

# ADVANCED SCIENCE

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## Supporting Information

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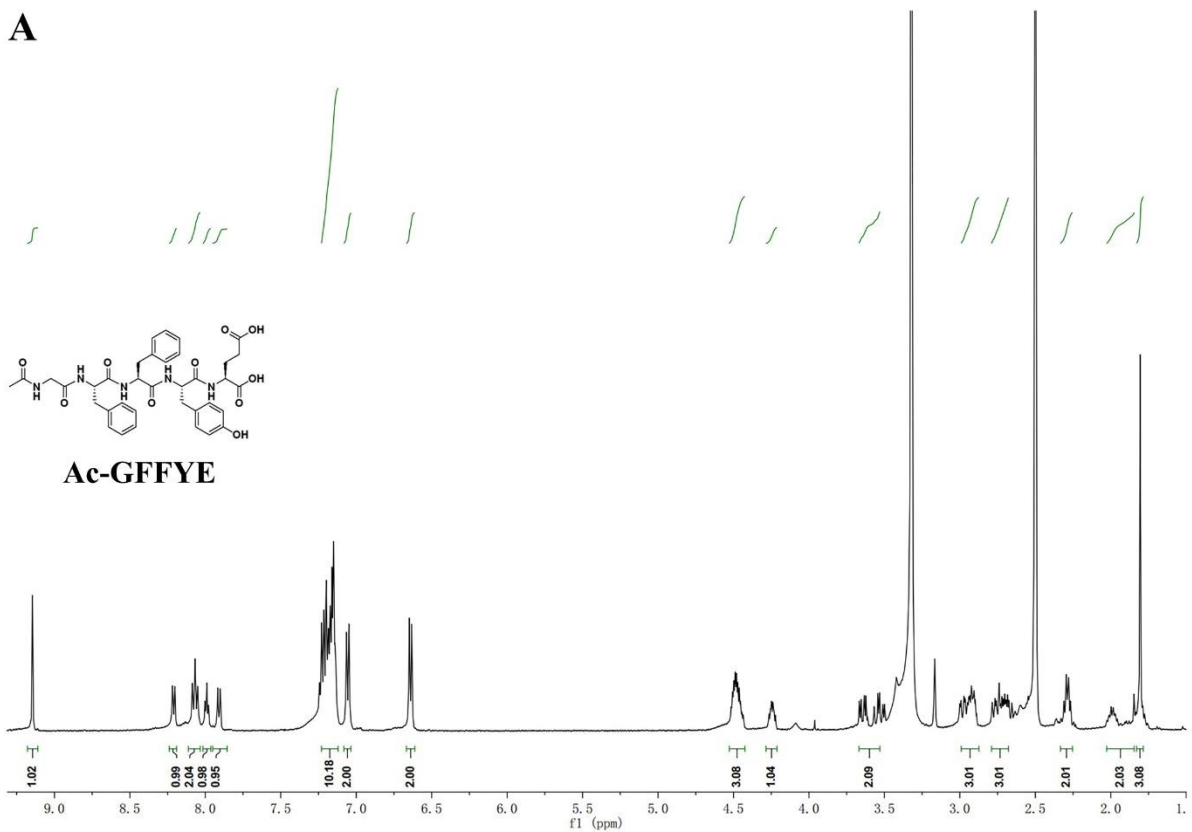
Retinal Microenvironment-Protected Rhein-GFFYE Nanofibers Attenuate Retinal Ischemia-Reperfusion Injury via Inhibiting Oxidative Stress and Regulating Microglial/Macrophage M1/M2 Polarization

*Zhuhong Zhang\**, *Shengjun Peng*, *Tengyan Xu*, *Jia Liu*, *Laien Zhao*, *Hui Xu*, *Wen Zhang*, *Yuanying Zhu* and *Zhimou Yang\**

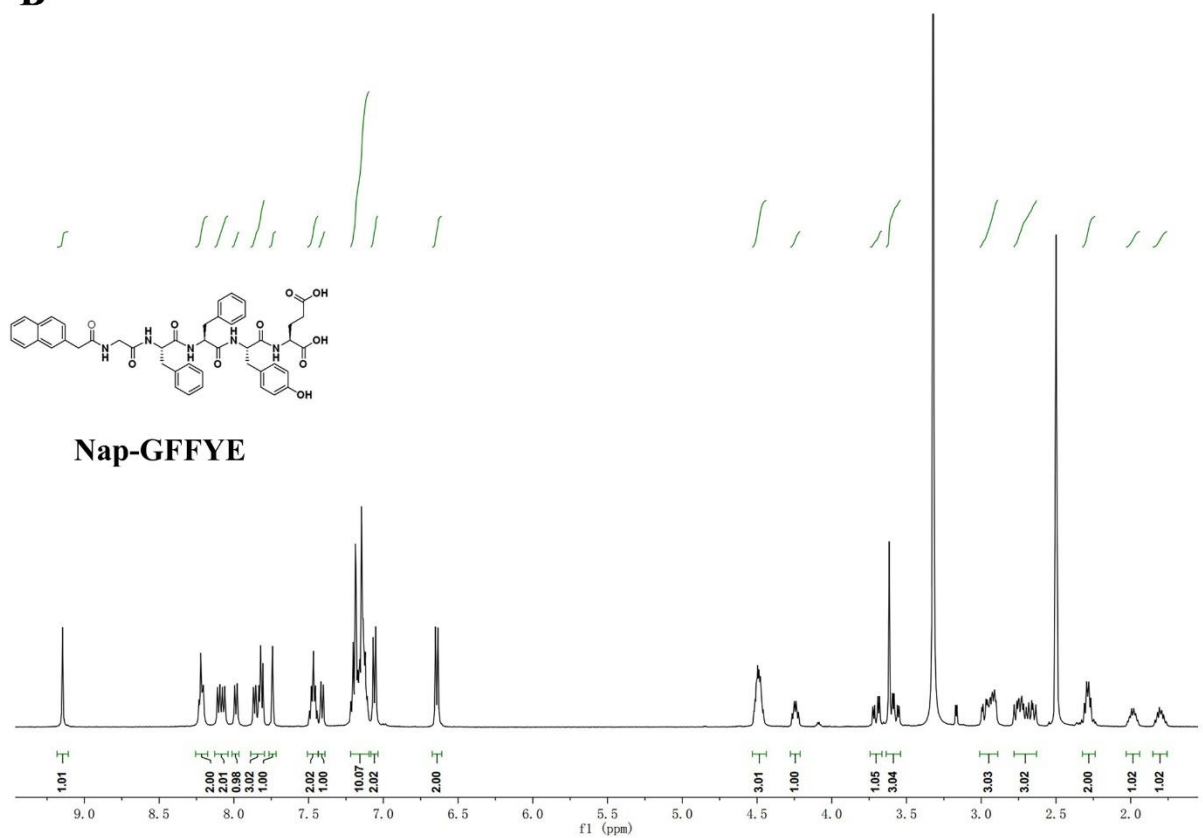
**Retinal microenvironment-protected rhein-GFFYE nanofibers attenuate  
retinal ischemia-reperfusion injury via inhibiting oxidative stress and  
regulating microglial/macrophage M1/M2 polarization**

*Zhuhong Zhang\**, *Shengjun Peng*, *Tengyan Xu*, *Jia Liu*, *Laien Zhao*, *Hui Xu*, *Wen Zhang*,  
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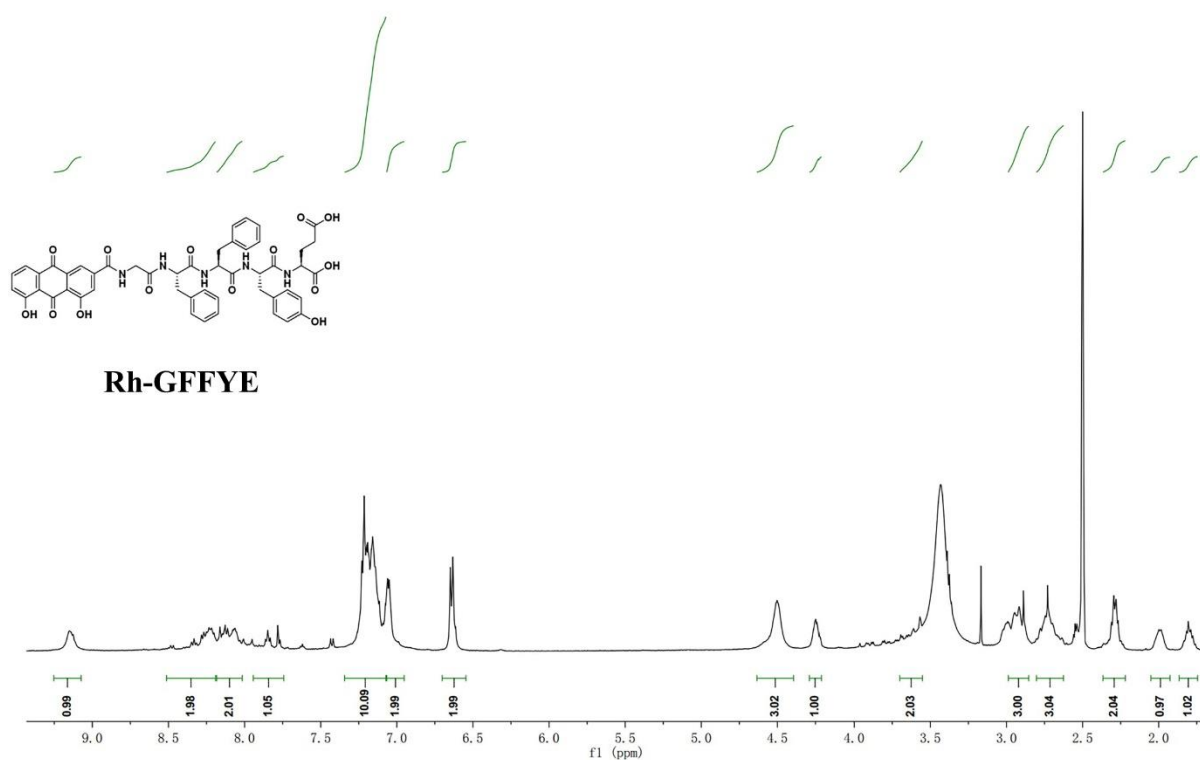
**A**



**B**

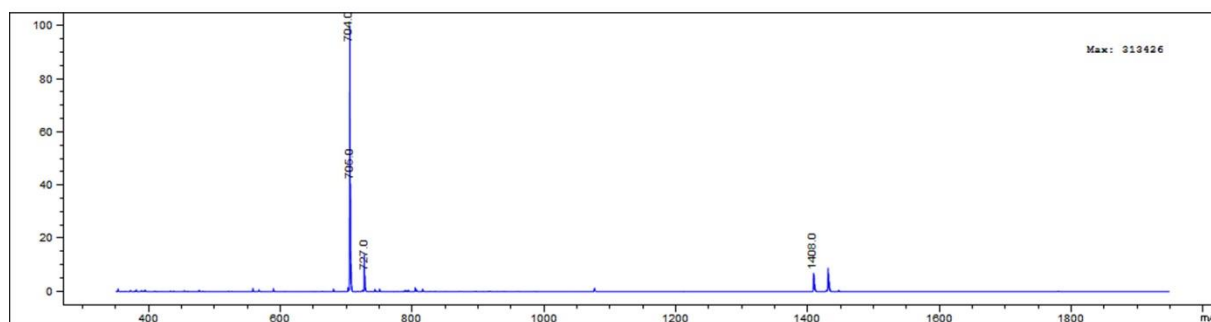


C



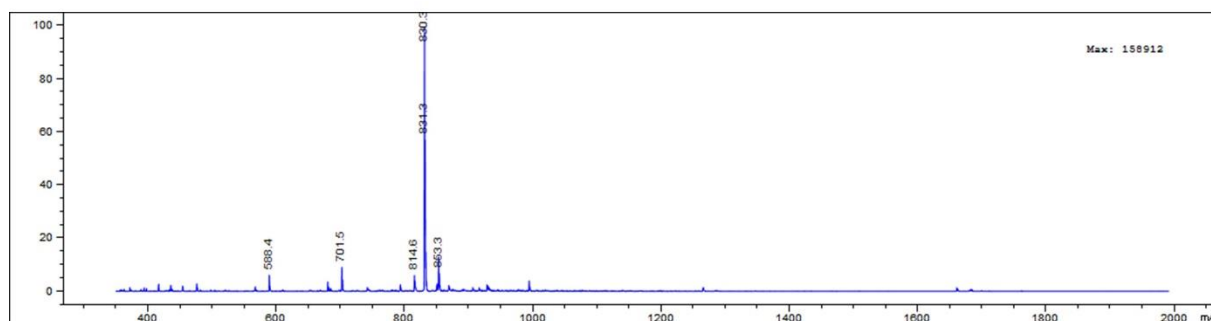
**Figure S1.  $^1\text{H}$  NMR spectrum of three compounds.** A)  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO-}d_6$ ) spectrum of Ac-GFFYE.  $\delta$  9.15 (s, 1H), 8.21 (d,  $J = 7.8$  Hz, 1H), 8.07 (t,  $J = 8.8$  Hz, 2H), 7.99 (t,  $J = 5.7$  Hz, 1H), 7.91 (d,  $J = 8.4$  Hz, 1H), 7.23 – 7.12 (m, 10H), 7.06 (d,  $J = 8.3$  Hz, 2H), 6.64 (d,  $J = 8.1$  Hz, 2H), 4.48 (dtt,  $J = 13.1, 8.7, 4.3$  Hz, 3H), 4.25 (s, 1H), 3.67 – 3.53 (m, 2H), 2.99 – 2.87 (m, 3H), 2.79 – 2.68 (m, 3H), 2.33 – 2.25 (m, 2H), 2.03 – 1.85 (m, 2H), 1.81 (s, 3H). B)  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO-}d_6$ ) spectrum of Nap-GFFYE.  $\delta$  9.15 (s, 1H), 8.22 (q,  $J = 4.7, 3.5$  Hz, 2H), 8.09 (dd,  $J = 16.2, 8.1$  Hz, 2H), 7.99 (d,  $J = 8.3$  Hz, 1H), 7.89 – 7.80 (m, 3H), 7.74 (s, 1H), 7.47 (tt,  $J = 7.0, 5.2$  Hz, 2H), 7.41 (dd,  $J = 8.4, 1.7$  Hz, 1H), 7.22 – 7.10 (m, 10H), 7.06 (d,  $J = 8.2$  Hz, 2H), 6.64 (d,  $J = 8.3$  Hz, 2H), 4.49 (qd,  $J = 9.7, 9.2, 4.1$  Hz, 3H), 4.24 (td,  $J = 8.3, 5.1$  Hz, 1H), 3.70 (dd,  $J = 16.7, 5.7$  Hz, 1H), 3.64 – 3.54 (m, 3H), 3.01 – 2.89 (m, 3H), 2.78 – 2.63 (m, 3H), 2.32 – 2.24 (m, 2H), 1.99 (dq,  $J = 13.7, 7.5, 6.8$  Hz, 1H), 1.85 – 1.76 (m, 1H). C)  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO-}d_6$ ) spectrum of Rh-GFFYE.  $\delta$  9.15 (s, 1H), 8.24 (td,  $J = 16.0, 15.6, 7.8$  Hz, 2H), 8.18 – 8.02 (m, 2H), 7.94 – 7.74 (m, 1H), 7.34 – 7.07 (m, 10H), 7.05 (q,  $J = 6.6, 4.6$  Hz, 2H), 6.63 (t,  $J = 8.8$  Hz, 2H), 4.63 – 4.39 (m, 3H), 4.25 (tt,  $J = 9.0, 4.8$  Hz, 1H), 3.70 – 3.55 (m, 2H), 2.99 – 2.85 (m, 3H), 2.80 – 2.63 (m, 3H), 2.37 – 2.22 (m, 2H), 1.99 (s, 1H), 1.81 (tt,  $J = 10.4, 6.1$  Hz, 1H).

A



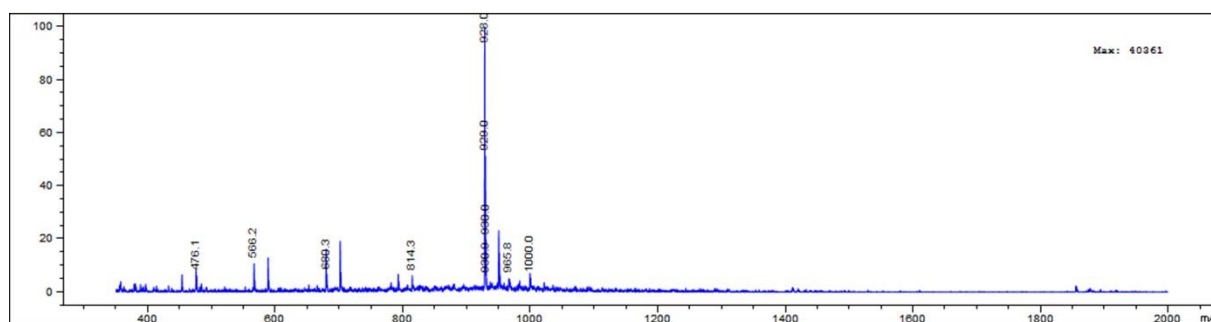
B

Ac-GFFYE



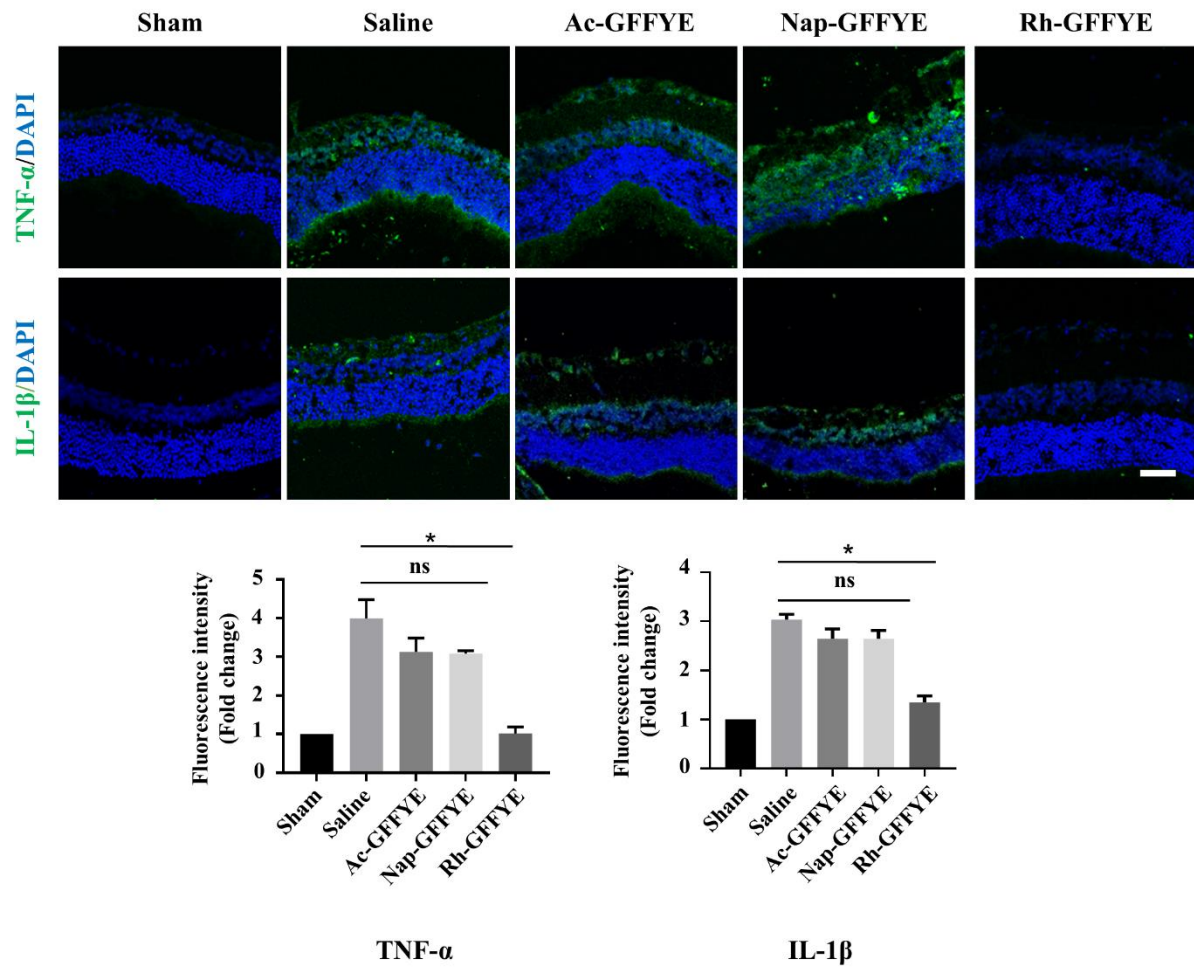
C

Nap-GFFYE

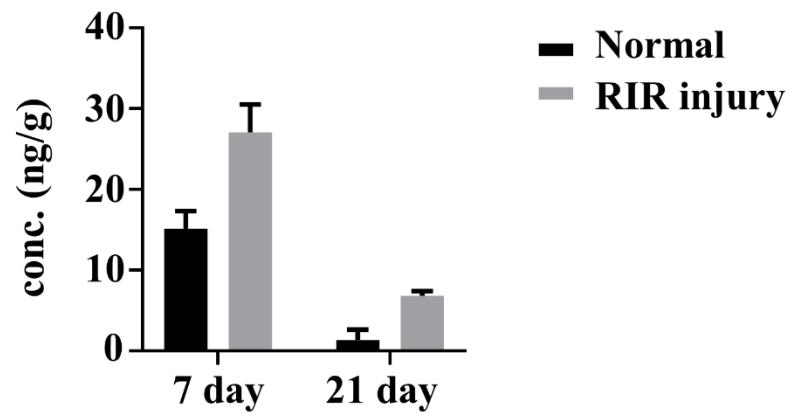


Rh-GFFYE

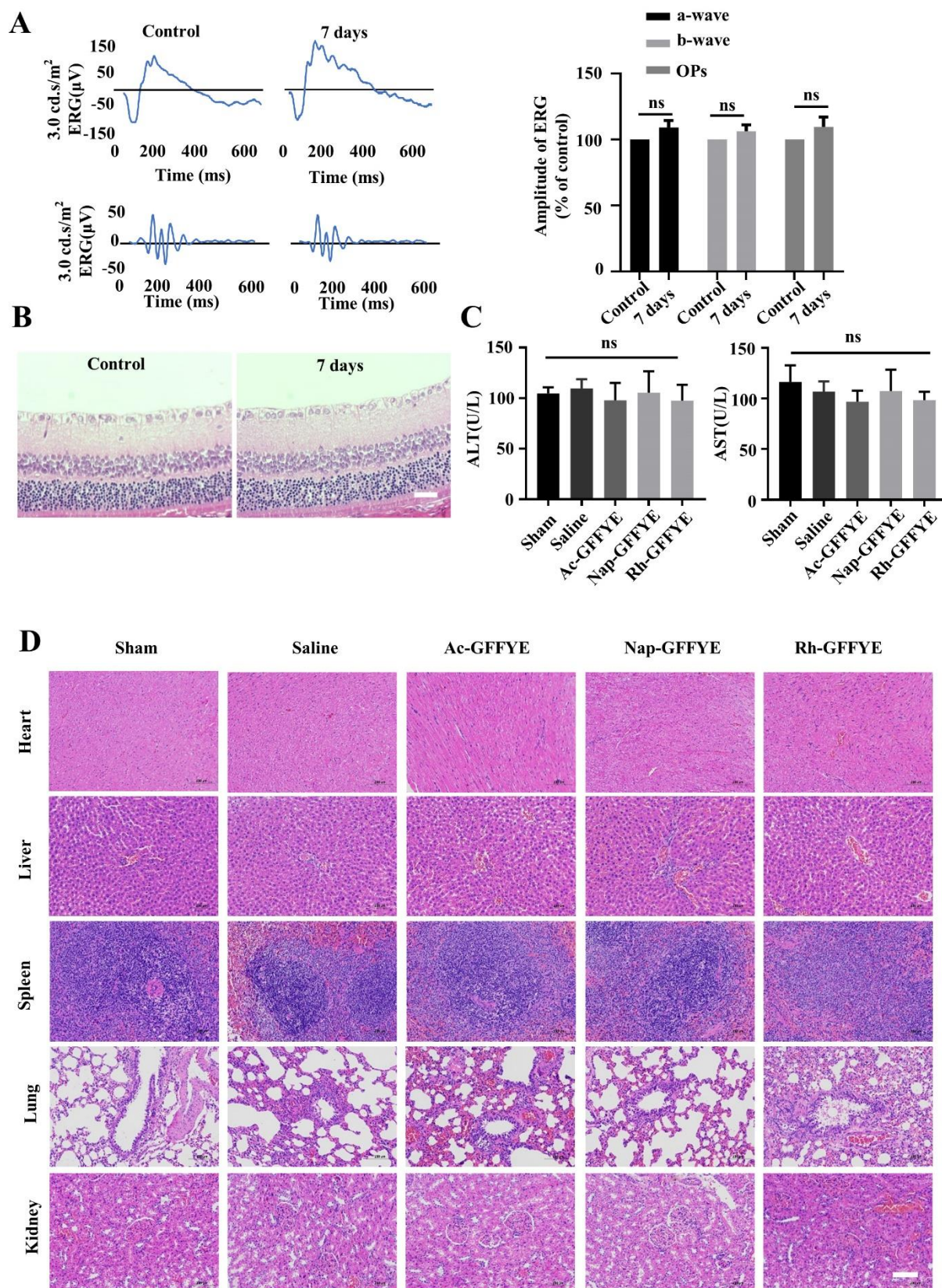
**Figure S2. Mass spectrometry (MS) spectrum of three compounds.** A) MS spectrum of Ac-GFFYE. MS: calcd.  $M = 703.7$ , obsvd.  $(M+H)^+ = 704$ . B) MS spectrum of Nap-GFFYE. MS: calcd.  $M = 829.9$ , obsvd.  $(M+H)^+ = 830.3$  C) MS spectrum of Rh-GFFYE. MS: calcd.  $M = 927.9$ , obsvd.  $(M+H)^+ = 928.0$ .



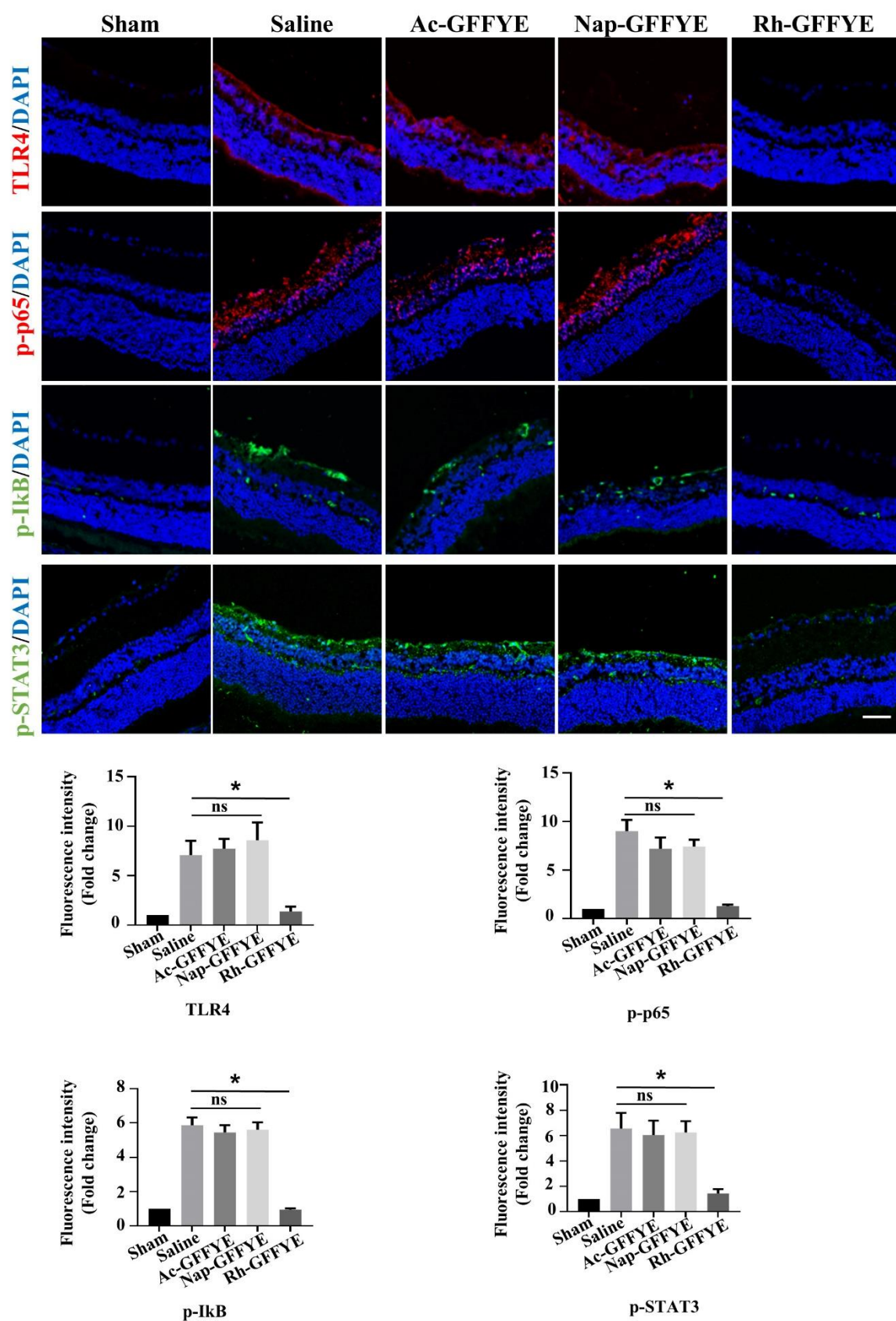
**Figure S3. Inhibition of RIR injury-induced secretion of inflammatory cytokines by Rh-GFFYE following intravitreal injection.** Representative CLSM images showing the levels of inflammatory factor TNF- $\alpha$  and IL-1 $\beta$  in retinal cryosections collected at 7 days after intravitreal injection. Fluorescence intensities of TNF- $\alpha$  and IL-1 $\beta$  quantified by Image J software. Scale bar = 50  $\mu$ m. The data were presented as the mean  $\pm$  SD of  $n = 6$ ; \* $P < 0.05$  compared with the Saline group.



**Figure S4. Distribution of rhein in normal and ischemia retinas.** Concentrations of rhein in normal and RIR injury retinas after 7 days and 21 days following intravitreal injection of Rh-GFFYE.

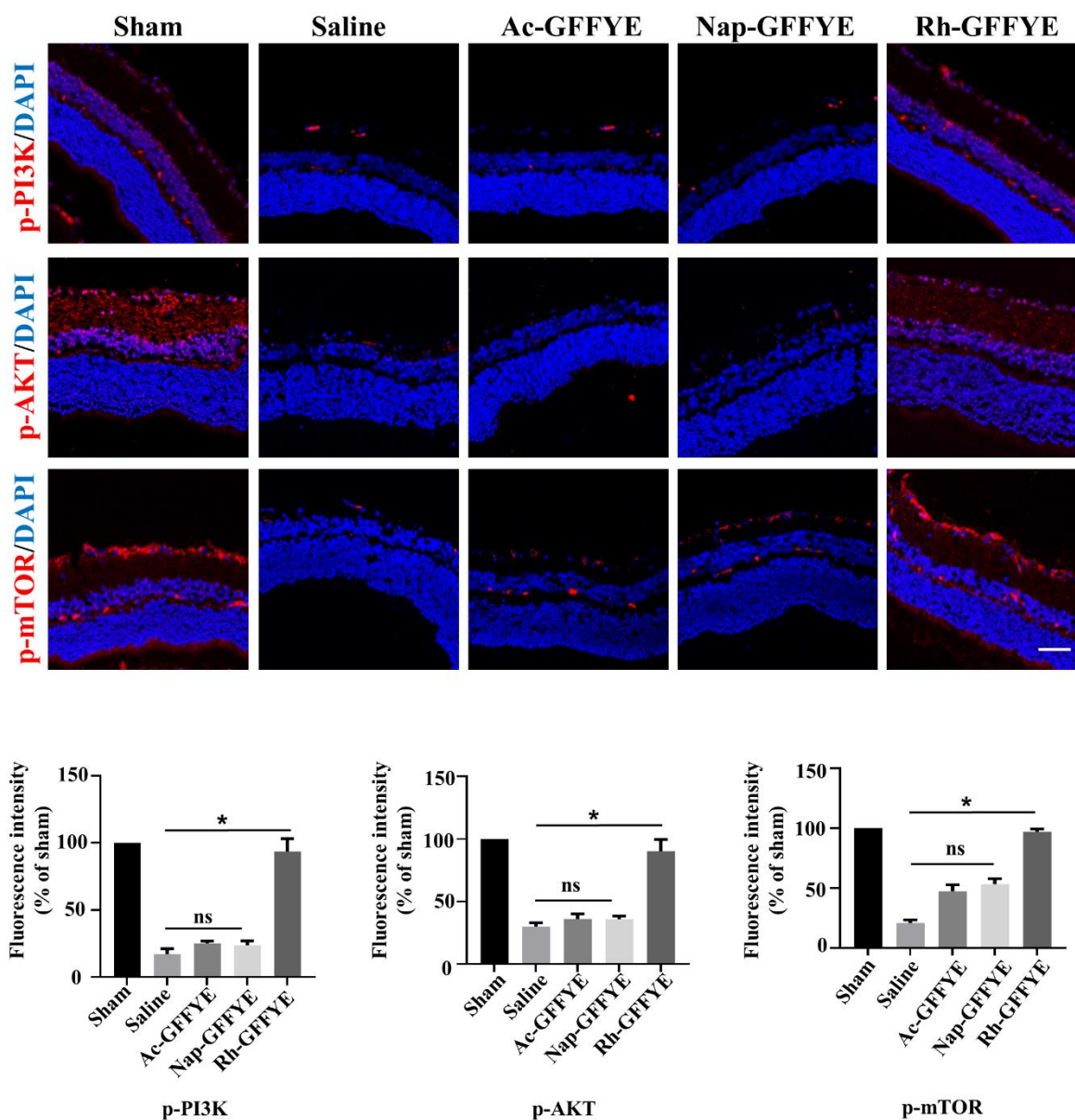


**Figure S5. Safety evaluation of Rh-GFFYE.** A) Representative ERG traces of normal rats treated with Rh-GFFYE after 7 days (lighting intensity =  $3.00 \text{ cd}\cdot\text{s}\cdot\text{m}^{-2}$ ), Amplitudes of the scotopic ERG a and b waves and sums of the OPs amplitudes at the light intensity of  $3.00 \text{ cd}\cdot\text{s}\cdot\text{m}^{-2}$ . The data were presented as the mean  $\pm$  SD of  $n = 6$ . B) H&E stained retina tissues collected at 7 days after intravitreal injection of Rh-GFFYE. Scale bar =  $50 \mu\text{m}$ . C) Measurement of serum AST and ALT activity in rats after intravitreal injection Rh-GFFYE for 7 days. The data were presented as the mean  $\pm$  SD of  $n = 6$ . D) H&E staining of main organs of heart, liver, spleen, lung, kidney. Scale bar =  $100 \mu\text{m}$ .



**Figure S6. Inhibition of RIR injury-induced upregulation of NF-κB and STAT3 signaling pathways by Rh-GFFYE following intravitreal injection.** Representative CLSM images of TLR4, p-NF-κB p65 (p-p65), p-IκB and p-STAT3 in retinal cryosections indicating that Rh-GFFYE-mediated reduction in the level of TLR4, p-

NF- $\kappa$ B p65, p-I $\kappa$ B and p-STAT3 in RIR-injured retina compared to Saline group. Fluorescence intensities of TLR4, p-NF- $\kappa$ B p65, p-I $\kappa$ B and p-STAT3 quantified by Image J software. Scale bar = 50  $\mu$ m. The data were presented as the mean  $\pm$  SD of  $n = 6$ . \* $p < 0.05$  compared with the Saline group.



**Figure S7. Upregulation of RIR injury-induced inhibition of PI3K/AKT/mTOR signaling pathway by Rh-GFFYE following intravitreal injection.** Representative CLSM images of p-PI3K, p-mTOR and p-AKT in retinal cryosections indicating that Rh-GFFYE-mediated enforcement in levels of p-PI3K, p-AKT and p-mTOR in RIR-injured retina compared to Saline group. Fluorescence intensities of p-PI3K, p-AKT and p-mTOR quantified by Image J software. Scale bar = 50  $\mu$ m. The data were presented as the mean  $\pm$  SD of  $n = 6$ . \* $p < 0.05$  compared with the Saline group.