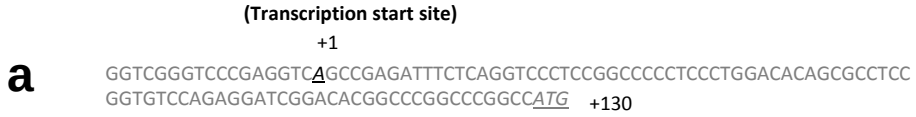
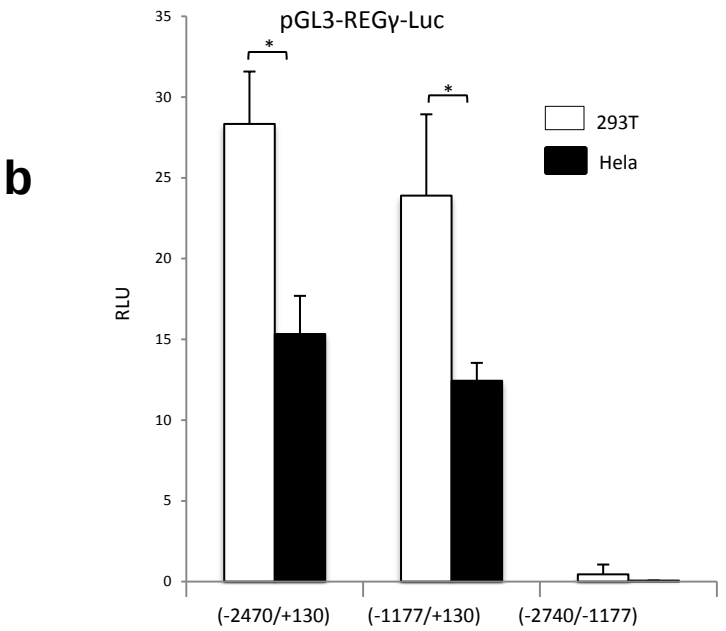
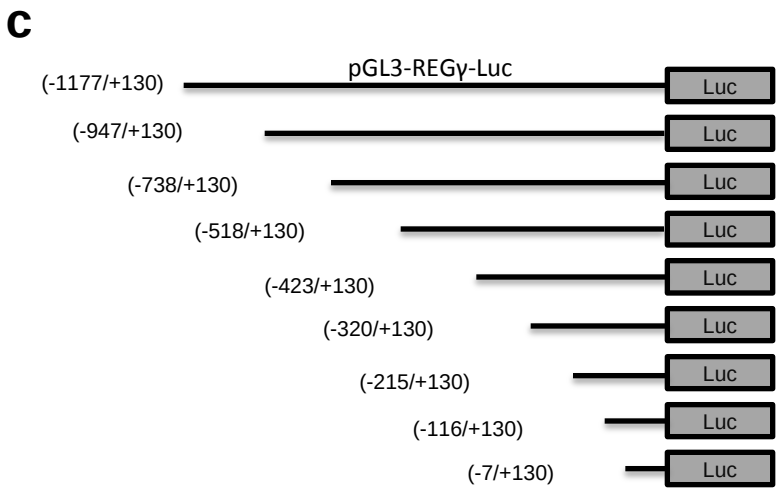
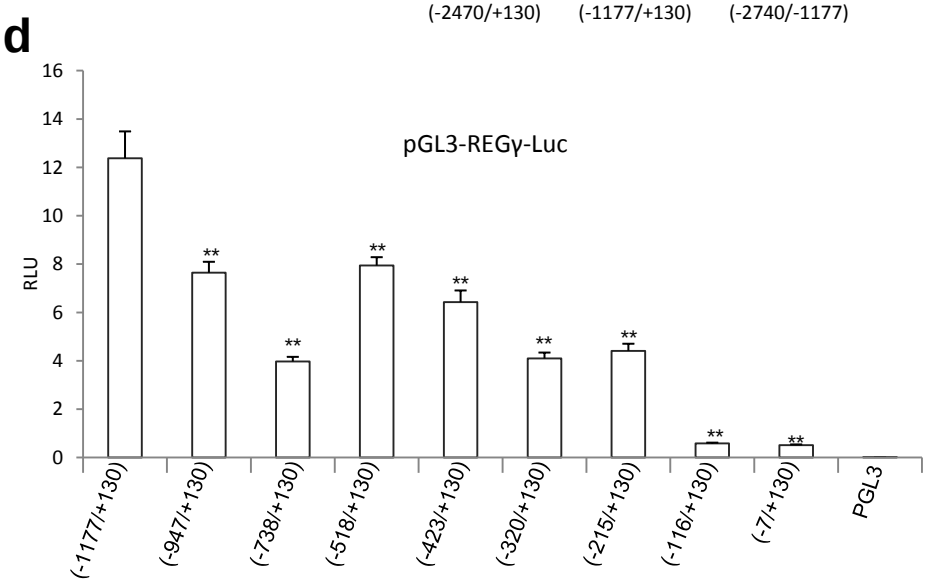
Supplementary Figure S1



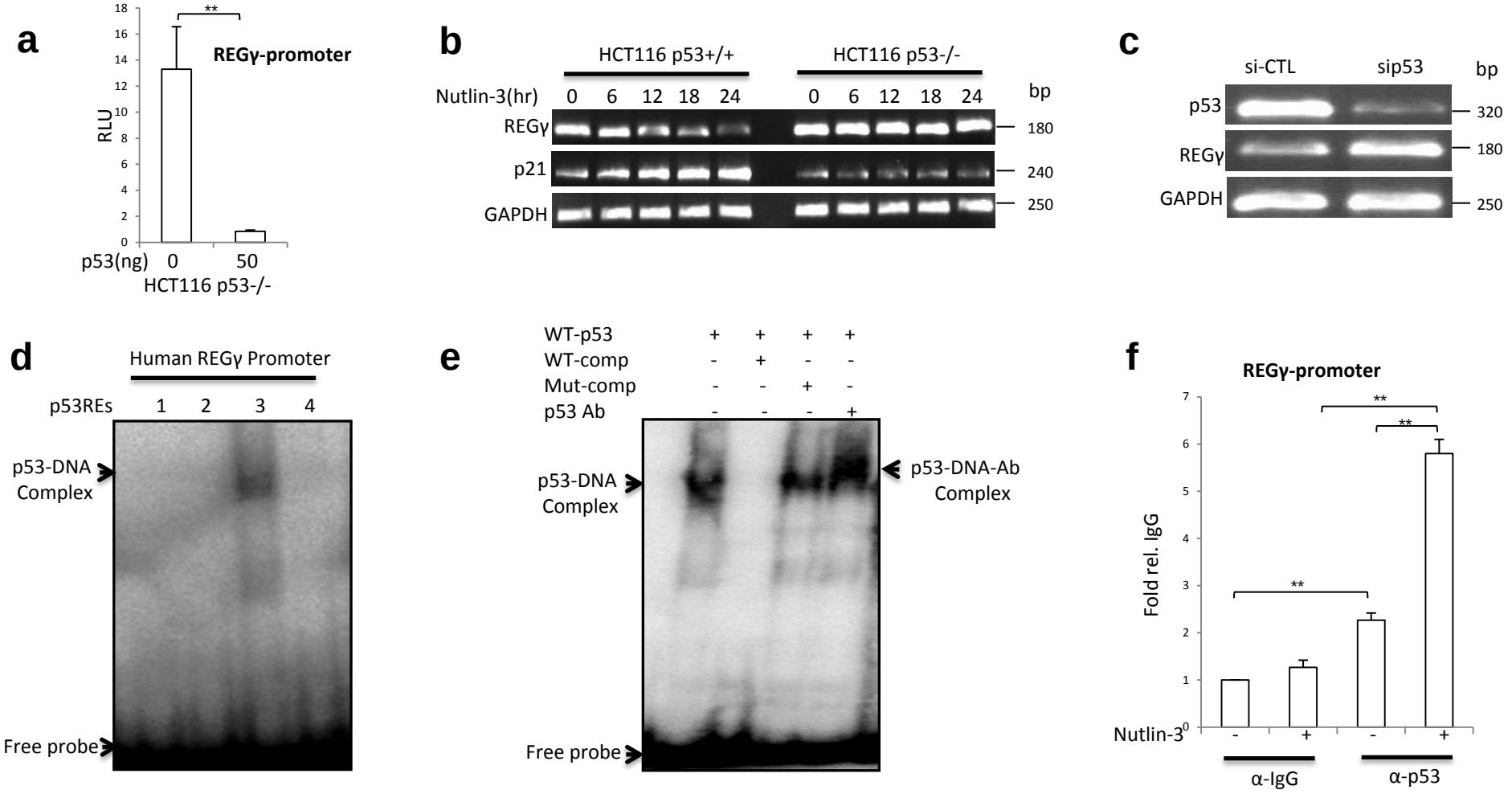






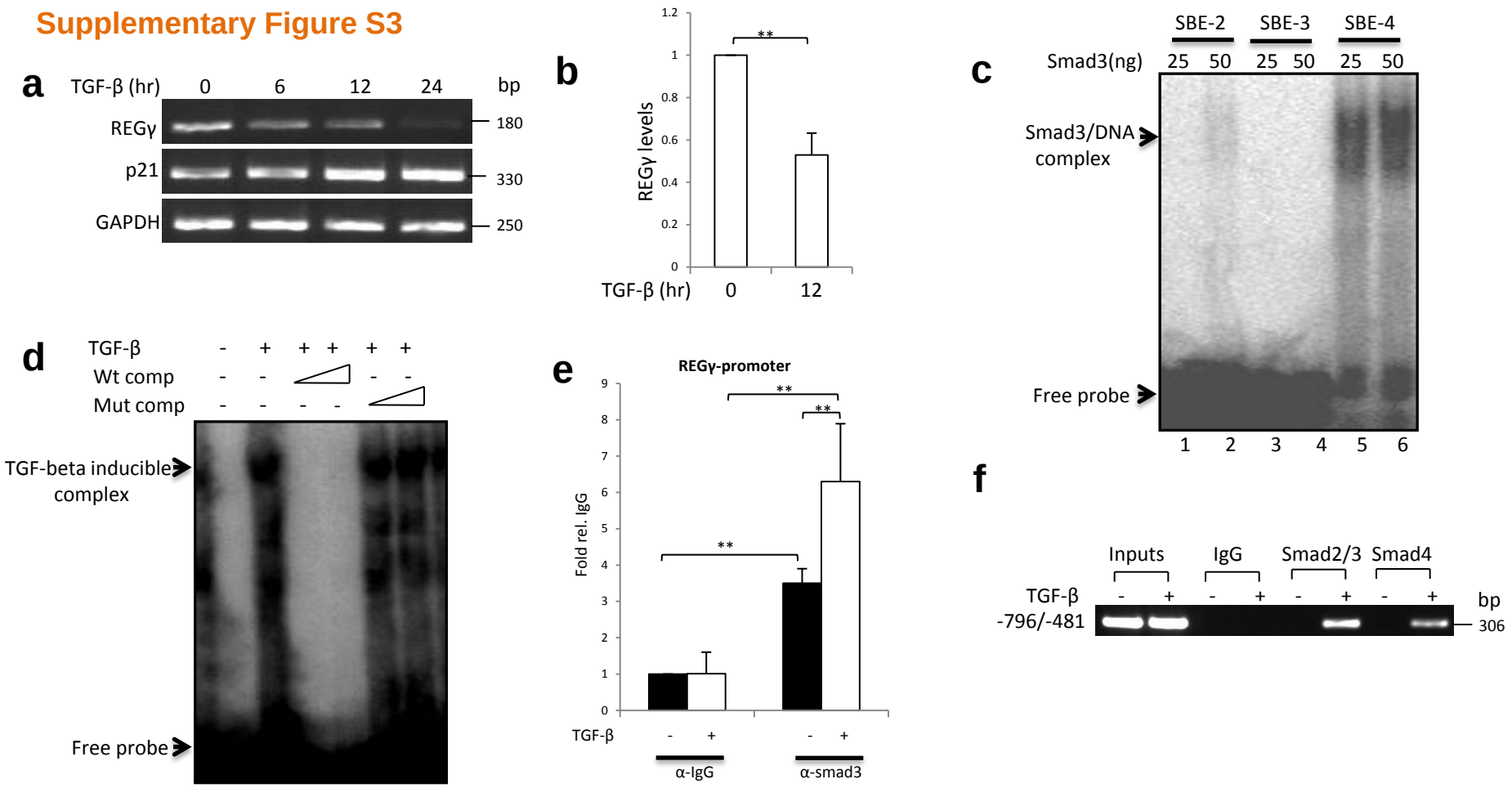
Supplementary Figure S1: REGγ promoter analysis indicates potential regulation by two tumor suppressor pathways. (a) TSS of the REGγ gene was determined by 5′RACE PCR. **(b)** A 2.6 KB clone and its two different truncated forms from the REGγ promoter were inserted into luciferase reporter expression vector and transfected into 293T and Hela cells for 24 hr, and then assayed for luciferase activity. Data are representative of three technical repeats with mean ± s.d. (Two-tailed Student's *t*-test, P*<0.05). **(c)** Scheme of deletion constructs of the 5′ flanking region of the REGγ luc-reporter. **(d)** Different deletion constructs of the 5′ flanking region of the REGγ promoter were transfected into 293T cells for 24 hrs, and then analyzed for luciferase activity. Error bars show the s.d. from three biological replicates (Two-tailed Student's *t*-test, P*<0.05, p**<0.005).

Supplementary Figure S2



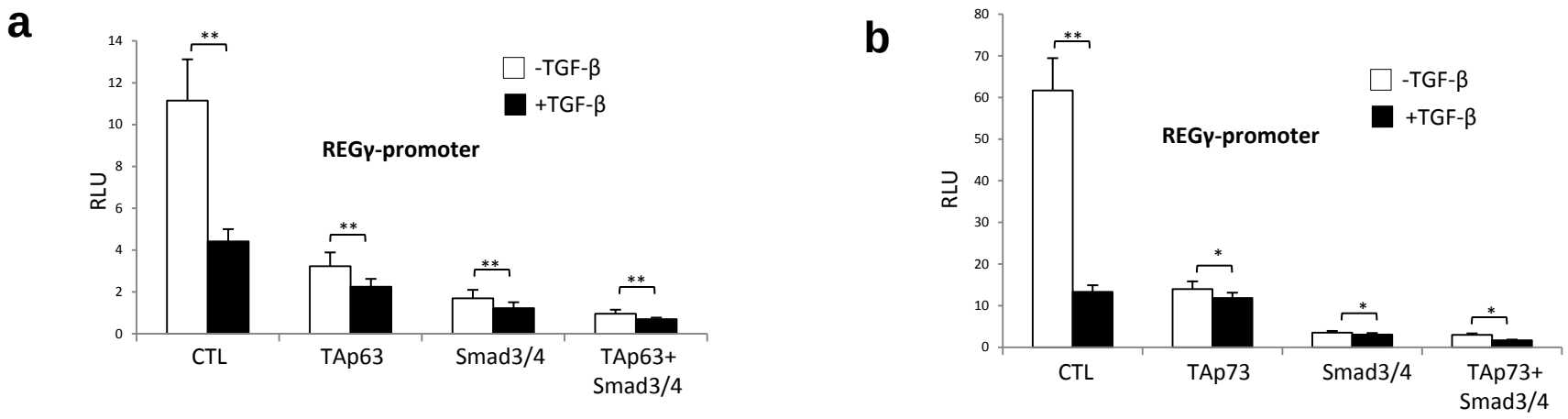
Supplementary Figure S2: p53 suppresses REGγ promoter activity via a p53 responsive element in REGγ promoter. (a) HCT116 p53 (-/-) cells were co-transfected with the REGγ reporter construct along with empty vector or p53 expression plasmid for 24 hr prior to lysis, and analyzed for luciferase activity. Error bars show the s.d. from three biological replicates (Two-tailed Student's t-test, p**<0.005). **(b)** HCT116 p53 (+/+) and HCT116 p53 (-/-) were treated with (10 μmol/L) Nutlin-3a for indicated time points and semi qRT-PCR was performed. **(c)** A549 cells were transfected with the siRNA specific for p53 (20nM) for 48 hr, and qRT-PCR was performed. **(d)** 50 ng of purified p53 was incubated with each of the probe corresponding to four different p53 REs from the REGγ promoter and in Vitro EMSA was carried out. **(e)** In Vitro EMSA was performed with the double-stranded oligonucleotides containing the p53 binding site from the REGγ promoter. **(f)** A549 cells were treated with (10 μmol/L) Nutlin-3 and analyzed by qChIP assay. Data are representative of three technical repeats with mean ± s.d. (Two-tailed Student's *t*-test, P*<0.05, p**<0.005).

Supplementary Figure S3



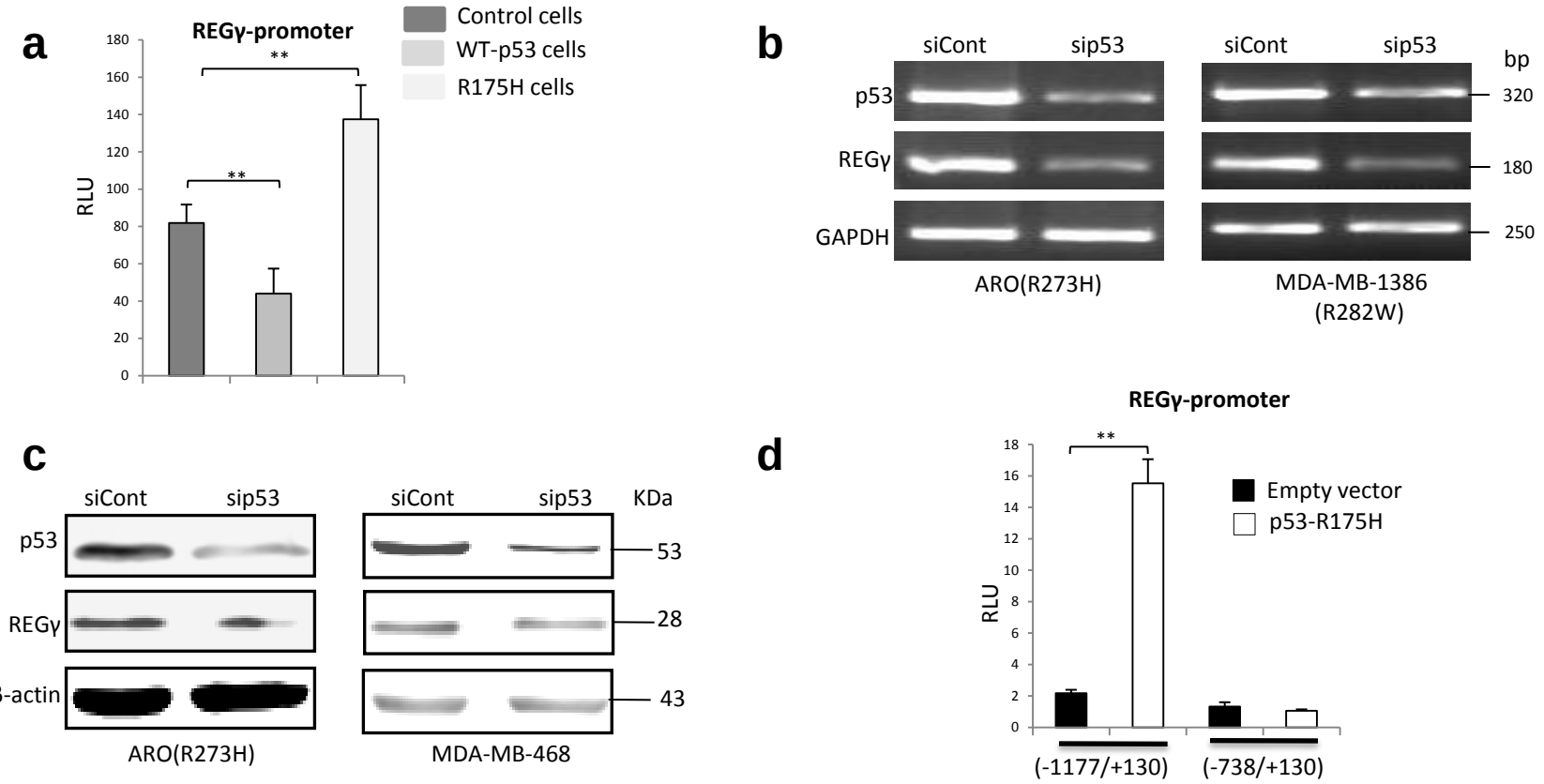
Supplementary Figure S3: TGF-β inhibits REGγ transcription through an activated Smad complex. (a) H1299 were incubated in the absence or presence of 5ng/ml TGF-β for indicated time points. Total RNA was isolated and subjected to semi qRT-PCR. **(b)** HaCaT cells were incubated in the absence or presence of 5ng/ml TGF-β for 12 hr. Total RNA was isolated and subjected to qRT-PCR. Data are representative of three technical replicates with mean ± s.d. (Two-tailed Student's *t*-test, P*<0.05, p**<0.005). **(c)** In vitro EMSA was performed using purified GST-Smad3 proteins. **(d)** H1299 cells were treated with 5ng/ml TGF-β for 24 hr, and then nuclear extracts were prepared. Equal amounts of ^{32}p-radiolabeled oligonucletide corresponding to SBE box from the REGγ promoter were incubated with the indicated nuclear extracts. **(e)** H1299 cells were treated with 5ng/ml TGF-β and analyzed by qChIP assay. Data are representative of three technical repeats with mean ± s.d. (Two-tailed Student's *t*-test, P*<0.05, p**<0.005). **(f)** H1299 cells were stimulated with 5ng/ml TGF-β for 24 hr, and ChIP was performed with the smad2/3 and Smad4 antibodies. IgG serve as a negative control.

Supplementary Figure S4



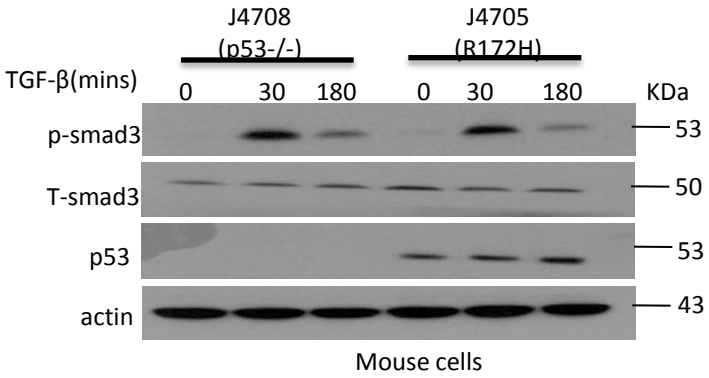
Supplementary Figure S4: p53 and TGF-β synergistically represses REGγ-proteasome pathway through p53RE/SBE region. (a) H1299 cells were co-transfected with expression vectors encoding TA-p63 or Smad3/4, both alone and in combination, left untreated or treated with 5ng/ml TGF-β for 24 hr before lyses, and analyzed for luciferase activity. CTL represent the transfection of empty vectors in H1299 cells. Data are representative of three technical repeats with mean ± s.d. (Two-tailed Student's *t*-test, P*<0.05, p**<0.005). **(b)** H1299 cells were co-transfected with expression vectors encoding TA-p73 or Smad3/4, both alone and in combination, left untreated or treated with 5ng/ml TGF-β for 24 hr before lyses, and analyzed for luciferase activity. CTL represent the transfection of empty vectors in H1299 cells. Data are representative of three technical repeats with mean ± s.d. (Two-tailed Student's *t*-test, P*<0.05, p**<0.005).

Supplementary Figure S5



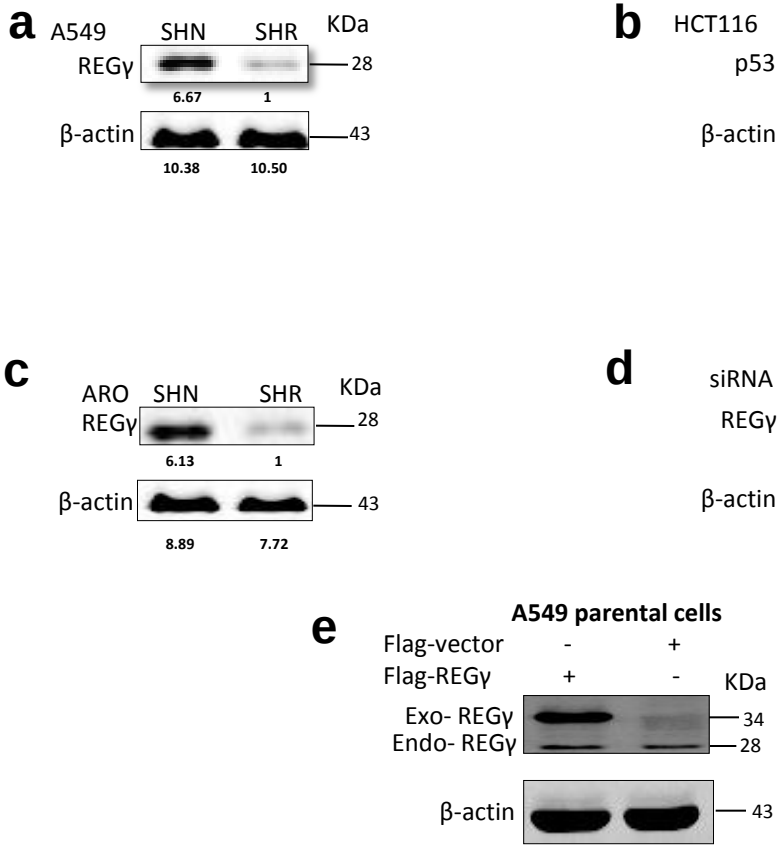
Supplementary Figure S5: REGγ expression is transcriptionally induced by mutant p53 proteins. (a) UMSSC-1cells stably expressing mutant p53 R175H and p53 respectively were transfected with REGγ promoter (2μg) for 24 hr, and analyzed for luciferase activity. Data are representative of three biological repeats with mean ± s.d. (Two-tailed Student's *t*-test, P*<0.05, p**<0.005). **(b)** ARO and MDA-MB-1386 cells were transfected independently with siRNA directed against p53 (20nM) for 48 hr, and subjected to semi qRT-PCR. **(c)** ARO and MDA-MB-468 cells were transfected with siRNA directed against p53 (20nM) for 72 hr, and subjected to western blot analysis with the antibodies specific for REGγ and p53. **(d)** Mutant-p53 transactivate the region between -1177/-738 on the REGγ promoter. Data are representative of three technical repeats with mean ± s.d. (Two-tailed Student's *t*-test, P*<0.05, p**<0.005).

Supplementary Figure S6



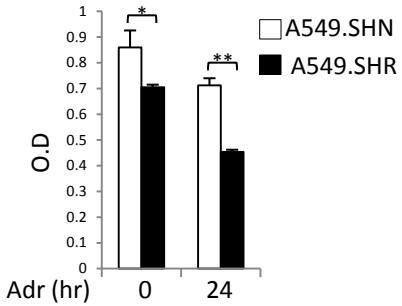
Supplementary Figure S6: Mutant p53 antagonize the recruitment of Smad/N-CoR complex on the REGγ promoter.
Mouse oral cancer cells expressing either empty vector J4708 (p53-/-) or mutant-p53 J4705 (p53-R172H) were left untreated or treated with 5ng/ml TGF-β at indicated time points and subjected to immunoblot analysis.

Supplementary Figure S7



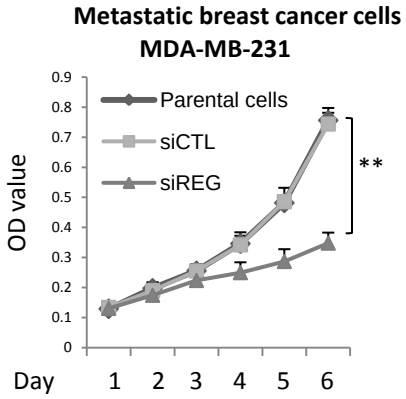
Supplementary Figure S7: Knockdown and overexpression efficiency of REGγ in multiple cell lines. **(a)** A549 cells stably integrated with control shRNA (A549.SHR) or with shRNA targeting REGγ (A549.SHR). **(b)** Expression of p53 in HCT116 cells lines. **(c)** ARO cells stably integrated with control shRNA (ARO.SHR) or with shRNA targeting REGγ (ARO.SHR)**. (d)** MDA-MB cells were transfected with 20nM siREGγ for 72 hrs. **(e)** Overexpression of REGγ in A549 Parental cells. Cells were transfected with either control flag-vector or flag-REGγ for 24hr, and analyzed by immunoblot.

Supplementary Figure S8



Supplementary Figure S8. Knockdown of REGγ decrease drug resistance in lung cancer cells. Knockdown of REGγ decrease drug resistance in A549 lungs cancer cells. A549 cells were treated with Adriamycin for 24 hr, and then analyzed by MTT assay. Data are representative of three biological repeats with mean ±s .d. (Two-tailed Student's *t*-test, P*<0.05, p**<0.005).

Supplementary Figure S9



Supplementary Figure S9: Knockdown of REGγ decrease cell proliferation in breast cancer cells. Knockdown of REGγ decrease cell proliferation in breast cancer cells. MDA-MB-231 cells were transfected with siRNA directed against REGγ (20 μM) for indicated time points and then analyzed by MTT assay. Data are representative of three technical repeats with mean ± s.d. (Two way ANOVA, p**<0.005).

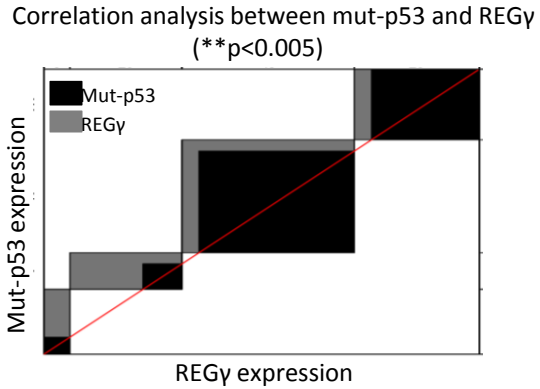
Supplementary Figure S10

a

The relationship between REGγ and Mutant-p53 expression

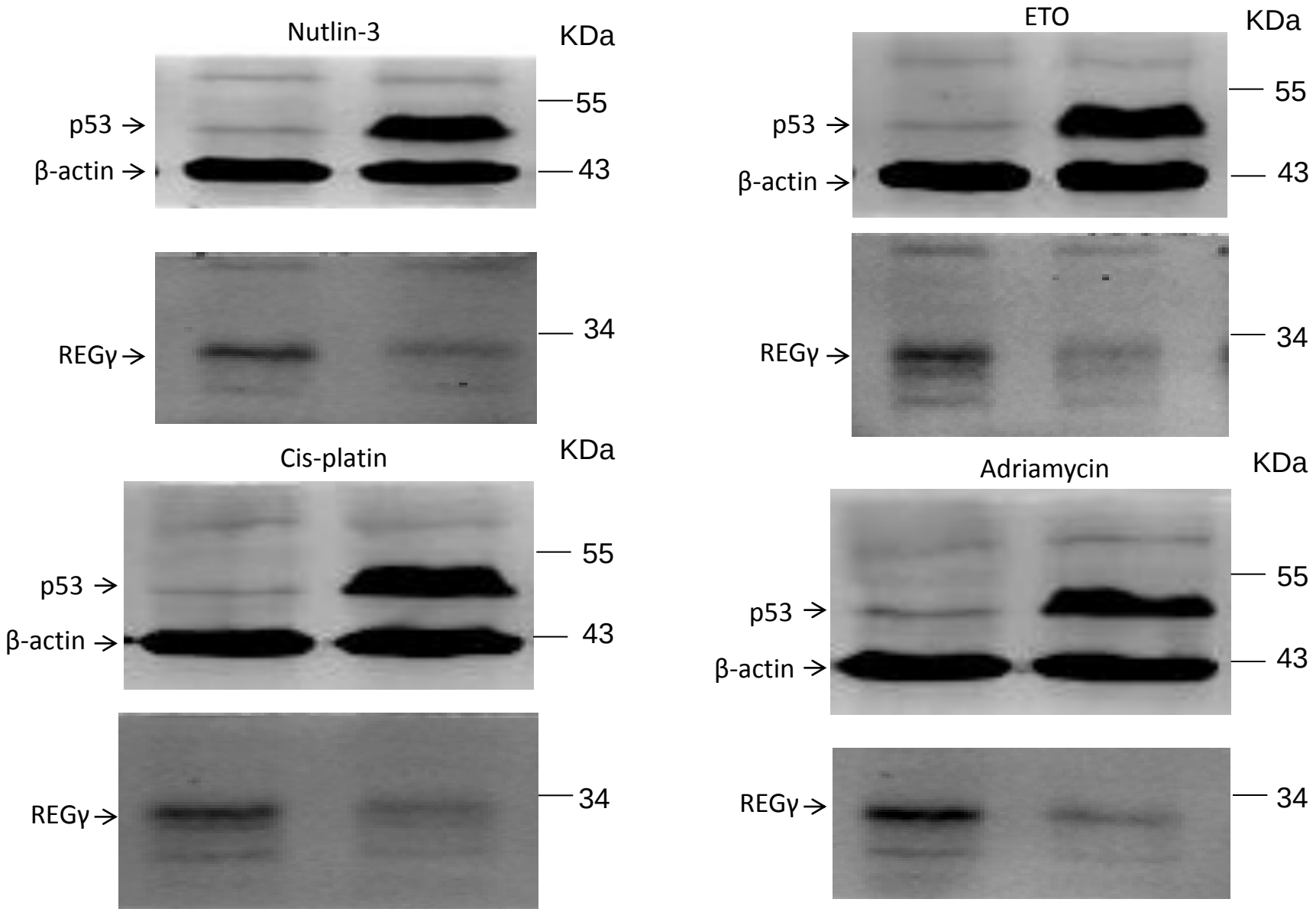
Gene	Neg(-)	+	++	+++	total cases	total %
REGγ	6(5.94%)	26(25.74%)	40(39.60%)	29(28.71%)	101	95/101(94%)
Mut-p53	23(22.77%)	13(12.87%)	40(39.60%)	25 (24.75%)	101	78/101(77%)

b



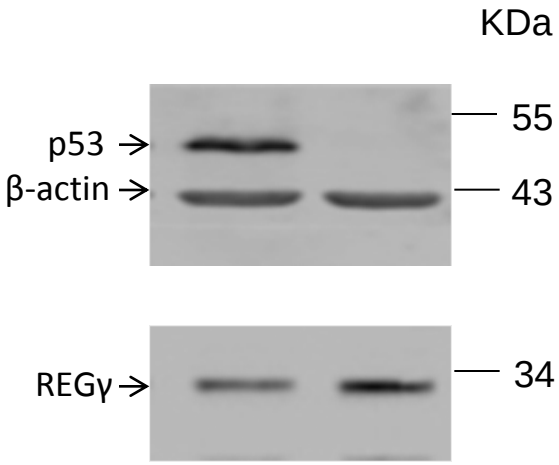
Supplementary Figure S10. Aberration in p53 and TGF-β signaling induces REGγ expression in multiple cancer cells. (a) Statistical analysis of REGγ and mutant p53 over expression. Table representing the REGγ and mutant p53 proteins staining statistical analysis in 101 different cancer tissues which was evaluated by IHC. **(b)** Mutant p53 and REGγ correlation in the same sets of tumors. Agreement plot analyses between REGγ and mutant p53 reveals that these two proteins express in the same set of tumor. The weighted kappa coefficient value is 0.7749755 (Two-tailed student‘t’ test, P**<0.005), which suggest that they are highly co-related.

Supplementary Figure S11



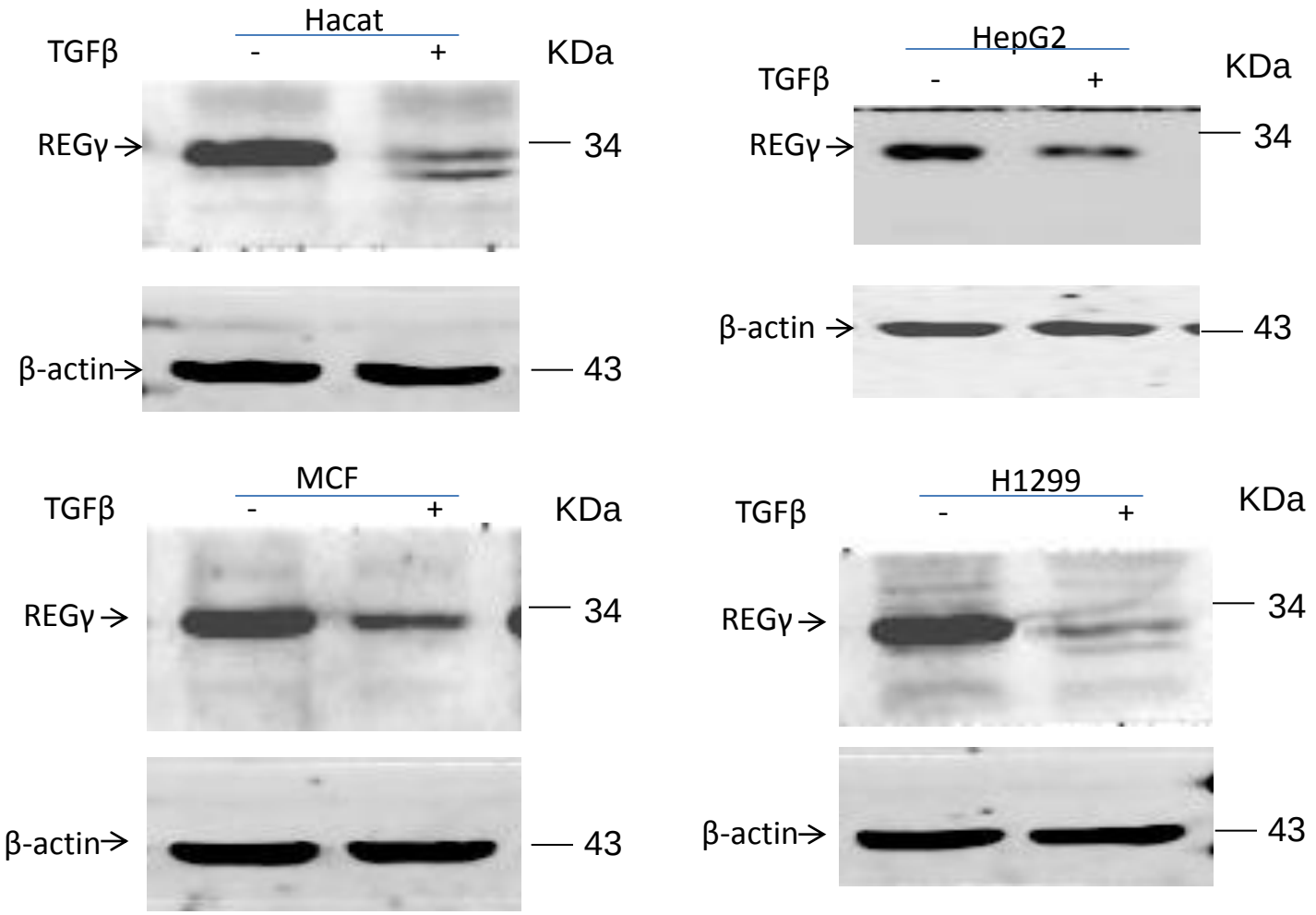
Supplementary Figure S11: Full-length blots of Fig. 1f. A549 cells were treated with different anti-cancer drugs such as Nutlin-3 (10 µmol/L), Cisplatin (5 µg/ml), Etoposide (10 µmol) and Adriamycin (1 µM) and analyzed by western blot.

Supplementary Figure S12



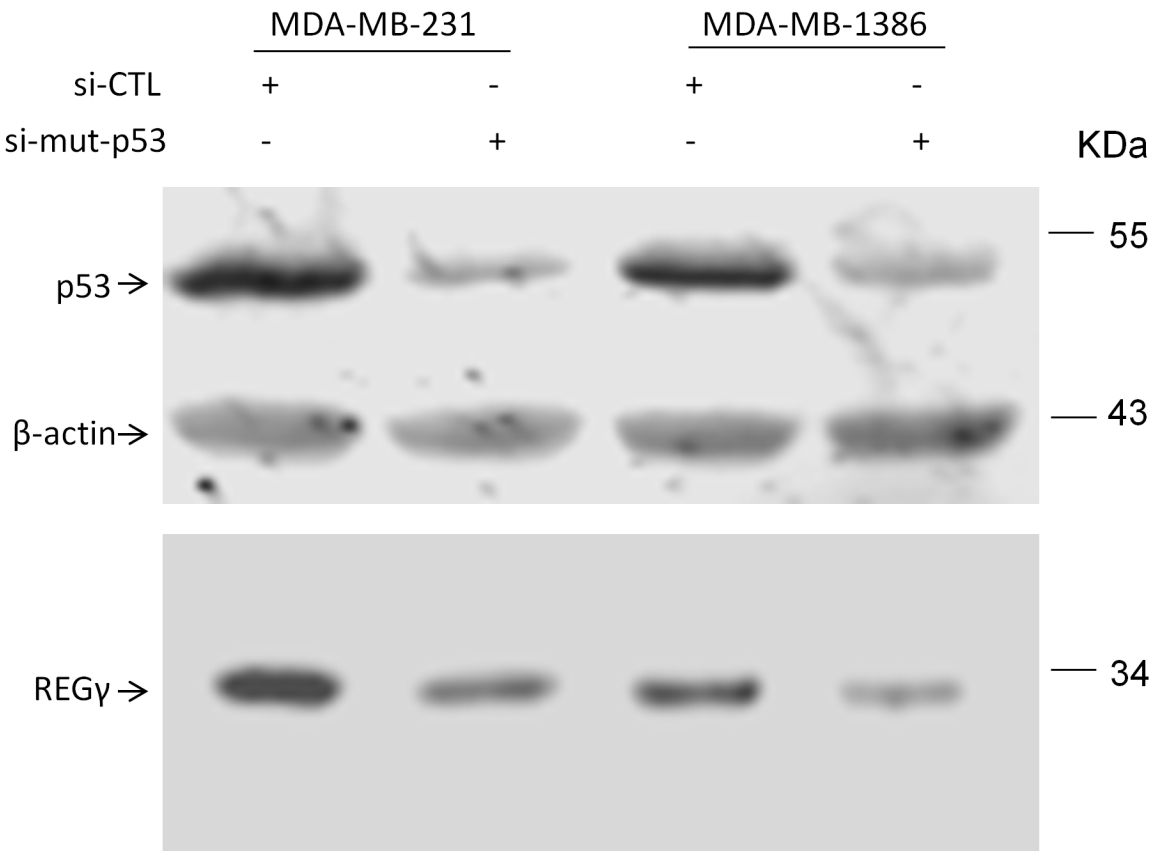
Supplementary Figure S12: Full-length blots of Fig. 1g. Comparative analysis of REGγ protein levels in MEF p53 (+/+) and MEF p53 (-/-) cells.

Supplementary Figure S13



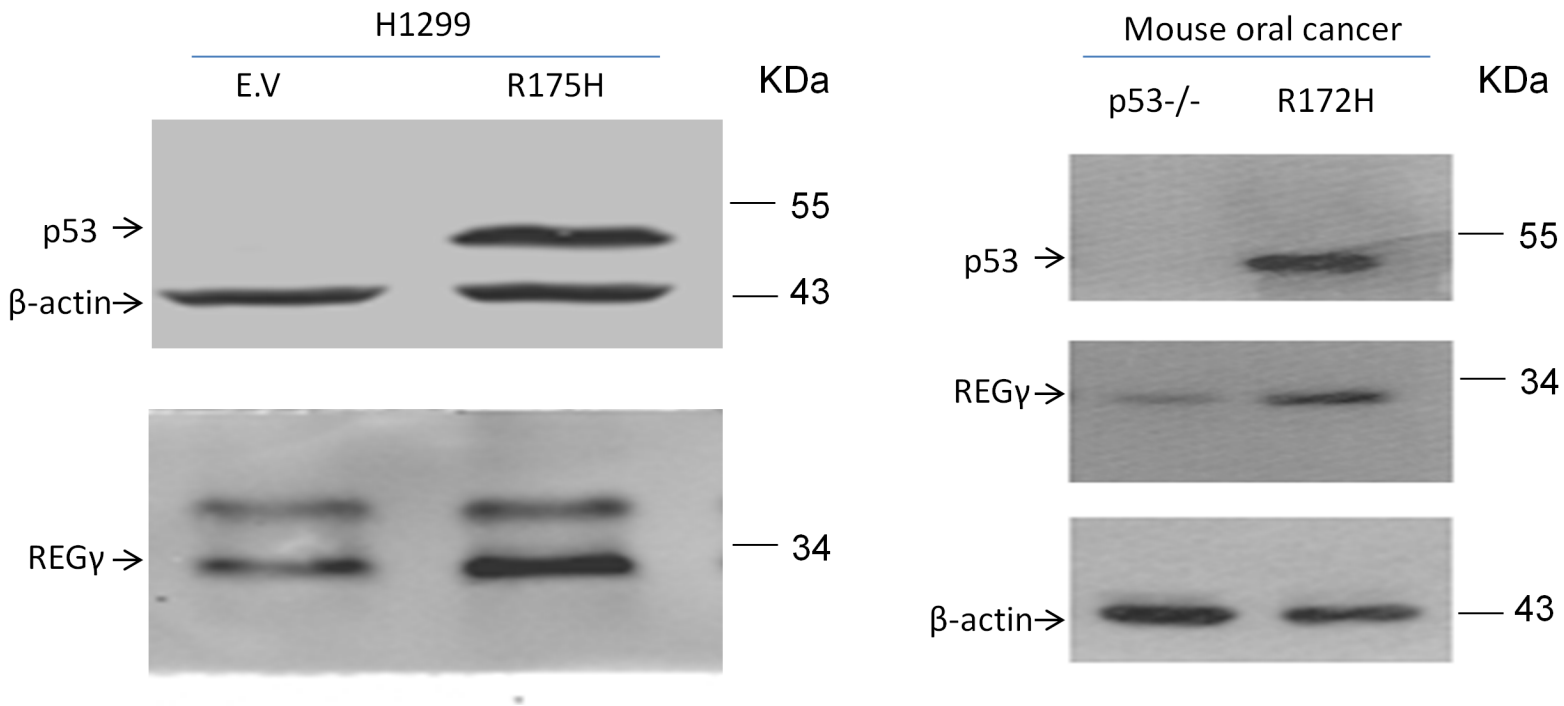
Supplementary Figure S13: Full-length blots of Fig. 2e. HaCaT, HepG2, MCF-7 and H1299 cells were treated with 5ng/ml TGF-β and analyzed by western blot.

Supplementary Figure S14



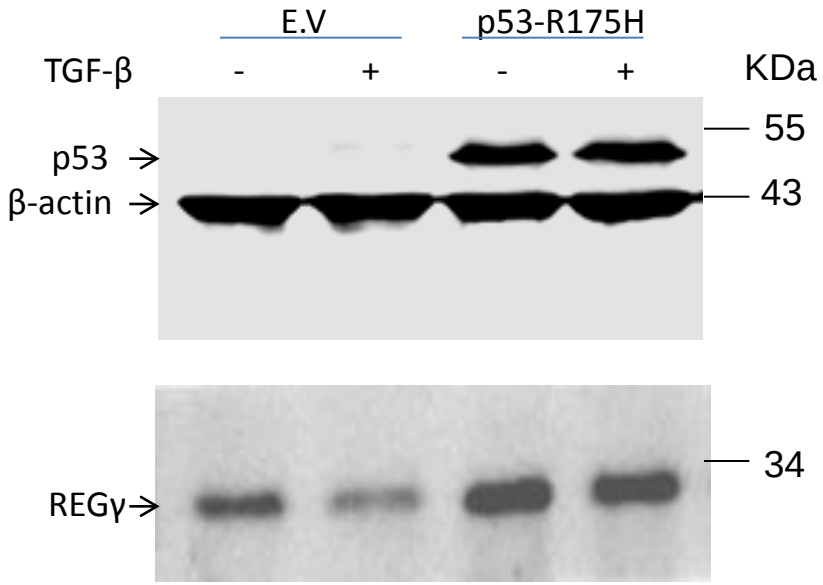
Supplementary Figure S14: Full-length blots of Fig. 4d. MDA-MB-231 and MDA-MB-1386 cells were transfected with siRNA directed against p53 (20nM) for 72 hr, and analyzed by Western blot.

Supplementary Figure S15



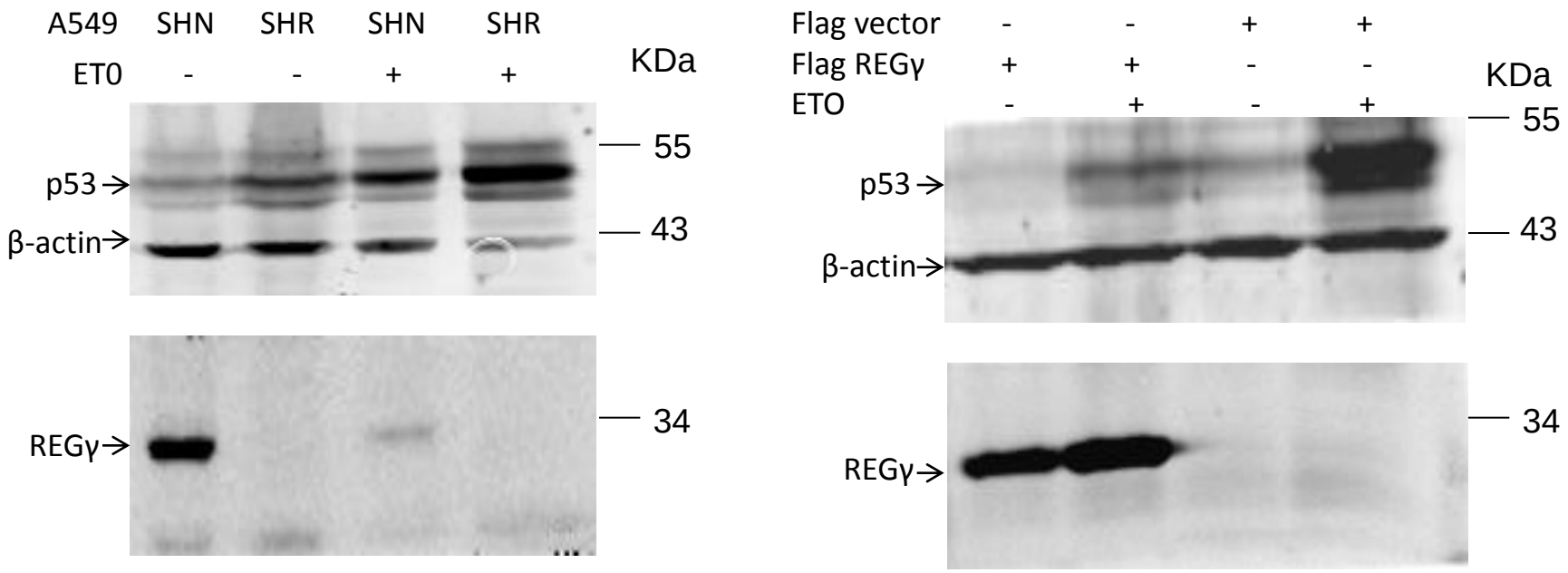
Supplementary Figure S15: Full-length blots of Fig. 4e. (Left panel) H1299 cells stably expressing either empty vector (E.V) or p53-R175H and (Right panel) Immortalized oral cancer cells from p53 (-/-) or p53-R172H mice were subjected to western blot analysis.

Supplementary Figure S16



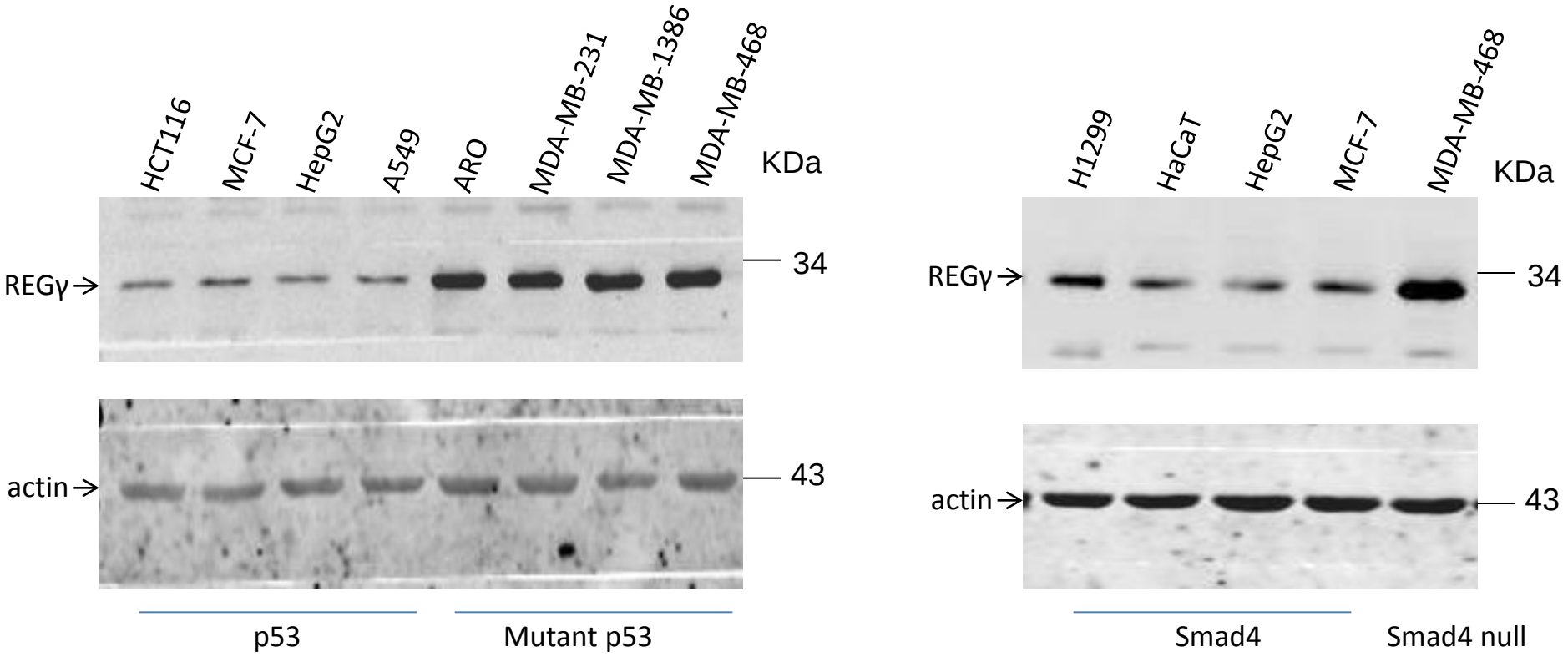
Supplementary Figure S16: Full-length blots of Fig. 5c. H1299 cells stably expressing either p53-R175H mutant or empty vectors (E.V) were left untreated or treated with 5ng/ml TGF-β and analyzed by western blot.

Supplementary Figure S17



Supplementary Figure S17: Full-length blots of Fig. 7f and 7g. Manipulation of REGγ and p53 protein levels mutually affects both protein expressions in cancer cells. (Left panel) A549 SHN and SHR cells were left untreated or treated with ETO (10 μmol/L) for 24 hr, and analyzed by Western blot. (Right panel) A549 cells were transfected with flag-REGγ for 24hr, and then left untreated or treated with ETO (10 μmol/L) for 24 hr, and analyzed by Western blot.

Supplementary Figure S18



Supplementary Figure S18: Full-length blots of Fig. 9a. (Left panel) Western blot analyses of cellular REGγ protein levels in p53 and mutant p53 expressing cancer cells which is correlated with p53 status. (Right panel) Smad4 null cancer cells show more REGγ protein levels in comparison with Smad4 containing cells.

Supplementary Table S1: EMSA probes sequences used in this study

Probes	Sequences (5'-3')
Human p53RE-3WT	5 ′GTACCAGGAAAGCTTGGTACAACGGCGCTTGTCTTTTTCGCTC 3 ′
Human p53RE-3Mutant	5 ′GTACCAGGAAAGATTTGTACAACGGCGATTCTCTTTTTCGCTC 3 ′
Mouse p53RE-WT	5 ′TCTGAAGACAAAGCAAGTTTTTGAGCATGAAGCCATTATTTCA 3 ′
Smad SBE-4WT	5 ′GCTTGGTACAACGGCGCTTGTCTTTTTCGCTCTAGCCTAAACAC 3 ′
Smad SBE-4Mutant	5 ′GCTTGGTACAACGGCGCTTATGTTTTTCGCTCTAGCCTAAACAC 3 ′
p53 RE/SBE	5 ′TGTACCAGGAAAGCTTGGTACAACGGCGCTT**GTCT**TTTTCGCTCT 3 ′
	Note: Underline letters represents p53RE-3, while the bold letters represent SBE-4.

Supplementary Table S2: Primers sequences used in ChIP Assay

Primers	Sequences (5'-3')
Human REGγ p53 forward	5´- ACTCTGGGGATCAATTTGCCA-3´
Human REGγ p53 reverse	5´- GGGCACTGTGTCAGATTCTAGA-3´
Human REGγ SBE forward	5´- AGAGAGATCGAATGAATGGGTG-3´
Human REGγ SBE reverse	5´- AACCCAGATCTCCTAACTTCTACGG-3´
REGγ mutant p53 forward	5´- CCAGAGCTGTGCTCTTCAATA-3´
REGγ mutant p53 reverse	5´- GTGACGTAGGCTCGGGGAAGT-3´
GAPDH forward	5´- AAAAGCGGGGAGAAAGTAGG-3´
GAPDH reverse	5´- AAGAAGATGCGGCTGACTGT-3´
Mouse REGγ p53 forward	5´- GGAAAACTTGTCAAGAGCACA-3´
Mouse REGγ p53 reverse	5´- TAACTTTCCATCTTACGGGA-3´

Supplementary Table S3: siRNA sequences used in this study

siRNA	Sequences (5'-3')
siSmad3	5´-GGATTGAGCTGCACCTGAATG-3´
sip53	5´-CCGCGCCATGGCCATCTACA-3´
siN-CoR	5′-TGCTACTTCTCGAGGAAACA-3´

Supplementary Table S4: Primers used in RT-PCR experiments

Primers	Sequences (5'-3')
Human REGγ forward	5´- ACAAGTGAGGCAGAAGAC-3´
Human REGγ reverse	5´- ATCATGGCTATTGGTGAG-3´
Smad3 forward	5´- AGAAGACGGGGCAGCTGGAC-3´
Smad3 reverse	5´- GACATCGG55ATTCGGGGATAG-3´
GAPDH forward	5´- GTCAGTGGTGGACCTGACCT-3´
GAPDH reverse	5´- CCCTGTTGCTGTAGCCAAAT-3´
Human p53 forward	5´- AATCTACTGGGACGGAACAGC-3´
Human p53 reverse	5´- GACCCTTTTTGGACTTCAGGTG-3´
Mouse REGγ forward	5´- TCTCCTCACCAATAGCCACG-3´
Mouse REGγ reverse	5´- CTCGATCAGCAGCCGAAT-3´
Mouse p53 forward	5´- CCTGTGCAGTTGTGGGTC-3´
Mouse p53 reverse	5´- TTCCAGATACTCGGGATACA-3´
N-CoR forward	5´- GGAATCGAAGCGACCACGT-3´
N-CoR reverse	5´- ACTAAAGGCAAAACCGCAGC-3´