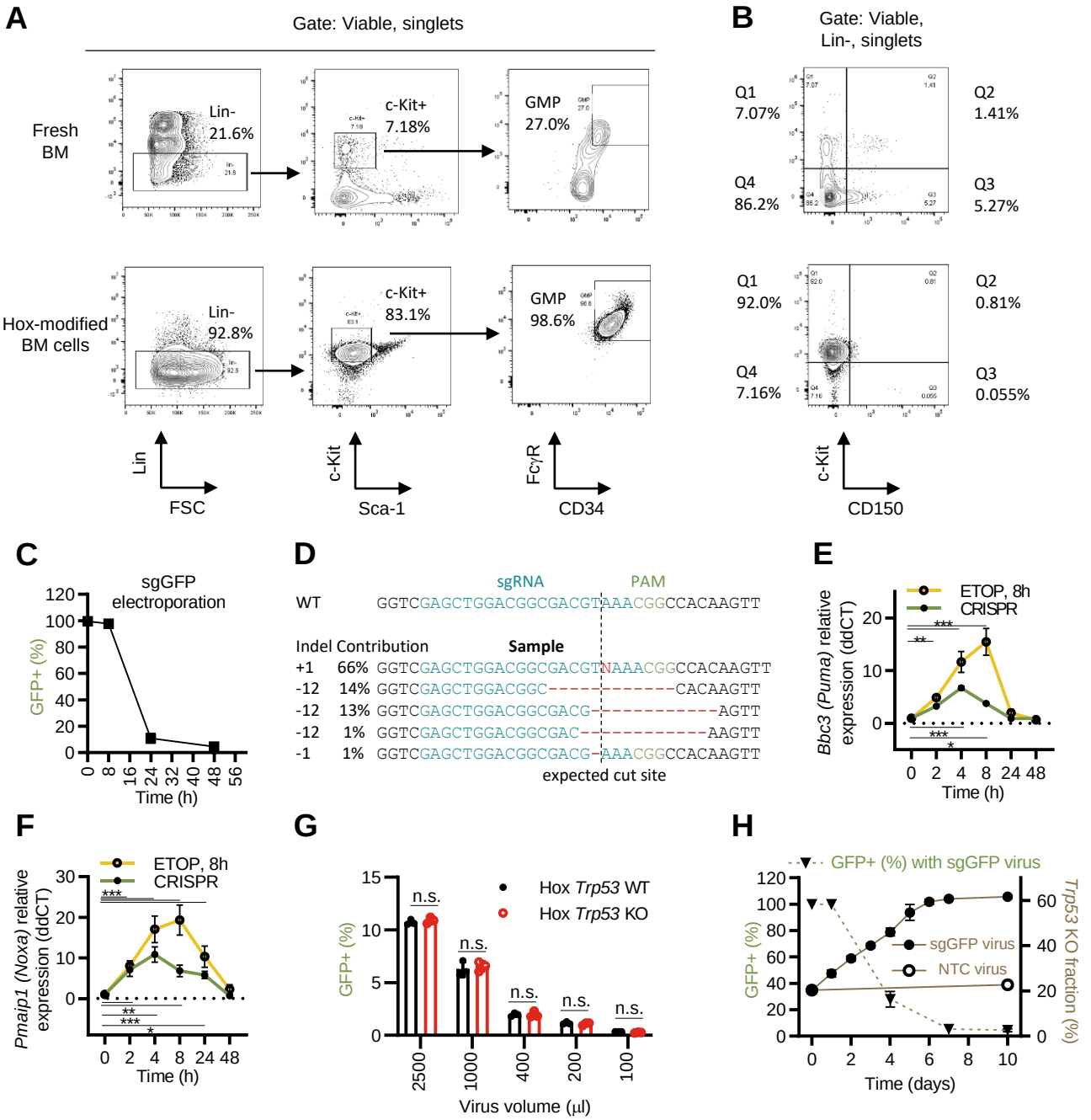
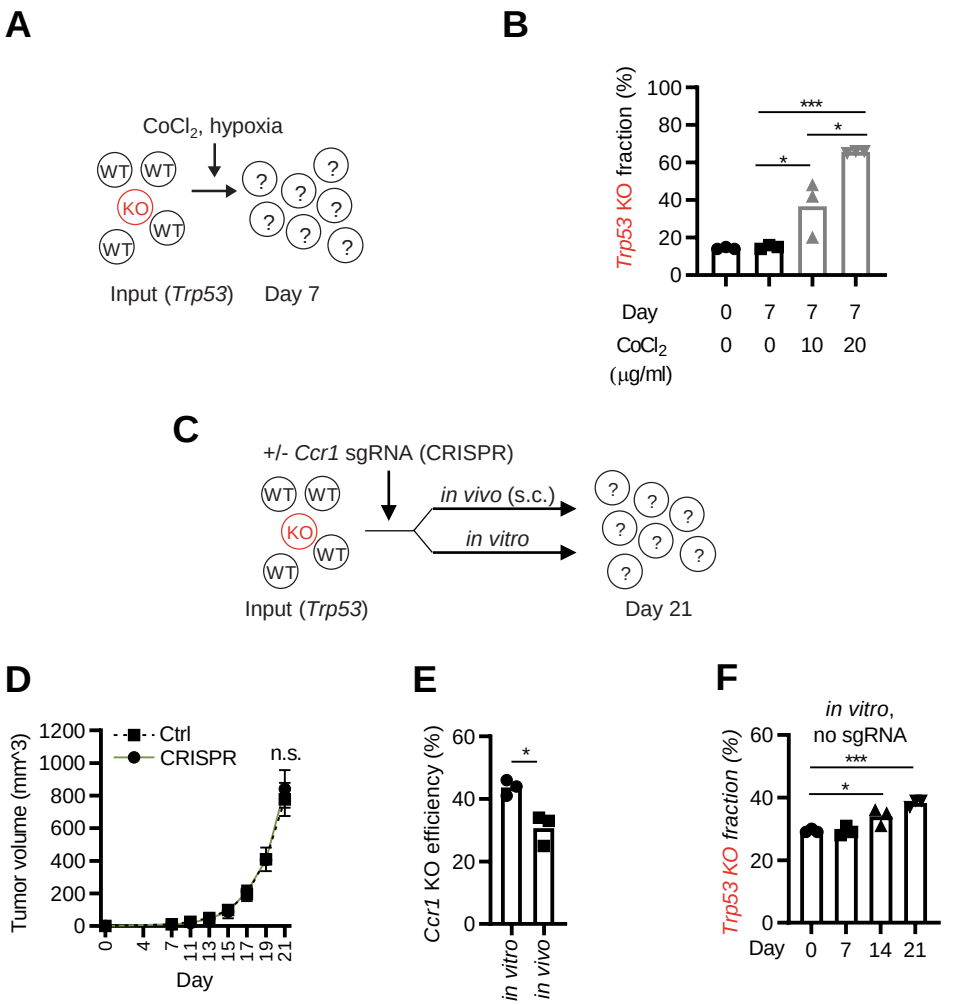


Supplementary Figure 1.
Hox cells, and CRISPR-mediated GFP inactivation. **A-B,** Flow cytometry analysis of fresh bone marrow (BM) and Hox expanded BM cells from C57BL/6 Cas9+ GFP+ mice. Hox cells display a Granulocyte-Monocyte Progenitor (GMP) phenotype (Lin-, c-kit+, Sca-1-, FcγR+, CD34+, CD150-). **C,** Kinetic flow cytometer analysis of GFP signal of Hox cells electroporated with a *GFP* targeting sgRNA (sgGFP). **D,** Sanger sequencing and ICE mutation analysis of the *GFP* targeted region in Hox cells 48 h after electroporation with sgGFP. **E-F**, Kinetic qPCR analysis of *Bbc3* (*Puma)* (E) and *Pmaip1* (*Noxa)* (F) expression of Hox cells exposed to CRISPR (electroporated with sgGFP) or ETOP. **G**, Lentivirus (carrying *eGFP*) titration for transduction of Hox *Trp53* WT or KO cells. GFP+ cells indicate successfully transduced cells (without puromycin selection). **H,** Kinetic flow cytometer analysis of GFP signal of Hox cells transduced with lentiviral particles carrying sgGFP. Triangles indicate the kinetics of *GFP* KO (left y-axis), showing the expected slower kinetics of *GFP* KO compared to sgRNA delivery by electroporation in (C). Circles indicate the kinetics of the enrichment of *Trp53* KO cells (right y-axis). Data presented as mean +/- SEM, n=3 (C, E-F, H), or mean and individual values, n=3 (G). Data is combined from three independently performed experiments (C, E-H). n.s. = non-significant, * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$ by two-way ANOVA and Tukey's post-test (E-G).



Supplementary Figure 2.
Enrichment of *Trp53* KO cells by $CoCl_2$ or by CRISPR *in vivo.* **A,** Model describing hypoxia experimental setup. **B,** WT and *Trp53* KO Hox cells were mixed and cultured for seven days in the presence of different concentrations of Cobalt Chloride ($CoCl_2$) that stabilizes hypoxia-inducible factor-1α, and thereby inducing chemical hypoxia. **C,** Model describing experimental setup where B16 cells (mix WT and *Trp53* KO) were transfected +/- *Ccr1* targeting sgRNA, injected s.c. to C57BL/6 mice or kept in culture for 21 days. **D,** Growth of tumors transfected +/- *Ccr1* sgRNA. **E,** % *Ccr1* KO of cells collected *in vitro*, or *in vivo* after 21 days. **F,** Kinetic analysis of enrichment of *Trp53* KO cells *in vitro*, in the absence of CRISPR. Data shown as mean and individual data, n=3 (B, E-F), or mean and SEM, n=3 (D). Data is combined from three independently performed experiments (B, D-F). n.s. = non-significant, * = $p < 0.05$ and *** = $p < 0.001$ by one-way ANOVA and Tukey´s post-test (B, F), two-way ANOVA and Tukey's post-test (D), or unpaired T-test (E).

A

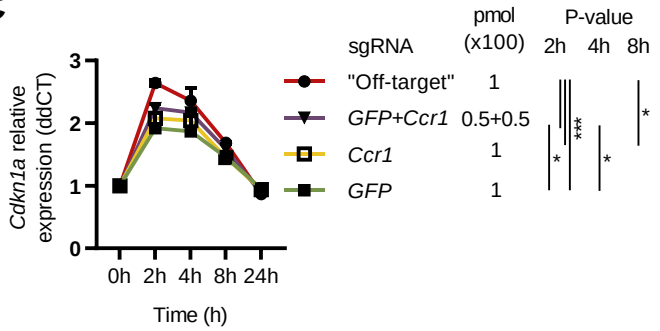
"Off-target" sgRNA (binding *Ccr1*)
AGUUCUAUAUCUCCAUCCAC

Off-target: 0, 5, 194, 3258, 4017

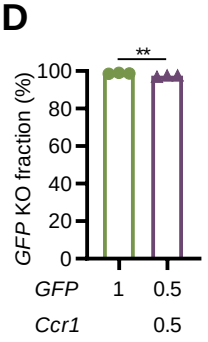
B

	sgRNA	PAM
Chr_9 - *Ccr1*	AGTTCTATATCTCCATCCAC	AGG
One mismatch:		
Chr_10:75866429	AGTTCTA**C**ATCTCCATCCAC	AGG
Chr_2:53999529	AGTTCTATATCTCCATCCA**A**	AGG
Chr_X:85552265	AGTTCT**C**TATCTCCATCCAA	AGG
Chr_14:5523456	AGTTCTA**C**ATCTCCATCCAC	AGG
Chr_3:153192533	AGTTCTACATCT**T**CATCCAC	AGG

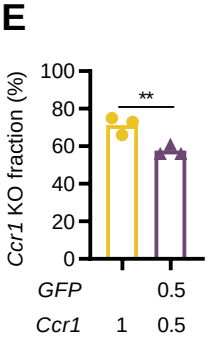
C



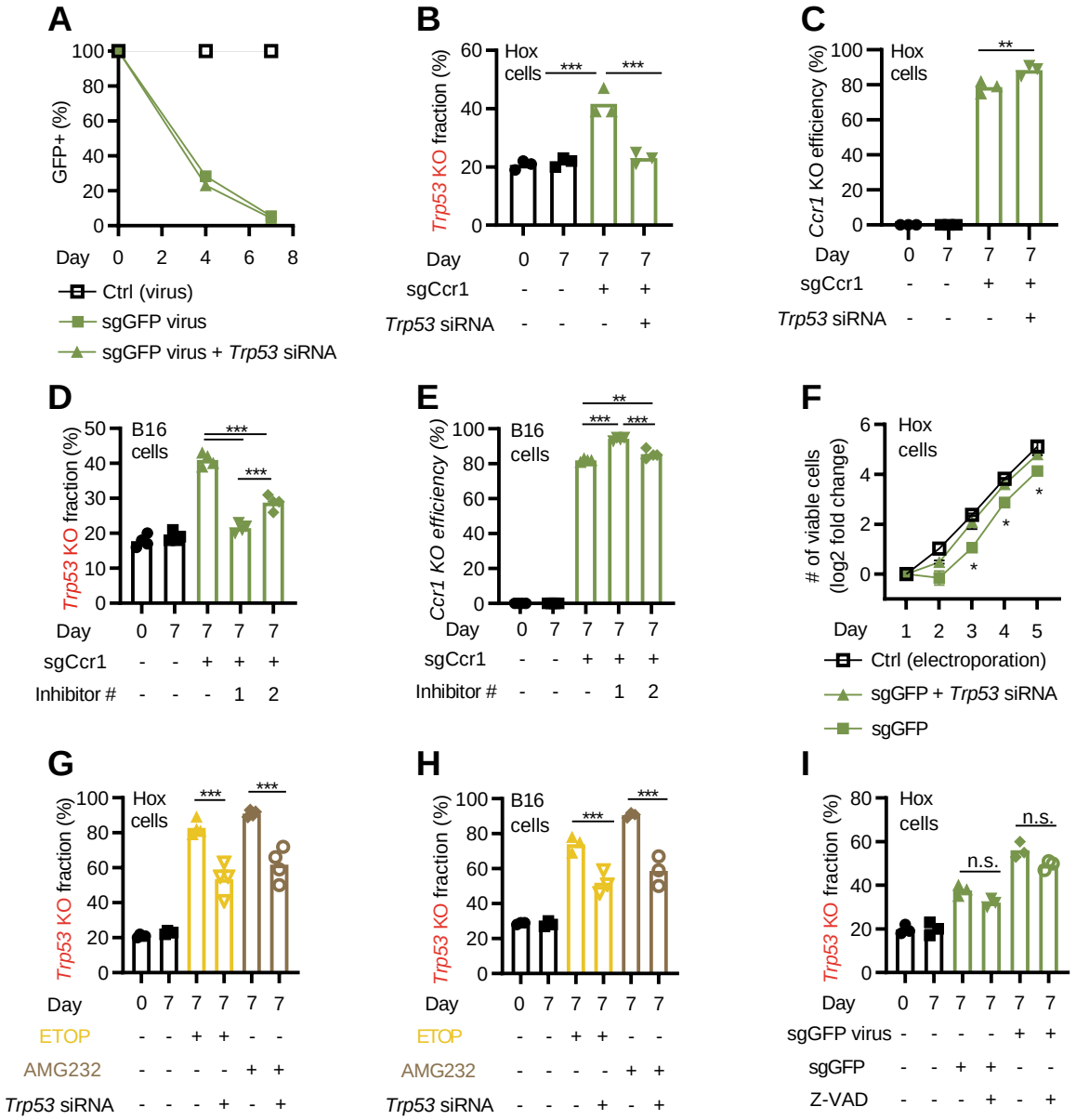
D



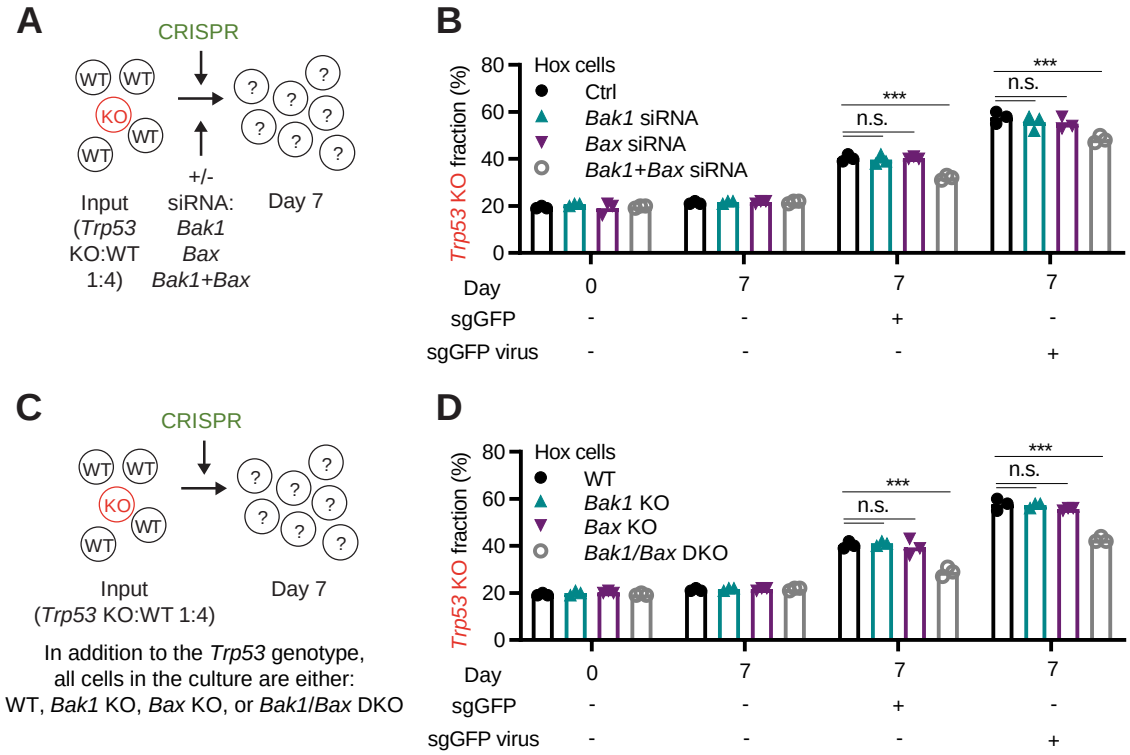
E



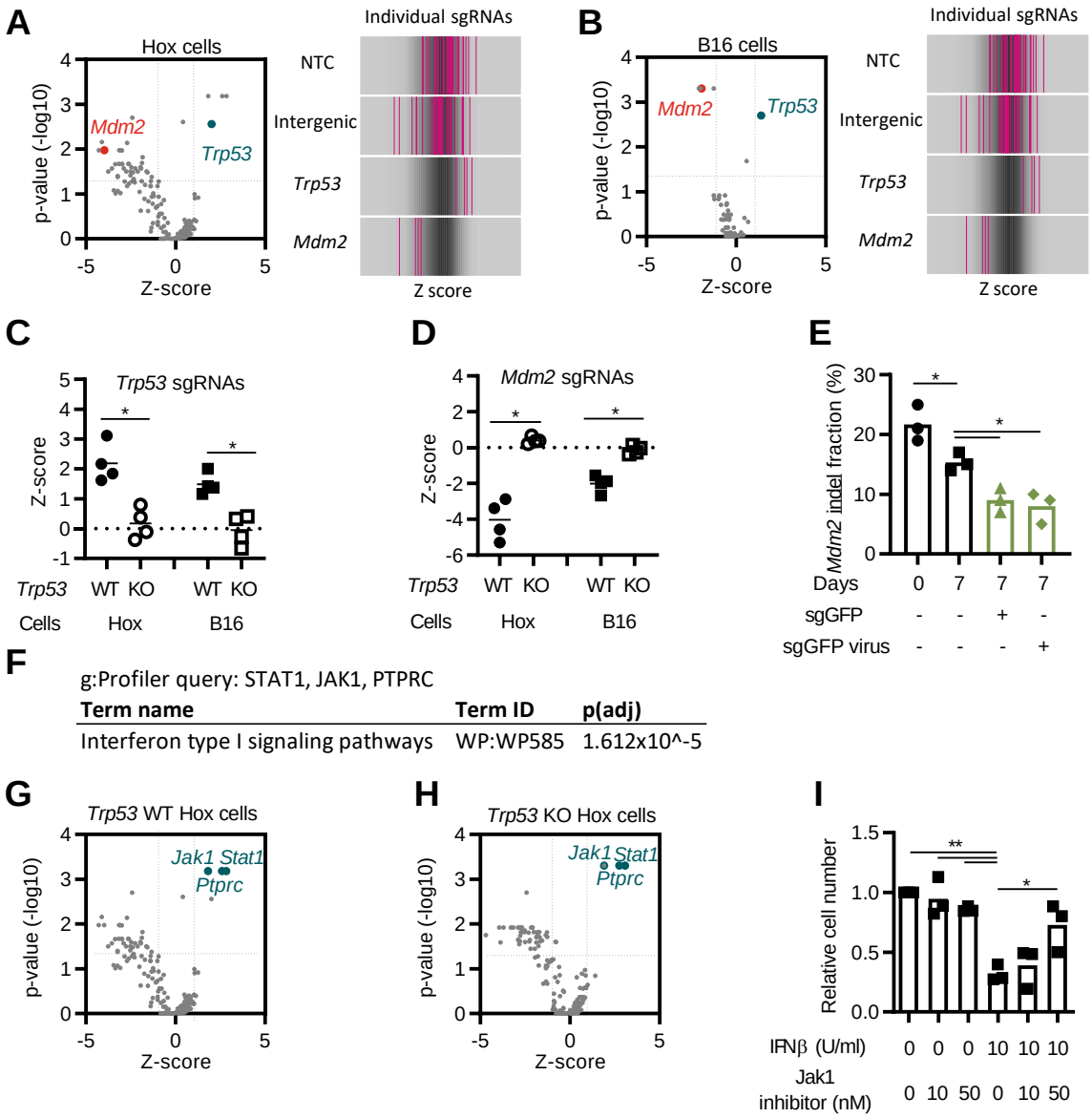
Supplementary Figure 3.
DNA damage response level induced by sgRNAs could be a parameter affecting the enrichment of *Trp53* KO cells. **A,** Information about "Off-target" sgRNA. Off-target indicates number of targets with 0, 1, 2, 3, and 4 mismatches to the mouse genome as identified by Cas-OFFinder. **B,** Examples of mismatches for the "Off-target" sgRNA. **C,** Hox cells (Cas9+ and GFP+) were electroporated by different sgRNAs at the indicated doses (pmol x 100), and cells collected for *Cdkn1a* qPCR (same data as shown in heatmap, Fig. 2B, but with quantification). **D-E,** Hox cells (Cas9+ and GFP+) were electroporated by different sgRNAs at the indicated doses (pmol x 100). Seven days later cells were collected for flow cytometry to determine the % of *GFP* KO cells (D), or for sequencing to determine the % of *Ccr1* KO sequences (E). Data presented as mean +/- SEM, n=3 (C), or mean and individual data, n=3 (D, E). Data is combined from three independently performed experiments (C-E). * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$ by two-way ANOVA and Tukey´s post-test (C), and unpaired T-test (D-E).



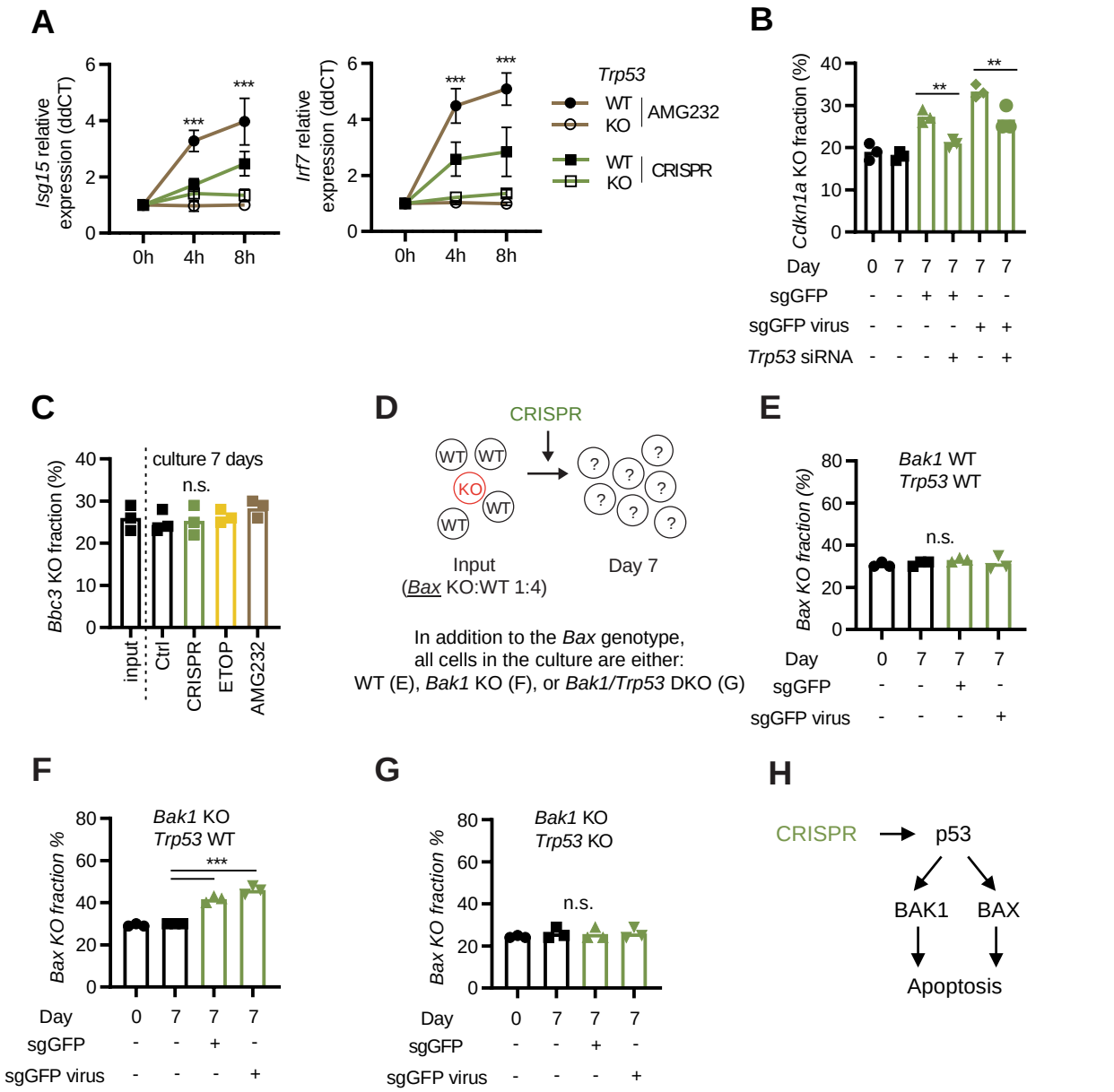
Supplementary Figure 4.
Inhibition of *Trp53* in B16 and Hox cells. **A**, Hox cells (Cas9+ and GFP+) were electroporated +/- *Trp53* siRNA and subsequently transduced with sgGFP virus. The KO efficiency of *GFP* was followed over time by flow cytometry. **B**, WT and *Trp53* KO Hox cells were mixed and electroporated with sgCcr1 +/- *Trp53* siRNA. Cells were cultured for seven days, followed by sequencing of *Trp53* to quantify the frequency of mutations. **C,** The *Ccr1* knockout efficiency was measured by sequencing *Ccr1* in Hox cells treated as in (B). **D,** WT and *Trp53* KO B16 cells were mixed and transfected with sgCcr1 +/- inhibitors (#1 = *Trp53* siRNA, #2 KU55933 inhibiting ATM). Cells were cultured for seven days, followed by sequencing of *Trp53* to quantify the frequency of mutations. **E,** The *Ccr1* knockout efficiency was measured by sequencing *Ccr1* in B16 cells treated as in (D). **F,** Growth characteristics of Cas9+ GFP+ Hox cells electroporated with sgGFP +/- *Trp53* siRNA. **G,** WT and *Trp53* KO Hox cells were mixed and incubated with an 8h pulse of ETOP or AMG232 in the presence or absence of *Trp53* siRNA. **H,** WT and *Trp53* KO B16 cells were mixed and incubated with an 8h pulse of ETOP or AMG232 in the presence or absence of *Trp53* siRNA. **I**, WT and *Trp53* KO Hox cells were mixed and transduced with sgGFP virus or electroporated with sgGFP in the presence of the caspase inhibitor Z-VAD. Cells were then cultured for seven days, followed by sequencing of *Trp53*, and the frequency of *Trp53* mutations quantified. Data is shown as mean +/- SEM, n=3 (A, F), or mean and individual values, n=3 (B-C, H-I) or n=4 (D-E, G). Data is combined from two independently experiments performed in duplicates (D-E, G) or three independently performed experiments (A-C, F, H-I) independent experiments. n.s. =non-significant, * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$ by one-way ANOVA and Tukey´s post-test (B-E, G-I), or two-way ANOVA and Tukey´s post-test (F, indicated significance relates to sgGFP +/- *Trp53* siRNA).



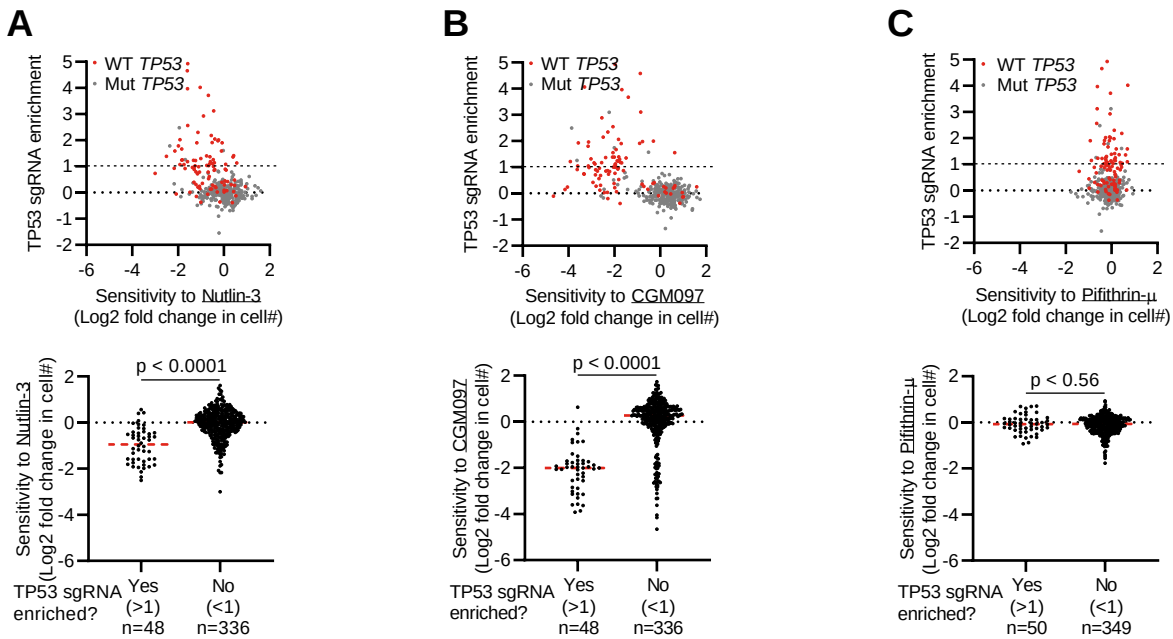
Supplementary Figure 5.
Bak1 and *Bax* are involved in the CRISPR mediated enrichment of Trp53 mutated cells. **A**, Model describing *Bak1/Bax* siRNA experimental setup. **B**, *Trp53* WT and KO Hox cells were mixed and electroporated with sgGFP or transduced with sgGFP virus +/- *Bak1* siRNA, *Bax* siRNA, or *Bak1+Bax* siRNA. Cells were cultured for seven days, followed by sequencing of *Trp53* to quantify the frequency of mutations. **C,** Model describing *Bak1/Bax* KO experimental setup. **D**, *Trp53* WT and KO Hox cells (additionally WT, *Bak1* KO, *Bax* KO or *Bak1*/*Bax* DKO for all cells in respective culture, in addition to the *Trp53* genotype) were mixed and electroporated with sgGFP or transduced with sgGFP virus. Cells were cultured for seven days, followed by sequencing of *Trp53* to quantify the frequency of mutations. Data is shown as mean and individual values, n=3 (B, D). Data is combined from three independently performed experiments (B, D). n.s. = non-significant and *** = $p < 0.001$ by two-way ANOVA and Tukey´s post-test (B, D).



Supplementary Figure 6.
p53 and MDM2 play a central role in the CRISPR-mediated DNA damage response, and JAK1/STAT1 signaling negatively affects Hox cell survival independently of p53 status. **A-B,** Exploratory CRISPR screen targeting 395 cell death-related genes and controls in Hox cells (A), and B16 cells (B). The sgRNA representation was analyzed by next-generation sequencing, and enrichment/depletion deconvoluted by MAGeCK. Genes (left) and individual sgRNAs of non-targeting controls (NTC), intergenic controls, *Trp53*, and *Mdm2* (right) enriched and depleted after seven days by the CRISPR-induced DNA damage. **C-D,** Enrichment and depletion of individual sgRNAs for *Trp53* (C), and *Mdm2* (D) in the CRISPR screen, comparing WT and *Trp53* KO Hox and B16 cells. **E,** Hox cells WT or with mutations (any indels, including insertions and deletion of 3 nucleotides) in *Mdm2* were mixed and exposed to CRISPR (sgRNA electroporation or sgRNA virus). Cells were cultured for seven days, followed by sequencing of *Mdm2* to quantify the frequency of indels. **F,** Top WikiPathways (WP) term identified by g:Profiler querying STAT1, JAK1, and PTPRC. **G-H,** Enrichment of *Jak1*, *Stat1*, and *Ptprc* sgRNAs in Hox WT (G), and Hox *Trp53* KO cells (H). (G) is the same data as (A), but indicating *Jak1*, *Stat1*, *Ptprc* sgRNAs. **I,** Hox cells cultured for five days with type I interferon (IFNβ) and Jak1 inhibitor. Figure shows relative cell number compared to the control (no IFNβ or Jak1 inhibitor). Data is presented as volcano plots with adjusted p-values and Z-score (log2 fold enrichment/depletion of sgRNAs) (A-B, G-H), or mean and individual values , n=4 (C-D), or n=3 (E, I). Data is combined from three independently performed experiments (E, I). * = $p < 0.05$ and ** = $p < 0.01$ by Mann-Whitney test (C, D), or one-way ANOVA and Tukey's post-test (E, I).



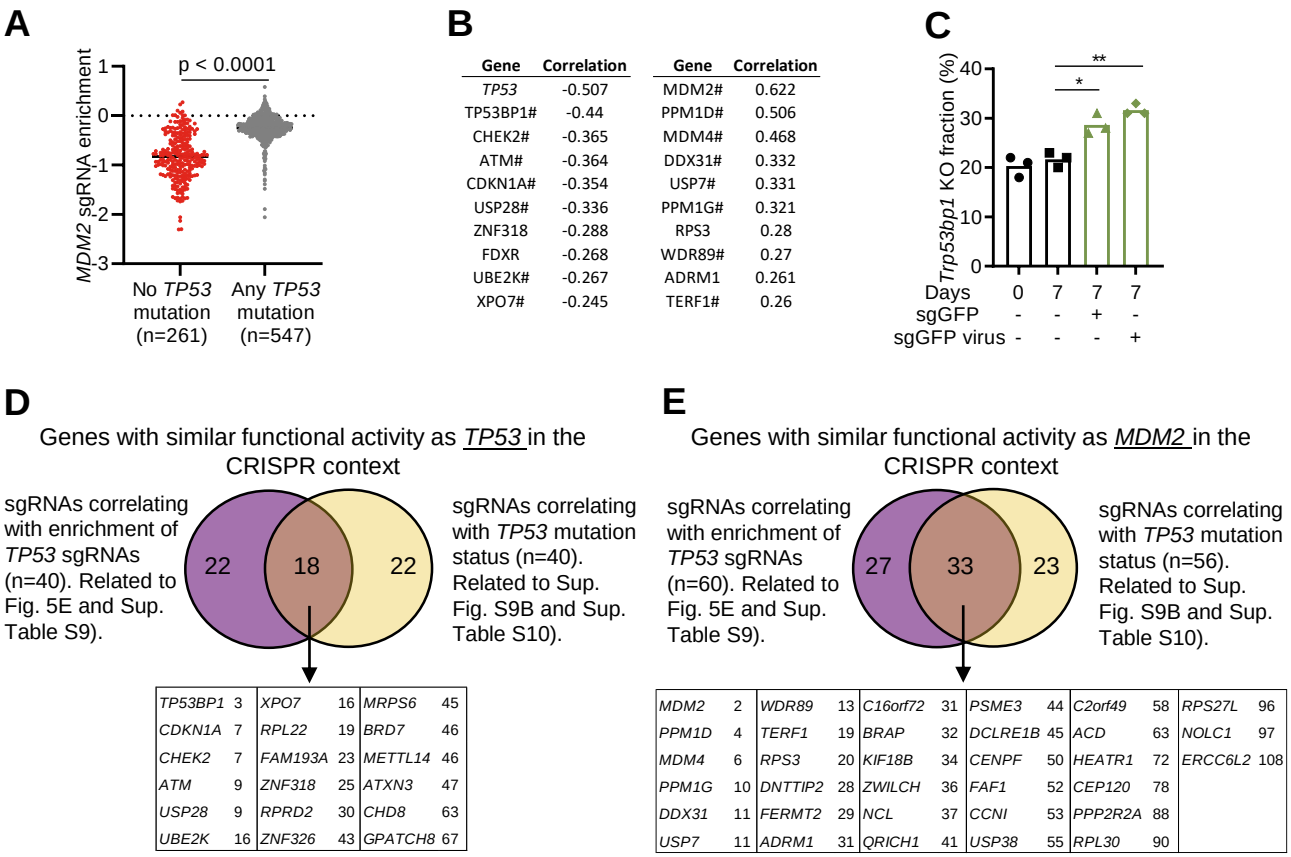
Supplementary Figure 7.
p53 activation causes upregulation of interferon-stimulated genes, enrichment of mutations in *Cdkn1a* but not *Bbc3* by CRISPR, and CRISPR enriches *Bax* mutated cells in *Bak1* KO cells **A,** WT and *Trp53* KO Hox cells were electroporated with sgGFP (CRISPR) or cultured in AMG232. Cells were collected at 0, 4 and 8h and expression of the interferon-stimulated genes *Isg15* (left) and *Irf7* (right) quantified by qPCR. **B,** WT and *Cdkn1a* KO Hox cells were mixed and electroporated with sgGFP +/- *Trp53* siRNA. Cells were cultured for seven days, followed by sequencing of *Cdkn1a* to quantify the frequency of mutations. **C,** WT and *Bbc3* KO Hox cells were mixed and electroporated with sgGFP or exposed to an 8h pulse with Etoposide or AMG232. Cells were subsequently cultured for seven days, followed by sequencing of *Bbc3* to quantify the frequency of mutations. **D**, Model describing experimental setup. **E-G**, *Bax* WT and KO Hox cells (additionally WT (E), *Bak1* KO (F), or *Bak1*/*Trp53* DKO (G) for all cells in respective culture) were mixed and electroporated with sgGFP or transduced with sgGFP virus. Cells were cultured for seven days, followed by sequencing of *Bax* to quantify the frequency of mutations. **H**, Model indicating *Bak1* and *Bax* playing redundant role in the CRISPR induced DNA damage response. Data is presented as mean and SEM, n=3 (A), or mean and individual values, n=3 (B-C, E-G). Data is combined from three independently performed experiments (A-C, E-G). n.s. = non-significant, ** = $p < 0.01$, and *** = $p < 0.001$ by two-way ANOVA and Tukey's post-test (A), or by one-way ANOVA and Tukey's post-test (B-C, E-G). Statistic in (A) indicates comparison between WT and *Trp53* KO cells treated with AMG232.



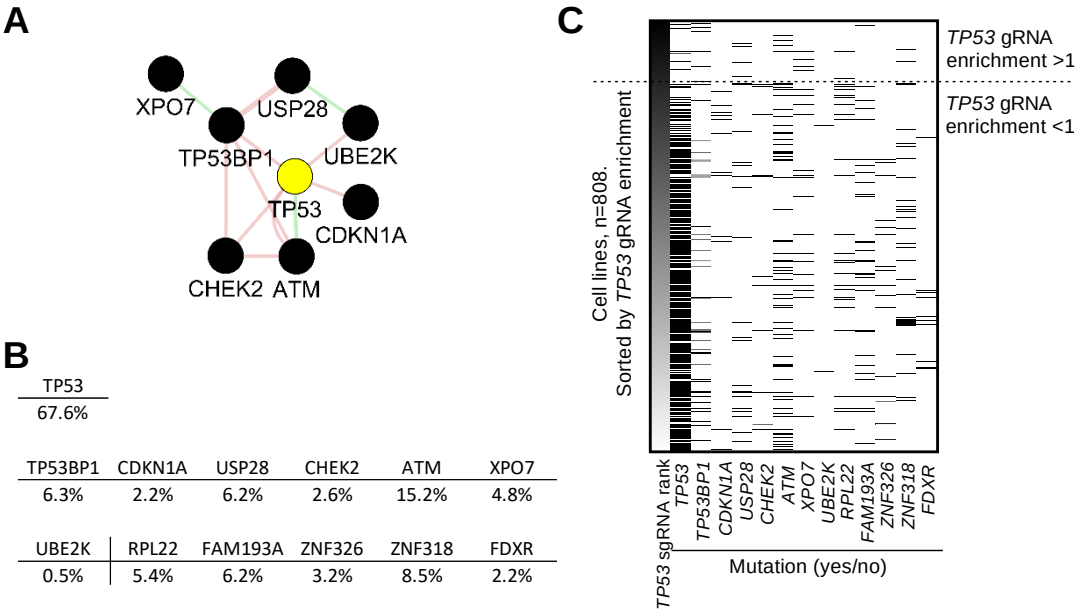
D

Term	Overlap	Adjusted P-value	Combined Score	Genes
TP53 CHEA	5/319	8.54E-04	235.3	CDKN1A;MDM2;XPO7;MDM4;PPM1D
TAF1 ENCODE	10/3346	0.02	36.9	USP7;USP38;RPL22;DDX31;MDM2;ZNF326;MDM4;PPM1D;WDR89;PPM1G
CREB1 CHEA	6/1444	0.05	33.7	CDKN1A;USP38;DDX31;ATM;TP53BP1;PPM1D
FOXA2 ENCODE	3/316	0.06	62.2	MDM2;XPO7;UBE2K
E2F4 ENCODE	4/710	0.06	36.4	CHEK2;ATM;PPM1D;TERF1
E2F6 ENCODE	8/3245	0.11	16.0	DDX31;MDM2;ZNF326;USP28;PPM1D;WDR89;TERF1;PPM1G
YY1 ENCODE	7/2753	0.13	14.4	USP7;RPL22;XPO7;ZNF326;MDM4;TERF1;UBE2K
FOXA1 ENCODE	2/205	0.15	43.7	MDM2;XPO7

Supplementary Figure 8.
The sensitivity of cell lines to Nutlin-3, CMG097, and Pifithrin-m, and transcription factor binding. **A-C,** *TP53* sgRNA enrichment score from the Depmap, and sensitivity to Nutlin-3 (A), CGM097 (B), and Pifithrin-μ (C). Sensitivity is defined as the number of cells after a five-day culture, compared to control-treated cells (log2 fold change). Upper graphs show both parameters and *TP53* mutation status (WT or mutated, Mut) indicated by color, lower graphs show sensitivity in cells stratified based on *TP53* sgRNA enrichment. Data includes all available data in the Depmap CRISPR (Avana) 20Q4, Mutation Public 20Q4, and Drug sensitivity (PRISM Repurposing Primary Screen) 19Q4 releases. Each dot represents one cell line. Statistics based on unpaired T-test (A-C). **D,** Transcription factor binding to the twenty genes presented in Fig. 5E analyzed by the Enrichr software: ENCODE and ChEA Consensus TFs from ChIP-X. Statistical analysis calculated by the Enrichr software of TFs linked to the gene set.



Supplementary Figure 9.
sgRNA enrichment/depletion that correlates with *TP53* mutation status. **A,** *MDM2* sgRNA enrichment score stratified based on *TP53* mutation status. **B,** Top ten negative and positive correlating genes comparing sgRNA enrichment in cells that are WT for *TP53* or that has any mutation in *TP53*. # indicates genes that overlap with the sgRNA correlation list in Figure 5E. **C,** WT and *Trp53bp1* KO Hox cells were mixed and electroporated with sgGFP or transduced with sgGFP virus. Cells were cultured for seven days, followed by sequencing of *Trp53bp1* to quantify the frequency of mutations. **D-E,** Venn diagrams showing overlap of genes identified to correlate with the two different approaches, divided into genes that functionally behave similarly as *TP53* (D), and *MDM2* (E), in the CRISPR context. Overlapping genes are sorted based on the combined ranking of the two different approaches, where lower numbers indicates stronger enrichment. TP53 is not included in (D), as TP53 is not found in Figure 5E due to the nature of the design of the analysis. Data includes all available data in the Depmap CRISPR Avana 20Q4, and Mutation Public 20Q4 releases. Each dot represents one cell line. Data is shown as mean and individual values, n=3 (C). Data is combined from three independently performed experiments (C). Statistics based on unpaired T-test (A), correlation calculated by Depmap (B), or one-way ANOVA and Tukey´s post-test (C), * = $p < 0.05$, ** = $p < 0.01$.



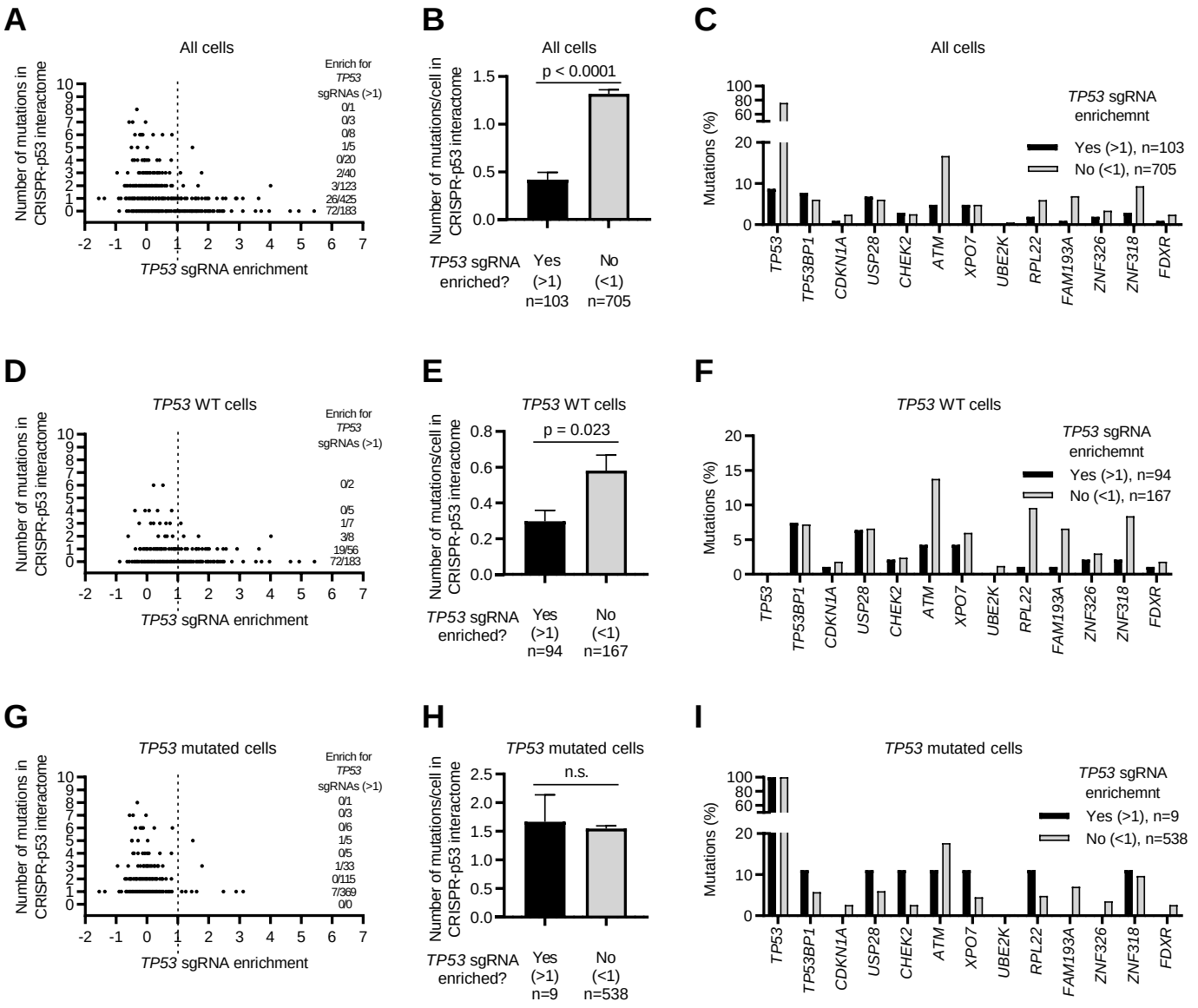
B

TP53
67.6%

TP53BP1	CDKN1A	USP28	CHEK2	ATM	XPO7
6.3%	2.2%	6.2%	2.6%	15.2%	4.8%

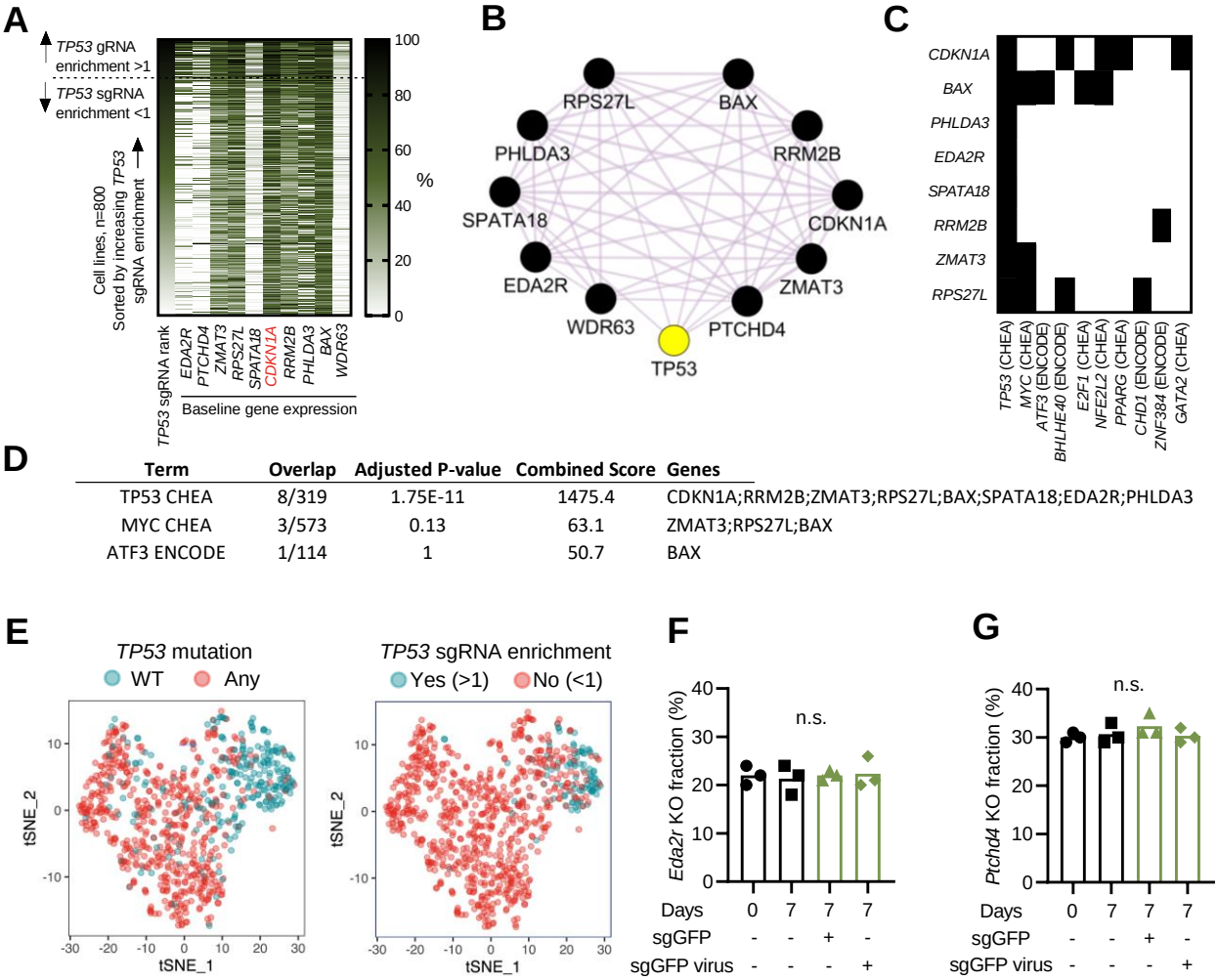
UBE2K	RPL22	FAM193A	ZNF326	ZNF318	FDXR
0.5%	5.4%	6.2%	3.2%	8.5%	2.2%

Supplementary Figure 10.
Mutation frequency in the core CRISPR-p53 tumor suppressor interactome. **A,** A core CRISPR-p53 tumor suppressor interactome based on experimental data and analysis of Depmap correlations related to *TP53* sgRNA enrichment and *TP53* mutation. Includes tumor suppressor genes overlapping in Fig. 5E and Supplementary Fig. S9B. Interactions identified by geneMANIA: Red = physical interaction, green = genetic interaction. **B,** Mutation frequency (% any mutation) in all cell lines where both CRISPR screen data and mutation data were available, n=808. Includes all genes identified in Fig. 5E and/or Supplementary Fig. S9B (the last five genes were only found in one of the top 10 lists generating a list of 13 genes – extended CRISPR-p53 tumor suppressor interactome). **C,** Heat map showing mutations (white = WT, black = any mutation) of the extended CRISPR-p53 tumor suppressor interactome (all genes identified in Fig. 5E and/or Supplementary Fig. S9B). Data is based on all available data in the Depmap CRISPR (Avana) 20Q4, and Mutation Public 20Q4 releases.



Supplementary Figure 11.

Analysis of the enrichment of *TP53* sgRNAs and mutation frequency in the extended CRISPR-p53 tumor suppressor interactome (13 genes defined in Sup. Fig. S10B-C). **A-C,** Analysis of all cell lines included in the Depmap data set (20Q4 release) showing; the number of mutations in the 13 genes in each cell line and *TP53* sgRNA enrichment (A); the total number of mutations/cell in the 13 genes stratified based on *TP53* sgRNA enrichment (B); and mutation frequency of each individual gene as frequency of cells that enrich (>1) or not (<1) for *TP53* sgRNAs (C). **D-F**, similar to A-C but only analyzing cells WT for *TP53*. **G-I**, similar to A-C but only analyzing cells with *TP53* mutations. Each dot indicates on cell line (A, D, G). The dotted lines in A, D and G are added to indicate cut-off (>1) used to define *TP53* sgRNA enrichment. Data is based on all available data in the Depmap CRISPR (Avana) 20Q4, and Mutation Public 20Q4 releases. Statistics based on unpaired T-test (B, E, H).



D

Term	Overlap	Adjusted P-value	Combined Score	Genes
TP53 CHEA	8/319	1.75E-11	1475.4	CDKN1A;RRM2B;ZMAT3;RPS27L;BAX;SPATA18;EDA2R;PHLDA3
MYC CHEA	3/573	0.13	63.1	ZMAT3;RPS27L;BAX
ATF3 ENCODE	1/114	1	50.7	BAX

Supplementary Figure 12.
p53 target gene expression, but not *TP53* expression, predicts *TP53* sgRNA enrichment. **A,** Baseline expression of the top 10 genes (identified in Fig. 6E) in 800 cell lines sorted by *TP53* sgRNA enrichment rank. For visualization purposes, the expression is normalized to 0-100%, where the highest expression of each gene is defined as 100% and the lowest as 0%. **B,** Interactions of the top 10 genes identified by geneMANIA: purple = co-expression. **C-D,** Transcription factor binding to the top 10 genes analyzed by the Enrichr software: ENCODE and ChEA Consensus TFs from ChIP-X. (C) Target genes (y-axis) for different transcription factors (TF, x-axis) indicated with black color. (D) Statistical analysis of TFs linked to the gene set as identified by the Enrichr software. **E,** tSNE dimensionality reduction analysis of cells based on the expression of the top 10 genes (same data as Fig. 6F but divided into two graphs for clarity). The colors indicate *TP53* mutation state (left) and TP53 sgRNA enrichment (right). **F-G,** WT and *Eda2r* (F), or *Ptchd4* (G) KO Hox cells (Cas9+ and GFP+) were mixed and electroporated with sgGFP or transduced with sgGFP virus. Cells were cultured for seven days, followed by sequencing of *Eda2r* (F), or *Ptchd4* (G) to quantify the frequency of mutations. Data presented as individual cell lines (each dot/line represents one human cell line), n=800 (A, E), or as mean and individual data, n=3 (F-G). Data includes all available overlapping data in the Depmap CRISPR (Avana), and Expression Public 20Q4 releases. Data is representative of three independent experiments (F-G). n.s. = non-significant by one-way ANOVA and Tukey's post-test (F-G).