Immature T-cell clustering and efficient differentiation require the polarity protein Scribble

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Abstract

T-cell polarization is required for cell migration and cell–cell interactions, cellular behaviors crucial for lymphocyte differentiation. Despite expression of the epithelial polarity network in T cells, neither its contribution to thymocyte polarity nor its requirement during development is known. We report here that depletion of the polarity protein Scribble in hematopoietic progenitor cells results in inefficient T-cell development characterized by a partial developmental block during the early double-negative (DN) stage of differentiation. Scribble-depleted hematopoietic progenitor cells exhibit a delayed transition into late CD44<sup>hi</sup>–CD25<sup>-</sup> DN3 cells, evidenced by the accumulation of early CD44<sup>int</sup>CD25<sup>+</sup> DN3 cells. As a consequence, a limited cellular expansion and a reduced frequency of intracellular T-cell receptor β-positive DN3 cells are observed among Scribble-deficient differentiating T cells. Moreover, whereas purified Scribble-depleted DN2 and DN3 cells do not exhibit compromised spontaneous motility, T-cell clustering and prolonged homotypic interactions among such cells are reduced. This deficiency correlates with a lack of polarization of the integrin LFA-1 during T-cell migration or on the initiation of T-cell–T-cell interactions. Scribble is therefore a critical contributor to the clustering of immature T cells, an event shown here to be necessary for efficient developmental progression.

Results

Depletion of Scribble Expression in Developing T Cells by RNA Interference. The Scribble-deficient murine models Circletail and Line 90 are perinatally lethal, which hinders the analysis of lymphopoiesis (17, 18). Moreover, gastroschisis, characterized by externalization of the liver and gut through the open abdominal wall, limits the use of hematopoietic progenitors derived from the fetal livers (FLs) of these mice (17). Likewise, the potential disruption of hematopoietic niches due to the loss of Scribble in alternate cell lineages calls into question the reliability of any analysis of fetal lymphocyte development. To circumvent these issues, we depleted Scribble gene expression in FL-derived hematopoietic progenitors by RNA interference (RNAi). Depleted progenitors were then differentiated on OP9-DL1 cells, a well-characterized in vitro model of T-cell development (19).

Scribble gene expression was attenuated by the retrovirial delivery of three separate RNAi vectors (Scrib1, Scrib2, and Scrib3) encoding Scribble-targeting short hairpin RNA (shRNA). A fourth RNAi vector targeting luciferase was used as a negative control. Expression of GFP from an independent promoter within each vector allowed for the monitoring of transduction efficiency (Figs. S14 and S24).

Generated viral supernatants were used to transduce enriched early HPCs (HSA<sup>low</sup>) isolated from murine FLs on day 14–15 of gestation. At 24 h after transduction, CD117<sup>+</sup> Sca-1<sup>+</sup> GFP<sup>+</sup> progenitors were FACS-sorted and then cocultured with OP9-DL1 cells. After 5 d and 10 d of culture, CD45<sup>+</sup> GFP<sup>+</sup> cells were FACS-sorted and total cell lysates were generated. The Scrib3 vector maintained stable Scribble depletion and GFP expression, with Scribble silencing of >98% (Fig S12). In contrast, whereas

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Scrib1 and Scrib2 vectors depleted Scribble expression (Fig. S2B), GFP expression driven by the Scrib1 and Scrib2 vectors was lost over time. Consequently, all data given herein were derived from the Scrib3 vector, although the same developmental phenomena were observed in CD45+ GFP+ Scribble1 knockdown (KD) cells and Scrib2 KD cells isolated from OP9-DL1 cocultures (Fig. S2C).

Depletion of Scribble in FL-Derived Hematopoietic Progenitors Results in Inefficient T-Cell Development. To characterize the developmental effects of Scribble depletion, cells were harvested, counted, and stained for the following surface markers demarking the DN and double-positive (DP) stages of T-cell differentiation: CD25, CD44, CD4, and CD8. On days 5 and 6 of coculture, the cellularity of Scrib3 KD cultures was reduced by an average of 30% (Fig. 1A). At this stage, developmental progression in Scrib3 KD cells was comparable to that in control Luc KD cells, with the majority of cells being CD44intCD25+ (Fig. 1B). However, by day 13, an accumulation of CD44hiCD25+ early DN3 cells was observed in Scrib3 KD cocultures. On average, 10–30% more CD44hiCD25+ early DN3 were detected among Scrib3 KD cells compared with those detected among Luc KD cells (Fig. 1C). Such an accumulation suggests compromised DN3 differentiation.

Given the reduced cellularity of Scrib3 KD cultures and the possible influence of cell density on differentiation, the DN2−DN3 transition was directly compared between equal numbers of Scrib3 KD and Luc KD DN2 cells. Toward this end, CD44hiCD25− Scrib3 KD and Luc KD DN2 cells were FACs-sorted, plated at equal cell densities on OP9-DL1 cells, and allowed to differentiate. Again, a higher proportion of Scrib3 KD cells were found to be CD44hiCD25+ early DN3 cells, confirming a partial block in DN3 differentiation (Fig. 1D). In addition, compromised differentiation of Scrib3 KD cells beyond the CD44hiCD25− early DN3 stage resulted in delayed CD44hiCD25+ DN4 development and inefficient production of CD4+CD8+ DP cells. The percentage of DP Scrib3 KD cells was an average of 5.3-fold lower than that of control Luc KD DP cells in eight independent experiments (Fig. 1E).

To promote the outgrowth of DP cells, the IL-7 concentration within cultures was reduced (20). On day 6 of coculture, IL-7 concentrations were reduced by fivefold, after which cultures were maintained for 6 more days. Analysis of cultures on day 12 demonstrated that in low IL-7 concentrations, the differentiation of Scrib3 KD DP cells remained less efficient than that of control cells (Fig. 1F).

Scrib3 KD Progenitors Are Not Redirected to the γδ T-Cell Lineage. In addition to supporting the differentiation of αβ T cells, OP9-DL1 cells also support differentiation of the γδ T-cell lineage (19). Given that the rearrangement of δ and γ loci is initiated at the DN2 stage of development (21), immediately preceding the observed developmental defect, we addressed the possibility that the depletion of Scribble resulted in a preferential commitment to the γδ lineage. Toward this end, after 12 d of culture, cells were stained for CD24 and γδTCR+ to follow the differentiation of γδ T cells. No apparent accumulation of γδ T cells was observed; rather, the percentage of γδ T cells was reduced on average by 1.5- to 2-fold, suggesting that γδ differentiation is affected by loss of Scribble as well (Fig. 1G).

Scribble Is Required for the DN3–DP Developmental Transition. Although Scribble depletion was found to delay the differentiation of late CD44hiCD25− DN3 cells, whether Scribble is also required for DN3–DP differentiation was unclear. Thus, CD44hiCD25− Scrib3 and Luc KD DN3 cells were FACs-sorted and plated at equal cell densities on OP9-DL1 cells. After 6 d of culture, the cells were harvested, counted, and phenotyped for developmental progression.

At 6 d postsorting, the cellularity of Scrib3 KD cultures was on average 65% that of control Luc KD cultures (Fig. 2A). However, cell cycle analysis of such cultures did not reveal any discernible difference in cycling rate or apoptosis (Fig. 2B). Thus, the expression of intracellular TCRβ (iTCRβ) and the size of DN3 cells were maintained for 6 more days. Analysis of cultures on day 12 demonstrated that in low IL-7 concentrations, the differentiation of Scrib3 KD DP cells remained less efficient than that of control cells (Fig. 1F).

On day 13 of coculture, harvested cells were analyzed for surface expression of CD4 and CD8. Representative contour plots of five independent experiments are shown. (F) After 6 d of culture, cells were harvested and reseeded in the presence of reduced IL-7 concentrations. The contour plots displaying CD4 and CD8 expression are representative of three independent experiments conducted on day 12. (G) Differentiation of γδ T cells was examined on day 11 of coculture. Representative contour plots are gated on GFP+ cells. Numbers in quadrants indicate the percentage of cells within each quadrant.
KD DN3 cultures (Fig. 2C). Analysis of forward scatter showed a lower proportion of iTCRβ− Scrib3 KD DN3 cells displaying an enlarged morphology typical of β selection-induced proliferation (Fig. 2C). As such, the reduction in cellularity that we noted is likely the result of fewer DN3 cells transitioning through β selection and undergoing proliferation before further differentiation.

Indeed, the reduced iTCRβ expression was correlated with a delay in further development. FACS-sorted late DN3 cells depleted of Scribble exhibited delayed down-regulation of CD25 and IL-7Ra (CD127) expression, indicating compromised differentiation beyond the DN3 stage of development (Fig. 2D). Likewise, the generation of DP cells was typically threefold less efficient in the absence of Scribble (Fig. 2D). This indicates that Scribble is not required solely for DN3 differentiation, but rather that is continuously required for the efficient generation of DP cells.

**Scribble Is Required for DP Generation in Fetal Thymic Organ Cultures.** The need for Scribble during DP differentiation was confirmed in fetal thymic organ cultures. Day-14.5 fetal thymocytes (FTs) were transduced, generating Luc KD and Scrib3 KD FTs, which were used to reconstitute 2-deoxyguanosine–treated fetal thymic lobes. After 12 d of standard fetal thymic organ culture, lobes were harvested, and the percentage of DP cells among GFP+ cells was determined by flow cytometry. Whereas Luc KD FTs readily differentiated to the DP stage, Scrib3 KD FTs displayed a marked reduction in DP differentiation. In three independent experiments, the average percentage of DP cells was 2.5- to 3-fold higher in Luc KD lobes compared with Scrib3 KD lobes (Fig. 2E).

**Loss of Scribble in DN Cells Limits T-Cell Clustering.** Developing T cells on OP9-DL1 cells are motile and engaged in cell–cell interactions, cellular behaviors that require cell polarization. We thus assessed the migratory and adhesive behaviors of Scrib3 KD cells on OP9-DL1 cells. On day 6 of OP9-DL1 coculture, live cocultures (>80% CD25−) were stained for CD45 expression and immediately imaged. Control Luc KD cells were localized in tightly packed T-cell clusters in which cells were spread onto underlying OP9-DL1 cells. In striking contrast, Scrib3 KD cultures exhibited infrequent, loosely packed clusters (Fig. 3A).

Given the delayed differentiation of DN3 cells, the tendency of purified DN2 and DN3 cells to cluster in the absence of Scribble was compared with that in control cells. DN2 and DN3 cells were purified by FACS and plated in triplicate on subconfluent OP9-DL1 cells to allow quantification of individual clusters on individual OP9-DL1 cells and avoid the merging of adjacent clusters. Clusters were allowed to form over a 16-h period. A cluster was considered a grouping of three or more T cells attached and spread on to the surface of an individual OP9-DL1 cell. Irrespective of cell density, Luc KD DN2 and DN3 cells formed on average sevenfold more T-cell clusters than Scrib3 KD DN2 or DN3 cells (Fig. 3B).

Time-lapse imaging was then used to evaluate the ability of Scrib3 KD cells to engage in T–T cell interactions to form clusters. As reported elsewhere, developing T cells on the OP9-DL1 cells were found to be highly motile, spontaneously migrating through the OP9-DL1 monolayer. In addition, extensive motility was observed within individual T-cell clusters. Clustered Luc KD cells displaced themselves within clusters and sampled the surface of adjacent cells (Movie S1). However, despite extensive motility within control clusters, cluster formation was maintained. In three independent experiments, during which more than 20 clusters were imaged over 10 min, >90% of control clusters remained intact (Fig. 3C). In contrast, Scrib3 KD cells polarized and migrated toward one another, but did not remain...
Depletion of Scribble Limits Polarization of LFA-1 During Cell Migration and at Sites of T-Cell–T-Cell Contact. OP9-DL1 cells secrete stromal-derived factor 1α, a chemokine that stimulates the redistribution of LFA-1 to polarized patches (22). Such cell surface clustering contributes to increased LFA-1 avidity, thereby enhancing T-cell adhesion (23). Thus, the localization of endogenous LFA-1 was compared in control and Scribble-depleted cocultures to determine whether Scribble depletion affects the polarization of this integrin implicated in cell clustering.

Between day 6 and day 10 of coculture, cultures were fixed and stained for surface LFA-1. Individual migrating cells, as well as cells initiating T-cell–T-cell interactions, were scored for LFA-1 polarization. Confocal images of 1-μm optical sections for a total of 10 μm were captured from identified cells. An extended-focus algorithm was used on the confocal stack, after which a look-up table was applied. A cell was scored as having polarized LFA-1 when a twofold higher density of LFA-1 staining was detected at the leading edge. A minimum of 100 Luc and Scrib3 KD cells were scored from three independent experiments. In Luc KD cultures, on average 74.5% of cells exhibited clearly polarized LFA-1 at the leading edge and at sites of initial cell–cell contact. In contrast, LFA-1 was more diffuse on the surface of Scrib3 KD cells, with only 23.8% of cells exhibiting a polarized accumulation of LFA-1 (Fig. 5 A and B). This observed difference in polarized LFA-1 redistribution did not result from differential expression levels of LFA-1 or its ligand ICAM-1 on immature T cells, as evidenced by flow cytometry staining (Fig. S3A).

Chemokine stimulation also induces the conversion of LFA-1 between a low-affinity state and a high-affinity state. An antibody that preferentially binds the low-affinity conformation of LFA-1 (24) was used to detect the surface expression of low-affinity LFA-1 by flow cytometry. Comparable levels of expression were detected on Scrib3 KD and Luc KD CD44−CD25+ DN2 and CD44+CD25+ DN3 cells (Fig. S3B), indicating that Scribble does not appear to influence the conversion between LFA-1 affinity states.

Discussion

We have presented evidence suggesting that the polarity protein Scribble plays a crucial role in promoting the cell–cell interactions required for T-cell development. Strikingly, depletion of Scribble in immature T cells did not abolish cell migration, but rather disrupted polarized distribution of surface LFA-1, compromised T-cell clustering, and limited the efficiency of T-cell development.

The developmental block occurring at the DN T-cell stage in Scribble-deficient cells was correlated with a reduced incidence of T-cell–T-cell clustering in DN2 and DN3 cells. Whereas Scribble-deficient immature T cells migrated toward one another, they did not engage in prolonged interactions, as did control cells. Control cells were observed to form stable interactions leading to the establishment of T-cell clusters that remained intact over periods exceeding 10 min. Thus, it can be concluded that in developing T cells, the scaffold Scribble contributes to the establishment and maintenance of T-cell clusters.

Homotypic interactions between immature developing T cells are required to specify lineage commitment and differentiation; for example, T-cell–T-cell adhesion promotes SLAM family member interactions required to drive natural killer T-cell dif-
ferentiation (25). Similarly, thymocyte–thymocyte interactions promote CD4 cell selection in the presence of MHC II–expressing thymocytes (26). The role of homotypic interactions during the early stages of αβ T-cell development has not been identified, however. Thus, although others have reported clustering of developing T cells on OP9-DL1 cells, our results provide evidence that this behavior is required for the efficient development of DP cells.

A parallel may be drawn with the large clusters of activated T cells that persist on dendritic cells. Homotypic interactions within such clusters enable direct polarized cytokine delivery to promote activation responses and are dependent on LFA-1 activity (27). Within the OP9-DL1 coculture system, LFA-1 is clustered at sites of contact between immature T cells. LFA-1 was not clustered at sites of contact between Scribble-depleted immature T cells, however, suggesting that Scribble is required to orchestrate molecular events directing LFA-1 localization to promote homotypic interactions between developing T cells.

Limited evidence supports a requirement for LFA-1 during the early stages of T-cell development. Thus, it must be considered that the early developmental defect that we have identified does not result solely from lost LFA-1 polarization. Rather, the lack of LFA-1 polarization is likely representative of a polarity defect among multiple molecules that cumulatively contribute to inefficient T-cell development. The identification of these molecules is clearly of importance.

Regardless, the role of Scribble in LFA-1 polarization is itself of interest. The Scrib complex includes Scribble, Dlg, and Lgl, which in epithelial cells act in concert to establish cell polarity. Dlg has been shown to form a dynamic complex with the LFA-1–binding membrane protein PTA-1 and the actin-binding protein 4.1G. This complex is proposed to provide the structural support to enable LFA-1 clustering (28). Thus, examining whether Scribble plays an active role in this pathway is of interest.

The role of Scribble in cell polarity has been described as cell context–specific, with varied functions including the regulation of directed cell migration and stabilization of adherens junctions.

**Fig. 5.** Scribble is required for LFA-1 polarization on immature T cells. (A) Localization of LFA-1 on immature T cells was visualized by fixation and surface staining of cocultures by staining for CD11a. Representative extended-focus images of polarized CD25+ immature T cells of either GFP or LFA-1 (Cy-3) are shown. A color look-up table was applied to all images of LFA-1 staining. White arrows identify sites of accumulated LFA-1. (B) The incidence of polarized LFA-1 among >100 cells from three independent experiments.

**Fig. 4.** The presence of WT T cells does not rescue clustering or efficient T-cell development. (A) FACS-sorted Scrib3 KD and Luc KD GFP+c-Kit+Sca-1+ HPCs were cultured in the presence of threefold excess of uninfected FACS-sorted GFP+c-Kit+Sca-1+ HPCs. Representative histograms of 10,000 events are shown. (B) Bright-field images of mixed cocultures compared with images of GFP fluorescence. (C) The proportion of GFP+ cells within 40 analyzed clusters in Scrib3 KD + WT or Luc KD + WT cocultures between day 8 and day 10 of culture. (D) Cultures were harvested on day 12 of coculture and phenotyped for developmental progression. The contour plots of CD44 and CD25 expression and dot plots of CD4 and CD8 expression shown are representative of three independent experiments. All plots are gated on GFP+ cells, and numbers indicate the percentage of cells within indicated gates or quadrants.
Recently however, the Scrib complex has been reported to be recruited to the leading edge of migrating epithelial cells during dorsal closure of the Drosophila embryo, whereas it is relocalized to the lateral membrane to promote the restoration of apico-basal polarity on closure. Through differential localization and binding partners, Scribble apparently acts as a molecular switch, allowing epithelial cells to alternate between an apical-basal adhesive state and a migratory state during epithelial-to-mesenchyme transition (29). In mature T cells, Scribble is localized to the uropod in migrating cells and temporarily relocates to sites of contact on interaction with antigen-presenting cells (12). We propose that in T cells, Scribble acts as a similar molecular switch, allowing migrating T cells to transition to an adherent state and engage in homotypic interactions, enabling the acquisition of differentiation signals.

Materials and Methods
Fetal Cell Isolation. FLs were harvested from CD-1 mice on day 14–15 of gestation. Single-cell suspensions were generated and aliquoted into 24-well plates (1 mL aliquoted per well). Harvested CD24−/− FL cells were supplemented with 1.5 h at room temperature.

Microscopy. All imaging experiments were conducted on the WaveFX Spinning Disk Confocal System (Quorum Technologies) using a Zeiss inverted Axiovert 200 microscope equipped with an ASI stage (Applied Scientific Instruments). Temperature and CO2 level were maintained at 37 °C and 5%, respectively, using the Live Cell System (Pathology Devices). Samples were imaged using a Zeiss C-Apochromat 63×/1.2 NA water immersion objective lens. Sample preparation is described in SI Materials and Methods.

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In Vitro T-Cell Differentiation. At 16 h after transduction, CD24−/− FL cells were FACS-sorted as c-kit+ScA-1+GFP+ and plated at 4,000–6,000 cells per well on a 24-well plate containing a confluent layer of OP9-DL1 cells. Hema-topoietic progenitors were differentiated as described originally. In brief, cocultures were maintained in alpha MEM supplemented with 20% FCS, 5 μg/mL of hFlt-3L, and 0.5 μg/mL of mL-7 and passed onto fresh confluent OP9-DL1 every 4 d.

Fetal Thymic Organ Culture. Fetal thymic lobes were isolated from day-14.5 CD-1 embryos, laid on insert filters, and cultured in 1.35 mM deoxyguanosine/DMEM. After 5 d of culture, lobes were rinsed for 2 h in DMEM and then placed in Terasaki plates in which 10,000 transfused TFS per well were aliquoted. The plates were then inverted and incubated for 48 h. Lobes were rescued and cultured on insert filters for 1–2 wk.
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: GATCCGGCATTCAGATCCTCAAGCTCAAGAGAGCTTGGAGATCTTGAAGTGCCTTTTTACGCGTG; Scrib1 reverse: AAATTCACGCGTAAAAAAGGCACTTCAAGATCTCCAAGC TTCTCTTGAGCTTGGAGATCTTGAAGTCGCCG; Scrib2 forward: GATCCGCTAGTGATGTTTGTA CAAGAGATGGTTGTTACCAAACATCAGTCTAGTTTTTACGCGTG; Scrib2 reverse: AAATTCACGCGTAAAAATCTAGTGATGTTGTTACCAAACATCAGTCTAGTTTTTACGCGTG. 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 fluorochrome 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.

**Microscopy.** 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).

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 Bl-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+CD25− DN2 and CD44−CD25+ DN3 cells.
Movie S1. Clustered Luc KD cells displaced themselves within clusters and sampled the surface of adjacent cells.

Table S1. Antibodies used for flow cytometry and immunoblotting

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