**Supplementary Data**

**Four Supplementary Figures**

**Supplementary Figure Legends**

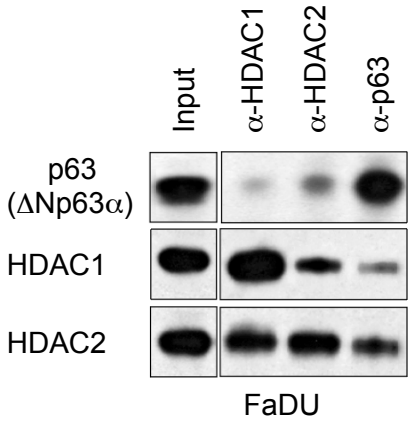
**Supplementary Table**

**Supplementary Materials and Methods**

**Supplementary Reference**

## Supplementary Figure 1

A

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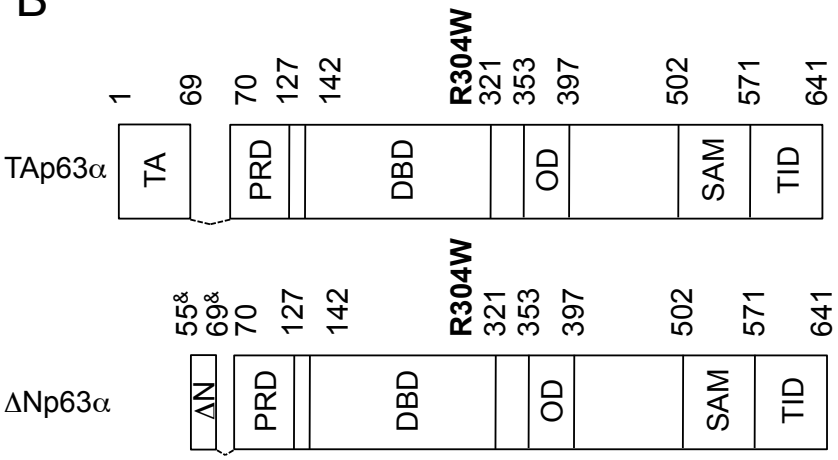
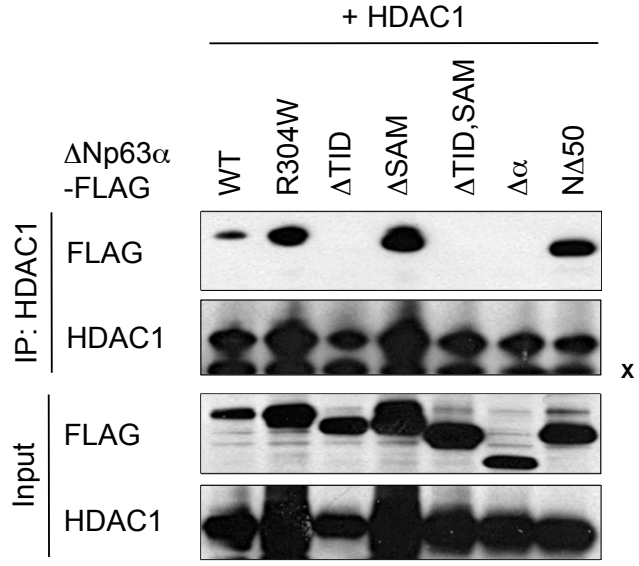
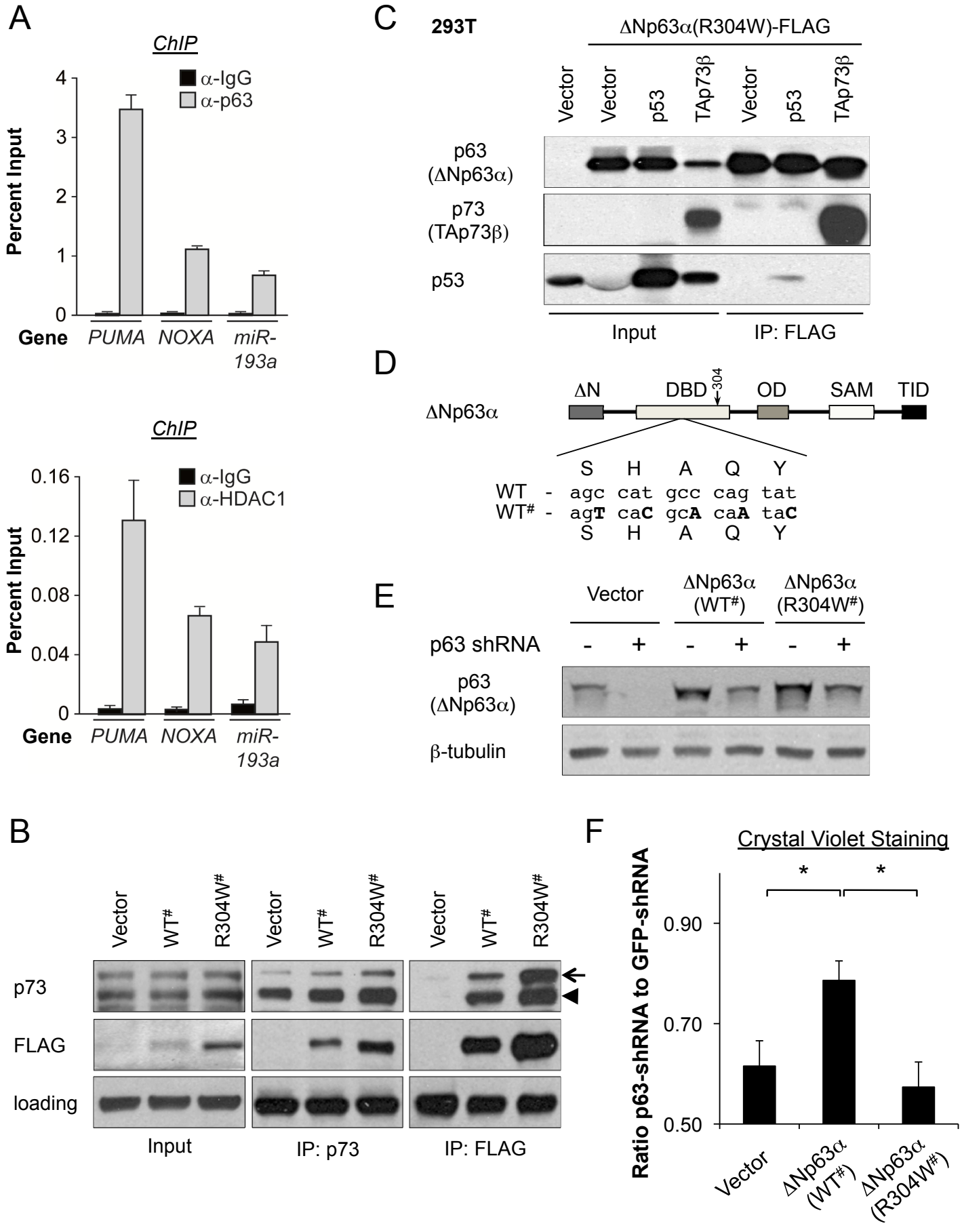



| Construct | Amino Acids |
|---|---|
| ΔNp63α (WT) | 55&-641 |
| ΔNp63α (ΔTID) | 55&-570 |
| ΔNp63α (ΔSAM) | 55&-491, 572-641 |
| ΔNp63α (ΔTID, SAM) | 55&-501 |
| ΔNp63α (Δα) | 55&-412 |
| ΔNp63α (NΔ50) | 106-641 |

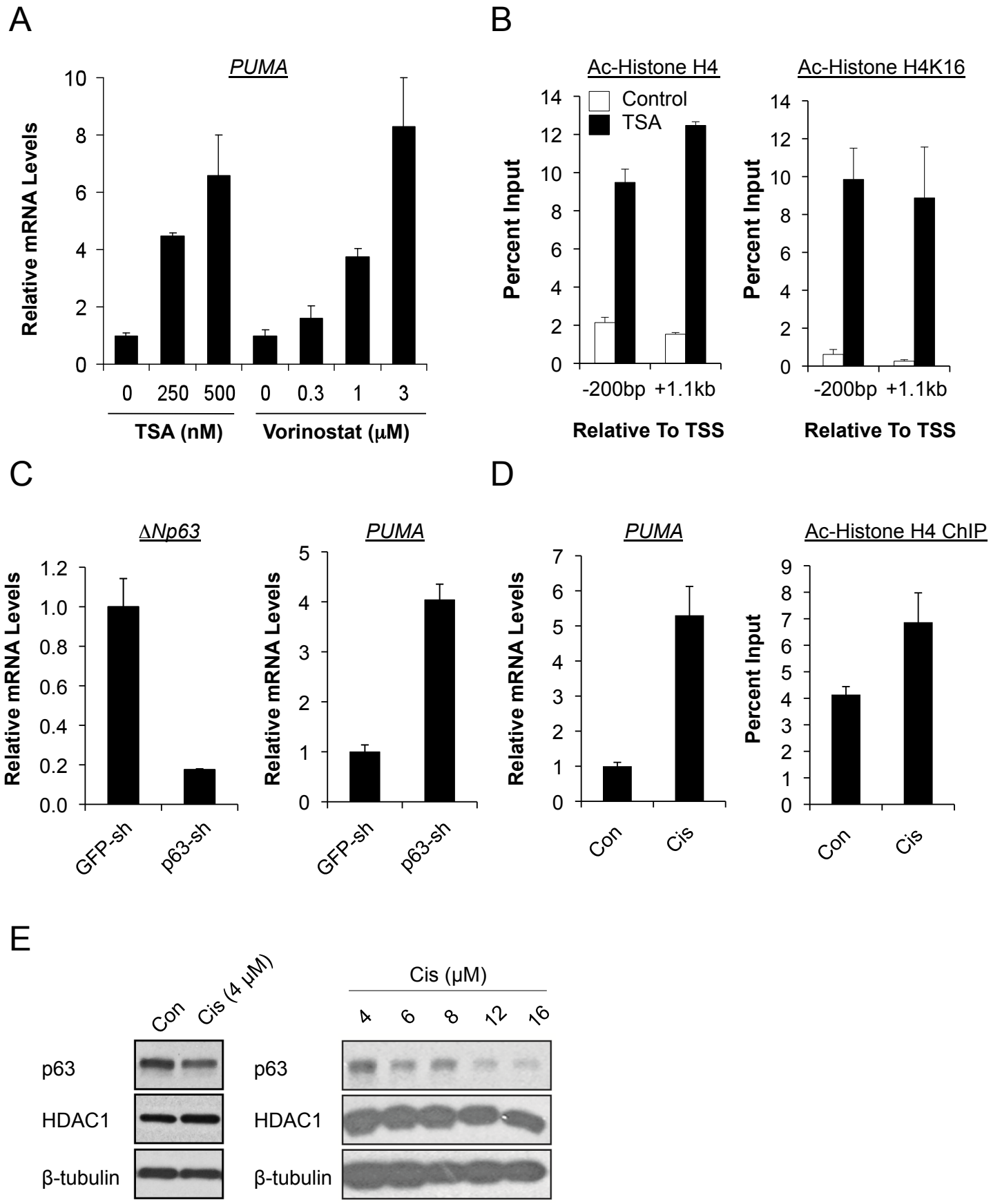
C



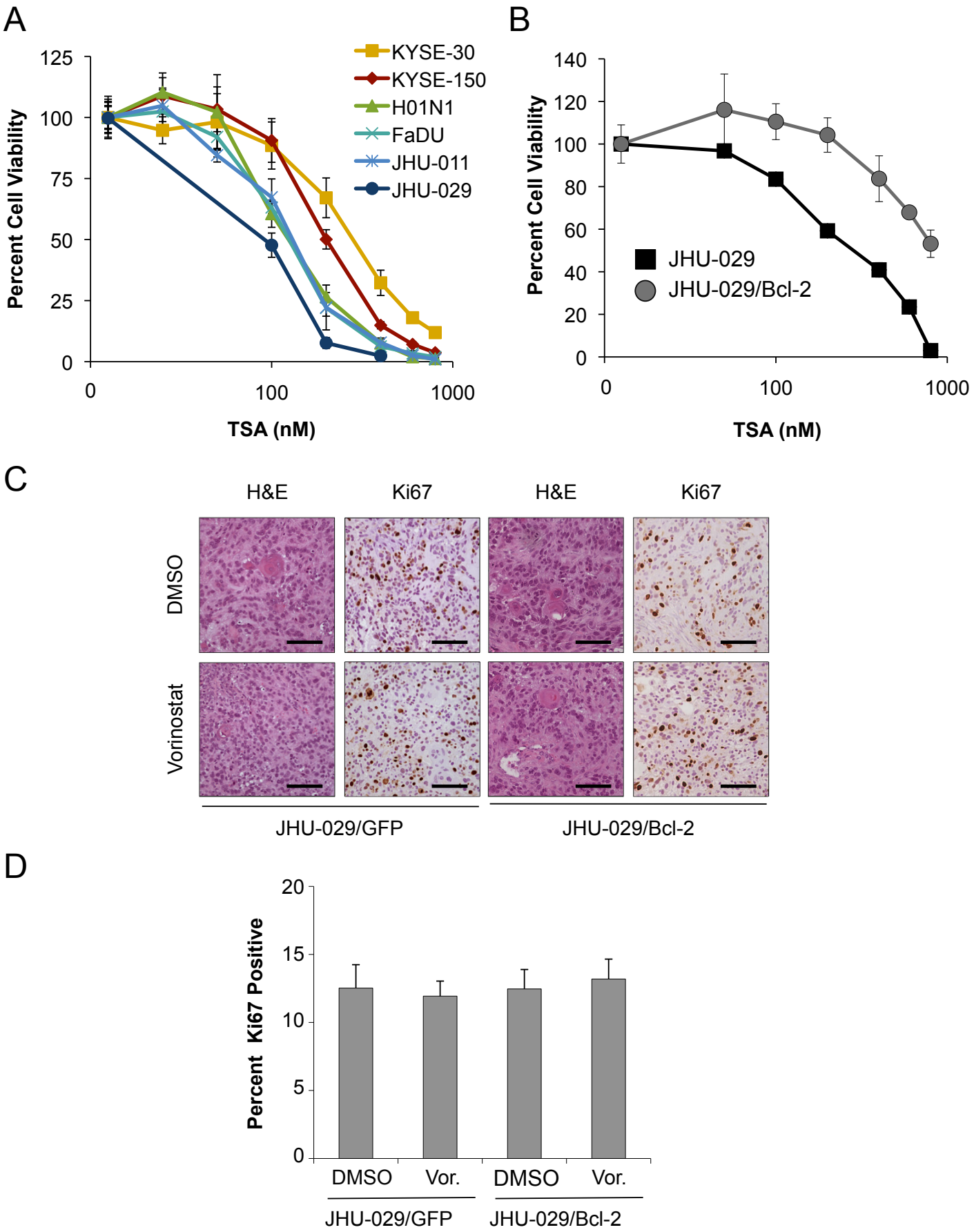
## Supplementary Figure 2



## Supplementary Figure 3



## Supplementary Figure 4



**Supplementary Figure Legends**

**Supplementary Figure 1**. **ΔNp63α and HDAC interact in HNSCC cells.**

*A*, Endogenous p63 associates with HDAC1 and HDAC2 in FaDU. Proteins were immunoprecipitated from nuclear extracts with indicated antibodies. *B*, Schematic of TAp63α (top) and ΔNp63α (bottom) deletion and point mutants. PRD, proline rich domain. OD, oligomerization domain. SAM, Sterile Alpha Motif. TID, Trans-Inhibitory Domain. Note that TAp63α and ΔNp63α N-terminal regions are transcribed from distinct promoters, and splice in-frame at amino acid 70 relative to TAp63α. ($^{\&}$) Designates amino acids unique to ΔNp63. *C*, The p63 TID domain is required for HDAC interaction. HDAC1 and ΔNp63α-FLAG were co-expressed in 293T cells, followed by IP with an α-HDAC1 antibody.

**Supplementary Figure 2**. **DNA binding by ΔNp63α is required, and p73 binding by ΔNp63α is insufficient to suppress cell death.**

*A*, ChIP showing binding of p63 (top) and HDAC1 (bottom) to the regulatory regions of three p63-regulated transcriptional target genes. *B*, Western blot (left) and IP/western (right) showing co-immunoprecipitation of endogenous p73 and FLAG epitope-tagged wild-type (WT$^{\#}$) or DNA binding-deficient point mutant (R304W$^{\#}$) ΔNp63α proteins stably expressed in JHU-029 cells. Arrow: TAp73α; arrowhead: TAp73β. Empty vector transduced cells serve as a negative control for specificity of the α-FLAG antibody. Loading controls are β-tubulin (western) and IgG heavy chain (IP/western). ($^{\#}$)Indicates shRNA-resistant protein. *C*, Western blot (left) and IP/western (right) showing p73 binds to ΔNp63α(R304W) with greater affinity than p53. 293T cells were transiently transfected with indicated expression plasmids and IP was performed with α-FLAG (M2) antibody. *D*, Nucleotide changes (bold capital) in the p63 shRNA target sequence within the DNA binding domain, which mediate resistance to p63 shRNA. Note that amino acid 232-236 sequence shown remains unchanged. DBD, DNA-binding domain.OD, oligomerization domain. SAM, Sterile Alpha Motif. TID, Trans-Inhibitory Domain. Position of R304W mutation is indicated. *E*, Western blot analysis showing equivalent

ΔNp63α protein levels following knock-down of endogenous p63 by lentiviral shRNA in cells expressing shRNA-resistant ΔNp63α wild-type ($WT^{\#}$) or mutant ($R304W^{\#}$) proteins. β-tubulin serves as a loading control. *F*, Cell death following endogenous p63 knockdown is rescued by exogenous shRNA-resistant wild-type but not R304W mutant ΔNp63α. Quantification of crystal violet staining of JHU-029 cells stably expressing the indicated ΔNp63α proteins 96 hours after lentiviral infection with control (GFP-directed) or p63-directed shRNA constructs. Values are expressed as a ratio of cell number in p63-shRNA wells over GFP-shRNA wells. N=4 for each sample. Error bars indicate +/- SEM. *$p<0.05$.

**Supplementary Figure 3. HDAC inhibition and cisplatin treatment activate the *PUMA* locus in SCC cells.**

*A*, Treatment of JHU-029 cells with the HDAC inhibitor TSA (left) or vorinostat (right) for 24 hrs increases *PUMA* mRNA levels in a similar dose-dependent manner. *B*, ChIP showing similar upregulation pattern of histone H4 acetylation and histone H4K16 acetylation at the *PUMA* locus (relative to Transcriptional Start Site) following TSA treatment (500 nM, 2hrs). *C*, Lentiviral shRNA mediated p63 knockdown leads to *PUMA* induction (48 hrs post viral infection). *D*, Cisplatin treatment (4 μM, 24 hrs) of JHU-029 cells leads to *PUMA* mRNA induction (left) and reversal of histone H4 acetylation as assessed by ChIP (right, -200bp). E, Western Blot analysis of JHU-029 cells showing that Cisplatin leads to dose-dependent degradation of p63 at 24 hours of treatment. β-tubulin serves as a loading control.

**Supplementary Figure 4**. **Inhibition of the p63/HDAC complex blocks tumor progression *in vivo*.**

*A*, Dose-response of SCC cell lines to TSA. Viability was measured at 72 hours after treatment by XTT assay. *B*, Dose-response curve of JHU-029 cells stably expressing BCL2 or control empty vector 24 hours after treatment with indicated dose of TSA. Error bars indicate +/- standard deviation. *C*, Representative histology (H&E) and proliferation (Ki67) in JHU-029/GFP Xenografts (Left) or JHU-029/BCL2 Xenografts (Right) treated with vehicle (DMSO) or 50 mg/kg vorinostat. *D*, Quantification of Ki67

in tumor sections from JHU-029/GFP treated with DMSO (n=8 sections, 7713 total cells) or vorinostat (n=8 sections, 6282 total cells) and JHU-029/BCL2 tumors treated with DMSO (n=7 sections, 5027 total cells) or vorinostat (n=7 sections, 5406 total cells). Error bars indicate +/- SEM. Scale bar = 50μM

# Supplementary Table 1

PCR primer sequences

| Target | Forward (5' → 3') | Reverse (5' → 3') |
|---|---|---|
| | **QRT-PCR** | |
| ΔNp63 | GGAAAACAATGCCCAGACTC | GTGGAATACGTCCAGGTGGC |
| β2-microtubulin | AGCTGTGCTCGCGCTACTCTC | CACACGGCAGGCATACTCATC |
| PUMA | ACGACCTCAACGCACAGTACGAG | AGTCCCATGATGAGATTGTACA |
| GAPDH | CACCCAGAAGACTGTGGATGG | GTCTACATGGCAACTGTGAGG |
| | **Chromatin IP** | |
| *PUMA* | | |
| -8 kb | GCCCGCATGCAGCCCTCA | TGTGTGCCTAGCCCAATGTTTTATTGT |
| -200bp | TGGCCTTGTGTCTGTGAGTAC | GGCCTAGCCCAAGGCAAGGAG |
| +1.1kb | CGTGTGTTGTCGGTGAGAGT | GCCTCAGCCAAGACAGAAAC |
| +8kb | GGCTCTGTGGCCCCTGGGTA | GAGACTGTCGCCTCGCTGCC |
| *ACTB* | CCAGGAATTCCGACTTTCAA | CAGGACTGGTGAAGTGCTCA |

**Supplementary Materials and Methods**

**Animal studies and Immnohistochemsitry.** All animals were housed and treated in accordance with protocols approved by the Subcommittee on Research Animal Care at the Massachusetts General Hospital. Xenograft tumors were generated by subcutaneous injection of 2 x $10^6$ JHU-029 tumor cells and $10^6$ NIH 3T3 cells suspended in 1:1 matrigel (BD Biosciences): RPMI. Mice were treated with 50 mg/kg vorinostat every day starting 13 days after cell injection. Tumor volumes were determined as previously described (1). Assistance in processing of Xenograft samples was provided by the Dana-Farber/Harvard Cancer Center Specialized Histopathology Core Facility. Five micron sections were cut and stained for Ki67 (rabbit polyclonal, NCL-Ki67p, Novacastra) using standard protocols. Staining for Cleaved Caspase 3 was performed using the SignalStain Cleaved Caspase-3 (Asp175) IHC detection kit (Cell Signaling) according to manufacturer's instructions.

**Protein extraction.** For total protein extraction, cells were lysed in RIPA buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% (w/v) sodium deoxycholate, 0.1% (w/v) SDS, 1% (v/v) NP40, proteinase inhibitor cocktail, phosphatase inhibitor cocktail) for 30 min at 4$^o$C.

**Chromatin Immunoprecipitation.** For α-p63 and α-HDAC1 samples cells were subjected to additional cross-linking protein-protein interaction with 2 mM EGS (Ethylene Gycol-bis (Succinimidylsuccinate)) in PBS for 40 minutes at room temperature followed by washing in PBS and cross-linking with 1% (v/v) formaldehyde at room temperature for 15 minutes. For all other reactions, the protein-DNA was cross-linked with 1% (v/v) formaldehyde at room temperature for 15 minutes. Cross-linking was stopped by adding 0.125 M glycine. Cells were then lysed in PIPES hypotonic buffer (5 mM PIPES pH 8, 85 mM KCl, 0.5% NP40) for 30 min at 4$^o$C, pelleted, then resuspended in RIPA buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% (w/v) sodium deoxycholate, 0.1% (w/v) SDS, 1% (v/v) NP40, proteinase inhibitor cocktail, phosphatase inhibitor cocktail, and 5 μM trichostatin and 5 mM sodium butyrate) and

incubated over night at 4$^{o}$C. Nuclear lysate were sonicated 7.5 min for 5 rounds in a bioruptor (Diagenode). Sonicated chromatin was precleared with ProteinG sepharose beads pre-blocked with BSA and sonicated salmon sperm DNA. Samples were incubated with 2 µg antibody for over night at 4$^{o}$C and with beads for additional 2 hrs. Beads were washed in washing buffer 1 (150 mM NaCl, 20 mM Tris pH 8.1, 2 mM EDTA, 0.1% (w/v) SDS, 1% (v/v) Triton-X-100), washing buffer 2 (500 mM NaCl, 20 mM Tris pH 8.1, 2 mM EDTA, 0.02% (w/v) SDS, 1% (v/v) Triton-X-100), washing buffer 3 (250 mM LiCl ,10 mM Tris pH 8.1, 1 mM EDTA, 1%(w/v) sodium deoxycholate, 1% (v/v) NP40), and 10 mM Tris pH 7.9, 1 mM EDTA. Beads were incubated for 3 hours at 55$^{o}$C, then overnight at 65$^{o}$C in 10 mM Tris pH 7.9, 1 mM EDTA), 0.5% (w/v) SDS, 10 µg RNase A, and 10 µg Proteinase K. DNA was purified via Qiaquick PCR purification kit (Qiagen) as per manufacturer's instructions. PCR was performed using IQ SYBR Green Supermix reagent (Bio-Rad) in a MX300P machine (Stratagene). Percentage of input from the IPs was calculated by using 1% input as standard. See Table S1 for primer sequences (in 5'-3' direction).

**Antibodies.** *TAP:* FLAG conjugated beads (M2, Sigma), HA conjugated beads (3F10, Roche). *Immunoprecipitation:* p63 (H-129, Santa Cruz), FLAG (M2, Sigma), HDAC1 (A300-713A, Bethyl Laboratories), HDAC2 (H-54, Santa Cruz), or IgG (Santa Cruz). *Protein Fractionation:* p63 (4A4, Sigma, 1:5000), HDAC1 (Bethyl Laboratories, 1:1000), HDAC2 (Santa Cruz, 1:500) and Lamin A/C (Cell Signaling, 1:1000). *Western Blot:* p63 (4A4, Sigma, 1:5000), p73 (Ab-2, Calbiochem, 1:1000), FLAG (M2, Sigma, 1:1000), β-tubulin (D-10, Santa Cruz, 1:1000), HDAC1 (A300-713A, Bethyl Laboratories, 1:1000), HDAC2 (H-54, Santa Cruz, 1:500), BCL2 (Santa Cruz, C2, 1:1000), PARP1 (Cell Signaling, 1:1000), GAPDH (Santa Cruz, FL-335, 1:1000). *ChIP:* FLAG (M2, Sigma), p63 (H-129, Santa Cruz), HDAC1 (A300-713A, Bethyl Laboratories), GAPDH (FL-335, Santa Cruz), pan-Acetylated histone H4 (06-866, Millipore), Acetyl-histone H4, Lys 16 (07-329, Millipore).

**Supplementary Reference**

1. Ory B, Ramsey MR, Wilson C, Vadysirisack DD, Forster N, Rocco JW, Rothenberg SM, and Ellisen LW. A microRNA-dependent program controls p53-independent survival and chemosensitivity in human and murine squamous cell carcinoma. J. Clin. Invest. 2011;121:809-20.