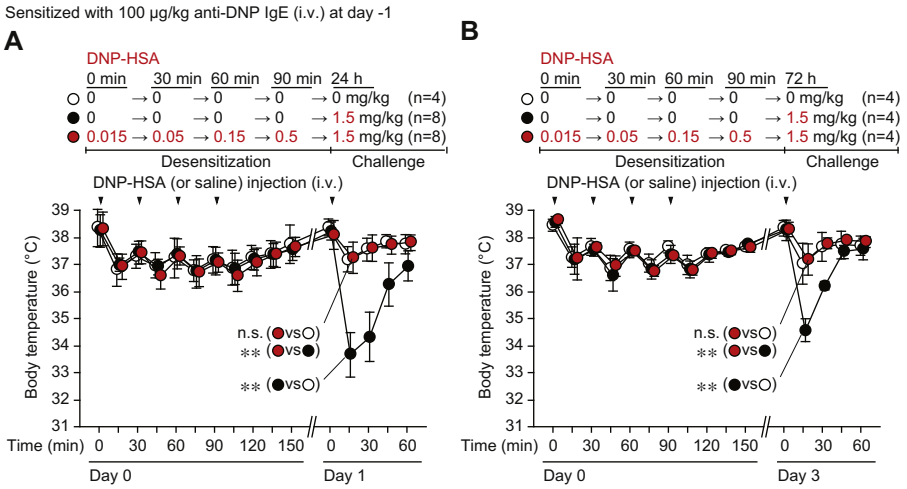


FIG E1. Rapid desensitization can prevent PSA reactions *in vivo* if mice are challenged with the target dose of antigen 1 or 3 days after the last desensitization dose. **A** and **B,** Mice were sensitized (intravenously) with 100 μg/kg anti-DNP IgE. Body temperature was measured at the indicated time points after injection (intravenously) with sequentially increasing amounts of DNP-HSA *(Desensitization)*. Body temperature was measured at the indicated time points after injection (intravenously) with a target dose of DNP-HSA *(Challenge)* injected 1 (Fig E1, *A*) or 3 (Fig E1, *B*) days after the last desensitization dose. N = 4 to 8 mice per group from 2 to 3 independent experiments. $^{**}P < .01$ and *n.s.* (not significant, $P > .05$). Body temperatures at 15 minutes after 1.5 mg/kg DNP-HSA challenge were compared by using 1-way ANOVA, followed by the Bonferroni test. *i.v.*, Intravenously.

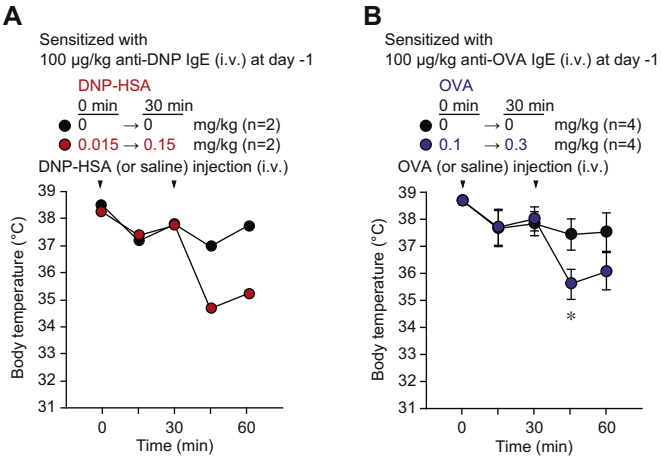
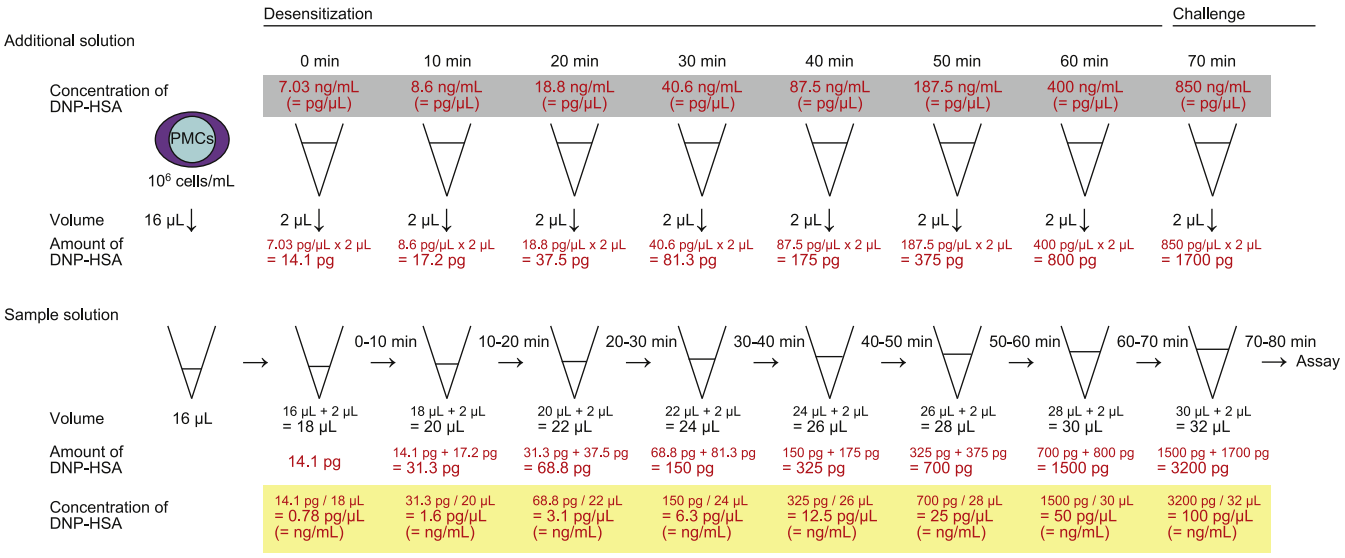


FIG E2. Evidence that exposure to appropriate, gradually increasing doses of antigen are needed to effectively desensitize mice to a target dose of antigen *in vivo*. Mice were sensitized (intravenously) with 100 μg/kg anti-DNP **(A)** or 100 μg/kg anti-OVA **(B)** IgE. The next day, body temperature was measured at the indicated time points after injection (intravenous) with the indicated concentrations of DNP-HSA (Fig E2, *A*) or OVA (Fig E2, *B*). N = 2 to 4 mice per group from 1 to 2 independent experiments. **P* < .05. Body temperatures at 45 minutes were compared by using the unpaired Student *t* test. *i.v.*, Intravenously.

A

Example of experimental protocol (DNP Desens. + DNP Challenge)



B

			Desensitization								
		Time	0	0-10	10-20	20-30	30-40	40-50	50-60	60-70	
No Desens.	Additional solution	volume (μL)	16	2	2	2	2	2	2	2	
		amount (pg)	0	0	0	0	0	0	0	0	
		concentration (ng/mL)	0	0	0	0	0	0	0	0	
	Sample solution	volume (μL)	16	18	20	22	24	26	28	30	
		amount (pg)	0	0	0	0	0	0	0	0	
		concentration (ng/mL)	0	0	0	0	0	0	0	0	
DNP Desens. (Alexa DNP Desens.)	Additional solution	volume (μL)	16	2	2	2	2	2	2	2	Fig 2, C, Fig 4, A, C, D, Fig 5, C Fig 6, B - E, FIg 7, A - D, Fig E8, Fig E9, A, Fig E10, Fig E11
		amount (pg)	0	14.1	17.2	37.5	81.3	175	375	800	
		concentration (ng/mL)	0	7.0	8.6	18.8	40.6	87.5	187.5	400	
	Sample solution	volume (μL)	16	18	20	22	24	26	28	30	
		amount (pg)	0	14.1	31.3	68.8	150	325	700	1500	
		concentration (ng/mL)	0	0.78	1.6	3.1	6.3	12.5	25	50	
OVA Desens.	Additional solution	volume (μL)	16	2	2	2	2	2	2	2	
		amount (pg)	0	2250	2750	6000	13000	28000	60000	128000	
		concentration (ng/mL)	0	1125	1375	3000	6500	14000	30000	64000	
	Sample solution	volume (μL)	16	18	20	22	24	26	28	30	
		amount (pg)	0	2250	5000	11000	24000	52000	112000	240000	Fig 4, F, Fig E9, B
		concentration (ng/mL)	0	125	250	500	1000	2000	4000	8000	

Red; DNP-HSA (or Alexa 633-labeled DNP-HSA) Blue; OVA

FIG E3. Details of *in vitro* experimental protocols. **A,** Example of the protocol for analyzing the desensitization, followed in some experiments by challenge, of mouse peritoneal mast cells (PMCs) *in vitro*. Samples of PMCs in Tyrode's buffer received sequentially, at the times indicated in the Figure, small volumes ("Additional solutions") of either Tyrode's buffer (as a control) or Tyrode's buffer containing antigen (DNP-HSA) (or, in other experiments shown in Fig E3, *B* or *C*, Alexa 633-labeled DNP-HSA, ovalbumin [OVA] or anti-IgE [α-IgE]). This yielded samples ("Sample solutions") of PMCs in Tyrode's buffer containing the final amounts and concentrations of antigen or α-IgE shown in *yellow*. **B** and **C,** Details of the *in vitro* experimental protocols used in various Figures.

C

			Desensitization								Challenge	
		Time	0	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	
No Desens. + No Challenge	Additional solution	volume (μL)	16	2	2	2	2	2	2	2	2	
		amount (pg)	0	0	0	0	0	0	0	0	0	
		concentration (ng/mL)	0	0	0	0	0	0	0	0	0	
	Sample solution	volume (μL)	16	18	20	22	24	26	28	30	32	
		amount (pg)	0	0	0	0	0	0	0	0	0	
		concentration (ng/mL)	0	0	0	0	0	0	0	0	0	Fig 2, C - G, M, P,
No Desens. + DNP Challenge	Additional solution	volume (μL)	16	2	2	2	2	2	2	2	2	Fig 4, A, C, E,
(No Desens. + Alexa DNP Challenge)		amount (pg)	0	0	0	0	0	0	0	0	3200	Fig 5, C
		concentration (ng/mL)	0	0	0	0	0	0	0	0	1600	Fig 6, B, C, E,
	Sample solution	volume (μL)	16	18	20	22	24	26	28	30	32	Fig 7, C, D,
		amount (pg)	0	0	0	0	0	0	0	0	3200	Fig E9, A,
		concentration (ng/mL)	0	0	0	0	0	0	0	0	100	Fig E10, Fig E11
DNP Desens. + DNP Challenge	Additional solution	volume (μL)	16	2	2	2	2	2	2	2	2	Fig 2, C - G, M, P,
(Alexa DNP Desens.		amount (pg)	0	14.1	17.2	37.5	81.3	175	375	800	1700	Fig 4, A, C, E,
+ Alexa DNP Challenge)		concentration (ng/mL)	0	7.0	8.6	18.8	40.6	87.5	187.5	400	850	Fig 5, C
	Sample solution	volume (μL)	16	18	20	22	24	26	28	30	32	Fig 6, B, C, E,
		amount (pg)	0	14.1	31.3	68.8	150	325	700	1500	3200	Fig 7, C, D,
		concentration (ng/mL)	0	0.78	1.6	3.1	6.3	12.5	25	50	100	Fig E9, A,
												Fig E10, Fig E11
No Desens. + OVA Challenge	Additional solution	volume (μL)	16	2	2	2	2	2	2	2	2	
		amount (pg)	0	0	0	0	0	0	0	0	512000	
		concentration (ng/mL)	0	0	0	0	0	0	0	0	256000	
	Sample solution	volume (μL)	16	18	20	22	24	26	28	30	32	Fig 2, K, M,
		amount (pg)	0	0	0	0	0	0	0	0	512000	Fig 4, F,
			0	0	0	0	0	0	0	0	16000	Fig E9, B
OVA Desens. + OVA Challenge	Additional solution	volume (μL)	16	2	2	2	2	2	2	2	2	
		amount (pg)	0	2250	2750	6000	13000	28000	60000	128000	272000	
		concentration (ng/mL)	0	1125	1375	3000	6500	14000	30000	64000	136000	
	Sample solution	volume (μL)	16	18	20	22	24	26	28	30	32	Fig 2, K, M,
		amount (pg)	0	2250	5000	11000	24000	52000	112000	240000	512000	Fig 4, F,
		concentration (ng/mL)	0	125	250	500	1000	2000	4000	8000	16000	Fig E9, B
OVA Desens. + DNP Challenge	Additional solution	volume (μL)	16	2	2	2	2	2	2	2	2	
		amount (pg)	0	2250	2750	6000	13000	28000	60000	128000	3200	
		concentration (ng/mL)	0	1125	1375	3000	6500	14000	30000	64000	1600	
	Sample solution	volume (μL)	16	18	20	22	24	26	28	30	32	
		amount (pg)	0	2250	5000	11000	24000	52000	112000	240000	3200	
		concentration (ng/mL)	0	125	250	500	1000	2000	4000	8000	100	Fig 2, M
DNP Desens. + OVA Challenge	Additional solution	volume (μL)	16	2	2	2	2	2	2	2	2	
		amount (pg)	0	14.1	17.2	37.5	81.3	175	375	800	512000	
		concentration (ng/mL)	0	7.0	8.6	18.8	40.6	87.5	187.5	400	256000	
	Sample solution	volume (μL)	16	18	20	22	24	26	28	30	32	
		amount (pg)	0	14.1	31.3	68.8	150	325	700	1500	512000	
		concentration (ng/mL)	0	0.78	1.6	3.1	6.3	12.5	25	50	16000	Fig 2, M
No Desens. + α-IgE Challenge	Additional solution	volume (μL)	16	2	2	2	2	2	2	2	2	
		amount (pg)	0	0	0	0	0	0	0	0	16000	
		concentration (ng/mL)	0	0	0	0	0	0	0	0	8000	
	Sample solution	volume (μL)	16	18	20	22	24	26	28	30	32	
		amount (pg)	0	0	0	0	0	0	0	0	16000	
		concentration (ng/mL)	0	0	0	0	0	0	0	0	500	Fig 2, P
α-IgE Desens. + α-IgE Challenge	Additional solution	volume (μL)	16	2	2	2	2	2	2	2	2	
		amount (pg)	0	70.3	85.9	187.5	406.3	875	1875	4000	8500	
		concentration (ng/mL)	0	35.2	43.0	93.8	203.1	437.5	937.5	2000	4250	
	Sample solution	volume (μL)	16	18	20	22	24	26	28	30	32	
		amount (pg)	0	70.3	156.3	343.8	750	1625	3500	7500	16000	
		concentration (ng/mL)	0	3.9	7.8	15.6	31.3	62.5	125	250	500	Fig 2, P
DNP Desens. + α-IgE Challenge	Additional solution	volume (μL)	16	2	2	2	2	2	2	2	2	
		amount (pg)	0	14.1	17.2	37.5	81.3	175	375	800	16000	
		concentration (ng/mL)	0	7.0	8.6	18.8	40.6	87.5	187.5	400	8000	
	Sample solution	volume (μL)	16	18	20	22	24	26	28	30	32	
		amount (pg)	0	14.1	31.3	68.8	150	325	700	1500	16000	
		concentration (ng/mL)	0	0.78	1.6	3.1	6.3	12.5	25	50	500	Fig 2, P
α-IgE Desens. + DNP Challenge	Additional solution	volume (μL)	16	2	2	2	2	2	2	2	2	
		amount (pg)	0	70.3	85.9	187.5	406.3	875	1875	4000	3200	
		concentration (ng/mL)	0	35.2	43.0	93.8	203.1	437.5	937.5	2000	1600	
	Sample solution	volume (μL)	16	18	20	22	24	26	28	30	32	
		amount (pg)	0	70.3	156.3	343.8	750	1625	3500	7500	3200	
		concentration (ng/mL)	0	3.9	7.8	15.6	31.3	62.5	125	250	100	Fig 2, P

			Desensitization							wash	Challenge	
		Time	0	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	
No Desens.	Additional solution	volume (μL)	16	2	2	2	2	2	2	2	32	
+ wash + DNP Challenge		amount (pg)	0	0	0	0	0	0	0	0	3200	
		concentration (ng/mL)	0	0	0	0	0	0	0	0	100	
	Sample solution	volume (μL)	16	18	20	22	24	26	28	30	32	
		amount (pg)	0	0	0	0	0	0	0	0	3200	
		concentration (ng/mL)	0	0	0	0	0	0	0	0	100	Fig 2, H
DNP Desens.	Additional solution	volume (μL)	16	2	2	2	2	2	2	2	32	
+ wash + DNP Challenge		amount (pg)	0	14.1	17.2	37.5	81.3	175	375	800	3200	
		concentration (ng/mL)	0	7.0	8.6	18.8	40.6	87.5	187.5	400	100	
	Sample solution	volume (μL)	16	18	20	22	24	26	28	30	32	
		amount (pg)	0	14.1	31.3	68.8	150	325	700	1500	3200	
		concentration (ng/mL)	0	0.78	1.6	3.1	6.3	12.5	25	50	100	Fig 2, H

Red; DNP-HSA (or Alexa 633-labeled DNP-HSA) Blue; OVA Green; α-IgE

FIG E3. *(Continued)*

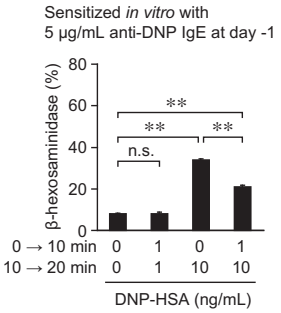


FIG E4. Evidence that exposure to appropriate, gradually increasing doses of antigen are needed to effectively desensitize mice to a target dose of antigen *in vitro*. Purified PMCs were sensitized with 5 μg/mL anti-DNP IgE and challenged in a 2-step protocol (without washing between) with the indicated concentrations of DNP-HSA. Percentage of β-hexosaminidase release was measured after 20 minutes. N = 4 per group. $^{**}P < .01$ and *n.s.* (not significant, $P > .05$).

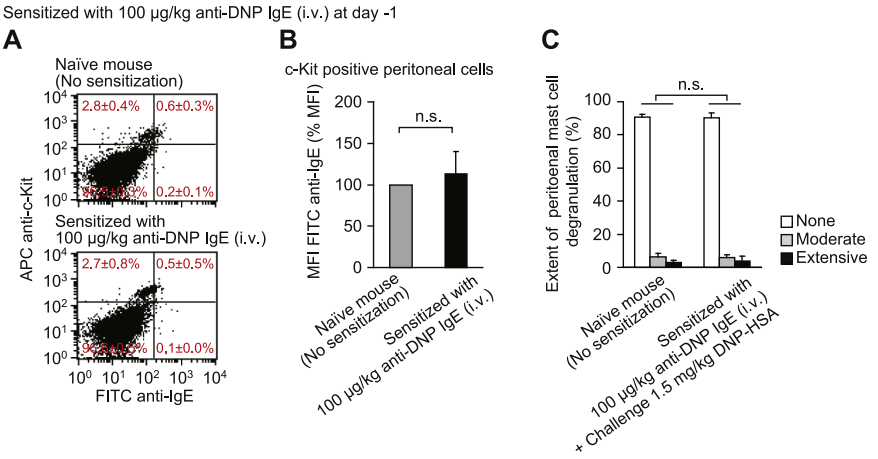


FIG E5. Intravenous injection of anti-DNP IgE (100 μg/kg) does not efficiently sensitize PMCs *in vivo*. **A,** Representative dot plots of surface IgE levels and c-Kit expression on peritoneal cells isolated from individual naive *(No sensitization)* or anti-DNP IgE–sensitized mice. Nine mice, each of which produced similar results, were tested for each group. The average percentage of cells in each quadrant from 9 individual mice is shown in red. **B,** MFI of cell-surface IgE molecules in c-Kit$^+$ PMCs in Fig E5, *A*. Data were normalized by using MFI from naive mice as 100%; to test for statistical significance, values were compared with a hypothetical value of 100% by using the 1-sample *t* test. **C,** Extent of PMC degranulation (percentage of MCs exhibiting extensive [>50% of granules in that cell exhibiting evidence of degranulation], moderate [10% to 50% of granules affected], or no [none; <10% granules affected] degranulation was quantified by using Giemsa-stained slides); comparisons between the indicated experimental conditions were tested for statistical significance by using the χ^2 test. **P* < .05 and *n.s.* (not significant, *P* > .05). *i.v.*, Intravenously.

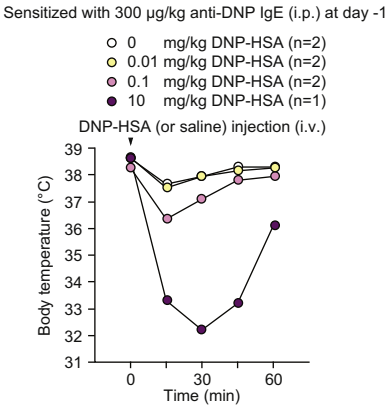


FIG E6. Pilot experiment testing responses to various doses of antigen challenge in sensitized mice. Mice were sensitized (intraperitoneally) with 300 μg/kg anti-DNP IgE. The next day, body temperature was measured after a single challenge (intravenously) with DNP-HSA. N = 1 to 2 mice per group from 1 experiment. *i.p.*, Intraperitoneally; *i.v.*, intravenously.

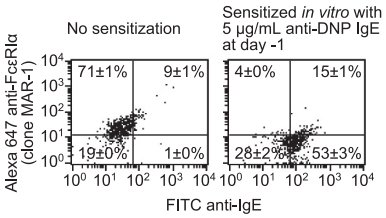


FIG E7. IgE sensitization blocks binding of anti-FcεRIα antibody (clone MAR-1) to FcεRIα. PMCs were cultured without *(left)* or with *(right)* 5 μg/mL anti-DNP IgE for 16 to 24 hours and then stained with FITC anti-IgE antibody and Alexa Fluor 647 anti-FcεRIα antibody. Representative *dot plots* of surface IgE and FcεRIα levels are shown. The average percentage of cells in each quadrant is shown; averages were calculated from 6 samples per group from 2 independent experiments, each using PMCs pooled from 3 to 5 mice.

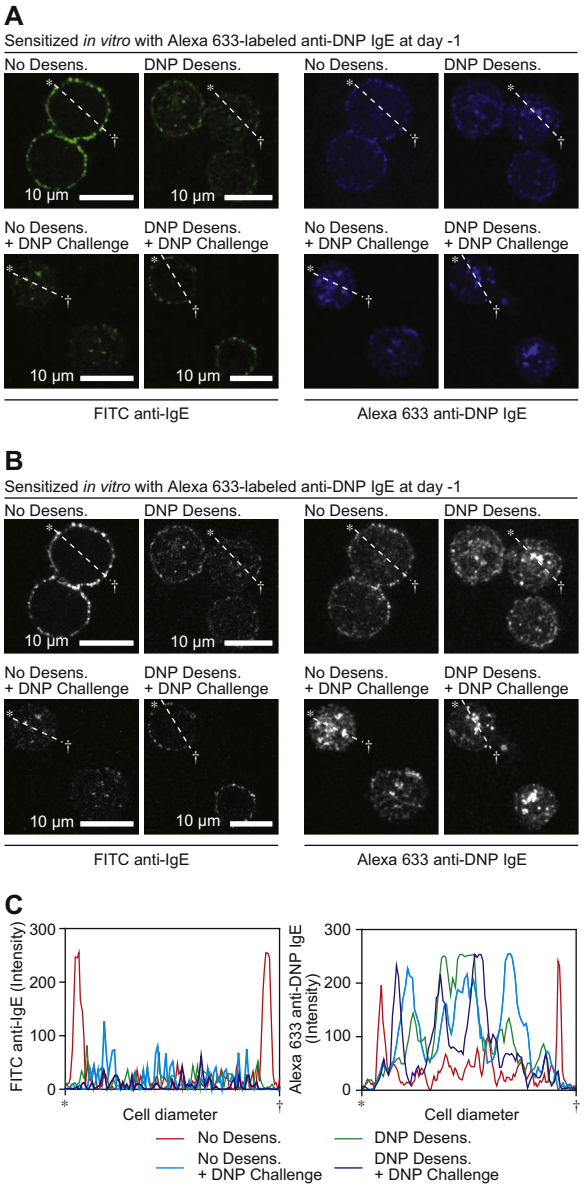


FIG E8. **A** and **B,** Confocal microscopic images of IgE internalization during rapid desensitization shown in color (Fig E8, *A*) or black and white (Fig E8, *B*; for easier visualization of signal). Primary isolated PMCs were sensitized with 5 μg/mL Alexa Fluor 633–labeled anti-DNP IgE and then desensitized with 0.78 → 50 ng/mL DNP-HSA. Representative confocal images showing staining for cell-surface IgE (detected by using FITC anti-IgE) and Alexa Fluor 633–labeled anti-DNP IgE; FITC *(left panels)* and Alexa Fluor 633 *(right panels)* signals are shown in separate panels to facilitate visualization of each fluorophore. Four images per group from 2 independent experiments, each using PMCs pooled from 3 to 5 mice, were examined; one representative image is shown in each panel. **C,** Line profiles of fluorescence intensity, depicting the intensity of fluorescence measured at various points along the cell transects shown in Fig E8, *A*.

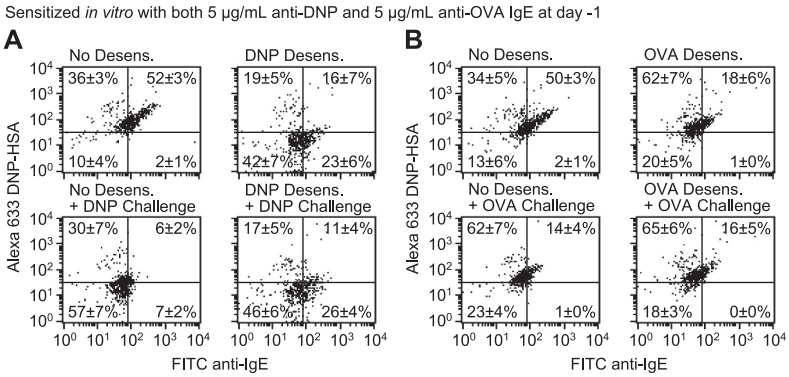


FIG E9. Internalization of antigen-specific IgE during rapid desensitization. Primary isolated PMCs were sensitized with both anti-DNP and anti-OVA IgE and then desensitized (0.78 → 50 ng/mL DNP-HSA **[A]** or 125 → 8,000 ng/mL OVA **[B]**) and challenged (100 ng/mL DNP-HSA [Fig E9, *A*] or 16,000 ng/mL OVA [Fig E9, *B*]) by using the protocol in Fig E3. After desensitization/challenge, the PMCs were stained with FITC anti-IgE antibody and Alexa Fluor 633–labeled DNP-HSA on ice. Representative *dot plots* show total IgE (FITC) and anti-DNP IgE (Alexa Fluor 633) levels on the cell surface. N = 6 per group from 3 independently isolated populations of PMCs (each pooled from 3-5 mice).

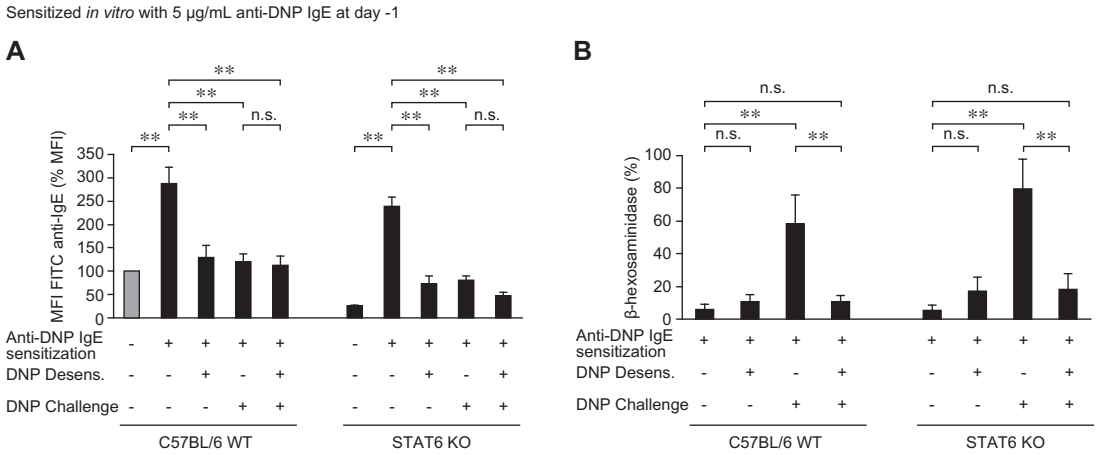


FIG E10. Rapid desensitization of STAT6 knockout PMCs *in vitro*. Primary isolated PMCs from C57BL/6 wild-type *(WT)* control mice and STAT6 knockout *(KO)* mice were sensitized with 5 μg/mL anti-DNP IgE and treated the next day with DNP-HSA by using the desensitization protocol shown in Fig 2 and Fig E3. **A,** MFI of cell-surface IgE normalized by using the MFI from WT unstimulated PMCs *(gray bar)* as 100%; values were compared with a hypothetical value of 100% by using the 1-sample *t* test to test for statistical significance. **B,** β-Hexosaminidase release. Fig E10, *A* and *B*, N = 6 samples per group from 2 independent experiments, each using PMCs pooled from 3 to 5 mice. ***P* < .01 and *n.s.* (not significant, *P* > .05).

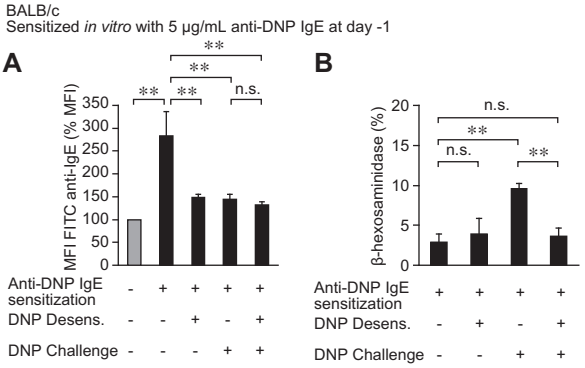


FIG E11. Rapid desensitization of BALB/c PMCs *in vitro*. Primary isolated PMCs from BALB/c mice were sensitized with 5 μg/mL anti-DNP IgE and treated the next day with DNP-HSA by using the desensitization protocol shown in Fig 2 and E3. **A,** The MFI of cell-surface IgE normalized by using the MFI from wild-type unstimulated PMCs *(gray bar)* as 100%; values were compared with a hypothetical value of 100% by using the 1-sample *t* test to test for statistical significance. **B,** β-Hexosaminidase release. N = 6 samples per group from 2 independent experiments, each using PMCs pooled from 3 to 5 mice. $^{**}P < .01$ and *n.s.* (not significant, $P > .05$).

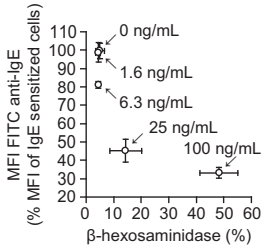


FIG E12. Susceptibility of PMCs to antigen-induced degranulation and IgE internalization. Purified PMCs were sensitized with 5 μg/mL anti-DNP IgE and then challenged with a single dose of DNP-HSA (0, 1.6, 6.3, 25, or 100 ng/mL). Percentage β-hexosaminidase release (from Fig 2, *A*) and cell-surface IgE levels (from Fig 4, *B*) are plotted on the same graph.

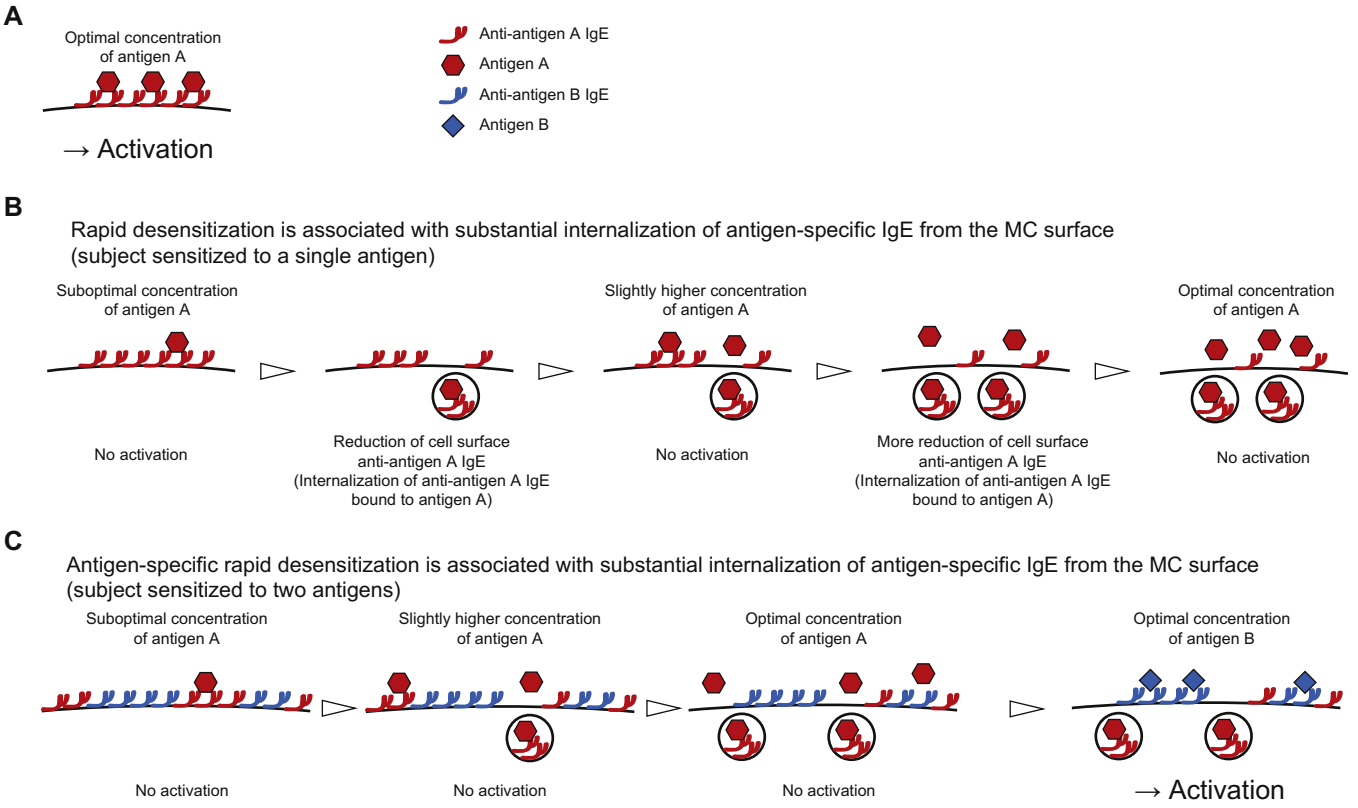


FIG E13. Our hypothesized mechanism of rapid desensitization of MCs. **A,** An optimal concentration of antigen A can activate MCs sensitized with anti–antigen A IgE. **B,** Sequentially increasing the concentration of antigen A reduces anti–antigen A IgE levels on the MC surface (through internalization of anti-antigen A IgE bound to antigen A). **C,** Rapid desensitization is associated with substantial internalization of IgE from the MC surface in an antigen-specific manner. Accordingly, in a subject sensitized to both antigen A and antigen B, antigen B can still activate MCs that have been desensitized to antigen A.

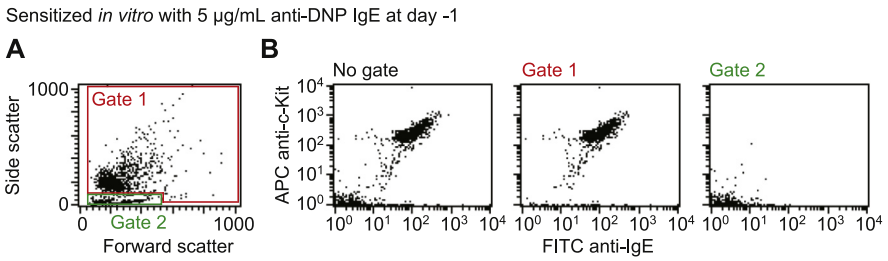


FIG E14. Flow cytometric PMC gating. For flow cytometric analysis of PMCs (Figs 4-7, E7, and E9-E12), samples were gated as shown in **(A)** (gate 1 in forward/side scatter plots) to select c-Kit and IgE double-positive PMCs. **B,** Greater than 99% of the cells in gate 1 (from Fig E14, *A*) are c-Kit$^+$ PMCs.