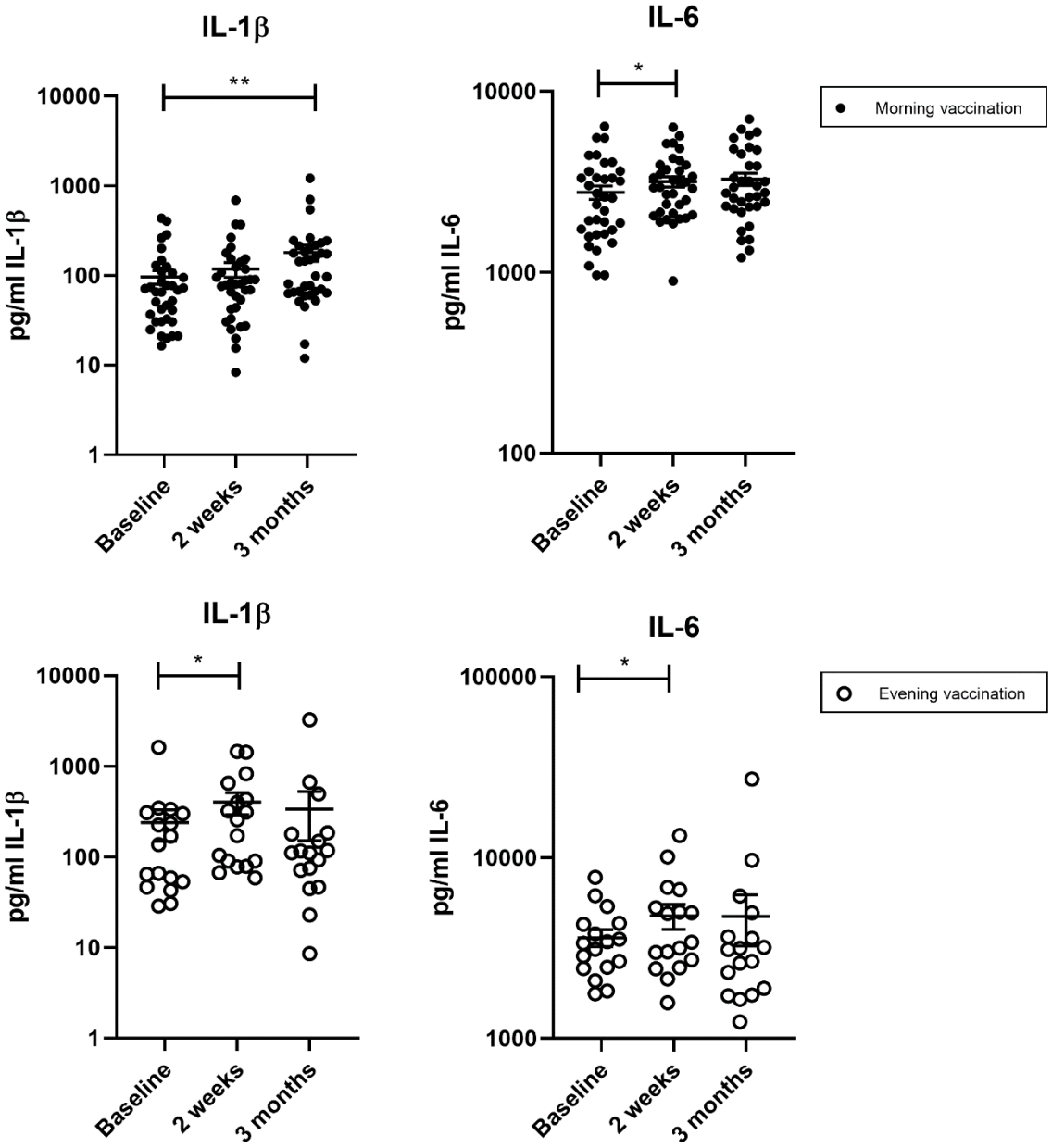
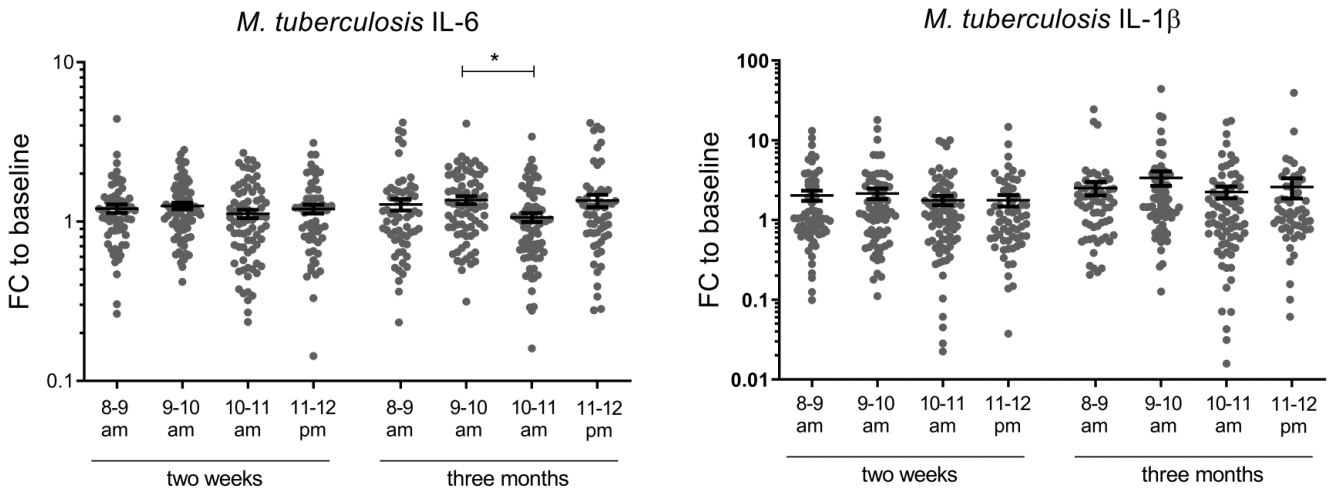
Supplementary figure 1



Supplementary figure 1

IL-1β and IL-6 production to *M. tuberculosis* stimulation two weeks and three months after BCG vaccination in morning and evening vaccinated individuals (Mean ±SEM, n=36 morning vaccinated, n=18 evening vaccinated, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, Friedman Test, Dunn multiple comparison test).

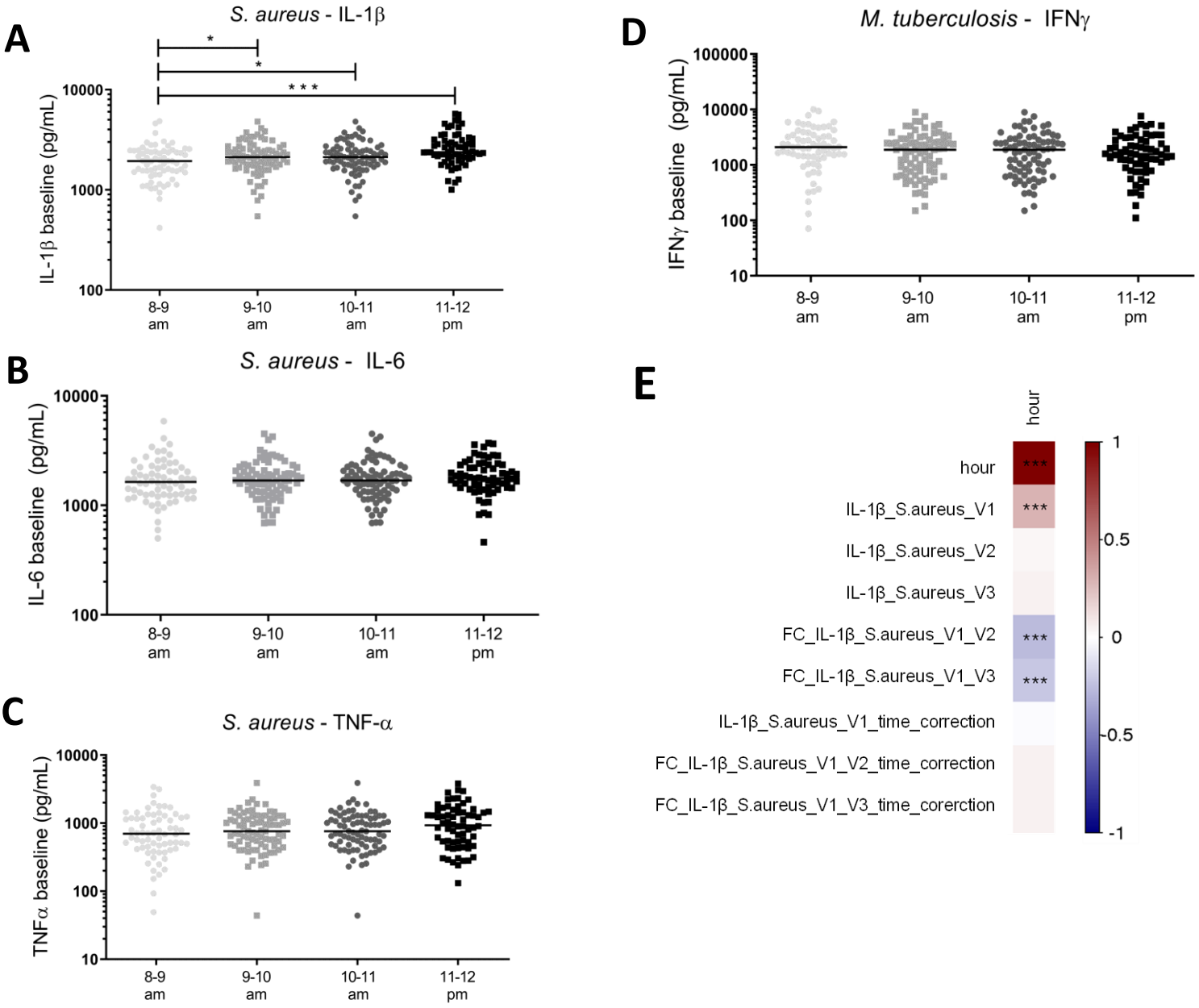
Supplementary figure 2



Supplementary figure 2

Fold changes (compared to baseline) of PBMC-derived IL-1β (A) and IL-6 (B) production to *M. tuberculosis* stimulation two weeks and three months after BCG vaccination are compared between 8am and 12pm (Mean ± SEM, n=68 vaccinated between 8am-9am, n=80 vaccinated between 9am-10am, n=84 vaccinated between 10am-11am, n=66 vaccinated between 11am-12pm, *p < 0.05, Kruskal-Wallis test, Dunn multiple comparison test).

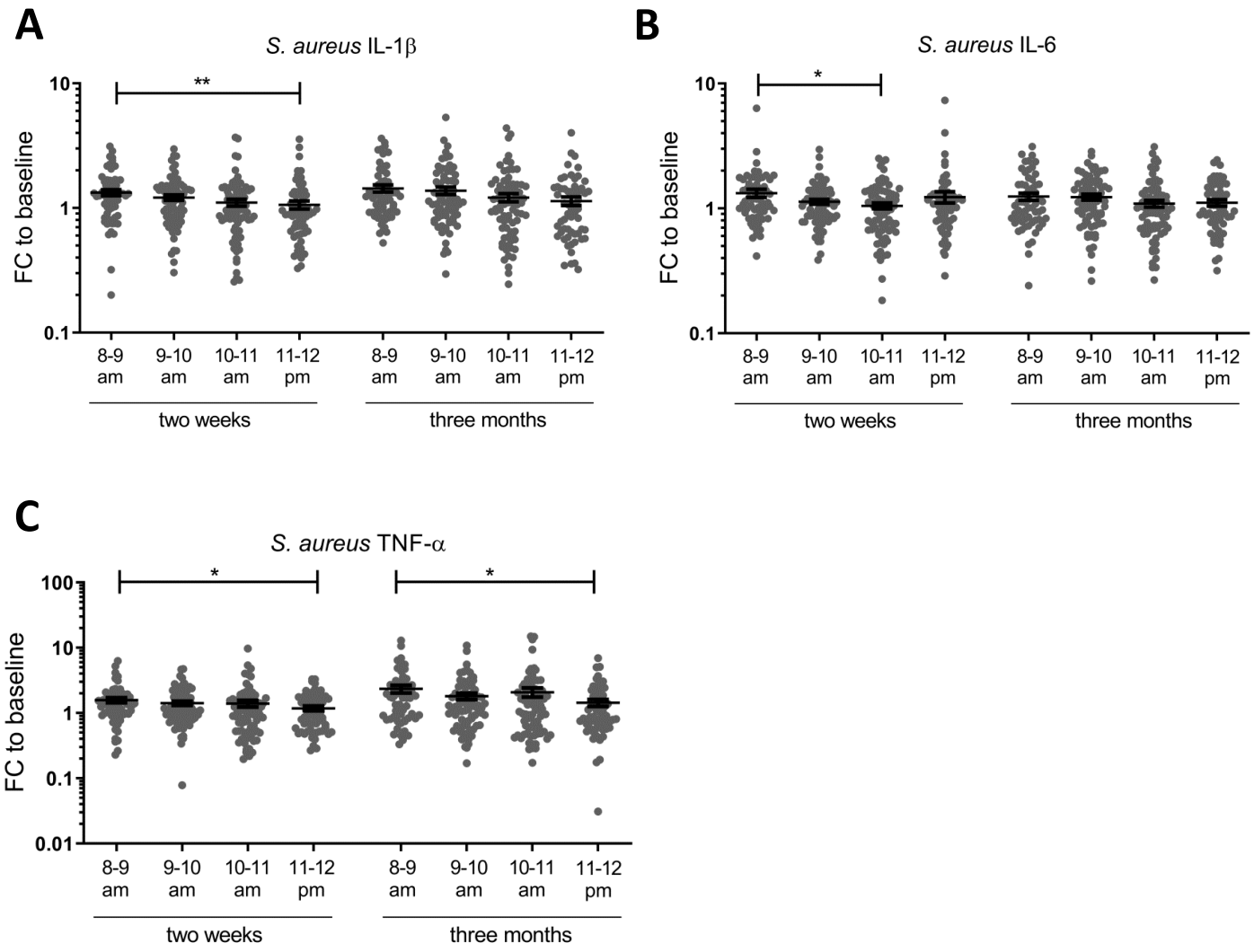
Supplementary figure 3



Supplementary figure 3

PBMC-derived *S. aureus*-induced IL-1β (A), IL-6 (B) and TNF-α (C) production, and *M. tuberculosis*-induced IFN-γ production (D) at baseline (before BCG vaccination) is compared between morning subgroups vaccinated between 8am and 12pm. (Median, n=68 vaccinated between 8am-9am, n=80 vaccinated between 9am-10am, n=84 vaccinated between 10am-11am, n=66 vaccinated between 11am-12pm, *** $p < 0.001$, * $p < 0.05$, Kruskal-Wallis test, Dunn multiple comparison test.) Spearman correlation of IL-1β production upon *S. aureus* stimulation corrected for time of blood sampling using a linear regression model (*** $p < 0.001$) (E).

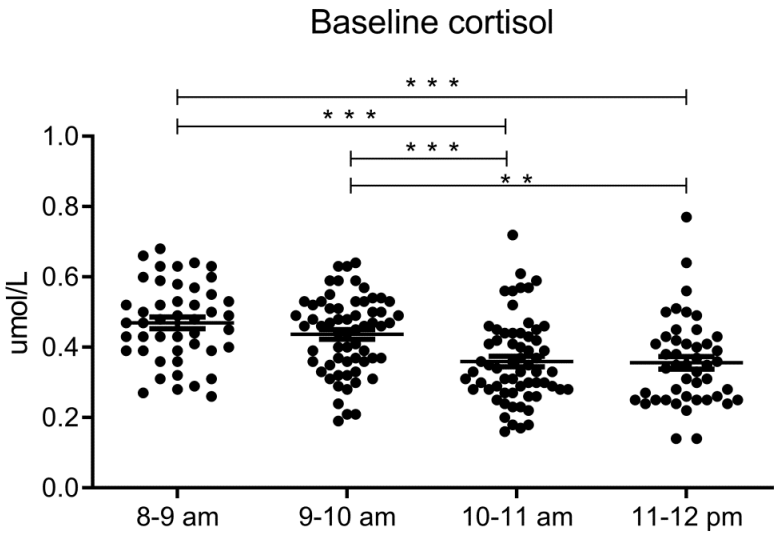
Supplementary figure 4



Supplementary figure 4

Comparisons of fold changes of IL-1β (A), IL-6 (B) and TNF-α (C) concentrations corrected for monocyte percentages within the PBMC fraction between morning vaccinated subgroups. (Mean ± SEM, n=68 vaccinated between 8am-9am, n=80 vaccinated between 9am-10am, n=84 vaccinated between 10am-11am, n=66 vaccinated between 11am-12pm, ** $p < 0.01$, * $p < 0.05$, Kruskal-Wallis test, Dunn multiple comparison test).

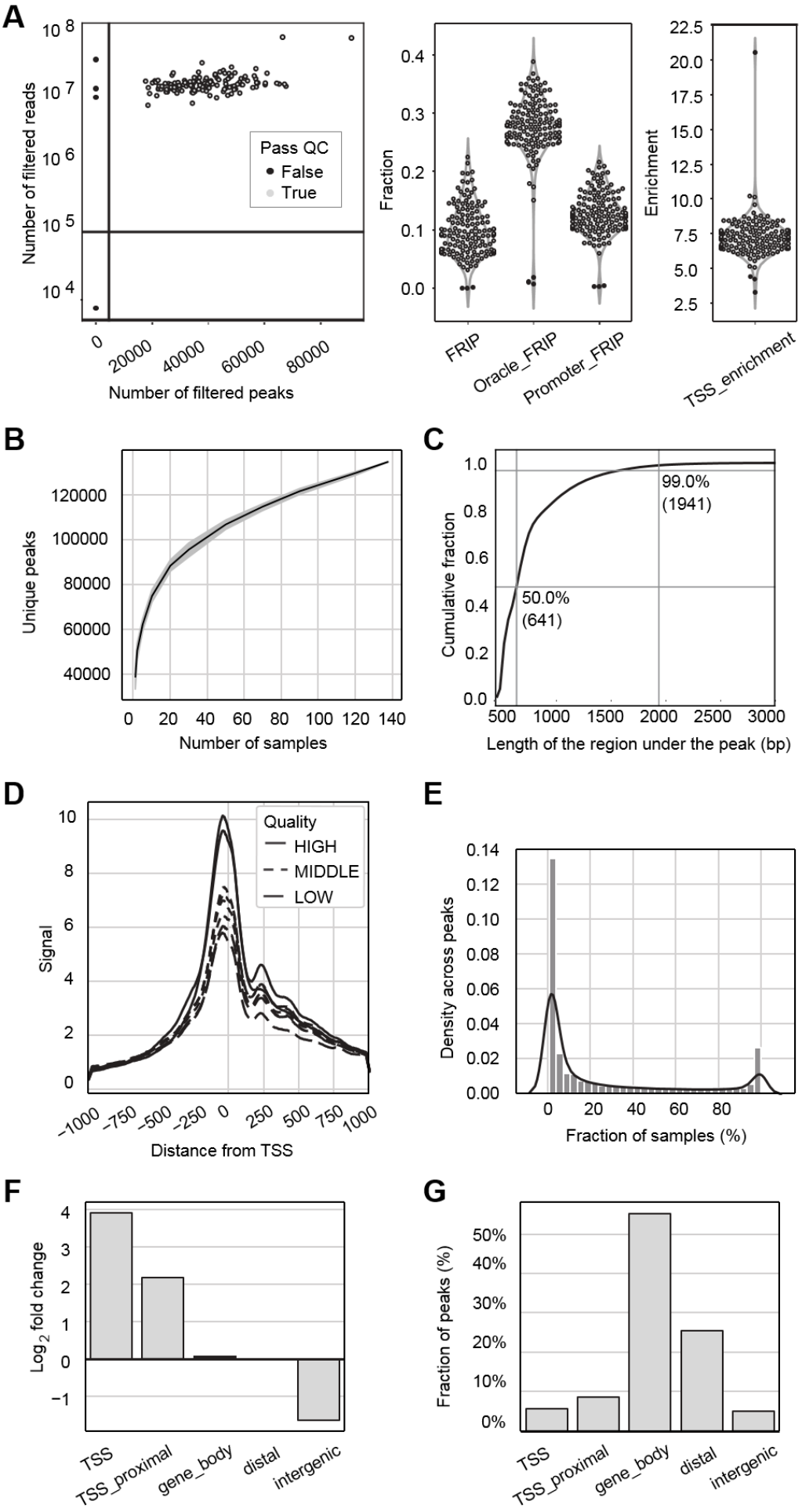
Supplementary figure 5



Supplementary figure 5

Baseline cortisol concentrations are compared between morning vaccinated subgroups (Mean ± SEM, n=68 vaccinated between 8am-9am, n=80 vaccinated between 9am-10am, n=84 vaccinated between 10am-11am, n=66 vaccinated between 11am-12pm, *** $p < 0.001$, ** $p < 0.01$, Kruskal-Wallis test, Dunn multiple comparison test).

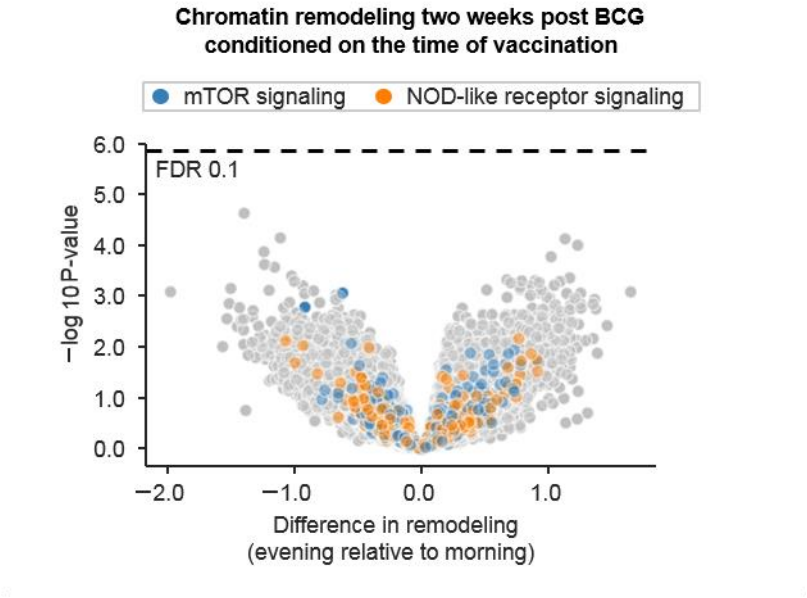
Supplementary figure 6



Supplementary figure 6

Sample quality control (QC) plots including a scatter plot of the number of filtered peaks versus the number of filtered reads (sequencing depth) with dashed lines highlighting thresholds to pass QC; and violin plots showing the distributions of QC statistics including FRIP, Oracle_FRIP, Promoter_FRIP, and TSS_enrichment (A). Saturation analysis showing the number of unique peaks detected across samples. The grey area around the curve indicates 95% confidence interval for samples added in random order (25 iterations per point) (B). Cumulative distribution of unique peaks lengths (C). Strength of ATAC-seq signal around TSSs. The plot is calculated as the height of the pileup of reads at TSSs ±1,000 bp divided by the average noise value at ±1,000 bp from the TSS. The plot shows two randomly selected HIGH quality samples (maximum value of TSS_enrichment at least 1 standard deviation above the mean), two randomly selected LOW quality samples (maximum value of TSS_enrichment at least 1 standard deviation below the mean) and four randomly selected MEDIUM quality samples. For all samples it is possible to identify a strong signal-to-noise ratio at the TSS and a characteristic pattern highlighting the first nucleosome. Overall, this is indicative of intact chromatin (D). Histogram showing the percentage of samples in which a given peak from the consensus peak set was detected (E). Peak annotation enrichment calculated as the log2 fold change against the mean of 100 comparable, randomly generated, peak sets (F). Peak annotation distribution (G).

Supplementary figure 7



Supplementary figure 7

Differential chromatin accessibility (DA) analysis of an interaction effect between BCG training (two weeks post BCG compared to baseline) and time of vaccination (evening compared to morning). (n=36 morning vaccinated, n=18 evening vaccinated; DA was performed with LIMMA which computes P-values with a moderated T-test; Benjamini-Hochberg procedure was used to control the FDR).

Table S1 participant characteristics 300BCG cohort.

	Total (n=302)
Sex, female	171 (57%)
Age, years	26 (10.8)
BMI	22.5 (2.6)
Current smoker	19 (6.2%)
BCG scar *#	272 (95%)
BCG scar size (cm) after three months *	0.42 (0.17)

Presented are characteristics of sex, age, BMI, current smoking status, and the BCG scar size measured three months after vaccination. Data are shown as n (%) or mean (±SD).
* scar sizes of 17 individuals were missing. #percentage calculated over 285 volunteers with available scar size data.

TREND Statement Checklist

Paper Section/ Topic	Item No	Descriptor	Reported? ✓	Reported? Pg #
Title and Abstract				
Title and Abstract	1	• Information on how unit were allocated to interventions	N.A.	
		• Structured abstract recommended	x	2
		• Information on target population or study sample	x	2
Introduction				
Background	2	• Scientific background and explanation of rationale	x	3-4
		• Theories used in designing behavioral interventions	N.A.	
Methods				
Participants	3	• Eligibility criteria for participants, including criteria at different levels in recruitment/sampling plan (e.g., cities, clinics, subjects)	x	15
		• Method of recruitment (e.g., referral, self-selection), including the sampling method if a systematic sampling plan was implemented	x	15
		• Recruitment setting	x	15
		• Settings and locations where the data were collected	x	15
Interventions	4	• Details of the interventions intended for each study condition and how and when they were actually administered, specifically including:		
		○ Content: what was given?	x	15
		○ Delivery method: how was the content given?	x	15
		○ Unit of delivery: how were the subjects grouped during delivery?	N.A.	
		○ Deliverer: who delivered the intervention?	x	15
		○ Setting: where was the intervention delivered?	x	15
		○ Exposure quantity and duration: how many sessions or episodes or events were intended to be delivered? How long were they intended to last?	x	15
		○ Time span: how long was it intended to take to deliver the intervention to each unit?	x	15
		○ Activities to increase compliance or adherence (e.g., incentives)	x	15
Objectives	5	• Specific objectives and hypotheses	x	4
Outcomes	6	• Clearly defined primary and secondary outcome measures	N.A.	
		• Methods used to collect data and any methods used to enhance the quality of measurements	x	15-21
		• Information on validated instruments such as psychometric and biometric properties	x	15-21
Sample Size	7	• How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules	x	15
Assignment Method	8	• Unit of assignment (the unit being assigned to study condition, e.g., individual, group, community)	N.A.	
		• Method used to assign units to study conditions, including details of any restriction (e.g., blocking, stratification, minimization)	N.A.	
		• Inclusion of aspects employed to help minimize potential bias induced due to non-randomization (e.g., matching)	N.A.	

TREND Statement Checklist

Blinding (masking)	9	• Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to study condition assignment; if so, statement regarding how the blinding was accomplished and how it was assessed.	N.A.	
Unit of Analysis	10	• Description of the smallest unit that is being analyzed to assess intervention effects (e.g., individual, group, or community)	x	15-21
		• If the unit of analysis differs from the unit of assignment, the analytical method used to account for this (e.g., adjusting the standard error estimates by the design effect or using multilevel analysis)	x	15-21
Statistical Methods	11	• Statistical methods used to compare study groups for primary methods outcome(s), including complex methods of correlated data	x	15-21
		• Statistical methods used for additional analyses, such as a subgroup analyses and adjusted analysis	x	15-21
		• Methods for imputing missing data, if used	x	15-21
		• Statistical software or programs used	x	15-21
Results				
Participant flow	12	• Flow of participants through each stage of the study: enrollment, assignment, allocation, and intervention exposure, follow-up, analysis (a diagram is strongly recommended)	x	Fig. 1A
		○ Enrollment: the numbers of participants screened for eligibility, found to be eligible or not eligible, declined to be enrolled, and enrolled in the study	x	Fig. 1A
		○ Assignment: the numbers of participants assigned to a study condition	x	Fig. 1A
		○ Allocation and intervention exposure: the number of participants assigned to each study condition and the number of participants who received each intervention	x	Fig. 1A
		○ Follow-up: the number of participants who completed the follow-up or did not complete the follow-up (i.e., lost to follow-up), by study condition	x	15 Fig. 1A
		○ Analysis: the number of participants included in or excluded from the main analysis, by study condition	x	15 Fig. 1A
		• Description of protocol deviations from study as planned, along with reasons	N.A.	
Recruitment	13	• Dates defining the periods of recruitment and follow-up	x	15
Baseline Data	14	• Baseline demographic and clinical characteristics of participants in each study condition	x	Fig. 2B 15
		• Baseline characteristics for each study condition relevant to specific disease prevention research	N.A.	
		• Baseline comparisons of those lost to follow-up and those retained, overall and by study condition	N.A.	
		• Comparison between study population at baseline and target population of interest	N.A.	
Baseline equivalence	15	• Data on study group equivalence at baseline and statistical methods used to control for baseline differences	N.A.	

TREND Statement Checklist

Numbers analyzed	16	• Number of participants (denominator) included in each analysis for each study condition, particularly when the denominators change for different outcomes; statement of the results in absolute numbers when feasible	x	5-9 (Fig. legends)
		• Indication of whether the analysis strategy was "intention to treat" or, if not, description of how non-compliers were treated in the analyses	N.A.	
Outcomes and estimation	17	• For each primary and secondary outcome, a summary of results for each estimation study condition, and the estimated effect size and a confidence interval to indicate the precision	N.A.	
		• Inclusion of null and negative findings	x	5-9 + 15-21
		• Inclusion of results from testing pre-specified causal pathways through which the intervention was intended to operate, if any	N.A.	
Ancillary analyses	18	• Summary of other analyses performed, including subgroup or restricted analyses, indicating which are pre-specified or exploratory	x	5-9
Adverse events	19	• Summary of all important adverse events or unintended effects in each study condition (including summary measures, effect size estimates, and confidence intervals)	N.A.	
DISCUSSION				
Interpretation	20	• Interpretation of the results, taking into account study hypotheses, sources of potential bias, imprecision of measures, multiplicative analyses, and other limitations or weaknesses of the study	x	10-14
		• Discussion of results taking into account the mechanism by which the intervention was intended to work (causal pathways) or alternative mechanisms or explanations	x	10-14
		• Discussion of the success of and barriers to implementing the intervention, fidelity of implementation	x	10-14
		• Discussion of research, programmatic, or policy implications	x	10-14
Generalizability	21	• Generalizability (external validity) of the trial findings, taking into account the study population, the characteristics of the intervention, length of follow-up, incentives, compliance rates, specific sites/settings involved in the study, and other contextual issues	x	10-14
Overall Evidence	22	• General interpretation of the results in the context of current evidence and current theory	x	10-14

From: Des Jarlais, D. C., Lyles, C., Crepaz, N., & the Trend Group (2004). Improving the reporting quality of nonrandomized evaluations of behavioral and public health interventions: The TREND statement. *American Journal of Public Health*, 94, 361-366. For more information, visit: http://www.cdc.gov/trendstatement/