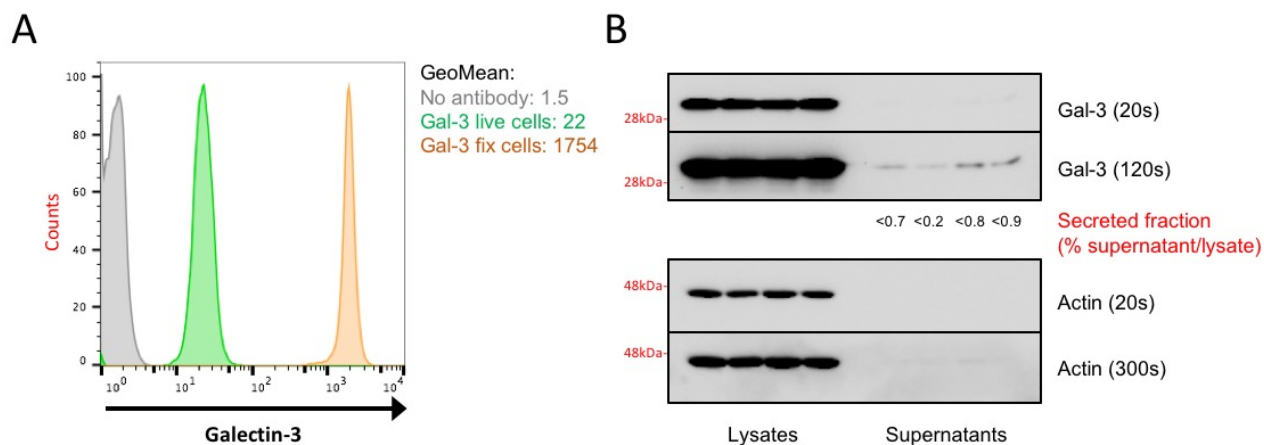


## Supplementary Information

### Figure S1. Gal-3 localisation in cytosol, cell surface and supernatant.

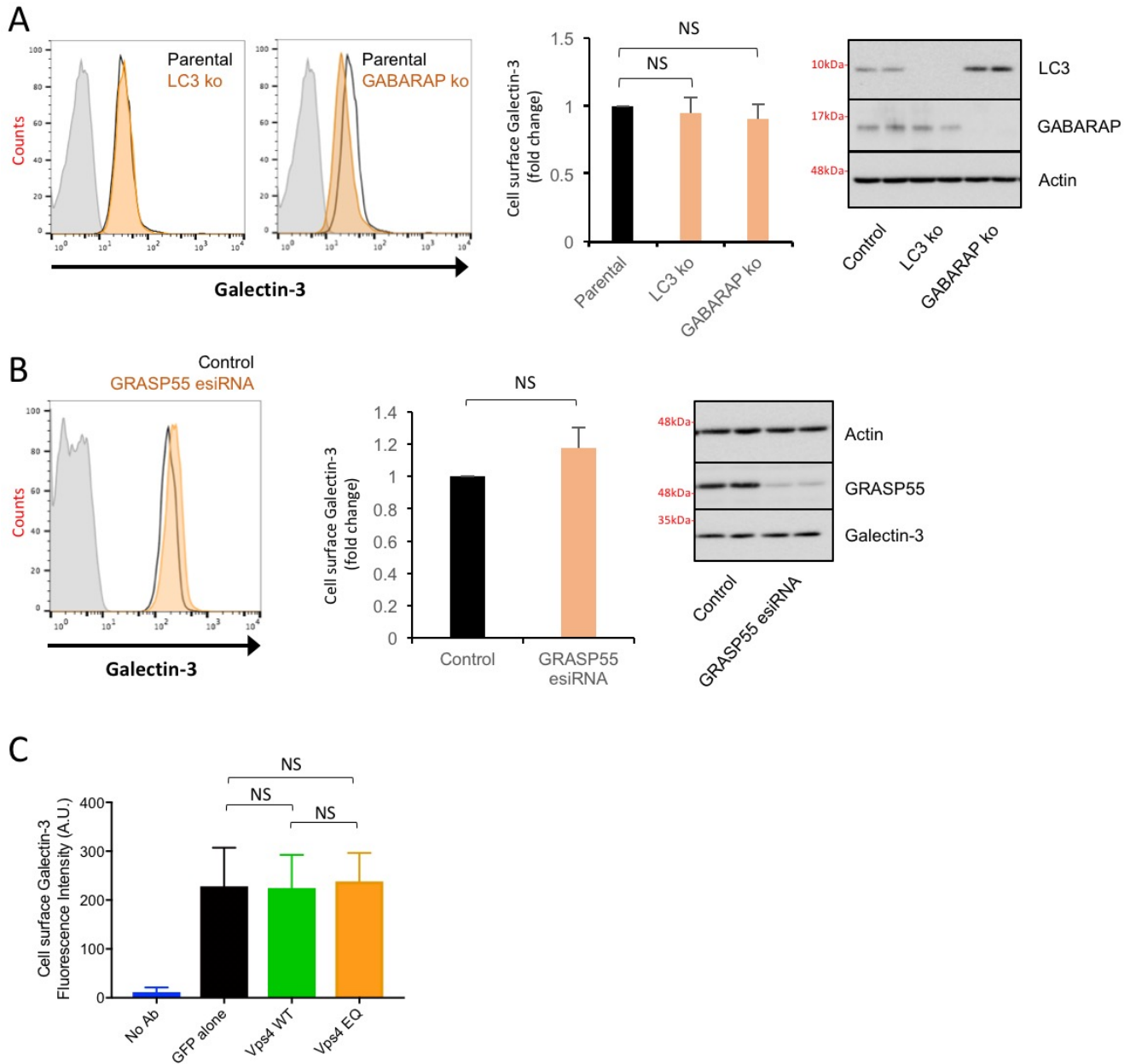


### Figure S1. Gal-3 localisation in cytosol, cell surface and supernatant.

A. Cell surface and intracellular levels of Gal-3 in sHeLa cells detected by flow cytometry. Cells were collected and stained for Gal-3 either as live cells or after fixation with methanol. The geometric mean of the fluorescence intensity is indicated for each treatment. This gives an indication of the proportion of Gal-3 on the cell surface relative to the entire pool of Gal-3

B. Level of free Gal-3 secreted by sHeLa cells at steady state. sHeLa cells were incubated in serum-free medium for 24 h and the cells and medium then assessed by western blot. Gal-3 was assessed in the lysate and medium (supernatant), where cells were lysed in an equal volume as the medium. An equal volume of each were assessed for Gal-3 and the proportion of Gal-3 detected in the supernatant compared to the lysate was calculated. Actin was used as a loading control and control for cell lysis. Exposure times are indicated to allow relative comparisons between blots to illustrate the large increase in Gal-3 in the supernatant compared to actin.

## Figure S2. LC3, Vps4 and GRASP55 do not regulate cell surface Gal-3.



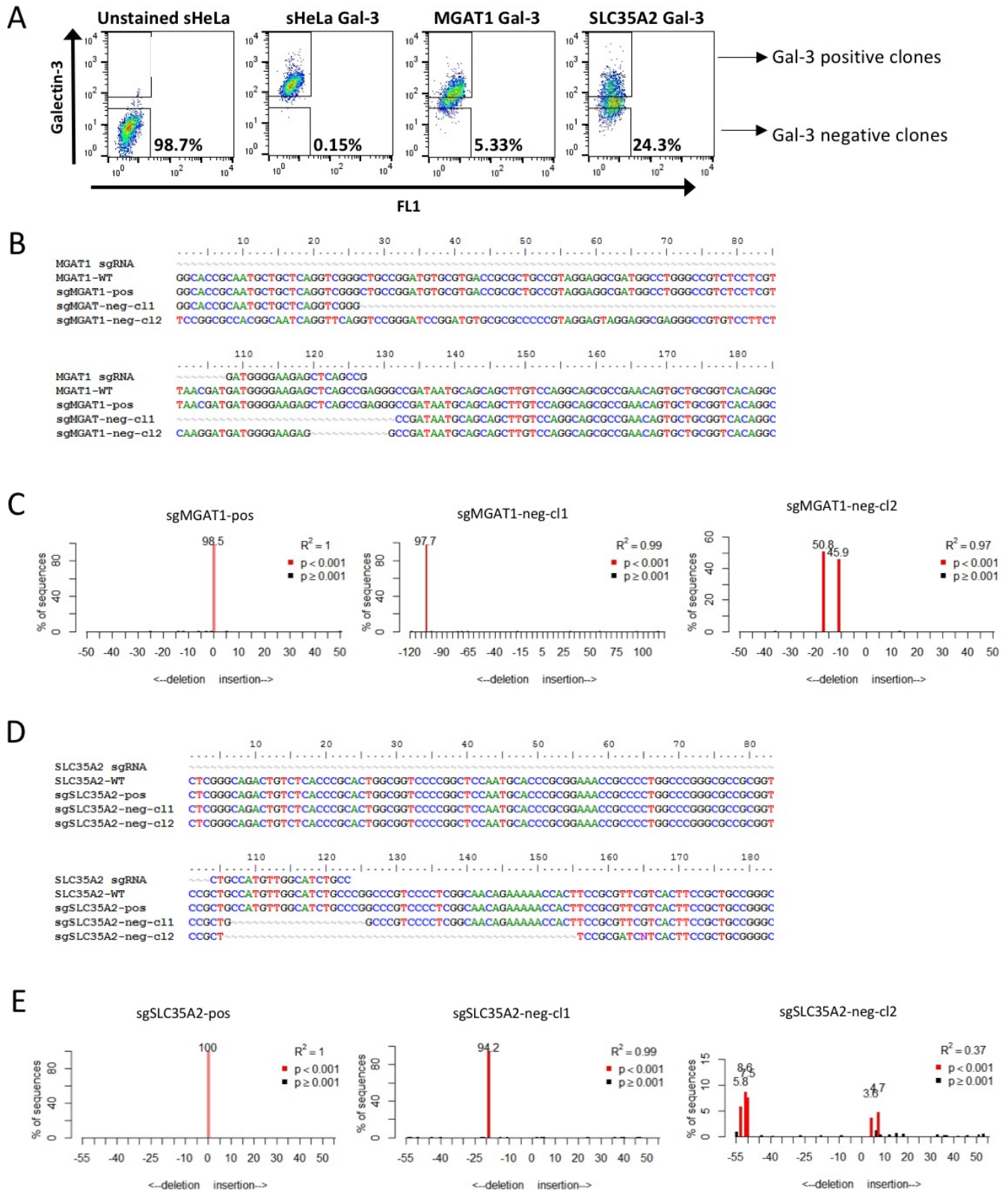
### Figure S2. LC3, Vps4 and GRASP55 do not regulate cell surface Gal-3.

A. Cell surface localization of Gal-3 in LC3 and GABARAP CRISPR/Cas9 targeted HeLa cells measured by flow cytometry. Gray line: no antibody; black: parental; orange: mutants. Quantification is shown. Error bars represent  $\pm$ s.e.m. from biological replicates ( $n = 3$ ); NS: not significant using a two sample Students *t*-test comparing the mutants to parental cells. Western blotting analysis of LC3 and GABARAP is shown on the right.

B. Cell surface localization of Gal-3 in GRASP55 esiRNA targeted HeLa cells measured by flow cytometry. Gray line: no antibody; black: control; orange: esiRNA. Quantification is shown. Error bars represent  $\pm$ s.e.m. from biological replicates ( $n = 3$ ); NS: not significant using a two sample Students *t*- test comparing the GRASP55 esiRNA to control cells. Western blotting analysis of GRASP55 and Gal-3 is shown on the right.

C. Cell surface localisation of Gal-3 on sHeLa expressing GFP alone, GFP tagged wild type Vps4 or GFP tagged Vps4 EQ mutant (dominant negative mutant). sHeLa cells were transiently transfected and 24 h post transfection cells expressing GFP were analysed for Gal-3 cell surface expression by flow cytometry. Error bars represent  $\pm$ s.e.m where  $n = 2$  for no Ab and GFP alone and  $n = 3$  for Vps4 wild type and EQ  $n=3$ ; NS: not significant using a two sample Students *t*-test.

## Figure S3. Generation of MGAT1 and SLC35A2 knockout sHeLa cells using CRISPR/Cas9.



**Figure S3. Generation of MGAT1 and SLC35A2 knockout sHeLa cells using CRISPR/Cas9.**

A. Cell surface localization of Gal-3 in polyclonal MGAT1 and SLC35A2 CRISPR/Cas9 targeted suspension HeLa cells measured by flow cytometry. Single clones were isolated from the Gal-3 positive and negative populations indicated.

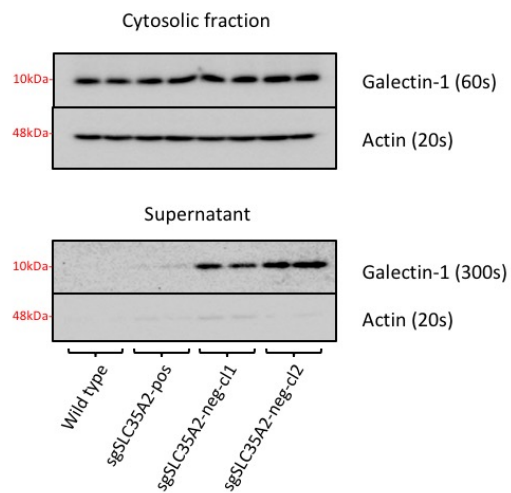
B. Gal-3 negative clones contain large deletions in the CRISPR/Cas9 targeted regions of MGAT1. Sequence alignment of wild type sHeLa, Gal-3 positive clone and Gal-3 negative clones 1 and 2. Sequencing analysis revealed that MGAT1 clone 1 contained a homozygous 105 bp deletion, whereas clone 2 resulted in a heterogenous phenotype, containing an 11 bp deletion and a 17 bp deletion in the MGAT1 gene.

C. TIDE analysis of MGAT1 Gal-3 positive and negative clones. TIDE analysis confirms the sequence analysis as in A and shows that the frequency of the two deletions in clone 2 was approximately 50% each, indicating heterozygous mutations had been induced in each allele.

D. Gal-3 negative clones contain large deletions in the CRISPR/Cas9 targeted regions of SLC35A2. Sequencing results for SLC35A2 clones showed that clone 1 contained a 19 bp deletion and clone 2 a 50 bp deletion, both of which were appear homozygous across both alleles.

E. TIDE analysis of SLC35A2 Gal-3 positive clone and negative clones. TIDE analysis on SLC35A2 clones confirm that the positive contains on insertions or deletions, clone 1 contains a homozygous 19 bp deletion and clone 2 contains a 50 bp deletion as indicated by sequencing, however, TIDE analysis clone 2 was less accurate due to the quality of the sequencing data.

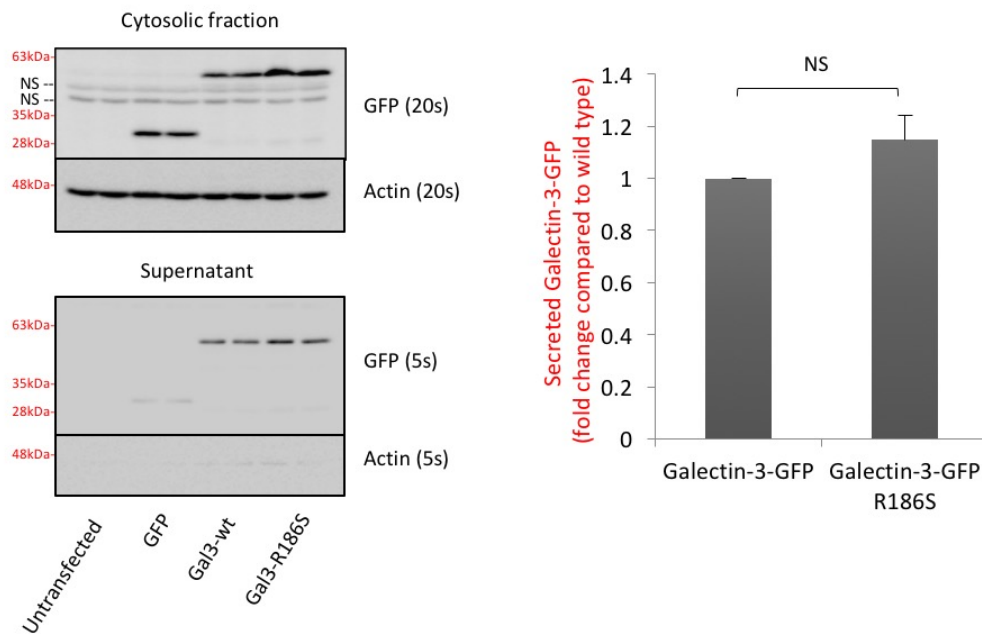
## Figure S4. Gal-1 is secreted from SLC35A2 deficient sHeLa.



### Figure S4. Gal-1 is secreted from SLC35A2 deficient sHeLa.

Gal-1 secreted from SLC35A2 deficient suspension HeLa cells after 24 h incubation in serum-free medium assessed by western blot.

## Figure S5. Gal-3 R186S mutant does not have a secretion defect.



### Figure S5. Gal-3 R186S mutant does not have a secretion defect.

Gal-3-GFP wildtype or R186S mutant secreted from sHeLa cells assessed by western blot after 24 h incubation in serum-free medium. Quantification is shown on the right. Error bars represent  $\pm$ s.e.m. from biological replicates ( $n = 4$ ); NS: not significant using a two sample Students *t*- test.

**Table S1. Enriched genes identified in the genome-wide CRISPR screen for Gal-3 cell surface localisation**

[Click here to Download Table S1](#)



**Table S2. primers used in this study**

Table S2. primers used in this study

Name	Oligonucleotide sequence 5'-3'	Application
MGAT1 sgRNA	GATGGGGAAGAGCTCAGCCG	sgRNA for CRISPR/Cas9 knockout
MGAT1_F	GGCGAGGAAATCTCGGTCAT	Amplification forward primer
MGAT1_R	CCTCACCCGGAAGTGATTC	Amplification and sequencing reverse primer
SLC35A2 sgRNA	GGCAGATGCCAACATGGCAG	sgRNA for CRISPR/Cas9 knockout
SLC35A2_F	TCAGAATGTTCTCTTCCCCGC	Amplification and sequencing forward primer
SLC35A2_R	TTCCTGACTCGCACCTGATG	Amplification reverse primer
<b>CRISPR screen analysis</b>		
sgRNA_outer_F	GCTTACCGTAACTTGAAAGTATTTTCG	Forward PCR1 primer
sgRNA_outer_R	GTCTGTTGCTATTATGTCTACTATTCTTTCC	Reverse PCR1 primer
P5-sgRNA_inner_F	AATGATACGGCGACCACCGAGATCTACACTC TCTTGTGGAAAGGACGAAACACCG	Forward PCR2 primer with Illumina P5
P7-index-sgRNA_inner_R	CAAGCAGAAGACGGCATAACGAGATACATCG GTGACTGGAGTTCAGACGTGTGCTCTTCCGA TCTTCTACTATTCTTCCCCTGCACTGT	Reverse PCR2 primer with Illumina P7 and index
Illumina sequencing primer	ACACTCTTGTGGAAAGGACGAAACACCG	Custom sgRNA sequencing primer