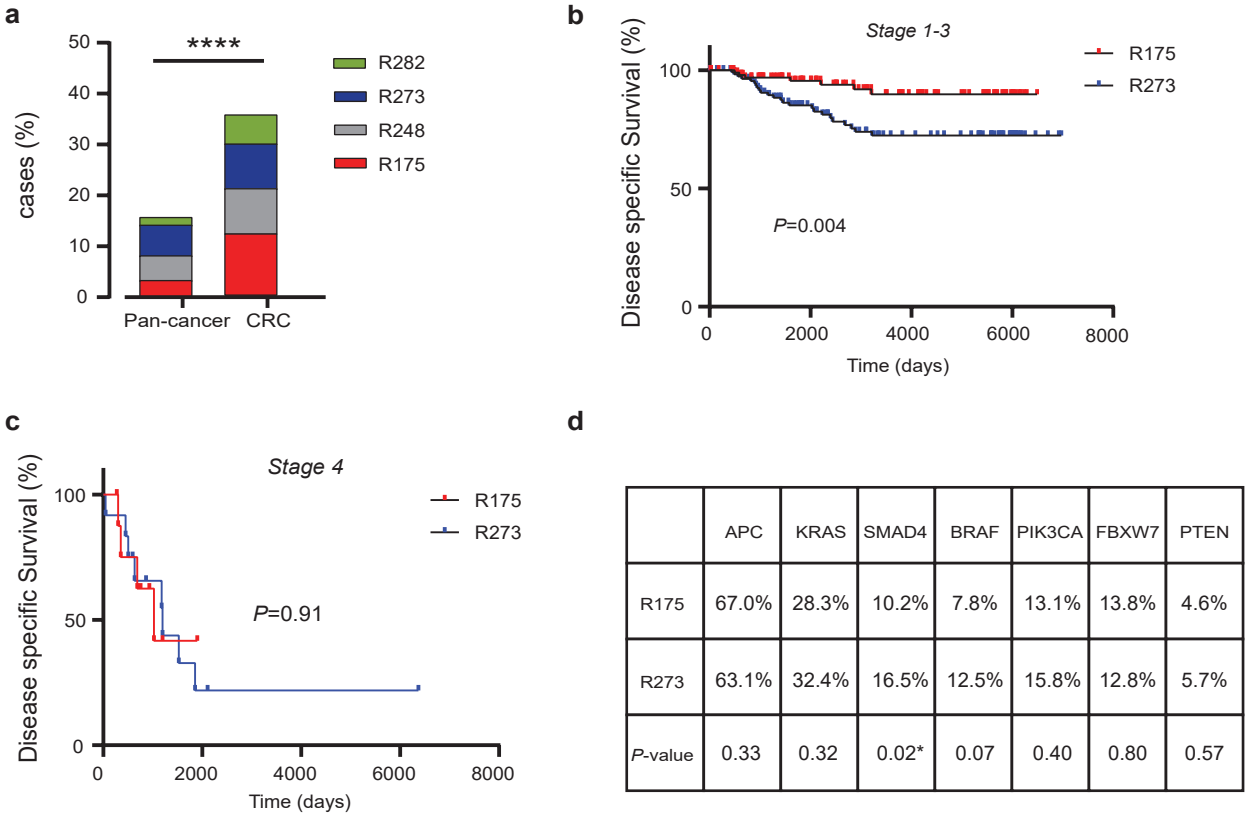
Fig S1.



	APC	KRAS	SMAD4	BRAF	PIK3CA	FBXW7	PTEN
R175	67.0%	28.3%	10.2%	7.8%	13.1%	13.8%	4.6%
R273	63.1%	32.4%	16.5%	12.5%	15.8%	12.8%	5.7%
P-value	0.33	0.32	0.02*	0.07	0.40	0.80	0.57

Fig S1. TP53 R273 mutations are associated with more aggressive colorectal tumors and shorter overall survival relative to R175 mutations

a, Relative abundance of mutations in the top four TP53 hotspot mutational sites in colorectal cancer (CRC, n=323) versus all other cancers (Pan-cancer, n=3396) in TCGA. Shown is the % of cases with mutations in each of the indicated hotspot residues out of all TP53-mutated cases. ****P-value <0.0001 (Fisher's exact test). b-c, Disease specific overall survival of patients with either R175 or R273 p53 mutations presenting in stages 1-3 (b) or stage 4 (c). Data from TCGA COAD-READ and published data (17). Log-rank test. d, Co-occurrence of CRC tumors harboring either R175 (N=282) or R273 (N=296) mutations with different driver mutations. For each gene, the percentage describes the number of CRC tumors harboring mutations in both genes together, out of the total number of tumors with TP53 mutations at the indicated residue (R175 or R273). Statistical significance for co-occurrence was calculated using Fisher's exact test. *P-value <0.05.

Fig S2.

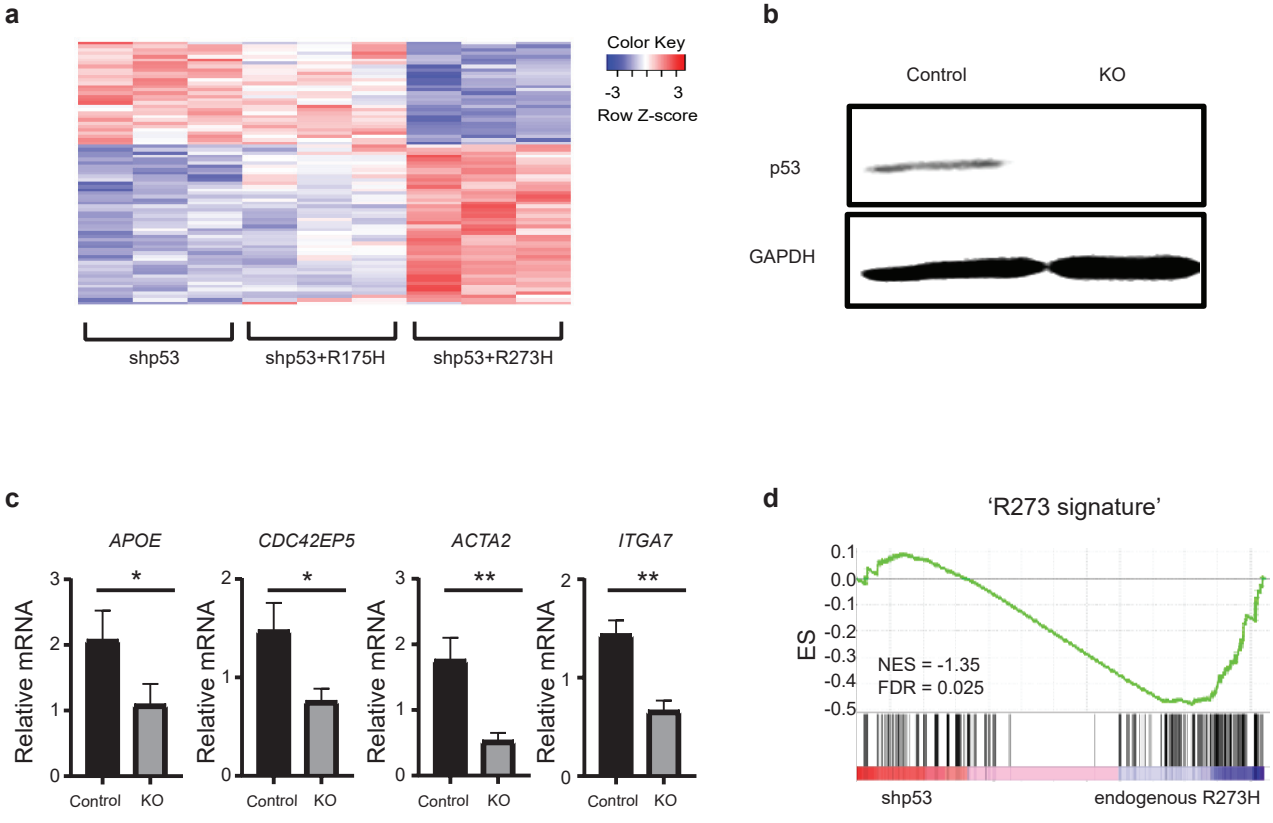


Fig S2. p53R273H orchestrates a distinct transcriptional signature

a, Heatmap of differentially expressed genes (fold change>1.5, Padj<0.05) in SW480 cells expressing p53 shRNA (shp53), without or with stable overexpression of shRNA-resistant p53R175H or p53R273H (n=3 for each condition). b, Western blot analysis of p53 protein in parental SW480 cells (Control) and SW480 cells after CRISPR/Cas9 TP53 knockout (KO). c, RT-qPCR analysis of representative R273 signature genes in parental SW480 cells and SW480 TP53 KO cells. Three biological repeats. *P-value<0.05; **P-value<0.01, unpaired two-tailed t-test. d, GSEA enrichment plot for SW480 RNA-seq data ranked by fold change upon mutant p53 depletion (23), using the R273H signature as the tested gene set. ES = enrichment score.

Fig S3.

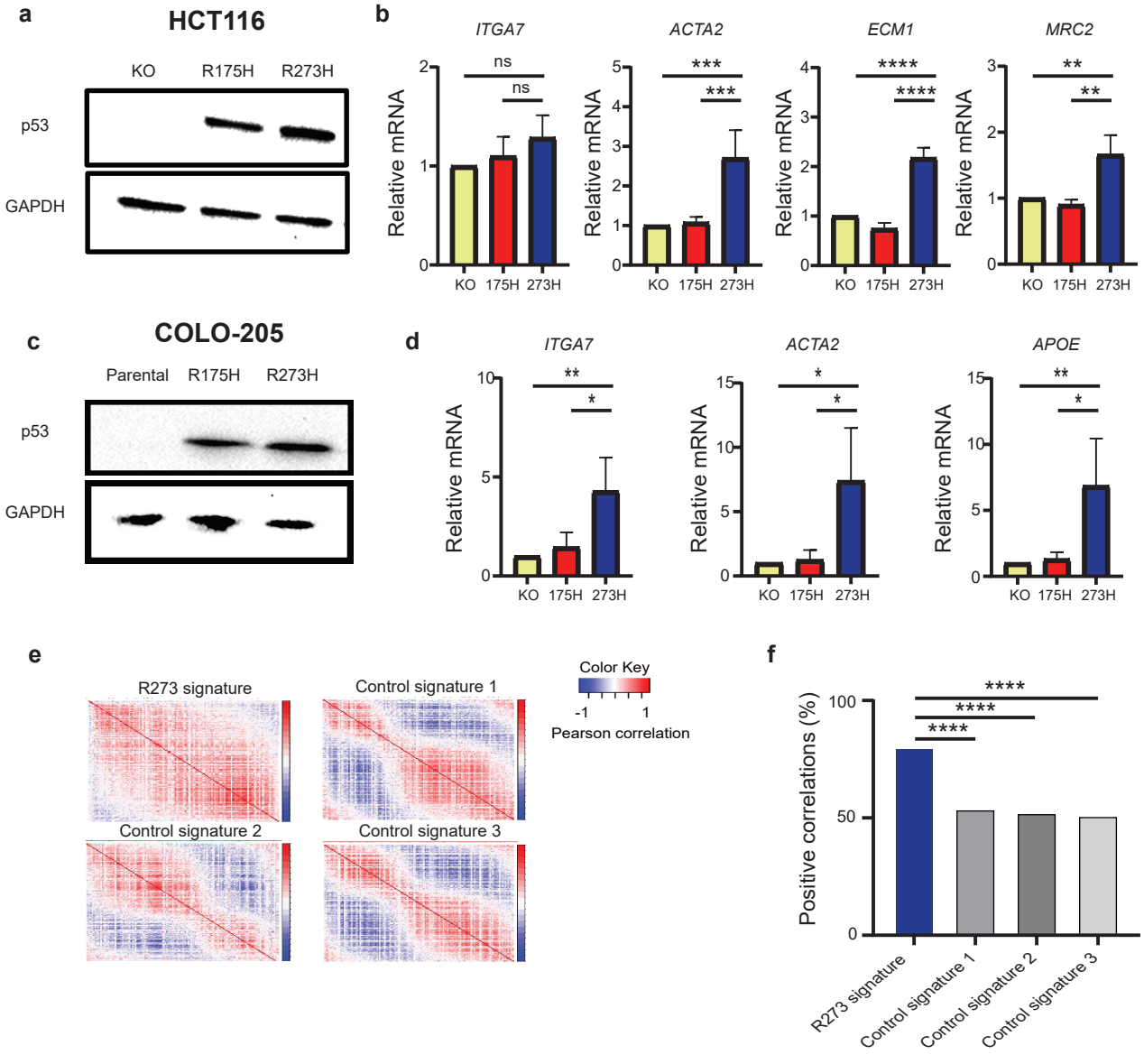


Fig S3. The R273 signature is upregulated in CRC cell lines and tumors

a, Western blot analysis of p53 in HCT116 TP53 KO cells and their derivatives stably transduced with p53R175H or p53R273H. b, RT-qPCR analysis of representative R273 signature genes in the cells in (a). Four independent biological repeats. One-way ANOVA and Tukey's post hoc test of the indicated comparisons. c. Western blot analysis of p53 in parental COLO-205 cells and their derivatives stably transduced with p53R175H or p53R273H. d, RT-qPCR analysis of representative R273 signature genes in the cells in (c), from four independent biological repeats. One-way ANOVA and Tukey's post hoc test. e, Correlogram of the genes in the R273 signature and in three independent control signatures in the TCGA colorectal cancer cohort (n=635), demonstrating extensive co-expression of the R273 signature genes. Correlation was calculated by Pearson coefficient (see expression ladder on right). Each control signature comprised an equal number of genes as the R273 signature, having an adjusted P-value>0.98 (non-differentially expressed genes) in our RNA-seq. f, Percentage of positive correlations out of the total correlations in the R273 signature and in the control signatures. Pearson correlation coefficient was used to calculate correlations between genes, and two sided Fisher's exact test was used to calculate statistical differences between groups (****P-value<0.0001).

Fig S4.

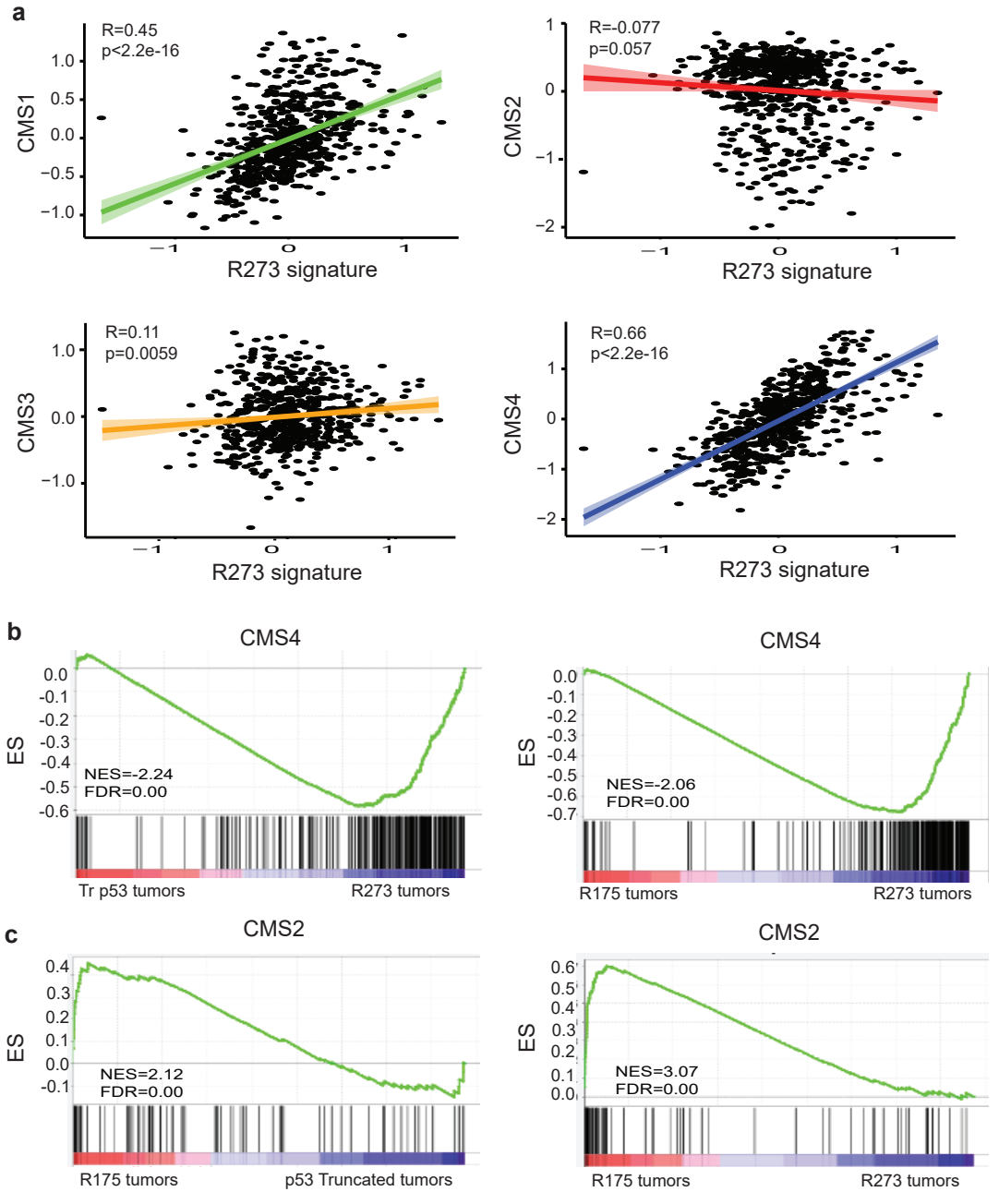


Fig S4. The R273 signature and R273-mutated CRC are preferentially associated with CMS4, while R175-mutated CRC is associated with CMS2

a.Pearson R correlation between the R273 signature and the cell-intrinsic gene signatures of the 4 CMS subtypes (Sveen et al, 2018). b, GSEA of CRC tumors harboring R273 mutations (n=28) compared to tumors harboring R175 (n=36) or truncating (Tr; n=28) mutations. For truncating mutations, we selected the 28 samples with the lowest p53 mRNA levels, to better approximate null mutations. Genes were ranked by fold change, and the CMS4 gene signature was used as the tested gene set. c, GSEA of CRC tumors harboring R175 mutations (n=36) compared to tumors harboring R273 (n=28) or truncating (Tr; n=28) mutations. Truncating mutations were as in (b). The CMS2 gene signature was used as the tested gene set.

Fig S5.

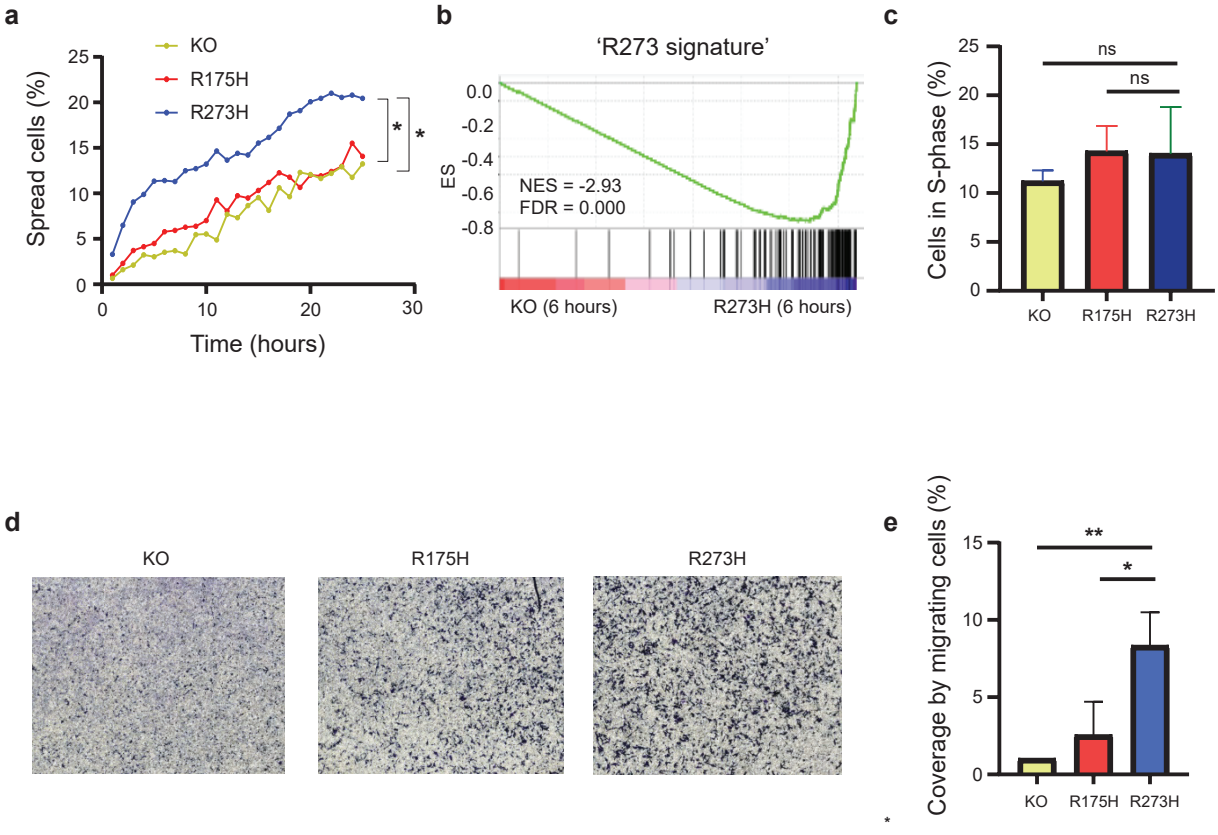


Fig S5. p53R273H promotes cell spreading, migration and invasion

a, Kinetics of spreading of RKO p53 KO cells (KO) and their derivatives stably expressing p53R175H or p53R273H. Analysis was as in Fig 4b. b, GSEA plot of the transcriptomes of SW480 KO cells and their derivatives stably expressing p53R273H, six hours post seeding. Genes was ranked by fold change upon p53R273H overexpression and the R273H signature was used as the tested gene set. c, SW480 TP53 KO cells and their derivatives stably expressing p53R175H or p53R273H were subjected to cell cycle profiling by BrdU and DAPI staining. The bar graph represents the average percentage of cells in S phase from two biological repeats. One-way ANOVA and Tukey's post hoc test. d, Representative images of transwell migration assays performed with RKO TP53 KO cells and their derivatives stably expressing p53R175H or p53R273H. e, Average percentage of area coverage (ImageJ) by migrating cells in transwell migration assays. Three biological repeats. One-way ANOVA and Tukey's post hoc test.

Fig S6.

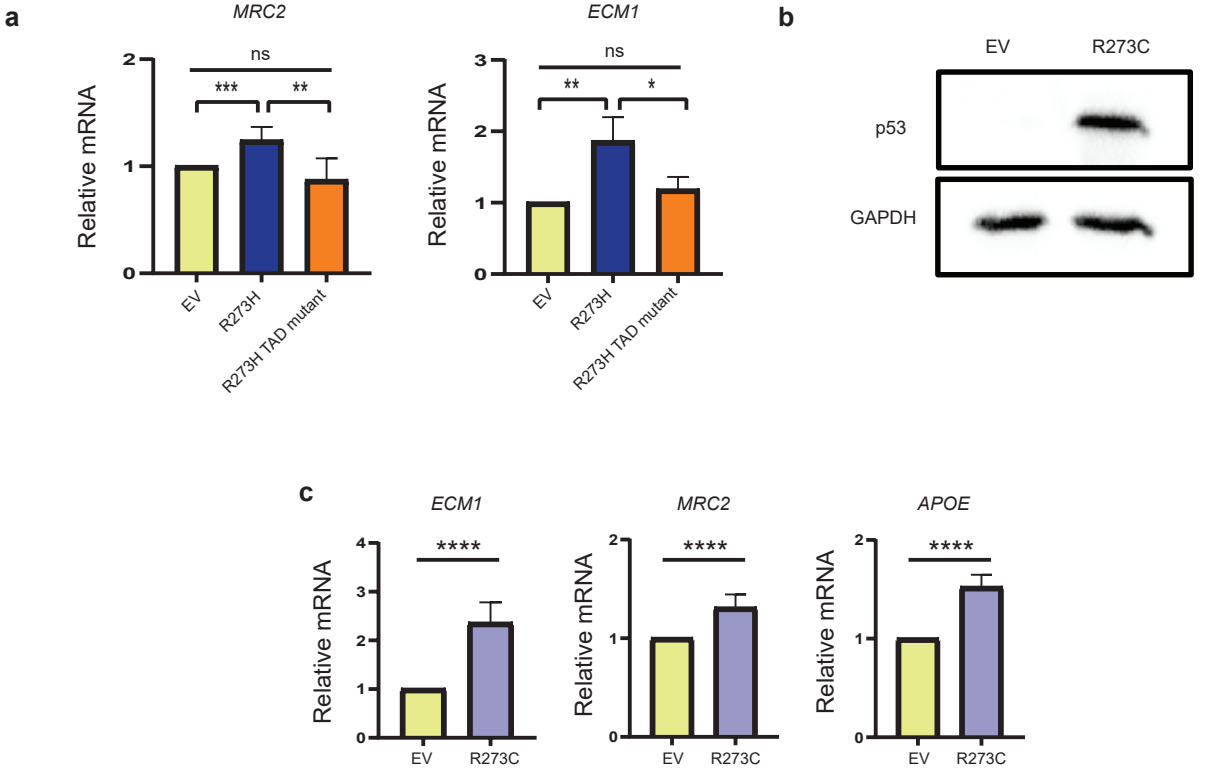


Fig S6. Regulation of R273 signature genes by p53R273H and p53R273C

a, RT-qPCR analysis of the expression of representative R273 signature genes in SW480 TP53 KO cells transiently transfected with empty vector (EV) or with DNA encoding intact p53R273H (R273H) or with p53R273H carrying a double mutation in residues 22,23 within the transactivation domain (R273H TAD mutant). Cells were harvested 48 hours post-transfection. Five biological repeats. One-way ANOVA and Tukey's post hoc test. b, Western blot analysis of p53 in SW480 KO cells, harvested 48 hours after transient transfection with empty vector (EV) or with DNA encoding p53R273C. c, RT-qPCR analysis of representative R273 signature genes cells as in (b). Five biological repeats. One-way ANOVA and Tukey's post hoc test.