SUPPLEMENTAL FIGURES
Figure S1:

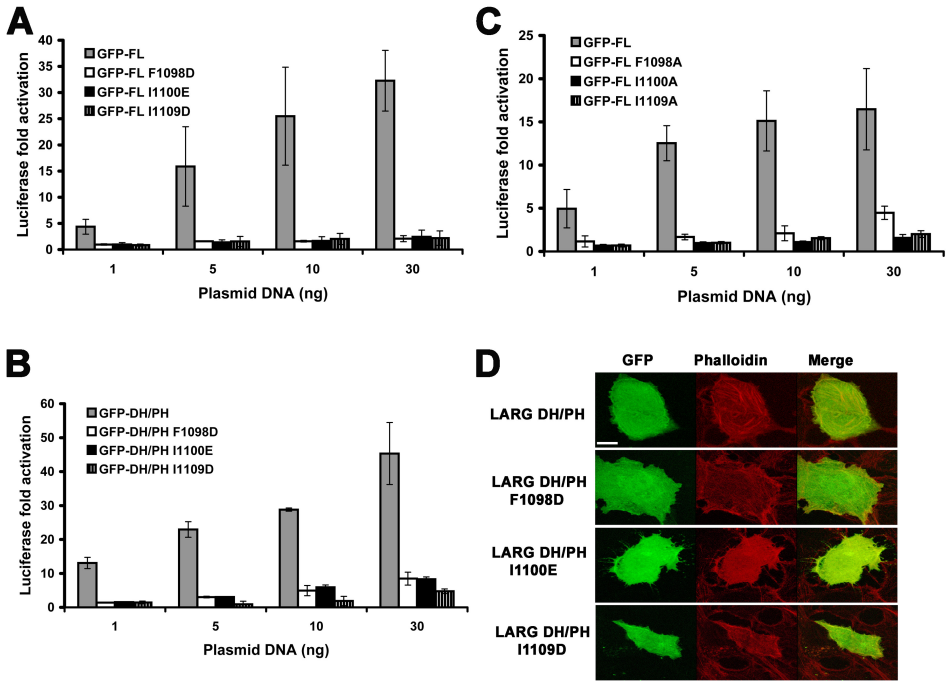

Figure S1: Hydrophobic patch mutants inhibit the activity of GFP-fusions of LARG in cells. GFP fusions of (A) FL or (B) DH/PH fragments of LARG (WT and hydrophobic patch mutants) were tested for their ability to induce RhoA activation in HEK293T cells by SRE.L luciferase reporter assay. In both contexts, the mutants greatly inhibit activity. Data represents the mean ± SD of 2 independent experiments, each measured in triplicate (C) SRE.L luciferase activities of GFP-LARG-F1098A, -I1100A, and -I1109A also show a defect relative to WT in RhoA activation. Data represents the mean ± SD of three experiments, each measured in triplicate. (D) Confocal microscopy of NIH3T3 cells expressing GFP-DH/PH proteins. Only WT GFP-DH/PH was able to induce stress fiber formation. Scale bar: 10 μm.

Figure S2:

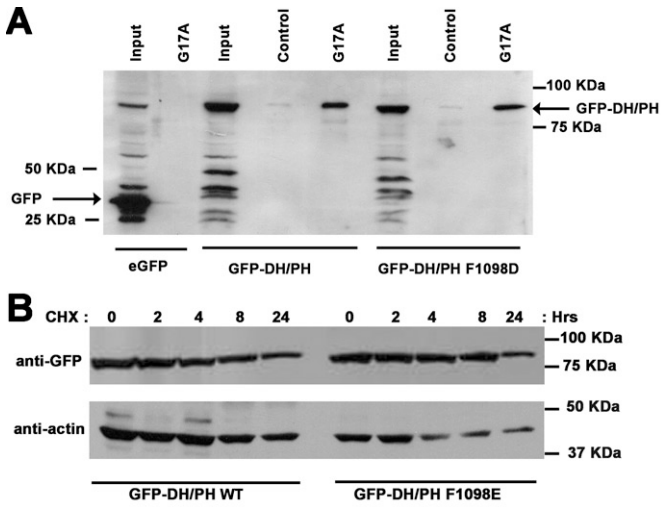

Figure S2: Hydrophobic patch mutants of LARG are structurally intact. (A) Purified, nucleotide-free RhoA-G17A was biotinylated, coupled to streptavidin beads, and used as bait in pull-downs from extracts from HEK293T cells expressing GFP alone or GFP-DH/PH (WT or F1098D). Samples were resolved by SDS-PAGE, and GFP and GFP-fusion proteins were detected by Western using an anti-GFP antibody. Lanes labeled as "input", "G17A", and "control" correspond to total lysate, bound RhoA-G17A after 3 washes, and blank beads, respectively. The data shown is representative of 2 experiments. (B) Stability of GFP-tagged LARG proteins. Lysates from HEK 293T cells transfected with the indicated constructs and treated with cycloheximide for the indicated times were analyzed by Western blot using anti-GFP (top panel) and anti-actin (bottom panel) antibodies. There is no significant difference in stability between WT and F1098D LARG DH/PH fragments. The data shown is representative of at least 2 experiments**.**

Figure S3:

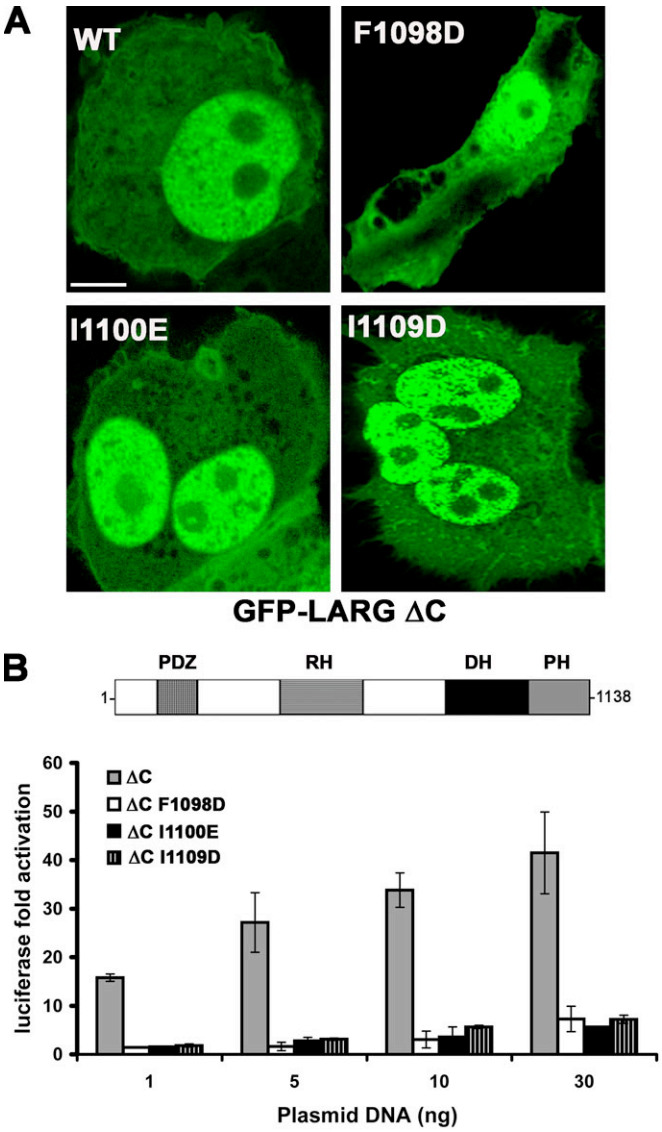

Figure S3: Mutation of the hydrophobic patch impairs RhoA activation by nuclear-localized LARG ΔC. (A) Confocal microscopy images of COS-7 cells transfected with GFP fusions of LARG ΔC (WT, F1098D, I1100E, or I1109D) showing nuclear distribution. Scale bar: 10 μm. (B) Normalized SRE.L luciferase activities of C-terminally truncated LARG (WT and hydrophobic patch mutants). Despite their predominantly nuclear localization, ΔC-F1098D, ΔC-I1100E, and ΔC-I1109D are still severely compromised in their ability to induce RhoA activation in cells relative to WT.

Data represents the mean ± SD of three independent experiments, each measured in triplicate.

Figure S4:

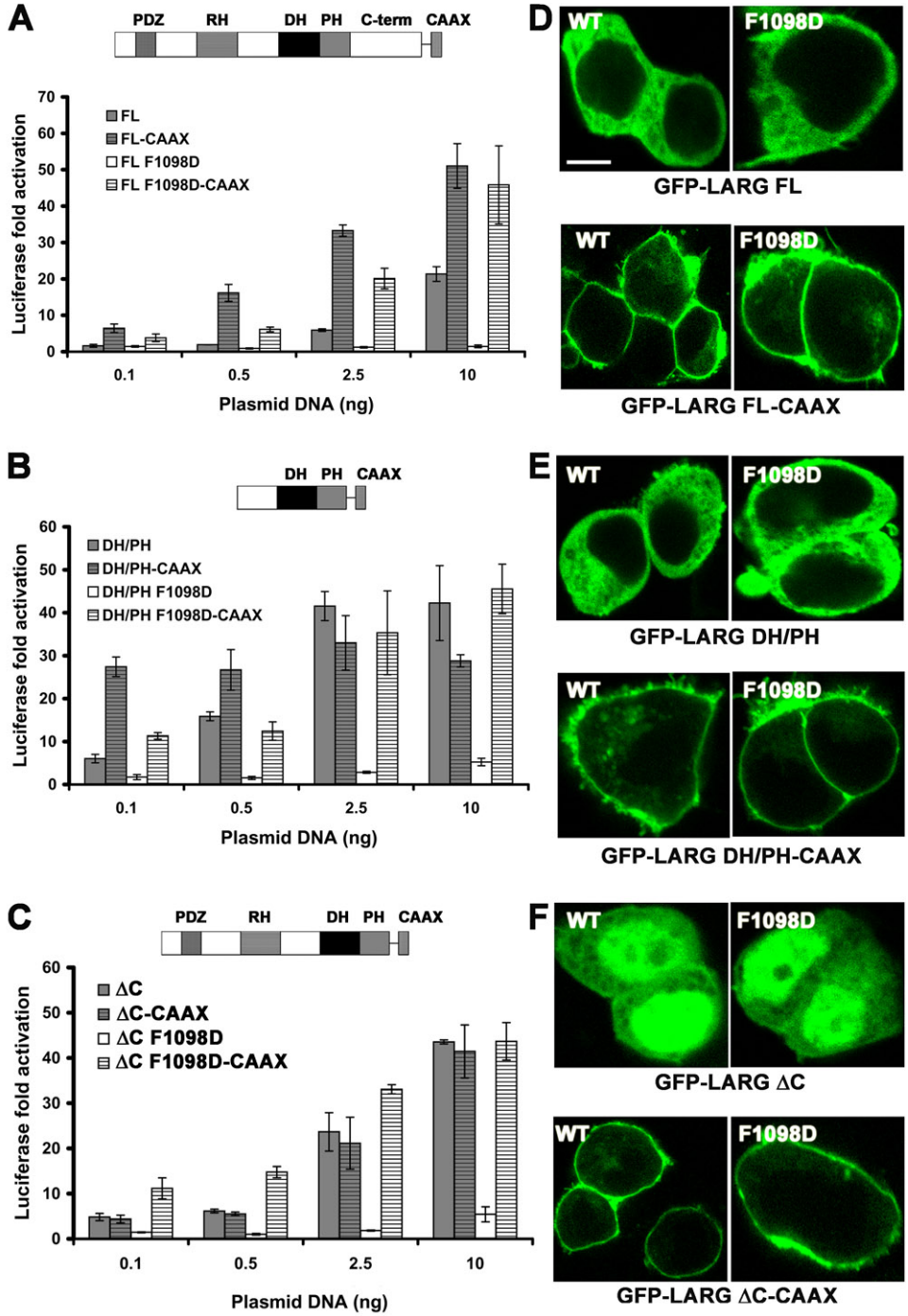

Figure S4: Fusion of LARG with the K-Ras C-terminus rescues the activity of the F1098D mutant. SRE.L luciferase activity of (A) FL, (B) DH/PH, and (C) ΔC LARG (WT and F1098D mutant) ± the membrane-targeting CAAX fusion. Each data point represents the mean ± SD of one experiment measured in triplicate. Confocal images of

(D) FL, (E) DH/PH, and (F) ΔC LARG expressed in HEK293T cells. The CAAX fusion redistributes LARG to the cell membrane, even in the case of the ΔC constructs, which are nuclear localized in the absence of the fusion. Scale bar: 10μm. Confocal images of some of the non-fused proteins in panels (E) and (F) are the same as in Fig. 6C and are shown here for comparison.

Figure S5:

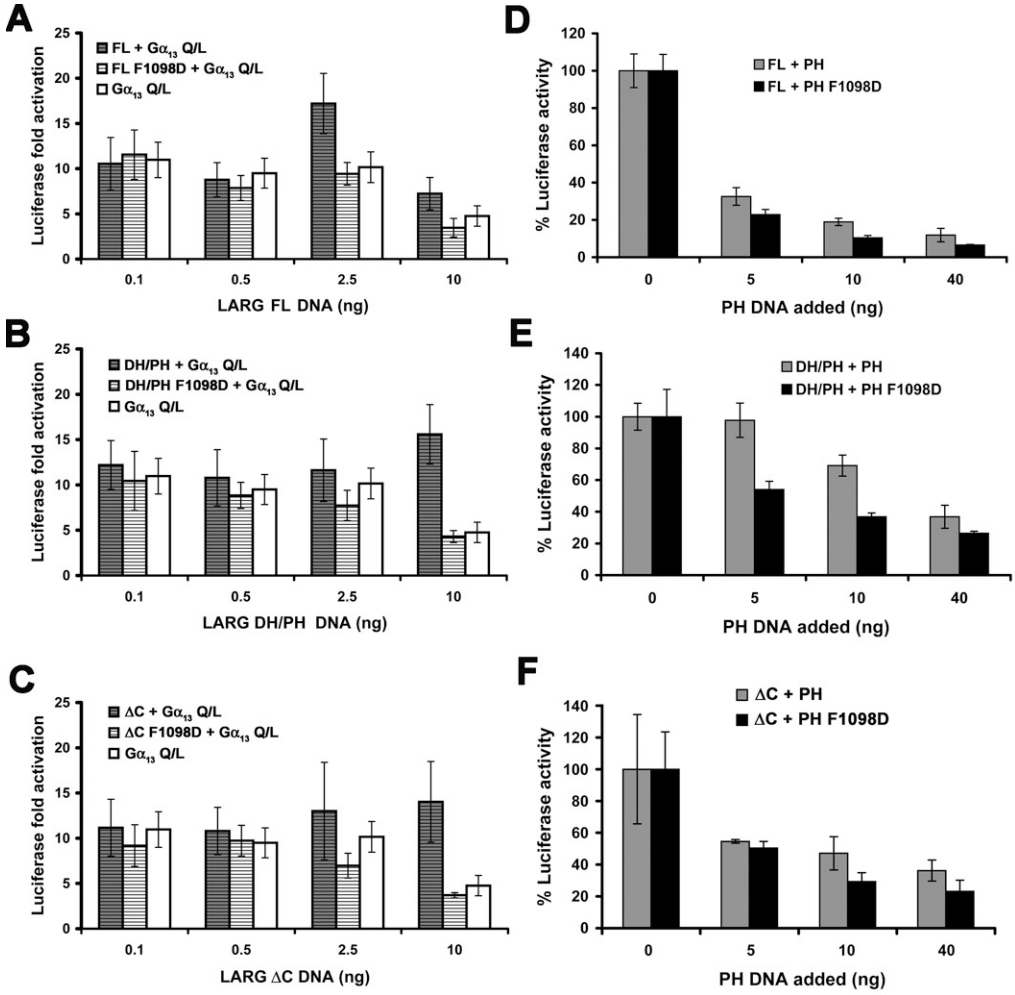

Figure S5: Co-transfection experiments. (A-C) Comparison of SRE.L luciferase activities of HEK293T cells co-transfected with 5 ng of $G\alpha_{13}$-Q226L and increasing amounts of LARG WT or F1098D, or GFP alone: (A) FL, (B) DH/PH and (C) ΔC. Data is representative of two independent experiments and error bars give the SD for each data point measured in triplicate. (D-F) Inhibition of SRE.L luciferase activities from HEK293T cells co-transfected with 10 ng DNA of (D) FL, (E) DH/PH or (F) ΔC and increasing DNA amounts of WT or F1098D LARG PH domain constructs. The hydrophobic patch does not appear to be involved in this inhibition because the F1098D

mutant causes similar inhibition to WT. The data shown represents the mean ± SD from one experiment measured in triplicate.