Supplementary methods

Analysis of FOXC2 expression by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted from LECs cultured on-chip and LECs cultured on a T75 flask (Thermo Fisher Scientific, Waltham, MA, USA) using TRIzol (Invitrogen, Waltham, MA, USA). At the end of the 4-day culture period, syringes were removed from the Luer connectors and culture medium was aspirated from the chips. To induce cell lysis, 100 µL of TRIzol were added to each Luer and 50 µL were added to the LEC channel (outlet) for each sample in the chip (3 per idenTx 3 device). Chips were incubated for 40 min on ice after which TRIzol was pipetted up-down before sample collection. Four samples were pooled to ensure sufficient RNA yield. For flask culture, at the end of the 4-day culture period cells were detached with TrypLE (Gibco, Life Technologies, Carlsbad, CA, USA) and 1 mL TRIzol was added to $0.5x10^6$ cells for RNA isolation. RNA was extracted following manufacturer's instructions using 10 µg of glycogen (Invitrogen) per extraction to facilitate RNA precipitation. RNA was quantified by UV absorbance using a Nanodrop 8000 spectrophotometer (Thermo Fisher Scientific).

Reverse transcription was carried out using the High-Capacity cDNA Reverse Transcription (RT) kit (Applied Biosystems, Waltham, MA, USA) with 100 ng of total RNA per sample in a 20 µL final reaction volume. First, a 2X RT master mix was prepared following manufacturer's instructions containing 10X RT buffer, 25X dNTP Mix (100 nM), 10X RT random primers, MultiScribe™ Reverse Transcriptase and nuclease free water. Then, 10 µL of this master mix were mixed with 10 µL RNA sample and the RT was conducted with the following cycle: 10 min at 25 °C, 120 min at 37 °C and 5 min at 85 °C, after which samples were held at 4 °C.

The resulting cDNA (10 ng) was used to assemble qRT-PCR reactions in a final volume of 10 µL containing TaqMan Fast Advanced Master mix and TaqMan FAM-MGB gene expression assays Hs00270951_s1 (FOXC2), Hs02786624_g1 (GAPDH) and Hs01060665_g1 (ACTB) (Applied Biosystems). The thermal cycling was done in a QuantStudio™ 7 Flex Real-Time PCR system (Thermo Fisher Scientific) performing a 20 s hold at 95 °C followed by 40 cycles of 1 s at 95 °C and 20 s at 60 °C. No template controls were used in each reaction as negative control. FOXC2 expression values in LECs on-chip were normalized to internal controls (β-actin and GAPDH) and presented as fold change relative to LECs cultured on flask using the comparative Ct method.

Supplementary figures

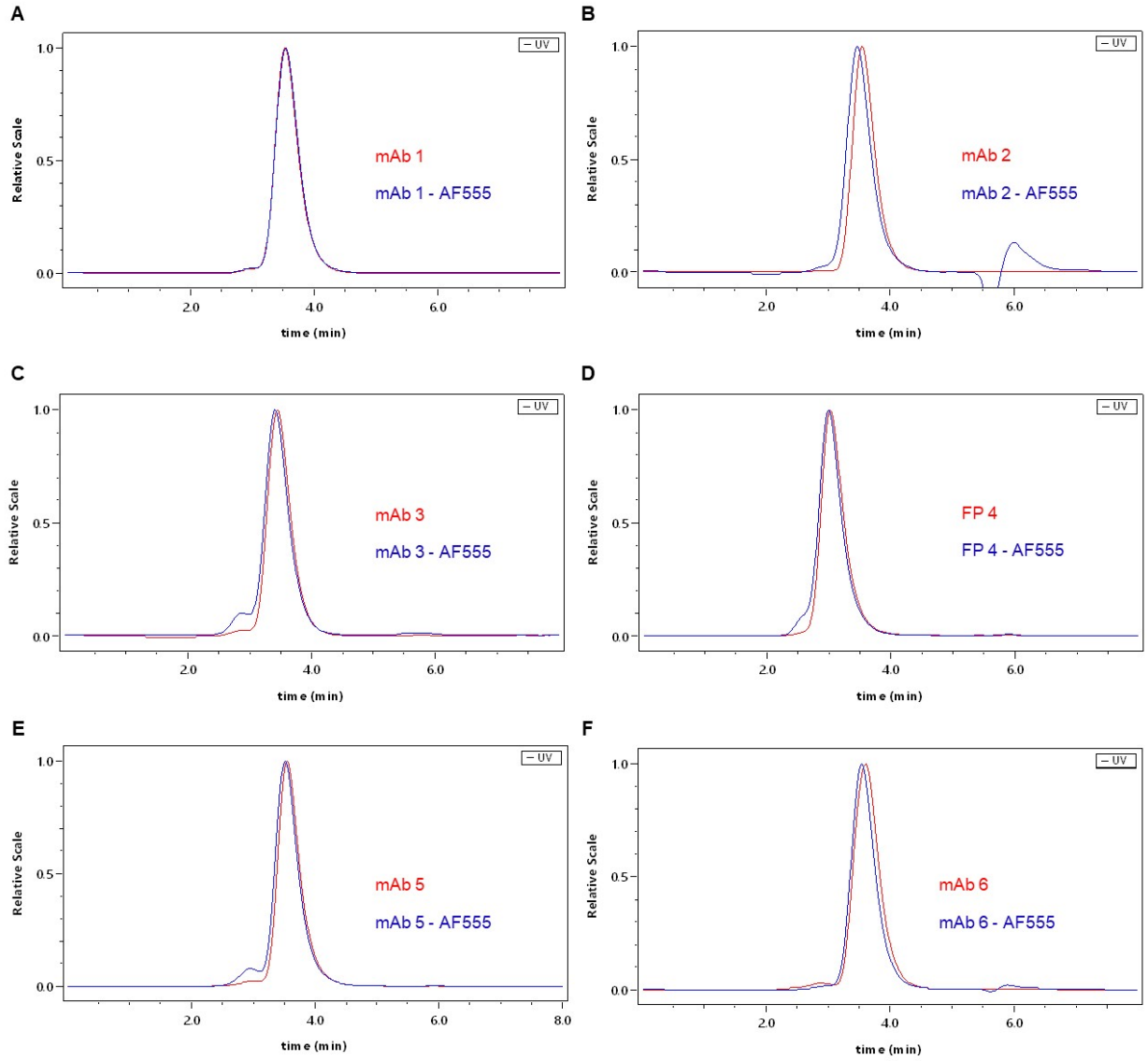

Fig. S1. Size exclusion chromatograms of monoclonal antibodies (mAb) and fusion protein (FP) before and after labeling. A-F: chromatograms of molecule list 1-6 showing the unconjugated and Alexa Fluor 555 (AF555) conjugated molecule in red and blue, respectively.

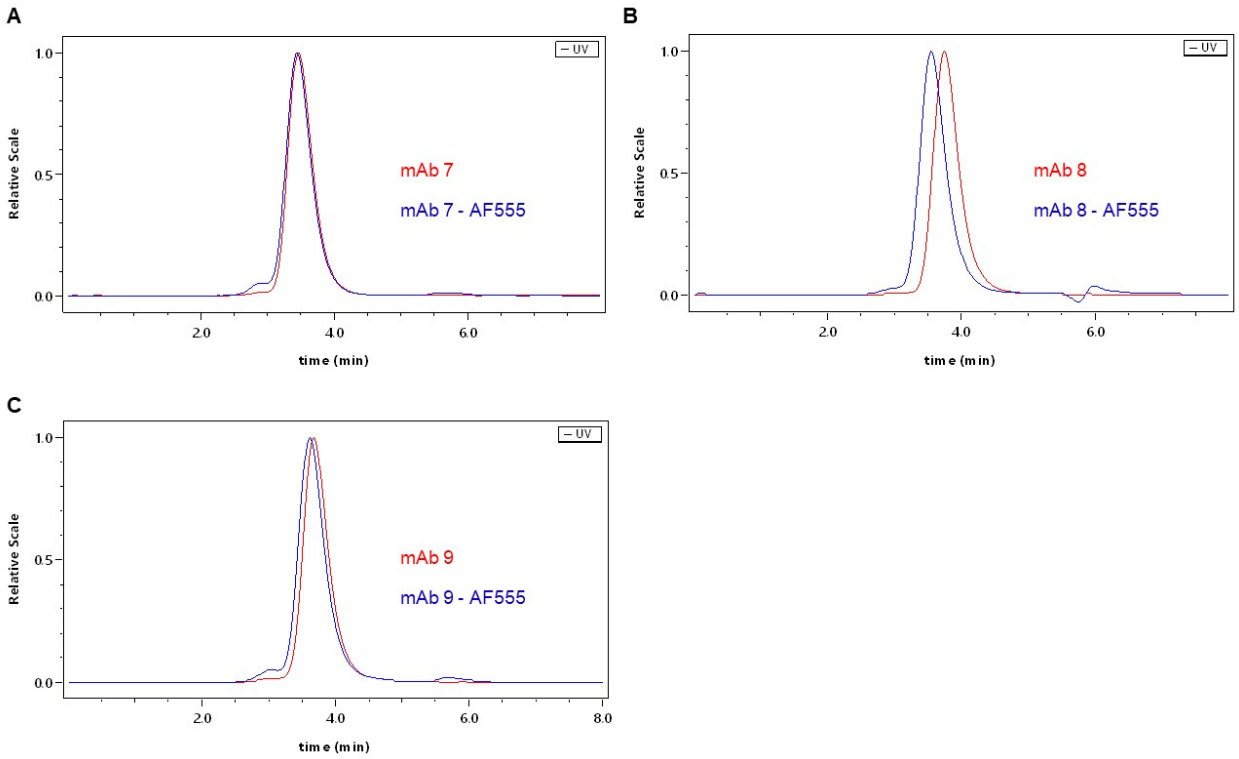

Fig. S2. Size exclusion chromatograms of monoclonal antibodies (mAb) and fusion protein (FP) before and after labeling. A-F: chromatograms of molecule list 7-9 showing the unconjugated and Alexa Fluor 555 (AF555) conjugated molecule in red and blue, respectively.

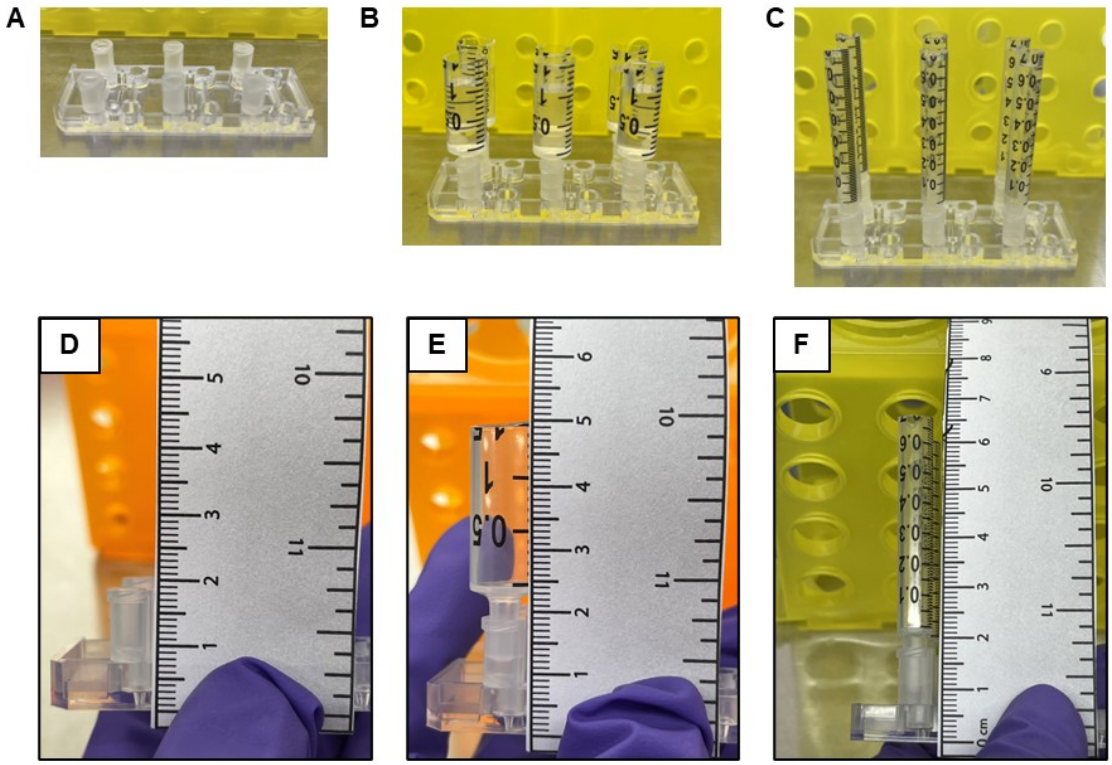

Fig. S3. Chip setups for hydrostatic pressure driven flow induction. A) Luer setup used for antibody transport measurements. B) 3 mL syringe setup used for lymphatic endothelial cell (LEC) growth. C) 1 mL syringe setup used for LEC growth. D) Pressure head in luer setup (~1.8 cm). E) Pressure head in 3 mL syringe setup (~4.2 cm). F) Pressure head in 1 mL syringe setup (~6.3 cm).

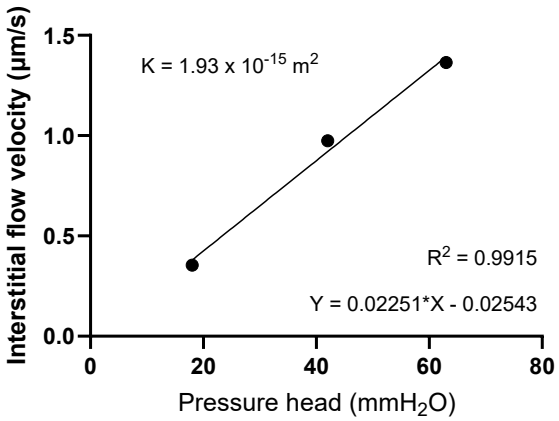

Fig. S4. Correlation analysis of pressure head and interstitial flow velocity. Linear regression analysis of the pressure head established by the different flow setups: luer (~18 mmH_2O), 3 mL syringe (~42 mmH_2O) and 1 mL syringe (~63 mmH_2O), and the resulting interstitial flow velocity within the extracellular matrix (R^2 = 0.9915). The resulting equation is used to calculate the hydraulic permeability at 18 mmH_2O as follows: Hydraulic permeability (K) = gel width (m) * viscosity (kg/m/s) * velocity (m/s) / pressure difference ($kg/m/s^2$) K= 1.3e-3 m * 6.9e-4 kg/m/s * 0.3797e-6 m/s / 176.58 $kg/m/s^2$ = 1.93e-15 m^2.

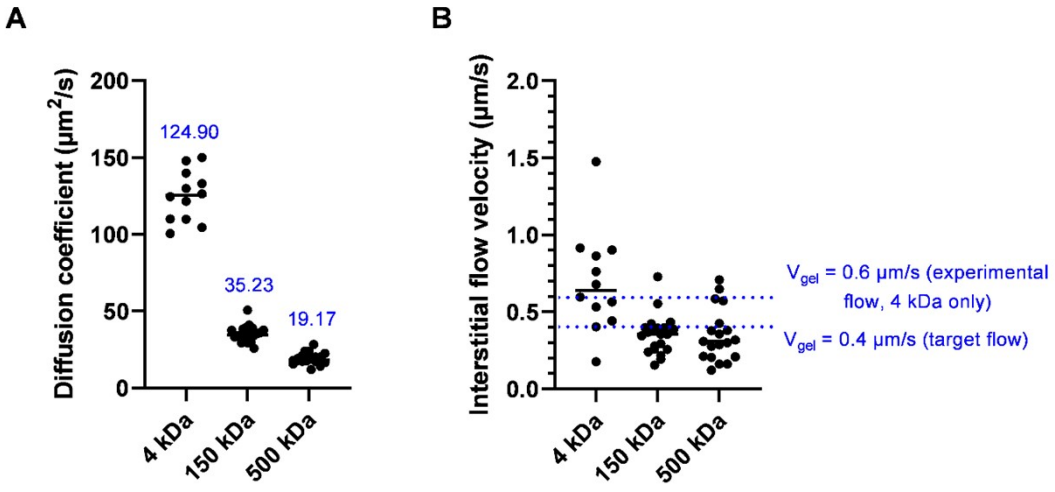

Fig. S5. Measured (black) vs simulated (blue) transport parameters for 4, 150 and 500 kDa FITC-dextran transport evaluations. A) Diffusion coefficients. B) Interstitial flow velocities. Experimental flow in simulations (v_{gel}=0.6 µm/s) was achieved by a 2-fold decrease in gel resistance as well as a 2-fold increase of the membrane conductivity compared to the conditions used for the simulation of target flow (v_{gel}= 0.4 µm/s).

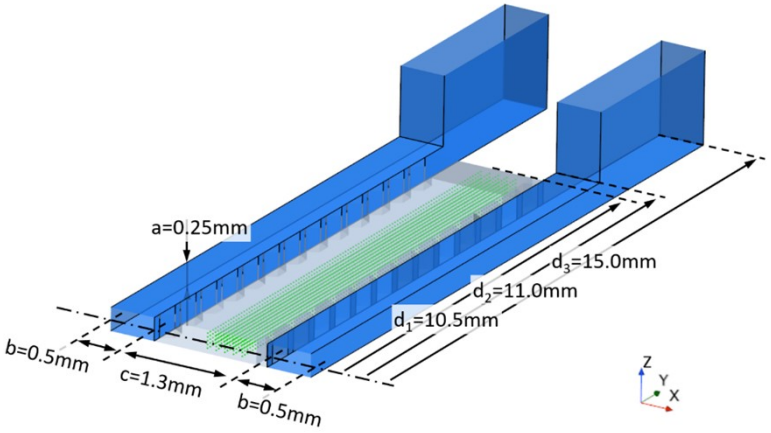

Fig. S6. Geometric dimensions of in- and outlet channel (blue) and gel matrix (grey) with lymph vessel locations (green). Only half-model is shown due to symmetry to the xz-plane (dashed dotted center line).

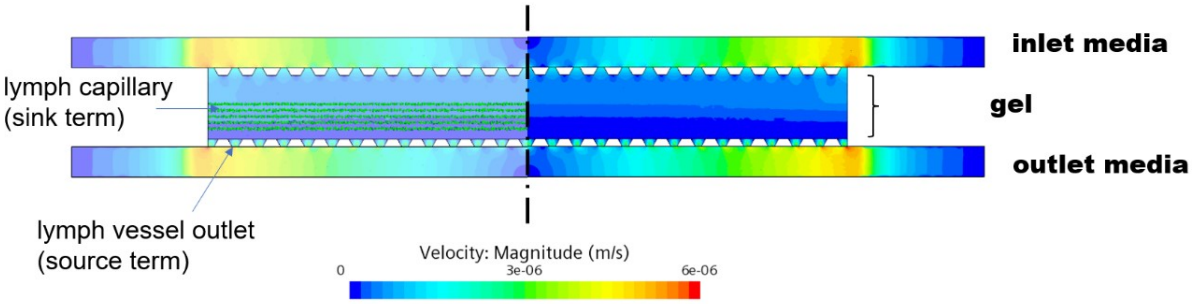

Fig. S7. Flow velocities in CFD simulation of the MPS with target gel flow velocity v_{gel}= 0.4 µm/s showing the reduced velocity in the gel at the absorption sites and the high flows at the locations of re-administration into the outlet channel. For better visibility of the contour plot the elements used for lymph capillary absorption are not visualized on the right-hand side of the graphic. With the dimensions a= 0.25 mm and b= 0.5 mm from **Fig. S6** the Reynolds number for maximum flow velocity 6 µm/s is calculated to Re= $\rho * v * D_h/\mu$=0.003 with D_h= 2ab/(a+b).

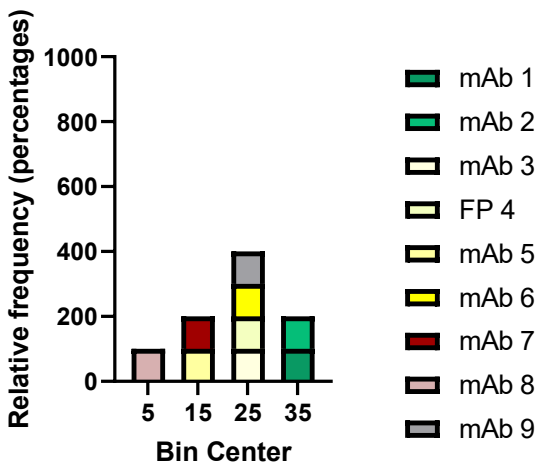

Fig. S8. Frequency distribution analysis of percentage monoclonal antibody (mAb) and fusion protein (FP) transport on-chip showing a 4-bin classification of the molecules. Bin width = 10; center of first bin = 5; center of last bin = 35.

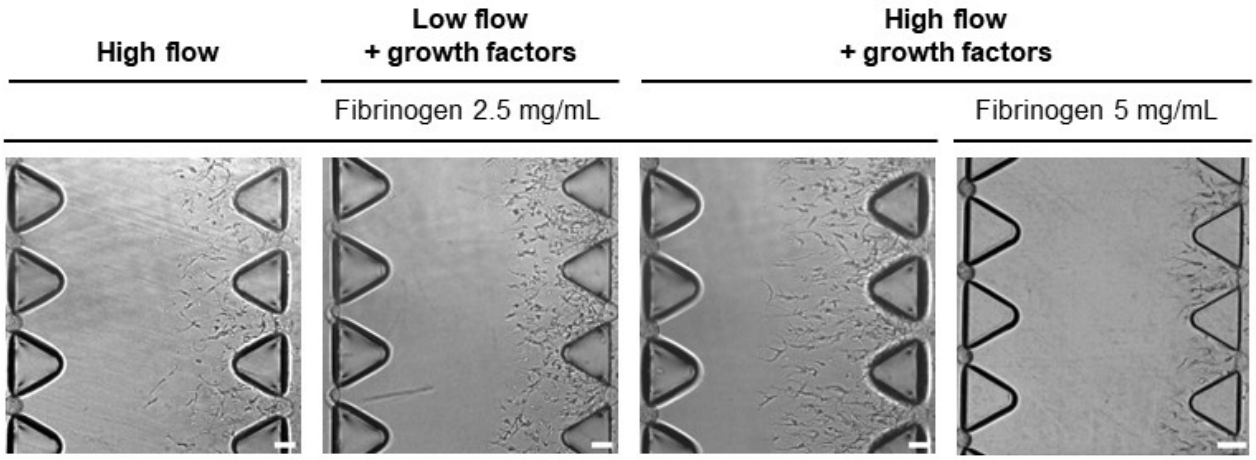

Fig. S9. Bright field images showing the morphology of lymphatic capillaries grown under high and low flow, in 2.5 and 5 mg/mL fibrin EMCs, with and without growth factor supplementation. Scale bar = 100 μm.

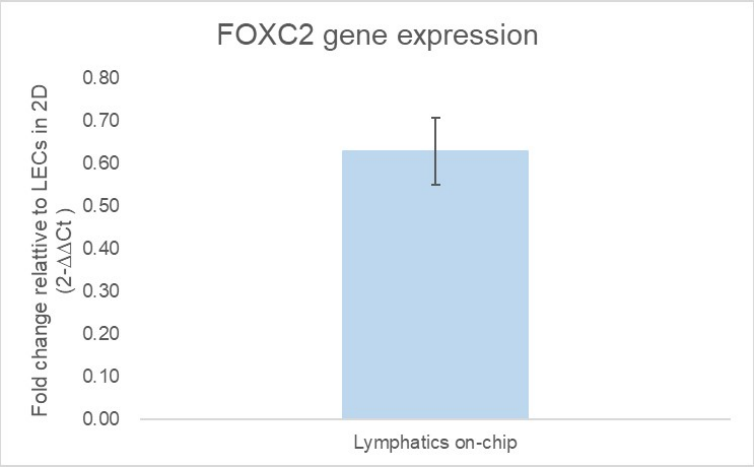

Fig. S10. FOXC2 mRNA expression in lymphatics on-chip. Expression values in LECs on-chip were normalized to internal controls (β-actin and GAPDH) and presented as fold change relative to LECs cultured on 2D flask using the comparative Ct method.

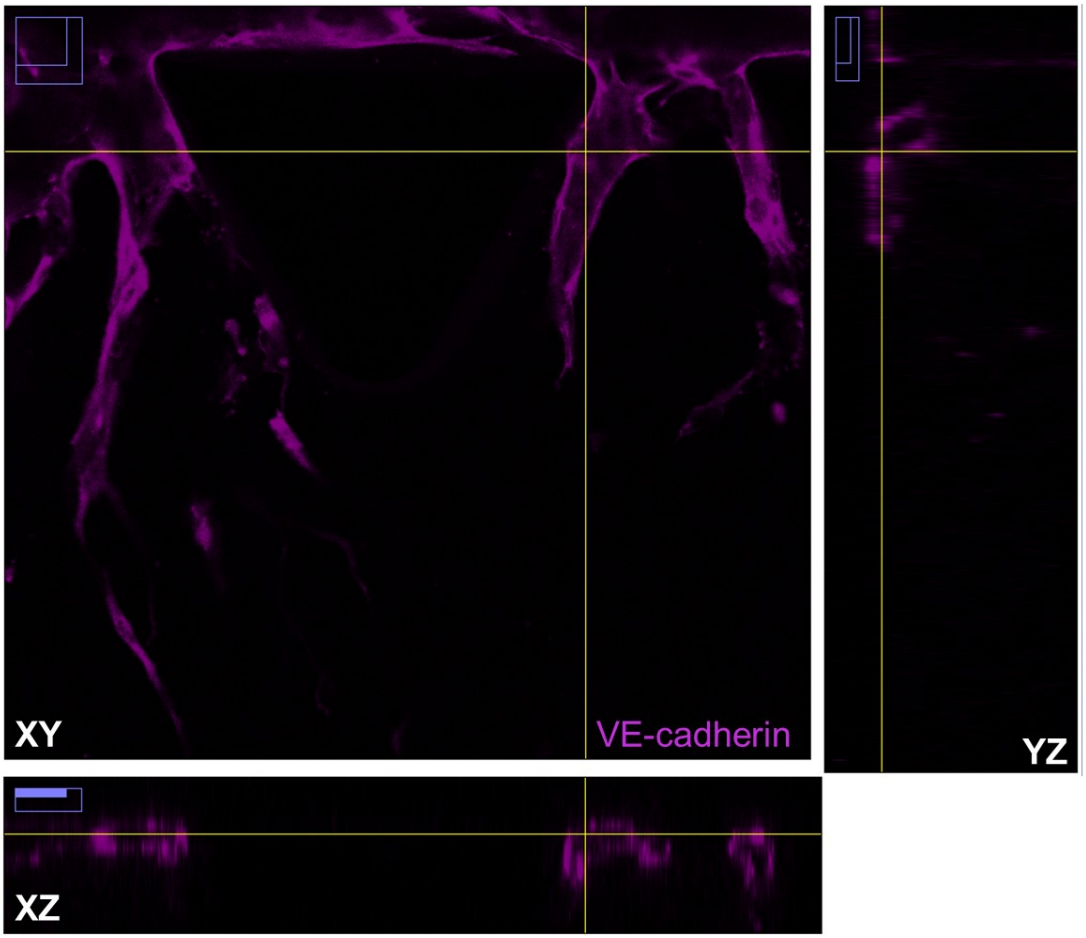

Fig. S11. Confocal cross-sections of VE-cadherin immunostaining showing lymphatic capillary lumens. XY image shows the morphology of lymphatic capillaries growing from the lymphatic tubule in the

outlet channel (top) into the extracellular matrix in the central channel (bottom). Cross-sections of the capillary lumens are shown in the XZ and YZ images, marked by the yellow lines. Image dimensions are XY: 708.49x708.49 µm; XZ: 708.49x253.29 µm and YZ: 253.29x708.49 µm.

Table S1. Material parameters for fluids, gel and membrane used for lymphatic chip simulations with computational fluid dynamics software.

Fluid Properties

Density (ρ, kg/m$^{3)}$	1000.0
Viscosity (µ, Pa*s)	6.9e-4
Diffusion coefficient for 4 kDa (D_4, µm^2/s)	124.9
Diffusion coefficient for 150 kDa (D_{150}, µm^2/s)	35.2

Gel Properties

Porosity (χ, --)	0.9
Porous viscous resistance for target flow (P_v, kg/m^3/s)	1e11
Porous viscous resistance for exp. flow (P_v, kg/m^3/s)	0.5e11

Membrane Properties

Thickness (Δx, µm)	1.0
Hydraulic conductivity for target flow (K_L, m^2)	1.0e-18
Hydraulic conductivity for exp. flow (K_L, m^2)	2.0e-18

Table S2. Fluorescence intensity values detected by confocal microscopy in the outlet and inlet (reference) chip channels and corresponding drainage rate values calculated for fluorescein isothiocyanate-dextran of 4 kDa.

Dextran 4 kDa						
Time (min)	**Mean fluorescence intensity (n=3)**			**Drainage rate (1/min) (n=3)**		
0	308.923	376.636	332.402			
2	285.783	353.295	340.518	-0.00042	-0.00043	0.000211
4	310.554	543.301	358.64	0.000452	0.003534	0.00047
6	456.827	1114.475	421.485	0.00267	0.010622	0.00163
8	817.793	2090.094	526.261	0.006589	0.018144	0.002718
10	1380.343	3215.411	738.919	0.010269	0.020928	0.005516
12	2210.958	4479.696	1083.197	0.015162	0.023512	0.00893
14	3312.04	5792.161	1562.312	0.020099	0.024408	0.012427
16	4499.643	7177.83	2277.098	0.021678	0.02577	0.01854
18	5666.84	8603.799	3175.466	0.021306	0.026519	0.023301
20	6741.724	9890.584	4054.231	0.019621	0.023931	0.022793
22	7806.882	11196.65	5117.516	0.019443	0.024289	0.027579
24	8902.579	12533.11	6370.354	0.020001	0.024855	0.032496
26	9929.354	13678.28	7565.425	0.018743	0.021297	0.030997
28	10940.692	14697.2	8650.904	0.018461	0.018949	0.028155
30	11980.47	15713.42	9911.693	0.01898	0.018899	0.032702
Reference	27391.444	26885.64	19277.067			

Table S3. Fluorescence intensity values detected by confocal microscopy in the outlet and inlet (reference) chip channels and corresponding drainage rate values calculated for fluorescein isothiocyanate-dextran of 150 kDa.

Dextran 150 kDa								
Time (min)	**Mean fluorescence intensity (n=4)**				**Drainage rate (1/min) (n=4)**			
0	314.455	313.407	252.072	281.143				
2	284.053	310.21	286.237	229.626	-0.00048	-0.0001	0.000751	-0.00118
4	289.455	339.219	244.337	228.278	8.54E-05	0.000912	-0.00092	-3.1E-05
6	341.125	383.712	260.07	239.074	0.000817	0.001399	0.000346	0.000246
8	512.779	464.268	289.967	294.381	0.002714	0.002533	0.000658	0.001262
10	923.163	620.215	366.659	480.345	0.006489	0.004904	0.001687	0.004244
12	1596.636	858.59	512.393	823.961	0.01065	0.007497	0.003205	0.007842
14	2500.848	1167.642	745.138	1374.949	0.014298	0.009719	0.005119	0.012574
16	3647.629	1609.417	1050.702	2089.123	0.018134	0.013893	0.00672	0.016298
18	5002.178	2162.725	1464.786	2985.897	0.021419	0.017401	0.009107	0.020465
20	6440.882	2752.866	1947.709	3962.381	0.02275	0.018559	0.010621	0.022284
22	7902.854	3413.98	2475.545	4925.954	0.023118	0.020791	0.011609	0.02199
24	9556.24	4174.561	3051.693	5902.047	0.026145	0.02392	0.012672	0.022275
26	11172.412	4957.157	3654.356	6978.572	0.025556	0.024612	0.013255	0.024567
28	12748.981	5716.462	4297.961	7941.059	0.02493	0.023879	0.014155	0.021965
30	14230.691	6457.991	4916.189	8677.694	0.02343	0.02332	0.013597	0.016811
Reference	31619.66	15898.75	22733.898	21909.6				

Table S4. Fluorescence intensity values detected by confocal microscopy in the outlet and inlet (reference) chip channels and corresponding drainage rate values calculated for fluorescein isothiocyanate-dextran of 500 kDa.

Dextran 500 kDa						
Time (min)	**Mean fluorescence intensity (n=3)**			**Drainage rate (1/min) (n=3)**		
0	266.232	297.724	319.84	-0.00039	-0.00025	0.001068
2	251.402	288.825	360.479	0.000399	5.36E-05	-0.00064
4	266.729	290.746	336.184	-5.9E-05	-0.00018	0.000494
6	264.467	284.306	354.973	-3.6E-05	5.3E-05	-4.6E-05
8	263.09	286.205	353.221	0.000115	0.00014	0.000903
10	267.515	291.222	387.6	0.00031	0.00064	0.001932
12	279.424	314.163	461.134	0.000784	0.002175	0.004525
14	309.504	392.082	633.377	0.001758	0.003983	0.008546
16	376.98	534.782	958.711	0.003177	0.008114	0.012788
18	498.914	825.465	1445.515	0.004496	0.011673	0.014196
20	671.489	1243.647	1985.942	0.005977	0.013831	0.01732
22	900.876	1739.121	2645.272	0.008473	0.015677	0.022227
24	1226.066	2300.725	3491.423	0.010029	0.022346	0.020903

26	1610.988	3101.225	4287.169	0.012277	0.025731	0.021153
28	2082.189	4023.012	5092.42	0.013949	0.027514	0.023066
30	2617.546	5008.66	5970.518			
Reference	19190.375	17911.76	19034.228			

Table S5. Fluorescence intensity values detected by confocal microscopy in the outlet and inlet (reference) chip channels and corresponding drainage rate values calculated for IgG-DyLight650.

IgG																	
Time (min)	**Mean fluorescence intensity (n=8)**								**Drainage rate (1/min) (n=8)**								
0	307.863	375.736	328.03	379.563	313.407	332.413	450.066	332.402									
2	316.567	361.751	325.065	378.296	310.21	331.319	444.351	340.518	0.000222	-0.00041	-7.768E-05	-3.4E-05	-0.0001	-2.8663E-05	-0.00015	0.000211	
4	335.321	397.065	346.791	394.575	339.219	341.363	476.075	358.64	0.000479	0.001047	0.00056922	0.000438	0.000912	0.000263153	0.000853	0.00047	
6	374.194	490.692	373.771	455.075	383.712	380.724	579.469	421.485	0.000992	0.002776	0.00070688	0.00163	0.001399	0.00103126	0.00278	0.00163	
8	448.693	659.014	447.603	570.285	464.268	455.67	795.92	526.261	0.001902	0.00499	0.0019344	0.003103	0.002533	0.001963588	0.005819	0.002718	
10	550.498	938.866	555.072	773.301	620.215	576.526	1164.063	738.919	0.002598	0.008296	0.00281569	0.005468	0.004904	0.003166432	0.009897	0.005516	
12	697.425	1357.337	736.257	1090.35	858.59	775.961	1712.528	1083.197	0.00375	0.012405	0.00474705	0.00854	0.007497	0.005225205	0.014744	0.00893	
14	879.339	1888.366	996.407	1480.439	1167.642	1120.939	2420.171	1562.312	0.004643	0.015742	0.00681594	0.010507	0.009719	0.009038437	0.019023	0.012427	
16	1133.355	2540.303	1358.515	2023.139	1609.417	1602.626	3277.55	2277.098	0.006484	0.019326	0.00948724	0.014618	0.013893	0.012620218	0.023049	0.01854	
18	1474.727	3317.276	1818.276	2651.202	2162.725	2177.577	4267.042	3175.466	0.008713	0.023033	0.01204576	0.016917	0.017401	0.015063739	0.0266	0.023301	
20	1823.747	4134.648	2361.931	3264.723	2752.866	2788.807	5399.748	4054.231	0.008908	0.02423	0.01424378	0.016525	0.018559	0.01601425	0.03045	0.022793	
22	2209.261	4952.557	2950.09	3962.124	3413.98	3463.279	6565.223	5117.516	0.00984	0.024246	0.01540979	0.018785	0.020791	0.017671193	0.031331	0.027579	
24	2682.965	5814.963	3595.309	4848.247	4174.561	4230.253	7740.432	6370.354	0.012091	0.025565	0.01690476	0.023868	0.02392	0.020094749	0.031593	0.032496	
26	3203.244	6675.988	4365.752	5597.874	4957.157	5032.213	8878.202	7565.425	0.01328	0.025525	0.02018564	0.020191	0.024612	0.021011383	0.030586	0.030997	
28	3764.721	7462.283	5276.342	6341.961	5716.462	5874.316	9974	8650.904	0.014331	0.023309	0.02385749	0.020042	0.023879	0.022063132	0.029458	0.028155	
30	4395.244	8280.324	6176.188	7214.08	6457.991	6800.116	10936.61	9911.693	0.016094	0.02425	0.023576	0.023491	0.02332	0.024255996	0.025878	0.032702	
Reference	19589.357	16866.61	19083.941	18563.14	15898.75	19083.94	18599.28	19277.07									

Table S6. Fluorescence intensity values detected by confocal microscopy in the outlet channel for fluorescein isothiocyanate-dextran of 4 kDa normalized to the values detected in the inlet (reference) channel.

Dextran 4 kDa			
Time (min)	**Mean fluorescence intensity normalized to reference (n=3)**		
0	0.011278	0.014009	0.017243391
2	0.010433	0.013141	0.017664409
4	0.011338	0.020208	0.01860449
6	0.016678	0.041452	0.021864581
8	0.029856	0.07774	0.027299848
10	0.050393	0.119596	0.038331506
12	0.080717	0.16662	0.056190965
14	0.120915	0.215437	0.081045109
16	0.164272	0.266976	0.118124713
18	0.206884	0.320015	0.164727653
20	0.246125	0.367876	0.210313685
22	0.285012	0.416455	0.265471713
24	0.325013	0.466164	0.330462824
26	0.362498	0.508758	0.392457265
28	0.39942	0.546656	0.44876661
30	0.43738	0.584454	0.51417018

Table S7. Fluorescence intensity values detected by confocal microscopy in the outlet channel for fluorescein isothiocyanate-dextran of 150 kDa normalized to the values detected in the inlet (reference) channel.

Dextran 150 kDa				
Time (min)	**Mean fluorescence intensity normalized to reference (n=4)**			
0	0.009945	0.019713	0.011087936	0.012832
2	0.008983	0.019512	0.012590758	0.010481
4	0.009154	0.021336	0.010747695	0.010419
6	0.010788	0.024135	0.011439745	0.010912
8	0.016217	0.029202	0.01275483	0.013436
10	0.029196	0.03901	0.016128294	0.021924
12	0.050495	0.054004	0.022538722	0.037607
14	0.079092	0.073442	0.032776517	0.062756
16	0.11536	0.101229	0.046217415	0.095352
18	0.158198	0.136031	0.064431801	0.136283
20	0.203699	0.17315	0.085674221	0.180851
22	0.249935	0.214733	0.108892237	0.224831
24	0.302225	0.262572	0.134235361	0.269382
26	0.353338	0.311795	0.160744805	0.318517
28	0.403198	0.359554	0.189055172	0.362447
30	0.450058	0.406195	0.216249277	0.396068

Table S8. Normalized fluorescence intensity values detected by confocal microscopy for fluorescein isothiocyanate-dextran of 4 kDa (**Table S6**) transformed to concentration.

Dextran 4 kDa			
Time (min)	**Mean fluorescence intensity transformed to concentration (mg/mL) (n=3)**		
0	0.000112781	0.00014	0.000172
2	0.000104333	0.000131	0.000177
4	0.000113376	0.000202	0.000186
6	0.000166777	0.000415	0.000219
8	0.000298558	0.000777	0.000273
10	0.000503932	0.001196	0.000383
12	0.000807171	0.001666	0.000562
14	0.001209151	0.002154	0.00081
16	0.001642718	0.00267	0.001181
18	0.002068836	0.0032	0.001647
20	0.002461252	0.003679	0.002103
22	0.002850117	0.004165	0.002655
24	0.003250131	0.004662	0.003305
26	0.003624984	0.005088	0.003925
28	0.003994201	0.005467	0.004488
30	0.0043738	0.005845	0.005142

Table S9. Normalized fluorescence intensity values detected by confocal microscopy for fluorescein isothiocyanate-dextran of 150 kDa (**Table S7**) transformed to concentration.

Dextran 150 kDa				
Time (min)	**Mean fluorescence intensity transformed to concentration (mg/mL) (n=4)**			
0	9.94492E-05	0.000197	0.000111	0.000128
2	8.98343E-05	0.000195	0.000126	0.000105
4	9.15427E-05	0.000213	0.000107	0.000104
6	0.000107884	0.000241	0.000114	0.000109
8	0.000162171	0.000292	0.000128	0.000134
10	0.000291959	0.00039	0.000161	0.000219
12	0.00050495	0.00054	0.000225	0.000376
14	0.000790916	0.000734	0.000328	0.000628
16	0.001153595	0.001012	0.000462	0.000954
18	0.001581983	0.00136	0.000644	0.001363
20	0.002036986	0.001731	0.000857	0.001809
22	0.002499348	0.002147	0.001089	0.002248
24	0.003022246	0.002626	0.001342	0.002694
26	0.003533375	0.003118	0.001607	0.003185
28	0.004031979	0.003596	0.001891	0.003624
30	0.004500583	0.004062	0.002162	0.003961