Compromised function of the ESCRT pathway promotes endolysosomal escape of tau seeds and propagation of tau aggregation

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Supporting Information:

Figure S1

Legends for:
Tables S1, S2
Movies S1 – S5.

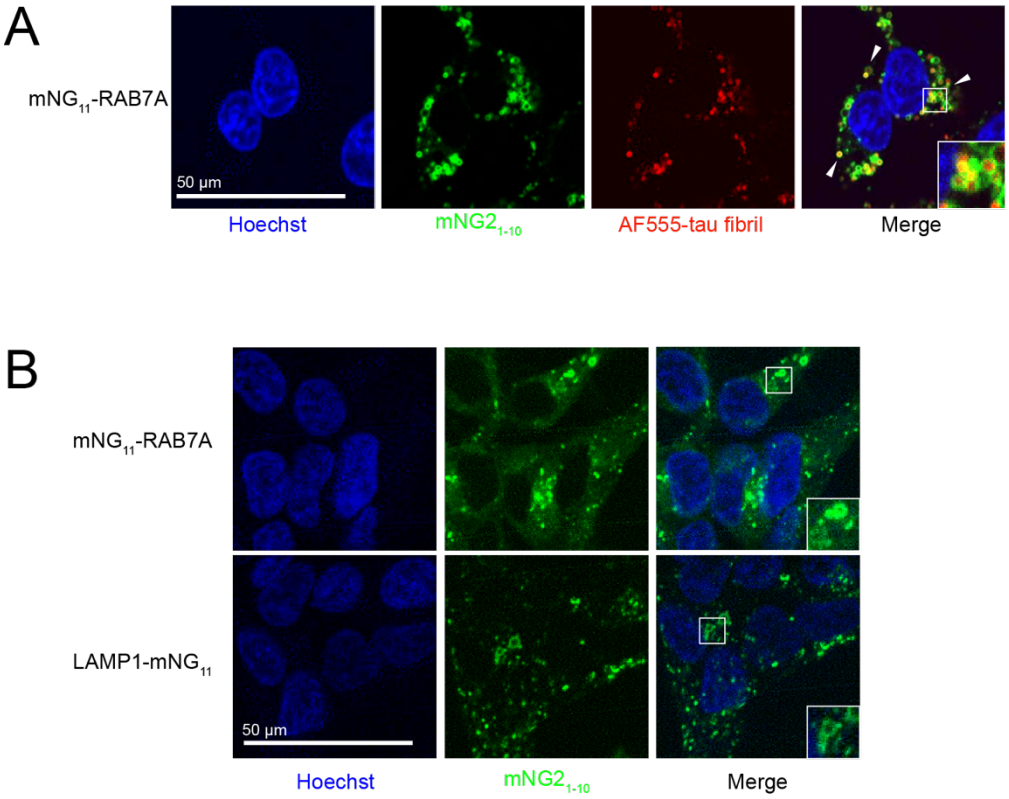

Figure. S1 – Endogenous tagging of endolysosomal compartments
(**A**) Representative fluorescence microscopy images of CRISPRi-HEK293T cells with RAB7A-mNG$_{11}$ endogenously labeled with the split-mNeonGreen system. Cells were treated with AF555-tau fibrils for 22 hours. (**B**) Representative fluorescence microscopy images of HEK293T cells with mNG$_{11}$-RAB7A (top) or LAMP1-mNG$_{11}$ (bottom) endogenously labeled with the split-mNeonGreen system.

Legends for Supplementary Tables and Movies

Table S1 – Gene epsilon and p values for CRISPRi screens
Excel spreadsheet containing the combined analyzed results for the CRISPRi screens performed with the Proteostasis-focused sgRNA libraries. The counts for each library screen were analyzed using our MaGecK-iNC pipeline (see materials and methods). (**Tab 1**) The columns list the gene index, epsilon (phenotype, where negative valudes indicate less aggregation, positive values indicate more aggregation, p-value, product (epsilon*–log10 p-value), and gene name. NTC indicate "quasi-genes" composed of random sets of 5 non-targeting negative control sgRNAs.

Table S2 – sgRNA sequences and validation
(**Tabs 1-3**) List of sgRNA targets and accompanying protospacers used for CRISPRi screens. The columns list the target and protospacer. (Tab 1) Endolysosome/Autophagy (Tab 2) Chaperones/Co-chaperonse (Tab 3) Ubiquitin Proteasome System. (**Tab 1,2**) Validation of target gene knockdown by CRISPRi. (Tab 4) Columns list sgRNA activity, which is expressed as percent knockdown, standard error from n=3 technical replicates, and sgRNA oligos used in experiments for single sgRNA analysis. The columns list the sgRNA, % knockdown efficiency, standard error from n=3 technical replicates, forward oligo, and reverse oligos (used for generating lentiviral sgRNA expression plasmids. (Tab 4) Oligonucleotides used for RT-qPCR analysis. The columns list the gene target, forward, and reverse primer.

Movie S1 – Time-lapse of tau aggregate formation in HEK293T cells expressing tau.K18(LM)-Clover2 and transduced with non-targeting control sgRNA
Time-lapse microscopy of cell entry of AF555-tau fibrils and resulting aggregation of the cytosolic tau-clover2 construct. CRISPRi-HEK293T cells expressing tau.K18(LM)-clover2 transduced with a non-targeting control. Each frame represents 20 minute intervals and is played at 5 frames per second; movie starts with the addition of fibrils.

Movie S2 - Time-lapse of tau aggregate formation in HEK293T cells expressing tau.K18(LM)-Clover2 and transduced with CHMP6 sgRNA
Time-lapse microscopy of cell entry of AF555-tau fibrils and resulting aggregation of the cytosolic tau-clover2 construct. CRISPRi-HEK293T cells expressing tau.K18(LM)-clover2 transduced with a CHMP6 sgRNA. Each frame represents 20 minute intervals and is played at 5 frames per second; movie starts with the addition of fibrils.

Movie S3 – Time-lapse of AF555-tau fibrils in cells expressing tagged mNG_{11}-RAB7A
Time-lapse microscopy of partial co-localization of AF555-tau fibrils with RAB7A tagged vesicles. HEK293T cells with tagged mNG_{11}-RAB7A were treated with AF555-tau fibrils for 22 hours before movies were acquired. Each frame represents 2.4 second intervals and is played at 5 frames per second.

Movie S4 – Time-lapse of AF647-tau fibrils in cells expressing tagged mNG_{11}-LAMP1 and a non-targeting sgRNA
Time-lapse microscopy of partial co-localization of AF647-tau fibrils with LAMP1 tagged vesicles. HEK293T cells with tagged mNG_{11}-LAMP1 were treated with AF647-tau fibrils for 48 hours before movies were acquired. Images were acquired at 15 minute intervals.

Movie S5 – Time-lapse of AF647-tau fibrils in cells expressing tagged mNG_{11}-LAMP1 and an sgRNA targeting CHMP6
Time-lapse microscopy of partial co-localization of AF647-tau fibrils with LAMP1 tagged vesicles. HEK293T cells with tagged mNG_{11}-LAMP1 were treated with AF647-tau fibrils for 48 hours before movies were acquired. Images were acquired at 15 minute intervals.