

P2X4 receptor stimulation enhances MrgprB2-mediated mast cell activation and pseudoallergic reactions in mice

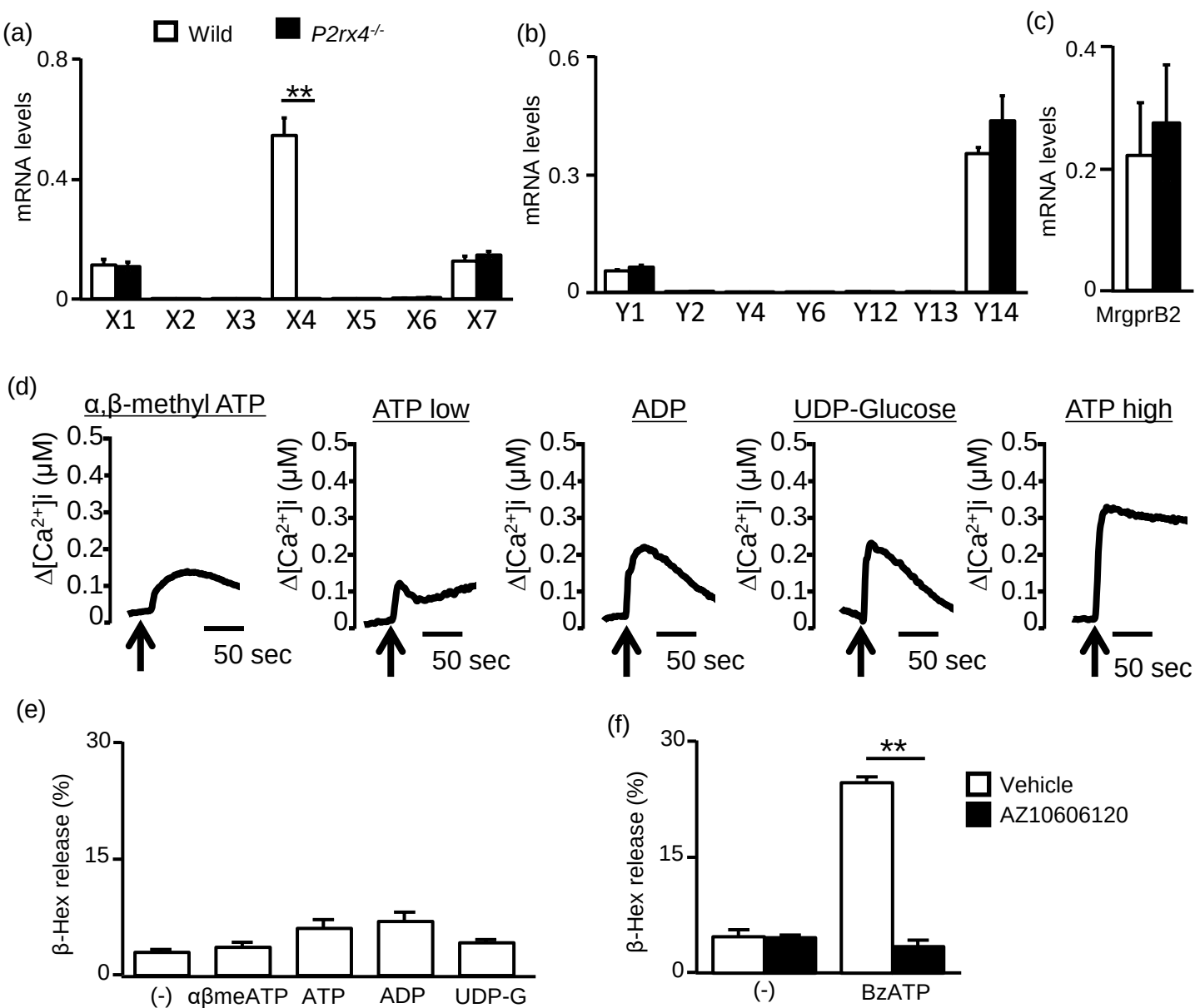
Kazuki Yoshida^{1*}, Shota Tanihara¹, Yuki Miyashita¹, Kosuke Obayashi¹, Masa-aki Ito¹, Kimiko Yamamoto², Toshiyashu Imai³, and Isao Matsuoka¹

¹ Laboratory of Pharmacology, Faculty of Pharmacy, Takasaki University of Health and Welfare, Takasaki-shi, Gunma 370-0033, Japan

² Department of Biomedical Engineering, Graduate School of Medicine, The University of Tokyo, Tokyo 113-0033, Japan

³ Discovery Research Laboratories, Nippon Chemiphar Co., Ltd., Misato, Saitama 341-0005, Japan

* Corresponding author: yoshida-k@takasaki-u.ac.jp

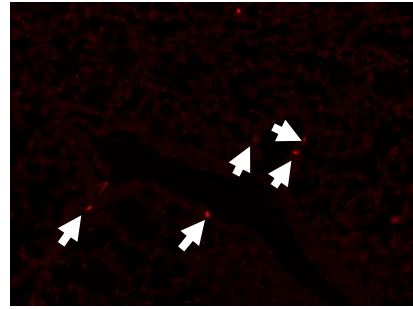
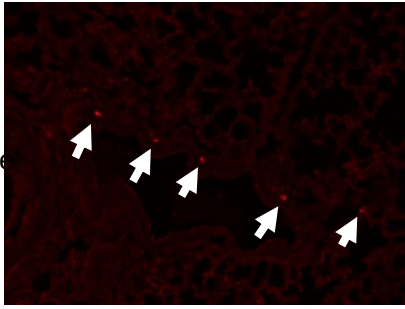


Supplementary Figure S1. Expression of P2 receptors mRNA and effect of purinergic receptor agonist on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) levels and degranulation in PMCs. P2X (a), P2Y (b) receptor and MrgprB2 (c) mRNA expression were analyzed by quantitative real time-PCR. mRNA levels were normalized by GAPDH (n=4). (d) PMCs preloaded with fura 2-AM were stimulated with P2X1 receptor agonist $\alpha\beta$ meATP (10 μ M), P2X receptor agonist ATP (100 μ M), P2Y₁ receptor agonist ADP (100 μ M), P2Y₁₄ receptor agonist UDP-G (100 μ M) or 1 mM ATP (ATP high). Traces of typical changes in $[Ca^{2+}]_i$ were shown as representative of 3-4 experiments. (e) PMCs were stimulated with $\alpha\beta$ meATP (10 μ M), ATP (100 μ M), ADP (100 μ M) and UDP-G (100 μ M) for 10 min, and β -hexosaminidase (β -Hex) released into the reaction medium were measured (n=3). (f) PMCs were stimulated with P2X7 receptor agonist BzATP (300 μ M) in the presence or absence of P2X7 receptor antagonist AZ10606120 (10 μ M) (n=3). Data are presented as the mean $\pm$ SEM. **P<0.01

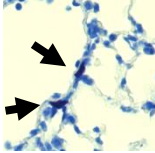
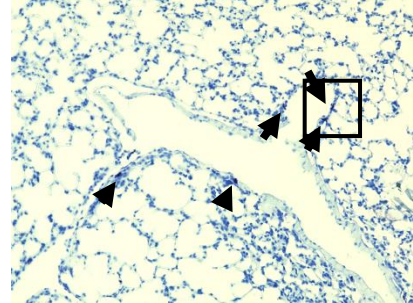
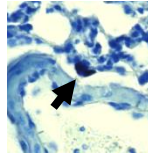
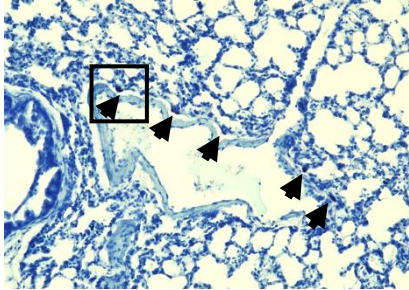
(a) WT BMMCs $\rightarrow$ *Kit*^{W-sh/W-sh}

(b) *P2rx4*^{-/-} BMMC $\rightarrow$ *Kit*^{W-sh/W-sh}

Avidin-rhodamine



Toluidine blue



100 μ m

Supplementary Figure S2. Tissue distribution of reconstituted MCs in *Kit*^{W-sh/W-sh} mice. MC deficient *Kit*^{W-sh/W-sh} mice were intravenously injected with BMMCs prepared from WT (a) - and *P2rx4*^{-/-} (b) mice. Four month later, lung sections were examined for reconstituted MC using avidin-rhodamine (upper panel) and toluidine blue stain (lower panel). Arrows indicate MCs.