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Supplementary Materials for

Temporal regulation of gene expression through integration of p53 dynamics and modifications

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Figure S1

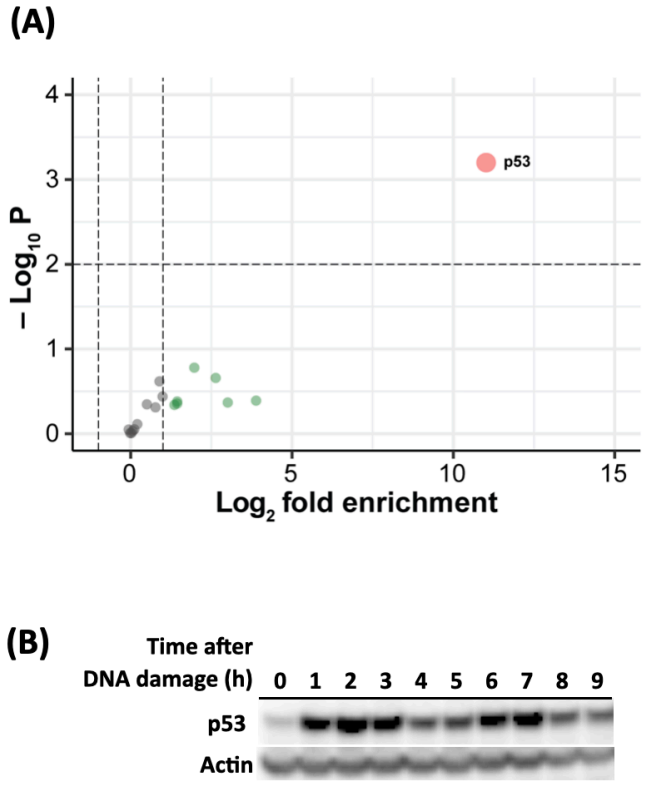

Fig. S1: p53 immunoprecipitation and dynamics in cells. (A) Magnetic beads were conjugated with either PAB1801 or an isotype control. Captured proteins were in-solution digested and peptides were analyzed by Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS). Abundance was measured by label free quantification. The fold enrichment was calculated for immunoprecipitations using anti-p53 antibody compared to isotype control. (n=3 biological replicates; p-value represents Bonferroni-corrected values following t-test of the abundance values for each protein). **(B)** Western blot of total p53 levels at the indicated time points after 10 Gy X-ray irradiation of MCF7 cells. Actin is shown as a loading control.

Figure S2

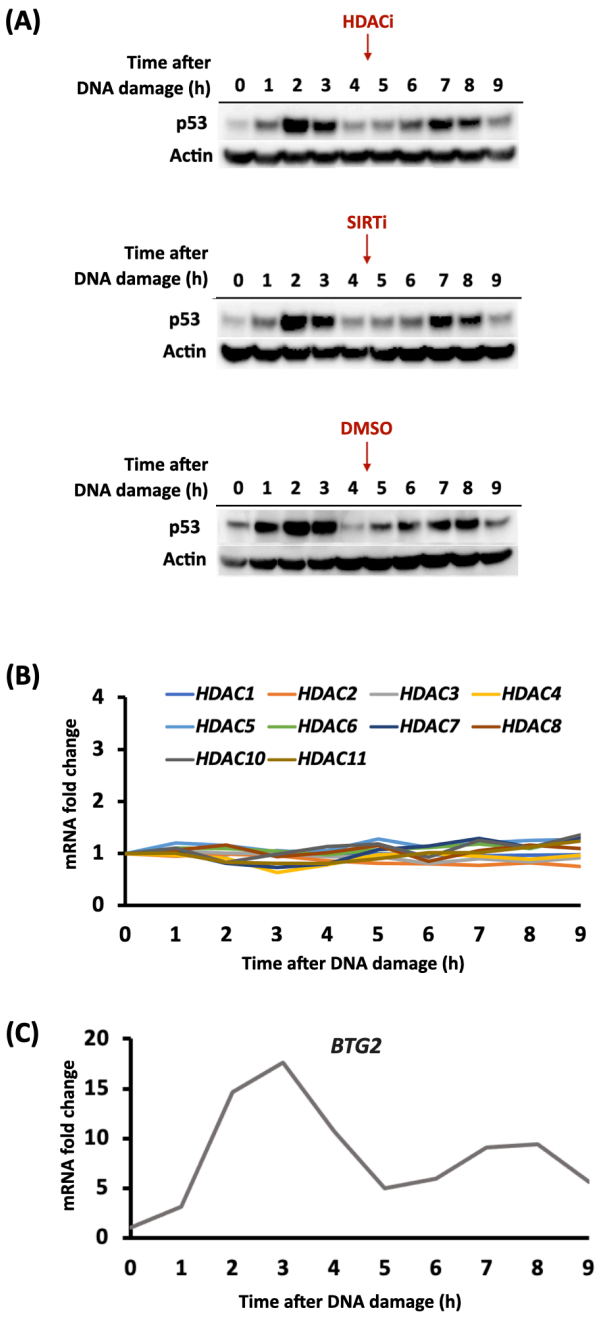

Fig. S2: Effects of deacetylase inhibitors on p53 dynamics, and expression patterns of acetylation regulators (A) Western blot of p53 levels at the indicated timepoints after 10 Gy X-ray irradiation. HDACi, SIRTi or DMSO control was added at 4.5 h, corresponding to the trough between the first two peaks of p53 expression. Actin is shown as a loading control. (**B-C**) Expression levels based on RNA-Seq of (**B**) HDAC family enzymes or (**C**) *BTG2* following DNA damage (source data obtained from Jiménez et al. 2022 (*22*)).

Figure S3

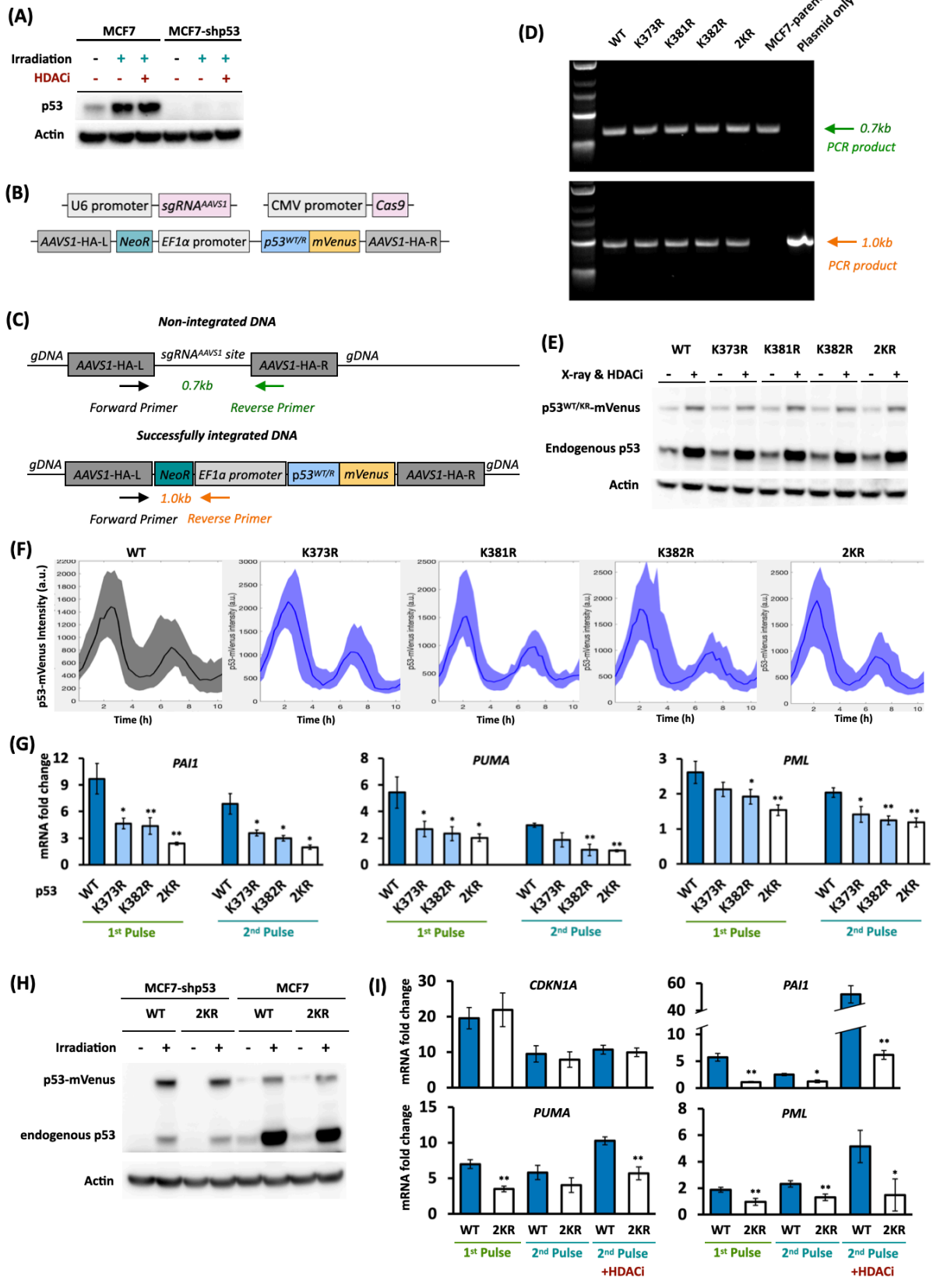
(A)
MCF7
MCF7-shp53
Irradiation
HDACi
p53
Actin
(B)
U6 promoter
sgRNA AAVS1
CMV promoter
Cas9
AAVS1-HA-L
NeoR
EF1α promoter
p53 WT/R
mVenus
AAVS1-HA-R
(C)
Non-integrated DNA
gDNA
AAVS1-HA-L
sgRNA AAVS1 site
AAVS1-HA-R
gDNA
0.7kb
Forward Primer
Reverse Primer
Successfully integrated DNA
gDNA
AAVS1-HA-L
NeoR
EF1a promoter
p53 WT/R
mVenus
AAVS1-HA-R
gDNA
1.0kb
Forward Primer
Reverse Primer
(D)
WT
K373R
K381R
K382R
2KR
MCF7-parental
Plasmid only
0.7kb
PCR product
1.0kb
PCR product
(E)
WT
K373R
K381R
K382R
2KR
X-ray & HDACi
p53 WT/KR-mVenus
Endogenous p53
Actin
(F)
WT
K373R
K381R
K382R
2KR
p53-mVenus Intensity (a.u.)
Time (h)
(G)
PAI1
PUMA
PML
mRNA fold change
p53
WT
K373R
K382R
2KR
1st Pulse
2nd Pulse
(H)
MCF7-shp53
MCF7
WT
2KR
WT
2KR
Irradiation
p53-mVenus
endogenous p53
Actin
(I)
CDKN1A
PAI1
PUMA
PML
mRNA fold change
WT
2KR
1st Pulse
2nd Pulse
2nd Pulse +HDACi

Fig. S3: WT or mutant p53 cell line generation and resulting effects on p53 dynamics and target gene expression (A) Western blot showing p53 levels at 7.5 hrs in control or shp53-expressing cells in the presence or absence of HDACi. Actin is shown as a loading control. **(B)** Design of plasmids used for CRISPR-Cas9 mediated knock-in of p53 variants into the genomic safe harbor site AAVS1. **(C)** Single cell clones were checked for successful integration of the construct into genomic DNA using the indicated primers. **(D)** PCR analysis of single cell clones harboring WT or mutant p53 constructs. Indicated bands depict expected products generated from PCR from panel B. Plasmid only refers to purified plasmid containing AAVS integration construct (lower panel from **A**) extracted from *E. coli* prior to transformation. **(E)** MCF7 cells expressing WT or mutant p53-fused to mVenus were subjected to 10 Gy X-ray and HDACi addition at 4.5 hrs following irradiation and their p53 levels analyzed by western blot 8 hrs post initial irradiation. **(F)** WT and mutant p53 levels were monitored by live cell fluorescence microscopy. Solid line represents the median while shaded area represents interquartile range. **(G)** qPCR analysis of the mRNA levels of the indicated genes at 3 and 8 hrs post-irradiation of WT or indicated mutant p53 expressing MCF7 cells, normalized to mRNA levels for unirradiated cells. Error bars represent standard deviation from 3 biological replicates. * $p< 0.05$, ** $p< 0.01$ by t-test. **(H)** MCF7 or MCF7-shp53 cells expressing WT or mutant p53-fused to mVenus were subjected or not to 10 Gy X-ray and their p53 levels analyzed by western blot 2.5 hrs post initial irradiation. **(I)** qPCR analysis of the mRNA levels of the indicated genes at 3 and 8 hrs post-irradiation with or without the addition of HDACi in WT or 2KR mutant p53-expressing MCF7-shp53 cells, normalized to mRNA levels for unirradiated cells. Error bars represent standard deviation from 3 biological replicates. * $p< 0.05$, ** $p< 0.01$ by t-test.

Figure S4

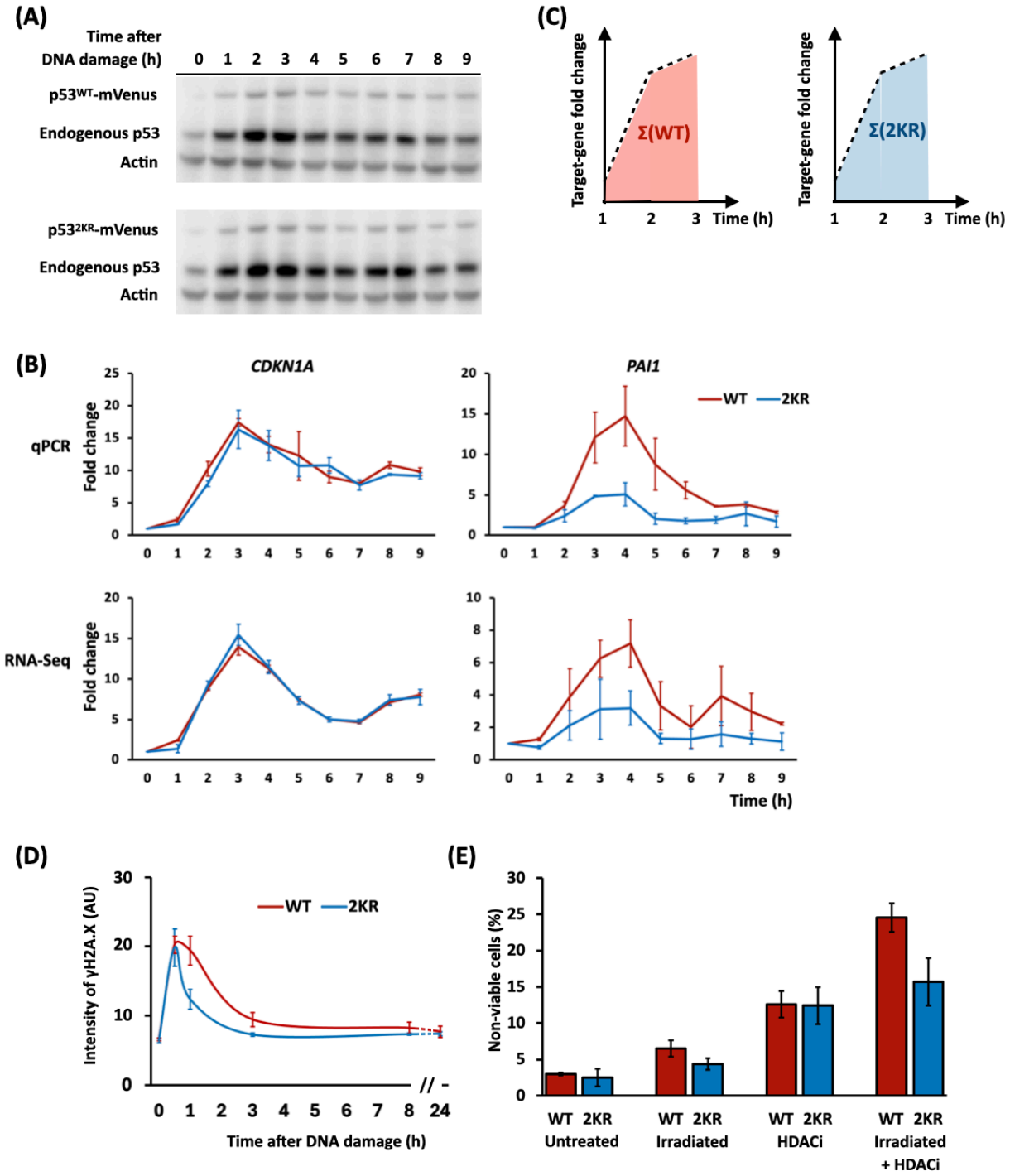

Fig. S4: Expression of WT and 2KR p53 and their effects on gene expression, DNA repair, and viability (A) Western-blot of cells expressing WT (top) or 2KR (bottom) p53-Venus fusions at the indicated timepoints following irradiation. Endogenous and exogenous p53 are indicated.

Actin is shown as a loading control. **(B)** qPCR analyses show same expression trends as RNA-Seq for the p53 targets *CDKN1A* and *PAI1*. **(C)** Schematic showing the calculation of the area under the curve for dynamic gene expression data (See Materials & Methods). **(D)** Quantification of mean γH2A.X intensity of top 10% p53-Venus expressing cells following irradiation. n >100 cells per condition per time point, error bars indicate standard deviation. **(E)** Cell viabilities were measured (detailed in Materials and Methods) by Trypan blue staining of WT and mutant p53-expressing MCF7 subjected to the indicated treatments. * $p < 0.05$, by t-test (n = 3 biological replicates per condition)

Table S1 (.csv file): Normalized enrichment score (NES) and p-values for each gene ontology (GO) function was calculated (detailed in Materials & Methods). NA represents situations where no gene within a given GO category was detectable at a given time point.

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