Article title: Corticospinal propagation of full-length TDP-43 toxicity drives brain-to-muscle pathology

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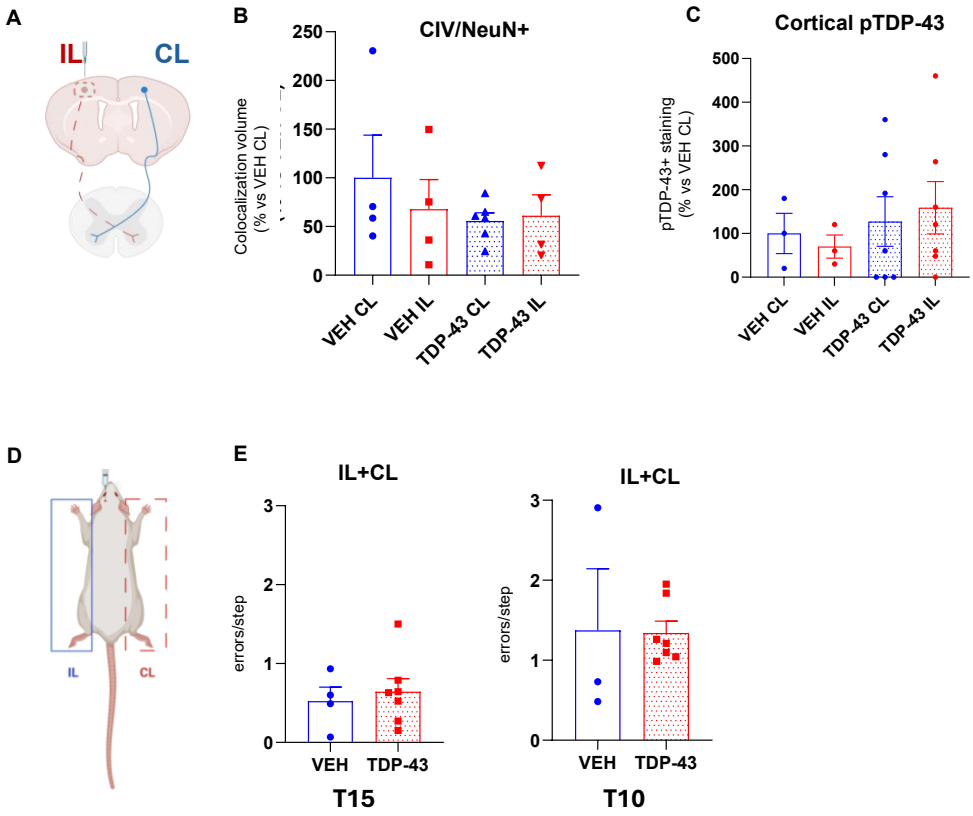
Supplementary Fig. 1



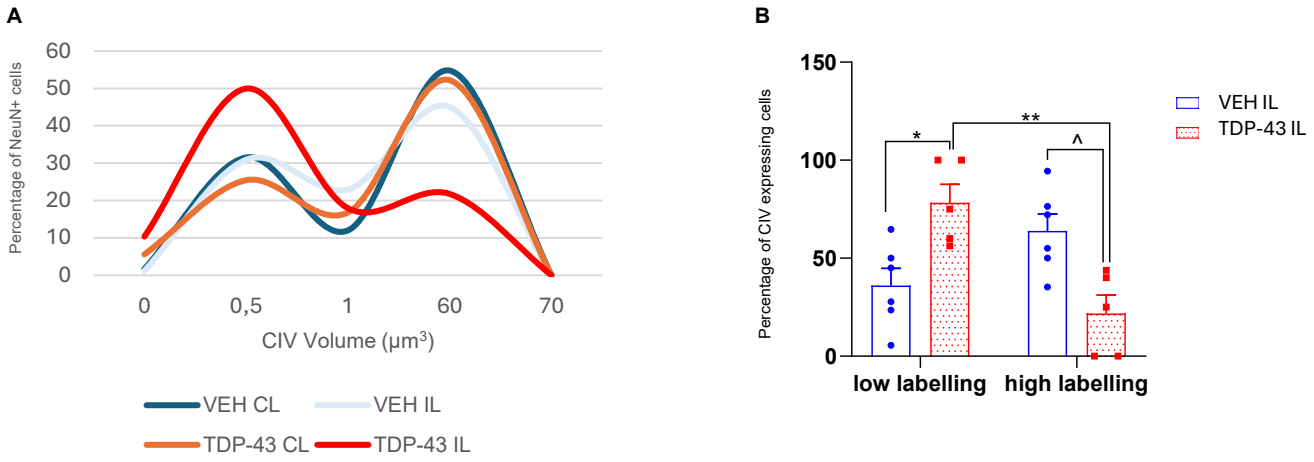
Supplementary Fig. 1 Mitochondria CIV, pTDP-43, and motor behavior are not altered one-month after FL TDP-43 infusion. A) Schematic representation of the intracortical infusion side of FL TDP-43 (IL, ipsilateral, dashed red line) and the corresponding contralateral motor cortex (CL; blue line). **B)** Total volume occupied by CIV colocalized with NeuN^{+} cells in the primary motor cortex. **C)** pTDP-43-positive staining in the primary motor cortex, expressed as a percentage of the VEH CL group. **D)** Schematic representation of the paws ipsilateral (IL, blue rectangle) and contralateral (CL, dashed red rectangle) to FL TDP-43 infusion side. **E)** Sensorimotor deficits were measured using the challenging beam walk test. T15 and T10 correspond to the 15 mm and 10 mm beams. Graphs show the cumulative number of errors/step by both ipsilateral and contralateral paws. Values represent mean ± SEM. FL = full length; VEH = vehicle

Supplementary Table 1

GENE	SPECIES	PRIMER SEQUENCE (5' → 3')
MT2A	Rat	GCAAAGAGGCTTCGGACAAG GGCTAGGTTCCTACGTTGTT
Hspb8	Rat	TCTCCAGAGGGTCTGCTCAT GCAGGTGACTTCCTGGTTGT
Bag3	Rat	TTCACTGCCCATCACCCATC CCTGGCTTGCTTTCTGGTTT
Hspa8	Rat	TGGAACTATTGCTGGCCTCA GAGCACATTCCTTTCAGCCC
Sqstm1	Rat	CAGAGCAGATGAAGAAGATAGCC GACAAATGCGTCCAGTCGTC
Stub1	Rat	TGGAGAGTTATGATGAGGCCA CGAAGGGCACTAGGAATATCATC
Gapdh	Rat	GGCTGCCTTCTCTTGTGACA TGAACTTGCCGTGGGTAGAG
Rpl13a	Rat	CGAAAGGTGGTGGTTGTACG GGTTGGTGTTCATCCGCTTT

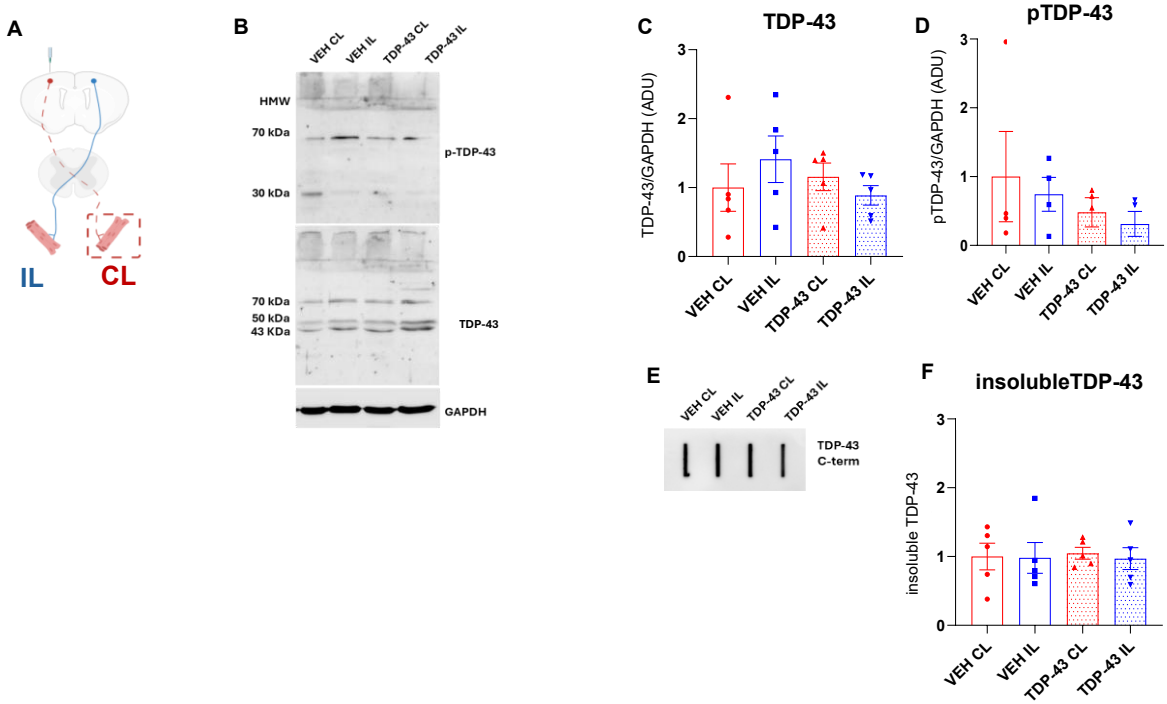
Supplementary Table 1 RT-qPCR primers list

Supplementary Fig. 2



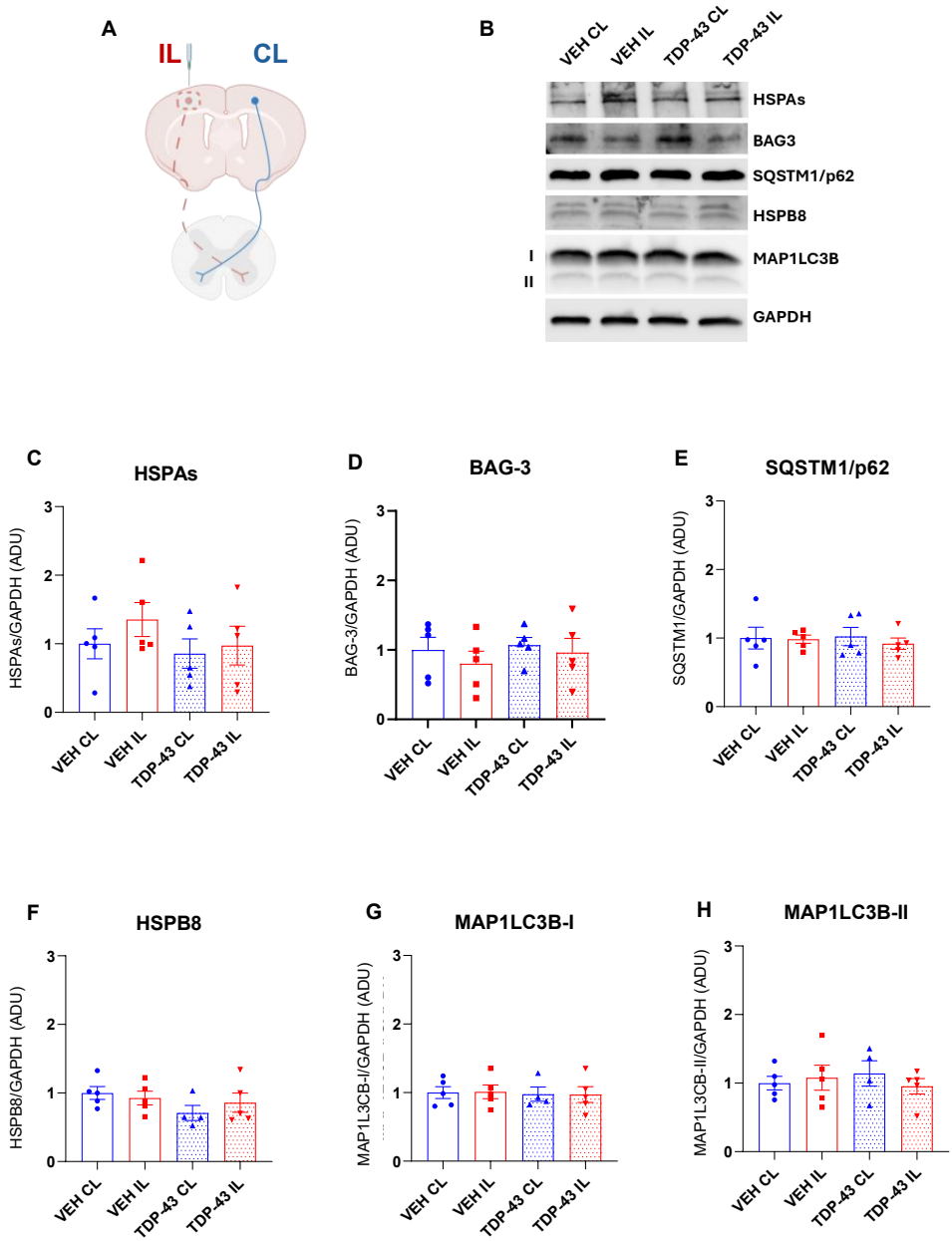
Supplementary Fig. 2 FL TDP-43 infusion drives changes in CIV expression in neurons of the primary motor cortex. A) Frequency distribution showing CIV colocalization within NeuN+ cells in the primary motor cortex. **B)** NeuN+ cells were categorized into two sub-populations, based on CIV expression, namely low labelling and high labelling. Values represent the mean ± SEM (one-way ANOVA followed by Tukey's post hoc test) *^$p < 0.05$, **$p < 0.001$. Note that dotted red columns in the graphs refer to the TDP-43-infused side. All measures were made four months after FL TDP-43 infusion. VEH = vehicle

Supplementary Fig. 3



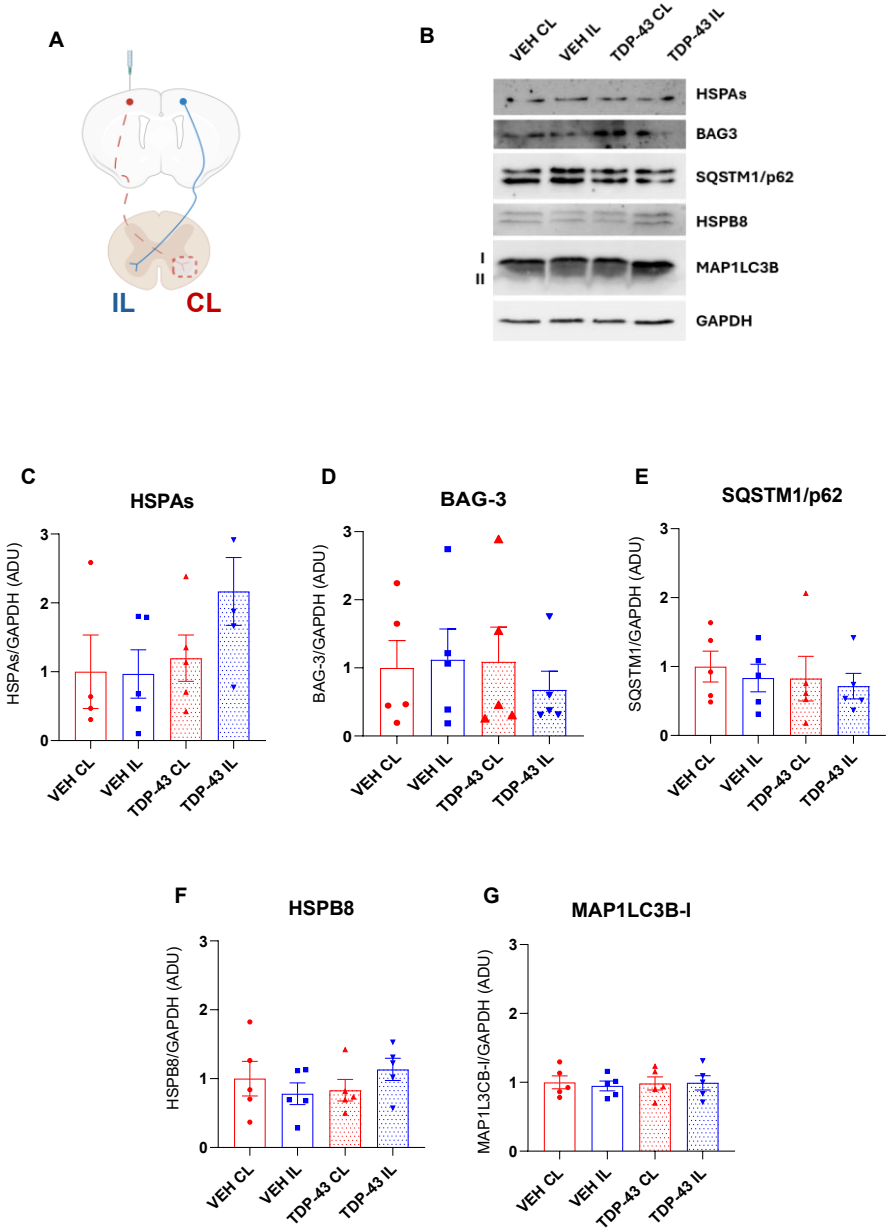
Supplementary Fig. 3 TDP-43 behavior in the gastrocnemius muscle. A) Schematic representation of the skeletal muscle anatomically connected with intracortical infusion side through the spinal cord after decussation. Gastrocnemius muscle ipsilateral (IL) to FL TDP-43-infused motor cortex is anatomically connected to the non-infused side of the motor cortex via the spinal cord (blue line). Gastrocnemius muscle contralateral (CL; dashed red square) is anatomically connected to the infused motor cortex (VEH- or FL TDP-43) via the spinal cord (dashed red line). **B-D**) Representative Western blot analysis (**B**) and relative quantifications of TDP-43 (**C**) and pTDP-43 (**D**) species. The graphs show the densitometric analyses of TDP-43 and pTDP-43 signals normalized using GAPDH as loading control. Data are expressed as arbitrary densitometric units (ADU). Each bar represents the mean ± SEM of five independent replicates run and processed in parallel (one-way ANOVA with Tukey's post hoc test among groups). **E-F**) Representative filter retardation assay (**E**) and relative densitometric analysis (**F**) of insoluble TDP-43 species. Value represents the mean ± SEM of five independent replicates (one-way ANOVA with Tukey's post hoc test among groups). Note that dotted red columns in the graphs refer to the muscle anatomically connected with the TDP-43-infused cortex. All measures were made four months after FL TDP-43 infusion. FL = full length; VEH = vehicle

Supplementary Fig. 4



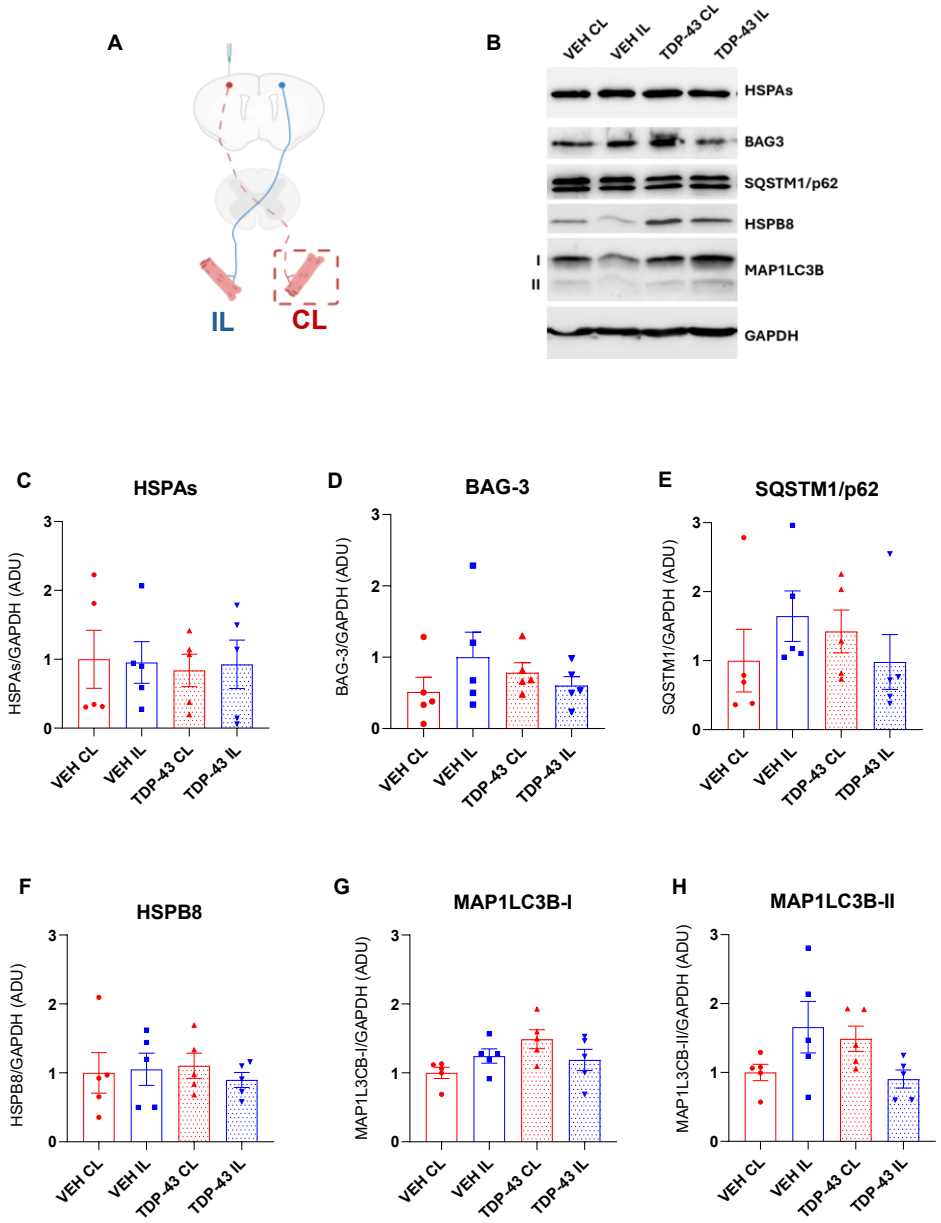
Supplementary Fig. 4 FL TDP-43 infusion does not affect the protein quality control system in the primary motor cortex. A) Schematic representation of the intracortical infusion side of FL TDP-43 (IL, ipsilateral, dashed red line) and the corresponding contralateral motor cortex (CL; blue line). Dashed red square in the scheme corresponds to the motor cortex side infused with either VEH- or FL-TDP-43. **B)** Representative Western blot analysis of the chaperone proteins HSPAs (both inducible and constitutive), the small heat shock protein HSPB8 and its co-chaperone BAG3, the autophagy receptor SQSTM1/p62 and the autophagy marker MAP1LC3B-I and its lipidated MAP1LC3-II form. **C-H**) Western blot quantifications of HSPAs (**C**), BAG3 (**D**), SQSTM1/p62 (**E**), HSPB8 (**F**), MAP1LC3B-I (**G**) and MAP1LC3B-II (**H**) protein levels. The graphs show the densitometric analyses of each protein normalized using GAPDH as loading control. Data are expressed as arbitrary densitometric units (ADU). Each bar represents the mean ± SEM of five independent replicates run and processed in parallel (one-way ANOVA with Tukey's post hoc test among groups). Note that dotted red columns in the graphs refer to the TDP-43-infused side. All measures were made four months after FL TDP-43 infusion. FL = full length; VEH = vehicle

Supplementary Fig. 5



Supplementary Fig. 5 FL TDP-43 infusion does not affect the protein quality control system in the cervical spinal cord. A) Schematic representation of the spinal cord anatomically connected with the intracortical infusion side after decussation. Spinal cord ipsilateral (IL) to FL TDP-43-infused motor cortex is anatomically connected to the non-infused side of the motor cortex (blue line). Spinal cord contralateral (CL; dashed red square) is anatomically connected to the infused motor cortex (VEH- or FL TDP-43, dashed red line). **B)** Representative Western blot analysis of the chaperone proteins HSPAs (both inducible and constitutive), the small heat shock protein HSPB8 and its co-chaperone BAG3, the autophagy receptor SQSTM1/p62 and the autophagy marker MAP1LC3B-I and its lipidated MAP1LC3-II form. **C-G**) Western blot quantifications of HSPAs (C), BAG3 (**D**), SQSTM1/p62 **(E)**, HSPB8 (**F**) and MAP1LC3B (**G**) protein levels. The graphs show the densitometric analyses of each protein normalized using GAPDH as loading control. Data are expressed as arbitrary densitometric units (ADU). Each bar represents the mean ± SEM of five independent replicates run and processed in parallel (one-way ANOVA with Tukey's post hoc test among groups). Note that dotted red columns in the graphs refer to the spinal cord side anatomically connected with the TDP-43-infused cortex. All measures were made four months after FL TDP-43 infusion. FL = full length; VEH = vehicle

Supplementary Fig. 6



Supplementary Fig. 6 FL TDP-43 infusion does not affect the PQC system in the gastrocnemius muscle. A) Schematic representation of the skeletal muscle anatomically connected with intracortical infusion side through the spinal cord after decussation. Gastrocnemius muscle ipsilateral (IL) to FL TDP-43-infused motor cortex is anatomically connected to the non-infused side of the motor cortex via the spinal cord (blue line). Gastrocnemius muscle contralateral (CL; dashed red square) is anatomically connected to the infused motor cortex (VEH- or FL TDP-43) via the spinal cord (dashed red line). **B)** Representative Western blot analysis of the chaperone proteins HSPAs (both inducible and constitutive), the small heat shock protein HSPB8 and its co-chaperone BAG3 the autophagy receptor SQSTM1/p62 and the autophagy marker MAP1LC3B-I and its lipidated MAP1LC3-II form. **C-H)** Western blot quantifications of HSPAs (**C**), **BAG3** (**D**), SQSTM1/p62 (**E**), HSPB8 (**F**) and MAP1LC3B-I (**G**) and MAP1LC3B-II (**H**) protein levels. The graphs show the densitometric analyses of each protein normalized using GAPDH as loading control. Data are expressed as arbitrary densitometric units (ADU). Each bar represents the mean ± SEM of five independent replicates run and processed in parallel (one-way ANOVA with Tukey's post hoc test among groups). Note that dotted red columns in the graphs refer to the muscle anatomically connected with the TDP-43-infused cortex. All measures were made four months after FL TDP-43 infusion. FL = full length; VEH = vehicle