SUPPLEMENTAL DATA

Analysis of the host microRNA response to *Salmonella* uncovers the control of major cytokines by the *let-7* family

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Content of Supplemental Data:

Tab S1 454 read mapping-efficiency

Tab S2 Read abundance of significantly regulated miRNAs (RAW 264.7)

Tab S3 Read abundance of significantly regulated miRNAs (HeLa)

Fig S1 Scatter plots 454 results (SPI-mutant infection)

Fig S2 Analysis of miRNA-expression over time (RAW 264.7 cells)

Fig S3 Regulation of *let-7* and miR-155 in HeLa cells

Fig S4 Apoptosis and cytotoxicity assays (RAW 264.7 cells)

Fig S5 *let-7* regulation in TLR4$^{-/-}$ macrophages exposed to *Salmonella*

Fig S6 Identification of *let-7* target sites within IL-6 and IL-10 3' UTRs

Fig S7 Cytokine expression upon ectopic *let-7* regulation (RAW 264.7)

Fig S8 *let-7* expression in MEF cells

Table S1. Small RNA libraries

Salmonella strain [a]	cell type [b]	time-point (h) [c]	total number of reads [d]	% of mapped reads [e]
-	RAW 264.7	0	43958	76.725
WT	RAW 264.7	4	47015	76.744
ΔSPI1	RAW 264.7	4	33712	75.917
ΔSPI2	RAW 264.7	4	40090	69.104
ΔSPI1/2	RAW 264.7	4	41276	79.763
WT	RAW 264.7	24	47932	72.766
ΔSPI1	RAW 264.7	24	42484	81.261
ΔSPI2	RAW 264.7	24	40434	79.032
ΔSPI1/2	RAW 264.7	24	41084	82.640
-	HeLa	0	48691	76.004
WT	HeLa	4	49258	74.260
WT	HeLa	8	40293	66.857
WT	HeLa	24	60039	51.566
-	HeLa	24	48319	82.175

[a] *Salmonella* strain used for infection

[b] infected host cell type

[c] time-point post infection at which samples for cDNA library preparation were taken

[d] obtained number of 454 reads per library

[e] percentage of reads in the library that could be mapped to Sanger miRBase sequences

Table S2. Abundance of significantly regulated miRNAs in RAW 264.7 cells

time-point [a]	0	24	24	24	24	24
strain [b]	-	WT	SPI1	SPI2	SPI1/2	-
			% of all reads [c]			
mmu-miR-155	0.41	10.51	9.20	8.53	9.81	0.54
mmu-miR-146a	0.12	0.49	0.30	0.25	0.41	0.14
mmu-miR-146b	0.11	0.42	0.41	0.35	0.45	0.12
mmu-miR-21	0.76	2.59	6.84	7.65	7.19	0.69
mmu-let-7a	1.33	0.51	0.52	0.55	0.04	1.01
mmu-let-7c	0.90	0.38	0.54	0.61	0.68	0.84
mmu-let-7d	0.64	0.30	0.30	0.35	0.24	0.66
mmu-let-7f	2.27	1.06	1.63	1.80	1.31	2.21
mmu-let-7g	1.20	0.60	0.82	0.82	0.70	1.27
mmu-let-7i	0.36	0.14	0.44	0.42	0.50	0.35
mmu-miR-98	0.14	0.06	0.07	0.06	0.05	0.15
mmu-miR-27b	5.59	6.25	3.89	3.60	4.35	7.61
mmu-miR-27a	3.75	1.32	4.39	4.17	4.21	5.14
mmu-miR-222	0.53	0.91	0.61	0.59	0.70	0.35
mmu-miR-1928	0.15	0.27	0.13	0.17	0.18	0.14
mmu-miR-30a	0.06	0.04	0.12	0.15	0.11	0.05
mmu-miR-101a	0.04	0.06	0.16	0.16	0.19	0.07
mmu-miR-151-3p	0.05	0.02	0.10	0.08	0.05	0.03
mmu-miR-142-5p	0.30	0.43	0.60	0.60	0.71	0.35
mmu-miR-182	0.25	0.32	0.44	0.51	0.42	0.23
mmu-miR-185	0.25	0.15	0.13	0.09	0.08	0.28
mmu-miR-190	0.01	0.00	0.00	0.00	0.00	0.01
mmu-miR-191	1.37	1.37	2.02	1.81	1.65	0.92
mmu-miR-30e	0.11	0.09	0.27	0.31	0.33	0.10
mmu-miR-19b	0.23	0.16	0.53	0.53	0.55	0.24
mmu-miR-22	0.16	0.35	1.04	1.10	1.05	0.25
mmu-miR-26b	1.37	0.68	0.55	0.48	0.67	1.05
mmu-miR-92a	1.98	0.83	0.72	0.66	0.50	1.13
mmu-miR-96	0.03	0.02	0.13	0.12	0.13	0.05
mmu-miR-340-5p	0.06	0.08	0.11	0.07	0.09	0.05
mmu-miR-320	0.30	0.18	0.10	0.10	0.09	0.20
mmu-miR-103	1.33	1.08	0.92	0.80	1.08	1.57
mmu-miR-107	0.61	0.43	0.38	0.34	0.39	0.70
mmu-miR-222	0.53	0.91	0.61	0.59	0.70	0.35
mmu-miR-7a	0.10	0.22	0.37	0.32	0.41	0.12
mmu-miR-362-3p	0.10	0.11	0.05	0.04	0.07	0.13
mmu-miR-378	0.51	0.25	0.24	0.22	0.17	0.45
mmu-miR-425	0.08	0.11	0.31	0.29	0.29	0.10
mmu-miR-1939	0.85	1.54	0.95	1.21	0.54	1.52
mmu-miR-1961	0.17	0.09	0.05	0.05	0.07	0.15
mmu-miR-805	0.21	0.01	0.02	0.00	0.01	0.09

[a] time-point post infection at which samples for cDNA-library preparation were taken

[b] applied *Salmonella* strain

[c] percent of reads in the respective library that match to the miRNA indicated; values in the shadowed fields below denote regulations greater two-fold

Table S3. Abundance of significantly regulated miRNAs in HeLa cells

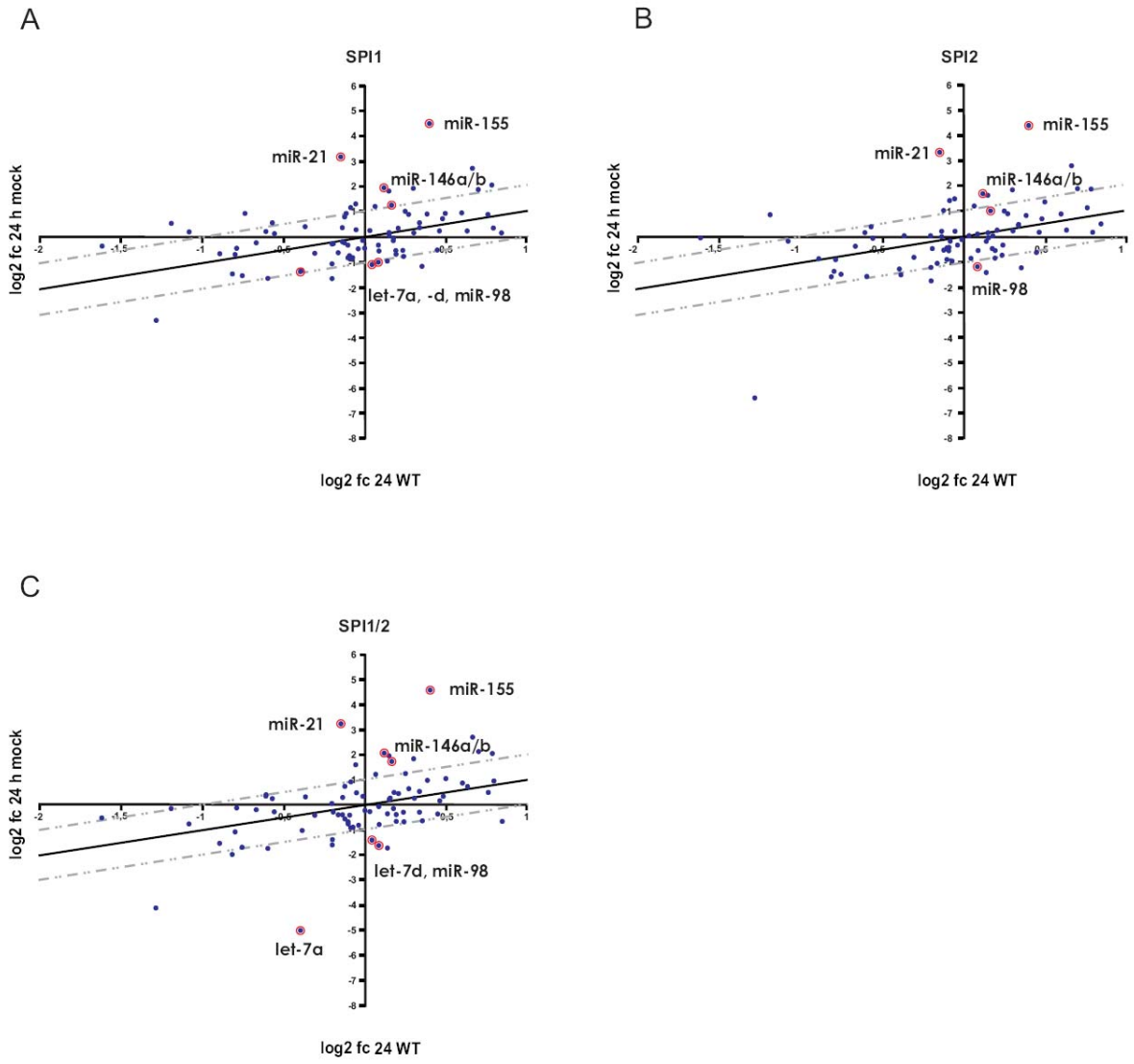
time-point [a]	0	24	24
strain [b]	-	WT	-
		% of all reads [c]	
hsa-miR-1308	0.075	**0.29**	0.10
hsa-let-7a	1.08	**0.59**	1.22
hsa-let-7b	0.43	**0.21**	0.50
hsa-let-7d	0.18	**0.08**	0.18
hsa-let-7f	0.92	**0.62**	0.96
hsa-let-7i	0.36	**0.18**	0.42
hsa-miR-16	8.43	**4.27**	9.10
hsa-miR-17	7.16	**3.09**	6.88
hsa-miR-20a	4.70	**3.41**	6.82
hsa-miR-93	0.81	**0.59**	1.18
hsa-miR-106a	0.09	**0.04**	0.10
hsa-miR-196a	0.52	**0.31**	0.65
hsa-miR-224	0.29	**0.26**	0.63
hsa-miR-151-5p	0.74	**0.39**	0.84
hsa-miR-886-5p	0.13	**0.02**	0.06
hsa-miR-1297	0.27	**0.16**	0.32

[a] time-point post infection at which samples for cDNA-library preparation were taken

[b] applied *Salmonella* strain

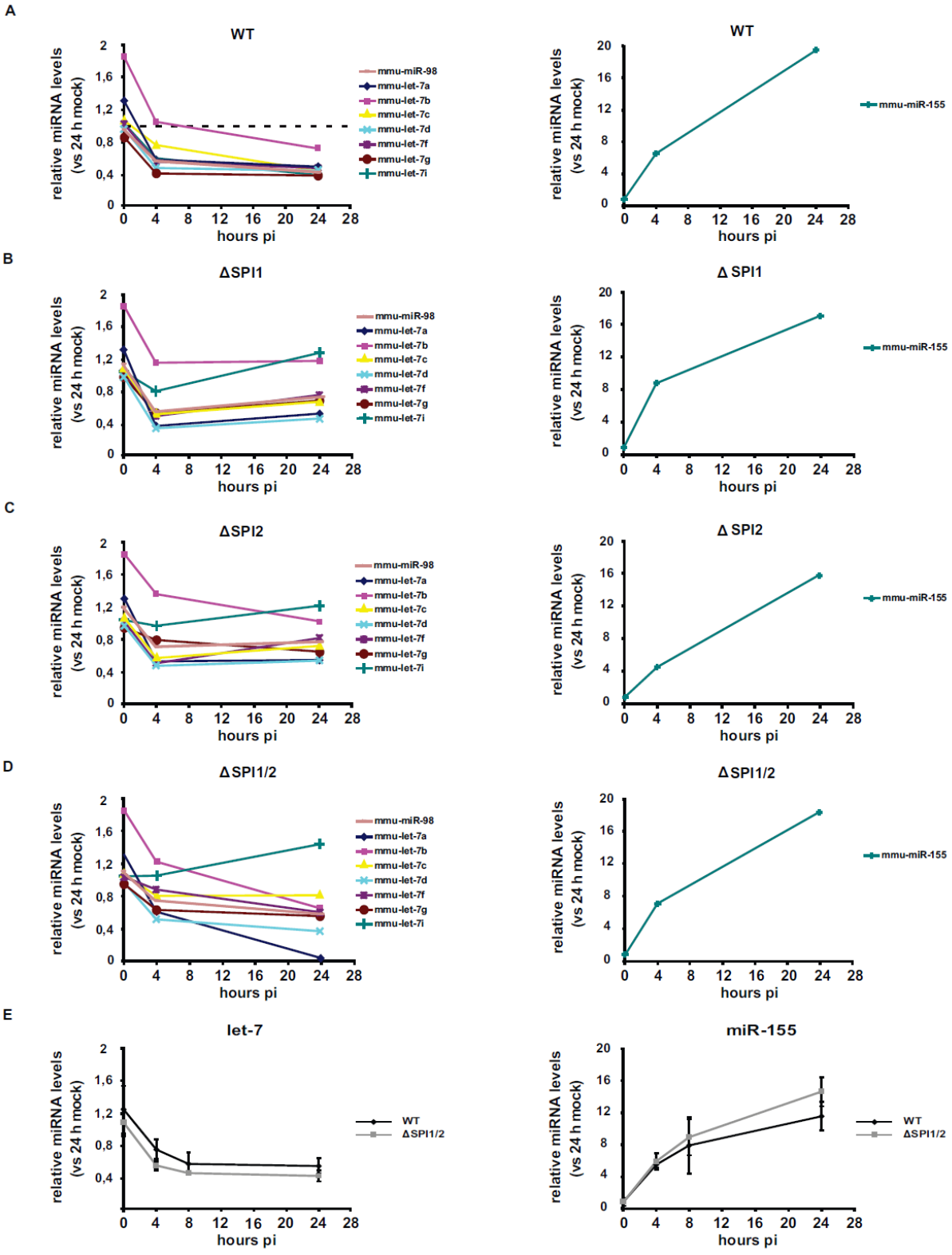
[c] percent of reads in the respective library that match to the miRNA indicated

Figure S1

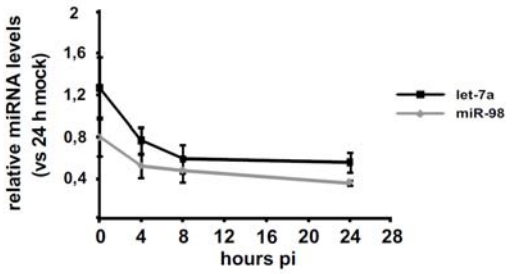


Infection of RAW 264.7 cells with attenuated *Salmonella* mutants induces a similar miRNA expression pattern as wild-type infection (see Fig 1). Infections were carried out using strains (A) ΔSPI1, (B) ΔSPI2, and (C) ΔSPI1/2. Small RNA libraries of uninfected cells at 0 h, cells infected with wild-type *Salmonella* at 24 h pi and mock treated cells at 24 hours p.i., were analyzed by 454 sequencing, and microRNA expression changes were calculated by comparison of cDNA hits in the libraries (24 h infection vs 0 h, 24 h mock vs 0 h). The graphs show log2 fold changes in infected cells (y-axis) versus log2 fold changes in mock-treated cells (x-axis).

Figure S2


A
WT
relative miRNA levels (vs 24 h mock)
hours pi
mmu-miR-98
mmu-let-7a
mmu-let-7b
mmu-let-7c
mmu-let-7d
mmu-let-7f
mmu-let-7g
mmu-let-7i
WT
mmu-miR-155
B
ΔSPI1
Δ SPI1
C
ΔSPI2
Δ SPI2
D
ΔSPI1/2
ΔSPI1/2
E
let-7
miR-155
WT
ΔSPI1/2

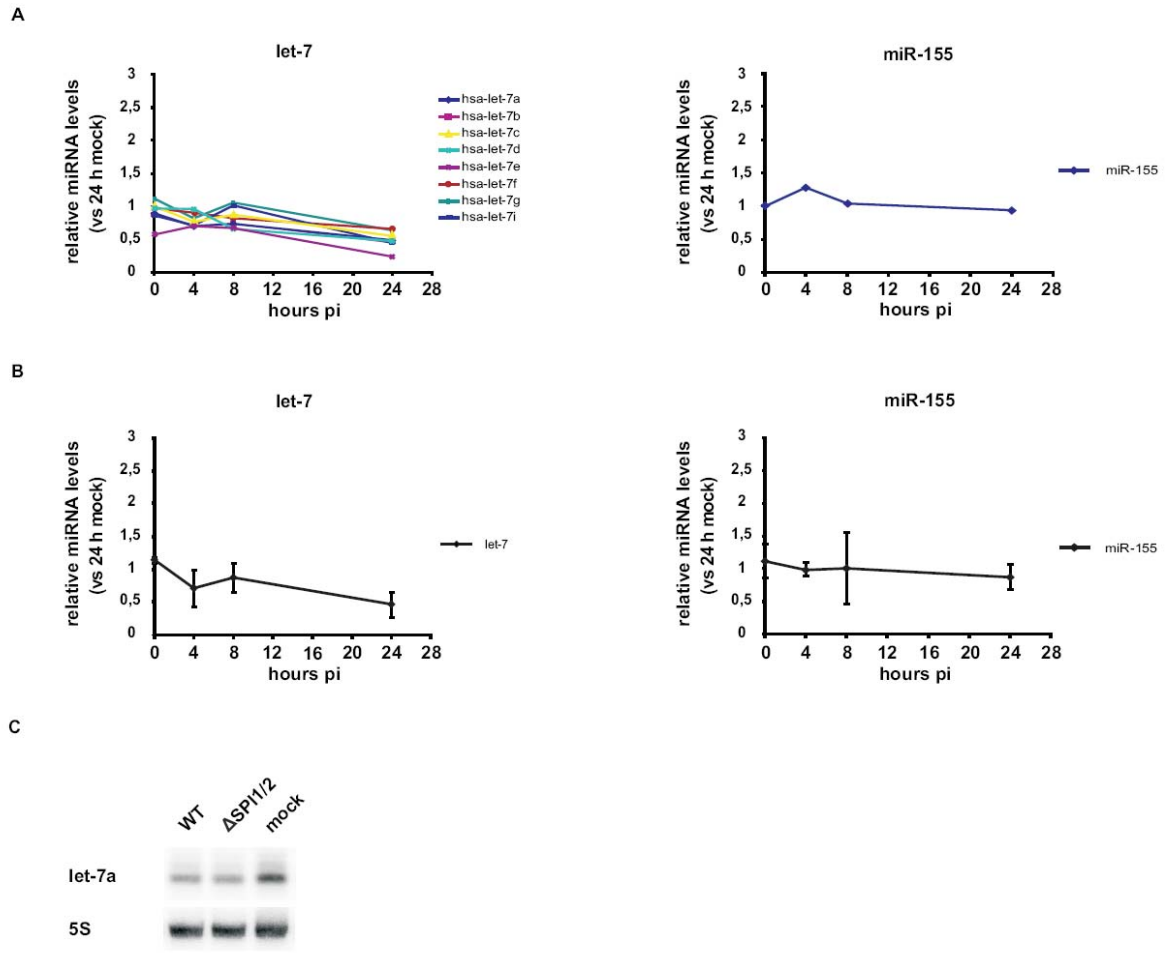
F



Time-course analysis of *let-7* and miR-155 expression in RAW 264.7 cells.

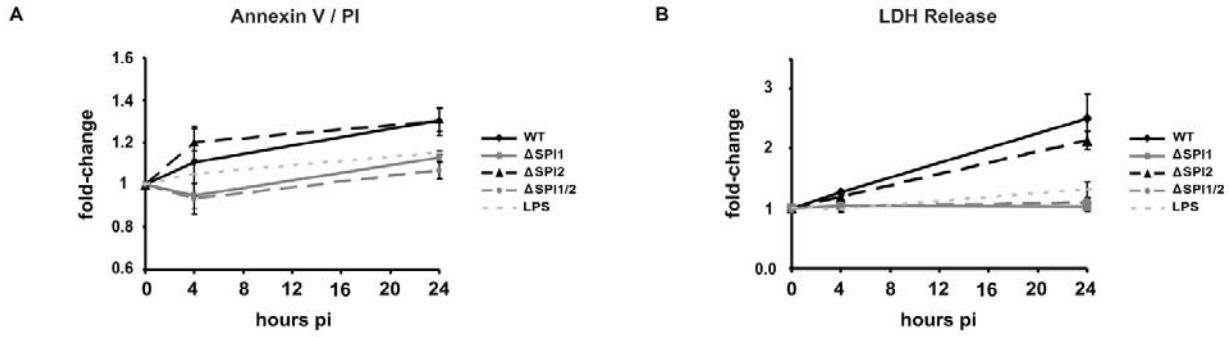
Comparative 454 sequencing (A-D) and qRT-PCR analysis (E) of miR-155 and *let-7a* at 4, 8 and 24 hours p.i. in RAW 264.7 cells. (F) Comparative qRT-PCR-analysis of let-7a and miR-98 expression at 4, 8 and 24 hours p.i. in RAW 264.7 cells. qRT-PCR results are shown as mean-values and standard deviations derived from three independent experiments. All fold-changes refer to the 24 h mock control.

Figure S3



Validation of let-7 regulation in HeLa cells. Comparative 454 sequencing (A) and qRT-PCR (B) analysis of miR-155 and *let-7* family at 4, 8 and 24 hours pi in HeLa cells. (C) Northern-blot validation of let-7 regulation upon infection of HeLa cells with wild-type or ΔSPI1/2 *Salmonella* for 24 h (compared to 24 h mock-treatment). All fold-changes refer to the 24 h mock control.

Figure S4

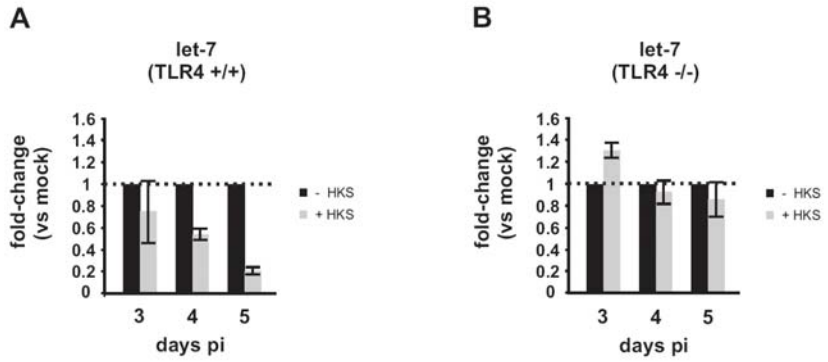


Effect of *Salmonella* TTSS mutants and miRNA-mimics on apoptosis and necrosis.

Induction of apoptosis (A) and cytotoxicity (B) in RAW 264.7 cells infected with the indicated *Salmonella* strains or stimulated with LPS. Fold-changes refer to the 0 h control.

Apoptosis induction was assessed by FACS-quantification of Annexin V-positive / PI-negative cells. Cytotoxicity was determined indirectly by photometric quantification of LDH-activity in cell-culture supernatants. Mean values and standard deviations derived from three independent experiments are shown. Significant induction of apoptosis and cytotoxicity (compared to mock-treatment) was observed for *Salmonella* wild-type and SPI2-mutant infection as well as for LPS-treatment (P-value < 0.05), however not for SPI1- and SPI1/2-mutant infection (P-value > 0.05).

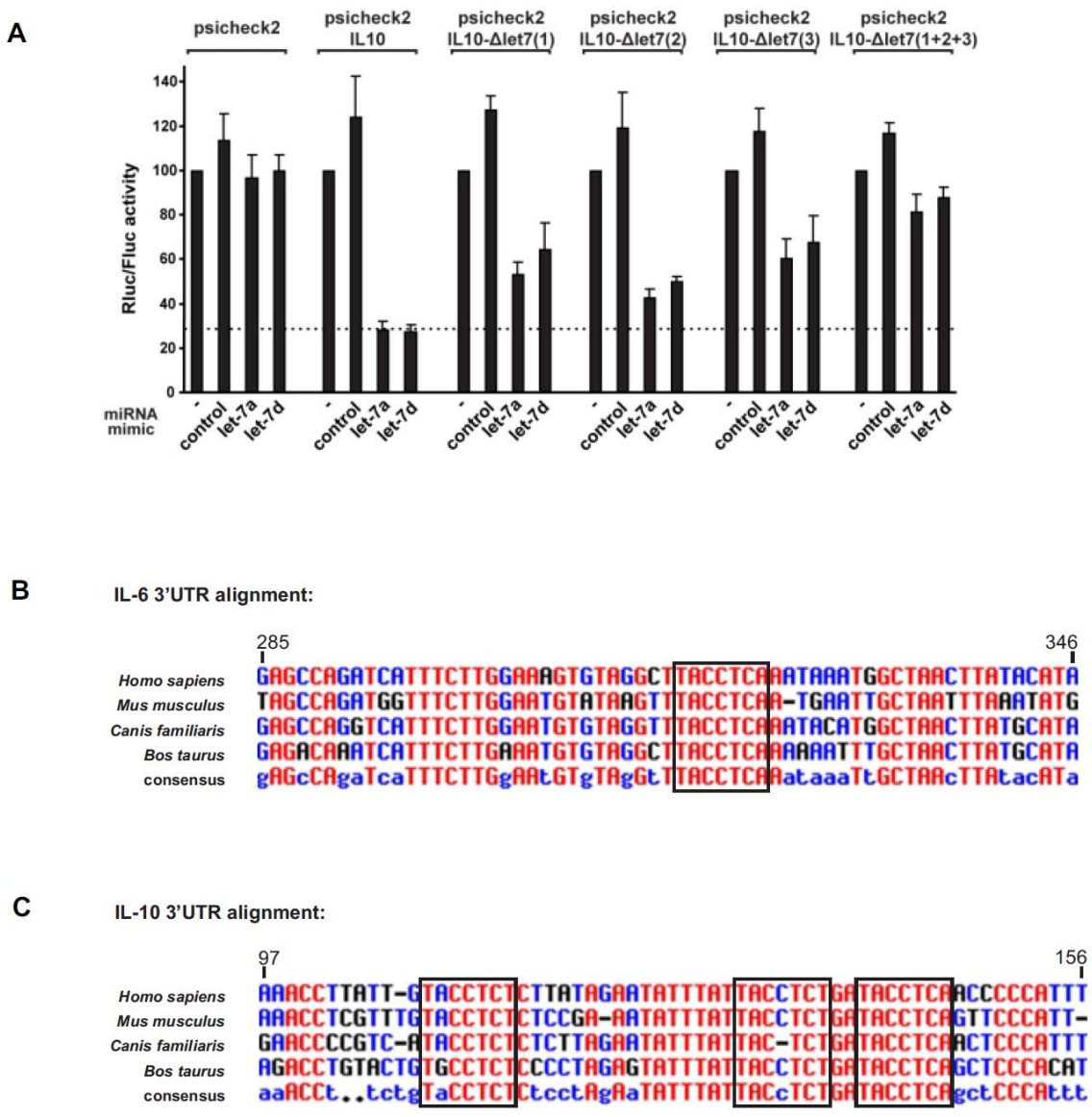
Figure S5



Expression of let-7 in *Salmonella*-exposed primary macrophages.

Expression of let-7 in bone-marrow derived primary macrophages obtained from wild-type (A) or TLR4-/- (B) animals as determined by qRT-PCR. Cells were differentiated with murine colony-stimulating factor (MCSF) alone (-HKS) or with MCSF + heat killed *Salmonella* (+HKS). Average fold-regulation of let-7 upon HKS-treatment and standard deviations derived from three independent experiments are shown. Let-7 regulation upon HKS-challenge of TLR4 +/+ cells was significantly different compared to the respective regulation observed in TLR4 -/- cells (*P*-values < 0.05).

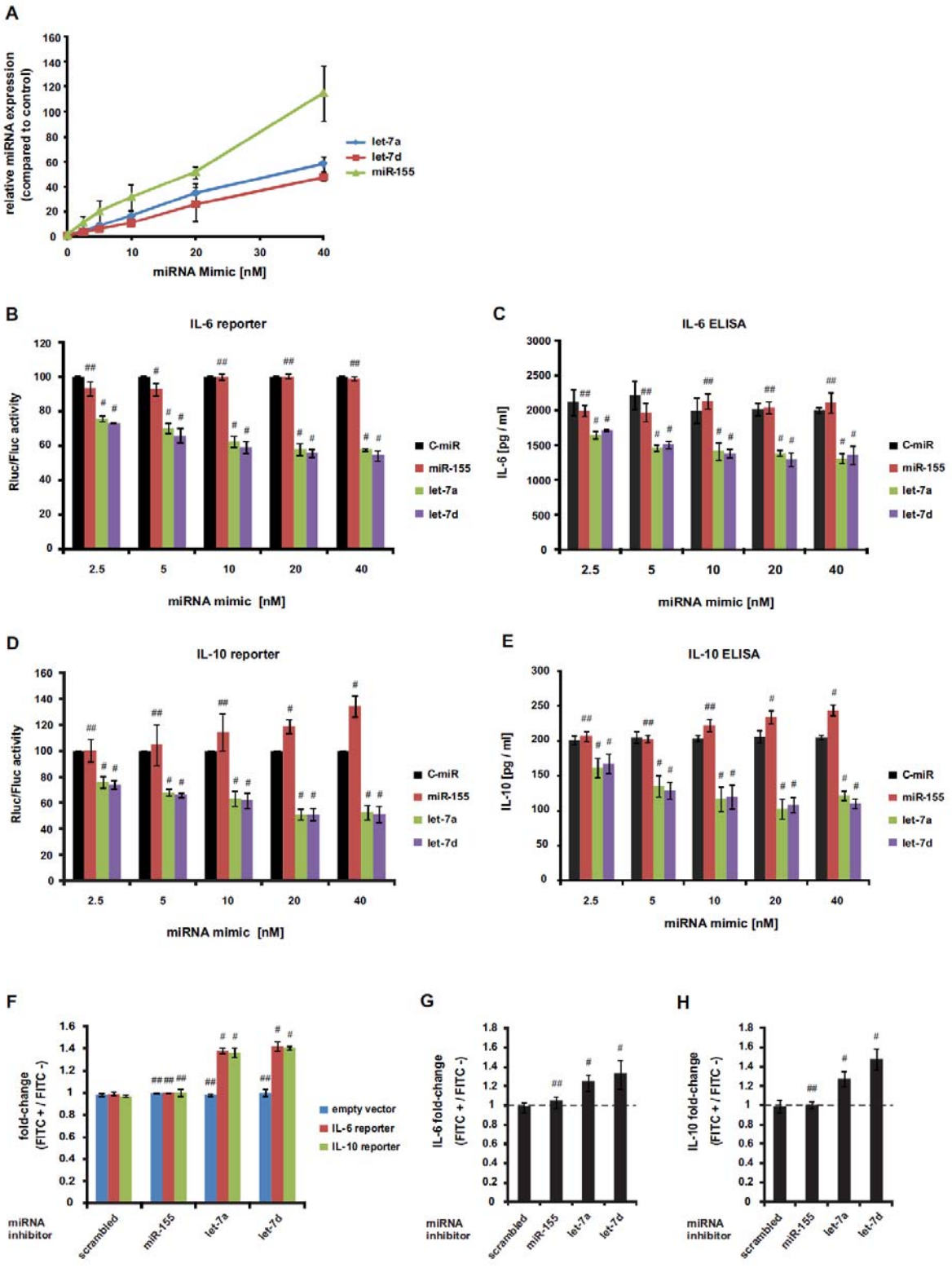
Figure S6



IL-6 and IL-10 3'UTRs contain binding sites for *let-7*.

MEF cells were transfected with a mixture containing the psicheck2 (control) plasmid or the *Renilla* luciferase reporter plasmids carrying the 3' UTR of IL-10, or mutants thereof, in combination with microRNA mimics (A). Let-7 binding sites within the IL-6 (B) and IL-10 (C) 3' UTRs are conserved among mammalian genomes. Numbers above the *Homo sapiens* sequence in both alignments refer to the nucleotide position within the human 3' UTR. Sequence alignment was performed using the Multalin web-interface (http://multalin.toulouse.inra.fr/multalin/; Corpet F. 1988 Multiple sequence alignment with hierarchical clustering. Nucleic Acids Research 16 (22): 10881-10890).

Figure S7

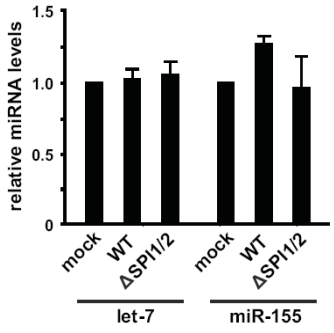


Analysis of IL-6 and IL-10 reporter regulation and cytokine secretion upon artificial modulation of let-7 and miR-155 levels in *Salmonella* infected RAW 264.7 cells. Overexpression of let-7a, let-7d and miR-155 using pre-miR precursor molecules was verified by

TaqMan qRT-PCR, compared to a lipofected control (A). let-7 over-expression caused repression of IL-6 and IL-10 reporters (B, D) and decrease of IL-6 and IL-10 abundance in cell-culture supernatants(C, E). # Significant difference compared to C-miR treatment. ## No significant difference compared to C-miR treatment.

Inhibition of miRNAs was carried out using fluorescein (FITC) labelled LNAs. Following separation of the FITC-positive and –negative fractions by cell-sorting, IL-6 and IL-10 reporter activity (F) and cytokine secretion (G, H) in the FITC-positive fraction (FITC+) was compared to the negative fraction (FITC-). # Significant difference compared to scrambled LNA treatment. ## No significant difference compared to scrambled LNA treatment.

Figure S8



Validation of let-7 and miR-155 expression in MEF cells. Expression of let-7a and miR-155 in MEF cells upon infection as detected 24 h post infection with wild-type or ΔSPI1/2 *Salmonella* by qRT-PCR. Fold-changes refer to the 24 h mock control.