Supplementary Information

Supplementary Tables

Supplementary Table S1. The relationship between ZNF711 and clinical pathological characteristics in 150 patients with EOC.

Parameters	Number of cases	ZNF711 expression		*P* values
		Low (n=84)	High (n=66)	
Age (years)				
< 62	80	44	36	0.869
≥ 62	70	40	30	
Histological type				
Serous	119	69	50	0.185
Endometrioid	17	8	9	
Mucinous	3	3	0	
Undifferentiated	11	4	7	
FIGO stage				
I or II	53	16	37	<0.001
III or IV	97	68	29	
Histologic grade				
1	17	16	1	<0.001
2	13	4	9	
3	120	64	56	
SLC31A1 expression				
Low	81	64	17	<0.001
High	69	20	49	
Chemo-response status				
Chemosensitivity	58	26	32	0.042
Chemoresistance	92	58	34	
Survival status				
Dead	107	73	34	< 0.001
Alive	43	11	32	

Supplementary Table S2. Univariate and multivariate analysis of factors associated with over-all survival in 150 EOC patients.

Characteristics	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* values	HR (95% CI)	*P* values
Age (years)	1.067 (0.730-1.560)	0.736		
Histological type	0.956 (0.768-1.190)	0.688		
FIGO stage	1.575 (1.036 -2.393)	0.033		
Histologic grade	0.840 (0.624-1.132)	0.253		
SLC31A1 expression	0.639 (0.434-0.942)	0.024		
Chemo-response status	2.742 (1.814-4.145)	< 0.001	2.428 (1.598-3.688)	< 0.001
ZNF711 expression	0.422 (0.280-0.635)	< 0.001	0.489 (0.323-0.740)	0.001

HR, hazard ratio; CI, confidence interval.

Supplementary Table S3. Primers and Oligonucleotides

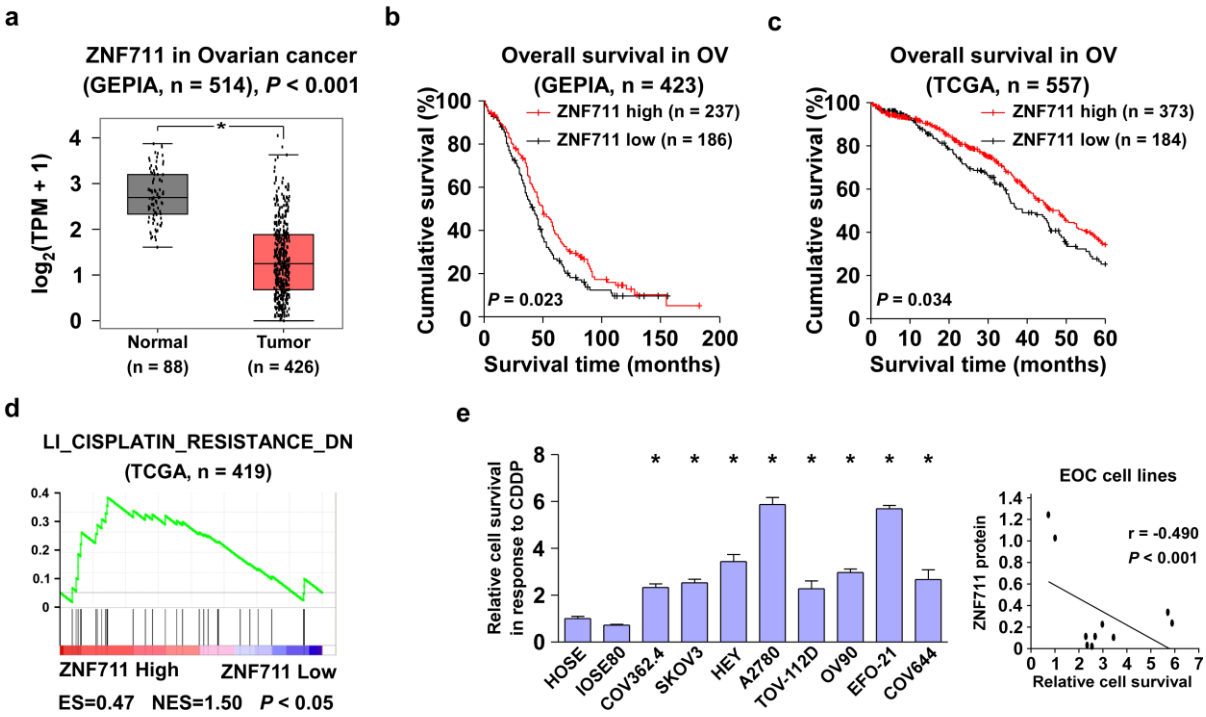
Used for sub-cloning and plasmid construction:	
Primer used for FLAG tagged ZNF711	
ZNF711-up	GccAGATCTgccATGGATTCAGGCGGTGGAAGTCTTG
ZNF711-dn	gccGAATTCgccTTACATAAGAGCCTCTTTGTGGTGC
Primer used for ZNF711 shRNA	
pSuper Retro ZNF711-RNAi#1-up	GATCCCCAUUUUCUUUGGGAAAACGCUC TTCAAGAGAUAAAAGAAACCCUUUUGCGAGTTTTTA
pSuper Retro ZNF711-RNAi#1-dn	AGCTTAAAAACUCGCAAAAGGGUUUCUUUUA TCTCTTGAAGAGCGUUUUCCCAAAGAAAAUGGG
pSuper Retro ZNF711-RNAi#2-up	GATCCCCUAAAAUCUGCAGCAAUUUCUU TTCAAGAGAAUUUUAGACGUCGUUAAAGAATTTTTA
pSuper Retro ZNF711-RNAi#2-dn	AGCTTAAAAAUUCUUUAACGACGUCUAAAAU TCTCTTGAAAAGAAAUUGCUGCAGAUUUUAGGG
Primer used for SLC31A1 promoter reporter construction	
P1-luc-up	GCCATGGACTACAAGGACGACGATGACAAGCCTCAGTAT GTCTTTTACCATCAAA
P1-luc-dn	AGTGCTAGCAAGCTTCATATCCTCCAGTCCCGAGCTCTTT CTCCT
P2-luc-up	GCCATGGACTACAAGGACGACGATGACAAGCGGGGTAG GAAGGGAACAGAGTGGT
P2-luc-dn	AGTGCTAGCAAGCTTCATATCCTCCAGTCCCGAGCTCTTT CTCCT
P3-luc-up	GCCATGGACTACAAGGACGACGATGACAAGGAACACCAT AAGCAGTCCCTGTCCG
P3-luc-dn	AGTGCTAGCAAGCTTCATATCCTCCAGTCCCGAGCTCTTT CTCCT
P4-luc-up	GCCATGGACTACAAGGACGACGATGACAAGGCACCGGA ATGTGGCAAGGGTCTCC
P4-luc-dn	AGTGCTAGCAAGCTTCATATCCTCCAGTCCCGAGCTCTTT CTCCT
P5-luc-up	GCCATGGACTACAAGGACGACGATGACAAGCCTCAGTAT GTCTTTTACCATCAAA
P5-luc-dn	AGTGCTAGCAAGCTTCATATGAGGAAGGGTTTTGTCTCTT TTAGT
P6-luc-up	GCCATGGACTACAAGGACGACGATGACAAGCAAGCTGTC CACTGGCACAACACAA

P6-luc-dn	AGTGCTAGCAAGCTTCATATTGGAGGGCGTGGCTGAGAG ATACCG
Primer used for qPCR	
ZNF711-up	ATGGCCCATACCATGATTATGC
ZNF711-dn	TCAGCTTCAAGTACCGCTTCA
SLC31A1-up	GGGGATGAGCTATATGGACTCC
SLC31A1-dn	TCACCAAACCGGAAAACAGTAG
JHDM2A-up	GTGCTCACGCTCGGAGAAA
JHDM2A -dn	GTGGGAAACAGCTCGAATGGT
siRNA	
SLC31A1 si #1	UUUUUUCCAGGAAAAGUUGAG
SLC31A1 si #2	AUUCUUAAAGCCAAAGUAGAA
JHDM2A si #1	UUCUUUUCCUCCAAGAUUCCC
JHDM2A si #2	UGUUCUACUAAAAAUGCUCUC
EHMT2 si #1	GGCAUCUCAGGAGGAUGCCAAUGAA
EHMT2 si #2	AAGAUUCUCAGAUUCAUCCCC
MSP primers for SLC31A1 promoter	
Set1/Methylated-up	TAAGAGAGATGTCGGGTTTCG
Set1/Methylated-dn	AAACCCTTACCACATTCCGATA
Set1/Unmethylated-up	TAAGAGAGATGTTGGGTTTTGAA
Set1/Unmethylated-dn	AAAAACCCTTACCACATTCCAATA
Set2/Methylated-up	TTCGTTAAGAGAGATGTCGGG
Set2/Methylated-dn	AACCCTTACCACATTCCGATA
Set2/Unmethylated-up	TTTGTTAAGAGAGATGTTGGG
Set2/Unmethylated-dn	AAAACCCTTACCACATTCCAATA
Set3/Methylated-up	TTCGTTAAGAGAGATGTCGGG
Set3/Methylated-dn	AAACCCTTACCACATTCCGATA
Set3/Unmethylated-up	TTTGTTAAGAGAGATGTTGGG
Set3/Unmethylated-dn	AAAACCCTTACCACATTCCAATA

Supplementary Table S4. Potential trans-regulatory factors were enriched on the SLC31A1 promoter in HEY/ZNF711 cells					
Protein	**Gene name**	**Log2(Fold change)**	**-10lg(P)**	**#Peptides**	**#Unique**
ZNF711_HUMAN	zinc finger protein 711	2.573	77.18	59	50
JHDM2A_HUMAN	JmjC-domain-containing histone demethylase 2A	1.789	53.68	40	32
CTNNB1_HUMAN	Catenin beta-1	1.427	42.81	32	28
POLR2A_HUMAN	RNA polymerase II subunit A	1.423	56.70	52	47
TBP_HUMAN	TATA-box binding protein	1.392	61.76	30	27
ANM1_HUMAN	Protein arginine N-methyltransferase 1	1.384	47.74	8	6
FLVCR1_HUMAN	FLVCR heme transporter 1	1.302	26.47	9	6
CHTOP_HUMAN	chromatin target of PRMT1	1.255	55.51	8	6
KMT2B_HUMAN	lysine methyltransferase 2B	1.149	14.46	5	3
CTNA1_HUMAN	catenin (cadherin-associated protein), alpha 1	1.134	32.01	5	2
SMAD5_HUMAN	SMAD family member 5	1.131	21.12	4	1
IFRD2_HUMAN	interferon related developmental regulator 2	1.130	23.89	4	1
TIF1B_HUMAN	tripartite motif containing 28	1.090	32.70	3	1
RPL36_HUMAN	60S ribosomal protein L36	1.077	19.69	3	1
KDM1A_HUMAN	lysine demethylase 1A	1.071	12.21	2	1

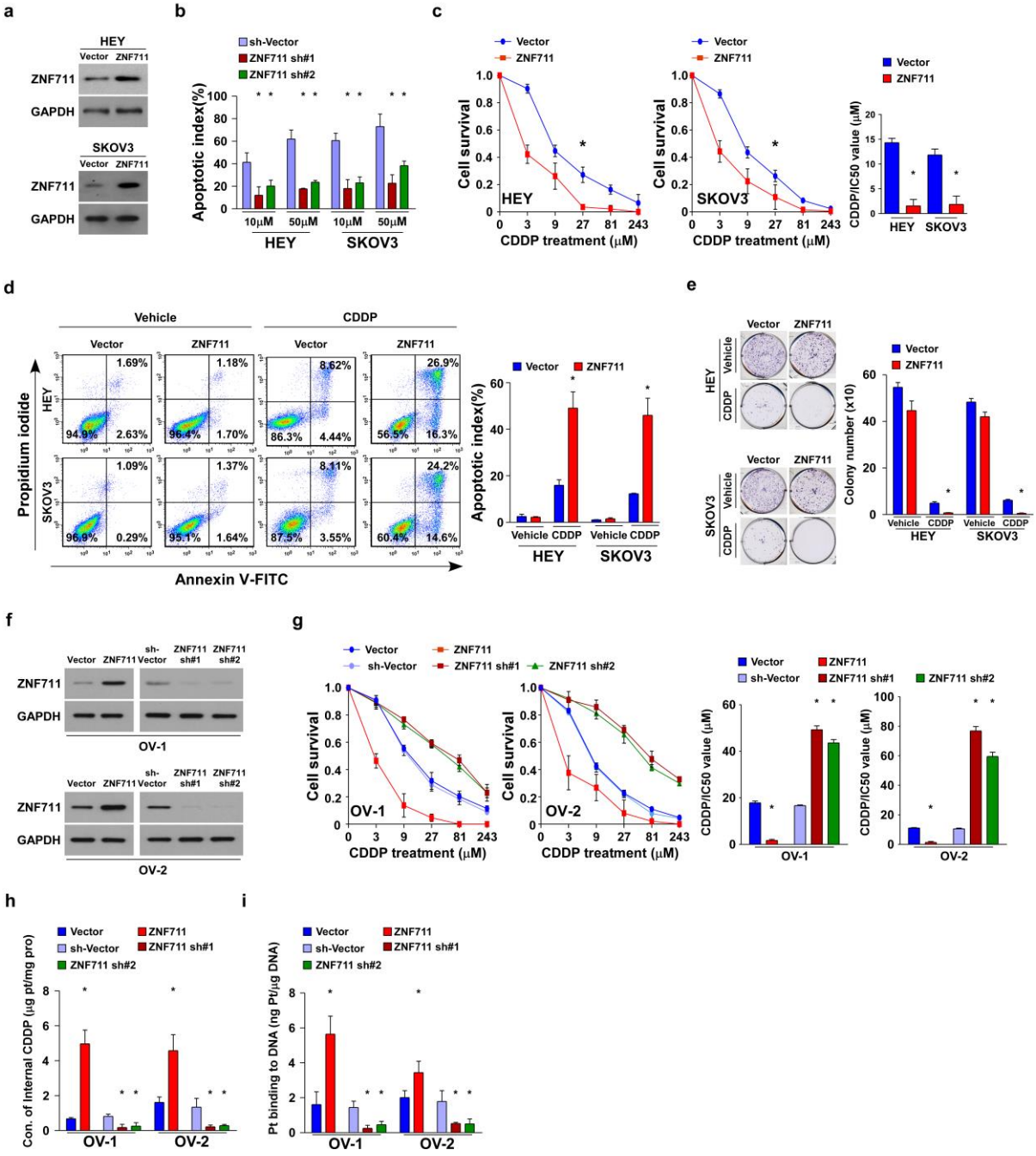
Supplemental Figures and Figure Legends

Supplemental Figure 1



Supplemental Fig. 1. ZNF711 was significantly downregulated in EOC cells and tissues. **(a)** According to the gene expression profiling interactive analysis (GEPIA) dataset, ZNF711 expression was significantly suppressed in ovarian cancer tissues compared with the GTEx data. **P* < 0.05 (Unpaired t test). **(b)** Analysis of overall survival of ovarian cancer from GEPIA database. **(c)** Kaplan-Meier analysis of overall survival of ovarian cancer patients from the Cancer Genome Atlas (TCGA) dataset. **(d)** The gene set enrichment analysis (GSEA) revealed that ZNF711 was inversely correlated with CDDP resistance. **(e)** The relative cell survival of indicated cells following CDDP treatment (5μM). Each bar shown in the figure represents the mean ± SD of three independent experiments. **P* < 0.05 (one-way ANOVA with Bonferroni's correction). The cut-off used to define low and high tumors in Supplemental Fig.1b and c was auto selection of best cut-off.

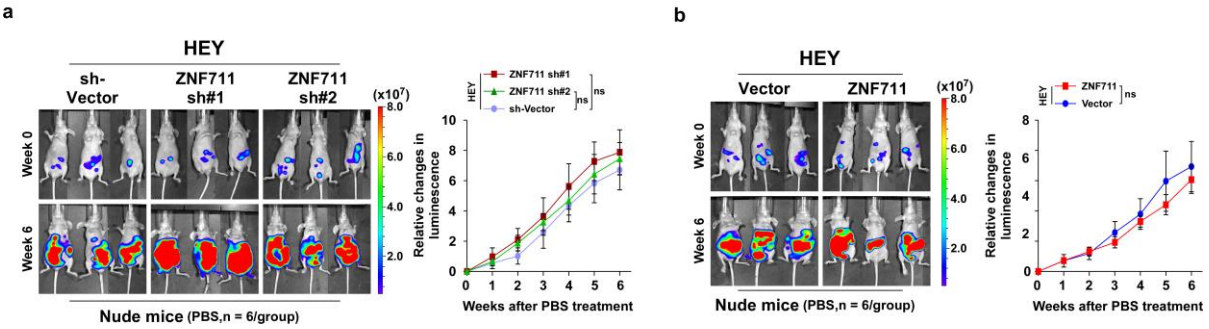
Supplemental Figure 2



Supplemental Fig.2. ZNF711 overexpression confers CDDP sensitivity in EOC cells *in vitro*. (a) Immunoblotting (IB) assays were performed to verify ZNF711 expression in the indicated stable HEY and SKOV3 cell lines. **(b)** The apoptotic percentage in indicated cells with higher doses of CDDP (10 μM and 50 μM, respectively). **(c)**An MTT cell viability assay (left) and half-maximal inhibitory concentration (IC_{50}) value of CDDP (right) in HEY and SKOV3 cells stably expressing ZNF711 compared to the cells expressing vector control. **(d)**

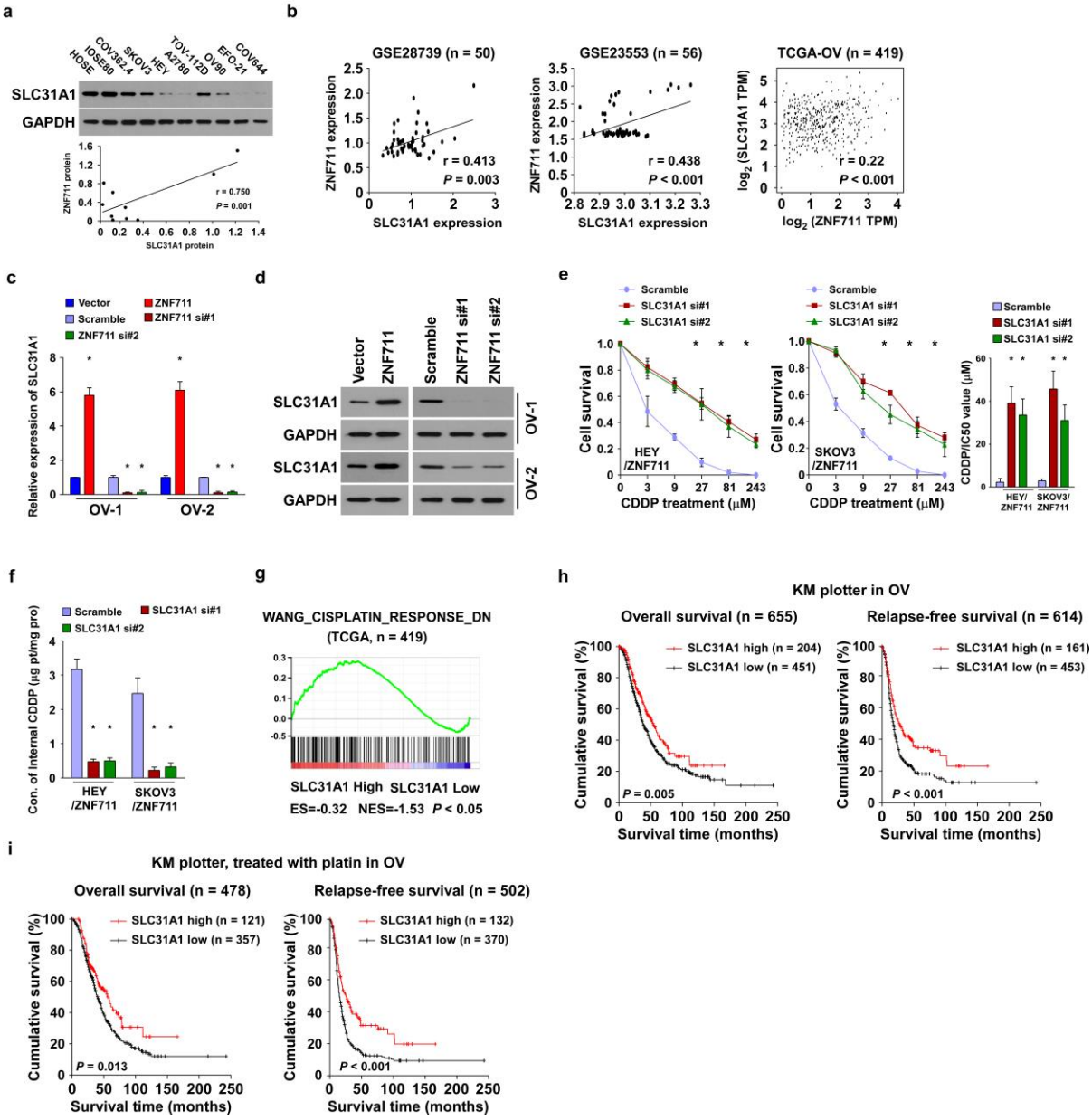
FACS analysis of Annexin V staining (left) and quantification (right) of indicated HEY and SKOV3 stable cell lines treated with vehicle or CDDP (5μM, 24 hrs). **(e)** Representative images (left) and quantification (right) of colony number of the indicated cells treated with vehicle or CDDP (5μM). **(f)** Immunoblotting (IB) assays were performed to verify ZNF711 expression in the indicated stable primary OV-1 and OV-2 cells. **(g)** An MTT cell viability assay (left) and half-maximal inhibitory concentration (IC_{50}) value of CDDP (right) in indicated cells. **(h)** Content of intercellular CDDP in the indicated OV-1 and OV-2 cells. **(i)** The quantification of DNA-bound CDDP (Pt) in the indicated OV-1 and OV-2 cells. Each bar shown in the figure represents the mean ± SD of three independent experiments. $*P < 0.05$ (unpaired t-test or one-way ANOVA with Bonferroni's correction).

Supplemental Figure 3



Supplemental Fig.3. PBS-treated xenograft tumor with atopic expression level of ZNF711 showed similar tumor growth kinetic. (a) Representative images of tumor-bearing nude mice were presented at the inititial time of PBS treatment (upper) and six weeks after PBS treatment (lower) (left panel, n = 6/group). Relative changes in the bioluminescence signal of intraperitoneal tumors in nude mice upon PBS chemotherapy at the indicated time points (right panel). (b) Representative images of tumor-bearing nude mice formed by indicated cells (left) and relative changes in the bioluminescence signal of intraperitoneal tumors (right). Each bar shown in the figure represents the mean ± SD of three independent experiments.

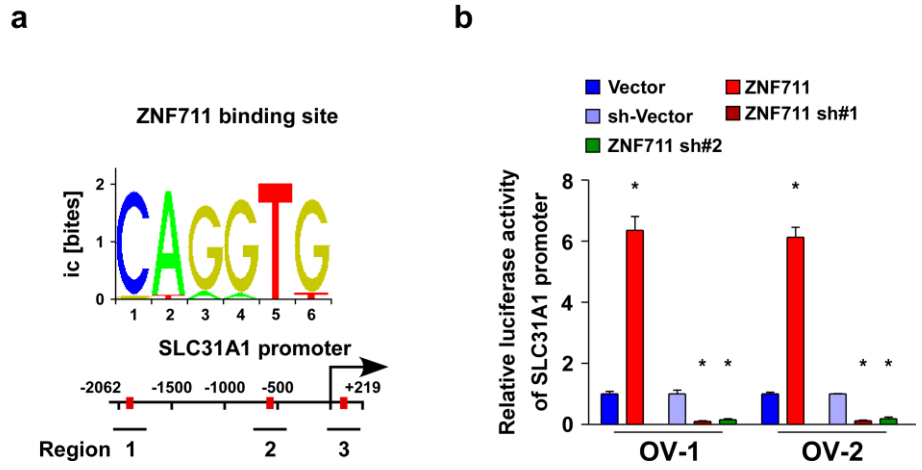
Supplemental Figure 4



Supplemental Fig. 4. ZNF711 modulates CDDP resistance via regulating SLC31A1 expression. (a) The protein expression of SLC31A1 in indicated cells (upper) and the correlation between the protein expression of ZNF711 and SLC31A1 in indicated cells (lower). **(b)** The correlation between ZNF711 and SLC31A1 in GSEA and TCGA database. **(c-d)** The mRNA (b) and protein (c) expression of SLC31A1 in OV-1 and OV-2 cells with different expression levels of ZNF711. **(e)** An MTT cell viability assay (left) and half-maximal inhibitory concentration (IC_{50}) value of CDDP (right) in indicated cells. **(f)** The

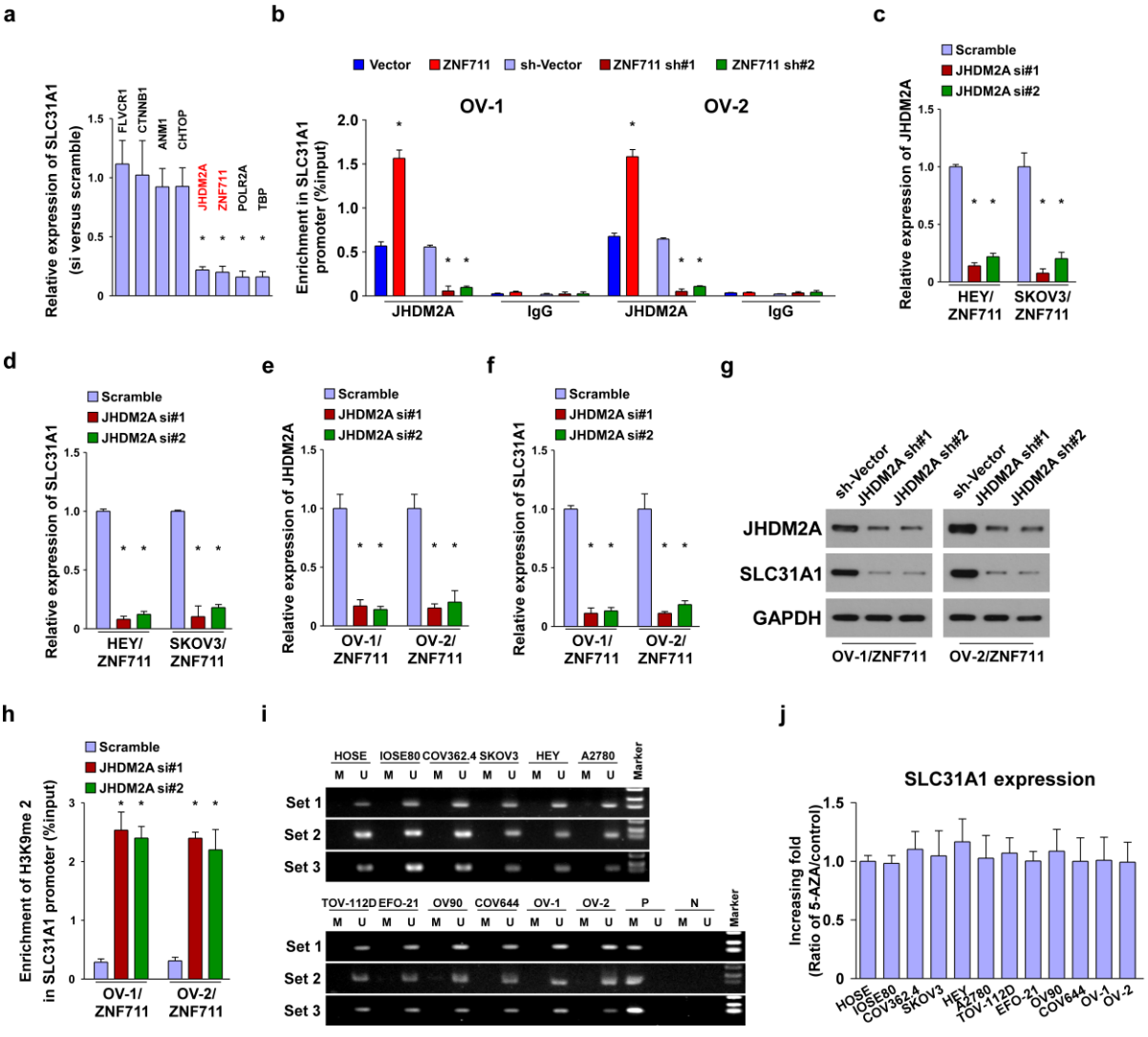
intracellular concentration of CDDP in indicated cells. **(g)** GSEA analysis revealed that the expression of SLC31A1 in EOC tissues was negatively correlated with CDDP resistance ($P < 0.05$). **(h-i)** Kaplan-Meier analysis of the overall and relapse-free survival in ovarian cancer patients (h) and ovarian cancer patients treated with platinum (i). Each bar shown in the figure represents the mean ± SD of three independent experiments. *$P < 0.05$ (one-way ANOVA with Bonferroni's correction).

Supplemental Figure 5



Supplemental Fig. 5. ZNF711 regulates SLC31A1 promoter activity in EOC cells. (a) The Schematic diagram of ZNF711 binding site and the promoter regions of SLC31A. **(b)** The luciferase signal of the SLC31A1 promoter luciferase reporter in indicated OV-1 and OV-2 cells. Each bar shown in the figure represents the mean ± SD of three independent experiments. *$P < 0.05$ (one-way ANOVA with Bonferroni's correction).

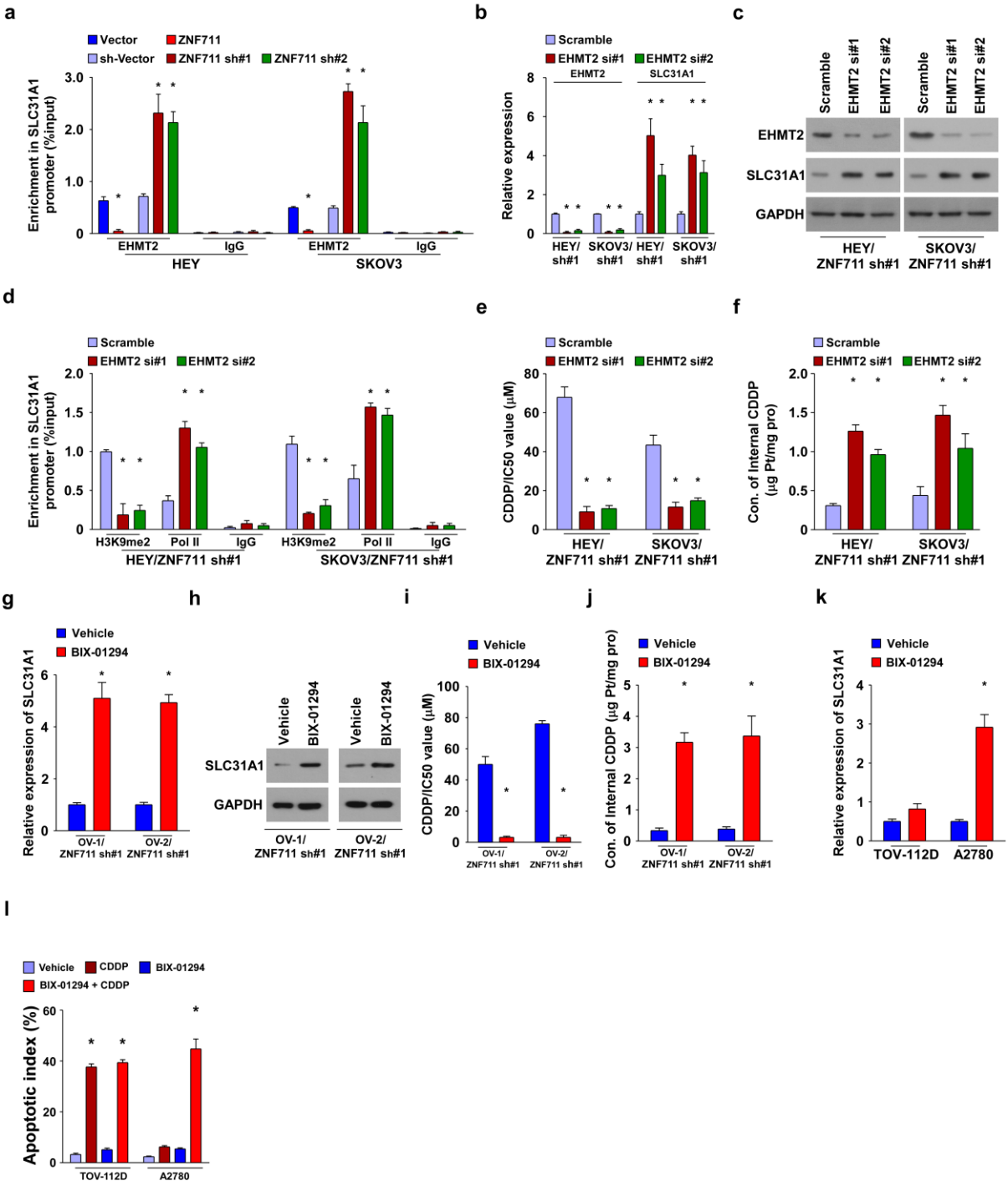
Supplemental Figure 6



Supplementary Fig. 6. JHDM2A and SLC31A1 expression in the indicated cells. (a) Relative expression of SLC31A1 in the indicated siRNA-transfected cells treated with CDDP. **(b)** JHDM2A mRNA expression was suppressed in the indicated EOC cells. **(c)** The level of SLC31A1 mRNA expression in the indicated cells. **(e-f)** The mRNA expression of JHDM2A and SLC31A1 in the indicated cells. **(g)** The protein expression JHDM2A and SLC31A1 in the indicated cells. **(h)** Enrichment of H3K9me2 in SLC31A1 promoter in indicated cells. **(i)** Electrophoresis of PCR products spanning the SLC31A1 promoter from bisulfite-treated DNA. Each lane contains products generated from separate PCR reactions using three sets of primers, each of which contains two paires of primers specific for

methylated (M) or unmethylated (U) DNA template, respectively. The genomic DNA of OV-1 cells treatment with SssI Methyltransferase was used as positive control and water (H2O) was used as a negative control for PCR. **(j).** Increasing fold of SLC31A1 expression in EOC cell lines and primary tumor cells compared with normal ovarian cells with the treatment of 5-AZA versus vehicle control. Each bar shown in the figure represents the mean ± SD of three independent experiments. *$P < 0.05$ (one-way ANOVA with Bonferroni's correction).

Supplemental Figure 7



Supplemental Fig. 7. ZNF711 suppression promotes EHMT2 enrichment in the SLC31A1 promoter. (a) EHMT2 enrichment in the SLC31A1 promoter was examined in the indicated cells. **(b and c)** The expression level of EHMT2 and SLC31A1 in the indicated cells. **(d)** H3K9me2 and Pol II enrichment in the SLC31A1 promoter in cells transfected with EHMT2 siRNA or the scramble. **(e)** IC50 value of CDDP in the indicated cells. **(f)** The

concentration of intracellular CDDP in the indicated cells. **(g-h)** The mRNA (g) and protein (h) expression of SLC31A1 in ZNF711-silenced OV-1 and OV-2 cells treated with BIX-01294 (5μM) and CDDP (5μM). (i) IC50 value of CDDP in the indicated cells. **(j)** The concentration of intracellular CDDP in indicated OV-1 and OV-2 cells. **(k)** Relative expression of SLC31A1 mRNA expression in TOV-112D and A2780 cells treated with BIX-01294 (5μM) and CDDP (5μM). **(l)** The apoptotic index of TOV-112D and A2780 cells treated with vehicle, CDDP, BIX-01294 (5μM) and BIX-01294 + CDDP (5μM), respectively. Each bar shown in the figure represents the mean ± SD of three independent experiments. *$P < 0.05$ (unpaired t-test or one-way ANOVA with Bonferroni's correction).

Supplemental Fig.8

Fig1b

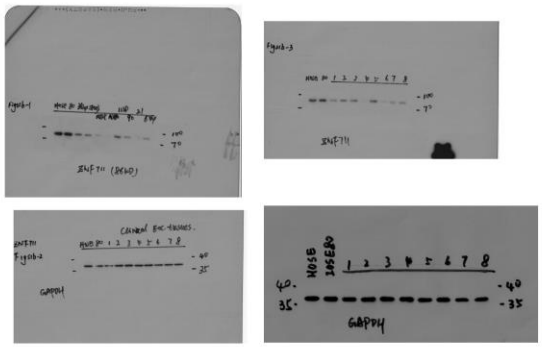


Fig2a

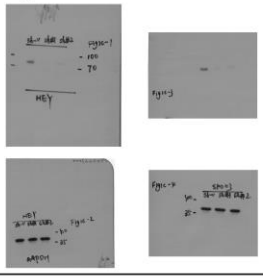


Fig4b

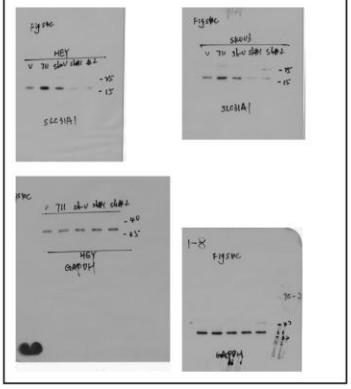


Fig4e

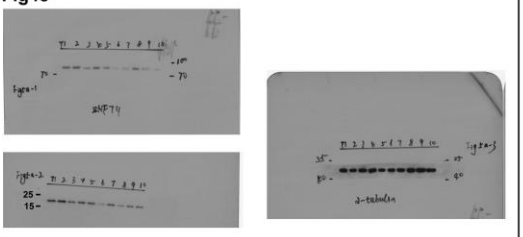


Fig6C

Fig6D

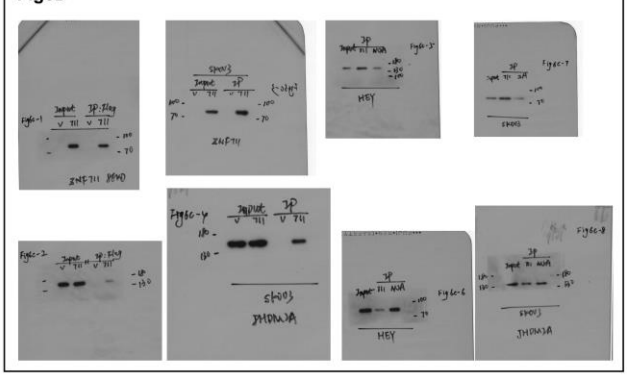
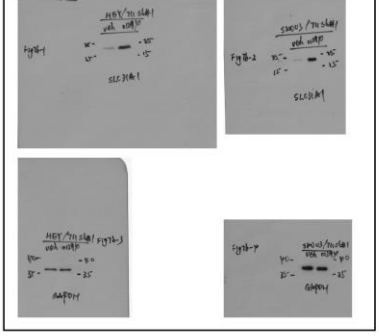
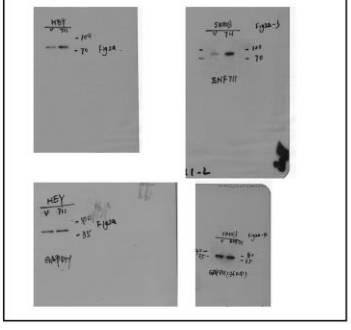
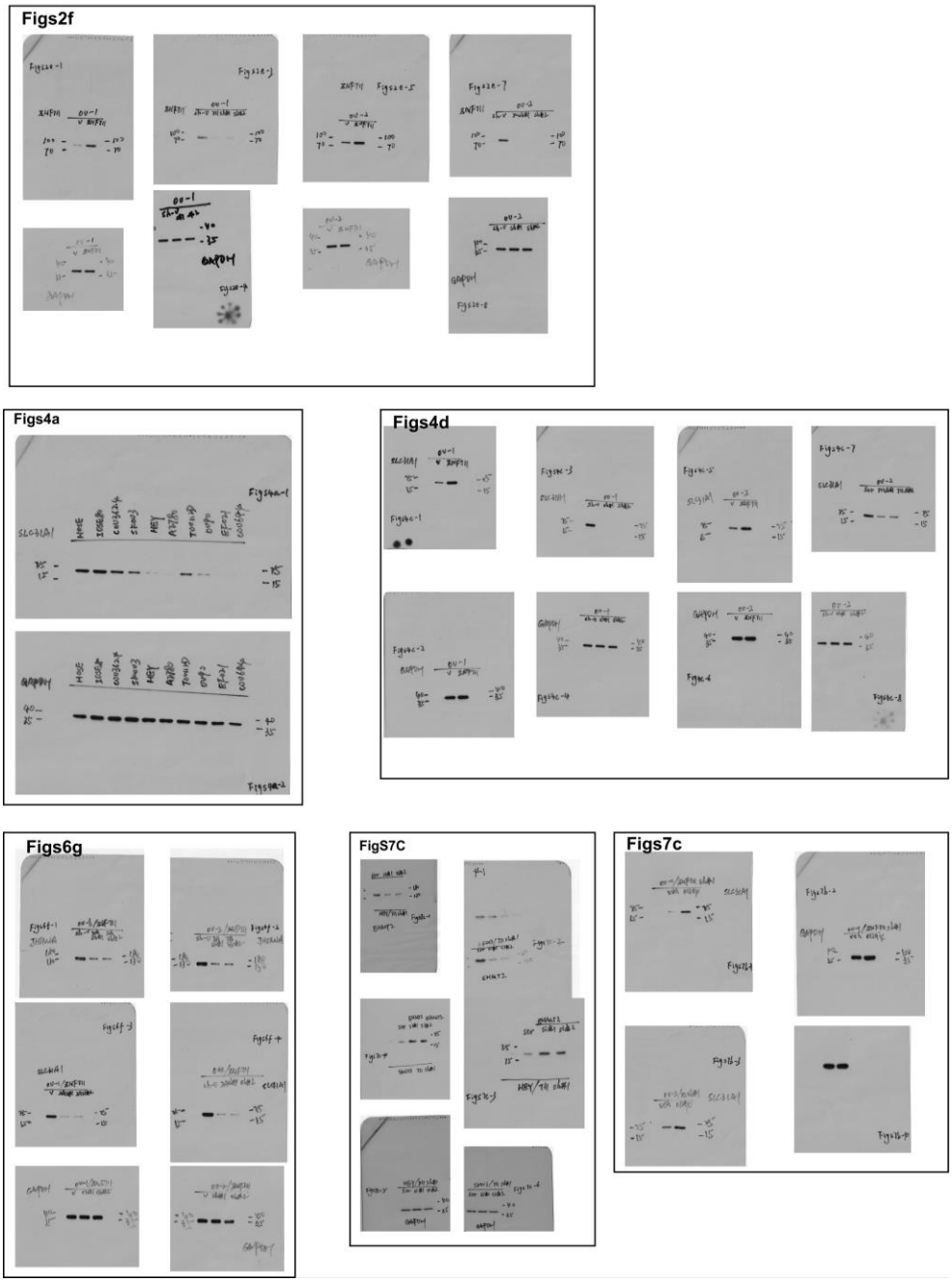


Fig7B



Figs2A





Supplemental Fig. 8. Uncut gel for Figures and Supplemental Figures