SUPPLEMENTAL MATERIAL

Contents

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Materials: All lectins were from Vector labs (Burlingame, CA), primary antibodies from ThermoFisher (Waltham, MA), recombinant human selectin-IgG fusion proteins from R&D Systems (Minneapolis, MN) and secondary Abs from Jackson Immunoresearch (West Grove, PA). HL60 cells were purchased from American Type Culture Collection (Manassas, VA). These cells were transduced with a virus generated using SiC-V1-scr (Kelkar, A., et al. 2020), and single cell sorted to obtain an isogenic clone called 'HL60-Cas9'. These cells express uniform high levels of the nuclease based on expression of the surrogate dTomato fluorescence reporter.

Dox-inducible sgRNA vector construction: The SiC-V2-Cas9G7 vector (Addgene 133043) was available from our previous study (Kelkar, A., Zhu, Y., et al. 2020). This vector expresses a tetracycline/Dox-regulated H1 promoter that drives sgRNA expression, a Tet-repressor to regulate this Pol III promoter, and a Cerulean (monomeric Cyan Fluorescence Protein/CFP) fluorescence reporter. Site directed mutagenesis was performed on SiC-V2-Cas9G7 to introduce a silent mutation that deactivates the single BsmBI site. This new plasmid is called "SiC-V2 mutBsmBI". The BsmBI-Stuffer-ChRNA-BsmBI region from LentiCRISPR V2 plasmid (Addgene 52961, (Sanjana, N.E., et al. 2014)) was amplified using AgeI/EcoRI handles and cloned into the AgeI/EcoRI site of "SiC-V2 mutBsmBI" just downstream of H1. Next, BFP (blue fluorescence protein) was PCR amplified from pKLV2-U6gRNA5(BbsI)-PGKpuro2ABFP-W (Addgene 67974, (Tzelepis, K., et al. 2016)), and it replaced the Cerulean encoding cassette in "SiC-V2 mutBsmBI". The resulting vector allows doxycycline-inducible expression of sgRNA from H1 promoter. A variant was also created where the H1 was replaced by the U6 promoter. These vectors are called 'H1-Dox-sgRNA', and 'U6-Dox-sgRNA'.

CRISPR guide selection and library construction: The glycoGene CRISPR library includes ~8-11 high potency sgRNA against 347 gene targets related to cellular glycosylation. These sgRNA were selected both from previously designed high on-target/low off-target 'second-generation sgRNA' that are part of other whole-genome CRISPR libraries (Doench, J.G., et al. 2016, Read, A., et al. 2017, Shalem, O., et al. 2014, Tzelepis, K., Koike-Yusa, H., et al. 2016, Wang, T., et al. 2015) and additional sequences generated using the Genetic Perturbation Platform (GPP) service (https://portals.broadinstitute.org/gpp/public/analysis-

tools/sgrna-design-help). The fitness of all sgRNA was evaluated using the stand-alone sgRNA potency scoring algorithm, Azimuth (Doench, J.G., Fusi, N., et al. 2016). This algorithm computes a score between 0 and 1, where 1 indicates a high likelihood of potency, and 0 indicates a low likelihood of potency. The score is computed by weighting the influence of base positions within the protospacer as well as its context bases (the bases preceding and succeeding the protospacer). Guides with a potency score >0.35 were considered to be active, and included in the glycoGene CRISPR library.

The library was synthesized as a pool of oligos (91 bases long) by CustomArray Inc. Each oligo consists of the 20-mer sgRNA sequence (Supplemental Table S2) flanked by BsmBI cutting sites, and a pair of PCR primer sequences at the termini (Primer 1F: TAAAGGGACGGTTAATCGCTCACC and Primer 105R: GGTCTGAACGTAAGGAAGTGTGGA). The library was amplified using Accuprimer PfX supermix with 1µl each of 100 µM 1F and 100 µM 105R in a 50 µl reaction. 5 µl amplicon was loaded on a 3% agarose gel to confirm product size and remaining amplicon was column purified using NucleoSpin gel and PCR clean-up kit (Macherey-Nagel).

The cloning vector plasmids (H1-Dox-sgRNA, and U6-Dox-sgRNA) were digested with BsmBI and the vector backbone was gel extracted. Golden gate ligation of sgRNA oligos with both vector plasmid backbones ('H1-Dox-sgRNA', and 'U6-Dox-sgRNA') was performed in two independent reactions, in the presence of Esp31, T7 DNA ligase and ATP using the following cycling conditions: 37ºC 2h, 9 cycles (20ºC, 20 min; 37ºC, 5 min) and 4ºC hold. After column purification, 2 µl of the ligation product was electroporated into NEB-10 beta electrocompetent cells using Bio-Rad MicroPulser. Electroporated cells were plated on a 24.5 cm^2 LB agar (+100 µg/ml Carbenicillin) plate and incubated overnight at 37ºC. The bacterial lawn was gently scraped from the plate using 10 ml LB medium and the process was repeated 2 more times to retrieve all the bacterial growth into a 30 ml pool that was incubated at 37ºC for 2 h for bacterial outgrowth. 2/3rd of the culture was used for making Glycerol stocks of the library and 1/3rd diluted into 150 ml of LB medium containing Carbenicillin. Following overnight incubation at 200 rpm, 37ºC, library plasmid was prepared from the bacterial culture using the NuceloBond Xtra Midi Kit (Macherey-Nagel).

Glycogene library virus preparation: Lentivirus was made by mixing 17 µg pooled library plasmid, 21 µg of psPAX2 packaging plasmid and 8.7 µg of pMD2.G envelope plasmid with 125mM Ca^{2+}, 1mL 2X HBS

(HEPES-buffered saline, 50mM HEPES+140mM NaCl, 10mM KCl, 1.5 mM Na_2HPO_4, pH 7.05), and sterile water to make a 2mL reaction mix. Reaction mix was added drop by drop onto a 150mm culture dish plated with Lenti-X 293T cells (Takara Bio, Mountain View, CA) at 60-70% confluence. 8h later, cell culture medium was changed to 20mL Opti-MEM™ (ThermoFisher). 18hrs later, the first batch of virus was harvested. 15mL fresh Opti-MEM™ was fed to the cells with 10mM sodium butyrate (MilliporeSigma, St. Louis, MO). 18hrs later, a second batch of virus was harvested. After spinning down at 1,000g for 2 min, supernatant was filtered using 0.45µm Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units with PES Membrane (Thermofisher). The filtered virus was pelleted at 50,000g for 2h at 4°C. The supernatant was removed and the final product was resuspended in 175µl volume (i.e. 200X concentrated virus). Aliquots of concentrated virus were stored at -80°C until use.

Synthesis of Cas9G7 sgRNA: A custom DNA oligo, 5'-**TTCTAATACGACTCACTATAG**TACGCCGGCTACATTGACGG**GTTTTAGAGCTAGA,** was purchased that contained the T7 promoter (red) followed by a 20bp guide sequence against Cas9 (green, called 'Cas9G7' (Kelkar, A., Zhu, Y., et al. 2020)) and the first several bases of the sgRNA scaffold (blue). The EnGen sgRNA Synthesis Kit (New England Biolabs) was used to produce the corresponding synthetic sgRNA. To this end, the above oligonucleotide was mixed with T7 enzyme and reaction mix provided with the kit for 2 h at 37ºC. The product formed was DNAase treated, and the RNA was purified using the RNA Clean & Concentrator kit (Zymo Research, Irvine, CA). The final product was stored at -80ºC until use.

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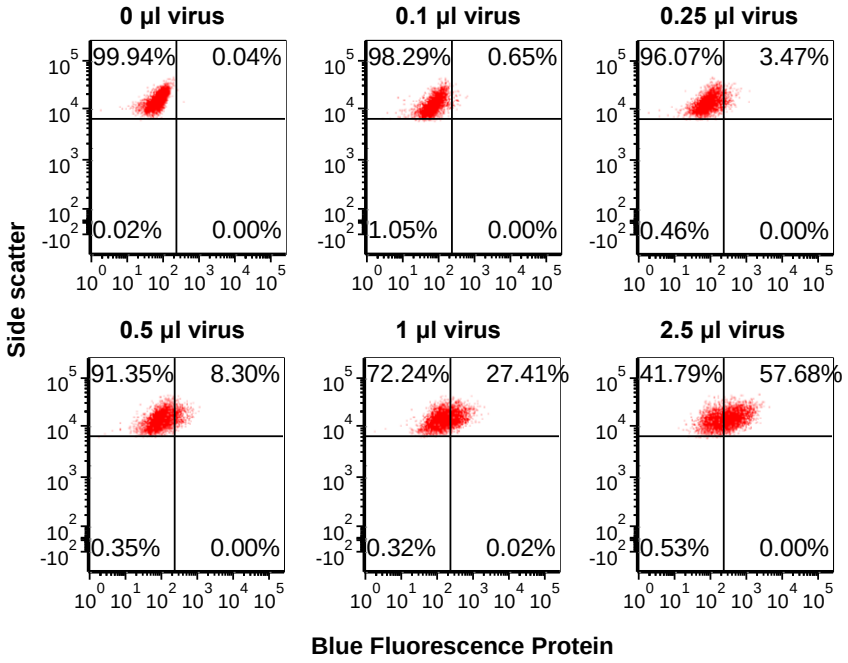
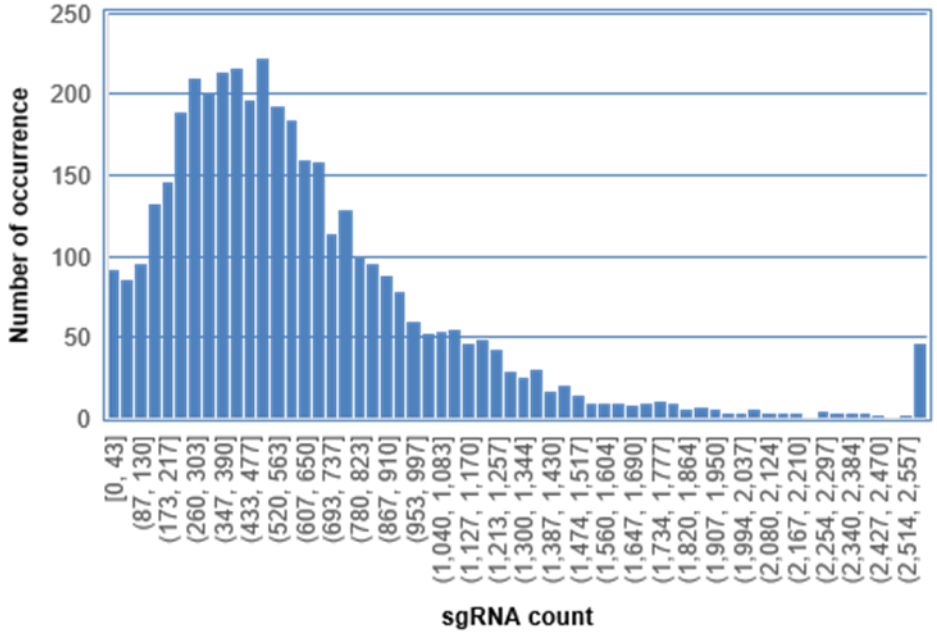


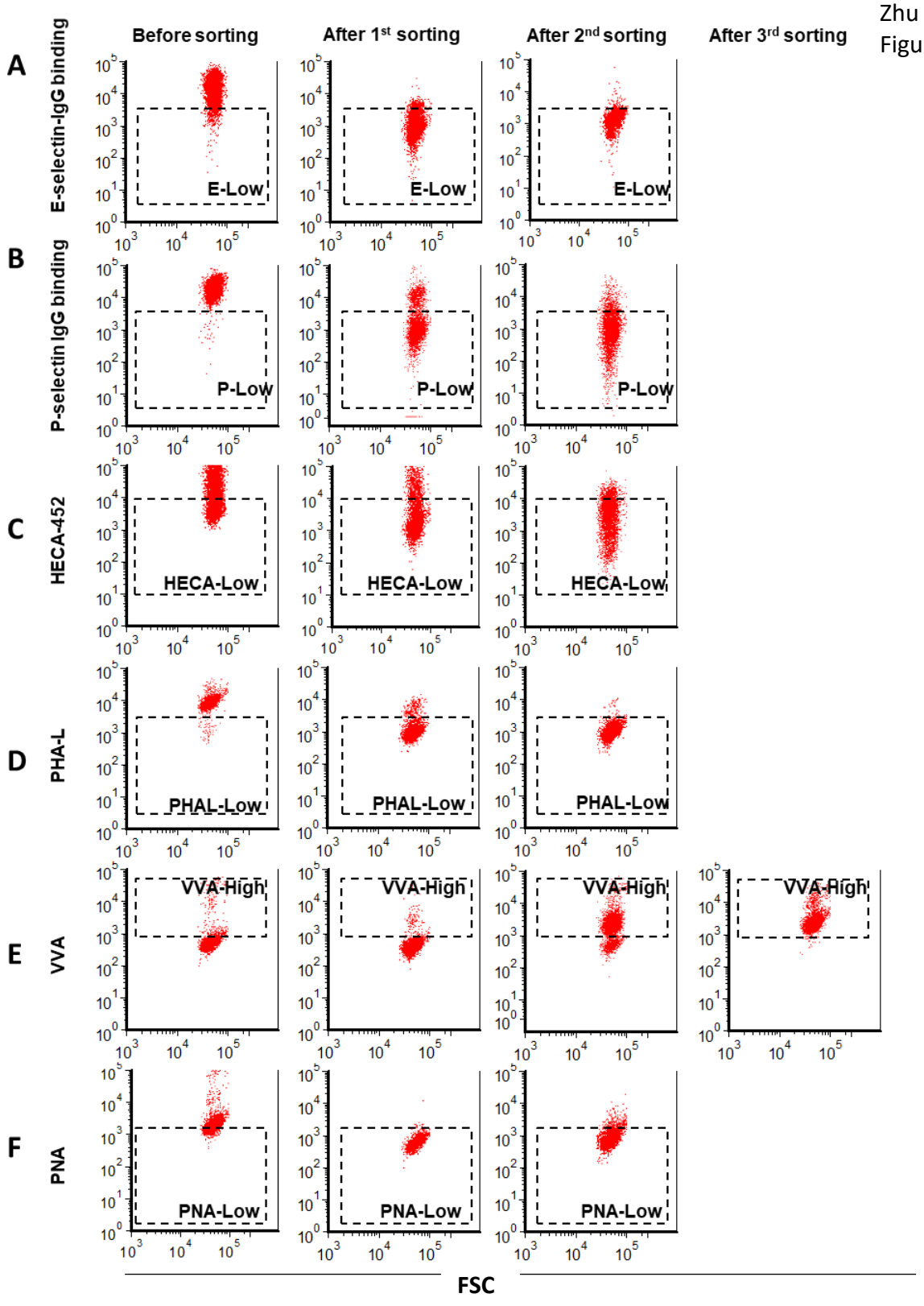
Supplementary Fig S1. Glycogene CRISPR virus titration. In a pilot run, 3×10^6 HL-60 cells were transduced in 6-well plates (3mL/well) using the glycoGene CRISPR library lentivirus that carries a BFP reporter. GlycoGene CRISPR virus concentrate volume was varied from 0-2.5µL, as shown in individual panels. % transduced cells varied from 0-57.68% on day 2. Based on this pilot study, 1µL virus (MOI~0.27) was applied in the final transduction that was scaled up and used to create the HL60-lib and enriched cell populations described in this manuscript.

Zhu et al.
Figure S2



Supplemental Fig. S2. Guide representation in HL60-lib. The full glycoGene CRISPR library contains 3976 sgRNA targeting 347 genes. The figure provides the sgRNA distribution in the HL60-lib cells based on Next Generation Sequencing (NGS). 80 of the sgRNA had low representation (<43 counts, left corner), while 45 genes had high representation (>2557 counts, piled at the right corner). Raw counts are presented in Supplemental Table S3.

Zhu et al.
Figure S3



Supplementary Fig S3. Serial sorting of transduced HL60s. HL60 glycogene transduced cell populations exhibiting low E-selectin binding (**panel A**), low P-selectin binding (**panel B**), low HECA-452 expression (**panel C**) and low PHA-L binding (**panel D**) were FACS sorted by gating on the box indicated using dashed line. Two serial sorts were performed as shown to enrich for these cell populations, with the first sort being performed on day 12 after viral transduction. Similar serial sorts were performed to enrich for cell populations exhibiting high VVA binding (**panel E,** 3-sorts) and low PNA recognition (**panel F**). In each case, a distinct low/high binding cell population was evident after the first sort, and this was progressively enriched in subsequent sorts.