Supplementary Information

Figure S1. ΔNp63α inhibits cell invasion. (**a**) Stable Hs-578T, H1299 and A549 cells were subjected to transwell assays for cell invasion. Twenty-four hours after plating, invading cells were fixed and stained with crystal violet and photographed under a light microscope. Representative pictures from three independent experiments are shown. Scale bars = 200 μm.

Figure S2. ΔNp63α affects gene expression in Hs-578T cells. Hs-578T cells stably expressing either vector control (C), wild type (WT) or mutant (C306R or C526W) ΔNp63α were subjected to gene expression profiling using Affymetrix human genome U133A 2.0 arrays. (**a**) Gene expression profile from Hs-578T stable cells was depicted as a heatmap. Heatmap shows genes significantly altered ($P < 0.05$) by ΔNp63α expression in two independent experiments. Blue: down-regulation; white: no change; orange: up-regulation. (**b**) CD82 expression in Hs-578T stable cells as assessed by gene array.

Figure S3. CD82 is essential for ΔNp63α-mediated inhibition of cell invasion. (**a**) Hs-578T cells were infected with retrovirus expressing CD82 or an empty vector control (C) and selected by puromycin resistance. Stable Hs-578T cells were subjected to transwell assays for cell invasion, as previously described. Representative images from three independent experiments are shown. Scale bars = 100 μm. (**b** – **d**) Hs-578T, H1299 and A549 cells were infected with recombinant retrovirus encoding wild type murine ΔNp63α or a vector control, and subsequently infected with either one of three independent lentivirus expressing shRNA against CD82 (shCD82-1, shCD82-2 and shCD28-3), or a control shRNA (shC). Stable cells were subjected to transwell assays for cell invasion, as previously described. Representative pictures from three independent experiments are shown. Scale bars = 200 µm. (**e** – **g**) Hs-578T cells expressing WT ΔNp63α were subsequently infected with two additional lentivirus expressing shRNA against CD82 (shCD82-4 and shCD28-5), or a control shRNA (shC). (**e**) Whole-cell lysates were subjected to western blotting, as indicated. (**f** – **g**) Stable cells were subjected to cell invasion assays as described above. (**f**) Invading cells were photographed. Scale bars = 100 µm. (**g**) Cell invasion results are presented as means and SE from three independent experiments.

Figure S4. CD82 mediates ΔNp63α-induced inhibition of cell invasion. Hs-578T and H1299 cells were infected with recombinant retrovirus encoding wild type murine ΔNp63α or a vector control. Stable cells were then infected with lentivirus expressing shRNA against CD82 (shCD82-1) or a control shRNA (shC). Cells expressing ΔNp63α and shCD82-1 were subsequently infected with lentivirus expressing CD82 in order to revert the rescue of cell invasion by CD82 ablation. (**a**) Whole-cell lysates were subjected to western blotting, as indicated. (**d**) Stable cells were subjected to transwell assays for cell invasion, as described previously. Invading cells were photographed (left panels) and quantitated (right panels). Results presented as representative images (left) or means and SE (right) from three independent experiments. Scale bars = 200 µm.

Figure S5. p63 ablation-induced cell invasion is partly mediated by CD82. (**a**) FaDu and MCF-10A cells were infected with lentivirus expressing shRNA against p63 (shp63) or a control shRNA (shC). (**a**) Cells were subjected to transwell cell invasion assays, as described previously. Representative images from three independent experiments are shown. (**b**) FaDu and MCF-10A cells expressing shp63 or shC were infected with retrovirus expressing CD82 or a vector control (Vec). Cells were subjected to transwell cell invasion assays, as described previously. Representative images from three independent experiments are shown. (**c**) GSK3β ablation induces cell invasion via down-regulation of CD82. FaDu cells were infected with lentivirus expressing shRNA against GSK3β (sh3β) or a control (shC), and subsequently infected with recombinant retrovirus expressing either CD82 or a vector control (C). Puromycin-resistant cells were subjected cell invasion assays, as described previously. Images representative of three independent experiments. Scale bars = 200 µm.

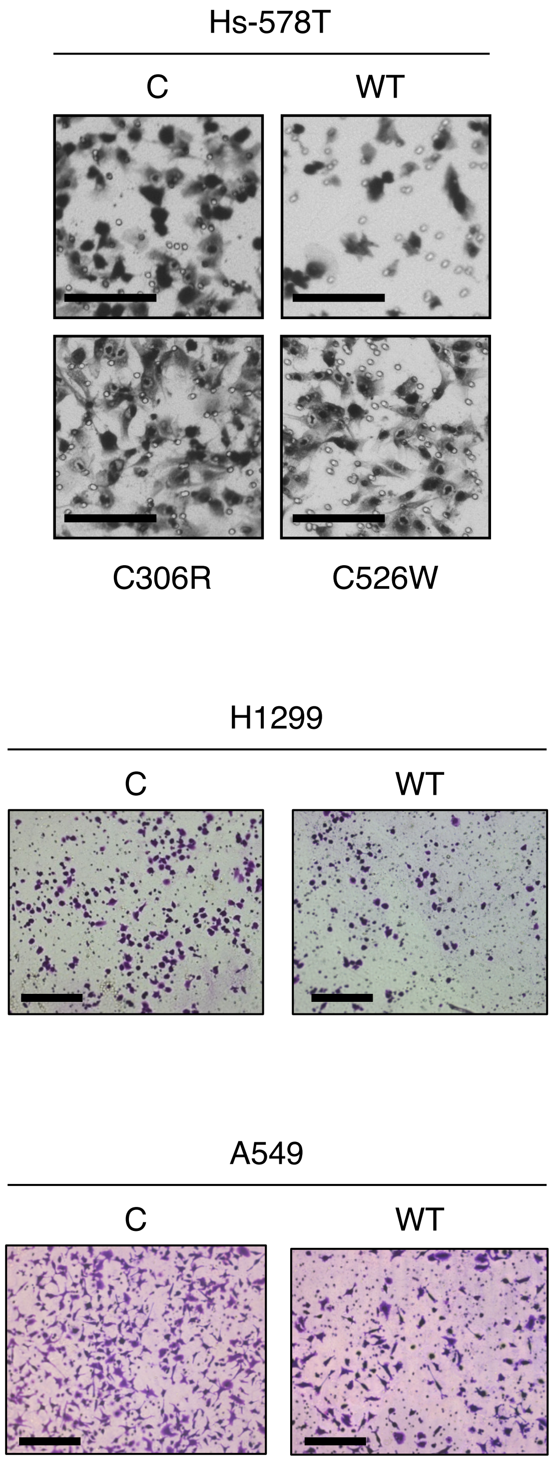
Table S1. List of known target genes regulated by ΔNp63α (fold change >2) detected by Affymetrix array. Experimental evidence from the literature include identification in global expression profile analyzes after altering p63 cellular levels, validation of gene expression changes at the transcriptional level, identification of candidate response elements with subsequent testing in transcriptional reporter assays, and/or chromatin immunoprecipitation studies to verify occupancy of p63 at genomic loci.

Table S2. Ontological classification of ΔNp63α-regulated genes. Hs-578T cells stably expressing ΔNp63α or a vector control were profiled by Affymetrix array (n = 2). Genes whose expression was two fold or more than that of the vector ($P < 0.05$) were classified ontologically using DAVID Bioinformatics Resources 6.7.

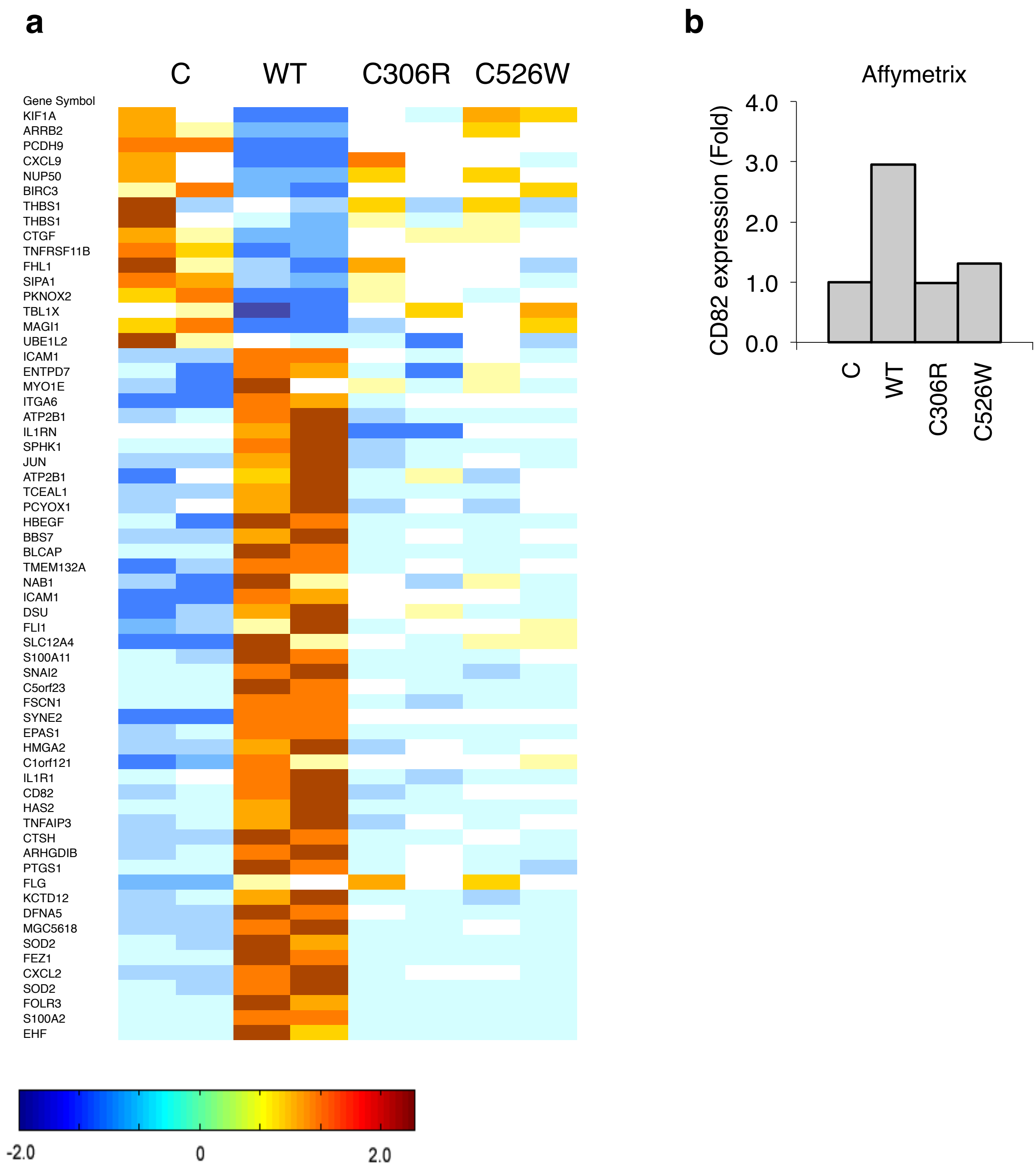
Table S3. List of genes up-regulated by wild type ΔNp63α classified by ontology. Hs-578T cells stably expressing ΔNp63α or a vector control were profiled by Affymetrix array (n = 2). Genes whose expression was two fold or more than that of the vector ($P < 0.05$) were classified ontologically using DAVID Bioinformatics Resources 6.7.

Table S4. Oligonuleotides used for mutageneis, ChIP, PCR, Q-PCR, siRNA and shRNA.

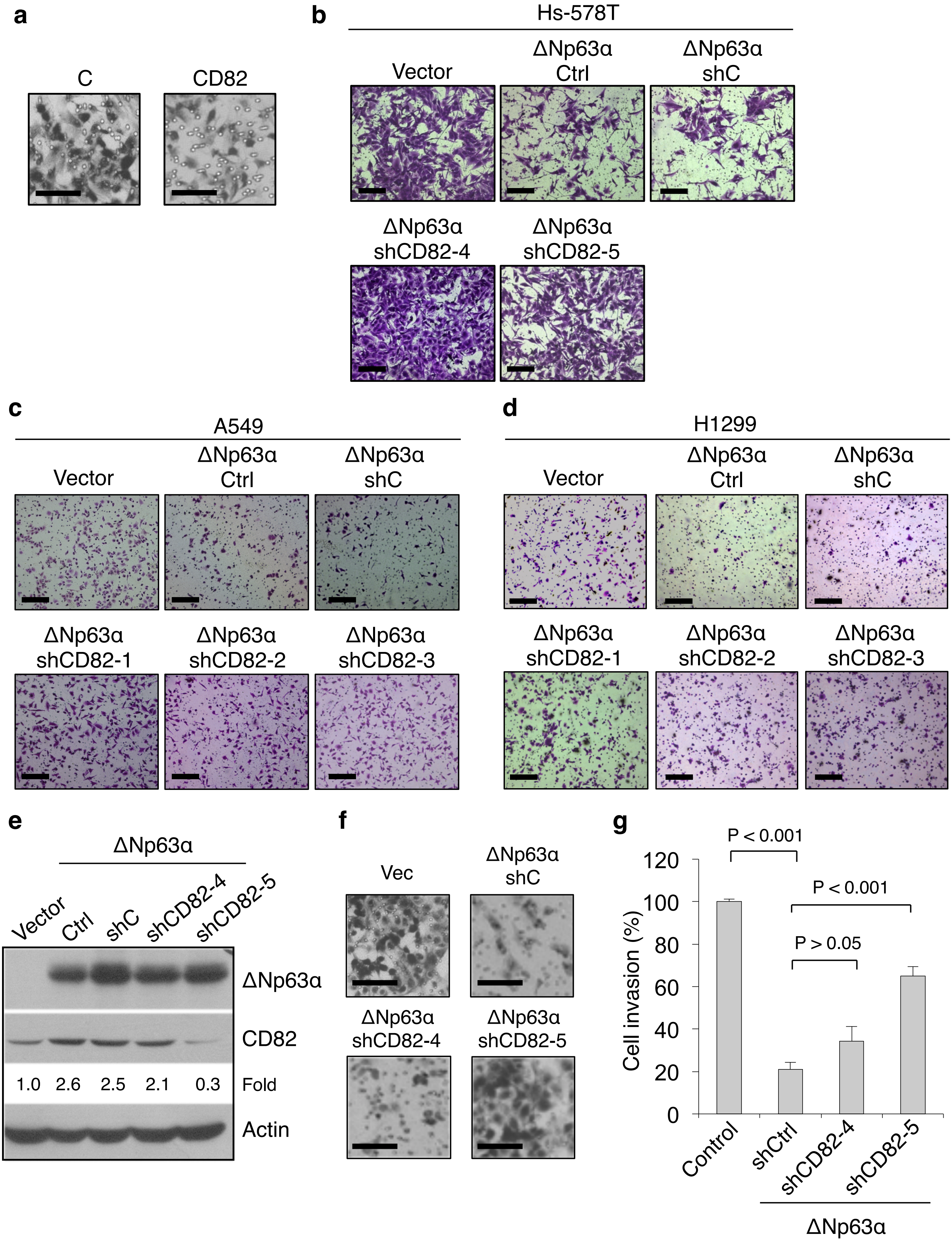
a



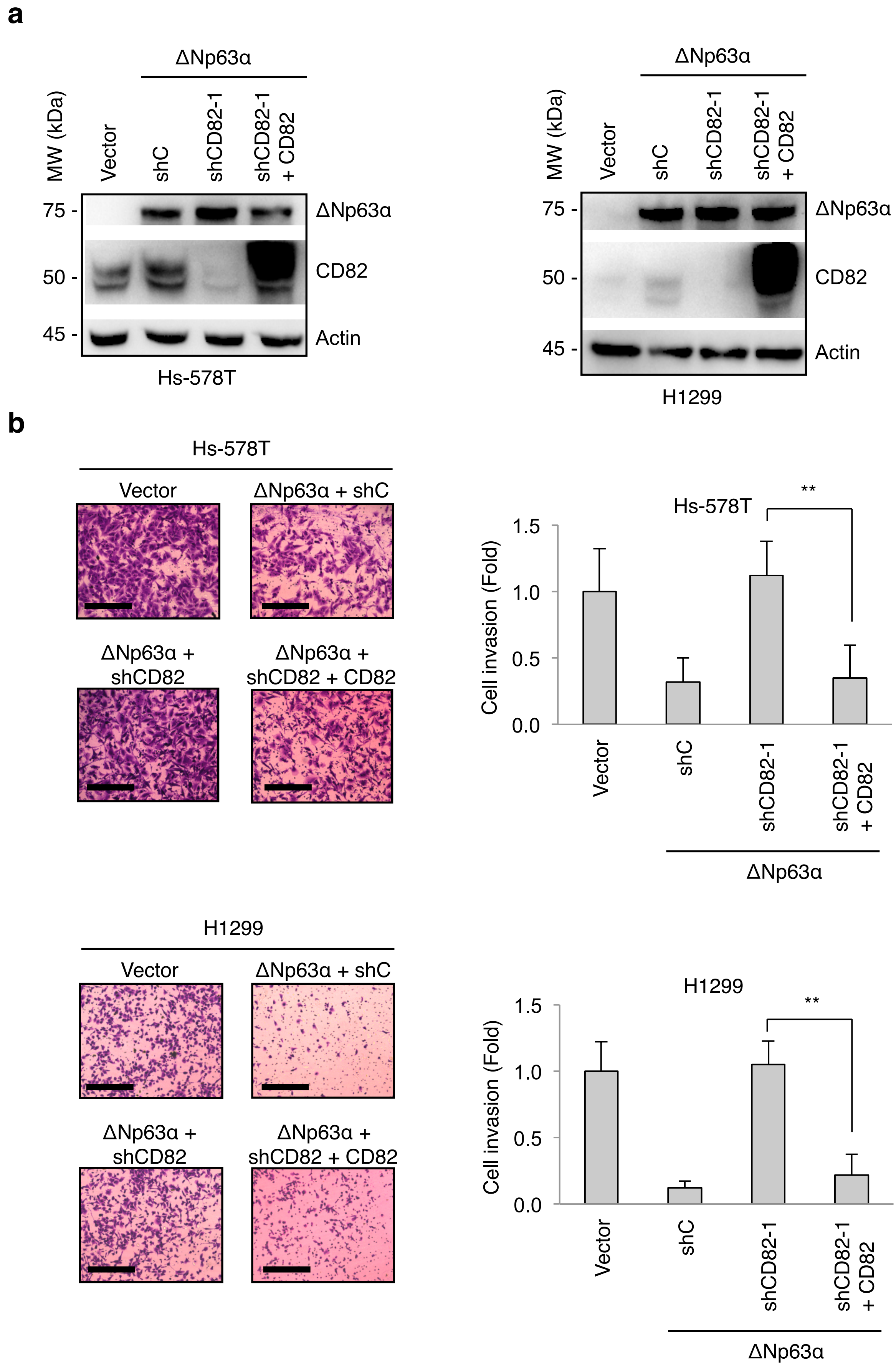
Supplemental Figure S1, Wu et al.



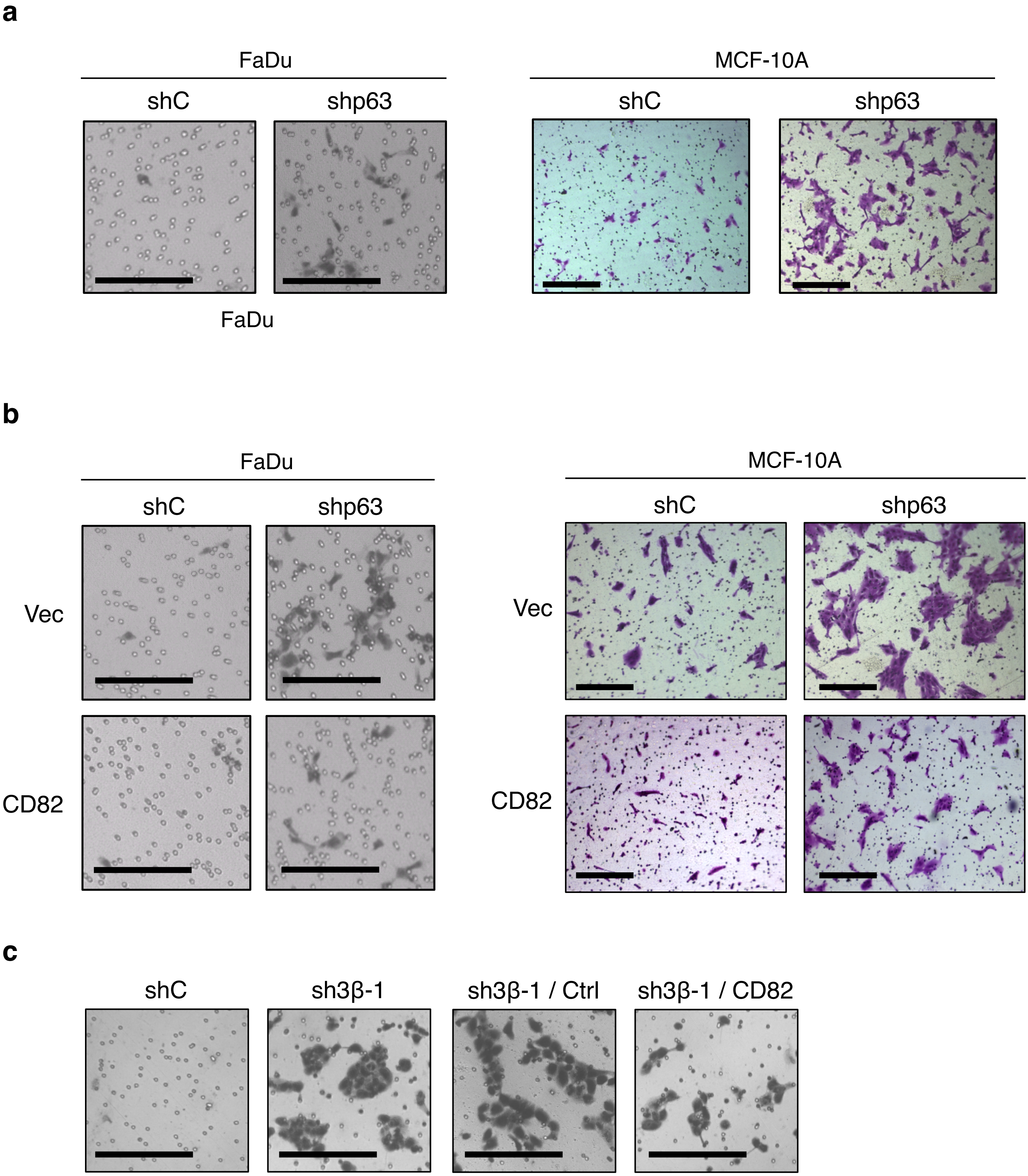
Supplemental Figure S2, Wu et al.



Supplemental Figure S3, Wu et al.



Supplemental Figure S4, Wu et al.



Supplemental Figure S5, Wu et al.

Table S1. List of known target genes regulated by ΔNp63α (fold change > 2).

Gene symbol	fold	reference	Gene symbol	fold	reference
ACP5	3.1	(Truong et al., 2006)	IL32	2.5	(Carroll et al., 2006)
AKR1B10	-5.6	(Truong et al., 2006)	ITGA6	2.0	(Carroll et al., 2006; Truong et al., 2006)
ANXA8	13.6	(Osada et al., 2005)	JUN	2.1	(Testoni et al., 2006)
ARHGDIB	4.0	(Carroll et al., 2006)	KCTD12	4.4	(Truong et al., 2006)
BHLHB3	2.3	(Adorno et al., 2009)	KRT14	31.6	(Boldrup et al., 2007; Candi et al., 2006; Romano et al., 2007)
CSTA	3.6	(Barbieri et al., 2006)	KYNU	2.4	(Truong et al., 2006)
CTGF	-2.5	(Carroll et al., 2006)	LAMC2	3.5	(Carroll et al., 2006)
DUSP1	3.0	(Truong et al., 2006)	LAMB3	2.0	(Carroll et al., 2006)
EGFR	2.1	Carroll et al., 2006; Nishi et al., 2001)	LPXN	2.4	(Carroll et al., 2006)
FABP4	19.7	(Truong et al., 2006)	MAST4	2.1	(Osada et al., 2005)
FEZ1	6.2	(Osada et al., 2005)	MT1X	2.9	(Sasaki et al., 2005; Truong et al., 2006)
FLG	4.1	(Barbieri et al., 2006; Candi et al., 2006; Truong et al., 2006)	NR4A2	-2.0	(Pozzi et al., 2009)
FST	2.1	(Barbieri et al., 2006)	NT5E	2.5	(Osada et al., 2005)
GM2A	2.0	(Truong et al., 2006)	PBX1	-2.1	(Barbieri et al., 2006; Truong et al., 2006)
HBEGF	2.4	(Wu et al., 2003)	PERP	2.4	(Ihrie et al., 2005)
HEY1	2.4		PKNOX2		(Testoni et al., 2006)
ICAM1	2.2	(Carroll et al., 2006; Kikuchi et al., 2004)	PLLP	3.7	(Osada et al., 2005)
IL1A	6.7	(Barbieri et al., 2006; Truong et al., 2006)	POSTN	-3.8	(Barbieri et al., 2006)
IL1B	4.8	(Truong et al., 2006)	PTGS1	6.7	(Truong et al., 2006)
IL1F5	10.3	(Truong et al., 2006)	S100A2	13.9	(Kirschner et al., 2008; Lapi et al., 2006)
IL1R1	3.7	(Truong et al., 2006)	SAA1	18.2	(Truong et al., 2006)
IL1RN	2.1	(Truong et al., 2006)	SLC7A11	2.0	(Wu et al., 2003)
IL32	2.5	(Carroll et al., 2006)	TNC	2.5	(Barbieri et al., 2006)

Table S2. Ontological classification of ΔNp63α-regulated genes.

Ontological classification	Up-regulated	Down-regulated
Cell communication	49	28
Development	48	18
Cell differentiation	29	6
Immune syst. process	22	6
Response to stress	21	9
Apoptosis	12	5
Cell proliferation	13	4
Cell cycle	8	5
Cell adhesion	19	9
Cell migration / motility	14	0

Table S3. Ontological classification of genes up-regulated by wild type ΔNp63α in Hs-578T cells.

Ontological Classification	Gene names			
Cell communication	ANGPTL2	DUSP6	INHBA	PBEF1
	ANGPTL4	EGFR	ITGA6	PITPNC1
	ARHGDIB	EPAS1	ITPR2	RAPGEF5
	BLNK	EPO	KLRC1	RASAL2
	C3	FST	KLRC2	S100A11
	CAP2	FZD3	LOX	SNAI2
	CD24	GRB14	LPXN	SOD2
	CLEC4A	HBEGF	MCTP2	SPHK1
	CSF2	HEY1	MT1X	TNC
	CXCL2	IL1A	NAB1	TNFAIP3
	CXCL3	IL1B	NEDD9	
	DIRAS3	IL1R1	NRG1	
	DUSP1	IL6R	P2RY6	
Development	ANGPTL2	EPAS1	IL6R	NRG1
	ANGPTL4	EPO	INHBA	PAPPA
	ARHGDIB	FEZ1	ITGA6	PERP
	BCL2A1	FLG	JUN	PLXDC1
	BLNK	FLI1	KRT14	PTGS1
	CAP2	FST	KRT17	RAPGEF5
	CD24	FZD3	LAMB3	SERPINB2
	CSF2	HBEGF	LAMC2	SNAI2
	CSTA	HEY1	LOX	SOD2
	DFNA5	HMGA2	NAB1	SPHK1
	EGFR	IL1A	NEDD9	TNC
	EHF	IL1B	NOV	TNFAIP3
Cell differentiation	ANGPTL4	EPAS1	INHBA	SERPINB2
	BCL2A1	EPO	JUN	SOD2
	BLNK	FEZ1	KRT14	SPHK1
	CD24	FLG	NAB1	TNC
	CSF2	FST	NRG1	TNFAIP3
	CSTA	HEY1	PAPPA	
	DFNA5	IL1A	PERP	
	EHF	IL1B	PTGS1	

Ontological Classification	Gene names			
Immune syst. process	ARHGDIB	CTSS	IL1B	INHBA
	BLNK	CXCL2	IL1F5	ITGA6
	C3	CXCL3	IL1R1	PODXL
	CD24	EPO	IL1RN	SAA1
	CLEC4A	FCGR2C	IL32	
	CSF2	IL1A	IL6R	
Response to stress	ANGPTL4	DUSP1	IL1A	SAA1
	BLNK	EGFR	IL1B	SERPINA1
	C3	EPAS1	IL1F5	SOD2
	CD24	EPO	IL1R1	
	CXCL2	FABP4	IL1RN	
	CXCL3	HBEGF	PTGS1	
Apoptosis	ANGPTL4	CSF2	INHBA	SOD2
	BCL2A1	IL1A	PERP	SPHK1
	CD24	IL1B	SERPINB2	TNFAIP3
Cell proliferation	CD24	FSCN1	IL1B	SPHK1
	CSF2	FZD3	IL6R	
	EGFR	HBEGF	PBEF1	
	EHF	IL1A	S100A11	
Cell cycle	DIRAS3	EGFR	IL1B	NEDD9
	DUSP6	IL1A	INHBA	SPHK1
Cell adhesion	ARHGDIB	FEZ1	LAMB3	PODXL
	CD24	FZD3	LAMC2	SAA1
	CD82	ICAM1	LPXN	TMEM8
	CLEC4A	IL32	NEDD9	TNC
	EGFR	ITGA6	PERP	
Cell migration/ motility	ARHGDIB	DUSP6	IL1B	SAA1
	CALD1	EGFR	ITGA6	SPHK1
	CD24	FEZ1	PODXL	
	CD82	HBEGF	S100A2	

Supplemental Table S3, Wu et al.

Table S4. Oligonuleotides used for mutagenesis, ChIP, PCR, Q-PCR, siRNA, and shRNA.		
Oligonucleotide	Sequence	Applicaiton
Human p63 shRNA	CCGTTTCGTCAGAACACACAT	shRNA
CD82 shRNA-1	GTTTCATCTCTGTCCTGCAAA	shRNA
CD82 shRNA-2	CTTCTACAACTGGACAGACAA	shRNA
CD82 shRNA-3	AAGAGCAGTTTCATCTCTGTC	shRNA
CD82 shRNA-5	CCTGGCCGACAAGAGCAGTTT	shRNA
GSK3β shRNA	AAGTGATTGGCAATGGAATGGCTCAT	shRNA
β-Catenin siRNA	AGCTGATATTGATGGACAGdTdT	siRNA
Lamin A/C siRNA	CUGGACUUCCAGAAGAACAdTdT	siRNA
P1 fwd	CTCATCAACCCACACCTCCT	ChIP
P1 rev	CTAGCCCTTGAATTCCCACA	ChIP
P2 fwd	ACAGGGTTTCATCCTGTTGC	ChIP
P2 rev	CCTACAGCCACCTCTTCGTC	ChIP
Human p63 fwd	GTTATCCGCGCCATGCCTGTCTAC	Q-PCR
Human p63 rev	TCCCCTCTACTCGAATCAAATG	Q-PCR
CD82 fwd	AGCGCGGAGCAGAAAGCAGAACC	Q-PCR
CD82 rev	GCCCCCACGCCGATGAAGACA	Q-PCR
β-Catenin fwd	GTGGAGGGGGTCCGCATGGAAGAA	Q-PCR
β-Catenin rev	GAGAATAAAGCAGCTGCACAAACAATGGA	Q-PCR
GAPDH fwd	GGGGAGCCAAAAGGGTCATCATCT	Q-PCR
GAPDH rev	GAGGGGCCATCCACAGTCTTCT	Q-PCR
Murine ΔNp63α-C306R	CTTTGAGGCCAGGATCCGTGCTTGCCCAGGA	Mutagenesis
Murine ΔNp63α-C526W	GGGCTGCTCATCATGGCTGGACTATTTCACG	Mutagenesis
Murine ΔNp63α fwd	GAAGATCTATGTTGTACCTGG	Cloning
Murine ΔNp63α rev	GTTAACTCATTCTCCTTCCTC	Cloning