

Supplementary Table 3
Supplementary Glycan Microarray Document
Based on [MIRAGE Glycan Microarray Guidelines](#)

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Classification	
1. Sample: Glycan Binding Sample	
Description of Sample	Recombinant soluble chimeric protein containing murine MelLec fused to the mutated Fc portion of human IgG1 was generated essentially as described (Graham et al., J Immunol Methods. 2006). Please see Methods part for more details. The preparation of FLAG-tagged human Langerin extracellular domain and the rat anti-FLAG detection antibody mAb L5 was essentially as described (Park et al., J Immunol Methods. 2008).
Sample modifications	no
Assay protocol	Described in Methods part under ‘Glycan microarray analyses’.
2. Glycan Library	
Glycan description for defined glycans	The microarray (in-house designation ‘Glycosciences Array Sets 32-39bis’) contained 496 lipid-linked glycan probes and the probe names and structures are in Extended data Table 1 . These were from the collection assembled in the course of research in the Glycosciences Laboratory (https://glycosciences.med.ic.ac.uk/glycanLibraryList.html).
Glycan description for undefined glycans	Not relevant.
Glycan modifications	Unless otherwise specified the neoglycolipids (NGLs) were prepared from reducing oligosaccharides by reductive amination with the amino lipid, 1,2-dihexadecyl-sn-glycero-3-phosphoethanolamine [(DHPE) (Chai et al., Methods Enzymol. 2003)]; AO, NGLs prepared from reducing oligosaccharides by oxime ligation with an aminoxy functionalized DHPE [(AOPE) (Liu et al., Chem. Biol. 2007)]. For full description of the lipid moieties of the glycan probes, please see https://glycosciences.med.ic.ac.uk/docs/lipids.pdf .
3. Printing Surface; e.g., Microarray Slide	
Description of surface	Nitrocellulose-coated glass microarray slides.
Manufacturer	16-Pad FAST® Slide from Whatman (Sanford, ME, US)
Custom preparation of surface	Not relevant.

Non-covalent Immobilization	The lipid-linked glycan probes were formulated as liposomes by adding carrier lipids, phosphatidylcholine and cholesterol (Liu et al., Methods Mol. Biol. 2012) for robotically arraying and non-covalent immobilization on nitrocellulose-coated glass slides.
4. Arrayer (Printer)	
Description of Arrayer	Piezorray (PerkinElmer LAS, Beaconsfield, UK)
Dispensing mechanism	Non-contact liquid delivery with four dispensing tips.
Glycan deposition	Approximate 0.33 nl was printed for each spot. Each glycan probe was printed at two levels (2 and 5 fmol per spot) in duplicate.
Printing conditions	The printing solutions contained 100 pmol/μl of phosphatidylcholine and cholesterol (both from SIGMA) as lipid carriers in addition to the lipid-linked glycan probes in water (HPLC grade). The concentrations of the lipid-linked glycan probes were 5 and 15 pmol/μl for the 2 and 5 fmol per spot levels, respectively.
5. Glycan Microarray with “Map”	
Array layout	Each array slide contained 16-pad subarrays of glycan probes printed at two levels in duplicate (four spots for one probe in a row) as detailed in Material and Methods.
Glycan identification and quality control	For quality control ‘Glycosciences Array Sets 32-39bis’ was previously analysed with various glycan-binding proteins including lectins and antibodies. These data are available on request. As an example, FLAG-tagged human Langerin showed binding as predicted to multiple glycans (Galustian et al., Int Immunol 2004 ; Extended data Fig. 3 and Extended data Table 1).
6. Detector and Data Processing	
Scanning hardware	ProScanArray microarray scanner (PerkinElmer LAS, Beaconsfield, UK)
Scanner settings	Scanning resolution: 10 μm / pixel (this resolution is adequate for the sizes of sample spots) Laser channel: Red (scan wavelength 633 nm) PMT gain 35 for ProScanArray Scan power: 90% for Fc-MeILec and 80% for FLAG-tagged human Langerin (no saturation of binding signals).
Image analysis software	ScanArray Express software (PerkinElmer)
Data processing	The ProScan file was entered into an in-house microarray database using software (designed by Dr Mark Stoll, http://www.beilstein-institut.de/en/publications/proceedings/glyco-2009) for data processing. No particular normalization method or statistical analysis was used.

7. Glycan Microarray Data Presentation

Data presentation	The microarray binding results are in Extended data Fig. 3 and Extended data Table 1 . The table includes the list of glycan probes present in the array, their sequences, fluorescence intensities at the 5 fmol per spot level and errors (half of the difference of signal intensities of duplicate spots for each glycan probe).
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8. Interpretation and Conclusion from Microarray Data

Data interpretation	No software or algorithms were used to interpret processed data.
Conclusions	MeILec did not elicit any significant binding signals in the glycan microarrays unlike the C-type lectin Langerin.