P2X7 receptor antagonism prevents IL-1β release from salivary epithelial cells and reduces inflammation in a mouse model of autoimmune exocrinopathy

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Running title: *Role of P2X7R in Salivary Gland Inflammation*

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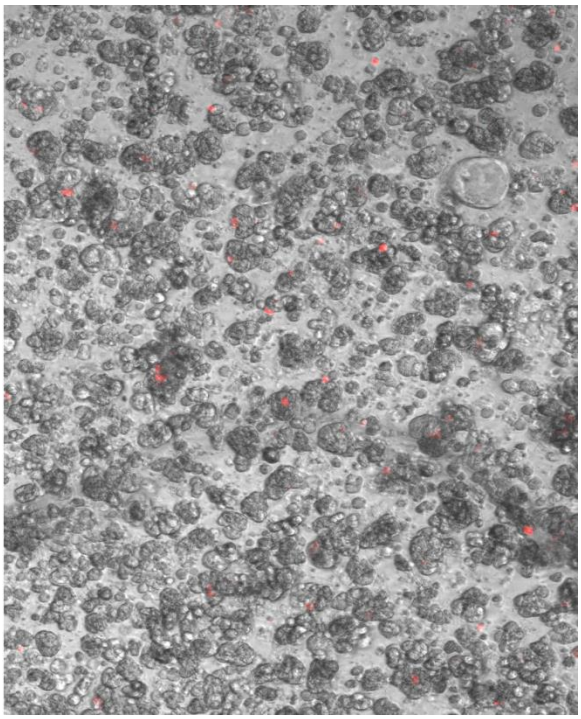
**Figure S1**

**(A)**



**(B)**

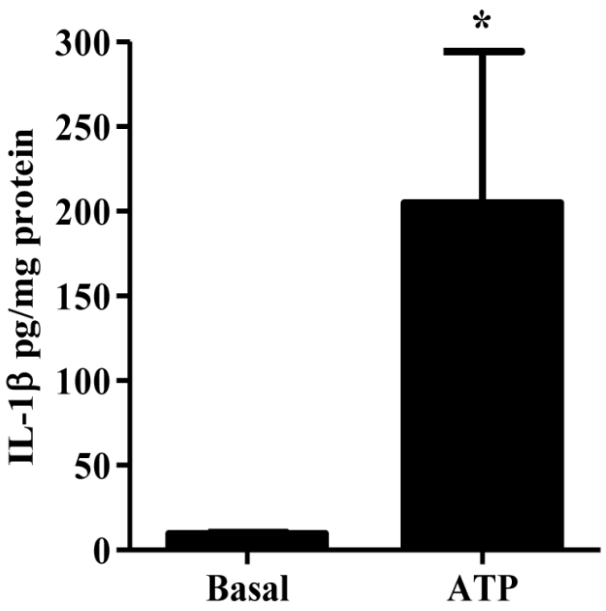


**Figure S1. ATP-induced IL-1β release in isolated epithelial cells—**Wild type dispersed SMG cell aggregates were suspended in PBS and non-specific antibody binding was blocked by incubation for 10 min at 4°C with anti-mouse CD16/32 antibody (1μg/$10^6$ cells in 100 μl, Mouse $F_C$ block, Cat. No. 553142, BD Biosciences, San Jose, CA). Then, SMG cells were incubated for 20 min at 4°C with Alexa Fluor 594-conjugated anti-mouse CD45 antibody (1:50 dilution, clone 30-F11, Biolegend, San Diego, CA) and washed 3 times in PBS. (A) Stained cells were placed on a 8-well coverslip and fluorescence was visualized using a Nikon TI-E inverted microscope equipped with appropriate filters. Images from 3 independent SMG preparations were analyzed for immune cell contamination by counting the number of $CD45^+$ cells (red) and the total number of cells. Epithelial cell purity of SMG cell preparations was 97-99%. (B) SMG epithelial cells were enriched using the magnetic bead-based EasySep Mouse Epithelial Cell Enrichment Kit to remove hematopoietic and endothelial cells and fibroblasts. The isolated epithelial cells were stimulated with ATP (3 mM) for 90 min at 37°C and then cells were collected by centrifugation and IL-1β was quantified in the supernatant using the IL-1β Quantikine ELISA kit. Data represent means ± S.E., where $*P < 0.05$ indicates a significant increase over basal levels.