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

Supplementary Information

PRESCIENT: Platform for the Rapid Evaluation of Antibody Success using Integrated Microfluidics Enabled Technology

Supplementary Figure S1	Experimental analysis comparing MHV infection rates between two different types of cell culture medias
Supplementary Figure S2	Experimental analysis comparing MHV infection in the presence of non-nAbs and nAbs
Supplementary Figure S3	Experimental analysis demonstrating levels of non-nAbs producing hybridoma cell viability in droplets
Supplementary Figure S4	Experimental micrographs demonstrating progression of MHV host cell infection in droplets
Supplementary Figure S5	Experimental analysis demonstrating single hybridoma cell droplets neutralizing MHV host cell infection

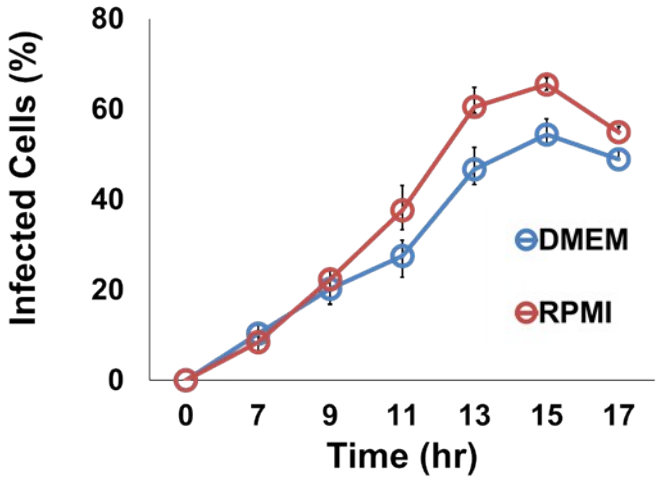

Figure S1. Experimental results comparing bulk infection between two different types of cell culture medias (RPMI 1640 and DMEM). Standard deviation denoted by error bars.

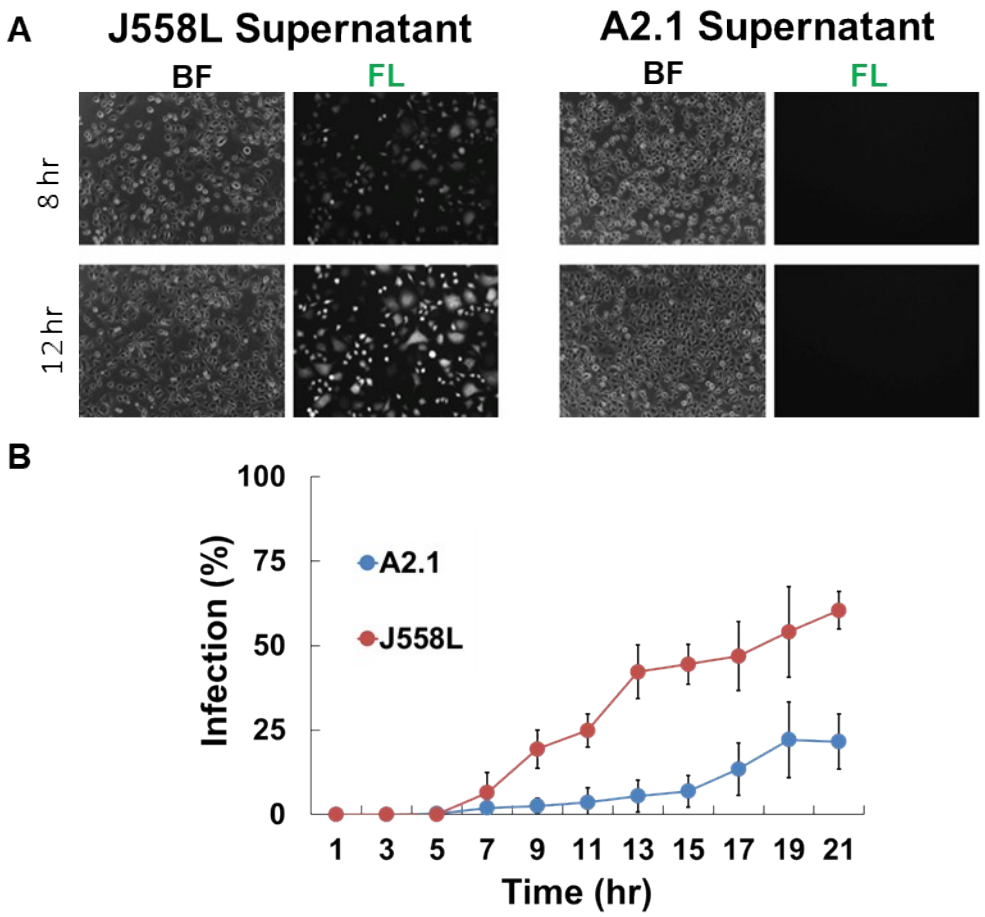

Figure S2. (A) Experimental micrographs comparing two timepoints between wells with non-nAbs (left) and nAbs (right) rich supernatant. Fluorescence shows the infected host cells. (B) Experimental results comparing the efficiency of the two types of antibodies at neutralizing MHV. Standard deviation denoted by error bars.

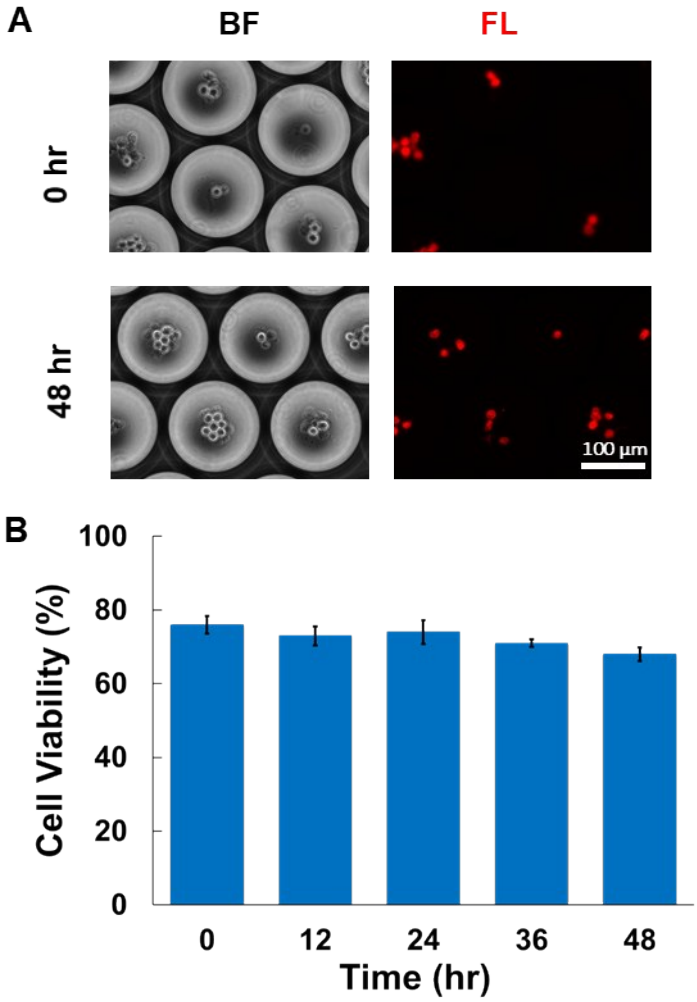

Figure S3. (A) Experimental results comparing viability of the non-nAbs producing hybridoma cells in RPMI media over 48 hours. Standard deviation denoted by error bars. (B) Experimental micrographs demonstrating level of cell death. Dead cells stained by propidium iodide dead cell stain (FL).

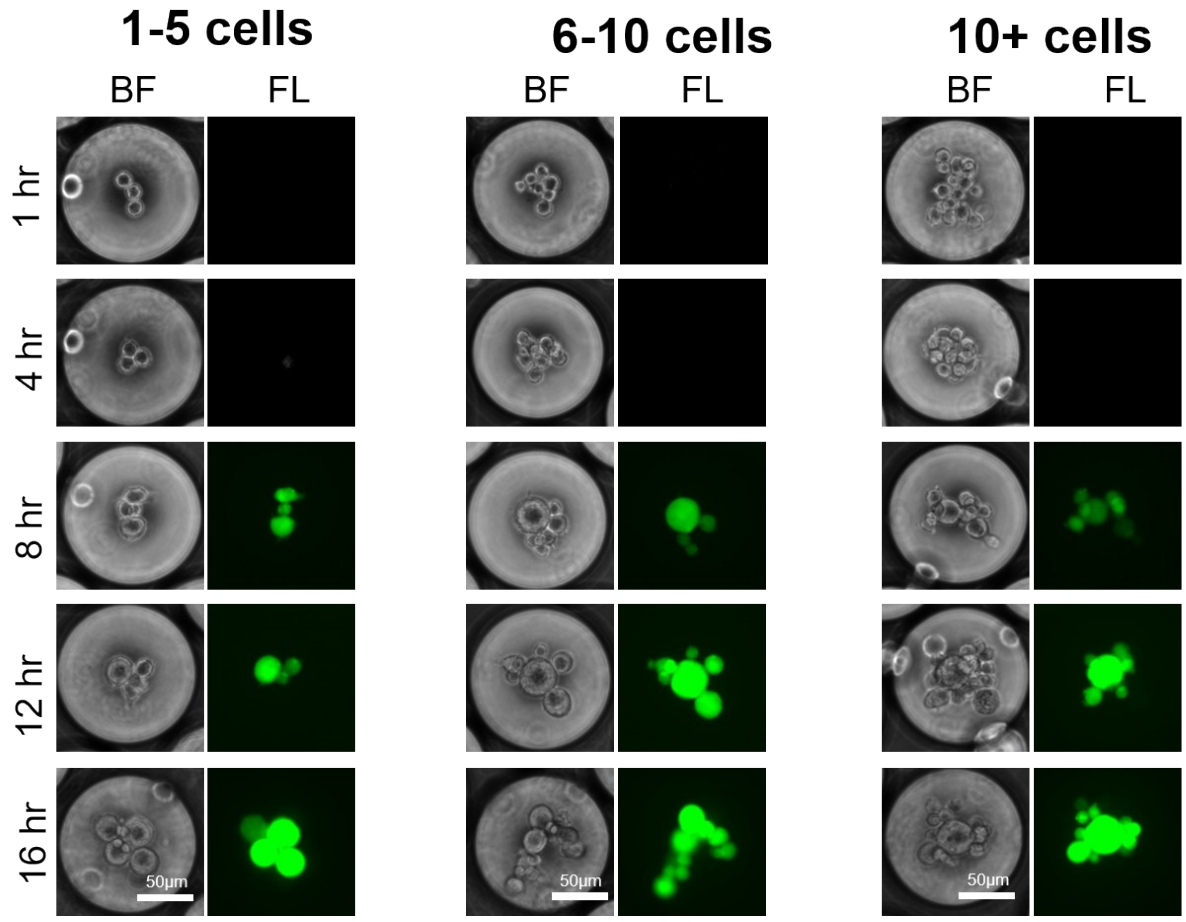

Figure S4. Experimental micrographs of the in droplet host cell infection assay.

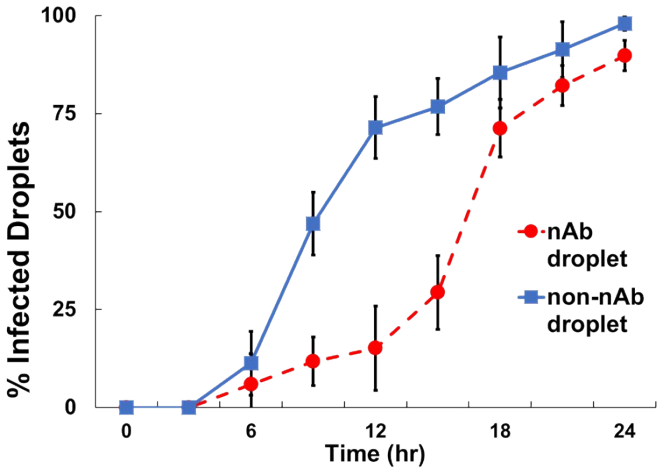

Figure S5. Percent of single hybridoma cell droplets showing infection after entire PRESCIENT assay. SEM denoted by error bars

Supplemental Materials:

Media Preparation:

For 1 L hybridoma cell media preparation, mix 880mL of liquid base media (Roswell Park Memorial Institute (RPMI) 1640 ATCC modification (Thermo Fisher # A1049101) with 100 mL Fetal Bovine Serum ('FBS', Thermo Fisher # 16000044). Next, add 10 mL MEM non-essential amino acids ('NEAA', Thermo Fisher # 11140050) along with 10 mL penicillin-streptomycin (Thermo Fisher # 1514022). Finally, add 12 mL GlutaMAX ™ Supplement (Thermo Fisher # 35050061). Filter sterilize the solution before using and maintain at 8C for long-term storage. For host cell media preparation, mix 880 mL of liquid base media (Dulbecco's Modified Eagle Media - High glucose, Thermo Fisher # 11995065) with 100 mL of FBS. Next, add 10 mL NEAA along with 10 mL penicillin-streptomycin. Finally, add 10 mL Sodium Pyruvate (Thermo Fisher # 11360070). Solution should be filter sterilized before usage and maintain at 8C for long-term storage.

Microfab:

Photoresist SU-8 2050 & SU-8 2075 (Microchem Corp., #Y111072 1000L1GL/ # Y111074 1000L1GL, MA) 3inch Silicon wafers (University Wafers #447, MA)

Medias: Roswell Park Memorial Institute (RPMI) 1640 ATCC modification (Thermo Fisher # A1049101), Dulbecco's Modified Eagle Media - High glucose (Thermo Fisher # 11995065)

Media supplements: PBS, pH 7.4 (Thermo Fisher # 10010023), MEM Non-Essential Amino Acids – 100x (Thermo Fisher 11140050), GlutaMAX ™ Supplement (Thermo Fisher # 35050061), Penicillin-Streptomycin – 10,000 U/mL (Thermo Fisher # 1514022), Trpysin-EDTA – 0.25% with phenol red (Thermo Fisher # 25200056), Sodium Pyruvate – 100mM (Thermo Fisher # 11360070), Fetal Bovine Serum – certified US origin (Thermo Fisher # 16000044)

Syringe: BD Luer-Lok (1mL & 5mL) (VWR # BD-309629 & VWR # BD 309603)

Syringe needles: Fisnar Blunt End Needles – Lavender 0.5Inx30GA (Ellsworth Adhesives # 5601135)

Syringe Pumps: Chemyx Fusion 400 (Chemyx #10060)

Tubing: Tygon ND-100-80 01.X.03X500 (VWR #89404-300)

PDMS: 184 Silicon Elastomer Kit 0.5KG Sylgard Elastomer (Ellsworth Adhesives)

Glass Slides: 75x50mm Corning (VWR #89092-008)

Metal for electrodes:

Surfactant: Pico-Surf™ 1, 5% in Novec 7500 (Dolomite #3200216), Aquapel (PPG Industries)

Oil: 3M™ Novec™ 7500 Engineered Fluid (3M # 98021229285)

Virus: MHV-A59-EGFP was originally constructed by Sarma et al (1). Virus was clonally isolated by limiting dilution and expanded to produce a stock which produced no detectable non-fluorescent plaques when plaque assayed on L2 cell monolayers (about 100 plaques).

Cells: L2 cells, a mouse lung tumor cell line, were originally obtained from Dr. Kathryn Holmes and were maintained in DMEM supplemented with 10% serum as described previously. The anti-MHV-A59 hybridoma with neutralizing activity, A2.1, was obtained from Dr. Wendy Gilmore and recognizes the spike protein (2). A2.1 cells were maintained in RPMI 1640 supplemented with fetal bovine serum. The J558L hybridoma was obtained from Koichi Kobayashi and maintained in RPMI 1640 supplemented with fetal bovine serum.

1. Das Sarma J, Scheen E, Seo S-h, Koval M, & Weiss SR (2002) Enhanced green fluorescent protein expression may be used to monitor murine coronavirus spread in vitro and in the mouse central nervous system. *Journal of NeuroVirology* 8(5):381-391.
2. Gilmore W, Fleming JO, Stohlman SA, & Weiner LP (1987) Characterization of the structural proteins of the murine coronavirus strain A59 using monoclonal antibodies. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.)* 185(2):177-186.