Gilden et al., http://www.jcb.org/cgi/content/full/jcb.201105127/DC1

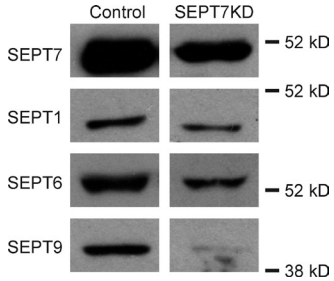


Figure S1. **Protein levels of a panel of septins after SEPT7 knockdown.** Western blots demonstrating loss of septin complexes in SEPT7KD cells.

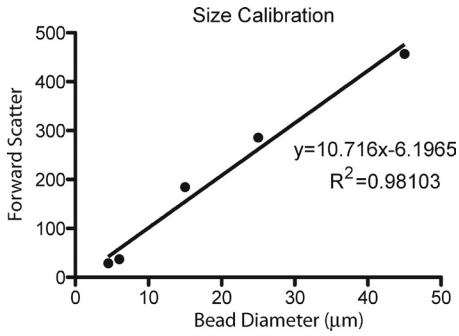
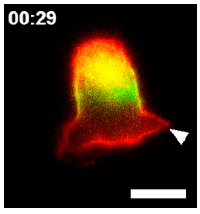
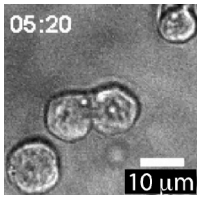


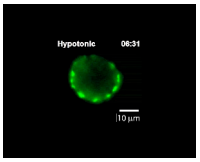
Figure S2. **Standard curve used for calibrating cell size in the flow cytometry volume-change assay.** Standard curve generated by recording forward scatter values of polystyrene microspheres using the same flow cytometer settings as hypotonic volume-change assay. The curve was used to calculate the approximate size of cells undergoing volume change.



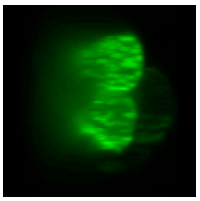
Video 1. **Time-lapse of a D10 T cell expressing SEPT6-GFP and GPI-linked mCherry and crawling on an ICAM-1–coated coverslip.** The white arrowhead indicates a leading edge protrusion that is retracted into the septin collar as the cell changes direction. Epifluorescence images were collected on an inverted microscope (Axiovert 200M). Time in minutes and seconds is indicated in the top left corner. Bar, 10 µm.



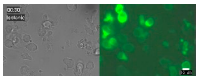
Video 2. **Time-lapse microscopy of control and SEPT7KD D10 T cells undergoing mitosis after release from mitotic block.** Note the relatively smooth surface of the control cell and small blebs on the surface of the SEPT7KD cell as they undergo cytokinesis. Images were collected on an inverted microscope (Axiovert 200M). Time in minutes and seconds is indicated in the top left corner.



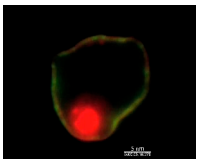
Video 3. **Time-lapse of a D10 T cell expressing SEPT6-GFP.** At the start of the video, the cell is in isotonic media but is shifted to 100 mOsm hypotonic media after 1 min. The cell undergoes hydrostatic expansion followed by RVD, during which SEPT6-GFP aggregates on the cortex in ringlike structures. Images were collected on an inverted microscope (Axiovert 200M). Time in minutes and seconds is indicated in the top right corner.



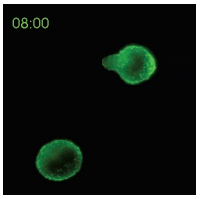
Video 4. **3D reconstruction of the top half of a set of SEPT6-GFP–expressing cells that have been subjected to 100 mOsm hypotonic conditions for 30 min and have undergone RVD.** Dramatic 1-µm SEPT6 rings stud the entire cell cortex. Images were collected on an inverted microscope (Axiovert 200M), and reconstruction was made in MetaMorph software.



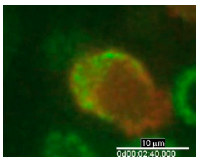
Video 5. **Time-lapse of a full field of D10 T cells expressing SEPT6-GFP undergoing hydrostatic expansion and RVD.** Initially, the cells are in isotonic media, but media is shifted to 100 mOsm after 1 min. In the majority of the cells, SEPT6-GFP aggregates on the cortex in ringlike structures. Images were collected on an inverted microscope (Axiovert 200M). Time in minutes and seconds is indicated in the top left corner.



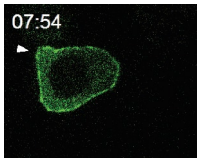
Video 6. **Time-lapse of a D10 T cell expressing SEPT6-GFP and GPI-linked mCherry.** After 1 min, media is shifted from isotonic to 100 mOsm hypotonic, and, after this, invaginations in the mCherry-marked membrane can be seen underlying SEPT6-GFP ring structures. Images were collected on an inverted microscope (Axiovert 200M).



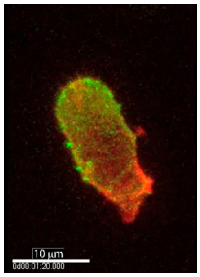
Video 7. **Time-lapse imaging of D10 T cells expressing SEPT6-GFP undergoing treatment with jasplakinolide.** When jasplakinolide is added at 100 nM, the elongated motile cell in the top right corner adopts the morphology and diffusely punctate septin distribution of the rounded cell at the bottom left. Images were collected on an inverted microscope (Axiovert 200M). Time in minutes and seconds is indicated in the top left corner.



Video 8. **Time-lapse imaging of D10 cells expressing SEPT6-GFP and LifeAct-Ruby.** Cells were shifted to hypotonic media after 1 min of imaging. After this time, actin-containing membrane ruffles appear, and SEPT6-GFP accumulates at the base of those ruffles during retraction. Confocal images were collected using a microscope (Axiovert 200M) fitted with a spinning disk (CSU10).



Video 9. **Time-lapse confocal imaging of a D10 T cell expressing SEPT6-GFP, which has been treated with dynasore to induce blebbing.** Initially, the cell is crawling normally, and SEPT6-GFP is enriched in the midzone of the cell and relatively low at the leading edge. When the cell begins to bleb at the leading edge (white arrowheads), SEPT6-GFP accumulates on those blebs. After a period of blebbing, the cell returns to normal motility, and midzone distribution of SEPT6-GFP is recovered. Confocal images were collected using a microscope (Axiovert 200M) fitted with a spinning disk (CSU10). Time in minutes and seconds is indicated in the top left corner.



Video 10. **Time-lapse confocal imaging of a crawling dynasore-treated cell.** Many blebs can be observed, demonstrating early recruitment of LifeAct-Ruby to the bleb followed by recruitment of SEPT6-GFP. Confocal images were collected using a microscope (Axiovert 200M) fitted with a spinning disk (CSU10).