Supplementary Information

Eucalyptus oil reduces allergic reactions and suppresses mast cell degranulation by downregulating IgE-FcεRI signalling

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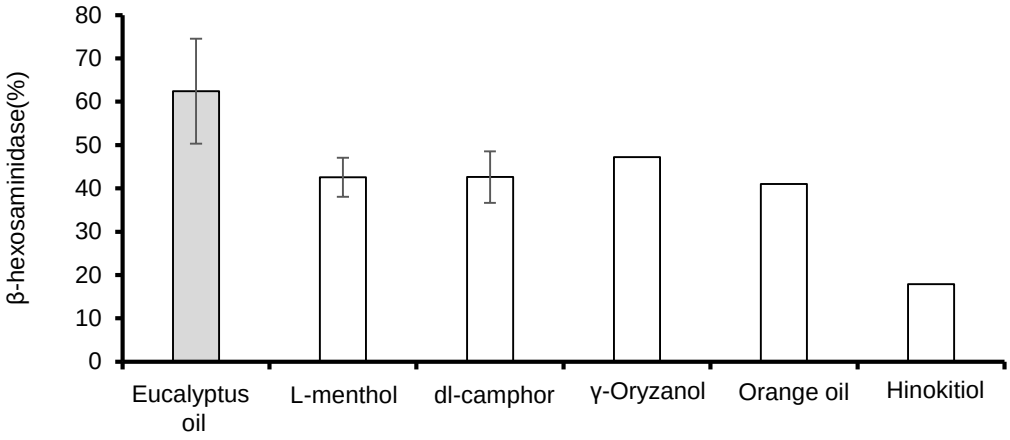
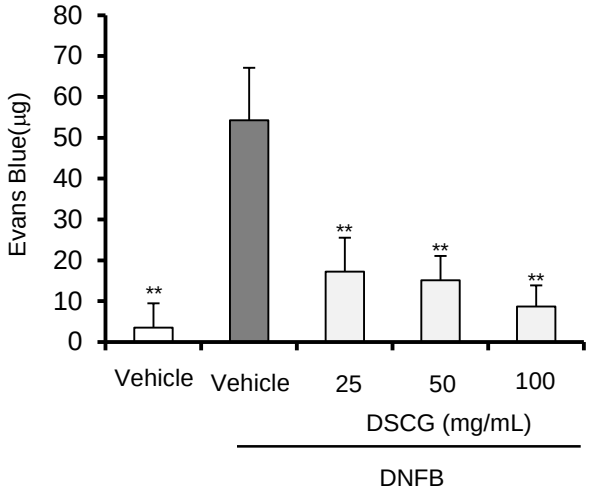
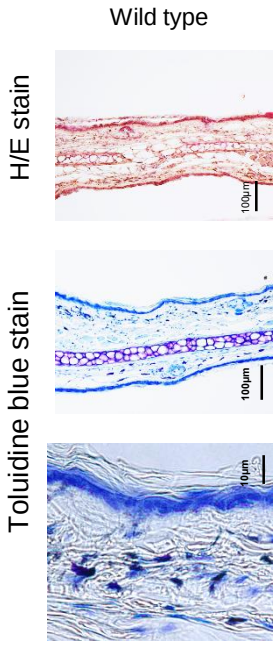


Supplementary Figure 1.
Screening of plant oil in degranulation inhibitory effect test of RBL-2H3 cells. β-HEX in RBL-2H3 cells. β-HEX valuesare expressed as percentage of total β-HEX present in the corresponding cell lysate.
Eucalyptus oil,L-menthol,dl-camphor: Data shown are means± SEM (N=3)
γ-Oryzanol, Orange oil, hinokitiol: Data shown are means (N=2)
Eucalyptus oil, L-menthol, dl-camphor and Orange oil were purchased from Nippon Terpene Chemicals.
Hinokitiol was purchased from Wako.
γ-Oryzanol was purchased from Tsuno.

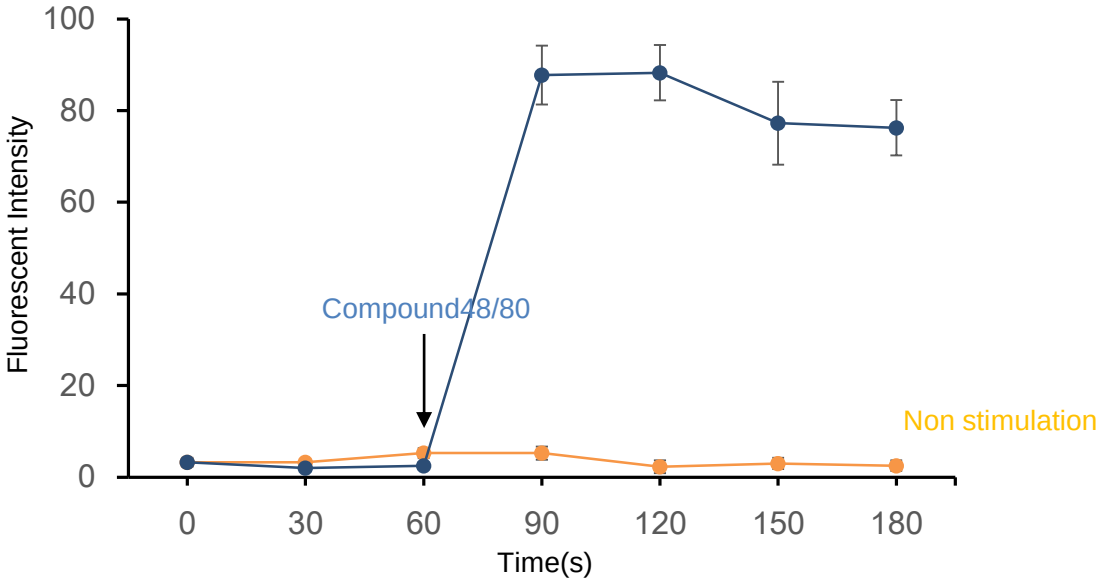


Supplementary Figure 2

Anti-DNP-IgE was injected intravenously 24 h before DNFB application. One hour before DNFB application, DSCG (sodium cromoglicate) or vehicle (PBS) was applied to the inside of each ear of all animals. DNFB was applied to the outside of the ear, followed by an immediate injection of Evans blue dye. Thirty minutes later, the ears were excised, and the absorbance of the dye was measured. Data are presented as the mean ± SEM (N = 6/group). The statistical significance of the differences was assessed using Dunnett's test (**P < 0.01 versus vehicle-DNFB).

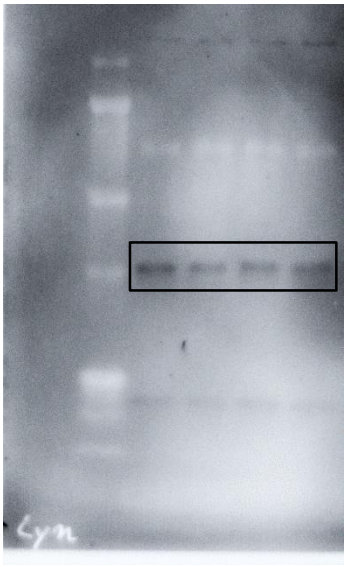


Supplementary Figure 3.
Haematoxylin and eosin-stained and toluidine blue-stained images ofear tissue in WBB6F1+/+ (wild-type) mouse.



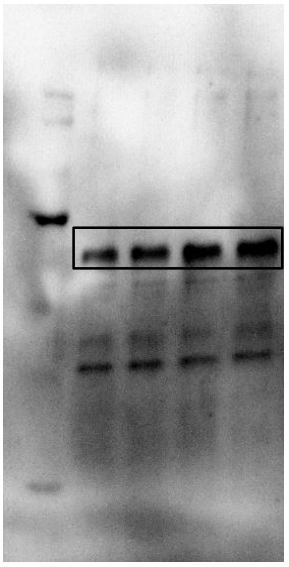
Supplementary Figure 4
Time course of intracellular Ca^{2+} concentration in BMMC. BMMCs were incubated in medium containing Fluo 4-AM. BMMCs were stimulated with Comp48/80 60s after the start of monitoring the fluorescence intensity. Data are displayed as mean ± SEM (N = 5).
Concentration of Compound 48/80 was as reported by Rothschild,A.M.(1970)
Rothschild,A.M. Mechanisms of histamine release by compound 48/80. Br J Pharmacol. 38.253-62 (1970)

Lyn:56kDa



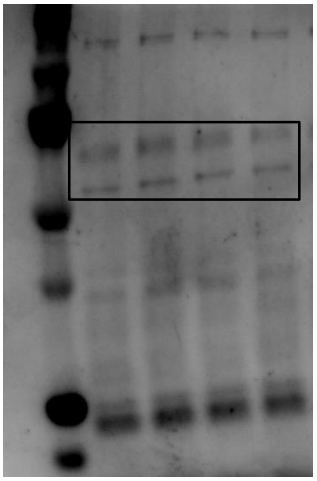
DNP-HSA(ng/ml)	0	0	50	50
1,8-Cineol (μg/ml)	0	0.5	0	0.5

p-Lyn:56kDa



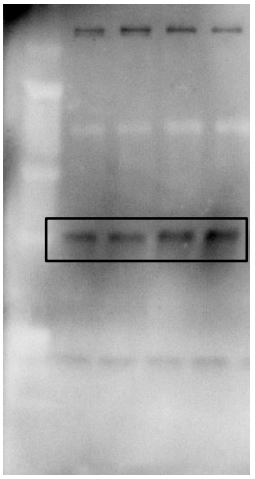
DNP-HSA(ng/ml)	0	0	50	50
1,8-Cineol (μg/ml)	0	0.5	0	0.5

Syk:72kDa



DNP-HSA(ng/ml)	0	0	50	50
1,8-Cineol (μg/ml)	0	0.5	0	0.5

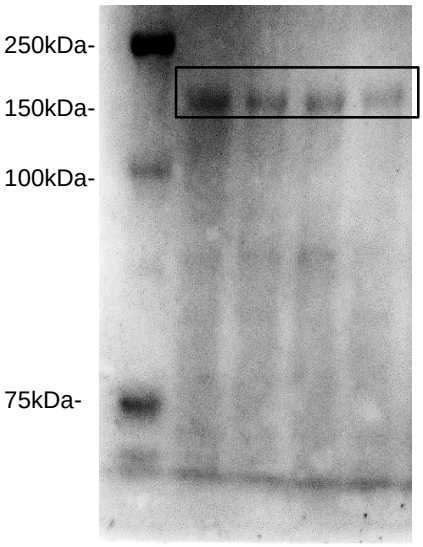
p-Syk:72kDa



DNP-HSA(ng/ml)	0	0	50	50
1,8-Cineol (μg/ml)	0	0.5	0	0.5

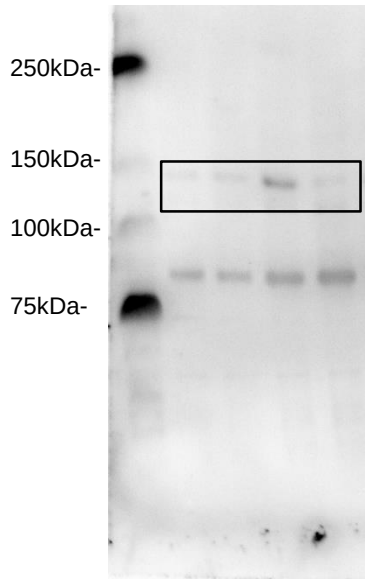
Supplementary figure5. Uncropped immunoblot used in Figure 6. (Effect of 1,8-cineol on the phosphorylation of Syk and Lyn.)

PLCγ :150kDa



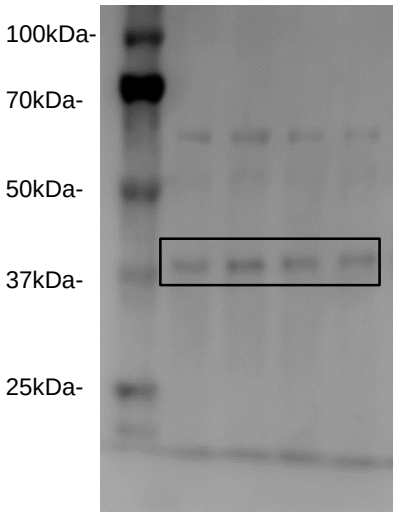
DNP-HSA(ng/ml)	0	0	50	50
1,8-Cineol (μg/ml)	0	0.5	0	0.5

p-PLCγ:150kDa



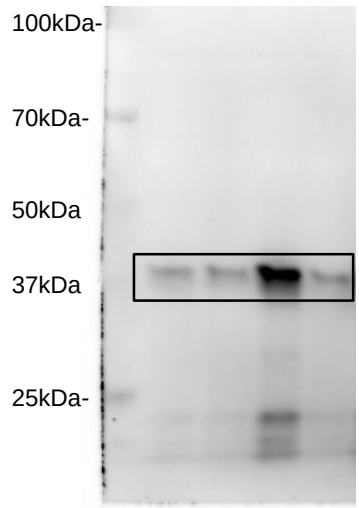
DNP-HSA(ng/ml)	0	0	50	50
1,8-Cineol (μg/ml)	0	0.5	0	0.5

p38:40kDa



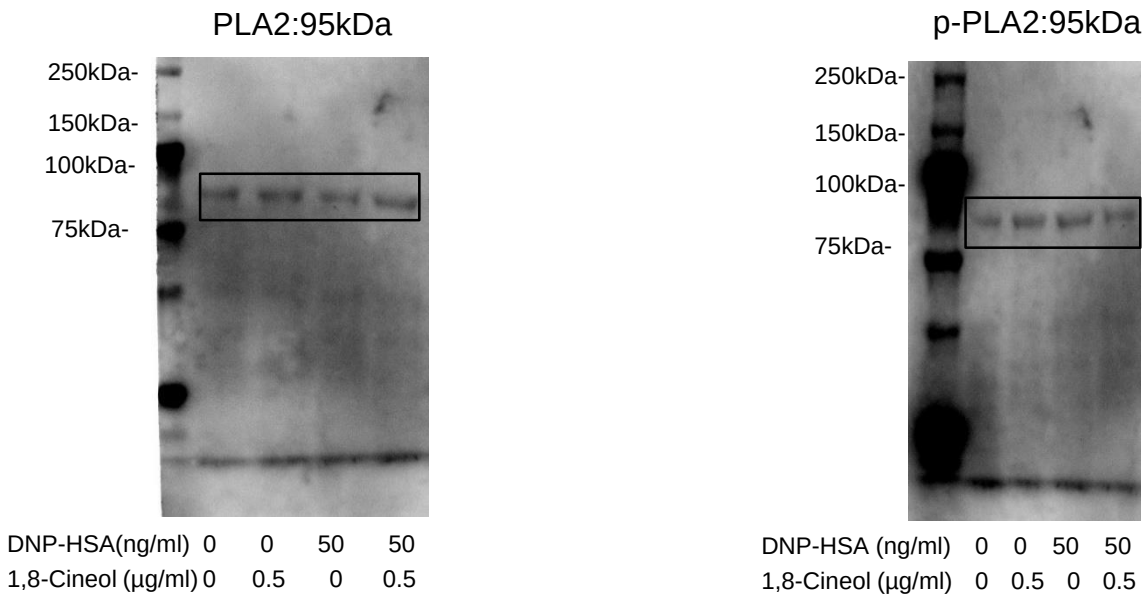
DNP-HSA(ng/ml)	0	0	50	50
1,8-Cineol (μg/ml)	0	0.5	0	0.5

p-p38:43kDa



DNP-HSA(ng/ml)	0	0	50	50
1,8-Cineol (μg/ml)	0	0.5	0	0.5

Supplementary figure6. Uncropped immunoblot used in Fig.7 a,b (Effect of 1,8-cineol on the phosphorylation of PLCγ, and p38)



Supplementary figure7. Uncropped immunoblot used in Fig.7 c (Effect of 1,8-cineol on the phosphorylation of PLA2)