Supplemental Data

The RNA-binding protein Tristetraprolin (TTP) is a critical negative regulator of NLRP3 expression

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Supplementary Figure legends

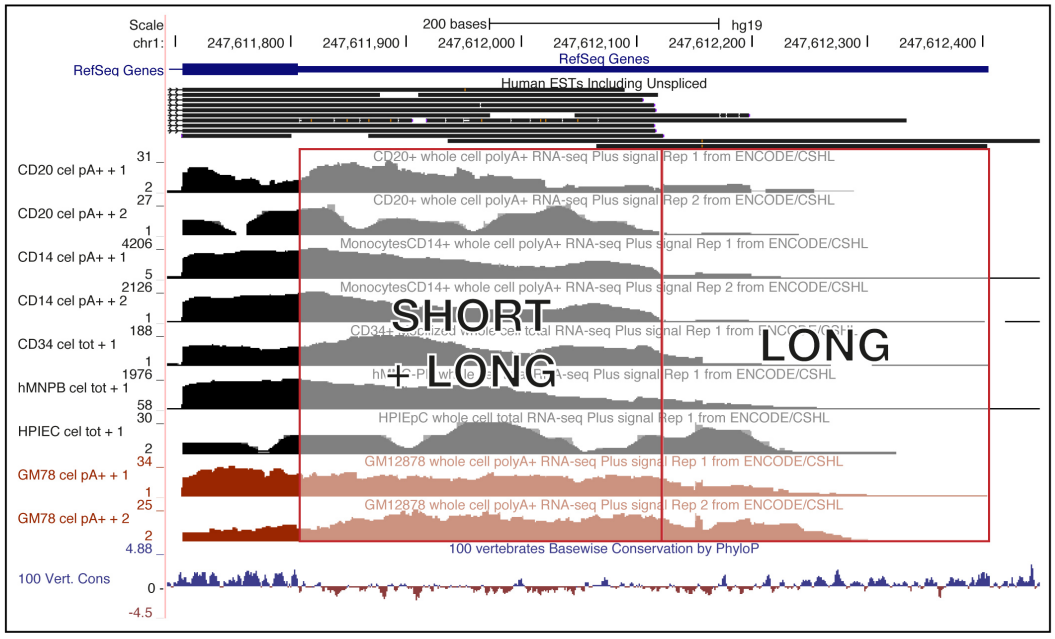
Supplementary Figure 1
Relative expression of NLRP3 3'UTR isoforms. Visualization of the expression tracks quantified in Fig. 1c in the UCSC genome browser. The windows that were used to quantify the average expression of the short and long 3'UTR isoform are indicated.

Supplementary Figure 2
Human and mouse NLRP3 3'UTR features. **(a)** Human and mouse NLRP3 3'UTR sequence with AUUUA pentamers highlighted and ARE*Score* calculation (20). Human short 3'UTR isoform is underlined. **(b)** Conservation of the proximal polyadenylation site. Multiple sequence alignment was performed using Clustal Omega (1.2.3) (63,64). **(c)** 3'RACE of the NLRP3 3'UTR in untreated BMDMs or BMDMs stimulated with 100 ng/ml LPS for 5 h. PCR products were analysed by agarose gel electrophoresis. **(d)** Close-up of the human 3'UTR in the UCSC genome browser (Fig. 1a) highlighting conservation of the miR-223 target site and potential ARE sequences.

Supplementary Figure 3
Complete results of the Zinc finger protein expression screen described in Fig. 2a. HEK293T cells were transfected with an empty vector or full-length NLRP3 3'UTR Firefly luciferase reporter constructs, an internal TK-Renilla control and ZFP expression vectors. Firefly luciferase readings were normalized to *Renilla* activity and the ratio between the expression of the NLRP3 3'UTR and empty vector relative to the Bluescript control of two independent experiments ± S.E.M. is shown.

Supplementary Figure 4
TTP represses NLRP3 inflammasome activity. **(a)** Primary human macrophages were transfected with 50 nM control (siScr_2) or TTP siRNA (siTTP_2). Starting from 24 hours after transfection, cells were primed with 10 ng/ml LPS for 3 hours, followed by inflammasome activation with 10 μM nigericin for 1 hour as indicated. Cleaved IL-1β and cleaved Caspase-1 in the supernatant (S/N) and NLRP3, Pro-IL-1β, Pro-Caspase-1, TTP and beta actin (ACTB) in the cell lysate (Lys) were analysed by western blot. **(d)** THP-1 cells were transfected with control (siScr_1) or TTP siRNA (siTTP_1/2). 24 hours after transfection, cells were primed with PMA for 8 hours, followed by inflammasome activation with 10 μM nigericin for 1 hour as indicated. Cleaved Caspase-1 in the supernatant (S/N) and NLRP3, TTP and beta actin (ACTB) in the cell lysate (Lys) were analyzed by western blot. Representative blots of at least three independent experiments are shown.

Supplementary Figure 1



Supplementary Figure 2

a

ARE*Score* (Spasic *et al.* PLoS Genetics 2012) analysis of human & mouse NLRP3 3'UTR (short isoform underlined & AREs highlighted):

HUMAN

GAGUGGAAACGGGGCUGCCAGACGCCAGUGUUCUCCGGUCCCUCCAGCUGGGGGCCCUCAGGUGGAGAGAGCUGCGAUCCAU
CCAGGCCAAGACCACAGCUCUGUGAUCCUUCCGGUGGAGUGUCGGAGAAGAGAGCUUGCCGACGAUGCCUUCCUGUGCAGAG
CUUGGGCAUCUCCUUUACGCCAGGGUGAGGAAGACACCAGGACAAUGACAGCAUCGGGUGUUGUUGUCAUCACAGCGCCUCA
GUUAGAGGAUGUUCCUCUUGGUGACCUCAUGUAAUUAGCUCAUUCAAUAAAGCACUUUCUUUAUUUUUCUCUUCUCUGUCUA
ACUUUCUUUUUCCUAUCUUUUUUCUUCUUUGUUCUGUUUACUUUUGCUCAUAUCAUCAUUCCCGCUAUCUUUCUAUUAACUG
ACCAUAACACAGAACUAGUUGACUAUAUAUUAUGUUGAAAUUUUAUGGCAGCU**auuuauuuauuua**AAUUUUUUGUAACAGU
UUUGUUUUCUAAUAAGAAAAAUCCAUGCUUUUUGUAGCUGGUUGAAAAUUCAGGAAUAUGUAAAACUUUUUGGU**auuua**AUU
AAAUUGAUUCCUUUUCUUAAUUUUAAAAAA
AREscore (long): 8.2
AREscore (short): 0

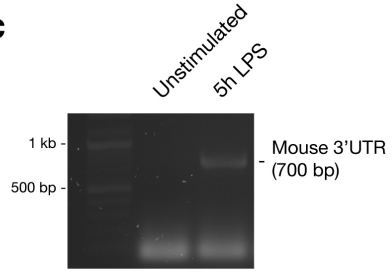
MOUSE

GCGUGGAAGCAGGACCACCAGGUGCCUCGGUCCUGCCCCAAGUCCUGCCCCAAGCCCCAGUGCGCACUGCUCUUCACUGCUA
UCAAGCCCUCCUUCACCAUCAGGAUCACAGCCGAGGCUCUUCUGGUAUAGGGUCUGGAGCAAAGGCUUGUGUGGGACCAAAU
AUUUUUCCUCACAUCGAUAACGUGAAACUGCCAGAGGCUGCCCUUCCCAUCAUAUCCUCAGUGGGCAAGGUGUUCCCUCUUG
GUGACUUCAUGGAAACAGCUUCAAGAAAACGCCUUUUCUGUCCUCCCCCGCCCUCCUCUUACUCCUGCCCCUCCUCCUCCUC
CUCCUCCCCUCCCCCCCCCUCCUCCUCCGCUUCUCCCCCCACCUGUCUUUCUCUCUCUGGGCCUCUGGUUUUUUGACCUUUG
CCCAUACCUUCAGUCUUGUCUUCCUGUUAACUGACCAUCCCGCAUAAGGAGCUGCCCGUGGGCUAGAUGGAAGGUUUGUGGC
AGCCUCUCAGCUACAUUGUUUGUUUUUAUUUUUUAAUAGUUAUGAUUUCUCUUUAGCUACCUGAAAACUCAGAG**auuua**UAA
AACCCAUUUUUGU**auuua**UUGUAUGUUUGUACUGCUUUCUUA**auuua**AAAAUGUAUCUAGAAUUCUUUUAAGUU**auuua**UCC
AAACUACUAAAAAUAAAUCAGUUUACAC**auuua**AAAU
AREscore: 6.5

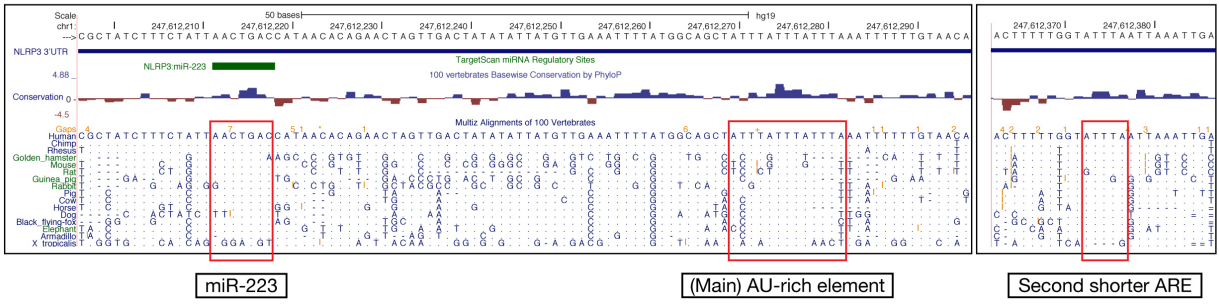
Median human/mouse ARE*Score*: 1.3

b

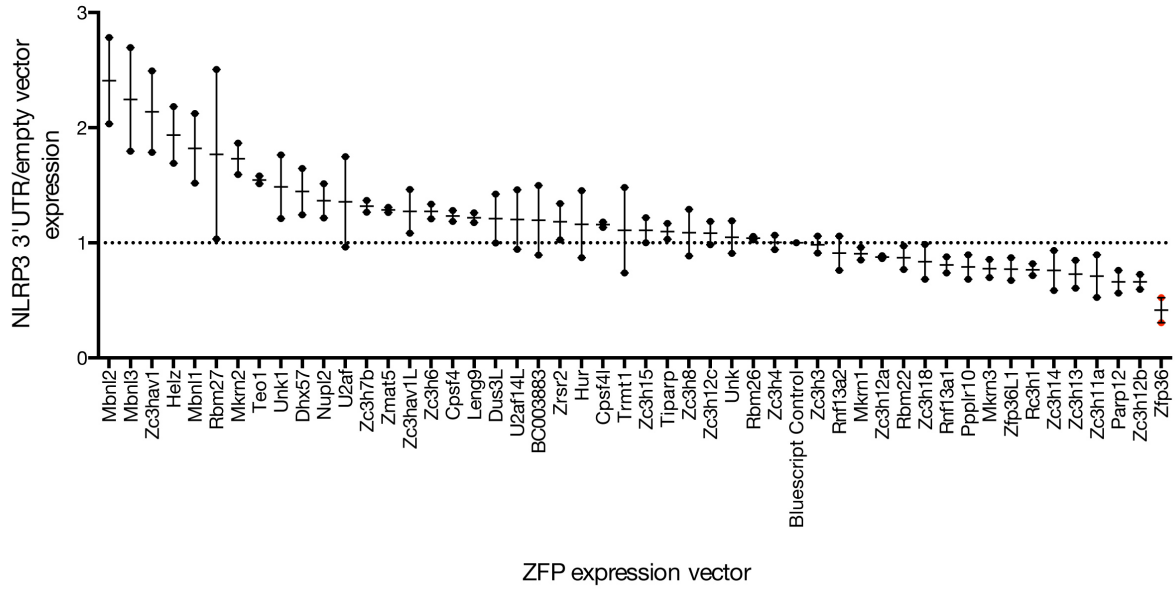
c



d



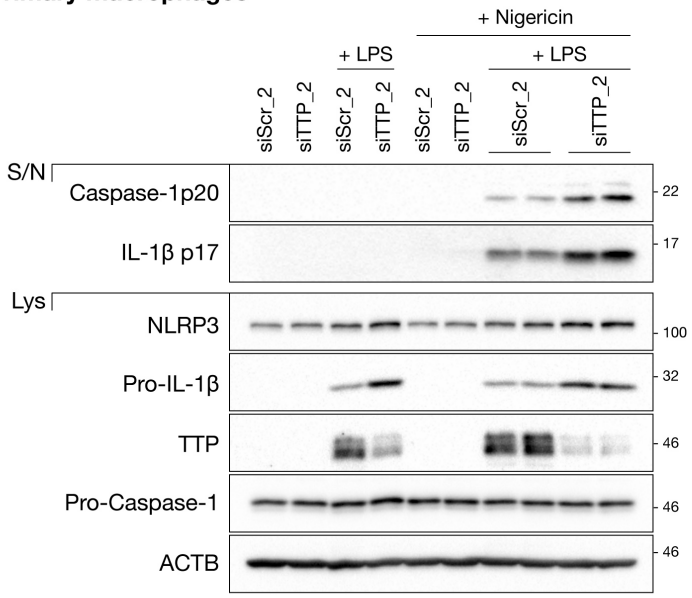
Supplementary Figure 3



Supplementary Figure 4

a

Primary macrophages



b

THP1