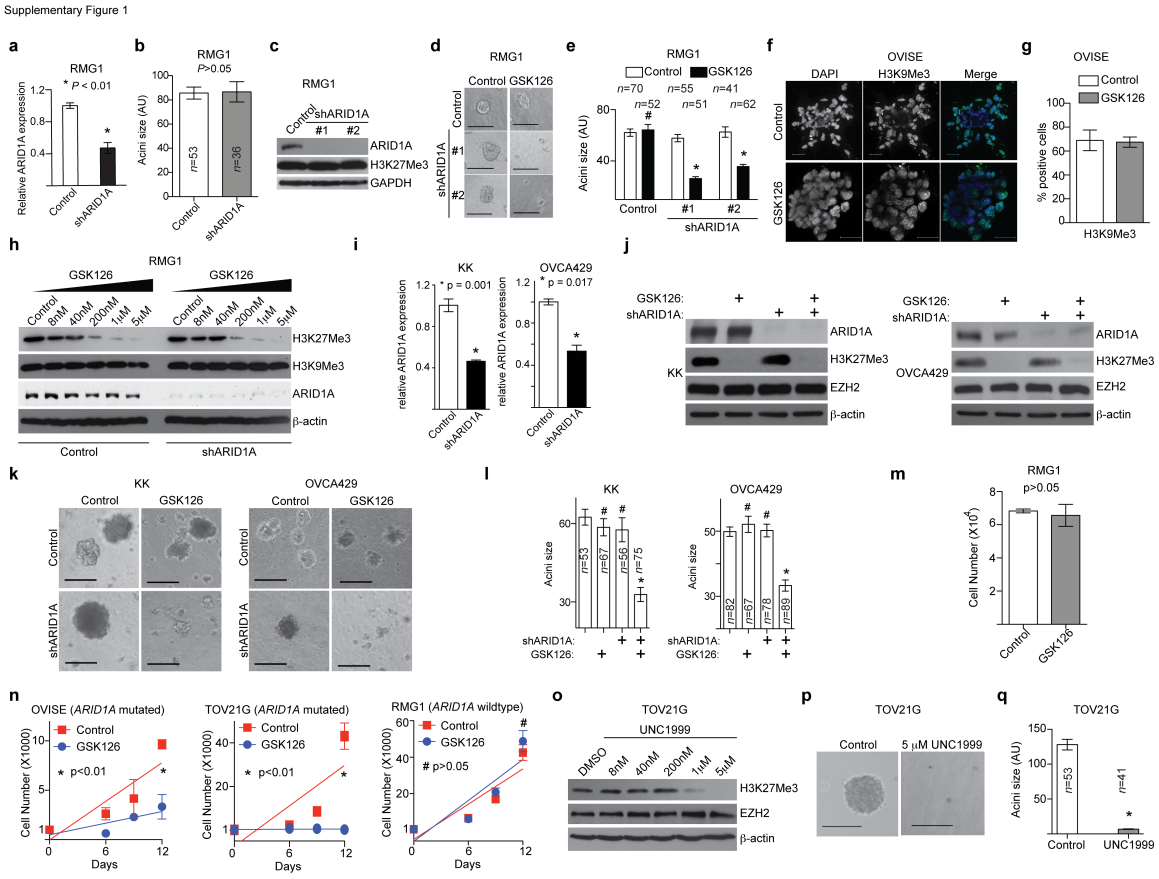
Supplementary Information

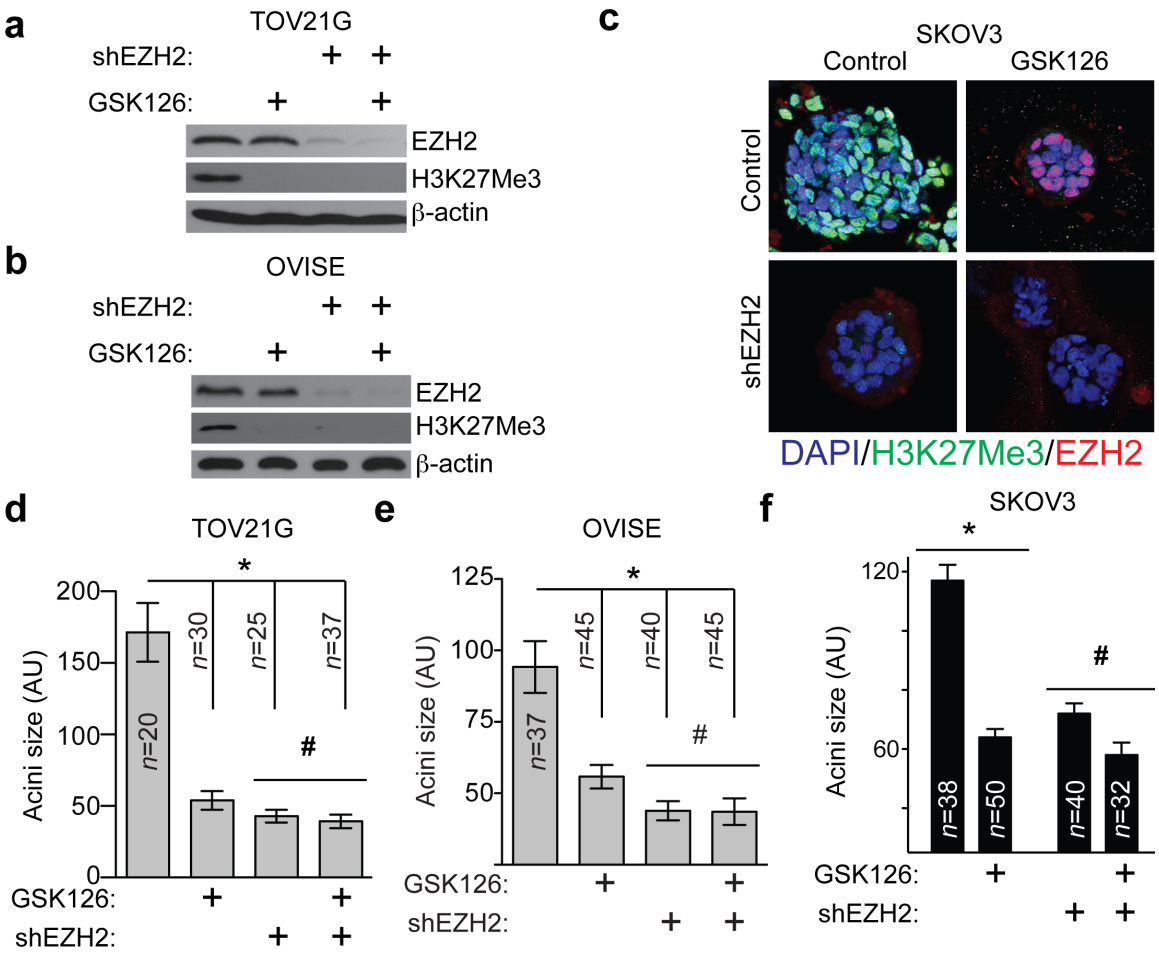
Supplementary Figures 1-6 and Tables 1-3



Supplementary Figure 1. EZH2 inhibitor selectively suppresses the growth of ARID1A inactivated OCCC cells. (**a**) *ARID1A* wild type ovarian clear cell cancer RMG1 cells were infected with lentivirus encoding control or shARID1A. Following drug-selection, ARID1A mRNA expression was determined by qRT-PCR. Mean of three repeats with s.d. (**b**) Same as (a). Drug-selected cells were grown on Matrigel for 12 days. Acini diameters were measured for control and shARID1A acini using NIH Image J software. Error bars represent s.e.m. (**c**) *ARID1A* wild type OCCC RMG1 cells were transduced with lentivirus encoding two individual shARID1As or control. Following drug selection, expression of ARID1A, H3K27Me3 and the loading control GAPDH in the indicated cells was determined by immunoblotting. (**d**) Same as (c), but the cells were plated onto Matrigel and treated with or without 5μM GSK126 or vehicle control. Representative images of acini from indicated cells. Scale Bars = 75 of measurable units (AU) using the NIH Image J software. (**e**) Quantitation of (d). (**P*<0.0001 and # *P*>0.05). (**f**) *ARID1A* mutated ovarian cancer OVISE cells were grown in 3D using Matrigel and

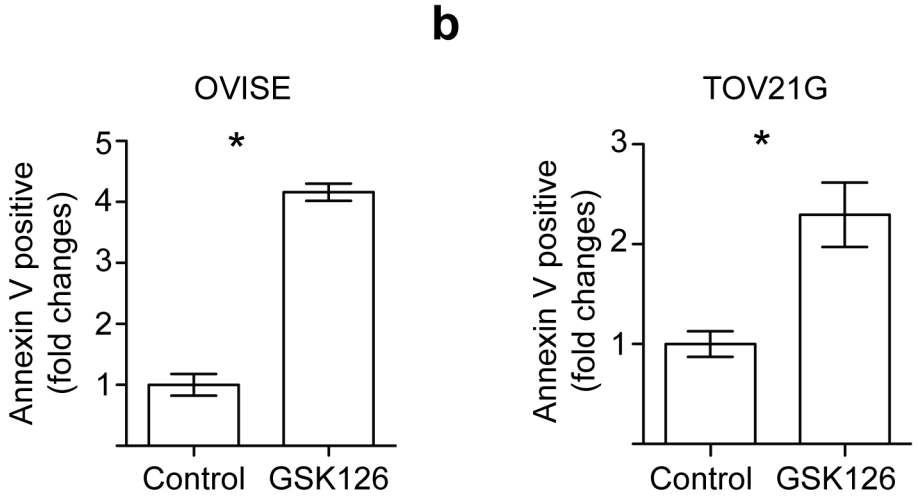
treated with or without 5μM GSK126 for 12 days. Acini formed by the indicated cells were stained for H3K9Me3 (green). DAPI counter staining was used to visualize cell nuclei (blue). (**g**) Same as (f), but quantified for H3K9Me3 positive cells (n=3). (**h**) Same as (a), but examined for expression of H3K27Me3, H3K9Me3, ARID1A and β-actin following treatment with the indicated concentration of GSK126 for 72 hours in the indicated control or shARID1A expressing RMG1 cells**.** (**i**) Same as (a), but for *ARID1A* wild type ovarian clear cell cancer KK and OVCA429 cells (n=3). (**j-l**) *ARID1A* wild type KK and OVCA429 cells expressing shARID1A or control treated with or without 5 μM GSK126 were examined for expression of ARID1A, EZH2, H3K27Me3 and β-actin by immunoblotting (j), shown in (k) are phase-contrast images of acini at day 12, which was quantified in (l). (# $P>0.05$ and * $P<0.0001$). (**m**) Same as (b), but the number of cells recovered from 3D culture were counted in the *ARID1A* wild type RMG1 cells (n=3). (**n**) An equal number of the indicated *ARID1A* mutated OVISE or TOV21G cells or *ARID1A* wild type RMG1 cells cultured using conventional 2D plastic tissue culture plates were treated with 5μM GSK126 or vehicle control. The number of cells at the indicated time points was counted. Mean of three repeats with SD and linear regression analysis. (**o**) Expression of H3K27Me3, EZH2 and β-actin was evaluated following treatment with the indicated concentration of UNC1999 for 72 hours in *ARID1*A mutated OCCC TOV21G cells**.** (**p**) Phase-contrast images of acini formed by *ARID1A* mutated OCCC TOV21G cells cultured in 3D conditions treated with or without 5 μM UNC1999 for 12 days. (**q**) Quantitation of (m). (* $P<0.0001$). Error bars represent s.e.m. and *n* is indicated on graphs unless otherwise specified.

Supplementary Figure 2



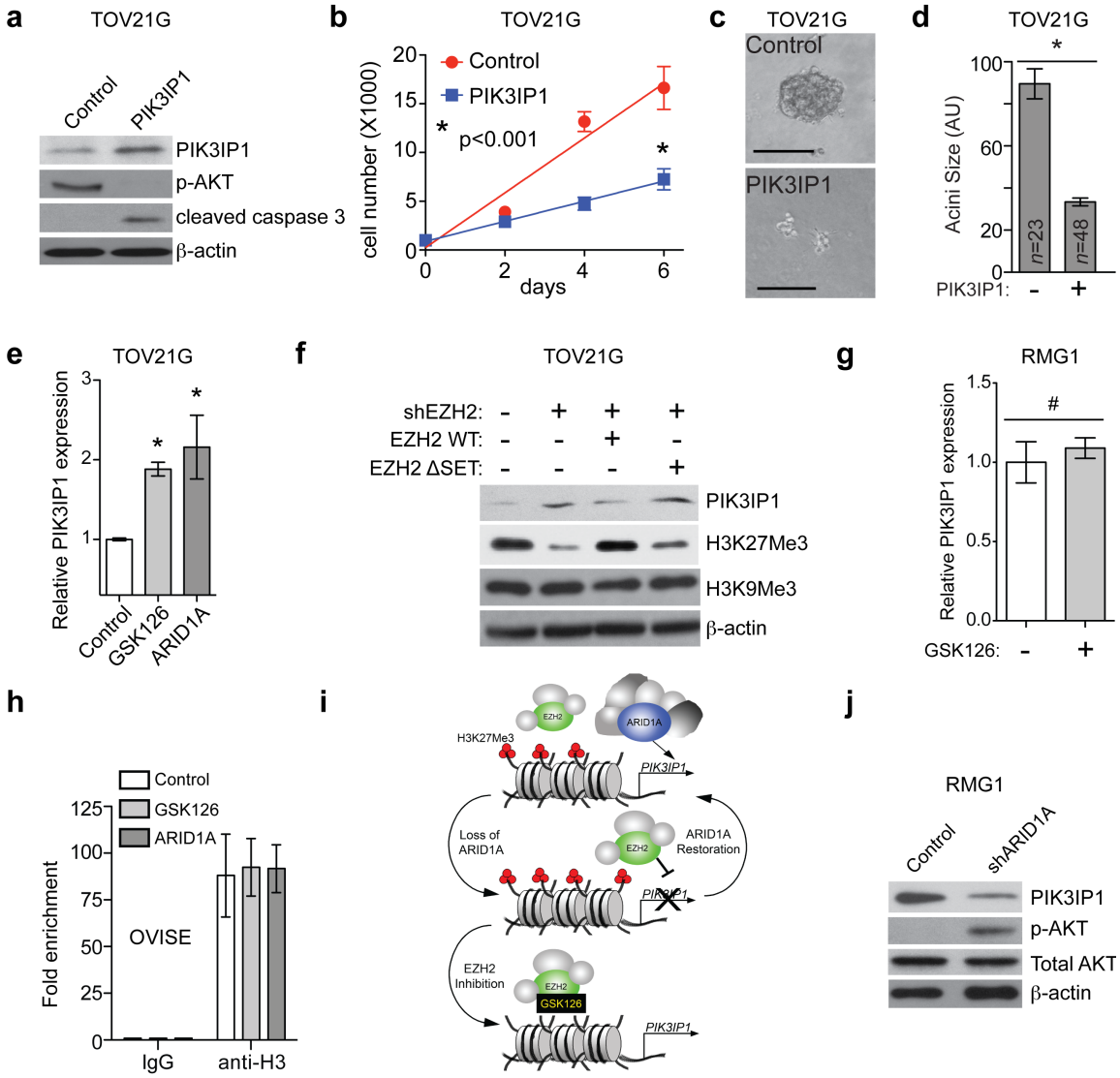
Supplementary Figure 2. GSK126 activity is EZH2 dependent. (**a**) *ARID1A* mutated OCCC TOV21G cells were infected with lentivirus encoding shEZH2 or control. Drug-selected cells were treated with or without 5 μM GSK126 for 12 days in 3D culture. Expression of EZH2, H3K27Me3 and loading control β-actin in the indicated cells recovered from 3D culture was determined by immunoblotting. (**b**) *ARID1A* mutated OCCC OVISE cells were infected with lentivirus encoding shEZH2 or control. Drug-selected cells were treated with or without 5 μM GSK126 for 12 days in 3D culture. Expression of EZH2, H3K27Me3 and loading control β-actin in the indicated cells recovered from 3D culture was determined by immunoblotting. (**c**) *ARID1A* mutated SKOV3 cells were infected with lentivirus encoding shEZH2 or control. Drug-selected cells were treated with or without 5μM GSK126 for 12 days in 3D culture. Acini formed by the indicated cells were stained for EZH2 (red) and H3K27Me3 (green). DAPI counter staining was used to visualize cell nuclei (blue). (**d**) Same as (a), but quantified for the diameter of the acini formed by the indicated TOV21G cells (# P=0.549, *P<0.0001). (**e**) Same as (b), but quantified for the diameter of the acini formed by the indicated OVISE cells (# P=0.549, *P<0.0001). (**f**) Same as (c), but quantified for the diameter of the acini formed by the indicated SKOV3 cells. (# P>0.05, *P<0.0001). Error bars represent s.e.m. and n is indicated on graphs.

Supplementary Figure 3



Supplementary Figure 3. GSK126 induces apoptosis of *ARID1A* mutated cells. *ARID1A* mutated clear cell ovarian cancer OVISE (**a**) and TOV21G (**b**) cells cultured in 3D using Matrigel were treated with 5 μM GSK126 or vehicle control. Cells recovered from 3D culture were subjected to Annexin V staining using Guava Nexin assay (n=3, * P<0.001). Error bars represent s.e.m.

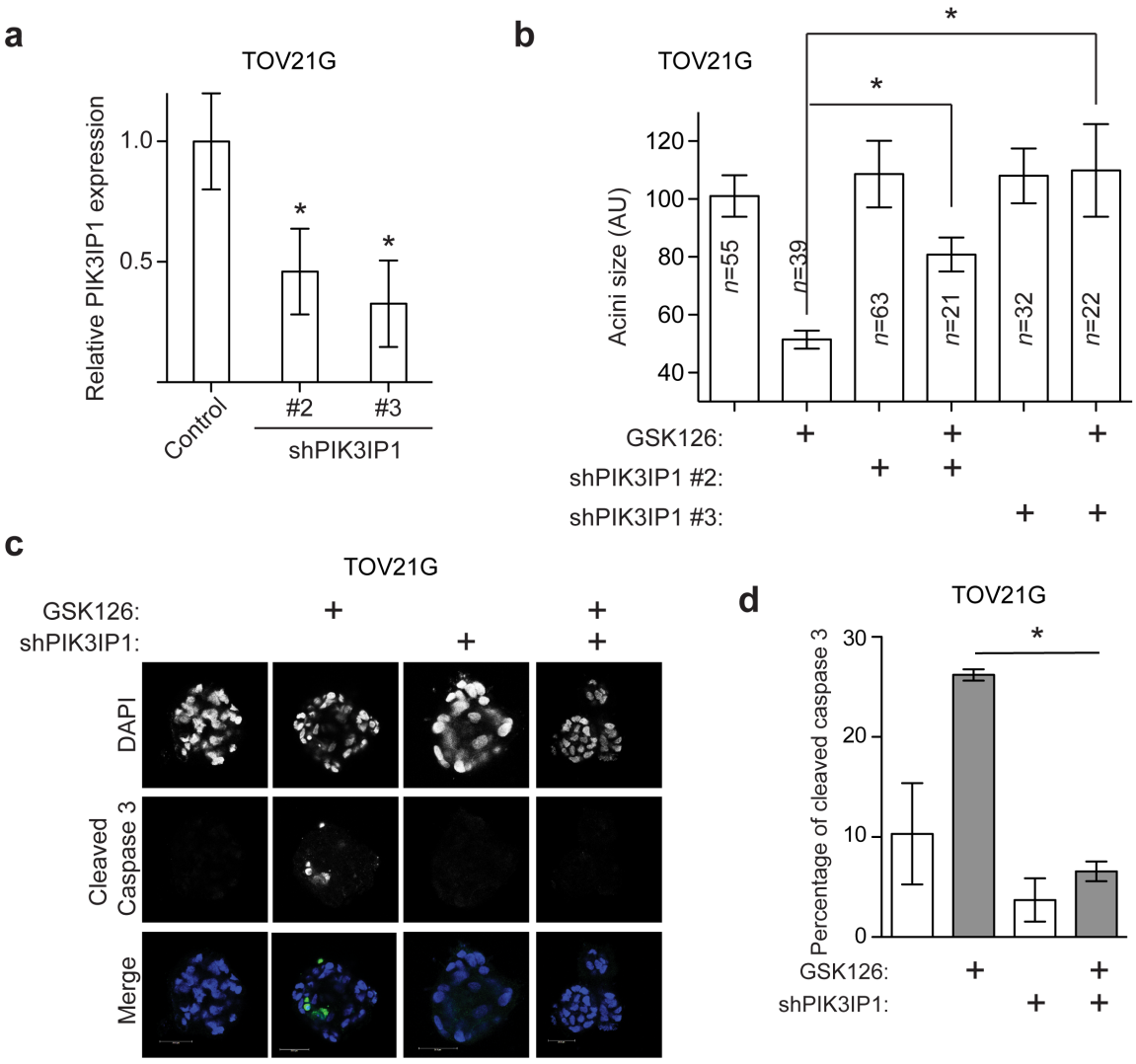
Supplementary Figure 4



Supplementary Figure 4. *PIK3IP1* is a novel ARID1A/EZH2 target gene. (**a**) *ARID1A* mutated TOV21G cells were transiently transfected with a plasmid encoding for PIK3IP1 or control. The indicated cells were examined for the expression of PIK3IP1, phospho-AKT, a marker of active PI3K/AKT signaling, cleaved caspase 3, an apoptotic marker, and loading control β-actin by immunoblotting. (**b**) Same as (a), but an equal number of the indicated cells were seeded. The number of cells at the indicated time points was counted. Mean of three repeats with s.d. and linear regression analysis. (**c**) Same as (b), but grown in 3D using Matrigel for 12 days. Phase-contrast images of the acini formed by the indicated cells. Scale Bars = 75 of measurable units (AU) using the NIH Image J software. (**d**) Quantitation of (c). Acini size were measured as diameter for control and shARID1A acini using NIH Image J software (n is indicated on the graphs, error bars represent s.e.m and *P<0.001). (**e**) *ARID1A* mutated ovarian cancer TOV21G cells were grown in 3D using Matrigel. RNA was extracted from cells recovered from the 3D culture and *PIK3IP1* expression was evaluated using qRT-PCR (n=3, *P<0.01). (**f**) *ARID1A* mutated TOV21G cells were infected with a lentivirus encoding shEZH2 targeting the 3' untranslated region (UTR) of the human *EZH2* gene together with a

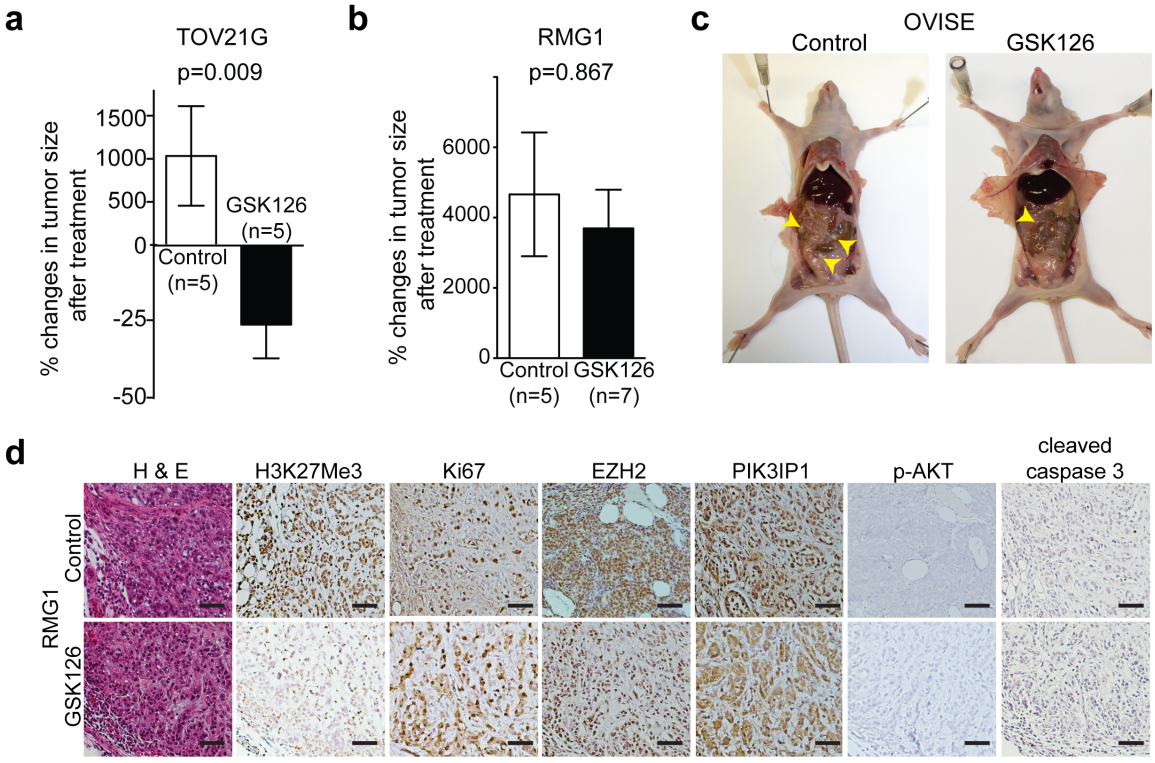
retrovirus encoding wild type EZH2 or a SET domain deleted EZH2 mutant (EZH2 ΔSET) to inactivate its methyltransferase activity. The indicated cells were examined for the expression of PIK3IP1, H3K27Me3, H3K9Me3 and loading control β-actin by immunoblotting. Please see Fig. 3b for confirmation of knockdown of endogenous EZH2 and expression of ectopic wild type EZH2 or EZH2 ΔSET. (**g**) *ARID1A* wild type OCCC RMG1 cells were treated with or without 5μM GSK126 and *PIK3IP1* expression was evaluated using qRT-PCR. (n=3, # *P*>0.05). (**h**) *ARID1A* mutated OVISE cells treated with or without 5 μM GSK126 or restored for ARID1A expression were subjected to ChIP analysis using an antibody against core histone H3. An isotype matched IgG was used as a control. ChIP products were subjected to quantitative PCR analysis using primers that amplify the human *PIK3IP1* promoter region to quantify the association of histone H3 with the *PIK3IP1* gene promoter in the indicated cells (n=3). (**i**) A model for the proposed regulation of the *PIK3IP1* gene by EZH2 and ARID1A. (**j**) *ARID1A* wild type ovarian clear cell cancer RMG1 cells expressing control or shARID1A were examined for the expression of PIK3IP1, phospho-AKT, a marker of active PI3K/AKT signaling, total AKT and loading control β-actin by immunoblotting.

Supplementary Figure 5



Supplementary Figure 5. *PIK3IP1* contributes to the observed synthetic lethality by GSK126. (**a**) *ARID1A* mutated OCCC TOV21G cells were infected with lentivirus encoding the indicated shPIK3IP1s or control and *PIK3IP1* expression was evaluated using qRT-PCR (n=3, error bars represent s.d. and **P*<0.006). (**b**) Same as (a), but an equal number of the indicated cells were grown in 3D using Matrigel and treated with or without 5μM GSK126. After 12 days, acini diameter was measured using NIH Image J software (n is indicated on the graphs, error bars represent s.e.m. and * *P*<0.0001). (**c**) Same as (b). Acini formed by the indicated cells were stained for cleaved-caspase 3 (green), a marker of cell apoptosis. DAPI counter staining was used to visualize cell nuclei (blue). (**d**) Quantification of (c). (n=3, **P*<0.001). Error bars represent s.e.m.

Supplementary Figure 6



Supplementary Figure 6. Inhibition of EZH2 activity causes the regression of the *ARID1A* mutated tumors. (**a**) 1×10^6 *ARID1A* mutated TOV21G cells were unilaterally injected into the peri-ovarian bursa sac of the nude immuno-compromised female mice. Tumors were allowed to establish for 4 weeks. Three mice were sacrificed and the size of the dissected tumors was used as baseline for comparison. The rest of the mice were randomized into two groups for GSK126 or vehicle control treatments (n=5 for each of the groups). Mice were treated daily with 50 mg/kg GSK126 or vehicle control for an additional 14 days by intraperitoneal injection. At necropsy, the size of the dissected tumors was measured by subtracting control counter lateral ovary size from that of the size from the tumor cell injected one to limit variation among different mice. The percentage of changes in tumor sizes was quantified by comparing tumor size after treatment with vehicle control or GSK126 with the baseline tumor size before treatment. *P* value was calculated by Wilcoxon rank-sum test. (**b**) 1×10^6 *ARID1A* wild type RMG1 cells were unilaterally injected into the peri-ovarian bursa sac of the nude immuno-compromised female mice. Tumors were allowed to establish for 4 weeks. Three mice were sacrificed and the size of the dissected tumors was used as baseline for comparison. The rest of the mice were randomized into two groups for GSK126 (n=7) or vehicle control treatments (n=5). Mice were treated daily with 50 mg/kg GSK126 or vehicle control for additional 14 days by intraperitoneal injection. At necropsy, the size of the dissected tumors was measured. The percentage of changes in tumor sizes was quantified by comparing tumor size after treatment with vehicle control or GSK126 with the baseline tumor size before treatment. *P* value was calculated by Wilcoxon rank-sum test. (**c**) 3×10^6 *ARID1A* mutated ovarian clear cell cancer OVISE cells were injected into the intraperitoneal cavity of nude immuno-compromised female mice. The injected tumor cells were allowed to grow for 4 days, and then the injected mice were randomly separated into two groups for GSK126 or vehicle control treatments. Mice were treated

daily with 50 mg/kg GSK126 or vehicle control by intraperitoneal injection for an additional 26 days. On day 30 mice were sacrificed. Shown are examples of mice from control and treated groups. Arrows point to tumor nodules formed in the intraperitoneal cavity. Error bars represent s.e.m. (**d**) Tumors dissected from GSK126 or vehicle control treated *ARID1A* wild type RMG1 tumor bearing mice were sectioned and subjected to immunohistochemical staining using antibodies against H3K27Me3, Ki67, EZH2, PIK3IP1, p-AKT and cleaved caspase 3. Bars= 50 μm.

Supplementary Table 1

Small Molecule	Target developed against	+ ARID1A (acini size)			- ARID1A (acini size)			% Change	*p-value*	FDR
		Mean	S.E.M.	n	Mean	S.E.M.	n			
GSK126	EZH2	66.01	2.45	76	28.40	1.62	84	-56.98%	0.0001	0.001
Dacinostat	HDAC	25.83	1.21	90	15.17	1.33	45	-41.28%	0.0001	0.001
MC1568	HDAC2	46.84	2.42	65	29.39	2.13	71	-37.24%	0.0001	0.001
Mocetinostat	HDAC1, 2, 3	11.96	1.24	34	9.52	0.75	28	-20.36%	0.0936	0.164
Belinostat	HDAC	53.73	2.11	77	45.85	2.89	52	-14.67%	0.0441	0.088
Control	N/A	72.72	2.50	82	69.44	2.90	93	-4.51%	0.1481	0.23
ITF2357	HDAC	55.06	2.08	57	52.83	2.57	58	-4.05%	0.5196	0.727
Entinostat	HDAC1	29.85	1.28	86	29.15	1.96	43	-2.32%	0.7559	0.882
Vorinostat	HDAC	53.45	2.46	71	52.40	3.23	54	-1.97%	0.8027	0.803
JNJ-26481585	HDAC	53.02	2.17	82	53.06	2.75	24	0.06%	0.9937	0.869
Droxinostat	HDAC3, 6, 8	50.12	1.90	80	51.12	3.89	52	2.00%	0.7926	0.854
Pracinostat	HDAC1	40.41	1.70	93	41.36	3.29	68	2.35%	0.8173	0.763
Panobinostat	HDAC	6.83	0.52	20	7.52	0.86	25	10.14%	0.52	0.662
CUDC-101	HDAC, EGFR, HER2	43.85	1.92	80	53.91	3.47	47	22.95%	0.0061	0.017
AR-42	HDAC	7.80	0.60	33	10.37	1.32	17	32.86%	0.0377	0.088
EX 527	Sirtuin	37.71	2.26	61	52.04	3.65	55	38.01%	0.0006	0.002

Supplementary Table 1. Identification of the EZH2 inhibitor GSK126 that selectively suppresses the growth of ARID1A knockdown OCCC cells. *ARID1A* wild type OCCC RMG1 cells expressing shARID1A or control were cultured in 3D conditions and treated with vehicle control (0.1% DMSO) or the indicated small molecule inhibitors with a final concentration of IC_{50} as previously established in the literature. After 12 days in culture, the diameters of acini formed from the indicated treatment groups or controls were measured using NIH Image J software. The list of small molecules is ranked by inhibitory rate.

Supplementary Table 2

Small Molecule	Target	Dose [nM]	Citation (PMID)
GSK126	EZH2	100	23051747
Dacinostat	HDAC	32	14744786
MC1568	HDAC2	22000	20639404
Mocetinostat	HDAC1, 2, 3	1660	18413790
Belinostat	HDAC	27	12939461
Control	N/A		
ITF2357	HDAC	16	16557334
Entinostat	HDAC1	300	10200307
Vorinostat	HDAC	5000	11016644
JNJ-26481585	HDAC	2.43	19861438
Droxinostat	HDAC3, 6, 8	1460	20053768
Pracinostat	HDAC1	52	20197387
Panobinostat	HDAC	5	19671764
CUDC-101	HDAC, EGFR, HER2	15.7	20143778
AR-42	HDAC	610	20532179
EX 527	Sirtuin	38	16354677

Supplementary Table 2. Epigenetic targeting small molecule panel used for evaluation. Identification of small molecules utilized in the evaluation, intended target, and IC_{50} utilized. To limit potential off-target effects, doses used for the evaluation were the IC_{50} of their intended target as determined based on previously published literature.

Supplementary Table 3

The list of genes that meet the prioritization criteria
ALDH1A2
ALDH1A1
EPHX2
PIK3IP1
RHOU
SULF2
REPS2
TSC22D3

Supplementary Table 3. List of ARID1A/EZH2 target genes identified using the integrative strategy. Common genes that were significantly upregulated by GSK126 treatment and wild type *ARID1A* restoration in ARID1A mutated OVISE ovarian clear cell cancer cells that are direct EZH2/H3K27Me3 target genes based on a published ChIP-seq database using *ARID1A* mutated ovarian cancer SKOV3 cells [18]. Further, these genes are significantly downregulated in laser capture and microdissected ovarian clear cell carcinomas compared with normal ovarian surface epithelial cells in a published database [19].

References:

18. Li, H.*, et al.* ALDH1A1 is a novel EZH2 target gene in epithelial ovarian cancer identified by genome-wide approaches. *Cancer Prev Res (Phila)* **5**, 484-491 (2012).
19. Stany, M.P.*, et al.* Identification of novel therapeutic targets in microdissected clear cell ovarian cancers. *PLoS One* **6**, e21121 (2011).