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Supplemental Material to:

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Palmitoylation regulates vesicular trafficking of R-Ras to membrane ruffles and effects on ruffling and cell spreading.

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Figure S1

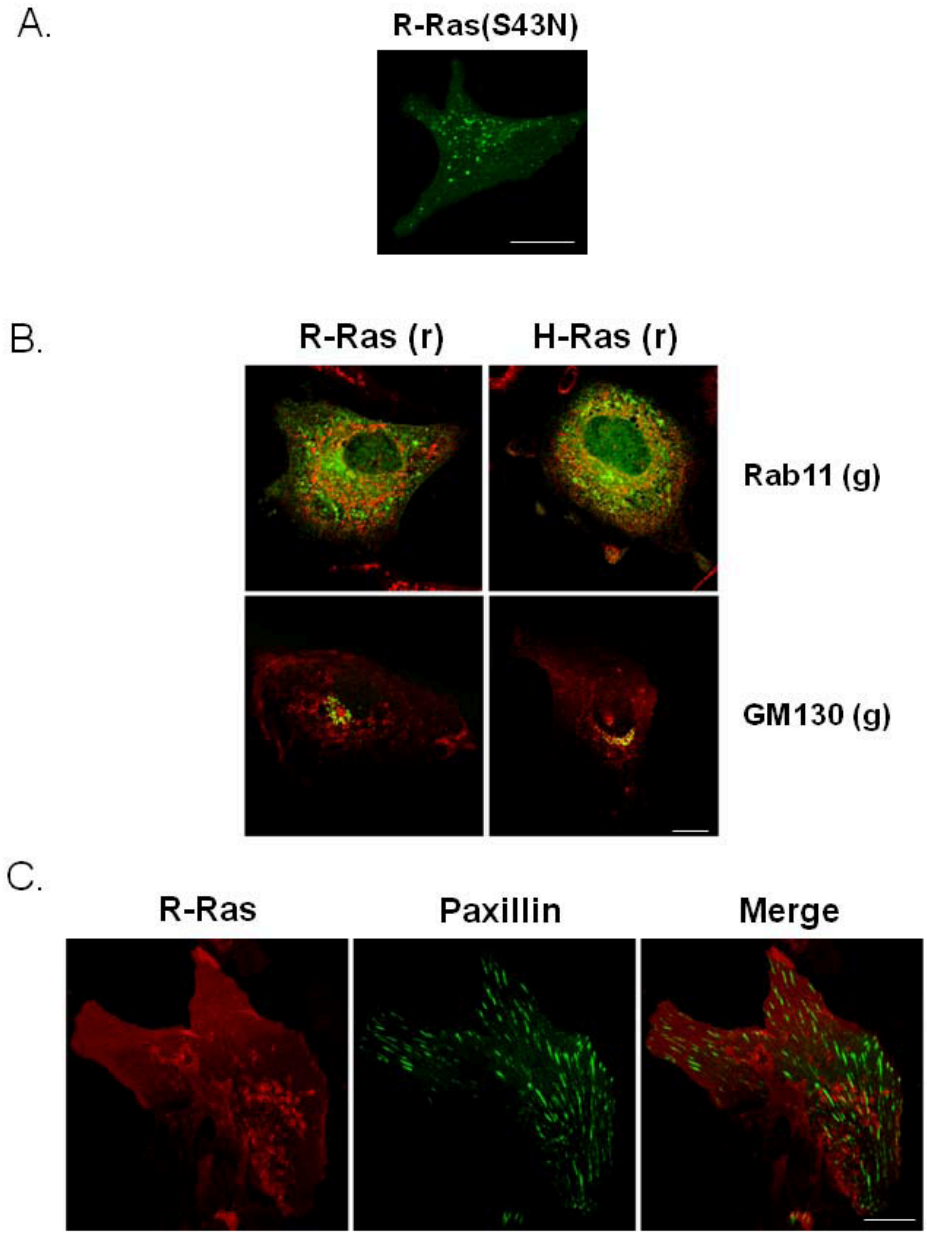

Figure S1. Dominant negative R-Ras is mistargeted in cells; endogenous R-Ras and H-Ras staining patterns; R-Ras does not localize to focal adhesions in NIH 3T3 cells. (A) GFP-R-Ras(S43N) imaged in a live cell. (B) Cells transfected with GFP-Rab11 (top) or GFP-GM130 (bottom, green) were fixed in formaldehyde and labeled with antibodies for R-Ras (left) or H-Ras (right) followed by fluorescent secondary antibody conjugates (red). (C) RFP-R-Ras(wt) (red) and GFP-paxillin (green) were co-transfected and imaged in a live cell seeded on a fibronectin-coated surface. Confocal laser scanning was performed in series for each fluorophore at the same z-axis plane (1 μm thick). Bars, 10 μm.

Figure S2

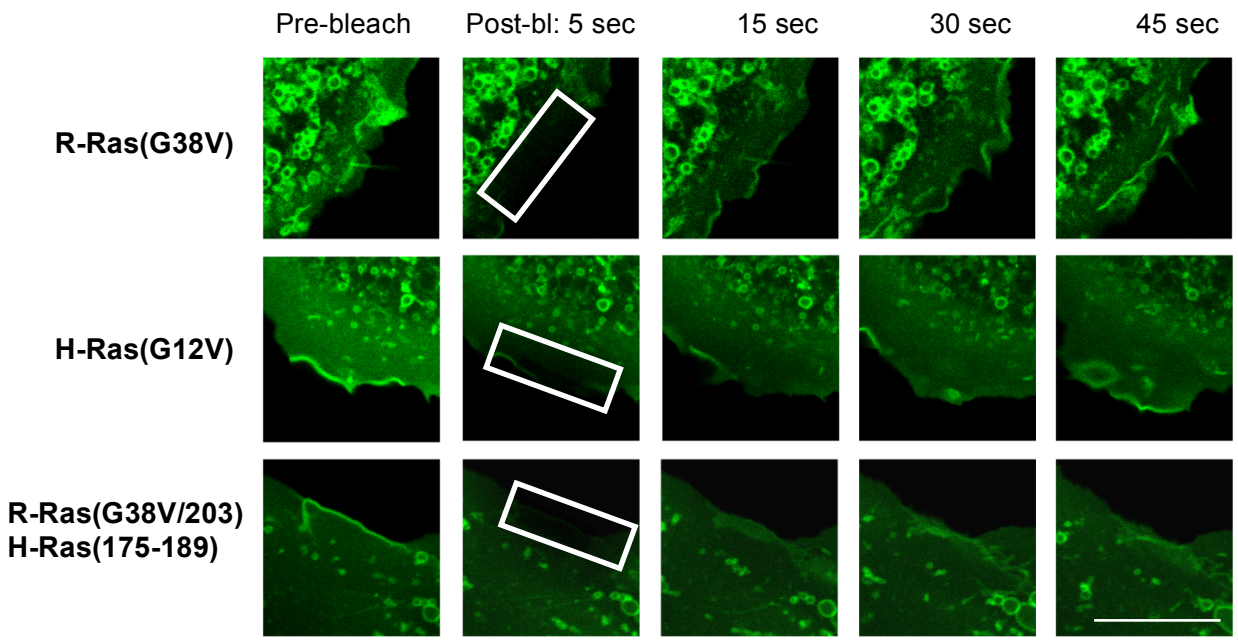

Figure S2. FRAP imaging for GFP-R-Ras or -H-Ras in migrating cells. Representative images from FRAP series in Figure 3. The white box shows the bleach area. Bar, 7.5 μm.

Video S1. R-Ras vesicular trafficking in migrating cells. RFP-R-Ras(G38V) (red) and GFP-Rab11 (green) were imaged by confocal fluorescence scanning in 30 sec intervals in living NIH 3T3 cells seeded on fibronectin-coated surfaces.

Video S2. R-Ras activation is required for anterograde vesicular trafficking. GFP-R-Ras(S43N) was imaged by confocal fluorescence scanning in 15 sec intervals in living NIH 3T3 cells seeded on fibronectin-coated surfaces.

Video S3. R-Ras(G38V) and R-Ras(G38V/203)H-Ras(175-189) vesicular trafficking and segregation in migrating cells. GFP-R-Ras(G38V) (green) and RFP-R-Ras(G38V/203)H-Ras(175-189) (red) were imaged by confocal fluorescence scanning in 30 sec intervals in living NIH 3T3 cells seeded on fibronectin-coated surfaces. The leading edge of the cell is to the lower right (now shown). Manual tracking of a R-Ras(G38V) vesicle (blue line) and a RFP-R-Ras(G38V/203)H-Ras(175-189) vesicle (green line) reveal the segregation and rapid anterograde trafficking of R-Ras(38V) from R-Ras containing the H-Ras HVR.

Video S4. R-Ras(G38V/203)H-Ras(175-189) vesicular trafficking in migrating cells. RFP-R-Ras(G38V/203)H-Ras(175-189) (red) and GFP-Rab11 (green) were imaged by confocal fluorescence scanning in 1 min intervals in living NIH 3T3 cells seeded on fibronectin-coated surfaces.

Video S5. R-Ras(G38V) and PH-Akt in membrane ruffles. RFP-R-Ras(G38V) (red, Vid. S5A) and GFP-PH-Akt (green, Vid. S5B) were imaged by confocal fluorescence microscopy in 30 sec intervals in living NIH 3T3 cells seeded on fibronectin-coated surfaces. Merged image sequence is shown in Video S5C.