Supplemental material

Chang et al., https://doi.org/10.1083/jcb.201902061

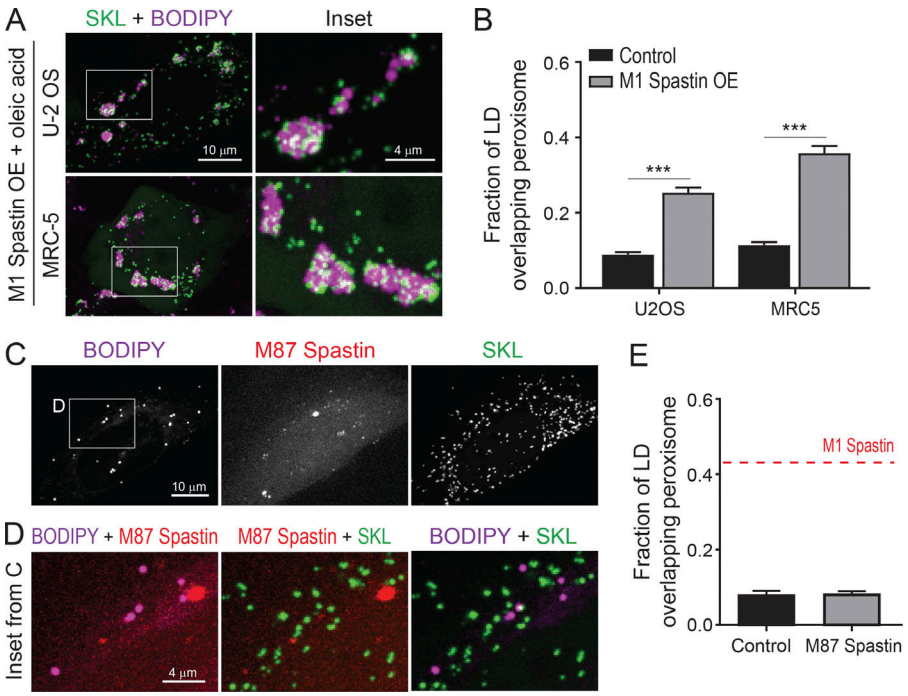


Figure S1. **M1 Spastin promotes LD–peroxisome contact formation in U2-OS and MRC-5 cells. (A)** Association between BODIPY-665/676–labeled LDs and mEmerald-SKL–labeled peroxisomes in U-2 OS (top) and MRC-5 (bottom) cells expressing mApple-M1 Spastin in the presence of 300 µM oleic acid treatment. Representative confocal MIP images are shown. OE, overexpression. **(B)** Fraction of LD overlapping peroxisome as described in A. Means ± SEM are shown (23–34 cells from three independent experiments). ***, P < 0.001. **(C and D)** Localization of BODIPY-665/676–labeled LDs and mEmerald-SKL–labeled peroxisomes in HeLa cells overexpressing mApple-M87 Spastin (C). Images from the inset (D). Representative confocal MIP images are shown. **(E)** Fraction of LD overlapping peroxisome in control and mApple-M87 Spastin-expressing HeLa cells. Red dashed line indicates the fraction of LD overlapping peroxisome as shown in Fig. 1 E. Means ± SEM are shown (20–25 cells from three independent experiments).

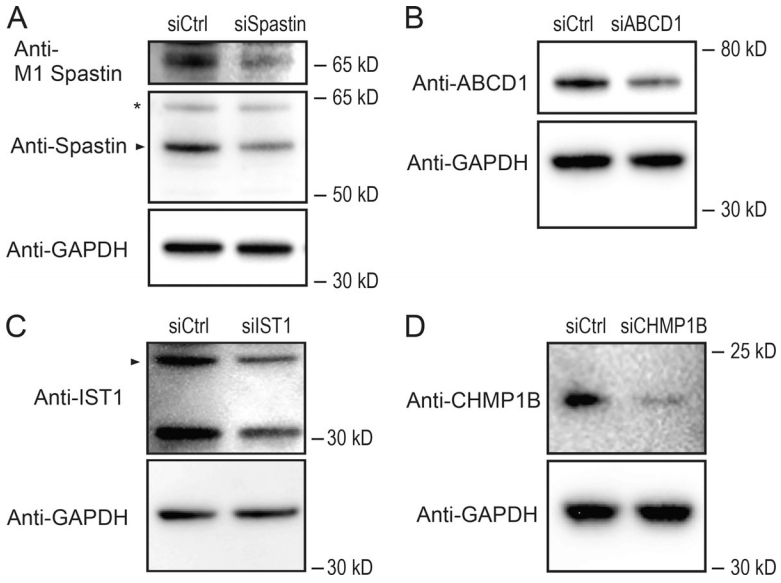


Figure S2. **Detecting endogenous protein levels following siRNA transfection. (A)** Endogenous Spastin protein levels detected by Western blotting using anti-M1 Spastin (top) and anti-Spastin (middle) antibodies in HeLa cells transfected with siCtrl or siSpastin. GAPDH protein levels serve as a loading control (bottom). Arrowhead and asterisk (middle panel) indicate M87 Spastin at expected molecular weight and a nonspecific band, respectively. **(B)** Endogenous ABCD1 protein levels detected by Western blotting using anti-ABCD1 antibody (top) in HeLa cells transfected with siCtrl or siABCD1. GAPDH protein levels serve as a loading control (bottom). **(C)** Endogenous IST1 protein levels detected by Western blotting using anti-IST1 antibody (top) in HeLa cells transfected with siCtrl or siIST1. Arrowhead (top panel) indicates IST1 at expected molecular weight. GAPDH protein levels serve as a loading control (bottom). **(D)** Endogenous CHMP1B protein levels detected by Western blotting using anti-CHMP1B antibody (top) in HeLa cells transfected with siCtrl or siCHMP1B. GAPDH protein levels serve as a loading control (bottom).

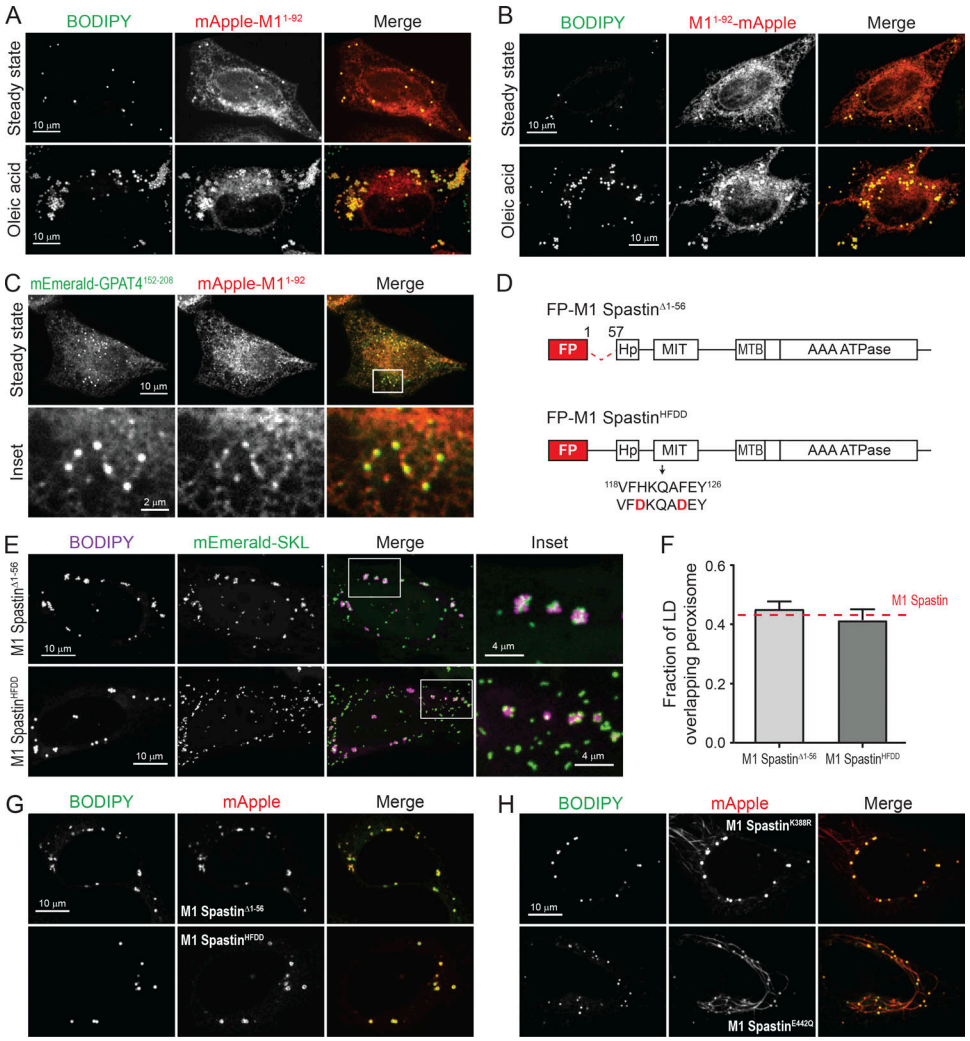


Figure S3. **Subcellular localization of M1 Spastin synthetic constructs and mutants respective to LDs and peroxisomes. (A and B)** Localization of mApple-M1$^{1–92}$ (A) and M1$^{1–92}$-mApple (B) and BODIPY-493/503–labeled LDs in HeLa cells in steady state (top) or following 300 µM oleic acid treatment for 16 h (bottom). Representative confocal images are shown. **(C)** mApple-M1$^{1–92}$ colocalizes with mEmerald-GPAT4$^{152–208}$ in steady-state HeLa cells. Representative confocal images are shown. **(D)** Diagrams of FP-M1 Spastin$^{\Delta1-56}$ and FP-M1 Spastin^{HFDD}. The red dashed line indicates domain deletion. Mutated residues are labeled in red. **(E)** The association between BODIPY-665/676–labeled LDs and mEmerald-SKL–labeled peroxisomes in HeLa cells expressing mApple-M1 Spastin$^{\Delta1–56}$ or mApple-M1 Spastin^{HFDD}. Representative confocal MIP images are shown. **(F)** Fraction of LD overlapping peroxisomes as described in E and in cells overexpressing mApple-M1 Spastin (red dashed line). Mean ± SEM is shown (28–34 cells from three independent experiments). **(G)** Colocalization of BODIPY-665/676–labeled LDs and mApple-M1 Spastin$^{\Delta1–56}$ or mApple-M1 Spastin^{HFDD} in HeLa cells. Representative confocal images are shown. **(H)** Colocalization of BODIPY-665/676–labeled LDs and mApple-M1 Spastin^{K388R} or mApple-M1 Spastin^{E442Q} in HeLa cells. Representative confocal images are shown.

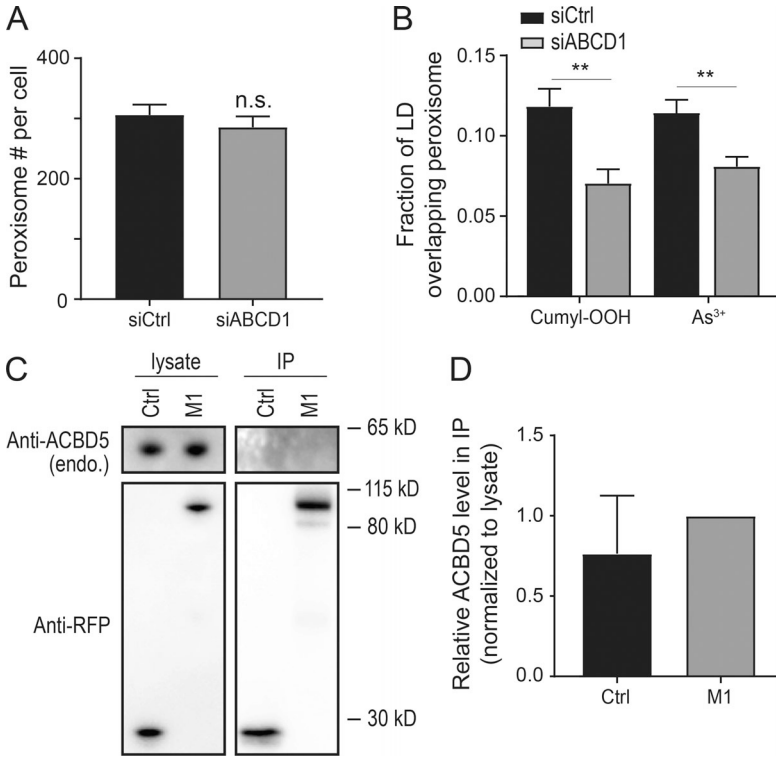
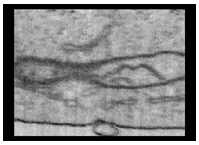


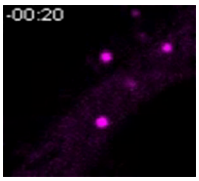
Figure S4. **ABCD1 supports LD–peroxisome contact formation. (A)** Number of mCherry-SKL–labeled peroxisome in HeLa cells transfected with siCtrl or siABCD1. Means ± SEM are shown (30–32 cells from four independent experiments). n.s., not significant. **(B)** Fraction of BODIPY-665/676–labeled LD overlapping mEmerald-SKL–labeled peroxisome in siCtrl and siABCD1-transfected HeLa cells treated with cumyl-OOH or with As^{3+} for 30 min. Means ± SEM are shown (25–27 cells from three independent experiments). ** $P < 0.01$. **(C)** IP of ACBD5 in HeLa cells transfected with mApple-C1 vector (Ctrl) and mApple-M1 Spastin (M1). Protein levels of endogenous ACBD5 and overexpressed constructs assessed by Western blotting. **(D)** Quantification of relative ACBD5 levels in the IP as described in C. The value of M1 is set as 1. Means ± SEM from five independent IP experiments are shown.

Table S1. **Oligonucleotide information**

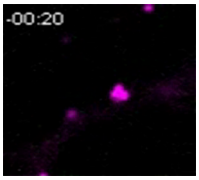
Name	Sequence (5′–3′)
M1 Spastin F	ATATGAATTCTCCGGGTGGAC (EcoRI)
M87 Spastin F	ATCGAATTCTATGGCAGCCAAGAGGAGCT (EcoRI)
Spastin R	ATCGGGATCCTTAAACAGTGGTATCTCCAAAGTC (BamHI)
M1 Spastin^{M87A} F	TTCTCCCGCGCCCTCGCGGCAGCCAAGAGGAGCTCC (mutagenesis)
M1 Spastin^{M87A} R	GCTCCTCTTGGCTGCCGCGAGGGCGCGGGAGAAGCG (mutagenesis)
FP-M1$^{1–92}$ F	Same as M1 Spastin F
FP-M1$^{1–92}$ R	ATCGGGATCCTTAGCTCCTCTTGGCTGCCATG (BamHI)
M1$^{1–92}$-FP F	ATCGAGATCTCACCATGAATTCTCCGGGTGGAC (BglII)
M1$^{1–92}$-FP R	ATCGAAGCTTGCTCCTCTTGGCTGCCATG (HindIII)
2xFKBP F	ATCGGCTAGCACCATGGGTGCTCTCGAGGGAGTGCA (NheI)
2xFKBP R	ATCGACCGGTAGTGCACTGCCTCCAGCTGA (AgeI)
M1 Spastin^{K388R} F	CCACCTGGGAATGGGAGGACAATGCTGGCTAAAGCAG (mutagenesis)
M1 Spastin^{K388R} R	TTTAGCCAGCATTGTCCTCCCATTCCCAGGTGGAC (mutagenesis)
M1 Spastin^{E442Q} F	ATAATTTTTATAGATCAAGTTGATAGCCTTTTGTGT (mutagenesis)
M1 Spastin^{E442Q} R	AAAGGCTATCAACTTGATCTATAAAAATTATAGAAGGTTGAA (mutagenesis)
M1 Spastin$^{\Delta1–56}$ F	ATCGGAATTCTCTGTTTGTAGGCTTCGCG (EcoRI)
M1 Spastin$^{\Delta1–56}$ R	Same as Spastin R
M1 Spastin^{HFDD} F	CGCGTCCGAGTCTTCGACAAACAGGCCGACGAGTACATCTCCATTGCCC (mutagenesis)
M1 Spastin^{HFDD} R	AATGGAGATGTACTCGTCGGCCTGTTTGTCGAAGACTCGGACGCGCTCG (mutagenesis)
M1$^{1–92}$-197–328 (PXI) F	GCAGCCAAGAGGAGCAAGATGCAACCAGTTTTGCC (In-Fusion cloning)
M1$^{1–92}$-197–328 (PXI) R	TTATCTAGATCCGGTGGATCCTTACTATATAAGGTTAGCAAGGTTGCT (In-Fusion cloning)
FP-197–328 (PXI) F	ATCGGAATTCTAAGATGCAACCAGTTTTGCC (EcoRI)
FP-197–328 (PXI) R	ATCGGGATCCTTACTATATAAGGTTAGCAAGGTTGCT (BamH)
GPAT4$^{152–208}$ F	ATCGAATTCTAACTTCCAGTACATCAGCCTT (EcoRI)
GPAT4$^{152–208}$ R	ATCGGGATCCTTAGAACTCCTTAAACCTCCCATT (BamHI)
IST1 F	ATCGGTCGACCTGGGCTCTGGATTTAAAG (SalI)
IST1 R	ATCGGGTACCCTATGTTTTCTTTTTCAGCTCTTC (KpnI)
CHMP1B F	ATCGGTCGACTCTAACATGGAGAAACACCTG (SalI)
CHMP1B R	ATCGGGTACCTCACACTTGATCCCGAAGG (KpnI)
siControl F	TAATACGACTCACTATAGGGTTTGCCTTCCTGTTTTTGCT
siControl R	TAATACGACTCACTATAGGGTTGCCGGGAAGCTAGAGTAA
siSpastin F	TAATACGACTCACTATAGGGTTTAAGGCCTTGCCTTGATG
siSpastin R	TAATACGACTCACTATAGGGTTTAAGGCCTTGCCTTGATG
siABCD1 F	TAATACGACTCACTATAGGGTGGAGCCCACAAAGTCTACC
siABCD1 R	TAATACGACTCACTATAGGGGGCTTGGTCAGGTTGGAGTA
siIST1 F	TAATACGACTCACTATAGGGCCAAAATCCTGGTGGAGAGA
siIST1 R	TAATACGACTCACTATAGGGTCCGGGAAAGATCATCAAAG
siCHMP1B F	TAATACGACTCACTATAGGGCGGCCAAAGAACTGAGTAG
siCHMP1B R	TAATACGACTCACTATAGGGTCACACTTGATCCCGAAGG



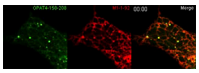
Video 1. **Volumetric LD–peroxisome contacts driven by M1 Spastin.** Animation of 3D rendering in Fig. 1 H. Video begins by sectioning through the z axis, then repeating with the annotated LDs (red) and peroxisomes (cyan), and once more with the 3D rendering of the LD and peroxisome segmentations. The 3D renderings are rocked about the x axis. A 360° rotation with all the encompassing organelles in the volume follows (green, mitochondria; blue, ER; gray, plasma membrane; purple, endosome; orange, multivesicular body). Bounding box is 1.2 × 1.2 × 1.6 µm. Video frame rate, 25 frames per second.



Video 2. **Dynamic association between LDs and peroxisomes.** Dynamic association of LDs (magenta) and peroxisomes (green) as described in Fig. 1 I. Acquisition rate, 20 s per frame; video frame rate, 5 frames per second.



Video 3. **Stable association between LDs and peroxisomes mediated by M1 Spastin overexpression.** Stable association of LDs (magenta) and peroxisomes (green) in M1 Spastin-overexpressing HeLa cells as described in Fig. 1 I. Acquisition rate, 20 s per frame; video frame rate, 5 frames per second.



Video 4. **Dynamic association between $M1^{1–92}$ and $GPAT4^{158–208}$.** Dynamic association of $M1^{1–92}$ (red) and $GPAT4^{158–208}$ (green) as described in Fig. S3 C. Confocal images of $M1^{1–92}$ and $GPAT4^{158–208}$ were acquired simultaneously. Acquisition rate, 5 s per frame; video frame rate, 6 frames per second.