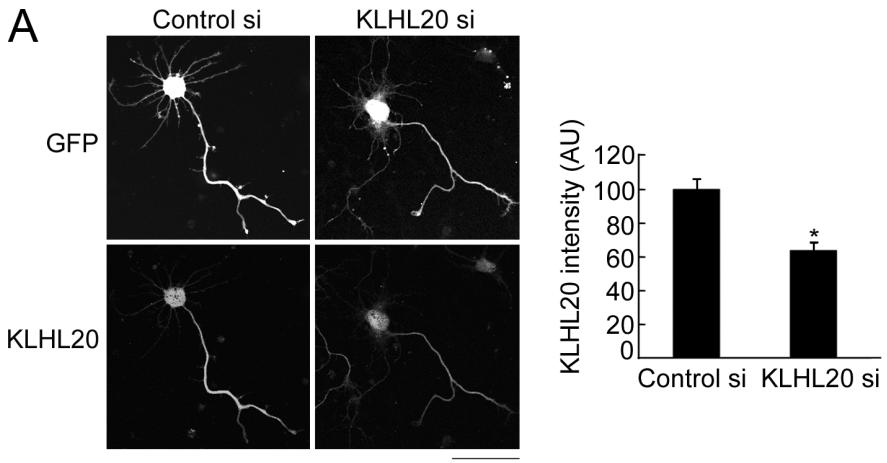
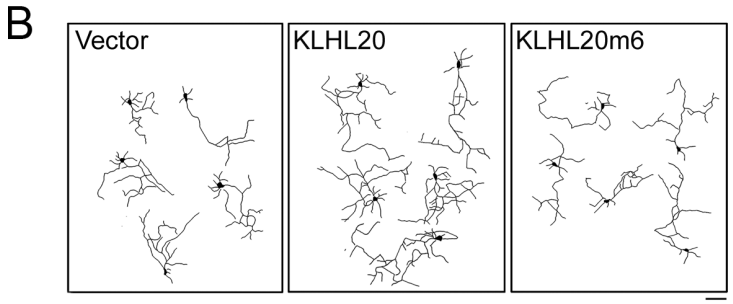
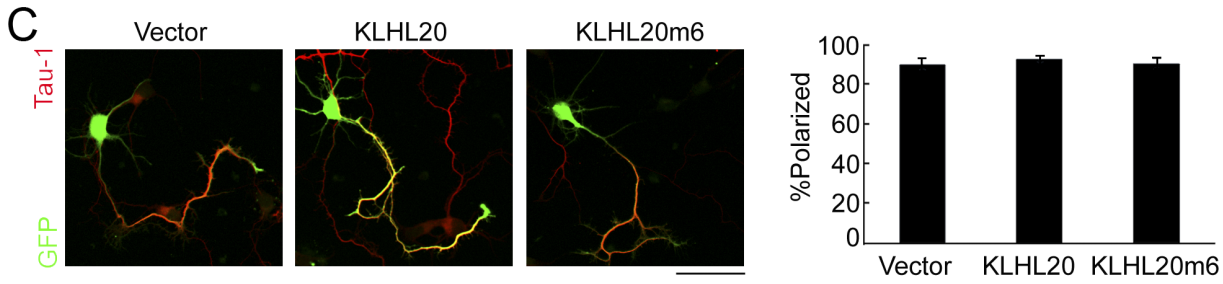
Lin et al., http://www.jcb.org/cgi/content/full/jcb.201103015/DC1







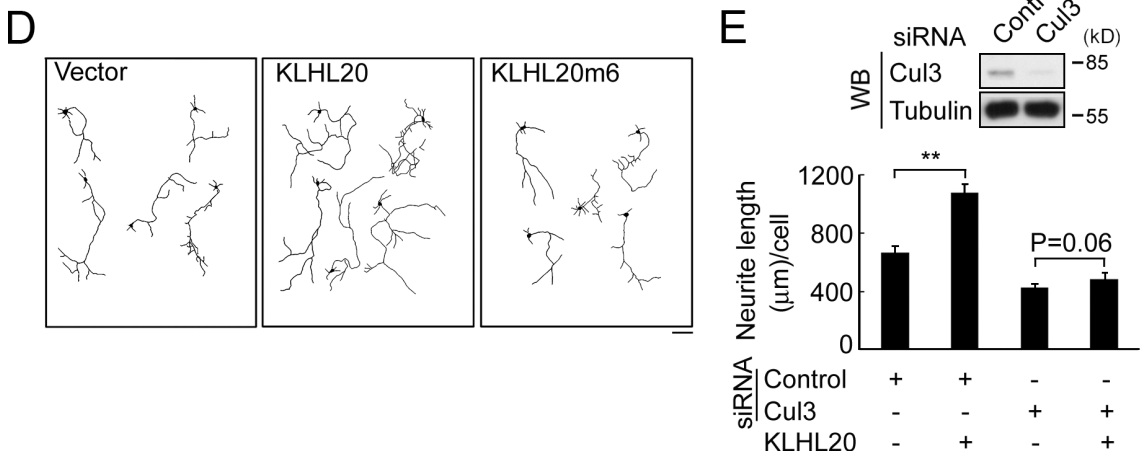


Figure S1. **The effects of KLHL20 on neuronal polarity and neurite outgrowth.** (A) Specificity of the KLHL20 antibody. Hippocampal neurons at DIV0 were transfected with control siRNA or KLHL20 siRNA together with GFP. Neurons were fixed and immunostained at DIV3, and the KLHL20 immunointensity was quantified as described in Materials and methods. Data represent means ± SEM from three independent experiments (*, $P < 0.05$; $n = 15$). (B and D) NeuronJ drawings of representative GFP-positive neurons as shown in Fig. 1 (C [panel B] and D [panel D]). (C) KLHL20 does not affect neuronal polarity. Hippocampal neurons at DIV0 were transfected with the indicated constructs and GFP at a ratio of 4:1. Neurons were fixed and stained with anti–Tau-1 at DIV3. The percentage of neurons with a single Tau-1–positive process ≥100 µm was quantified and plotted. Data represent means ± SEM from three independent experiments, and ≥30 GFP-positive neurons were analyzed for each transfection. (E) Cul3 depletion blocks KLHL20-induced neurite outgrowth. Hippocampal neurons at DIV0 were transfected with the indicated plasmid and/or siRNA together with GFP and were monitored for neurite outgrowth at DIV4. Data represent means ± SEM from three independent experiments (**, $P < 0.005$; $n \geq 35$). The knockdown efficiency of Cul3 siRNA is shown at the top. AU, arbitrary unit; si, siRNA; WB, Western blot. Bars, 50 µm.

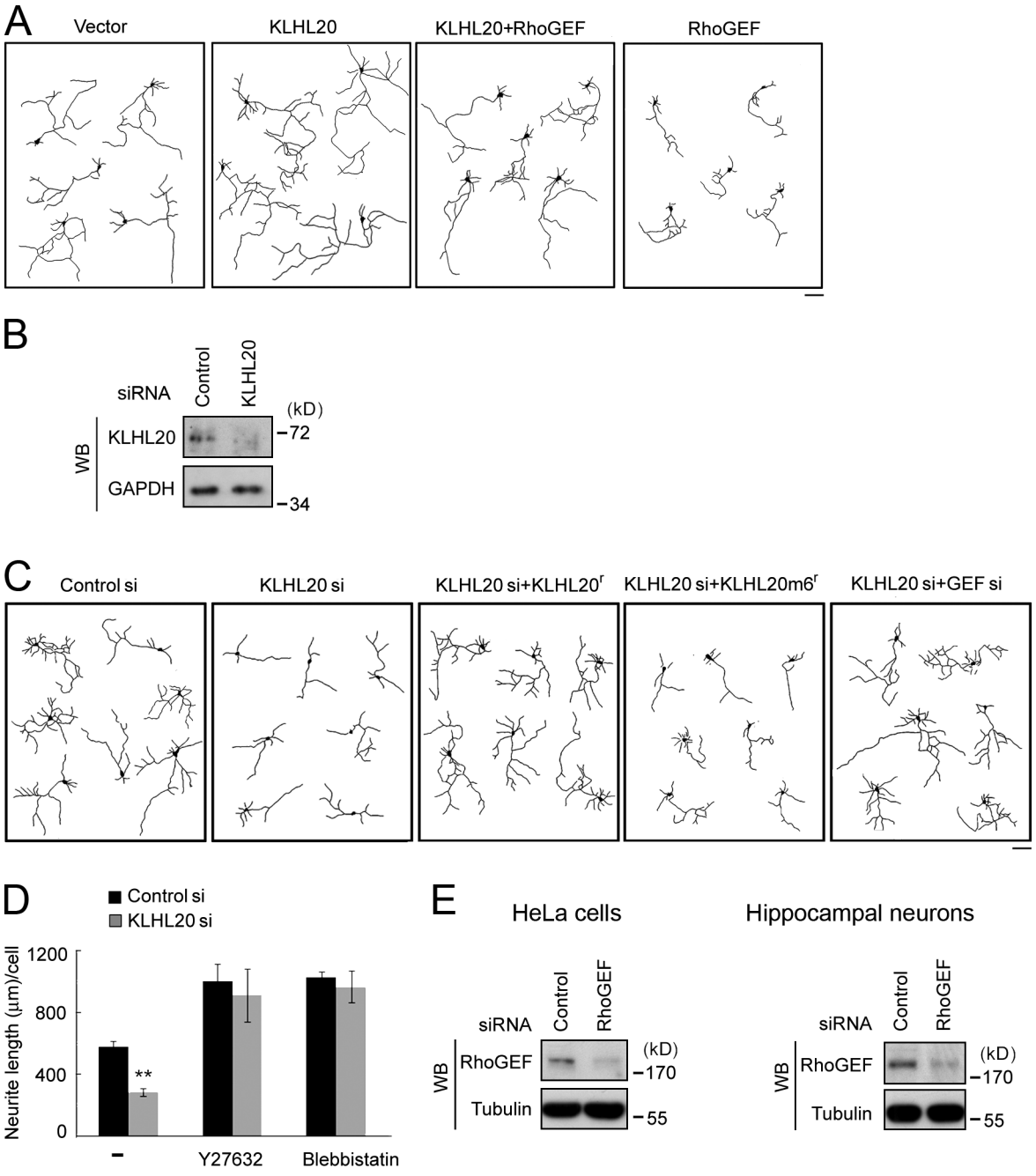


Figure S2. **KLHL20 promotes neurite outgrowth by down-regulating the PDZ-RhoGEF/Rho/ROCK/myosin II signaling axis.** (A and C) NeuronJ drawings of representative GFP-positive neurons as in Fig. 4 (A and B; DIV5), respectively. Bars, 50 μm. (B) The efficacy of KLHL20 siRNA. Hippocampal neurons were transfected with KLHL20 siRNA (si) or control siRNA and then analyzed by Western blotting (WB). (D) Blockage of ROCK or myosin II abrogates the effect of KLHL20 on neurite outgrowth. Hippocampal neurons at DIV0 were transfected with indicated siRNA together with GFP. Neurons were treated with 10 μM of the indicated inhibitor at DIV2 and assayed for neurite length at DIV4. Data represent means ± SEM from three independent experiments (**, $P < 0.005$; $n \geq 35$). The minus sign indicates the treatment of cells with DMSO. (E) The efficacy of PDZ-RhoGEF siRNA. HeLa cells (left) or hippocampal neurons (right) transfected with PDZ-RhoGEF siRNA or control siRNA were lysed and analyzed by Western blotting. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

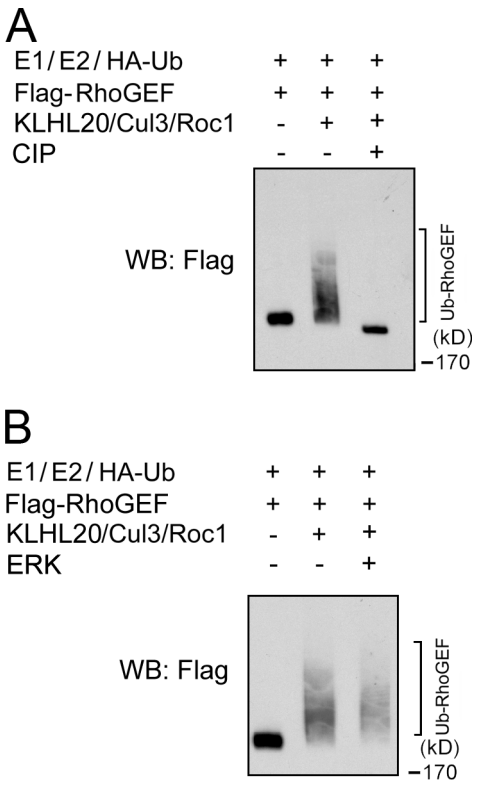


Figure S3. **The effects of calf intestine phosphatase and ERK on PDZ-RhoGEF ubiquitination.** (A) Phosphorylation is required for PDZ-RhoGEF ubiquitination by KLHL20–Cul3–Roc1. Baculovirally purified PDZ-RhoGEF was treated with or without calf intestine phosphatase (CIP) and then subject to in vitro ubiquitination assay as in Fig. 3 C. (B) ERK does not promote PDZ-RhoGEF ubiquitination by KLHL20-based E3 ligase. Purified PDZ-RhoGEF was phosphorylated by ERK1 and then subject to in vitro ubiquitination assay.