

depleting 6A6-IgG2b antibodies were sialylated as described above. (B) Sialylated 6A6-IgG2b antibodies were administered to mice and platelet counts determined 0, 4, and 24 hours following treatment. Mean and standard deviation of 5 mice per group are plotted; \* $p < 0.05$  as determined by Anova followed by Tukey's post hoc.

**Figure 4.** Recombinant, sialylated IgG Fc fragments are anti-inflammatory. Recombinant human IgG1 was digested with papain and Fcs were purified by HPLC followed by protein G purification. The recombinant Fcs (rFc) were galactosylated and sialylated *in vitro* with  $\alpha 2,6$  sialyltransferase. A. Glycosylation was confirmed by lectin blotting for terminal galactose with ECL (top panel),  $\alpha 2,6$  sialic acid with SNA (middle panel), and coomassie loading controls are shown in the bottom panel. B. Mice were administered 1g/kg IVIG, 0.033g/kg SNA<sup>+</sup> IVIG Fcs, or 0.33g/kg sialylated rFc (2,6ST rFc) 1 hour prior to K/BxN sera, and footpad swelling was monitored over the next several days. Mean and standard deviation of clinical scores of 4-5 mice per group are plotted; \*denotes  $p < 0.05$  as determined by Kruskal-Wallis Anova followed by Dunn's post hoc.

**Supplemental figure 1.** Linkage-specific sialidase digestions of IVIG. Linkage-specific sialidase digestion conditions were optimized using fetuin, a high-sialylated protein. A. Fetuin was incubated with an  $\alpha 2,3/2,6$  sialidase (top two panels) or an  $\alpha 2,3$  sialidase (bottom two panels) for different intervals, and digestions assessed by lectin blotting for  $\alpha 2,6$  (SNA) and  $\alpha 2,3$  (MAL I) linkages. Four hour incubation with each enzyme specifically removed the appropriate

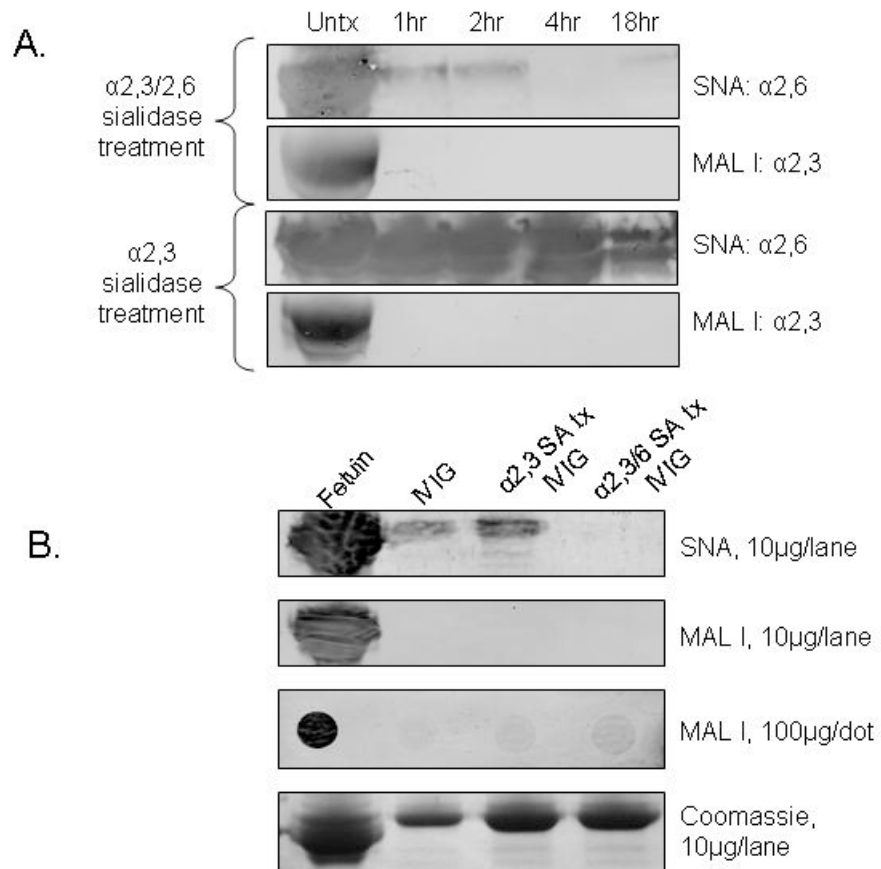
linkages compared to untreated controls. Therefore, these optimized conditions were then applied to IVIG, and the digestions verified by lectin blotting. B. Fetuin, untreated IVIG and  $\alpha$ 2,3 SA tx IVIG contained  $\alpha$ 2,6 linkages (SNA, top panel). While fetuin stained strongly with MAL I (middle panels) for  $\alpha$ 2,3 linkages, these linkages were not detected on any IVIG preparation in conventional lectin blots where 10 $\mu$ g per lane nor in dot blots where 100 $\mu$ g of sample were spotted. Coomassie stained loading controls are shown below.

**Supplemental Figure 2.** *In vitro* sialylation of Fcs. A. To indirectly evaluate sialylation efficiency, these glycosylated preparations were verified by lectin blotting for terminal galactose (ECL). B. Intensities were normalized by coomassie loading controls; the  $\beta$ 1,4GT treated Fc sample was considered to be 100% galactosylated, while the 2,6ST treated sample was calibrated to 0 based on the ECL lectin blot. The sialylated Fc samples were adjusted accordingly.

**Supplemental Table 1. Doses of IVIG preparations.**

<b>IVIG prep</b>	<b>IVIG</b>	<b>IVIG Fc</b>	<b>SNA<sup>+</sup> IVIG</b>	<b>SNA<sup>+</sup> IVIG Fc</b>	<b>2,3ST IVIG Fc</b>	<b>2,6ST IVIG Fc</b>	<b>2,6ST rFc</b>
<b>Dose</b>	1g/kg	0.33g/kg	0.1g/kg	0.033g/kg	0.033g/kg	0.033g/kg	0.033g/kg
<b>Amount/ mouse injection</b>	20mg	6.66mg	2mg	0.66mg	0.66mg	0.66mg	0.66mg

# Supplemental Figure 1



Supplemental Figure 2

