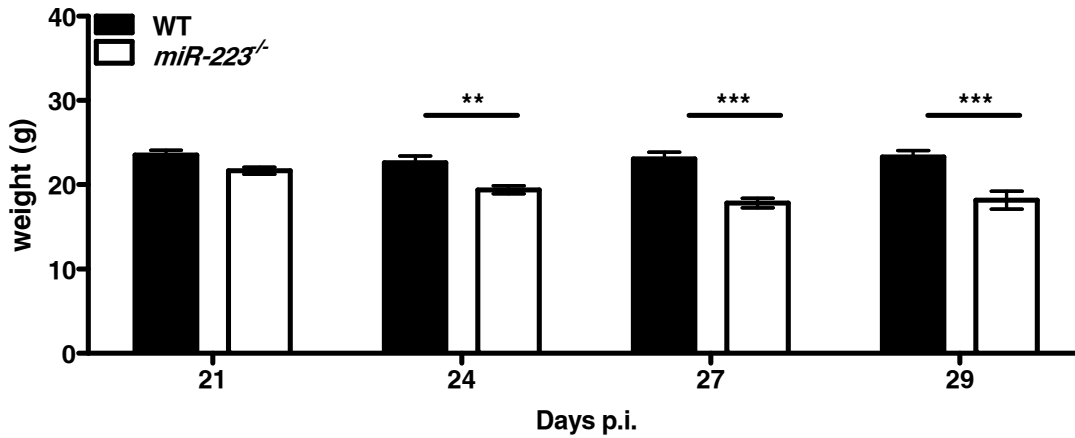
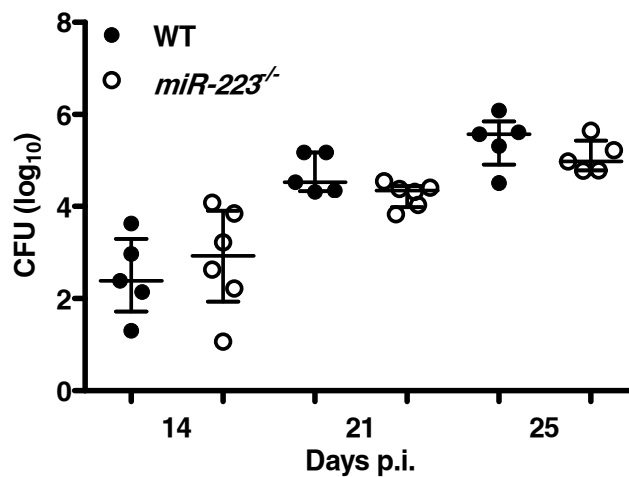
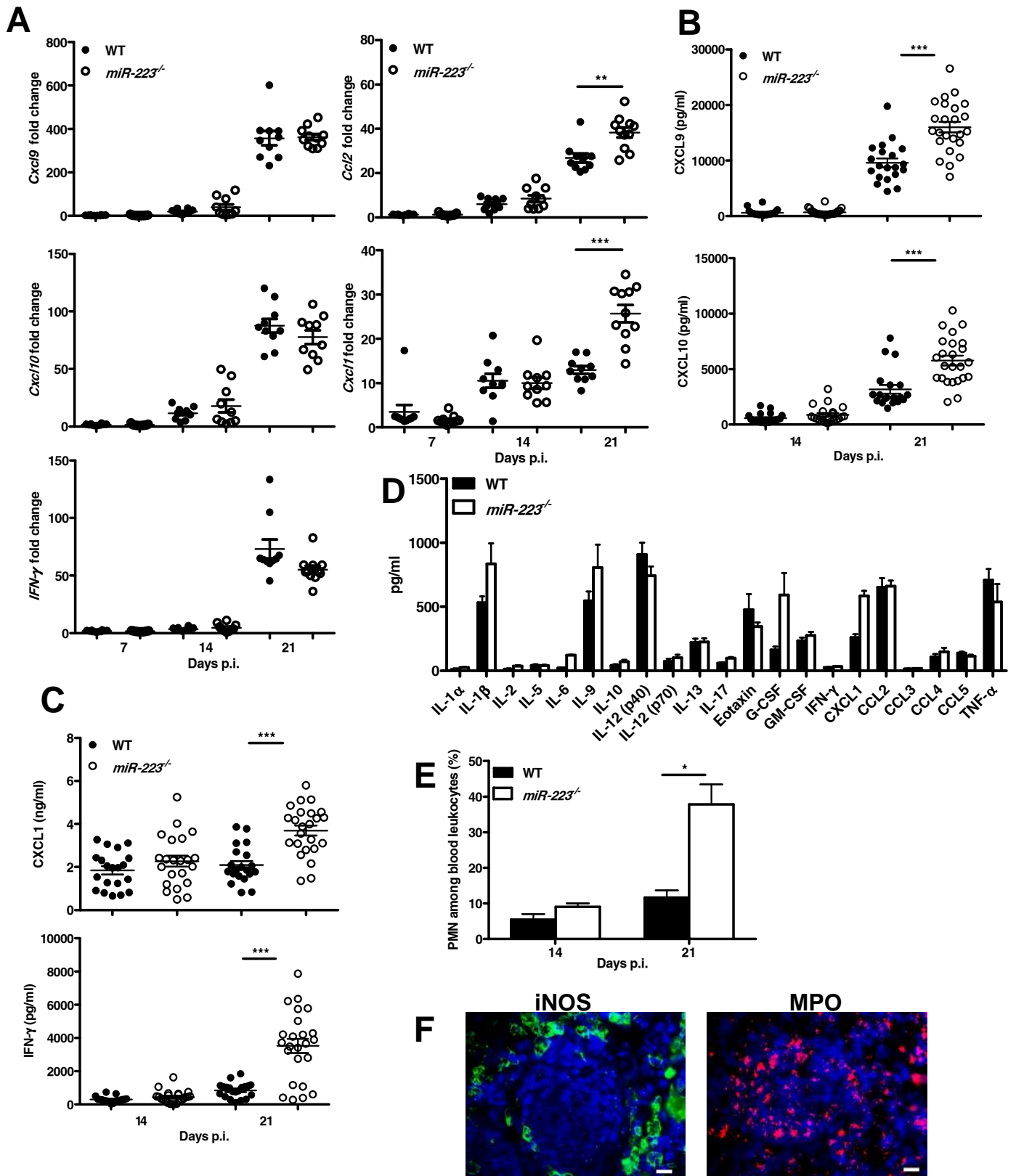


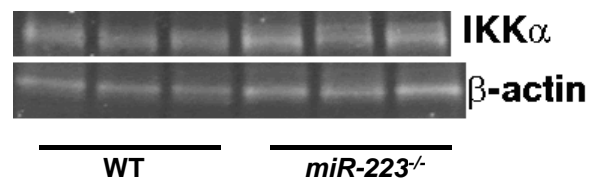
**A****B****Figure S1**

Wasting disease in *miR-223*<sup>-/-</sup> mice infected with *Mtb* in absence of increased bacterial dissemination. **(A)** Body weights of C57BL/6 (WT) and *miR-223*<sup>-/-</sup> mice recorded at various time-points following infection. Data are presented as mean  $\pm$  SEM and results are representative of two independent experiments, ANOVA with Bonferroni post-test, (n=10). **(B)** Bacterial loads in spleens of WT and *miR-223*<sup>-/-</sup> mice. Data are presented as median  $\pm$  iQR and results are representative of three independent experiments (n=5-6). \*\*p<0.01, \*\*\*p<0.001.



**Figure S2**

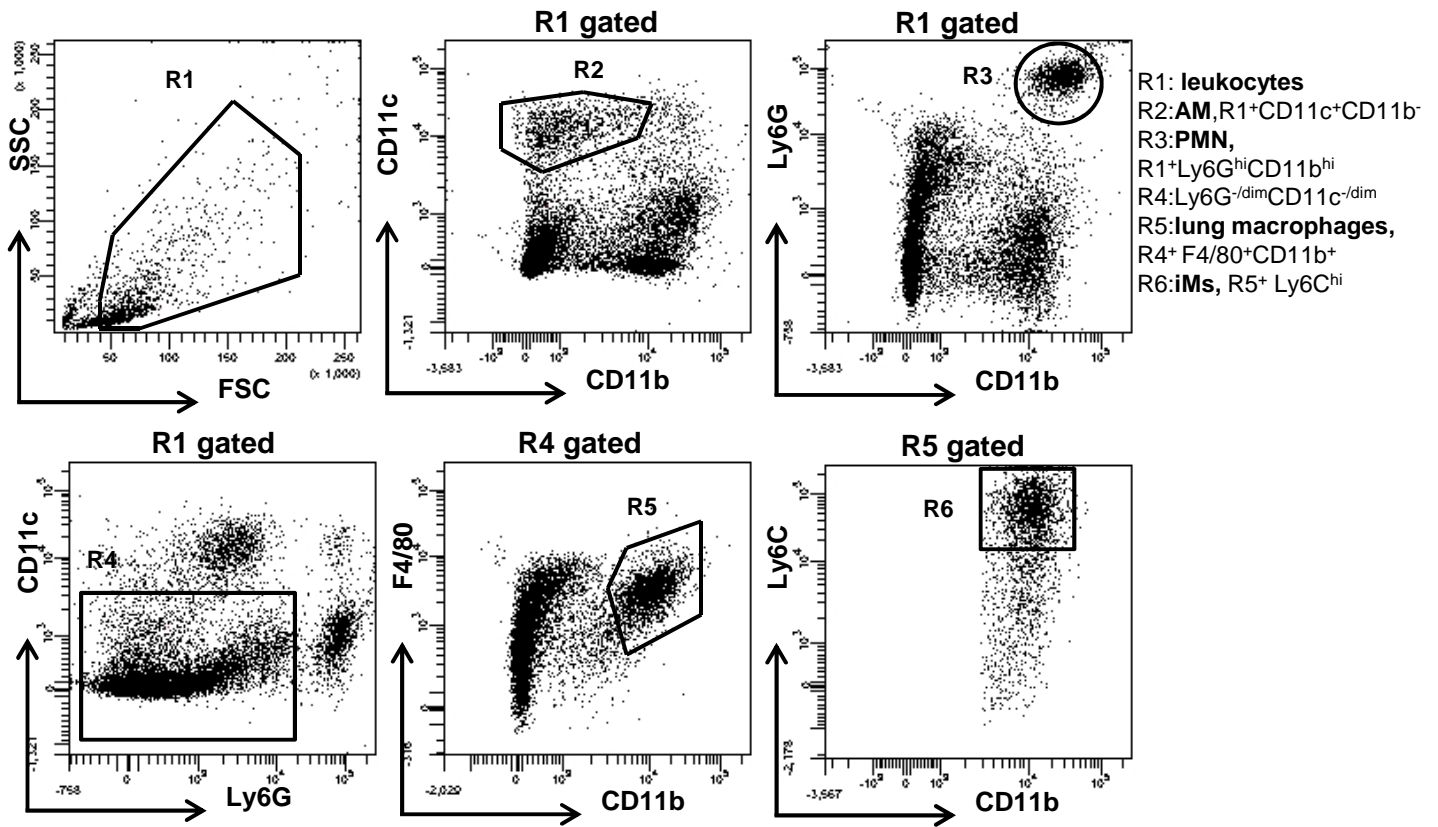
Cellular and molecular immune parameters in *Mtb*-infected C57BL/6 (WT) and *miR-223*<sup>-/-</sup> mice. **(A)** qRT-PCR of chemokine and cytokine genes in respiratory parenchyma. *Gapd* was used as reference gene and data were normalized to uninfected mice. Data are presented as mean  $\pm$  SEM and are representative of two independent experiments, Student's t-test ( $n=9-11$ ). **(B,C)** Protein concentrations of Cxcl9, Cxcl10, Cxcl1 and IFN- $\gamma$  were measured using multiplex immunoassay in lung homogenates collected at 21 days p.i. Data are presented as mean  $\pm$  SEM and are pooled from four independent experiments, ANOVA with Bonferroni post-test ( $n=18-24$ ). **(D)** Serum concentration of immune mediators. Data are presented as mean  $\pm$  SEM ( $n=5-6$ ). **(E)** Neutrophils were enumerated in blood smears at 21 days p.i. Data are presented as mean  $\pm$  SEM and are representative of two independent experiments, Student t-test ( $n=3$ ). **(F)** Immunohistochemistry for inducible NOS (iNOS) and myeloperoxidase (MPO) in lung tissue of *miR-223*<sup>-/-</sup> mice at 25 days p.i. Pictures are representative of two independent experiments ( $n=3$ ). Scale bar: 10  $\mu$ m. \*  $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ .



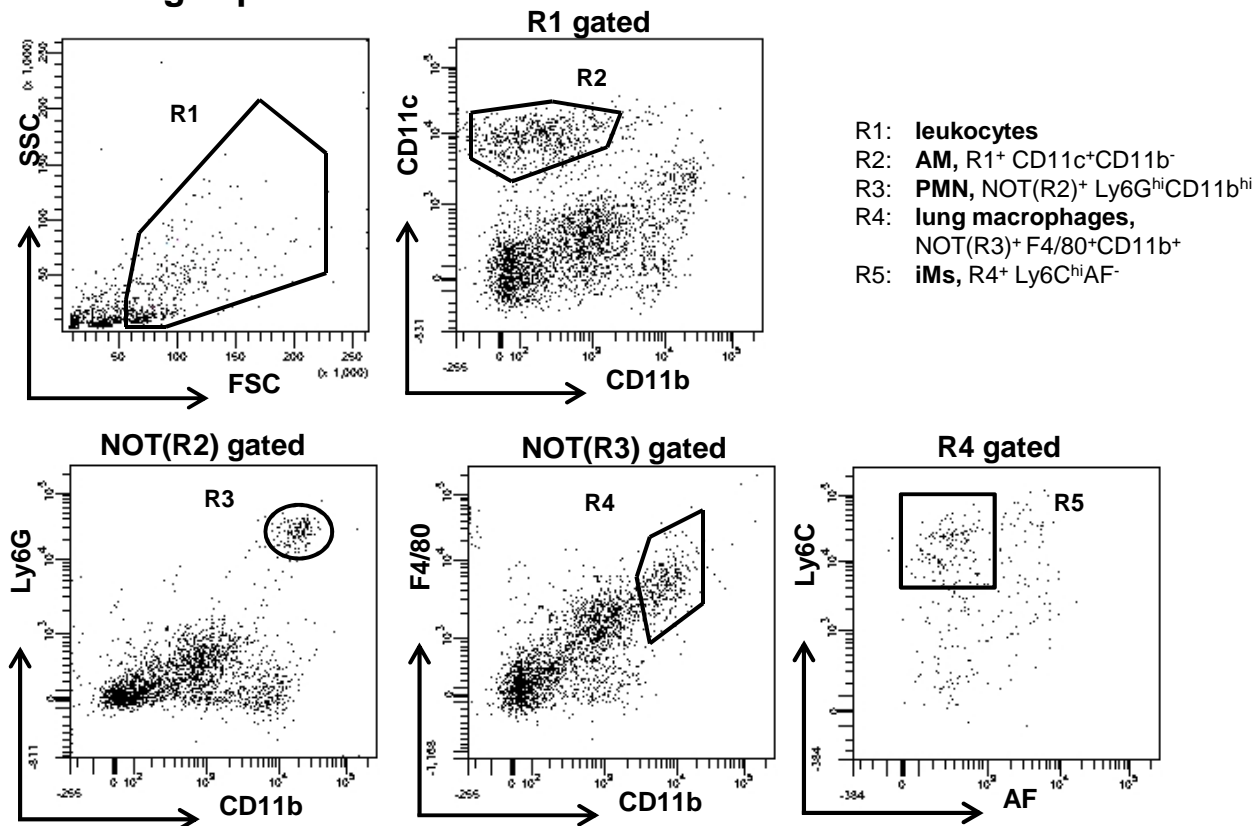
**Figure S3**

Western blot for total IKK $\alpha$  and  $\beta$ -actin in lung homogenates from C57BL/6 (WT) and *miR-223*<sup>-/-</sup> mice at 21 days p.i. Data are representative of two independent experiments (n=5).

## Flow cytometry experiments

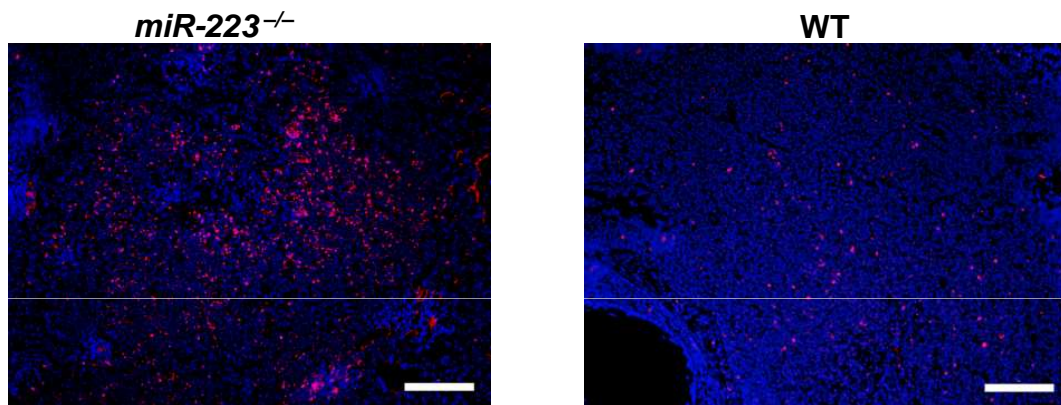


## Sorting experiments



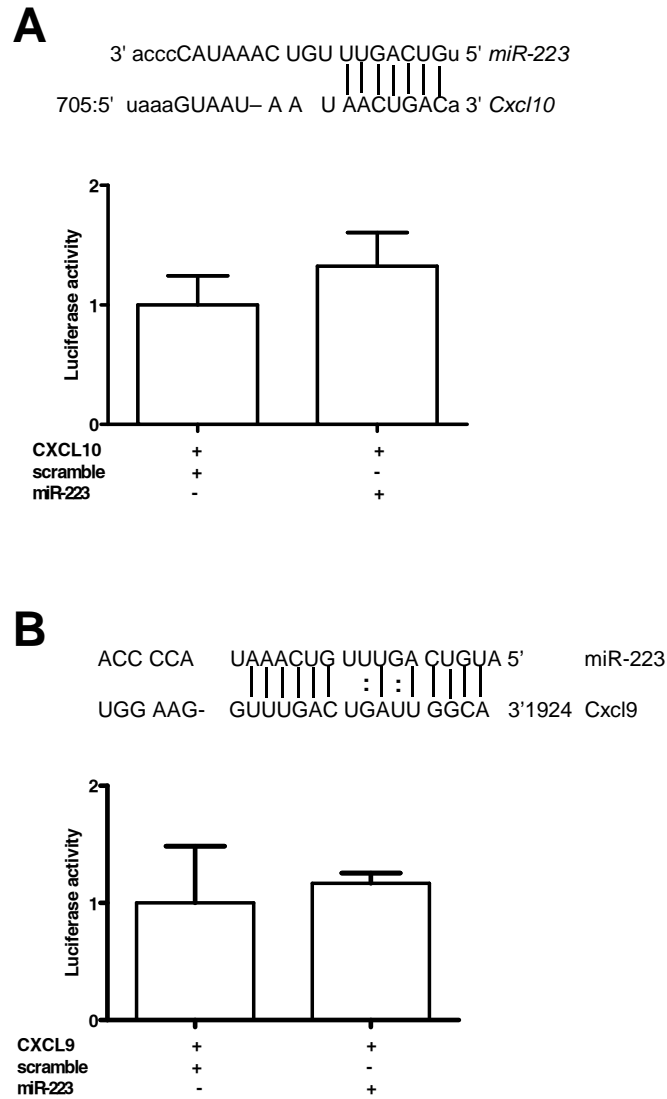
**Figure S4**

Gating strategies applied for **(A)** identification of innate cell populations: AMs [leukocyte gate (R1)<sup>+</sup> CD11c<sup>hi</sup>CD11b<sup>-</sup>], PMNs [leukocyte gate (R1)<sup>+</sup> Ly6G<sup>hi</sup>CD11b<sup>hi</sup>], lung macrophages [leukocyte gate (R1)<sup>+</sup> CD11c<sup>-</sup>Ly6G<sup>-</sup> F4/80<sup>+</sup>CD11b<sup>+</sup>], iMs [leukocyte gate (R1)<sup>+</sup> CD11c<sup>-</sup>Ly6G<sup>-</sup> F4/80<sup>+</sup>CD11b<sup>+</sup> Ly6C<sup>hi</sup>] and **(B)** sorting of innate cell populations: AM [leukocyte gate (R1)<sup>+</sup> CD11c<sup>hi</sup>CD11b<sup>-</sup>], PMNs [leukocyte gate (R1)<sup>+</sup> NOT(AM)<sup>+</sup> Ly6G<sup>hi</sup>CD11b<sup>hi</sup>], lung macrophages [leukocyte gate (R1)<sup>+</sup> NOT(AM)<sup>+</sup> NOT(PMN)<sup>+</sup> CD11c<sup>-</sup>Ly6G<sup>-</sup> F4/80<sup>+</sup>CD11b<sup>+</sup>], iMs [leukocyte gate (R1)<sup>+</sup> NOT(AM)<sup>+</sup> NOT(PMN)<sup>+</sup> CD11c<sup>-</sup>Ly6G<sup>-</sup> F4/80<sup>+</sup>CD11b<sup>+</sup> Ly6C<sup>hi</sup>].



**Figure S5**

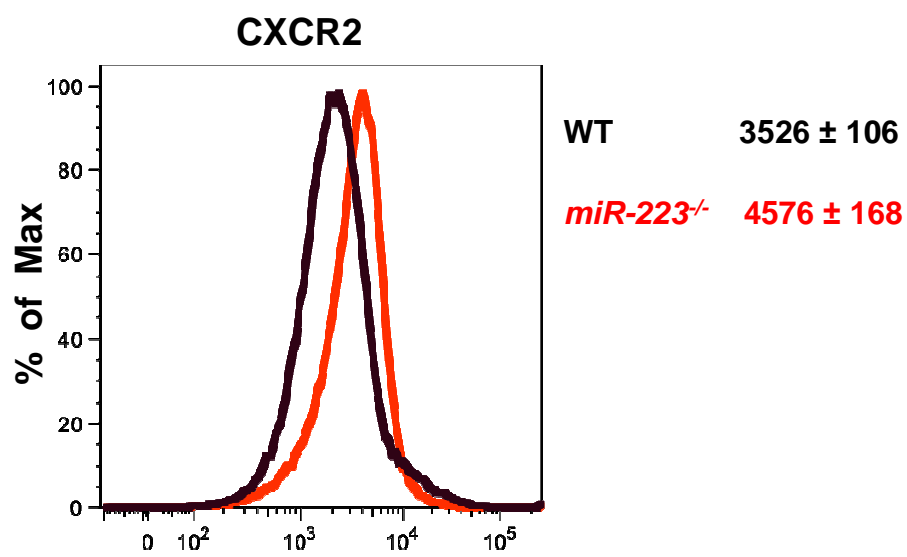
Cell death events in lungs of WT (C57BL/6) and *miR-223*<sup>-/-</sup> mice during TB. Lung tissue collected at day 21 p.i. was TUNEL-stained (Roche) to evaluate the proportion of apoptotic cells in situ. Pictures are representative of two independent experiments (n=5). Scale bar: 100  $\mu$ m.



**Figure S6**

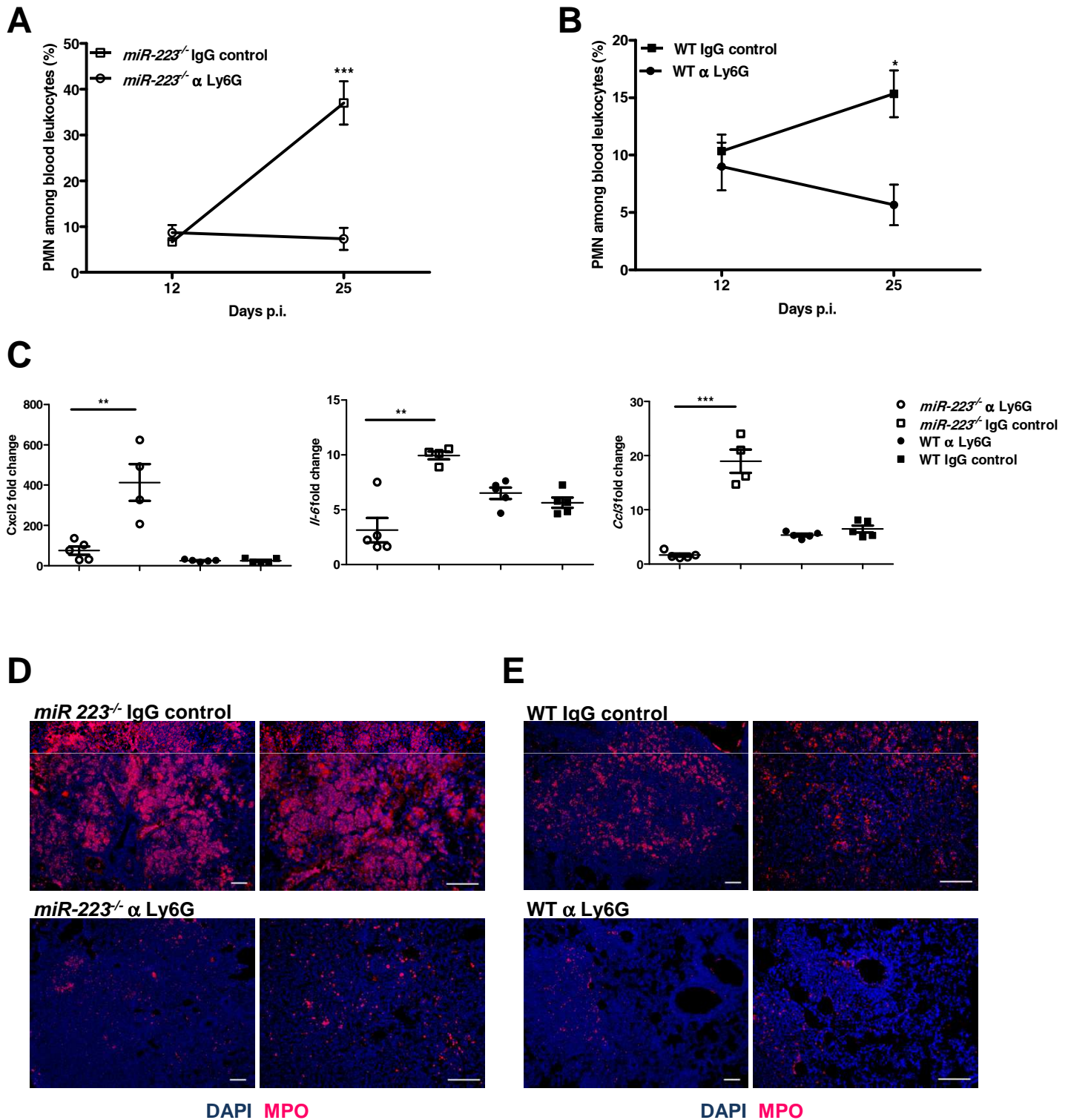
MiR-223 does not target the chemokines Cxcl9 and Cxcl10. **(A)** Luciferase activity of HeLa cells transfected with 3'UTR of *cxcl9* plus scramble and miR-223 mimics. **(B)** Luciferase activity of HeLa cells transfected with 3'UTR of *cxcl10* plus scramble and miR-223 mimics. Data are presented as mean  $\pm$  SEM and results are representative of three experiments with three replicates each.

**A**



**Figure S7**

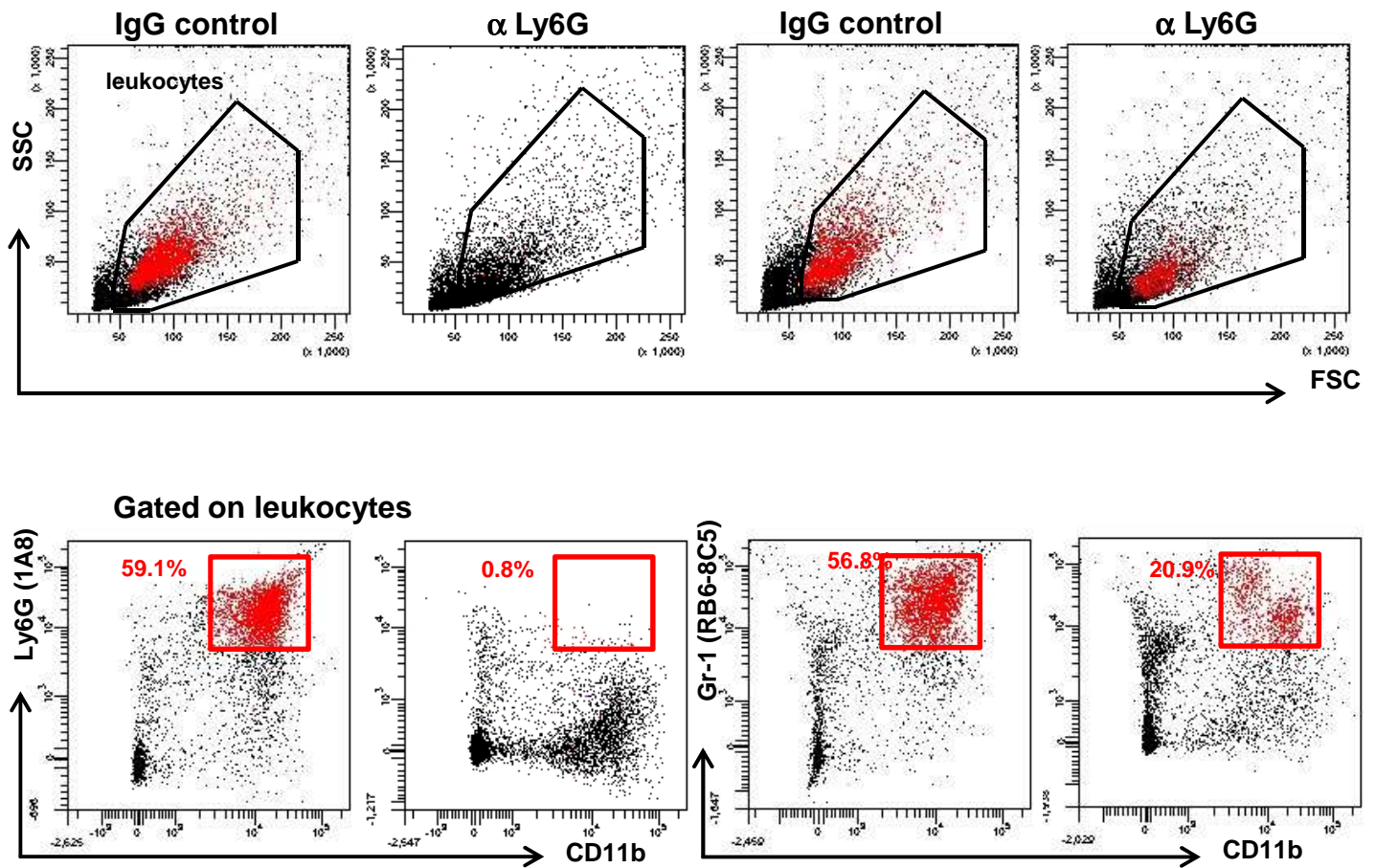
CXCR2 cell surface expression on PMNs. PMNs from C57BL/6 (WT) and *miR-223*<sup>-/-</sup> mice were purified from the bone marrow and stained for CXCR2 followed by analysis by FACS. Numbers represent the mean fluorescence  $\pm$  SEM (n=4) Data are representative of two experiments with four replicates each.



**Figure S8**

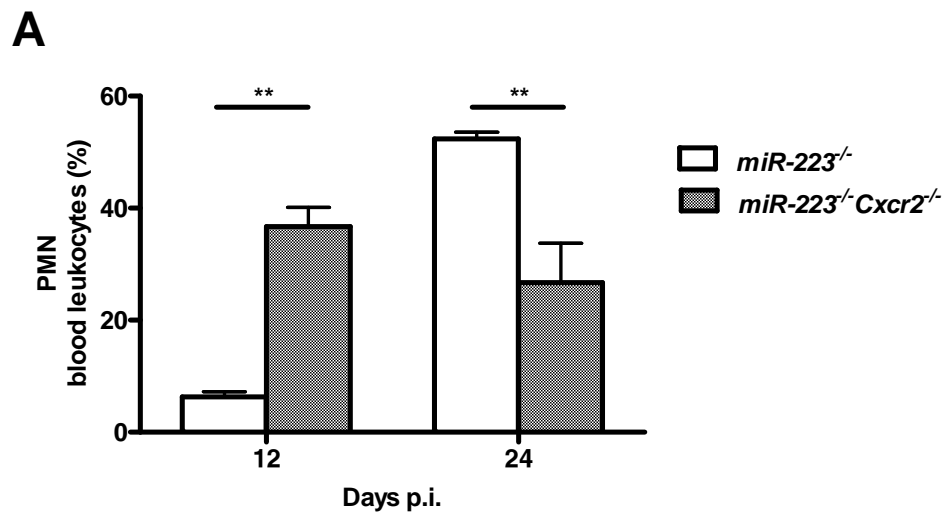
Treatment with mAb against Ly6G affects the expression of genes targeted by *miR-223* during TB. **(A)** *MiR-223*<sup>-/-</sup> mice were treated with mAb against Ly6G (PMN-specific) or with control IgG. PMN frequency was evaluated by analysis of blood smears at indicated times p.i. Data are presented as mean ± SEM and are representative of two independent experiments, ANOVA with Bonferroni post-test (n=3). **(B)** C57BL/6 (WT) mice were treated with mAb against Ly6G (PMN specific) or with control IgG. PMN frequency was evaluated by analysis of blood smears at indicated times p.i. Data are presented as mean ± SEM, ANOVA with Bonferroni post-test (n=3). **(C)** *Cxcl2*, *Il-6* and *Ccl3* were quantified using qRT-PCR in lung tissue collected at 25 days p.i. from C57BL/6 (WT) and *miR-223*<sup>-/-</sup> mice treated as indicated. U6 snRNA was used as reference gene and data were normalized to uninfected mice. Data are presented as mean ± SEM and are representative of two experiments (n=3–5). **(D, E)** Immunohistochemistry for neutrophils and inflammatory monocytes/macrophages (MPO) in the lung tissue collected at day 25 p.i. Data are representative of two independent experiment (n=5). Scale bar 100 μm. \* p<0.05, \*\*p<0.01, \*\*\*p<0.001.





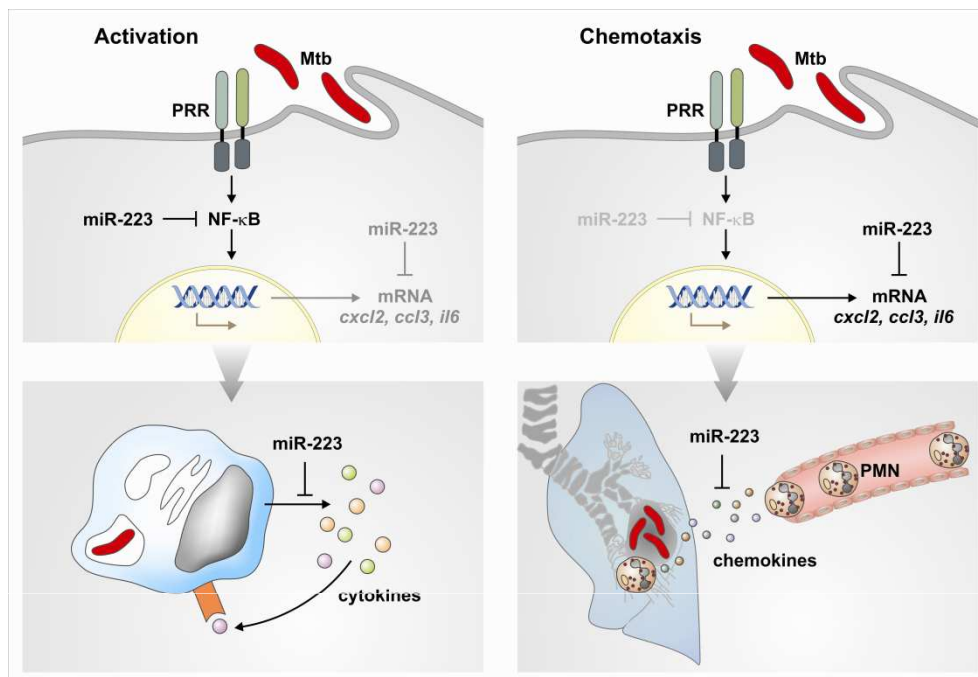
**Figure S9**

Flow cytometric analysis of lung cells upon PMN-specific treatment. Mice were treated with control antibody (control) or PMN-specific Ly6G mAb (1A8) and consequently lung tissue was processed for flow cytometric investigations. Representative dot plots indicate the effect of the treatment on lung PMN frequency (n=5). Data are representative of two experiments.



**Figure S10**

Frequencies of blood PMNs during TB in *miR-223*<sup>-/-</sup> and *Cxcr2*<sup>-/-</sup> *miR-223*<sup>-/-</sup> mice. Blood was processed for haematologic analysis at 12 and 24 days p.i. Data are presented as mean ± SEM and are from one experiment. ANOVA with Bonferroni post-test (n=3). \*\*p<0.01.



### Figure S11

Proposed model for miR-223 activity during *Mtb* infection. MiR-223 fine-tunes myeloid cell activation during *Mtb* infection by modulating NF- $\kappa$ B activation, and thus in mononuclear cells, primarily controls gene transcription. In addition miR-223 targets Il-6 and the chemokines Cxcl2 and Ccl3 post-transcriptionally: in this way miR-223 affects PMN dynamics at the site of mycobacterial infection.

Primary sequence name	Fold change	<i>P</i> value
hsa-miR-335*	4.58655	0.00007
hsa-miR-197	2.39823	0.00007
hsa-miR-223	1.71582	0.00192
hsa-miR-625	1.54236	0.03436
hsa-miR-22	1.47565	0.04672
hsa-miR-629	1.35915	0.04027
hsa-miR-451	-1.03788	0.045936
hsa-miR-148b	-1.49055	0.04583
hsa-miR-19b	-1.61769	0.00312
hsa-miR-501-3p	-1.73841	0.0032
hsa-miR-940	-1.83695	0.00956
hsa-miR-296-5p	-1.87385	0.00145
hsa-miR-33b*	-2.07159	0.03662
hsa-miR-92b	-2.83205	0.01852
hsa-miR-744	-3.28177	0.00065
hsa-miR-1238	-4.07883	0.00032

**Table S1**

Differentially regulated mRNAs in whole blood of active TB patients compared to healthy latent TB-infected individuals.

Fold change	P value	Primary sequence name	Accession #	miR-223 predicted target
74.92	2.15 E-07	Hnrn	NM_133698	N
74.40	1.67 E-07	Serpinb3b	NM_198680	Y
38.75	0.00007	Myot	NM_021484	N
37.34	7.84 E-10	Sprri5	NM_026822	N
36.25	4.85 E-08	Krt2-4	NM_008475	N
35.78	0.00003	Crisp1	NM_009638	N
33.76	1.44 E-07	Sprr3	NM_011478	N
31.17	5.86 E-10	Calm4	NM_020036	N
30.72	9.08 E-10	Il1f6	NM_019450	N
27.79	1.71 E-09	Serpinb3a	NM_009126	N
27.70	5.72 E-07	Krt1-13	NM_010662	N
25.47	9.35 E-07	Myh4	NM_010855	N
25.04	1.48 E-09	Dmkn	NM_172899	Y
23.97	1.51 E-09	Asah3	NM_175731	N
22.16	2.90 E-11	Mt4	NM_008631	N
21.79	0.00021	Serpinb5	NM_009257	N
21.46	0.00045	Klk7	NM_011872	Y
21.21	1.98 E-09	Serpinb3c	NM_201363	Y
19.88	2.47 E-09	Hnrn	NM_133698	N
18.34	6.57 E-08	Pkp1	NM_019645	Y
15.33	0.00003	Gpr87	NM_032399	N
15.26	1.56 E-09	Serpinb3a	NM_009126	N
14.01	0.00098	Sprri1	NM_033175	N
14.00	6.97 E-09	Myh8	NM_177369	N
12.55	1.52 E-12	Sprri7	NM_027137	N
12.16	1.19 E-07	Dsc1	NM_013504	N
12.10	8.52 E-06	Pla2g4e	NM_177845	N
11.41	1.09 E-07	Myh2	NM_144961	N
10.88	8.35 E-07	Pvalb	NM_013645	N
9.79	1.22 E-12	Sprri9	NM_026335	N
9.48	3.85 E-12	Sim2	NM_011377	N
7.09	7.40 E-07	Jsrp1	NM_028001	N
6.69	2.99 E-09	Alox12b	NM_009659	N
6.56	6.63 E-09	Il1f5	NM_019451	Y
6.44	0.00028	Them5	NM_025416	N
5.32	0.00023	Gsdm1	NM_021347	N
5.29	3.74 E-12	Tbx15	NM_009323	Y
5.10	1.32 E-29	Cyp1a1	NM_009992	N
4.67	0.00011	Casq1	NM_009813	N
4.45	0.00002	Hsd17b2	NM_008290	N
4.16	0.00011	S100a15	NM_199422	N
3.40	0.0008	Defb6	NM_054074	N
3.39	0	Ces1	NM_021456	Y
3.36	0	Ces1	NM_021456	Y
3.33	0.00074	Smtnl1	NM_024230	N
3.11	0.00029	Cdsn	NM_001008424	N
3.10	1.44 E-07	Mybph	NM_016749	N
2.56	0.00046	Prom2	NM_138750	N
2.26	0.00002	Cpa4	NM_027926	Y
2.14	0.00016	Pdk4	NM_013743	Y
2.10	2.43 E-10	Il1f9	NM_153511	N
2.03	0.00004	Myom2	NM_008664	N

**Table S2**

List of upregulated genes in lung tissue of *miR-223*<sup>-/-</sup> mice at day 14 post-*Mtb*-infection.

Fold change	P value	Primary sequence name	Accession #	miR-223 predicted target
9.93	2.36 E-06	Cxcl2	NM_009140	Y
7.56	0.00009	Olf633	NM_146354	N
7.13	5.86 E-11	Retnlb	NM_023881	N
6.96	7.41 E-23	Il1f9	NM_153511	N
6.90	5.54 E-42	Csf3	NM_009971	Y
6.70	2.84 E-11	Ccl3	NM_011337	Y
6.60	1.55 E-10	Olfm4	NM_001030294	N
6.08	6.27 E-14	Mmp10	NM_019471	N
5.82	0.00042	Myh4	NM_010855	N
5.78	1.46 E-13	Il11	NM_008350	N
5.42	9.86 E-17	Gzmf	NM_010374	N
5.40	3.66 E-11	Trem1	NM_021406	Y
5.40	1.40 E-12	Olfm4	NM_001030294	N
5.25	2.94 E-11	Ccl4	NM_013652	N
5.18	3.57 E-08	Orm2	NM_011016	N
4.64	1.19 E-06	Orm1	NM_008768	N
4.33	3.91 E-06	Grem1	NM_011824	Y
4.33	3.16 E-10	Il6	NM_031168	Y
4.14	2.20 E-07	BC055107	NM_183187	N
4.02	0.00039	Pou5f1	NM_013633	N
3.95	1.84 E-12	Scrg1	NM_009136	N
3.88	9.31 E-10	Dmbt1	NM_007769	N
3.87	0.00003	lpf1	NM_008814	N
3.87	5.66 E-15	Tnfrsf23	NM_024290	N
3.85	8.60 E-36	Hspa1a	NM_010479	Y
3.82	2.12 E-14	Hspa1a	NM_010479	Y
3.79	8.21 E-10	Gpr109a	NM_030701	N
3.69	4.82 E-07	Il1f6	NM_019450	N
3.64	5.35 E-27	Arg1	NM_007482	N
3.62	3.71 E-07	Ms4a3	NM_133246	N
3.55	0.00078	Stfa3	NM_025288	N
3.49	1.20 E-06	Klra17	NM_133203	N
3.43	0	Il22	NM_016971	N
3.40	0	Orm3	NM_013623	N
3.30	7.89 E-06	Gm1960	NM_203320	N
3.29	0	Hspa1a	NM_010479	Y
3.29	3.77 E-24	Etos1	NM_053258	N
3.29	2.94 E-11	Stfa1	NM_001001332	N
3.24	0	Il22	NM_016971	N
3.20	0.00008	Stfa1	NM_001001332	N
3.17	0.0001	Sfn3	NM_011409	N
3.14	0.00005	Serpina3m	NM_009253	N
3.06	6.23 E-08	Ceacam10	NM_007675	Y
3.01	0.00009	Akr1c14	NM_134072	N
3.00	1.16 E-35	Ngp	NM_008694	N
2.97	2.14 E-11	Saa1	NM_009117	N
2.96	0.00008	Stfa1	NM_001001332	N
2.95	1.75E-07	Saa2	NM_011314	N
2.86	0.00021	Il1rn	NM_031167	N
2.84	0.00003	Retnla	NM_020509	N
2.79	0.00006	Retnla	NM_020509	N
2.79	1.39 E-20	Trem1	NM_021406	Y
2.75	0.00016	Mt2	NM_008630	Y
2.69	0.00048	Onecut2	NM_194268	Y
2.68	1.75 E-14	Fosl1	NM_010235	N
2.68	1.06 E-22	Slpi	NM_011414	N
2.66	0.00001	Itgb2l	NM_008405	N
2.61	1.26 E-18	Ctsg	NM_007800	N
2.57	0.00002	Lphn3	NM_198702	Y
2.53	1.35 E-07	Cd14	NM_009841	N
2.53	0	Rhov	NM_145530	Y
2.50	9.72 E-12	Pram1	NM_001002842	N
2.47	4.55 E-07	Sectm1	NM_026907	N
2.47	6.47 E-24	Hspa1a	NM_010479	Y
2.47	2.17 E-10	Osm	NM_001013365	Y

2.46	3.76 E-18	Mxd1	NM_010751	Y
2.41	4.26 E-06	Ptprn	NM_008985	N
2.41	3.63 E-09	Map3k6	NM_016693	N
2.40	9.80 E-08	Saa3	NM_011315	N
2.40	0.00006	Syt8	NM_018802	N
2.37	0.00005	Saa4	NM_011316	N
2.36	4.43 E-08	Map3k6	NM_016693	N
2.36	0.000233	Ccl2	NM_011333	Y
2.36	0.00038	Pappa	NM_021362	Y
2.34	1.90 E-07	F13a1	NM_028784	N
2.34	1.67 E-07	Ptx3	NM_008987	N
2.33	2.38 E-10	Serpina3n	NM_009252	Y
2.28	4.67 E-22	Tlr6	NM_011604	Y
2.27	1.61 E-10	Saa1	NM_009117	N
2.24	2.78 E-06	Ltf	NM_008522	N
2.24	0.00002	Ltf	NM_008522	N
2.23	3.97 E-10	Pdzd3	NM_133226	N
2.23	1.24 E-04	Cxcl9	NM_008599	Y
2.22	2.78 E-29	G0s2	NM_008059	Y
2.21	0.0008751	Cxcl10	NM_021274	Y
2.20	0.0001	Inhba	NM_008380	N
2.19	1.66 E-09	Bcl2l11	NM_207680	Y
2.19	0.00007	Prtn3	NM_011178	N
2.18	0.00041	Acsl6	NM_144823	N
2.17	6.11 E-20	Osm	NM_001013365	Y
2.16	0.00016	MGC58177	NM_198666	N
2.16	0.00031	Fpr1	NM_013521	N
2.15	3.32 E-08	Aldh1a3	NM_053080	Y
2.14	1.24 E-11	Arg2	NM_009705	N
2.13	5.60 E-06	Ifng	NM_008337	Y
2.13	0.00081	Serpina2	NM_011111	N
2.13	9.66 E-07	Pla1a	NM_134102	N
2.13	4.46 E-06	Mybph	NM_016749	N
2.12	1.91 E-09	Sult1a1	NM_133670	Y
2.12	5.85 E-07	Mpo	NM_010824	N
2.10	1.05 E-09	Sectm1	NM_026907	N
2.10	0.00022475	Cxcl1	NM_008176	Y
2.10	0.00012	Speer4a	NM_029376	N
2.09	2.05 E-30	Ltb4r1	NM_008519	N
2.09	1.35 E-25	Has1	NM_008215	N
2.09	3.59 E-07	Timp1	NM_011593	N
2.08	4.42 E-35	Wdfy1	NM_027057	N
2.07	9.84 E-06	Sphk1	NM_025367	N
2.05	0.00028	Ces6	NM_133960	N
2.05	1.74 E-06	Gda	NM_010266	N
2.05	1.25 E-12	Slc5a9	NM_145551	N
2.05	8.41 E-07	Speer4d	NM_025759	N
2.05	5.59 E-06	Fkbp5	NM_010220	Y
2.04	0.00003	Cxcl13	NM_018866	N
2.04	2.62 E-16	Mefv	NM_019453	N
2.04	1.52 E-09	Chl1	NM_007697	Y
2.03	1.13 E-30	Rnf36	NM_080510	N
2.02	1.55 E-09	Timp1	NM_011593	N
2.01	6.05 E-06	Dio2	NM_010050	Y
2.01	1.12 E-10	Sphk1	NM_025367	N
2.01	0.00026	Tnc	NM_011607	N
2.01	7.95 E-06	Ptx3	NM_008987	N

**Table S3**

List of upregulated genes in lung tissue of *miR-223*<sup>-/-</sup> mice at day 21 post-infection.

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Gene name <sup>b</sup>	Mouse genotype	Lung tissue <sup>a</sup>		
		Day 7	Day 14	Day 21
<i>Imo2</i> <sup>(39)</sup>	WT	0.89 ± 0.11	1.00 ± 0.18	0.62 ± 0.26
	<i>miR-223</i> <sup>-/-</sup>	0.78 ± 0.11	0.81 ± 0.12	0.55 ± 0.42
<i>mef2c</i> <sup>(24)</sup>	WT	1.26 ± 0.27	1.16 ± 0.07	0.46 ± 0.08
	<i>miR-223</i> <sup>-/-</sup>	1.24 ± 0.07	1.15 ± 0.13	0.35 ± 0.08
<i>ikk-a</i> <sup>(26)</sup>	WT	0.72 ± 0.05	0.80 ± 0.10	0.53 ± 0.16
	<i>miR-223</i> <sup>-/-</sup>	0.84 ± 0.10	0.84 ± 0.09	0.39 ± 0.03
<i>CEBP-b</i> <sup>(39)</sup>	WT	0.34 ± 0.19	0.64 ± 0.32	0.58 ± 0.20
	<i>miR-223</i> <sup>-/-</sup>	0.61 ± 0.18	0.84 ± 0.68	0.89 ± 0.27

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<sup>a</sup> Fold change value normalized using *Gapd* and compared to uninfected samples. Data are representative of 2 independent experiments with 5 animals/group.

<sup>b</sup> Predicted miR-223 target (see references)

#### Table S4

Transcriptional activity of known *miR-223* targets in lung tissue of C57BL/6 (WT) and *miR-223*<sup>-/-</sup> mice during TB. qRT-PCR of *Imo2*, *mef2c*, *ikka*, and *CEBP-β* in lung parenchyma at different time points during TB.



<b>Primer name</b>	<b>Primer sequence 5'→3'</b>
<b>CXCL2 3'UTR For</b>	CTCGAG <sup>1</sup> gccttaagatacccactaaca
<b>CXCL2 3'UTR Rev</b>	GCGGCCGC <sup>2</sup> acatgaataaataaatgtgtcca
<b>CCL3 3'UTR For</b>	CTCGAG <sup>1</sup> gagtcttgaggcagcgaggaacccc
<b>CCL3 3'UTR Rev</b>	GCGGCCGC <sup>2</sup> gcatattattacttctctgg
<b>IL6 3'UTR For</b>	CTCGAG <sup>1</sup> tcggtatgcctaagcatatc
<b>IL6 3'UTR Rev</b>	GCGGCCGC <sup>2</sup> acattataaaaatacatcacaag
<b>CXCL9 3'UTR For</b>	CTCGAG <sup>1</sup> agaccattactttaccaacaag
<b>CXC9 3'UTR Rev</b>	GCGGCCGC <sup>2</sup> gaatttagtcactttactga
<b>CXCL10 3'UTR For</b>	CTCGAG <sup>1</sup> ctggagtgaagccacgcaca
<b>CXCL10 3'UTR Rev</b>	GCGGCCGC <sup>2</sup> gtctcctaccaccccaacttct
<b>CXCL2 mut 1</b>	gggggtggggacacctaccugcagtcggatggct
<b>CXCL2 mut 2</b>	agccatccgactgcaggtaggtgtccccacccc
<b>CCL3 mut 1</b>	tgatgacaaagtcatgggctttcaaagtacaatagatgct
<b>CCL3 mut 2</b>	agcatctattgtacattgaaagcccatgactttgtcatca
<b>IL6 mut 1</b>	atgttgtctctacgaagcccggatagttgggacactatfff
<b>IL6 mut 2</b>	aaaatagtgccaactatccgggcttcgtagagaacaacat

<sup>1</sup> XhoI restriction sequence

<sup>2</sup> NotI restriction sequence

### **Table S5**

Primers used for generation of 3'-UTR luciferase reporter constructs.