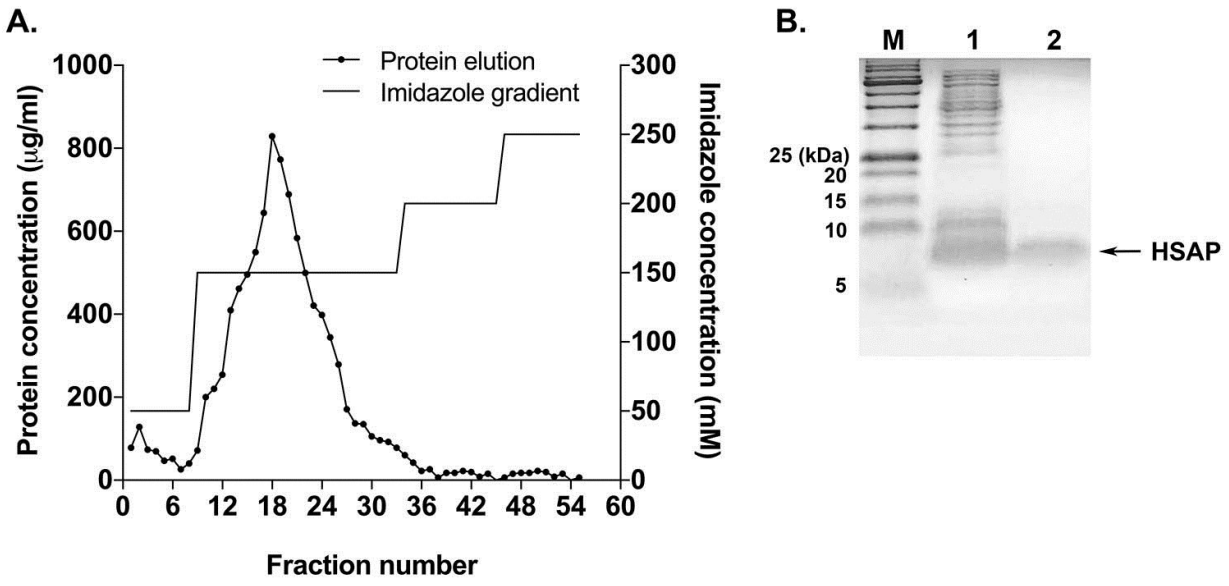
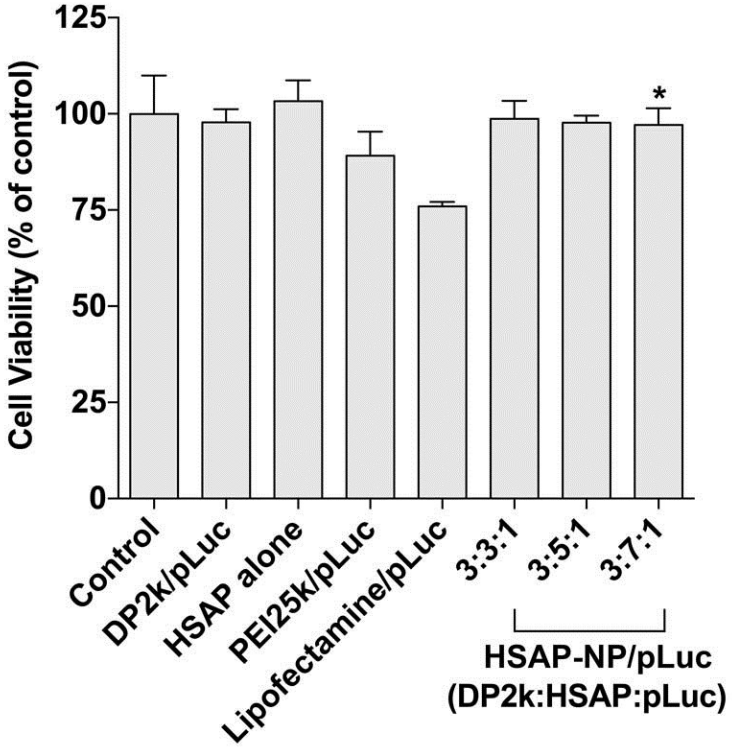
Electronic Supplementary Material (ESI) for Biomaterials Science.



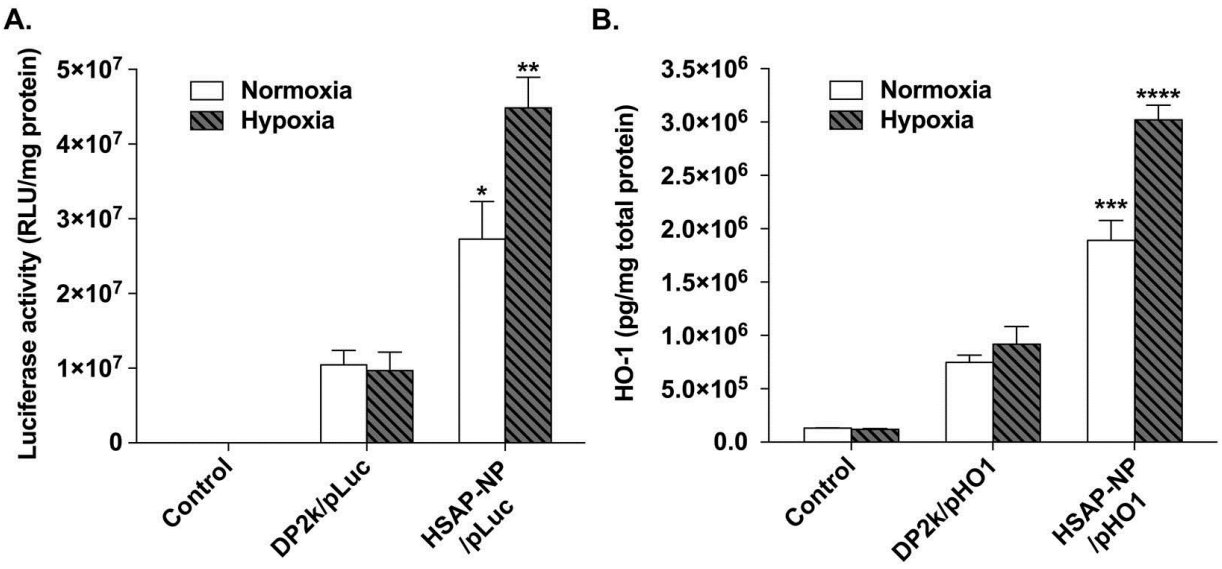
Supplementary Fig. 1. Purification of HSAP.

(A) Nickel-chelate affinity chromatography. HSAP was purified by nickel-chelate affinity chromatography using step gradients of imidazole. HSAP was eluted at a concentration of 150 mM imidazole. **(B) SDS-PAGE.** Purified HSAP was analyzed by SDS-PAGE. M: molecular weight markers, 1: crude extracts, 2: purified HSAP.

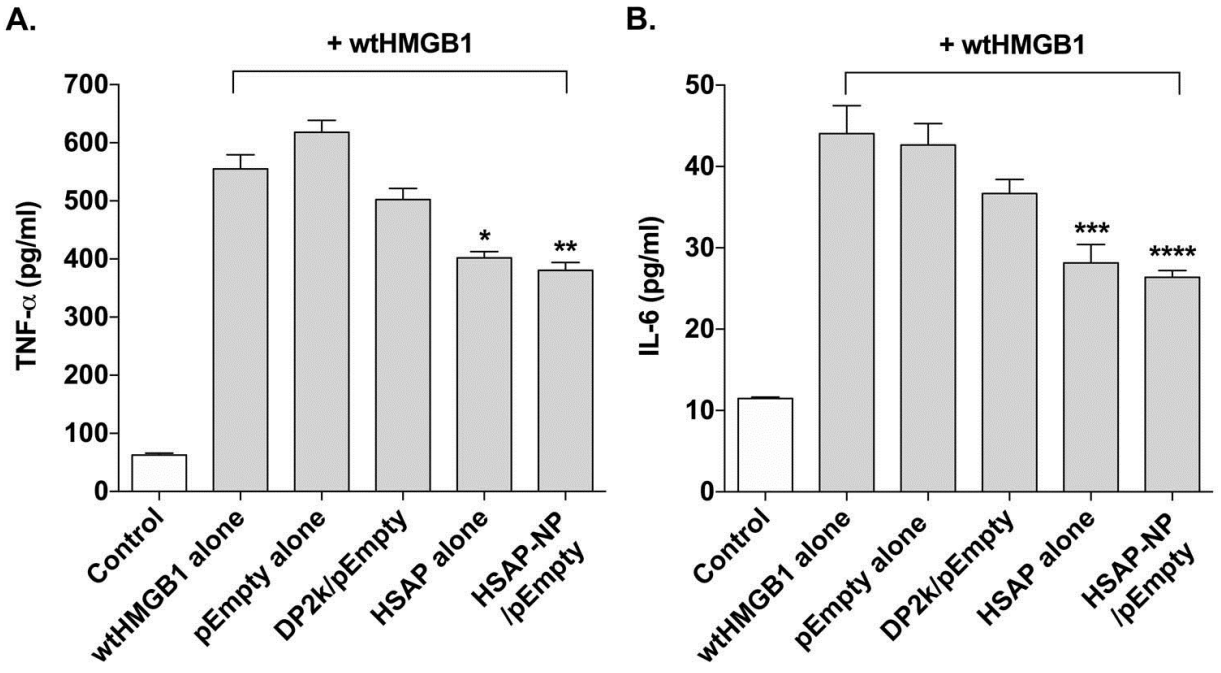


Supplementary Fig. 2. Cytotoxicity of HSAP-NP in Neuro2A cells.

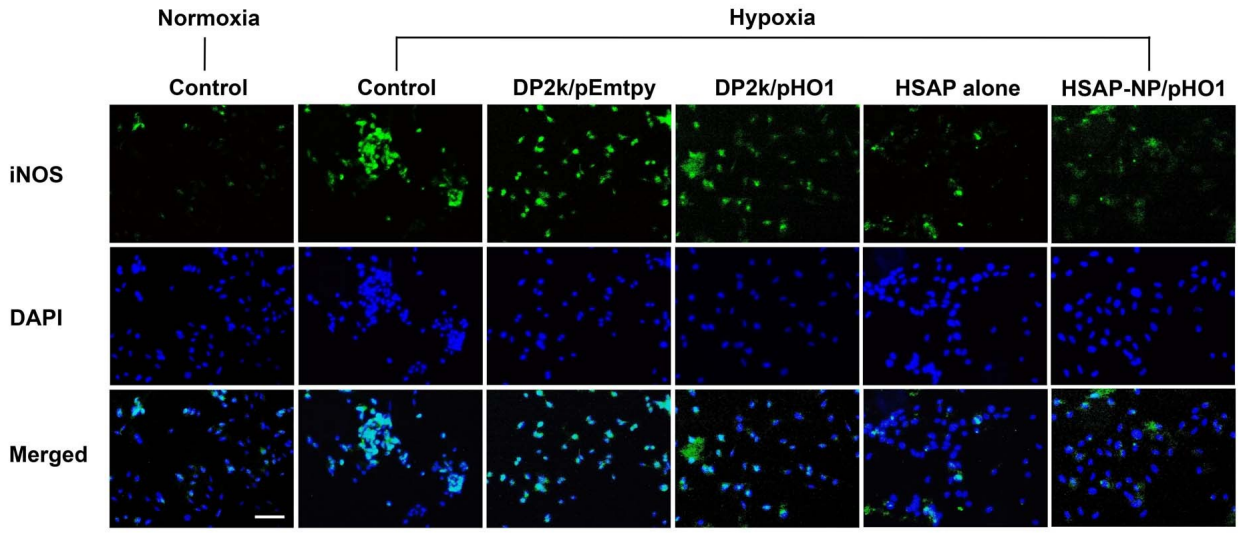
DP2k/pLuc complex, PEI25k/pLuc, and Lipofectamine/pLuc complexes were prepared at their optimal weight ratios for transfection. To prepare the HSAP-NP, DP2k/pβ-Luc complex at a fixed weight ratio of 3:1 was mixed with various amounts of HSAP. DP2k/pLuc, PEI25k/pLuc, Lipofectamine/pLuc complexes, HSAP alone, and HSAP-NP were transfected to Neuro2A cells. After 24 h, toxicity of the reagent was measured by MTT assay. The data are presented as the mean value ± standard deviation of quadruplicate experiments. *$p < 0.05$ compared with Lipofectamine/pLuc.



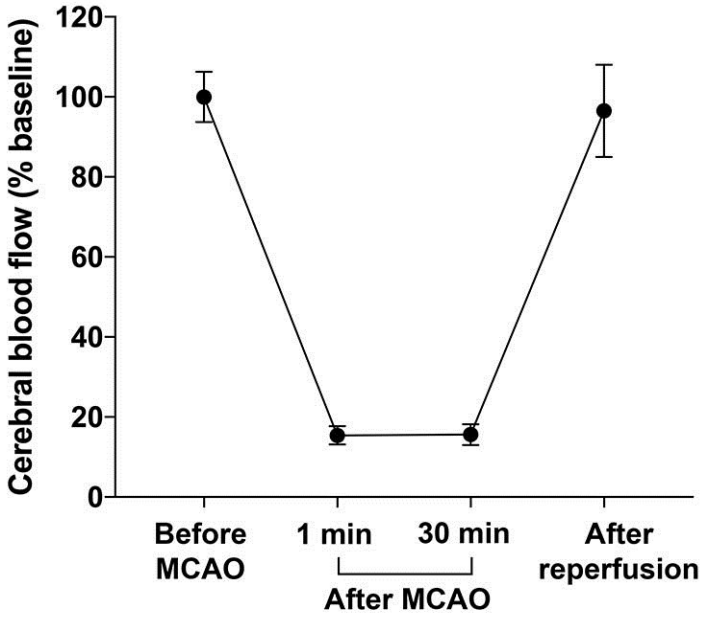
Supplementary Fig. 3. Gene delivery efficiency of HSAP-NP in C6 rat glial cells. (A) Luciferase assay. DP2k/pβ-Luc complex and HSAP-NP/pLuc were prepared at their optimal weight ratios and transfected into C6 cells. The cells were incubated under normoxia or hypoxia conditions for 24 h. Transfection efficiency was measured by luciferase assay. The data are presented as the mean value ± standard deviation of triplicate experiments. *, **$p < 0.05$ compared to the other groups. **(B) HO1 ELISA.** DP2k/pHO1 complex and HSAP-NP/pHO1 were prepared and transfected into C6 cells. The cells were incubated under normoxia or hypoxia conditions for 24 h. HO-1 expression was measured by HO1 ELISA. The data are presented as the mean value ± standard deviation of triplicate experiments. ***, ****$p < 0.05$ compared to the other groups.



Supplementary Fig. 4. Anti-inflammatory effects of HSAP-NP. Raw264.7 mouse macrophage cells were seeded at 24 h before transfection. After 24 h, the cells were incubated with wtHMGB1 for 4 h. Next, pEmpty alone, DP2k/pEmpty, HSAP alone, and HSAP-NP/pEmpty were transfected into the cells. After 24 h, the cell culture media were harvested, and pro-inflammatory cytokines were analyzed by ELISA. **(A) TNF-α ELISA. (B) IL-6 ELISA.** The data are presented as the mean value ± standard deviation of triplicate experiments. *p, **p, ***p, ****p < 0.05 compared to the control, wtHMGB1 alone, pEmpty alone, and DP2k/pEmpty.



Supplementary Fig. 5. Down-regulation of iNOS in hypoxic condition by HSAP-NP. DP2k/pEmpty, DP2k/pHO1, HSAP alone, and HSAP-NP/pHO1 were transfected into C6 rat glioblastoma cells. After 24 h, the cells were harvested and iNOS immunofluorescence staining was performed. The cells were stained with anti-iNOS antibody (green) and nuclei were stained with DAPI (blue). Scale bar is 100 μm.



Supplementary Fig. 6. CBF in the MCAO model. CBF was analyzed by laser doppler flowmetry. After preparing the MCAO models, CBF was decreased and maintained the low blood flow for 60 min. CBF was recovered after reperfusion. The data are presented as the mean value ± standard deviation of triplicate experiments.

Supplementary Table 1. Confirmation of HSAP-NP formation: Pulldown assay with anti-His-tag antibody. HSAP-NP was prepared with Cy5-labeled DP2k at their optimal weight ratios for transfection. HSAP was immunoprecipitated with anti-His-tag antibody. Co-precipitated DP2k was measured by fluorescence, as DP2k was Cy5-labeled. Co-precipitated pDNA was measured by fluorescence after staining with StaySafe nucleic acid detection reagent.

	pDNA alone	DP2k/pDNA complex	HSAP-NP/pDNA
Fluorescence of labeled pDNA (ex/em = 514/537 nm)	116.2 ± 5.6	131.7 ± 7.5	170 ± 17.2
Fluorescence of labeled DP2k (ex/em = 648/663 nm)	1186 ± 112	1393 ± 169	2359 ± 587