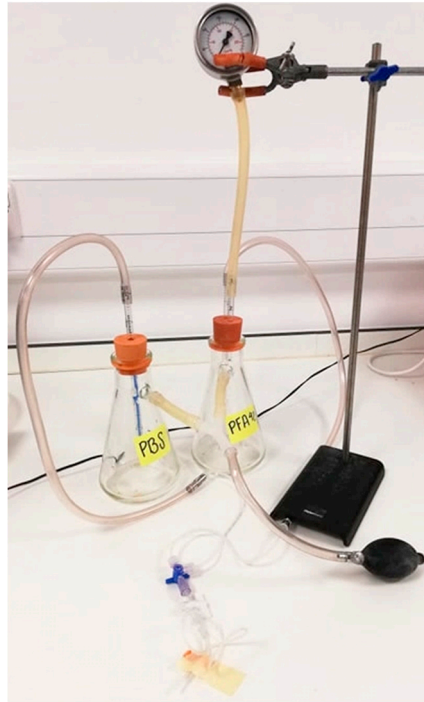
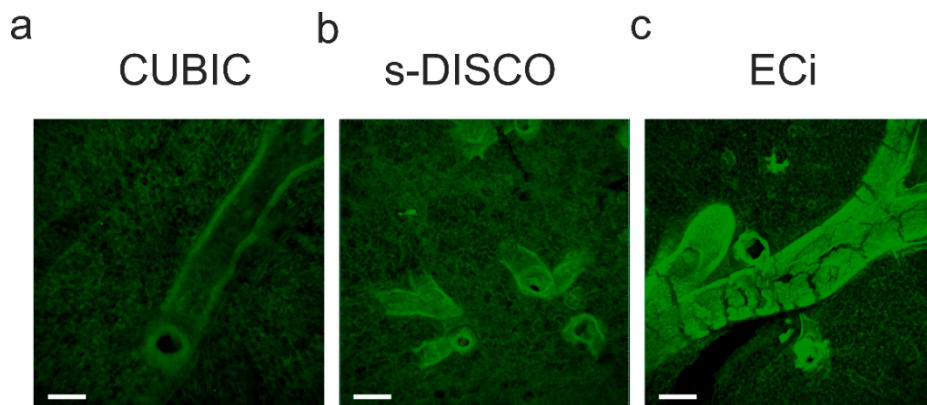


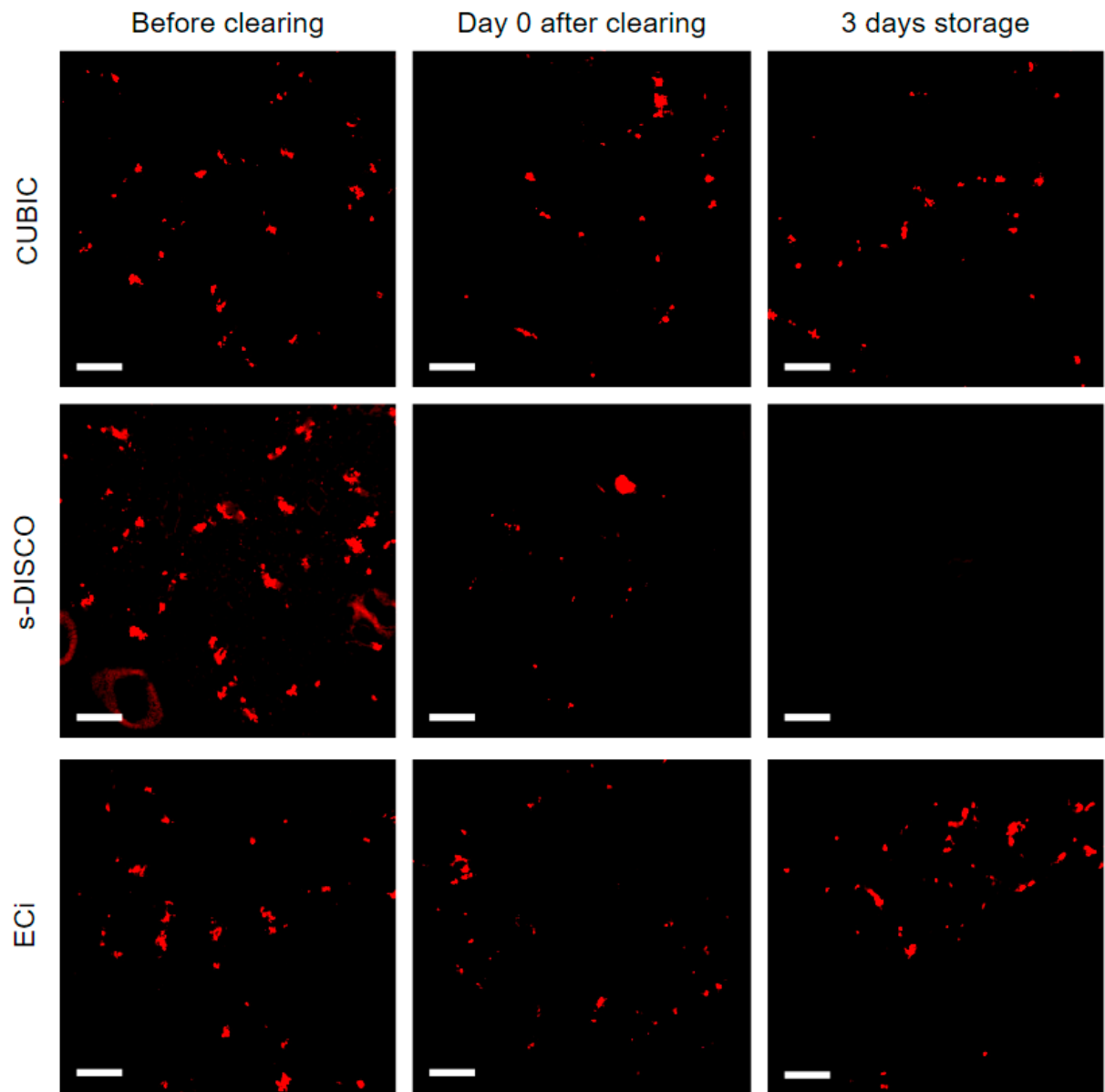
Supplementary figures



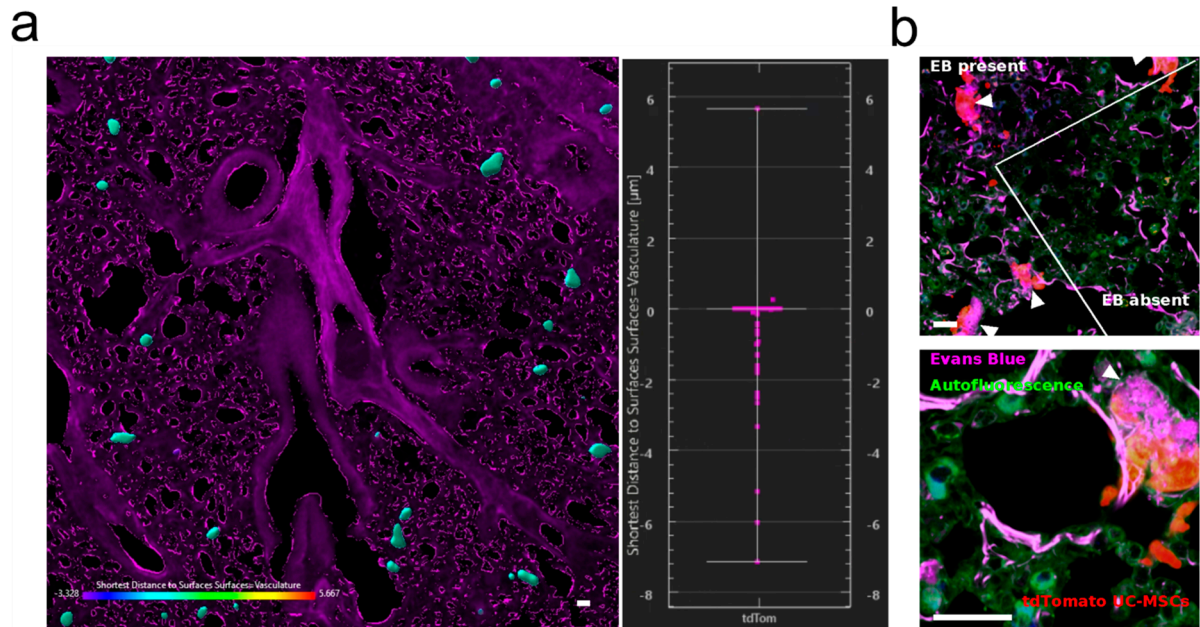
Supplementary figure S1. Perfusion pump. Two Erlenmeyer flasks, filled with either PBS or 4% PFA, are joined through tubes connected to their sidearms, which are connected to a manual bulb that helps pressurize the system. A pressure gauge is connected to one of the bottles to monitor and maintain pressure. Pipettes extend through rubber stoppers and down into the fluid in each flask. The pressure flowing through the sidearm pumps the liquid from each bottle into these pipettes and out of the flasks, where a stopcock allows fluid to flow from one flask at a time.



Supplementary figure S2. Lung morphology after optical tissue clearing. a) CUBIC cleared lung. b) s-DISCO cleared lung. c) ECI cleared lung. Scale bar = 150 μm . MIPs before image reconstruction were obtained by confocal imaging on a Leica DMI8 with Andor Dragonfly spinning disk, coupled to an EMCCD camera using a 10x/0.45 air objective. Z-stacks were captured using the 488 nm laser line and 525/50 emission filter.



Supplementary figure S3. Fluorescence preservation of tdTomato (red) after clearing 100 μ m thick lung sections. Impact of CUBIC, s-DISCO and ECi clearing on the fluorescence of tdTomato before, immediately after, and 3 days after of storage in RI solution.



Supplementary figure S4. Evans Blue vascular staining. a) Lung 30 μm cryosections. IMARIS 3D reconstruction and shortest distance calculation analysis. Distance analysis performed between the tdTomato hUC-MSCs (cyan) and the vasculature (magenta). $n = 9$. b) tdTomato hUC-MSCs obstructing EB flow through vasculature. Arrowheads indicate areas of EB accumulation. Scale bar 25 μm .

Supplementary methods

Solvent based optical tissue clearing of 100 μm lung sections.

Supplementary table S1. Solvent based optical tissue clearing of 100 μm lung sections.

	100 μm sections	
	s-DISCO	ECi
1-propanol 50%	3 min	3 min
1-propanol 80%	3 min	3 min
1-propanol 100%	3 x 3 min	3 x 3 min
DCM	1 min	-
DBE w/ 0.4% propyl gallate	storage	-
ECi	-	storage