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Supplemental Information

Fluoxetine ameliorates mucopolysaccharidosis type IIIA

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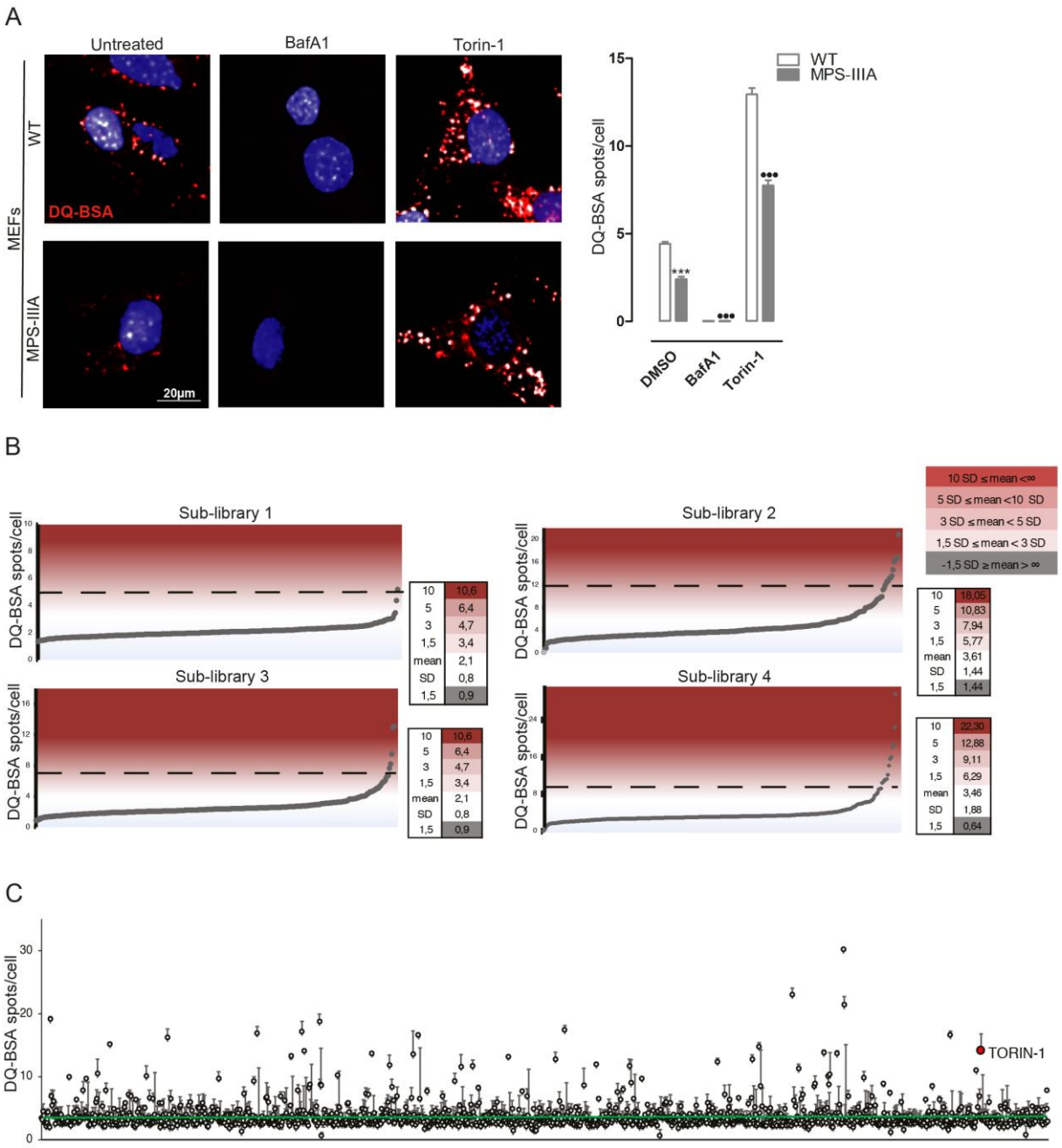
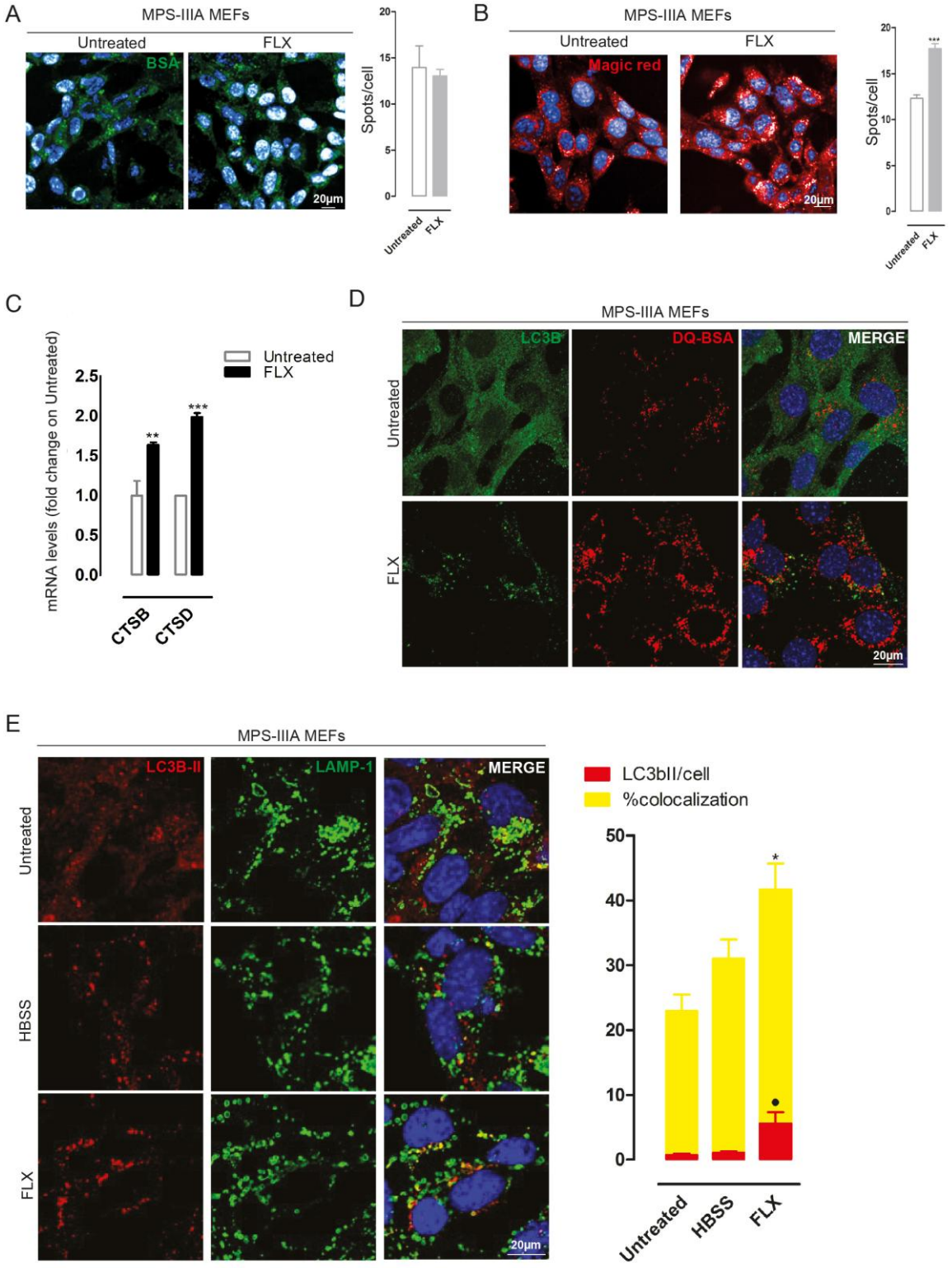


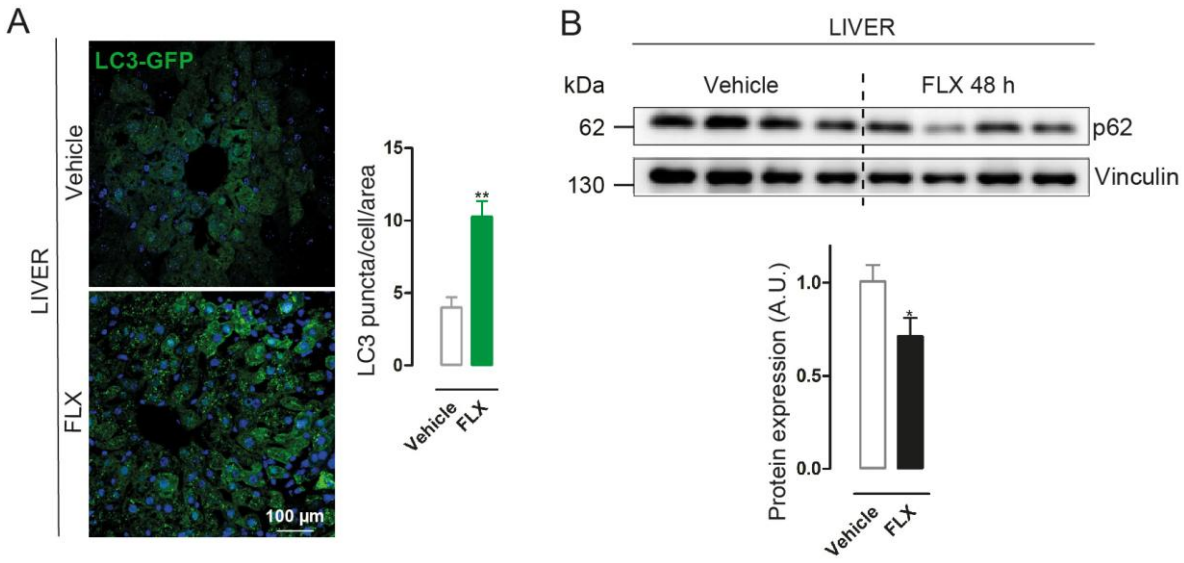
Supplementary figure 1. A) Setting of the DQ-BSA assay in WT and MPS-IIIA MEFs untreated, treated for 3 hours with Bafilomycin (BafA1) or Torin-1. The plot shows "DQ-BSA spots per cell" values ± SEM of n > 100 cells pooled from 3 independent experiments; ANOVA test ***p < 0.001 vs WT; •••p < 0.01 vs DMSO. B) Heatmaps showing the analysis of hit selection from the four sub-libraries tested. C) The plot summarizes the activity of

compounds in the DQ-BSA assay in MPS-IIIA MEFs; the green line represents the media of DMSO response and the red spot corresponds to the positive control Torin-1.

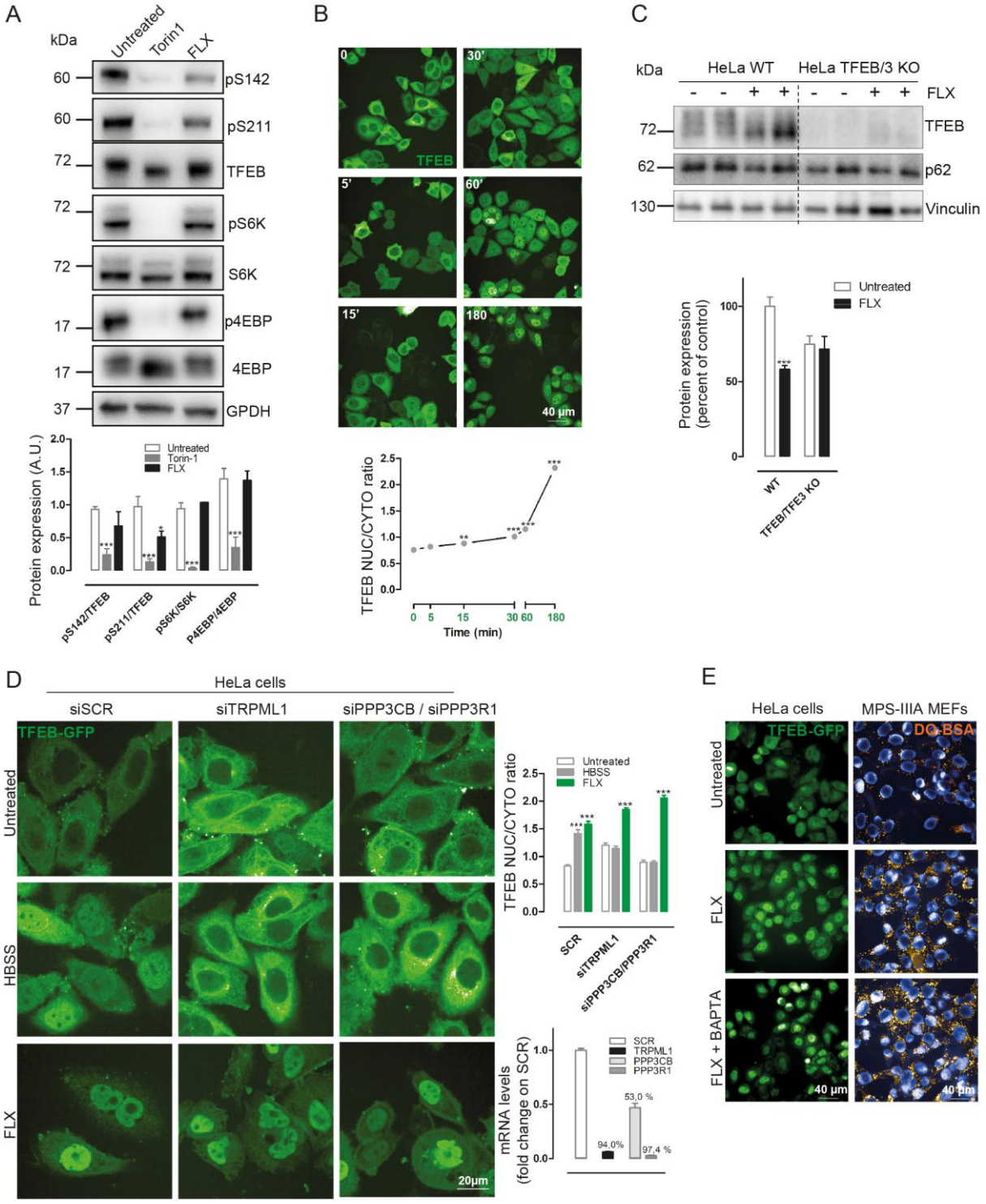


Supplementary figure 2. A) Representative HC-images of untreated and FLX-treated MPS-IIIA MEFs incubated with BSA Alexa Fluor 488 conjugated. Plot shows the spots per cell

values ± SEM of n > 100 cells pooled from 3 independent experiments B) Representative HC-images of Magic red assay in untreated and FLX-treated MPS-IIIA MEFs; the plot show the spots per cell ± SEM of n > 100 cells pooled from 3 independent experiments T-test ***p < 0.001 vs Untreated. C) Quantitative RT-qPCR showing the mRNA levels of Cathepsin B and D (CTSB, CTSD) genes in MPS-IIIA MEFS untreated or upon 24 h of FLX treatment. The plot shows the mean values ± SEM n=4 samples per condition. T test **p < 0,01 vs untreated; ***p < 0.001 vs untreated. D) Representative confocal images showing the co-localization of the endolysosomal compartment, marked with DQ-BSA probe, with autophagosomes marked using LC3B antibodies upon 3 hours of treatment with FLX in MPS-IIIA MEFs. E) Representative confocal images of MPS-IIIA MEFs untreated, starved (HBSS) or treated with FLX showing LC3b puncta formation and its co-localization with lysosomal compartment marked with LAMP-1. The plot shows both LC3B puncta per cell ± SEM and percentage of co-localization of n > 100 cells pooled from 3 independent experiments *p < 0.05 vs Untreated/ % colocalization; •p < 0.05 vs Untreated/ LC3BII/cell.

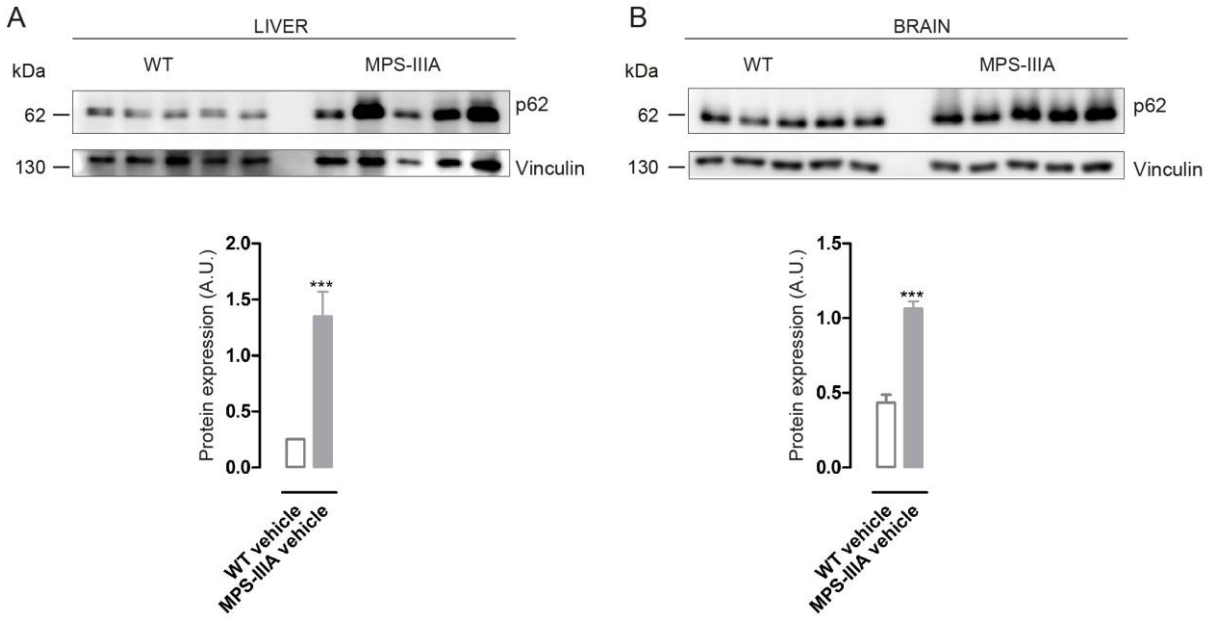


Supplementary figure 3. A) Representative confocal images of liver section from GFP-LC3 mice treated with vehicle or FLX; the plot shows the number of LC3B puncta per cell ± SEM of the vehicle-treated and FLX-treated mice (n=4 per group) T-test **p < 0.01 vs vehicle. B) Representative image of immunoblot analysis of p62 in liver protein extracts from vehicle-treated and FLX-treated GFP-LC3 mice. The plot shows the densitometry of p62 band normalized to Vinculin in vehicle-treated and FLX-treated mice (n=4 per group) as mean values ± SEM.; T-test *p < 0.05 vs vehicle.

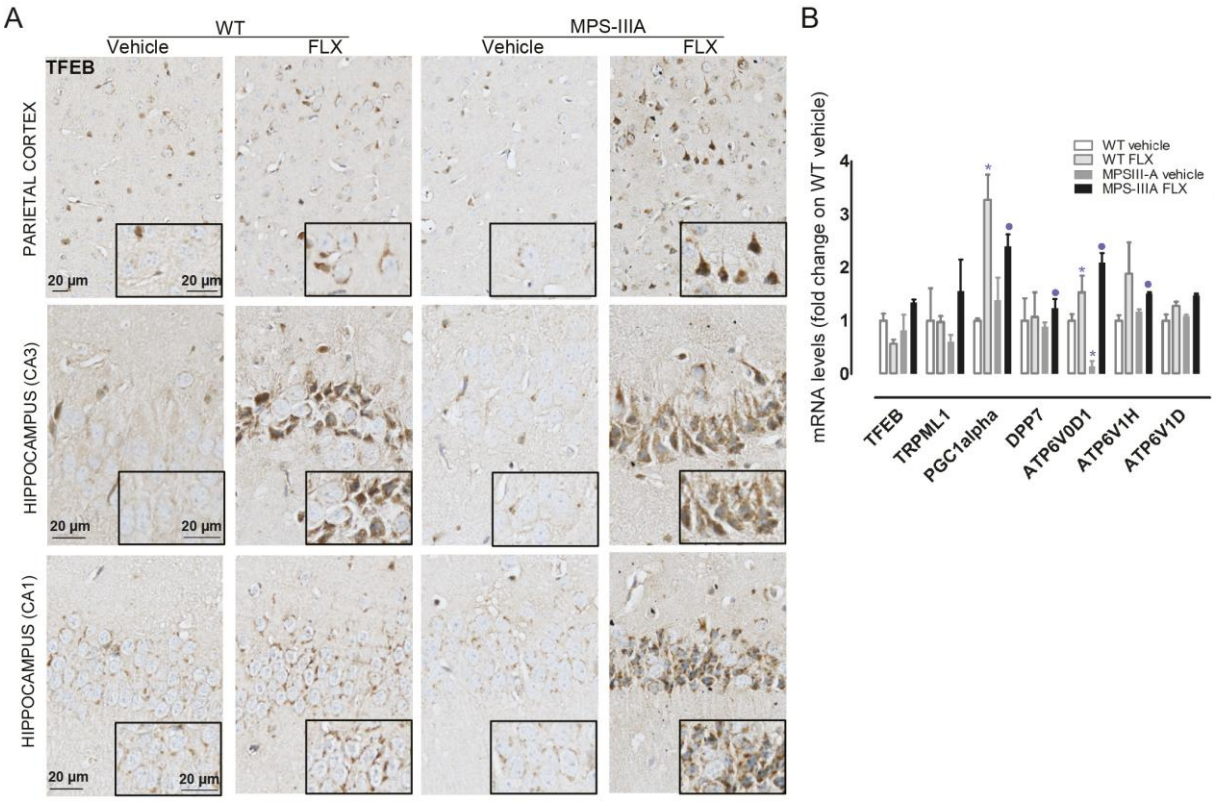


Supplementary figure 4. A) IB analysis of endogenous phosphorylation of p70 S6 Kinase (PS6k), p4EBP and of TFEB (pS142 and pS211) upon FLX treatment for 3 hours in HeLa

cells stably expressing TFEB-GFP plasmid. The plot shows the densitometry of bands normalized to GPDH as mean values ± SEM. of n=3 lysates per condition pooled from 3 independent experiments; ANOVA test *p < 0.05 vs Untreated; ***p < 0.001 vs Untreated . B) Representative images of HeLa cells stably expressing TFEB-GFP in untreated, starved (HBSS), or FLX-treated conditions at different time points (5-180 min); the plot shows the kinetics of TFEB nuclear translocation expressed as the ratio of nuclear to cytoplasmic fraction ± SEM. of n > 100 cells pooled from 3 independent experiments; ANOVA test **p < 0.01 vs time 0; *** p < 0.001 vs time 0. C) Representative image of immunoblot analysis of endogenous TFEB and p62 in HeLa WT and TFEB/TFE3 KO untreated or treated with FLX for 3 hours. The plot shows the densitometry of the p62 band normalized to Vinculin as mean values ± SEM. of n=6 lysates per condition pooled from 3 independent experiments; ANOVA test ***p < 0.001 vs Untreated; D) E) Representative high content images of HeLa stably expressing TFEB-GFP treated with siRNAs targeting TRPML1 channel or Calcineurin (siRNAs targeting PPP3CB and PPP3R1 subunits were used in together) or a scrambled sequence (SCR) in untreated, starved (HBSS) and Fluoxetine (FLX) treatment. The above plot reports the ratio between nuclear and cytosolic TFEB. Values are means ± SEM. of n > 1000 cells pooled from 3 independent experiments; ANOVA test ***p < 0.001 vs Untreated. The below plot shows the mRNA expression levels of TRPML1, PPP3CB, and PPP3R1 in silenced cells compared to SCR (and the relative percentage of silencing). F) Fluoxetine activity upon calcium chelation. On the left, representative high content images of HeLa stably expressing TFEB-GFP untreated, treated with Fluoxetine alone or in combination with BAPTA-AM. On right, representative HC- images of DQ-BSA assay in MPS-IIIA MEFS untreated, treated with Fluoxetine alone or in combination with BAPTA-AM.



Supplementary Figure 5. A) Representative images of immunoblot analysis of p62 in liver protein extracts from MPS-IIIA mice compared to age-matched WT. The plot show densitometry of P62 on Vinculin (n=5) as mean values ± SEM; T-test *p < 0.05 vs WT. B) Representative images of immunoblot analysis of p62 in brain protein extracts from MPS-IIIA mice compared to age-matched WT. The plot show densitometry of P62 on Vinculin (n=5) as mean values ± SEM; T-test *p < 0.05 vs WT.



Supplementary Figure 6. A) Representative immunostaining of TFEB in parietal cortex and hippocampus (CA3, CA1) sections from FLX treated and vehicle-treated- WT and MPS-IIIA mice. Nuclei were stained with hematoxylin II (blue). B) Graph of quantitative RT-qPCR showing the mRNA levels of a subset of TFEB target genes in brain samples from vehicle and FLX WT and MPS-IIIA mice (n=5). The data in the graphs are mean values ± SEM T-test *p < 0.05 vs WT vehicle: •p < 0.05 vs MPS-IIIA vehicle