Transition of mesenchymal and epithelial cancer cells depends on α1-4 galactosyltransferase-mediated glycosphingolipids

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Supplementary information

SUPPLEMENTARY MATERIAL AND METHODS

Reagents, antibodies and enzymes: Antibodies for detection of cell surface-associated GSLs were applied as described previously (1). Additional antibodies were applied; primary antibodies E-Cadherin (#3195), Tubulin (#2148), H3K4m3 (#9727), and Slug (#9585), secondary antibodies HRP-anti-rabbit IgG (#7074) and HRP-anti-mouse IgG (#7076) all obtained from Cell Signaling Technology (BioConcept Ltd, Allschwil, Switzerland). Vimentin (#MS-129-P1) antibody was purchased from Thermo Fisher Scientific (Life technologies, Reinach, Switzerland). Anti-human CD44-PE (#130-098-108) and CD24-PE-Cy7 (#561646) antibodies were obtained from MACS Miltenyi Biotec (Bergisch Gladbach, Germany) and BD Pharmingen (Allschwil, Switzerland), respectively.

Endoglycoceramidase II (EGCase II, recombinant clone derived from *Rhodococcus* sp and expressed in *Escherichia coli*) was purchased from Sigma-Aldrich (MO, USA). Glycosphingolipid standards from Matreya LLC (Neutral Glycosphingolipid Qualmix 1505, globotriosylceramide (CTH), Monosialoganglioside Mixture 1508, Gangliotetraosylceramide 1064) were obtained from Adelab Scientific (SA, Australia). Immobilin-P Polyvinylidene Difluoride (PVDF, 0.2 μm) was obtained from Millipore (MA, USA). Microtiter plates (Corning 96-well clear flat bottom, COR3364) were purchased from Invitro (Vic, Australia). Cation exchange resin beads (AG50W-X8) was obtained from BioRad (Hercules, CA, USA) and PerfectPure C18 Zip Tips were obtained from Eppendorf (Hamburg, Germany). Potassium hydroxide and sodium borohydride were obtained from Sigma-Aldrich (MO, USA). Other reagents and solvents such as methanol, ethanol, chloroform and acetonitrile were of HPLC or LC/MS grade.

Single-guided RNA design and vector construction: Single guided RNAs (sgRNA) targeting exon 3 of *A4GALT* were designed using the web tool of the Zhang laboratory (http://crispr.mit.edu) (2). sgRNA1 and sgRNA2 (Supplementary Table S3, Figure 2) with scores of 91 and 85, respectively, were selected for gene editing of the entire open reading frame (ORF) of *A4GALT*. Intended oligo pairs encoding 20nt targeted sequences (Supplementary table S3) with overhangs (both 5' and 3') from *BsbI* restriction site were

ordered, annealed and finally cloned into either pSpCas9(BB)-2A-GFP (addgene, #PX458) or pSpCas9(BB)-2A-puro (addgene, #PX459) *via BsbI* restriction site using T4-DNA ligase (Promega, Dübendorf, Switzerland). Constructs were transformed into DH5alpha *E. coli* strains and sequenced for confirmation of the sgRNA inserted into PX458 by Sanger DNA sequencing using Primer human U6.

Transfection and single cell sorting: IGROV1, BG1 and SKOV3 cell lines were grown in 6-well plate ($3x10^5$ - $5x10^5$ cells/well) for 24 h and transiently transfected using Viafect® transfection reagent (Promega) with 2.5 µg of sgRNA containing either PX458 (Addgene # 48138) or PX459 (Addgene # 62988) donor plasmid to generate homozygous *ΔA4GALT*. Cells were washed with PBS, harvested using cell dissociation buffer (non-enzymatic, Sigma-Aldrich, Buchs, Switzerland) and finally re-suspended in RPMI containing 10% FCS 72 h after transfection. Following single cell sorting was performed on a BD FACS Aria Cell Sorter (BD Bioscience) sorting for single $DAPI^-$ and GFP^+ cells into 96-well flat-bottom plates with pre-warmed RPMI containing 10% FCS. Plates were incubated for up to 3 weeks following transfer to 48-well plates and genomic DNA isolated for genotyping PCR to characterize single cell clones.

Genotyping PCR: Selected clones were characterized to identify homozygous knockout by using three independent PCR primer pairs (Supplementary Table S3). PCR was performed using 2.5x GoTaq Green Master Mix (Promega), 300nM primer, 30ng genomic DNA (gDNA). PCR conditions were 95°C for 2 min, then 32 cycles of 94°C for 15 sec, 58.9°C for 15 sec, 72°C for 30-45 sec (depending on the amplicon length), finished with 1 cycle at 72°C for 5 min. Amplicons were visualized on a 1.5 % agarose gel.

DNA sequencing: PCR products corresponding to genomic modifications were purified and cloned into the pGEM®-T Easy Vector System (Promega) according to the manufacturer's protocol and sequenced using T7-F (Supplementary Table S3) primer by Sanger DNA sequencing service from Source Bioscience Life Sciences (Berlin, Germany). Samples were shipped at a concentration of 100 ng/µl with specific primer (if required) of 3.2 pg/µl.

Analysis of glycans released from GSLs using PGC LC-ESI-MS/MS: The extraction of GSLs from cell lines was carried out with some modifications based on our previously described method (3). Briefly, cells (1 x 10^7) were harvested, washed thrice in 10 ml of phosphate buffered saline and pelleted through centrifugation at 1800 g for 20 min. A total of 5 ml of chloroform: methanol (2:1) was added to the cell pellet and incubated overnight in a 4^0C incubator shaker. The supernatant was collected after centrifugation at 1800xg for 20 min and the pellet was re-extracted again as described above. The combined supernatants were evaporated to dryness under vacuum and the dried glycosphingolipid mixture was re-dissolved in 50 µl of chloroform: methanol (2:1).

Subsequently, GSLs were spotted onto a polyvinylidene diflouride (PVDF) membrane to immobilize the GSLs. PVDF spots were cut and placed in a chloroform-compatible 96-well microplate, to which 50 µl of re-dissolved glycosphingolipid mixtures from cell line samples were spotted. 10 µl of neutral and acidic glycosphingolipid standards were also on individual PVDF spots and the 96-well microplate was air-dried at room temperature to ensure proper binding of the GSLs. To release the glycans from membrane-bound glycosphingolipids, 2 µl (4mU) of Endoglycoceramidase II in 50 µl of 0.05 M sodium acetate buffer, pH 5.0 was added to each sample well and incubated for 16 h at 37^{o}C. Approximately 50 µl of released glycans was recovered from the individual sample wells and transferred to Eppendorf tubes containing 1ml of chloroform: methanol: water (8:4:3). The sample well was then washed with 50 µl of water and residual glycans were added to the Eppendorf tube. The upper methanol: water layer containing the released oligosaccharides (~400 µl) was dried and kept for subsequent analysis.

The released glycans were reduced to alditols with 20 µl of 200 mM sodium borohydride in 50 mM potassium hydroxide at 50^{o}C for 2 h. The reaction mixture was quenched using 2 µl of 100% glacial acetic acid. The glycan alditols (~20 µl) were applied to individually prepared cation exchange columns which consist of 45 µl of cation exchange resin beads (AG50W-X8) deposited onto reversed phase µC18 ZipTips (Perfect Pure, Millipore) and placed in microfuge tubes. The columns were conditioned by a series of pre-washing steps

with a) 50 µl of 1 M HCl, b) 50 µl of methanol and c) 50 µl of water as previously described (4). Glycan alditols were eluted with Milli Q water (50 µl, twice) and dried. Subsequent drying steps with methanol (100 µl, thrice) were performed to remove residual borate. Purified glycan alditols were re-suspended in 15 µl of water prior to MS analysis.

The reduced GSL glycans were analyzed by a capillary LC (Dionex) connected to a linear ion-trap mass spectrometer (LTQ Velos Pro, Thermo Scientific). 3 µl of the sample was injected onto a Hypercarb porous graphitized carbon (PGC) capillary column (5 µm Hypercarb KAPPA, 180 µm x 100 mm, Thermo Hypersil, Runcorn, UK). The separation of glycans was carried out over a linear HPLC gradient of 0-70 % (v/v) acetonitrile /10 mM ammonium bicarbonate for 45 mins followed by a 10 min wash-step using 90 % (v/v) acetonitrile /10 mM ammonium bicarbonate at a flow rate of 4 µl/min. The MS spectra were obtained within the mass range of m/z 400 - m/z 1500. The temperature of the transfer capillary was maintained at 325 ^{o}C and the capillary voltage was set at 3.2 kV. Neutral and acidic glycans were detected in the negative ion mode as $[M-H]^{1-}$ and $[M-2H]^{2-}$ ions and their signal intensities and fragmentation were analyzed using the Thermo Xcalibur Qualitative Analysis (Versions 2.2) software.

***A4GALT* rescue, mutagenesis and cloning of E-cadherin constructs:** To rescue the A4GALT in *ΔA4GALT* cells, we utilized IGROV1 genomic DNA as template and designed primer to amplify the *A4GALT* open reading frame. The PCR was performed using 2 U *Pfu* DNA polymerase (Promega), 1 x *Pfu* DNA polymerase buffer, 300nM forward and reverse primer, 30 ng genomic DNA (gDNA), 200 µM dNTPs and nuclease free water under following conditions: 95°C for 2 min followed by 32 cycles of 94°C for 15 sec, 58°C for 15 sec, 72°C for 3min, and finished with 1 cycle at 72°C for 5 min. Amplicons were visualized on 1 % agarose gel and purified by Wizard SV gel and PCR Clean-Up System (Promega) and finally cloned into plasmid pcDNA3.1 (Addgene #V790-20) which was used as mutagenesis template. After DNA Sanger sequencing the mutagenesis PCR was performed as previously described (5). The PCR amplifications were carried out with Expand^{Tm} High Fidelity PCR system (Roche, Switzerland) in a 50 µl reaction volume, which was carried out with 100 ng

pcDNA3.1*A4GALT* open reading frame as template, 1 µM primer pair, 200 µM dNTPs and 2 U of DNA polymerase. The PCR was performed under following conditions: 94°C for 5 min; 16 cycles of 94°C for 1 min, 52°C for 1 min and 72°C for 16 min; followed by 72°C for 1 h. The Amplicons were then evaluated by agarose gel electrophoresis and purified by Wizard SV gel and PCR Clean-Up System (Promega) and further treated with restriction enzyme *DpnI* (NEB). An aliquot of 1 µl PCR product was transformed into DH5α and plated on LB plate containing 100µg/ml ampicillin. Positive mutants were selected by *BsmI* restriction digestion. The desired *A4GALT* open reading frame was then introduced into pUltra (Addgene #24129, (6)) and pCW57.1 (Addgene #41393) *via NheI* and *XhoI*/*SalI* cloning procedure for further lentiviral transduction. In parallel, full length E-cadherin (addgene #45769) and IL2R/E-cadherin cytotail (addgene #45773) were amplified *via* Quick Fusion Cloning Kit (bimake.com, Munich, Germany) for cloning into desired lentiviral vector. All primers are listed in Supplementary Table S3. For constitutive and bicistronic expression of E-cadherin C-terminal tagged with EGFP (addgene #28009) and A4GALT DXD/DXA, we removed EGFP from pUltra (addgene #24129) by *AgeI* and *XbaI* following infusion cloning as described above using primer InFusion_E-cadGFP_F and InFusion_E-cadGFP_R. All plasmids were partly sequenced for verification by Sanger DNA sequencing (Microsynth).

Lentivirus production and transduction: HEK293T cells were cultured as described above. One day prior to transfection, cells were seeded at ~40% confluence in a T75cm^2 flask. Cells were transfected when they reached 70–80% confluence. For each flask, 4 µg of plasmid pUltra (Addgene #24129, (6)) and pCW57.1 (Addgene #41393) encoding the genes of interest (A4GALT (DXD), A4GALT (DXA)), full length E-cadherin, or IL2/E-cadherin cytotail) and 2 µg of pMD2.G (Addgene #12259) and 2 µg of pCMVR8.74 (Addgene #22036) were transfected using 24 µl of jetPEI reagent and 1 ml of 150 mM NaCl solution (Polyplus-transfection, Chemie Brunschwig AG, Basel, Switzerland). Media was changed 24 h after transfection. Virus supernatant was collected 48 h later and filtered with a 0.45 µm polyvinylidene fluoride filter (Millipore), and stored at -80°C. IGROV1 *ΔA4GALT* cells were

transduced with pUltra and pCW57.1 lentiviral supernatant in 4 ml of media supplemented with 8 µg/ml polybrene (Sigma) in T25cm^2 flask and selected after 3 passages by GFP enrichment and 5 µg/ml puromycin treatment.

MTT assay: To identify the proliferation rate cells were seeded at a density of 20'000 cells/ well in 96-well plates and incubated for 24 h to 120 h. At each time point MTT dye (Sigma-Aldrich) was added at a final concentration of 500 µg/mL and incubated for 3 h. After removal of supernatant 200 µl of DMSO was added to dissolve the crystals. The optical density (OD, absorbance at 540 nm) was measured with a Synergy H1 Hybrid Reader (Biotek, Basel, Switzerland). The measurement was performed in quadruplets and experiments were repeated thrice.

Colony formation assay: Cells were harvested and adjusted in cell number for seeding of 500 cells per well of a 12-well plate. The standard culture medium was replaced every 3 days. On the 10th day after seeding, colonies were counted only if they contained more than 50 cells. Finally, the cells were stained using crystal violet and images were taken.

Soft agar assay: Cells were grown in medium containing 1 % base agar and 0.3 % top agar. Additional culture media were overlaid every 3-4 days. After 10-14 days of culture, colonies were enumerated and pictures were taken. The experiments were independently repeated at least three times.

Aggregation or anoikis assay: To evaluate anchorage-independent growth, cells were grown in ultra-low attachment T25cm^2 flasks. Cells were than harvested at day 4, 7 and 10, washed with PBS and stained with propidium iodide (PI) following the manufactures instructions. The percentage of PI positive cells was analyzed by flow cytometry using BD Accuri^{TM} C6.

Cell motility (migration and invasion): Directed cell motility assay was performed as described previously (7). In brief, sub-confluent cells were grown in serum-free media for 24 h and 7.5 x 10^5 cells were seeded into the upper chamber of each insert following incubation at 37°C for 18 h allowing cells to migrate to the chemo-attractant (medium containing 10 % FBS). After incubation, media in the interior part of the insert was removed and the insert was immersed in 0.2% crystal violet/ 10% ethanol for 20 min. The insert was intensively washed

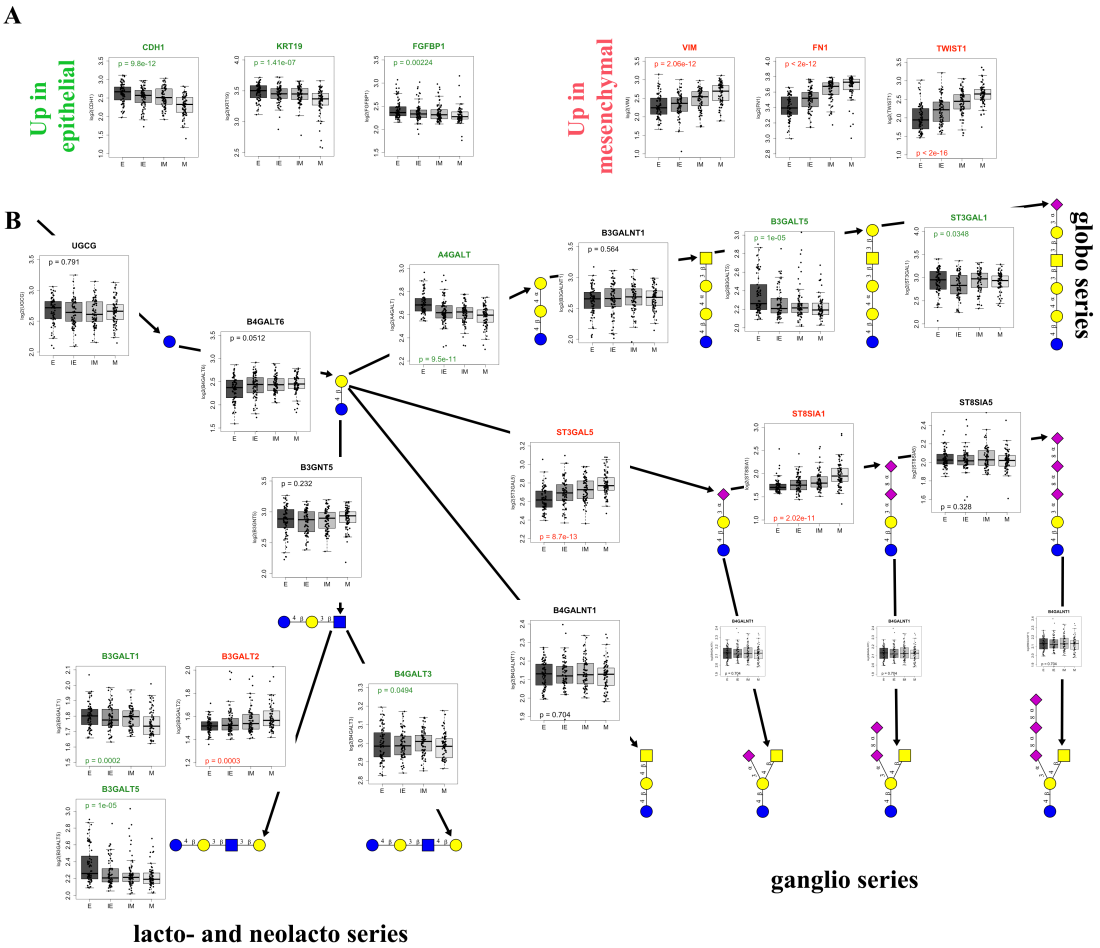
and non-migrated cell in the interior of the insert were removed using a cotton-tip swab. Five random areas of the inserts were photographed for migrated cells, and cell counts were performed.

In regards to invasion assay, trans-well inserts were covered with a final volume of 100 µl Matrigel (diluted with media, 1:80) for 1 h at room temperature to settle down and polymerized. The plate was then placed in the incubator at 37°C for overnight. Cells were then processed according to the previously described cell migration protocol mentioned above and after incubation at 37°C for 24 h, media was removed and the insert was immersed in crystal violet for 20 min and followed the same protocol as described before.

SUPPLEMENTARY FIGURES

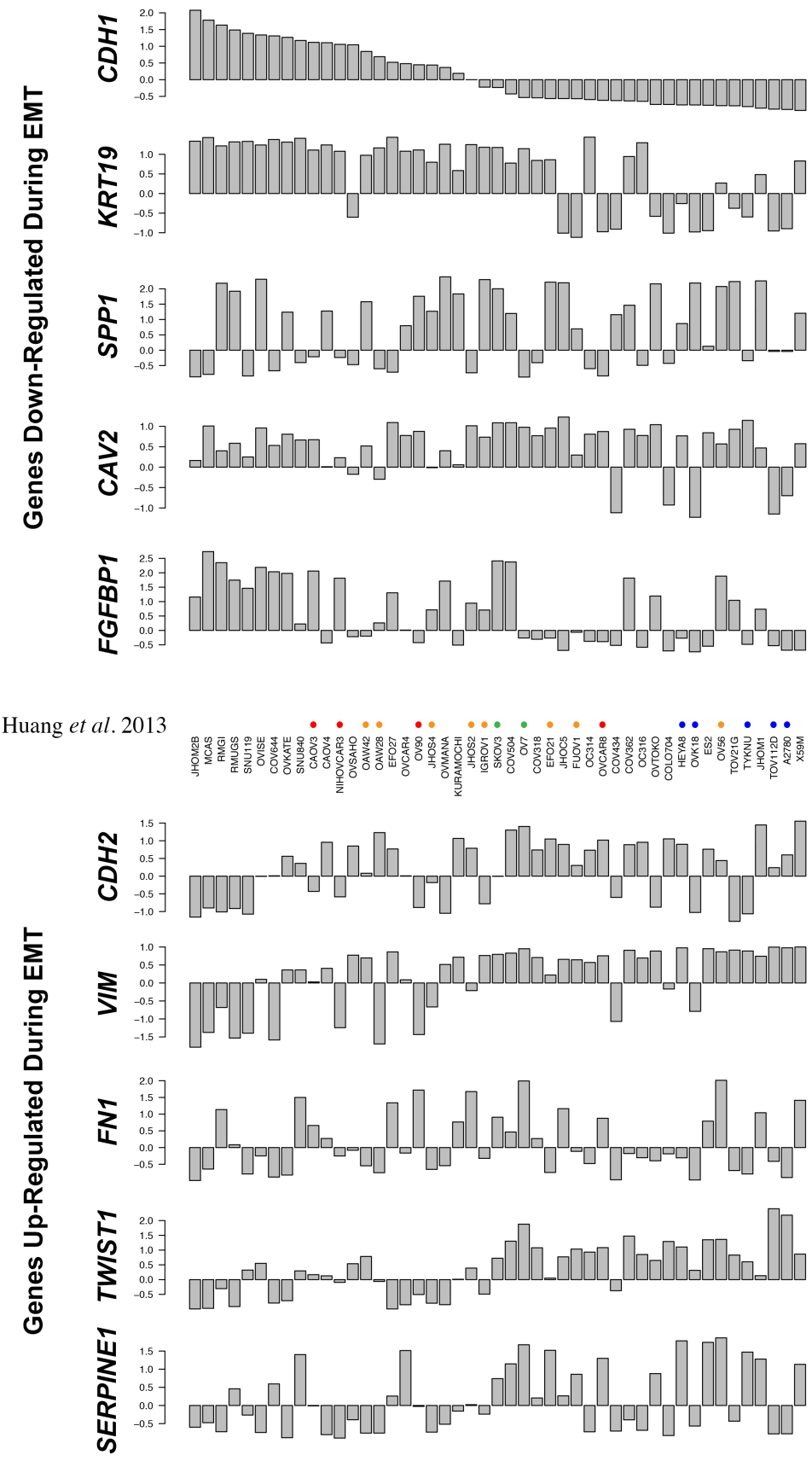
Supplementary Figure S1

Globoside GSL synthesizing glycosyltransferases show elevated gene expression in epithelial tissue samples. Tothill transcriptomic data set was divided into four EMT states: epithelial (E), intermediated epithelial (IE), intermediate mesenchymal (IM), and mesenchymal based on previously developed EMT score (8). **A)** Upregulated EMT markers in epithelial (green) and mesenchymal (red) cells among four EMT states, respectively. **B)** KEGG annotated glycosyltransferases involved in glucosylceramide-related glycosphingolipids divided into three major series- globo, (neo-) lacto, and ganglio series glycosphingolipids. Boxplot showing expression of glycosyltransferase-encoding genes significantly up-regulated in epithelial (green), in mesenchymal (red), or without significant changes (black). Black solid arrows indicate elongation of glycan structures.



Supplementary Figure S2

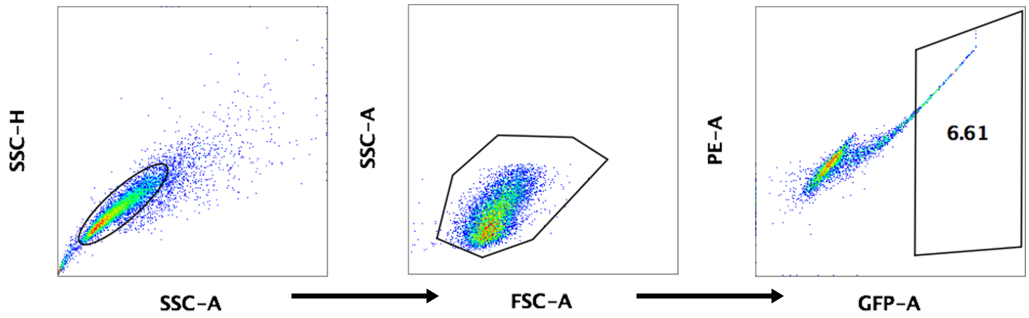
EMT marker gene expression in 47 ovarian cancer cell lines. Normalized transcriptomic data were accessed *via* the http://www.cbioportal.org. The cell line CCLE Broad Cancer cell line encyclopedia (Novartis/ Broad, (9)) containing mRNA expression Z-scores (microarray) were sorted in descending order for *CDH1* expression in all available ovarian cancer cell lines (n=47) including IGROV1. Five corresponding epithelial markers (*KRT19*, *SPP1*, *CAV2*, and *FGFBP1*) and mesenchymal markers (*CDH2*, *VIM*, *FN1*, *TWIST1*, and *SERPINE1*) were plotted in bar charts accordingly. In addition, findings on EMT states in ovarian cancer cells published in Huang *et al.* 2013 (10) were highlighted for cell lines matching in both data sets (color code and legend below the figure).



Huang *et al*. 2013

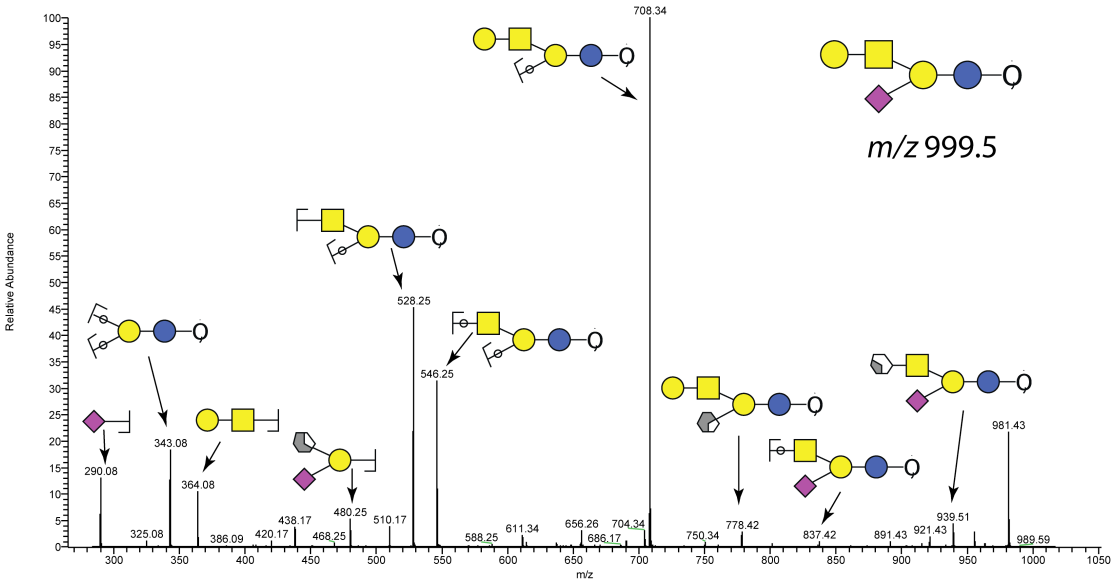
Supplementary Figure S3

Single cell sorting strategy after transient transfection of sgRNA CRISPR-*Cas9* constructs. Cancer cell lines were transfected with equal amount of PX458 incorporating two different *A4GALT*-specifci sgRNA in addition to *Cas9* and GFP. Single cell sorting was performed as exemplified for IGROV1 ovarian cancer cells.



Supplementary Figure S4

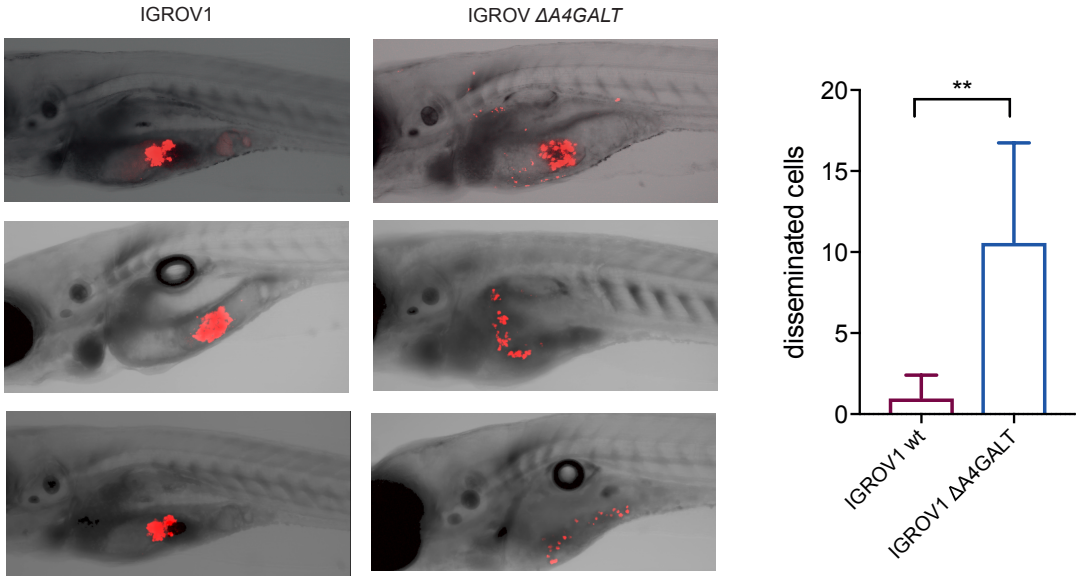
Quantification of glycans released from glycosphingolipids in wildtype and *ΔA4GALT* ovarian cancer cells using LC-ESI-MS/MS. Proposed GSL-glycan structures and their relative abundance as detected on the glycolipid membranes of ovarian epithelial cancer cells IGROV1 wild type and the knockout. GSL-glycan structures were enzymatically released from glycolipids and separated by PGC-LC-ESI MS/MS analysis. Structures were assigned based on MS/MS fragmentation (where possible) and biological GSL pathway constraints. Structures are depicted according to the CFG (Consortium of Functional Glycomics).



Supplementary Figure S5

Dissemination of *ΔA4GALT* compared to IGROV1 wildtype cells.

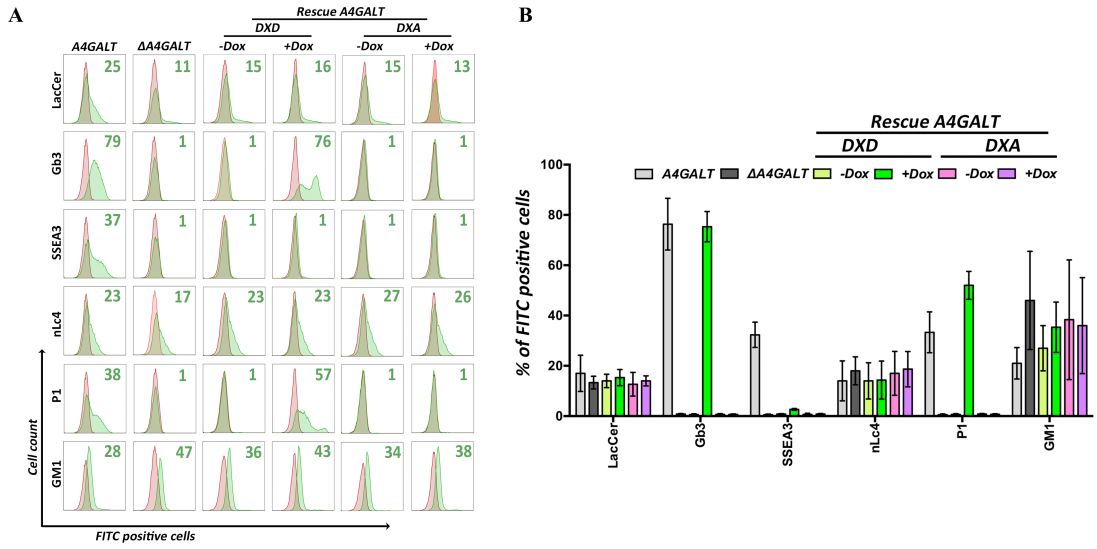
Cells were transplanted in the yolk of 48 hpf zebrafish embryos and followed for 2-3 days. Note that *ΔA4GALT* cells generally show a more dispersed phenotype, while control cells "cluster" at the injection area. Three representative zebrafish embryos are shown for each group (of a total of n=10 embryos analysed in n=2 independent biological experiments). The graph on the right summarizes the number of cells that disseminate from the injection area in each condition. A Mann-Whitney U test was used to test for statistical significance.



Supplementary Figure S6

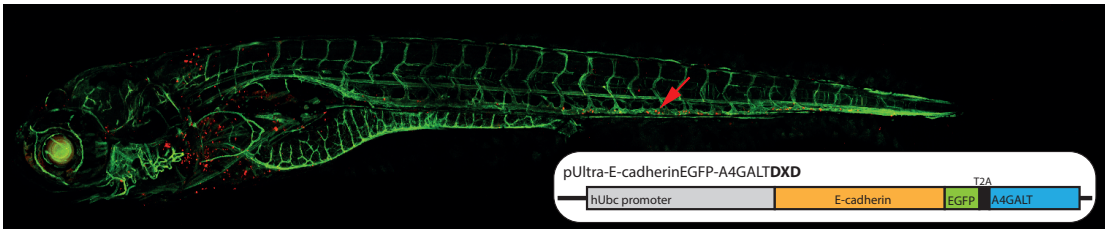
The aspartate-any residue-aspartate motif is required for enzymatic activity of A4GALT.

A) Representative histogram of flow cytometry data on IGROV1 and sub-clones stained for lactosylceramide (LacCer), globosides (Gb3, SSEA), neo-lacto series GSLs (paragloboside nLc4 and P_1), and ganglioside GM1. Red histogram depicts the negative control. Values within each plot show the percentage of $FITC^+$ cells in this experiment. **B)** Bar-chart summarizes the level of GSLs (% of $FITC^+$ positive cells) in different ovarian cancer cell lines IGROV1 (*A4GALT, ΔA4GALT*, Rescue (DXD) and Rescue mutant (DXD)) cell lines. Mean and standard deviation are based on three independent experiments.

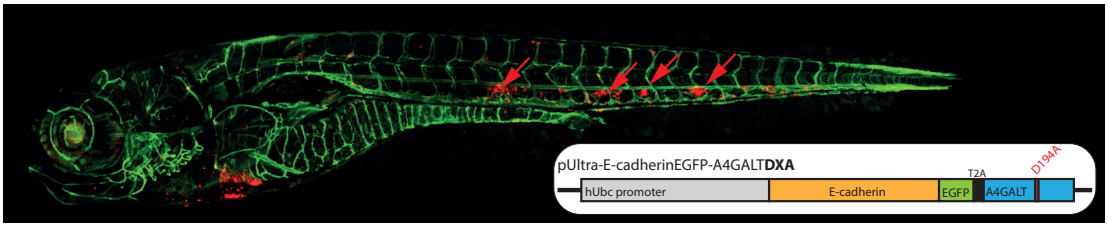


Supplementary Figure S7

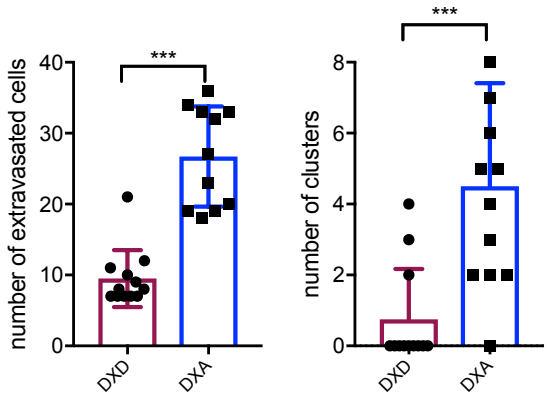
Bicistronic expression of E-cadherinEGFP and A4GALT DXA shows enhanced extravasation *in vivo*. IGROV1 *ΔA4GALT* cells re-expressing either wildtype A4GALT (DXD) or mutant (DXA) together with E-cadherin C-terminal tagged with EGFP were implanted into the Duct of Cuvier of *Tg(kdrl-eGFP)*^{la116} zebrafish embryos and quantified for numbers of extravasated cells and cluster formation (red arrows). Corresponding quantitations are shown next to representative fluorescence images. Shown are representative pictures from each group and quantitated results from a total number of n=12 embryos analysed in n=2 independent biological experiments.



Tg(kdrl-eGFP)^{la116} IGROV1 *ΔA4GALT*



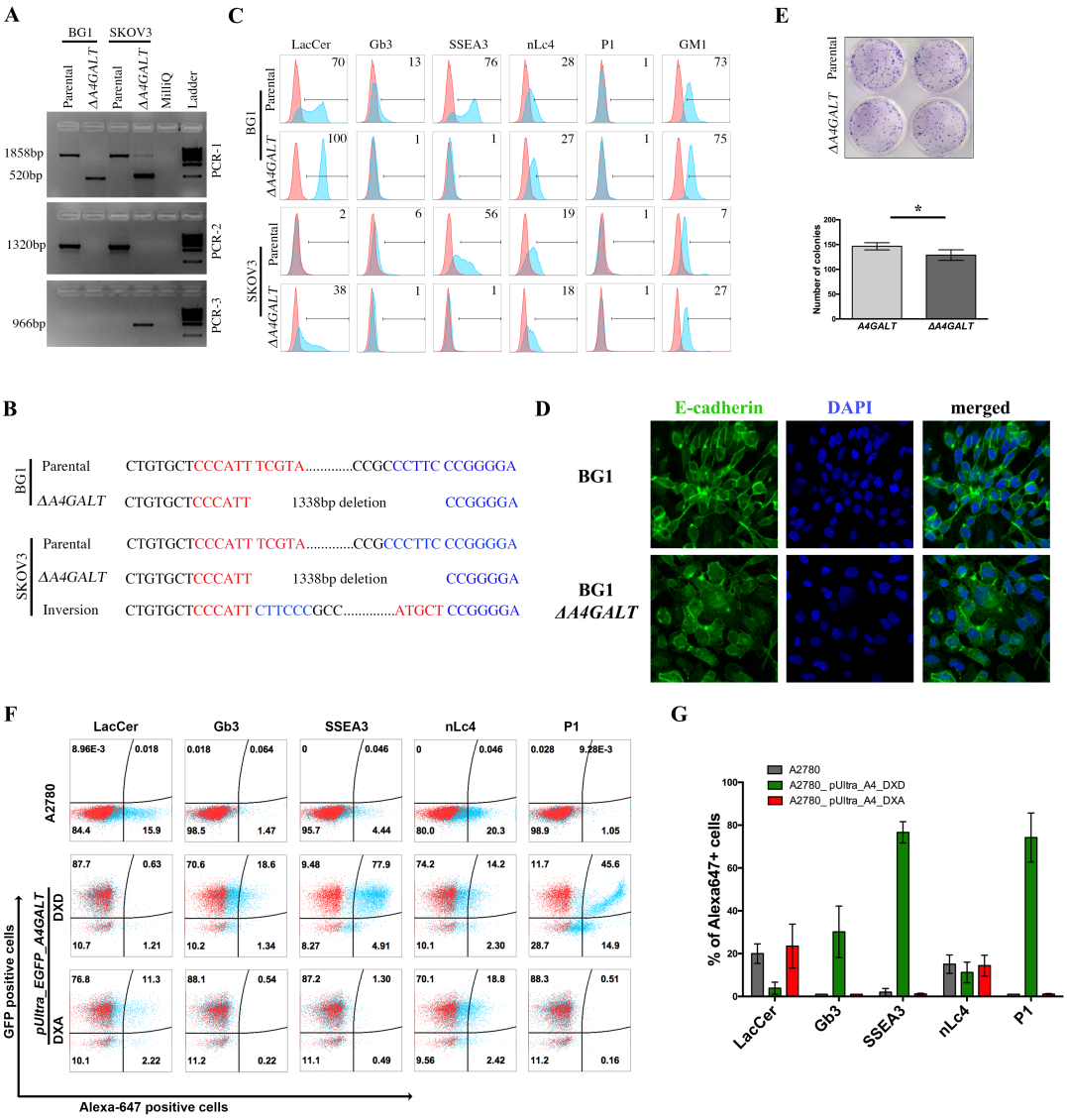
Tg(kdrl-eGFP)^{la116} IGROV1 *ΔA4GALT*



Supplementary Figure S8

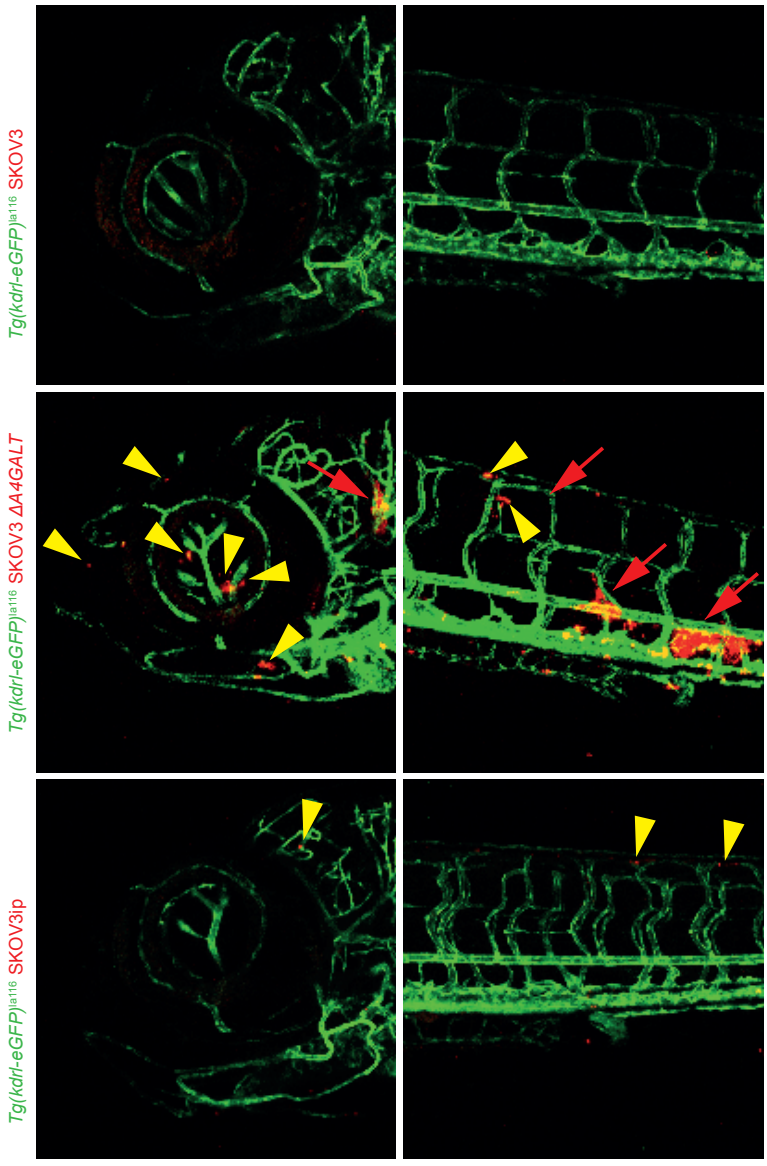
Genetic depletion of *A4GALT* correlates with the absence of globosides in ovarian cancer cells representing the four different states of EMT.

A) PCR for initial verification of selected *ΔA4GALT* clones obtained from BG1 and SKOV3. **B)** Sanger DNA sequencing results for selected clones at the *Cas9*-targeted region showing deletion and inversion in two additional genome edited cell lines. **C)** Corresponding flow cytometry results shown as representative counter plots for validation of GSL expression in selected *ΔA4GALT*, mock (red), stained (blue). **D)** Corresponding fluorescence images showing E-cadherin expression in BG1 wildtype and *ΔA4GALT* cells. **E)** Anchorage-dependent growth investigated by colony formation assay in SKOV3 and BG1 cells and their corresponding *ΔA4GALT* cell clones. **F)** Overexpression of A4GALT in A2780 cells negative for GSL expression. Counter plots display expression of GSL expression in A2780 cells stably expressing A4GALT cloned into pUltra (co-expression with EGFP), mock (red), stained (blue). **G)** Barchart summarizing three independent experiments for overexpression of A4GALT in A28780 cells.


A
BG1
SKOV3
Parental
ΔA4GALT
Parental
ΔA4GALT
MilliQ
Ladder
1858bp
520bp
PCR-1
1320bp
PCR-2
966bp
PCR-3
C
LacCer
Gb3
SSEA3
nLc4
P1
GM1
BG1
Parental
ΔA4GALT
SKOV3
Parental
ΔA4GALT
70
13
76
28
1
73
100
1
1
27
1
75
2
6
56
19
1
7
38
1
1
18
1
27
E
Parental
ΔA4GALT
*
Number of colonies
200
150
100
50
0
A4GALT
ΔA4GALT
B
BG1
Parental
CTGTGCTCCCATT TCGTA.....CCGCCCTTC CCGGGGA
ΔA4GALT
CTGTGCTCCCATT
1338bp deletion
CCGGGGA
SKOV3
Parental
CTGTGCTCCCATT TCGTA.....CCGCCCTTC CCGGGGA
ΔA4GALT
CTGTGCTCCCATT
1338bp deletion
CCGGGGA
Inversion
CTGTGCTCCCATT CTTCCCGCC.....ATGCT CCGGGGA
D
E-cadherin
DAPI
merged
BG1
BG1
ΔA4GALT
F
LacCer
Gb3
SSEA3
nLc4
P1
A2780
8.96E-3
0.018
84.4
15.9
0.018
0.064
98.5
1.47
0
0.046
95.7
4.44
0
0.046
80.0
20.3
0.028
9.28E-3
98.9
1.05
pUltra_EGFP_A4GALT
DXD
87.7
0.63
10.7
1.21
70.6
18.6
10.2
1.34
9.48
77.9
8.27
4.91
74.2
14.2
10.1
2.30
11.7
45.6
28.7
14.9
DXA
76.8
11.3
10.1
2.22
88.1
0.54
11.2
0.22
87.2
1.30
11.1
0.49
70.1
18.8
9.56
2.42
88.3
0.51
11.2
0.16
GFP positive cells
Alexa-647 positive cells
G
A2780
A2780_ pUltra_A4_DXD
A2780_ pUltra_A4_DXA
% of Alexa647+ cells
100
80
60
40
20
0
LacCer
Gb3
SSEA3
nLc4
P1

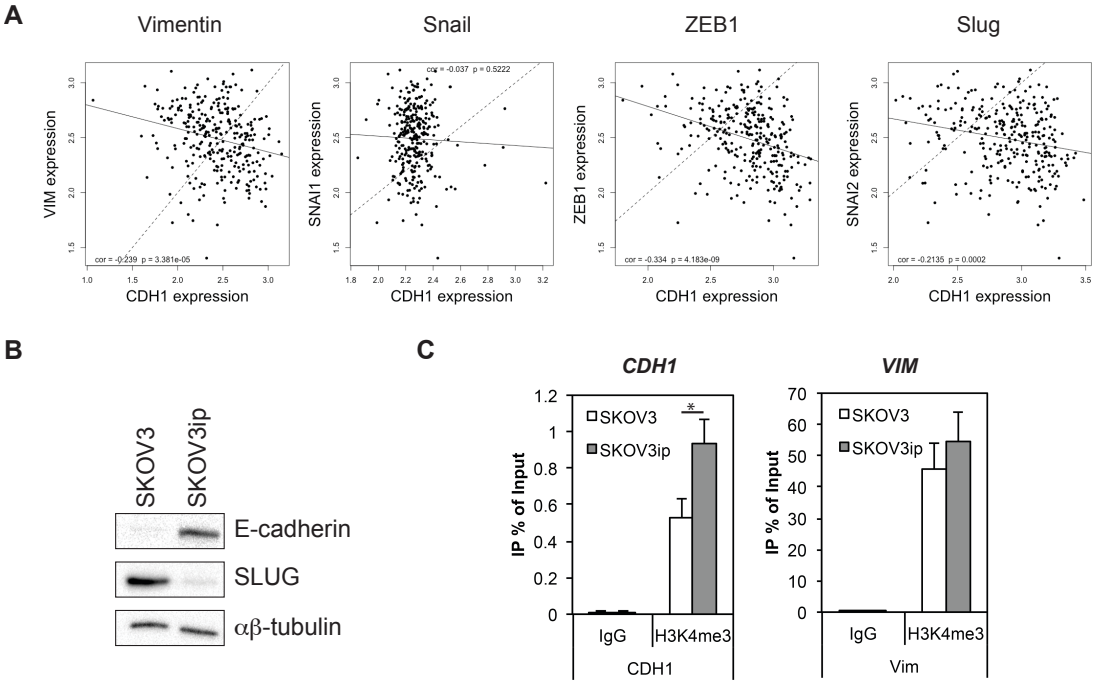
Supplementary Figure S9

Fluorescently labelled cells were transplanted into the Duct of Cuvier of 48 hpf zebrafish embryos and cells followed for 2-3 days. Representative close-ups for each condition showing extravasated cells (yellow arrowheads) and clusters of cancer cells (red arrows) outside the zebrafish vasculature. Images of corresponding whole embryos and quantifications are shown in Figure 5F, n =12-16 embryos were analysed in n=3 independent biological experiments.



Supplementary Figure S10

Increased E-cadherin in SKOV3ip coincides with SLUG expression and histone modifications. A) Correlation of *CDH1* expression with classical EMT markers in Tothill transcriptomic data set. **B)** Representative Western blot for differential expression of E-cadherin and SLUG in both cell lines. **C)** ChIP-qPCR analysis of H3K4me3 status at the promoter of *CDH1* and *Vimentin* in SKOV3 and SKOV3ip ovarian cancer cells. Results are represented as a percentage of immunoprecipitated DNA (IP) compared to input. *Vimentin* promoter was a positive control of H3K4me3 ChIP in both of cell lines. Error bars indicate standard deviation of 2 biological and 2 technical replicates. Bar chart with mean ± s.d., **p*-value < 0.05.



SUPPLEMENTARY TABLES

Supplementary Table S1

Cell lines used in this study. Table provides details of all cell lines including type of cells, name of the cell lines, culture media conditions, and supplements.

Cell types	Cell Lines	Culture media	Supplements
Normal	FT190	DMEM-Ham's F12 without HEPES Buffer, penicillin (100U/ml) and streptomycin (Sigma-Aldrich)	2% Ultroser™ (PALL, Life Science)
	FT133tag		
	FT237		
	FT240		
	HOSE6.3	RPMI-1640 supplemented with 10% FBS, penicillin (100U/ml) and streptomycin (100µg/ml) (Sigma-Aldrich, Buchs, Switzerland)	
	HOSE17.1		
Ovarian Cancer	IGROV1		
	A2780		
	SKOV3		
	OVCAR3		
	OVCAR4		
	OVCAR5		
	OVCAR8		
	BG1		
	OAW42		
	CAOV3		
	TOV21G		
	OVSAHO		
	KURAMOCHI		
	Tyk-Nu		
	EFO27		1mM Sodium pyruvate (Sigma- Aldrich)
Breast Cancer	MCF7		
	T47D		
	MDA-MB-231		
Colon Cancer	HCT16		
	HCT116		
	HT29		
	LS174T		
Leukemia	THP-1		
	K562		
Cervical cancer	HeLa		
	ME-180		
Kidney	HEK293T		
Neuroblastoma	U521		

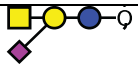
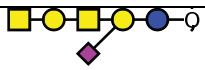
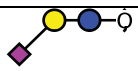
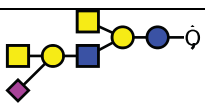
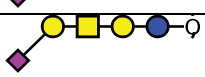
Supplementary Table S2

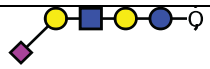
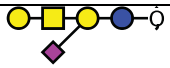
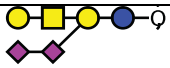
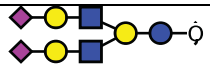
Table providing details of oligonucleotides used in this study. Name of oligonucleotides, DNA sequence, and method applying particular oligonucleotides are provided. RT-qPCR primer were established as recently described (11).

Oligonucoleotide name	Sequences (5′–3′)	Method
sgRNA1_Forward	caccgTGGCTGCTCACCTACGAAATGGG	CRISPR-*Cas9*
sgRNA1_Reverse	aaacATTTCGTAGGTGAGCAGCCAC	CRISPR-*Cas9*
sgRNA2_Forward	caccgCAATCTTGCCTCCCCGGGAA	CRISPR-*Cas9*
sgRNA2_Reverse	aaacTTCCCGGGGAGGCAAGATTGC	CRISPR-*Cas9*
Human U6_Forward	GAGGGCCTATTTCCCATGATTCC	Cloning
PCR_1,2_Forward	ACCAGGAAAGAGAGGTCTAATG	Genotyping *A4GALT*
PCR_1,3_Reverse	CTGTTGAGGAGCTGTGGGAG	Genotyping *A4GALT*
PCR_2,3_Reverse	CTCAACGGCGCGTTCCTGG	Genotyping *A4GALT*
T7_F	TAATACGACTCACTATAGGG	Cloning
Sequencing_A4_Forward	GGACCACTACAACGGCTGGAT	DNA Sequencing
Sequencing_A4_Reverse	ACGAAGTCCCGCATGCACA	DNA Sequencing
A4_qPCR_Forward	GTCTGCACCCTGTTCATCA	RT-qPCR
A4_qPCR_Reverse	AGAGCTGCCCTTTCTCCTTG	RT-qPCR
CDH1_Forward	GCCTCCTGAAAAGAGAGTGGAAG	RT-qPCR
CDH1_Reverse	TGGCAGTGTCTCTCCAAATCCG	RT-qPCR
Vimentin_Forward	TGCAGGAGGAGATGCTTCAGAG	RT-qPCR
Vimentin_Reverse	CAGAGACGCATTGTCAACATCCTG	RT-qPCR
HSPCB_Forward	TCTGGGTATCGGAAAGCAAGCC	RT-qPCR
HSPCB_Reverse	GTGCACTTCCTCAGGCATCTTG	RT-qPCR
YWHAZ Forward	ACTTTTGGTACATTGTGGCTTCAA	RT-qPCR
YWHAZ Reverse	CCGCCAGGACAAACCAGTAT	RT-qPCR
SDHA_Forward	TGGGAACAAGAGGGCATCTG	RT-qPCR
SDHA_Reverse	CCACCACTGCATCAAATTCATG	RT-qPCR
A4GALT_Exon3_NheI_F	CATGCTAGCGGATACCATGTCCAAGCC	Cloning
A4GALT_Exon3_XhoI_R1	CATCTCGAGTCACAAGTACATTTTCATGGC	Cloning
CMV_F	CAAATGGGCGGTAGGCGTG	Cloning
BGH_R	TAGAAGGCACAGTCGAGG	Cloning
A4GALT_DXA_forward	GTTCGGCGGCATCTAACTGGACACGGCATTCATTG	Mutagenesis PCR
A4GALT_DXA_reverse	CCGCAGGTTCTTGAGAACAATGAAGTCCGTGTCCAGGTAG	Mutagenesis PCR
InFusion_CDH1_F	CGCCTGGAGAATTGGATGGGCCCTTGGAGCCGC	Cloning
InFusion_CDH1_R	ATCCAGTCACTATGGCTAGTCGTCCTCGCCGCC	Cloning
InFusionIL2RCDH1_F	CGCCTGGAGAATTGGATGGATTCATACCTGCTGAT	Cloning
InFusionIL2RCDH1_R	ATCCAGTCACTATGGCTAGTGGTCCTCGCCGCC	Cloning
CDH1_Bis_1F	ATTTTAGTAATTTTAGGTTAGAGGG	Bisulfite Sequencing
CDH1_Bis_1R	TCCAAAAACCCATAACTAACC	Bisulfite Sequencing
CDH1_Bis_2F	AGTAATTTTAGGTTAGAGGGTT	Bisulfite Sequencing
CDH1_Bis_2R	CTAAAATCTAAACTAACTTC	Bisulfite Sequencing
CDH1_Chip_F	AGAGGAGGTTGAGGGCACTT	ChIP
CDH1_Chip_R	CCCACCAGGTTTTTGCAGTC	Chip
VIM_Chip_F	AAAGCGCAATTATGCCCTGC	Chip
VIM_Chip_R	AACTTGCTGGCTCCATTCCA	Chip
InFusion_E-cadGFP_F	TGCAGGTCCGATCCAATGGGCCCTTGGAGCCGC	Cloning
InFusion_E-cadGFP_R	CCGGAGCCGGATCCTCTTGATCAGCTCGTCCATG	Cloning

Supplementary Table S3

Quantification of glycans released from glycosphingolipids in wildtype and *ΔA4GALT* ovarian cancer cells using LC-ESI-MS/MS. Proposed GSL-glycan structures and their relative abundance as detected on the glycolipid membranes of ovarian epithelial cancer cells IGROV1 wild type and the knockout. GSL-glycan structures were enzymatically released from glycolipids and separated by PGC-LC-ESI MS/MS analysis. Structures were assigned based on MS/MS fragmentation (where possible) and biological GSL pathway constraints. Structures are depicted according to the CFG (Consortium of Functional Glycomics). Not detected (n.d.)

	$[M-H]^{1-}$ ($[M-2H]^{2-}$)	**Proposed structure**	**Wildtype Relative abundance % (SE)**	***ΔA4GALT* Relative abundance % (SE)**
1	505.3		3.8 (0.32)	n.d.
2a	708.3		3.3 (0.24)	2.6 (0.12)
2b	708.2		1.3 (0.08)	2.1 (0.03)
3	626.2		6.9 (0.37)	0.8 (0.03)
4	837.4		0.3 (0.01)	0.4 (0.02)
5	911.4		2.6 (0.43)	0.4 (0.03)
6a	1202.5 (600.8)		2.2 (0.33)	1.7 (0.09)
6b	1202.5 (600.8)		4.6 (0.17)	2.4 (0.21)
7	634.2		13.9 (1.32)	17.0 (1.45)
8	1405.6 (702.3)		3.6 (0.43)	1.6 (0.23)
9a	999.5		1.6 (0.16)	0.1 (0.01)

9b	999.5		0.0	0.6 (0.03)
9c	999.5		13.4 (1.05)	29.4 (2.65)
10a	1290.6 (644.8)		40.4 (1.89)	38.5 (1.65)
11	1655.8 (827.4)		2.3 (0.24)	2.4 (0.17)

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