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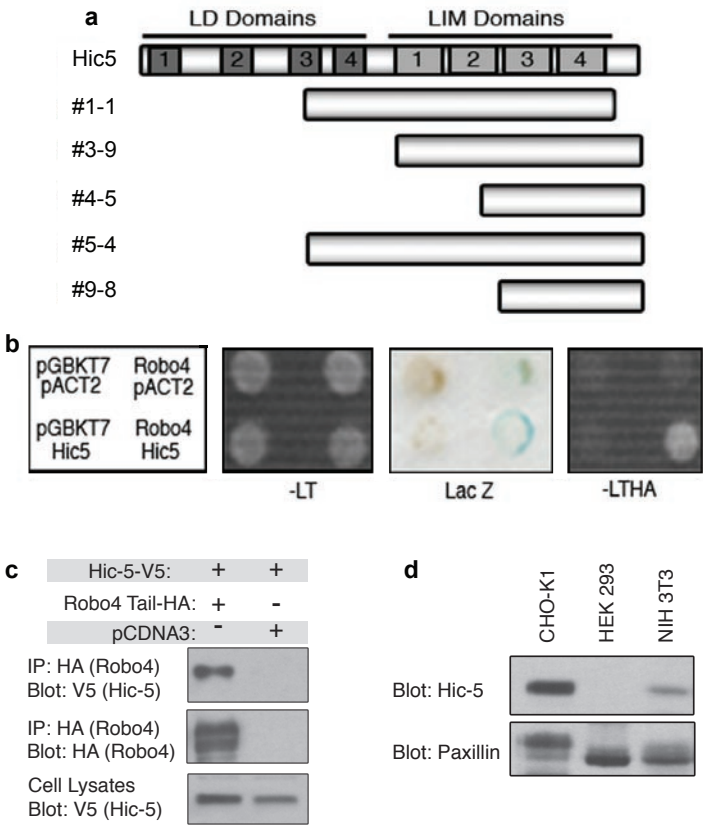

Figure S1 Hic-5 is a Robo4-interacting protein. (**a**) Schematic representation of full-length Hic-5 and the cDNA clones recovered from the yeast two-hybrid screen. (**b**) *S. cerevisiae* strain PJ694-A was transformed with the indicated plasmids and plated to synthetic media lacking Leucine and Tryptophan, or Leucine, Tryptophan, Histidine and Alanine. Colonies capable of growing on nutrient deficient media were spotted onto the same media, replica plated, and either photographed or used for the beta-galactosidase assay. (**c**) Lysates from HEK 293 cells expressing Robo4 cytoplasmic tail-HA and Hic-5-V5, or empty vector (pcDNA3) and Hic-5-V5 were immunoprecipitated with HA antibodies and immunoblotted with V5 antibodies. (**d**) Total cell lysates from Cho-K1, HEK 293 and NIH 3T3 cells were immunoblotted with antibodies to Hic-5 and paxillin.

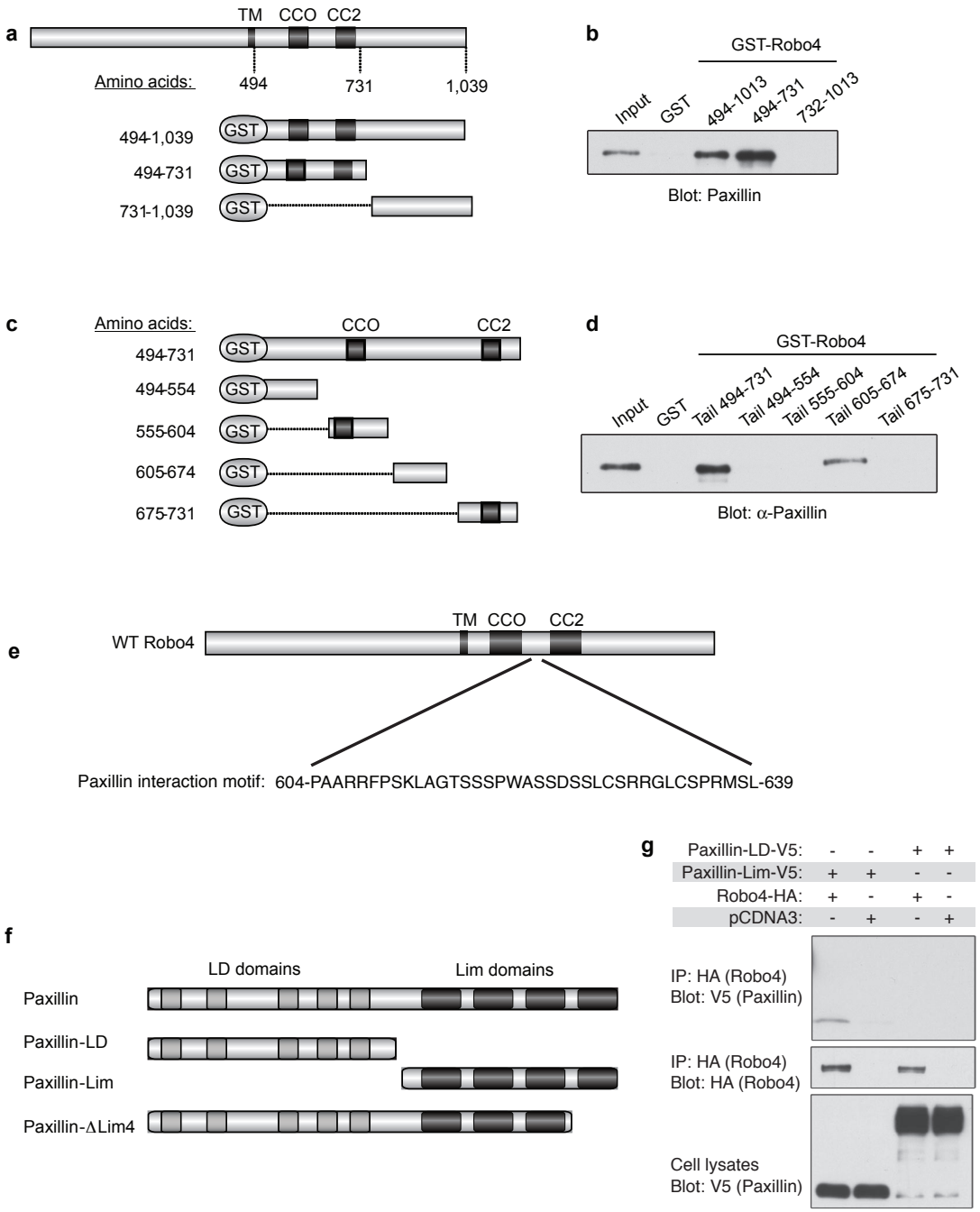

Figure S2 Identification of the Robo4 – paxillin interaction interface. (**a**, **c**) Schematic representation of GST-Robo4 fusion proteins used in pull down assays in **b** and **d**. (**b**, **d**) Purified GST-Robo4 fusion proteins were incubated with recombinant purified paxillin, precipitated with glutathione agarose and immunoblotted with paxillin antibodies. **(e)** A schematic representation of the murine Robo4 protein and illustration of the amino acids comprising the paxillin interaction motif (PIM). **(f)** Schematic representation of paxillin constructs. (**g**) Lysates of HEK 293 cells expressing Robo4 cytoplasmic tail-HA and the indicated paxillin constructs were immunoprecipitated from with HA antibodies and immunoblotted with V5 antibodies

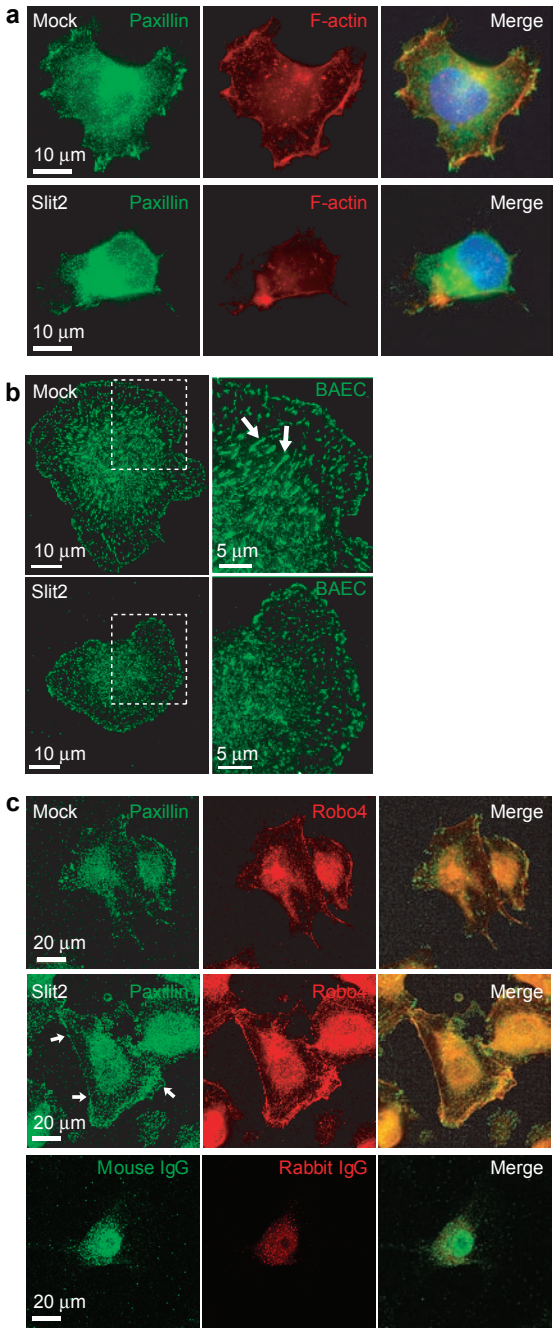

Figure S3 Slit2 redirects paxillin from focal adhesions to the cell surface. (**a**) HEK 293 cells expressing Robo4 were plated on fibronectin in the absence and presence of Slit2, and processed for indirect immunofluorescence using paxillin antibodies. (**b**) Bovine aortic endothelial (BAE) cells were plated on fibronectin in the absence and presence of Slit2, and processed for indirect immunofluorescence using paxillin antibodies. Arrows indicate mature focal adhesion complexes. (**c**) EAHY endothelial cells expressing Robo4 were plated on fibronectin incubated in the absence and presence of Slit2, and processed for indirect immunofluorescence using paxillin and Robo4 antibodies. Arrows indicate paxillin localized at the cell surface.

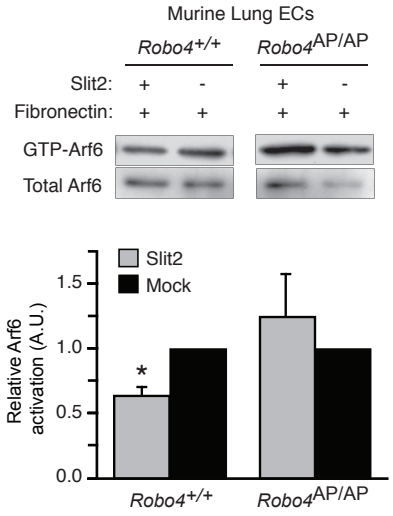

Figure S4 Robo4 is required for Slit2-mediated inhibition of Arf6 activity. Lysates from murine lung endothelial cells plated on fibronectin, in the absence and presence of Slit2, were precipitated with GST-GGA3 and immunoblotted with Arf6 antibodies ($n = 4$). *p<0.05.

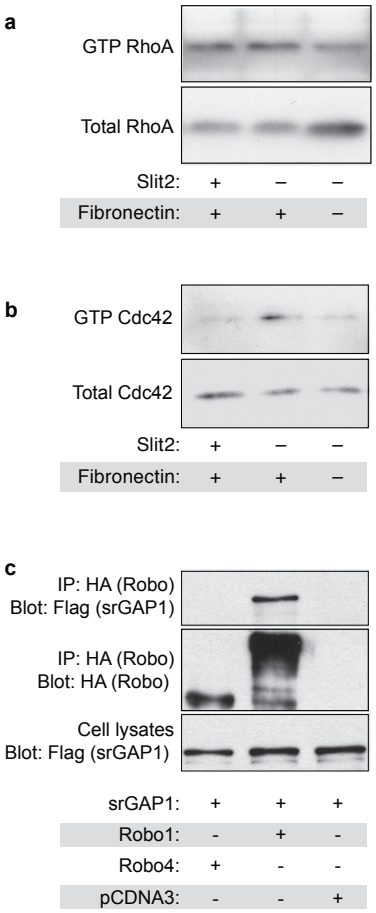

Figure S5 Robo4 inhibits Cdc42 activation but does not interact with srGAP1. (**a**, **b**) Lysates from endothelial cells plated on fibronectin, in the absence and presence of Slit2, were precipitated with (**a**) GST-RBD and immunoblotted with RhoA antibodies, or (**b**) GST-PBD and immunoblotted with Cdc42 antibodies. (**c**) Lysates from HEK 293 cells expressing the indicated plasmids were immunoprecipitated with HA antibodies and immunoblotted with Flag antibodies.

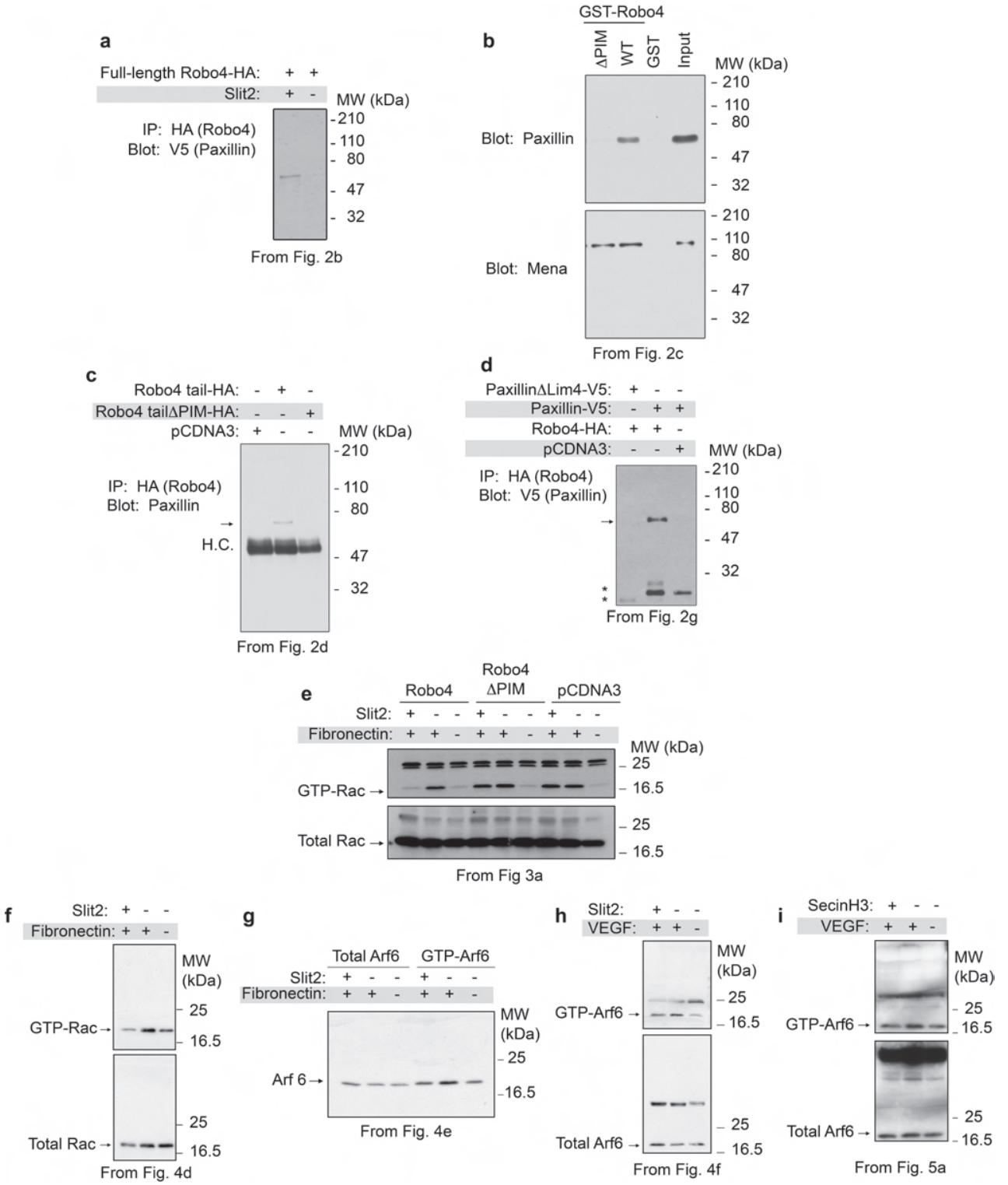

Figure S6 Selected representative full scans. (**a**) Figure 2b, Slit2 enhances Robo4-paxillin interaction. (**b**, **c**) Figure 2c and 2d, deletion of the paxillin interaction motif (PIM) results in loss of Robo4-paxillin interaction in vitro (**c**) and in mammalian cells (**d**). Figure 2g, deletion of Lim4 abrogates binding between paxillin and Robo4. (**e**) Figure 3a, the Robo4 PIM is necessary for Slit2-mediated inhibition of Rac activity. (**f**, **g**) Figure 4d and 4e, Slit2 blocks fibronectin-induced Rac (**f**) and Arf6 activation (**g**) in endothelial cells. (**h**) Figure 4f, Slit2 blocks VEGF-induced Arf6 activation in endothelial cells. (**i**) Figure 5a, SecinH3 blocks VEGF-induced Arf6 activation in endothelial cells. Arrows indicate relevant band. Asterisks indicate V5-tagged paxillin degradation products.