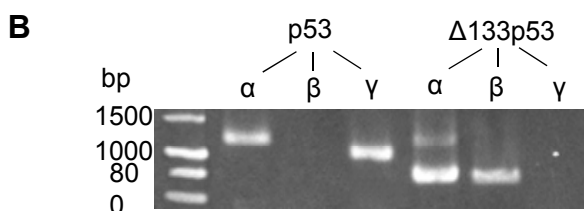
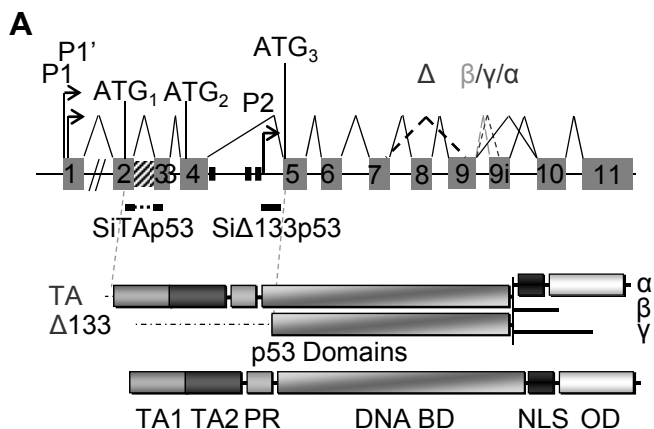
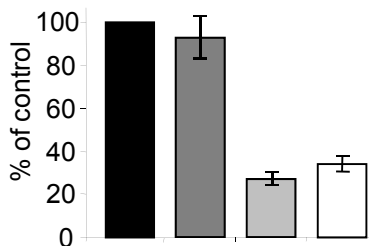


Bernard et al, Figure 1.

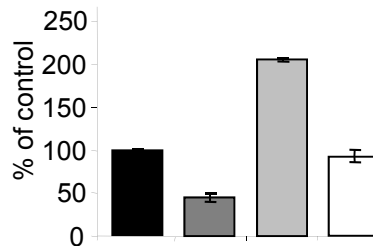


C1 Δ133p53 mRNA expression



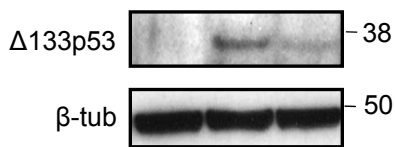
SiNON	+	-	-	-
SiTAp53	-	+	-	+
SiΔ133p53	-	-	+	+

C2 p53 mRNA expression



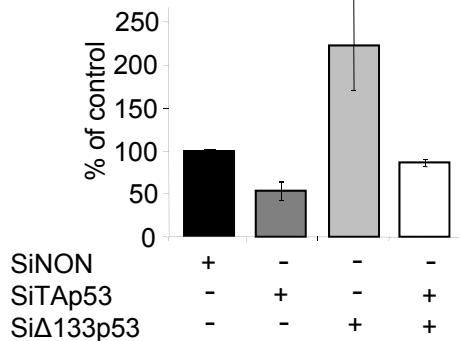
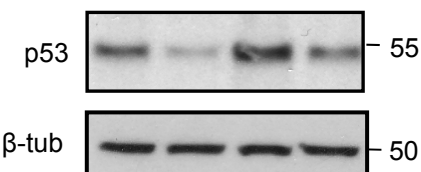
SiNON	+	-	-	-
SiTAp53	-	+	-	+
SiΔ133p53	-	-	+	+

D1 Δ133p53 protein expression



SiNON	+	-	-
SiΔ133p53	-	-	+
Δ133p53	-	+	+

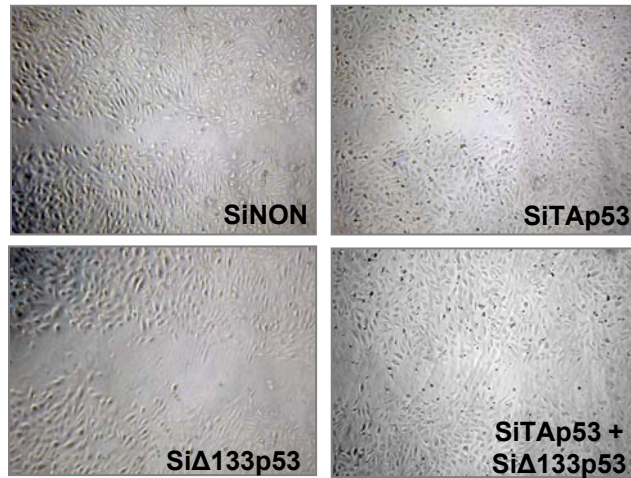
D2 p53 protein expression



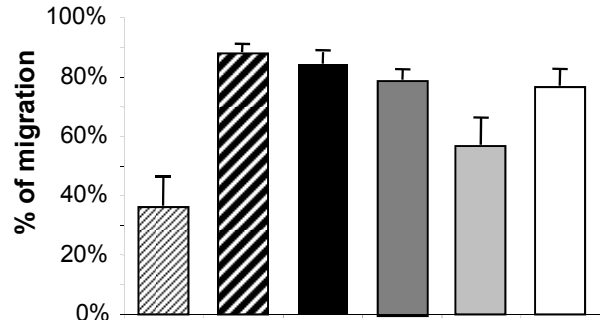
SiNON	+	-	-	-
SiTAp53	-	+	-	+
SiΔ133p53	-	-	+	+

Bernard et al, Figure 2.

A1

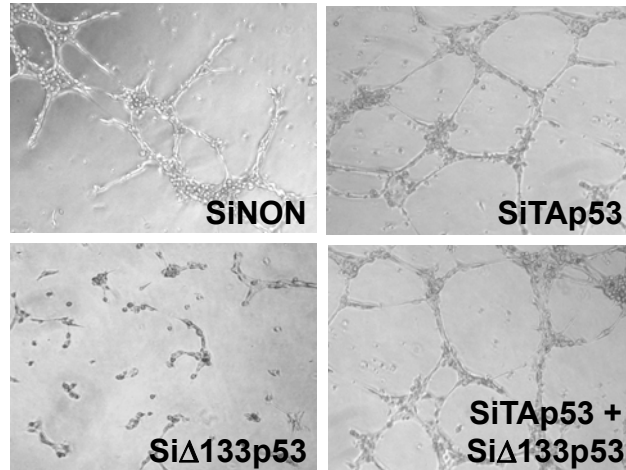


A2 Migration quantification

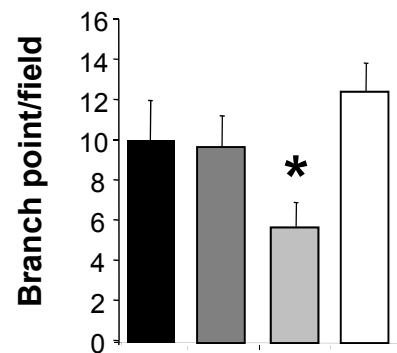


C + (FGF-2)	-	+	-	-	-	-
C -	-	-	-	-	-	-
SiNON	-	-	+	-	-	-
SiTAp53	-	-	-	+	-	+
SiΔ133p53	-	-	-	-	+	+

B1



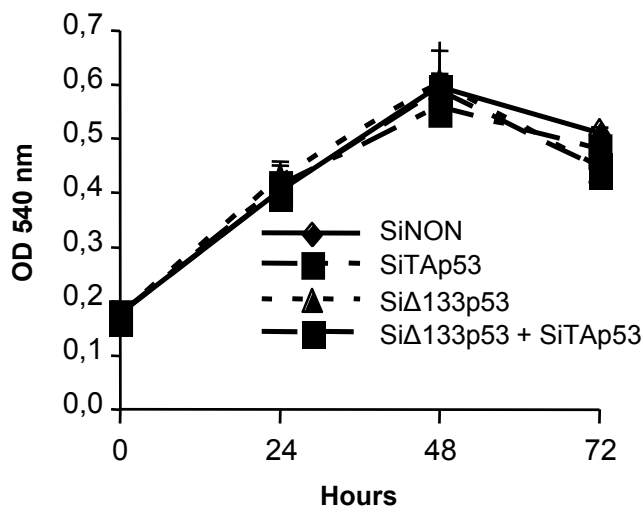
B2 Tubulogenesis quantification (U87 conditioned media)



SiNON	+	-	-	-
SiTAp53	-	+	-	+
SiΔ133p53	-	-	+	+

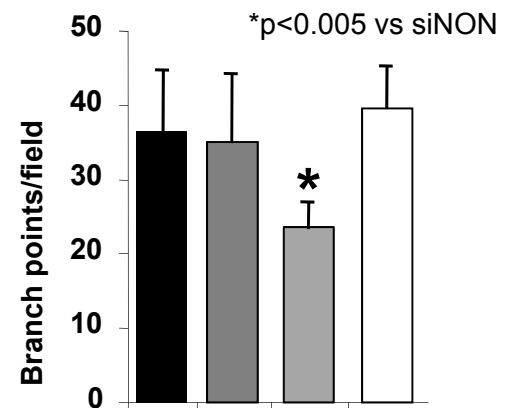
C

HUVEC Proliferation



D

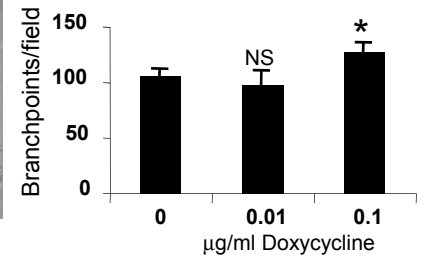
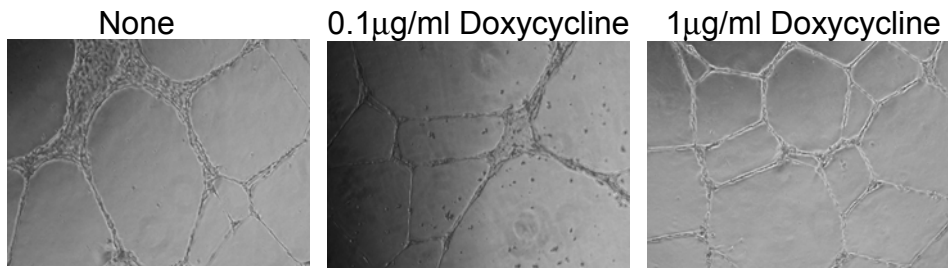
Tubulogenesis quantification (U2OS conditioned media)



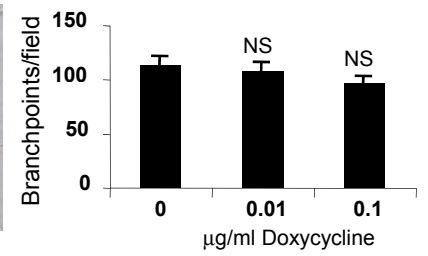
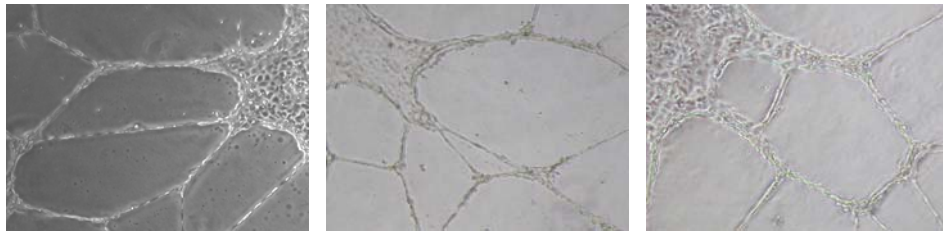
SiNON	+	-	-	-
SiTAp53	-	+	-	+
SiΔ133p53	-	-	+	+

Bernard et al, Figure 3.

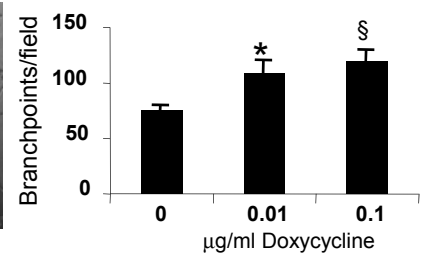
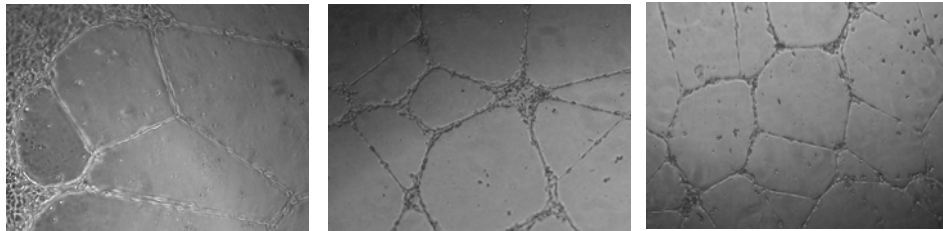
A $\Delta 133p53\alpha$



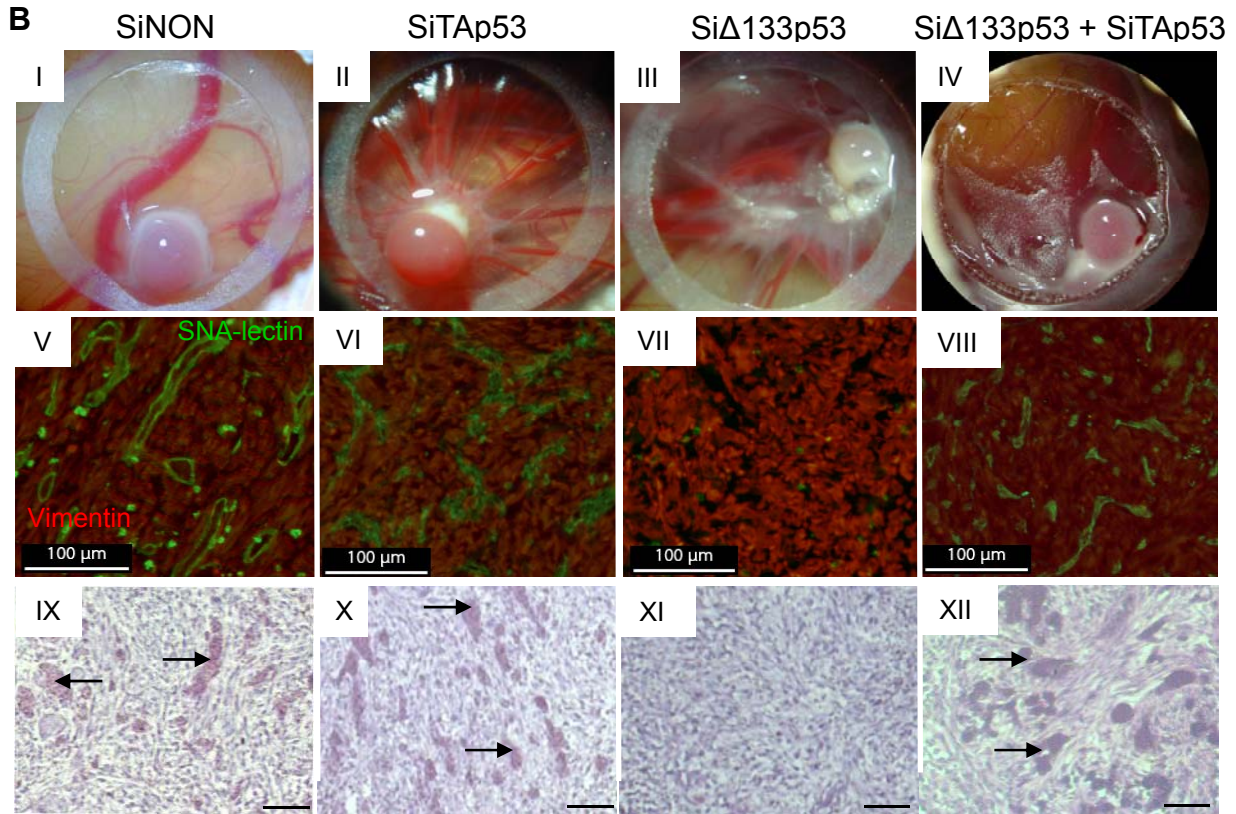
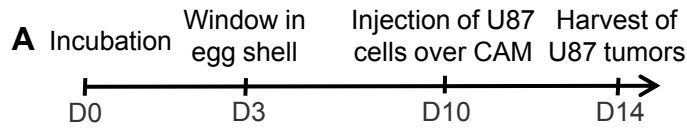
B $\Delta 133p53\beta$



C $\Delta 133p53\gamma$



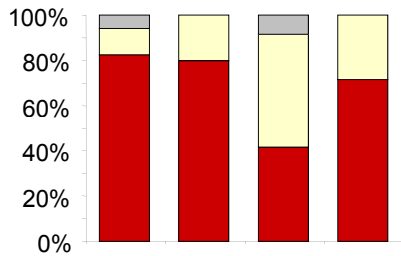
Bernard et al, Figure 4.



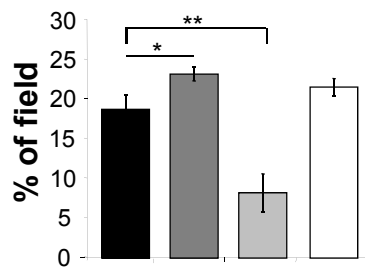
C

Angiogenic appearance

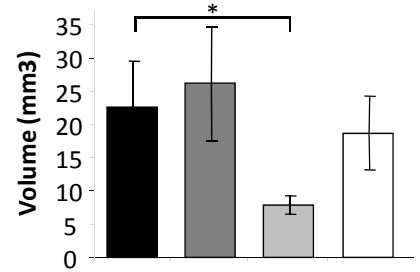
■ no tumor □ avascular ■ vascular



Vessel quantification



Tumor volume



SiNON (nM)

SiTAp53

SiΔ133p53

+

-

-

-

-

+

-

+

-

+

-

+

-

+

-

+

-

+

-

+

-

+

-

+

-

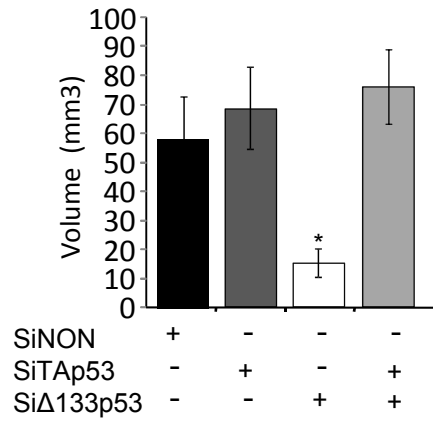
+

-

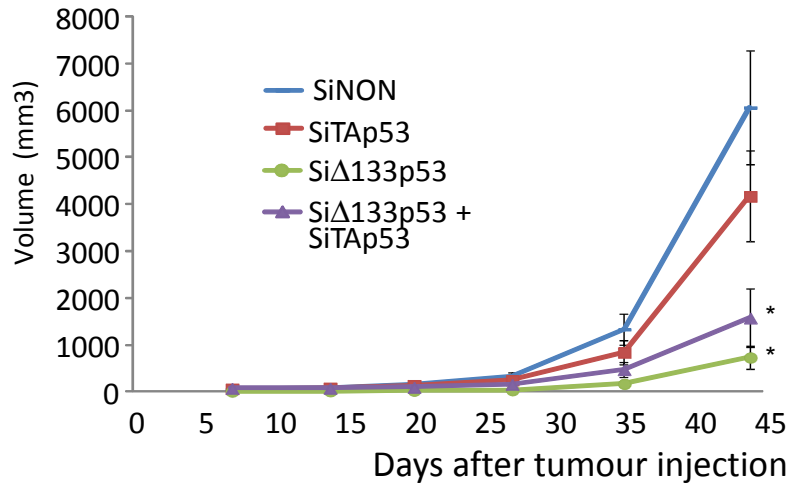
+

Bernard et al, Figure 5.

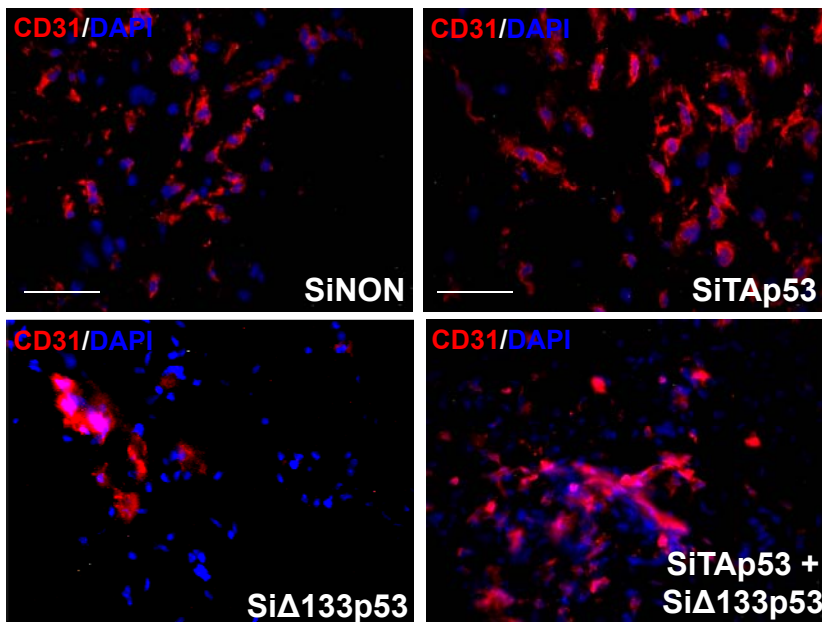
A Tumour volume
Day 7



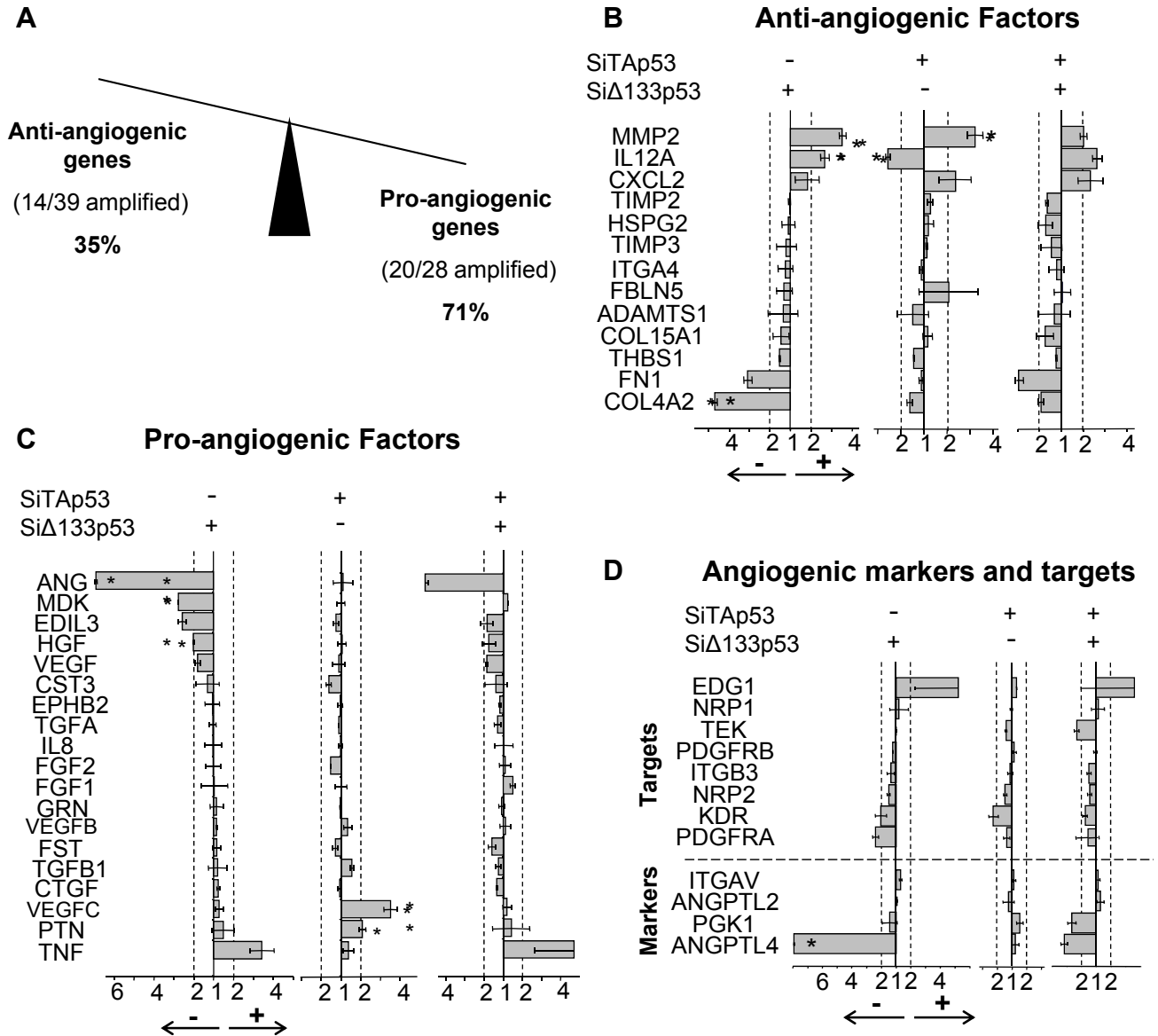
B Tumour growth



C Tumour angiogenesis
Day 7



Bernard et al, Figure 6.

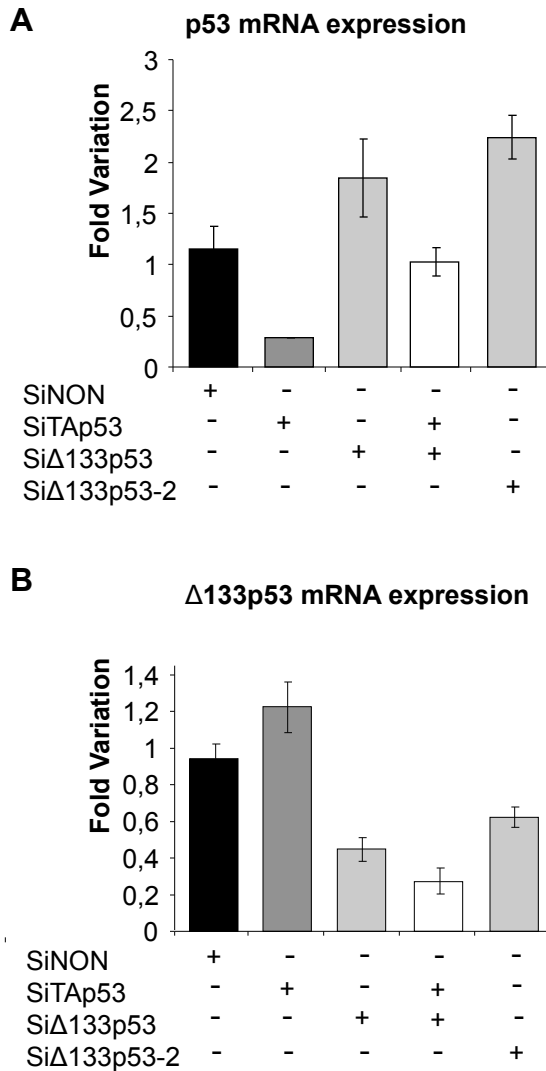


		Time (days)			
		SiNON	SiTAp53	SiΔ133	SiΔ133 + SiTAp53
Day 7	Average	58	68	15	76
	SEM	14	14	5	13
	p value		0,3060	0,0095	0,1904
Day 14	Average	79	86	20	76
	SEM	19	19	8	13
	p value		0,4026	0,0095	0,4471
Day 20	Average	143	140	31	97
	SEM	38	30	13	11
	p value		0,4750	0,0107	0,1356
Day 27	Average	321	252	44	161
	SEM	83	49	13	38
	p value		0,2431	0,0060	0,0564
Day 35	Average	1336	841	173	474
	SEM	335	258	53	159
	p value		0,1317	0,0051	0,0220
Day 44	Average	6062	4171	736	1577
	SEM	1206	974	239	614
	p value		0,1218	0,0014	0,0039

Table I . Effect of siΔ133p53 treatment on tumour growth in nude mice. U87 cells transfected with siNON, siTAp53, siD133p53 or siTAp53 + siD133p53 were xenografted into nude mice. Tumour volumes were quantified from day 7 to day 44 (8 tumors / condition).

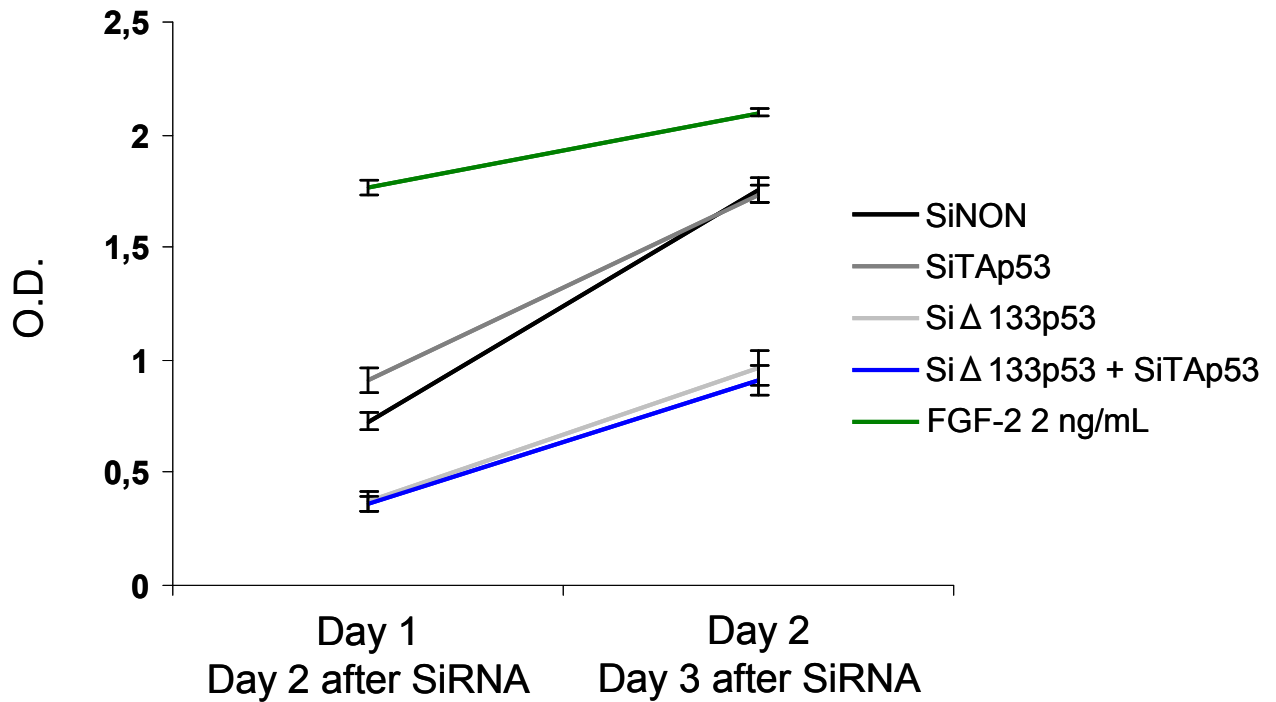
Table II: Angiogenesis Taqman Low Density Array of $\Delta 133p53$ and p53 knockdown.

	Si_ 133p53		SiTAp53		Si_ 133p53 + SiTAp 53	
	Moy	p	Moy	p	Moy	p
<i>Pro-Angiogenic factors</i>						
TNF	3.43	0.08	1.37	0.20	4.70	0.16
ANGPT2	2.15	0.23	6.94	0.25	-3.12	0.13
PTN	1.48	0.28	2.06	0.05	1.43	0.37
VEGFC	1.28	0.21	3.50	0.04	1.20	0.27
CTGF	1.23	0.10	-1.07	0.28	-1.35	0.03
TGFB1	1.21	0.37	1.55	0.06	-1.25	0.20
FST	1.16	0.30	-1.28	0.17	-1.60	0.15
VEGFB	1.15	0.06	1.33	0.16	1.11	0.38
GRN	1.15	0.36	-1.04	0.08	-1.09	0.34
FGF1	1.03	0.48	1.00	0.50	1.50	0.08
FGF2	-1.03	0.48	-1.53	0.00	-0.90	0.36
IL8	-1.03	0.48	-1.02	0.43	1.02	0.48
TGFA	-1.04	0.43	-1.12	0.04	-1.30	0.21
EPHB2	-1.07	0.45	-1.04	0.39	-1.17	0.14
CST3	-1.31	0.38	-1.62	0.10	-1.38	0.36
VEGF	-1.80	0.09	-1.12	0.39	-1.86	0.04
HGF	-2.01	0.01	1.06	0.41	-1.75	0.22
EDIL3	-2.58	0.10	-1.25	0.18	-1.84	0.20
MDK	-2.77	0.01	-1.01	0.49	1.24	0.01
ANG	-6.91	0.02	1.10	0.44	-5.08	0.07
<i>Anti-angiogenic factors</i>						
VASH1	3.70	0.21	4.84	0.24	1.84	0.13
MMP2	3.51	0.02	3.21	0.05	2.02	0.20
IL12A	2.64	0.04	-2.55	0.05	2.63	0.17
CXCL2	1.78	0.20	2.35	0.15	2.34	0.20
TIMP2	-1.05	0.27	1.27	0.13	-1.65	0.04
HSPG2	-1.12	0.40	1.20	0.26	-1.72	0.19
TIMP3	-1.21	0.34	1.11	0.12	-1.45	0.16
ITGA4	-1.27	0.37	-1.12	0.23	-1.22	0.37
FBLN5	-1.32	0.17	2.07	0.28	1.05	0.49
ADAMTS1	-1.38	0.13	-1.48	0.05	-1.32	0.30
COL15A1	-1.46	0.28	1.16	0.29	-1.74	0.14
THBS1	-1.53	0.03	-1.44	0.02	-1.24	0.25
FN1	-3.08	0.09	-1.13	0.21	-2.94	0.09
COL4A2	-4.70	0.05	-1.61	0.10	-1.93	0.06
<i>Angiogenesis targets</i>						
EDG1	5.23	0.15	1.29	0.38	3.62	0.25
NRP1	1.19	0.37	-1.04	0.32	1.14	0.36
TEK	-1.01	0.33	-1.35	0.04	-2.32	0.06
PDGFRB	-1.22	0.01	1.15	0.17	-1.04	0.34
ITGB3	-1.36	0.18	-1.10	0.26	-1.48	0.09
NRP2	-1.51	0.06	-1.41	0.08	-1.42	0.08
KDR	-2.04	0.15	-2.15	0.12	-1.74	0.07
PDGFRA	-2.39	0.08	-1.32	0.17	-1.55	0.31
<i>Angiogenesis markers</i>						
ITGAV	1.30	0.06	1.13	0.16	1.12	0.19
ANGPTL2	1.07	0.09	-1.20	0.31	1.32	0.14
PGK1	-1.44	0.28	1.49	0.09	-2.65	0.09
ANGPTL4	-7.93	0.02	1.22	0.20	-3.15	0.07



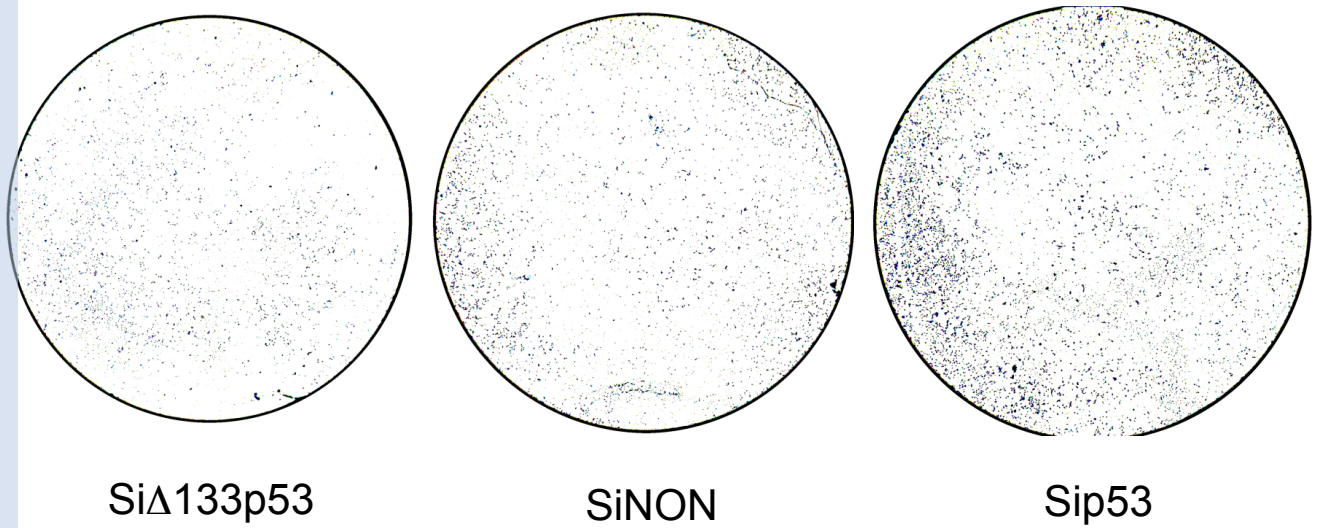
Supplementary Figure S1. SiΔ133p53-2 knocks down Δ133p53 in U87 cells.

p53 (A) and Δ133p53 mRNAs (B) relative expression following siRNA treatment (qRT-PCR). Experiments were repeated twice in duplicate. Sample quantity is normalized with 18S rRNA using the $2^{-\Delta\Delta Ct}$ method and set to percentage of control.

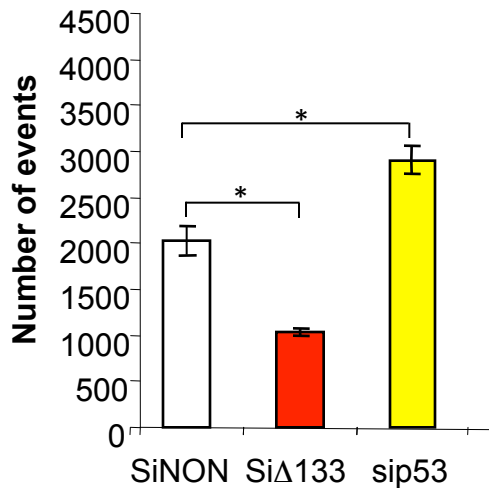


Supplementary Figure S2. U87 cell proliferation after siRNA treatment.

MTT proliferation assay in U87 cells treated by SiRNAs or FGF-2 (as positive control). SiRNA treatment was performed as described in Mat & Meth.

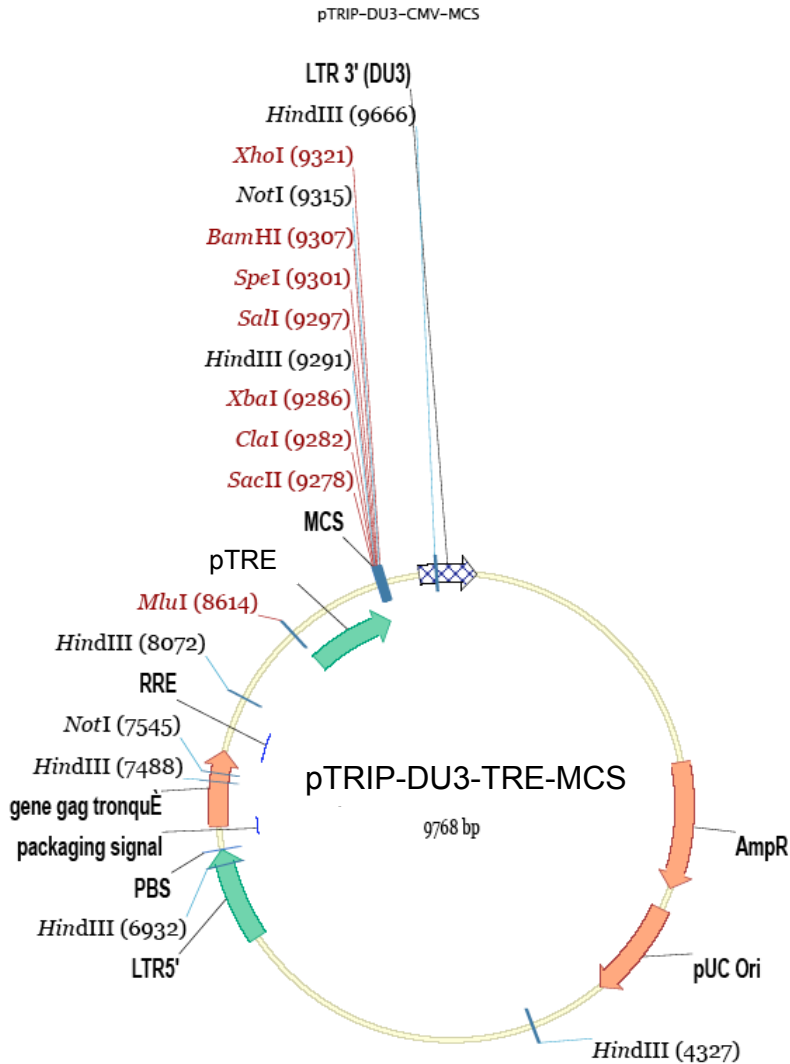


ABAE positive cells 6h after treatment



Supplementary Figure S3. Transwell migration assay in Boyden Chambers.

Conditioned media from siRNA-transfected U87 cells were used to assess ABAE cell migration in a Boyden Chamber assay. (A) Micrographs of Boyden chamber assays of ABAE cells treated during 6 hours with conditioned media from U87 cells transfected with siRNAs as indicated in Mat & Meth. (B) Quantification of ABAE migration. Asterisk indicates statistical significance. *, $P < 0.05$ vs control (conditioned medium from siNON-transfected U87 cells).



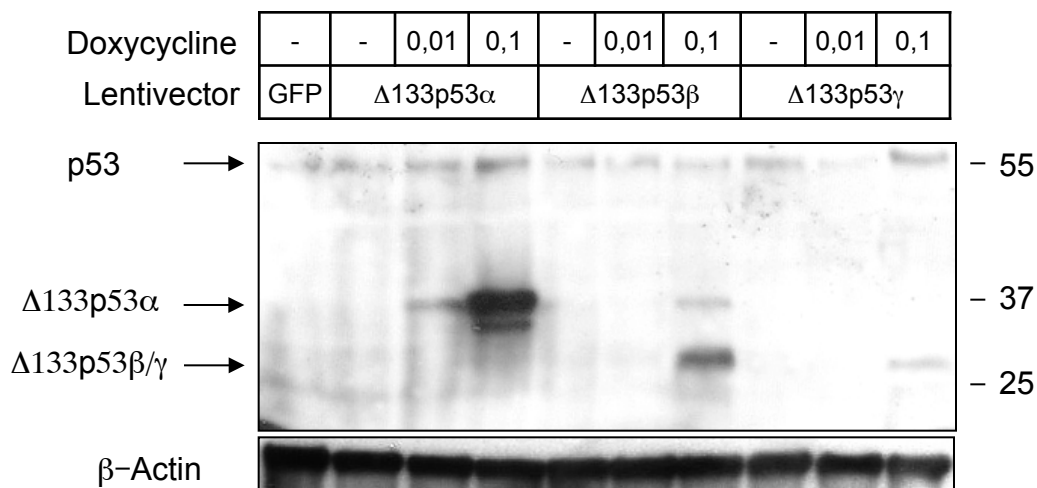
Supplementary Figure S4. The pTRIP-DU3-TRE-MCS lentivector.

The lentivector pTRIP-DU3-TRE-MCS was derived from the self-inactivating lentivector pTRIP-DU3-EF1a-EGFP (1). The EF1a promoter was replaced by the tetracycline-inducible pTRE promoter from the vector pTRE-tight (Clontech) and a multiple cloning site (MCS) was inserted in place of the GFP gene.

The cDNAs coding $\Delta 133P53\alpha$, $\Delta 133P53\beta$, or $\Delta 133P53\gamma$ (XhoI-BamHI fragments), respectively, were subcloned into the SalI-BamHI sites of pTRIP-DU3-TRE-MCS. The resulting lentivector plasmids were pTRIP-TRE- $\Delta 133P53\alpha$, - $\Delta 133P53\beta$, and - $\Delta 133P53\gamma$.

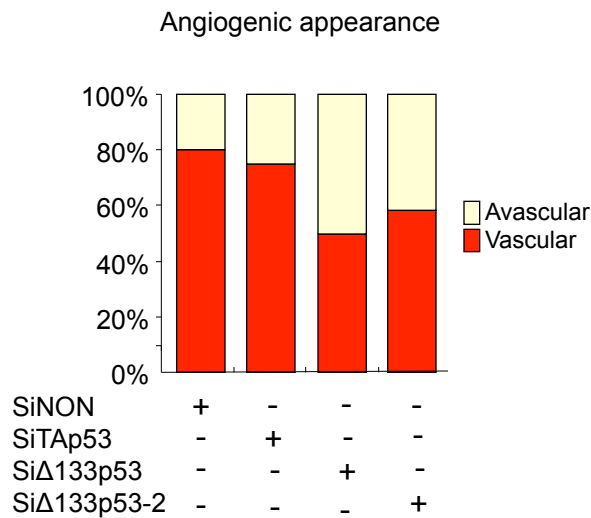
1. Sirven A, Ravet E, Charneau P, et al. Enhanced transgene expression in cord blood CD34(+)-derived hematopoietic cells, including developing T cells and NOD/SCID mouse repopulating cells, following transduction with modified trip lentiviral vectors. *Mol Ther* 2001;3(4):438-48.

U87 overexpressing lentivector-based doxycycline-induced p53 isoforms



Supplementary Figure S5. Induction of $\Delta 133p53\alpha$, β or γ in U87 TetOn cells transduced by lentivectors.

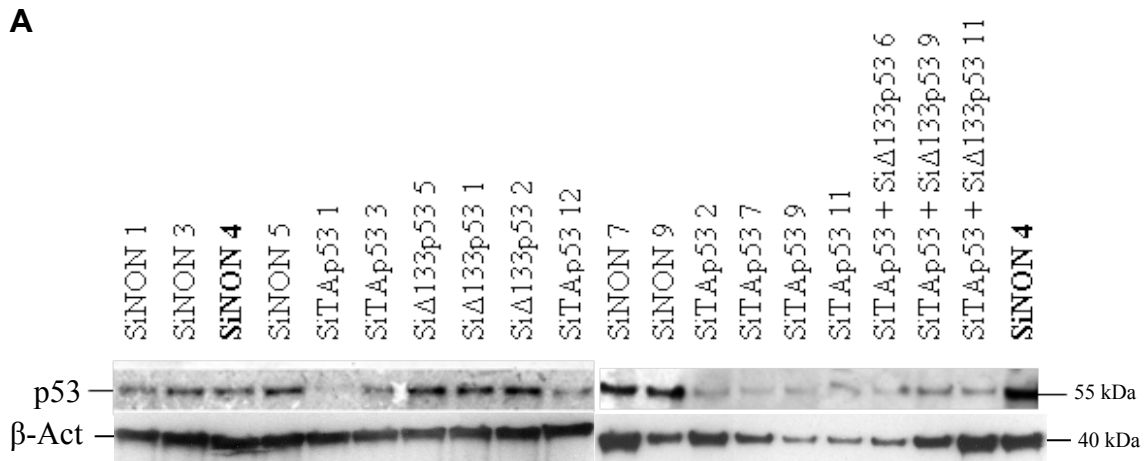
U87 cells were transduced by the lentivector pTRIP-rTTA-TETON expressing the tetracycline-inducible transactivator rTTA-advanced (Clontech), than these U87 TetOn cells were transduced by the lentivectors pTRIP-TRE- $\Delta 133P53\alpha$, - $\Delta 133P53\beta$, and - $\Delta 133P53\gamma$ (plot dilution at 1/8 provides 100% transduction) and treated 48 hours later with doxycycline at 0.01 or 0.1 $\mu\text{g/ml}$. Cells were harvested 48h after induction for Western blot analysis using anti-p53 antibody.



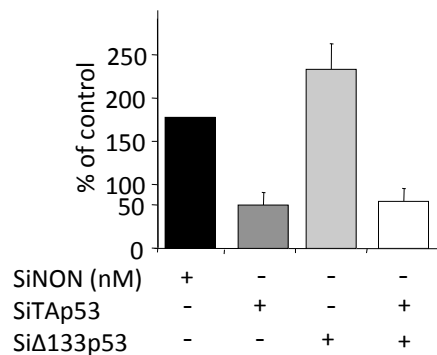
Supplementary Figure S6. SiΔ133P53-2 is anti-angiogenic in CAM.

U87 tumors were analysed in CAM following Treatment with siNON, siTAp53, siΔ133P53 or siΔ133P53-2. Tumor analysis was performed as in Fig. 4.

Angiogenic appearance is presented as the percentage of vascular or avascular tumors following treatments with the different siRNAs (8-10 tumors for each siRNA treatment).



B Quantification of P53 protein expression in CAM tumors



Supplementary Figure S7. p53 protein expression in CAM tumors.

- (A) Western blot from tumors of U87 cells treated with the different siRNAs 4 days after implantation in CAM (indicated on the top of each lane). The samples were analysed on two different gels on which the same amount of SiNON4 sample was deposited.
- (B) Quantification of p53 signal from Western blots after normalization with β -actin (we did not take into account lanes 5-7 of the right gel where actin detection was low).