

Figure S1

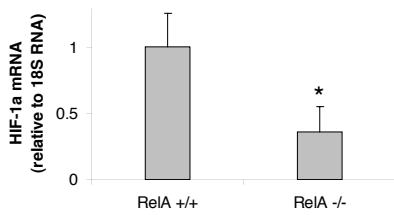
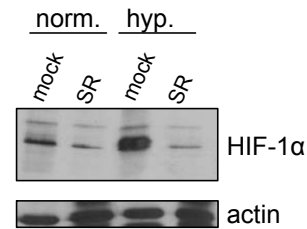


Figure S1: RelA is required for HIF-1α mRNA expression. RNA from either *RelA*^{+/+} or *RelA*^{-/-} MEFs cultured under normoxic conditions was analyzed by Q-RT-PCR. Results are expressed as means±SEM. p<0.05: *, vs *RelA*^{+/+} cells.
Figure S2: NF-κB is required for HIF-1α accumulation under hypoxia. HEK293

Figure S2



cells were transfected with a non-degradable IκBα mutant (IκB superrepressor-SR) or a control vector (mock). 48 hrs after transfection cells were cultured under normoxia or hypoxia (O₂ = 0.5%) for 2 hrs. HIF-1α protein expression was analyzed by immunoblotting.

Figure S3

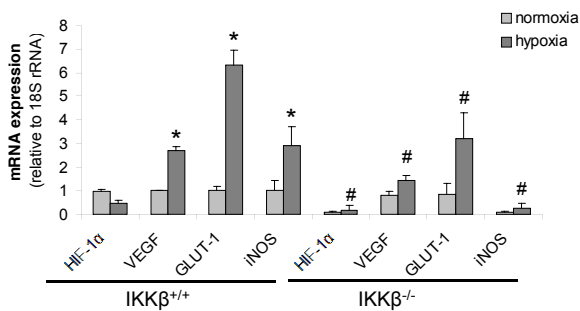
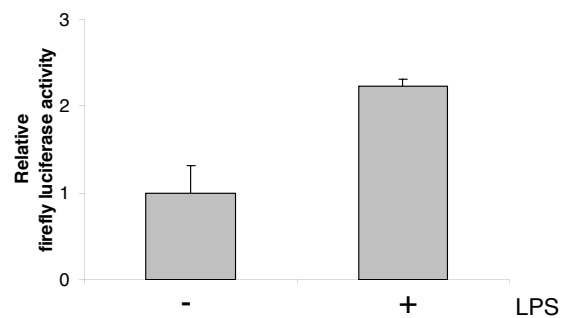


Figure S3: IKKβ is required for hypoxia-induced HIF-1α and target gene expression. MEF from either *Ikkβ*^{+/+} or *Ikkβ*^{-/-} embryos were cultured under normoxia or hypoxia (O₂ = 0.5%) for 4 hrs and mRNA expression was analyzed by Q-RT-PCR. Results are means of three separate experiments done in triplicates. Results are expressed as means±SEM. p<0.05: *, vs normoxic *Ikkβ*^{+/+}

Figure S4



cells; #, vs hypoxic *Ikkβ*^{+/+} cells.

Figure S4: LPS stimulates HIF-1α promoter activity. MEF were transfected with a luciferase reporter gene driven by the HIF-1α promoter. After 36 hrs the cells were incubated for 2 hrs without or with LPS (1 mg/mL). Results are averages ±SEM. (n=3)

Figure S5

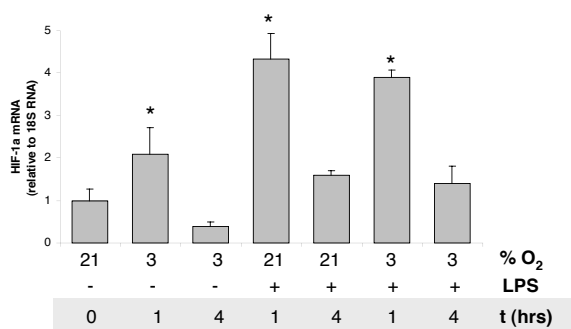
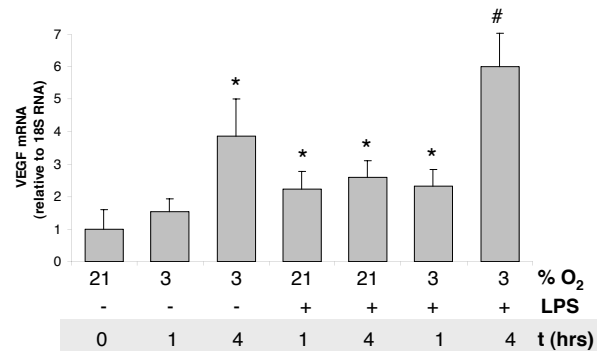


Figure S5: LPS potentiates HIF-1α and VEGF mRNA expression. RAW264.7 cells were cultured in the absence or presence of LPS (1 mg/mL) under normoxia (O₂ = 21%) or hypoxia (O₂ = 3%). At the indicated time-points RNA was extracted and



was analyzed by Q-RT-PCR. Results are expressed as means±SEM. p<0.05: *, vs untreated normoxic cells; #, vs untreated hypoxic cells.

Figure S6

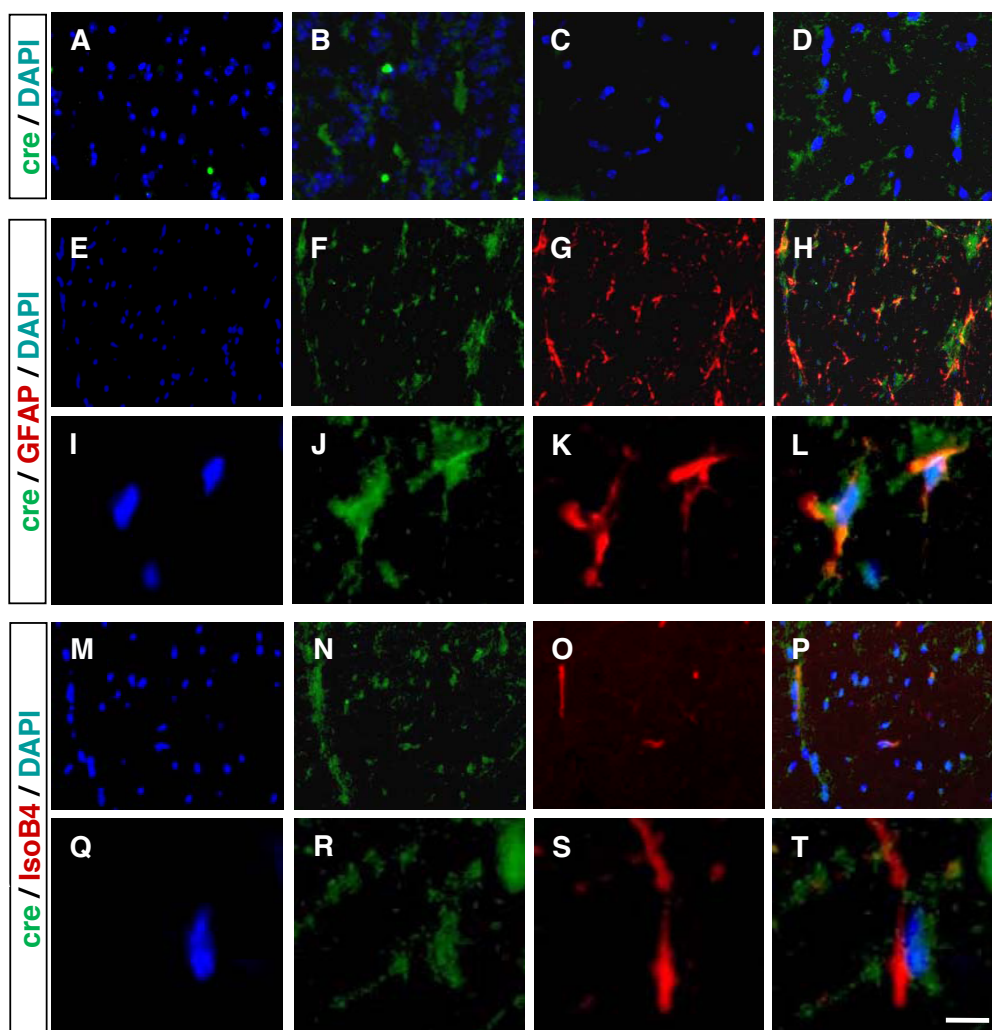


Figure S6: CRE is mainly expressed in astrocytes in brain of poly (I:C) injected *Mx1Cre* mice. *Ikk $\beta^{fl/fl}$ /Mx1Cre* mice were either left untreated or were injected with 250 μ g poly(I:C) (Sigma) on three alternate days. Twenty four hrs after the last injection mice were sacrificed and their brains collected and snap frozen. Brain sections (10 μ m) were processed for immunohistochemistry. Immunostaining of control mouse brains (A, C) or poly(I:C) injected mouse brains (B, D) with anti-CRE antibody revealed CRE expression (green) in the thalamus (B, D) and cerebellum (D) of poly(I:C) injected mice. Staining for CRE (F) (green) and GFAP (G) (red), an astrocyte marker, revealed co-localization (yellow) in astrocytes of poly(I:C) injected *Ikk $\beta^{fl/fl}$ /Mx1Cre* mice (H). High

magnification images (I-L) show nuclear localization of CRE in astrocytes of poly(I:C) injected mice. CRE (N) and IsoB4, a marker for endothelial cells and microglia (O) immunostaining in poly(I:C) injected *Ikk $\beta^{fl/fl}$ /Mx1Cre* mice show different patterns of expression (P). High magnification images show a CRE-negative endothelial cell in close proximity to a CRE-positive glial cell with astrocytic morphology (Q-T). Nuclei are stained blue with DAPI. Scale bar: A-D 25 μ m; E-H 50 μ m; I-L 8.3 μ m; M-P 25 μ m; Q-T 6.2 μ m. Primary antibodies used were rabbit anti-CRE (1:1000; Novagen, Madison, WI, USA), rat anti-GFAP (1:1000; Zymed, San Francisco, CA, USA) and anti-Isolectin B4 (1:300; Sigma, Saint Louis, MO, USA).

Figure S7

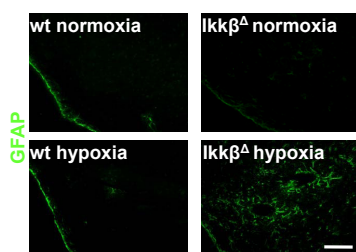


Figure S7: IKK β deficiency results in increased astrogliosis in brains of hypoxic mice. Mice of the indicated genotypes were kept under normoxia or hypoxia ($O_2 = 8\%$) for 24 hrs. After this period the mice were perfused with a fixative and the brain was collected and frozen. Brain sections at the cerebellar region (10 μ m) were stained with an antibody against GFAP (an astrocyte marker). Scale bar: 35 μ m.

Supporting references

1. Greten, F. R. et al. IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* **118**, 285-296 (2004).
2. Rius, J., Martinez-Gonzalez, J., Crespo, J. & Badimon, L. Involvement of neuron-derived orphan receptor-1 (NOR-1) in LDL-induced mitogenic stimulus in vascular smooth muscle cells: role of CREB. *Arterioscler Thromb Vasc Biol* **24**, 697-702 (2004).