Expanded View Figures

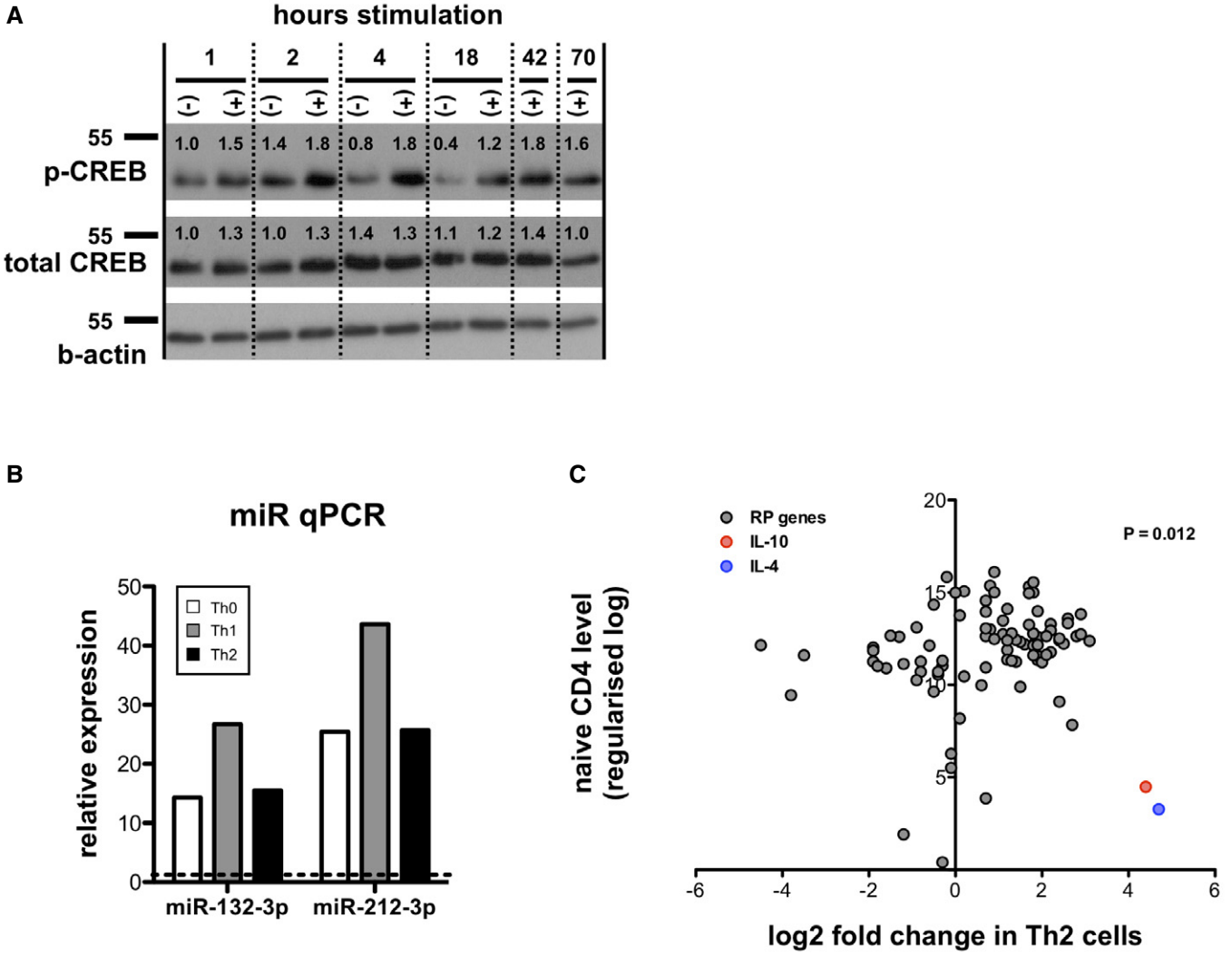


Figure EV1. The miR-132/212 cluster regulates RP mRNA levels in CD4$^+$ T cells from chronically infected spleens.

A Expression of phosphorylated CREB (Ser133), total CREB and β-actin loading control in naïve CD4$^+$ T cells cultured for indicated number of hours in presence (+) or absence (–) of anti-CD3/anti-CD28, as determined by Western blot. Numbers indicate intensity normalised to 1-h unstimulated samples (lane 1) and corrected by β-actin loading control. Representative of two independent experiments from 3 pooled mice each.

B Relative expression of miR-132-3p and miR-212-3p determined by qPCR in naïve mouse CD4$^+$ T cells stimulated with anti-CD3/anti-CD28 for 18 h under Th0 (non-polarising; white), Th1 (rIL-12/anti-IL-4; grey) or Th2 conditions (rIL-4/anti-IFNγ; black) relative to level in naïve cells prior to stimulation.

C Fold change of all RP transcripts (grey) in Th2 cells compared to naïve CD4 T cells. Data taken from RNA sequencing experiments described in reference 24. Fold changes in IL-10 (red) and IL-4 (blue) indicated for comparison. The statistical significance of the observed up-regulation of RP transcripts in Th1 cells is determined by chi-squared test.

Source data are available online for this figure.

Figure EV2. The B-TFIID cofactor BTAF1 is a direct miR-132 target in CD4$^+$ T cells.

A Volcano plot of RNA-seq gene expression in purified CD62L$^+$ CD44$^-$ naïve WT cells before and after 1 day (18 h) stimulation with anti-CD3/anti-CD28. Fold change determined as log2 mean FPKM (stimulated/pre-stimulation) from 4 WT mice. Transcripts significantly different ($P < 0.05$) are shown in red.

B Volcano plot of RNA-seq gene expression in purified CD62L$^+$ CD44$^-$ naïve *miR-132*$^{-/-}$ cells before and after 1 day (18 h) stimulation with anti-CD3/anti-CD28. Fold change determined as log2 mean FPKM (stimulated/pre-stimulation) from 4 WT mice. Transcripts significantly different ($P < 0.05$) are shown in red.

C RNA-seq gene expression levels of BTAF1 from pre-stimulation (d0), 18 h anti-CD3/anti-CD28 (d1) and the spleen of d28 *L. donovani* infection (Ld), from WT (blue) and *miR-132*$^{-/-}$ (red) mice (n = 4–5 mice per group). Significance determined by unpaired t-test as indicated.

D Schematic of miR-212/132-3p 7mer-m8 site in the 3′UTR of *BTAF1* transcript, showing conservation in human, mouse and chimp. The site is also conserved in, rhesus, squirrel, rabbit, pig, cow, cat, dog, brown bat, elephant, opossum, macaw and chicken, but not rat or lizard.

E Relative luciferase activity in mouse 3T3 cells transfected with plasmid containing WT (white) or miR-212/132-mutant (grey) *BTAF1* 3′UTR immediately downstream of Renilla luciferase, in the presence of miR-132-3p or miR-212-3p mimics. Error bars indicate SEM from eight replicate treatments. Significance determined by unpaired t-test.

F Nucleotide sequences of mouse mature miRNA derived from miR-212/132 cluster. Seed sequences indicated in bold.

G Volcano plots of RNA-seq gene expression for transcripts containing a poorly conserved miR-132-5p site (upper panels) or a broadly evolutionary conserved miR-212-5p site (lower panels). Fold change determined as log2 mean FPKM *miR-132*$^{-/-}$/WT) from 4 WT and *miR-132*$^{-/-}$ mice. Transcripts significantly different between WT and miR-132$^{-/-}$ ($P < 0.05$) are shown in red. Data compare pre-stimulation naïve CD4 T cells (d0, left panels); after 18 h *in vitro* stimulation with anti-CD3/anti-CD28 (d1, middle panels); and from the spleens of d28 *L. donovani*-infected mice. Transcripts that are significantly different ($P < 0.05$) and show > 2 fold change in expression are indicated.

H RNA levels of BACH2 (based on RNA-seq) from pre-stimulation (d0), 18 h anti-CD3/anti-CD28 (d1) and the spleen of d28 *L. donovani* infection (Ld), from WT (blue) and *miR-132*$^{-/-}$ (red) mice. Significance determined by unpaired t-test as indicated (n = 4–5 mice per group).

I Log2 fold change (LFC) in RP genes after 18 h *in vitro* stimulation of WT (blue) or miR-132$^{-/-}$ naïve CD4$^+$ T cells with anti-CD3/anti-CD28. Percentages of up-regulated and down-regulated transcripts in WT (40%) and *miR-132*$^{-/-}$ (61%) cells are shown. Statistical significance is determined with chi-squared test.

J DeltaLFC (LFC$^{\text{miR-132}-/-}$ − LFC$^{\text{WT}}$) after 18 h *in vitro* stimulation of WT (blue) or *miR-132*$^{-/-}$ naïve CD4$^+$ T cells with anti-CD3/anti-CD28. Significance is determined with chi-squared test. Data information: *$P < 0.05$, **$P < 0.01$.

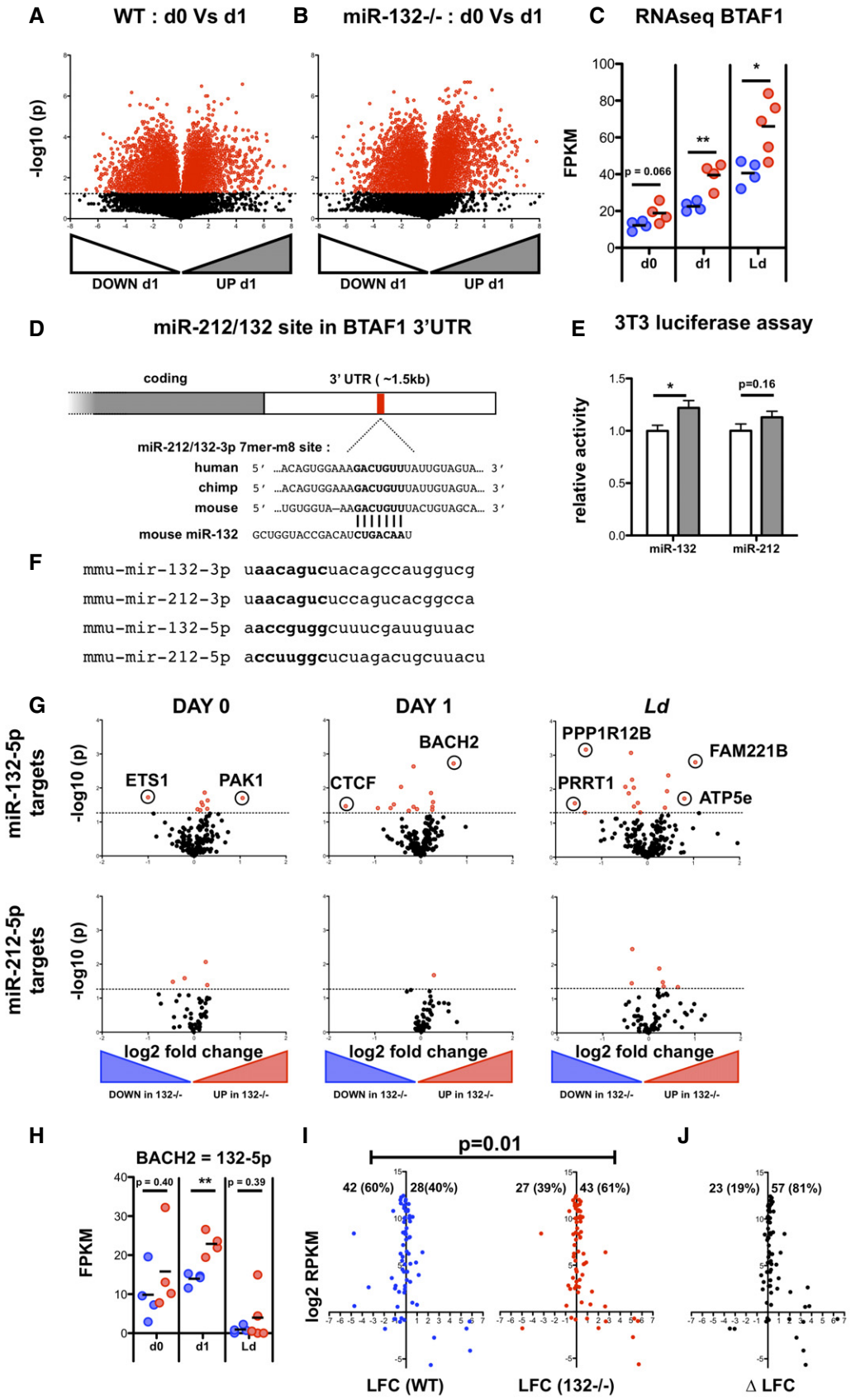
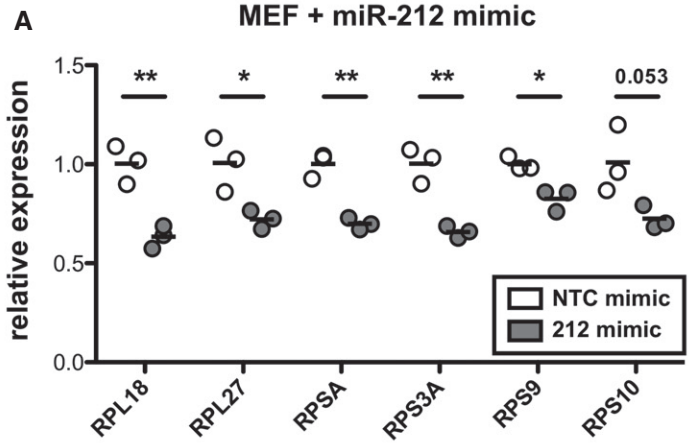
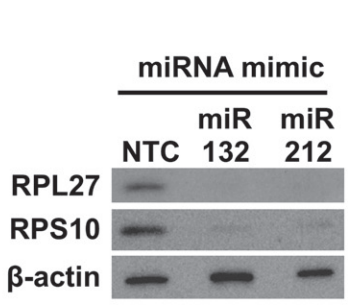

A
WT : d0 Vs d1
B
miR-132-/- : d0 Vs d1
C
RNAseq BTAF1
-log10 (p)
DOWN d1
UP d1
FPKM
p = 0.066
d0
d1
Ld
D
miR-212/132 site in BTAF1 3'UTR
coding
3' UTR (~1.5kb)
miR-212/132-3p 7mer-m8 site :
human 5' ...ACAGUGGAAAGACUGUUUAUUGUAGUA... 3'
chimp 5' ...ACAGUGGAAAGACUGUUUAUUGUAGUA... 3'
mouse 5' ...UGUGGUA--AAGACUGUUUACUGUAGCA... 3'
mouse miR-132 GCUGGUACCGACAUCUGACAAU
E
3T3 luciferase assay
relative activity
p=0.16
miR-132
miR-212
F
mmu-mir-132-3p uaacagucuacagccauggucg
mmu-mir-212-3p uaacagucuccagucacggcca
mmu-mir-132-5p aaccguggcuuucgauuguuac
mmu-mir-212-5p accuuggcucuagacugcuuacu
G
DAY 0
DAY 1
Ld
miR-132-5p targets
miR-212-5p targets
ETS1
PAK1
CTCF
BACH2
PPP1R12B
FAM221B
PRRT1
ATP5e
log2 fold change
DOWN in 132-/-
UP in 132-/-
H
BACH2 = 132-5p
I
p=0.01
42 (60%)
28(40%)
27 (39%)
43 (61%)
log2 RPKM
LFC (WT)
LFC (132-/-)
J
23 (19%)
57 (81%)
Δ LFC

Figure EV2.





C

Molecular Pathway	FDR q-value
GO NUCLEIC ACID BINDING TRANSCRIPTION FACTOR ACTIVITY	3.83E-20
GO REGULATORY REGION NUCLEIC ACID BINDING	6.39E-16
GO SEQUENCE SPECIFIC DNA BINDING	2.55E-13
GO RNA POLYMERASE II TRANSCRIPTION FACTOR ACTIVITY SEQUENCE SPECIFIC DNA BINDING	4.55E-13
GO TRANSCRIPTION FACTOR ACTIVITY RNA POLYMERASE II CORE PROMOTER PROXIMAL REGION SEQUENCE SPECIFIC BINDING	2.99E-12
GO MACROMOLECULAR COMPLEX BINDING	4.98E-11
GO DOUBLE STRANDED DNA BINDING	1.41E-10
GO RNA BINDING	1.97E-10
GO CHROMATIN BINDING	1.97E-10
GO CORE PROMOTER PROXIMAL REGION DNA BINDING	1.69E-09

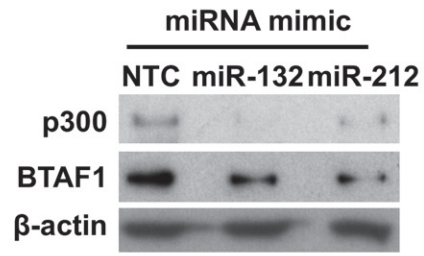


Figure EV3. miR-132 and its targets p300 and BTAF1 control RP expression.

A mRNA levels of indicated RP transcripts determined by qRT–PCR in MEFs transfected with NTC mimics (white) or miR-212-3p mimics (grey). Cultures performed in triplicate. Statistical significance determined by *t*-test.

B Protein levels determined by Western blot of RPL27 and RPS10 in MEFs transfected with NTC, miR-132-3p or miR-212-3p mimics for 48 h.

C Top enriched molecular function GO terms for miR-132/212-3p predicted target genes. Predictions retrieved from Targetscan, total context score < -0.1.

D Protein levels determined by Western blot of BTAF1 and p300 in EL4 cells transfected with NTC, miR-132-3p, or miR-212-3p mimics for 48 h.

Data information: $*P < 0.05$, $**P < 0.01$.

Source data are available online for this figure.

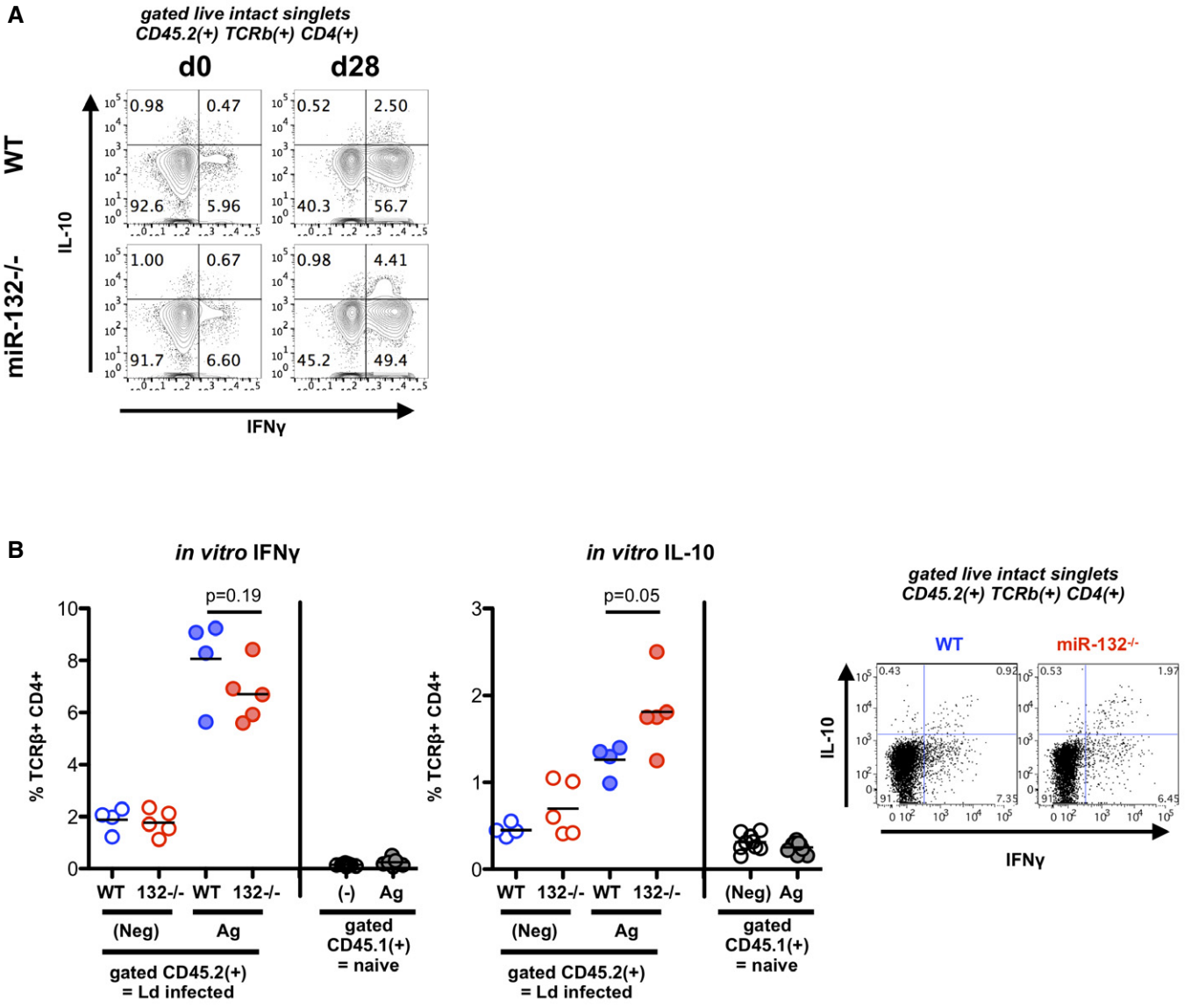


Figure EV4. miR-132 controls the balance between IL-10 and IFNγ production in CD4$^+$ T cells.

A Intracellular cytokine staining of WT or *miR-132*$^{-/-}$ splenic live CD45.2$^+$ TCRβ$^+$ CD4$^+$ cells for IFNγ and IL-10 from day(d) 0 naïve and d28 *L. donovani*-infected mice following *ex vivo* stimulation (4 h) with PMA and ionomycin.

B Antigen-specific IFNγ and IL-10 production by splenic CD4$^+$ T cells from CD45.2$^+$ *L. donovani*-infected WT (blue) or *miR-132*$^{-/-}$ (red) mice was assessed as described in Materials and Methods. Cells were cultured for 3 days in the absence of exogenous stimulation ("Neg", open circles) or with parasite antigen ("Ag", closed circles), after which cytokine production by CD4$^+$ T cells from infected ("CD45.2") or naïve mice ("CD45.1") was determined. Representative FACS plots for antigen-stimulated CD45.2$^+$ cells are shown. Significance determined by unpaired *t*-test, purified CD4$^+$ T cells from 4 to 5 mice per group.

Figure EV5. miR-132 promotes protective immunity to *L. donovani*.

A Percentage of IL10$^+$ splenic live CD45.2$^+$ TCRβ$^+$ CD4$^+$ cells for IL-10 in day (d)0 naïve and d21 in *L. donovani*-infected WT (blue), *IL-10*$^{+/-}$ (open green) and *IL-10*$^{-/-}$ (filled green) mice. d21 used due to accelerated parasite clearance and immune resolution in *IL-10*$^{-/-}$ mice. Significance determined by ANOVA compared to WT group (n = 3–5 per group).

B Percentage of IFNγ$^+$ splenic live CD45.2$^+$ TCRβ$^+$ CD4$^+$ cells for IL-10 in d0 naïve and d21 in *L. donovani*-infected WT (blue), *IL-10*$^{+/-}$ (open green) and *IL-10*$^{-/-}$ (filled green) mice. d21 used due to accelerated parasite clearance and immune resolution in *IL-10*$^{-/-}$ mice. Significance determined by ANOVA compared to WT group.

C Gating strategy for defining distinct myeloid populations in infected mice shown in C. NBNT = non-B non-T, i.e. B220$^-$ CD3$^-$ in d0 naïve and d28 *Ld*-infected WT mice.

D Total spleen cell numbers or of indicated myeloid populations in d0 naïve and d28 *Ld*-infected WT (blue) or *miR-132*$^{-/-}$ (red) mice. Myeloid cells gated as live CD45.2$^+$ CD3$^-$ B220$^-$ Ly6G$^-$ SS^{lo} singlets then; DC (CD11c$^+$ F4/80$^-$ MHCII$^+$); MφA (CD11b$^+$ F4/80$^+$ CD11c$^-$); MφB (CD11c$^+$ F4/80$^+$ CD11b^{lo}); and MφC (CD11c$^+$ F4/80$^+$ CD11b^{hi}). Bars show mean + SEM. Data pooled from two independent experiments (n = 4–5 per group for each experiment). Significance determined by unpaired t-test as indicated.

E Total spleen cell numbers or of indicated myeloid populations in d0 naïve and d21 *Ld*-infected WT (blue), *IL-10*$^{+/-}$ (open green) and *IL-10*$^{-/-}$ (filled green) mice (n = 3–5 per group). Myeloid cells gated as in (D). Bars show SEM. Significance determined by one-way ANOVA and is shown compared to WT group.

F Spontaneous and LPS-induced IL-10 production by indicated spleen myeloid populations (as in D) from N (naive) and *Ld*-infected mice (d28), determined by intracellular cytokine staining. n.d., not detected, i.e. cell type absent in naïve mice. Significance determined by unpaired t-test as indicated, and data pooled from two independent experiments each with 3–5 mice per group. Bars show mean + SEM.

G Day 21 liver parasite burdens expressed as LDU (Leishman-Donovan units) in WT (blue) and *miR-132*$^{-/-}$ (red) mice. Each data point represents an individual mouse. Significance determined by unpaired t-test.

H Left-hand panel: Day 28 liver parasite burdens expressed as LDU (Leishman-Donovan units) in WT (blue) and *miR-132*$^{-/-}$ (red) mice. Right-hand panel shows same data expressed relative to WT levels (WT mean = 1). Data from four independent infection experiments with 4–5 mice per group per experiment. Significance determined by unpaired t-test.

I Day 42 liver parasite burdens expressed as LDU (Leishman-Donovan units) in WT (blue) and *miR-132*$^{-/-}$ (red) mice. Each data point represents an individual mouse. Significance determined by unpaired t-test.

J Spleen size expressed as % body weight for naïve (= 0 parasite dose) or d28 *L. donovani*-infected WT (blue) and *miR-132*$^{-/-}$ (red) mice. Mice were infected with 10, 30 or 100 × 10^6 *L. donovani* amastigotes. Data pooled from two independent experiments with 3–5 mice per group. Significance determined by unpaired t-test.

K Liver size expressed as % body weight for naïve (=0 parasite dose) or d28 *L. donovani*-infected WT (blue) and *miR-132*$^{-/-}$ (red) mice. Mice were infected with 10, 30 or 100 × 10^6 *L. donovani* amastigotes. Data pooled from two independent experiments with 3–5 mice per group. Significance determined by unpaired t-test. Boxes for (J and K) extend from 25th to 75th percentile, whiskers are minimum and maximum values, and horizontal lines indicate median.

Data information: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

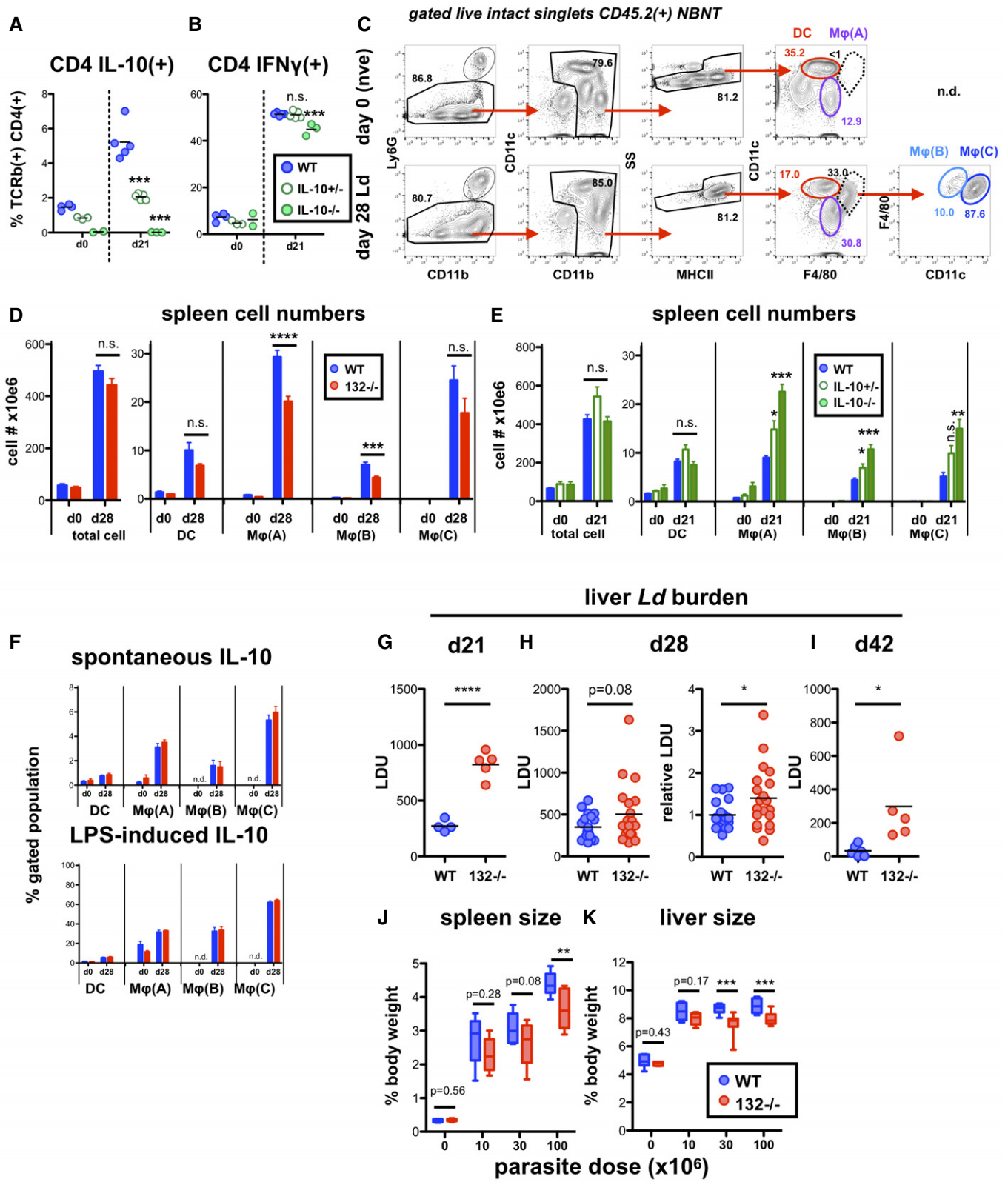

A
CD4 IL-10(+)
% TCRb(+) CD4(+)
d0
d21
B
CD4 IFNγ(+)
n.s.
***
WT
IL-10+/-
IL-10-/-
C
gated live intact singlets CD45.2(+) NBNT
day 0 (nve)
day 28 Ld
Ly6G
CD11b
CD11c
SS
MHCII
F4/80
DC
Mφ(A)
Mφ(B)
Mφ(C)
n.d.
86.8
79.6
81.2
35.2
<1
12.9
80.7
85.0
17.0
33.0
30.8
10.0
87.6
D
spleen cell numbers
cell # x10e6
total cell
DC
Mφ(A)
Mφ(B)
Mφ(C)
d0 d28
WT
132-/-
****
E
spleen cell numbers
d0 d21
IL-10+/-
IL-10-/-
F
spontaneous IL-10
% gated population
LPS-induced IL-10
n.d.
liver Ld burden
G
d21
H
d28
I
d42
LDU
relative LDU
p=0.08
p=0.56
p=0.28
p=0.17
p=0.43
J
spleen size
K
liver size
% body weight
parasite dose (x10⁶)

Figure EV5.