Figure E1

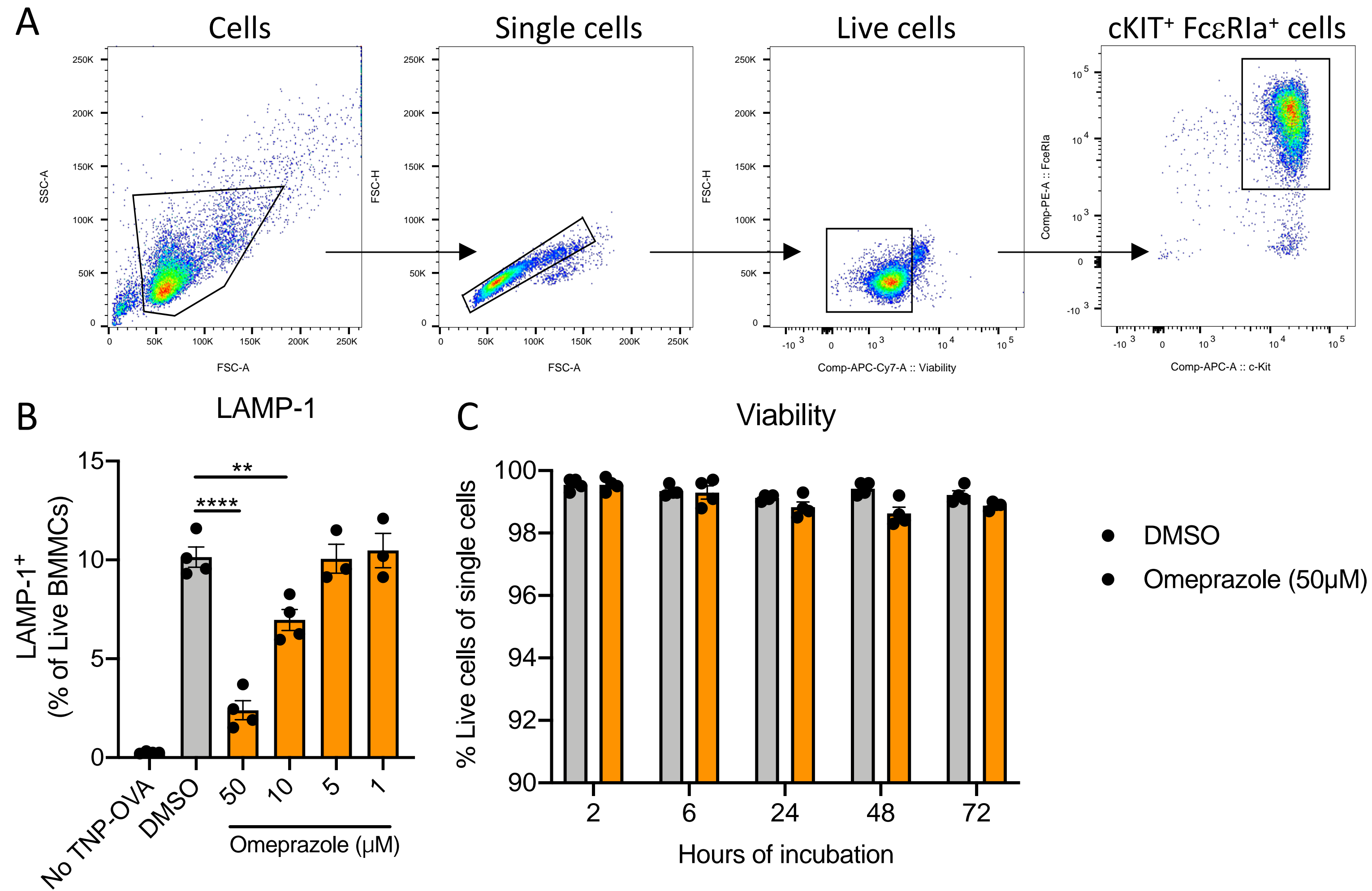


Figure E2

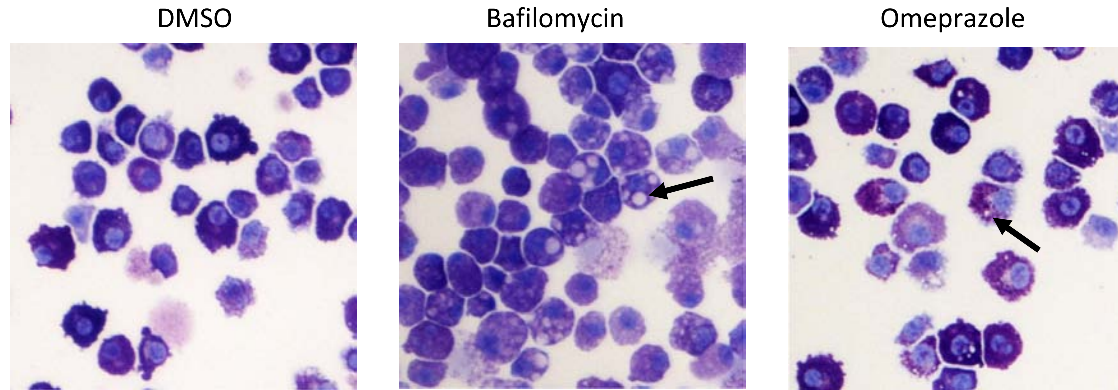
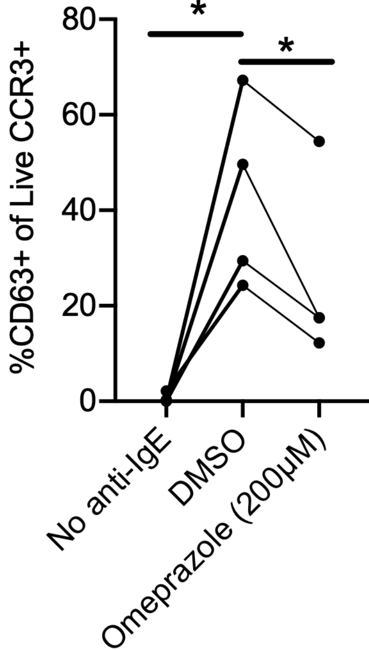


Figure E3



1 **ONLINE REPOSITORY**

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3 Omeprazole inhibits IgE-mediated mast cell activation and allergic inflammation induced by

4 ingested allergen in mice

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25 **Methods**
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27 **Murine mast cell LAMP-1 assay.** 10^5 bone marrow-derived mast cells (BMMCs) in 100µl were
28 incubated with anti-TNP IgE (500ng/ml). Unbound IgE was washed away and cells were treated
29 with omeprazole at 50 µM for two hours. Following treatment, cells were stimulated with TNP-
30 OVA (100ng/ml) for 10 minutes at 37ºC while also being stained for cell surface marker c-Kit
31 (APC), degranulation marker LAMP-1 (PE), and viability (Viability-APCCy7). The reaction was
32 stopped with cold FACS buffer. Cells were pelleted and resuspended in FACS buffer prior to
33 acquisition on an LSR Fortessa (BD Biosciences, Franklin Lakes, NJ). Mast cells were identified
34 as live cells that are $cKit^+$. The expression of LAMP-1 on the cell surface was used as a
35 measure of mast cell degranulation. For omeprazole treatment, cells were incubated with the
36 drug (50µM) for two hours, then stimulated with TNP-OVA.
37 For adenosine-mediated degranulation, cells were not incubated with IgE prior to treatment.
38 Cells were stimulated with 100µM adenosine (Sigma) in the presence of staining antibodies.
39 **Human mast cell LAMP-1 assay.** 10^5 cord blood mast cells (CBMCs) in 100µl were incubated
40 with human myeloma IgE (100ng/ml; Chemicon International, Tecaluma, CA). Unbound IgE was
41 washed away and cells were treated with omeprazole at various doses (50, 100, and 200µM) for
42 two hours. Following treatment, cells were stimulated with rabbit anti-human IgE (100ng/ml) for
43 10 minutes in the presence of viability dye (Viability-APCCy7), anti-LAMP-1 (PE), and anti-cKit
44 (APC). Cells were pelleted and resuspended in FACS buffer prior to acquisition on an LSR
45 Fortessa (BD). Mast cells were identified as live cells that are $cKit^+$. The expression of LAMP-1
46 on the cell surface was used as a measure of mast cell degranulation. For omeprazole
47 treatment, cells were incubated with the drug (50µM) for two hours, then stimulated with anti-
48 IgE.
49 **Histamine ELISA.** Histamine concentration in plasma or cell culture supernatants was
50 measured using the histamine ELISA kit from SPI bio (Cayman Chemical, Ann Arbor, Michigan)

51 following the manufacturer's instructions. For measurement of histamine concentration in
52 plasma, blood was collected 5 minutes after antigen challenge from mice. For measurement of
53 histamine concentration in cell culture, supernatants were collected 5 minutes after stimulation.
54 **Cytokine release from BMMCs.** BMMCs were sensitized with 500ng/mL anti-TNP IgE and
55 treated with omeprazole (50μM) for two hours. Following treatment, cells were stimulated with
56 100ng/mL of TNP-OVA for six hours. Supernatants were collected and immediately assayed for
57 to measure cytokine concentration.
58 **Human mast cell cytokine secretion.** CBMCs were sensitized with human myeloma IgE
59 (100ng/mL). Unbound IgE was washed away and cells were treated with omeprazole at various
60 doses (50, 100, and 200μM) for two hours. Following treatment, cells were stimulated with rabbit
61 anti-human IgE (100ng/ml) for 6 hours then supernatants were collected. Human mast cell IL-5
62 and IL-13 secretion was measured using a Cytometric Bead Array from BD Biosciences (San
63 Jose, California), according to the manufacturer's instructions.
64 **Transcriptional profiling of BMMCs by NanoString.** BMMCs (10^6 cells in 1mL) were
65 sensitized overnight with 500ng/ml anti-TNP IgE. Unbound IgE was removed and cells were
66 treated with 50μM Omeprazole for 2 hours. Cells were then stimulated with 100ng/ml TNP-OVA
67 for 2h. Stimulation was stopped by washing with ice cold PBS, prior to lysis and homogenization
68 in RTL buffer (Qiagen) with β-mercaptoethanol (Sigma). RNA was isolated using the Qiagen
69 RNeasy mini kit according to the manufacturer's instructions. Purified RNA (100 ng) was
70 hybridized with customized nCounter gene expression code sets as previously established for
71 unbiased analysis of Th2 inflammation[1]. Direct mRNA counts were determined by the
72 nCounter® Digital Analyzer System (NanoString Technologies, Seattle, WA;
73 www.nanostring.com) according to the manufacturer's protocol. Direct mRNA counts were
74 normalized to internal positive and negative controls and 5 house-keeping genes according to
75 manufacturer's recommendations. Data is presented as standard deviation from the row mean.

76 **PGD2 assay.** PGD2 concentration in cell culture supernatants was measured using the
77 commercially available ELISA kit from Cayman Chemical following the manufacturer's
78 instructions (Cayman). Samples to be assayed were prepared in the same manner as for
79 human mast cell cytokine secretion. **Phosphoprotein detection.** For each time point, 10^6
80 BMMCs were sensitized overnight with anti-TNP IgE (500ng/ml). Following sensitization, cells
81 treated with control or omeprazole for two hours then stimulated with 100ng/ml of TNP-OVA.
82 Stimulation was stopped with cold FACS buffer and cells were pelleted and lysed with
83 radioimmunoprecipitation assay (RIPA) buffer (Sigma) containing a cocktail of protease and
84 phosphatase inhibitors (Roche). Lysed cells were spun to collect protein supernatant. 10µl of
85 lysate was resolved on a 12% MiniProtean TGX precast gel (Biorad) and electrophoretically
86 transferred to a PVDF membrane (Millipore). Membranes were blocked with 5% BSA in TBST
87 for at least one hour. Membranes were probed with primary antibody overnight, washed with
88 TBST then probed with appropriate peroxidase conjugated secondary antibodies for one hour.
89 Membranes were developed with Supersignal chemiluminescent substrate reagent and imaged
90 on an iBright Western Blot scanner.
91 **Calcium assay.** BMMCs sensitized with anti-TNP IgE were treated with omeprazole (50µM) or
92 control. Intracellular calcium was stained using the Fluo 4NW calcium assay kit following the
93 manufacturer's instructions. Change in the fluorescence of the calcium indicator was measured
94 using a Tecan Spark plate reader. Baseline fluorescence was measured for 30 seconds and
95 cells were then stimulated with 100ng/mL of TNP-OVA and changes in fluorescence were
96 measured for five minutes.
97 **pH Assays.** PPI-treated BMMCs (10^6 cells/mL) were incubated with FITC-Dextran overnight
98 (100µg/mL). Cells were washed and stained for cell surface markers c-Kit and FcεRIα, and for
99 viability. Cells were acquired by flow cytometry, and the intensity of the FITC signal of live c-Kit
100 and FcεRIα double-positive cells was assessed. Lysosensor Blue DND 167 (ThermoFisher) was

101 used to measure the changes in pH of acidic organelles following PPI treatment. Briefly, PPI-
102 treated BMMCs were washed and incubated with 1μM Lysosensor Blue for one hour. Cells were
103 washed and resuspended in media and the fluorescence intensity of the Lysosensor Blue was
104 measured using a Tecan Spark plate reader. To measure changes in intracellular pH following
105 treatment of BMMCs with PPI, pHRodoGreen AM intracellular pH indicator was used following
106 the manufacturer's instructions. Briefly, PPI-treated cells were washed with live cell imaging
107 solution (ThermoFisher) then incubated with a mixture of pHRodoGreen and PowerLoad for 30
108 minutes. Cells were washed and resuspended in FACS buffer then stained for viability and cell
109 surface markers (c-Kit and FcεRIα). BMMCs were acquired on an LSR Fortessa and the
110 intensity of the intracellular pH indicator was measured in the population of live c-Kit and FcεRIα
111 double-positive cells.
112 **Enzyme linked immunosorbent assay (ELISA).** MCPT-1, OVA-IgE, and total IgE were
113 measured in serum isolated from mouse blood collected by intracardiac puncture. MCPT-1 was
114 measured using the MCPT-1 ELISA (ThermoFisher) following the manufacturer's instructions.
115 Total IgE was measured using a direct sandwich ELISA using mouse IgE capture antibody
116 (Southern Biotech, #11101-01) and HRP-conjugated anti-mouse IgE (Southern Biotech, #1110-
117 05). OVA-IgE was measured using a direct sandwich ELISA using mouse IgE capture antibody
118 (Southern Biotech, #11101-01), and detected using biotinylated-OVA (US Biologicals, #O8075-
119 01) and Streptavidin-HRP. All ELISAs were developed with TMB and reactions were stopped
120 with 2N H_2SO_4. Cytokines in cell culture supernatants were measured using IL-6, IL-13, and
121 TNF-a Ready SET-Go! kits (ThermoFisher) following the manufacturer's instructions. IL-4 in cell
122 culture supernatants was measured using the ELISA MAX Standard Set Mouse IL-4 kit
123 following the manufacturer's instructions (Biolegend).
124 **RT-qPCR.** Jejunum sections were collected from mice and stored in RNA*later* (ThermoFisher)
125 solution at 4°C overnight before freezing tissue at -80°C. RNA was extracted from 20 to 30mg of

126 tissue using the Qiagen RNeasy mini kit according to the manufacturer's instructions. RNA
127 concentration was measured using a Nanodrop 2000. One μg of RNA was reverse transcribed
128 into cDNA using iScript cDNA Synthesis Kit (Biorad) following the manufacturer's instructions.
129 Gene expression was measured using a QuantStudio 3 real time qPCR instrument
130 (ThermoFisher), Taqman Fast Advanced Mastermix (Applied Biosystems) and predesigned
131 probes (Life Technologies, Applied Biosystems). Expression of all genes was normalized to
132 *Gapdh* and calculated using the Cq values and the 2^{-ddCq} method.
133 **Intestinal cell isolation.** Jejunum segments were isolated and flushed with PBS containing
134 10% FBS. The intestine was cut longitudinally and chopped into 0.5cm segments then
135 incubated in buffer containing 10mM EDTA and 1mM DTE for 20 minutes to remove the
136 epithelial layer. Tissue was finely chopped using a razor blade then digested with 971U/ml
137 collagenase VIII and 5μg/mL DNAse I (Applichem) in complete RPMI for 30 minutes. Cell
138 fraction was resuspended in 40% Percoll and overlaid above a 70% Percoll fraction. Cells were
139 centrifuged at 600xg for 20 minutes and leukocytes at the interface were collected.
140 **Histology.** Jejunums were collected from mice and segments of approximately 1cm were fixed
141 in 4% paraformaldehyde for a minimum of 48 hours. Dehydration, paraffin embedding,
142 sectioning and staining were done by the Harvard Medical Area Rodent Histopathology Core
143 (Harvard, Boston, MA). Images were captured on a Nikon E800 microscope (Nikon, Melville,
144 NY).
145 **May Grünwald Giemsa Staining.** BMMCs were treated with bafilomycin or omeprazole or drug
146 vehicle. 300,000 cells were adhered onto poly-lysine coated slides by cytospin (Shandon
147 Cytospin 3). Slides were stained with May Grünwald solution (Sigma) for 5 minutes, washed 3
148 times in deionized water, stained with Giemsa solution (Sigma) for 15 minutes then washed 6
149 times in deionized water. Slides were air dried then fixed with Permount (Sigma). Images were
150 captured on a Nikon E800 microscope (Nikon, Melville, NY).

151 **Human basophil activation test.** Human basophil activation tests were performed as
152 previously described [2] using the FLOW CAST Basophil activation test kit (Bühlmann
153 Laboratories, Schönenbuch, Switzerland). Briefly, 50µL aliquots of whole blood from healthy
154 donors were incubated with omeprazole (200µM) for two hours in a 37°C water bath. Following
155 treatment, samples were stimulated with 200ng/mL anti-IgE (Southern biotech) and
156 simultaneously stained for cell viability (Viability-APCCy7), anti-CCR3 (PE), anti-CD63 (FITC)
157 for 15 minutes at 37 °C. Red blood cells were lysed, and cells were washed and acquired on an
158 LSR Fortessa (BD). Basophils were identified as SSC^{low} and $CCR3^{+}$. The expression of CD63
159 on the cell surface was used as a measure of basophil activation.
160

161 **Figure legends**

162 **Figure E1.** *Gating strategy for identification of bone marrow derived mast cells by flow*
163 *cytometry, dose responsive effect of omeprazole on mast cell degranulation, and viability of*
164 *cells following incubation with omeprazole*. A) Cell population, and subsequently single cells,
165 are first identified based on forward and side scatter. Live cells are then selected from the single
166 cell gate based on negative staining for the viability dye. Live cells expressing cKIT and FcεRIa
167 are defined as bone marrow mast cells (BMMCs). B) LAMP-1 expression of IgE anti-TNP bound
168 BMMCs following incubation with various doses of omeprazole and degranulation with TNP-
169 OVA. C) Viability of BMMCs following incubation with omeprazole. Viability staining is applied to
170 all BMMC culture experiments analyzed by flow cytometry. For panel B, data are shown from
171 one experiment representative of two experiments. Statistical analysis by one-way analysis of
172 variance. $**P<0.01$, $****P<0.0001$

173 **Figure E2.** *Alterations in granule morphology following incubations with bafilomycin and*
174 *omeprazole*. May Grünwald/Giemsa staining of cytospin preparations of BMMCs following
175 incubation with DMSO, bafilomycin (20 nM) or omeprazole (50 μM) for 24 hours.

176 **Figure E3.** *Omeprazole blocks activation of basophils.* Activation of basophils in whole blood
177 using anti-IgE following treatment with 200 μM omeprazole or vehicle for 2 hours. Basophil
178 activation was quantified by flow cytometry. Basophils were identified as SSC^{low}, negative
179 staining for viability dye, and $CCR3^{+}$. The expression of CD63 on the cell surface was used as a
180 measure of basophil activation. Data points represent four different individuals assayed in
181 duplicates for each condition. Statistical analysis between treatment groups was done using a
182 paired t-test. $*P<0.05$

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184 **References**

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