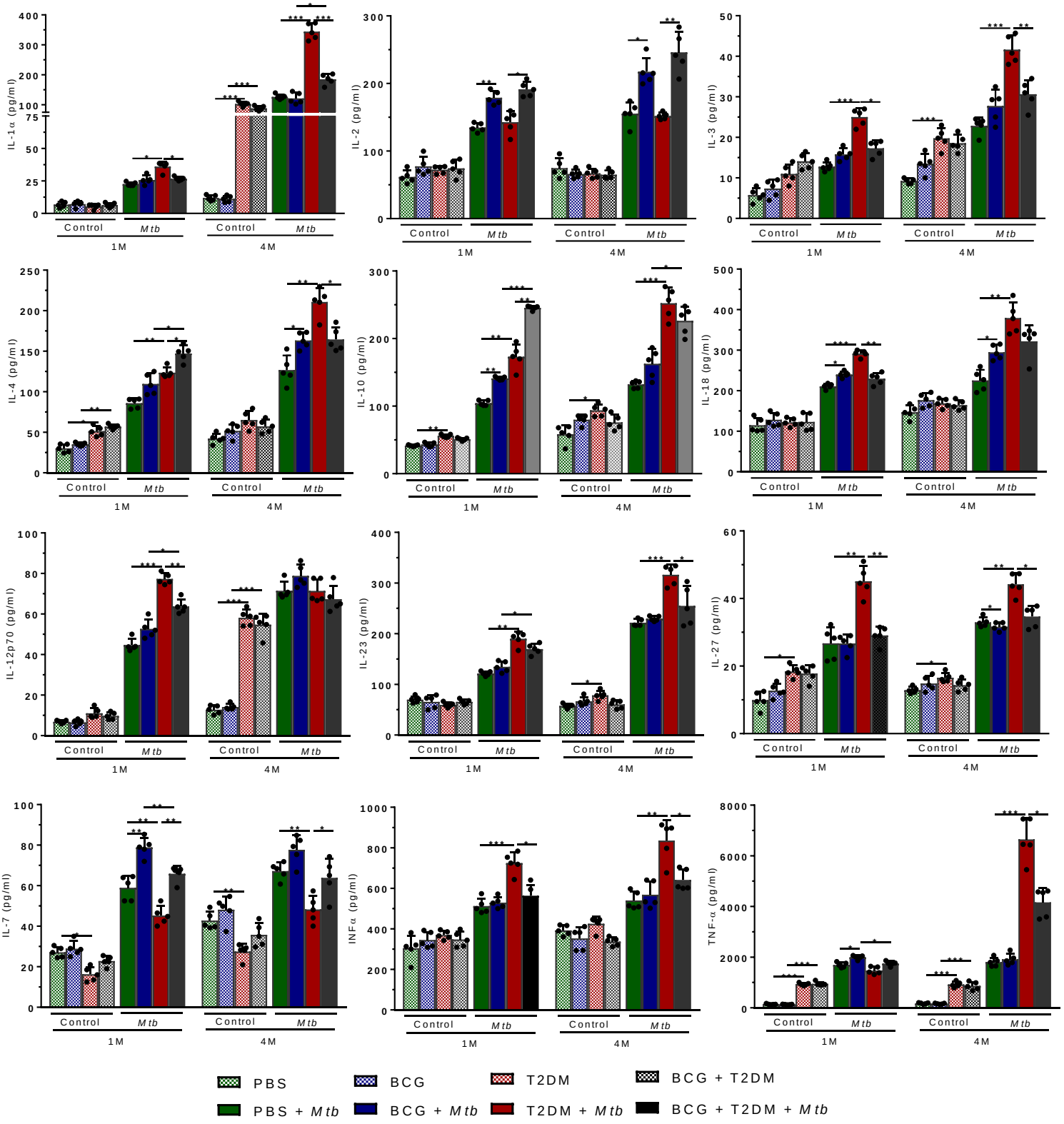
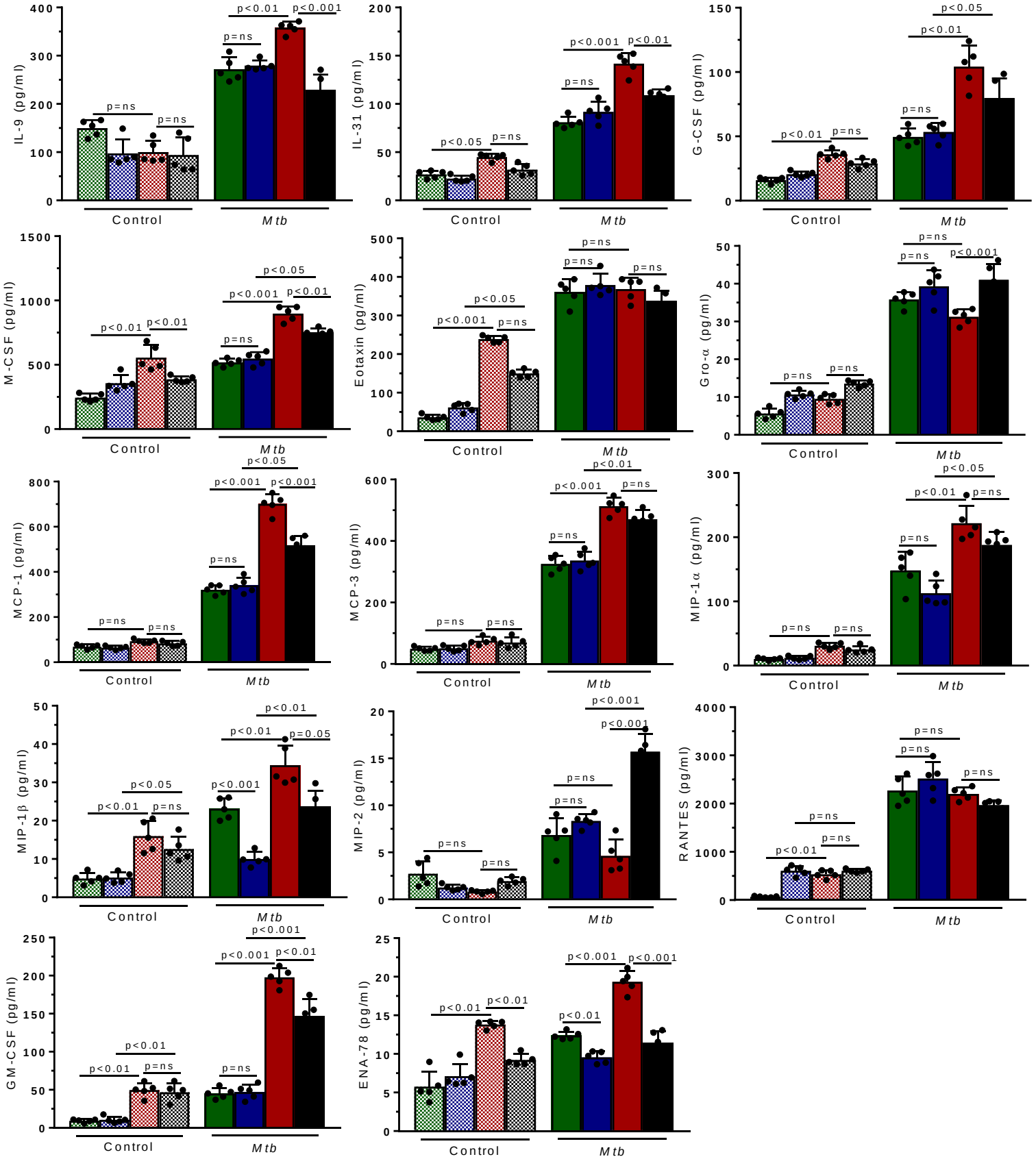
Supplementary Figures and Tables

Supplementary Figure 1



Supplementary Fig. 1. BCG vaccination alters cytokine levels in T2DM mice infected with *Mtb*.
PBS control, BCG-vaccinated, T2DM or BCG-vaccinated T2DM mice were infected with ~100 CFU of aerosolized *Mtb* H37Rv. At one and 4 months p.i., lung homogenates from PBS control, BCG-vaccinated control, T2DM, BCG-vaccinated T2DM, infected control, infected T2DM, infected BCG-vaccinated control and infected BCG-vaccinated T2DM mice were collected, and cytokine levels were measured in a multiplex ELISA. Experiments were performed two times and each time 2 to 3 mice per group were used. The data are shown as mean ± SDs of *n=5* mice per group. The statistical analysis was performed by one-way ANOVA followed Tukey's multiple comparisons test. *, $p<0.05$; **, $p<0.01$ and ***, $p<0.001$.

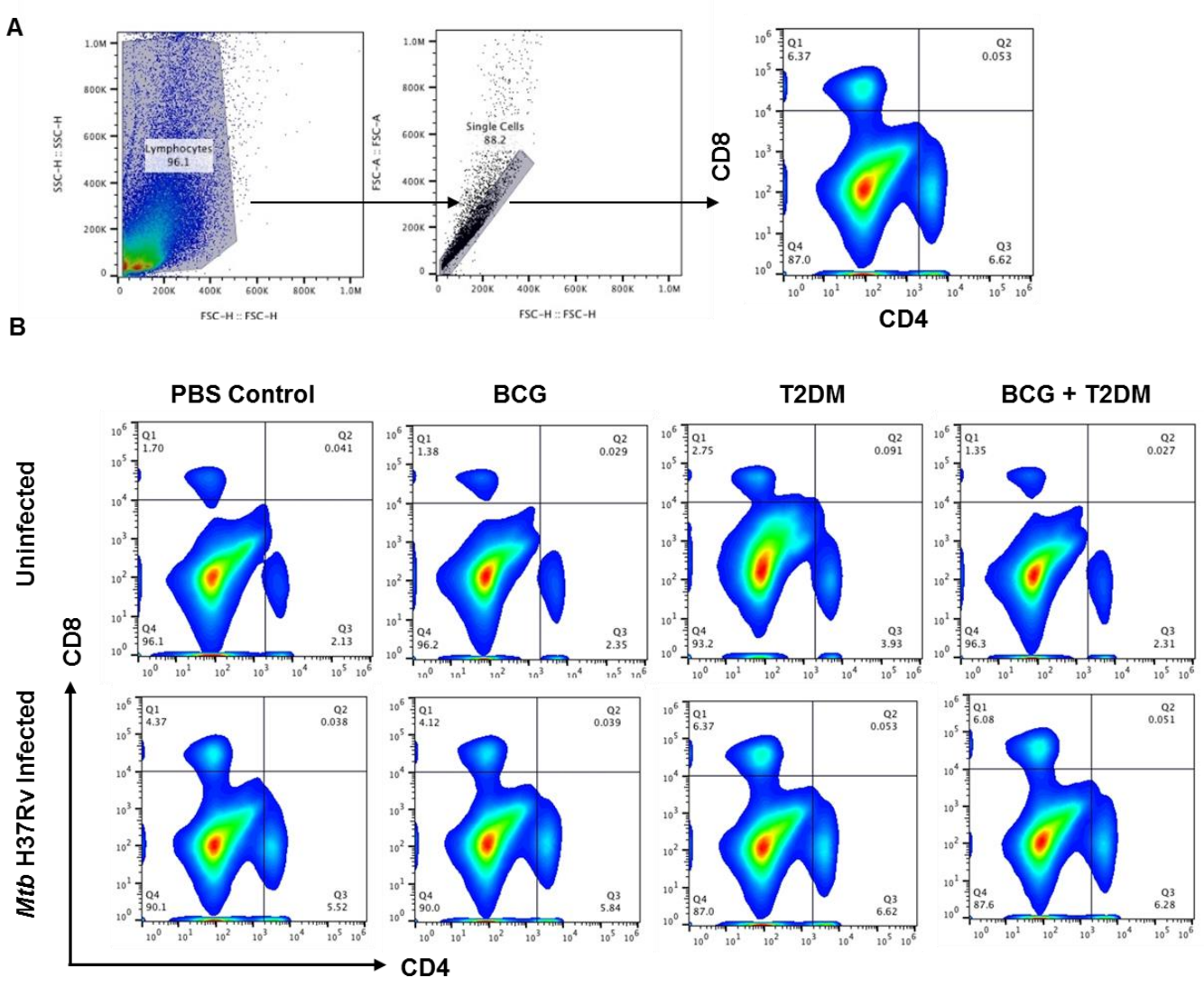
Supplementary Figure 2



Supplementary Fig. 2. BCG vaccination alters various cytokine and chemokine levels in T2DM mice infected with Mtb at four months p.i.

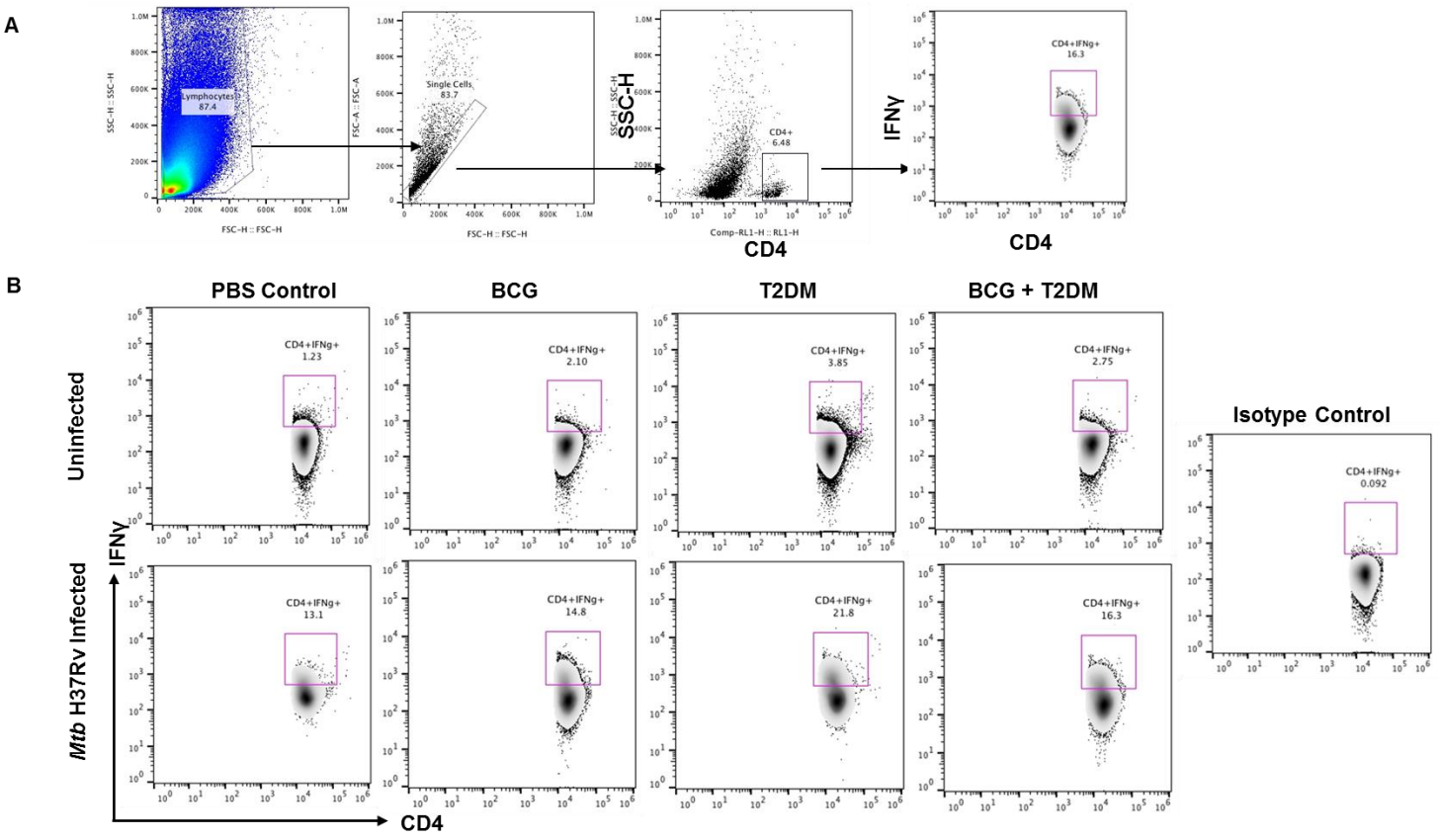
PBS control, BCG-vaccinated, T2DM or BCG-vaccinated T2DM mice were infected with ~100 CFU of aerosolized *Mtb* H37Rv. A. At 4 months p.i., lung homogenates from PBS control, BCG-vaccinated control, T2DM, BCG-vaccinated T2DM, infected control, infected T2DM, infected BCG-vaccinated control and infected BCG-vaccinated T2DM mice were collected, and chemokine levels were measured in a multiplex ELISA. Experiments were performed two times and each time 2 to 3 mice per group were used. The data are shown as mean ± SDs of *n=5* mice per group. The statistical analysis was performed by one-way ANOVA followed Tukey's multiple comparisons test. *, $p<0.05$; **, $p<0.01$ and ***, $p<0.001$.

Supplementary Figure 3



Supplementary Fig. 3. Gating strategies to determine CD4+ and CD8+ cell populations in the lungs.
PBS control, BCG-vaccinated, T2DM or BCG-vaccinated T2DM mice were infected with ~100 CFU of aerosolized *Mtb* H37Rv. A. At one and 4 months p.i., the lungs from PBS control, BCG-vaccinated control, T2DM, BCG-vaccinated T2DM, infected control, infected T2DM, infected BCG-vaccinated control and infected BCG-vaccinated T2DM mice were isolated. Lung cells were prepared for flow cytometry as described in the methods sections. (**A-B**) Representative gating strategies for CD4+ and CD8+ cells are shown for four months p.i. Experiments were performed using *n=5* mice per group.

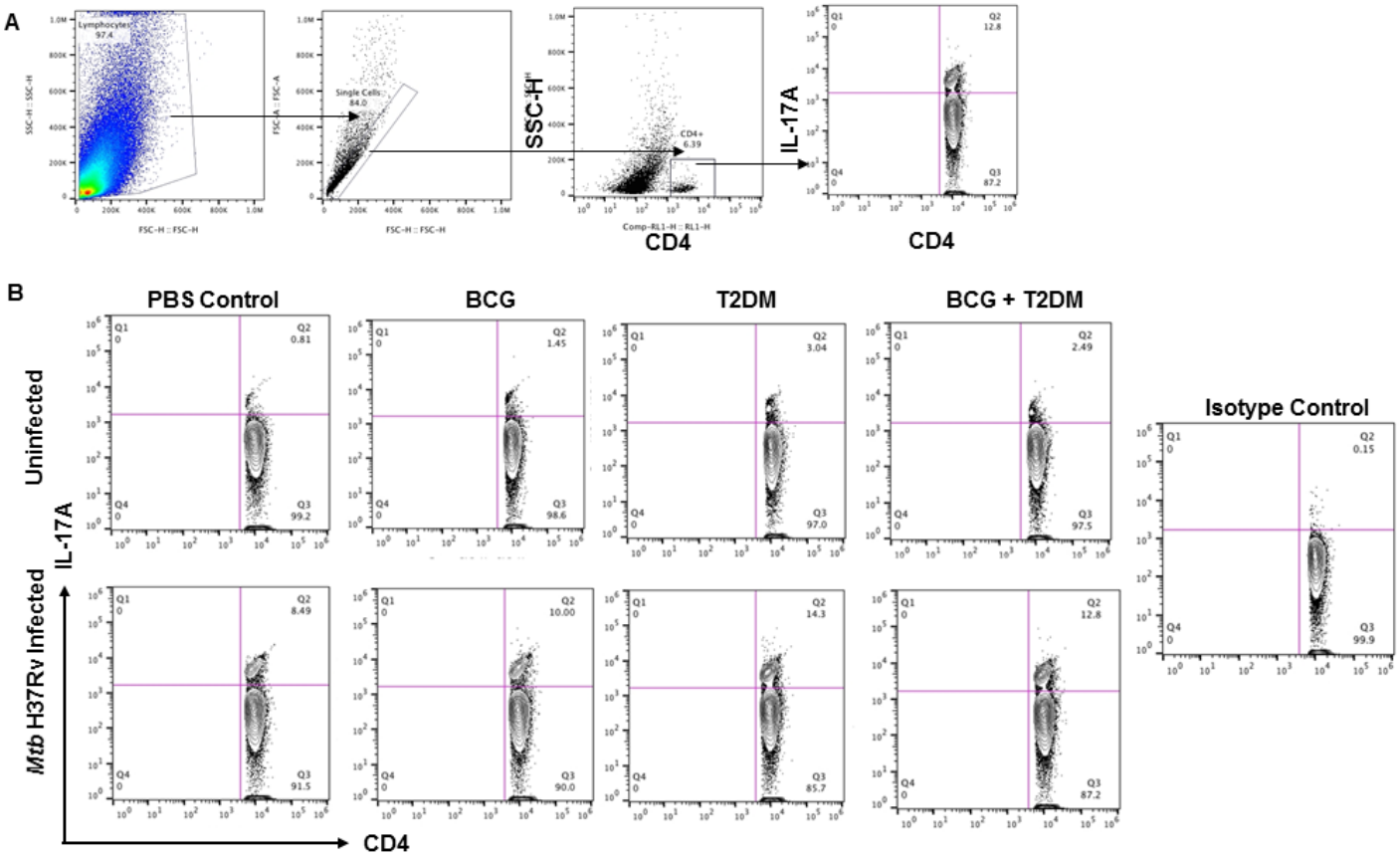
Supplementary Figure 4



Supplementary Fig. 4. Gating strategies to determine CD4+IFN-γ+ cells in the lungs.

PBS control, BCG-vaccinated, T2DM or BCG-vaccinated T2DM mice were infected with ~100 CFU of aerosolized *Mtb* H37Rv. A. At one and 4 months p.i., the lungs from PBS control, BCG-vaccinated control, T2DM, BCG-vaccinated T2DM, infected control, infected T2DM, infected BCG-vaccinated control and infected BCG-vaccinated T2DM mice were isolated. Lung cells were prepared for flow cytometry by surface staining followed by intracellular staining as described in the methods section. (**A-B**) Representative gating strategies for CD4+INF-γ+ cells are shown for four months p.i. Experiments were performed using *n=5* mice per group.

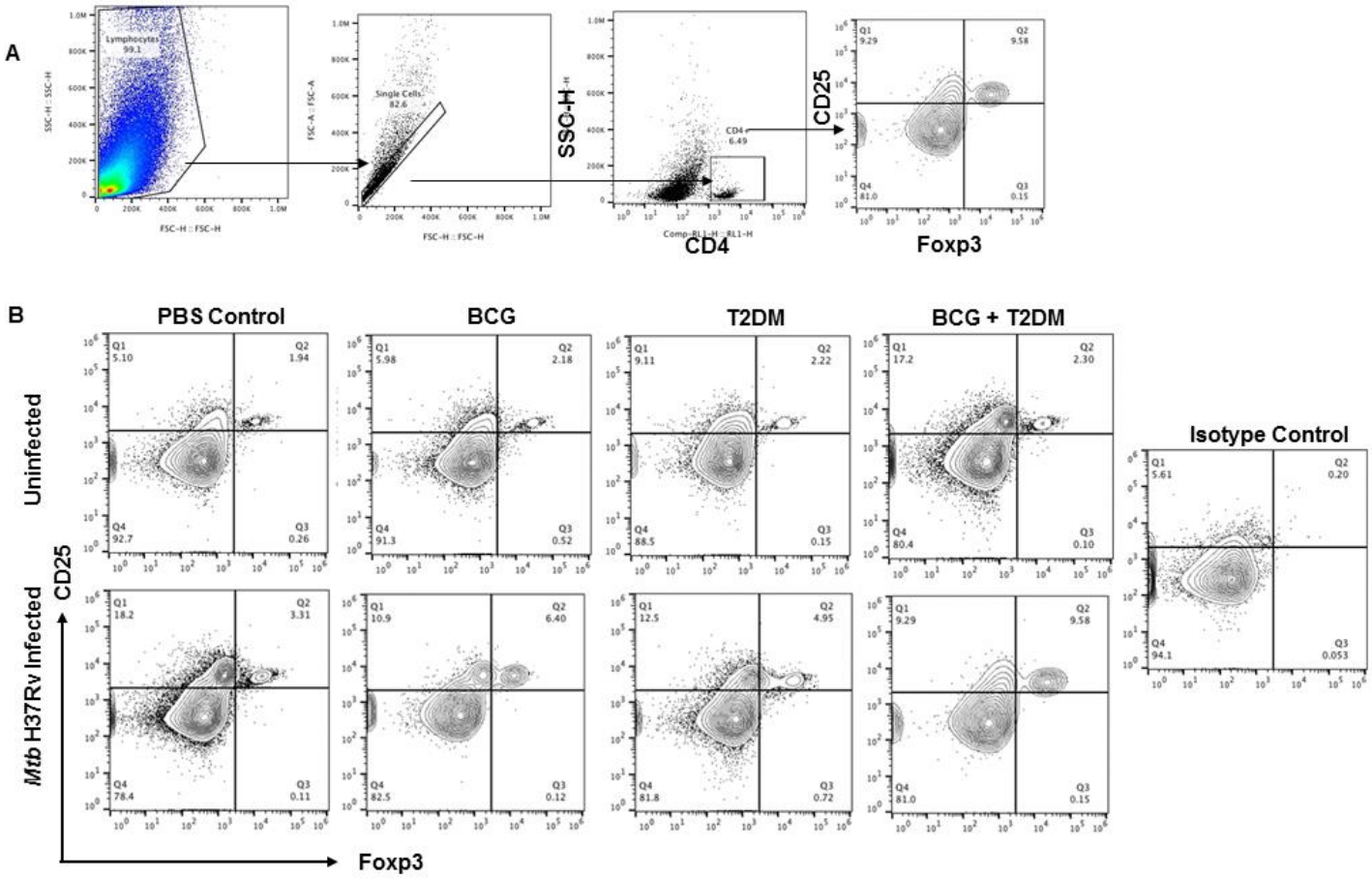
Supplementary Figure 5



Supplementary Fig. 5. Gating strategies to determine CD4+IL-17A+ cells in the lungs.

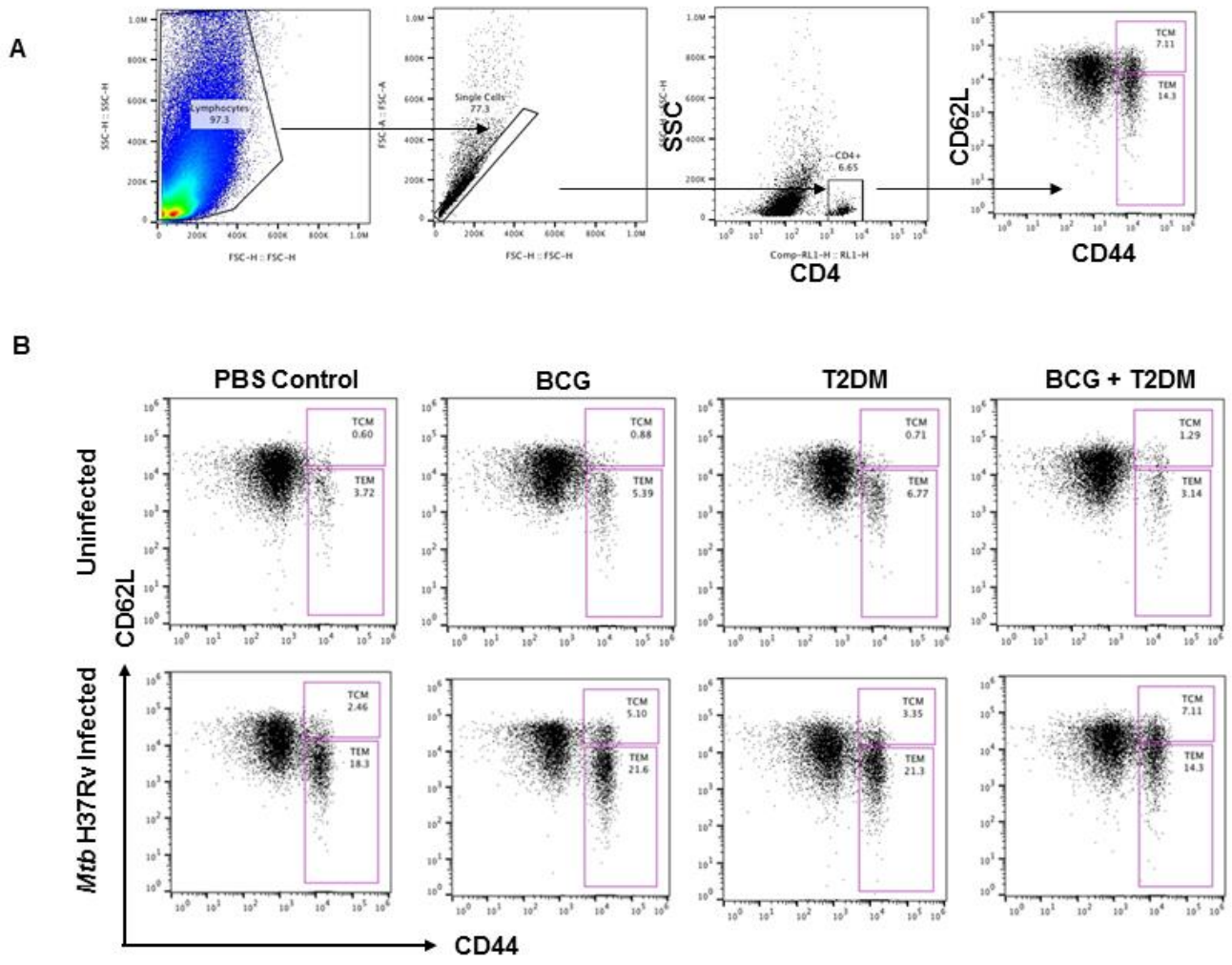
PBS control, BCG-vaccinated, T2DM or BCG-vaccinated T2DM mice were infected with ~100 CFU of aerosolized *Mtb* H37Rv. A. At one and 4 months p.i., the lungs from PBS control, BCG-vaccinated control, T2DM, BCG-vaccinated T2DM, infected control, infected T2DM, infected BCG-vaccinated control and infected BCG-vaccinated T2DM mice were isolated. Lung cells were prepared for flow cytometry by surface staining followed by intracellular staining of IL-17A+ cells. (**A-B**) Representative gating strategies for CD4+IL-17A+ cells are shown for four months p.i. Experiments were performed using *n=5* mice per group.

Supplementary Figure 6



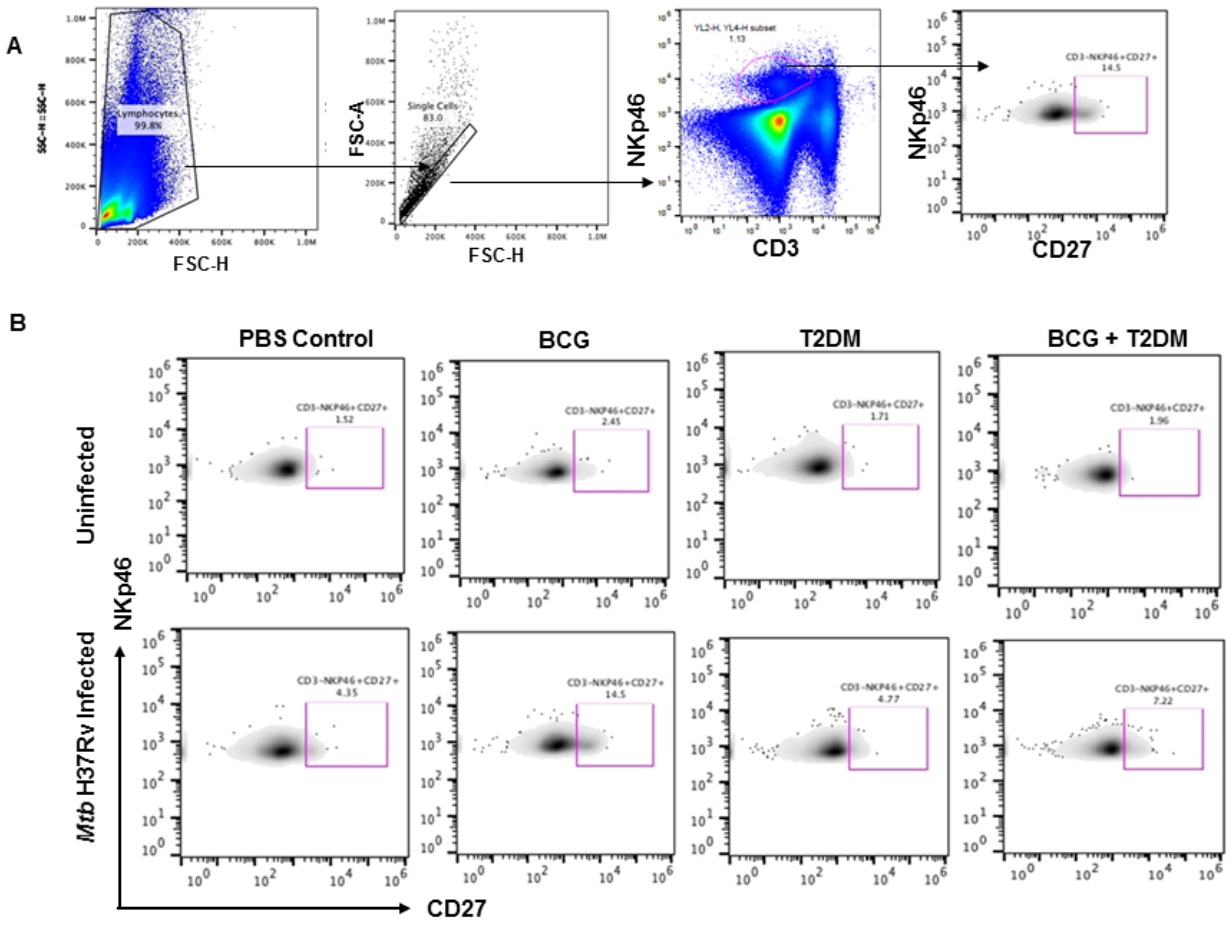
Supplementary Fig. 6. Gating strategies to determine CD4+CD25+Foxp3+ T-regulatory cells in the lungs.
PBS control, BCG-vaccinated, T2DM or BCG-vaccinated T2DM mice were infected with ~100 CFU of aerosolized *Mtb* H37Rv. A. At one and 4 months p.i., the lungs from PBS control, BCG-vaccinated control, T2DM, BCG-vaccinated T2DM, infected control, infected T2DM, infected BCG-vaccinated control and infected BCG-vaccinated T2DM mice were isolated. Lung cells were prepared for flow cytometry by surface staining (CD4+CD25+) followed by intracellular staining of Foxp3+ cells. (**A-B**) Representative gating strategies for CD4+CD25+Foxp3+ cells are shown for four months p.i. Experiments were performed using *n=5* mice per group.

Supplementary Figure 7



Supplementary Fig. 7. Gating strategies to determine CD4+CD44+CD62L+ memory T cells in the lungs.
PBS control, BCG-vaccinated, T2DM or BCG-vaccinated T2DM mice were infected with ~100 CFU of aerosolized *Mtb* H37Rv. A. At one and 4 months p.i., the lungs from PBS control, BCG-vaccinated control, T2DM, BCG-vaccinated T2DM, infected control, infected T2DM, infected BCG-vaccinated control and infected BCG-vaccinated T2DM mice were isolated. Lung cells were prepared for flow cytometry as described in the methods sections. (**A-B**) Representative gating strategies for CD4+CD44+CD62L+ cells are shown for four months p.i. Experiments were performed using *n=5* mice per group.

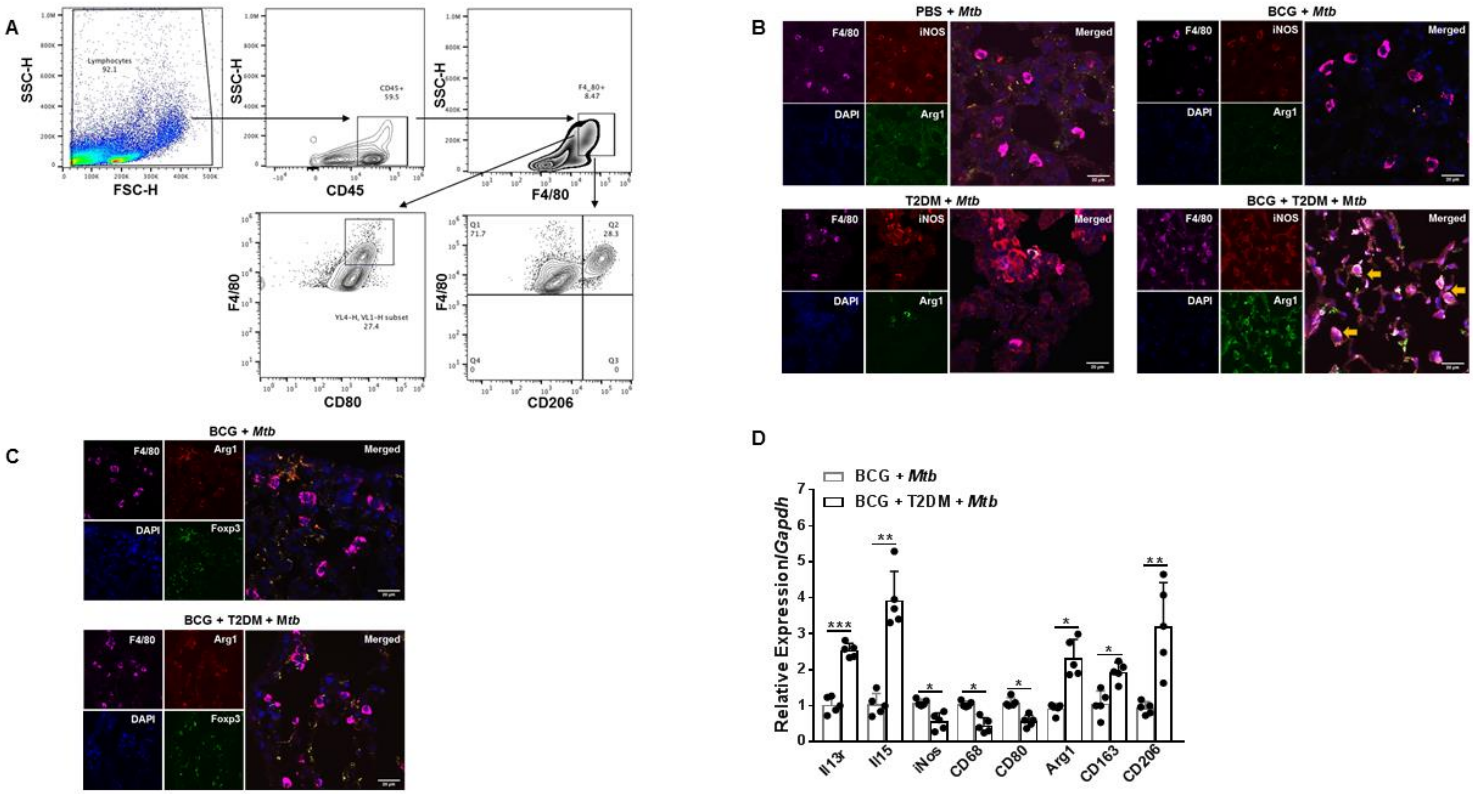
Supplementary Figure 8



Supplementary Fig. 8. Gating strategies to determine CD3-NKp46+CD27+ memory NK cells in the lungs.

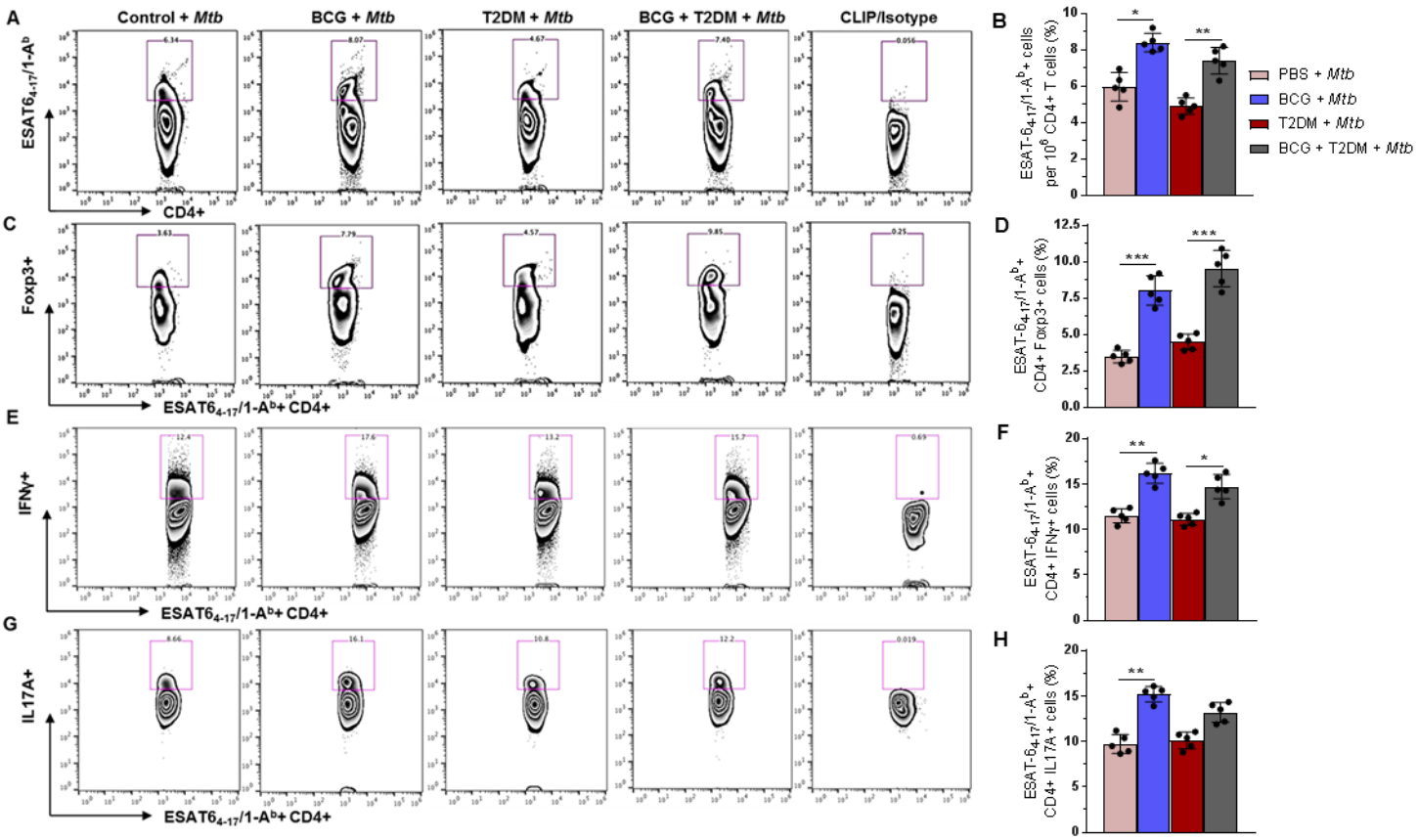
PBS control, BCG-vaccinated, T2DM or BCG-vaccinated T2DM mice were infected with ~100 CFU of aerosolized *Mtb* H37Rv. A. At one and 4 months p.i., the lungs from PBS control, BCG-vaccinated control, T2DM, BCG-vaccinated T2DM, infected control, infected T2DM, infected BCG-vaccinated control and infected BCG-vaccinated T2DM mice were isolated. Lung cells were prepared for flow cytometry as described in the methods sections. (**A-B**) Representative gating strategies for CD3-NKp46+CD27+ memory NK cells are shown for four months p.i. Experiments were performed using *n=5* mice per group.

Supplementary Figure 9



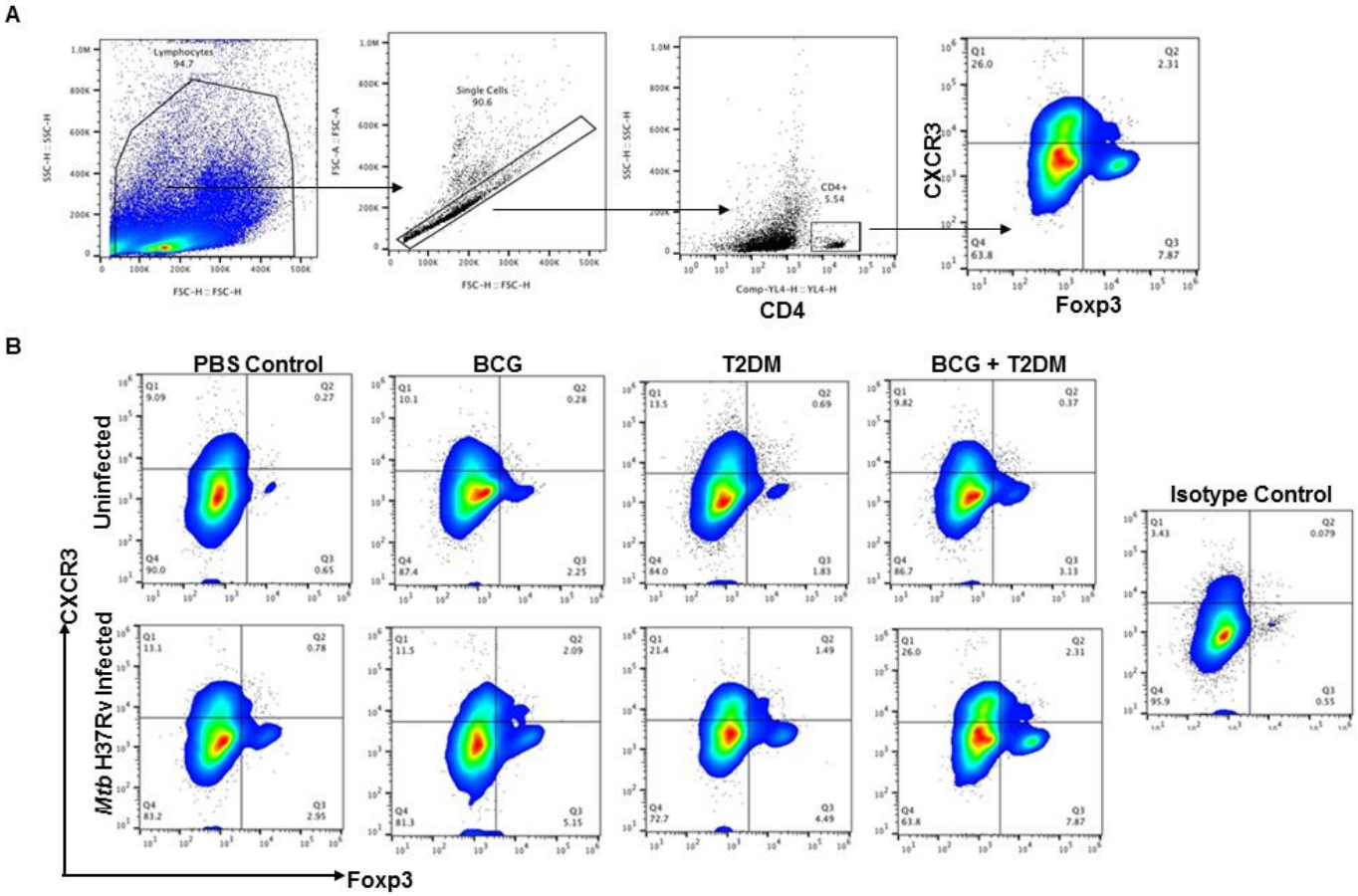
Supplementary Fig. 9. BCG vaccination induces increased infiltration of T-regulatory cells and M2 macrophages in the lungs of *Mtb*-infected T2DM mice. PBS control, BCG-vaccinated, T2DM or BCG-vaccinated T2DM mice were infected with ~100 CFU of aerosolized *Mtb* H37Rv. A. At one and 4 months p.i., the lungs from PBS control, BCG-vaccinated control, T2DM, BCG-vaccinated T2DM, infected control, infected T2DM, infected BCG-vaccinated control and infected BCG-vaccinated T2DM mice were isolated. Lung cells were prepared for flow cytometry as described in the methods sections. (**A**) Representative gating strategies for CD45+F4/80+CD80+ cells or CD45+F4/80+CD206+ cells are shown for four months p.i. At four-months post-infection (p.i.), lungs from infected control, infected T2DM, infected BCG-vaccinated control and infected BCG-vaccinated T2DM mice were isolated and formalin fixed. Paraffin-embedded tissue sections were prepared and analyzed by confocal microscopy for (**B**) F4/80+iNOS+Arg1+ cells and (**C**) F4/80+Foxp3+Arg1+ cells. Representative images of staining patterns were taken from multiple fields at 63X with oil immersion. Scale bar: 20 µm. (**D**) At 4 months p.i., total RNA from the lungs of BCG-vaccinated nondiabetic and BCG-vaccinated T2DM mice infected with *Mtb* were isolated, and cDNA was prepared. Quantitative real-time PCR analysis shows *IL-13R*, *IL-15*, *iNOS*, *CD68*, *CD80*, *Arg1*, *CD163* and *CD206* gene expression. Experiments were performed two times and each time 2 to 3 mice per group were used. For statistical analysis unpaired t test (two tailed) was performed. The data are shown as mean ± SDs of *n=5* mice per group.*, *p<0.05*; **, *p<0.01* and ***, *p<0.001*.

Supplementary Figure 10



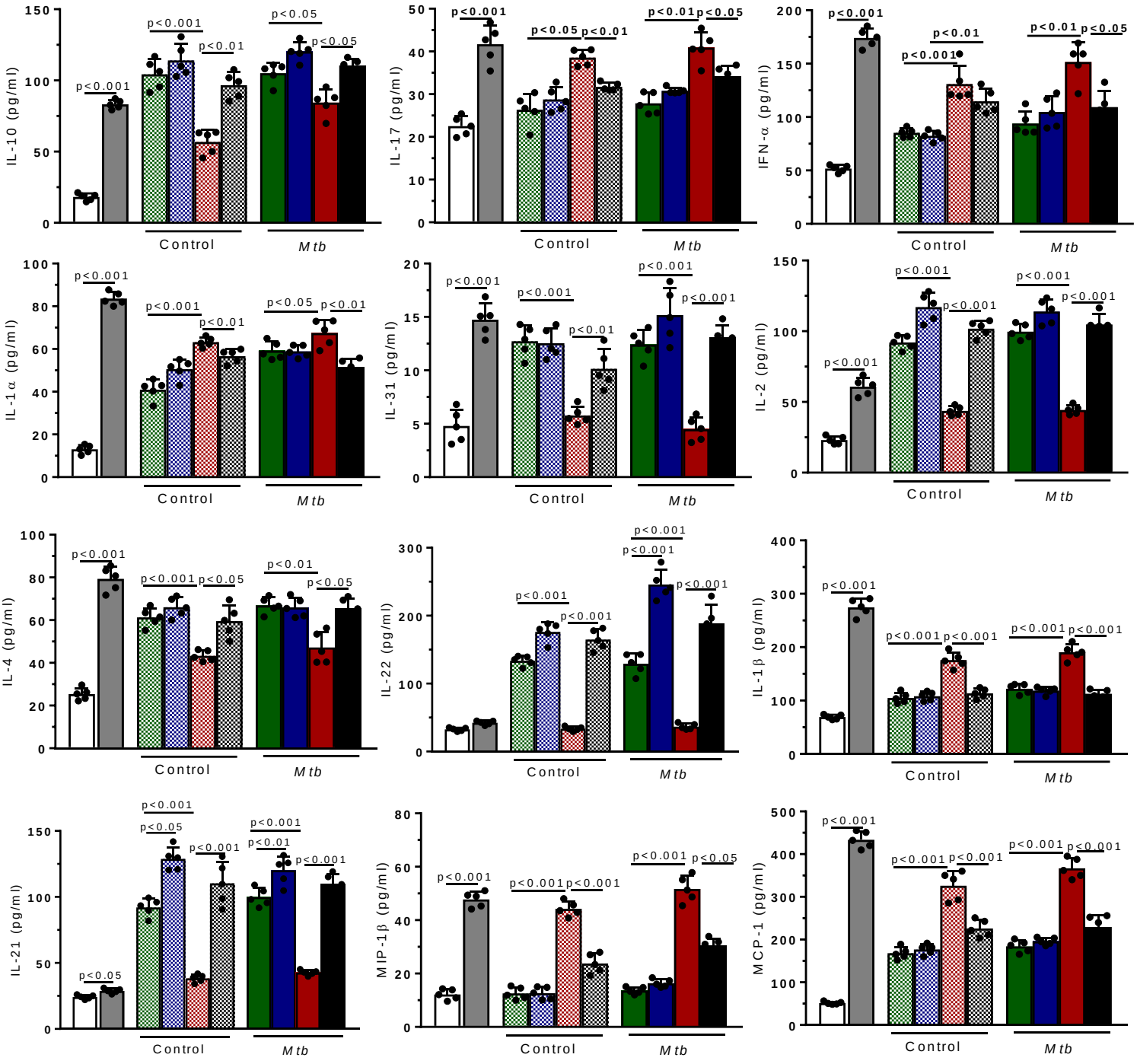
Supplementary Fig. 10. Antigen-specific responses of BCG-vaccinated T2DM mice infected with *Mtb*.
PBS control, BCG-vaccinated, T2DM or BCG-vaccinated T2DM mice were infected with *Mtb* as described in Fig. 1. Four months after infection, lung cells were prepared as described in the methods section. **(A and B)** Percentage of tetramer$^+$ CD4+ T cells in the lung and representative plots of ESAT-6$_{4-17}$/I-A^b+ MHC II tetramer staining gated on CD4+ T cells. **(C-D)** Percentage of tetramer$^+$ CD4+ T and Foxp3+ cells in the lung and representative plots of Foxp3 staining gated on tetramer$^+$ CD4+ T cells. **(E-F)** Percentage of IFN-γ-producing tetramer$^+$ lung CD4+ T cells and representative plots of IFN-γ staining gated on tetramer$^+$ lung CD4+ T cells. **(G-H)** Percentage of IL-17A-producing tetramer$^+$ lung CD4+ T cells and representative plots of IL-17A staining gated on tetramer$^+$ lung CD4+ T cells. Experiments were performed two times and each time 2 to 3 mice per group were used. The data are shown as mean ± SDs of *n=5* mice per group. The statistical analysis was performed by one-way ANOVA followed Tukey's multiple comparisons test. *, $p<0.05$; **, $p<0.01$ and ***, $p<0.001$.

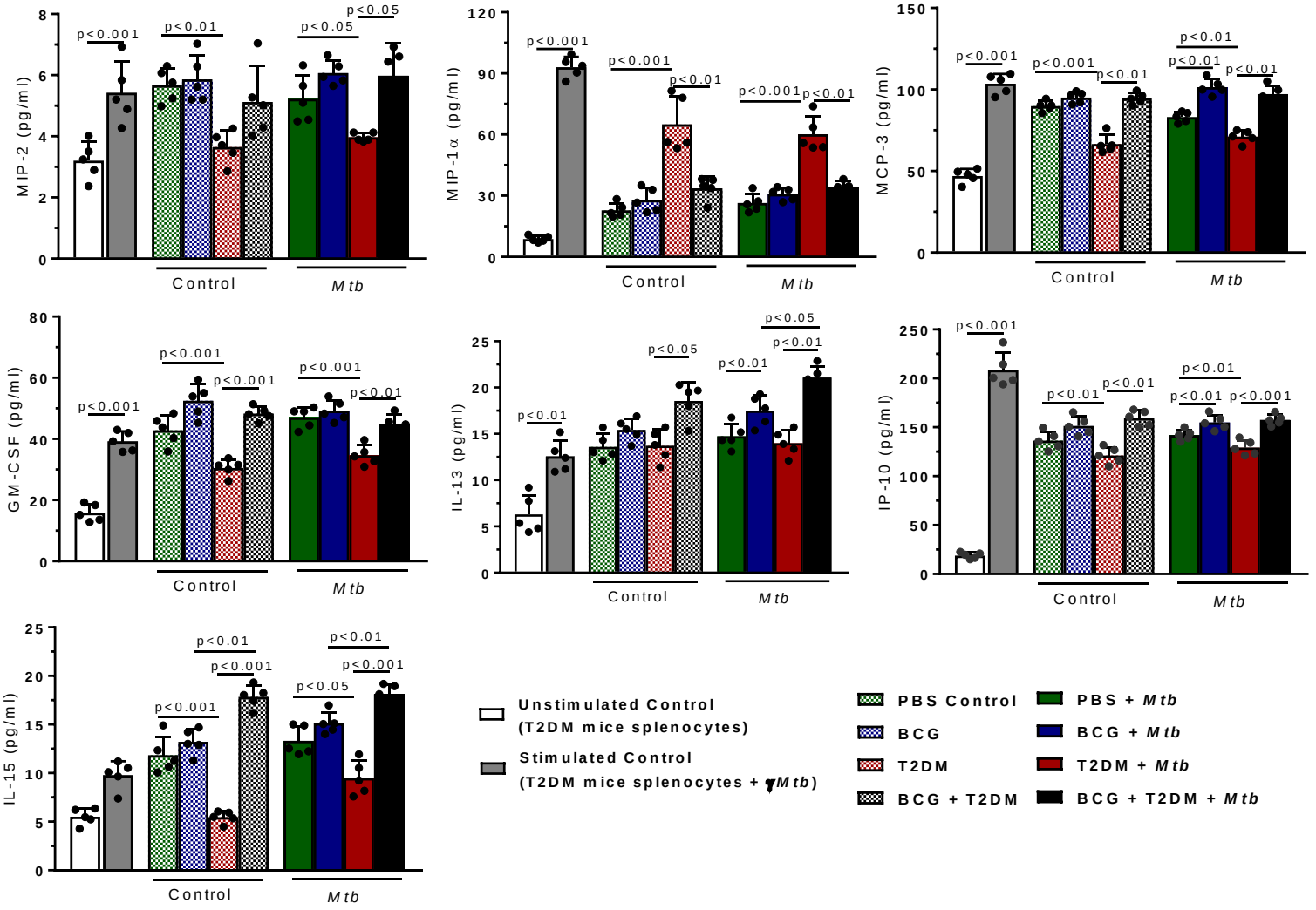
Supplementary Figure 11



Supplementary Fig. 11. Gating strategies to determine CD4+Foxp3+CXCR3+ T-regulatory cells in the lungs.
PBS control, BCG-vaccinated, T2DM or BCG-vaccinated T2DM mice were infected with ~100 CFU of aerosolized *Mtb* H37Rv. A. At 4 months p.i., the lungs from PBS control, BCG-vaccinated control, T2DM, BCG-vaccinated T2DM, infected control, infected T2DM, infected BCG-vaccinated control and infected BCG-vaccinated T2DM mice were isolated. Lung cells were prepared as described in the methods section. Lung cells were gated on CD4+Foxp3+CXCR3+ cells. (**A-B**) Representative gating strategies for CD4+Foxp3+CXCR3+ cells are shown. Experiments were performed using *n=5* mice per group.

Supplementary Figure 12

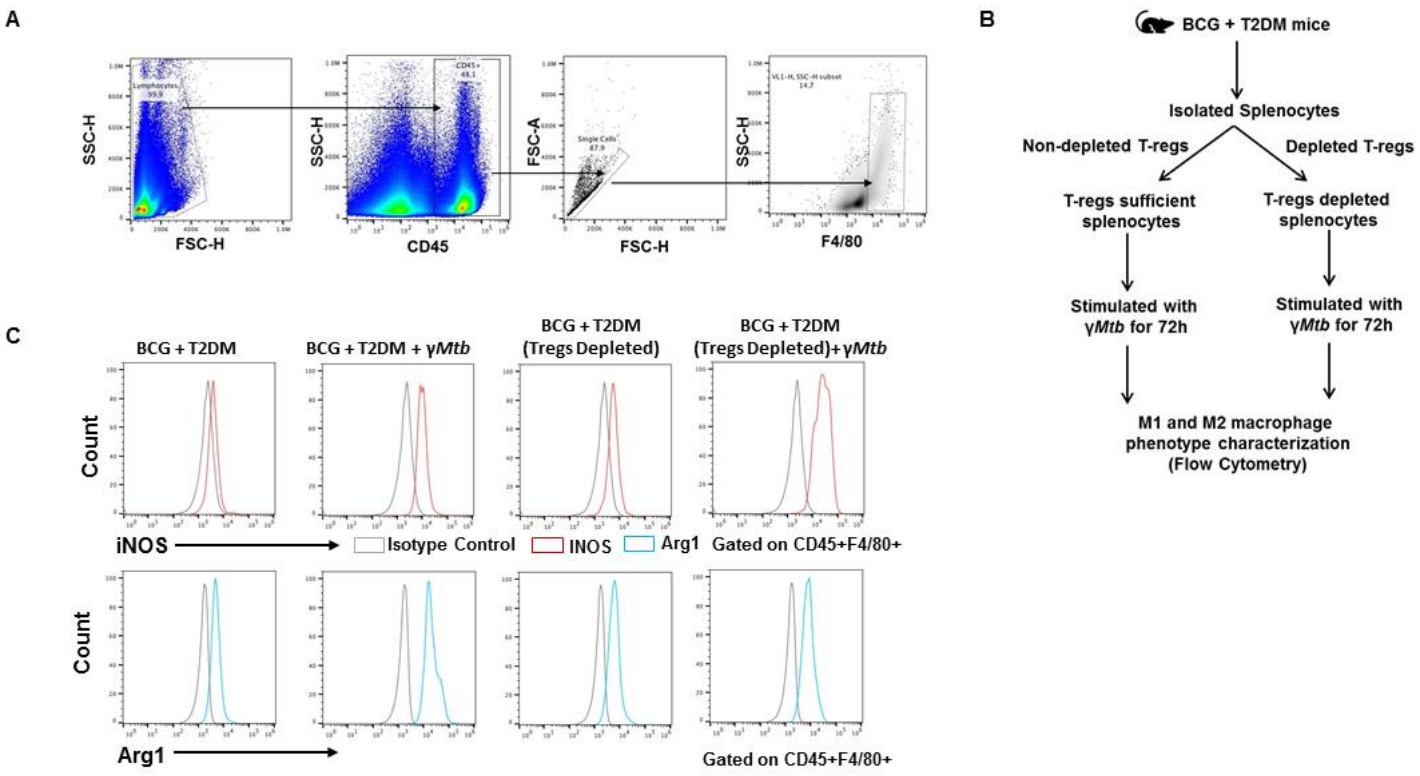




Supplementary Fig. 12. T-regulatory cells from T2DM mice are less immunosuppressive.

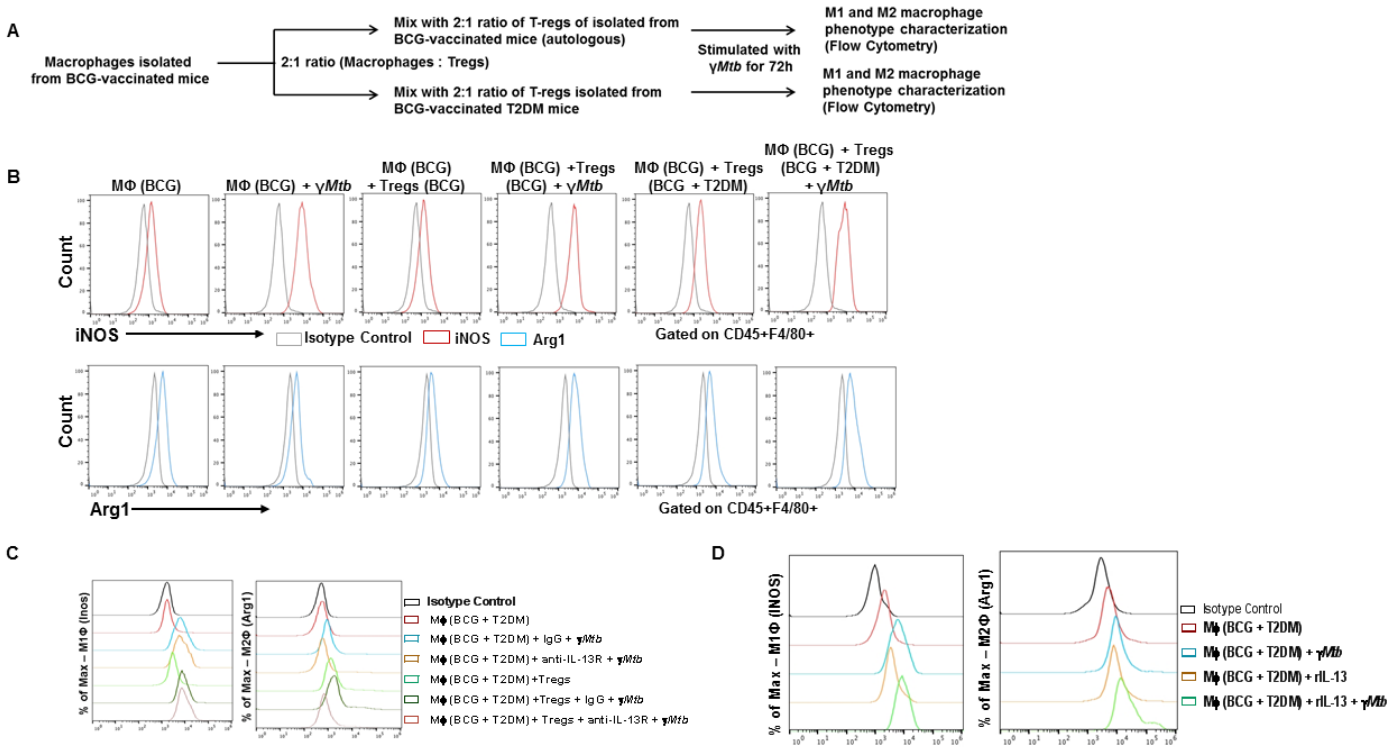
PBS control, BCG-vaccinated, T2DM or BCG-vaccinated T2DM mice were infected with ~100 CFU of aerosolized *Mtb* H37Rv. At 4 months p.i., T-regulatory cells were isolated from the lungs of PBS control, BCG-vaccinated control, T2DM, BCG-vaccinated T2DM, infected control, infected T2DM, infected BCG-vaccinated control and infected BCG-vaccinated T2DM mice. T-regulatory cell-depleted splenocytes were isolated from *Mtb*-infected T2DM mice, labeled with CFSE and cultured with γ-irradiated *Mtb* H37Rv (10 µg/ml) in the presence of T-regulatory cells from all the above groups of mice at a 1:1 ratio. After 72 h, supernatants were collected, and cytokines and chemokine levels were measured in a multiplex ELISA. Experiments were performed two times and each time 2 to 3 mice per group were used. The data are shown as mean ± SDs of *n=5* mice per group. The statistical analysis was performed by one-way ANOVA followed Tukey's multiple comparisons test. *, $p<0.05$; **, $p<0.01$ and ***, $p<0.001$.

Supplementary Figure 13



Supplementary Fig. 13. Gating strategies for CD45+F4/80+iNOS+ or Arg1+ cells. BCG-vaccinated or BCG-vaccinated T2DM mouse lungs or splenocytes were isolated. Cells were prepared as described in the methods section and stained for CD45+F4/80+iNOS+ or CD45+F4/80+Arg1+ cells. **(A)** A representative gating strategy is shown. **(B)** A schematic representation of the isolation of splenocytes from BCG-vaccinated T2DM mice, depletion of T-regulatory cells and stimulation with γ-irradiated *Mtb* is shown. **(C)** BCG-vaccinated T2DM mouse splenocytes were isolated and cultured in 12-well plates at 1×10^6 cells/well in RPMI-1640 containing penicillin (Life Technologies) and 10% heat-inactivated FCS, with or without γ-irradiated *Mtb* (10 µg/ml) at 37°C and 5% CO_2. In some wells, T reg-depleted splenocytes were stimulated with γ-irradiated *Mtb* (10 µg/ml). After 72 h, the cells were collected and phenotypically characterized for M1/M2 macrophages. Experiments were performed using *n=5* mice per group.

Supplementary Figure 14



Supplementary Fig. 14. ***T-regulatory cells from*** **BCG-vaccinated T2DM mice** ***suppress inflammation through IL-13R signaling.***
(A) Macrophages isolated from BCG-vaccinated mice were cocultured with autologous T-regulatory cells or T-regulatory cells isolated from BCG-vaccinated T2DM mice and stimulated with γ-irradiated *Mtb* (10 µg/ml). After 72 h of incubation, the macrophages were analyzed for M1 and M2 phenotypes by flow cytometry. (**B**) Representative histogram showing the CD45+F4/80+iNos+ cells and CD45+F4/80+Arg1+ cells. **(C)** For neutralization studies, BCG-vaccinated T2DM mouse macrophages were stimulated with γ-irradiated *Mtb* (10 µg/ml) with or without autologous T-regulatory cells, and in some wells, IgG control or IL-13R (10 µg/ml) antibodies were added. After 72 h of incubation, the M1 and M2 phenotypes of cultured macrophages were determined by flow cytometry. **(C)** Recombinant IL-13 (10 ng/ml) was added to the macrophages from BCG-vaccinated T2DM mice and stimulated with γ-irradiated *Mtb* (10 µg/ml). After 72 h of incubation, the M1 and M2 phenotypes of cultured macrophages were determined by flow cytometry, and representative histogram plots for CD45+F4/80+iNos+ cells and CD45+F4/80+Arg1+ cells are shown. Experiments were performed using *n=5* mice per group.

Supplemental Table 1: Leukocyte populations in the lungs of control and *Mtb*-infected mice.

	Months	PBS Control	BCG	T2DM	BCG + T2DM	PBS + *Mtb*	BCG + *Mtb*	T2DM + *Mtb*	BCG + T2DM + *Mtb*
CD3+ Cells	**1**	21850 ± 305	24998 ± 1547	28949 ± 495	36149 ± 275	96301 ± 6144	117244 ± 3681	146425 ± 6251	182733 ± 3484 **@#**
	4	25171 ± 296	26202 ± 554	25081 ± 401	24718 ± 769	87711 ± 2535	106926 ± 2329	121147 ± 2424	110609 ± 5045 ***@**
CD4+ Cells	**1**	11422 ± 480	11835 ± 435	15515 ± 680	18392 ± 543	34461 ± 1531	50158 ± 1662	85198 ± 4665	92424 ± 2349 ***@#**
	4	16388 ± 575	17094 ± 545	32078 ± 682	23599 ± 1364	45577 ± 1591	48213 ± 1023	66198 ± 1379	53660 ± 1073 ***@**
Ly6G+ Neutrophils	**1**	2450 ± 74	2778 ± 135	6717 ± 120	9877 ± 435	102527± 6473	98521 ± 1491	86414 ± 992	91860 ± 1615
	4	2029 ± 102	2250 ± 64	5609 ± 312	4389 ± 65	58527 ± 1059	60021 ± 2003	79425 ± 2060	66360 ± 2386 **@$**
CD3-NK1.1+	**1**	907 ± 28	1171 ± 29	902 ± 17	1127 ± 79	3438 ± 126	7701 ± 157	3510 ± 62	5279 ± 347 ***#$**
	4	611 ± 13	1027 ± 70	789 ± 64	910 ± 53	3286 ± 101	7167 ± 117	3779 ± 218	5622 ± 290 ***@#$**
CD11b+	**1**	8514 ± 499	9072 ± 61	10679 ± 339	11019 ± 427	16609 ± 912	18370 ± 1012	20901 ± 901	27101 ± 1009 **@#$**
	4	9766 ± 1751	10486 ± 568	13185 ± 1161	12294 ± 1060	16136 ± 1588	19889 ± 1435	22096 ± 2072	21022 ± 2325 ***@**
CD11c+	**1**	10027 ± 135	10782 ± 395	11907 ± 611	15037 ± 321	60520 ± 1679	69302 ± 1109	76819 ± 1600	81099 ± 1091 ***@#$**
	4	11110 ± 1865	11966 ± 1402	12465 ± 889	12514 ± 1838	54806 ± 1321	64848 ± 4694	91347 ± 5841	73174 ± 3150 ***@#$**
CD11c+F4/80+	**1**	3591 ± 31	3552 ± 301	5460 ± 221	5581 ± 37	8757 ± 484	9519 ± 79	15090 ± 509	14031 ± 429 **@#$**
	4	3214 ± 150	3276 ± 158	5213 ± 174	4538 ± 76	7530 ± 315	8236 ± 220	12941 ± 2414	11056 ± 2091 **@**
B220+Igm+	**1**	13066 ± 491	14343 ± 1109	16975 ± 765	17663 ± 620	19248 ± 524	22912 ± 1400	21057± 966	21488 ± 1605 *****
	4	11873 ± 659	13626 ± 552	17982 ± 544	15357 ± 325	17076 ± 391	20481 ± 879	20684 ± 1715	20465 ± 767 ***@**

Supplementary Table 1. Leukocyte populations in the lungs of control and *Mtb*-infected mice.
PBS control, BCG-vaccinated, T2DM or BCG-vaccinated T2DM mice were infected with ~100 CFU of aerosolized *Mtb* H37Rv. A. At one and 4 months p.i., the lungs from PBS control, BCG-vaccinated control, T2DM, BCG-vaccinated T2DM, infected control, infected T2DM, infected BCG-vaccinated control and infected BCG-vaccinated T2DM mice were isolated. The absolute numbers and frequencies of various leukocytes per 10^6 total lung cell populations were determined by flow cytometry. Experiments were performed two times and each time 2 to 3 mice per group were used. The data are shown as mean ± SEM of *n=5* mice per group. Statistical analysis was performed by one-way ANOVA followed Tukey's multiple comparisons test with, $p<0.05$ was considered significant. Comparisons between the groups as follows,
***** PBS + *Mtb vs*. BCG + *Mtb***;**
@ PBS + *Mtb vs*. T2DM + *Mtb*;
BCG + *Mtb vs*. BCG + T2DM + *Mtb*;
$ T2DM + *Mtb vs*. BCG+ T2DM + *Mtb.*

Supplemental Table 2. List of primers used for the qRT-PCR analysis in this study

Gene Name	Mouse Primer Sequences (5'…3')	
	Forward	**Reverse**
Bcl2	CATGTGTGTGGAGAGCGTCA	GCATGCTGGGGCCATATAGT
klrg1	ACATTTCCGGACAACCAGGG	TGTTCCTCAAGCCGATCCAG
Itgae	GAGCAAAACCAAGCCTCAGC	AGGAGAAACAGTCGGAAGGAAA
Stat3	CGTGGAGCTGTTCAGAAACTT	AACTGGACACCAGTCTTGATGA
Tbet	TCAACCAGCACCAGACAGAG	AAACATCCTGTAATGGCTTGTG
Rorγt	TTCACCCCACCTCCACTG	TGCAAGGGATCACTTCAATTT
Irf4	ACCCCATGACAGCACCTTAT	GGGTGGCATCATGTAGTTATGA
Gata3	CATTACCACCTATCCGCCCTATG	CACACACTCCCTGCCTTCTGT
Tnfrsf18	GTTTGGCTTCCGGTGTGTTG	TTCTCTGGTGGTCACTGCAA
Gzmb	GAAGCCAGGAGATGTGTGCT	GCACGTTTGGTCTTTGGGTC
Il10	GCTCTTGCACTACCAAAGCC	CTGCTGATCCTCATGCCAGT
Lag3	TGGCTGGAAAGAGTGGCAAT	TGCTACAGGTGTAGGTCCCA
Tgfb1	CATGGAGCTGGTGAAACGGA	GGCGAGCCTTAGTTTGGACA
Ctla4	ATCCTGGAAACAAACTGGATCA	TGGGCTTCAGATAACGGCTG
Pd1	CAGACTGAAAAACAGGCCGC	GTATGATCTGGAAGCGGGCA
Bcl6	GAACCACGATCCGCACTAT	CGTGCCGGGTAAACTGGATA
Foxp3	AGAGGTATTGAGGGTGGGTGT	TTTCTTCTGTCTGGAGTGGC
Cxcr3	GCAACTGTGGTCGAGAAAGC	GGCATAGAGCAGCGGATTGA
Il18	GCCTCAAACCTTCCAAATCA	TGGATCCATTTCCTCAAAGG
Il13	CCTGGCTCTTGCTTGCCTT	GGTCTTGTGTGATGTTGCTCA
Cxcr5	ACTGGCCTTCTACAGTAACAGC	CAGCACCAGGATGTTTCCCA
Cxcr6	GAGTGGGTCTTTGGCACAGT	CGATCCACTGTGATGCAGGT
Il2ra	AACACCACCGATTTCTGGCT	CGTTAGGTGAATGCTTGGCG
Tnfrsf1b	GCATCTGTAGCATCCTGGCT	GTGGGCTCTGGCTGAGATAC

Ccr7	TGTGATTTCTACAGCCCCCAG	TGACAAGGAGAGCCACCACC
Ido	GATGCTGGGACATTCCTTCAGT	GAACATCGTCATCCCCTCGG
Tnfrsf9	TGGTGAGCTTCTCTCCCAGTA	TAAGGACCTGCAAGGAGTGC
Ox40	ACAAGTGCTGTCGTGAGTGC	CAAGGTGGGTGGAGAGGGCAA
Il13r	GAATTTGAGCGTCTCTGTCGAA	GGTTATGCCAAATGCACTTGAG
Il15	CTCTGTTGACAAGCAATGAGACG	TCTTCAGTATGTCTAGCCCCTG
Inos	GCAGAATGTGACCATCATGG	ACAACCTTGGTGTTGAAGGC
Arg1	GGAATCTGCATGGGCAACCTGTGT	AGGGTCTACGTCTCGCAAGCCA
CD68	TTGCTAGGACCGCTTATAG	AAGGATGGCAGGAGAGTA
CD163	AAGCATTACTGTCATCATAG	CTCCACCTACAAGTCTAA
CD206	ATCCACTCTATCCACCTTCA	TGCTTGTTCATATCTGTCTTCA
F4/80	AAGCATCCGAGACACACACAGTCT	TGACTGTACCCACATGGCTGATGA
CD80	CTAGTTTCTCTTTTTCAGGTTGTGA	GTATGTGCCCCGGTCTGAAA
Gapdh	AGGTCGGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
βactin	TTACAGGAAGTCCCTCACCC	ACACAGAAGCAATGCTGTCAC