

Supplementary Information

Elk-1 regulates retinal ganglion cell axon regeneration after injury

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3 Supplemental Figures

Figure S1. Validation of Elk-1 and REST constructs.

Figure S2. Full-length western blots of those presented in the main figures.

Figure S3. Full length western blots of those presented in Figure S1.

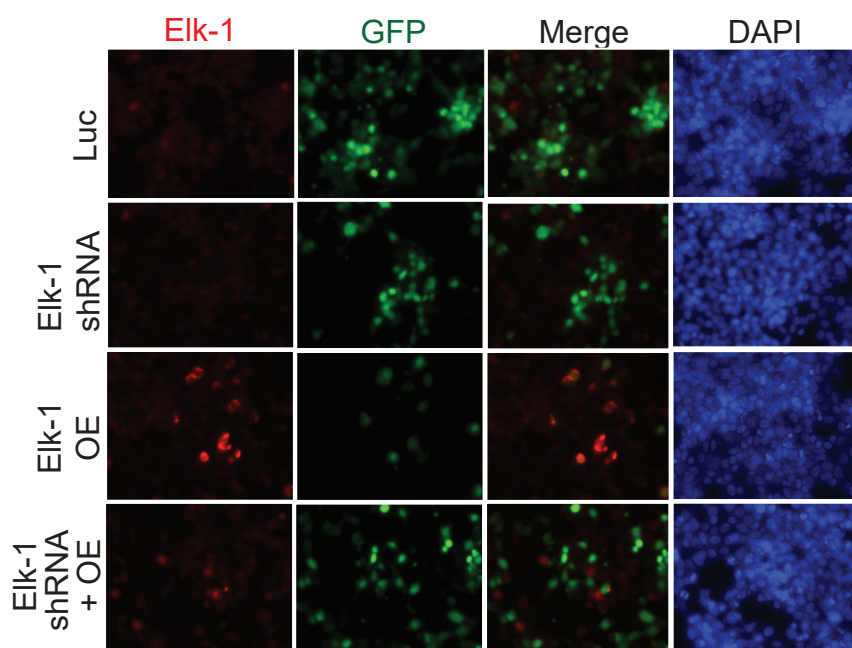
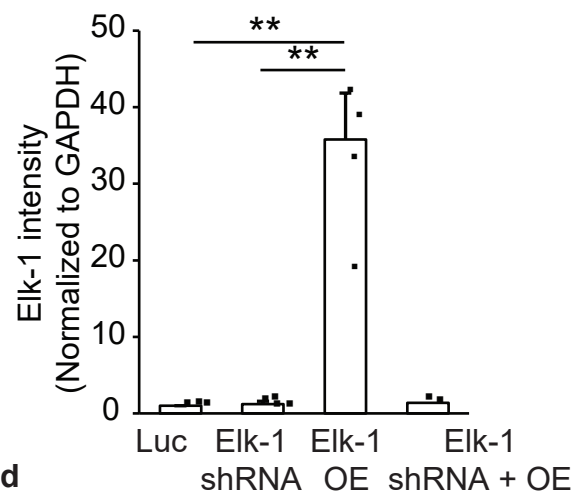
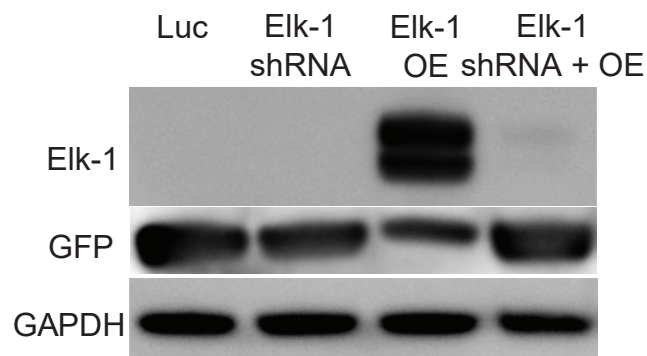
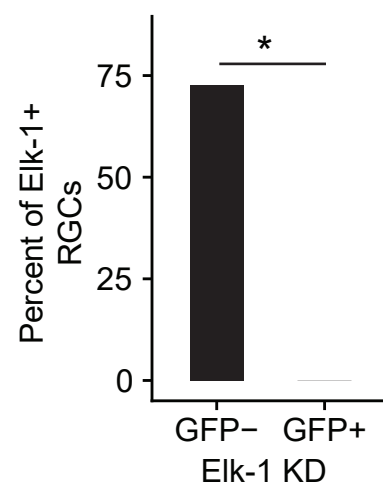
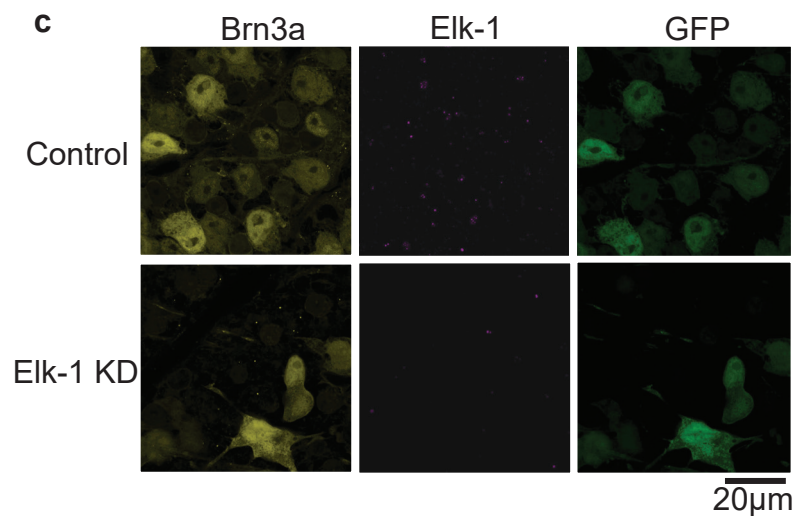
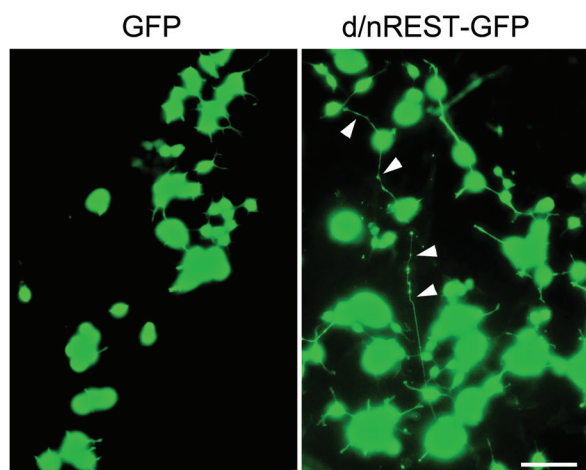
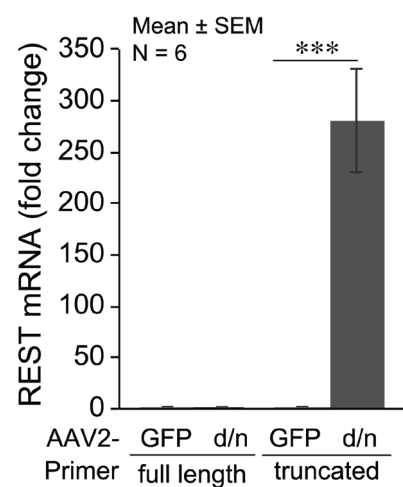
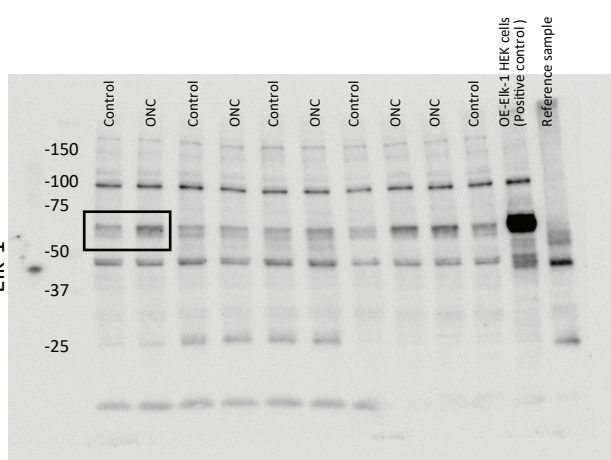
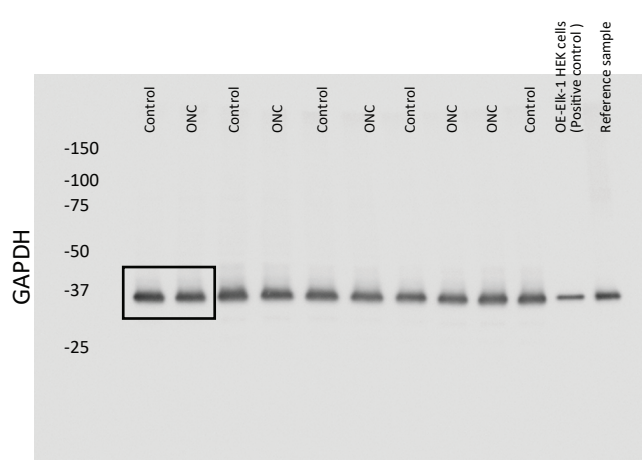
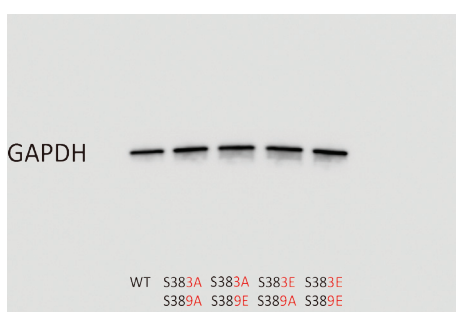
a**Elk-1 shRNA and OE in HEK 293T cells****b****d****Elk-1 shRNA in flatmount mouse retina****c****e****REST D/N in HEK 293T cells****f**

Figure S1. Validation of Elk-1 and REST constructs.

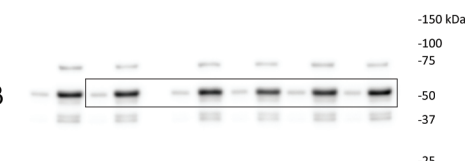
(a) Expression of luciferase (Luc), Elk-1 shRNA, Elk-1 overexpression (OE), or combined Elk-1 shRNA and Elk-1 OE plasmids in HEK 293T cells. Cells were stained with Elk-1 antibody and imaged for Elk-1 and GFP expression. **(b)** Western blot against Elk-1, GFP, and GAPDH for HEK 293T cells as described in (a). Relative quantification shown below. $**P<0.01$, Kruskal-Wallis test, followed by two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli to correct for multiple testing. **(c)** Retinal flatmounts prepared 2 weeks after AAV2-scramble-GFP vs AAV2-Elk-1-shRNA intravitreal injection in wildtype mouse eyes, demonstrates decreased Elk-1 expression in RGCs after knockdown. **(d)** Quantification of Elk-1 positivity in RGCs shows significant loss of Elk-1 expression in RGCs transduced with knockdown virus compared to neighboring cells not transduced with knockdown virus. $*p<0.05$, Fisher's exact test. **(e)** Expression of REST D/N compared to GFP control in HEK 293T cells. White arrows demonstrate formation of extensions in these cells after REST D/N expression. **(f)** qPCR of HEK 293T cells transduced with either AAV2-REST D/N or AAV2-GFP. Primers targeted for full-length REST did not show amplification. Primers targeting truncated REST showed significant increase in REST expression compared to GFP control. $***P<0.001$, two-tailed T-test.

a**b****c**

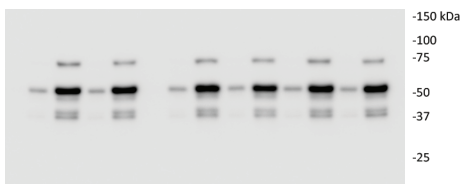
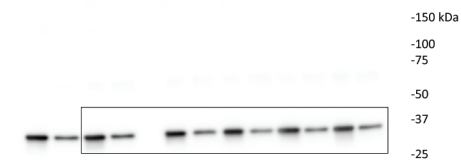
Protein Standards
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Cytoplasmic
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Lap2β



GAPDH



Flag

(GAPDH)

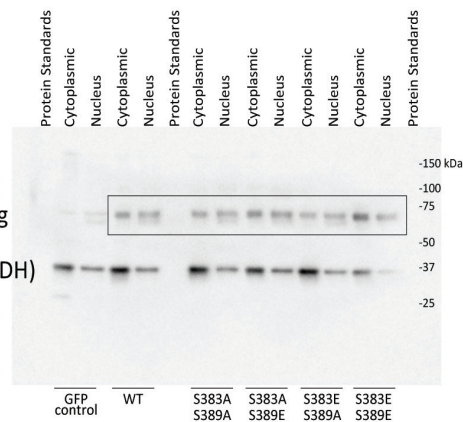
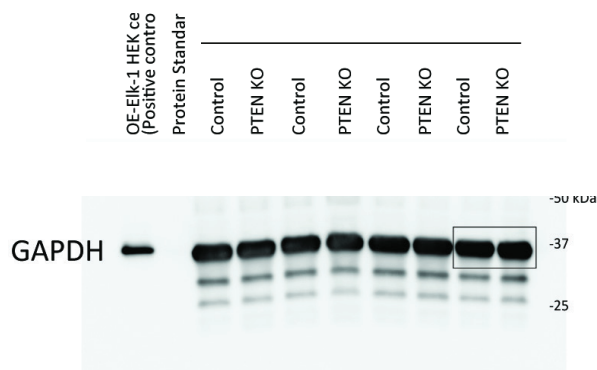
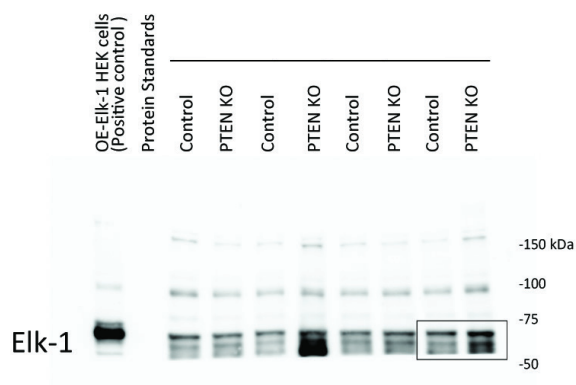
**d**

Figure S2. Full length western blots of those presented in the main figures.

(a) Full length blots for Figure 2a. **(b)** Full length blots for Figure 3a. **(c)** Full length blots for Figure 3b, with alternative exposures included. **(d)** Full length blots for Figure 4a.

a

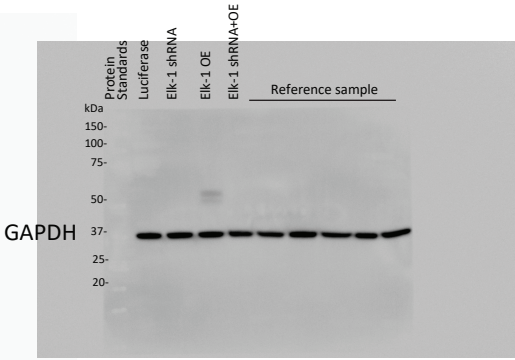
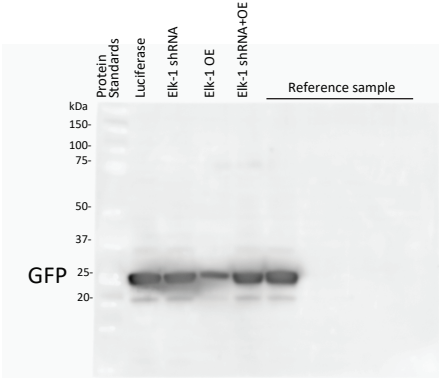
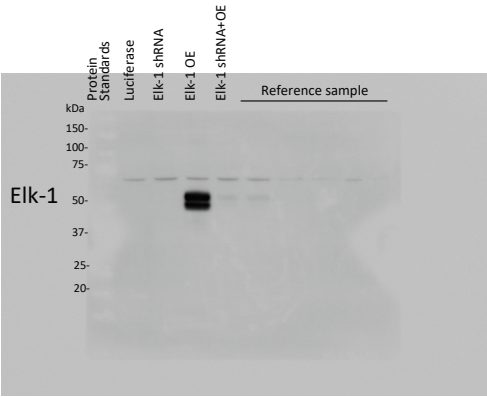


Figure S3. Full length western blots of those presented in Figure S1.

(a) Full length blots for Figure S1b.