

FIGURE S2

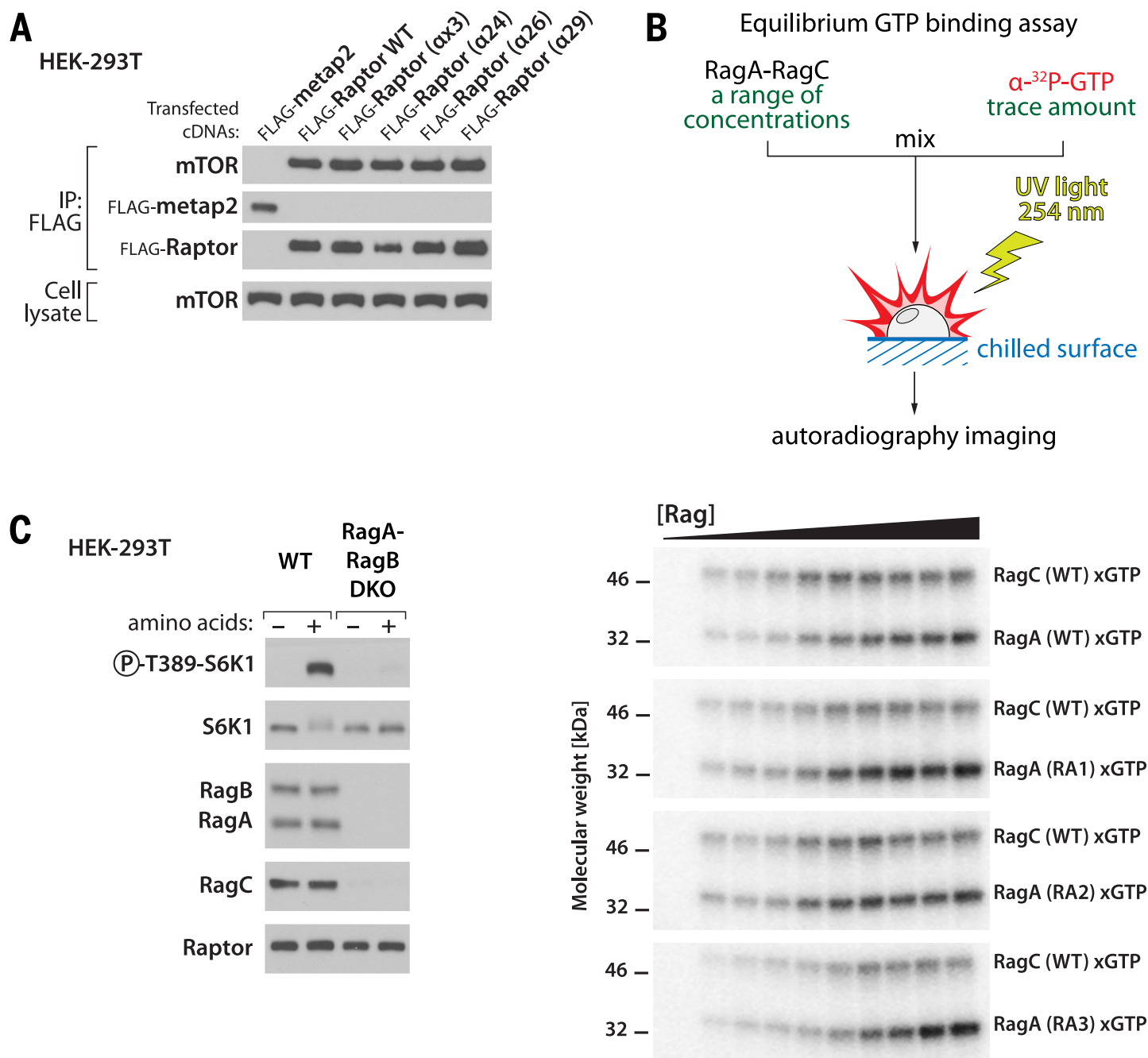


Fig. S2. (A) Raptor mutants that cannot bind RagA, are still able to co-immunoprecipitate mTOR. Flag-metap2 was used as a negative control protein.

(B) An equilibrium GTP binding assay was set up such that increasing amounts of Rag GTPases were mixed with a trace amount of radiolabeled GTP before being cross-linked with UV light (top panel). The resulting protein-nucleotide cross-links were analyzed by SDS-PAGE and phosphor imaging (see methods). RagA mutations in its Raptor-interacting region do not interfere with the capacity of RagA to bind GTP (bottom panel).

(C) Characterization of RagA-RagB double knockout (DKO) HEK-293T cells. These cells have a partial loss of RagC because as an obligate binding partner of RagA/B it becomes destabilized in their absence.