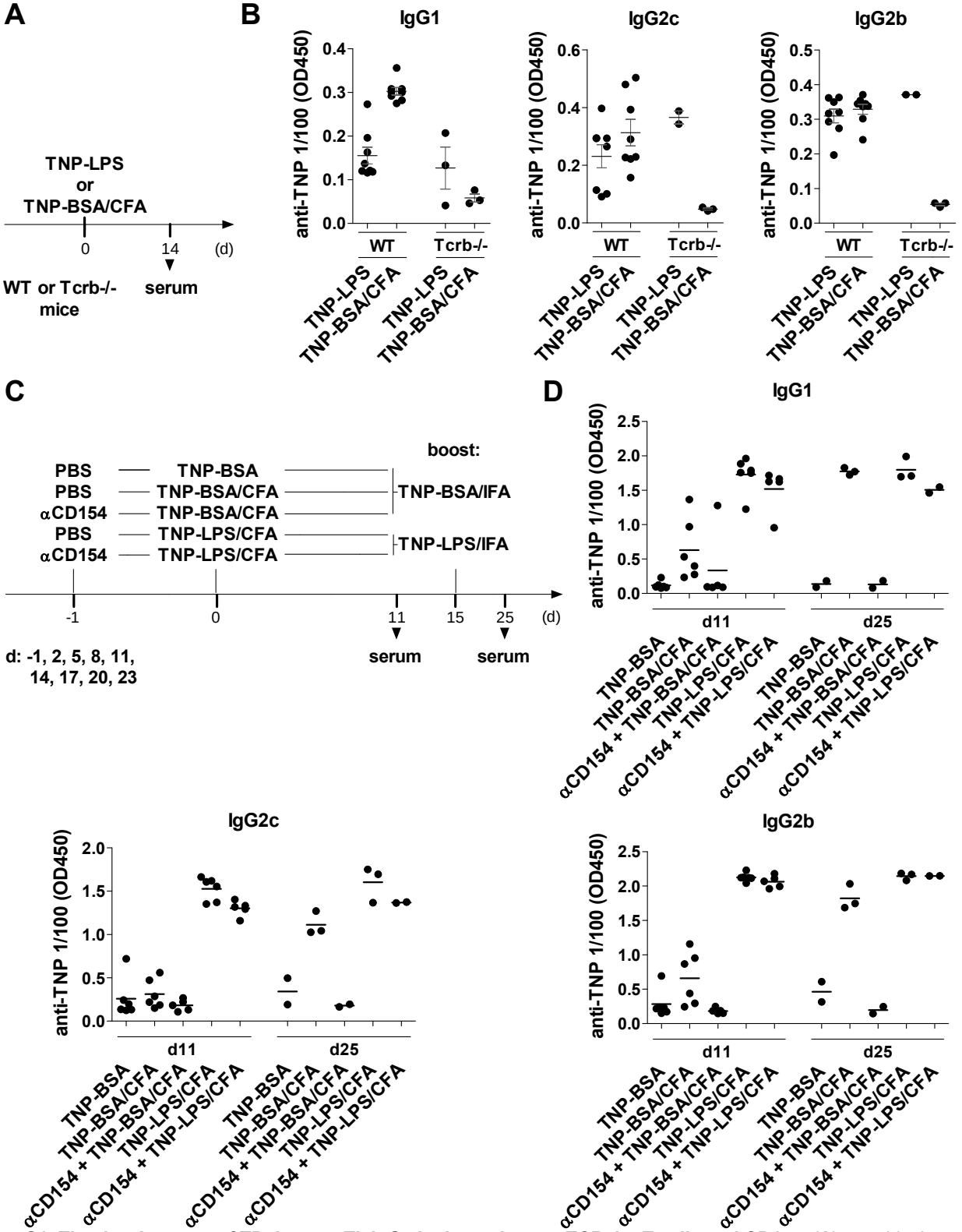
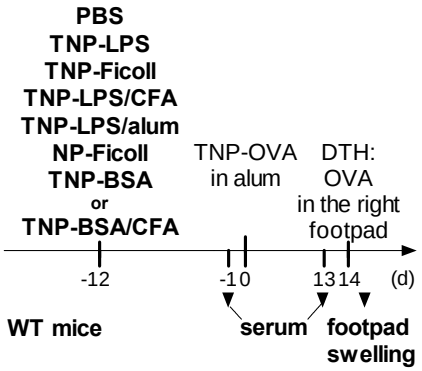
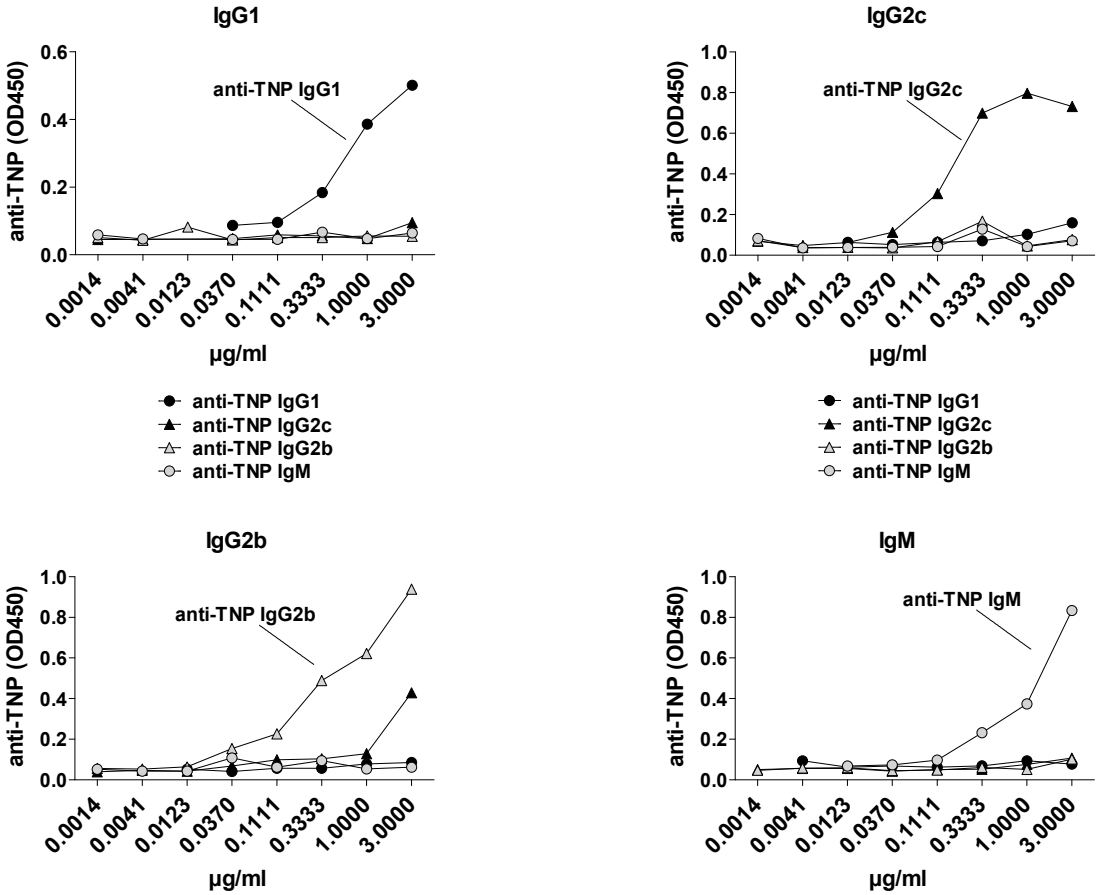


Figure S1. The development of TD, but not TI, IgGs is dependent on TCRab+ T cells and CD154. (A) Graphical representation of the experimental approach followed in **B**. WT or *Tcrb-/-* mice were injected with 50 µg of TNP-LPS alone or TNP-BSA in CFA on d 0. **(B)** Anti-TNP IgG1, IgG2c and IgG2b serum Ab levels on d 14 were determined via ELISA. The symbols represent data from individual animals. Horizontal lines represent means + SEM. One representative of 2 independent experiments is shown. **(C)** Schematic plan of the experimental approach followed in **D**. WT mice were injected with 50 µg of TNP-BSA alone or in CFA on d 0 and boosted with 50 µg of TNP-BSA in IFA on d 15 or injected with 50 µg of TNP-LPS in CFA on d 0 and boosted with 50 µg of TNP-LPS in IFA on d 15. The mice were additionally treated with PBS or aCD154 Abs every third day starting on d -1. **(D)** Anti-TNP IgG1, IgG2c and IgG2b serum Ab levels on d 11 and 25 were determined via ELISA. One representative of 2 independent experiments is shown.

A


PBS
TNP-LPS
TNP-Ficoll
TNP-LPS/CFA
TNP-LPS/alum
NP-Ficoll
TNP-BSA
or
TNP-BSA/CFA
TNP-OVA
in alum
DTH:
OVA
in the right
footpad
-12
-10
13
14
(d)
WT mice
serum
footpad
swelling

B


IgG1
anti-TNP IgG1
anti-TNP (OD450)
0.0
0.2
0.4
0.6
0.0014
0.0041
0.0123
0.0370
0.1111
0.3333
1.0000
3.0000
µg/ml
IgG2c
anti-TNP IgG2c
anti-TNP (OD450)
0.0
0.2
0.4
0.6
0.8
1.0
0.0014
0.0041
0.0123
0.0370
0.1111
0.3333
1.0000
3.0000
µg/ml
anti-TNP IgG1
anti-TNP IgG2c
anti-TNP IgG2b
anti-TNP IgM
anti-TNP IgG1
anti-TNP IgG2c
anti-TNP IgG2b
anti-TNP IgM
IgG2b
anti-TNP IgG2b
anti-TNP (OD450)
0.0
0.2
0.4
0.6
0.8
1.0
0.0014
0.0041
0.0123
0.0370
0.1111
0.3333
1.0000
3.0000
µg/ml
IgM
anti-TNP IgM
anti-TNP (OD450)
0.0
0.2
0.4
0.6
0.8
1.0
0.0014
0.0041
0.0123
0.0370
0.1111
0.3333
1.0000
3.0000
µg/ml

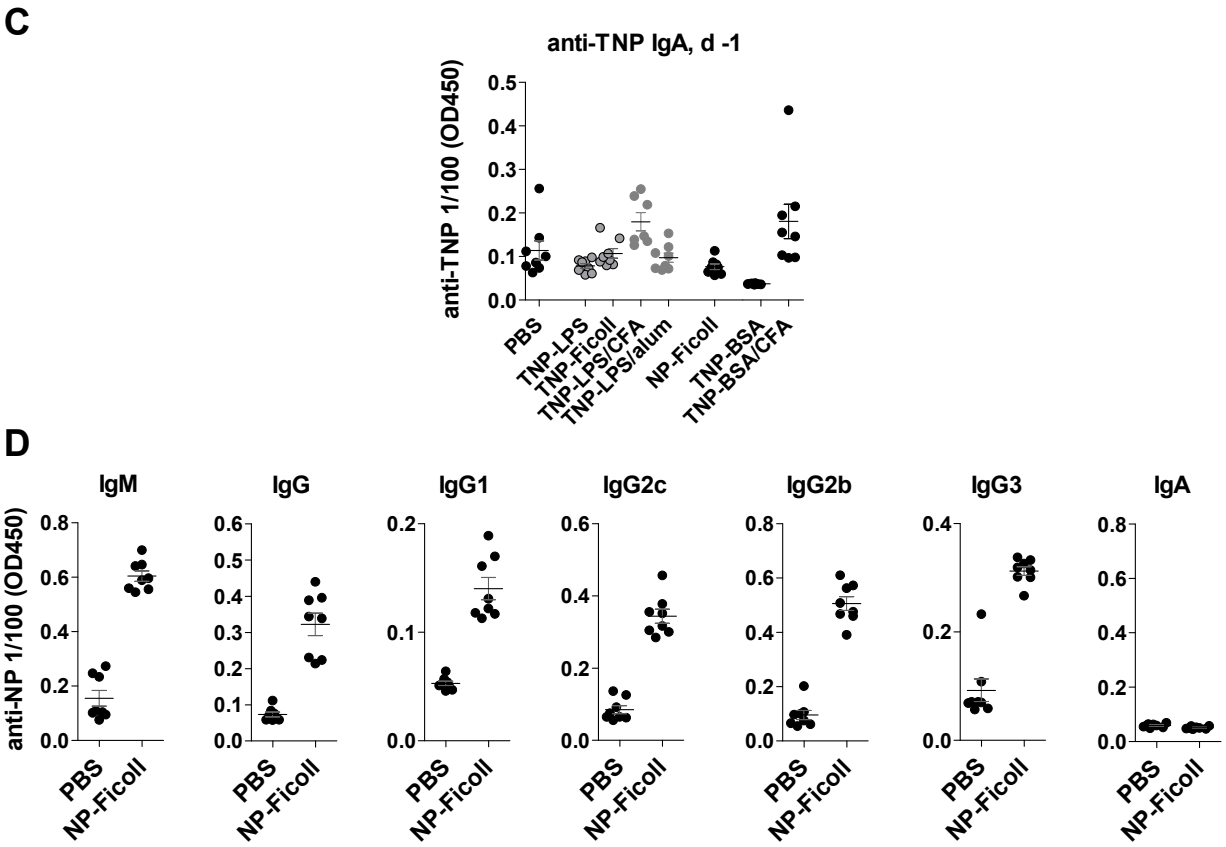


Figure S2. TI antigen-specific B cell activation reduces a subsequent antigen-induced DTH reaction. (A) Graphical representation of the experimental approach followed in **B-D** and described in **Figure 1**. WT mice were injected i.p. with either PBS or 50 µg of TNP-Ficoll, TNP-LPS or TNP-LPS in CFA or alum or NP-Ficoll or TNP-BSA alone or TNP-BSA in CFA on d -12. DTH was induced through i.p. injection of TNP-OVA in alum on d 0 and OVA in the right footpad on d 14. **(B)** The TNP-reactivity of cloned anti-TNP monoclonal IgG1, IgG2c, IgG2b and IgM standard Abs with identical VDJ and VJ regions is shown, in addition to the specificity validation of secondary HRP-coupled anti-IgG1, anti-IgG2c, anti-IgG2b and anti-IgM Abs used in the anti-TNP ELISA experiments to calculate the anti-TNP Ab concentrations in **Figure 1**. Each ELISA was repeated at least 2 times. **(C)** Anti-TNP IgA Ab levels on d -1 were determined via ELISA. The symbols represent data from individual animals. Horizontal lines represent means + SEM. **(D)** Anti-NP serum isotype and IgG subclass Ab levels on day -1 induced with TI NP-Ficoll were determined via ELISA. One representative of 3 independent experiments is shown.

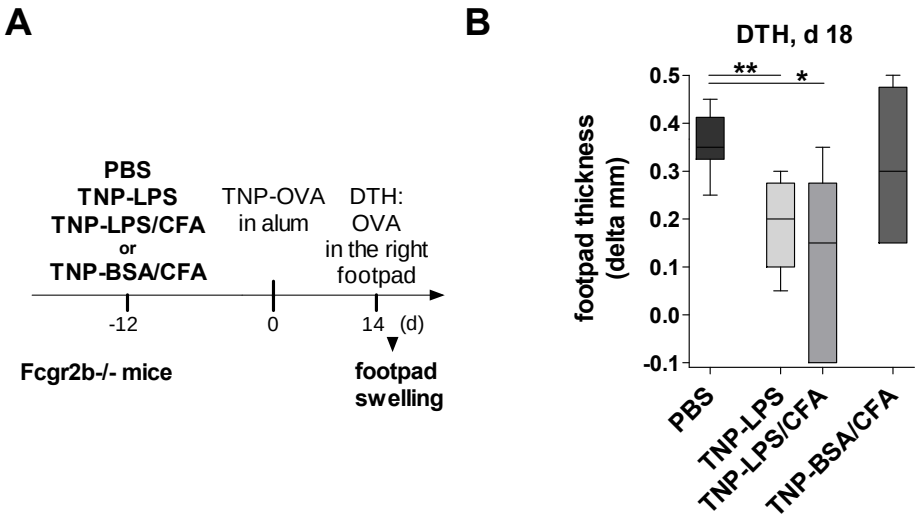
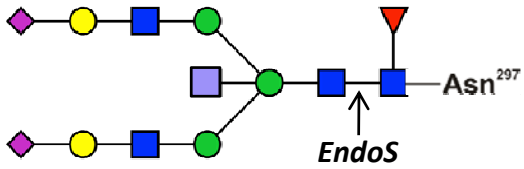


Figure S3. TI antigen-specific B cell activation reduces a subsequent antigen-induced DTH reaction independent of FcgRIIB. (A) Graphical representation of the experimental approach followed in **B**. Fcgr2b-/- mice were injected i.p. with either PBS (n=6) or 50 µg of TNP-LPS (n=5), TNP-LPS in CFA (n=6), or TNP-BSA in CFA (n=5) on d -12. DTH was induced through i.p. injection of TNP-OVA in alum on d 0 and OVA in the right footpad on d 14. **(B)** The differences in footpad thickness between the right and left footpad of the indicated *Fcgr2b-/-* mice were determined at 4 days after local DTH induction. A box-and-wisker diagram with median and sample minimum and maximum is shown. One representative of 3 independent experiments is shown.

A

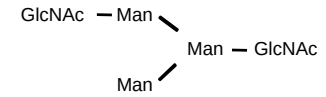
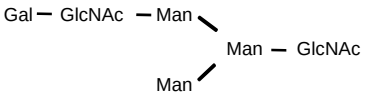
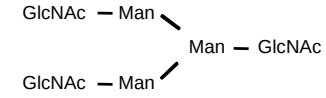
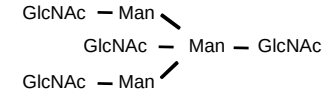
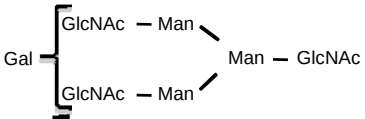
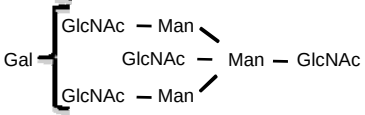
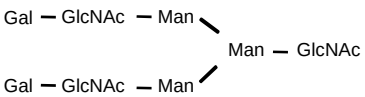
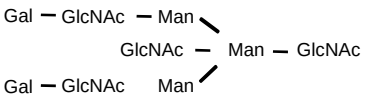
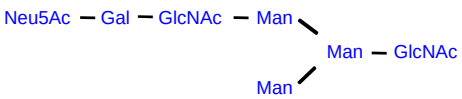
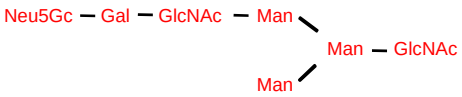
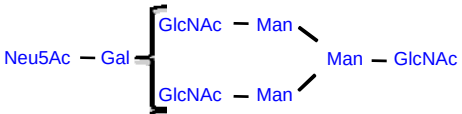
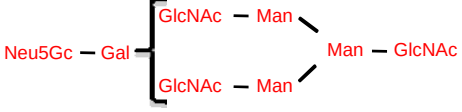


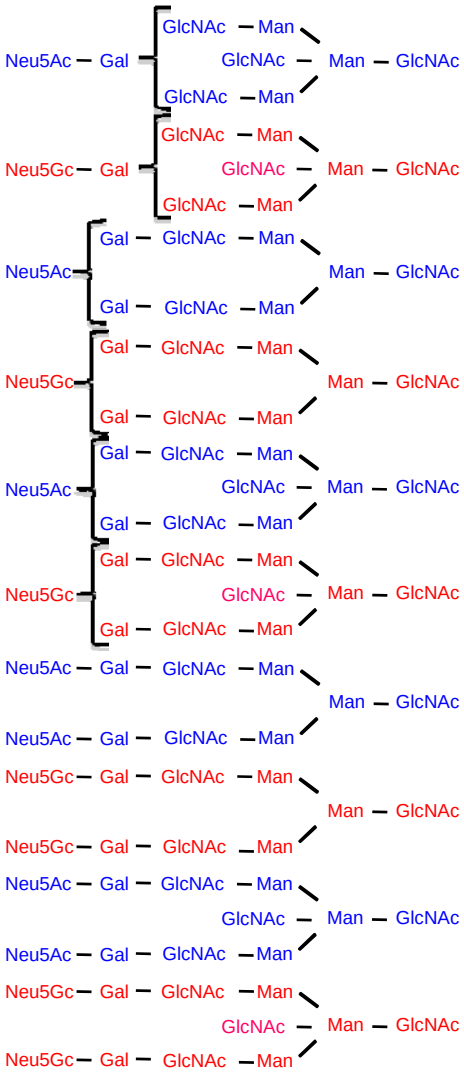
■ , □ : GlcNAc ▼ : fucose

● : mannose (Man) ○ : galactose (Gal; G)

◆ : sialic acid (Neu5Ac or Neu5Gc; S)

B

Human and mouse IgG Fc glycan structures (EndoS)	mass (m/z)	abbreviation
	1172	G0 (human and mouse)
	1376	G1
	1417	G0
	1662	G0
	1621	G1
	1866	G2
	1825	G2
	2070	G2
	1737	G1 S1 (human)
	1767	G1 S1 (mouse)
	1982	G1 S1
	2012	G1 S1

Human and mouse IgG Fc glycan structures (EndoS)	mass (m/z)	abbreviation
	2227	G1 S1
	2257	G1 S1
	2186	G2 S1
	2216	G2 S1
	2431	G2 S1
	2461	G2 S1
	2547	G2 S2
	2607	G2 S2
	2792	G2 S2
	2852	G2 S2

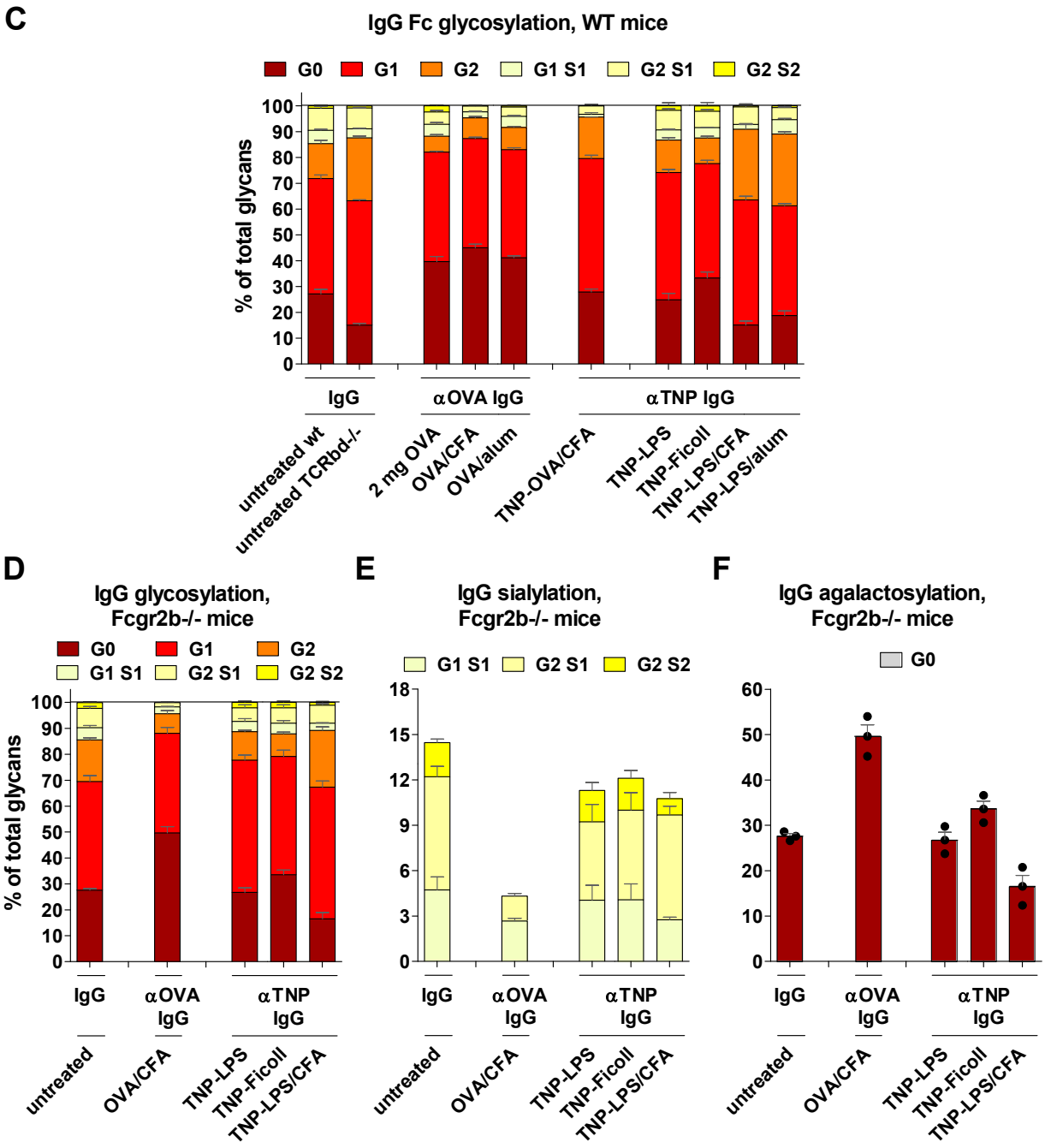


Figure S4. TI immunization induces sialylated IgG Abs. (A) The largest IgG Fc glycan coupled to Asn 297 with the cleavage site of EndoS is depicted. Sialic acid: human N-acetylneuraminic acid [Neu5Ac] or murine N-glycolylneuraminic acid [Neu5Gc] depending on the source of the IgG Ab: murine serum or supernatant from human HEK 293 cells. **(B)** Potential human and murine Fc glycan structures released with EndoS from Asn297. The numbers show the molecular masses (m/z) of the potential human and mouse Fc glycan structures (permethylated) determined through MALDI-TOF MS. **(C)** The Fc glycosylation (the same analyses as in **Figure 3, B and C**) of purified total IgGs from the pooled sera of three 10 wk-old untreated WT (independent experiments, n=11) or *Tcrbd-/-* (n=3) mice or purified OVA- or TNP-specific IgGs from the pooled sera of 6-10 10-wk-old WT mice i.p. injected 14 days before with 2 mg of pure OVA (n=3); or 100 µg of OVA in CFA (n=18) or alum (n=3) or TNP-OVA in CFA (n=3); or 50 µg of TNP-LPS (n=4), TNP-Ficoll (n=5) or TNP-LPS in CFA (n=3) or alum (n=3) was analyzed. . The bars indicate the frequency of glycan structures with 0, 1 or 2 galactose (G) and 0, 1 or 2 sialic acid (S) residues and represent means + SEM for all independent experiments for each group. **(D)** The Fc glycosylation, **(E)** sialylation and **(F)** agalactosylation (G0) of purified total IgGs from the pooled sera of three 10 wk-old untreated *Fcgr2b-/-* mice (n=3) or purified OVA- or TNP-reactive IgGs from the pooled sera of six 10-wk-old *Fcgr2b-/-* mice i.p. injected 14 days before with 100 µg of OVA in CFA (n=3) or 50 µg of TNP-Ficoll (n=3), TNP-LPS (n=3) or TNP-LPS in CFA (n=3) were analyzed. The symbols represent data from individual animals.

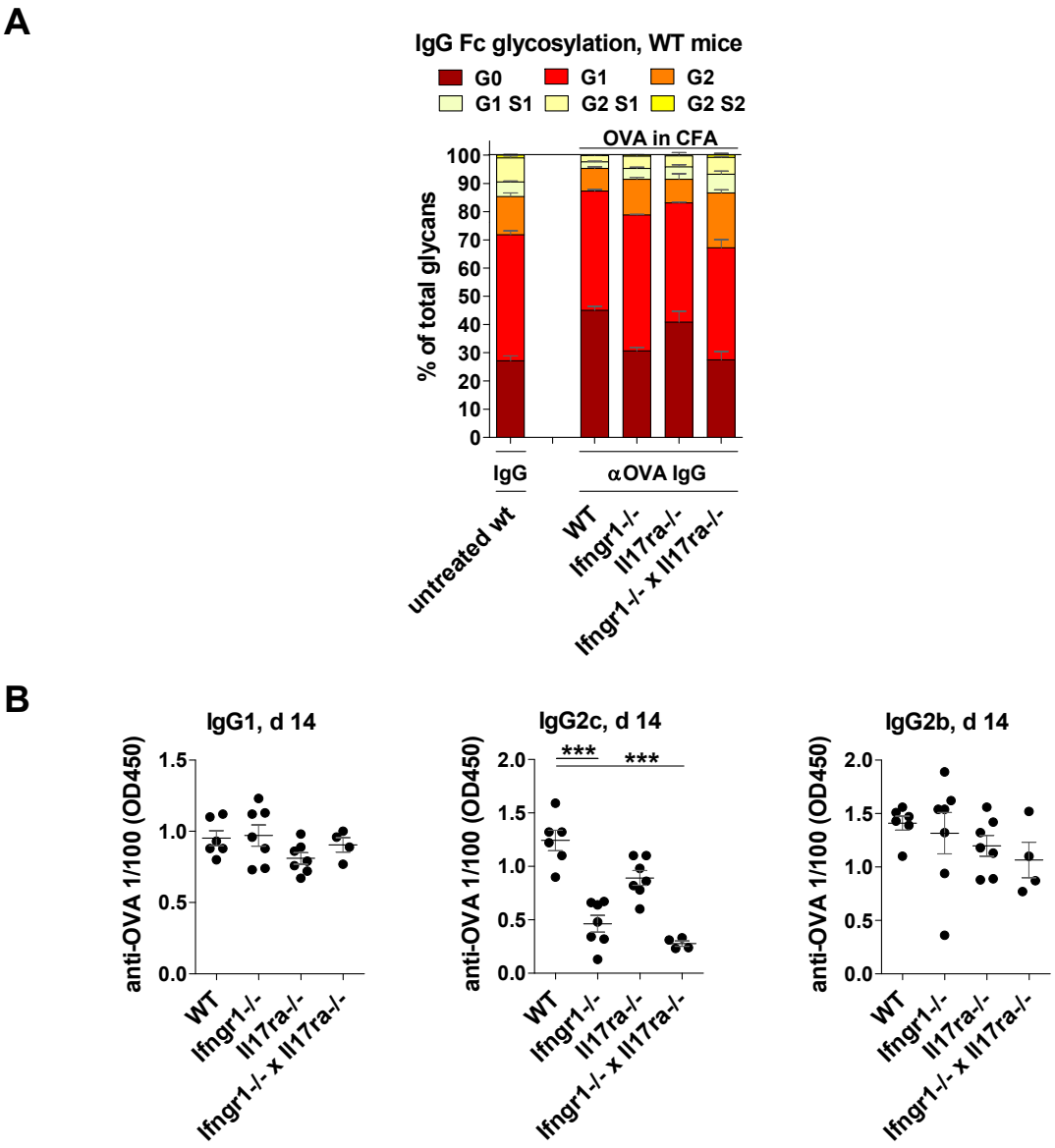
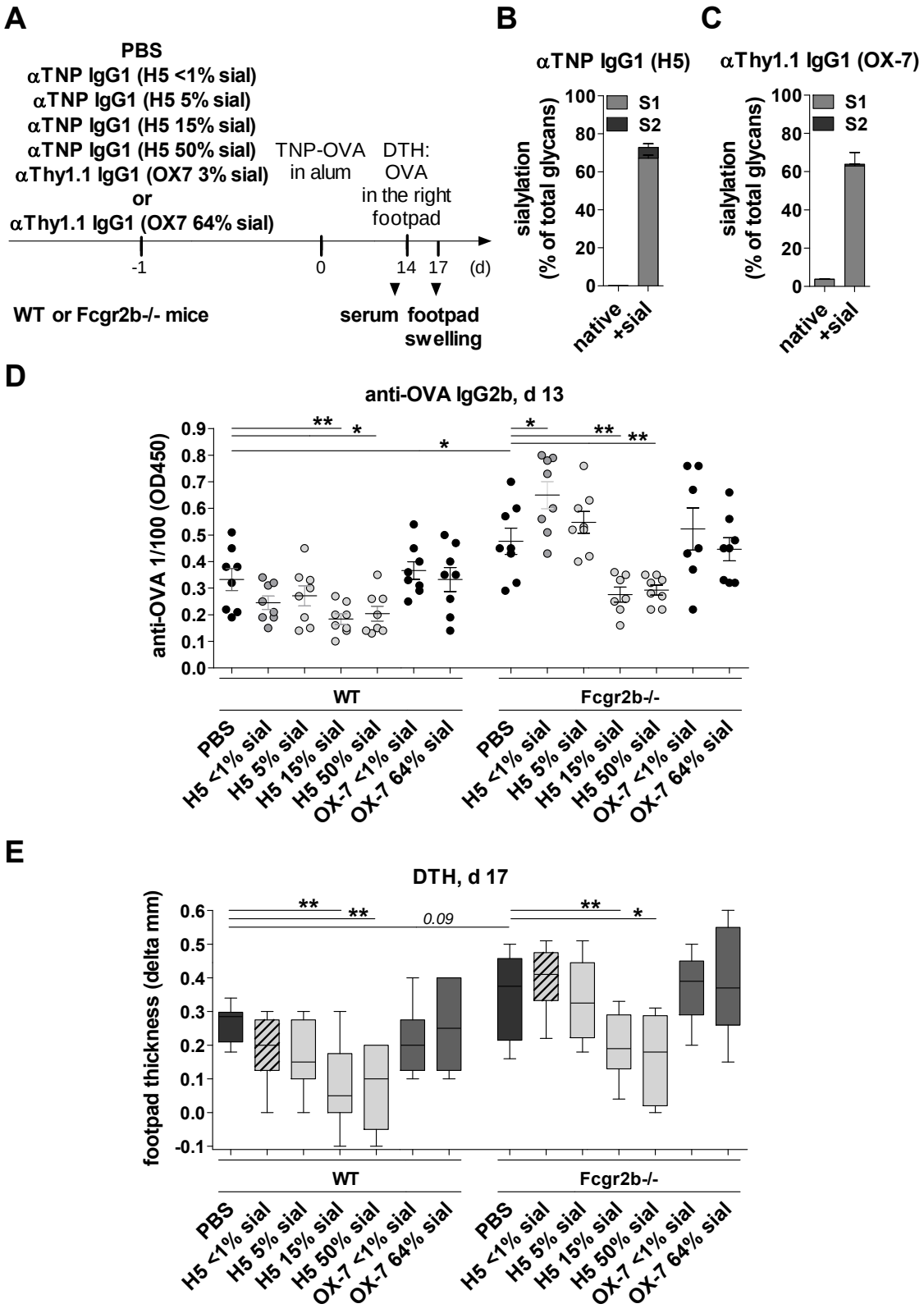


Figure S5. IFNgRI and IL-17RA are important for the development of inflammatory asialylated IgGs. (A) The Fc glycosylation (the same analyses as in **Figure 3, D and E**). of purified total IgGs from the pooled sera of three 10-wk-old untreated WT mice (independent experiments; n=11) or purified OVA-specific IgGs from the pooled sera of six 10-wk-old WT (n=18), *Ifngr1-/-* (n=5), *Il17ra-/-* (n=3) and *Ifngr1-/- x Il17ra-/-* (n=3) mice i.p. injected 14 days before with 100 µg of OVA in CFA was analyzed through EndoS-treatment and MALDI-TOF MS (**Supplemental Figure 4, A and B**). The bars indicate the frequency of glycan structures with 0, 1 or 2 galactose (G) and 0, 1 or 2 sialic acid (S) residues and represent the means + SEM for all independent experiments for each group. The data from untreated and treated WT mice were used from our recent studies (**13**) **(B)** Anti-OVA serum IgG1, IgG2c and IgG2b levels on d 14 of the indicated groups were determined via ELISA. The symbols represent data from individual animals. Horizontal lines represent means + SEM. One representative of 3 ELISA experiments is shown.


A
PBS
αTNP IgG1 (H5 <1% sial)
αTNP IgG1 (H5 5% sial)
αTNP IgG1 (H5 15% sial)
αTNP IgG1 (H5 50% sial)
αThy1.1 IgG1 (OX7 3% sial)
or
αThy1.1 IgG1 (OX7 64% sial)
TNP-OVA in alum
DTH: OVA in the right footpad
-1
0
14
17
(d)
WT or Fcgr2b-/- mice
serum
footpad swelling
B
αTNP IgG1 (H5)
sialylation (% of total glycans)
S1
S2
native
+sial
C
αThy1.1 IgG1 (OX-7)
D
anti-OVA IgG2b, d 13
anti-OVA 1/100 (OD450)
WT
Fcgr2b-/-
PBS
H5 <1% sial
H5 5% sial
H5 15% sial
H5 50% sial
OX-7 <1% sial
OX-7 64% sial
E
DTH, d 17
footpad thickness (delta mm)
0.09

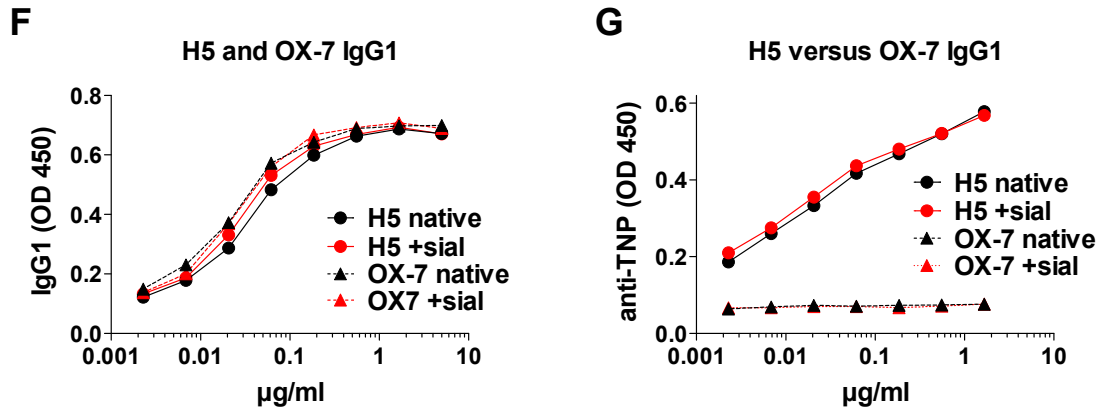


Figure S6. Transfer of antigen-specific sialylated monoclonal IgGs reduces a subsequent antigen-induced DTH response. (A) Graphical representation of the experimental approach followed in **B-G**. WT and *Fcgr2b-/-* mice were simultaneously injected i.v. on d -1 with either PBS or 100 µg of native non-sialylated (<1% sial) or 5%, 15% or 50% sialylated (sial; mix of native and in vitro sialylated H5 (70% sialylation; B)) monoclonal anti-TNP murine IgG1 (hybridoma clone H5) or with 100 µg of native low-sialylated (3% sial) or 64% in vitro sialylated antigen-unspecific anti-Thy1.1 murine IgG1 (hybridoma clone OX-7; C) (each group, n=7-8). DTH responses were induced through the injection of TNP-OVA in alum on d 0 and OVA in the right footpad on d 14. **(B and C)** The frequency of Fc sialic acid (S) modifications (**Supplemental Figure 4**) on native or in vitro sialylated **(B)** anti-TNP murine IgG1 (clone H5) or **(C)** anti-Thy1.1 murine IgG1 (clone OX-7) was determined through EndoS-treatment and MALDI-TOF MS. The bars represent means + SEM. **(D)** Serum IgG2b anti-OVA Ab levels on d 13 were determined via ELISA. Symbols represent data from individual animals. Horizontal lines represent means + SEM. **(E)** The differences in footpad thickness between the right and left footpad were determined at 3 days after local DTH induction. A box-and-wisker diagram with median and sample minimum and maximum is shown. One representative out of two independent experiments is shown. **(F and G)** Altered IgG glycosylation of anti-TNP murine IgG1 does not influence antigen binding. **(F)** Murine IgG1 ELISA and **(G)** TNP reactivity of native non-sialylated anti-TNP IgG1 (H5 native) and anti-Thy1.1 IgG1 (OX-7 native) Abs and their in vitro sialylated variants (H5 +sial; OX-7 +sial). One representative of 2 independent ELISA experiments is shown in F and G.

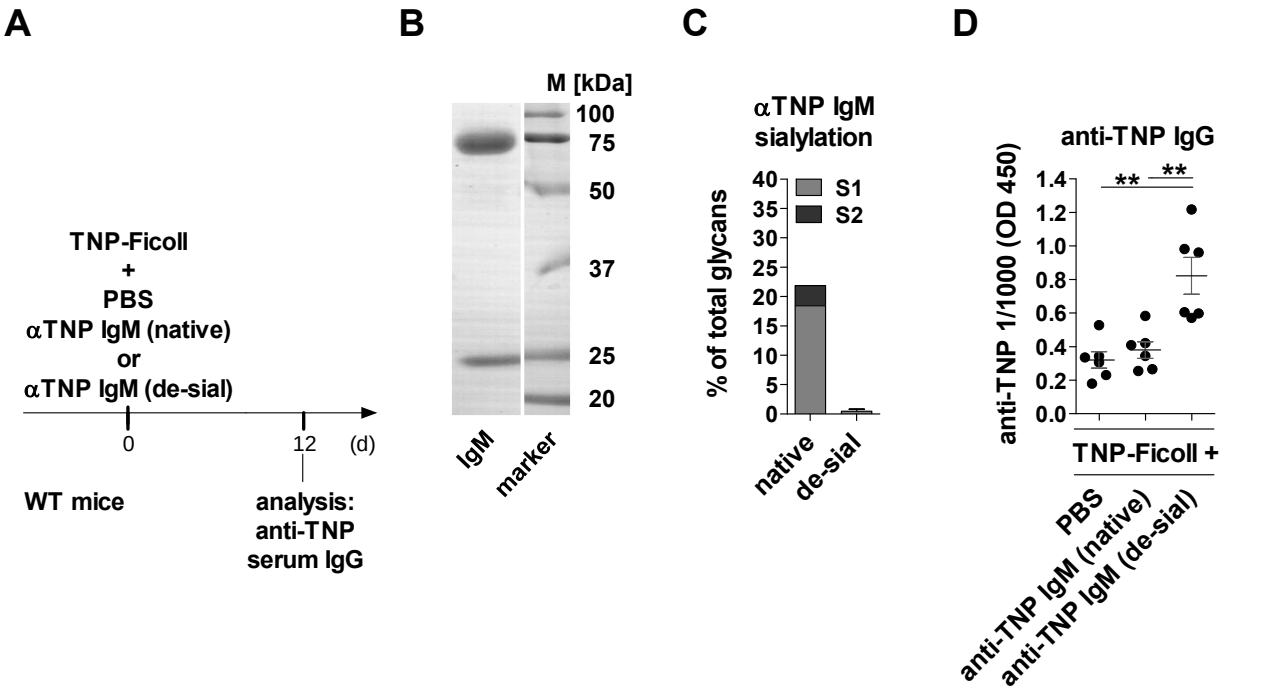
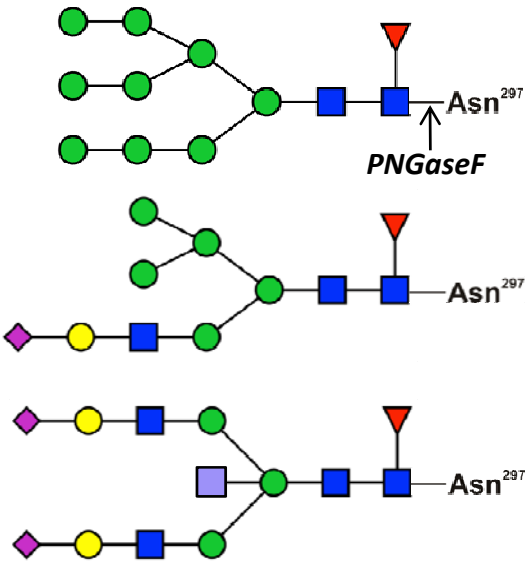


Figure S7. IgM Ab sialylation regulates antigen-induced B cell activation. (A) Graphical representation of the experimental approach followed in **B-D**. WT mice were injected i.p. with ICs containing 100 µg of TNP-Ficoll and 50 µg of either native sialylated or sialidase-treated (de-sial) monoclonal anti-TNP IgM (each group, n=6). The positive control group received PBS and TNP-Ficoll (no IC). **(B)** Coomassie blue staining of a SDS PAGE gel with 10 µg of heat-denatured native monoclonal anti-TNP IgM Ab. The lanes were run on the same gel but were noncontiguous. **(C)** The frequency of sialic acid (S) containing glycan structures on the indicated IgM Abs were determined through PNGaseF-treatment and MALDI-TOF MS **(Supplemental Figure 8**). The bars represent the means. **(D)** Anti-TNP serum IgG levels on d 12 were determined via ELISA. One representative of 2 independent experiments is shown.

A



2 GlcNAc — high-mannose structures

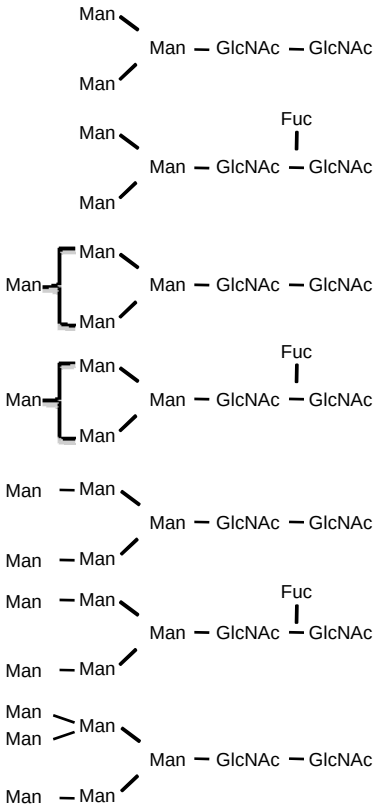
3 GlcNAc — hybrid structures

4 GlcNAc — complex structures

B

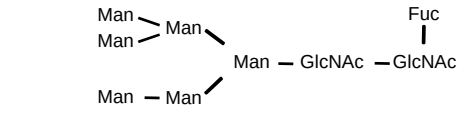
Human IgG Fc glycan structures (PNGaseF)	mass (m/z)	abbreviation

High-mannose structures

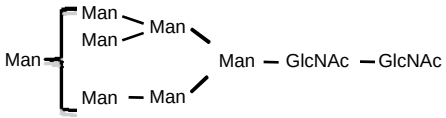


Human IgG Fc glycan structures (PNGaseF)	mass (m/z)	abbreviation

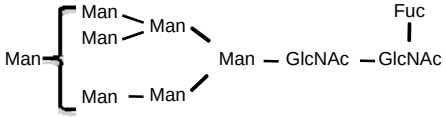
High-mannose structures



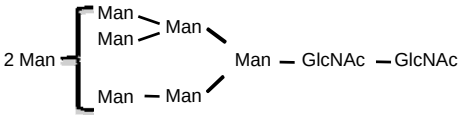
	1958	F M6



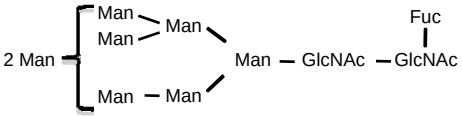
	1988	M7



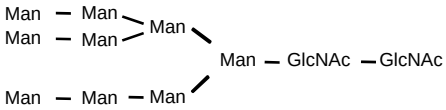
	2162	F M7



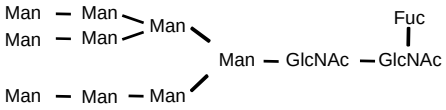
	2192	M8



	2366	F M8

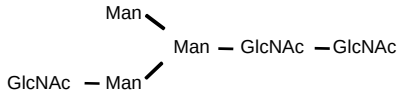


	2396	M9



	2570	F M9

Hybrid structures



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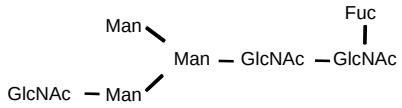

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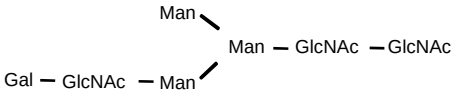

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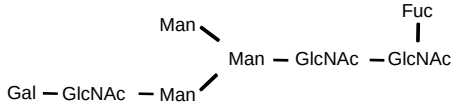
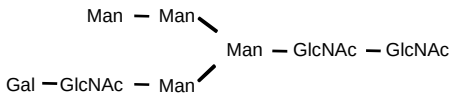


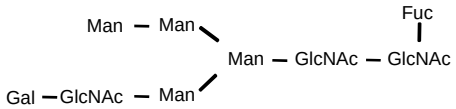
	1795	F M3 A1 G1

Human IgG Fc glycan structures (PNGaseF)	**mass (m/z)**	**abbreviation**

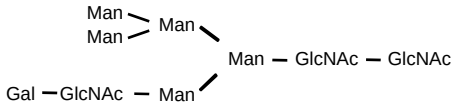
Hybrid structures



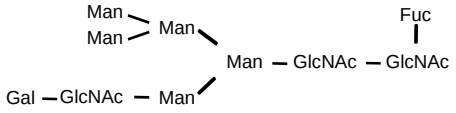
1825 M4 A1 G1



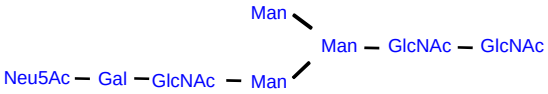
1999 F M4 A1 G1



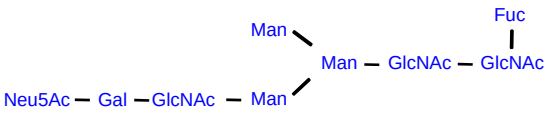
2029 M5 A1 G1



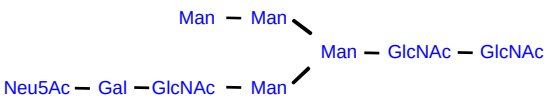
2203 F M5 A1 G1



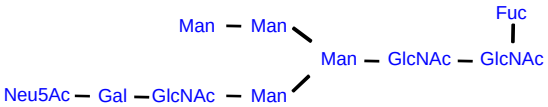
1982 M3 A1 G1 S1



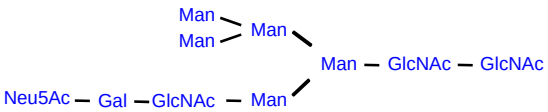
2156 F M3 A1 G1 S1



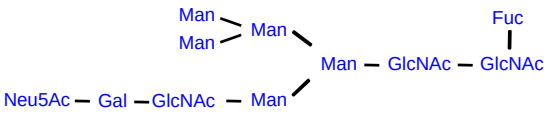
2186 M4 A1 G1 S1



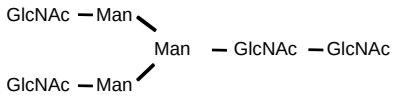
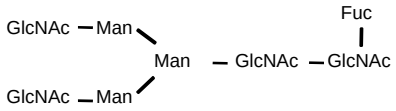
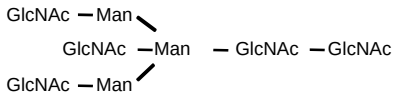
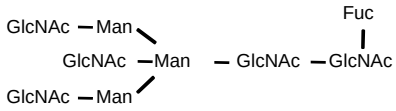
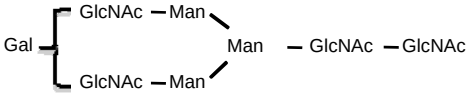
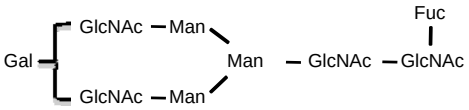
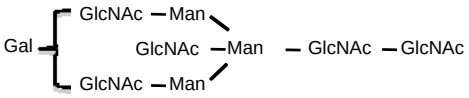
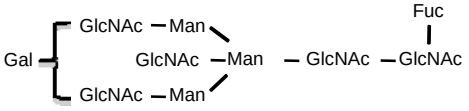
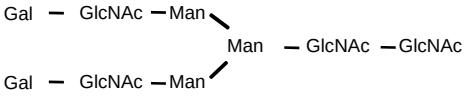
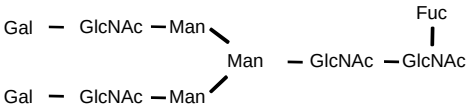
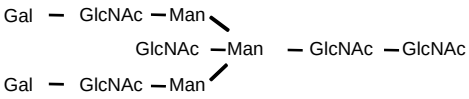
2360 F M4 A1 G1 S1



2390 M5 A1 G1 S1

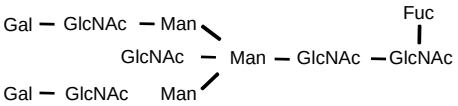
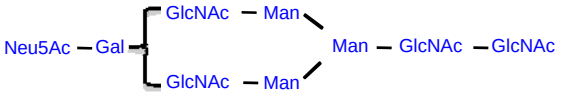
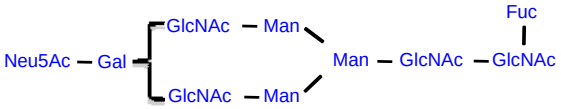
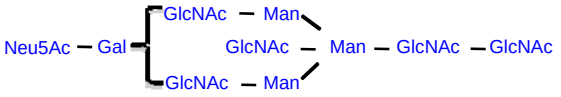
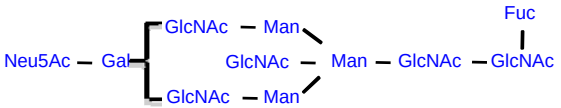
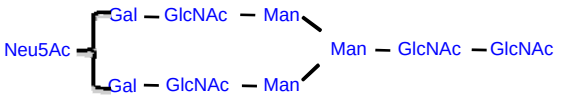
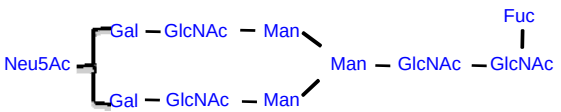
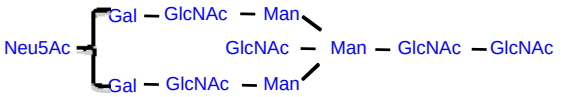
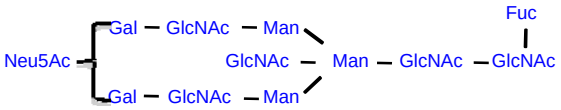
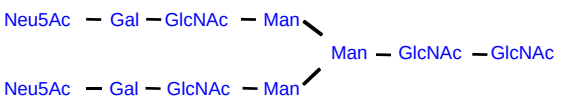
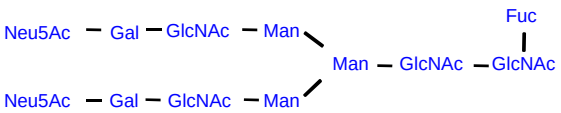


2564 F M5 A1 G1 S1

Human IgG Fc glycan structures (PNGaseF)	**mass (m/z)**	**abbreviation**
Complex structures		
	1662	A2
	1836	F A2
	1907	A2 B
	2081	F A2 B
	1866	A2 G1
	2040	F A2 G1
	2111	A2 B G1
	2285	F A2 B G1
	2070	A2 G2
	2244	F A2 G2
	2315	A2 B G2

Human IgG Fc glycan structures (PNGaseF)	**mass (m/z)**	**abbreviation**

Complex structures

	2489	F A2 B G2
	2227	A2 G1 S1
	2401	F A2 G1 S1
	2472	A2 B G1 S1
	2646	F A2 B G1 S1
	2431	A2 G2 S1
	2605	F A2 G2 S1
	2676	A2 B G2 S1
	2850	F A2 B G2 S1
	2792	A2 G2 S2
	2967	F A2 G2 S2

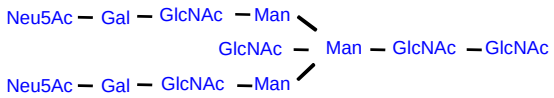
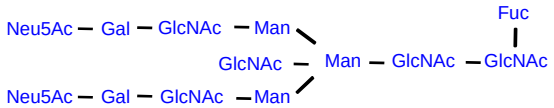
Human IgG Fc glycan structures (PNGaseF)	mass (m/z)	abbreviation
Complex structures		
	3038	A2 B G2 S2
	3212	F A2 B G2 S2

Figure S8. Possible N-glycans linked to IgM. (A) IgM Abs contain 5 conserved glycosylation sites, and 2 of these sites bear oligo-mannose glycans (**65**). The largest N-glycans bearing high-mannose, hybrid or complex structures with 2, 3 or 4 terminal GlcNAcs, respectively, are depicted. Dark-blue: N-acetyl-glucosamine (GlcNAc; A); green: mannose (Man; M); light-blue: bisecting GlcNAc (B); red: fucose (Fuc; F); yellow: galactose (Gal; G); pink: sialic acid (Neu5Ac; S). The monoclonal IgM contains only human Neu5Ac and no murine Neu5Gc because it was produced in human HEK 293 cells. MALDI-TOF MS was performed from PNGaseF-released N-glycans. The cleavage site of PNGaseF is indicated with an arrow. **(B)** Potential IgM N-glycan structures released with PNGaseF. The numbers show the molecular masses (m/z) of the possible N-glycan structures (permethylated).

Tables

Supplementary Tables

Table S1: Primer.

anti-TNP variable VDJ heavy chain part, forward primer:	5´ tctaccggtgtacattccgaggtgcagcttcaggagtca
anti-TNP variable VDJ heavy chain part, reverse primer:	5´ gcagggctagctgcagagacagtgaccagagtccc
anti-TNP complete VJ kappa light chain, forward primer:	5´ gtcaccggtgtacattcagacattgtgatgtcacagtct
anti-TNP complete VJ kappa light chain, reverse primer:	5´ ttattcggaagctttcaacactcattcctgttgaag
C57BL/6 IgG1 forward primer (NheI):	5´ cctcgcgctagcacgacacccccatctgtctatccac
C57BL/6 IgG1 reverse primer (BsiWI):	5´ ttattcggcgtacgcgtcatttaccaggagagtgggag
C57BL/6 IgG2c forward primer (NheI):	5´ cctcgcgctagcacaacagccccatcggtctatccac
C57BL/6 IgG2c reverse primer (BsiWI):	5´ ttattcggcgtacgcgtcatttacccagagaccgggag
C57BL/6 IgG2b forward primer (NheI):	5´ cctcgcgctagcacaacacccccatcagtctatccac
C57BL/6 IgG2b reverse primer (BsiWI):	5´ ttattcggcgtacgcgtcatttacccggagaccgggag
C57BL/6 IgM forward primer (NheI):	5´ cctcgcgctagccagtccttcccaaatgtcttccccctcg
C57BL/6 IgM reverse primer (BsiWI):	5´ ttattcggcgtacgcgtcaatagcaggtgccgcctgtg