Supporting Information to:

CD33 recruitment inhibits IgE-mediated anaphylaxis and desensitizes mast cells to allergen

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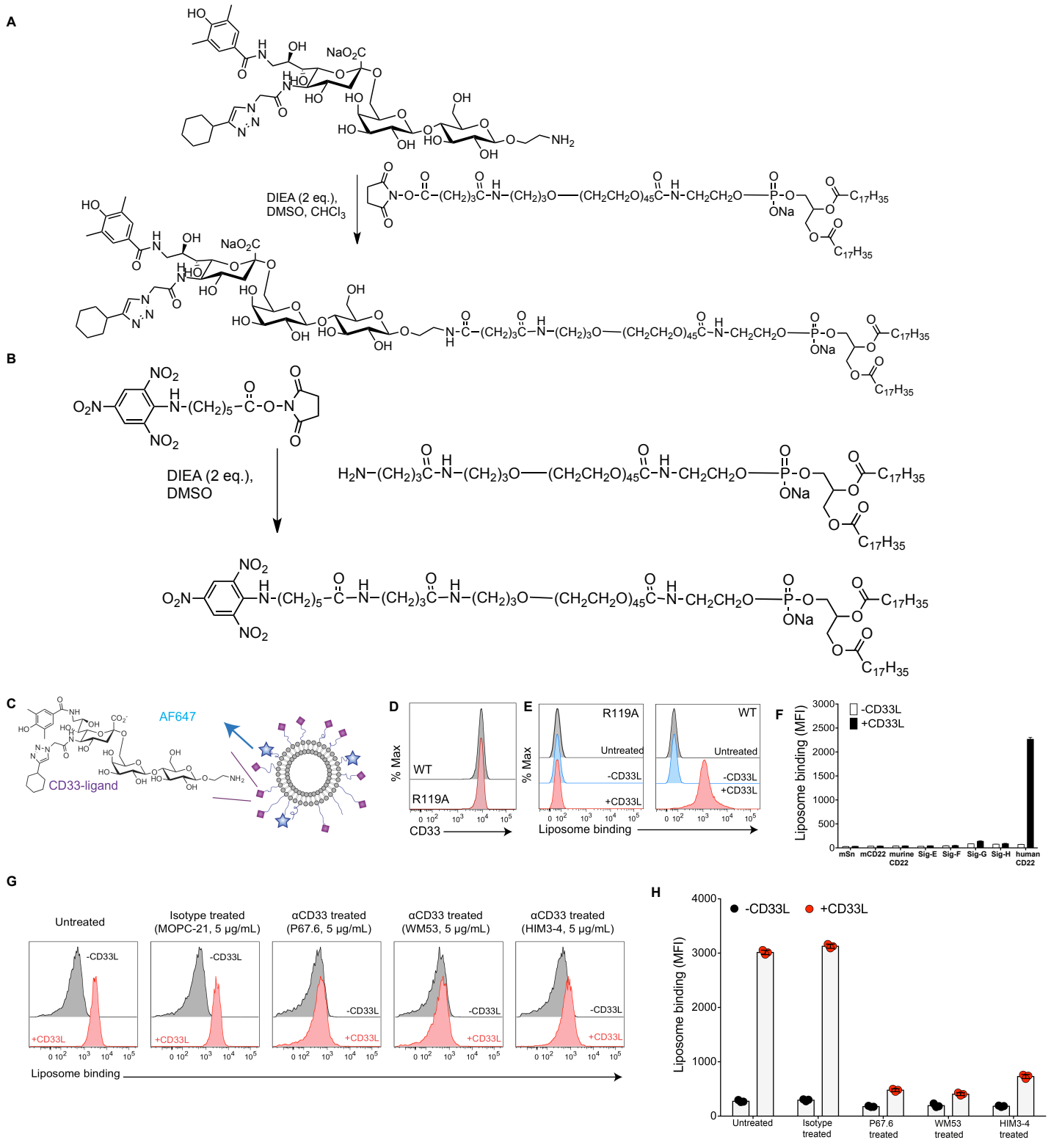
Supplementary Materials:

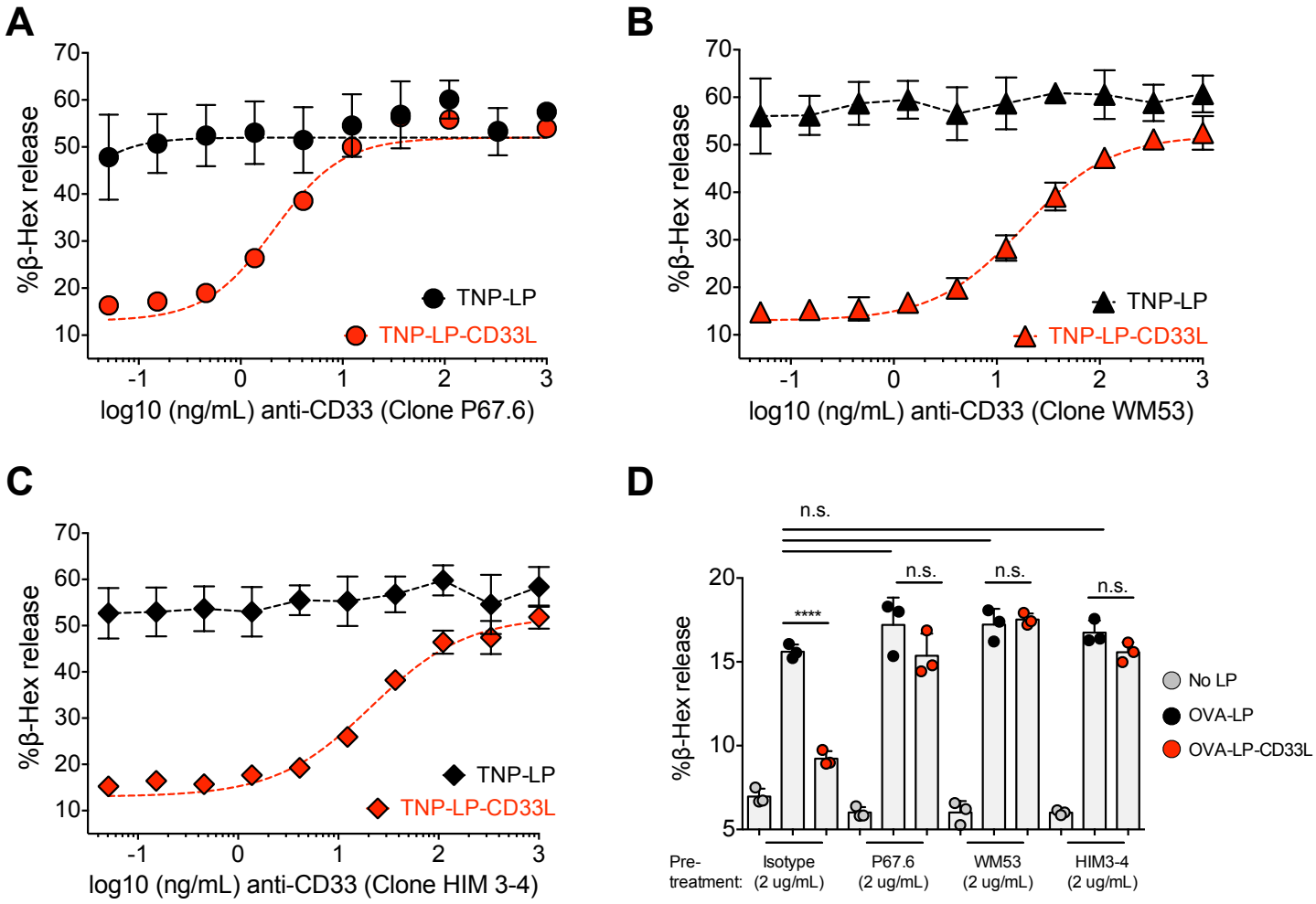


Supplemental Figure 1. Formulation and characterization of antigenic liposomes displaying CD33 ligand.

(**A**) Reaction condition used to couple human CD33-ligand to PEGylated lipid.

(**B**) Reaction condition used to couple tri-nitrophenol (TNP, Biosearch technology, # N-1010-100) to PEGylated lipid.

(**C**) A schematic representation of a fluorescent liposome (AF647) formulated with CD33L only (LP-CD33L).
(**D**) Anti-CD33 (Clone WM53) staining of CHO cells transfected with WT or R119A CD33.
(**E**) Binding or fluorescent liposome +/- CD33L (20 μM) to CHO cells transfected with WT or R119A CD33.
(**F**) Binding of fluorescent liposomes +/- CD33L (20 μM) to CHO cell lines transfected with different Siglecs. N = 3 per condition, values were plotted as means ± S.D.
(**G**) Binding of fluorescent TNP-LP or TNP-LP-CD33L (20 μM) to un-sensitized LAD2 cells in the presence of isotype control or different clones of anti-CD33 antibodies (5 μg/mL).
(**H**) Quantification of liposome binding to LAD2 cells in G. (**G, H**) n=3 per condition. Shown are representative from three independent experiments.
Shown are representative from two (**D-F**) or three (**G, H**) independent experiments.



Supplemental Figure 2. Degranulation of LAD2 cells induced by antigenic liposomes in the presence of anti-CD33 antibodies.

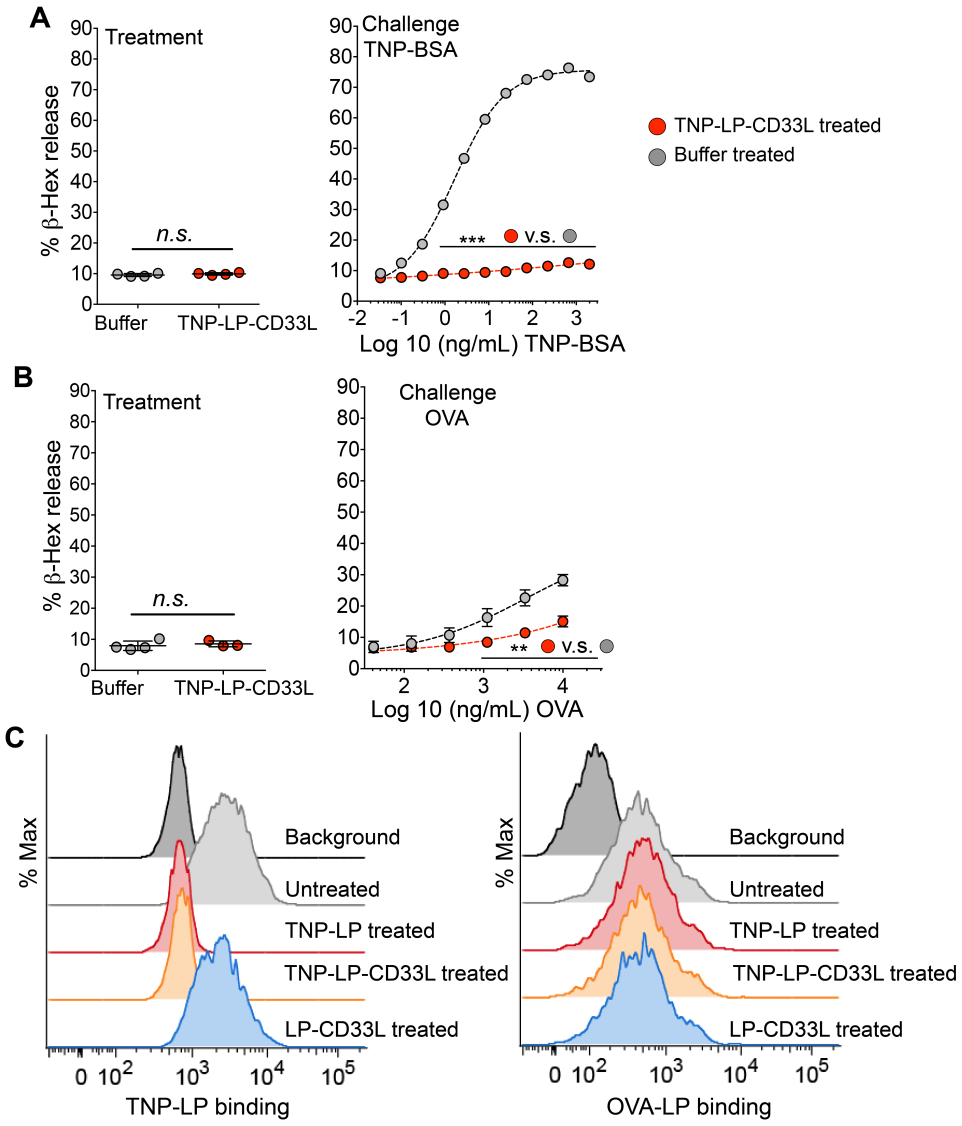
(**A-C**) Degranulation induced by TNP-LP or TNP-LP-CD33L (5 μM) in the presence of isotype or anti-CD33 antibodies. (**A**) Clone 67.6. (**B**) Clone WM53. (**C**) Clone HIM 3-4. N = 4 per condition, values were plotted as means ± S.D.

(**D**) Degranulation induced by OVA-LP or OVA-LP-CD33L (30 μM liposome containing 1.4 μg/mL of OVA) in the presence of isotype or CD33 antibodies (2 μg/mL). N = 3 per condition.

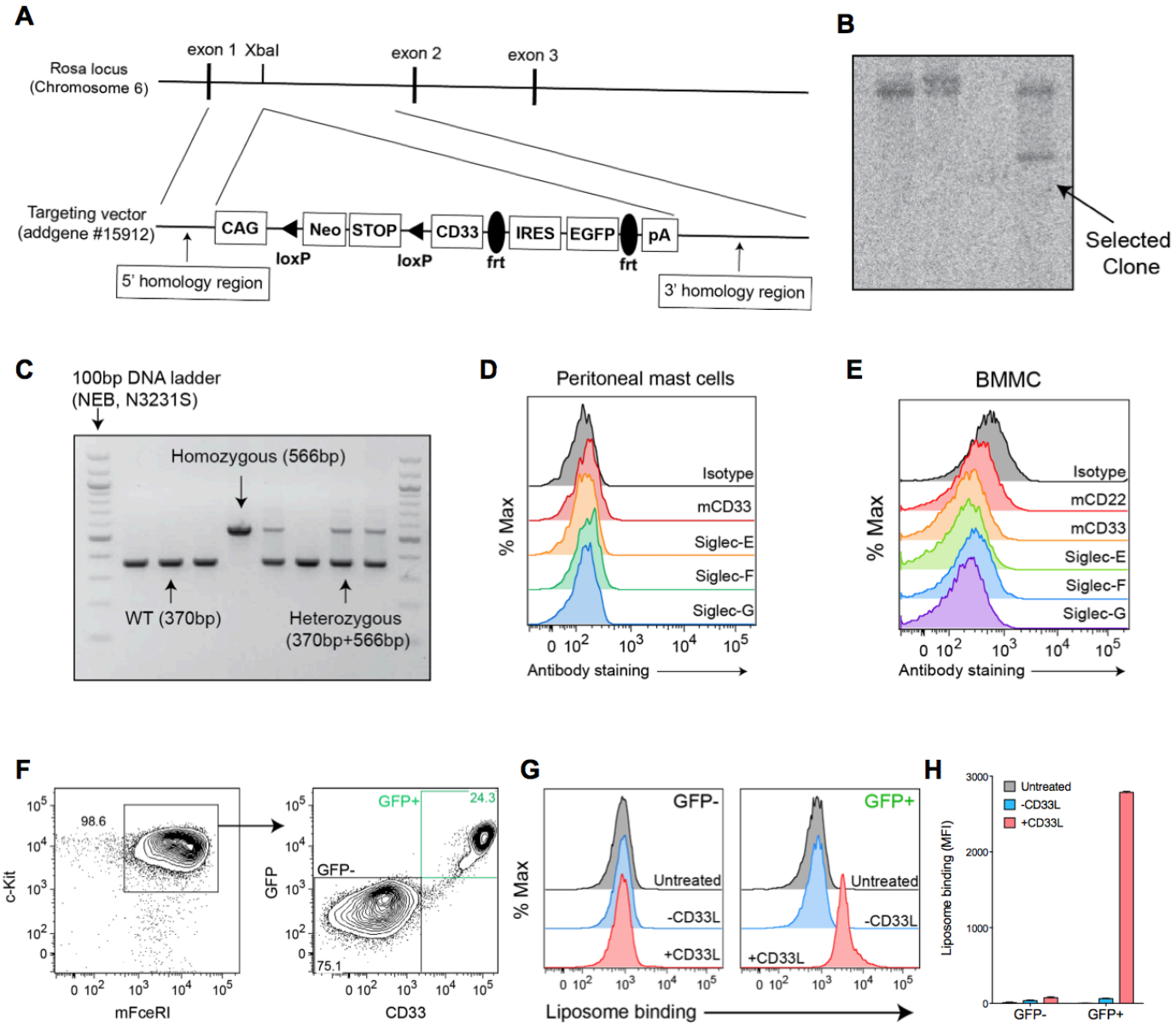
(**A-C**) LAD2 cells were sensitized with anti-TNP-IgE (1 μg/mL) overnight. (**D**) LAD2 cells were sensitized with anti-OVA-IgE (Clone EC1 and PMP68, 1 μg/mL each clone) overnight.

Shown are representative data of three (**A-C**) or two (**D**) independent experiments.

(**D**) was analyzed by 1-way ANOVA followed by Tukey's test: **** $P < 0.0001$; n.s., not significant ($P > 0.05$).



Supplemental Figure 3. Antigenlic liposomes with CD33L desensitize mast cells in vitro.
(**A**) Degranulation induced by treatment with buffer or TNP-LP-CD33L (5 μM, *left*). Degranulation induced by subsequent challenge of TNP^{31}BSA (Biosearch technology, *right*).
(**B**) Degranulation induced by treatment with buffer or TNP-LP-CD33L (5 μM, *left*). Degranulation induced by subsequent challenge with ovalbumin (OVA, Worthington, *right*). (**K** and **L**, n=4 per condition, values were plotted as means ± S.D.)
(**C**) Binding of fluorescent TNP-LP (20 μM) or fluorescent OVA-LP (20 μM) to LAD2 cells treated with TNP-LP, TNP-LP-CD33L or LP-CD33L (10 μM). Background of liposome binding was determined using untreated LAD2 cells.
LAD2 cells were pre-sensitized overnight with αTNP-IgE, and αOVA-IgEs (Clone MEB38, PMP-68 and E-C1 each at 500 ng/mL). Results are representative of at least two independent experiments. Data were analyzed by unpaired two-tailed Student's t tests: ** $P < 0.01$; *** $P < 0.001$; n.s., not significant ($P > 0.05$).



Supplemental Figure 4. Development of Rosa26-Stop$^{fl/fl}$-CD33 transgenic mice and characterization murine mast cells.

(**A**) A schematic scheme of a targeting vector containing cDNA encoding full length CD33 incorporated into the Rosa26-locus through homologous recombination.

(**B**) Southern blot analysis of PRX embryonic stem cells transfected with the targeting vector. Arrow indicated selected clone with targeting vector inserted into the Rosa locus.

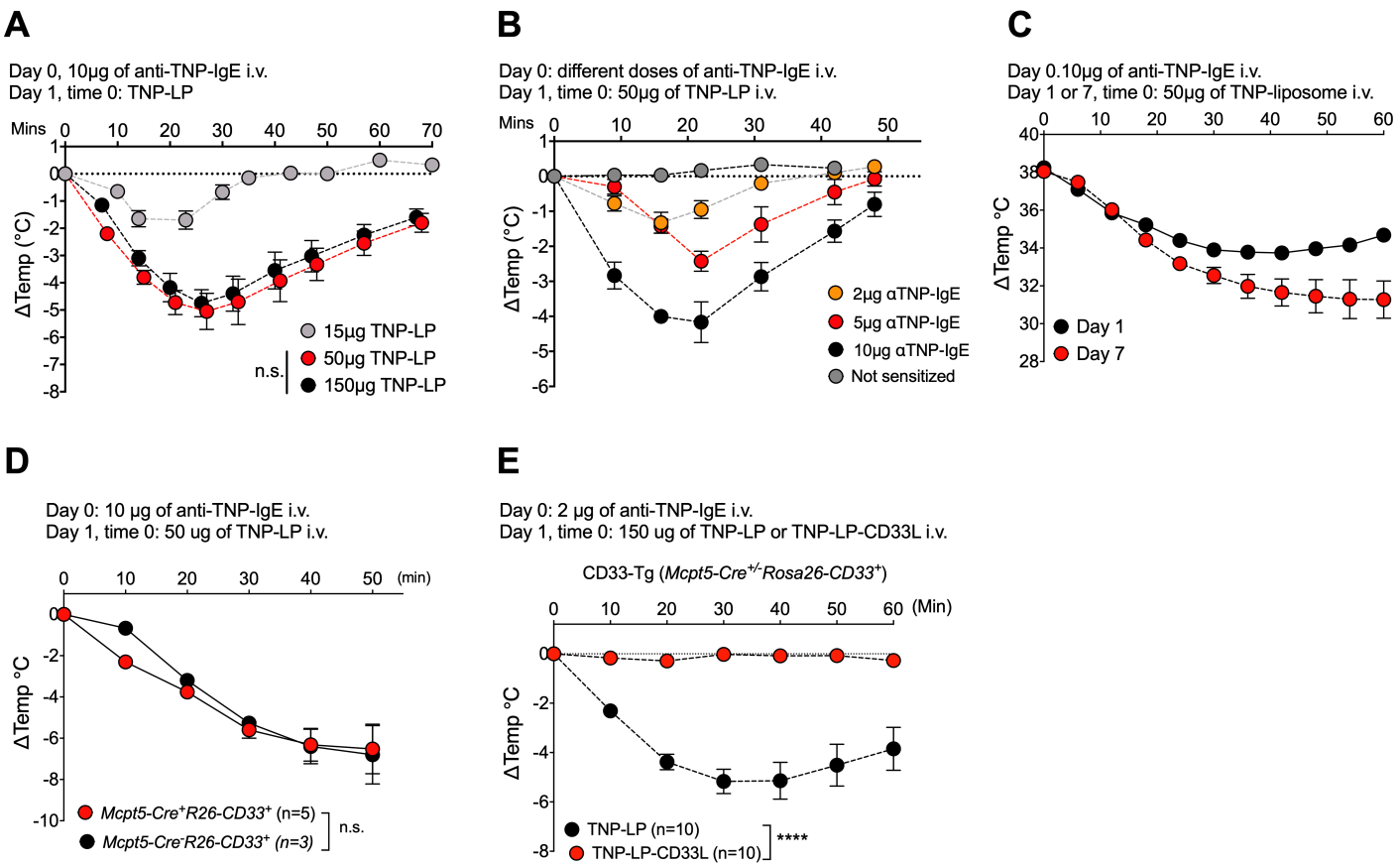
(**C**) PCR amplification of WT, heterozygous or homozygous Rosa26-Stop$^{fl/fl}$-CD33 transgenic mice. WT=370bp, homozygous=566bp, and heterozygous=370bp+566bp.

(**D**) Antibody staining of murine Siglecs on peritoneal cells harvested from a C57BL/6J mice.

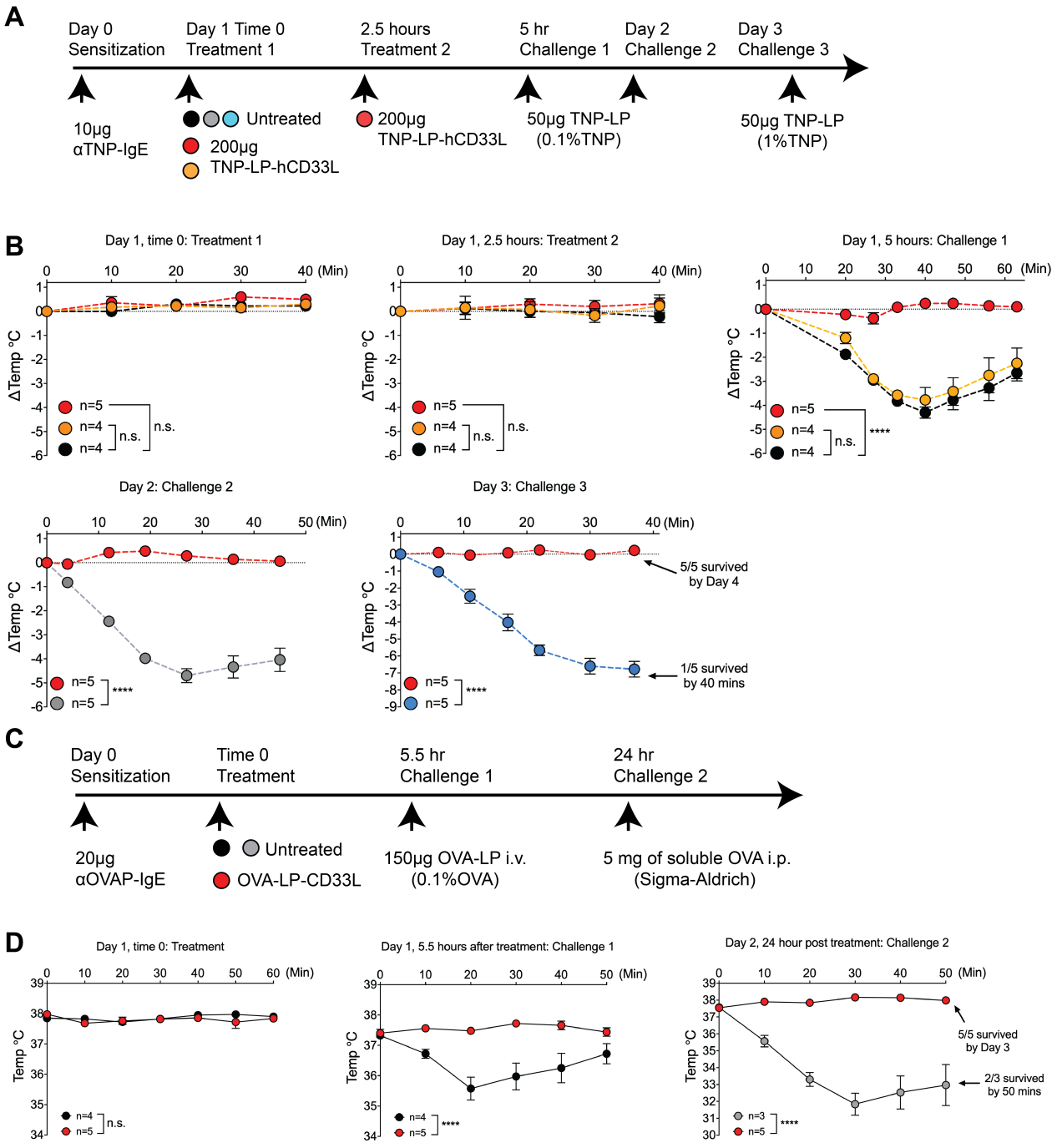
(**E**) Antibody staining of murine Siglecs on mast cells derived from bone marrow of a C57BL/6J mice.

(**F**) Flow cytometry analysis of bone marrow derived mast cells (BMMCs) cultured from CD33-Tg mice.

(**G, H**) Binding of fluorescent liposome +/- CD33L (20 μM) to GFP^{+} or GFP^{-} BMMCs. Binding data was representative from at least three independent experiments. (**h**) Quantification of mean fluorescent intensity quantified from (**G**). (**H**) N = 2 per data point. (**F-H**) Shown are representative data from three independent culture or experiments.



Supplemental Figure 5. Anaphylaxis induced by TNP-LP or TNP-LP-CD33L.
(**A**) Dose response of anaphylaxis induced by TNP-LP in mice intravenously sensitized with αTNP-IgE (10 µg), n = 4/group.
(**B**) Rectal temperature drop induced by TNP-LP (50 µg) in mice sensitized with no IgE, 2, 5, or 10 µg of anti-TNP-IgE), n= 3 or 4/group.
(**C**) Rectal temperature drop induced by TNP-LP (50 µg) 1 or 7 days after sensitization with αTNP-IgE (10 µg, Clone MEB-38). N = 4 or 5/group.
C57BL/6J mice were used in **A** and **B**. CD33-Tg and Control-Tg mice were used in **C**.
(**D**) Decrease of rectal temperature induced by TNP-LP (50 µg, 200uL of 0.33mM liposome) in Control-Tg or CD33-Tg. Sensitized with 10 µg of anti-TNP-IgE.
(**E**) Decrease of rectal temperature induced by TNP-LP or TNP-LP-CD33L (150 µg or 200uL of 1mM liposome) in CD33 Tg previously sensitized with 2 µg of anti-TNP-IgE.
(**A-E**) values were plotted as means ± s.e.m. (**A**, **D** and **E**) were analyzed by repeated measures 2-way ANOVA: **** P <0.0001; n.s., not significant (P >0.05).



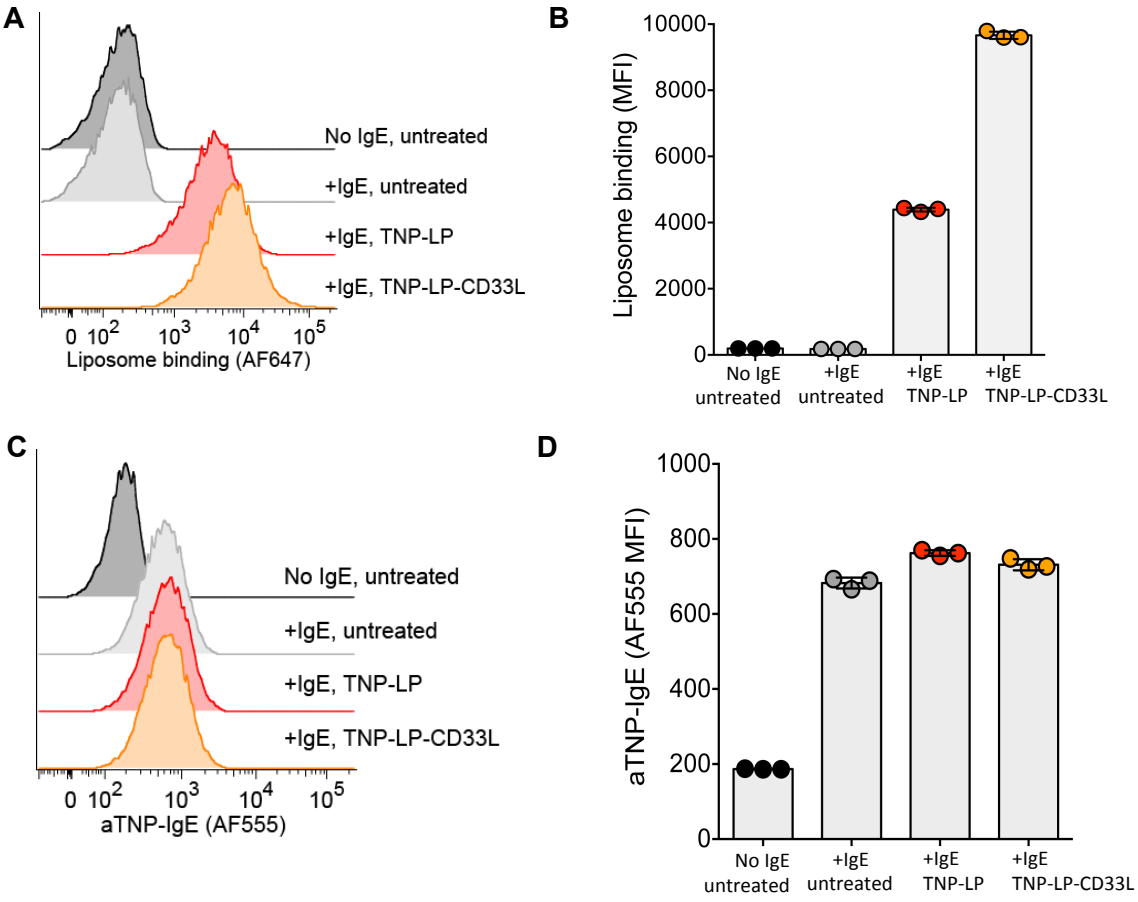
Supplemental Figure 6. Antigenic liposomes with CD33L desensitize mice to subsequent antigen challenges.

(**A**) Injection scheme to determine the dose requirement for TNP-LP-CD33L to desensitize mice. CD33-Tg mice were used in TNP-LP-CD33L treated group (*red, orange*). CD33-Tg and Control-Tg mice were used in the three untreated groups (*black*, *grey,* and *blue*) for the subsequent challenges at 5h, Day 2 and Day 3.

(**B**) Rectal temperature induced by indicated by treatment or challenges described in **A**. The 2-injection scheme (*red*) was representative of three n = 4 or 5 experiments.

(**C**) Injection scheme using OVA-LP-CD33L to desensitize mice. CD33-Tg mice were used in OVA-LP-CD33L treated group (*red*). CD33-Tg and Control-Tg mice were used in the two untreated

groups (*black*, and *grey*) for the subsequent challenges at 5.5h, and Day 2. All mice were intraveneously sensitized with 20 μg of anti-OVA-IgE (Clone EC1 and PMP68, 10 μg each). (**D**) Rectal temperature induced by indicated by treatment or challenges described in **C.** Data shown was representative of two n=5 experiments.Values were plotted as means ± s.e.m. Interaction P values in (**B** and **D**) were determined by repeated measures 2-way ANOVA followed by Tukey's test: **** P <0.0001; n.s., not significant (P >0.05).



Supplemental Figure 7. Impact of TNP-LP or TNP-LP-CD33L on IgE.
LAD2 cells were sensitized with AF555 labeled anti-TNP-IgE (500 ng/mL) overnight.
(A) Binding of TNP-LP or TNP-LP-CD33L (20 μM) to LAD2 cells.
(B) Quantification of mean fluorescent intensity of liposome binding from A,
(C) Signal from AF555 labeled IgE on LAD2 cells.
(D) Quantification of mean fluorescent intensity of anti-TNP-IgE. N=3 per condition.

Supplemental Table 1. Antibodies used in flow cytometry.

Reactivity	Antigen	Clone or Cat #	Fluorophor	Vendor	Working concentration
Mouse	c-Kit	2B8	APC-Cy7	Biolegend	1-2μg/mL
Mouse	FcεRI	1-Mar	PE/Cy7	Biolegend	1-2μg/mL
Mouse	CD22	OX-97	APC	Biolegend	1-2μg/mL
Mouse	CD33	9A11	APC	eBioscience	1-2μg/mL
Mouse	Siglec-E	#750620	AF647	R&D	1-2μg/mL
Mouse	Siglec-F	9C7(1)	AF647	In house	1-2μg/mL
Mouse	Siglec-G	SH2.1	AF647	eBioscience	1-2μg/mL
N.A.	Isotype (Rat IgG1)	RTK2071	AF647	Biolegend	1-2μg/mL
N.A.	Isotype (Rat IgG2a)	RTK2758	AF647	Biolegend	1-2μg/mL
N.A.	Isotype (Rat IgG2b)	RTK4530	AF647	Biolegend	1-2μg/mL
Mouse	CD45	30-F11	BV 605	Biolegend	1-2μg/mL
Mouse	F4/80	BM8	PE/Cy7	Biolegend	1-2μg/mL
Mouse	Siglec-F	E50-2440	BV421	BD	1-2μg/mL
Mouse	CD11b	M1/70	BV650	Biolegend	1-2μg/mL
Mouse	Fc Blocker	# 101320	N.A.	Biolegend	2μg/mL
Human	CD33	WM53	PE or PE/Cy7	Biolegend	1-2μg/mL
Human	CD33	P67.6	PE	Biolegend	1-2μg/mL
Human	FcεRI	CRA-1	BV 421	Biolegend	1μg/mL
Human	c-Kit	104D2	AF488	Biolegend	1μg/mL
Human	CD45	HI30	APC-Cy7	Biolegend	1μg/mL
Human	CD3	HIT3a	PE/Cy7	Biolegend	1μg/mL
Human	CD19	H1B19	PE/Cy7	Biolegend	1μg/mL
Human	CD56	HCD56	BV510	Biolegend	1μg/mL
Human	Siglec-1 (CD169)	7-239	PE	Biolegend	2μg/mL
Human	CD22	HIB22	PE	Biolegend	2μg/mL
Human	Siglec-5	IA5	PE	Biolegend	2μg/mL
Human	Siglec-6	# FAB2859P	PE	R&D System	50 X
Human	Siglec-7	6-434	PE	Biolegend	2μg/mL
Human	Siglec-8	7C9	PE	Biolegend	2μg/mL
Human	Siglec-9	K8	PE	Biolegend	2μg/mL
Human	Siglec-10	5G6	PE	Biolegend	2μg/mL
Human	Fc Blocker	# 422302	N.A.	Biolegned	2μg/mL
N.A.	Unknown (Isotype control)	MOPC-21	PE or PE/Cy7	Biolegend	2μg/mL

Supplemental Table 2. Antibodies used in western blotting.

Antigen	Residue	Clone/Cat #	Vendor	Dilution Factor
P-Syk	Tyr352	#2701	Cell signaling	1000
Syk		#13198	Cell Signaling	2000
P-PLCγ1	Tyr783	D6M9S /#14008	Cell Signaling	1000
P-PLCγ2	Tyr1217	#3871	Cell Signaling	1000
P-JNK (SAPK/JNK)	Thr183/Tyr185	81E11/ #4668	Cell Signaling	2000
P-Akt	Ser473	D9E/ #4060	Cell Signaling	2000
P-MEK 1/2	Ser217/221	41G9/#9154	Cell Signaling	2000
P-Erk 1/2 (p44/42 MAPK)	Thr202/Tyr204	D13.14.4E/ #4370	Cell Signaling	2000
Erk 1/2 (p44/42 MAPK)		137F5/# 4695	Cell Signaling	5000
Anti-rabbit IgG, HRP-linked		#7074	Cell Signaling	2000

Reference:

1. Gicheva N, Macauley MS, Arlian BM, Paulson JC, and Kawasaki N. Siglec-F is a novel intestinal M cell marker. *Biochemical and biophysical research communications.* 2016;479(1):1-4.