Ribet et al., https://doi.org/10.1083/jcb.201703096

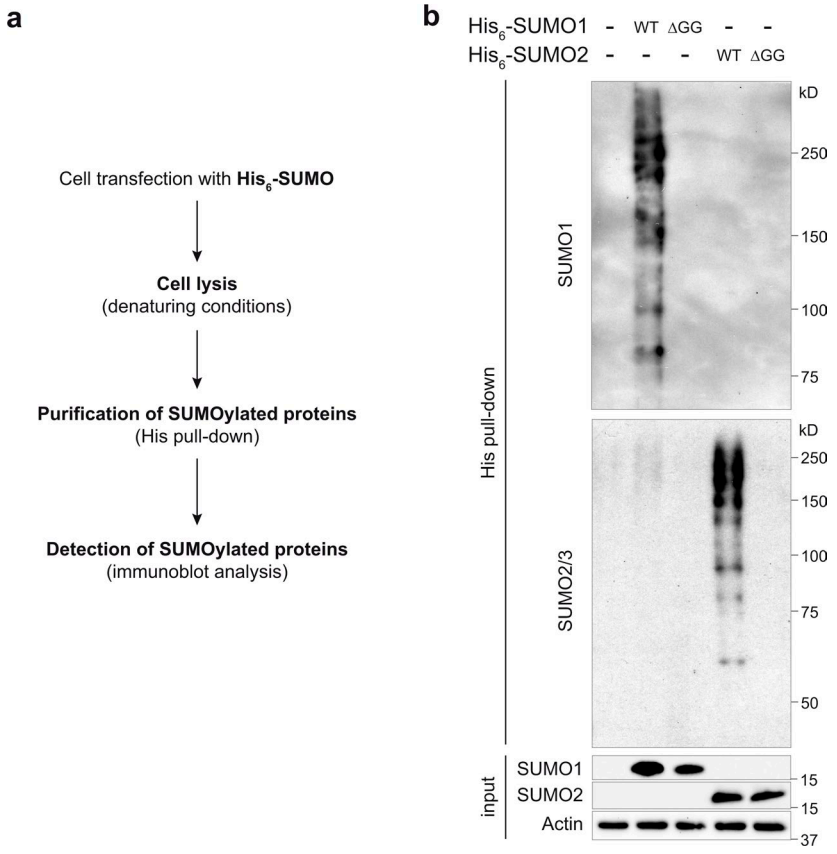

Figure S1. **Septin SUMOylation assay.** (a) Schematic representation of SUMOylated protein detection assay. (b) Immunoblot analysis, using anti-SUMO1 or anti-SUMO2/3 antibodies, of SUMOylated proteins from His pull-down fractions from HeLa cells transfected with WT His_6-SUMO or nonconjugatable ΔGG mutants. Proteins conjugated to SUMO1 or SUMO2 were detected only when WT His_6-SUMO1 or His_6-SUMO2 were expressed, respectively (and not with the corresponding ΔGG mutants). Input fractions are shown as controls.

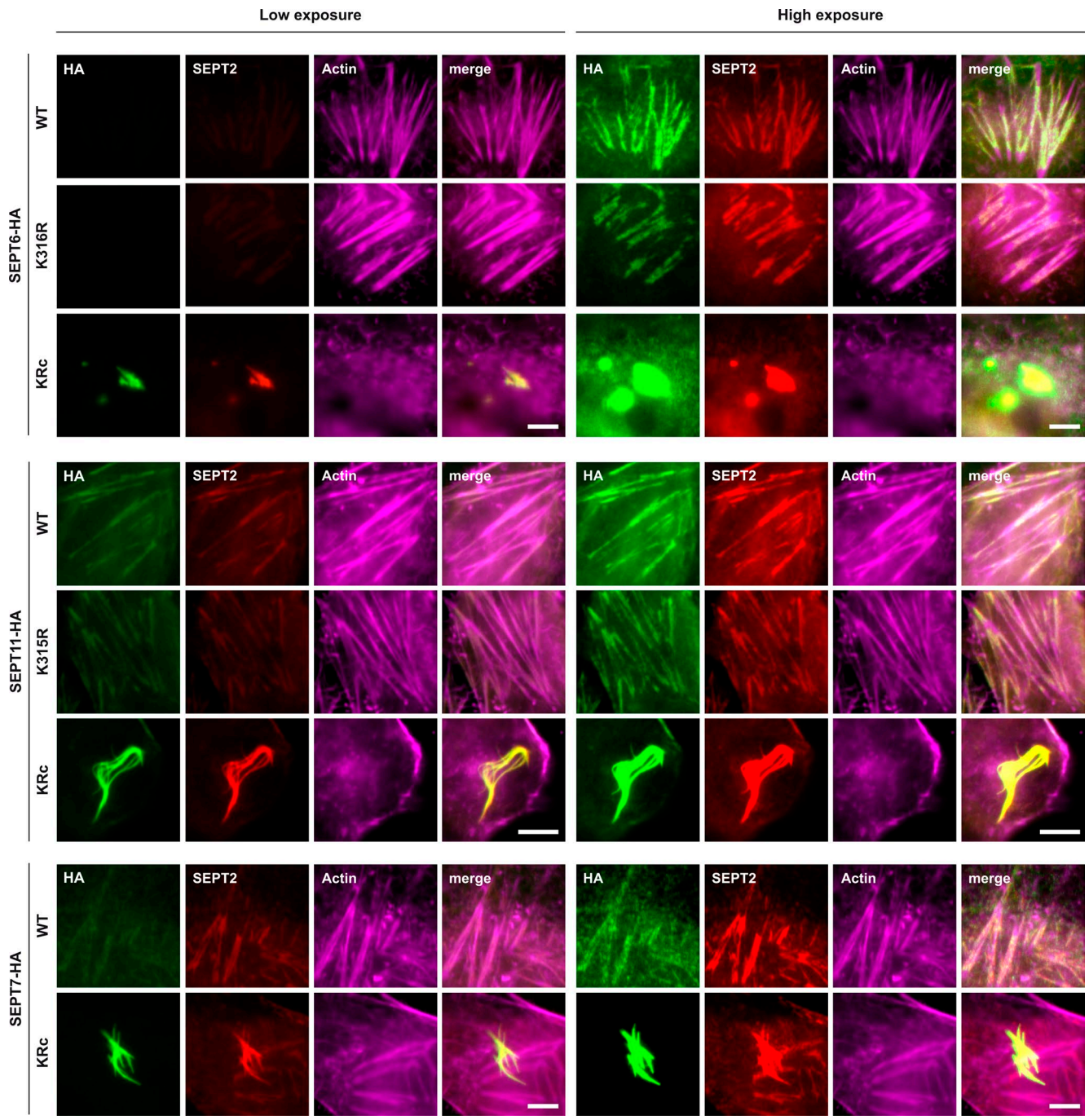

Figure S2. **Colocalization between HA-tagged septins, endogenous SEPT2, and actin filaments.** Fluorescent light microscopy images of HeLa cells transfected with HA-tagged WT or mutant septins. Cells were stained for HA-tagged septins (anti-HA antibodies, green), endogenous SEPT2 (anti-SEPT2 antibodies, red), and actin (phalloidin, magenta). Bars, 5 µm. Aberrant bundles formed by non-SUMOylatable septins colocalize with endogenous SEPT2, but not with actin, in contrast to filaments formed by WT septins.

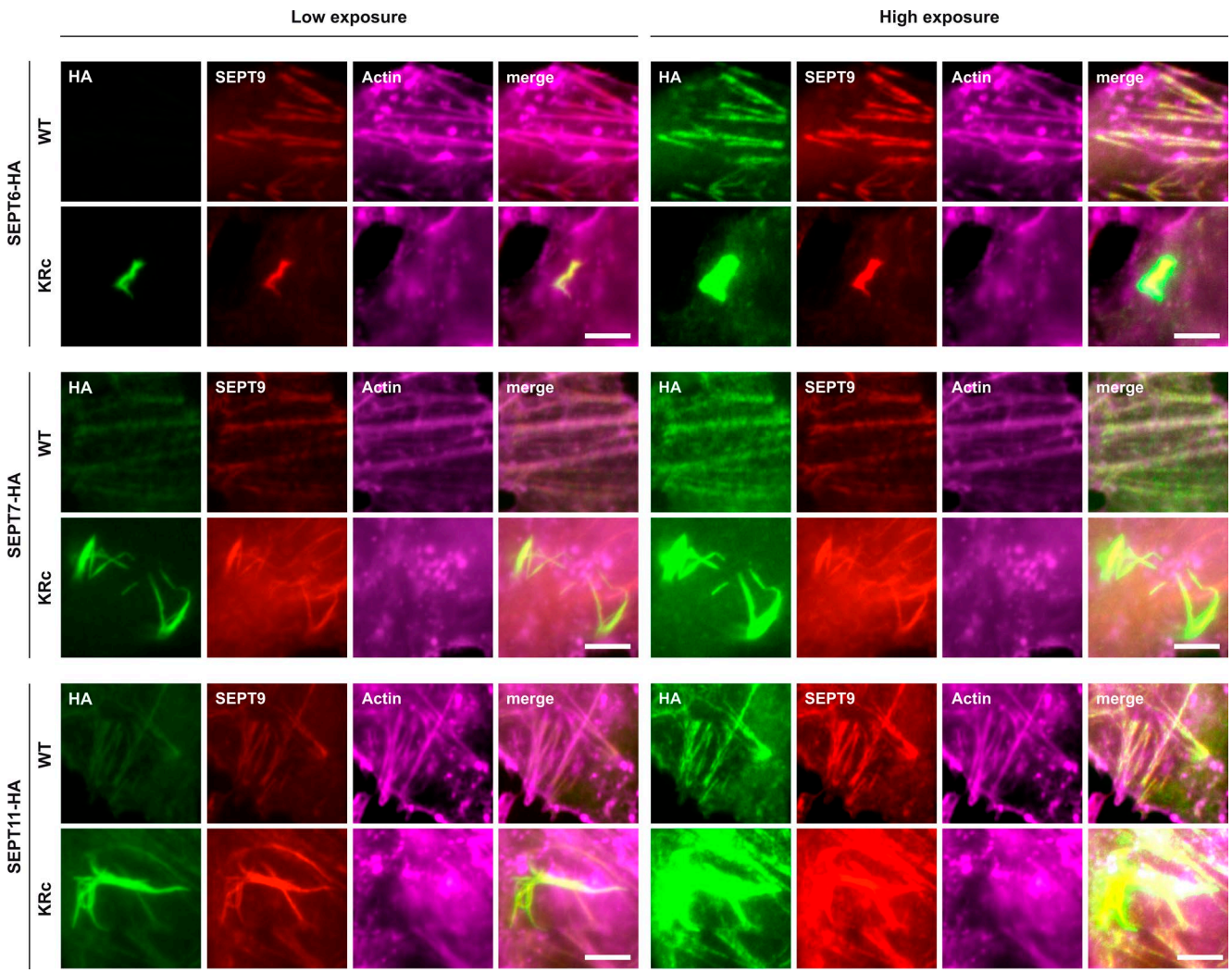

Figure S3. **Colocalization between HA-tagged septins, endogenous SEPT9, and actin filaments.** Fluorescent light microscopy images of HeLa cells transfected with HA-tagged WT or non-SUMOylatable (KRc) septins. Cells were stained for HA-tagged septins (anti-HA antibodies, green), endogenous SEPT9 (anti-SEPT9 antibodies, red), and actin (phalloidin, magenta). Bars, 5 μm. Aberrant bundles formed by non-SUMOylatable septins colocalize with endogenous SEPT9, but not with actin, in contrast to filaments formed by WT septins.

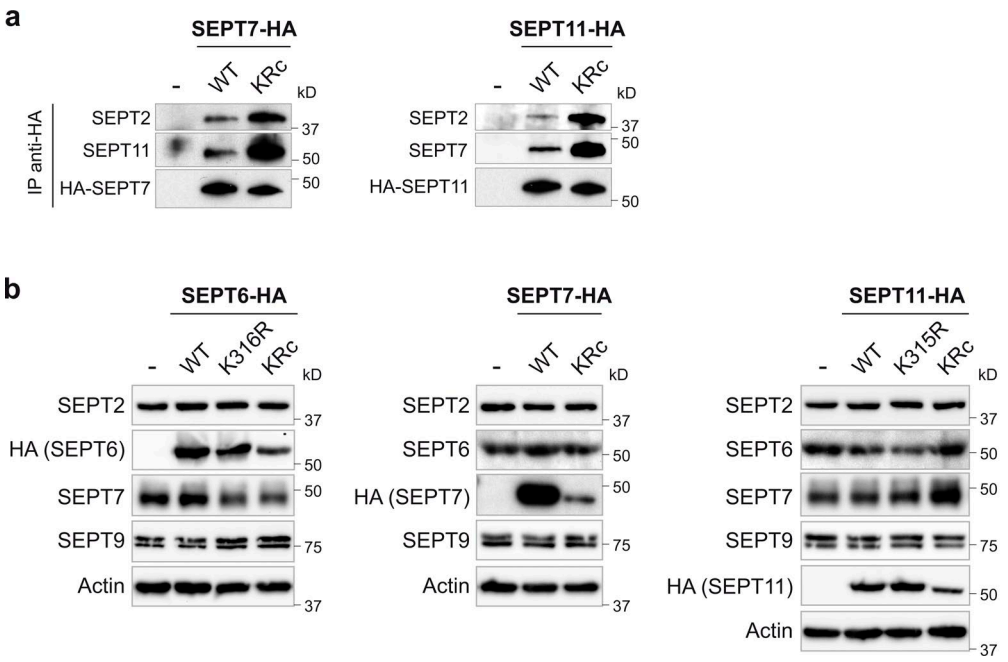

Figure S4. **Coimmunoprecipitation of HA-tagged septins with endogenous septins.** (a) Immunoblot analysis of coimmunoprecipitation experiments between HA-tagged SEPT7 and SEPT11 and endogenous septins. Endogenous septins coimmunoprecipitate with both WT and non-SUMOylatable HA-tagged septins, indicating that a lack of SUMOylation does not inhibit SEPT7 or SEPT11's ability to form complexes with septins from other septin groups. IP, immunoprecipitation. (b) Immunoblot analysis of endogenous septin levels in whole cell lysates from HeLa cells transfected with WT or mutant HA-tagged SEPT6, SEPT7, and SEPT11. Antibodies against SEPT2, SEPT6, SEPT7, and SEPT9 were used to monitor changes in endogenous septin levels. Actin is shown as a loading control. Expression of the different septin mutants does not alter endogenous SEPT2, SEPT6, SEPT7, or SEPT9 protein levels.

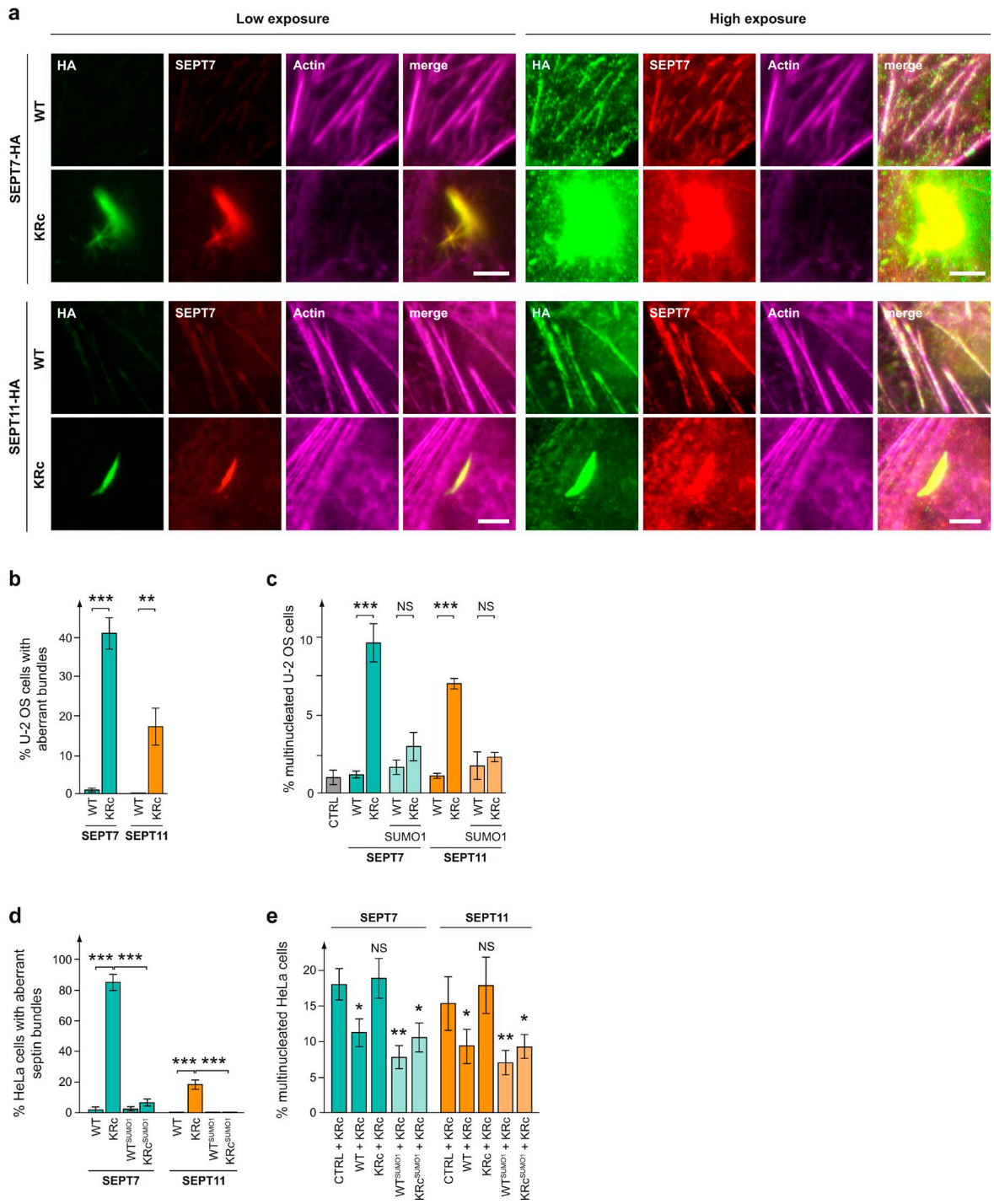

Figure S5. **Lack of SEPT7 and SEPT11 SUMOylation induces cell division defects and aberrant septin bundle formation in HeLa and U-2 OS cells.** (a) Fluorescent light microscopy images of septin filaments in U-2 OS cells transfected with HA-tagged WT or non-SUMOylatable (KRc) SEPT7 and SEPT11 variants. Cells were stained with HA-tagged septins (anti-HA antibodies, green), endogenous septins (anti-SEPT7 antibodies, red), and actin (phalloidin, magenta). Bars, 5 µm. (b) Percentage of U-2 OS cells exhibiting aberrant septin bundles after transfection with WT or mutant SEPT7 or SEPT11. (c) Percentage of U-2 OS cells exhibiting multinucleation after transfection with SEPT7 and SEPT11 variants. As already observed in HeLa cells, expression of non-SUMOylatable SEPT7 and SEPT11 variants leads to the formation of aberrant septin bundles, which colocalize with endogenous SEPT7, and to cytokinesis defects resulting in the formation of multinucleated cells. These phenotypes can be reverted by the artificial fusion of SUMO at the end of SEPT7 and SEPT11 non-SUMOylatable variants, highlighting the essential role of septin SUMOylation in both filament organization and cytokinesis in U-2 OS cells. (d) Percentage of HeLa cells displaying aberrant septin bundles after transfection with WT, non-SUMOylatable, or constitutively SUMOylated SEPT7 and SEPT11. Constitutively SUMOylated SEPT7 and SEPT11 variants do not form aberrant septin bundles in contrast to non-SUMOylatable variants. (b–d) Mean from three independent experiments. (e) Percentage of HeLa cells exhibiting multinucleation after cotransfection with equivalent amount of expression vectors for non-SUMOylatable SEPT KRc mutant and either control plasmid (pCDNA.3; CTRL) or WT, KRc, WT^{SUMO1}, or KRc^{SUMO1} septin variants (mean from three to five experiments; error bars, SD; * $P < 0.05$; ** $P < 0.01$; ***, $P < 0.001$). Cotransfection of SEPT KRc^{SUMO1} septin variants partially restores the cell division defects induced by SEPT KRc mutants alone. This indicates that constitutively SUMOylated septins can functionally restore defects induced by non-SUMOylatable septin mutants.

Table S1. **Bioinformatic search for SIMs**

Septin	UniProt accession number	Isoform[a]	Nb SIM with score <6[b]	Nb SIM with score >6[b]	Best score[c]
SEPT1	Q8WYJ6	Isoform 1	2	0	2.957
		Isoform 2	1	0	2.587
SEPT2	Q15019	Isoform 1	3	0	3.978
		Isoform 2	3	0	3.978
SEPT3	Q9UH03	Isoform 1	3	0	3.609
		Isoform 2	3	0	3.609
		Isoform 3	1	0	2.587
SEPT4	O43236	Isoform 1	0	0	–
		Isoform 2	0	0	–
		Isoform 3	0	0	–
		Isoform 4	1	0	3.022
		Isoform 5	0	0	–
SEPT5	Q99719	Isoform 1	5	0	2.978
		Isoform 2	4	0	2.978
SEPT6	Q14141	Isoform ii	2	0	3.739
		Isoform i	2	0	3.739
		Isoform iv	2	0	3.739
		Isoform v	2	0	3.739
SEPT7	Q16181	Isoform 1	5	0	5.130
		Isoform 2	5	0	5.130
SEPT8	Q92599	Isoform 1	3	0	3.739
		Isoform 2	3	0	3.739
		Isoform 3	3	0	3.739
SEPT9	Q9UHD8	Isoform 1	4	0	3.696
		Isoform 2	4	0	3.696
		Isoform 3	3	0	3.283
		Isoform 4	3	0	3.283
		Isoform 5	4	0	3.696
		Isoform 7	4	0	3.696
		Isoform 8	4	0	3.761
		Isoform 9	3	0	3.283
SEPT10	Q9P0V9	Isoform 1	2	0	3.413
		Isoform 2	2	0	3.413
		Isoform 3	2	0	3.413
SEPT11	Q9NVA2	Isoform 1	3	0	3.739
		Isoform 2	3	0	3.739
SEPT12	Q8IYM1	Isoform 1	5	0	4.739
		Isoform 2	4	0	4.739
SEPT14	Q6ZU15	Isoform 1	2	0	3.587
PML[d]	P29590	Isoform i	0	1	10.739

Dashes, not applicable.
[a]Isoform reference according to the UniProt database.
[b]Number of predicted SIMs according to GPS-SBM 1.0 software (http://sumosp.biocuckoo.org; score threshold: 2.5). Only predicted SIMs with scores >6 are considered to be confident.
[c]Score of the best SIM predicted.
[d]Example of a protein with one established functional SIM (Shen et al., 2006).

Table S2. **Plasmids used in this study**

Reference	Name	Gene (UniProt ID)	Mutation	Tag	Origin
BUG 3452	pCDNA3-FLAG-Ubc9	*Ubc9*		FLAG (N-term)	This study
BUG 3127	pSG5-His_6 SUMO1 WT	*SUMO1*		His_6 (N-term)	Impens et al., 2014
BUG 3100	pSG5-His_6 SUMO1 ΔGG	*SUMO1*	ΔGG	His_6 (N-term)	This study
BUG 3128	pSG5-His_6 SUMO2 WT	*SUMO2*		His_6 (N-term)	Impens et al., 2014
BUG 3129	pSG5-His_6 SUMO2 ΔGG	*SUMO2*	ΔGG	His_6 (N-term)	This study
BUG 3101	pCDNA3-SEPT2 WT-HA	*SEPT2* isoform 1 (Q15019-1)		HA (C-term)	This study
BUG 3453	pCDNA3-SEPT2 KRn-HA	*SEPT2* isoform 1 (Q15019-1)	K3R	HA (C-term)	This study
BUG 3409	pCDNA3-SEPT2 KRc-HA	*SEPT2* isoform 1 (Q15019-1)	K310, 318, 325R	HA (C-term)	This study
BUG 3102	pCDNA3-SEPT6 WT-HA	*SEPT6* isoform II (Q14141-1)		HA (C-term)	This study
BUG 3373	pCDNA3-SEPT6 K316R-HA	*SEPT6* isoform II (Q14141-1)	K316R	HA (C-term)	This study
BUG 3374	pCDNA3-SEPT6 KRc-HA	*SEPT6* isoform II (Q14141-1)	K316, 327, 337, 338, 351, 353, 358, 362, 367, 372, 373, 379, 380, 381, 385, 386, 387, 397, 400, 420, 423, 425, 426R	HA (C-term)	This study
BUG 3105	pCDNA3-SEPT7 WT-HA	*SEPT7* isoform I (Q16181-1)		HA (C-term)	This study
BUG 3375	pCDNA3-SEPT7 KRc-HA	*SEPT7* isoform I (Q16181-1)	K313, 326, 328, 333, 349, 351, 352, 364, 366, 368, 371, 373, 387, 388, 395, 400, 408, 429, 431, 432, 433, 435R	HA (C-term)	This study
BUG 3383	pCDNA3-SEPT7 WT-HA-SUMO1	*SEPT7* isoform I (Q16181-1)	SEPT7 WT fused to SUMO1 in C-term	HA (C-term)	This study
BUG 3384	pCDNA3-SEPT7 KRc-HA-SUMO1	*SEPT7* isoform I (Q16181-1)	SEPT7 KRc fused to SUMO1 in C-term	HA (C-term)	This study
BUG 3107	pCDNA3-SEPT9 WT-HA	*SEPT9* isoform I (Q9UHD8-1)		HA (C-term)	This study
BUG 3412	pCDNA3-SEPT9 KRn-HA	*SEPT9* isoform I (Q9UHD8-1)	K2, 3, 28, 62, 69, 87, 99, 117, 133, 141, 156, 171, 186, 194, 226, 262, 271R	HA (C-term)	This study
BUG 3411	pCDNA3-SEPT9 KRc-HA	*SEPT9* isoform I (Q9UHD8-1)	K579R	HA (C-term)	This study
BUG 3108	pCDNA3-SEPT11 WT-HA	*SEPT11* isoform I (Q9NVA2-1)		HA (C-term)	This study
BUG 3376	pCDNA3-SEPT11 K315R-HA	*SEPT11* isoform I (Q9NVA2-1)	K315R	HA (C-term)	This study
BUG 3377	pCDNA3-SEPT11 KRn+m-HA	*SEPT11* isoform I (Q9NVA2-1)	K34, 54, 65, 81, 95, 110, 115, 134, 136, 162, 170, 171, 175, 184, 190, 195, 197, 199, 244, 248, 251, 272, 301, 308R	HA (C-term)	This study
BUG 3378	pCDNA3-SEPT11 KRc-HA	*SEPT11* isoform I (Q9NVA2-1)	K315, 326, 336, 337, 350, 352, 357, 361, 366, 371, 378, 379, 380, 384, 385, 386, 397, 398, 399, 418, 419, 421, 423, 424R	HA (C-term)	This study
BUG 3387	pCDNA3-SEPT11 WT-HA-SUMO1	*SEPT11* isoform I (Q9NVA2-1)	SEPT11 WT fused to SUMO1 in C-term	HA	This study
BUG 3388	pCDNA3-SEPT11 KRc-HA-SUMO1	*SEPT11* isoform I (Q9NVA2-1)	SEPT11 KRc fused to SUMO1 in C-term	HA	This study
BUG 3471	pCDNA3-SEPT11 WT-HA-SUMO2	*SEPT11* isoform I (Q9NVA2-1)	SEPT11 WT fused to SUMO2 in C-term	HA	This study
BUG 3472	pCDNA3-SEPT11 KRc-HA-SUMO2	*SEPT11* isoform I (Q9NVA2-1)	SEPT11 KRc fused to SUMO2 in C-term	HA	This study
BUG 4169	pGFP-SEPT11 WT	*SEPT11* isoform I (Q9NVA2-1)		GFP (N-term)	This study
BUG 4170	pGFP-SEPT11 KRc	*SEPT11* isoform I (Q9NVA2-1)	K315, 326, 336, 337, 350, 352, 357, 361, 366, 371, 378, 379, 380, 384, 385, 386, 397, 398, 399, 418, 419, 421, 423, 424R	GFP (N-term)	This study

Table S3. **Primary antibody information**

Targeted protein	Assay (dilution)	Species	Source	Reference
HA tag	WB (1:1,000) IF (1:200)	Mouse	Cell Signaling Technology	6E2; 2367
HA tag	WB (1:1,000)	Rabbit	Santa Cruz Biotechnologies	Y11; sc-805
FLAG	WB (1:1,000)	Mouse	Sigma-Aldrich	M2; F3165
Ubc9	WB (1:5,000)	Rabbit	Homemade	R202; this study
SUMO1	WB (1:1,000)	Rabbit	Cell Signaling Technology	4930
	WB (1:1,000)	Rabbit	Homemade	R204; Ribet et al., 2017
SUMO2/3	WB (1:5,000)	Rabbit	Homemade	R205; Ribet et al., 2017
RanGAP1	WB (1:1,000)	Rabbit	Sigma-Aldrich	R0155
Actin	WB (1:10,000)	Mouse	Sigma-Aldrich	AC-15; R5441
SEPT2	WB (1:1,000) IF (1:200)	Rabbit	Homemade	R170; Mostowy et al., 2010
SEPT6	WB (1:1,000)	Rabbit	Homemade	R136; this study
SEPT7	WB (1:1,000) IF (1:200)	Rabbit	Homemade	R171; Mostowy et al., 2010
SEPT9	WB (1:1,000) IF (1:200)	Rabbit	Homemade	R69; Pizarro-Cerdá et al., 2002
SEPT11	WB (1:1,000)	Rabbit	Homemade	R274; this study
KIF20A/Rabkinesin-6	IF (1:500)	Rabbit	Homemade	Echard et al., 1998

WB, Western blot; IF, immunofluorescence.

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