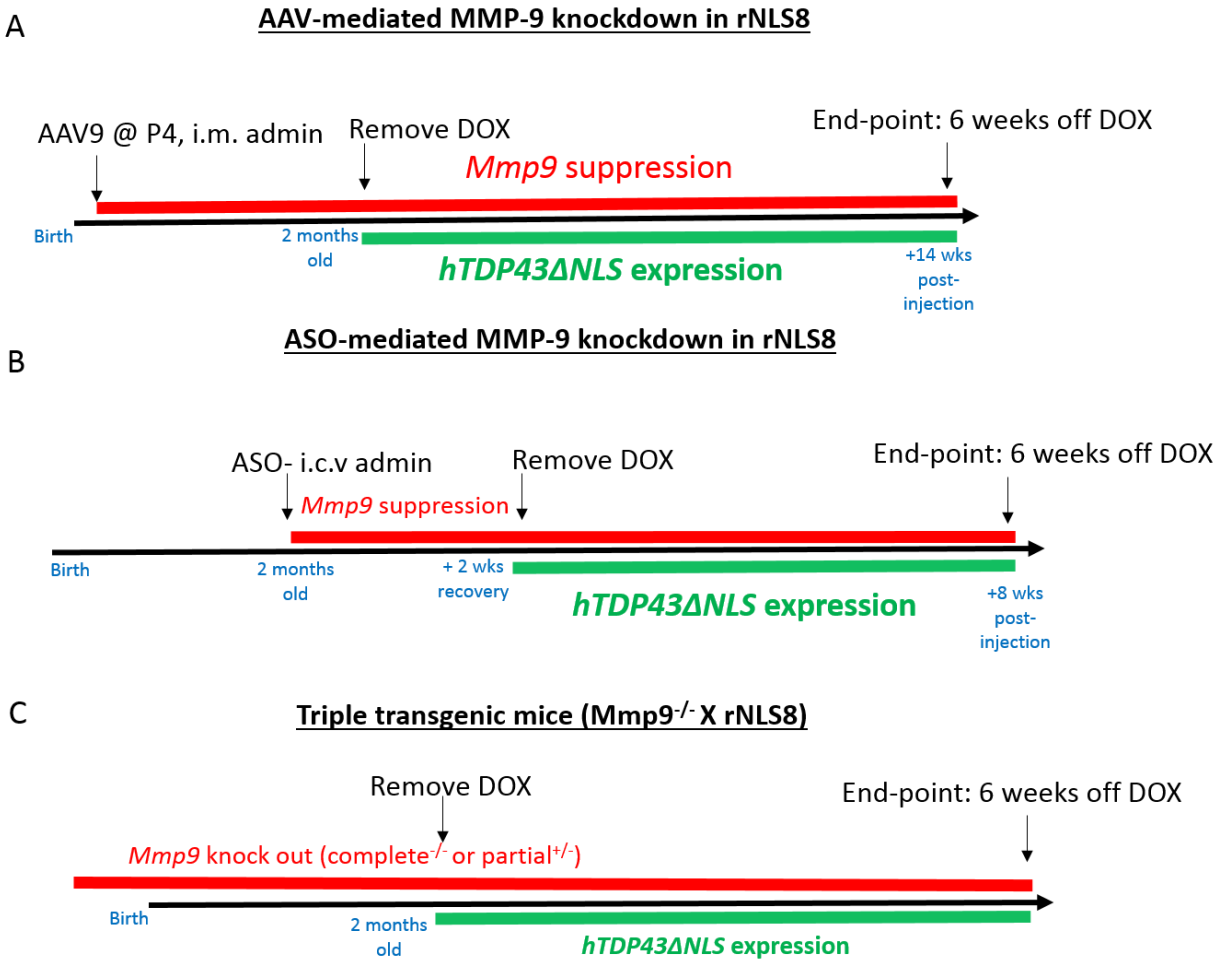
Supplemental Results

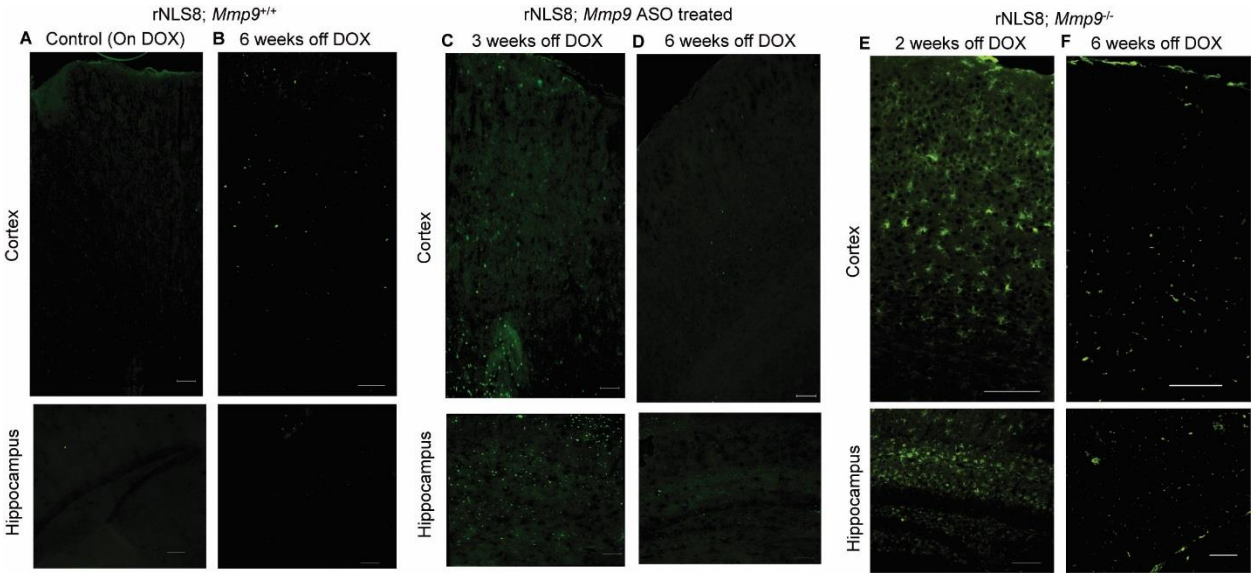
Supplementary Movie 1. Approximately 1/3 of rNLS8 mice that were injected with ASOxMMP9 displayed a jumpy, hyperactive set of behaviors consistent with seizure activity between 2 and 3 weeks off DOX. Age- and DOX treatment- matched rNLS8 mice injected with ASO-cntrl had a typical disease course compared to rNLS8 mice without any ASO treatment. Finally, nTg mice treated with ASOxMMP9 appeared normal and had no motor defects or seizure-like behaviors, suggesting that it is an interaction between MMP-9 reduction and TDP-43-triggered disease processes that negative effects rNLS8 mice, rather than ASO-treatment itself.

Supplementary Figure 1



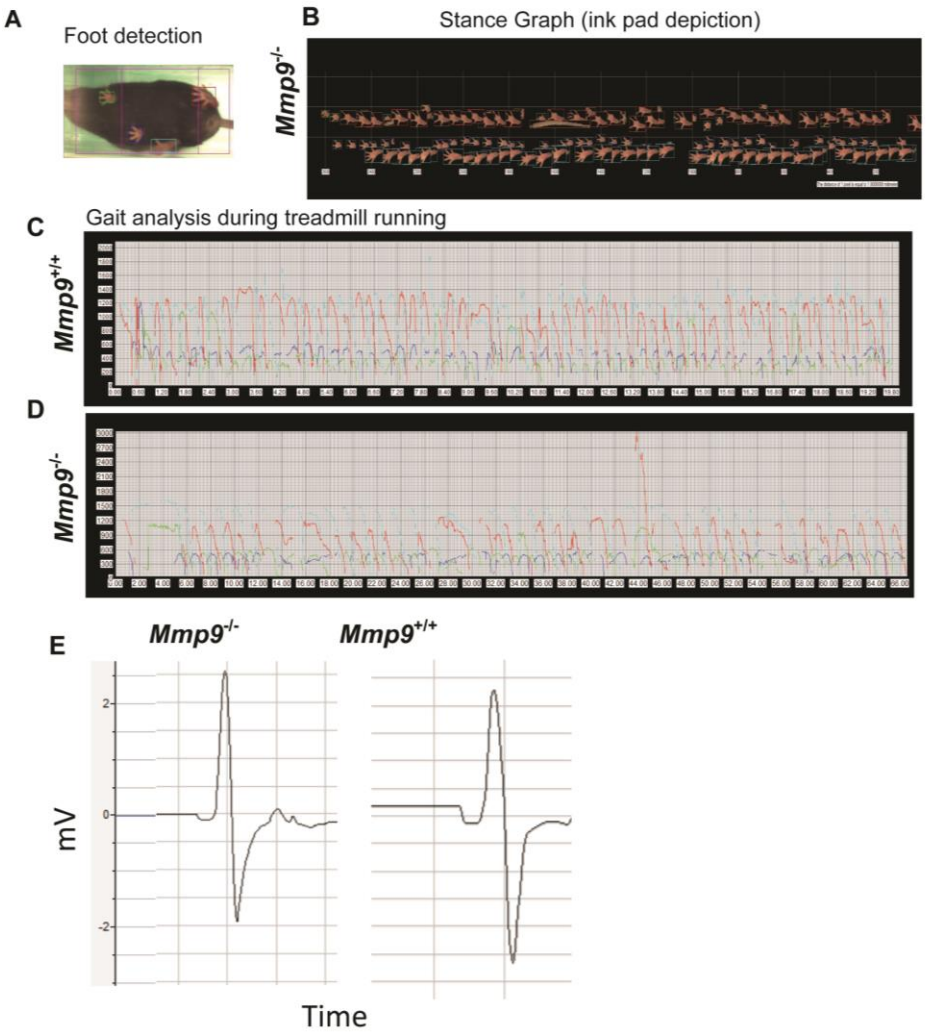
Supplementary Fig. 1. Experimental timelines for studies targeting MMP-9 in rNLS8 mice. A. shRNA against *Mmp9* was delivered to MNs that connect to the TA muscle on one side of an rNLS8 mouse pup at 4 days old, lowering MMP-9 expression in that motor pool from the first week of life on (red line). When the injected mice were 2 months old, DOX was removed from their diets to induce transgene expression (green line). Mice were euthanized 6 weeks later for tissue analysis. **B.** ASOs were delivered into adult mice to reduce MMP-9 expression throughout the central nervous system for 9-13 weeks (red line), starting around 2 months of age. 2 weeks after ASO administration, DOX was removed from the rNLS8 mice's diet to induce transgene expression (green line). Mice were euthanized 6 weeks later. **C**. Mmp9$^{-/-}$ mice were crossed with rNLS8 mice, to produce *Mmp9*$^{-/-}$;rNLS8 that never had MMP-9 expressed in any tissue. These mice were maintained on DOX until they were 2 months old, and then it was removed to induce transgene expression (green line). Mice were euthanized at 6 weeks off DOX for tissue analysis.

Supplementary Figure 2



Supplementary Fig. 2. C-fos expression in cortex and hippocampus of ASO-xMMP9 and rNLS8;*Mmp9*$^{-/-}$ mice suggest that lowering *Mmp9* levels in the brain triggers an increase in seizure activity after about 2 weeks of *hTDP43ΔNLS* expression. Representative immunostaining with c-fos (green) of cryosections from cortex (top row) and hippocampus (bottom row) of rNLS8 mice show very low c-fos expression in animals with MMP-9 intact either prior to transgene expression (**A**, control on DOX) or after 6 weeks off DOX (**B**). In contrast, when MMP-9 levels are reduced either using ASOs or genetically, a subset of animals die early and have increased c-fos levels, especially in the hippocampus (**C, E**), though not in all animals off DOX (**D,F**). Scale bars= 100 µm.

Supplementary Figure 3



Supplementary Fig. 3. *Mmp9*$^{-/-}$ mice have no apparent motor defects compared to WT controls. (**A-D**) The GaitScan system was used to compare *Mmp9*$^{+/+}$ and *Mmp9*$^{-/-}$ littermates. Videos were taken of the mice running on a transparent belt treadmill and the software identified the footprints of the mice as they ran from the ventral view (**A-B**). The software then analyzed the videos, and determined various characteristic parameters that are related to the pathophysiological conditions, and displayed their overall performance on stance graphs (**C-D**). No significant differences were detected between *Mmp9*$^{+/+}$ and *Mmp9*$^{-/-}$ mice, with both groups of animals able to run at the maximum track speed (18 cm per second) with average stride frequencies > 2 Hz. (**E**) Correspondingly, we did not detect any significant differences in the maximum evoked CMAP from the gastrocnemius muscles of *Mmp9*$^{-/-}$ (left) or *Mmp9*$^{+/+}$ (right) mice. Representative traces are shown. This is consistent with the almost complete TA muscle innervation detected in adult *Mmp9*$^{-/-}$ mice (avg: 92%).