Grotsky et al., http://www.jcb.org/cgi/content/full/jcb.201204053/DC1

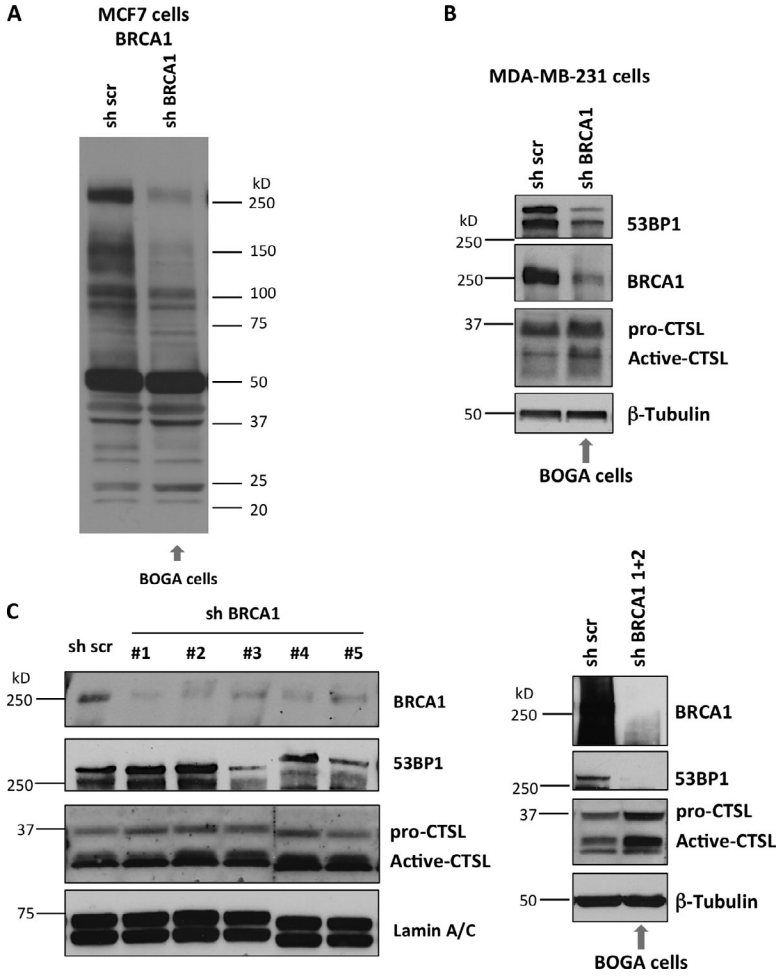

Figure S1. **Activation of CTSL-mediated degradation of 53BP1 in BRCA1- deficient breast cancer cells.** (A) Western blot showing the marked depletion of BRCA1 by one shRNA generated by J. Zhang. Note the main decrease in signal at 250 kD. The antibody detected nonspecific bands of lower molecular mass. (B) MDA-MB-231 breast cancer cells were lentivirally transduced with shRNA control (sh scr) or shBRCA1 as in A. Shortly after selection, BRCA1-deficient cells underwent growth arrest. Approximately 10 d later, MDA-MB-231 cells resumed proliferation. Western blots performed in cells that bypass growth arrest (BOGA cells) show up-regulation of CTSL and degradation of 53BP1. (C) MCF7 cells were lentivirally transduced with five different shRNAs specific for BRCA1 or shRNA control (sh scr). Western blots show a reduction of BRCA1 with all hairpins, increased CTSL levels with four hairpins, and degradation of 53BP1 with hairpins 3 and 5 (left). However, the depletion of BRCA1 and the activation of CTSL-mediated degradation of 53BP1 are modest (left). As such, none of the five cell lines fully growth arrested. Thus, we combined shRNAs 1 and 2 for depletion of BRCA1. We achieved a marked depletion of BRCA1 and a clear growth arrest. Importantly, BOGA cells generated with these hairpins activated CTSL-mediated degradation of 53BP1 (right). Overall, loss of BRCA1 with different shRNAs activates CTSL-mediated degradation of 53BP1 in different types of breast cancer cells.

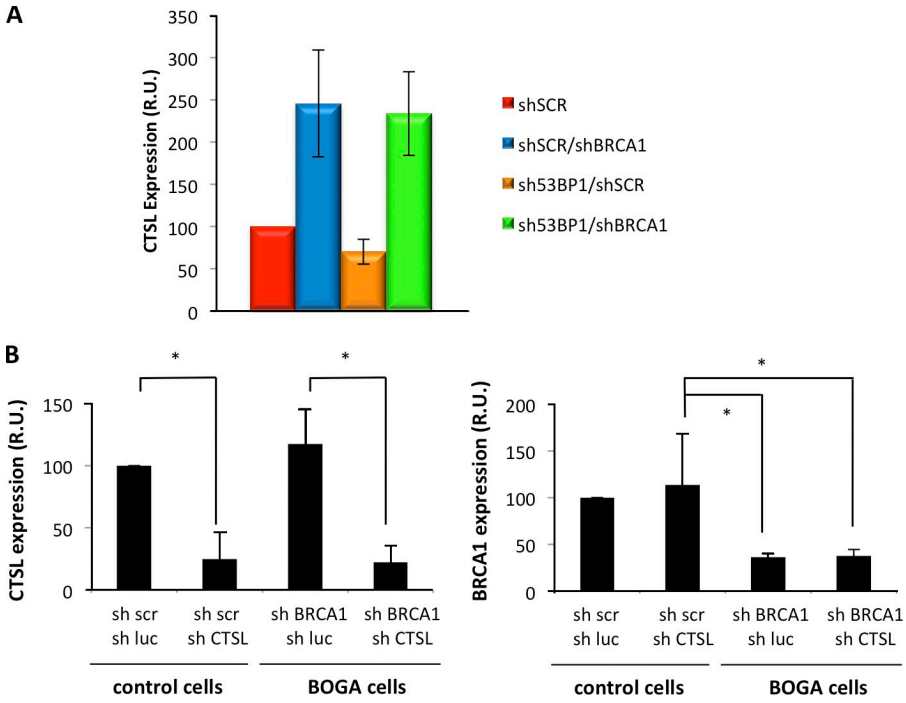

Figure S2. **Transcript levels of CTSL and BRCA1 in cells double deficient in BRCA1/53BP1 or BRCA1/CTSL.** (A) MCF7 cells were lentivirally transduced with shRNA specific for depletion of 53BP1 or shRNA control (sh scr). After selection, cells were transduced with shRNA specific for BRCA1. After double selection (puromycin and G418), transcript levels of CTSL were monitored in the different cell lines. Note how CTSL is up-regulated in cells deficient in both 53BP1 and BRCA1 (see Fig. 2 A). The mean of two independent experiments is presented. (B) MCF7 control and BOGA cells were lentivirally transduced with shRNA specific for depletion of CTSL or shRNA control. Graphs show the relative expression of CTSL (left) and BRCA1 (right) in the different cell lines as determined by qRT-PCR. Note the marked down-regulation of CTSL transcripts in control and BOGA cells transduced with shCTSL. The mean and standard deviation of three independent experiments is shown. Asterisk shows the p-value of statistical significance (*, $P \leq 0.05$).

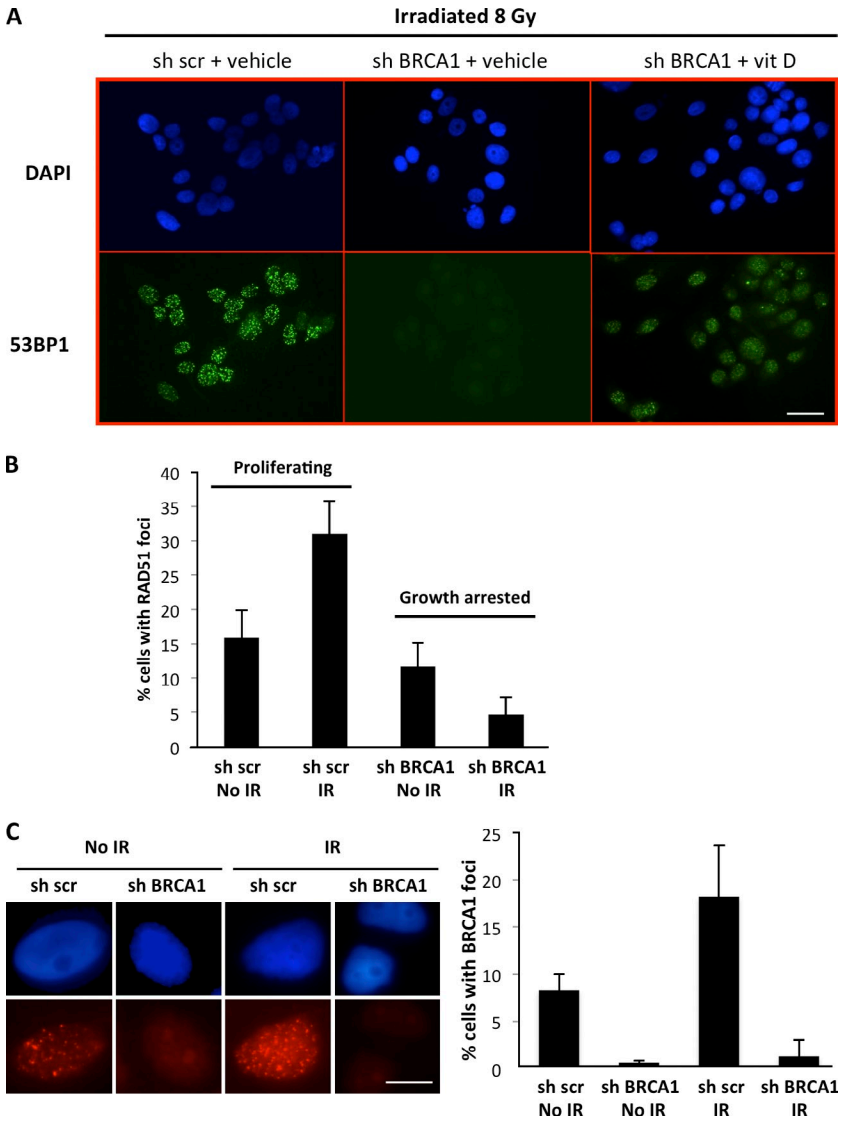

Figure S3. **Vitamin D rescues 53BP1 IRIF and inhibits RAD51 IRIF in the context of BRCA1 deficiency.** (A) BOGA cells were treated with vehicle or vitamin D for 24 h before IR with 8 Gy. Cells were fixed 6 h after IR and immunofluorescence was performed with 53BP1 antibody. More than 1,000 cells were counted per condition. The images of large fields show that the effect is not restricted to a few cells, but rather is representative of the whole population of cells. Bar, 50 µm. (B) MCF7 cells lentivirally transduced with shRNA control (sh scr) or shBRCA1 were irradiated with 8 Gy and subjected to immunofluorescence with RAD51 antibody after arrest of BRCA1-deficient cells. Graph shows the quantitation of percentage of cells positive for RAD51 foci formation. The mean ± SD of three independent experiments is shown. Note how growth-arrested BRCA1-depleted cells are deficient in RAD51 IRIF. (C) Control and BOGA cells were subjected to immunofluorescence with BRCA1 antibody after IR with 8 Gy. Images show the lack of staining in BOGA cells. Graph shows the quantitation of percentage of cells positive for BRCA1 IRIF. The mean ± SD of three independent experiments is shown. Note how BOGA cells are unable to form BRCA1 IRIF. These data demonstrate that BOGA cells remain BRCA1 deficient. Bar, 10 µm.

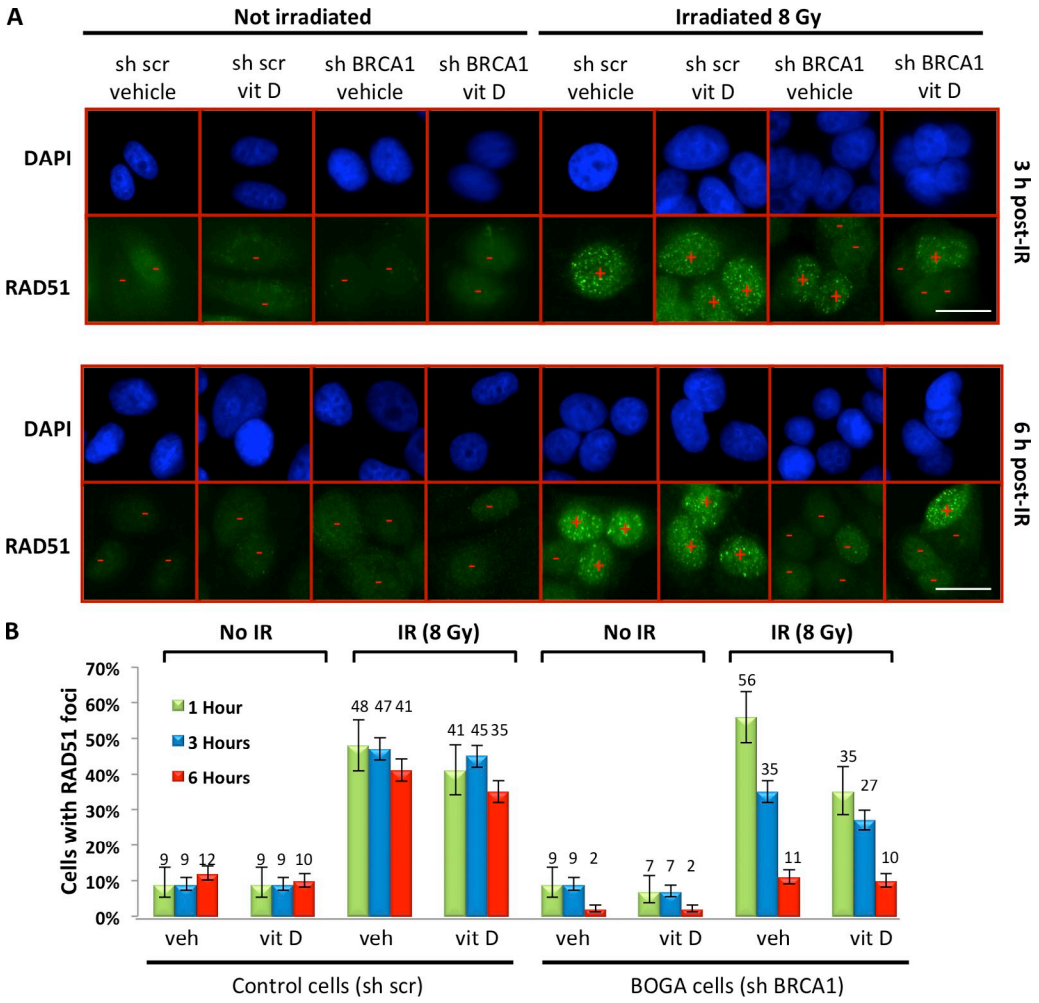

Figure S4. **Formation of RAD51 IRIF over time in MCF7 control and BOGA cells.** (A) Immunofluorescence images showing the formation of RAD51 IRIF in control and BOGA cells at 3 and 6 h after IR with 8 Gy. Each of the cell lines was treated with vitamin D or vehicle 24 h before IR. Note the decrease in RAD51 foci formation at 3 and 6 h after IR. Plus denotes positive cells (>10 IRIF) and minus negative cells. (B) Graph shows quantitation of percentage of control and BOGA cells positive for RAD51 IRIF 1, 3, or 6 h after IR. Note that although vitamin D impacts RAD51 foci formation at 1 h after IR, it does not exacerbate the defects at later time points. At least 1,000 cells were counted per condition. Error bars were calculated using the exact binomial test. Bars, 10 µm.

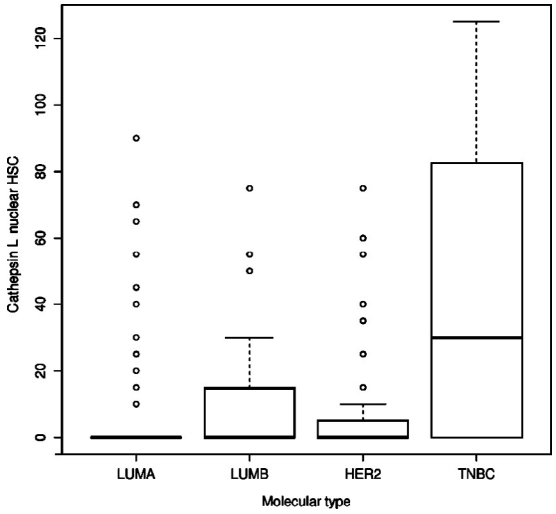

Figure S5. **Boxplot with the distribution of CTSL nuclear expression depending on breast cancer molecular type.** Note how the distribution of CTSL nuclear expression for TNBC is different from the rest of breast cancer molecular types. Error bars represent interquartile range. This figure highlights that all cases of luminal A molecular type with positive CTSL expression (HSC >0) are outliers, whereas TNBC show higher expression and no outlier given the high variability of positive expression among cases of this molecular type.