

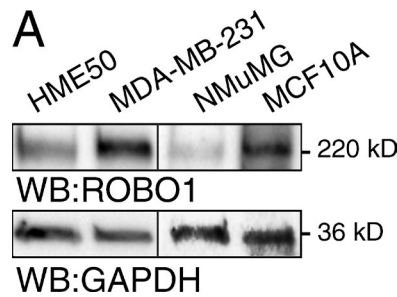
Le et al., <http://www.jcb.org/cgi/content/full/jcb.201507054/DC1>

Figure S1. **Mammary cells express ROBO1.** (A) Representative blot for ROBO1 and GAPDH showing expression of ROBO1 in HME50, MDA-MB-231, NMuMG, and MCF10A cells.

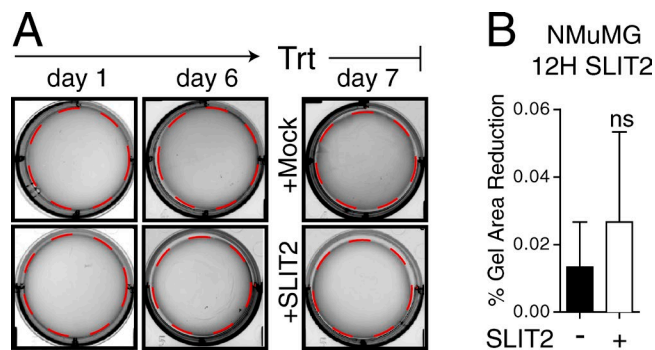


Figure S2. **HD collagen gels resist contractile forces from NMuMG cells even in the presence of SLIT2.** (A) Image of HD gels embedded with NMuMG cells and treated for 12 h with SLIT2 or Mock on day 6. Dashed lines encircle the gel area. (B) Quantification of percent gel reduction. ns, no significance by *t* test.

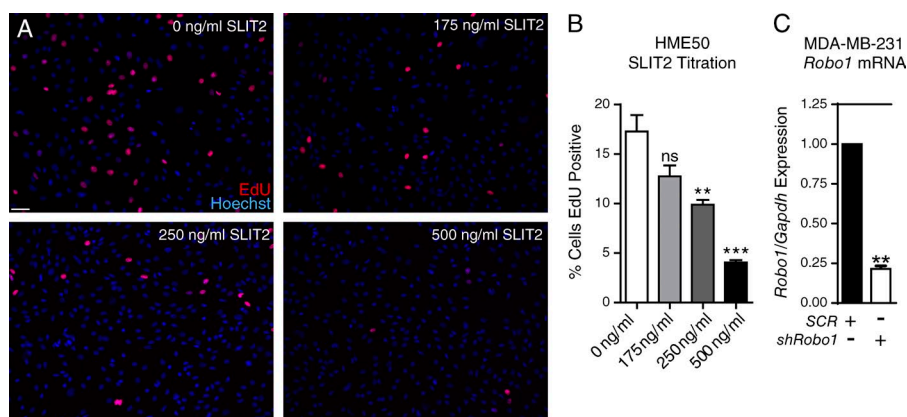


Figure S3. **SLIT2 inhibits mammary cell proliferation.** (A) HME50 cells treated with SLIT2 and assessed for EdU (red) labeling. Bar, 100 μ m. (B) Quantification of EdU-labeled cells at different SLIT2 concentrations. Asterisks denote significance; ns, no significance by *t* test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. (C) Knockdown of *Robo1* in MDA-MB-231 cells via lentivirus.

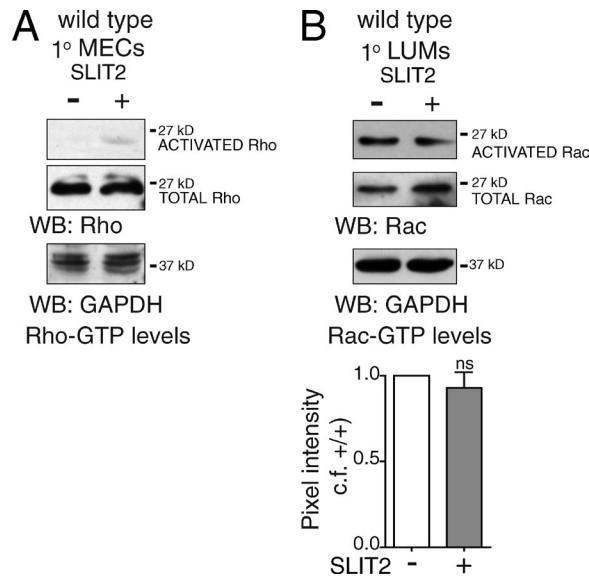


Figure S4. **SLIT2/ROBO1 modestly, but irreproducibly, activates Rho in MECs and does not activate Rac in luminal MECs.** (A and B) Activated GTPase levels were determined by pull-down assays using rotekin (A) or PAK (B) binding domains. Representative blots for GTP-Rho, total Rho, and GAPDH (A) and GTP-Rac, total Rac, and GAPDH (B) in extracts generated from primary MEC cells (A) and primary luminal MECs (LUMs; B). (B, bottom) Quantification of GTPase activation ($n = 3$ experiments).

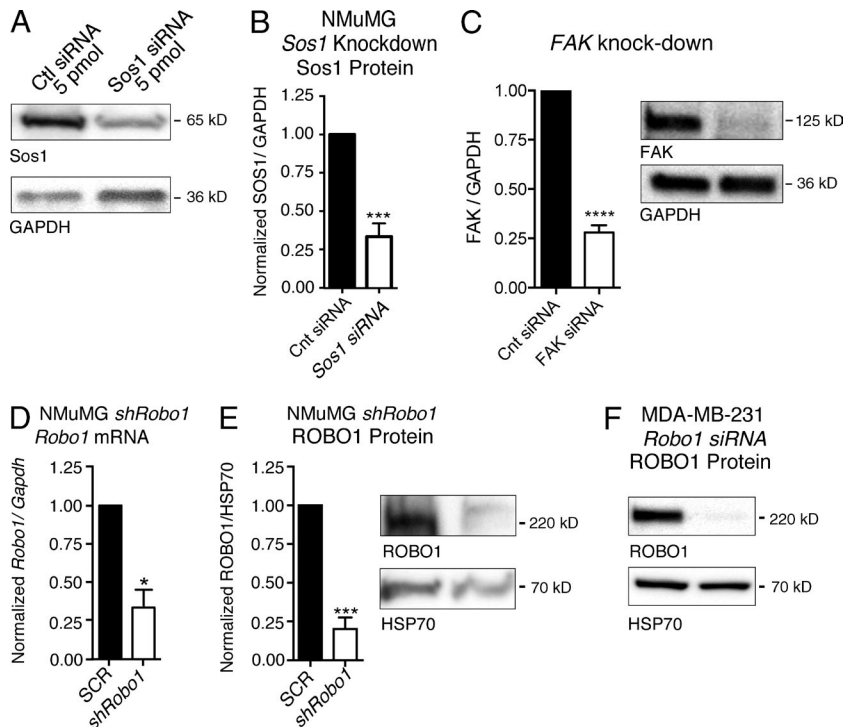


Figure S5. **Knockdown of Robo1, FAK, or Sos1 via lentivirus or siRNA.** (A and B) Blots (A) and quantification (B) of FAK and GAPDH from NMuMG cells transfected with Ctl or FAK siRNA. (C and D) Analysis of *Robo1* mRNA (C) and ROBO1 protein (D) in NMuMG cells infected with shRNA lentivirus targeting *Robo1* (*shRobo1*) or scrambled sequence (SCR). (D, bottom) Blots of ROBO1 and loading control HSP70. (E and F) Blots (E) and quantification (F) of SOS1 and GAPDH from NMuMG cells transfected with Ctl or *Sos1* siRNA. Asterisks denote significance by *t* test: *, $P < 0.05$; ***, $P < 0.001$; ****, $P < 0.0001$.