Supporting Information for:

Nuclear repartitioning of galectin-1 by an extracellular glycan switch regulates mammary morphogenesis

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Materials and Methods

Chemicals and antibodies

All of the chemical reagents were of analytical grade, obtained from commercial suppliers, and used without further purification, unless otherwise noted. All of the reactions were performed in a N_2 atmosphere. Liquid reagents were added with a syringe. For measuring the levels of Gal-1, we used the rabbit polyclonal α-Gal-1 antibody (Abcam ab25138) (1) for all experiments except subnuclear colocalization studies with Gemin-4. In the latter, as well as to confirm the staining of Gal-1 in paraffin-embedded mammary gland sections, we used mouse monoclonal α-Gal-1 antibody (sc-271819). The following antibodies and culture reagents were obtained as indicated: anti-Lamin A/C (sc-6215) (Santa Cruz Biotechnology), anti-α6-integrin (MAB1378, Chemicon), anti-ZO-1 (61-7300, Thermo Scientific), Matrigel® (#354230, BD Biosciences); anti-total Erk1/2 (#137F5, Cell Signaling), protease inhibitor cocktail 1 (#539131, CalBiochem), anti-α-smooth muscle actin (clone 1A4, Sigma Aldrich), Alexa Fluor 488/568/633 conjugated phalloidin (Life Technologies), fluorescein-conjugated lectins (Vector Labs), anti-Gemin 4 antibody (rabbit polyclonal ab31581; Abcam), carrier free recombinant human GAL-1 and GAL-3 (R&D systems) and Calcein AM dye (Life Technologies).

Immunofluorescence

Inguinal mammary glands were freshly dissected from C57BL/6 female mice at different stages of their developmental history. Glands were mounted on glass slides and fixed with 4% paraformaldehyde for 15 minutes followed by fixation in Carnoy's fixative (75% ethanol and 25% glacial acetic acid) overnight. Following fixation, the glands were dehydrated by treatment with increasing concentrations of ethanol (from 70% to 100%) and then treated with xylene overnight. Hematoxylin and Eosin stained and unstained 5 µm tissue paraffin embedded sections were prepared by the UCSF Helen Diller Family Comprehensive Cancer Center Mouse Pathology Core. Deparaffinized sections were subjected to microwave-based antigen retrieval using citrate buffer (pH 6.0) for 10 min. The sections were permeabilized with PBS containing 0.1% Triton X-100 for 30 min, blocked with PBS containing 5% normal goat serum, 1% BSA and 0.05% Tween-20 for

1 hour at room temperature and followed by incubation with α-Gal-1 antibody, diluted 1:200, overnight at 4°C. All images were obtained using a laser scanning confocal microscopy LSM710 (Zeiss).

Immunostaining of mammary whole-mounts

Mammary glands were freshly dissected from 5-week-old (35 days postpartum) C57BL/6 mice. The mammary tissue was processed as previously described (2) with some modifications in order to perform immunostaining. The antigen retrieval was performed as described earlier. Then, the mammary whole-mount was incubated with Triton X-100 (0.1%) for 25 min and blocked (5 % normal goat serum + 1% BSA + 0.05% Tween-20) overnight at 4°C. This was followed by incubation with α-Gal-1 antibody, diluted at 1:50, or FITC-conjugated plant lectins ECA and SNA (Vector Labs) diluted at 1:100 in blocking buffer, overnight at 4°C. The whole mounts were washed with blocking buffer every 3 hours for 24 hours followed by incubation with Alexa Fluor-633-conjugated goat anti-rabbit antibody (1:100) overnight. The whole mounts were again subjected to a 24-hour wash in blocking buffer followed by dehydration and mounting using Permount (Fisher Scientific, PA). Images were generated using a confocal microscope (LSM710, Zeiss) using a 10X objective.

Cell culture and preparation of cell clusters

Functionally normal mouse mammary epithelial cells, EpH4, were kindly provided by E. Reichmann (Institut Suisse de Recherches, Epalinges, Switzerland)(3). Cells were maintained in 1:1 DMEM/F12 (University of California San Francisco Cell Culture Facility), supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) and 50 μg/ml gentamycin. EpH4 cells between passage 10 and 20 were processed for further experiments. S1 and T4-2 cells were grown in tissue culture monolayers (two-dimensional) using Falcon tissue culture plastic or three-dimensional laminin-rich extracellular matrix (lrECM; Matrigel, BD Biosciences) in defined medium as described previously(4, 5).

Three dimensional (3D) Type-1 collagen (CL-1) culture

For 3D cell cultures, EpH4 cell clusters were embedded in CL-1 gels as previously described (6). Briefly 8 volumes of acid-extracted bovine Type 1 collagen, Cellagen^{TM} (IAC-50, Koken, Tokyo, Japan) was gently mixed with 1 volume of 10X DMEM/F12, and appropriate volume of 0.1N sodium hydroxide to adjust the pH to 7.4 on ice. Two layers of collagen were poured into each well: a basal layer consisting only of CL-1 and an upper layer containing EpH4 cells suspended as single cells. This was followed by gelation at 37 °C for 15 min. After gelation, 300 µL of Mammary Epithelial Cell Growth Medium (MEGM^{TM}, Lonza) was added to the cultures and replaced every other day. Where indicated, Calcein AM dye (Life Technologies) prepared according to manufacturer's instructions was used to assess for cell viability. For exogenous treatments, we used 25 µM Ac_4ManNAc (controls were treated with an appropriate volume of vehicle, DMSO). In experiments with exogenous Gal-1, we used human recombinant GAL-1, either unconjugated, or conjugated with Alexa Fluor-633 using a protein labeling kit (Life Technologies) according to the manufacturer's instructions.

Analysis of branching morphogenesis

The branching phenotype of EpH4 clusters embedded in CL-1 gel (1 mg/ml) was determined after a 5-day culture period. The branching phenotype was defined as a cell cluster having at least two processes, which were at least half of the diameter of the central body, extending from its central body. Quantification of EpH4 cell branching was carried out by determining the percentage of branching clusters in each well. In addition, we analyzed the number of branches in each cluster. All experiments were repeated at least three times.

shRNA and constructs of expression

To transduce FLAG-tagged human GAL-1, NLS-GAL-1 (possessing the nuclear localization signal (NLS) sequence, $_{197}$PHPPKRLRSDPDAC$_{210}$, from human Gemin-4 (GenBank ID: AAF35283.1) as predicted by cNLSMapper(7), on the C-terminus of GAL-1), NES-GAL-1F (possessing the nuclear export signal (NES) sequence, 4INQMFSVQLSL14, from human Staufen-2 (GenBank ID:AAH08370.1), on the C-terminus of GAL-1), SEC-GAL-1 (possessing the signal peptide sequence,

1MYSMQLASCVTLTLVLLVNS20, from murine Interleukin-2 (GenBank ID: EDL35098.1), on N-terminus of GAL-1), N46D GAL-1 (possessing the Asn46 to Asp mutation), and myc-tagged GNE (possessing the sequence, 31EQKLISEEDL40, from human c-myc (GenBank ID: CAA46984.1) on the N-terminus of GNE), each cDNA was made by PCR, confirmed by sequencing and ligated into pLenti-EF1α-Puro (8), generated in our laboratory.

Lentivirus plasmids containing shRNA (Mission shRNA; Sigma, St Louis, USA) against murine Gal-1, Gemin-4, ST6Gal1 and GNE were used for gene silencing experiments. To packaging lentivirus, each lentiviral plasmid with other components (pLP1, pLP2 and pVSVG) were transfected into 293FT cells using FuGene6 (Roche, Basel, Switzerland) (see SI Appendix, Fig. S12 for hairpin sequences). Transfected cells were cultured in DMEM containing 5% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin. Culture media were replaced after 24 hours with fresh growth media. 48 hours later, lentiviral particles were concentrated from culture media filtrated with 0.45 µm filters by using Lenti-X Concentrator (Clontech). Aliquots were stored at -80 °C until use. To transduce EpH4 cells, 1.0×10^5 cells were plated in each well of a six-well plate, infected with the lentivirus, treated with polybrene for 24 hrs and selected by adding 5 µg/ml puromycin to the growth medium for 4 days. Lentivirus with scrambled sequence was used as shRNA control (Sigma). Target sequences for Gal-1 and Gemin-4 are outlined in Extended Data Fig. 11. To visualize GAL-1 in cells, C-terminus of GAL-1F and mutant Gal-1F cDNAs were tagged with monomeric Ypet (8) or mCherry (Clontech), respectively, by PCR. To visualize GNE, N-terminus of myc-tagged GNE was tagged with mCherry. For control constructs, NLS, NES and SEC peptide sequences (described above) were tagged with mYPet.

Gal-1$^{-/-}$ mice (MGI)

Lgals1-null (*Gal-1$^{-/-}$*) mice were obtained from the Consortium for Functional Glycomics (Scripps Research Institute, La Jolla, CA) and bred with C57BL/6 mice from The Jackson Laboratory in order to generate wild-type (WT) littermates(9). Genotyping of tail end biopsy specimens was performed by Polymerase Chain Reaction (PCR) using WT

forward primer: 5'-GACCCCATCCCTACACCCCAG-3'; Gal-1-null forward primer: 5'-CTATCAGGACATAGCGTTGG-3'; and common antisense primer: 5'-AAACTTCAGCCGGGAGAAAGG-3'. Animal use protocol was obtained and procedures were followed in strict accordance with guidelines established by Stanford University institutional animal care and use committee (IACUC).

Ex-vivo Organoid Assay

Primary organoids were isolated from 8 week-old virgin C57BL/6 mice as well as 8 week-old *Gal-1*$^{-/-}$ mice as described previously. Briefly, the fourth (inguinal) mammary glands were dissected out after lymph nodes were removed, then minced and digested in a collagenase/trypsin mixture (0.2% trypsin, 0.2% type-IV collagenase, 5% FBS, 5 µg/mL insulin in DMEM/F-12) for 30 min at 150 rpm at 37°C. After centrifugation, the fatty supernatant was discarded, and the cell pellet was resuspended in DMEM/F-12 and pelleted twice. After resuspending pellet in DMEM/F-12 containing 80 U of DNase I (Sigma), for 5 min at room temperature with occasional shaking, the suspension was spun at 80 x g for 10 min, and differential centrifuged in DMEM/F-12 was performed in order to concentrate primary organoids. The final pellet was resuspended in the desired amount of medium. For viral transduction, the organoids were seeded in 24-well poly(HEMA)-coated plates at a density of 1000 organoids per well and infected with lentivirus in the presence of 8 µg/mL polybrene for 24 h.

Western blotting

To verify Gal-1 depletion and overexpression, EpH4 cells were cultured in CL-1 gel (3 mg/ml) for 48 hours in serum-free media (MEGM, Lonza). For protein isolation, media was removed; the cells were washed with PBS and lysed with a buffer containing 20 mM Tris (pH 7.5), 150 mM NaCl, 0.5% Tween-20 with protease and phosphatase inhibitor cocktails (EMD Millipore, Billerica, USA). Protein concentration from samples was determined using the BCA Protein Assay kit (Thermo Scientific, Waltham, USA) following the manufacturer's instructions. Protein samples were then mixed with 10X Laemmli sample buffer and heated at 95 °C for 5 minutes. Samples were loaded into a pre-cast 4-20% tris-glycine polyacrylamide gel (Invitrogen) using the NOVEX system

(Invitrogen). Resolved proteins were transferred to a nitrocellulose membrane (Whatman, Maidstone, UK) and followed by blocking in PBS, 0.05% Tween-20 with 3% BSA for 1 hour at room temperature. Membranes were incubated overnight at 4°C with 5% BSA, 0.1% Tween-20 in PBS containing anti-Gal-1 antibody (Abcam) and anti-α-tubulin antibody (Sigma). Primary antibodies were detected with the Pierce SuperSignal detection kit (Rockford, IL) and signal was captured with the FluorChem 8900 analysis system (Alpha Innotech, San Leandro, CA).

Glycopolymer synthesis

Soluble lactose- and cellobiose-GPs were synthesized as previously described.[19] Briefly, polymerizations were carried out using standard Schlenk techniques. S-Ethyl-S'-(α,α'-dimethyl-α''-acetic acid)trithiocarbonate (4.09 mg, 4.55 μmol), lactosyl ethyl acrylamide or cellobiosyl ethyl acrylamide (0.100 g, 228 μmol), 4,4′-azobis(4-cyanovaleric acid) (319 μg, 1.14 μmol), DMF (200 μl) and H_2O (800 μl) were added to the Schlenk tube. The solution was degassed by sparging with N_2 for 30 min. The Schlenk flask was placed in a 70 °C bath to start the polymerization. After 16 h, the reactions were stopped by exposure to oxygen. The conversion for the glycosyl monomer was calculated by comparing the integrals of the alkene proton peaks of the monomer (5.7 ppm, 1H) and those of the backbone methylene protons on the glycopolymer (1.87- 1.00 ppm, 2H). Glycopolymers were purified by dialysis against H_2O for 72 h and lyophilized. ^{1}H spectra were obtained with a 500 MHz Bruker spectrometer. Chemical shifts (δ) are reported in parts per million and standardized against solvent peaks. Aqueous size exclusion chromatography was performed using a Viscotek TDA 302 SEC fitted with a Shodex SB-803 HQ column and using differential refractive index detection. The mobile phase used was an aqueous solution of $NaNO_3$ (0.1M) at a flow rate of 1.0 mL min^{-1} and at a constant temperature of 40 °C. Conventional calibration was achieved using narrow PEG standards. Polydispersity indices were determined using the Omnisec for Windows software.

Lactose-GP. According to the above procedures, **lactose-GP** was obtained as a light yellow solid (90.2 mg, 89%). Conversion (NMR) = quant. PDI (SEC) = 1.1. ^{1}H NMR (500

MHz, D_2O): δ 4.61 – 4.33 (m, 100H), 4.12 – 3.20 (m, 800H), 2.33 – 1.87 (m, 47H), 1.87 – 1.38 (m, 77H), 1.10 (s, 12H). (See SI Appendix, Fig. S13)

Cellobiose-GP. Cellobiose-GP was prepared using the above procedure to yield a light yellow solid (87.9 mg, 87%). Conversion (NMR) = quant. PDI (SEC) = 1.2. ^{1}H NMR (500 MHz, D_2O): δ 4.65 – 4.36 (m, 100H), 4.15 – 3.12 (m, 800H), 2.35 – 1.87 (m, 41H), 1.87 – 1.38 (m, 73H), 1.38 – 1.09 (m, 26H). (See SI Appendix, Fig. S13)

Treatment of cells with Glycopolymers

EpH4 cells cultured on top of a layer of 1 mg/ml CL-1 gel or embedded within CL-1 gels were incubated with lactose-GP, cellobiose-GP, or no GP for 1 - 3 days. After 1 day, EpH4 cells on top of CL-1 gels were treated with a α-Gal-1 antibody (Abcam) and imaged using confocal fluorescence microscopy. After 3 days, EpH4 cells embedded within CL-1 were washed, fixed, washed extensively and stained with α-Gal-1 antibody (Abcam), Phalloidin- Alexa Fluor 633 (Life Technologies), and DAPI to assay Gal-1 levels. The cells were then imaged using confocal fluorescence microscopy (LSM710, Zeiss).

Visualization of Intranuclear bodies

Visualization of Gem bodies was performed as previously described(10). Briefly, after washing cells with ice-cold PBS, cells were extracted for 10 min with ice-cold cytoskeletal (CSK) buffer (10 mM PIPES, 300 mM sucrose, 100 mM NaCl, 3 mM $MgCl_2$, 0.5% Triton X-100, pH 6.8) with protease inhibitors (Roche). Next, DNA was digested by DNase I (Boehringer, Indianapolis, IN) in CSK buffer (50 mM NaCl) for 1 h at 37°C, and chromatin was washed out with CSK buffer. Chromatin removal was confirmed by staining with DAPI. Cells were then washed with 0.25 M ammonium sulfate in CSK followed by a second wash with 2 M NaCl. After washing three times with PBS, fixation was performed in 4% ultrapure paraformaldehyde (EMS) and samples were immunostained for Gal-1 and Gemin-4, as described above. Samples were then imaged with a laser scanning confocal microscope (LSM710, Zeiss) using a 40X water immersion lens.

RT-qPCR

Total RNA extracted from control EpH4 cells, EpH4 cells with Gal-1 KD, and EpH4 cells with Gal-1 KD ectopically expressing NLS-GAL-1 was subjected to RT-PCR (iScript One-Step RT-PCR Kit, Bio-Rad) on a CFX96 Touch Real-Time PCR Detection System. After addition of the master mix to each sample, the following program was executed on three replicates for each condition: 50 °C for 10 min for cDNA synthesis, 90 °C for 1 min, then a 90 °C for 10 sec and 65 °C for 30 sec polymerase sequence was repeated for 39 cycles. The melting temperature for each amplicon generated was subsequently verified following RT-PCR. At least one primer for each gene traversed two exons. Mapk1/3 mRNA levels were normalized to the internal control β-actin transcript level for each condition (see Fig. S12 for primer sequences).

Statistics

For all experiments analyzing nuclear to extranuclear ratios of Gal-1, cells from at least three independent fields for each of three independent experiments were counted. The ratios were then calculated using ImageJ (National Institute of Health) as follows: using corresponding DAPI images, both nuclear and extranuclear regions were identified and mean fluorescence was determined. Data were recorded in Excel (Microsoft), and histograms were plotted. Data were analyzed using the unpaired student's t test and error bars indicate the standard error of the mean.

References

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SI Figure Legends

Figure S1. Gal-1 levels at different developmental stages of mammary gland morphogenesis

Immunofluorescence micrographs of paraffin-embedded sections of mammary glands stained for Gal-1 (upper panel) and DNA (lower panel) from C57BL/6 *Gal-1*$^{-/-}$ mice (top left) and C57BL/6 wt mice (top right and bottom rows) during distinct stages of development, at 5 weeks (35 days postpartum) (top right represents isotype control), at 10 weeks (70 days postpartum), mid pregnancy, lactation, and involution. Gal-1 levels are low after branching is complete and during pregnancy but increase moderately during lactation and during involution within mammary stromal cells. The Gal-1 antibody used for staining the top left and bottom row of sections is ab25138. Scale bar, 100 μm.

Figure S2. Knockout of Gal-1 impairs branching of mammary glands *in vivo*

Autofluorescence micrographs of carmine-stained mammary glands from 60 days postpartum C57BL/6 wild type (left) and *Gal-1*$^{-/-}$ mice (right) showing epithelial architecture (left panels). Scale bar, 100 μm. Quantification of the number of branch points in wild type and *Gal-1*$^{-/-}$ murine mammary glands (N = 8, mice chosen from three separate litters). For bar graph, error bars represent s.e.m. Statistical significance is given by ****$P < 0.0001$.

Figure S3. Gal-1 is concentrated in invading branches of EpH4 cells in 3D culture

EpH4 cells, which exhibit branching and migration when cultured in 3D CL-1 gels, were stained with α-Gal-1 antibody and DAPI. The epithelia constituting the branches (bounded by trapezoids) show high levels of Gal-1 when compared with the non-branching core (bounded by oval). Scale bar, 70 μm.

Figure S4. shRNA-based Gal-1 knockdown and rescue

(*A*) Immunoblot showing Gal-1 knockdown in lysates from EpH4 cells using one scrambled control shRNA and two separate shRNA clones against Gal-1. Ponceau staining indicates uniform loading. Graph showing quantification of Gal-1 levels in cells with Gal-1 knockdown. Error bars represent s.e.m. (*B*) Immunofluorescence

micrographs of control wt cells and cells treated with Gal-1 shRNA show effective knockdown of Gal-1. Scale bar, 50 μm.

Figure S5. Localization of GAL-1 constructs and branching of EpH4 cells expressing GAL-1 constructs

(*A*) Schematic depiction of overexpression constructs used to test the effect of subcellular localization of GAL-1 on mammary epithelial branching. Blue box refers to the FLAG peptide sequence. (*B*) Immunoblot showing levels of endogenous Gal-1 (bottom) and ectopically expressed GAL-1 (top) in control (scrambled shRNA) cells, Gal-1 KD cells, Gal-1 KD cells ectopically expressing GAL-1, Gal-1 KD cells ectopically expressing NLS-GAL-1, and GAL-1 KD cells ectopically expressing SEC-GAL-1 (from left to right). (*C*) Brightfield micrographs of Gal-1 KD EpH4 cells ectopically expressing GAL-1-mYPet (top, left), mYPet (top, right), NLS-mYPet (middle, left), NES-mYPet (middle, right), SEC-GAL-1-mYPet (bottom left) or SEC-mYPet (bottom right) were cultured within 3D CL-1 scaffolds. No branching was observed except with the constructs contained GAL-1. Scale bar, 50 μm. Inset fluorescence micrographs indicate the subcellular localization of each mYPet fusion construct (DNA (blue) and mYPet (green)) Scale bar, 5 μm. (*D*) Micrograph of EpH4 cell in 3D treated with recombinant GAL-1-Alexa Fluor 633 (green) were fixed, permeabilized and stained for DNA (blue) and F-actin (red). Strong nuclear localization of exogenously added GAL-1 was observed. Scale bar, 20 μm.

Figure S6. Secreted GAL-1 translocates to mammary epithelial nuclei

Gal-1 KD EpH4 cells with (right) and without (left) ectopic expression of SEC-GAL-1-mYpet were grown above permeable co-culture membrane inserts that allow exchange of secreted factors but are not conducive to cell migration. GAL-1 KD EpH4 cells ectopically expressing NLS-mCherry were co-cultured below the membrane inserts. Fluorescent micrographs show presence of SEC-GAL-1-mYPet signal in the nuclei of NLS-mcherry expressing cells suggesting nuclear translocation of secreted GAL-1. Scale bar, 50 μm.

Figure S7. Extracellular glycans control nuclear localization of GAL-1
Fluorescence micrographs of EpH4 cells with knockdown of endogenous Gal-1 and overexpression of GAL-1-mYPet. Cells were grown on top of CL-I gels (left) and treated with either a soluble lactose-GP (middle), which binds Gal-1, or a soluble cellobiose-GP (right). Scale bar, 50 µm.

Figure S8. Gal-1 colocalizes with terminal LacNAc and laminin in mammary ductal epithelia
Immunofluorescence micrographs of paraffin-embedded mammary gland section from 60 days postpartum C57BL/6 mice showing mammary ductal epithelia stained with DAPI (top, left; white), ECL (top, right; green), α-pan-laminin antibody (bottom, right; blue), and α-Gal-1 antibody (bottom, left red). The merged image shows colocalization of Gal-1, laminin and LacNAc in the basement membrane (white arrowhead). Scale bar, 20 µm.

Figure S9. Effect of sialoside overexpression on nuclear levels of Gal-1 in mammary epithelia
(*A*) Fluorescence micrographs of EpH4 cells overexpressing GNE (middle panel), the rate limiting enzyme in sialic acid biosynthesis, or treated with the small molecule Ac_4ManNAc (right panel), which is processed to CMP-sialic acid. EpH4 cells were stained with *Sambucus nigra* lectin (SNA), which is specific for α2,6 linked SA, and with an α-Gal-1 antibody. Scale bar, 50 µm. (*B*) Quantification of α2,6-linked SA levels in EpH4 cells as a result of overexpression of GNE and Ac_4ManNAc treatment. (*C*) Quantification of Gal-1 nuclear:extranuclear ratio in EpH4 cells as a result of overexpression of GNE and Ac_4ManNAc treatment. (*D*) Fluorescence micrographs of control wt EpH4 cells and cells with GNE knockdown cultured in 3D CL-1 gel and stained for α2,6-linked SA (white) and DNA (blue). Branching and migration is absent in EpH4 cells lacking SA residues. Scale bar, 50 µm. (*E*) Fluorescence micrographs of EpH4 control cells (left), cells with ST6Gal1 knockdown (middle) and cells overexpressing ST6GAL1 (right) stained with SNA (white), indicating that knockdown and overexpression of ST6GAL1 modulates α2,6 SA content. Scale bar, 100 µm.

Figure S10. Association of Gal-1 and Gemin-4 in mammary epithelial nuclei
(*A*) Immunofluorescence micrographs of EpH4 cells depleted of cytoplasm, soluble nuclear chromatin, and DNA and then stained with an α-Gal-1 antibody and an α-Gemin-4 antibody. Scale bar, 200 μm. (*B*) Immunofluoresence micrographs of EpH4 cells, control (left) and with Gemin-4 knockdown (right), cultured in 2D and stained with DAPI (top) and α-Gemin-4 antibody (bottom). Scale bar, 50 μm. (*C*) Immunofluorescence micrographs of control (left) and Gemin-4 KD EpH4 cells (right) cultured in 3D and stained with Phalloidin and DAPI (top) to assess branching morphology and Calcein AM vital dye (bottom) to assess viability. Scale bar, 200 μm.

Figure S11. Nuclear Gal-1 enhances Erk1/2 expression
Table of ΔC_q values for Mapk1/3 mRNA transcripts determined by RT-qPCR. Total RNA was extracted from control EpH4 cells, EpH4 cells with Gal-1 KD, and EpH4 cells with Gal-1 KD ectopically expressing NLS-GAL-1, and RT-qPCR was performed. β-actin was used as a housekeeping gene for normalization. mRNA levels of Mapk1/3 correlate with nuclear Gal-1 levels.

Fig. S12 Hairpin sequences for shRNA-based knockdown and primer sequences for RT-qPCR

Fig. S13 ^{1}H NMR Spectra for lactose-GP and cellobiose-GP

Figure S1

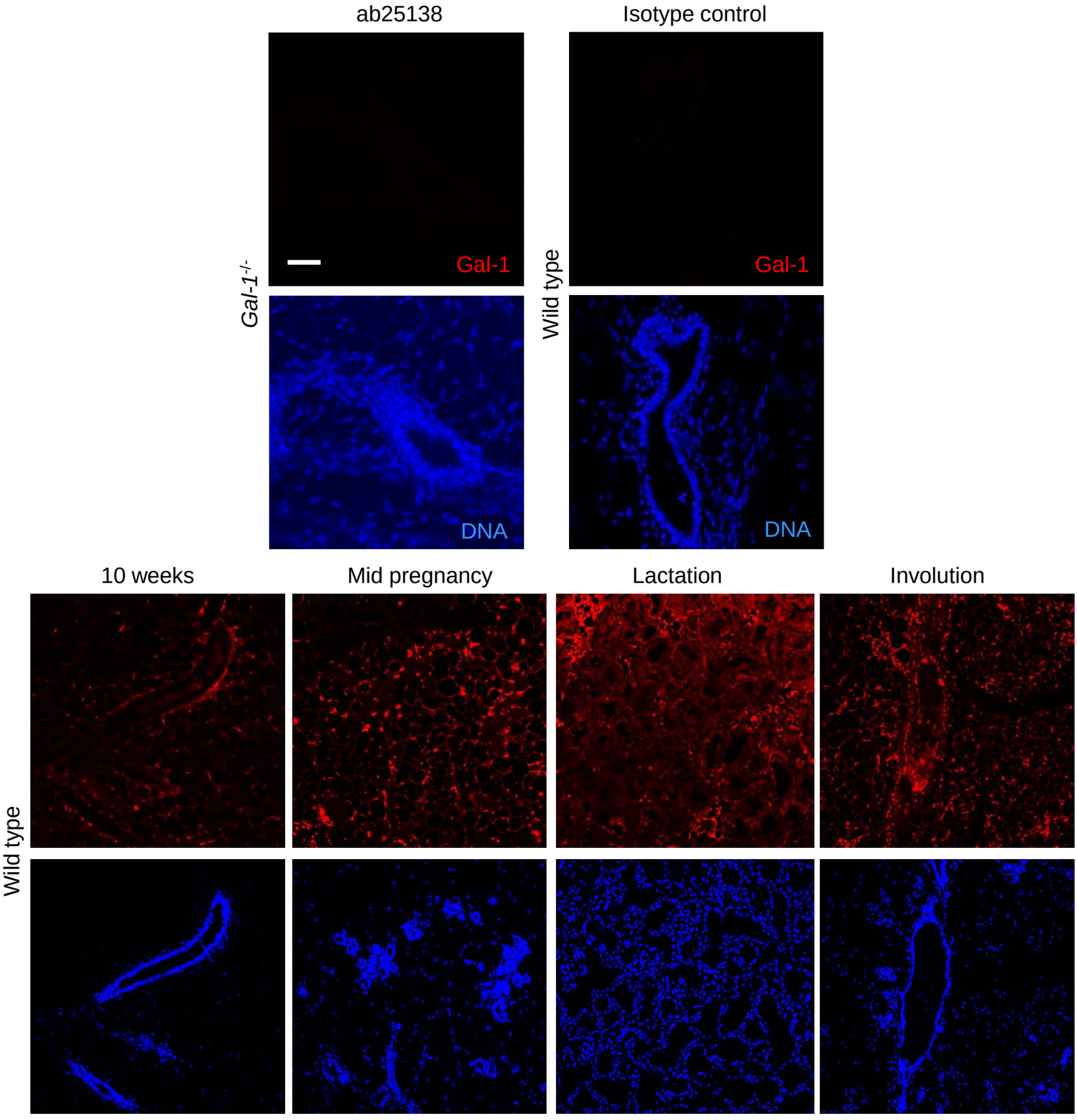
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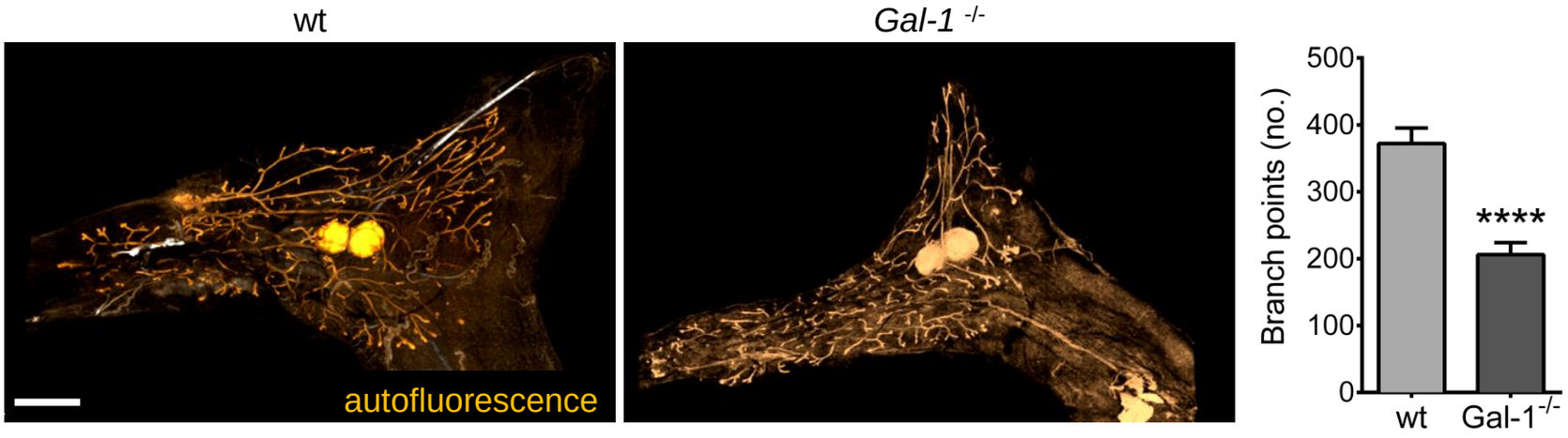
Figure S2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wt
Gal-1-/-

Figure S3

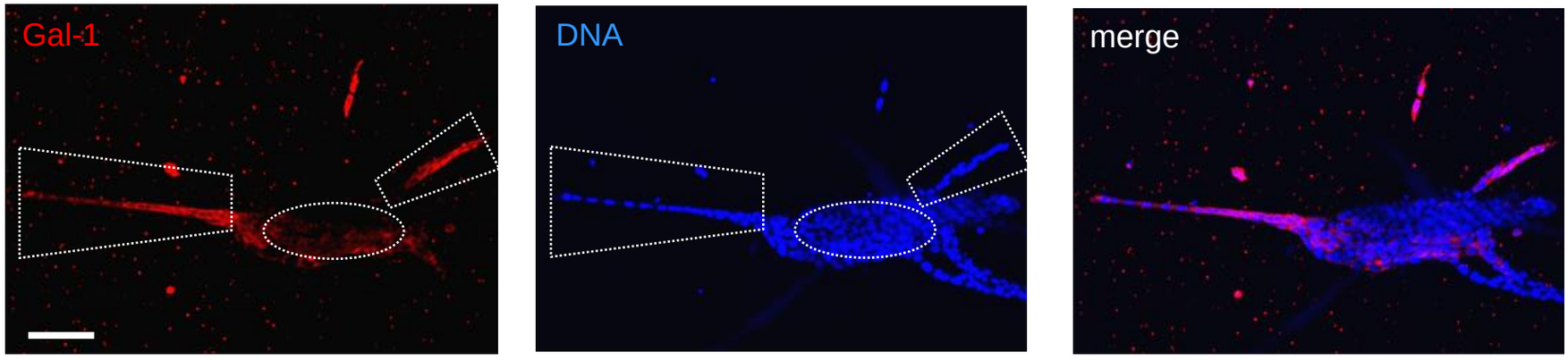
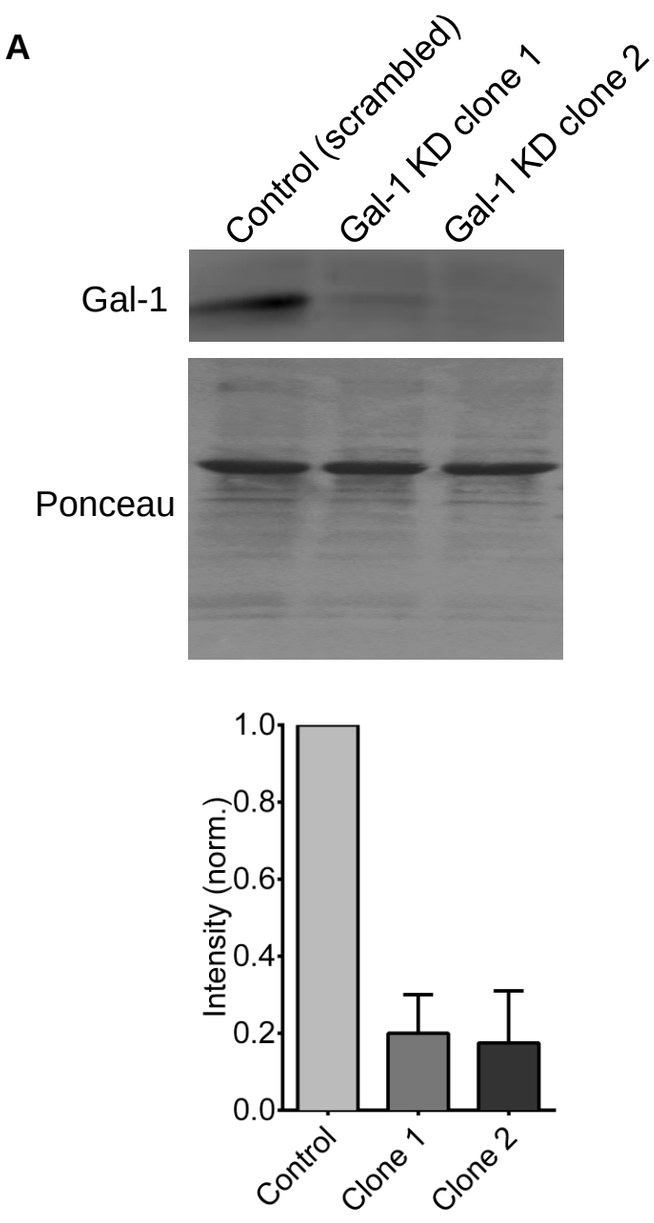


Figure S4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Clone 1
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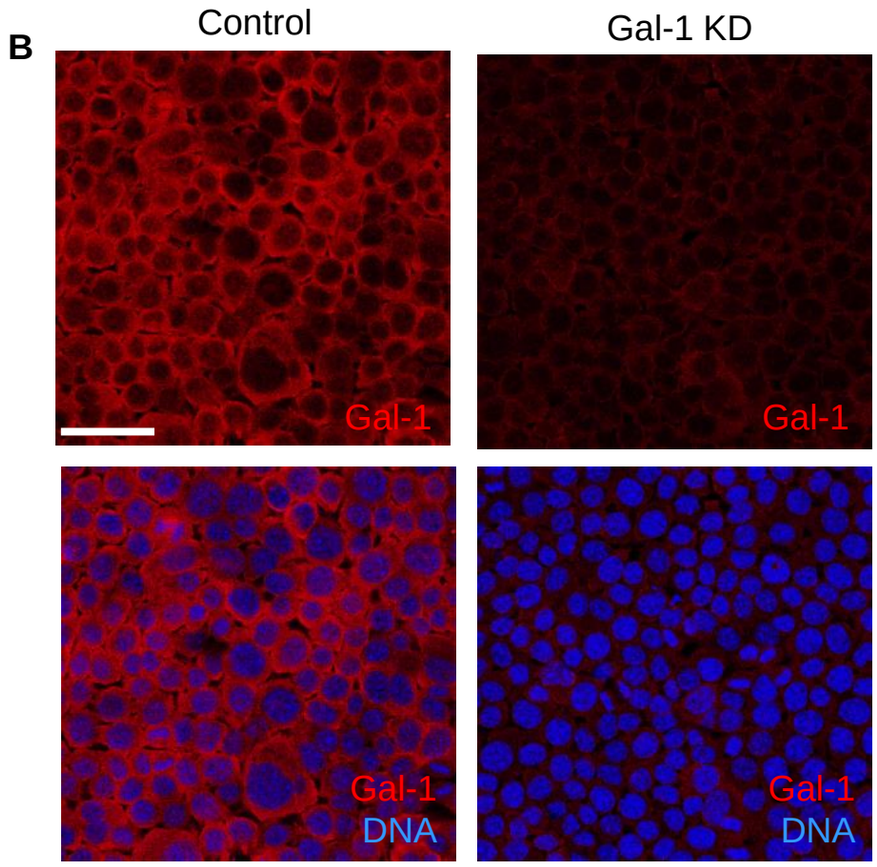
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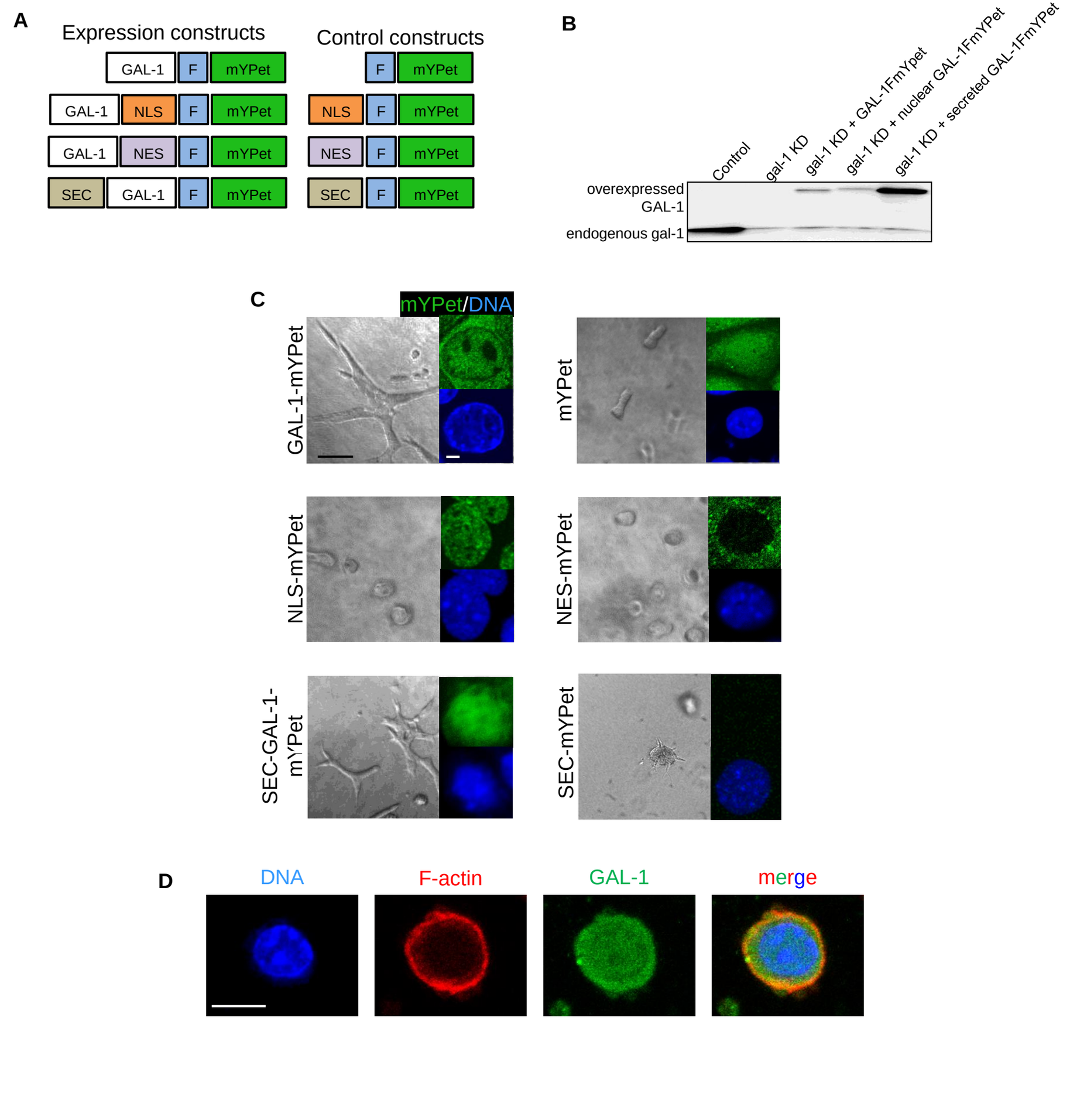
Figure S5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GAL-1-mYPet
mYPet
NLS-mYPet
NES-mYPet
SEC-GAL-1-mYPet
SEC-mYPet
D
DNA
F-actin
GAL-1
merge

Figure S6

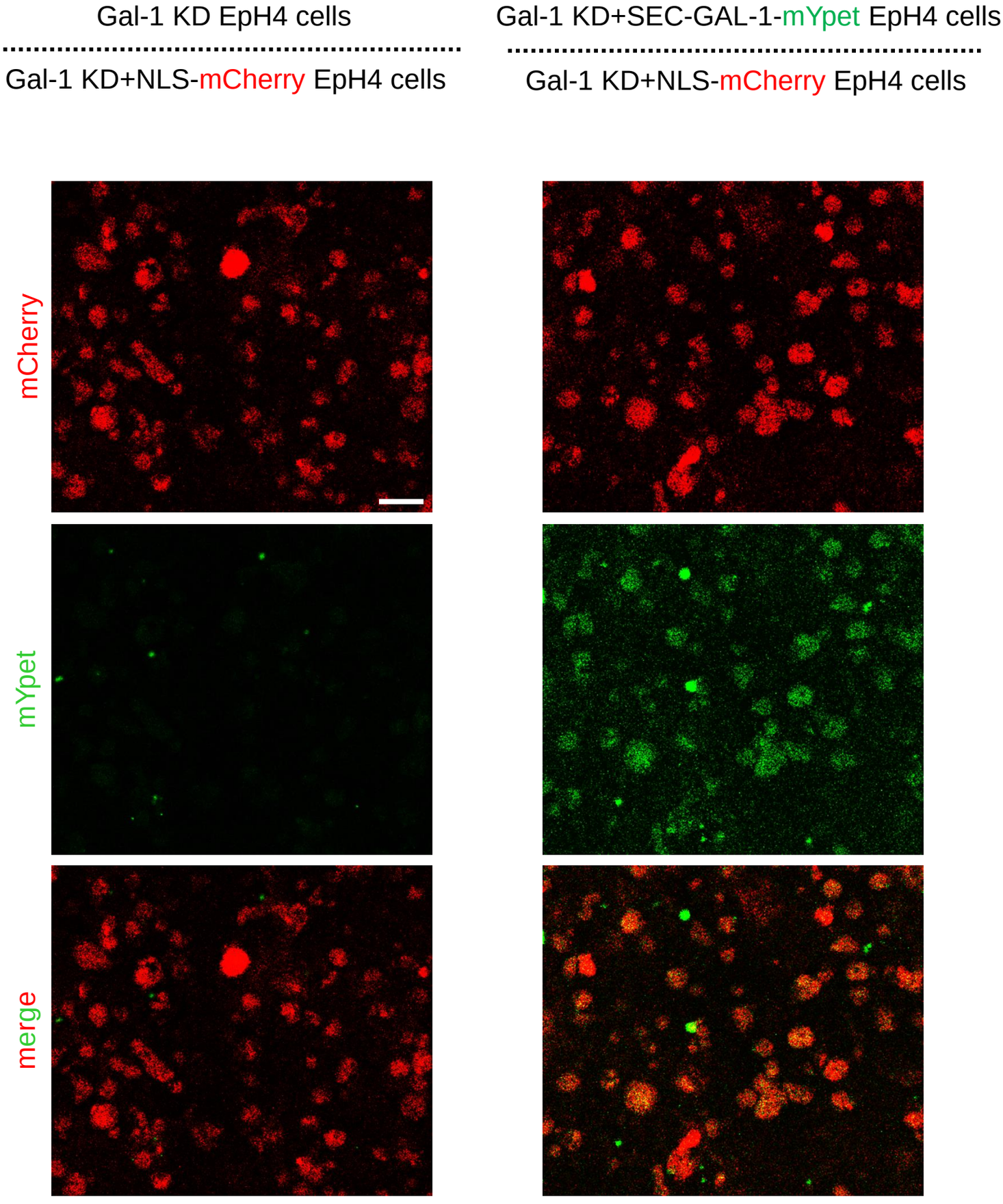

Gal-1 KD EpH4 cells
Gal-1 KD+NLS-mCherry EpH4 cells
Gal-1 KD+SEC-GAL-1-mYpet EpH4 cells
Gal-1 KD+NLS-mCherry EpH4 cells
mCherry
mYpet
merge

Figure S7

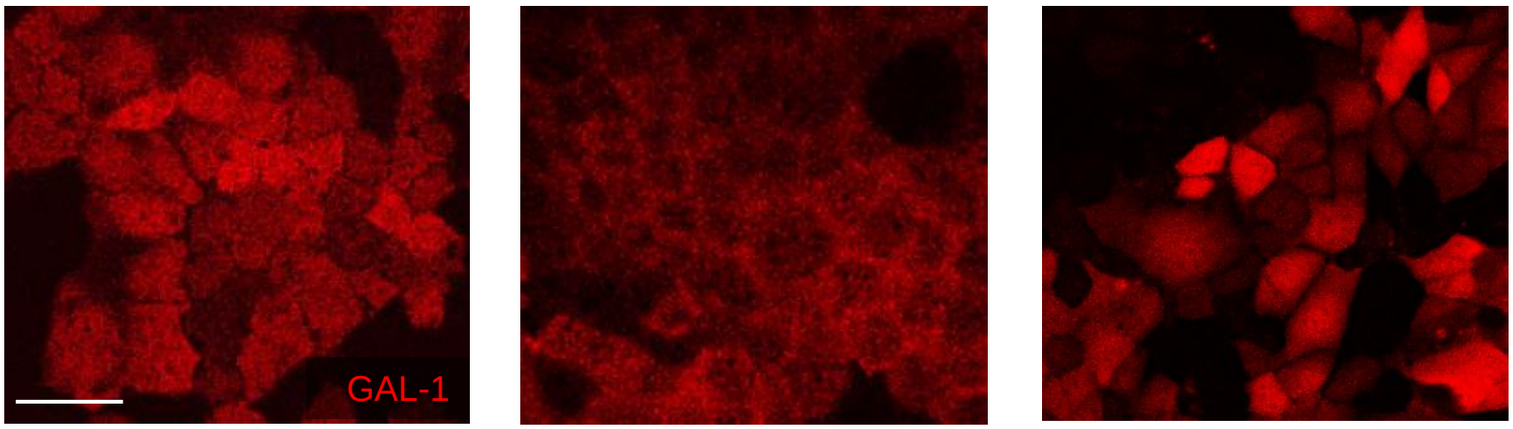


Figure S8

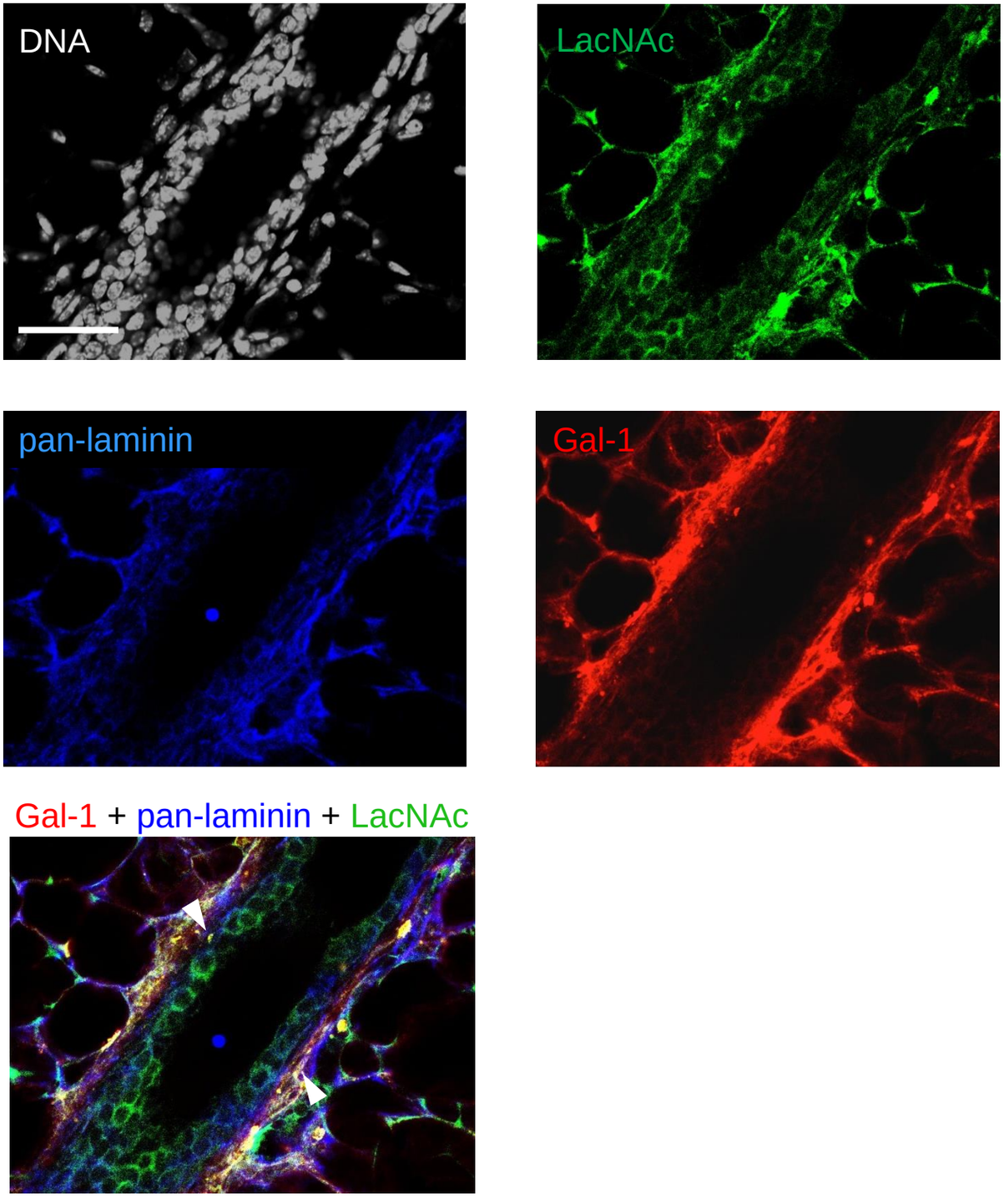


Figure S9

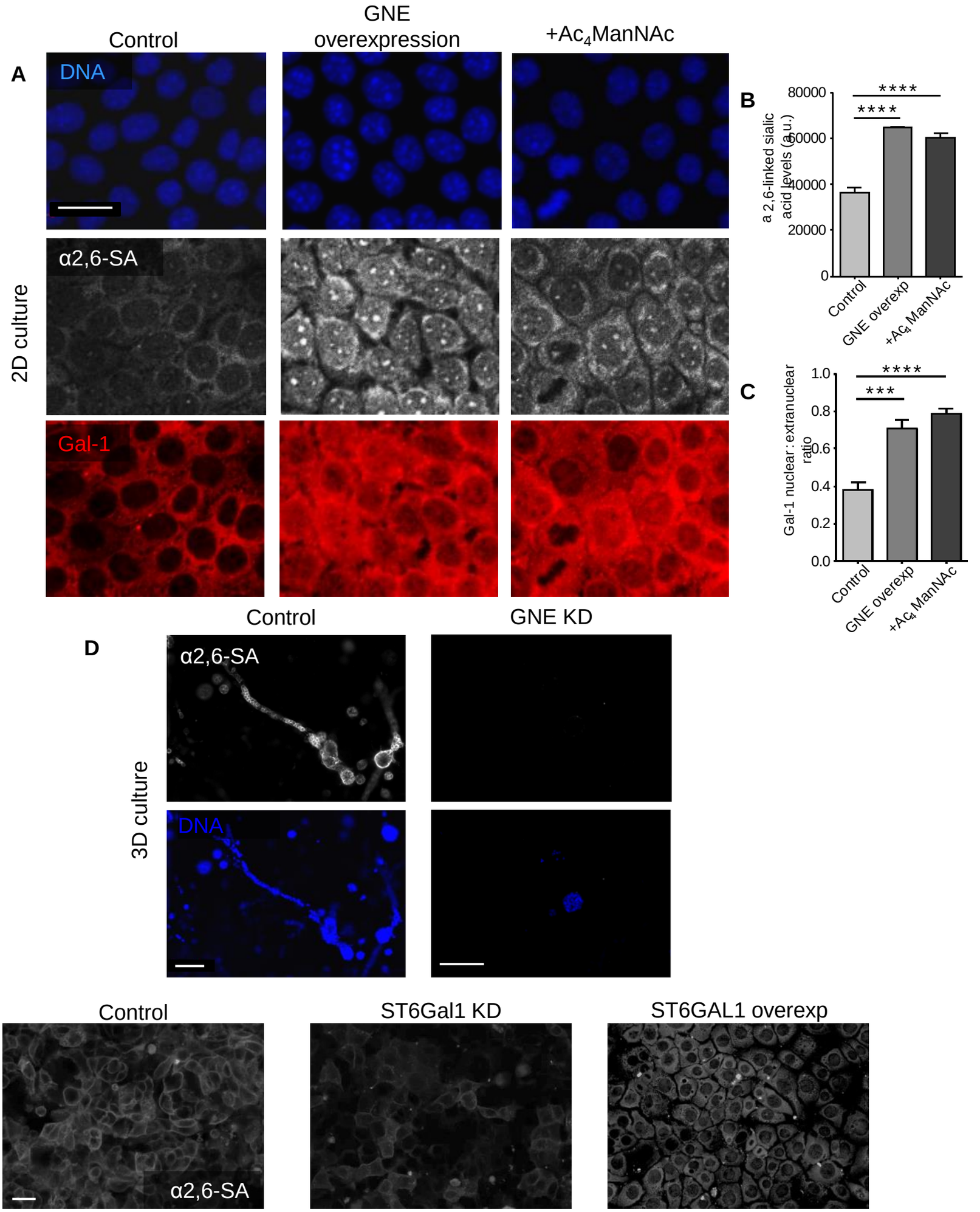
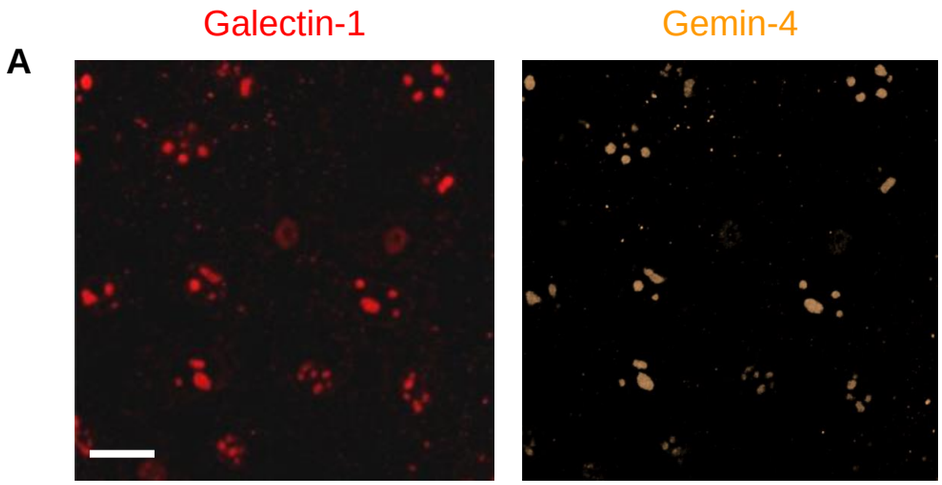
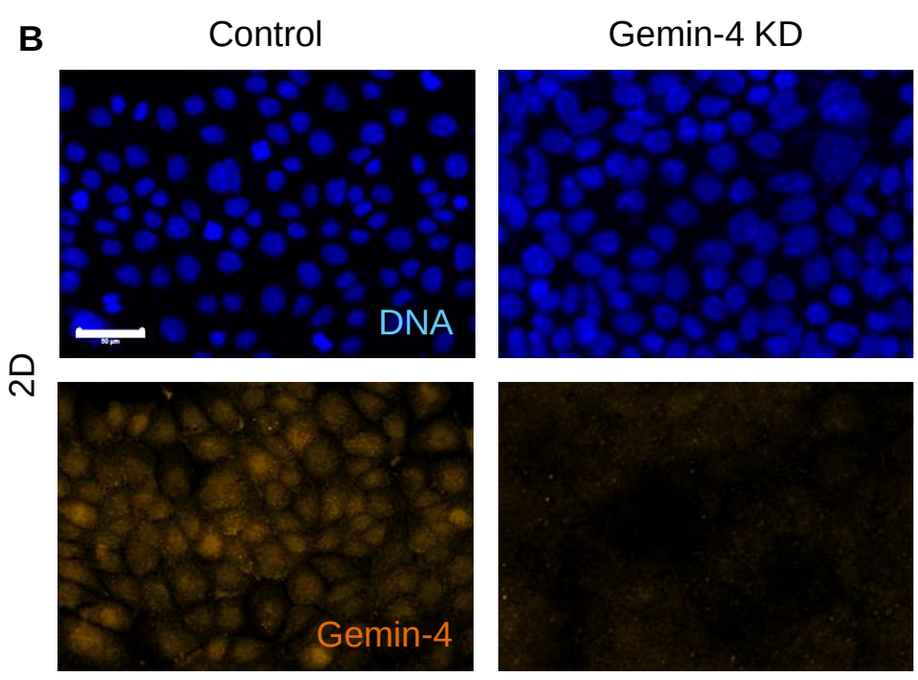

A
Control
GNE overexpression
+Ac4ManNAc
DNA
α2,6-SA
Gal-1
2D culture
B
a 2,6-linked sialic acid levels (a.u.)
80000
60000
40000
20000
0
****
****
Control
GNE overexp
+Ac4 ManNAc
C
Gal-1 nuclear : extranuclear ratio
1.0
0.8
0.6
0.4
0.2
0.0
****
***
Control
GNE overexp
+Ac4 ManNAc
D
Control
GNE KD
3D culture
α2,6-SA
DNA
E
Control
ST6Gal1 KD
ST6GAL1 overexp
α2,6-SA

Figure S10


A
Galectin-1
Gemin-4


B
Control
Gemin-4 KD
2D
DNA
Gemin-4

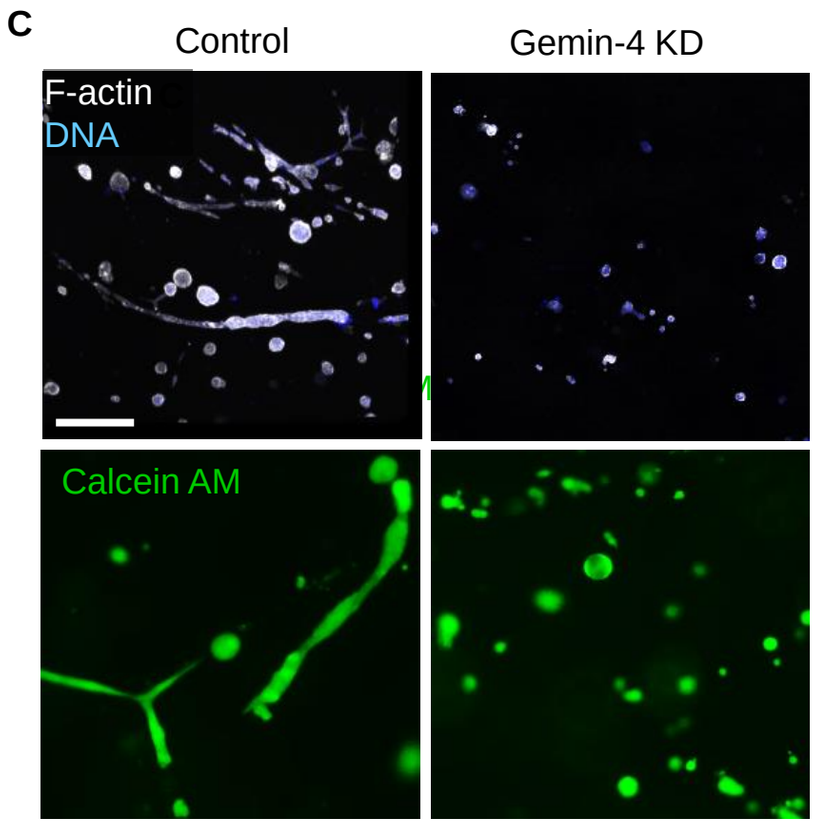

C
Control
Gemin-4 KD
F-actin
DNA
Calcein AM

Figure S11

Samples			
Gene	**ΔC_q Exp.***		
sample	*wt*	*Gal-1 KD*	*Gal-1 KD* + *NLS-GAL-1*
Mapk1 mRNA	0.6127	0.2327	0.7186
Mapk3 mRNA	0.3179	0.0580	0.1426

* ΔC_q Expression is calculated using β-actin mRNA as internal control.

Figure S12

Mouse Gal-1 hairpin sequences:
#1: CCGGGTGTGTAACACCAAGGAAGATCTCGAGATCTTCCTTGGTGTTACACACTTTTT
#2: CCGGAGACGGACATGAATTCAAGTTCTCGAGAACTTGAATTCATGTCCGTCTTTTTT
#3: CCGGCGCCAAGAGCTTTGTGCTGAACTCGAGTTCAGCACAAAGCTCTTGGCGTTTTT
#4: CCGGCCTGACCATCAAGCTGCCAGACTCGAGTCTGGCAGCTTGATGGTCAGGTTTTT

Gemin-4 hairpin sequences:
#1: CCGGGCCTCAGATCAAGCAGGTAATCTCGAGATTACCTGCTTGATCTGAGGCTTTTT
#2: CCGGCCCAGAGCTAAGAACAAACAACTCGAGTTGTTTGTTCTTAGCTCTGGGTTTTT
#3: CCGGGCTGACATGCTGAGTGTGTTTCTCGAGAAACACACTCAGCATGTCAGCTTTTT

Human GAL-1 hairpin sequences:
#1:CCGGCAACCTGTGCCTGCACTTCAACTCGAGTTGAAGTGCAGGCACAGGTTGTTTTTTG
#2:CCGGGTGTTGCAGAGGTGTGCATCACTCGAGTGATGCACACCTCTGCAACACTTTTTTG
#3:CCGGACGGTGACTTCAAGATCAAATCTCGAGATTTGATCTTGAAGTCACCGTTTTTTTG

ST6Gal1 hairpin sequences:
#1:CCGGCGAGAGATTGATAATCATGATCTCGAGATCATGATTATCAATCTCTCGTTTTT
#2:CCGGCCAGACTACAACTTCTTCGAACTCGAGTTCGAAGAAGTTGTAGTCTGGTTTTT
#3:CCGGCGCTCCTCTTCGAGAAGAATACTCGAGTATTCTTCTCGAAGAGGAGCGTTTTT

GNE hairpin sequences:
#1:CCGGGTTTCTGCGAAGGCAAATTTGCTCGAGCAAATTTGCCTTCGCAGAAACTTTTTG
#2:CCGGGCCAAGAACAAAGACTATATGCTCGAGCATATAGTCTTTGTTCTTGGCTTTTTG
#3:CCGGGATGCCCTGATCTCGTTTAACCTCGAGGTTAAACGAGATCAGGGCATCTTTTTG

PCR Primers:

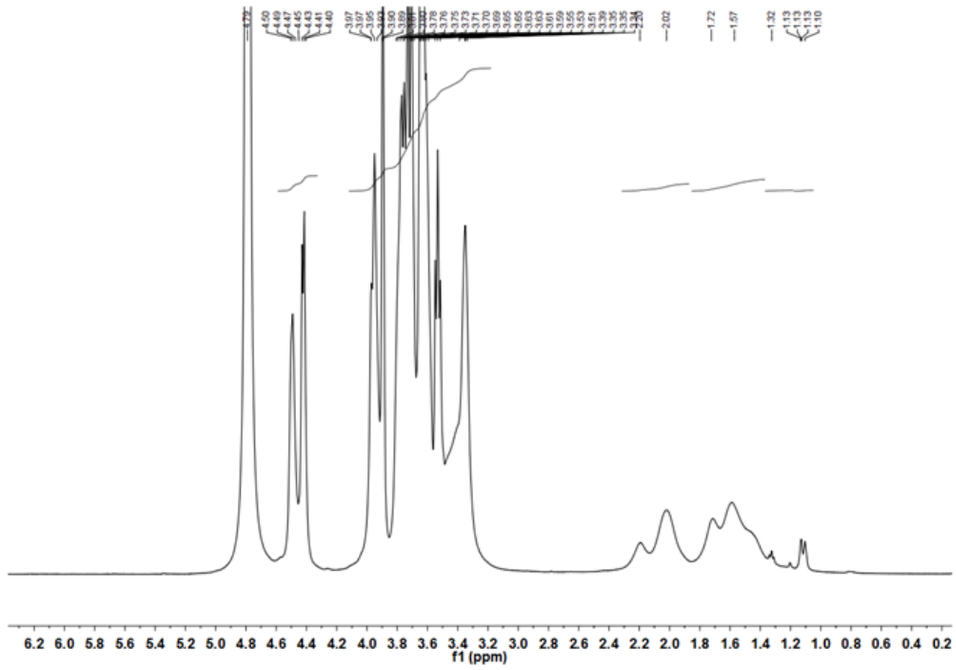
Mapk1:
Forward: TACGGCATGGTTTGCTCTGC
Reverse: GACAGTAGGTCTGGTGCTCA

Mapk3:
Forward: GTACGGCATGGTCAGCTCAG
Reverse: ATCTGGATCTCCCTCAGCGT

βactin:
Forward: CACACCCGCCACCAGTTC
Reverse: CGATGGAGGGGAATACAGCC

Figure S13

^{1}H NMR for **lactosyl GP**:



^{1}H NMR for **cellobiosyl GP**: