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Supplemental Material to:

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Analysis of centrosome localization of BRCA1 and its activity in suppressing centrosomal aster formation

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Fig. S1

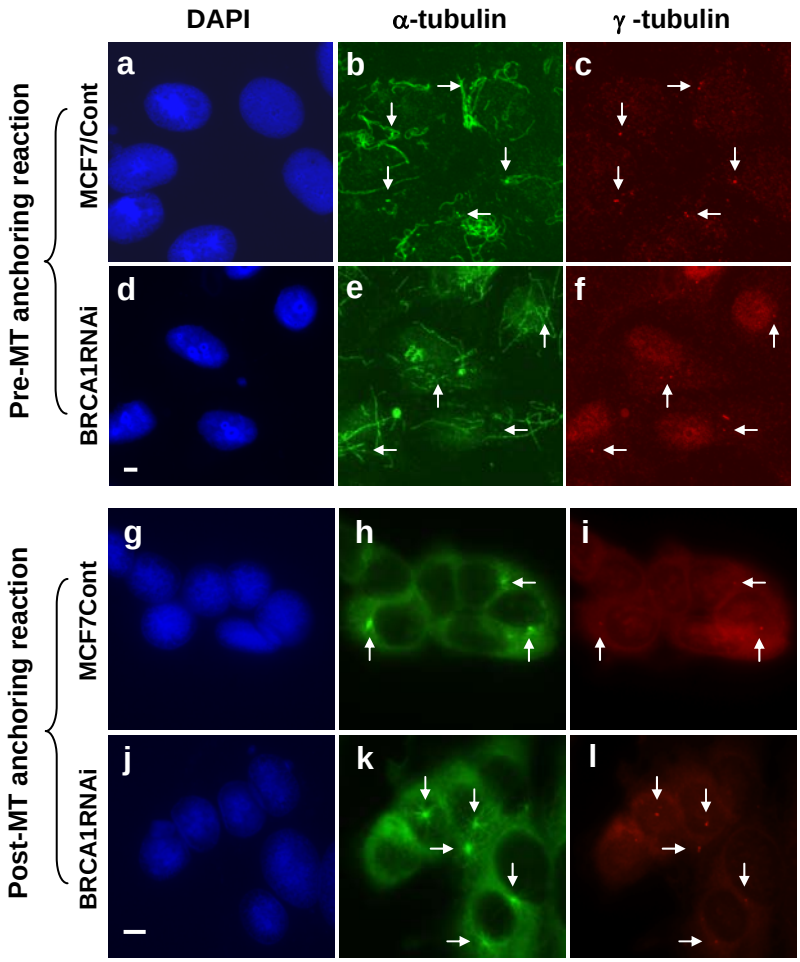

Fig. S1. (a-f) MCF7/control and BRCA1RNAi cells were treated with nocodazole (1.5 μg/ml) for 40 min on ice, and co-immunostained for MTs with anti-α-tubulin antibody and centrosomes with anti-γ-tubulin antibody. Arrows point to the positions of centrosomes. (g-l) MCF7/control and BRCA1RNAi cells in parallel cultures were incubated in fresh warm media for 7 min to allow for MT re-growth. Cells were then co-immunostained for MTs and centrosomes. Arrows point to the positions of centrosomes. Scale bar, 10 μm.

Fig. S2

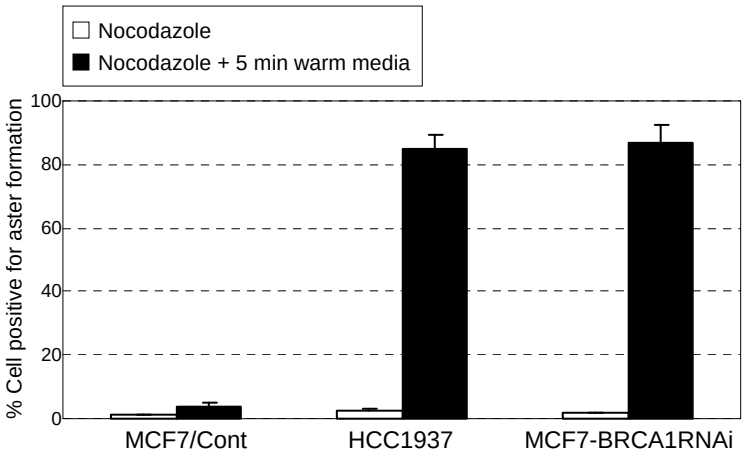

Fig. S2. MCF7/control, MCF7-BRCA1RNAi, and HCC1937 cells were treated with nocodazole on ice, and incubated in fresh warm media for 5 min to allow for MT re-growth. Cells were then co-immunostained for MTs with anti-α-tubulin antibody and centrosomes with anti-γ-tubulin antibody. The aster forming activity of centrosomes was determined as positive if centrosomes had a MT aster with >30 MTs (> 4 μm long). The results are shown as the average $\pm$ standard error from three experiments. For each experiment, >200 cells were examined.

Fig. S3

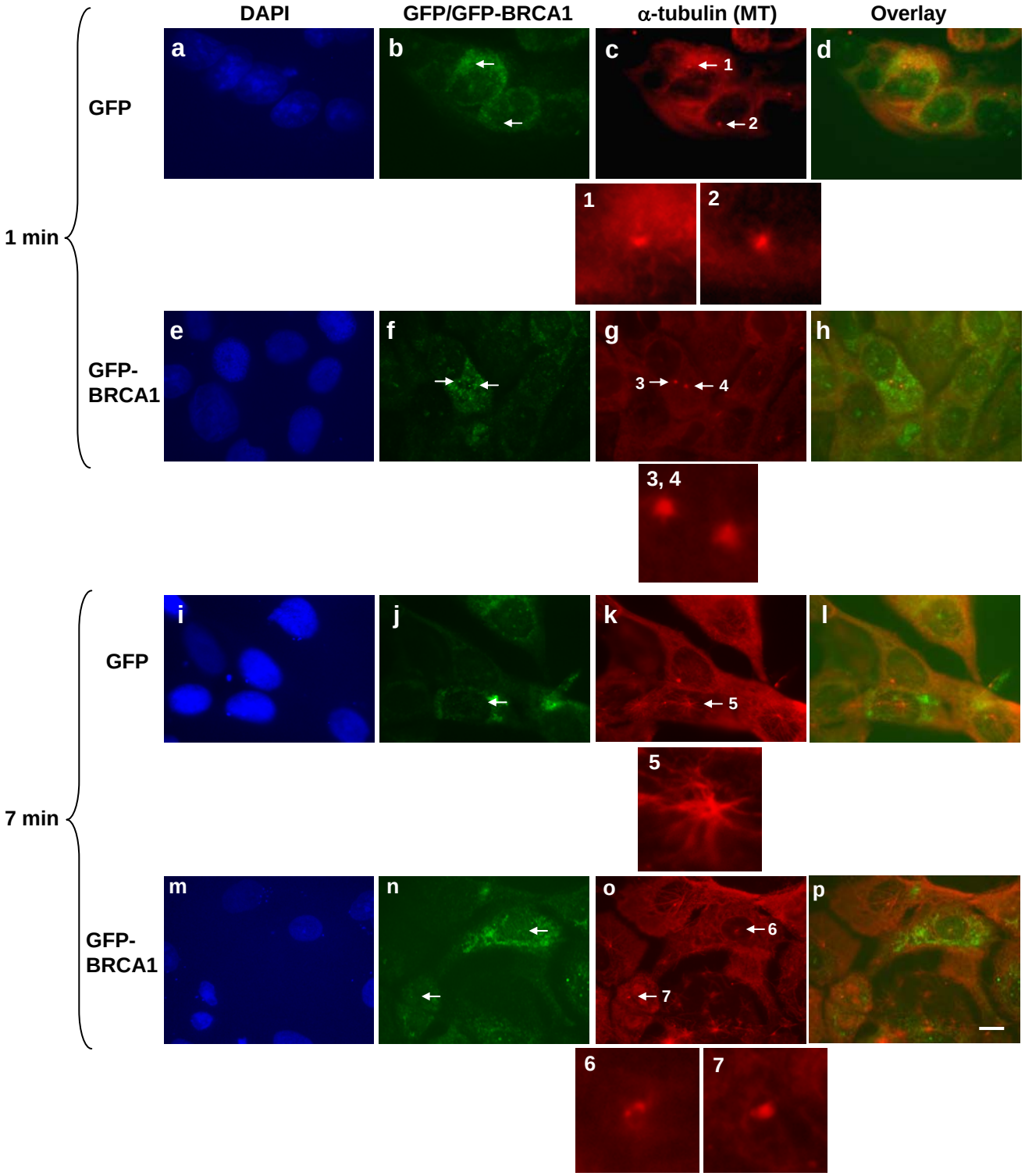

Fig. S3. HCC1937 cells were transfected with either GFP-vector or GFP-wt BRCA1, and were subjected to aster formation assay with either 1 or 7 min MT re-growth period. Cells were co-immunostained with anti-GFP and anti-α-tubulin antibodies. The centrosome areas are indicated by arrows Scale bar, 10 μm.

Figure S4

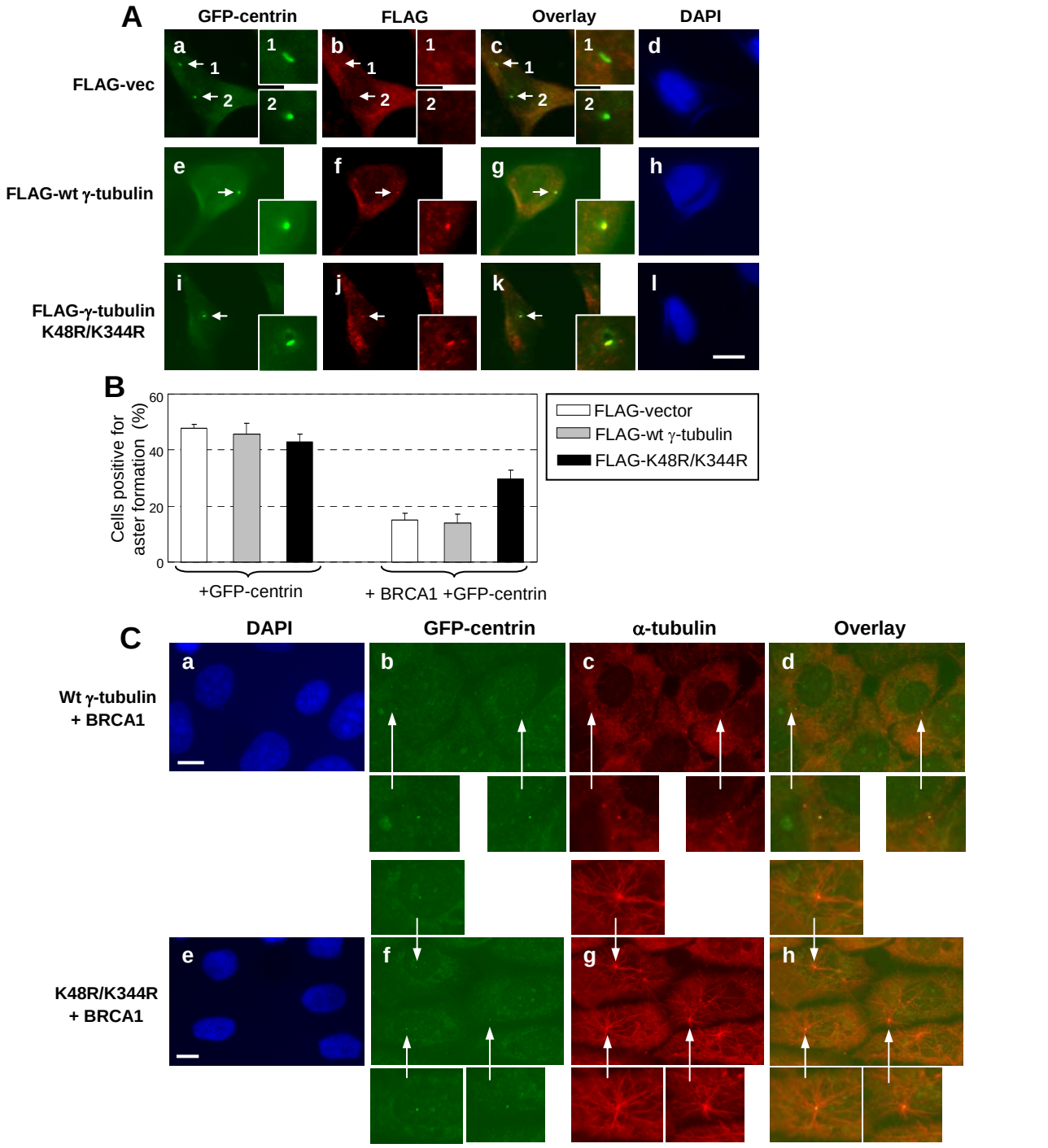

Fig. S4. **Role of γ-tubulin ubiquitination in the BRCA1-mediated suppression of centrosomal aster formation.** (A) HCC1937 cells were co-transfected with BRCA1 and GFP-centrin together with a FLAG-vector (control), wt γ-tubulin, or K48R/K344R. The transfected cells were immunostained with anti-FLAG and anti-GFP antibodies. The centrosomes identified by GFP-centrin signals are indicated by arrows (panel a, e, i). The panels on the right show the magnified images of the indicated areas. Scale bar, 10 μm. (B) The transfected cells were subjected to aster formation assay, and the results are shown as the average ± standard error from three experiments. For each experiment, >200 cells were examined. The representative immunostaining images of the cells co-transfected with BRCA1 and either wt γ-tubulin or K48R/K344R mutant are shown in (C). Arrows point to the centrosomes. The sub-panels show the magnified images of the indicated areas. Scale bar, 10 μm.