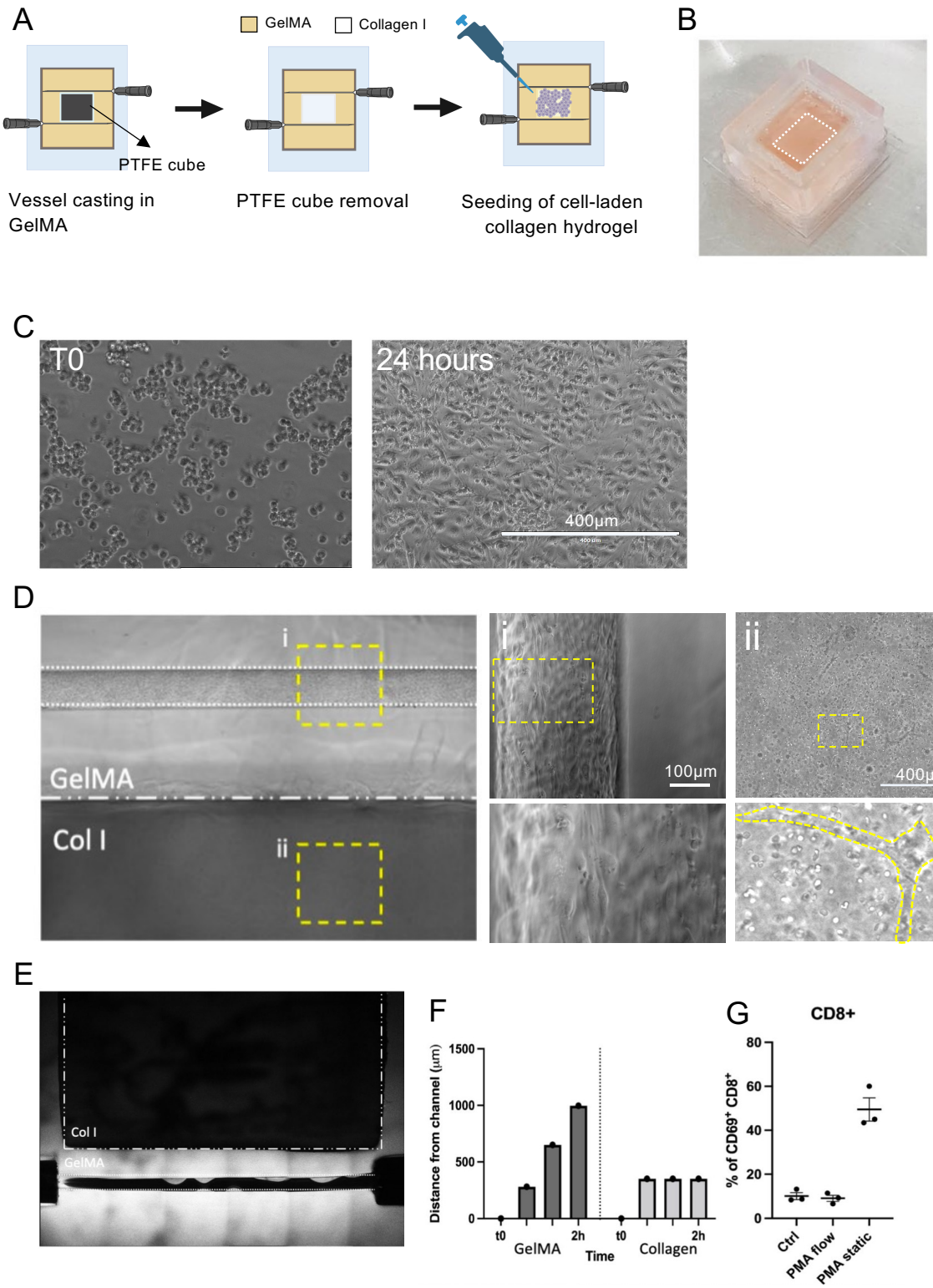


**Figure S1. eLN iteration with central compartment.** A) Schematic showing fabrication process of the LN device with central compartment. Within a PDMS mold, needles and a PTFE cube are used to create channel and central compartment voids respectively. GelMA (yellow) is then cast into the device and polymerized before the PTFE cube is removed and the central, collagen-based (white) cell-laden compartment is seeded. B) Representative photograph of a device: a PDMS chamber filled with GelMA and cell-laden collagen gel (demarked by dotted line) C) Murine LECs cultured on GelMA rapidly form a monolayer. D) Representative tilescan image showing a full needle-casted lymphatic vessel in GelMA, the clean interface

between GelMA and central compartment after optimization of the magnetic PTFE cube, ‘vessel’ channel (i) and cell-laden collagen central compartment (ii). Inset i) Representative zoomed images of a needle cast lymphatic vessel in GelMA and ii) stretched murine FRCs (outlined in yellow dashed line) with lymphocytes in the collagen central compartment. Scale bar = 400μm. E) Representative tile-scan image obtained with a confocal microscope shows convection of 70kDa dextran from a perfused acellular channel into GelMA but not into the Collagen I central compartment after 2h. Dextran shown in greyscale. Black represents lack of dextran within central collagen matrix. F) Quantification of fluorescent dextran front speed, calculated in the GelMA and at the device midpoint for 2h. G) Flow cytometry quantification of activated $CD69^{+}$ $CD8^{+}$ T cells after perfusion of PMA through the channel (PMA flow), or in static conditions bathing the gel (PMA static) vs. static control (ctrl). Each dot (n=3) represents an independent experiment. Data are mean ± SEM. Scale bars = 400 μm

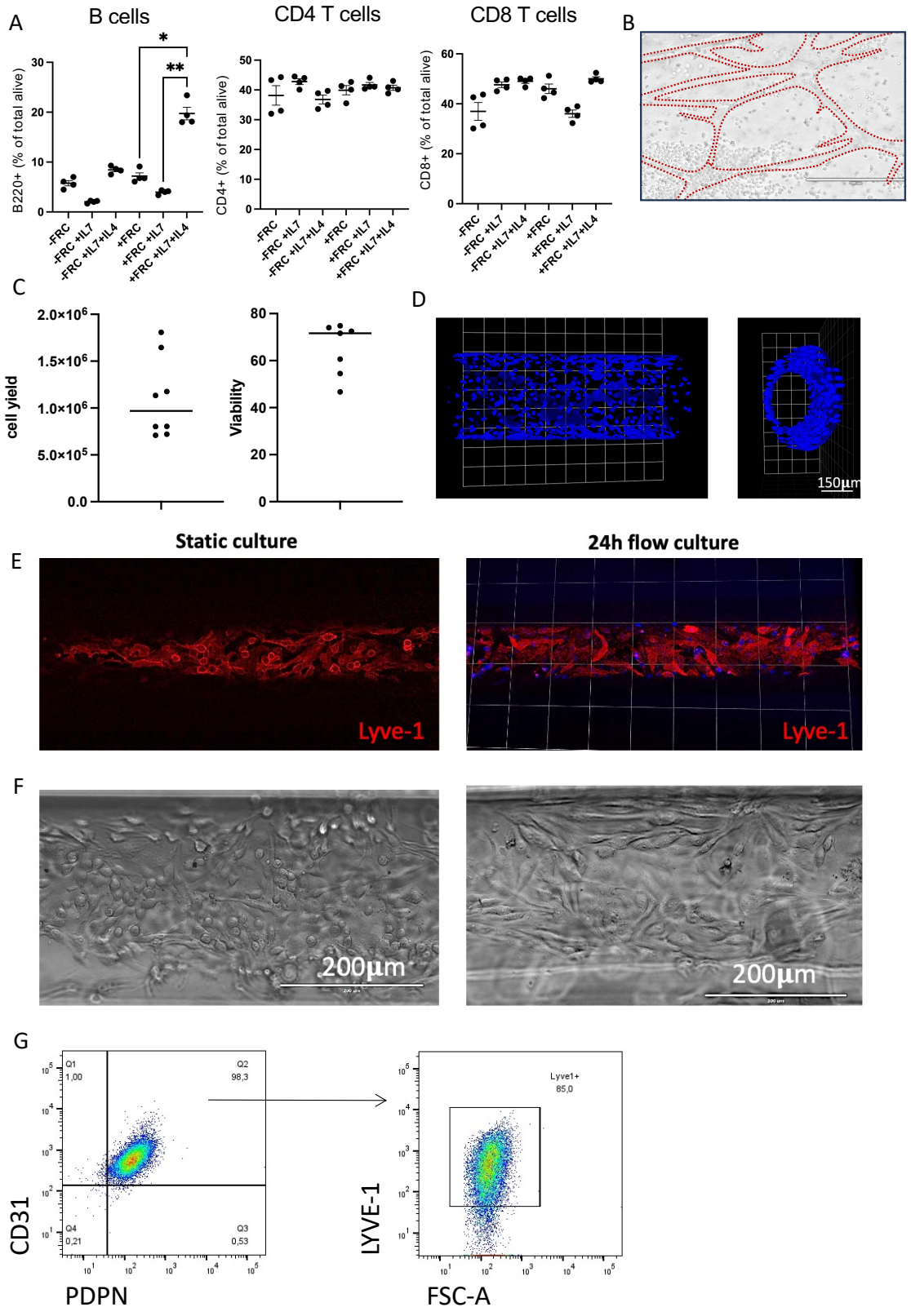


**Figure S2.** A) Flow cytometry quantification of murine immune cell abundance, expressed as % of total live cells 48 hours after seeding into hydrogel blends supplemented with different cytokines. N= 3 independent experiments. B) Representation of Figure 2E highlighting FRC network with red dotted lines C) Flow cytometry quantification of cell yield and viability from

hydrogel blend-based devices after gel digestion. Data shown as median n= 8 individual devices. D) DAPI staining of endothelial-lined channel. E) Representative LYVE-1 staining of LECs after static culture (left panel) and 24h of flow (right panel). F) Representative brightfield image of LECs in static culture (left panel) and showing elongated morphology after 24h flow culture (right panel). Scale bar = 200μm. G) Flow cytometry gating strategy for LECs characterization. LECs were identified in the $CD31^{+}/PDPN^{+}$ population and were LYVE-1 positive.

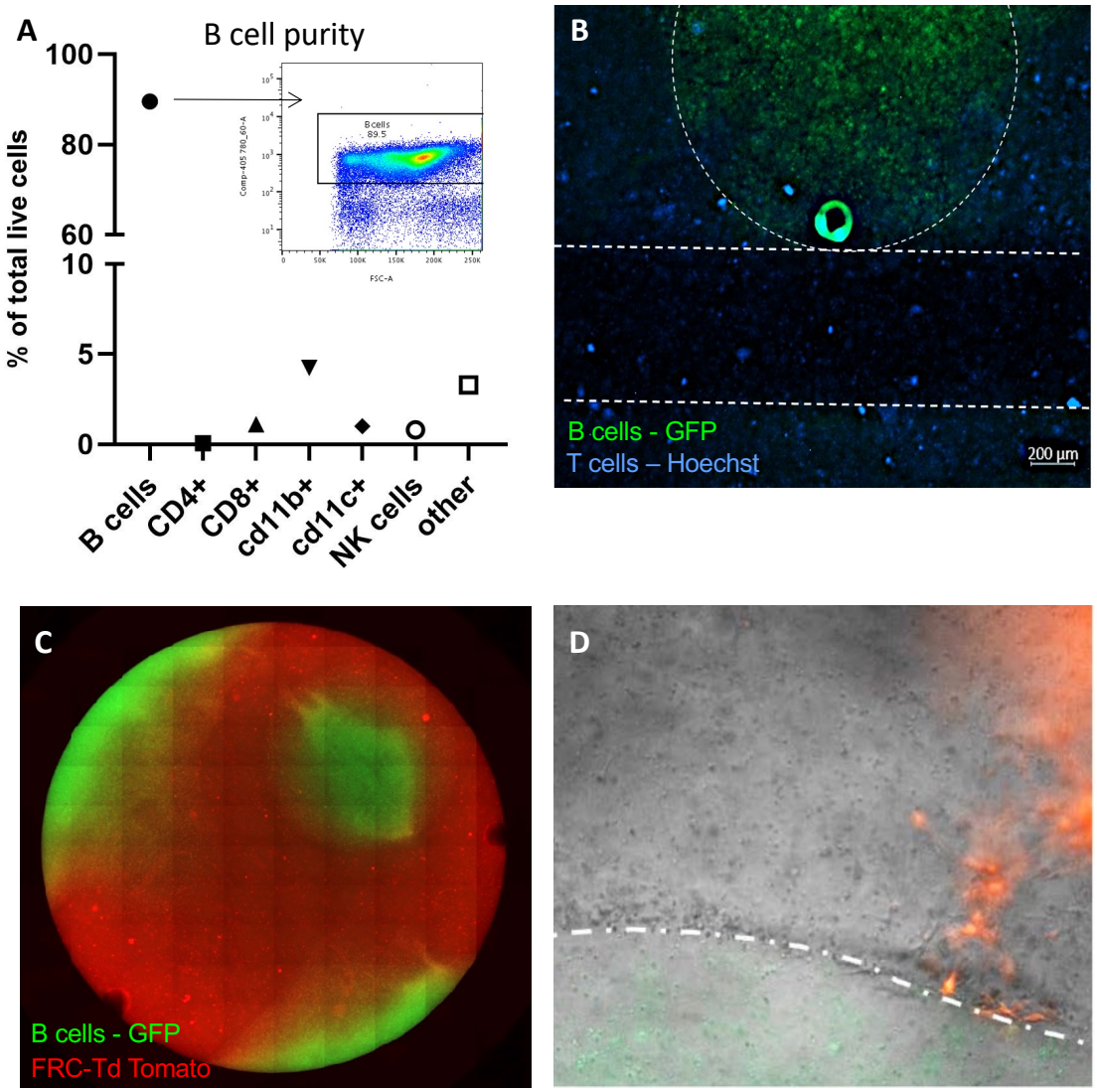


**Figure S3. A**) Post-purification quantification of murine immune populations. Eluate was analyzed by flow cytometry and B cells, CD4+, CD8+, cd11b+, cd11c+, NK cells were measured. B) Representative confocal image showing formation of B-cell-like structure (in green, with unlabeled FRC) surrounded by a T cell zone (in blue). Dotted line denotes the T-B boundary. Dashed line representative SCS channel boundaries. Scale bar = 200µm. C) Representative tilescan of a whole well with immune compartmentalization after first crosslinking the B cell zones then the whole gel; B cell follicles in green and TRCs in red mixed with unlabeled lymphocytes from the negative fraction. Scale bar = 1mm. D) Merged brightfield image showing a defined interface between the B cell follicle (green) and the T cell zone laden with TRC (orange) after 24h of culture. Cells of the T cell zone were unable to penetrate the follicle to interact.

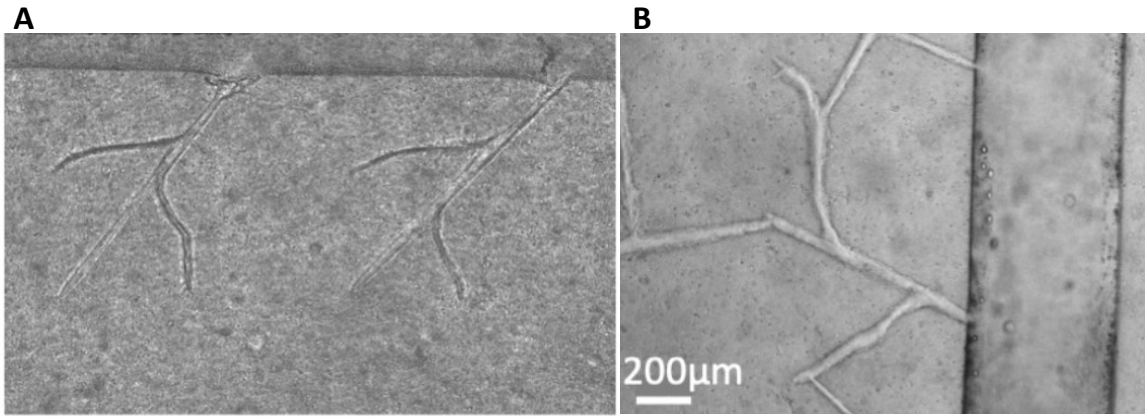


**Figure S4.** Conduits were successfully laser-carved via photoablation in a range of formations. A) Representative brightfield images showing repeated microchannels pattern along the main casted channel. B) Repeating, branched laser-carved conduits connected to each other Scale bar 200µm.

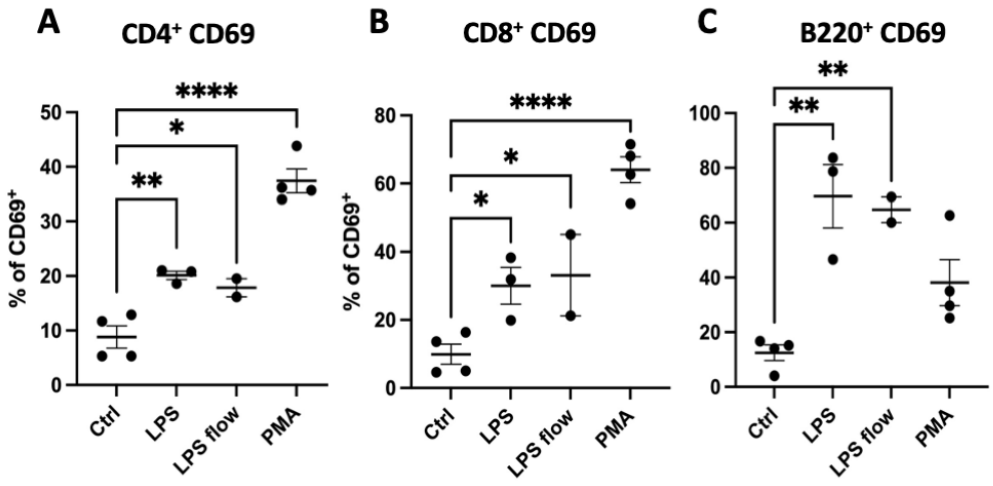


**Figure S5.** The LN device mounts an innate immune response to LPS. Flow cytometry quantification of the expression of CD69 in murine CD4+(A), CD8+ (B) and B220+ (C). Samples were either treated with LPS, statically and with flow, PMA/ionomycin, or normal cell culture medium. Each dot represents independent experiment (at least n=2). One-way ANOVA and Tukey's multiple comparisons tests were conducted. ns = not significant ($p > 0.05$), * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$.

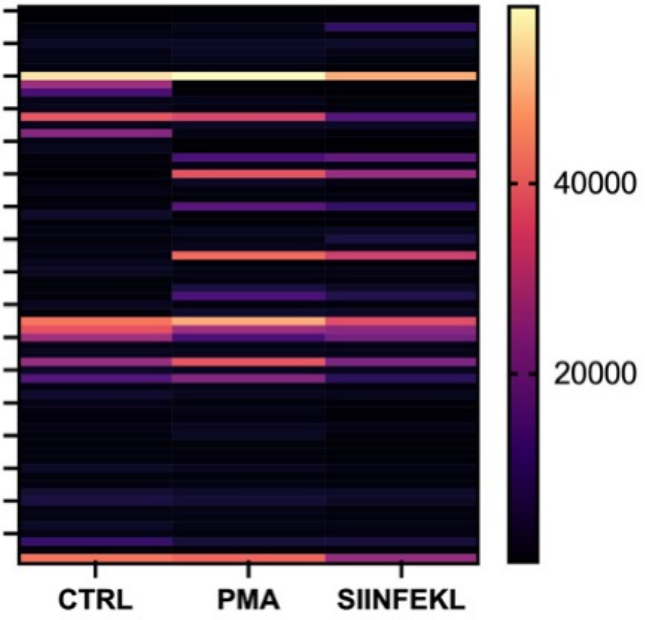


**Figure S6.** Heatmap of the cytokine array quantification obtained with ImageJ Macro 'Protein Array Analyzer'.

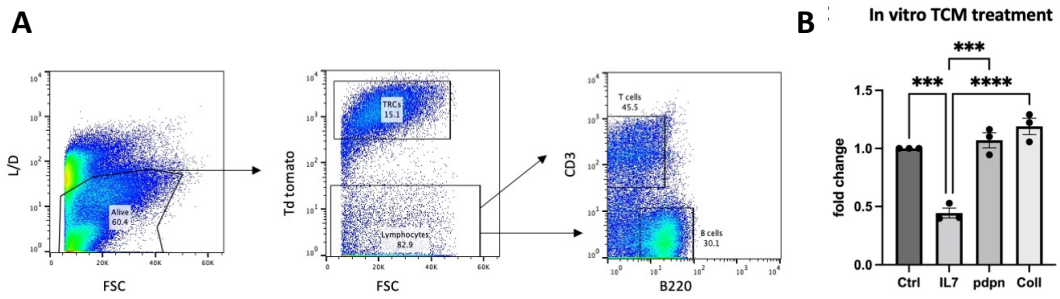


**Figure S7.** TCM causes changes in the gene expression of FRCs. A) Gating strategy used for cell sorting of the LN device after TCM treatment. Td tomato+ cells were separated by Td tomato- portion in which CD3 and B220 were used to pull out T and B cells. (B) Quantitative RT-PCR analysis of Il-7, Pdpn and Col1a1, showed as fold change. Each dot (n=3) represents an independent experiment. One-way ANOVA and Tukey's multiple comparisons tests were conducted. ns = not significant ($p > 0.05$), * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$.

**Table S1**

**Flow Cytometry Antibodies**. Table detailing the clone, species, company, dilution and conjugates for each of the primary antibodies used for flow cytometric studies.

| Antibody | Clone | Species | Dilution | Conjugate | Company (Cat. No.) |
|---|---|---|---|---|---|
| **B220** | RA3-6B2 | Rat | 1:200 | Biotin, APC-Cy7 | Biolegend (103203), (103224) |
| **CD3ε** | 17A2, 1452C11 | Armenian Hamster | 1:200 | AF488, PE | Biolegend (100210), (100308) |
| **CD4** | GK1.5 | Rat | 1:200 | BV-785, AF488, PE, PE-Cy7 | Biolegend (100453), (100423), (100408) Invitrogen (25-0041-82) |
| **CD44** | IM7 | Rat | 1:200 | APC-eFluor 780 | eBiosciences (47-0441) |
| **CD62L** | MEL-14 | Rat | 1:200 | APC, PE-Cy7 | Biolegend (104412), (103318) |
| **CD69** | H1.2F3 | Hamster | 1:200 | APC | BD Pharmingen (560689) |
| **CD8a** | 53-6.7 | Rat | 1:200 | BV-785, PE, APC eFluro780 | Biolegend (100750), (100758) Invitrogen (47-0081-80) |

**Movies**

**Movie S1.** Lymphocytes moving in the collagen/GelMA blend hydrogel around FRCs (in orange).

**Movie S2.** FRCs (in orange) unable to enter the B cell follicle (in green) due to the hard interface with the surrounding matrix.