Supplementary Figure legends

Fig.S1. Establishment of mouse embryonic fibroblast cell lines that stably overexpress RNF168 or 53BP1. (A) Wild-type, BRCA1$^{\Delta 11/\Delta 11}$ and BRCA1$^{\Delta 11/\Delta 11}$53BP1$^{-/-}$ MEFs were stably transduced with retroviral vectors encoding RNF168^{WT} or RNF168^{R57D} and maintained under antibiotic selection (2 μg/ml puromycin). Samples were collected for Western blot analysis. (B) Similar to A, except cells were stably transduced with retroviral vector encoding 53BP1^{DB}. (C) WT and BRCA1$^{\Delta 11/\Delta 11}$ MEFs were stably transduced with retroviral vector encoding 53BP1^{DN}. Cells were irradiated at 2 Gy and processed 1 h later for standard immunofluorescence. Samples were stained for HA (red) and imaged at 63x magnification. Note that anti-HA antibodies yield a cross-reacting, non-specific band at a molecular weight identical to that predicted for 53BP1^{DN}, thus ruling out unambiguous detection by Western blot; 53BP1^{DN} is not recognized by any commercially available anti-53BP1 antibody. (D) 53BP1$^{-/-}$ MEFs stably transduced with retroviral vector encoding 53BP1^{DB} were treated with 1 μM PARPi (24 h) and harvested for preparation of metaphase spreads. Dot plots indicate the total amount of aberrations per cell in two independent experiments. At least 100 metaphases were analyzed for each condition.

Fig.S2. RNF168 overexpression results in persistently active 53BP1. BRCA1$^{\Delta 11/\Delta 11}$ MEFs were stably transduced with retroviral vectors encoding RNF168^{WT} or RNF168^{R57D}. (A) BRCA1$^{\Delta 11/\Delta 11}$ MEFs stably transduced with retroviral vectors encoding RNF168^{WT} or RNF168^{R57D} were irradiated with 10 Gy and fixed 4 h later. Samples were stained for conjugated ubiquitin (FK2, green) and imaged at 63x magnification. (B-D) BRCA1$^{\Delta 11/\Delta 11}$ MEFs stably transduced with retroviral vectors encoding RNF168^{WT} or RNF168^{R57D} were irradiated with 2 Gy and fixed at the indicated time-points post-IR and processed for standard immunofluorescence. Samples were stained for 53BP1 (red) and imaged at 63x magnification. A representative experiment is shown in B. (C) Quantification of 53BP1 foci. (D) Histograms show the percentages of cells that contain >5 foci of 53BP1 (left panel) or RIF1 (right panel) in three independent experiments. At least 100 cells were scored for each sample and treatment condition. Statistical significance was determined with two-tailed unpaired Student's *t*-test; *, $P < 0.05$ compared to empty vector-transduced cells.

Fig.S3. 53BP1 overexpression antagonizes end resection. (A, B) BRCA1$^{\Delta 11/\Delta 11}$ MEFs were stably transduced with retroviral vectors encoding 53BP1^{DB} or 53BP1^{DN}. (A) BRCA1$^{\Delta 11/\Delta 11}$ cells were irradiated (10 Gy, 4 h) or treated with 1 μM PARPi for 24 h. Samples were processed for standard immunofluorescence. Left panel: cells were co-stained for HA-tag (green) and RAD51 (red) and imaged at 63x magnification. A representative experiment is shown. Right panel: the percentage of cells that contain >10 RAD51 foci in three independent experiments. At least 200 cells were scored for each sample and treatment condition. (B) BRCA1$^{\Delta 11/\Delta 11}$ cells were

irradiated with 10 Gy and fixed 4 h later for RPA2 staining followed by high-throughput automated imaging; mean integrated nuclear intensities for chromatin-bound RPA2 was determined for each of >5,000 individual cells. (C) Wild-type cells were stably transduced with retroviral vectors encoding $53BP1^{DB}$ and irradiated (10 Gy, 4 h) or treated with 1 μM PARPi for 24 h. Samples were processed for standard immunofluorescence. Left panel: cells were co-stained for HA-tag (green) and RAD51 (red) and imaged at 63x magnification. A representative experiment is shown. Right panel: the percentage of cells that contain >10 RAD51 foci in two independent experiments. At least 100 cells were scored for each sample and treatment condition. For A and B, statistical significance was determined with two-tailed unpaired and paired Student's *t*-test; *, $P < 0.05$ compared to empty vector-transduced cells.

Fig.S4. Overexpression of RNF168 or 53BP1 does not alter cell cycle progression. $BRCA1^{\Delta 11/\Delta 11}$ MEFs stably transduced with retroviral vectors encoding $RNF168^{WT}$, $RNF168^{R57D}$, $53BP1^{DB}$ or $53BP1^{DN}$ were irradiated with 10 Gy and fixed at indicated time points post-IR for cell cycle analysis. Alternatively, cells were treated with 1 μM PARPi for 24 h. Histograms show the percentage of cells in each cell cycle phase from three independent experiments.

Fig.S5 RNF168 and 53BP1 are not limiting factors for CSR in B cells. Wild-type splenic B cells were transduced with retroviral vectors encoding $RNF168^{WT}$, $RNF168^{R57D}$ or $53BP1^{DB}$ and cultured in the presence of LPS/IL-4/RP105 to stimulate CSR. Histograms depict the relative frequency of IgG_1 expression in B220-positive B cells from three independent experiments.

Supplementary References

1. Cancer Genome Atlas, N. (2012) Comprehensive molecular portraits of human breast tumours. *Nature*, **490**, 61-70.
2. Cancer Genome Atlas Research, N. (2011) Integrated genomic analyses of ovarian carcinoma. *Nature*, **474**, 609-615.
3. Cerami, E., Gao, J., Dogrusoz, U., Gross, B.E., Sumer, S.O., Aksoy, B.A., Jacobsen, A., Byrne, C.J., Heuer, M.L., Larsson, E. *et al.* (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer discovery*, **2**, 401-404.

4. Gao, J., Aksoy, B.A., Dogrusoz, U., Dresdner, G., Gross, B., Sumer, S.O., Sun, Y., Jacobsen, A., Sinha, R., Larsson, E. *et al.* (2013) Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Science signaling*, **6**, pl1