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LETTER

CXCL17 induces activation of human mast cells via MRGPRX2

To the Editor,

Mast cells (MCs) play critical roles in allergic disease, canonically by activation through the IgE-dependent pathway. However, MCs can also be activated by IgE-independent mechanisms with the Mas-related G protein-coupled receptor X2 (MRGPRX2) being extensively studied. MRGPRX2 is found on connective tissue MCs with abundant expression in the skin. Human MRGPRX2 is activated by a range of endogenous polycationic inflammatory peptides such as host defence peptides and neuropeptides (e.g., LL-37 and substance P) but with relatively low potency.[1] Thus, it is plausible that additional endogenous agonists at MRGPRX2 have important physiological/pathophysiological roles.

The novel chemokine CXCL17 is expressed in mucosal tissues and has suggested antimicrobial roles besides being a regulator of cell chemotaxis and inflammation.[2] A previous study suggested that the orphan G protein-coupled receptor (GPCR) GPR35 acts as the receptor for CXCL17, but this observation remains controversial.[2] Given the presence of polycationic regions within CXCL17, we hypothesized that it might act as a MRGPRX2 agonist and that this action might be of importance in inflammatory conditions where CXCL17 expression is upregulated.

To determine whether CXCL17 activates human MCs via the MRGPRX2 pathway, we utilized the LAD2 MC line that natively express MRGPRX2 and FcεRI receptors, and *MRGPRX2* knockdown LAD2 cells (*MRGPRX2*-KD; Figure S1A). CXCL17, MRGPRX2 agonists LL-37 and compound 48/80 (C48/80), induced calcium mobilization (Figure 1A) and degranulation measured by β-hexosaminidase release (Figure 1B) and enhanced surface expression of CD63 (Figure S1B) in wild-type (WT) LAD2 cells with responses being markedly dampened in *MRGPRX2*-KD cells. Antigen (NIP-BSA)-induced IgE-dependent responses were unaltered in *MRGPRX2*-KD LAD2 cells (Figure 1A,B; Figure S1). The MRGPRX2 dependency of this effect was also confirmed through the inhibitory action of the MRGPRX2 inverse agonist compound C9[3] (Figure 1C). In addition, CXCL17 strongly synergized with the alarmin IL-33, a known MC activator that is upregulated in psoriasis, to induce CCL2 release from LAD2 cells (Figure 1D). CXCL17 also triggered β-hexosaminidase and histamine release from purified rat peritoneal MCs, presumably via the rat homologue of *MRGPRX2*, *MrgprB3* (Figure S2).

The mRNA levels of *CXCL17* and *MRGPRX2* have been reported to be increased in psoriatic skin compared with healthy

Abbreviations: C48/80, compound 48/80; GPCRs, G protein-coupled receptors; MCs, mast cells; MRGPRX2, Mas-related G protein-coupled receptor X2; MRGPRX2-KD, MRGPRX2 knockdown; NIP, 4-hydroxy-3-iodo-5-nitrophenylacetyl; WT, wild type.

skin biopsies.[4,5] We therefore performed immunohistochemistry and conducted an analysis of spatial transcriptomic data from biopsies from non-lesional and lesional psoriatic skin to establish the in vivo connection between CXCL17 and MRGPRX2. By immunohistochemistry, the expression of CXCL17 was increased in psoriatic lesional skin compared with non-lesional skin, mainly in the epidermis (Figure 2). Mast cells, as identified by tryptase and MRGPRX2 staining, were clearly observed in both lesional and non-lesional skin sections (Figure 2A). In addition, spatial transcriptomics analysis (Figure 2B; Figure S3) showed enhanced expression of the MC marker tryptase β2 (*TPSB2*) in lesional psoriatic skin compared with non-lesional skin, suggesting increased MC numbers or increased *TPSB2* gene expression perhaps indicative of MC activation. Concordant with the immunohistochemistry data, spatial transcriptomics also showed localization of *CXCL17* expression to tissue areas containing MCs (as identified by *TPSB2* expression) largely within the epidermis (*see supplementary information for a more comprehensive description of the spatial transcriptomics data analysis*). In summary, we demonstrate that CXCL17 activates human MCs in a concentration-dependent manner, via the MRGPRX2 pathway. CXCL17 is one of the more potent MRGPRX2 agonists identified among the family of known MRGPRX2-activating antimicrobial peptides. We also demonstrate that CXCL17 expression is proximal to that of MRGPRX2-positive MCs in psoriatic skin. Our data suggest that in psoriasis, CXCL17 release from keratinocytes causes the activation of human MCs via MRGPRX2. CXCL17 might also act synergistically with other MC stimuli enhancing the importance of the CXCL17-MC axis in features of psoriasis such as itch in which MC MRGPRX2 has been highlighted.[6] Other psoriasis-associated antimicrobial peptide mediators[7] that are known MRGPRX2 agonists (e.g., LL-37 and β-defensins) may also contribute to this pathway. Further studies are required to characterize the unique pathobiological importance of CXCL17-induced MC activation in psoriasis and other non-communicable inflammatory skin diseases.

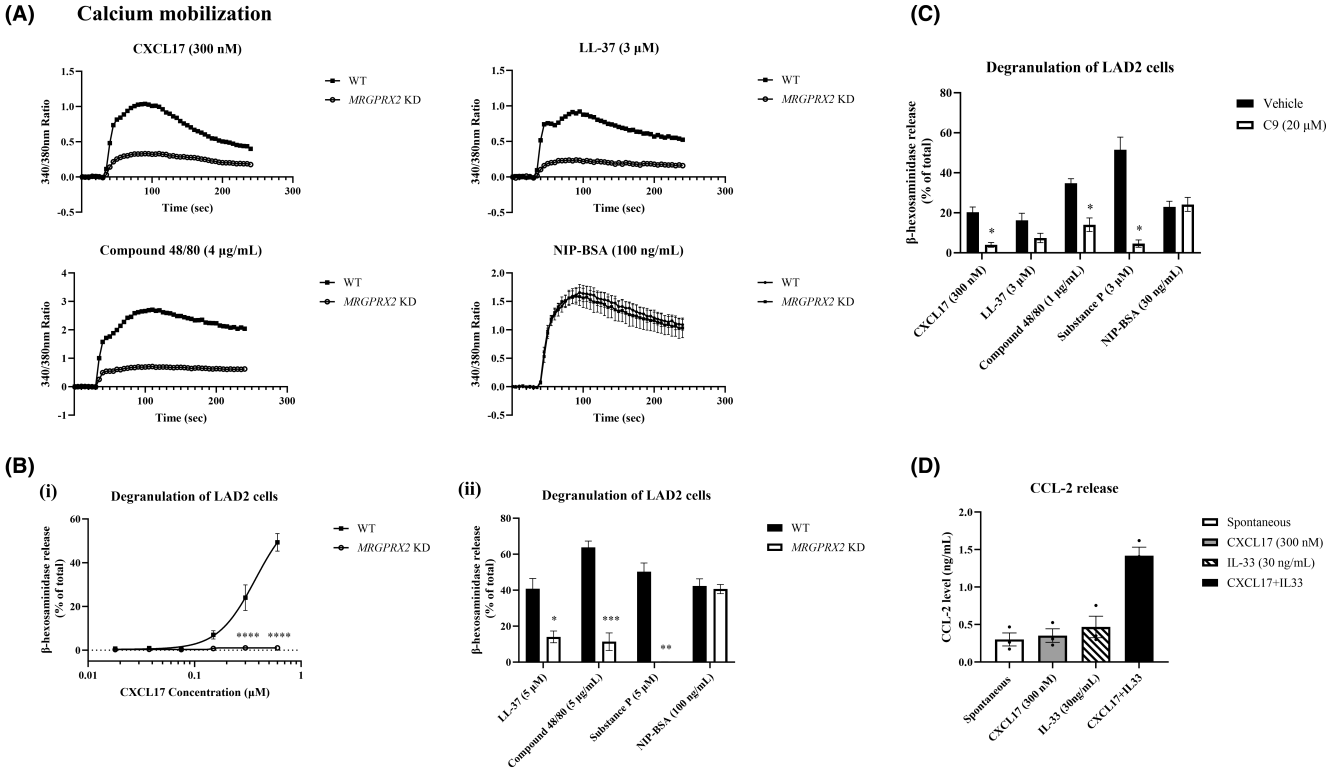

FIGURE 1 CXCL17 induces human mast cell activation via MRGPRX2. CXCL17, LL-37, C48/80 and NIP-BSA triggered (A) calcium mobilization (Fura-2) and (B) β-hexosaminidase release in wild-type LAD2 cells (WT). CXCL17-, LL-37- and C48/80-induced responses were reduced in *MRGPRX2*-knockdown LAD2 cells (KD). (C) Compound C9 inhibits CXCL17-, LL-37- and C48/80-induced β-hexosaminidase release in wild-type LAD2 cells. Data represent mean ± SEM, $n = 4$. Statistical significance was determined by (B) (i) two-way ANOVA with Sidak's multiple comparisons test, $^{****}p < .0001$. (B) (ii) and (C) unpaired *t* test with the Welch correction, $^{*}p < .01$, $^{**}p < .001$, $^{***}p < .0001$. (D) CXCL17 and IL33 work synergistically to induce CCL2 release in LAD2 cells. Mean ± SEM, $n = 3$.

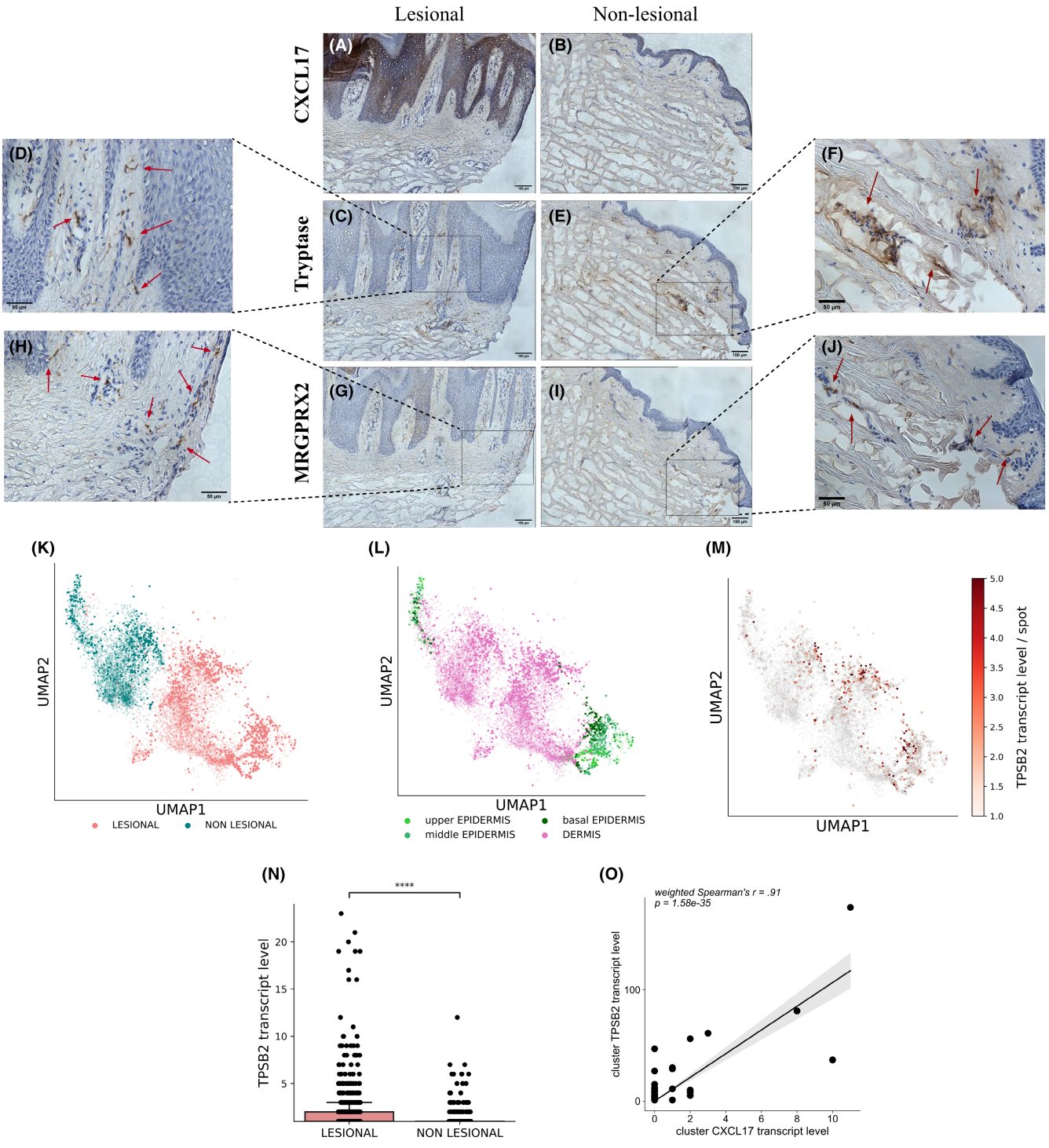

FIGURE 2 Increased expression of CXCL17 in psoriatic skin is spatially proximal to mast cells. (A–J) Representative immunohistochemistry images of lesional and non-lesional skin from one of the two patients with psoriasis, with tile images at 20× magnification and digitally zoomed areas of interest. The expression of CXCL17 was dramatically elevated in lesional psoriasis skin with MRGPRX2-positive and tryptase-positive mast cells localized proximally. Scale bar (tile images) = 100 μm. Scale bar (zoomed in images) = 50 μm. Red arrows show tryptase- and MRGPRX2-positive staining. Spatial transcriptomics analysis of paired lesional and non-lesional skin biopsies from three patients with psoriasis. UMAP plots of (K) biopsy type; (L) anatomical layer; (M) localization of mast cells (as identified by *TPSB2* expression). (N) Mast cell counts are increased in lesional skin compared with non-lesional skin; statistical significance was determined by the one-sided Mann–Whitney–Wilcoxon test, ****$p = 6.421e\text{-}06$. (O) *CXCL17* gene expression positively correlates to MC-tryptase (*TPSB2*) expression.

AUTHOR CONTRIBUTIONS

GAM and JD designed and planned the study; JD conducted experiments and collected the majority of data; CH performed spatial transcriptomics analysis; CWW, NAF, HA, JSK and MPM generated key tools, reagents and clinical samples and provided expertise on their use; JD and GAM wrote the draft manuscript; CWW, CH, MPM, HA and JSK provided critical insight into the generated data and revised the draft manuscript. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest. CWW is now an employee of Dimerix Ltd, which had no involvement in or contribution to the project.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.