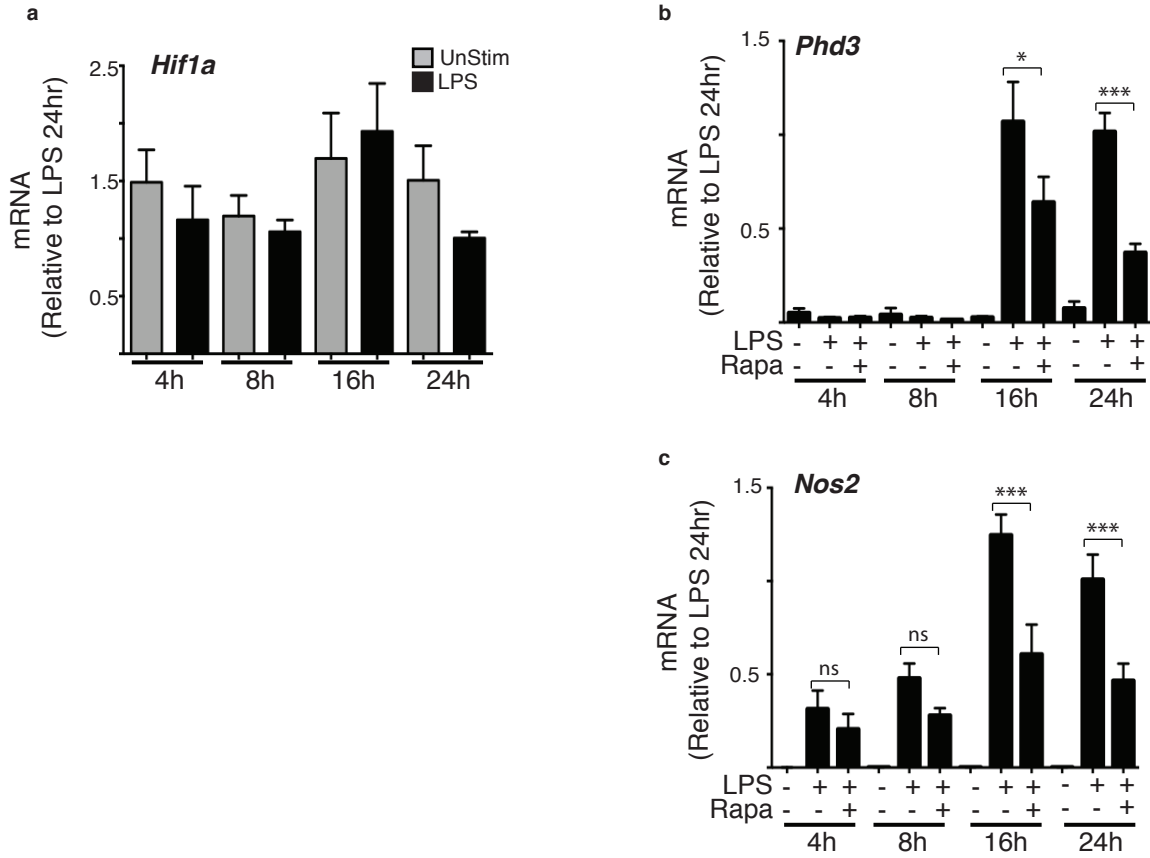


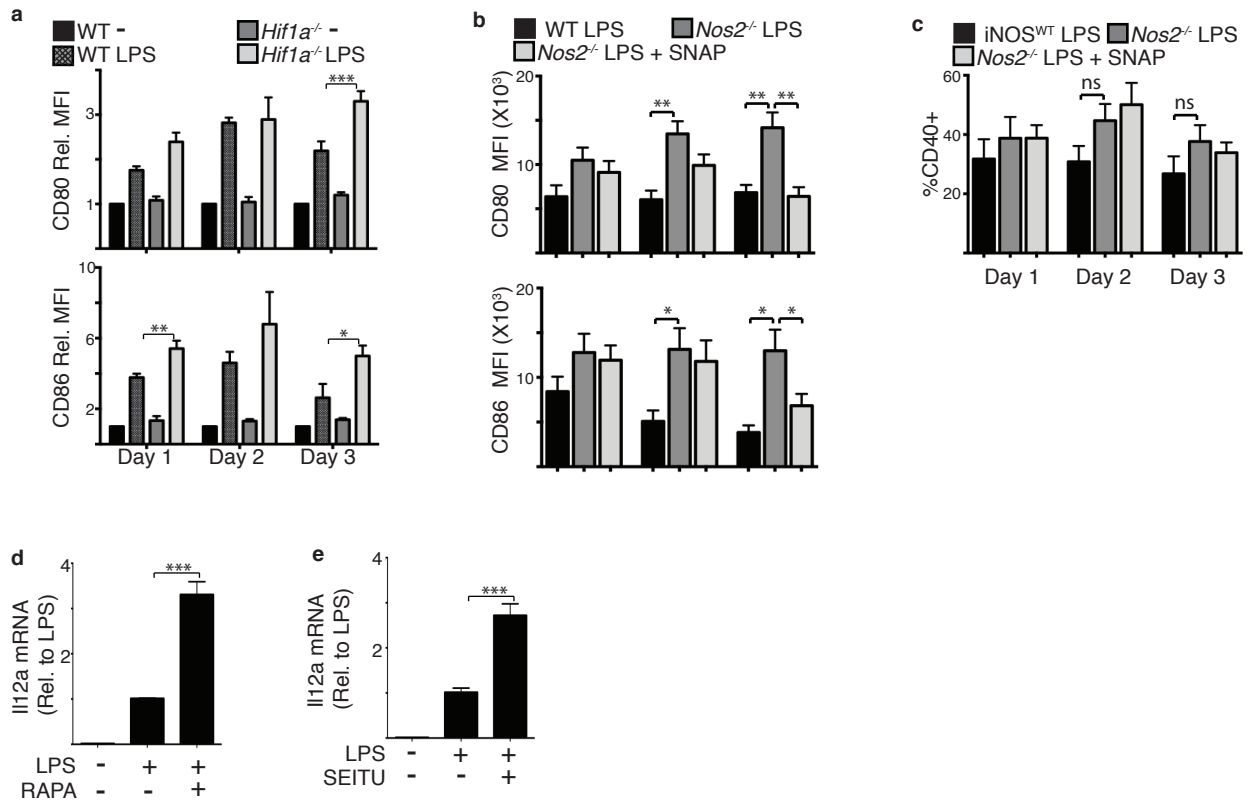
Supplementary Figure 1.

GM-DCs were pulsed with SIINFEKL peptide for 8 hours +/- LPS (100 ng/ml), washed and placed in media containing either 10 mM glucose (Glc), 10mM galactose (Gal) or 10 mM glucose plus 10mM galactose (Glc+Gal). GM-DCs were then maintained in these culture conditions for 2 or 3 days. On the indicated day post LPS stimulation GM-DCs were washed and normal media containing 10 mM glucose was added before the addition of purified CFSE labelled OT-I T cells. After a co-culture period of 48 hours the OT-I T cells were analysed by flow cytometry for proliferation as measured by CFSE dilution. Data is mean +/- range of two separate experiments.



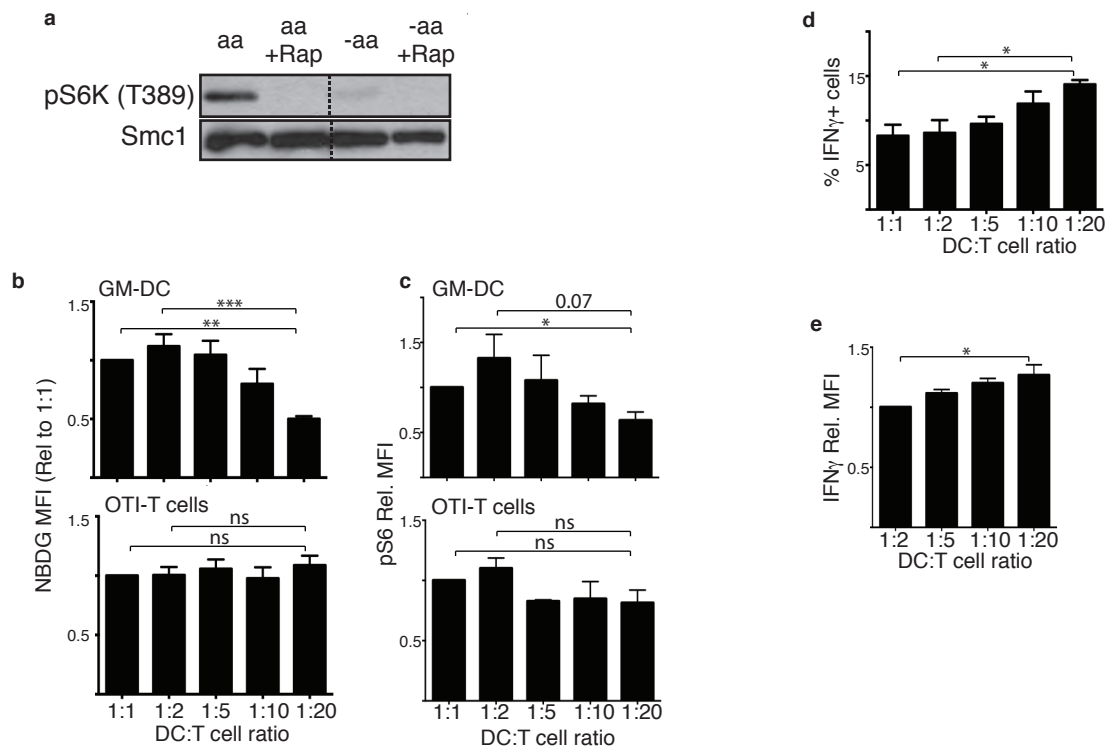
Supplementary Figure 2.

GM-DC were left unstimulated (UnStim) or stimulated with LPS (100 ng/ml) in the presence or absence of rapamycin (20 nM) (as indicated) for 4, 6, 16 and 24 hours, then analysed by qPCR for the expression of (a) *Hif1a* (b) *Phd3* or (c) *Nos2* mRNA. Data is mean +/- SEM of three experiments. Data was analysed using a 1-way ANOVA with a Tukey post-test (*p<0.05, ***p<0.001, ns = not significant)



Supplementary Figure 3.

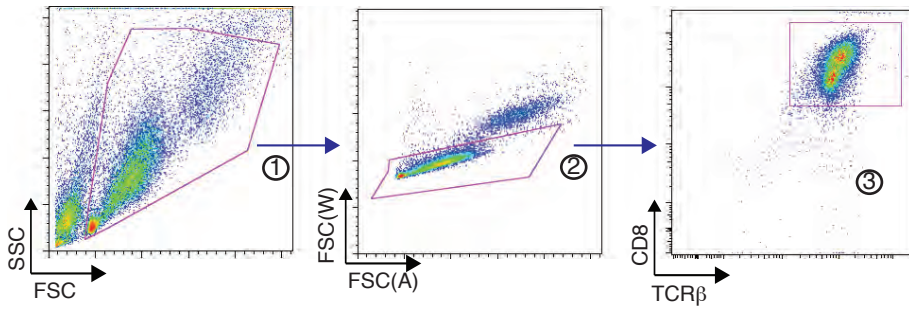
(a) HIF-1 $\alpha^{\text{flox/flox}}$ (WT) or HIF-1 $\alpha^{\text{flox/flox}}$ VavCre (*Hif1a*^{-/-}) GM-DCs were pulsed with SIINFEKL peptide +/- LPS (100 ng/ml) for 1, 2 or 3 days. CD80 and CD86 expression (MFI, mean fluorescent intensity) was analysed by flow cytometry. (b,c) WT and iNOS-null (*Nos2*^{-/-}) GM-DCs were pulsed with SIINFEKL peptide +/- LPS (100 ng/ml) for 1, 2 or 3 days. GM-DCs were treated with SNAP (500 μ M) every 5 hours, where indicated, and CD80 and CD86 (b) and CD40 (c) expression was analysed by flow cytometry. (d,e) GM-DCs were left unstimulated or stimulated for 20 hours in the presence or absence of (d) rapamycin (20 nM) or (e) SEITU (500 μ M) and *IL12a* mRNA was analysed by qPCR. Data is mean +/- SEM of three (d,e), six (a) or seven (b,c) separate cultures. Data was analysed using a 1-way ANOVA with a Tukey post-test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)



Supplementary Figure 4.

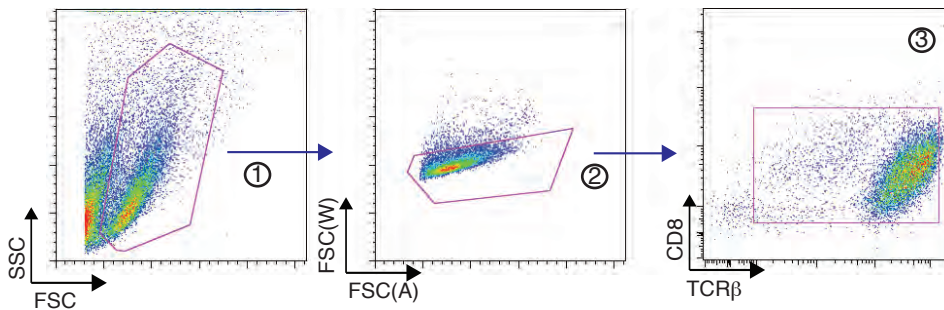
(a) GM-DC were left in complete media +/- rapamycin (20 nM) or deprived of amino acids for 1 hour +/- rapamycin and analysed by immunoblot analysis for the levels of phosphorylated p70 S6-kinase on threonine 389 (pS6K-T389). Structural Maintenance Of Chromosomes protein (Smc1) expression was assessed as a loading control. **(b-e)** GM-DCs were pulsed with SIINFEKL peptide for 6 h +/- LPS (100 ng/ml), washed and purified OT-I T cells were added at different T:DC ratios for 18 h. **(b)** NBDG uptake and **(c)** pS6 levels in GM-DC (top) and CD8 T cells (bottom) was measured by flow cytometry. **(d,e)** IFN γ production was assessed in CD8 T cells by intracellular flow cytometry; % IFN γ positive T cells (d) and the mean fluorescent intensity (MFI) of IFN γ expression in IFN γ positive T cells (e) is shown. Data is representative of two separate experiments (a) and mean +/- SEM five separate experiments (b-e). Data was analysed using a 1-way ANOVA with a Tukey post-test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

a Gating Scheme for T cell analysis



1. Cell gate
2. Doublet discrimination
3. TCRβ+ CD8+ OTI T cells

b Gating Scheme for GM-DC analysis

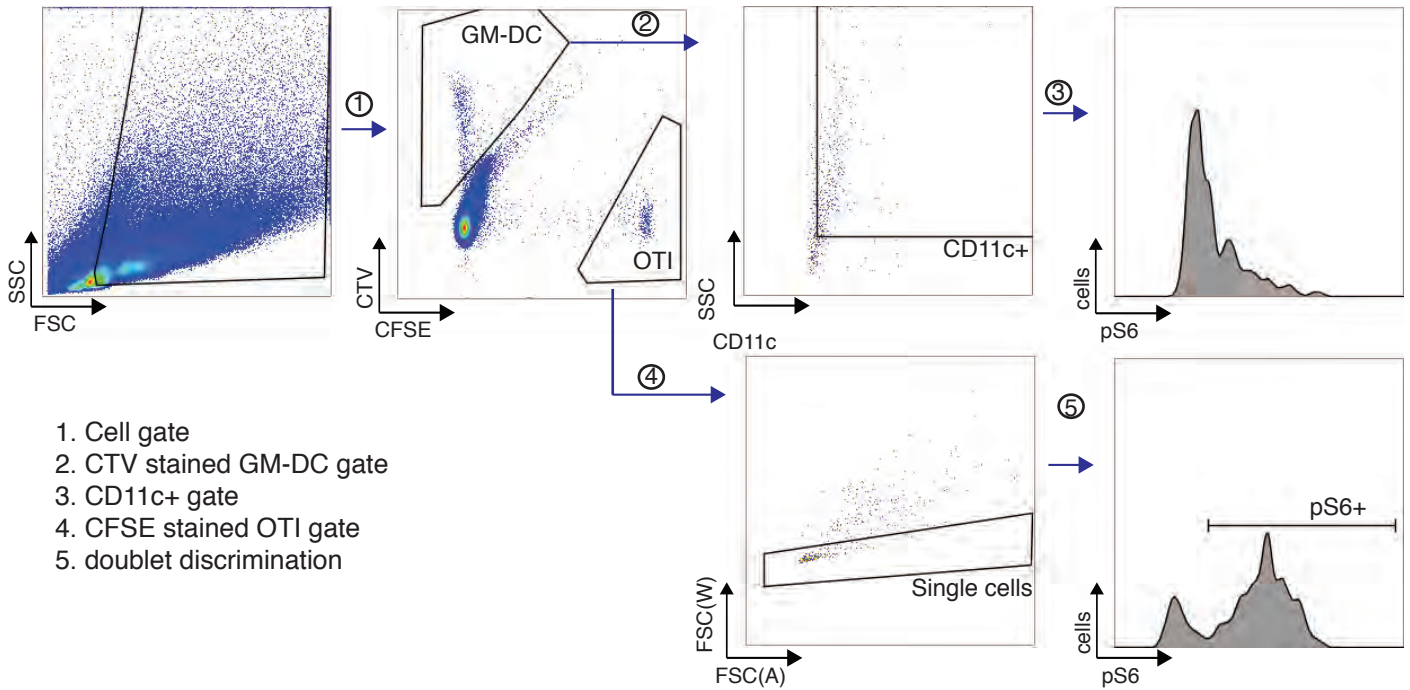


1. Cell gate
2. Doublet discrimination
3. CD11c+ MHCII+ GM-DC

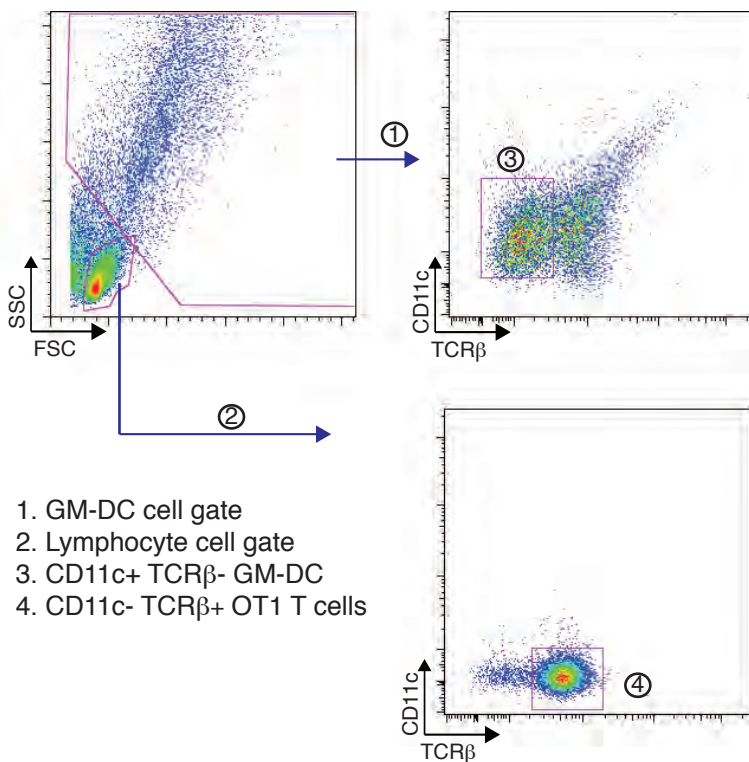
Supplementary Figure 5.

(a) Gating strategy for the analysis of OTI CD8 T cells in Fig.2, Fig. 7i-l, Fig. 8h-j, Supplementary Figure 1 and Supplementary Figure 4d, (b) Gating strategy for the analysis of GM-DC in Fig. 1b-d, Fig. 7a-d and Supplementary Figure 3a-c

a
Gating Scheme for *in vivo* experiments



b
Gating Scheme for DC/T cell co-cultures



Supplementary Figure 6.

(a) Gating strategy for the analysis of adoptively transferred GM-DC and OTI CD8 T cells in Fig.8k-m. (b) Gating strategy for the analysis of GM-DC and OTI CD8 T cells in co-culture experiments in Fig.8b-c and Supplementary Figure 4b-c.

Supplementary Figure 7

Fig 3c



Fig 3g

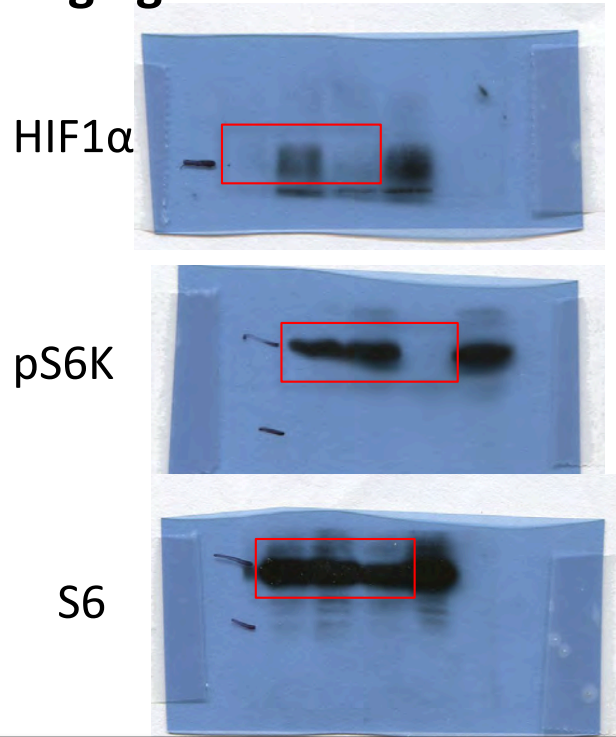
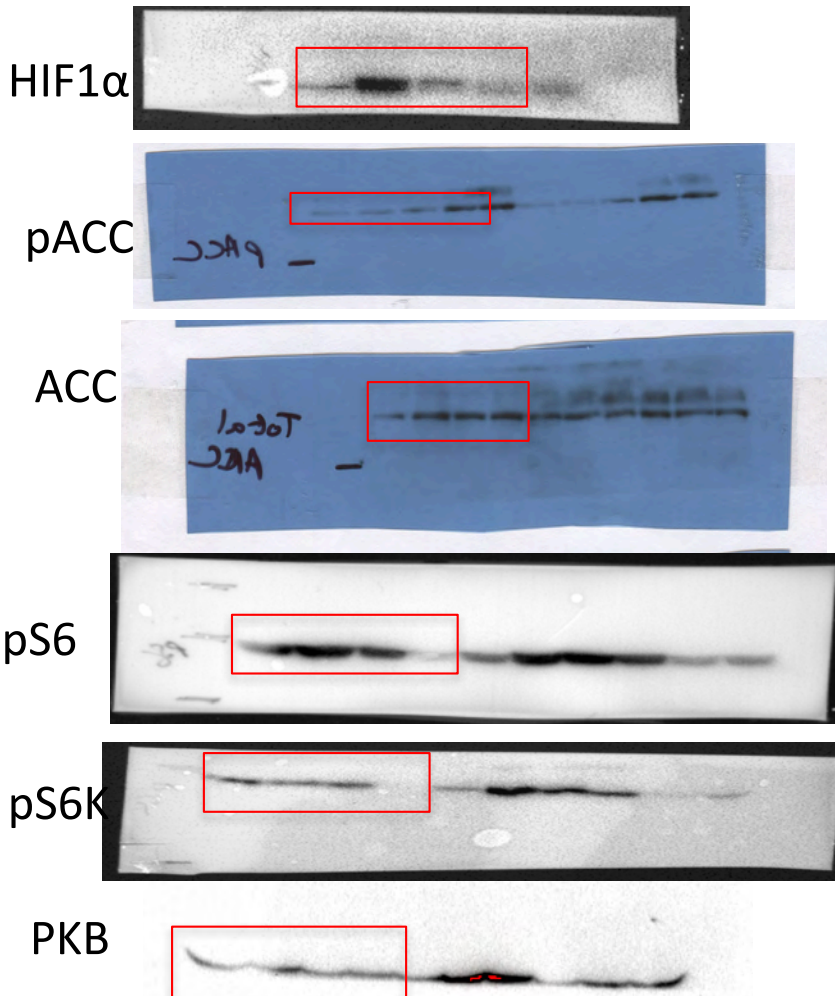


Fig 3e



Supplementary Figure 7

Fig 6a

27-11-15
-, <, <, SEITK

pS6 pS6



HIF-1α HIF-1α



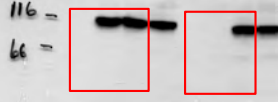
PKB PKB



Fig 6f

WT 1 2 3 4 5 6 7 8
US LPS LPS LPS LPS LPS LPS LPS
+ SAMP + SAMP + SAMP + SAMP + SAMP + SAMP

Hif-1α



β-actin

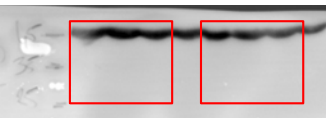
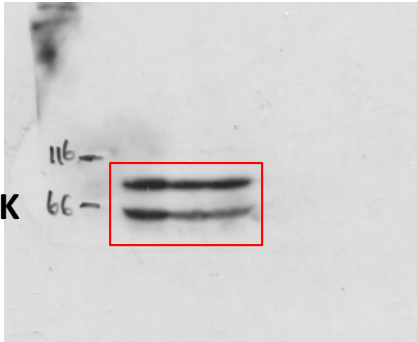
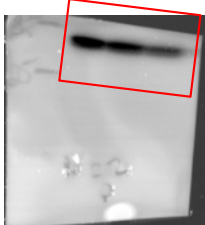


Fig 6c

pS6K



pS6



S6K

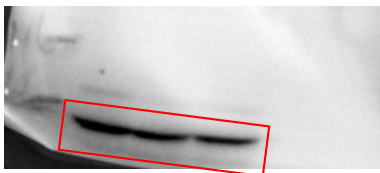
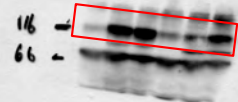


Fig 6g

HIF-1α



β-actin

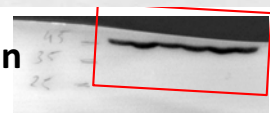
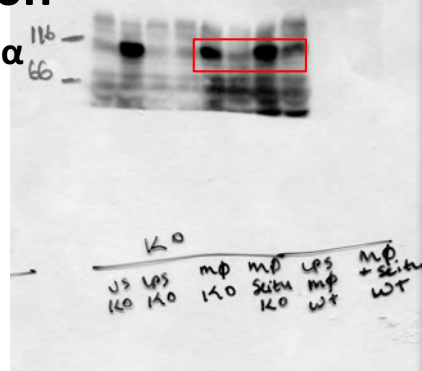
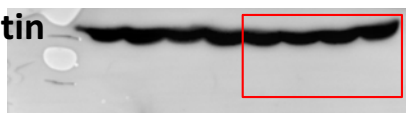


Fig 6h

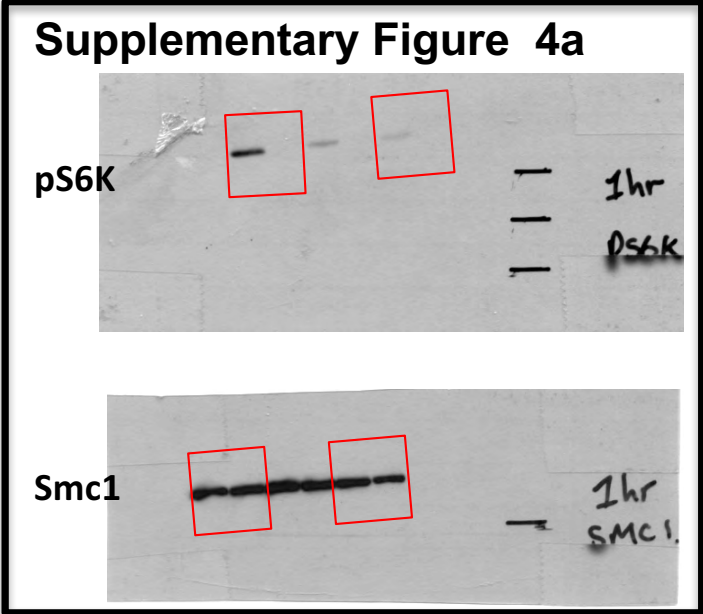
HIF-1α



β-actin



Supplementary Figure 7



Supplementary Table 1:**RTPCR primers**

Gene	Forward sequence 5'-3'	Reverse sequence 5'-3'
<i>Phd3</i>	CTGGTCCTGTACTGCGGGAG	GACCCCTCCGTGTAACCTTG
<i>Nos2</i>	GTCAACTGCAAGAGAACGGAGAAC	CTGCGGGGAGCCATTTTG
<i>Pkf</i>	GCGGCGTGTGTTTCATTGTAG	CACTTCTCGTTCCGGAGCAC
<i>Pkm2</i>	GCTATTCGAGGAACTCCGCC	AAGGTACAGGCACTACACGC
<i>Slc2a1</i>	GGAATCGTCGTTGGCATCCT	CGAAGCTTCTTCAGCACACTC
<i>Il12a</i>	AGTCCCGAAACCTGCTGAAG	GCTCCCTCTTGTTGTGGAAGA
<i>Rplp0</i>	CATGTCGCTCCGAGGGAAG	CAGCAGCTGGCACCTTATTG
<i>Il10</i>	GGACAACATACTGCTAACCGAC	AGAAATCGATGACAGCGCCTC
<i>TNF</i>	AGG CAC TCC CCC AAA AGA TG	TGG TTT GTG AGT GTG AGG GTC