Supplemental Experimental Procedures

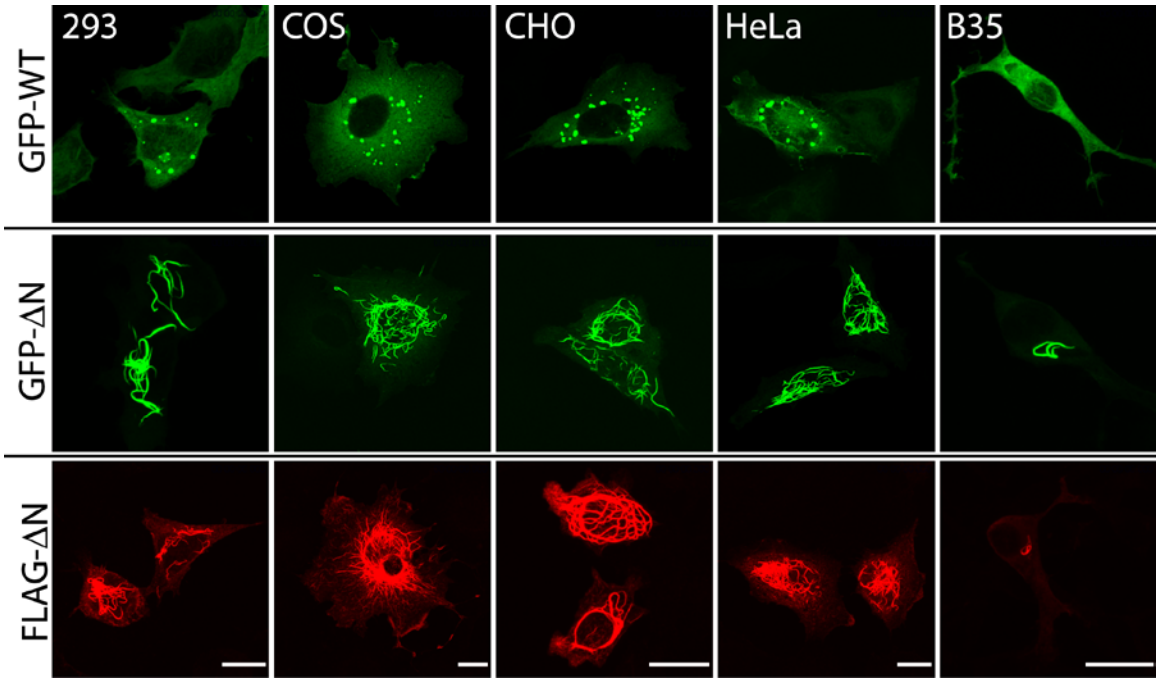
Plasmid Construction
FLAG-HIS and FLAG-MBP vectors were constructed as previously described (Kim et al, 2011). SEPT2 constructs were subcloned into these vectors using EcoRI and XhoI.

Pulldown Assay
HEK293 cells were sub-cultured in DMEM+10% FBS, and seeded onto 6-well plates. Cells were transfected with Lipofectamine 2000 (Invitrogen). For high level-expression of septin pairs, 2ug of each septin plasmid were co-transfected. For lower-expression, only 10% (or 200ng) of plasmid was transfected and supplemented with 1.8ug of an inert plasmid (pcDNA3.0). Twenty-four hours later, HEK293 cells were washed with ice-cold phosphate buffered saline (PBS). Cells were lysed using 1% Triton X-100 and 20mM imidazole in PBS with protease inhibitor tablet (Roche). Lysates were clarified in a microcentrifuge, 13,000xg at 4°C. Supernatants were incubated with Ni-NTA agarose (Qiagen) for 1 hour. Beads were washed three times in 0.1% Triton X-100 and 20mM imidazole in PBS. Washed beads were resuspended in 1X SDS-loading buffer and run on 10% SDS-PAGE. Gels were transferred onto PVDF and immunoblotted for mouse FLAG (Sigma), rabbit SEPT6 (Proteintech), rabbit SEPT7 (Proteintech), and rabbit SEPT9 (Kim et al, Estey et al) at the following dilutions: 1:7500, 1:5000, 1:5000 and 1:250.

Supplemental Figure 1. SEPT2 Δ15 is filamentous in multiple cell lines
(A) Localisation of GFP-SEPT2 to perinuclear puncta and cytosol (top row). Filamentous structures of GFP-SEPT2 Δ15 (middle row). Filamentous structures of FLAG- SEPT2 Δ15 (bottom row). Transfections were performed in 5 different cell lines, including HEK293 (first column), COS (second column), CHO (third column), HeLa (fourth column) and B35 (last column). Scale bar indicates 8μm.

Supplemental Figure 2. Overexpressed SEPT2-SEPT2 pair does not pulldown endogenous septins.
(A) High expression of the SEPT2-SEPT2 pair does not pulldown endogenous septins. The SEPT2-SEPT2 pair was overexpressed in HEK293 cells and pulled down using Ni-NTA agarose. Similar to the septin-septin pairwise interactions from GFP immunoprecipitates described in Figure 6, the SEPT2 G mutant did not interact with WT SEPT2. The resultant pulldowns were immunoblotted for SEPT6, SEPT7, and SEPT9 to determine if endogenous septins could facilitate SEPT2-SEPT2 interactions. Asterisk denotes non-specific band.
(B) Low expression of SEPT2 can incorporate into endogenous septin complexes. Transfecting only 10% of HIS-SEPT2 plasmid (200ng) results in efficient incorporation of endogenous SEPT6, SEPT7 and SEPT9.

Supplemental Figure 1



Supplemental Figure 2