## Supplementary Figures



### Supplementary figure S1

Aqua Live-dead flow cytometric viability analysis of unstimulated PMNs after 4 hours in culture (A, n=9 combined from several experiments) and including chloroquine (CQ) 30 min pre-incubation (B, n=5-9). (C) as in B but including CpG stimulation and ELISA analysis as indicated (n=7). (D) TNF release (triplicate ELISA) of WT (n=4) vs. *Unc93b13d/3d* (n=3) murine BM-PMN stimulated as indicated for 4 h. A-D represent combined data (mean+SD) from ‘n’ biological replicates (each dot represents one donor/mouse). \* p<0.05 according to Wilcoxon signed rank sum (B) and one-way ANOVA with Sidak correction (C, D).



### Supplementary figure S2

Luminex multiplex cytokine analysis of PMN supernatants (screening analysis). Mean values of TNF-α, IL-1β, IL-6, IL-16 and MIP-1β for n=2 donors shown.



### Supplementary figure S3

ELISA of IL-8 (A) or MIP-1β (B) secreted from psoriasis PMNs (n=3) or PMNs from sex-and age-matched healthy donors (n=3-10) in response to LPS treatment. Combined data (mean+SD) from ‘n’ biological replicates (each dot represents one donor). \* p<0.05 according to Kruskall-Wallis test with Dunn’s correction (A, B).



### Supplementary figure S4

Flow cytometric cell count of migrated CD4 T cells (A), CD8 T cells (B) and CD14+HLA-DR+ monocytes (C) quantified in transwell assays with total PBMCs in the upper and SDF-1α (positive control) in the lower compartment (A-C, n=6-7, p>0.05 for treatments vs. media according to Friedman or one-way ANOVA test, respectively). A-C represent combined data (mean+SD) from ‘n’ biological replicates (each dot represents one donor). \* p<0.05 according to Friedmann test with Dunn’s correction (A, B), or one-way ANOVA with Dunnett’s correction for multiple testing (C).



### Supplementary figure S5

Specificity of SYTO RNAselect staining: Fixed NETs were treated with RNase A or buffer control (n=4) and quantification of pixel values of nuclear or NET events scored by a blinded observer in the RNA (A) or DNA channels (B) is shown for one representative experiment. Microscopy analysis of PMN stimulated with either RNA-Alexa647 and unlabeled LL37 (C) or unlabeled RNA and LL37-Atto488 (D) to distinguish exogenously added RNA (red in C) or LL37 (green in D) from endogenously released RNA (green in C, SYTO RNAselect) or LL37 (red in D, anti-LL37 and Alexa647 conjugated secondary Ab). n=2 for both experiments. (E) Staining for RNA in granule-like patterns observed for selected PMNs. In A-E one representative of ‘n’ replicates is shown. \* p<0.05 according to one-way ANOVA with Holm-Sidak’s correction (A) or Kruskal-Wallis test with Dunn’s correction (B) to adjust for multiple testing.



### Supplementary figure S6

NF-κB dual luciferase reporter assay in HEK293 cells, transfected with NF-κB firefly luciferase reporter, *Renilla* control reporter and plasmids for either TLR9 (A), empty vector (B) or MyD88 (C), subsequently stimulated with CpG ODN (A), recombinant TNF (B) or left unstimulated (C), without (arrow) or with IRS661, IRS954, IRS869 and IRS546 (n=2 each). (D) Aqua Live-dead flow cytometric viability analysis of PMNs treated with IRS661 and IRS954 only (4 h, n=5-9). (E) IL-8 (release from PMNs stimulated with LPS (E, n=4-6) with or without IRS661 (1 nM), IRS954 (50 nM) pre-incubation (30 min) quantified by ELISA. (E) as in D but stimulation with RNA-LL37 and pre-incubation with chloroquine and measuring MIP-1β release (CQ, 10 µM, n=6). D-F represent combined data (mean+SD) from ‘n’ biological replicates (each dot represents one donor). In A-C one representative of ‘n’ replicates is shown (mean+SD). \* p<0.05 according to two-way ANOVA (A), one-way ANOVA with Dunnett’s correction (only TNF or MyD88 group, respectively, B, C), Kruskall-Wallis test with Dunn’s correction (D) or one-way ANOVA with Sidak correction (E, F).



### Supplementary figure S7

Graphical summary of key findings.

## Supplementary Tables

**Table S1: Summary of primary PMN responses across experiments.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Analyte | LPS | PMA | R848 | RNA | Pam2 | Pam3 | CpG DNA | genomic DNA/ssDNA | RNA  + LL37 | genomic DNA/ssDNA + LL37 |
| IL-8 | ++ | +++ | ++ | +/- | +++ | ++ | +++ | - | ++ | - |
| CD62L | +++ | +++ | - | - | +++ | ++ | +++ | - | + | - |
| ROS | +/- | +++ | - | - | - | - | - | - | - | - |

### Table S2: Commercial TLR ligands and inhibitors

|  |  |  |
| --- | --- | --- |
| Component | company | Product no. |
| LL37 | InvivoGen | tlrl-l37 |
| LPS-EK (ultrapure) | InvivoGen | tlrl-peklps |
| R848 (Resiquimod) | InvivoGen | tlrl-r848-5 |
| Chloroquine | InvivoGen | tlrl-chq |
| PMA | InvivoGen | tlrl-pma |
| Pam2CSK4 | InvivoGen | tlrl-pm2s-1 |
| Pam3CSK4 | InvivoGen | tlrl-pms |

### Table S3: RNA/DNA and inhibitors

|  |  |  |
| --- | --- | --- |
| Component | Sequence | company |
| CpG2006 | 5’TsCsGsTsCsGsTsTsTsTsGsTsCsGsTsTsTsTsGsTsCsGsTsT3’ | TIB MOLBIOL |
| RNA40 | 5’GsCsCsCsGsUsCsUsGsUsUsGsUsGsUsGsAsCsUsC3’ | iba |
| ssDNA60 | 5’AC(AC)28AC3’ | TIB MOLBIOL |
| IRS546 | 5’TsCsCsTsGsCsAsGsGsTsTsAsAsGsT3’ | TIB MOLBIOL |
| IRS661 | 5’TsGsCsTsTsGsCsAsAsGsCsTsTsGsCsAsAsGsCsA3’ | TIB MOLBIOL |
| IRS869 | 5’TsCsCsTsGsGsAsGsGsGsGsTsTsGsT3’ | TIB MOLBIOL |
| IRS954 | 5’TsGsCsTsCsCsTsGsGsAsGsGsGsGsTsTsGsT3’ | TIB MOLBIOL |
| ssRNA40 was obtained from IBA. The backbone is phosphorothioate. | | |

### Table S4: Antibodies and recombinant proteins

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Item | fluorophore | species | isotype | company | Product no. |
| Isotype control | PE | mouse | IgG1 kappa | eBioscience | 12471442 |
| Isotype control | FITC | mouse | IgM | BioLegend | 401605 |
| Isotype control | APC | mouse | IgG1 kappa | BD Bioscience | 550854 |
| Isotype control | BV421 | mouse | IgG1 kappa | BioLegend | 400157 |
| Isotype control | AF488 | mouse | IgG2a kappa | BioLegend | 400233 |
| Isotype control | FITC | mouse | IgG2b | BioLegend | 401206 |
| Anti-hCD15 | PE | mouse | IgG1 kappa | BioLegend | 323006 |
| Anti-hCD66b | FITC | mouse | IgG1 kappa | BioLegend | 305103 |
| Anti-hCD62L | BV421 | mouse | IgG1 kappa | BioLegend | 30482 |
| Anti-hCD14 | PE | mouse | IgG1 kappa | ImmunoTools | 21620144 |
| Anti-hCD3 | AF488 | mouse | IgG2a | BioLegend | 317310 |
| Anti-hCD4 | PE | mouse | IgG1 kappa | BioLegend | 300508 |
| Anti-hCD8 | APC | mouse | IgG1 kappa | ImmunoTools | 21810086 |
| Anti-hHLA-DR | FITC | mouse | IgG2b kappa | BioLegend | 327006 |
| Recombinant hMIP-1β | - | - | - | ImmunoTools | 11343223 |
| Recombinant hIL-16 | - | - | - | ImmunoTools | 11340163 |
| Recombinant hSDF-1α | - | - | - | ImmunoTools | 11343363 |
| SYTO RNAselect | n/a | - | - | ThermoFisher | S32703 |
| Anti-hLL37 | unconjugated | rabbit | IgG | LSBio | LS-B6696-500 |
| Anti-hNeutrophil Elastase | unconjugated | mouse | IgG1 | Novus Biologicals | MAB91671-100 |
| Anti-rabbit | AF647 | chicken | IgG | ThermoFisher | A-21443 |
| Anti-mouse | AF594 | chicken | IgG1 | ThermoFisher | A-21201 |

### Table S5: Plamids used for HEK293T transfection

|  |  |  |
| --- | --- | --- |
| Plasmid name Insert | Vector backbone | Insert |
| EGFP | pC1-EGFP | EGFP |
| NF-κB reporter | pGL3 | 6x NFKB response element |
| Renilla | pRL-TK | Renilla |
| hTLR7 | pcDNA3.1 (+) | hTLR7 |
| hTLR8 | pcDNA3.1 (+) | hTLR8 |
| hTLR9 | pEF-SEM | hTLR9 |
| MyD88 | pTO-N-SH Streptag  N-terminal Gateway | hMyD88 FL aa13-296  L265P, Stop-codon |
| pcDNA3.1 | pcDNA3.1 (+) | Empty |
| pEF-SEM | pEF-SEM | Empty |

### Table S6: 10x Ammoniumchloride erythrocyte lysis buffer

|  |  |  |
| --- | --- | --- |
| Compound | company | Product no. |
| 1.54 M NH4Cl | Roth | 5470.1 |
| 100 mM KHCO3 | Fluka | 60220 |
| 1 mM EDTA; pH 8 | ThermoFisher | 15575020 |
| dissolved in Ampuwa water | Fresenius Kabi | 1833 |
| pH adjusted to 7.3, sterile filtered (0.22 µm) |  |  |