Saraiva et al., http://www.jcb.org/cgi/content/full/jcb.201301016/DC1



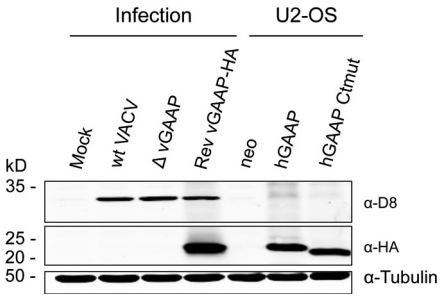



Figure S1. **hGAAP protein levels in U2-OS cells overexpressing hGAAP are comparable to vGAAP protein levels after viral infection.** U2-OS neo cells were infected with wild-type (wt) vGAAP, deletion (Δ) vGAAP, or revertant (Rev) vGAAP-HA VACV and harvested after 6 h. The IB using anti-HA Ab compares GAAP expression in equivalent numbers of VACV-infected cells and the cells overexpressing hGAAP. The VACV protein D8 was used as a control for viral infection, and tubulin was used as a control for cell loading. The results are typical of three similar analyses.

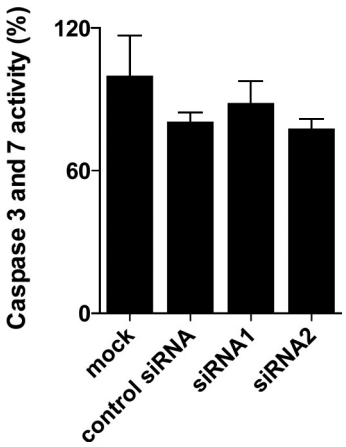



Figure S2. **hGAAP knockdown does not stimulate caspase activity.** U2-OS neo cells were transfected with control siRNA or siRNAs (1 and 2) specific for hGAAP. The activities of caspases 3 and 7 were measured after 36 h. Results show caspase activity as a percentage of that detected in mock-transfected cells. Data are representative of three experiments. Error bars are means ± SEM.

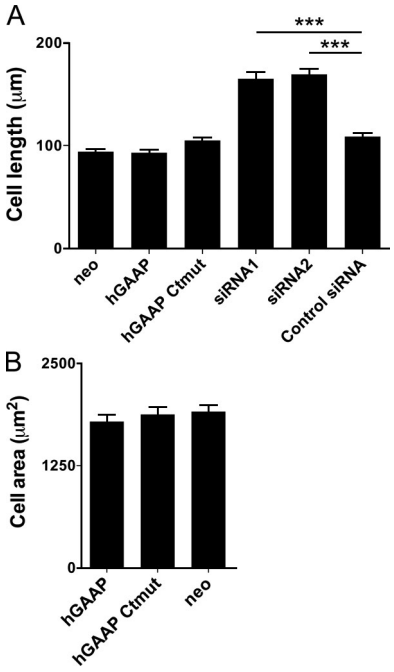



Figure S3. **Loss of hGAAP causes cell elongation, whereas overexpression of hGAAP affects neither cell length nor area.** (A) U2-OS cells overexpressing hGAAPs, or transfected with siRNA to reduce hGAAP expression, were seeded on fibronectin at low confluence and fixed after 24 h, and the lengths of cells were measured from confocal images. (B) Similar experiments with cells overexpressing hGAAP or hGAAP Ctmut were fixed after 18 h and used to determine cell areas. Results (A and B) are means ± SEM from 80 cells. ***, $P < 0.001$ (Student's *t* test).

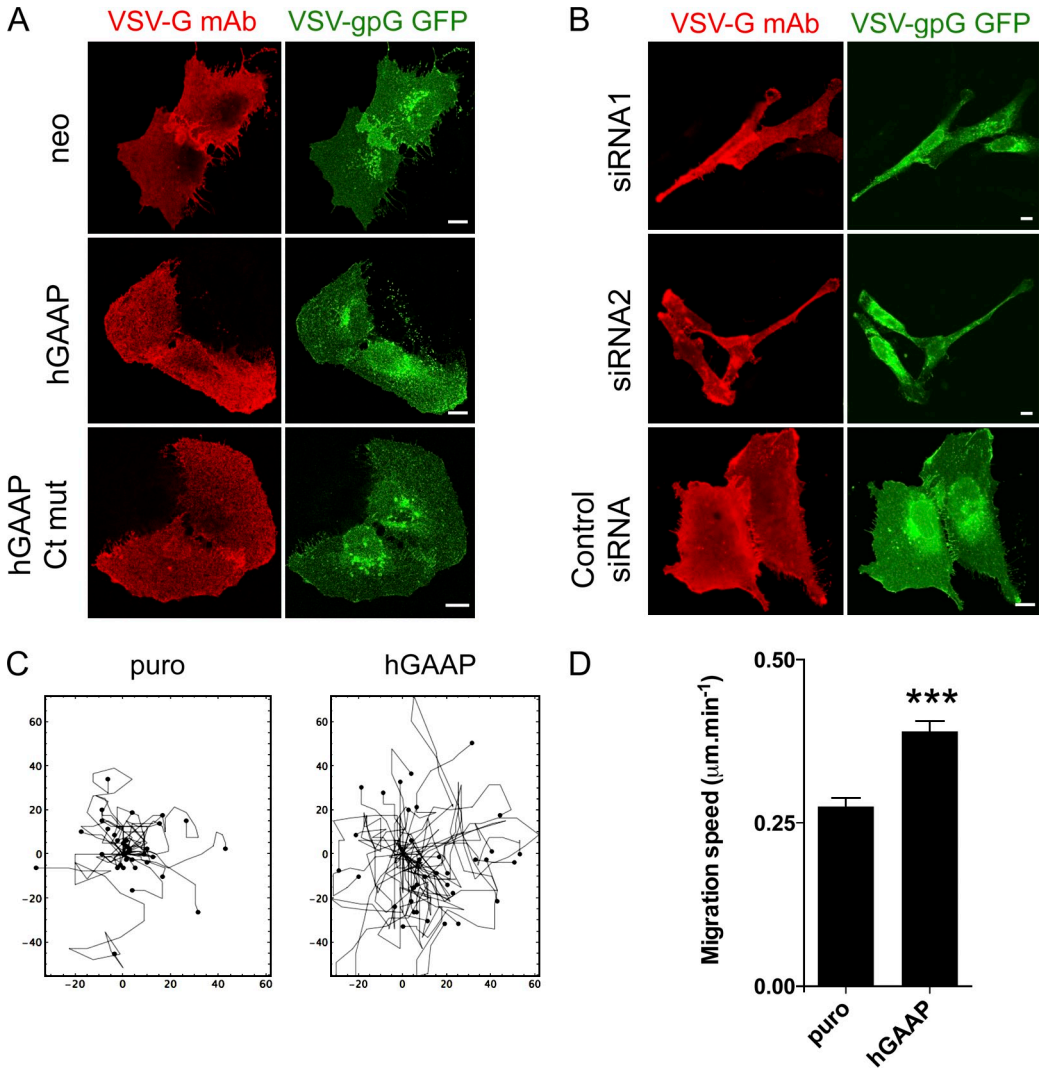



Figure S4. **Overexpression or knockdown of hGAAP does not prevent protein trafficking to the plasma membrane.** (A and B) U2-OS cells overexpressing hGAAP-HA or hGAAP Ctmut-HA (A) or transfected with siRNAs for 18 h (B) were transfected with a plasmid encoding VSV-G–GFP and incubated for a further 18 h. Cells were fixed, but not permeabilized, and immunostained with an anti–VSV-G Ab. Cells were imaged for total VSV-G (GFP signal) and cell surface VSV-G (Ab). Bars, 10 µm. Results are typical of three similar analyses. hGAAP overexpression increases the speed of HeLa cell migration. (C and D) HeLa cells overexpressing hGAAP or control puro cells were seeded at low density on fibronectin-coated dishes. Individual cells were imaged at 5-min intervals for 18 h. Tracks of individual cells ($n$ = 30) are shown in C. (D) Migration rates are shown as means ± SEM from 30 cells. ***, $P < 0.001$ (Student's $t$ test, relative to puro cells).

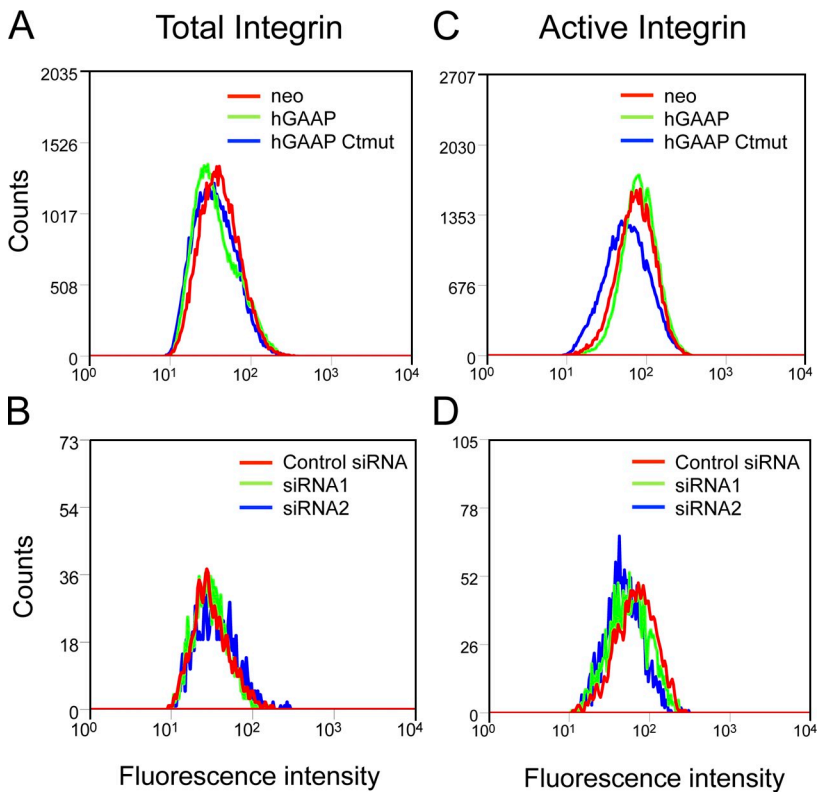
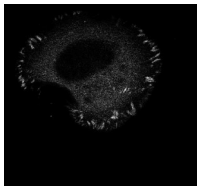
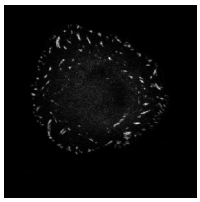


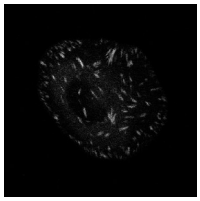

Figure S5. **Cell surface expression of integrins is unaffected by overexpression or knockdown of hGAAP.** (A–D) Cell surface expression of total (A and B) or active (C and D) β1 integrin in U2-OS cells was determined by FACS using antibodies specific to active or total human β1 integrin. Cells were transfected as indicated with control plasmid (neo), plasmids encoding hGAAP or hGAAP Ctmut, or with hGAAP-specific or control siRNAs. Results are representative of three independent experiments.



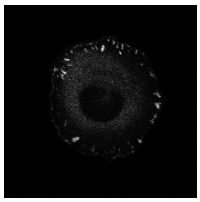
Video 1. **hGAAP stimulates turnover of focal adhesions: Control cells.** U2-OS cells expressing vinculin-GFP were imaged by time-lapse fluorescence microscopy using a laser-scanning confocal microscope (LSM 510 META). Images were acquired at 2-min intervals for 90 min. Playback rate is 15 frames/s.



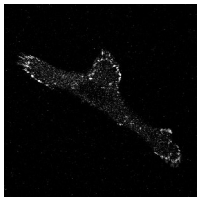
Video 2. **hGAAP stimulates turnover of focal adhesions: Cells overexpressing hGAAP.** U2-OS cells stably overexpressing hGAAP and transiently expressing vinculin-GFP were imaged by time-lapse fluorescence microscopy using a laser-scanning confocal microscope (LSM 510 META). Images were acquired at 2-min intervals for 90 min. Playback rate is 15 frames/s.



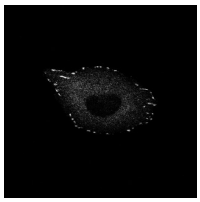
Video 3. **hGAAP stimulates turnover of focal adhesions: Cells expressing hGAAP Ctmut.** U2-OS cells stably expressing hGAAP Ctmut and transiently expressing vinculin-GFP were imaged by time-lapse fluorescence microscopy using a laser-scanning confocal microscope (LSM 510 META). Images were acquired at 2-min intervals for 90 min. Playback rate is 15 frames/s.



Video 4. **hGAAP stimulates turnover of focal adhesions: Cells transfected with control siRNA.** U2-OS cells transfected with control siRNA followed by expression of vinculin-GFP were imaged by time-lapse fluorescence microscopy using a laser-scanning confocal microscope (LSM 510 META). Images were acquired at 2-min intervals for 90 min. Playback rate is 15 frames/s.



Video 5. **hGAAP stimulates turnover of focal adhesions: Cells transfected with siRNA1 against hGAAP.** U2-OS cells transfected with siRNA1 against hGAAP followed by expression of vinculin-GFP were imaged by time-lapse fluorescence microscopy using a laser-scanning confocal microscope (LSM 510 META). Images were acquired at 2-min intervals for 90 min. Playback rate is 15 frames/s.



Video 6. **hGAAP stimulates turnover of focal adhesions: Cells transfected with siRNA2 against hGAAP.** U2-OS cells transfected with siRNA2 against hGAAP followed by expression of vinculin-GFP were imaged by time-lapse fluorescence microscopy using a laser-scanning confocal microscope (LSM 510 META). Images were acquired at 2-min intervals for 90 min. Playback rate is 15 frames/s.