

Supplemental tables

Supplemental table 1: Overview of antibodies and membrane dyes used for flow cytometry.

Antibody	Clone	Conjugate	Dilution or concentration	Supplier	Reference
Anti-human TCR V δ 2	B6	PE	1:200	BioLegend	331408
Anti-human CD16	3G8	BV605	1:500	BioLegend	302040
Anti-human CD56	NCAM16.2	BV510	1:100	BD Horizon	563041
Anti-human CD3	UCHT1	BV510	1:200	BD Horizon	563109
Anti-human CD25	2A3	BV605	1:100	BD Horizon	562660
Anti-human CD69	FN50	BUV395	1:100	BD Biosciences	564364
Anti-human/mouse Granzyme B	GB11	FITC	1:100	BD Biosciences	560211
Anti-HLA-I/MHC-I	W6/32	PerCP-eFluor 710	1:250	Invitrogen	46-9983-42
Anti-human CD11b	Bear1	PE	1:100	Beckman Coulter	IM2581U
Anti-human ICAM1	15.2	AF546	1:10 (saturating) 1:100 (non-saturating)	Santa Cruz	Sc-107 AF546
Goat anti mouse IgG	polyclonal	AF647	1:200	Invitrogen	A21236
Goat anti mouse IgG	M1310G05	APC	1:200	Biolegend	410712
LIVE/DEAD™ Fixable Near-IR			1:800	Invitrogen	L34976A
VPD450			1:400 (1,0 mg/ml stock)	BD Horizon	562158
DiO lipophilic membrane dye			1:200 (5 μ M stock)	Invitrogen	V22886
DIL stain			1:400 (2,5 mg/ml stock)	Invitrogen	D3911
AF350 NHS Ester			1:100 (1,0 mg/ml stock)	Invitrogen	A10168
Cell trace violet			1:400 (1 μ M stock)	Thermo Fisher Scientific	C34557
CFSE			125nM	Invitrogen	C34554

Supplemental table 2: Antibody panel figure 5 and 6.

Target	Clone	Conjugate	Source	Reference
CD9	FMC56		Leucocyte typing workshop	21-23
	CLB-thromb/8		Leucocyte typing workshop	21-23
	Gi7		Leucocyte typing workshop	21-23
	TP82		Leucocyte typing workshop	21-23
	PHN200		Leucocyte typing workshop	21-23
	MEM-61		Leucocyte typing workshop	21-23
	MB16		Leucocyte typing workshop	21-23
	BU-16		Leucocyte typing workshop	21-23
	BA2		Leucocyte typing workshop	21-23
	MM2/356		Leucocyte typing workshop	21-23
	M-L13		Leucocyte typing workshop	21-23
	TI117		Leucocyte typing workshop	21-23
	DS21		Leucocyte typing workshop	21-23
	1AA2.H9		Leucocyte typing workshop	21-23
	GR2110		Leucocyte typing workshop	21-23
	MEM-192		Leucocyte typing workshop	21-23
	SN4		Leucocyte typing workshop	21-23
	PHN33		Leucocyte typing workshop	21-23
	ALB6	FITC	Beckman Coulter	IM1755U
	CD13	DU-ALL1		Leucocyte typing workshop
SN4d			Leucocyte typing workshop	21-23
Li 9.4			Leucocyte typing workshop	21-23
MB61			Leucocyte typing workshop	21-23
L138		PE	BD	347406
JP34-10			Leucocyte typing workshop	21-23
Tük1			Leucocyte typing workshop	21-23
MoU48			Leucocyte typing workshop	21-23
CD15	CLB-mono-gran-2		Leucocyte typing workshop	21-23
	GF.26.7.3		Leucocyte typing workshop	21-23
	VIM10		Leucocyte typing workshop	21-23
	U1-4B4		Leucocyte typing workshop	21-23
CD29	U1-58		Leucocyte typing workshop	21-23
	K20	FITC	Beckman Coulter	IM0791U
	A-1A5		Leucocyte typing workshop	21-23
	JBS5		Leucocyte typing workshop	21-23

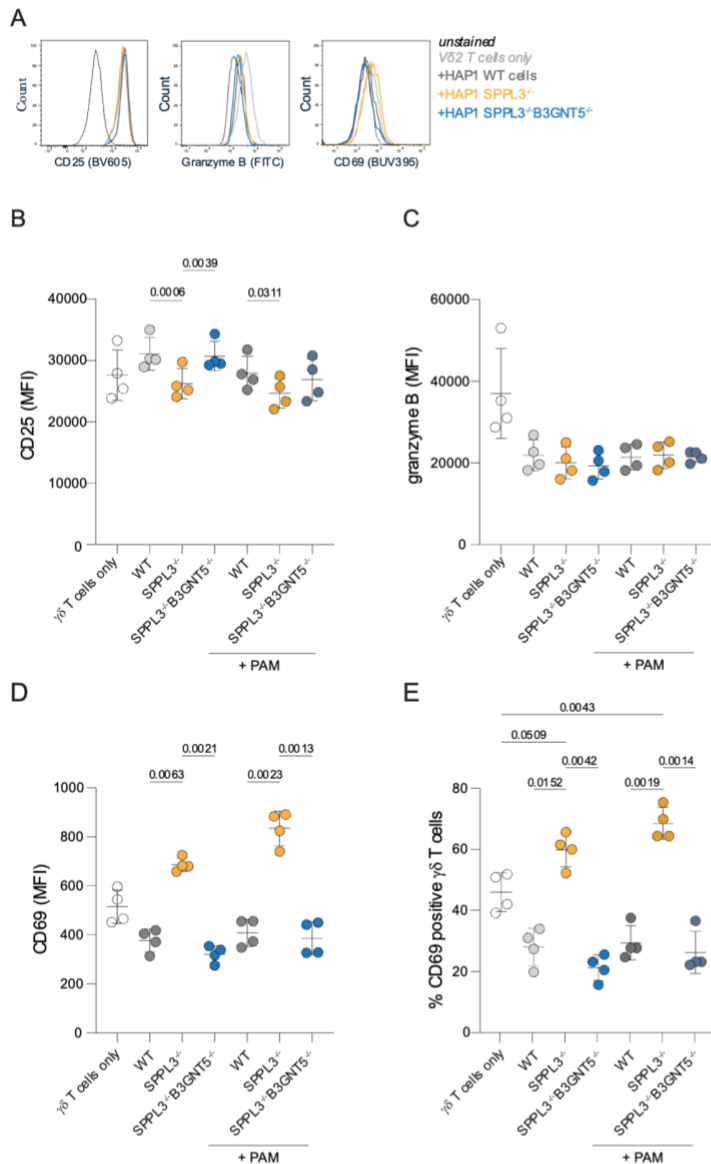
	4B4		Leucocyte typing workshop	21-23
	13		Leucocyte typing workshop	21-23
CD36	131		Leucocyte typing workshop	21-23
	CLB-IVC7	FITC	CLB	M1613
	L103		Leucocyte typing workshop	21-23
CD46	46-1		In house produced (complement group)	
	46-2		In house produced (complement group)	
	46-3		In house produced (complement group)	
	46-4		In house produced (complement group)	
	46-5		In house produced (complement group)	
	46-6		In house produced (complement group)	
	46-7		In house produced (complement group)	
	46-8		In house produced (complement group)	
	46-9		In house produced (complement group)	
	46-10		In house produced (complement group)	
	46-11		In house produced (complement group)	
	GB24		In house produced (complement group)	
CD47	B6H12		Leucocyte typing workshop	21-23
	BRIC125		Leucocyte typing workshop	21-23
	MP30-1, UMRh		Leucocyte typing workshop	21-23
	CC2C6	FITC	Biologend	323106
	BRIC126		Leucocyte typing workshop	21-23
	2D3	FITC	Thermo Fisher Scientific	11-0478-42
CD49b	6F1		Leucocyte typing workshop	21-23
	Gi9		Leucocyte typing workshop	21-23
	CLB-thromb/4, 10G11		Pelicluster	M1540
	AK-7		Biologend	314306
	HAS3		Leucocyte typing workshop	21-23
	5e8		Leucocyte typing workshop	21-23
	Gi14		Leucocyte typing workshop	21-23
CD49d	HP1/7		Leucocyte typing workshop	21-23
	8F2		Leucocyte typing workshop	21-23
	9F10	APC	BD	559881
CD49e	X6		Leucocyte typing workshop	21-23
	3D3		Leucocyte typing workshop	21-23
	2H6		Leucocyte typing workshop	21-23
	SAM1	FITC	Beckman Coulter	IM1854U
	P4-7		Leucocyte typing workshop	21-23
	16		Leucocyte typing workshop	21-23
CD49f	BQ16		Leucocyte typing workshop	21-23
	D1:EF		Leucocyte typing workshop	21-23
	J8H		Leucocyte typing workshop	21-23
	135-13C		Leucocyte typing workshop	21-23
CD51	AMF7		Leucocyte typing workshop	21-23
	NKI-M9		Leucocyte typing workshop	21-23
	LM142		Leucocyte typing workshop	21-23
	NKI-M7		Leucocyte typing workshop	21-23
	13C2		Leucocyte typing workshop	21-23
CD55	BRIC110	FITC	CLB	M2192
	CD55 1		In house produced (complement group)	
	CD55 2		In house produced (complement group)	
	CD55-3		In house produced (complement group)	
	CD55-4		In house produced (complement group)	
	CD55-5		In house produced (complement group)	
CD59	VJ1/12.2	Pacific blue	Abcam	AB117734
	6H3		Leucocyte typing workshop	21-23
	MEM43	FITC	CLB	M2191
	OV9A2	PE	eBioscience	12-0596-42
	MEM43		Leucocyte typing workshop	21-23
	p282 (H19)	BV421	BD Biosciences	564329
CD70	HNE.51	FITC	DAKO	F7204
	HNC.142		Leucocyte typing workshop	21-23
	CLB-2F2	FITC	CLB	M1705
	Ki-24	PE	BD	555835

	LD6		Leucocyte typing workshop	21-23
CD81	1D6		Leucocyte typing workshop	21-23
	5A6		Leucocyte typing workshop	21-23
CD92	VIM15b		Leucocyte typing workshop	21-23
	VIM15		Leucocyte typing workshop	21-23
CD98	GRVI		Leucocyte typing workshop	21-23
	4F2		Leucocyte typing workshop	21-23
CD146	PIH12	PE	BD	550315
	F432G-3		Leucocyte typing workshop	21-23
	F439E10		Leucocyte typing workshop	21-23
CD147	UM-8D6		Leucocyte typing workshop	21-23
CD151	14A2 H1		Leucocyte typing workshop	21-23
	11B1 G4		Leucocyte typing workshop	21-23
CD155	D171		Leucocyte typing workshop	21-23
CD172a (SIRPa)	12C4		In house produced	44
	4G5			
	602411		R&D systems	MAB4546
	72		In house produced	
CD236	NaM19-3C4		Leucocyte typing workshop	21-23
	BRAC1		Leucocyte typing workshop	21-23
	RB8		Leucocyte typing workshop	21-23
	BRIC10		Leucocyte typing workshop	21-23
	RB8		Leucocyte typing workshop	21-23
	BRIC4		Leucocyte typing workshop	21-23
CD321	F11		Leucocyte typing workshop	21-23
HLA-I (MHC-I)	BB7.2	APC	eBioscience	17-9876-41
	B1.23.2	APC	eBioscience	17-5935-42
	W6/32	PerCP- efluoro710	eBioscience	46-9983-42

Supplemental tables

Supplemental Figures

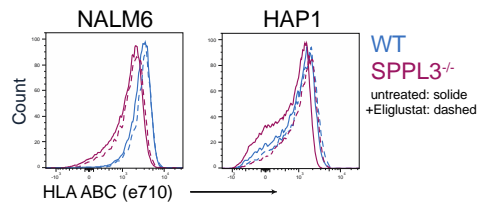
Supplemental Figure 1



Supplemental figure 1. Coculture of NK and $\gamma\delta$ T cells with WT or SPPL3^{-/-} tumor cells.

(A) Histograms showing expression of CD25, granzyme B and CD69 by the $\gamma\delta$ T cells after coculture with the HAP1 target cells. The MFI of CD25, granzyme B and CD69 positive $\gamma\delta$ T cells (B and C) and percentage of CD69 positive cells (E) after coculture with HAP1 WT, SPPL3^{-/-} or SPPL3^{-/-}B3GNT5^{-/-} cells. Data shown are representative from four experiments with four donors per experiment and three technical replicates per datapoint. A one-way ANOVA was used to assess statistical significances.

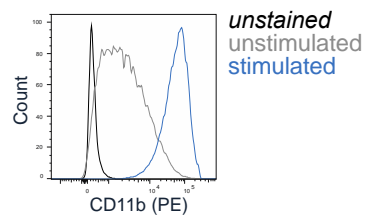
Supplemental Figure 2



Supplemental figure 2. HLA-I staining of NALM6 cells treated with a UGCG inhibitor

Histograms of HAP1 and NALM6 WT (blue) and SPPL3^{-/-} (yellow) cells stained for HLA-I either untreated (dashed line) or treated with UGCG inhibitor Eliglustat (solid line) for three days. Data shown represent two different batches and three technical replicates.

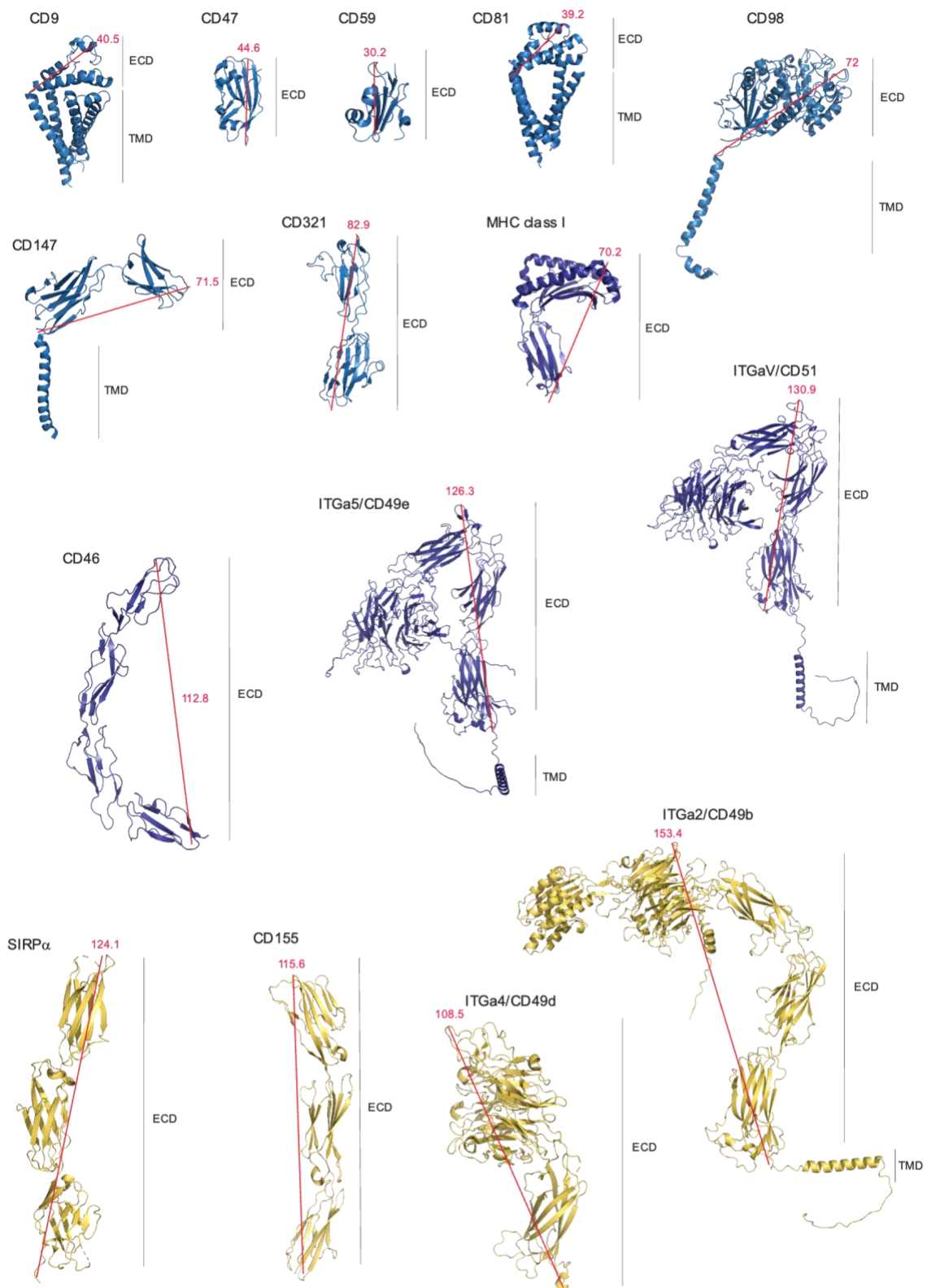
Supplemental Figure 3



Supplemental figure 3. CD11b expression by matured NB4 cells.

Flow cytometry plot demonstrating the CD11b staining of NB4 cells after seven days of stimulation with ATRA. Data shown represent four experiments with two technical replicates.

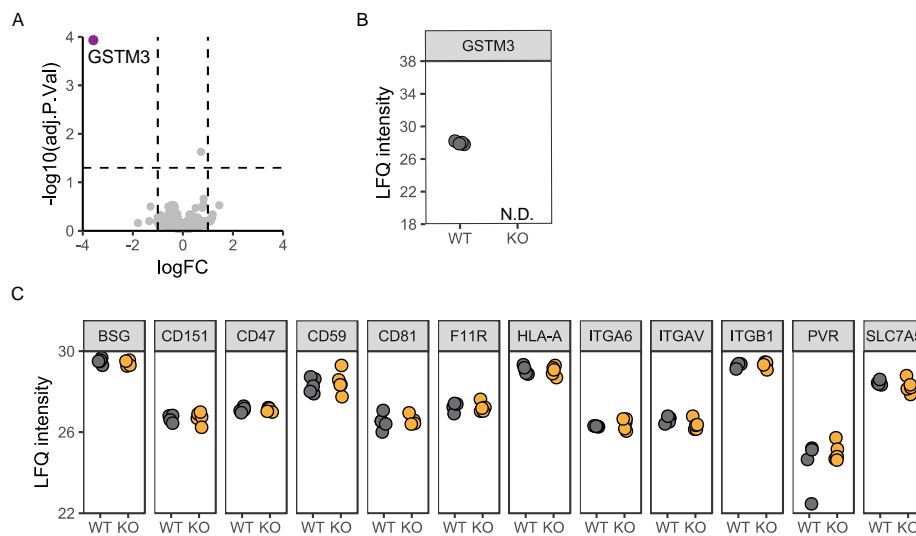
Supplemental Figure 4



Supplemental figure 4. Protein structures of proteins with and without epitopes identified to be affected by nsGSLs.

Available protein structures were obtained from the Alphafold database for the proteins that contained epitopes that were affected (blue), for which at least one antibody was affected (purple) or not affected (yellow) by nsGSLs. The distance between the alpha carbon of the first amino acid of the extracellular domain to all other amino acids were determined in Armstrong (Å) using PyMOL and the longest theoretical distance is shown (red line). ECD: extracellular domain. TMD: transmembrane domain.

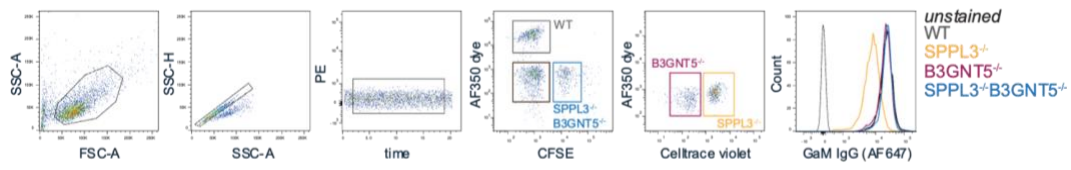
Supplemental Figure 5



Supplemental figure 5. Analysis of the protein abundance of HAP1 WT and SPPL3^{-/-} cells from LC-MS/MS.

(A) Differentially abundant proteins ($FDR < 0.05$ & $|\log_2 \text{fold change}| > 1$, $n = 5$) between HAP1 WT and SPPL3^{-/-} cells. (B) LFQ intensity for GSTM3. (C) LFQ intensities for detected proteins from the antibody panel shown in figure 5. Differential abundance (\log_2 fold change) is plotted against the adjusted p-value ($-\log_{10}$ values). Data shown represent one experiment with five biological replicates.

Supplemental Figure 6



Supplemental figure 6. Gating strategy

HAP1 WT, SPPL3^{-/-} and SPPL3^{-/-}B3GNT5^{-/-} cells were given a membrane dye, mixed and incubated with CD147 targeting antibodies.