nature portfolio

https://doi.org/10.1038/s41586-025-08796-4

Supplementary information

Bifidobacteria support optimal infant vaccine responses

In the format provided by the authors and unedited

Supplementary Information for Ryan *et al.* 2025 "*Bifidobacteria support optimal infant vaccine responses*".

Supplementary Discussion

Our data extend upon several important previous studies that have investigated the impact of antibiotics on vaccine responses. In adults, for example, participants that were randomised to antibiotics prior to immunisation with trivalent influenza vaccine had significantly impaired neutralising and binding antibody titres, however, this was not observed in adults with pre-existing immunity to influenza[11] and in a separate study, oral rotavirus vaccine immunogenicity was not found to be reduced in adults randomised to antibiotics[12]. The data are becoming clearer in infants, where two important studies have found that cumulative antibiotic exposure over the first year of life is associated with an impaired response to vaccination[2-4]. Interestingly, we did not detect an effect of antibiotic exposure on serum IgA responses to the oral rotavirus vaccine. This contrasts with a recent study assessing rotavirus seropositivity at 7 months of age in Brazil, Peru, and South Africa, which found that the probability of seropositivity was 40% higher among children who had at least one course of antibiotics[70]. This previous study did consider a longer period of antibiotic exposure than in our study, which could explain the discrepancy between these studies. Future research should assess anti-rotavirus vaccine IgA responses at mucosal surfaces, which may be more relevant to protective immunity.

The antibiotics administered directly to neonates in our study were predominantly a combination of benzylpenicillin and gentamicin. This combination targets both gram positive and gram negative organisms commonly associated with neonatal sepsis, although it is important to note that none of the infants in our study were subsequently confirmed to have sepsis (confirmed infant sepsis was an exclusion criterion). Given that neonatal sepsis is a life-threatening condition that necessitates rapid intervention, reducing antibiotic usage in this

context may be challenging. Although clinical sepsis risk calculators are proving beneficial[71], other interventions will be needed to mitigate any deleterious effects of neonatal antibiotics on the microbiota and developing immune system where antibiotic exposure cannot be avoided. An important implication of our study therefore is that administering probiotics that contain *Bifidobacterium* species to infants exposed to neonatal antibiotics may be a feasible, cheap, and safe intervention to enhance responses to vaccination in these infants. The use of probiotics to enhance vaccine responses has been investigated previously, however, only ~50% of these RCTs reporting a beneficial effect[72]. The RCTs conducted to date have been small and have investigated many different probiotics. Most importantly, none of the trials to date specifically recruited participants with a disrupted microbiota. We think it unlikely that administering probiotics to infants with an already healthy gut microbiota would have a beneficial effect on vaccine responses. Instead, future work aiming to utilise probiotics to improve vaccine responses should carefully consider the inclusion criteria, the choice of probiotic, the timing of the administration of the probiotic and the availability of human milk oligosaccharides (HMOs) or other prebiotics to nourish key species.

Interestingly, intrapartum antibiotic exposure was not associated with reduced antibody responses to vaccination in our study. We did, however, find that intrapartum antibiotic exposure was associated with significant changes to the composition, predicted functional capacity and antimicrobial resistance profile of the gut microbiota in the first week of life. We also identified an association between intrapartum antibiotic exposure and altered transcriptional profiles and the frequency of $CD45RA^-$ regulatory T cells in blood. Intrapartum antibiotic exposure has also been associated with poorer health outcomes in other contexts[73-75]. Additionally, comparatively less attention has been paid to the effects that postpartum antibiotic exposure via breastfeeding might have on the developing microbiota and immune system[76]; our finding that PN-ABX infants had significantly impaired IgG responses against

several serotypes in the PCV13 vaccine at either 7 or 15 months, suggests the need for further research in this area.

A unique aspect of our study was the implementation of an extensive metagenomic, transcriptomic, serological and cellular assessment of longitudinally collected stool and blood samples. RNA sequencing revealed subtle but coordinated changes in the baseline transcriptome in the blood of antibiotic exposed infants including the increased activity of multiple inflammation-related transcriptional modules pre-vaccination in infants exposed directly to antibiotics in the neonatal period. Several of these modules were correlated with subsequent responses to vaccination suggesting that altered immune status pre-vaccination may, at least in part, explain why infants exposed directly to antibiotics in the neonatal period had suboptimal responses to vaccination.

Additional References:

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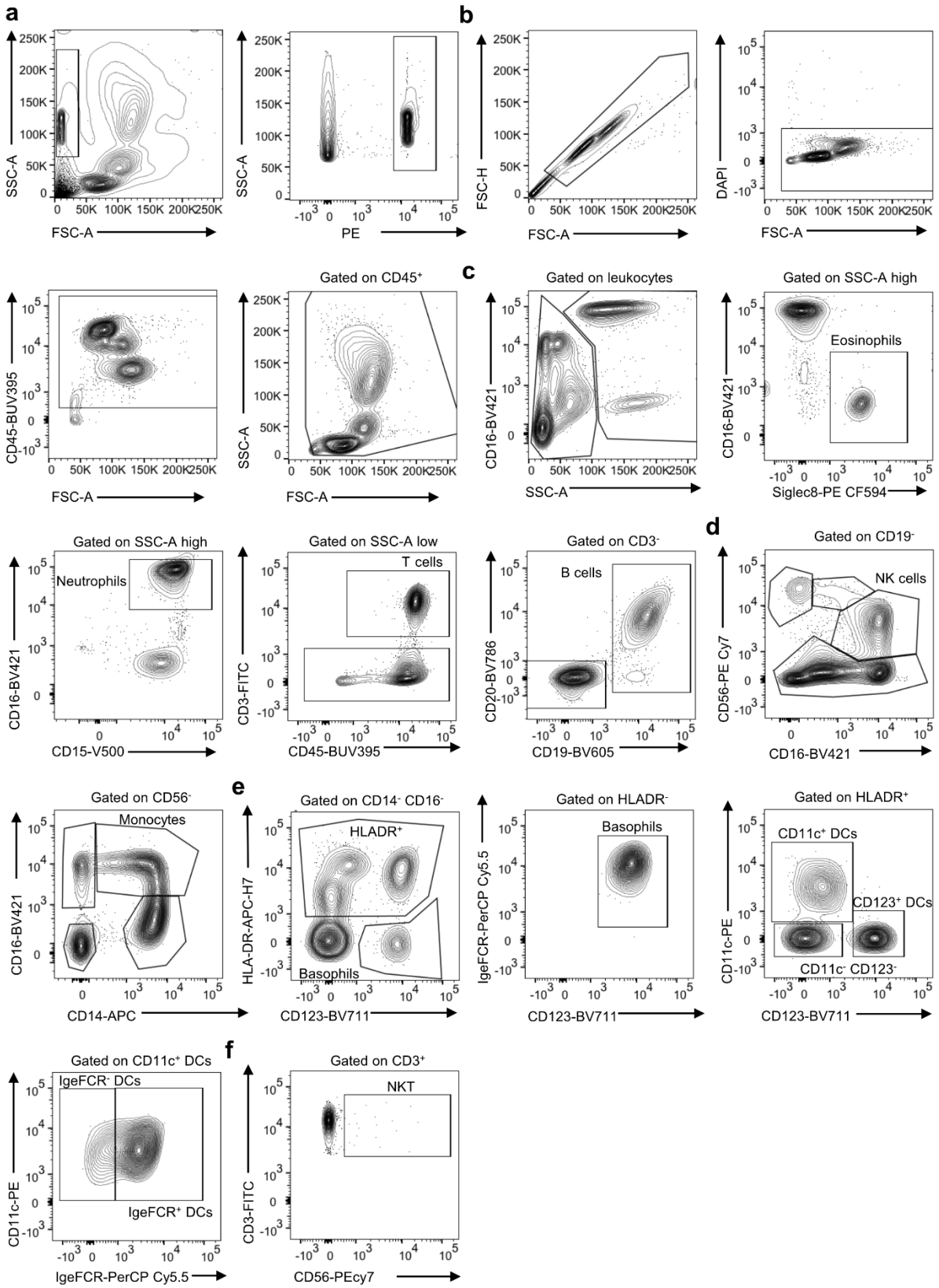
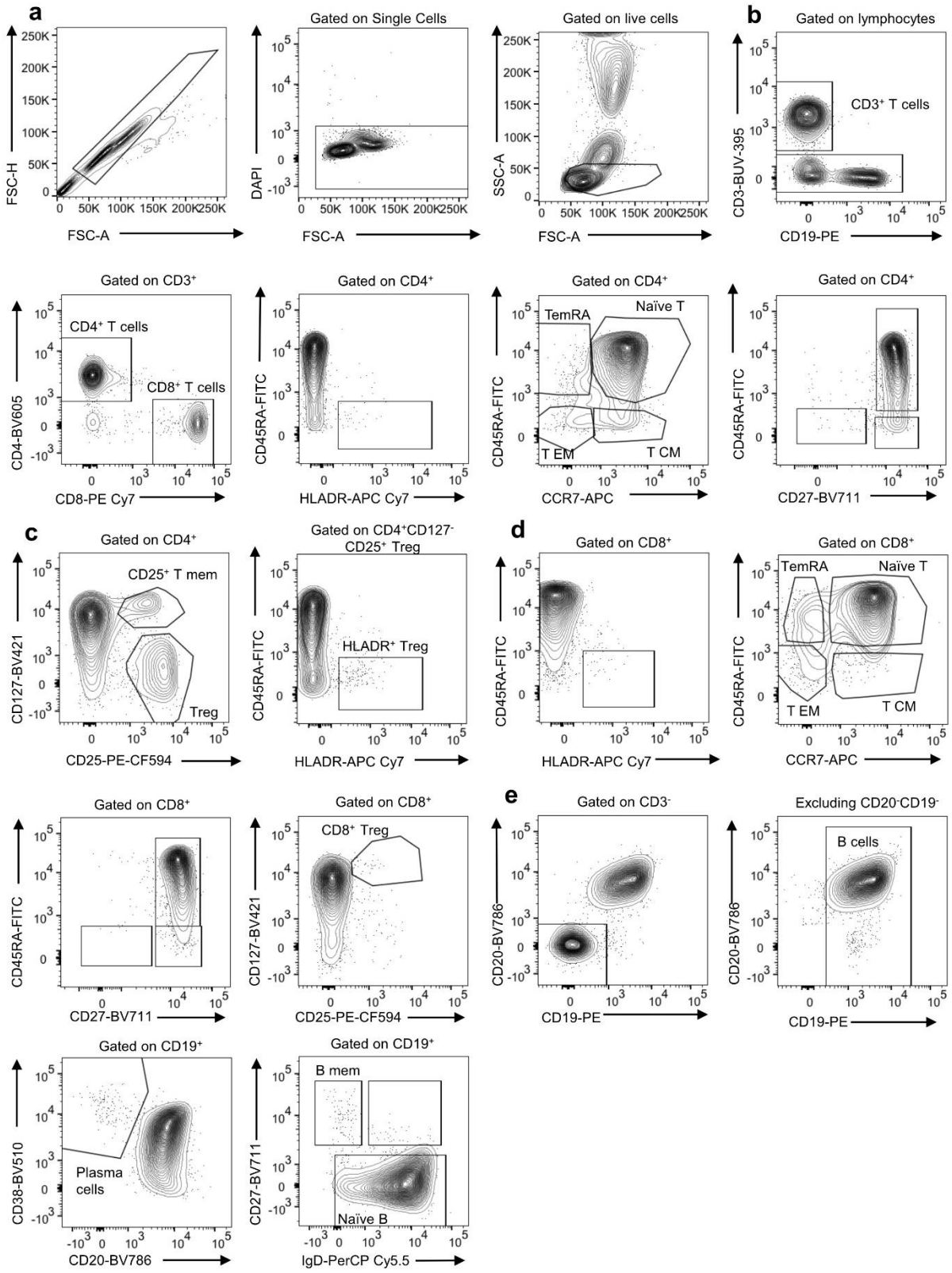


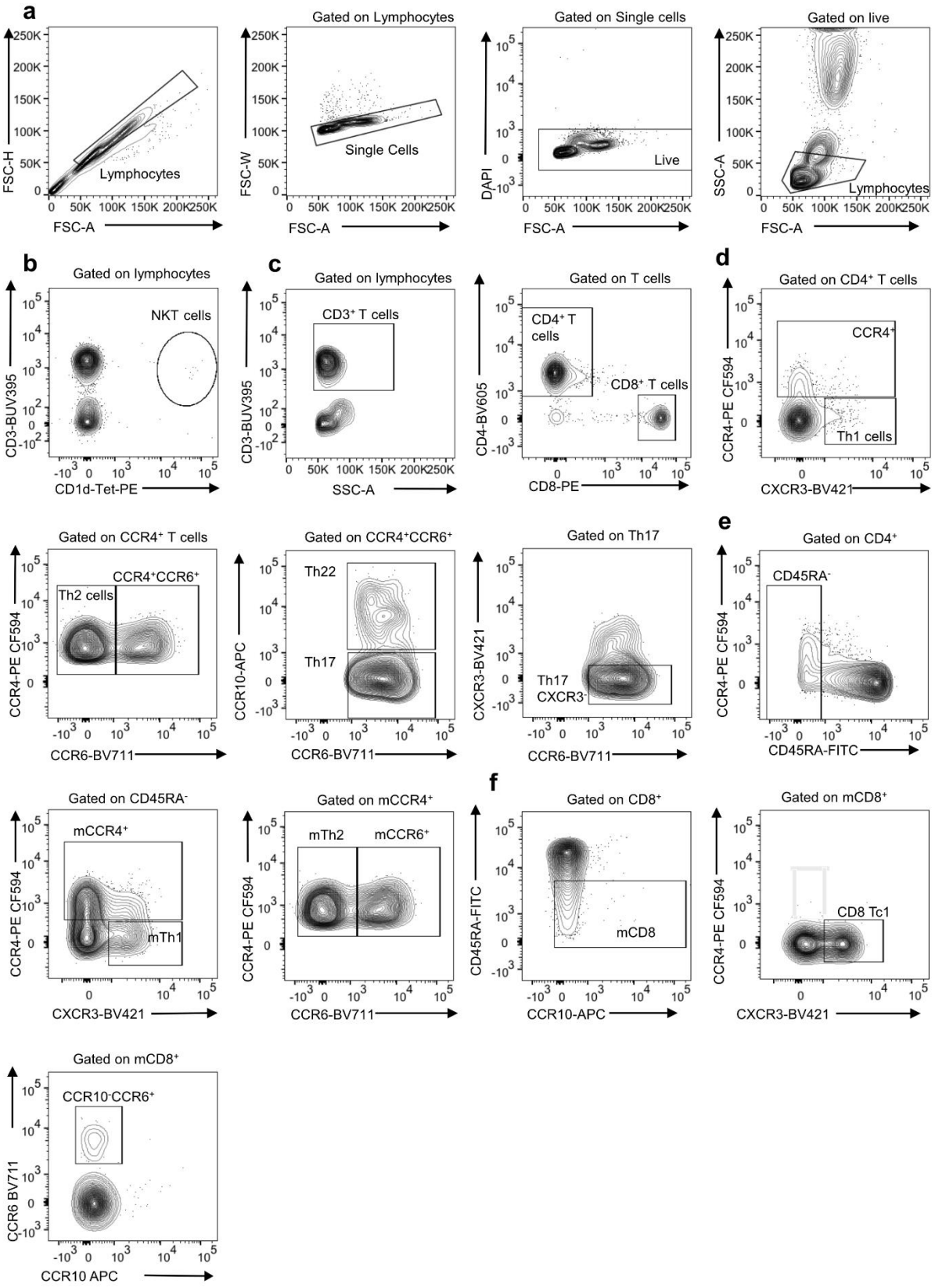
Supplementary Fig 1. Flow cytometry analysis representative gating strategy for the pan-leukocyte panel. (a) Beads from each Trucount tube were recorded from FSCxSSC and then PE^{hi}. **(b)** For all analyses, cells were gated by singlet discrimination, followed by live, CD45^{+} and leukocytes. (**c)** Leukocytes were split into SSC high and low populations. Eosinophils and neutrophils were gated on SSC low and high populations, respectively. T cells were gated on

SSC low $CD3^{hi}$ and $CD3^{-}$ cells were further defined as $CD19^{+}CD20^{+}$ B cells. (**d)** The $CD19^{-}$ population was gated on to define $CD16^{-}CD56^{+}$ NK cells. $CD56^{-}$ cells were further gated on CD16 and CD14 expression to identify monocyte populations. (**e)** Remaining $CD14^{-}CD16^{-}$ cells were further gated on HLA-DR and CD123 expression to identify $CD123^{+}$HLA-DR^{-} Basophils. HLA-DR^{+} cells were gated on CD11c and CD123 in order to identify various DC populations. (**f)** $CD3^{+}$ T cells were gated further on their CD56 expression to identify $CD56^{+}CD3^{+}$ NKT-like cells.



Supplementary Fig. 2. Flow cytometry analysis representative gating strategy for the B and T cell panel. (a) For all analyses, cells were gated by singlet discrimination, live and for leukocytes using SSCxFSC. **(b)** $CD3^+$ T cells were gated on $CD4^+$ and $CD8^+$ expression. $CD4^+$ T cells were then defined as $CD45RA^-HLA\text{-}DR^+$ $CD4^+$ T cells, $CCR7^+CD45RA^+$ Naïve $CD4^+$ T cells, $CCR7^+CD45RA^-$ $CD4^+$ central memory T cells, $CCR7^-CD45RA^-$ CD4 effector memory T cells. **(c)** $CD4^+$ T cells were also gated on CD127 and CD25 expression to identify

$CD127^{-}CD25^{+}$ Tregs, which were further gated for CD45RA and HLA-DR expression to identify $CD45RA^{-}HLADR^{+}$ Tregs. **(d)** $CD8^{+}$ T cells were then defined as $CD45RA^{-}HLADR^{+}$ $CD8^{+}$ T cells, $CCR7^{+}CD45RA^{+}$ naïve $CD8^{+}$ T cells (naïve T cells), $CCR7^{+}CD45RA^{-}$ $CD8^{+}$ central memory T cells (Tcm), and $CCR7^{-}CD45RA^{-}$ CD8 effector memory T cells (Tem). **(e)** The $CD3^{-}$ population from **b** was gated further on CD20 and CD19 so that $CD20^{-}CD19^{-}$ cells could be gated out and $CD19^{+}$ B cells could then be gated for. These were further divided into a CD38 and CD20 gate to identify $CD38^{+}CD20^{-}$ plasma cells and an IgD and CD27 gate to identify $IgD^{-}CD27^{+}$ Memory B cells and IgD^{+} naïve B cells.



Supplementary Fig. 3. **Flow cytometry analysis representative gating strategy for the extended B and T cell panel**. **(a)** Cells were gated by singlet discrimination, live cells and for lymphocytes using SSCxFSC. **(b)** NKT cells were identified using a tetramer specific for CD1d. **(c)** $CD3^+$ T cells were gated on $CD4^+$ and $CD8^+$ expression. **(d)** $CD4^+$ T cells were then defined as $CCR4^+$ $CCR3^+$ Th1 cells and $CCR4^+$ CD4 T cells. This $CCR4^+$ gate was used to

define $CCR4^{+}CCR6^{-}$ Th2 cells and $CCR4^{+}CCR6^{+}$ cells. The $CCR4^{+}CCR6^{+}$ cells were further gated to identify $CCR10^{+}CCR6^{+}$ Th22 cells and $CCR10^{-}CCR6^{+}$ Th17 cells. Th17 cells could be further distinguished by identifying the $CXCR3^{-}$ population of Th17 cells. **(e)** $CD45RA^{-}$ T cells were identified using the $CD4^{+}$ T cell gate. This $CD45RA^{-}$ T cell gate was used to identify $CCR4^{-}CXCR3^{+}$ Th1 memory cells (mTh1) and a $CCR4^{+}$ memory population that was further gated to identify $CCR4^{+}CCR6^{-}$ Th2 memory cells (mTh2). (**f)** The $CD8^{+}$ T cell gate was further gated on the $CD45RA^{-}$ population to identify $CD8^{+}$ memory T cells (mCD8). This population was further gated to identify $CCR10^{+}CCR4^{-}$ $CD8^{+}$ mTc1 cells. Finally, the mCD8 population was also gated to identify a $CCR6^{+}CCR10^{-}$ population.