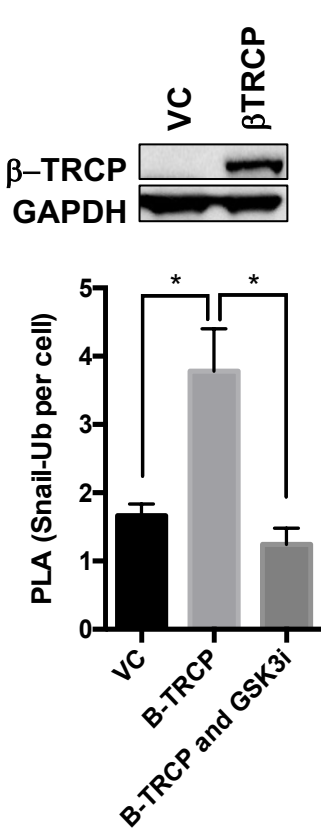
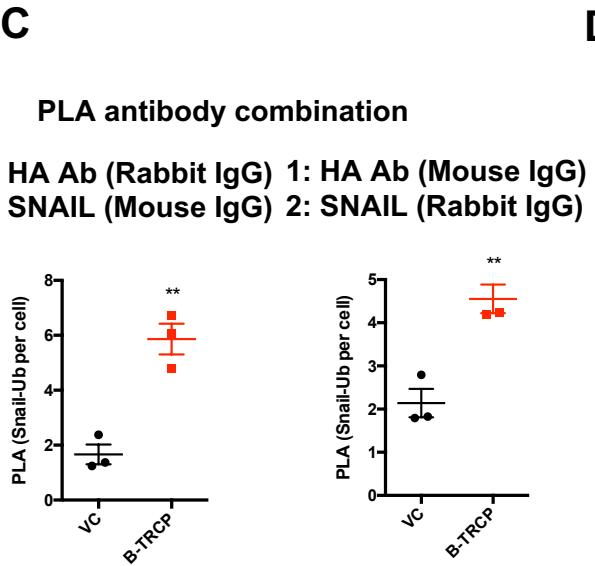
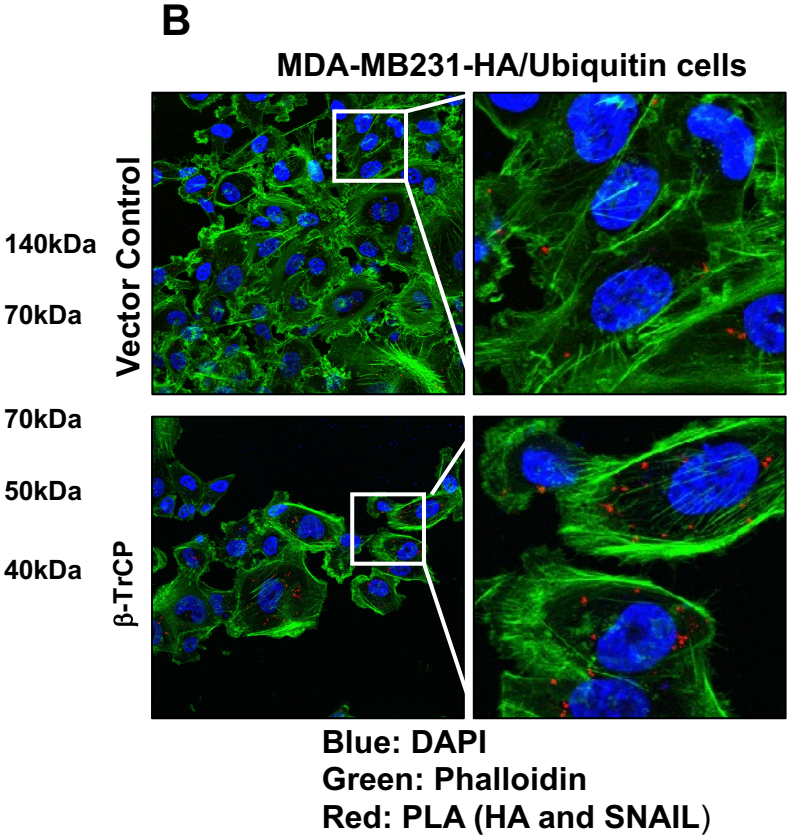
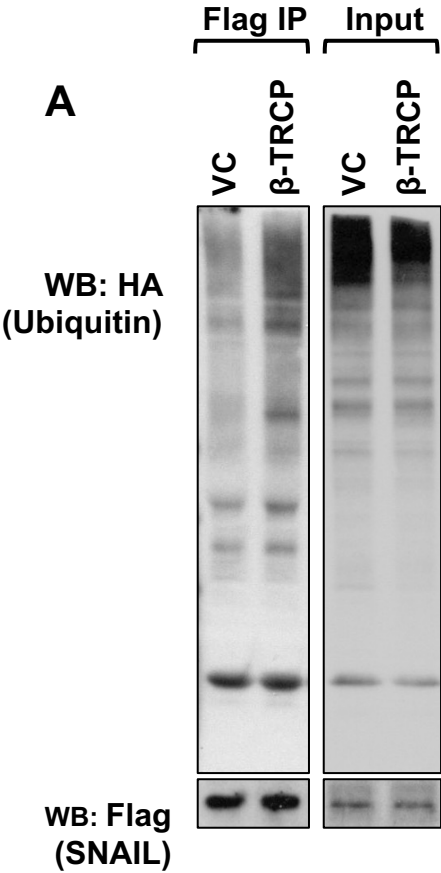


# Supplemental Figure 3



**Supplemental Figure 3.** Validation of PLA as a method to detect protein ubiquitination. A)  $\beta$ -TRCP, an established SNAIL E3 ligase, promotes ubiquitination of immunoprecipitated FLAG-SNAIL. MDA-MB231 cells co-expressing HA-Ubiquitin, FLAG-SNAIL and vector control or  $\beta$ TRCP were treated with MG132 and lysed. Anti-FLAG antibody was used to immunoprecipitate SNAIL and immunoprecipitates were blotted for Ubiquitin using anti-HA antibody. B) Representative images of proximity ligation assay (PLA) performed on MG132-treated MDA-MB231 cells co-expressing HA-ubiquitin and vector control or  $\beta$ TRCP, using anti-HA (Ubiquitin) and anti-SNAIL antibodies. C) Quantitation of PLA signals/cell using different isotype combinations of anti-HA (Ubiquitin) and anti-SNAIL antibodies. At least 90 cells were counted. D) GSK3 $\beta$  inhibitor (TWS119) treatment of  $\beta$ -TRCP overexpressing cells decreases SNAIL/ubiquitin PLA signals.