Supplemental Figure 1. Gradient formation in the well of an 8-well slide. (A) Theoretical diffusion of a small molecule dye, and of rAprA, at 20 minutes after introduction to the corner of a well as a point source. The observed distribution of dye from experiments as in panel B at the indicated times is also shown. Data values are mean ± SEM, n=3. (B) Images of methylene blue dye, added to the top left corner of the well, forming a gradient in the well. Images were taken at the top left corner (left column), middle (middle column) and bottom right corner (right column) of the well. Bar is 0.1 cm.
**Supplemental Figure 2.** rAprA does not inhibit Ras cortical activation in *grlH*− cells, and does not alter total Ras levels within 60 minutes. (A) *grlH*− cells expressing Raf1-RBD-GFP (green) were incubated in 0 (control) or 300 ng/ml rAprA for 0 or 30 minutes then imaged. Image pairs are fluorescence on top and combined fluorescence and DIC below. Bars are 10 µm. Images are representative of cells from 3 independent experiments. (B and C) Whole cell lysates of wild type Ax2 cells incubated with or without 300 ng/ml rAprA for the indicated times were run on SDS–polyacrylamide gels. Gels were stained with Coomassie (B), or Western blots of the gels were stained with anti-Ras antibodies (C). Images are representative of 3 independent experiments. (D) Ax2 cells were incubated in the indicated concentrations of rAprA for 6 hours. Whole cell lysates were run on duplicate SDS–polyacrylamide gels. One gel was stained with Coomassie and a western blot of the other gel was stained with anti-Ras antibodies. For quantitation of the immunoblot, bands were first normalized to their respective lane on the Coomassie gel and then normalized to the 6-hour control (no rAprA). Values are mean ± SEM, n=3. *** indicates p < 0.001 compared to control (2-way ANOVA, Holm-Šídák’s test).

**Supplemental Figure 3.** Levels of Ras in mutant cells. Ax2, *rasC*−, *rasG*−, *rasG*/C−, and *rasD*− cells were incubated without rAprA. (A) The cells were then fixed and stained with anti-Ras antibodies. Images are representative of cells from 3 independent experiments. Bars are 10 µm. (B) Lysates of the indicated cell types were used for a Coomassie-stained gel (top). Aliquots of the lysates were electrophoresed on SDS-polyacrylamide gels, and western blots were stained with anti-Ras antibodies (bottom). (C) The band intensity in the Ras western blot was normalized to the integrated intensity in the corresponding lane of the Coomassie-stained gel. Graph shows the normalized Ras levels. Values are mean ± SEM of 3 independent experiments.
Supplemental Figure 4. AprA inhibition of Ras cortical activation in aprA− cells. (A) aprA− cells expressing Raf1-RBD-GFP were incubated in 300 ng/ ml rAprA and imaged. Graph shows Raf1-RBD-GFP (green) intensity at the cortical region of the cells, normalized to the 0-minute control. Values are mean ± SEM of the averages from 3 independent experiments, with at least 40 randomly chosen cells examined for each point in each experiment. (B) aprA− cells were incubated as in panel A, and total cell lysates or Raf1-RBD affinity bead pull-down samples were run on SDS-polyacrylamide gels. Gels were stained with Coomassie, or western blots of the gels were stained with anti-Ras antibody. Graph shows the levels of GTP-bound Ras normalized to the 0-minute control. Values are mean ± SEM from 3 independent experiments. ** indicates p < 0.01, *** p < 0.001 compared to the 0-minute control (Unpaired t-tests, Welch’s correction).
**Supplemental Figure 5.** High cell density decreases Ras levels in cells at the center of colonies. The indicated numbers of wildtype Ax2 cells were spotted onto glass wells and incubated in HL5 for 6 hours. The cells were then fixed and stained with anti-Ras antibodies. Bars are 0.5 mm. Stitched images are representative of 3 independent experiments.

**Supplemental Figure 6.** AprA inhibits Ras activation at the cortical region in some mutants. Live cells of the indicated cell types expressing Raf1-RBD-GFP (green) were imaged at 0 and 30 minutes of exposure to a uniform concentration of 300 ng/ml of rAprA. Image shows combined fluorescence and DIC. Bar is 10 µm. Images are representative of 3 independent experiments.
Supplemental Figure 7. Erk2 is required for rAprA induced chemorepulsion. The indicated strains were imaged for 40 minutes after 20 minutes of exposure in growth medium (control) or in a rAprA gradient in growth medium in Insall chambers. A positive forward migration index (FMI) indicates chemorepulsion from the AprA and a negative FMI indicates chemotraction. Values are mean ± SEM of the averages of 4 independent experiments with at least 45 randomly chosen cells examined in each experiment. On the left side of the graph, *** indicates p < 0.001, **** p < 0.0001 compared with control for that genotype (Unpaired t-tests, Welch’s correction). On the right side of the graph, * indicates p < 0.05 compared to Ax2 (Unpaired t-tests, Welch’s correction).

Supplemental Figure 8. rAprA redistributes active Ras to the region of the cell away from a source of rAprA in some mutants. Images are of live cells expressing Raf1-RBD-GFP (green) in a rAprA or buffer gradient. * indicates the direction from the cell where rAprA was added. Image shows combined fluorescence and DIC. Bar is 10 µm. Images are representative of 3 independent experiments.
Supplemental Figure 9. Ras cortical activation in mutant cells at the edge of a colony. Colonies of the indicated strains expressing Raf1-RBD-GFP were incubated in HL5 for 6 hours. Live images of the cells were then taken. Graph shows the percent of cells at the edges of colonies that have Raf1-RBD-GFP at the cortical region of the cell. Values are mean ± SEM of the averages from 4 independent experiments, with at least 30 randomly chosen cells examined in each experiment. * indicates p < 0.05, ** p < 0.01, *** p < 0.001 compared to Ax2 (Unpaired t-tests, Welch’s correction).