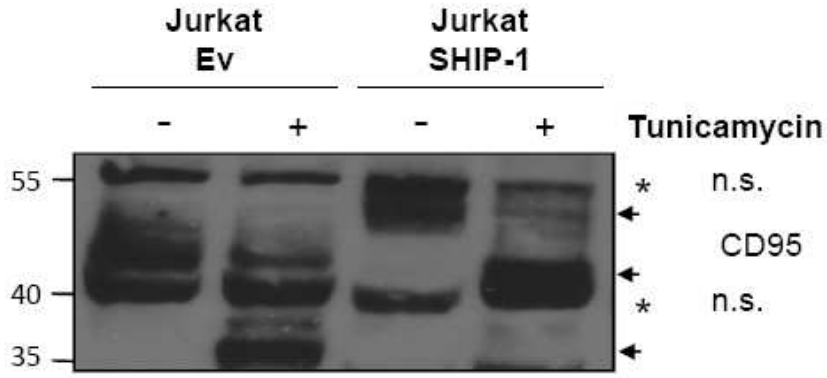
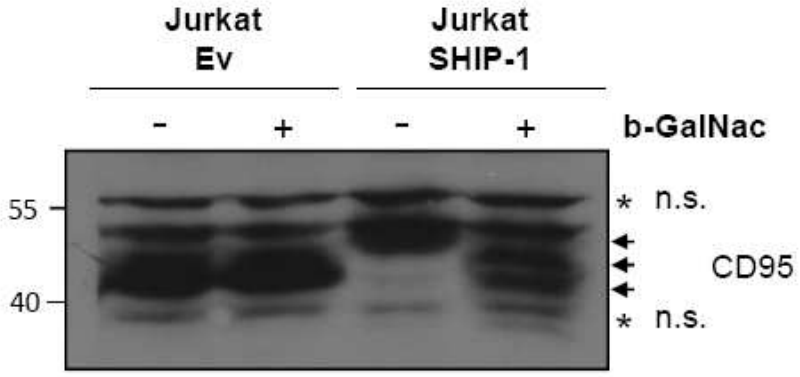
Supplementary Data: Figure 1a and 1b, Figure 2, Table 1 and Table 2.

Supplementary Figure 1. SHIP-1 modifies CD95 glycosylation. (a) Tunicamycin (TN) (2µg/mL) or **(b)** benzyl-GalNac (b-GalNac) (4mM) were added to Jurkat and Jurkat SHIP-1 cell cultures for two days prior analysis. Cell lysates were next analyzed by Western blotting, using an antibody directed against CD95 protein.

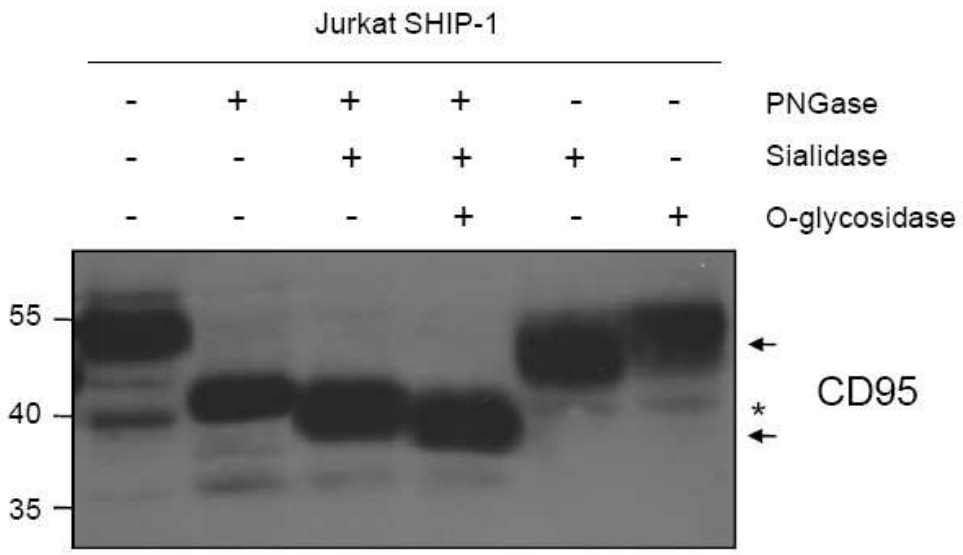
A.



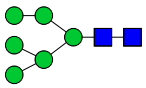
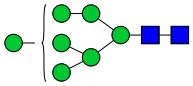
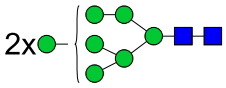
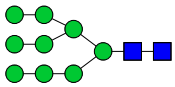
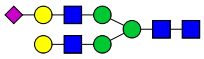
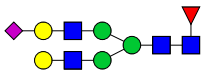
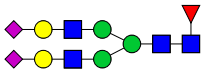
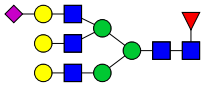
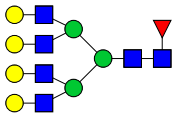
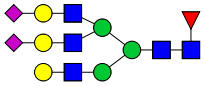
B.



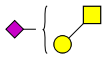
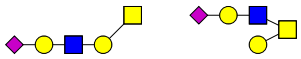
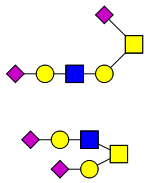
Supplementary Figure 2. CD95 from Jurkat SHIP-1 cells contains sialylated core type-1 glycans. Sequential digestion of Jurkat SHIP-1 cell lysate with PNGase, sialidase and O-glycanase was performed at 37°C. Digested samples were next subjected to Western blotting analyses using an antibody directed against CD95 protein.



Supplementary Table 1. Compositions and relative intensities of the 10 most abundant *N*-glycans derived from Jurkat, Jurkat SHIP-1 and CEM cells released using PNGase-F. Samples were permethylated prior to analysis. The m/z values for the molecular ions observed in the MALDI spectra are shown in the first column (the m/z values are rounded up to whole numbers for the ease of comparison) and structures defined from compositional, biosynthetic and MS/MS information are shown in column 2. Relative intensities were obtained by peak height measurements which were normalised to the most abundant glycan which was designated 100%. Blue square: N-Acetylglucosamine; Green round: Mannose; Yellow round: Galactose; Red triangle: Fucose; Violet rhombus: Sialic acid.

Signal (m/z)	Cartoon depiction of proposed structure	Relative intensities (%)		
		Jurkat	Jurkat SHIP-1	CEM
1784		100	100	100
1988		89	89	51
2192		95	75	30
2396		90	36	25
2431		20	13	5
2605		14	9	6
2966		10	6	4
3054		11	2	3
3142		9	1	1
3146		9	1	2

Supplementary Table 2. Compositions and relative intensities of the *O*-glycans derived from Jurkat, Jurkat SHIP-1 and CEM cells by reductive elimination. Samples were permethylated prior to analysis. The m/z values for the molecular ions observed in the MALDI spectra are shown in the first column (the m/z values are rounded up to whole numbers for the ease of comparison) and structures defined from compositional, biosynthetic and MS/MS information are shown in column 2. Relative intensities were obtained by peak height measurements which were normalised to the most abundant glycan which was designated 100%. Yellow square: *N*-Acetylgalactosamine; Blue square: N-Acetylglucosamine; Yellow round: Galactose; Violet rhombus: Sialic acid.

Signal (m/z)	Cartoon depiction of proposed structure	Relative intensities (%)		
		Jurkat	Jurkat SHIP-1	CEM
330		100	Not detected	Not detected
895		3	35	20
1256		Not detected	100	100
1344		Not detected	32	Not detected
1705		Not detected	63	33

Materials and Methods

Reagents. Tunicamycin and b-GalNAc inhibitors were purchased from Sigma-Aldrich (UK). PNGase, O-glycanase and sialidase were supplied by Calbiochem (La Jolla, CA, USA).

Glycomic Analysis of Jurkat, Jurkat SHIP-1 and CEM cells. Cells were subjected to sonication, reduction, carboxymethylation, and tryptic digestion as described previously (1) . *N*-glycans were released using peptide *N*-glycosidase F (PNGase-F) of the tryptic glycopeptides. The reaction was carried out in 50 mM ammonium bicarbonate, pH 8.5, for 20 h at 37 °C with 3 units of enzyme (Roche Applied Science, UK). The released N-glycans were purified by using a Sep-Pak C18 cartridge (Waters Corp.) as described (1). *O*-glycans were released by direct reductive elimination of the tryptic glycopeptides using KBH_4 in KOH and subsequently purified using Dowex columns (Sigma-Aldrich, UK). The purified native *N*-glycans and *O*-glycan samples were permethylated and then further purified with Sep-Pak cartridges. Glycans were eluted in aqueous acetonitrile fractions and then lyophilised. Glycans were eluted in the 35% and/or 50% acetonitrile fractions and were analysed by MALDI-TOF-TOF MS/MS as follows. Permethylated samples were dissolved in 10 µl of methanol and 1 µl was pre-mixed with 1 µl of matrix (20 mg/ml 2,5-dihydroxybenzoic acid (DHB) in 70% (v/v) aqueous methanol) and spotted onto a target plate. MS data were acquired on a 4800 MALDI-TOF/TOF mass spectrometer (Applied Biosystems) in the reflectron mode. The potential difference between the source acceleration voltage and the collision cell was set to 1 kV and argon was used as collision gas. The 4700 Calibration Standard kit, calmix (Applied Biosystems), was used as the external calibrant for the MS mode and [Glu1]fibrinopeptide B human (Sigma-Aldrich, UK) was used as an external calibrant for the MS/MS mode.

References

1. Jang-Lee J, North SJ, Sutton-Smith M, Goldberg D, Panico M, Morris H, *et al*. Glycomic profiling of cells and tissues by mass spectrometry: fingerprinting and sequencing methodologies. *Methods Enzymol* 2006; **415:** 59-86.