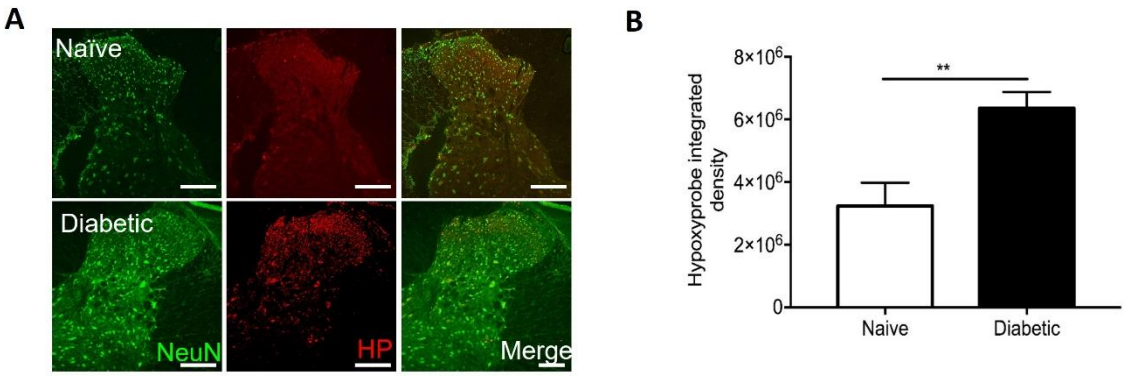
Supplemental Figures

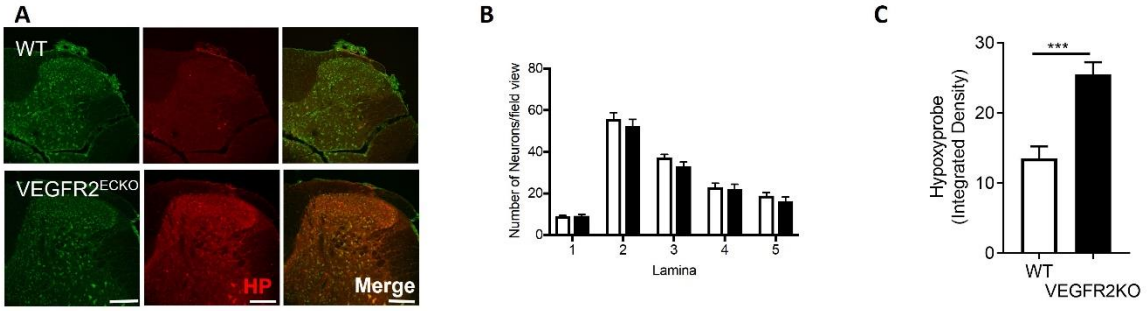
Supplemental Figure. 1



Supplemental Figures 1 – *Diabetes induced hypoxia in the dorsal horn*

[A] Low power Confocal microscopy of the dorsal horn was used to identify hypoxyprobe (HP) labelled sensory neurons (low power images scale bar = 100μm) in Naïve and Diabetic Sprague Dawley female rats. [B] In the diabetic rat dorsal horn demonstrated increased levels of hypoxyprobe fluorescence compared to Naïve control animals (*P<0.05, Unpaired T Test, n=5 per group).

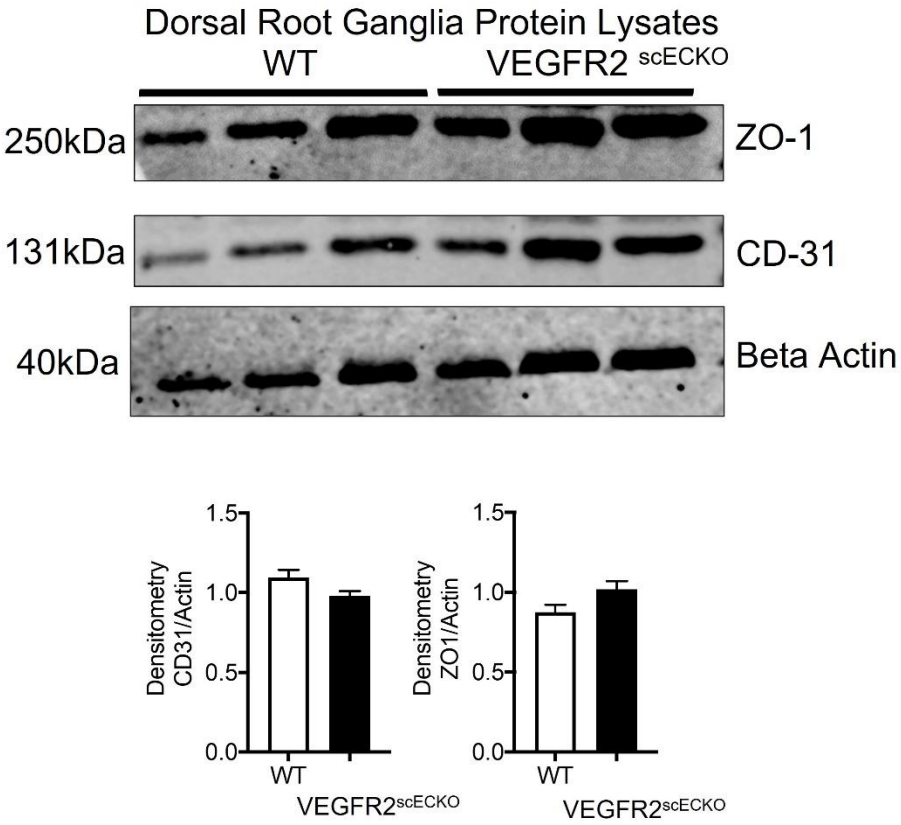
Supplemental Figure. 2



Supplemental Figures 2 – Endothelial *VEGFR2 Knock-Out induced hypoxia in the dorsal horn*

[A] Low power confocal microscopy of the dorsal horn was used to identify hypoxyprobe (HP) labelled sensory neurons (low power images scale bar 100μm) in WT and VEGFR2^{ECKO} mice. [B] There were no alterations in neuronal number in the dorsal horn of the spinal cord in either the WT or VEGFR2^{ECKO} mice (white bars=WT, black bars = VEGFR2^{ECKO}). [C] In VEGFR2^{ECKO} mice demonstrated increased level of hypoxyprobe fluorescence compared to naive control animals (**P<0.01, Unpaired T Test, n=5 per group).

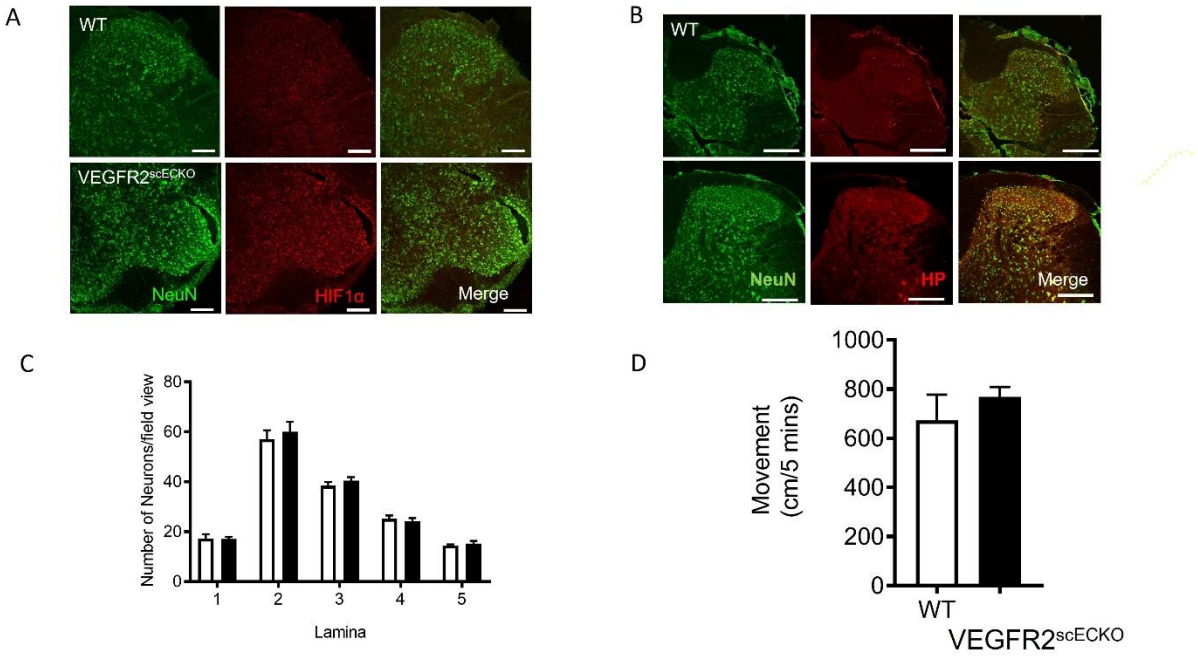
Supplemental Figure. 3



Supplemental Figure 3 – *DRG vasculature was unaffected by intrathecal OHT injection*

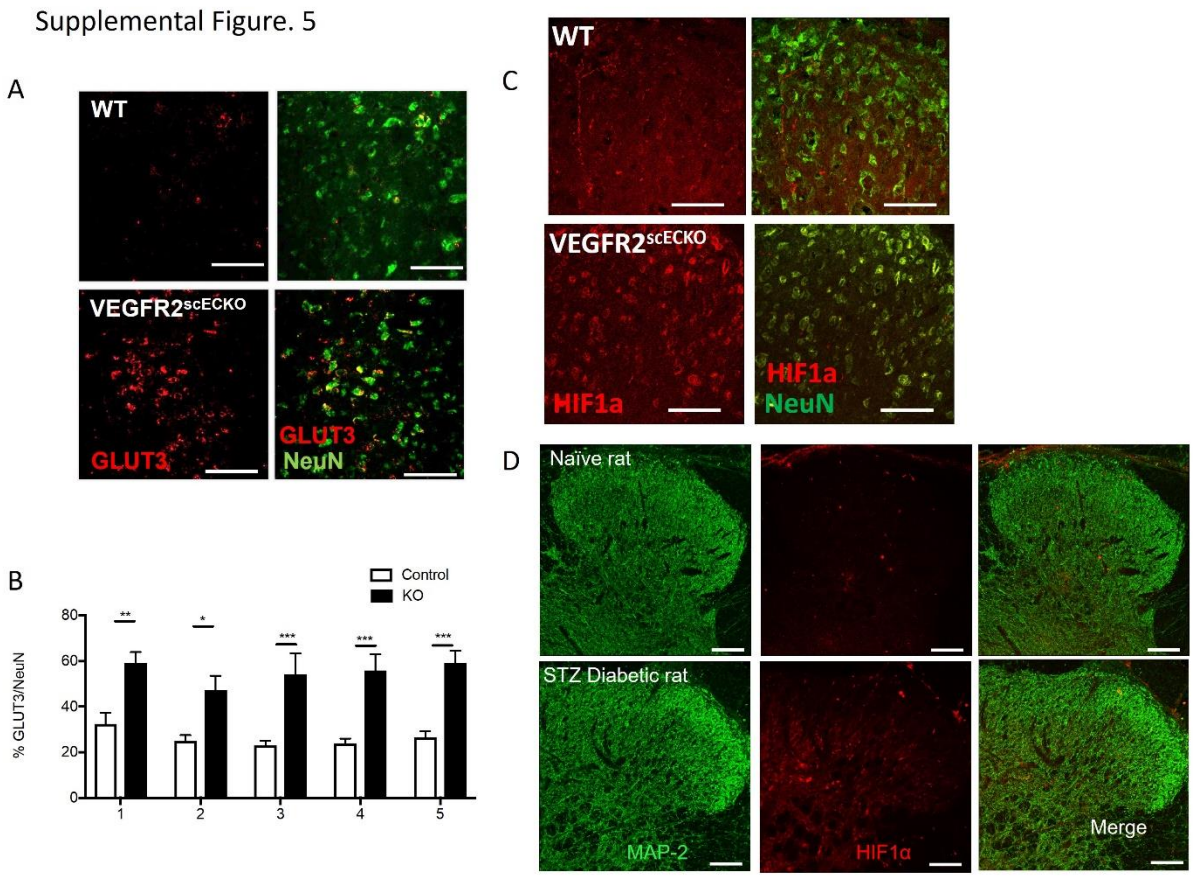
OHT treatment led to no alterations in protein expression of endothelial markers in the dorsal root ganglion extracted from WT or itVEGFR2 mice. Densitometry quantification of protein samples extracted from dorsal root ganglion (pooled dorsal root ganglion from lumbar 3, 4 and 5) demonstrated no change in ZO1 or CD31 expression in either WT or itVEGFR2KO mice.

Supplemental Figure. 4



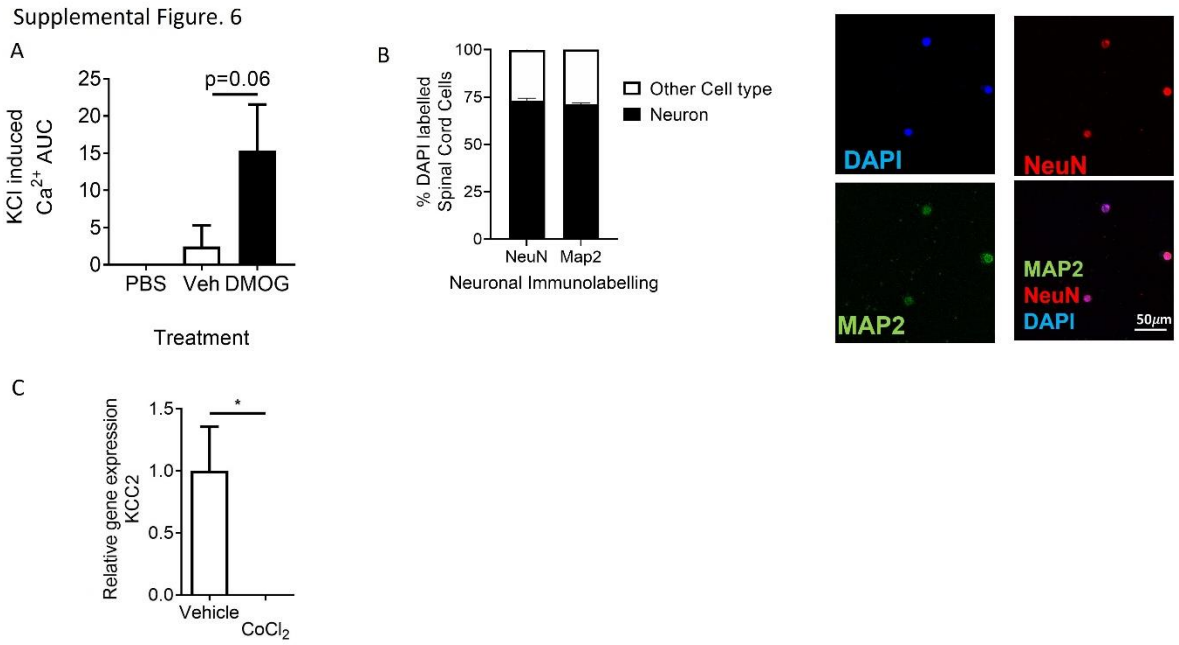
Supplemental Figure 4 – *Spinal cord vasculopathy induces hypoxia and no impact upon motor behaviour*

[A] 8 days after OHT treatment HIF1α protein immunoreactivity was increased in neurons of the dorsal horn in VEGFR2^{scECKO} mice (Representative confocal images of WT and VEGFR2^{scECKO} co-labelled with NeuN, low power images (main) scale bar = 100μm). [B] Representative confocal images of Hypoxyprobe labelled sensory neurons in the dorsal horn of WT and VEGFR2^{scECKO} mice (Red; co-labelled with NeuN (Green)lowpower images scale bar = 100 μm). [C] There were no alterations in neuronal number in the dorsal horn of the spinal cord in either the WT or VEGFR2^{scECKO} mice (white bars=WT, black bars = VEGFR2KO). [D] There were no alterations in motor behavior with WT and VEGFR2^{scECKO} mice moving the same distance in the testing environment (n=5 per group).



Supplemental Figure 5 – *Spinal cord vasculopathy induces GLUT3 and HIF1α expression in sensory neurons in the dorsal horn*

[A] 8 days following OHT treatment GLUT3 protein immunoreactivity was increased in sensory neurons of the dorsal horn in VEGFR2^{scECKO} mice (Representative confocal images of WT and VEGFR2^{scECKO} co-labelled with NeuN, high power images scale bar = 50 μm). [B] There was an increase in the percentage of dorsal horn sensory neurons expressing GLUT3 in VEGFR2^{scECKO} mice when compared to WT mice (*P<0.05, **P<0.01, ***P<0.001 Two way ANOVA with post Bonferroni test, n= 5 per group). [C] 8 days following OHT treatment HIF1α protein immunoreactivity was increased in sensory neurons of the dorsal horn in VEGFR2^{scECKO} mice (Representative confocal images of WT and VEGFR2^{scECKO} co-labelled with NeuN, high power images scale bar = 50 μm). [D] HIF1α protein immunoreactivity was increased in sensory neurons of the dorsal horn in STZ induced diabetic Sprague Dawley rats versus age matched control rats (Representative confocal images of WT and VEGFR2^{scECKO} co-labelled with NeuN, low power images scale bar = 100μm,).



Supplemental Figure 6 – *Hypoxia induced dorsal horn neuron activation*

[A] Isolated spinal cord neurons treated (24hrs) with either chemical induction of hypoxia through 1mM DMOG treatment versus vehicle control demonstrated increased neuronal response following stimulation with KCL (p=0.06 One Way ANOVA). [B] Lumbar spinal cord neurons were isolated from C57bl6 mice (labelled with neuronal markers NeuN and MAP2 as well as nuclei marker DAPI; scale bar = 50μm). [C] Isolated neurons were exposed to normoxic and hypoxic conditions. Hypoxia led to reduced expression of KCC2 (*P<0.05, **P<0.01, Unpaired T Test). [C]