Klement et al., http://www.jcb.org/cgi/content/full/jcb.201405077/DC1

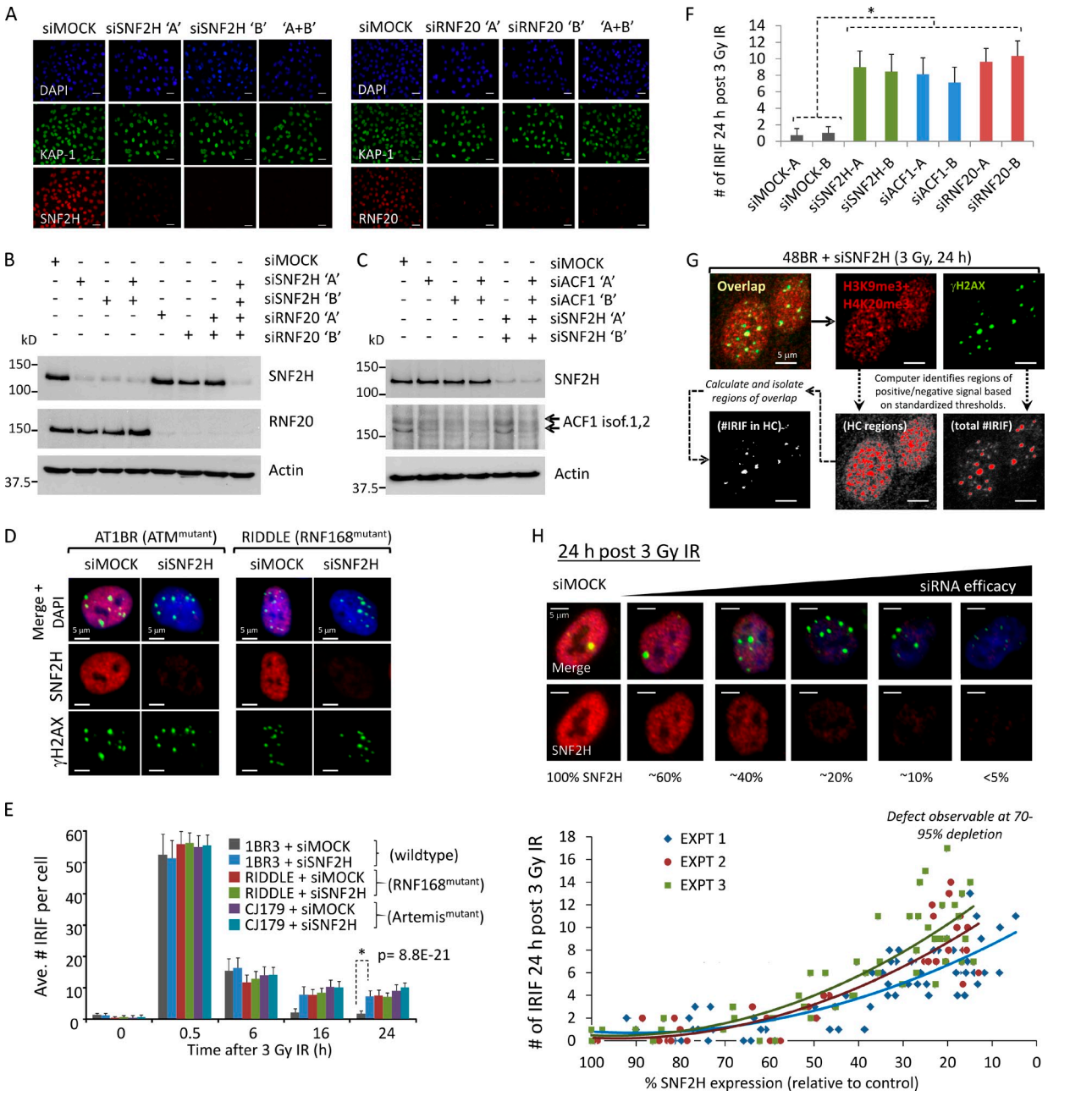

Figure S1. **Controls for SNF2H/RNF20/ACF1 depletion by siRNA and expression constructs.** (A) 48BR primary fibroblasts were treated with the indicated siRNAs toward either SNF2H or RNF20 and, 72 h later, immunostained for the indicated proteins. Bars, 40 µm. (B and C) 48BR cells were transfected with the indicated siRNAs and immunoblotted for indicated proteins. (D) SNF2H was depleted by siRNA (siMock = scrambled siRNA) in wild-type (1BR3), ATM mutant (AT1BR), RNF168 mutant (RIDDLE), or Artemis mutant (CJ179) quiescent primary human fibroblasts. Cells were irradiated with 3 Gy IR and immunostained 24 h later for SNF2H (red), γ-H2AX (green), and DAPI (blue). Bars, 5 µm. (E) The mean number of γ-H2AX per nucleus from cells prepared as in D and harvested at 0.5, 6, 16, and 24 h after 3 Gy IR was enumerated for three independent experiments. Error bars show SD. P-values (standard two-tailed Student's *t* test) are indicated. (F) Wild-type 48BR cells (as in Fig. 1, A and B) were transfected with individual siRNAs toward SNF2H, ACF1, or RNF20 as indicated. 72 h later, cells were irradiated with 3 Gy IR, stained with SNF2H/RNF20, γ-H2AX, and DAPI; γ-H2AX foci were enumerated as in E. Error bars show SD. The asterisk indicates statistical significance. (G) Methodology for examining γ-H2AX selectively within heterochromatic (HC) regions of primary human fibroblasts. Quiescent 48BR cells were treated with scrambled (mock) or SNF2H siRNA, irradiated, and harvested as indicated. Cells were then stained with γ-H2AX and H3K9me3 + H4K20me3 and imaged by confocal microscopy. Standardized regions of green-positive and red-positive signal were identified by software, which isolated overlap equating with γ-H2AX foci located in an H3K9me3 + H4K20me3–positive (heterochromatic) region. Bars, 5 µm. (H, top) A selection of 48BR cells with differing levels of knockdown after transfection with SNF2H siRNA, irradiated with 3 Gy, and immunostained for SNF2H and γ-H2AX 24 h later. Bars, 5 µm. (bottom) A quantification of number of γ-H2AX foci relative to quantified SNF2H expression. DSB repair defect were observed at 70–95% SNF2H depletion, and although the size of γ-H2AX decreased in cells with >95% SNF2H loss, the overall number of γ-H2AX foci were unaltered. Lines indicate second order polynomial trendlines. EXPT, experiment.

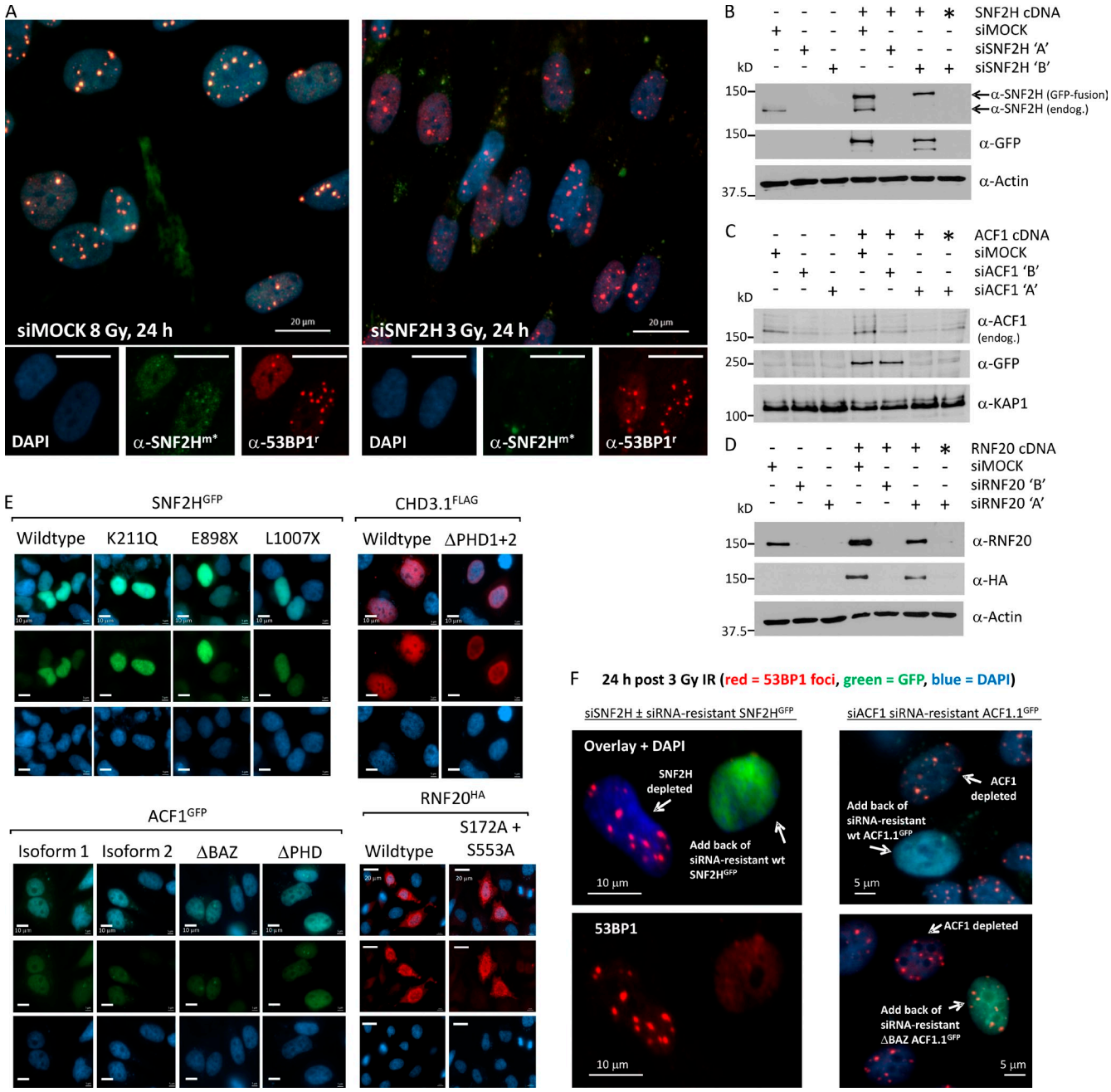

Figure S2. **Controls for SNF2H/RNF20/ACF1 depletion by siRNA and expression constructs.** (A) 48BR cells were transfected with Mock or SNF2H siRNA and, 72 h later, were irradiated with the indicated IR doses and immunostained for SNF2H (green), 53BP1 (red), and DAPI (blue). Bars, 20 µm. Asterisks indicate that the α-SNF2H mouse antibody (ab33747; Abcam) cross reacts weakly with an unknown protein present in IRIF. Please note that the specific signal for SNF2H is pannuclear only; in contrast, the α-SNF2H rabbit antibody (ab72499; Abcam) shown in Fig. 1 A (and used to detect SNF2H in all immunoblots) shows only pannuclear signal (no foci) that may be ablated by the same SNF2H siRNA used in Fig. S1 (A–C) and described in the Materials and methods. (B–D) HeLa cells were transfected with the indicated siRNAs and siRNA-resistant plasmids (SNF2H resistant to siRNA B, ACF1 resistant to siRNA A, and RNF20 resistant to siRNA B). The asterisks indicate the use of natural sequence cDNA, which would be susceptible to siRNA-mediated knockdown. 24 h after transfection, cells were extracted, and 50 µg of extract was resolved by PAGE and immunoblotted for the indicated proteins. siMOCK, mock siRNA. (E) Representative images of HeLa cells transfected with the indicated plasmids, demonstrating clear nuclear localization. In the case of RNF20 expression, in addition to a clear nuclear signal, a perinuclear staining was evident for all constructs as reported previously (Moyal et al., 2011). Bars, 10 µm. (F, left) An example image of SNF2H-depleted cells 24 h after 3 Gy IR (and stained for 53BP1) with and without SNF2H^{GFP} expression. Cells with the wild-type SNF2H^{GFP} displayed markedly less persisting IRIF relative to neighboring cells lacking wild-type SNF2H^{GFP}. (right) An example image of ACF1-depleted cells 24 h after 3 Gy IR (and stained for 53BP1) with and without ACF1.1^{GFP} expression. Cells with the wild-type ACF1.1^{GFP} displayed markedly less persisting IRIF relative to neighboring cells lacking wild-type ACF1.1^{GFP}, whereas expression of BAZ domain deleted ACF1.1^{GFP} showed a similar number of IRIF.

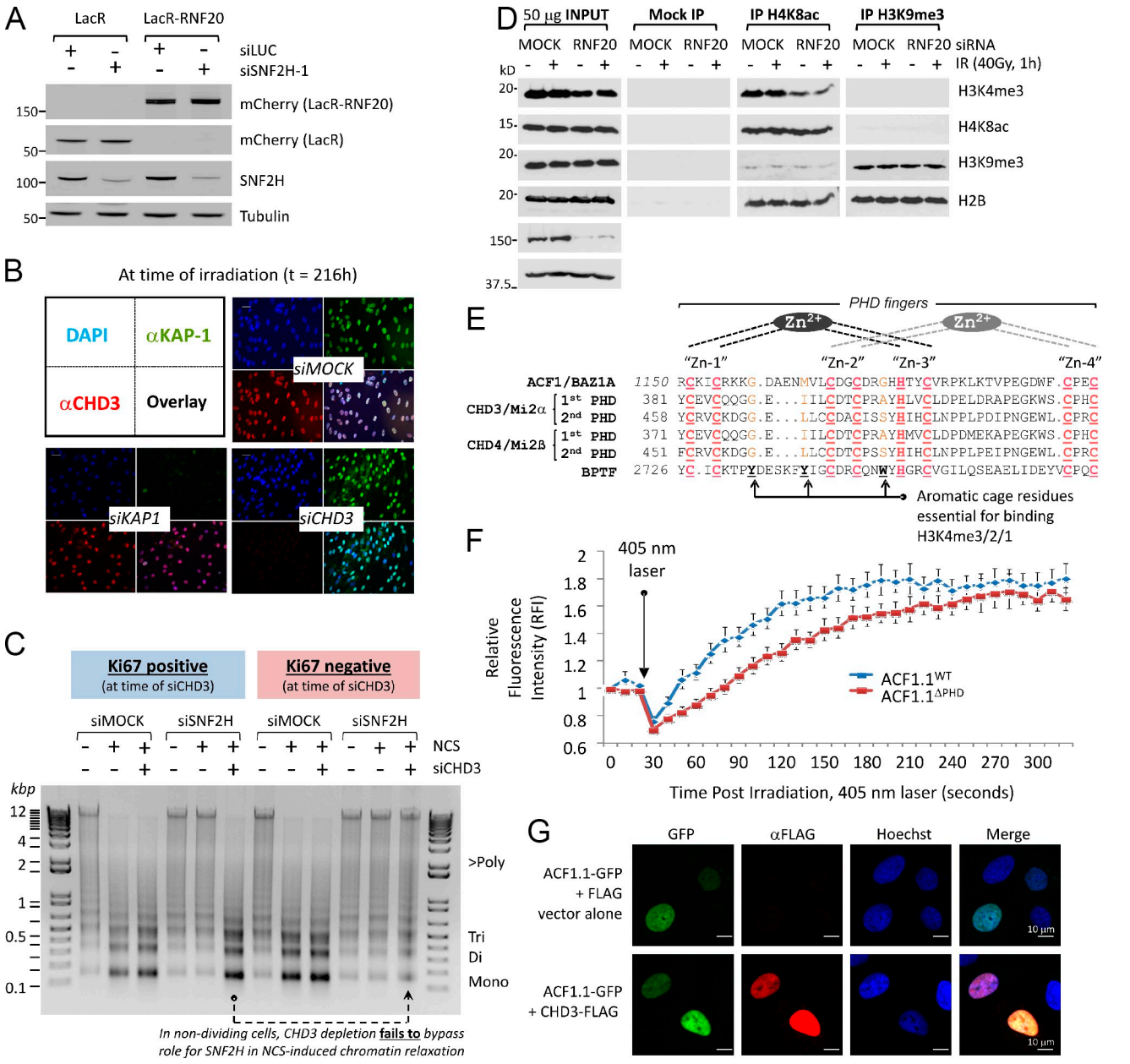

Figure S3. **Controls for LacR expression constructs, siRNA controls for CHD3.1 and KAP-1 knockdown, representative gels of MNase assays using primary fibroblasts, IP experiments using anti-H3K9me3 and anti-H4K8ac, alignments of the PHD fingers of ACF1 with CHD3.1, CHD4, and BPTF, and 405-nm laser microirradiation data with expression construct controls.** (A) Immunoblot controls for SNF2H knockdown for experiment described in Fig. 4 (D–G). siLUC, luciferase siRNA. (B) 48BR cells (as in Fig. 5) treated with either mock, CHD3, or KAP-1 siRNA were harvested (120 h time point from Fig. 5 A) and stained for CHD3, KAP-1, and DAPI to confirm efficacy of the initial siRNA treatment. Bars, 40 μm. (C) A representative agarose gel for the data presented in Fig. 5 B (i–iv). (D) HeLa cells were treated ±RNF20 siRNA and, 48 h later, irradiated as indicated, harvested, and processed into nucleosome-soluble extracts as outlined in methods. 500 μg extract was immunoprecipitated with either anti-H4K8ac (to isolate euchromatic nucleosomes) or anti-H3K9me3 (to isolate heterochromatic nucleosomes) and immunoblotted for the indicated proteins alongside 50 μg of whole cell extract as input. (D) Sample images of cells from the experiment described in Fig. 7 F, showing 53BP1 (green) and DAPI (blue) overlap staining. (E) Amino acid sequence alignment of the PHD fingers from human ACF1, CHD3, CHD4, and BPTF, indicating key zinc-coordination residues in red. Orange letters refer to sites of absent aromatic cage residues in ACF1, CHD3, and CHD4 relative to BPTF. (F) HeLa cells were transfected as in (Fig. 7 B) with either wild-type or PHD mutant ACF1.1 and incubated with Hoechst before irradiation with a directed 405-nm laser. GFP signal was imaged live over time. The relative fluorescence intensity increase for GFP signal obtained in D was quantified. Error bars show SD. (G) Representative images of cells from F showing coexpression of constructs. Bars, 10 μm.

Reference

Moyal, L., Y. Lerenthal, M. Gana-Weisz, G. Mass, S. So, S.Y. Wang, B. Eppink, Y.M. Chung, G. Shalev, E. Shema, et al. 2011. Requirement of ATM-dependent monoubiquitylation of histone H2B for timely repair of DNA double-strand breaks. *Mol. Cell.* 41:529–542. http://dx.doi.org/10.1016/j.molcel.2011.02.015