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ΔNp63α utilizes multiple mechanisms to repress transcription in squamous cell carcinoma cells

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ΔNp63α is a potent oncogene in squamous cell carcinomas (SCCs) and a pro-proliferative factor expressed by basal epithelial cells. ΔNp63α functions both as a transcriptional repressor and activator, but it is not clear how these activities contribute to its oncogenic potential. ΔNp63α was proposed to function as a dominant negative of the related factor p53. Additionally, ΔNp63α was shown to inactivate its family member TAp73 and mediate recruitment of repressive histone deacetylase (HDAC) complexes to chromatin. Recently, we identified a new mechanism of repression involving recruitment of histone H2A/H2A.Z exchange complexes and H2A.Z deposition at ΔNp63α target genes. Here, we aimed to define the possible co-occurrence of the various repressive mechanisms. In lung SCC cells expressing ΔNp63α, p53 and TAp73, we found that ΔNp63α exerts its pro-proliferative and transcriptional repressive effects in a manner independent of p53, TAp73 and histone H3 and H4 deacetylation. Instead, ΔNp63α target genes are differentiated from non-target genes within the p53 network by incorporation and accumulation of acetylated H2A.Z. These results indicate that ΔNp63α utilizes multiple mechanisms of repression in diverse epithelial and SCC cells.

Keywords: transcriptional repression, histone acetylation, p53, p73, H2A.Z, squamous cell carcinoma, oncogene

Abbreviations: AcH2A.Z, acetylated histone variant H2A.Z; AcH4, acetylated histone H4; ChIP, chromatin immunoprecipitation; H3K9Ac, histone H3 acetylated on lysine 9; HAT, histone acetyltransferase; HDAC, histone deacetylase; Q-PCR, quantitative polymerase chain reaction; Q-RT-PCR, quantitative reverse transcriptase polymerase chain reaction; SCC, squamous cell carcinoma; shp63, shRNA targeting p63; shp73, shRNA targeting p73; SRB, sulforhodamine B

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Introduction

ΔNp63α is a member of the p53 family of transcriptional regulators that functions as a potent oncogene in squamous cell carcinomas (SCCs) of various origins.[1-3] ΔNp63α overexpression is an indicator of poor prognosis for carcinomas of the lung, breast, cervix, prostate and urinary tract, and it is well established that ΔNp63α promotes cell proliferation and blocks apoptosis.[4-7] Despite its importance in cancer biology, the mechanism of action of ΔNp63α remains poorly understood. ΔNp63α has been shown to function as a transcriptional repressor of various genes within the p53 network, although the set of ΔNp63α transcriptional targets appears to vary significantly from cell type to cell type.[5,8-10] Furthermore, the mechanisms by which ΔNp63α affects target gene expression and promotes cell proliferation are diverse. Specific functions of ΔNp63α may be p53-dependent[4] and/or p73-dependent[5,10] and may be mediated through the recruitment of histone deacetylases (HDACs)[11,12] and/or deposition of histone variant H2A.Z.[13] In this study, we have determined that the precise contribution of these various mechanisms differs in a cell type-specific manner. We utilized H226 lung SCC cells, which express wild type p53, ΔNp63α and TAp73, to define the role of p73, HDACs and H2A.Z in mediating transcriptional repression by ΔNp63α. Using an isogenic cell system, we found that knockdown of p73 does not affect the response of H226 SCC cells to ΔNp63α depletion, indicating that ΔNp63α acts in a p73-independent manner in these cells. Furthermore, ΔNp63α knockdown does not affect histone H3 or H4 acetylation at enhancer sites, demonstrating that histone deacetylation is not essential for ΔNp63α-mediated transcriptional repression in these cells. Finally, deposition of histone variant H2A.Z and acetylated H2A.Z does discriminate

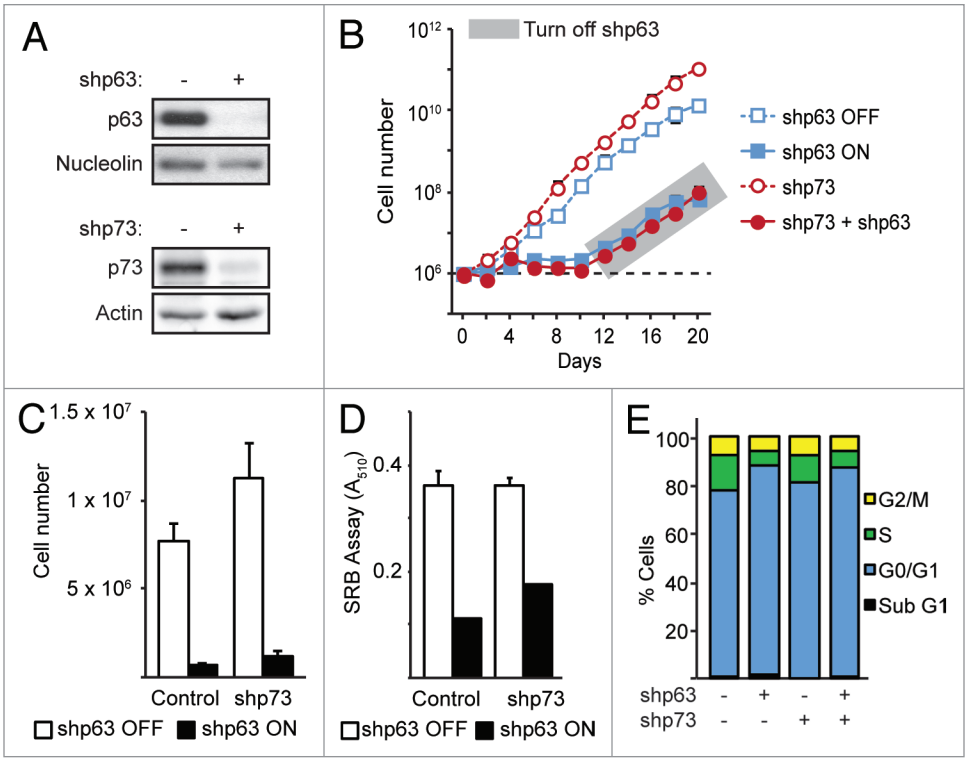

Figure 1. ΔNp63α drives H226 SCC cell proliferation independently of p73. (**A**) Western blot of H226 cell extracts following 48 h ΔNp63α knockdown or following stable p73 knockdown. Nucleolin and actin serve as loading controls. (**B and C**) Cell proliferation assays performed by direct cell counting. Cells carrying stably integrated shRNAs against ΔNp63α and p73 were pretreated with doxycycline for 5 d prior to seeding to induce ΔNp63α knockdown. In (**B**), 1×10^6 cells were seeded at day 0, and doxycycline was removed from the media at day 10 to allow re-expression of ΔNp63α. In (**C**), 5×10^5 cells were seeded at day 0, and cells were counted after 5 d without any disturbance. (**D**) Sulforhodamine B (SRB) assay of cell proliferation following 5 d of knockdown of ΔNp63α and/or p73. (**E**) Cell cycle profile following 5 d of knockdown of ΔNp63α and/or p73.

between classes of genes that are affected by ΔNp63α knockdown and those within the p53 network that are not. In conjunction with other published studies, these results indicate that ΔNp63α utilizes multiple mechanisms to affect gene expression and promote cell proliferation in epithelial and SCC cells, and that the relative contributions of each of these mechanisms vary substantially from cell type to cell type.

Results and Discussion

ΔNp63α drives proliferation of SCC cells independently of p73 status. ΔNp63α is a potent pro-proliferative factor in basal keratinocytes and epithelial stem cells,[3] and it functions as a potent oncogene in squamous cell carcinomas (SCCs) of diverse origins.[5,13,14] Loss of ΔNp63α expression in keratinocytes and SCC cells results in decreased cell proliferation via cell cycle arrest, senescence and/or apoptosis, depending on the cell type.[4,5,13,15] Little is known, however, about the relative contributions of the p53 family members, p53 and p73, to ΔNp63α-dependent cell proliferation. Previous studies have shown cell type-specific responses; ΔNp63α expression is required to allow for continued proliferation of cells of epithelial origin, yet this phenomenon may be either p53-dependent[4] or p53-independent[5,10] and either p73-dependent[5,10] or p73-independent[10] in normal keratinocytes and various SCC cells.

We chose to investigate the role of the various family members in ΔNp63α-dependent cell proliferation in rare SCC cells expressing wild type versions of p53, ΔNp63α and TAp73: H226 lung SCC cells. To test for a functional interaction between these proteins, we generated isogenic cell lines stably expressing shRNAs targeting each mRNA (Gallant-Behm et al.;[13] **Fig. 1A**). The shRNA targeting ΔNp63α (referred to as shp63) is tetracycline-inducible, allowing us to toggle cells between ΔNp63α-competent and ΔNp63α-deficient states by adding or removing doxycycline from the media. As described previously, H226 cells undergo a reversible cell cycle arrest following the loss of ΔNp63α expression (**Fig. 1B–E**). Our recent studies have demonstrated that H226 cell cycle arrest following ΔNp63α knockdown is p53-independent.[13] Similarly, concomitant knockdown of p73 using a constitutive shRNA (shp73) does not rescue the proliferation arrest caused by ΔNp63α depletion (**Fig. 1B–E**). However, p73 knockdown alone does increase the proliferation rate of H226 cells, confirming the anti-proliferative effects of TAp73 in this cell line. Taken together, these results demonstrate that ΔNp63α drives proliferation of SCC cells independently of p53 and p73.

ΔNp63α and p53 target gene expression is not affected by p73 status in H226 SCC cells. ΔNp63α has been shown to function as a transcriptional repressor of a number of genes within the p53 network.[5,8-10] In fact, the DNA response elements to which ΔNp63α and p73 bind are

indistinguishable from one another,[16] and they are highly similar to that of p53.[17-19] Thus it has been proposed that ΔNp63α acts as a dominant-negative to the transcriptional activators p53 and/or TAp73, either by direct binding and inactivation or by preventing their access to DNA. With respect to p53, both hypotheses have been refuted; several groups have established that p53 and ΔNp63α do not hetero-oligomerize,[20,21] and our recent work using H226 SCC cells has shown that ΔNp63α does not prevent p53 occupancy at its enhancer sites, nor does it prevent p53 from activating transcription of its target genes.[13] Furthermore, we found that the two factors affect the expression of largely non-overlapping gene sets, indicating that ΔNp63α utilizes other mechanisms of transcriptional repression in SCC cells.[13] With respect to TAp73, work in the Ellisen laboratory has demonstrated that ΔNp63α repression of the p53 target genes PUMA and NOXA in JHU-029 SCC cells is TAp73β-dependent.[5,10] Several groups have confirmed that ΔNp63α-TAp73β hetero-oligomerization does occur in vitro, in vivo and on chromatin.[5,16,20-22] Additionally, ΔNp63α mRNA,[10] total protein[5,16] and chromatin-bound protein[16] levels were found to exceed that of TAp73 in SCC cells and primary SCC tumors, suggesting that the more abundant ΔNp63α may occupy enhancer sites as homo-tetramers, thereby preventing TAp73 occupancy (model presented in **Fig. 5A**).

In order to determine whether transcriptional repression by ΔNp63α is p73-dependent in H226 SCC cells, we utilized our isogenic cell lines expressing an inducible shRNA targeting p63 with or without a constitutively expressed shRNA targeting p73. Our previous studies using H226 cells established three "gene classes."[13] Class I genes are those that are induced upon p53 activation using Nutlin-3 or other stimuli; class III genes are induced upon ΔNp63α knockdown; and class II genes are induced under both conditions (**Fig. 2A**). In this study, we proceeded to investigate the mRNA expression patterns of several class I, II and III genes under circumstances of p53 activation, ΔNp63α knockdown, or the combination of the two, in cells with either wild

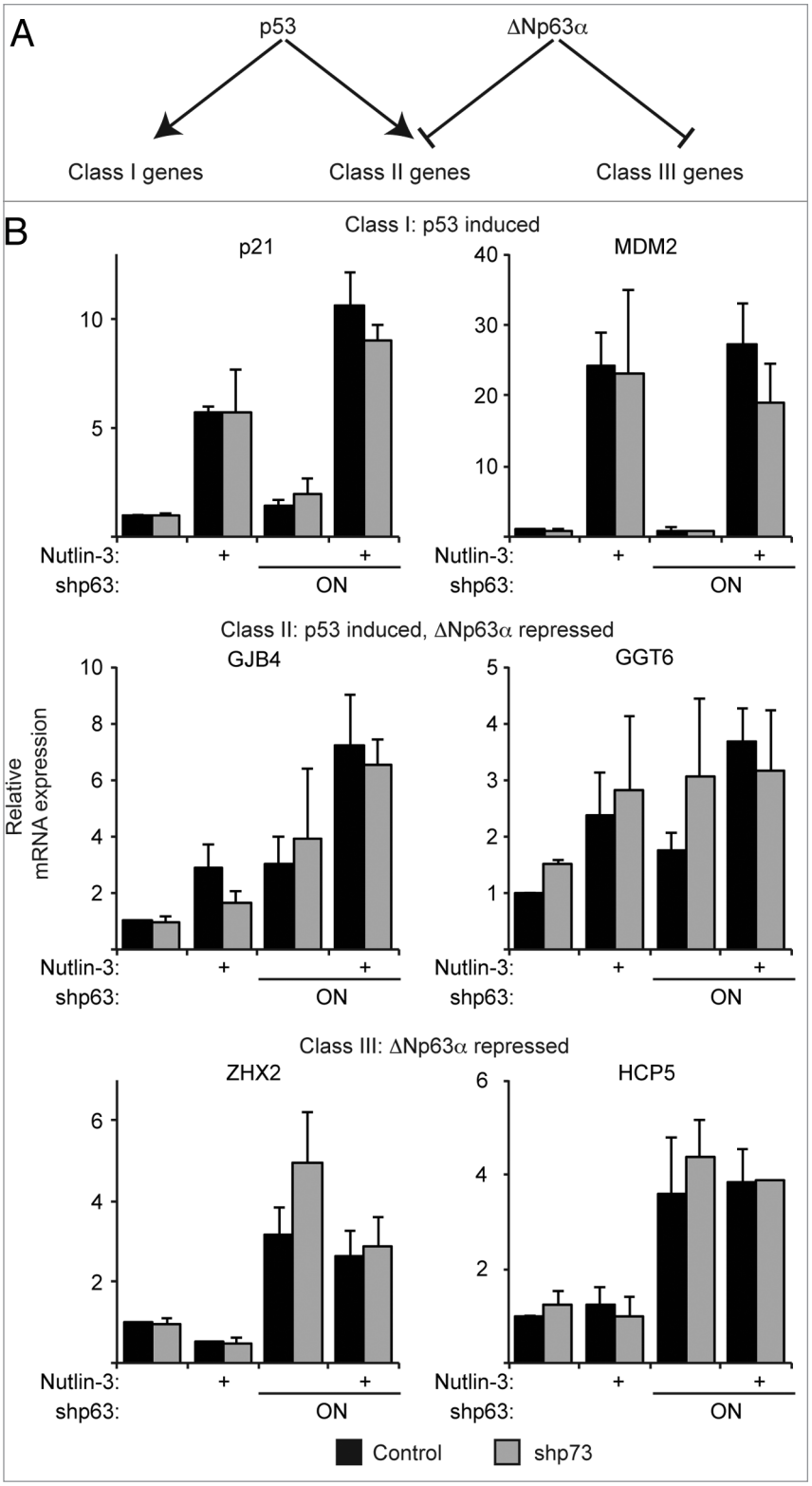

Figure 2. ΔNp63α regulates gene expression in H226 SCC cells independently of p73. (**A**) Flowchart defining gene classes based on microarray and Q-RT-PCR data published in Gallant-Behm et al.[13] Genes with an increased expression following p53 activation are referred to hereafter as "Class I." Genes which are upregulated following ΔNp63α knockdown are "Class III." Genes with an increased expression following both treatments are "Class II." (**B**) Quantitative RT-PCR analysis of selected Class I, II and III target genes following 12 h of 10 μM Nutlin-3 treatment, 48 h of ΔNp63α knockdown, or combination treatment, in cells expressing high levels (black bars) or low levels (gray bars) of p73.

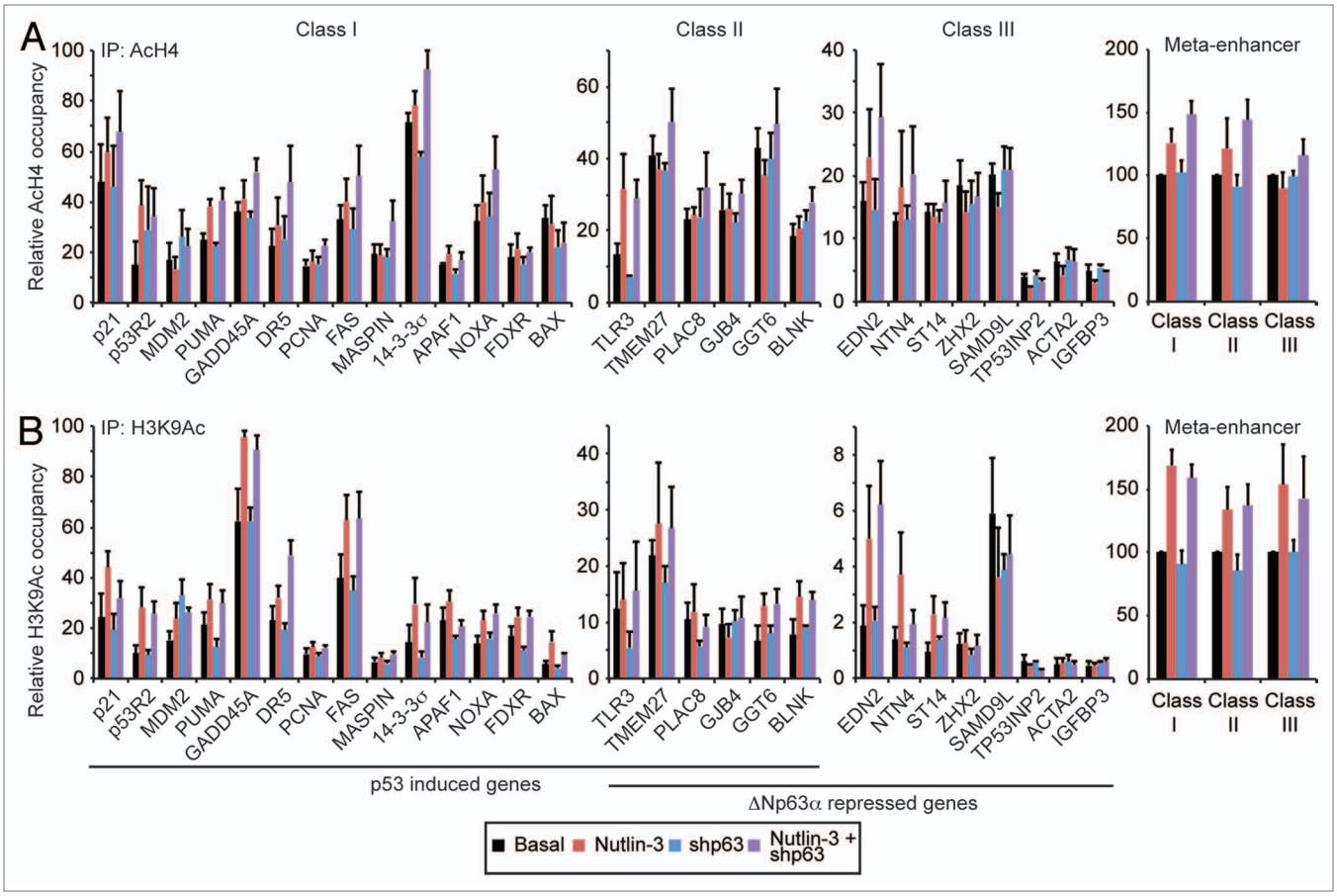

Figure 3. ΔNp63α does not significantly affect histone acetylation at p53/ΔNp63α gene enhancers in H226 SCC cells. ChIP assays were performed with whole-cell extracts from control cells (black bars), following 12 h of 10 μM Nutlin-3 treatment (red bars), 48 h of ΔNp63α knockdown (blue bars), or the combination of Nutlin-3 treatment and ΔNp63α knockdown (purple bars). Antibodies specific for acetylated histone H4 (**A**) and acetylated H3 lysine 9 (H3K9Ac) (**B**) were used. ChIP-enriched DNA was quantified by Q-PCR for the p53/p63 response elements of each indicated gene. See Gallant-Behm et al.[13] for gene maps and amplicon locations. Meta-enhancer values were calculated as the average PCR signal for each treatment group relative to basal values for all response elements tested within a gene class.

type TAp73 or p73 knockdown (**Fig. 2B**). As expected, the class I genes p21 and MDM2 were upregulated upon p53 activation and were not significantly affected by ΔNp63α knockdown, irrespective of p73 status. Interestingly, the expression patterns of the class II and III genes were likewise unaffected by p73 status; the class II genes GJB4 and GGT6 were upregulated by both p53 activation and ΔNp63α knockdown, and the class III genes ZHX2 and HCP5 were only upregulated following the loss of ΔNp63α, with and without p73 knockdown. These results demonstrate that ΔNp63α represses expression of its target genes independent of both p53 and p73 in H226 SCC cells. Taken in conjunction with our cell proliferation studies and other previously published data, this indicates that ΔNp63α utilizes cell type-specific mechanisms of action; while others have shown that ΔNp63α represses TAp73 in its role as a transcriptional activator of pro-apoptotic genes in JHU-029 and JHU-011 SCC cells,[10] we have herein demonstrated that ΔNp63α functions as a transcriptional repressor and oncogene in H226 SCC cells by p73-independent mechanisms.

ΔNp63α does not affect histone H3 and H4 acetylation at p53/p63/p73 enhancer sites. DNA-binding transactivators and repressors exert many of their effects via site-directed recruitment of chromatin-modifying factors. For example, p53 recruits histone acetyl-transferases (HATs) to the chromatin of its target genes, and histone hyper-acetylation is required for p53 transactivation.[23] Recent studies in two independent laboratories have suggested that ΔNp63α can function as a transcriptional repressor by recruiting histone deacetylases HDAC1 and HDAC2 to its target genes. LeBoeuf et al.[11] have demonstrated that HDAC1 and HDAC2 bind to and are specifically active at ΔNp63α-repressed promoters in undifferentiated primary keratinocytes, and that HDAC inhibition results in increased H3K9 acetylation at the p53/p63/p73 enhancer site of ΔNp63α-repressed genes. Furthermore, HDAC1 and HDAC2 double-knockout mice phenocopy ΔNp63α-knockout mice, suggesting that ΔNp63α utilizes HDACs to repress the expression of anti-proliferative genes, thereby promoting epithelial proliferation.[11] HDACs also play a role in ΔNp63α-mediated repression of pro-apoptotic genes in select SCC cells. Ramsey et al.[12] have recently

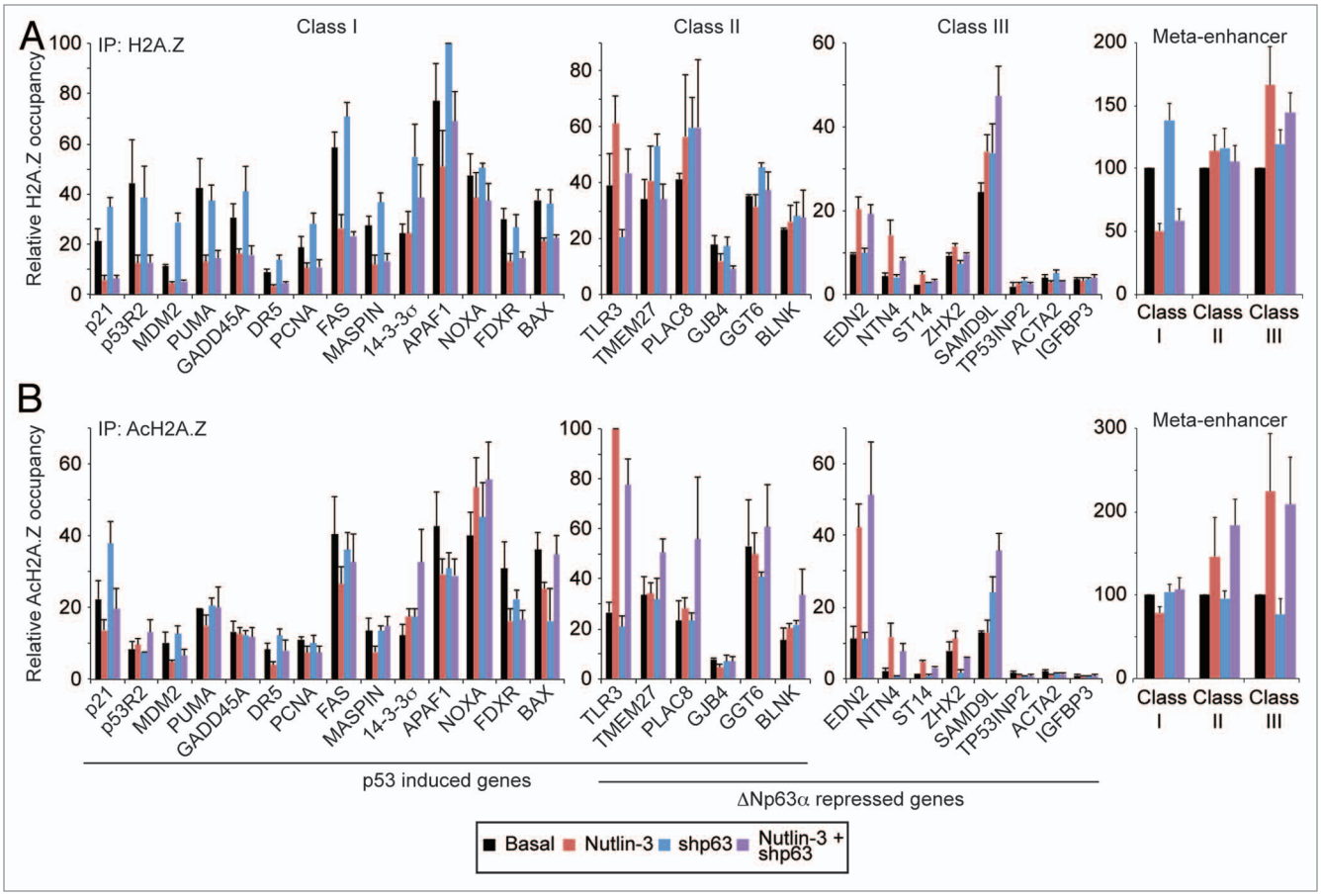

Figure 4. ΔNp63α differentially affects histone variant H2A.Z deposition and acetylation at p53 and ΔNp63α target gene enhancers. ChIP assays were performed with whole-cell extracts from control cells (black bars), following 12 h of 10 μM Nutlin-3 treatment (red bars), 48 h of ΔNp63α knockdown (blue bars), or the combination of Nutlin-3 treatment and ΔNp63α knockdown (purple bars). Antibodies specific for histone variant H2A.Z (**A**) and acetylated H2A.Z (**B**) were used. ChIP-enriched DNA was quantified by real-time PCR for the p53/p63 response elements of each indicated gene. See Gallant-Behm et al.[13] for gene maps and amplicon locations. Meta-enhancer values were calculated as the average PCR signal for each treatment group relative to basal values for all response elements tested within a gene class.

confirmed that in JHU-029 SCC cells, ΔNp63α directly interacts with HDAC1 and HDAC2 in a large molecular weight complex, that ΔNp63α and HDAC1 co-localize to the enhancer site of the pro-apoptotic gene PUMA, and that ΔNp63α knockdown results in increased histone H4 acetylation at the PUMA enhancer site[12] (see model in **Fig. 5B**).

As HDAC recruitment and histone acetylation at select p53/p63/p73 enhancer sites regulate gene expression and proliferation in keratinocytes and JHU-029 cells, we decided to investigate whether transcriptional repression by ΔNp63α in H226 cells is mediated by histone deacetylation. Specifically, we postulated that the antagonistic effects of p53 and ΔNp63α on histone acetylation could vary across gene classes, with class I genes being more sensitive to p53-driven hyperacetylation and class III genes being more prone to ΔNp63α-mediated deacetylation. We therefore utilized chromatin immunoprecipitation (ChIP) to evaluate histone H3 and H4 acetylation at the enhancer sites of a broad selection of class I, II and III genes following p53 activation and/or ΔNp63α depletion (**Fig. 3**). Interestingly, our analysis revealed that histone acetylation is not a diagnostic marker of p53-dependent activation or ΔNp63α-dependent repression. As anticipated, histone H4 acetylation (AcH4) at the enhancer sites of p53-activated genes (class I and II) is increased upon p53 activation with Nutlin-3 (**Fig. 3A**, compare red and purple bars to black control bars). Similarly, histone 3 acetylation on lysine 9 (H3K9Ac) is increased at class I and II genes following p53 activation (**Fig. 3B**). However, it is noteworthy that several class III genes, such as EDN2, NTN4 and ST14, show increased H3 K9 acetylation upon p53 activation, indicating that p53 may still be able to recruit HATs to these enhancers, although these genes remain refractory to transactivation by p53. Surprisingly, neither AcH4 nor H3K9Ac is consistently increased upon ΔNp63α knockdown at these enhancers (compare blue bars to black bars), indicating that ΔNp63α does not inhibit the histone acetylation of its target genes in H226 squamous cell carcinoma cells. In fact, of the 28 enhancers tested, only MDM2 showed a significant increase in H3K9Ac levels following ΔNp63α depletion. MDM2 shows the strongest occupancy for ΔNp63α;[13] however, ΔNp63α does not

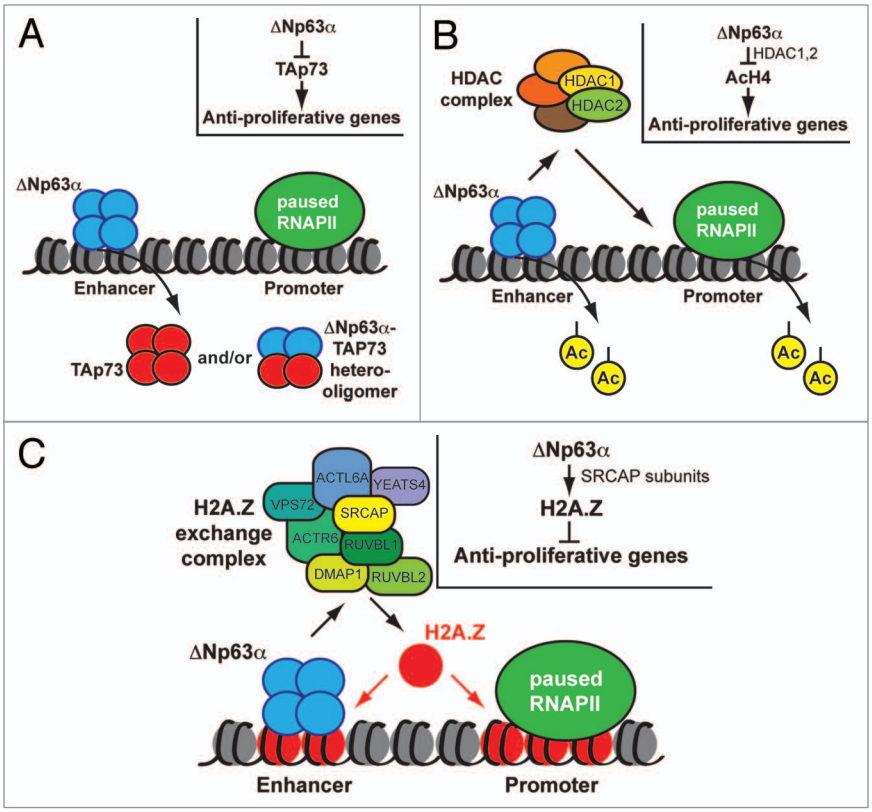

Figure 5. Model of different mechanisms by which ΔNp63α represses transcription in various SCC cells. (**A**) ΔNp63α may evict or exclude TAp73 isoforms from the enhancer site of anti-proliferative genes, thereby acting as a dominant-negative to p73. This mechanism has been observed in primary human keratinocytes,[10] JHU-011 head and neck SCC cells[10] and JHU-029 head and neck SCC cells.[5] (**B**) ΔNp63α may recruit the histone deacetylases HDAC1 and HDAC2 to chromatin, causing the deacetylation of histone H4 at the p53 enhancer and promoter site. This mechanism has been observed in JHU-029 SCC cells.[12] (**C**) ΔNp63α may recruit subunits of the SRCAP complex and mediate H2A.Z incorporation to repress RNAPII at anti-proliferative genes in a mechanism that is autonomous of p53, p73 and HDACs. This mechanism has been observed in H226 SCC cells.[13]

repress MDM2 (**Fig. 2B**), so the observed increase in H3K9Ac has no significant role in MDM2 expression. Although as a group class III enhancers display lower levels of histone acetylation than class I and class II (compare Y axis values), many class I genes have equally low levels, so the correlation is not absolute.

Taken together, these results indicate that in H226 cells, gene-specific regulation by p53 and ΔNp63α cannot be explained by the differential action of their known interacting HATs and HDACs. These findings further illustrate that the mechanisms of transcriptional repression by ΔNp63α are cell type-specific; while ΔNp63α mediates histone deacetylation of enhancer sites in keratinocytes and JHU-029 SCC cells, this mechanism of action is not conserved across all SCC cells.

Differential deposition of histone variant H2A.Z at p53/p63/p73 enhancer sites of p53 and ΔNp63α target genes. We have recently identified a new mechanism by which ΔNp63α represses expression of its target genes; ΔNp63α interacts with and recruits members of the SRCAP chromatin remodeling complex, which, in turn, deposits histone variant H2A.Z to the transcriptional start site of ΔNp63α target genes.[13] As H2A.Z has been shown to act as a transcriptional repressor when localized at or adjacent to transcriptional start sites,[24,25] this offers a mechanism by which ΔNp63α may directly modulate the chromatin environment of its target genes to repress transcription (see model in **Fig. 5C**).

In order to better understand the role of ΔNp63α in regulating H2A.Z deposition at target genes, we performed a ChIP analysis of H2A.Z occupancy at p53/p63/p73 enhancer sites (**Fig. 4A**). Consistent with H2A.Z's proposed role in transcriptional repression,[26] class I gene enhancers show a notable decrease in H2A.Z occupancy following p53 activation (compare black vs. red bars in **Fig. 4A**, Meta-enhancer). Conversely, class II gene enhancers show little change, and class III gene enhancers show an increase in H2A.Z occupancy following p53 activation. Furthermore, ΔNp63α depletion leads to increased H2A.Z occupancy at most class I enhancers, but not consistently at class II and class III enhancers (compare black to blue bars in Meta-enhancer).

It has been shown that H2A.Z acetylation and exchange are coupled molecular processes,[27,28] and that H2A.Z acetylation can affect the stability of histone octamers.[29] Therefore, we next investigated the level of acetylated H2A.Z at enhancer sites (AcH2A.Z, **Fig. 4B**). Interestingly, the levels of AcH2A.Z clearly discriminate between genes that are repressed by ΔNp63α (class II and III), and those that are not (class I). Notably, p53 activation (red bars) leads to an increase in AcH2A.Z occupancy at several class II and III but not class I enhancers, and ΔNp63α depletion (blue bars) leads to a slight decrease in AcH2A.Z occupancy in class III enhancers only. Altogether, these observations indicate that whereas class I enhancers undergo effective acetylation-coupled H2A.Z removal upon p53 activation, class II-III enhancers do not. In combination with our previous findings that depletion of H2A.Z leads to de-repression of ΔNp63α (class III) target genes,[13] these data suggest that in H226 cells, ΔNp63α represses the transcription of its target genes by a mechanism that is dependent on H2A.Z deposition at target gene enhancers and/or transcriptional start sites.

Final comments. Altogether, our results in combination with other studies indicate that there are multiple mechanisms by which ΔNp63α may repress its target gene expression: (1) ΔNp63α may bind to and inactivate TAp73 or may prevent its binding to enhancer sites;[5,10,16] (2) ΔNp63α may recruit HDACs and thereby modulate H3 and H4 acetylation at enhancer sites;[11,12] and (3) ΔNp63α may recruit histone variant H2A.Z to

enhancers and transcriptional start sites of its target genes[13] (**Fig. 5**). Notably, these mechanisms are utilized by proliferating keratinocytes and SCC cells in a combinatorial fashion and in a cell type-specific manner.

Materials and Methods

Cell proliferation assays. All cell proliferation assays were performed as described in Gallant-Behm et al.[13] Briefly, H226 lung SCC cells were transduced with a doxycycline inducible shRNA targeting p63 (pTRIPZ vector, CCG AGC TAT GTC AGT ACT ATT) with or without a constitutively expressed shRNA targeting p73 (pLL3.7 vector, GCC CCG GTT AAT TTG CAT ATA). Cell proliferation was assessed by direct cell counting at a single or multiple time points, by sulforhodamine B (SRB) assay, or via cell cycle analysis as described previously.[13]

Q-RT-PCR. Cells transduced with one or both shRNAs were treated with 10 μM Nutlin-3 (Cayman) for 12 h to activate p53 or 1 μg/mL doxycycline (Sigma-Aldrich) for 48 h to induce expression of shp63, as described in Gallant-Behm et al.[13] Total RNA was then extracted and subjected to quantitative RT-PCR for select p53 and p63 target genes. All primer sequences have been published in Gallant-Behm et al.,[13] with the exception of GGT6 (forward primer: TAC CAC CAC GGT GCC ATC ATC A, reverse primer: AAA ACA TGG CAC CTA GCC CCG T) and HCP5 (forward primer: CCA AGG TTG CGG GTC ATG GAG T, reverse primer: ATT GTA ATC TGC CCA GGT CTG GAT CA).

Chromatin immunoprecipitation. All ChIP analyses were performed as described previously.[30] Briefly, subconfluent H226 cells transduced with the p63 shRNA were treated with 1 μg/mL doxycycline for 48 h and/or 10 μM Nutlin-3 for 12 h and cross-linked with 1% formaldehyde prior to preparation of whole-cell lysates. Cell lysates (1 mg protein) were subjected to ChIP using the indicated antibodies, followed by DNA purification. ChIP-enriched DNA was analyzed by Q-PCR using an absolute quantification method as detailed previously,[30] with the indicated primer sets. All antibodies and primers used were previously published in Gallant-Behm et al.[13]

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References

1. Mills AA. p63: oncogene or tumor suppressor? Curr Opin Genet Dev 2006; 16:38-44; PMID:16359856; http://dx.doi.org/10.1016/j.gde.2005.12.001
2. Koster MI, Dai D, Roop DR. Conflicting roles for p63 in skin development and carcinogenesis. Cell Cycle 2007; 6:269-73; PMID:17224652; http://dx.doi.org/10.4161/cc.6.3.3792
3. Senoo M, Pinto F, Crum CP, McKeon F. p63 Is essential for the proliferative potential of stem cells in stratified epithelia. Cell 2007; 129:523-36; PMID:17482546; http://dx.doi.org/10.1016/j.cell.2007.02.045
4. Truong AB, Kretz M, Ridky TW, Kimmel R, Khavari PA. p63 regulates proliferation and differentiation of developmentally mature keratinocytes. Genes Dev 2006; 20:3185-97; PMID:17114587; http://dx.doi.org/10.1101/gad.1463206
5. Rocco JW, Leong CO, Kuperwasser N, DeYoung MP, Ellisen LW. p63 mediates survival in squamous cell carcinoma by suppression of p73-dependent apoptosis. Cancer Cell 2006; 9:45-56; PMID:16413471; http://dx.doi.org/10.1016/j.ccr.2005.12.013
6. Moergel M, Abt E, Stockinger M, Kunkel M. Overexpression of p63 is associated with radiation resistance and prognosis in oral squamous cell carcinoma. Oral Oncol 2010; 46:667-71; PMID:20656547; http://dx.doi.org/10.1016/j.oraloncology.2010.06.012
7. Garcia S, Dalès JP, Charafe-Jauffret E, Carpentier-Meunier S, Andrac-Meyer L, Jacquemier J, et al. Poor prognosis in breast carcinomas correlates with increased expression of targetable CD146 and c-Met and with proteomic basal-like phenotype. Hum Pathol 2007; 38:830-41; PMID:17316758; http://dx.doi.org/10.1016/j.humpath.2006.11.015
8. Westfall MD, Mays DJ, Sniezek JC, Pietenpol JA. The Delta Np63 alpha phosphoprotein binds the p21 and 14-3-3 sigma promoters in vivo and has transcriptional repressor activity that is reduced by Hay-Wells syndrome-derived mutations. Mol Cell Biol 2003; 23:2264-76; PMID:12640112; http://dx.doi.org/10.1128/MCB.23.7.2264-2276.2003
9. Mundt HM, Stremmel W, Melino G, Krammer PH, Schilling T, Müller M. Dominant negative (DeltaN) p63alpha induces drug resistance in hepatocellular carcinoma by interference with apoptosis signaling pathways. Biochem Biophys Res Commun 2010; 396:335-41; PMID:20403333; http://dx.doi.org/10.1016/j.bbrc.2010.04.093
10. DeYoung MP, Johannessen CM, Leong CO, Faquin W, Rocco JW, Ellisen LW. Tumor-specific p73 up-regulation mediates p63 dependence in squamous cell carcinoma. Cancer Res 2006; 66:9362-8; PMID:17018588; http://dx.doi.org/10.1158/0008-5472.CAN-06-1619
11. LeBoeuf M, Terrell A, Trivedi S, Sinha S, Epstein JA, Olson EN, et al. Hdac1 and Hdac2 act redundantly to control p63 and p53 functions in epidermal progenitor cells. Dev Cell 2010; 19:807-18; PMID:21093383; http://dx.doi.org/10.1016/j.devcel.2010.10.015
12. Ramsey MR, He L, Forster N, Ory B, Ellisen LW. Physical association of HDAC1 and HDAC2 with p63 mediates transcriptional repression and tumor maintenance in squamous cell carcinoma. Cancer Res 2011; 71:4373-9; PMID:21527555; http://dx.doi.org/10.1158/0008-5472.CAN-11-0046
13. Gallant-Behm CL, Ramsey MR, Bensard CL, Nojek I, Tran J, Liu M, et al. ΔNp63α represses anti-proliferative genes via H2A.Z deposition. Genes Dev 2012; 26:2325-36; PMID:23019126; http://dx.doi.org/10.1101/gad.198069.112
14. Parsa R, Yang A, McKeon F, Green H. Association of p63 with proliferative potential in normal and neoplastic human keratinocytes. J Invest Dermatol 1999; 113:1099-105; PMID:10594758; http://dx.doi.org/10.1046/j.1523-1747.1999.00780.x
15. Rivetti di Val Cervo P, Lena AM, Nicoloso M, Rossi S, Mancini M, Zhou H, et al. p63-microRNA feedback in keratinocyte senescence. Proc Natl Acad Sci USA 2012; 109:1133-8; PMID:22228303; http://dx.doi.org/10.1073/pnas.1112257109
16. Yang A, Zhu Z, Kettenbach A, Kapranov P, McKeon F, Gingeras TR, et al. Genome-wide mapping indicates that p73 and p63 co-occupy target sites and have similar dna-binding profiles in vivo. PLoS ONE 2010; 5:e11572; PMID:20644729; http://dx.doi.org/10.1371/journal.pone.0011572
17. Ortt K, Sinha S. Derivation of the consensus DNA-binding sequence for p63 reveals unique requirements that are distinct from p53. FEBS Lett 2006; 580:4544-50; PMID:16870177; http://dx.doi.org/10.1016/j.febslet.2006.07.004
18. Perez CA, Ott J, Mays DJ, Pietenpol JA. p63 consensus DNA-binding site: identification, analysis and application into a p63MH algorithm. Oncogene 2007; 26:7363-70; PMID:17563751; http://dx.doi.org/10.1038/sj.onc.1210561
19. Kouwenhoven EN, van Heeringen SJ, Tena JJ, Oti M, Dutilh BE, Alonso ME, et al. Genome-wide profiling of p63 DNA-binding sites identifies an element that regulates gene expression during limb development in the 7q21 SHFM1 locus. PLoS Genet 2010; 6:e1001065; PMID:20808887; http://dx.doi.org/10.1371/journal.pgen.1001065
20. Davison TS, Vagner C, Kaghad M, Ayed A, Caput D, Arrowsmith CH. p73 and p63 are homotetramers capable of weak heterotypic interactions with each other but not with p53. J Biol Chem 1999; 274:18709-14; PMID:10373484; http://dx.doi.org/10.1074/jbc.274.26.18709
21. Joerger AC, Rajagopalan S, Natan E, Veprintsev DB, Robinson CV, Fersht AR. Structural evolution of p53, p63, and p73: implication for heterotetramer formation. Proc Natl Acad Sci USA 2009; 106:17705-10; PMID:19815500; http://dx.doi.org/10.1073/pnas.0905867106
22. Chan WM, Siu WY, Lau A, Poon RY. How many mutant p53 molecules are needed to inactivate a tetramer? Mol Cell Biol 2004; 24:3536-51; PMID:15060172; http://dx.doi.org/10.1128/MCB.24.8.3536-3551.2004
23. Espinosa JM, Emerson BM. Transcriptional repression by p53 through intrinsic DNA/chromatin binding and site-directed cofactor recruitment. Mol Cell 2001; 8:57-69; PMID:11511360; http://dx.doi.org/10.1016/S1097-2765(01)00283-0
24. Marques M, Laflamme L, Gervais AL, Gaudreau L. Reconciling the positive and negative roles of histone H2A.Z in gene transcription. Epigenetics 2010; 5:267-72; PMID:20364108; http://dx.doi.org/10.4161/epi.5.4.11520

25. Gévry N, Chan HM, Laflamme L, Livingston DM, Gaudreau L. p21 transcription is regulated by differential localization of histone H2A.Z. Genes Dev 2007; 21:1869-81; PMID:17671089; http://dx.doi.org/10.1101/gad.1545707
26. Guillemette B, Bataille AR, Gévry N, Adam M, Blanchette M, Robert F, et al. Variant histone H2A.Z is globally localized to the promoters of inactive yeast genes and regulates nucleosome positioning. PLoS Biol 2005; 3:e384; PMID:16248679; http://dx.doi.org/10.1371/journal.pbio.0030384
27. Choi J, Heo K, An W. Cooperative action of TIP48 and TIP49 in H2A.Z exchange catalyzed by acetylation of nucleosomal H2A. Nucleic Acids Res 2009; 37:5993-6007; PMID:19696079; http://dx.doi.org/10.1093/nar/gkp660
28. Altaf M, Auger A, Monnet-Saksouk J, Brodeur J, Piquet S, Cramet M, et al. NuA4-dependent acetylation of nucleosomal histones H4 and H2A directly stimulates incorporation of H2A.Z by the SWR1 complex. J Biol Chem 2010; 285:15966-77; PMID:20332092; http://dx.doi.org/10.1074/jbc.M110.117069
29. Ishibashi T, Dryhurst D, Rose KL, Shabanowitz J, Hunt DF, Ausió J. Acetylation of vertebrate H2A.Z and its effect on the structure of the nucleosome. Biochemistry 2009; 48:5007-17; PMID:19385636; http://dx.doi.org/10.1021/bi900196c
30. Gomes NP, Bjerke G, Llorente B, Szostek SA, Emerson BM, Espinosa JM. Gene-specific requirement for P-TEFb activity and RNA polymerase II phosphorylation within the p53 transcriptional program. Genes Dev 2006; 20:601-12; PMID:16510875; http://dx.doi.org/10.1101/gad.1398206