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1029 **Figure S1. Genes enriched in the first-round screen of mCherry-negative cells.**

1030 The x-axis represents genes targeted by the Brunello library, grouped by GO analysis.

1031 The y-axis shows the enrichment score $[-\log_{10}]$ of each gene based on MAGeCK analysis of the

1032 sgRNA reads in gDNA^{Sort1} vs gDNA^{sort0} (**Table S2**). Each circle represents a gene, with its size

1033 indicating the statistical significance $[-\log_{10}]$ of enrichment when comparing gDNA^{Sort1} to

1034 gDNA^{Sort0}. The color of each circle represents the function of the genes. Only genes with an

1035 enrichment score greater than 10^4 are shown.

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1037 **Figure S2. SLC35A1 KO in HAE-ALI culture.**

1038 **(A) Generation of HAE-ALI^{SLC35A1-KO} cultures.** Human airway epithelial cell line CuFi-8

1039 cells were transduced with a gRNA/Cas9 lentivirus. The puromycin resistant cells were seeded

1040 onto Transwell inserts and differentiated at an ALI for 4 weeks. **(B) Validation of SLC35A1**

1041 **expression in HAE-ALI cultures.** Western blotting detected SLC35A1 expression in cultures

1042 derived from the scramble control but none in the cultures from *SLC35A1* KO cells. β -actin was

1043 detected as a loading control. **(C) Transepithelial electrical resistance (TEER) measurement.**

1044 HAE-ALI cultures, Scramble control, SLC35A1-KO, KIAA0319L-KO, and the Scramble control
1045 treated with NA were detected for TEER values.

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1047 **Figure S3. SIA expression in wide-type or mutant *SLC35A1* expression HEK293^{SLC35A1-KO}**
1048 **cells.**

1049 HEK293^{SLC35A1-KO} cells were mock-treated or transduced with lentiviral vectors
1050 expressing SLC35A1 WT, T128A and Δ C Tail mutants, as indicated, followed by selection of
1051 blasticidin (at 10 μ g/ml) for 2 weeks. The cells were fixed with 4% PFA and then permeabilized
1052 with 0.1% Triton X-100 for intracellular staining. Biotinylated SNA and MAL II lectins were used
1053 to stain glycan expression in HEK293^{SLC35A1-KO} cells. SNA (**A**) and MAL II (**B**) stained cells were
1054 incubated with DyLight 649-conjugated streptavidin for visualization under a confocal
1055 microscope (CSU-W1 SoRa, Nikon) at 60 \times . HEK293 Scramble cells were used as a control.

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1057 **Table S1. A list of genes enriched in the second round (FD400210-FD400208) of the**
1058 **sorted mCherry-negative cells and ranked by the $-\log_{10}$ enrichment score.**

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1060 **Table S2. A list of genes enriched in the first round (FD400209-FD400208) of the sorted**
1061 **mCherry-negative cells and ranked by the $-\log_{10}$ enrichment score.**

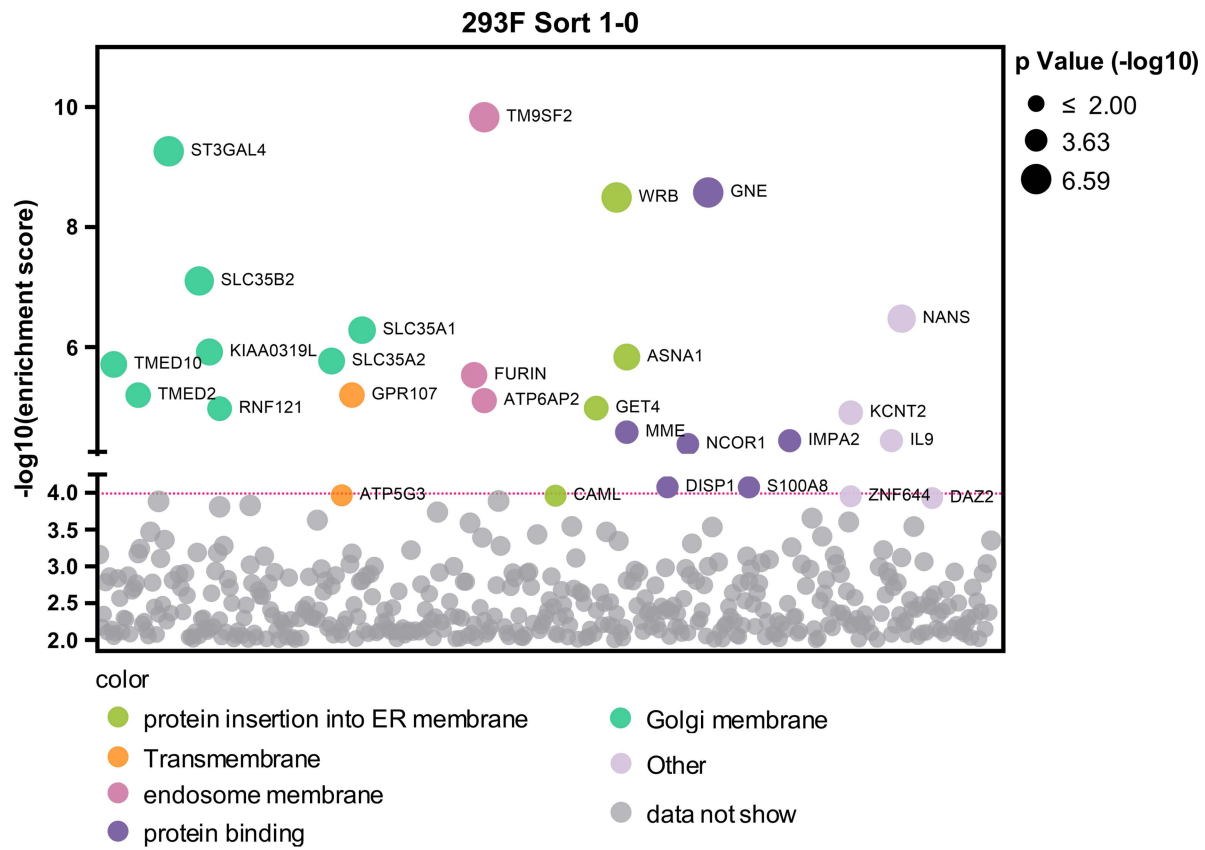


Figure S1

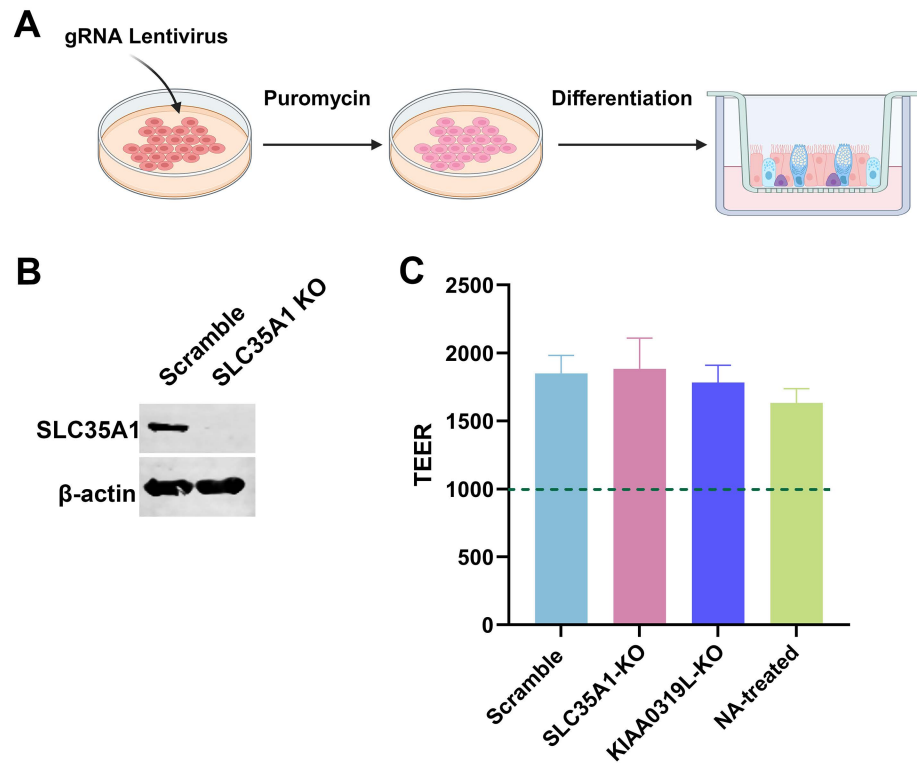


Figure S2

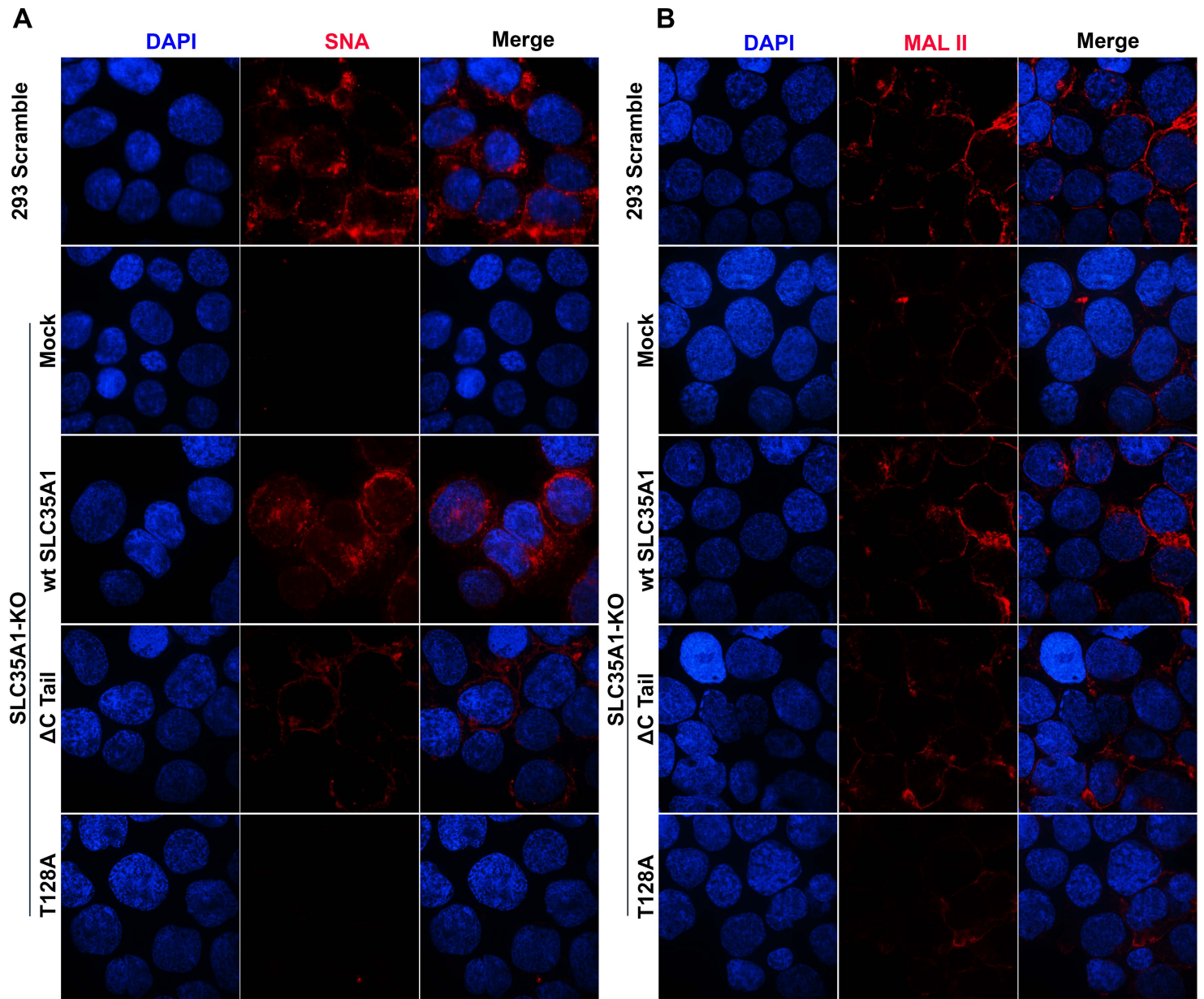


Figure S3