**Supplementary** **Materials**



**Figure S1. Isolation of Skin Blister ILC2**. **A.** Flow cytometry antibody stain for skin blister ILC2. ILC2 are CD45+/CD3-/Lineage-/CRTH2+/IL-7Rα+. **B.** Full gating strategy for isolation and analysis of blood ILC2. **C.** Isotype controls for strategy of isolation and analysis of ILC2. **D.** Representative flow cytometry plots for ILC2 uptake of MDP-Rhodamine. ILC2 were pre-stimulated as indicated with Pam3CSK4 (10µg/ml)or IL-33 and PGD2 (50ng/ml and 100nM) (U: unstimulated) for 1 hour prior to addition of MDP-rhodamine (MDP-Rho, 5µg/ml for 3 hours), unconjugated rhodamine (rhodamine, 5µg/ml for 3 hours) or media alone (- control).

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**Figure S2. NOD2 expression analysis of ILC2.** **A.** Flow cytometry analysis of NOD2 protein in ILC2 from different sources, (HC: *ex vivo* blood analysis, Skin: *ex vivo* skin analysis and Cultured ILC2: ILC2 which have been isolated, expanded and cultured for 6-8 weeks). ILC2 were stimulated for 24 hours with Pam3CSK4 (10µg/ml) or IL-33 (50ng/ml) and PGD2 (100nM) (n=7, one-way ANOVA with Tukey’s multiple comparison test, data representative of 5 independent experiments). **B.** Flow cytometry analysis of NOD2 protein in ILC subsets *ex vivo* within PBMC and skin biopsy-derived mononuclear cells. (n=4 blood donors (black) and n=3 skin donors (red), one-way ANOVA with Tukey’s multiple comparison test, data representative of 3 independent experiments).



**Figure S3. Imaging of MDP-rhodamine uptake by activated ILC2.** Representative confocal microscopy images of blood-derived ILC2 cytospins. ILC2 were pre-stimulated for 1 hour as indicated with Pam3CSK4 (10µg/ml) or IL-33 (50ng/ml) and PGD2 (100nM) and then incubated for 3 hours with MDP-rhodamine (5µg/ml, or unconjugated rhodamine control).

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**Figure S4. NOD2 and TLR2 stimulation of ILC2.** Real-time PCR analysis of **A.** cytokine or **B.**  NOD2 gene expression by ILC2 following 6 hour stimulation with MDP (1µg/ml) and/or Pam3CSK4 (10µg/ml). Gene expression normalized to *GAPDH*. (n=5-13, one-way ANOVA with Tukey’s multiple comparison test, data representative of 5 independent experiments).



**Figure S5. Skin resident bacterial preparation stimulation of ILC2.** **A.** Full gating strategy for analysis of skin biopsy ILC2. **B.** Representative flow cytometry plots of ILC2 cytokine protein production assessed *ex vivo* by intracellular flow cytometry after 24 hour stimulation of skin biopsy-derived mononuclear cells with heat-killed preparations of *S. aureus* or *S. epidermidis*, or IL-33 (50ng/ml). **C.** Isotype FMO control and representative flow cytometry plots of gating strategy for detection of ILC2 cytokine production following 24 hour stimulation with MDP (1µg/ml) and/or Pam3CSK4 (10µg/ml) measured by intracellular flow cytometry in PBMCs from a healthy volunteer.



**Figure S6. ILC2 NOD2 mutation analysis. A.** CLA ILC2 surface expression measured by flow cytometry following 24 hour stimulation with MDP (1µg/ml), Pam3CSK4 (10µg/ml), IL-33 (50ng/ml) or PGD2 (100nM), in PBMC from healthy volunteers with wild type *NOD2* gene expression (WT *NOD2*) or patients with loss of function *NOD2* mutations (*NOD2* SNPs). (n=4-6, two-way ANOVA with Dunnett’s multiple comparison test, data representative of at least 6 independent experiments). **B.** Allelic discrimination plots of R702W mutation *NOD2* Taqman genotyping assay of AD patients (upper plot n=11) and total samples (lower plot n=11 AD patients and n=7 healthy volunteers). **C.** Table of genotyping results for R702W mutation *NOD2* Taqman genotyping assay. **D.** Induction of ILC2-derived IL-6 following 24 hour stimulation with MDP (1µg/ml), Pam3CSK4 (10µg/ml), heat-killed preparations of SA (*S. aureus*), SE (*S. epidermidis*) or IL-33 (50ng/ml) and PGD2 (100nM), measured by intracellular flow cytometry in PBMC from atopic dermatitis (AD) patients with heterozygous R702W mutation or wild type (WT) *NOD2* alleles. (n=4 WT and n=3 R702W), one-way ANOVA with Sidak’s multiple comparison test, data representative of 2 independent experiments.

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**Figure S7. Skin and blood ILC2 may be capable of autophagy.** Autophagy-associated gene expression of skin blister and blood derived ILC2 determined by RNA-Seq and measured in RPKM following 24 hour HDM blister challenge.



**Figure S8. ILC2 LC3-II gating strategy.** Isotype FMO control and representative flow cytometry plots gating strategy for detection of ILC2 LC3-II following 24 hour stimulation with MDP (1µg/ml), Pam3CSK4 (10µg/ml), heat-killed preparations of SA (*S. aureus*), SE (*S. epidermidis*), PA (*P. aeruginosa*) or IL-33 (50ng/ml) measured by intracellular flow cytometry in ILC2 isolated and expanded from healthy volunteer blood.