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Supplemental Information

Guanidinylated Neomycin Conjugation Enhances Intranasal Enzyme Replacement in the Brain

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Figure S1. GNeo structure and purification of GNeo-conjugated recombinant enzymes. A Structure of *N*-hydroxysuccinimide activated ester of guanidinylated neomycin (GNeo-NHS).[48] **B-D** GNeo conjugated enzymes were purified by affinity chromatography on heparin-Sepharose. The elution positions of conjugated and unconjugated enzyme are shown. Unconjugated SGSH did not bind to column. Chromatograms show elution profile of **B** GNeo-AP-IDUA, **C** GNeo-IDUA and **D** GNeo-SGSH.

Figure S2. GNeo-conjugation does not affect enzyme stability. A AP-IDUA and GNeo-AP-IDUA were incubated at 45 ^{o}C for the indicated time and then assayed for enzyme activity. The values were scaled to samples that had not been heat-treated. **B** MPS I mice (*Idua*$^{-/-}$) were treated intravenously with 5 mg/kg GNeo-IDUA. Plasma was collected at 2, 5, 10, 20, 40 and 60 minutes following treatment. IDUA enzyme activity was measured in the plasma of N = 3 mice.

Figure S3. GNeo conjugation does not enhance nose-to-brain delivery of recombinant enzymes. A *Idua*$^{-/-}$ mice (20-36 weeks) were treated intranasally with PBS, 1 mg/kg IDUA or 1 mg/kg GNeo-IDUA. **B** *Sgs*$^{+/-}$ mice (6-9 weeks old) were treated intranasally with 1 mg/kg Myc-His_6-SGSH or 1 mg/kg GNeo- Myc-His_6-SGSH. Animals were perfused with PBS and whole brains were collected 1 hr following treatment. The amount of IDUA mass was determined by measuring enzyme activity against a standard curve of enzyme activity vs. Aldurazyme® mass. The amount of Myc-His_6-SGSH in brain extracts was measured by ELISA using known amounts of Myc-His_6-SGSH to create a standard curve.

Figure S4. Pharmacokinetic analysis of intranasally administered GNeo-conjugated sulfamidase. *Sgsh*$^{+/-}$ mice (8-9 weeks old) were treated intranasally with 1 mg/kg GNeo-His_6-SGSH and perfused with PBS at 0, 0.5, 1, 2, and 4 hrs following treatment. His_6-SGSH enzyme was collected from whole brain extracts using Ni^{2+} beads and enzyme activity was measured and normalized to a standard curve generated with known amounts of His_6-SGSH. N = 3–5 mice per bar.

Figure S5. GNeo-IDUA reduces GAG storage in the *Idua*$^{-/-}$ mouse brain. *Idua*$^{-/-}$ mice were treated intranasally with 1 mg/kg IDUA or GNeo-IDUA every-other-day. Uronic acid levels were measured by carbazole assay in GAG preparations from whole brain lysate. **A** 14 d treatment, **B** 30 d treatment, **C** 60 d treatment.

Figure S6. GNeo-SGSH reduces the accumulation of the MPS IIIA biomarker the brain. *Sgsh*$^{h/h}$ mice were treated intranasally with 1 mg/kg SGSH or GNeo-SGSH every other day for 30 d. Non-reducing end biomarkers were measured in GAG preparations from whole brain homogenates.

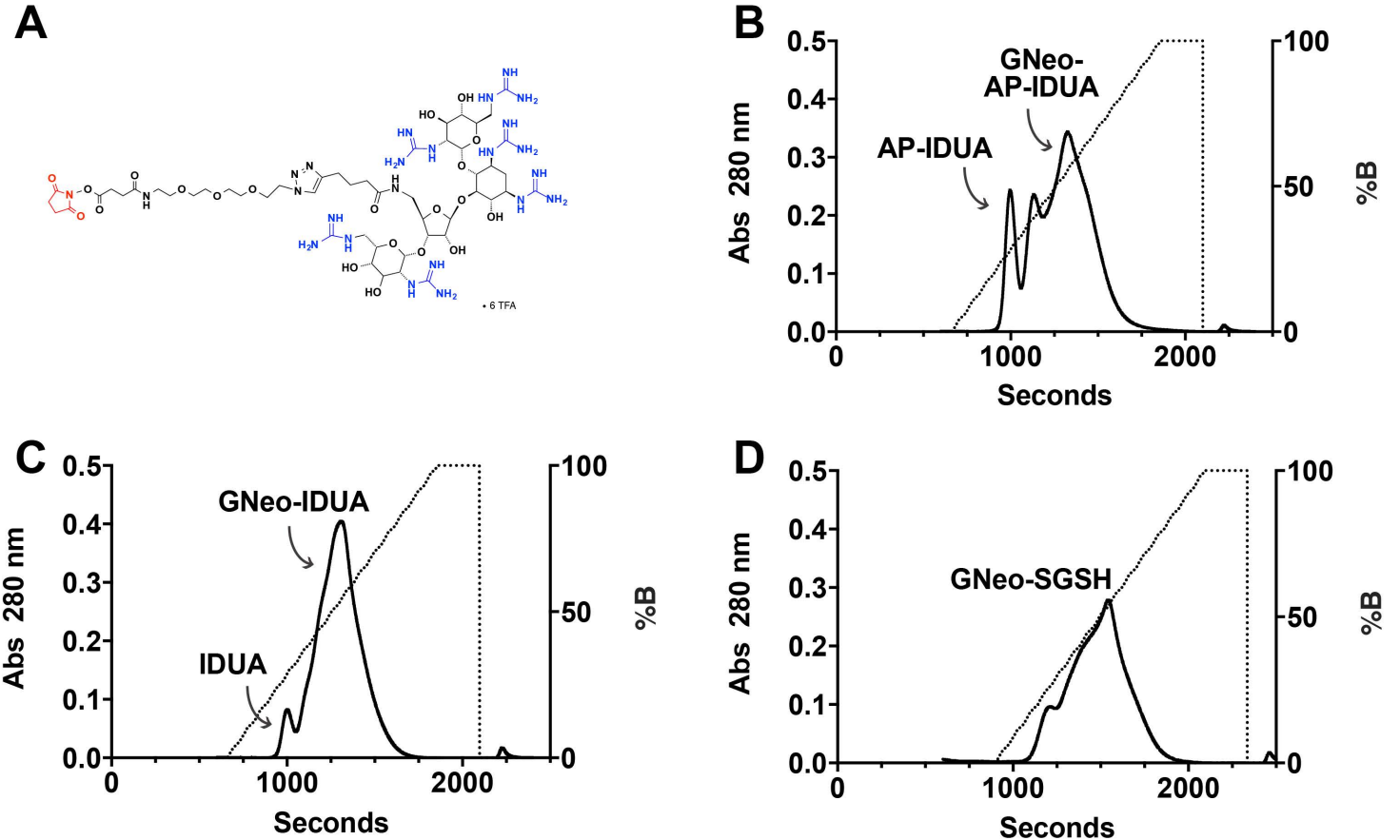


Fig. S1 Tong et al.

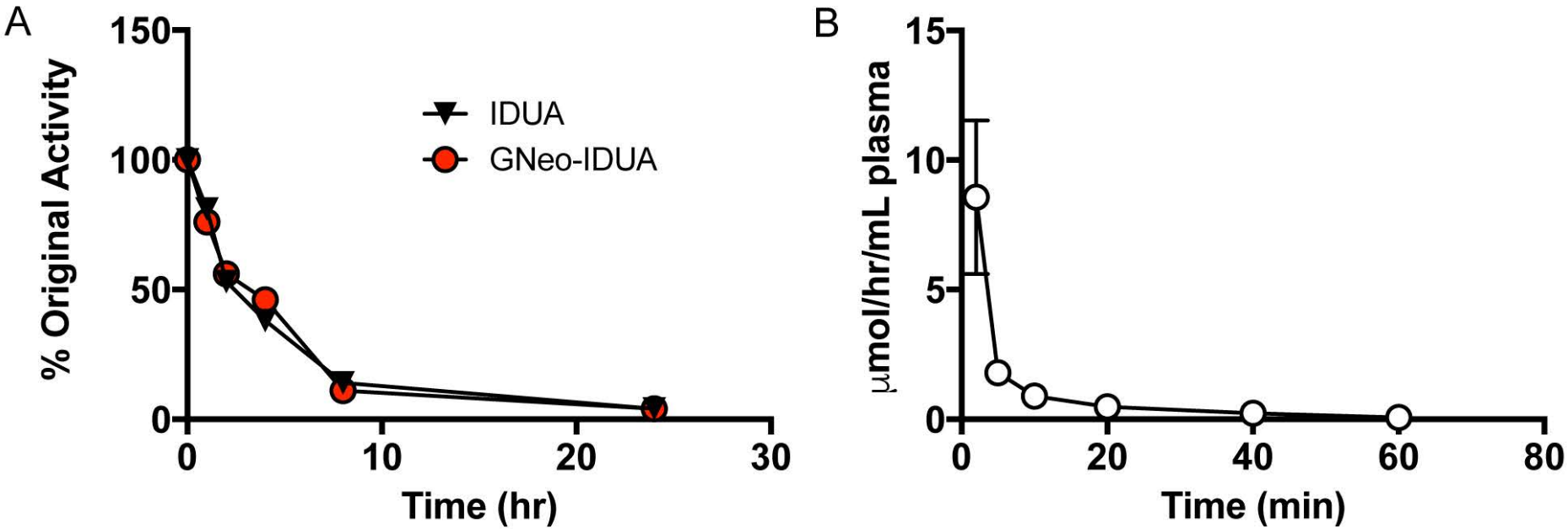


Fig. S2 Tong et al.

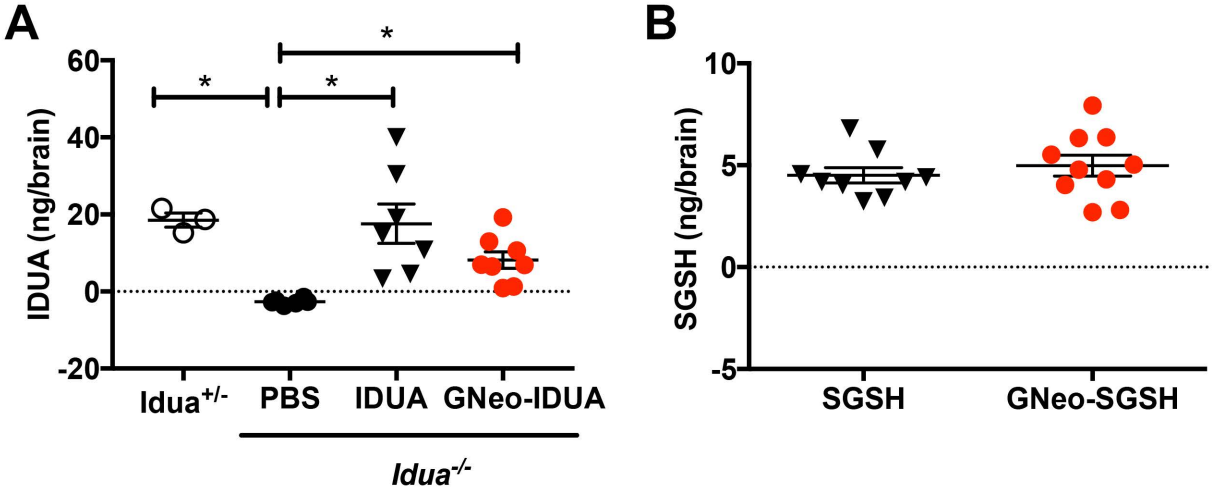
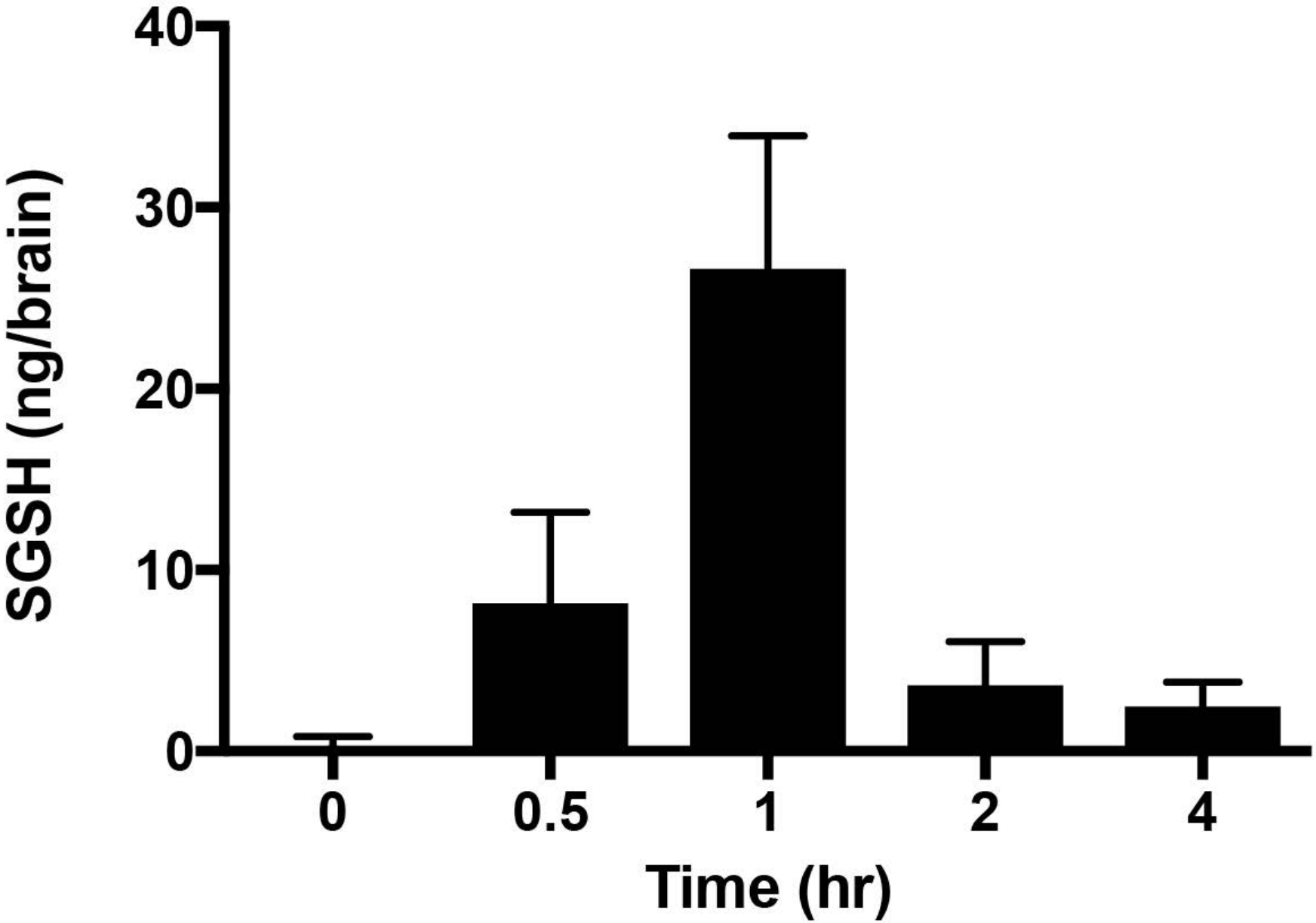


Fig. S3 Tong et al.



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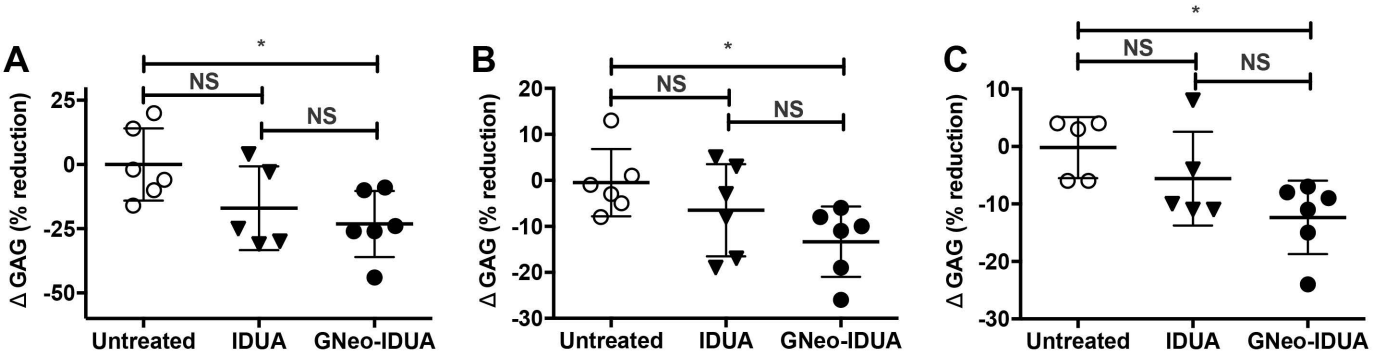

Fig. S5 Tong et al.

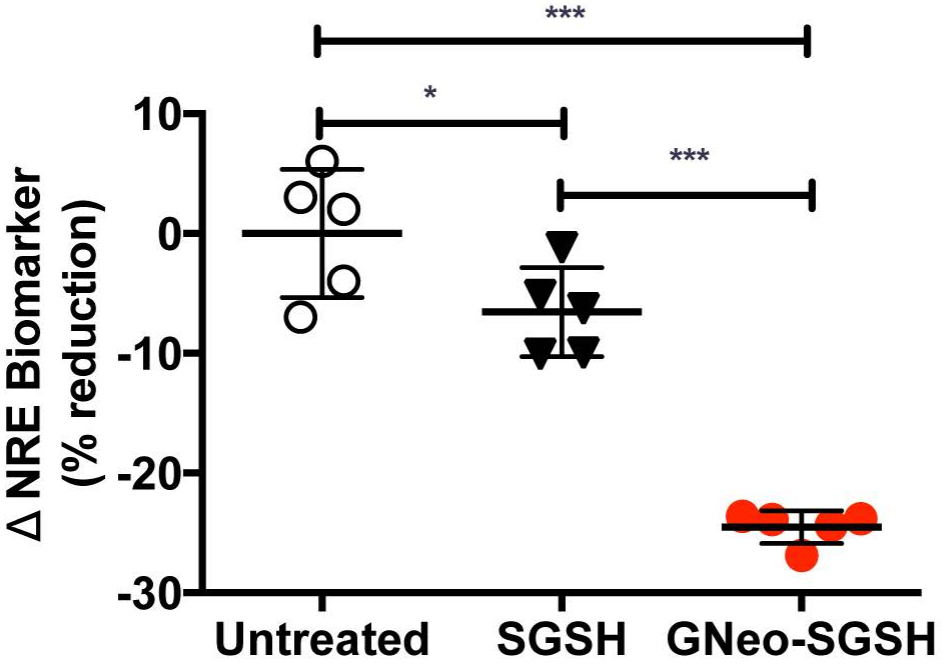

Fig. S6 Tong et al.