Supplemental Table 1

cDNA primers

GAPDH	5’-GTC AAC GGA TTT GGT CGT ATT-3’
	5’- GAT CTC GCT CCT GGA AGA TGG-3’
mus GAPDH	5’-CCA GCC TCG TCC CGT AGA CA-3’
	5'- GCC TCA CCC CAT TTG ATG TTA GTG -3'
mus p53	5’-CAC GTG CTC ACC CTG GCT AA-3’
	5’-CTC AAC ATC CTG GGG CAG CA-3’
p53	5’-AAG GAA ATT TGC GTG TGG AGT-3’
	5’-AAA GCT GTT CCG TCC CAG TA-3’
CHEK 1	5’-GAA AGG GGC AAA AAG G-3’
	5’-ATG TAT GAG GGG CTG GTA-3’
mus CHEK1	5’-GGT TCA GGG CAT CAG TTT-3’
	5’-GGT CTC TTT CAG GCA TTG-3’
CCNA2	5’-GAC GGC GCT CCA AGA GG-3’
	5’-AAT GGT GAA CGC AGG CTG TT-3’
mus CCNA2	5’-CCC CAG AAG TAG CAG AGT TT-3’
	5’-GGT ACG GGT CAG CAT CTA TC-3’
CCNE 1	5’-CAG TAT CCC CAG CAA ATC T-3’
	5’-AGT TCT CTA TGT CGC ACC AC-3’
CDC25A	5’-GGC AGG GGA GAA GAG CAA-3’
	5’-CAG GGA CAG AAG AGG CGT AG-3’
SHH	5’-TCT GCT GCT AGT CCT CGT CT-3’
	5’-TGT CGG GGT TGT AAT TGG GG-3’

Cloning primers

CHEK1 promoter	5’- GCTCTCCCCGCCTGTTCTTTG-3’
	5’-GCAGAAAACGCCTGCGGCAGCG- 3’

ChIP primers

CCNA2	5’-CCT TTG GTT TAC CCT TCA CTC G -3’
	5’- CCA AAG AAT AGT CGT AGC CG-3’
CHEK 1	5’- GTA CCA GGA GGT TCC CGT TG-3’
	5’-GGG TCT GGG GAA GAG AGG AA -3’

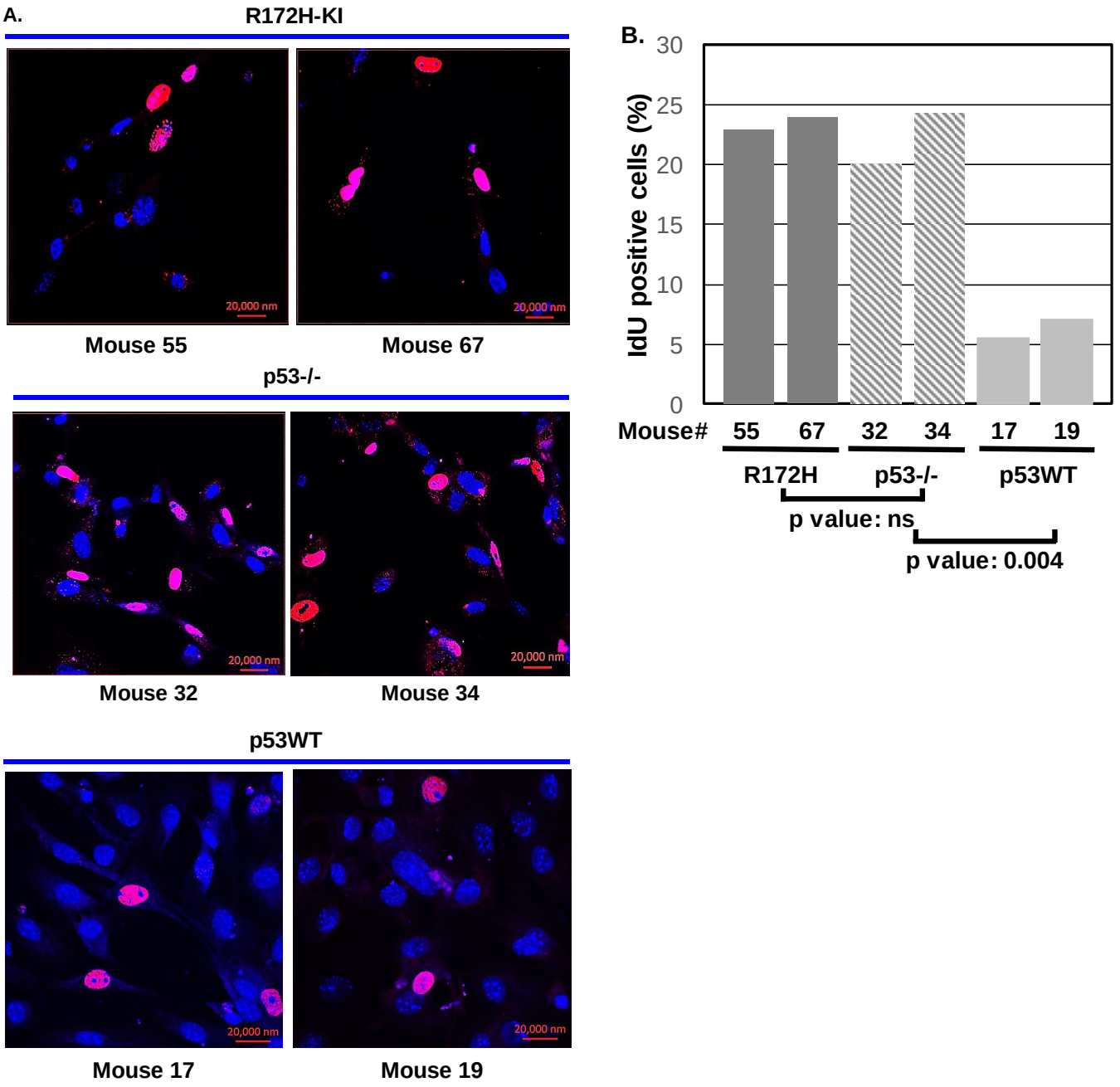


Figure S1: GOF p53 mutants do not hasten the S phase entry of cells.

The figure shows representative images of lung cells from (A) p53R172H-KI (R172H), p53-/- and WT (p53 WT) mice pulse-labeled with IdU at 12 hours after confluence-arrest and replating. Cells were immunostained by Alexa 594 tagged IdU antibody, stained with DAPI and analyzed by confocal microscopy (20X). Lung cells from two different mice for each construct were analyzed. Two hundred nuclei were scored in each experiment. Percent of nuclei incorporating IdU in lung cells from p53R172H-KI, p53-/- or p53 WT mice are shown by bar graphs. The p-values calculated using Student's *t* test is shown at the bottom of the bar graph. ns: Not significant. Data represents three independent experiments.

p53-/-

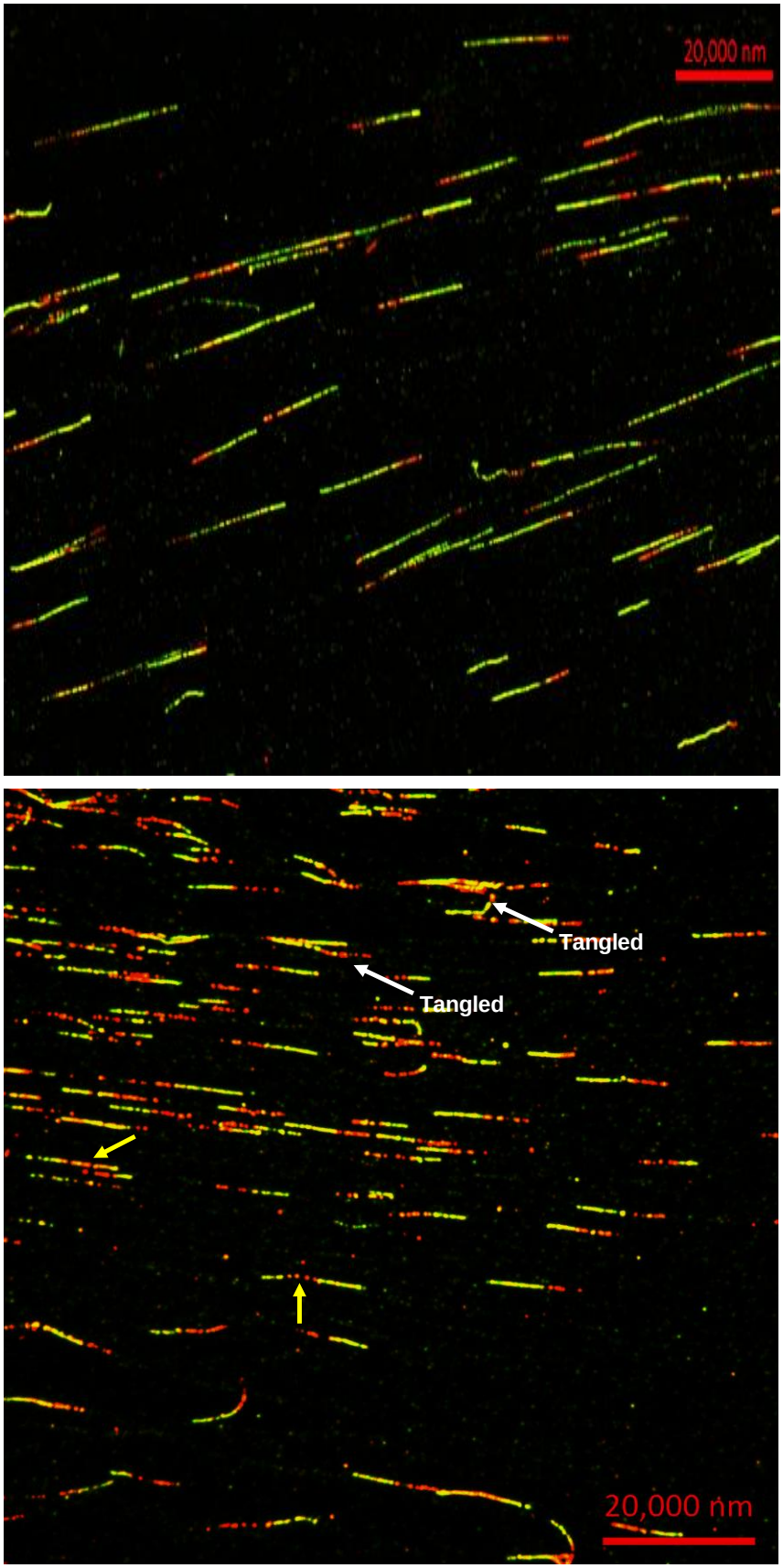


Figure S2A: Representative fiber images of replicating DNA fibers generated from lung cells of p53-/- mice. Origins and examples of tangled fibers are indicated by arrows

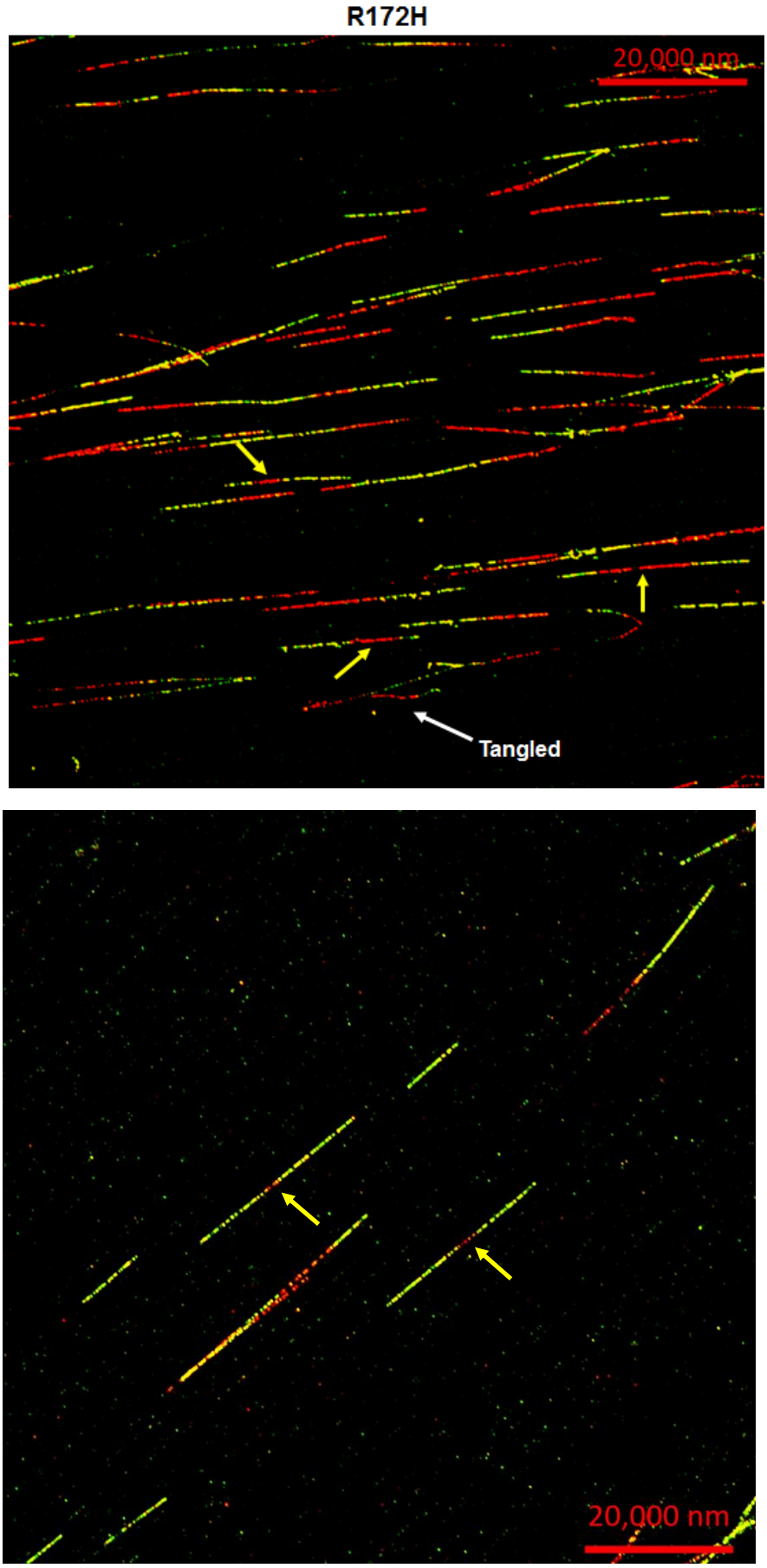


Figure S2B: Representative fiber images of replicating DNA fibers generated from lung cells of p53 R172H-KI (R172H) mice. Origins and an example of tangled fiber are indicated by arrows

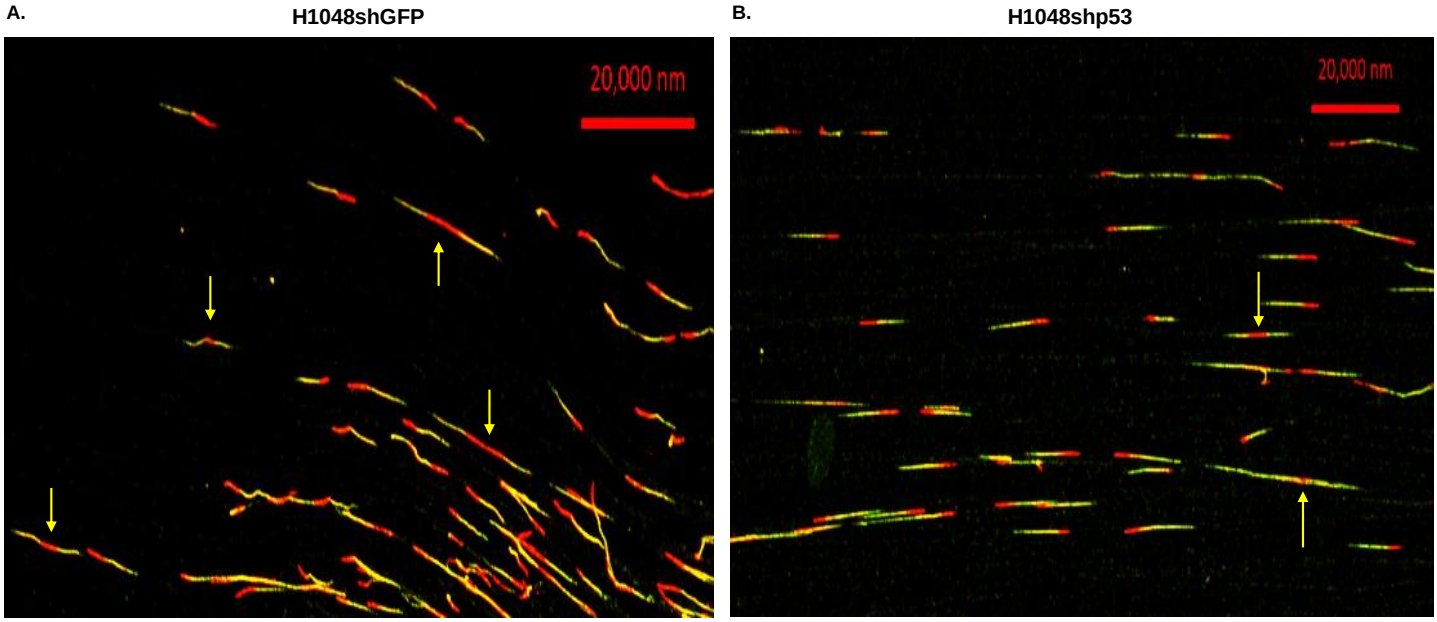


Figure S3: Representative images of replicating DNA fibers generated from H1048 cells stably expressing shGFP or shp53. Origins are indicated by arrows.

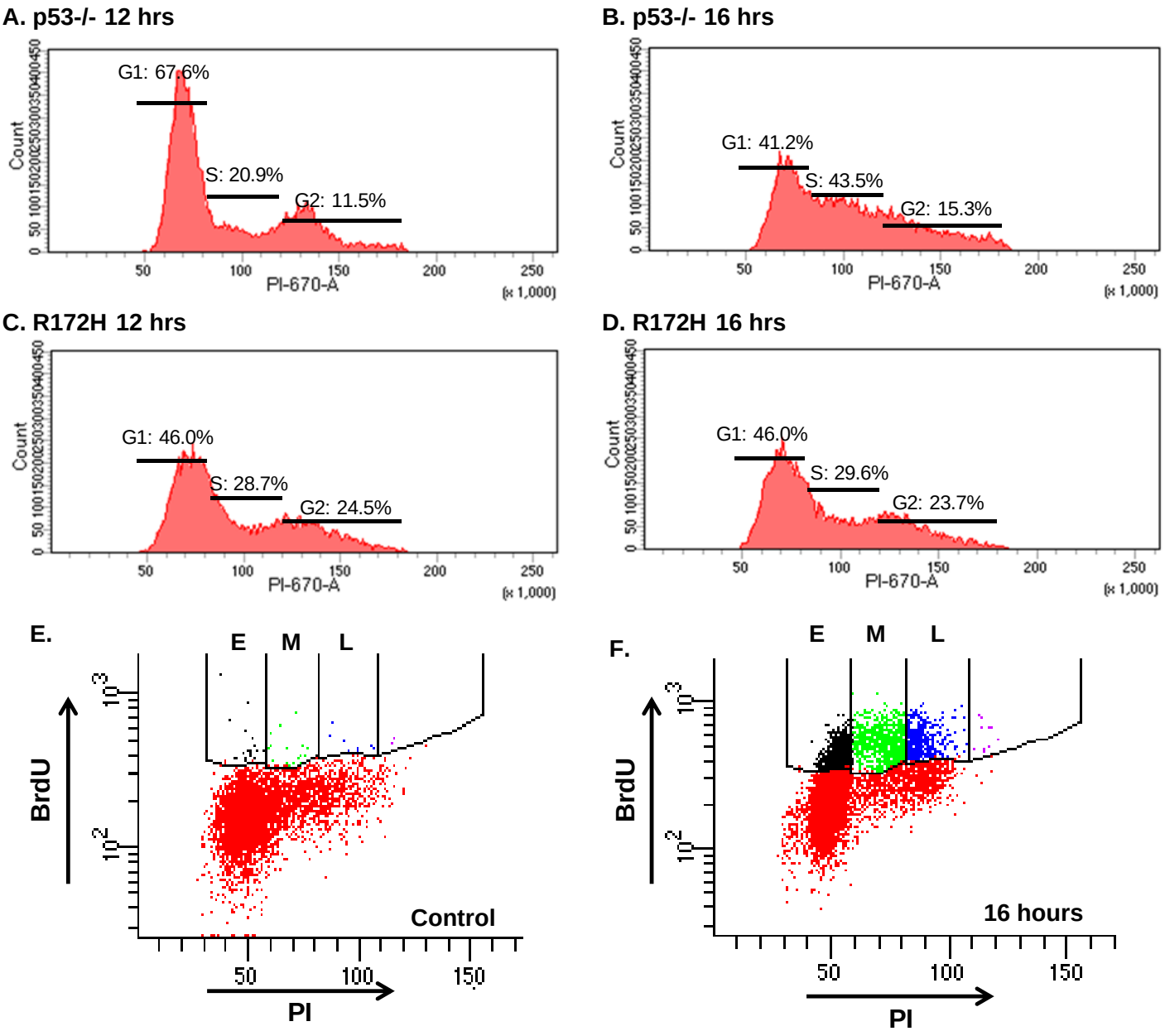
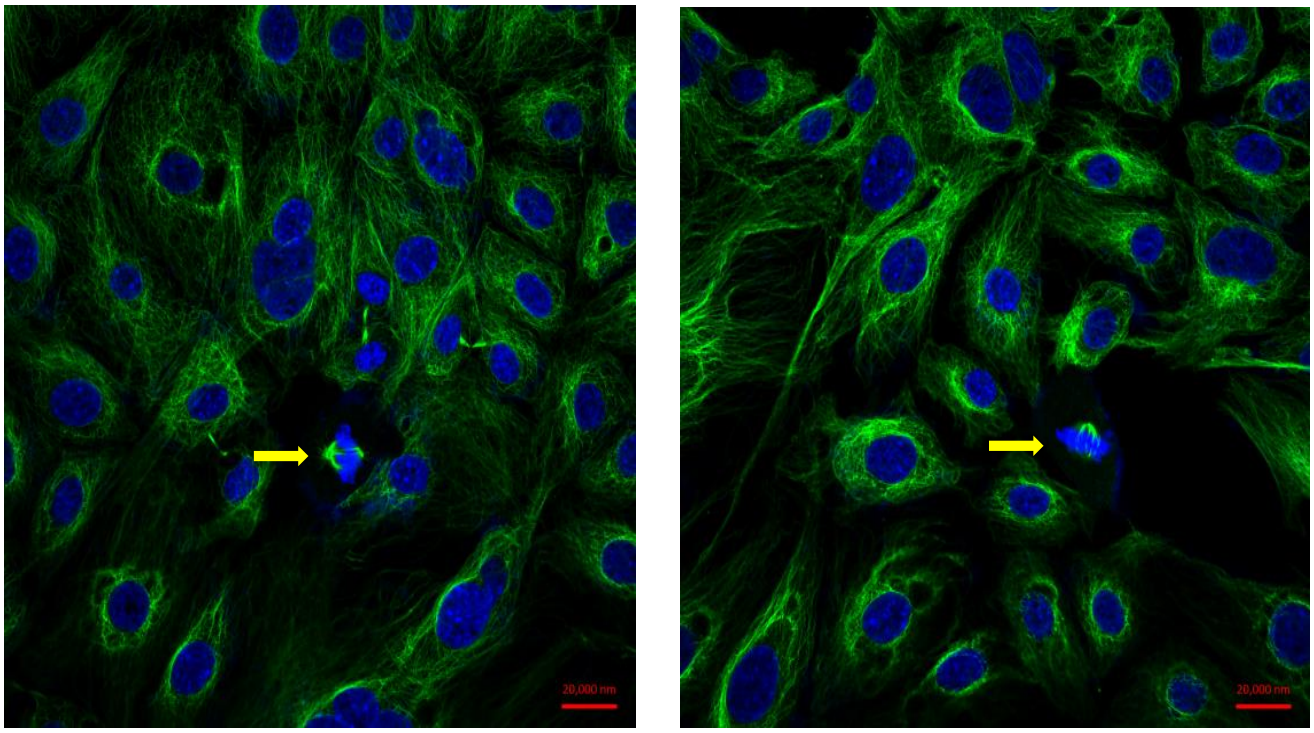


Figure S4: Flow cytometric gating of lung cells from p53-/- and p53R172H-KI (R172H) mice.

DNA histogram (A-D) show percentages of cells in G1, S, G2/M phases at 12 (A, C) and 16 hours (B, D) after density arrest and replating. Representative bivariate dot plot generated by flow cytometry of lung cells after BrDU incorporation, immunostaining and PI staining at 16 hours after releasing cell cycle arrest (E, F). As a background control, antibody immunostaining and PI staining were performed without BrDU incorporation (E). Gating to identify BrDU positive cells in early (E), mid (M), and late (L) S phase of the cell cycle is indicated.

p53-/-



R172H

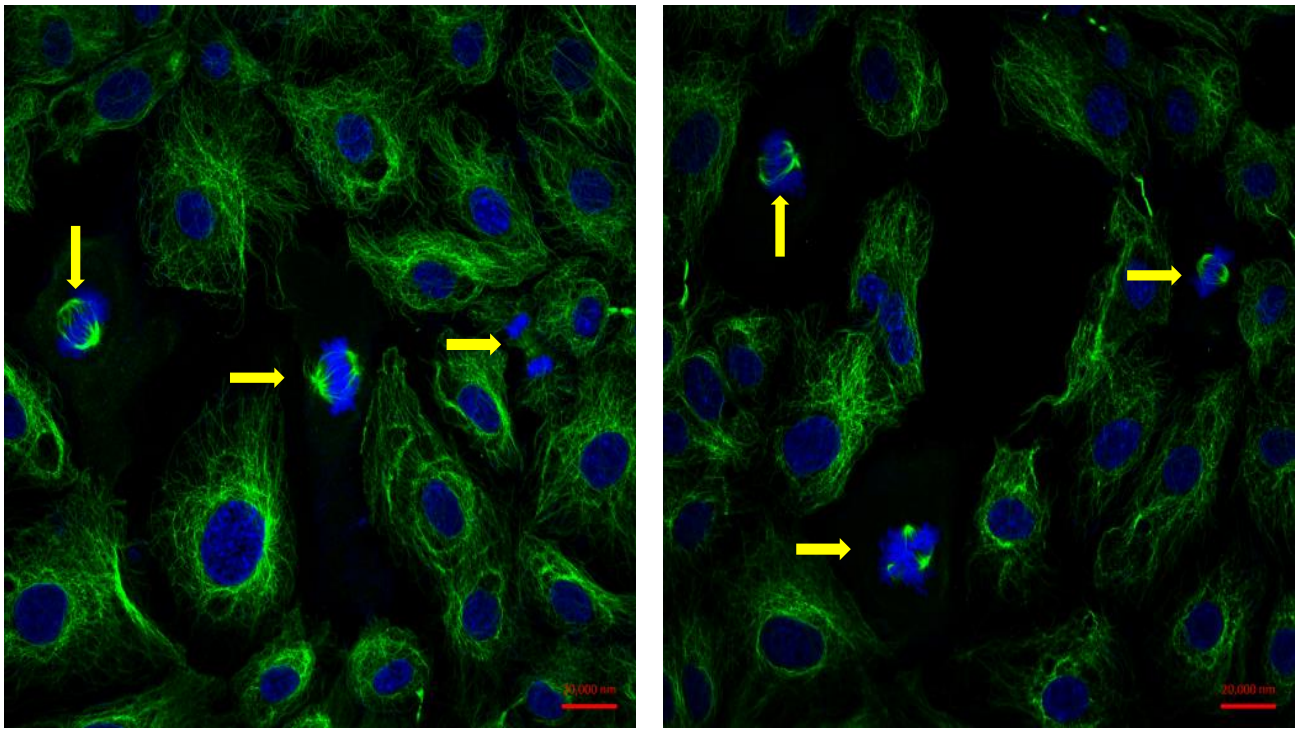


Figure S5: Representative images to show mitotic lung cells from p53-/- and p53R172H-KI (R172H) mice. Cells undergoing mitosis are indicated by arrows.

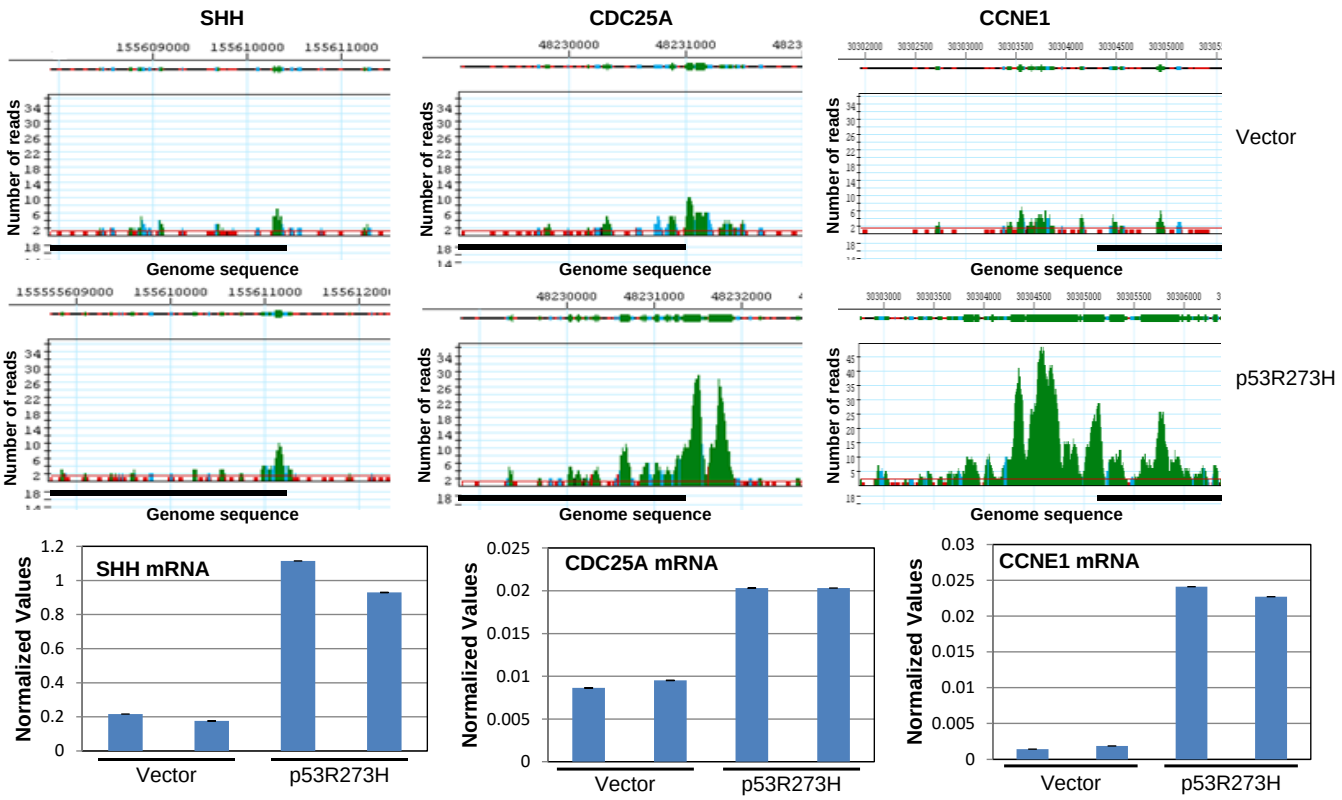


Figure S6: p53R273H localizes on the upstream sequences of SHH, CDC25A and CCNE1 genes and upregulate their transcription.

The upper panels show peaks representing areas of maximal p53R273H binding detected by next generation sequence analysis of DNA fragments recovered from ChIP of H1299 cells stably transfected with control vector (Vector) or stably expressing p53R273H (p53R273H) using anti-p53 antibody. Sequences of the major peaks where mutant p53 binds are underlined. Lower panel shows transcript levels of SHH, CDC25A and CCNE1 genes determined by RT-QPCR analysis, and were picked arbitrarily to confirm the RNA-seq data. Assays from duplicate independent preparations were performed and plotted as mean ± SEM.

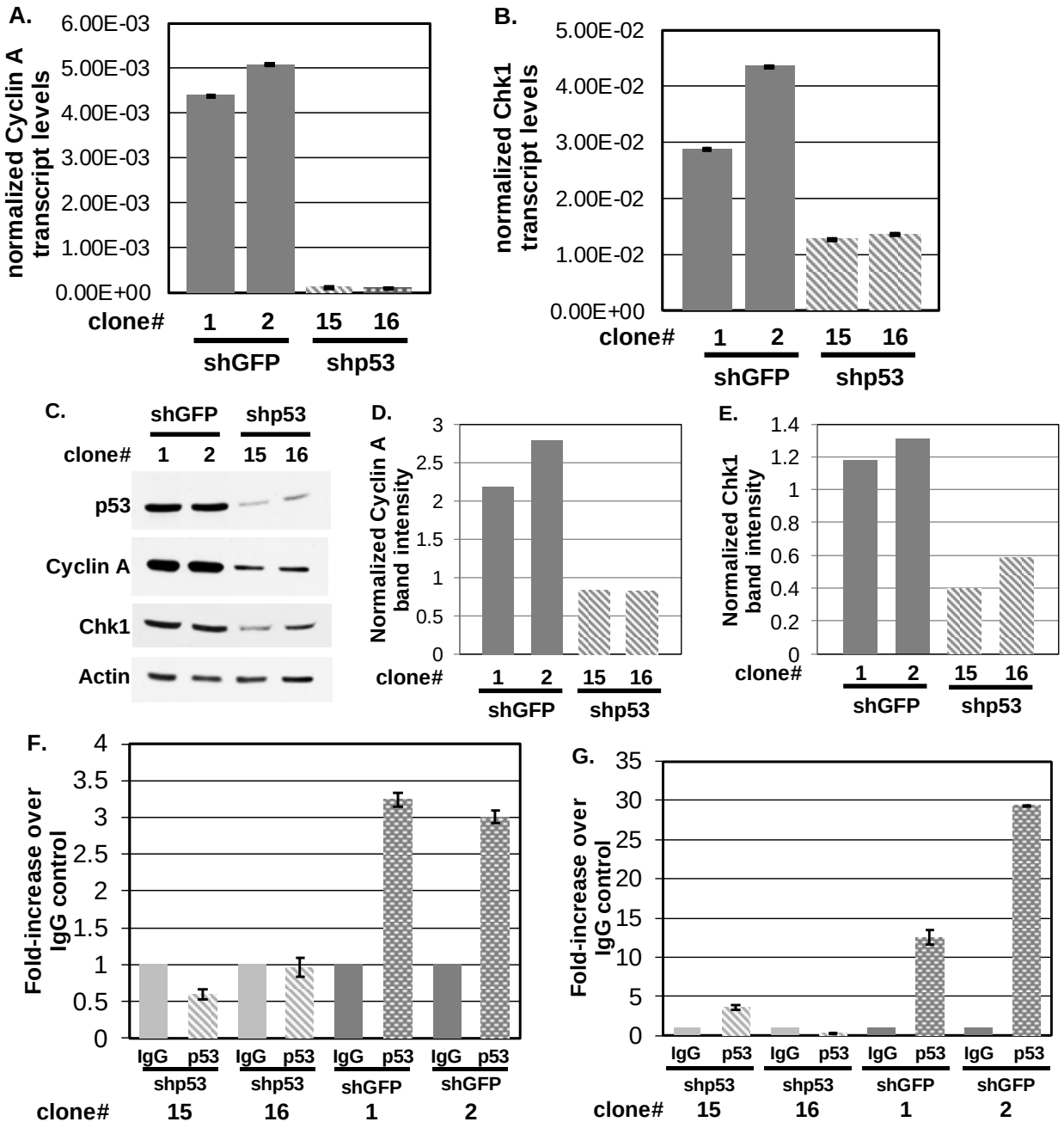


Figure S7: Knockdown of p53 (R175H) in human lung cancer VMRC cells reduces Cyclin A and Chk1 expression and localization on *CCNA2* and *CHEK1* promoter. Transcript levels of Cyclin A (A) and Chk1(B) in VMRC cells stably expressing shGFP or shp53 from a lentiviral vector were determined by RT-QPCR. Data represents QPCR values normalized to GAPDH levels and plotted as mean ± SEM. Cyclin A and Chk1 protein levels were determined by immunoblot analysis (C). Band intensities of Cyclin A (D) and Chk1 (E) were determined by densitometry and normalized by Actin loading control. p53 ChIP analyses of *CCNA2* (F) and *CHEK1* (G) promoters in p53-depleted (shp53) and mock-depleted (shGFP) VMRC cells. Fold increase over IgG control are plotted as mean ± SEM. Two clones of each construct were used in all experiments. The data represents two independent experiments.

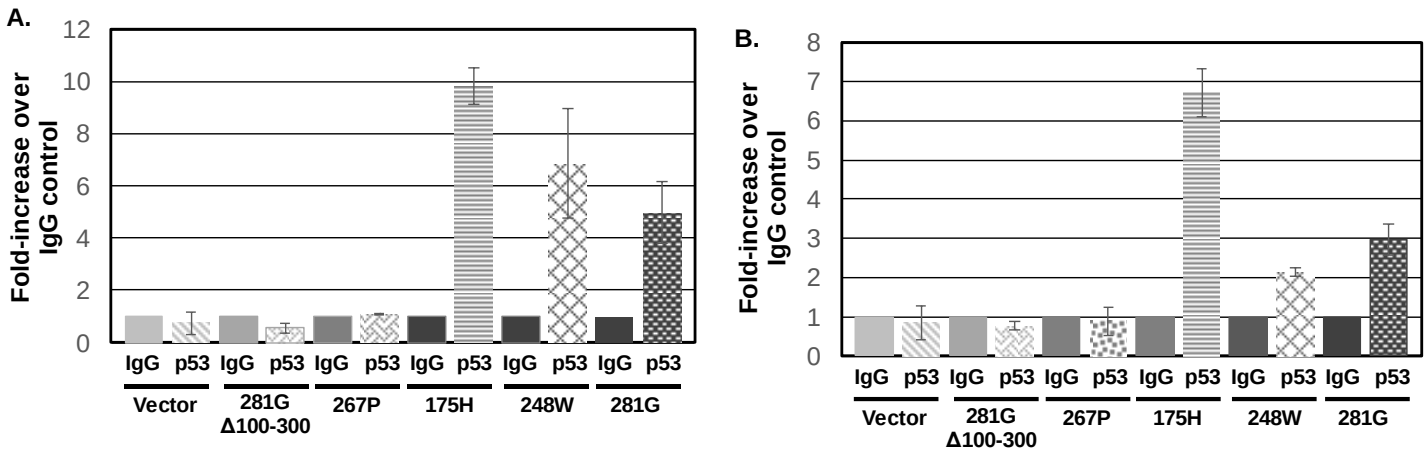


Figure S8: p53 mutants with compromised tumor formation ability do not show localization on *CCNA2* (A) and *CHEK1* (B) promoters. p53 ChIP analysis *CCNA2* and *CHEK1* promoters from H1299 cells stably expressing p53 mutants with (175H, 248W, 281G) and without (267P, 281GΔ100-300) GOF activity. Increase in immunoprecipitated promoter fragments by anti-p53 antibody over control IgG are shown by bar graphs. H1299 cell line stably expressing empty vector was used as control. Data is shown in bar graphs and plotted as mean ± SEM. The data represent three independent experiments.

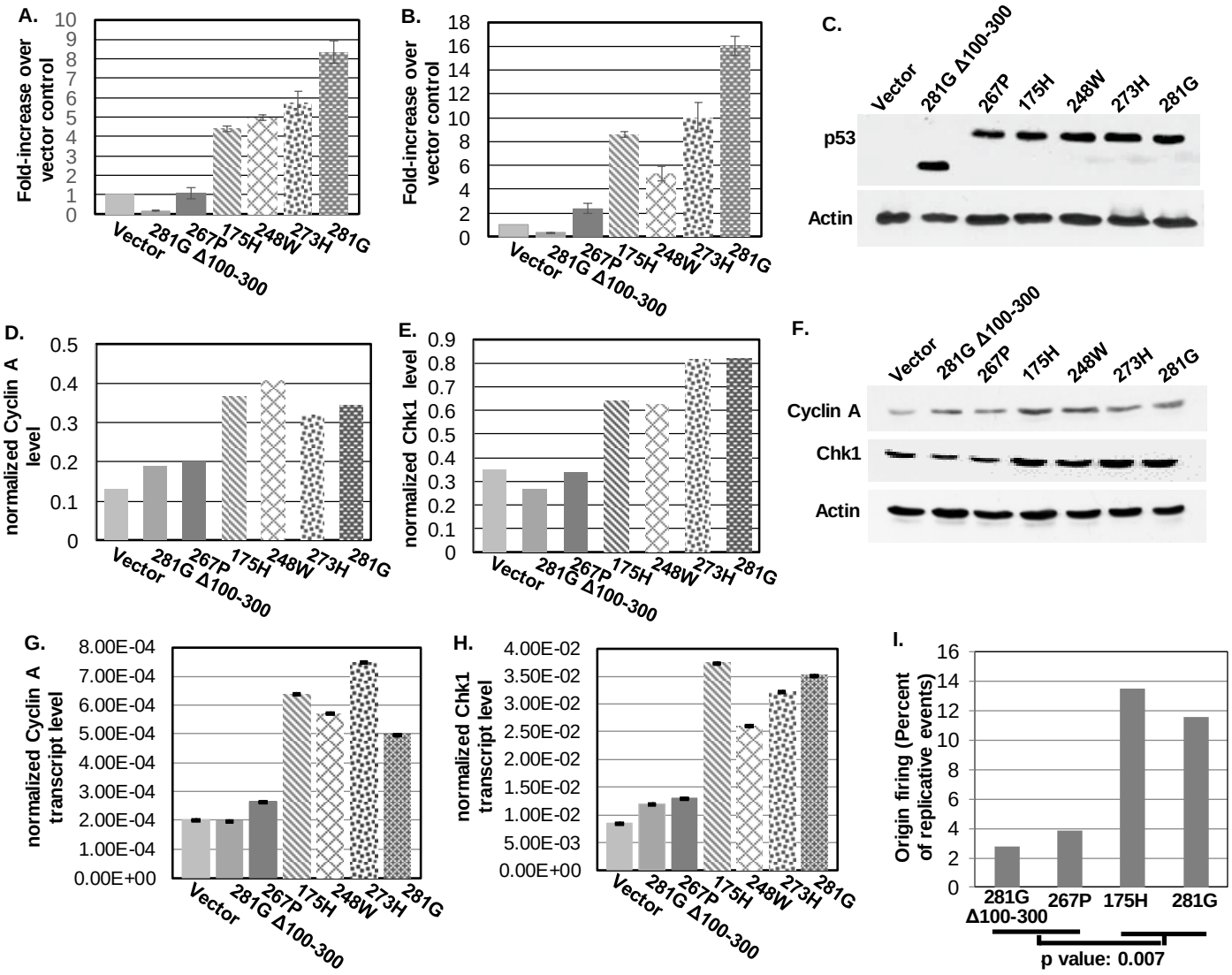


Figure S9: p53 mutants with compromised tumor formation ability do not upregulate of Cyclin A and Chk1 promoter activity (A-C) or expression of Cyclin A and Chk1 protein (D-F) and transcripts (G, H), and do not induce firing of DNA replication origins (F) in H1299 lung cancer cells. CCNA2 (A) and CHEK1 (B) promoter activities in the presence of p53 mutants with and without GOF activity were determined by transient transfection analysis using luciferase reporter. Increase in luciferase activities over vector control are shown by bar graphs as mean ± SEM of triplicate experiments. p53 expression was determined by immunoblot analysis (C). Actin is a loading control. Expression of Cyclin A and Chk1 protein were determined by immunoblot analysis and quantified by densitometry (D-F). The bar graphs (D, E) show densitometric values normalized to loading control (actin) of a representative experiment. Cyclin A and Chk1 transcripts (G, H) were determined by RT-QPCR. Data shown in bar graphs (G, H) are values normalized to GAPDH levels plotted as mean ± SEM of a representative experiment. Experiments were repeated three times. Origin firing in early S phase by p53 mutants in H1299 cells were determined by fiber analysis of replicating DNA as

described in the text and are shown by bar graphs as percent of origins in replicating fibers in each sample (F). Two non-GOFp53 mutants (281G Δ100-300, 267P) and two GOFp53 mutants (175H, 281G) were analyzed. Approximately 200 untangled fibers from each experiment were scored. The p-values are shown at the bottom of the bar graph calculated using Student's *t* test.

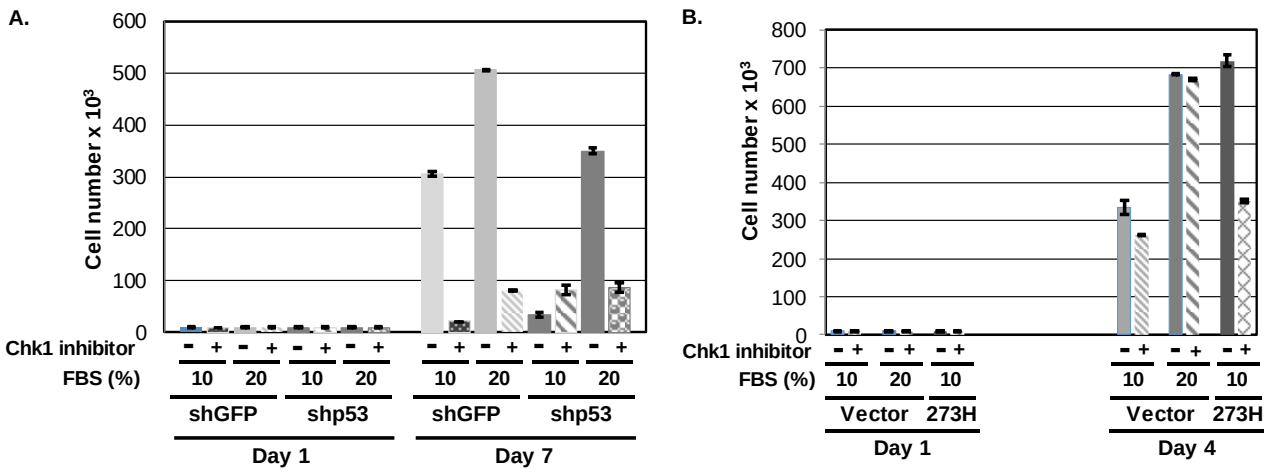


Figure S10: Artificial growth acceleration does not sensitize p53-null or GOF p53 expressing lung cancer cells to Chk1 inhibitor PF0047736. Proliferation of mock-depleted or p53-depleted H1048 lung cancer cells (A) or H1299 cells stably expressing p53R273H (273H) or a vector control (B) in the presence of Chk1 inhibitor PF00477736 (100nM), or only solvent (DMSO) and growth medium with 10% or 20% FBS. Data is shown as mean ± SD from triplicate experiments.

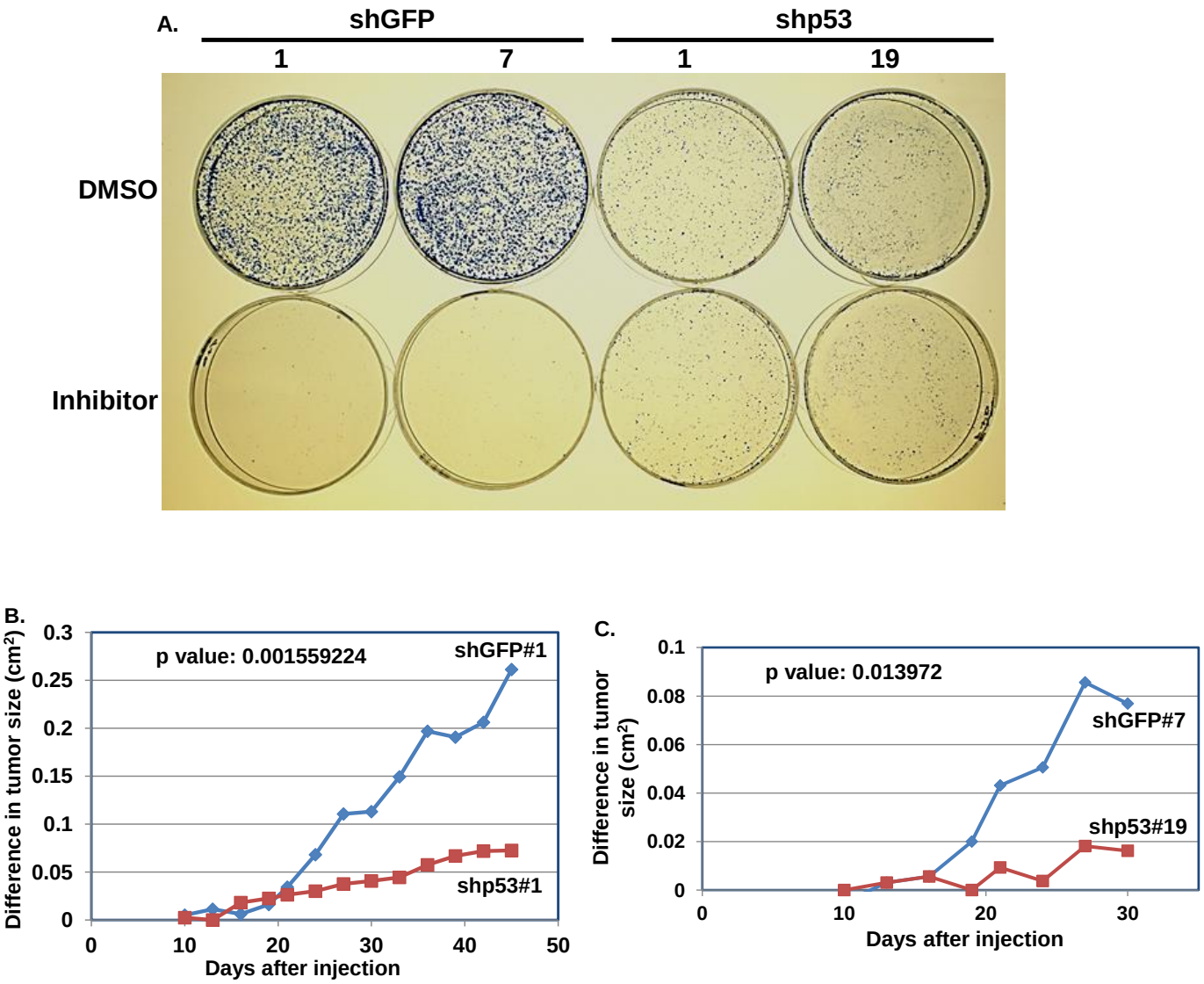


Figure S11: A small molecule inhibitor of Chk1, PF0047736 (100nM), (Inhibitor), selectively inhibits proliferation of H1048 lung cancer cells expressing shGFP as opposed to H1048 cells expressing shp53 and reduces tumor size.

A. Equal number (10,000) of cells were plated in each dish, and treated with PF0047736 (100nM) 24 hours after plating cells. Cells were fixed with methanol and stained with methylene blue on 7th day.

B, C. Extent of reduction in size of tumors generated by H1048 cells expressing shGFP (shGFP) or shp53 (shp53) after treatment with PF0047736 (10mg per Kg) with increase in time. Two different clone sets were injected. p values calculated using Student's *t* test are shown in the graphs.