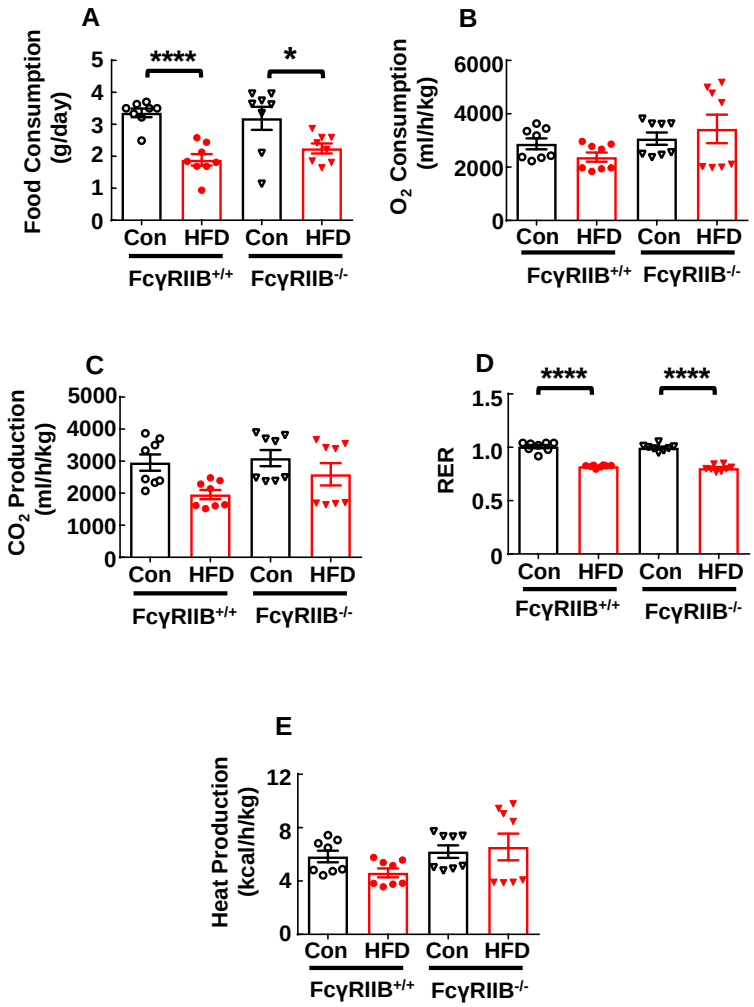


Figure S1. Related to Figure 1. Food consumption and energy expenditure in control diet- and HFD-fed mice are unaffected by global FcγRIIB deletion. (A-E) Beginning at 5 weeks of age, male FcγRIIB$^{+/+}$ and FcγRIIB$^{-/-}$ mice were fed a control diet (Con) or high-fat diet (HFD) for 10 weeks, and food consumption (A), O_2 consumption (B), CO_2 production (C), respiratory exchange rate (RER, D), and heat production (E) were quantified. In A-E, values are mean±SEM, N=8, *p<0.05, ****p<0.001. One-way ANOVA with Tukey's posthoc testing was used.

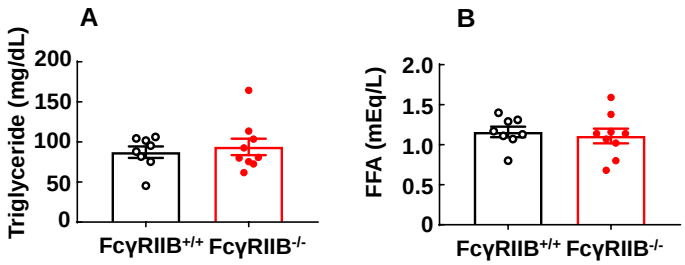


Figure S2. Related to Figure 1. Plasma levels of triglyceride and free fatty acid (FAA) in HFD-fed mice are unaffected by global FcγRIIB deletion. Beginning at 5 weeks of age, male FcγRIIB$^{+/+}$ and FcγRIIB$^{-/-}$ mice were fed a high-fat diet for 12 weeks, and plasma TG (A) and FFA (B) concentrations were measured. Values are mean±SEM, N=8,9. Student's t-test was used.

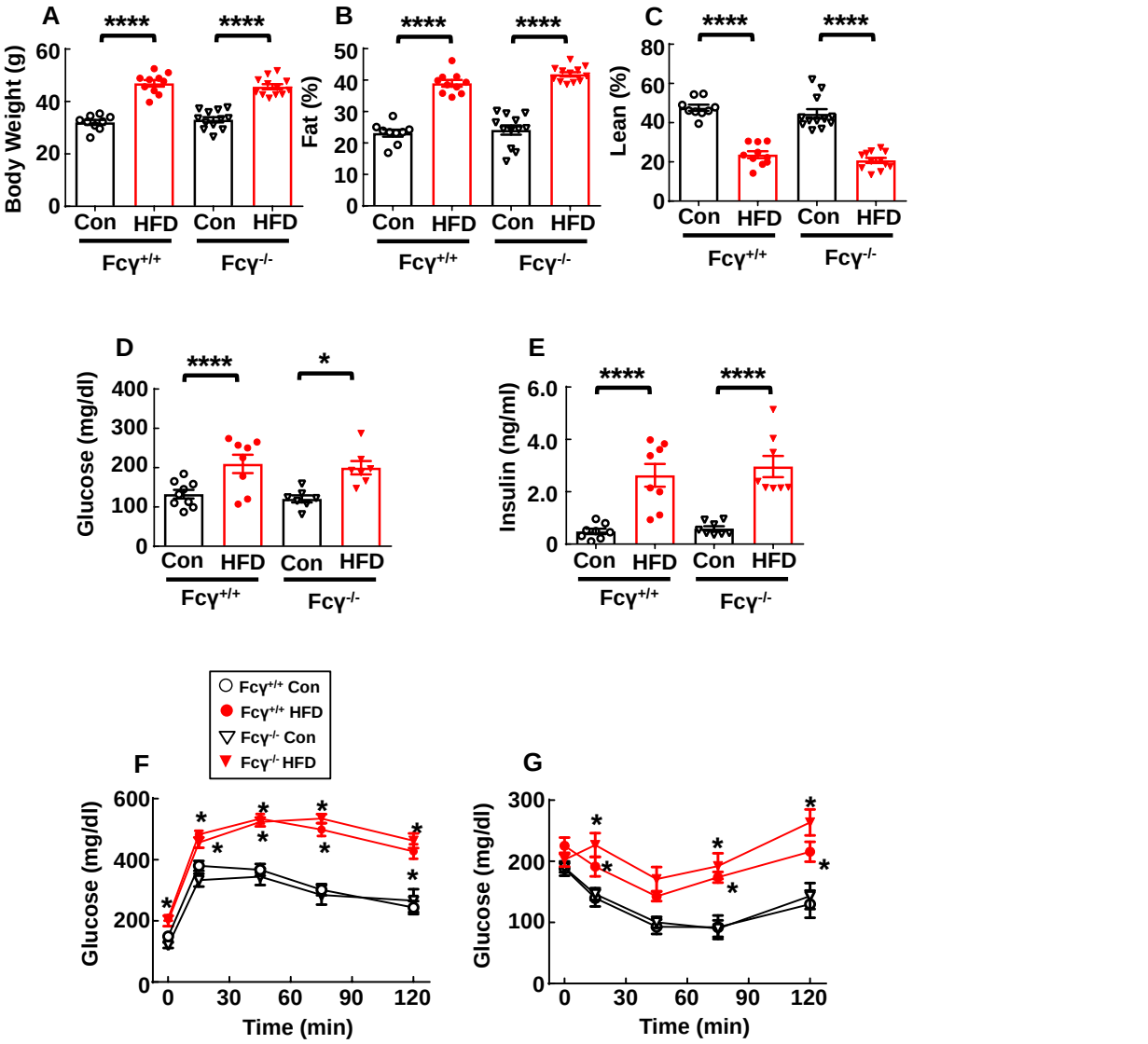


Figure S3. Related to Figure 1. Mice lacking Fc γ chain ($Fc\gamma^{-/-}$) are not protected from obesity-induced glucose intolerance or insulin resistance. (A-C) Beginning at 5 weeks of age, male $Fc\gamma^{+/+}$ and $Fc\gamma^{-/-}$ mice were fed a control diet (Con) or high-fat diet (HFD) for 12 weeks, and body weight (A), fat and lean mass (B, C) were evaluated. (D, E) The mice were fasted for 4-6 h, blood glucose (D) and insulin levels (E) were measured (N=7-9), and GTT (F) was performed. Following one week recovery while continuing the assigned diets, the mice were fasted for 4-6 h, and ITT (G) was performed. In F and G, N=7-13. Values are mean±SEM, *p<0.05, ****p<0.001. In A-E, one-way ANOVA with Tukey's posthoc testing was used. In F and G, two-way ANOVA with Tukey's posthoc testing was used.

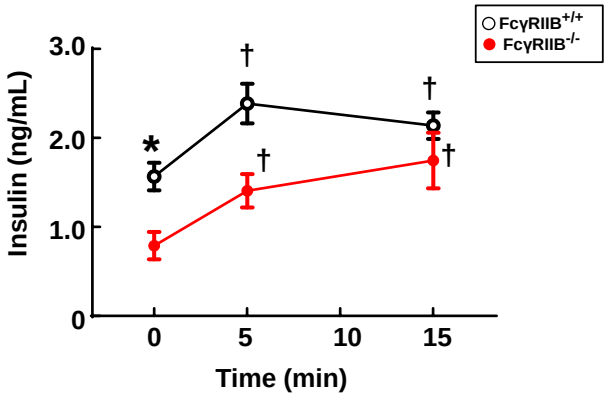


Figure S4. Related to Figure 1. Increase in plasma insulin in response to glucose in HFD-fed mice is unaffected by global FcγRIIB deletion. Beginning at 5 weeks of age, male FcγRIIB$^{+/+}$ and FcγRIIB$^{-/-}$ mice were fed a high-fat diet for 12 weeks, and plasma insulin concentrations were measured before and following IP administration of D-glucose (2g/kg body weight). Values are mean±SEM, N=9, *p<0.05 vs.FcγRIIB$^{-/-}$, †p<0.05 vs. Time 0. Two-way ANOVA with Tukey's posthoc testing was used.

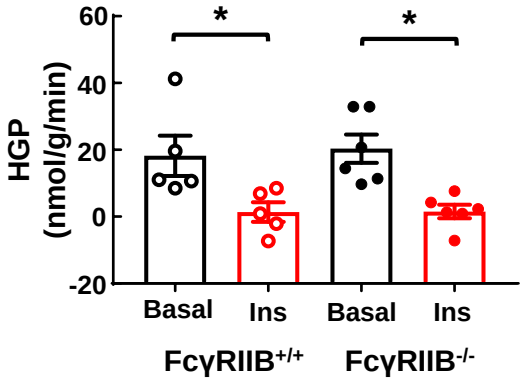


Figure S5. Related to Figure 1. Hepatic glucose production (HGP) during a hyperinsulinemic euglycemic clamp in HFD-fed mice is unaffected by global FcγRIIB deletion. Beginning at 5 weeks of age, male FcγRIIB$^{+/+}$ and FcγRIIB$^{-/-}$ mice were fed a high-fat diet for 12 weeks, and hyperinsulinemic euglycemic clamps were performed. Values are mean±SEM, N=5-6, *p<0.05. One-way ANOVA with Tukey's posthoc testing was used.

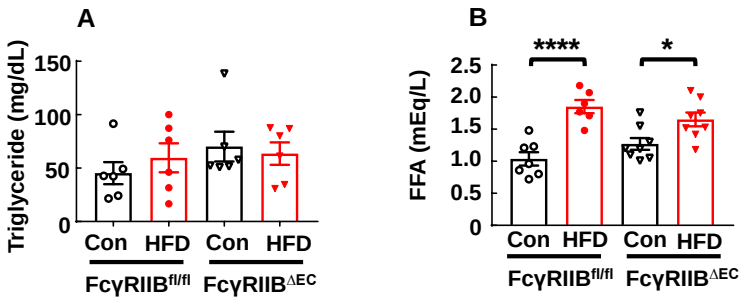


Figure S6. Related to Figure 2. Plasma levels of triglyceride (TG) and free fatty acid (FAA) in HFD-fed mice are unaffected by endothelial FcγRIIB deletion. Beginning at 5 weeks of age, male FcγRIIB$^{fl/fl}$ and FcγRIIB$^{\Delta EC}$ mice were fed a control diet (Con) or high-fat diet (HFD) for 12 weeks, and plasma TG (A) and FFA (B) concentrations were measured. Values are mean±SEM, N=6-8. *p<0.05, ****p<0.001. One-way ANOVA with Tukey's posthoc testing was used.

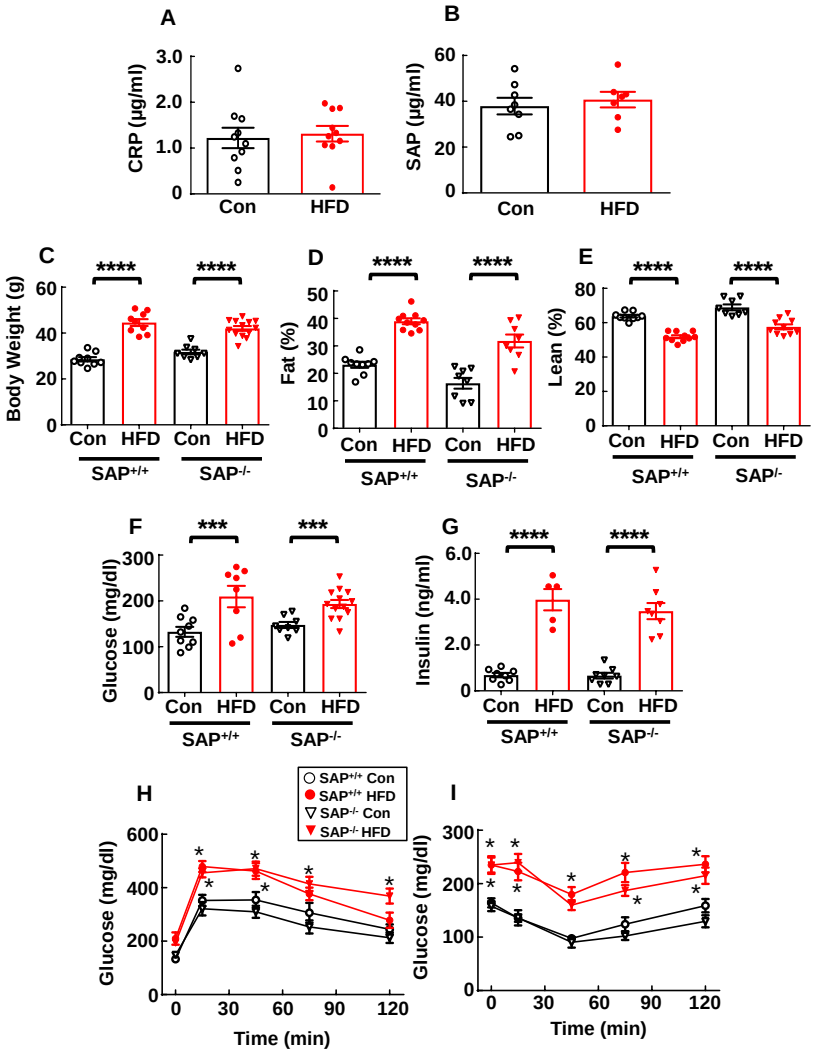


Figure S7. Related to Figure 3. Diet-induced obesity does not affect circulating CRP or SAP levels, and SAP$^{-/-}$ mice are not protected from obesity-induced glucose intolerance or insulin resistance. (A,B) Male wild-type mice were fed a control diet or HFD for 12 weeks beginning at 5 weeks of age, and plasma CRP (A) and SAP (B) levels were measured by ELISA. In A and B, N=7-8. (C-E) Beginning at 5 weeks of age, male SAP$^{+/+}$ or SAP$^{-/-}$ mice were fed a control diet or HFD for 12 weeks, and body weight (C), fat mass (D) and lean mass (E) were measured. (F-I) Mice were fasted for 4-6 h, plasma fasting glucose (F) and insulin (G) were assessed (N=5-13), and GTT (H) was performed. Following one week recovery while continuing the assigned diets, the mice were fasted for 4-6 h and ITT (I) was performed. Values are mean±SEM, In A-G, ***p<0.005., ****p<0.0001. In H and I, N=8-13. *p<0.05 vs. Con. In A-G, one-way ANOVA with Tukey's posthoc testing was used. In H and I, two-way ANOVA with Tukey's posthoc testing was used.

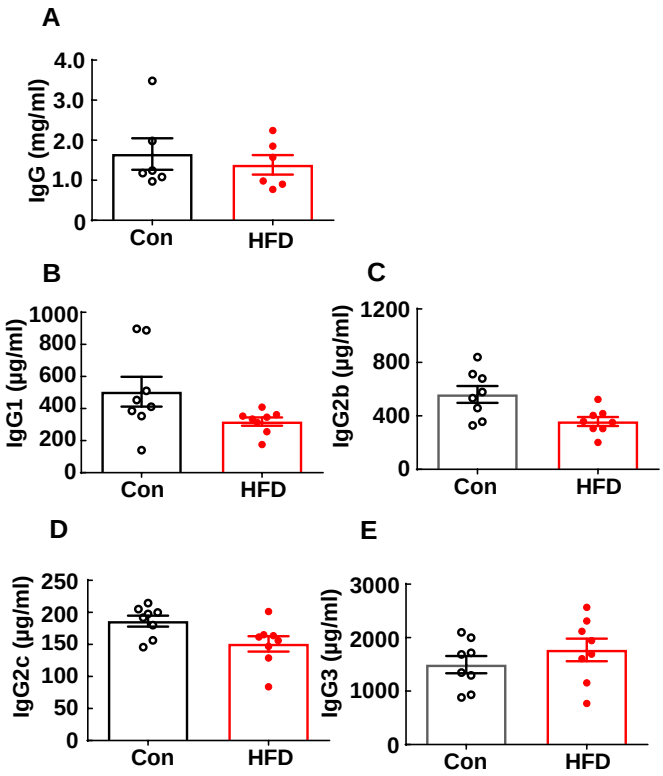


Figure S8. Related to Figure 3. Plasma levels of total IgG and IgG subclasses are unaffected by diet-induced obesity. Beginning at 5 weeks of age, mice were fed a control diet or HFD for 12 weeks, and plasma levels of total IgG (A), IgG1 (B), IgG2b (C), IgG2c (D) or IgG3 (E) were measured by ELISA. Values are mean±SEM, N=6-8. Student's t test was used.

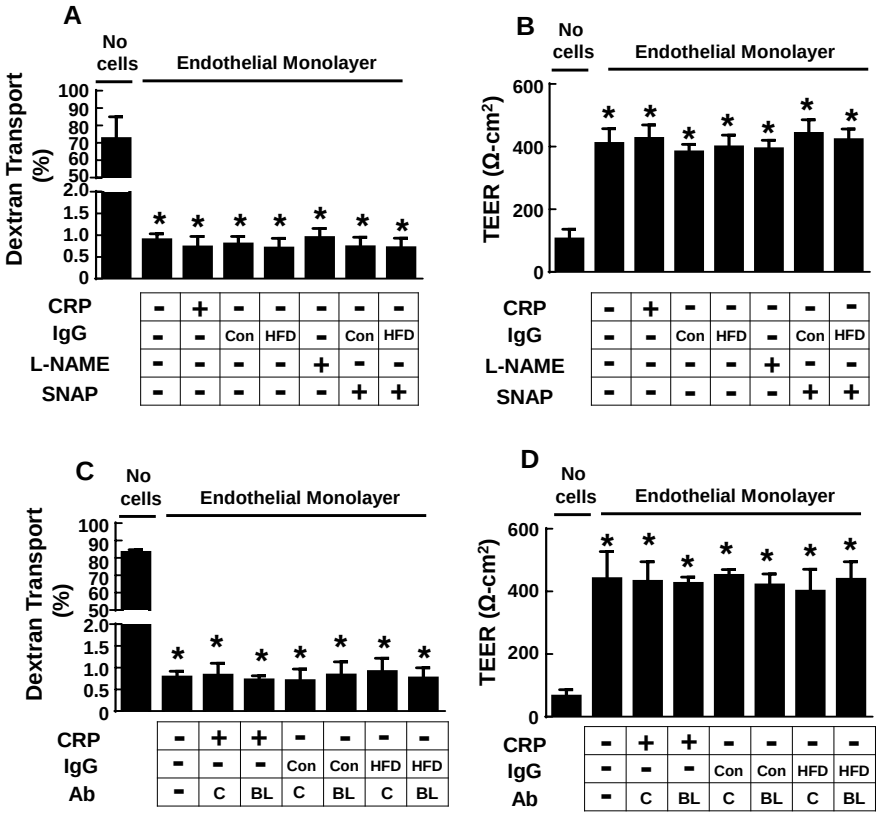


Figure S9. Related to Figure 5. Interventions in studies of insulin transcytosis do not affect endothelial monolayer integrity. Media alone (No cells) or endothelial cells were seeded on transwells, and following the cell treatments described in Figure 5E and F, FITC-dextran (MW 4000) transfer across the monolayer was evaluated over 2h (A,C), or transendothelial electrical resistance (TEER) was measured (B,D). Values are mean±SEM, N=6-7, *p<0.05 vs. no cells. One-way ANOVA woth Tukey's posthoc testing was used.

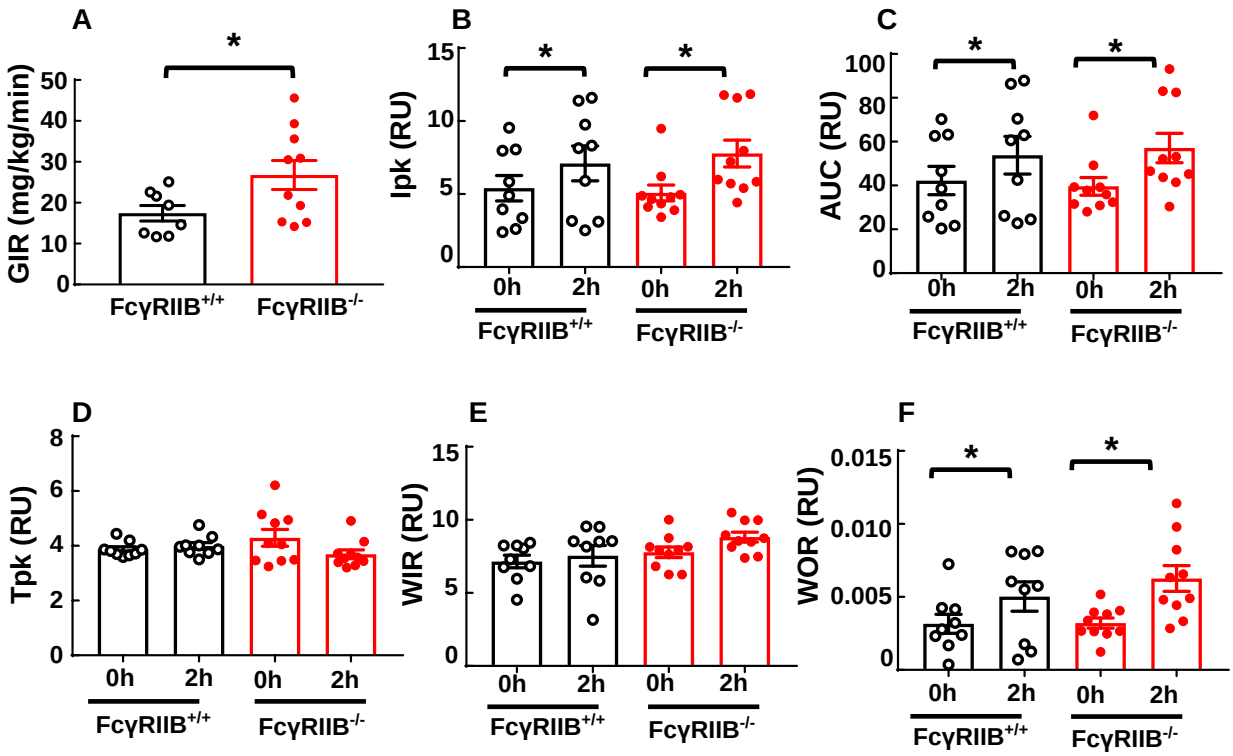


Figure S10. Related to Figure 5. FcγRIIB does not impact skeletal muscle capillary blood volume (CBV) or microvascular blood flow (MBF) responses to insulin in obese mice. Beginning at 5 weeks of age, FcγRIIB$^{+/+}$ and FcγRIIB$^{-/-}$ male mice were fed HFD for 12 weeks, and dynamic contrast-enhanced ultrasound (DCE-US) images were then captured on the proximal hindlimb adductor muscle group using a lipid shelled perfluorocarbon-based microbubble contrast agent. Images were obtained at baseline (0 h), and in response to insulin infusion (20 mU/Kg/min) at the end of a 2h hyperinsulinemic-euglycemic clamp. (A) Glucose infusion rate (GIR) during the clamp. Comparison was done by unpaired Student's t-test. (B-F) Regions-of-interest were defined, and average time-intensity curves were generated and analyzed to extract measures of CBV (B, peak intensity, Ipk), MBF (D, time-to-peak intensity, Tpk, and E, wash-in-rate, WIR), and both CBV and MBF (C, area under the curve, AUC, and F, wash-out rate, WOR). Values are mean±SEM, expressed as relative units (RU). Paired Student's t tests were used to compare values at 0h and 2h, and unpaired Student's t-tests were done to compare parameters in FcγRIIB$^{+/+}$ versus FcγRIIB$^{-/-}$ mice. P*<0.05.

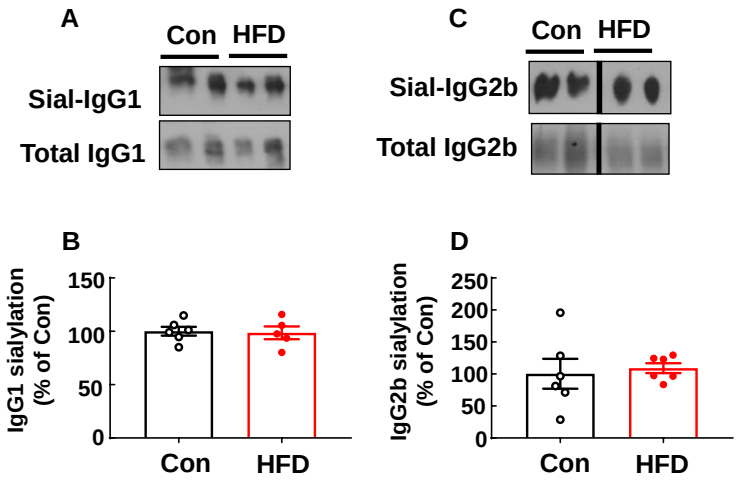


Figure S11. Related to Figure 6. HFD feeding does not affect sialylation of IgG1 or IgG2b. Male wild-type mice (C57BL/6) were fed a control diet (Con) or high-fat diet (HFD) for 12 weeks, IgG subclasses were isolated and sialylation levels of IgG1 (A, B) or IgG2b (C, D) were evaluated. Representative SNA-lectin blots are in A and C, and summary data are in B and D. N=5-6. Values are mean±SEM. Student's t test was used.

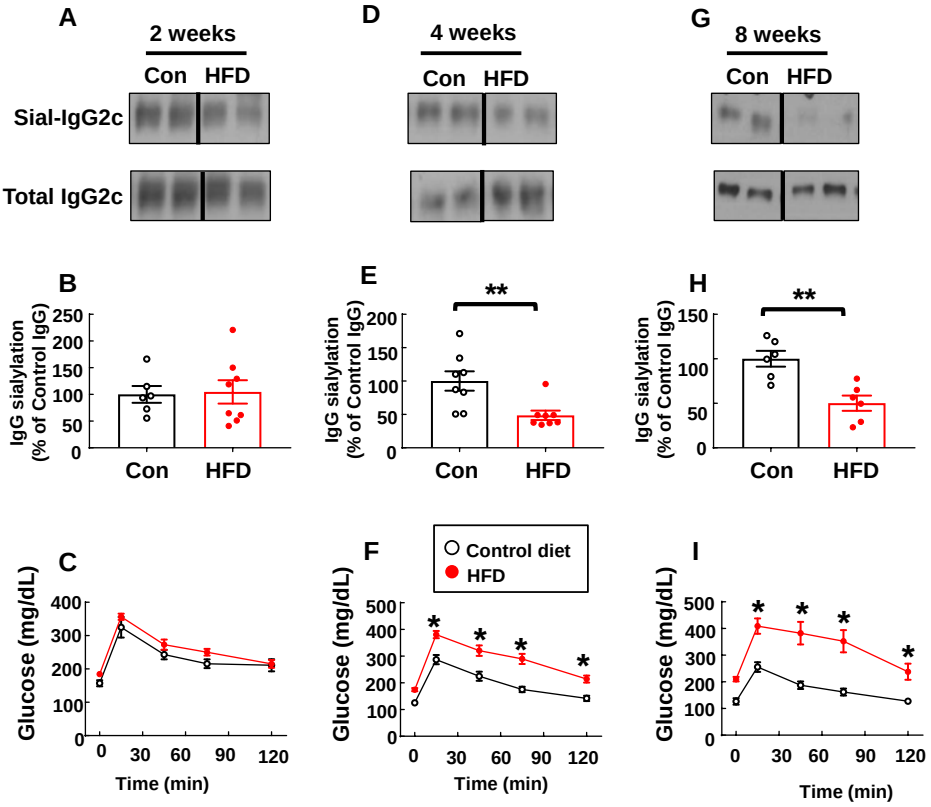


Figure S12. Related to Figure 6. Hyposialylation of IgG2c and glucose intolerance are evident at 4 weeks of HFD feeding. Male wild-type mice (C57BL/6) were fed a control diet (Con) or high-fat diet (HFD) for 2, 4 and 8 weeks, and sialylation of IgG2c and GTT were evaluated. Representative SNA-lectin blots are in A, D, G, summary data for relative sialylation are in B, E, and H, and GTTs are in C, F and I. N=6-8. Values are mean±SEM, In B, E, and H, **p<0.005. In C, F and I, *p<0.05 vs. Control diet. In B, E and H, Student's t test was used. In C, F and I, two-way ANOVA with Tukey's posthoc testing was used.

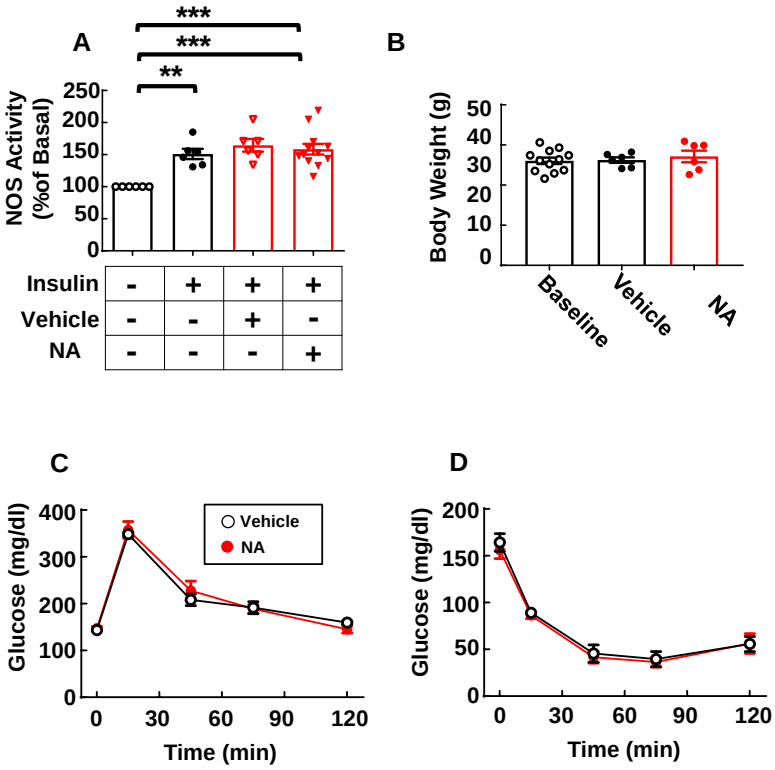


Figure S13. Related to Figure 6. Neuraminidase treatment alone does not affect insulin activation of eNOS in cultured endothelial cells or glucose tolerance or insulin sensitivity in mice. (A) Endothelial cells were pre-treated with vehicle or neuraminidase (NA, 2 units/ml) for 30 min, and eNOS activation by insulin (100nM) was assessed. N=6-12. **p<0.01, ***p<0.005. (B-D) Male wild-type mice were fed a HFD for 12 weeks starting at 5 weeks of age, and while continuing HFD they were injected with vehicle or neuraminidase (NA, 30 units/mouse) for 2 times/week for 2 weeks. Body weight was evaluated before (baseline), and after treatment (B). GTT (C) and ITT (D) were performed after 1 and 2 weeks of treatment, respectively. In B-D, N=6-12. Values are mean±SEM. In A and B, one-way ANOVA with Tukey's posthoc testing was used. In C and D, two-way ANOVA with Tukey's posthoc testing was used.

	Control	T2DM	P-value
n	6	6	
Gender (male/female)	2/4	1/5	
Age (years)	38.50±2.907	60.00 ±1.751	<0.0001
Body Weight (kg)	82.22 ±3.750	95.37±4.829	0.057
BMI	30.13±1.502	35.53 ±1.748	0.041
HbA1c	5.250 ± 0.09574	7.683 ± 1.126	0.056
SI	6.243 ± 1.331	1.205 ± 0.1896	0.0038
DI	2677 ± 218.2	127.4 ± 61.06	<0.0001

HbA1c: Hemoglobin A1c
SI: Sensitivity index
DI: Disposition index

Table S1. Related to Figure 4. Characteristics of control and T2DM human subjects.

Peptide Sequence			Control Diet		HFD	
		Glycoform	Intensity	Percentage	Intensity	Percentage
EDYNSTLR	Non-glycosylated	No	45953	1.9%	5497	1.7%
	Glycosylated with sialic acid	$Fuc_1Hex_4HexNAc_4NeuGc_1$	132259	5.5%	n.d.	n.d.
		$Fuc_1Hex_5HexNAc_4NeuGc_1$	330222	13.7%	n.d.	n.d.
	Glycosylated without sialic acid	$Fuc_1Hex_3HexNAc_3$	8294	0.3%	n.d.	n.d.
		$Fuc_1Hex_3HexNAc_4$	306926	12.8%	51917	15.8%
		$Fuc_1Hex_4HexNAc_4$	1084351	45.2%	182865	55.7%
		$Fuc_1Hex_5HexNAc_4$	493955	20.6%	87817	26.8%

No: no glycan, n.d.: not detected

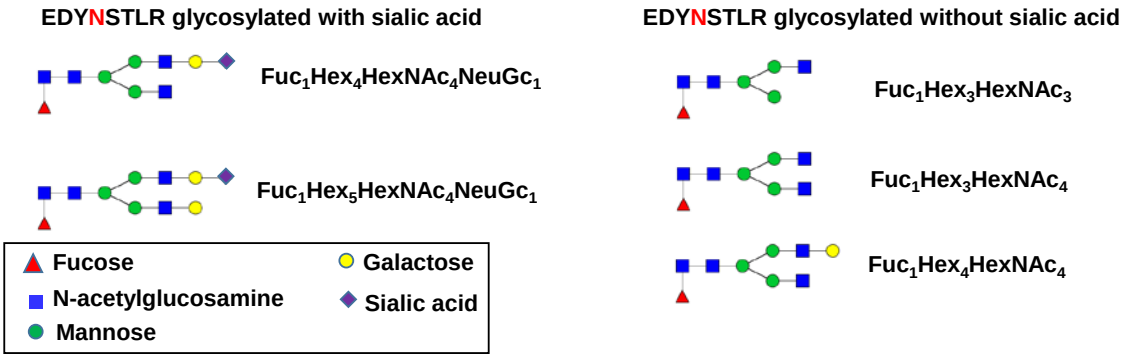
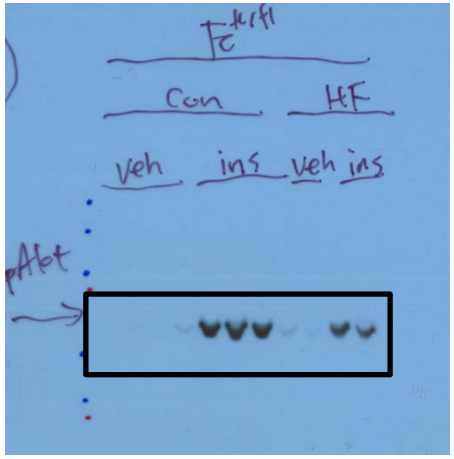
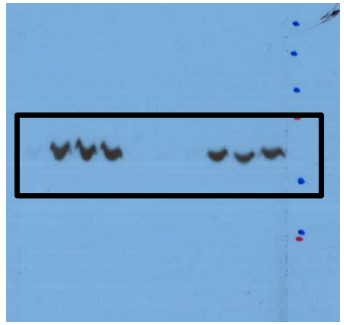


Table S2. Related to Figure 6. Diet-induced obesity in mice causes a loss of terminal sialylation of the IgG2c Fc Asn297-associated glycan.

Fig. 2K

pAkt





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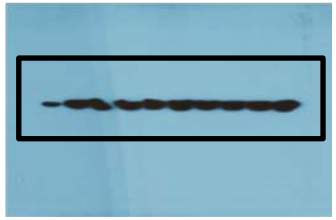


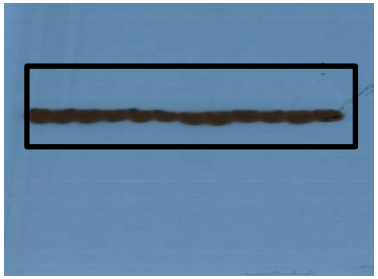
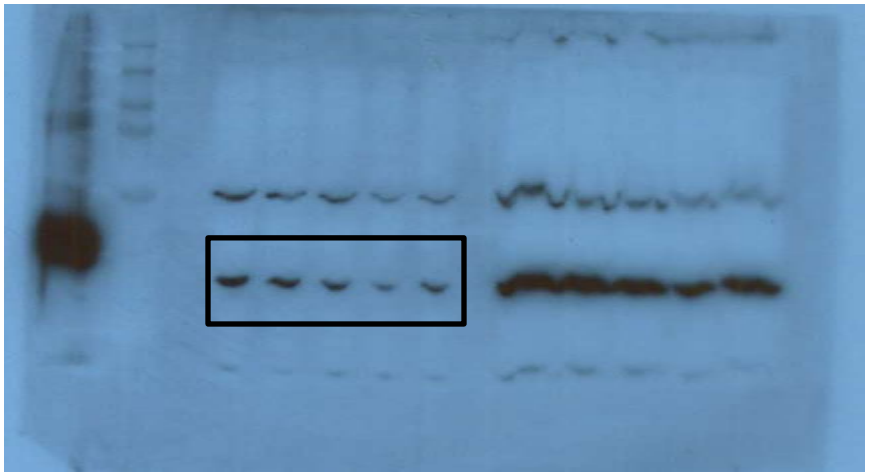


Fig. 5B

FcγRIIB



eNOS

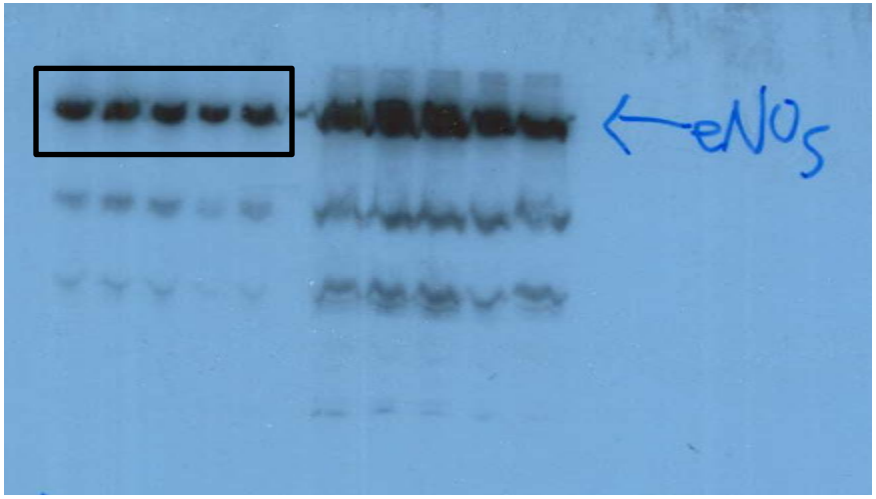
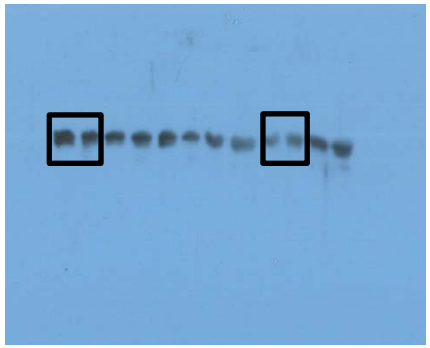


Fig. 6-D

Sial-IgG



Total IgG

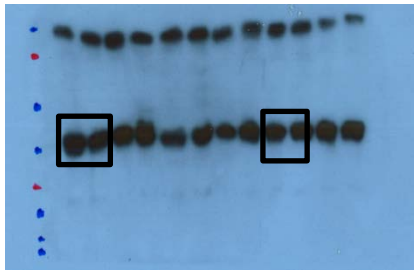
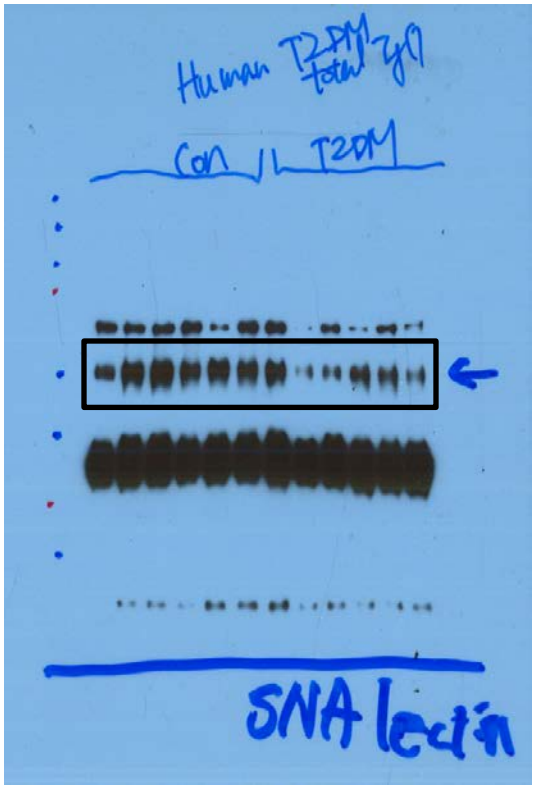


Fig. 6-E



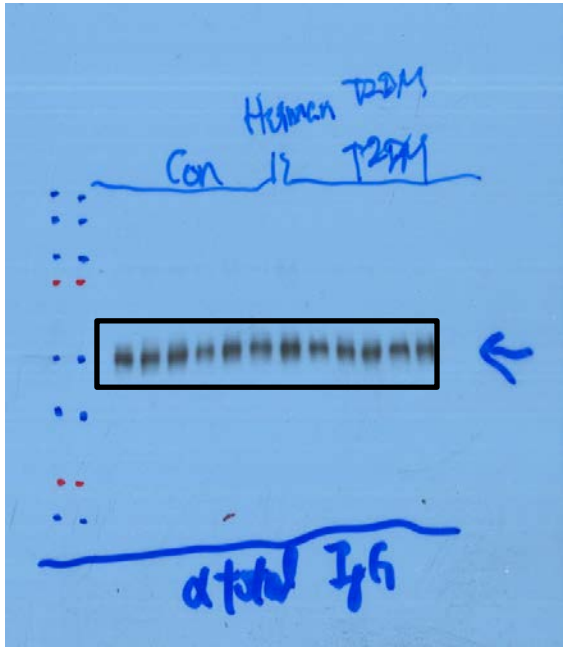
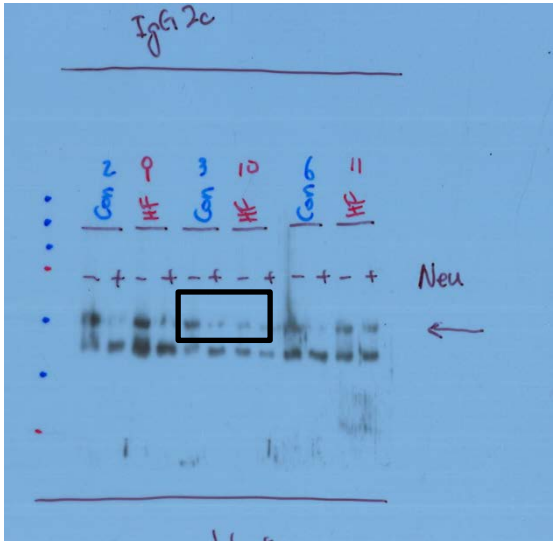


Fig. 6-F

Sial-IgG



Total IgG

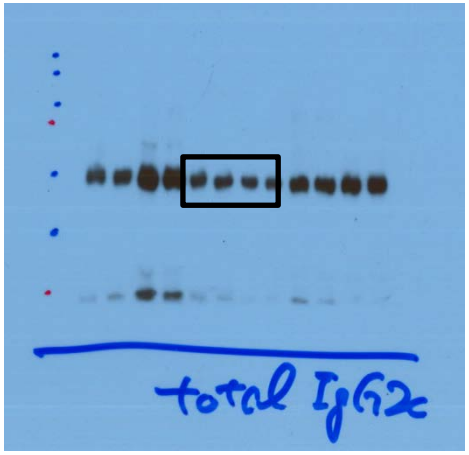
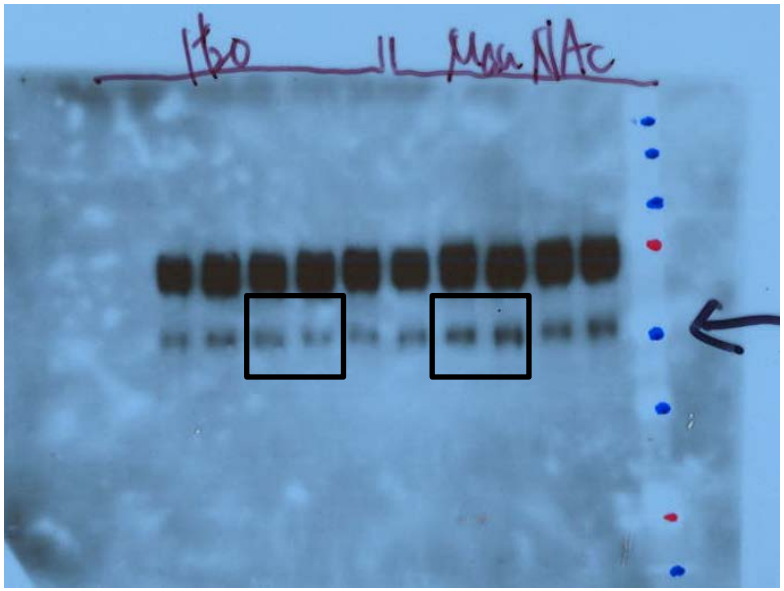


Fig. 7A

Sial-IgG



Total-IgG

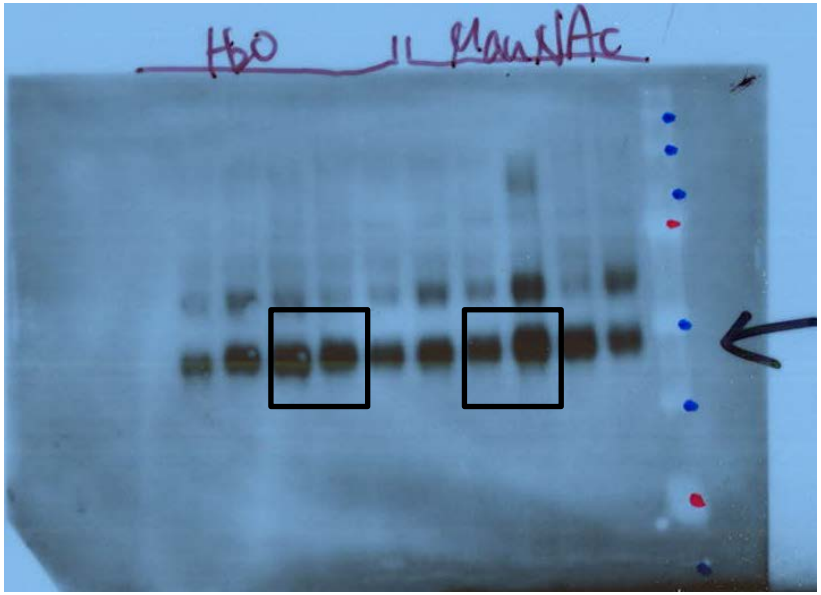
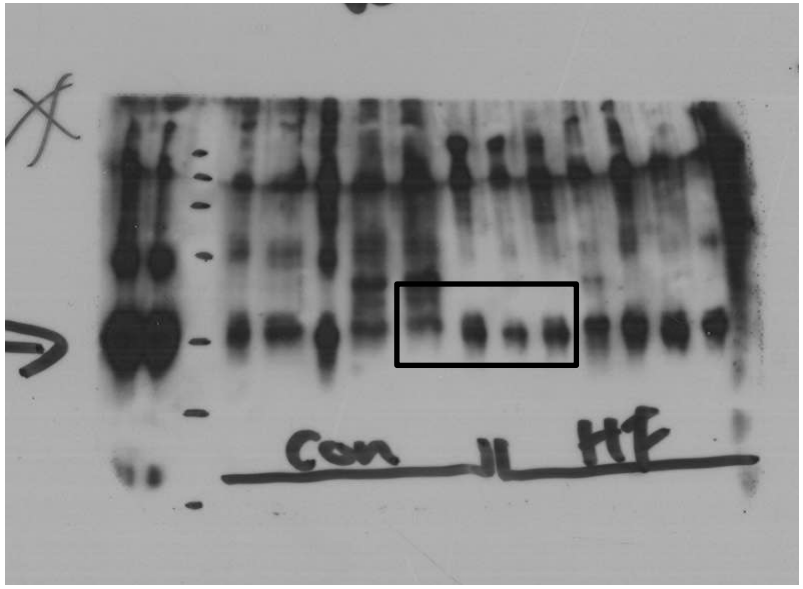


Fig. S11A



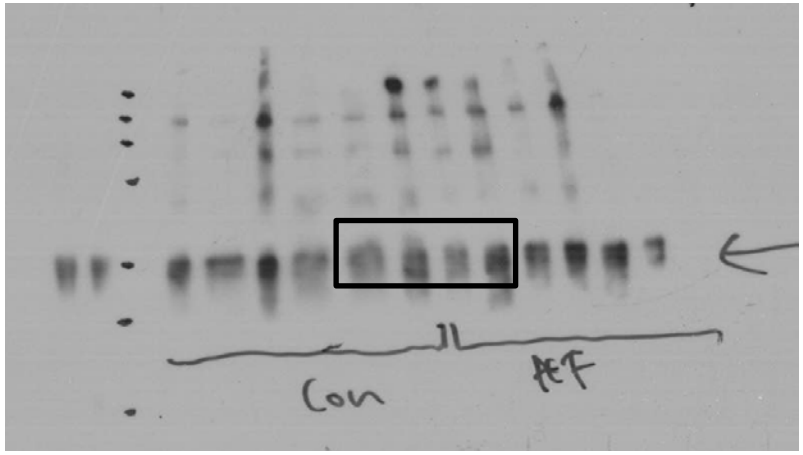
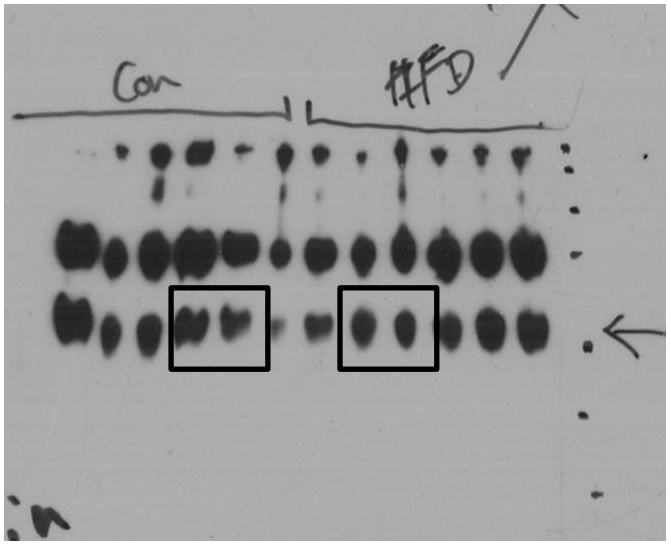


Fig. S11C



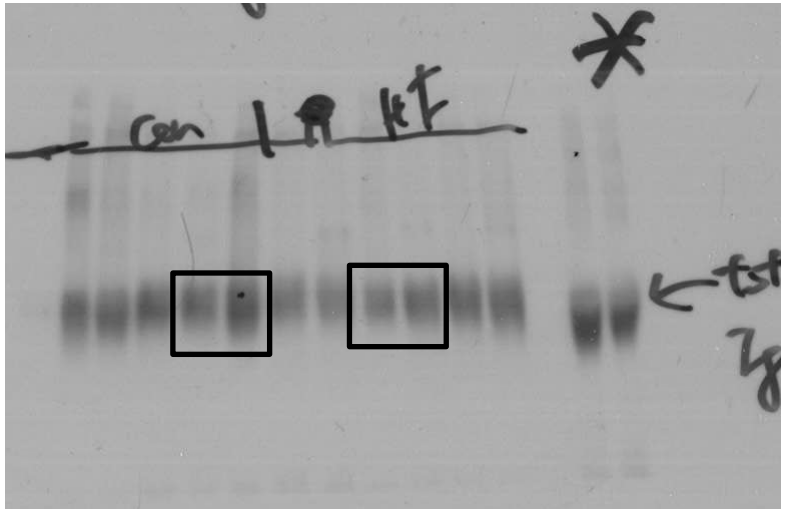
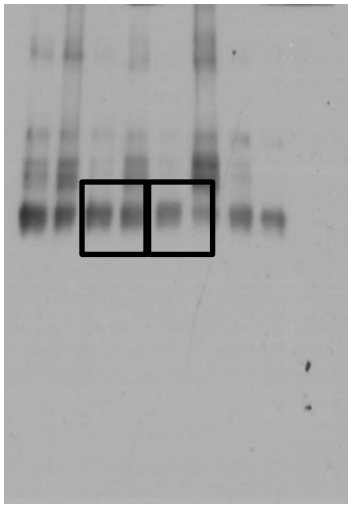


Fig. S12A

Sial-IgG2c



Total IgG2c

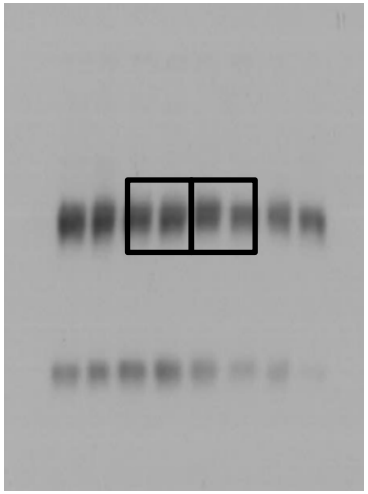
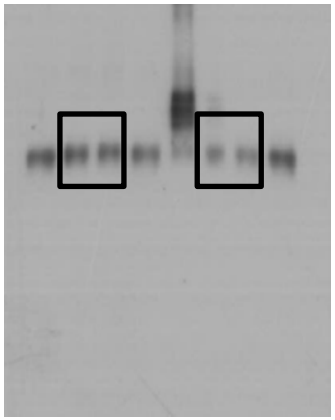


Fig. S12D



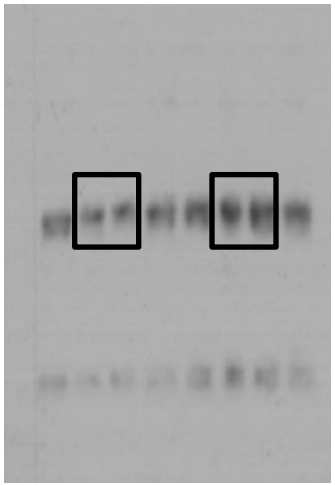


Fig. S12G