Supplemental Figure

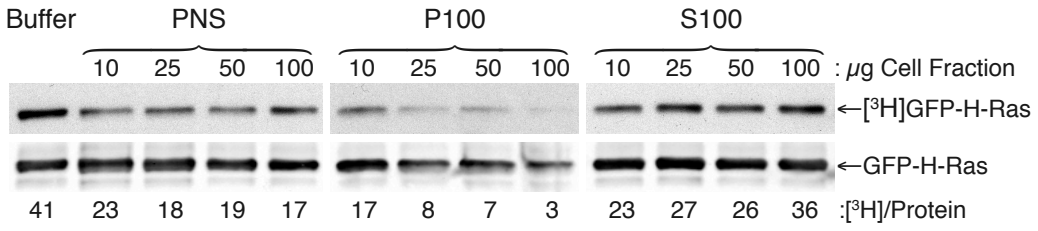

Figure S1

SUPPLEMENTAL FIGURE LEGEND

Figure S1. H-Ras Depalmitoylation Activity Resides in Membranes.

Lysates of Cos1 cells transiently transfected with GFP-H-Ras and metabolically labeled with [^{3}H]palmitic acid were immunoprecipitated with Y13-259AC. Beads containing immunoprecipitated protein were washed extensively and rotated at 30°C with the indicated amounts of either PNS, S100, or P100 cellular material from Hela S2 cell for 5min.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

In vitro Depalmitoylation

COS-1 cells transfected with GFP-H-Ras were metabolically labeled with 0.25 mCi/ml [^{3}H]palmitic acid in Labeling Media. Cells were lysed in RIPA buffer and immunoprecipitated with Y13-259AC at 4°C. Beads were then extensively washed with 1 ml RIPA Buffer. To collect cellular fractions, Hela S2 cells were disrupted by nitrogen cavitation and the cavitate was clarified at 500 x *g* for 10 min. The resulting post-nuclear supernatant (PNS) was reserved or subjected to ultracentrifugation to obtain cytosol (S100) and membrane (P100) fractions. Protein content of these fractions was estimated by BCA assay. Equivalent amounts of protein from each fraction was then diluted in Reaction Buffer (10 mM Hepes, pH 7.3, 100 mM KCl, 3 mM NaCl, 3.5 mM $MgCl_2$, 1 mM DTT, and protease inhibitors without EDTA) and rotated with beads containing the immunoprecipitated [^{3}H] palmitate-labeled GFP-H-Ras at 30°C for 5min. Beads were immediately pelleted and 2x Laemmli buffer containing 5mM DTT was added before SDS-PAGE, western blotting, and autoradiography.