Supplementary Materials

Intranasal delivery of anti-apoptotic siRNA complexed with Fas-signaling blocking peptides attenuates cellular apoptosis in brain ischemia

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Total Supplementary Figures (S1~S5)

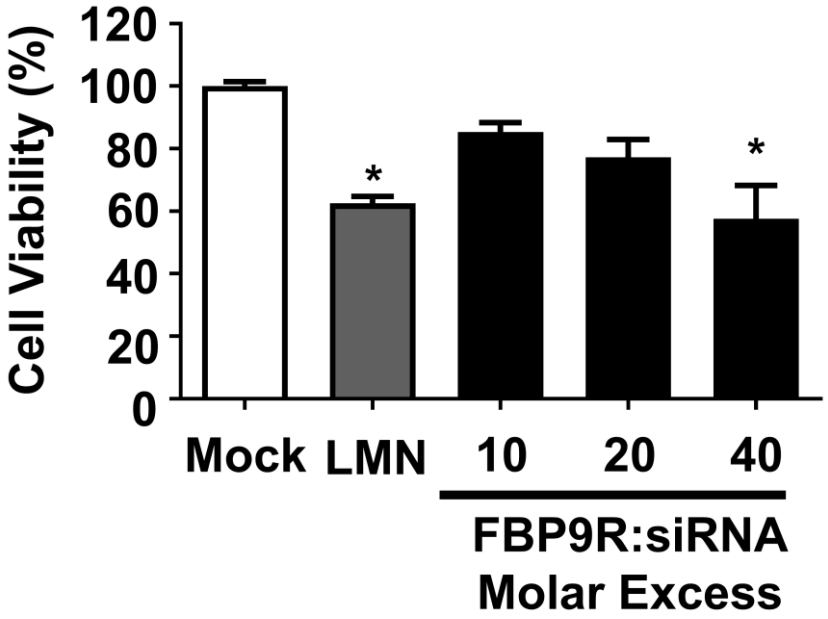

Figure S1. Cytotoxicity measured by the CCK-8 viability assay of Jurkat cells. Cytotoxicity assessed 24 hr post-transfection of Jurkat cells with FBP9RC/siRNA nanocomplexes. FBP9RC peptides were complexed with 100 pmole of siRNA at the indicated peptide:siRNA molar excess. Relative percent cell viability was normalized with the mock group. *$p<0.05$ verse 10 group.

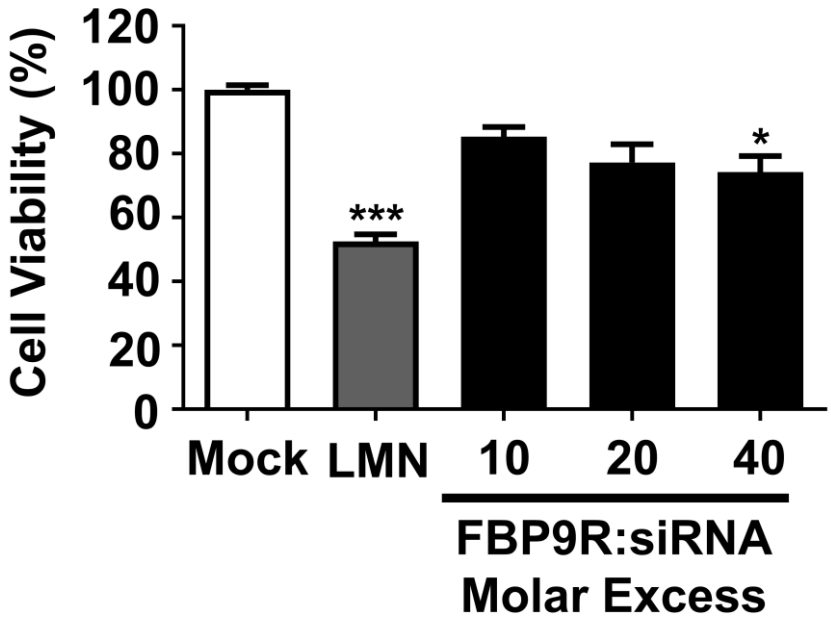

Figure S2. Cytotoxicity measured by the CCK-8 viability assay of Neuro-2a cells. Cytotoxicity assessed 24 hr post transfection of Neuro-2a cells with FBP9RC/siRNA nanocomplexes. FBP9RC peptides were complexed with 100 pmole of siRNA at the indicated peptide:siRNA molar excess. Relative percent cell viability was normalized with mock group. *$p<0.05$, ***$p<0.001$ verse 10 group.

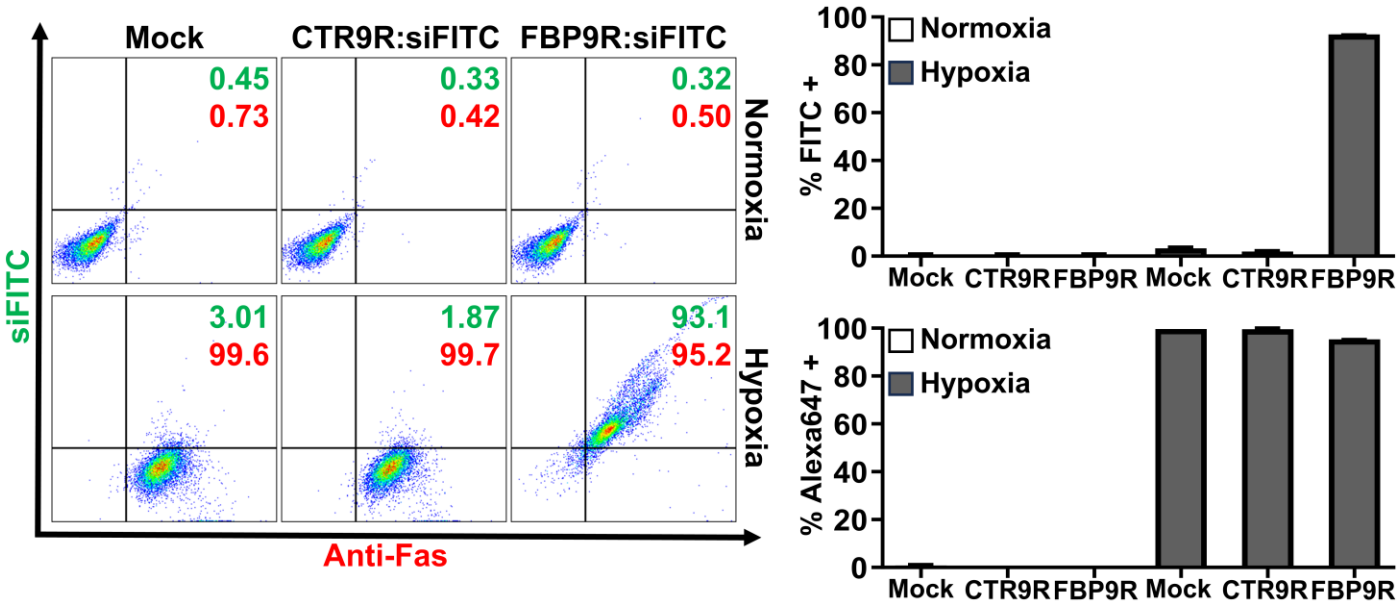

Figure S3. Fas induction in Neuro-2a cells under hypoxic conditions. Flow cytometry analysis for Fas and FBP9R binding in hypoxia Neuro-2a cells. Hypoxia induced Fas and FBP9R binding exclusively to hypoxic cells. In hypoxia, scrambled peptide (CTR9R) did not bind to Fas, although cells expressed Fas. Representative dot-plot (left panel) and cumulative data for percent of Fas-positive cells (right panel). *p<0.05, **p<0.01 versus mock group. All data were obtained from three independent experiments and shown as mean ± SD.

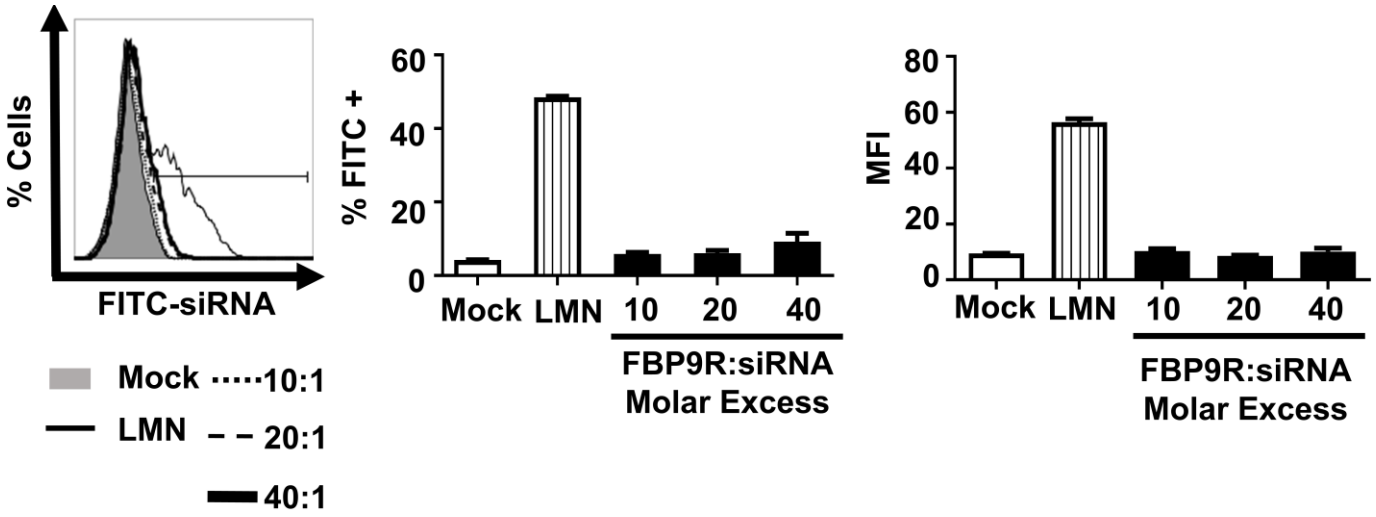

Figure S4. Ineffectiveness of FBP9R in delivering siRNA into normoxia Neuro-2a cells. Flow cytometry analysis for the efficacy of siRNA delivery by FBP9R/siRNA nanocomplexes in hypoxia Neuro-2a cells. FBP9R peptides were complexed with 200 pmole of FITC-labeled siRNA at the indicated peptide:siRNA molar excess. Representative histogram (left panel) and cumulative data for percent of FITC-positive cells (middle panel), mean fluorescence intensity (right panel). *$p<0.05$, **$p<0.01$ versus mock group. All data were obtained from three independent experiments and shown as mean ± SD.

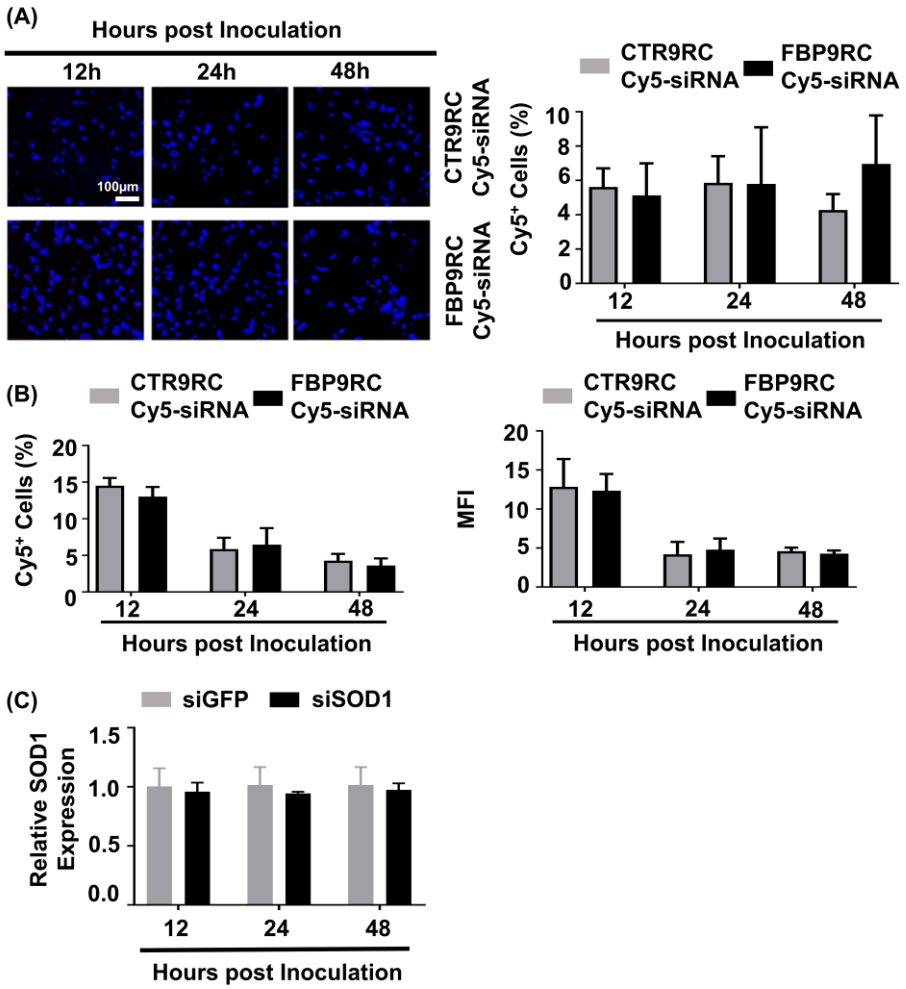

Figure S5. Lack of localization of intranasally delivered FBP9R/siRNA nanocomplexes to normoxia brain regions in the rat model of brain ischemia. (A) Immunohistochemistry of intranasally delivered Cy5-labeled siRNA in the normal hemisphere at indicated hours post-injection. Representative images (left panel) and cumulative data from six independent animals per groups (right panel) indicating percent of Cy5-positive cells. Scale bar indicates 50 μm. (B) Flow cytometry analysis of Cy5-positive cells in the infarcted hemisphere. Cumulative data for percent of Cy5-positive cells (left panel) or mean fluorescence intensity (right panel) obtained from six independent animals per groups. (C) Gene silencing efficiency of intranasally delivered FBP9R/siRNA nanocomplexes in the normal hemisphere from six independent animals.