Supplementary Figures

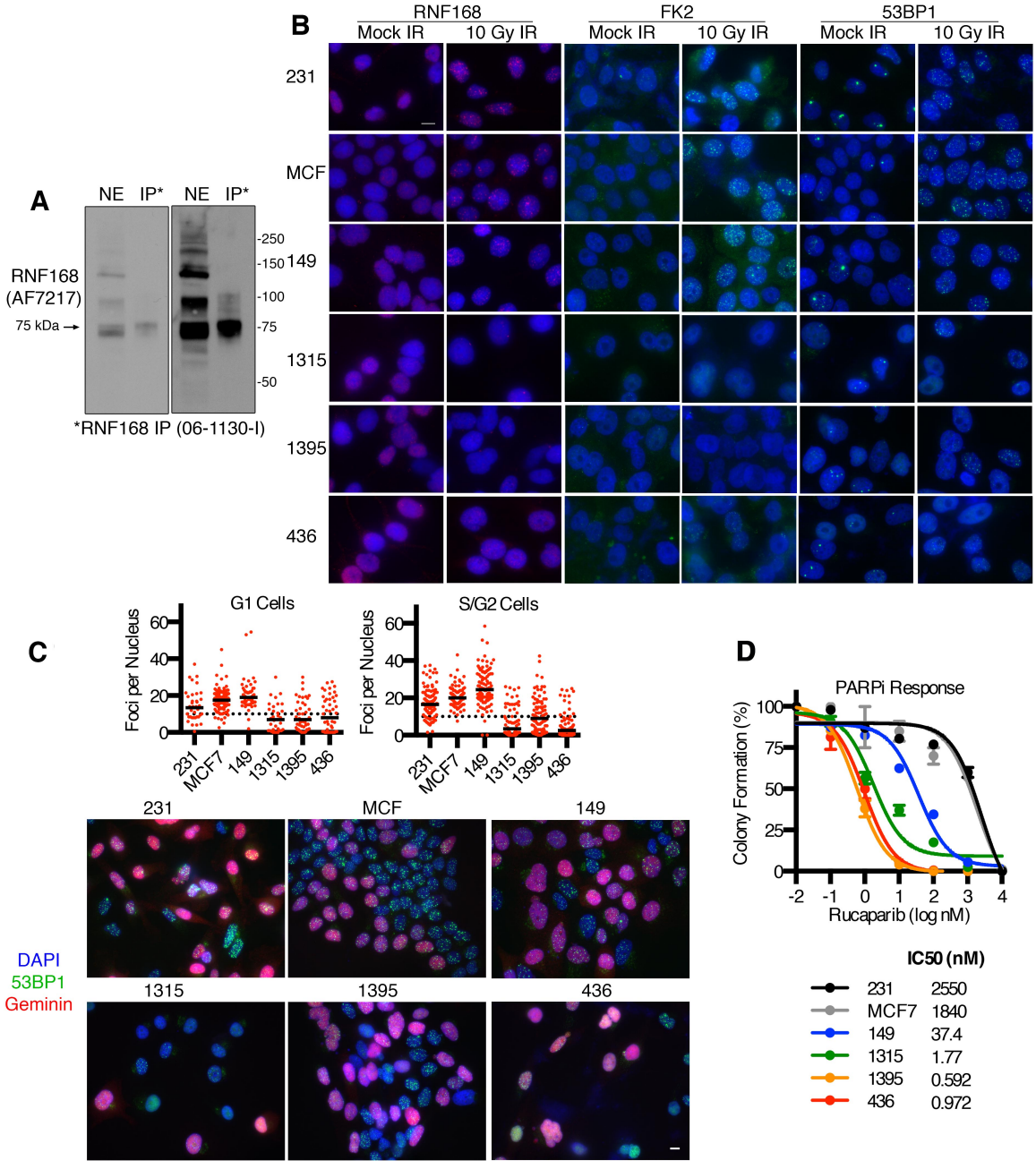

Figure S1. Cell line panel IRIF images

(**A**) RNF168 was immunoprecipated from MCF7 cells using polyclonal anti-RNF168 antibody 06-1130-I which uses full length RNF168 as the immunogen. The nuclear extracts (NE) and precipitated protein (IP) were separated for Western blotting and probed with anti-RNF168

antibody AF7217 which detects amino acids 423-565. Light (left) and dark (right) exposures and M.W. markers are shown. The presence of the band in the IP sample suggests RNF168 migrates at approximately 75 kDa.

(**B**) Representative images of cells assessed for RNF168, ubiquitin with FK2 antibody, and 53BP1 from **Fig. 1C-E**. Scale bar indicates 10 μm.

(**C**) Cell lines were evaluated for 53BP1 foci 8h post 10 Gy IR and co-stained for geminin to assess potential differences in IRIF that could be attributed to cell cycle phases. 53BP1 foci per nucleus were quantified for each nucleus separately for G1 cells (geminin negative) and S/G2 cells (geminin positive) for a minimum of 100 nuclei. Black solid lines indicate median value; black dotted line indicate 10 foci per nucleus, typically used to score foci positive cells. Representative images are shown, 10 μm scale bar.

(**D**) Cell lines indicated were incubated with PARPi and colony formation assays performed, IC50 values shown. BRCA1 wild-type MDA-MB-231 and MCF7 cell lines exhibited resistance to PARPi, while BRCA1 deficient SUM1315MO2, HCC1395, and MDA-MB-436 cells displayed sensitivity to PARPi. BRCA1-Δ11q expressing SUM149PT cells exhibited intermediate resistance to PARPi, in line with previous data that shows partial HR functionality of the Δ11q isoform[1].

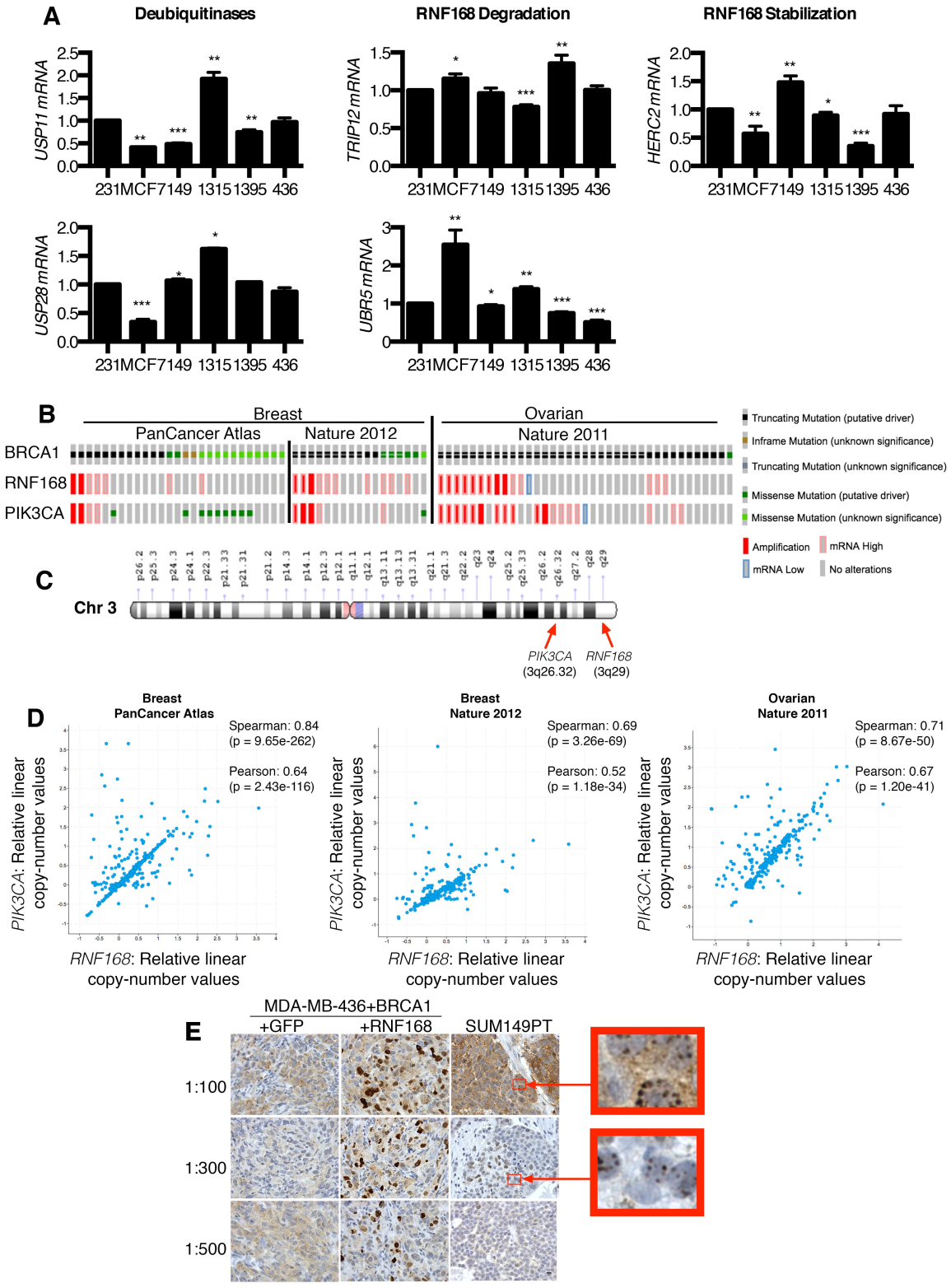
A
Deubiquitinases
RNF168 Degradation
RNF168 Stabilization
USP11 mRNA
TRIP12 mRNA
HERC2 mRNA
USP28 mRNA
UBR5 mRNA
231MCF7149 13151395 436
B
Breast
PanCancer Atlas
Nature 2012
Ovarian
Nature 2011
BRCA1
RNF168
PIK3CA
Truncating Mutation (putative driver)
Inframe Mutation (unknown significance)
Truncating Mutation (unknown significance)
Missense Mutation (putative driver)
Missense Mutation (unknown significance)
Amplification
mRNA High
mRNA Low
No alterations
C
Chr 3
PIK3CA (3q26.32)
RNF168 (3q29)
D
Breast PanCancer Atlas
Spearman: 0.84
(p = 9.65e-262)
Pearson: 0.64
(p = 2.43e-116)
Breast Nature 2012
Spearman: 0.69
(p = 3.26e-69)
Pearson: 0.52
(p = 1.18e-34)
Ovarian Nature 2011
Spearman: 0.71
(p = 8.67e-50)
Pearson: 0.67
(p = 1.20e-41)
PIK3CA: Relative linear copy-number values
RNF168: Relative linear copy-number values
E
MDA-MB-436+BRCA1
+GFP
+RNF168
SUM149PT
1:100
1:300
1:500

Figure S2. Assessment of RNF168 in tumors

(**A**) Cell lines were analyzed for the indicated mRNA levels by q-RT-PCR. Values are normalized to *HPRT* gene expression and presented as a fraction of expression in MDA-MB-231 cells. Mean ± S.D. is shown, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (unpaired t-test) compared to MDA-MB-231 cells.

(**B**) *BRCA1*, *RNF168*, and *PIK3CA* mutations, mRNA, and copy number alterations were examined in *BRCA1* mutant patient cohorts in the following datasets using cBioPortal: Breast Invasive Carcinoma (TCGA, PanCancer Atlas), Breast Invasive Carcinoma (TCGA, Nature 2012), Ovarian Serous Cystadenocarcinoma[2]. The results are based upon data generated by the TCGA Research Network[3,4]: https://www.cancer.gov/tcga.

(**C**) *RNF168* and *PIK3CA* gene locations on chromosome 3 are indicated. The close proximity of *RNF168* to known oncogenic driver and frequently amplified *PIK3CA* raises the possibility that *RNF168* amplifications may occur as passenger events.

(**D**) Relative linear copy-number values are plotted and correlation assessed for *PIK3CA* and *RNF168* from the complete datasets described in (B).

(**E**) RNF168 IHC was optimized using MDA-MB-436 xenografts with BRCA1 expression and GFP expression as a negative control and RNF168 expression as a positive control. SUM149PT xenografts were used as a control for endogenous levels of RNF168. RNF168 antibody dilutions of 1:100, 1:300, and 1:500 provided an optimal range of detection. The dilution of 1:300 was selected and utilized for additional experiments. Representative images are shown, 10 μm scale bar. Selected regions were enlarged to show nuclear localization of RNF168 and the presence of foci within the nucleus.

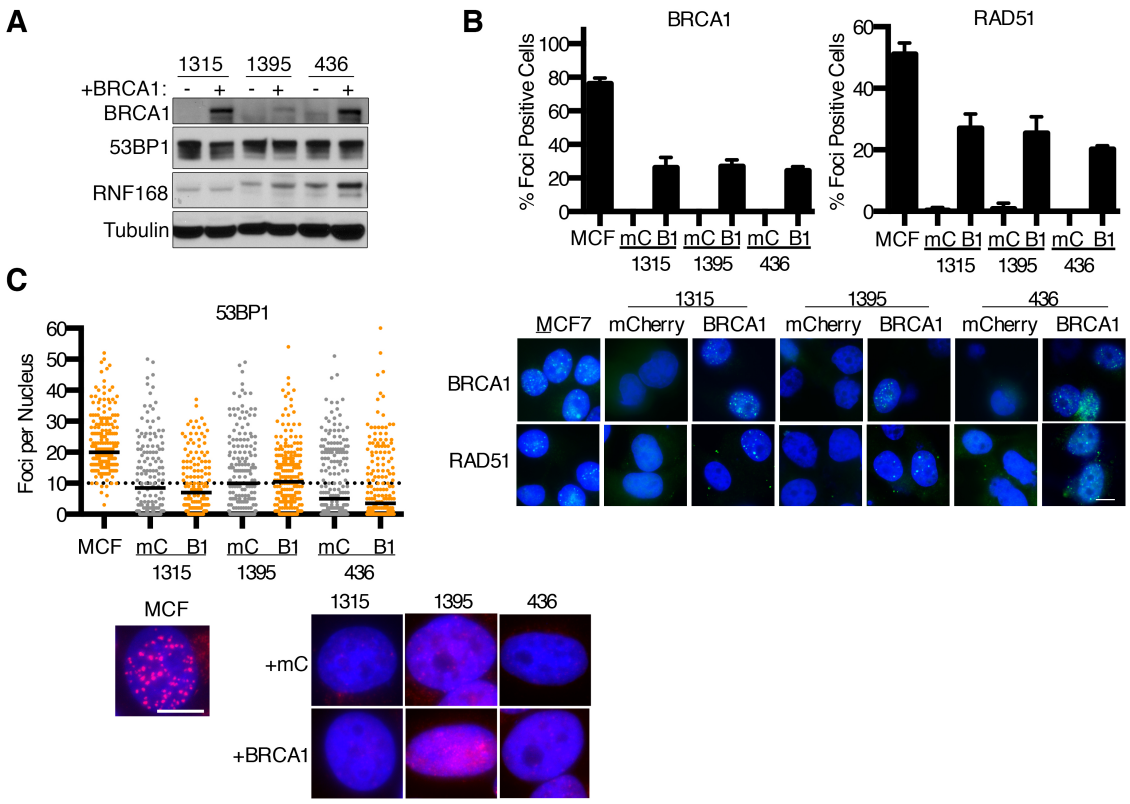

Figure S3. BRCA1 add back rescues RAD51 foci but not RNF168 expression

(**A**) BRCA1 deficient cell lines SUM1315MO2, HCC1395, and MDA-MB-436 were transduced for expression of wild-type BRCA1 or mCherry control with an IRES-GFP. Cells were sorted for GFP expression and routinely assessed for BRCA1 expression by Western blotting. 53BP1 and RNF168 protein levels were also assessed.

(**B**) Cells from (A) were assessed for BRCA1 localization and function by IRIF analysis for BRCA1 and RAD51 at 8h post 10 Gy IR. Foci positivity was quantified and mean ± S.D. shown. Wild-type BRCA1 expressing MCF7 cells served as a positive control. Representative images are shown, 10 μm scale bar.

(**C**) Cells were analyzed for 53BP1 IRIF. The number of foci per nucleus is shown, a minimum of 100 nuclei were assessed. Black solid lines indicate median value; black dotted line indicates 10 foci per nucleus. Representative nuclei are shown, 10 μm scale bar.

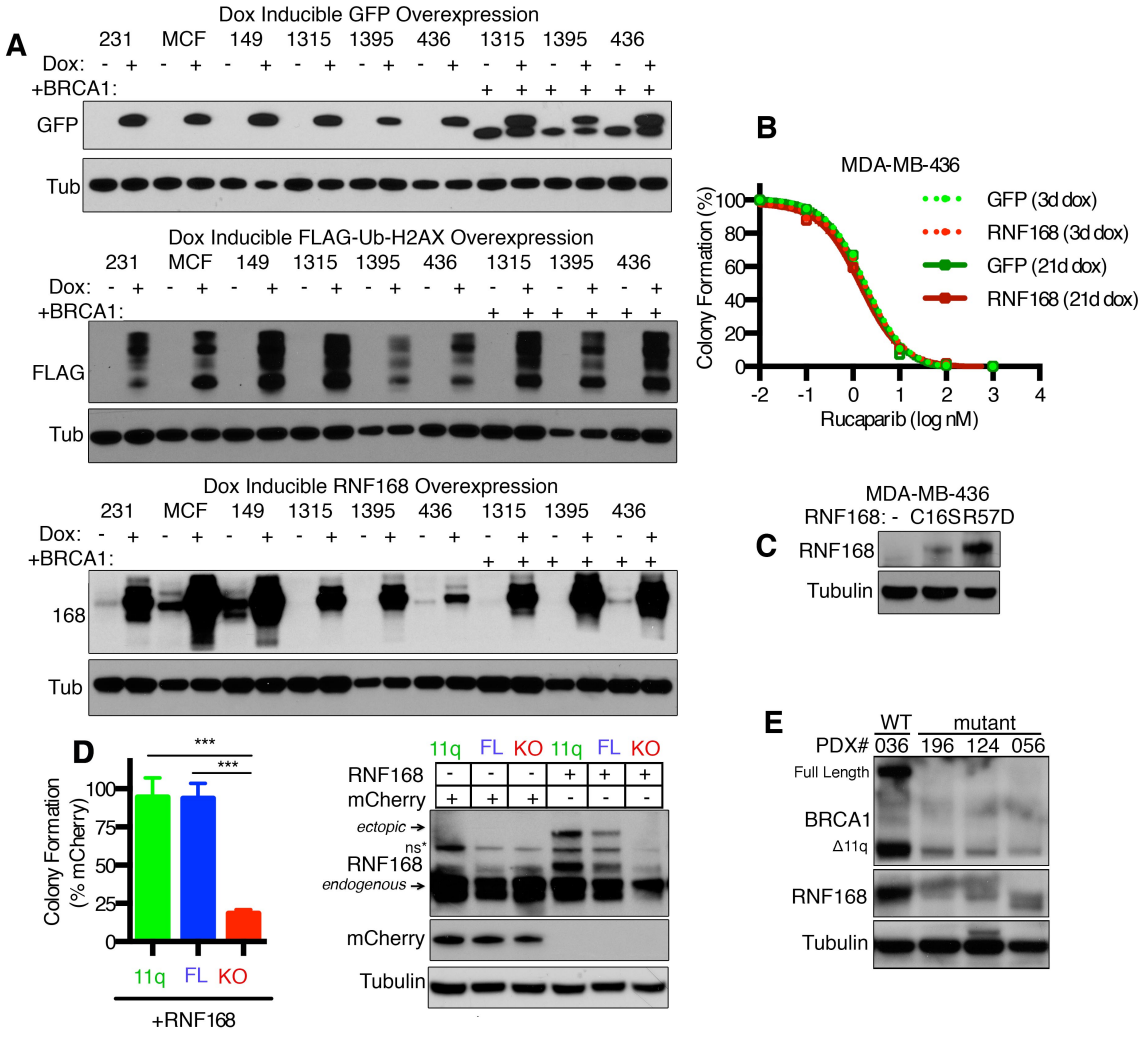

Figure S4. Generation of dox-inducible GFP, ub-H2AX, and RNF168 expressing cells

(**A**) Lentivirus containing constructs for dox-controlled expression of GFP, ub-H2AX, and RNF168 were transduced into MDA-MB-231, MCF7, SUM149PT, SUM1315MO2, HCC1395, and MDA-MB-436 cell lines as well as ectopic BRCA1 expressing SUM1315MO2, HCC1395, and MDA-MB-436 as isogenic controls. Transduced cells were selected with puromycin then cultured in the presence or absence of 4 μg/ml dox for 3 days, collected, and lysates subjected to Western blotting. Dox minus cells were maintained in media containing TET-free FBS. Expression was achieved in all cell lines but ub-H2AX and RNF168 expression was generally lower in BRCA1 deficient cell lines. See **Fig. 3** for more details.

(**B**) MDA-MB-436 cell lines expressing dox-inducible GFP or RNF168 were incubated with dox for 3 or 21 days prior to seeding for colony formation assays in the presence of PARPi.

(**C**) Wild type RNF168 and C16S and R57D constructs were expressed in MDA-MB-436 cells. C16S is a RING domain mutation that significantly reduces the E3 ligase activity of RNF168. The R57D mutation prevents the ubiquitination of H2AX. MDA-MB-436 cells with constitutive expression of wild-type RNF168 did not survive blasticidin selection and no lysate was able to be collected. However, $RNF168^{C16S}$ and $RNF168^{R57D}$ expressing MDA-MB-436 cells selected for stable integration using blasticidin and lysates collected for Western blotting.

(**D**) CRISPR/Cas9 was previously used to generate SUM149PT cell lines with additional BRCA1 mutations that restore the reading frame and expression of the full-length BRCA1 protein (FL) or block expression of the full-length and BRCA1-Δ11q isoforms (KO), or control sgGFP that maintain endogenous expression of BRCA1-Δ11q (11q)[1]. The 11q, FL, and KO SUM149PT derivatives were transduced with mCherry or mCherry-tagged RNF168, sorted, and assessed for colony formation. Colony formation was normalized to mCherry controls, mean ± S.D. is shown, ***$p < 0.001$ (unpaired t-test). Inset, cells cultured for 2 weeks post sorting were subjected to Western blotting for the indicated proteins.

(**E**) *BRCA1* WT PDX036 and *BRCA1* mutant PDX196 (2080delA), PDX124 (2080delA,) PDX056 (895delGT) tumors were assessed for BRCA1 and RNF168 protein expression by Western blot. BRCA1 full-length and Δ11q isoforms are indicated. Subsequent analyses were performed in **Fig. 4C-D**.

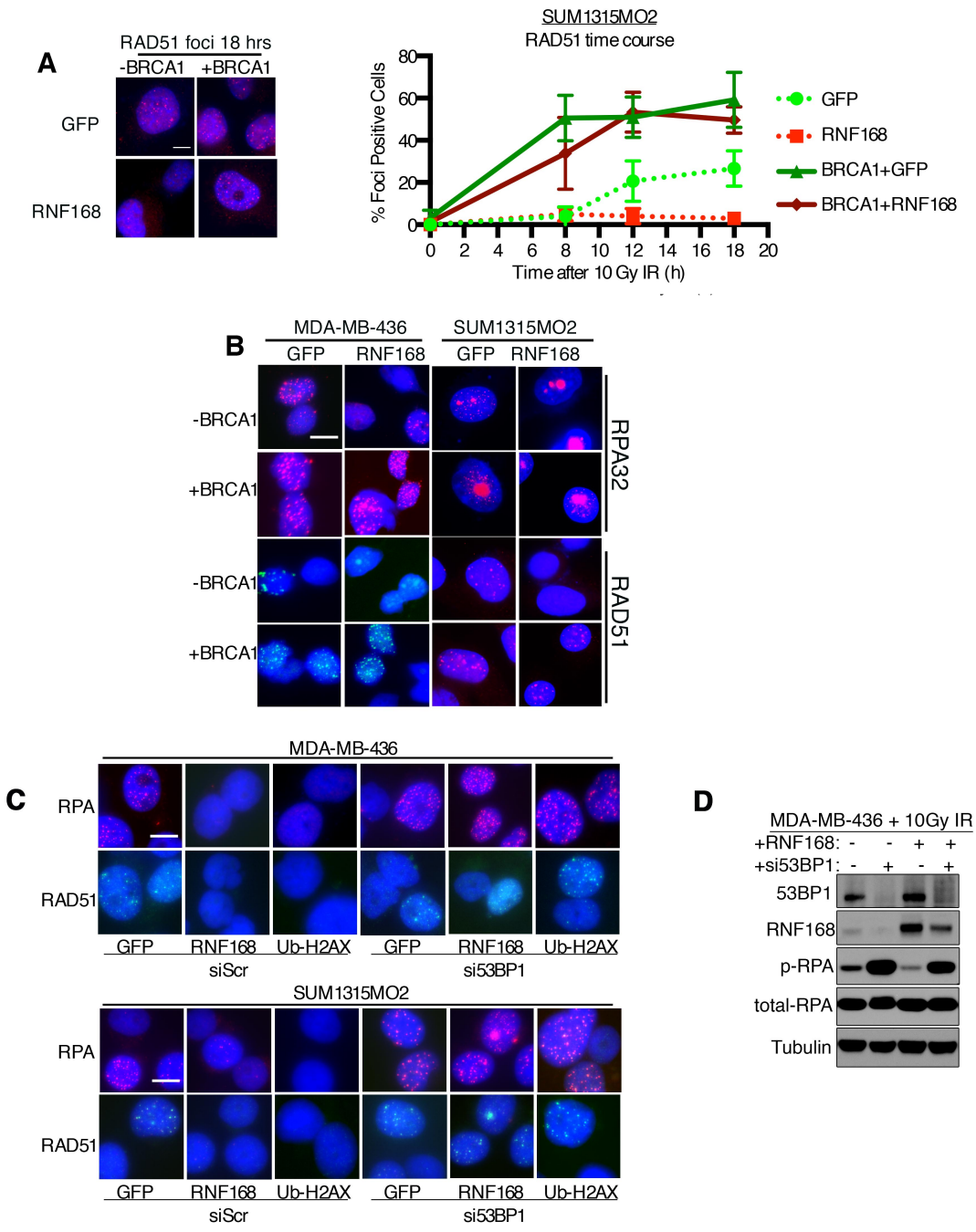

Figure S5. RPA32 and RAD51 foci in RNF168-expressing cells

(**A**) Dox-inducible GFP and RNF168 SUM1315MO2 +/- BRCA1 cells were assessed for RAD51 foci formation at time points from 0 to 18h post 10 Gy IR. Nuclei were scored as foci positive with 5 or more foci, mean ± S.D. shown. Representative nuclei are shown from the 18h time point, 10 μm scale bar. BRCA1 expressing cells exhibited RAD51 foci formation earlier and at higher levels

then BRCA1 deficient cells, regardless of RNF168 expression. However, BRCA1-deficient cells that expressed the GFP control exhibited detectable RAD51 foci at 18h after irradiation that was substantially reduced in RNF168 expressing cells.

(**B**) Representative images of cells assessed for RPA32 and RAD51 foci from **Fig. 5B**, 10 μm scale bar.

(**C**) Representative images of cells assessed for RPA32 and RAD51 foci from **Fig. 6A-B**, 10 μm scale bar.

(**D**) Dox-inducible GFP and RNF168 MDA-MB-436 cells were treated with scrambled (Scr) or 53BP1 targeted siRNA and subjected to 10 Gy IR. Cells were collected after 8h and lysates subjected to Western blotting for the indicated proteins. Phosphorylated S4/8 RPA32 (p-RPA) is an indicator of resection, and controlled for by total RPA levels. 53BP1 knockdown dramatically enhanced p-RPA, however p-RPA was still observed in GFP expressing MDA-MB-436 cells with a substantial decrease exhibited by RNF168 expressing cells. This supports IRIF based observations that RNF168 reduces resection via 53BP1 recruitment.

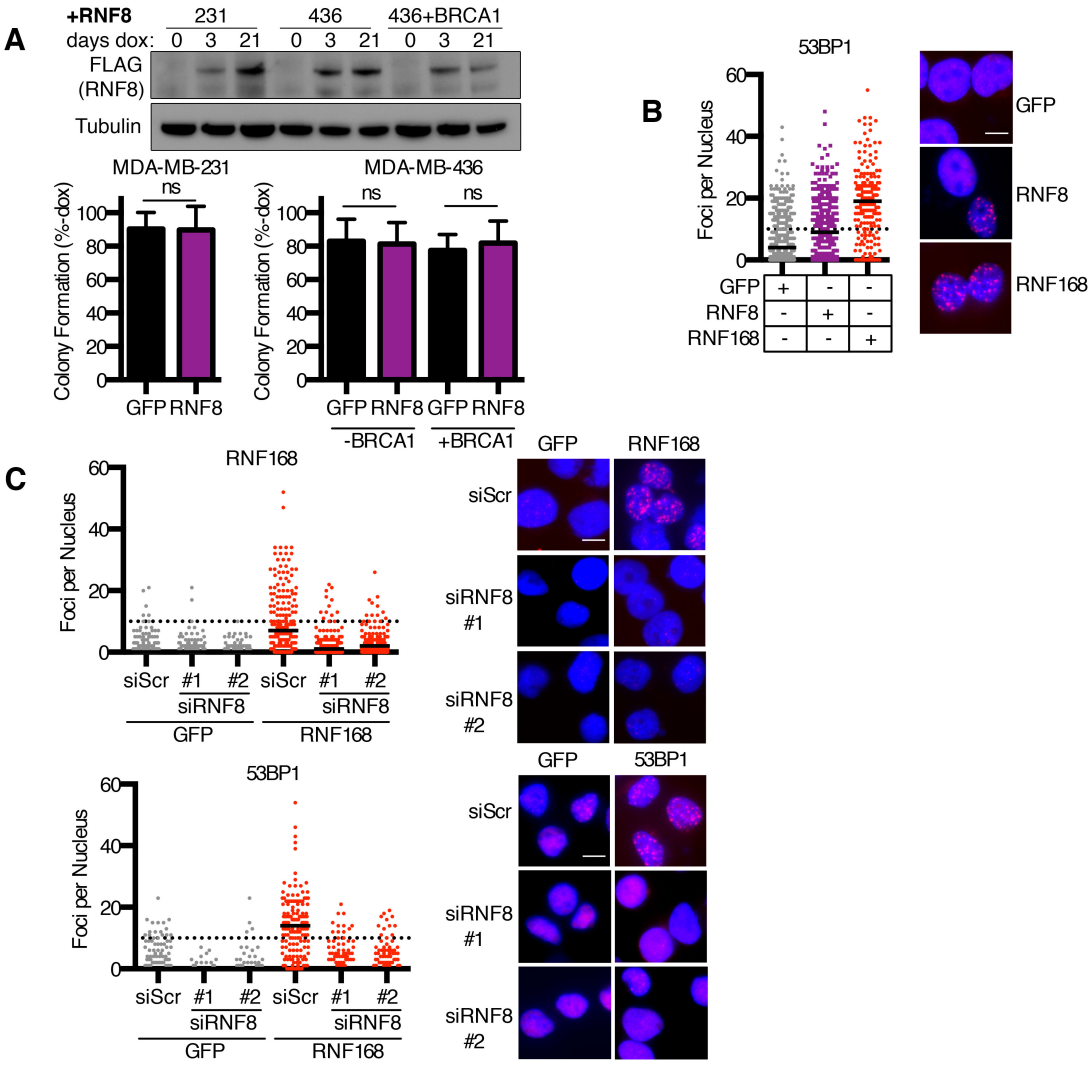

Figure S6. Effects of ectopic RNF8 on proliferation and IRIF

(**A**) RNF8 was overexpressed under dox control in MDA-MB-231, MDA-MB-436 and MDA-MB-436+BRCA1 cell lines. Expression was evaluated by Western blot at 0, 3, and 21 days of dox incubation. *Below*, cell lines were assessed for colony formation in the absence or presence of dox. Colony formation of dox treated cells is shown as a percentage of colonies formed in the absence of dox. Mean ± S.D. is shown, (ns), not significant (unpaired t-test).

(**B**) 53BP1 foci per nucleus was quantified in MDA-MB-436 cells overexpressing GFP, RNF8, and RNF168. Representative images are shown. Scale bar indicates 10 μm.

(**C**) MDA-MB-436 cells expressing dox-inducible GFP or RNF168 were treated with scrambled (Scr) or RNF8 targeting siRNA for 72h and assessed for RNF168 and 53BP1 IRIF. Foci per nucleus values and representative images are shown. Black solid lines indicate median value; black dotted line indicate 10 foci per nucleus. Scale bar indicates 10 μm.

Supplemental References

1 Wang, Y. *et al.* The BRCA1-Delta11q Alternative Splice Isoform Bypasses Germline Mutations and Promotes Therapeutic Resistance to PARP Inhibition and Cisplatin. *Cancer Res* **76**, 2778-2790, doi:10.1158/0008-5472.CAN-16-0186 (2016).

2 Cancer Genome Atlas Research, N. Integrated genomic analyses of ovarian carcinoma. *Nature* **474**, 609-615, doi:10.1038/nature10166 (2011).

3 Gao, J. *et al.* Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* **6**, pl1, doi:10.1126/scisignal.2004088 (2013).

4 Cerami, E. *et al.* The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* **2**, 401-404, doi:10.1158/2159-8290.CD-12-0095 (2012).