

Cell Host & Microbe, Volume 28

Supplemental Information

BCG Vaccination in Humans

Elicits Trained Immunity

via the Hematopoietic Progenitor Compartment

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Supplemental Figures

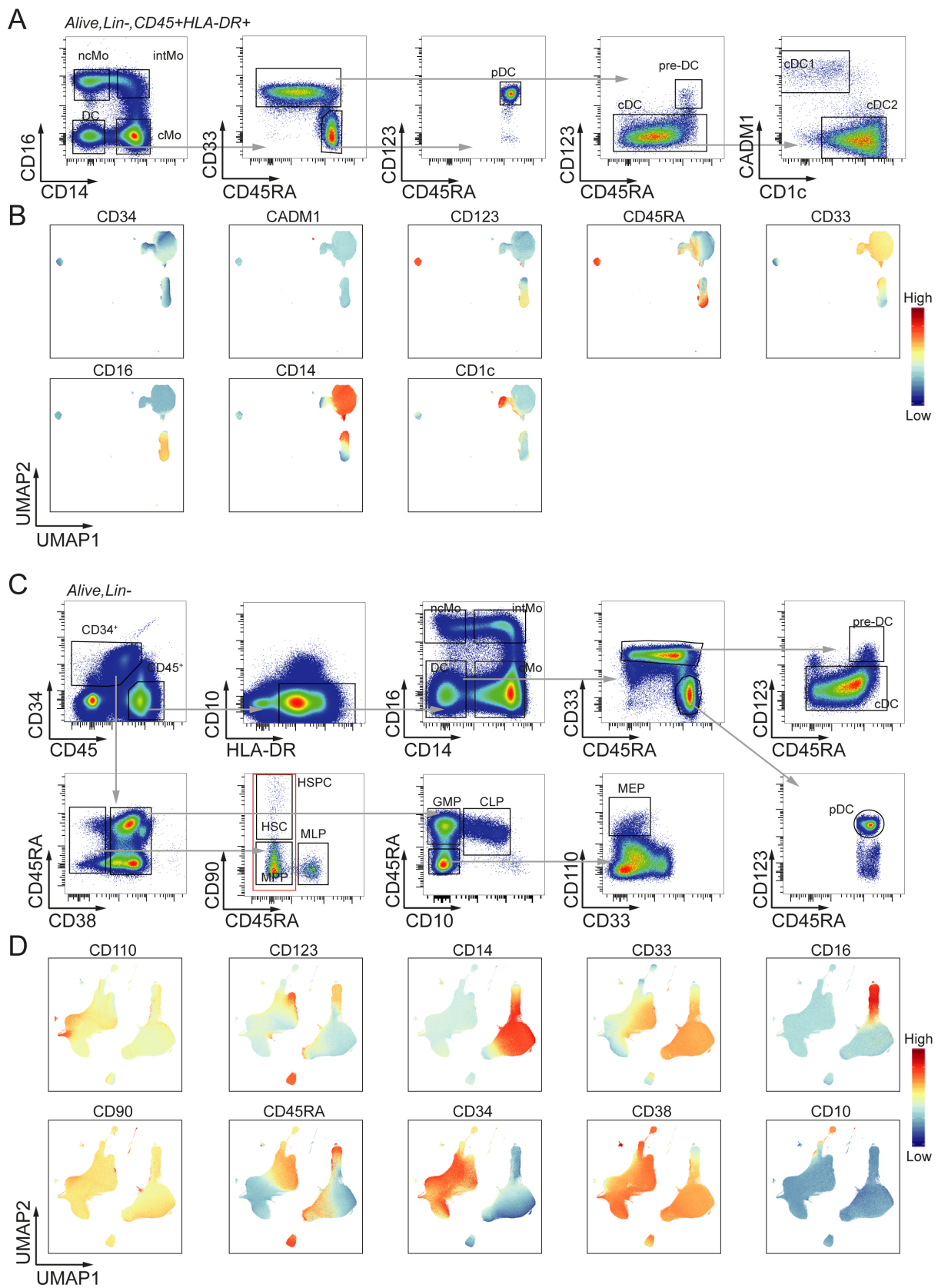


Fig. S1 related to Figure 1. Gating strategy and surface marker expression. (A) Representative manual gating strategy to identify cell lineages in PBMCs and region annotation in the low dimensional space. **(B)** Color-coded expression levels overlaid in the reduced low dimensional space (UMAP) of the PBMC. **(C)** Representative

manual gating strategy to identify cell lineages in the MNC BM fraction and region annotation in the low dimensional space. Combined HSC/MPP (HSPC) was sorted for transcriptomic analysis. **(D)** Colour-coded expression levels overlayed in the reduced low dimensional space (UMAP) of the MNC BM compartment for the individual surface markers used for flow cytometric analysis.

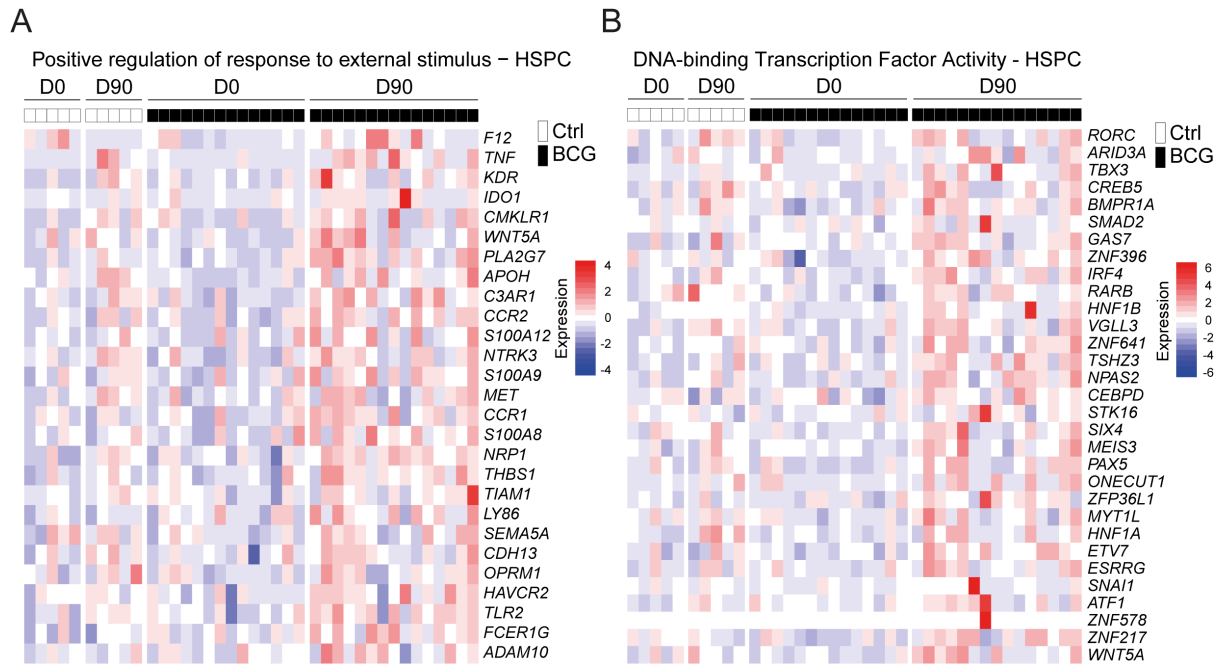


Fig. S2 related to Figure 3 and 4. Genes associated to gene ontology terms in HSPCs DEG from BCG-vaccinated individuals. (A) Expression heatmap of HSPCs for the enriched term “Positive regulation of response to external stimulus” (GO:0032103). **(B)** Expression heatmap of genes overlapping upregulated DEGs in HSPCs and genes associated with the term “DNA binding transcription factor activity” (GO:0003700).

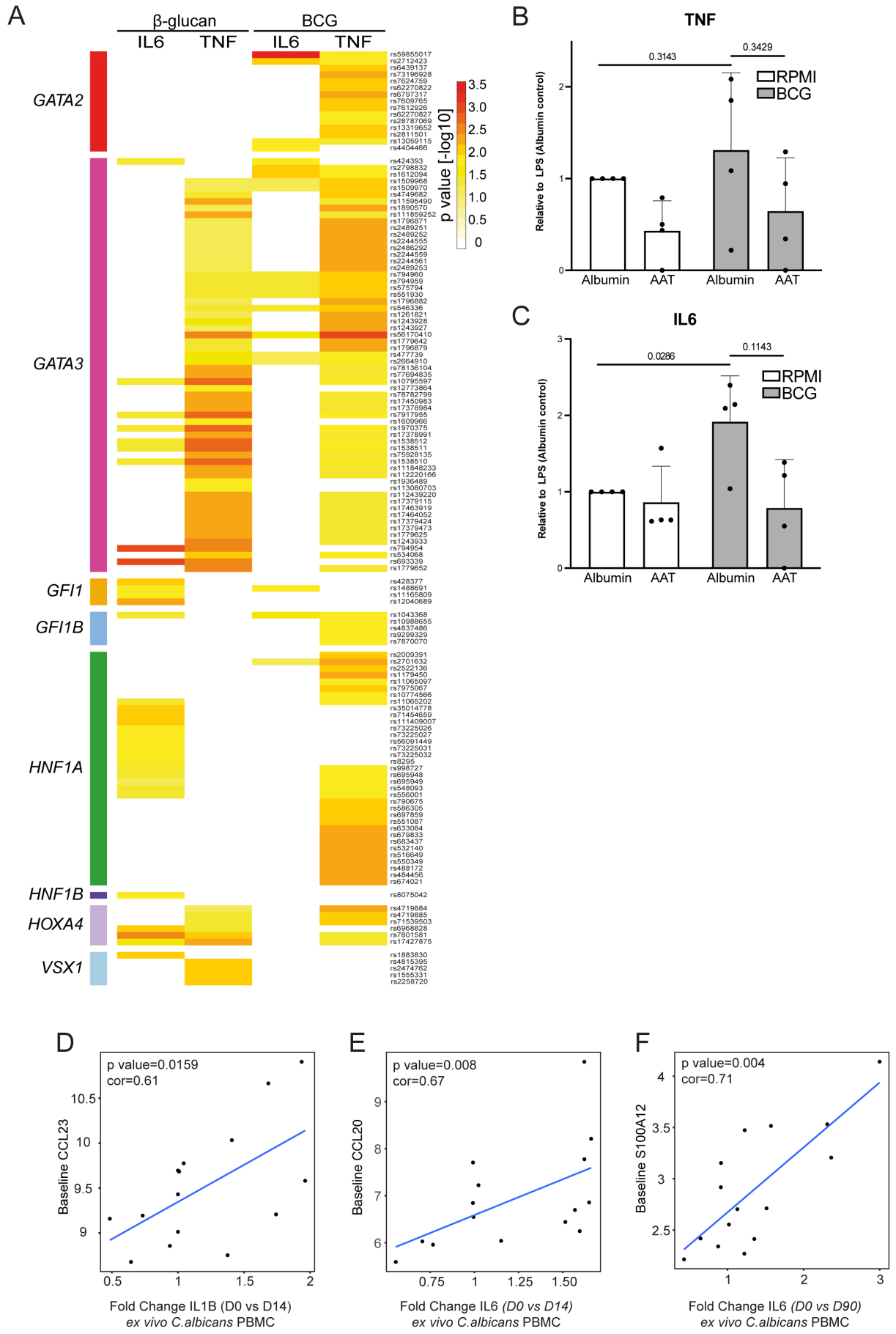


Fig. S3 related to Figure 5. Transcription factors associated with BCG-induced trained immunity. (A) Ex vivo training of PBMCs with β -glucan or BCG and IL6/TNF cytokine measurements after LPS challenge. SNPs \pm 250kb within the genomic locus of all the transcription factors corresponding to the enriched TFBS gene signatures and variants thereof significantly leading to a change in cytokine production for any of the conditions are listed. Threshold for inclusion, p value $<$ 0.01. SNP association to the transcription factors is indicated by color coding in the first column of the heatmap. (B/C). Human primary monocytes were trained in-vitro with BCG for 24h, in the presence of either albumin (as control) or AAT (both at 100 g/ml). After washing the cells and resting them in culture medium for 5 days, restimulation with LPS (10 ng/ml) was done for another 24 h, after which TNF (C) or IL6 (D) were measured by ELISA. (D) Fold changes of IL1B after ex vivo *C. albicans* restimulation and serum CCL23 (D0 vs D14) correlated in the same individuals. Data points from BCG-vaccinated or Ctrl-treated individuals are shown in black or grey, respectively. (E) Fold changes of IL6 after ex vivo *C. albicans* restimulation and serum CCL29 (D0 vs D14) correlated in the same individuals. Data points from BCG-vaccinated or Ctrl-treated individuals are shown in black or grey, respectively. (F) Fold changes of IL6 after ex vivo *C. albicans* restimulation and serum S100A12 (D0 vs D90) correlated in the same individuals. Data points from BCG-vaccinated or Ctrl-treated individuals are shown in black or grey, respectively. See also Table S14.

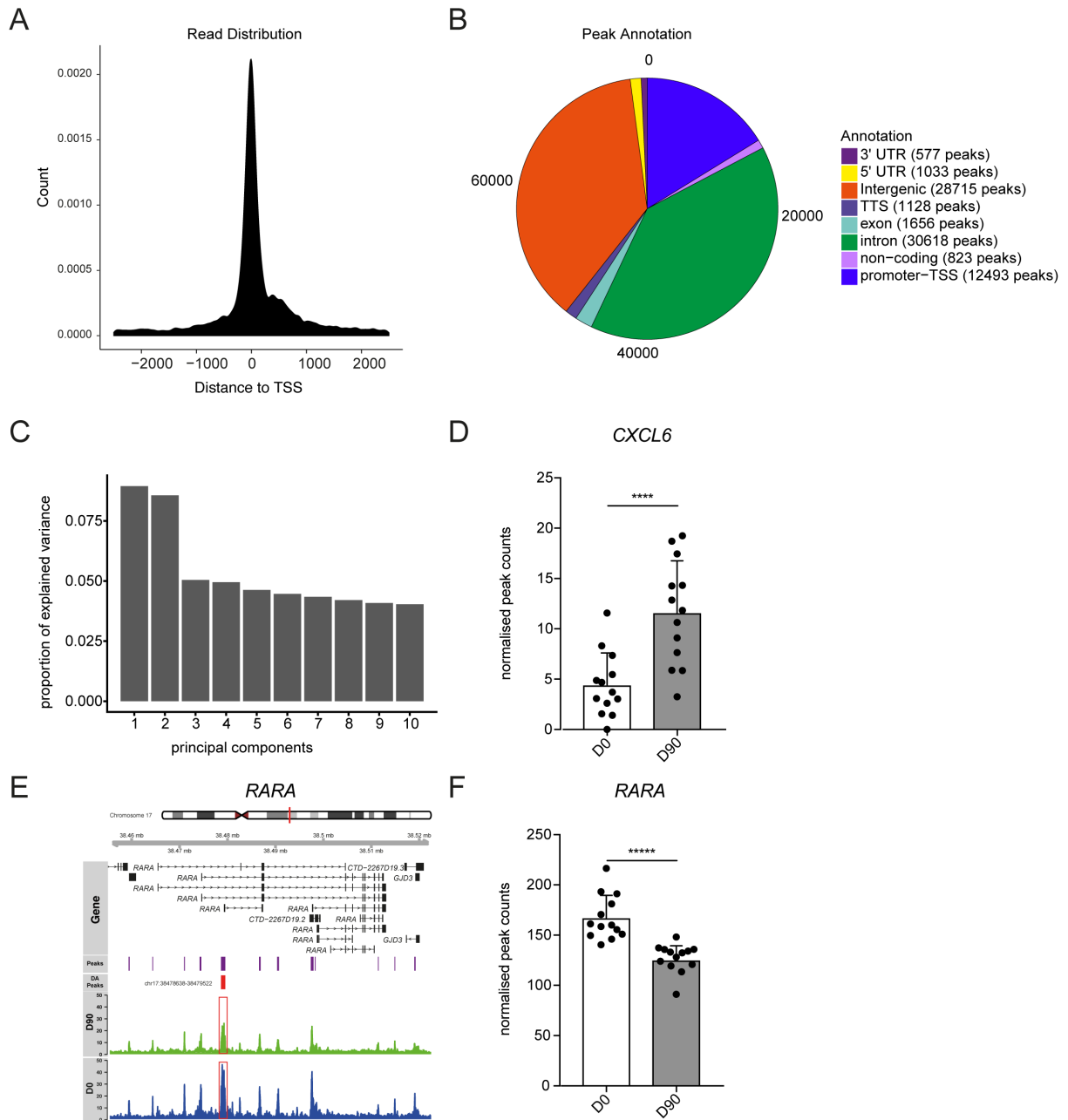


Fig. S4 related to Figure 6. BCG-induced alterations in open chromatin regions in CD14⁺ monocytes. (A) Distribution of reads relative to the transcription start site (TSS) of the closest gene. **(B)** Classification of all detected peaks according to genomic functional elements. Associated number of peaks are displayed in brackets. **(C)** Contribution of the principal components 1 - 10 to the explained variance. **(D)** Normalised peak counts of the DA peak associated with the *CXCL6* genomic region. **(E)** Example genome track surrounding the *RARA* region. Called peaks are visualized in violet and differentially accessible (DA) peaks in red. Summarized reads from CD14⁺ monocytes before and after vaccination are shown in blue and green, respectively. **(F)** Normalised peak counts of the DA peak associated with the *RARA* genomic region (n = 13, per group; ****, p value < 10⁻⁴; *****, p value < 10⁻⁷). UTR, untranslated region.

Supplemental Tables

Table S1. Raw cytokine data. Related to Figure 1.

Raw cytokine concentrations from supernatants of PBMCs cultured in presence of *C.albicans* for 24h [pg/ml]

Donor	Condition	Timepoint	IL10	IL1B	IL1RA	IL6	IFNA	IFNG	TNFA
1	BCG	D0	205.0	18000.0	56515.1	20524.2	1.1	36211.1	6645.1
2	BCG	D0	202.6	17255.9	59932.6	14085.5	7.5	23157.2	5574.9
3	Control	D0	212.4	14329.8	45704.6	15899.3	3.1	20648.7	3537.8
5	BCG	D0	207.5	13755.8	75033.1	25517.6	1.1	40232.0	6752.3
6	BCG	D0	496.7	18000.0	56547.6	44112.9	6.4	11658.4	7283.4
7	Control	D0	159.9	18000.0	75164.7	50846.7	1.1	63158.1	7681.0
8	BCG	D0	230.6	6250.6	23704.4	27507.2	1.1	4681.3	2484.3
9	BCG	D0	374.5	13080.5	78447.3	10166.7	1.1	8924.8	3916.0
10	BCG	D0	154.4	10331.6	45810.7	29154.5	1.1	5152.0	2882.5
11	Control	D0	301.1	14250.5	51245.8	36899.4	7.9	19322.5	4370.8
12	BCG	D0	207.8	7427.0	48878.0	23363.0	1.1	9725.7	2443.5
13	Control	D0	110.3	8466.0	30593.9	21823.6	1.1	9647.9	1757.9
14	BCG	D0	338.8	8289.7	68207.1	29917.1	1.1	4659.2	2162.9
15	BCG	D0	290.9	9245.4	51601.4	20197.4	1.1	2522.0	1256.7
16	BCG	D0	152.5	12190.5	86836.7	10972.2	1.1	9033.7	879.8
17	BCG	D0	552.1	18000.0	77851.3	50563.1	1.1	12510.5	8962.8
18	Control	D0	307.7	18000.0	52019.3	24763.1	9.5	8172.3	1442.2
19	BCG	D0	600.1	12626.7	88402.3	45540.1	7.8	1015.2	3877.7
20	BCG	D0	209.8	18000.0	58265.5	25680.6	4.2	61236.9	9916.8
21	BCG	D0	102.4	16636.9	40056.0	20660.0	1.1	96000.0	6337.5
1	BCG	D14	322.2	18000.0	81313.0	33278.6	3.1	16974.2	9146.5
2	BCG	D14	263.0	18000.0	78309.3	22473.0	1.1	31104.3	7699.2
3	Control	D14	164.4	16023.2	39821.0	10360.2	6.1	56809.1	4546.1
5	BCG	D14	184.4	6657.4	48484.3	14178.6	1.1	21913.4	3700.3
6	BCG	D14	591.7	13191.3	29658.6	33806.0	7.6	17161.5	11244.4
7	Control	D14	151.6	16002.3	46344.9	33974.4	1.1	33662.2	8253.6
8	BCG	D14	197.4	10524.3	38164.0	27372.3	1.1	11846.0	4151.6
9	BCG	D14	355.1	17990.5	77350.9	16488.8	2.2	18109.8	2253.9
10	BCG	D14	397.6	18000.0	78539.4	48066.2	1.1	17387.1	5879.6
11	Control	D14	383.0	14372.7	53109.1	37284.8	12.5	96000.0	6450.1
12	BCG	D14	401.4	14577.2	87043.6	26896.6	6.4	8722.3	5266.0
13	Control	D14	109.2	9582.8	26961.1	23043.4	1.1	8409.1	3230.3
14	BCG	D14	287.7	16051.2	72706.9	46914.5	3.0	31482.0	4508.0
15	BCG	D14	147.8	9324.7	34397.3	14258.4	1.1	6779.2	3577.2
16	BCG	D14	132.2	11443.7	51157.2	10882.4	1.1	15441.5	6685.5
17	BCG	D14	591.5	18000.0	94088.8	50170.2	3.5	3578.2	8437.1
18	Control	D14	192.1	17517.4	47816.4	20434.4	8.7	22377.9	4833.5
19	BCG	D14	525.3	17775.7	74901.8	75600.0	35.6	2487.3	2754.9

20	BCG	D14	483.7	18000.0	87251.1	38888.9	4.5	96000.0	8807.9
21	BCG	D14	228.8	10720.9	35468.1	21137.0	1.1	19678.7	6495.9
1	BCG	D90	241.7	18000.0	59042.0	23202.5	3.8	37800.1	6954.6
2	BCG	D90	117.0	15343.0	51989.3	9105.9	1.1	15588.6	5525.8
3	Control	D90	70.1	18000.0	54691.3	7478.0	2.4	44098.0	6942.9
5	BCG	D90	416.0	16768.9	108674.8	31313.2	1.1	34918.8	5481.5
6	BCG	D90	469.0	18000.0	51869.6	38757.5	5.5	4655.6	8023.0
7	Control	D90	256.4	10442.5	38481.5	30895.2	1.3	50522.8	4759.9
8	BCG	D90	433.2	9260.4	46130.6	11845.1	9.9	12279.4	5331.6
9	BCG	D90	439.3	18000.0	69807.7	15387.6	5.5	35722.5	7606.9
10	BCG	D90	199.1	18000.0	73559.1	26711.7	4.3	25290.6	7403.8
11	Control	D90	438.5	13738.3	53354.2	29081.0	8.6	30318.9	6266.1
12	BCG	D90	737.3	13399.1	127075.8	70049.5	42.2	8183.4	5525.8
13	Control	D90	120.1	6746.7	29918.3	17278.8	1.6	15213.5	2812.6
14	BCG	D90	520.2	18000.0	75120.8	40479.9	0.9	38971.8	5018.8
15	BCG	D90	340.0	11410.6	64523.2	18459.7	1.1	11352.3	4291.1
16	BCG	D90	664.0	18000.0	120515.8	25927.8	8.1	74883.4	11024.5
17	BCG	D90	570.6	18000.0	61826.6	61795.5	4.1	8593.5	10659.8
18	Control	D90	413.9	18000.0	67265.7	30735.6	31.2	30732.7	8225.8
19	BCG	D90	617.8	18000.0	100945.0	46465.8	14.3	8432.0	6488.8
20	BCG	D90	464.9	18000.0	105305.5	59351.2	10.4	96000.0	11942.6
21	BCG	D90	397.5	18000.0	81745.7	32474.0	2.7	77717.6	7073.0

Table S4. Gene Ontology Enrichment (Biological Processes) in upregulated genes, HSPC D0 vs D90.
Related to Figure 4.

GO Term	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue
GO:0036230	granulocyte activation	51/643	491/16618	1.32E-10	2.24E-07	1.89E-07
GO:0002446	neutrophil mediated immunity	51/643	494/16618	1.65E-10	2.24E-07	1.89E-07
GO:0002283	neutrophil activation involved in immune response	50/643	481/16618	1.97E-10	2.24E-07	1.89E-07
GO:0043312	neutrophil degranulation	50/643	481/16618	1.97E-10	2.24E-07	1.89E-07
GO:0042119	neutrophil activation	50/643	486/16618	2.84E-10	2.58E-07	2.17E-07
GO:0060326	cell chemotaxis	29/643	248/16618	1.14E-07	8.65E-05	7.28E-05
GO:0044057	regulation of system process	41/643	487/16618	2.63E-06	1.71E-03	1.44E-03
GO:0032103	positive regulation of response to external stimulus	27/643	261/16618	3.45E-06	1.96E-03	1.65E-03
GO:0050922	negative regulation of chemotaxis	9/643	35/16618	5.26E-06	2.65E-03	2.23E-03
GO:0061387	regulation of extent of cell growth	14/643	89/16618	7.64E-06	3.47E-03	2.92E-03
GO:0019932	second-messenger-mediated signaling	24/643	229/16618	9.58E-06	3.95E-03	3.33E-03
GO:0050920	regulation of chemotaxis	20/643	172/16618	1.14E-05	4.33E-03	3.65E-03
GO:0060560	developmental growth involved in morphogenesis	22/643	204/16618	1.43E-05	4.98E-03	4.19E-03
GO:0071675	regulation of mononuclear cell migration	9/643	40/16618	1.71E-05	5.54E-03	4.67E-03
GO:1902668	negative regulation of axon guidance	5/643	10/16618	1.83E-05	5.54E-03	4.67E-03
GO:0090066	regulation of anatomical structure size	36/643	442/16618	2.20E-05	6.25E-03	5.27E-03
GO:0006836	neurotransmitter transport	20/643	184/16618	3.09E-05	8.23E-03	6.93E-03
GO:0030516	regulation of axon extension	12/643	76/16618	3.26E-05	8.23E-03	6.93E-03

GO:0045761	regulation of adenylate cyclase activity	12/643	77/16618	3.73E-05	8.92E-03	7.51E-03
GO:0048638	regulation of developmental growth	26/643	282/16618	3.97E-05	9.02E-03	7.59E-03
GO:0031279	regulation of cyclase activity	13/643	92/16618	5.18E-05	9.16E-03	7.71E-03
GO:0002430	complement receptor mediated signaling pathway	5/643	12/16618	5.39E-05	9.16E-03	7.71E-03
GO:0042430	indole-containing compound metabolic process	7/643	27/16618	5.67E-05	9.16E-03	7.71E-03
GO:0006586	indolalkylamine metabolic process	6/643	19/16618	5.78E-05	9.16E-03	7.71E-03
GO:0086014	atrial cardiac muscle cell action potential	6/643	19/16618	5.78E-05	9.16E-03	7.71E-03
GO:0086026	atrial cardiac muscle cell to AV node cell signaling	6/643	19/16618	5.78E-05	9.16E-03	7.71E-03
GO:0086066	atrial cardiac muscle cell to AV node cell communication	6/643	19/16618	5.78E-05	9.16E-03	7.71E-03
GO:0051339	regulation of lyase activity	13/643	93/16618	5.81E-05	9.16E-03	7.71E-03
GO:0032760	positive regulation of tumor necrosis factor production	10/643	57/16618	5.85E-05	9.16E-03	7.71E-03
GO:0097529	myeloid leukocyte migration	18/643	164/16618	6.70E-05	1.00E-02	8.42E-03
GO:0086003	cardiac muscle cell contraction	10/643	58/16618	6.82E-05	1.00E-02	8.42E-03

Table S5. BCG-induced changes in full blood cell count parameters from the MIS-BAIR study.
Related to Figure4.

Parameter	BCG-vaccinated (n=385)	BCG-naïve (n=409)	Adjusted ^a	
			Difference ^b (95% CI) in mean	p-value ^c
White cell count (x10⁹/L)				
Mean (sd)	14.32 (4.65)	13.85 (4.41)	0.50 (-0.10 – 1.09)	0.100
Median (IQR)	13.4 (11.2 – 16.5)	13.1 (11.1 – 15.8)		
Neutrophil count (x10⁹/L)				
Mean (sd)	7.29 (3.72)	6.80 (3.49)	0.51 (0.06 – 0.96)	0.026
Median (IQR)	6.8 (4.6 – 9.2)	6.1 (4.4 – 8.6)		
Lymphocyte count (x10⁹/L)				
Mean (sd)	4.63 (1.73)	4.56 (1.56)	0.07 (-0.15 – 0.29)	0.544
Median (IQR)	4.3 (3.5 – 5.5)	4.4 (3.4 – 5.4)		
Monocyte count (x10⁹/L)				
Mean (sd)	1.59 (0.79)	1.59 (0.82)	-0.01 (-0.12 – 0.10)	0.917
Median (IQR)	1.4 (1.1 – 2)	1.5 (1 – 2)		

a) Adjusted for the following (potential confounders identified using bivariate regressions):

- WCC – age at the time of examination
- Neutrophils – age at the time of examination
- Lymphocytes – sex, age at the time of examination
- Monocytes – age at the time of examination

b) Mean complete blood count parameter value in BCG-vaccinated group – mean complete blood count parameter value in BCG-naïve group

c) Means compared using multivariate regression analysis

Table S6. Baseline variables of the MIS-BAIR cohort. Related to Figure 4

Parameter	BCG-vaccinated (n=385)	BCG-naïve (n=409)
Sex		
Male	190 (49.4%)	194 (47.4%)
Birth method		
Vaginal	248 (64.4%)	256 (62.6%)
Caesarean	137 (35.6%)	153 (37.4%)
Gestational age (weeks)		
Mean (sd)	39.5 (1.3)	39.3 (1.2)
Median (IQR)	39.4 (38.5 – 40.5)	39.3 (38.4 – 40.3)
Birth weight (g)		
Mean (sd)	3422 (440)	3423 (453)
Median (IQR)	3430 (3130 – 3700)	3390 (3130 – 3740)
Age at FBE (hours)		
Mean (sd)	76.6 (81.8)	75.4 (70.8)
Median (IQR)	51.0 (48.4 – 64.6)	51.2 (48.4 – 64.9)
Time between randomisation & FBE (hours)		
Mean (sd)	42.7 (73.8)	39.9 (61.3)
Median (IQR)	24.5 (7.6 – 42.1)	24.4 (5.4 – 43.9)

Table S7. Effect of potential confounders on neutrophil count in the MIS-BAIR cohort.

Related to Figure 4.

Parameters	Difference (95% CI) in mean neutrophil count (x10E9/L)	p-value
Female Sex	0.25 (-0.26 – 0.75)	0.34
Caesarean Birth method	-0.26 (-0.78 – 0.26)	0.325
Gestational age (weeks)	0.03 (-0.17 – 0.23)	0.781
Birth weight (g)	-0.0001 (-0.0006 – 0.0005)	0.823
Age at FBE (hours)	-0.02 ^a (-0.02 – -0.02)	<0.001
Time between randomisation & FBE (hours)	-0.02 (-0.02 – -0.02)	<0.001

a) i.e neutrophil count decreases with age by 0.02 x10⁹/L per hour

Table S9. ATAC-sequencing alignment statistics and peak calls. Related to Figure 6.

Sample Name	Sample nr	Donor	Time point	read Nr	one alignment	failed alignment	Suppressed alignment	Peak Nr
S1Td0	3904	1	D0	93147328	56235249	32324701	4587378	14195
S1Tm3	3905	1	D90	22657866	14230772	7300268	1126826	23336
S2Td0	3906	2	D0	20152114	12423961	6711341	1016812	29669
S2Tm3	3907	2	D90	137207180	47726096	85350217	4130867	27430
S5Td0	3910	5	D0	21277803	5075424	15758455	443924	3513
S5Tm3	3911	5	D90	20731170	13507420	6139199	1084551	3430
S8Td0	3916	8	D0	35627324	21504396	12433288	1689640	39393
S8Tm3	3917	8	D90	56393806	23938253	30602138	1853415	10520
S9Td0	3918	9	D0	17027823	10364803	5798871	864149	18926
S9Tm3	3919	9	D90	26792118	6572971	19666841	552306	2219
S10Td0	3920	10	D0	16979891	7430930	9042480	506481	6642
S10Tm3	3921	10	D90	81294788	32847495	45648344	2798949	24252
S12Td0	3924	12	D0	13883512	9029369	4195436	658707	8298
S12Tm3	3925	12	D90	139826704	41469901	94962963	3393840	27851
S14Tdo	3928	14	D0	19723687	15191229	3466721	1065737	21920
S14Tm3	3929	14	D90	362450317	98517998	255753253	8179066	55202
S16Td0	3932	16	D0	22649770	13505333	8093293	1051144	26304
S16Tm3	3933	16	D90	47461683	28968659	16228026	2264998	19826
S17Td0	3934	17	D0	34096880	20904292	11488559	1704029	17201
S17Tm3	3935	17	D90	17527738	11379669	5271475	876594	7250
S19Td0	3938	19	D0	19177190	11745295	6484450	947445	12957
S19Tm3	3939	19	D90	63824163	17721373	44596616	1506174	17416
S20Td0	3940	20	D0	15914647	9910524	5201102	803021	22056
S20Tm3	3941	20	D90	21983401	14002305	6819209	1161887	10553
S21Td0	3942	21	D0	40965843	20446301	18816958	1702584	24391
S21Tm3	3943	21	D90	26426022	16240969	8825666	1359387	12297

Table S11. Enriched TFBS motifs in opening peaks (D0 vs D90) vs sampled background peaks in CD14⁺ monocytes. Related to Figure 6.

TFBS Motif	#positive Hits (per 1000bp)	#Hits in Background set (per 1000bp)	Site p value	Site FDR
FOXP2.1	35.33	26.61	0	0
TBP.1	30.40	23.59	0	0
SOX-6.1	63.93	49.95	0	0
B-ATF.1	53.94	43.87	0	0
FOXA2.1	18.27	13.59	4.2331E-290	1.3334E-288
KLF1.1	5.48	3.51	5.0517E-190	1.273E-188
POU5F1;SOX-2.1	7.63	5.25	1.6552E-187	3.476E-186
FOXA1.1	11.06	8.23	7.7918E-176	1.4025E-174
SOX-2.1	16.04	12.67	1.1595E-168	1.8263E-167
FOXH1.1	2.48	1.35	1.2096E-149	1.6935E-148
FOXO1.1	7.10	5.05	1.1098E-148	1.3984E-147
CEBPdelta.1	29.91	25.60	3.0999E-140	3.5508E-139
GATA-4.1	15.50	12.51	1.5453E-137	1.6226E-136
CEBPbeta.1	28.48	24.85	7.5604E-104	7.3278E-103
IRF-4.1	37.53	33.40	3.9136E-100	3.5222E-99
Mxi-1.1	3.09	2.02	1.2014E-99	1.00919E-98
NANOG.1	54.87	49.95	9.08868E-97	7.15734E-96
GATA-1.1	11.99	9.92	4.01315E-85	2.97446E-84
c-Jun.1	15.39	13.18	2.15968E-73	1.51177E-72
FOXA3.1	4.50	3.36	2.33898E-71	1.55111E-70
SMAD1.1	6.45	5.10	2.27916E-68	1.43587E-67
GATA-2.1	4.55	3.45	8.24929E-67	4.94957E-66
VDR.1	0.20	0.04	7.41055E-66	4.24422E-65
PRDM1.1	0.14	0.02	2.81795E-62	1.54375E-61
PU1.1	46.36	42.84	3.17184E-59	1.66522E-58
TCF-7L2.1	0.20	0.04	5.81875E-59	2.93265E-58
TCF-7L1.1	0.22	0.05	1.02693E-58	4.97667E-58
FOXP1.1	6.60	5.36	7.02168E-55	3.27678E-54
MEF-2C.1	0.13	0.02	1.09186E-50	4.91336E-50
MEF-2A.1	0.15	0.03	5.52439E-47	2.40025E-46

Table S12. List of antibodies and secondary staining reagents for flow cytometric analysis.

Related to Methods section.

Name	Fluorophore	Clone	Source	Use
CADM1	-(Biotin)	3E1	MBL	PBMC
CD10	APC/Cy7	HI10a	BioLegend	PBMC (Lin)
CD10	APC	HI10a	BioLegend	BM
CD110	PE-CF594	1.6.1	BD Biosciences	BM
CD115	PE/Cy7	12-3A3-1B10	ThermoFisher Scientific	PBMC
CD116	BV421	hGM-CSFR-M1	BD Biosciences	PBMC
CD123	BV785	6H6	BioLegend	BM, PBMC
CD135	PE	4G8	BD Biosciences	BM
CD14	BV711	M5E2	BioLegend	PBMC
CD14	BV421	HCD14	BioLegend	BM
CD15	APC/Fire 750	W6D3	BioLegend	BM, PBMC (Lin)
CD16	BV510	3G8	BioLegend	PBMC
CD16	PE/Cy7	3G8	BioLegend	BM
CD19	APC/Cy7	SJ25C1	BioLegend	BM, PBMC (Lin)
CD1c	PE/Dazzle 594	L161	BioLegend	PBMC
CD20	APC/Cy7	2H7	BioLegend	BM, PBMC (Lin)
CD3	APC/Cy7	OKT3	BioLegend	BM, PBMC (Lin)
CD33	BV650	WM53	BioLegend	PBMC
CD34	AF700	581	BioLegend	BM, PBMC
CD38	BV510	HB-7	BioLegend	BM
CD45	PerCP	HI30	BioLegend	BM, PBMC
CD45RA	FITC	HI100	BioLegend	BM, PBMC
CD7	APC-eFluor 780	eBio124-1D1	ThermoFisher Scientific	BM, PBMC (Lin)
CD90	BV711	5E10	BD Biosciences	BM
CX3CR1	APC	2A9-1	BioLegend	PBMC
HLA-DR	BV570	L243	BioLegend	BM, PBMC
Streptavidin	PE	-	BioLegend	PBMC
DRAQ7	DRAQ7	-	Biolegend	BM, PBMC