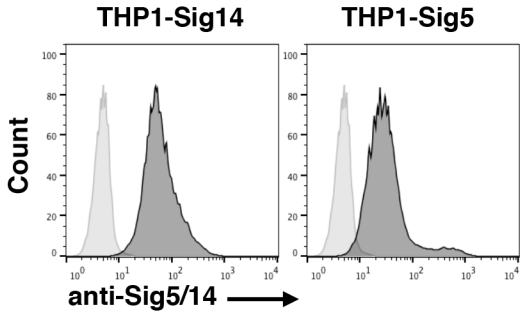
a

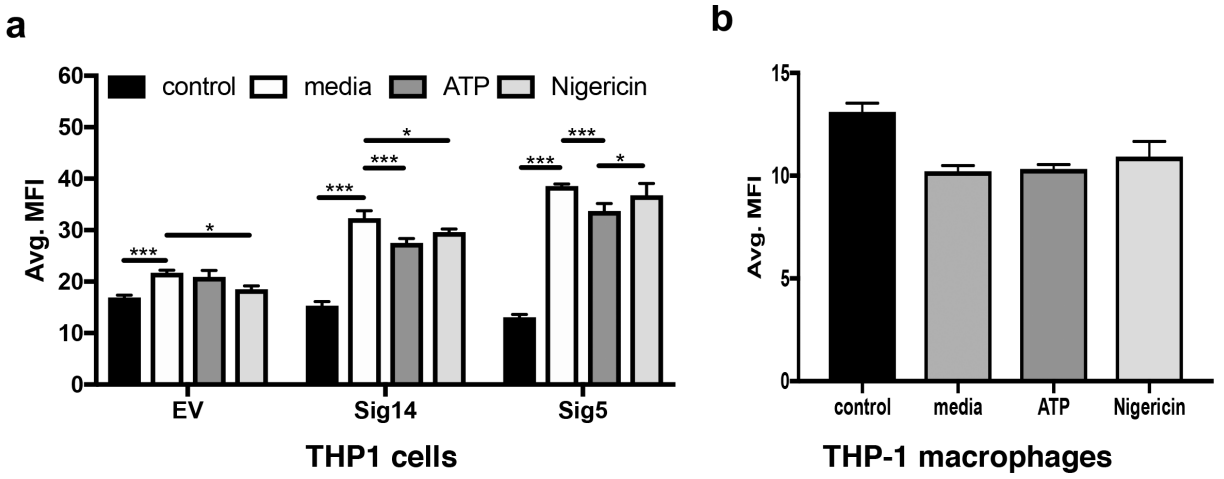


b

TNF-α (pg/ml)			
	EV	Sig14	Sig5
media	n.d	n.d	n.d
5 mM ATP	n.d	n.d	n.d
10 μM nigericin	n.d	n.d	n.d

IL-6 (pg/ml)			
	EV	Sig14	Sig5
media	n.d	n.d	n.d
5 mM ATP	n.d	n.d	n.d
10 μM nigericin	n.d	n.d	n.d

Fig. S1. Characterization of THP1-Sig14 and THP-1-Sig5 cells. **a** Cell surface expression of Siglec-5/14 was detected using an antibody that recognizes Siglec-5 and Siglec-14 and flow cytometry analysis. **b** THP-1 EV, Sig5, or Sig14 cells were stimulated with 5 mM ATP or 10 μM nigericin for 30 min then incubated for additional 2 h. TNF-α and IL-6 production was assessed by ELISA. n.d. indicates cytokine was not detected.



c

Sig5/14 (pg/ml)				
Cells:	THP-1	EV	Sig14	Sig5
media	n.d	n.d	n.d	n.d
5 mM ATP	n.d	n.d	n.d	n.d
10 μM nigericin	n.d	n.d	n.d	n.d

Fig. S2. Analysis of cell surface and secreted Siglec-5/14 upon treatment with NLRP3 inflammasome activators. THP-1 cells were stimulated with 5 mM ATP or 10 μM nigericin for 30 min and then incubated for additional 2 h. (**a**, **b**) Cell surface Siglec-5/14 was detected using an anti-Siglec-5 antibody (Biolegend, Clone 1A5) and flow cytometry analysis. Average mean fluorescence intensity (MFI) is shown compared to media-treated cells stained with an isotype control. **c** Soluble Siglec-5/14 in cell supernatants was analyzed by ELISA. n.d. indicates secreted Siglec-5/14 protein was not detected. Data are representative from 2 independent experiments performed in triplicate. Error bars denote the SD, * $p < 0.05$, *** $p < 0.001$.

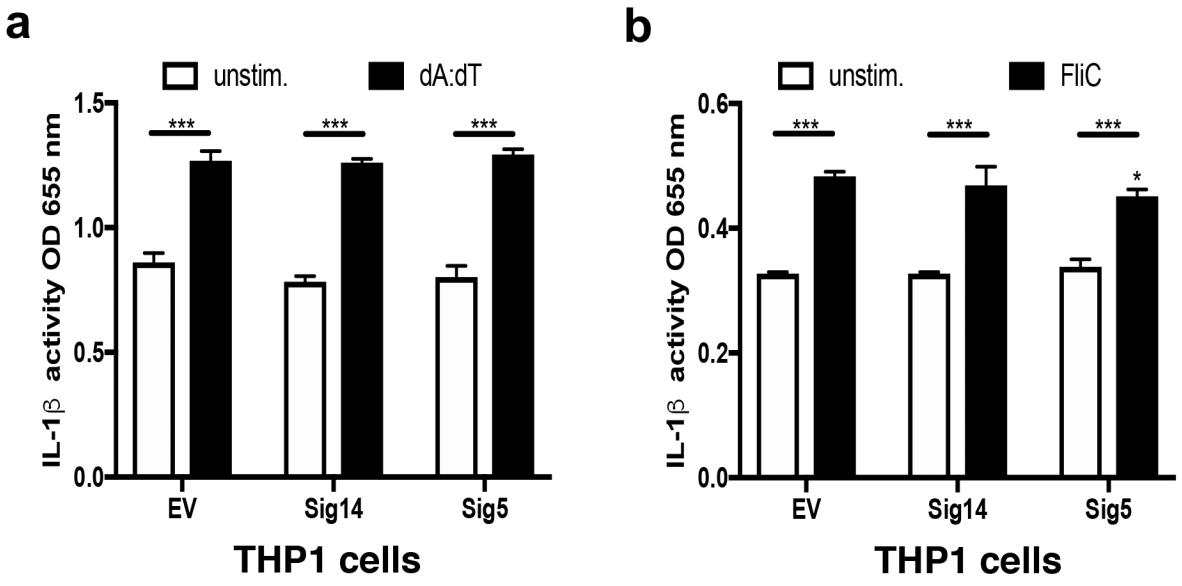


Fig. S3. Analysis of bioactive IL-1β release upon AIM2 and NLRC4 inflammasome ligand stimulation of THP-Siglec cells. THP1-EV, THP1-Sig14 and THP1-Sig5 cells were differentiated with 25 nM of phorbol-12-myristate-13-acetate (PMA) and 10 ug/ml LPS for 18 h and stimulated with (**a**) Poly(dA:dT) or (**b**) Flagellin for 2 h. The amount of secreted and mature IL-1β in supernatants was determined using the IL-1β sensor cells (InvivoGen). Data is representative from 3 independent experiments performed in triplicate. Error bars denote the SD, ***$p < 0.001$.

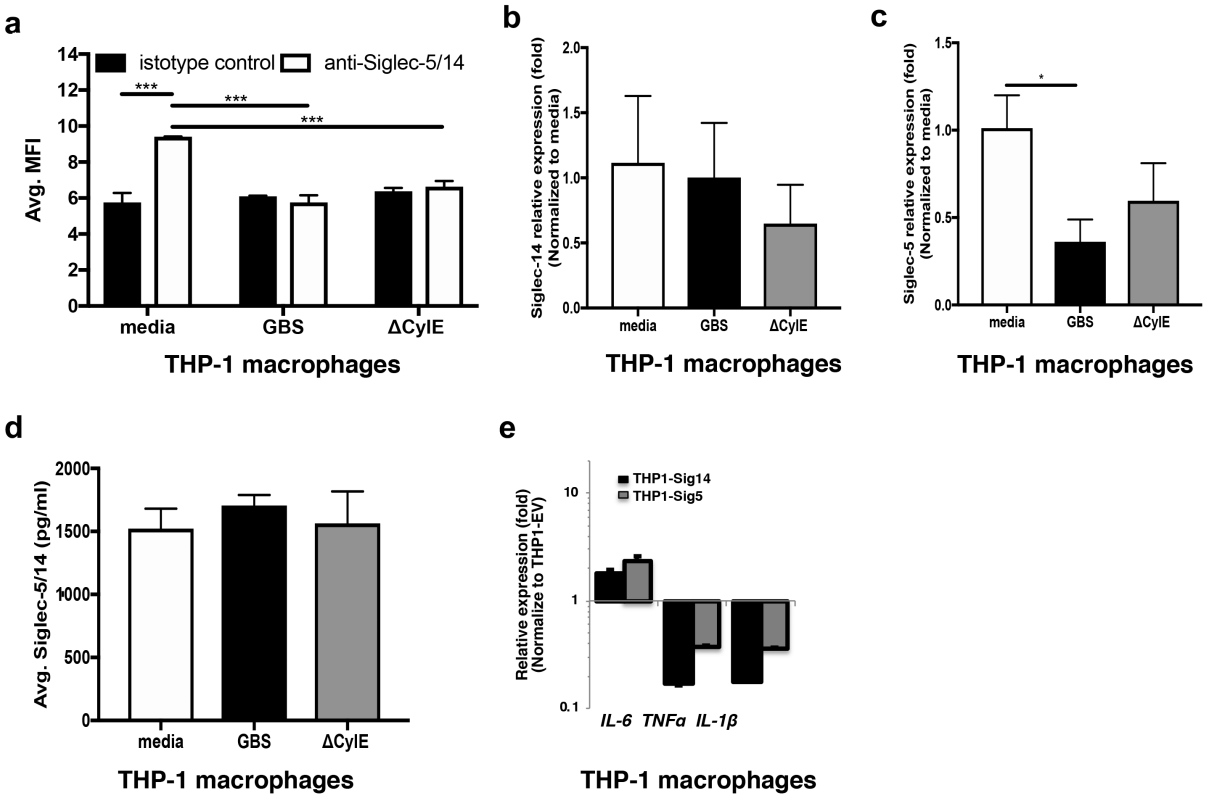


Fig. S4. Analysis of Siglec-5 and Siglec-14 expression upon THP-1 cell exposure to GBS. THP-1 cells were exposed to GBS WT and GBS Δ*cylE* mutant bacteria at an MOI = 10 bacteria/cell and incubated for 16 h. **a** Cell surface Siglec-5/14 expression was detected using an anti-Siglec-5 antibody (Biolegend, Clone 1A5) and flow cytometry analysis. Average mean fluorescence intensity (MFI) is shown compared to media-treated cells stained with an isotype control. (**b, c**) Siglec-14 and Siglec-5 mRNA expression was analyzed by qPCR. **d** Soluble Siglec-5/14 in cell supernatants was analyzed by ELISA. **e** IL-6, TNF-α, and IL-1β expression was quantified by qPCR analysis. Data are representative from 2 independent experiments performed in triplicate. Error bars denote the SD, ***$p < 0.001$.

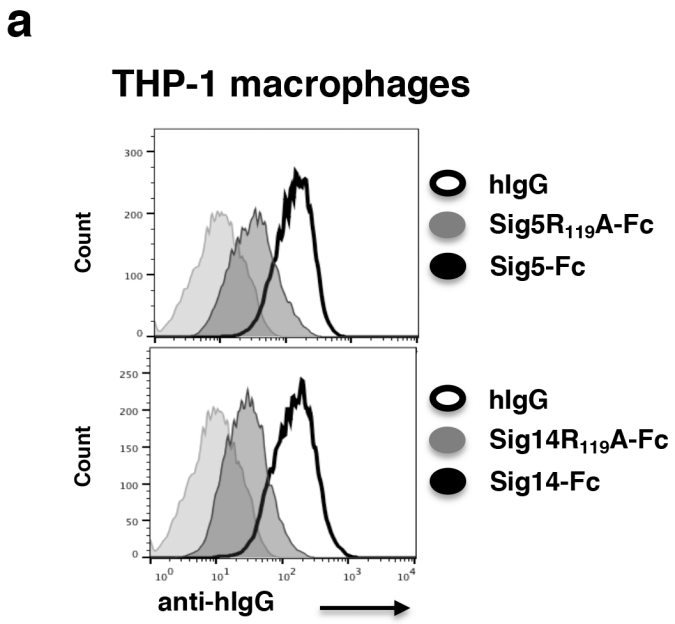


Fig. S5. Flow cytometry detection of sialic acid-dependent and independent binding by Siglec-14-Fc and Siglec-5-Fc recombinant proteins to THP-1 cells. Cells were incubated with the indicated recombinant Siglec-Fc proteins and stained with an anti-human IgG antibody to detect Siglec-Fc binding. Histograms display the fluorescence intensity distribution. Black line: Siglec-5-Fc or Siglec-14-Fc; filled histograms (dark grey): sialic acid binding mutant, Siglec-5R119A-Fc or Siglec-14R119A-Fc; filled histograms (light grey): hIgG-Fc control.

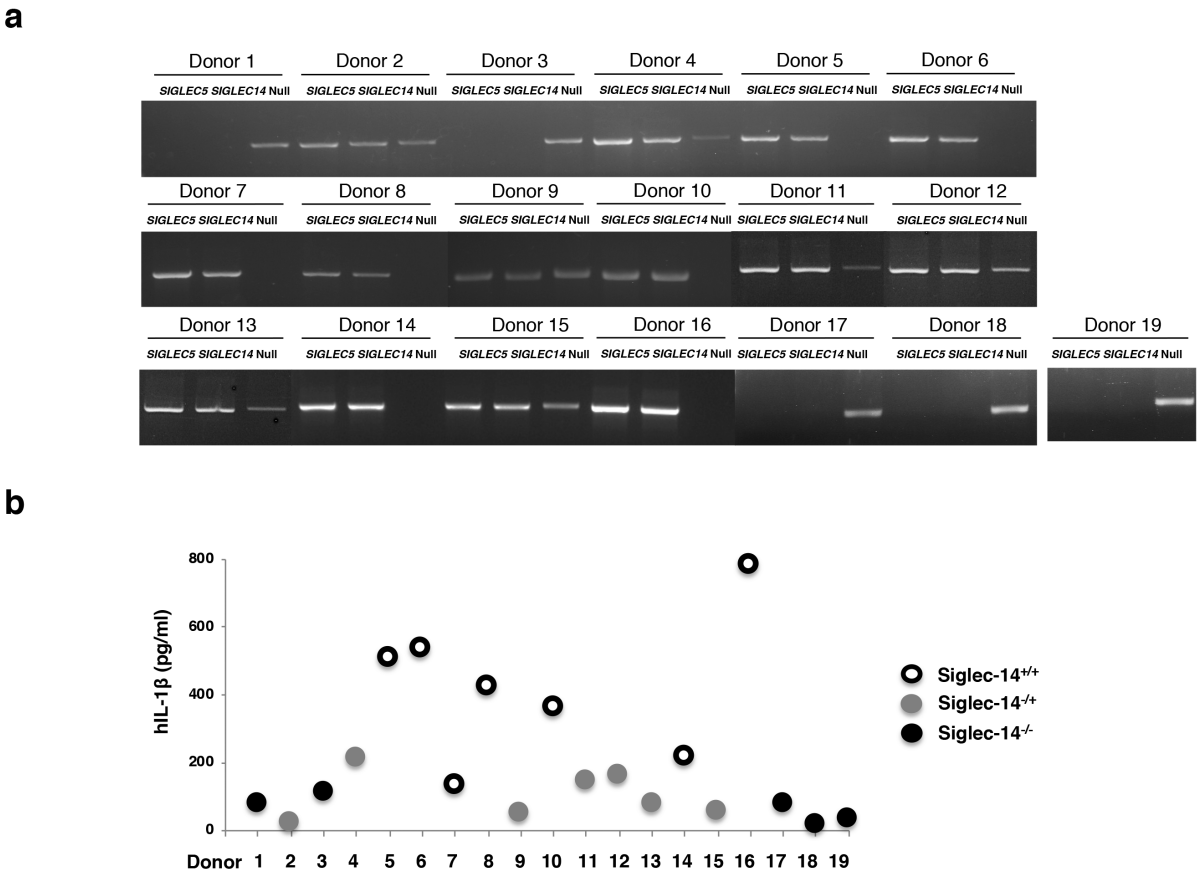


Fig. S6. Siglec-5 and Siglec-14 genotype analysis and association with IL-1β response to GBS. **a** Genomic PCR results of *SIGLEC5* (left lane), *SIGLEC14* (middle lane), and *SIGLEC14/5* fusion gene (Null, right lane) of human monocyte-derived macrophages isolated from 19 donors. **b** Cells of the indicated genotypes were infected with WT GBS and IL-1β production was measured by ELISA (graph displays all the results presented in Fig. 4).