

1 **Title**

2 Serotonin hormonally regulates lacrimal gland secretory function via serotonin type 3a
3 receptor

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1 **Supplementary Information**

2 **Supplementary Materials and Methods**

3 **Protein secretion from lacrimal gland (LG) acinar cells**

4 Rats were euthanized with an overdose of pentobarbital sodium. The excised
5 LG was digested by collagenase type 3 (Worthington, USA) and incubated in saline
6 solution (140 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, 10
7 mM dextrose [pH 7.4]) with 5-hydroxytryptamine (5-HT) for 60 minutes at 37°C. The
8 preparations were centrifuged at 800 g for 3 minutes and the protein concentration in the
9 supernatant measured using Bradford reagent (Sigma-Aldrich, USA), with bovine
10 serum albumin (BSA) as the standard. The protein secretion rate was calculated as a
11 percentage of that before stimulation. Solutions of 5-HT (0.1, 1, 10, 100, and 1,000 μM)
12 were diluted to the desired concentrations with saline solution and used as stimulants.
13 Ondansetron (10 μM) was appropriately prepared in saline solution before use. For each
14 experiment, six LGs were used in each group.

15

16 ***In situ* hybridization (ISH) in mouse lacrimal gland**

17 Mice were euthanized with an overdose of pentobarbital sodium and their LGs
18 rapidly dissected. *In situ* hybridization was performed as described under Methods.

1 **Supplementary Figure Legends**

2 **Supplementary Figure 1. Effect of Trp-free diet on tear secretion in male mice**

3 Mice were fed either a standard (control group) or a Trp-free diet (Trp-free group) for 7
4 days after the acclimatization period. (A) Changes in tear secretion. Each measurement
5 was performed during 7 days of feeding with either a standard or a Trp-free diet.
6 Atrophic alterations in the LG size (B) and weight (C) were recorded on day 7 after the
7 switch to the Trp-free diet. The dotted line indicates LG (B, the scale bar is 2 mm). Each
8 panel shows the control (upper) and Trp-free diet (lower) groups. Open and solid
9 symbols indicate the control and Trp-free groups, respectively (A, C). Data represent
10 the mean \pm SD, n=5 mice. *** $P < 0.001$ versus control, ### $P < 0.001$ versus day 0.

11

12 **Supplementary Figure 2. Recovery of tear secretion and lacrimal gland weight**
13 **after reverse switch from Trp-free to standard diet**

14 Mice were given a Trp-free diet for 7 days followed by a reverse switch to the standard
15 diet. (A) Recovery of tear secretion (n=7 mice). (B) Recovery of LG weight (n=8-10
16 LG). Right photographs show the typical gross appearance of LG after 3 (upper) and 7
17 (lower) days of reverse switch to the standard diet from Trp-free diet (the scale bar is
18 2 mm). The dotted line indicates LG. All data represent the mean \pm SD, ** $P < 0.01$,

1 *** $P < 0.001$ versus day 0.

2

3 **Supplementary Figure 3. Effect of Trp-free diet on peripheral tissue weight**

4 (A) Changing ratio of tissue weight on day 7 after the switch to Trp-free diet. Data show
5 the ratio of tissue weight of the Trp-free diet group to that of the standard diet group
6 (control, red dotted line). Mean \pm SD, n = 4-8. * $P < 0.05$, *** $P < 0.001$ versus
7 corresponding control, ††† $P < 0.001$ versus liver, §§§ $P < 0.001$ versus submandibular
8 gland. (B) Alteration in the size of the liver (upper) and submandibular gland (lower) at
9 day 7 after the switch to Trp-free diet. Each photograph shows the control (left) and
10 Trp-free diet (right) groups. The scale bar is 5 mm.

11

12 **Supplementary Figure 4. Effects of ondansetron infusion on lacrimal gland**
13 **autophagy**

14 Mice were infused with either saline (vehicle) or ondansetron for 7 days via an osmotic
15 pump. (A) Alteration in LG size. The dotted line indicates LG (the scale bar is 2 mm).
16 (B) Changes in the expression of autophagy-related proteins. Data show the
17 representative western blot images and quantitative analysis (bar chart) at day 7 after
18 systemic infusion of either the vehicle or ondansetron. Open and solid columns indicate

1 the vehicle and ondansetron groups, respectively. All data represent the mean \pm SD, n=5
2 LG. *** $P < 0.001$ versus corresponding vehicle.

3

4 **Supplementary Figure 5. Stimulated tear secretion by serotonin and SR57227A in**
5 **mice with blood serotonin concentration under the normal level**

6 Mice were intraperitoneally injected with either saline (vehicle, open circle), 5-HT
7 (closed circle), or SR57227A (closed triangle). Tear secretion was measured 5 minutes
8 before and every 5 minutes for 30 minutes after injection. Data represent the mean \pm SD,
9 n=5 mice. * $P < 0.05$ versus 0 min.

10

11 **Supplementary Figure 6. Protein release from isolated rat lacrimal gland acinar**
12 **cells after stimulation with 5-HT**

13 Exocytotic release of protein was determined as the amount of total protein release from
14 LG acinar cells isolated from normal rat. (A) Total protein release from LG acinar cells
15 induced by 5-HT stimulation. The LG was stimulated with 5-HT at dosage ranging from
16 0.1 to 1,000 μ M. (B) Inhibitory effects of ondansetron (10 μ M) on total protein release
17 from LG acinar cells by 5-HT (100 μ M). All data represent the mean \pm SD, n=5 LG.
18 *** $P < 0.001$ versus control.

1

2 **Supplementary Figure 7. In situ hybridization analysis of 5-HTR mRNA**
3 **expression in mouse lacrimal gland**

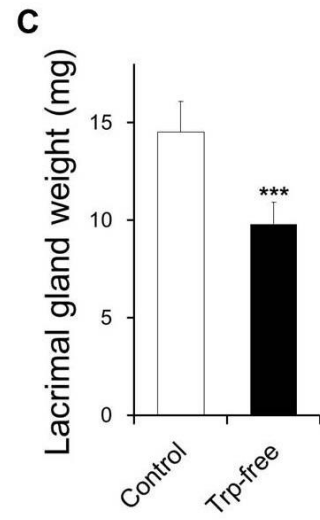
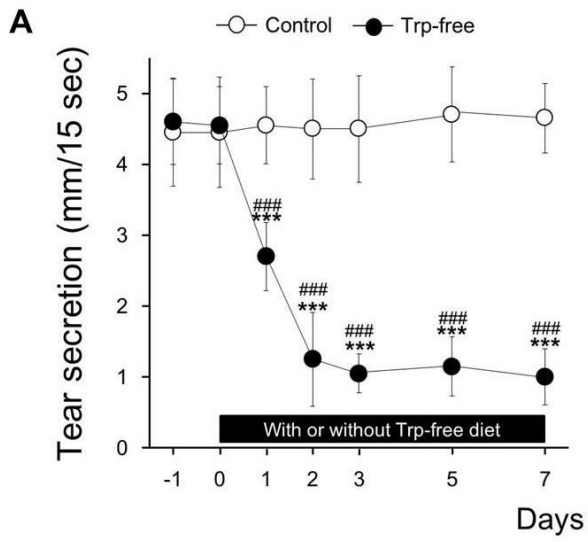
4 The mRNA expression of the 5-HTR subtype was determined in normal mouse LG. In
5 situ hybridizations are shown for the indicated 5-HTR and M3R probes. Each panel
6 shows LG acinar cells. Nuclear fast red was used as the counter stain. The scale bar is
7 10 μm .

8

9 **Supplementary Figure 8. Morphological changes in ductal cells of the lacrimal**
10 **gland**

11 Morphological changes in LG ductal cells by 5-HT₃ receptor antagonist/agonist
12 infusion (A), and parasympathetic post-ganglionic denervation (PGD) combined with a
13 Trp-free diet (B). Ondansetron, a 5-HT₃ receptor antagonist, was infused into mice for 7
14 days via an osmotic pump. SR57227A, a 5-HT₃ receptor agonist, was infused into mice
15 under conditions in which blood 5-HT levels were decreased by a Trp-free diet. PGD
16 was performed 3 days after feeding a Trp-free diet or a standard diet. Morphological
17 changes in LG were evaluated 1 day after PGD. Saline infusion was used as vehicle
18 control. Scale bar = 10 μm .

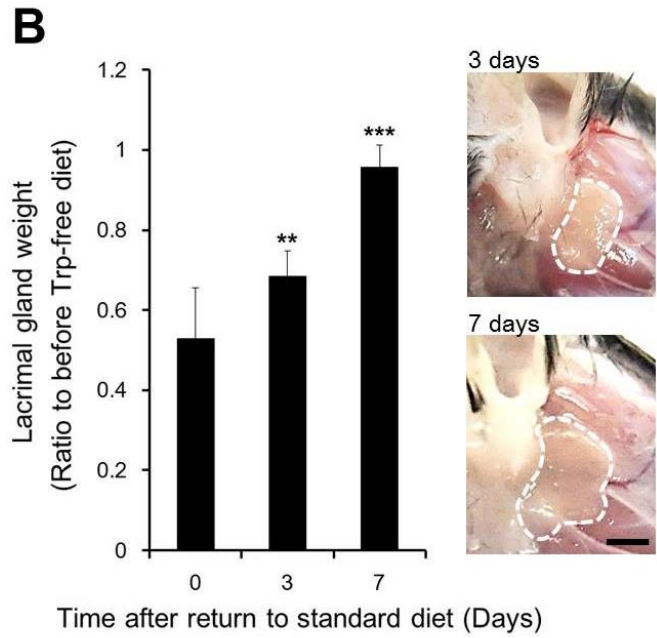
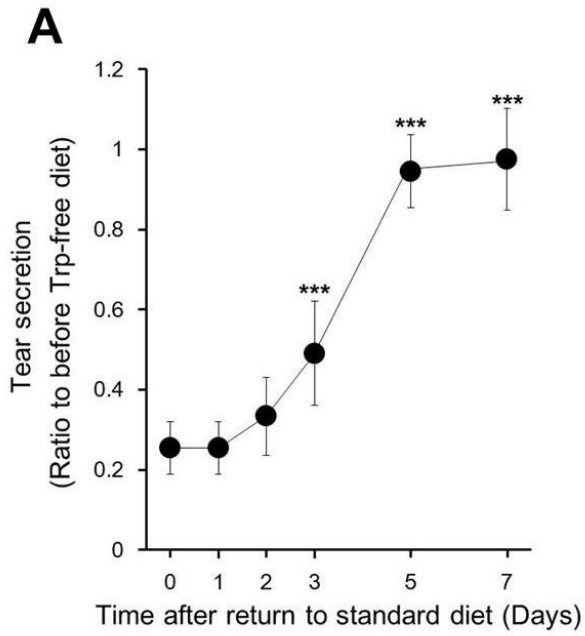
1 Supplementary Figure 1



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1 **Supplementary Figure 2**



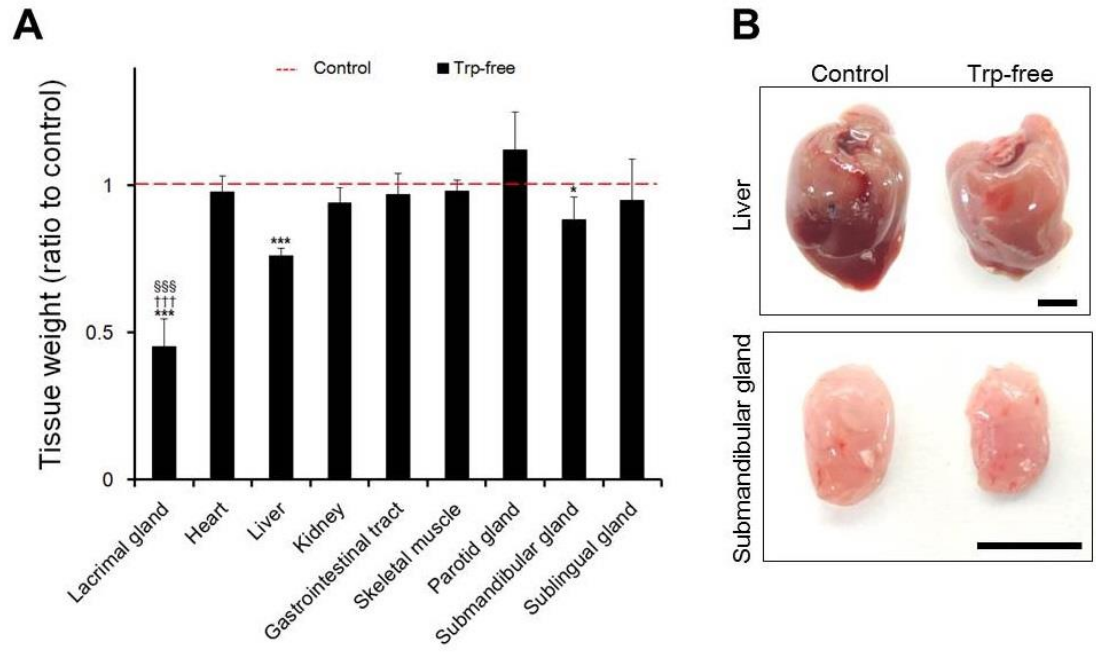
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1 **Supplementary Figure 3**

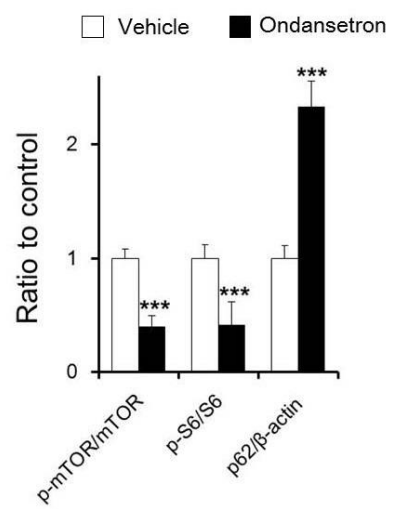
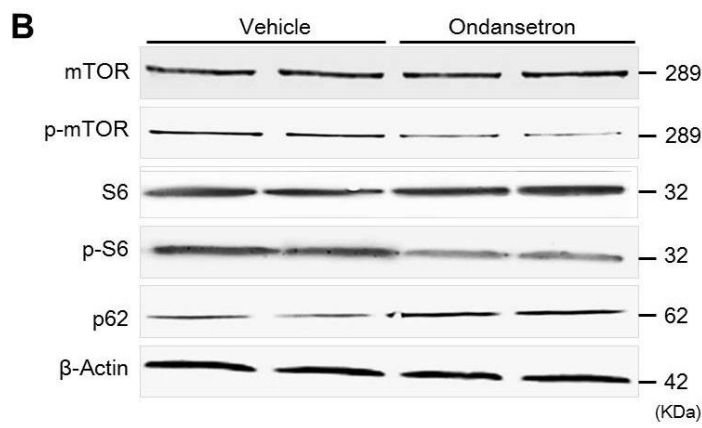
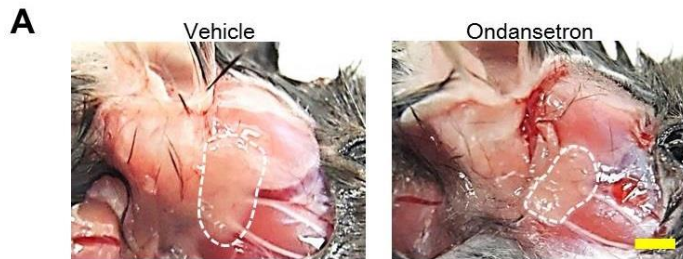
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1 Supplementary Figure 4



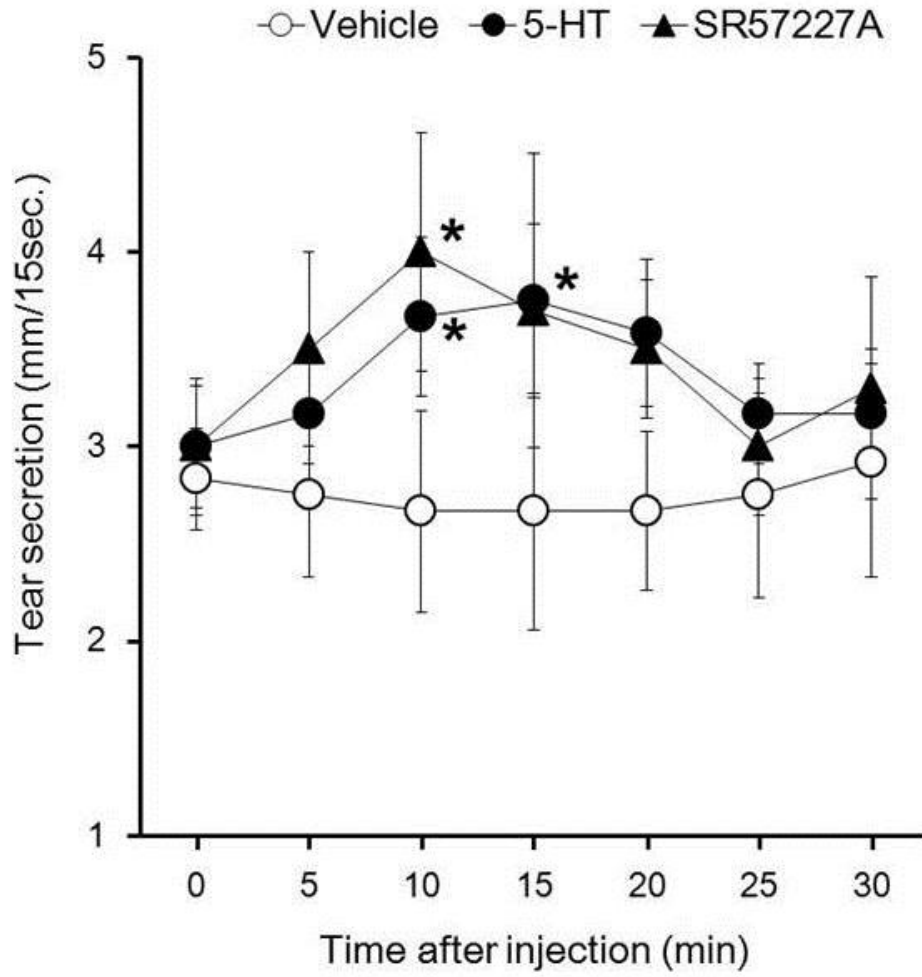
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1 **Supplementary Figure 5**

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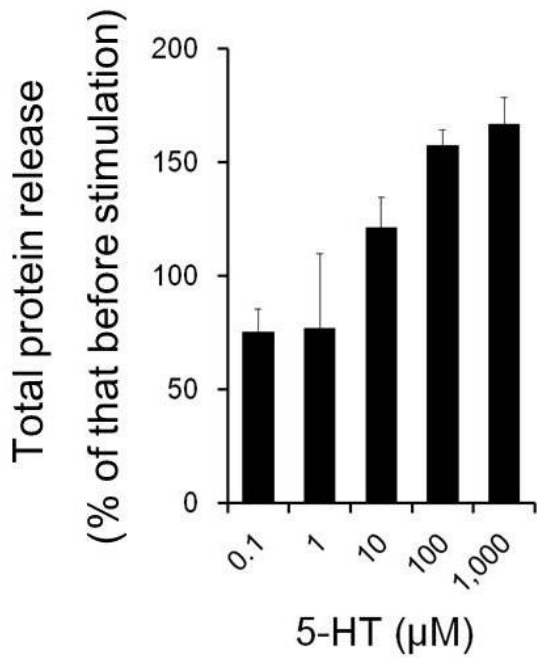
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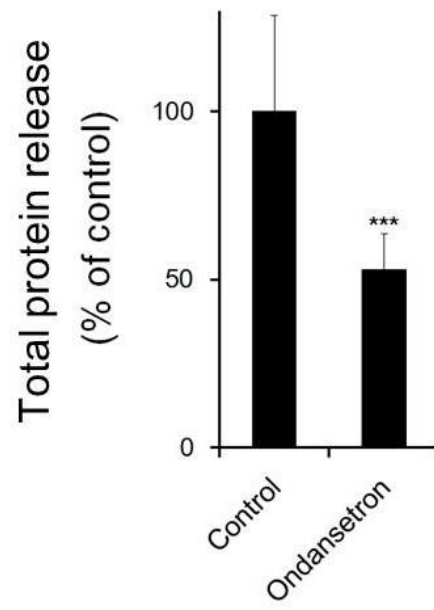
1 **Supplementary Figure 6**

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A



B



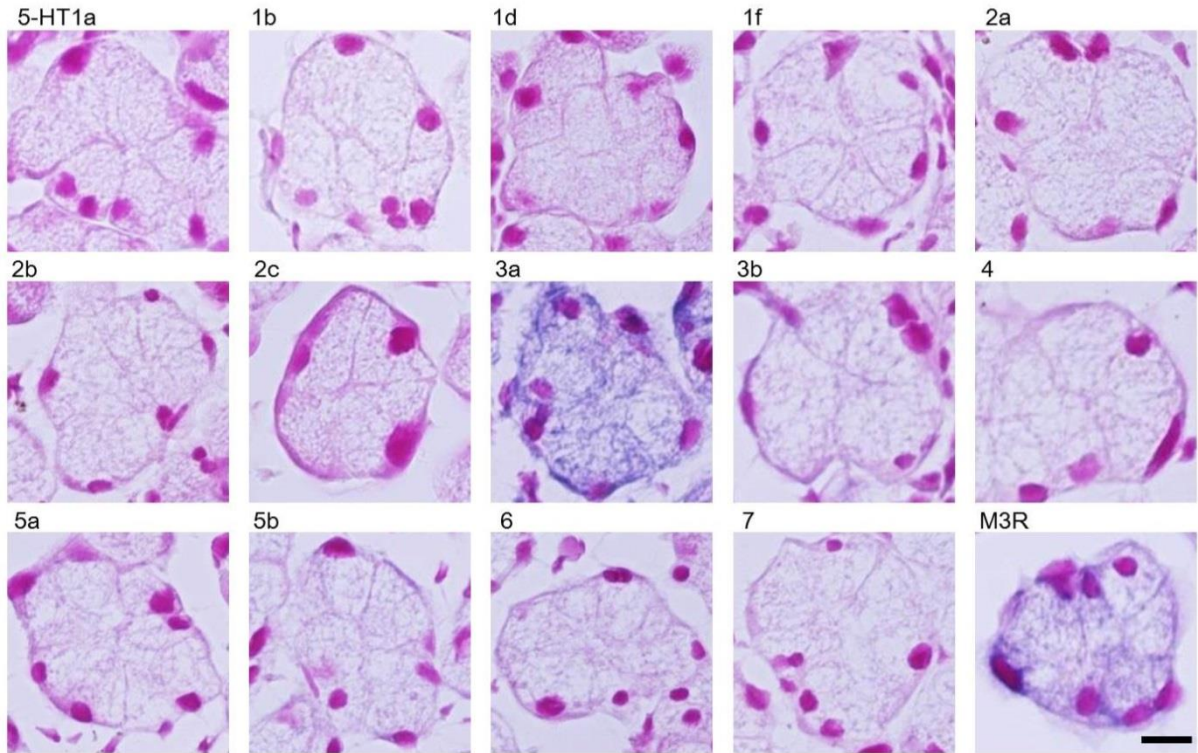
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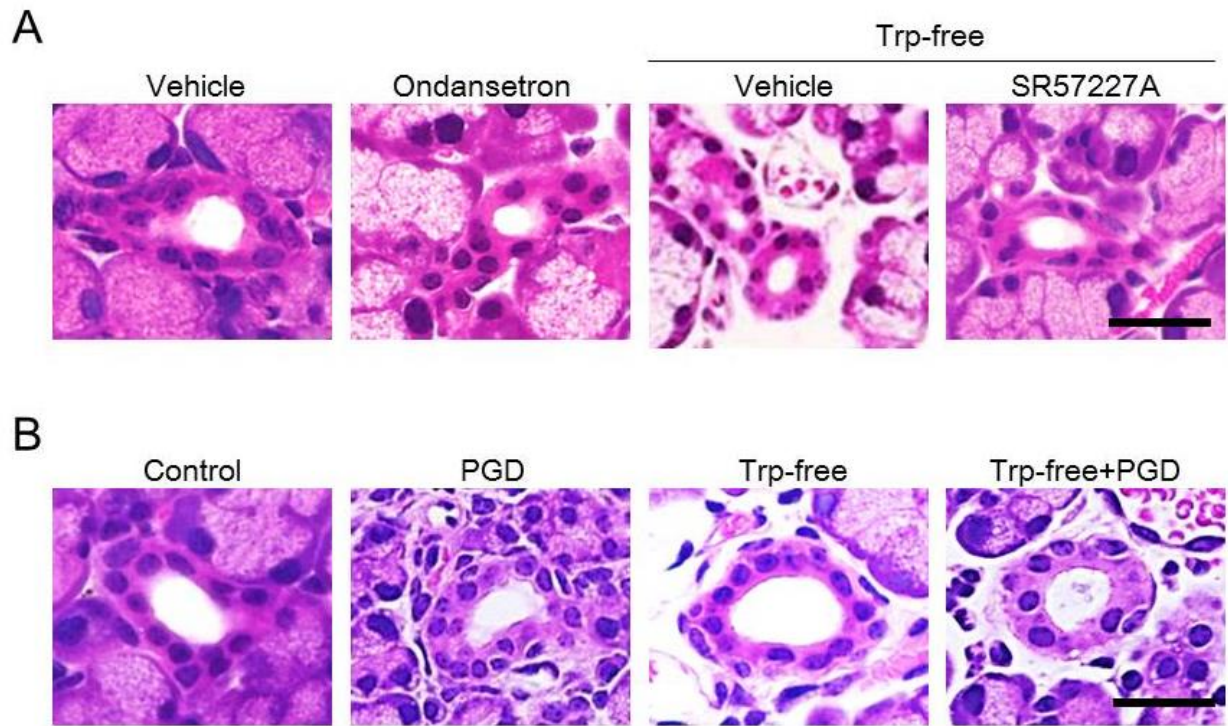
1 **Supplementary Figure 7**



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1 **Supplementary Figure 8**



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