A Grainyhead-Like 2/Ovo-Like 2 Pathway Regulates Renal Epithelial Barrier Function and Lumen Expansion

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Supplements

Materials and Methods

Human tissue samples

Paraffin material was chosen from the archive of the Institute of Pathology of the Charité Berlin and used when adequate tissue was available. The study was undertaken according to approved institutional review board protocols. It was conducted within the framework of the declaration of Helsinki.

Mouse nephric duct samples

Paraffin-embedded E10.0 embryos were cut into 5µm sections. For lumen area quantification, HE stained sections were analyzed at the level of the posterior part of the mesonephros where mesonephric tubules were visible but not connected to the nephric duct. The measurements were performed using ImageJ.

Antibodies

The following antibodies were used: Anti-Grhl2 (HPA004820, Sigma-Aldrich, Munich, Germany), anti-Aquaporin 2 (SC-9882, Santa Cruz Biotechnology, Heidelberg, Germany), Anti-Podocalyxin (AF1556, R&D Systems, Abingdon, United Kingdom), Anti-a-Tubulin (T9026, SIGMA-Aldrich), Anti-Claudin4 (36-4800, Life technologies, Darmstadt, Germany), Anti-E-Cadherin (610182, BD Bioscience, Heidelberg, Germany), Anti-Par-3 (07-330, Merck Millipore, Darmstadt, Germany), Anti-Rab25 (R8407, SIGMA-Aldrich), Ant-b-Catenin (610154, BD Bioscience) and Anti-Cytokeratin 8 (TROMA-I, Developmental Studies Hybridoma Bank, DSHB), that was developed by Philippe Brulet and Rolf Kemler under the auspices of the NICHD and maintained by the University of Iowa, Department of Biology, Iowa City.

Cloning procedure/ lentiviral particles

All plasmids for the production of lentiviral particles were purchased from Addgene (Cambridge, USA) Lentiviral particles were generated using the 2nd generation packaging system, comprising the envelope plasmid psPAX2 (Addgene plasmid 12260) and packaging plasmid pMD2.G (Addgene plasmid 12259) established in the laboratories of Didier Trono (EPFL, Lausanne, Switzerland). [1] The Wiznerowicz laboratories (lentiweb.com) provided protocols for the production and titration of lentiviral particles. To establish a lentiviral mediated KD of Grhl2, H1 promoter-shRNA cassettes were cloned from pSUPER directly into the vector plasmid pLVTHM (Addgene plasmid 12247) using EcoRI and ClaI restriction sites. shRNAs directed against Grhl2 target the sequence 5´-GACTTCCCCTGATGATTCA-3´. A shRNA targeting 5′-CATCACGTACGCGGAATACT-3′ of firefly luciferase was used as a negative control.

Full-length cDNA of Cldn4 and Rab25 were amplified from IMCD3 cDNA using Phusion High-Fidelity DNA Polymerase (New England BioLabs, Frankfurt am Main, Germany). Full-length cDNA of Ovol2A was generated from cDNA of wild-type kidney. The coding sequence of Cdh1 was kindly provided from the Daniel Besser laboratories.[2] A Grhl2 rescue construct, which is resistant to the Grhl2-shRNA was generated in our lab.[3] We used full-length cDNA of click beetle luciferase as control cDNA. Vector plasmid pLVUT-tTR-KRAB (Addgene plasmid 11651) was utilized to produce lentiviral particles to integrate expression cassettes of cDNAs in IMCD3 Grhl2-KD cells. The GFP-EMCV IRES-tetR-KRAB domain was replaced by cDNA-IRES-mCherry.

Cell culture/ three-dimensional (3D) cultivation of IMCD-3 cells

IMCD-3 cells were purchased from ATCC (CRL-2123, Manassas, USA) and maintained in DMEM+GlutaMAX (Life technologies) supplemented with 10% fetal bovine serum (FBS) and penicillin/ streptomycin (100 Units/ ml/ 100 µg/ ml). Cells were sub-cultured two to three times per week in a ratio of 1:4 to 1:8 using 0.25% trypsin, 0.5 mM EDTA in 1xPBS.

IMCD3 cells were transduced with lentiviral particles generated from pLVTHMshRNA at a multiplicity of infection (MOI) of 5. At the same time cells were infected with a second type of lentiviral particle, generated from pLVUT-cDNA-mCherry (MOI of 0.5). Cells were expanded for at least six days and EGFP- and mCherry-expressing cells were isolated using Fluorescence-activated cell sorting (FACS, BD FACSAriaII, BD Biosciences).

For 3D cultivation, IMCD-3 Grhl2-Con, Grhl2-KD, Grhl2-KD;Grhl2-Res were trypsinized and centrifuged to remove the complete growth medium. A suspension of 1.5×10^4 cells in 150µl serum-free medium were given to 150 µl Matrigel (Basement Membrane Matrix, BD Biosciences) and plated in Nunc® LabTek® 8-well chamber slides (Thermo Fisher Scientific, Bonn, Germany). After incubation for 30 min at 37°C complete growth medium was added to cover the Matrigel. Medium was changed every second day.

RNA extraction and cDNA synthesis

RNA was extracted from IMCD-3 cells using RNeasy Mini-kit (Qiagen, Hilden, Germany) according to manufacturer's instructions including treatment with RNase-free DNase I (Qiagen). 500 ng total RNA of each sample was utilized for cDNA synthesis using RevertAid First Strand cDNA Synthesis Kit (Fermentas, St. Leon-Rot, Germany) according to manufacturer's instructions.

Real-time PCR

Quantitative Real-time PCR was performed using FastStart Universal SYBR Green Master (Rox) (Roche Diagnostics, Mannheim, Germany) with cDNA as template and the primers listed in Table S4. Relative mRNA expression was normalized to β-actin and calculated according to ΔΔCt method.

Microarray

Illumina TotalPrep RNA Amplification kit (Life technologies) was used to synthesis cRNA out of RNA isolated from IMCD3 cells according to manufacturer's instructions. Quality of RNA and cRNA was evaluated using Agilent 2100 Bioanalyzer and the Agilent RNA 6000 Nano kit (Agilent Technologies, Böblingen, Germany). We used MouseWG-6 v2.0 Expression BeadChips (Illumina, Essex, UK) to analyze genome-wide gene expression profiles.

Chromatin Immunoprecipitation Sequencing (ChIP-Seq)

We performed ChIP analysis using an antibody directed against H3K4me3 (pAB-003-010, diagenode, Seraing (Ougrée), Belgium) on IMCD-3 Grhl2-KD and Grhl2-Con using the iDeal ChIP-seq kit (diagenode) according to manufacturer's instructions. The protocol was modified as follows. Cells were grown to confluence and cowered with DMEM. Cross-linking was done with 1% formaldehyde on a shaking platform for 10 min at room temperature (RT). The reaction was quenched with 125 mM glycine. DMEM was removed from the plates and cells were washed with ice-cold 1xPBS than following the kit's manual. ChIP experiments were performed on IMCD-3 cells, embryonic (embryonic day E15.5) and adult kidney of mice using an antibody against Grhl2. IMCD-3 cells were grown to 100% confluence, 5×10^6 cells were cross-linked with 1% formaldehyde for 20 min at room temperature (RT). Kidneys were cut into small pieces and homogenized in Lysis Buffer (15mM HEPES (pH=7.8), 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM EDTA, 1 mM DTT, 10% Glycerol) with 0.5% IGEPAL and proteinase inhibitors. Nuclei were fixed in Lysis Buffer with 1% formaldehyde for 20 min (RT). Chromatin was fragmented to an average size of 300-500 bp. Chromatin (100 µg) and 5 µg of antibody was used per assay using Chromatin Immunoprecipitation (ChIP) Assay Kit (Merck Millipore, Darmstadt, Germany). IP'd DNA concentration was determined using the Qubit system (Qubit ds DNA HS Assay kit, Molecular probes/ Life Technologies) and quality was controlled using Agilent 2100 Bioanalyzer and the Agilent High Sensitivity DNA kit (Agilent Technologies). For library preparation the TruSeq ChIP-Seq Sample Prep Kit (illumina) was used according to manufacturer's instructions. Flow cell was sequenced using illumina HiSeq 2000 (50 bp reads). Primers used for quantitative ChIP-PCR are listed in Table S5.

Immunofluorescence/ immunohistochemical staining

Immunohistochemical staining on human tissue was performed on 5 µm paraffin-embedded, dewaxed and rehydrated sections. Samples were blocked in 5% bovine serum albumin (BSA) in 1xTBS, 0.025% Tween20 and stained with primary antibodies and secondary antibodies labelled to alkaline phosphatase. Enzyme activity was detected using SIGMA FAST^{TM} Fast Red TR/Naphthol AS-MX Alkaline Phosphatase Substrate Tablets Set (SIGMA-Aldrich) according to manufacturer's instructions.

Embryonic (E18) or adult (P30) kidneys of C57BL/6 mice were cut in slices and fixed in 4% paraformaldehyde (PFA). 16 µm cryosections were incubated with 1% bovine serum albumin (BSA), 0.1% Triton X-100, stained with primary antibodies and secondary antibodies labeled with Cy2, Cy3, Cy5, Alexa488 or Alexa 647 (JacksonImmunoResearch, Newmarket, UK). Staining for Grhl2 required preliminary treatment with PBS/citric acid (10 mM).

IMCD-3 cysts grown in 3D culture were fixed with 4% PFA for 30 min on ice while rotating gently. After incubation with 50mM NH_4Cl, cells were permeabilized with 0.5% TritonX-100 in 1xPBS. Cysts were blocked with 1%BSA, 0.25% TritonX-100 in 1xPBS and incubated with primary antibodies and secondary antibodies in combination with nuclei counterstaining substance SYTOX® Green (Life Technologies). Cells were mounted using Dako Mounting Medium (Agilent

Technologies).

Confocal images were obtained with an inverted Zeiss LSM700 (Carl Zeiss Microscopy, Jena, Germany) or TCS SP5 Tandem Microscope (Leica microsystems, Wetzlar, Germany).

Western Blot

Whole-cell lysates of IMCD-3 cells were prepared using RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 1% IGEPAL, 0.5% sodium deoxycholate, 0.1% SDS, 1X Protease inhibitor from Roche Diagnostics, Mannheim, Germany). Total protein was quantitated using Pierce BCA Protein Assay kit (Thermo Fisher Scientific) according to manufacturer's instructions.

Protein samples were incubated with NuPAGE loading buffer (10% β-mercaptoethanol added) at 95°C for 10 min. For sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), samples were loaded on Novex Bis-Tris 4-12% precast gels and electrophoresis was performed according to the manufacturer's instructions using MOPS SDS running buffer (50 mM MOPS, 50 mM TrisBase, 3.5 mM SDS, 1.0 mM EDTA) and the XCell SureLock® Mini-Cell (Life technologies). Proteins were transferred onto PVDF membrane using 1.25 mM Bicine, 1.25 mM BisTris, 0.05 mM EDTA, 10% methanol. Blocking was performed in 1xTris-buffered saline (TBS, 50 mM Tris-Cl, pH 7.6, 150 mM NaCl) and 0.05% Tween20. Membranes were incubated with primary antibodies and secondary antibodies conjugated to horseradish peroxidase. Chemilumenscence was detected using the SuperSignal West Femto Chemiluminescent Substrate (Thermo Fisher Scientific). Relative signal intensity was analyzed using Adope Photoshop.

Transepithelial Resistance (TER) Measurements

IMCD-3 cell were grown on 0.4 µm PCF membranes. TER measurements were done using Ussing chambers and chopsick electrodes as described previously.[4] Statistical analysis was done using Student's t-test.

Statistical analysis

Data represent analysis of at least three independent experiments. Statistical analysis was done using Student's t-test or Mann-Whitney-U Test.

Microarray data analysis

After quantile normalization of the data, we considered genes as differentially expressed if a fold change larger than or equal to 1.4 (control vs. KD) and a p-value less than 0.05 using Student's t test were present. Heatmap visualization of the microarray data was done using MultiExperiment Viewer (http://www.tm4.org/). Heatmap visualized data has been maximum normalized on a per-gene basis. Gene names are according to the used Illumina microarray chip (MouseWG-6 v2). A gene was considered “successfully rescued” in one of the rescue constructs, if:

1.) the p-value after t-test control vs. rescue expression values was <= 0.05 [no significant difference between control and rescue] or

2.) p-value t-test rescue vs. Grhl2-KD <0.05 and average(rescue)/average(control)>=1/1.4 for differential genes downregulated in KD (that is, average(rescue)/average(control)>=1.4) [significant difference between rescue and KD + right fold change tendency for DE genes downregulated in KD] or

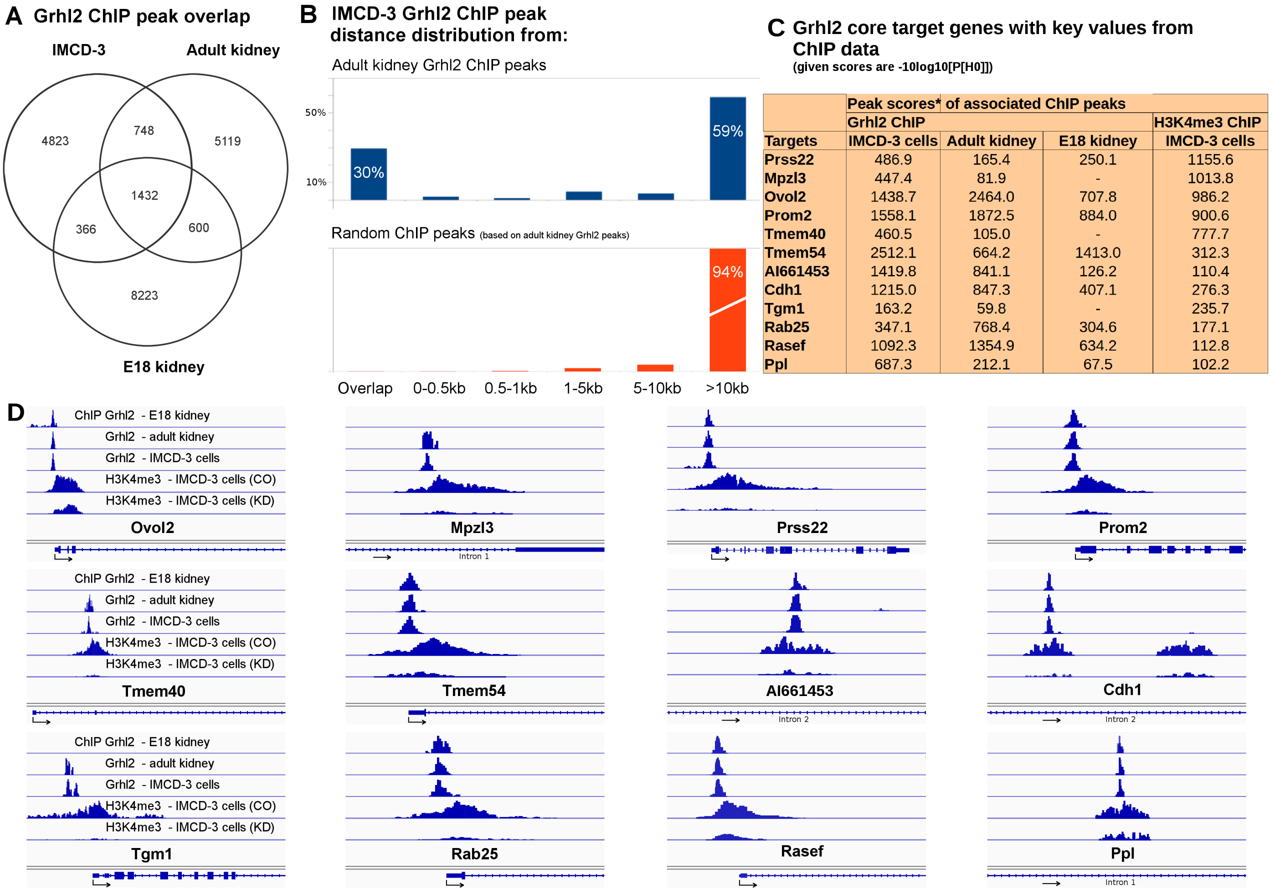
3.) p-value t-test rescue vs. Grhl2-KD <0.05 and average(rescue)/average(control)<=1.4 for differential genes upregulated in KD (that is, average(rescue)/average(control)<=1/1.4) [significant difference between rescue and KD + right fold change tendency for DE genes upregulated in KD].

ChIP data analysis

The alignment was done using Bowtie2. The reads were then filtered by applying a mapping quality threshold (MAPQ>20), removing PCR duplicates, and considering only uniquely mapping reads with less than 1bp mismatch. We used MACS for peak calling with default parameters (P<1e-05). Foreground and background models used for peak finding are mentioned in the main paper as well as total peak numbers. The peaks were ranked according to the intrinsic scores provided by MACS (negative log10 transform of the peak's p-value). Motif finding was then performed using MEME on the top 10% of all peaks in a 50 bp window around the peak's summit and optimized for de novo detection of 8-9 base pair motifs.[5] Motif enrichment in a given sequence set was done using MAST and FIMO.[6,7] Visualization of the ChIP data was carried out using the Integrative Genome Viewer provided by the Broad Institute.[8,9]

Miscellaneous Bioinformatics

Most of the plots of the bioinformatics related figures were generated with custom-made python scripts using matplotlib.[10] Operations on genomic intervals (e.g. generation of random peaks, distance calculations) were based on bedtools and its python interface pybedtools.[11,12] Analagously, operations on sam and bam files were performed with samtools and pysam,[13] respectively. Gene ontology and ChIP peak annotation was done using HOMER.[14]



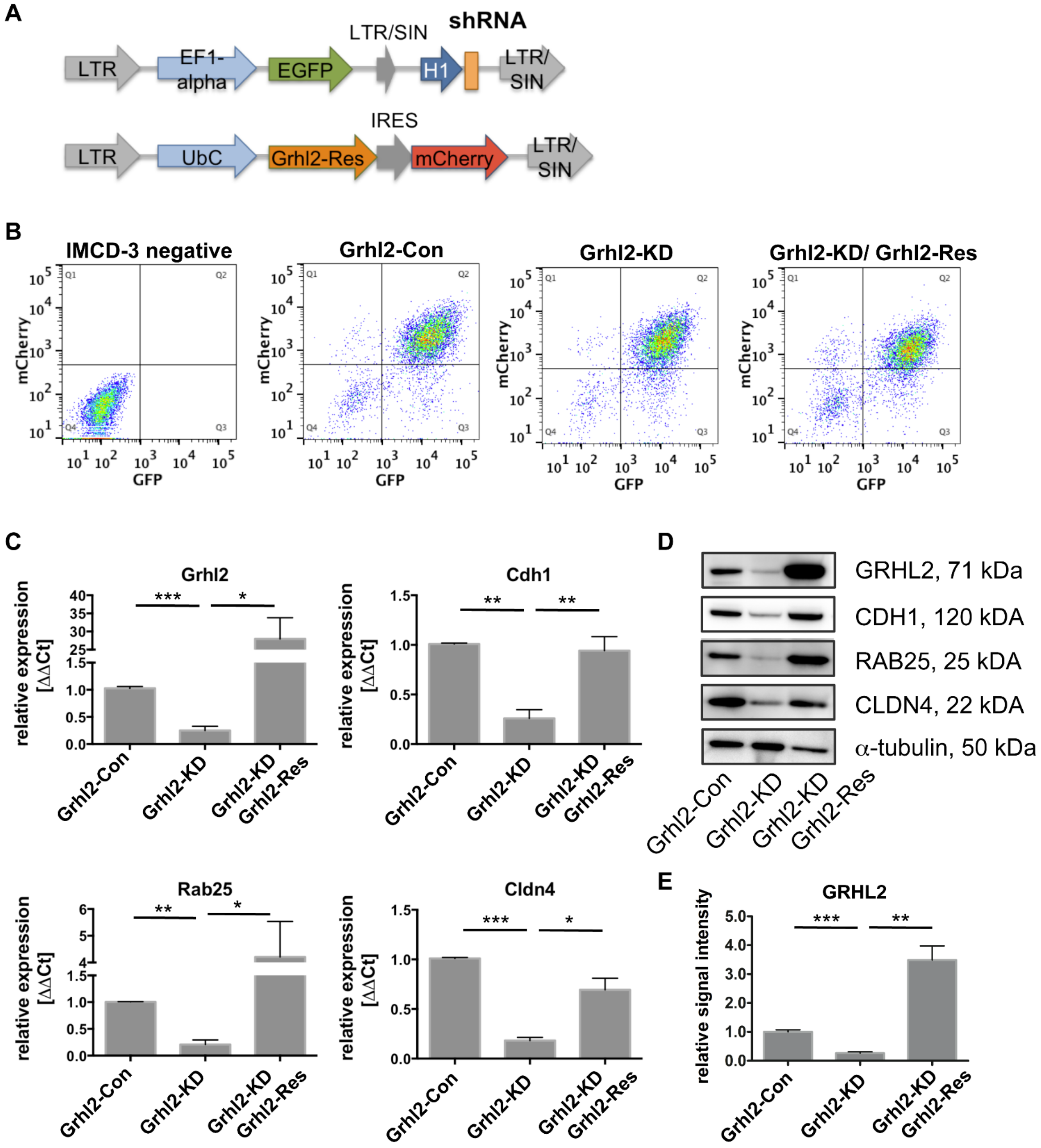
C Grhl2 core target genes with key values from ChIP data
(given scores are -10log10[P[H0]])

	Peak scores* of associated ChIP peaks			
	Grhl2 ChIP			H3K4me3 ChIP
Targets	IMCD-3 cells	Adult kidney	E18 kidney	IMCD-3 cells
Prss22	486.9	165.4	250.1	1155.6
Mpzl3	447.4	81.9	-	1013.8
Ovol2	1438.7	2464.0	707.8	986.2
Prom2	1558.1	1872.5	884.0	900.6
Tmem40	460.5	105.0	-	777.7
Tmem54	2512.1	664.2	1413.0	312.3
AI661453	1419.8	841.1	126.2	110.4
Cdh1	1215.0	847.3	407.1	276.3
Tgm1	163.2	59.8	-	235.7
Rab25	347.1	768.4	304.6	177.1
Rasef	1092.3	1354.9	634.2	112.8
Ppl	687.3	212.1	67.5	102.2

Supplemental Figure 1

Grhl2 binding through ChIP-seq and derived target genes by combining ChIP-seq and gene expression data.

A Venn diagram for all Grhl2 ChIP peaks shows a significant overlap between IMCD-3 cells, adult, and E18 kidney. **B** The Distance distribution of IMCD-3 Grhl2 ChIP peaks from adult kidney Grhl2 ChIP peaks reveal two distinct groups with peaks that either intersect or occur at larger distances (>10 kb) from each other. The bottom orange subplot shows the distance distribution from a random set of control peaks based on adult kidney Grhl2 ChIP peaks. **C** Overview of the Grhl2 target gene set with scores and key values from ChIP-seq experiments. The Grhl2 peak score is referring to MACS generic peak score. In case of multiple peak occurrences, the scores refer to the most significant peaks fulfilling the criteria mentioned in the main text. **D** Grhl2 target genes with ChIP tracks at the Grhl2 and differential H3K4me3 peak areas which have been used for Grhl2 target identification. Target genes were obtained based on differential expression in microarray data, differential H3K4 trimethylation, and Grhl2 ChIP peak occurrence (with Grhl2 binding motif) in IMCD-3 cells and adult kidney (differential H3K4me3 peak occuring ±500bp from TSS and/or within genomic region, Grhl2 ChIP peaks intersecting each other and H3K4me3 peak area). Note that most of the targets also exhibit a ChIP peak in E18 kidney.

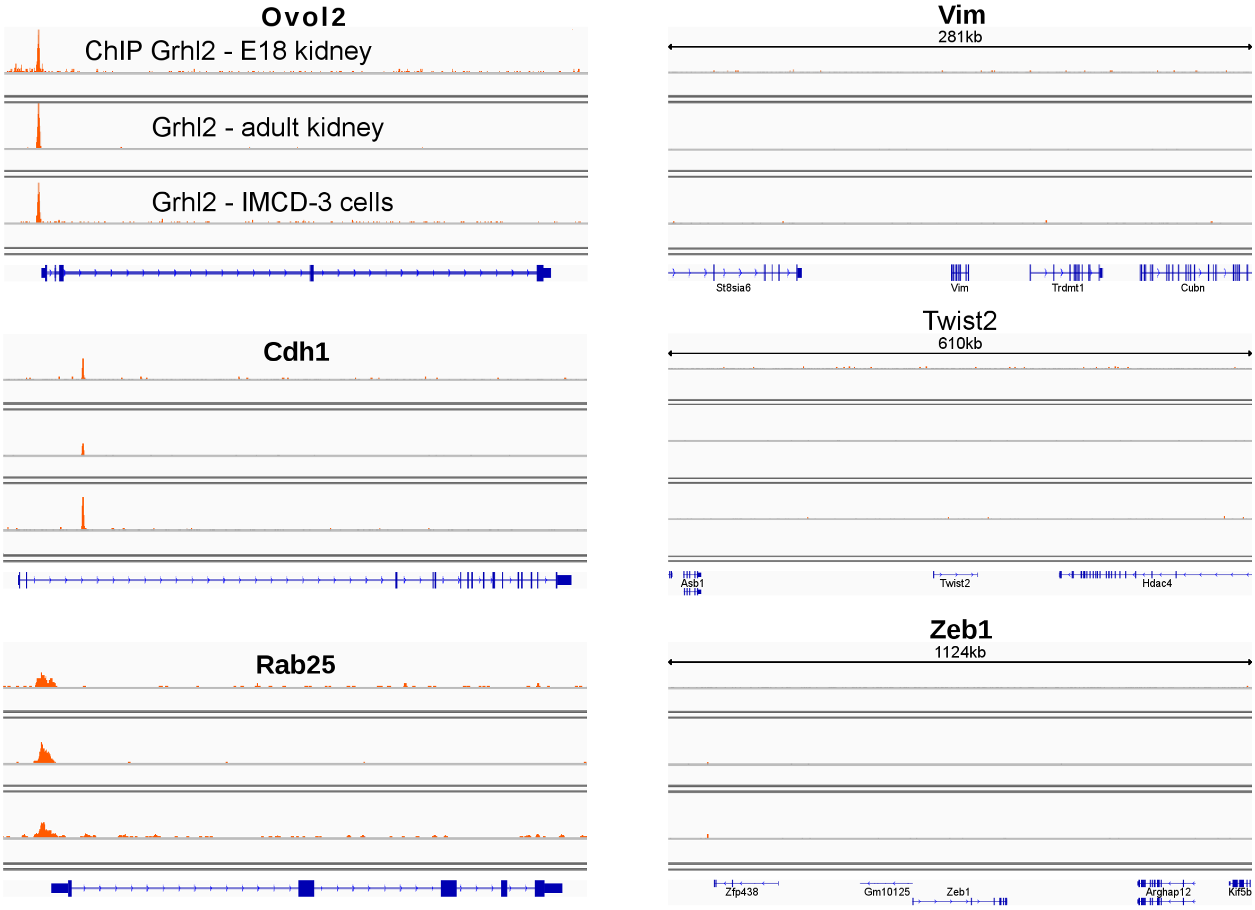


Supplemental Figure 2

Validation of IMCD-3 Grhl2-KD cells.

IMCD-3 cells were infected with lentiviral particles to stably integrate expression cassettes for a Grhl2 shRNA (or control shRNA) and the marker gene GFP **A**. Lentiviral particles were used in a multiplicity of infection (MOI) of 5. At the same time, cells were transduced to integrate a cassette for synthesis of a Grhl2 rescue (Grhl2-Res) construct (or control cDNA), followed by mCherry (MOI: 0.5). **B** At least six days after infection, GFP- and mCherry-expressing cells were isolated using Fluorescence-activated cell sorting (FACS; IMCD-3 negative: non-infected cells; Control (Grhl2-Con): control shRNA, GFP plus control cDNA and mCherry; Grhl2-KD: Grhl2 shRNA, GFP plus cDNA and mCherry; Grhl2-KD/Grhl2-Res: Grhl2 shRNA, GFP plus Grhl2-Res and mCherry).

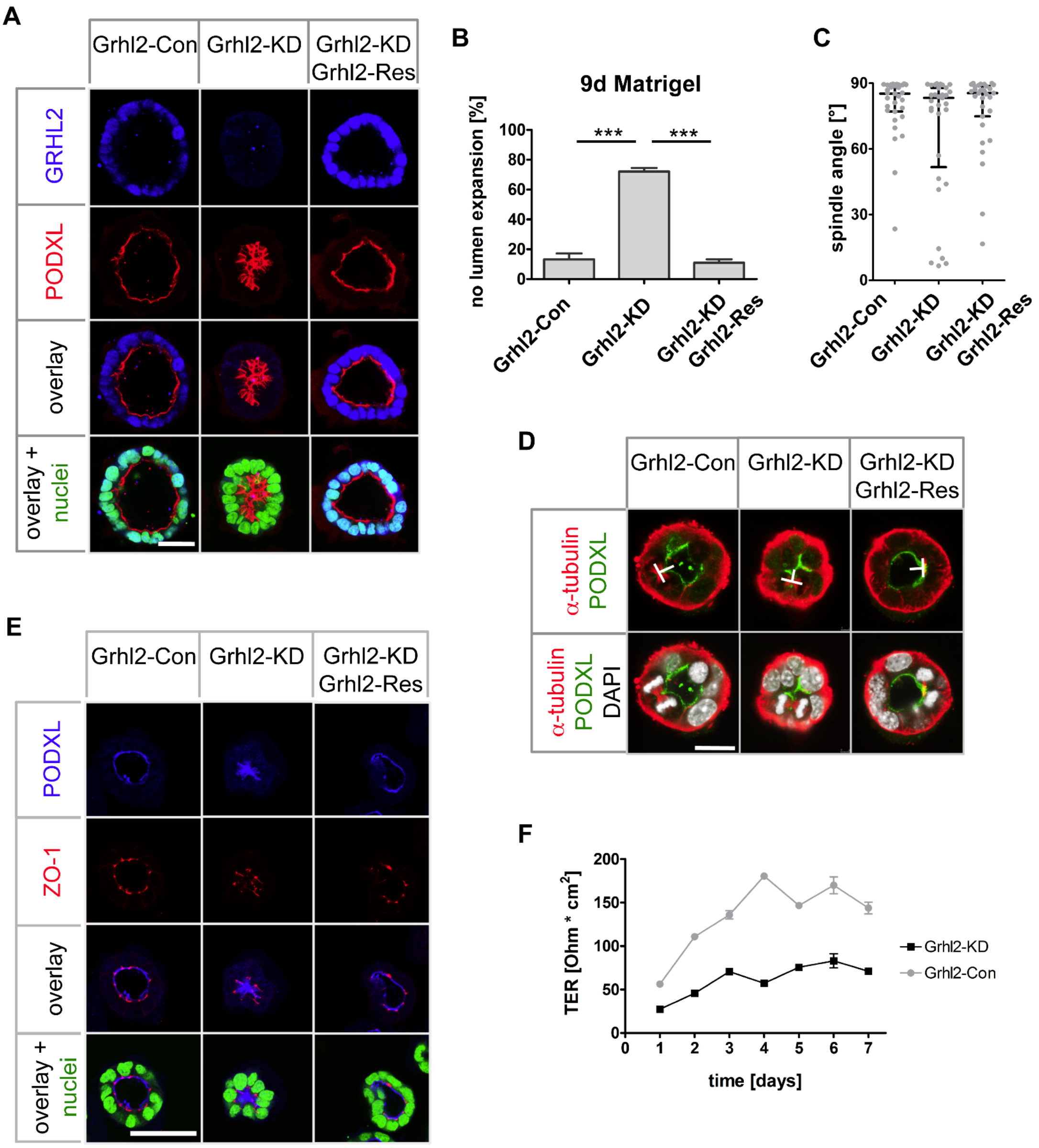
Isolated cells were expanded and used for further investigations. Expression of Grhl2 and known targets Cdh1, Cldn4, and Rab25 was confirmed in IMCD-3 Grhl2-KD, control cells (Grhl2-Con), and Grhl2-KD;Grhl2-Res cells using quantitative RT-PCR **C** and Western blots **D**. **E** Shown is a quantification of GRHL2 expression from Western blots (mean ± SD, ***p<0.001, **p<0.01, *p<0.05).



Supplemental Figure 3

Grhl2 ChIP tracks at selected core targets and EMT genes.

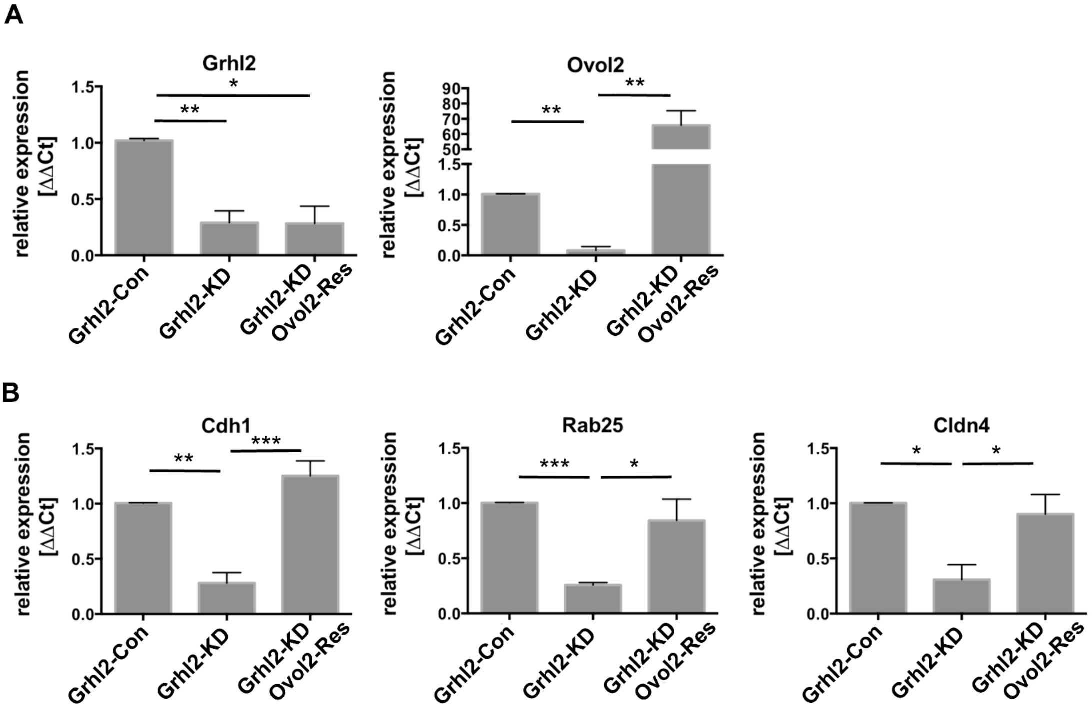
IGV snapshots of raw read count tracks from Grhl2 ChIP-seq experiments for murine E18, adult kidney, and IMCD-3 cells. The plots on the left side show 3 genes of the Grhl2 core target module (Ovol2, Cdh1, Rab25). On the right, raw read count tracks for Vim, Twist2, and Zeb1 are displayed with read count signal at noise level even at long distance around the respective gene. All tracks are scaled to the peak height of the Ovol2 tracks.



Supplemental Figure 4

Characterization of IMCD-3 Grhl2-KD cysts at several time points.

A Culture period of Grhl2-Con, Grhl2-KD, and Grhl2-KD;Grhl2-Res was prolonged to nine days in Matrigel. Cysts were immunofluorescence-stained with antibodies against GRHL2 and podocalyxin (PODXL). Nuclei were counterstained using SYTOXGreen; scale bar: 25 µm. **B** Still most of Grhl2-KD cysts did not form open lumen cysts compared to Grhl2-Con and Grhl2-KD;Grhl2-Res (mean ± SEM). **C, D** Grhl2-Con, Grhl2-KD, and Grhl2-KD; Grhl2-Res cells were grown in Matrigel for four days. Cells were immunofluorescence-stained using antibodies against α-tubulin and PODXL. Nuclei were counterstained using DAPI. **C** Orientation of the mitotic spindle towards the lumen, for Grhl2-Con and Grhl2-KD-Res cysts, or the podocalyxin-marked center of the whole cyst, for Grhl2-KD cysts, were analyzed (median and IQR). **D** Representative mitotic spindles in Grhl2-Con, Grhl2-KD and Grhl2-KD, Grhl2-Res cysts are shown; scale bar: 15 µm. **E** Grhl2-Con, Grhl2-KD, and Grhl2-KD;Grhl2-Res cells were grown in Matrigel for five days and immunofluorescence-stained using antibodies against PODXL and tight junction protein zonula occludens-1 (ZO-1). Staining revealed no overt abnormalities in ZO-1 localization in Grhl2-KD cysts, compared to Grhl2-Con and Grhl2-KD;Grhl2-Res cysts; scale bar: 38 µm. **F** Grhl2-Con and Grhl2-KD cells were grown on polycarbonate membranes. Measurements of transepithelial resistances (TER) are shown for seven days (mean ± SEM).



Supplemental Figure 5

Validation of IMCD-3 Grhl2-KD;Ovol2-Res cells.

A, B IMCD-3 cells were infected with lentiviral particles to integrate expression cassettes for a Grhl2 shRNA, or control shRNA, and for the marker gene GFP. At the same time, cells were transduced to integrate a cassette for synthesis of full-length cDNA of Ovol2A (Grhl2-KD; Ovol2-Res), or Luciferase control gene, followed by mCherry. GFP- and mCherry-expressing cells were isolated using FACS. **A** Expression of Grhl2 and Ovol2 were confirmed in Grhl2-Con, Grhl2-KD, and Grhl2-KD;Ovol2 using quantitative RT-PCR. **B** Shown are expression data for Cdh1, Rab25, and Cldn4 in Grhl2-Con, Grhl2-KD, and Grhl2-KD;Ovol2-Res using quantitative RT-PCR (mean ±SD, ***p<0.001, **p<0.01, *p<0.05).

Supplemental Table 1

Differentially expressed genes between Grhl2-Con and Grhl2-KD.

List of differentially expressed genes with expression levels in Grhl2-Con, Grhl2-KD, and the rescue constructs for Grhl2 and Ovol2.

	Averages				Fold changes				Rescue success in	
Gene name (Illumina)	Grhl2-Con	Grhl2-KD	Grhl2-Res	Ovol2-Res	Con/KD	Ovol2-Res/ Con	Grhl2-Res/ Con	ttest Con vs. KD	Grhl2-Res	Ovol2-Res
Atp8b1	596.64	244.42	586.35	225.71	2.44	0.38	0.98	0.0001	1	0
Calm3	9050.18	17566.95	17994.21	16783.07	0.52	1.85	1.99	0.0002	0	0
2310040B03Rik	562.49	898.48	540.07	712.44	0.63	1.27	0.96	0.0002	1	1
Ovol2	258.80	179.12	302.73	385.12	1.44	1.49	1.17	0.0002	1	1
Il28ra	845.52	588.41	1002.23	1101.72	1.44	1.30	1.19	0.0003	1	1
Atg9b	4526.00	1231.47	4313.38	6713.05	3.68	1.48	0.95	0.0003	1	1
LOC100046254	285.01	178.00	233.31	200.42	1.60	0.70	0.82	0.0004	1	0
Ap1m2	1015.24	312.64	967.75	1595.49	3.25	1.57	0.95	0.0004	1	1
Tmem54	3272.00	476.29	4298.71	487.02	6.87	0.15	1.31	0.0005	1	0
EG432448	6816.56	13124.74	11778.28	11879.01	0.52	1.74	1.73	0.0005	0	0
Prss8	1277.87	740.15	1045.18	1049.30	1.73	0.82	0.82	0.0005	1	1
Meis2	2043.83	2910.19	1827.38	1967.63	0.70	0.96	0.89	0.0006	1	1
Sprr1a	967.41	465.55	321.26	2090.60	2.08	2.16	0.33	0.0006	0	1
Bhlhb2	4033.83	2365.93	4860.47	5796.33	1.70	1.44	1.20	0.0006	1	1
Wisp2	159.86	241.61	238.43	155.26	0.66	0.97	1.49	0.0007	1	1
LOC100046802	282.11	598.72	187.63	633.44	0.47	2.25	0.67	0.0008	1	0
Eno3	500.11	881.53	352.73	1201.21	0.57	2.40	0.71	0.0008	1	0
Galnt3	232.26	158.01	224.47	290.53	1.47	1.25	0.97	0.0008	1	1
LOC100046211	5101.68	8774.14	6738.59	8947.54	0.58	1.75	1.32	0.0010	1	0
LOC214575	235.98	161.40	277.71	199.46	1.46	0.85	1.18	0.0010	1	1
Slc44a2	3517.47	2498.94	2938.12	2293.49	1.41	0.65	0.84	0.0010	1	0
Npal2	2019.51	769.11	1500.12	1417.14	2.63	0.70	0.74	0.0011	1	1
LOC100047167	1249.91	2194.93	1482.30	2136.44	0.57	1.71	1.19	0.0012	1	0
Dkk3	822.68	1510.65	706.57	665.69	0.54	0.81	0.86	0.0012	1	1
Ocrl	1017.34	687.22	937.51	748.83	1.48	0.74	0.92	0.0012	1	0
Marveld1	427.63	754.20	359.14	273.95	0.57	0.64	0.84	0.0012	1	1
Sc4mol	11040.51	7306.50	7110.08	6910.16	1.51	0.63	0.64	0.0013	1	0
Pi4k2b	1904.97	2769.45	1798.75	2185.47	0.69	1.15	0.94	0.0013	1	1
Ccdc68	700.59	281.21	338.72	508.67	2.49	0.73	0.48	0.0013	0	1
Syt8	426.54	184.71	214.19	242.04	2.31	0.57	0.50	0.0013	0	0
Gpbar1	190.71	559.89	168.89	322.36	0.34	1.69	0.89	0.0014	1	0
9930039L23Rik	4195.62	2144.35	3092.12	2745.79	1.96	0.65	0.74	0.0014	1	0
Mal	4346.98	1448.58	3173.67	3903.48	3.00	0.90	0.73	0.0015	1	1
Otud7b	570.81	398.92	504.76	510.43	1.43	0.89	0.88	0.0016	1	1
Epha1	548.09	261.79	701.32	488.67	2.09	0.89	1.28	0.0016	1	1
Stard10	2780.30	1382.27	3934.59	1372.92	2.01	0.49	1.42	0.0016	1	0
Inadl	404.91	279.45	453.05	410.20	1.45	1.01	1.12	0.0017	1	1
Ptpla	394.19	653.10	322.44	318.36	0.60	0.81	0.82	0.0017	1	1
Il17re	573.30	182.59	1346.34	151.87	3.14	0.26	2.35	0.0018	1	0
B3gnt3	567.92	331.33	444.73	635.51	1.71	1.12	0.78	0.0018	1	1
Phldb1	555.14	833.17	443.43	547.29	0.67	0.99	0.80	0.0019	1	1
Pcyt1b	1023.66	651.25	725.16	891.54	1.57	0.87	0.71	0.0021	0	1
Eps8l1	289.24	190.36	361.10	399.94	1.52	1.38	1.25	0.0022	1	1

	Averages				Fold changes				Rescue success in	
Gene name (Illumina)	Grhl2-Con	Grhl2-KD	Grhl2-Res	Ovol2-Res	Con/KD	Ovol2-Res/ Con	Grhl2-Res/ Con	ttest Con vs. KD	Grhl2-Res	Ovol2-Res
Rasef	230.72	157.31	292.30	158.48	1.47	0.69	1.27	0.0023	1	0
Usp43	404.42	271.88	443.56	440.57	1.49	1.09	1.10	0.0023	1	1
Cpe	9908.38	16885.12	6390.62	5632.92	0.59	0.57	0.64	0.0023	1	1
Zscan21	1264.11	1781.93	417.25	841.92	0.71	0.67	0.33	0.0023	1	1
2010004A03Rik	788.32	541.24	466.08	876.73	1.46	1.11	0.59	0.0024	1	1
Pkia	3440.06	5614.56	3303.00	3032.40	0.61	0.88	0.96	0.0025	1	1
Thbs3	286.07	427.83	435.12	377.53	0.67	1.32	1.52	0.0025	1	1
Myo9a	419.37	632.50	432.58	312.26	0.66	0.74	1.03	0.0025	1	1
scl0003547.1_6	339.60	561.07	298.56	283.26	0.61	0.83	0.88	0.0026	1	1
Rrad	653.75	974.58	460.03	985.36	0.67	1.51	0.70	0.0026	1	1
Cdh6	3737.95	6336.54	4940.14	3722.85	0.59	1.00	1.32	0.0027	1	1
Cldn4	27144.28	9168.79	27597.50	30441.99	2.96	1.12	1.02	0.0028	1	1
AI427122	1160.55	571.63	780.03	1134.80	2.03	0.98	0.67	0.0029	0	1
Batf3	182.82	268.33	205.33	206.49	0.68	1.13	1.12	0.0029	1	1
Cxcl16	2034.03	726.45	2609.12	2240.99	2.80	1.10	1.28	0.0029	1	1
Anapc5	9000.87	13215.64	10385.50	13060.71	0.68	1.45	1.15	0.0031	1	0
Marveld3	1183.92	622.90	1119.24	883.91	1.90	0.75	0.95	0.0031	1	1
Mobk1b	1656.47	1092.48	1308.97	1024.31	1.52	0.62	0.79	0.0032	0	0
Zeb1	343.33	1340.83	214.35	294.80	0.26	0.86	0.62	0.0032	1	1
Tnk1	1930.21	1052.81	2571.52	2947.82	1.83	1.53	1.33	0.0033	1	1
Tspan18	230.51	409.81	150.32	429.74	0.56	1.86	0.65	0.0034	1	0
Itgb4	4544.50	2472.02	5434.25	7267.41	1.84	1.60	1.20	0.0034	1	1
3110043J09Rik	548.60	373.42	521.41	486.33	1.47	0.89	0.95	0.0035	1	1
Hnt	496.51	710.05	332.28	280.48	0.70	0.56	0.67	0.0036	1	1
Cdh1	1354.63	515.19	1190.31	1637.61	2.63	1.21	0.88	0.0037	1	1
Stx2	395.34	627.37	319.75	379.97	0.63	0.96	0.81	0.0037	1	1
Sult2b1	537.83	374.16	1112.66	987.47	1.44	1.84	2.07	0.0038	1	1
Mvd	2754.42	1802.94	2016.96	1817.13	1.53	0.66	0.73	0.0038	0	0
Mapk13	2036.04	1033.38	4640.18	2696.44	1.97	1.32	2.28	0.0041	1	1
1700012H17Rik	1361.16	843.95	1287.04	558.86	1.61	0.41	0.95	0.0041	1	0
St8sia2	196.98	286.13	167.21	135.57	0.69	0.69	0.85	0.0042	1	1
Mtap7	1064.54	566.15	1133.79	1423.52	1.88	1.34	1.07	0.0042	1	1
A430088H15Rik	2610.05	1541.27	1939.54	1679.72	1.69	0.64	0.74	0.0043	1	1
Flrt3	552.09	293.56	316.37	297.44	1.88	0.54	0.57	0.0044	0	0
Cldn23	551.40	267.41	1387.03	636.26	2.06	1.15	2.52	0.0044	1	1
Bahcc1	1408.25	2068.36	1792.27	2184.47	0.68	1.55	1.27	0.0045	1	0
Kif21a	459.52	319.74	443.89	305.92	1.44	0.67	0.97	0.0045	1	0
Ehd2	193.09	274.07	149.48	238.41	0.70	1.23	0.77	0.0045	1	1
LOC100048721	398.12	229.50	232.32	227.36	1.73	0.57	0.58	0.0046	0	0
Slco4a1	2139.00	1461.05	1856.76	1973.61	1.46	0.92	0.87	0.0046	1	1
Gmfg	304.87	206.58	317.38	372.32	1.48	1.22	1.04	0.0049	1	1
Smox	1897.36	1073.07	2302.64	2528.74	1.77	1.33	1.21	0.0050	1	1
Gper	320.34	978.00	259.84	535.30	0.33	1.67	0.81	0.0050	1	1
Nfix	666.31	1482.63	836.19	1023.91	0.45	1.54	1.25	0.0051	1	1
Fut10	738.94	1101.89	687.15	398.69	0.67	0.54	0.93	0.0052	1	1
Foxc1	3368.75	5012.78	3653.60	3048.94	0.67	0.91	1.08	0.0053	1	1
St14	1679.98	712.99	1789.77	1754.53	2.36	1.04	1.07	0.0054	1	1

	Averages				Fold changes				Rescue success in	
Gene name (Illumina)	**Grhl2-Con**	**Grhl2-KD**	**Grhl2-Res**	**Ovol2-Res**	**Con/KD**	**Ovol2-Res/ Con**	**Grhl2-Res/ Con**	**ttest Con vs. KD**	**Grhl2-Res**	**Ovol2-Res**
LOC100040736	146.62	208.25	153.93	175.37	0.70	1.20	1.05	0.0054	1	1
Gsto1	23254.76	9391.20	15580.07	23633.54	2.48	1.02	0.67	0.0054	0	1
6430573F11Rik	513.11	823.49	533.84	328.08	0.62	0.64	1.04	0.0055	1	1
Nr2c1	503.45	345.85	365.95	359.45	1.46	0.71	0.73	0.0055	0	0
Pkd2	2167.22	3271.71	1470.09	1973.43	0.66	0.91	0.68	0.0057	1	1
Aacs	4020.60	5630.80	5926.12	4751.25	0.71	1.18	1.47	0.0058	0	1
Fmnl2	1452.34	2105.23	601.31	851.44	0.69	0.59	0.41	0.0058	1	1
H2-K1	556.98	395.41	706.21	706.44	1.41	1.27	1.27	0.0061	1	1
Car12	711.76	1329.96	614.44	478.84	0.54	0.67	0.86	0.0061	1	1
Ank	1219.68	2825.28	1186.97	1671.80	0.43	1.37	0.97	0.0062	1	1
Slc4a11	385.33	220.53	334.08	511.03	1.75	1.33	0.87	0.0063	1	1
Fam110b	1138.50	696.90	1043.03	483.96	1.63	0.43	0.92	0.0064	1	0
LOC100044622	369.56	521.14	400.15	251.52	0.71	0.68	1.08	0.0065	1	1
Trp53i11	330.84	575.72	349.01	647.42	0.57	1.96	1.05	0.0065	1	0
9530095P18Rik	808.27	425.90	632.19	607.53	1.90	0.75	0.78	0.0066	1	1
Ctla2b	191.41	131.61	128.54	167.32	1.45	0.87	0.67	0.0067	0	1
BC046418	477.99	322.42	436.77	497.65	1.48	1.04	0.91	0.0067	1	1
Ankrd56	2807.70	1906.27	3351.17	3022.00	1.47	1.08	1.19	0.0072	1	1
Slc29a1	648.06	1007.04	752.45	955.72	0.64	1.47	1.16	0.0076	1	1
Ppp1r14d	221.13	156.74	211.63	208.43	1.41	0.94	0.96	0.0078	1	1
A730040I05Rik	365.09	235.57	258.29	395.57	1.55	1.08	0.71	0.0079	0	1
Dclk2	387.38	684.60	305.44	268.99	0.57	0.69	0.79	0.0079	1	1
4732474O15Rik	1166.75	418.94	1025.16	714.23	2.78	0.61	0.88	0.0084	1	0
Rpl18a	13054.97	8693.33	14155.53	12170.41	1.50	0.93	1.08	0.0084	1	1
1700047I17Rik1	1711.35	1207.20	2179.28	1599.96	1.42	0.93	1.27	0.0084	1	1
9530027K23Rik	1776.84	904.25	1916.29	1161.14	1.96	0.65	1.08	0.0085	1	0
Anxa1	1946.66	882.37	1418.48	1185.35	2.21	0.61	0.73	0.0085	1	0
Rgl1	449.09	668.07	405.17	538.99	0.67	1.20	0.90	0.0085	1	1
Cldn7	1280.72	650.90	1346.79	1819.74	1.97	1.42	1.05	0.0086	1	1
Krt7	14541.59	5269.03	8977.73	16283.77	2.76	1.12	0.62	0.0086	0	1
Kntc1	2772.97	3923.74	3305.99	3712.30	0.71	1.34	1.19	0.0087	1	0
OTTMUSG00000015762	338.80	206.41	248.83	380.40	1.64	1.12	0.73	0.0087	1	1
Stat1	663.54	456.30	747.46	438.98	1.45	0.66	1.13	0.0088	1	0
Bspry	810.78	347.77	965.53	748.49	2.33	0.92	1.19	0.0088	1	1
Mpp2	194.65	288.46	143.48	159.89	0.67	0.82	0.74	0.0090	1	1
Rcan1	422.62	911.91	656.28	268.80	0.46	0.64	1.55	0.0091	1	1
Ildr1	285.38	183.43	276.33	292.12	1.56	1.02	0.97	0.0091	1	1
Rbm35a	7197.20	3190.41	7469.76	7954.26	2.26	1.11	1.04	0.0092	1	1
Nt5dc2	3876.46	7943.51	4585.02	4998.34	0.49	1.29	1.18	0.0095	1	1
Mpzl3	240.11	132.83	227.65	162.42	1.81	0.68	0.95	0.0097	1	0
Hr	512.99	758.04	2686.92	1915.88	0.68	3.73	5.24	0.0098	0	0
Sgpp2	498.43	312.60	203.98	739.11	1.59	1.48	0.41	0.0098	0	1
3110050N22Rik	2042.55	2869.51	1541.58	1838.36	0.71	0.90	0.75	0.0101	1	1
Cgn	860.91	451.12	633.11	852.97	1.91	0.99	0.74	0.0101	1	1
Sox12	954.35	1431.35	902.58	641.14	0.67	0.67	0.95	0.0103	1	1
Fam110c	1154.08	644.89	1020.14	1059.51	1.79	0.92	0.88	0.0105	1	1
Casp7	1181.16	1725.75	1154.15	1152.15	0.68	0.98	0.98	0.0106	1	1

	Averages				Fold changes				Rescue success in	
Gene name (Illumina)	Grhl2-Con	Grhl2-KD	Grhl2-Res	Ovol2-Res	Con/KD	Ovol2-Res/ Con	Grhl2-Res/ Con	ttest Con vs. KD	Grhl2-Res	Ovol2-Res
Krt19	6846.32	3086.42	5865.22	10099.60	2.22	1.48	0.86	0.0108	1	1
Lpin3	448.39	309.85	437.76	392.53	1.45	0.88	0.98	0.0108	1	1
Cda	511.55	301.82	408.36	449.99	1.69	0.88	0.80	0.0109	1	1
EG635702	234.96	162.65	224.52	138.87	1.44	0.59	0.96	0.0110	1	0
Rab25	7624.17	1739.95	14198.88	6976.77	4.38	0.92	1.86	0.0110	1	1
Mcam	708.62	1157.16	388.85	558.35	0.61	0.79	0.55	0.0111	1	1
D17H6S56E-5	804.21	515.90	825.44	669.46	1.56	0.83	1.03	0.0112	1	1
Ppbp	7756.87	3373.04	7125.99	5290.39	2.30	0.68	0.92	0.0113	1	1
Wnt2	169.82	389.45	151.43	165.07	0.44	0.97	0.89	0.0113	1	1
9830001H06Rik	540.76	971.76	238.45	423.77	0.56	0.78	0.44	0.0113	1	1
Chrnb1	5625.06	3532.76	5389.02	3639.63	1.59	0.65	0.96	0.0114	1	0
Atp2a1	268.07	182.51	234.33	527.22	1.47	1.97	0.87	0.0114	1	1
Arg1	235.66	162.07	767.98	171.08	1.45	0.73	3.26	0.0114	1	0
Gfm1	1728.48	1182.99	1379.82	1500.81	1.46	0.87	0.80	0.0115	0	1
Fbxo30	336.85	527.56	359.06	445.24	0.64	1.32	1.07	0.0115	1	0
Efemp2	3484.96	5485.39	3243.45	4073.13	0.64	1.17	0.93	0.0115	1	1
Stxbp1	727.98	1148.20	1693.08	428.65	0.63	0.59	2.33	0.0116	0	1
Wipf1	380.24	753.46	203.73	465.26	0.50	1.22	0.54	0.0119	1	1
Sfn	813.02	393.14	1137.71	1019.45	2.07	1.25	1.40	0.0120	1	1
Ttyh3	1754.95	3178.24	1127.70	1872.42	0.55	1.07	0.64	0.0120	1	1
AW548124	265.15	547.77	174.41	253.84	0.48	0.96	0.66	0.0120	1	1
8430415N23Rik	580.44	1332.95	1519.38	1083.15	0.44	1.87	2.62	0.0120	0	0
Maml1	330.28	499.07	376.26	404.97	0.66	1.23	1.14	0.0120	1	1
Tmem38b	2175.49	1541.80	1882.36	1559.23	1.41	0.72	0.87	0.0122	1	0
Msln	1046.06	423.53	742.98	1024.77	2.47	0.98	0.71	0.0125	1	1
6330578E17Rik	1597.45	2507.57	2687.42	2851.25	0.64	1.78	1.68	0.0125	0	0
scl0002694.1_23	350.25	204.18	323.37	357.34	1.72	1.02	0.92	0.0129	1	1
6330549D23Rik	289.87	500.96	197.67	209.36	0.58	0.72	0.68	0.0131	1	1
LOC383576	921.37	537.53	1002.49	649.04	1.71	0.70	1.09	0.0131	1	0
9930023K05Rik	923.04	231.48	541.46	1925.81	3.99	2.09	0.59	0.0131	0	1
Ccng1	2870.86	2042.54	3480.84	2699.94	1.41	0.94	1.21	0.0133	1	1
Ly6a	5516.55	1838.57	1687.62	8257.38	3.00	1.50	0.31	0.0133	0	1
4632417N05Rik	597.03	345.33	1171.98	263.19	1.73	0.44	1.96	0.0133	1	0
Klk8	1087.94	322.95	1047.39	2647.99	3.37	2.43	0.96	0.0134	1	1
Nacc2	1498.18	3546.54	855.73	2078.40	0.42	1.39	0.57	0.0135	1	1
Krt18	15048.22	7231.22	17235.49	16752.19	2.08	1.11	1.15	0.0138	1	1
Twist2	1714.36	4515.89	820.99	1473.57	0.38	0.86	0.48	0.0139	1	1
Tmem184a	1355.37	702.33	1841.21	1586.71	1.93	1.17	1.36	0.0140	1	1
Krt20	906.16	327.08	476.00	2518.75	2.77	2.78	0.53	0.0140	1	1
Fzd2	1084.02	1611.16	822.57	1030.47	0.67	0.95	0.76	0.0140	1	1
C920027I18Rik	1168.08	719.22	619.90	909.99	1.62	0.78	0.53	0.0141	0	0
Ube2e3	1320.44	1902.96	1114.20	1320.47	0.69	1.00	0.84	0.0141	1	1
Agl	596.58	888.50	715.98	544.11	0.67	0.91	1.20	0.0142	1	1
D11Bwg0414e	9131.16	13346.19	10854.49	9371.49	0.68	1.03	1.19	0.0144	1	1
Ctsh	1675.07	701.31	1487.47	1663.13	2.39	0.99	0.89	0.0146	1	1
Prkch	283.95	166.85	359.85	242.22	1.70	0.85	1.27	0.0147	1	1
Cep170	1586.57	2301.57	834.72	932.67	0.69	0.59	0.53	0.0148	1	1

	Averages				Fold changes				Rescue success in	
Gene name (Illumina)	Grhl2-Con	Grhl2-KD	Grhl2-Res	Ovol2-Res	Con/KD	Ovol2-Res/ Con	Grhl2-Res/ Con	ttest Con vs. KD	Grhl2-Res	Ovol2-Res
2310007B03Rik	1966.73	307.45	1724.94	2361.37	6.40	1.20	0.88	0.0148	1	1
Tgfbr1	811.67	1482.95	1172.55	844.37	0.55	1.04	1.44	0.0151	1	1
Tmem40	826.55	228.12	1022.14	353.44	3.62	0.43	1.24	0.0152	1	0
LOC100045981	2143.92	3407.51	3123.91	4094.27	0.63	1.91	1.46	0.0155	1	0
Centd1	1181.36	610.49	1389.89	772.64	1.94	0.65	1.18	0.0155	1	0
Pou6f1	1367.29	2314.23	510.72	1043.18	0.59	0.76	0.37	0.0161	1	1
Kctd15	1023.42	1787.73	1085.50	1019.52	0.57	1.00	1.06	0.0162	1	1
Vgll3	466.29	204.08	368.32	334.51	2.28	0.72	0.79	0.0164	1	1
D130071O13Rik	396.65	584.48	223.09	312.97	0.68	0.79	0.56	0.0166	1	1
Bnc2	189.65	270.54	236.13	162.38	0.70	0.86	1.25	0.0167	1	1
Smarcd3	285.71	810.69	218.19	421.78	0.35	1.48	0.76	0.0169	1	0
Sept5	707.15	1108.52	561.63	989.85	0.64	1.40	0.79	0.0169	1	0
Ccbl1	1393.60	912.26	2440.33	1000.53	1.53	0.72	1.75	0.0172	1	0
Fosl2	1251.35	1975.83	1187.17	1549.13	0.63	1.24	0.95	0.0172	1	1
Htr5b	229.76	145.63	500.23	119.43	1.58	0.52	2.18	0.0173	1	0
Samd14	1832.30	2720.44	1726.71	1766.89	0.67	0.96	0.94	0.0174	1	1
Wnt7a	2319.23	668.78	1829.66	4527.93	3.47	1.95	0.79	0.0175	1	1
EG629383	989.51	1680.71	1906.11	1894.07	0.59	1.91	1.93	0.0176	0	0
Sparc	2321.54	4608.30	602.03	2437.60	0.50	1.05	0.26	0.0177	1	1
Ppp1r9b	748.08	1219.26	986.87	795.13	0.61	1.06	1.32	0.0177	1	1
Ctsa	344.64	504.22	331.06	314.90	0.68	0.91	0.96	0.0177	1	1
Zkscan1	521.96	731.94	553.62	573.73	0.71	1.10	1.06	0.0180	1	1
Gm22	366.21	603.67	626.28	791.15	0.61	2.16	1.71	0.0181	1	0
Tmie	439.81	282.76	532.07	482.08	1.56	1.10	1.21	0.0182	1	1
Dlx1	191.07	342.82	156.33	178.13	0.56	0.93	0.82	0.0184	1	1
Met	591.39	956.12	685.27	704.25	0.62	1.19	1.16	0.0185	1	1
Ripk4	6244.91	3818.70	5820.57	6463.02	1.64	1.03	0.93	0.0186	1	1
Tgfb1i1	225.83	341.22	219.18	396.90	0.66	1.76	0.97	0.0187	1	0
Tnc	261.11	494.71	1046.94	242.67	0.53	0.93	4.01	0.0189	0	1
Casp14	1859.67	840.16	318.09	2095.38	2.21	1.13	0.17	0.0192	0	1
4933428A15Rik	2742.81	1235.04	2454.46	928.70	2.22	0.34	0.89	0.0194	1	0
Dennd2a	2154.00	4814.95	1146.30	2816.45	0.45	1.31	0.53	0.0194	1	1
Abi2	1643.30	2572.67	2299.50	1450.12	0.64	0.88	1.40	0.0196	0	1
2300008H03Rik	319.65	220.45	252.22	234.33	1.45	0.73	0.79	0.0197	1	0
Fgf13	462.99	227.94	455.50	258.61	2.03	0.56	0.98	0.0197	1	0
Leprel1	397.12	691.22	253.02	286.71	0.57	0.72	0.64	0.0199	1	1
Rasl11b	12934.72	19759.72	9767.98	12571.04	0.65	0.97	0.76	0.0203	1	1
H2-Bl	182.64	124.52	284.22	199.25	1.47	1.09	1.56	0.0203	1	1
Mcf2l	209.13	313.32	216.09	232.69	0.67	1.11	1.03	0.0206	1	1
Serpinb6b	190.11	134.71	162.17	154.48	1.41	0.81	0.85	0.0212	1	1
Cttnbp2nl	3189.12	2209.78	3362.29	5675.13	1.44	1.78	1.05	0.0214	1	1
4933428D01Rik	197.26	139.35	159.96	143.59	1.42	0.73	0.81	0.0214	1	0
Serpina3h	397.46	1797.36	400.87	420.16	0.22	1.06	1.01	0.0215	1	1
Mslnl	254.09	156.41	414.18	421.85	1.62	1.66	1.63	0.0216	1	1
6330408J11Rik	511.67	326.79	545.99	584.53	1.57	1.14	1.07	0.0217	1	1
Pemt	539.55	379.19	580.20	397.21	1.42	0.74	1.08	0.0218	1	0
1600002K03Rik	2433.90	1669.39	2419.73	2311.54	1.46	0.95	0.99	0.0218	1	1

	Averages				Fold changes				Rescue success in	
Gene name (Illumina)	Grhl2-Con	Grhl2-KD	Grhl2-Res	Ovol2-Res	Con/KD	Ovol2-Res/ Con	Grhl2-Res/ Con	ttest Con vs. KD	Grhl2-Res	Ovol2-Res
Upk3b	500.98	314.05	2750.03	401.62	1.60	0.80	5.49	0.0219	1	1
Tox	256.95	410.79	207.39	208.00	0.63	0.81	0.81	0.0221	1	1
Scly	716.11	1283.78	806.80	756.22	0.56	1.06	1.13	0.0223	1	1
Depdc1b	726.82	1083.18	957.83	887.57	0.67	1.22	1.32	0.0225	1	1
Grhl2	2023.61	672.93	387.48	1006.21	3.01	0.50	0.19	0.0227	0	0
Mxd3	343.90	674.19	317.66	406.70	0.51	1.18	0.92	0.0227	1	1
LOC100044221	1639.90	1041.22	1865.41	1951.48	1.57	1.19	1.14	0.0227	1	1
Akr1c12	511.36	917.29	379.69	689.48	0.56	1.35	0.74	0.0228	1	1
9130211I03Rik	235.20	427.15	285.14	256.61	0.55	1.09	1.21	0.0229	1	1
1700016K19Rik	267.93	175.21	285.54	296.39	1.53	1.11	1.07	0.0231	1	1
1810019J16Rik	544.23	298.48	588.60	660.23	1.82	1.21	1.08	0.0232	1	1
Btn1a1	289.47	168.32	169.21	374.72	1.72	1.29	0.58	0.0235	0	1
Tmprss2	4776.12	1428.19	3370.01	2362.57	3.34	0.49	0.71	0.0238	1	0
Tmem102	855.06	566.65	1259.43	831.80	1.51	0.97	1.47	0.0238	1	1
Sav1	7734.73	12696.80	8378.33	8067.03	0.61	1.04	1.08	0.0239	1	1
Stxbp5	841.74	538.20	867.86	631.68	1.56	0.75	1.03	0.0239	1	1
Sgms2	640.73	413.67	534.56	849.05	1.55	1.33	0.83	0.0239	1	1
Apbb2	818.43	1160.26	703.69	922.70	0.71	1.13	0.86	0.0240	1	1
Tbc1d2	804.01	342.96	1062.19	867.51	2.34	1.08	1.32	0.0240	1	1
Pmvk	1825.48	1293.48	1572.16	1229.82	1.41	0.67	0.86	0.0241	1	0
A430106B04Rik	374.97	539.60	321.91	539.15	0.69	1.44	0.86	0.0242	1	0
D930046M13Rik	400.30	709.10	287.26	486.67	0.56	1.22	0.72	0.0243	1	1
Apcdd1	150.39	307.40	178.02	156.72	0.49	1.04	1.18	0.0243	1	1
Cdkn1a	4892.51	2405.32	4595.29	5408.69	2.03	1.11	0.94	0.0245	1	1
1700105P06Rik	236.64	352.19	287.70	242.69	0.67	1.03	1.22	0.0245	0	1
Prom2	476.68	169.81	567.14	176.57	2.81	0.37	1.19	0.0249	1	0
Moxd1	301.50	193.77	706.94	491.94	1.56	1.63	2.34	0.0251	1	1
Cic	391.61	631.37	446.68	405.04	0.62	1.03	1.14	0.0254	1	1
Dysf	481.50	739.55	481.89	323.23	0.65	0.67	1.00	0.0256	1	1
2310028O11Rik	250.85	354.97	315.84	298.98	0.71	1.19	1.26	0.0258	1	1
Ramp2	373.61	772.45	274.98	423.97	0.48	1.13	0.74	0.0259	1	1
4632428D17Rik	236.01	388.95	141.46	242.94	0.61	1.03	0.60	0.0259	1	1
EG666756	8374.86	5691.37	6766.79	7390.58	1.47	0.88	0.81	0.0261	1	1
Fam102a	2420.97	1476.93	2848.61	1980.33	1.64	0.82	1.18	0.0261	1	1
Fermt2	2004.72	2980.48	2112.09	2096.07	0.67	1.05	1.05	0.0267	1	1
1500002O10Rik	272.34	435.05	191.12	233.20	0.63	0.86	0.70	0.0269	1	1
Nnmt	845.14	1647.67	234.63	1156.05	0.51	1.37	0.28	0.0270	1	1
Sephs1	224.82	370.43	286.82	321.25	0.61	1.43	1.28	0.0270	0	0
Vim	919.22	3957.28	408.97	1463.84	0.23	1.59	0.44	0.0270	1	1
Rhpn2	685.37	341.07	259.24	857.26	2.01	1.25	0.38	0.0272	0	1
1110012J17Rik	1101.81	2026.12	451.71	692.50	0.54	0.63	0.41	0.0272	1	1
1110018J23Rik	1699.53	3157.95	1301.49	1387.02	0.54	0.82	0.77	0.0273	1	1
Dcbld1	1561.79	898.61	1740.85	1344.43	1.74	0.86	1.11	0.0274	1	1
Pus7l	1946.70	1277.82	2242.30	1869.28	1.52	0.96	1.15	0.0278	1	1
BC004044	377.28	764.12	334.65	381.01	0.49	1.01	0.89	0.0280	1	1
4631426J05Rik	246.41	516.22	212.79	201.89	0.48	0.82	0.86	0.0280	1	1
Npy	181.50	356.70	196.24	155.49	0.51	0.86	1.08	0.0280	1	1

	Averages				Fold changes				Rescue success in	
Gene name (Illumina)	**Grhl2-Con**	**Grhl2-KD**	**Grhl2-Res**	**Ovol2-Res**	**Con/KD**	**Ovol2-Res/ Con**	**Grhl2-Res/ Con**	**ttest Con vs. KD**	**Grhl2-Res**	**Ovol2-Res**
Zdhhc13	3346.01	2340.81	2472.83	2686.93	1.43	0.80	0.74	0.0281	0	1
Ctxn1	1389.75	2479.68	1550.83	2133.74	0.56	1.54	1.12	0.0281	1	1
6430514L14Rik	242.16	363.67	194.84	285.21	0.67	1.18	0.80	0.0282	1	1
Tnni2	527.57	204.56	255.29	301.13	2.58	0.57	0.48	0.0283	0	1
Slc45a3	671.08	477.24	761.03	809.35	1.41	1.21	1.13	0.0284	1	1
Plek2	1258.45	857.27	1694.90	2179.23	1.47	1.73	1.35	0.0285	1	1
Axud1	2286.68	1604.15	2641.86	2619.53	1.43	1.15	1.16	0.0285	1	1
Sgk2	280.15	186.42	219.17	202.94	1.50	0.72	0.78	0.0285	1	0
Tmem62	1427.00	981.45	1073.67	1085.40	1.45	0.76	0.75	0.0286	1	0
Klf5	3920.03	2047.78	4198.52	5677.42	1.91	1.45	1.07	0.0287	1	1
Adi1	3227.26	2289.06	3099.96	2046.32	1.41	0.63	0.96	0.0288	1	0
Rnf144a	599.36	1180.74	325.02	827.59	0.51	1.38	0.54	0.0289	1	1
Bex4	8089.15	4746.78	10412.28	6612.33	1.70	0.82	1.29	0.0290	1	1
G430005B15Rik	809.38	1167.71	437.46	718.11	0.69	0.89	0.54	0.0292	1	1
LOC545481	523.98	236.42	399.35	748.78	2.22	1.43	0.76	0.0293	1	1
3110018K01Rik	205.85	333.42	133.03	195.54	0.62	0.95	0.65	0.0294	1	1
Hebp2	817.86	567.47	1370.58	486.41	1.44	0.59	1.68	0.0294	1	0
LOC670044	995.10	1452.86	1117.95	1205.43	0.68	1.21	1.12	0.0294	1	1
Sim2	6282.45	12179.20	6061.98	5243.90	0.52	0.83	0.96	0.0299	1	1
Zbtb32	214.95	141.08	155.87	298.51	1.52	1.39	0.73	0.0299	0	1
2210011C24Rik	830.91	541.96	427.85	1155.73	1.53	1.39	0.51	0.0301	0	1
Ppif	6250.40	4046.84	7818.45	7113.82	1.54	1.14	1.25	0.0302	1	1
Plau	5915.56	1783.24	2892.10	4596.35	3.32	0.78	0.49	0.0306	1	1
Enc1	3678.34	5346.09	4435.33	3775.26	0.69	1.03	1.21	0.0307	1	1
Pdgfa	6602.75	9350.71	7950.47	6072.48	0.71	0.92	1.20	0.0311	1	1
Tmem79	3309.14	1869.32	6212.54	3096.39	1.77	0.94	1.88	0.0313	1	1
Lama3	1421.67	400.34	1226.41	2987.41	3.55	2.10	0.86	0.0313	1	1
Fkbp5	758.37	1071.89	894.58	641.93	0.71	0.85	1.18	0.0316	1	1
Itpk1	1491.08	2136.05	2161.23	1618.69	0.70	1.09	1.45	0.0319	1	1
Rhob	2290.39	3947.34	1895.14	3470.66	0.58	1.52	0.83	0.0319	1	1
Ivns1abp	3852.91	5721.81	4010.62	3877.99	0.67	1.01	1.04	0.0320	1	1
Grb10	611.85	1124.39	321.92	326.37	0.54	0.53	0.53	0.0321	1	1
Spsb2	555.36	851.43	627.47	581.57	0.65	1.05	1.13	0.0321	1	1
Spag1	462.66	323.31	965.25	436.06	1.43	0.94	2.09	0.0323	1	1
2610528J11Rik	334.17	155.78	325.76	505.09	2.15	1.51	0.97	0.0326	1	1
scl0002540.1_6	416.74	817.88	191.00	377.34	0.51	0.91	0.46	0.0326	1	1
Lhfpl2	2359.54	3787.57	1426.15	2159.25	0.62	0.92	0.60	0.0326	1	1
D10Ertd516e	799.26	1157.94	893.72	854.82	0.69	1.07	1.12	0.0330	1	1
Slc2a1	12256.50	7680.47	12396.61	17975.46	1.60	1.47	1.01	0.0337	1	1
Gipc2	1620.04	1025.61	1518.52	467.98	1.58	0.29	0.94	0.0340	1	0
LOC638301	454.15	264.34	355.61	404.03	1.72	0.89	0.78	0.0341	1	1
2610009E16Rik	3146.91	5630.57	3089.30	3093.67	0.56	0.98	0.98	0.0342	1	1
9130204C11Rik	322.68	480.02	341.17	464.58	0.67	1.44	1.06	0.0343	1	0
LOC100046395	3378.39	2341.54	2619.57	2359.80	1.44	0.70	0.78	0.0344	0	0
5930429A15Rik	276.98	450.29	208.67	369.58	0.62	1.33	0.75	0.0350	1	1
Dscr1	1526.76	2403.64	2086.57	1434.78	0.64	0.94	1.37	0.0350	1	1
Oas1g	475.39	299.44	751.39	456.27	1.59	0.96	1.58	0.0353	1	1

	Averages				Fold changes				Rescue success in	
Gene name (Illumina)	Grhl2-Con	Grhl2-KD	Grhl2-Res	Ovol2-Res	Con/KD	Ovol2-Res/ Con	Grhl2-Res/ Con	ttest Con vs. KD	Grhl2-Res	Ovol2-Res
Tgm1	1074.94	364.15	737.07	2315.24	2.95	2.15	0.69	0.0353	1	1
4930445K14Rik	441.63	658.24	498.27	565.67	0.67	1.28	1.13	0.0354	1	1
Ifit3	5213.28	9967.33	4937.22	4405.54	0.52	0.85	0.95	0.0354	1	1
Ddr1	2063.41	2939.59	1744.97	1951.65	0.70	0.95	0.85	0.0357	1	1
Dpysl2	2214.80	3334.80	1510.31	1804.15	0.66	0.81	0.68	0.0358	1	1
Fgd6	454.12	282.54	450.36	550.65	1.61	1.21	0.99	0.0359	1	1
Pias3	1657.76	2593.21	1468.32	1664.86	0.64	1.00	0.89	0.0361	1	1
Nedd4b	151.18	219.60	168.48	157.05	0.69	1.04	1.11	0.0361	1	1
4833409N03Rik	1800.93	2719.13	2065.25	1595.25	0.66	0.89	1.15	0.0361	1	1
Elfn1	169.82	310.07	287.23	185.24	0.55	1.09	1.69	0.0361	0	1
Ggt7	161.81	267.34	197.86	199.24	0.61	1.23	1.22	0.0363	1	1
2610528B01Rik	336.63	480.99	340.25	353.29	0.70	1.05	1.01	0.0364	1	1
Col16a1	481.10	826.13	564.94	626.59	0.58	1.30	1.17	0.0364	1	1
2310043J07Rik	1388.71	482.71	1609.16	1325.01	2.88	0.95	1.16	0.0366	1	1
Plekho2	371.52	577.34	321.06	409.28	0.64	1.10	0.86	0.0369	1	1
Itga2	199.87	139.75	242.32	239.23	1.43	1.20	1.21	0.0369	1	1
4933436C20Rik	314.51	540.34	267.13	169.57	0.58	0.54	0.85	0.0370	1	1
Smad6	624.42	1026.61	688.87	709.96	0.61	1.14	1.10	0.0371	1	1
Tgfb3	358.83	553.83	428.95	298.97	0.65	0.83	1.20	0.0375	1	1
Ankrd13d	374.06	573.42	886.61	377.83	0.65	1.01	2.37	0.0377	0	1
Tmem159	677.14	432.24	691.94	427.14	1.57	0.63	1.02	0.0378	1	0
Serpina3g	169.49	445.09	145.38	161.06	0.38	0.95	0.86	0.0381	1	1
AI480653	978.19	1423.48	1252.58	1209.51	0.69	1.24	1.28	0.0381	1	1
Sdcbp2	391.18	161.49	382.84	1217.35	2.42	3.11	0.98	0.0381	1	1
C130057N11Rik	583.49	824.52	670.41	670.89	0.71	1.15	1.15	0.0381	1	1
Mb	204.31	144.81	333.20	172.23	1.41	0.84	1.63	0.0382	1	1
Lbh	1397.14	2887.46	868.49	2227.01	0.48	1.59	0.62	0.0383	1	1
Cox7a2l	3208.95	5682.85	6005.51	5362.96	0.56	1.67	1.87	0.0386	0	0
C130073O12Rik	383.46	245.55	411.92	257.60	1.56	0.67	1.07	0.0386	1	1
Col4a3	1555.53	1009.28	1830.66	1042.78	1.54	0.67	1.18	0.0387	1	0
Cysltr1	205.14	129.08	222.59	149.40	1.59	0.73	1.09	0.0388	1	1
Lepre1	208.73	299.71	193.85	178.97	0.70	0.86	0.93	0.0388	1	1
Gata3	211.53	384.27	390.28	241.07	0.55	1.14	1.85	0.0389	1	1
Cdcp1	447.83	291.59	380.24	376.46	1.54	0.84	0.85	0.0389	1	1
Chst7	371.89	257.29	851.67	359.37	1.45	0.97	2.29	0.0390	1	1
1500005K14Rik	1152.89	719.88	1499.50	1099.76	1.60	0.95	1.30	0.0390	1	1
St3gal2	334.03	491.03	278.83	287.40	0.68	0.86	0.83	0.0391	1	1
Ppap2c	14917.20	10191.82	12215.81	12591.07	1.46	0.84	0.82	0.0392	1	1
Pmepa1	514.94	355.81	446.65	427.09	1.45	0.83	0.87	0.0393	1	1
Ddit4	4412.13	1921.04	4665.58	4184.31	2.30	0.95	1.06	0.0394	1	1
Sprr2g	611.67	308.18	381.77	937.99	1.98	1.53	0.62	0.0394	1	1
BC025546	4778.02	3311.96	3609.35	4158.56	1.44	0.87	0.76	0.0395	0	1
Gjb3	4662.03	2083.38	3929.04	4831.35	2.24	1.04	0.84	0.0397	1	1
Ckmt1	237.95	156.58	259.20	359.92	1.52	1.51	1.09	0.0397	1	1
Mcc	363.91	578.94	448.07	292.60	0.63	0.80	1.23	0.0398	1	1
4432405B04Rik	499.37	916.23	530.37	531.11	0.55	1.06	1.06	0.0399	1	1
Ntn4	217.85	318.28	248.77	191.35	0.68	0.88	1.14	0.0400	1	1

	Averages				Fold changes				Rescue success in	
Gene name (Illumina)	Grhl2-Con	Grhl2-KD	Grhl2-Res	Ovol2-Res	Con/KD	Ovol2-Res/ Con	Grhl2-Res/ Con	ttest Con vs. KD	Grhl2-Res	Ovol2-Res
4732458O05Rik	437.47	625.27	441.19	1058.37	0.70	2.42	1.01	0.0400	1	0
Sh3rf2	251.85	156.86	238.94	192.16	1.61	0.76	0.95	0.0402	1	1
Fbxo33	1000.06	713.12	972.64	771.58	1.40	0.77	0.97	0.0404	1	1
Usp12	326.14	480.16	368.00	351.82	0.68	1.08	1.13	0.0405	1	1
Ramp3	1462.19	781.85	1272.85	1756.43	1.87	1.20	0.87	0.0406	1	1
Ankrd47	1510.70	748.16	1277.63	1511.36	2.02	1.00	0.85	0.0409	1	1
Sema5a	961.14	1930.65	699.01	563.87	0.50	0.59	0.73	0.0410	1	1
Trim47	824.30	1278.63	1347.47	1124.21	0.64	1.36	1.63	0.0412	0	1
Cxcl10	774.86	527.15	696.72	1045.00	1.47	1.35	0.90	0.0414	1	1
6430598A04Rik	247.90	349.30	300.35	355.03	0.71	1.43	1.21	0.0414	0	0
2700084L06Rik	1941.54	2970.74	1926.84	2106.73	0.65	1.09	0.99	0.0414	1	1
Palm	871.77	1413.69	747.77	952.47	0.62	1.09	0.86	0.0417	1	1
Gcnt3	304.84	179.64	215.19	192.99	1.70	0.63	0.71	0.0420	1	1
Cdon	456.57	722.60	447.60	386.90	0.63	0.85	0.98	0.0423	1	1
LOC100038882	2020.76	1029.22	3186.11	1704.64	1.96	0.84	1.58	0.0430	1	1
Cacnb3	624.56	911.62	657.71	746.73	0.69	1.20	1.05	0.0432	1	1
Pcolce	1717.19	4416.04	771.30	1990.61	0.39	1.16	0.45	0.0432	1	1
Marcks	3781.91	6033.78	6367.38	3994.79	0.63	1.06	1.68	0.0432	1	1
Col6a1	230.99	828.54	162.82	314.13	0.28	1.36	0.70	0.0436	1	1
Atoh8	184.58	506.81	210.69	150.45	0.36	0.82	1.14	0.0439	1	1
Id3	1495.49	2125.23	1375.94	1525.31	0.70	1.02	0.92	0.0440	1	1
Mpnd	2163.74	3153.35	2248.71	2100.41	0.69	0.97	1.04	0.0440	1	1
Prss22	1787.42	206.48	2601.04	631.79	8.66	0.35	1.46	0.0442	1	1
Krt17	333.32	216.55	636.61	2740.65	1.54	8.22	1.91	0.0442	1	1
2610202E01Rik	517.51	866.94	548.06	555.55	0.60	1.07	1.06	0.0444	1	1
Figf	165.88	365.97	142.91	192.16	0.45	1.16	0.86	0.0445	1	1
Pdlim4	4340.38	6490.67	5648.01	5893.55	0.67	1.36	1.30	0.0447	1	1
Ocln	411.90	263.49	440.68	401.92	1.56	0.98	1.07	0.0450	1	1
Cd40	519.04	344.84	1078.62	741.84	1.51	1.43	2.08	0.0451	1	1
Ece1	963.16	1507.64	1148.27	900.12	0.64	0.93	1.19	0.0455	1	1
9330132O05Rik	586.64	918.98	520.85	708.97	0.64	1.21	0.89	0.0459	1	1
LOC381770	210.73	300.65	201.13	175.69	0.70	0.83	0.95	0.0463	1	1
Tacstd2	17451.86	8508.10	16845.38	14238.21	2.05	0.82	0.97	0.0464	1	1
Trim8	6494.15	11981.84	7499.35	7097.34	0.54	1.09	1.15	0.0467	1	1
S100a14	677.09	295.37	873.37	1477.00	2.29	2.18	1.29	0.0471	1	1
Rab11fip5	5169.74	3314.83	5075.62	8175.23	1.56	1.58	0.98	0.0474	1	1
Apc2	158.24	226.35	167.41	179.36	0.70	1.13	1.06	0.0476	1	1
AI661453	460.60	304.20	485.69	631.81	1.51	1.37	1.05	0.0481	1	1
Ptms	1099.12	1649.92	1156.90	1169.85	0.67	1.06	1.05	0.0483	1	1
LOC236311	315.95	174.45	182.62	333.12	1.81	1.05	0.58	0.0483	1	1
Hmgcr	608.93	386.42	388.79	389.90	1.58	0.64	0.64	0.0486	0	1
Ppl	2239.14	1473.39	1744.24	2002.74	1.52	0.89	0.78	0.0486	1	1
5830411G16Rik	1441.91	2022.66	2189.38	2332.49	0.71	1.62	1.52	0.0488	0	0
Btbd6	2128.73	3222.50	2462.71	2553.08	0.66	1.20	1.16	0.0489	1	1
Nupr1	13745.86	8663.44	8245.42	12653.05	1.59	0.92	0.60	0.0490	0	1
Sox9	3251.60	1562.51	3588.43	5224.90	2.08	1.61	1.10	0.0491	1	1
Myl9	175.05	248.93	233.72	257.03	0.70	1.47	1.34	0.0493	1	0

	Averages				Fold changes				Rescue success in	
Gene name (Illumina)	Grhl2-Con	Grhl2-KD	Grhl2-Res	Ovol2-Res	Con/KD	Ovol2-Res/ Con	Grhl2-Res/ Con	ttest Con vs. KD	Grhl2-Res	Ovol2-Res
Sdc2	1073.55	1974.42	1182.44	742.40	0.54	0.69	1.10	0.0496	1	1
Gm1673	2286.69	3853.44	1388.55	1434.51	0.59	0.63	0.61	0.0496	1	1

Supplemental Table 2

Gene ontology analysis on differentially expressed genes.

Gene ontology analysis for differentially expressed genes between Grhl2-Con and Grhl2-KD using the HOMER software package. GO terms with more than 500 genes or less than 2 genes in term have been excluded from the analysis as well as categories with less than 2 target genes in term.

TermID	Term	p-value	logP	Genes in Term	Target Genes in Term	Gene Symbols
GO:0030855	epithelial cell differentiation	<0.0001	-14.5	391	21	Sfn, Tmem79, Cdh1, Sprr2g, Sprr1a, Tgfb1i1, Ppl, Vim, Gata3, Fzd2, Stx2, Fam20c, Tgm1, Sav1, Id3, Klf5, Krt17, Map7, Sox9, Wnt7a, Anxa1
GO:0032880	regulation of protein localization	<0.0001	-12.4	448	21	Pkia, Sfn, Pkdcc, Cdh1, Tmem184a, Krt20, Tgfb3, Rab11fip5, Gata3, Tgfbr1, Ramp3, Btn1a1, Sema5a, Cd40, Apc2, Gper1, Wipf1, Cdkn1a, Pkd2, Nedd4l, Anxa1
GO:0007507	heart development	<0.0001	-12.3	413	20	Rbm20, Casp7, Ovol2, Mb, Cpe, Foxc1, Maml1, Tgfbr1, Gata3, Fzd2, Smarcd3, Ece1, Grhl2, Sav1, Id3, Smad6, Ramp2, Wnt2, Pkd2, Sox9
GO:0043588	skin development	<0.0001	-12.1	249	15	Sfn, Tmem79, Tgm1, Apcdd1, Pdgfa, Sav1, Itga2, Sprr1a, Sprr2g, Prss8, Krt17, Sox9, Wnt7a, Anxa1, Ppl
GO:0031424	keratinization	<0.0001	-11.5	34	6	Sprr1a, Sprr2g, Sfn, Krt17, Tgm1, Ppl
GO:0030216	keratinocyte differentiation	<0.0001	-11.5	71	8	Sfn, Tgm1, Sav1, Sprr2g, Sprr1a, Krt17, Ppl, Anxa1
GO:2000027	regulation of organ morphogenesis	<0.0001	-11.2	147	11	Fzd2, Plau, Met, Cdh1, Pdgfa, Wnt2, Tacstd2, Sox9, Foxc1, Ntn4, Gata3
GO:0060693	regulation of branching involved in salivary gland morphogenesis	<0.0001	-11.1	11	4	Met, Cdh1, Pdgfa, Ntn4
GO:0070201	regulation of establishment of protein localization	<0.0001	-11.1	375	18	Pkia, Sfn, Pkdcc, Cdh1, Krt20, Tgfb3, Gata3, Tgfbr1, Ramp3, Btn1a1, Sema5a, Cd40, Apc2, Gper1, Wipf1, Cdkn1a, Pkd2, Anxa1
GO:0050678	regulation of epithelial cell proliferation	<0.0001	-11.1	239	14	Arg1, Plau, Cdh1, Sav1, Ovol2, Vash1, Wnt2, Stat1, Sox9, Twist2, Mcc, Wnt7a, Gata3, Tgfbr1
GO:0060688	regulation of morphogenesis of a branching structure	<0.0001	-10.9	56	7	Wnt2, Met, Cdh1, Tacstd2, Sox9, Pdgfa, Ntn4
GO:0072659	protein localization to plasma membrane	<0.0001	-10.9	77	8	Cdh1, Ehd2, Fgf13, Palm, Krt18, Ramp2, Map7, Ramp3
GO:0035295	tube development	<0.0001	-10.8	459	20	Pkdcc, Met, Cdh1, Pdgfa, Ovol2, Tgfb3, Ddr1, Stat1, Ctsh, Foxc1, Gata3, Sparc, Sema5a, Grhl2, Sav1, Zeb1, Sim2, Pkd2, Sox9, Wnt7a
GO:0051272	positive regulation of cellular component movement	<0.0001	-10.6	284	15	Cxcl10, Plau, Cxcl16, Pdgfa, Fam110c, Figf, Gper1, S100a14, Itga2, Epha1, Sox9, Ctsh, Gata3, Tgfbr1, Mcam

TermID	Term	p-value	logP	Genes in Term	Target Genes in Term	Gene Symbols
GO:0032386	regulation of intracellular transport	<0.0001	-10.5	320	16	Pkia, Tmem102, Sfn, Pkdcc, Gsto1, Cdh1, Ehd2, Tgfb3, Tgfbr1, Sema5a, Cxcl10, Apc2, Gper1, Wipf1, Cdkn1a, Pkd2
GO:0030334	regulation of cell migration	<0.0001	-10.4	471	20	Cdh1, Cxcl16, Pdgfa, Fam110c, Figf, Itga2, Epha1, Tacstd2, Vim, Ctsh, Gata3, Mcam, Lama3, Cxcl10, Plau, Gper1, S100a14, Vash1, Sox9, Mcc
GO:2000351	regulation of endothelial cell apoptotic process	<0.0001	-10.0	27	5	Ramp2, Sema5a, Cd40, Gper1, Gata3
GO:0007009	plasma membrane organization	<0.0001	-10.0	112	9	Cdh1, Ehd2, Dysf, Fgf13, Palm, Krt18, Ramp2, Map7, Ramp3
GO:2000145	regulation of cell motility	0.0001	-9.9	490	20	Cdh1, Cxcl16, Pdgfa, Fam110c, Figf, Itga2, Epha1, Tacstd2, Vim, Ctsh, Gata3, Mcam, Lama3, Cxcl10, Plau, Gper1, S100a14, Vash1, Sox9, Mcc
GO:0051223	regulation of protein transport	0.0001	-9.8	339	16	Pkia, Sfn, Pkdcc, Cdh1, Krt20, Tgfb3, Gata3, Tgfbr1, Btn1a1, Sema5a, Cd40, Apc2, Wipf1, Cdkn1a, Pkd2, Anxa1
GO:0003206	cardiac chamber morphogenesis	0.0001	-9.8	115	9	Fzd2, Smarcd3, Grhl2, Sav1, Ovol2, Wnt2, Cpe, Foxc1, Gata3
GO:0030335	positive regulation of cell migration	0.0001	-9.7	271	14	Cxcl10, Plau, Cxcl16, Pdgfa, Fam110c, Figf, Gper1, S100a14, Itga2, Epha1, Sox9, Ctsh, Gata3, Mcam
GO:0031344	regulation of cell projection organization	0.0001	-9.6	346	16	Met, Enc1, Klk8, Fam110c, Itga2, Tgfb3, Tacstd2, Vim, Sdc2, Gata3, Tgfbr1, Ntm, Fgf13, Palm, Klf5, Wnt7a
GO:2000147	positive regulation of cell motility	0.0001	-9.5	276	14	Cxcl10, Plau, Cxcl16, Pdgfa, Fam110c, Figf, Gper1, S100a14, Itga2, Epha1, Sox9, Ctsh, Gata3, Mcam
GO:0008544	epidermis development	0.0001	-9.4	211	12	Sfn, Tgm1, Apcdd1, Pdgfa, Sav1, Sprr2g, Sprr1a, Prss8, Krt17, Sox9, Anxa1, Ppl
GO:0040017	positive regulation of locomotion	0.0001	-9.1	288	14	Cxcl10, Plau, Cxcl16, Pdgfa, Fam110c, Figf, Gper1, S100a14, Itga2, Epha1, Sox9, Ctsh, Gata3, Mcam
GO:0033157	regulation of intracellular protein transport	0.0001	-8.9	189	11	Pkia, Sfn, Pkdcc, Sema5a, Cdh1, Apc2, Wipf1, Cdkn1a, Tgfb3, Pkd2, Tgfbr1
GO:0050680	negative regulation of epithelial cell proliferation	0.0001	-8.8	102	8	Cdh1, Sav1, Vash1, Stat1, Sox9, Mcc, Tgfbr1, Gata3
GO:0003205	cardiac chamber development	0.0002	-8.7	132	9	Fzd2, Smarcd3, Grhl2, Sav1, Ovol2, Wnt2, Cpe, Foxc1, Gata3
GO:0034330	cell junction organization	0.0002	-8.6	105	8	Lama3, Marveld3, Cdh1, Fermt2, Ramp2, Tgfb3, Ocln, Itgb4
GO:0046822	regulation of nucleocytoplasmic transport	0.0002	-8.5	166	10	Pkia, Sfn, Sema5a, Cdh1, Apc2, Wipf1, Cdkn1a, Tgfb3, Pkd2, Tgfbr1
GO:0060021	palate development	0.0002	-8.5	81	7	Tgfb3, Fzd2, Pkdcc, Csrnp1, Bnc2, Wnt7a, Tgfbr1

TermID	Term	p-value	logP	Genes in Term	Target Genes in Term	Gene Symbols
GO:0072657	protein localization to membrane	0.0002	-8.4	168	10	Cdh1, Ehd2, Fermt2, Fgf13, Palm, Krt18, Ramp2, Map7, Cpe, Ramp3
GO:0045765	regulation of angiogenesis	0.0003	-8.2	172	10	Cxcl10, Sema5a, Rhob, Col4a3, Ramp2, Vash1, Epha1, Stat1, Cysltr1, Ctsh
GO:0003007	heart morphogenesis	0.0003	-8.2	206	11	Fzd2, Smarcd3, Grhl2, Sav1, Ovol2, Pkd2, Wnt2, Sox9, Foxc1, Cpe, Gata3
GO:0009913	epidermal cell differentiation	0.0003	-8.1	114	8	Sfn, Tgm1, Sav1, Sprr2g, Sprr1a, Krt17, Ppl, Anxa1
GO:0001558	regulation of cell growth	0.0005	-7.6	294	13	Wisp2, Sfn, Cda, Cxcl16, Fgf13, Ppp1r9b, Cdkn1a, Rrad, Ddr1, Krt17, Tnk1, Apbb2, Tgfbr1
GO:0007167	enzyme linked receptor protein signaling pathway	0.0005	-7.6	413	16	Ddit4, Met, Csrnp1, Fermt2, Figf, Pdgfa, Tgfb3, Tgfb1i1, Epha1, Ddr1, Foxc1, Tgfbr1, Smad6, Sox9, Grb10, Stub1
GO:1901342	regulation of vasculature development	0.0005	-7.5	188	10	Cxcl10, Sema5a, Rhob, Col4a3, Ramp2, Vash1, Epha1, Stat1, Cysltr1, Ctsh
GO:0001568	blood vessel development	0.0005	-7.5	416	16	Figf, Pdgfa, Ovol2, Epha1, Foxc1, Tgfbr1, Sema5a, Plau, Rhob, Smad6, Klf5, Ramp2, Pkd2, Wnt2, Vash1, Wnt7a
GO:0001944	vasculature development	0.0006	-7.5	458	17	Figf, Pdgfa, Ovol2, Epha1, Foxc1, Tgfbr1, Fzd2, Sema5a, Plau, Rhob, Klf5, Smad6, Ramp2, Vash1, Wnt2, Pkd2, Wnt7a
GO:0044802	single-organism membrane organization	0.0006	-7.4	338	14	Cdh1, Ehd2, Fermt2, Dysf, Fgf13, Palm, Gper1, Krt18, Ramp2, Ppif, Chrnb1, Map7, Cpe, Ramp3
GO:0001501	skeletal system development	0.0006	-7.4	379	15	Dlx1, Sparc, Pkdcc, Thbs3, Csrnp1, Grhl2, Fam20c, Bnc2, Zeb1, Tgfb3, Sox9, Twist2, Wnt7a, Foxc1, Tgfbr1
GO:0060491	regulation of cell projection assembly	0.0006	-7.4	70	6	Tgfb3, Tacstd2, Fam110c, Palm, Klf5, Tgfbr1
GO:0032410	negative regulation of transporter activity	0.0006	-7.3	27	4	Ppif, Pkd2, Nedd4l, Gsto1
GO:0061061	muscle structure development	0.0007	-7.3	383	15	Cdon, Smarcd3, Krt19, Met, Nupr1, Id3, Klf5, Smad6, Ramp2, Wnt2, Bcl9, Chrnb1, Rcan1, Foxc1, Maml1
GO:0048514	blood vessel morphogenesis	0.0007	-7.2	346	14	Sema5a, Plau, Rhob, Pdgfa, Figf, Klf5, Smad6, Ovol2, Ramp2, Vash1, Epha1, Foxc1, Wnt7a, Tgfbr1
GO:0032388	positive regulation of intracellular transport	0.0008	-7.2	163	9	Sfn, Cxcl10, Sema5a, Cdh1, Ehd2, Gper1, Wipf1, Tgfb3, Tgfbr1
GO:0048565	digestive tract development	0.0008	-7.1	102	7	Ovol2, Pkdcc, Cdh1, Stx2, Pdgfa, Sav1, Klf5
GO:0010632	regulation of epithelial cell migration	0.0010	-6.9	105	7	Itga2, Vash1, Tacstd2, Sox9, Mcc, Ctsh, Gata3
GO:0006140	regulation of nucleotide metabolic process	0.0010	-6.9	483	17	Cda, Ppif, Arap2, Epha1, Tbc1d2, Fzd2, Cxcl10, Ocrl, Cd40, Ece1, Palm, Gper1, Ramp2, Pkd2, Mcf2l, Dennd2a, Arhgap8
GO:0042306	regulation of protein import into nucleus	0.0010	-6.9	136	8	Pkia, Sema5a, Cdh1, Apc2, Cdkn1a, Pkd2, Tgfb3, Tgfbr1
GO:0014812	muscle cell migration	0.0011	-6.9	14	3	Ddr1, Plau, Met

TermID	Term	p-value	logP	Genes in Term	Target Genes in Term	Gene Symbols
GO:0060037	pharyngeal system development	0.0011	-6.9	14	3	Ece1, Tgfbr1, Gata3
GO:0046165	alcohol biosynthetic process	0.0011	-6.8	78	6	Mvd, Hmgcr, Sc4mol, Sgms2, Pmvk, Pemt
GO:0090287	regulation of cellular response to growth factor stimulus	0.0011	-6.8	138	8	Fam20c, Figf, Zeb1, Smad6, Tgfb3, Tgfb1i1, Grb10, Gata3
GO:0040007	growth	0.0011	-6.8	361	14	Thbs3, Pkdcc, Nupr1, Plau, Grhl2, Lepre1, Bnc2, Tgfb3, Bcl9, Map7, Sox9, Wnt7a, Foxc1, Gata3
GO:0048705	skeletal system morphogenesis	0.0011	-6.8	207	10	Thbs3, Csrnp1, Grhl2, Bnc2, Zeb1, Tgfb3, Sox9, Twist2, Wnt7a, Tgfbr1
GO:0003231	cardiac ventricle development	0.0012	-6.8	108	7	Fzd2, Smarcd3, Grhl2, Cpe, Sav1, Foxc1, Gata3
GO:0044087	regulation of cellular component biogenesis	0.0012	-6.7	406	15	Prkch, Stxbp1, Cand1, Fam110c, Palm, Klf5, Kank3, Smad6, Tgfb3, Epha1, Nedd4l, Tacstd2, Wnt7a, Stub1, Tgfbr1
GO:0016126	sterol biosynthetic process	0.0012	-6.7	32	4	Mvd, Hmgcr, Sc4mol, Pmvk
GO:2000008	regulation of protein localization to cell surface	0.0013	-6.6	15	3	Nedd4l, Cdh1, Rab11fip5
GO:0046824	positive regulation of nucleocytoplasmic transport	0.0013	-6.6	81	6	Tgfb3, Sfn, Sema5a, Cdh1, Tgfbr1, Wipf1
GO:0042692	muscle cell differentiation	0.0014	-6.6	213	10	Cdon, Smarcd3, Krt19, Met, Smad6, Ramp2, Bcl9, Chrnb1, Rcan1, Maml1
GO:0055123	digestive system development	0.0014	-6.5	112	7	Ovol2, Pkdcc, Cdh1, Stx2, Pdgfa, Sav1, Klf5
GO:0010830	regulation of myotube differentiation	0.0014	-6.5	56	5	Cdon, Ehd2, Sox9, Id3, Maml1
GO:0001525	angiogenesis	0.0015	-6.5	252	11	Sema5a, Plau, Rhob, Pdgfa, Figf, Klf5, Ovol2, Ramp2, Vash1, Epha1, Tgfbr1
GO:1900180	regulation of protein localization to nucleus	0.0015	-6.5	145	8	Pkia, Sema5a, Cdh1, Apc2, Cdkn1a, Pkd2, Tgfb3, Tgfbr1
GO:0090102	cochlea development	0.0016	-6.5	34	4	Fzd2, Cdh1, Sox9, Zeb1
GO:0043403	skeletal muscle tissue regeneration	0.0016	-6.5	16	3	Bcl9, Plau, Wnt7a
GO:0060575	intestinal epithelial cell differentiation	0.0016	-6.5	16	3	Cdh1, Sav1, Klf5
GO:2000352	negative regulation of endothelial cell apoptotic process	0.0016	-6.5	16	3	Ramp2, Sema5a, Gata3
GO:0048568	embryonic organ development	0.0016	-6.4	418	15	Fzd2, Pkdcc, Krt19, Tmie, Grhl2, Zeb1, Ovol2, Wnt2, Tgfb3, Pkd2, Sox9, Twist2, Foxc1, Gata3, Tgfbr1
GO:0006970	response to osmotic stress	0.0017	-6.4	58	5	Itga2, Mapk13, Pkd2, Nedd4l, Map7
GO:0010942	positive regulation of cell death	0.0017	-6.4	379	14	Ddit4, Nupr1, Cd40, Rhob, Sav1, Gper1, Id3, Casp7, Cdkn1a, Ppif, Tgfb3, Hmgcr, Anxa1, Apbb2
GO:1902580	single-organism cellular localization	0.0018	-6.3	220	10	Cdh1, Ehd2, Fermt2, Fgf13, Palm, Krt18, Ramp2, Map7, Cpe, Ramp3
GO:1902578	single-organism localization	0.0018	-6.3	220	10	Cdh1, Ehd2, Fermt2, Fgf13, Palm, Krt18, Ramp2, Map7, Cpe, Ramp3
GO:1901617	organic hydroxy compound biosynthetic process	0.0018	-6.3	116	7	Mvd, Hmgcr, Sc4mol, Sgms2, Pmvk, Pemt, Gata3
GO:0030799	regulation of cyclic nucleotide metabolic process	0.0019	-6.3	117	7	Ramp2, Pkd2, Fzd2, Cxcl10, Ece1, Palm, Gper1
GO:0030178	negative regulation of Wnt signaling pathway	0.0019	-6.2	118	7	Cdh1, Sox9, Apcdd1, Apc2, Grb10, Mcc, Dkk3
GO:0042303	molting cycle	0.0021	-6.1	89	6	Prss8, Sox9, Pdgfa, Mpzl3, Apcdd1, Sav1

TermID	Term	p-value	logP	Genes in Term	Target Genes in Term	Gene Symbols
GO:0042633	hair cycle	0.0021	-6.1	89	6	Prss8, Sox9, Pdgfa, Mpzl3, Apcdd1, Sav1
GO:0002009	morphogenesis of an epithelium	0.0022	-6.1	388	14	Sema5a, Met, Grhl2, Ovol2, Pkd2, Ripk4, Tgfb1i1, Ddr1, Tnc, Krt17, Sox9, Wnt7a, Ctsh, Gata3
GO:1900542	regulation of purine nucleotide metabolic process	0.0023	-6.1	478	16	Ppif, Arap2, Epha1, Tbc1d2, Fzd2, Cxcl10, Cd40, Ocrl, Ece1, Palm, Gper1, Ramp2, Pkd2, Mcf2l, Dennd2a, Arhgap8
GO:0042312	regulation of vasodilation	0.0024	-6.0	38	4	Hmgcr, Ece1, Gper1, Smad6
GO:0061024	membrane organization	0.0025	-6.0	395	14	Cdh1, Ehd2, Fermt2, Dysf, Fgf13, Palm, Gper1, Krt18, Ramp2, Ppif, Chrnb1, Map7, Cpe, Ramp3
GO:0030111	regulation of Wnt signaling pathway	0.0026	-6.0	194	9	Cdh1, Apcdd1, Apc2, Wnt2, Sox9, Grb10, Mcc, Dkk3, Wnt7a
GO:0032413	negative regulation of ion transmembrane transporter activity	0.0026	-5.9	19	3	Pkd2, Nedd4l, Gsto1
GO:2001259	positive regulation of cation channel activity	0.0026	-5.9	19	3	Pkd2, Nedd4l, Gsto1
GO:0048608	reproductive structure development	0.0027	-5.9	233	10	Fzd2, Serpinb6b, Nupr1, Pcyt1b, Tnc, Sox9, Map7, Wnt7a, Foxc1, Gata3
GO:0030198	extracellular matrix organization	0.0028	-5.9	160	8	Lepre1, Col4a3, Ramp2, Sox9, Mpzl3, Foxc1, Tgfbr1, Apbb2
GO:0014031	mesenchymal cell development	0.0028	-5.9	94	6	Ovol2, Fam101b, Wnt2, Sox9, Foxc1, Tgfbr1
GO:0030814	regulation of cAMP metabolic process	0.0028	-5.9	94	6	Ramp2, Pkd2, Cxcl10, Ece1, Palm, Gper1
GO:0045216	cell-cell junction organization	0.0028	-5.9	94	6	Ramp2, Marveld3, Ocln, Tgfb3, Cdh1, Fermt2
GO:0014902	myotube differentiation	0.0029	-5.9	40	4	Cdon, Bcl9, Met, Rcan1
GO:0043062	extracellular structure organization	0.0029	-5.8	161	8	Lepre1, Col4a3, Ramp2, Sox9, Mpzl3, Foxc1, Tgfbr1, Apbb2
GO:0030856	regulation of epithelial cell differentiation	0.0030	-5.8	95	6	Prkch, Ovol2, Sox9, Zeb1, Gata3, Tgfbr1
GO:0061458	reproductive system development	0.0031	-5.8	238	10	Fzd2, Nupr1, Serpinb6b, Pcyt1b, Tnc, Map7, Sox9, Foxc1, Wnt7a, Gata3
GO:0010563	negative regulation of phosphorus metabolic process	0.0033	-5.7	321	12	Pkia, Ddit4, Cda, Met, Pdgfa, Palm, Smad6, Cdkn1a, Ppif, Hmgcr, Epha1, Grb10
GO:0045936	negative regulation of phosphate metabolic process	0.0033	-5.7	321	12	Pkia, Ddit4, Cda, Met, Pdgfa, Palm, Smad6, Cdkn1a, Ppif, Hmgcr, Epha1, Grb10
GO:0008406	gonad development	0.0034	-5.7	165	8	Fzd2, Serpinb6b, Nupr1, Pcyt1b, Map7, Sox9, Foxc1, Gata3
GO:0060324	face development	0.0034	-5.7	42	4	Tgfb3, Csrnp1, Grhl2, Twist2
GO:0060485	mesenchyme development	0.0035	-5.7	131	7	Ovol2, Fam101b, Wnt2, Sox9, Bnc2, Foxc1, Tgfbr1
GO:0045684	positive regulation of epidermis development	0.0036	-5.6	21	3	Prkch, Tmem79, Krt17
GO:0045109	intermediate filament organization	0.0036	-5.6	21	3	Krt20, Krt17, Vim
GO:0048562	embryonic organ morphogenesis	0.0036	-5.6	283	11	Fzd2, Tmie, Grhl2, Zeb1, Ovol2, Pkd2, Tgfb3, Sox9, Twist2, Gata3, Tgfbr1
GO:0043627	response to estrogen	0.0036	-5.6	99	6	Tgfb3, Sprr2g, Wnt7a, Gper1, Tgfbr1, Ramp3
GO:0003208	cardiac ventricle morphogenesis	0.0036	-5.6	69	5	Smarcd3, Grhl2, Foxc1, Cpe, Gata3

TermID	Term	p-value	logP	Genes in Term	Target Genes in Term	Gene Symbols
GO:0048145	regulation of fibroblast proliferation	0.0036	-5.6	69	5	Cdkn1a, Wnt2, Nupr1, Fosl2, Pdgfa
GO:0034329	cell junction assembly	0.0036	-5.6	69	5	Lama3, Ramp2, Itgb4, Cdh1, Fermt2
GO:0051241	negative regulation of multicellular organismal process	0.0037	-5.6	368	13	Plau, Stxbp1, Klk8, Atp2a1, Pdgfa, Sav1, Smad6, Hmgcr, Tgfb3, Tacstd2, Sox9, Twist2, Gata3
GO:0001837	epithelial to mesenchymal transition	0.0038	-5.6	43	4	Fam101b, Wnt2, Sox9, Tgfbr1
GO:0010464	regulation of mesenchymal cell proliferation	0.0038	-5.6	43	4	Wnt2, Sox9, Pdgfa, Zeb1
GO:0048545	response to steroid hormone	0.0040	-5.5	207	9	Gper1, Agl, Sprr2g, Tgfb3, Wnt7a, Anxa1, Ramp3, Nr2c1, Tgfbr1
GO:0051147	regulation of muscle cell differentiation	0.0040	-5.5	101	6	Cdon, Ehd2, Sox9, Zeb1, Id3, Maml1
GO:0042246	tissue regeneration	0.0041	-5.5	22	3	Bcl9, Plau, Wnt7a
GO:0051491	positive regulation of filopodium assembly	0.0041	-5.5	22	3	Tgfb3, Palm, Tgfbr1
GO:0090103	cochlea morphogenesis	0.0041	-5.5	22	3	Fzd2, Sox9, Zeb1
GO:0048701	embryonic cranial skeleton morphogenesis	0.0041	-5.5	44	4	Tgfb3, Grhl2, Twist2, Tgfbr1
GO:0045766	positive regulation of angiogenesis	0.0042	-5.5	102	6	Ramp2, Epha1, Sema5a, Rhob, Cysltr1, Ctsh
GO:0008277	regulation of G-protein coupled receptor protein signaling pathway	0.0042	-5.5	102	6	Ramp2, Ece1, Palm, Gper1, Ppp1r9b, Ramp3
GO:0050808	synapse organization	0.0044	-5.4	103	6	Tnc, Klk8, Cdh1, Palm, Elfn1, Wnt7a
GO:0048146	positive regulation of fibroblast proliferation	0.0044	-5.4	45	4	Cdkn1a, Wnt2, Fosl2, Pdgfa
GO:0045682	regulation of epidermis development	0.0044	-5.4	45	4	Prkch, Ovol2, Tmem79, Krt17
GO:0070838	divalent metal ion transport	0.0045	-5.4	211	9	Bspry, Zdhhc13, Atp2a1, Ramp2, Cacnb3, Pkd2, Nipal2, Cysltr1, Ramp3
GO:0010171	body morphogenesis	0.0048	-5.3	46	4	Tgfb3, Cdon, Csrnp1, Twist2
GO:0055001	muscle cell development	0.0049	-5.3	105	6	Ramp2, Krt19, Rcan1, Chrnb1, Maml1, Smad6
GO:0000904	cell morphogenesis involved in differentiation	0.0050	-5.3	426	14	Fam101b, Fzd2, Sema5a, Cdh1, Stxbp1, Fermt2, Flrt3, Wnt2, Epha1, Sox9, Tgfbr1, Stxbp5, Apbb2, Gata3
GO:0045664	regulation of neuron differentiation	0.0050	-5.3	426	14	Cdon, Klk8, Met, Enc1, Fgf13, Palm, Dpysl2, Zeb1, Sox9, Wnt7a, Vim, Ntm, Sdc2, Gata3
GO:0048762	mesenchymal cell differentiation	0.0051	-5.3	106	6	Ovol2, Fam101b, Wnt2, Sox9, Foxc1, Tgfbr1
GO:0045786	negative regulation of cell cycle	0.0053	-5.2	178	8	Nupr1, Rhob, Cdkn1a, Vash1, Pkd2, Foxc1, Apbb2, Gata3
GO:0007169	transmembrane receptor protein tyrosine kinase signaling pathway	0.0053	-5.2	257	10	Ddit4, Met, Csrnp1, Figf, Pdgfa, Epha1, Ddr1, Sox9, Grb10, Foxc1
GO:0002064	epithelial cell development	0.0054	-5.2	142	7	Tmem79, Cdh1, Sox9, Map7, Wnt7a, Klf5, Gata3
GO:0060828	regulation of canonical Wnt signaling pathway	0.0054	-5.2	142	7	Wnt2, Cdh1, Sox9, Apc2, Mcc, Wnt7a, Dkk3
GO:2000736	regulation of stem cell differentiation	0.0055	-5.2	76	5	Tgfb3, Tgfb1i1, Tacstd2, Sox9, Sav1
GO:0090316	positive regulation of intracellular protein transport	0.0058	-5.1	109	6	Tgfb3, Sfn, Sema5a, Cdh1, Tgfbr1, Wipf1
GO:0051224	negative regulation of protein transport	0.0058	-5.1	109	6	Btn1a1, Pkd2, Pkia, Pkdcc, Apc2, Anxa1
GO:0030308	negative regulation of cell growth	0.0059	-5.1	144	7	Cdkn1a, Rrad, Cda, Fgf13, Tnk1, Apbb2, Ppp1r9b
GO:0008299	isoprenoid biosynthetic process	0.0059	-5.1	25	3	Mvd, Hmgcr, Pmvk

TermID	Term	p-value	logP	Genes in Term	Target Genes in Term	Gene Symbols
GO:0045165	cell fate commitment	0.0060	-5.1	221	9	Dlx1, Cdon, Fgf13, Wnt2, Tgfb1i1, Sox9, Wnt7a, Gata3, Tgfbr1
GO:0090257	regulation of muscle system process	0.0061	-5.1	145	7	Itga2, Tnni2, Nedd4l, Ece1, Atp2a1, Cald1, Gper1
GO:0071559	response to transforming growth factor beta	0.0061	-5.1	78	5	Tgfb3, Fermt2, Sox9, Smad6, Tgfbr1
GO:0030801	positive regulation of cyclic nucleotide metabolic process	0.0061	-5.1	78	5	Ramp2, Fzd2, Cxcl10, Ece1, Gper1
GO:0071560	cellular response to transforming growth factor beta stimulus	0.0061	-5.1	78	5	Tgfb3, Sox9, Fermt2, Smad6, Tgfbr1
GO:0045596	negative regulation of cell differentiation	0.0064	-5.1	485	15	Dlx1, Fgf13, Zeb1, Gper1, Id3, Ovol2, Meis2, Bcl9, Tgfb1i1, Tacstd2, Sox9, Twist2, Wnt7a, Gata3, Tgfbr1
GO:0001936	regulation of endothelial cell proliferation	0.0065	-5.0	79	5	Arg1, Wnt2, Vash1, Stat1, Tgfbr1
GO:0071363	cellular response to growth factor stimulus	0.0066	-5.0	224	9	Sparc, Ddit4, Fermt2, Smad6, Ramp2, Tgfb3, Sox9, Tgfbr1, Gata3
GO:0072511	divalent inorganic cation transport	0.0066	-5.0	224	9	Bspry, Zdhhc13, Atp2a1, Ramp2, Cacnb3, Pkd2, Nipal2, Cysltr1, Ramp3
GO:0048708	astrocyte differentiation	0.0066	-5.0	26	3	Nfix, Sox9, Vim
GO:0061326	renal tubule development	0.0066	-5.0	26	3	Pkd2, Stat1, Sox9
GO:0001937	negative regulation of endothelial cell proliferation	0.0066	-5.0	26	3	Vash1, Stat1, Tgfbr1
GO:0018149	peptide cross-linking	0.0066	-5.0	26	3	Sprr1a, Tgm1, Anxa1
GO:0030324	lung development	0.0069	-5.0	186	8	Sparc, Pkdcc, Grhl2, Pdgfa, Sav1, Tgfb3, Sim2, Ctsh
GO:0006937	regulation of muscle contraction	0.0069	-5.0	113	6	Itga2, Tnni2, Nedd4l, Atp2a1, Cald1, Gper1
GO:0043068	positive regulation of programmed cell death	0.0069	-5.0	353	12	Nupr1, Cd40, Rhob, Sav1, Gper1, Id3, Casp7, Cdkn1a, Tgfb3, Hmgcr, Anxa1, Apbb2
GO:0051056	regulation of small GTPase mediated signal transduction	0.0071	-5.0	354	12	Rgl1, Cdon, Ocrl, Fgd6, Cd40, Arap2, Mcf2l, Epha1, Tbc1d2, Dennd2a, Tnk1, Arhgap8
GO:0030323	respiratory tube development	0.0073	-4.9	188	8	Sparc, Pkdcc, Grhl2, Pdgfa, Sav1, Tgfb3, Sim2, Ctsh
GO:0007044	cell-substrate junction assembly	0.0073	-4.9	27	3	Lama3, Itgb4, Fermt2
GO:0006695	cholesterol biosynthetic process	0.0073	-4.9	27	3	Mvd, Hmgcr, Pmvk
GO:0090183	regulation of kidney development	0.0074	-4.9	52	4	Tacstd2, Sox9, Pdgfa, Gata3
GO:0021700	developmental maturation	0.0075	-4.9	189	8	Thbs3, Tmem79, Stxbp1, Fam20c, Palm, Ntn4, Gata3, Tdrd5
GO:0031099	regeneration	0.0079	-4.8	53	4	Bcl9, Tnc, Plau, Wnt7a
GO:0045981	positive regulation of nucleotide metabolic process	0.0080	-4.8	83	5	Ramp2, Fzd2, Cxcl10, Ece1, Gper1
GO:0001942	hair follicle development	0.0080	-4.8	83	5	Prss8, Sox9, Apcdd1, Pdgfa, Sav1
GO:0051153	regulation of striated muscle cell differentiation	0.0080	-4.8	83	5	Cdon, Ehd2, Sox9, Id3, Maml1
GO:1900544	positive regulation of purine nucleotide metabolic process	0.0080	-4.8	83	5	Ramp2, Fzd2, Cxcl10, Ece1, Gper1
GO:0045137	development of primary sexual characteristics	0.0082	-4.8	192	8	Fzd2, Serpinb6b, Nupr1, Pcyt1b, Map7, Sox9, Foxc1, Gata3
GO:0051146	striated muscle cell differentiation	0.0084	-4.8	154	7	Cdon, Bcl9, Krt19, Met, Rcan1, Chrnb1, Maml1
GO:0022405	hair cycle process	0.0084	-4.8	84	5	Prss8, Sox9, Pdgfa, Apcdd1, Sav1

TermID	Term	p-value	logP	Genes in Term	Target Genes in Term	Gene Symbols
GO:0022404	molting cycle process	0.0084	-4.8	84	5	Prss8, Sox9, Pdgfa, Apcdd1, Sav1
GO:0007517	muscle organ development	0.0084	-4.8	233	9	Cdon, Met, Nupr1, Id3, Klf5, Wnt2, Bcl9, Rcan1, Foxc1
GO:0070848	response to growth factor	0.0084	-4.8	233	9	Sparc, Ddit4, Fermt2, Smad6, Ramp2, Tgfb3, Sox9, Tgfbr1, Gata3
GO:0035239	tube morphogenesis	0.0084	-4.8	318	11	Sema5a, Met, Cdh1, Grhl2, Zeb1, Ovol2, Pkd2, Ddr1, Sox9, Ctsh, Gata3
GO:0060322	head development	0.0085	-4.8	54	4	Tgfb3, Csrnp1, Grhl2, Twist2
GO:0090090	negative regulation of canonical Wnt signaling pathway	0.0088	-4.7	85	5	Cdh1, Sox9, Apc2, Mcc, Dkk3
GO:0031345	negative regulation of cell projection organization	0.0088	-4.7	85	5	Klk8, Tacstd2, Fgf13, Vim, Ntm
GO:0097305	response to alcohol	0.0088	-4.7	119	6	Sprr2g, Cdh1, Aacs, Gper1, Tgfbr1, Ramp3
GO:0007519	skeletal muscle tissue development	0.0088	-4.7	119	6	Cdon, Bcl9, Met, Rcan1, Nupr1, Klf5
GO:0030857	negative regulation of epithelial cell differentiation	0.0089	-4.7	29	3	Ovol2, Zeb1, Tgfbr1
GO:0071333	cellular response to glucose stimulus	0.0089	-4.7	29	3	Aacs, Rab11fip5, Gper1
GO:0048864	stem cell development	0.0090	-4.7	195	8	Fam101b, Ovol2, Wnt2, Bcl9, Sox9, Wnt7a, Foxc1, Tgfbr1
GO:0009880	embryonic pattern specification	0.0090	-4.7	55	4	Sim2, Wnt7a, Smad6, Tdrd5
GO:0001654	eye development	0.0092	-4.7	322	11	Cdon, Grhl2, Zeb1, Meis2, Sox9, Twist2, Foxc1, Vim, Gata3, Abi2, Tgfbr1
GO:0072091	regulation of stem cell proliferation	0.0092	-4.7	86	5	Wnt2, Smarcd3, Sox9, Pdgfa, Zeb1
GO:0009615	response to virus	0.0092	-4.7	157	7	Cxcl10, Ddit4, Cd40, Ifnlr1, Isg15, Ifit3, Batf3
GO:0043010	camera-type eye development	0.0095	-4.7	280	10	Cdon, Grhl2, Zeb1, Sox9, Twist2, Foxc1, Vim, Gata3, Abi2, Tgfbr1
GO:0051051	negative regulation of transport	0.0096	-4.6	324	11	Btn1a1, Pkia, Pkdcc, Gsto1, Apc2, Ppif, Pkd2, Hmgcr, Nedd4l, Grb10, Anxa1
GO:0008283	cell proliferation	0.0098	-4.6	462	14	Sfn, Ddit4, Sema5a, Purb, Met, Klk8, Fgf13, Figf, Col4a3, Zbtb32, Map7, Sox9, Wnt7a, Foxc1
GO:0042771	intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator	0.0098	-4.6	30	3	Cdkn1a, Ddit4, Nupr1
GO:0007389	pattern specification process	0.0099	-4.6	416	13	Dlx1, Sema5a, Cdon, Smarcd3, Zeb1, Smad6, Ovol2, Sim2, Pkd2, Wnt7a, Foxc1, Tgfbr1, Tdrd5

Supplemental Table 3

Grhl2 core target module.

Table explaining the generation of the Grhl2 core target module.

The following table contains genes that fulfill the major criteria for our Grhl2 core target set. This includes:

to be differentially expressed (control vs. KD),

to have a differential H3K4me3 ChIP peak in IMCD-3 cells intersecting with the gene body or within +-500bp from TSS,

to have a Grhl2 ChIP peak in IMCD3 cells intersecting with the mentioned diff. H3K4me3 peak,

to have a ChIP peak in adult kidney intersecting with the Grhl2 ChIP peak from IMCD-3 cells.

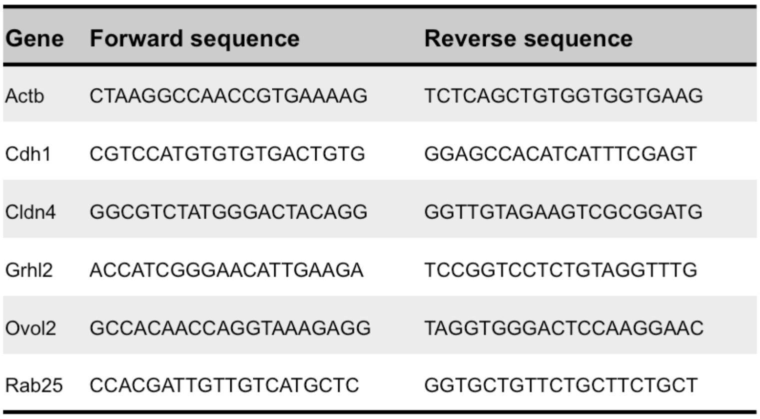
⬜	**no peak in adult kidney OR no rescue in Grhl-Res**
🟧	**no peak center related Grhl2 binding motif**
🟥	**final core target set**

				Grhl2 ChIP peak occurrence consistent with Grhl2 ChIP peak in IMCD-3 cells in:		Associated Grhl2 ChIP peak in IMCD-3 cells				Associated diff. H3K4me3 ChIP peak in IMCD-3 ce				Motif occurrence in 50bp window around peak summit (p-value<1e-03)		
Name	**Entrez ID**	**Down- or upreg. in KD**	**Success-fully rescued in Grhl2-Res**	**Adult kidney**	**E18 kidney**		**Start**	**End**	**MACS peak score**		**Start**	**End**	**MACS peak score**		**p-value (FIMO)**	**Sequence**
🟥 Prss22	70835	DOWN	YES	YES	YES	chr17	24134882	24135365	486.88	chr17	24132298	24135860	1155.62	YES	0.000128	GGACCAGTC
🟥 Tmem54	66260	DOWN	YES	YES	YES	chr4	128782536	128782999	2512.06	chr4	128782297	128784223	312.3	YES	0.000370	GGACCGGCT
⬜ 2310007B03Rik	71874	DOWN	YES	NO	YES	chr1	95054266	95054542	146.89	chr1	95053725	95057685	1390.51	YES	0.000119	AGACCAGCC
🟥 Rab25	53868	DOWN	YES	YES	YES	chr3	88352030	88352452	347.13	chr3	88350717	88353006	177.1	YES	0.000133	TAACCTGTT
🟥 Tmem40	94346	DOWN	YES	YES	NO	chr6	115708850	115709040	51.48	chr6	115707783	115710545	777.65	YES	0.000293	TGACCAGTC
⬜ Lama3	16774	DOWN	YES	NO	NO	chr18	12662427	12662735	112.58	chr18	12660937	12664719	787.32	YES	0.000750	ATACCTGCC
🟧 Il17re	57890	DOWN	YES	YES	YES	chr6	113408498	113408875	346.9	chr6	113408356	113409883	535.47	NO		
🟥 Tgm1	21816	DOWN	YES	YES	NO	chr14	56332795	56332985	144.56	chr14	56331144	56334628	235.74	YES	0.000263	GAGCCAGTT
🟥 Prom2	192212	DOWN	YES	YES	YES	chr2	127366959	127367430	1558.07	chr2	127365786	127367795	900.57	YES	0.000018	AAACCTGTC
🟥 Cdh1	12550	DOWN	YES	YES	YES	chr8	109134806	109135281	1215.01	chr8	109134232	109135862	276.33	YES	0.000005	AAACCAGTT
🟧 Ctsh	13036	DOWN	YES	YES	YES	chr9	89964274	89964634	907.72	chr9	89963827	89965216	145.43	NO		
⬜ Syt8	55925	DOWN	NO	YES	YES	chr7	149620101	149620413	2161.46	chr7	149620003	149623228	838.76	YES	0.000240	TGACCAGTT
🟧 Arhgap40	545481	DOWN	YES	YES	NO	chr2	158348878	158349223	1575.98	chr2	158348457	158349800	65	NO		
⬜ Sprr1a	20753	DOWN	NO	NO	YES	chr3	92290303	92290585	272.11	chr3	92289025	92291064	64.95	YES	0.000030	AAACCTGCT
🟥 Mpzl3	319742	DOWN	YES	YES	NO	chr9	44868176	44868442	447.43	chr9	44867641	44870275	1013.82	YES	0.000387	GAACCAGAT
🟧 Dcbld1	66686	DOWN	YES	YES	NO	chr10	51997822	51998226	59.46	chr10	51997215	51998581	71.25	NO		
⬜ Fam110b	242297	DOWN	YES	NO	YES	chr4	5695796	5696142	248.05	chr4	5695820	5697132	272.84		0.000010	AAACCTGTT
🟥 Ppl	19041	DOWN	YES	YES	YES	chr16	5119594	5119928	687.32	chr16	5118800	5120523	102.2	YES	0.000260	CAACCTGCC
🟧 Eps8l1	67425	DOWN	YES	YES	YES	chr7	4416901	4417184	66.19	chr7	4415317	4418035	298.33	NO		

				Grhl2 ChIP peak occurrence consistent with Grhl2 ChIP peak in IMCD-3 cells in:		Associated Grhl2 ChIP peak in IMCD-3 cells				Associated diff. H3K4me3 ChIP peak in IMCD-3 ce				Motif occurrence in 50bp window around peak summit (p-value<1e-03)		
Name	**Entrez ID**	**Down- or upreg. in KD**	**Success-fully rescued in Grhl2-Res**	**Adult kidney**	**E18 kidney**		**Start**	**End**	**MACS peak score**		**Start**	**End**	**MACS peak score**		**p-value (FIMO)**	**Sequence**
AI661453	224833	DOWN	YES	YES	YES	chr17	47588025	47588457	1419.88	chr17	47587526	47589113	110.4	YES	0.000069	AGACCTGTC
Rasef	242505	DOWN	YES	YES	YES	chr4	73436734	73437120	1092.34	chr4	73435069	73437509	112.83	YES	0.000195	GGACCTGCT
Ovol2	107586	DOWN	YES	YES	YES	chr2	144157800	144158064	1438.74	chr2	144156034	144158556	986.16	YES	0.000122	GAACCGGTC
Otud7b	229603	DOWN	YES	NO	NO	chr3	95937325	95937653	54.68	chr3	95936534	95939733	70.49	YES	0.000312	GAGCCAGTC
Apbb2	11787	UP	YES	NO	NO	chr5	66995044	66995359	71.29	chr5	66994269	66995651	71.78	NO		
Nedd4l	83814	UP	YES	NO	NO	chr18	65196669	65197350	634.54	chr18	65196294	65197210	64.17	NO		

Supplemental Table 4

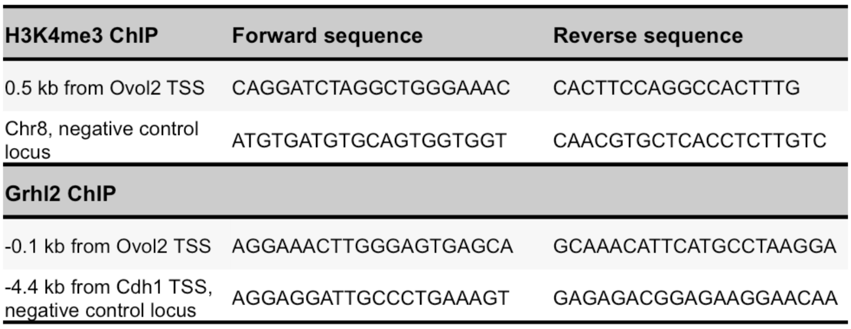
Primers used for quantitative RT-PCR.



Gene	Forward sequence	Reverse sequence
Actb	CTAAGGCCAACCGTGAAAAG	TCTCAGCTGTGGTGGTGAAG
Cdh1	CGTCCATGTGTGTGACTGTG	GGAGCCACATCATTTCGAGT
Cldn4	GGCGTCTATGGGACTACAGG	GGTTGTAGAAGTCGCGGATG
Grhl2	ACCATCGGGAACATTGAAGA	TCCGGTCCTCTGTAGGTTTG
Ovol2	GCCACAACCAGGTAAAGAGG	TAGGTGGGACTCCAAGGAAC
Rab25	CCACGATTGTTGTCATGCTC	GGTGCTGTTCTGCTTCTGCT

Supplemental Table 5

Primers used for quantitative ChIP-PCR.



H3K4me3 ChIP	Forward sequence	Reverse sequence
0.5 kb from Ovol2 TSS	CAGGATCTAGGCTGGGAAAC	CACTTCCAGGCCACTTTG
Chr8, negative control locus	ATGTGATGTGCAGTGGTGGT	CAACGTGCTCACCTCTTGTC
Grhl2 ChIP		
-0.1 kb from Ovol2 TSS	AGGAAACTTGGGAGTGAGCA	GCAAACATTCATGCCTAAGGA
-4.4 kb from Cdh1 TSS, negative control locus	AGGAGGATTGCCCTGAAAGT	GAGAGACGGAGAAGGAACAA

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