

Figure legends supplemental figures

Supplemental figure 1.

(a) Staining pattern of cre-protein is similar to GFAP-staining of astrocytes but does not resemble NeuN-staining of neurons in mouse hippocampus. (b) Cre protein does not colocalize with NeuN-positive neurons (scalebar 100 μ m) (c) Upper: GFAPcre+/VHL^{+f/+f} are smaller than wildtype littermates. Lower: A dome-shaped head in GFAPcre+/VHL^{+f/+f} mice (arrowhead) indicates the presence of hydrocephalus in those animals. (d) Analysis of Evans blue (EB) leakage into the CNS indicates a significantly impaired blood-brain barrier function in GFAPcre+/VHL^{+f/+f} (1.73 \pm 0.2-fold increase (n=7) of OD₆₂₀/g brain) and GFAPcre+/VHL^{+f/+f}/HIF-1 α ^{+f/+f} (1.46 \pm 0.2-fold increase (n=4)) compared to GFAPcre-negative mice. GFAPcre+/VHL^{+f/+f}/HIF-2 α ^{+f/+f} mice have no significantly altered blood-brain barrier permeability (1.22 \pm 0.1-fold change (n=5; Mann-Whitney-test)) (e) Upper graph: hearts of GFAPcre+/VHL^{+f/+f} (167.1 \pm 10.8 mg (n=7)) and of GFAPcre+/VHL^{+f/+f}/HIF-1 α ^{+f/+f} (158.0 \pm 7.9 (n=5)) are significantly larger than hearts of control (GFAPcre-; 130.0 \pm 2.5 mg (n=9)) and of GFAPcre+/VHL^{+f/+f}/HIF-2 α ^{+f/+f} mice (135.0 \pm 8.9 mg (n=4; Mann-Whitney-test)). Lower graph: spleens are significantly enlarged in GFAPcre+/VHL^{+f/+f} (244.2 \pm 31.9 mg (n=6)) and in GFAPcre+/VHL^{+f/+f}/HIF-1 α ^{+f/+f} animals (173.0 \pm 14.5 mg (n=5)) compared to control mice (GFAPcre-: 77.7 \pm 8.4 mg (n=9)). Spleens are not significantly different in GFAPcre+/VHL^{+f/+f}/HIF-2 α ^{+f/+f} animals (88 \pm 0.01 mg (n=4); data represents means \pm S.E.M.). (f) Hydrocephaly is detectable in three week old GFAPcre+/VHL^{+f/+f} and GFAPcre+/VHL^{+f/+f}/HIF-1 α ^{+f/+f} animals. In GFAPcre+/VHL^{+f/+f}/HIF-2 α ^{+f/+f} mice ventricles are not enlarged (representative Nissl staining, 50x magnification).

Supplemental figure 2.

Expression of VEGF mRNA (a) and GLUT-1 mRNA (b) in livers is not significantly different between control (GFAPcre-; n=11), GFAPcre+/VHL^{+f/+f} (n=6), GFAPcre+/VHL^{+f/+f}/HIF-1 α ^{+f/+f} (n=6) and GFAPcre+/VHL^{+f/+f}/HIF-2 α ^{+f/+f} (n=5) animals (data represents means \pm S.E.M.). (c) VEGF mRNA is significantly upregulated in brains of GFAPcre+/VHL^{+f/+f} (n=10) and GFAPcre+/VHL^{+f/+f}/HIF-1 α ^{+f/+f} mice (n=6) compared to control animals (GFAPcre-; n=8). Double knockout of VHL and HIF-1 α does not change VEGF expression significantly. In contrast, double knockout of VHL and HIF-2 α (n=10) significantly reduces VEGF upregulation, and double knockout of VHL and VEGF (n=5) reduces it further to wildtype levels (data represent means \pm S.E.M.). (d) Immunofluorescence staining in the hippocampus shows that VEGF upregulation in GFAPcre+/ GFAPcre+/VHL^{+f/+f} mice increases vessel numbers as visualized by the endothelial marker CD34 (red). Double knockout of VHL and HIF-2 α reduces vessel numbers whereas double knockout of VHL and HIF-1 α has essentially no effect. In GFAPcre+/VHL^{+f/+f}/VEGF^{+f/+f} mice vessel number is further reduced (representative picture of 3 animals each genotype; astrocytes are stained with anti-GFAP (green); nuclei are stained with DAPI; scalebars 100 μ m). (e) 2-Photon microscopy shows the increased cerebral vessel number in GFAPcre+/VHL^{+f/+f} and GFAPcre+/VHL^{+f/+f}/HIF-1 α ^{+f/+f} mutant animals. No difference is observed between wildtype and GFAPcre+/VHL^{+f/+f}/HIF-2 α ^{+f/+f} animals (representative images of cranial windows; larger image 400x, scalebar 50 μ m; inset 200x, scalebar 200 μ m). (f) In vivo measurements of brain capillary diameter (right) and the blood velocity in the brain capillaries (left) by 2-photon microscopy

demonstrate that both GFAPcre+/VHL^{+f/+f} and GFAPcre+/VHL^{+f/+f}/HIF-1 α ^{+f/+f} animals have significantly thicker capillaries and that bloodflow is significantly slower compared to wildtype (GFAPcre-) and GFAPcre+/VHL^{+f/+f}/HIF-2 α ^{+f/+f} mice (n=4 each genotype; Kruskal-Wallis-test).

Supplemental figure 3.

(a) Survival of GFAPcre+/VHL^{+f/+f}/VEGF^{+f/+f} mice (n=19) is significantly reduced compared to wildtype mice (GFAPcre-; n=62), but extended compared to GFAPcre+/VHL^{+f/+f} animals (data represents median survival (weeks)). (b) The hydrocephaly of the GFAPcre+/VHL^{+f/+f} mice is completely rescued in GFAPcre+/VHL^{+f/+f}/VEGF^{+f/+f} double knockout animals (representative T1 weighed fMRI picture).

Supplementary Methods:

Primer Sequences for RNA analyses:

EPO: (F): 5'-TGCGACAGTCGAGTTCTGGA-3'

(R): 5'-TCTGCACAACCCATCGTGAC-3'

Probe: 5'-(6~FAM)AGGTACATCTTAGAGGCCAAGGAGGCAGAAA-(BHQ)-3'

VEGF (F): 5'-ATCCGCATGATCTGCATGG-3'

(R): 5'-AGTCCCATGAAGTGATCAAGTTCA-3'

Probe: 5'-(6~FAM)TGCCACGTCAGAGAGCAACATCAC-(BHQ)-3'

Primer Sequences for DNA recombination analyses:

Location of the primers see (42)

VHL:

VHL F1: 5'-CTGGTACCCACGAAACTGTC-3'

VHL F2: 5'-CTAGGCACCGAGCTTAGAGGTTTGCG-3'

VHL R: 5'-CTGACTTCCACTGATGCTTGTCACAG-3'

Product size: VHL 2-lox allele: 460bp, 1-lox allele: 260bp; wt allele 290bp

HIF-1 α :

HIF-1 α F1: 5'-TTGGGGATGAAAACATCTGC-3'

HIF-1 α F2: 5'-GCAGTTAAGAGCACTAGTTG-3'

HIF-1 α R: 5'-GGAGCTATCTCTCTAGACC-3'

Product size: HIF-1 α 1-lox allele: 270bp; 2-lox allele 260bp; wt allele 240bp

HIF-2 α :

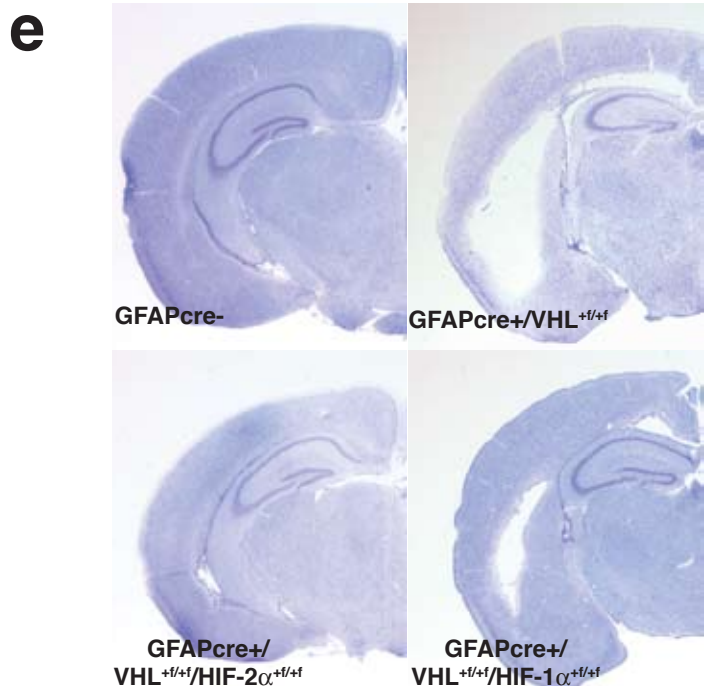
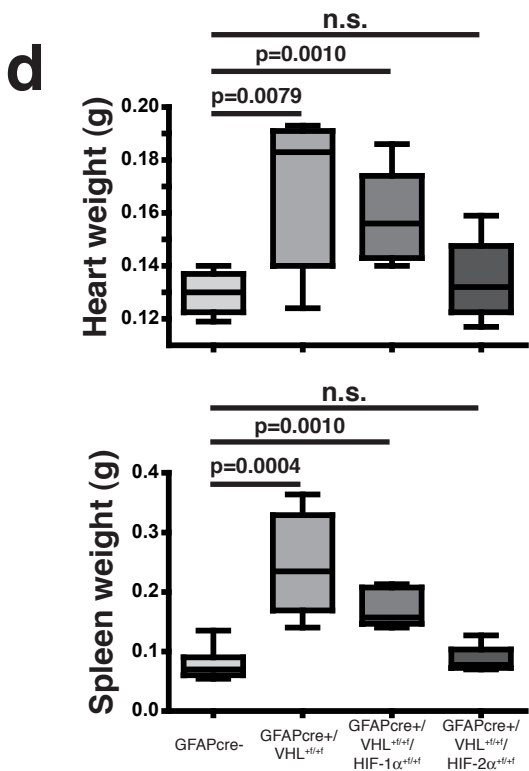
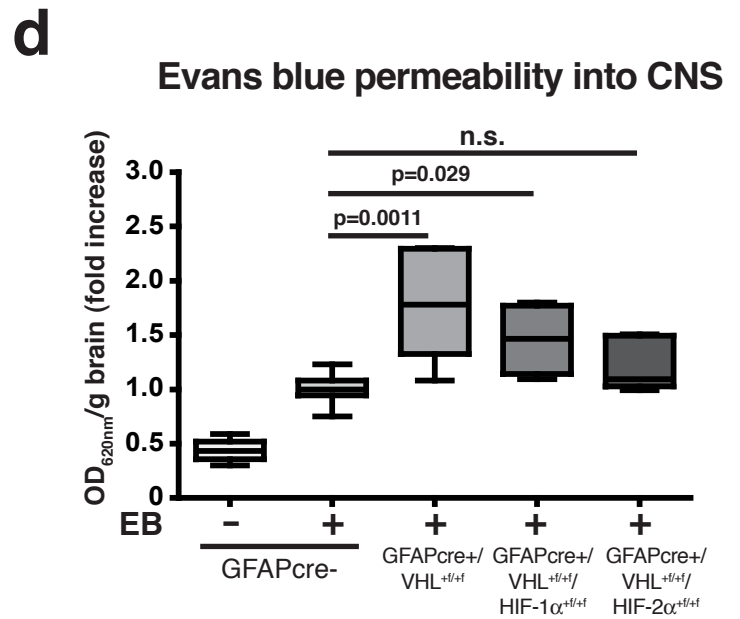
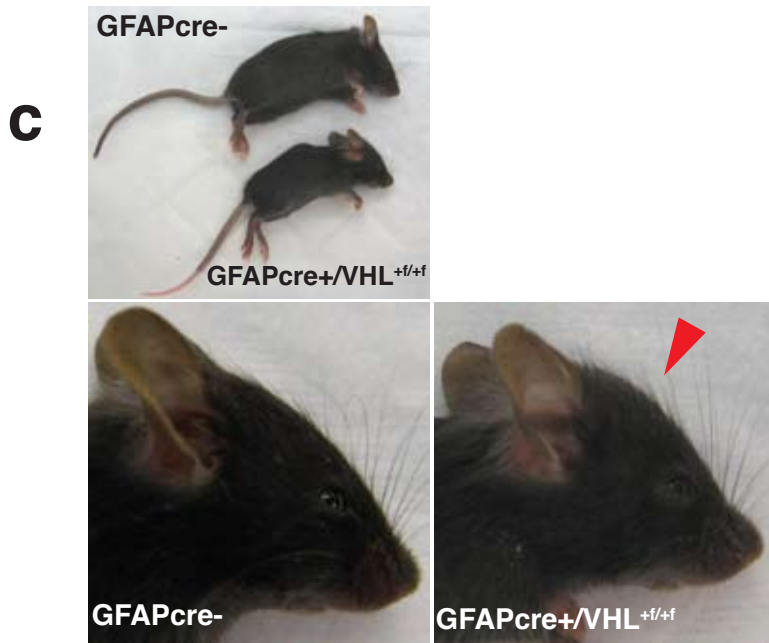
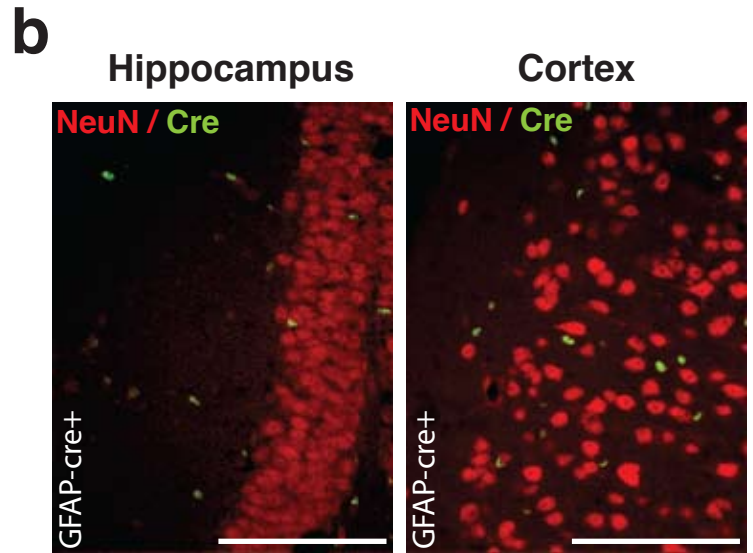
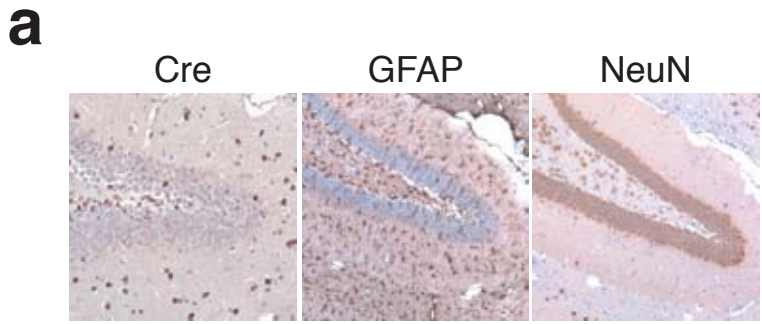
HIF-2 α F1: 5'-CAGGCAGTATGCCTGGCTAAT TCCAGTT-3'

HIF-2 α F2: 5'-CTTCTTCCATCATCTGGGATCTGGGACT-3'

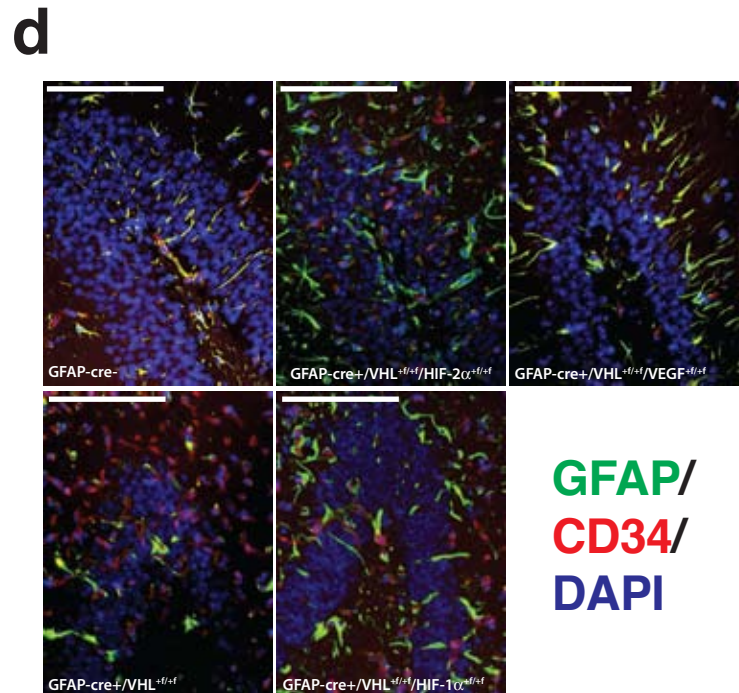
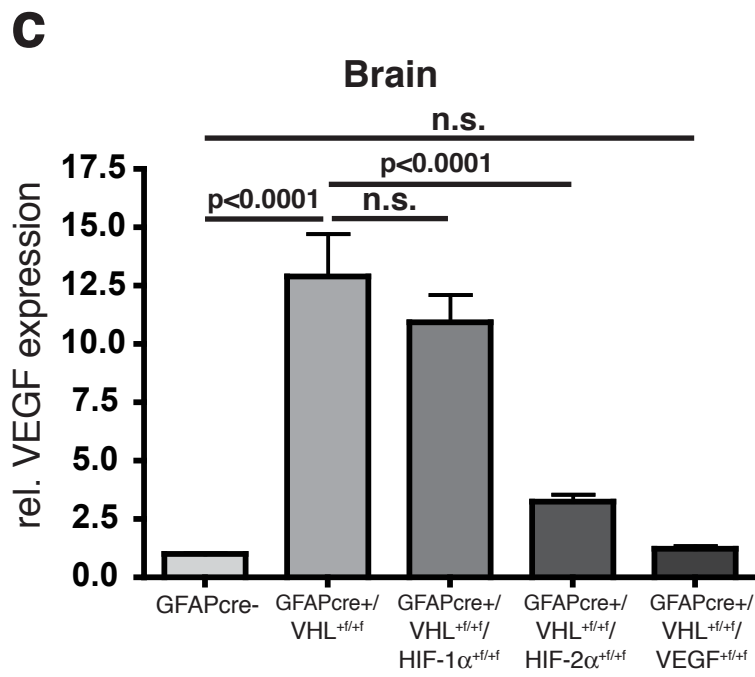
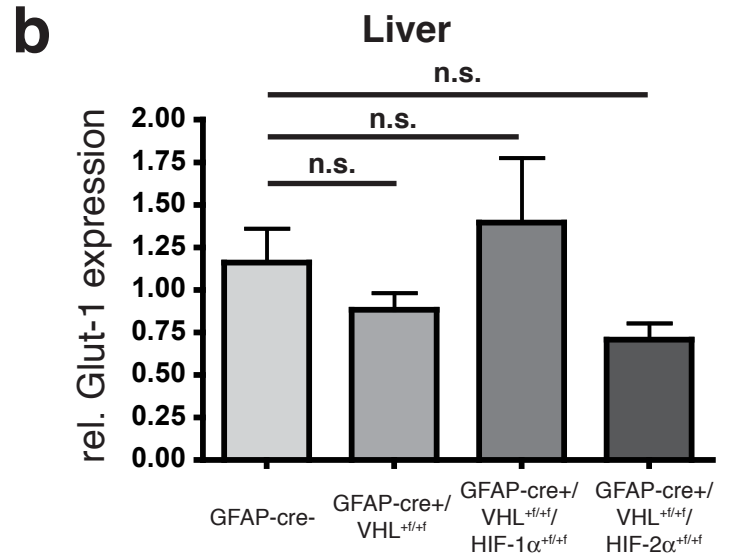
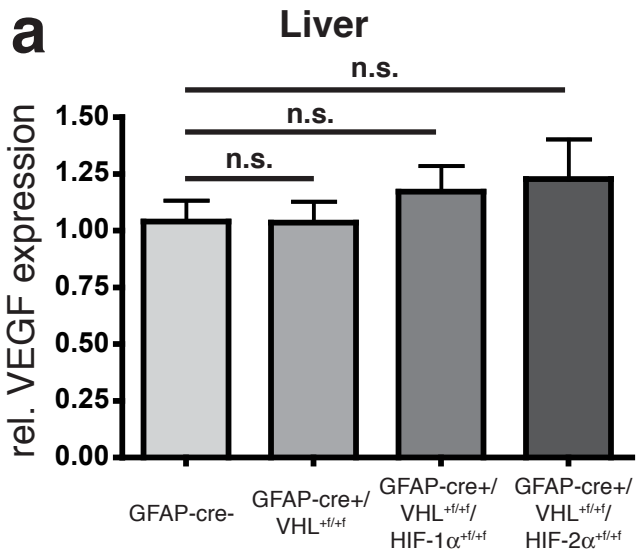
HIF-2 α R: 5'-GCTAACACTGTACTGTCTGAAAGAGTAGC-3'

Product size: HIF-2 α 1-lox allele: 360bp; 2-lox allele 460bp; wt allele 430bp

Supplemental Figure 1

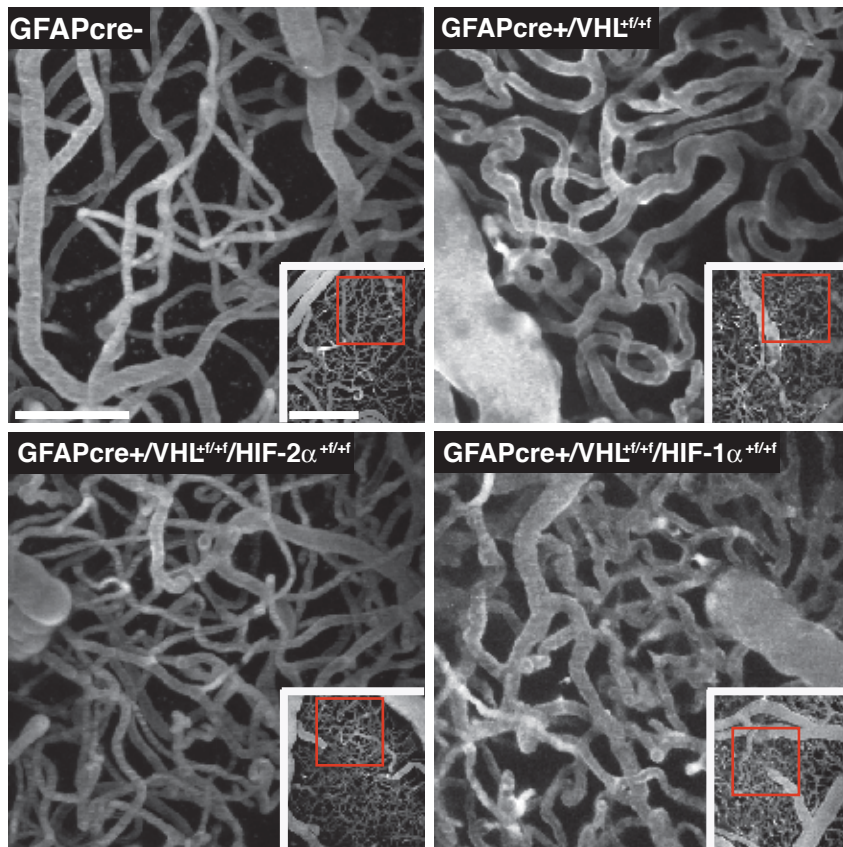


Supplemental Figure 2

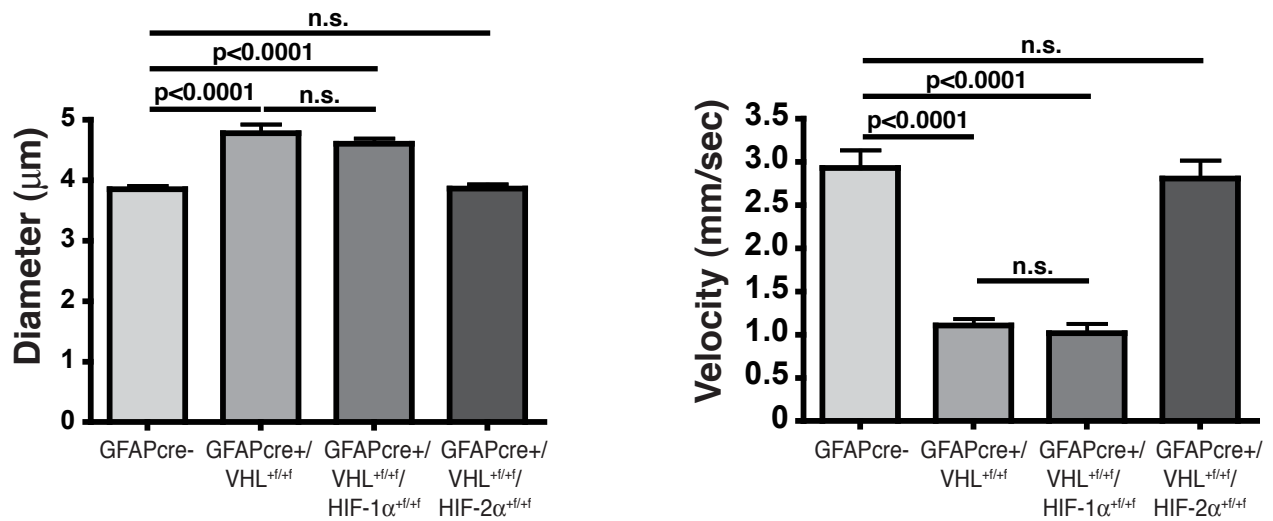


Supplemental Figure 2

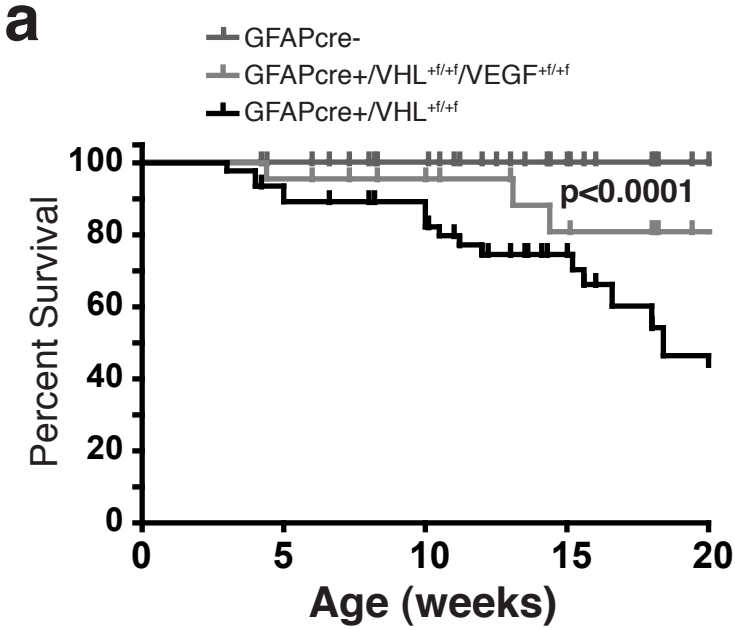
e



f



Supplemental figure 3



b

