SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. Elicitation and analysis of IgE-dependent PCA reactions in rats and mice. Female rats or male mice injected i.d. (in dorsal skin or ear pinnae) with vehicle (saline) or anti-DNP IgE (2.5 or 10 ng/site) were challenged i.v. with DNP-HSA (1 or 10 mg/kg) in the presence of Evans blue (10 or 100 mg/kg). In mice, substantial extravasation of Evans blue occurred only when their ear pinnae were sensitized with the higher concentration of IgE (i.e., 10 ng/site) and challenged with the higher concentration of DNP-HSA (i.e., 10 mg/kg) in the presence of Evans blue at 100 mg/kg. N = 4 - 5 per group from one of 2 - 3 independent experiments, each of which gave similar result. ** $p < 0.01$; n.s.: $p > 0.05$.

Supplementary Figure S2. Effect of cromolyn on IgE-dependent PCA reactions in female mice. Female mice injected i.d. with vehicle (saline) or anti-DNP IgE (10 ng/site) were challenged i.v. with DNP-HSA (10 mg/kg) in the presence of Evans blue (100 mg/kg) with or without cromolyn (injected simultaneously). We detected no inhibitory effect of cromolyn (at 10 or 100 mg/kg) on PCA-associated Evans blue extravasation. N = 5 per group from 1 experiment. ** $p < 0.01$; n.s.: $p > 0.05$.

Supplementary Figure S3. Plasma histamine concentrations during IgE-dependent PSA reactions in rats and mice. Female rats (**a**) or male mice (**b**) sensitized i.v. with anti-DNP IgE (1 μg/kg) were challenged i.v. with DNP-HSA (1 mg/kg). Histamine concentrations were measured in plasma 90 sec after DNP-HSA challenge. Unlike in rats, in mice there was no significant increase in blood histamine after treating the animals with the same concentrations of IgE and antigen. N = 4 per group from one of 2 - 3 independent experiments, each of which gave similar result. ** $p < 0.01$; n.s.: $p > 0.05$.

Supplementary Figure S4. Effect of repeated treatment with cromolyn on IgE-dependent PCA or PSA reactions in mice *in vivo*. Male mice received i.p. vehicle (0 mg/kg) or 100 mg/kg cromolyn every 12 h for 2.5 d (Experimental regimen: **a**). Mice injected with saline or anti-DNP IgE (10 ng/site i.d. in **b** and **c**; 100 μg/kg i.v. in **d** and **e**) were challenged i.v., 30 min after the last administration of cromolyn (or vehicle), with DNP-HSA (10 mg/kg) in the presence (**b**) or absence (**c - e**) of Evans blue (100 mg/kg); we then measured Evans blue extravasation (**b**), ear swelling (**c**), plasma histamine concentrations (**d**) or plasma mMCP-1 concentrations (**e**). N = 4 (**b**), 5 (**c**), 5

(**d**) or 10 (**e**) per group from 1 (**b - d**) or 2 (**e**) independent experiments. ** $p < 0.01$, * $p < 0.05$; n.s.: $p > 0.05$.

Supplementary Figure S5. Effect of IgE concentrations on antigen-induced degranulation of rat and mouse PMCs *in vitro*. PMCs from female rats (**a**) or mice (**b**) sensitized with various concentrations of anti-DNP IgE were stimulated with DNP-HSA (100 ng/mL), and β-hexosaminidase release was measured. Relatively higher concentrations of IgE were needed to elicit robust β-hexosaminidase release in mouse as opposed to rat PMCs. N = 3 per group from one of 2 - 3 independent experiments, each of which gave similar result. ** $p < 0.01$ versus values for groups not treated with anti-DNP IgE (0 ng/mL).

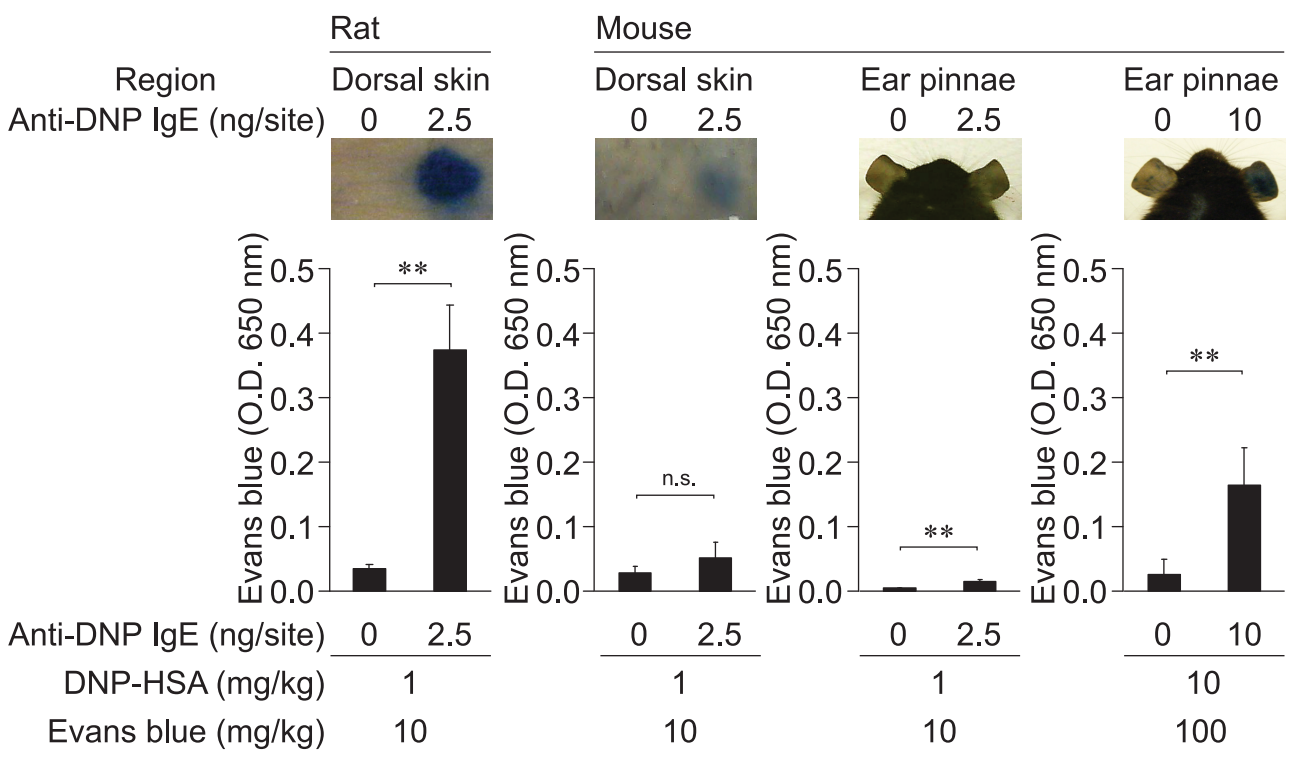
Supplementary Figure S6. Effect of cromolyn pretreatment on its inhibitory effect on activation of rat PMCs. IgE (50 ng/mL)-sensitized PMCs from female rats were stimulated with DNP-HSA (100 ng/mL) for 3 min with or without cromolyn pretreatment (0 min indicates simultaneous treatment) *in vitro*, and we measured β-hexosaminidase in the supernatant. A 5 - 10 min pretreatment with cromolyn reduced the inhibitory effect of cromolyn on β-hexosaminidase release. N = 3 per group from

one of 3 independent experiments, each of which gave similar result. ** $p < 0.01$; n.s.: $p > 0.05$.

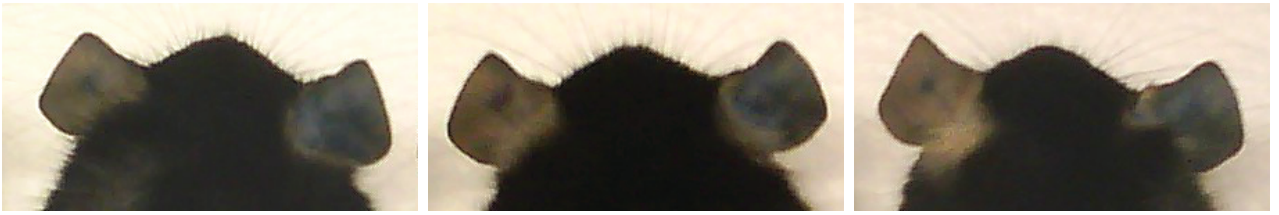
Supplementary Figure S7. Effect of cromolyn on IgE-dependent degranulation of mouse BMCMCs *in vitro*. BMCMCs from female mice were cultured as previously described (Akahoshi M, et al. J Clin Invest 2011;121:4180-4191). Briefly, bone marrow cells from C57BL/6 mice were cultured in IL-3-containing (WEHI-conditioned) medium for 4 weeks to generate cell populations that contained more than 95% mast cells. IgE (5 μg/mL)-sensitized mouse BMCMCs were stimulated with DNP-HSA (10 ng/mL) for 3 min with or without cromolyn pretreatment *in vitro*, and β-hexosaminidase in the supernatant was measured. N = 3 per group from one of the 2 independent experiments performed, each of which gave similar results. ** $p < 0.01$; n.s.: $p > 0.05$. Similar results also were obtained in 2 additional experiments in which β-hexosaminidase release was assessed at 1 h after antigen challenge (data not shown).

Supplementary Figure S8. Effect of cromolyn on LPS-induced TNF production in cells from mast cell- and basophil- deficient *Cpa3-Cre*; *Mcl-1*$^{fl/fl}$ mice versus littermate control mice *in vitro*. Spleen cells (**a** and **b**) or peritoneal cells (**c** and **d**) from male

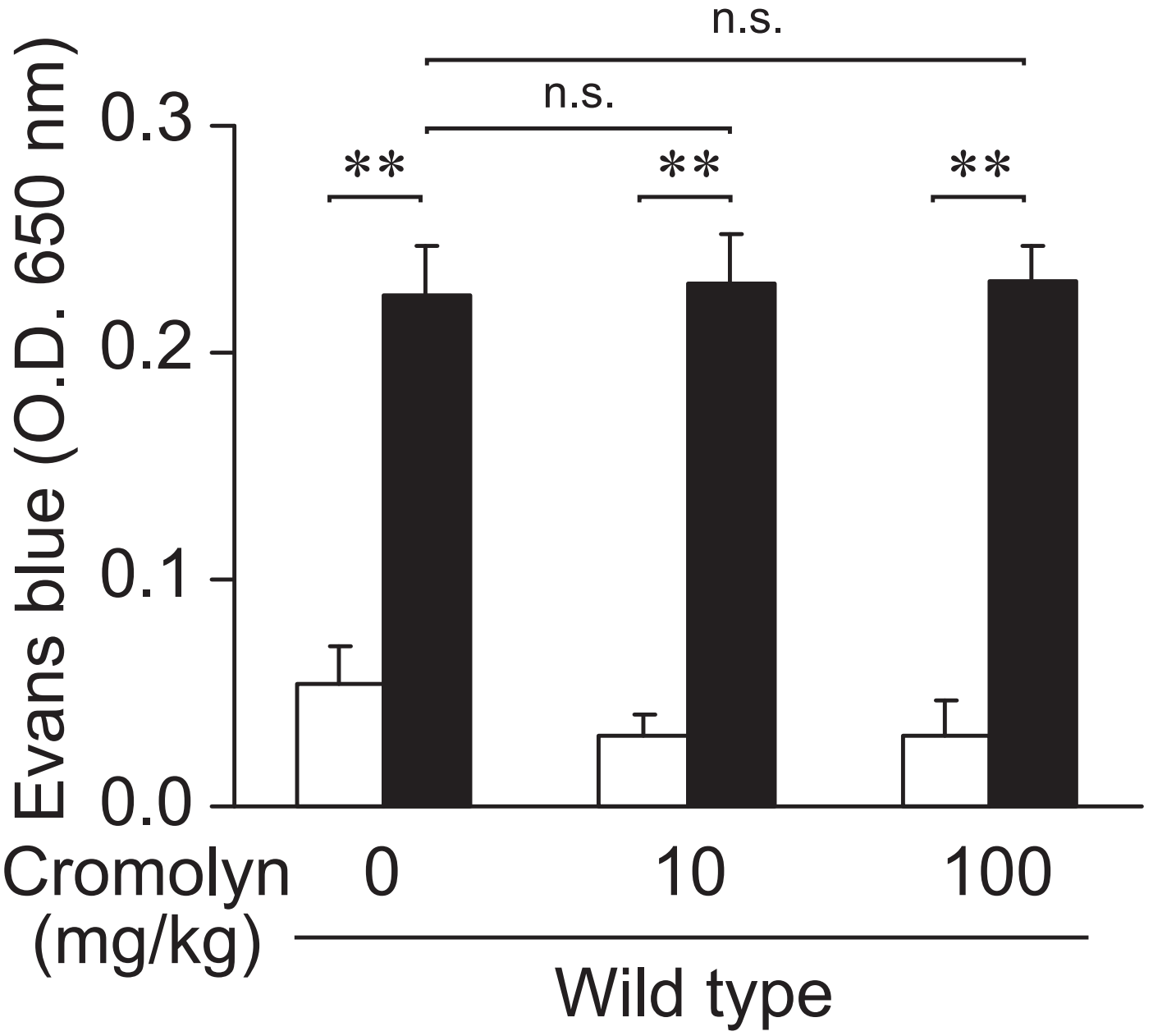
Cpa3-Cre; *Mcl-1*$^{+/+}$ (control littermate; **a** and **c**) or *Cpa3-Cre*; *Mcl-1*$^{fl/fl}$ (**b** and **d**) mice incubated for 15 min with or without cromolyn were stimulated with 1 ng/mL LPS for 3 h, and TNF concentrations in the supernatants were measured. The results are similar to those obtained with wild type versus mast cell-deficient *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice (Figure 5). N = 4 per group from 1 experiment. ** $p < 0.01$, * $p < 0.05$.

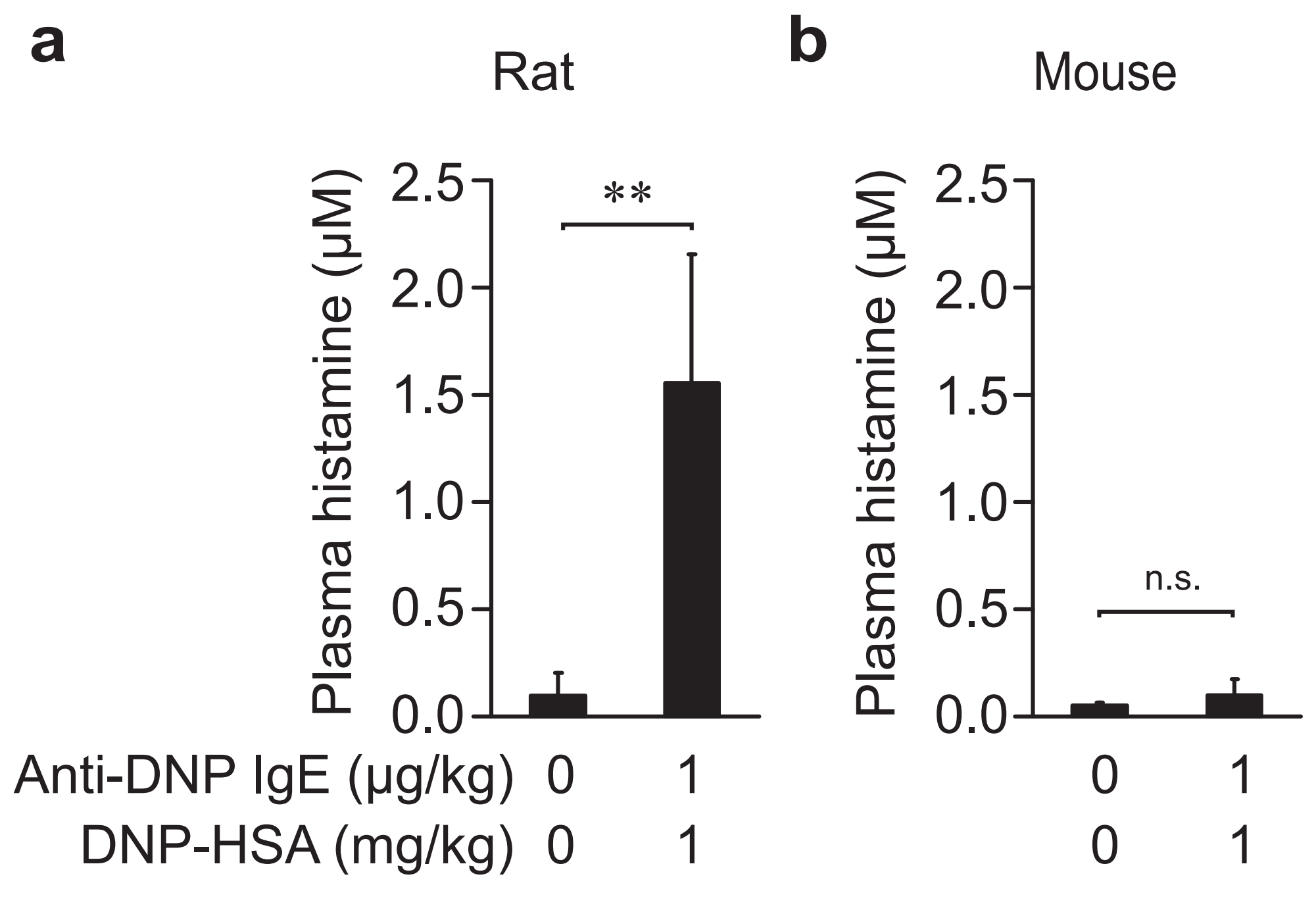

Rat
Mouse
Region
Dorsal skin
Dorsal skin
Ear pinnae
Ear pinnae
Anti-DNP IgE (ng/site)
0
2.5
0
2.5
0
2.5
0
10
Evans blue (O.D. 650 nm)
0.5
0.4
0.3
0.2
0.1
0.0
**
n.s.
**
**
Anti-DNP IgE (ng/site)
0
2.5
0
2.5
0
2.5
0
10
DNP-HSA (mg/kg)
1
1
1
10
Evans blue (mg/kg)
10
10
10
100

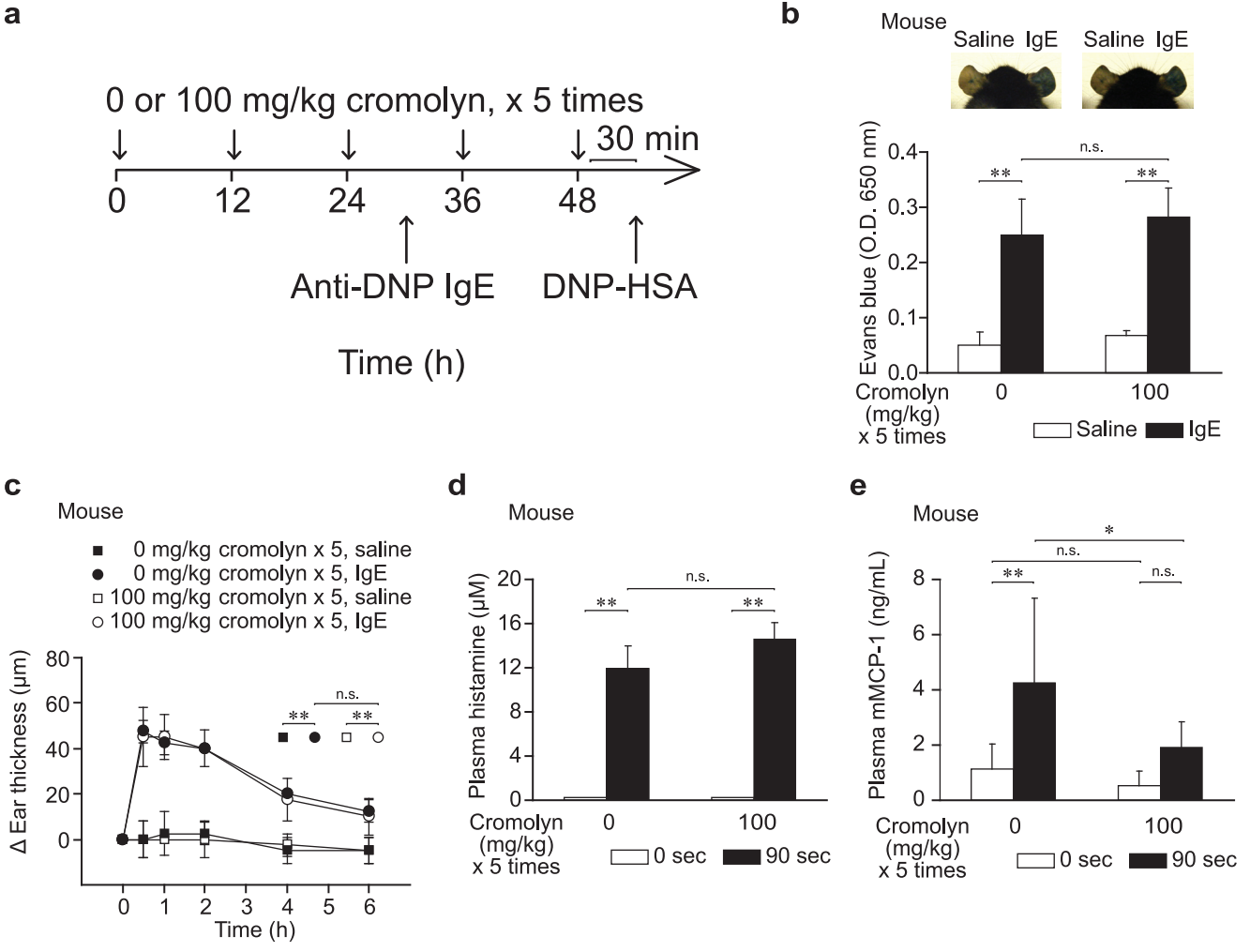
Female mice

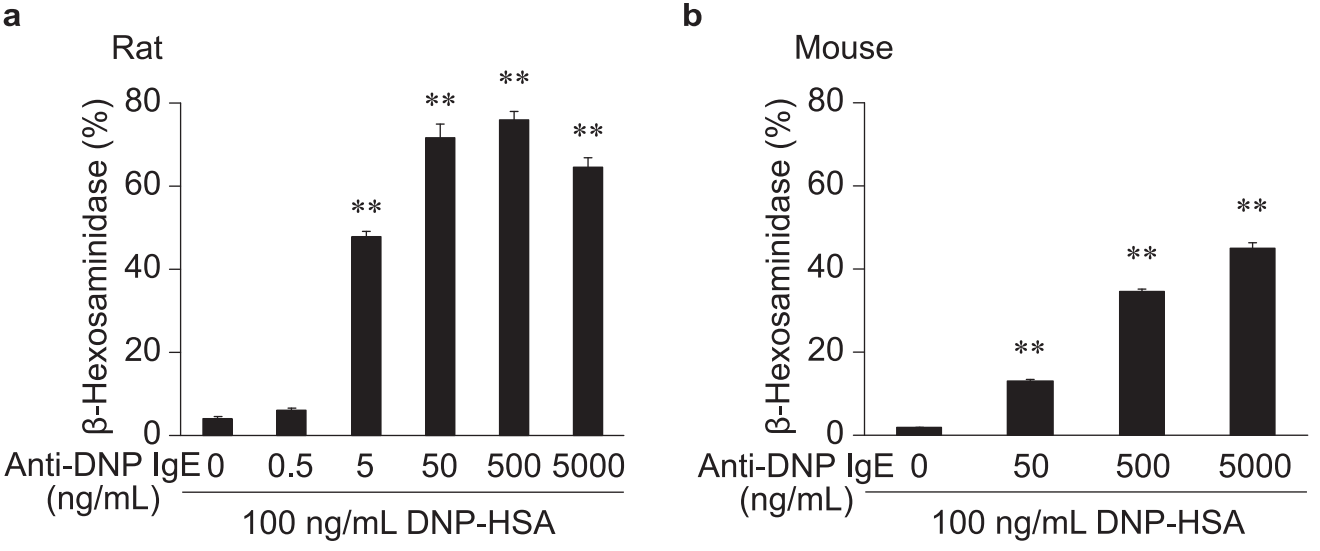


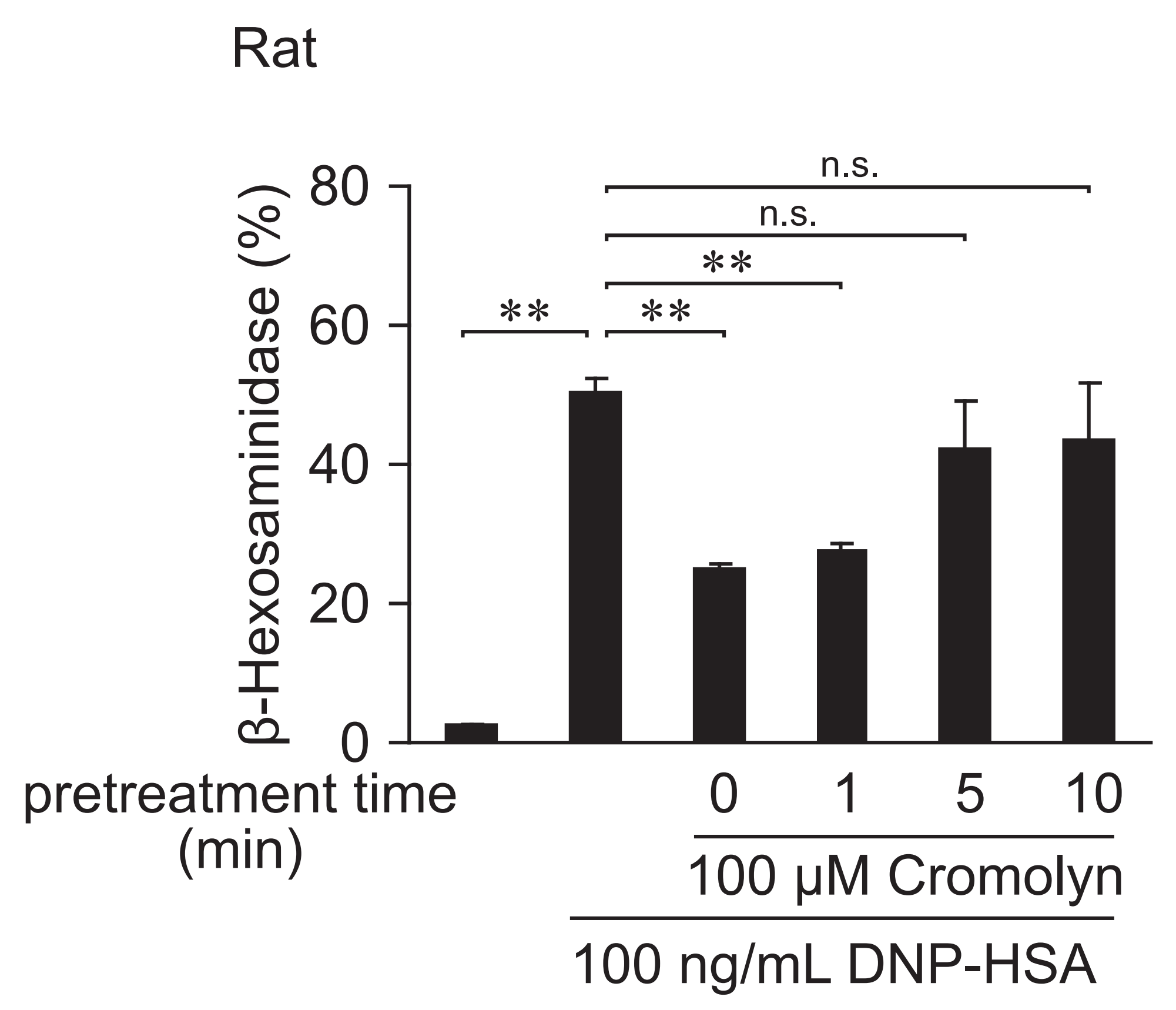
Cromolyn (mg/kg) 0 10 100

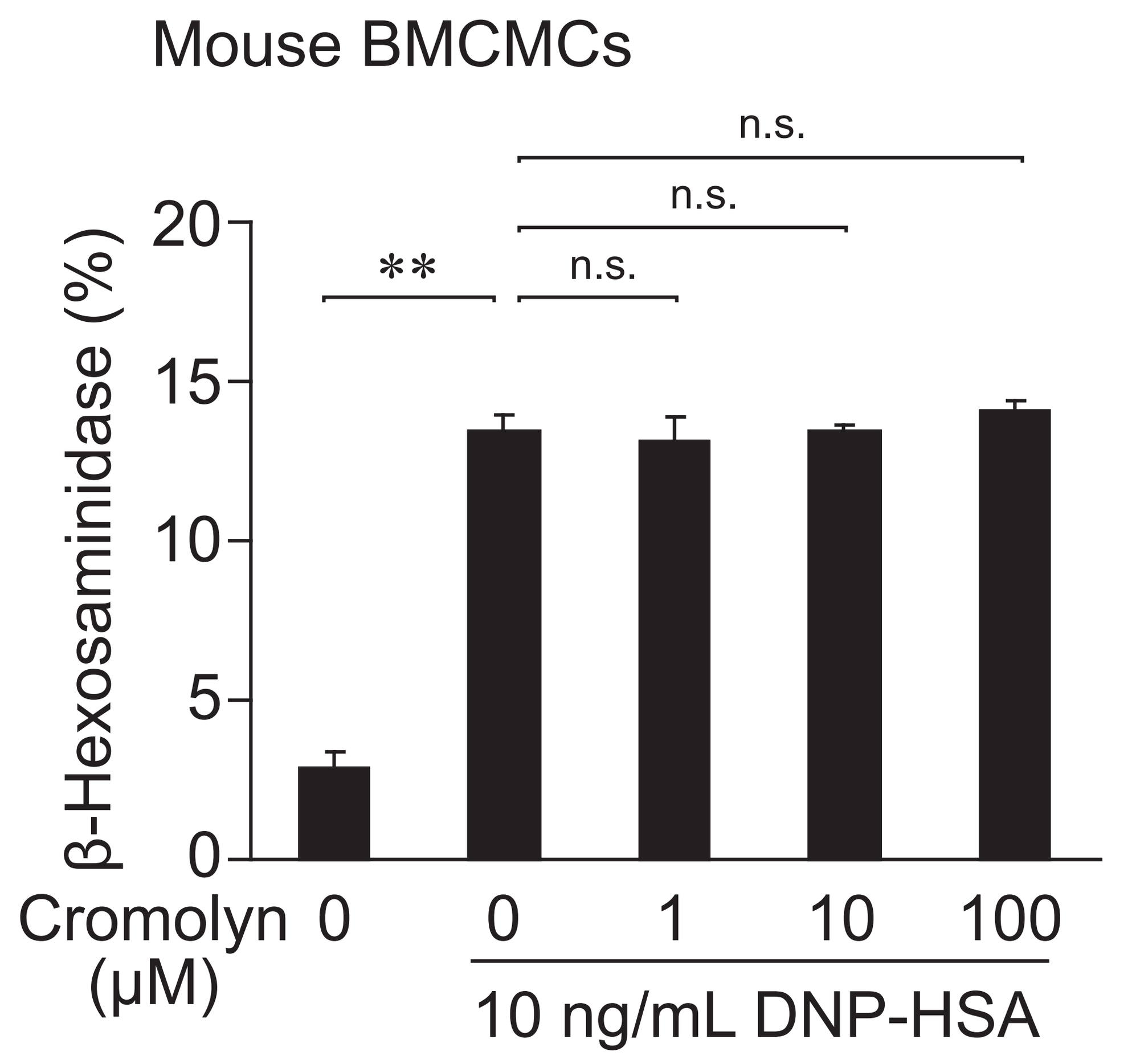


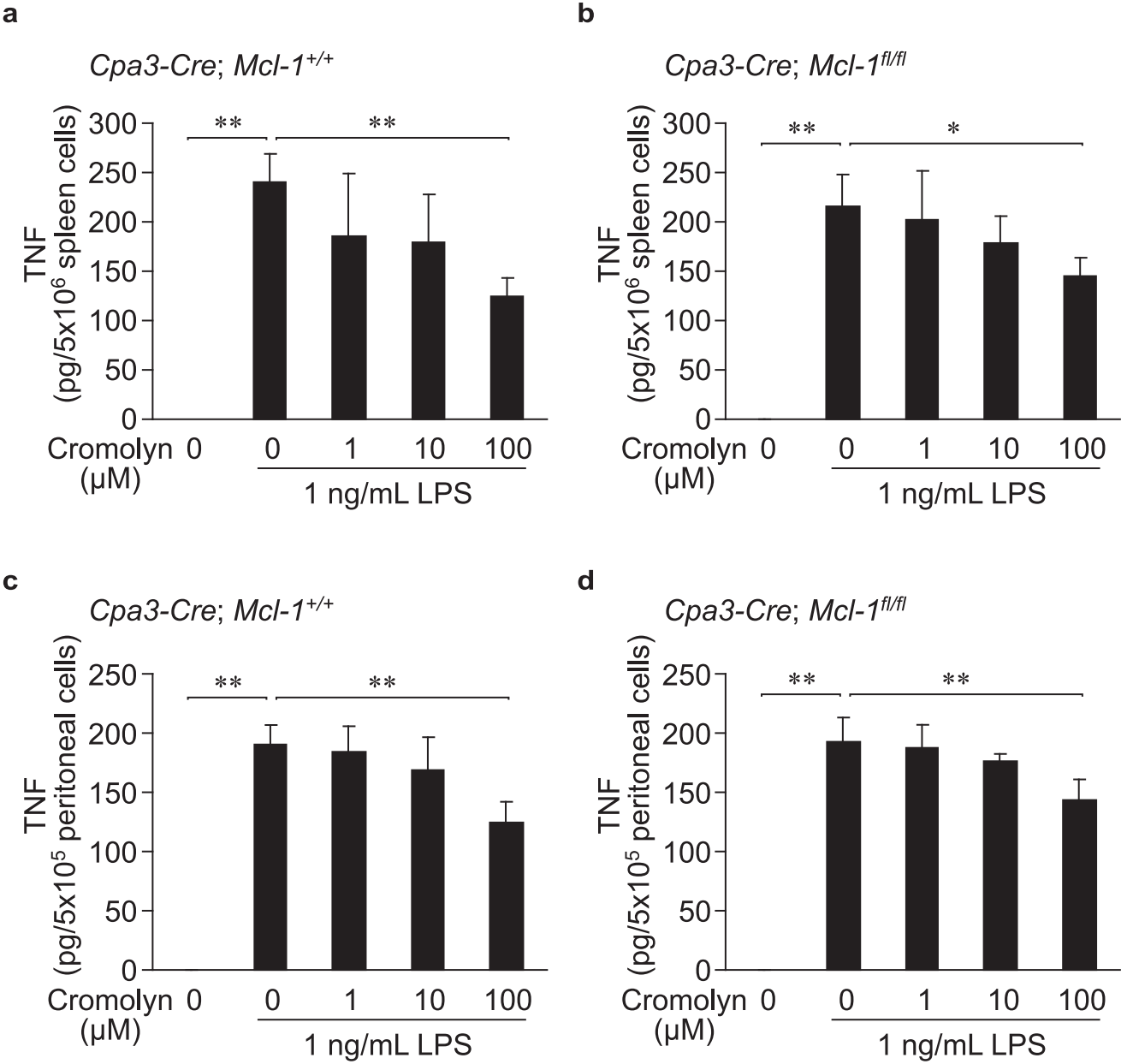

a
Rat
b
Mouse
Plasma histamine (μM)
2.5
2.0
1.5
1.0
0.5
0.0
**
n.s.
Anti-DNP IgE (μg/kg) 0 1 0 1
DNP-HSA (mg/kg) 0 1 0 1


a
0 or 100 mg/kg cromolyn, x 5 times
30 min
0
12
24
36
48
Anti-DNP IgE
DNP-HSA
Time (h)
b
Mouse
Saline IgE
Saline IgE
Evans blue (O.D. 650 nm)
0.4
0.3
0.2
0.1
0.0
n.s.
**
**
Cromolyn (mg/kg) x 5 times
0
100
Saline
IgE
c
Mouse
0 mg/kg cromolyn x 5, saline
0 mg/kg cromolyn x 5, IgE
100 mg/kg cromolyn x 5, saline
100 mg/kg cromolyn x 5, IgE
Δ Ear thickness (μm)
80
60
40
20
0
n.s.
**
**
0
1
2
3
4
5
6
Time (h)
d
Mouse
Plasma histamine (μM)
20
16
12
8
4
0
n.s.
**
**
Cromolyn (mg/kg) x 5 times
0
100
0 sec
90 sec
e
Mouse
Plasma mMCP-1 (ng/mL)
8
6
4
2
0
*
n.s.
**
n.s.
Cromolyn (mg/kg) x 5 times
0
100
0 sec
90 sec


a
Rat
β-Hexosaminidase (%)
0
20
40
60
80
**
**
**
**
Anti-DNP IgE (ng/mL)
0
0.5
5
50
500
5000
100 ng/mL DNP-HSA
b
Mouse
β-Hexosaminidase (%)
0
20
40
60
80
**
**
**
Anti-DNP IgE (ng/mL)
0
50
500
5000
100 ng/mL DNP-HSA


Rat
β-Hexosaminidase (%)
80
60
40
20
0
n.s.
n.s.
**
**
**
pretreatment time (min)
0
1
5
10
100 μM Cromolyn
100 ng/mL DNP-HSA


Mouse BMCMCs
n.s.
n.s.
**
n.s.
β-Hexosaminidase (%)
20
15
10
5
0
Cromolyn (μM)
0
0
1
10
100
10 ng/mL DNP-HSA


a
Cpa3-Cre; Mcl-1+/+
TNF (pg/5x10^6 spleen cells)
300
250
200
150
100
50
0
**
**
Cromolyn (μM)
0
0
1
10
100
1 ng/mL LPS
b
Cpa3-Cre; Mcl-1fl/fl
TNF (pg/5x10^6 spleen cells)
**
*
Cromolyn (μM)
0
0
1
10
100
1 ng/mL LPS
c
Cpa3-Cre; Mcl-1+/+
TNF (pg/5x10^5 peritoneal cells)
250
200
150
100
50
0
**
**
Cromolyn (μM)
0
0
1
10
100
1 ng/mL LPS
d
Cpa3-Cre; Mcl-1fl/fl
TNF (pg/5x10^5 peritoneal cells)
**
**
Cromolyn (μM)
0
0
1
10
100
1 ng/mL LPS