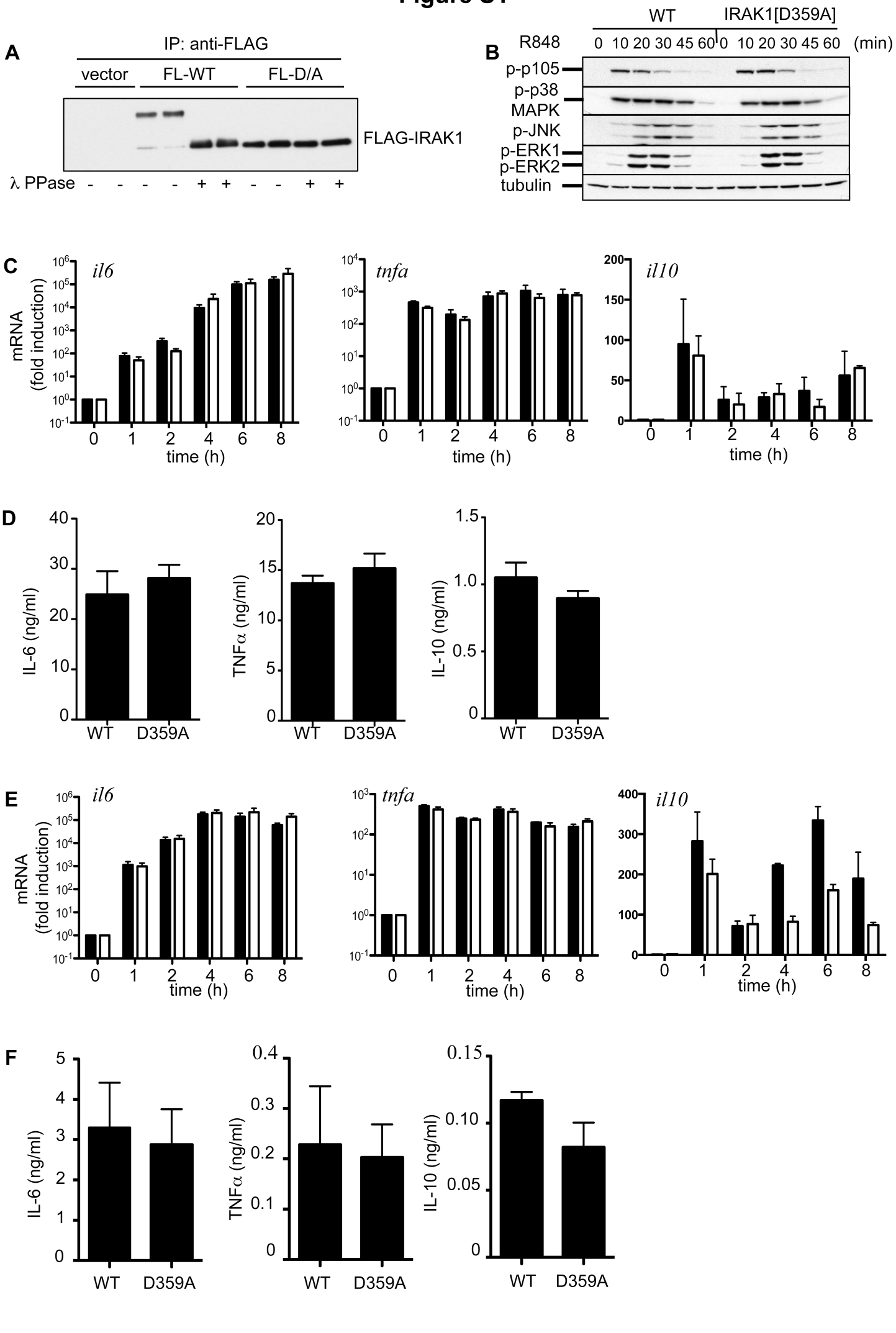
Figure S1


A
IP: anti-FLAG
vector
FL-WT
FL-D/A
FLAG-IRAK1
λ PPase - - - - + + - - + +
B
WT
IRAK1[D359A]
R848
0 10 20 30 45 60 0 10 20 30 45 60 (min)
p-p105
p-p38
MAPK
p-JNK
p-ERK1
p-ERK2
tubulin
C
il6
tnfa
il10
mRNA (fold induction)
time (h)
D
IL-6 (ng/ml)
TNFα (ng/ml)
IL-10 (ng/ml)
WT
D359A
E
il6
tnfa
il10
mRNA (fold induction)
time (h)
F
IL-6 (ng/ml)
TNFα (ng/ml)
IL-10 (ng/ml)
WT
D359A

Supplementary Table I

IRAK2 primers	
primer	sequence
Primers for vector cloning	
Arm1 sense	gagcggccgcGTACTAACCACAGTGTAGTACAATCAGGCTTGAAAGATCC
Arm1 antisense	aaggccggccatatatcctgcaggTACCAACCCACCCTAGCAGACACATTTC
Arm2 sense	tcctgcaggataacttcgtataatgtatgctatacgaagttaTGGTATATCCTGACTGAGCATATTAGC
Arm2 antisense	ttggccggcCAGCACTTGGGAGGCAGAAACAGG
Arm3 sense	agggcgcgccataacttcgtataatgtatgctatacgaagttatctagaGGATTAAAGGTGTGCGCCACTACGC
Arm3 antisense	ttctcgagGCATGTCATAGCTTATGCCGTTGCAGG
Splice donor sense	aaccggtgtgccATGATGGCATCGTGCCCAGGGG
Splice donor antisense	taccggttaattaacatatgCAGCACTTGGGAGGCAGAAACAGG
IRAK2 screening / genotyping primers	
RNA sense (p1)	CCTCGGTGCACATGCTTTACATGTG
RNA antsinse (p2)	AGAGTACGATGTTCTCCATCAGTCTC
	Positive clones give 317bp band
5' loxP sense (p3)	TGAGATTTATTTCCCCAGACCAGCAAG
5' loxP antisense	CCTAAAGCTGGCCCTCAAGCTATTC
(p4)	*300bp wild type, 350bp targeted*
3' loxP sense (p5)	TCCTCTCCACTCTTCACGGGAC
3' loxP antisense (p6)	GCGTAGTGGCGCACACCTTTAATC
	500bp wild type, 600bp neo excised targeted
IRAK1 genotyping primers	
Sense	CAATGAACAGCTCTGTTCTGAGTGTC
Antisense (floxed)	CCAAATGGTCCTGATAAACTGGACTAG
Antisense (knockout)	ACCAAAACCATGTACTCAGATGCTAAGC
	273bp wild type, 389bp floxed, 490bp knockout

Sequences of primers used for generating IRAK2[E525A] mice and routine genotyping of IRAK2[E525A] and IRAK1[D359A] knock-in mice.

Supplementary Figure

Figure S1. Signalling and cytokine production in BMDM from IRAK1[D359A] mice. (A) IL-1R cells were transfected with plasmids encoding FLAG-tagged wild type human IRAK1 (FL-WT) or the IRAK1[D358A] mutant (FL-D/A) or empty vector (vector). The cells were lysed after 24 h and IRAK1 immunoprecipitated from 0.25 mg of cell extract protein with anti-FLAG. The Immunoprecipitates were resuspended and incubated in the absence or presence of phage λ phosphatase (λPPase) (see Methods), followed by SDS-PAGE and immuno-blotting with an anti-FLAG antibody. **(B)** Primary BMDM from wild-type and IRAK1[D359A] mice were stimulated for the times indicated with 1 μg/ml R848 and lysed. The extracts were denatured in SDS, subjected to SDS-PAGE, and immunoblotted with the antibodies indicated. **(C)** BMDM from wild-type (black bars) and IRAK1[D359A] (white bars) mice were stimulated for the times indicated with 1.0 μg/ml R848. RNA was extracted from the cells and *il6* (left hand panels), *tnfa* (middle panels) or *il10* (right hand panels) mRNA measured by qPCR. The results are plotted as fold-increase in mRNA relative to the level determined in unstimulated cells. Error bars represent the mean ± SEM for experiments with BMDM from three mice of each genotype. The data shown is representative of two independent experiments. (**D**) BMDM from wild-type and IRAK1[D359A] mice were stimulated for 24 h with 1 μg/ml R848 for the times indicated. The cell culture medium was collected and IL-6, TNFα and IL-10 were measured by ELISA. Error bars represent the mean ± SD for experiments with BMDM from three mice of each genotype. The data shown is representative of three independent experiments. (**E**) As in C, except that the cells were stimulated with LPS. (**F**) As in D, except that the cells were stimulated with LPS.