

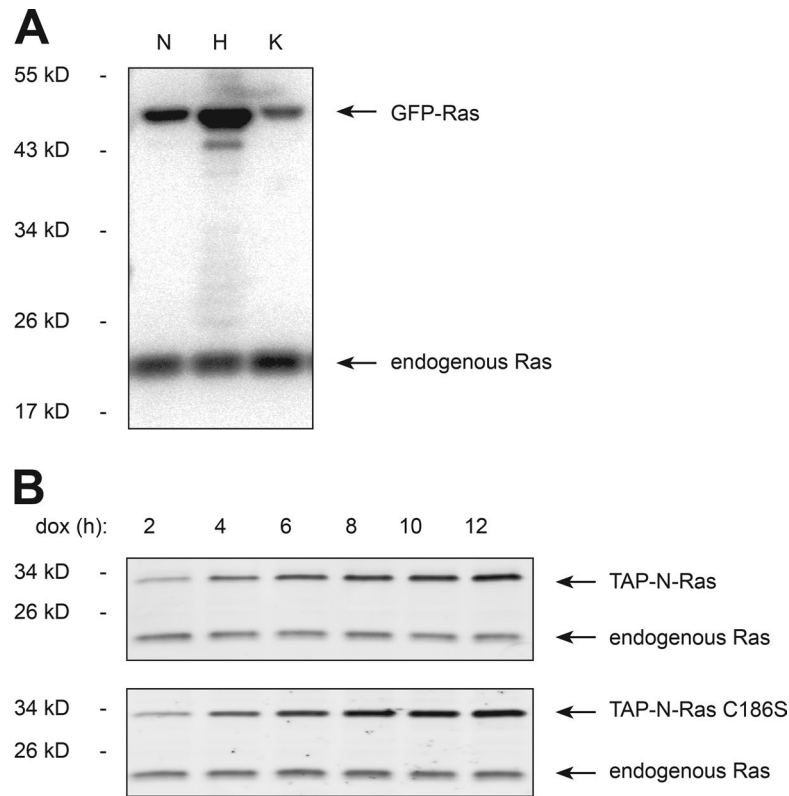
Zhou et al., <http://www.jcb.org/cgi/content/full/jcb.201604061/DC1>

Figure S1. **Ras expression levels in stably transfected cell lines.** (A) MDCK cells stably expressing GFP-tagged N- (N), H- (H), and K-Ras4B (K) at near-endogenous levels were generated by transfection with pEGFP-Ras and selection with G418. (B) Flp-In T-Rex HEK293 cells stably expressing tetracycline-inducible TAP-N-Ras (wild type or C186S). Cells were treated with doxycycline (dox) for the indicated time. (A and B) Ras protein levels were analyzed by immunoblot.

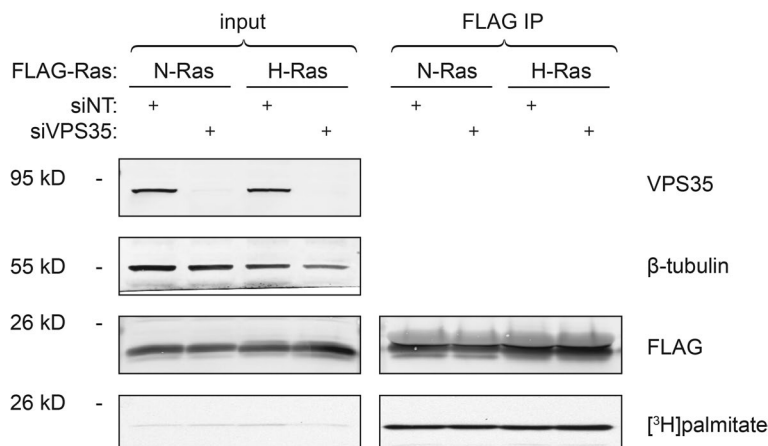


Figure S2. **Silencing VPS35 does not affect palmitoylation of Ras.** HEK293 cells were transiently transfected with FLAG-tagged N- or H-Ras and untargeted siRNA (NT) or a validated pool targeting VPS35 and incubated with 0.5 mCi ml^{-1} [³H]palmitate for 1 h. Whole-cell lysates (input) were immunoprecipitated with an anti-FLAG antibody (FLAG immunoprecipitation [IP]), and palmitoylation levels were analyzed by fluorimetry.

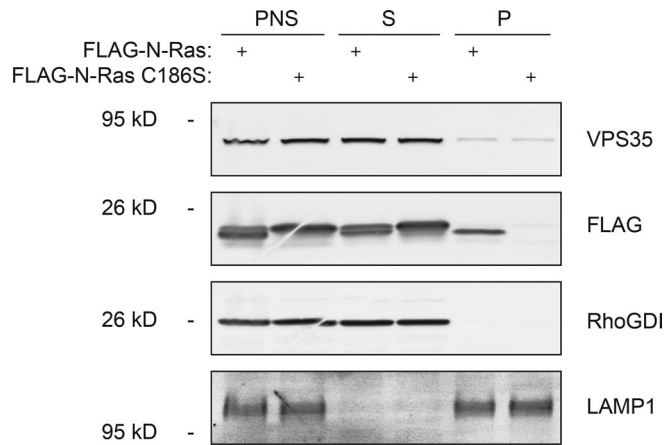


Figure S3. **The bulk of endogenous VPS35 is cytosolic.** Cell equivalents from postnuclear supernatant (PNS), S350 (S), and P350 (P) were analyzed for the indicated proteins by immunoblot. RhoGDI and LAMP1 served as markers of cytosol and membrane, respectively.

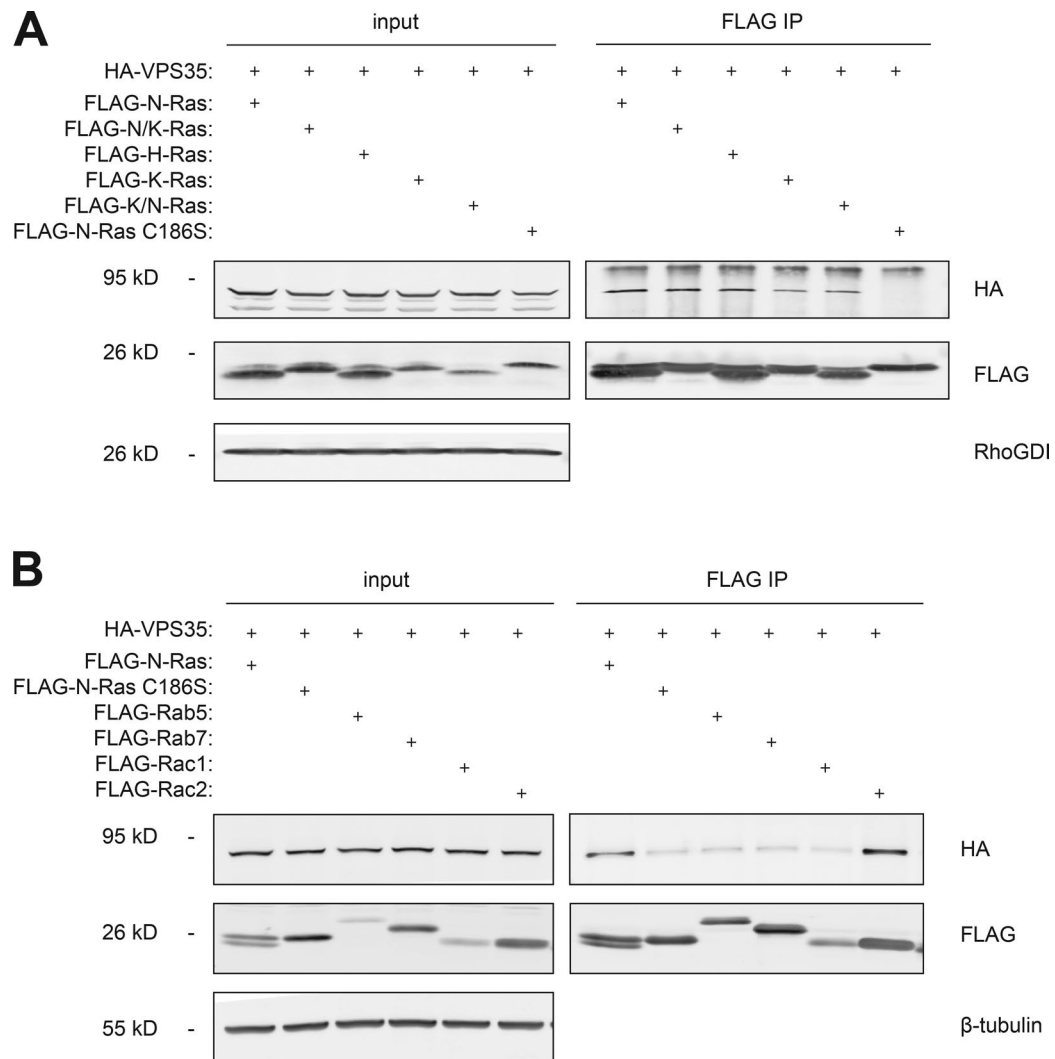


Figure S4. **VPS35 coimmunoprecipitates with Ras proteins and some, but not all, Ras-related small GTPases.** HEK293 cells were transiently transfected with plasmids directing expression of the indicated proteins (N/K-Ras, N-Ras with the HVR of K-Ras4B; K/N-Ras, K-Ras4B with the HVR of N-Ras). Whole-cell lysates were analyzed as in Fig. 2. Data shown are representative of at least three independent experiments.

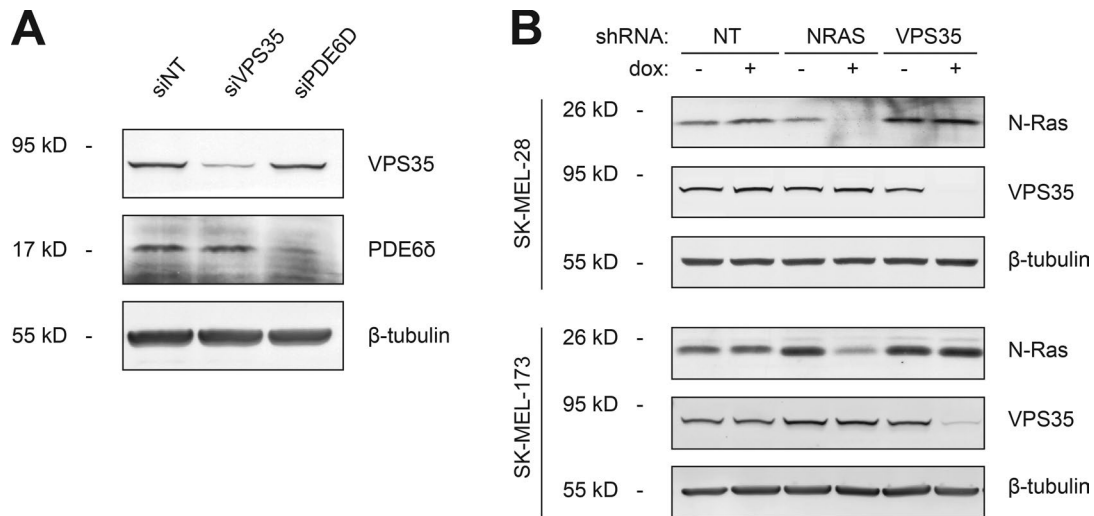


Figure S5. **Efficiency of silencing VPS35, PDE6D, and NRAS.** (A) U2OS cells were transiently transfected with untargeted Dharmacon SMARTpool siRNAs (NT) or a validated pool targeting *PDE6D* or *VPS35*. (B) SK-MEL-28 and SK-MEL-173 cells stably transfected with untargeted shRNAs (NT) or shRNA targeting *NRAS* and *VPS35*. Immunoblot shows efficiency of silencing of *PDE6D*, *VPS35*, and *NRAS* after 3 d of siRNA or doxycycline.

Table S1 is provided as an Excel file and provides data on mass spectrometry identification of cytosolic protein affinity purified by Nras and/or NrasC186S.