Supplemental Methods

Preparation of human tissues

Tonsil and spleen specimens were obtained through an institutional arrangement with the National Disease Research Interchange, NDRI. Upon receipt, tonsil or spleen biopsies were placed in a 100 mm sterile petri dish containing 10 mL of sterile medium and minced using sterile scissors and forceps. The cell suspension and medium was passed through a 40 μm sterile strainer (BD Falcon) into a 50 mL sterile conical tube to eliminate large debris from the cell suspension. The minced tissue was rinsed one to three times (until the medium did not appear cloudy) with 10 mL of medium to recover single cells. After each wash, the medium and cell suspension was passed through the strainer and collected into a 50 mL conical tube. The cell suspension was centrifuged for 5 minutes at 400-600xG. Cells were treated with 10- 35 mL of sterile ammonium chloride solution (0.8% NH_4Cl with 0.1 mM EDTA, StemCell Technologies, or ACK lysing buffer, ThermoFisher) for 10-15 minutes at room temperature to remove erythrocytes, followed by neutralization with 15 ml of complete medium. The pellet was finally variously re-suspended at 1-100 $x10^6$ cells/mL using FACS-buffer depending on experimental requirements.

Human blood was collected from healthy donors according to the MedImmune institutional Research Specimen Collection Program and informed consent policy. 4-5 tubes of blood per donor were collected in Vacutainer CPT™ (BD Biosciences); peripheral blood mononuclear cells (PBMC) were obtained by spinning tubes at 1700xG for 25 minutes. Plasma was aspirated off and the PBMC monolayer was gently collected into a separate 50ml conical tube. Cells were washed with FACS buffer and counted. If needed, cells were lysed to removed erythrocytes as described above. After counting cells were variously suspended at 1-100$x10^6$ per mL.

Flow Cytometry Immunophenotyping

All antibodies against cell-surface antigens (CD20-BV421 clone 2H7 and CD27-BV786 clone O323 from BioLegend, CD38-BUV395 clone HB7, CD19-APC clone SJ25C1, IgD-PerCP-Cy5.5 clone IA6-2, CD3-BV510 clone UCHT1, CD14-BV510 clone MφP9 and CD15-BV510 clone W6D3 from BD Biosciences) were prepared in 50 μL BD Biosciences brilliant violet stain buffer. $1x10^6$—$5x10^6$ per test were aliquoted in 50 μL to the pre-mixed antibodies for a total stain volume of 100 μL, and incubated for 20-30 minutes. Cells were washed once with d-PBS, and further

incubated with fixable live/dead blue (Invitrogen) prepared at 1:500 for 30 minutes and washed again. For detection of cytoplasmic immunoglobulins or the nuclear proliferation-associated antigen Ki67, cells were then fixed with 1X Fix-Perm buffer (eBioscience) for 30 minutes, followed by a wash with 1X Permeabilization/Wash buffer (eBioscience). Fixed and permeabilized cells were re-suspended in 100 μL Permeabilization/Wash buffer, and further incubated with antibodies to cytoplasmic antigens for 20-30 minutes (IgG-FITC clone G18-145, IgM-FITC clone G20-127 from BD Biosciences, IgA-FITC pAb from Invitrogen, or Ki67-PE clone Ki67 from BioLegend), washed a final time with d-PBS, and suspended in staining buffer for data collection on either BD Biosciences LSRII or Fortessa cytometers, followed by analysis using FlowJo. Due to scarcity of ASC, we used 95% Poisson statistical cutoff as our method for acquisition of rare cell subset data. For example, for our BM phenotyping data we measured a median ASC ($CD27^{+}CD38^{high}$) frequency of 0.238% (n=17), were the median number of total events measured was 501,187, with gated event ASC median 842 which fulfill the Poisson statistical criteria of less than 5% coefficient of variation essential for accurate rare event detection[24]. Our intracellular cytoplasmic staining method on average accounted for 85% of CD19 positive and negative immunoglobulin secretion in both BM and spleen. We were not able to detect IgE secreting ASC. Our IgE detection assay was validated on a multiple myeloma cell line (U266-source ATCC) known to secrete IgE.

Supplemental Methods Table 1

Reagent	Vendor	Part #	Volume
LIVE/DEAD Fixable Blue Dead Cell Stain Kit	Invitrogen	L-23105	Prepare 1:500 from DMSO stock
Foxp3 / Transcription Factor Staining Buffer Set (for Fix/Perm),	eBioscience	00-5523-00	Prepared 1X Fix and Perm according to kit directions
CD20 BV421	BioLegend	302330	1.5uL
CD27 BV785	BioLegend	302831	1.5uL
CD38 BUV395	BDBiosciences	563811	1uL
CD19 APC	BDBiosciences	340437	5uL

IgD PCPC5.5	BDBiosciences	561315	1.5uL
CD3, CD14, CD15 BV510	BDBiosciences	563109, 563079, 563141	1.5uL
IgG FITC	BDBiosciences	555786	1.5uL
IgM FITC	BDBiosciences	562029	1.5uL
IgA FITC	Invitrogen	H14001	1:40, then use 1uL
Ki67 PE	BioLegend	350514	1:5, then use 1uL

Cell Sorting

For sorting cells into ELISpot plates, we used rigorous sorting methods we previously described by Evans et al[24] for manufacturing using the BD Biosciences Influx platform. Briefly, the sorter drop delay profile was tested and validated using fluorescent particles (Flow-Check Fluorospheres, Beckman Coulter, Inc.) sorted onto slides and visually evaluated under a fluorescent microscope before and after each sort. Accurate well-deposition was optimized daily with fluorescent particles sorted onto the lid of a covered 96-well ELISpot plate.

Detecting immunoglobulin secretion by Elispot

Mabtech (Mariemont, OH) Human IgG ELISpotBASIC (No. 3850-2H), Human IgA ELISpotBASIC (No. 3860-2H), and Human IgM ELISpotBASIC (No. 3880-2H) kits were used for assessing functional immunoglobulin secreting capacity of plasma cells. ELISpot wells were pre-wet using 100µL of 70% ethanol for 2 minutes. Plates were washed with distilled deionized water (200µL per well, 5 times) using a plate washer (BioTek, Winooski, VT). Anti-IgG, Anti-IgA, or Anti-IgM capture antibody was diluted to 15µg/mL with sterile PBS (pH 7.4) and added to wells at 100 µL per well. In some experiments, anti-IgG, IgA and IgM were combined within the same wells to assess the efficiency of ELISpot total immunoglobulin detection. Our ELISpot detection method accounted on average for 75% and 73% of CD19 positive and negative immunoglobulin secretion from both BM and spleen respectively. Detection of antigen specific immunoglobulins was performed as described by Sasaki et al[27] with modifications. For detection of antigen specific immunoglobulins, coating was with the vaccine products Fluzone®, Daptacel, IPOL (Sanofi Pasteur), Varivax®, or MMR (Merck). Fluzone® was diluted to 22µg per 100µL per well. Daptacel, IPOL, Varivax® and MMR were diluted in a similar volume though protein

concentrations were not provided in product literature. Coated plates were then incubated at 4C overnight. Before use, plates were washed with PBS using the plate washer. Wells were blocked using 200μL of complete medium (RPMI with 10% FBS) for at least 30 minutes at room temperature. Cells were labeled with CD20-BV421 clone 2H7, CD27-FITC clone O323, CD38-PE-Cy7 clone HB7, CD19-APC clone SJ25C1, and IgD-PerCP-Cy5.5 clone IA6-2. Cells were then sorted into wells; specifically, among the $CD38^{high}CD27^{+}$ cell subset, either the total population or $CD19^{+}$ and CD19 negative were selected for sorting, and non-$CD38^{high}CD27^{+}$ $CD19^{+}$ B cells were sorted directly into wells, and upon completion of sorting, the plates were incubated overnight at 37C. Following incubation, plates were washed with PBS using the plate washer. Biotinylated detection antibody was diluted to 1μg/mL in PBS with 0.5% FBS, and added at 100μL per well, and incubated for 2 hours at room temperature. Plates were washed with PBS on the plate washer, and 100μL per well of Streptavidin-HRP diluted 1:1000 was added and incubated for 1 hour at room temperature. Plates were washed with PBS a final time and TMB substrate was added to the wells at 200μL per well to develop spots. Once spots were seen in the wells, plates were washed in tap water and air dried. After drying, plates were read using an AID ELISpot Reader (Strassberg, Germany). Wells were visually inspected to confirm the spot counts determined by software.

Vaccines and vaccine antigens

Use of the vaccines to identify vaccine-antigen immunoglobulin binding from ASC subsets is described mainly by vaccine name in the text, but sometimes by vaccine target. Fluzone® (flu, influenza vaccine), Daptacel (Tetanus, Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed), IPOL (Polio, Poliovirus Vaccine Inactivated), Varivax® (Chickenpox, Varicella Virus Vaccine Live), or MMR (Measles, Mumps, and Rubella).

Flow cytometric detection of RNA

ASC and B cell-associated RNAs were detected by flow cytometry using the PrimeFlow™ kit (ThermoFisher) and strictly following the manufacturer's recommended protocol. In brief, cells were first stained with fluor-conjugated mAbs to surface antigens to allow selective identification of B cell subsets and plasma cells (CD19-PE, CD20-BV421, CD27-BV786, IgD-FITC and CD38-PECy7), followed by washing, fixation and permeablization. Cells were incubated with gene-

specific probes and incubated for 2 hours at 40C for hybridization. Bound probes were detected with further hybridization reactions at 40C for 1.5 hours with PrimeFlow™ complimentary pre-amplifier and AlexaFluor-647-labeled amplifier probes that form branched complexes capable of 8000-fold amplification of a single RNA copy. Data were collected on BD Biosciences LSRII or Fortessa cytometers, and analyzed with FlowJo and are presented graphically as Median Fluorescence Intensity (MFI) values with the background MFI of the no-probe control subtracted.

Microarray and gene expression analysis

BM from healthy donors was obtained from the Lonza Group Ltd., (No. 1M-125A) and processed similar to that described by Karnell et al[25]. Staining and cell sorting method was similar to that described above: Cells were sorted using Becton Dickinson (BD Biosciences) FACS ARIA™ II or BD Influx™ cell sorter equipped with 4-way sorting feature. A 100μm nozzle was used for both sorters. Droplet delay calculation was performed using Accudrop and DIVA software (Aria) or Sortware (Influx). Influx drop delay calculations were always verified by sorting Beckman Coulter Flow-Check™ Pro, 10μm beads by sorting and counting defined bead numbers repeatedly on a microscope slide and verifying microscopically. Of viable cells ($DAPI^-$ Sigma Aldrich No. 9532), ASC were defined as $CD38^{high}CD27^+CD138^+$ and then gated on $CD19^+$ or negative ASC. Two B cell populations were also sorted based on positive and negative CD20 expression, they were defined as $CD19^+CD20^+$ (Boolean "not" logical gating excluded $CD38^{high}CD27^+$) or $CD19^+CD20^-$ (Boolean "not" logical gating excluded $CD38^{high}CD27^+$). The two ASC fractions were placed on the outside collection when performing 4-way cell sorting in order to reduce the possibility of contamination. All sorted fractions were collected in FACS buffer and centrifuged, and the resulting cell pellet was suspended in RNA lysis buffer (Ambion).

Total RNA was extracted using PAXgene Blood RNA kit (Qiagen). RNA was quantified spectrophotometrically to ensure that the absorbance at 260 nm/280 nm was >1.9. RNA quality was assessed on an Agilent 2100 Bioanalyzer using an RNA 6000 Nano LabChip. For whole-genome microarray, biotin-labeled amplified complementary RNA (cRNA) was generated with Affymetrix GeneChip One-Cycle cDNA Synthesis kit and Affymetrix GeneChip IVT Labeling kit, and hybridized on Affymetrix Human Genome U133 Plus 2.0 GeneCHip array as described by Streicher et al[26]. Data capture and quality assessments were performed with the GeneCHip

Operating Software tool. Expression values were calculated as probe-level summaries, with frozen robust multi-array average analysis using R. Group comparison were performed using a Bayesian linear model based approach (Limma package in R). Genes with expression difference of fold change ≥ 2 and a false discovery rate of ≤ 0.05 were defined as significant. Principle component analysis, volcano plot and heatmap were generated using R. All whole-genome microarray data have been deposited into GEO (accession numbers GSE107683)

B-cell V(D)J sequencing methods

B cell immunoglobulin repertoires were profiled at the single cell level using the 10X Genomics Single Cell V(D)J Reagent Kit (10X Genomics, Pleasanton, CA, USA). CD19 positive and negative cells were FACS sorted, washed and diluted to 1000 cells/ul in 1X PBS with 0.04% BSA. 13,000-20,000 cells were loaded per reaction. The direct target enrichment step two utilized 10 PCR cycles.

BCR libraries were quantified on the QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) using the Kapa Library Quantification Kit for Illumina Platforms with ROX Low qPCR Mastermix (Kapa Biosystems, Wilmington, MA, USA). Libraries were diluted to 4nM and pooled in equal volumes. The pool was diluted to 1pM with 1% PhiX spike-in, and sequenced on the NextSeq 500 System (Illumina, San Diego, CA, USA) with 150bp paired-end reads.

Subsequently, Cell Ranger (v2.1, 10X Genomics) was used for fastq generation, V(D)J gene alignment and annotation. For each sample, CDR-H3 and CDR-L3 were annotated and extracted to count for unique clones. Clones with the same CDR-H3 across samples were considered as identical clones and pairwise clonotype overlap was calculated as the number of identical clones between two given samples. Heatmap visualization of the overlap was plotted using pheatmap.

Statistical analysis

Unless otherwise stated, the analysis of unpaired students t-tests were performed using GraphPad Prism version 7.0c for Mac, GraphPad Software, La Jolla California USA, www.graphpad.com.

Likewise, all bar graphs and scatter plots were generated by GraphPad Prizm® version 7.0c for Mac.