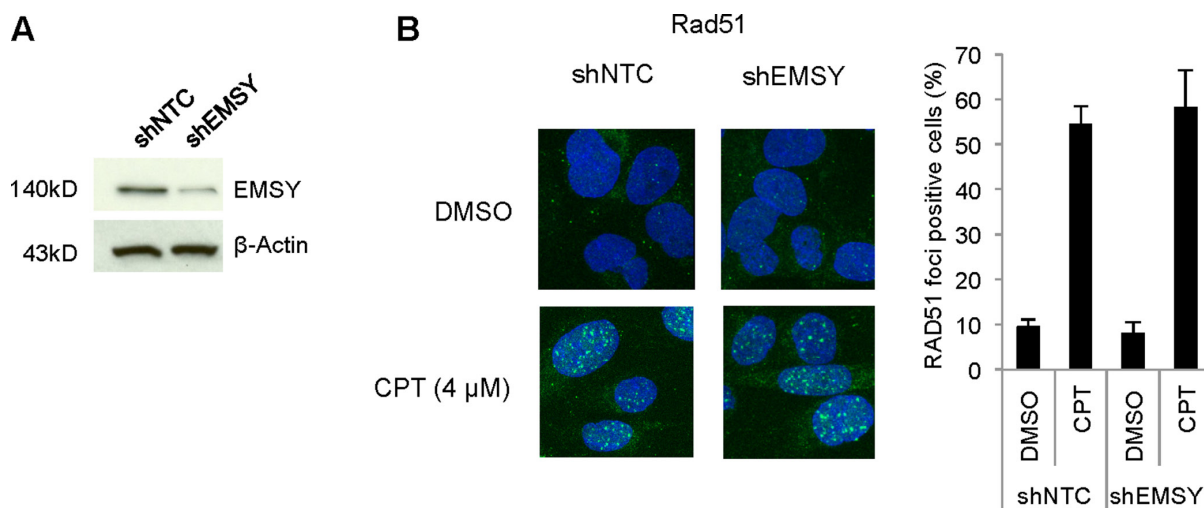
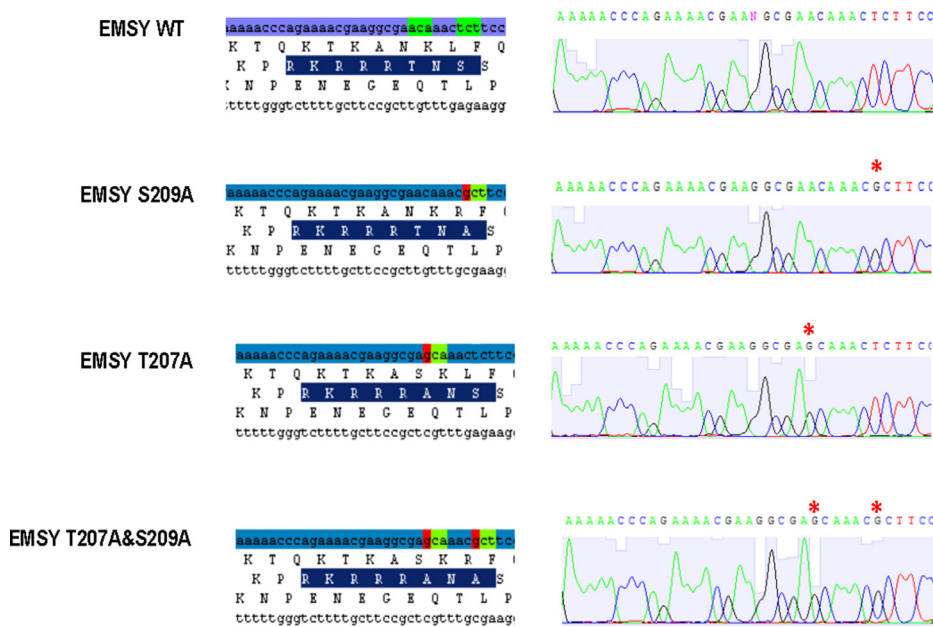


The EMSY threonine 207 phospho-site is required for EMSY-driven suppression of DNA damage repair

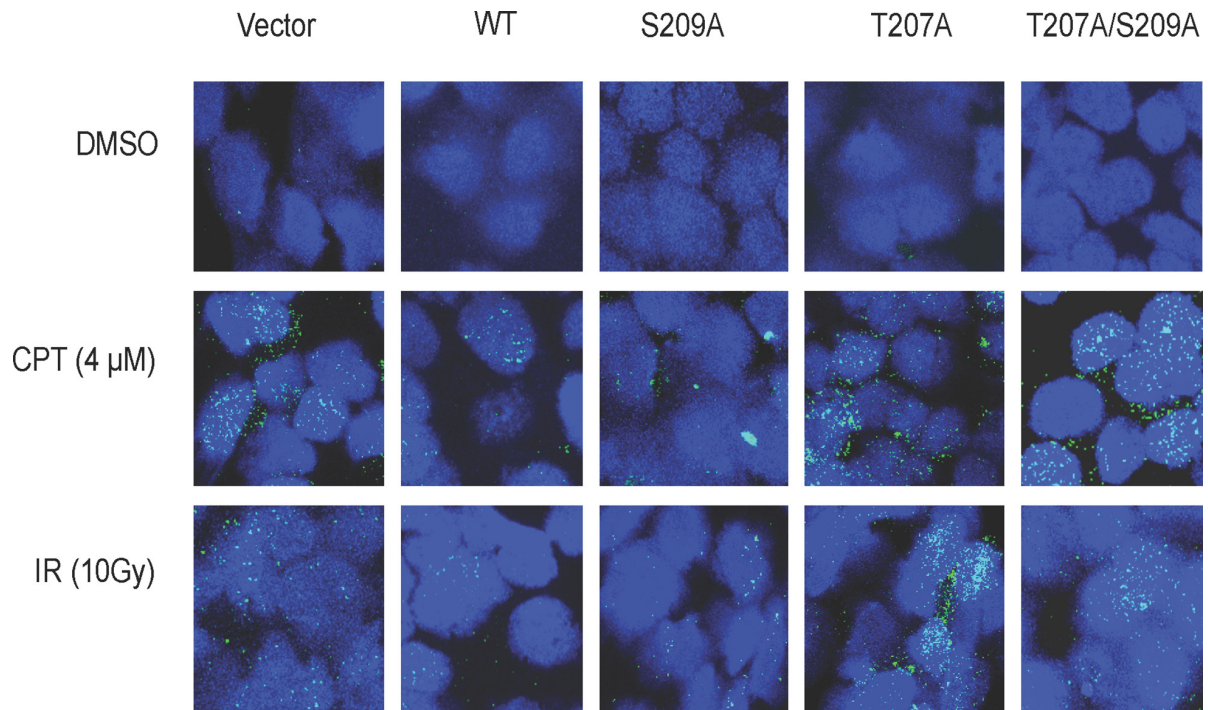
Supplementary Materials



Supplementary Figure 1: RAD51 foci formation assessment in EMSY KD OVCAR3 cells. (A) Western blot showing OVCAR3 cells stably depleted for EMSY with shRNA (shEMSY). shNTC (non-template control) was used as a negative control. (B) RAD51 foci formation in CPT treated cells with representative images and quantification.

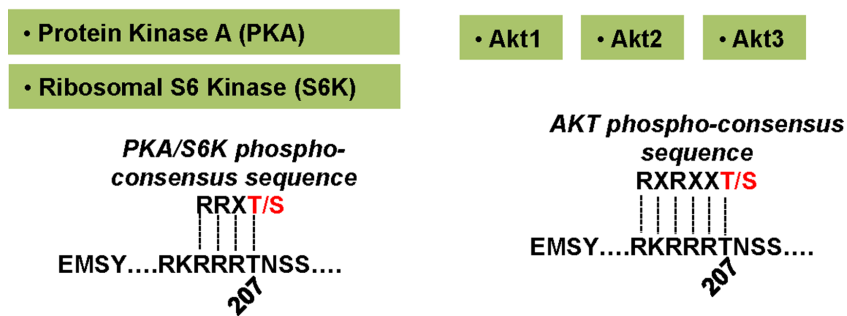


Supplementary Figure 2: Confirmation of EMSY mutations with Sanger sequencing.

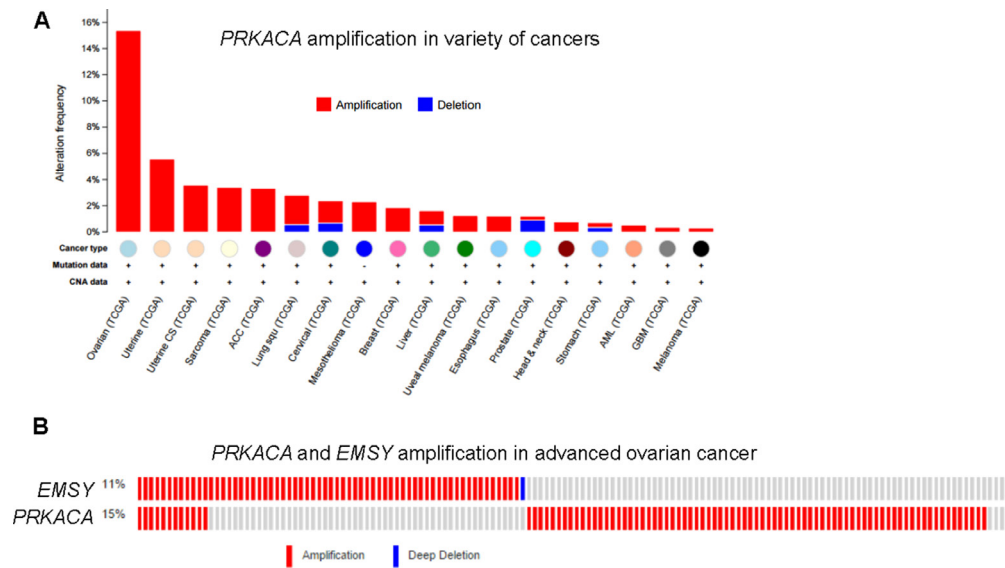


Supplementary Figure 3: RAD51 foci formation immunocytochemistry in OVCAR8 cells overexpressing wild type and mutant EMSY. Cells were treated with either 4 μM camptothecin (CPT) or irradiation (10 Gy). DMSO-treated cells were used as a control. Cells with five or more RAD51 foci were counted as positive.

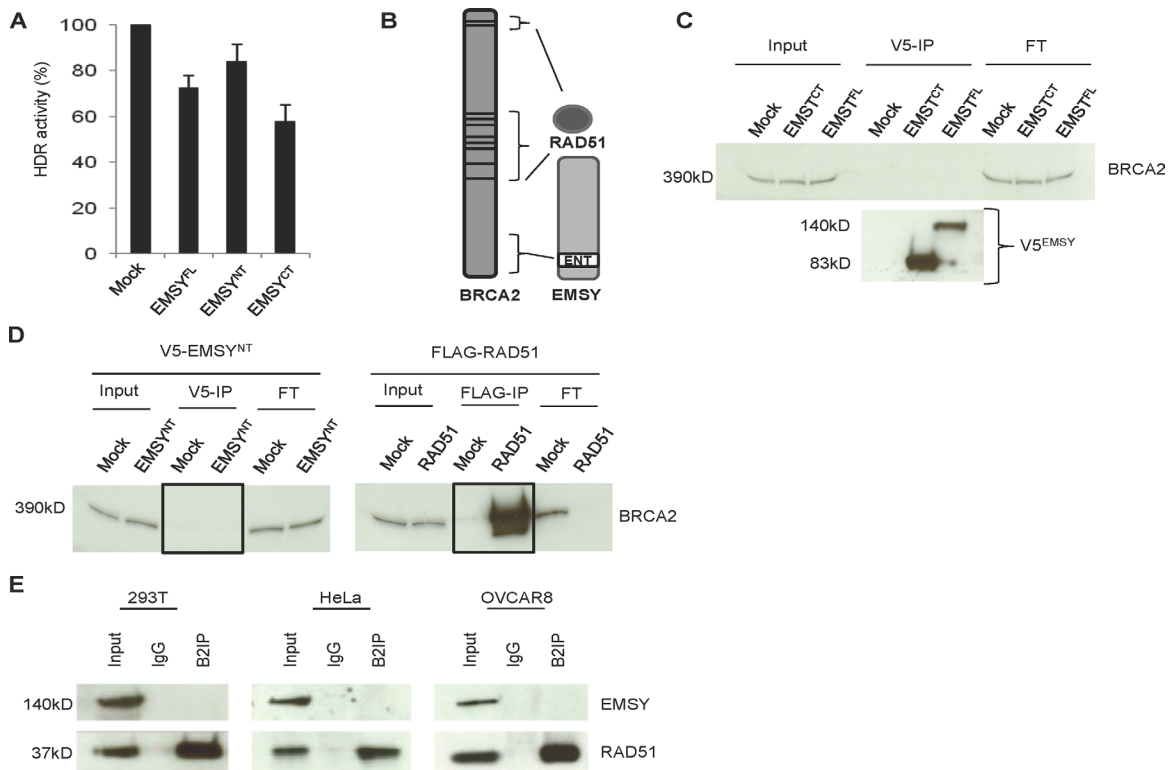
Protein kinases predicted to target EMSY T207



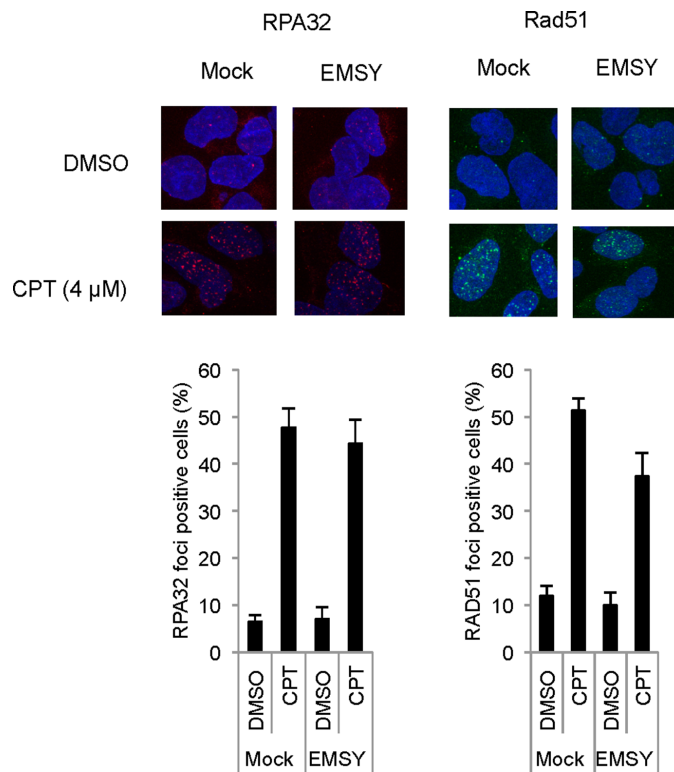
Supplementary Figure 4: Protein kinases predicted to target the EMSY T207 phospho-site by group-based prediction system for protein kinase-specific prediction (<http://gps.biocuckoo.org>). Consensus sequences are shown below the corresponding kinases.



Supplementary Figure 5: *PRKACA*, encoding PKA, amplification in advanced ovarian carcinoma (source: cbioportal.mskcc.org). (A) *PRKACA* amplification is most frequent in advanced ovarian carcinoma. (B) Frequent amplification of both *PRKACA* and *EMSY* in advanced ovarian carcinoma.



Supplementary Figure 6: *EMSY* does not interact with *BRCA2*. (A) The DR-GFP assay in cells overexpressing the full-length, N-terminal, and C-terminal *EMSY*. HDR activity is normalized to mock controls (empty vector-transfected cells). (B) Schematic representation of *BRCA2* interactions with *RAD51* and *EMSY*. (C) Immunoprecipitation with the V5 antibody in cells overexpressing the full-length or C-terminal *EMSY*. The blots are probed with *BRCA2* antibody. The flow-through (FT) represents the lysates after the immunoprecipitation (IP) reaction. Overexpressed *EMSY* is detected with the V5 antibody (V5^{EMSY}). (D) IP with the V5 and FLAG antibodies in cells overexpressing V5-tagged N-terminal *EMSY* or FLAG-tagged *RAD51* (positive control). FLAG-IP indicates successful IP; *BRCA2* is co-immunoprecipitated with the overexpressed *RAD51*. (E) Endogenous IP in 293T, HeLa, and OVCAR8 cell lines with *BRCA2* antibody (B2IP). Non-specific IgG served as an IP control. *RAD51* served as a positive control.



Supplementary Figure 7: No change in RPA32 foci formation upon EMSY overexpression. RPA32 and RAD51 foci formation in CPT-treated OVCAR8 cells. Quantification of RAD51-positive cells is shown below the images.

Supplementary Table 1: RPPA antibody list. See_Supplementary_Table_1