Thrombin-derived host defence peptide modulates neutrophil rolling and migration *in vitro* and functional response *in vivo*

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Supplementary Information

Supplementary Figure 1: Effects of GKY25 on PMN toxicity and functional ROS response. (A) PMNs (5 X 10^5 cells/ml) were treated with either IVE25 or GKY25 for up to 4 hrs at 37^{o}C before LDH activity measurement (n = 3). **(B)** PMNs (1 X 10^6 cells/ml) were pre-treated with either LPS, IVE25 or GKY25 for 1hr at 37^{o}C before being subjected to 25 nM PMA stimulation for ROS response (n = 5). One-way ANOVA. Figures are representative of 2 independent experiments.

Supplementary Figure 2: Assessment of GKY25 inhibition of LPS-induced ROS generation by flow cytometry. PMNs (1 X 10^6 cells/ml) pre-incubated with 10 µM H_2DCFDA for 30 min at 37^{o}C were treated with 25 nM PMA for 15 min as positive control or co-treated with 5 µM IVE25 or 5 µM GKY25 with 10 ng/ml LPS for 1 hr at 37^{o}C before flow cytometry assessment for **(A)** H_2DCFDA, **(B)** CD11b or **(C)** CD62L. (---) Untreated baseline. One-way ANOVA. Figures are representative of 2 independent experiments.

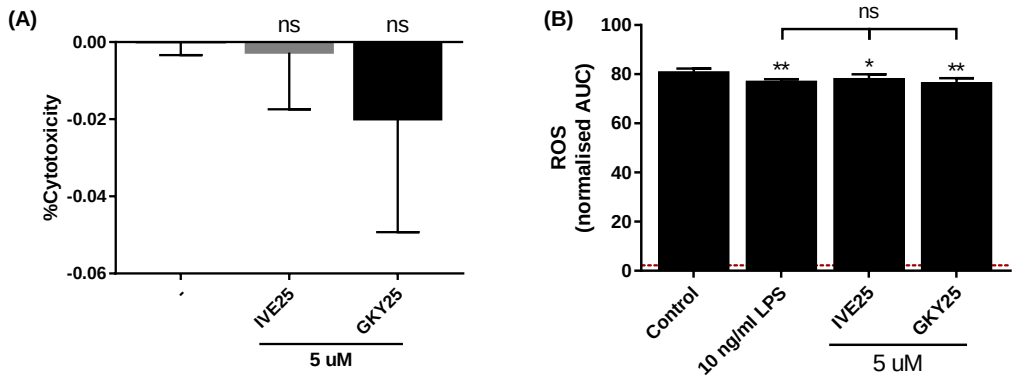
Supplementary Figure 3: Modulation of PSGL1 by GKY25. PMNs (5 X 10^5 cells/ml) isolated from 3 individuals were treated with 5 µM GKY25 for 1 hr at 37^{o}C before flow cytometry. Multiple T-tests.

Supplementary Figure 4: Rolling frequencies of PMNs on E-selectin coated PDMS microdevice. PMNs (1 X 10^6 cells/ml) were treated with 5 µM IVE25 or GKY25 before being subjected to microfluidics flow assessment. Rolling frequencies of GKY25-treated PMNs were

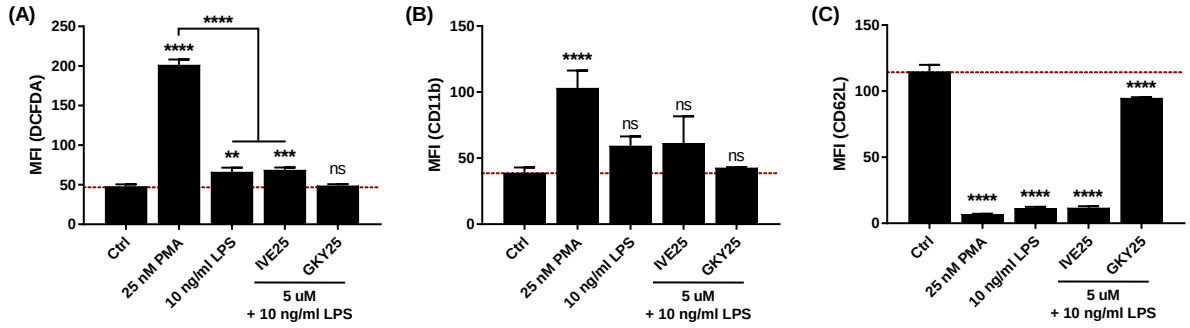
plotted with **(A)** untreated PMNs and **(B)** IVE25-treated PMNs. Figures were representative of 3 independent experiments.

Supplementary Figure 5: Neutrophil infiltration *in vivo*. (A-B) Mice were administered with LPS in the absence or presence of GKY25 before being sacrificed after 1 hr (n = 4). **(C-D)** Mice were also pre-treated with GKY25 intraperitoneally before LPS administration subcutaneously (n = 3). **(A, C)** Skin sections were stained with anti-neutrophil antibody (NIMP-R14, red) and DAPI (blue) and **(B, D)** used for scoring for neutrophil infiltration. Unpaired T-test.

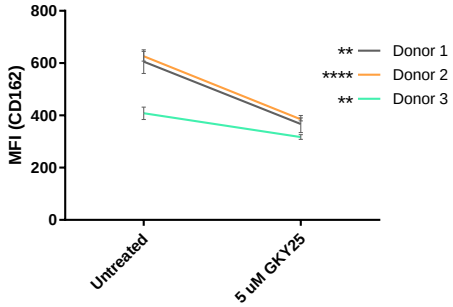
Supplementary Figure 1



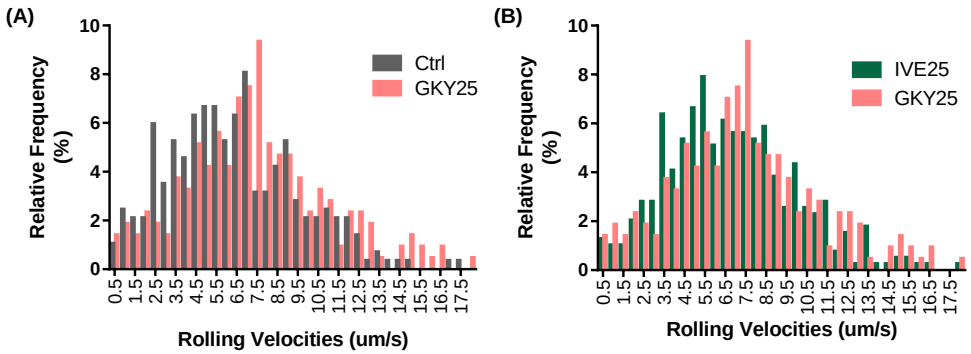
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5