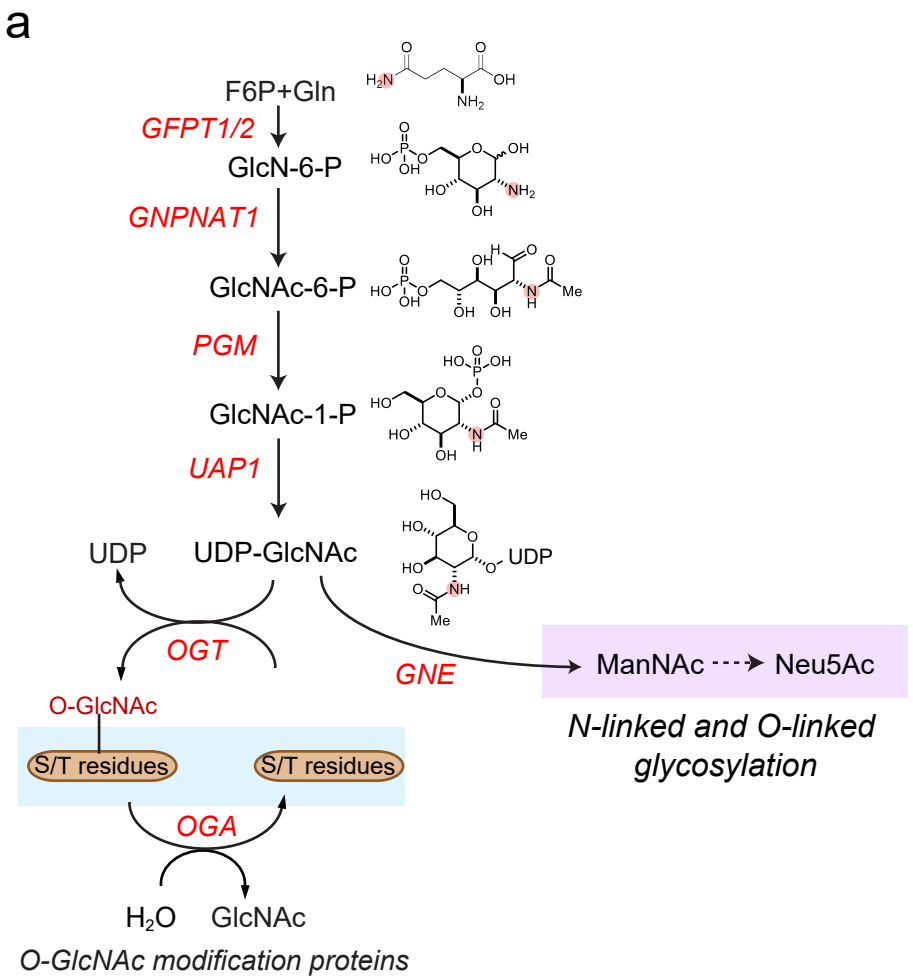
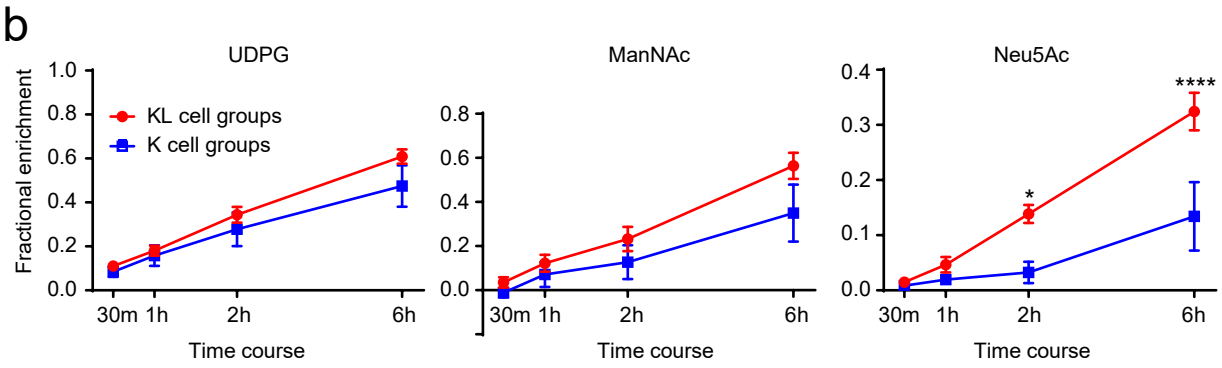
Supplementary Figure Legend.

Supplementary Fig. 1. a, Schematic of the hexosamine biosynthesis pathway and ^{15}N incorporation from [γ-^{15}N]glutamine (labeled in pink) into the HBP intermediates. **b,** Time course of ^{15}N labeling in UDP-HexNAc, ManNAc, and Neu5Ac in K and KL cells cultured in medium containing [γ-^{15}N]glutamine (30m, 1hr, 2hrs, and 6hrs). Individual data points represent the average value of each cell line (3 replicates/cell line). Statistical significance was assessed using two-way ANOVA followed by Sidak's multiple comparisons test. *p<0.05; ****p<0.0001. The stable isotope labeling experiment was performed once.

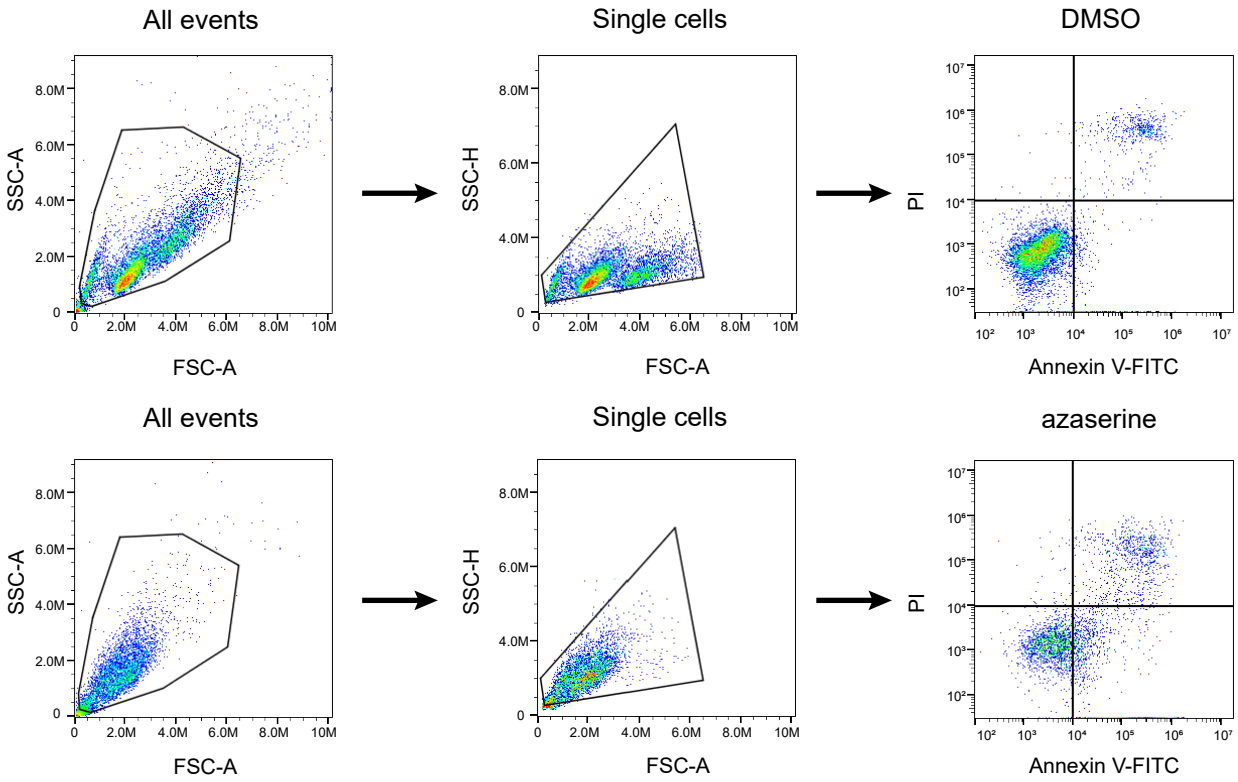
Supplementary Fig. 2. Gating strategies used throughout the study. a, Gating strategy for cell death (PI-Annexin V assays). **b,** Gating strategy for lectin binding assays.

Supplementary Fig. 3. Representative flow cytometry plots of lectin binding assays. a and b, Representative plots of the L-PHA binding (**a**) and LEA binding (**b**) assays shown in Fig **2e**. The oncogenotypes of each cell line and MFI of the dot plot are shown. FACS analyses were performed twice.


a
F6P+Gln
GFPT1/2
GlcN-6-P
GNPNAT1
GlcNAc-6-P
PGM
GlcNAc-1-P
UAP1
UDP
UDP-GlcNAc
OGT
GNE
ManNAc
Neu5Ac
N-linked and O-linked glycosylation
O-GlcNAc
S/T residues
S/T residues
OGA
H2O
GlcNAc
O-GlcNAc modification proteins

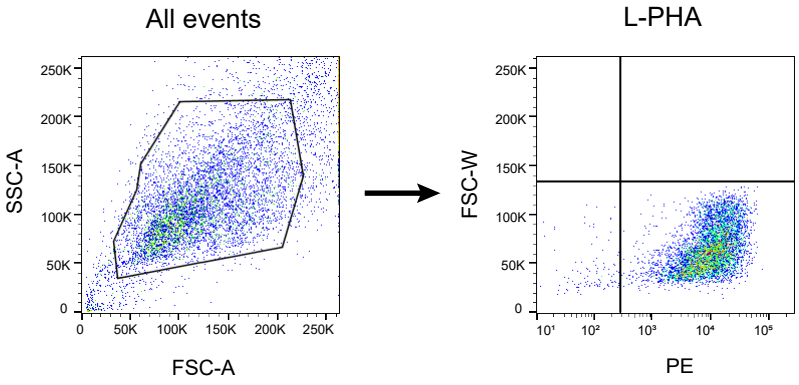

b
UDPG
ManNAc
Neu5Ac
Fractional enrichment
KL cell groups
K cell groups
Time course
30m
1h
2h
6h
*
****

a. PI/Annexin V-FITC staining

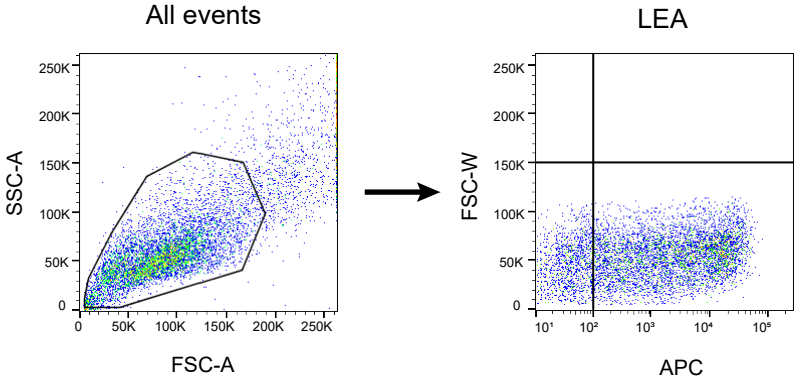


b. Lectin binding assay

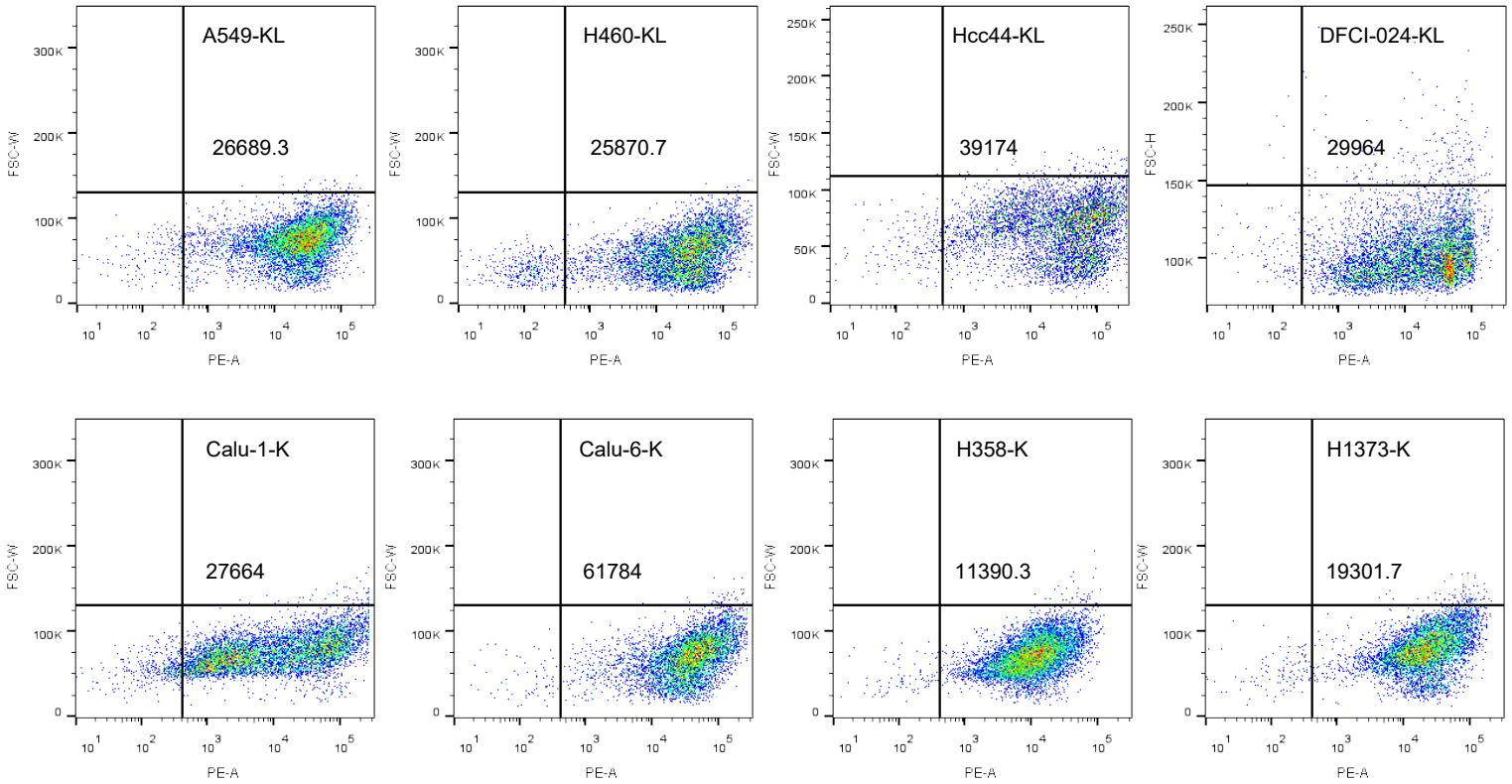
L-PHA



LEA



a. L-PHA representative dot plots



b. LEA representative dot plots