Supplemental material

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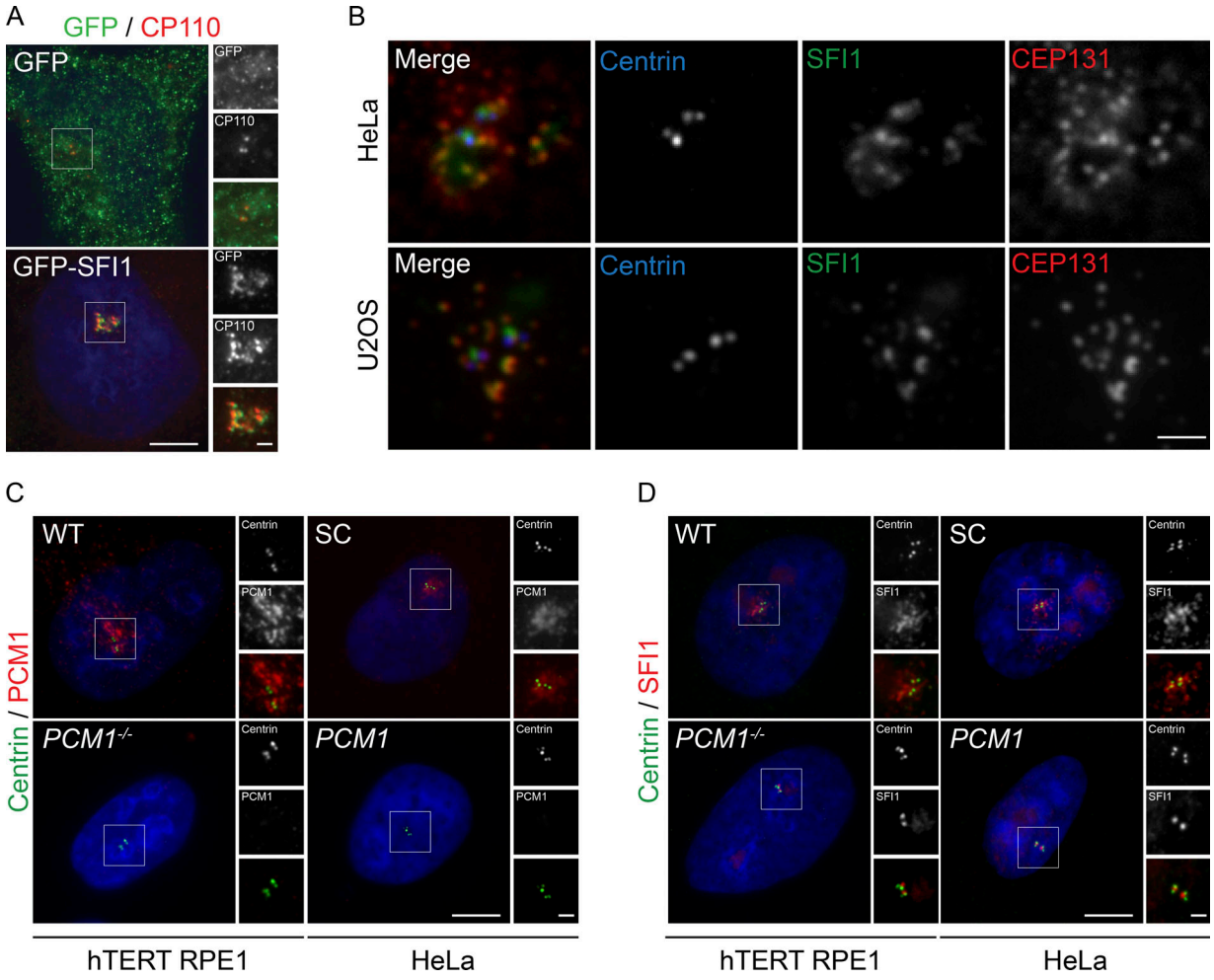

Figure S1. **SFI1 localizes to centriolar satellites during S phase. (A)** HeLa cells transfected with GFP or GFP-SFI1 were costained for GFP (green) and the centriole distal end component CP110 (red). **(B)** Airyscan maximum projections of HeLa and U2OS cells costained with antibodies to Centrin (blue), SFI1 (green), and CEP131 (red). Scale bars represent 5 µm for larger images and 1 µm for inset images. **(C and D)** S phase hTERT-RPE1 WT and *PCM1* knockout cells and HeLa cells transfected with SC or *PCM1* siRNA costained with Centrin (Green) and PCM1 (red) or SFI1 (red). Scale bars represent 5 µm for larger images and 1 µm for inset images.

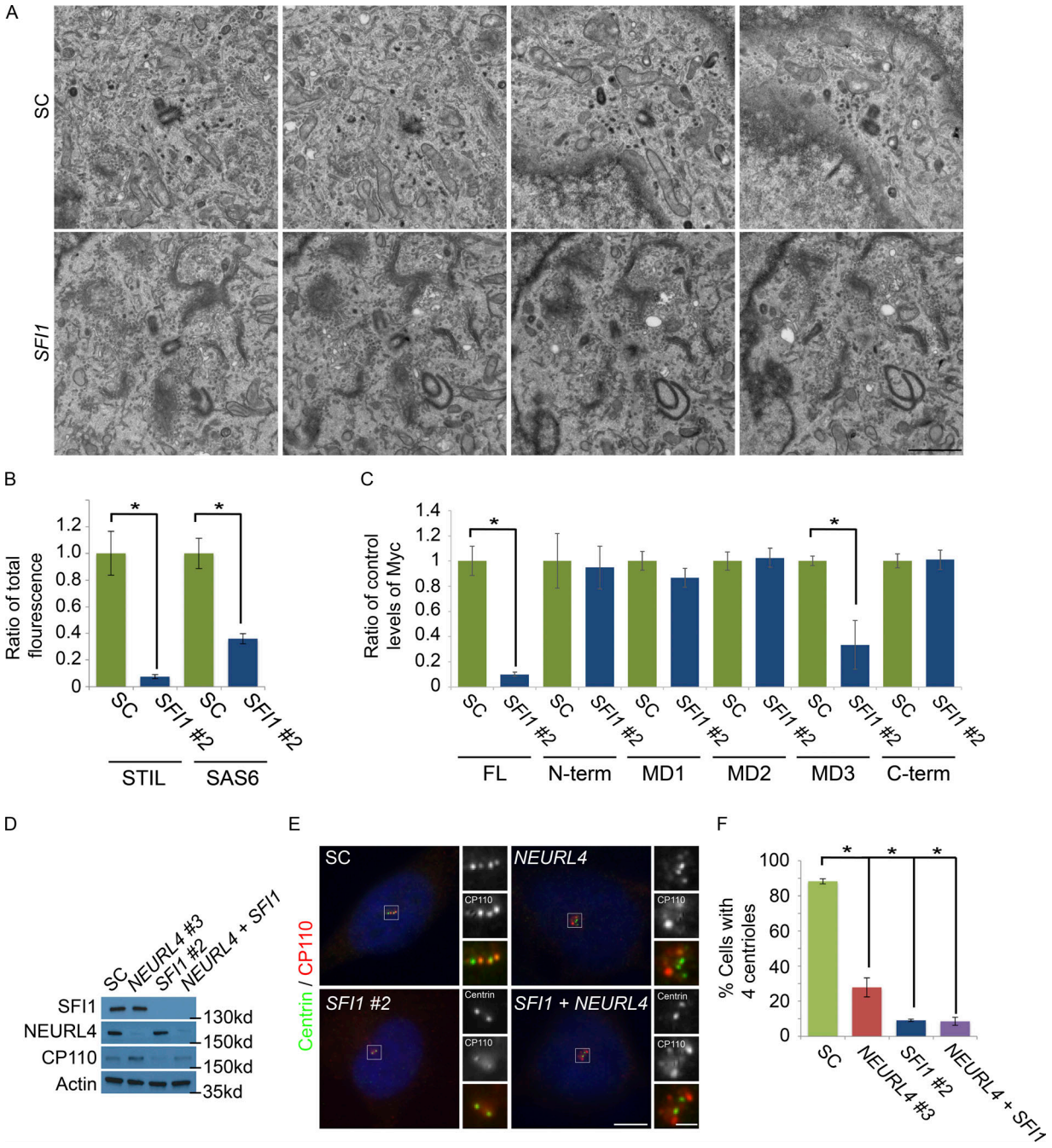

Figure S2. **CP110 stability is not required for SFI1-dependent centriole duplication. (A)** Uncropped electron micrograph images of SC and *SFI1*-depleted HeLa cells (associated with Fig. 2 C). Scale bar represents 2 µm. **(B)** Quantification of the mean fluorescence intensities ± SD of centrosomal STIL and SAS6 in SC and *SFI1* siRNA-treated cells as expressed as the mean proportion ± SD of the fluorescence intensities of SC cells. For all quantifications, 10 cells were analyzed per experiment (n = 3). *P < 0.005 (t test). **(C)** Quantification of the protein levels of full-length, N-terminus, MD1, MD2, MD3, and C-terminus of STIL in SC and *SFI1* siRNA-treated DLD1 cells expressed as a ratio of the control cells. For quantifications, three independent experiments were analyzed (n = 3). *P < 0.005 (t test). **(D)** Total cell lysates of SC, *SFI1 #2*, *NEURL4* #3, or *SFI1* #2 and *NEURL4* #3 siRNA transfected HeLa cells were immunoblotted for SFI1, NEURL4, and CP110. Actin served as a loading control. **(E)** S phase HeLa cells transfected with SC siRNA or siRNA *SFI1 #2*, *NEURL4* #3, or *SFI1* #2 and *NEURL4* #3 co-stained for Centrin (green) and CP110 (red). **(F)** Percentage of S phase SC, *SFI1 #2*, *NEURL4*, or *SFI1 #2* or *NEURL4 #1* siRNA transfected HeLa cells with four centrioles.

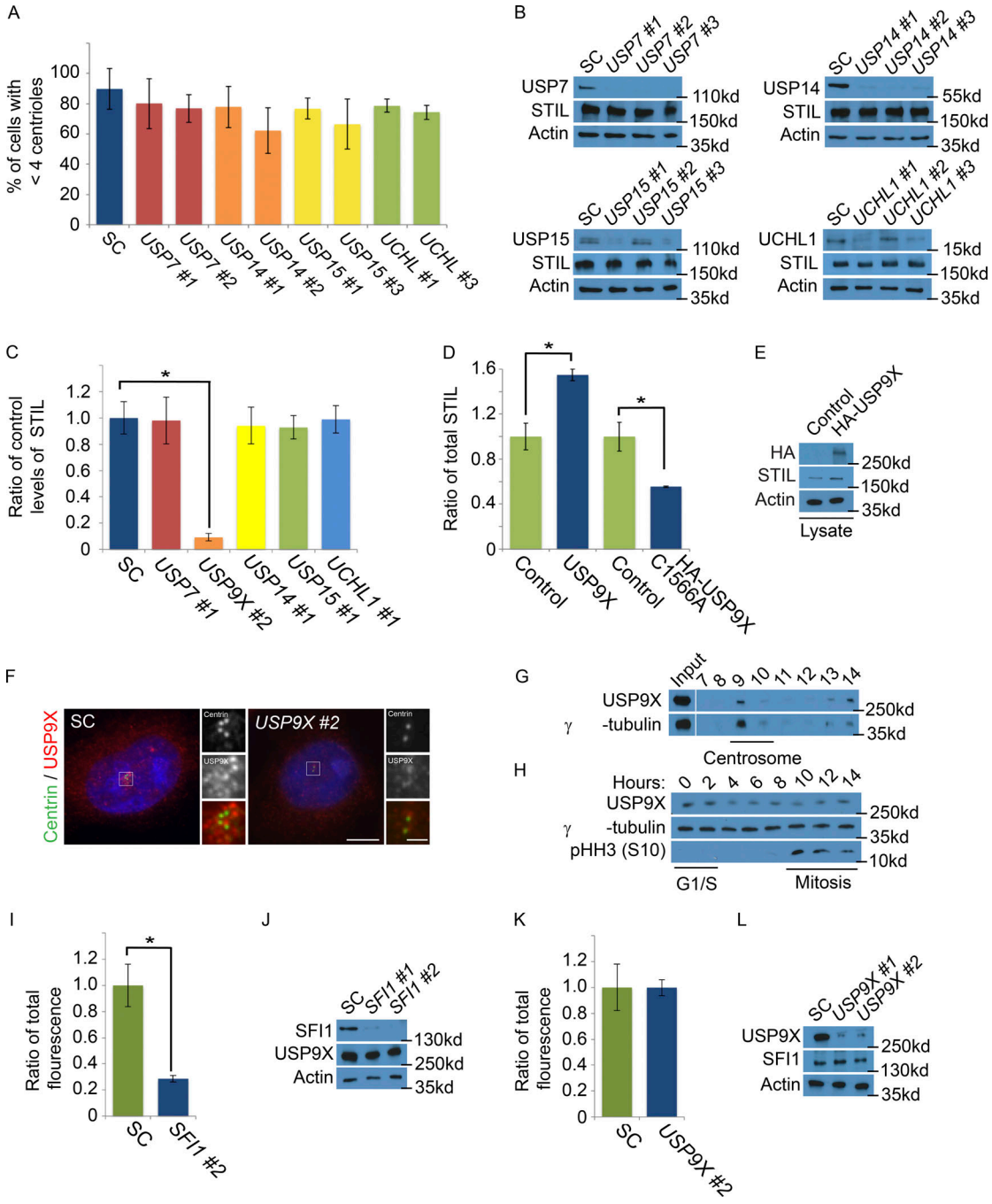

Figure S3. **USP9X localizes to the centrosome during S phase. (A)** Percentage of S phase cells with four centrioles in SC, *USP7 #1*, *USP7 #2*, *USP14 #1*, *USP14 #2*, *USP15 #1*, *USP15 #3*, *UCHL #1*, and *UCHL #3* siRNA-treated HeLa cells. For all quantifications, ≥100 cells were counted per experiment (n = 3); *P < 0.005 (paired t test). Error bars represent ± SD. **(B)** Total cell lysates from HeLa cells transfected with SC or one of three nonoverlapping siRNAs to *USP7*, *USP14*, *USP15*, or *UCHL1* immunoblotted for STIL, USP7, USP14, USP15, and UCHL1. Actin served as a loading control. **(C)** Quantification of centrosomal STIL in SC, *USP7 #1*, *USP9X #2*, *USP14 #1*, *USP15 #1*, or *UCHL1 #1* siRNA-treated cells as expressed as the STIL mean fluorescence intensities ± SDs as compared with the fluorescence intensities of SC cells. For all quantifications, 10 cells were analyzed per experiment (n = 3). *P < 0.005 (t test). **(D)** Quantification of the protein levels of STIL in control, HA-USP9X, or HA-USP9X C1566A transfected HeLa cells. For quantifications, three independent experiments were analyzed (n = 3). *P < 0.005 (t test). **(E)** Total cell lysates from HeLa cells 6 h after transfection with control or HA-USP9X immunoblotted for HA or STIL. Actin served as a loading control. **(F)** S phase HeLa cells transfected with SC or *USP9X #2* siRNA costained for Centrin (green) and USP9X (red). **(G)** Sucrose gradient fractions of HeLa cell lysates were immunoblotted for USP9X and γ-tubulin. Dashed line represents spliced blot. **(H)** Total cell lysates from double thymidine-synchronized HeLa cells were immunoblotted for USP9X, γ-tubulin, and phospho-Histone H3 (serine 10). Cells were harvested at indicated time points after release from thymidine block. **(I)** Quantification of centrosomal USP9X fluorescence ± SDs in SC and *SFI1* siRNA-treated S phase HeLa cells, expressed as the proportion of the centrosomal fluorescence of SC-treated cells. **(J)** SC-treated and *SFI1*-depleted HeLa cells immunoblotted for USP9X and SFI1. Actin served as a loading control. **(K)** Quantification of SFI1 fluorescence ± SDs in SC- and *USP9X*-depleted HeLa cells, expressed as the proportion of the centrosomal fluorescence of SC-treated cells. **(L)** Total cell lysates of HeLa cells transfected with SC or *USP9X* siRNA were immunoblotted for USP9X and SFI1. Actin served as a loading control.

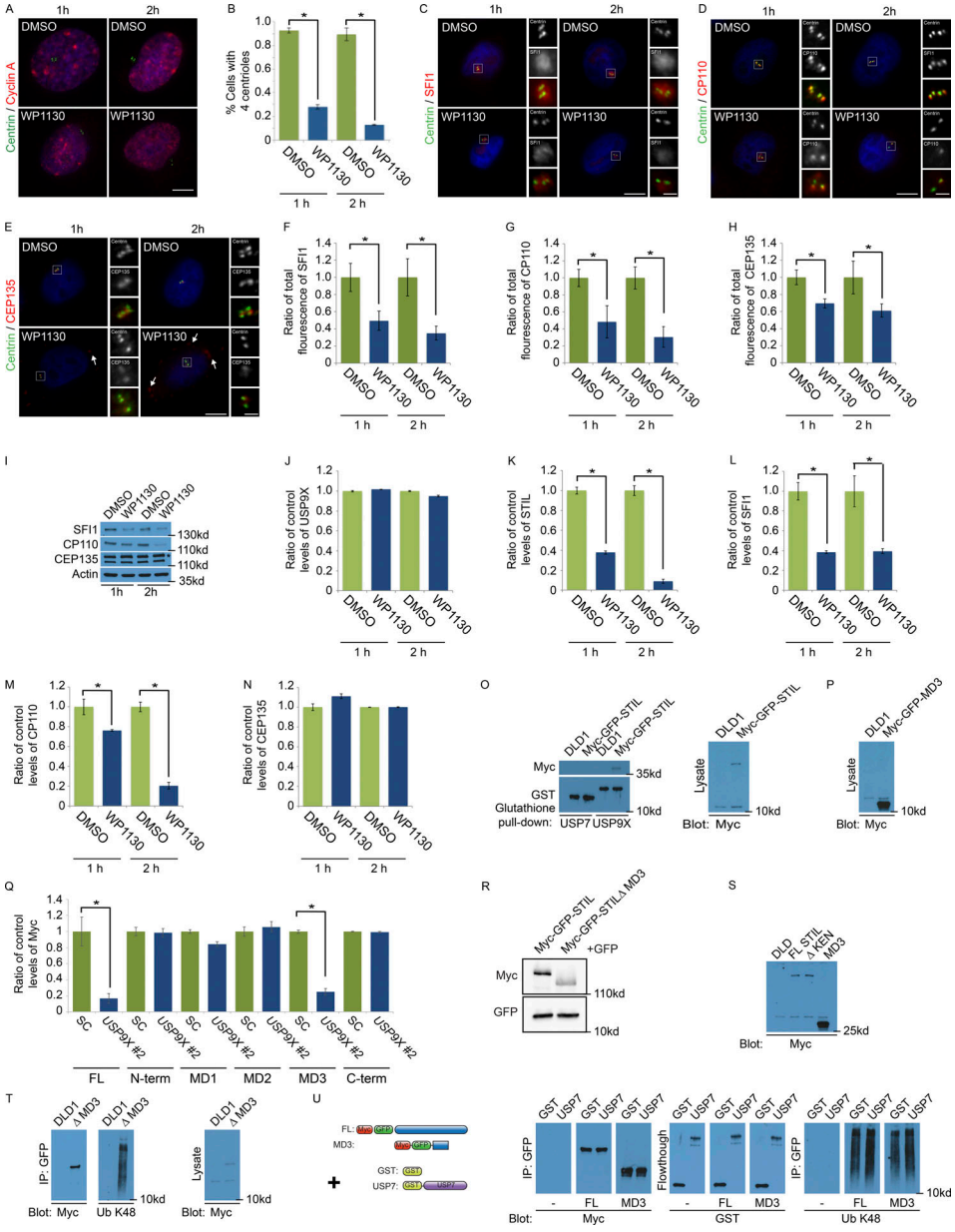

Figure S4. **USP9X function is required to stabilize STIL. (A)** S phase cells treated with DMSO or WP1130 for the indicated time periods and costained for Centrin (green) and Cyclin A (red). **(B)** Percentage of S phase cells with four centrioles in DMSO- or WP110-treated HeLa cells after 1 or 2 h of treatment. **(C–E)** HeLa cells treated with DMSO or WP1130 for 1 or 2 h were stained for Centrin (green) and SFI1, CP110, or CEP135 (red). Scale bars represent 5 µm for larger images and 1 µm for inset images. Arrows indicate cytoplasmic accumulations of CEP135 upon treatment with WP1130. **(F–H)** Quantification of the fluorescence intensity of SFI1, CP110, and CEP135 in HeLa cells treated with DMSO or WP1130 for the indicated time periods and expressed as the proportion of the centrosomal fluorescence of DMSO-treated cells. For all quantifications, 10 cells were analyzed per experiment (n = 3). *P < 0.005 (t test). **(I)** Total cell lysates from HeLa cells treated with DMSO or WP1130 for 1 or 2 h and immunoblotted for SFI1, CP110, and CEP135. Actin served as a loading control. Asterisk denotes specific band. **(J–N)** Quantification of the protein levels of USP9X, STIL, SFI1, CP110, and CEP135 from DMSO and WP1130 treated cells at the indicated time points and expressed as a proportion of the DMSO-treated control. For quantifications, three independent experiments were analyzed (n = 3). *P < 0.005 (t test). **(O)** Total cell lysates from doxycycline-induced control DLD-1 or cells expressing Myc-GFP–tagged STIL were incubated with recombinant USP7 or USP9X. Proteins that precipitated with GST-USP7 or USP9X^{CD} were analyzed by immunoblotting for Myc and GST. **(P)** Total cell lysates from doxycycline-induced control DLD-1 and cells expressing GFP-Myc–tagged MD3 cells were immunoblotted for Myc. **(Q)** Quantification of the protein levels of full-length, N-terminus, MD1, MD2, MD3, and C-terminus STIL in SC- and USP9X-depleted cells expressed as a ratio to that of control cells. For quantifications, three independent experiments were analyzed (n = 3). *P < 0.005 (t test). **(R)** Total cell lysates from HEK293T cells transfected with GFP and Myc-GFP-STIL or Myc-GFP-STILΔMD3 were immunoblotted for Myc and GFP. **(S)** Total lysates from doxycycline-induced control DLD-1 and cells expressing Myc-GFP–tagged STIL, STILΔKEN, or MD3 were immunoblotted for Myc. **(T)** Total cell lysates of doxycycline-induced control DLD-1 or DLD-1 cells expressing Myc-GFP–tagged STILΔMD3 were immunoprecipitated with GFP-trap beads. Precipitating proteins were detected with antibodies to Myc or K48-linked ubiquitin. Total cell lysate was probed with an antibody to Myc. **(U)** Total cell lysates of doxycycline-induced control DLD-1 or DLD-1 cells expressing full-length (FL) Myc-GFP-STIL or the Myc-GFP-MD3 fragment of STIL were immunoprecipitated with GFP-trap beads, incubated with recombinant GST or GST-USP7, and immunoblotted for Myc, GST, and ubiquitylated (Ub) K48.

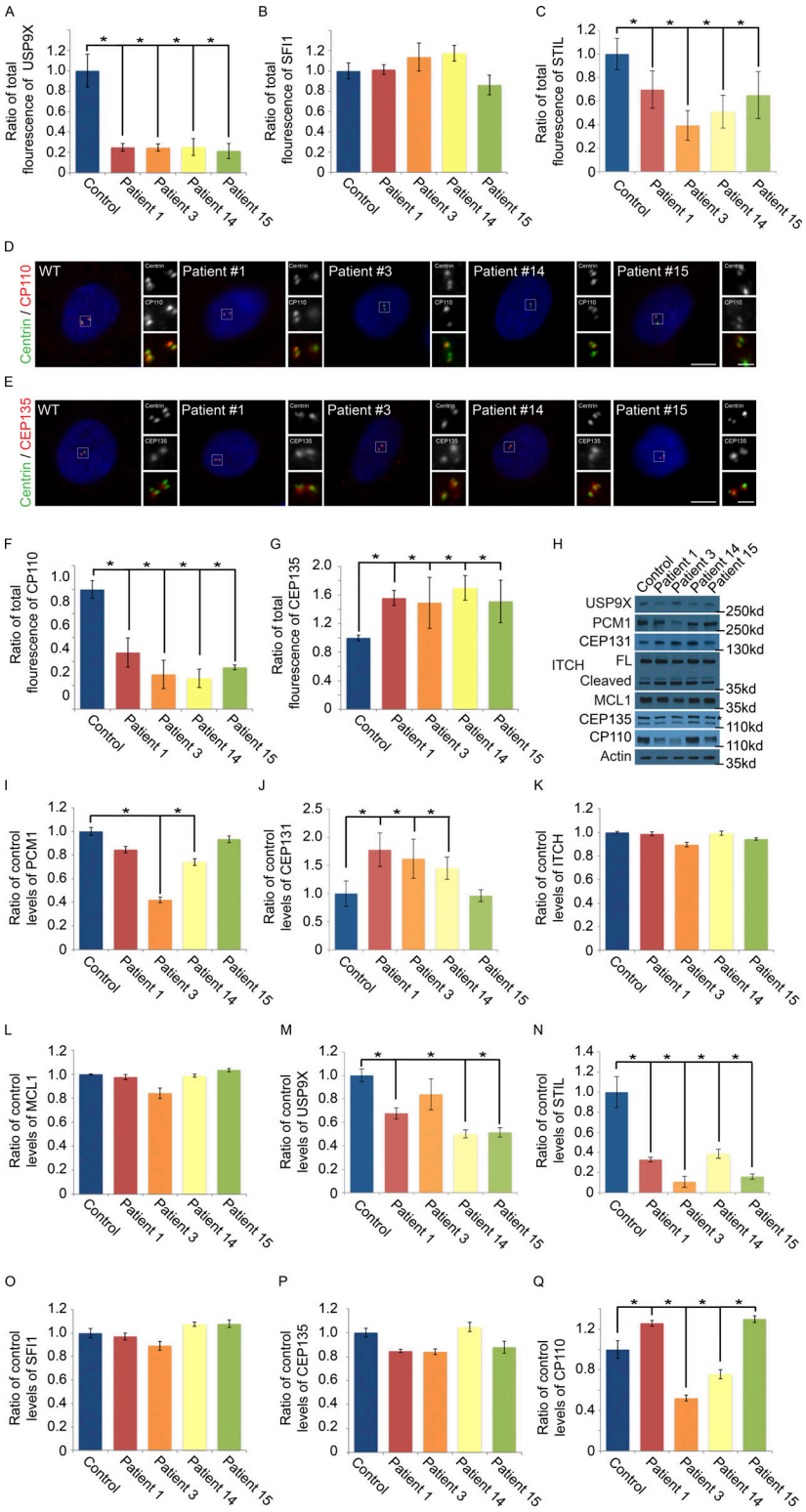

Figure S5. **MRXSF99-associated mutations do not alter the stability of PCM1, CEP131, ITCH, and MCL1. (A–C)** Quantification of the centrosomal fluorescence intensity of USP9X, SFI1, or STIL in control or MRXS99F cells expressed as the mean proportion ± SD of the fluorescence intensities of control cells. For all quantifications, 10 cells were analyzed per experiment (n = 3). *P < 0.005 (t test). **(D and E)** WT and MRXS99F patient fibroblasts in S phase were costained for Centrin (green) and CP110 or CEP135 (red). Scale bars represent 5 µm for larger images and 1 µm for inset images. **(F and G)** Quantifications of the centrosomal fluorescence intensities of CP110 or CEP135 in control and MRXS99F patient fibroblast cells, expressed as a ratio to that of control cells. **(H)** Total cell lysates from control and MRXS99F patient fibroblasts were immunoblotted for USP9X, PCM1, CEP131, ITCH, MCL1, CEP135, and CP110. Actin served as a loading control. Asterisk denotes specific band. **(I–Q)** Quantification of the protein levels of PCM1, CEP131, ITCH, MCL1, USP9X, SFI1, STIL, CEP135, and CP110 from control and MRXS99F fibroblasts, expressed as a ratio to that of control cells. For quantifications, three independent experiments were analyzed (n = 3). *P < 0.005 (t test).

Table S1. **Antibodies used**

Target	Dilution	Species	Source and catalog number
Centrin	1:1,000	Mouse	Millipore 04-1624
SFI1 79	1:1,000	Rabbit	Self
SFI1 81	1:500 (WB)	Rabbit	Self
SFI1	1:1,000; 1:1,100 (WB)	Rabbit	Proteintech 13550-1-AP
β-Actin	1:1,000 (WB)	Rabbit	Proteintech 20536-1-AP
γ-Tubulin	1:2,000 (WB)	Rabbit	Sigma T5192
pHH3	1:500 (WB)	Rabbit	Cell Signaling 9701S
GFP	1:1,000; 1:2,500 (WB)	Mouse	Roche 11814460001
CEP135	1:2,000; 1:1,000 (WB)	Rabbit	Proteintech 24428-1-AP
CP110	1:1,000; 1:2000 (WB)	Rabbit	Proteintech 12780-1-AP
Cyclin A	1:500	Rabbit	Santa Cruz SC-751
NEURL4	1:1,000 (WB)	Rabbit	Proteintech 55345-1-AP
STIL	1:1,000	Rabbit	Dr. Andrew Holland and self
STIL	1:2,000 (WB)	Rabbit	Bethyl A302-441
c-Myc	1:1,000	Mouse	Millipore 05-724
SAS6	1:1,000	Rabbit	Dr. Alexander Dammermann, University of Vienna, Vienna Biocenter, Vienna, Austria
SAS6	1:2,000 (WB)	Mouse	Santa Cruz SC-81431
USP7	1:1,000 (WB)	Rabbit	Cell Signaling 4833S
USP9X	1:500	Rabbit	Bethyl A301-351A
USP9X	1:10,000 (WB)	Rabbit	Proteintech 55054-1-AP
USP14	1:1,000 (WB)	Rabbit	Cell Signaling 11931S
USP15	1:1,000 (WB)	Rabbit	Proteintech 14354-1-AP
UCHL1	1:1,000 (WB)	Rabbit	Millipore MABN48
CEP131	1:2,000 (WB/IF)	Guinea pig	Kodani et al., 2015
PCM1	1:5,000 (WB/IF)	Rabbit	Proteintech 19856-1-AP
MCL1	1:10,000 (WB)	Rabbit	Proteintech 16225-1-AP
ITCH	1:10,000 (WB)	Rabbit	Proteintech 20920-1-AP
HA-HRP	1:1,000 (WB)	Mouse	Cell Signaling 2999S
Ub K48	1:500 (WB)	Rabbit	Millipore 05-1307
Ub K48	1:200 (WB)	Rabbit	Abcam ab140601
GST-HRP	1:1,000 (WB)	Rabbit	Cell Signaling 5475S

IF, immunofluorescence; Ub, ubiquitin; WB, Western blot.

Table S2. **siRNA targeting sequences**

siRNA names	Antisense sequence (5′ to 3′)
SC	AAACUAAACUGAGGCAAUGCC
SFI1 #1	GCGCCUUUACUCACUGGAAACACUA
SFI1 #2	CAACAAGAAGUCUUCUGCAUCCUUU
PCM1	GGGCUCUAAACGUGCCUCC
NEURL4	CAACACCAUCCUGUCUGCCUACAAU
USP7 #1	UUUACAUUCUUCUAACAGGUCCCGG
USP7 #2	UCAAAUUUACACCAUUUGCCAUCCC
USP7 #3	UAUCGAGCAACACUCGACAAAGCUC
USP9X #1	AAACGAAGCAAUUUGACGGUUUCCU
USP9X #2	AAGUCUGGCUCAUUGCCUGCAUUGG
USP9X #3	UAUUUAGGCUGCUACCUAAGUCUGC
USP14 #1	UACCAUUGGAGGUUCAUCUGUAUUC
USP14 #2	UAGAGUAGAACGUAAGCGAUAUGCC
USP14 #3	AUAUCUUCUGGUGUUACGAUGCUGA
USP15 #1	UUGAUCUCCCAUCUGGUAUUUGUCC
USP15 #2	UUCUUCGGCAACCACCUUAUCUGGC
USP15 #3	UACCAUUGGAGGUUCAUCUGUAUUC
UCHL1 #1	ACAGCACUUUGUUCAGCAUCUCGGG
UCHL1 #2	AACUGAUCCAUCCUCAAAUCCCAGU
UCHL1 #3	AGGACUAACUUCUUGUCCCUUCAGC

Table S3. ***USP9X* patient fibroblasts**

Patient	Mutation
Female 1	c. 2554 C>T (p. Arg852*)
Female 3	c.3028-2A>G
Female 14	c.3709del (p. Cys1237fs)
Female 15	ChrX:40998091–41057271 del

Reference

Kodani, A., T.W. Yu, J.R. Johnson, D. Jayaraman, T.L. Johnson, L. Al-Gazali, L. Sztriha, J.N. Partlow, H. Kim, A.L. Krup, et al. 2015. Centriolar satellites assemble centrosomal microcephaly proteins to recruit CDK2 and promote centriole duplication. *eLife.* 4:e07519. https://doi.org/10.7554/eLife.07519