Supplemental material

Ratcliffe et al., https://doi.org/10.1083/jcb.201804106

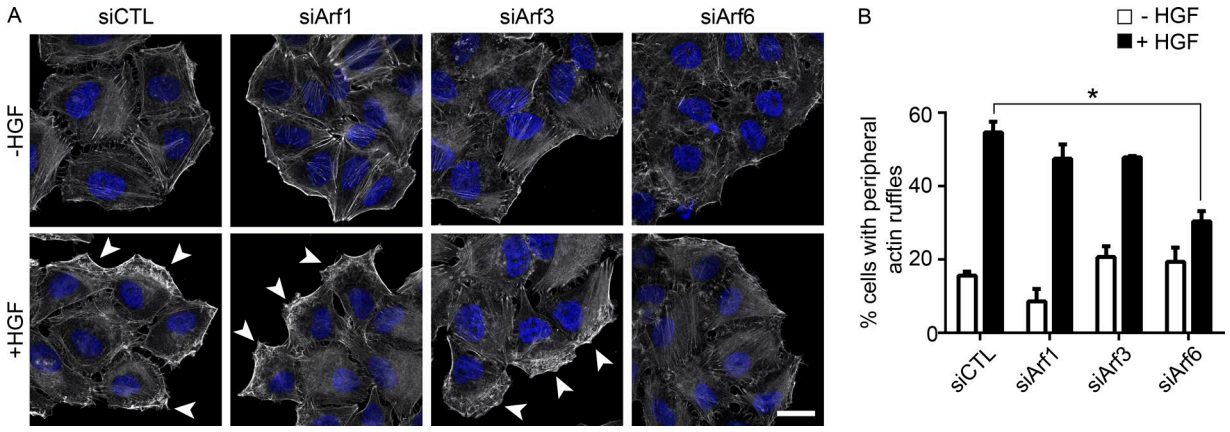


Figure S1. **Arf6 regulates HGF-dependent actin remodeling. (A)** Confocal images of HeLa cells counterstained with phalloidin (F-actin) and DAPI and treated with or without HGF. **(B)** Quantification of experiments shown in A. Scale bar, 20 µm. Arrowheads indicate peripheral membrane ruffles. All quantified data indicate mean ± SEM from three independent experiments. *, P < 0.05.

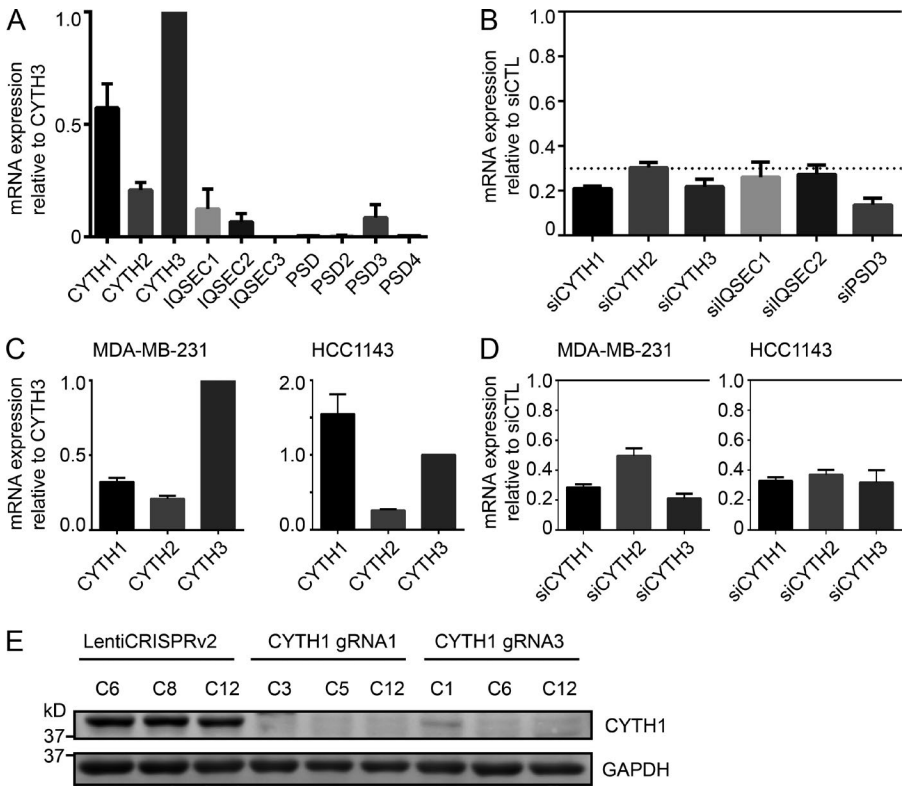


Figure S2. **Expression and depletion of Arf GEFs and cytohesin-1 isoforms and mutants in HeLa cells. (A)** Relative levels of known Arf GEFs from HeLa cell lysates measured by quantitative RT-PCR. **(B)** Relative levels of siRNA-mediated depletion of Arf GEFs expressed in HeLa cells measured by quantitative RT-PCR. **(C)** Relative levels of cytohesin family members from MDA-MB-231 and HCC1143 cell lysates measured by quantitative RT-PCR. **(D)** Relative levels of siRNA-mediated depletion of cytohesin family members in MDA-MB-231 and HCC1143 cells measured by quantitative RT-PCR. **(E)** Western blot analysis of cytohesin-1 protein levels in empty vector or CYTH1 gRNA expressing DO clones generated using the CRISPR/Cas9 LentiCRISPR v2 system. All quantified data indicate mean ± SEM from three independent experiments.

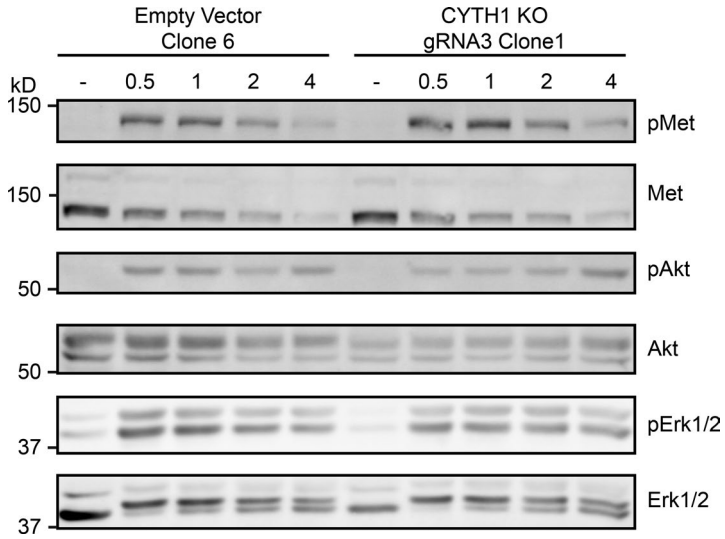


Figure S3. **CYTH1 KO does not affect Met stability or HGF-dependent Akt or Erk1/2 signaling.** Control or CYTH1 KO cells were stimulated with HGF in the presence of cycloheximide for the times indicated. Western blot of lysates is shown.

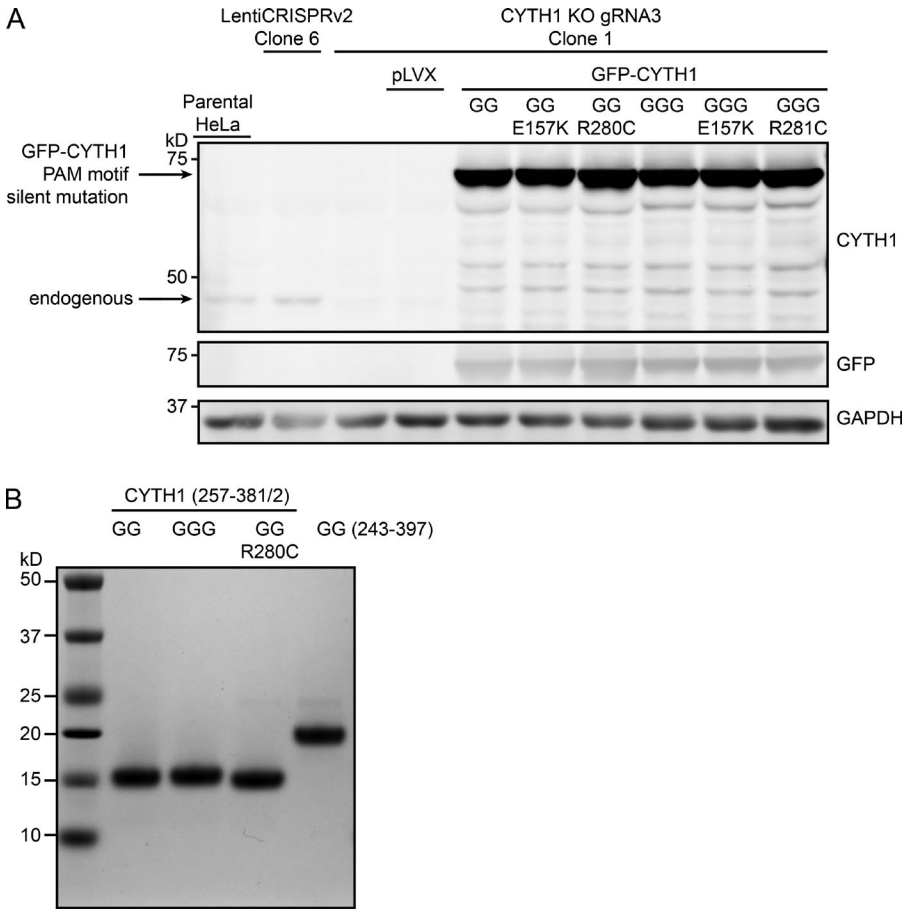


Figure S4. **Expression of cytohesin-1 isoforms and mutants. (A)** Western blot analysis of populations of expressing Cas9-resistant EGFP-CYTH1 isoforms (diglycine GG or triglycine GGG) and mutants were generated from CYTH1 KO gRNA3 clone 1. **(B)** Purification of cytohesin-1 PH domain variants. Coomassie-stained acrylamide gel of purified cytohesin-1 PH domain variants.

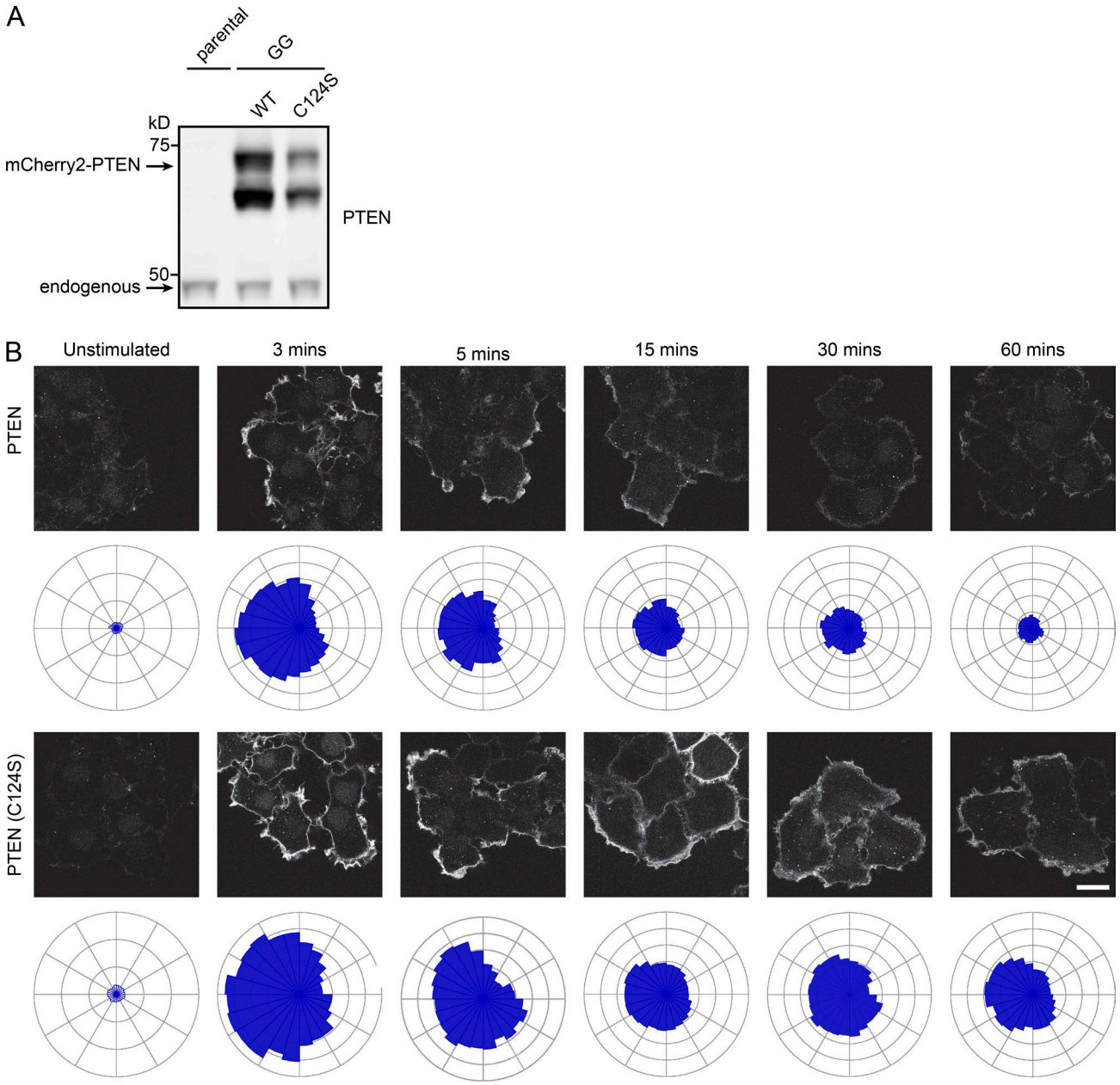
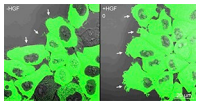


Figure S5. **PTEN activity attenuates diglycine cytohesin-1 membrane recruitment. (A)** Western blot analysis of cells expressing mCherry2-PTEN WT or C124S mutant. **(B)** HeLa cells stably expressing EGFP-tagged diglycine CYTH1 and mCherry-PTEN were either not treated (–, unfilled) or treated (+, filled) with HGF for the indicated time points, permeabilized with ice-cold 0.05% saponin in Pipes buffer and imaged by confocal microscopy. Scale bar, 20 µm.



Video 1. **Diglycine EGFP-CYTH1 localizes to the plasma membrane in response to HGF.** HeLa cells stably expressing EGFP-tagged diglycine CYTH1 were either untreated or treated with HGF for 15 min, permeabilized with ice-cold 0.05% saponin in Pipes buffer, and imaged by confocal microscopy. Transmitted light and confocal fluorescence images were captured every 4 s for 40 s after addition of permeabilization buffer, overlayed, and displayed at 1/5 s per frame. Scale bar, 20 µm.