, 2002). Hence, six different isoforms of p63 are present in cells, at different levels of expression (Testoni and Mantovani, 2006). The molecular complexity of p63 is attributed to complex structure that gives rise to six different variants which either act as transcriptional activators or repressors. The differential expression of different p63 isoforms regulates the complex biochemical activities, implying its role during development and carcinogenesis.

2. p63 in development:

p63 is expressed in basal cells of the skin, cervix, tongue, esophagus, mammary glands and prostate. Unlike p53 knockout mice which developed spontaneous tumors, p63 knock out mouse was found to have several developmental defects and died within a day of its birth owing to dehydration and maternal neglect (Donehower et al., 1992; Mills et al., 1999; Yang et al., 1999). Mice lacking p63 display severe developmental defects such as limb truncations, abnormal skin and absence of hair follicles, teeth and mammary glands. These defects in organs of epithelial origin demonstrated that p63 plays a pivotal role in embryonic development. The surface epithelium of p63 null mice consists of single cell layer that fails to proliferate and differentiate into mature epithelium (Mills et al., 1999; Yang et al., 1999). Recent studies indicate p63 as a critical factor for molecular switch of epithelial stratification. However, role of p63 in development and differentiation of epithelial stem cell still remains controversial and the center of debate. While some argue that p63 is essential for proliferative potential of already committed epithelial stem cell, but not essential for commitment of simple ectoderm to epithelial stem cell (Yang et al., 1999), others believe that p63 is essential for both commitment and differentiation of simple ectoderm to simple epithelial lineage (Mills et al., 1999). However recent findings have suggested the involvement of p63 not only for commitment and differentiation of ectoderm to simple epithelium but also for proliferative potential (Koster et al., 2007). The argument in favor is that this dual role of p63 is due to the existence of six different variants which act in a differential manner. To further explicate the role of p63 in epidermal development, studies were undertaken to see the individual role of TAp63α and ΔNp63α using transgenic mice complementation studies (Candi et al., 2006). The TAp63α complemented mouse has a similar phenotype like p63 knock out mouse, with no epidermis. In

p53 DNA consensus
(10-mer or half site)

	purine-rich	core	pyrimidine-rich
El Diery *et al*	R R R	C A/T A/T G	Y Y Y
Inga *et al*	R R R	C A/T A/T G	Y Y Y

p63 DNA consensus
(10-mer or half site)

	purine-rich	core	pyrimidine-rich
Osada *et al*	R R R	C G T G	Y Y Y
Ortt *et al*	AT-rich T/A A/T A	core C A/T T G	AT-rich T T/A T
Perez *et al*	R R R	C A/G T/A G	Y Y Y

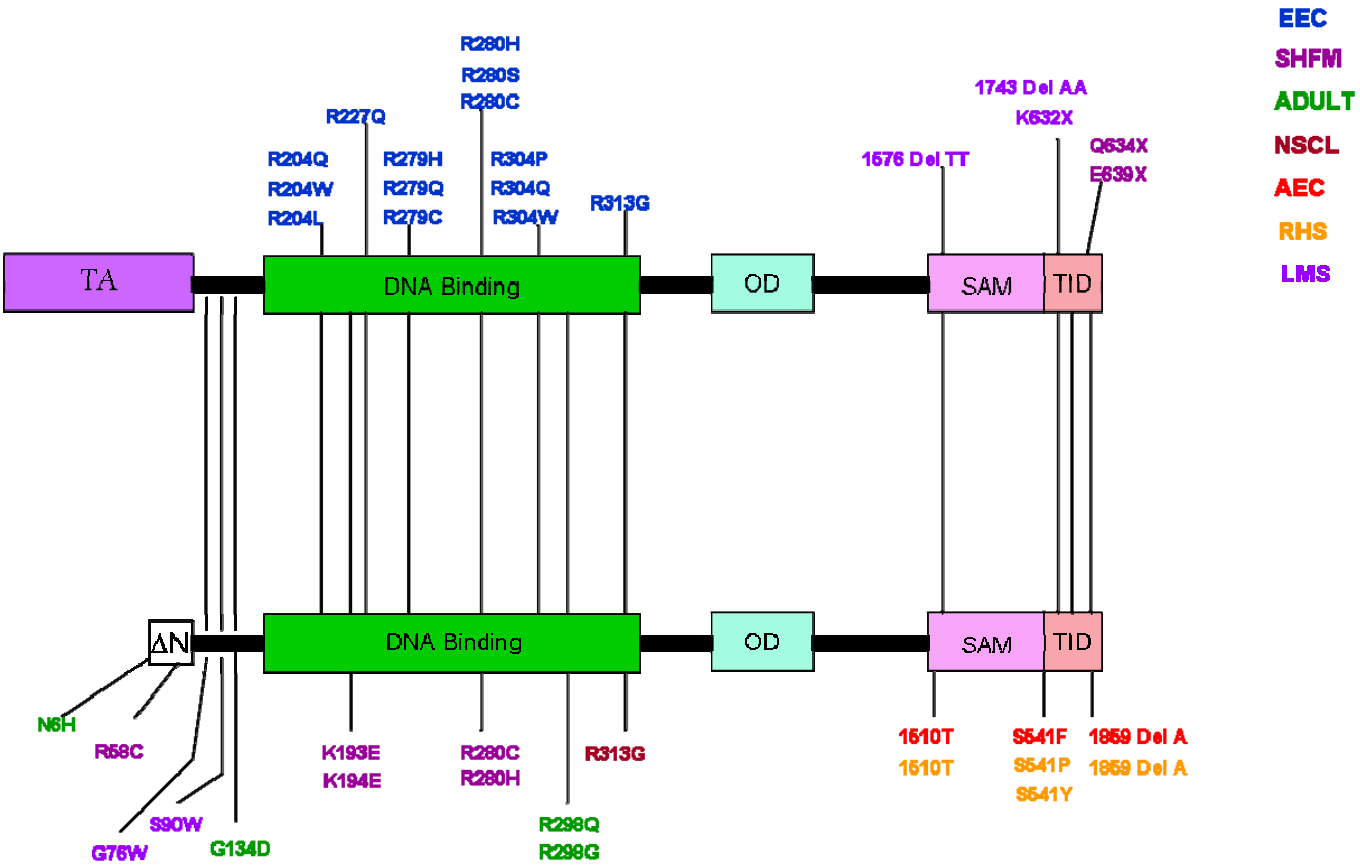
Figure 2 : *DNA binding sites in p53 and p63 identified by different approaches.* p63 binds to DNA binding motif with unique characteristics distinct from the p53 DNA consensus site. Osada *et al* identified that p63 has higher specificity for responsive elements containing the half site 5'-RRRCGTGYYY-3'. p63 specific DNA consensus site is characterized by the presence of a G in the 5^{th} or 6^{th} position in the core domain and a relatively high number of mismatches in the purine and pyrimidine rich flanking regions (adapted from Perez *et al*, 2007).

sharp contrast, the ΔNp63α transgenic complemented mice had more epidermis. The ΔN complimented animals expressed greater amounts of basal layer proteins K5 and K14 than either the p63-/- and p63-/-; TA-/-. However, both the p63-/-; TA and p63-/-; ΔN mice died within a few hours of birth. This study suggested that exogenous introduction of the either of the p63 isoform does not compensate for the absence of endogenous p63.

3. Role of p63 in human diseases:

Mutations in p63 have been documented in several different human developmental disorders (Figure 3A). These p63 related disorders observed in human beings have certain phenotypic characters similar to p63 knock out mice. Ectodermal dysplasia, Split foot/hand malformation and craniofacial defects are the hallmark features observed in p63 mutations. Ectrodactyly, ectodermal dysplasia and cleft-lip palate (EEC) syndrome is characterized by ectodermal dysplasia with developmental anomalies reflecting perturbation of skin, limbs, hair, teeth & apocrine glands. The severe defects that are associated with the EEC mutants are dependent on the nature of the mutation (Rinne et al., 2007). The amino acids in the DNA binding domain are very crucial for the interaction with DNA, therefore mutations of these amino acids may have an unfavorable effect on their DNA binding, also leading to a decrease in their transactivation ability (van Bokhoven et al., 2001). Strikingly most of the germline mutations observed in EEC mutations have similar locations as the hot spot mutation

A)


EEC
SHFM
ADULT
NSCL
AEC
RHS
LMS
R280H
R280S
R280C
R227Q
R204Q
R204W
R204L
R279H
R279Q
R279C
R304P
R304Q
R304W
R313G
1743 Del AA
K632X
1676 Del TT
Q634X
E639X
TA
DNA Binding
OD
SAM
TID
ΔN
DNA Binding
OD
SAM
TID
N6H
R58C
S90W
G75W
G134D
K193E
K194E
R280C
R280H
R313G
R298Q
R298G
1610T
1610T
S541F
S541P
S541Y
1959 Del A
1959 Del A

B)

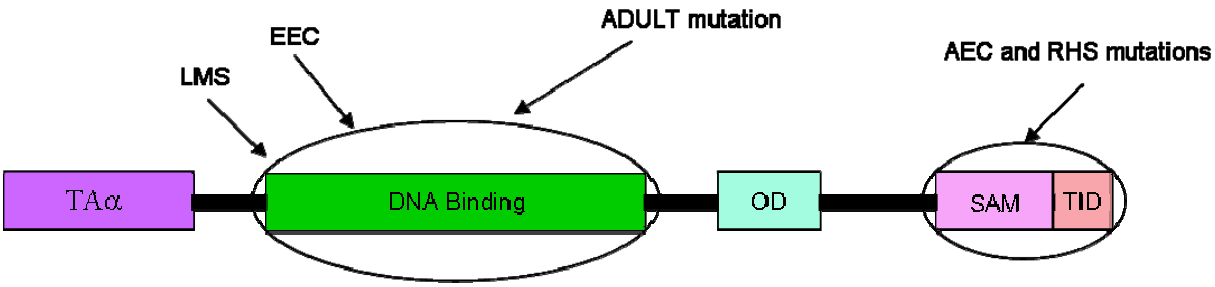

ADULT mutation
EEC
LMS
AEC and RHS mutations
TAα
DNA Binding
OD
SAM
TID

Figure 3: *p63 mutations associated with human developmental syndromes*. A) Distribution of the p63 mutations identified in different human developmental disorders. Mutations in EEC and SHFM syndrome are localized to the DNA binding domain that are expected to abrogate interaction with DNA and lower transactivation activity. RHS and AEC are localized to the SAM domain. LMS mutations are frame shift mutations in exons 13 and 14. Abbreviations: EEC, ectrodactyly, ectodermal dysplasia, clefting; AEC, ankyloblepharon, ectodermal dysplasia, clefting; ADULT, acro-dermato-ungual-lacrimal-tooth; SHFM, split hand/foot malformation; LMS, limb-mammary syndrome; RHS, Rapp Hodgkin syndrome; NSCL, Non syndromic cleft palate (adapted from Rinne *et al*, 2007). B) The syndrome phenotype is determined by the position of the mutation. Strong genotype-phenotype co-relation is seen especially in LMS, EEC and ADULT syndrome as mutations observed in these conditions are clustered in the DNA binding domain. Also, AEC and RHS syndrome mutations are clustered in the same domains (SAM and TID) and give rise to very similar clinical phenotype.

sites in p53 gene. The arginine codon mutation in p63 like R204, R279H, R304, R280 correspond to the hotspot mutations in p53 like R175, R248, R273 and R249 respectively, which also lead to loss of DNA binding capacity (Li and Prives, 2007). Ankyloblepharon, ectodermal dysplasia, clefting (AEC) or Hay Wells syndrome is characterized by ankyloblepharon or fused eyelids and lack of limbs. Ectodermal dysplasia is more pronounced in AEC than in EEC. Mutations in AEC syndrome are localized to the SAM domain so it only affects the alpha isoforms (Figure 3). These mutations disrupt the protein-protein interaction function leading to the loss of normal biological activity associated with this domain. Rapp-Hodgkin syndrome (RHS) is manifested with alopecia, hypodontia and dry skin. Similar to AEC syndrome, this syndrome lacks the orofacial clefting and limb defects. Also the mutations in RHS are point mutations and deletion mutations in the SAM and TID domain. Limb-mammary syndrome (LMS) is characterized by mammary gland and nipple hypoplasia, no hair and skin. Acro-dermato-ungual-lacrimal-tooth syndrome (ADULT) is distinguished by neurodermitic signs, excessive freckling and exfoliative dermatitis. Protein modeling data suggests that R298Q mutation which resides in the DNA binding domain of p63 does not lead to the loss of the transactivation potential. Split hand-split foot malformation (SHFM), a limb malformation is characterized with absence of median digital rays of the hand and feet and Syndactyly of digits (Czeizel et al., 1993). NSCL (Non-syndromic cleft lip/palate) is characterized by orofacial clefting (Rinne et al., 2007).

Combined p63 mutation data for these syndromes indicate extensive genotype-phenotype correlations with each of these syndromes having a distinct pattern and type of mutations (Celli et al., 1999; Rinne et al., 2006). For example, majority of mutations observed in EEC syndrome

are missense mutations generating amino-acid substitutions in the residues involved in DNA binding, thereby affecting all p63 isoforms (Kantaputra et al., 2003; Ying et al., 2005) . On the other hand, mutations causing AEC and RHS syndrome are all missense mutations coding for the SAM domains, thus only p63α isoforms are affected (Figure 3B). It is interesting to note that EEC mutations, and not AEC mutations, cause limb defects. Similarly LMS mutations cause mammary gland dysplasia but mutations in EEC or SHFM rarely do. The differences in part could be due to different sets of genes regulated by p63 and p63 mutants. Mutations in p63 could lead to loss of function or gain of function which results from a combination of specific protein-protein interactions as well as activation or repression of specific target genes. Since, p63 is a transcription factor, the molecular targets of p63, both normal and specific to p63 mutants will provide insight on the mechanisms underlying its role in development.

4. p63 in cancer:

The role of p63 in tumorigenesis still remains unclear and controversial. Even though p63 mutations are rarely detected in human cancers, several studies have implicated that p63 might play a role in cancer progression. Loss of p63 and p73 has been associated with aggressive tumor progression and poor prognosis (Koga et al., 2003; Park et al., 2000; *Urist et al., 2002; Urist et al., 2002; Wang et al., 2002). In addition, loss of p63 expression has also been correlated with cancer progression in various cancers including prostate and bladder cancers (Park et al., 2004; Parsons et al., 2001; Urist et al., 2002).

TAp63 isoforms have been shown to promote growth arrest by inducing anti proliferative genes like *Insulin Growth Factor Binding Protein-3* (IGFBP-3), BAX, *Vitamin D receptor* (VDR) and Maspin (Kommagani et al., 2006; Senoo et al., 2002; Shimada et al., 1999; Spiesbach et al., 2005; Wu et al., 2005), and inhibiting the pro proliferative genes like Vascular Endothelial Growth Factor (VEGF) & Heat Shock protein 70 (HSP70) (Senoo et al., 2002; Wu et al., 2005). Although loss of p63 co-operates with loss of p53 in tumor development, the exact mechanism for this action is still unclear (Flores et al., 2005). Several studies have suggested the possible role for p63 in several different apoptotic pathways. TAp63α protein has been shown to induce apoptosis by activating both mitochondrial and death receptor apoptotic pathways which sensitizes the cancer cells towards chemotherapy (Gressner et al., 2005b). Endogenous p63 has been shown to be induced by many chemotherapeutic agents and blocking this function by p63 mutants might confer chemoresistance (Petitjean et al., 2005). In addition, TAp63 isoforms have been shown to be critical for mitochondrial apoptotic pathway upon NGF withdrawal in developing sympathetic neurons (Jacobs et al., 2005). Further, TAp63 isoforms were shown to accumulate upon DNA damage induced by Topoisomerase II inhibitors (Katoh et al., 2000; Okada et al., 2002; Petitjean et al., 2005). The role of p63 in tumorigenesis has been supported by studies using p63 involving heterozygous mice which were predisposed to tumor formation and displayed loss of heterozygosity (LOH) for the wildtype allele (Flores et al., 2005). In contrast, various reports refuted the LOH for p63 in heterozygous mice, and argued against the notion that p63 might act as a tumor suppressor (Keyes et al., 2006; Koster et al., 2006). However, TAp63 isoforms have been shown to promote apoptosis during embryonic development possibly by coordinating with other counterparts of the p53 family (Flores et al., 2002; Jacobs et al., 2005). In contrast, ΔNp63α isoform, that lacks the TA domain represses the

expression of IGFBP-3 and induces expression of HSP70 and VEGF to promote proliferation in squamous carcinomas. Several studies indicate the role of ΔNp63α as an oncogene since, it is over expressed in various cancer types and has been shown to induce the pro survival proteins (Casciano et al., 2002; Choi et al., 2002; Hu et al., 2002; Park et al., 2000; Senoo et al., 2002; Sniezek et al., 2004; Wu et al., 2005). ΔNp63α has also been shown to repress p21 and 14-3-3σ genes, thereby inhibiting the p53 mediated growth arrest and apoptosis (Westfall et al., 2003). Additionally, ΔNp63α isoform act in a dominant negative manner towards p53 and TAp63 isoforms by inhibiting their target genes, to maintain the proliferative potential of epithelial stem cells. Together, all these studies point towards the relevance of studying the biological effects of p63 mutants. Even though the role of p63 in tumorigenesis remains unclear, general consensus is that ΔNp63α promotes proliferation hence acting as an oncogene, whereas TA isoforms promotes cell cycle arrest and apoptosis thereby performing tumor suppressive functions.

5. *Rationale:*

Since the discovery of p63 as a p53 family member, it has been investigated as a back up tumor suppressor gene. The importance of p63 was demonstrated by the p63 knock out studies where it was shown to play an important role in development. p63 can induce cell cycle arrest and apoptosis by activating p53 specific target genes. Loss of p63 and p73, another member of p53 family, results in failure of cells with functional p53 to undergo apoptosis in response to DNA damage (Flores et al., 2002). Due to sequence and structure homology in the oligomerization domain of p53 and p63, like p53, p63 also functions as a tetramer. Also, the human developmental syndromes associated with p63 mutations indicate genotype-phenotype correlations with each syndrome having a distinct pattern and type of mutation. However, little is known about the precise pathogenetic mechanism that underlies the phenotypic specificity observed in different mutational classes. In this dissertation research we tried to address two specific aims. Firstly, we investigated the effect of naturally occurring TAp63γ mutants on biological activity of wildtype TAp63γ. We chose TAp63γ as it has been shown to be the most potent transactivator of all other p63 isoforms (Shimada et al., 1999). Also, the mutations that we included in our studies are germline mutations localized in the DNA binding domain which are common to all isoforms of p63. Our hypothesis was that p63 mutants interact with wildtype p63, abolishing its DNA binding capacity and disrupting its activity. Thus, the binding of p63 mutants to wildtype p63 can lead to inhibition of its transcriptional activity which might potentially result in a subsequent loss of its biological functions like growth arrest, apoptosis and differentiation. Therefore, these p63 mutations can gain additional functions by regulating wildtype p63 function and thus actively contribute to cancer and development. Secondly, we performed gene

expression studies, to identify novel target genes regulated by these mutations. We hypothesized that the distinct phenotype observed in human developmental syndromes associated with specific p63 mutations is due to differences in the target genes regulated by these mutants. In this aim we investigated the differential regulation of target genes by each of the p63 mutants by analyzing the gene expression profile using DNA microarray following overexpression of these mutants in a mammalian cell line.

II. Materials and Methods:

1. *Cell lines and plasmids:* H1299, a human non-small lung carcinoma cell line (obtained from ATCC) and HCT 116 p53-/-, a colon epithelial cell line (a generous gift from Dr Steven Berberich, Wright State University) which are devoid of p53 were maintained in Dulbecco's modified eagle medium (DMEM) supplemented with 10% Fetal Bovine calf Serum (FBS) and 1% PS (Penicillin and Streptomycin) at 37^{o}C, in a humidified 5% CO_2. Expression plasmids encoding GST-TAp63γ and HA-TAp63γ were constructed by cloning the coding sequence of TAp63γ (p51A), into pcDNA3.1myc and pcDNA3.1GST (Invitrogen) (Kadakia et al., 2001). The p51A cDNA was a kind gift from Shuntaro Ikawa (Tohuku University, Japan). GST tagged TAp63γ mutants were created using PCR based site-directed mutagenesis method using sense and antisense primers. The primer sets for the mutants included in this study are 1) R279H sense (5'GGAGGGATGAACCACCGTCCAATTTTAATC3') and antisense (5'GATTA-AAATTGACGGTGGTTCATCCCTCC3') 2) K194E sense (5'CATGCCTGT-CTACAAAGAAGCTGAGCACGTCAC3') and antisense (5'GTGACGTGCTCAG-CTTCTTTGTAGACAGGCATG3') 3) R204W sense (5'GGAGGTGGTGAAG-TGGTGCCCCAACCATG3') and antisense (5'CATGGTTGGGGCACCA-CTTCACCACCTCC3') 4) R227Q sense (5'CTCCTAGTCATTTGATTCAAG-TAGAGGGGAACAGC3') and antisense (5'GCTGTTCCCCTCTACTTGAATCAAAT-GACTAGGAG3') 5) R280C sense (5'GGAGGGATGAACCGCTGTCCAATTTT-AATCATTGTTACT3') and antisense (5'AGTAACAATGATTAAAATTGGACA-GCGGTTCATCCCTCC3') 6) C306R sense (5'GGCCCGGATCCGTGCTTGCCCAG3')

and antisense (5'CTGGGCAAGCACGGATCCGGGCC3') 7) R298Q sense (5'GCAAG-TCCTGGGCCAACGCTGCTTTGAGG3') and antisense (5'CCTCAAAGCAGCGTT-GGCCCAGGACTTGC3'). PG13-Luc reporter plasmid containing 13 copies of p53 binding DNA consensus sequence was obtained from Dr. Steven Berberich (Wright State University, USA). Other reporters, Maspin-Luc and Hdm2-Luc were kind gifts from Dr Lindsey Mayo (Indiana University, USA). Shh full length promoter construct was constructed as reported earlier (Caserta et al., 2006). Membrane bound hybrid GFP plasmid, PAB35 was a kind gift from Dr Lynn Enquist (Princeton University, USA).

2. *Transactivation studies: :* To measure the PG13-Luc, Hdm2-Luc, Maspin-Luc and Shh-Luc reporter activities, cells were seeded in 24-well plates at 5 x 10^4 cells/well (for H1299) and 1 x 10^5 cells/well (for HCT p53-/-). At 24 hr after seeding, cells were transfected with 100 ng of reporter constructs and a constant amount of CMV-Renilla Luc plasmid, along with desired plasmids as indicated using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). All transfections were done in duplicate. After 5 hr incubation the medium was replaced with DMEM medium supplemented with 10% FBS and 1% PS. At 24 hr post transfection, cells were washed once with 1X PBS and whole cell extracts were made by adding 100 μL of Passive Lysis buffer (Promega, Madison, WI) directly onto the plate and put on a rocker at room temperature for 30 min. The lysates were then transferred into 1.7 mL eppendorf tubes. Dual luciferase assay was performed to detect both firefly and Renilla luciferase activity using Dual-Luciferase Reporter 1000 Assay System as per manufacturer's protocol (Promega, Madison, WI).

Briefly, to measure reporter activity 2 μL of each sample was taken in separate tube and 50 μL of LASII reagent was added to the tube and mixed well by pipetting and immediately placed in a luminometer to get the reading for firefly luciferase. This was followed by addition of 50 μL of Stop and Glow reagent to the same tube and vortexing, to allow proper mixing, the sample was read again to get the Renilla luciferase reading. The relative luciferase activity was measured by calculating ratio of Firefly luciferase activity to Renilla luciferase activity. The average of the ratios from duplicate samples was then plotted in a graph where error bars represented standard deviations.

3. *Protein Isolation and Immunoblotting studies:* H1299 and HCT116 p53-/- cells were seeded onto 6 well plates with 2.5 x 10^5 cells/well and 5 x 10^5 cells/well respectively. At 24 hr post seeding, cells were transiently transfected with desired plasmids using Lipofectamine 2000 (Invitrogen, Carlsbad, CA), in serum and antibiotic free DMEM. After 5 hrs incubation the medium was replaced with DMEM medium supplemented with 10% FBS and 1% PS. At 24 hr post-transfection, cells were first washed with 1X PBS and harvested in Radio Immunoprecipitation Assay (RIPA) buffer (0.5% sodium deoxycholate, 1% NP-40, 0.1% SDS, phosphate buffered saline, pH 7.4). The buffer with 1% protease inhibitor cocktail (Sigma) was added onto the plates directly and placed on ice for 30 min. The cells were then scraped and transferred to 1.7 mL eppendorf tubes. The extracts were placed for an additional 30 min on ice, to allow complete lysis of the cells. The samples were then centrifuged for 5 min at 14,000 rpm at 4^{o}C. Protein concentration was determined using BCA reagent (Pierce, Rockford, IL), using a 96 well plate. Standard curve was generated using bovine serum albumin (BSA) ranging from 1

μg/μL to 13 μg/μL. For each of the sample 2μL of sample and 98 μL of sterile distilled water were mixed properly and 100 μL of BCA reagent added to each well and incubated at 37^{o}C for 15 min. The absorbance was measured using a spectrophotometer at the wavelength of 562 nm and the protein concentration calculated using a standard curve. Equal amount of protein was used from all samples and mixed with 5X SDS loading dye (0.5 M DTT, 0.3 M Tris (pH 6.8), 10% SDS, 50% glycerol and 0.05% bromophenol blue) and heated at 97^{o}C for 5 min prior to loading. Protein extracts were run on 10% SDS-PAGE gel using 1X SDS buffer (25 mM Tris pH 8.3, 250 mM glycine, 0.1% SDS) for about 4 hr at 200 constant volts and transferred onto PVDF membrane (Millipore Corporation, Billerica, MA) using transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol and pH 8.3) at 1.10 Amps for 1 hr using a Transblot system (Bio-Rad) and blocked with 5% blocking milk solution (1M Tris pH 7.4, 5M NaCl, 0.05% Tween-20 and 5% non fat dry milk). The membrane was then subjected to immunoblotting at room temperature (in 5% non fat dry milk made in 1X TTBS (Tris-Tween 20 Buffered Saline) using antibodies to detect specific proteins overnight. Mouse monoclonal anti-VDR D-6 (1:2000), rabbit polyclonal anti-p21 C-19 (1:2000), rabbit polyclonal anti-Shh H160 (1:250), mouse monoclonal anti-p63 4A4 (1:4000) (Santa Cruz Biotechnology, Santa Cruz, CA), mouse monoclonal anti-Mdm2 (1:500) (Calbiochem, San Diego, CA) and mouse monoclonal anti-β-actin (1:25,000) (Sigma, St. Louis, MO) antibodies were used to detect VDR, p21, Shh, p63, Hdm2 and β-actin expression respectively. Appropriate IgG conjugated with horseradish peroxidase was used as secondary antibody (Promega, Madison, WI). The membrane was washed thrice with 1X TTBS for 15 min each and then exposed to Super-signal West Pico Chemiluminescent Substrate Kit (Pierce,

Rockford, IL) for 1 min to detect the chemiluminescent signal. The protein was visualized using FUJI FILM LAS3000 image reader. The antibodies were stripped from the membrane by incubating the blot in Western Stripping Buffer (25 mM glycine, 1% SDS, pH 2.0) for 30 min twice, at room temperature on a rocker. This was followed by washing the blot for 5 min twice with 1X PBS. The membrane was then blocked with 5% blocking milk followed by subsequent immunoblotting with another primary antibody according to the method indicated.

4. *RNA isolation and TaqMan based real time PCR:* For RNA studies, cells were transfected with desired expression plasmid. At 24 hr post-transfection, cells were washed with 1X PBS and lysed directly on the culture plate using the RNAeasy method as per manufacturer's protocol (Qiagen, Valencia, CA). The RNA was quantified by diluting 2μL of RNA sample with 98μL of TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0). This sample mix was then transferred into a quartz cuvette; the absorbance was measured using spectrophotometer at A_{260}/A_{280} nm. The readings were taken relative to the blank TE. The RNA concentration was determined by using the following relation: RNA_{conc} = (40 μg/ mL)*(A_{260})*(dilution factor)*(1 mL/1000 μL), assuming A_{260} of 1 corresponds to 40 μg of RNA per mL. The purity of RNA was estimated by the ratio of A_{260}/A_{280}, with pure RNA having a ratio from 1.8-2.0. Each RNA sample was reverse transcribed individually using random hexamers to create cDNA. Briefly, 1 μg of total RNA was mixed with 2.5 μL of 10X TaqMan RT buffer, 5.5 μL of 25 mM $MgCl_2$, 5.0 μL of deoxyNTPs, 1.25 μL of random hexamers, 0.5 μL of RNase inhibitor, 0.625 μL of Multiscribe reverse transcriptase, and RNase-free water for a total reaction volume of 25

μL to synthesize cDNA by using TaqMan reverse transcription kit (Applied Biosystems, Foster city, CA).The reverse transcription (RT) reaction was performed in a Perkin Elmer Gene Amp PCR System 2400 programmed to sequentially cycle as follows: initial 10 min incubation at 25^{o}C, 30 min RT step at 48^{o}C, 5 min inactivation step at 95^{o}C, and an infinite hold at 4^{o}C. After the RT reaction was complete, cDNA was diluted 1:2 by adding 25 μL sterile DNAse/RNAse free water to each sample prior to storage at -20^{o}C. Quantitative real-time PCR analysis was performed in a 96 well micro titer plate format on an ABI Prism 7900HT sequence detection system using TaqMan Universal master mix and Assay on Demand reagents. Briefly, a 20 μL reaction was prepared by mixing 2 μL of cDNA sample, 10 μL of TaqMan Master Mix, 7 μL of DNAse/RNAse water and 1 μL of AOD (Applied Biosystems) containing forward and reverse primers and a fluorescent TaqMan probe, designed and optimized for gene of interest for use in a 96 well plate format. The PCR conditions used were 2 min hold at 50^{o}C, 10 min hold at 95^{o}C and 40 cycles of 15 sec 95^{o}C denaturation step and 1 min 60^{o}C annealing and elongation step. Each sample was analyzed using SDS 2.0 software (ABI) in triplicate for target gene specific for VDR (Hs_ 0017213_m1), p21 (Hs_00355782_m1), Hdm2 (Hs_ 00242813_m1) and Shh ((Hs_ 00179843_m1) (PE Applied Biosystems, Foster City, CA). GAPDH was used as an internal normalization control. These primers were designed by ABI, to span intron-exon junction, eliminating the possibility of detecting genomic DNA. Each well was monitored for fluorescent dye and signals were considered significant if the fluorescence intensity significantly exceeded the standard deviation of the basic fluorescence, defined as the threshold cycle (C_T). Relative mRNA quantitation was performed using the comparative ΔΔCt method (Caserta et al., 2006). Briefly, any

sample that deviated by more than half a CT was excluded from the analysis. The RQ value for each sample from the triplicate conditions was determined and compared to the average GAPDH values. The average RQ values were graphed; the error bars represent the standard deviation from the triplicate conditions.

5. *Immunoprecipitation Assay:* H1299 cells were seeded at a density of 4.5 x 10^5 cells/6 cm plate. At 24 hr after plating the cells, expression plasmids encoding HA tagged wildtype TAp63γ and GST tagged TAp63γ mutants were transiently transfected either alone or in combination, as indicated. Cells were washed with 1X DPBS and then harvested for total protein using RIPA (radioimmunoprecipitation assay) buffer (0.5% sodium deoxycholate, 1% NP40, 0.1% SDS, PBS, pH 7.4) mixed with 1% PIC (Sigma). Briefly, total protein was pre cleared with 20 μL of recombinant-protein G-sepharose beads (Invitrogen, Carlsbad, CA) for 1 hr at 4^{o}C. After pre clearing beads were removed by centrifugation at 13,000 rpm for 1 min. The total protein was incubated with rotation for O/N at 4^{o}C with 1 μg of monoclonal anti-HA 12CA5 antibody (Roche Diagnostics, Indianapolis, IN). Next day immunoprecipitated samples were incubated with rec-protein G-sepharose beads for 1 hr followed by four washes with RIPA buffer to remove the unbound proteins. Immunoprecipitated samples with beads were run on 10% SDS gel and immunoblotted with rabbit polyclonal anti-GST Z5 antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

6. *Immunofluorescence studies:* H1299 cells were plated on sterilized coverslips at a density of 1.5 x 10^5cells/well of a 6 well plate. At 24 hr after seeding, expression plasmids

encoding HA-tagged wildtype TAp63γ or GST tagged TAp63γ mutants were transiently transfected either alone or in combination. For immunofluorescence staining, after washing with 1X DPBS, colonies were fixed for 8 min with 3% paraformaldehyde and permeabilized for 20 min with 1.0% Triton X-100. Cells were blocked with 0.5% normal goat serum (NGS) and incubated with primary antibodies for 1 hr at room temperature. Primary antibodies used to detect HA-TAp63γ wildtype and GST tagged mutants were mouse monoclonal anti-HA 12CA5 (Roche Diagnostics, Indianapolis, IN) at a dilution of 1:100 and rabbit polyclonal anti-GST Z5 (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:200. After three washes with 0.5% (NGS), cells were incubated with secondary goat anti-rabbit, fluorescein isothiocyanate (FITC)-conjugated immunoglobulin G (IgG) antibody (Jackson Immunoresearch, West Grove, PA, USA) at a dilution of 1:250, and secondary donkey anti-mouse, texas red (TR) dye-conjugated IgG antibody (Jackson Immunoresearch, West Grove, PA, USA) at a dilution of 1:275 for 1 hr at room temperature. Hoechst dye 33342 (Sigma, St. Louis, MO) was used for nuclear staining. Preparations were examined using fluorescence microscopy.

7. *Flow cytometry:* H1299 cells were plated at a density of 2.25 x 10^5 cells/6 well plate and co-transfected with expression plasmids encoding membrane bound hybrid-US9GFP (PAB35) with either TAp63γ or TAp63γ mutants or empty vector using Lipofectamine 2000. Membrane bound GFP plasmid (PAB35) was cotransfected along with either TAp63γ or TAp63γ mutants, to distinguish the transfected cells from the non-transfected cells. Healthy cells were used as a control for this experiment. At 48 hr post transfection, cells were harvested for flow cytometry. Cells were collected by trypsinization using

0.25% Trypsin-EDTA (Gibco), pelleted by centrifugation at 12,000 rpm for 5 min and resuspended in phosphate-buffered saline (1X PBS). Two volumes of cold, 70% ethanol were added dropwise, while vortexing and the samples were stored at 20°C until the day of analysis. At that time, cells were pelleted as mentioned above and resuspended in staining solution (50 μg/mL Propidium Iodide, 32 μg/mL RNase A (Sigma, St Louis, MO) in PBS). Samples were moved to flow tubes and stored at 4°C for at least 1 hr in the dark prior to analysis. Flow cytometric analysis for GFP and Propidium Iodide (PI) fluorescence was performed using CellQuest software (Becton Dickinson Immunocytometry Systems (BDIS), San Jose, CA). For each analysis 10,000 gated events were collected to permit cell cycle analysis of all cells and GFP cell subpopulations. The GFP and PI fluorescence signals were separated with a 560 shortpass dichroic mirror and collected with a 530/30 bandpass (FL1, GFP) and 572/26 bandpass (FL2, PI). Data analysis was performed using CellQuest (BDIS). The GFP fluorescence was collected on a logarithmic scale and the PI fluorescence was collected on a linear scale.

8. *Colony Formation Assay:* H1299 cells were seeded at a density of $2.5x10^5$ cells/well in a 6 well plate and transfected with expression plasmids encoding TAp63γ, TAp63γ mutants or vector using Lipofectamine 2000 as indicated. At 24 hr post transfection, cells were trypsinized in 500 μL of 0.25% Trypsin-EDTA (Gibco), pelleted and resuspended in fresh complete media for counting. From each condition 1000 cells were counted on a hemocytometer and then replated in each well of a 6 well plate, media was changed after every 2 days. After 15 days of seeding and monitoring cell growth, the media was

aspirated and cells were washed with 1X DPBS and 1 mL of crystal violet dye (0.1% crystal violet in 10% ethanol) was added to each well for 5 min. The plates were subsequently washed with water twice and left to dry at room temperature for 2 days. At this point the pictures for each of the samples were taken. Subsequently, 1 mL of 10% acetic acid was added to destain the cells for 30 min at RT. 100 µL of this destained solution from each well was transferred to 96 well plate and read at 590 nm using spectrophotometer.

9. *RNA isolation/cRNA preparation:* Cells were lysed directly in culture plate and total RNA was isolated using the RNAeasy method as per manufacturer's protocol (Qiagen, Valencia, CA). The RNA was quantified by spectrophotometer reading at 260 nm and integrity of the total RNA was determined by agarose gel electrophoresis and by spectrophotometer reading ratio at 260/280. A ratio of 1.8-2.0 was considered optimum for further analysis. First strand cDNA synthesis was performed with 5 µg of total RNA, 100 pmols of T7-oligo (dT) primer and 200 units of Superscript II enzyme (Invitrogen). RNase H-dependent second strand synthesis was performed using 10 units of DNA ligase and 40 units of DNA polymerase I. The double-stranded cDNA was purified using GeneChip Sample Cleanup Module (Affymetrix, Inc., Santa Clara, CA). Biotin-labeled cRNA was prepared employing GeneChip IVT Labeling Kit (Affymetrix) according to manufacturer's protocol, using T7 RNA polymerase and biotinylated nucleotides to produce a labeled single stranded cRNA. The cRNA was purified using GeneChip Sample Cleanup Module and quantified using Agilent (PE Applied Biosystems, Foster City, CA) Bioanalyzer. 20 µg of cRNA was used for fragmentation utilizing GeneChip Sample Cleanup Module as per manufacturer's protocol. A small portion of the

fragmented and non fragmented cRNA was subjected to agarose gel electrophoresis to assess quality of cRNA and fragmentation reaction.

10. *Array hybridization and scanning:* 15 μg of fragmented cRNA along with non eukaryotic spike controls were hybridized to Affymetrix HG-U133A GeneChips containing 39,000 transcript variants. Hybridization was performed for 16 hr in an Affymetrix GeneChip Hybridization oven 640 at 45^{o}C using constant rotation at 60 rpm. GeneChips were subjected to washing and staining on Affymetrix GeneChip Fluidics Station 400 according to manufacturer's protocol. Immediately after staining, the GeneChips were scanned at 570 nm on an Affymetrix Scanner 3000 following protocols developed by Affymetrix for the HG-U133A arrays. The digitized images from the scanned chips were processed using Affymetrix Microarray Suite (MAS) version 5.0 and global scaling to a target intensity of 150 was applied to all chips prior to analysis.

11. *Data Mining:* All analyses were performed using Affymetrix MAS5.0 and GeneSpring version GX7.3.1. The Affymetrix CHP files were first imported into GeneSpring. In GeneSpring, the chips were normalized to the fiftieth percentile and each gene normalized to its median relative expression. To identify the genes which were modulated by each of the TAp63γ mutants when compared to wildtype TAp63γ, the following data mining approach was employed. First, only those genes that were identified as being present or marginal in at least two of the three replicates in the condition where expression is expected using Filter on Flags were included in the lists. Next, gene lists containing genes that showed at least a 2 fold or greater increases or

decreases relative to wildtype TAp63γ using Filter on Fold Change were created. Based on these criteria the genes that passed through both these filters were identified using Venn diagrams from GeneSpring. The subset of genes that made it to both these lists were then passed through ANOVA statistical tool for a statistical significance of $p \leq 0.05$. This approach made our data analysis more stringent. We also utilized NCBI PubMed and OMIM for getting more functional description on these genes.

12. *Functional pathway analysis:* The list of genes with significant changes in gene expression (both increases and decreases) based on the microarray experiments were exported from GeneSpring into Ingenuity Pathways Analysis (IPA) 5.0 (Ingenuity Systems, Redwood City, CA) to create pathway maps of interacting genes. The core analysis identified the pathways from the canonical pathways that were significant to our data sets. Genes from the data sets that were associated with a canonical pathway in the Ingenuity knowledge base were considered for analysis.

III. *Results:*

Differential effect of TAp63γ mutants on p53/p63 targets: We examined the effect of wildtype TAp63γ and naturally occurring TAp63γ mutants on PG13-Luc, Hdm2-Luc and Maspin-Luc reporters in H1299 (p53 -/-) and HCT116 (p53-/-) cells, all 3 targets shown to be regulated by both p63 and p53 (Figure 4). As expected the wildtype TAp63γ significantly upregulated the reporter activity of all reporters tested in both these cell lines. In addition, TAp63γ (R227Q) and TAp63γ (R298Q) exerted similar effects as wildtype TAp63γ and showed significant upregulation of all the reporters. Interestingly, TAp63γ (K194E) observed only in SHFM syndrome led to a significant increase in all the 3 reporters tested. On the other hand, TAp63γ (R280C) observed both in SHFM and EEC also upregulated PG13-Luc and Hdm2-Luc but did not affect the Maspin-Luc reporter activity. Finally, TAp63γ (R279H), TAp63γ (R204W) and TAp63γ (C306R) all observed only in EEC syndrome had little or no effect on the reporter activity of all reporters tested. A summary chart of the results obtained from transactivation of these 3 reporters in both H1299 and HCT 116 (p53-/-) cell lines indicating the fold change relative to vector control is included (Table 1).

Next, we examined the effect of these mutants on endogenous transcript levels of Hdm2 and p21 (Figure 5A). H1299 cells were transfected with TAp63γ and TAp63γ mutants or empty vector backbone and the relative expression of these genes was assessed using TaqMan based real time

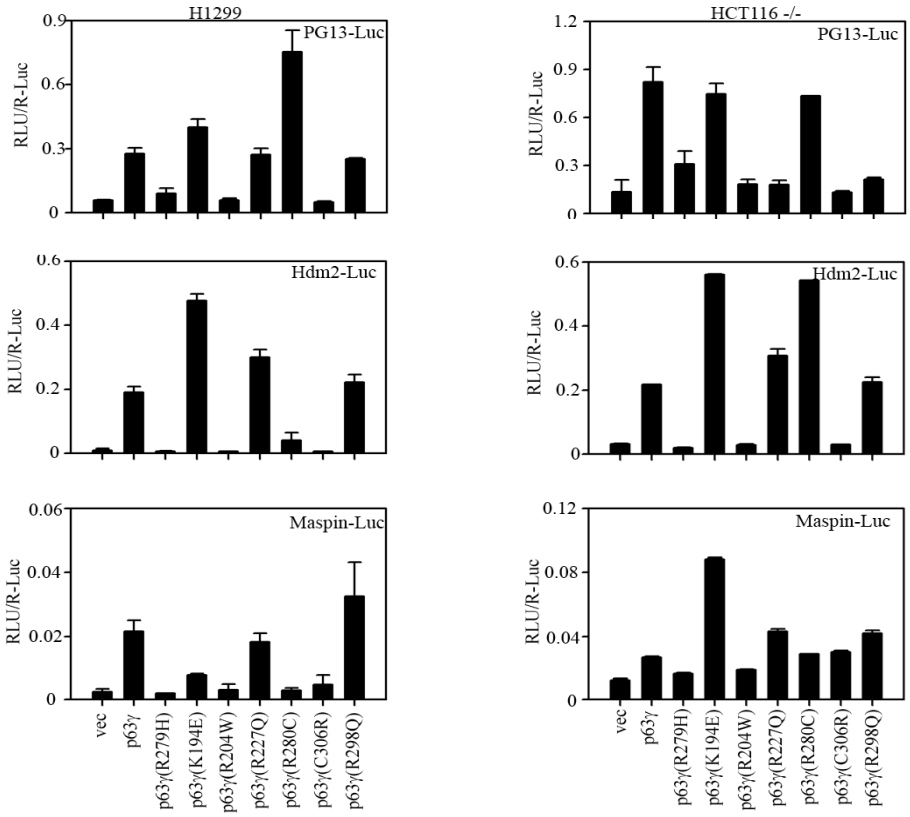

H1299
HCT116 -/-
PG13-Luc
Hdm2-Luc
Maspin-Luc
RLU/R-Luc
0.9
0.6
0.3
0
1.2
0.9
0.6
0.3
0
0.6
0.4
0.2
0
0.6
0.4
0.2
0
0.06
0.04
0.02
0
0.12
0.08
0.04
0
vec
p63γ
p63γ(R279H)
p63γ(K194E)
p63γ(R204W)
p63γ(R227Q)
p63γ(R280C)
p63γ(C306R)
p63γ(R298Q)

Figure 4: *Differential effect of TAp63γ mutants on transactivation of p53/p63 targets.* H1299 and HCT116 p53-/- cells were transfected with PG13-Luc, Maspin-Luc and Hdm2-Luc reporter alone (100 ng) or along with wildtype TAp63γ or TAp63γ mutants (1 μg) and CMV-R-Luc (10 ng) plasmids using Lipofectamine 2000. At 24 hr post transfection cells were harvested and subjected to dual luciferase assay as per manufacturer's protocol. Y-axis represents RLU/R-Luc relative luciferase units normalized for transfection efficiency. Error bars represent standard deviations.

	PG13-Luc		Hdm2 Luc		Maspin Luc	
Samples	**H1299**	**HCT -/-**	**H1299**	**HCT -/-**	**H1299**	**HCT -/-**
Vector	**1.0 ± 0.0**	**1.0 ± 0.1**	**1.0 ± 0.0**	**1.0 ± 0.0**	**1.0 ± 0.0**	**1.0 ± 0.0**
WTp63	**4.7 ± 0.0**	**6.1 ± 0.1**	**23.4 ± 0.0**	**7.0 ± 0.0**	**9.0 ± 0.0**	**2.2 ± 0.0**
R279H	**1.5 ± 0.0**	**2.3 ± 0.1**	**0.7 ± 0.0**	**0.6 ± 0.0**	**0.8 ± 0.0**	**1.3 ± 0.0**
K194E	**6.8 ± 0.0**	**5.6 ± 0.1**	**58.6 ± 0.0**	**18.1 ± 0.0**	**3.3 ± 0.0**	**7.1 ± 0.0**
R204W	**1.0 ± 0.0**	**1.4 ± 0.0**	**0.7 ± 0.0**	**0.9 ± 0.0**	**1.3 ± 0.0**	**1.5 ± 0.0**
R227Q	**4.6 ± 0.0**	**1.3 ± 0.0**	**36.8 ± 0.0**	**9.9 ± 0.0**	**7.6 ± 0.0**	**3.5 ± 0.0**
R280C	**12.8 ± 0.1**	**5.5 ± 0.0**	**4.9 ± 0.0**	**17.5 ± 0.0**	**1.2 ± 0.0**	**2.3 ± 0.0**
C306R	**0.8 ± 0.0**	**1.0 ± 0.0**	**0.7 ± 0.0**	**0.9 ± 0.0**	**1.9 ± 0.0**	**2.4 ± 0.0**
R298Q	**4.3 ± 0.0**	**1.6 ± 0.0**	**27.2 ± 0.0**	**7.2 ± 0.0**	**13.6 ± 0.0**	**3.4 ± 0.0**

Table 1: *Summary of the transactivation data indicating the differential effect of TAp63γ mutants on p53/p63 target genes.* The numbers in the column represent the fold change relative to empty vector control. The numbers in red and blue represent SHFM mutants (K194E and R280C) and mutants that mimic wildtype p63 (R227Q and R298Q) respectively.

PCR. Consistent with our results from the transactivation data (Figure 4) cells transfected with TAp63γ, TAp63γ(R227Q), TAp63γ(R298Q), TAp63γ(K194E) and TAp63γ(R280C) showed a significant increase in the Hdm2 and p21 transcript levels. Once again, TAp63γ(R279H), TAp63γ (R204W) and TAp63γ(C306R) had little or no effect on the transcript levels of both p21 and Hdm2. Similar results were observed in HCT (p53-/-) (Figure 5). To correlate the increase in transcript level of Hdm2 and p21 to s e results demonstrate that while TAp63γ(R227Q) and TAp63γ(R298Q) mutants behave like wildtype p63, the mutants observed in SHFM syndrome, TAp63γ (K194E and R280C) also significantly induce targets specific for both p53/p63. teady state protein levels, we examined the effects of mutant p63 on the endogenous Hdm2 and p21 protein levels using immunoblot analysis. We observed a significant increase in both Hdm2 and p21 protein expression levels in cells transfected with wildtype TAp63γ, TAp63γ (K194E), TAp63γ (R227Q) and TAp63γ (R298Q) and a modest increase with TAp63γ(R280C) mutant (Fig 5B). As shown in Figure 5 wildtype TAp63γ is highly unstable and both TAp63γ (R227Q) and TAp63γ (R298Q) mutants (Figure 4) mimic wildtype TAp63γ not only in its ability to transactivate target genes but are as unstable as its wildtype counterpart (Figure 5B).

TAp63γ(R227Q) and TAp63γ(R298Q) mimic wildtype TAp63γ in regulation of a p63 specific target: Having demonstrated the differential effect of these naturally occurring mutants on gene targets regulated by both p53 and p63, we then tested the ability of TAp63γ and TAp63γ mutants to regulate Shh, previously shown by our laboratory to be a p63 specific target gene (Caserta et al., 2006). We examined the effects of wildtype and mutant TAp63γ on the transcripts and protein levels of Shh. As shown in Figure 6A and 6B, TAp63γ (R227Q) and TAp63γ (R298Q)

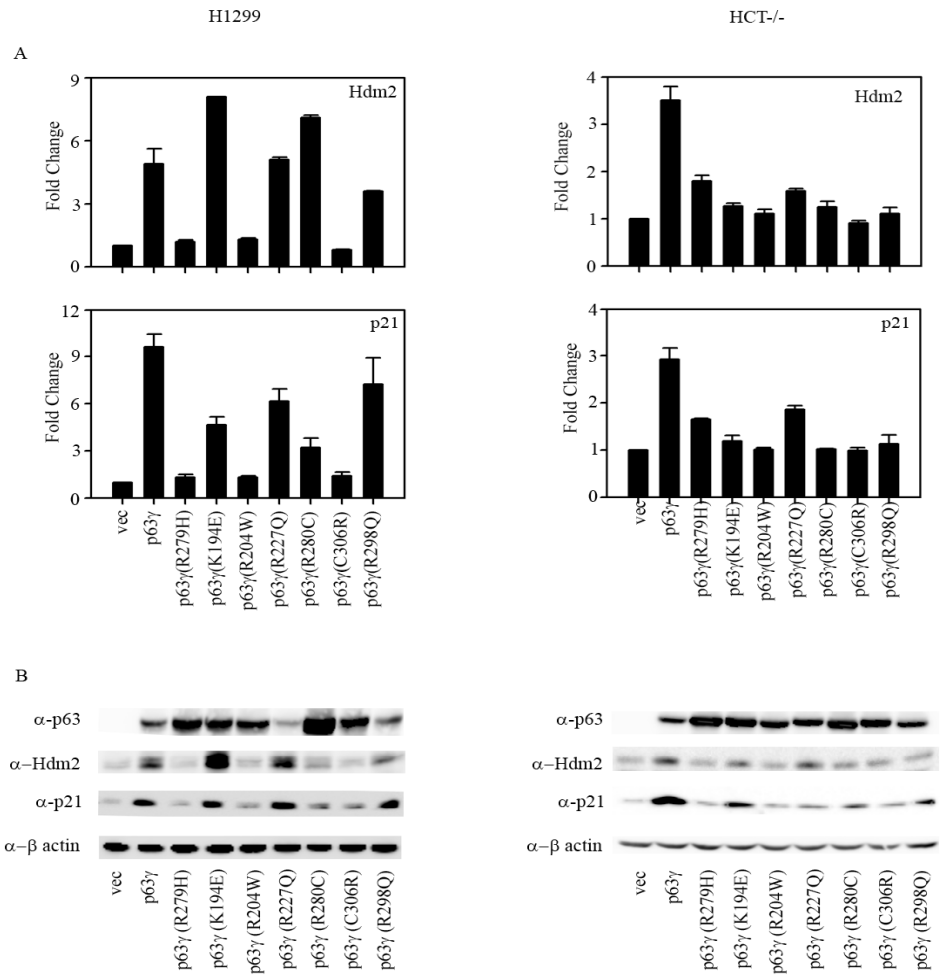

H1299
HCT-/-
A
Hdm2
p21
Fold Change
vec
p63γ
p63γ(R279H)
p63γ(K194E)
p63γ(R204W)
p63γ(R227Q)
p63γ(R280C)
p63γ(C306R)
p63γ(R298Q)
B
α-p63
α–Hdm2
α-p21
α–β actin

Figure 5: *Differential effects of TAp63γ mutants on p53/p63 target genes at transcript and protein levels.* A) To determine the effect of wildtype TAp63γ and TAp63γ mutants on endogenous target gene expression using TaqMan based real time-PCR, H1299 and HCT p53-/- cell lines were transfected with 3 μg of TAp63γ or TAp63γ mutants or empty vector alone. At 24 hr post transfection, total RNA was extracted and target gene expression was evaluated using RT-PCR. Y-axis represents fold change in Hdm2 and p21 transcript levels relative to vector transfected cells. B) Immunoblot analysis was performed to confirm overexpression of p63 and endogenous expression of p53 and p63 specific targets, Hdm2 and p21. Immunoblotting for β-actin served as the loading control.

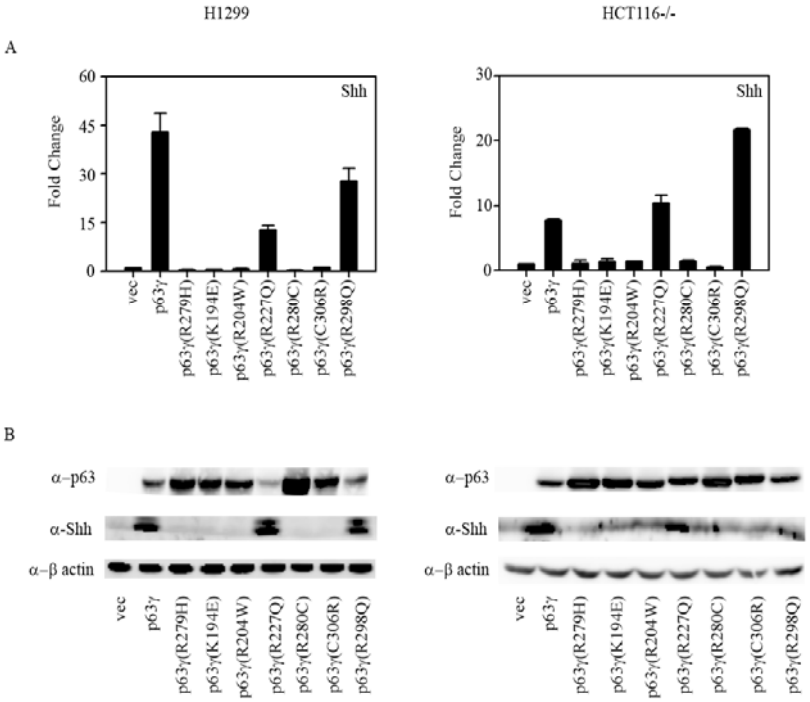

H1299
HCT116-/-
A
Shh
Fold Change
60
45
30
15
0
30
20
10
0
vec
p63γ
p63γ(R279H)
p63γ(K194E)
p63γ(R204W)
p63γ(R227Q)
p63γ(R280C)
p63γ(C306R)
p63γ(R298Q)
B
α–p63
α-Shh
α–β actin

Figure 6: *TAp63γ(R227Q) and TAp63γ(R298Q) mimic wildtype TAp63γ in their ability to induce Shh.* A) Shh transcript level in H1299 and HCT-/- cells transfected with TAp63γ or TAp63γ mutant's expression plasmids was detected using TaqMan real time-PCR. The plasmid were used at a concentration of 3 μg. Y-axis represents fold change in Shh expression relative to vector transfected cells. B) Immunoblot analysis was performed to confirm the overexpression of p63 and endogenous expression of p63 specific target, Shh. Immunoblotting for β-actin served as the loading control.

along with wildtype TAp63γ led to a significant increase in the Shh transcript levels and a corresponding increase in the protein levels of Shh. These results further demonstrate that TAp63γ(R227Q) and TAp63γ(R298Q) retain the transactivation ability of wildtype TAp63γ.

TAp63γ mutants do not affect TAp63γ mediated regulation of Hdm2: In order to assess the effects of TAp63γ mutants on wildtype TAp63γ mediated transactivation of p53 responsive genes, H1299 cells were transfected with Hdm2-Luc reporter along with TAp63γ alone or with increasing doses of TAp63γ mutants as indicated (Figure 7). As expected, TAp63γ led to a significant increase in Hdm2 reporter activity. Interestingly the TAp63γ(R279H), TAp63γ(R204W) and TAp63γ(C306R) mutants which were unable to transactivate Hdm2 by themselves, did not inhibit wildtype TAp63γ mediated transactivation of Hdm2-Luc (Figure 7A, 7C and 7F). Mutants TAp63γ(K194E), TAp63γ(R227Q) and TAp63γ(R298Q) which by themselves can induce Hdm2-Luc reporter activity did not result in a significant synergistic effect when co-transfected with wildtype TAp63γ (Figure 7B, 7D and 7G). Finally, TAp63γ(R280C) which induces a modest increase in Hdm2-Luc reporter activity led to a dose dependent synergistic increase in Hdm2-Luc reporter activity when co-transfected with wildtype TAp63γ (Figure 7E).

Next we examined the effects of TAp63γ mutants on wildtype mediated induction of endogenous transcript levels of Hdm2 and p21 (Figure 8A). Consistent with our results from the transactivation data (Figure 7) none of the TAp63γ mutants had any affect on TAp63γ mediated

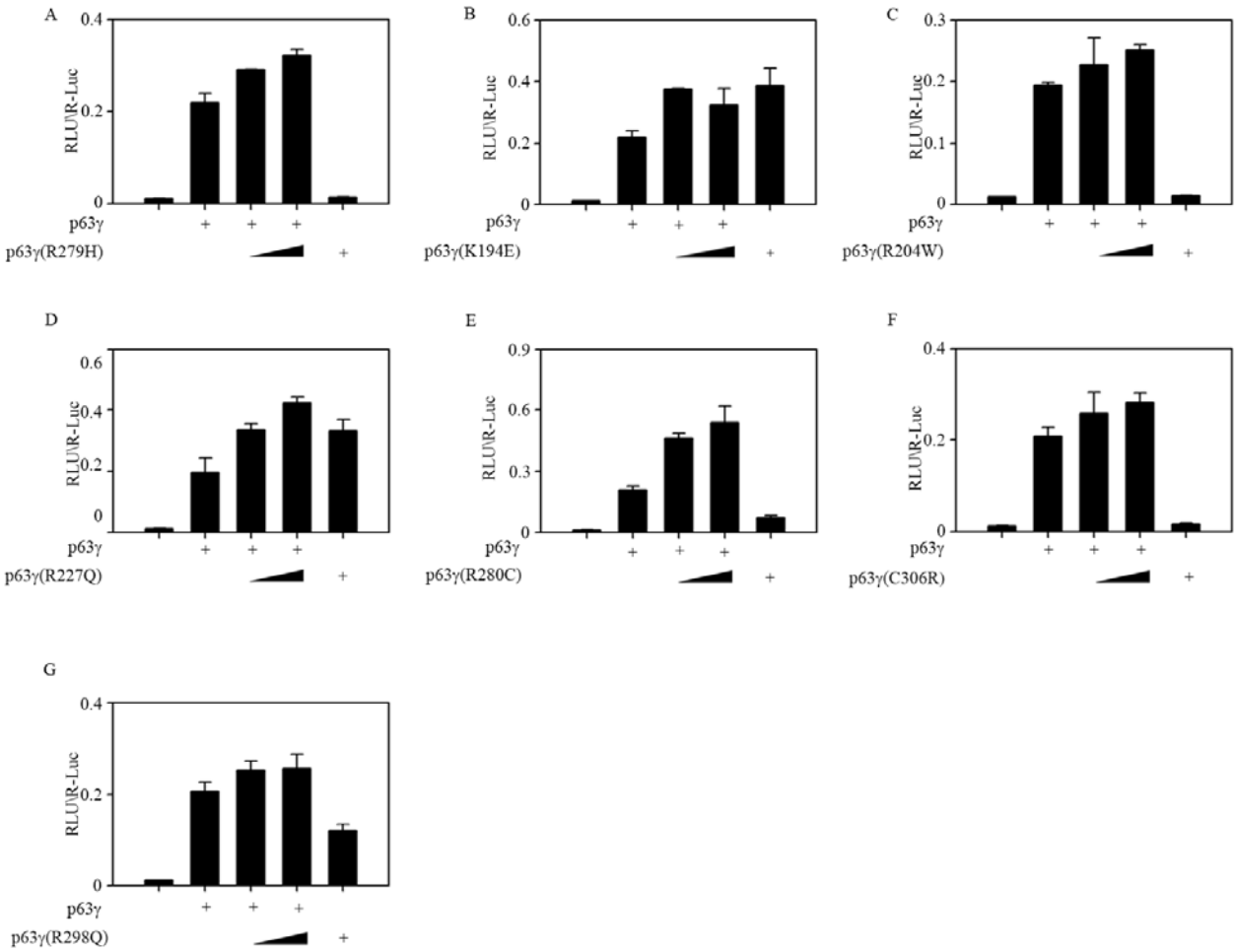
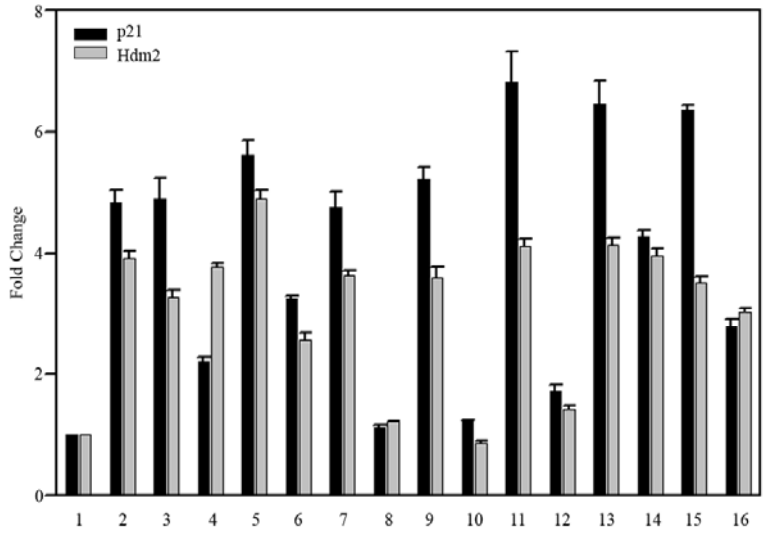

A
RLU/R-Luc
0.4
0.2
0
p63γ
+ + +
p63γ(R279H)
+
B
RLU/R-Luc
0.6
0.4
0.2
0
p63γ
+ + +
p63γ(K194E)
+
C
RLU/R-Luc
0.3
0.2
0.1
0
p63γ
+ + +
p63γ(R204W)
+
D
RLU/R-Luc
0.6
0.4
0.2
0
p63γ
+ + +
p63γ(R227Q)
+
E
RLU/R-Luc
0.9
0.6
0.3
0
p63γ
+ + +
p63γ(R280C)
+
F
RLU/R-Luc
0.4
0.2
0
p63γ
+ + +
p63γ(C306R)
+
G
RLU/R-Luc
0.4
0.2
0
p63γ
+ + +
p63γ(R298Q)
+

Figure 7: *TAp63γ mutants do not affect the TAp63γ mediated transactivation of Hdm2 and p21 (p53/p63 common target).* H1299 cell lines were co-transfected with the 100 ng of Hdm2-Luc reporter alone or with wildtype HA-TAp63γ and GST-TAp63γ mutants alone or in combination (in 1:2 and 1:4 ratios, where 1 corresponds to 200 ng of plasmid) as indicated using Lipofectamine 2000. At 24 hr post transfection, whole cell extracts were subjected to Dual Luciferase Assay. Y axis represents relative luciferase units normalized to transfection efficiency.

regulation of Hdm2 and p21 transcript levels. To correlate the effects of TAp63γ mutants on wildtype mediated induction of transcript levels to steady state protein levels, we examined the effects of mutant p63 on wildtype TAp63γ mediated induction of Hdm2 and p21 protein expression levels. As shown in Figure 8B, none of the mutants affected wildtype p63 mediated induction of Hdm2 and p21. Immunoblotting confirmed the overexpression of GST-TAp63γ mutants and wildtype HA-TAp63γ. Immunoblotting for actin was used as a loading control. Altogether, our data shows that TAp63γ mutants do not inhibit the wildtype TAp63γ mediated induction of p53/p63 gene.

TAp63γ mutants observed in some EEC syndrome inhibit TAp63γ mediated Shh induction: We examined the effects of TAp63γ mutants on wildtype TAp63γ mediated Shh-Luc reporter activity and Shh protein expression, a target gene regulated by p63 but not p53 (Caserta et al., 2006). This was achieved by transfecting H1299 cells with Shh-Luc reporter, wildtype TAp63γ and TAp63γ mutants as indicated in Figure 8. We observed that TAp63γ induced transactivation of Shh reporter was significantly inhibited by TAp63γ(R279H), TAp63γ(R204W) and TAp63γ(R280C) mutants in a dose dependent manner (Figure 9A, 9C and 9E). Interestingly, once again the TAp63γ(R227Q) and TAp63γ(R298Q) mutants (Figure 9D and 9G) show only a modest increase in the transactivation of Shh reporter when co-transfected with wildtype TAp63γ. Finally, although the TAp63γ(C306R) and TAp63γ(K194E) by themselves do not induce Shh reporter activity, they do not affect the ability of wildtype TAp63γ to induce Shh reporter activity (Figure 9B and 9F).

A


p21
Hdm2
Fold Change
0
2
4
6
8
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

B

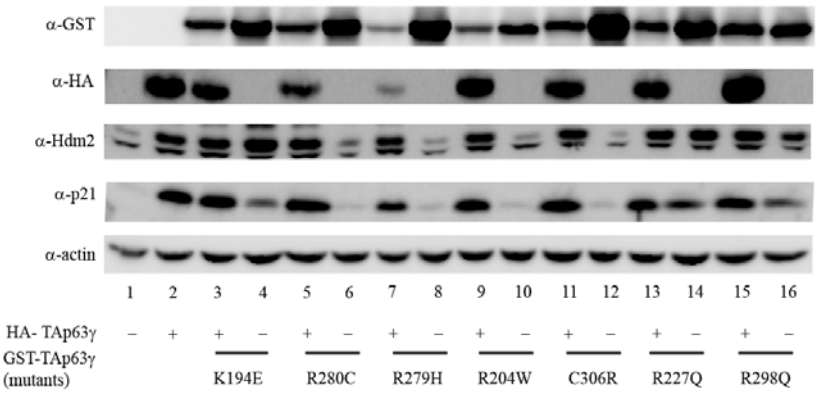

α-GST
α-HA
α-Hdm2
α-p21
α-actin
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
HA- TAp63γ
GST-TAp63γ (mutants)
K194E
R280C
R279H
R204W
C306R
R227Q
R298Q

Figure 8: *TAp63γ mutants do not affect the TAp63γ mediated effects on its target gene expression at transcript and protein levels*. H1299 cells were transfected with either GST-TAp63γ mutants alone or in combination with wildtype HA-TAp63γ in equal amounts (1.5 μg) using Lipofectamine 2000 as indicated. A) Hdm2 and p21 transcript levels were detected using TaqMan based real time PCR. Y axis represents the fold change in transcript levels relative to vector transfected cells. B) Immunoblot analysis of cell extracts harvested at 24 hr post transfection was resolved on SDS-PAGE and probed using anti-GST, anti-HA, anti-Hdm2, anti-p21 and anti-actin.

Next we examined the effects of these mutants on endogenous expression of Shh and VDR (Figure 10A). For this H1299 cells were transfected with either GST-TAp63γ mutants alone or in combination with wildtype HA-TAp63γ and the relative expression of these genes was assessed using TaqMan based real time PCR. Consistent with our results from the transactivation data (Figure 9) cells transfected with TAp63γ(R279H), TAp63γ(R204W) and TAp63γ(R280C) significantly inhibited the wildtype TAp63γ mediated induction of Shh and VDR transcript levels.

To correlate the decrease in the transcript level of Shh steady state protein level, we then examined the effect of mutant p63 on endogenous Shh protein level using immunoblot analysis. We observed a significant inhibition of the Shh protein expression levels in cells transfected with TAp63γ(R279H), TAp63γ(R204W) and TAp63γ(R280C) and a modest increase with TAp63γ(R227Q) and TAp63γ(R298Q) as shown in Figure 10B. Also, the mutants TAp63γ(K194E) and TAp63γ(C306R) as expected did not affect Shh expression both at transcript and protein levels. Immunoblot analysis confirmed the overexpression of HA tagged TAp63γ and GST tagged TAp63γ mutants. p21 was used as a positive control in immunoblot analysis. Taken together our data shows that TAp63γ mutants observed in EEC syndrome significantly inhibited the wildtype TAp63γ mediated induction of specific p63 gene (Shh).

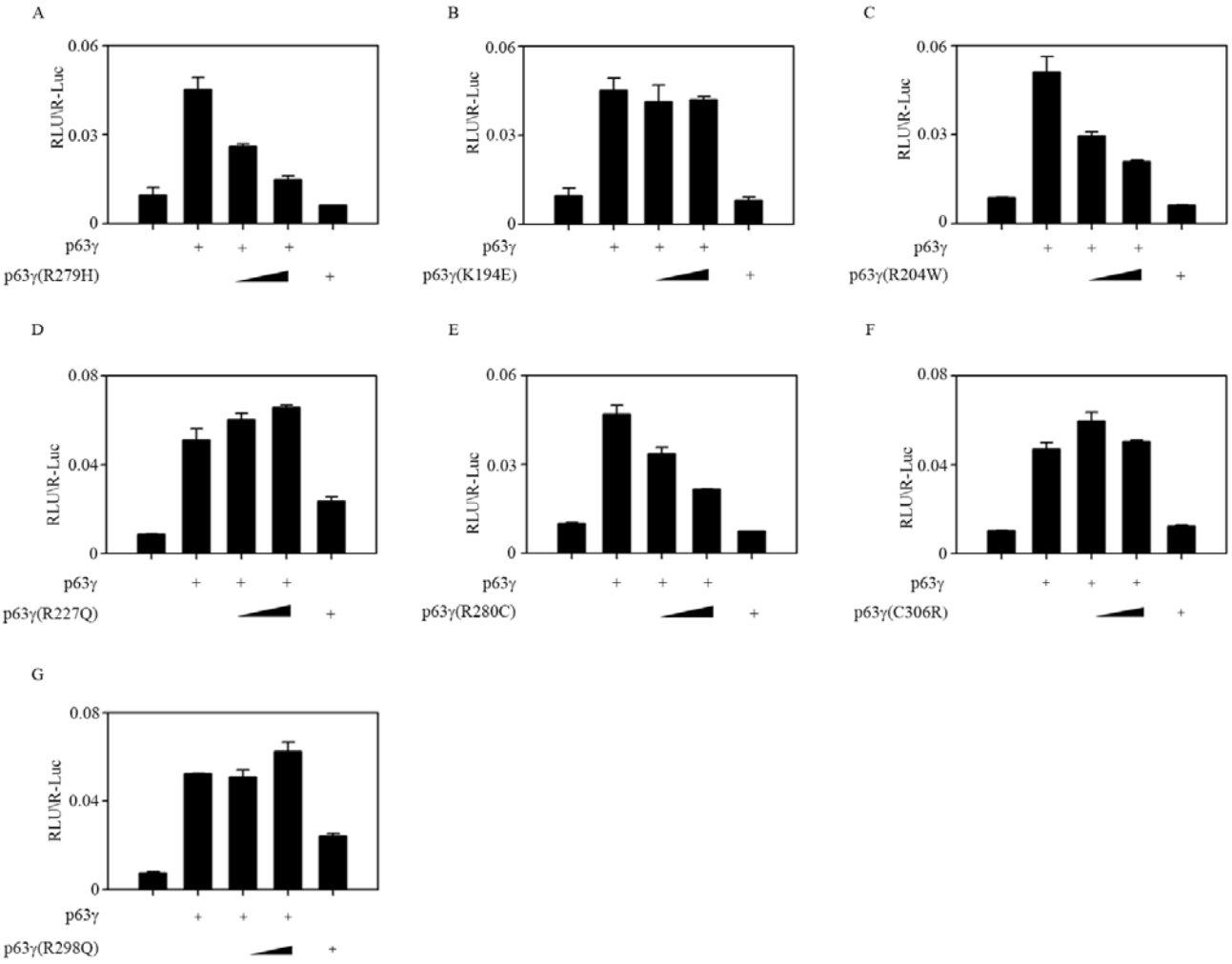
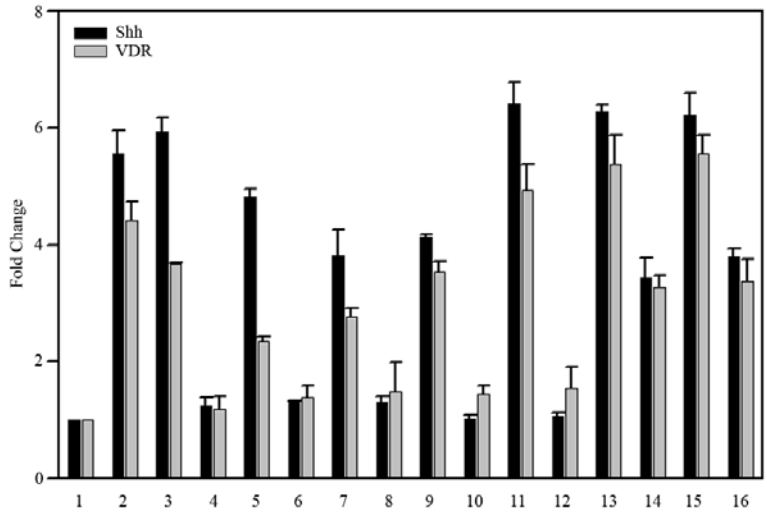

A
B
C
D
E
F
G
RLU/R-Luc
0.06
0.03
0
0.08
0.04
p63γ
+
p63γ(R279H)
p63γ(K194E)
p63γ(R204W)
p63γ(R227Q)
p63γ(R280C)
p63γ(C306R)
p63γ(R298Q)

Figure 9: *EEC mutants inhibit the wildtype TAp63γ mediated induction of Shh.* H1299 cells were co-transfected with the 100 ng of Shh-Luc reporter plasmid alone or with wildtype HA-TAp63γ alone or with increasing concentration of GST-TAp63γ mutants (1:2 and 1:4 ratios, where 1 corresponds to 200 ng of plasmid) as indicated, using Lipofectamine 2000. At 24 hr post transfection, whole cell extracts were subjected to Dual Luciferase Assay. Y axis represents relative luciferase units normalized to transfection efficiency relative to empty vector transfected cells.

A


Shh
VDR
Fold Change
0
2
4
6
8
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

B

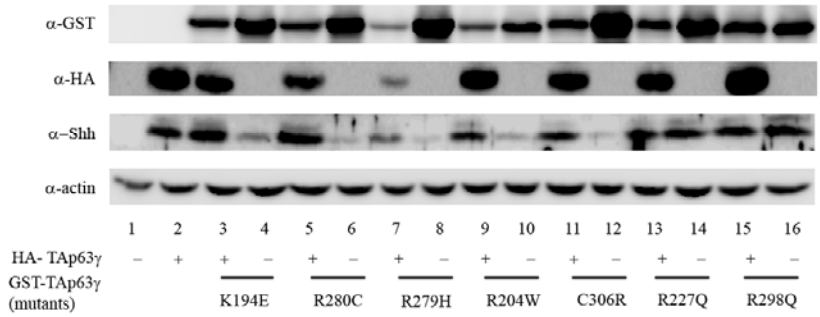

α-GST
α-HA
α−Shh
α-actin
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
HA- TAp63γ
GST-TAp63γ (mutants)
K194E
R280C
R279H
R204W
C306R
R227Q
R298Q

Figure 10: *EEC mutants inhibit the wildtype TAp63γ mediated induction of p63 specific target genes.* H1299 cells were transfected with either GST-TAp63γ mutants alone or in combination with wildtype HA-TAp63γ in equal amounts (1.5 μg) using Lipofectamine 2000 as indicated. A) Shh and VDR transcript levels were detected using TaqMan based real time PCR. Y axis represents the fold change in transcript levels relative to vector transfected cells. B) Immunoblot analysis of cell extracts harvested at 24 hr post transfection was resolved on SDS-PAGE and probed using anti-GST, anti-HA, anti-Shh and anti-actin.

TAp63γ mutants do not affect the localization of wildtype TAp63γ: Having observed the differential effect of TAp63γ mutants on the p53/p63 and p63 specific genes and the ability of EEC mutants to inhibit wildtype mediated effects on p63 specific targets we next assessed whether the effects of p63 mutants on induction of these genes could be as a result of effects of these mutants on the localization of wildtype TAp63γ. For this, we co-transfected H1299 cells with HA tagged TAp63γ alone or along with different GST tagged TAp63γ mutants. At 24 hr post-transfection, immunofluorescence assays were performed to study the localization of both wildtype and mutant p63. TAp63γ by itself was localized in the nucleus. The EEC mutants TAp63γ(R279H and R204W) that lead to a dose dependent decrease in TAp63γ mediated induction of Shh, a p63 specific target (Caserta et al., 2006), and TAp63γ(C306R) do not affect the localization of wildtype TAp63γ (Figure 11). Additionally, TAp63γ(R280C) mutant which is observed in both SHFM and EEC syndrome and also leads to a dose dependent decrease of wildtype p63 mediated Shh induction, did not have any effect on the localization of wildtype TAp63γ. Interestingly, TAp63γ(K194E) observed specifically in the SHFM syndrome, is the only mutant that showed partial cytoplasmic localization, however it also did not affect the localization of wildtype TAp63γ (Figure 12). Furthermore, TAp63γ(R227Q and R298Q) mutants that mimic wildtype, when co-transfected with wildtype TAp63γ also did not affect its localization (Figure 13). Taken together, we show that all the TAp63γ mutants by itself localized to the nucleus and when co-transfected with wildtype TAp63γ did not affect the nuclear localization of wildtype TAp63γ.

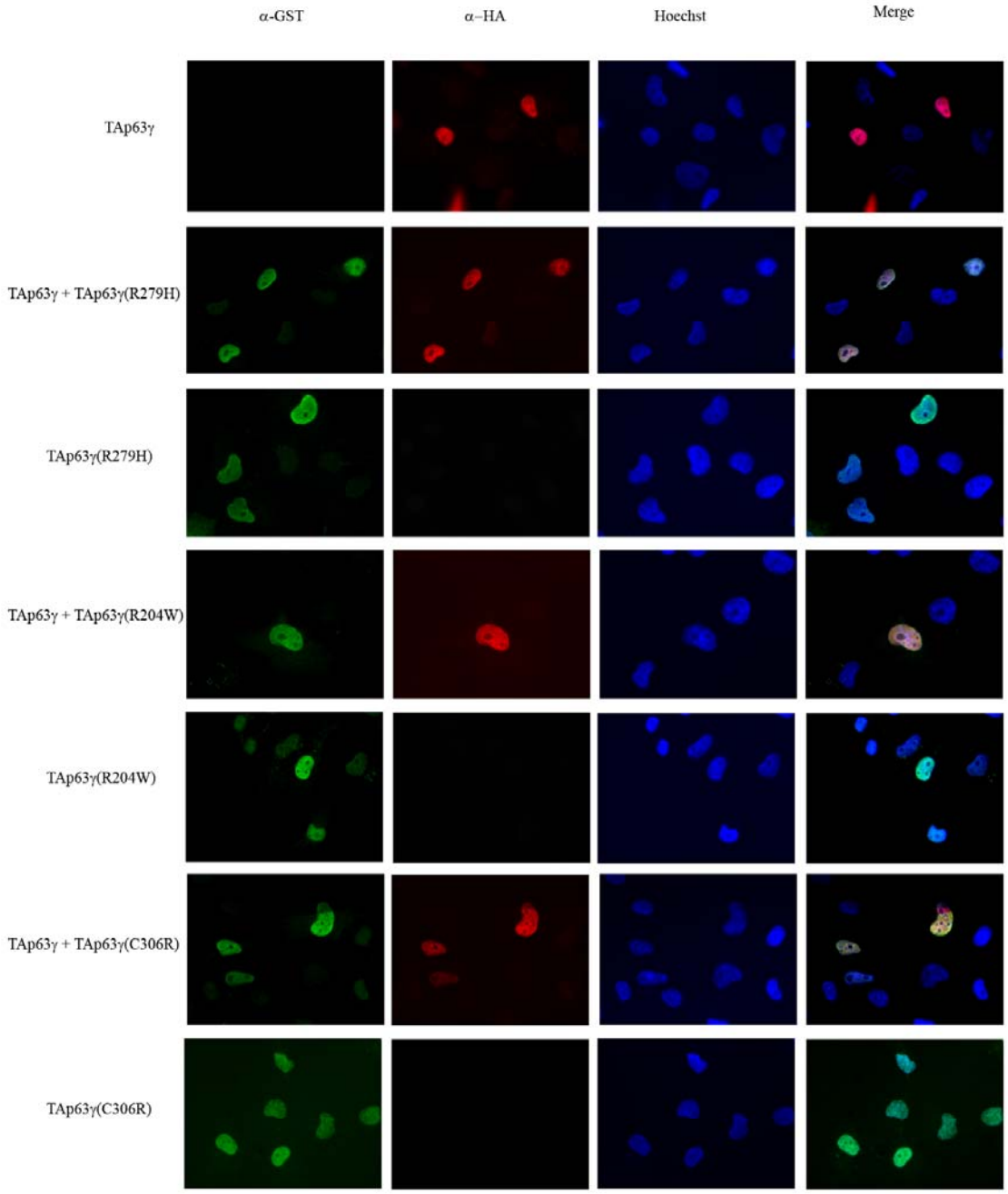

α-GST
α−HA
Hoechst
Merge
TAp63γ
TAp63γ + TAp63γ(R279H)
TAp63γ(R279H)
TAp63γ + TAp63γ(R204W)
TAp63γ(R204W)
TAp63γ + TAp63γ(C306R)
TAp63γ(C306R)

Figure 11: *TAp63γ mutants observed in EEC syndrome do not affect the localization of wildtype TAp63γ.* H1299 cells were transfected with GST tagged TAp63γ mutants alone (1 μg) or along with HA-tagged wildtype TAp63γ (0.5 μg). At 24 hr post transfection, cells were fixed with 3% paraformaldehyde. HA-TAp63γ and GST-TAp63γ mutant expression was detected using mouse anti-HA and rabbit anti-GST primary antibodies and corresponding anti-mouse Texas Red and anti-rabbit FITC-conjugated secondary antibodies. The nucleus was stained with Hoechst 33342, and the cells were examined under fluorescence microscope.

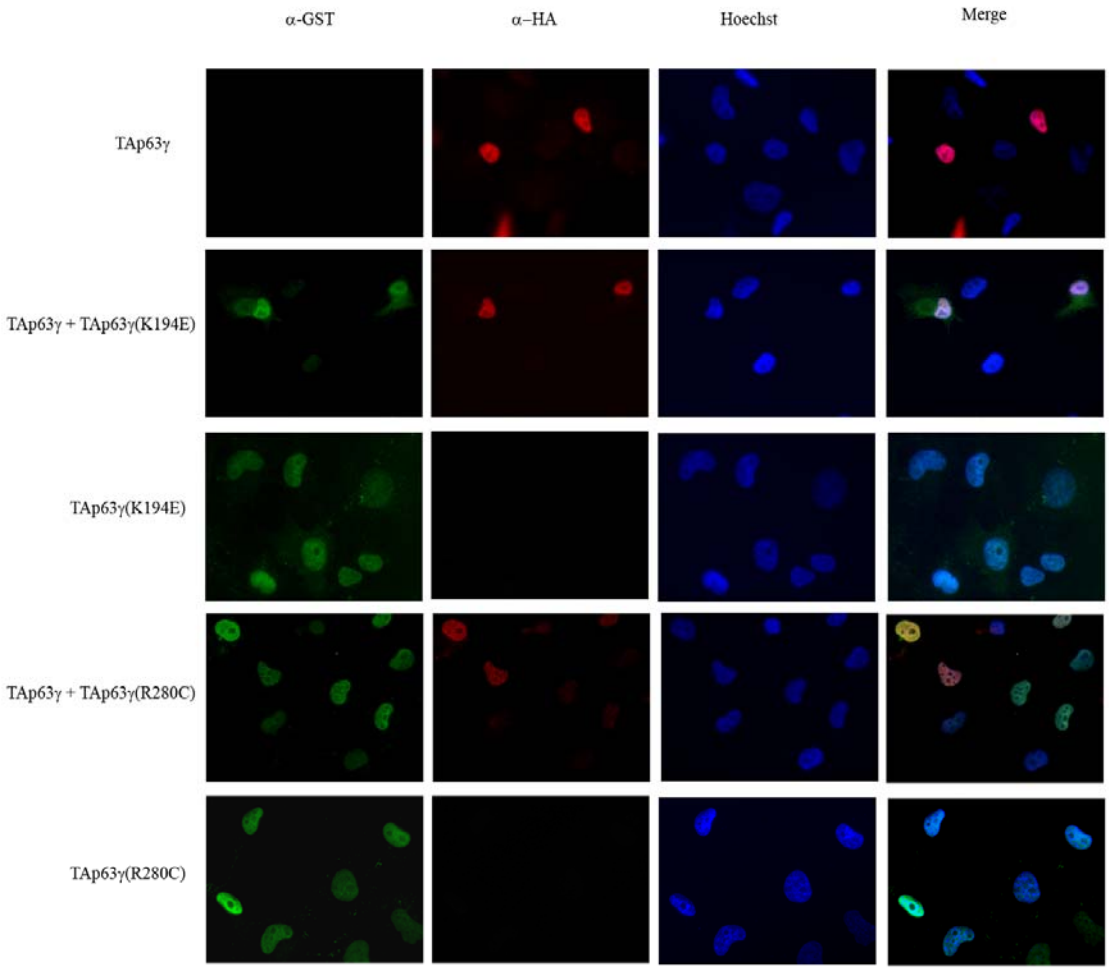

α-GST
α−HA
Hoechst
Merge
TAp63γ
TAp63γ + TAp63γ(K194E)
TAp63γ(K194E)
TAp63γ + TAp63γ(R280C)
TAp63γ(R280C)

Figure 12: *TAp63γ mutants observed in SHFM syndrome do not affect the localization of wildtype TAp63γ.* H1299 cells were transfected with GST tagged TAp63γ mutants alone (1 μg) or along with HA-tagged wildtype TAp63γ (0.5 μg). At 24 hr post transfection, cells were fixed with 3% paraformaldehyde. HA-TAp63γ and GST-TAp63γ mutant expression was detected using mouse anti-HA and rabbit anti-GST primary antibodies and corresponding anti-mouse Texas Red and anti-rabbit FITC-conjugated secondary antibodies. The nucleus was stained with Hoechst 33342, and the cells were examined under fluorescence microscope.

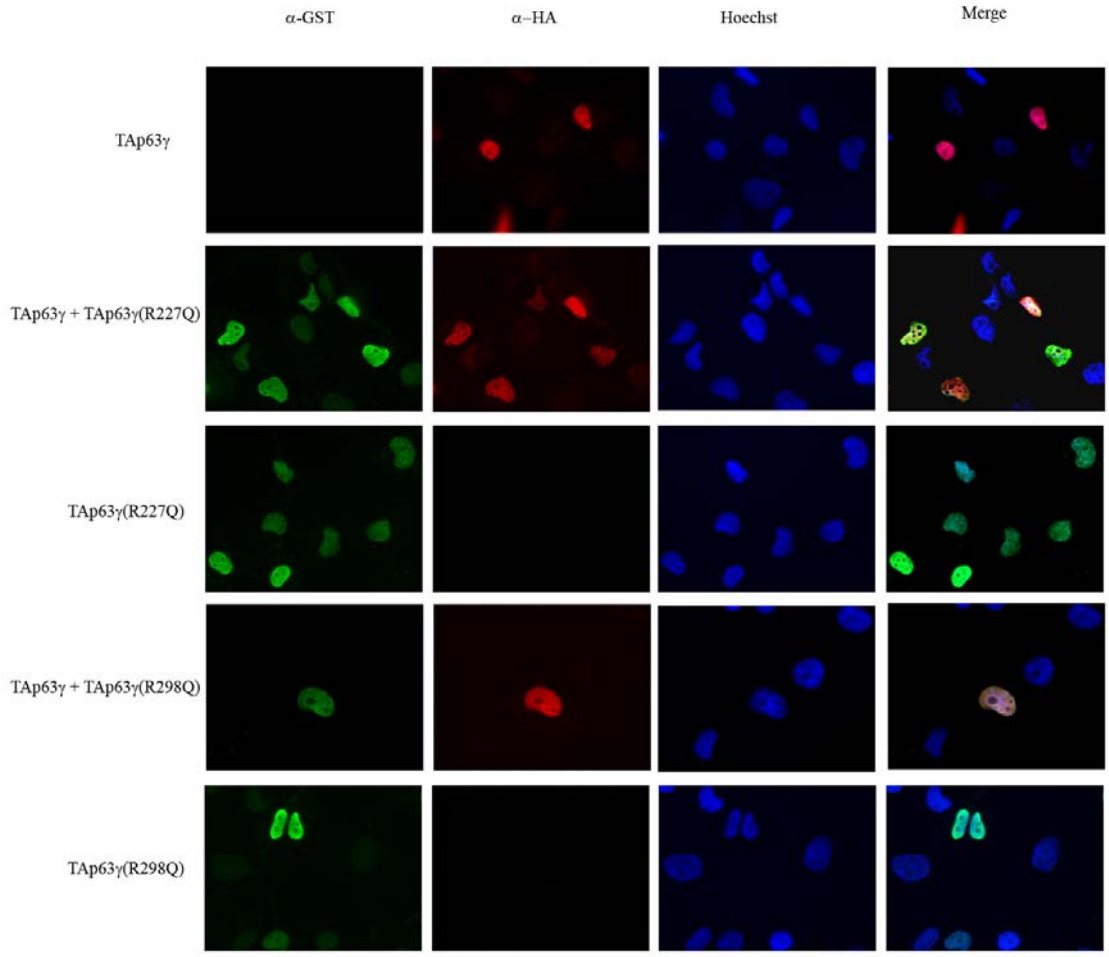

α-GST
α−HA
Hoechst
Merge
TAp63γ
TAp63γ + TAp63γ(R227Q)
TAp63γ(R227Q)
TAp63γ + TAp63γ(R298Q)
TAp63γ(R298Q)

Figure 13: *TAp63γ(R227Q) and TAp63γ(R298Q) mutants that mimic wildtype TAp63γ do not affect its localization.* H1299 cells were transfected with GST tagged TAp63γ mutants alone (1 μg) or along with HA-tagged wildtype TAp63γ (0.5 μg). At 24 hr post transfection, cells were fixed with 3% paraformaldehyde. HA-TAp63γ and GST-TAp63γ mutant expression was detected using mouse anti-HA and rabbit anti-GST primary antibodies and corresponding anti-mouse Texas Red and anti-rabbit FITC-conjugated secondary antibodies. The nucleus was stained with Hoechst 33342, and the cells were examined under fluorescence microscope.

Wildtype TAp63γ interacts with TAp63γ mutants: In order to confirm that the lack of effect on localization of wildtype p63 was not in part due to the inability of the mutant p63 to interact with wildtype p63, we next examined whether the mutant p63 can associate with wildtype p63. This will also enable us to determine whether the p63γ mutants included in our study form hetero-tetramer complexes with wildtype p63. For this, H1299 cells were transfected with expression vectors encoding GST-TAp63γ mutants in the presence or absence of wildtype HA- TAp63γ as shown in Figure 14. Whole cell extracts were subjected to immunoprecipitation experiments using anti-HA antibody against wildtype HA tagged TAp63γ (panel A) and subsequently immunoblotted with anti-GST to detect GST tagged TAp63γ mutants. Subsequently, these blots were also immunoblotted with anti-HA to confirm immunoprecipitation of wildtype HA tagged TAp63γ. Our results clearly demonstrate that all the p63 mutants associated with wildtype p63. Panel B represents whole cell extracts immunoblotted with anti-HA and anti-GST antibodies to confirm the overexpression of the wildtype and mutant p63. In addition, we observed that TAp63γ(R279H), TAp63γ(R204W), TAp63γ(C306R) and TAp63γ(R298Q) stabilized wildtype TAp63γ (Figure 14B). Taken together, these results demonstrate that all the TAp63γ mutants tested in this study interact with wildtype TAp63γ.

Differential effects of TAp63γ mutants on cell growth: TAp63 isoforms have been reported to be involved in promoting apoptosis during development and cancer progression. In particular, p63 has been shown to be required for p53 mediated apoptosis in mouse embryonic fibroblasts (Flores et al., 2002; Gressner et al., 2005a). Hence, the phenotypic features like syndactyly and cleft lip palate observed in p63 mutation associated developmental syndromes might be due to the perturbations in the apoptotic signaling pathways. To address that, we assessed the effects of

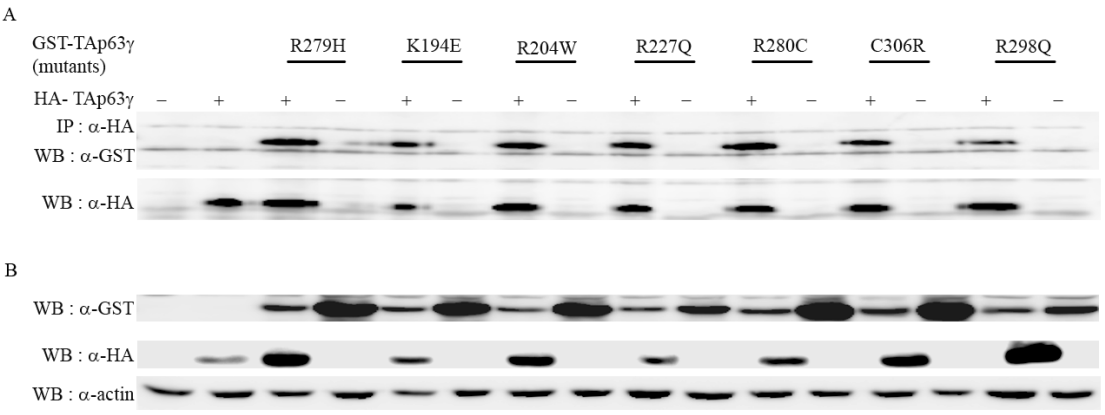

A
GST-TAp63γ (mutants)
R279H
K194E
R204W
R227Q
R280C
C306R
R298Q
HA- TAp63γ
– + + – + – + – + – + – + – + –
IP : α-HA
WB : α-GST
WB : α-HA
B
WB : α-GST
WB : α-HA
WB : α-actin

Figure 14: *Association between TAp63γ and TAp63γ mutants.* A) H1299 cell were transfected with either wildtype HA-TAp63γ alone (2.5 μg), GST-TAp63γ mutants alone (2.5 μg) or along with HA-TAp63γ. At 24 hr post transfection, whole cell lysates were made. Aliquots containing 300 μg of protein were subjected to Immunoprecipitation using anti-HA mouse antibody. Immunoprecipitates were resolved by SDS-PAGE gel and immunoblotted with anti-GST rabbit antibody. B) To confirm the overexpression, of wildtype and mutant proteins, equivalent amounts of protein from each transfection was fractionated onto a SDS PAGE and immunoblotted with anti-HA mouse and anti-GST rabbit antibody

TAp63γ mutants on cell survival and apoptosis, using colony formation assay and flow cytometry, respectively. As expected based on transactivation data, TAp63γ(R227Q) and TAp63γ(K194E) led to reduced levels of cell proliferation similar to wildtype TAp63γ (Figure 15A and 15B). In contrast, TAp63γ(R279H) mutant showed an increased cell growth when compared to wildtype TAp63γ (Figure 13A & 13B). Consistent with colony formation assay data, TAp63γ(R227Q) and wildtype showed a significant induction of apoptosis relative to vector (Figure 15C). In contrast, compared to wildtype p63, mutant TAp63γ(R279H) showed significantly reduced levels of apoptosis, supporting our colony formation assay results which shows that TAp63γ(R279H) promotes cell proliferation (Figure 15). Together, our data demonstrates that TAp63γ mutants exert differential effects on cell survival and growth inhibition, which might explain the phenotypic variations observed within p63 associated diseases.

GeneChip data analysis using GeneSpring: The precise role of p63 mutations has not been well defined but the distinct phenotypes observed in human developmental syndromes associated with p63 mutants might be due to the differences in target genes regulated by these mutants. We performed gene expression studies, to study if p63 missense mutations result in gain or loss of function. Specifically, we investigated the differential regulation of target genes by these mutants in H1299 cell lines by studying the gene expression profile and determining if the phenotype associated with a specific p63 mutant occurs as a result of its effect on gene expression.

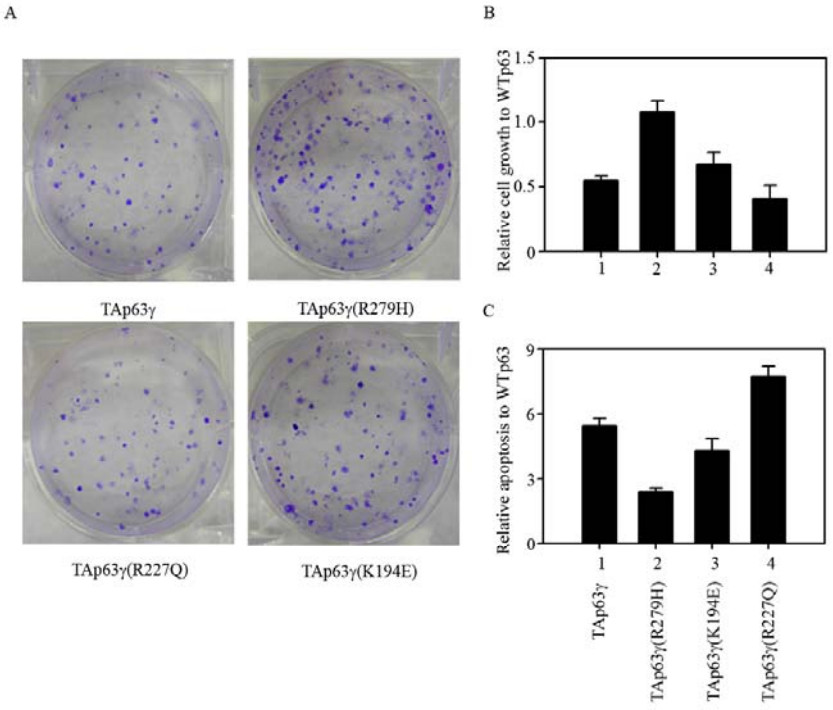

A
TAp63γ
TAp63γ(R279H)
TAp63γ(R227Q)
TAp63γ(K194E)
B
Relative cell growth to WTp63
1.5
1.0
0.5
0
1
2
3
4
C
Relative apoptosis to WTp63
9
6
3
0
1
2
3
4
TAp63γ
TAp63γ(R279H)
TAp63γ(K194E)
TAp63γ(R227Q)

Figure 15: *Differential effect on TAp63γ mutants on cell growth.* H1299 cells were transfected with either 3 μg of TAp63γ or representative TAp63γ mutants as indicated. A) Schematic representation of the colonies observed in these conditions after staining with crystal violet B) Quantitative representation of the destained crystal violet using 1% acetic acid and absorbance read at 590nm from the duplicate samples. Y axis represents percentage of cell growth relative to wildtype TAp63γ. C) H1299 cells were co-transfected with either TAp63γ mutants or wild TAp63γ and membrane bound hybrid-US9GFP (PAB35). Cells were harvested at 48 hr post transfection, fixed in 70% ethanol and DNA stained with propidium iodide solution, as described in Materials and Methods. DNA distribution was analyzed using Flow cytometry using CellQuest Program. Graph represents fold apoptosis of the sub G1 cells positive for PI and PAB35 (membrane bound hybrid GFP). Y –axis represents % apoptosis relative to wildtype TAp63γ.

Treatment	Induced	Suppressed
K194E_v_p63γ	29	72
R280C_v_p63γ	141	112
R204W_v_p63γ	31	110
C306R_v_p63γ	41	202
R279H_v_p63γ	117	72
R227Q_v_p63γ	30	75
R298Q_v_p63γ	38	90

Table 2: Summary of the number of genes that were either upregulated or downregulated by TAp63γ mutants when compared to wildtype TAp63γ.

Data Mining was performed as described in the Materials and Methods section. The total number of genes that were modulated with each of TAp63γ mutants relative to wildtype p63 control is summarized in Table 2. The genes that made it to the decreased list are indicative of loss of function while the genes that made it to the increased list indicate gain of function. Interestingly, the EEC mutant TAp63γ(C306R) was the had approximately 200 genes suppressed when compared wildtype p63. In addition, more than 100 genes were induced by TAp63γ(R280C) and TAp63γ(R279H) mutants respectively when compared to wildtype TAp63γ.

Common gene alterations associated with TAp63γ mutants relative to wildtype TAp63γ: Analysis of the gene targets clearly demonstrated that TAp63γ mutants induced numerous genes that are known to play an important role in cell cycle control, apoptosis, differentiation, development, proliferation, transcription control and signaling. Our data clearly shows that TAp63γ mutants are involved in multiple signaling pathways. We first identified target genes that were regulated by at least 4-7/7 TAp63γ mutants included in our study. By comparing genes that were suppressed by TAp63γ mutants, we identified approximately 40 overlapping genes that were suppressed in at least 4/7 when compared to wildtype TAp63γ (Tables 3, 4, 6 and 7). Approximately, 20% of the genes that made it to our list are identified p63 targets - *Cyclin dependent kinase inhibitor 1C* (CDKN1C), *Insulin growth factor binding protein 3* (IGFBP3), *Ferredoxin reductase* (FDXR) and *Aquaporin3* (AQP3) (Perez and Pietenpol, 2007; Trink et al., 2007). Of the genes not identified as p63 targets: C*hemokine (C-X-C motif) receptor 4* (CXCR4), *Distal-less homeo box 5* (DLX5) and *Nuclear receptor subfamily 2, group F, member 1* (NR2F1) were of particular interest, since they are involved in different development processes (Chin et al., 2007; Zhou et al., 2000; Zou et al., 1998). Our analysis of the genes that were regulated by all

			Fold Change relative to wildtype TAp63γ								
Gene Name	Gene Symbol	Description	K194E	R280C	R204W	C306R	R279H	R227Q	R298Q	Identified as p63 targets	Functions
207768_at	EGR4	early growth response 4	0.0922	0.185	0.174	0.329	0.212	0.11	0.155	-	apoptosis, atrophy, proliferation
213348_at	CDKN1C	Cyclin-dependent kinase inhibitor 1C (p57, Kip2)	0.249	0.32	0.241	0.417	0.134	0.386	0.45	+	apoptosis, G1 phase, growth, proliferation, S phase, cell cycle progression, transformation, cell viability, morphology, senescence
219750_at	TMEM144	transmembrane protein 144	0.476	0.312	0.343	0.16	0.409	0.401	0.382	-	integral to membrane
39248_at	AQP3	aquaporin 3	0.22	0.207	0.173	0.198	0.184	0.199	0.168	+	osmotic water permeability, survival, glycerol permeability, water permeability, permeability

Table 3: Genes that were downregulated in all 7 mutants compared to wildtype TAp63γ

			Fold Change relative to wildtype TAp63γ								
Gene Name	Gene Symbol	Description	K194E	R280C	R204W	C306R	R279H	R227Q	R298Q	Identified as p63 targets	Functions
204249_s_at	LMO2	LIM domain only 2 (rhombotin-like 1)	0.376	0.184	0.265	0.225	0.239	NR	0.491	-	differentiation, sprouting
204364_s_at	REEP1	receptor accessory protein 1	0.0676	0.202	0.0791	0.255	0.178	NR	0.467	-	protein insertion into membrane
209569_x_at	D4S234E	DNA segment on chromosome 4 (unique) 234 expressed sequence	0.44	0.437	0.345	0.497	0.262	NR	0.342	-	dopamine receptor signaling pathway
216248_s_at	NR4A2	nuclear receptor subfamily 4, group A, member 2	0.416	0.358	0.245	0.38	0.332	NR	0.345	-	apoptosis, differentiation, anoikis, growth, maturation, cell cycle progression
217028_at	CXCR4	chemokine (C-X-C motif) receptor 4	0.102	0.147	0.115	0.268	NR	0.218	0.44	-	migration, chemotaxis, development, homing, fusion, proliferation, apoptosis, retention

Table 4: Genes that were downregulated in at least 6/7 mutants compared to wildtype TAp63γ. Abbreviation NR stands for 'not regulated'.

7 TAp63γ mutants relative to wildtype TAp63γ identified 4 genes (*Early growth response 4* (EGR4), CDKN1C, *Transmembrane protein 144* (TMEM144) and AQP3) of which 2 genes (AQP3 and CDKN1C) are identified p63 targets while EGR4 and TMEM144 are not known to be regulated by p63 (Table 3). Furthermore, we identified 5 genes (*LIM domain only 3* (LMO2), *receptor accessory protein 1* (REEP1), *DNA segment on chromosome 4 234 expressed sequence* (D4S234E), *nuclear receptor subfamily 4, group A, member 2* (NR4A2) and CXCR4) to be downregulated by at least 6/7 p63 mutants in comparison to wildtype, none of these genes have been shown to be regulated by p63 (Table 4). Analysis of genes that were induced by at least 4 of 7 TAp63γ mutants led to the identification of only 7 overlapping genes (*Protein tyrosine phosphatase, non receptor type 11* (PTPN11), *zinc finger protein 37B* (ZNF37B), Chac cation *transport regulator homolog 1* (CHAC2), *zinc finger protein 236* (ZNF236), *BCL2 binding component 3* (BBC3), *erythropoietin receptor* (EPOR) and *coiled-coil domain containing 71* (CCDC71)) when compared to wildtype TAp63γ (Appendix Table 8). In particular, the only gene identified to be induced by 6/7 mutants was PTPN11, a bonafide oncogene that is mutated in several cancer types ; is hyperactivated in solid tumors and is also required for Ras-Erk cascade (Chan and Feng, 2007; Mohi and Neel, 2007) (Table 5). Additionally, BBC3(PUMA), a known p63 target gene that can modulate cellular apoptosis through intrinsic pathway via binding to Bcl-2 on mitochondria was induced by 4/7 TAp63γ mutants (Rocco et al., 2006). EPOR another gene induced by 4 TAp63γ mutants, is a regulator of RBC formation and is present on tumor cells (Udupa, 2006). EPO signaling inhibits apoptosis and promote cell proliferation, differentiation and cell survival (Sytkowski, 2007). Taken together our analysis suggested that the TAp63γ mutants significantly suppress gene involved in cell cycle arrest and induce the genes involved in proliferation. The greater number of overlapping genes in the

			Fold Change relative to wildtype TAp63γ								
Gene Name	Gene Symbol	Description	K194E	R280C	R204W	C306R	R279H	R227Q	R298Q	Identified as p63 targets	Functions
205867_at	PTPN11	protein tyrosine phosphatase, non-receptor type 11 (Noonan syndrome 1)	3.997	2.035	3.834	NR	2.616	2.324	4.172	-	apoptosis, differentiation, morphology, growth, proliferation, adhesion, G2 phase, transformation, chemotaxis

Table 5: Genes that were upregulated by at least 6/7 mutants compared to wildtype TAp63γ

decreased list when compared to wildtype TAp63γ is indicative of a loss of function being more predominant with the p63 mutations.

Mutant specific alteration of genes regulated by TAp63γ mutants: To understand the mechanisms of p63 related disorders, we generated a list of genes that were regulated by each of these mutants specifically compared to wildtype TAp63γ using GeneSpring. Focusing on these genes will help us identify the different genes that might be specifically regulated by these mutants which would give us insight into the differential effect of these mutants exerted towards p53 and/or p63 target genes. We manually assigned functional categories to each gene using information available at NCBI, Affymetrix (Netaffx), Gene Ontology browser from GeneSpring and Ingenuity Pathway Analysis. The manual classification allowed us to uncover some new potential targets of p63 mutants.

SHFM syndrome mutants:

K194E mutant: Our analysis of specific genes regulated by TAp63γ(K194E) mutant, observed only in SHFM syndrome, stressed the role of K194E in regulation of genes involved in apoptosis, cell proliferation, differentiation and development. We identified a total of 21 genes, of which 9 were induced while the remaining suppressed compared to wildtype p63. Some of the striking genes identified to be downregulated by K194E were *T-box 2* (TBX2) and *fibroblast growth factor receptor 1* (FGFR1) (Appendix Table 9). The T-box of transcription factor, Fibroblast Growth factor (FGFs) and its receptor, FGFR play an important role in embryogenesis and limb development (King et al., 2006; Li et al., 2005; Xu et al., 1999). Tbx2 is expressed in

the forelimb and the hindlimb in the mouse and involved in cell-type specification and morphogenesis of mammary gland (Gibson-Brown et al., 1998; King et al., 2006; Naiche et al., 2005; Rowley et al., 2004). Fgfr1 is primarily expressed in the mesenchyme of developing limb buds, craniofacial bone and mammary gland development (Dillon et al., 2004; Li et al., 2005; Rice et al., 2003; Xu et al., 1999). Overall, our analysis provides an insight into some target genes that might play a pivotal role in the developmental deformities associated with the SHFM mutants.

R280C (SHFM/EEC) mutant: This p63 mutation is observed in two different developmental syndromes EEC and SHFM. We identified approximately 135 genes specifically regulated by this mutant; almost 100 of these genes were induced while the remaining genes were suppressed when compared to wildtype TAp63γ (Appendix Table 10). The more number of increases suggest that this mutant has a gain of function effect. Analysis of these gene targets demonstrated the role of R280C in regulating genes implicated in metabolic processes, apoptosis, development, immune response, differentiation, cell viability and signaling. Although pro apoptotic genes: *Interferon gamma-inducible protein 16* (IFI16), *protein tyrosine phosphatase receptor type, O* (PTPRO), *Calpain 5* (CAPN5), *Septin 4* (SEPT4) (Lee et al., 2006), etc were induced in the screening for genes regulated by TAp63γ(R280C), genes related to cell viability like *Thyroid hormone receptor alpha* (THRA), matrix *metallopeptidase 7 protein* (MMP7), etc also increased when compared to wildtype TAp63γ. However, it should be noted that the pro-apoptotic genes are more abundant in the list of TAp63γ(R280C) regulated genes (Appendix Table 9). Interestingly, most of these genes are also involved in development. IFI16 is a gene known to modulate apoptosis and inhibit cell cycle progression and loss of this gene results in

deregulation of p53-mediated apoptosis, leading to tumor disposition (Alimirah et al., 2007; Kwak et al., 2003; Zhang et al., 2007). CAPN5, SEPT5 and PTPRO are highly expressed during embryogenesis and have tumor suppressor like ability (Beltran et al., 2003; Dear and Boehm, 1999; Larisch, 2004; Motiwala et al., 2004). MMP7 is expressed in epithelial cells and has been implicated in mammary gland tumorigenesis (Lynch et al., 2007; Sorrell et al., 2005). Thyroid hormone receptors play a role in brain development, inhibition of cell death and stimulation of cell growth which imparts oncogenic potential to this gene (Bernal, 2007; Thormeyer and Baniahmad, 1999; Yoshioka et al., 2006). In addition to these, we also observed other developmental genes like *Transcription factor 3* (TCF3) (Kim et al., 2007) and *laminin, alpha 4* (LAMA4) (Salmivirta and Ekblom, 1998) also upregulated by TAp63γ(R280C). The genes that stood out amongst those downregulated when compared to wildtype TAp63γ were anti apoptotic genes (*V-yes Yamaguchi sarcoma viral oncogene homolog 1* (YES1), *Ras guanyl releasing protein 1* (RASGRP1) and *FYN oncogene related YES* (FYN)) and pro-apoptotic genes (*S100 calcium binding protein A2* (S100A2), *Cbp/p300 interacting transactivator, with Glu/Asp rich carboxy-terminal domain, 2* (CITED2) and *TNF receptor superfamily, member 6* (FAS)) (Arnaud et al., 2003; Feng et al., 2001; Oki-Idouchi and Lorenzo, 2007; Wang et al., 2007). S100A2 and CITED2 are both known targets of p63 and have been shown to play a role in keratinocyte differentiation (Lapi et al., 2006; Vigano et al., 2006). Taken together our analysis classified R280C as a gain of function mutant involved in regulation of genes involved in different cellular processes.

EEC syndrome mutants:

R204W mutant: TAp63γ(R204W) mutant which is observed in EEC syndrome regulates about 40 genes, 31 of which are downregulated while 9 are upregulated when compared to wildtype TAp63γ (Appendix Table 11). Many genes involved in regulation of transcription factors (*ELK4 ETS domain protein* (ELK4), *Regulatory factor X,3 (*RFX3), *TEA domain family member 1* (TEAD1), *Amyotrophic lateral sclerosis 2 chromosome region, candidate 8* (ALS2CR8), etc) and metabolic processes (*Matrix metallopeptidase 1* (MMP1), *Iduronate 2-sulphatase* (IDS), *Glycine amidinotransferase* (GATM), Dehydrogenase E1 and *transketolase domain containing 1* (DHTKD1), *ABO blood group transferase A alpha 1-3 galactosyltranferase* (ABO), *Dehydrogenase/reductase SDR family, member 9* (DHRS9), etc) are downregulated specifically by TAp63γ(R204W). *Jagged 2* (JAG2) which is a ligand for NOTCH signaling, involved in apoptosis, proliferation and differentiation in many different tissues and is a known target of p63 is also downregulated by R204W (Sasaki et al., 2001). JAG2, plays a primary role in cleft palate, oral differentiation and craniofacial development observed in EEC patients (Casey et al., 2006). MMP1 is a zinc dependent protease, involved in invasiveness, proliferation and malignancy by degrading extracellular matrix (Seiki, 2003). Moreover, *Tumor necrosis factor receptor superfamily member 11B* (TNFRSF11B), that plays a role in cell viability, anoikis and differentiation is the gene positively regulated by TAp63γ(R204W) (Holen and Shipman, 2006).

C306R mutant: TAp63γ(C306R) observed in EEC syndrome is the most dominant negative mutant with approximately 120 genes downregulated and only about 20 genes upregulated when compared to wildtype TAp63γ, suggesting that it is a loss of function mutant (Appendix Table 12). Amongst the other developmentally related genes the most prominent genes downregulated by this mutant are *Folate receptor 1* (FOLR1), *Transforming growth factor, beta 2* (TGFB2) and

UDP-glucose ceramide glucosyltransferase (UGCG), which are involved in early embryonic development with cleft palate and cleft lip with or without cleft palate and hypodontia (Bianchi et al., 2000; Piedrahita et al., 1999; Slayton et al., 2003). The suppression of TGFB2 and FOLR1 is particularly intriguing, since p63 knock out mice also shows craniofacial and limb defects, as well as corneal epithelial abnormalities similar to the knock out phenotype of these two genes. Many receptors (FOLR1, Coagulation factor 2 (F2R), *RPA interacting protein olfactory receptor, family 5, subfamily T, member 2* (RPAIN) and *Nuclear factor receptor subfamily 2, group F, member 2* (NR2F2), etc) and kinases (G protein coupled receptor 6 (GRK6), *Adrenergic beta receptor kinase 2* (ADRBK2) , *PI3 kinase regulatory subunit 3* (PIK3R3), etc) are downregulated by TAp63γ(C306R) mutant. There were almost similar number of decreases in genes involved in survival (FOLR1, *Growth differentiation factor 15* (GDF15), *Integrin alpha V* (ITGAV), etc) and apoptosis (Fibronectin 1 (FN1), *Mixed lineage leukemia* (MLL), *Activating transcription factor 2* (ATF2), *Cullin2* (CUL2), *Cyclin dependent kinase 6* (CDK6), etc). In addition to these genes, other genes involved in metabolic processes, transport and immune response were also downregulated by C306R mutant. Taken together our data suggests that C306R mutant downregulates more genes than any other mutant making it a loss of function mutation, involved in multiple signaling pathways.

R279H mutant: TAp63γ(R279H) which is observed exclusively in EEC related disorders and is known for its dominant negative effects on wildtype mediated TAp63γ mediated induction of its target genes, surprisingly had more number of genes induced than repressed, suggesting it is a gain of function mutant. We identified approximately 80 genes that increased and only 6 genes that decreased when compared to wildtype TAp63γ (Appendix Table 13). The most striking gene

upregulated by this mutant was *GLI- Kruppel family member 2* (GLI2), which is involved in development of cleft palate, hair follicles, mammary gland and limbs (Slayton et al., 2003). The knock out phenotype of GLI2 mice is similar to the phenotype observed with p63 knock out. Another gene that was induced by R279H mutant specifically was *Bcl2 related protein A1* (BCL2A1), an anti-apoptotic gene which has been shown to suppress apoptosis induced by p53 tumor suppressor gene (D'Sa-Eipper et al., 1996). The genes involved in cell cycle progression and cell viability (*Thioredoxin interacting protein* (TXNIP), *Calbindin 1* (CALB1), *Androgen receptor* (AR), etc) were also induced in our analysis in comparison to wildtype TAp63γ supporting or results from cell proliferation assays where TAp63γ(R279H) had enhanced cell survival and decreased cell death when compared to wildtype TAp63γ. The genes that were downregulated by R279H mutant when compared to wildtype TAp63γ included *Matrix metallopeptidase 12* (MMP12), *Inhibin alpha* (INHA), *Bone morphogenetic protein 1* (BMP1), etc which are involved in cellular processes like invasiveness, proliferation and differentiation. Overall, our data indicates that the R279H mutant is not simply a dominant negative, but also upregulates numerous genes when compared to wildtype p63, making it a gain of function mutant.

R227Q mutant: TAp63γ(R227Q) is localized to the DNA binding domain and belongs to the EEC syndrome category. However, it is different from the other EEC mutants observed within this syndrome owing to its ability to exert similar effects are wildtype TAp63γ. Also, it lacks some distinctive phenotypic characters like orofacial clefting, observed in other EEC mutation phenotype. About 24 genes were downregulated and 19 genes were upregulated specifically by TAp63γ(R227Q) when compared to wildtype TAp63γ (Appendix Table 14). The genes

suppressed by this mutant are involved in development, regulation of transcription, metabolite transport and differentiation. The key functional categories among the genes that were upregulated by TAp63γ(R227Q) compared to wildtype TAp63γ, involved those playing a role in signal transduction, apoptosis and cell cycle arrest. It is interesting to note that the genes involved in development (*Retinoic acid receptor, alpha* (RARA), *Acrosomal vesicle protein 1* (ACRV1), *Laminin alpha 5* (LAMA5), etc) were suppressed by this mutant, suggesting the impact of this regulation in relation with the developmental defects associated with the p63 mutant phenotype. The most interesting gene identified is RARA due to its involvement in craniofacial development which is a hallmark feature observed in EEC syndrome patients (Houdayer and Bahuau, 1998). Furthermore, LAMA5 has been shown to play a crucial role in kidney and dental embryonic development and hair morphogenesis (Fukumoto et al., 2006; Kikkawa and Miner, 2006; Li et al., 2003). In agreement with our results from cell proliferation and cell death assays wherein TAp63γ(R227Q) mutant behave like wildtype TAp63γ in its ability to induce apoptosis, we identified genes with tumor suppressive abilities (*Glucocorticoid receptor DNA binding factor 1* (GRLF1) and *Mitochondrial tumor suppressor 1* (MTUS1)) to be induced by this mutant when compared to its wildtype counterpart (Yu et al., 2005). Both GRLF1 and MTUS1 have been shown to restrict the growth of malignant glioma and pancreatic tumors respectively (Seibold et al., 2003; Tikoo et al., 2000).

ADULT syndrome mutant R298Q: TAp63γ(R298Q) mutation is observed only in ADULT syndrome. We identified approximately 25 genes which were downregulated and 10 genes upregulated when compared to wildtype TAp63γ (Appendix Table 15). In addition to anti-apoptotic genes (*Deoxyribonuclease I-like 3* (DNASE1L3), *Interleukin 1, alpha* (IL1A) and

protein phosphatase 2, regulatory subunit B, beta isoform (PPP2R2B), etc) and pro-apoptotic genes *(Sin3-associated polypeptide, 18kDa* (SAP18) and *Phospholipase A2, group VII* (PLA2G7), etc), we also identified genes involved in development (*B cell CLL/lymphoma 11A* (BCL11A) and *Lysyl oxidase-like 1* (LOXL1)) in the decreased lists. Interestingly, although both pro apoptotic and anti apoptotic genes are repressed by R298Q mutant when compared to wildtype TAp63γ, the former is more abundant than the later. BCL11A, a kruppel like zinc finger transcription factor, is not only required for normal skeletal and lymphoid development but is a proto-oncogene involved in different malignancies (Ganss and Jheon, 2004; Liu et al., 2003; Satterwhite et al., 2001). LOXL1 acts as a tumor suppressor gene owing to its ability to antagonize the ability of Ras-Erk to promote cell survival and is also involved in notochord development (Gansner et al., 2007; Wu et al., 2007). Amongst the genes that was identified to be induced, *Plexin domain containing 1* (PLXDC1) plays a role in development and tumor invasiveness and poor survival of osteogenic sarcoma patients (Fuchs et al., 2007).

Ingenuity pathway analysis: Furthermore, the combined list of genes that were regulated by at least 4/7 TAp63γ mutants when compared to wildtype TAp63γ with GeneSpring was analyzed using Ingenuity Pathway Analysis (IPA) software to identify unique networks involving these genes. These networks represented relationships between the genes identified in our analysis and other genes thereby giving us information on possible genes upstream or downstream of the targets identified in this study. Several different pathway maps were created from this gene list which helped us identify unique interactions between genes and proteins that they might encode. The network represented in Figure 16 includes the genes which not only includes the greatest number of the gene targets identified by our data mining strategy but also showed greatest fold

changes of gene expression in either direction. We also ran the list of genes that were specifically regulated by each mutant to get an idea of the signaling pathways important for the role of p63 in development. Here, we represent a map from R279H, a gain of function mutation (Figure 17). It is interesting to see the network is coded red for the most part suggesting the gain of function. Also, the network contains numerous genes that are involved in different developmental signaling pathways.

Network 1 : mutants vs WT test - 2007-10-08 01:38 PM : mutants vs WT.xls

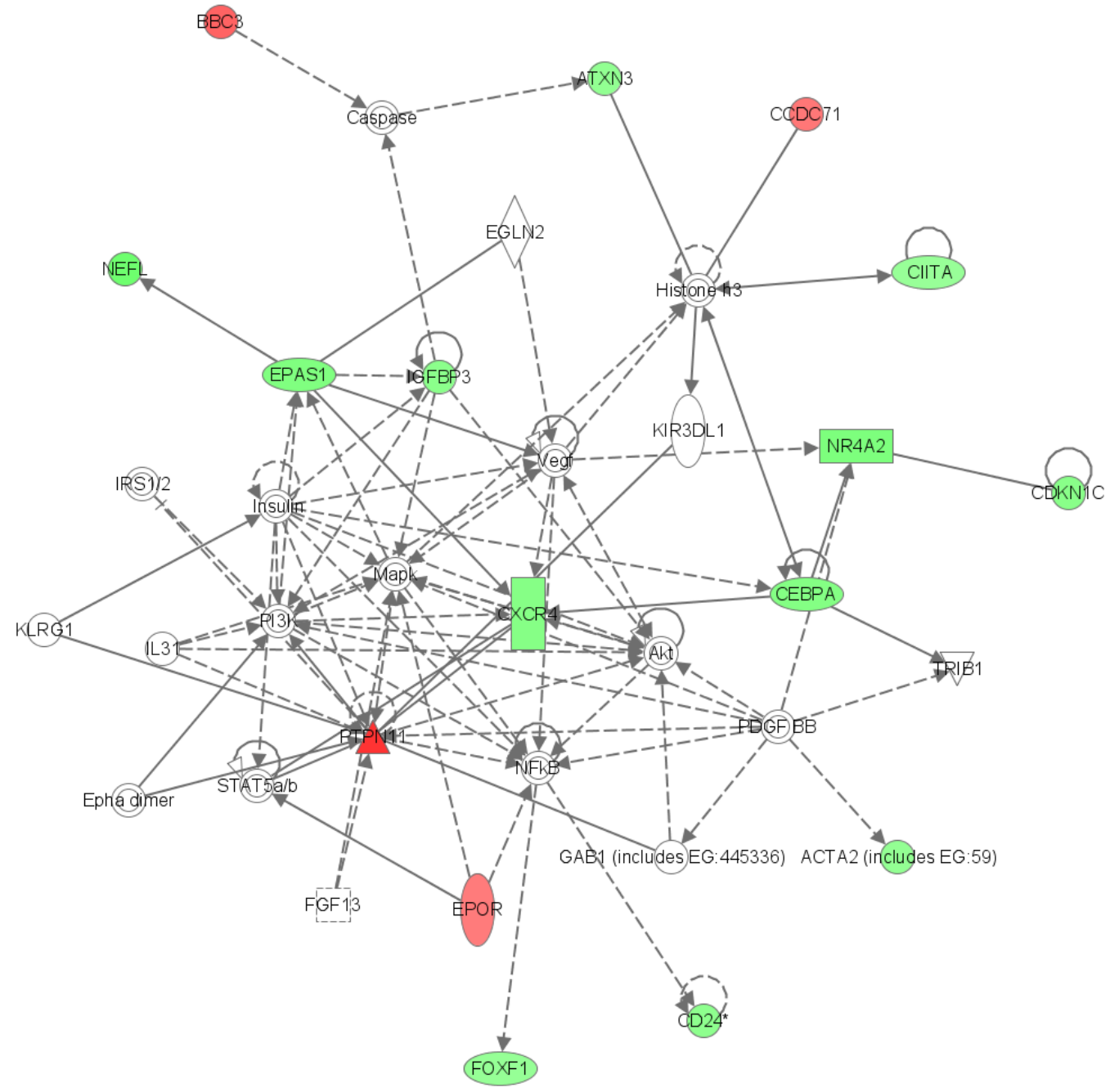


Figure 16: *IPA of the relationships between the genes that were both upregulated and downregulated by TAp63γ mutants when compared to wildtype p63.* The network shown here is a graphical representation of the molecular relationships between genes/gene products. The color scheme represents green (decreases) and red (increases). The higher intensity of color represents a greater fold change. The lines connecting the nodes are curated from the literature. Solid lines are direct relationships and the dashes lines are indirect relationships.

Network 1 : R279H specific v wt - 2007-10-24 11:29 AM : R279H specific v wt.xls

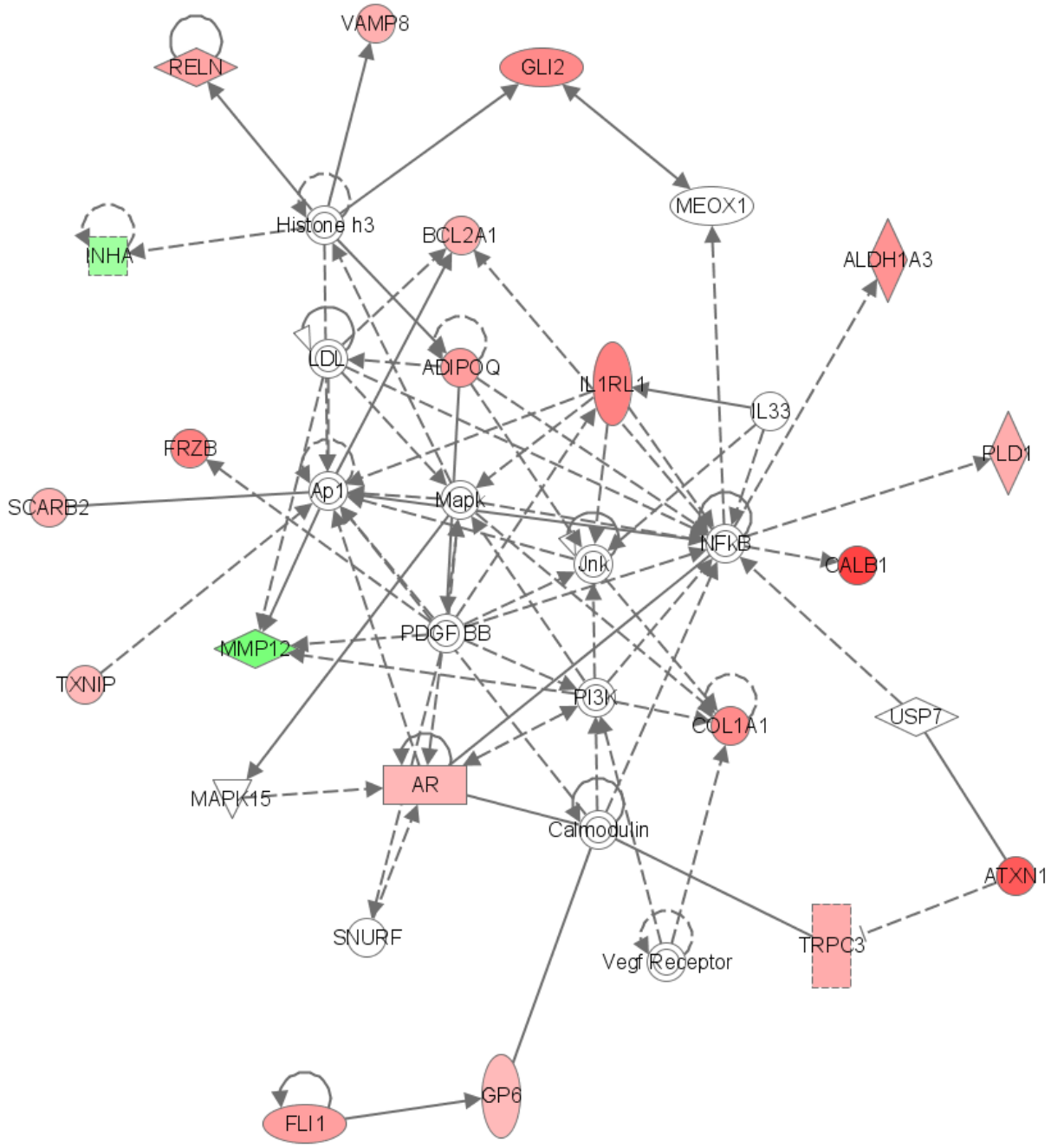


Figure 17: *IPA of the relationships between the genes that were both upregulated and downregulated by R279H mutant when compared to wildtype p63.* The network shown here is a graphical representation of the molecular relationships between genes/gene products. The color scheme represents green (decreases) and red (increases). The color intensity is proportional to the fold change. The greater the fold changes the higher the color intensity. The more number of reds indicate a gain of function. The lines connecting the nodes are curated from the literature. Solid lines are direct relationships and the dashes lines are indirect relationships.

IV. *Discussion:*

p63 plays an indispensable role in epithelial morphogenesis and cancer progression (Yang et al., 1999). Pathogenic mutations of p63 are shown to be responsible for several human syndromes exhibiting developmental defects. Although distinct p63 mutational patterns are observed with each developmental syndrome suggesting a genotype-phenotype relationship, the impact of these p63 mutations on gene expression and physiology of cell during development and cancer progression are still not clear.

It has previously been reported that arginine codons 204, 227, 279 and 280 of p63 are important for specific and nonspecific interactions with DNA target sequences, and mutations within those residues are highly detrimental to DNA binding and transactivation activity (Celli et al., 1999). Our studies also demonstrate that arginine mutants TAp63γ (R279H), TAp63γ (R204W) and TAp63γ (C306R) lack the transactivation activity based on their inability to induce the expression of Hdm2 and p21. Interestingly, p63 heterozygous mutant mice are more predisposed to tumor formation (Flores, 2007; Flores et al., 2005). It is therefore possible that the inability of TAp63γ (R279H), TAp63γ (R204W) and TAp63γ (C306R) mutant to induce cell cycle arrest genes might predispose the patients harboring these mutations to a greater incidence of tumor formation. Interestingly, many of these p63 mutations observed in EEC syndrome correspond exactly to the hotspot mutations in p53 genes: p63 R204, R279, R280 are analogous to p53 R175, R248 and R249 respectively (Li and Prives, 2007) an exception being R227, which is exclusive to p63. Alternatively, TAp63γ(R227Q) and TAp63γ(R298Q) mutants mimic its wildtype counterpart in their ability to transactivate both p63/p53 and p63 specific targets, and

therefore is less likely to increase the risk of these patients towards cancer development. The apparent distinguishable transcriptional activity of TAp63γ(R227Q) is also supported by the fact that, TAp63γ(R227Q) is a rare EEC mutation observed in only 1/227 EEC patients, lacks orofacial clefting and has fewer limb defects than typically observed in EEC syndrome (Rinne et al., 2006). Similarly, in spite of missense mutation in the DNA binding domain, the ability of ADULT syndrome specific TAp63γ(R298Q) mutant to retain wildtype activity might be due to presence of a second transactivation domain (Duijf et al., 2002; Propping et al., 2000; Reisler et al., 2006). Additionally, a distinct phenotypic overlap between ADULT (R298Q) and EEC (R227Q) syndromes has been reported (Reisler et al., 2006), which might reflect their ability to retain the transcriptional potential of the wildtype TAp63γ.

Differential regulation of p53/p63 and p63 specific target genes by mutants associated with EEC syndrome demonstrates that the molecular basis of phenotypic variation observed within the EEC syndrome could be as a result of perturbation of different signaling pathways normally regulated by p63. Our results showed that, while R279H mutant was unable to affect the TAp63γ mediated induction of p53/p63 target genes; it significantly inhibited the wildtype p63 mediated induction of p63 specific genes. This suggests that EEC mutant TAp63γ(R279H) may not always act in a dominant negative fashion towards all target genes. Adding to this complexity, our results showed that, while TAp63γ(R280C) and TAp63γ (K194E) mutants significantly induced the p53/p63 responsive genes, these mutants did not induce the p63 specific target genes. Our results are consistent with the observation that Arg 280 and Lys 194 amino acid residues although involved in the maintenance of the overall structure of the DNA binding domain, when mutated only has subtle effects on DNA-binding capacity of p63 (Ianakiev et al., 2000). It is

therefore possible that subtle differences in the transactivation ability of these mutants might be critical not only for the clinical variability observed in the same syndrome, but also other pathogenic conditions observed with p53 and p63.

Furthermore, we demonstrated that TAp63γ(R279H), TAp63γ(R204W) and TAp63γ(R280C) mutants act in dominant negative manner to inhibit the wildtype TAp63γ mediated transactivation of p63 specific target genes. In contrast, TAp63γ(R227Q) and TAp63γ(R298Q) significantly enhanced the wildtype p63 mediated transactivation. Wildtype p63 interacts with all the mutants tested and localization of wildtype was not affected by any of the mutant which suggests that the ability of mutants to inhibit wildtype activity might not be simply forming heterotetramers, but could be due to the ability of dominant negative mutants to compete with wildtype to bind to p63 specific responsive elements. Additionally, results from our interaction studies (Figure 14) indicate that the mutants may be responsible in stabilizing the wildtype p63 which thereby leads to increased effects of wildtype p63 on its target genes when co-transfected with p63 mutants.

Previous reports indicated that, exogenous TAp63 can activate genes involved in cell cycle arrest and apoptosis (Fan et al., 2007b; Yang and McKeon, 2000). Consistent with transactivation results, we demonstrated the differential ability of p63 mutants in promoting apoptosis. While TAp63γ(R227Q) and TAp63γ(K194E) mutants significantly promoted cell death, TAp63γ(R279H) mutant significantly promoted the proliferation. The differential effect of

mutants on cell survival may also dictate the complex phenotypic variation in p63 associated diseases.

In order to understand the developmental program managed by p63 and the different p63 mutations observed in patients, the identification of transcriptional target genes is indispensable. We performed GeneChip experiments in H1299 cells by overexpressing TAp63γ mutants to identify genes that are commonly and specifically regulated by the mutants. H1299 cells were used for these experiments because they lack endogenous p53 and any detectable levels of p63 isoforms. However, there is a limitation due to potentially non-physiologic amount of overexpresssed protein that might positively influence genes that might not be activated by lower levels of p63, or also repress genes by indirect mechanism. To date very little information is available on the genes that are regulated by p63 in epithelial cells or ectodermal signaling, but there is a good chance that there might be overlapping network of genes governing the various phenotypic outcomes associated with p63 related syndrome. Our goal was to identify if the distinct phenotype associated with the p63 mutations are due to regulation of some common and different target genes by these mutants. In addition to the identification of some new potential targets, our analysis confirmed some previously described targets as expected.

The mutation patterns observed in developmental syndrome associated with p63 have a remarkable specificity. All these syndromes are associated with point mutations in the single p63 allele and are not a result of haploinsufficiency, as indicated from the knock out mouse model. Our analysis of genes that were regulated by all mutants identified many genes suppressed by p63 mutants when compared to wildtype p63 while only few genes were induced. Among the

master regulators that were identified in the decreases compared to wildtype p63 were known targets like CDKN1C (p57, Kip2), AQP3 and IGFBP3. Studies show that AQP3 is highly expressed in the keratinocyte plasma membrane (Sougrat et al., 2002; Trink et al., 2007). The downregulation of this gene by overexpression of the p63 mutants could lead to the gross epithelial abnormalities observed in patients harboring p63 mutations. One particular observation amongst the gene that were suppressed by at least 6/7 mutants (LMO2, REEP1, D4S234E, NR4A2 and CXCR4) was that R227Q did not have any effect on these gene compared to wildtype, once again supporting the previous reports, categorizing it as a rare mutation with slightly different phenotype than the other EEC syndrome mutants. Additionally, FOXF1 and CITED1 genes involved in proliferation were downregulated by SHFM and all EEC mutants except R227Q and ADULT mutation R298Q. Interestingly, IGFBP3, another known target of both p53 and p63 was downregulated by all mutants except R227Q and R298Q once again suggesting that these two mutants retain the wildtype's ability to induce its target gene and supporting the observation that R227Q could be a ADULT syndrome mutant due to its phenotypic overlap with R298Q (Reisler et al., 2006).

The rare patients with heterozygous deletion of single allele of p63, display no characteristic signs of EEC syndrome (van Bokhoven et al., 2001), thereby suggesting that EEC syndrome mutations could have a gain of function or loss of function effect. Our analysis identified EEC syndrome mutations R279H and R280C as gain of function with greater number of genes increased. IPA analysis of R279H mutant identified a network of genes that were all increased by this mutant, thereby providing us with target genes that are already known to play a role in development and provides information on the network of genes regulated by this p63 mutant.

The other mutation identified to have a gain of function effect is R280C which is observed in both EEC and SHFM having more genes induced than suppressed when compared to wildtype p63. We identified genes involved in various metabolic processes, transport, development and apoptosis to be regulated by this mutant. The unexpected functional category identified here was immunity. It will be interesting to further investigate the role of p63 in this field, since p63 has been shown to be expressed in the immune system (Yang et al., 1998). In agreement with our earlier observation of R280C being able to induce p21, a cell death kinase inhibitor, we identified various genes involved in cell cycle control to be specifically upregulated by R280C.

The most striking contribution of p63 relates to its importance in squamous cell differentiation, skin renewal and development. Studies with p63 null mice clearly suggested the role of p63 is epithelial morphogenesis. Our analysis showed C306R belonging to EEC syndrome as a loss of function of mutant downregulating genes involved in different cellular processes like development, cell death, differentiation and metabolic processes. Although the suppression of these genes doesn't necessarily indicate a complete blockade in the downstream signaling cascade, it does result in some defective outcomes as observed in the EEC syndrome patients. The NOTCH signaling is a critical regulator of differentiation and proliferation. Given the role of JAG2 in cleft palate, oral differentiation and craniofacial development, an interesting observation was that R204W mutation classified as a specific EEC syndrome downregulated this gene. One of the reasons for the gross abnormalities observed in this particular mutant phenotype could be the suppression of this signaling pathway. R204W suppresses multiple transcription factors; the repression of these genes could be a basic event in the deregulation of developmental program regulated by wildtype p63.

NSCLP is one of the most congenital anomalies associated with p63 mutations. Retinoic acid which is a derivative of Vitamin A is known to play an important role in development during embryogenesis (Fan et al., 2007a). The dominant negative mutations of RA result in developmental abnormalities including craniofacial defects (Houdayer and Bahuau, 1998). RA interacts with RARA (Retinoic acid receptor, alpha) or RXR, therefore the regulation of this gene by R227Q mutant observed in EEC syndrome suggests that it might be one of the critical regulators of p63 signaling and the repression of this signaling pathway leads to the defective phenotype observed in EEC syndrome. Furthermore, LAMA5 which was also downregulated by R227Q has also been shown to play a crucial role in development of dental placodes and in hair morphogenesis (Fukumoto et al., 2006; Kikkawa and Miner, 2006; Li et al., 2003). R298Q observed in ADULT syndrome also regulated genes involved in various processes involved in development, immune response, transport, apoptosis and cell viability. We identified Tbx2 as one the genes downregulated by K194E, a SHFM syndrome mutant. Tbx2 has been shown to play a role in limb morphogenesis (Manning et al., 2006); (Nissim et al., 2007). The suppression of Tbx2 by K194E might lead to defects in limb development, a characteristic of SHFM syndrome. Understanding the molecular biology of K194E mutant in regulating genes which are important for normal limb and mammary gland development will aid in our understanding of the phenotype observed in SHFM related developmental anomaly.

In conclusion, our data demonstrates that the different TAp63γ mutants vary in their ability to transactivate p53/p63 and p63 target genes. Our analysis confirms that p63 plays a central role in development by impinging on multiple pathways at the cross roads of development and

apoptosis. Taken together our analysis provides information on the network of genes that are regulated by the p63 mutants. Future direction will aim at validation of the target genes identified by GeneChip analysis using real-time PCR and immunoblotting. The phenotypic variations observed within p63 related syndromes could in part be due to the differential effects of these mutants on canonical and non-canonical downstream signaling pathways of p63. Molecular description of these developmental syndromes involving p63 will aid in finding strategies for their recognition and alleviation. The identification of genes regulated by specific p63 mutants will help in the linking of p63 into signaling pathways involved in limb morphogenesis, differentiation and apoptosis. Undoubtedly, a better understanding of the effects exerted by these mutants may improve our comprehension of developmental and cancer biology and aid in better therapeutic strategies at least in cancers wherein over expression of ΔNp63α has been reported.

V. *Appendix*

			Fold Change relative to wildtype TAp63γ								
Gene Name	Gene Symbol	Description	K194E	R280C	R204W	C306R	R279H	R227Q	R298Q	Identified as p63 targets	Functions
200974_at	ACTA2	actin, alpha 2, smooth muscle, aorta	NR	0.437	0.359	0.45	0.382	NR	0.483	+	morphology, adhesion, polarization, disassembly, cell spreading, expansion, morphogenesis, maturation
203304_at	BAMBI	BMP and activin membrane-bound inhibitor homolog (Xenopus laevis)	0.434	NR	0.44	NR	0.435	0.377	0.157	-	colony formation
204529_s_at	TOX	thymus high mobility group box protein TOX	0.102	0.341	NR	0.327	0.474	NR	0.137	-	expansion, commitment
205935_at	FOXF1	forkhead box F1	0.125	0.427	0.233	0.491	0.0996	NR	NR	+	Proliferation
206752_s_at	DFFB	DNA fragmentation factor, 40kDa, beta polypeptide (caspase-activated DNase)	NR	0.411	0.404	0.121	NR	0.471	0.46	+	condensation, degradation, instability, cell death, transformation
207144_s_at	CITED1	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1	0.185	0.35	0.398	0.428	0.362	NR	NR	-	aggregation, growth, differentiation, cell death
207813_s_at	FDXR	ferredoxin reductase	NR	0.296	0.268	0.234	0.312	NR	0.478	+	apoptosis, survival, growth, permeabilization
208054_at	HERC4	hect domain and RLD 4	0.464	0.486	0.14	NR	0.266	0.427	NR	-	ubiquitin protein ligase activity

			Fold Change relative to wildtype TAp63γ								
Gene Name	Gene Symbol	Description	K194E	R280C	R204W	C306R	R279H	R227Q	R298Q	Identified as p63 targets	Functions
213707_s_at	DLX5	distal-less homeo box 5	0.238	NR	0.409	0.353	0.38	NR	0.126	+	production, morphogenesis, development, differentiation
214984_at	SMG1	PI-3-kinase-related kinase SMG-1	NR	0.215	0.464	0.191	0.333	0.203	NR	-	DNA repair, amino acid transport
215957_at	UBE2D1	ubiquitin-conjugating enzyme E2D 1 (UBC4/5 homolog, yeast)	NR	0.154	0.449	NR	0.275	0.254	0.0897	-	protein modification process; ubiquitin-dependent protein catabolic process
216657_at	ATXN3	ataxin 3	0.37	0.376	NR	0.478	NR	0.474	0.276	-	cell death, endoplasmic reticulum stress response
217904_s_at	BACE1	beta-site APP-cleaving enzyme 1	0.392	NR	0.369	0.339	NR	0.459	0.464	-	regulation, cell death, degeneration, deposition
217983_s_at	RNASET2	ribonuclease T2	NR	0.371	0.477	0.379	0.484	NR	0.461	-	unknown

Table 6: Genes that were downregulated in at least 5/7 mutants compared to wildtype TAp63γ

			Fold Change relative to wildtype TAp63γ								
Gene Name	Gene Symbol	Description	K194E	R280C	R204W	C306R	R279H	R227Q	R298Q	Identified as p63 targets	Functions
200878_at	EPAS1	endothelial PAS domain protein 1	0.196	0.423	0.393	NR	NR	0.244	NR	-	ciliogenesis, colony formation, morphology, accumulation, adhesion, differentiation
204039_at	CEBPA	CCAAT/enhancer binding protein (C/EBP), alpha	NR	NR	0.378	0.333	0.379	NR	0.434	-	differentiation, proliferation, adipogenesis, cell cycle progression, maturation, apoptosis, expansion, morphology
205156_s_at	ACCN2	amiloride-sensitive cation channel 2, neuronal	0.139	0.212	0.444	NR	0.396	NR	NR	-	damage response
205535_s_at	PCDH7	BH-protocadherin (brain-heart)	NR	0.288	0.474	NR	NR	0.459	0.414	-	interaction
207038_at	SLC16A6	solute carrier family 16 (monocarboxylic acid transporters), member 6	0.458	NR	0.395	0.474	NR	NR	0.424	-	monocarboxylic acid transport

			Fold Change relative to wildtype TAp63γ								
Gene Name	Gene Symbol	Description	K194E	R280C	R204W	C306R	R279H	R227Q	R298Q	Identified as p63 targets	Functions
207141_s_at	KCNJ3	potassium inwardly-rectifying channel, subfamily J, member 3	NR	0.433	NR	0.449	0.35	0.468	NR	-	potassium ion transport
209505_at	NR2F1	Nuclear receptor subfamily 2, group F, member 1	NR	0.442	0.443	0.342	NR	NR	0.484	-	apoptosis, development, migration, projection, innervation, cytostasis, extension, differentiation
209771_x_at	CD24	CD24 antigen (small cell lung carcinoma cluster 4 antigen)	0.309	NR	0.465	NR	0.223	0.373	NR	+	binding, apoptosis, adhesion, proliferation, motility, invasiveness, rolling, expansion
210239_at	IRX5	iroquois homeobox protein 5	NR	0.444	0.449	0.447	0.471	NR	NR	-	development and cell death
210387_at	HIST1H2BG	histone 1, H2bg	0.418	0.466	NR	0.49	NR	0.467	NR	-	nucleosome assembly, chromosome organization and biogenesis
210609_s_at	TP53I3	tumor protein p53 inducible protein 3	NR	0.419	0.417	0.411	0.349	NR	NR	+	induction of apoptosis by oxidative stress

			Fold Change relative to wildtype TAp63γ								
Gene Name	Gene Symbol	Description	K194E	R280C	R204W	C306R	R279H	R227Q	R298Q	Identified as p63 targets	Functions
210925_at	CIITA	class II, major histocompatibility complex, transactivator	0.495	0.47	0.417	NR	0.5	NR	NR	-	activation-induced cell death
214586_at	GPR37	G protein-coupled receptor 37 (endothelin receptor type B-like)	0.492	0.313	NR	NR	0.453	0.386	NR	-	cell death, endoplasmic reticulum stress response, hyperpolarization
214850_at	GUSBP1	glucourinidase, beta pseudogene 1	NR	NR	0.44	0.37	NR	0.484	0.261	-	carbohydrate metabolic process
215342_s_at	RABGAP1L	RAB GTPase activating protein 1-like	NR	0.255	NR	0.47	0.115	NR	0.284	-	regulation of Rab GTPase activity
216379_x_at	CD24	CD24 antigen (small cell lung carcinoma cluster 4 antigen)	0.479	0.437	0.463	NR	0.369	NR	NR	+	binding, apoptosis adhesion, proliferation, motility, cell spreading, invasiveness, rolling, expansion
217551_at	LOC441453	similar to olfactory receptor, family 7, subfamily A, member 17	0.439	NR	0.219	0.388	0.212	NR	NR	-	signal transduction, GPCR protein signaling

			Fold Change relative to wildtype TAp63γ								
Gene Name	Gene Symbol	Description	K194E	R280C	R204W	C306R	R279H	R227Q	R298Q	Identified as p63 targets	Functions
218706_s_at	NS3TP2	HCV NS3-transactivated protein 2	NR	0.398	0.253	0.483	NR	NR	0.388	-	Unknown
219179_at	DACT1	dapper, antagonist of beta-catenin, homolog 1 (Xenopus laevis)	0.445	NR	0.141	0.473	NR	0.403	NR	-	Wnt signaling pathway, multicellular organism development
219358_s_at	CENTA2	centaurin, alpha 2	NR	0.439	0.37	0.445	0.468	NR	NR	-	GTPase activator activity
220860_at	PURG	purine-rich element binding protein G	0.366	0.323	0.357	0.292	NR	NR	NR	-	DNA binding
221805_at	NEFL	neurofilament, light polypeptide 68kDa	0.286	0.371	0.334	NR	NR	0.174	NR	-	organization, biogenesis, assembly, dissociation, clustering, apoptosis, size

Table 7: Genes that were downregulated in at least 4/7 mutants compared to wildtype TAp63γ

			Fold Change relative to wildtype TAp63γ									
Gene Name	Gene Symbol	Description	K194E	R280C	R204W	C306R	R279H	R227Q	R298Q	No. of mutants in which the gene expression increased	Identified as p63 targets	Functions
215358_x_at	ZNF37B	zinc finger protein 37b (KOX 21)	2.946	2.959	NR	NR	2.436	3.146	3.7	5	-	unknown
219270_at	CHAC2	Chac, cation tranport regulator homolog 1 (E.coli)	2.32	NR	2.571	2.713	3.383	NR	2.543	5	-	protein binding
222227_at	ZNF236	zinc finger protein 236	2.049	2.474	2.576	3.38	2.513	NR	NR	5	-	DNA dependent regulation of transcription
211692_s_at	BBC3	BCL2 binding component 3 ; BCL2 binding component 3	2.697	NR	2.963	NR	2.065	NR	2.393	4	+	apoptosis, DNA damage response, permeabilization, survival, cell death
215054_at	EPOR	erythropoietin receptor	2.51	NR	2.489	NR	NR	2.026	2.242	4	-	proliferation, differentiation, mitogenesis, growth, development, G1 phase, morphology, apoptosis, G2/M phase transition,
219893_at	CCDC71	coiled-coil domain containing 71	2.04	NR	2.574	NR	2.064	2.068	NR	4	-	unknown

Table 8: Genes that were upregulated by at least 4/7 mutants when compared to wildtype TAp63γ

Gene Name	Gene Symbol	K194E Fold Change	Description	Functions
207901_at	IL12B	2.901	interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40)	proliferation, activation, differentiation, cytotoxicity, stimulation, development, polarization, apoptosis
205655_at	MDM4	2.446	Mdm4, transformed 3T3 cell double minute 4, p53 binding protein (mouse)	proliferation, apoptosis, growth, cell death, cell cycle progression, transformation, colony formation
219503_s_at	TMEM40	3.123	transmembrane protein 40	integral to membrane
206385_s_at	ANK3	2.314	ankyrin 3, node of Ranvier (ankyrin G)	biogenesis, stability, fragmentation, deformability, length, activation, loss, contraction, anchoring
209689_at	CCDC93	2.442	coiled-coil domain containing 93	Unknown
213496_at	LPPR4	2.296	plasticity related gene 1	Development
216519_s_at	PROSC	2.408	Proline synthetase co-transcribed homolog	Enzyme
219080_s_at	CTPS2	2.017	CTP synthase II	pyrimidine nucleotide biosynthetic process
221915_s_at	RANBP1	2.101	RAN binding protein 1	biogenesis, association, growth
40560_at	**TBX2**	**0.472**	**T-box 2**	**immortalization, proliferation, aging, growth, senescence, fate determination**
204036_at	EDG2	0.411	endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2	apoptosis, morphology, proliferation, formation, migration, motility, cell spreading, chemotaxis
201069_at	**MMP2**	**0.454**	**matrix metallopeptidase 2**	**invasion, migration, growth, proliferation, apoptosis, invasiveness, differentiation, chemotaxis, tubulogenesis, malignancy**
204483_at	ENO3	0.474	enolase 3 (beta, muscle)	Glycolysis
205841_at	JAK2	0.222	Janus Kinase 2	proliferation, apoptosis, growth, transformation, differentiation, mitogenesis, cell viability, binding, G1 phase, survival
206508_at	CD70	0.445	CD70 molecule	differentiation, apoptosis, proliferation, depletion, necrosis, signaling, interaction, cell cycle progression
206615_s_at	ADAM22	0.477	ADAM metalopeptidase domain 22	proliferation, migration
210803_at	TXNRD2	0.328	Thioredoxin reductase 2	cell death, hematopoiesis

Gene Name	Gene Symbol	K194E Fold Change	Deascription	Functions
214337_at	COPA	0.455	coatomer protein complex, subunit alpha	Phagocytosis
215404_x_at	**FGFR1**	**0.445**	**fibroblast growth factor receptor 1**	**proliferation, outgrowth, mitogenesis, growth, survival, apoptosis, maturation, migration, cell death, cell movement**
215783_s_at	ALPL	0.309	Alkaline phosphatase, liver/bone/kidney	Mineralization
217632_at	GNL3L	0.496	guanine nucleotide binding protein-like 3 (nucleolar)-like	nucleotide binding

Table 9: Genes that were specifically regulated by TAp63γ(K194E) mutant alone when compared to wildtype TAp63γ. The fold change values above 2.0 indicate increases; while the values less than 0.5 indicate decreases with K194E mutant when compared to wildtype p63. The highlighted genes are the genes discussed in the result section.

Gene Name	Gene Symbol	R280C Fold Change	Description	Function
208596_s_at	UGT1A10	5.069	UDP glucuronosyltransferase 1 family, polypeptide A10	metabolic process
202342_s_at	TRIM2	5.485	tripartite motif-containing 2	protein binding
203399_x_at	PSG3	4.065	Pregnancy specific beta-1-glycoprotein 3	defense response
208966_x_at	**IFI16**	**3.98**	**interferon, gamma-inducible protein 16**	**differentiation, proliferation, cell cycle progression, apoptosis, contact growth inhibition, morphology, accumulation, DNA damage response, G1/S phase transition, osteogenesis**
217578_at	XPO1	4.738	Exportin 1 (CRM1 homolog, yeast)	intracellular protein transport
221778_at	JHDM1D	2.336	jumonji C domain-containing histone demethylase 1 homolog D (S. cerevisiae)	Unknown
207142_at	KCNJ3	3.223	potassium inwardly-rectifying channel, subfamily J, member 3	potassium ion transport
222005_s_at	GNG3	3.664	Guanine nucleotide binding protein (G protein), gamma 3	aggregation, growth, adhesion
207051_at	SLC17A4	2.063	solute carrier family 17 (sodium phosphate), member 4	Transport
219761_at	CLEC1A	2.746	C-type lectin domain family 1, member A	cell surface receptor linked signal transduction
207602_at	TMPRSS11D	2.946	transmembrane protease, serine 11D	proteolysis; respiratory gaseous exchange
216415_at	DNAH3	2.191	Dynein, axonemal, heavy polypeptide 3	ciliary or flagellar motility; microtubule-based movement
219172_at	UBTD1	2.014	ubiquitin domain containing 1	protein modification
216818_s_at	OR2J2	6.463	olfactory receptor, family 2, subfamily J, member 2	signal transduction
215671_at	PDE4B	2.928	Phosphodiesterase 4B, cAMP-specific (phosphodiesterase E4 dunce homolog, Drosophila)	catalytic activity
220735_s_at	SENP7	2.208	SUMO1/sentrin specific peptidase 7	proteolysis, ubiquitin cycle
217319_x_at	CYP4A11 ; CYP4A22	4.097	cytochrome P450, family 4, subfamily A, polypeptide 11 ; cytochrome P450, family 4, subfamily A, polypeptide 22	electron transport; fatty acid metabolic process

Gene Name	Gene Symbol	R280C Fold Change	Description	Function
220047_at	SIRT4	2.396	sirtuin (silent mating type information regulation 2 homolog) 4 (S. cerevisiae)	chromatin silencing
1316_at	**THRA**	**2.339**	**thyroid hormone receptor, alpha (erythroblastic leukemia viral (v-erb-a) oncogene homolog, avian)**	**differentiation, proliferation, fusion, morphology, transformation, production, activity, size, apoptosis**
205552_s_at	OAS1	6.225	2',5'-oligoadenylate synthetase 1, 40/46kDa	Apoptosis
219938_s_at	PSTPIP2	4.91	proline-serine-threonine phosphatase interacting protein 2	Unknown
216647_at	**TCF3**	**2.282**	**Transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)**	**apoptosis, development, proliferation, differentiation, growth, cell cycle progression, lymphopoiesis, morphology, G1 phase**
210198_s_at	PLP1	13.8	proteolipid protein 1 (Pelizaeus-Merzbacher disease, spastic paraplegia 2, uncomplicated)	proliferation, differentiation, hyperproliferation, antiviral response, activation, survival, degeneration, apoptosis, damage
210772_at	FPRL1	3.528	formyl peptide receptor-like 1 ; formyl peptide receptor-like 1	chemotaxis, migration, adhesion, infiltration, cell movement, proliferation
215231_at	PRKAG2	3.821	Protein kinase, AMP-activated, gamma 2 non-catalytic subunit	fatty acid metabolism
216402_at	SEC14L4	2.693	SEC14-like 4 (S. cerevisiae)	Transport
202202_s_at	**LAMA4**	**3.506**	**laminin, alpha 4**	**migration, branching, adhesion, binding, alignment, development, elongation, degeneration, proliferation**
203961_at	NEBL	2.194	nebulette	ion transport; regulation of actin filament length
204259_at	**MMP7**	**2.161**	**matrix metallopeptidase 7 (matrilysin, uterine)**	**apoptosis, invasion, proliferation, aggregation, migration, malignancy, cell movement, invasiveness, differentiation, survival**
205092_x_at	ZBTB1	2.828	zinc finger and BTB domain containing 1	differentiation, proliferation, fusion, morphology, transformation, production, activity, size, apoptosis

Gene Name	Gene Symbol	R280C Fold Change	Description	Function
205166_at	**CAPN5**	**2.693**	**calpain 5**	**apoptosis, invasion, cell death, adhesion, motility, proliferation, migration, formation, chemotaxis, chemokinesis**
205355_at	ACADSB	2.242	acyl-Coenzyme A dehydrogenase, short/branched chain	metabolic process
205513_at	TCN1	2.866	transcobalamin I (vitamin B12 binding protein, R binder family)	cobalt ion transport
205552_s_at	OAS1	6.225	2',5'-oligoadenylate synthetase 1, 40/46kDa	immune response
205636_at	SH3GL3	2.63	SH3-domain GRB2-like 3	formation, detachment, endocytosis, migration
206826_at	PMP2	2.229	peripheral myelin protein 2	activation, transport
206830_at	SLC4A10	5.34	solute carrier family 4, sodium bicarbonate transporter-like, member 10	Transport
207096_at	SAA4	2.841	serum amyloid A4, constitutive	acute-phase response
207128_s_at	ZNF223	2.08	Zinc finger protein 223	regulation of transcription
207362_at	SLC30A4	2.264	solute carrier family 30 (zinc transporter), member 4	Transport
207449_s_at	POFUT2	2.2	protein O-fucosyltransferase 2	metabolic process
207815_at	PF4V1	2.933	platelet factor 4 variant 1	immune response
208026_at	HIST1H4F	2.664	histone 1, H4f	Unknown
208121_s_at	**PTPRO**	**2.059**	**protein tyrosine phosphatase, receptor type, O**	**apoptosis, growth, cell cycle progression, survival, proliferation, presence, cell movement**
208261_x_at	IFNA10	2.258	interferon, alpha 10	defense response
209244_s_at	KIF1C	2.28	kinesin family member 1C	transport, dynamics, depletion, redistribution, cell viability, motility
209483_s_at	NSL1	2.049	NSL1, MIND kinetochore complex component, homolog (S. cerevisiae)	cell cycle; chromosome segregation; mitosis; methylation; cell division
210198_s_at	PLP1	13.8	proteolipid protein 1 (Pelizaeus-Merzbacher disease, spastic paraplegia 2, uncomplicated)	synaptic transmission, depolarization, damage, proliferation
210272_at	CYP2B7P1	2.092	cytochrome P450, family 2, subfamily B, polypeptide 7 pseudogene 1	electron transport

Gene Name	Gene Symbol	R280C Fold Change	Description	Function
210302_s_at	MAB21L2	9.488	mab-21-like 2 (C. elegans)	nervous system development
210583_at	POLDIP3	2.745	polymerase (DNA-directed), delta interacting protein 3	Unknown
210657_s_at	**SEPT4**	**3.748**	**septin 4**	**cell death, development, capacitation, apoptosis**
210661_at	GLRA3	6.111	glycine receptor, alpha 3	Unknown
210680_s_at	MASP1	2.002	mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive factor)	complement activation, classical pathway; innate immune response
211141_s_at	CNOT3	2.213	CCR4-NOT transcription complex, subunit 3	DNA-dependent; regulation of transcription
211144_x_at	TRGC2	2.723	T cell receptor gamma constant 2	immune response
211479_s_at	HTR2C	2.833	5-hydroxytryptamine (serotonin) receptor 2C	transformation, aggregation, formation
211718_at	MGC2889	2.439	hypothetical protein MGC2889	Unknown
211907_s_at	PARD6B	5.744	par-6 partitioning defective 6 homolog beta (C. elegans)	reassembly, polarization, assembly, structure, development, transformation, migration
212354_at	SULF1	2.079	sulfatase 1	Apoptosis
213113_s_at	SLC43A3	2.538	solute carrier family 43, member 3	Transport
213421_x_at	PRSS3	2.204	protease, serine, 3 (mesotrypsin)	migration, desensitization
213802_at	PRSS12	2.106	Protease, serine, 12 (neurotrypsin, motopsin)	migration, desensitization
214411_x_at	CTRB2	2.349	chymotrypsinogen B2	Proteolysis
214587_at	COL8A1	2.64	collagen, type VIII, alpha 1	Proliferation
214945_at	NY-REN-7 ; LOC389347	3.941	NY-REN-7 antigen ; similar to KIAA0752 protein	Translation
215199_at	CALD1	2.716	caldesmon 1	formation, assembly, cytokinesis, morphology, translocation, cell cycle progression, G2/M phase transition, size, cell movement
215231_at	PRKAG2	3.821	Protein kinase, AMP-activated, gamma 2 non-catalytic subunit	fatty acid synthesis
215417_at	EXOC6B	2.249	exocyst complex component 6B	protein transport

Gene Name	Gene Symbol	R280C Fold Change	Description	Function
215430_at	GK2	3.697	glycerol kinase 2	glycerol-3-phosphate metabolic process
215659_at	GSDML	2.154	Gasdermin-like	Unknown
215671_at	PDE4B	2.928	Phosphodiesterase 4B, cAMP-specific (phosphodiesterase E4 dunce homolog, Drosophila)	migration, growth, apoptosis, G1 phase, G2/M phase transition, chemotaxis, proliferation, differentiation
215674_at	KIAA1659	6.587	KIAA1659 protein	Unknown
215771_x_at	RET	4.334	ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1, Hirschsprung disease)	transformation, proliferation, survival, apoptosis, differentiation, colony formation, migration, growth, scattering, mitogenesis
216170_at	EEF1G	4.255	Eukaryotic translation elongation factor 1 gamma	translational elongation
216197_at	ATF7IP	3.027	activating transcription factor 7 interacting protein	regulation of transcription
216545_at	LOC441886	2.842	similar to Aspartate aminotransferase, mitochondrial precursor (Transaminase A) (Glutamate oxaloacetate transaminase-2)	Unknown
216557_x_at	IGHA1	2.742	immunoglobulin heavy constant alpha 1	proliferation, apoptosis, phagocytosis, binding, growth, cytolysis, differentiation, mitogenesis, infiltration
216566_at	IGLC2	5.48	Immunoglobulin lambda joining 3	Unknown
216639_at	SRPX2	2.201	sushi-repeat-containing protein, X-linked 2	Unknown
216722_at	VENTXP1	2.21	VENT homeobox (Xenopus laevis) pseudogene 1	Unknown
216895_at	GABRG3	2.018	gamma-aminobutyric acid (GABA) A receptor, gamma 3	ion transport
219115_s_at	IL20RA	3.102	interleukin 20 receptor, alpha	blood coagulation
219671_at	HPCAL4	2.066	hippocalcin like 4	central nervous system development
219761_at	CLEC1A	2.746	C-type lectin domain family 1, member A	defense response
219841_at	AICDA	4.321	activation-induced cytidine deaminase	accumulation, proliferation, activation
219898_at	GPR85	4.489	G protein-coupled receptor 85	signal transduction

Gene Name	Gene Symbol	R280C Fold Change	Description	Function
219938_s_at	PSTPIP2	4.91	proline-serine-threonine phosphatase interacting protein 2	Cytokinesis
220035_at	NUP210	3.291	nucleoporin 210kDa	protein targeting
220210_at	CHRNA10	2.188	cholinergic receptor, nicotinic, alpha polypeptide 10	synaptic transmission, depolarization, damage, proliferation
220290_at	AIM1L	4.425	absent in melanoma 1-like	Unknown
220327_at	VGL-3	2.237	vestigial-like 3	regulation of transcription
220623_s_at	TSGA10	2.215	testis specific, 10	Spermatogenesis
221136_at	GDF2	2.269	Growth differentiation factor 2	Proliferation
221491_x_at	HLA-DRB1	2.001	major histocompatibility complex, class II, DR beta 1	cell death, proliferation, apoptosis, adhesion, binding, activation, inhibition
221633_at	NCAPH2	2.051	Non-SMC condensin II complex, subunit H2	Segregation
221857_s_at	TJAP1	2.292	Tight junction associated protein 1 (peripheral)	Growth
222137_at	CC2D1A	2.083	Coiled-coil and C2 domain containing 1A	regulation of transcription
222196_at	LOC286434	2.616	hypothetical protein LOC286434	Unknown
222293_at	IGSF4C	2.034	immunoglobulin superfamily, member 4C	immune response
220673_s_at	KIAA1622	0.372	KIAA1622	Unknown
212486_s_at	**FYN**	**0.498**	**FYN oncogene related to SRC, FGR, YES**	**proliferation, morphology, apoptosis, adhesion, myelination, activation, migration, development, degranulation**
219263_at	RNF128	0.257	Ring finger protein 128	regulation of transcription
204268_at	S100A2	0.488	S100 calcium binding protein A2	endothelial cell migration, tumor suppressor, differentiation
219985_at	**HS3ST3A1**	**0.491**	**Heparin sulfate (glucosamine) 3-O-sulfotransferase 3A1**	**transferase activity**
205590_at	**RASGRP1**	**0.449**	**RAS guanyl releasing protein 1 (calcium and DAG-regulated)**	**proliferation, maturation, transformation, migration, differentiation, apoptosis**

Gene Name	Gene Symbol	R280C Fold Change	Description	Function
215719_x_at	**FAS**	**0.444**	**Fas (TNF receptor superfamily, member 6)**	**apoptosis, immune response, signal transduction**
203066_at	GALNAC4S-6ST	0.363	B cell RAG associated protein	hexose biosynthetic process
219262_at	SUV39H2	0.378	suppressor of variegation 3-9 homolog 2 (Drosophila)	cell differentiation, chromatin assembly, regulation of transcription, cell cycle, chromatin modification
202783_at	NNT	0.46	nicotinamide nucleotide transhydrogenase	electron transport
204841_s_at	EEA1	0.474	early endosome antigen 1, 162kD	vesicle fusion
207029_at	KITLG	0.442	KIT ligand	proliferation, colony formation, apoptosis, differentiation, growth, survival, migration, degranulation, chemotaxis
208384_s_at	MID2	0.484	midline 2	Unknown
209357_at	**CITED2**	**0.415**	**Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2**	**proliferation, morphology, apoptosis**
209802_at	PHLDA2	0.445	pleckstrin homology-like domain, family A, member 2	Growth
209910_at	SLC25A16	0.47	solute carrier family 25 (mitochondrial carrier; Graves disease autoantigen), member 16	Transport
210985_s_at	SP100	0.34	nuclear antigen Sp100	invasion, migration
213297_at	RMND5B	0.451	equired for meiotic nuclear division 5 homolog B (S. cerevisiae)	Meiosis
213307_at	SHANK2	0.5	SH3 and multiple ankyrin repeat domains 2	intracellular signaling cascade
213935_at	ABHD5	0.392	abhydrolase domain containing 5	proteolysis; aromatic compound metabolic process
214230_at	CDC42	0.499	cell division cycle 42 (GTP binding protein, 25kDa)	transformation, apoptosis, cell cycle progression, growth, cell spreading, morphology, migration, outgrowth
215095_at	ESD	0.493	Esterase D/formylglutathione hydrolase	Unknown
215118_s_at	IGHA1	0.444	Translocation associated fusion protein IRTA1/IGA1 (IRTA1/IGHA1)	immune response
215470_at	GTF2H2	0.498	General transcription factor IIH, polypeptide 2, 44kDa	regulation of transcription

Gene Name	Gene Symbol	R280C Fold Change	Description	Function
218309_at	CAMK2N1	0.307	Calcium/calmodulin-dependent protein kinase II inhibitor 1	adhesion, survival
219262_at	SUV39H2	0.378	suppressor of variegation 3-9 homolog 2 (Drosophila)	remodeling, length, assembly, binding, mitosis
219263_at	RNF128	0.257	Ring finger protein 128	ubiquitin cycle; negative regulation of cytokine biosynthetic process
219973_at	ARSJ	0.485	arylsulfatase J	Unknown
219985_at	HS3ST3A1	0.491	Heparin sulfate (glucosamine) 3-O-sulfotransferase 3A1	transferase activity
219999_at	MAN2A2	0.491	mannosidase, alpha, class 2A, member 2	metabolic process
220216_at	C8orf44	0.425	chromosome 8 open reading frame 44	Unknown
220321_s_at	CCDC121	0.458	coiled coil domain containing 21	Unknown
221350_at	HOXC8	0.367	homeo box C8	regulation of transcription
222180_at	**YES1**	**0.478**	**V-yes-1 Yamaguchi sarcoma viral oncogene homolog 1**	**neuritogenesis, assembly, disassembly, invasion, anoikis, transformation, apoptosis**

Table 10: Genes that were specifically regulated by TAp63γ(R280C) mutant alone when compared to wildtype TAp63γ

Gene Name	Gene Symbol	R204W Fold Change	Description	Functions
209784_s_at	**JAG2**	**0.453**	**jagged 2**	**differentiation, proliferation, apoptosis, adhesion, survival, selection**
214156_at	MYRIP	0.376	myosin VIIA and Rab interacting protein	intracellular protein transport
204475_at	**MMP1**	**0.358**	**matrix metallopeptidase 1 (interstitial collagenase)**	**invasion, invasiveness, aggregation, proliferation, migration, differentiation, apoptosis, malignancy, growth, cell movement**
201843_s_at	EFEMP1	0.285	EGF-containing fibulin-like extracellular matrix protein 1	endoplasmic reticulum stress response
202438_x_at	**IDS**	**0.382**	**iduronate 2-sulfatase (Hunter syndrome)**	**metabolic process**
202871_at	TRAF4	0.387	TNF receptor-associated factor 4	colony formation, apoptosis, growth
202895_s_at	SIRPA	0.399	signal-regulatory protein alpha	adhesion, phagocytosis, cell spreading, migration, apoptosis, survival, polarization, attachment
203178_at	**GATM**	**0.493**	**glycine amidinotransferase (L-arginine:glycine amidinotransferase)**	**creatine biosynthetic process**
203563_at	AFAP1	0.284	actin filament associated protein	cross-linkage, organization
204840_s_at	EEA1	0.477	early endosome antigen 1, 162kD	vesicle fusion
204845_s_at	ENPEP	0.434	glutamyl aminopeptidase (aminopeptidase A)	signaling, proliferation, activity
206268_at	LEFTY1	0.415	left-right determination factor 1	migration, proliferation
206919_at	**ELK4**	**0.148**	**ELK4, ETS-domain protein (SRF accessory protein 1)**	**regulation of transcription**
209312_x_at	HLA-DRB1	0.483	major histocompatibility complex, class II, DR beta 1	activation, proliferation, apoptosis, interaction, inhibition, binding, negative selection, positive selection, conversion

Gene Name	Gene Symbol	R204W Fold Change	Description	Functions
209916_at	**DHTKD1**	**0.499**	**dehydrogenase E1 and transketolase domain containing 1**	**metabolic process**
210218_s_at	SP100	0.465	nuclear antigen Sp100	invasion, migration
210675_s_at	PTPRR	0.382	protein tyrosine phosphatase, receptor type, R	Proliferation
211440_x_at	CYP3A43	0.425	cytochrome P450, family 3, subfamily A, polypeptide 43	electron transport
212394_at	KIAA0090	0.494	KIAA0090	protein binding
212991_at	FBXO9	0.153	F-box protein 9	protein ubiquitination
214600_at	**TEAD1**	**0.404**	**TEA domain family member 1 (SV40 transcriptional enhancer factor)**	**regulation of transcription**
214934_at	ATP9B	0.329	ATPase, Class II, type 9B	Transport
216716_at	**ABO**	**0.493**	**ABO blood group (transferase A, alpha 1-3-N-acetylgalactosaminyltransferase; transferase B, alpha 1-3-galactosyltransferase)**	**metabolic process**
217020_at	**RARB**	**0.407**	**retinoic acid receptor, beta**	**differentiation, growth, apoptosis, cell cycle progression, cytostasis, morphology, proliferation**
217671_at	**RFX3**	**0.268**	**Regulatory factor X, 3 (influences HLA class II expression)**	**regulation of transcription**
218174_s_at	C10orf57	0.297	chromosome 10 open reading frame 57	Unknown
219334_s_at	OBFC2A	0.5	oligonucleotide/oligosaccharide-binding fold containing 2A	Unknown
219799_s_at	**DHRS9**	**0.479**	**dehydrogenase/reductase (SDR family) member 9**	**metabolic process**
219834_at	**ALS2CR8**	**0.441**	**amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 8**	**regulation of transcription**
221874_at	KIAA1324	0.45	KIAA1324	Unknown

Gene Name	Gene Symbol	R204W Fold Change	Description	Functions
117_at	HSPA6	5.087	heat shock 70kDa protein 6 (HSP70B')	apoptosis, cell death, growth, cell viability, proliferation, colony, differentiation, endoplasmic reticulum stress response, survival, condensation
200795_at	SPARCL1	5.095	SPARC-like 1 (mast9, hevin)	Apoptosis
213418_at	HSPA6	11.85	heat shock 70kDa protein 6 (HSP70B')	Chaperone
222153_at	MYEF2	2.056	myelin expression factor 2	Commitment
204932_at	**TNFRSF11B**	**2.434**	**tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)**	**apoptosis, differentiation, osteoclastogenesis, proliferation, activity, activation, anoikis, cell viability**
211513_s_at	OGFR	2.628	opioid growth factor receptor	Growth
211753_s_at	RLN1	2.313	relaxin 1	signal transduction; female pregnancy
212523_s_at	KIAA0146	2.284	KIAA0146 protein	Unknown
222121_at	SGEF	2.202	Src homology 3 domain-containing guanine nucleotide exchange factor	signal transduction

Table 11: Genes that were specifically regulated by TAp63γ(R204W) mutant alone when compared to wildtype TAp63γ

Gene Name	Gene Symbol	C306R Fold Change	Description	Functions
203989_x_at	**F2R**	**0.45**	**coagulation factor II (thrombin) receptor**	**aggregation, proliferation, activation, invasion, morphology, shape change, transformation, cell cycle progression, cell movement**
209250_at	DEGS1	0.476	degenerative spermatocyte homolog 1, lipid desaturase (Drosophila)	growth, sub-G1 phase
215407_s_at	ASTN2	0.183	astrotactin 2	Unknown
203634_s_at	CPT1A	0.333	carnitine palmitoyltransferase 1A (liver)	cell death
212815_at	ASCC3	0.495	Activating signal cointegrator 1 complex subunit 3	Unknown
214764_at	RRP15	0.467	ribosomal RNA processing 15 homolog (S. cerevisiae)	protein binding
211074_at	**FOLR1**	**0.492**	**folate receptor 1 (adult) ; folate receptor 1 (adult)**	**growth, colony formation, proliferation, cell viability**
209277_at	TFPI2	0.435	Tissue factor pathway inhibitor 2	proliferation, invasion, attachment
222235_s_at	GALNACT-2	0.483	chondroitin sulfate GalNAcT-2	Unknown
203049_s_at	KIAA0372	0.496	KIAA0372	Unknown
210495_x_at	**FN1**	**0.467**	**fibronectin 1**	**migration, adhesion, cell spreading, apoptosis, proliferation, attachment, assembly, survival**
221765_at	**UGCG**	**0.468**	**UDP-glucose ceramide glucosyltransferase**	**epidermis development, biosynthetic process**
221841_s_at	KLF4	0.452	Kruppel-like factor 4 (gut)	proliferation, migration, colony formation, invasion, cell cycle progression, growth, size, morphology, amplification
204925_at	CTNS	0.441	cystinosis, nephropathic	Transport
212538_at	DOCK9	0.468	dedicator of cytokinesis 9	Unknown
221577_x_at	**GDF15**	**0.377**	**growth differentiation factor 15**	**growth, apoptosis, signaling, rotation, survival, invasiveness, cell viability, morphology, G1 phase**
210466_s_at	SERBP1	0.469	SERPINE1 mRNA binding protein 1	Binding, apoptosis

Gene Name	Gene Symbol	C306R Fold Change	Description	Functions
209676_at	TFPI	0.349	tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	blood coagulation
201213_at	PPP1R7	0.351	protein phosphatase 1, regulatory subunit 7	Unknown
202848_s_at	**GRK6**	**0.467**	**G protein-coupled receptor kinase 6**	**chemotaxis, influx, migration**
203875_at	SMARCA1	0.354	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1	remodeling, differentiation, outgrowth
208661_s_at	TTC3	0.479	tetratricopeptide repeat domain 3	Unknown
221766_s_at	FAM46A	0.492	family with sequence similarity 46, member A	Unknown
212079_s_at	**MLL**	**0.462**	**Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)**	**transformation, growth, differentiation, colony formation, immortalization, apoptosis, maturation, self-renewal, development, proliferation**
60474_at	C20orf42	0.394	chromosome 20 open reading frame 42	Unknown
209712_at	SLC35D1	0.445	solute carrier family 35 (UDP-glucuronic acid/UDP-N-acetylgalactosamine dual transporter), member D1	Transport
203216_s_at	MYO6	0.344	myosin VI	differentiation, apoptosis, plasticity, morphogenesis, integrity, DNA damage response
212984_at	**ATF2**	**0.432**	**Activating transcription factor 2**	**growth, differentiation, colony formation, cell death, G2/M phase transition, survival, synaptic transmission, recovery, double-stranded DNA break repair**
202284_s_at	CDKN1A	0.469	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	apoptosis, growth, proliferation, cell cycle progression, G1 phase, cell death, senescence, differentiation, S phase, G2 phase
205370_x_at	DBT	0.466	dihydrolipoamide branched chain transacylase E2	metabolic process
201141_at	GPNMB	0.415	glycoprotein (transmembrane) nmb	adhesion, proliferation
201200_at	CREG1	0.398	Cellular repressor of E1A-stimulated genes 1	growth, differentiation, G1/S phase transition, proliferation

Gene Name	Gene Symbol	C306R Fold Change	Description	Functions
201299_s_at	MOBK1B	0.478	MOB1, Mps One Binder kinase activator-like 1B (yeast)	Telophase
201534_s_at	UBL3	0.447	ubiquitin-like 3	protein modification process
201617_x_at	CALD1	0.457	caldesmon 1	assembly, cytokinesis, morphology, translocation, cell cycle progression, G2/M phase transition, size, cell movement
201939_at	PLK2	0.486	polo-like kinase 2 (Drosophila)	apoptosis, growth, S phase, survival
202127_at	PRPF4B	0.351	PRP4 pre-mRNA processing factor 4 homolog B (yeast)	mRNA processing
202351_at	**ITGAV**	**0.476**	**integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)**	**adhesion, binding, migration, invasion, proliferation, apoptosis, growth, motility, survival, cell spreading**
202890_at	MAP7	0.425	microtubule-associated protein 7	binding, deformation, morphology, biogenesis
203049_s_at	KIAA0372	0.496	KIAA0372	Unknown
203078_at	**CUL2**	**0.327**	**cullin 2**	**G1/S phase transition, proliferation, cell cycle progression, growth, apoptosis**
203455_s_at	SAT1	0.458	spermidine/spermine N1-acetyltransferase	growth, cytostasis, apoptosis
203671_at	TPMT	0.423	Thiopurine S-methyltransferase	Proliferation
203710_at	ITPR1	0.339	Inositol 1,4,5-triphosphate receptor, type 1	apoptosis, release, leakage, cell death, calcium oscillation, depolarization, extension, long term depression
204056_s_at	MVK	0.44	mevalonate kinase (mevalonic aciduria)	biosynthetic process
204184_s_at	**ADRBK2**	**0.491**	**Adrenergic, beta, receptor kinase 2**	**Mitogenesis**
204545_at	PEX6	0.343	peroxisomal biogenesis factor 6	Biogenesis
204761_at	USP6NL	0.496	USP6 N-terminal like	regulation of Rab GTPase activity
204793_at	GPRASP1	0.414	G protein-coupled receptor associated sorting protein 1	Unknown
204821_at	BTN3A3	0.373	butyrophilin, subfamily 3, member A3	Unknown
204970_s_at	MAFG	0.436	v-maf musculoaponeurotic fibrosarcoma oncogene homolog G (avian)	proliferation, dysfunction, differentiation

Gene Name	Gene Symbol	C306R Fold Change	Description	Functions
205575_at	C1QL1	0.432	complement component 1, q subcomponent-like 1	Unknown
206141_at	MOCS3	0.288	molybdenum cofactor synthesis 3	Unknown
206440_at	LIN7A	0.487	lin-7 homolog A (C. elegans)	Transport
206526_at	RIBC2	0.488	RIB43A domain with coiled-coils 2	Unknown
206613_s_at	TAF1A	0.454	TATA box binding protein (TBP)-associated factor, RNA polymerase I, A, 48kDa	regulation of transcription
206857_s_at	FKBP1B	0.36	FK506 binding protein 1B, 12.6 kDa	proliferation, cell viability
206942_s_at	PMCH	0.451	pro-melanin-concentrating hormone	hyperpolarization, plasticity
207143_at	CDK6	0.417	Cyclin-dependent kinase 6	cell cycle progression, G1 phase, G1/S phase transition, proliferation, growth, transformation, apoptosis, checkpoint control, lifespan
207455_at	P2RY1	0.259	Purinergic receptor P2Y, G-protein coupled, 1	aggregation, binding
207565_s_at	MR1	0.483	Major histocompatibility complex, class I-related	antigen processing
207598_x_at	XRCC2	0.489	X-ray repair complementing defective repair in Chinese hamster cells 2	stability, sister chromatid exchange, exchange, aberration, cell death, aneuploid, growth, apoptosis, survival
208325_s_at	AKAP13	0.397	A kinase (PRKA) anchor protein 13	cell rounding, survival, apoptosis, growth, decondensation, assembly, transformation, proliferation
208806_at	CHD3	0.337	chromodomain helicase DNA binding protein 3	remodeling, assembly, biogenesis
209016_s_at	KRT7	0.467	keratin 7	cytoskeleton organization and biogenesis
209040_s_at	PSMB8	0.399	proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional peptidase 7)	transmembrane potential, endoplasmic reticulum stress response, lysis, activation, replication
209119_x_at	**NR2F2**	**0.5**	**Nuclear receptor subfamily 2, group F, member 2**	**Migration**
209909_s_at	**TGFB2**	**0.486**	**transforming growth factor, beta 2**	**proliferation, apoptosis, growth, differentiation, cytostasis, cell cycle progression, cell death, colony formation, adhesion, stimulation**

Gene Name	Gene Symbol	C306R Fold Change	Description	Functions
210268_at	NFX1	0.38	nuclear transcription factor, X-box binding 1	Unknown
210655_s_at	FOXO3A	0.414	forkhead box O3A	apoptosis, cell cycle progression, proliferation, survival, cell death, DNA damage response, checkpoint control, accumulation, morphology, transformation
210867_at	CNOT4	0.347	CCR4-NOT transcription complex, subunit 4	regulation of transcription
211580_s_at	**PIK3R3**	**0.343**	**phosphoinositide-3-kinase, regulatory subunit 3 (p55, gamma)**	**apoptosis, survival, proliferation, growth, morphology, chemotaxis, cell spreading, cell cycle progression, S phase, haptotaxis**
211673_s_at	MOCS1	0.472	molybdenum cofactor synthesis 1	metabolic process
211828_s_at	TNIK	0.464	TRAF2 and NCK interacting kinase	cell spreading
211965_at	ZFP36L1	0.444	zinc finger protein 36, C3H type-like 1	Proliferation
212310_at	MIA3	0.339	melanoma inhibitory activity family, member 3	Unknown
212315_s_at	NUP210	0.276	nucleoporin 210kDa	cell viability
212325_at	DKFZP686A01247	0.283	hypothetical protein	Unknown
212930_at	ATP2B1	0.328	ATPase, Ca++ transporting, plasma membrane 1	proliferation, cell cycle progression, extension, cell death
213353_at	ABCA5	0.445	ATP-binding cassette, sub-family A (ABC1), member 5	Transport
213572_s_at	SERPINB1	0.384	serpin peptidase inhibitor, clade B (ovalbumin), member 1	Unknown
213900_at	C9orf61	0.466	chromosome 9 open reading frame 61	Unknown
214130_s_at	PDE4DIP	0.375	phosphodiesterase 4D interacting protein (myomegalin)	cytoskeleton organization and biogenesis
214330_at	ATPAF2	0.468	ATP synthase mitochondrial F1 complex assembly factor 2	protein folding

Gene Name	Gene Symbol	C306R Fold Change	Description	Functions
214724_at	DIXDC1	0.443	DIX domain containing 1	multicellular organismal development
214993_at	ASPHD1	0.471	aspartate beta-hydroxylase domain containing 1	peptidyl-amino acid modification
215030_at	GRSF1	0.437	G-rich RNA sequence binding factor 1	morphogenesis of embryonic epithelium
215069_at	NMT2	0.429	N-myristoyltransferase 2	Binding
215150_at	YOD1	0.485	YOD1 OTU deubiquinating enzyme 1 homolog (yeast)	ubiquitin cycle
215646_s_at	VCAN	0.246	versican	adhesion, apoptosis, recognition, attachment, cell movement, growth, proliferation, elastogenesis, differentiation, outgrowth
215886_x_at	USP12	0.47	ubiquitin specific peptidase 12	Unknown
216218_s_at	PLCL2	0.409	phospholipase C-like 2	binding, activation, hyperproliferation, migration, development, signaling, apoptosis
216531_at	MBTPS2 ; YY2	0.355	membrane-bound transcription factor peptidase, site 2 ; YY2 transcription factor	Unknown
216841_s_at	SOD2	0.392	superoxide dismutase 2, mitochondrial	apoptosis, cell death, proliferation, transmembrane potential, survival, growth, mitogenesis, response, migration
216962_at	**OR5T2 RPAIN**	**0.399**	**RPA interacting protein olfactory receptor, family 5, subfamily T, member 2**	**signal transduction**
217607_x_at	EIF4G2	0.433	eukaryotic translation initiation factor 4 gamma, 2	growth, apoptosis, transformation, cell death, cell cycle progression, binding, shunting, morphology, differentiation
218031_s_at	FOXN3	0.495	forkhead box N3	G2/M phase
218166_s_at	RSF1	0.5	remodeling and spacing factor 1	positioning, assembly, remodeling
218183_at	C16orf5	0.44	chromosome 16 open reading frame 5	Unknown
218502_s_at	TRPS1	0.388	trichorhinophalangeal syndrome I	Apoptosis
218935_at	EHD3	0.455	EH-domain containing 3	Unknown
219012_s_at	C11orf30	0.318	chromosome 11 open reading frame 30	DNA repair

Gene Name	Gene Symbol	C306R Fold Change	Description	Functions
219094_at	ARMC8	0.437	armadillo repeat containing 8	Unknown
219174_at	IFT74	0.414	ntraflagellar transport 74 homolog (Chlamydomonas)	Unknown
219274_at	TSPAN12	0.413	tetraspanin 12	Unknown
219346_at	LRFN3	0.428	leucine rich repeat and fibronectin type III domain containing 3	Unknown
219387_at	CCDC88A	0.465	coiled-coil domain containing 88A	proliferation, migration, replication, phosphorylation, biogenesis
219460_s_at	TMEM127	0.307	transmembrane protein 127	Unknown
219499_at	SEC61A2	0.466	Sec61 alpha 2 subunit (S. cerevisiae)	protein transport
220039_s_at	CDKAL1	0.496	CDK5 regulatory subunit associated protein 1-like 1	metabolic process
220474_at	SLC25A21	0.286	solute carrier family 25 (mitochondrial oxodicarboxylate carrier), member 21	Transport
220764_at	PPP4R2	0.347	protein phosphatase 4, regulatory subunit 2	protein modification process
220776_at	KCNJ14	0.344	potassium inwardly-rectifying channel, subfamily J, member 14	hyperpolarization, plasticity
220940_at	KIAA1641	0.421	KIAA1641	Unknown
221683_s_at	Cep290	0.386	centrosome protein cep290	Unknown
221276_s_at	SYNC1	0.431	Syncoilin, intermediate filament 1	Unknown
222018_at	NACA ; NACAP1	0.394	Nascent-polypeptide-associated complex alpha polypeptide	transport, transcription, translation
222031_at	LOC286434	0.449	hypothetical protein LOC286434 ; similar to Serine/threonine-protein kinase PRKX (Protein kinase PKX1)	Unknown
222263_at	SLC35E1	0.49	solute carrier family 35, member E1	Transport
51228_at	RBM12B	0.475	RNA binding motif protein 12B	Unknown
204697_s_at	CHGA	3.233	chromogranin A (parathyroid secretory protein 1)	Activation, injury, biogenesis
204777_s_at	MAL	3.067	mal, T-cell differentiation protein	polarization, development, differentiation
214974_x_at	CXCL5	17.62	chemokine (C-X-C motif) ligand 5	chemotaxis, transmigration, signaling, stimulation, proliferation, activation
219080_s_at	CTPS2	2.818	CTP synthase II	Unknown
204770_at	TAP2	2.569	transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	Segregation
212806_at	KIAA0367	6.454	KIAA0367	Apoptosis

Gene Name	Gene Symbol	C306R Fold Change	Description	Functions
202987_at	TRAF3IP2	2.297	TRAF3 interacting protein 2	Apoptosis
203741_s_at	ADCY7	2.294	adenylate cyclase 7	growth, apoptosis, G1 phase, G2/M phase transition
204276_at	TK2	2.35	thymidine kinase 2, mitochondrial	Transformation
205110_s_at	FGF13	2.036	fibroblast growth factor 13	proliferation, migration, angiogenesis, invasion, signaling, volume, cell death, differentiation
205669_at	NCAM2	3.577	neural cell adhesion molecule 2	fasciculation, lamination, long-term potentiation, development, adhesion, morphology
206094_x_at	UGT1A6	2.377	UDP glucuronosyltransferase 1 family, polypeptide A6	metabolic process
206181_at	SLAMF1	2.467	Signaling lymphocytic activation molecule family member 1	proliferation, co-stimulation, activation, polarization
210176_at	TLR1	2.256	toll-like receptor 1	inflammatory response
210969_at	PKN2	3.023	protein kinase N2	apoptosis, cell-cell adhesion, differentiation, reorganization
210999_s_at	GRB10	2.262	growth factor receptor-bound protein 10	growth, apoptosis, transformation, G2 phase, S phase, signaling
211315_s_at	CACNA1G	2.372	calcium channel, voltage-dependent, alpha 1G subunit	proliferation, neuritogenesis, cell death
212425_at	SCAMP1	2.024	Secretory carrier membrane protein 1	Transport
213382_at	MST1	12.32	macrophage stimulating 1 (hepatocyte growth factor-like)	migration, growth, morphology, scattering, stimulation, motility, activation, atrophy
215783_s_at	ALPL	2.163	alkaline phosphatase, liver/bone/kidney	Mineralization
215844_at	TNPO2	3.063	transportin 2 (importin 3, karyopherin beta 2b)	Transport
216077_s_at	L3MBTL	2.029	l(3)mbt-like (Drosophila)	Cytokinesis
216565_x_at	LOC391020	2.054	similar to Interferon-induced transmembrane protein 3 (Interferon-inducible protein 1-8U)	Unknown
218468_s_at	GREM1	2.552	gremlin 1, cysteine knot superfamily, homolog (Xenopus laevis)	signaling, growth, migration
219501_at	ENOX1	2.191	ecto-NOX disulfide-thiol exchanger 1	Unknown

Gene Name	Gene Symbol	C306R Fold Change	Description	Functions
220115_s_at	CDH10	2.817	cadherin 10, type 2 (T2-cadherin)	cell-cell adhesion
220591_s_at	EFHC2	2.039	EF-hand domain (C-terminal) containing 2	Unknown
220686_s_at	PIWIL2	3.347	piwi-like 2 (Drosophila)	zygotene, prophase, early pachytene stage, development
221169_s_at	HRH4	2.246	histamine receptor H4	binding, chemotaxis

Table 12: Genes that were specifically regulated by TAp63γ(C306R) mutant alone when compared to wildtype TAp63γ

Gene Name	Gene Symbol	R279H Fold Change	Description	Functions
206763_at	FKBP6	2.303	FK506 binding protein 6, 36kDa	misalignment, homologous pairing
222313_at	CNOT2	2.197	CCR4-NOT transcription complex, subunit 2	Growth
201884_at	CEACAM5	2.381	carcinoembryonic antigen-related cell adhesion molecule 5	aggregation, binding, colony formation, cytotoxicity, stimulation, differentiation, activation, anoikis
202311_s_at	COL1A1	3.253	collagen, type I, alpha 1	aggregation, migration, proliferation, binding, adhesion, morphology, invasion, cell spreading, degranulation, growth
208142_at	FAM12A	3.16	family with sequence similarity 12, member A	Unknown
219945_at	DDX25	2.455	DEAD (Asp-Glu-Ala-Asp) box polypeptide 25	Spermatogenesis
215457_at	ARPC1A	3.09	Actin related protein 2/3 complex, subunit 1A, 41kDa	polymerization, assembly, nucleation, organization, stabilization, polarization, rearrangement, biogenesis
205625_s_at	**CALB1**	**6.805**	**calbindin 1, 28kDa**	**apoptosis, cytotoxic reaction, survival, paired-pulse facilitation, function, fragmentation, plasticity**
215723_s_at	PLD1	2.306	phospholipase D1, phophatidylcholine-specific	fusion, migration, invasion, size, attachment, elongation, cell flattening, morphology, budding
201010_s_at	**TXNIP**	**2.03**	**thioredoxin interacting protein**	**proliferation, apoptosis, survival, sub-G1 phase, development, growth, activity, response, differentiation**
202157_s_at	CUGBP2	2.115	CUG triplet repeat, RNA binding protein 2	Unknown
202546_at	VAMP8	2.237	vesicle-associated membrane protein 8 (endobrevin)	exocytosis, secretion, fusion, growth

Gene Name	Gene Symbol	R279H Fold Change	Description	Functions
202751_at	TFIP11	2.292	tuftelin interacting protein 11	Unknown
203231_s_at	ATXN1	5.369	ataxin 1	vacuolation, loss, synaptic transmission, morphology, apoptosis
203240_at	FCGBP	2.114	Fc fragment of IgG binding protein	immune response
203698_s_at	FRZB	3.597	frizzled-related protein	Growth
203962_s_at	NEBL	2.875	nebulette	ion transport; regulation of actin filament length
203969_at	LOC153914	2.326	hypothetical protein LOC153914	Unknown
204008_at	DNAL4	2.011	dynein, axonemal, light polypeptide 4	microtubule motor activity
204124_at	SLC34A2	3.131	solute carrier family 34 (sodium phosphate), member 2	transmembrane potential
204310_s_at	NPR2	2.654	natriuretic peptide receptor B	intracellular signaling cascade
204719_at	ABCA8	2.811	ATP-binding cassette, sub-family A (ABC1), member 8	Transport
205285_s_at	FYB	2.742	FYN binding protein (FYB-120/130)	proliferation, binding, adhesion, migration, activation
205431_s_at	BMP5	3.23	bone morphogenetic protein 5	differentiation, neurogenesis, apoptosis, cell viability
205668_at	LY75	2.073	lymphocyte antigen 75	immune response
205681_at	**BCL2A1**	**2.288**	**BCL2-related protein A1**	**apoptosis, survival, transformation, cell death, growth, cell cycle progression, necrosis, proliferation, cell viability**
205923_at	RELN	2.515	reelin	migration, positioning, morphogenesis, positive selection, compaction, detachment, branching, binding
206198_s_at	CEACAM7	2.422	carcinoembryonic antigen-related cell adhesion molecule 7	Unknown
206331_at	CALCRL	2.55	calcitonin receptor-like	proliferation, migration, activation, binding, apoptosis

Gene Name	Gene Symbol	R279H Fold Change	Description	Functions
206420_at	IGSF6	2.981	immunoglobulin superfamily, member 6	immune response
206609_at	MAGEC1	2.372	melanoma antigen family C, 1	unknown
206733_at	TULP2	2.086	tubby like protein 2	visual perception
207056_s_at	SLC4A8	3.099	solute carrier family 4, sodium bicarbonate cotransporter, member 8	ion transport
207175_at	ADIPOQ	2.693	adiponectin, C1Q and collagen domain containing	proliferation, migration, differentiation, colony formation, binding, apoptosis, generation, size
207526_s_at	IL1RL1	3.556	interleukin 1 receptor-like 1	activation, differentiation, proliferation, volume, infiltration, growth, recruitment, apoptosis
207638_at	PRSS7	2.75	protease, serine, 7 (enterokinase)	proteolysis
207820_at	ADH1A	2.069	alcohol dehydrogenase 1A (class I), alpha polypeptide	metabolic process
208057_s_at	**GLI2**	**3.3**	**GLI-Kruppel family member GLI2**	**proliferation, differentiation, transformation, development, clustering, projection, cell death, branching morphogenesis**
208154_at	LOC51336	2.276	mesenchymal stem cell protein DSCD28	unknown
208245_at	RAB9P1	2.351	RAB9, member RAS oncogene family, pseudogene 1	unknown
209696_at	FBP1	2.877	fructose-1,6-bisphosphatase 1	metabolic process
209763_at	CHRDL1	2.387	chordin-like 1	differentiation, commitment
210602_s_at	CDH6	3.193	cadherin 6, type 2, K-cadherin (fetal kidney)	adhesion, interaction, organization, dissociation, shape change, segregation, binding, outgrowth, assembly

Gene Name	Gene Symbol	R279H Fold Change	Description	Functions
210814_at	TRPC3	2.332	transient receptor potential cation channel, subfamily C, member 3	transmembrane potential, binding
211184_s_at	USH1C	2.121	Usher syndrome 1C (autosomal recessive, severe)	function, differentiation
211239_s_at	ADAM7	8.023	ADAM metallopeptidase domain 7	Proteolysis
211349_at	SLC15A1	2.524	solute carrier family 15 (oligopeptide transporter), member 1	oligopeptide transport
211621_at	**AR**	**2.075**	**androgen receptor (dihydrotestosterone receptor; testicular feminization; spinal and bulbar muscular atrophy; Kennedy disease)**	**growth, proliferation, apoptosis, migration, binding, invasion, cell death, mitogenesis, cell cycle progression**
211825_s_at	FLI1	2.638	Friend leukemia virus integration 1	growth, apoptosis, colony formation, differentiation, transformation, morphology, proliferation
212705_x_at	PNPLA2	2.349	patatin-like phospholipase domain containing 2	metabolic process
213130_at	ZNF473	2.235	zinc finger protein 473	regulation of transcription
213725_x_at	XYLTI1	4.039	xylosyltransferase I	glycosaminoglycan biosynthetic process
213866_at	SAMD14	2.416	sterile alpha motif domain containing 14	Unknown
215151_at	DOCK10	6.949	dedicator of cytokinesis 10	Unknown
215591_at	SATB2	2.327	SATB family member 2	regulation of transcription
215754_at	SCARB2	2.192	scavenger receptor class B, member 2	cell adhesion
216489_at	TRPM3	2.368	transient receptor potential cation channel, subfamily M, member 3	ion transport
216874_at	DKFZp686O1327	2.049	Homo sapiens, clone IMAGE:5538654, mRNA	Unknown
217315_s_at	KLK13	2.055	kallikrein 13	Proteolysis
217525_at	OLFML1	5.858	olfactomedin-like 1	Unknown
218029_at	FAM65A	2.08	family with sequence similarity 65, member A	DNA directed RNA polymerase activity

Gene Name	Gene Symbol	R279H Fold Change	Description	Functions
218747_s_at	TAPBPL	2.456	TAP binding protein-like	antigen processing
219044_at	FLJ10916	2.108	hypothetical protein FLJ10916	metabolic process
219278_at	MAP3K6	3.279	mitogen-activated protein kinase kinase kinase 6	Unknown
219945_at	DDX25	2.455	DEAD (Asp-Glu-Ala-Asp) box polypeptide 25	Spermatogenesis
220002_at	KIF26B	4.157	kinesin family member 26B	microtubule-based movement
220336_s_at	GP6	2.021	glycoprotein VI (platelet)	aggregation, activation, adhesion, binding, inhibition, secretion
220803_at	AMSH-LP	5.725	Associated molecule with the SH3 domain of STAM (AMSH) like protein	ubiquitin cycle
221304_at	UGT1A10	2.264	UDP glucuronosyltransferase 1 family, polypeptide A10	metabolic process
221319_at	PCDHB8	3.59	protocadherin beta 8	cell adhesion
222128_at	NSUN6	3.023	NOL1/NOP2/Sun domain family, member 6	Unknown
222168_at	ALDH1A3	2.991	Aldehyde dehydrogenase 1 family, member A3	Apoptosis
48031_r_at	C5orf4	2.279	chromosome 5 open reading frame 4	metabolic process
56748_at	TRIM10	2.169	tripartite motif-containing 10	Hemopoiesis
61297_at	CASKIN2	2.304	CASK interacting protein 2	Unknown
204580_at	**MMP12**	**0.359**	**matrix metallopeptidase 12 (macrophage elastase)**	**invasion, proliferation, binding, growth, recovery, migration, malignancy**
206650_at	IQCC	0.355	IQ motif containing C	Unknown
207595_s_at	**BMP1**	**0.448**	**bone morphogenetic protein 1**	**differentiation, neurogenesis, growth**
210141_s_at	**INHA**	**0.484**	**inhibin, alpha**	**proliferation, necrosis, stimulation, differentiation, growth, signaling**
218589_at	P2RY5	0.18	purinergic receptor P2Y, G-protein coupled, 5	signal transduction
37802_r_at	FAM63B	0.246	family with sequence similarity 63, member B	Transport

Table 13: Genes that were regulated specifically by TAp63γ(R279H) mutant alone when compared to wildtype TAp63γ

Gene Name	Gene Symbol	R227Q Fold Change	Description	Functions
220149_at	FLJ22671	0.448	hypothetical protein FLJ22671	unknown
220119_at	EPB41L4A	0.241	erythrocyte membrane protein band 4.1 like 4A	unknown
220073_s_at	PLEKHG6	0.434	pleckstrin homology domain containing, family G (with RhoGef domain) member 6	regulation of transcription
202057_at	KPNA1	0.476	karyopherin alpha 1 (importin alpha 5)	binding, apoptosis
202463_s_at	MBD3	0.462	methyl-CpG binding domain protein 3	growth
203749_s_at	**RARA**	**0.488**	**retinoic acid receptor, alpha**	**differentiation, apoptosis, maturation, growth, proliferation, expansion, endoplasmic reticulum stress response**
204182_s_at	ZBTB43	0.199	zinc finger and BTB domain containing 43	regulation of transcription
204230_s_at	SLC17A7	0.4	solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 7	neurotransmission
205547_s_at	TAGLN	0.256	transgelin	invasiveness, biogenesis
206776_x_at	**ACRV1**	**0.434**	**acrosomal vesicle protein 1**	**multicellular organismal development**
206971_at	GPR161	0.287	G protein-coupled receptor 161	proliferation, migration
207101_at	VAMP1	0.467	vesicle-associated membrane protein 1 (synaptobrevin 1)	Exocytosis, transport
207597_at	ADAM18	0.37	ADAM metallopeptidase domain 18	proliferation, migration
208349_at	TRPA1	0.483	transient receptor potential cation channel, subfamily A, member 1	ion transport
209293_x_at	ID4	0.349	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein	proliferation, differentiation, colony formation, survival
209859_at	TRIM9	0.428	tripartite motif-containing 9	unknown
210150_s_at	**LAMA5**	**0.475**	**laminin, alpha 5**	**migration, proliferation, growth, adhesion, elongation, apoptosis, cell spreading, invasion, morphology**

Gene Name	Gene Symbol	R227Q Fold Change	Description	Functions
210876_at	ANXA2P1	0.337	Annexin A2 pseudogene 1	unknown
212636_at	QKI	0.359	quaking homolog, KH domain RNA binding (mouse)	apoptosis, differentiation
214664_at	PAICS	0.335	phosphoribosylaminoimidazole succinocarboxamide synthetase	purine biosynthesis
215512_at	MARCH6	0.418	membrane-associated ring finger (C3HC4) 6	ubiquitin cycle
215930_s_at	CTAGE5	0.498	CTAGE family, member 5	enzyme activator activity
216180_s_at	SYNJ2	0.473	synaptojanin 2	Clustering
218800_at	SRD5A2L	0.447	steroid 5 alpha-reductase 2-like	Unknown
221978_at	HLA-F	0.431	major histocompatibility complex, class I, F	immune response
207754_at	RASSF8	2.013	Ras association (RalGDS/AF-6) domain family 8	signal transduction
215855_s_at	TMF1	2.382	TATA element modulatory factor 1	regulation of transcription
217519_at	MACF1	3.232	Glycine-rich protein (GRP3S)	depolymerization, stabilization, stability, cell movement
201187_s_at	ITPR3	2.515	inositol 1,4,5-triphosphate receptor, type 3	depolarization, extension, apoptosis, release
201667_at	GJA1	2.141	gap junction protein, alpha 1, 43kDa (connexin 43)	signaling, proliferation, growth, apoptosis, assembly, contact growth inhibition, response
202046_s_at	**GRLF1**	**2.235**	**glucocorticoid receptor DNA binding factor 1**	**neuritogenesis, shape change, morphology, development**
204530_s_at	TOX	3.08	thymus high mobility group box protein TOX	regulation of transcription
204920_at	CPS1	2.197	carbamoyl-phosphate synthetase 1, mitochondrial	metabolic process
209936_at	RBM5	3.167	RNA binding motif protein 5	apoptosis, proliferation, growth
210755_at	HGF	2.519	hepatocyte growth factor (hepapoietin A; scatter factor)	migration, proliferation, apoptosis, invasion, growth, morphogenesis

Gene Name	Gene Symbol	R227Q Fold Change	Description	Functions
212093_s_at	**MTUS1**	**2.199**	**mitochondrial tumor suppressor 1**	**Proliferation**
213056_at	FRMD4B	2.007	FERM domain containing 4B	Unknown
215064_at	SC5DL	2.23	Sterol-C5-desaturase (ERG3 delta-5-desaturase homolog, fungal)-like	metabolic process
215538_at	LARGE	4.537	like-glycosyltransferase	Unknown
216740_at	TRERF1	2.644	Transcriptional regulating factor 1	regulation of transcription

Table 14: Genes that were specifically regulated by TAp63γ(R227Q) mutant alone when compared to wildtype TAp63γ

Gene Name	Gene Symbol	R298Q Fold Change	Description	Functions
205060_at	PARG	0.496	poly (ADP-ribose) glycohydrolase	single-stranded DNA break repair, cytostasis, DNA damage response, recognition
212392_s_at	PDE4DIP	0.401	phosphodiesterase 4D interacting protein (myomegalin)	cytoskeleton organization and biogenesis
214152_at	CCPG1	0.453	cell cycle progression 1	Unknown
222227_at	ZNF236	0.488	zinc finger protein 236	regulation of transcription
203570_at	**LOXL1**	**0.349**	**lysyl oxidase-like 1**	**colony formation, cell death, development**
204909_at	DDX6	0.473	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6	Unknown
205554_s_at	**DNASE1L3**	**0.396**	**deoxyribonuclease I-like 3**	**Survival**
205647_at	RAD52	0.33	RAD52 homolog (S. cerevisiae)	homologous recombination repair, development
206214_at	**PLA2G7**	**0.42**	**phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)**	**fertilization, motility, infiltration, apoptosis**
206573_at	KCNQ3	0.346	potassium voltage-gated channel, KQT-like subfamily, member 3	Hyperpolarization
207323_s_at	MBP	0.451	myelin basic protein	activation, proliferation, stimulation, outgrowth, priming, infiltration, myelination, development, organization, stability
207401_at	PROX1	0.489	prospero-related homeobox 1	budding, tubulation, motility, differentiation, sprouting, migration, commitment, proliferation, chemotaxis
208200_at	**IL1A**	**0.498**	**interleukin 1, alpha**	**activation, apoptosis, proliferation, stimulation, differentiation, growth, survival, adhesion, response**
208741_at	**SAP18**	**0.481**	**sin3-associated polypeptide, 18kDa**	**cell death**
210347_s_at	**BCL11A**	**0.499**	**B-cell CLL/lymphoma 11A (zinc finger protein)**	**apoptosis, transformation, cell death, differentiation, growth**

Gene Name	Gene Symbol	R298Q Fold Change	Description	Functions
212477_at	CENTB2	0.486	centaurin, beta 2	regulation of GTPase activity
213849_s_at	**PPP2R2B**	**0.493**	**protein phosphatase 2, regulatory subunit B , beta isoform**	**apoptosis, survival**
214291_at	RPL17	0.469	ribosomal protein L17	regulation of translation
214668_at	C13orf1	0.469	chromosome 13 open reading frame 1	Unknown
217127_at	CTH	0.464	cystathionase (cystathionine gamma-lyase)	proliferation, apoptosis
218901_at	PLSCR4	0.495	phospholipid scramblase 4	blood coagulation
219509_at	MYOZ1	0.424	myozenin 1	Assembly
219694_at	FAM105A	0.486	family with sequence similarity 105, member A	Unknown
219747_at	C4orf31	0.34	chromosome 11 open reading frame 1	Unknown
221016_s_at	TCF7L1	0.31	transcription factor 7-like 1 (T-cell specific, HMG-box)	differentiation, survival
36612_at	KIAA0280	0.471	KIAA0280 protein	Unknown
205817_at	SIX1	2.828	sine oculis homeobox homolog 1 (Drosophila)	proliferation, apoptosis, differentiation, disorganization, migration
219996_at	ASB7	2.06	Ankyrin repeat and SOCS box-containing 7	intracellular signaling
214081_at	PLXDC1	2.759	plexin domain containing 1	multicellular organism development
202665_s_at	WIPF1	2.953	WAS/WASL interacting protein family, member 1	morphology, proliferation, endocytosis, degranulation, cytostasis, polarization
204667_at	FOXA1	2.054	forkhead box A1	development, differentiation
206112_at	ANKRD7	2.128	ankyrin repeat domain 7	intracellular signaling
211207_s_at	ACSL6	2.312	acyl-CoA synthetase long-chain family member 6	length, proliferation
213206_at	GOSR2	2.099	golgi SNAP receptor complex member 2	transport, fusion
214814_at	YT521	2.23	Splicing factor YT521-B	Unknown
216683_at	TBCA	2.822	Tubulin-specific chaperone a	Unknown
218925_s_at	C11orf1	5.29	chromosome 11 open reading frame 1	Unknown

Table 15: Genes that are specifically regulated by TAp63γ(R298Q) mutant alone when compared to wildtype TAp63γ

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