S1. Partitioning of individual septins between soluble and insoluble fractions of cells permeabilized under various conditions. K562 cells were permeabilized by saponin at either 0 °C or 20 °C as indicated. The dependence of ionic strength was evaluated by permeabilization in 1xPEM, 0.5xPEM or 0.25xPEM as outlined under “Materials and Methods”. The released (Supernatant, S) and cell associated (Pellet, P) proteins were analyzed by western blot using the indicated antibodies. It should be noted that similar septin partitioning was obtained in the presence of 1% of the non-ionic detergent Triton X-100 to buffers (data not shown), which solubilized most cellular membranes. This indicates that the insoluble state of septins at low ionic strength does not depend on membrane binding.
S2. Quantitative and qualitative analyses of septin pools derived from SEPT7-deficient Jurkat cells.

An RNA interference shuttle vector directing shRNA-SEPT7 expression was transfected into Jurkat cells followed by counter-selection with hygromycin for one week. Septins released from permeabilized cells (protocol as in Figure 2A) were analyzed by western blot (A) or resolved by density gradient centrifugation (B). The distribution of septins in fractions was analyzed by western-blot using the indicated antibodies. Note that the corresponding distributions of septins derived from control Jurkat cells are presented in Figure 3C.

Western blots reveal efficient SEPT7-depletion (>90 %), while SEPT2, SEPT6 and SEPT9 isoforms were reduced by 60 – 80 %. In accordance with SEPT7-deficient K562 cells (see Figure 5), sedimentation profiles show that the bulk of residual SEPT2 and SEPT6 are contained within aberrantly small complexes, while isoforms of SEPT9 do not co-exist with other septins in the absence of SEPT7.
S3. Quantitative and qualitative analyses of septin pools derived from K562 cells depleted of either SEPT2 or SEPT5.

K562 cells were transfected with Vector-Co (A, which is the same panel as in Figure 6B), shRNA-SEPT2 (B) or shRNA-SEPT5 (C) and counter-selected with hygromycin as in Figure 6. Septins released from permeabilized cells were analyzed by western-blot (data shown in Figure 6A together with SEPT2/SEPT5 co-depleted cells) or resolved by gel filtration chromatography. The elution profiles of SEPT6, SEPT7 and SEPT9\textsuperscript{75kDa} is shown.
Western blots in Figure 6A show that depletion of either SEPT2 or SEPT5 alone results in only a modest decrease in the content of other septins, while co-depletion results in ~75% reduction of all other septins except SEPT9. The data in Figure 6C shows that the diminishing heteromer content in SEPT2-subgroup depleted cells is not associated with aberrantly small complexes, which contrasts to the consequences of SEPT7-depletion (see Figure 5E). In accordance with these findings, depletion of either SEPT2 or SEPT5 alone reveals only heteromers within the normal size-range. It is notable that the modest decrease of heteromer content in SEPT2-deficient cells is associated with a noticeable increase in the relative SEPT9 content within the heteromer pool. The corresponding effects of single depletion of SEPT5 are very minor. This predicts that SEPT5 is expressed at lower levels than SEPT2, which is consistent with the estimated mRNA-levels of these two SEPT2-subgroup members (see Figure 1B).

Our results are consistent with SEPT2 and SEPT5 – the major SEPT2-subgroup septins expressed in K562 cells – sharing a subgroup-specific function that is essential for initiation of heteromer assembly. In addition, the data also suggest that SEPT9 isoforms in K562 cells are normally present in sub-stoichiometric amounts relative to the total pool of septin heteromers in this cell model system, which is consistent with data shown in Figure 8C.
Supplemental Material:

Construction of pMEP and shRNA shuttle vector derivatives

To construct the pMEP4 shuttle vector directing inducible expression of Flag epitope-tagged SEPT6 (pMEP-SEPT6-Flag), a PCR approach was used with a 5’ primer containing 21 nucleotides from the 5’ untranslated region of Op18 (primer sequence: 5’- GGA CCC AAG CTT GTC GCT TGT CTT CTA TTC ACC ATG GCA GCG ACC GAT ATA GCT CG) and a 3’ primer encoding the 8 amino acids Flag epitope tag (primer sequence: 5’-GG CCG CGG ATC CTA CTT GTC GTC ATC GTC CTT GTA GTC ATT TTT CTT CTC TTT GTC TCT CTT C). The purpose of fusing the initiator Met-codon of SEPT6 with the untranslated 5’ Op18 sequence was to optimize protein expression in human cells. The PCR fragment was digested with Hind III and BamHI I and used to replace the corresponding fragment of the pMEP4 vector. The pMEP4 shuttle vector directing inducible expression of Flag epitope-tagged SEPT7 (pMEP-SEPT7-Flag) was constructed by an analogous strategy (primer sequences: 5’- GGA CCC AAG CTT GTC GCT TGT CTT CTA TTC ACC ATG TCG GTC AGT GCG AGA TCC GC; 5’- GG CCG CGG ATC CTA CTT GTC GTC ATC GTC CTT GTA GTC AAA GAT CTT CCC TTT CTT CTT G). Templates used were pCMV6-XL5-SEPT6 (SC128089, Origene) and pK-myc-SEPT7 (Sheffield, P.J., Oliver, C.J., Kremer, B.E., Sheng, S., Shao, Z., and Macara, I.G. (2003). J Biol Chem 278, 3483). The general strategy behind construction of replicating Epstein-Barr virus-based shuttle vectors directing constitutive expression of short hairpin RNA (shRNA) has been described earlier (Holmfeldt, P., Stenmark, S., and Gullberg, M. (2004). EMBO J 23, 627-637). In our search for optimal targeting sequences, at least four sequences for each septin family member were tested. The most efficient targeting sequences, which were used in this study, were as follows: SEPT2 (accession NM_001008491.1): CAGCCAACTCAGTTTATAA; SEPT5 (accession NM_002688.4): CCGAGACTGAGAAGCTTAT; SEPT7 (accession NM_001788.4): CTTGCAGCTGTGACTTATA; SEPT9 (accession NM_001113491.1): GTCCATCAGCAGCATATT. A BLAST search of the NCBI database ensured specific targeting of the cognate mRNA. In cases where several isoforms of a given septin have been identified the targeting sequences were selected in regions common to all transcripts. For phenotypic complementation, a 6xHis-tagged SEPT7 derivative (pMEP-immHisSEPT7) was used, which was immune to shRNA-SEPT7 mediated suppression due to 6 silent mutations within the 19-nt targeting sequence (nt 938-960 in the SEPT7 ORF, 5’-CTCGCCGCAGTTACCTACA). The coding sequences of all PCR-generated fragments were confirmed by nucleotide sequence analysis.