Extracellular ATP and IL-23 form a local inflammatory circuit leading to the development of a neutrophil-dependent psoriasiform dermatitis

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Supplemental Data

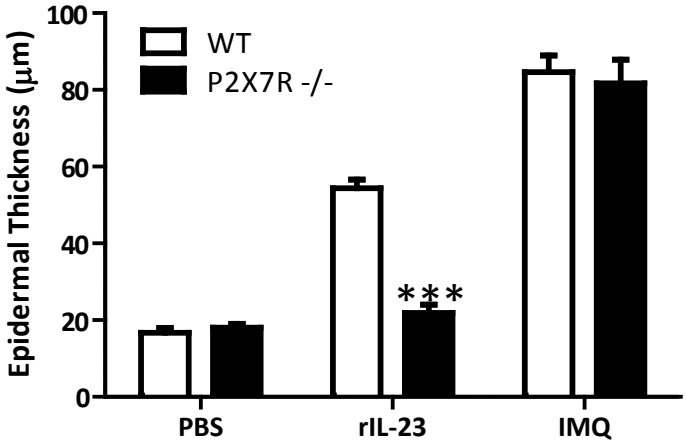

Figure S1. P2X7R signaling is necessary for the induction of acute psoriasiform inflammation induced by rIL-23 but not IMQ. rIL-23 and IMQ were utilized to induce inflammation in P2X7R -/- mice or age-matched C57BL/6 (WT) mice. On day 6 skin samples were collected and stained with H&E. Epidermal thickness was quantitated. Bars represent the mean ± SEM (n=6, combined from two independent experiments), ten independent high powered field (HPF) measurements were averaged from each mouse. Asterisks indicate a significant difference compared to WT mice treated with rIL-23. *** = $p < 0.001$.

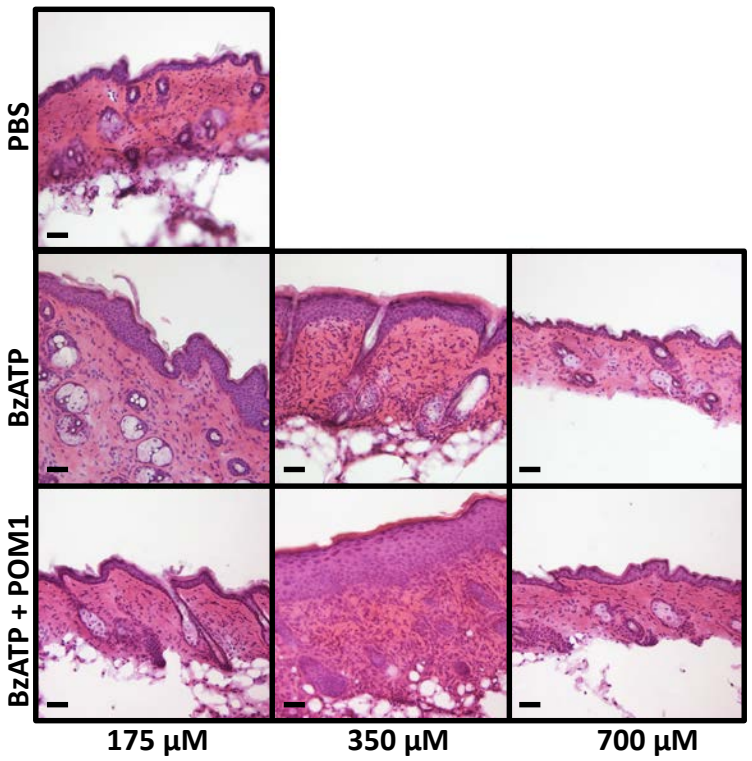

Figure S2. Titration of BzATP. WT mice were injected daily for 4 d with BzATP (175 µM, 350µM, or 700 µM) in 100 µl PBS in the presence or absence of POM1. On day 5 skin samples were collected and stained with H&E to assess histological phenotype, measure bar = 100 µm (n=4).

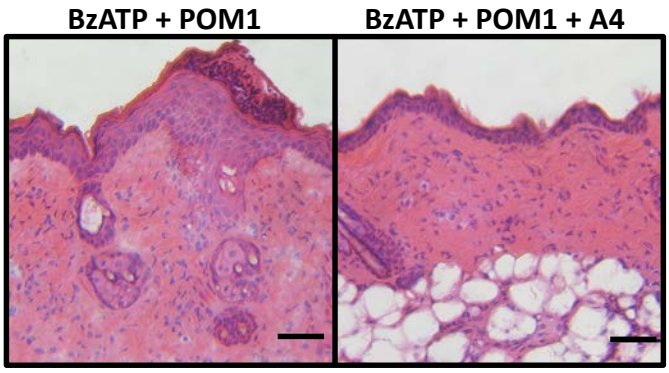

Figure S3. Specific blockade of the P2X7R inhibits the development of psoriasis-like lesions. WT mice were treated daily with BzATP + POM1 ± A438079 (A4; 80 µmol/kg), a competitive P2X7R antagonist that is inactive at other P2 receptors. On day 5 skin samples were collected and stained with H&E, measure bar = 100 µm (n=4).

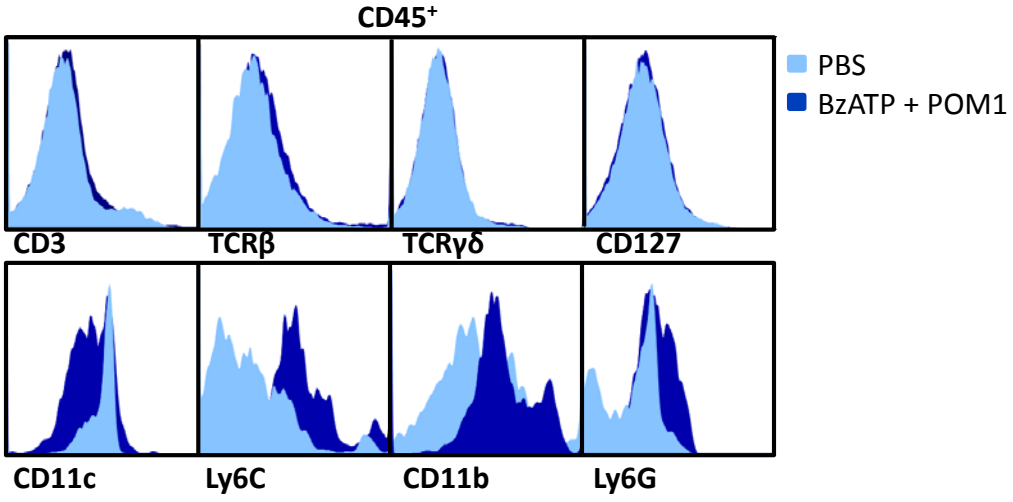

Figure S4. Inflammatory infiltrate induced following P2X7R stimulation. Expression of CD3, TCRβ, TCR γδ, CD127, CD11c, CD11b, LY6C, and LY6G (Gated on $CD45^+$ cells) on cutaneous inflammatory infiltrates following PBS or BzATP + POM1 treatments on day 5. Four mice per treatment group were divided into two tubes for staining. One representative of two independent experiments.

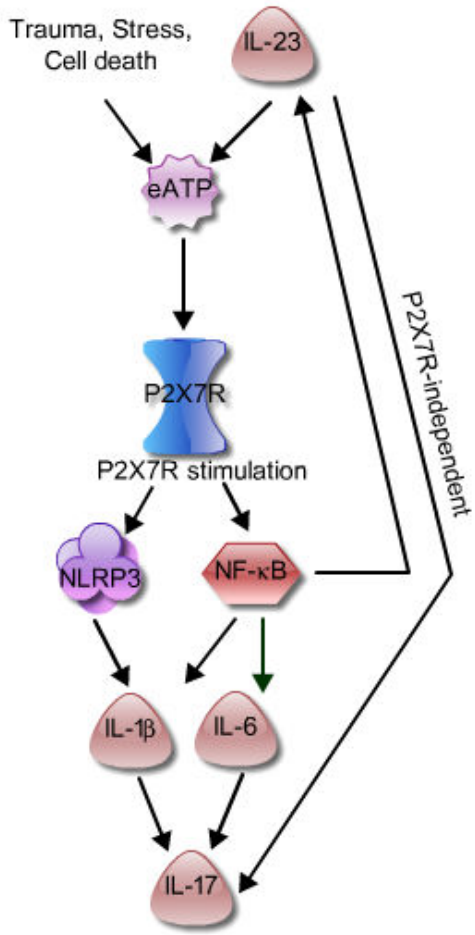

Figure S5. IL-23/ATP inflammatory pathway. ATP is released into the extracellular (eATP) microenvironment following initial trauma, stress, or cell death. Additionally, IL-23 can also lead to the secretion of eATP, likely following DC activation. eATP then activates the P2X7R stimulating a variety of signaling pathways, including the activation of the NLRP3 inflammasome and NF-κB translocation. Together these pathways induce the secretion of IL-1β, IL-6, and IL-23, which can then lead to the expression of IL-17. Moreover, there are likely P2X7R-independent pathways that lead to IL-17 expression following IL-23 secretion. For instance, the IL-1α or IL-36 pathways may induce an alternative inflammatory circuit.

Supplemental Materials and Methods

Antibody	Clone	Dye	Manufacturer
CD3	145-2C11	BUV395	BD Biosciences
CD127	A7R34	PE	eBiosciences
CD45.2	104	PerCP/Cy5.5	BD Biosciences
CD11b	M1/70	V450	BD Biosciences
CD11c	HL3	PE/Cy7	BD Biosciences
TCRβ	H57-597	BV421	Biolegend
TCRλδ	GL3	PE/Cy7	Biolegend
Ly6C	HK1.4	Alexa 488	Biolegend
Ly6G	IA8	Alexa 647	Biolegend
IL-17a	TC11-18H10	V450	BD Biosciences
Lineage	CD3/GR-1/CD11b /B220/Ter-119	Fitc	Biolegend