Supporting Information

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SI Materials and Methods

Generation of RNAi Retroviral Vectors. Scrib1, Scrib2, and a control Luc shRNA were cloned into the RNAi-Ready–pSIREN–RetroQ–ZsGreen (Clontech) vector. Complementary shRNA oligonucleotides, designed to include 5′BamHI and 3′EcoRI overhangs, were annealed and ligated into BamHI/EcoR1 linearized vector. Scrib1 targets a Scribble coding region, whereas Scrib2 targets the Scribble 3′ UTR. Oligonucleotides were designed as follows: Scrib1 forward: GATCCGGCACTTCAAGATCTCCAAGCTCAAGAGAAGCTTGGAGATCTTGAAGTGCCTTTTTTACGCGTG; Scrib1 reverse: AATTCACGCGTAAAAAAGGCACTTCAAGATCTCCAAGCTTCTCTTGAGCTTGGAGATCTTGAAGTGCCG; Scrib2 forward: GATCCGCTAGTGATGTTTGTACAACCACAAGAGATGGTTGTACAAACATCACTAGTTTTTTACGCGTG; Scrib2 reverse: AATTCACGCGTAAAAAACTAGTGATGTTTGTACAACCATCTCTTGTGGTTGTACAAACATCACTAGCG. Underlined sequences correspond to hairpin loops. The Scrib3 vector and the corresponding Luc control shRNA vector are retroviral miRNA-based RNAi vectors (pMSCVmiR30) kindly provided by Dr. S. K. Muthuswamy (Ontario Cancer Institute, Toronto, ON, Canada) (1).

Flow Cytometry. Surface antigens were detected using fluochrome conjugated antibodies, specific for CD25, CD44, CD4, CD8, CD24, CD5, and CD127 (Table S1). Intracellular proteins were detected by initially fixing and permeabilizing cells with BD Cytofix/Cytoperm (BD Biosciences). Intracellular TCRβ was then detected using a PE-conjugated TCRβ-specific antibody (BD Biosciences), and Scribble expression was monitored using a Scribble specific antibody (H-300; Santa Cruz Biotechnology) visualized with a PE- conjugated goat anti-rabbit antibody (Southern Biotech). For monitoring of cell cycle progression, 1×10^6 cells were fixed in 1% paraformaldehyde and stored in 70% ethanol at −20 °C. Pelleted cells were then resuspended in 0.5 mL of PI/RNase staining buffer (BD Biosciences) and incubated for 15 min at room temperature before sample collection. All samples were collected on a FACSCalibur instrument (BD Biosciences) and subsequently analyzed with FlowJo software (Tree Star). FACS sorting was performed on a FACSAria (BD Biosciences) to a purity of >95% as determined by postsorting analysis.

Microsopy. OP9-DL1 cells were first plated on 35-mm glass-bottomed culture dishes (MatTek) previously coated with 0.1% gelatin. Once OP9-DL1 cells reached confluence, day 5–6 OP9-DL1 cocultures were passaged onto the stroma and allowed to adhere and spread over 24–48 h. Clusters were imaged every 30 s for 10 min. A *z*-stack was collected over a 10-μm distance at 1-μm intervals. For LFA-1 localization, cultures were fixed in a 4% paraformaldehyde–PBS solution for 10 min and then gently washed with PBS alone. After 30 min of blocking in PBS containing 4% goat serum, cultures were incubated for 30 min with a biotinylated anti–LFA-1 antibody (M17/4; eBioscience), which was then detected using streptavidin-conjugated Cy3 (Jackson ImmunoResearch).

1. Zhan L, et al. (2008) Deregulation of Scribble promotes mammary tumorigenesis and reveals a role for cell polarity in carcinoma. *Cell* 135:865–878.

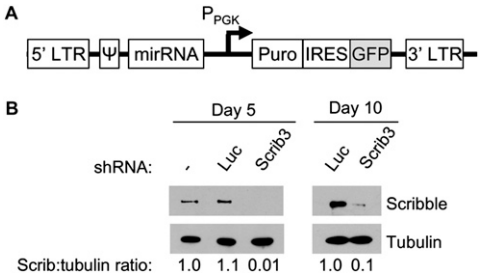


Fig. S1. Depletion of Scribble gene expression during T-cell development. (*A*) A schematic representation of the retroviral microRNA30 (mirRNA) based RNA interference vector in which Scrib3 and Luc shRNA were cloned (kindly provided by Dr. Muthuswamy, Ontario Cancer Institute, Toronto, Ontario) is shown. (*B*) Cell lysates generated from GFP^+CD45^+ FACS sorted cells were resolved by SDS-PAGE. Scribble protein expression levels are normalized to tubulin and quantified by densitometry to determine the efficiency of KD.

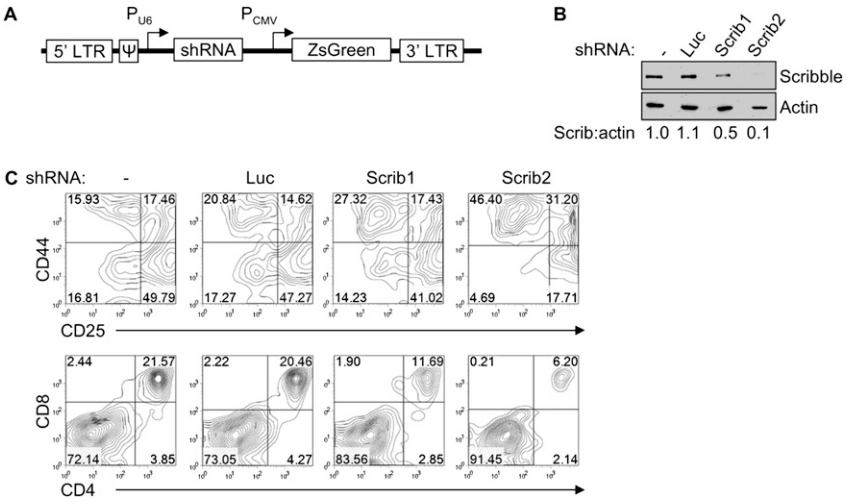


Fig. S2. Scribble depletion using alternate targeting vectors results in efficient T-cell development. (*A*) Schematic representation of RNAi-Ready–pSIREN–RetroQ–ZsGreen vectors used to retrovirally deliver Luc, Scrib1, and Scrib2 shRNA into FL-derived hematopoietic progenitors. (*B*) Scribble expression was quantified in BI-141 T cells stably expressing Luc, Scrib1, or Scrib2 shRNA by resolving the corresponding cell lysates and immunoblotting, followed by normalization to actin expression levels by densitometry. (*C*) Developmental progression of FL-derived progenitors was monitored after 8 d of OP9-DL1 coculture. Representative contour plots of 10 experiments are shown. All plots are gated on GFP$^+$ cells.

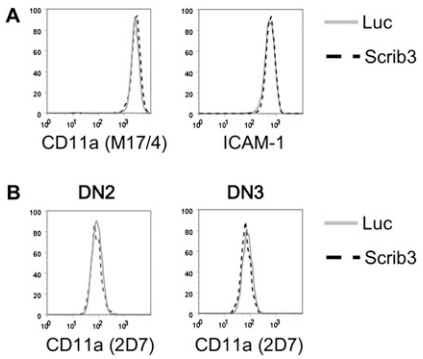
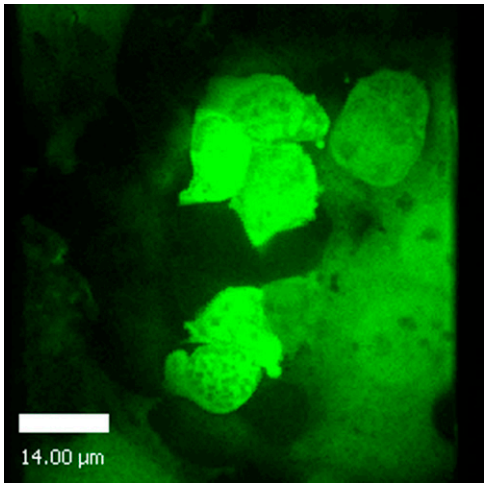


Fig. S3. Surface expression of LFA-1. (*A*) Flow cytometry analysis of LFA-1 (CD11a) and ICAM-1 surface expression on GFP$^+$CD45$^+$ Luc KD or Scrib3 KD cells. (*B*) Representative graphs of low-affinity LFA-1 (CD11a) surface expression on Luc and Scrib3 KD, as detected by the anti-CD11a antibody clone 2D7, gated on CD44^{HIGH}CD25$^+$ DN2 and CD44$^{lo/-}$CD25$^+$ DN3 cells.

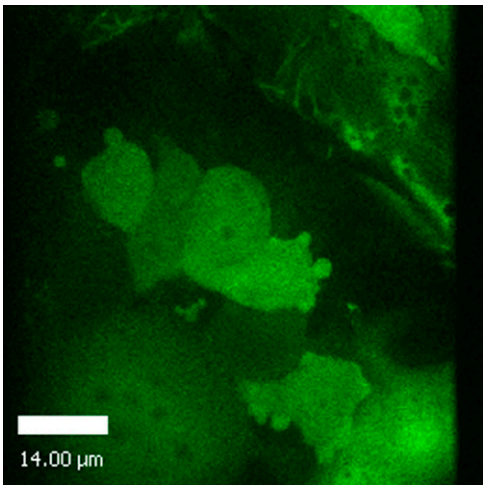
Table S1. Antibodies used for flow cytometry and immunoblotting

Antibody	Clone	Vendor
CD4	RM4-5	BD Biosciences
CD8	53-6.7	BD Biosciences
CD11a	M17/4	eBioscience
CD11a	2D7	eBioscience
CD24	30-F1	eBioscience
CD25	PC61	BD Biosciences
CD44	1M7	eBioscience
CD45	30-F11	BD Biosciences
CD54	3E2	BD Biosciences
CD117	2B8	eBioscience
CD127	A7R34	eBioscience
Sca-1	D7	eBioscience
γδ	RBioGL3	eBioscience
TCRβ	H57-597	eBioscience
Flag	M2	Sigma-Aldrich
Scribble	H-300	Santa Cruz Biotechnology
βPix	Cat. #07-01450	Millipore
αPix	Cat. #4573	Cell Signaling
Pak1	Cat. #2602	Cell Signaling
Git1/2	13/P95 PKL	BD Biosciences
phospho-Erk	Cat. #9101	Cell Signaling
Erk	Cat. #9102	Cell Signaling
Rac1	Cat. #240106	Cell Biolabs
Tubulin	DM1A	Sigma-Aldrich



Movie S1. Clustered Luc KD cells displaced themselves within clusters and sampled the surface of adjacent cells.

Movie S1



Movie S2. Scrib3 KD cells polarized and migrated toward one another, but did not remain engaged in prolonged T-cell–T-cell interactions.

Movie S2