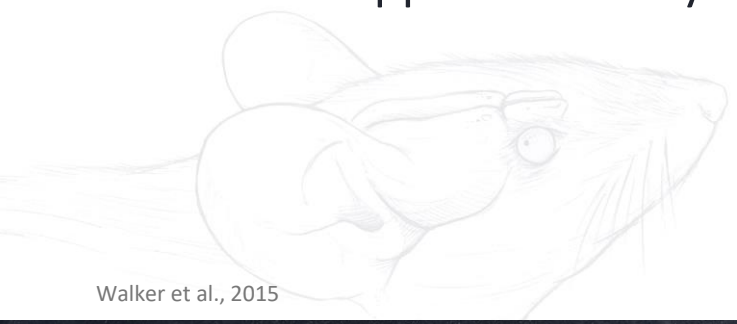
PsychoGenics

Redefining Drug Discovery Through Innovation

Amyotrophic Lateral Sclerosis Models
TDP43

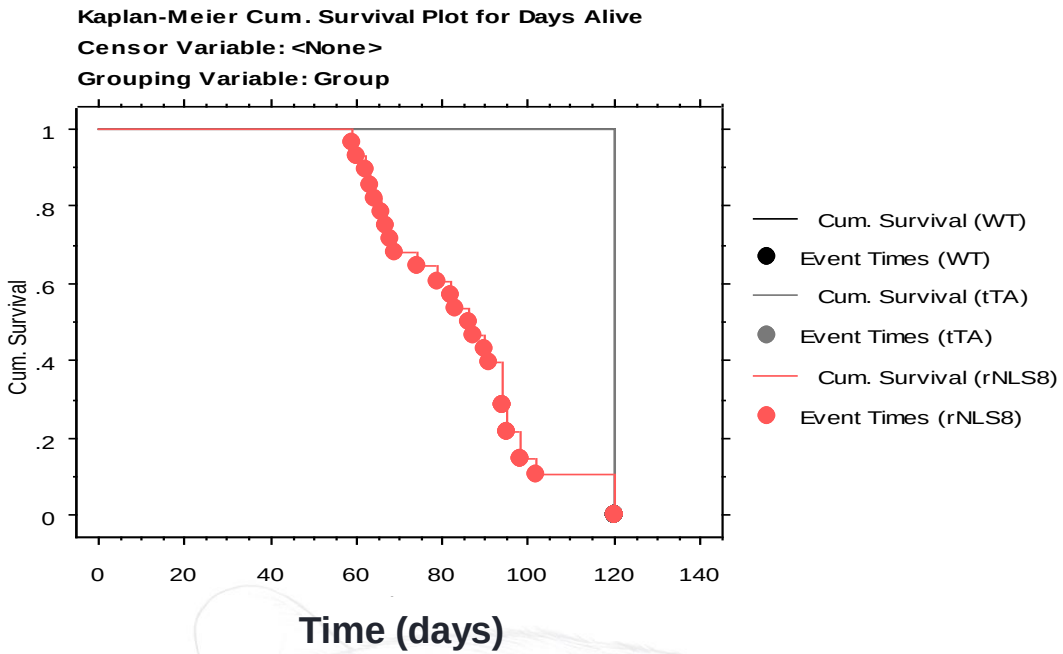
Introduction

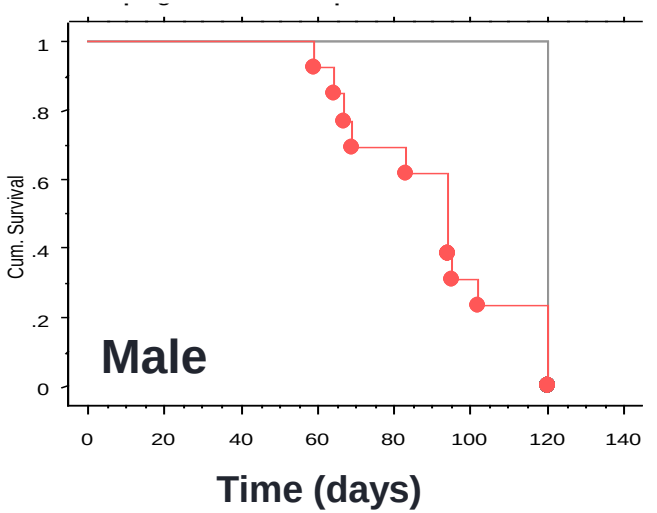
- It is estimated that ~97 % of ALS patients display a common phenotype in disease-affected tissues, namely the deposition of the TAR-DNA binding protein (TDP)-43
- Bigenic rNLS8 mice were generated in Virginial Lee's lab with doxycycline-suppressible expression of hTDP-43 harboring a defective nuclear localization signal (ΔNLS) under the control of the *neurofilament heavy chain* promoter.
- Expression of hTDP-43ΔNLS in these mice resulted in the accumulation of insoluble, phosphorylated cytoplasmic TDP-43 in brain and spinal cord, loss of endogenous nuclear mouse TDP-43, brain atrophy, muscle denervation, dramatic motor neuron loss, and progressive motor impairments leading to death.
- DOX will suppress these symptoms

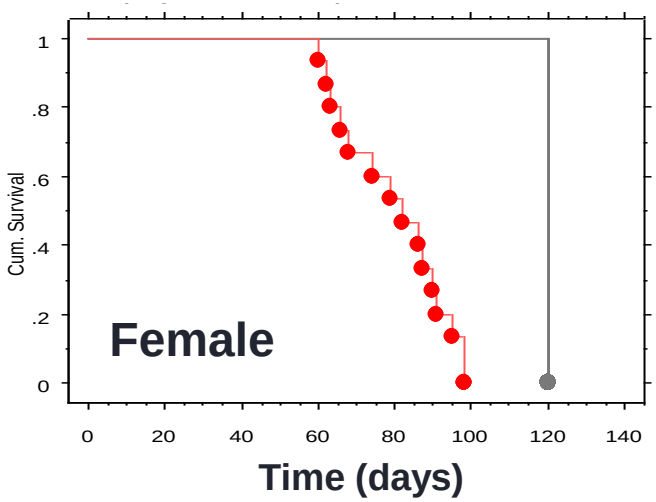


Walker et al., 2015

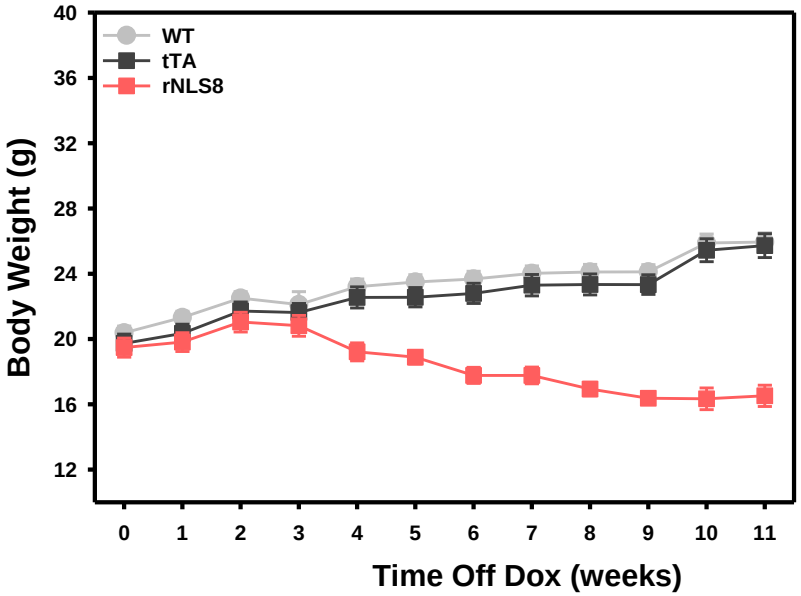
Decrease in Survival Time in rNLS8 mice

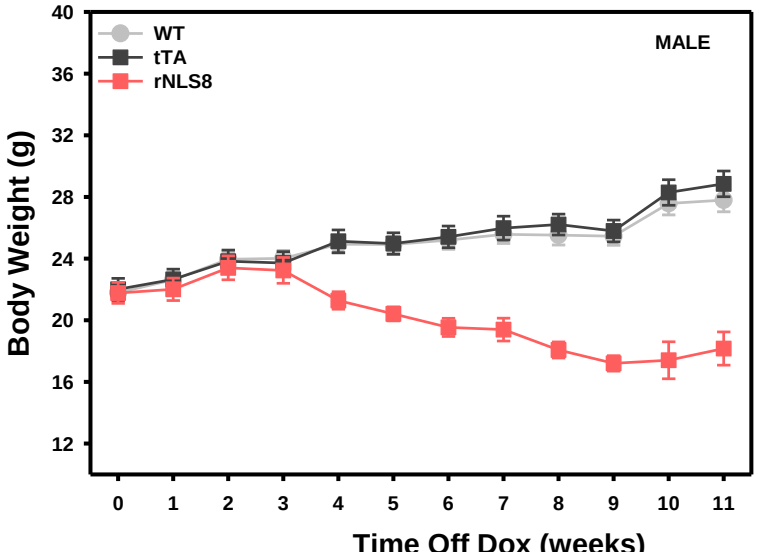


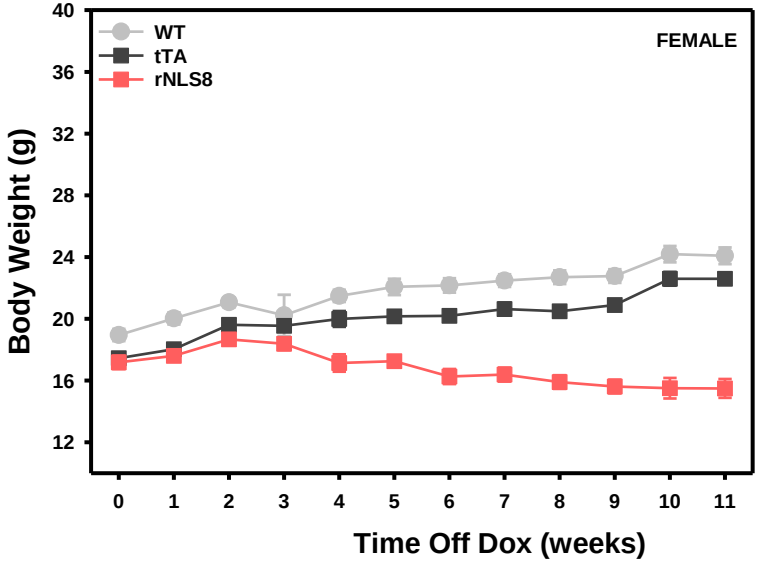




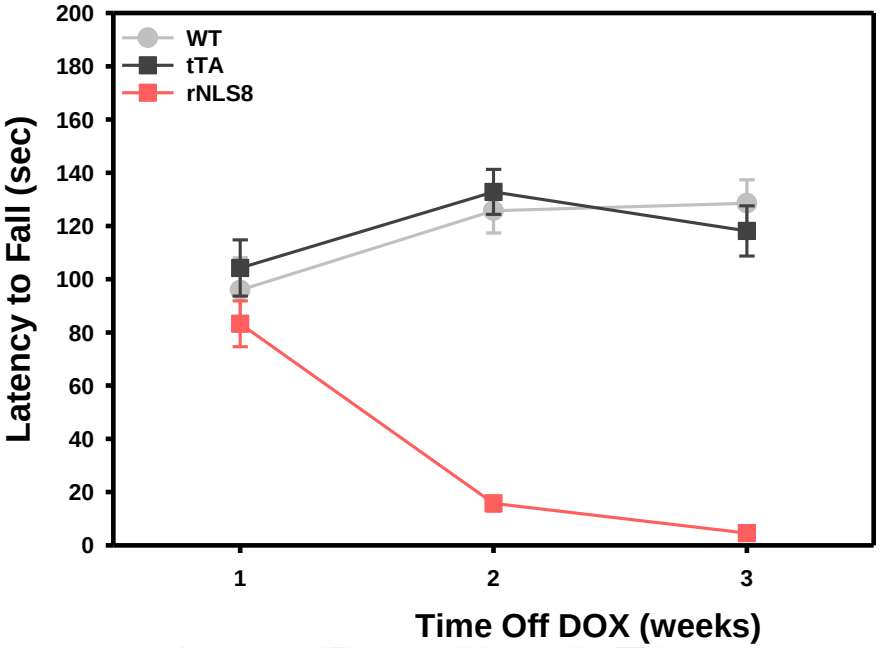
Decline in body weight with disease progression in rNLS8 mice

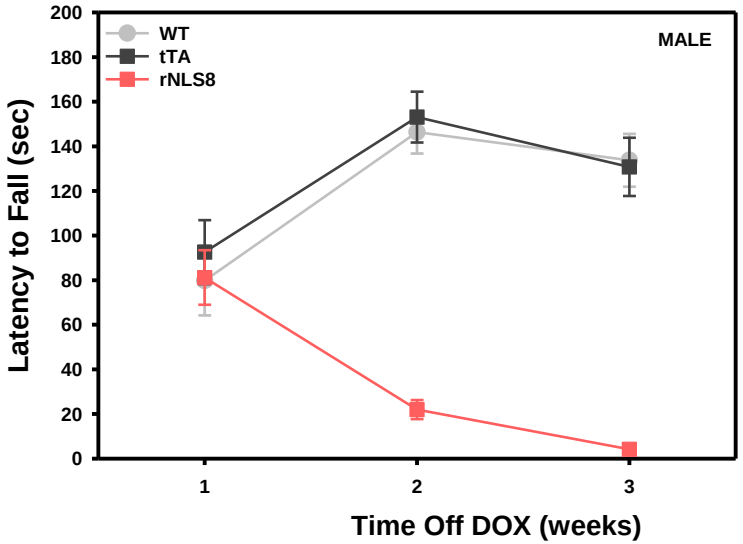


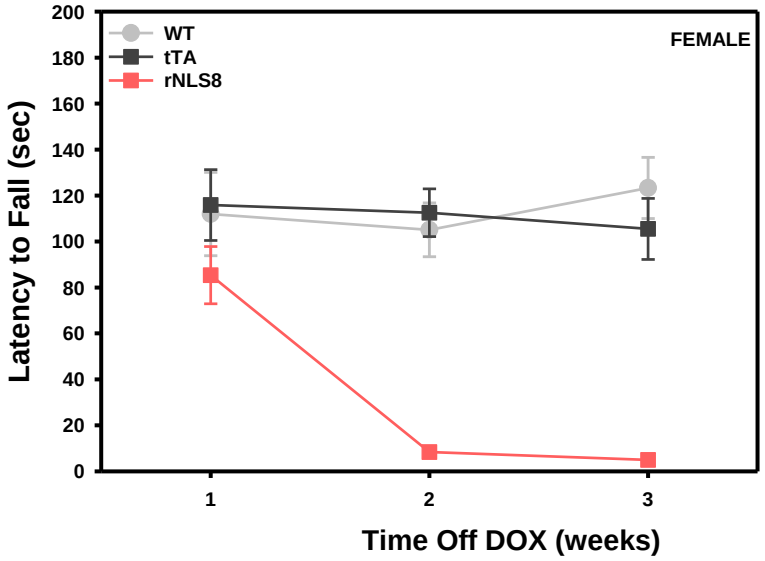




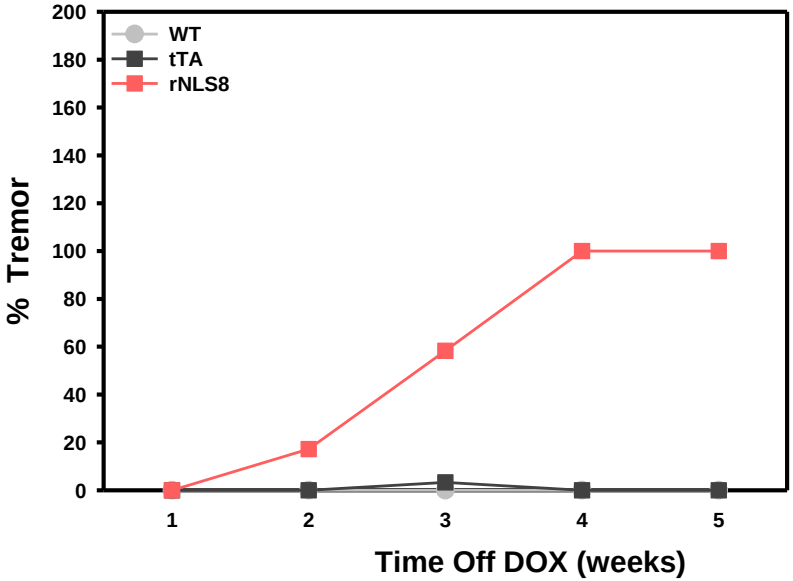
Reduced latency to fall (wire hang) with disease progression in rNLS8 mice

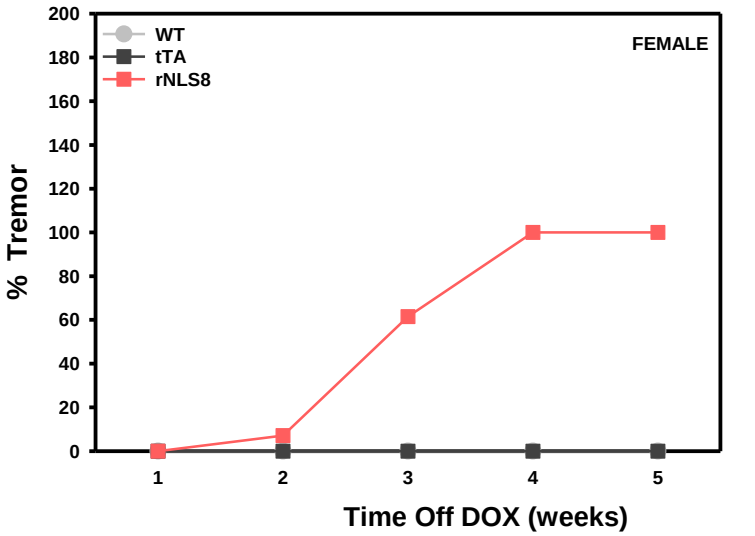


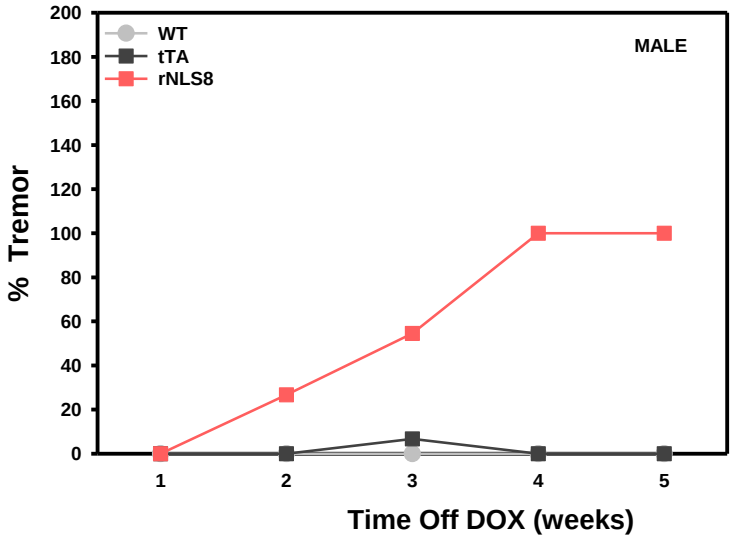




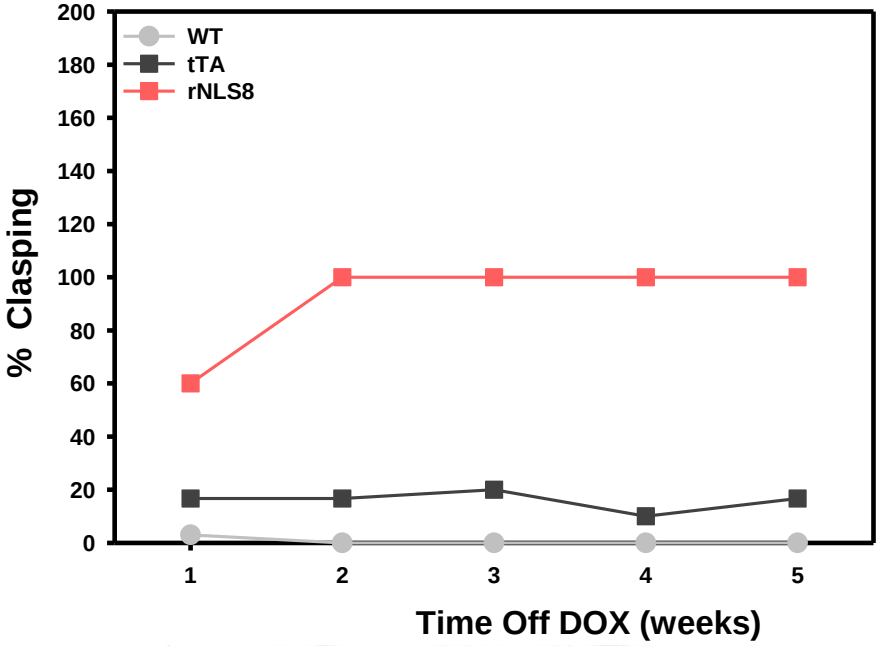
Increased tremors with disease progression in rNLS8 mice

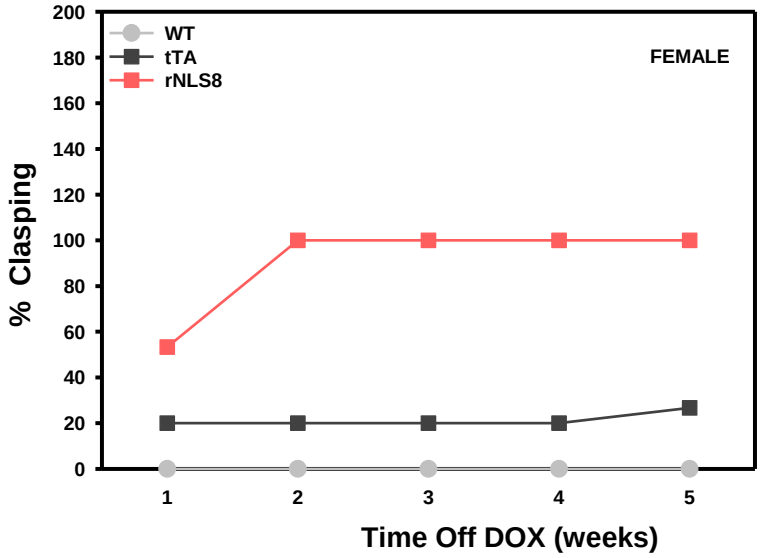


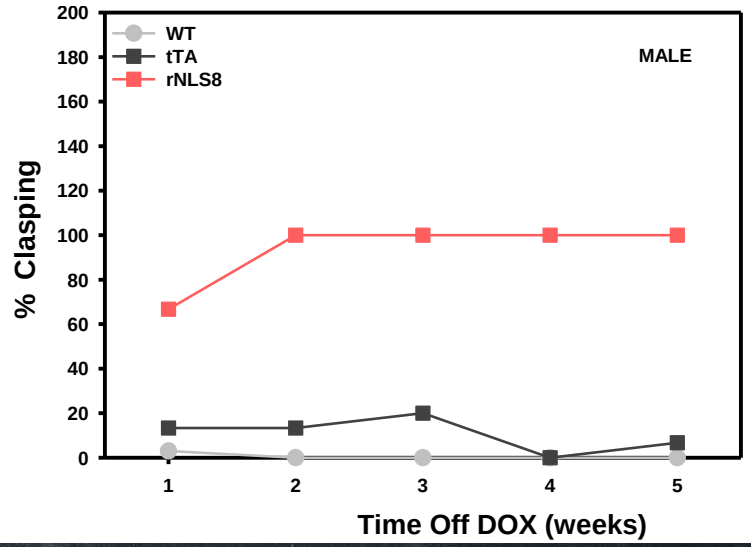




Increased clasping with disease progression in rNLS8 mice



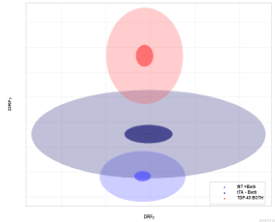


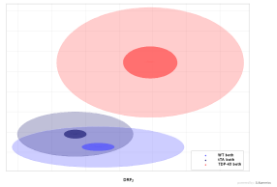


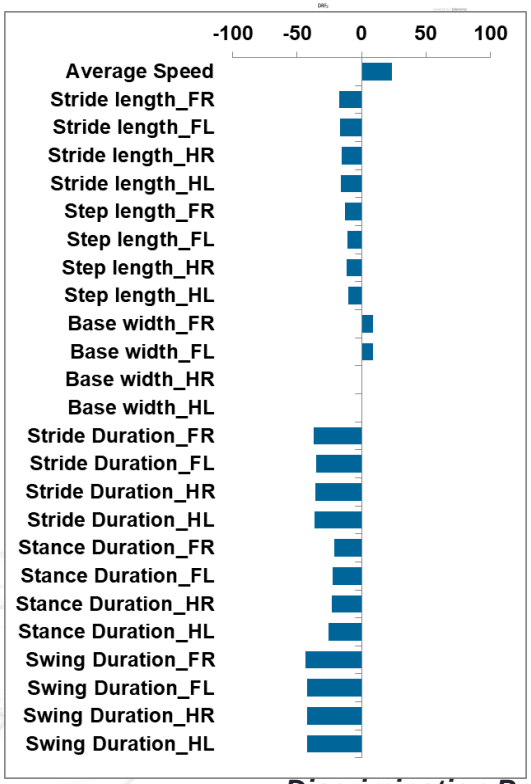
NeuroCube® Comprehensive Gait Analysis: Genders Combined

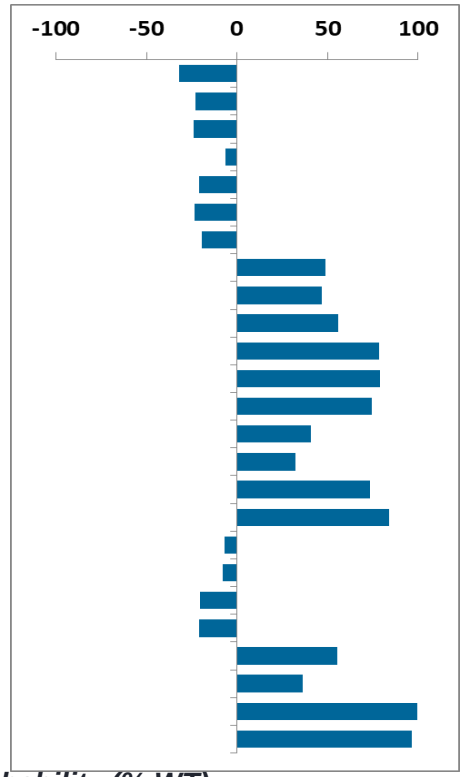
Time off Dox:	1 week	3 weeks
Discrimination vs WT:	96%*	90%*
Discrimination vs tTA:	76%*	94%*

- WT
- tTA
- rNLS8







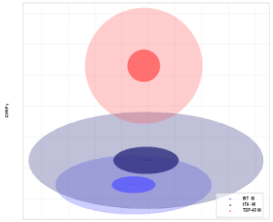


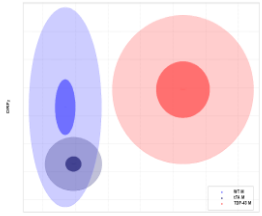
Discrimination Probability (% WT)

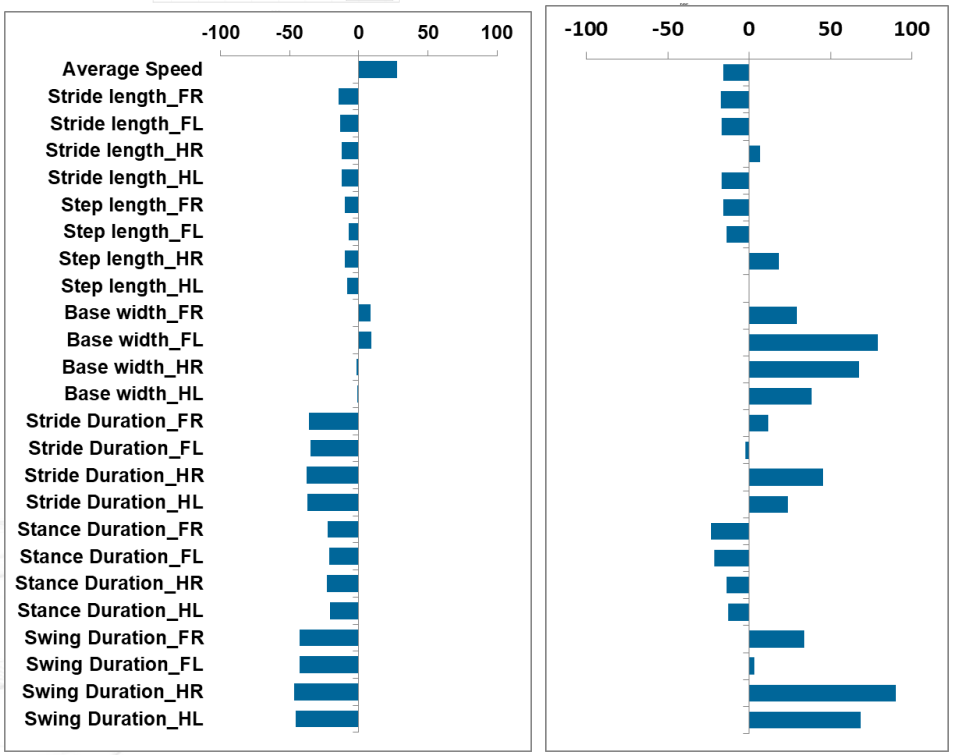
NeuroCube® Comprehensive Gait Analysis: Male

Time off Dox:	1 week	3 weeks
Discrimination vs WT:	93%*	89%*
Discrimination vs tTA:	82%*	85%*

- WT
- tTA
- rNLS8





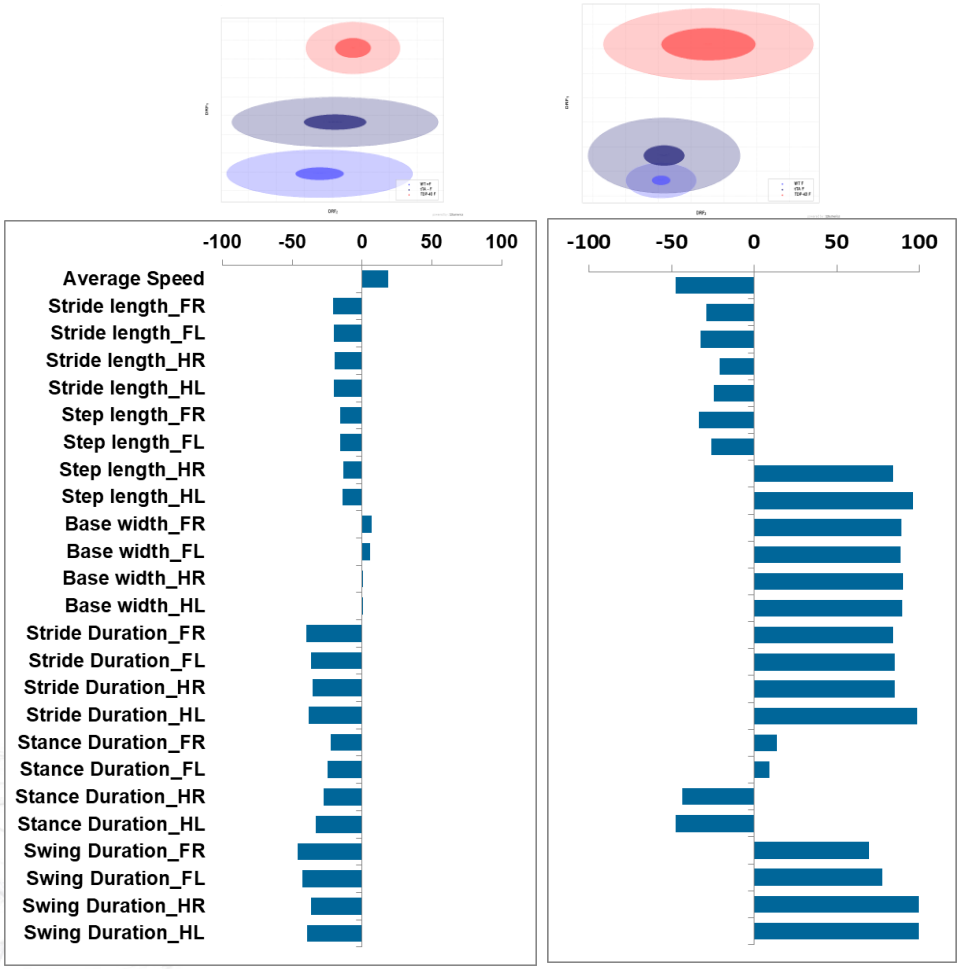


Discrimination Probability (% WT)

NeuroCube® Comprehensive Gait Analysis: Female

Time off Dox:	1 week	3 weeks
Discrimination vs WT:	100%*	100%*
Discrimination vs tTA:	93%*	89%*

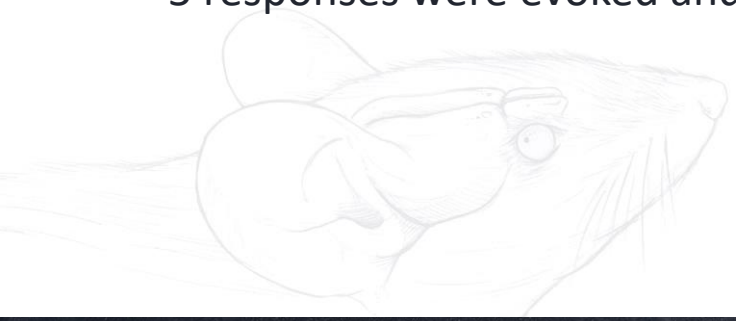
- WT
- tTA
- rNLS8



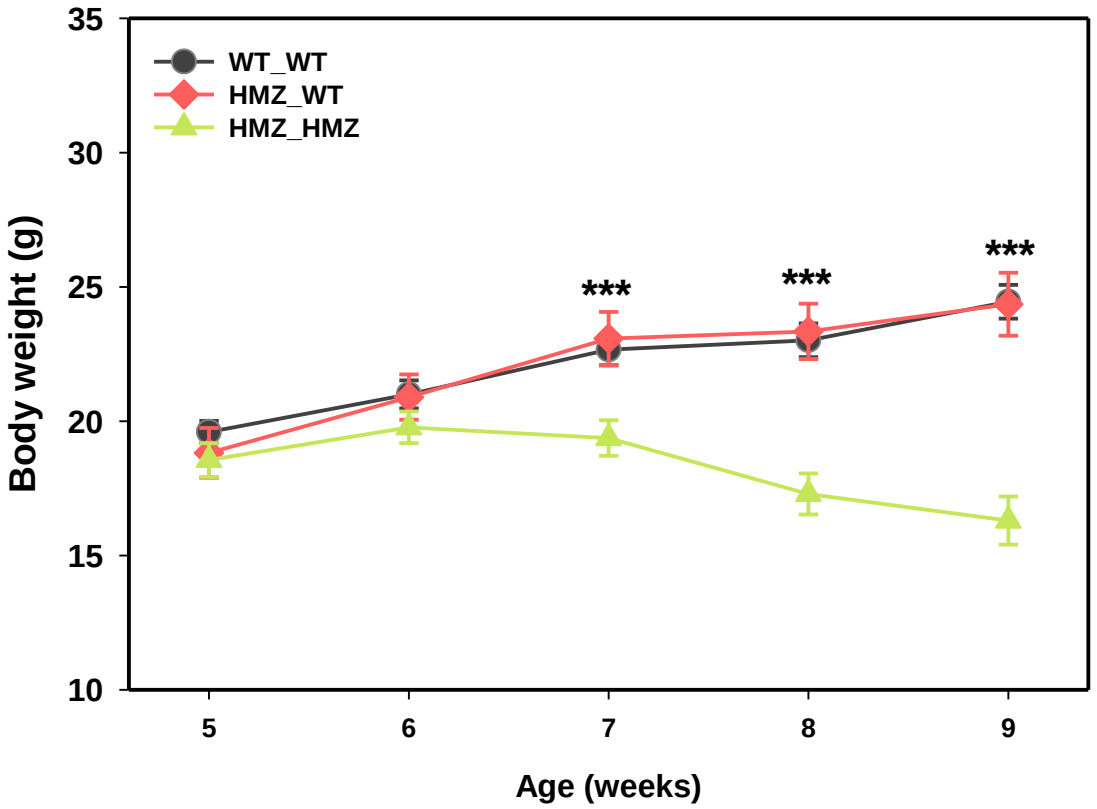
Discrimination Probability (% WT)

Compound Muscle Action Potential

- Male and female mice, 5 weeks old were used
- Dox-diet until five weeks of age for all groups, then all groups taken off dox onward
- Mice were anesthetized and maintained using isoflurane for the recordings
- Compound muscle action potential (CMAP) recordings performed in the gastrocnemius where the muscle is at maximum diameter
  - The stimulating electrodes are placed along the axis of the sciatic nerve.
- Stimulus was gradually increased until a maximal response was reliably evoked.
  - 5 responses were evoked and averaged to generate smooth averaged responses



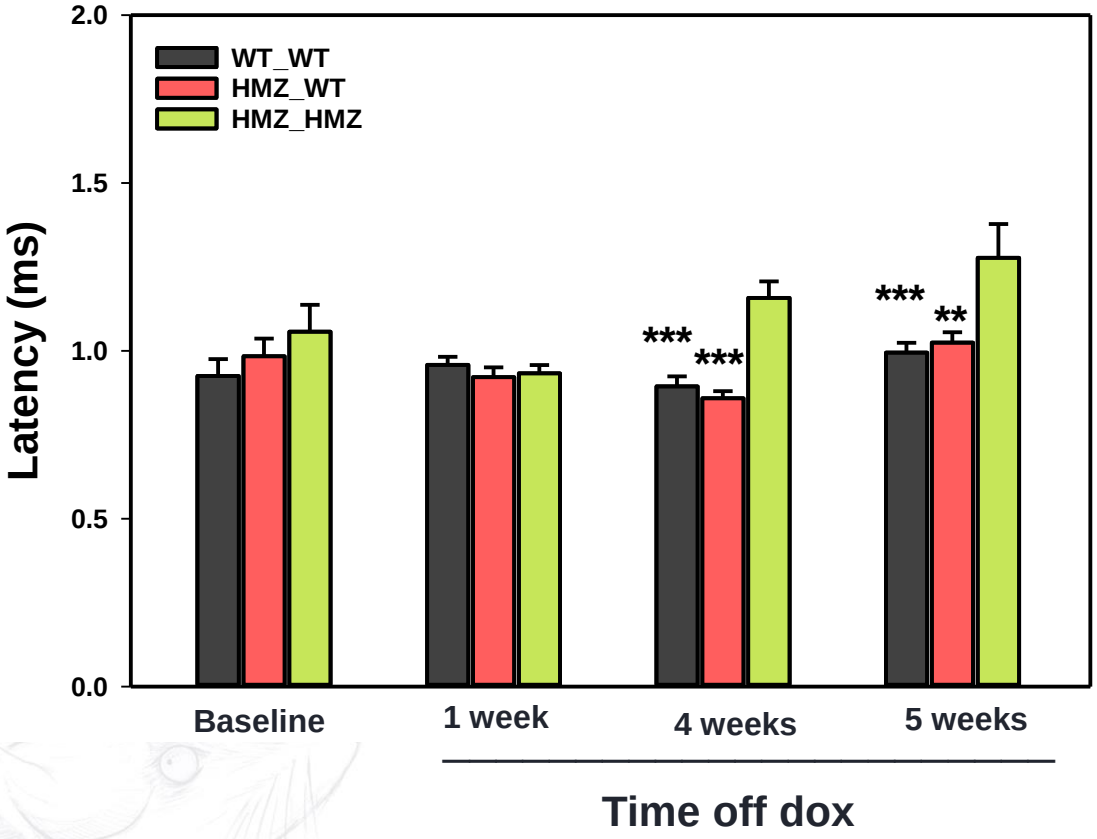
Body weights



*** p<0.001 WT_WT and WT_HMZ compared to HMZ_HMZ, RM ANOVA

Onset latency

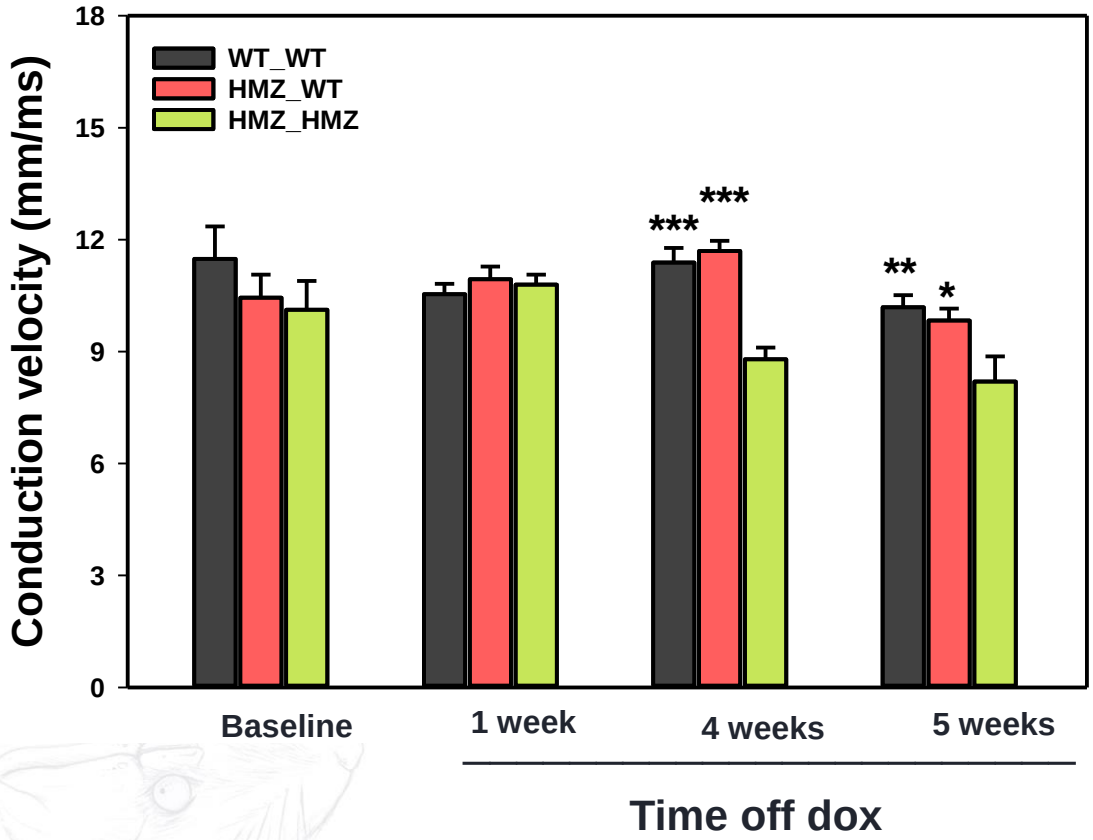
- significant increase in the onset latency of muscle (gastrocnemius) activation in response to motor nerve stimulation



***p<0.001,**p<0.01 compared to HMZ_HMZ

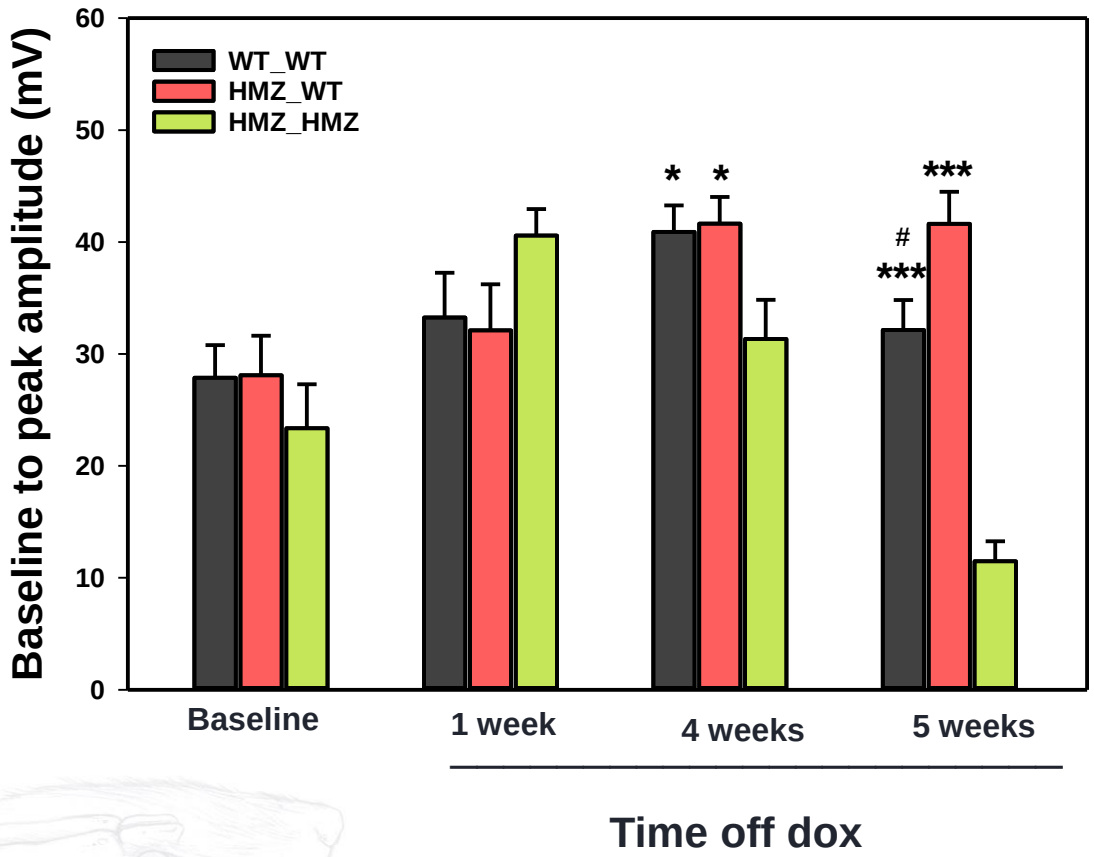
Nerve conduction velocity

- decrease in nerve conduction velocity at 4 and 5 weeks after removal of Dox



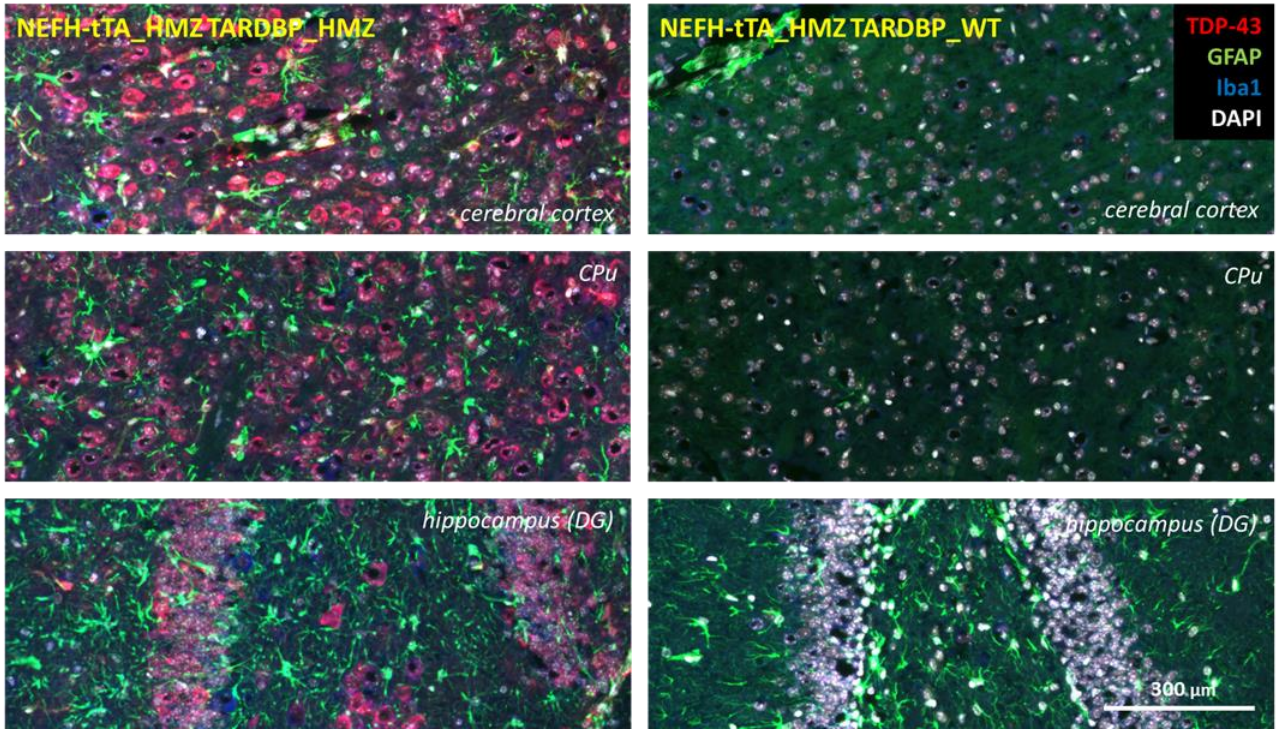
***p<0.001, **p<0.01, *p<0.05 compared to HMZ_HMZ

TDP43 mice show a decrease in CMAP Amplitude



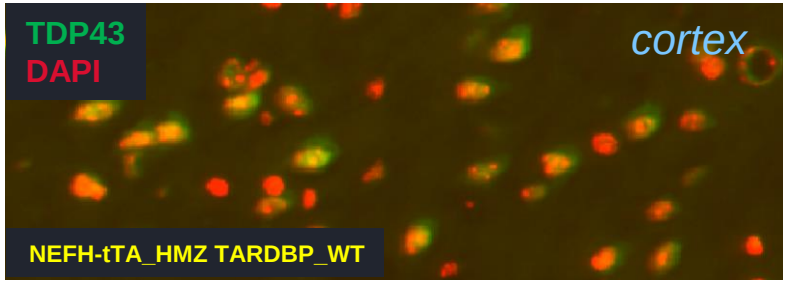
***p<0.001, *p<0.05 compared HMZ_HMZ ; #p<0.05 compared to HMZ_WT , one-way ANOVA

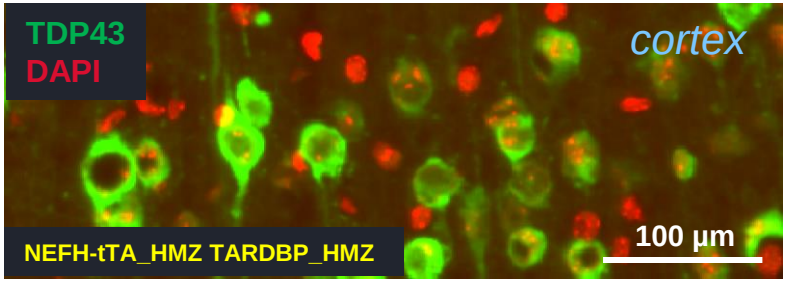
Histology in brain samples

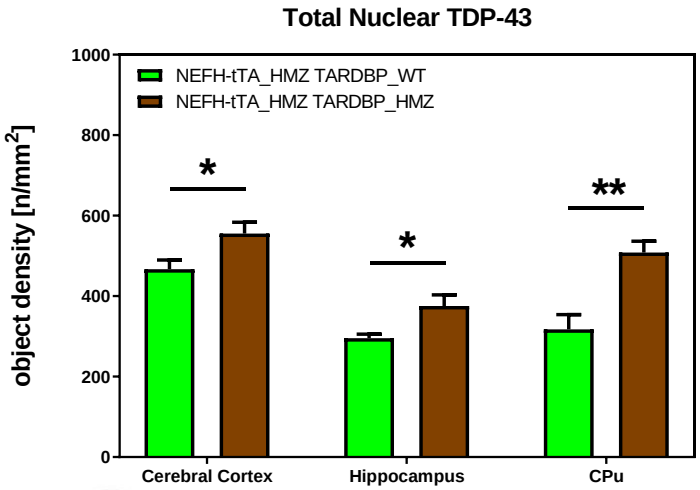


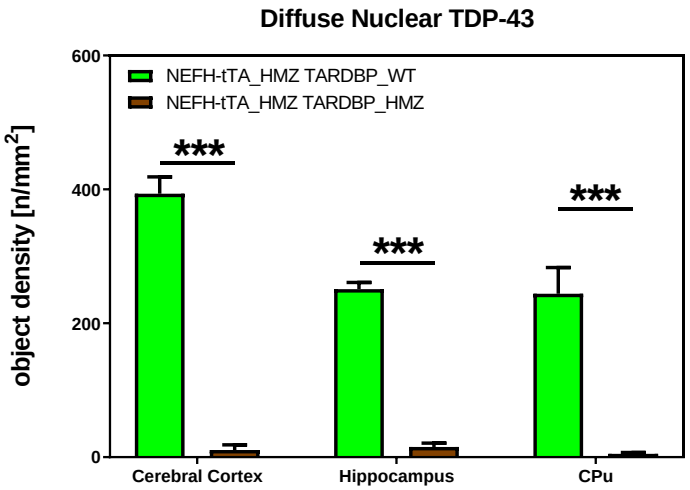
We examined brain TDP pathology as well as neuroinflammation in TDP 43 mice compared to littermate controls. TDP43 mice show a strong overexpression of TDP protein with perinuclear cytoplasmic accumulation of TARDBP protein and focal inclusions. Affected brain regions are the cerebral cortex, hippocampus, basal ganglia and cerebellum. Astrogliosis accompanies the overexpression in these regions as well as increased microglial activation.

TDP-43 Quantification – density on nuclei



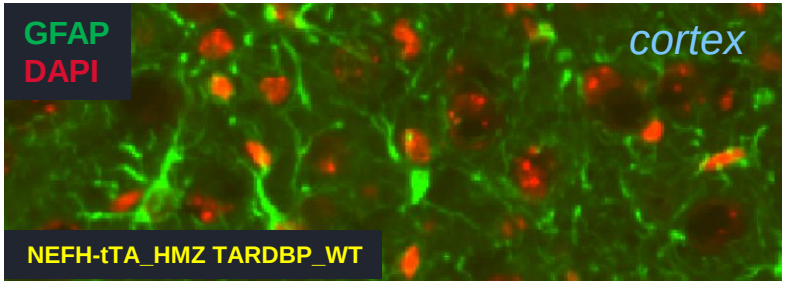


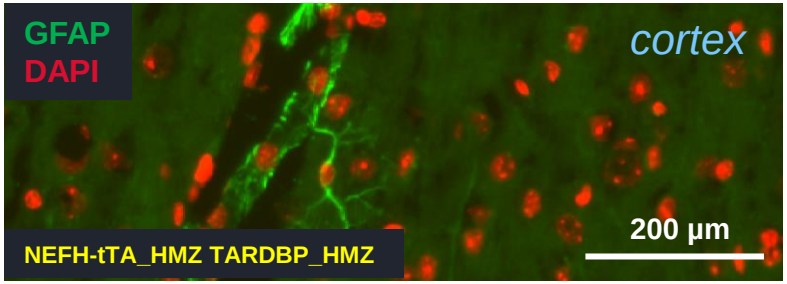


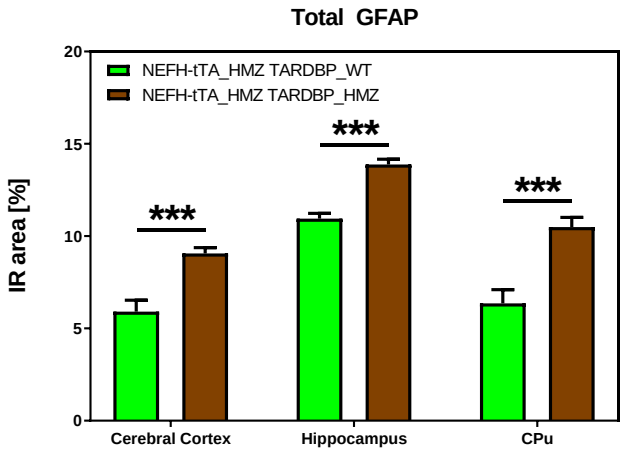


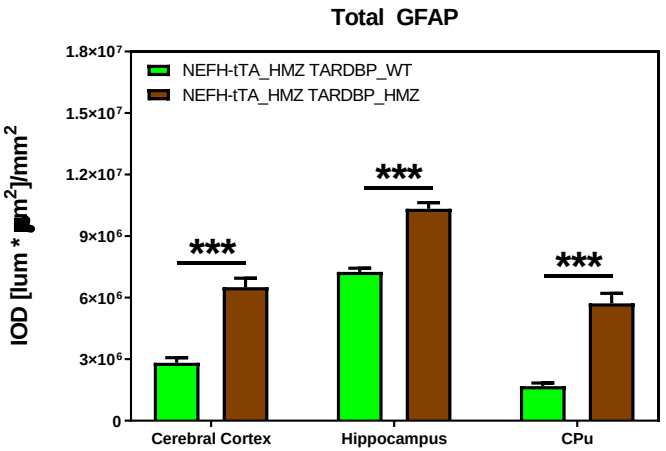
- Overall nuclear object density is greater in HMX compared to WT
- Diffuse signal at lower intensity as found in WT is close to absent in HMZ, since TDP-43 spreads from nucleus to cytoplasm.

GFAP quantification – integrated optical density





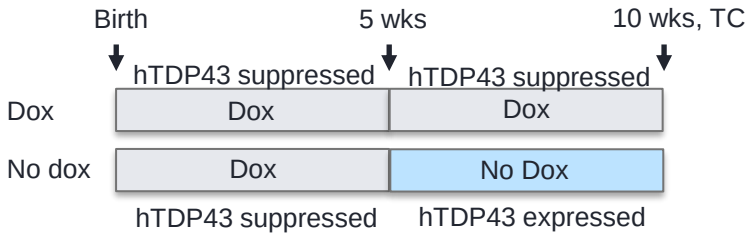




- Astrogliosis is significant in the cerebral cortex, hippocampus and caudate putamen (dorsal striatum).

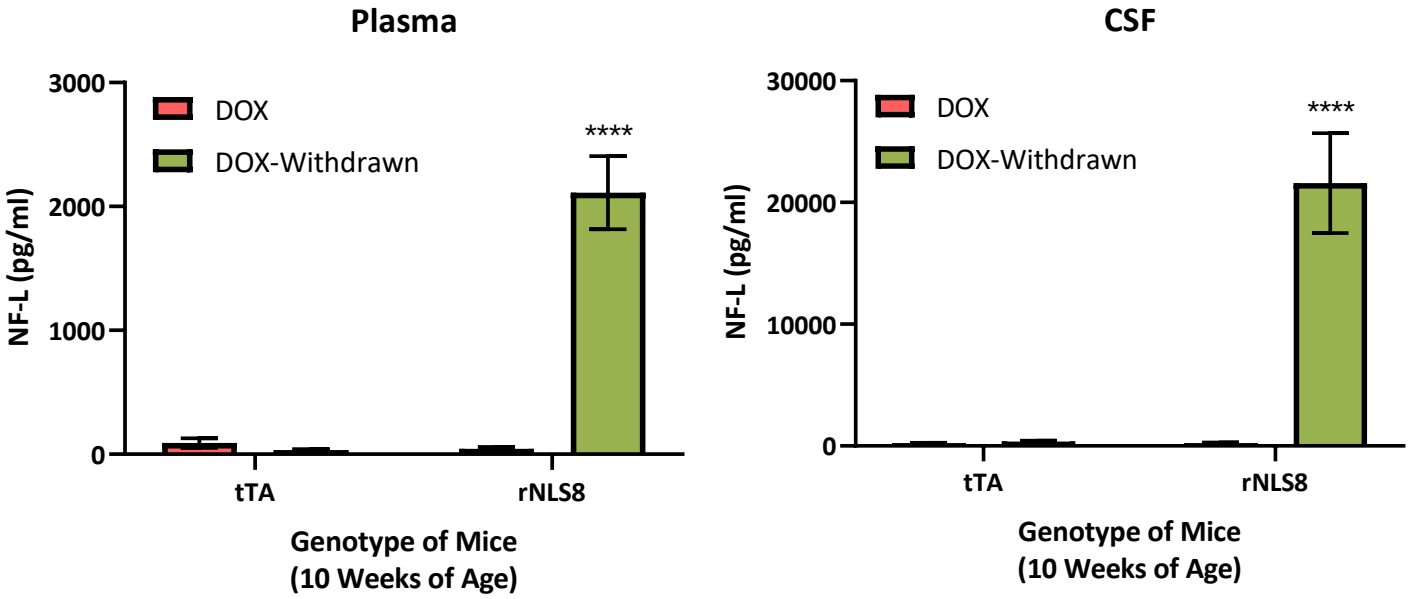
Biomarker

- First 5 weeks on dox
  - Dox group given dox until tissue collection
  - Non-dox group taken off dox at 5 weeks
  - Both groups TC at 10 weeks



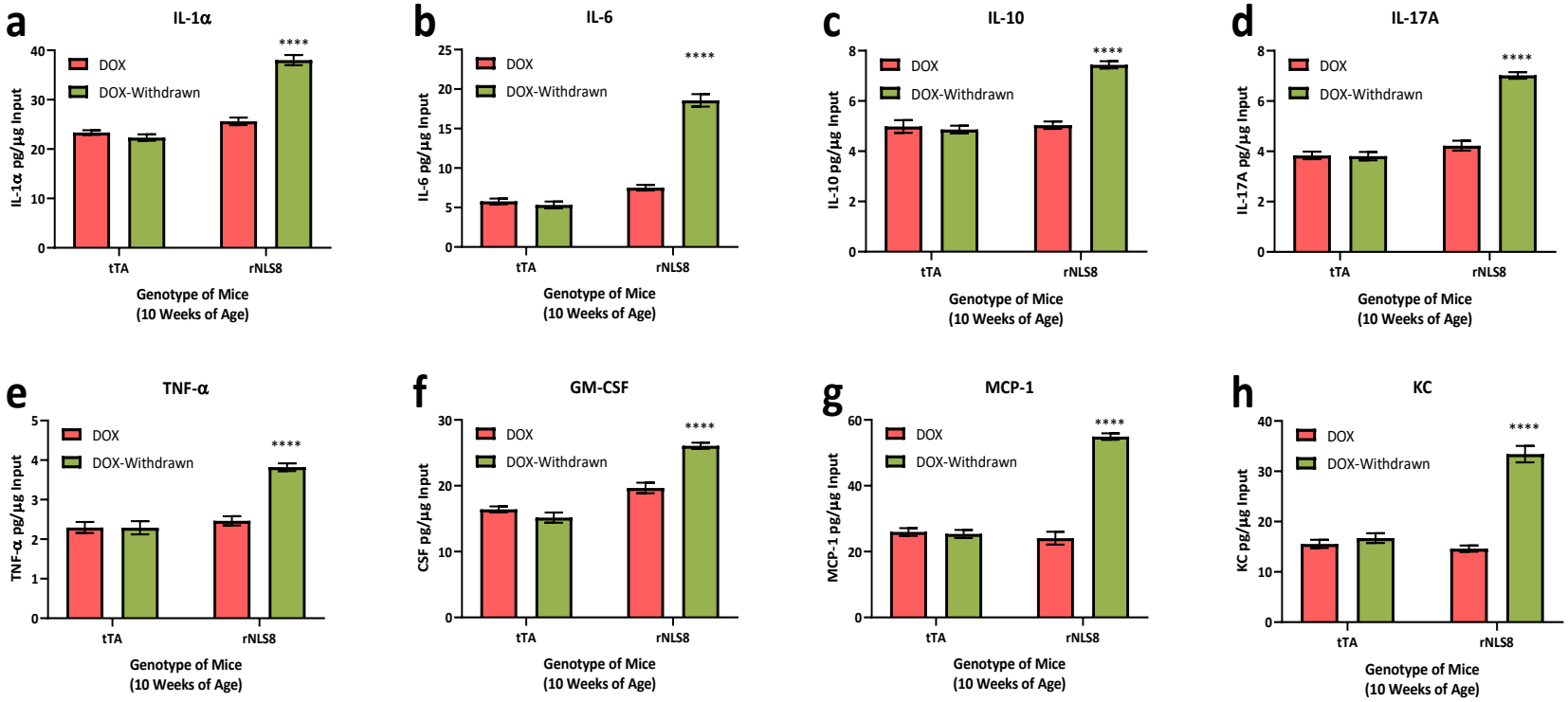
- Terminal plasma, CSF, brain, spinal cord collected NFL assay on Quanterix – biomarker for neurodegeneration
- Inflammatory transcripts by QPCR
- Inflammatory protein markers by multiplex Luminex assay

Neurofilament Light Chain (NFL)



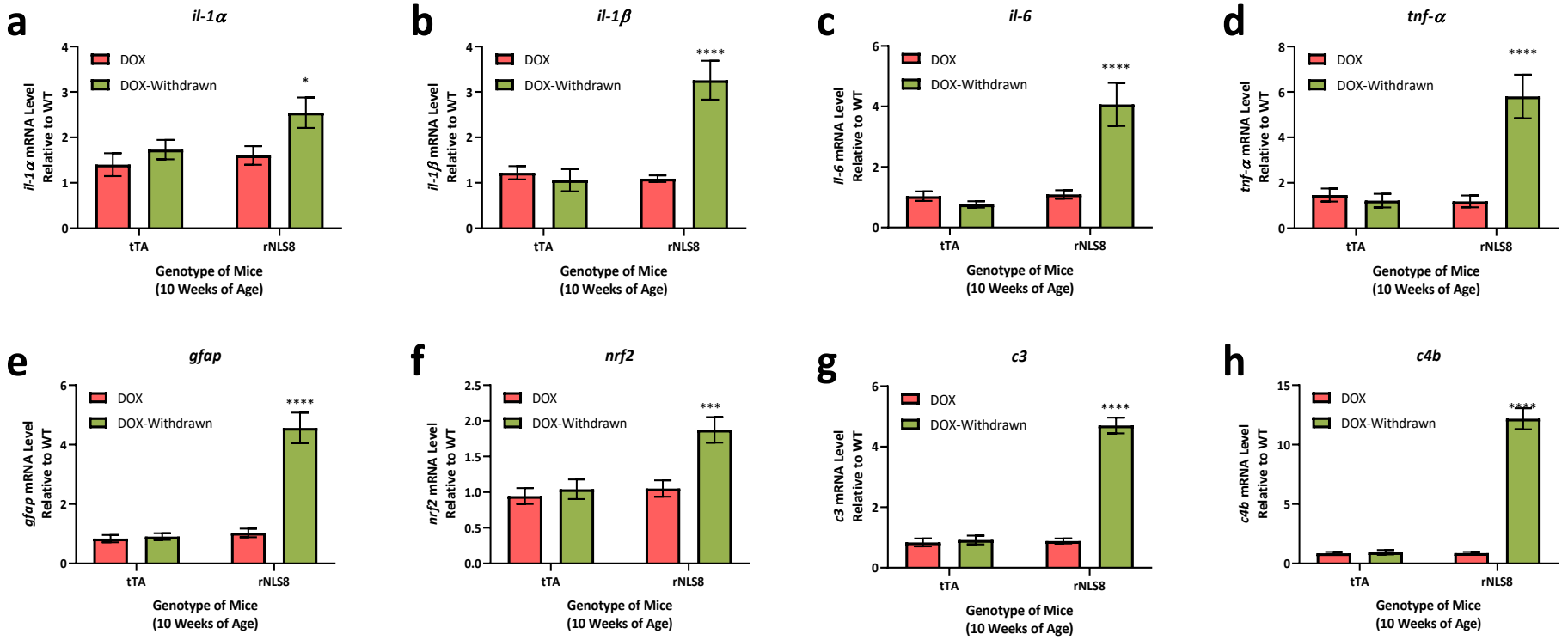
Plasma and CSF NFL concentrations were significantly increased in 5 weeks old rNLS8 mice that were maintained off doxycycline for 5 weeks, as compared to those maintained on Doxycycline treatment for the same time. NFL levels were measured by SIMOA Quanterix technology. DOX withdrawal had no statistically significant effect on tTA control mice (n=8).

Increased Inflammatory Protein Markers in rNLS8 mice



Inflammatory protein, IL-1a, IL-6, IL-10, IL-17A, TNF-α, GM-CSF, MCP-1 and KC, levels were significantly elevated in cortical of rNLS8-Dox off mice as compared to those of DOX on mice. Proteins were measured by multiplex Luminex assay and presented as normalized to the input protein

Increased Inflammatory Markers in rNLS8 mice



Transcript expression levels for inflammatory markers il-1α, il-1ß, il-6, tnf-α, gfap, nrf2, c3 and c4b were significantly elevated in cortical lysates of rNLS8 Dox off group compared to those of DOX on group. mRNA expression levels were measured by RT-qPCR and presented as relative to WT cohort