Genetic Deletion of SEPT7 Reveals a Cell Type-Specific Role of Septins in Microtubule Destabilization for the Completion of Cytokinesis

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Published Version</td>
<td>doi:10.1371/journal.pgen.1004558</td>
</tr>
<tr>
<td>Citable link</td>
<td><a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:12785892">http://nrs.harvard.edu/urn-3:HUL.InstRepos:12785892</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA">http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA</a></td>
</tr>
</tbody>
</table>
Genetic Deletion of SEPT7 Reveals a Cell Type-Specific Role of Septins in Microtubule Destabilization for the Completion of Cytokinesis

Manoj B. Menon1*, Akihiro Sawada2, Anuhar Chaturvedi3, Pooja Mishra4, Karin Schuster-Gossler5, Melanie Gallia6, Axel Schambach6,7, Achim Gossler5, Reinhold Förster4, Michael Heuser3, Alexey Kotlyarov1, Makoto Kinoshita2*, Matthias Gaestel1*

1 Institute of Physiological Chemistry, Hannover Medical School, Hannover, Germany, 2 Department of Molecular Biology, Division of Biological Science, Nagoya University Graduate School of Science, Furo, Chikusa, Nagoya, Japan, 3 Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany, 4 Institute of Immunology, Hannover Medical School, Hannover, Germany, 5 Institute of Molecular Biology, Hannover Medical School, Hannover, Germany, 6 Institute of Experimental Hematology, Hannover Medical School, Hannover, Germany, 7 Division of Hematology/Oncology, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts, United States of America

Abstract

Cytokinesis terminates mitosis, resulting in separation of the two sister cells. Septins, a conserved family of GTP-binding cytoskeletal proteins, are an absolute requirement for cytokinesis in budding yeast. We demonstrate that septin-dependence of mammalian cytokinesis differs greatly between cell types: genetic loss of the pivotal septin subunit SEPT7 in vivo reveals that septins are indispensable for cytokinesis in fibroblasts, but expendable in cells of the hematopoietic system. SEPT7-deficient mouse embryos fail to gastrulate, and septin-deficient fibroblasts exhibit pleiotropic defects in the major cytokinetic machinery, including hyperacetylation/stabilization of microtubules and stalled midbody abscission, leading to constitutive multinucleation. We identified the microtubule depolymerizing protein stathmin as a key molecule aiding in septin-independent cytokinesis, demonstrated that stathmin supplementation is sufficient to override cytokinesis failure in SEPT7-null fibroblasts, and that knockdown of stathmin makes proliferation of a hematopoietic cell line sensitive to the septin inhibitor forchlorfenuron. Identification of septin-independent cytokinesis in the hematopoietic system could serve as a key to identify solid tumor-specific molecular targets for inhibition of cell proliferation.

Introduction

Cytokinesis as final step of cell division is essential for cell proliferation, but there is a considerable degree of diversity in its underlying mechanisms among eukaryotes. Even within one organism, such as the amoeba Dictostelium discoideum, cytokinesis may proceed by different mechanisms for cells growing in suspension or in an attachment-dependent manner. This has been impressively demonstrated for the myosin II-deletion mutant of D. discoideum, which could not complete cytokinesis in suspension but successfully proliferates when attached to surfaces [1]. Hence, it could be speculated that other cells also confine different molecular requirements for attachment-dependent and -independent cytokinesis, although there is little molecular proof for this idea in mammalian cells. Recent support for this idea comes from the observation that in lymphocytes the hematopoietic lineage-specific Rho-GAP ARHGAP19 is essential for cytoskeleton remodeling resulting in cell division [2] while in most other cells M-phase GAP (MP-GAP) is the major factor restraining RhoA during cell division [3].

Septins, a conserved family of polymerizing GTP-binding proteins regarded as the forth component of the cytoskeleton [4], organize a ring that serves as a submembranous scaffold and diffusion barrier for various molecules, which is an absolute requirement for cytokinesis in budding yeast [5,6]. In metazoans, septins associate with the mitotic spindle, contractile ring, intercellular bridge and midbody at varying degrees [7,8]. For example, anillin-dependent recruitment of septins to the intercellular bridge is required for constriction site formation and ingestion in HeLa cells [9], maturation of the midbody ring in Drosophila melanogaster requires septin-dependent removal of anillin via its C-terminal PH-domain [10], and septins are required for the release of midbody and midbody ring into daughter cells during the subsequent cell division in Caenorhabditis elegans [11]. Perturbation or depletion of one of the major septin subunits, such as the pivotal subunit SEPT7 [12,13], affects multiple steps in mitosis [4,14]. In vitro studies with mammalian cell lines have revealed pleiotropic defects in mitotic spindle organization and chromosome alignment [15], cleavage furrow...
Cytokinesis is the finalizing step of the complex scenario of mitosis, leading to separation of two sister cells. The cellular mechanism of cytokinesis in eukaryotes differs at least between yeasts, plants and animals. So far, it is also not clear whether all mammalian cells follow the same mechanistic rules of cytokinesis. Here, we demonstrate that, depending on the mammalian cell type, two different pathways could result in completion of cytokinesis, a septin-dependent pathway and a distinct mechanism, which does not require septins prevalent in the hematopoietic system. Using multiple conditional knockouts, we demonstrate this cell type specificity in vitro and in vivo, and present evidence for the involvement of cell-type specific alteration of the microtubule cytoskeleton. Our data, together with the previously available septin knockdown data in cancer cell lines, suggest septins as plausible antitumor targets with high therapeutic index due to lack of off-target effects on hematopoiesis.

Results

Deletion of Sept7 causes embryonic lethality

We floxed Sept7 gene (exon 4, encoding the GTP-binding P-loop) in the mouse genome using the Cre-loxP system (Figure 1A). The Sept7lox allele was converted to Sept7−/− (null) allele by using oocyte-specific expression of Cre-recombinase (ZP3-Cre). Sept7−/− (KO) embryos were found in utero up to embryonic day 6.5 (E6.5)-E7.0, but not after E10.5, indicating early embryonic lethality (Figure 1B). As the genetic loss of SEPT9 or SEPT11 causes embryonic death by E10 [21] and E13 [22] respectively, SEPT7 deficiency revealed absence of persistent chromatin bridges as shown by LAP2 staining (Figure S5). However, Sept7−/− cells were often accompanied by unresolved α-tubulin aggregates (arrowheads in Figure 3C) and about two-fold hyperacetylation of α-tubulin (Figure 3D and Figure S3C, S3E). These data indicate hyperstabilization of microtubules in Sept7−/− cells, as has been observed in interphase HeLa cells [23] and postmitotic primary neurons [26]. Anillin, a contractile ring organizer which interacts with actomyosin and septins, was reduced in interphase nuclei of Sept7−/− cells (Figure 3E, cf. Figure 2C and Figure S1). However, Sept7 was dispensable for the targeting of anillin to the cleavage furrow (Figure 3F). Thus, genetic loss of SEPT7 in fibroblasts appeared to affect mitotic spindle and midbody rather than the contractile ring.

SEPT7 is dispensable for the cytokinesis of myeloid and lymphoid cells

Next, we examined the aforementioned presumed dispensability of SEPT7 in non-adherent cell lines. We introduced a bidirectional γ-retroviral mCherry-Cre construct [27] (Figure S6A, 6b) into Sept7−/−lox/lox bone marrow cells, which successfully induced recombination. An interleukin (IL)-3/IL-6/SCF-dependent myeloid colony formation assay (Figure S6C) revealed that each subpopulation of the Sept7−/− leukocytes exhibited subnormal but sufficient proliferative activity in vitro (Figure 4B). Given that most of these Sept7−/− cells (Figure 4C) had undergone more than 10 replication cycles, SEPT7 protein carried over from the original Sept7lox/lox cell had been eliminated. These data indicate that the resistance to the loss of SEPT7 in mitosis is a common trait of the myeloid lineage.

To corroborate the dispensability of SEPT7 in myeloid cell mitosis in vivo, we generated lymphocyte-specific Sept7−/− mice, by intercrossing Sept7lox/lox and CD2-Cre lines [28]. We detected efficient recombination in the bone marrow (Figure S7A), spleen, thymus, and lymph nodes (Figure 4D) with recognizable volume loss in the spleen and thymus (Figure 4E). Flow cytometric analysis demonstrated complete loss of SEPT7 in cells collected from thymus, while those from spleen contained a minor population that fully expressed SEPT7 (Figure 4F). Viability of lymphocytes from spleen, peripheral lymph nodes (Figure 4G), thymus (Figure 4H), bone marrow (Figure S7B) and a...
Figure 1. Generation of Sept7 floxed mice and characterization of embryonic lethality of the Sept7 knockout. 

A, Strategy for conditional targeting of Sept7. A neomycin cassette with flanking FRT and lox-P sites was incorporated by homologous recombination in ES cells. The resulting mice were crossed with Flippase expressing mice to remove the neomycin cassette retaining the lox-P flanked (floxed) exon 4. Cre expression leads to excision of exon 4 and a downstream frame shift.

B, Analysis of embryonic lethality in Sept7 knockout mice. Analysis of progeny by

<table>
<thead>
<tr>
<th></th>
<th>Sept7/ Sept7</th>
<th>Sept7/Δ</th>
<th>Δ/Δ</th>
<th>$\chi^2$ (p&lt;0.05, test 5.99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E 6.5-7.0</td>
<td>7</td>
<td>9</td>
<td>4</td>
<td>1.100</td>
</tr>
<tr>
<td>E 10.5</td>
<td>8</td>
<td>16</td>
<td>-</td>
<td>8.000</td>
</tr>
<tr>
<td>P1</td>
<td>9</td>
<td>28</td>
<td>-</td>
<td>13.82</td>
</tr>
</tbody>
</table>

Figure 1. Generation of Sept7 floxed mice and characterization of embryonic lethality of the Sept7 knockout. A, Strategy for conditional targeting of Sept7. A neomycin cassette with flanking FRT and lox-P sites was incorporated by homologous recombination in ES cells. The resulting mice were crossed with Flippase expressing mice to remove the neomycin cassette retaining the lox-P flanked (floxed) exon 4. Cre expression leads to excision of exon 4 and a downstream frame shift. B, Analysis of embryonic lethality in Sept7 knockout mice. Analysis of progeny by
Discussion

This study has revealed two distinct types of mammalian cytokinesis which vary by the requirement for SEPT7/septins. Consistent with previous studies [5,16,18] our findings indicate that cell division requires septins in two spatiotemporally distinct processes, first for the organization of the contractile ring and later for midbody abscission. The former became known early on due to the high prominence and its evolutionarily conservation from budding yeast to humans, while the latter had remained unknown due to its cell-type-dependence. Fibroblasts, typical adherent cells, divide in contact with other cells and/or connective tissue in vivo and extracellular matrices and artificial substrate in vitro. In contrast, amoeboid hematopoietic cells grow planktonically in vitro and divide individually in suspension. Our study confirm the role of septins in the recruitment of the microtubule cleaving machinery (multi-protein membrane associated abscission machinery probably including spastin for local microtubule destabilization) [7,8] to the midbody for final microtubules scission. This system seems to be inactive in the absence of SEPT7 in fibroblasts, leading to midbody stabilization. In the hematopoietic system the abundance of stathmin leads to a passive rescue due to general microtubule destabilization and thus cytokinesis proceeds in a septin independent manner. The supplementation of stathmin is sufficient for fibroblasts to override the loss of SEPT7 and to complete cytokinesis. The abundant expression of stathmin in early embryo [33,34] may account for the dispensability of septins up to midgestation. These data indicate that the synergy between septins and stathmin, among other microtubule-regulating proteins, is critical for completion of cytokinesis and midbody abscission. The entire process should depend not only on the quantitative balance of tubulin/stathmin/septin but also on the phosphorylation level of stathmin [29,30]. Of note, β1-integrin-blocking antibodies can inhibit cytokinesis of adherent cells, but not their cytokinesis in suspension [35]. Given these and our findings, it is conceivable that non-adherent cells develop less cytoskeletal network than adherent cells, which should reduce the burden for midbody abscission. Conversely, myosin II-deficient Dictyostelium cells can complete cytokinesis on a substrate but not in suspension [36], indicating that microtubule is not a critical determinant in this case. A recent study with Drosophila revealed that the SEPT7 ortholog peanut (Pnut) and other septins are required for planar cell cytokinesis but dispensable for orthogonal cell division in the single-layered neuroepithelium of the dorsal thorax [37]. This finding supports our notion that SEPT7/septins play a context-dependent role in mammalian cytokinesis. Accordingly, SEPT7 is a promising target for the development of solid tumor-selective anti-proliferative therapy without damaging...
Figure 2. Cre-induced deletion of SEPT7 in fibroblasts leads to proliferation block and obligate multinucleation. 

A, Adenoviral Cre-expression in Sept7^flox/flox primary mouse embryonic fibroblasts (MEFs) [26] leads to deletion of SEPT7 and, as judged by immunofluorescence, to a strong reduction of expression of the other core-septins SEPT6/2. 

B, Retroviral Cre-transduction of Sept7^flox/flox immortalized mouse tail fibroblasts causes complete deletion of the Sept7 gene in two different experiments (#1, #2- independently immortalized lines) (control: non-integrating control virus). Genotyping by PCR as indicated in Supporting Figure S1. 

C, Western blot detection of multiplicity of infection (MOI)-dependent reduced SEPT7 levels in Sept7^flox/flox immortalized mouse tail fibroblasts. Expression of SEPT6/2/9 and anillin is significantly reduced. 

D, E, Adenoviral Cre-expression in primary Sept7^flox/flox MEFs decreases immunofluorescence detection of the proliferation marker Ki67 (D) and increases histone H3 phosphorylation (E). The percentage of positive cells is indicated. 

F, SEPT7-depleted Sept7^flox/flox immortalized mouse tail fibroblasts fail to proliferate (3 days post transduction). 

G, Retroviral Cre-transduced SEPT7-depleted cells are overgrown by non-depleted cells, as indicated by the relative increase in the floxed allele after 12 days in culture. 

H, Immunofluorescence analysis showing obligatory multi-nucleation of Sept7^KO tail fibroblasts after 11 days post transduction. DAPI is used for nuclear staining and the WGA as counter stain. Arrowheads indicate multi-nucleated, SEPT7-negative cells.

doi:10.1371/journal.pgen.1004558.g002

Figure 3. Defective cytokinesis and unresolved midbody in SEPT7-deficient fibroblasts. 

A, Time lapse differential interference contrast (DIC) microscopy of Cre-transduced floxed Sept7 tail fibroblasts. The green arrow indicates a SEPT7-positive, normal dividing cell (about 70% of cells). The process of cell division is completed in less than 120 min and two daughter cells appear. The red arrow indicates a SEPT7-negative cell which could not complete cytokinesis after nuclear division (about 30% of cells). Even after 400 min the daughter cells do not separate and the cell becomes multi-nucleated. 

B, Localization of microtubules (α-tubulin) and microfilaments (phalloidin) by immunofluorescence in the midbody zone of dividing fibroblasts in the absence (SEPT7^−/−) or presence of SEPT7 (WT). Two different cells each were analyzed. Arrowheads indicate the midbodies. 

C, Detection of unresolved midbody tubulin bundles (indicated by arrowheads) in multinucleated SEPT7-deficient cells. 

D, E, Immunofluorescence of tail fibroblasts transduced with pRbid-Cre-mCherry. D, About two-fold increased acetyl-tubulin detection and E, about 2–3-fold decreased nuclear anillin staining in mCherry-Cre-positive, SEPT7-negative cells. 

F, Intact recruitment of the remaining anillin to the midbody zone in mCherry-positive, SEPT7-negative cells.

doi:10.1371/journal.pgen.1004558.g003
hematopoietic cells. Reciprocally, stathmin could be selectively targeted in hematopoietic malignancies and p53-compromised cancer [38,39].

Materials and Methods

Generation of SEPT7 conditional knockout mice

Two independently developed Sept7 floxed mice strains were used in this study, both targeting exon 4 of mouse Sept7 gene using similar targeting strategies. Sept7floxed/foxed mice (Sept7foxedfoxed) were generated as indicated in Figure 1A. Briefly, the targeting vector containing lox sites and FRT sites flanked neomycin cassette was linearized and electroporated in 129Ola ES-cells. Two positive cell clones (42A3 and 44A1) obtained by PCR screen were injected into blastocysts for the generation of chimeric mice. Agouti germ line pups were derived from the mating of chimeric male mice, obtained following the blastocyst injection of Sept7 targeted ES-cell clone 44A1, with C57Bl/6 Flp females. The resulting sept7foxedneo mice were crossed with C57BL/6-(C3-TgPcgk1FLPo)10Syk/Flippase-expressing mice [40] to delete the neomycin cassette retaining thelox-P-flanked (floxed) exon 4 leading to Sept7foxed mice. Subsequent Cre-recombinase expression will catalyze exon 4 excision resulting in an additional frame-shift mutation downstream to this exon. For generation of Oocyte specific knockout animals, Sept7 homozygous floxed mice were crossed with B6-Zp3CreERT2/CAGCre[41]. Sept7foxedfoxed-Zp3Cre mice were bred to generate Sept7foxedfoxed mice. Lymphocyte-specific Sept7 knockout mice were generated by mating floxed animals with B6-hCD2-iCre mice [28]. In animal experiments age and sex matched, Cre-expressing Sept7 wt and floxed mice were used.

DNA isolation and genotyping

Tail biopsies, cells and colonies were overnight digested at 53°C in lysis buffer (50 mM Tris-Cl (pH 8.0), 100 mM EDTA, 100 mM NaCl and 1% SDS) containing protease-K (0.5 mg/ml). For tissue samples proteins were salted out with extra NaCl. DNA was precipitated with isopropanol, washed with 70% ethanol and dissolved in water. Genotyping PCR were performed with Hotstar Taq (Qiagen) with extra Mg2+ with controlling annealing temperature at 53°C. The primers used were: Sept7-p1 (5’- GGT ATA GGG GAC TTT GGG G-3’), Sept7-p2 (5’- CTT TGC ACA TAT GAC TAA GC -3’), Sept7-p3 (5’- GCC TCT TCT TTT ATG TCA AGG AGG-3’), Cre-sense (5’- GAA CCT GAT CCA CAT GTT CAG G-3’), Cre-antisense (5’- AGT GGG TTC GAA CGC TAG AGC CTG T-3’), Cre-fw1 (5’- AGA TGC CAG CAG AAT CAT AGG AGC ATG-3’), iCre-rev (5’- ATG AGC AGC ACC AAC CAC ACA CAG AAT G-3’), iCre-fw2 (5’- CTA GGC CAG AGA ATT GAA AGA TCT-3’), IL2-rev (5’- GTA GGT GGA AAT TCT AGC ATC ATC G-3’), Myo-fwd (5’- TTG CCT TGA TGG ACA GC -3’), Myo-rev (5’- TGG GCT GGG TGT TAG CCT TA -3’), Myogenin and IL2 gene fragments were amplified as controls for Cre and iCre genotyping respectively. PCR reactions were separated on 2% agarose gels and images acquired using INTAS Gel documentation system.

Embryonic lethality analysis

Sept7foxedfoxed mice were mated and plug checked for embryo analysis. Pregnant mice were sacrificed between embryonic day 6–7.0 or 10.5 days. The embryos were dissected out in cold PBS and cleaned up from extra-embryonic tissues. Whole embryos were overnight digested for DNA isolation and genotyping. Deviations from Mendelian ratios were calculated by Chi-squared test.

Cell culture methods

Sept7 floxed mouse embryonic fibroblasts were generated from E15 day embryos and maintained under standard conditions. Sept7 floxed adult tail fibroblasts (TFs) were isolated from 6–8 weeks old mice tail tips. Minced tail tips were sequentially digested with collagenase and trypsin at 37°C and plated on collagen coated dishes in DMEM supplemented with 20% serum, non-essential amino acids and antibiotics. The cells were split 1:4 and maintained in the same growth medium without coated dishes. To immortalize primary TFs, cells were co-transfected with pSV40Tag encoding simian virus 40 large T antigen and pREP8 plasmid (Invitrogen) in a 10:1 mixture; colonies were selected with 2 mM histidinol (Sigma). Jurkat cells were maintained in RPMI-1640 medium supplemented with 15% serum, 1 mM pyruvate and antibiotics. Post electroporation cells were additionally supported by 2 ng/ml IL2.

Antibodies and reagents

Antibody against SEPT7 was from IBL international (#JP18991), Rabbit anti-anillin antibodies were reported earlier (Watanabe et al., 2010). Antibodies used for western blot analysis were SEPT2 (#11397-1-AP, Acris), SEPT9 (#10769-1-AP, Acris), SEPT6c (sc-20180, Santa Cruz Biotech), SEPT18 (sc-48937, Santa Cruz Biotech), EF2 (sc-13004-R, Santa Cruz Biotech), GAPDH (#MAB374, Millipore), GFP (sc-9996, Santa Cruz Biotech) and Stathmin (#3352, Cell Signaling Technology). Antibodies used for Immunofluorescence staining were rabbit anti SEPT7, SEPT2, SEPT6 [23], K67, phospho-Histone-H3, cleaved caspase-3 (Cell Signaling Technology), tubulin-α (T6199, Sigma and sc-31779, Santa Cruz Biotech), LAP2 (#611000, BD Transduction lab) and acetylated tubulin (T6793, Sigma). All alexa-dye labeled secondary antibodies, tetramethyl rhodamine-conjugated WGA (#W849) and Alexa fluor-647-conjugated phalloidin (#A22877) were from Invitrogen. DAPI for DNA staining was from Cal Roth.
Polybrene (H9268), doxycycline (D9891), RNAse A (R4875) and propidium iodide (P4170) were from Sigma. Forchlorfenuron (FCF) was obtained from Santa Cruz Biotech. IL2 was from ImmunoTools. IL3, IL6 and SCF were from Peprotech.

Virus transduction

Primary MEFs were transduced with commercially available adenoviral Cre particles (AxCANCre2, TaKaRa, Japan).Gamma-retroviral particles (SF91-nlsCre and pRBid-Cre) were packaged as described previously [27]. Doxycycline inducible retroviral particles were generated as described previously [27].

Figure 5. Stathmin expression levels correlate with SEPT7 dependence on cytokinesis. A, Lysates prepared from mouse embryonic fibroblasts (MEF), tail fibroblasts (TF), thymocytes (Thy) and bone marrow cells (BM) were probed with indicated antibodies. B, SEPT7−/− MEFs transduced with inducible stathmin expression construct were treated with 1 μg/ml doxycycline, to induce stathmin expression similar to thymocytes (Thy). C, Cre-induced SEPT7 depletion in SEPT7−/− cells in the presence and absence of doxycycline induced stathmin expression (5 days). D, WST-1 assay showing the rescue of Cre-transduction-induced proliferation defect in fibroblasts by doxycycline-induced stathmin expression. E, Effect of doxycycline induced stathmin expression on Rbid-Cre induced multinucleation analyzed and quantified from fluorescent images as indicated in Figure S12. F, G, Knockdown of stathmin in Jurkat cells. F, Stathmin expression was reduced to about 28% by knockdown. G, Stathmin knockdown renders Jurkat cell proliferation sensitive to the septin inhibitor forchlorfenuron (FCF) applied at two different concentrations (50 and 100 μM).

doi:10.1371/journal.pgen.1004558.g005
Cells were lysed directly in SDS gel loading dye and western blotting was performed as previously described using gradient SDS-PAGE gels [43].

### Immunofluorescence staining

Cells were grown on glass coverslips and fixed with 4% paraformaldehyde (PFA) in PBS. Fixation was performed for 2–5 min at room temperature (RT) followed by 20 min at 4°C. Cells were permeabilized with 0.25% Triton X-100–PBS for 30 min at RT. Blocking was done using 4% bovine serum albumin (BSA) for 1 h at 4°C. Primary antibodies were used at a 1:50 to 1:200 dilution in 1% BSA–PBS for 1–2 h. Secondary antibodies or Alexa Fluor 647-conjugated phallolidin/tetramethyl rhodamine conjugated WGA was used at a 1:500 dilution in 1% BSA–PBS. Imaging was performed using a Leica TCS SP2 confocal microscope with standard settings. Fluorescent intensities were quantified using Image J program (NIH- http://rsb.info.nih.gov/ij/).

### Flow cytometry analysis

For immunophenotyping analysis of hematopoietic cells, spleen, thymus and bone marrow cells were isolated, RBC lysed (Pharmlyse, BD Biorad) and analyzed for surface staining with PE-CD3 (Clone 17A2) [44], FITC-CD4 (Clone RM4-5, Biolegend), and PE-CD8 (Pharmlyse, BD Biosciences) and analyzed for surface staining with PE CD4-PerCP (Clone RM4-5, Biolegend), and PE-CD8-Cy5 (Clone RmCD8-2) [45]. Samples were analyzed using an LSRII (BD Biosciences).

For SEPT7, acetyl tubulin and propidium iodide staining, thymocytes/splenocytes were fixed with 3x by volume PFA (4%) at RT for 30 min. Washed and resuspended in PBS and absolute methanol was added to 90% concentration final with constant mixing. The methanol permeabilization was continued for 30 min on ice. After 2x PBS wash cells were resuspended in 4% BSA-PBS and blocked at 4°C for 30 min. Cells were stained with primary antibodies (1:100 in 1%BSA-PBS) at RT for 30 min. After 1x PBS wash, samples were resuspended in secondary antibody dilution (anti rabbit Alexa fluor-488/anti mouse Alexa fluor-546 - 1:500 diluted in 1% BSA-PBS) and incubated for additional 30 min before PBS wash and FACS analysis. For analysis of DNA content fixed cells were treated with nuclear stain solution (1x PBS, 100 μg/mL propidium iodide, 100 μg/mL RNase-A) at RT for 15 min and analyzed by flow cytometry in Accuri- C6 flow cytometer.

### Time lapse-DIC microscopy

Cells were grown on 8 well chamber slides and time-lapse DIC images were acquired (1 per 10 min×16 h) using OLYMPUS FV1000 microscope fitted with 37°C/humid chamber.

### Methyl cellulose colony formation (CFC) assay

Bone marrow lineage negative cells were isolated by MACS separation (Miltenyl Biotec) and cells cultured for 2 days in the presence of IL3/SCF/IL6 medium. Cell were transduced on day 3 and 4 and left in suspension culture for another 4days. mCherry positive cells were sorted and seeded in 3 cm plates with methyl cellulose medium (IL3/IL6/SCF/1000cells/ml/plate) as described previously [45]. Colonies were photographed, counted and genotyped after 2 weeks of growth.

### Peripheral blood analysis

Blood samples were collected in lithium-heparin tubes (BD Microtainer- LH tubes) and subjected to differential blood count and analysis with Vet ABC hematology analyzer (Scil animal care company GmbH, Viernheim, Germany).

**In vitro proliferation assay for splenocytes and fibroblasts**

Spleens were aseptically isolated in RPMI medium (10% foetal calf serum, non-essential amino acids, antibiotics and 50 μM 2-mercapto ethanol) and splenocyte suspension obtained by passing through a 10 μm cell strainer. After RBC lysis cells/spleen were plated in a 6 cm plate and incubated at 37°C for 1 h to remove adherent cells. The suspension cells were collected and counted. 5–6×10^5 cells/100 μl medium/well were seeded in 96 well plates in the presence or absence of 5 μg/ml concanavalin A and 10 ng/ml murine IL2. Cell proliferation was assayed using WST1 reagent (Roche Applied Sciences) as per manufacturer protocol. For measuring fibroblast proliferation, 500cells/100 μl/well were seeded in 96 well plates. Viable cells were quantified daily using WST1 reagent as per manufacturer protocol.

**Stathmin knockdown analysis in Jurkat cells**

Jurkat cells were microporated with control siRNA (Allstars negative control siRNA- Qigaren) or siRNA against human Stathmin (Hs_STMN1_1: 5’-GCCGAGGGCUUGAAGCGCTT-3’-Qigaren) using a Microporator MP-100 system. 200 picomoles of siRNA were used per one million cells microporated at 1400 V/20 msec/ single pulse following the standard manufacturer’s protocol. 24 h post transfection cells were counted and re-seeded at 3x10^5/ml density in the presence or absence of FGF in v-bottom 96well plates (triplicate wells). After 48 h of treatment cells were collected and viable cell numbers quantified by flow cytometry in the presence of 2 μg/ml propidium iodide and 2 mM EDTA.

**Cell migration assay**

Quantitative microplate scratch assays were performed with mitomycin-C treated fibroblasts as described previously [46]. Stathmin-IRE5-EGFP transduced and sorted Sept7flox/flx cells were stained and seeded in 96 well plates in the presence or absence of 2 μg/ml doxycycline and scratches were made 24 h later after mitomycin pre-treatment. Infrared fluorescent images were acquired using a Li-COR odyssey scanner at 0 h, 6 h and 18 h. Migration indices were calculated and plotted.

### Ethics statement

All mice experiments were conducted according to German and international guidelines and were approved by the ethics committee of Hannover Medical School (MHH).

### Supporting Information

**Figure S1** Down-regulation of anillin in SEPT7-depleted fibroblasts. Band intensities for SEPT7 and anillin blots presented in figure 1C were quantified and normalized to GAPDH. The data for two different fixed lines are presented as percentage of non-transduced control.

(PDF)
Figure S2  Sept7 deletion does not induce enhanced cell death. Adenoviral Cre-transduced or control treated Sept7<sup>flox/flox</sup> primary MEFs were analyzed for apoptotic cells by cleaved caspase-3 staining. Cells with typical apoptotic morphology and cleaved caspase-3 staining (shown in set) were counted and plotted. (PDF)

Figure S3  Microfilament and microtubule architecture in the Sept7-deficient fibroblasts. A, Immortalized Sept7<sup>flox/flox</sup> fibroblasts transduced with retroviral Cre showing unaltered F-actin staining in the absence of Sept7. B, Sept7 knockout primary MEFs showing unaltered F-actin staining and C, enhanced microtubule acetylation. D, General microtubule architecture is unaffected in Sept7-deficient immortalized fibroblasts as shown by α-tubulin staining. E, Intensity of acetyl tubulin (green)/SEPT7 (red) staining were quantified from individual cells using ‘Color histogram’ plugin of Image J program (n = 10). Representative images used for analysis are shown in the right panel with the quantified intensity values. (PDF)

Figure S4  Dynamics of cell division in SEPT7-deficient fibroblasts. Time-lapse images were acquired for Cre-transduced Sept7<sup>flox/flox</sup> tail fibroblasts as described in methods, Figure S3A and supporting video S1. Total time taken for individual cells to complete cytokinesis was calculated. A, Sample time-lapse analysis showing a cell (indicated by red arrow) undergoing the complete process from cell detachment to complete abscission in 80 min. B, Similar analysis of all successful divisions in 39 distinct cells followed by time lapse. C, Classification of mitotic cells followed by time-lapse- including cells completing division (compiled from b) and cells failing to complete cell division. (PDF)

Figure S5  Staining for LAP2 in unresolved midbody structures in Sept7 KO fibroblasts. Upper and lower panel show two representative Sept7<sup>flox</sup> floxed tail fibroblasts transduced with mCherry-Cre and stained with indicated antibodies. LAP2/ Tubulin/DNA triple staining revealed the presence of unresolved midbody structures lacking chromosome bridges. (PDF)

Figure S6  Bidirectional retroviral vector for expression of Cre and mCherry. A, Expression cassette and important features in the bidirectional Cre-mCherry expression vector used in the study. B, mCherry positive cells show efficient Sept7-deletion as shown by SEPT7 co staining in Cre-transduced cells. C, Representative images of mCherry positive hematopoietic cell colonies genotyped and enumerated in Figure 4. (PDF)

Figure S7  Analysis of bone marrow in the Lymphocyte specific Sept7 KO. A, Sept7 genotyping showing partial deletion in CD2iCre mouse bone marrow. Tail biopsy DNA is shown as a control tissue. B, Lymphocytes: T cells (CD3<sup>+</sup>) and B cells (B220<sup>+</sup>) in the bone marrow from CD2iCre mice (n = 2) were analyzed by surface-staining and flow cytometry. (PDF)

Figure S8  Analysis of peripheral blood from lymphocyte specific Sept7 KO mice. Peripheral blood samples from Sept7<sup>flox/flox</sup> CD2-iCre mice (2x) and Sept7<sup>flox/flox::CD2-iCre</sup> mice (3x) were analyzed using an ABC Vet Automated Blood counter (Sci1 animal care company GmbH, Viernheim, Germany). (PDF)

Figure S9  Co-depletion of other septins in Sept7 KO thymocytes. Similar to fibroblasts, Sept7 deletion (CD2iCre) in thymocytes lead to deelopment of SEPT2/6/9. (PDF)

Figure S10  Analysis of tubulin acetylation in Sept7 KO thymocytes. A, Thymocytes from Sept7<sup>flox/flox::CD2-iCre</sup> mice (KO1 and KO2) and Sept7<sup>flox/flox::CD2-iCre</sup> mice (WT1) were analyzed by indirect fluorescence staining and flow cytometry analysis for acetylated tubulin. Labeled Secondary antibody only staining is shown as control. B, As positive control for flow-cytometric detection of acetyl tubulin, similar staining was performed with control and taxol (2 μM for 2 h) treated Jurkat cells. (PDF)

Figure S11  Effect of stathmin expression on fibroblasts migration. Sept7 floxed MEFs inducibly expressing stathmin were subjected to scratch wound healing assay. A, Representative fluorescent scans of wells showing scratch wound healing. B, Calculated migration index for 6 and 18 h wound healing (n = 19). (PDF)

Figure S12  Analysis of multinucleation in stathmin expressing Sept7 KO fibroblasts. Sept7 floxed MEFs inducibly expressing stathmin were transduced with Rbid-Cre, maintained in the presence or absence of 2 μg/ml doxycycline and were fixed and stained with DAPI. The extent of multinucleation in mCherry-positive cells in the presence or absence of doxycycline-induced stathmin expression was quantified and is presented in Figure 5E. (PDF)

Video S1  Stalled abscission and cytokinetic failure in Sept7 KO fibroblasts. Sequence from the time-lapse differential interference contrast (DIC) microscopy of Cre-transduced Sept7 floxed tail fibroblasts corresponding to Figure S3A. The cell attempting division (middle right) cannot resolve the intercellular bridge and does not complete cytokinesis after nuclear division. Even after 300 min the daughter cells do not separate and the cell becomes multi-nucleated. (AVI)

Acknowledgments

We thank Dr. S. Selbert (Polygene Transgenics) for help with the generation of the floxed Sept7 allele, Dr. B. Weinhold (HHF) for the ZP3-Cre, Dr. A. Kruger (HHF) for the hCD2<i>+</i>Cre strains, Dr. R. Bauerfeind (HHF) for support in confocal imaging and T. Yakovleva (HHF) for genotyping and breeding the mice. We thank H. Nakashima and D. Kamiya for help in the early part of MEF experiments.

Author Contributions

Conceived and designed the experiments: MBM. Performed the experiments: MBM AsA AK AC PM KSG. Analyzed the data: MBM AK AC PM MH AG RF. Contributed reagents/materials/analysis tools: ASc MGal. Contributed to the writing of the manuscript: MGae MK MM. Provided conceptual insight: MGae AK MK.

References