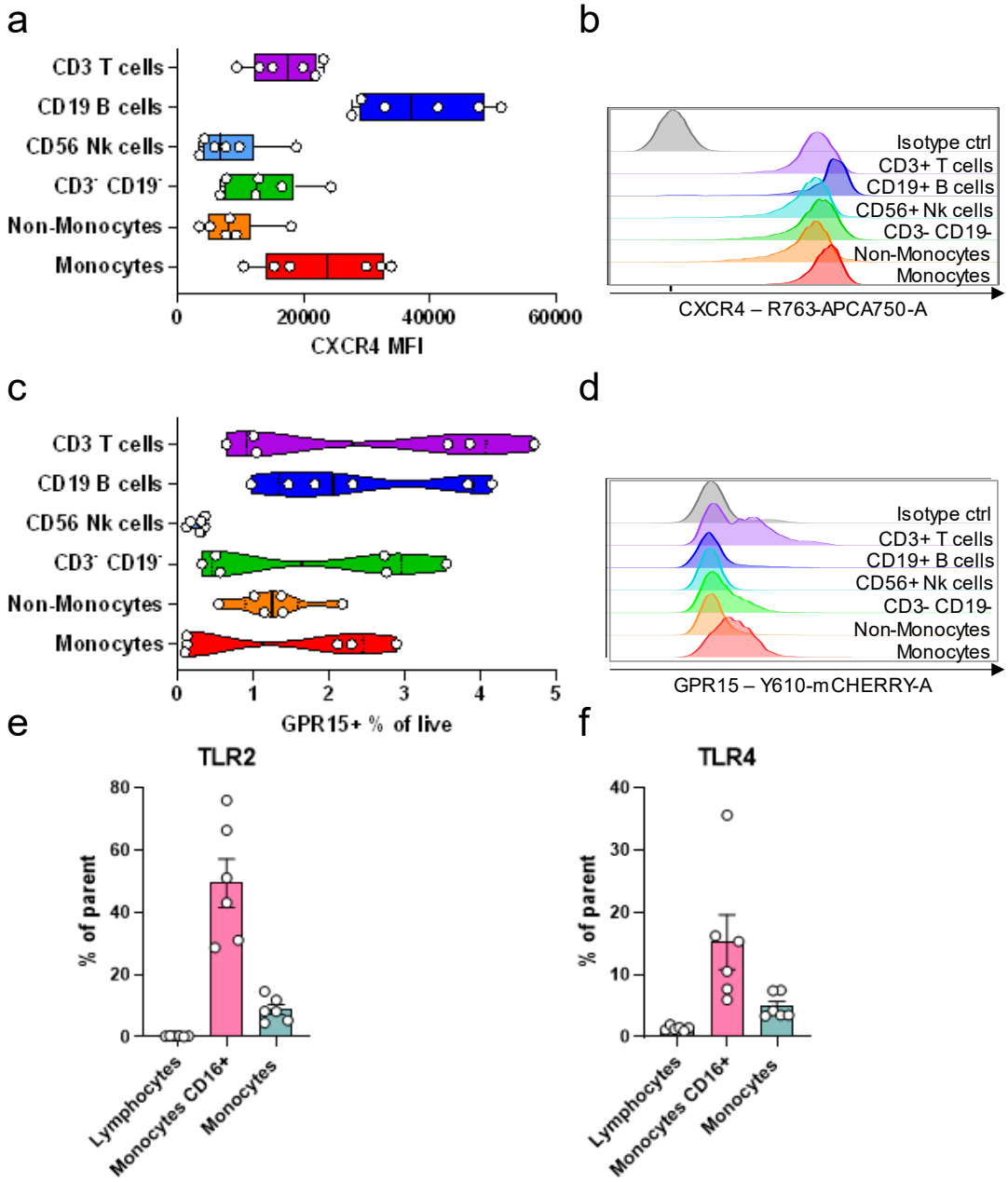
Supplementary Material

Supplementary Table 1: Flow Cytometry Antibody Overview

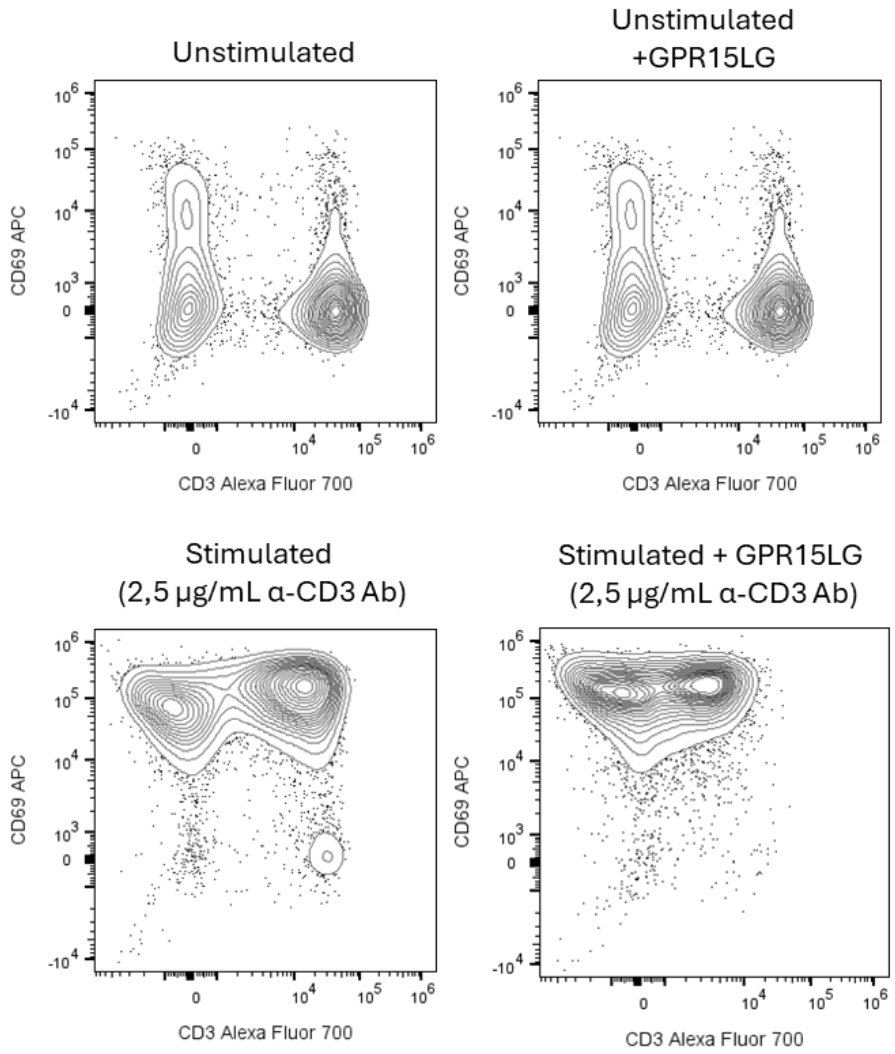
Target Antigen	Clone	Fluorochrome	Manufacturer	Catalogue Number	Working Dilution
CD3	OKT3	AF700	Biolegend	317340	1:100
CD4	OKT4	BV605	Biolegend	317438	1:100
CD8a	RPA-T8	PerCP/Cy5.5	Biolegend	301032	1:200
CD14	63D3	BV785	Biolegend	367142	1:33
CD16	3G8	BV421	Biolegend	302037	1:100
CD19	HIB19	BV421	Biolegend	302234	1:50
CD19	HIB19	FITC	Biolegend	302206	1:50
CD27	O323	BV785	Biolegend	302831	1:20
CD56	HCD56	BV650	Biolegend	318344	1:20
CD184 (CXCR4)	12G5	APC-Cy7	Biolegend	306528	1:33
GPR15	SA302A10	PE-Dazzle594	Biolegend	373012	1:20
CD80	2D10	PE	Biolegend	305207	1:100
CD69	FN50	PE/Dazzle594	Biolegend	310942	1:100
CD25	BC96	APC/Cyanine7	Biolegend	302614	1:100
CD163	GHI/61	APC	Biolegend	333610	1:33
CD11b	LM2	PerCP/Cy5.5	Biolegend	393106	1:33
HLA-DR	L243	PE-Cy7	Biolegend	307018	1:2500
CD107a	eBioH4A3	PE	Invitrogen	12-1079-42	1:20

Supplementary Table 2: IncuCyte Segmentation Parameters for the Phagocytosis Assay

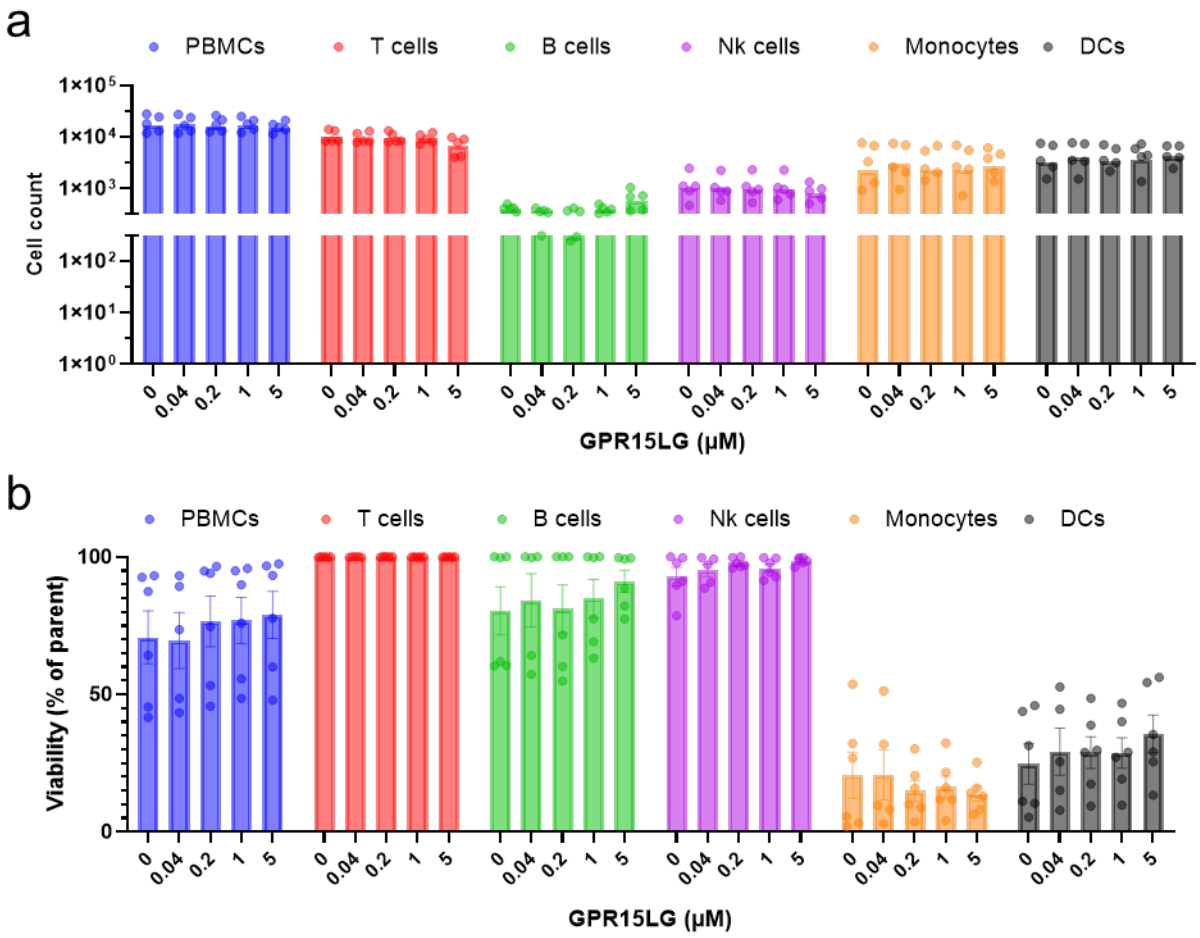
Parameter Name	Value
Basic Analyzer Type	Surface fit
Threshold	2
Edge split	on
Edge sensitivity	-30
Clean-up and hole fill	10 μm^2
Minimum red object area	300 μm^2
Adherent Cell-by-Cell Type	Boundary
Segmentation adjustment	0.6
Hole fill	100 μm^2
Cell detection sensitivity	2
Cell contrast	2
Cell morphology	3
Minimum area	300 μm^2
Minimum eccentricity	0.3



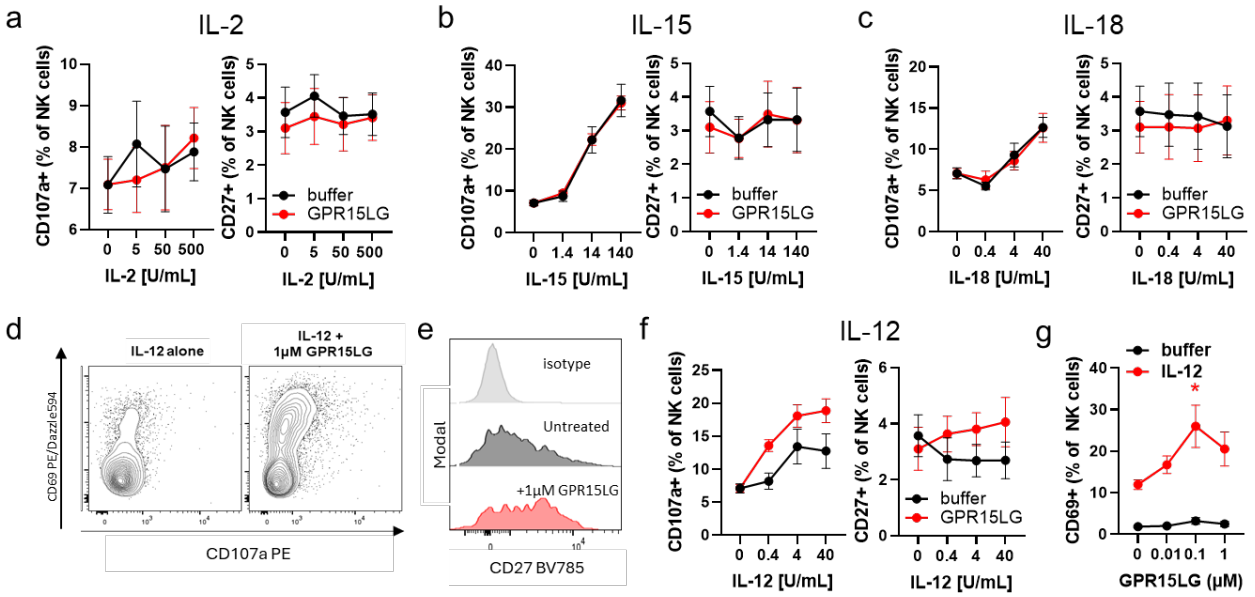
Supplementary Figure 1. Surface expression of CXCR4, GPR15 and TLRs on human peripheral blood immune-cell populations. Surface expression of CXCR4 (a,b), GPR15 (c,d) and TLRs (e,f) was analyzed by flow cytometry in the indicated PBMC populations, including CD4+ T cells, NK cells and monocytes. a,c) Representative histograms show receptor staining compared with the corresponding isotype control staining. b,d,e,f) Quantification of individual donors.



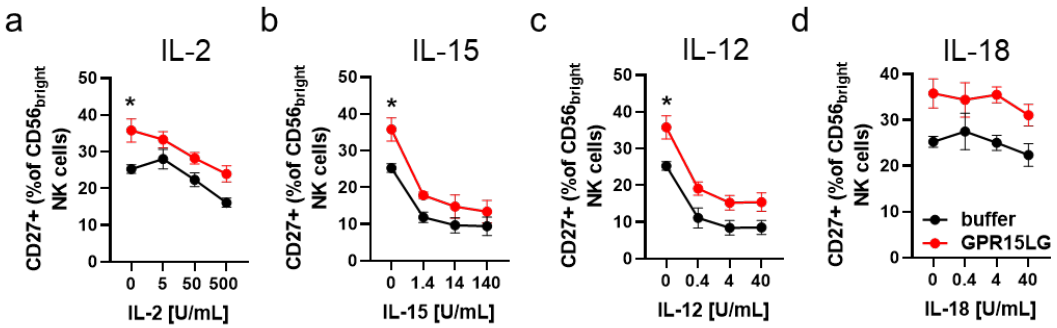
Supplementary Figure 2. GPR15LG leads to decreased CD3 surface presentation after CD3/CD28 stimulation. Representative contour plots of live singlet PBMCs CD69 and CD3 expression; cells were either left unstimulated or stimulated with 2.5 µg/mL α-CD3-Ab and α-CD28-sAb with or without 1 µM GPR15LG for 24 hours and markers were stained and analyzed by flow cytometry.



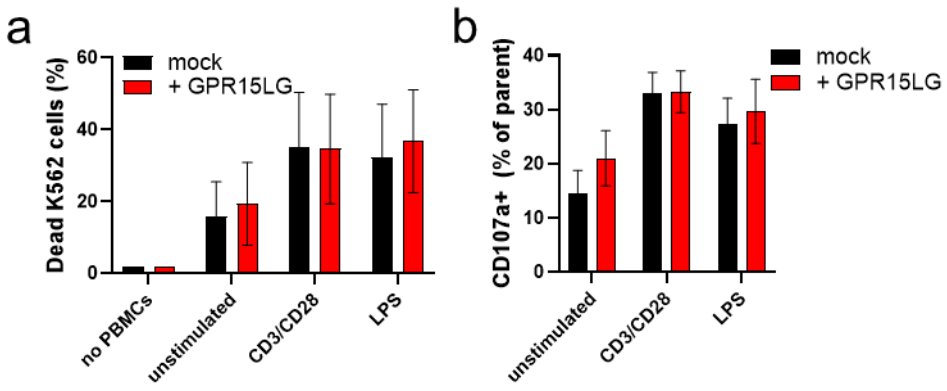
Supplementary Figure 3. GPR15LG is not toxic in cell culture at high concentrations. Viability from PBMC stimulation experiments was determined by fixable viability stain and flow cytometer analysis. n = 6 individual donors.



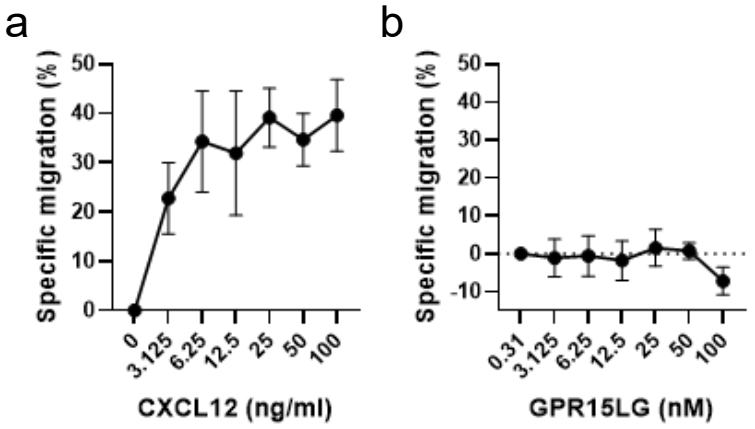
Supplementary Figure 4. GPR15LG enhances IL-12-induced NK cell activation. Freshly isolated NK cells were stimulated with titrations of recombinant cytokines IL-2, IL-15, IL-12 and IL-18 with and without 1µM GPR15LG (a-g). GPR15LG significantly enhanced IL-12-mediated activation, as measured by CD69, CD107a and CD27 expression and shown in representative plots in d,e. CD107a and CD27 expression were boosted in presence of GPR15LG following IL-12 stimulation (f). GPR15LG-induced activation enhancement was dose-dependent (g). N=3 donors, ±SEMs. (Statistics = two-way ANOVA with Šídák's multiple comparisons test).



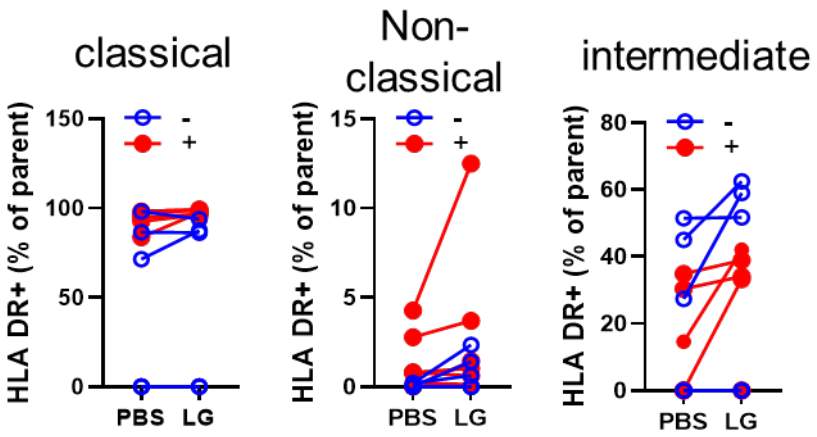
Supplementary Figure 5. GPR15LG enhances CD27 expression on CD56bright NK cells independent of activation. 1µM GPR15LG was applied to NK cells stimulated with rising concentrations of classical NK-activating cytokines: IL-2 (a), IL-15 (b), IL-12 (c) or IL-18 (d) and % of CD27+ cells was determined among CD56bright NK cells. N=3 donors, ±SEMs. (Statistics = two-way ANOVA with Šídák's multiple comparisons test).



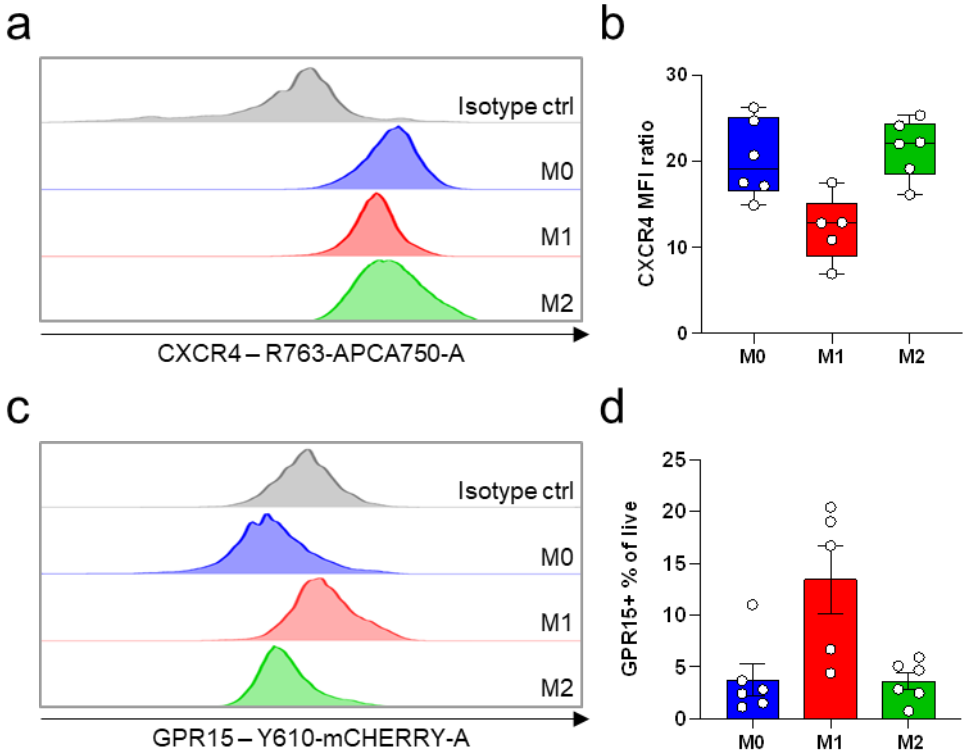
Supplementary Figure 6. GPR15LG does not affect cancer cell killing by NK cells. 8000 CellTrace Violet-prestained target cells (K562) were combined with 10x numbers of PBMCs and different stimuli and co-incubated overnight with or without GPR15LG to induce NK-mediated cell killing. After ~18h, cell co-cultures were stained and target cell viability as well as % of degranulated NK cells (CD107a+ of CD56+) cells was analyzed via flow cytometry. N= 3 donors measured in triplicates, bars show means ± SEMs. (Statistics = two-way ANOVA with Šídák's multiple comparisons test).



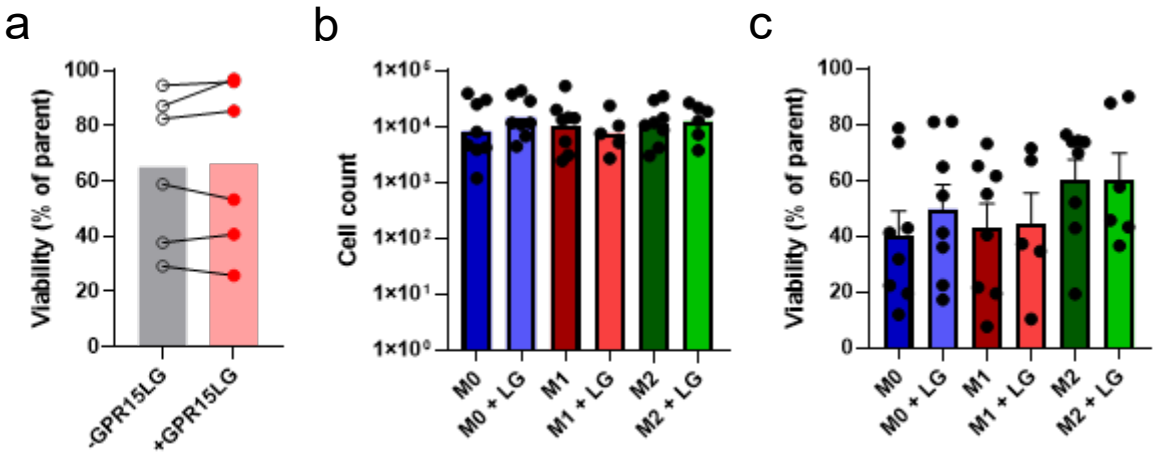
Supplementary Figure 7. GPR15LG does not induce NK cell migration. Enriched NK cells were allowed to migrate to either CXCL12 (a) or GPR15LG (b) in a transwell migration assay over 4 hours. N=5-6 donors, measured in triplicates, means ± SEMs.



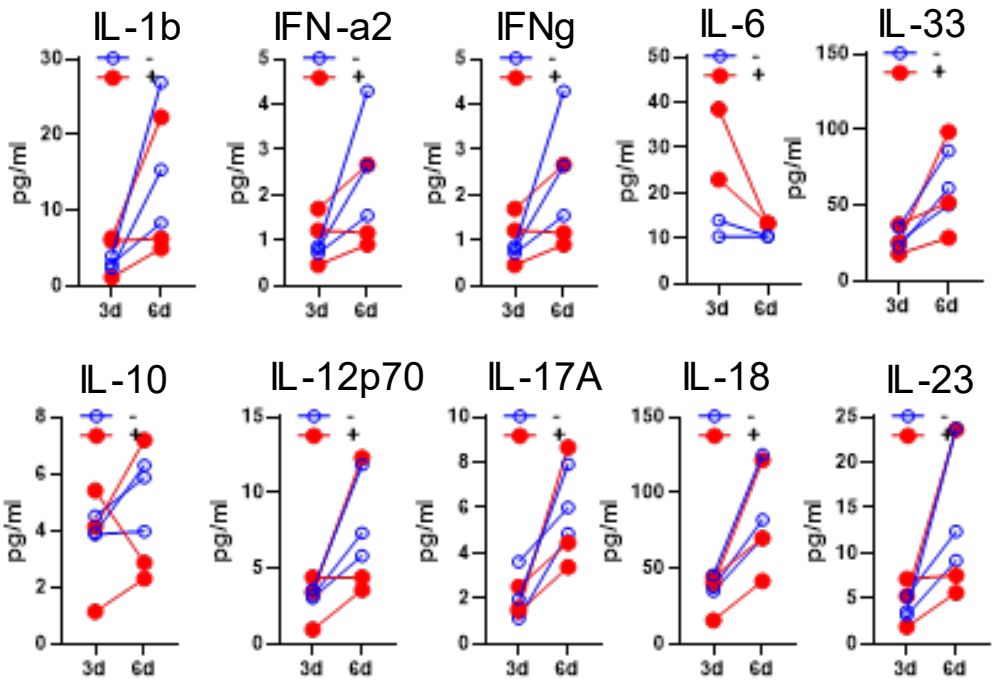
Supplementary Figure 8. GPR15LG does not alter HLA DR surface presentation on monocyte subpopulations. PBMCs were stimulated with 5 µg/mL α-CD3 antibody and 5 µg/mL α-CD28 antibody in presence or absence of 5 µM GPR15LG for 24 hours. Afterwards, HLA-DR expression on monocyte subsets was analyzed via flow cytometry. N=4, shown are means ± SEMs.



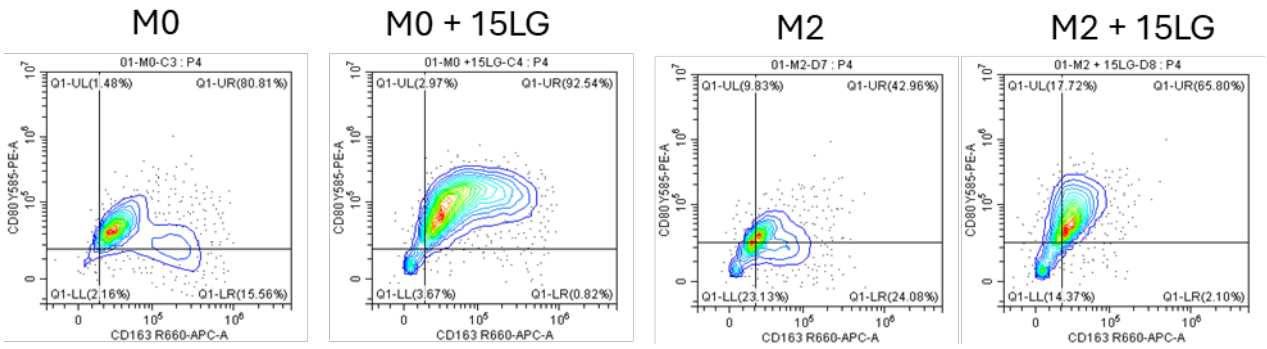
Supplementary Figure 9. CXCR4 and GPR15 surface expression on monocytes and monocyte-derived macrophages. Monocyte-derived macrophages polarized towards M0, M1 and M2 were stained for surface CXCR4 and GPR15 and analyzed by flow cytometry. a,c) Representative histogram of CXCR4 and GPR15 staining compared with the isotype control. b) CXCR4 MFI ratio relative to the isotype control. d) Frequency of GPR15+ cells among CD14+CD16+ monocytes. n = 5–6 donors, shown as median ± range or mean ± SEM.



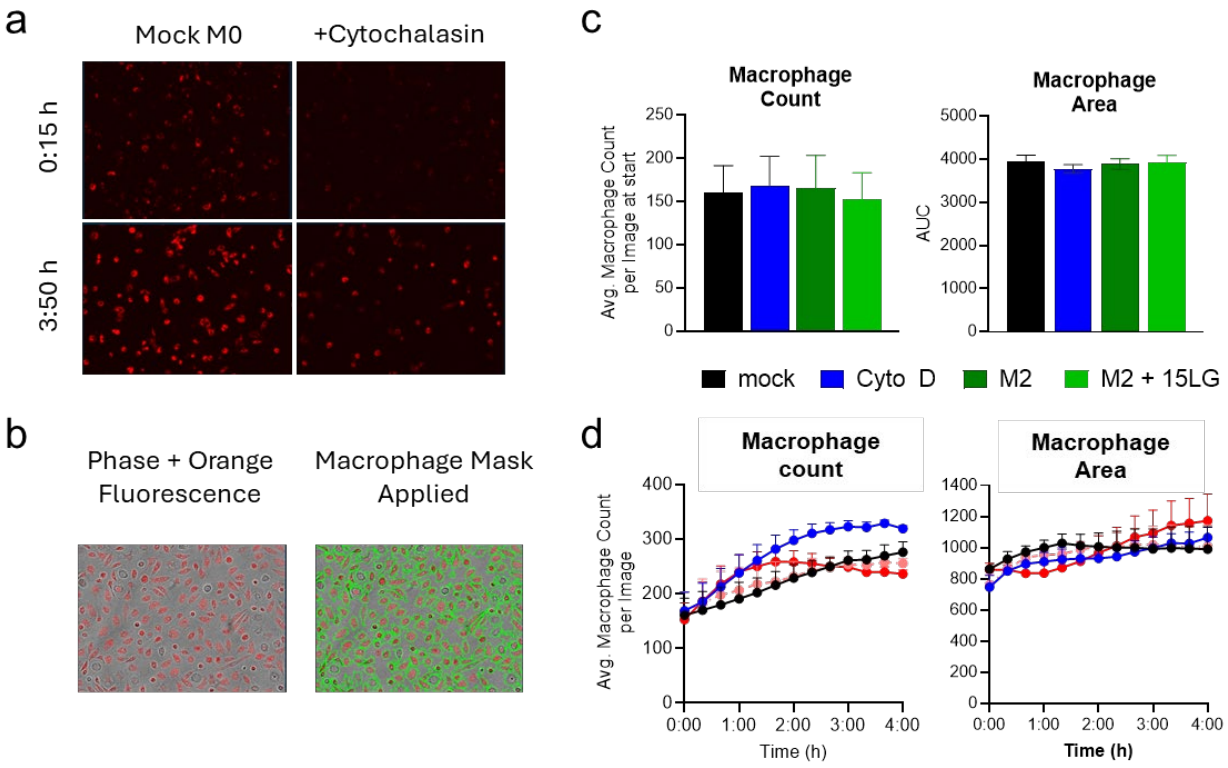
Supplementary Figure 10. GPR15LG is not toxic for monocyte-derived macrophages. Viability from stimulation experiments was determined by fixable viability stain and flow cytometer analysis. n = 6 individual donors.



Supplementary Figure 11. GPR15LG does not significantly alter chemokine release by macrophages. PBMCs were seeded at a density of 7.5 × 10^5 cells per well in 1 mL medium and cultured under two different conditions: macrophage differentiation medium (MDM; growth medium supplemented with M-CSF) as mock control and MDM additionally supplemented with GPR15LG 5µM. MDM contained M-CSF at a concentration of 15 ng/mL. On day 3 and 6, the culture medium was replaced with fresh medium supplemented with 1% penicillin/streptomycin and M-CSF. Supernatants were collected on days 3 and 6 and cytokine content was measured with a human inflammation panel 1 LEGENDPlex kit by flow cytometry. N=3 donors, dots represent means of duplicate measurements. (Statistics = two way ANOVA with Šídák's multiple comparisons test)



Supplementary Figure 12. GPR15LG restricts resolution-associated M2 polarization upon prolonged exposure, raw data. GPR15LG was supplied to fully differentiated MDMs during the 3-day-long polarization window at 5 µM concentration. Polarized macrophages were detached and stained and marker expression was analyzed by flow cytometry.



Supplementary Figure 13. GPR15LG does not affect macrophage size or counts. Macrophage phagocytosis was measured using the Sartorius Incucyte Imaging instrument. E.coli pHRodo Red Bioparticles were added to primary MDMs polarized either with or without GPR15LG. Cytochalasin D was used at 1µM to inhibit phagocytosis as an internal control (a). b) A macrophage mask was defined at the Incucyte analysis software to determine equal macrophage counts and areas at the beginning of the assay (c). Macrophage count and area were also observed over the duration of the assay to ensure cell health (d). N=3-7 donors, each sample represents a mean of 4-9 images made per well, bars and dots show means ± SEMs.