

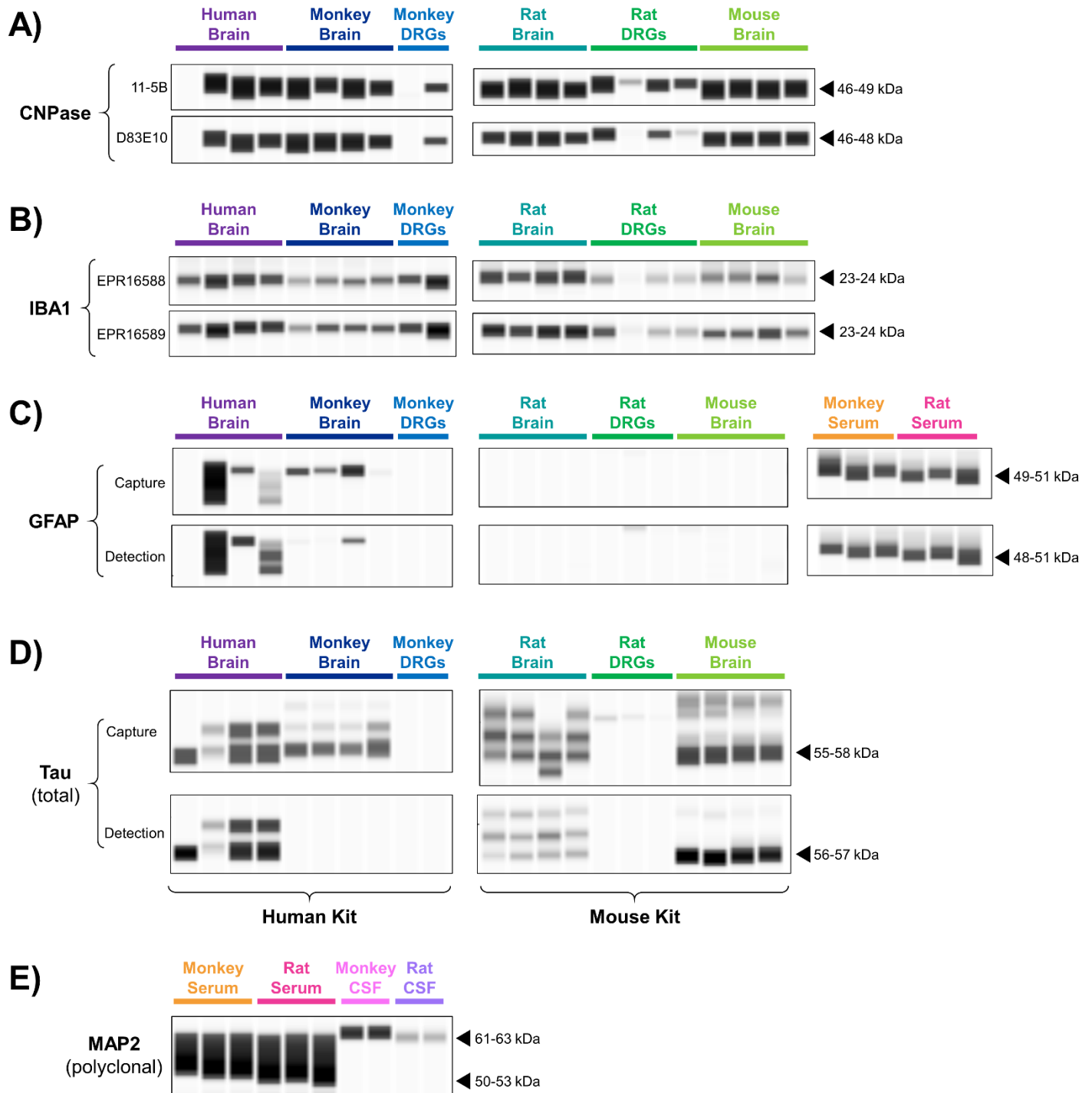
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**Supplemental information**

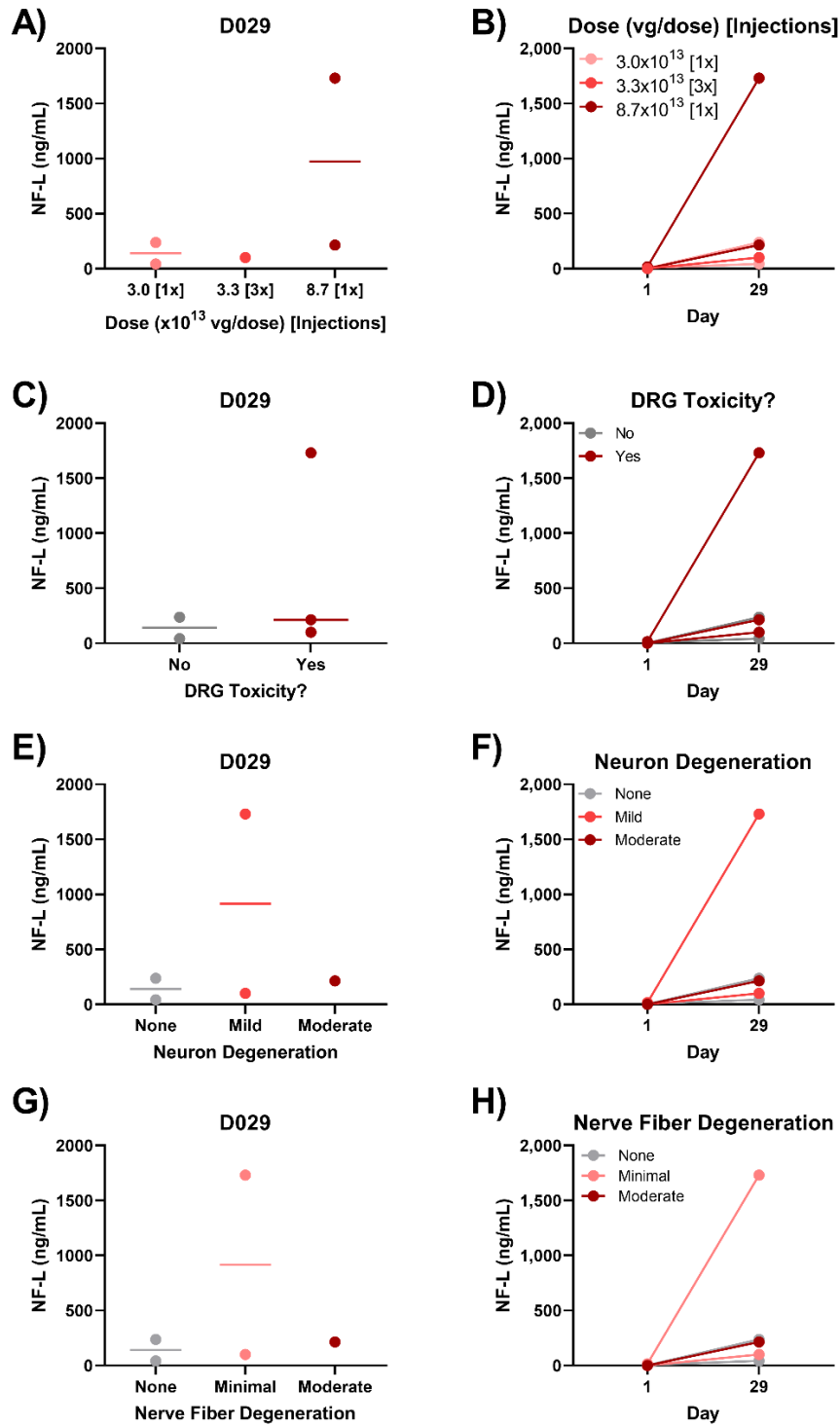
**Circulating neurofilament light chain as a promising  
biomarker of AAV-induced dorsal root ganglia  
toxicity in nonclinical toxicology species**

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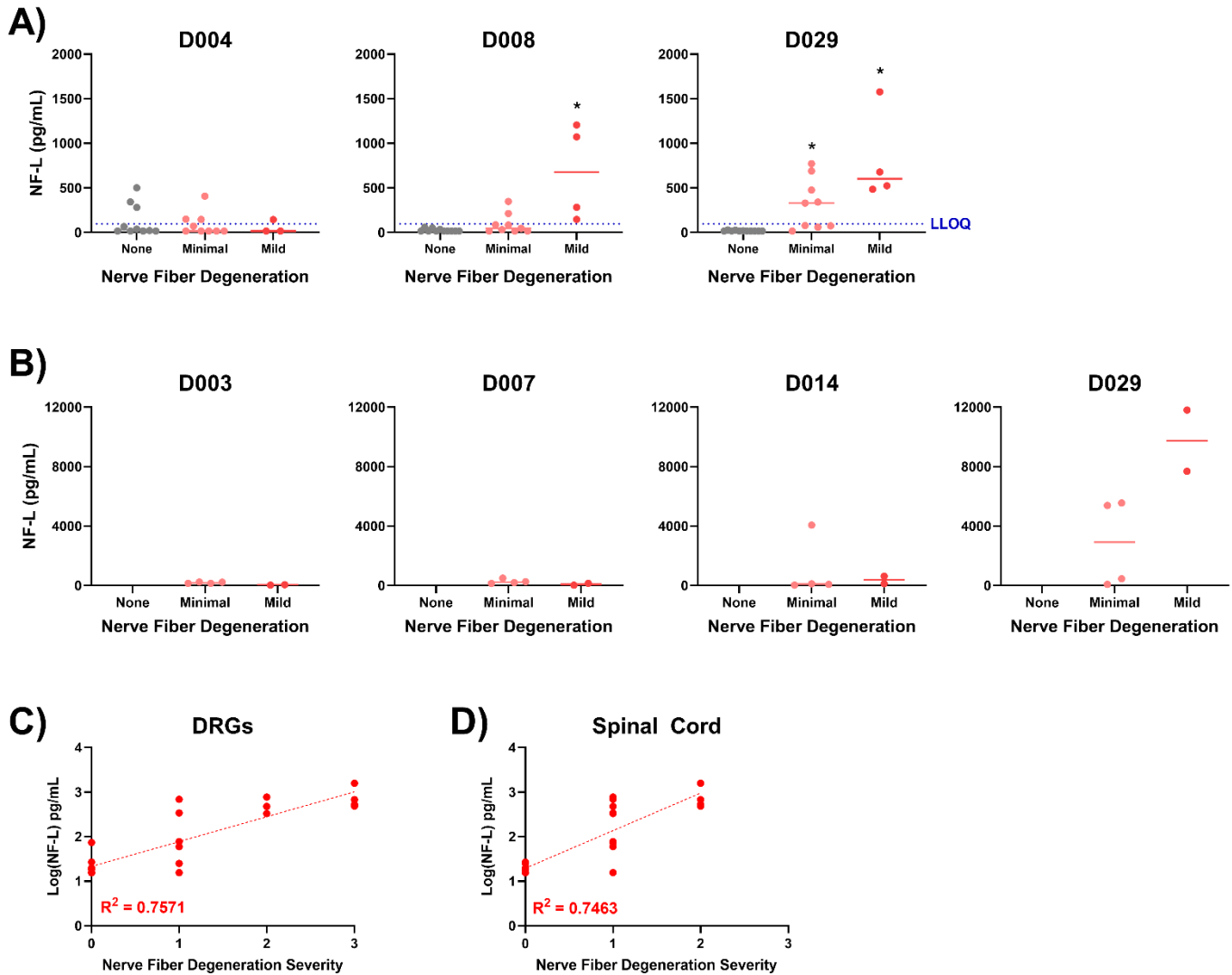
## SUPPLEMENTARY FIGURES



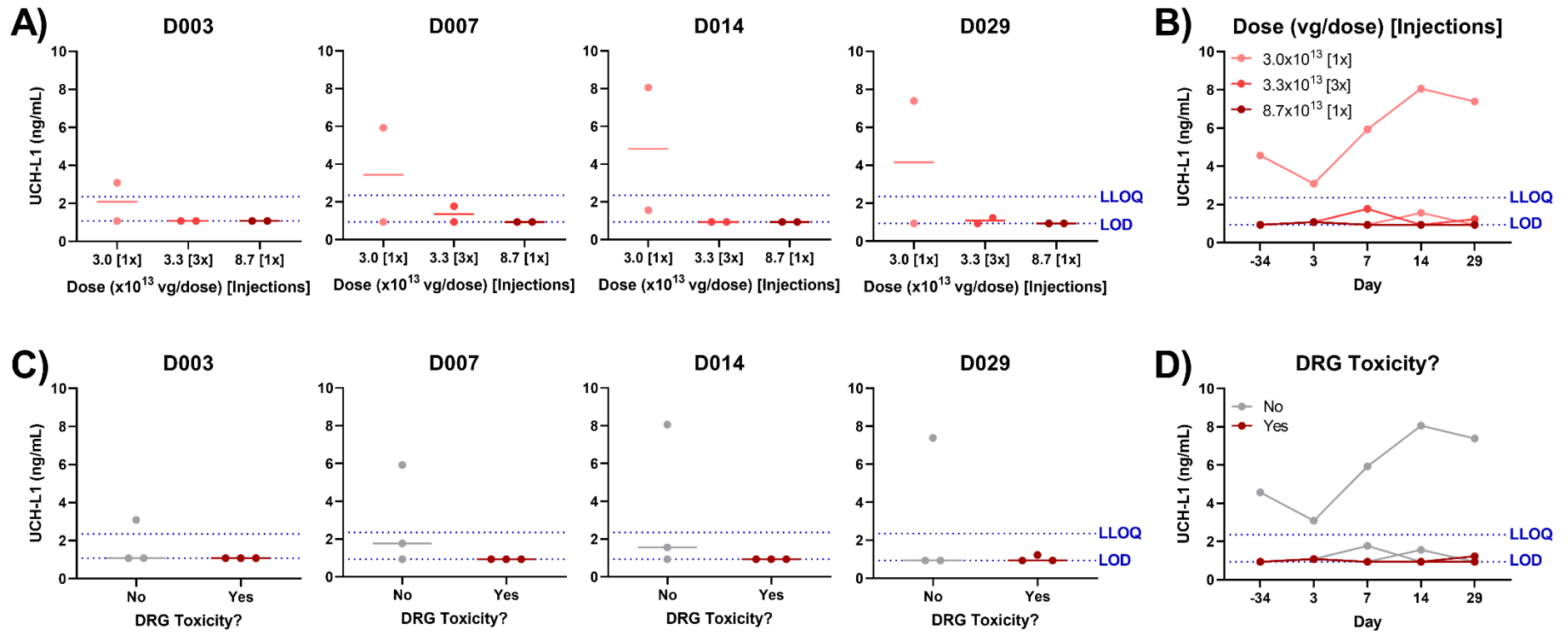
**Supplementary Figure S1: Detection of candidate biomarkers in brain, dorsal root ganglia (DRG), and biofluids from human and nonclinical species.** Wes capillary electrophoresis (ProteinSimple) was used to evaluate antibody cross-reactivity against brain homogenate (n=4) from human, cynomolgus monkey, Wistar Han rat, and CD1 mouse. Dissected DRGs (n=2-4), serum (n=3), and/or CSF (n=2) from monkey and rat were also assessed. A) CNPase antibody clones 11-5B and D83E10 were obtained from Millipore (MAB326) and Cell Signaling Technologies (5664), respectively. B) IBA1 antibody clones EPR16588 and ERP16589 were obtained from Abcam (ab178846 and ab178847, respectively). Meso Scale Discovery (MSD) R-PLEX kits were available for C) GFAP (human: F211M) and D) total Tau (human: F218D; mouse: F228E). E) The polyclonal antibody against MAP2 was obtained from PhosphoSolutions (1099-MAP2). Chemiluminescence signals were analyzed with Compass software (ProteinSimple), where image contrast was adjusted separately for each antibody to visualize the presence vs. absence of the target peak(s).



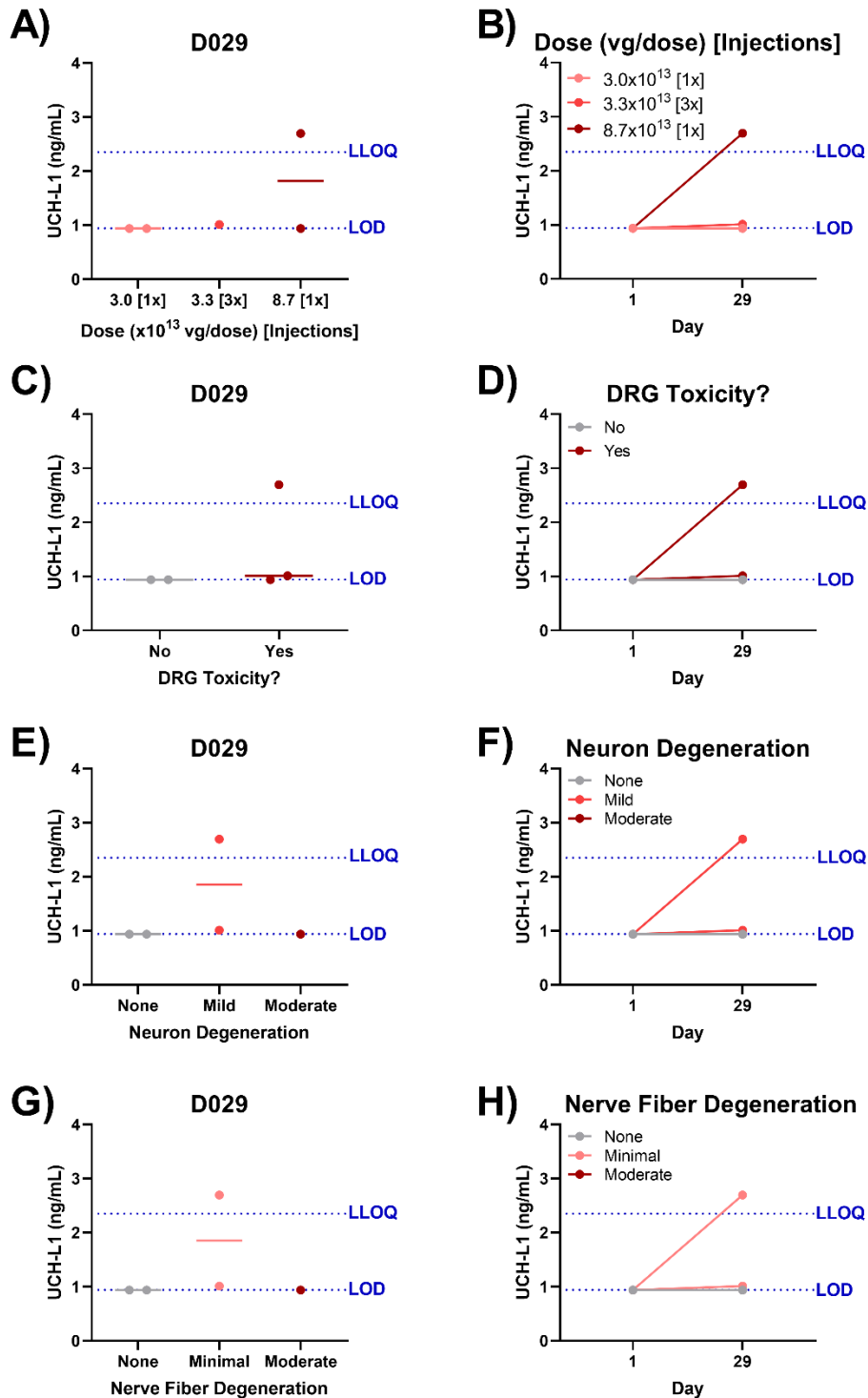
**Supplementary Figure S2: NF-L in cerebrospinal fluid (CSF) from cynomolgus monkeys exhibiting AAV-induced dorsal root ganglia (DRG) toxicity.** Male cynomolgus macaques ( $n=2$  per group) were injected with  $3.0 \times 10^{13}$  [1x],  $3.3 \times 10^{13}$  [3x], or  $8.7 \times 10^{13}$  [1x] vg/dose AAV vector via intrathecal catheter. CSF was collected on Day 1 (pre-dose) and 29, while histologic DRG lesions were assessed on Day 29. NF-L in CSF was quantified using an in-house duplex Meso Scale Discovery (MSD) assay with a functional lower limit of quantification (LLOQ) of 144 pg/mL. NF-L was plotted against A) AAV dose, C) presence/absence of DRG toxicity (neuronal and/or nerve fiber degeneration), E) severity of DRG neuron degeneration/necrosis, and G) severity of DRG nerve fiber degeneration. Statistical significance ( $p \leq 0.05$ ) was evaluated through one-way ANOVA analysis followed by Dunnett's multiple comparisons test (A, E, G) or t-test (C) performed in GraphPad Prism 9.0.0. NF-L for each animal has also been plotted by B) AAV dose, D) presence/absence of DRG toxicity (neuronal and/or nerve fiber degeneration), F) severity of DRG neuron degeneration/necrosis, and H) severity of DRG nerve fiber degeneration. Any values which fell below the limit of detection (LOD) were plotted as the functional LOD for that plate.



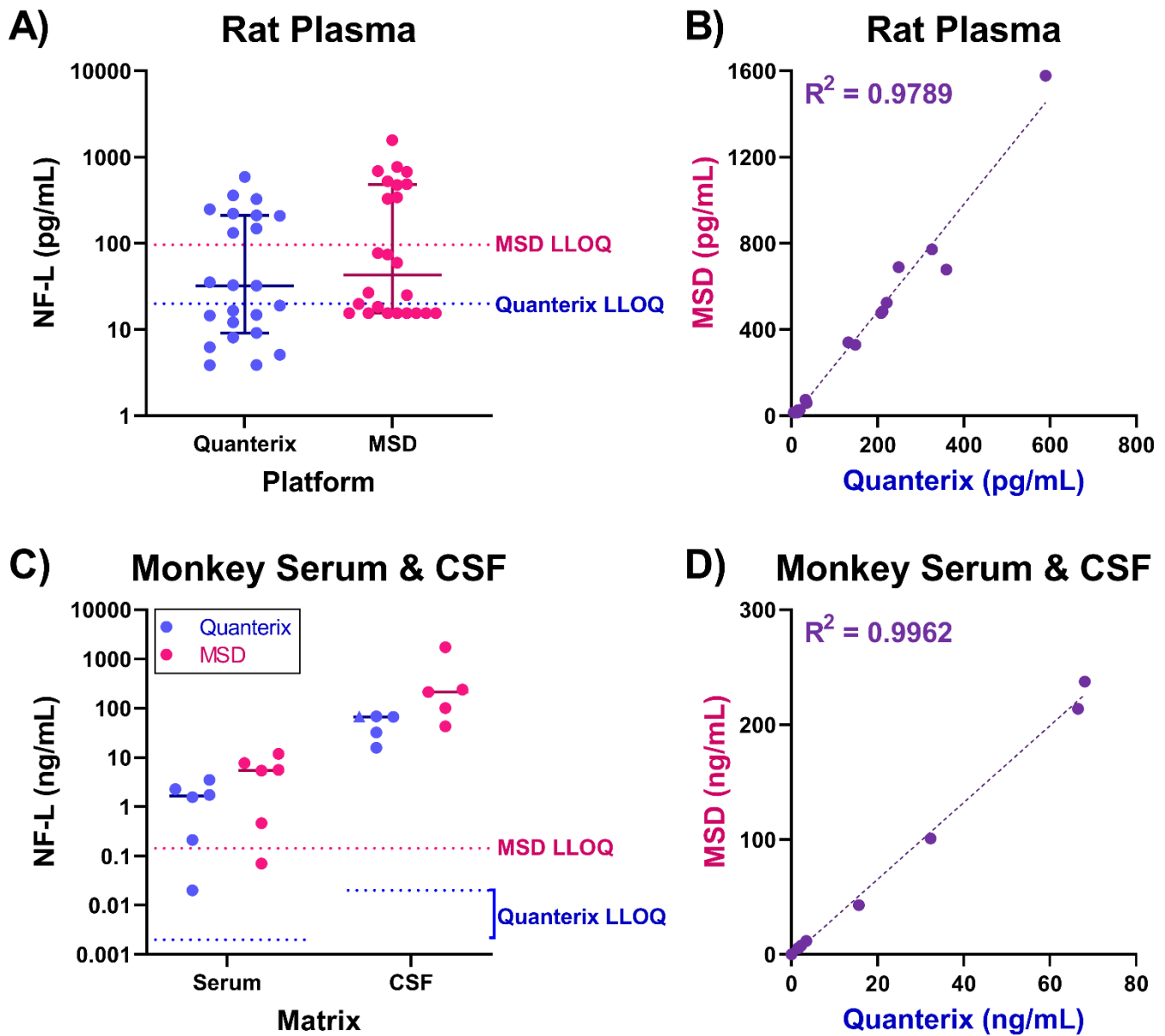
**Supplementary Figure S3: Circulating NF-L is associated with the severity of nerve fiber degeneration in the spinal cord.** A) Male Wistar Han rats ( $n=6$ ) were intravenously injected with a bolus dose of 0,  $2 \times 10^{13}$ ,  $5 \times 10^{13}$ , or  $1 \times 10^{14}$  vg/kg AAV vector. Plasma was collected 4, 8, and 29 days after injection, while histologic spinal cord lesions were assessed on Day 29. NF-L in plasma was quantified using an in-house duplex Meso Scale Discovery (MSD) assay with a functional lower limit of quantification (LLOQ) of 96 pg/mL. B) Male cynomolgus macaques ( $n=2$ ) were injected with  $3.0 \times 10^{13}$  [1x],  $3.3 \times 10^{13}$  [3x], or  $8.7 \times 10^{13}$  [1x] vg/dose AAV vector via intrathecal catheter. Serum was collected 3, 7, 14, and 29 days after injection, while histologic spinal cord lesions were assessed on Day 29. NF-L in serum was quantified using an in-house duplex Meso Scale Discovery (MSD) assay with a functional LLOQ of 144 pg/mL. For each species, NF-L has been plotted against the severity of nerve fiber degeneration in the spinal cord. Statistical significance ( $* p \leq 0.05$ ) was evaluated through a Kruskal-Wallis test followed by Dunn's multiple comparison test (A) or t-test (B) performed in GraphPad Prism 9.0.0. Any values which fell below the limit of detection (LOD) were plotted as the functional LOD for that plate. For rats, the correlation between  $\log(\text{NF-L})$  and nerve fiber degeneration is shown for C) dorsal root ganglia (DRGs) and D) the spinal cord, where the coefficient of determination ( $R^2$ ) was determined through linear regression analysis. Severity grades of none, minimal, mild, and moderate have been assigned numerical scores of 0, 1, 2, and 3, respectively.



**Supplementary Figure S4: UCH-L1 in serum from cynomolgus monkeys exhibiting AAV-induced dorsal root ganglia (DRG) toxicity.** Male cynomolgus macaques ( $n=2$  per group) were injected with  $3.0 \times 10^{13}$  [1x],  $3.3 \times 10^{13}$  [3x], or  $8.7 \times 10^{13}$  [1x] vg/dose AAV vector via intrathecal catheter. Serum was collected 3, 7, 14, and 29 days after injection, while histologic DRG lesions were assessed on Day 29. UCH-L1 in serum was quantified using an in-house duplex Meso Scale Discovery (MSD) assay. The blue dashed lines indicate the limit of detect (LOD) and lower limit of quantification (LLOQ) for the assay. UCH-L1 was plotted against A) AAV dose and C) presence/absence of DRG toxicity (neuronal and/or nerve fiber degeneration). Statistical significance ( $p \leq 0.05$ ) was evaluated through one-way ANOVA analysis followed by Dunnett's multiple comparisons test (A) or t-test (C) performed in GraphPad Prism 9.0.0. The UCH-L1 profile for each animal was also plotted by B) AAV dose and D) presence/absence of DRG toxicity (neuronal and/or nerve fiber degeneration). Any values which fell below the limit of detection (LOD) were plotted as the functional LOD for that plate.



**Supplementary Figure S5: UCH-L1 in cerebrospinal fluid (CSF) from cynomolgus monkeys exhibiting AAV-induced dorsal root ganglia (DRG) toxicity.** Male cynomolgus macaques (n=2 per group) were injected with  $3.0 \times 10^{13}$  [1x],  $3.3 \times 10^{13}$  [3x], or  $8.7 \times 10^{13}$  [1x] vg/dose AAV vector via intrathecal catheter. CSF was collected on Day 1 (pre-dose) and 29, while histologic DRG lesions were assessed on Day 29. UCH-L1 in CSF was quantified using an in-house duplex Meso Scale Discovery (MSD) assay. The blue dashed lines indicate the limit of detect (LOD) and lower limit of quantification (LLOQ) for the assay. UCH-L1 was plotted against A) AAV dose, C) presence/absence of DRG toxicity (neuronal and/or nerve fiber degeneration), E) severity of DRG neuron degeneration/necrosis, and G) severity of DRG nerve fiber degeneration. Statistical significance ( $p \leq 0.05$ ) was evaluated through one-way ANOVA analysis followed by Dunnett's multiple comparisons test (A, E, G) or t-test (C) performed in GraphPad Prism 9.0.0. The UCH-L1 profile for each animal was also plotted by B) AAV dose, D) presence/absence of DRG toxicity (neuronal and/or nerve fiber degeneration), F) severity of DRG neuron degeneration/necrosis, and H) severity of DRG nerve fiber degeneration. Any values which fell below the limit of detection (LOD) were plotted as the functional LOD for that plate.



**Supplementary Figure S6: NF-L values detected with an in-house Meso Scale Discovery (MSD) assay are highly correlated with Quanterix Simoa HD-X values.** NF-L in A) rat plasma and C) cynomolgus monkey serum and cerebrospinal fluid (CSF) was evaluated using both the Quanterix Simoa Neurology 4-Plex Panel (blue) and an in-house duplex MSD assay (pink). The blue and pink dashed lines indicate the lower limit of quantification (LLOQ) for the Quanterix and MSD assays, respectively. The correlation between Quanterix and MSD values is shown for B) rats and D) monkeys, where the coefficient of determination ( $R^2$ ) was determined through linear regression analysis. Any values which fell below the limit of detection (LOD) were plotted as the functional LOD for that assay. Note: The triangular data point (plot C) represents a sample which saturated the assay signal and thus has been plotted as the highest value obtained on the plate which did not saturate the signal. This sample was excluded from the correlation analysis (D).

## SUPPLEMENTARY TABLES

**Supplementary Table S1:** Sensitivity of Meso Scale Discovery (MSD) duplex assay

<b>Parameter</b>	<b>NF-L (pg/mL)</b>	<b>UCH-L1 (pg/mL)</b>
<b>Limit of Detection (LOD)</b>		
Analytical (average)	7.37	213
Functional (DF = 4)	29.50	851
Functional (DF=6)	44.24	1,277
<b>Lower Limit of Quantification (LLOQ)</b>		
Analytical	24	391
Functional (DF = 4)	96	1,564
Functional (DF=6)	144	2,346
<b>Upper Limit of Quantification (ULOQ)</b>		
Analytical	25,000	400,000
Functional (DF = 4)	100,000	1,600,000
Functional (DF=6)	150,000	2,400,000





**Supplementary Table S3:** 'Fit-for-purpose' validation criteria

<b>Test</b>	<b>Replicates</b>	<b>Samples</b>	<b>Criteria</b>
<b>INTRA-assay Precision &amp; Accuracy</b>	1 plate ≥ 3 replicates	<b>QC samples in matrix</b>	<b>For QCs:</b> CV ≤ 25% between replicates Bias = ± 25%
		<ul style="list-style-type: none"> <li>• High [ ]</li> <li>• Mid [ ]</li> <li>• Low [ ]</li> </ul>	
		<b>Detection limits in diluent</b>	<b>For ULOQ &amp; LLOQ:</b> CV ≤ 25% between replicates Bias = ± 30%
		<ul style="list-style-type: none"> <li>• ULOQ</li> <li>• LLOQ</li> </ul>	
<b>INTER-assay Precision &amp; Accuracy</b>	3 plates ≥ 3 replicates	<b>QC samples in matrix</b>	<b>For QCs:</b> CV ≤ 25% between plates Bias = ± 25%
		<ul style="list-style-type: none"> <li>• High [ ]</li> <li>• Mid [ ]</li> <li>• Low [ ]</li> </ul>	
		<b>Detection limits in diluent</b>	<b>For ULOQ &amp; LLOQ:</b> CV ≤ 25% between plates Bias = ± 30%
		<ul style="list-style-type: none"> <li>• ULOQ</li> <li>• LLOQ</li> </ul>	
<b>Freeze/Thaw Stability</b>	1 plate 3 replicates	1-4 freeze/thaw cycles of High, Mid, and Low QC samples	Recovery vs. Initial Thaw = 75-125%
<b>Dilution Linearity</b>	1 plate 3 replicates	Serial dilution (2- to 64-fold) in matrix	R <sup>2</sup> ≥ 0.9