Supplementary Materials and Methods

siRNA depletion of BCA2

siRNA sequences: Dharmacon ON-TARGET plus SMARTpool siRNA RNF115 (#L-006974-00-0005, Fisher Scientific) and non-targeting siRNA control (GFP): 5'-GGCUACGUCCAGGAGCGCAdTdT-3' (MWG)

MCF-7 cells (250,000) were seeded in 6-well plates and incubated overnight in RPMI supplemented with 10% FBS. The following day the transfection mixture was prepared by mixing: 2.4 µL Dharmafect1 transfection reagent in 237.6 µL of Serum Free Media (SFM) and incubating at room temperature for 5 min. Meanwhile 12 µL of 5 µM siRNA (either non-targeting GFP siRNA or BCA2 SMARTpool siRNA) was mixed with 228 µL SFM. The diluted Dharmafect1 was then added to the diluted siRNA and the mixture was incubated at room temperature for 30 min at room temperature. The cell media was aspirated and replaced with 1920 µL of compete media. After 30 min incubation 480 µL transfection mixture was added to each well, giving a final siRNA concentration of 25 nM. Cells were incubated with the siRNA for 48 hr before further experiments were performed.

Co-transfection of HA-BCA2 and EGFP-Rab constructs

Plasmid constructs: pEGFP-C1 containing Rab7 and Rab5 were used in the double-transfection / co-localisation experiments (20).

0.5 µg of pHA-BCA2 and 0.5 µg of EGFP-Rab5 or EGFP-Rab7 were used to transfect the cells according to the Fugene 6 transfection protocol outlined in Materials and Methods.

Dual-Immunofluorescence labelling of HA and BCA2 in transfected HeLa cells

HeLa cells were transfected with HA-BCA2 and subjected to single and dual-immunofluorescence labelling for HA and for BCA2 according to the protocols for these procedures outlined in Materials and Methods. The anti-BCA2 (#SAB2500854, Sigma Aldrich) antibody was used at a dilution of 1:5000.

Supplementary Figures

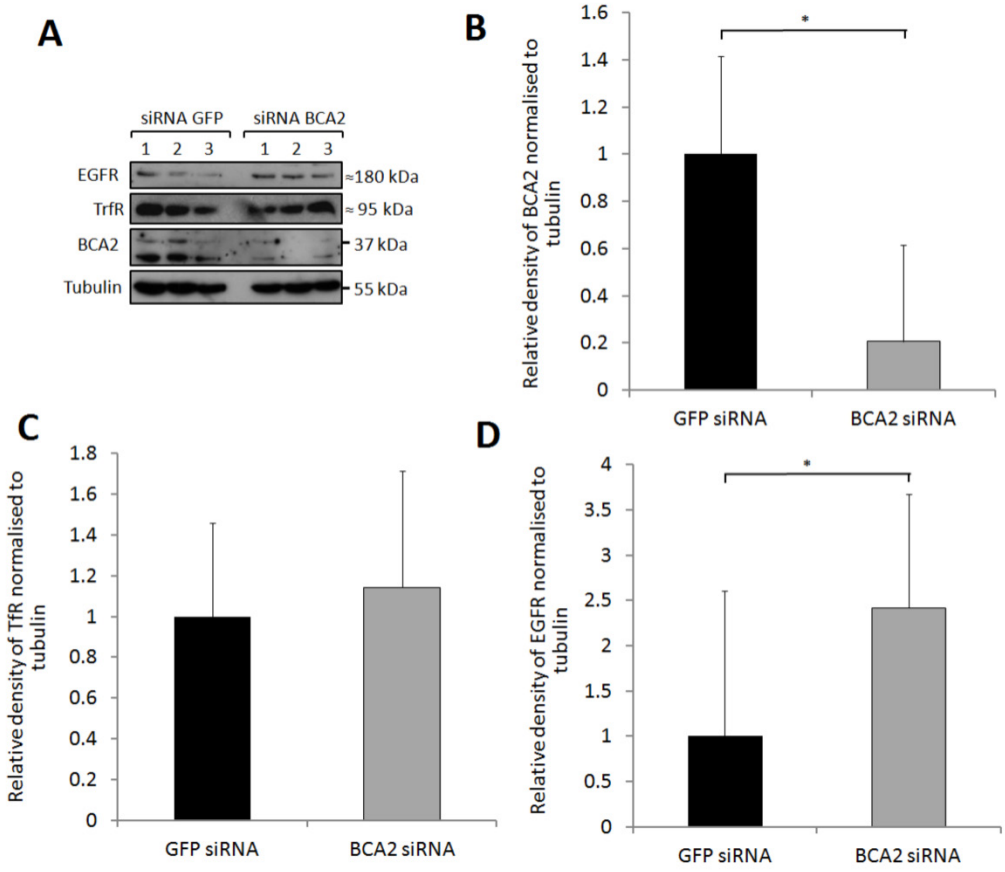

Figure S1: BCA2 depletion increases EGFR levels in MCF-7 cells. A) Immunoblots for BCA2 and selected recptors in cells treated with BCA2 SMARTpool siRNA or GFP non-targeting control siRNA. B, C and D) Relative protein expression was quantified with ImageJ software and normalised to tubulin loading control. *Results from three independent, single experiments are shown. Error bars represent 95% confidence intervals. * statistical significance $p \leq 0.05$.*

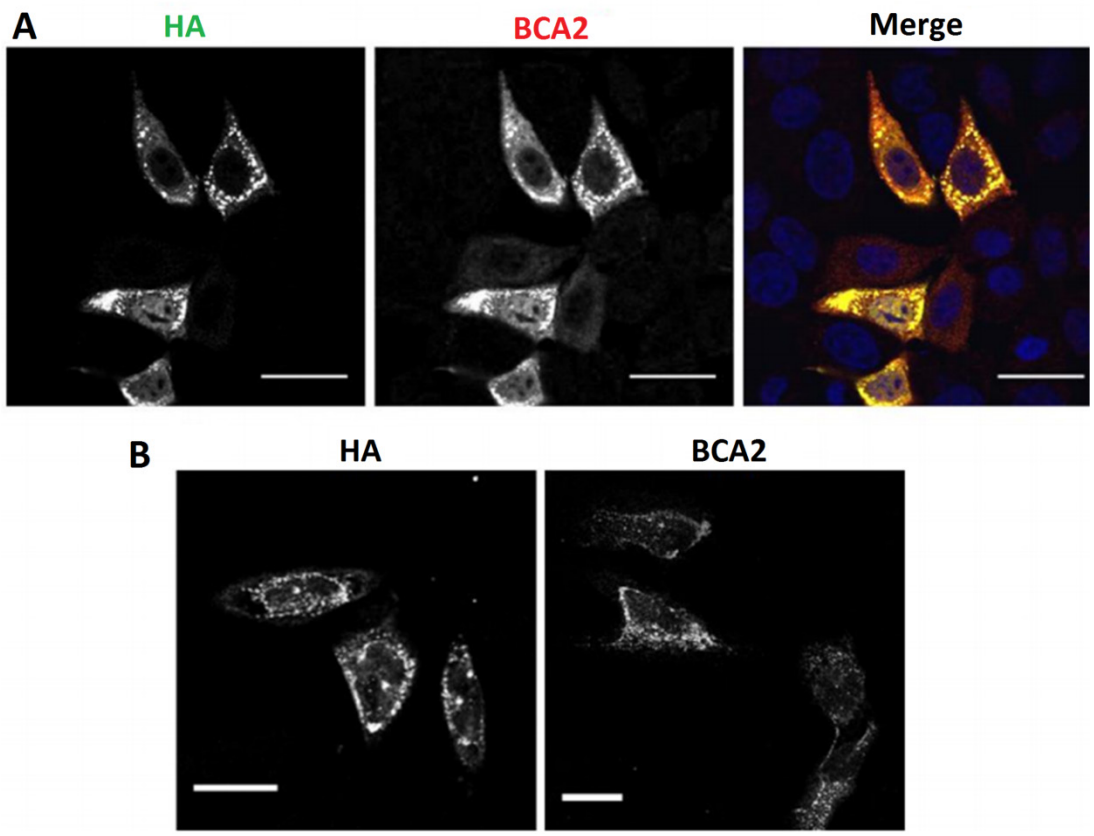

Figure S2: Anti-HA and Anti-BCA2 IF in HA-BCA2 Transfected MCF-7 cells. A) Sequential double IF was performed in HA-BCA2 transfected cells using the goat anti-BCA2 antibody and mouse anti-HA. Nuclei were counterstained with Hoechst. B) Separate anti-HA and anti-BCA2 IF was performed. Scale bars = 25 μm

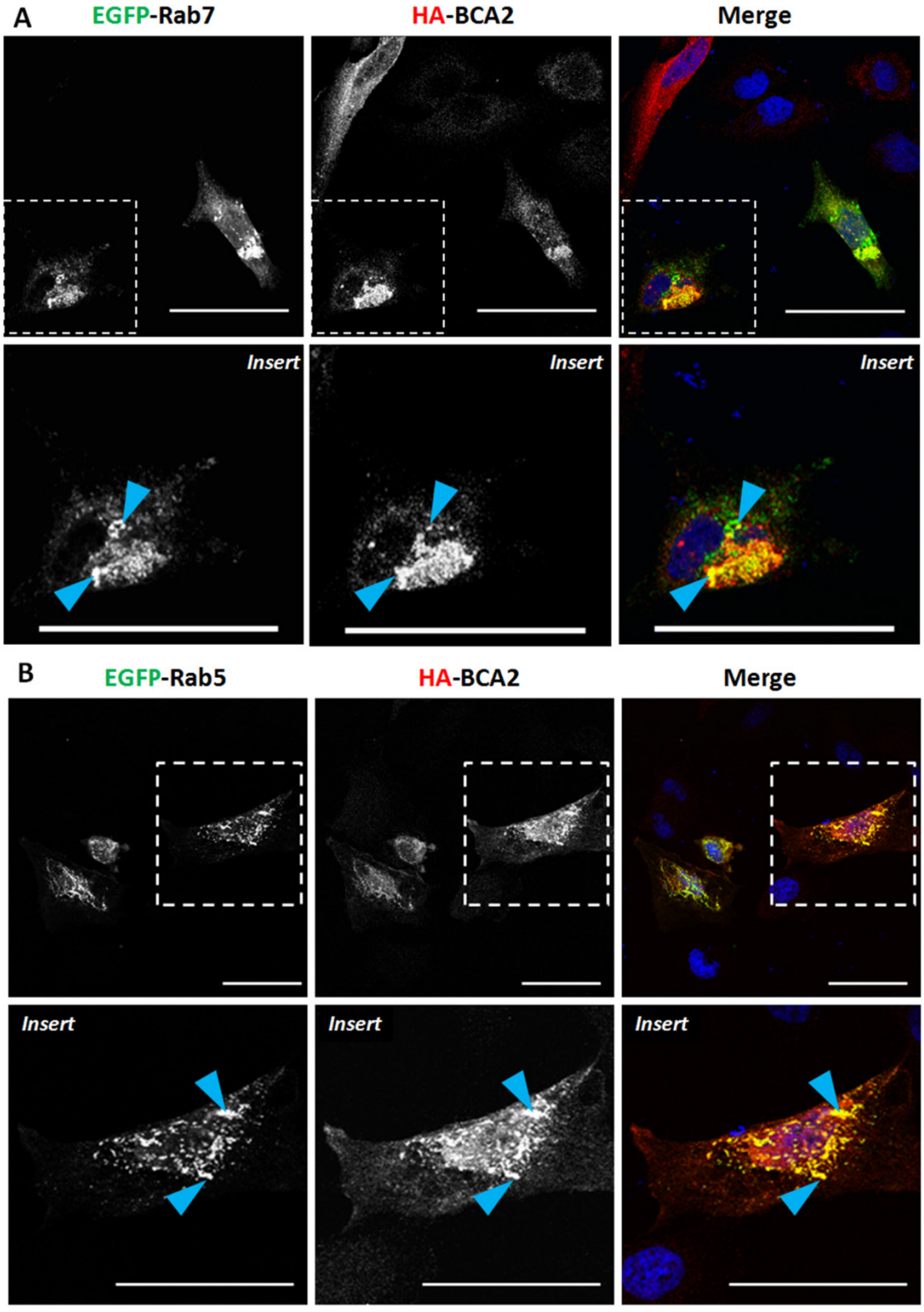

Figure S3: Confocal microscopy images of HeLa cells cotransfected with HA-BCA2 and selected EGFP-tagged Rab proteins. Cells were transiently transfected with HA-BCA2 and A) EGFP-Rab7, B) EGFP-Rab5. IF was performed for HA (red) and nuclei were stained with Hoechst (blue). *Blue arrowheads indicate colocalisation. Scale bars = 50 µm.*

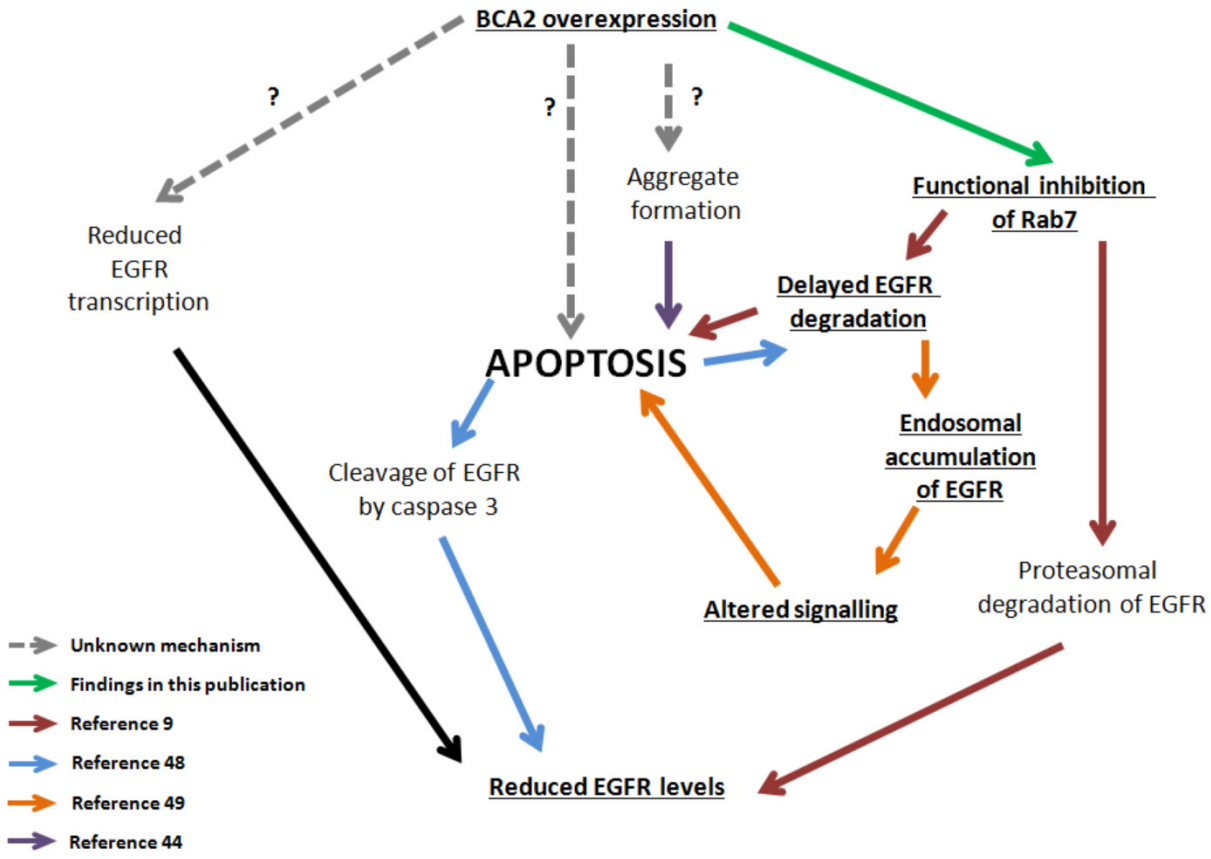

Figure S4: Summary of the possible mechanisms to explain BCA2 mediated effects on cell survival and on total receptor levels and trafficking. *Novel or confirmatory BCA2 findings from our studies are underlined and in bold.*