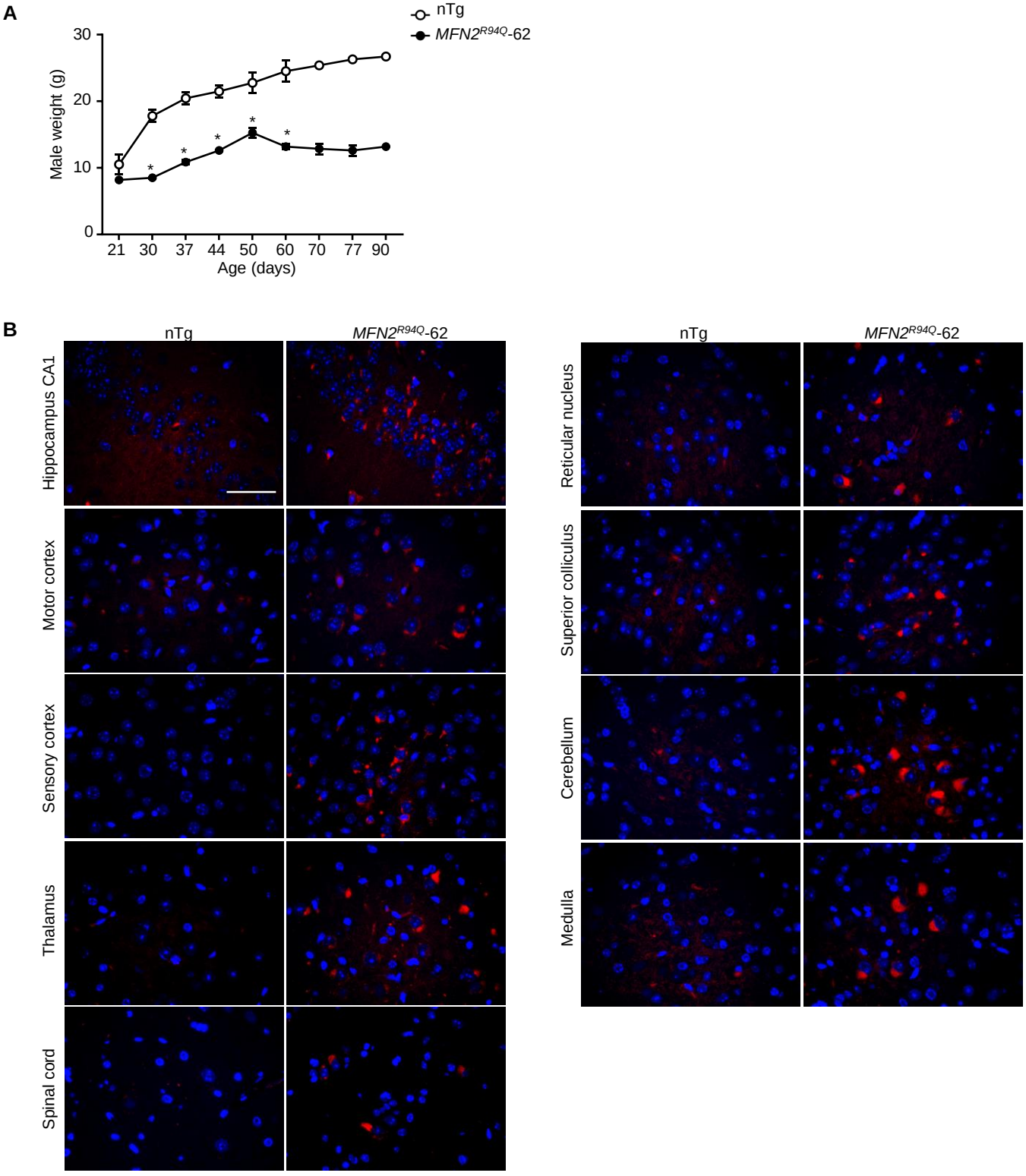
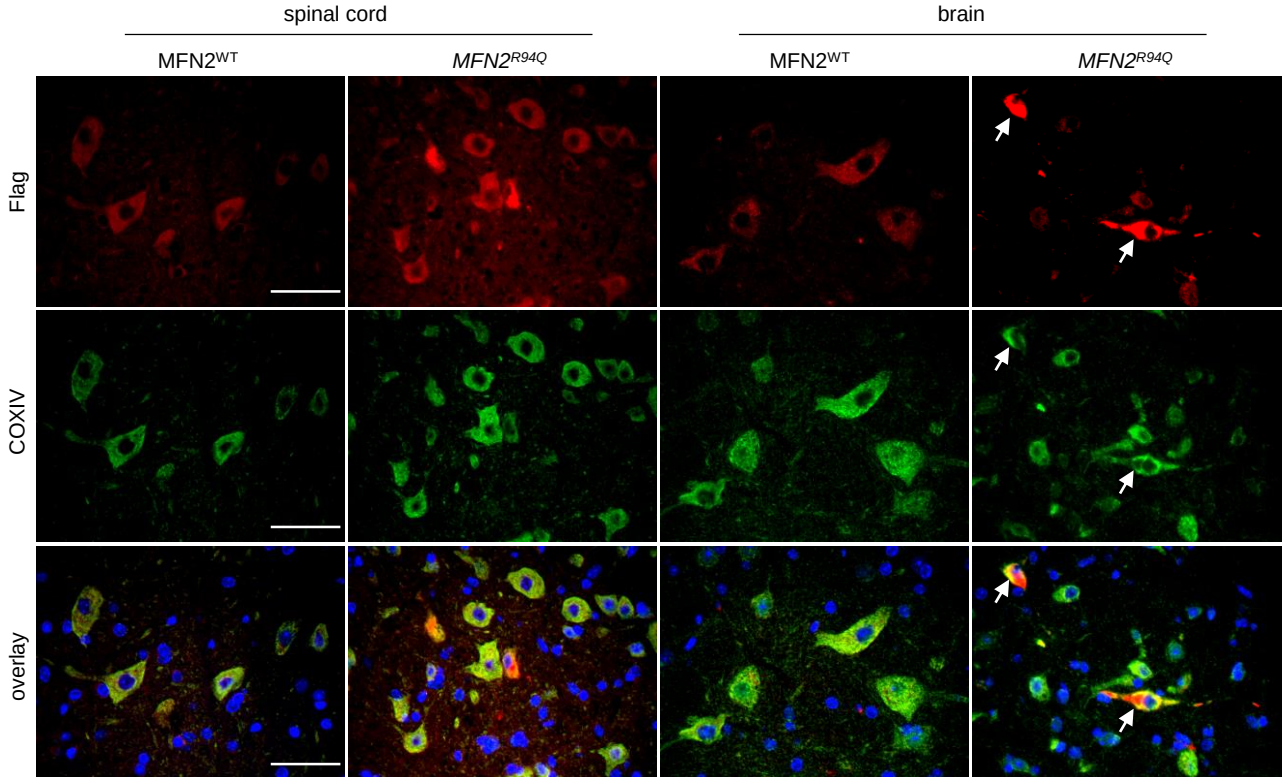
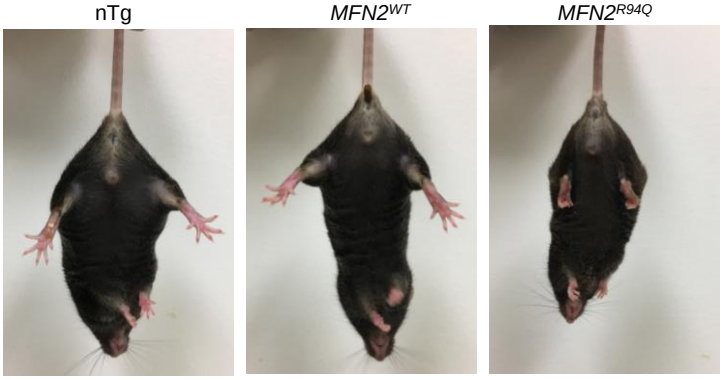
Supplemental Figure 1



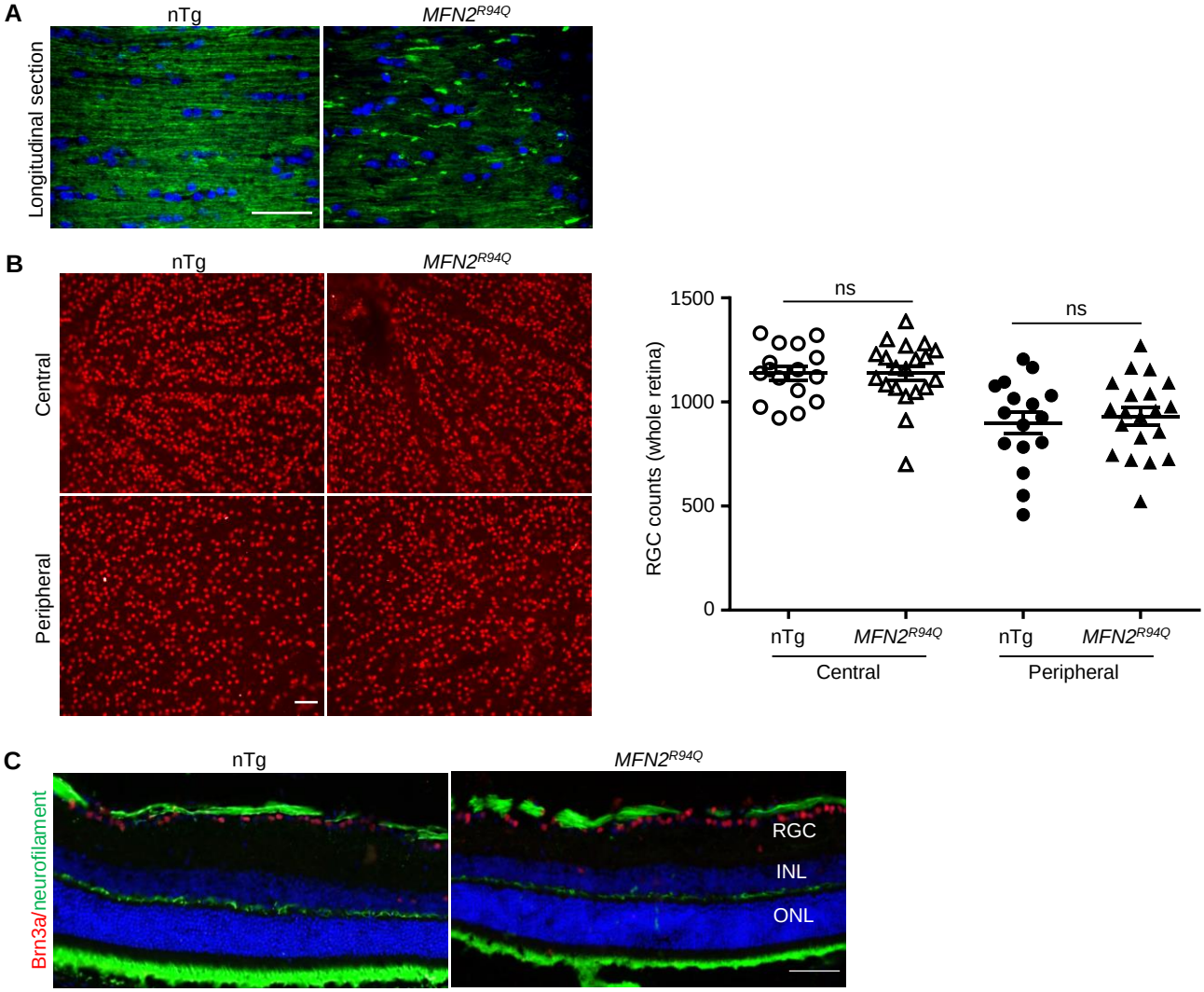
Supplemental Figure 2



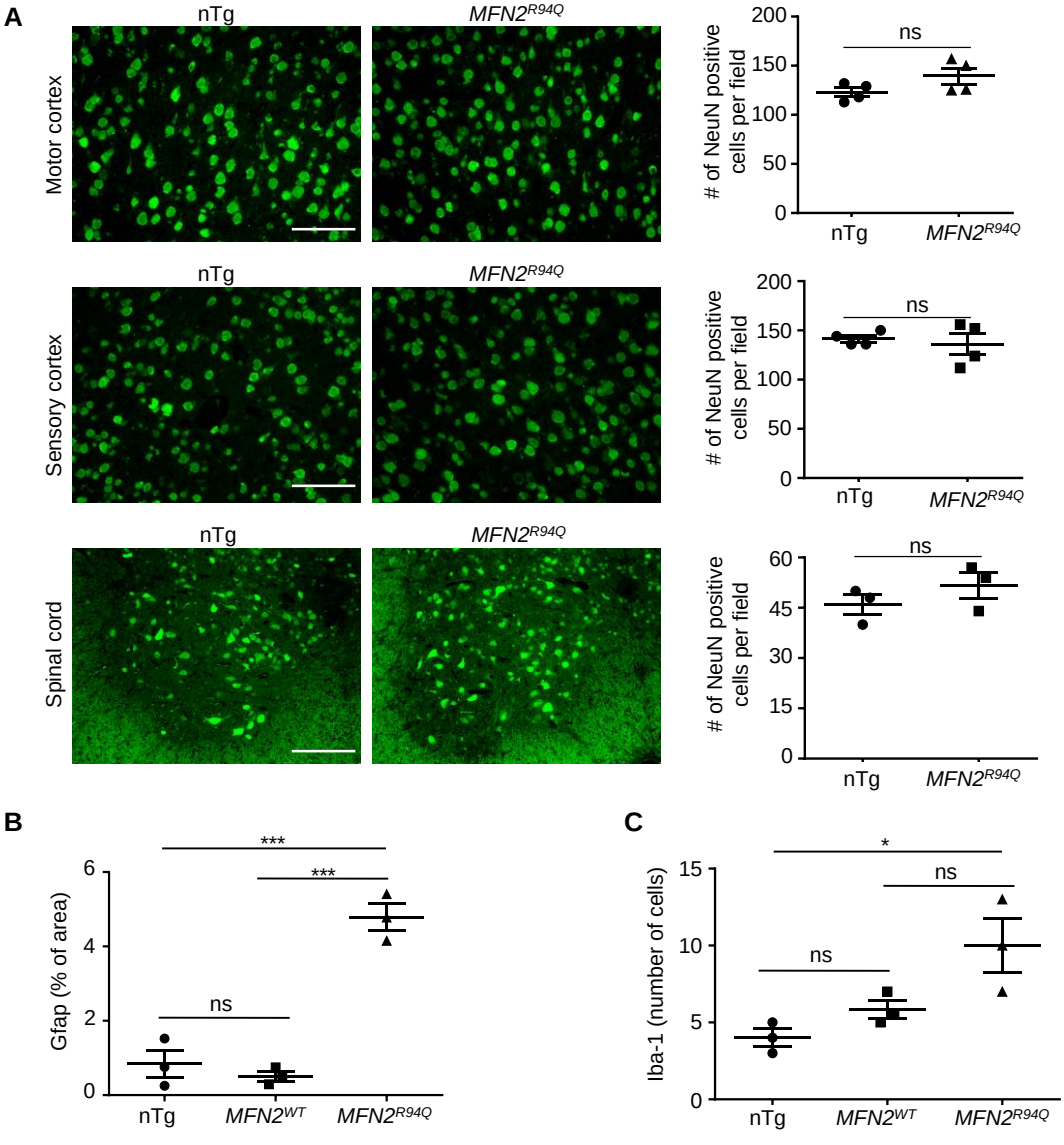
Supplemental Figure 3



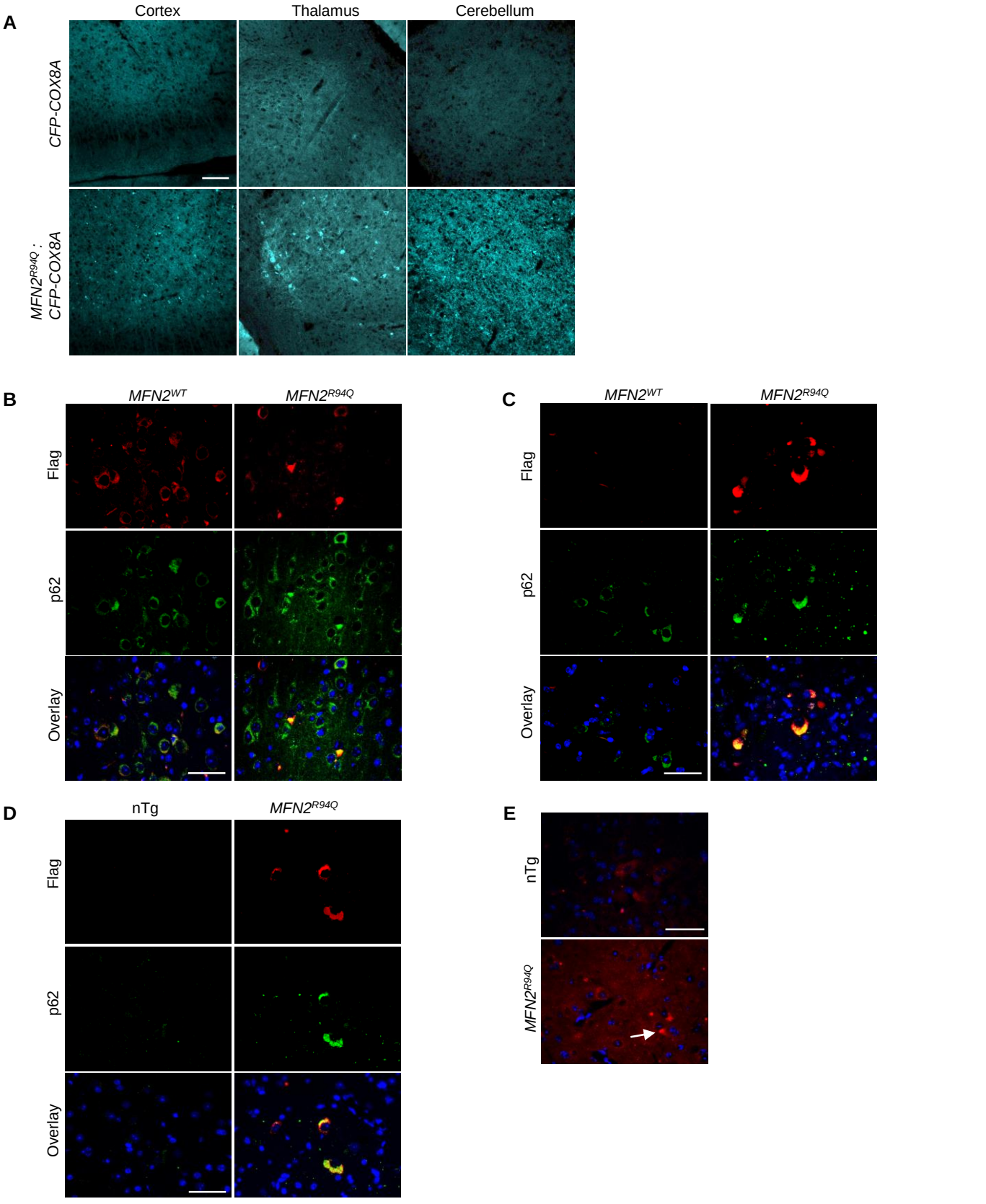
Supplemental Figure 4



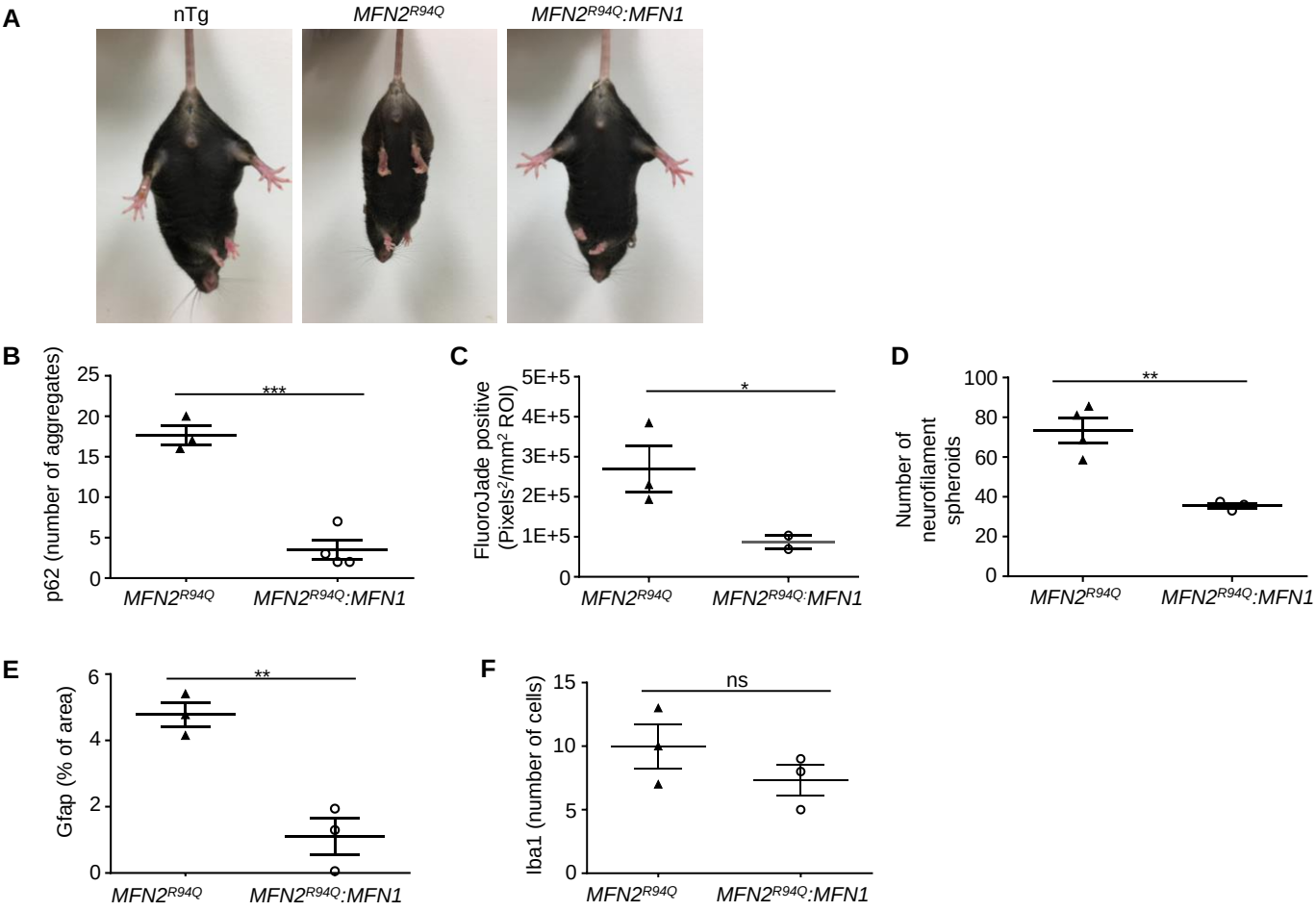
Supplemental Figure 5



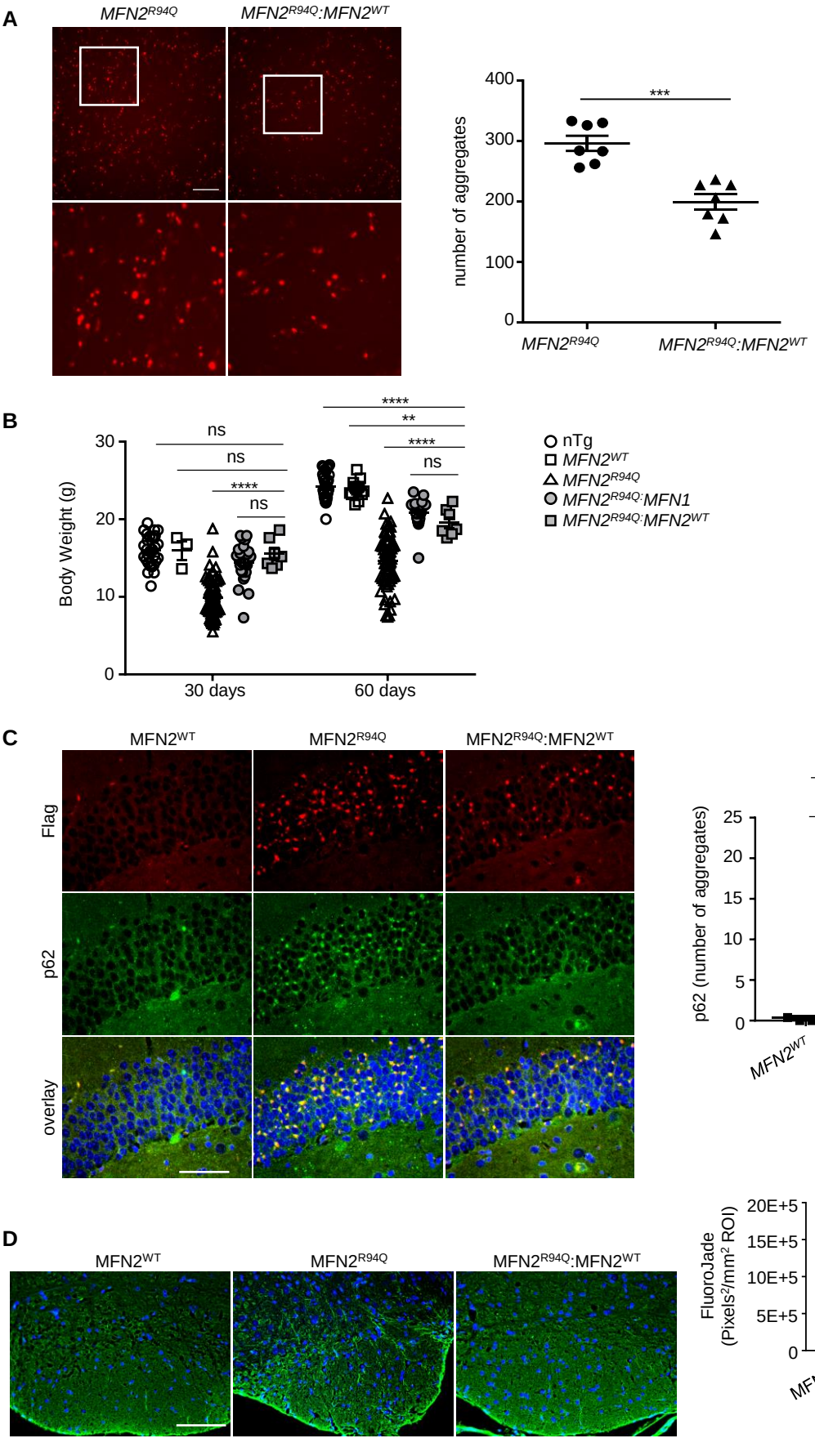
Supplemental Figure 6



Supplemental Figure 7



Supplemental Figure 8



Supplemental Figure 1. *MFN2^{R94Q}*-62 line. (**A**) *MFN2^{R94Q}*-62 line showed significant growth defects similar to the *MFN2^{R94Q}*-44 line. Body weight is presented as mean±SD. n=2-9 per genotype per time point. Student's two-tailed *t* test (nTg *vs. MFN2^{R94Q}*), $p<0.05$. (**B**) Mitochondrial clustering was observed in *MFN2^{R94Q}*-62 line in neuronal cytoplasm and proximal axons, similar to the *MFN2^{R94Q}*-44 line. Flag-tagged MFN2^{R94Q} protein expression and localization in mouse brain or spinal cord (3-month-old mice) was detected by immunostaining with anti-Flag antibody and counterstained with DAPI to label nuclei. Scale bar=50 μM. n=2 mice/genotype.

Supplemental Figure 2. Flag-MFN2 co-stained with mitochondrial marker COXIV. Transgenically expressed Flag-MFN2^{WT} or Flag-MFN2^{R94Q} (red) is colocalized with mitochondrial marker COXIV (green) in mouse spinal cord and brain (5-months). Areas where COXIV staining was diminished were present only at the center of the most dense mitochondrial clusters of MFN2^{R94Q}, which are indicated by white arrows in the figure. These likely represent degradation of mitochondrial components at the center of the cluster. Scale bar=50 μM. n=3 mice/genotype.

Supplemental Figure 3. The clasping phenotype in *MFN2^{R94Q}* mice. Clasping was observed in *MFN2^{R94Q}* mice but not the nTg or *MFN2^{WT}* control (10-month-old mice shown).

Supplemental Figure 4. Optic nerve neurofilament loss without retinal ganglion cell (RGC) loss in *MFN2^{R94Q}* mice. (**A**) Loss of neurofilament staining and axonal spheroids in the longitudinal sections of optic nerve (8 months). Neurofilament is stained with SMI32 antibody. Scale bar=50 μM. n=2-3/genotype. (**B**) RGC stained with Brn3a in the retinal whole mount preparation (8 months old). Scale bar=50 μM. Graph represents the quantification of RGC. For each animal, four images in the center and in the peripheral area were taken, respectively. n=4-5 mice/genotype . All the data points were pooled together for quantification. Data=mean±SEM, Student's two-tailed *t* test, ns=not significant. (**C**) No structural changes or RGC loss was seen on retinal cross sections (8 months old). Brn3a (red) stains RGC, and RT97 antibody stains neurofilament (green). INL: inner nuclear cell layer; ONL: outer nuclear cell layer. Scale bar=50 μM. n=2 mice/genotype.

Supplemental Figure 5. Quantification of neuronal cell bodies and gliosis in *MFN2^{R94Q}* mice. (**A**) No neuronal cell body loss was observed in *MFN2^{R94Q}* mice. NeuN staining of mouse cortex and lumbar spinal cord (14-month-old mice). Scale bar=100 μM for brain, =200 μM for spinal cord. Graph represents the quantification of NeuN positive cells as number of cells per field of view. For each animal, the average cell count of three images per area was used for quantification. 3-4 mice/genotype. Data=mean±SEM, Student's two-tailed *t* test, ns=not significant. (**B**) Quantification of astrogliosis in the brain stem, example images are shown in Figure 2F. Graph represents Gfap positive fluorescence as a percentage of area. (**C**) Quantification of microgliosis in cortex as shown in Figure 2G. Graph represents number of Iba1 positive cells per field. In both (**B**) and (**C**), Data=mean±SEM, n=3, Student's two-tailed *t* test, ns=not significant, *=$p<0.05$, ***=$p<0.001$.

Supplemental Figure 6. Mitochondrial accumulation in *MFN2^{R94Q}* mice. (**A**) Mitochondrial accumulation labelled with CFP-COX8A in vivo in the cortex, thalamus and cerebellum of

MFN2^{R94Q}*:CFP-COX8A* double transgenic mice (7 months old). Scale bar=100 μM. n=2 mice/genotype. (**B-D**) Mitochondrial accumulations were colocalized with p62 protein in the cortex (**B**), reticular nucleus (**C**) and spinal cord (**D**) of *MFN2*^{R94Q} mice. No mitochondrial accumulations were observed in *MFN2*^{WT} mice or non-transgenic (nTg) control mice. 5-month-old mice shown. Anti-Flag staining labels transgenic Flag-MFN2. Scale bar=50 μM. n=3 mice/genotype. (**E**) Mitochondrial accumulations in *MFN2*^{R94Q} mice also labeled with ubiquitin. Spinal cord, 5-month-old mice. Scale bar=50 μM. n=2 mice/genotype.

Supplemental Figure 7. Increased expression of MFN1 rescued the MFN2^{R94Q} phenotypes. (**A**) The clasping phenotype was rescued in *MFN2*^{R94Q}*: MFN1* transgenic mice**.** nTg: non-transgenic. 10 months old. The pictures of nTg and *MFN2*^{R94Q} mice are the same as supplementary Figure 3. (**B**) Quantification of p62 positive mitochondrial accumulations in Figure 6B. Graph represents number of p62 aggregates per region of interest (0.01mm^2). (**C**) Quantification of degenerating axons stained with Fluoro-Jade in Figure 6C. Graph represents Fluoro-Jade positive signal as pixel^2 per mm^2 region of interest (ROI) subtracted from background. (**D)** Quantification of neurofilament spheroids in optic nerve in Figure 6D. (**E**) Quantification of astrogliosis in Figure 6E. Graph represents Gfap positive signals as percentage of area in the field of view. (**F**) Quantification of microgliosis in Figure 6F. Graph represents number of Iba1 positive cells. There was a trend toward decreased Iba1 staining in *MFN2*^{R94Q}*:MFN1* double transgenic animals compared to *MFN2*^{R94Q} mice. In (**B-F**), data are represented as mean±SEM, Student's two-tailed *t* test, ns=not significant, *=p<0.05, **=p<0.001, ***=p<0.001, ****=p<0.0001.

Supplemental Figure 8. Increased expression of wildtype MFN2 rescued MFN2^{R94Q} phenotypes in vitro and in vivo. (**A**) MFN2^{R94Q} induced mitochondrial aggregation was rescued by increased MFN2^{WT} expression in a neuronal cell line (SH-SY5Y cells). Lentiviruses were used to transduce cells, MFN2^{R94Q} lentivirus also expressed IRES-mito-RFP to visualize mitochondria. Top panels are 20x images, with inset boxes shown at higher magnification below. Scale bar=100 μM. Graph on right is the quantification. Data are presented as mean±SEM (n=7). One-way ANOVA with Tukey's test was used for multiple comparison. ***=p<0.001. (**B**) Body weight of indicated genotypes. Data are presented as mean±SEM (n=3-64). Two-way ANOVA, Tukey's test was used for multiple comparison. **=p<0.01, ****=p<0.0001. ns=not significant. (**C**) Images of p62 labeled mitochondria in hippocampus in the indicated genotypes. p62 positive mitochondrial accumulations were significantly decreased in *MFN2*^{R94Q}*: MFN2*^{WT} mice. Anti-Flag staining labels transgenic MFN2. DAPI labels nuclei. 5-month-old mice. Scale bar=50 μM. Graph on right represents number of p62 aggregates per region of interest (0.01mm^2). Data= mean±SEM (n=2-5). Student's two-tailed *t* test, ****=p<0.0001. (**D**) Degenerating axons labeled with Fluoro-Jade staining in the brain pyramidal tracts (5 months old). Scale bar=50 μM. Graph represents the quantification of Fluoro-Jade positive signal as pixel^2 per mm^2 region of interest (ROI). Data= mean±SEM (n=3-4). One-way ANOVA with Tukey's test was used for multiple comparison. **=p<0.01,***=p<0.001,****=p<0.0001.

Supplemental Movie 1. Video showing non-transgenic (nTg), *MFN2*^{WT} and *MFN2*^{R94Q} mice. nTg (one black mark on tail, picked up at 33s), *MFN2*^{WT} (two black marks on tail, picked

up at 44s) and *MFN2*^{R94Q} (no tail marker, picked up at 57s) mice, 5 months old mice. Note the smaller size, wobbling gait, and impaired rearing of the *MFN2*^{R94Q} mice.

Supplemental Movie 2. Video showing non-transgenic (nTg), *MFN2*^{R94Q} transgenic and *MFN2*^{R94Q}*:MFN1* double transgenic mice. nTg (one black mark on tail, picked up at 33s), *MFN2*^{R94Q} transgenic (two black marks on tail, picked up at 45s) and *MFN2*^{R94Q}*:MFN1* double transgenic (no tail marker, picked up at 56s), 5 months old mice. The *MFN2*^{R94Q}*:MFN1* double transgenic were larger and appeared normal visually than *MFN2*^{R94Q} mice.