Supplemental Figure Legends

Figure S1. The effect of GCS-100 on sensitization to death is dependent on cell surface gal-3. (A) Three additional cell lines derived from patients with DLBCL were evaluated for total cellular gal-3 by immunoblot. Gal-3 was not detected in the whole cell lysate of SUDHL-8 cells, whereas gal-3 was detected in the whole cell lysates of SUDHL-16 and BCBL1 cells. (B) Cell surface gal-3 expression was assessed by flow cytometry, as described in the Methods. Gal-3 was not detected on the cell surface of SUDHL-8 cells, whereas gal-3 could be detected on the surface of SUDHL-16 cells. Although BCBL1 cells expressed cytoplasmic gal-3 as detected by immunoblot, little gal-3 was detected on the cell surface of BCBL1 cells, indicating that gal-3 is not secreted from or retained on the surface of BCBL1 cells. (C) Cell death following dex, GCS-100, or GCS-100 + dex was determined by annexin V and PI staining, as described in the Materials and Methods. While GCS-100 did not sensitize the cell surface gal-3- cell lines SUDHL-8 and BCBL1 to death, GCS-100 did sensitize the cell surface gal-3+ cell line SUDHL-16 to death (*** $p<.0001$). In C, values are mean ± SD of triplicate samples from 1 representative of 2 independent replicate experiments.

Figure S2. N-glycans are not responsible for gal-3 binding to CD45 on DLBCL cells. (A) SUDHL-6 and SUDHL-9 cells express the same set of CD45 transcriptional products, detected by RT-PCR, indicating that the two cell lines express the same splice variants of CD45: CD45RB (236 bp), CD45RBC (380 bp), and CD45RABC (577 bp). (B) The abundance of complex N-glycans on the surface of SUDHL-6 and SUDHL-9 cells was determined by binding of biotinylated PHA-L, detected with avidin-FITC. (C)

Reduction in complex N-glycans on SUDHL-6 cells did not reduce the amount of endogenous gal-3 at the cell surface. SUDHL-6 cells were treated with the mannosidase inhibitor DMNJ; DMNJ efficacy was confirmed by decreased PHA-L binding (left panel). SUDHL-6 cells treated with or without DMNJ were analyzed for cell surface gal-3 by flow cytometry (right panel); although DMNJ reduced complex N-glycans on the cell surface, there was no decrease in endogenous cell surface gal-3. (D) Reduction in complex N-glycans and core 2 O-glycans on SUDHL-6 cells reduced the heterogeneity of CD45. SUDHL-6 cells were treated with or without DMNJ and C2GnT-1 siRNA alone or in combination, and CD45 heterogeneity was evaluated by immunoblot. We observed a collapse in the CD45 band in SUDHL-6 cells treated with DMNJ alone, C2GnT-1 siRNA alone, and SUDHL-6 cells treated with both DMNJ and C2GnT-1 siRNA as compared to control treated cells, indicating that CD45 heterogeneity resulted from N- and O-glycosylation.

Figure S3. Gal-3 binds to a subset of CD45 on DLBCL cells. (A) Exogenous gal-3 binds to SUDHL-9 cells. Exogenous gal-3 was added to SUDHL-9 cells and bound for 30 minutes at 37 °C. Cells were fixed and bound gal-3 was detected by flow cytometry. SUDHL-9 does not express endogenous gal-3 (thin line) compared to the isotype control (gray filled). However, exogenous gal-3 binds to the surface of SUDHL-9 cells (thick line). (B) SUDHL-6 and SUDHL-9 cells were incubated with exogenous recombinant gal-3 (+) or buffer control (-) for 30 minutes at 37 ^{o}C, fixed with DTSSP and solubilized as described in Methods. Gal-3 was immunoprecipitated from lysates, precipitates were separated by SDS-PAGE, and CD45 and gal-3 were detected by immunoblotting. As

expected, endogenous and exogenous gal-3 co-precipitated with CD45 on SUDHL-6 cells. As SUDHL-9 cells do not express endogenous gal-3, CD45 did not co-precipitate with the anti-gal-3 antibody. However, when exogenous recombinant gal-3 was added to SUDHL-9 cells, gal-3 was immunoprecipitated but no CD45 was detected in the complexes. This indicates that, while CD45 on SUDHL-6 cells displays glycan ligands that permit gal-3 binding, CD45 on SUDHL-9 cells does not display glycan ligands that permit gal-3 binding.

Figure S4. Removal of cell surface gal-3 alters downstream signaling regulated by via CD45 phosphatase. SUDHL-6 cells were treated with 75 μg/mL GCS-100 or buffer control for 1 hour. Lyn was immunoprecipitated from cell lysates, and precipitates immunoblotted with anti-phospho-Lyn (pY507) and anti-Lyn to determine Lyn activation. Levels of phospho-Lyn and Lyn were determined by densitometry and levels of phospho-Lyn were normalized to Lyn expression. Decreased Lyn phosphorylation was seen in GCS-100 treated cells.

Figure S5. Expression of gal-3 and CD45 in primary DLBCL. Frozen lymph node tissue from four patients with DLBCL was homogenized in 5 mL modified RIPA lysis buffer (50 mM Tris pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 1 mM EDTA, 0.15 M NaCl, and protease inhibitors) with a mortar and pestle. Tissue homogenate was incubated for 1 hour at 4^{o}C to achieve optimal tissue solubilization, and then cleared by centrifugation. CD45 and gal-3 expression were examined from 1 μg of tissue lysate per sample (labeled 1-4) and resolved on a 3-8% Tris-Acetate gel. Whole cell lysates from

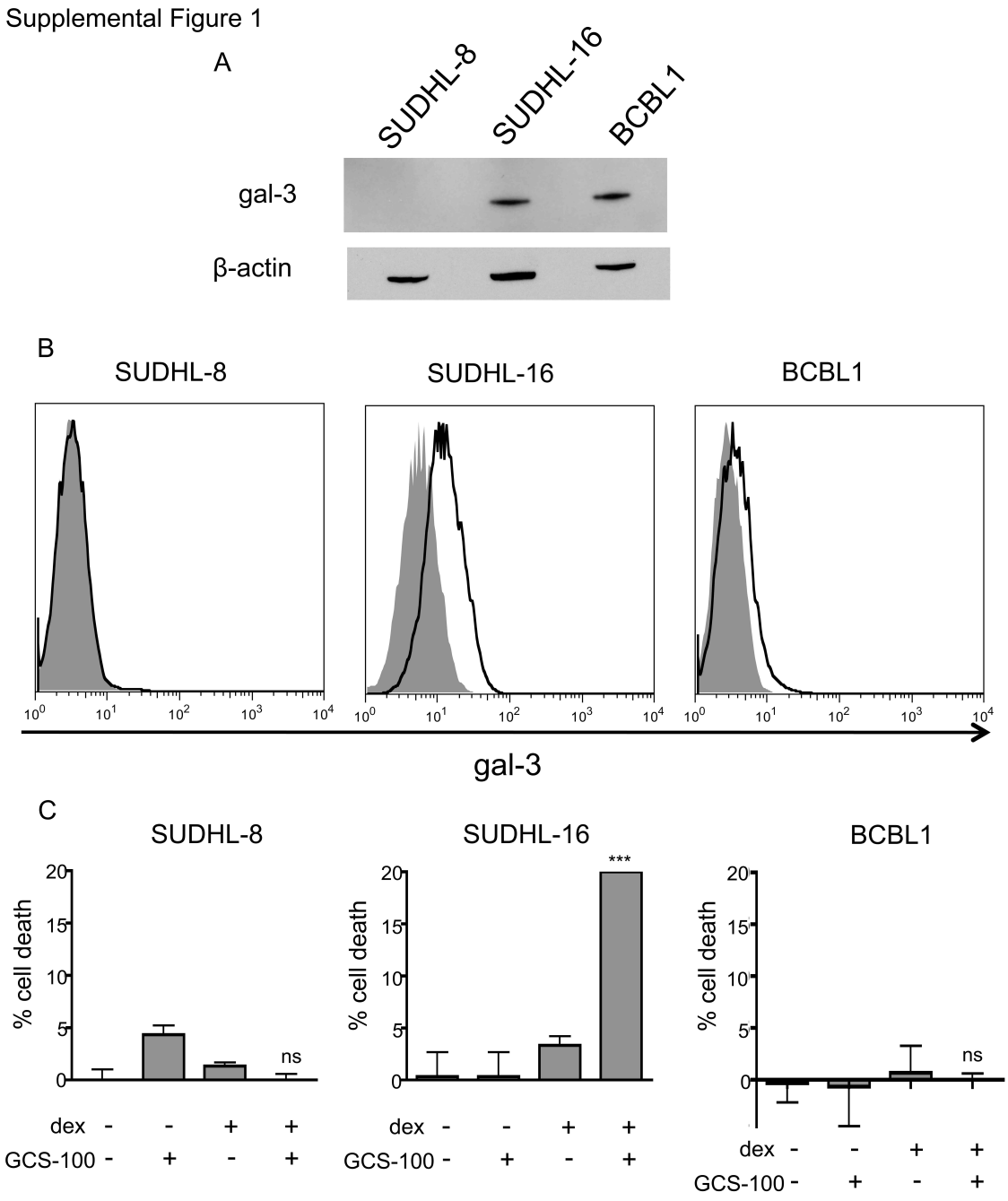
SUDHL-6 (S-6) and SUDHL-9 (S-9) cells are shown for comparison. (B) Phosphatase activity was examined in DLBCL tissue lysates. 10 μg of DLBCL tissue samples 3 and 4 were assayed for phosphatase activity as described in the Methods.

Table S1. Gal-3 expression by DLBCL cell lines detected by immunoblot

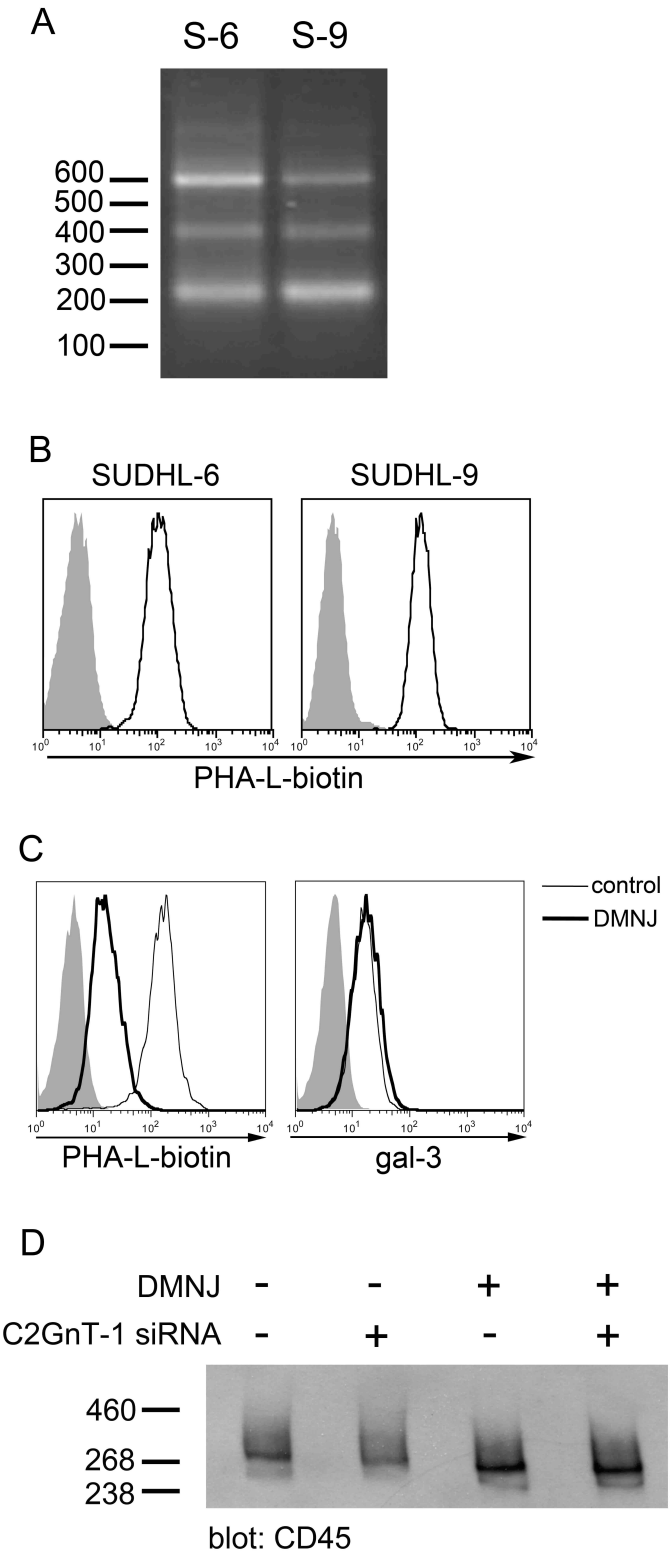
Cell Line	Gal-3 expression
SUDHL-4	-
SUDHL-6	+
SUDHL-8	-
SUDHL-9	-
SUDHL-16	+
HBL1	+
BCBL-1[1]	+
E[2]	+
KS-2[2]	+
NU-DHL-1[2]	-
R[2]	+
RRBL[2]	+

[1]No cell surface gal-3 detected by flow cytometry
[2]Observed in reference (6)

Supplemental Figure 1

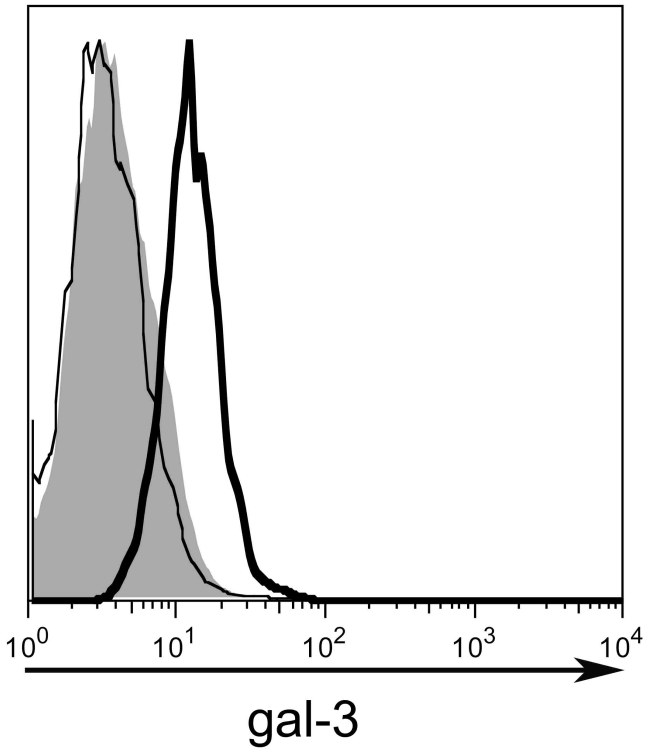


Supplemental Figure 2

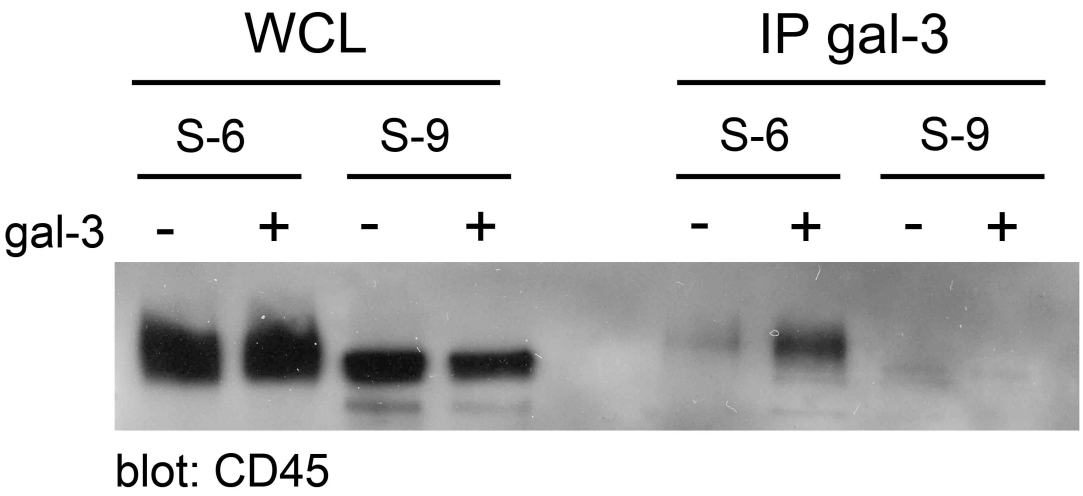


Supplemental Figure 3

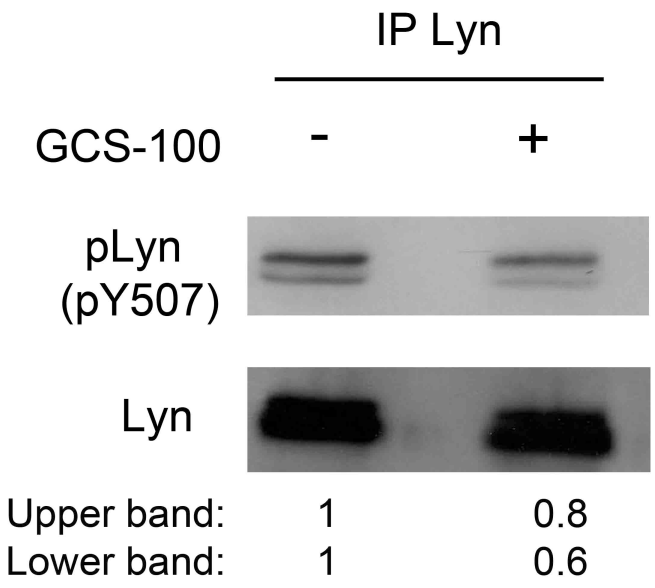
A



B



Supplemental Figure 4



Supplemental Figure 5