GRHL2-miR-200-ZEB1 maintains the epithelial status of ovarian cancer through transcriptional regulation and histone modification.

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Supplementary Results

GRHL2 knockdown up-regulates N-cadherin expression and does not affect cytokeratin expression

For mesenchymal markers, such as N-cadherin (*CDH2*) and vimentin (*VIM*), the mRNA expression was up-regulated after GRHL2 knockdown, especially in OVCA429 cells (Supplementary Figure S2a). For N-cadherin, the increment in protein expression was observed only in the OVCA429 shGRHL2 #10 cells (Supplementary Figure S2c). Cytokeratins were present in all the control and GRHL2-knockdown cells tested, as shown by immunofluorescence stainings (Supplementary Figure S2d).

Supplementary Methods

Anoikis assay

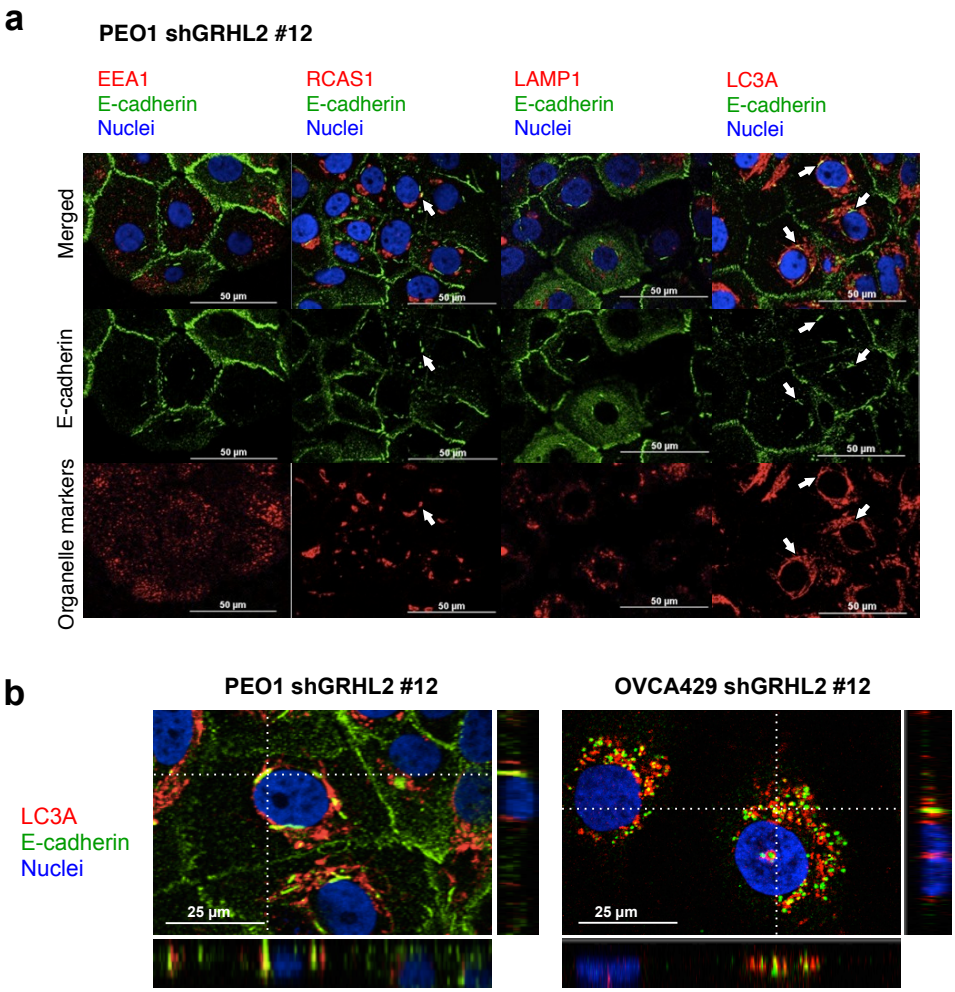
Anoikis resistance of control and GRHL2-knockdown OVCA429 cells was tested by seeding 3000, 6000 or 12,000 cells into each well of a 96-well plate of ultra-low attachment grade (3474, Corning). Cells were incubated at 37°C in 5% CO_2, and MTS assays (G5430, Promega) were performed at day 0, day 2, day 4 and day 6 according to a standard protocol. The cells were incubated with MTS reagent mix for 2 h and absorbance was read using a microplate reader (Tecan Infinite 200).

Spheroid invasion assay

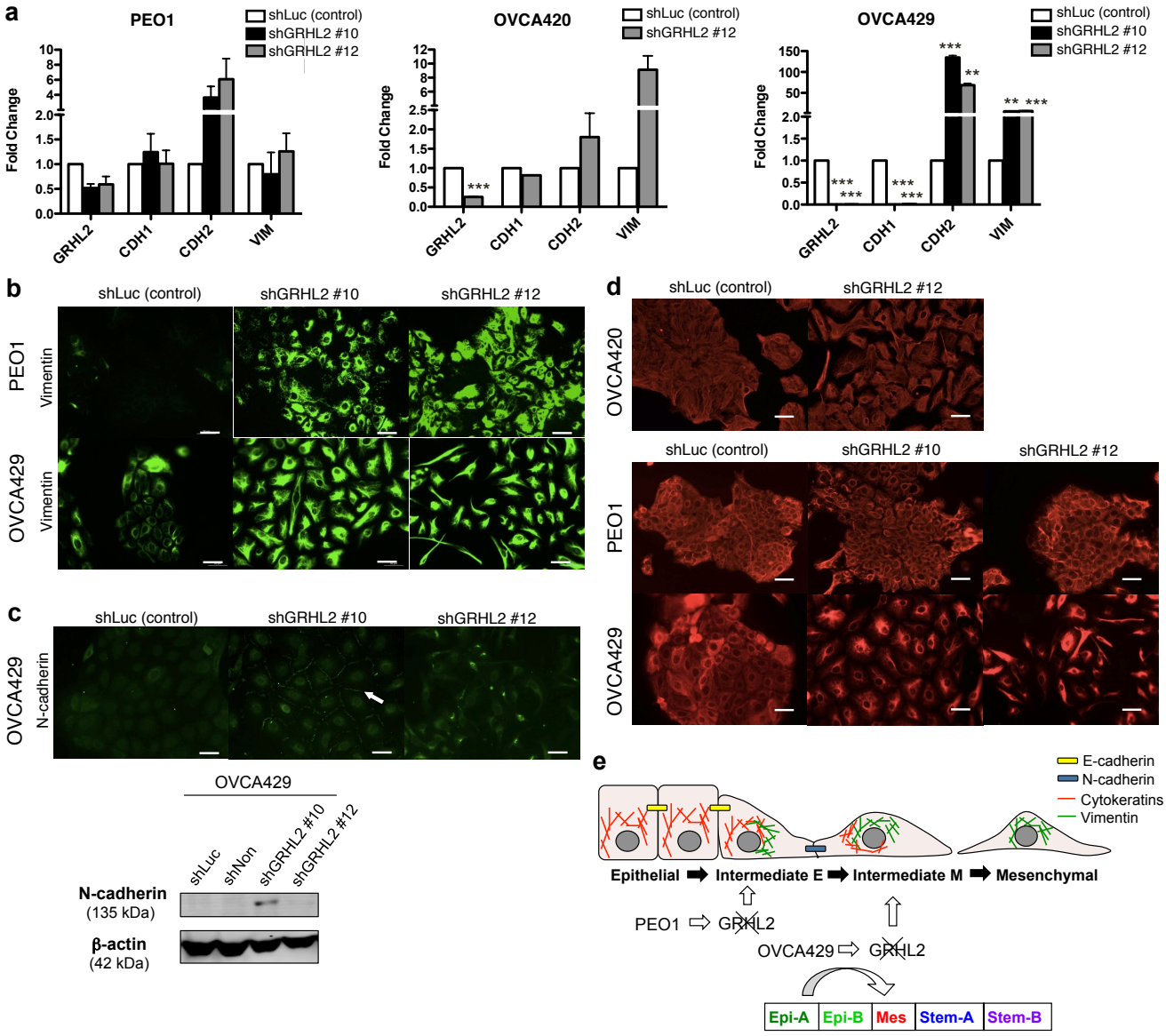
Control and GRHL2-knockdown OVCA429 cells were grown as spheroids following the protocol of a 96-well 3D Spheroid BME Cell Invasion Assay from Cultrex (3500-096-K), with modifications. Approximately 5000 cells were mixed with Spheroid Formation ECM and seeded into each well of a round-bottom, ultra-

low attachment, 96-well plate (7007, Corning). The plate was centrifuged at 200 ×*g* for 3 min and incubated at 37°C for 3 days to induce spheroid growth. After the assembled cells formed spheroids, BME matrix (Basement Membrane Extract) was added to each well (50 µl) and the plate centrifuged at 300 × *g* at 4°C for 5 min. The plate was incubated at 37°C for 1 h to allow for gel formation, before the addition of 100 µl of cell culture media. The spheroids were grown under normal cell culture condition (37°C, 5% CO_2) and were monitored every 3 or 4 days. Cells were stained for 1 h with Calcein AM (C3100MP, Life Technologies) to check for viability, and with Ethidium Homodimer-1 (E1169, Life Technologies) to check for dead cells. Images were taken using a Zeiss AxioImager M2 epifluorescence imaging system.

Supplementary Figures

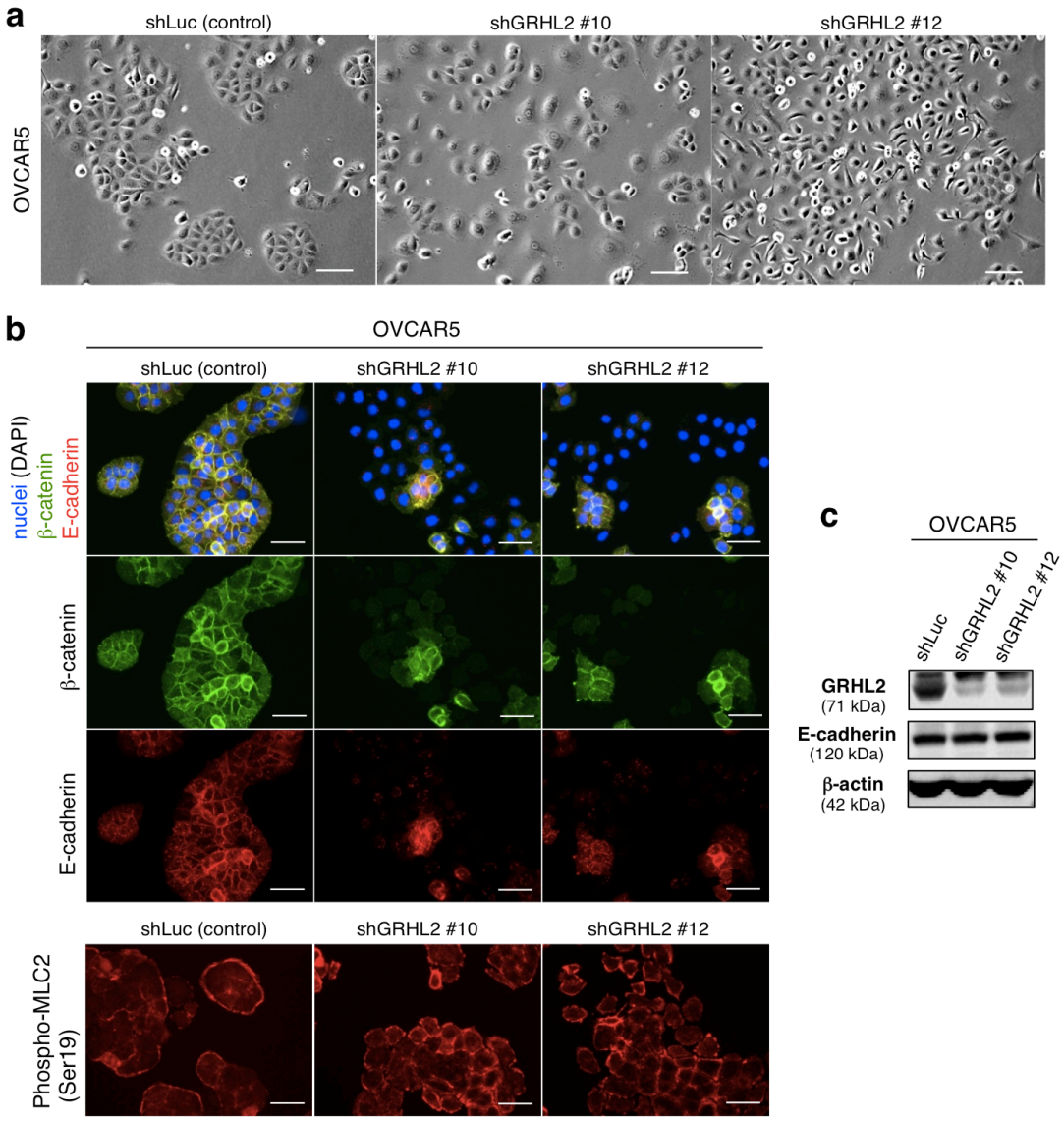


Supplementary Figure S1. Co-localization of cytoplasmic E-cadherin with various organelle markers in GRHL2-knockdown EOC cells.
(**a**) Immunofluorescence stainings of E-cadherin (green) and four organelle markers (red) in PEO1 shGRHL2 #12 cells. EEA1 (far left) marks the early endosome; RCAS1 (middle left) (also known as EBAG9) marks the Golgi apparatus; LAMP1 (middle right) marks lysosomes; LC3A (far right) marks autophagosomes. White arrows indicate co-localization (yellow). Scale = 50 µm. (**b**) Z-stack slices showing the co-localization (yellow) of LC3A (red) and cytoplasmic E-cadherin (green) in GRHL2-knockdown cells of PEO1 and OVCA429. Scale = 25 µm.

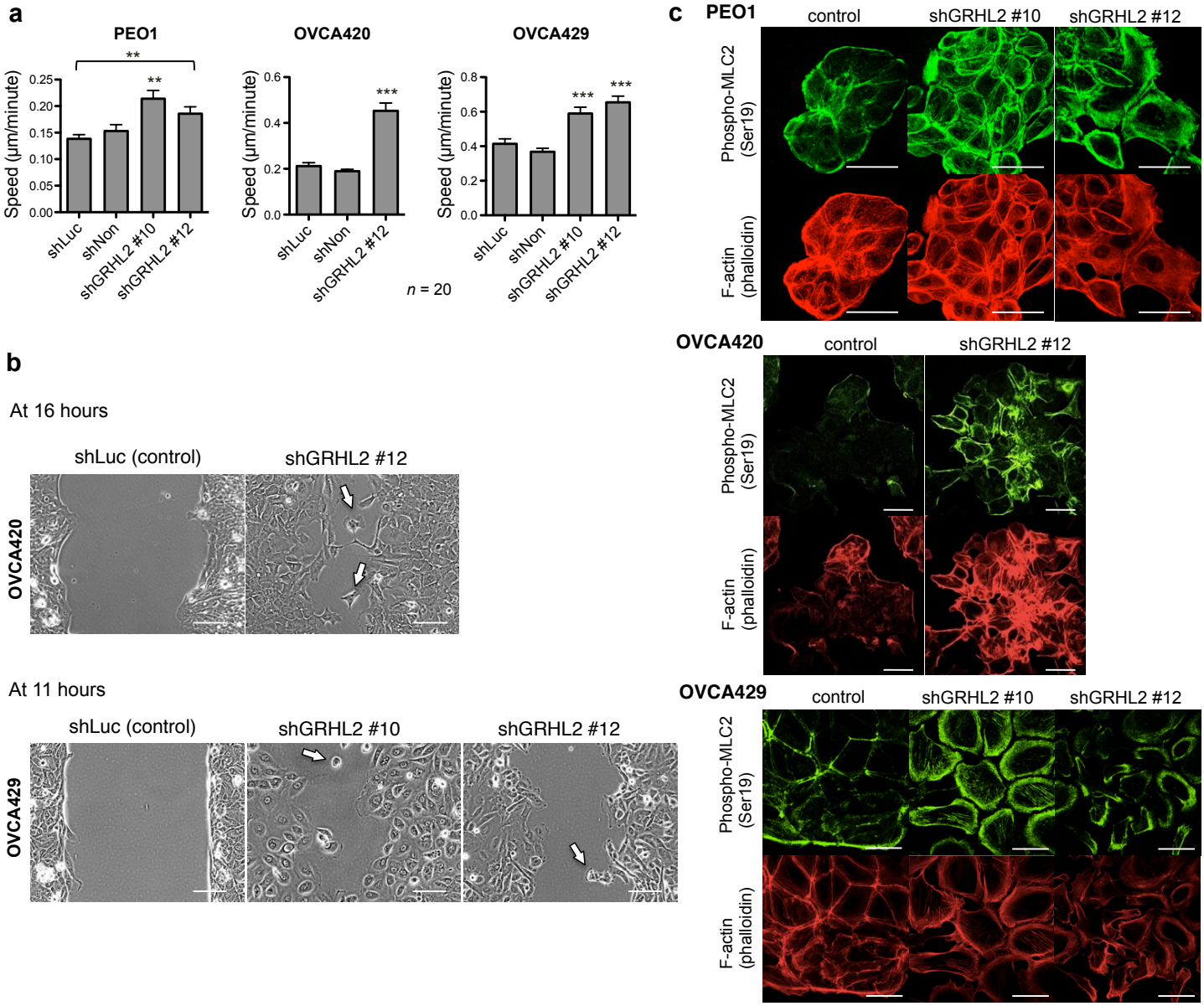


Supplementary Figure S2. Knockdown of GRHL2 results in the up-regulation of vimentin and N-cadherin.

(**a**) mRNA expression fold change (y-axis) in GRHL2, E- cadherin (*CDH1*), N-cadherin (*CDH2*) and vimentin (*VIM*), as measured by RT-qPCR in shLuc control (white bars), shGRHL2 #10 (black bars) and shGRHL2 #12 (grey bars) cells of PEO1, OVCA420 and OVCA429. Unpaired *t*-tests were performed on the $2^{-\Delta Ct}$ values for each gene, ** $p < 0.01$; *** $p < 0.001$. (**b**) Immunofluorescence stainings of vimentin in control (shLuc, shNon) and GRHL2-knockdown (shGRHL2 #10, #12) cells of PEO1 and OVCA429. Scale = 50 µm. (**c**) Immunofluorescence stainings (upper) of N-cadherin in control (shLuc, shNon) and GRHL2-knockdown (shGRHL2 #10, #12) OVCA429 cells. White arrow indicates junctional N-cadherin observed in shGRHL2 #10 OVCA429 cells. Scale = 50 µm. Western blots (lower) of N-cadherin and β-actin in the stable cell lines of OVCA429. Full-length blots are presented in Supplementary Figure S10. (**d**) Immunofluorescence stainings of pan-cytokeratin in the stable cell lines of PEO1, OVCA420 and OVCA429. Scale = 50 µm. (**e**) A simplified representation of the stepwise changes that occur in the phenotype (PEO1, OVCA429) and molecular subtype (OVCA429) of GRHL2-knockdown cells involving EMT markers E-cadherin, N-cadherin, cytokeratin and vimentin.

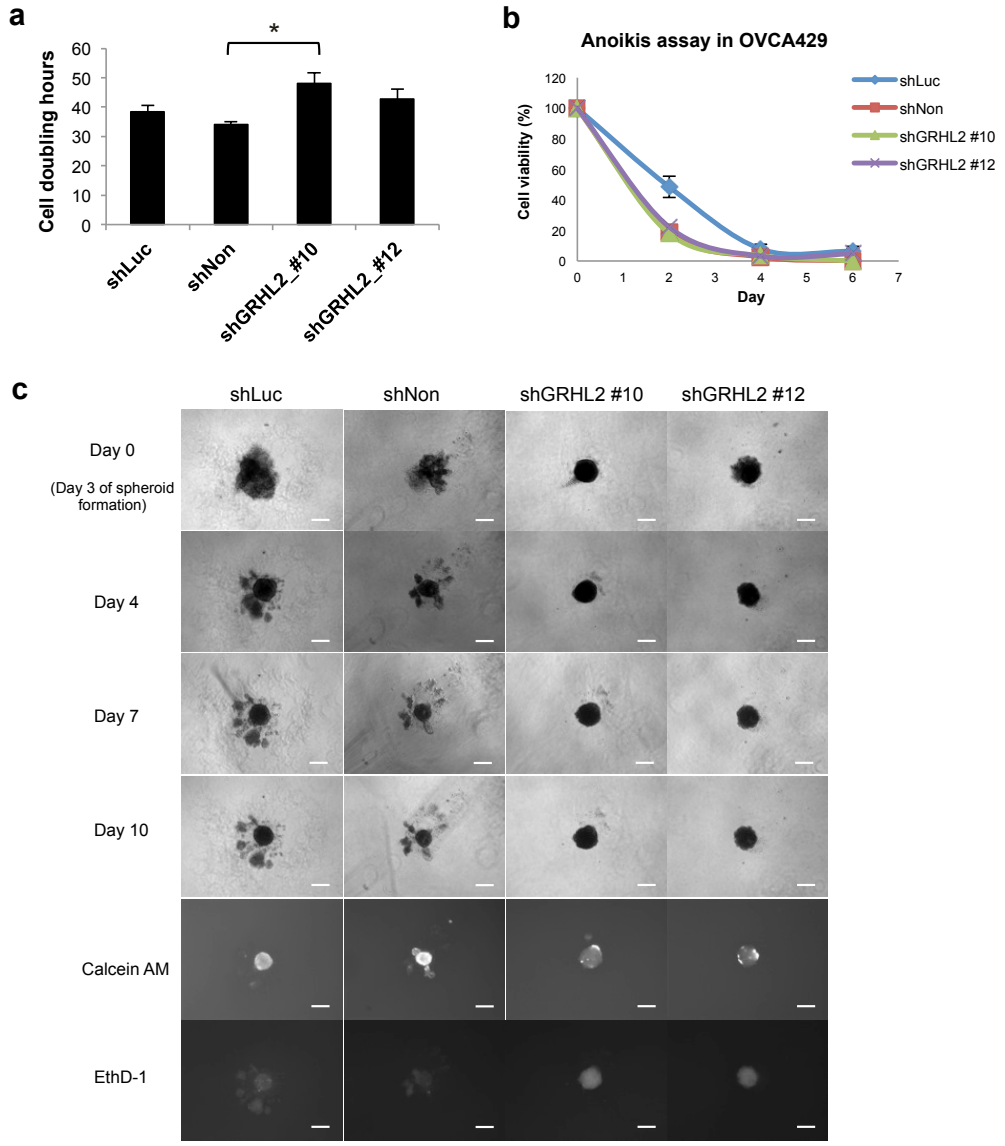


Supplementary Figure S3. Effects of GRHL2 knockdown in OVCAR5 cells.
(**a**) The morphologies of OVCAR5 cells infected with control shRNA (shLuc) and GRHL2-targeting shRNAs (shGRHL2 #10, shGRHL2 #12). Scale = 100 μm. (**b**) Immunofluorescence stainings of E-cadherin (red) and β-catenin (green) in the infected OVCAR5 cells. Nuclei were stained blue (DAPI). Immunofluorescence stainings (bottom) of phosphorylated myosin light chain 2 (ser19) (phospho-MLC2) in OVCAR5 cells. Scale = 50 μm. (**c**) Western blots of GRHL2, E-cadherin and β-actin in OVCAR5 cells. Upper bands in the GRHL2 panel were deemed non-specific. Full-length blots are presented in Supplementary Figure S10.



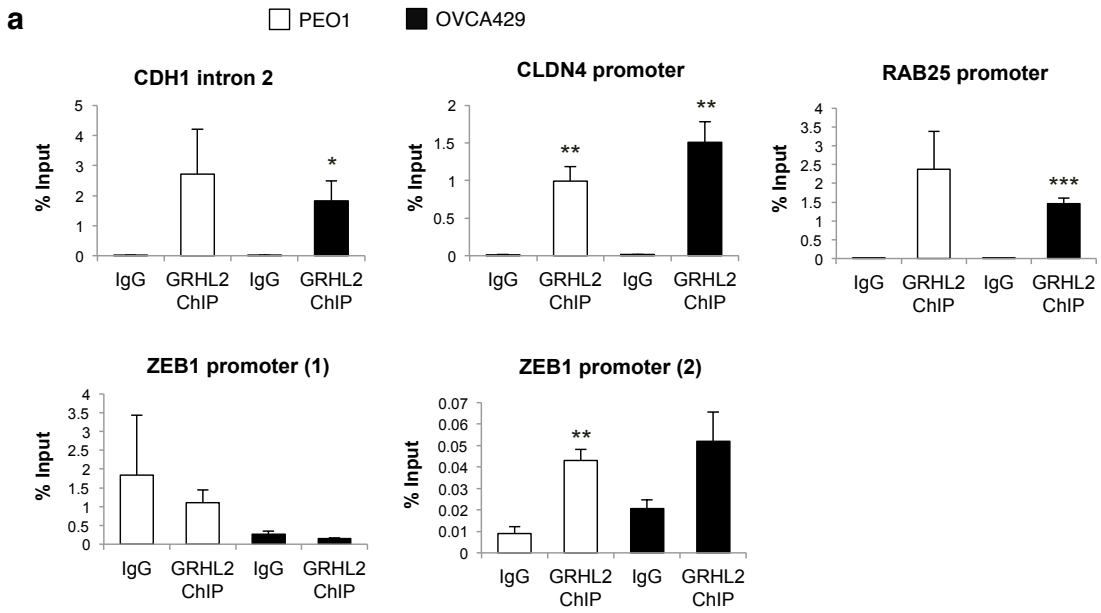
Supplementary Figure S4. Knockdown of GRHL2 promotes cell motility and scattering in EOC.

(**a**) The motility of control and GRHL2-knockdown PEO1, OVCA420, and OVCA429 cells as observed by time-lapse imaging (n = 20). Bar charts show the average speed of cell locomotion over the course of 11 to 18 h. Unpaired t-tests. ** $p < 0.01$; *** $p < 0.001$. (**b**) Phase-contrast images showing the migrating front of control and GRHL2-knockdown OVCA420 and OVCA429 cells during gap closure. Arrows indicate scattered, detached cells. Scale = 100 µm. (**c**) Immunofluorescence images showing phosphorylation of myosin light chain 2 (ser19) (phospho-MLC2) and F-actin (phalloidin) staining in control and GRHL2-knockdown PEO1 (upper panels), OVCA420 (middle panels), and OVCA429 (lower panels) cells. Scale = 50 µm.



Supplementary Figure S5. Effects of GRHL2 knockdown on cell doubling, anoikis and spheroid growth.

(**a**) Bar charts showing the doubling hours of control (shLuc, shNon) and GRHL2-knockdown (shGRHL2 #10, #12) OVCA429 cells. Statistical significance was determined by unpaired *t*-tests. * $p < 0.05$. (**b**) Line graph showing the anoikis resistance of OVCA429 stable cell lines grown on ultra-low attachment grade culture plates, presented as the percentage of viability (*y*-axis) examined by MTS assays, at Days 0, 2, 4 and 6 (*x*-axis). (**c**) Phase contrast images of OVCA429 stable cell lines grown as spheroids for three days on ultra-low attachment culture plates (Day 0) and subsequently embedded in basement membrane extract. Serial images of spheroids at Days 4, 7, and 10 are shown. At Day 15, the spheroids were stained with calcein AM to detect viable cells and ethidium homodimer-1 (EthD-1) to label dying/dead cells. Scale = 200 μm.

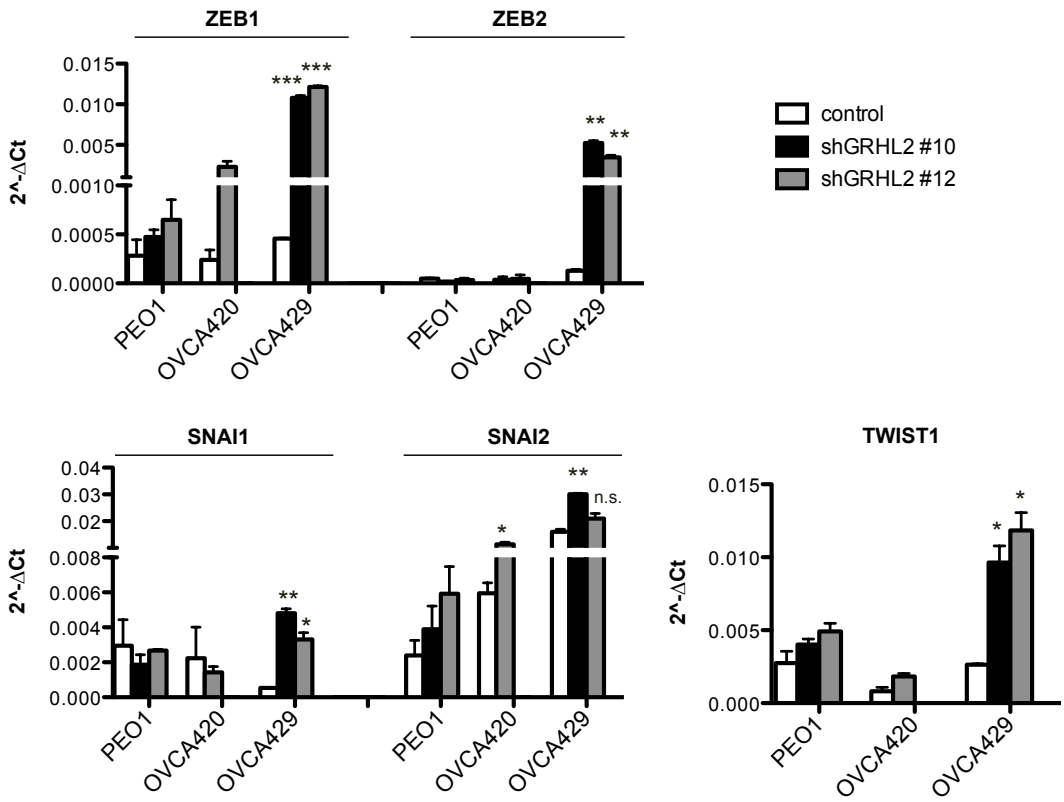


b

CDH1 intron 2	**+7573**	**+7607** **+7771**
	TTCAAAAGATC-------CAGC**AAACCAGCCAAACCAGT**TTCT-------GTTGTGGCTT	
CLDN4 promoter	**-87**	**+30** **+55**
	GTGACCTCAGCAT ----------AGG**AATCCAGAGAAACTGGTC**AGGAGGAG	
RAB25 promoter	**-191**	**-94** **-10**
	CAGTGGGC-------ACACC**AACCTGTCGAACCTGTC**TGACGTC--------TGTTCTCAG	
ZEB1 promoter	**primer pair (1):**	**-2091 / -128** **-2008 / -45** **-1908 / +56**
	CGGTCCCT--------TGTCGT**AAACCAGGT**GCGG--------CACAAGCGA	
	primer pair (2):	**-2261 / -298** **-2080 / -117**
	GCCGCCGAGCCTCCAACTT--------AACCGCCCGGTCCCTAGCA	

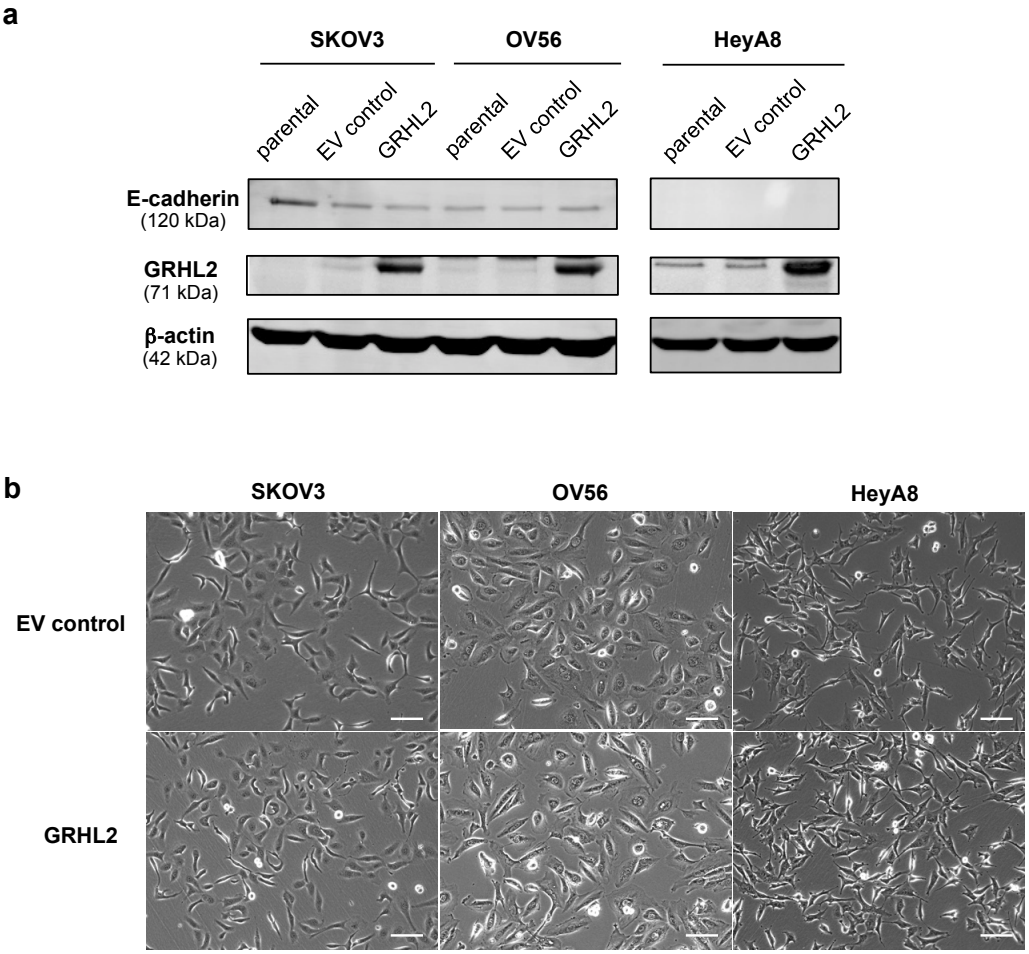
Supplementary Figure S6. The binding of GRHL2 at the regulatory elements of *CDH1, CLDN4, RAB25* and *ZEB1* genes.

(**a**) GRHL2 ChIP-qPCR in PEO1 and OVCA429 at intron 2 of *CDH1* and the promoter regions of *CLDN4*, *RAB25* and *ZEB1*. Signals of IgG control and ChIP samples were normalized to input DNA and presented as % input with SEM from three independent experiments. % input of GRHL2 ChIP samples were compared with IgG control using unpaired *t*-tests. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Two primer pairs were tested for ZEB1 promoter and no enrichment was detected by primer pair 1. (**b**) DNA sequences (enhancer/promoter) of different genes flanked by the primer pairs used in ChIP-qPCR. The GRHL2 binding motifs are labelled in red. Numbers refer to the distance (in base pairs) relative to the transcription start site (TSS) of each gene. For *ZEB1*, the distance relative to the TSS of both NM_001128128 and NM_030751 gene transcripts are indicated.



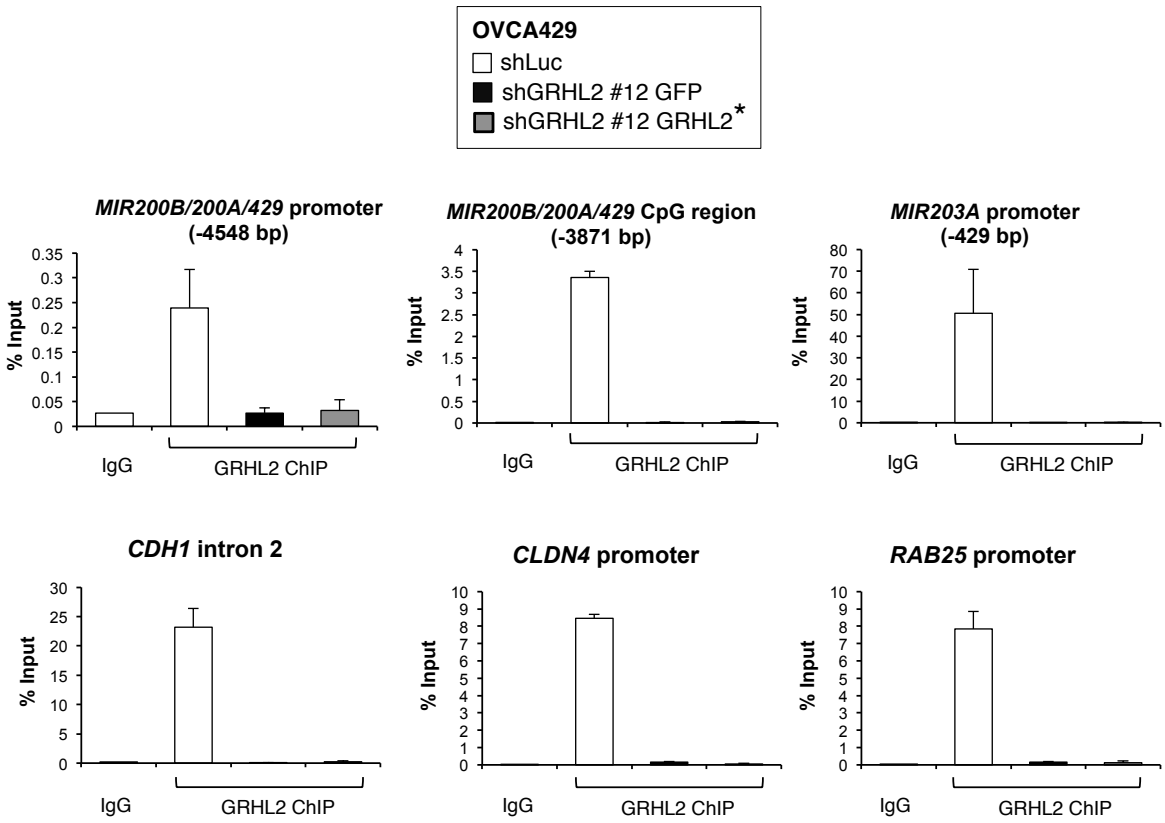
Supplementary Figure S7. Knockdown of GRHL2 affects the mRNA expression of other EMT transcription factors.

Bar charts showing the normalized mRNA expression ($2^{-\Delta Ct}$) (*y*-axis) of five EMT transcriptional drivers (ZEB1, ZEB2, SNAI1, SNAI2 and TWIST1) measured by RT-qPCR in control (white bars), shGRHL2 #10 (black bars), and shGRHL2 #12 (grey bars) cells of PEO1, OVCA420 and OVCA429. Unpaired *t*-tests were performed for statistical significance. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

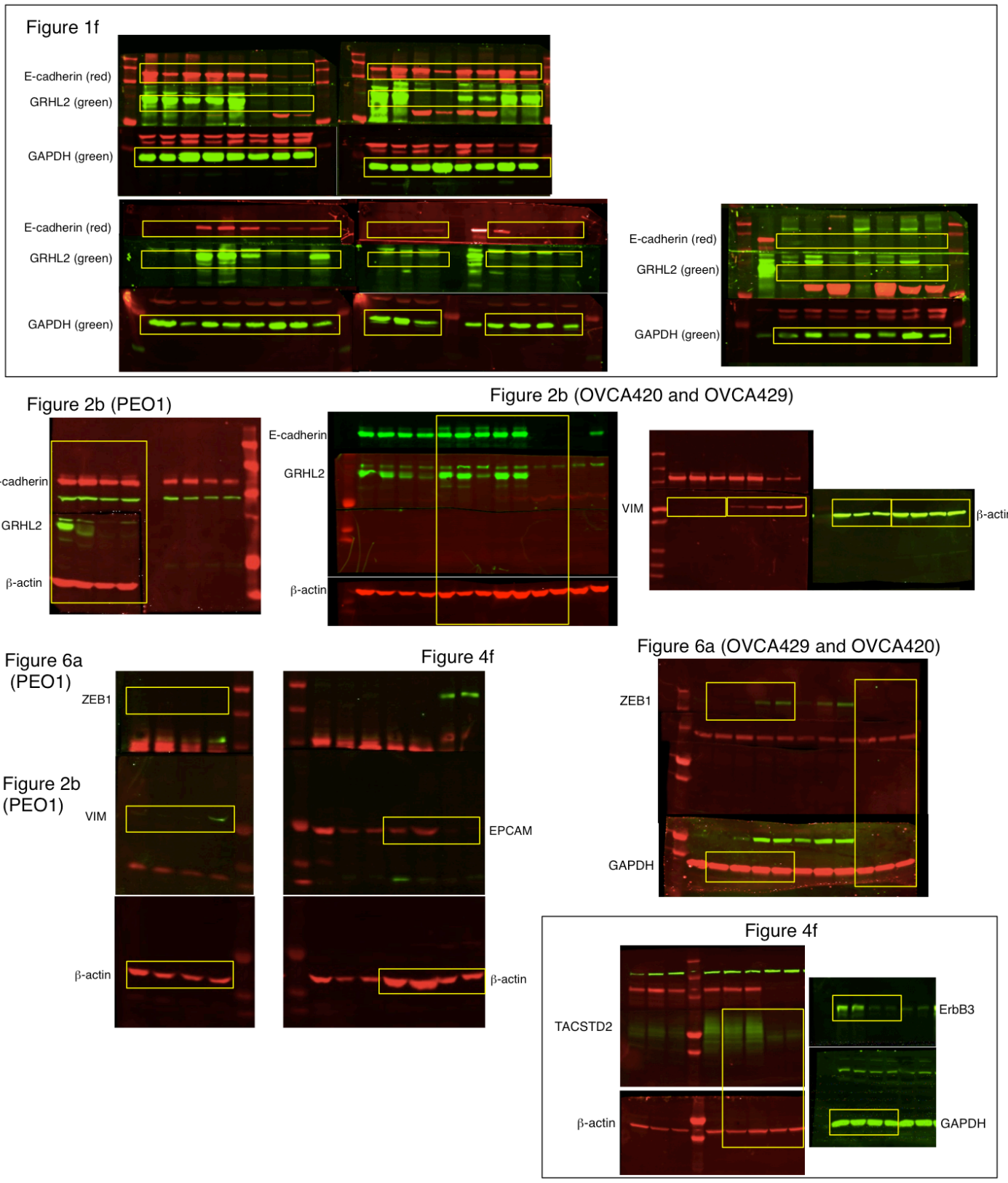


Supplementary Figure S8. Ectopic overexpression of GRHL2 in SKOV3, OV56 and HeyA8 cell lines.

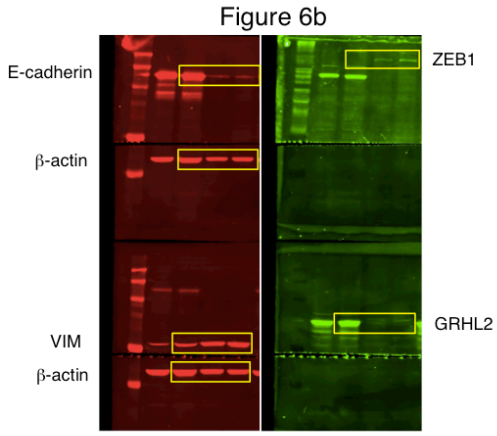
(**a**) Western blots of E-cadherin, GRHL2 and β-actin in parental, empty vector (EV) control, and GRHL2-overexpressing cells of three cell lines: SKOV3, OV56, HeyA8. Full-length blots are presented in Supplementary Figure S10. (**b**) Phase-contrast images showing the cell morphologies of SKOV3, OV56, HeyA8 cells infected with empty vector (EV) control and GRHL2-overexpressing plasmids. Scale = 100 μm.

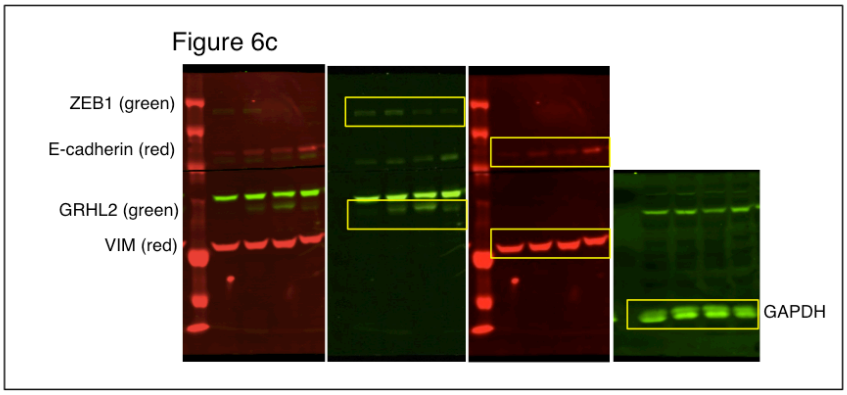


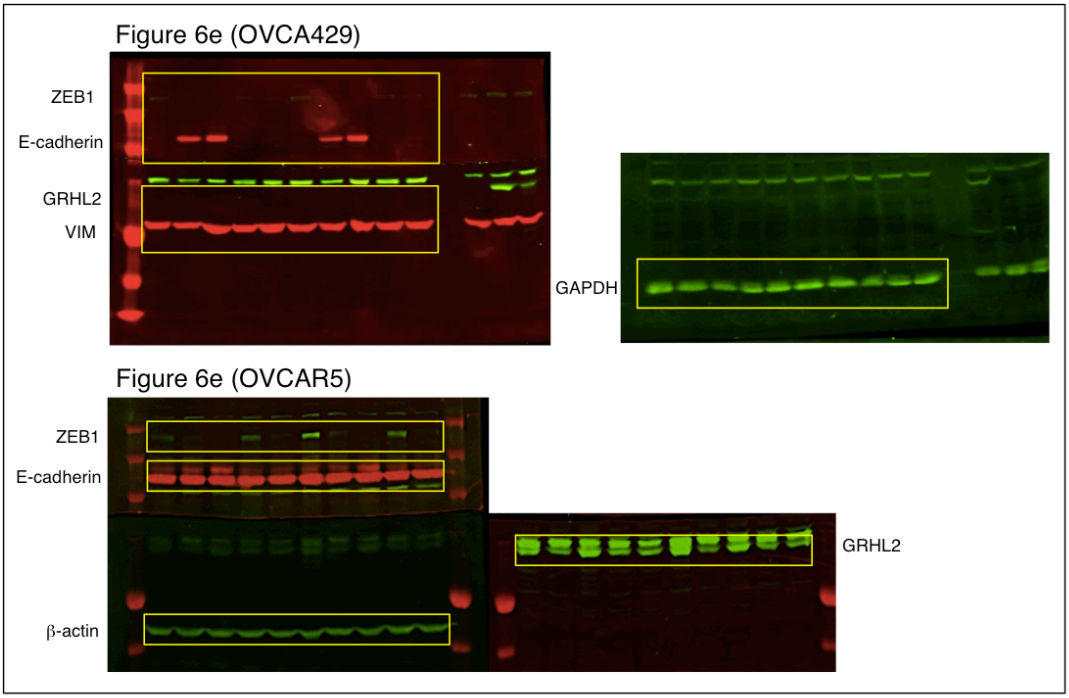
Supplementary Figure S9. The binding of GRHL2 at its target sites in OVCA429 control, shGRHL2 #12 GFP and GRHL2*-overexpressed shGRHL2 #12 cells. GRHL2 ChIP-qPCR in OVCA429 shLuc, shGRHL2 #12 GFP and shGRHL2 #12 GRHL2* cells at the regulatory elements of *MIR200B/200A/429*, *MIR203A*, *CDH1*, *CLDN4*, and *RAB25*. Signals of IgG control and ChIP samples were normalized to input DNA and presented as % input.

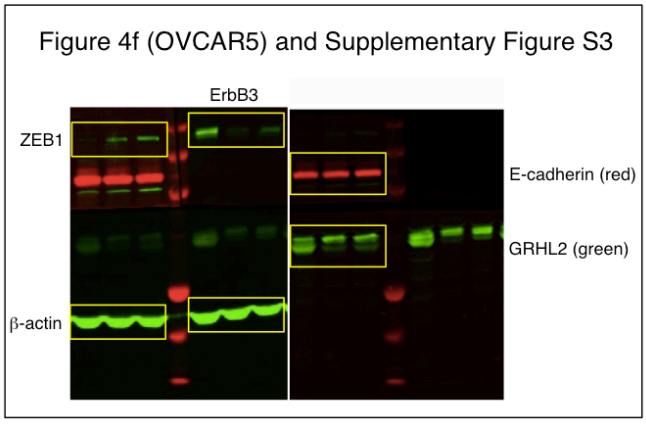


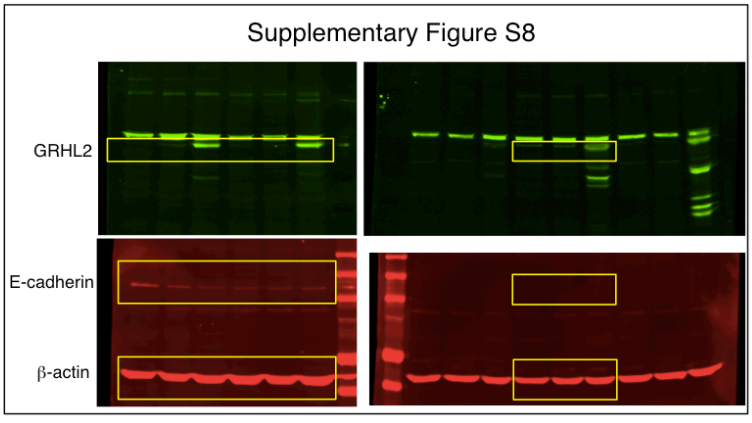
Supplementary Figure S10. Full-length blots.











Supplementary Figure S10 (continued).

Supplementary Tables

Supplementary Table S1. Expression changes of molecular subtype and EMT signature genes in GRHL2-knockdown OVCA429.

Table shows the mean log2 fold change of molecular subtype and EMT signature genes in shGRHL2 #10 and shGRHL2 #12 (5 samples in total) OVCA429 cells compared to control (shNon) based on GeneChip® Human Gene 2.0 ST Array.

probeset	Gene	Mean log2 fold change	Consistency in 5 samples	Subtype signatures	EMT signatures
16703715	*ZEB1*	0.379867472	5	Mes_Up	Mes
17049676	*SERPINE1*	1.360756325	5	Mes_Up	---
16897719	*CCDC88A*	1.368775499	5	EpiA_Dn	Mes
17078610	*IMPA1*	0.538482868	5	EpiA_Dn	Mes
16755878	*CHPT1*	0.43543463	5	EpiA_Dn	---
16832147	*ALDH3A2*	0.312655046	5	EpiA_Dn	---
17014839	*C6orf120*	0.640869627	5	---	Mes
17005138	*CAP2*	0.297158459	5	---	Mes
16854437	*CDH2*	0.793885546	5	---	Mes
16800871	*EID1*	0.614386046	5	---	Mes
16970404	*FGF2*	1.191353807	5	---	Mes
16906835	*PGAP1*	0.660551645	5	---	Mes
16691046	*PHTF1*	0.406282278	5	---	Mes
16792226	*SEC23A*	0.371906483	5	---	Mes
17113980	*SMARCA1*	0.836271972	5	---	Mes
16790267	*SUPT16H*	0.463567083	5	---	Mes
16702767	*VIM*	0.73864527	5	---	Mes
16968331	*FGF5*	0.389371311	4	Mes_Up	---
16896346	*FEZ2*	0.220701263	4	Mes_Up	---
17050591	*MET*	0.568297792	4	EpiA_Up	---
17056426	*PDE1C*	0.415867346	4	EpiA_Up	---
16878414	*MRPL33*	0.235583493	4	EpiA_Up	---
16780808	*KDELC1*	0.26957135	4	EpiA_Dn	Mes
16850477	*TYMS*	1.173964775	4	EpiA_Dn	---
16828450	*CFDP1*	0.474727562	4	EpiA_Dn	---
16812717	*BTBD1*	0.292358516	4	EpiA_Dn	---
16943681	*DZIP3*	0.268736522	4	EpiA_Dn	---
17117484	*TACC2*	0.246212394	4	EpiA_Dn	---
16905292	*CHN1*	0.310677891	4	---	Mes
16966649	*DCUN1D4*	0.487320578	4	---	Mes
16847321	*HEATR6*	0.341561859	4	---	Mes
16981364	*NEK1*	0.271764004	4	---	Mes
16806914	*NOP10*	0.40776221	4	---	Mes
16772493	*STX2*	0.550816438	4	---	Mes
16675334	*TROVE2*	0.583041148	4	---	Mes
16946513	*TRPC1*	0.43825924	4	---	Mes
16919567	*SDC4*	0.393103945	4	---	Epi
16754373	*GLIPR1*	0.271395018	3	Mes_Up	---
16840318	*NLRP1*	0.231051027	3	Mes_Up	---
16731461	*NNMT*	0.166710594	3	Mes_Up	---
16950989	*WNT7A*	0.213921448	3	EpiA_Up	---
16765389	*ATP5G2*	0.258602723	3	EpiA_Dn	Mes
16708179	*CUTC*	0.254735074	3	EpiA_Dn	Mes
16812011	*HMG20A*	0.190601096	3	EpiA_Dn	Mes
16955511	*PDHB*	0.21342514	3	EpiA_Dn	Mes
17022529	*WASF1*	0.329399999	3	EpiA_Dn	Mes
16781695	*ARHGEF40*	0.227221572	3	---	Mes
16840902	*AURKB*	0.293902814	3	---	Mes
16868130	*CD320*	0.157148038	3	---	Mes
16939247	*EXOG*	0.144691024	3	---	Mes
16690139	*EXTL2*	0.239413911	3	---	Mes

17087191	*HABP4*	0.451069501	3	---	Mes
16701324	*HNRNPU*	0.300494888	3	---	Mes
16703068	*MLLT10*	0.216686901	3	---	Mes
17022333	*OSTM1*	0.350622677	3	---	Mes
16970673	*PHF17*	0.135257405	3	---	Mes
16855026	*PIAS2*	0.465384287	3	---	Mes
16987125	*POLR3G*	0.302812326	3	---	Mes
17014562	*QKI*	0.188964432	3	---	Mes
16721861	*SWAP70*	0.487974308	3	---	Mes
16866724	*TCF3*	0.253140361	3	---	Mes
16869876	*WIZ*	0.089881887	3	---	Mes
17097375	*ZFP37*	0.217851497	3	---	Mes
16663051	*ZNF643*	0.246162986	3	---	Mes
16961616	*TNFSF10*	0.302585532	3	---	Epi
16846157	*PRR15L*	0.263856245	3	---	Epi
16841662	*TRIM16*	0.099878654	3	---	Epi
17084669	*RUSC2*	-0.174938576	-3	Mes_Up	---
16865590	*EPS8L1*	-0.123828372	-3	EpiA_Up	Epi
16799690	*SPINT1*	-0.153667178	-3	EpiA_Up	Epi
16733473	*ST14*	-0.160362514	-3	EpiA_Up	Epi
16991991	*WWC1*	-0.184467935	-3	EpiA_Up	Epi
16727232	*CST6*	-0.250536375	-3	EpiA_Up	Epi
17079477	*STK3*	-0.15998884	-3	EpiA_Up	---
16666336	*ST6GALNAC5*	-0.16982067	-3	EpiA_Up	---
16918755	*FER1L4*	-0.187311181	-3	EpiA_Up	---
16919399	*JPH2*	-0.220328153	-3	EpiA_Up	---
16702351	*GATA3*	-0.23649814	-3	EpiA_Up	---
16823719	*FAM86A*	-0.150107505	-3	EpiA_Dn	---
16705051	*CISD1*	-0.185121804	-3	EpiA_Dn	---
16719088	*NSMCE4A*	-0.235934966	-3	EpiA_Dn	---
16840284	*C1QBP*	-0.213699476	-3	---	Mes
16845219	*CCR10*	-0.257962531	-3	---	Mes
16924719	*CCT8*	-0.169012479	-3	---	Mes
16712991	*MTPAP*	-0.181114525	-3	---	Mes
16672779	*PPOX*	-0.163075447	-3	---	Mes
17015277	*RPP40*	-0.22916957	-3	---	Mes
16977016	*SDAD1*	-0.320691444	-3	---	Mes
17009193	*SLC29A1*	-0.201386037	-3	---	Mes
16867088	*TBXA2R*	-0.189541146	-3	---	Mes
16823666	*PPL*	-0.144019778	-3	---	Epi
16898788	*TGFA*	-0.147428052	-3	---	Epi
16753030	*PIP4K2C*	-0.172588124	-3	---	Epi
16720268	*EPS8L2*	-0.177340409	-3	---	Epi
17030426	*DDR1*	-0.193237412	-3	---	Epi
17033131	*DDR1*	-0.193949128	-3	---	Epi
17037907	*DDR1*	-0.193949128	-3	---	Epi
17027581	*DDR1*	-0.21066188	-3	---	Epi
16861630	*SPINT2*	-0.210694707	-3	---	Epi
16695490	*F11R*	-0.265460921	-3	---	Epi
16745002	*MPZL2*	-0.287830396	-3	---	Epi
17050522	*TES*	-0.319898883	-3	---	Epi
16662322	*GJB3*	-0.215511195	-4	EpiA_Up	Epi
16664218	*TSPAN1*	-0.231627778	-4	EpiA_Up	Epi
16893709	*SH3YL1*	-0.261561085	-4	EpiA_Up	Epi
16840599	*CLDN7*	-0.304040841	-4	EpiA_Up	Epi
17010461	*MYO6*	-0.777588228	-4	EpiA_Up	Epi
16869643	*GIPC1*	-0.294236919	-4	EpiA_Up	---
16764817	*KRT81*	-0.479493411	-4	EpiA_Up	---
16692724	*ANP32E*	-0.225426717	-4	---	Mes
16660503	*CDC42*	-0.24501568	-4	---	Mes
16778688	*NUFIP1*	-0.286294865	-4	---	Mes
16769007	*UHRF1BP1L*	-0.263837071	-4	---	Mes
16833876	*GRB7*	-0.179310548	-4	---	Epi
16908338	*TMBIM1*	-0.187492717	-4	---	Epi

16661314	*SFN*	-0.201540248	-4	---	Epi
16733288	*ST3GAL4*	-0.210965169	-4	---	Epi
17024187	*PERP*	-0.247570088	-4	---	Epi
16674130	*KIAA0040*	-0.257499416	-4	---	Epi
16844775	*KRT19*	-0.307649391	-4	---	Epi
16968213	*ANXA3*	-0.554525896	-4	---	Epi
16860946	*LSR*	-0.351938743	-5	Mes_Dn	Epi
17058601	*CLDN3*	-0.260280288	-5	EpiA_Up	Epi
17071497	*GRHL2*	-0.281223533	-5	EpiA_Up	Epi
16874693	*KLK6*	-0.288243544	-5	EpiA_Up	Epi
17107309	*VGLL1*	-0.380250773	-5	EpiA_Up	Epi
16904551	*GALNT3*	-0.444640436	-5	EpiA_Up	Epi
16665373	*INADL*	-0.490899515	-5	EpiA_Up	Epi
16957304	*ZBED2*	-0.519165498	-5	EpiA_Up	Epi
17070949	*ESRP1*	-0.523708165	-5	EpiA_Up	Epi
16820486	*CDH1*	-0.579192164	-5	EpiA_Up	Epi
17089525	*LCN2*	-0.817789812	-5	EpiA_Up	Epi
16904193	*ITGB6*	-1.874703719	-5	EpiA_Up	Epi
16778849	*LCP1*	-0.706980921	-5	EpiA_Up	---
17043982	*ITGB8*	-1.043347754	-5	EpiA_Up	---
16984689	*ITGA2*	-1.068027801	-5	EpiA_Up	---
16858321	*CARM1*	-0.233360651	-5	---	Mes
16820737	*DDX19A*	-0.514021193	-5	---	Mes
16671901	*RAB25*	-0.242807794	-5	---	Epi
16965915	*C4orf19*	-0.2486128	-5	---	Epi
16748529	*GPRC5A*	-0.299486836	-5	---	Epi
17024053	*MAP7*	-0.333257656	-5	---	Epi
16820463	*CDH3*	-0.368637439	-5	---	Epi
16723569	*EHF*	-0.382435719	-5	---	Epi
16838330	*SYNGR2*	-0.471763344	-5	---	Epi
16775546	*SCEL*	-0.873303915	-5	---	Epi
16689869	*F3*	-0.895222509	-5	---	Epi
16687847	*TACSTD2*	-1.012206028	-5	---	Epi
17011302	*AIM1*	-1.401691436	-5	---	Epi
17117110	*CD24*	-1.819563843	-5	---	Epi
16879863	*EPCAM*	-2.117373855	-5	---	Epi

Supplementary Table S2. GRHL2 target genes with their respective GRHL2 binding sites identified by ChIP-seq in EOC cells.

Table shows the GRHL2 ChIP-seq peaks associated with *ARHGEF19, CDH1, CLDN4, DRAM1, EEA1, ELF3, EPCAM, EPS8, ERBB3, ESRP1/2, GRHL1/2, MAL2, PRSS8, SLC44A2, SPINT1, RAB25, ST14, TACSTD2* and *ZEB1* genes detected in OVCAR3, PEO1 and OVCA429, with their respective peak length, MACS (Model-based analysis of ChIP-seq) score, fold enrichment, distance to TSS and genomic annotation. ChIP-seq peaks validated by ChIP-qPCR (Supplementary Figure S5) are marked ■.

Gene	Cell line	No. of Peaks	Peak Length	Score	Enrichment	Distance to TSS (base)	Genomic Annotation	
ARHGEF19	OVCAR3	1	292	71	54.62	-138	Promoter	
	PEO1	1	293	41	75.26	-137	Promoter	
	OVCA429	1	486	136	65.95	-147	Promoter	
CDH1	OVCAR3	1	282	25	68.02	7602	Intron 2	■
	PEO1	1	594	126	231.28	7621	Intron 2	■
	OVCA429	3	749	179	260.42	7624	Intron 2	■
			616	35	51.96	41716	Intron 2	
			253	32	39.12	52942	Intron 2	
CLDN4	OVCAR3	2	205	33	50.77	-5672	Intergenic	
			328	37	56.93	1525	3' UTR	
	PEO1	4	326	73	58.37	-5668	Intergenic	
			658	62	51.32	45	5' UTR	■
			578	203	162.32	1514	3' UTR	
			299	34	34.06	2659	TTS	
	OVCA429	5	395	158	173.75	-5680	Intergenic	
			317	73	65.97	-1140	Intergenic	
			678	126	87.29	20	5' UTR	■
			586	174	84.38	1509	3' UTR	
			488	25	25.85	2639	TTS	
DRAM1	PEO1	2	280	38	24.93	194	5' UTR	
			482	72	112.75	17581	Intron 1	
	OVCA429	3	392	64	55.42	191	5' UTR	
			398	69	50.19	17597	Intron 1	
			193	26	37.83	31251	Intron 4	
EEA1	OVCAR3	1	233	27	41.54	-488	Promoter	
	PEO1	1	505	109	87.16	-493	Promoter	
	OVCA429	1	608	149	54.19	-474	Promoter	
ELF3	OVCAR3	4	241	67	103.09	-2240	Intergenic	
			490	45	69.24	-158	Promoter	
			355	92	70.78	24416	Intergenic	
			377	132	187.1	35892	Intergenic	
	PEO1	4	607	191	114.54	-2235	Intergenic	
			705	111	80.39	-155	Promoter	
			342	195	154.07	24414	Intergenic	
			493	220	75.39	35895	Intergenic	
	OVCA429	6	541	205	99.42	-2239	Intergenic	
			499	118	116.79	-149	Promoter	
			453	149	54.19	24416	Intergenic	
			543	241	175.31	35890	Intergenic	
			455	29	21.1	46803	Intergenic	

			252	40	51.96	90650	Intergenic
EPCAM	PEO1	1	675	87	100.82	-29143	Intergenic
	OVCA429	1	664	82	114.38	-29133	Intergenic
EPS8	PEO1	1	574	108	43.18	-136	Promoter
	OVCA429	1	784	114	82.93	-145	Promoter
ERBB3	OVCAR3	1	617	31	60.64	-213	Promoter
	PEO1	1	1167	149	132.82	-248	Promoter
	OVCA429	2	1224	197	157.48	-247	Promoter
			334	59	72.99	6197	TTS (variant s)
ESRP1	OVCA429	1	290	52	25.22	16087	Intron 4
ESRP2	OVCAR3	1	585	119	176.05	2019	Intron 3
	PEO1	1	575	101	121.14	2031	Intron 3
	OVCA429	1	819	158	114.94	2029	Intron 3
GRHL1	OVCAR3	1	284	67	34.36	147	5'UTR
	PEO1	3	358	177	84.92	-9874	Intergenic
			327	78	31.18	141	5'UTR
			250	72	59.6	40585	Intron 11
	OVCA429	4	352	42	48.5	-9864	Intergenic
			322	80	29.1	80	5'UTR
			316	43	59.19	19600	Intron 8
			335	61	72.86	40581	Intron 11
GRHL2	OVCAR3	3	361	119	202.65	-55188	Intergenic
			339	68	108.76	-53813	Intergenic
			468	133	204.63	182	5'UTR
	PEO1	4	667	186	111.54	-55176	Intergenic
			264	25	14.49	-53809	Intergenic
			1001	271	108.35	228	5'UTR
			279	30	17.99	12293	Intron 1
	OVCA429	3	637	135	175.36	-55149	Intergenic
			411	33	36.1	-53863	Intergenic
			763	248	257.72	250	5'UTR
MAL2	PEO1	4	458	31	29.13	-64135	Intergenic
			428	73	35.02	-7461	Intergenic
			672	145	69.56	-3015	Intergenic
			254	40	23.99	-1070	Intergenic
	OVCA429	1	403	66	76.21	-3024	Intergenic
PRSS8	OVCAR3	2	281	59	171.3	4795	TTS of *PRSS8*
			439	35	53.85	-3238	TTS of *PRSS36*
	PEO1	2	837	216	64.77	4819	TTS of *PRSS8*
			534	113	105.04	-3238	TTS of *PRSS36*
	OVCA429	2	958	184	239.01	4816	TTS of *PRSS8*
			800	176	250.55	-3243	TTS of *PRSS36*
SLC44A2	OVCAR3	1	345	183	270.73	-4	Promoter
	PEO1	2	528	147	88.16	-7599	Intron 1 of isoform 2
			990	275	131.93	-9	Promoter
	OVCA429	3	595	64	95.01	-21967	Intron 1 of isoform 2
			521	127	184.77	-7592	Intron 1 of isoform 2
			780	278	67.41	-4	Promoter
SPINT1	OVCAR3	3	478	72	110.78	13297	3'UTR of *SPINT1*

			280	35	26.93	27523	Intergenic	
			290	54	70.23	27956	3'UTR of *RHOV*	
	PEO1	4	620	180	136.63	13296	3'UTR of *SPINT1*	
			389	31	18.59	21614	Intergenic	
			137	31	18.59	27524	Intergenic	
			281	40	23.99	27961	3'UTR of *RHOV*	
	OVCA429	2	611	179	130.21	13308	3'UTR of *SPINT1*	
			803	67	89.84	27956	3'UTR of *RHOV*	
RAB25	OVCAR3	1	379	113	86.93	-82	Promoter	■
	PEO1	1	466	174	163.49	-92	Promoter	■
	OVCA429	1	520	205	149.12	-87	Promoter	■
ST14	OVCAR3	1	269	39	60.01	412	Intron 1	
	PEO1	3	360	42	50.35	-13185	Intergenic	
			421	73	57.68	391	Intron 1	
			311	27	21.59	6790	Intron 1	
	OVCA429	3	466	46	45.53	-13216	Intergenic	
			359	67	32.49	413	Intron 1	
			273	26	30.7	6793	Intron 1	
TACSTD2	OVCAR3	1	328	91	70.01	209	5' UTR	
	PEO1	3	226	40	46.35	4607	Intergenic	
			611	240	143.86	192	5' UTR	
			230	28	13.43	-15203	Intergenic	
	OVCA429	3	292	53	44.06	4614	Intergenic	
			796	272	176.66	195	5' UTR	
			362	38	37.61	-42930	Intergenic	
ZEB1	OVCA429	1	286	25	18.19	32704	Intron 1	

TSS=Transcription start site; TTS=Transcription termination site; UTR=Untranslated region

Supplementary Table S3. MicroRNA genes with their respective GRHL2 binding sites identified by ChIP-seq in EOC cells.

Table shows the GRHL2 ChIP-seq peaks in the vicinity of *MIR203A*, *MIR200B*, *MIR205*, *MIR205HG*, *MIR21*, *MIRLET7G*, *MIR1908*, *MIR4284* genes in OVCAR3, PEO1 and OVCA429 cells with their respective peak length, MACS (Model-based analysis of ChIP-seq) score, fold enrichment, distance to miRNA gene and genomic annotation. ChIP-seq peaks validated by ChIP-qPCR (Figure 5) are marked ■.

Gene	Cell line	No. of Peaks	Peak Length	Score	Enrichment	Distance to miRNA hairpin (base)	Genomic Annotation	
MIR203A	OVCAR3	2	253	54	41.54	-993	Promoter	
			551	194	208.73	-369	Promoter	■
	PEO1	1	1090	223	133.73	-375	Promoter	■
	OVCA429	1	1189	205	163.87	-372	Promoter	■
MIR200B	OVCAR3	2	325	45	11.54	-3846	CpG	■
			202	25	12.82	-2296	CpG	
	PEO1	1	458	51	61.17	-3859	CpG	■
	OVCA429	1	653	41	29.82	-3863	CpG	■
MIR205	PEO1	1	258	33	60.57	1259	TTS of MIR205HG	
	OVCA429	1	291	59	21.46	1273		
MIR205HG	OVCA429	3	343	65	89.47	-68539	Intergenic	
			294	41	38.73	-18767	Intergenic	
			446	38	44.62	-12752	Intergenic	
MIR205HG/ MIR205	OVCA429	1	399	37	38.45	23 (MIR205HG)	Promoter of *MIR205HG*	
						-3287 (MIR205)		
MIR21	OVCAR3	1	189	33	60.09	-3796	Intron 10 of gene *VMP1*	
	PEO1	2	801	127	233.12	-3798		
			337	38	45.58	5180	Intergenic	
	OVCA429	4	521	37	59.15	-11695	Intergenic	
			884	230	334.62	-3793	Intron 10 of gene *VMP1*	
			349	88	99.94	5167	Intergenic	
			417	43	20.85	16100	Intergenic	
MIRLET7G	PEO1	1	243	39	46.78	2821	Intron 3 of gene *WDR82*	
	OVCA429	1	500	148	255.93	2837		
MIR1908	OVCA429	1	467	38	40.3	-117	Promoter of gene *FADS2*	
MIR4284	OVCA429	1	293	27	19.35	-4685	Intron 3 of gene *STX1A*	

TTS=Transcription termination site; HG=Host Gene

Supplementary Table S4. Differential expression of miR-203, miR-205 and miR-200 family in OVCA429 control and GRHL2-knockdown cells.

Table shows the log2 fold expression changes (<0 means down-regulation) of miR-200 family members, miR-203 and miR-205, detected from GeneChip® miRNA 2.0 microarray in shGRHL2 #10 and shGRHL2 #12 OVCA429 cells with respect to control (shNon). Only probesets with good detection above background (DABG) signals (p <0.05) are shown. Correlation with GRHL2 expression was checked by Spearman's correlation tests.

miRNA	Probeset ID	Mean log2 fold change shGRHL2 #10	Mean log2 fold change shGRHL2 #12	Mean log2 fold change (combined)	Spearman Rho
miR-200a-3p	hsa-miR-200a_st	-2.5501	-4.4951	-3.5226	0.9143
miR-200a-5p	hsa-miR-200a-star_st	-2.9038	-1.8138	-2.3588	0.3143
miR-200b-3p	hsa-miR-200b_st	-3.3550	-3.4100	-3.3825	0.8286
precursor of miR-200b	hp_hsa-mir-200b_x_st	-1.2102	-1.0752	-1.1427	0.7714
miR-429	hsa-miR-429_st	-0.5309	-0.5409	-0.5359	0.4571
miR-200c-3p	hsa-miR-200c_st	-2.2310	-2.7360	-2.4835	0.7333
miR-200c-5p	hsa-miR-200c-star_st	-2.1956	-2.6356	-2.4156	0.9143
precursor of miR-200c	hp_hsa-mir-200c_st	-1.4083	-2.0183	-1.7133	0.8286
miR-141	hsa-miR-141_st	-4.6551	-5.6601	-5.1576	0.8286
precursor of miR-141	hp_hsa-mir-141_st	-0.4612	-0.6362	-0.5487	0.8286
miR-203	hsa-miR-203_st	-3.0891	-5.5841	-4.3366	0.8286
miR-205-5p	hsa-miR-205_st	-4.2439	-2.7839	-3.5139	0.4952
miR-205-3p	hsa-miR-205-star_st	-1.2111	-1.4111	-1.3111	0.6667
precursor of miR-205	hp_hsa-mir-205_st	-0.5031	-0.2231	-0.3631	0.5810

Supplementary Table S5. EOC cell lines with their respective phenotypes and culture media.

Cell lines	EMT Phenotype	Media
A1847	Mesenchymal	RPMI 1640, 10% FBS, 10 µg/ml insulin
A2008	Epithelial	RPMI 1640, 10% FBS
A2780	Mesenchymal	RPMI 1640, 10% FBS
BG1	Intermediate Mesenchymal	DMEM, 10% FBS, 1 µg/ml insulin
C13	Epithelial	RPMI 1640, 10% FBS
Caov2	Intermediate Epithelial	RPMI 1640, 10% FBS
Caov3	Epithelial	DMEM, 10% FBS
CH1	Intermediate Mesenchymal	DMEM, 10% FBS
COLO720E	Mesenchymal	RPMI 1640, 10% FBS
DOV13	Intermediate Mesenchymal	DMEM, 10% FBS
EFO21	Intermediate Epithelial	RPMI 1640, 20% FBS, 1x NEAA
FUOV1	Intermediate Epithelial	DMEM/F12 (1:1), 10% FBS
Hey	Intermediate Mesenchymal	RPMI 1640, 10% FBS
HeyA8	Mesenchymal	RPMI 1640, 10% FBS
HeyC2	Intermediate Mesenchymal	RPMI 1640, 10% FBS
IGROV1	Intermediate Epithelial	RPMI 1640, 10% FBS
JHOS2	Intermediate Epithelial	DMEM/F12 (1:1), 10% FBS, 0.1 mM NEAA
JHOS3	Intermediate Epithelial	DMEM/F12 (1:1), 10% FBS, 0.1 mM NEAA
JHOS4	Intermediate Epithelial	DMEM/F12 (1:1), 10% FBS, 0.1 mM NEAA
OAW28	Intermediate Epithelial	DMEM, 10% FBS, 0.7 µg/ml insulin
OAW42	Intermediate Epithelial	DMEM, 10% FBS, 0.7 µg/ml insulin
OV17R	Intermediate Epithelial	DMEM/F12 (1:1), 5% FBS, 0.4 µg/ml hyrocortisone, 10 µg/ml insulin
OV2008	Epithelial	RPMI 1640, 10% FBS, 1x NEAA
OV56	Intermediate Epithelial	DMEM/F12 (1:1), 5% FBS, 0.5 µg/ml hyrocortisone, 10 µg/ml insulin
OV7	Intermediate Mesenchymal	DMEM/F12 (1:1), 5% FBS, 0.5 µg/ml hyrocortisone, 10 µg/ml insulin
OV90	Epithelial	MCDB105/M199 (1:1), 10% FBS
OVCA420	Epithelial	DMEM, 10% FBS
OVCA429	Intermediate Epithelial	DMEM, 10% FBS
OVCA432	Intermediate Epithelial	DMEM, 10% FBS
OVCA433	Intermediate Epithelial	DMEM, 10% FBS
OVCAR10	Mesenchymal	RPMI 1640, 10% FBS, 10 µg/ml insulin
OVCAR2	Intermediate Epithelial	RPMI 1640, 10% FBS, 10 µg/ml insulin
OVCAR3	Epithelial	RPMI 1640, 20% FBS, 10 µg/ml insulin
OVCAR5	Intermediate Epithelial	RPMI 1640, 10% FBS, 10 µg/ml insulin
OVCAR8	Epithelial	RPMI 1640, 10% FBS, 10 µg/ml insulin
OVK18	Mesenchymal	DMEM, 10% FBS
PEO1	Epithelial	RPMI 1640, 10% FBS
PEO4	Intermediate Epithelial	RPMI 1640, 10% FBS, 2.5 µg/ml insulin, 1x NEAA
SKOV3	Intermediate Mesenchymal	DMEM, 10% FBS
TOV112D	Mesenchymal	MCDB105/M199 (1:1), 10% FBS
TykNu	Mesenchymal	DMEM, 10% FBS
UWB1.289	Intermediate Epithelial	RPMI 1640/ MEGM (1:1), 3% FBS

Supplementary Table S6. 44 EOC patient samples and their respective molecular subtypes.

Tumour Sample	Molecular Subtype
JPKO 001	EpiA
JPKO 002	StemA
JPKO 005	Mes
JPKO 007	StemB
JPKO 008	EpiA
JPKO 009	EpiA
JPKO 010	EpiA
JPKO 013	StemB
JPKO 021	StemB
JPKO 023	StemB
JPKO 024	EpiA
JPKO 025	EpiA
JPKO 027	EpiA
JPKO 031	StemB
JPKO 033	StemB
JPKO 036	StemA
JPKO 045	StemA
JPKO 049	StemA
JPKO 055	EpiA
JPKO 056	EpiA
JPKO 060	StemB
JPKO 061	EpiB

Tumour Sample	Molecular Subtype
JPKO 063	StemB
JPKO 064	StemB
JPKO 065	EpiB
JPKO 069	StemA
JPKO 070	EpiA
JPKO 079	Mes
JPKO 080	StemA
JPKO 081	StemA
JPKO 082	EpiB
JPKO 083	Mes
JPKO 085	EpiB
JPKO 089	Mes
JPKO 095	StemB
JPKO 098	Mes
JPKO 099	StemA
JPKO 101	Mes
JPKO 102	Mes
JPKO 103	EpiB
JPKO 104	Mes
JPKO 105	Mes
JPKO 106	EpiB
JPKO 110	EpiB

Supplementary Table S7. RT-qPCR primers.
Primers purchased from Qiagen and used for RT-qPCR in this study.

Gene	Catalog no.	Ref Seq Accession no.
ACTB	PPH00073E	NM_001101.3
B2M	PPH01094E	NM_004048.2
GAPDH	PPH00150E	NM_002046.3
HPRT1	PPH01018B	NM_000194.2
RPL13A	PPH01020B	NM_012423.2
GRHL1	PPH19832A	NM_198182.2
GRHL2	PPH18929F	NM_024915.3
GRHL3	PPH12149A	NM_198174.2
ZEB1	PPH01922A	NM_030751.5
ZEB2	PPH09021B	NM_014795
SNAI1	PPH02459B	NM_005985
SNAI2	PPH02475A	NM_003068
TWIST1	PPH02132A	NM_000474.3
CDH1	PPH00135E	NM_004360.3
CDH2	PPH00636F	NM_001792.3
VIM	PPH00417E	NM_003380.3
CLDN4	PPH07330D	NM_001305.3
RAB25	PPH12800E	NM_020387.2
TACSTD2	PPH05688A	NM_002353.2
ERBB3	PPH00463B	NM_001982.3
ELF3	PPH09786C	NM_004433.4
EPS8	PPH07120A	NM_004447
ST14	PPH09550A	NM_021978.3
EPCAM	PPH05720A	NM_002354.2
MAL2	PPH10025A	NM_052886.2
ESRP1	PPH088556A	NM_017697.3
ESRP2	PPH18162A	NM_024939.2
EEA1	PPH07165A	NM_003566
DRAM1	PPH19768F	NM_018370.2
ARHGEF19	PPH22922A	NM_153213.3

Gene	Name of gene product	Catalog no.
RNU6-6P	U6 snRNA 6, pseudogene	MS00033740
MIR200A	hsa-miR-200a-3p	MS00003738
MIR200B	hsa-miR-200b-3p	MS00009016
	hsa-miR-200b-5p	MS00009023
MIR200C	hsa-miR-200c-3p	MS00003752
MIR203A	hsa-miR-203a-3p	MS00003766
MIR203B	hsa-miR-203b-5p	MS00042217
MIR205	hsa-miR-205-5p	MS00003780

Supplementary Table S8. ChIP-qPCR primers.

CDH1 intron 2	(forward) 5' TTCAAAAGATCCCCTGCGCT 3'
	(reverse) 5' AAGCCACAACAAACCCGTTC 3'
RAB25 promoter	(forward) 5' CAGTGGGCTGTCTCTGAAGG 3'
	(reverse) 5' CTGAGAACAGGAAGAGCGGG 3'
CLDN4 promoter	(forward) 5' GTGACCTCAGCATGGGCTTTGA 3’
	(reverse) 5' CTCCTCCTGACCAGTTTCTCTG 3’
ZEB1 promoter_1	(forward) 5' CGGTCCCTAGCAACAAGGTT 3’
	(reverse) 5' TCGCTTGTGTCTAAATGCTCG 3'
ZEB1 promoter_2	(forward) 5' GCCGCCGAGCCTCCAACTTT 3'
	(reverse) 5' TGCTAGGGACCGGGCGGTTT 3'
MIR200B/200A/429 promoter (-4548 bp)	
	(forward) 5’AGGTGGAGAGGCGAGAGTTGC 3’
	(reverse) 5’ CCAGGATGGGAAGGCTTCTGTG 3’
MIR200B/200A/429 CpG region (-3871 bp)	
	(forward) 5’ CGCAGCAGTGGAACCTGT 3’
	(reverse) 5’ GACAGCCCATCTGTCACCTG 3’
MIR203A promoter (-429 bp)	
	(forward) 5’ GGCCGTGGAGGATCAGTCG 3’
	(reverse) 5’ CTTCCCGGCGCCGGAATGT 3’