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SUPPLEMENTARY ONLINE DATA

Polyphenols differentially inhibit degranulation of distinct subsets of vesicles in mast cells by specific interaction with granule-type-dependent SNARE complexes

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a

Subclass (Color code)	Compounds (2 letter code)	Subclass (Color code)	Compounds (2 letter code)
Flavanol	(+)-Catechin (CT) (-)-Epicatechin (EP) EGCG (EG)	Flavonol	Kaempferol (KP) Quercetin (QT) Quercetrin (QR) Rutin (RT) Fisetin (FS) Myricetin (MR)
Flavanone	Naringin (NA) Hesperetin (HT) Hesperidin (HD) Taxifolin (TX)	Flavone	Apigenin (AP) Luteolin (LT) Chrysin (CR)
Isoflavone	Genistein (GS) Daidzein (DA)	Tannin	Ellagic acid (EL)
Chalcone	Butein (BT)	Dihydro-chalcone	Phloridzin (PH)
Antho-cyanidins	Cyanidin (CY) Pelargonidin (PG) Delphinidin (DL)	Lignan	Gomisin A (GA) Gomisin N (GN) Schizandrin (SC)
Benzoic acid derivatives	Ferulic acid (FA) Caffeic acid (CA) p-Coumaric acid (CU) Chlorogenic acid (CL) Trans-cinnamic acid (TC)	PI3 kinase inhibitor	Ly294002 (LY)

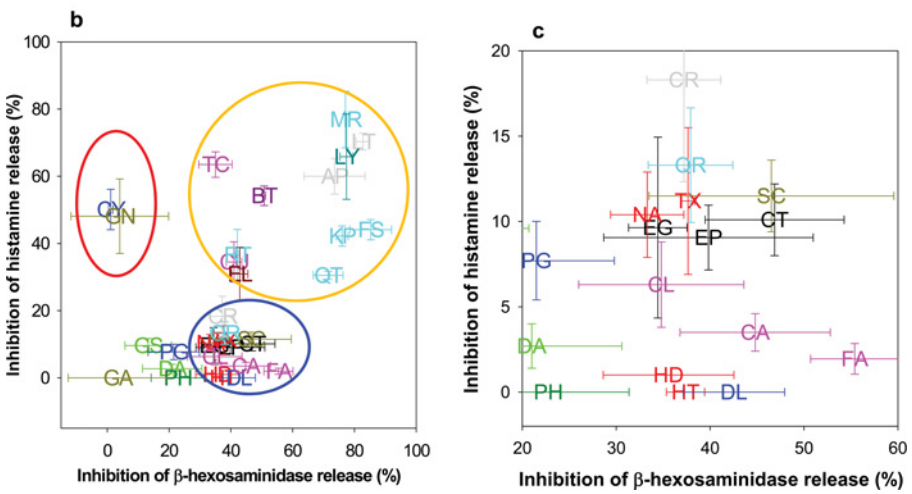

Figure S1 Effect of polyphenols on DNP-BSA-induced mast cell degranulation

(**a**) All 32 phenolic compounds used representing 11 subgroups and their abbreviations. (**b** and **c**) Inhibitory effect of polyphenols on β-hexosaminidase and histamine release from the RBL-2H3 cells. RBL-2H3 cells were treated with or without 10 μM polyphenols. Results are means $\pm$ S.D. from five independent experiments. Dense area of (**b**) is shown enlarged in (**c**) for clarity.

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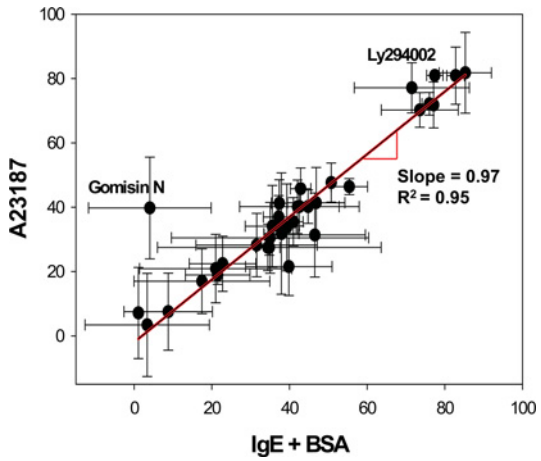

Figure S2 Effect of polyphenols on mast cell degranulation is independent of stimulation method

β-Hexosaminidase release from RBL-2H3 cells was stimulated by either FcεRI cross-linking or calcium ionophore A23187 in the presence or absence of polyphenols.

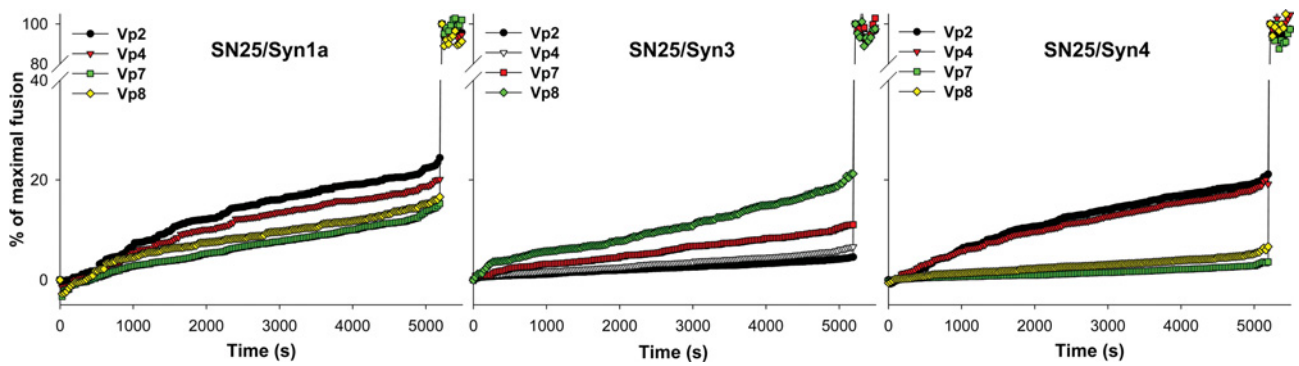

Figure S3 The lipid mixing ability of various SN25-containing SNARE complexes

SNAP-25 or Syn1a could induce lipid mixing with various VAMPs, but these proteins are not expressed in mast cells. SN25, 25 kDa synaptosome-associated protein; Vp, vesicle-associated membrane protein.

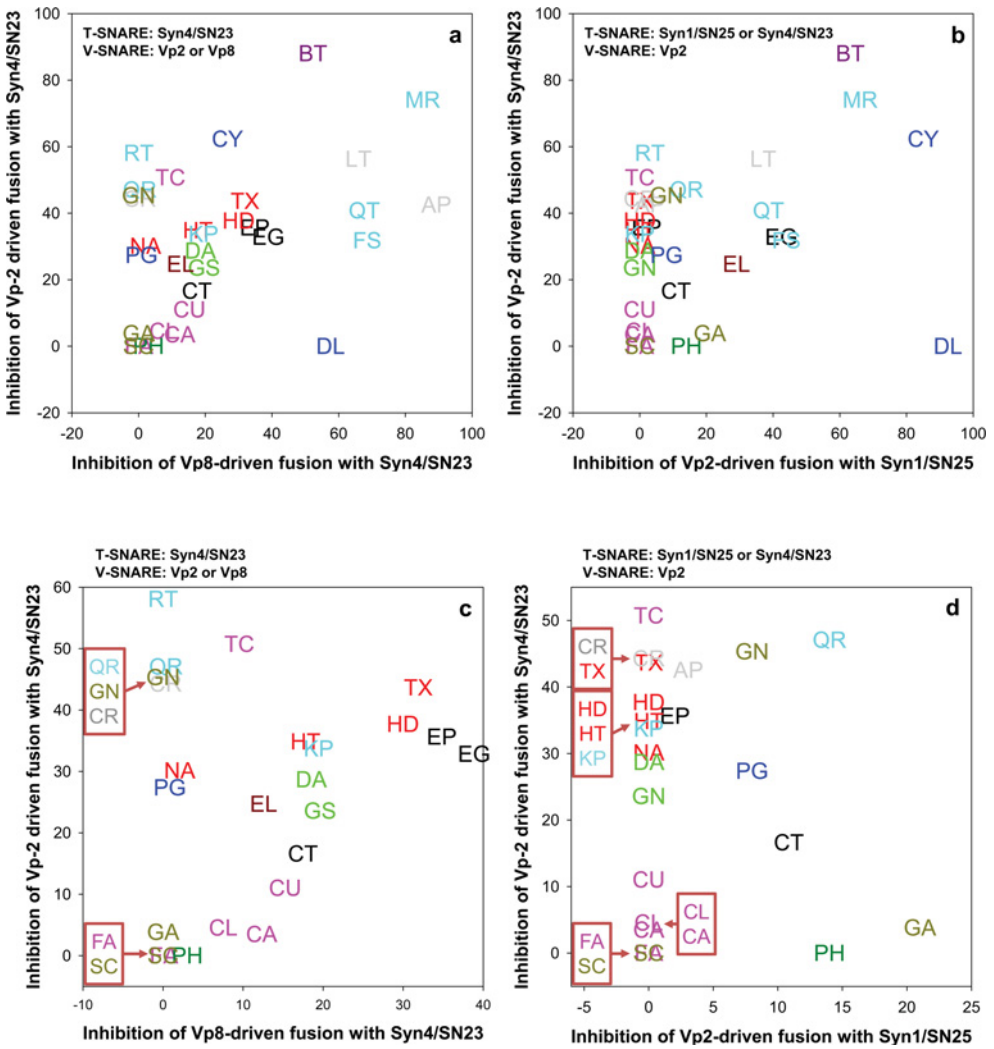

Figure S4 Inhibitory effect of 32 polyphenols on membrane fusion driven by three SNARE complexes

(**a**) SNAP-23/Syn4/VAMP2 compared with SNAP-23/Syn4/VAMP8. (**b**) SNAP-23/Syn4/VAMP2 compared with SNAP-25/Syn1/VAMP2. Dense areas of (**a** and **b**) are shown enlarged in (**c**) and (**d**) respectively for better resolution. SN23/25, 23/25 kDa synaptosome-associated protein; Vp, vesicle-associated membrane protein.

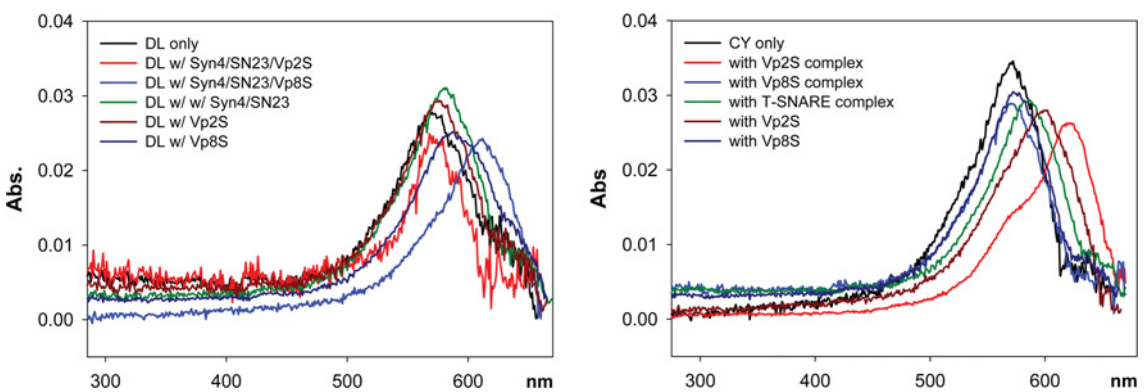

Figure S5 UV absorption spectra of polyphenols DL and CY with SNARE proteins

Soluble fragments of SNARE proteins (Vp2S and Vp8S) and t-SNARE complex were mixed with polyphenols and UV spectra were measured. Abs., absorbance; CY, cyanidin; DL, delphinidin; SN23/25, 23/25 kDa synaptosome-associated protein; Vp, vesicle-associated membrane protein.

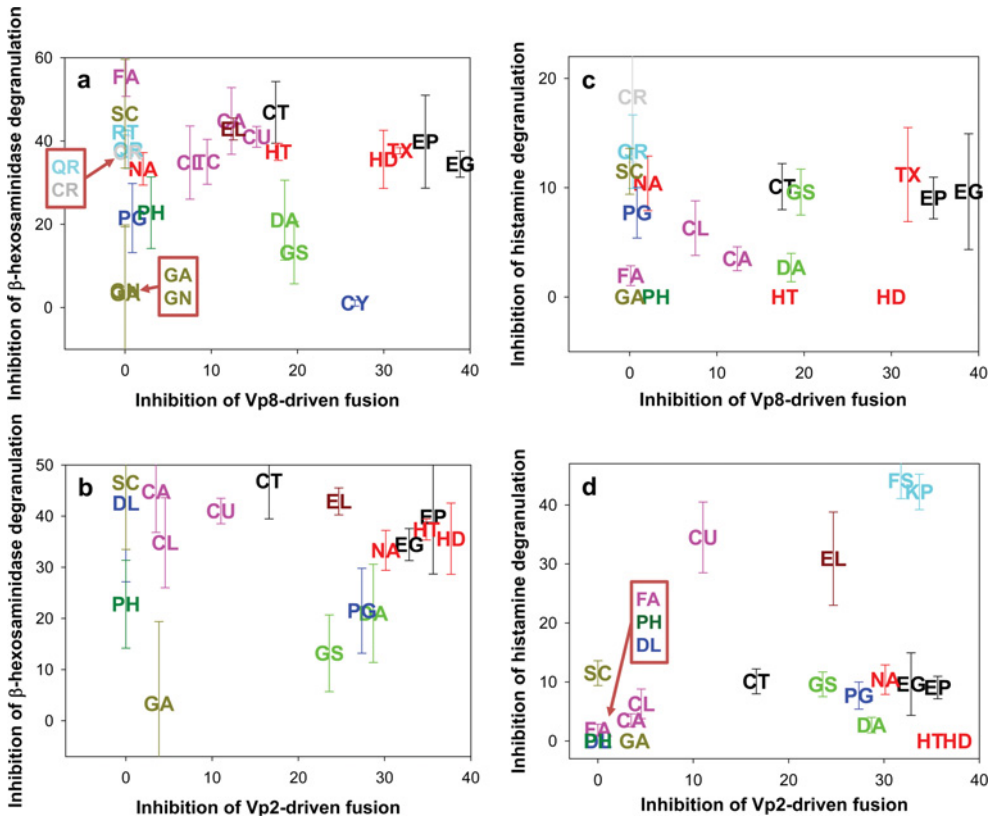

Figure S6 Dense areas of Figure 4 of the main text are shown enlarged

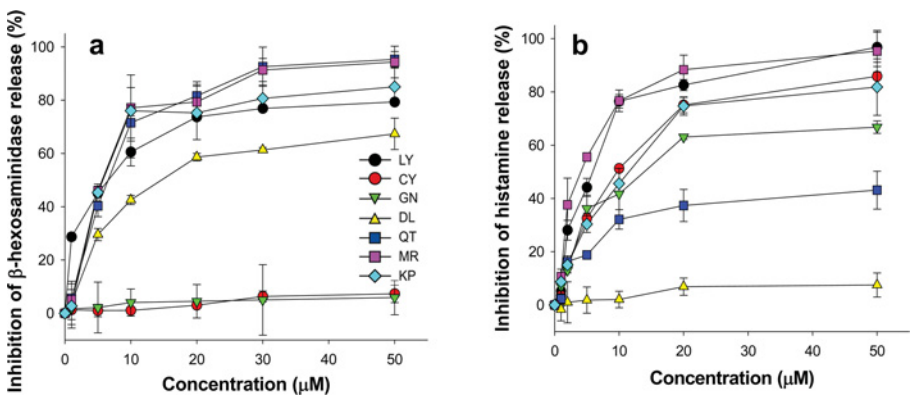

Figure S7 Determination of IC_{50} values of polyphenols for histamine and β-hexosaminidase release from RBL-2H3 cells

Table S1 Primers and condition for PCR analysis of RBL-2H3 cells

F, forward; R, reverse.

Type	Gene	Primer type	Primer sequence (5′→3′)	Annealing temperature (°C)	Number of cycles	Amplicon size (bp)
v-SNAREs	VAMP2	F	ATGTCGGCTACCGCTGCCAC	58	28	348
		R	ATCGTTTACTTCAGCACT			
	VAMP8	F	ATGGAGGCCAGTGGGAGTGC	58	28	300
		R	GGCACCATCCCCACT			
	VAMP8S	F	ATGGAGGCCAGTGGGAGTGC	58	28	222
		R	GTTCTGGTGGAAGAATGTGAAG			
	VAMP4	F	ATGCCTCCCAAGTTCAAGCG	58	30	423
		R	TAGTTGTGAAATACCGTACT			
	VAMP7	F	ATGGCCATTCTTTTTGCCGTTG	58	28	660
		R	GGCCAAGCTGTGTGAAGAAA			
t-SNAREs	SNAP-23	F	ATGGATGATCTATCACCAGA	52	30	630
		R	AGAGCAAAGAAACTCATTGACAGC			
	SNAP-25	F	ATGGCCGAGGACGCAGACAT	52	40	618
		R	AAGATGCTGGGAAGTGGT			
	Syntaxin 1a	F	ATGAAGGACCGAACCCAGGA	56	40	864
		R	ACCATCGGGGGCATCTTTGGA			
	Syntaxin 4	F	ATGCGCGACAGGACCCATGA	56	40	894
		R	CATCACCATAACCGTTGGA			
	Syntaxin 5	F	ATGTCCTGCCGGGATCGGAC	56	40	903
		R	ATCTTTGTGGTCTTCCTTGCC			
	β-Actin	F	ATGGGTCAGAAGGACTCCTACG	58	18	470
		R	CATTGTGATGGACTCCGGAGA			

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