Smart Liposomal Nanocarrier Enhanced the Treatment of Ischemic Stroke Through Neutrophil Extracellular Traps and Cyclic Guanosine Monophosphate-Adenosine Monophosphate Synthase-Stimulator of Interferon Genes (cGAS-STING) Pathway Inhibition of Ischemic Penumbra

Shanbo Sun[a#], Wei Lv[b#], Shengnan Li[a], Qi Zhang[a], Weichong He[a], Zhiyi Min[a], Chuanhui Teng[a], Yuqin Chen[a], Linfeng Liu[a], Jiaqing Yin[a], Baoli Zhu[c, e], Ming Xu[c, f*], Dongwei Dai[d*], Hongliang Xin[a*]

[a] Department of Pharmaceutics, School of pharmacy, Nanjing Medical University, Nanjing 211166, China

[b] Department of Pharmacy, The Jiangyin Clinical College of Xuzhou Medical University, Wuxi 214400, China

[c] Jiangsu Engineering Research Center of Health Emergency, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing 210009, China

[d] Department of Neurosurgery, The First Affiliated Hospital of Naval Medical University, Changhai Hospital of Shanghai, Shanghai 200433, China

[e] Center for Global Health, School of Public Health, Nanjing Medical University, Nanjing 211166, China

[f] School of Public Health, Nanjing Medical University, Nanjing 211166, China

*Corresponding Authors:

Prof. Hongliang Xin (E-mail: xhl@njmu.edu.cn);

Prof. Dongwei Dai (E-mail: chstroke@126.com)

Prof. Ming Xu (E-mail: sosolou@jscdc.cn)

[#] These authors contributed equally to this work.

Table S1 Particle size, PDI and ζ-potential of different formulations. The results are presented as the means ± SD (n = 3).

Formulation	Size [nm]	PDI	ζ-potential [mV]
C-Lipo	169.33 ± 3.93	0.16 ± 0.01	-6.69 ± 0.55
Lipo-CA	162.27 ± 0.73	0.15 ± 0.01	-9.45 ± 0.35
C-Lipo-CA	174.36 ± 1.07	0.16 ± 0.01	-12.237 ± 0.83

Table S2 Encapsulation efficiency and drug loading of C-Lipo/CA (n = 3).

Formulation	Encapsulation Efficiency（%）	Loading Capacity（%）
C-Lipo-CA	39.55 ± 1.94	4.15 ± 0.21

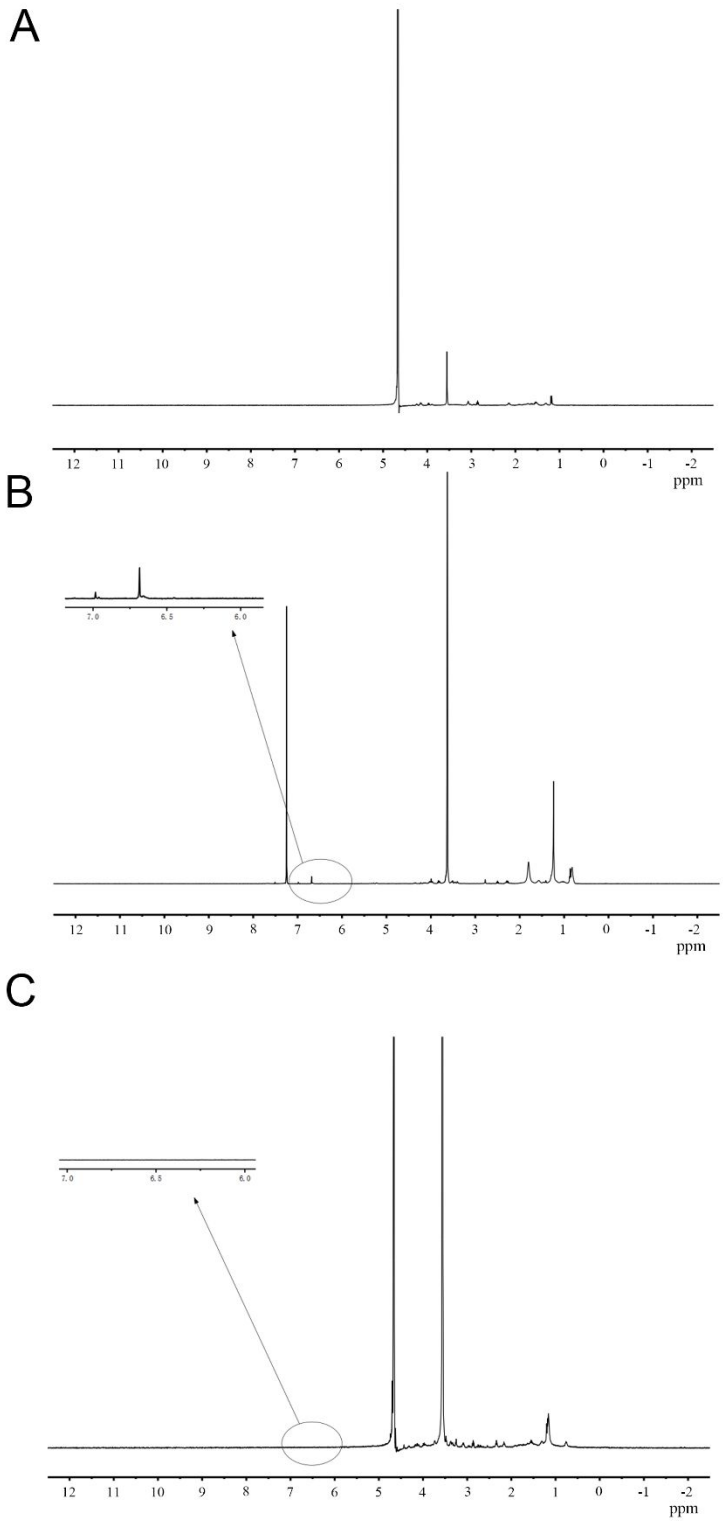

Figure S1 1H-NMR spectrum of **(A)** CREKA **(B)** Mal-PEG_{2000}-DSPE and **(C)** CREKA-PEG_{2000}-DSPE

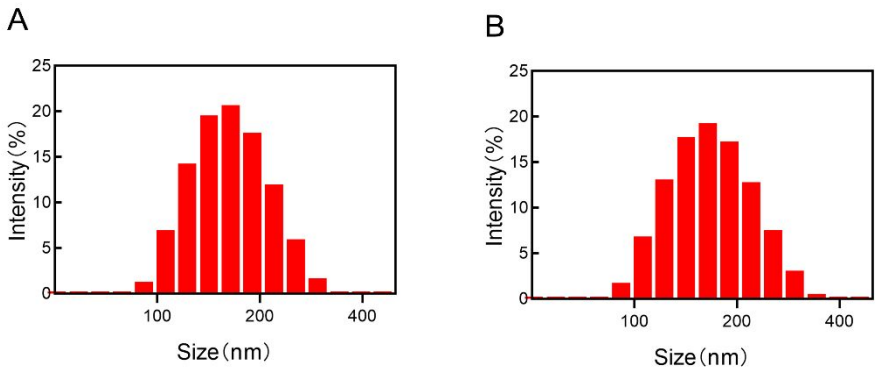

Figure S2 Size distribution of **(A)** Lipo-CA and **(B)** C-Lipo. Scale bar: 200 nm.

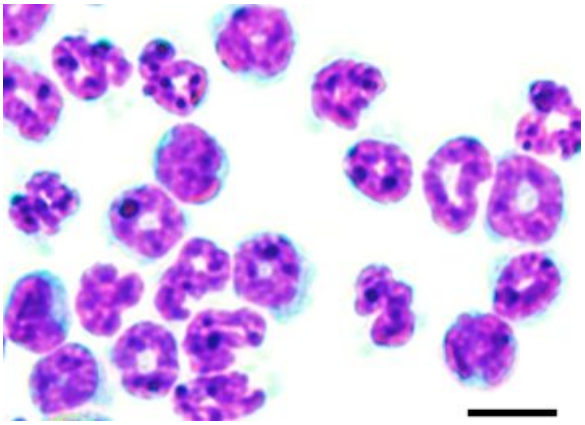

Figure S3. Morphological images of neutrophil cells stained with Wright-Giemsa. Scale bar, 10 μm

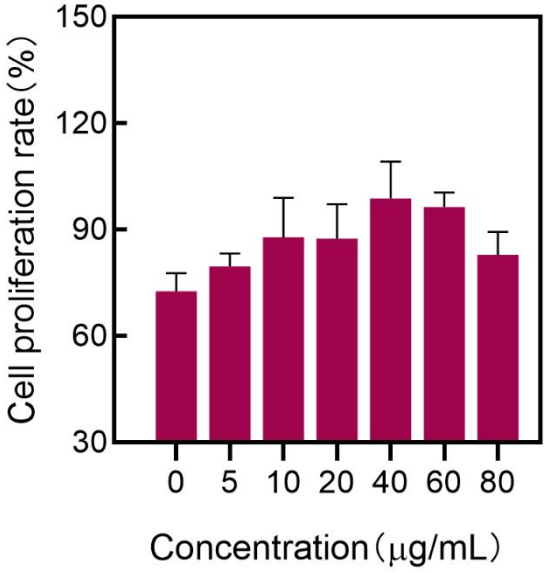

Figure S4 Cell proliferation rate of PC-12 cells incubated with supernatant of neutrophil-stimulated treated with different concentrations of Cl-amidine. The results are reported as the means ± SD (n = 4).

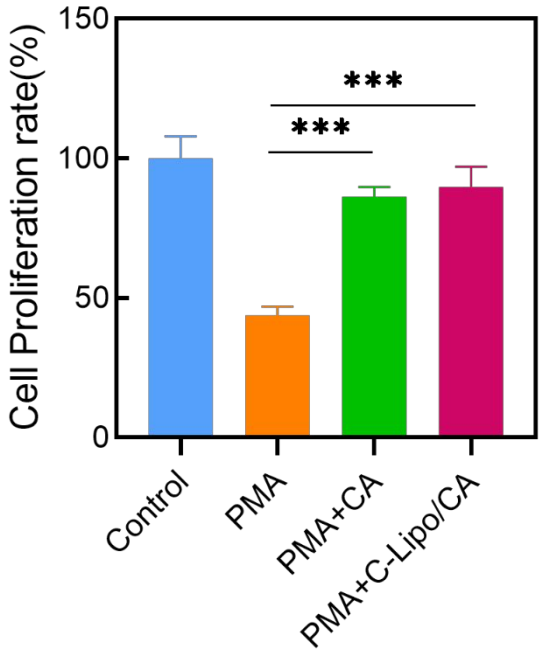

Figure S5 Cell proliferation rate of PC-12 cells treated with different treatments. The results are reported as the means ± SD (n = 5, ***P < 0.01).

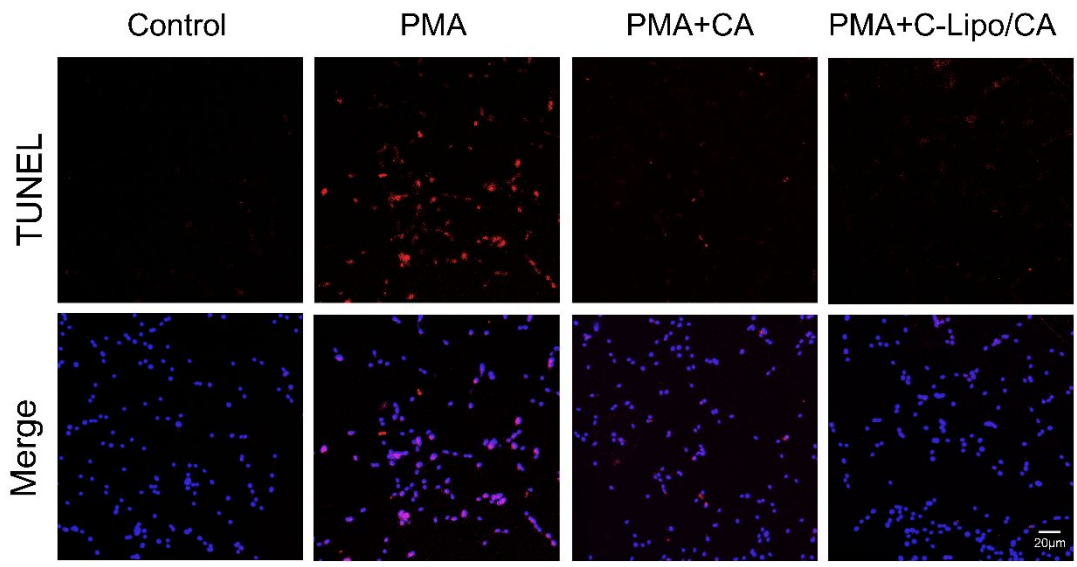

Figure S6 Representative images of TUNEL staining in PC-12 cells treated with different formations. Scale bar: 20 μm.

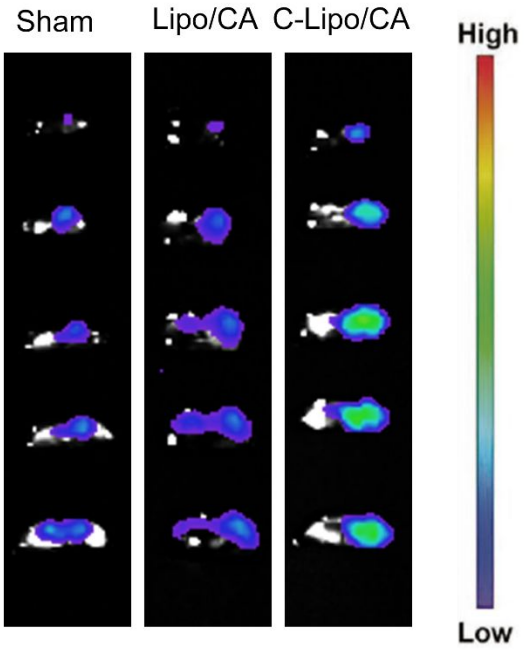

Figure S7 Representative fluorescence image of the brain slices excised after IVIS imaging

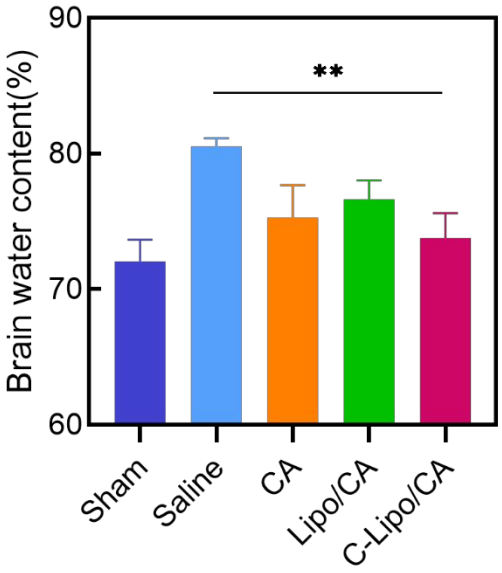

Figure S8 Brain water content of MCAO mice treated with different formulations. The results are reported as the means ± SD (n = 3). Significant differences between the indicated groups: ** $P < 0.01$

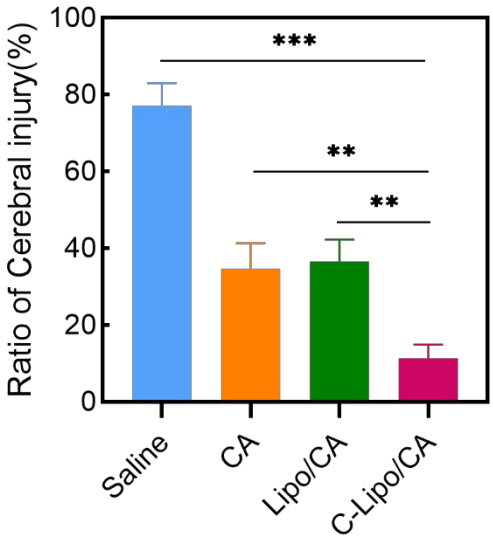

Figure S9 Cerebral injury ratio of MCAO mice treated with different formulations. The results are reported as the means ± SD (n = 3). Significant differences between the indicated groups: ** $P < 0.01$, *** $P < 0.001$.

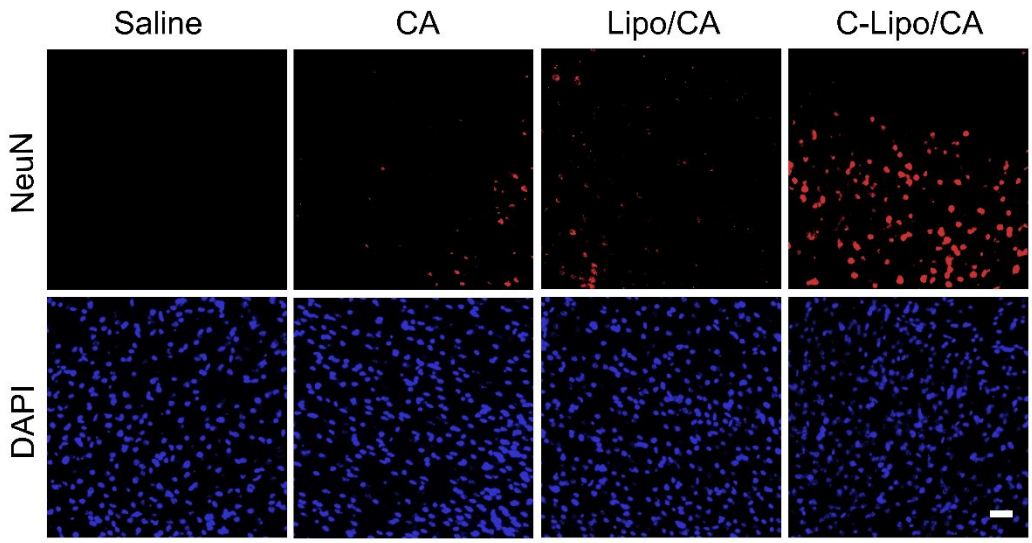

Figure S10 Representative images of NeuN staining in ischemic penumbra after treatment of different formulations. Scale bar: 20 μm

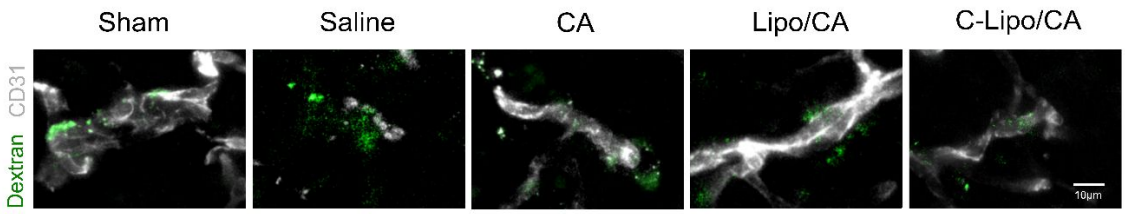

Figure S11 Extravascular dextran fluorescence in MCAO mice after treatment with different formulations. Scale bar: 10 μm

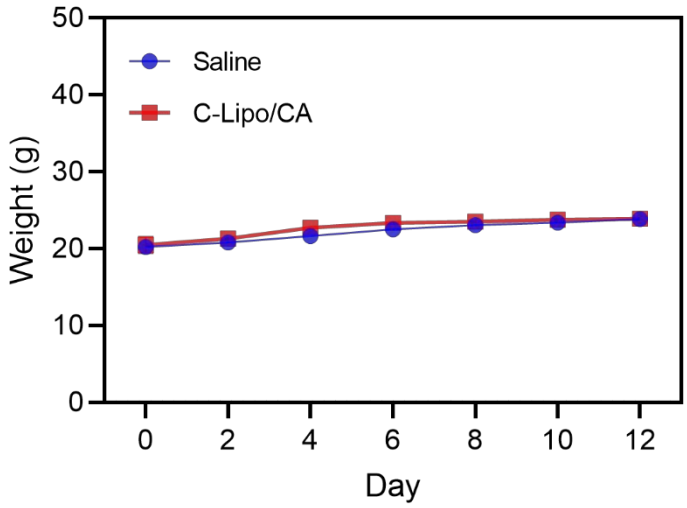

Figure S12 Body weight changes of mice within 12 days treated with different formulations. The results are reported as the means ± SD (n = 3)