Supplemental Fig. 1. IFN-γ differentially regulates LPS-induced IL-12 and IL-23 mRNA expression in mouse peritoneal macrophage



Supplemental Fig. 2. Mouse IL-23 p19 complete cDNA

1	cgcttagaag	tcggactaca	gagttagact	cagaaccaaa	ggaggtggat	agggggtcca
61	caggcctggt	gcagatcaca	gagccagcca	gatctgagaa	gcagggaaca	agATGCTGGA
121	TTGCAGAGCA	GTAATAATGC	TATGGCTGTT	GCCCTGGGTC	ACTCAGGGCC	TGGCTGTGCC
181	TAGGAGTAGC	AGTCCTGACT	GGGCTCAGTG	CCAGCAGCTC	TCTCGGAATC	TCTGCATGCT
241	AGCCTGGAAC	GCACATGCAC	CAGCGGGACA	TATGAATCTA	CTAAGAGAAG	AAGAGGATGA
301	AGAGACTAAA	AATAATGTGC	CCCGTATCCA	GTGTGAAGAT	GGTTGTGACC	CACAAGGACT
361	CAAGGACAAC	AGCCAGTTCT	GCTTGCAAAG	GATCCGCCAA	GGTCTGGCTT	TTTATAAGCA
421	CCTGCTTGAC	TCTGACATCT	TCAAAGGGGA	GCCTGCTCTA	CTCCCTGATA	GCCCCATGGA
481	GCAACTTCAC	ACCTCCCTAC	TAGGACTCAG	CCAACTCCTC	CAGCCAGAGG	ATCACCCCCG
541	GGAGACCCAA	CAGATGCCCA	GCCTGAGTTC	TAGTCAGCAG	TGGCAGCGCC	CCCTTCTCCG
601	TTCCAAGATC	CTTCGAAGCC	TCCAGGCCTT	TTTGGCCATA	GCTGCCCGGG	TCTTTGCCCA
661	CGGAGCAGCA	ACTCTGACTG	AGCCCTTAGT	GCCAACAGCT	TAAggatgcc	caggttccca
721	tggctaccat	gataagacta	atctatcagc	ccagacatct	accagttaat	taacccatta
781	ggacttgtgc	tgttcttgtt	tcgtttgttt	tgcgtgaagg	gcaaggacac	cattattaaa
841	gagaaaagaa	acaaacccca	gagcaggcag	ctggctagag	aaaggagctg	gagaagaaga
901	ataaagtctc	gagcccttgg	ccttggaagc	gggcaagcag	ctgcgtggcc	tgaggggaag
961	ggggcggtgg	catcgagaaa	ctgtgagaaa	acccagagca	tcagaaaaag	tgagcccagg
1021	ctttggccat	tatctgtaag	aaaaacaaga	aaaggggaac	attatacttt	cctgggtggc
1081	tcagggaaat	gtgcagatgc	acagtactcc	agacagcagc	tctgtacctg	cctgctctgt
1141	ccctcagttc	taacagaatc	tagtcactaa	gaactaacag	gactaccaat	acgaactgac
1201	aaatactacc	actatgacct	gtgacaaagc	tgcatattta	ttaagtggga	agggaacttt
1261	tgatatt <mark>att</mark>	<mark>ta</mark> tccttgta	acagtataga	tgatggtt <mark>at</mark>	ttattctatt	<mark>ta</mark> taaggaat
1321	tatgtatttt	tttttcaata	aagatttatt	<mark>ta</mark> tgtggc		

Characters labeled in red color : the coding sequence Characters labeled in green color : the putative AU-rich elements (AREs) Supplemental Fig. 3. LPS-induced IL-23 p19 expression is dependent on MyD88/c-Rel pathway in macrophages



Supplemental Fig. 4. The inhibitory effect of IFN-γ on LPS-induced p19 expression does not need de novo protein synthesis



Supplemental Fig. 5. Spontaneous joint inflammation in TTP knockout mice



Supplemental figure legends

Supplemental figure 1. Differential effects of IFN- γ on p19 and p35 mRNA expression in mouse peritoneal macrophages. Total RNA was isolated from peritoneal macrophages of wild type C57BL/6 mice and subjected to measure p19 (A), p40 (B) and p35 (C) mRNA expression by qRT-PCR. Data were normalized relative to GAPDH mRNA expression levels in each respective sample and further normalized to the results from the un-treated group (Med), which was set as 1. Data are representative of one of three separate experiments with similar results.

Supplemental figure 2. Schematic of mouse IL-23 p19 3'UTR. Listed is the complete sequence of IL-23 p19 mRNA (accession number: AF301619). The translated region starting with ATG is labeled in red color, whereas both 5'UTR and 3'UTR are represented in black and white. Within the 3'UTR region, there are six putative ARE sites labeled in green adjacent to the 3'end.

Supplemental figure 3. MyD88 and NF-kB c-Rel are critical for IL-23 p19 expression. Total RNA was extracted from peritoneal macrophages of WT, MyD88^{-/-} (A), and c-Rel^{-/-} (B) mice treated with IFN- γ , LPS, or IFN- γ plus LPS for 4 h and subjected to RT-PCR analysis for p19 mRNA expression. GAPDH was served as loading control. Data represented pooled RNA from four mice.

Supplemental figure 4. New protein synthesis is not required for IFN- γ -mediated p19 mRNA inhibition. 3×10^6 peritoneal macrophages isolated from C57BL/6 mice were cultured in 2 ml RPMI1640 complete medium with 10% FBS and first stimulated with 1 µg/ml LPS for 30 min, followed by addition of 3 µl cycloheximide (10 µg/ml) for additional 30 min. Then 10

ng/ml IFN- γ was added into the designed groups for 4 h as indicated. Same amount of DMSO (3 μ l) was added to cells treated with LPS or IFN- γ plus LPS as negative controls. Total RNA was extracted and p19 (**A**) and p40 (**B**) mRNA expression measured by qRT-PCR. Data were normalized relative to GAPDH mRNA expression levels in each respective sample and further normalized to the results from the un-treated group, which was set as 1. Results shown are one of two independent experiments with similar results.

Supplemental figure 5. Spontaneous arthritis happens in TTP knockout mice. For TTP knockout and wild type mice, the diameter of hind ankles and body weight were monitored every 2-3 days. The ankle sizes in diameters in two dimensions (horizontal and vertical) (**A**) and body weight (**B**) are shown for WT and TTP knockout mice at age 56 days. Data represent 7 mice in each group with standard deviations (SD). ***: p<0.0001 between two groups.