Extracellular ATP mediates mast cell–dependent intestinal inflammation through P2X7 purinoceptors

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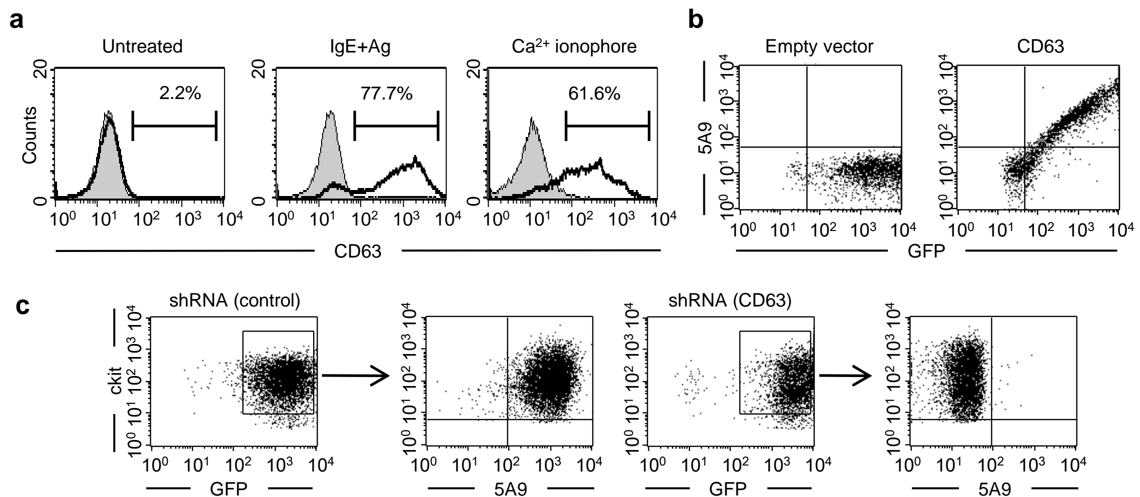
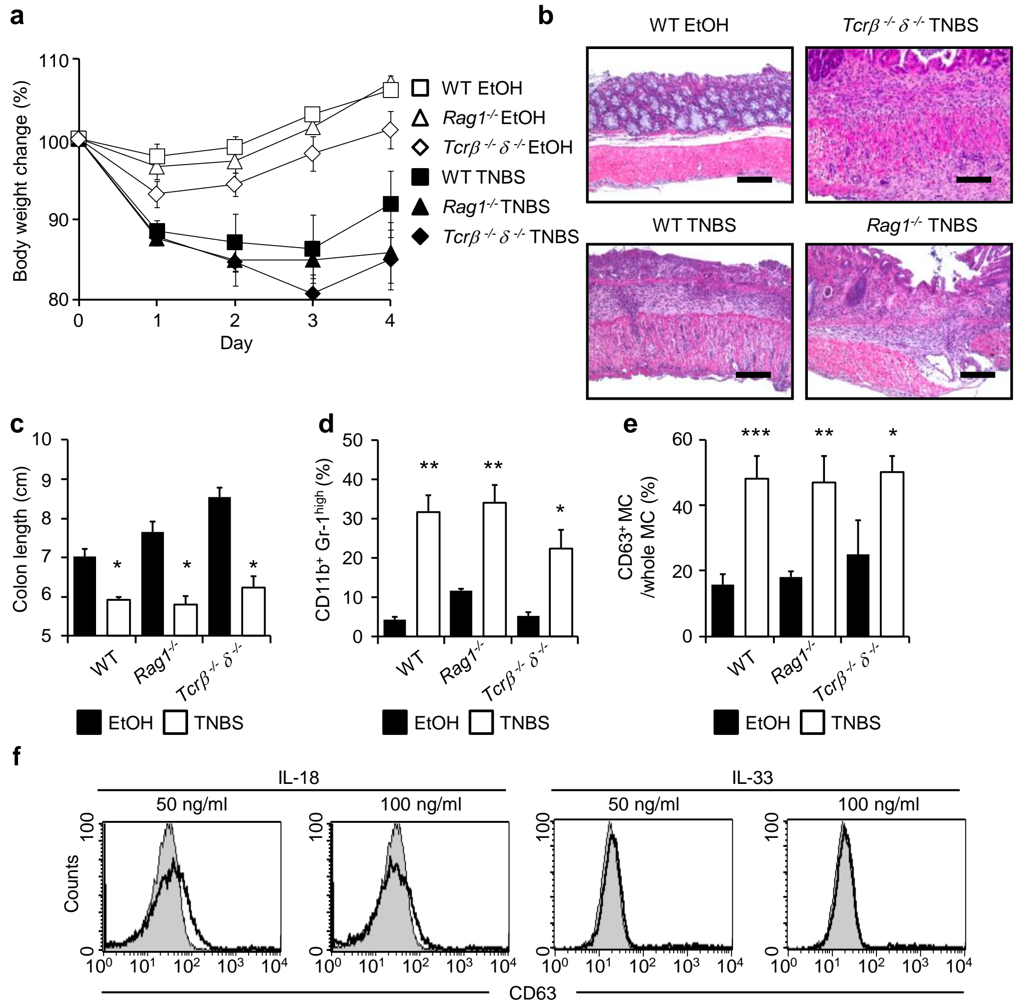
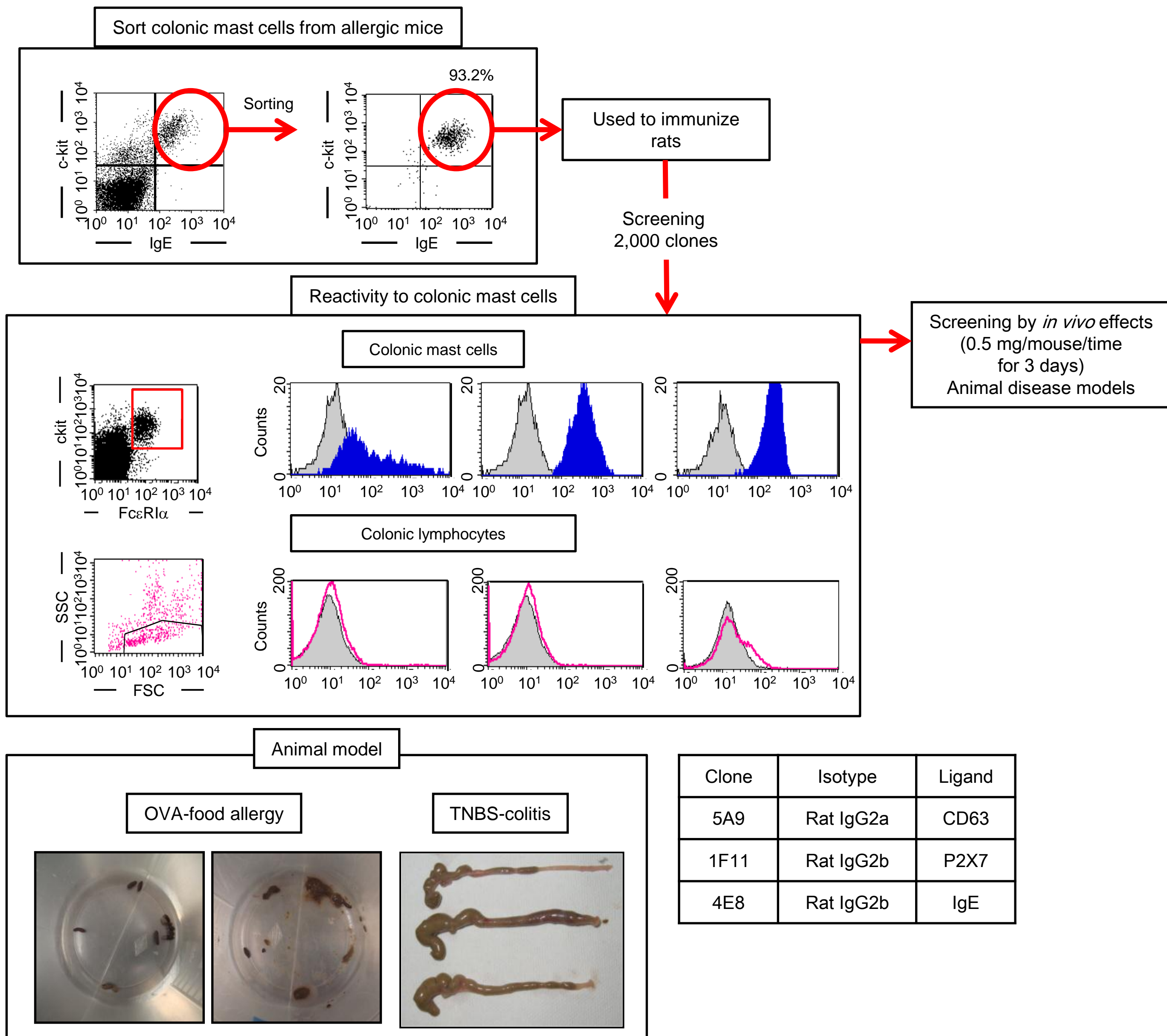


Supplementary Figure S1. Specific recognition of activated MCs by an anti-CD63 mAb. (a) BM-derived MCs were treated with anti-DNP-IgE plus DNP-HSA or calcium ionophore and stained with an anti-CD63 mAb (5A9) for flow cytometry. (b) CD63-transfected CHO cells were stained with an anti-CD63 mAb (5A9). (c) CD63-knocked down MC/9 cells were stimulated with calcium ionophore and stained with an anti-CD63 mAb (5A9)



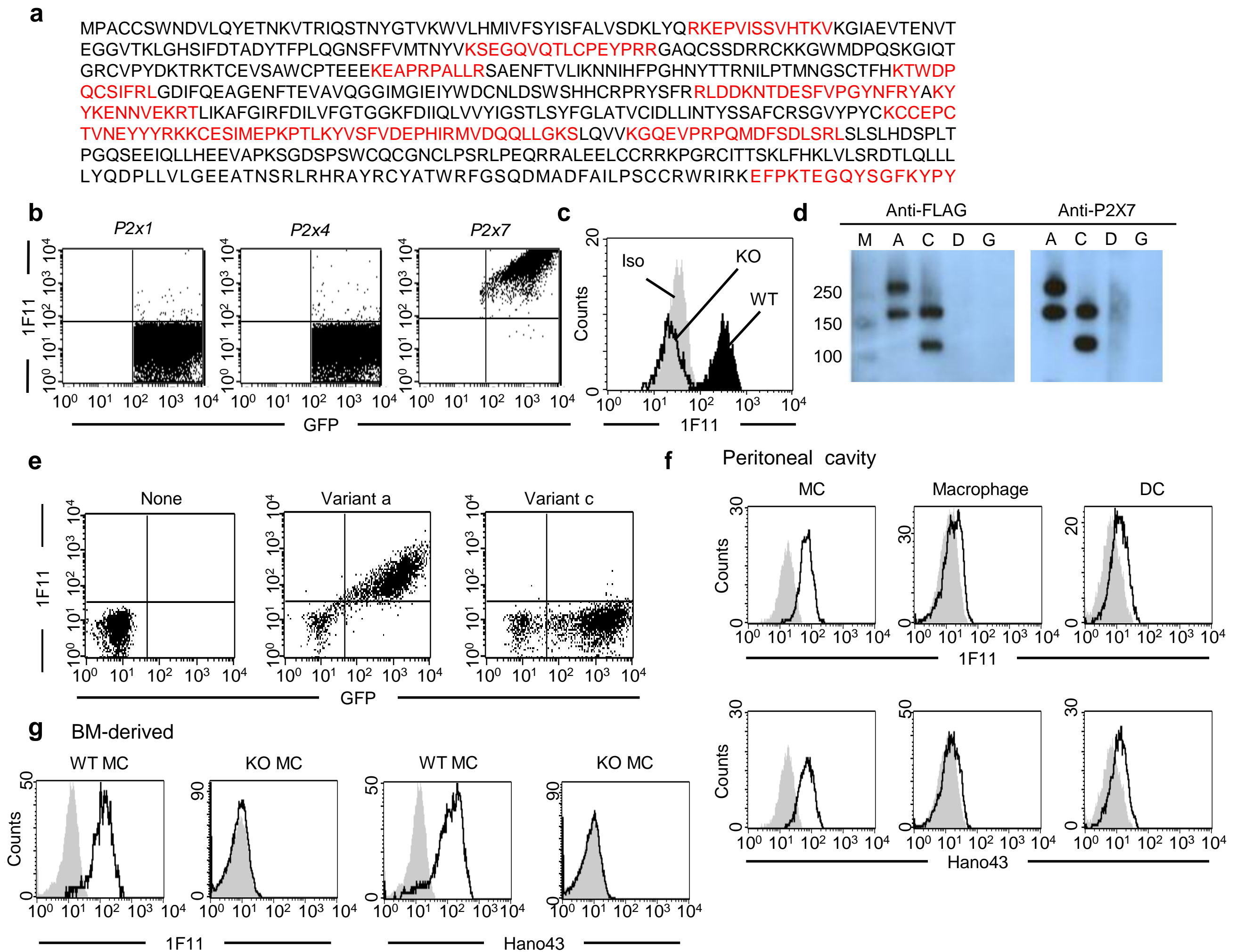
Supplementary Figure S2. Intestinal MCs mediate gut inflammation in a manner independent of T- and B- cells and IL-18 and IL-33.

Wild-type (n = 6), *Rag-1*$^{-/-}$ (n = 6), and *Tcrβ* $^{-/-}$ *δ* $^{-/-}$ (n = 5) mice were treated with TNBS. Control groups received 50% EtOH [wild-type (n = 6); *Rag-1*$^{-/-}$ (n = 5); and *Tcrβ* $^{-/-}$ *δ* $^{-/-}$ (n = 3)]. (a) Body weight changes were measured as percentages of baseline weight. (b) Tissue sections with H&E staining are representative of at least three mice per group. Scale bar, 100 μm. (c) Colon length was measured 4 days after TNBS treatment. *P < 0.003 (two-tailed Student's *t*-test). (d) Infiltration of CD11b^{+} Gr-1^{high} neutrophils into the colon was measured with flow cytometry. Data are shown as means ± s.e.m. (n = 3 to 6). *P = 0.0166 (Welch's *t*-test); ** P < 0.01 (Welch's *t*-test). (e) The percentage of CD63^{+} MCs in all c-kit^{+} FcεRIα^{+} MCs was determined with flow cytometry. Data are shown as means ± s.e.m. (n = 3 to 6), *P = 0.0315 (two-tailed Student's *t*-test); **P = 0.0343 (two-tailed Student's *t*-test). ***P = 0.0014 (Welch's *t*-test). (f) BM-derived MCs were stimulated with 50 or 100 ng ml^{-1} IL-18 or IL-33 for 3 h and then stained with an anti-CD63 mAb. Control staining with rat IgG2a is shown in gray. Data are representative of three experiments.

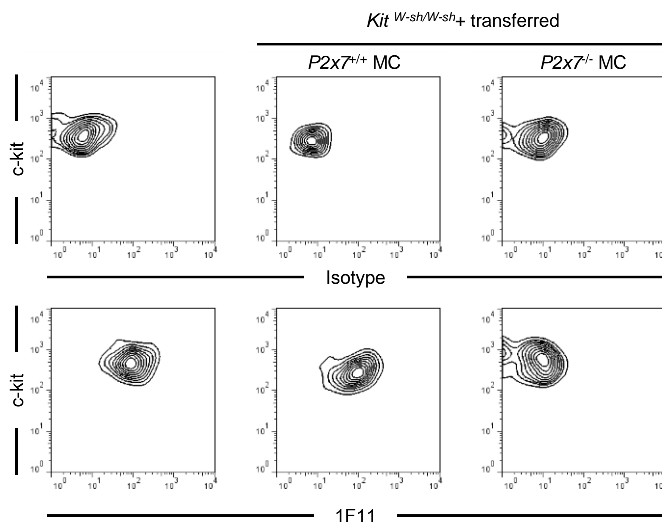


Clone	Isotype	Ligand
5A9	Rat IgG2a	CD63
1F11	Rat IgG2b	P2X7
4E8	Rat IgG2b	IgE

Supplementary Figure S3. Experimental procedure to generate anti-MC mAbs. MCs were purified from the colonic lamina propria of allergic mice and used to immunize rats. B-cell hybridomas were obtained by conventional methods and their specific reactivity to MCs was examined by flow cytometry. Hybridomas were further selected on the basis of their *in vivo* ability to inhibit OVA-induced food allergy or TNBS-induced colitis. Names, immunoglobulin isotypes, and ligands of the established mAbs are listed.

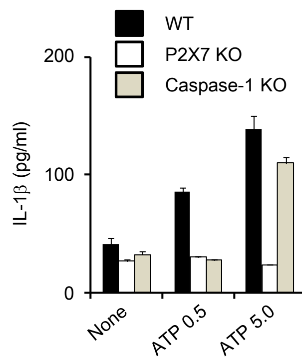


Supplementary Figure S4. Specific recognition of P2X7 purinoceptors by 1F11 mAb.
(a) The amino acid sequence of immunoprecipitants with the 1F11 mAb was determined by using LC-MS/MS. Red indicates the sequence covered by MS analysis. (b) The reactivity of 1F11 mAb to CHO cells expressing murine P2X1, P2X4, and P2X7 was examined. (c) Reactivity of 1F11 mAb to *P2x7*$^{+/+}$ (filled) and *P2x7*$^{-/-}$ (line) BM-derived MCs. Control staining with rat IgG2b is shown in gray. (d) CHO cells expressing flag-tagged variants a, c, and d of the P2X7 purinoceptors were immunoprecipitated with 1F11 mAb and subjected to western blotting with an anti-Flag or anti-polyclonal P2X7 antibody. (e) The reactivity of 1F11 mAb to *P2x7*$^{-/-}$ BM-derived MCs transfected with variants a and c of the P2X7 purinoceptors was examined with flow cytometry. (f) Peritoneal cells and (g) *P2x7*$^{+/+}$ or *P2x7*$^{-/-}$ BM-derived MCs were stained with two different anti-P2X7mAbs, 1F11 mAb and Hano43. Control staining with rat IgG2b is shown in gray.

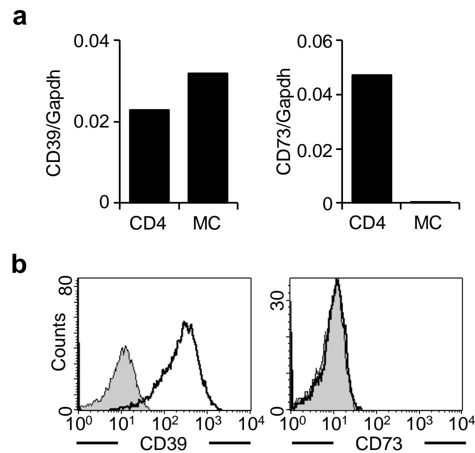


Supplementary Figure S5. P2X7 expression by colonic MCs in reconstituted *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice.

After reconstitution of *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice with *P2x7*$^{+/+}$ or *P2x7*$^{-/-}$ bone marrow derived mast cells, P2X7 expression by colonic c-kit^{+} FcεRIα^{+} MCs was examined by using flow cytometry.

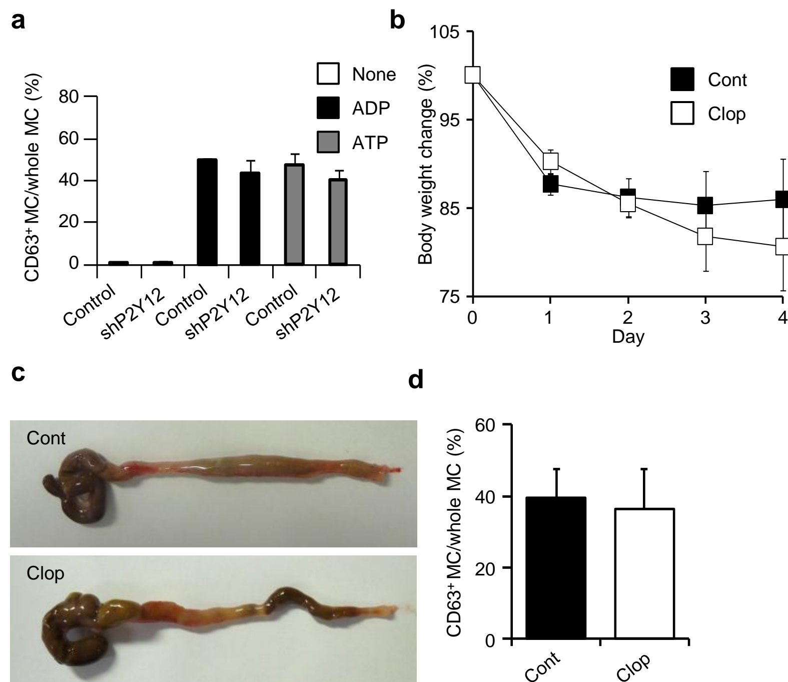


Supplementary Figure S6. Caspase-1-independent IL-1β production in MCs.
BM-derived MCs from wild-type, *P2x7*$^{-/-}$, and *caspase-1*$^{-/-}$ mice were stimulated with LPS followed by various concentrations of ATP. Production of IL-1β was measured by ELISA (n = 3). Data are shown as means ± s.e.m.

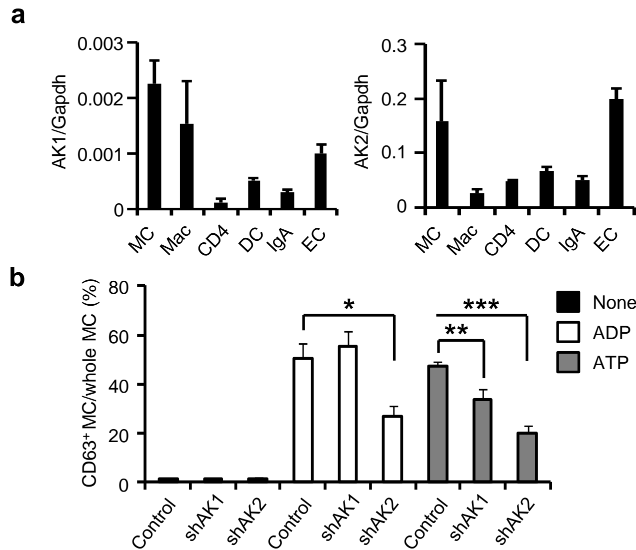


Supplementary Figure S7. Expression of CD39 and CD73 in colonic MCs.

(a) mRNA expression of CD39 and CD73 in colonic CD4 and MCs was determined by using quantitative RT-PCR. (b) CD39 and CD73 expression on colonic MCs was determined with flow cytometry. Control staining is shown in gray.

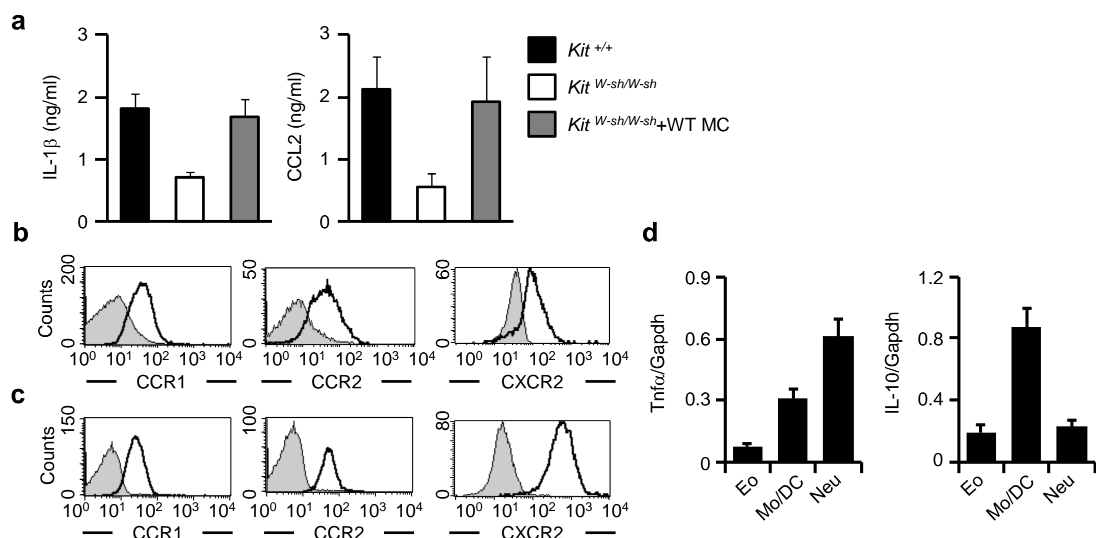


Supplementary Figure S8. P2Y12 inhibition does not affect colitis development.
(a) BM-derived MCs were transduced with shRNA expression lentivirus vector for P2Y12 or were mock-transduced and then stimulated with ADP or ATP for flow cytometric analysis of CD63 expression (n = 4). Data are shown as means ± s.e.m. (b–d) Mice received the P2Y12 inhibitor clopidogrel (Clop) or control vehicle (Cont) during colitis induction. (b) Body weight changes were measured. Data are shown as mean percentages of baseline weight ± s.e.m. (n = 3 for control; n = 5 for Clop). (c, d) Representative pictures of whole colons (c) and percentages of $CD63^{+}$ MCs in all MCs (d) 4 days after TNBS administration. Data are shown as means ± s.e.m.



Supplementary Figure S9. Involvement of adenylate kinase in P2X7-dependent MC activation.

(a) Expression of adenylate kinase 1 (AK1, left) and 2 (AK1, right) in colonic MCs, macrophages (Mac), $CD4^+$ T cells (CD4), DCs, IgA^+ plasma cells (IgA), and epithelial cells (EC) was measured by using quantitative RT-PCR (n = 4). Data are shown as means ± s.e.m. (b) BM-derived MCs were transduced with shRNA expression lentivirus vector for AK1 or AK2 or were mock-transduced and then stimulated with ADP or ATP for flow cytometric analysis of CD63 expression (n = 4). * $P = 0.0409$. ** $P = 0.0072$, *** $P < 0.0001$ (two-tailed Student's *t*-test). Data are shown as means ± s.e.m.



Supplementary Figure S10. Inflammatory cytokine and chemokine production in MC-deficient and MC-reconstituted mice.

(a) MC-deficient or MC-reconstituted mice were intrarectally administered TNBS and their colon tissues were incubated for 1 day to measure the production of IL-1β (left; n = 10) and CCL2 (right; n = 7) in the culture supernatant. Data are shown as means ± s.e.m. (b, c) CCR1, CCR2, and CXCR2 expression in $CD11b^+$ $Gr\text{-}1^{high}$ neutrophils in the colons (b) and blood (c) of TNBS-treated mice was examined by flow cytometry. Data are representative of three experiments. (d) TNFα (left) and IL-10 (right) expression in purified colonic eosinophils (SSC^{high} $CD11b^+$ $Gr\text{-}1^{neg}$), monocytes and dendritic cells (Mo/DC; SSC^{low} $CD11b^+$ $Gr\text{-}1^+$), and neutrophils (SSC^{low} $CD11b^+$ $Gr\text{-}1^{high+}$) was determined by using quantitative RT-PCR. Data are shown as means ± s.e.m. (n = 4).

Supplementary Table S1. Primer sequences

Gene	Forward 5' – 3'	Reverse 5' – 3'
P2Y1	CTGTGTGGACCCCATTCTTT	TCGGGACAGTCTCCTTCTGA
P2Y2	GGCCTGTGCATATGTGAGTG	TCCAGGTCTGCTGCCATT
P2Y4	CGGCGACTGTATCGACCT	GAGAGAACGGAGCCGAGAA
P2Y6	GCAGTCTTTGCTGCCACA	GTGGGCTCAGGTCGTAGC
P2Y12	CCCGGAGACACTCATATCCTT	TTGTAGTCTCTGACGCACAGG
P2Y13	ATGTGTGAGATGGGGAAAGG	CTGACTACTGTGGTGGTCTTCG
Pannexin1	AGACCAAGGGAGAGGACCA	GCTGCTCAGGTCCAAATCTT
Connexin32	ACCCATTTCGGACCAACC	AATCCATCTTGTCCTCTGGATG
Connexin43	TCCTTTGACTTCAGCCTCCA	CCATGTCTGGGCACCTCT
CCL2	CATCCACGTGTTGGCTCA	GATCATCTTGCTGGTGAATGAGT
CCL7	TTCTGTGCCTGCTGCTCATA	TTGACATAGCAGCATGTGGAT
CXCL2	AAAATCATCCAAAAGATACTGAACAA	CTTTGGTTCTTCCGTTGAGG
CD39	GCAAGCAGAGACAGCAAAAAC	GCAAAATCTCTTCACCTTAGAATCC
CD73	ATGAACATCCTGGGCTACGA	GTCCTTCCACACCGTTATCAA
Adenylate kinase1	TCCTGATCGACGGCTACC	AGTGTGGGCTGTCCAATCTT
Adenylate kinase2	TCCAAGACTCGCTGCTGAT	GGTAGGACCGGCCACTCT
TNFα	TGCCTATGTCTCAGCCTCTTC	GAGGCCATTTGGGAACTTCT
IL10	CAGAGCCACATGCTCCTAGA	TGTCCAGCTGGTCCTTTGTT
GAPDH	TGAACGGGAAGCTCACTGG	TCCACCACCCTGTTGCTGTA