## Supplemental Figures

**Figure S1 Septin RNA and protein expression in murine T cells. (a)** RNA was isolated from D10 T cells and whole cell extracts from mouse spleen and brain for positive controls. Septin expression was screened using reverse-transcriptase PCR (RT). Primers used for septin screening are shown in Supplemental Table 1. Primers for Hypoxanthine-guanine phosphoribosyltransferase (5' – CTCGAAGTGTTGGATACAGGC – 3' and 5' – GATAAGCGACAATCTACCAGAG – 3') were used as a positive control to confirm cDNA synthesis was successful. Transcripts for all septins tested except Septins 3 and 5 were present in D10 T cells. Screening primers were designed based on sequence information obtained from the Ensembl database (Supplemental Table 1). Regions common to all known transcripts of individual septins were chosen in order to amplify any possible isoforms expressed in T cells. When multiple transcripts were present, sequences were compared against transcripts found in the GenBank database. Partial mRNA sequence for Septin 12 is present in GenBank (gi:21312733). However, primers designed against that sequence did not amplify a fragment in any of the samples tested. A murine homolog of Septin 13 could not be found via sequence analysis. A putative gene with significant homology to other murine septins was found on chromosome 5 (Ensembl gene ID: ENSMUSG00000034219); however, the expression of this gene was not tested in these samples.(**b**) D10 T cells express Septins 1, 6, 7, 8 and 9. A panel of anti-septin specific polyclonal antibodies was used to screen lysate from D10 T cells in order to determine the septins that were expressed at the protein level. D10 T cells expressed at least two isoforms of Sept8 and Sept9.

**Figure S2. Septin staining is not associated with membrane density and is variably strand-like.** (**a)** D10 T cells were stained with anti-Sept7 and the membrane intercalating dye DiO. Poor colocalization indicates that Septin staining was not a result of local variations in membrane densities. (**b)** D10 T cells were stained with anti-Sept6 antibodies. Staining had less fibrous and more punctuate distributions. (**c)** A rare cell with an extra leading edge protrusion shows enrichment of Sept6 in the protrusion. (**d**) A round, nonmotile cell stained for Sept6 demonstrates a punctate, but evenly distributed distribution across the cell cortex. **(e)** A motile primary T cell stained for Sept6 shows a similar cortical staining to that of D10 cells. Scale bars represent 10 μm.

**Figure S3. Sept7 knock-down (KD) D10 T cells are not defective in cell division or survival.**(**a**) DNA content was examined in Sept7KD and control treated T cells 72 hours post-

transfection to determine if Sept7KD T cells had any defects in cell division. Cells were incubated with 10 μg/ml Hoechst 33342 for 45-90 minutes at 37°C and analyzed on a BD LSR II flow cytometer equipped with a UV laser. Profiles of DNA content of Sept7KD T cells, KD #1 (solid line) and KD #2 (dashed line, indicated by arrow) were almost identical to control treated cells (bold line), indicating D10 T cells divide normally in the absence of septin complexes. (**b**) Sorted control (bold line) and Sept7KD cells (solid and dotted lines) were stained for Annexin V-PE (BD) according to the manufacturer's instructions.

**Figure S4. Sept7KD T cells are not defective in integrin-adhesion but demonstrate inefficient motility under laminar flow.** (**a**) Control and Sept7KD cells adhere equivalently to ICAM-1. Cells were plated on ICAM-1 coated plastic and allowed to adhere for 1 hour, washed gently, then removed with EDTA and counted by flow cytometry. (**b**) Activated control or Sept7KD DO11 T cells we allowed to adhere on ICAM-coated glass coverslips for 3 minutes and then subjected to shear flow of 1 dyne/cm$^3$ for 15 minutes while images were collected. Similar to non-flow conditions, Sept7KD cells traveled slower than control cells, though the small difference in their displacements over time did not achieve statistical significance. Data was pooled from two independent experiments and was analyzed with a Mann-Whitney U-test.

**Figure S5. Full-size gel blots demonstrate specificity of antibodies.** Full images of gel blots used for analysis in Figures 1a, 2a, 2b, 4e, and 5f (**a, b, c**, **d,** and **e**, respectively.) Images vary in size because many membranes were cut for blotting of multiple proteins.

**Supplemental Table T1.** Primer pairs used for Septin RT-PCR analysis shown in Supplemental Figure S1.

**Supplemental Movies**

**Movie SM1** Three-dimensional renderings of annular and cortical septin bundles. D10 T cells, stained with anti-Septin7 and subjected to confocal imaging as in Figure 1d, were rendered using a maximal intensity projection algorithm to generate a three-dimensional reconstruction. Two cells are shown.

**Movie SM2** Crawling of control treated D10 T cells. In order to examine membrane dynamics in control treated T cells, time-lapse images were acquired at 5–10 sec intervals for at least 10 min. Imaging was performed in 0.25% low-melting point agarose to limit drifting of non-adherent cells.

**Movie SM3** Membrane blebbing in Sept7KD D10 T cells. In order to examine membrane dynamics in septin deficient T cells, time-lapse images were acquired at 5–10 sec intervals for at least 10 min. Membrane blebbing is indicated by arrows during the movie. Imaging was performed in 0.25% low-melting point agarose.

**Movie SM4** Excess protrusions in Sept7KD D10 T cells. In order to examine membrane dynamics in septin deficient T cells, time-lapse images were acquired at 5–10 second intervals for at least 10 min. Excess protrusions were observed for two different cells during the movie and are indicated by arrows. Imaging was performed in 0.25% low-melting point agarose.

**Movie SM5** Myosin II activity was required for cortical and mid-zone defects in Sept7KD D10 T cells. In order to determine if myosin II activity was still required for uropod formation in septin deficient T cells, control and Sept7KD T cells were treated with the myosin II inhibitor Blebbistatin. Time-lapse images were acquired at 30-sec intervals immediately after addition of the drug. Imaging was performed in 0.10% low-melting point agarose.

**Movie SM6** ROCK activity was required for cortical and mid-zone defects in Sept7KD D10 T cells. In order to determine if ROCK activity was still required for uropod formation in septin deficient T cells, control and Sept7KD T cells were treated with the ROCK inhibitor Y-27632. Time-lapse images were acquired at 30-sec intervals with addition of the drug after time-point five. Imaging was performed in 0.10% low-melting point agarose to limit drifting of non-adherent cells.

## Supplemental Table T1.

| Mouse Septin Gene/Protein | Ensembl Gene ID/ Transcript ID | Chromosome | PCR Primers | Primer Location | Fragment Size (mRNA)) |
|---|---|---|---|---|---|
| *SEPT1*/SEPT1 | ENSMUSG00000000486<br>ENSMUST00000000497 | Chr. 7 | For: 5' - GTATCAAGGTGAAGTTGACCTTGGTGG - 3'<br>Rev: 5' - TCCTCATCAGAGTCACACTCTGGG - 3' | Exon 3<br>Exon 8 | 388 bp |
| *SEPT2*/SEPT2 | ENSMUSG00000026276<br>ENSMUST00000027495 | Chr. 1 | For: 5' - TTCGACTGTTGAGATTGAAGAGCGG - 3'<br>Rev: 5' - TTCAATCAACTGGTTGGAGCCAACC - 3' | Exon 5<br>Exon 9 | 493 bp |
| *SEPT3*/SEPT3 | ENSMUSG00000022456<br>ENSMUST00000077804 | Chr. 15 | For: 5' - AAGTCAACACTGGTCAACACCCTC - 3'<br>Rev: 5' - TGACAGGAATGATGTTCACCACTTTGC - 3' | Exon 2<br>Exon 5 | 397 bp |
| *SEPT4*/SEPT4 | ENSMUSG00000020486<br>ENSMUST00000018544 | Chr. 11 | For: 5' - AAGGAGTATGTGGGCTTTGCAACC - 3'<br>Rev: 5' - TGTTGTTGACTGCATCCCCAAATCC - 3' | Exon 3<br>Exon 5 | 292 bp |
| *SEPT5*/SEPT5 | ENSMUSG00000072214<br>ENSMUST00000096987 | Chr. 16 | For: 5' - TGAAACACACCGTCGACATTGAGG - 3'<br>Rev: 5' - TGGAAACTGGTACACGTGGATCC - 3' | Exon 5<br>Exon 8 | 398 bp |
| *SEPT6*/SEPT6 | ENSMUSG00000050379<br>ENSMUST00000060474 | Chr. X | For: 5' -CAGCTGGTGAATAAGTCAGTCAGCC - 3'<br>Rev: 5' - TGATCTTGAACTTTGCCAGCTCACTC - 3' | Exon 2<br>Exon 5 | 508 bp |
| *SEPT7*/SEPT7 | ENSMUSG00000001833<br>ENSMUST00000060080 | Chr. 9 | For: 5' - TCCAGGATTTGGAGATGCAGTGG - 3'<br>Rev: 5' - CAAGGATACTGCCTTCCTCTGACTC - 3' | Exon 5<br>Exon 9 | 465 bp |
| *SEPT8*/SEPT8 | ENSMUSG00000018398<br>ENSMUST00000018542 | Chr. 11 | For: 5' - TATCTGCAGGAGGAGTTGAAGATCCG - 3'<br>Rev: 5' - GCTTAGGAACTCCTTCCTCTTTGCC - 3' | Exon 4<br>Exon 8 | 615 bp |
| *SEPT9*/SEPT9 | ENSMUSG00000059248<br>ENSMUST00000019038 | Chr. 11 | For: 5' - AGACGATCGAAATCAAGTCGATCACC - 3'<br>Rev: 5' - TCCTCATCAAACTCCTTCTGCGG - 3' | Exon 4<br>Exon 8 | 430 bp |
| *SEPT10*/SEPT10 | ENSMUSG00000019917<br>ENSMUST00000089092 | Chr. 10 | For: 5' - AGGAAGAACTGAAGATCAAGCGTGC - 3'<br>Rev: 5' - TAGTGCCTCATATGTGTCTGCTCCC - 3' | Exon 3<br>Exon 6 | 493 bp |
| *SEPT11*/SEPT11 | ENSMUSG00000058013<br>ENSMUST00000074733 | Chr. 5 | For: 5' - ATGACACAAGGATTCACGCCTGC - 3'<br>Rev: 5' - ATACAACTCGTAGTGGCGAGTGTGC - 3' | Exon 4<br>Exon 7 | 464 bp |