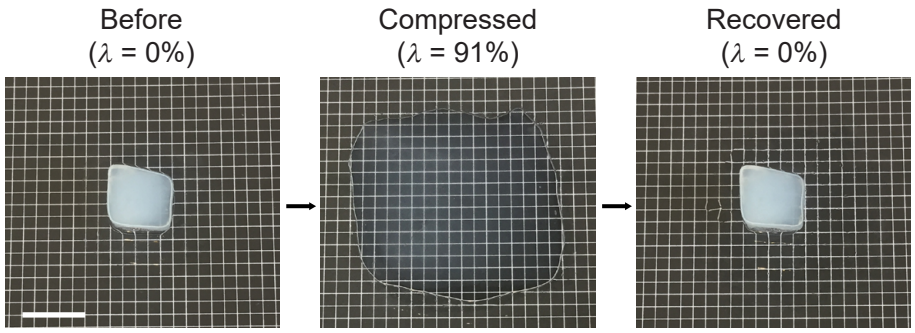
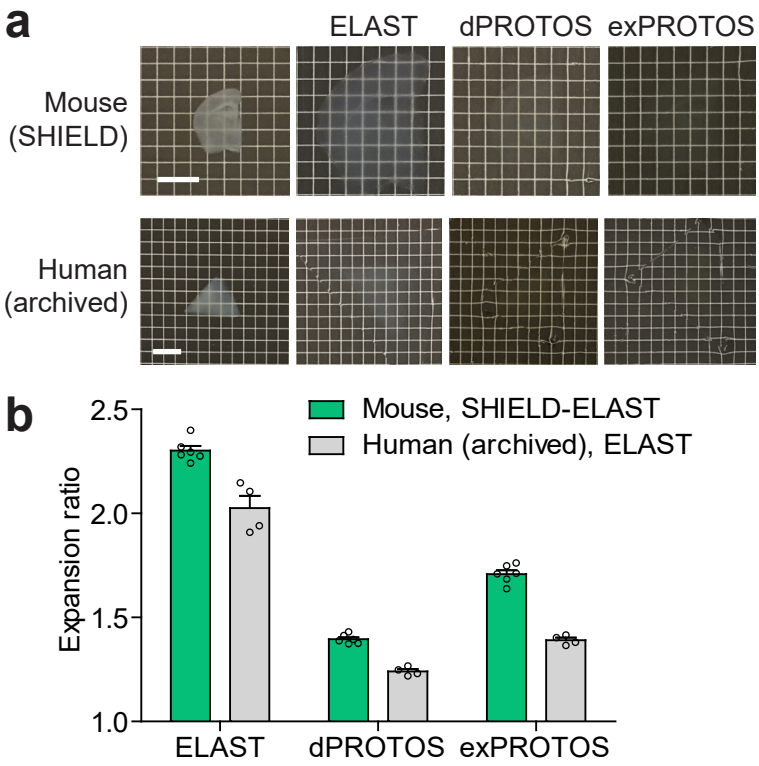
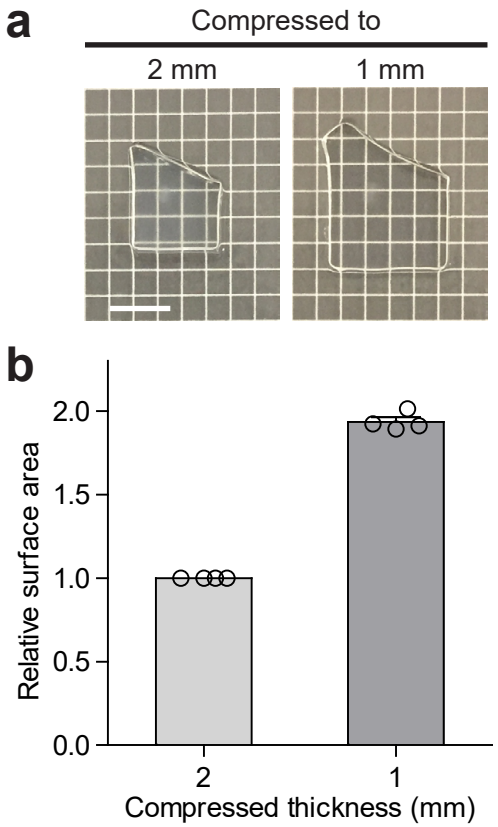


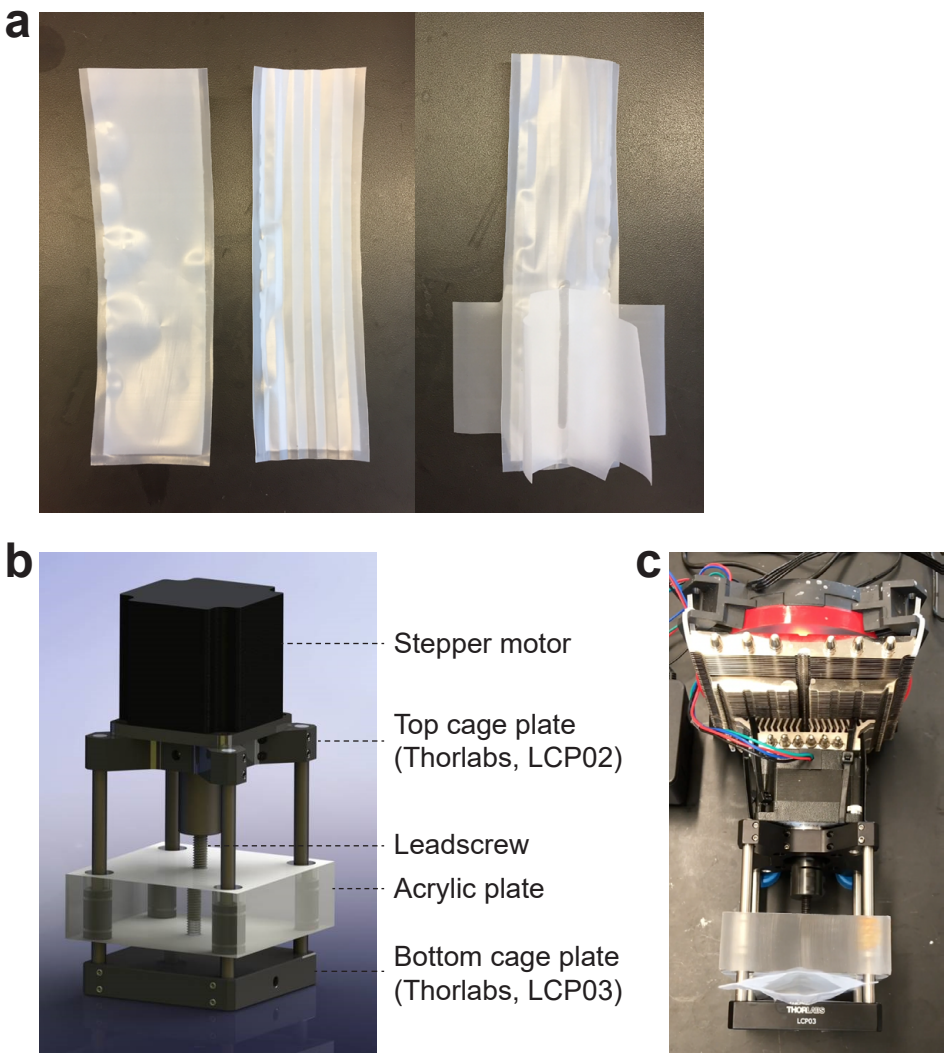
Supplementary Fig. 1 | Compression of ELASTicized human brain tissue. A 4.5-mm-thick ELASTicized human brain cortical block showing resistance to a compression by 11 times. λ, compression strain. Scale bar, 1 cm. Experiment was repeated two times with similar results.



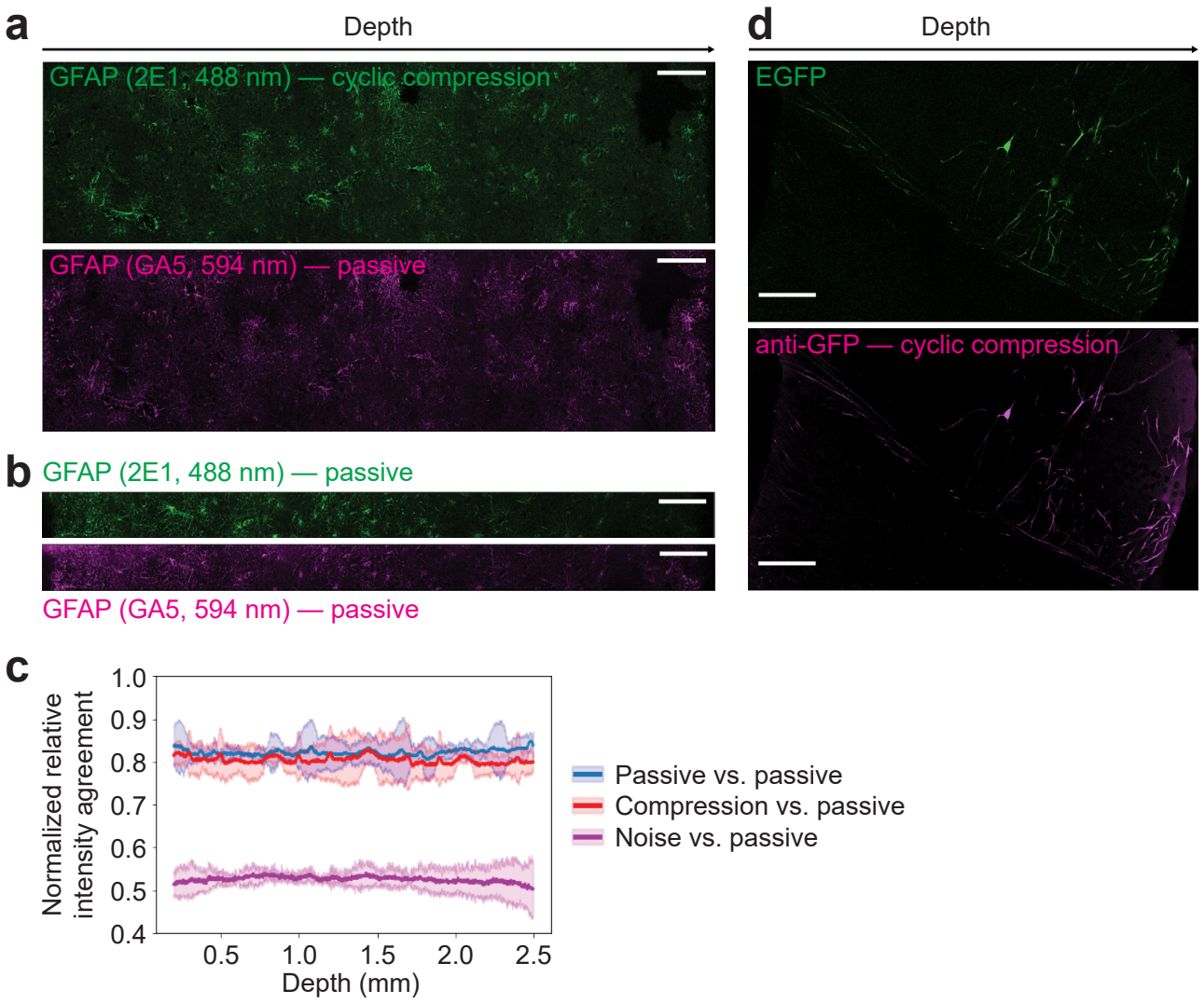
Supplementary Fig. 2 | Tissue size changes by ELASTicization. (**a**) Representative photos of mouse (*n* = 6 samples) and human (*n* = 4 samples) brain slices before and after ELASTicization and in two different RI-matching media. The mouse sample was perfused, fixed, and cleared using the SHIELD protocol, and the archived human sample that was long-term fixed in a formalin solution was permeabilized with an ionic detergent before ELASTicization. Surrounding hydrogels were left to help identify the sample shapes in RI-matching media. Scale bars, 5 mm. (**b**) Quantification of the tissue sizes (mean with SE; *n* = 6 samples for mouse, *n* = 4 samples for human).



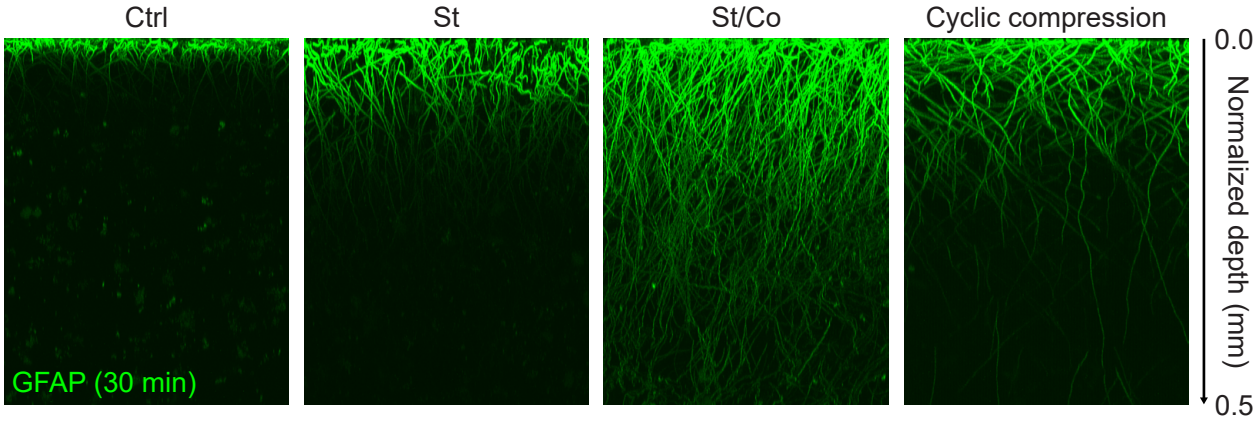
Supplementary Fig. 3 | The degree of lateral expansion by compression. (**a**) A 2.5-mm-thick ELASTicized mouse brain slice was manually compressed between two glass plates. The sample was first gently compressed to 2 mm to make the sample surfaces in full contact with the plates, and then further compressed to 1 mm. Representative images from four repeated samples are shown. Scale bar, 5 mm. (**b**) A comparison of the relative surface areas between compression to 2 mm and 1 mm (*n* = 4 samples). The plot shows mean with SE.



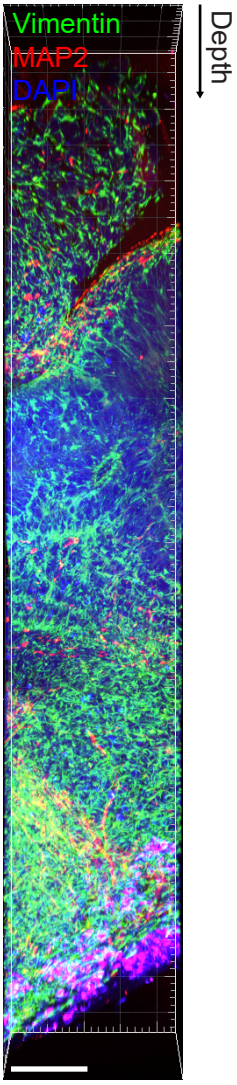
Supplementary Fig. 4 | The cyclic compression device. (**a**) Photos of sample bags before (left) and after patterning with grooves (middle) and after heat-fusion with wings to firmly attach the bag to the device (right). (**b**) A CAD drawing of the cyclic compression device with annotations for the parts. (**c**) A photo of a complete device during its operation. A computer CPU fan was attached to the stepper motor for cooling.



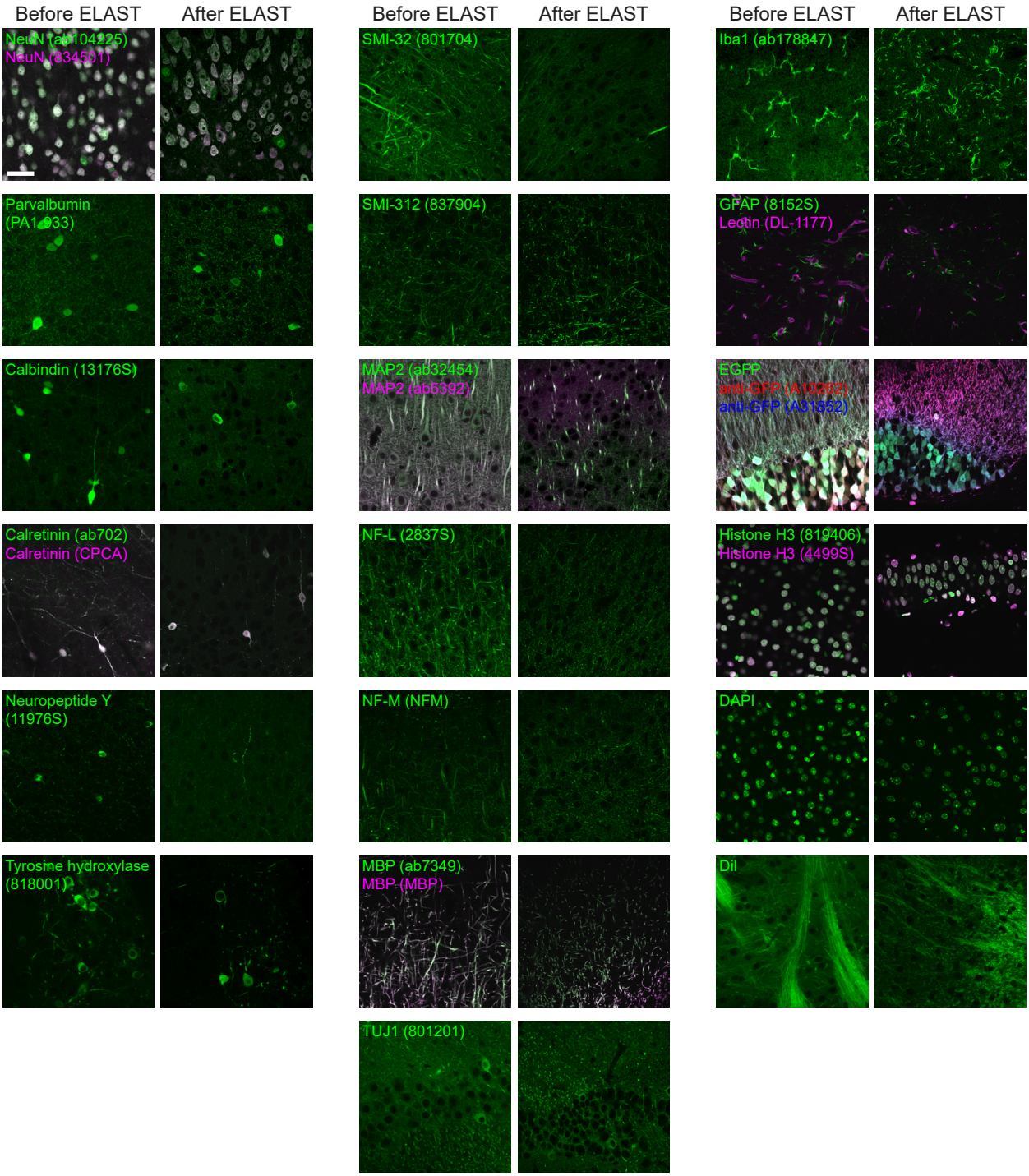
Supplementary Fig. 5 | Depth-wise immunoreactivity by cyclic compression. (**a**,**b**) Representative optical cross-sections showing depth-wise staining agreement of double-GFAP stained ELASTicized human samples (repeated three times). Two anti-GFAP antibodies conjugated with either Alexa Fluor 488 (clone 2E1; 644704, BioLegend) or Alexa Fluor 594 (clone GA5; 8152, Cell Signaling Technologies) were used. Scale bars, 200 μm. (**a**) Compression-stained GFAP-488 vs. passive-stained GFAP-594. (**b**) Simultaneous passively stained GFAP-488 vs. GFAP-594 (repeated three times). (**c**) Normalized relative intensity between GFAP-488 cyclic compression staining and GFAP-594 passive staining of 1.5-mm-thick ELASTicized human tissue (n = 3) remained nearly constant throughout the depth of tissue (red) (average coefficient of variation = 1.66%). No statistically significant difference was found between the average normalized relative intensity agreements of the compressed samples in the treatment group and that of the passively double-stained GFAP-488 / GFAP-594 control group (blue, n = 3) based on a two-tailed student t test (t = 1.34, P = 0.253). Both of these groups displayed statistically significantly larger relative signal intensity agreement than the agreement between randomly generated “noise” images (purple, n = 3) and the control group passively stained GFAP-594 images (vs. control, t = 27.2, ***P = 1.09 × 10^{-5}; vs. treatment, t = 21.2, ***P = 2.95 × 10^{-5}). Plots are means with 95% confidence intervals. (**d**) Representative optical cross-sections comparing anti-GFP compression stained signal to endogenously expressed GFP signal throughout the depth of an ELASTicized mouse tissue of 1-mm thickness (repeated three times). Scale bars, 200 μm.



Supplementary Fig. 6 | A comparison of immunolabeling depths among thinning methods. The same images in **Figure 3h** for Ctrl, St, and St/Co are shown to compare with a result by cyclic compression. The same sample preparation and image acquisition settings were used for all cases. Cyclic compression was performed by manual sixfold compression with a cycling of 50-s compression and 10-s release. Experiment was repeated two times with similar results.



Supplementary Fig. 7 | Rapid immunolabeling of a whole cerebral organoid using cyclic compression. A whole ELASTicized organoid was stained with anti-vimentin antibody, anti-MAP2 antibody, and DAPI. The stained organoid was cut in half, and one cut surface was imaged to show structural integrity and full antibody penetration through the whole thickness. Experiment was not repeated. Scale bar, 200 μm.



Supplementary Fig. 8 | Images of mouse brain tissues labeled with antibodies and dyes before and after ELASTicization. Antibody and lectin catalog numbers are shown in parentheses. Experiment was repeated two times with similar results. Scale bar (adjusted to match the original tissue dimensions), 50 μm.

Supplementary Table 1. Tissue sample preparation and image acquisition setting.

Figure	Sample source and thickness[a]	ELASTicization[b]	Labeling and RI-matching[b]
	Pre-processing[b]	Post-processing[b]	Microscopy condition
2a,b	Banked human brain (~1-cm-thick slab in formalin) Final thickness, 2 mm	Monomer infusion (4 °C, 9 d) Gelation (36 °C, 6 h)	exPROTOS (37 °C, 2 d)
	Stored in PBS upon receiving Vibratome-slicing (2 mm) Permeabilization (70 °C, o/n; 56 °C, 4 d) Washing (37 °C, 4 d)	Delipidation (37 °C, 2 d) Washing (37 °C, 2 d)	
2c	The same source with **2a,b** Final thickness, 2 mm	Monomer infusion (4 °C, 8 d) Gelation (33 °C, o/n)	
	Permeabilization (70 °C, o/n; 56 °C, 9 d) Washing (37 °C, 5 d)	Delipidation (56 °C, 2 d) Washing (37 °C, 2 d)	
2d	Banked human brain (~1-cm-thick slab in formalin) Final thickness, 0.1 mm	Monomer infusion (4 °C, o/n) Gelation (35 °C, 6 h)	Primary antibody (37 °C, o/n) Washing (37 °C, 8 h) Secondary antibody (37 °C, o/n) Washing (37 °C, 6 h)
	Stored in PBS upon receiving Manual slicing (~1.5 mm) Permeabilization (70 °C, o/n) Washing (37 °C, 6 h)	Delipidation (37 °C, o/n) Washing (37 °C, 1 d) Vibratome-sectioning (0.2 mm)	Leica TCS SP8 confocal Coverslip #1 63×/1.3 glycerol (for synapse) 25×/0.95 water (for all others)
2e	Thy1-EGFP-M mouse brain Final thickness, ~1.5 mm	Monomer infusion (4 °C, 5 d) Gelation (25–33 °C, 6 h)	See the FISH section of the Online Methods
	SHIELD-protection (perfusion, fixation, washing; 5 d) Splitting into hemispheres Delipidation (37 °C, 14 d) Washing (PBST with 0.02% thimerosal; 37 °C, 3 d)	Delipidation (37 °C, 9 d) Washing (37 °C, 2 d) Vibratome-sectioning (~3 mm) Manual cutting (2-mm width)	Leica TCS SP8 confocal Coverslip #1.5 25×/0.95 water
2f–h	Banked human brain (~1-cm-thick slab in formalin) Final thickness, 1 mm	Monomer infusion (4 °C, o/n) Gelation (35 °C, 6 h)	Antibody and lectin (37 °C, o/n) Washing (37 °C, 8 h)
	Stored in PBS upon receiving Vibratome-slicing (1 mm) Permeabilization (70 °C, o/n) Washing (37 °C, 6 h)	Delipidation (37 °C, o/n) Washing (37 °C, 1 d)	Olympus FV1200MPE confocal Coverslip #1.5 20×/1.0 water[c]
2i–k	Thy1-EGFP-M mouse brain Final thickness, 0.6 mm	Monomer infusion (4 °C, 5 d) Gelation (25–33 °C, 6 h)	dPROTOS (37 °C, o/n)
	SHIELD-protection (perfusion, fixation, washing; 5 d) Splitting into hemispheres Delipidation (45 °C, 10 d) Washing (37 °C, 3 d)	Delipidation (37 °C, 9 d) Washing (37 °C, 2 d) Vibratome-sectioning (1.4 mm)	Olympus FV1200MPE confocal Coverslip #1.5 20×/1.0 water[d]
3f–i **S6**	The same preparation with **2f–h**		Antibody (RT, 30 min) Washing (RT, 30 min)
			Olympus FV1200MPE confocal Coverslip #1.5 20×/1.0 water[c]
3l,m	Thy1-EGFP-M mouse brain Final thickness, ~3 mm	Monomer infusion (4 °C, 6 d) Gelation (26–35 °C, 6 h)	dPROTOS (37 °C, 1 d) Cyclic compression (16–20%, 42 s:12 s, RT, 1 d) dPROTOS (37 °C, 1 d)
	SHIELD-protection (perfusion, fixation, washing; 5 d) Splitting into hemispheres Delipidation (37 °C, 13 d) Washing (37 °C, 3 d)	Delipidation (37 °C, 7 d) Washing (37 °C, 2 d) Manual slicing (~6 mm)	Olympus FV1200MPE confocal Coverslip #1.5 10×/0.6 IMM

Supplementary Table 1. (*continued*)

3n	The same source with **2a,b** Final thickness, 5 mm	Monomer infusion (4 °C, 5 d) Gelation (26–35 °C, 6 h)	Photobleaching (4 °C, 3 d) Blocking (37 °C, 1 d) Antibody with cyclic compression (16%, 40 s:10 s, 37 °C, 1 d) Washing (37 °C, o/n) Antibody fixation (37 °C, 6 h) Washing (37 °C, 4 h) dPROTOS (37 °C, 1 d)
	Manual slicing (5 mm) Permeabilization (70 °C, o/n; 56 °C, 5 d) Washing (37 °C, 3 d)	Delipidation (56 °C, 5 d) Washing (37 °C, 3 d)	Olympus FV1200MPE confocal Coverslip #1.5 10×/0.6 IMM
3o	The same preparation with **2f–h**		Primary antibody in 35% 1,2-dimethoxyethane (37 °C, 4 h) Washing with 35% 1,2-dimethoxyethane (37 °C, 1 h) Washing with PBST (37 °C, 1 h) Secondary antibody (37 °C, o/n) Washing (37 °C, 2 h)
			Olympus FV1200MPE confocal Coverslip #1.5 20×/1.0 water[c]
ED2a	Thy1-EGFP-M mouse organs	Monomer infusion (4 °C, 3 d) Gelation (23–35 °C, 6 h)	
	SHIELD-protection (perfusion, fixation, washing; 5 d) Delipidation (45 °C, 2 d; 56 °C, 3 d) Washing (37 °C, 2 d)	Delipidation (56 °C, 4 d) Washing (37 °C, 2 d)	
ED2b **S7**	Cerebral organoid Final thickness, ~1 mm	Monomer infusion (4 °C, 1 d) Gelation (35 °C, 6 h)	Blocking with cyclic compression (25%, 36 s:9 s, RT, 4 h) Antibodies and DAPI with the same cyclic compression (12 h) Washing with the same cyclic compression (12 h)
	SHIELD-protection (fixation, washing; 3 d) Delipidation (55 °C, 2 d) Washing (37 °C , 0.5 d)	Delipidation (37 °C, 3 d) Washing (37 °C, 1 d)	Leica TCS SP8 confocal Coverslip #1 25×/0.95 water
ED3a	The sample used for **S4** (M) Final thickness, 1 mm		Blocking (37 °C, 6 h) Primary antibody (37 °C, 2 d) Washing (37 °C, o/n) Secondary antibody (37 °C, o/n) Washing (37 °C, 8 h)
	Washing (RT) until use		Zeiss LSM 780 confocal Coverslip #1 10×/0.45 dry
ED3b	The sample used for **S11a** Final thickness, 10 μm		H&E staining Slide glass mounting and sealing
	Cryo-sectioning (10 μm)[a]		Leica DM6000 B bright field Coverslip #1 2.5×/0.07 dry
S2 (M) **S3**	Thy1-EGFP-M mouse brain Final thickness, 1 mm	Monomer infusion (4 °C, o/n) Gelation (35 °C, 6 h)	(**S4**) dPROTOS (37 °C, 1 d) (**S4**) exPROTOS (37 °C, 1 d)
	SHIELD-protection (perfusion, fixation, washing; 5 d) Vibratome-slicing (1 mm) Delipidation (45 °C, 1.5 d) Washing (37 °C , 0.5 d)	Delipidation (37 °C, o/n) Washing (37 °C, 1 d) (**S5**) Manual chopping	

Supplementary Table 1. (*continued*)

S2 (H)	The same source with **2f–h** Final thickness, 2–3 mm	Monomer infusion (4 °C, 2 d) Gelation (29–31 °C, 6 h)	dPROTOS (37 °C , 1 d) exPROTOS (37 °C, 1 d)
	Manual slicing (1–2 mm) Permeabilization (70 °C, o/n; 56 °C, 1 d) Washing (37 °C, 1 d)	Delipidation (56 °C, 1 d) Washing (37 °C, o/n)	
S5a–c	The same source with **2a,b** Final thickness, 1 mm	Monomer infusion (4 °C, 6 d) Gelation (33 °C, o/n)	(**a**) Blocking (37 °C, 6 h) (**a**) Cyclic compression: antibody (20%, 40 s:10 s, RT, 6 h) (**a**) Washing (37 °C, 3 h) Vertical cut of tissue (**b**) Blocking (37 °C, 6 h) Passive antibody (37 °C, 3 h) Washing (37 °C, 1 h)
	Permeabilization (70 °C, o/n; 56 °C, 4 d) Washing (37 °C, 4 d)	Delipidation (56 °C, 2 d) Washing (37 °C, 3 d)	Leica TCS SP8 confocal Coverslip #1 20×/0.5 water
S5d	Thy1-EGFP-M mouse brain Final thickness, 1 mm	Monomer infusion (4 °C, 1 d) Gelation (35 °C, 6 h)	Blocking (37 °C, 6 h) Primary antibody with cyclic compression (20%, 40 s:10 s, 37 °C, 3 h) Washing (37 °C, 3 h) Secondary antibody with cyclic compression (20%, 40 s:10 s, 37 °C, 3 h) Washing (37 °C, 1 h)
	SHIELD-protection (perfusion, fixation, washing; 5 d) Vibratome-slicing (1 mm) Delipidation (45 °C, 2 d) Washing (37 °C , 1 d)	Delipidation (37 °C, 2 d) Washing (37 °C, 1 d)	Leica TCS SP8 confocal Coverslip #1 20×/0.5 water
S8	Thy1-EGFP-M mouse brain Final thickness, 100 μm	ShE: monomer infusion (4 °C, o/n) ShE: gelation (35 °C, 6 h)	ShE: blocking (37 °C, 3 h) Primary antibody (37 °C, o/n) Washing (37 °C, 8 h) Secondary antibody (37 °C, o/n) Washing (37 °C, 6 h)
	SHIELD-protection (perfusion, fixation, washing; 5 d) Vibratome-slicing (Sh: 100 μm; ShE: 1 mm) Permeabilization/delipidation (45 °C, 1.5 d) Washing (37 °C, 0.5 d)	ShE: hydration in PBS (o/n) ShE: vibratome-slicing (100 μm)[a] ShE: delipidation (37 °C, 8 h) ShE: washing (37 °C, o/n)	Olympus FV1200MPE confocal Coverslip #1 20×/1.0 water[c]

[a] Final thicknesses are presented in original dimensions before sample processing and ELASTicization.
[b] All the same solutions were used for each procedure, unless otherwise noted. The solution compositions and detailed protocols are described in the first section of the Online Methods.
[c] Note that a coverslip was used with the objective designed for use without coverslip. However, only the sample surfaces were imaged where aberration minimally affects imaging. Alternatively, no noticeable axial signal decay was detected based on depth-wise intensity of autofluorescence signal under a constant laser power.
[d] Note that the RIs between sample and objective immersion media mismatch, in addition to the use of a coverslip (see c above). Despite such suboptimal imaging conditions, two images before and after a compression test were obtained with identical imaging settings and sample orientations, which makes any possible aberrations present similarly in both images for a fair comparison.

Supplementary Table 2. Antibody and probe compatibility with ELASTicized mouse brain.

Antibody target or probe name	Catalog #	Vendor	Host species	Clonality or probe type	Sh[j]	ShE[k]
NeuN	ab104225	Abcam[a]	Rabbit	Polyclonal	O	O
	834501	BioLegend[b]	Mouse	Monoclonal	O	O
	MAB377	Millipore[c]	Mouse	Monoclonal	O	O
Calbindin	13176S	CST[d]	Rabbit	Monoclonal	O	O
Calretinin	ab702	Abcam[a]	Rabbit	Polyclonal	O	O
	CPCA-Calret	EnCor[e]	Chicken	Polyclonal	O	O
Parvalbumin	PA1-933	Invitrogen[f]	Rabbit	Polyclonal	O	O
Tyrosine hydroxylase	13106S	CST[d]	Rabbit	Monoclonal	O	O
	818001	BioLegend[b]	Mouse	Monoclonal	O	O
Neuropeptide Y	11976S	CST[d]	Rabbit	Monoclonal	O	O
SMI-32	801704	BioLegend[b]	Mouse	Monoclonal	O	O
SMI-312	837904	BioLegend[b]	Mouse	Monoclonal	O	O
Neurofilament light chain	2837S	CST[d]	Rabbit	Monoclonal	O	O
Neurofilament medium chain	NFM	Aves[g]	Chicken	Polyclonal	O	O
MAP2	ab32454	Abcam[a]	Rabbit	Polyclonal	O	O
	ab5392	Abcam[a]	Chicken	Polyclonal	O	O
Myelin basic protein	ab7349	Abcam[a]	Rat	Monoclonal	O	O
	MBP	Aves[g]	Chicken	Polyclonal	O	O
TUJ1	801201	BioLegend[b]	Mouse	Monoclonal	O	O
Iba1	ab178847	Abcam[a]	Rabbit	Monoclonal	O	O
GFAP	8152S	CST[d]	Mouse	Monoclonal	O	O
GFP	A10262	Invitrogen[f]	Chicken	Polyclonal	O	O
	A31852	Invitrogen[f]	Rabbit	Polyclonal	O	O
Histone H3	819406	BioLegend[b]	Mouse	Monoclonal	O	O
	4499S	CST[d]	Rabbit	Monoclonal	O	O
Lectin	DL-1177	Vector[i]	Tomato	Glycoprotein	O	O
DAPI	D1306	Invitrogen[f]		Chemical	O	O
DiI	D282	Invitrogen[f]		Chemical	O	O

[a] Abcam (Cambridge, MA, USA).
[b] BioLegend (San Diego, CA, USA).
[c] MilliporeSigma (Burlington, MA, USA).
[d] Cell Signaling Technologies (Danvers, MA, USA).
[e] EnCor Biotechnology (Gainesville, FL, USA).
[f] Life Technologies (Carlsbad, CA, USA).
[g] Aves Labs (Tigard, OR, USA).
[h] Novus Biologicals (Centennial, CO, USA).
[i] Vector Laboratories (Burlingame, CA, USA).
[j] Compatibility with SHIELD-protected samples before ELASTicization.
[k] Compatibility with SHIELD-protected samples after ELASTicization.

Supplementary Table 3. A summary of reagents used for labeling archived human brain specimens.

Antibody or probe	Catalog #	Vendor	Host species	Clonality	Dilution (μL/μL)
Anti-NeuN	ab104225	Abcam	Rabbit	Polyclonal	1:300[c]
Anti-MAP2	822501	BioLegend	Chicken	Polyclonal	1:300[c]
Anti-parvalbumin	PA1-933	Invitrogen	Rabbit	Polyclonal	1:300[c]
Anti-SMI-312	837904	BioLegend	Mouse	Monoclonal	2:300[c]
Anti-calretinin	ab702	Abcam	Rabbit	Polyclonal	10:1,000[d]
Anti-neurofilament light chain	NFL	Aves	Chicken	Polyclonal	60:1,000[d]
Anti-calbindin	13176	CST	Rabbit	Monoclonal	1:300[c]
Anti-tyrosine hydroxylase	818001	BioLegend	Mouse	Monoclonal	1:300[c]
Anti-neuropeptide Y	11976	CST	Rabbit	Monoclonal	1:300[c]
Anti-myelin basic protein	ab7349	Abcam	Rat	Monoclonal	2:300[c]
Anti-synapsin 1/2	106002	SYSY[a]	Rabbit	Polyclonal	1:300[c]
Anti-PSD-95	75-028	NeuroMab[b]	Mouse	Monoclonal	1:300[c]
Anti-GFAP (Alexa Fluor 594)	8152	CST	Mouse	Monoclonal (GA5)	10:1,000[e,f] 50:5,000[g] 3:300[h]
Anti-GFAP (Alexa Fluor 488)	644704	BioLegend	Mouse	Monoclonal (2E1)	3:300[h] 20:2,000[i]
Lectin (DyLight 488)	DL-1174	Vector	Tomato		10:1,000[f]
Anti-rabbit IgG (Alexa Fluor Plus 488)	A32731	Invitrogen	Goat	Polyclonal	1:300[c] 10:1,000[d]
Anti-mouse IgG (Alexa Fluor Plus 647)	A32728	Invitrogen	Goat	Polyclonal	1:300[c]
Anti-chicken IgY (Alexa Fluor 647)	ab150175	Abcam	Goat	Polyclonal	1:300[c] 20:1,000[d]
Anti-rat IgG (Alexa Fluor 647)	ab150151	Abcam	Donkey	Polyclonal	1:300[c]

[a] Synaptic Systems (Goettingen, Germany).
[b] UC Davis/NIH NeuroMab Facility (Davis, CA, USA).
[c] Antibody compatibility experiment with one 200-μm-thick ELASTicized human section piece.
[d] Double-immunolabeling of an ELASTicized 1-mm-thick human sample.
[e] Antibody delivery speed experiment with one ELASTicized 1-mm-thick human sample.
[f] Stretching-related deformation with two or three ELASTicized 1-mm-thick human samples.
[g] Immunolabeling of one ELASTicized 5-mm-thick human sample in a blocking solution.
[h] Passive staining for comparisons of depth-wise immunolabeling qualities.
[i] Staining with cyclic compression for a comparison of depth-wise immunolabeling qualities.