doi: 10.1038/nature06905

Figure S1

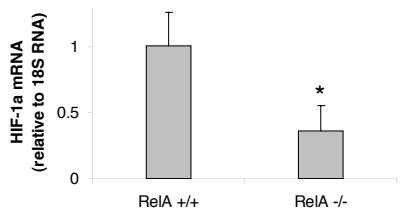

Figure S1: RelA is required for HIF-1α mRNA expression. RNA from either *RelA*$^{+/+}$ or *RelA*$^{-/-}$ MEFs cultured under normoxic conditions was analyzed by Q-RT-PCR. Results are expressed as means±SEM. p<0.05: *, *vs RelA*$^{+/+}$ cells.

Figure S2

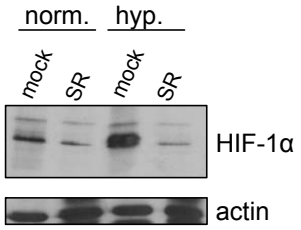

Figure S2: NF-κB is required for HIF-1α accumulation under hypoxia. HEK293 cells were transfected with a non-degradable IκBα mutant (IκB superrepressor-SR) or a control vector (mock). 48 hrs after transfection cells were cultured under normoxia or hypoxia (O_2 = 0.5%) for 2 hrs. HIF-1α protein expression was analyzed by immunoblotting.

Figure S3

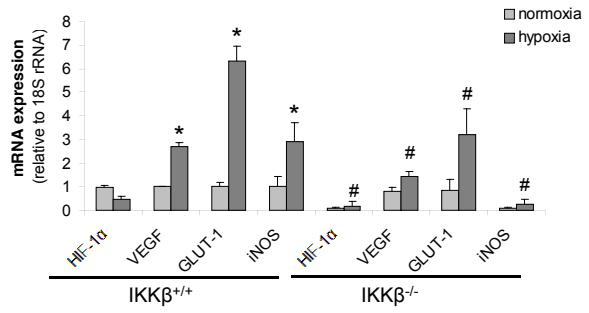

Figure S3:IKKβ is required for hypoxia-induced HIF-1α and target gene expression. MEF from either *Ikkβ*$^{+/+}$ or *Ikkβ*$^{-/-}$ embryos were cultured under normoxia or hypoxia (O_2 = 0.5%) for 4 hrs and mRNA expression was analyzed by Q-RT-PCR. Results are means of three separate experiments done in triplicates. Results are expressed as means±SEM. p<0.05: *, *vs* normoxic *Ikkβ*$^{+/+}$ cells; #, *vs* hypoxic *Ikkβ*$^{+/+}$ cells.

Figure S4

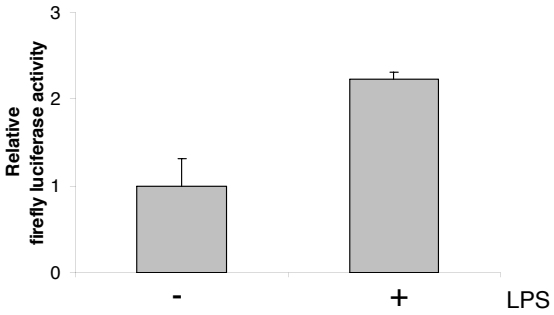

Figure S4: LPS stimulates HIF-1Ð promoter activity. MEF were transfected with a luciferase reporter gene driven by the HIF-1α promoter. After 36 hrs the cells were incubated for 2 hrs without or with LPS (1 mg/mL). Results are averages ±SEM. (n=3)

Figure S5

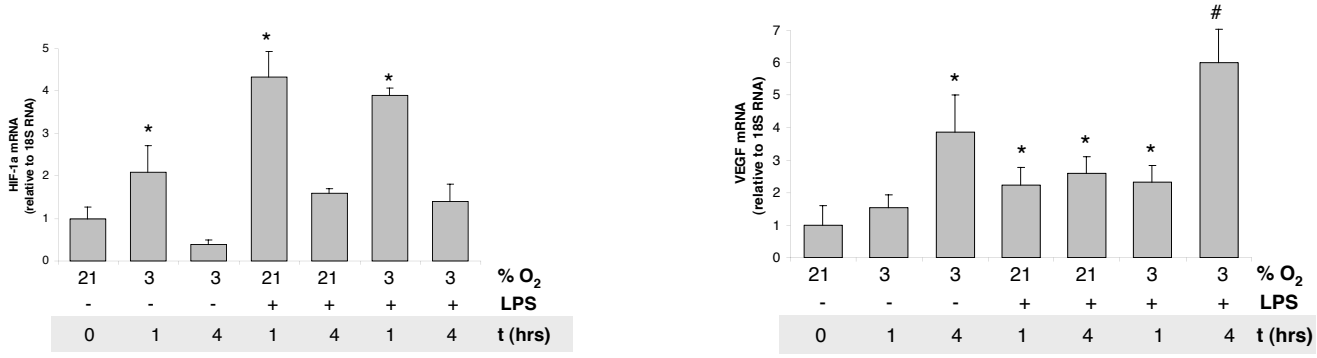

Figure S5: LPS potentiates HIF-1α and VEGF mRNA expression. RAW264.7 cells were cultured in the absence or presence of LPS (1 mg/mL) under normoxia (O_2= 21%) or hypoxia (O_2= 3%). At the indicated time-points RNA was extracted and was analyzed by Q-RT-PCR. Results are expressed as means±SEM. p<0.05: *, *vs* untreated normoxic cells; #, *vs* untreated hypoxic cells.

Figure S6

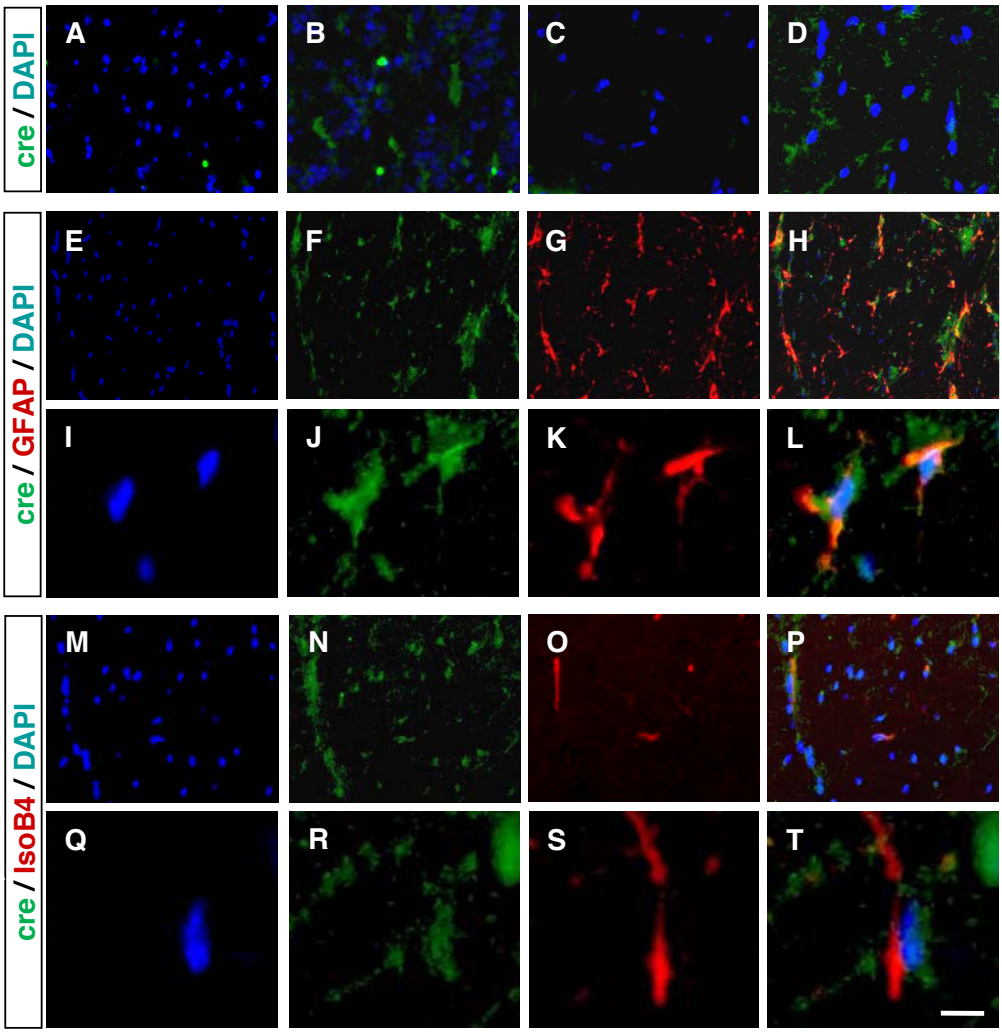

Figure S6: CRE is mainly expressed in astrocytes in brain of poly (I:C) injected *Mx1Cre* mice. *Ikkβ*$^{F/F}$*/Mx1Cre* mice were either left untreated or were injected with 250 μg poly(I:C) (Sigma) on three alternate days. Twenty four hrs after the last injection mice were sacrificed and their brains collected and snap frozen. Brain sections (10 μm) 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) 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 *Ikkβ*$^{F/F}$*/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 *Ikkβ*$^{F/F}$*/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 μm; E-H 50 μm; I-L 8.3 μm; M-P 25 μm; Q-T 6.2 μm. 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-Isolecitin B4 (1:300; Sigma, Saint Louis, MO, USA).

Figure S7

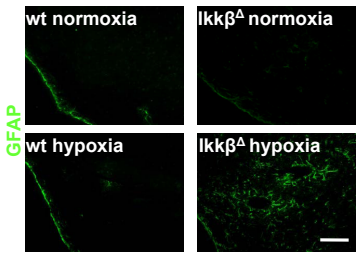

Figure S7: IKKβ deficiency results in increased astrogliosis in brains of hypoxic mice. Mice of the indicated genotypes were kept under normoxia or hypoxia (O_2 = 8%) for 24 hrs. After this period the mice were perfused with a fixative and the brain was collected and frozen. Brain sections at the cerebellar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

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).