Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Data

Microbubble Array Diffusion Assay for the Detection of Cell Secreting Factors

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Table S1 Primers for Immunoglobin Genes

Heavy:	Name	Sequence
Forward 5’	VH1 LEADER-A	ATGGACTGGACCTGGAGGAT
Forward 5’	VH1 LEADER-B	ATGGACTGGACCTGGAGCAT
Forward 5’	VH1 LEADER-C	ATGGACTGGACCTGGAGAAT
Forward 5’	VH1 LEADER-D	GGTTCCTCTTTGTGGTGGC
Forward 5’	VH1 LEADER-E	ATGGACTGGACCTGGAGGGT
Forward 5’	VH1-LEADER-F	ATGGACTGGATTTGGAGGAT
Forward 5’	VH1-LEADER-G	AGGTTCCTCTTTGTGGTGGCAG
Forward 5’	VH2Ext	CATACTTTGTTCCACGCTCC
Forward 5’	VH3 LEADER-A	TAAAAGGTGTCCAGTGT
Forward 5’	VH3 LEADER-B	TAAGAGGTGTCCAGTGT
Forward 5’	VH3 LEADER-C	TAGAAGGTGTCCAGTGT
Forward 5’	VH3 LEADER-D	GCTATTTTTAAAGGTGTCCAGTGT
Forward 5’	VH3 LEADER-E	TACAAGGTGTCCAGTGT
Forward 5’	VH3 LEADER-F	TTAAAGCTGTCCAGTGT
Forward 5’	VH4 LEADER-A	ATGAAACACCTGTGGTTCTTCC
Forward 5’	VH4 LEADER-B	ATGAAACACCTGTGGTTCTT
Forward 5’	VH4 LEADER-C	ATGAAGCACCTGTGGTTCTT
Forward 5’	VH4 LEADER-D	ATGAAACATCTGTGGTTCTT
Forward 5’	VH5 LEADER-A	TTCTCCAAGGAGTCTGT
Forward 5’	VH5 LEADER-B	CCTCCACAGTGAGAGTCTG
Forward 5’	VH6 LEADER-A	ATGTCTGTCTCCTTCCTCATC
Forward 5’	VH7 LEADER-A	GGCAGCAGCAACAGGTGCCCA
External 3’	IgMExt1	GTGATGGAGTCGGGAAGGAA
External 3’	IgAExt	GTGTAGTGCTTCACGTGGCA
External 3’	IgGExt	GAGTCCTGAGGACTGTAGGA
Internal 3’	IgMInt1	CGACGGGGAATTCTCACAGG
Internal 3’	IgAInt	GGCATGTCACGGACTTGCCG
Internal 3’	IgGInt	GCGCCTGAGTTCCACGACAC
KAPPA:		
External 5’	5' L VK1/2	ATGAGGSTCCCYGCTCAGCTGCTGG
External 5’	5' L VK 3	CTCTTCCTCCTGCTACTCTGGCTCCC
External 5’	5' L VK4	ATTTCTCTGTTGCTCTGGATCTCTG
External 3’	3' CK 543	GTTTCTCGTAGTCTGCTTTGCTCA
Internal 5’	5' PAN VK	ATGACCCAGWCTCCABYCWCCCTG
Internal 3’	3' CK 494	GTGCTGTCCTTGCTGTCCTGCT
LAMBDA:		
External 5’	5'L VL1	GGTCCTGGGCCCAGTCTGTGCTG
External 5’	5'L VL 2	GGTCCTGGGCCCAGTCTGCCCTG
External 5’	5'L VL 3	GCTCTGTGACCTCCTATGAGCTG
External 5’	5'L VL 4/5	GGTCTCTCTCSCAGCYTGTGCTG

External 5’	5' L VL6	GTTCTTGGGCCAATTTTATGCTG
External 5’	5'L VL 7	GGTCCAATTCYCAGGCTGTGGTG
External 5’	5'L VL8	GAGTGGATTCTCAGACTGTGGTG
External 3’	3' CL	CACCAGTGTGGCCTTGTTGCCTTG
Internal 5’	5' AGEI VL 1	CTGCTACCGGTTCCTGGGCCCAGTC
Internal 5’	5' AGEI VL 2	CTGCTACCGGTTCCTGGGCCCAGTC
Internal 5’	5' AGEI VL 3	CTGCTACCGGTTCTGTGACCTCCTAT
Internal 5’	5' AGEI VL 4/5	CTGCTACCGGTTCTCTCTCSCAGCYT
Internal 5’	5' AGEI VL 6	CTGCTACCGGTTCTTGGGCCAATTTT
Internal 5’	5' AGEI VL 7/8	CTGCTACCGGTTCCAATTCYCAGRCT
Internal 3’	3' XHOI CL	CTCCTCACTCGAGGGYGGGAACAGA

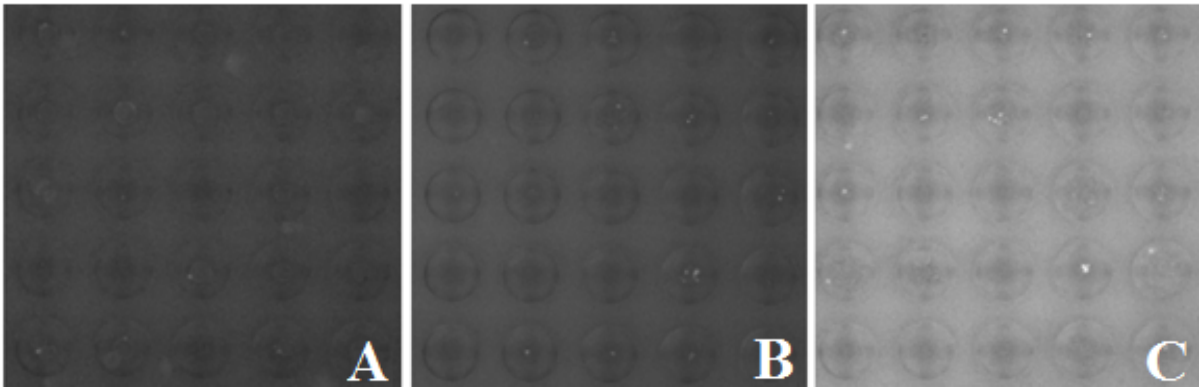

Figure S1: Tetanus Toxoid Detection via Immunoprecipitation – SA13 cells seeded in MB arrays and imaged under fluorescence showing little to no fluorescence detection of tetanus specific Ab. Varying AlexaFluor-Toxoid concentration A) 1 µg/mL, B) 3 µg/mL, C) 6 µg/mL. At concentrations reaching 6x the normal antigen concentration there is no IP, despite the lack of IP the images continually got brighter due to the background fluorescence associated with the Alexa fluorophore at higher concentrations in the media.

Figure S2: Precipitate Formation in Cell Culture Supernatant – SA13 supernatant was mixed with A) FITC α-IgG and B) Alexa488 tetanus toxoid. Arrows show precipitate formation with the α-IgG after 4 hours, while the tetanus toxoid shows no signs of precipitate formation.

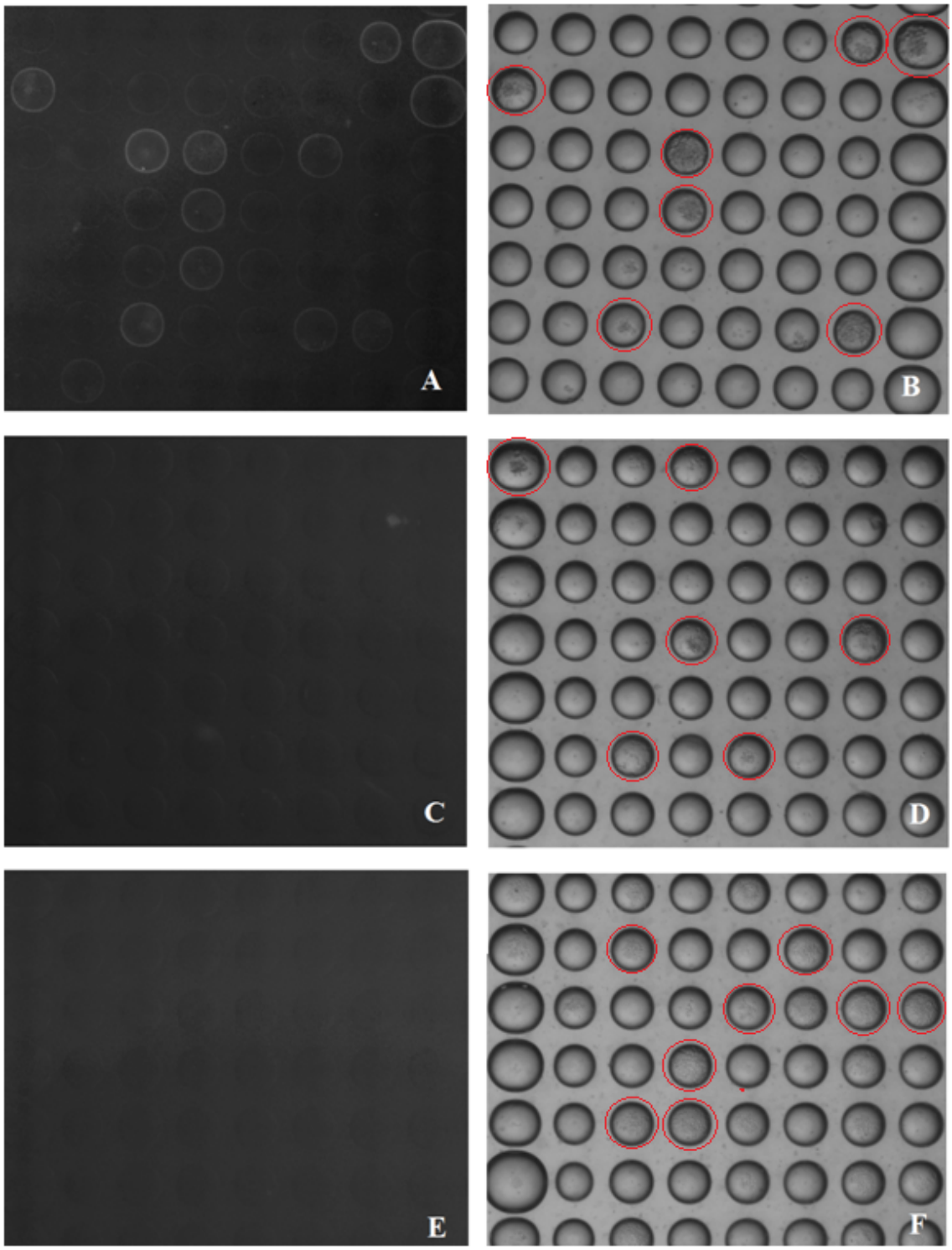

Figure S3 : Antigen Specific Detection Cell Line Comparison – Three cell lines were used to validate the detection specificity. A and B) SA13 fluorescent and bright-field images. C and D) Negative control ARH-77 fluorescent and bright-field images. E and F) Negative control CCL-119 fluorescent and bright-field images. Fluorescent images show toxoid specific detection for SA13 cells only. Red circles indicate MB wells with >20 cells present.

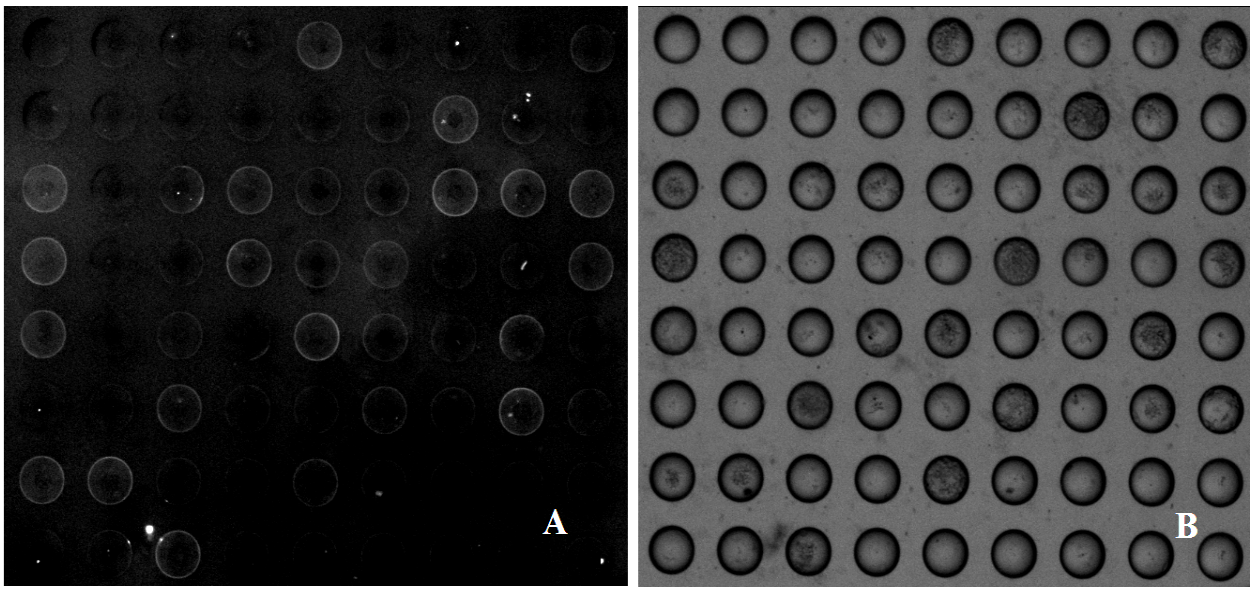

Figure S4: Ring formation using Affinity Capture Coating for IgG Detection – Ring formation using capture coating and FITC-α-IgG detection of SA13 cells culture in MB wells. (A) Fluorescence (B) Bright-field

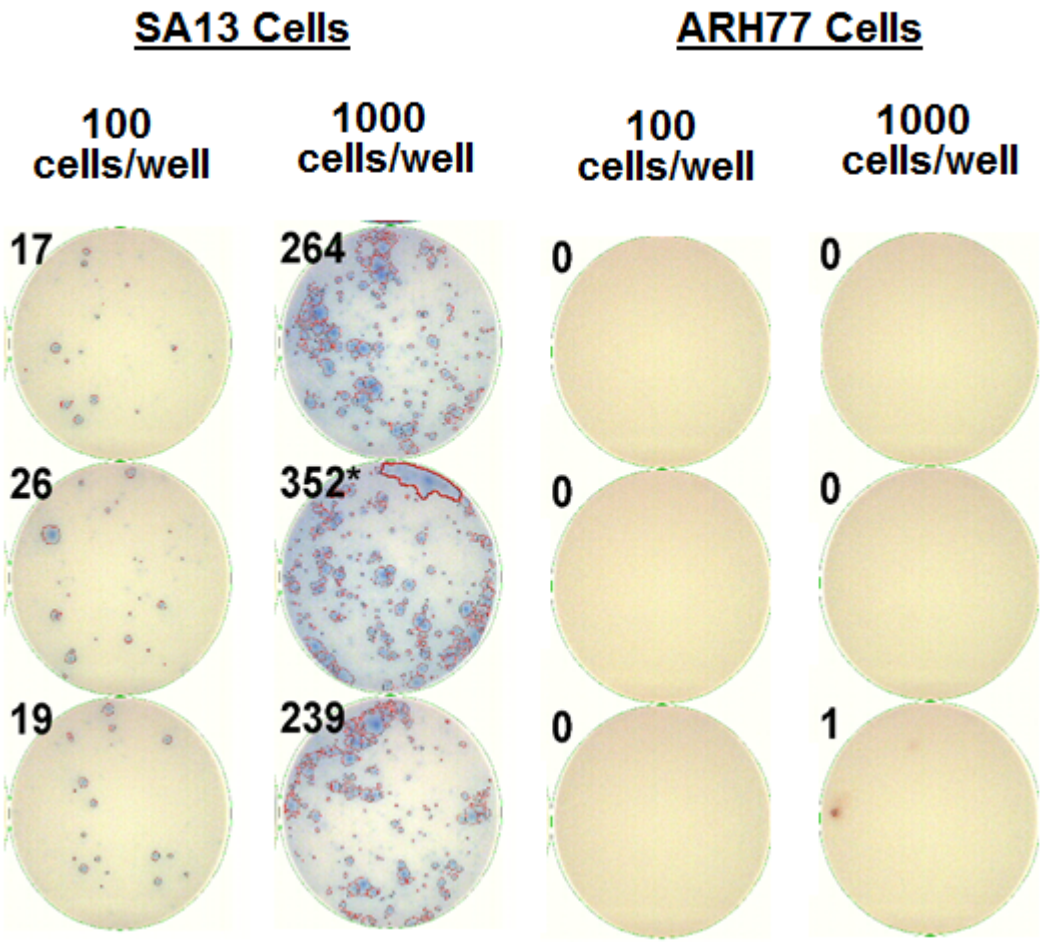

Figure S5 : Tetanus Toxoid Specific IgG Detection ELISpot Results – Number represent number of spots in well. Average of 21±5% and 0% for the SA13 and ARH77 sample, respectively

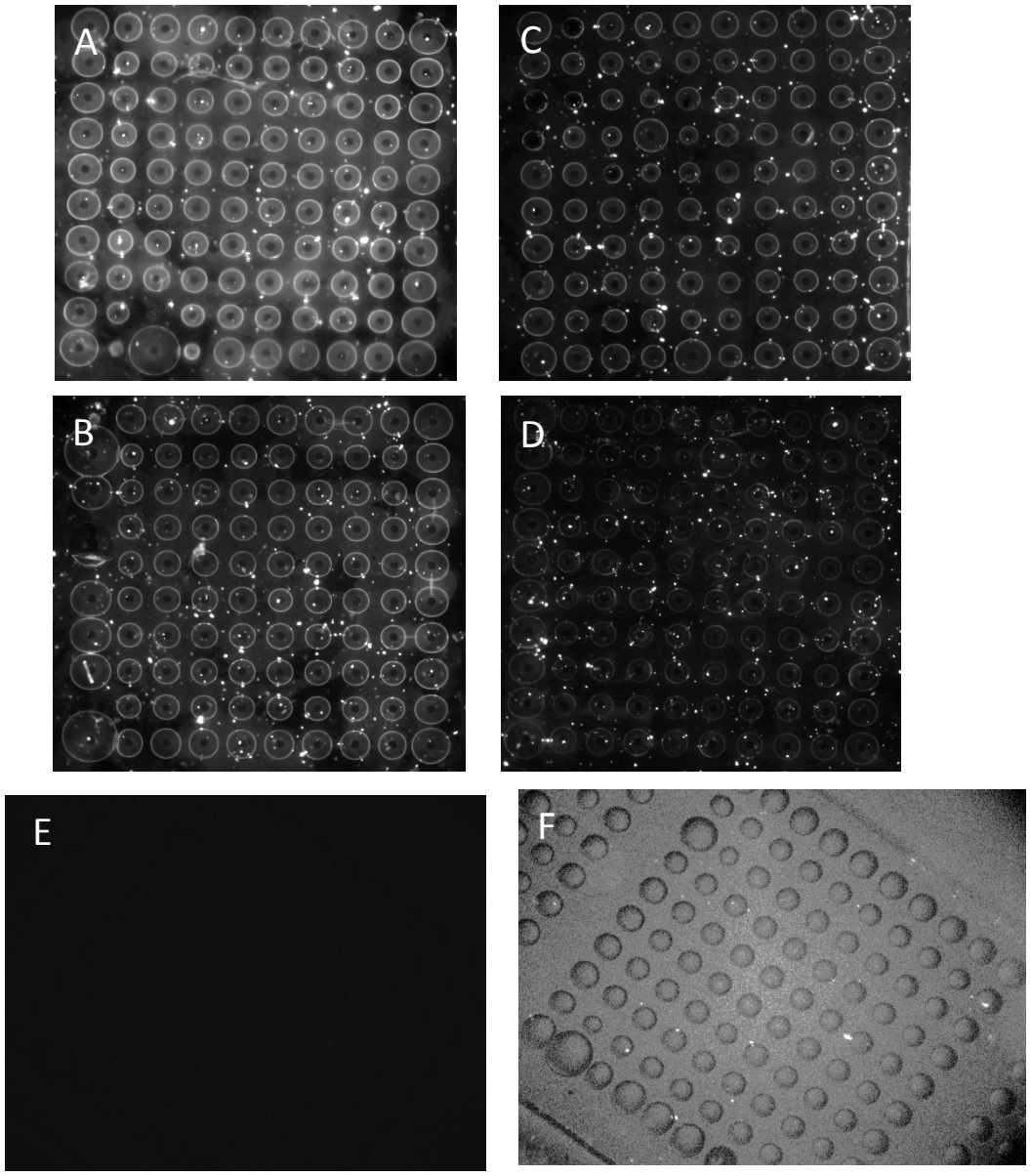

Figure S6: IgG Detection Threshold – Chips were coated with anti-human IgG and exposed to human IgG at (A) 1 μg/ml, (B) 0.1 μg/ml (C) 0.01 μg/ml (D) 0.001 μg/ml. Fluorescent images were taken after treatment with labeled anti-IgG for 12 hr and imaged enhanced to emphasize the presence of fluorescent rings. (E) Raw fluorescent image of a non-IgG exposed chip. (F) image shown in (E) enhanced to show lack of ring fluorescence. Camera integration time for all images was 250 ms. All images were equally enhanced using the contrast function in Image J.

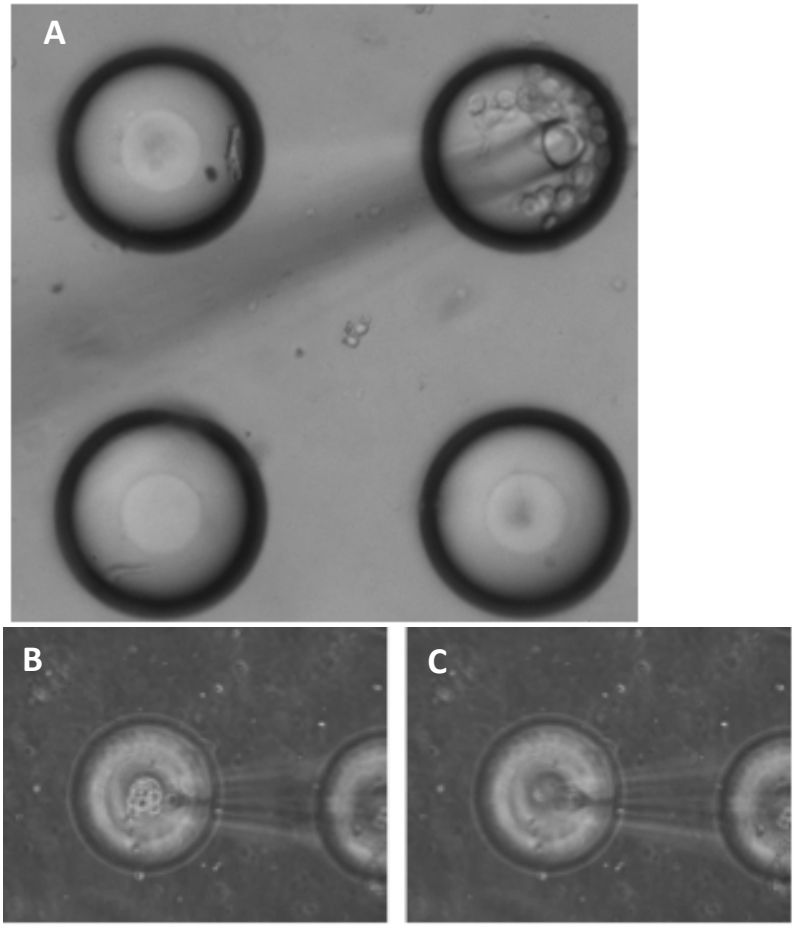

Figure S7 : Cell Recovery from MB wells – (A) shows pipet tip positioned inside MB well. (B and C) Shows MB well before and after aspirating cells.

Supplemental Figure S8. The Ig heavy chain variable region (VH) was sequenced from MB1 and analyzed by V-Quest (imgt.org) to determine Ig gene usage.

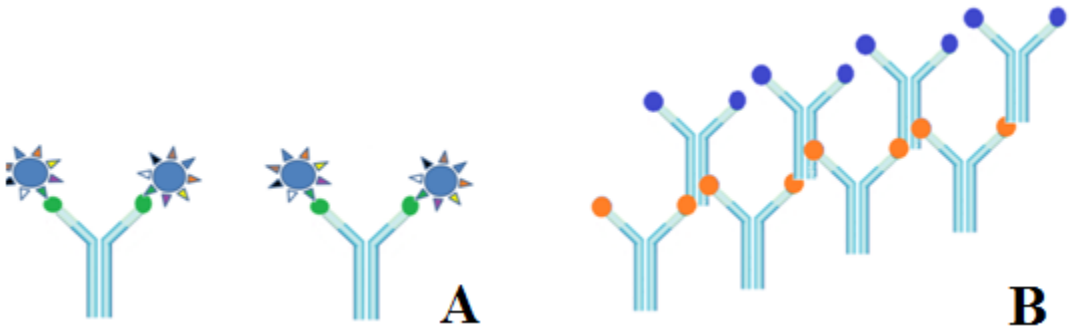

Figure S9: Precipitate Formation Diagram – Precipitate formation comparison between mAb – Antigen and mAb – α-IgG. A) MAb bound to unique epitope on antigen surface. B) FITC α-IgG (Orange) bound to mAb (Blue), leading to the precipitation of fluorescence. As diagramed in Figure 7A, mAb with an affinity to one unique epitope on an antigen bind that epitope and cease polymerization, while mixtures of IgG and α-IgG, as pictured in Figure 7B lead to the binding of the stalk region on the IgG by the α-IgG resulting in polymerization and precipitation of insoluble aggregates that fluoresce under the correct wavelength.

Figure S10: CnP approximations at various cell seeding concentrations – To approximate cell seeding distribution in large MB well arrays a probability term coined "C_nP" was developed which is a statistical approximation/best fit function using previous data collected on the effect of seeding concentration on cell entrapment. The equation returns a percent which can be used to estimate the number of MB wells per condition. Likewise, the previously determined percentages themselves can be used directly in place of the CnP term.

Cell Seeding Concentration (cells/cm2)	Corresponding C_nP
10,000	$y = 0.007x^3 - 0.0538x^2 + 0.1243x - 0.0024$
15,000	$y = 0.04x^3 - 0.2826x^2 + 0.4931x + 0.0067$
20,000	$y = 0.0064x^3 - 0.1277x^2 + 0.4535x - 0.0079$
25,000	$y = 0.008x^3 - 0.1154x^2 + 0.4334x - 0.0176$

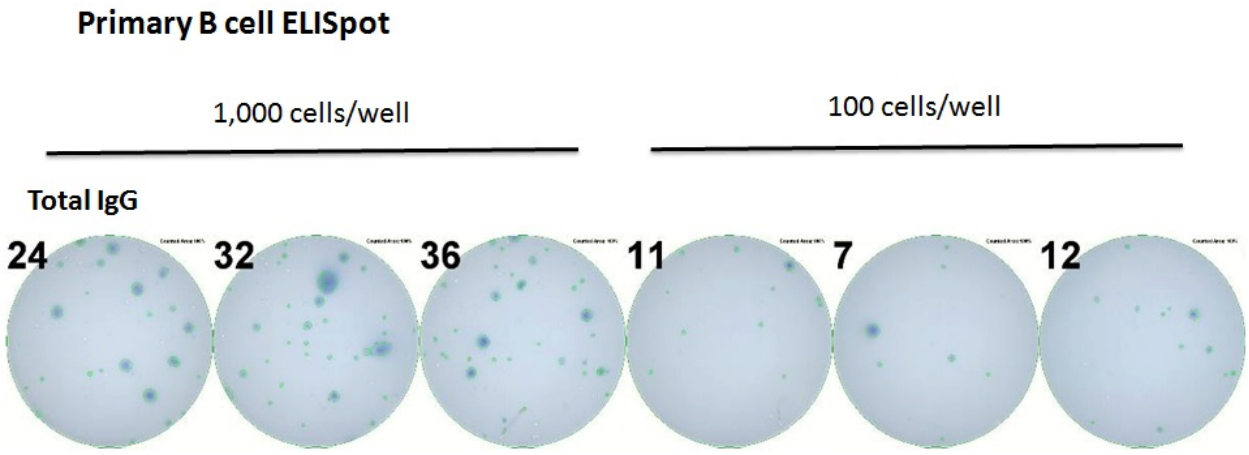

Figure S11 : Primary B cell IgG Detection ELISpot Results – Number represent number of spots in well. Averaging results from wells seeded with 1000 and 100 cells indicates 6.5 ± 4.2% of the primary B cells secrete IgG.