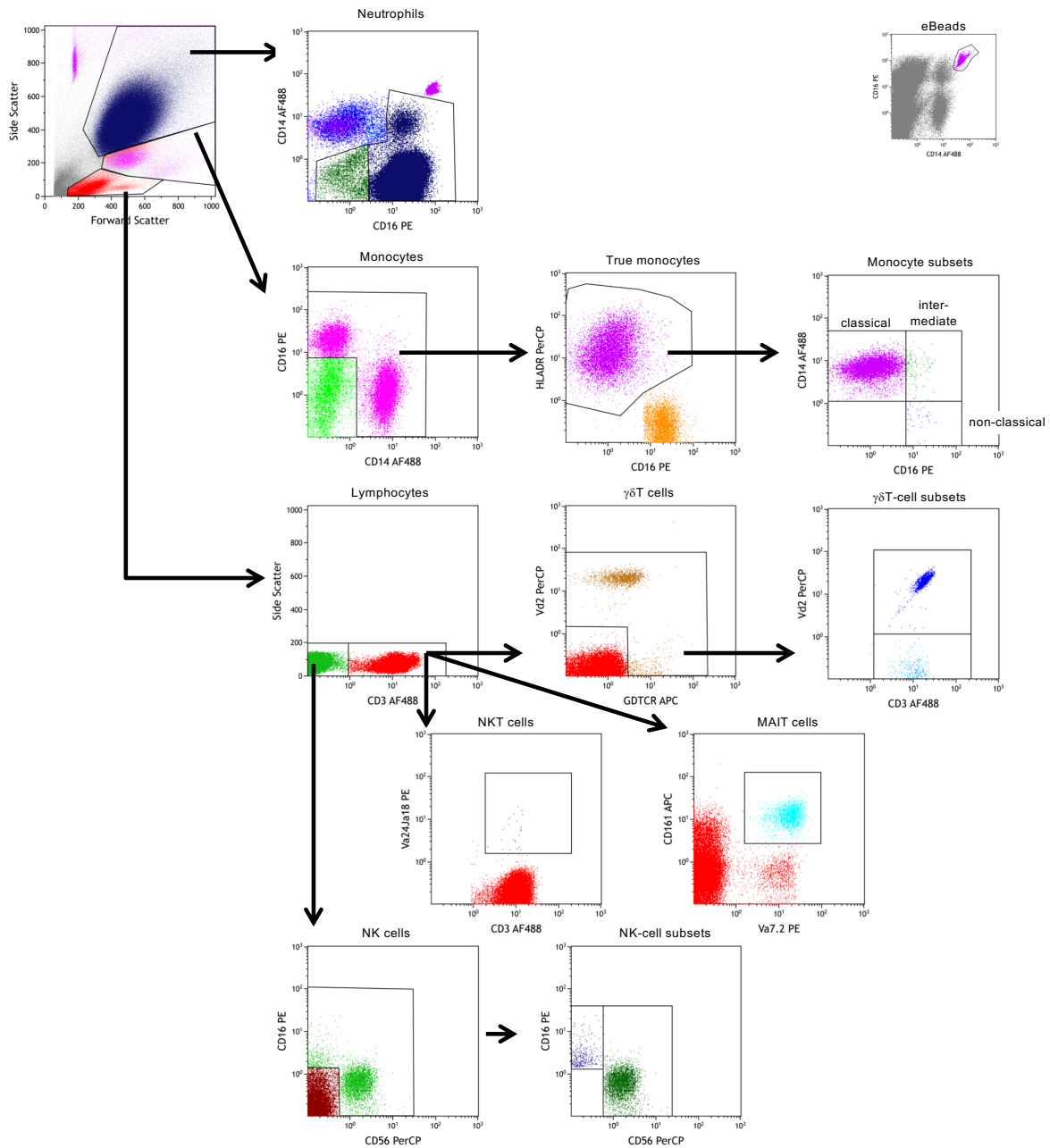Supplementary figures to

"Immune cell characteristics and cytokine responses in adult HIV-negative

*tuberculous meningitis: an observational cohort study"*

Arjan van Laarhoven, Sofiati Dian, Suzanne van Dorp, Feby Purnama, Valerie ACM Koeken,

Emira Diandini, Fitria Utami, Resvi Livia, Lika Apriani, Edwin Ardiansyah, Rob ter Horst,

Mihai G Netea, Tri Hanggono Achmad, Philip C Hill, Rovina Ruslami, Bachti Alisjahbana,

James E Ussher, Agnes Indrati, Ayesha Verrall, Ahmad Rizal Ganiem, Reinout van Crevel

Supplementary figure 1 (A) flow cytometry gating strategy. αβT cells are calculated by subtracting NKT cells, γδT cells and MAIT cells from the total number of CD3+ lymphocytes.
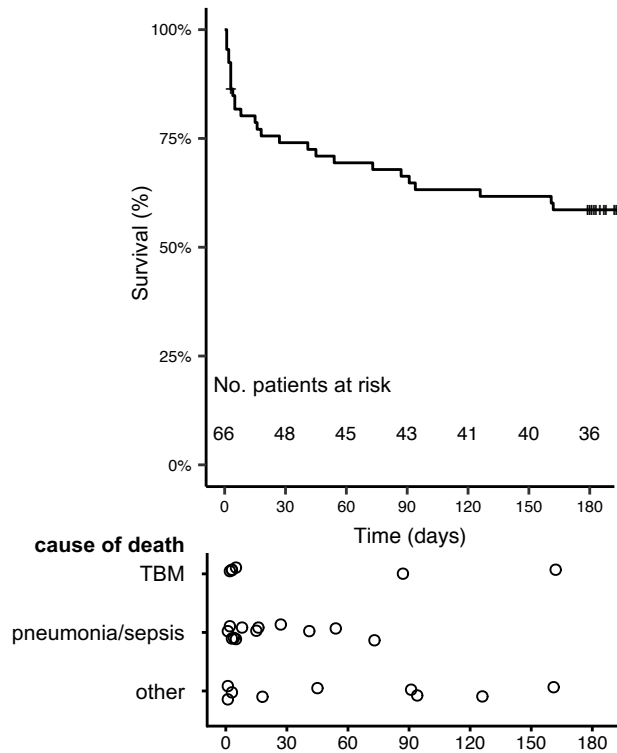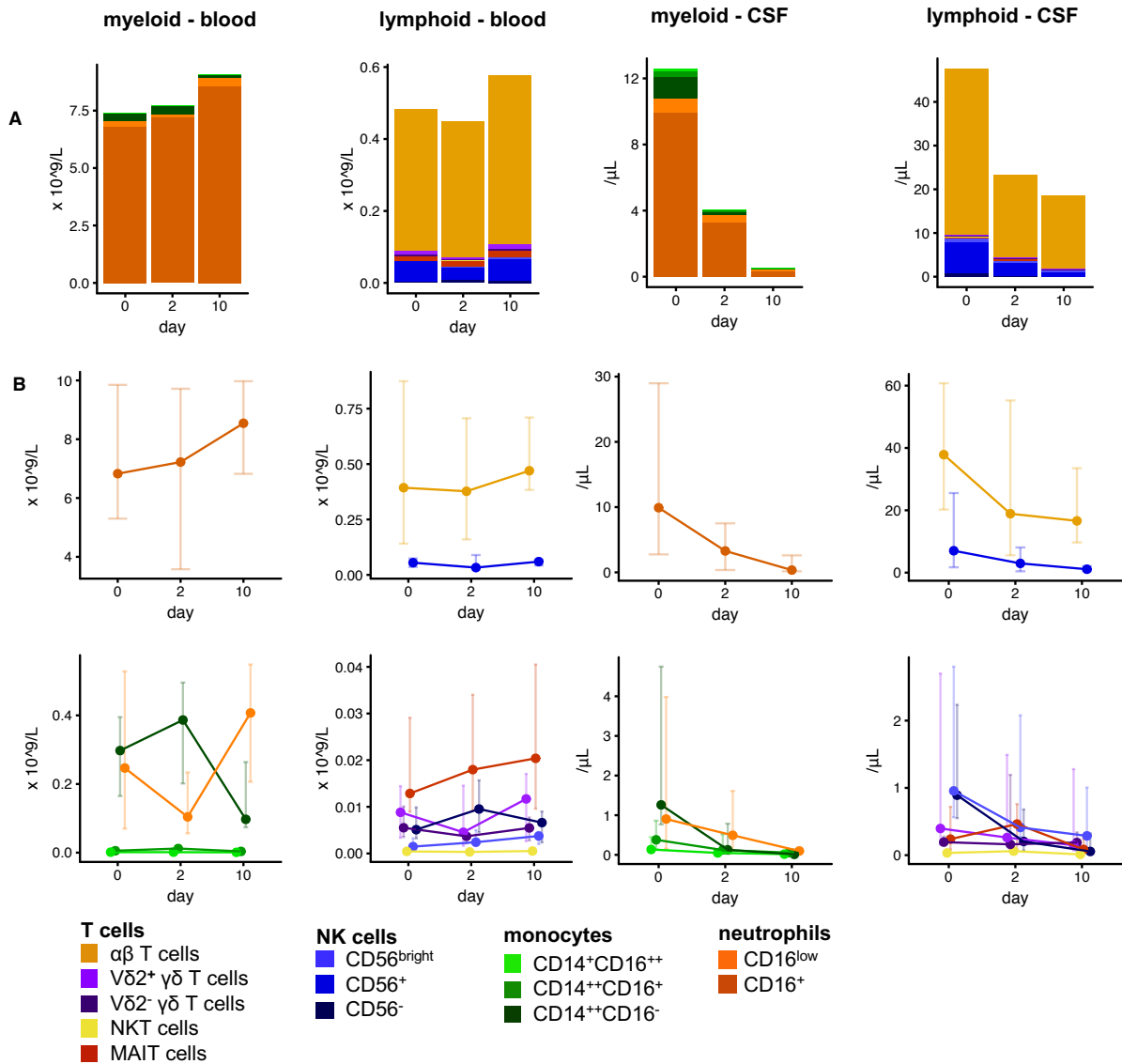
(B) Mortality of tuberculous meningitis patients in the first 180 days of follow-up. Outcome data were missing for one patient.

Presumptive cause of death is depicted below the Kaplan-Meier graph in three categories: 1) tuberculous meningitis-related

causes: 4 because of increased intracranial pressure, 2 because of a paradoxical reaction, 2) pneumonia (8) or sepsis (4), and

3) miscellaneous: 3 aspiration pneumonia, 3 suspected cardiac arrest or pulmonary embolism, 1 head injury, 1 metabolic

encephalopathy and 1 with unknown cause.
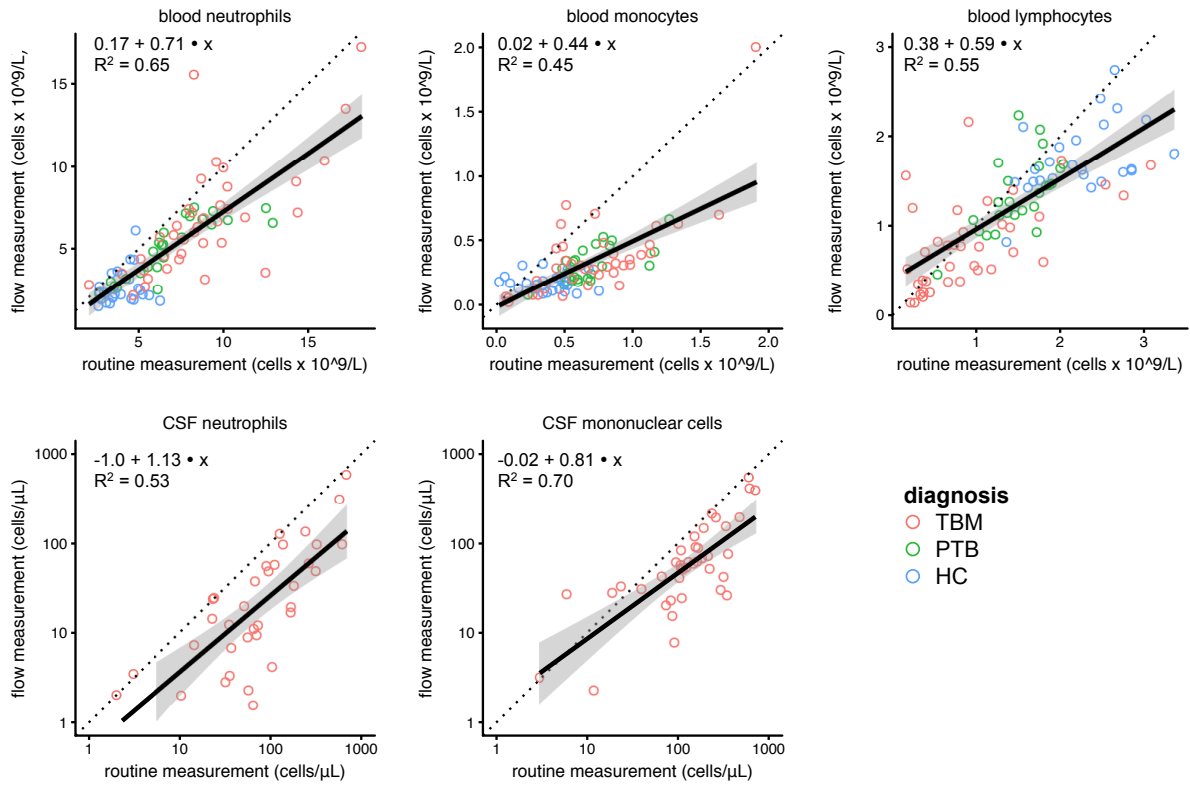
(C) Regression lines with 95% confidence intervals for the correlation between cell counts as measured routinely (x-axis), and with flow cytometry (y-axis) for blood and CSF. Regression formulas and regression coefficients (R^2) are printed on the graphs.

Note: CSF cells are plotted on a ^{10}log-axis and regression coefficients are ^{10}log-transformed.

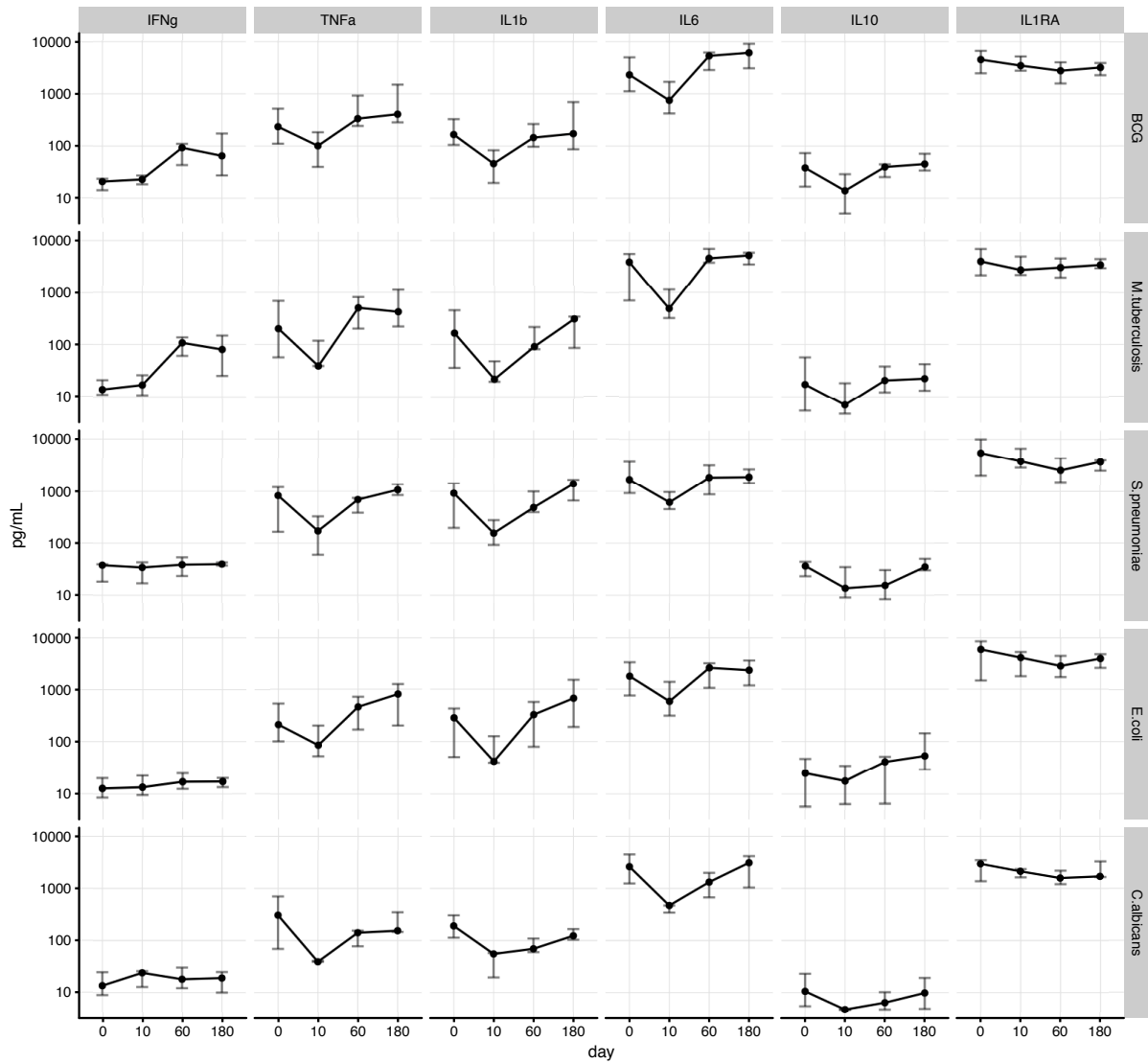**Supplementary figure 2** Flow cytometry follow-up study results of tuberculous meningitis patients who survived past day 10 (n = 15). Results show median (A) or median with interquartile range (B) for day 0, 2 and 10 for myeloid and lymphoid cell types separately in blood (left) and CSF (right). Note the different y-axis limits. Data is ≥ 80% complete for all time-points for blood and is ≥ 87% complete for CSF.
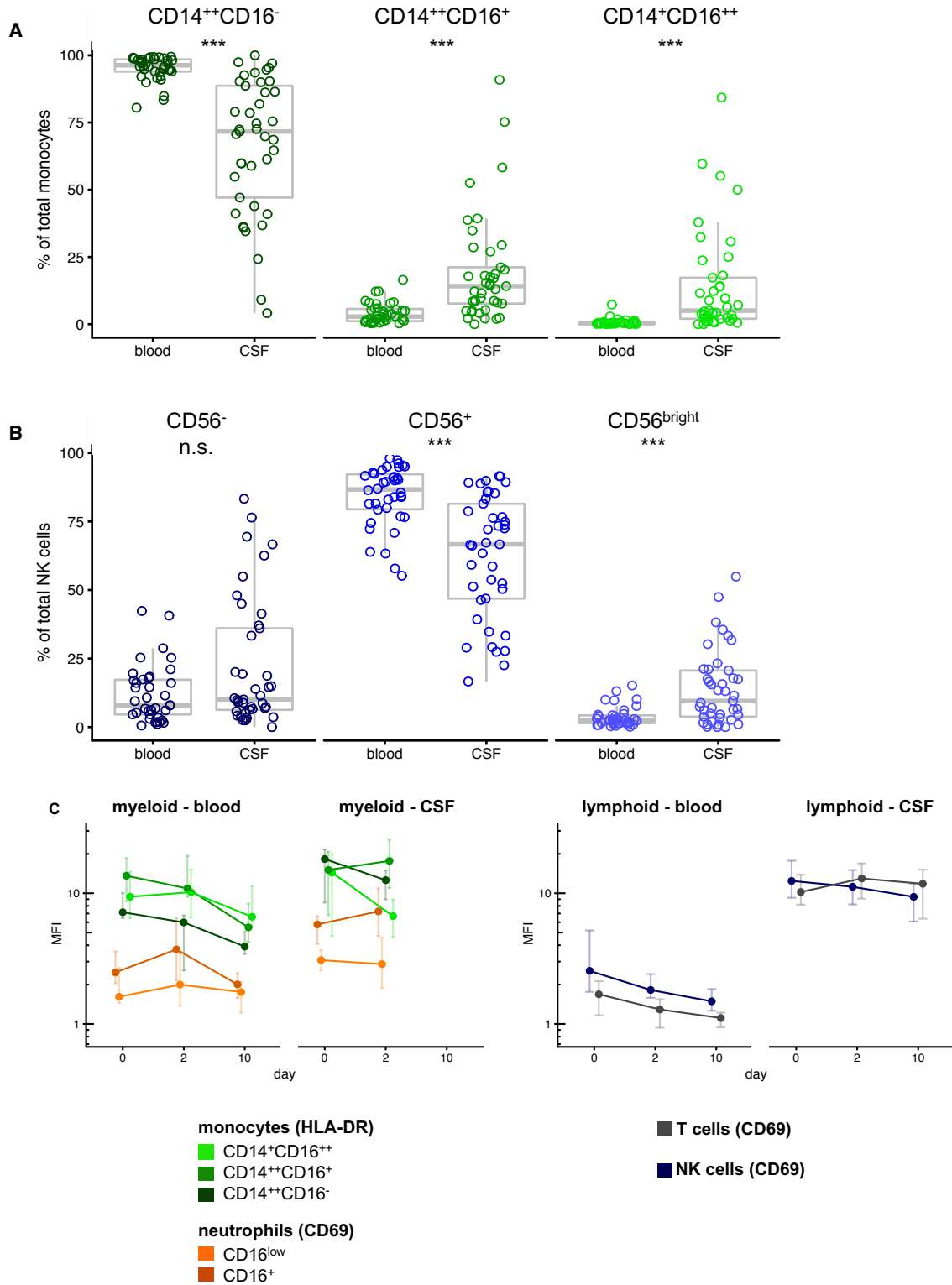
Supplementary figure 3 Flow cytometry results of proportions of monocyte (A) and NK cell (B) subtypes in blood versus CSF of tuberculous meningitis patients. *** p<0.001. (C) Activation marker expression by indicated cell populations during patient follow-up, all of whom survived past day 10 (n = 15). Data is ≥ 80% complete for all time-points for CSF and blood. Note: day 10 myeloid activation markers in CSF could not reliably be measured because of the low cell counts.
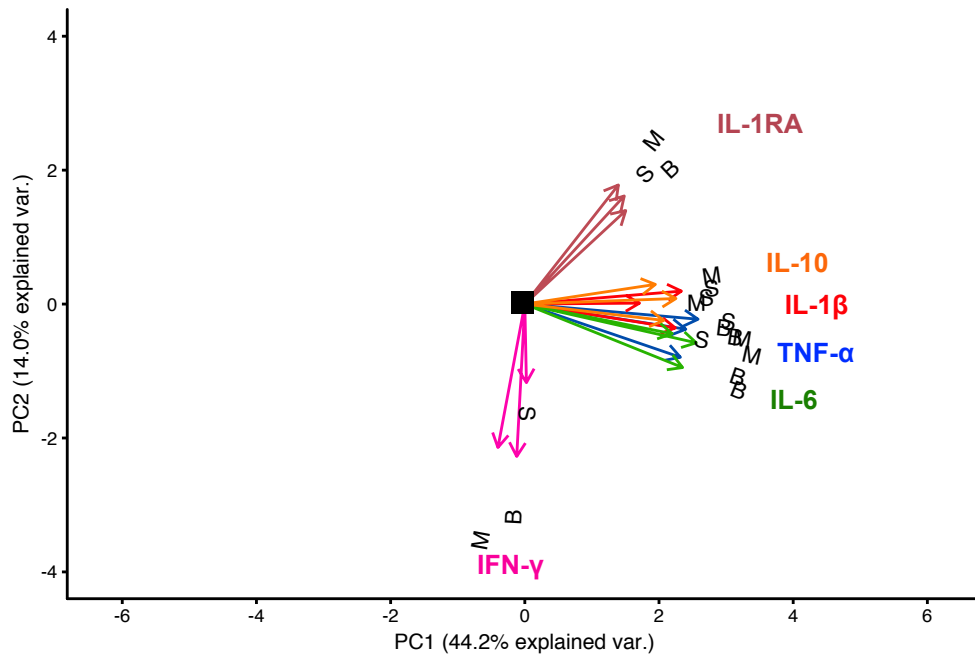
Supplementary figure 4 (A) Loadings for principal component analysis on ex-vivo cytokine data. B = BCG, M = *M. tuberculosis* and S = *S. pneumoniae*.

(B) Ex-vivo whole blood cytokine results for tuberculous meningitis patients in the follow-up study who survived past day 180

(n = 15). Results show median with interquartile range for all five stimuli (ordered vertically) for all six measured cytokines

(horizontally). Patients were selected who had complete data at day 0 and missed maximally one of the follow-up time-points.

Data is 93% complete for day 10, 73% for day 60 and 53% for day 180. Missing data were due to technical errors.
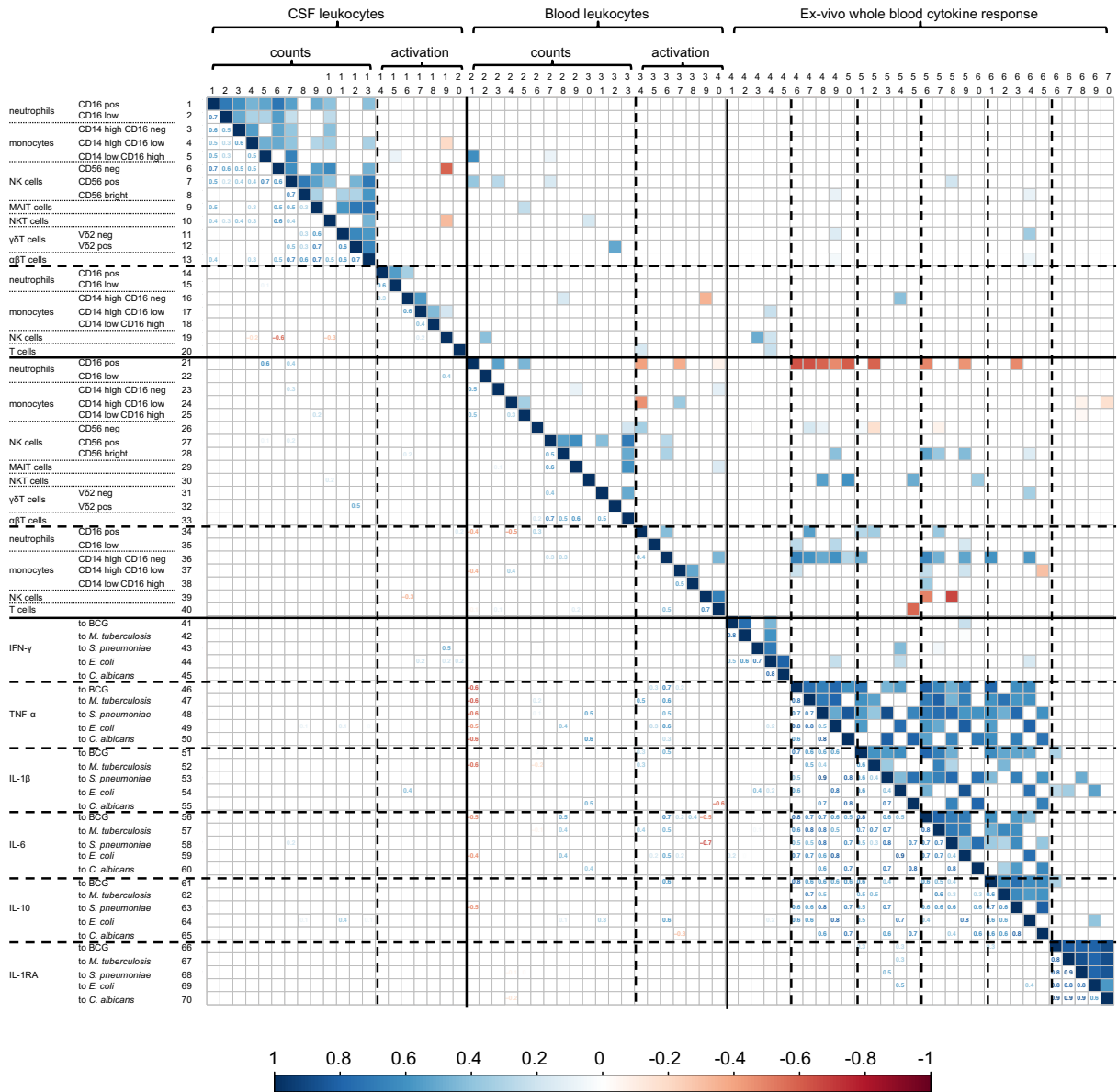
Supplementary figure 5 Correlation matrix for tuberculous meningitis patient CSF and blood cell counts and activation markers,

as well as blood ex-vivo whole blood response using Spearman correlation. Pair-wise complete data was included.

Supplementary table

Supplementary table 1: Clinical, cerebrospinal fluid and blood parameters of tuberculous meningitis patients

Variable	n/N or median	% or IQR
Clinical features		
Sex	37/67	(56%)
Age	27	(21–38)
Duration of main complaint	4	(2–7)
Duration of the first neurological symptom	14	(7–21)
Tuberculous meningitis grade		
I	2/67	(3%)
II	58/67	(87%)
III	7/67	(10%)
Temperature, °C	37.8	(37.0–38.2)
Glasgow Coma Scale score	13	(11–14)
Seizures present	1/66	(2%)
Motor abnormalities present	38/66	(57%)
Cranial nerve palsy present	51/66	(6%)
Abnormal chest radiograph findings	50/67	(75%)
Cerebrospinal fluid		
CSF to blood glucose ratio	0.14	(0.09–0.20)
Protein, mg/dL	221	(150–386)
Culture, % positive	46/67	(69%)
Blood		
Haemoglobin, g/dL	12.5	(10.5–13.8)
Thrombocytes, x 10^9/L	298	(234–379)
Outcomes		
Length of hospital stay in days	21	(12,23)
Alive at discharge	51/66	(76%)
Outcome at day 180		
Alive	39/71	(58%)
Deceased	27/67	(40%)
Lost to follow-up	1/67	(2%)

Continuous variables are presented as median with interquartile range and categorical variables as number and proportion.

IQR = interquartile range. Additional routine inflammatory markers can be found in Table 1.