

Supplementary Information for

A SPLICS reporter reveals α -synuclein regulation of lysosome-mitochondria contacts which affects TFEB nuclear translocation

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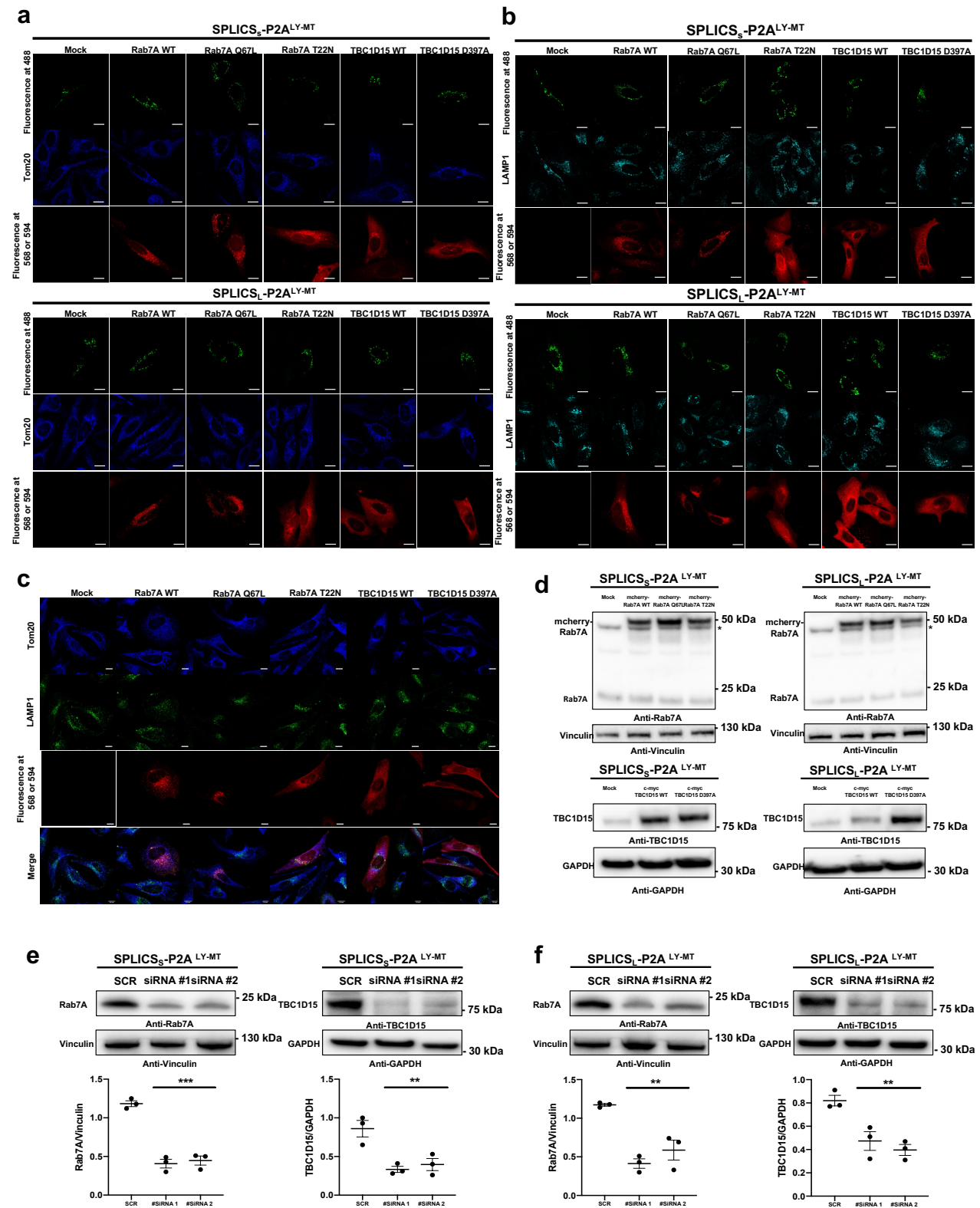
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These authors contributed equally

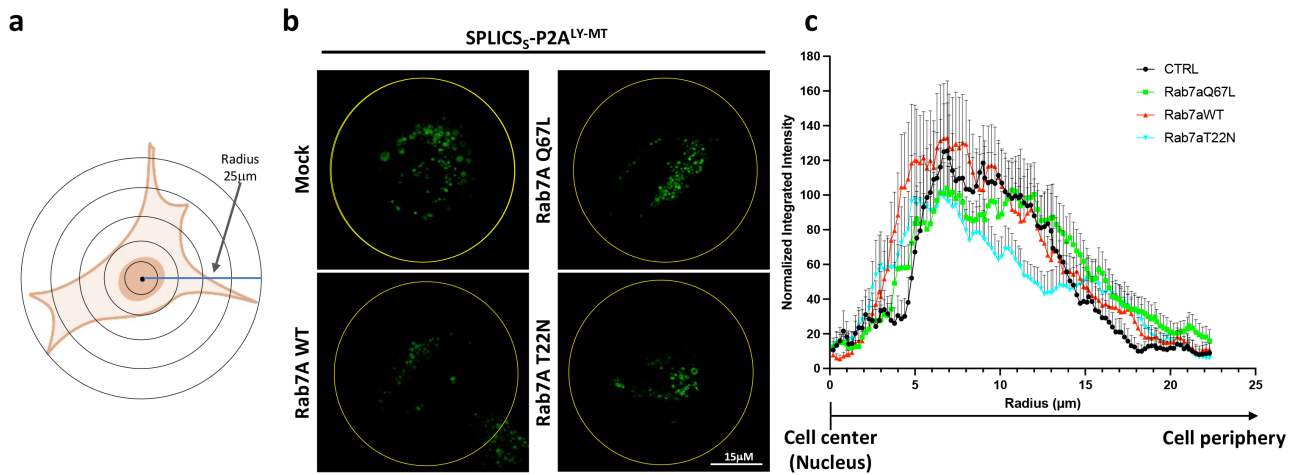
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Supplementary Figure 1



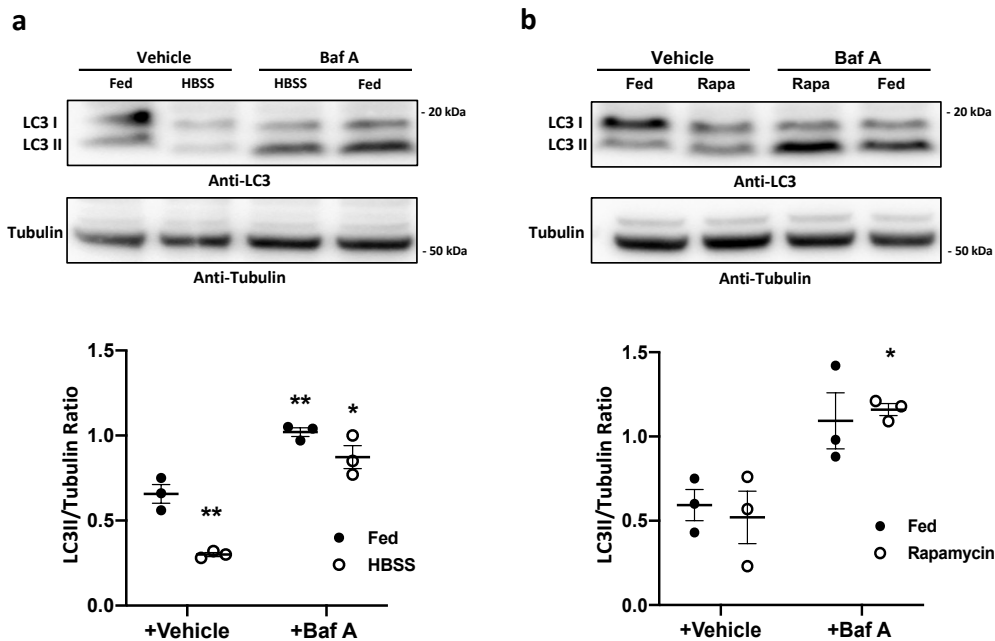
Supplementary Figure 1 Characterization of the Ly-Mt reporter. **a-b** Representative Z-projection images of HeLa cells transfected with SPLICS_{S/L}- P2A^{LY-MT} alone or plus Rab7A WT or Rab7A Q67L or Rab7A T22N or TBC1D15 WT or TBC1D15 D397A. Single channel of merged image reported in Fig. 2. SPLICS_{S/L}- P2A^{LY-MT} were represented by fluorescence “dots” upon excitation at 488 nm, mcherry -Rab7A WT or -Rab7A Q67L or -Rab7A T22N were represented upon excitation at 568 nm while c-myc -TBC1D15 WT or -TBC1D15 D397A by anti-cmyc upon excitation at 594 nm, mitochondria were detected by anti-Tom20 upon excitation at 405 nm. **b** Representative Z-projection images of HeLa cells transfected with SPLICS_{S/L}- P2A^{LY-MT} alone or plus Rab7A WT or Rab7A Q67L or Rab7A T22N or TBC1D15 WT or TBC1D15 D397A. Single channel of merged image reported in Fig. 2. SPLICS_{S/L}- P2A^{LY-MT} were represented by fluorescence “dots” upon excitation at 488 nm, mcherry -Rab7A WT or -Rab7A Q67L or -Rab7A T22N were represented upon excitation at 568 nm while c-myc -TBC1D15 WT or -TBC1D15 D397A by anti-cmyc upon excitation at 594 nm, lysosomes were detected by anti-LAMP1 upon excitation at 405 nm. **c** Representative Z-projection images of HeLa cells transfected with Rab7A WT or Rab7A Q67L or Rab7A T22N or TBC1D15 WT or TBC1D15 D397A. Mcherry -Rab7A WT or -Rab7A Q67L or -Rab7A T22N were represented upon excitation at 568 nm while c-myc -TBC1D15 WT or -TBC1D15 D397A by anti-cmyc upon excitation at 594 nm, mitochondria were detected by anti-Tom20 upon excitation at 405 nm and lysosomes were detected by anti-LAMP1 upon excitation at 488 nm. **d** Expression levels of overexpressed mcherry -Rab7A WT or c-myc -TBC1D15 WT were analyzed by Western blotting with anti-Rab7A or anti-TBC1D15 in HeLa cells transfected with SPLICS_{S/L}- P2A^{LY-MT} alone or plus Rab7A WT or Rab7A Q67L or Rab7A T22N or TBC1D15 WT or TBC1D15 D397A. Equal amount of total loaded lysate was verified by incubation with anti- vinculin or anti-GAPDH antibodies. * is aspecific band. **e-f** Expression levels of endogenous Rab7A WT or TBC1D15 WT were analyzed by Western blotting with anti-Rab7A or anti-TBC1D15 in HeLa cells transfected with SPLICS_{S/L}- P2A^{LY-MT} plus scramble or siRNA Rab7A #1 or siRNA Rab7A #2 or siRNA TBC1D15 #1 or siRNA TBC1D15 #2. Equal amount of total loaded lysate was verified by incubation with anti- vinculin or anti-GAPDH antibodies, mean $\pm$ SEM. Scale bar 10 μ m. The data were obtained from three independent transfections. (**p $\leq$ 0.01, ***p $\leq$ 0.001 one-way ANOVA)

Supplementary Figure 2



Supplementary Figure 2 Radial analysis of the SPLICS signal. **a.** Cartoon showing an example of the radial fluorescence analysis of cellular zones from the center of the nucleus to the cell periphery. **b.** Radial analysis (profile plot of normalized integrated intensities around concentric circles as a function of 25 μm distance from the center of the cell nucleus) of HeLa cells expressing SPLICS_S-P2A^{LY-MT} either alone or in combination with the indicated Rab7 expression plasmids was performed as in ⁹¹ by using the ImageJ plugin Radial_Profile.class. The yellow circle has a radius of 25 μm . The normalized integrated intensity of the SPLICS signal over the radius in μm , mean $\pm$ SEM from the center of the nucleus is shown in **c**. The data were obtained from two independent transfections.

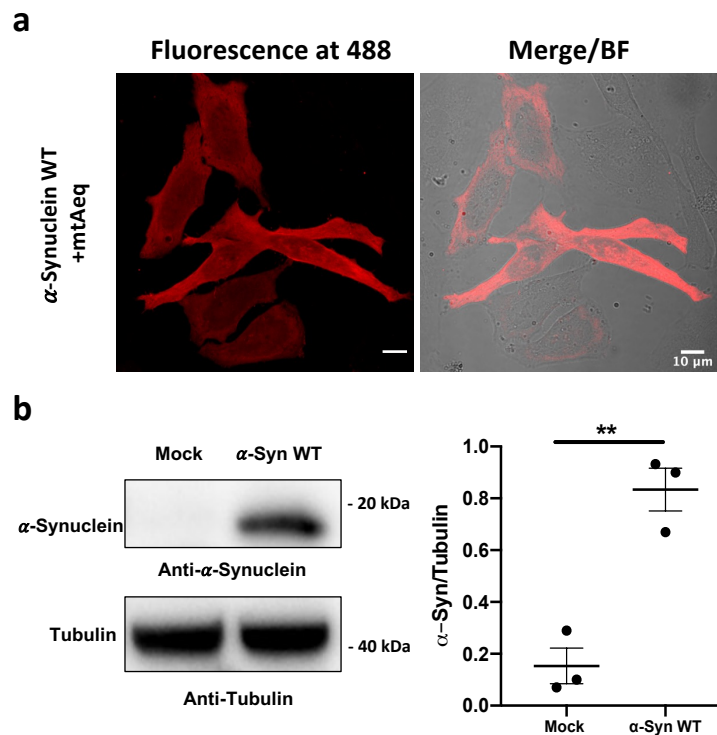
Supplementary Figure 3



Supplementary Figure 3 Evaluation of starvation induction. **a** Expression levels of endogenous LC3I/LC3II were analyzed by Western blotting with anti-LC3 in HeLa cells untreated (Fed) or incubated in HBSS for 3h, in presence of Bafilomycin A (Baf A) or DMSO (Vehicle). Equal amount of total loaded lysate was verified by incubation with anti- β tubulin antibody. The graphs represent the densitometric quantification of LC3II signal over the tubulin signal (LC3II/tubulin Ratio), mean $\pm$ SEM. **b** Expression levels of endogenous LC3I/LC3II were analyzed by Western blotting with anti-LC3 in HeLa cells untreated (Fed) or incubated with 1 μ M rapamycin for 5h, in presence of Bafilomycin A (Baf A) or DMSO (Vehicle). Equal amount of total loaded lysate was verified by incubation with anti- β tubulin antibody. The graph represents the densitometric quantification of LC3II signal over the tubulin signal (LC3II/tubulin Ratio), mean $\pm$ SEM.

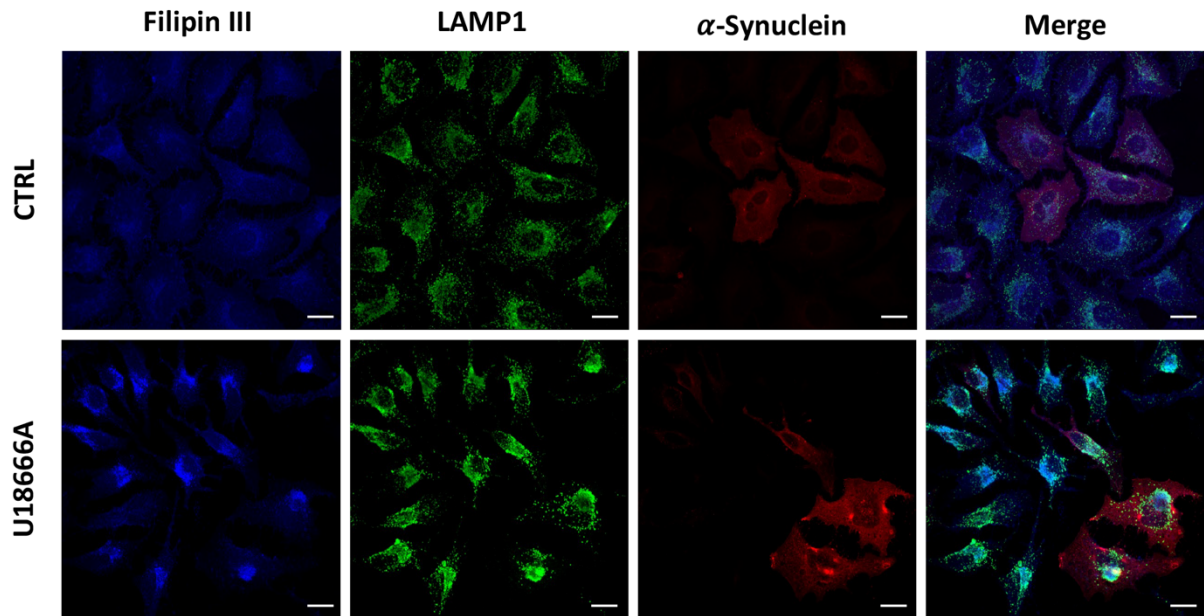
The data were obtained from three independent transfections. (* $p \leq 0.05$, ** $p \leq 0.01$ unpaired two-tailed t-test)

Supplementary Figure 4



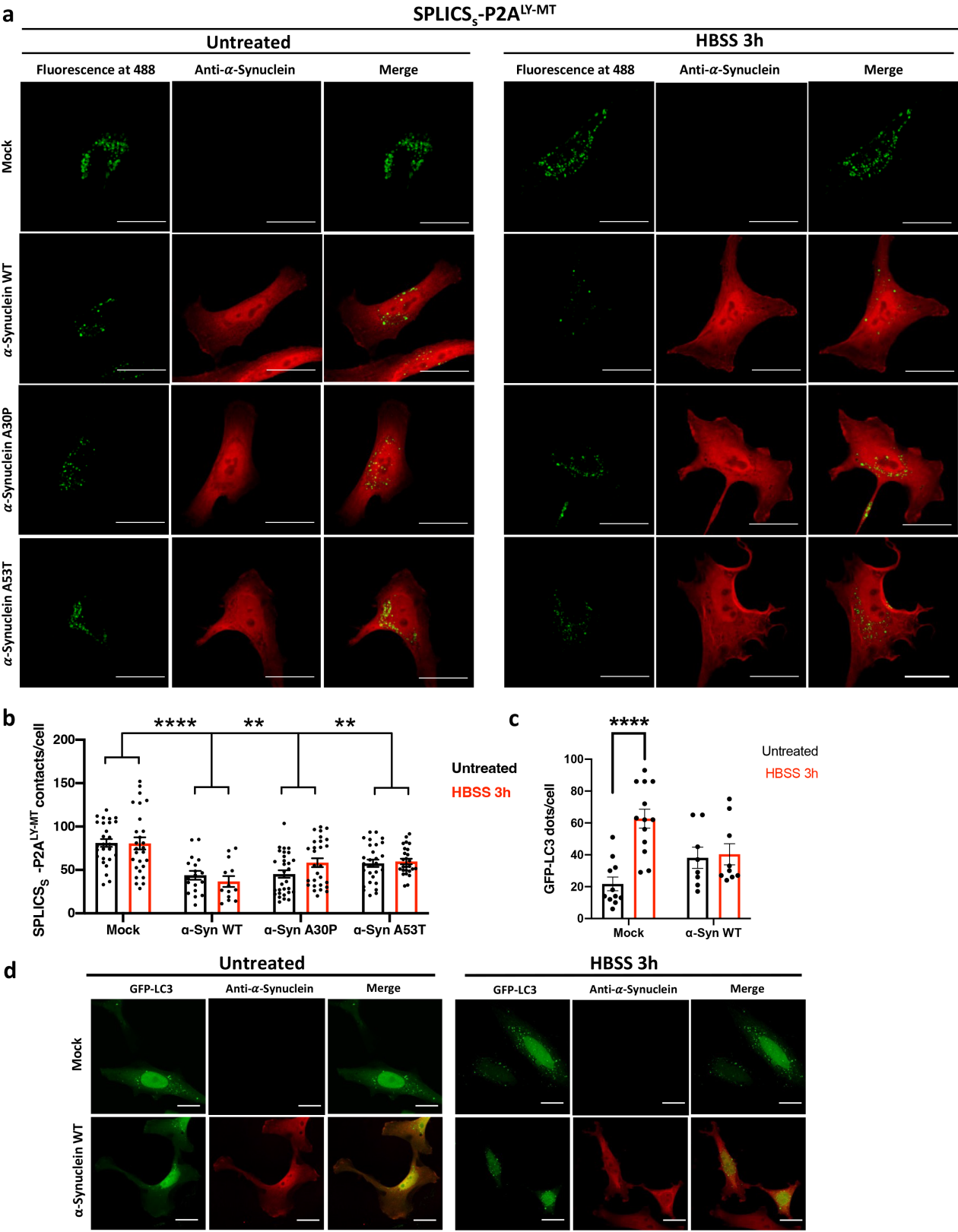
Supplementary Figure 4 Evaluation of α -synuclein overexpression. **a** Representative Z-projection images of HeLa cells co-transfected with mtAeqwt and α -Syn WT. α -synuclein was detected by anti- α -synuclein upon excitation at 633 nm (red) and merged with bright fields (BF). **b** Expression levels of overexpressed α -synuclein was analyzed by Western blotting with anti- α -synuclein in HeLa cells transfected with mtAeqwt alone (Mock) or plus α -Syn WT. Equal amount of total loaded lysate was verified by incubation with anti- β tubulin antibody. The graph represents the densitometric quantification of α -Syn/tubulin signal, mean $\pm$ SEM. Scale bar 10 μ m. The data were obtained from three independent transfections. (**p = 0.01 unpaired two-tailed t-test)

Supplementary Figure 5



Supplementary Figure 5 α -synuclein overexpression does not impinge on U1866A-induced cholesterol accumulation. Representative Immunofluorescence of control HeLa cells and cells transfected with α -Syn WT. Filipin III staining was detected upon excitation at 380nm (blue), LAMP1 was detected by anti-LAMP1 upon excitation at 488nm (green), α -synuclein was detected by anti- α -synuclein upon excitation at 633 nm (red). Scale bar 10 μ m. The data were obtained from three independent transfections.

Supplementary Figure 6

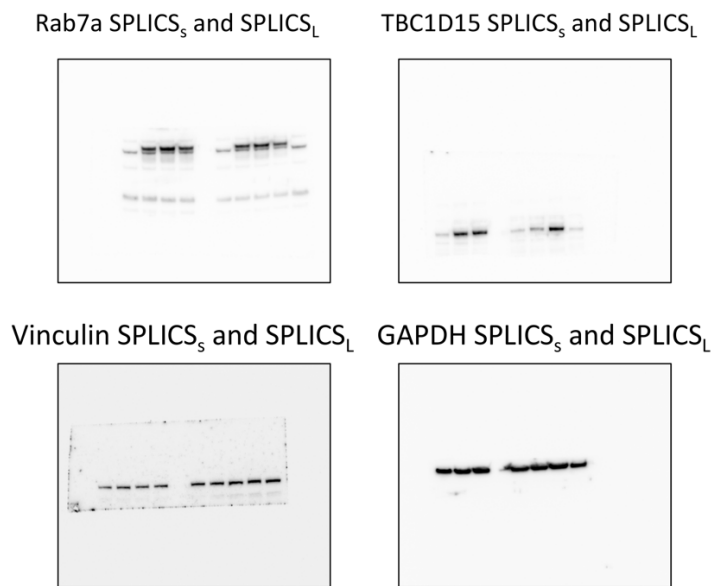


Supplementary Figure 6 α -Synuclein overexpression counteracts Ly-Mt short interactions under starvation. **a** Representative Z-projection images of HeLa cells transfected with SPLICS_S-

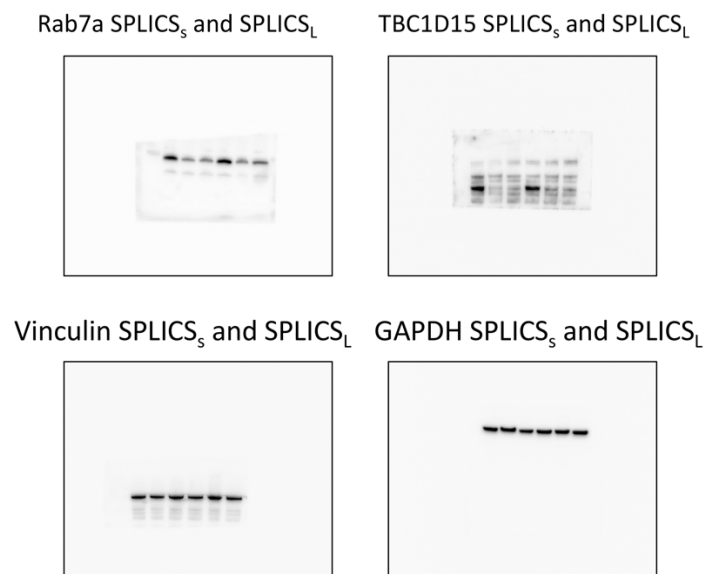
P2A^{LY-MT} (Mock) or co-transfected with SPLICSS- P2A^{LY-MT} and α -Syn WT or α -Syn A30P or α -Syn A53T in untreated condition or under HBSS treatment. SPLICSS- P2A^{LY-MT} was represented by fluorescent “dots” upon excitation at 488 nm (green) and α -synuclein was detected by anti- α -synuclein upon excitation at 633 nm (red). Merge panels are shown. **b** Quantification of short LY-MT contacts (Mean $\pm$ SEM: Untreated: SPLICSS-P2A^{LY-MT} + Mock 81.05 ± 4.54 n=28; SPLICSS-P2A^{LY-MT} + WT 43.83 ± 5.05 n=18 ****p ≤ 0.0001 ; SPLICSS-P2A^{LY-MT} + A30P 45.26 ± 4.27 n=31**p ≤ 0.01 ; SPLICSS- P2A^{LY-MT} + A53T 57.59 ± 4.25 n=28 **p ≤ 0.01 . HBSS treated: SPLICSS- P2A^{LY-MT} + Mock 80.57 ± 7.06 n=27; SPLICSS-P2A^{LY-MT} + WT 38.80 ± 7.17 n=15 ****p ≤ 0.0001 ; SPLICSS-P2A^{LY-MT} + A30P 58.39 ± 5.09 n=28 **p ≤ 0.01 ; SPLICSS-P2A^{LY-MT} + A53T 59.71 ± 3.19 n=26 **p ≤ 0.01). **c** Quantification of GFP-LC3 dots per cell in untreated or HBSS treated cells co-transfected with pcDNA3 (Mock) or α -Syn WT and GFP-LC3 (Mean $\pm$ SEM: Mock Untreated 21.72 ± 4.28 n=11 vs HBSS treated 62.69 ± 5.95 n=13 ****p ≤ 0.0001 ; α -Syn WT Untreated 38.12 ± 6.63 n=8 vs HBSS treated 40.33 ± 6.61 n=9). **d** Representative Z-projection images of HeLa cells untreated or HBSS treated overexpressing pcDNA3 (Mock) or α -SYN WT in presence of GFP-LC3. The SPLICSS- P2A^{LY-MT} contacts were quantified from the 3D rendering of a complete Z-stack, mean $\pm$ SEM. Scale bar 10 μ m. The data were obtained from three independent transfections. (**p ≤ 0.01 , ****p ≤ 0.0001 two-way ANOVA)

Full Scan for Supplementary Information

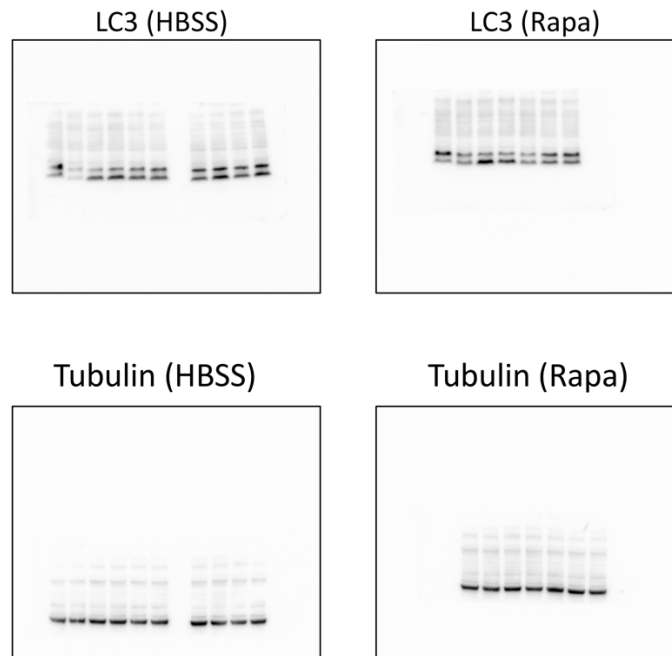
Rab7a and TBC1D15 Overexpression Supplementary Figure 1d



Rab7a and TBC1D15 SiRNA Supplementary Figure 1e and f



LC3
Supplementary Figure 3**a** and **b**



Synuclein
Supplementary Figure 4**b**

