Supplementary Materials for

Neuronal Mitochondrial Disaggregase CLPB Ameliorates Huntington's Disease Pathology in Mice

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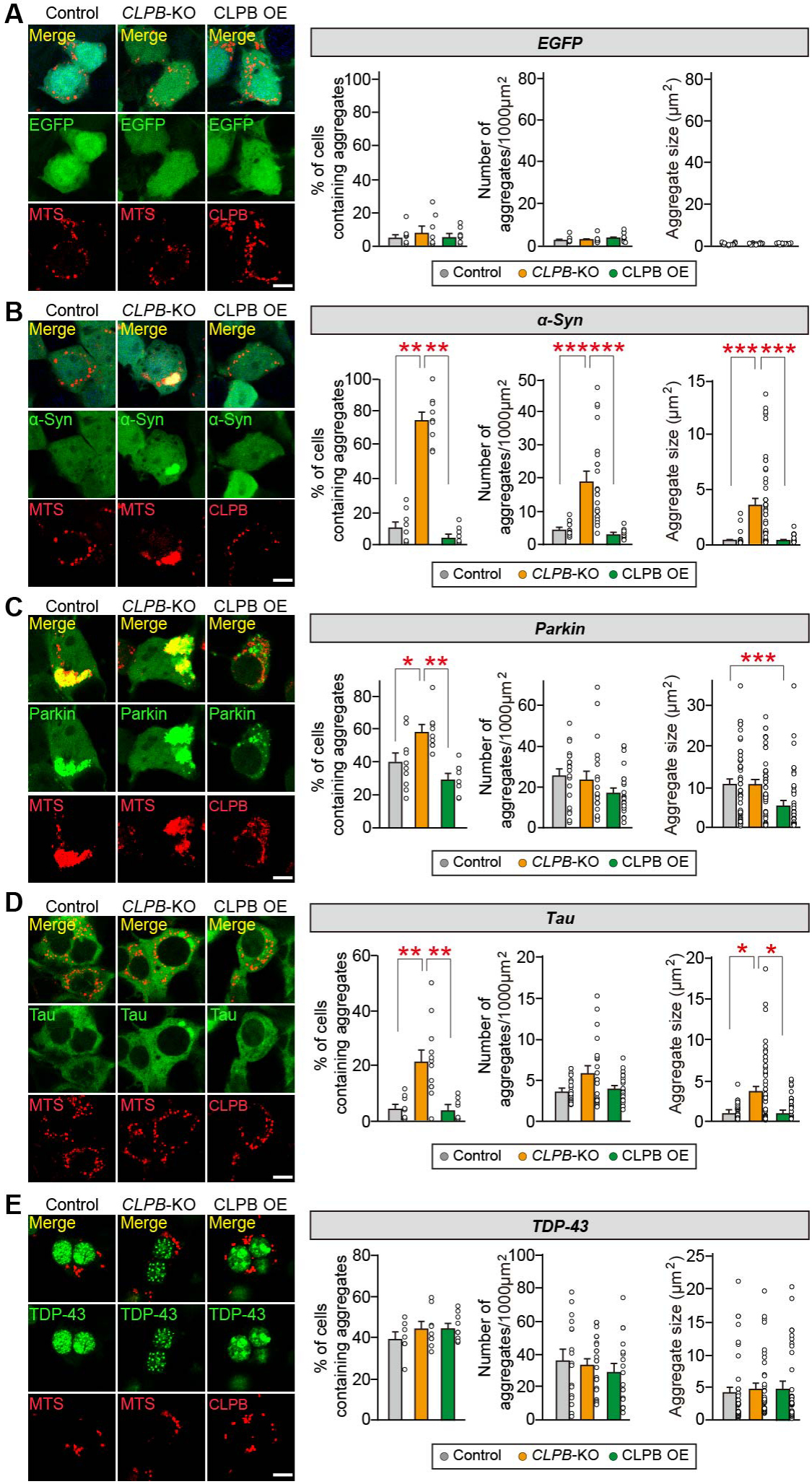
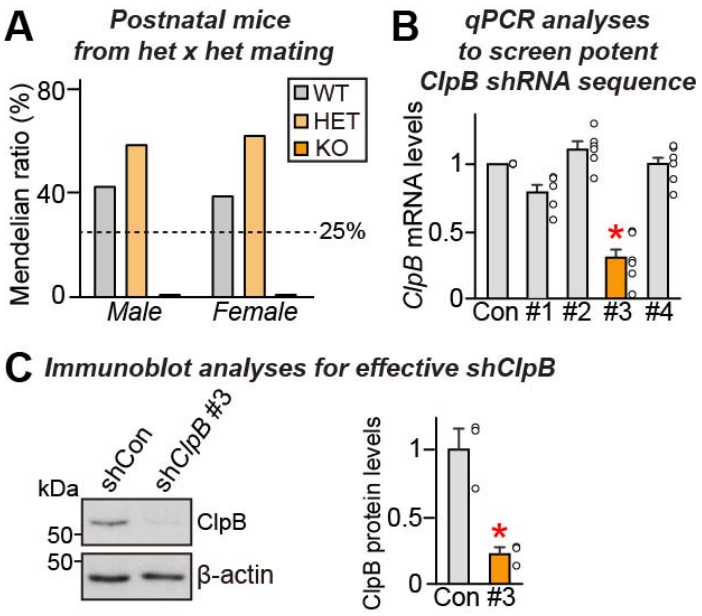
This file includes:
Supplementary figure S1–S6


A
Control
CLPB-KO
CLPB OE
Merge
EGFP
MTS
CLPB
EGFP
% of cells containing aggregates
Number of aggregates/1000μm²
Aggregate size (μm²)
Control
CLPB-KO
CLPB OE
B
α-Syn
C
Parkin
D
Tau
E
TDP-43

Supplementary Fig 1. *CLPB* deletion induces aberrant aggregation of physiological forms of disease-associated proteins

HEK293T WT or *CLPB* KO cells were transfected with EGFP alone (**A**) or EGFP-tagged α-synuclein (**B**), Parkin (**C**), Tau (**D**), or TDP-43 (**E**), together with either MTS (mitochondrial targeting sequence)-mCherry or FLAG-tagged CLPB. Cells were subjected to immunofluorescent imaging to visualize EGFP (green), mCherry (red), or FLAG (red). Scale bars: 10 μm. Quantification of the aggregate-containing cell percentage (**left**), aggregate density (**middle**), and average aggregate size (**right**). Data are presented as means ± SEMs. Statistical significance was assessed using the Kruskal–Wallis test followed by Dunn's *post-hoc* test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

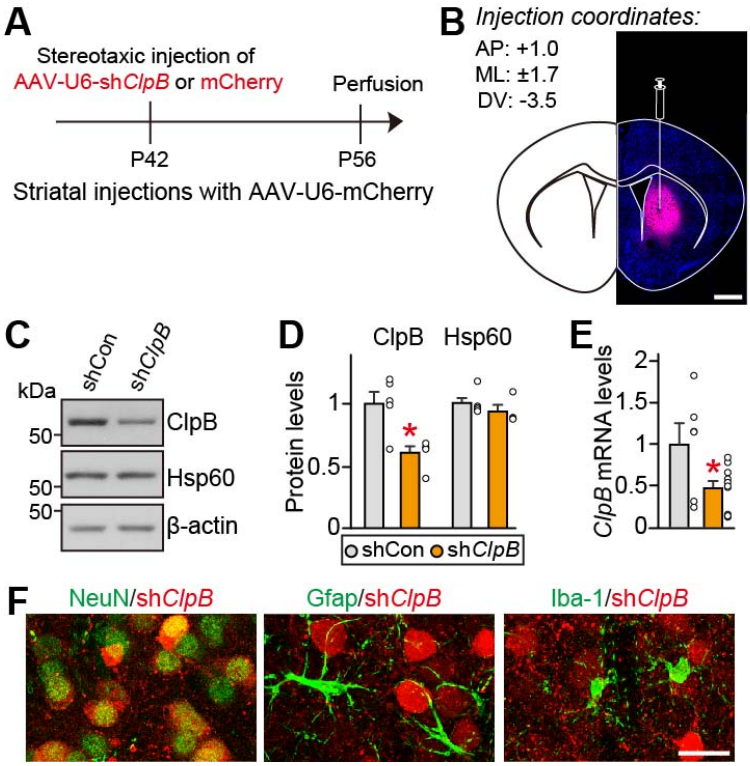


Supplementary Fig 2. Validation of a *ClpB* shRNA construct in cultured neurons

A Mendelian distribution of WT, heterozygous (*ClpB*$^{+/-}$), and homozygous (*ClpB*$^{-/-}$) postnatal mice.

B *ClpB* mRNA levels were measured by qRT-PCR in cultured cortical neurons infected at DIV3 with lentiviruses expressing four *ClpB*-targeting shRNAs (#1–#4). mRNA was prepared at DIV10. Note that shRNA#3 was the most effective at reducing *ClpB* mRNA expression.

C Cultured cortical neurons were infected at DIV3 with lentiviruses expressing *ClpB* shRNA#3, and protein lysates were collected at DIV10 for immunoblotting using the indicated antibodies. The uncropped blot images are provided in **Figure S6**. Quantification of ClpB levels normalized to the control is shown on the right.



Supplementary Fig 3. Validation of a *ClpB* shRNA construct in the mouse brain

A Experimental schematics.

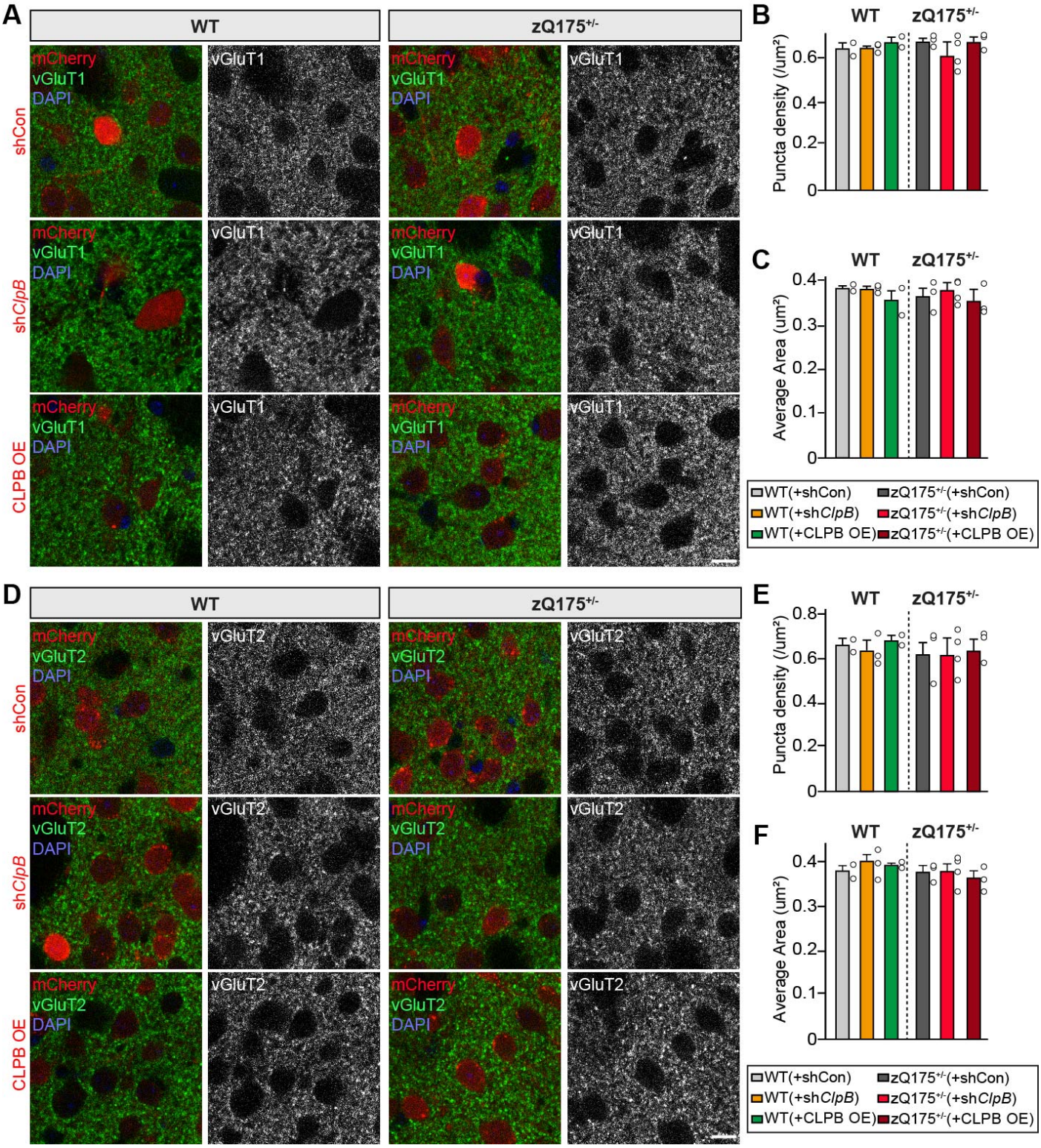
B Representative image showing the AAV injection site in the striatum. Scale bar: 500 μm.

C Representative immunoblot images of striatal tissues collected 2 weeks after stereotactic injection of AAV-sh*ClpB*#3 into WT mice. The uncropped blot images are provided in **Figure S6**.

D Quantification of ClpB and HSP60 protein levels from striatal tissues following AAV-sh*ClpB* injection. Data are presented as means ± SEMs from 3–5 independent experiments.

E Quantification of *ClpB* mRNA levels in striatal tissues following AAV-sh*ClpB* injection. Data are presented as means ± SEMs from 5–7 independent experiments.

F Representative images showing expression of NeuN, Gfap, Iba-1 (green), and/or sh*ClpB* (red) in the striatum following AAV-sh*ClpB* injection. Scale bar: 20 μm (applies to all images).



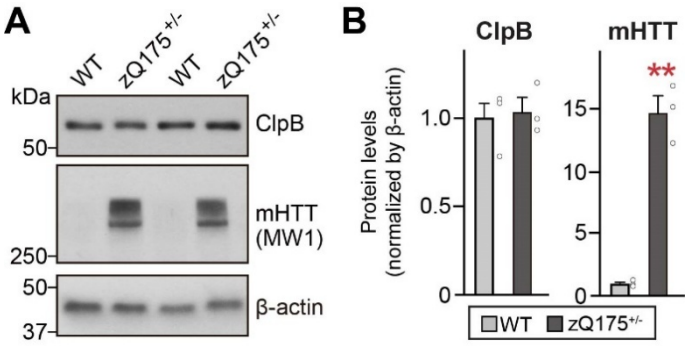
Supplementary Fig 4. zQ175 mice do not exhibit altered excitatory synaptic structure

A Representative images of the striatum 2 weeks after AAV injection into WT or zQ175$^{+/-}$ mice, immunostained for the corticostriatal excitatory synapse marker, vGluT1. Scale bar: 20 μm.

B and **C** Quantification of the density (**B**) and average size (**C**) of vGluT1^{+} synaptic puncta. Data are presented as means ± SEMs.

D Representative images of the striatum 2 weeks after AAV injection into WT or zQ175$^{+/-}$ mice, immunostained for the thalamostriatal excitatory synapse marker, vGluT2. Scale bar: 20 μm.

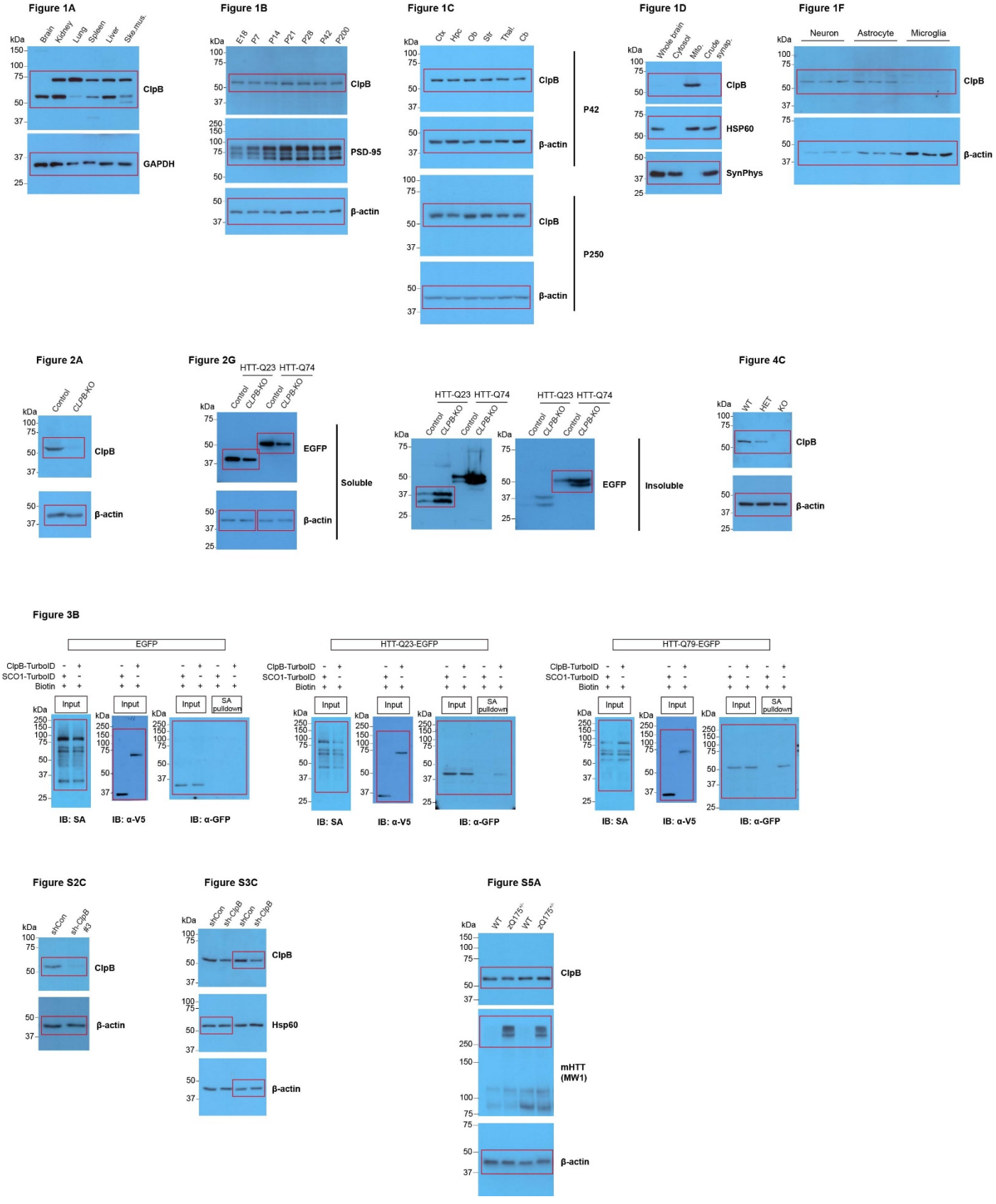
E and **F** Quantification of the density (**E**) and average size (**F**) of vGluT2-positive synaptic puncta. Data are presented as means ± SEMs.



Supplementary Fig. 5. ClpB expression remains stable in aged zQ175 mice

A Representative immunoblots showing protein levels of ClpB and mutant HTT (mHTT, detected by the MW1 antibody) in striatal lysates from aged zQ175 +/– and wild-type (WT) mice. β-actin was used as a loading control. The uncropped blot images are provided in **Figure S6**.

B Quantification of ClpB and mHTT protein levels normalized to β-actin. Data are presented as means ± SEMs (n = 3 mice per group; Student's t-test).



Supplementary Fig 6. Uncropped scanned images of representative immunoblots in this study