Supplementary Information for

High-throughput Monoclonal Antibody Screening from Immunized Rabbits via Droplet Microfluidics

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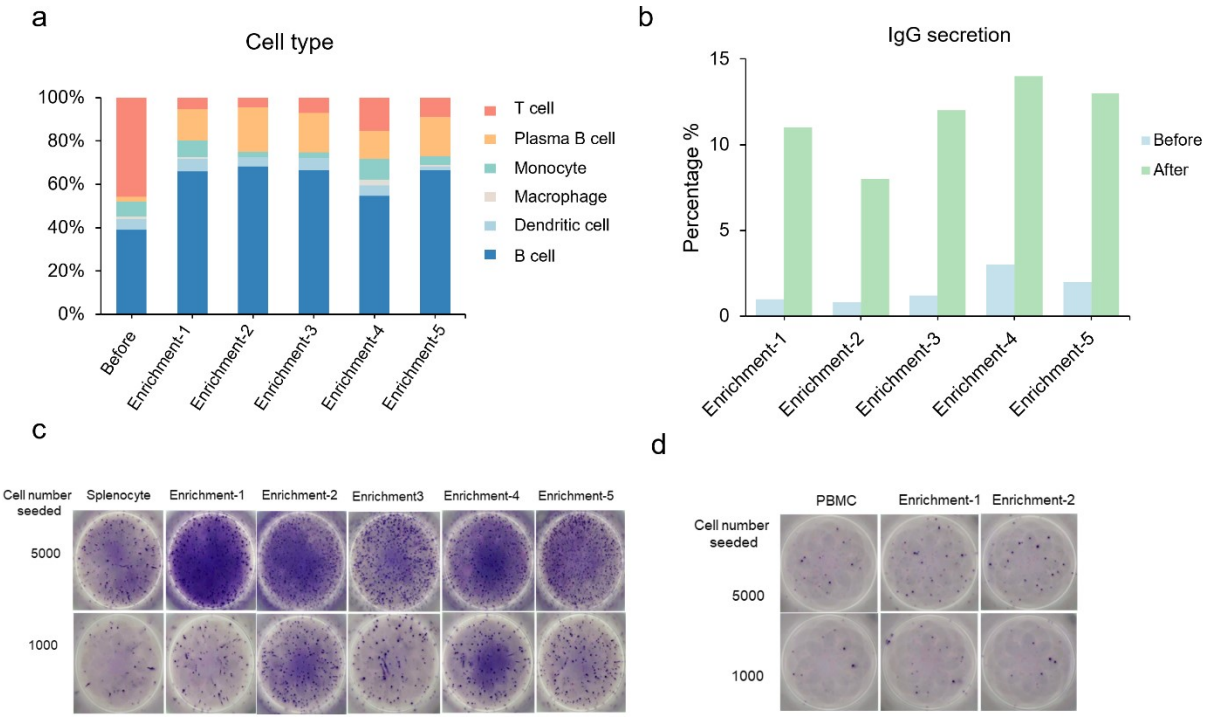

Figure. S1. a. Cell type composition of rabbit spleen samples before and after enrichment across five protocols (Enrichment-1 to Enrichment-5), determined by single-cell RNA sequencing (scRNA-seq). b. Percentage of IgG-secreting cells in spleen samples before and after enrichment for the five protocols measured by ELISpot, showing increased IgG secretion post-enrichment. c. Representative ELISpot images of splenocytes before and after enrichment (Enrichment-1 to Enrichment-5), seeded at 5,000 and 1,000 cells per well. d. Representative ELISpot images of peripheral blood mononuclear cells (PBMC) before and after enrichment (Enrichment-1 and Enrichment-2), seeded at 5,000 and 1,000 cells per well.

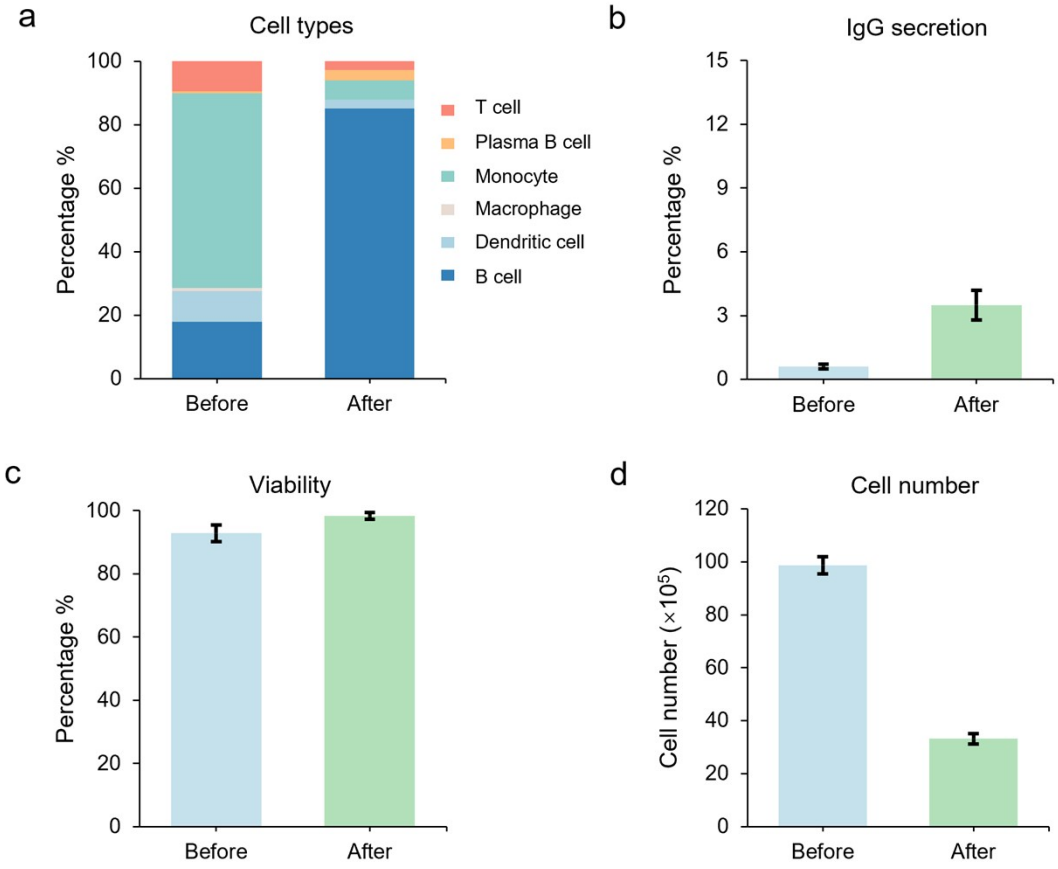

Figure. S2. Plasma B cell enrichment by magnetic negative depletion for PBMC sample from rabbits a. Cell types in the PBMC sample before and after enrichment measured by single cell sequencing, a sixfold enrichment of plasma B cells was achieved. b. IgG secretion of the PBMC sample before and after enrichment measured by ELISpot, a sixfold increase in IgG secretion ratio was observed. c. Cell viability remained high (>90%) before and after cell enrichment. d. Cell number drastically decreased by ~70% after cell enrichment.

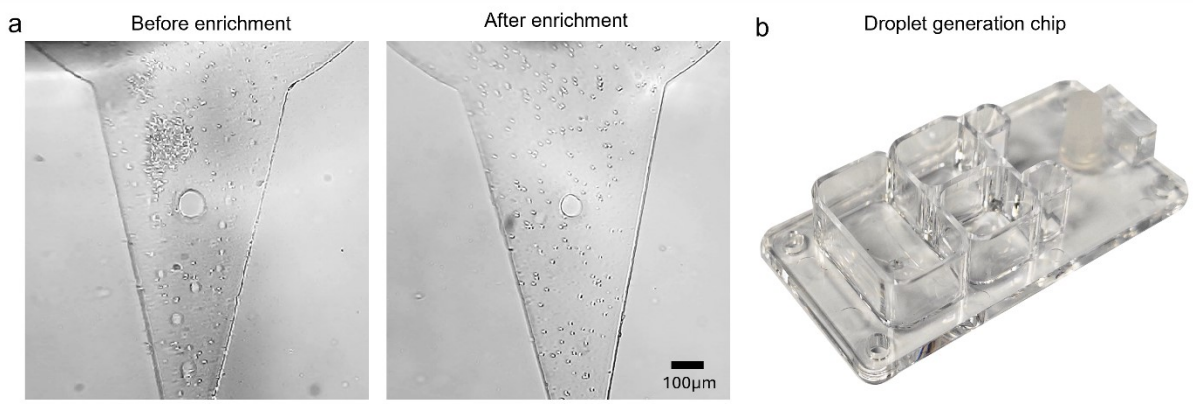

Figure. S3. a. Microscopy view of the cell sample being loaded into the droplet generation chip, showing that the enrichment significantly reduced cell aggregation, facilitating single cell encapsulation in water-in-oil droplets. b. Image of the droplet generation chip.

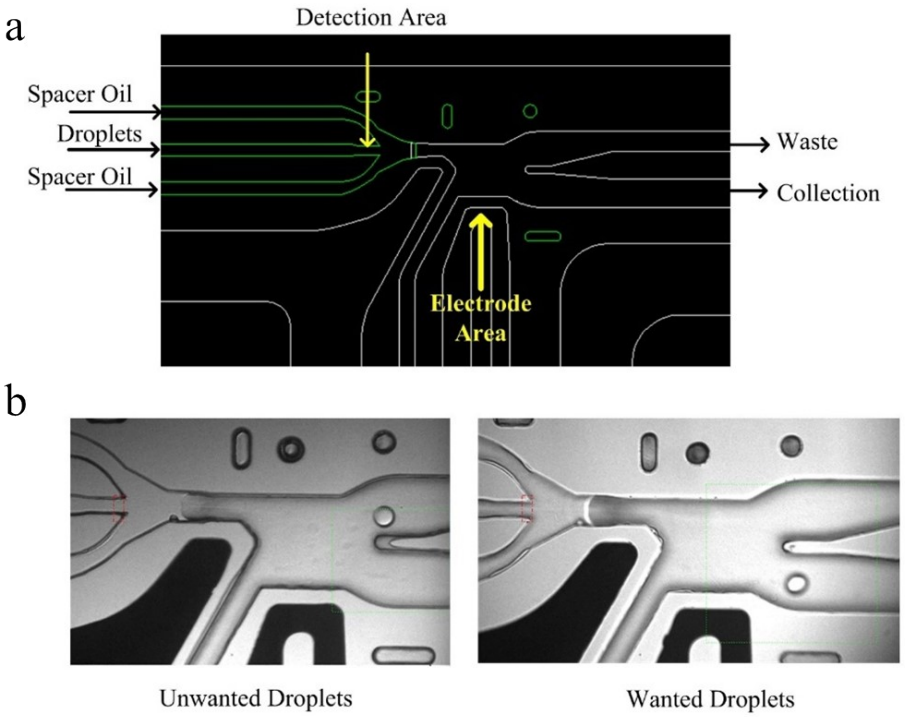

Figure. S4. Sorting chip design. a. In the sorting chip, the introduced droplets are separated by spacer oil to ensure sequential processing. Subsequently, these droplets pass through the detection area, where the droplets are compressed and elongated to improve the sensitivity of detection. b. For the droplets considered positive upon analysis via the FPGA built-in in the sorting instrument, the paired electrodes are excited, and droplets are pulled into the positive channel under the action of dielectrophoretic force. Otherwise, the droplets would enter the waste channel. In this fashion, target droplets are sorted and collected.

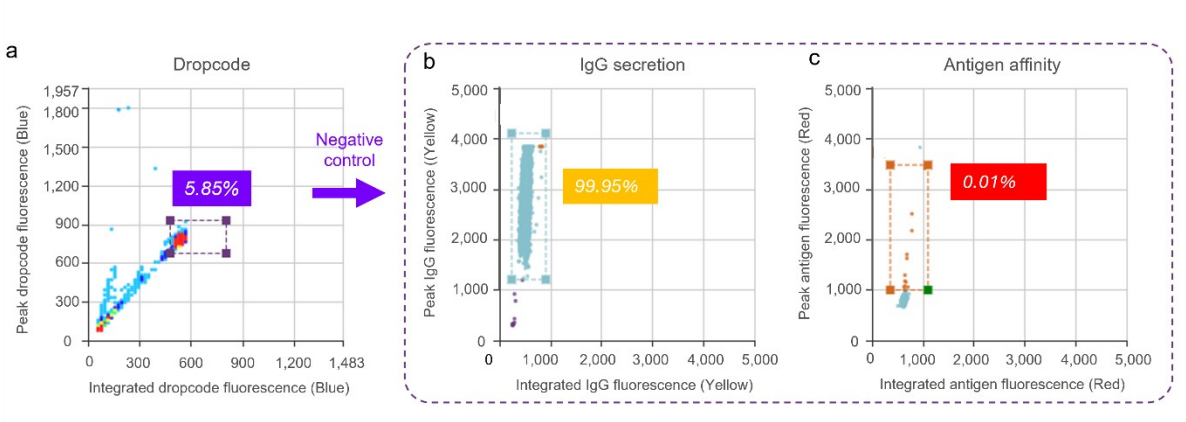

Figure. S5. For the particle aggregation-based assay, a negative control group was barcoded with Alexa Fluor 405 carboxylic acid (250 nM). a. The gated portion defines the negative control group, making up 5.85% of the droplets in total, while the rest belongs to the sample group. b. 99.95% of the negative control group exhibited IgG secretion. c. The gating threshold was set so that no more than 0.01% of the droplets exhibited non-specific binding signals.

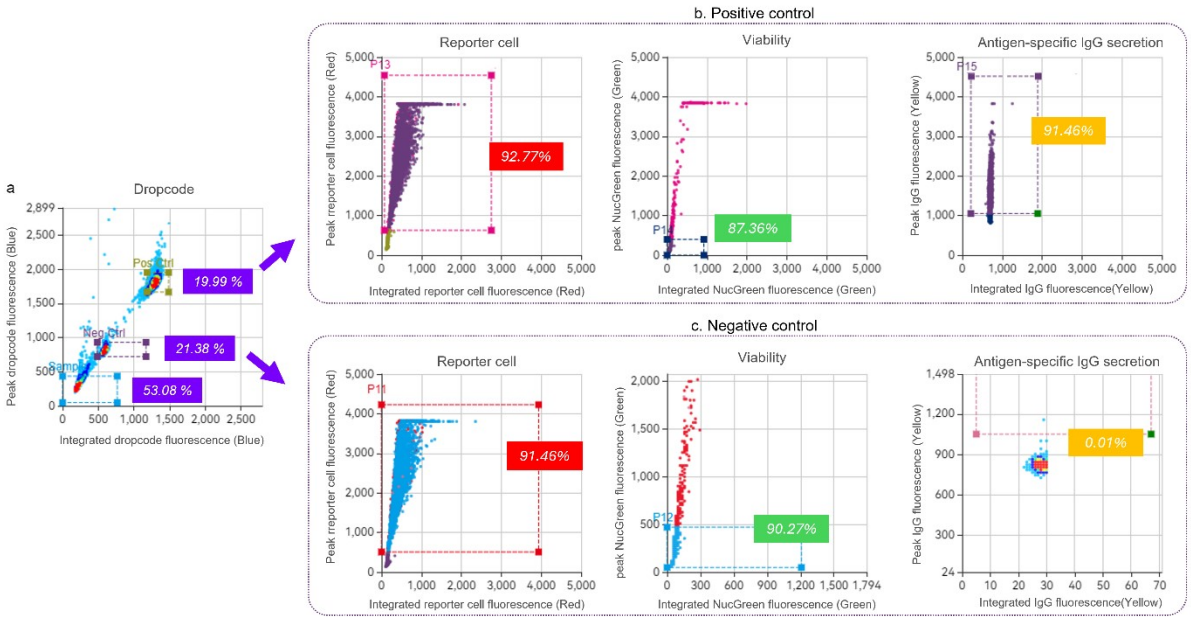

Figure. S6. For the reporter cell-based assay, a negative control group and a positive control group were barcoded with Alexa Fluor 405 carboxylic acid (1000 nM, 250 nM for the positive, negative control group respectively). a. Three gates were applied, each defining the positive control group (19.99%), negative control group (21.38%), and sample group (53.08%). b, c. The positive and negative control group were sequentially subjected to 1. reporter cell signal gate, 2. Cell viability gate, 3. Antigen-specific IgG signal gate. In the positive control group, 91.46% exhibited significant antigen-specific IgG secretion, whereas only 0.01% of the droplets in the negative control group exhibited non-specific binding signals.

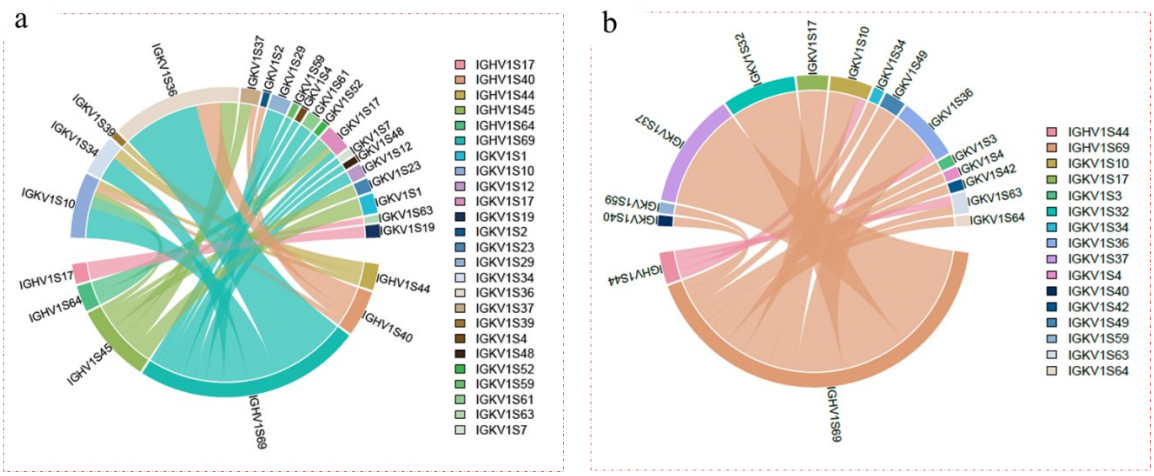

Figure. S7. Circos plot of paired heavy and light chain sequences from rabbit B cells for OVA(a) and hCD82(b), with arcs showing paring information, reflecting the diversity and specificity of single cell sequencing results. Clonal expansion analysis reveals a predominant heavy chain family, with IGHV1S69 showing extensive diversification through multiple pairings with different light chain partners.

Table S1. Fluorescence data and affinity result of selected positive droplets for OVA antigen

Antibody ID	FL1-(NucGreen)(Green)	FL2- (PE anti-Rabbit IgG Fc) (Yellow)	FL3- (OVA-AF647) (Red)	Affinity result（Y/N）
Ab-1	323.954	3056.89	2368.6	Y
Ab-2	248.878	1782.59	2378.37	Y
Ab-3	360.118	2224.04	2243.57	Y
Ab-4	223.548	1894.8	2139.1	Y
Ab-5	323.344	2845.24	1515.55	Y
Ab-6	310.831	2172.77	1129.64	N
Ab-7	312.815	3013.55	1725.97	Y
Ab-8	352.641	2530.59	2508.53	Y
Ab-9	291.452	2881.41	3455.89	Y
Ab-10	261.544	2271.19	1988.03	Y

Table S2. Fluorescence data and affinity result of selected positive droplets for hCD82

Antibody ID	FL1-(NucGreen)(Green)	FL2- (PE anti-Rabbit IgG Fc) (Yellow)	FL3- (CellTrace Far Red Stained Reporter cell) (Red)	Affinity result（Y/N）
Ab-1	305.033	2442.4	1385.69	Y
Ab-2	272.225	1313.15	3395.76	N
Ab-3	343.333	2425.76	1859.19	Y
Ab-4	267.647	1562.13	2508.99	N
Ab-5	315.561	2741.33	3666.31	Y
Ab-6	329.447	4117.4	1738.18	Y
Ab-7	356.304	3465.53	1891.38	Y
Ab-8	315.104	1153.63	2614.58	N
Ab-9	232.246	2935.58	2501.66	Y
Ab-10	328.227	2264.26	2642.05	Y

Table S3. Oligonucleotide primers for PCR amplification & Sanger sequencing

Primer ID	Sequence (5' > 3')
2nd VH forward 1	ctgggttccaggttccactggtgacCAGGAGCAGCWGAWGGARTC
2nd VH forward 2	ctgggttccaggttccactggtgacCAGTCASTGARGGAGTCCGR
2nd VH reverse	cgatgggcccttggtggaggCTGARGAGAYGGTGACSAGGGT
2nd VL forward	ctgggttccaggttccactggtgacGCHSYYRTGMTGACCCAGAC
2nd VL reverse	tgctcatcagatggcgggaaAGCTGGTGGGAAGAKGAGGACAG
VH/VL Seq forward	tgggttccaggttccactgg
VH Seq reverse	cgatgggcccttggtggagg
VL Seq reverse	tgctcatcagatggcgggaa

Table S4. Oligonucleotide primers for linear expression cassettes

Primer ID	Sequence (5' > 3')
CMV forward	agtaatcaattacggggtcattagttcatag
CMV reverse	gtcaccagtggaacctggaacccag
IgHC forward	cctccaccaagggcccatcg
IgLC forward	ttcccgccatctgatgagca
IgHC/IgLC reverse	tccccagcatgcctgctattgtc