Data supplements

Polarization and migration of hematopoietic stem and progenitor cells rely on RhoA/ROCK I pathway and an active reorganization of the microtubule network
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Supplemental Figures

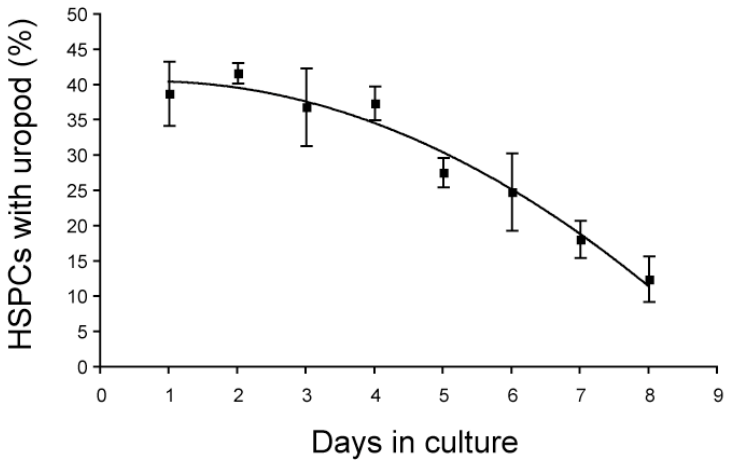

Figure S1. Number of hematopoietic progenitors with a uropod decreases upon cultivation. HSPCs cultured on MSCs for the indicated period (day) were observed by bright field microscopy. Number of cells harbouring a uropod is expressed as the percentage of total HSPCs (200 cells were counted for each day point; n = 3).

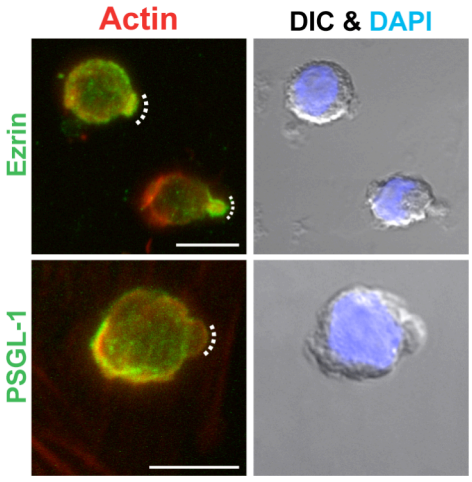

Figure S2. Microtubule depolymerization reduces the polarization of uropod-associated proteins. Nocodazole-treated HSPCs cultured on MSCs for 3 days were analyzed by indirect immunofluorescence upon permeabilization for ezrin (green) and PSGL-1 (green). Actin (red) and nuclei (blue) were visualized with Phalloidin and DAPI labeling, respectively. Differential interference contrast (DIC) images are shown (right panels).

Dashed curved line shows remnant of a uropod that can either contain ezrin and/or PSGL-1 or not. Scale bars, 10 μm.

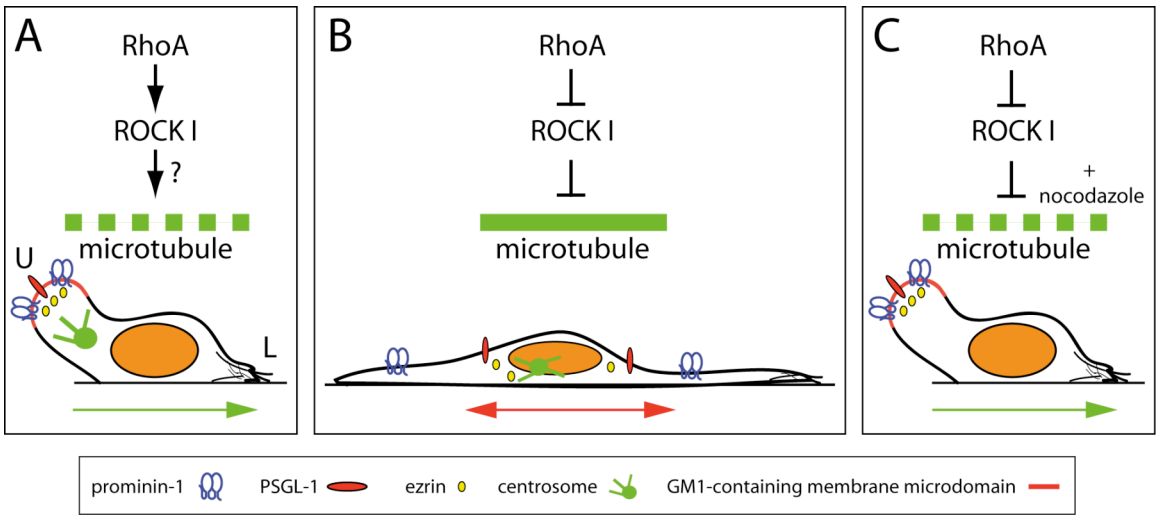

Figure S3. Schematic representation of a migrating hematopoietic progenitor and the role of RhoA/ROCK1 pathway and microtubule network in its polarization. (A) A migrating HSPC displays a uropod (U) at the rear pole and a lamellipodium (L) at the front edge. Centrosome is disposed asymmetrically. Several adhesion molecules including P-selectin glycoprotein ligand-1 (PSGL-1), and the stem cell marker prominin-1 (CD133) are selectively concentrated in the uropod (2,6). Ezrin, a member of the actin-binding ERM protein family, is found therein as well. Specific membrane microdomains particularly those containing ganglioside GM1 (red line; 6,9) might participate in this complex organization. From the cytoplasmic perspective, the activity (↓) of RhoA and its effector ROCK I contributes to the formation of the uropod, and hence polarization and migration, of HSPCs. The downstream (direct or indirect) targets (?) remain to be identified, but it may engage a protein involved in the microtubule destabilization (dashed thick green line). (B) Inhibition (⊥) of ROCKs using Y-27632 or the specific knockdown of ROCK I or its upstream regulator RhoA by means of RNA interference results either in a transient spherical (not shown) or elongated (as depicted) morphology. In both scenarios, the uropod structure is lost. In cells with elongated morphology, one to three long and thin plasma membrane protrusions could be observed. Prominin-1, PSGL-1 and ezrin are redistributed. These cells show an impairment of migration. (C) Microtubule depolymerization triggered by the addition (+) of nocodazole restores the proper polarization and migration of HSPCs. Green and red arrows indicate the direction of migration.

See attached document movies (mov):

Video S1. Video analysis of a migrating hematopoietic progenitor. HSPCs were cultured for 3 days on MSCs prior recording. Arrow and arrowhead indicate lamellipodium and uropod at the front and rear pole, respectively. Total video time: 19 min.

Video S2. Video analysis of Y-27632-treated hematopoietic progenitors. HSPCs cultured for 3 days on MSCs were treated with Rho kinase inhibitor Y-27632 prior recording. Arrows indicate thin plasma membrane protrusions emerging from a HSPC. Total video time: 18 min.

Video S3. Video analysis of nocodazole-treated hematopoietic progenitors. HSPCs cultured for 3 days on MSCs were treated with nocodazole prior recording. Arrowheads indicate numerous membrane blebs, which are formed transiently. Total video time: 18 min.

Video S4. Video analysis of nocodazole/Y-27632-treated hematopoietic progenitors. HSPCs cultured for 3 days on MSCs were treated with Rho kinase inhibitor Y-27632 and nocodazole prior recording. Arrow and arrowhead indicate lamellipodium and uropod at the front and rear pole, respectively, which is the expected morphology of a migrating HSPC. Total video time: 19.5 min.