

Supplementary Information

Holographic focused ultrasound enables multitarget EV and MSC delivery for neurovascular repair after ischemic stroke

Jia-Ji Pan^{1*#}, Chenyu Li^{1,2*}, Xinya Yao^{1*}, Yan Wei¹, Lan Shen¹, Xiangkun Piao¹, Mingyu Wang¹, Ji Hu³, Guo-Yuan Yang^{4#}, Yanbo Pei^{5#}, and Bingbing Cheng^{1,2,6#}

¹TRUTH Lab, School of Biomedical Engineering, ShanghaiTech University, Shanghai 201210, China.

²Shanghai Clinical Research and Trial Center, ShanghaiTech University, Shanghai 201210, China.

³School of Life Science and Technology, ShanghaiTech University, Shanghai 201210, China.

⁴Med-X Research Institute and School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai 200030, China.

⁵School of Physics, Harbin Institute of Technology, Harbin 150001, China.

⁶State Key Laboratory of Advanced Medical Materials and Devices, ShanghaiTech University, Shanghai 201210, China.

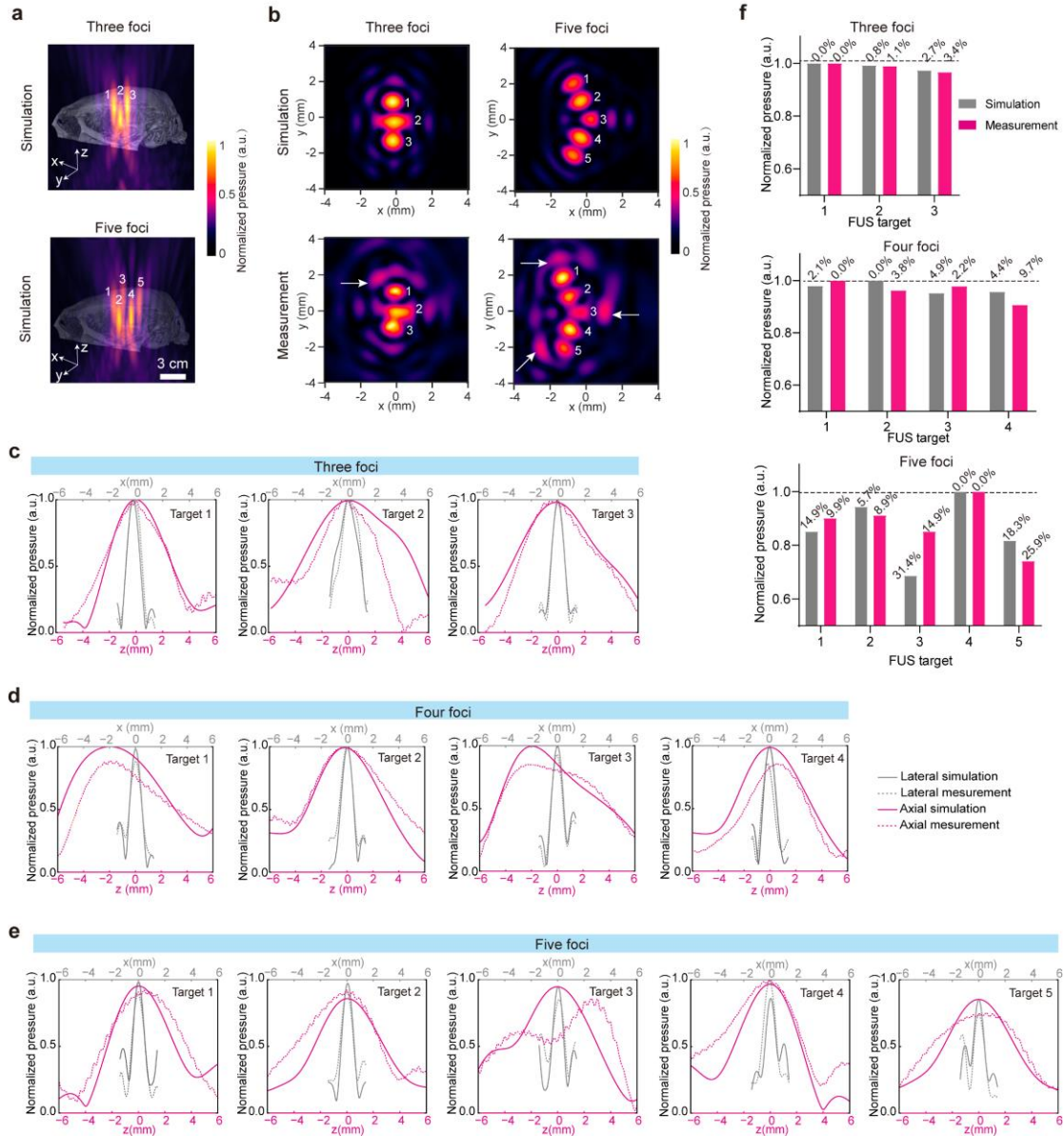
*These authors contributed equally to this work.

#Corresponding authors are B.C. (chengbb@shanghaitech.edu.cn), Y.P. (peiyabo@hit.edu.cn), G.Y. (gyyang@sjtu.edu.cn), and J.P. (panjj@shanghaitech.edu.cn).

Supplementary Figures 1–11

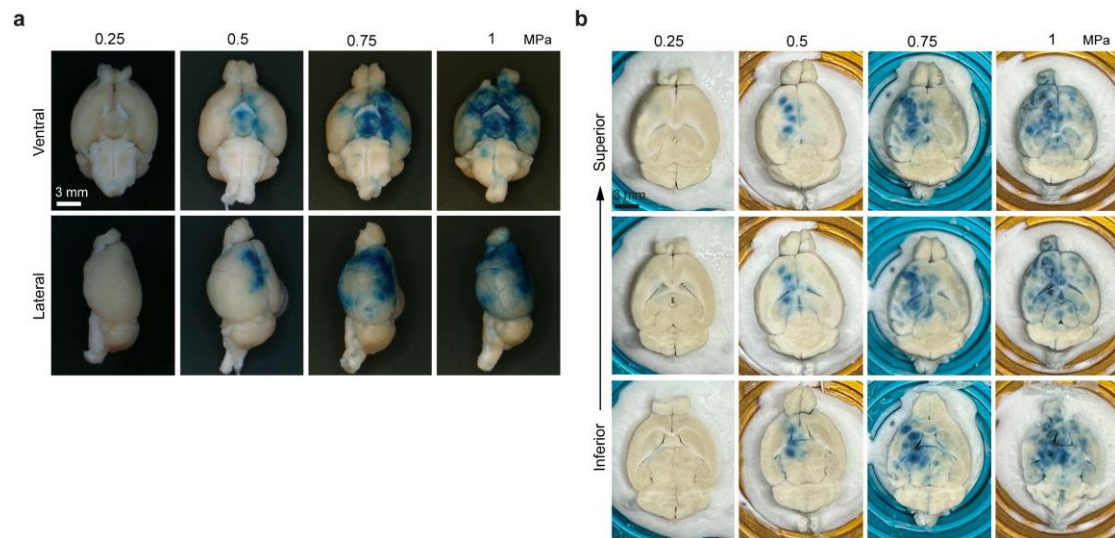
Supplementary Tables 1–4

Supplementary Movie 1



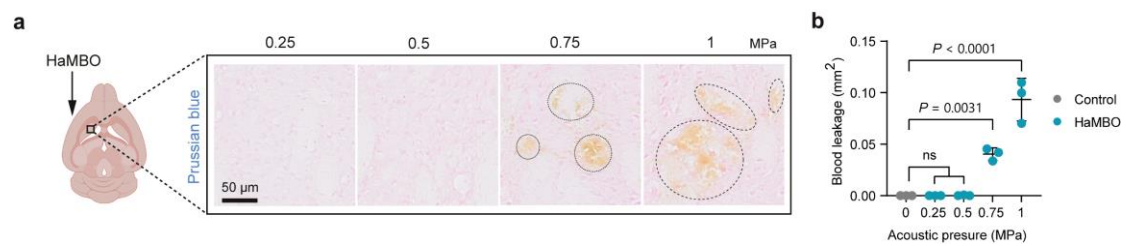
Supplementary Fig. 1. Acoustic characterization of multifocal holograms.

a, The 3D-simulated acoustic fields of three- and five-focus holograms incorporating mouse skull models derived from CT imaging. Numbers indicate FUS foci. b, Simulated and hydrophone-measured acoustic fields for three- and five-focus holograms. Arrows indicate side lobes with an acoustic pressure exceeding half of the main lobes. c–e, Acoustic pressure profiles across the beam centers of three- (c), four- (d), and five-focus (e) holograms. f, Normalized peak acoustic pressure at each focus for three-, four-, and five-focus holograms, with variations indicated above the bar columns. The dashed line indicates a normalized pressure value of 1. a.u., arbitrary units. Source data are provided as a Source Data file.



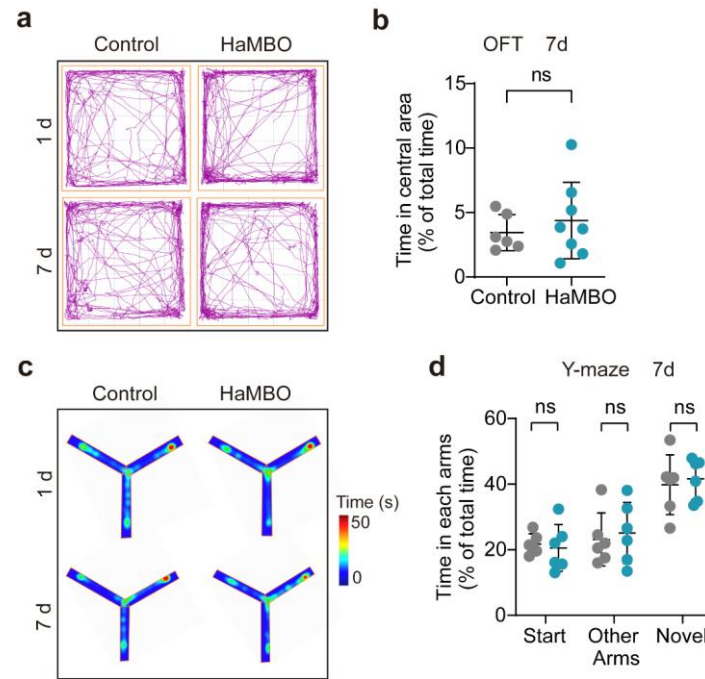
Supplementary Fig. 2. HaMBO-mediated EB delivery conforms to the peri-infarct geometry.

a, Bright-field images of ventral and lateral mouse brains showing superficial EB (blue) extravasation at 30 min post-HaMBO under 0.25, 0.5, 0.75, or 1 MPa. $n = 3$ mice per group. b, Corresponding horizontal brain sections illustrating EB (blue) extravasation into the brain parenchyma.



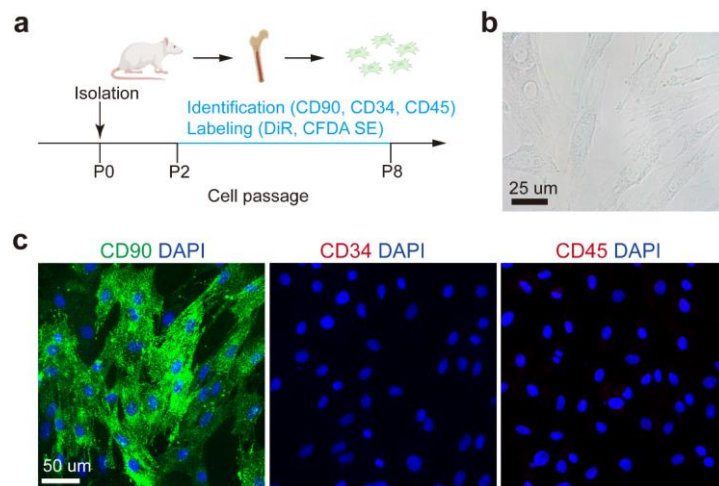
Supplementary Fig. 3. Assessment of blood leakage by Prussian blue staining.

a, Prussian blue staining of brain sections at 30 min following HaMBO under 0 (contralateral untreated brain region), 0.25, 0.5, 0.75, or 1 MPa. Dashed circles indicate fresh erythrocyte extravasation. b, Quantification of blood leakage. $n = 3$ mice per group. All data are presented as mean $\pm$ SD. Statistical analyses: one-way ANOVA with Tukey's *post-hoc* test (b). ns, not significant. Source data are provided as a Source Data file. Parts of panel a was created in BioRender. Pan, J. (2026) <https://BioRender.com/b9a3vj6>.



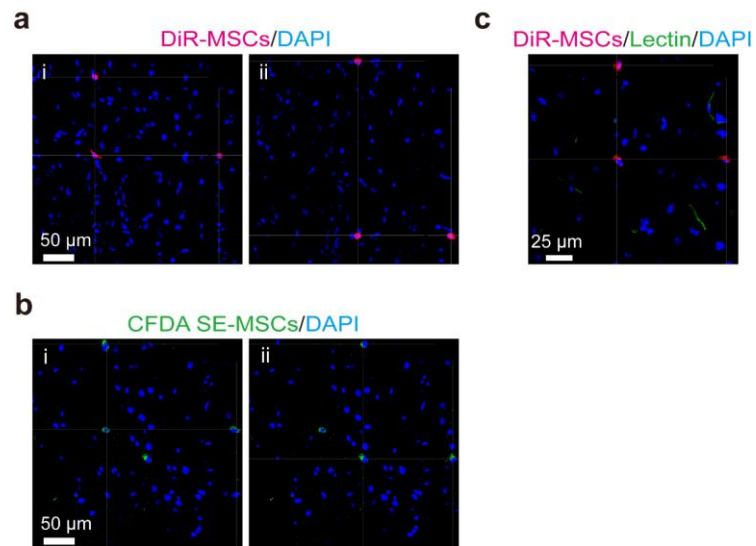
Supplementary Fig. 4. OFT and Y-maze performance following HaMBO.

a, OFT at 1 d and 7 d post-HaMBO. b, Quantification of OFT performance at 7 d post-HaMBO. $n = 6$ and 8 mice in the control and HaMBO groups, respectively. c, Y-maze test at 1 d and 7 d post-HaMBO. d, Quantification of Y-maze performance at 7 d post-HaMBO. $n = 6$ mice per group. All data are presented as mean $\pm$ SD. Statistical analyses: unpaired two-tailed Student's t test (b, d). ns, not significant. Source data are provided as a Source Data file.

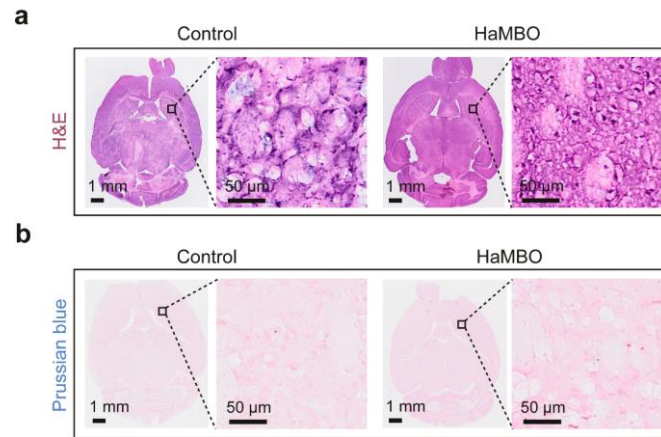


Supplementary Fig. 5. Isolation and characterization of MSCs.

a, Rat bone marrow-derived MSCs from passages 2 to 8 (P2–P8) used for subsequent experiments. b, Bright-field image showing spindle-shaped, fibroblast-like MSC morphology. c, Immunofluorescence staining confirming MSC identity: CD90⁺ and CD34⁻ /CD45⁻. Parts of panel a was created in BioRender. Pan, J. (2026) <https://BioRender.com/5gski21>.

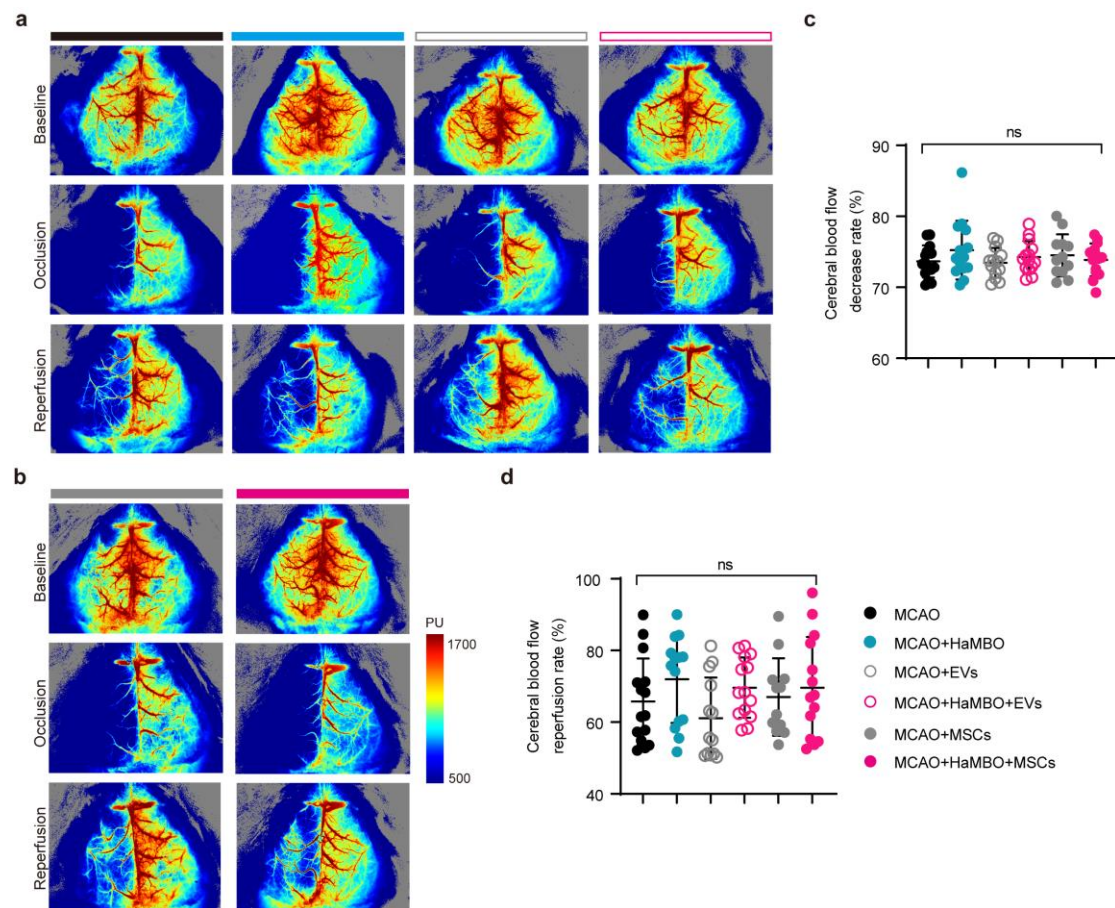


Supplementary Fig. 6. Z-stack confocal images of HaMBO-assisted MSC delivery. a, Z-stack confocal images of DiR-MSCs (magenta) from Supplementary Movie 1. b, Z-stack confocal images of CFDA SE-MSCs (green) shown in Fig. 4h. c, Z-stack confocal images of DiR-MSCs (magenta) with lectin-labeled blood vessels (green) from Fig. 4h. i–ii indicate delivered MSCs with DiR or CFDA SE signals surrounding the nuclei.



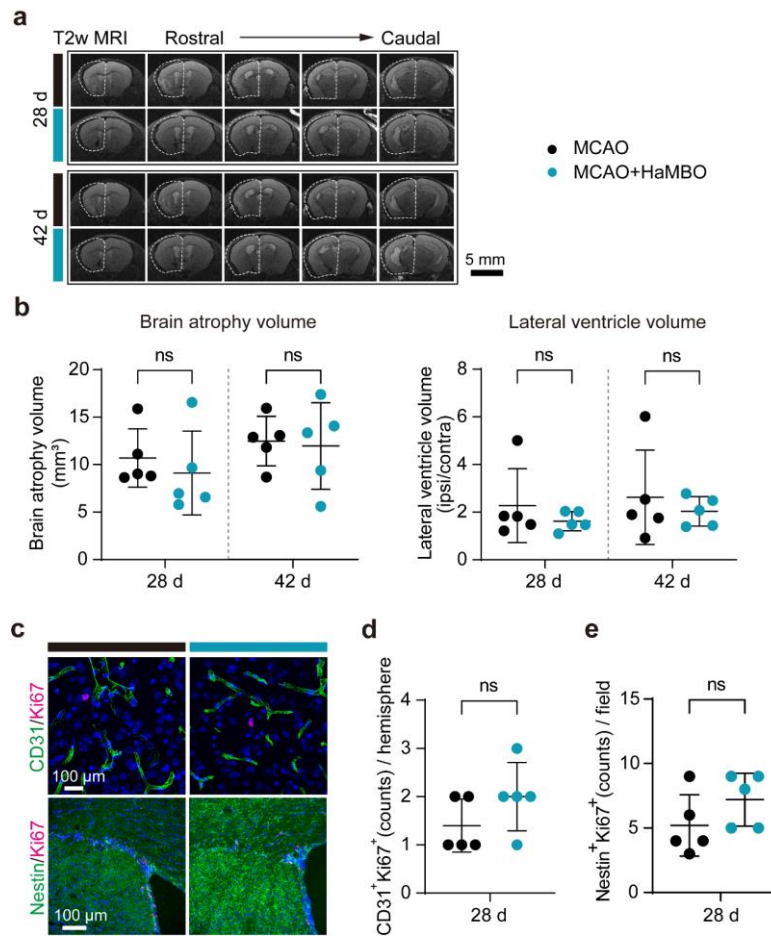
Supplementary Fig. 7. Histological analysis following combined HaMBO treatment and MSC delivery.

a, Representative H&E images from the control and HaMBO groups. n = 3 mice per group. b, Representative Prussian blue images from the control and HaMBO groups. n = 3 mice per group.



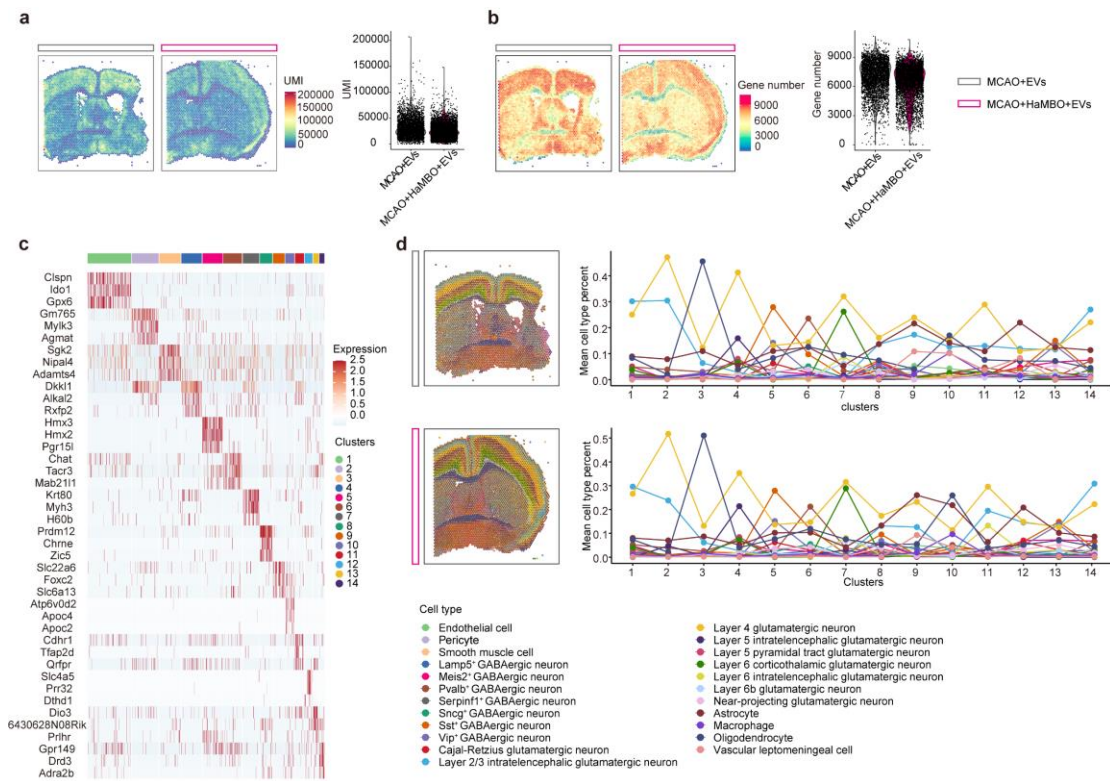
Supplementary Fig. 8. CBF monitoring during MCAO surgery.

a–b, Representative laser speckle contrast images showing CBF at baseline, occlusion, and reperfusion. c, Quantification of CBF reduction during occlusion. Sample size for the MCAO, MCAO+HaMBO, MCAO+EVs, MCAO+HaMBO+EVs, MCAO+MSCs and MCAO+HaMBO+MSCs groups were $n = 15, 14, 13, 14, 12,$ and 14 mice, respectively. d, Quantification of CBF reperfusion rate. Sample size for the MCAO, MCAO+HaMBO, MCAO+EVs, MCAO+HaMBO+EVs, MCAO+MSCs and MCAO+HaMBO+MSCs groups were $n = 15, 14, 13, 14, 12,$ and 14 mice, respectively. All data are presented as mean $\pm$ SD. Statistical analyses: one-way ANOVA with Tukey's *post-hoc* tests (c); Kruskal-Wallis test with Dunn's *post-hoc* test (d). ns, not significant. CBF, cerebral blood flow. PU, perfusion units. Source data are provided as a Source Data file.



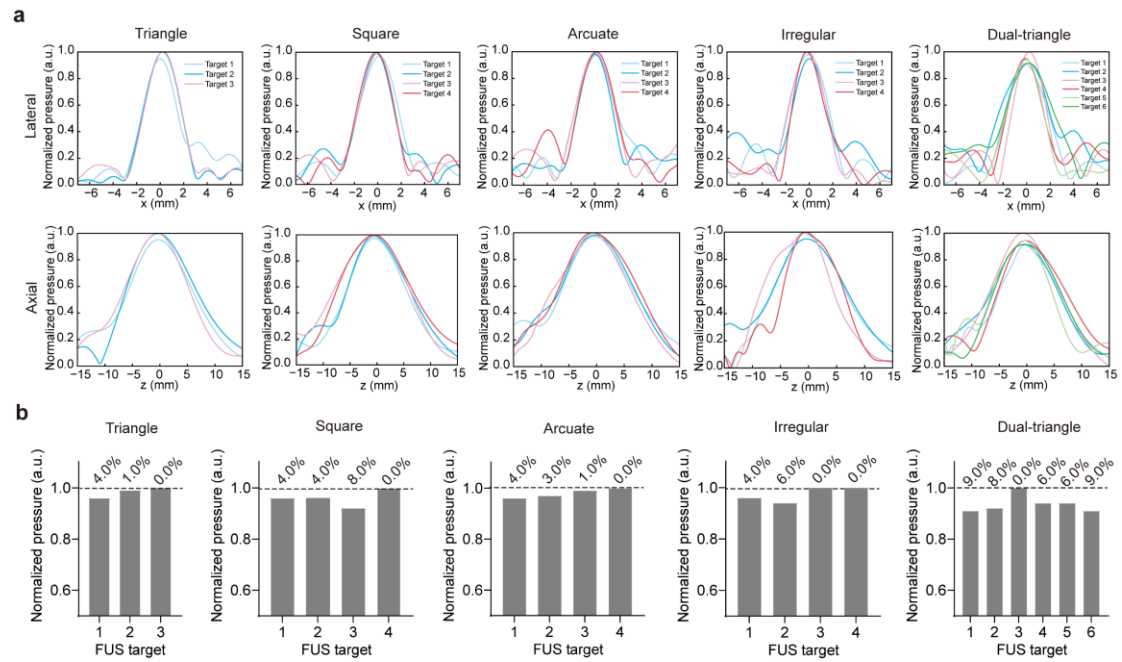
Supplementary Fig. 9. HaMBO alone shows neutral efficacy post-MCAO.

a, Representative axial T2w MRI images obtained at 28 d and 42 d post-MCAO. Dotted lines depict contralateral brain regions. b, Quantification of brain atrophy volume (left) and lateral ventricle volume (right). $n = 5$ mice per group. c, Immunofluorescence staining for CD31 (green), Nestin (green), Ki67 (cerise), and DAPI (blue) at 28 d post-MCAO. d, Quantification of angiogenesis. $n = 5$ mice per group. e, Quantification of neural progenitor proliferation. $n = 5$ mice per group. All data are presented as mean $\pm$ SD. Statistical analyses: unpaired two-tailed Student's t test (b, brain atrophy volume at 42 d; e), Mann–Whitney U test (b, except brain atrophy volume at 42 d; d). ns, not significant. ipsi/contra, ipsilateral/contralateral. Source data are provided as a Source Data file.



Supplementary Fig. 10. Additional spatial transcriptomic analyses.

a–b, Spatial feature plots and violin plots showing UMI counts (a) and gene counts (b) in MCAO+EVs and MCAO+HaMBO+EVs groups. c, Heatmap showing the top three marker genes for each cluster. UMI, unique molecular identifier. d, RCTD analysis showing the cellular composition of each spot. Source data are provided as a Source Data file.



Supplementary Fig. 11. Acoustic characterizations in human-skull simulations.

a, Lateral and axial acoustic pressure profiles across the beam centers of triangle, square, arcuate, irregular, and dual-triangle designs. b, Normalized peak acoustic pressure at each focus for each design, with variations indicated above the bar columns. The dashed line indicates a normalized pressure value of 1. Source data are provided as a Source Data file.

Supplementary Table 1. Coordinates of FUS foci for mouse hologram designs.

Hologram	Target	X (mm)	Y (mm)
Three foci	1	-0.1	0.8
	2	0.0	0.4
	3	-0.1	-1.4
Four foci	1	-0.7	1.7
	2	-0.1	0.6
	3	-0.1	0.7
	4	0.7	-1.8
Five foci	1	-0.7	1.9
	2	-0.2	0.9
	3	0.0	-0.3
	4	-0.2	-1.0
	5	-0.6	2.0

Supplementary Table 2. FWHM of FUS foci for mouse holograms.

Hologram	Target	Lateral		Axial	
		Simulation (mm)	Measurement (mm)	Simulation (mm)	Measurement (mm)
Three foci	1	1.11	1.22	5.04	5.56
	2	1.84	1.39	8.78	8.79
	3	1.13	1.09	7.8	6.13
Four foci	1	1.02	1.11	8.89	8.61
	2	1.14	1.19	6.38	7.61
	3	1.07	1.04	8.86	9.59
	4	1.02	1.15	6.39	5.73
Five foci	1	1.06	1.01	6.29	6.63
	2	1.1	1.19	6.05	7.25
	3	1.19	1.24	7.79	9.78
	4	1.03	1.14	5.24	7.15
	5	1.17	1.13	7.01	8.79

Supplementary Table 3. Coordinates of FUS foci in human simulations.

Design	Target	X (mm)	Y (mm)
Triangle	1	0.0	13.0
	2	-13.5	-7.0
	3	14.5	-7.5
Square	1	-11.0	10.5
	2	10.5	10.0
	3	-10.5	-11.0
	4	10.5	-11.5
Arcuate	1	-3.0	20.5
	2	3.0	7.5
	3	5.5	-6.0
	4	-0.5	-19.0
Irregular	1	-3.4	3.5
	2	-1.3	-14.5
	3	11.0	-11.0
	4	31.0	-16.5
Dual-triangle	1	-30.5	12.5
	2	-15.0	36.0
	3	-0.5	11.5
	4	0.5	-12.0
	5	29.5	-12.0
	6	15.5	13.5

Supplementary Table 4. FWHM of FUS foci in human simulations.

Design	Target	Lateral (mm)	Axial (mm)
Triangle	1	3.40	13.75
	2	3.50	14.25
	3	3.50	13.35
Square	1	3.85	12.85
	2	3.40	13.35
	3	3.65	15.75
	4	3.45	15.85
Arcuate	1	4.00	15.00
	2	3.30	15.60
	3	3.75	16.05
	4	3.60	16.30
Irregular	1	3.90	14.70
	2	3.40	15.45
	3	3.55	14.20
	4	3.30	12.10
Dual-triangle	1	4.20	12.70
	2	4.90	14.50
	3	3.20	14.20
	4	3.45	14.30
	5	3.55	12.20
	6	4.85	14.30