Supplemental Information

Profilin1 delivery tunes cytoskeleton dynamics towards CNS axon regeneration

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Supplemental Figures and Legends
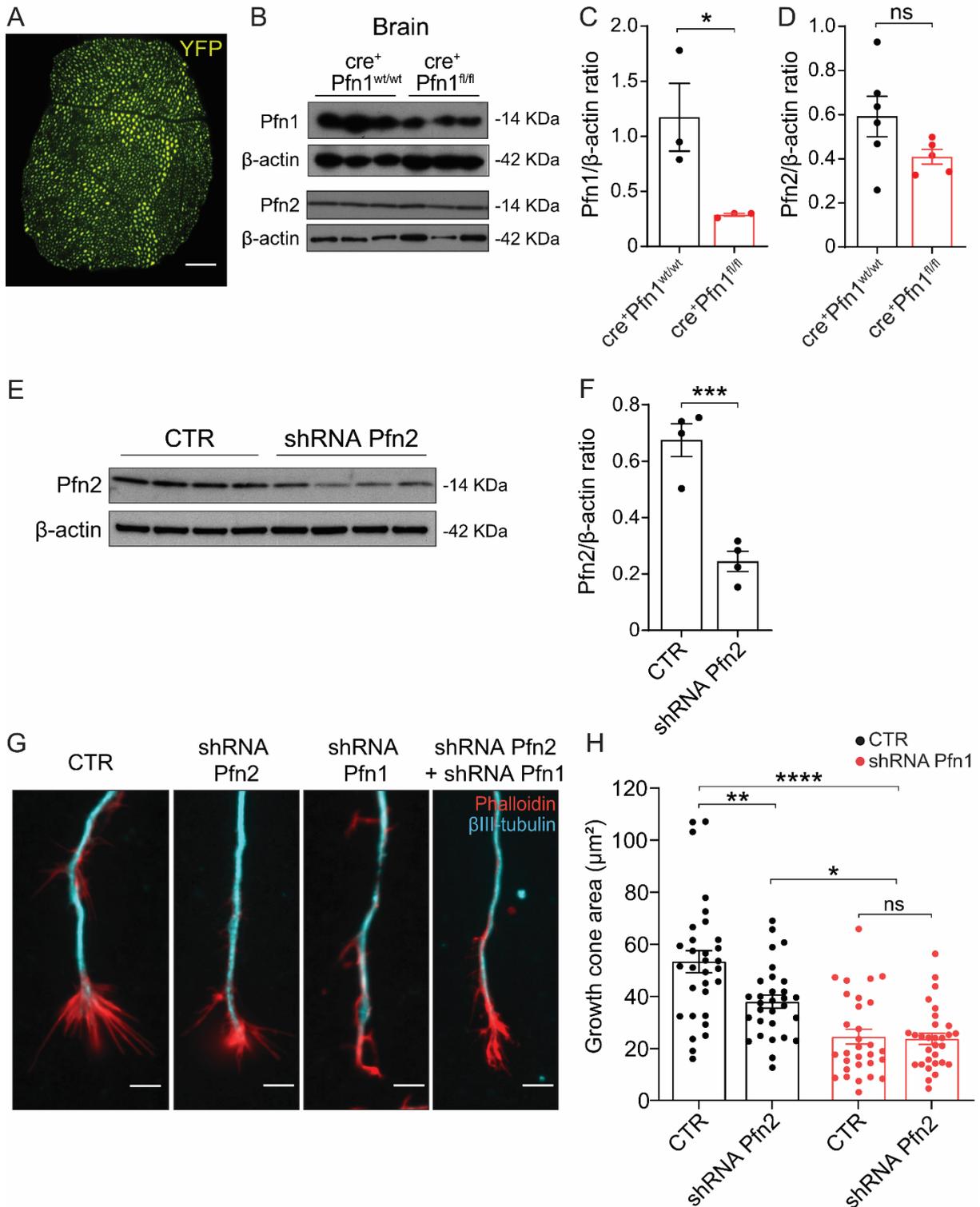
Supplemental Figure 1. Analysis of cre⁺Pfn^wt/wt and cre⁺Pfn^fl/fl mice. (A) Representative
image of a sciatic nerve cross section collected from a cre⁺Pfn1^wt/wt mouse expressing YFP in
myelinated axons. Scale bar: 50 μm. **(B)** Western blot and respective quantification by
densitometry showing **(C)** Pfn1 and **(D)** Pfn2 levels in brains of cre⁺Pfn1^wt/wt and cre⁺Pfn1^fl/fl
mice. β-actin signal was used as a loading control. Data represent mean ± SEM (*P < 0.05,
ns: not significant, t-test, *n* = 5-6 animals/condition). **(E)** Western blot analysis of Pfn2
knockdown in CAD cells 48 hr after transfection with a plasmid expressing shRNA Pfn2. β-

actin signal was used as a loading control. (**F**) Quantification of (E). Data represent mean ± SEM (***$P < 0.001$, t-test). (**G**) βIII-tubulin (cyan) and actin (red) in growth cones of hippocampal neurons co-nucleofected with a GFP and either/both Pfn1 shRNA and Pfn2 shRNA expressing plasmids. Scale bar: 4 μm. (**H**) Quantification of growth cone area related to (G). Only GFP^{+} neurons were chosen for quantification. Data represent mean ± SEM (*$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$, ns: not significant, one-way ANOVA Tukey's posttest, *n* = 30-31 neurons/condition).
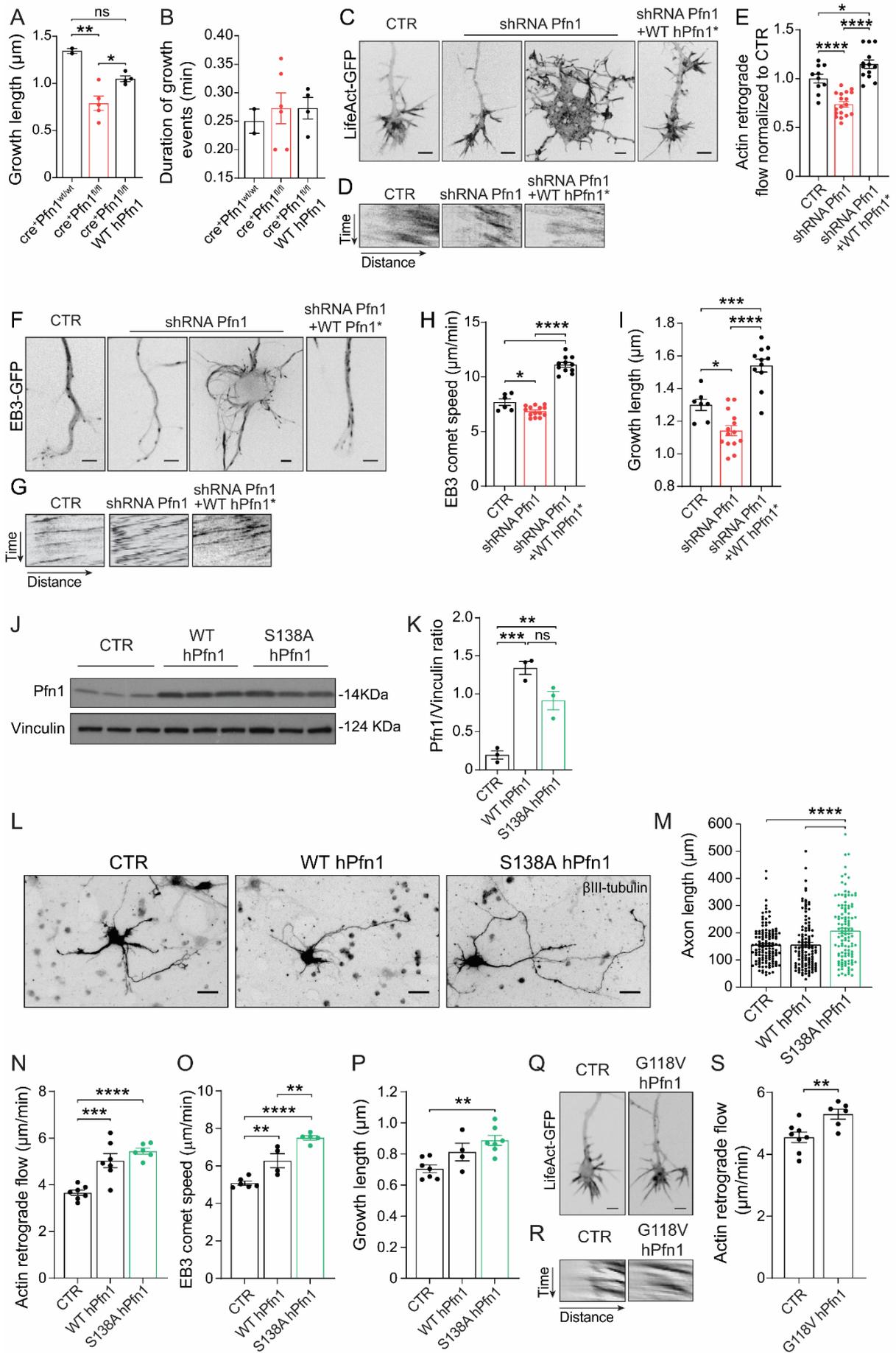
Supplemental Figure 2. Pfn1 levels regulate actin and MT dynamics. (**A**) Quantification of growth length and (**B**) duration in cre[+]Pfn1 DRG neurons. Data represent mean ± SEM (*P < 0.05, **P < 0.01, ns: not significant, one-way ANOVA Tukey's posttest, n = 2-6 growth cones/condition). (**C**) LifeAct-GFP imaging in hippocampal neurons expressing empty (CTR) or shRNA Pfn1 plasmids. Middle panels (shRNA Pfn1) show representative stage 3 (left) and stage 1 (right) growth cones; Scale bar: 4 µm. (**D**) Kymographs and (**E**) actin flow quantification related to (C). (**F**) EB3-GFP imaging related to (C). Scale bar: 3 µm. (**G**) Kymographs and (**H**) quantification of EB3 comet speed and (**I**) growth length, related to (F). Data in (E), (H) and (I) represent mean ± SEM (*P < 0.05, ***P < 0.001, ****P < 0.0001, one-way ANOVA Tukey's posttest, n = 6-18 growth cones/condition). (**J**) Western blot and (**K**) densitometry of CAD cells expressing WT or S138A hPfn1. Vinculin was used as control. Data represent mean ± SEM (**P < 0.01, ***P < 0.001, ns: not significant, one-way ANOVA Tukey's posttest). (**L**) βIII-tubulin staining of WT and S138A hPfn1 expressing hippocampal neurons. Scale bar: 30 µm. (**M**) Quantification of axon length related to (L). Data represent mean ± SEM (****P < 0.0001, one-way ANOVA Tukey's posttest, n = 117-126 neurons/condition). (**N**) Quantification of actin retrograde flow, (**O**) EB3 comet speed and (**P**) growth length related to (L). Data represent mean ± SEM (**P < 0.01, ***P < 0.001, ****P < 0.0001, one-way ANOVA Tukey's posttest, n = 4-7 growth cones/condition). (**Q**) LifeAct-GFP imaging in hippocampal neurons expressing empty (CTR) or G118V hPfn1 plasmids. (**R**) Kymographs and (**S**) actin flow quantification related to (Q). Data represent mean ± SEM (**P < 0.01, t-test, n = 6-8 growth cones/condition).

A

pAAV-GFP   pAAV-GFP.P2A.   pAAV-GFP.P2A.
            S138A hPfn1   H134S/S138A hPfn1

Pfn1   -14KDa

Vinculin   -124 KDa

B

Pfn1/vinculin ratio

ns

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AAV-GFP
AAV-GFP.P2A.
S138A hPfn1
AAV-GFP.P2A.
H134S/S138A hPfn1

C Brain

AAV-GFP   GFP

Spinal cord

AAV-GFP   d
           v   GFP

D Brain (zoom in)

GFP

Spinal cord (zoom in)

GFP

E DRG

GFP

F   GFP
         d
       r ─┼─ c
         v

AAV-GFP

AAV-GFP.P2A.
S138AhPfn1

1
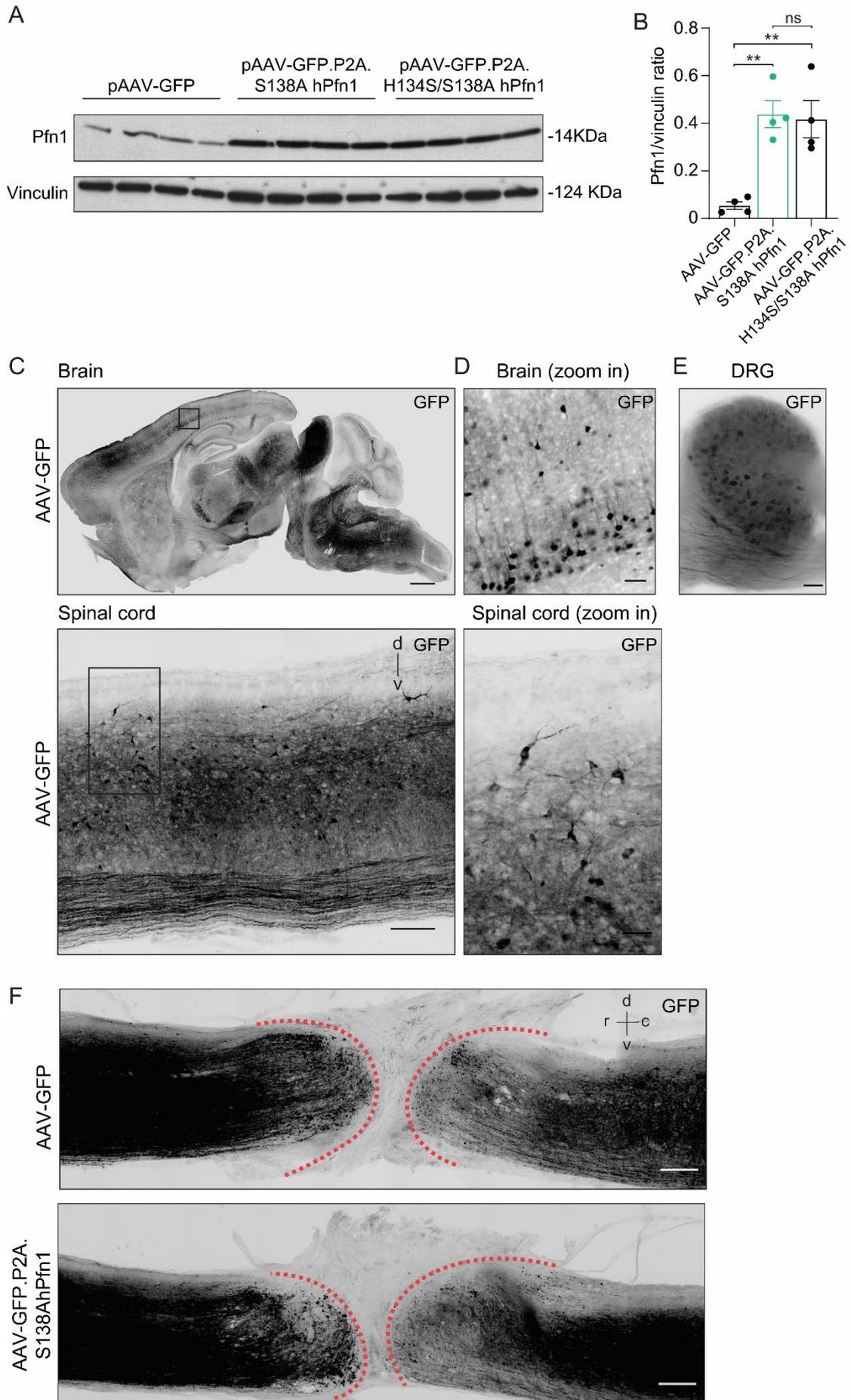
1 **Supplemental Figure 3. Validation of pAAV.GFP.P2A.S138A hPfn1 and**
2 **pAAV.GFP.P2A.H134S/S138A hPfn1 expression in vitro, and in vivo after systemic**
3 **viral delivery.** (**A**) Western blot analysis of Pfn1 expression in CAD 48 hr after transfection
4 with either pAAV-GFP, pAAV-GFP.P2A.S138A hPfn1 or pAAV-GFP.P2A.H134S/S138A
5 hPfn1plasmids. (**B**) Quantification of (A). Data represent mean ± SEM (**$P < 0.01$, ns: not
6 significant, one-way ANOVA Tukey's posttest). (**C**) GFP expression in brain and spinal cord,
7 2 weeks after systemic delivery of AAV-PHP.eB viral particles. Scale bars -brain: 800 µm; -
8 spinal cord: 200 µm; *d*:dorsal, *v*:ventral. (**D**) Zoom-ins of (C). Scale bar: 50 µm. (**E**) GFP
9 expression in DRG, 2 weeks after systemic delivery of AAV-PHP.eB viral particles. Scale
10 bar: 100 µm. (**F**) Low-magnification images of injured spinal cords shown in Figure 6K. Scale
11 bar: 300 µm.