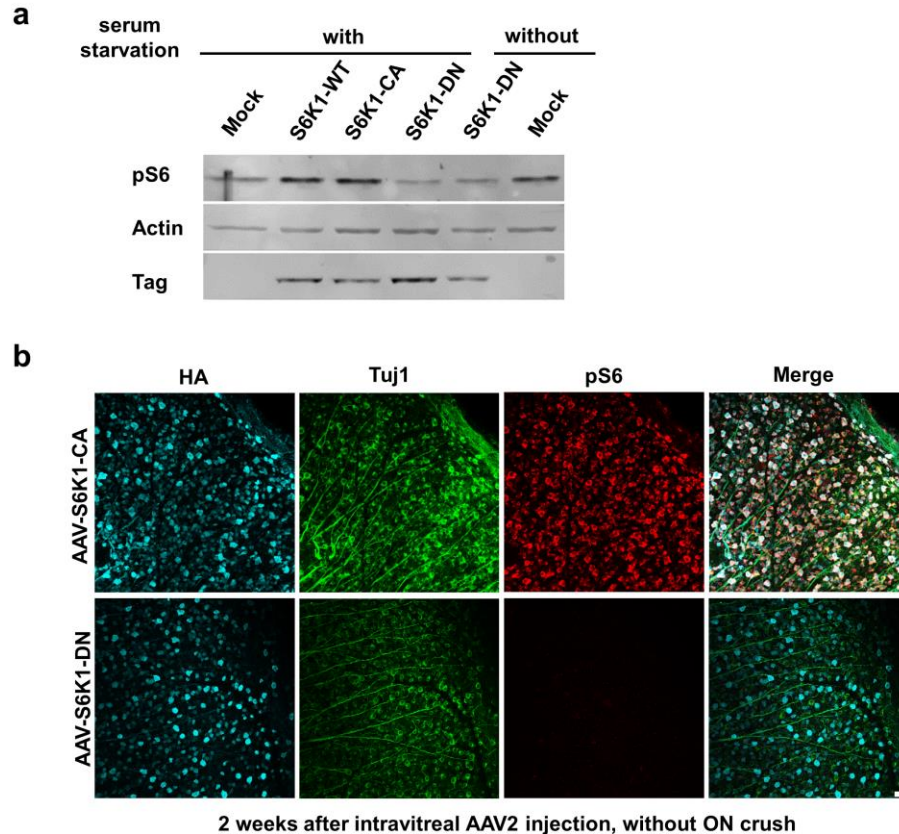


Supplementary Data

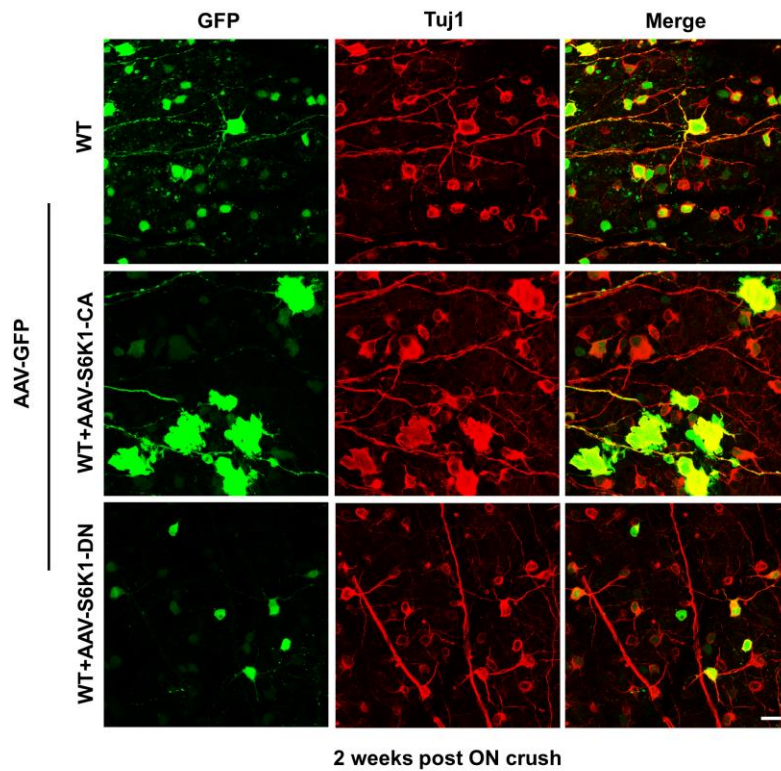
The mTORC1 Effectors S6K1 and 4E-BP Play Different Roles in CNS Axon Regeneration

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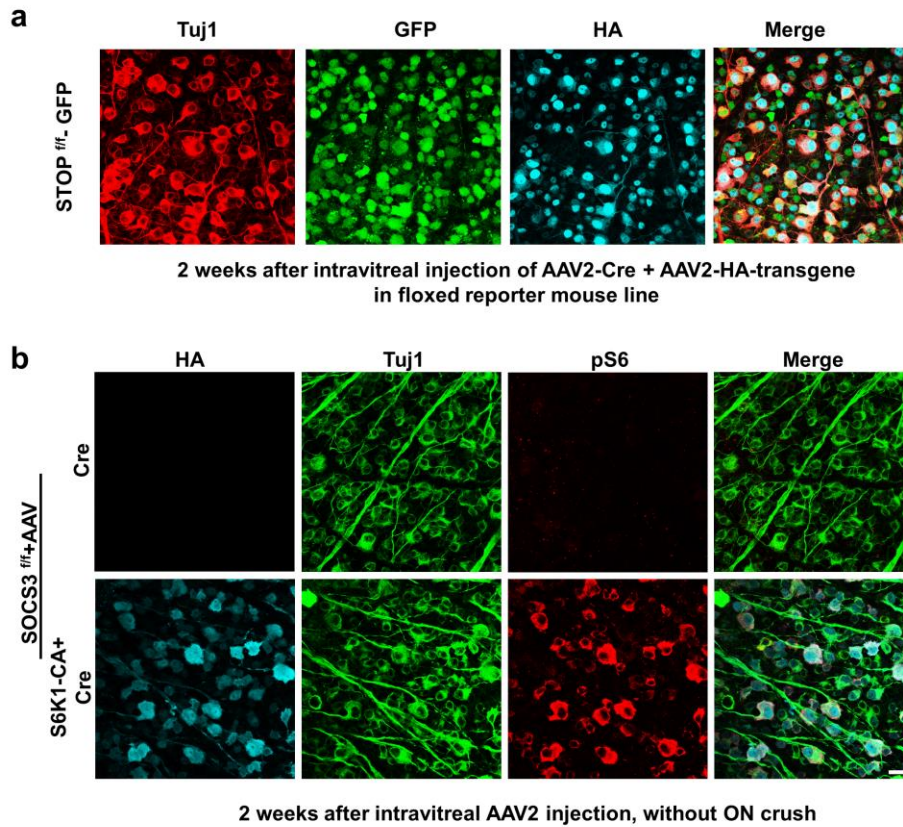
Supplementary Fig. 1

Supplementary Figure 1 S6K1-CA activates and S6K1-DN inhibits S6K1 activity in vitro and in vivo. (a) Western blot detection of protein expression after transient transfection of HEK293T cells with S6K1-WT, S6K1-CA or S6K1-DN, with or without serum starvation. (b) Representative confocal images of whole-mount retinas showing HA tag, blue; TUJ1 positive RGCs, green; pS6, red; merged images; 2 weeks after injection with AAV-S6K1-CA or AAV-S6K1-DN. Scale bar, 20 μ m.



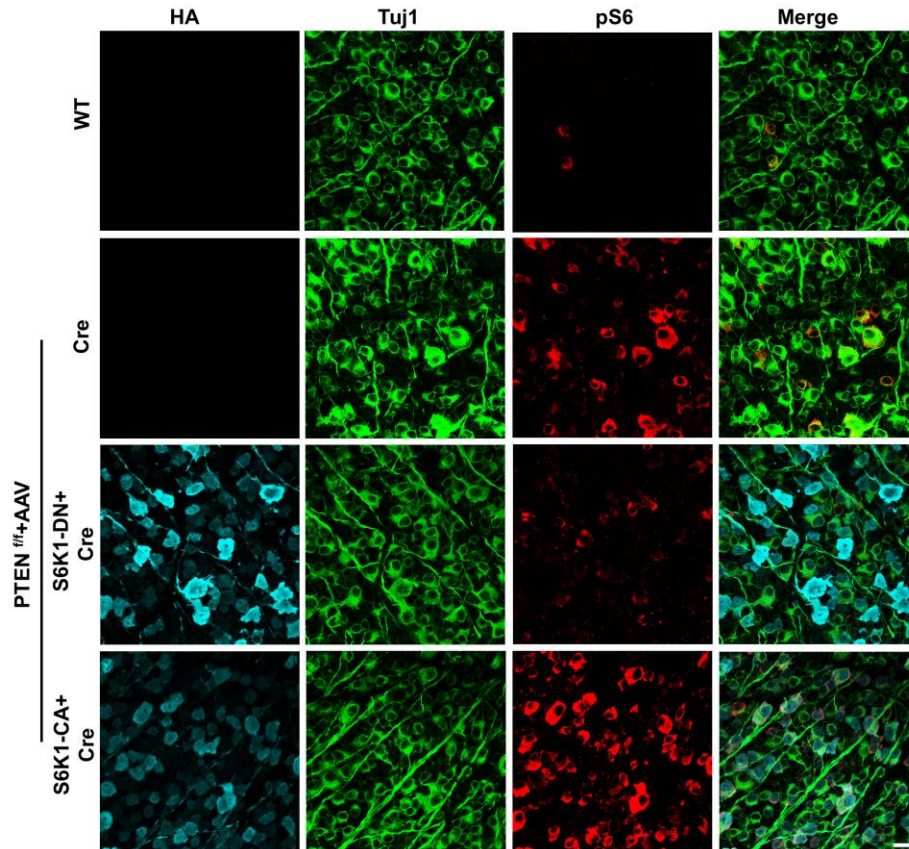
Supplementary Fig. 2

Supplementary Figure 2 S6K1 regulates RGC size. Representative confocal images of RGCs in whole-mount retinas showing GFP, green; Tuj1, red; merged images; 2 weeks after ON crush, GFP signal indicates enlarged RGC bodies in WT mice injected with AAV-S6K1-CA (middle row) and smaller RGC bodies with injection of AAV-S6K1-DN (bottom row). Scale bar, 20 μ m.



Supplementary Fig. 3

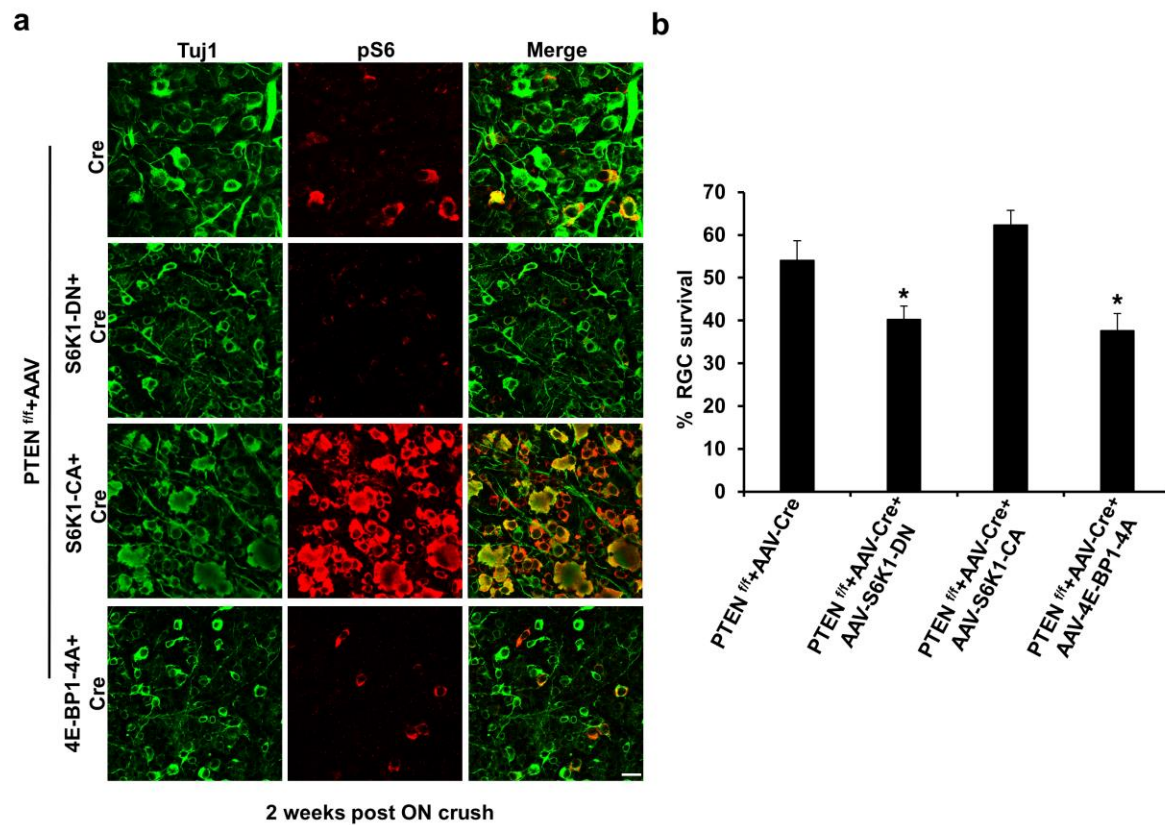
Supplementary Figure 3 Mixed injection of AAV-Cre + AAV-HA-transgene achieves high transgenic efficiency *in vivo*. (a) Representative confocal images of whole-mount retinas showing Tuj1 positive RGCs, red; GFP expression after Cre expression, green; HA tag, blue; merged images; 2 weeks after injection with AAV-Cre + AAV-HA-transgene in flox-stop-flox-GFP reporter mouse line. Scale bar, 20 μm . (b) Representative confocal images of whole-mount retinas showing Tuj1 positive RGCs, the expression of HA-S6K1 and pS6 level, 2 weeks after intravitreal injection with AAV-Cre + AAV-HA-S6K1 in SOCS3 floxed mouse line. Scale bar, 20 μm .



2 weeks after intravitreal AAV2 injection, without ON crush

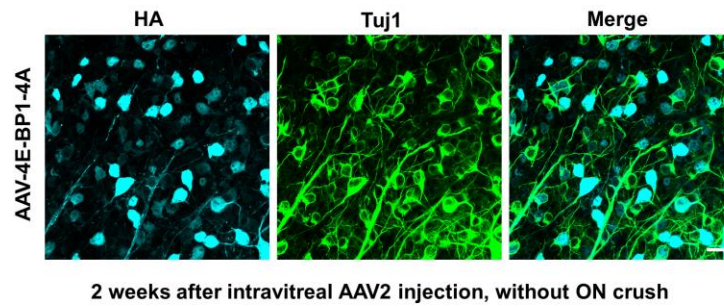
Supplementary Fig. 4

Supplementary Figure 4. S6K1 activation and inhibition in PTEN KO mice by mixed injection of AAV-Cre+AAV-S6K1-CA and AAV-Cre+AAV-S6K1-DN in PTEN^{fl/fl} mice. Representative confocal images of whole-mount retinas showing HA tag, blue; Tuj1 positive RGCs, green; pS6, red; merged images; in WT mice; 2 weeks after injection with AAV-Cre alone or AAV-Cre + AAV-S6K1-DN or AAV-Cre + AAV-S6K1-CA in PTEN floxed mice, Scale bar, 20 μ m.



Supplementary Fig. 5

Supplementary Figure 5. S6K1 inhibition and 4E-BP activation decrease RGC survival in PTEN KO mice (a) Confocal images of whole-mount retinas showing surviving Tuj1 positive RGCs and p-S6 immunostaining, 2 weeks after ON crush. Scale bar, 20 μ m. (b) Quantification of surviving RGCs, represented as percentage of Tuj1 positive RGCs in the injured eye, compared to the intact contralateral eye, 2 weeks after ON crush. *: $p < 0.05$. Data are presented as means \pm s.e.m, $n = 8-12$.



Supplementary Fig. 6

Supplementary Figure 6. HA-tagged AAV-4E-BP1-4A achieves high infection efficiency *in vivo*. Representative confocal images of RGCs in whole-mount retinas showing HA expression, blue; Tuj1, green; merged image; in WT mice, 2 weeks after injection with AAV-HA-4E-BP1-4A, Scale bar, 20 μm