**Twist1 induces distinct cell states depending on TGFBR1-activation**

**Supplemental Materials and Methods**

**A**

Sort 3 day analysis

CD44

CD24

CD24$^\text{high}$ CD44$^+$

**B**

Smad-dependent TGFβ signaling (HEK293T cells)

| Treatment | RLU |
|-----------|-----|
| Ctrl      | 0.5 |
| TGFβ      | 3.5 |
| TGFβ + A83| 0.2 |

**C**

Smad-dependent TGFβ signaling (HMLE-Twist1-ER cells)

| Treatment | RLU |
|-----------|-----|
| Ctrl      | 20  |
| TAM       | 80  |
| TAM + A83 | 3.5 |

**D**

DAPI E-cad Vim

**E**

TGFR1

Relative Gene Expression

| Treatment | 10^1 |
|-----------|------|
| sh-nt     | 1    |
| sh-1      | 10   |
| sh-2      | 10^2 |

**F**

Relative Gene Expression

| Gene | Ctrl | TAM | TAM + A83 |
|------|------|-----|-----------|
| FN   | $10^4$ | $10^3$ | $10^2$ |
| Wnt5a| $10^3$ | $10^2$ | $10^1$ |
| ZEB1 | $10^2$ | $10^1$ | $10^0$ |
| ZEB2 | $10^1$ | $10^0$ | $10^{-1}$ |

**G**

| Treatment | p-p65 | t-p65 | β-actin |
|-----------|-------|-------|---------|
| Ctrl      |       |       |         |
| TAM       |       |       |         |
| TAM + A83 |       |       |         |

**H**

Relative Gene Expression

| Gene | Ctrl | TAM | TAM + A83 |
|------|------|-----|-----------|
| E-cad| $10^4$ | $10^3$ | $10^2$ |
| Vim  | $10^3$ | $10^2$ | $10^1$ |
| FN   | $10^2$ | $10^1$ | $10^0$ |
| Wnt5a| $10^1$ | $10^0$ | $10^{-1}$ |
| ZEB1 | $10^0$ | $10^{-1}$ | $10^{-2}$ |
| ZEB2 | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ |

| Gene | Ctrl | TAM | TAM + Ax |
|------|------|-----|---------|
| Slug | $10^0$ | $10^{-1}$ | $10^{-2}$ |
Figure S1. Twist1 requires TGFBR1-activation for EMT-induction

(related to Figure 1)

(A) Fluorescent Activated Cell Sorting (FACS): CD44 and CD24 cell-surface staining and postsort analysis after 3 days in culture.

(B) Reporter assay: HEK293T cells were transfected with a reporter plasmid harboring smad binding elements (SBE) and a TGFβ1-expressing plasmid, and treated with TGFBR1-inhibitor A83-01 (A83). Reporter activity was measured after 24h. n=6.

(C) Reporter assay: HMLE-Twist1-ER cells treated with A83-01, 4-hydroxy Tamoxifen (TAM) and TAM+A83 for 16 days prior transfection with a reporter plasmid harboring smad binding elements (SBE). Recombinant TGFβ1 was added 24 hours after transfection and reporter activity was measured after another 24h. n=6.

(D) Immunofluorescence: E-cadherin (green), Vimentin (red), DAPI (blue). HMLE-Twist1-ER cells treated for 16 days with TAM, TAM+XAV939 (XAV, canonical Wnt signaling-inhibitor), TAM+SP600125 (SP, JNK-inhibitor). Scale bar: 200 µm.

(E) RT-PCR: TGFBR1. HMLE-Twist-ER cells HMEL-Twist1-ER cells transduced with non-targeting control (sh-nt) or sh-RNAs targeting TGFBR1 (sh-1 or sh-2). n=3.

(F) RT-PCR: Fibronectin (FN), Wnt5a, ZEB1, ZEB2, Slug. Cells as described in (E), 8 days post-induction with TAM. n=3

(G) Immunoblot: phosphorylated (p-) p65, total (t-) p65, β-actin. HMLE-Twist1-ER were treated for 16 days with TAM or TAM+A83-01 (A83).

(H) RT-PCR: E-cadherin, Vimentin, Fibronectin (FN), ZEB1, ZEB2 and Slug. HMLE-Twist1-ER cells treated with TAM or TAM+Axitinib (Ax) for 16 days. n=3.

Data are presented as mean ± SEM.
Figure S2. TGFBR1-activation directs Twist1-binding to a ZEB1-enhancer region (related to Fig. 2)

(A) RT-PCR: miR-141, miR-200a, miR-200b and miR-200c. HMLE-Twist1-ER treated with TGFBR1-inhibitor A83-01 (A83), recombinant TGFβ, 4-Hydroxy-Tamoxifen (TAM), TAM+A83 and TAM+TGFβ, at 6, 24, 48 and 72 hours post induction (dpi). Timepoint 0 was artificially set at value 1. Control (Ctrl) = untreated. miR expression was normalized to control cells for each timepoint. n=3.

(B) Chromatin Immunoprecipitation: Twist1-binding within the coding region of the ZEB1 gene (= Negative site, refer to Figure 2D) in HMLE-Twist1-ER cells treated analogous to (A) at 3dpi and 8dpi. IgG was used as antibody control. n=3.

(C) Chromatin Immunoprecipitation-sequencing, imported from data set GSM934616, visualized with IGB (http://bioviz.org/igb/index.html): Smad3-binding upstream and within the coding region of ZEB1 and JUNB.

Data are presented as mean ± SEM.
Figure S3. TGFβ accelerates Twist1-induced EMT (related to Figure 3)

(A) RT-PCR: E-cadherin, Fibronectin (FN), Wnt5a, ZEB1, ZEB2, Slug. A549-Twist1-ER cells treated with recombinant TGFβ, 4-Hydroxy-Tamoxifen (TAM), TAM+TGFβ and
TAM+TGFR1-inhibitor A83-01 (A83), at 16 days post induction (dpi). Control (Ctrl) = untreated. n=3.

(B) Immunofluorescence: E-cadherin (green), Vimentin (red), DAPI (blue). HMLE-Twist1-ER cells treated with TAM for 16 days, followed by treatment with TAM+TGFB1-inhibitor A83-01 (A83) for 6 days. Scale bar: 100 μm.

(C) RT-PCR: E-cadherin, N-cadherin, Vimentin, ZEB1 and ZEB2. HMLE-Twist1-ER cells treated with TAM for 16 days, followed by treatment with TAM+A83-01 (A83) with controls as indicated. n=3.

(D) RT-PCR: miR-141, miR-200a, miR-200b and miR-200c. HMLE-Twist1-ER cells were treated for 16 days with A83-01 (A83), TAM and TAM+A83. Control (Ctrl) = untreated. n=3.

Data are presented as mean ± SEM.
Figure 4. Twist1 induces collective invasion independently of TGFBR1-activation (related to Figure 4)

(A) Immunofluorescence: ZEB1 (green), DAPI (blue). HMLE-Twist1-ER cells treated with 4-Hydroxy-Tamoxifen (TAM), TGFBR1-inhibitor A83-01 (A83) and TAM+A83.
for 16 days, then seeded into 3D-collagen gels and cultured for 8 days without further treatment. Control (Ctrl) = untreated. Scale bar: 100 µm.

(B) Immunofluorescence: F-actin (Phalloidin, white), DAPI (blue). Cells treated as described in (A). Scale bar: 100 µm.

(C) Migration assay: quantification of migrated HMLE-Twist1-ER cells treated as described in (A). The number of cells migrated in the TAM-condition was set as 100%. n=3.

(D) Live cell imaging: quantification of average cell speed. Cells were treated as described in (A). Single cells were tracked between 10 and 13 dpi. n=563 cells for Ctrl, n=210 cells for TAM, n=338 cells for TAM+A83. One representative experiment from 3 biological replicates is shown. *p<0.001.

(E) Live cell imaging: quantification of average cell speed over five generations. Cells were treated as described in (A), tracked and quantified as described in (D). One representative experiment from 3 biological replicates is shown.

(F) Live cell imaging: quantification of cell-cycle duration (= duplication time). Cells were treated as described in (A) and tracked as described in (D). n=274 cells for Ctrl, n=84 cells for TAM, n=158 cells for TAM+A83. One representative experiment from 3 biological replicates is shown. *p<0.01, **p<0.001.

(G) Proliferation Assay: HMLE-Twist1-ER cells treated with A83-01 (A83), TAM+A83 for 21 days. Control (Ctrl) = untreated. Proliferation was measured between 18 and 21 days post induction. n=6.

Data are presented as mean ± SEM.
Supplemental Experimental Procedures

Fluorescence-Activated Cell Sorting and Flow Cytometry

Single cell suspensions were suspended in 0.1% BSA/PBS on ice prior to FACS. Cells were stained with the following antibodies: APC Mouse Anti-Human CD44 (clone G44-26; BD Biosciences: #559942) and FITC Mouse Anti-Human CD24 (clone ML5; BD Biosciences: #555427). Dead cells were excluded by 7-AAD (BD Biosciences). Cells were sorted and analyzed on a BD FACS AriaI, followed by data processing with FlowJoV10 software.

Reporter Assay

HEK293T cells were transfected with SBE4-luc [1] using X-tremeGENE HP DNA Transfection Reagent (Roche). 1x10⁴ cells were transfected with 90 ng reporter plasmid and 10 ng pGL-SV40-Renilla luciferase control plasmid. Reporter activities were measured using the Dual-Luciferase Reporter Assay System (Promega).

Migration Assay

2.5x10⁴ cells were plated in 24-well culture inserts with 8 μm pores (BD Falcon). After 24 hours, non-migrated cells were removed from the upper side of the insert. For visualization, migrated cells were stained with the Hemacolor Rapid staining Set (Merck) according to the manufacturer’s instructions.
**Proliferation Assay**

3000 cells per well were seeded in white polystyrene 96-well plates. After 24h, drugs were added every 24h for a total duration of 3 days. Viability of cells was measured every 24h using Cell Titer Glo (Promega) according to the manufacturer's instructions.

**Live cell imaging**

1x10⁴ cells per well were seeded in 24-well plates. After 24h, cells were imaged every 5min at 10x magnification with an Axio Observer.Z1 microscope using in-house software over a period of 3 days.

**Cell tracking and data analysis**

Individual cells were tracked manually using Timm’s Tracking Tool (TTT) [2,3]. Each cell was tracked until either one of the following events occurred: (i) division (resulting in two new cells), (ii) apoptosis, (iii) cell loss (i.e. the tracked cell left the observable area) or (iv) the end of the imaging period has been reached. For cell cycle duration computation, only cells that were observed from origin (division of its parent) to division were considered. The cell cycle time is the time between these two events. For the calculation of cell speed, the displacement between two consecutive observations was calculated as Euclidian distance and divided by the time between two observations (5 mins). Average cell speed represents the mean displacement of a cell over all observations. This measure was calculated for all tracked cells. For statistical analysis a Kruskal-Willis test with post-hoc pairwise comparisons using Nemenyi-test: p<001 was employed.
RNA Preparation and RT-PCR Analysis

cDNA: mRNA was isolated using the mRNeasy Mini Kit (Qiagen), cDNA synthesis was performed with EasyScriptPlus Kit and Power SYBR Green-PCR Master Mix (Applied Biosystems) was used for PCR. Transcript levels of RPL32 were used as a control for normalization.

Micro-RNAs (miRNA): The miRNeasy Mini Kit (Qiagen) was used to isolate total RNA, reverse transcription of miRNAs was performed with the miScript RT Kit (Qiagen). PCR was performed using Power SYBR Green-PCR Master Mix (Applied Biosystems) and miScript Primer Assays (control/normalization = HS-RNU6-2_11, miR-141 = HS-miR-141_1, miR-200a = HS-miR-200a_1, miR-200b = HS-miR-200b_3, miR-200c = HS-miR200c_1, Qiagen).

Samples were run on a QuantStudio 12K Flex qPCR system (Life Technologies). Analysis was described previously [4].

**Primers used for RT-PCR:**

| Gene     | Forward          | Reverse                      |
|----------|------------------|------------------------------|
| CD31     | AACAGTGTGGGACATGAAGAGCC | TGTAAACACGCACGTCATCCTT       |
| E-cadherin | TGCCAGAAAATGAAAAAGG | GTGTATGTGGCAATGCGTTC         |
| Fibronectin | CAGTGGGAGACCTCGAGAAG | TCCCTCGGAACATCGAAAC          |
| FoxC2    | GCCTAGAGGACCTGTTGAAGC | TTGACGAAGCACTCGTTGAG         |
| N-cadherin | ACAGTGCCACCTACAAGGG | CCGAGATGGGGTGATATG          |
| RPL32    | CAGGGTTCTGTAAGAGATTCAAGGG | CTTGGAGGAAACATTGTGACGGTC     |
Chromatin Immunoprecipitation (ChIP)

ChIP analysis was performed as described [5] with minor modifications. Briefly, cells were cross-linked with 1% formaldehyde for 20 min at room temperature and quenched with glycine. Following nuclei isolation with nuclear preparation buffer, these were sonicated in equal volumes of sonication buffers I and II using Bioruptor Plus (Diagenode SA). Next, chromatin extracts were cleared by centrifugation, diluted, pre-cleared with 50% sepharose 4B (Sigma-Aldrich, USA) slurry, and used for immunoprecipitation with 1 µg of anti-ERα antibody (HC20) (Santa Cruz: # sc-543) or IgG control antibody (Abcam: # ab37415). The immune complexes were pulled down using blocked protein A sepharose beads and the ChIP immune complexes were washed twice with IP buffer, wash buffer and with 1X TE buffer. The washed ChIP immune complexes and inputs (10%) were treated with RNase A (0.2 µg/µl) at 37°C and reverse cross-linked with Proteinase K (20 µg/µl, Invitrogen) overnight at 65°C. The phenol-chloroform-isoamyl alcohol extraction method was used to purify DNA, followed by precipitation with LiCl and linear polyacrylamide. The precipitated DNA was dissolved in nuclease free water and amplified by PCR. PCR-reaction was performed as previously described [6]. Briefly, the reaction was carried out in 14 µl of PCR mix, 1.5 µl of 5 µM primers, 1 µl of purified DNA and 8.5 µl of dH₂O. Input DNA was used for standard curves and normalization.

### Primers used for ChIP analysis:

| Gene  | Forward          | Reverse          |
|-------|------------------|------------------|
| Slug  | GGGGAGAAGCCTTTTTCTTG | TCCTCATGTGGTGCAAGGAG |
| TGFBR1 | ACGGCGTTACAGTTTCTG   | GCACATACAAACGCTATCTC |
| VEGFR2 | CAAGACAGGAAGACAGAAAACAC | GGTGCCACACGCTCTAGGA |
| Vimentin | GAGAACTTTTGCCGTTGAAGC | GCTTCCTGTAAGGGCAATC |
| Wnt5a  | ATGGCTGGAAGTGCAATGTCT | ATACCTAGCGACCAAGAA |
| ZEB1   | GCACAAGAGAGCCACAGTAG | GCAAGACAGTTTCAAGGTTTC |
| ZEB2   | TTCCTGGGCTACGACCATAC | TGTGCTCCATCAAGCAATTC |
### Western Blot Analysis

Whole protein lysates were prepared using RIPA buffer. Nuclear and cytoplasmic cell fractionations were done using Tween 20 lysis buffer. Protein lysates were resolved on a 10-12% SDS gel, transferred to PVDF membranes and probed with primary antibodies: E-cadherin (clone EP700Y; Biozol: #GTX61329), Fibronectin (BD Biosciences: #610078), NF-κB p65 (clone D14E12; Cell Signaling: #8242), Phospho-NF-κB p65 (Ser536) (clone 93H1; Cell signaling: #3033), Phospho-Smad2 (Ser465/467)/Smad3 (Ser423/425) (clone D27F4; Cell signaling: #8828), Slug (clone C19G7; Cell signaling: #9585), Smad 2/3 (clone D7G7; Cell signaling: #8685), Twist1 (clone Twist2C1a; Santa Cruz: #sc-81417), Vimentin (clone D21H3; Cell signaling: #5741) and ZEB1 (clone H-102; Santa Cruz: #sc-25388). β-actin (Abcam: # ab6276), α-tubulin (clone B-5-1-2; Sigma: #T5168) and Histone H3 (abcam: # ab1791) were used as internal controls. Following incubation with HRP-linked secondary antibodies (Jackson Immunoresearch: #111-036-045 and #115-036-062) proteins were visualized with ECL reagent (GE Healthcare). Blots were visualized using the ChemiDoc System (Bio-Rad). Acquired images quantified using ImageJ software to calculate the signal intensity based on area and pixel density. Relative values were determined by subtracting the background from the lane of interest, followed by normalization to the loading control.
**Immunofluorescence**

Cells were cultured on poly-D-lysine (Sigma) coated coverslips or in 3D collagen gels, fixed with 4% paraformaldehyde and permeabilized with 0.2% Triton X-100/PBS. After blocking with 10% goat serum (Biozol) in 0.1% BSA (Sigma), coverslips/gels were incubated overnight with primary antibodies in 0.1% BSA as following: α6-integrin (clone GOH3; Santa Cruz: #sc-19622), β-catenin (Clone 14; BD Biosciences: #610153), CD31 (clone WM59; BioLegend: #303102), E-cadherin (clone EP700Y; Biozol: #GTX61329), E-cadherin-Alexa 488 (clone 24E10; New England Biolabs: #3199), Laminin (clone L9393; Sigma-Aldrich: #L9393), Ki-67 (Abcam: #ab15580), Phalloidin–Atto 647N (Sigma: #65906), Vimentin (clone V9; Abnova: #MAB3578) and ZEB1 (H-102; Santa Cruz: #sc-25388). Secondary antibodies were goat-anti-rabbit, -rat and -mouse coupled to Alexa 488 or -594 (Life Technologies). Cell nuclei were visualized with 40,6-diamidino-2-phenylindol-dihydrochloride (DAPI, Sigma). Slides were mounted with Aqua-Poly/Mount reagent (Polysciences).

**Proteomics analysis**

Glycosyl residues on intact cells were labelled with aminooxybiotin under mild oxidative conditions as described before [7,8] and after cell lysis, glycosylated cell surface proteins were enriched with streptavidin beads (IBA). After stringent washing steps, proteins were on-bead proteolysed with trypsin, followed by deglycosylation with PNGase F (NEB). Eluted peptides were combined, acidified and directly used for analysis on a LTQ-OrbitrapXL connected with an Ultimate3000 nano HPLC system.
(Thermo Fisher Scientific) as described [9]. The full-scan MS spectra were acquired in the Orbitrap with a resolution of 60,000 and up to 10 most abundant peptide ions were selected for fragmentation in the linear ion trap. Peptides were identified and quantified using the Progenesis QI software (Nonlinear, Waters) and the Mascot search algorithm with the Ensembl Human public database as described [7-9].
Table S1: Cell surface proteomics analysis (related to Figure 5).

| Gene      | 3D Ratio TAM+A83/Ctrl | 2D Ctrl | 3D TAM+A83 | 2D Ctrl | 3D TAM | 2D TAM+A83 |
|-----------|------------------------|---------|------------|---------|--------|------------|
| ENPP1     | 28.8                   | 8       | 229        | 0       | 112728 | 1388       |
| PCDH7     | 26.2                   | 72      | 1874       | 5689    | 3821   | 663        |
| CD31      | 10.3                   | 3620    | 37155      | 4952    | 3377   | 46122      |
| CNTNAP2   | 7.0                    | 3006    | 20941      | 5022    | 0      | 51168      |
| SMAGP     | 6.0                    | 6928    | 41460      | 2931    | 56365  | 57863      |
| PNP       | 5.1                    | 248     | 1264       | 12309   | 3222   | 5178       |
| CD99      | 4.9                    | 1711    | 8322       | 19251   | 33107  | 87071      |
| IGSF8     | 4.7                    | 1520    | 7074       | 32350   | 25862  | 67722      |
| ACAT2     | 4.3                    | 201     | 868        | 16221   | 18152  | 14736      |
| SLC3A2    | 4.2                    | 10316   | 43463      | 126433  | 16686  | 85000      |
| SLC1A4    | 4.2                    | 2650    | 11124      | 47149   | 16061  | 24416      |
| SLC31A1   | 3.9                    | 27338   | 105362     | 99896   | 189796 | 91398      |
| XPO7      | 3.8                    | 5550    | 20916      | 14471   | 36507  | 30511      |
| ITGA10    | 3.7                    | 1643    | 6062       | 1747    | 31058  | 7992       |
| TRIP13    | 3.4                    | 834     | 2840       | 14533   | 7963   | 6929       |
| PPAP2A    | 3.3                    | 14292   | 47531      | 14347   | 275985 | 149722     |
| MPZL2     | 3.3                    | 7590    | 24992      | 4749    | 6021   | 19258      |
| METTL7A   | 3.3                    | 586     | 1915       | 3323    | 20452  | 14260      |
| EMP3      | 3.2                    | 175098  | 552127     | 134181  | 1438640 | 455855     |
| KBTBD8    | 3.1                    | 9346    | 29351      | 40664   | 34851  | 42462      |
| VPS29     | 3.0                    | 598     | 1809       | 10696   | 13180  | 13420      |
| HLA-F     | 3.0                    | 107     | 321        | 116     | 394    | 634        |
| FZD2      | 3.0                    | 394     | 1187       | 17485   | 56462  | 11380      |
| TMEM30A   | 2.9                    | 8242    | 24123      | 16591   | 39772  | 18198      |
| PTPRE     | 2.9                    | 1489    | 4343       | 6031    | 4579   | 7990       |
| ANO6      | 2.8                    | 64871   | 183765     | 78410   | 120341 | 94878      |
| SLC9A6    | 2.8                    | 2879    | 8031       | 4677    | 10122  | 3647       |
| F11R      | 2.8                    | 60498   | 168428     | 70872   | 15371  | 147765     |
| ENTPD2    | 2.8                    | 334207  | 928083     | 166011  | 43624  | 662929     |
| RAB38     | 2.8                    | 4258    | 11797      | 5205    | 517    | 6323       |
| CSE1L      | 2.8                    | 567064  | 1564572    | 1201389 | 1017165 | 1260009    |
| SUSD2     | 2.7                    | 19537   | 53666      | 17861   | 344487 | 834627     |
| ARL6IP5   | 2.7                    | 8558    | 23390      | 22150   | 87450  | 56160      |
| SCAMP3    | 2.7                    | 15882   | 42574      | 336188  | 25037  | 560759     |
| NIPR       | 2.7                    | 15882   | 42574      | 336188  | 25037  | 560759     |
| YIPF6     | 2.7                    | 3193    | 8478       | 20371   | 8827   | 16425      |
| SLC5A6    | 2.6                    | 32329   | 85279      | 226922  | 79071  | 90610      |
| Gene  | Fold Change |
|-------|-------------|
| SLC39A10 | 2.6 |
| DDR1 | 2.6 |
| CD9 | 2.6 |
| MCCC2 | 2.6 |
| THBD | 2.6 |
| SIRPA | 2.6 |
| SLC44A2 | 2.6 |
| YWHAG | 2.6 |
| HLA-A | 2.6 |
| ADGRE2 | 2.5 |
| FZD6 | 2.5 |
| YIPF5 | 2.5 |
| PROCR | 2.5 |
| FAT2 | 2.5 |
| DFNA5 | 2.5 |
| TALDO1 | 2.5 |
| AGTRAP | 2.4 |
| NME1 | 2.4 |
| EMB | 2.4 |
| ATP5O | 2.4 |
| PCCB | 2.4 |
| TEO2 | 2.4 |
| NOTCH1 | 2.4 |
| CTPS1 | 2.3 |
| XPO5 | 2.3 |
| SLC7A5 | 2.3 |
| ATP5B | 2.3 |
| CAP1 | 2.3 |
| ITGB4 | 2.3 |
| TTYH3 | 2.3 |
| TOMM22 | 2.3 |
| NRP1 | 2.3 |
| TMEM165 | 2.3 |
| FAS | 2.3 |
| PTK7 | 2.3 |
| SLC16A3 | 2.3 |
| SLC43A3 | 2.3 |
| PVR | 2.2 |
| CLND1 | 2.2 |
| CD44 | 2.2 |
| SLC9A1 | 2.2 |
| Gene   | Log2FC | Log2FC | Log2FC | Log2FC | Log2FC |
|--------|--------|--------|--------|--------|--------|
| IPO9   | 2.2    | 79155  | 177244 | 70451  | 128282 | 99049  |
| MCCC1  | 2.2    | 58759  | 131246 | 67567  | 41677  | 76899  |
| EPHB4  | 2.2    | 12185  | 27050  | 12336  | 66834  | 66571  |
| TNPO1  | 2.2    | 89727  | 199041 | 172321 | 179401 | 131659 |
| ITGA6  | 2.2    | 324385 | 716340 | 479073 | 315151 | 649297 |
| ACACA  | 2.2    | 187699 | 412916 | 112618 | 442562 | 707621 |
| CA9    | 2.2    | 798075 | 174332 | 121814 | 26162  | 420829 |
| ITGA3  | 2.2    | 395999 | 864060 | 989041 | 105995 | 121804 |
| SLC3A2 | 2.2    | 51595  | 112249 | 257209 | 42871  | 149888 |
| XPO1   | 2.2    | 165924 | 358990 | 252534 | 310341 | 252565 |
| CD276  | 2.2    | 123174 | 266444 | 134154 | 93303  | 123526 |
| OTUB1  | 2.2    | 11220  | 24122  | 72384  | 71486  | 76829  |
| STOML2 | 2.1    | 27981  | 60135  | 44177  | 80845  | 72669  |
| MPZL1  | 2.1    | 98441  | 211483 | 59819  | 232305 | 256733 |
| S100A14| 2.1    | 38195  | 81807  | 68350  | 3292   | 85429  |
| SLC1A5 | 2.1    | 262655 | 561424 | 472643 | 279915 | 386020 |
| IPO5   | 2.1    | 228311 | 486979 | 378732 | 351264 | 292435 |
| SLC16A1| 2.1    | 3827   | 8161   | 28579  | 6750   | 12025  |
| TKT    | 2.1    | 15105  | 32257  | 93370  | 91954  | 127740 |
| ABCC3  | 2.1    | 39424  | 83738  | 17578  | 66548  | 62646  |
| NPTN   | 2.1    | 976239 | 206726 | 120680 | 220740 | 132579 |
| PSMC1  | 2.1    | 794    | 1678   | 3773   | 8357   | 2987   |
| MPDU1  | 2.1    | 10899  | 23045  | 34255  | 44818  | 54175  |
| VAMP3  | 2.1    | 5872   | 12402  | 38799  | 38639  | 38228  |
| HSPA8  | 2.1    | 150089 | 315121 | 221296 | 167312 | 170240 |
| GNAI2  | 2.1    | 86298  | 180618 | 77482  | 130073 | 85013  |
| MST1R  | 2.1    | 156371 | 326325 | 236591 | 59545  | 260668 |
| VDAC1  | 2.1    | 244678 | 508728 | 989194 | 989772 | 914283 |
| ENG    | 2.1    | 7088   | 14692  | 37538  | 502    | 302    |
| ADGRE5 | 2.1    | 264334 | 544050 | 182522 | 136279 | 744713 |
| IPO7   | 2.1    | 327593 | 674113 | 487523 | 360577 | 367422 |
| ITGB1  | 2.1    | 987279 | 203150 | 350573 | 325610 | 281872 |
| ATP1B3 | 2.1    | 144731 | 297557 | 165222 | 158468 | 262195 |
| TUBA1C | 2.1    | 132739 | 272278 | 96232  | 217990 | 184232 |
| SLC26A2| 2.0    | 69582  | 142081 | 107473 | 297853 | 130708 |
| IGF1R  | 2.0    | 62119  | 126772 | 133807 | 60387  | 68568  |
| EPCAM  | 2.0    | 106868 | 217948 | 403828 | 13208  | 213803 |
| SLC12A2| 2.0    | 65638  | 133769 | 199970 | 690374 | 258181 |
| GALNT2 | 2.0    | 22823  | 46286  | 13275  | 42986  | 35680  |
| ATL3   | 2.0    | 5642   | 11394  | 21210  | 55512  | 37737  |
| GBP2   | 2.0    | 319    | 642    | 498    | 8742   | 4122   |
| Genes   | 2.0 | 75495 | 151870 | 218667 | 210921 | 208640 |
|---------|-----|-------|--------|--------|--------|--------|
| SEC61A1 | 2.0 | 1001655 | 2009974 | 970957 | 603979 | 863078 |
| CTNNA1  | 2.0 | 197596 | 396459 | 389180 | 46414 | 131235 |
| SCARB1  | 2.0 | 16042 | 32058 | 384 | 0 | 754 |
| SLCO4A1 | 2.0 | 10998 | 21977 | 52890 | 81242 | 23973 |
| NOTCH2  | 2.0 | 1028762 | 2052502 | 3180044 | 1735163 | 3203320 |
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