SUPPLEMENTAL INFORMATION

Mutant p53 Induces a Hypoxia Transcriptional Program in Gastric and Esophageal Adenocarcinoma

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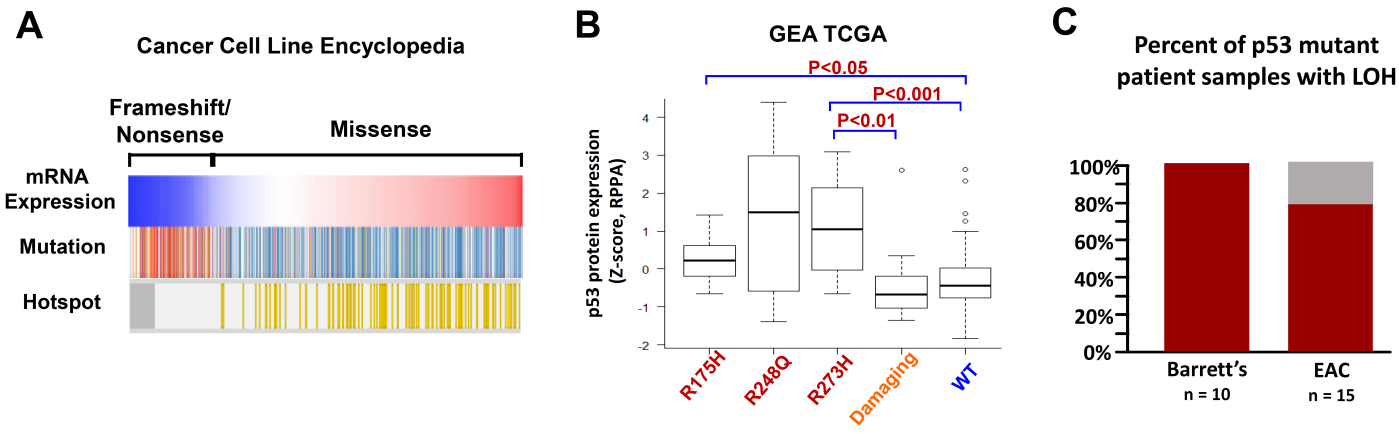
INVENTORY

Supplemental Information contains the Supplemental Data (including 10 figures),

SUPPLEMENTAL FIGURES

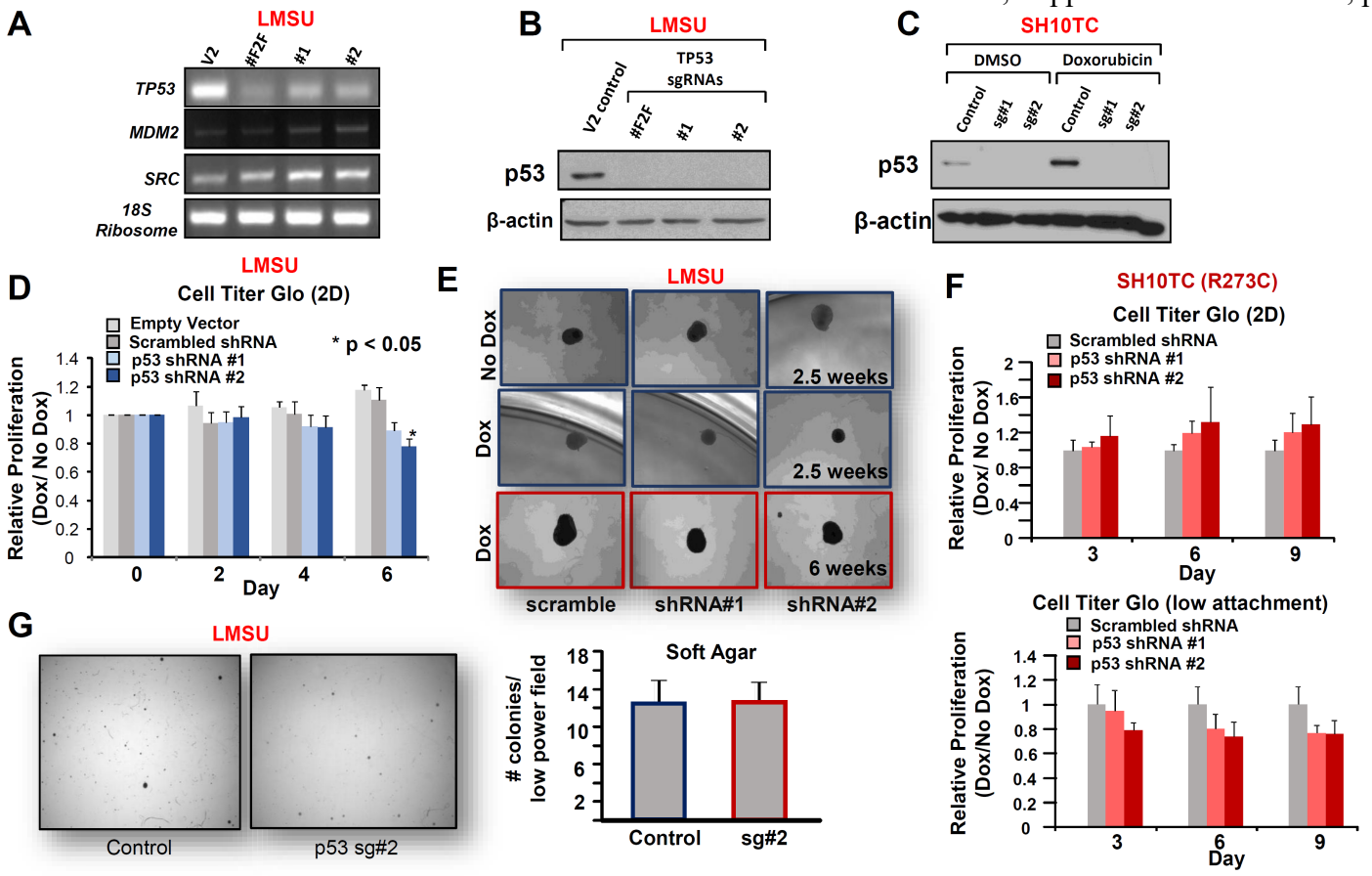


Supplemental Figure 1. p53 is frequently mutated and overexpressed in human gastroesophageal cancer

(A) mRNA expression of *TP53* across all cell lines found in CCLE annotated by type of p53 mutation (nonsense and frameshift mutations are red and orange, respectively, and missense mutations are blue); hotspot mutation (R175C/H, R248Q/W, R273C/H, bottom panel)

(B) Box plot showing protein expression levels of p53 in TCGA gastric cancer patients annotated by p53 mutation status. Damaging mutation include Nonsense or frameshift in earlier codons (<175). P-values are of pairwise comparison using Wilcoxon rank-sum test with Bonferroni correction.

(C) Percent of patients with p53 mutant Barrett's Esophagus or Esophageal Adenocarcinoma that exhibit loss of heterozygosity (LOH)



Supplemental Figure 2. Mutant p53 is expendable for primary tumor properties of gastroesophageal cancer cells

(A) RT-PCR showing cDNA expression levels of *TP53*, *MDM2*, *SRC*, and *18S Ribosome* (loading control) in LMSU gastric adenocarcinoma cell line, which harbors an endogenous R175H p53 mutation, stably expressing a Cas9 control vector (V2) or three distinct targeting sgRNAs in addition to Cas9.

(B) Immunoblot showing protein expression levels of p53 in LMSU gastric cancer cells expressing a Cas9 control vector (V2) or three distinct targeting sgRNAs in addition to Cas9.

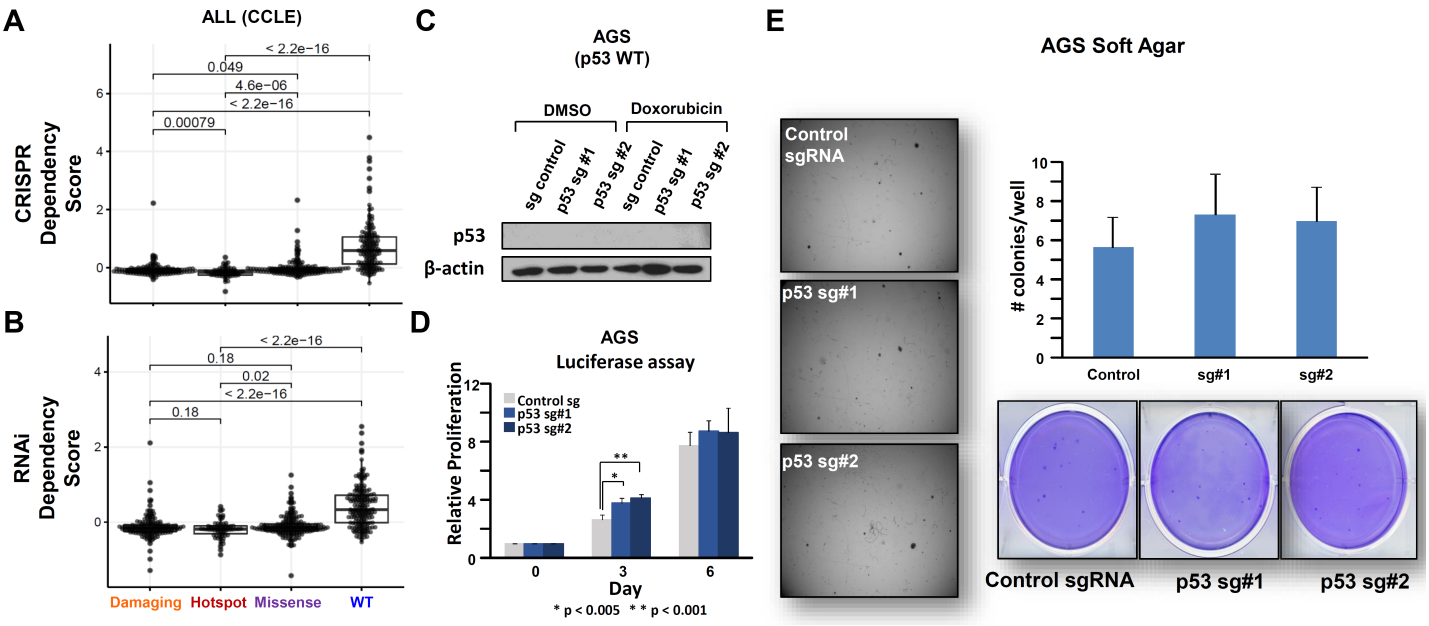
(C) Immunoblot showing protein expression levels of p53 in SH10TC esophageal adenocarcinoma cells (R273C) expressing a Cas9 control vector (V2) or two distinct targeting sgRNAs in addition to Cas9 in the presence or absence of doxorubicin.

(D) Proliferation of LMSU gastric adenocarcinoma cells (R175H) expressing an inducible vector control, scrambled control, or two targeting shRNAs shown as a ratio of with doxycycline/without doxycycline using CellTiterGlo.

(E) Low-attachment colony formation assay of LMSU gastric adenocarcinoma cells (R175H) expressing a scrambled control, or two targeting shRNAs at 2.5 weeks and 6 weeks.

(F) Proliferation of SH10TC esophageal adenocarcinoma cells (R273C) expressing a constitutive vector control or two *TP53* targeting shRNAs shown as a ratio of with doxycycline/without doxycycline using CellTiterGlo on adherent (top panel) and low attachment culture (bottom panel).

(G) Soft agar colony formation assay of LSMU gastric adenocarcinoma cells (R175H) expressing a control or p53 targeting sgRNA with representative images (left panel) and quantification (right panel).



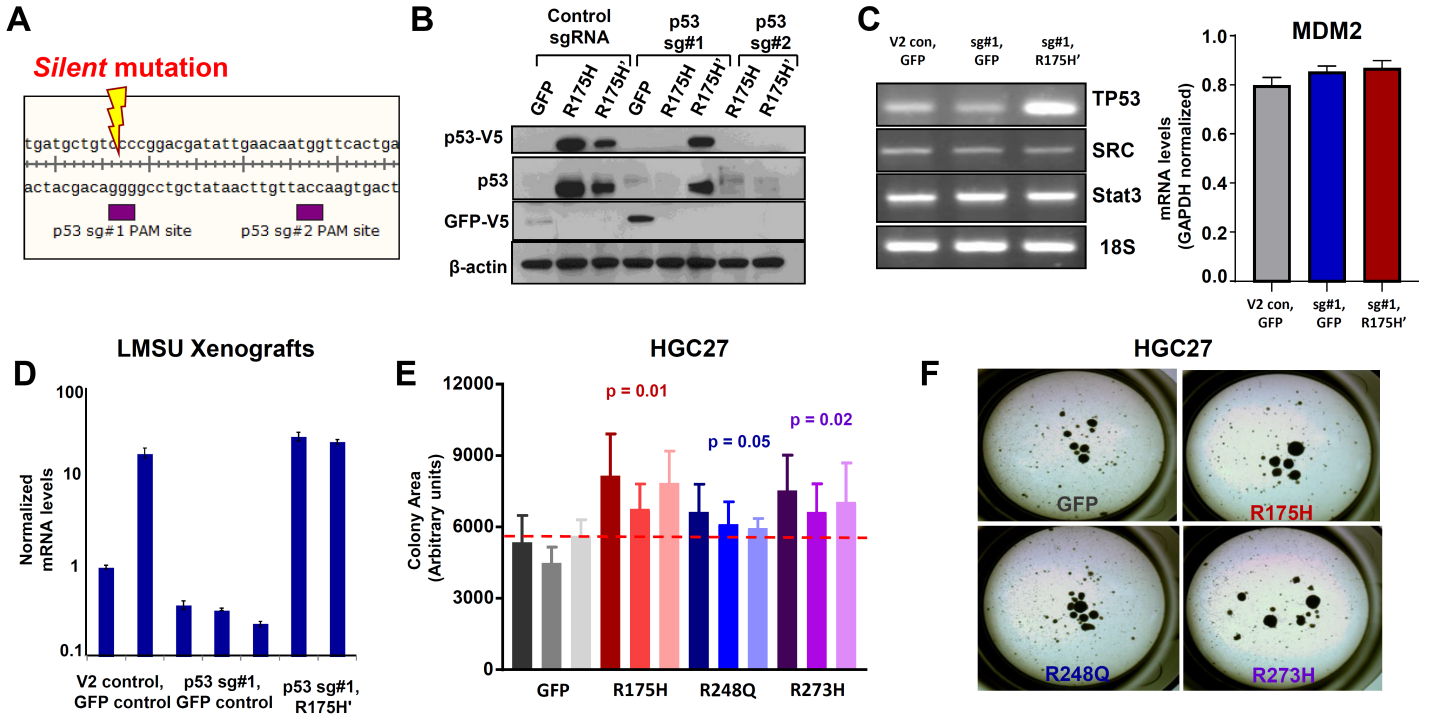
Supplemental Figure 3. Deletion of WT p53 in AGS gastric cancer cell line does not impact primary tumor growth properties

TP53 dependency scores for all CCLE cell lines with damaging (nonsense/frameshift) mutations, hotspot (R175H, R248Q/W, R273C/H) mutations, missense mutations, or wildtype for *TP53* using **(A)** CRISPR and **(B)** RNAi dependency data.

(C) Immunoblot showing WT p53 protein expression in AGS gastric adenocarcinoma cells expressing a Cas9 control vector (V2) or two distinct p53 targeting sgRNAs in addition to Cas9 in the presence or absence of doxorubicin.

(D) Proliferation of AGS-luciferase labelled cells expressing a control or two distinct p53 sgRNAs by luciferase assay

(E) Soft agar colony formation assay of AGS gastric adenocarcinoma cells expressing a control or two distinct p53 targeting sgRNAs as shown by representative phase contrast images (left panel), quantification of colonies (top right panel), and crystal violet staining.



Supplemental Figure 4. Overexpression of mutant p53 promotes primary tumor growth in gastric cancer

(A) Design of sgRNAs against TP53. Mutant p53-R175H' contains the silent point mutation in sgRNA#1 PAM site and is insensitive to TP53 sgRNA#1-mediated DNA editing.

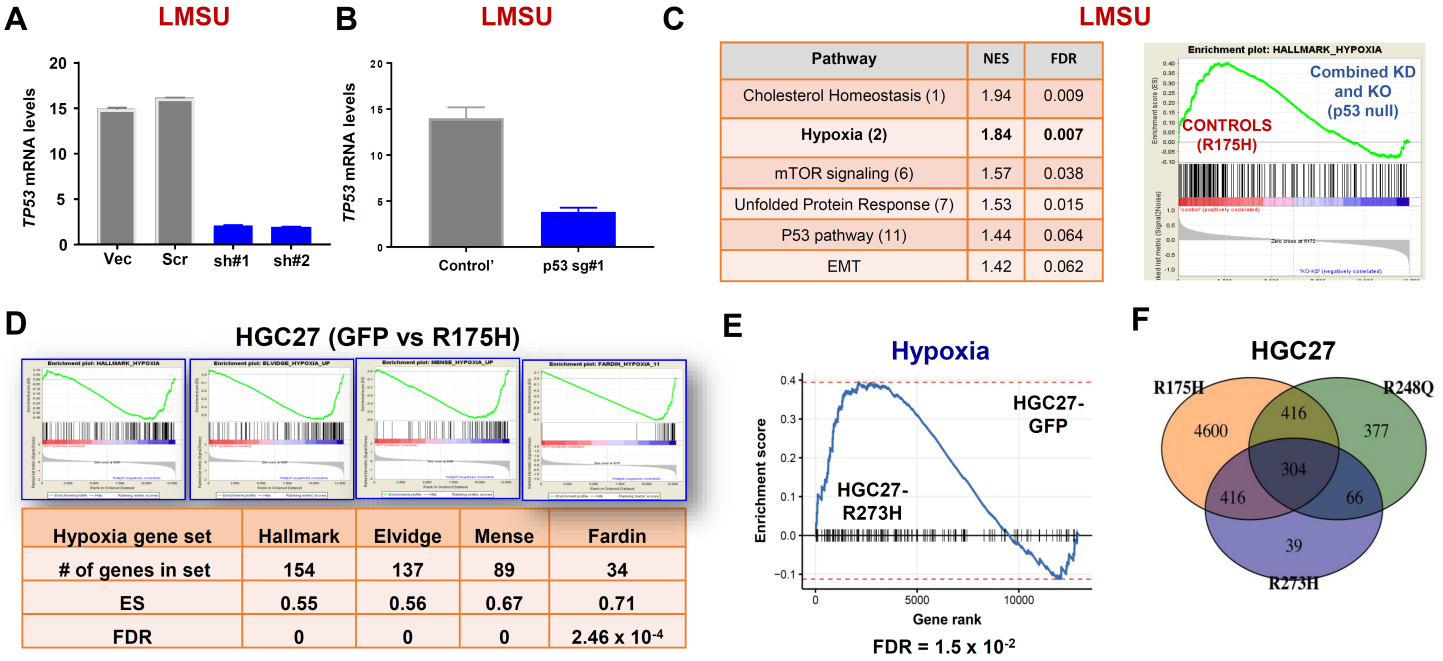
(B) Immunoblot showing control, sgRNA#1 and sgRNA#2 activity in GEA cells overexpressing GFP, R175H or R175H' (with silent mutation).

(C) cDNA expression levels of *TP53, SRC*, *STAT3* and *18S Ribosome* (loading control) of LMSU cells with sgTP53 and/or R175H' ORF by PCR. *MDM2* mRNA expression levels by RT-PCR in the same samples.

(D) Mutant R175H *TP53* gene expression in GFP, V2 control, GFP, p53 sg#1, and p53 sgRNA#1, R175H' OE primary tumor xenografts

(E) Quantification of colony area from HGC27 gastric cancer cells expressing either GFP control or indicated mutant p53 isoforms cultured under ultra-low attachment conditions. p-values calculated by Student's t-test of three biological replicates

(F) Representative mages of ultra-low attachment colonies from **(E)**



Pathway	NES	FDR
Cholesterol Homeostasis (1)	1.94	0.009
Hypoxia (2)	**1.84**	**0.007**
mTOR signaling (6)	1.57	0.038
Unfolded Protein Response (7)	1.53	0.015
P53 pathway (11)	1.44	0.064
EMT	1.42	0.062

Hypoxia gene set	Hallmark	Elvidge	Mense	Fardin
# of genes in set	154	137	89	34
ES	0.55	0.56	0.67	0.71
FDR	0	0	0	2.46 x 10^{-4}

Supplemental Figure 5. Mutant p53 regulates a hypoxia transcriptional program in gastric cancer cells

(A) mRNA expression of *TP53* in LMSU cells expressing either vector control, scrambled shRNA control, p53 shRNA#1, or p53 shRNA#2.

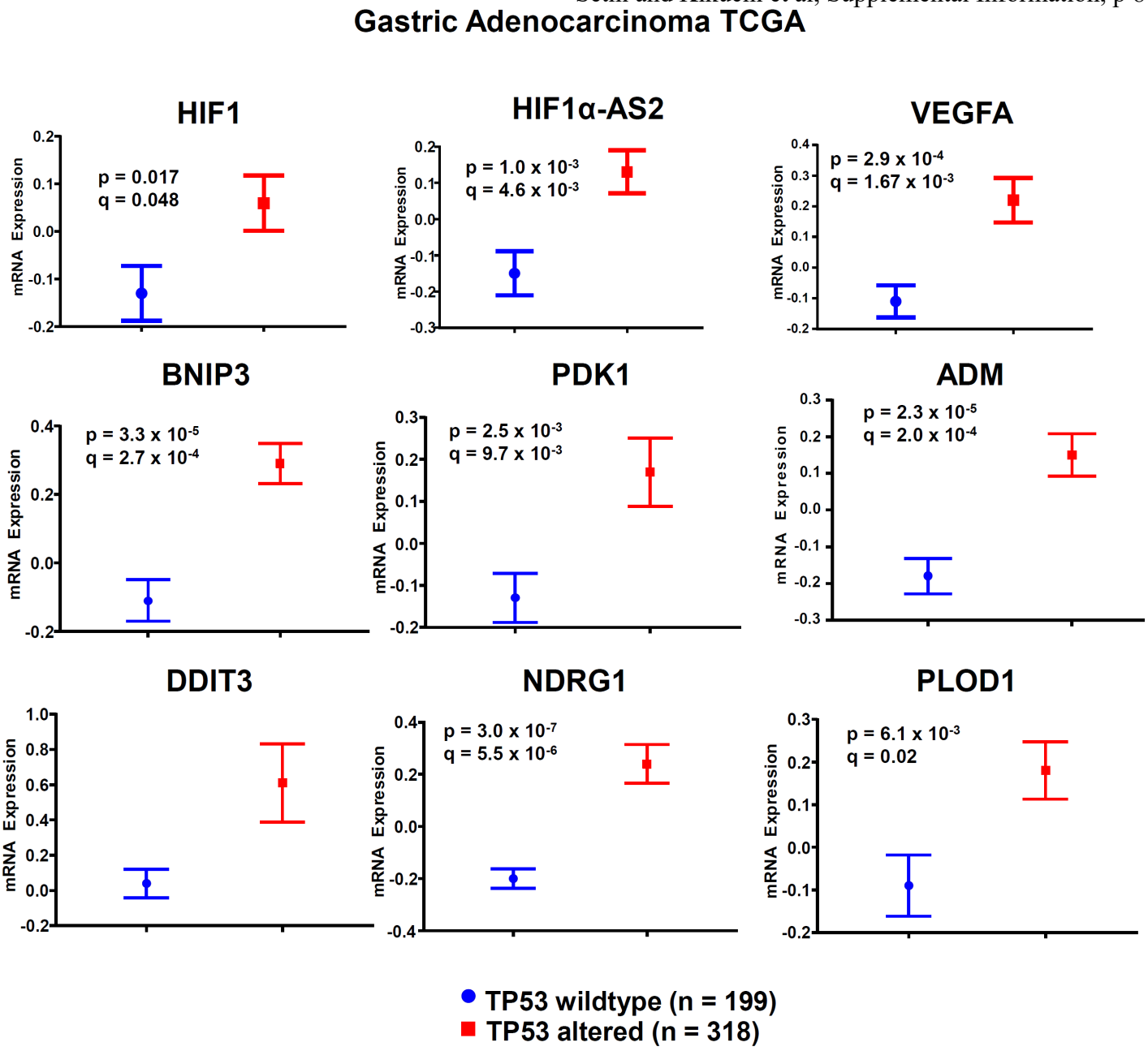
(B) mRNA expression of *TP53* in LMSU cells with control or sgRNA #1 against TP53

(C) Gene set enrichment analysis (GSEA) of mRNA sequencing data: Table of pathways enriched in p53-R175H expressing LMSU cells compared to knockdown controls (left panel) and GSEA plot for Hallmark hypoxia (right panel)

(D) GSEA analysis of distinct validated hypoxia gene-sets in HGC27 cells expressing p53-R175H compared to controls: GSEA plots (top panel); Table of hypoxia gene-set details (bottom panel)

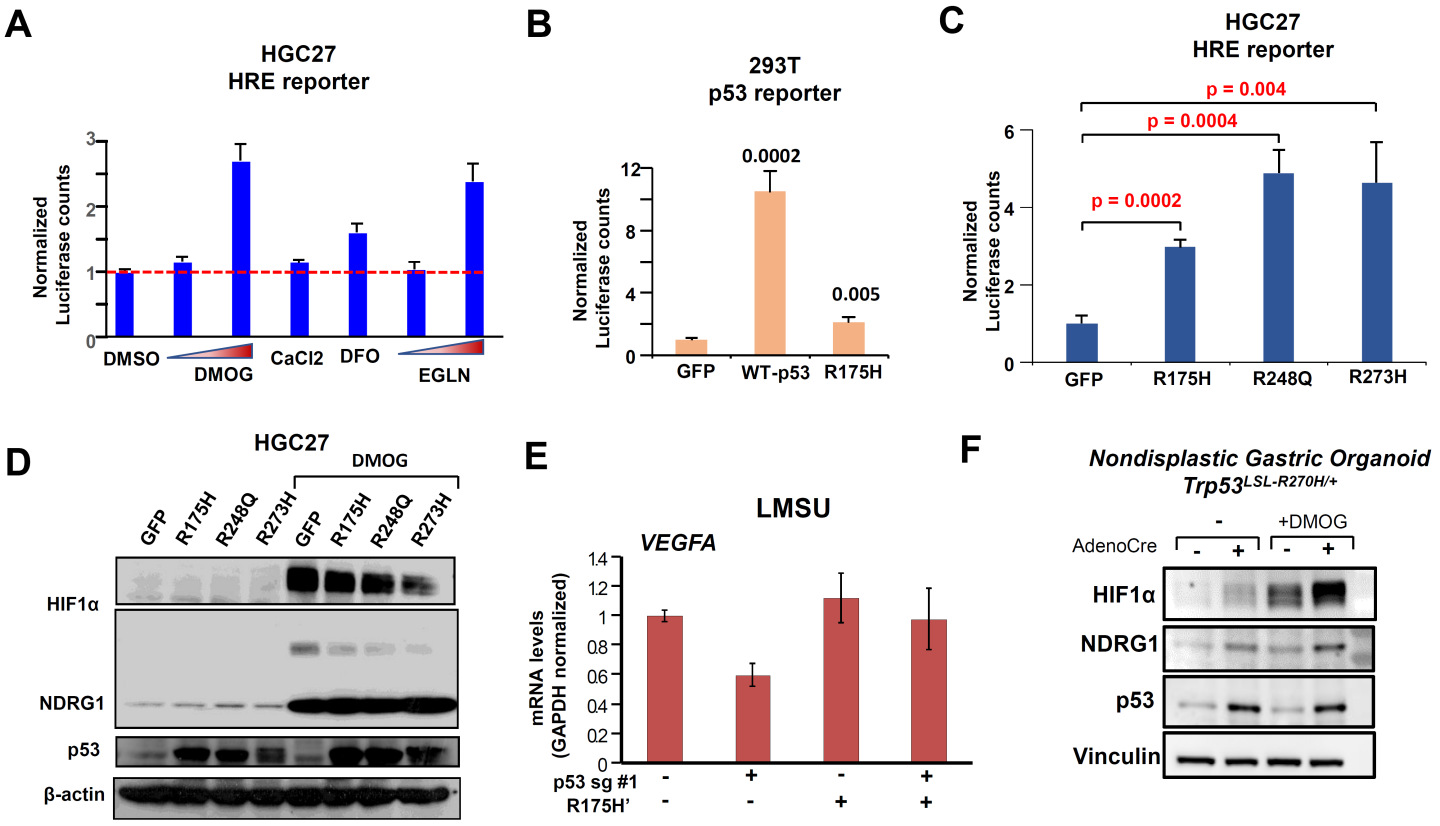
(E) GSEA plot of Hallmark hypoxia signature in R273H-expressing HGC27 cells versus GFP controls

(F) Venn-diagram of differentially expressed genes in HGC27 cells expressing the indicated mutant p53 compared to GFP controls



Supplemental Figure 6. *TP53* alterations are associated with induction of hypoxia genes in human gastric cancer

Expression levels of select hypoxia genes in patients with *TP53* altered (n=318) or wildtype (n=199) gastric cancer



Supplemental Figure 7. Mutant p53 activates hypoxia genes in gastric cancer cells *in vitro*

(A) HRE reporter luciferase activity in HGC27 cells treated with indicated chemical agents. Luciferase activity was calculated as a ratio of Firefly luciferase counts (HRE) divided by constitutive renilla luciferase counts.

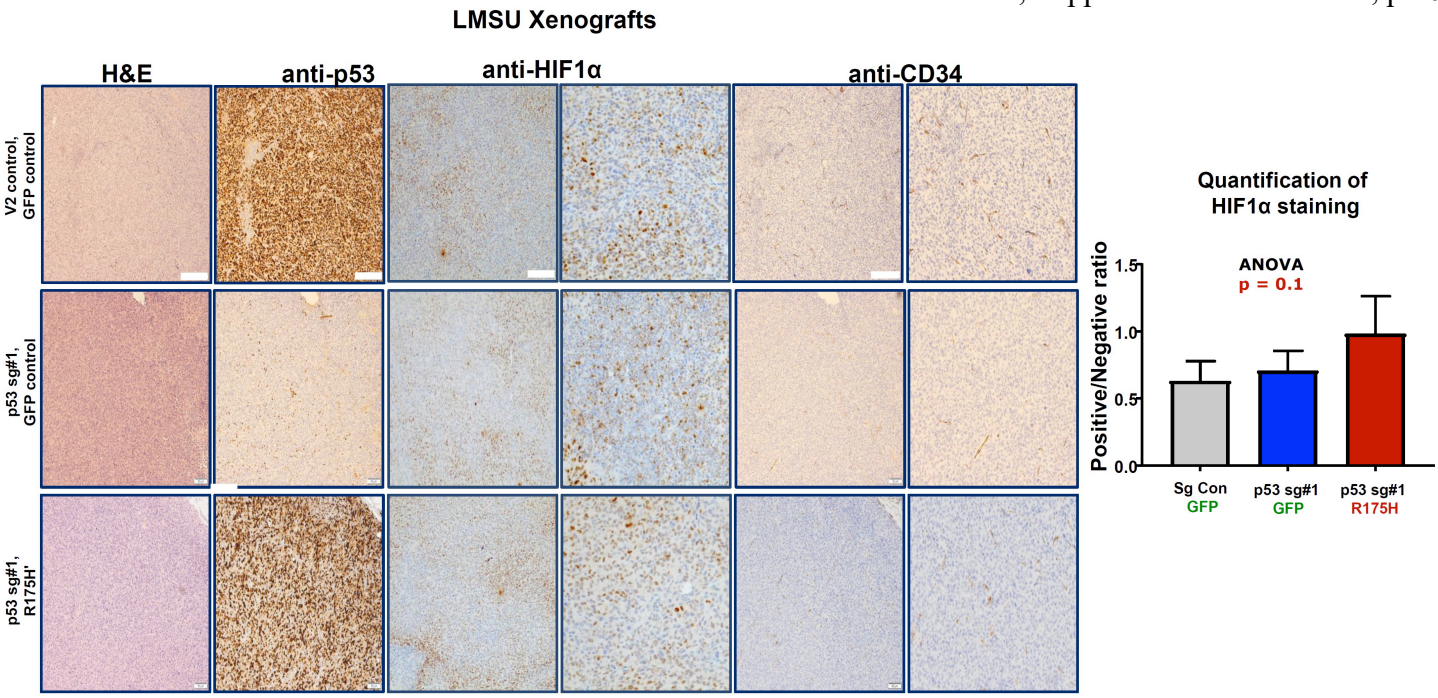
(B) p53 reporter luciferase assay of HEK293T cells co-transfected with GFP control, wildtype p53, or mutant R175H. Luciferase activity was calculated as firefly luciferase counts (p53 reporter) divided by constitutive renilla luciferase counts.

(C) HIF-responsive promoter (HRE) firefly luciferase reporter activity in HGC27 cells transiently transfected with indicated mutant p53 constructs; 1 mM DMOG treatment was used as a positive control. Data were normalized to co-transfected constitutively active Renilla luciferase activity. Results are shown as the average ± SD.

(D) Immunoblot showing HIF1α, NDRG1, and mutant p53 protein levels in HGC27 cells with and without 1mM DMOG treatment.

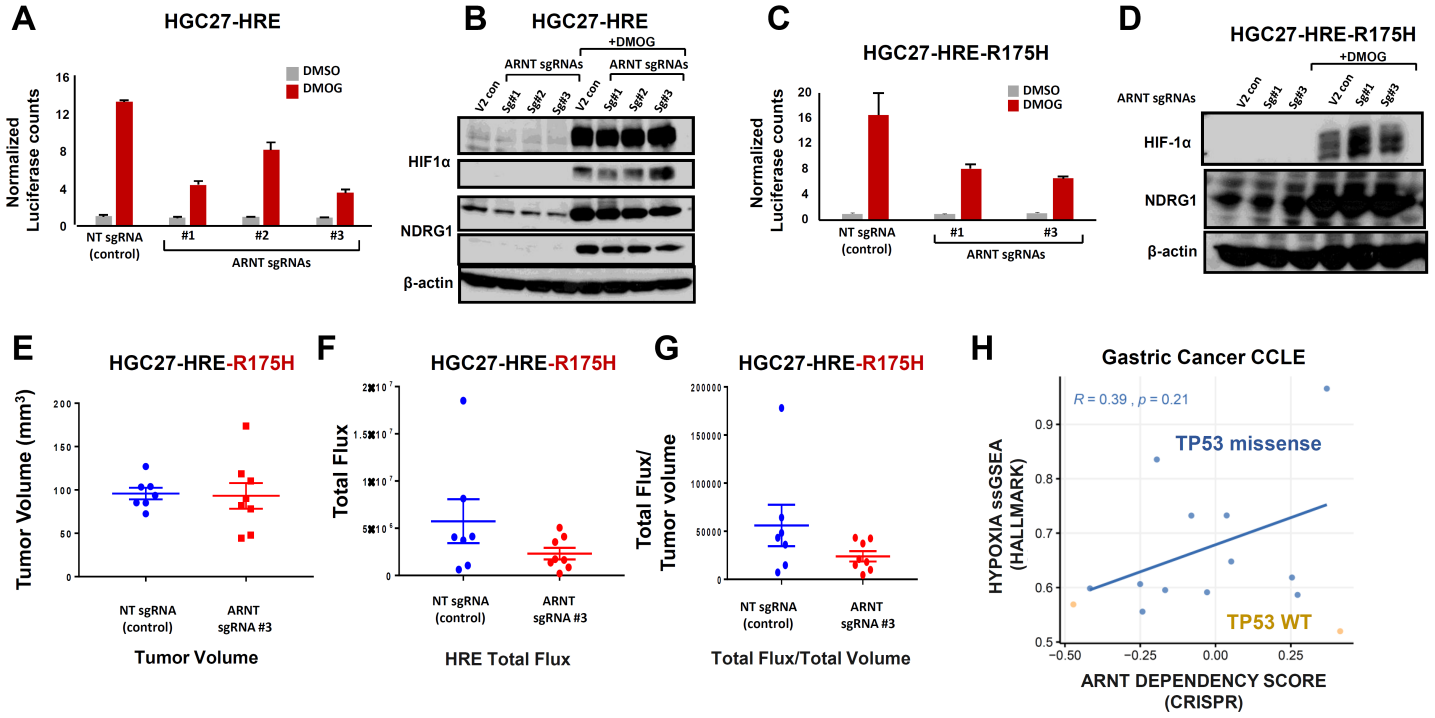
(E) Gene expression levels of *GAPDH*-normalized *VEGFA* levels in LMSU cells with indicated p53-R175H' and/or p53 sgRNA#1 expression.

(F) Protein expression of HIF-1α and p53 in conditional *Trp53*$^{LSL\text{-}R270H/+}$ gastric organoids with and without AdenoCre virus (AdCre) in the presence or absence of DFO or DMOG by immunoblot.



Supplemental Figure 8. Enforced expression of mutant p53 is associated with elevated HIF1α levels in gastric cancer primary tumor xenografts

Histological and immunohistochemical analysis of LMSU xenograft tumors expressing indicated control, p53 sgRNA, and p53-R175H'OE. Panels from left to right show representative images of H&E staining, p53 immunohistochemistry (IHC), HIF-1α IHC, and CD34 IHC. Scale bar, 100 μm.



Supplemental Figure 9. Inhibition of HIF co-factor ARNT does not impact primary tumor growth of mutant p53 gastric cancer

(A) Luciferase assay of HGC27-HRE cells stably expressing either control or ARNT sgRNAs in the presence and absence of 1 mM DMOG.

(B) Immunoblot of HIF-1α and NDRG1 expression levels in HGC27-HRE cells stably expressing either control or ARNT sgRNAs in the presence and absence of 1 mM DMOG. The top band is a darker exposure whereas the bottom band is a lighter exposure for HIF-1α and NDRG1.

(C) Luciferase assay of HGC27-HRE-R175H cells stably expressing either control or ARNT sgRNAs in the presence and absence of 1 mM DMOG.

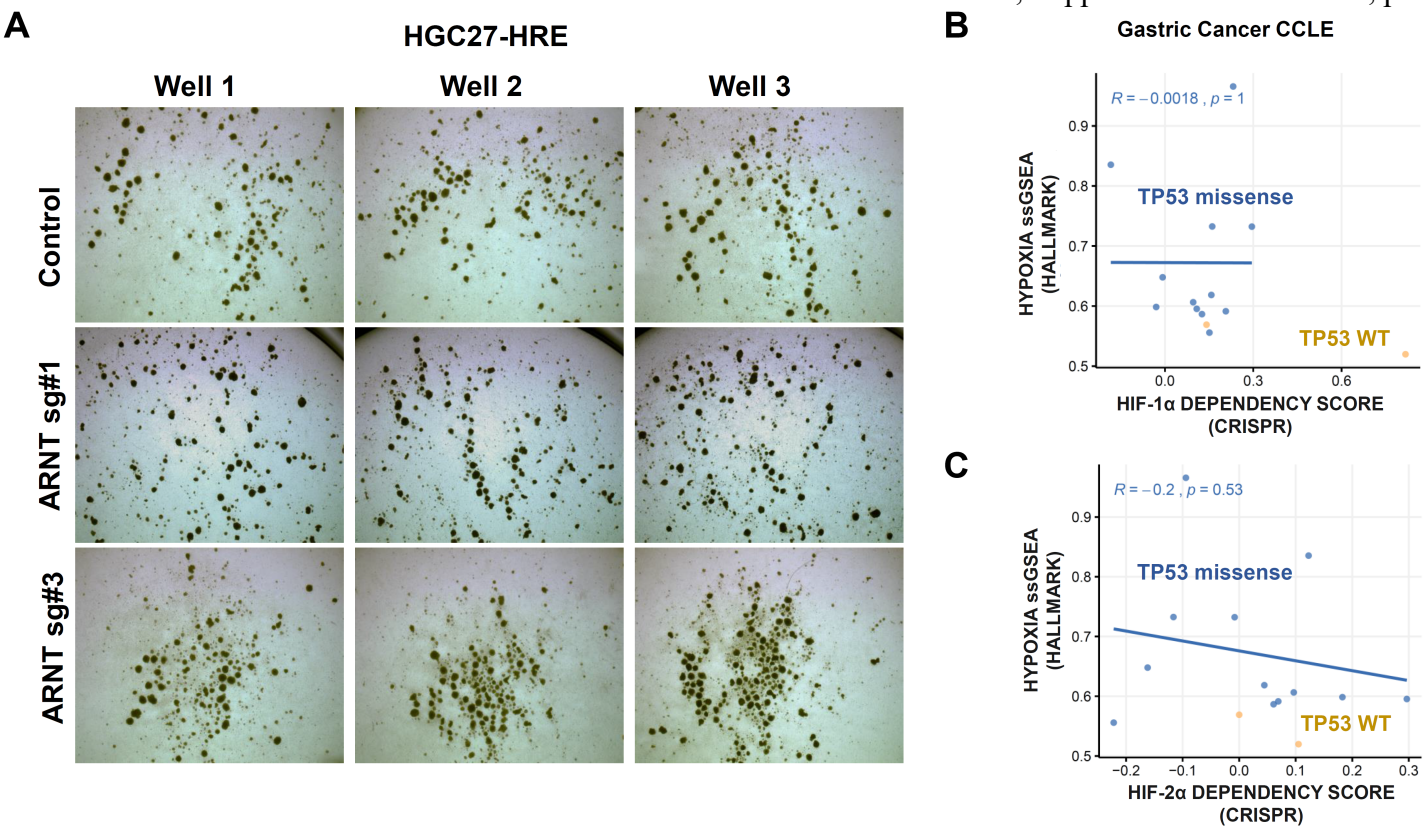
(D) Immunoblot of HIF-1α and NDRG1 expression levels in HGC27-HRE-R175H cells stably expressing either control or ARNT sgRNAs in the presence and absence of 1 mM DMOG

(E) Tumor volume of HGC27-HRE-R175H expressing control or ARNT sgRNA#3 xenografts at 2-week time point

(F) Total HRE Flux of HGC27-HRE-R175H expressing control or ARNT sgRNA#3 xenografts at 2-week time point

(G) Total flux (HRE-reporter activity) to tumor volume ratio of HGC27-HRE-R175H expressing control or ARNT sgRNA#3 xenografts at 2-week time point

(H) Scatter plot showing the association between ARNT CRISPR dependency and Hypoxia GSEA in gastric cancer cell lines annotated by *TP53* mutation status



Supplemental Figure 10. ARNT is not required for ultra-low attachment culture of mutant p53 gastric cancer cells

(A) Representative images of ultra-low attachment colonies of HGC27-HRE-R175H cells expressing either control or two distinct ARNT sgRNAs.

(B) Scatter plot showing the association between HIF1α CRISPR dependency and Hypoxia GSEA in gastric cancer cell lines annotated by *TP53* mutation status

(C) Scatter plot showing the association between HIF2α CRISPR dependency and Hypoxia GSEA in gastric cancer cell lines annotated by *TP53* mutation status