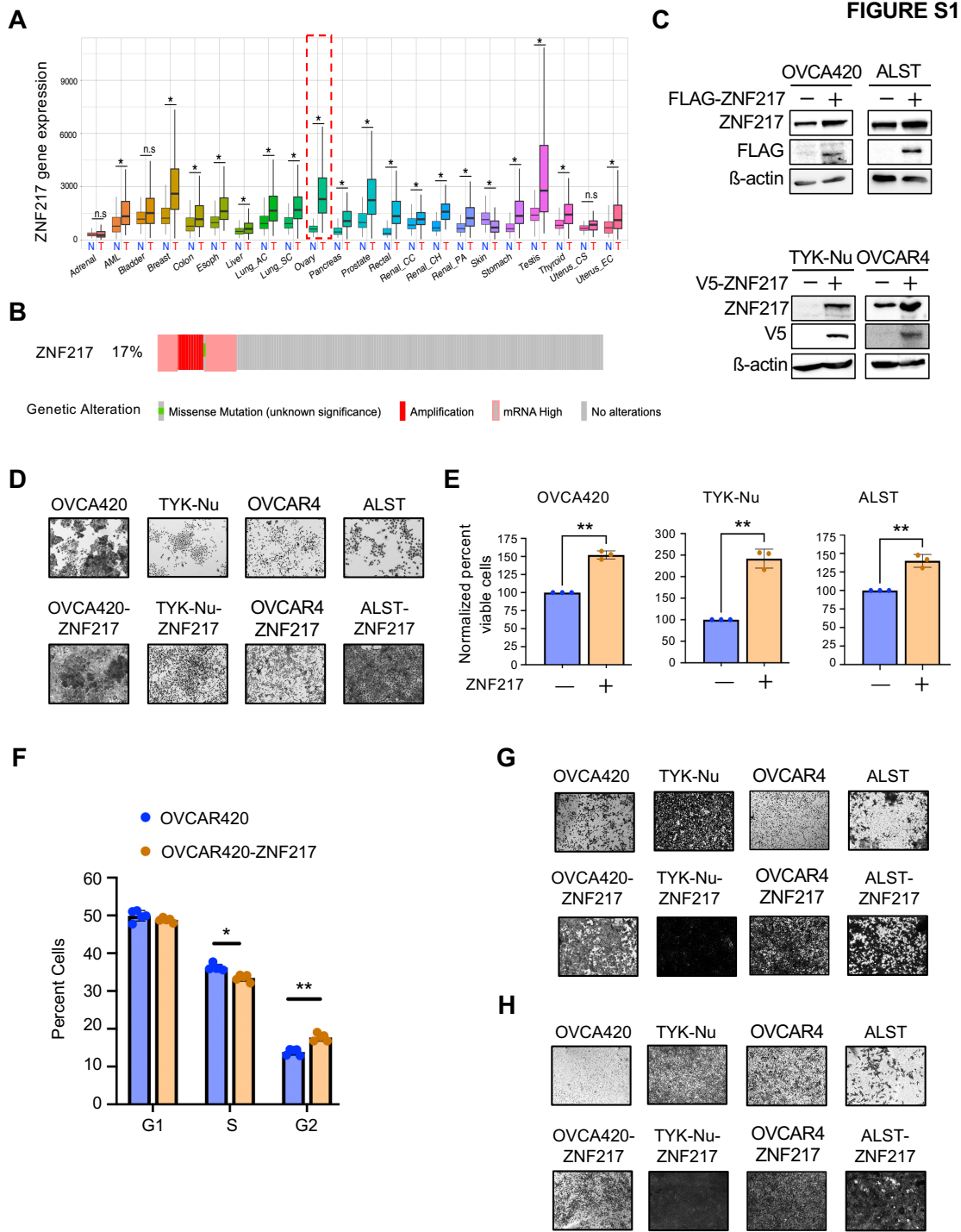


SUPPLEMENTAL FIGURES AND FIGURE LEGENDS



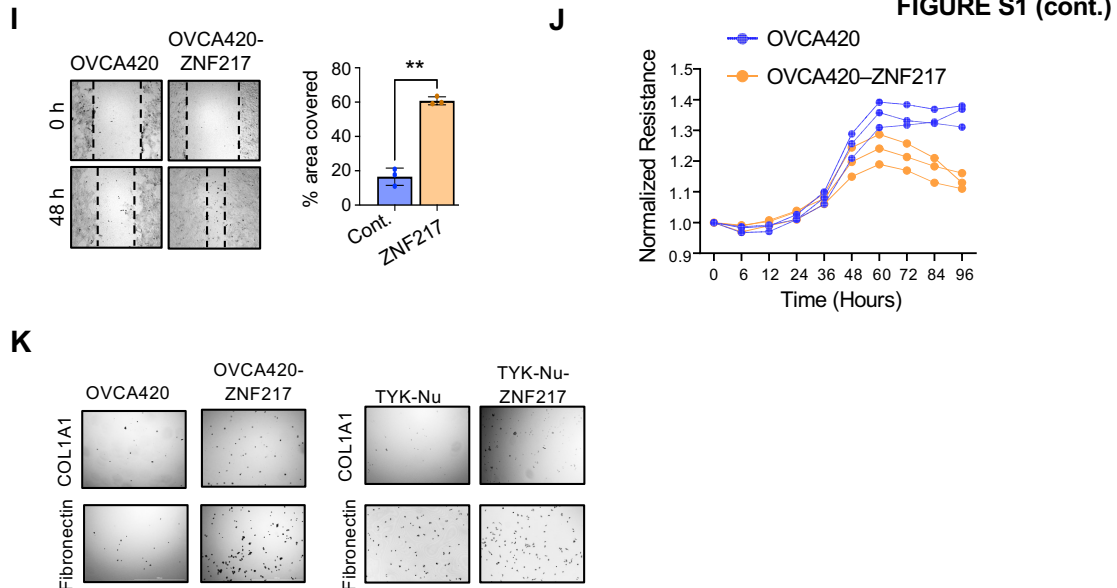
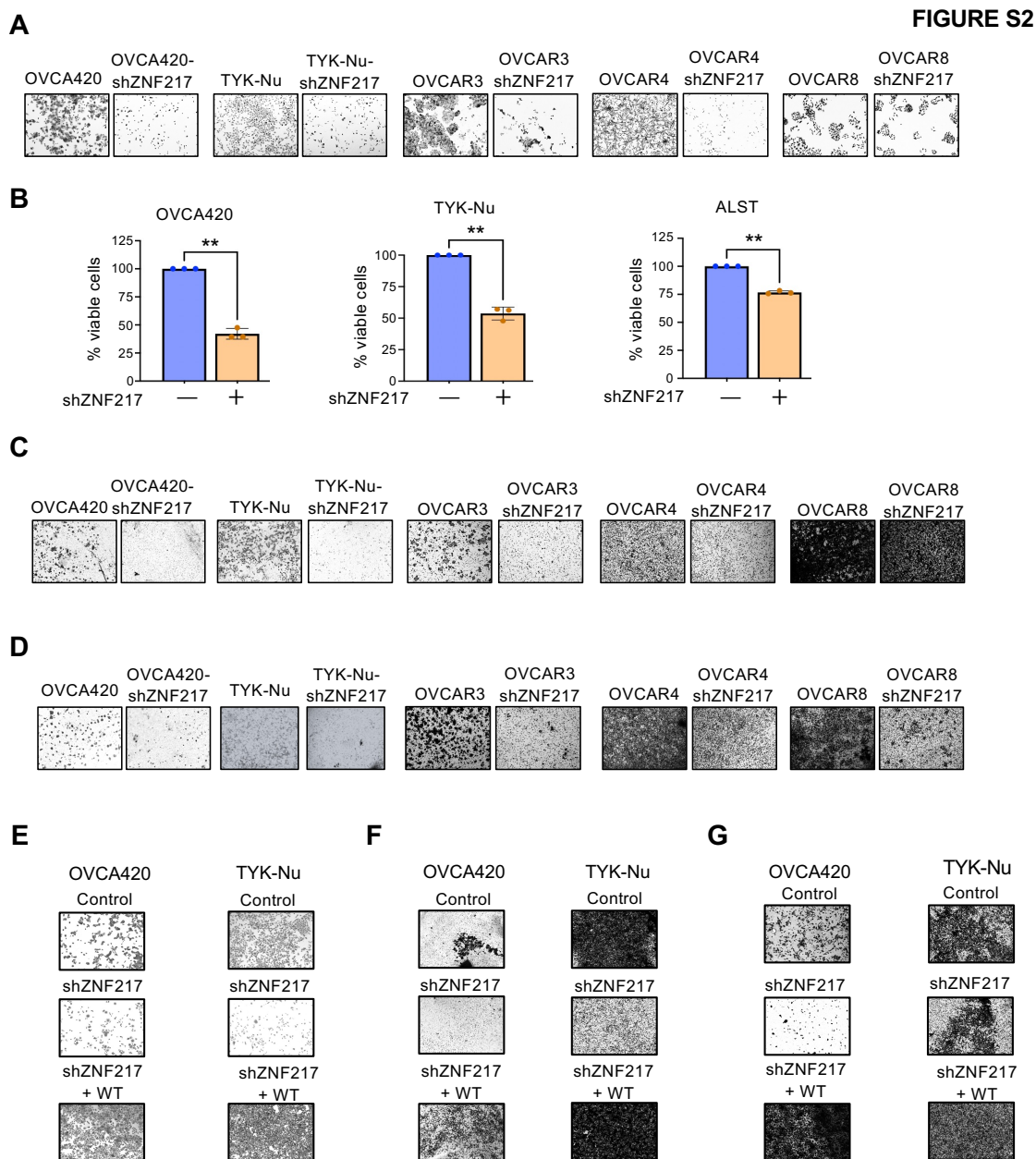


Figure S1. ZNF217 overexpression drives proliferation and metastatic phenotypes in ovarian cancer cells in vitro.

- A. ZNF217 mRNA is elevated in multiple cancers. Data retrieved from TNMplot.
- B. Data from The Cancer Genome Atlas Database (Firehose Legacy dataset) show that ZNF217 is amplified or overexpressed in 17 percent ovarian cancer cases.
- C. Western blot confirming stable ZNF217 overexpression in OVCA420, ALST, TYK-Nu, and OVCAR4 cells.
- D. Representative bright field images showing the effect of stable ZNF217 overexpression on cell proliferation in ovarian cancer cells.
- E. WST-1 cell proliferation assay showing ZNF217 overexpression increases proliferation in ovarian cancer cells (n=3).
- F. Cell cycle analysis using cells in sub-confluent culture showing differences in cell cycle distribution between OVCA420 and OVCA420-ZNF217 cells (n=5).

- 18 G. Representative bright field images showing the effect of stable ZNF217
19 overexpression on cell migration (transwell migration assay) in ovarian cancer cells.
- 20 H. Representative bright field images showing the effect of stable ZNF217
21 overexpression on the ability of ovarian cancer cells to invade through Matrigel
22 (Matrigel invasion assay).
- 23 I. Bright field representative images and quantification (n=3) at 0 and 48 hours from a
24 wound healing assay showing that ZNF217 increases cell migration in OVCA420 cells
25 that are treated with proliferation inhibitor, mitomycin C (MMC).
- 26 J. Individual runs from ECIS assay that was used to plot Fig. 2G.
- 27 K. Representative bright field images of control and ZNF217 overexpressing (stable
28 overexpression) OVCA420 and TYK-Nu cells on tissue culture plates coated with
29 fibronectin and COL1A1. The cells were allowed to attach to these plates for 45 min
30 before taking these images.

31



32

33 **Figure S2. ZNF217 depletion impairs ovarian cancer cell proliferation and**
 34 **metastatic potential in vitro.**

35 A. Representative bright field images showing the effect of stable ZNF217 knockdown on
 36 cell proliferation in ovarian cancer cells.

- 37 B. WST-1 cell proliferation assay showing ZNF217 knockdown decreases proliferation in
38 ovarian cancer cells (n=3).
- 39 C. Representative bright field images showing the effect of stable ZNF217 knockdown on
40 cell migration (transwell migration assay) in ovarian cancer cells.
- 41 D. Representative bright field images showing the effect of stable ZNF217 knockdown on
42 the ability of ovarian cancer cells to invade through Matrigel (Matrigel invasion assay).
- 43 E. Representative bright field images showing the effect of rescuing ZNF217 expression
44 using shRNA-resistant ZNF217 in ZNF217 knockdown ovarian cancer cells on cell
45 proliferation.
- 46 F. Representative bright field images showing the effect of rescuing ZNF217 expression
47 using shRNA-resistant ZNF217 in ZNF217 knockdown ovarian cancer cells on cell
48 migration (transwell migration assay).
- 49 G. Representative bright field images showing the effect of rescuing ZNF217 expression
50 using shRNA-resistant ZNF217 in ZNF217 knockdown ovarian cancer cells on
51 invasion (Matrigel invasion assay).

FIGURE S3

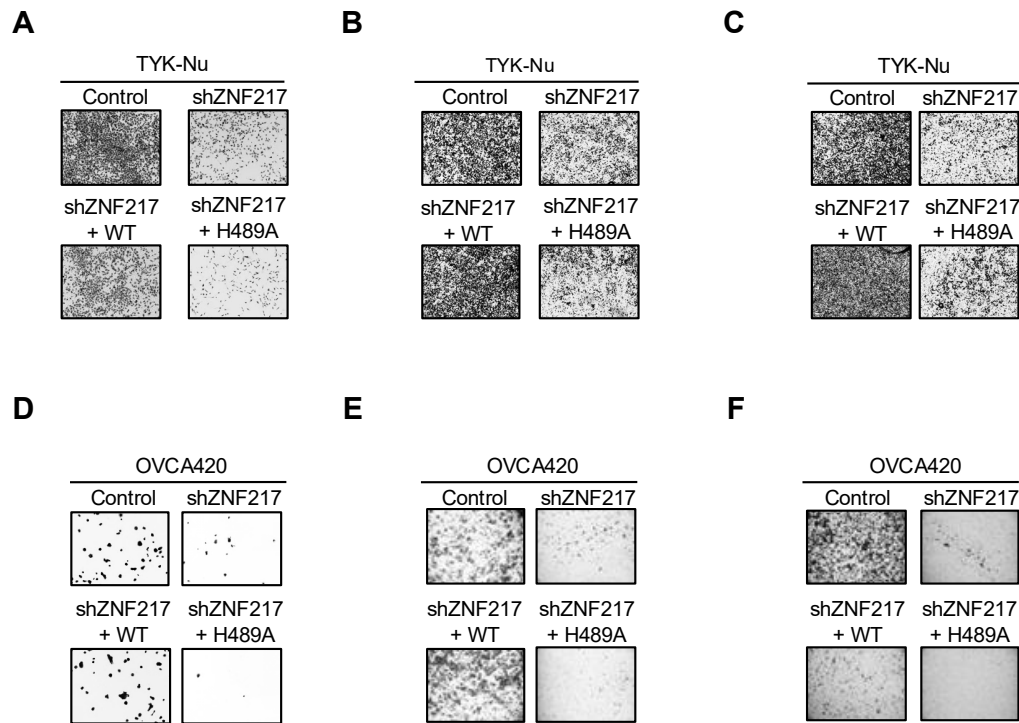


Figure S3. Oncogenic activity of ZNF217 in ovarian cancer cells is dependent on its ability to bind DNA.

- A. Representative bright field images showing that proliferation defects due to ZNF217 depletion in TYK-Nu cells can be rescued by an shRNA-resistant ZNF217-WT expression, but not ZNF217-H489A.
- B. Representative bright field images of transwell migration assay showing that ectopic expression of an shRNA-resistant ZNF217-WT, but not ZNF217-H489A, rescues defects in TYK-Nu migration induced upon ZNF217 knockdown.
- C. Representative bright field images showing that unlike ZNF217-WT, ZNF217-H489A mutant does not rescue the impact of ZNF217 depletion on the ability of TYK-Nu cells to invade through the Matrigel.

- D. Representative bright field images showing that proliferation defects due to ZNF217 depletion in OVCA420 cells can be rescued by an shRNA-resistant ZNF217-WT expression, but not ZNF217-H489A.
- E. Representative bright field images of transwell migration assay showing that ectopic expression of an shRNA-resistant ZNF217-WT, but not ZNF217-H489A, rescues defects in OVCA420 migration induced upon ZNF217 knockdown.
- F. Representative bright field images showing that unlike ZNF217-WT, ZNF217-H489A mutant does not rescue the impact of ZNF217 depletion on the ability of OVCA420 cells to invade through the Matrigel.

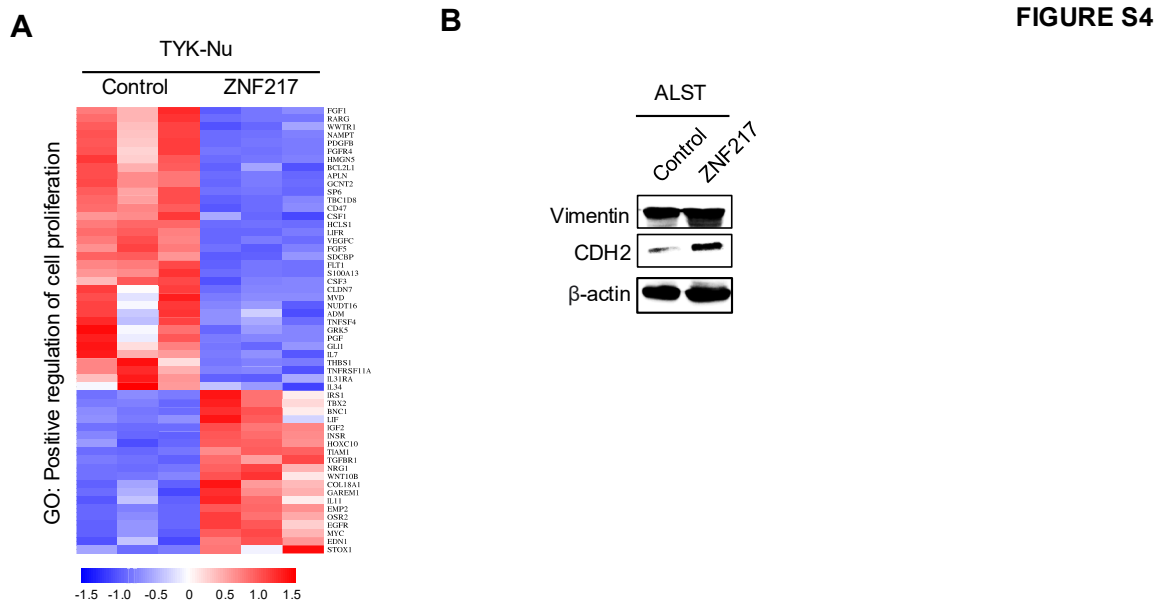
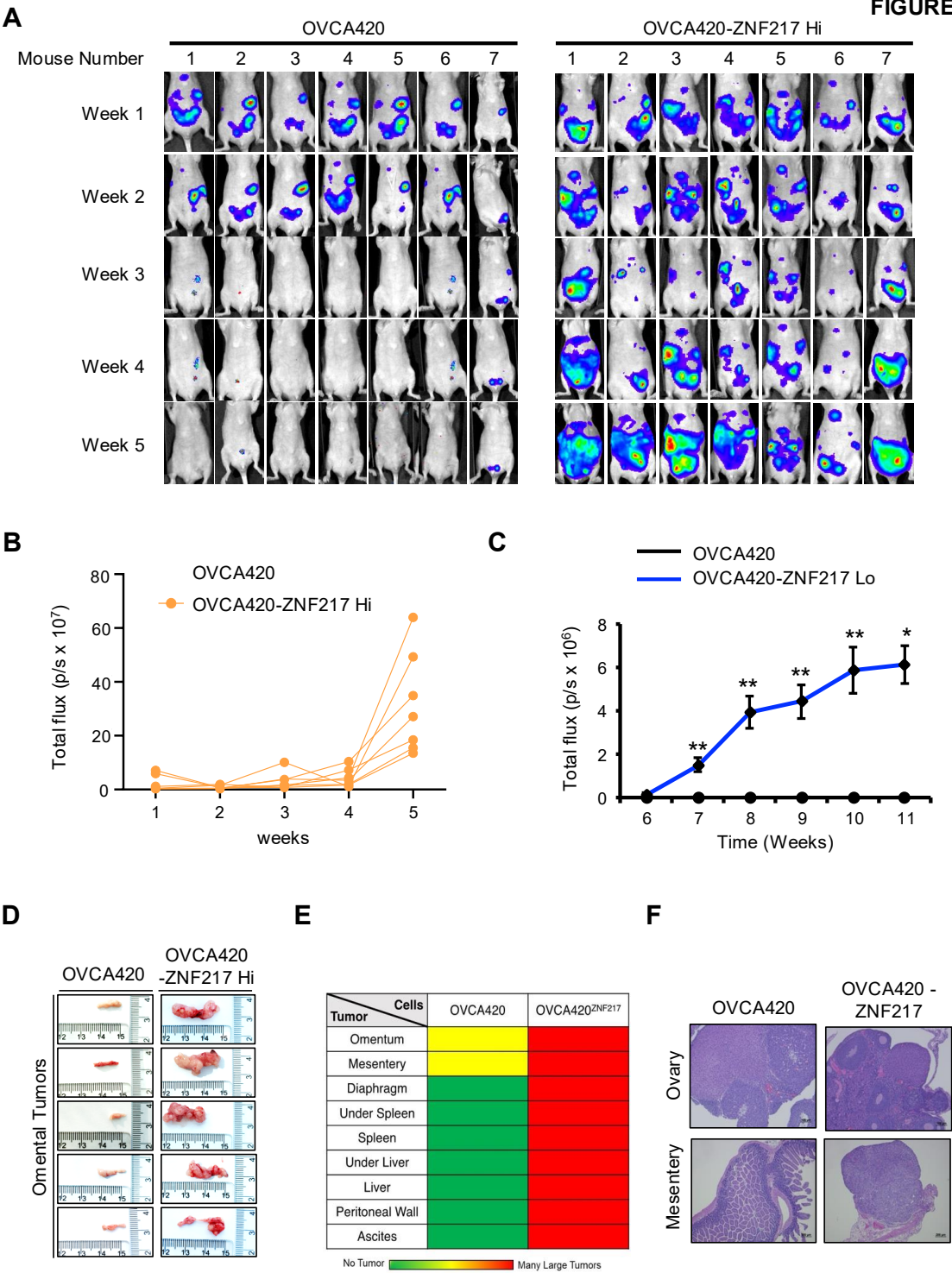


Figure S4. ZNF217 overexpression impacts transcription of genes important in driving ovarian cancer cell proliferation and EMT.

A. Heatmap showing differentially regulated genes in the gene ontology terms positive regulation of cell proliferation.

B. Western blot showing the stable ZNF217 overexpression causes an increase in the levels of vimentin and N-cadherin (CDH2) in ALST cells. Both these proteins are associated with a more mesenchymal phenotype.

FIGURE S5



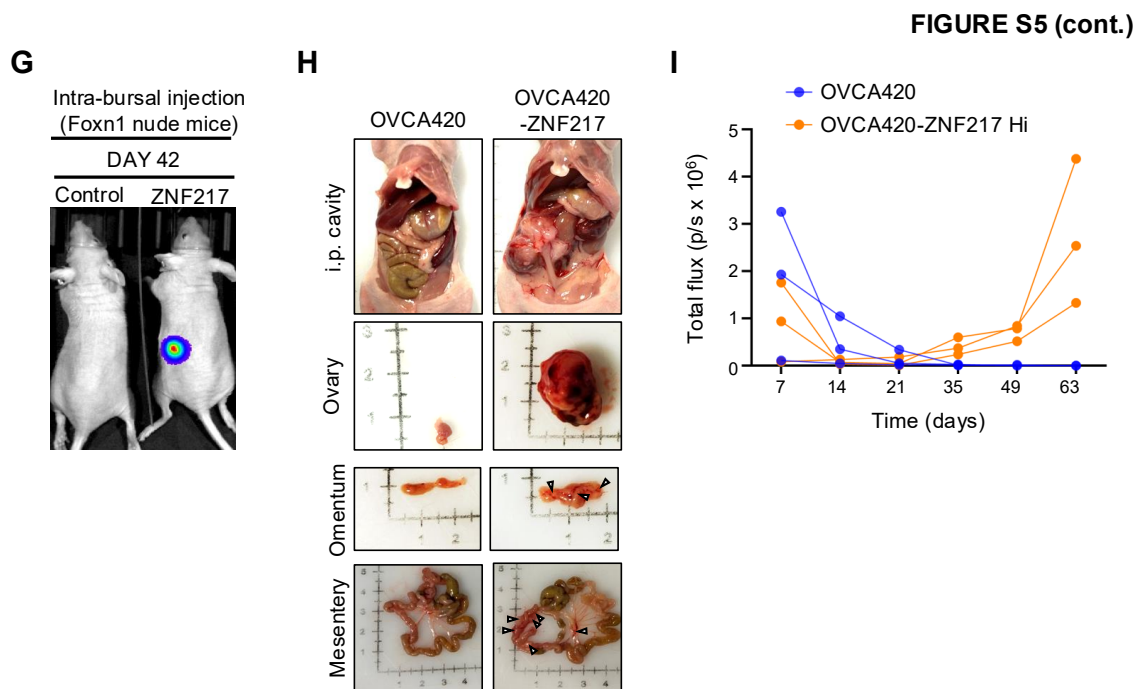


Figure S5. ZNF217 overexpression promotes ovarian cancer metastasis in vivo.

A. Non-invasive luciferase imaging using IVIS shows increased tumor burden in female foxn1 nude mice i.p injected with OVCA420-ZNF217 Hi cells compared control OVCA420 cells.

B. Quantification of luciferase intensity over time for individual mice i.p injected with OVCA420 and OVCA40-ZNF217 Hi cells. Cumulative representation of this data is shown in Figure 6C.

C. Quantification of luciferase intensity acquired through in vivo imaging over time reveals that in Foxn1 nude mice i.p injected with control and OVCA420-ZNF217 Lo cells, ZNF217 expression results in significantly higher metastatic tumor burden over time (n=6). The kinetics of progression is slower in OVCA420-ZNF217 Lo tumors compared to OVCA420-ZNF217 Hi tumors (see Figure 6C).

- D. Images of metastatic tumors on omentum in Foxn1 nude mice i.p injected with OVCA420 and OVCA420-ZNF217 Hi cells.
- E. The tumor burden in common ovarian cancer metastatic sites in Foxn1 nude mice i.p injected with OVCA420 and OVCA420-ZNF217 Hi cells.
- F. Hematoxylin and eosin (H&E) stain of OVCA420 control and ZNF217-high expressing tumors reveal minimal tumor burden in control. By contrast large metastatic lesions are observed in ZNF217 expressing cohorts. H&E for tumors on ovary and mesentery are shown.
- G. Representative in vivo imaging of luciferase intensity on day 42 in female Foxn1 nude mice injected intrabursally with OVCA420 control and OVCA420-ZNF217-Hi cells. Intrabursal injection of OVCA420-ZNF217-Hi cells results in larger tumor burden.
- H. ZNF217 expression in OVCA420 cells increases tumor forming ability in the peritoneal cavity, ovary, omentum, and mesentery in an ovarian intrabursal injection model using female immunodeficient Foxn1 nude mice.
- I. Quantification of luciferase intensity over time for individual mice i.b injected with OVCA420 and OVCA40-ZNF217 Hi cells. Cumulative representation of this data is shown in Fig. 6M.

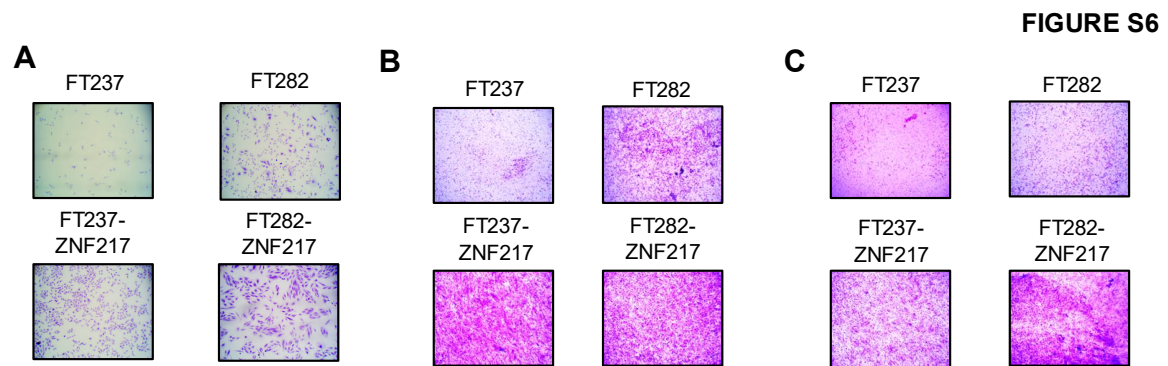


Figure S6. ZNF217 overexpression causes oncogenic transformation in immortalized fallopian tube secretory epithelial cells.

- A. Representative bright field images showing the effect of stable ZNF217 overexpression on cell proliferation in FT237 and FT282 cells.
- B. Representative bright field images showing the effect of stable ZNF217 overexpression on cell migration (transwell migration assay) in FT237 and FT282 cells.
- C. Representative bright field images showing the effect of stable ZNF217 overexpression on the ability of FT237 and FT282 cells to invade through Matrigel (Matrigel invasion assay).

A

	ALST	ALST-ZNF217
Carboplatin (IC50; μ M)	139.5	312.6
Paclitaxel (IC50; nM)	11.14	24.79

	OVCA420	OVCA420-ZNF217
Carboplatin (IC50; μ M)	597.9	6.248e+45
Doxorubicin (IC50; μ M)	0.33	0.66

	TYK-Nu	TYK-Nu-ZNF217
Carboplatin (IC50; μ M)	42.55	82.03
Doxorubicin (IC50; μ M)	0.111	0.164

B

	ALST	ALST-ZNF KD
Carboplatin (IC50; μ M)	205.8	38.31
Paclitaxel (IC50; nM)	38.61	4.573
Doxorubicin (IC50; μ M)	0.634	0.1443

	OVCA420	OVCA420-ZNF217 KD
Carboplatin (IC50; μ M)	8.563e+56	524.9

	TYK-Nu	TYK-Nu-ZNF217 KD
Doxorubicin (IC50; μ M)	0.673	0.351

FIGURE S7

Figure S7. ZNF217 promotes chemotherapeutic resistance in ovarian cancer cells.

- A. IC50 values for carboplatin, paclitaxel, and doxorubicin in control and ZNF217 overexpressing ALST, OVCA420 and TYK-Nu cells (N=3).
- B. IC50 values for carboplatin, paclitaxel, and doxorubicin in control and ZNF217 knockdown ALST, OVCA420 and TYK-Nu cells (N=3).

SUPPLEMENTARY TABLES

Table S1. List of differentially expressed genes in OVCA420 and OVCA420-ZNF217.

See attached excel file names Table S1.

Table S2. List of differentially expressed genes in TYK-Nu and TYK-Nu-ZNF217. See attached excel file names Table S2.

Table S3. List of common genes that are differentially regulated in both OVCA420 and TYK-Nu cells upon ZNF217 overexpression. See attached excel file names Table S3.

Table S4. List of genes that are differentially regulated in OVCA420 and TYK-Nu cells that have been identified as direct ZNF217 transcriptional targets in MCF-7 cells. See attached excel file named Table S4.

167

shRNA sequence	Source	Identifier
pLKO.1 puro-shZNF217-1 - CCCAAATCTATTGTTGTCTTA	Sigma (Mission shRNA)	TRCN0000013058
pLKO.1 puro-shZNF217-2 - ACACATTCAGAAGACCTTAAT	Sigma (Mission shRNA)	TRCN0000369268
pLKO.1 puro-shZNF217-3 - TGTAACCTGCTTGTTCAAAT	Sigma (Mission shRNA)	TRCN0000364635
pLKO.1 puro-shZNF217-4 - CCGGCAATTTCTTTGAGTAAA	Sigma (Mission shRNA)	TRCN0000013061
pLKO.1 puro-shZNF217-5 - GCCAACTCAATCCCTCTTAAT	Sigma (Mission shRNA)	TRCN0000013060

168

169 **Table S5.** ZNF217 shRNA sequence. ZNF217 shRNA was ordered from Sigma-Aldrich
 170 Mission shRNA. These sequences were purchased as a plasmid in the pLKO.1-puro
 171 plasmid.

172

Antibody	SOURCE	IDENTIFIER
β-actin	Sigma	A2228
E-cadherin (CDH1)	Proteintech	20874
FLAG	Sigma	F1804
KRT7	Proteintech	22208
N-cadherin (CDH2)	Proteintech	22018
PAX8	Proteintech	10336
SNAI1	Proteintech	13099
TWIST1	Proteintech	25465
V5 tag	Proteintech	14440
VCAM1	Proteintech	11444
Vimentin	Proteintech	10366
ZEB1	Proteintech	21544
ZNF217	Thermo Fisher	720352

173

174 **Table S6.** List of antibodies used in this study.

Gene	Primer Sequence
ZNF217	Fwd: AAACATGCCAACTCAATCCCTC Rev: GGAATGGAACAACAGCGGT
β -actin	Fwd: GCTCTTTTCCAGCCTTCCTT Rev: CGGATGTCAACGTCACACT
SNAI1	Fwd: TGCCCTCAAGATGCACATCCGA Rev: GGGACAGGAGAAGGGCTTCTC

175 **Table S7.** List of RT-qPCR primers used in this study.

Construct	Primer	Sequence	NEB Enzyme
FU-CRW_FLAG-ZNF217	FLAG-ZNF217 Forward	GTACTCTAGAATGGACTACAAAGACG ATGACGA	Xba1
	ZNF217 Reverse	CATGGAATTCTCAAGTTTTTTTGTCAAT TGGTC	EcoR1
PLVX-IRES-BERKY3:FLAG-ZNF217	FLAG-ZNF217 Forward	TGGCACCGGTATGGACTACAAAGACG ATGACGACAAGCAATCGAAAGTG	Age1
	ZNF217 Reverse	GCGGCCGCTCTATCAAGTTTTTTTGTGTC	Not1
PLVX-IRES-BERKY3:FLAG-ZNF217-H489A site directed mutagenesis	ZNF217-H489A Forward	CAAATTATTACCTCAATATTGCCCTCAG AACGCATACAGGTG	N/A
	ZNF217-H489A Reverse	CACCTGTATGCGTTCTGAGGGCAATAT TGAGGTAATAATTTG	N/A
V5-ZNF217_pLX307	N/A	Purchased from Addgene, plasmid #98384 ¹	N/A

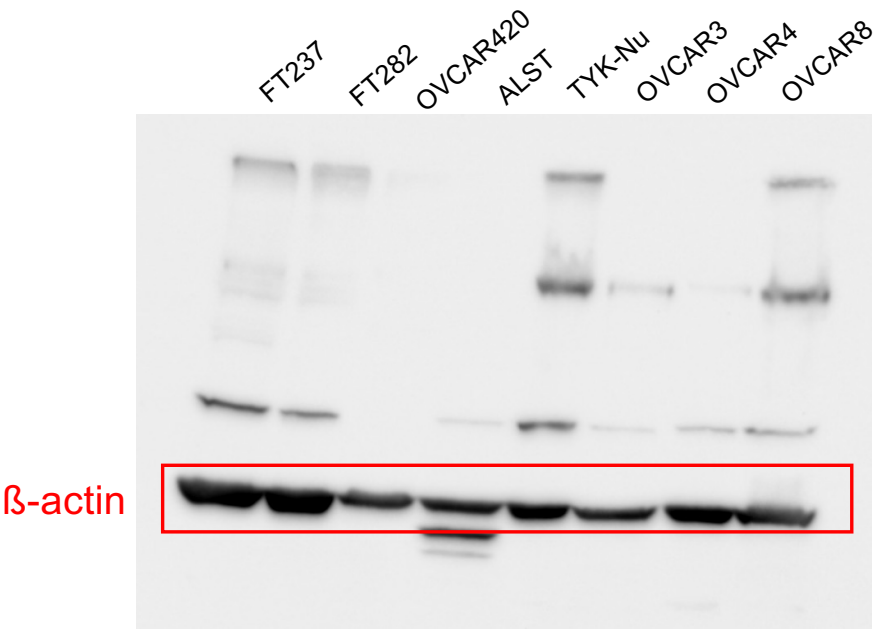
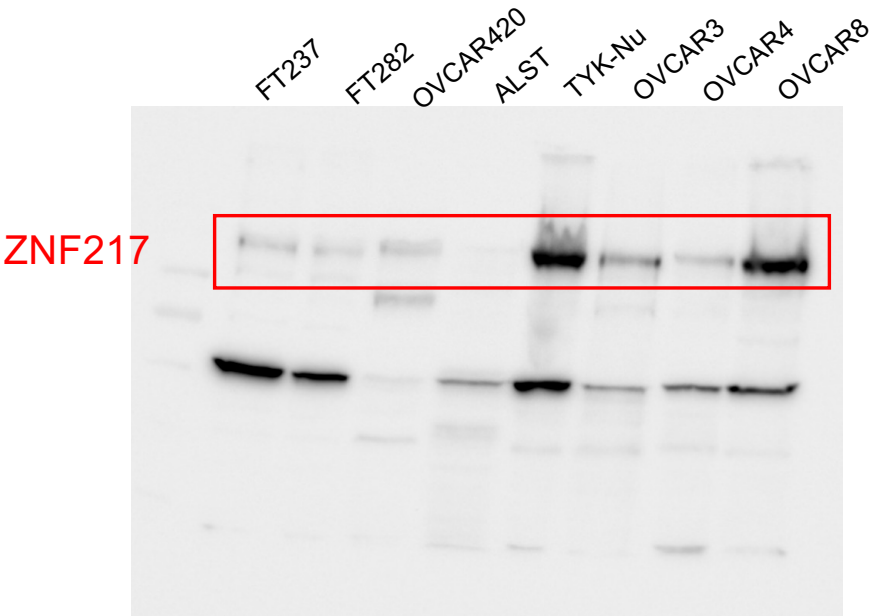
Table S8. List of primers used for molecular cloning.

Full Unedited, Annotated Blots
Main Figures

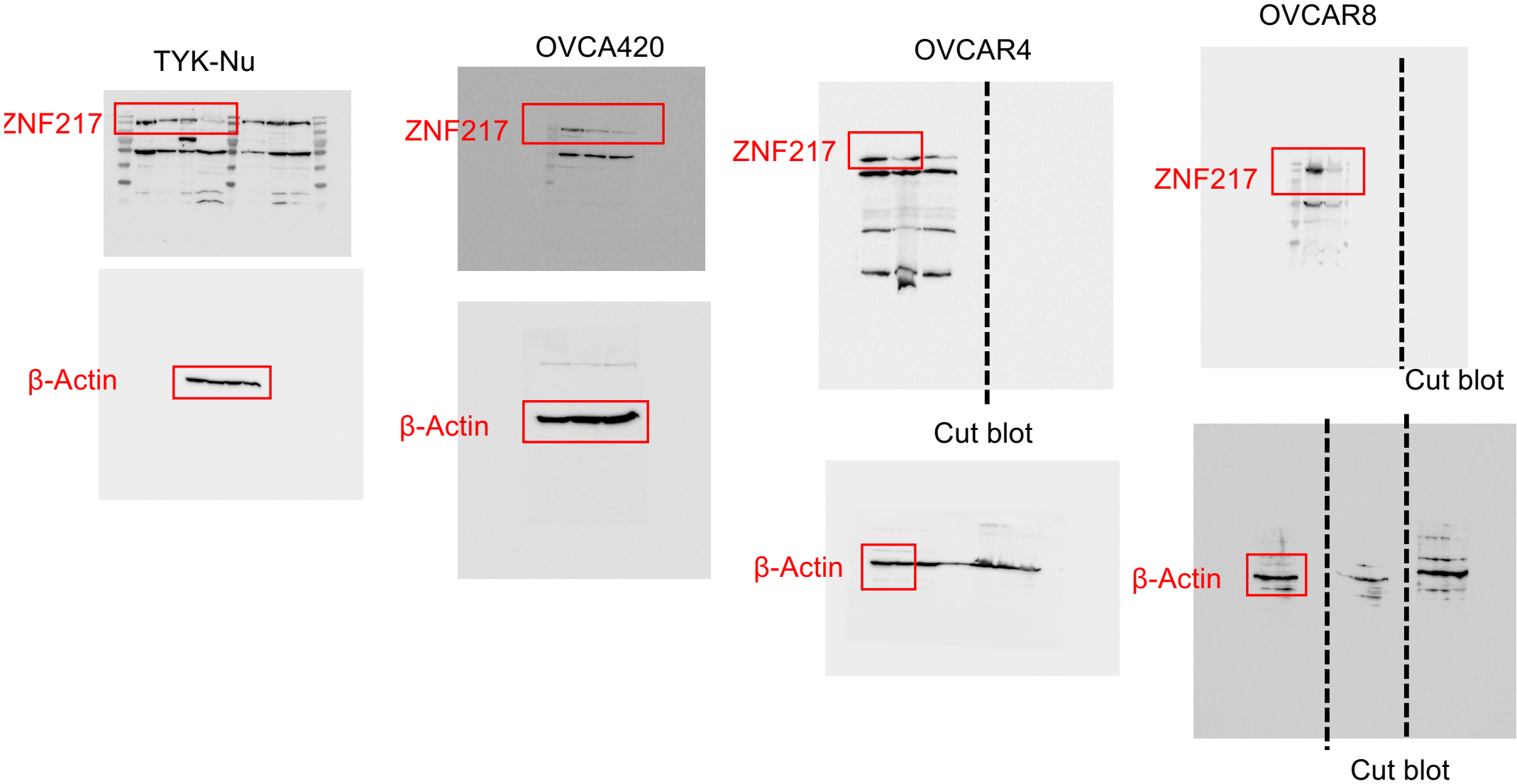
**ZNF217 promotes ovarian cancer progression by impacting multiple pivotal steps
in the metastatic process**

Wardrup KC et al.

Full Unedited Blots: Figure 1C

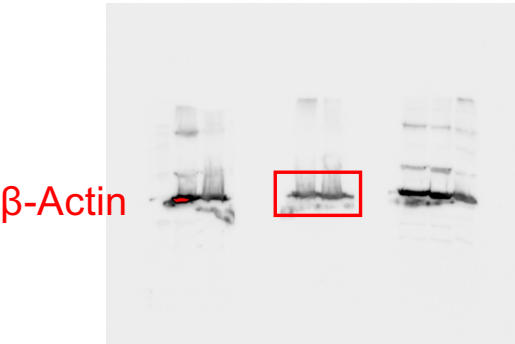
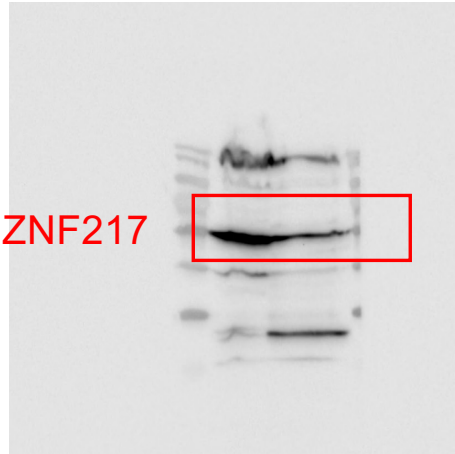


Full Unedited Blots: Figure 2A



Full Unedited Blots: Figure 2A (Cont).

OVCAR3



Full Unedited Blots: Figure 2G

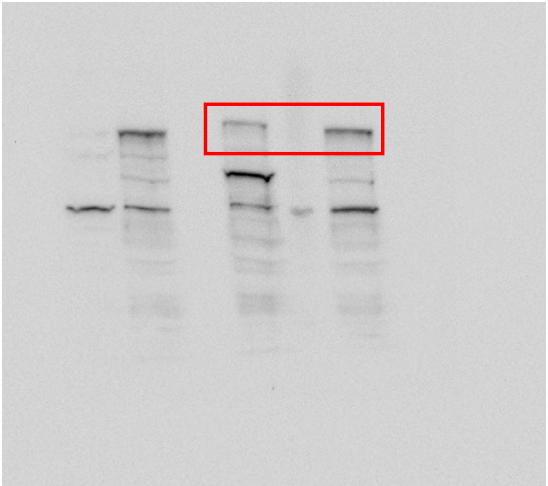
TYK-Nu

ZNF217



OVCA420

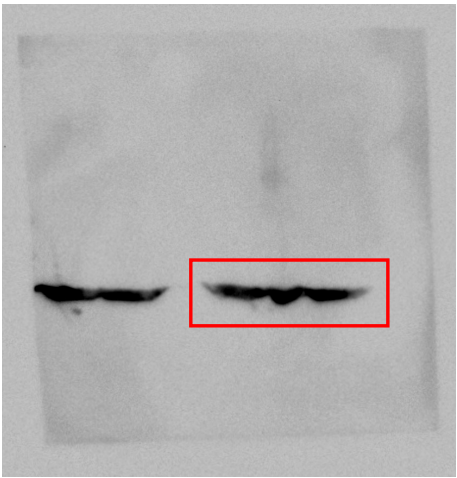
ZNF217



β -Actin

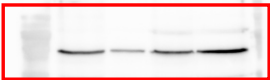


β -Actin

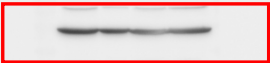


Full Unedited Blots: Figure 3B

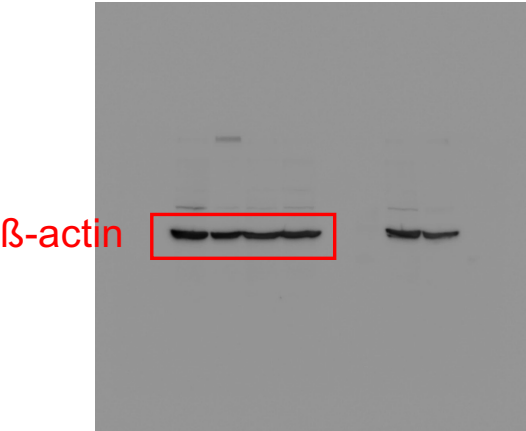
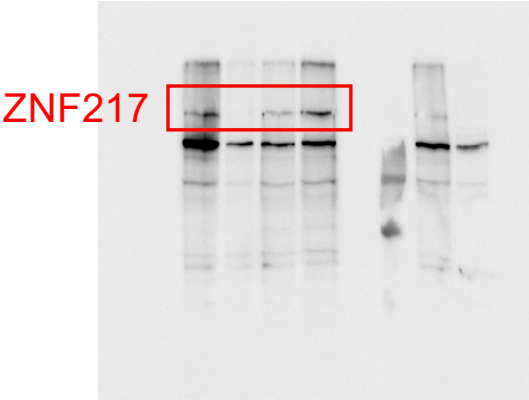
ZNF217



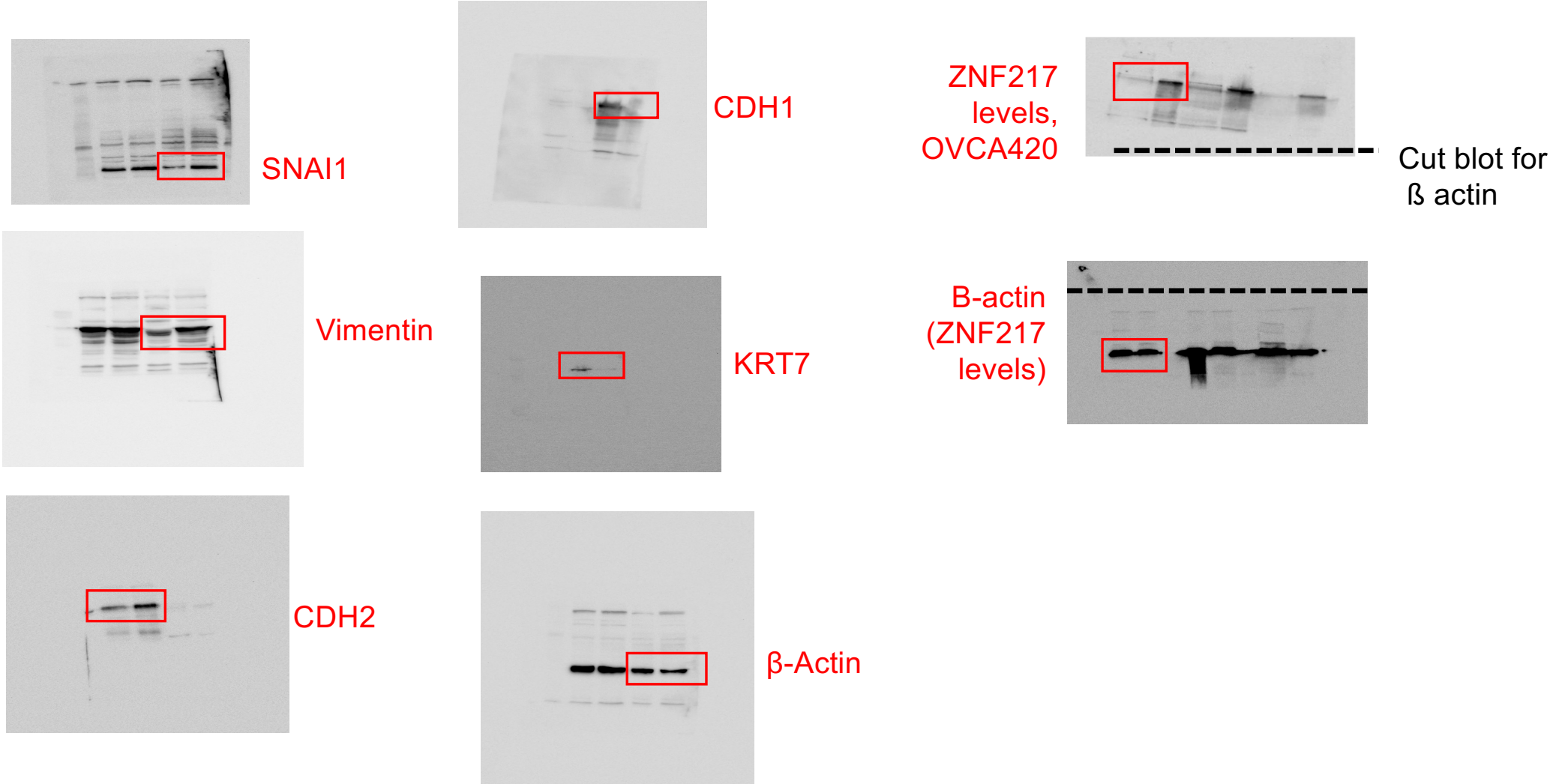
β -Actin



Full Unedited Blots: Figure 3G

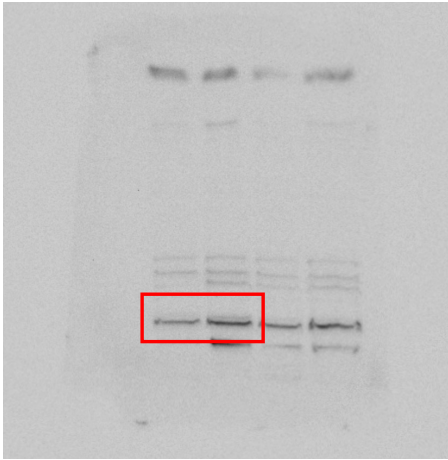


Full Unedited Blots: Figure 4H



Full Unedited Blots: Figure 4I

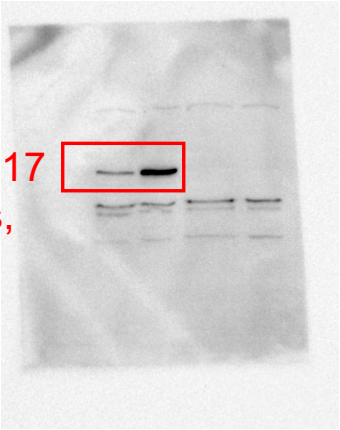
Snai1



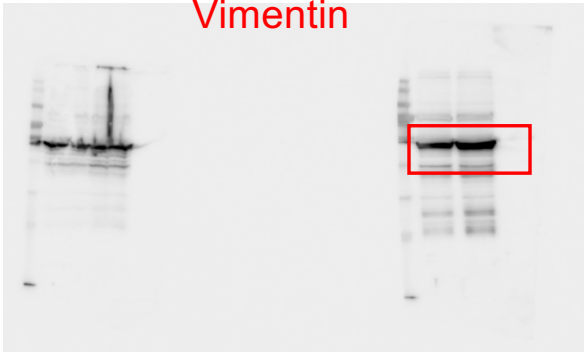
PAX8



ZNF217
levels,
tyknu



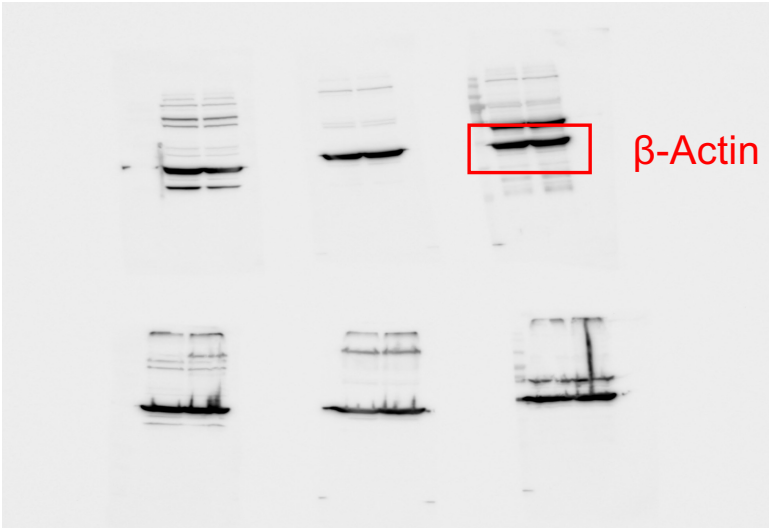
Vimentin



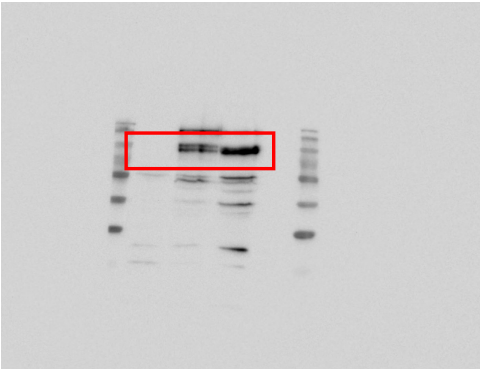
CDH2



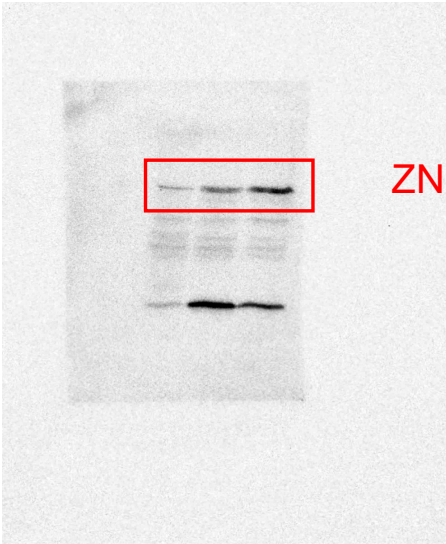
β -Actin



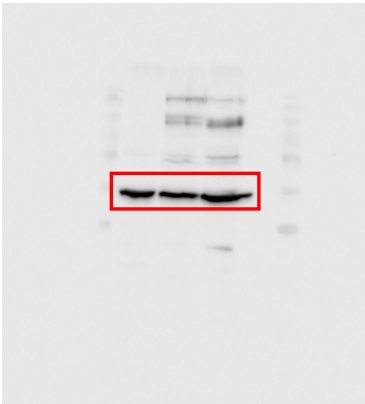
Full Unedited Blots: Figure 5A



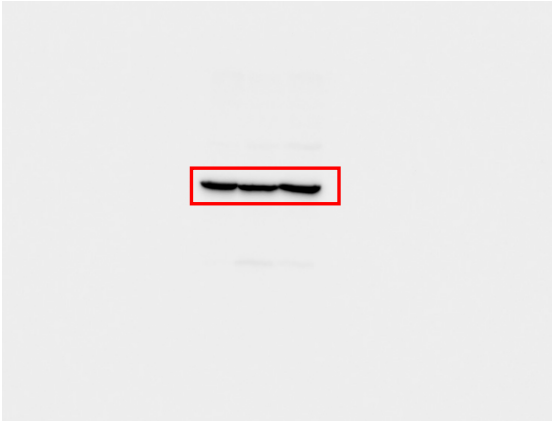
FLAG



ZNF217



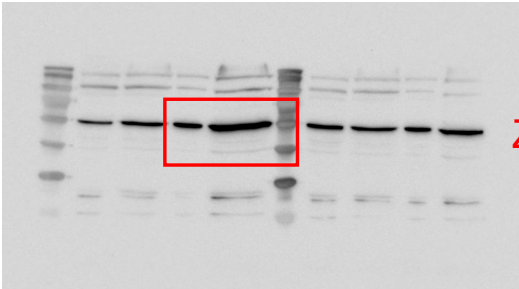
β -Actin



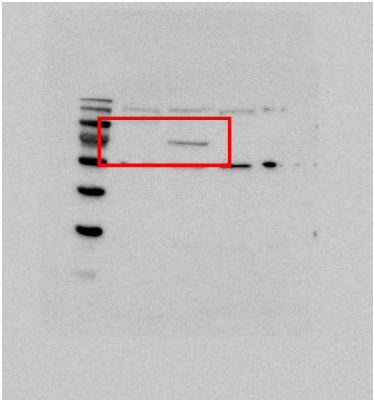
β -Actin

Full Unedited Blots: Figure 6A

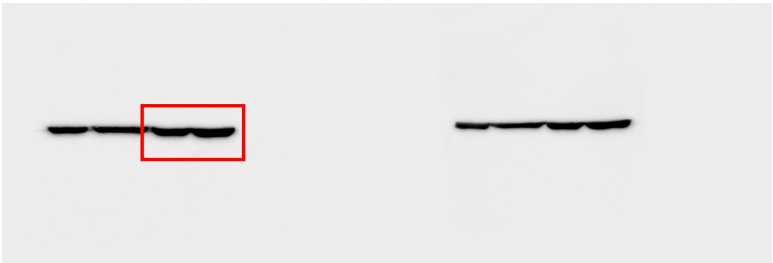
FT237



ZNF217

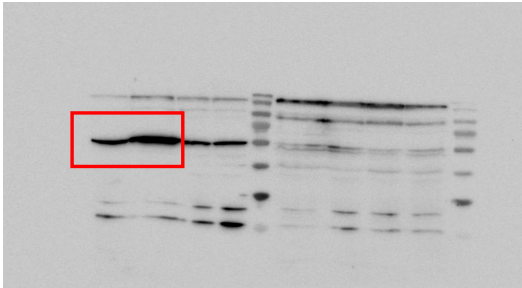


V5

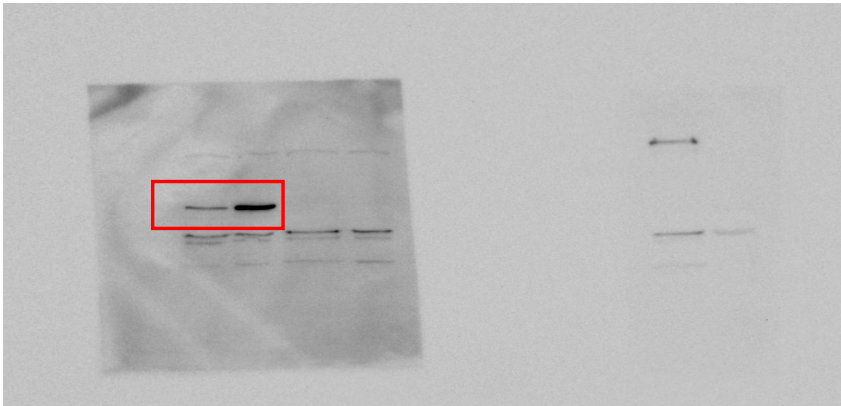


β -Actin

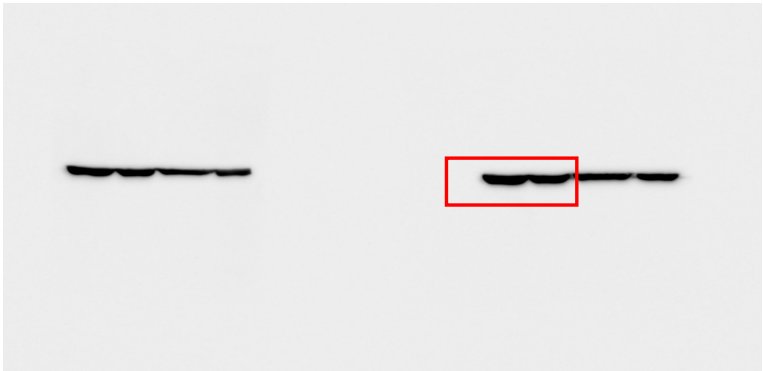
FT282



ZNF217



V5

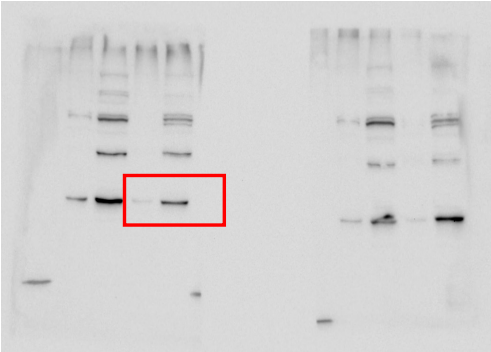


β -Actin

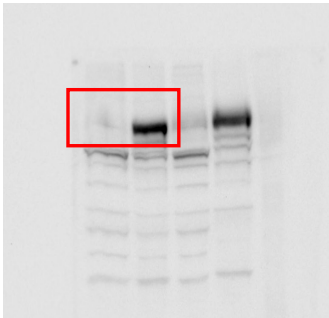
Full Unedited Blots: Figure 6F

FT237

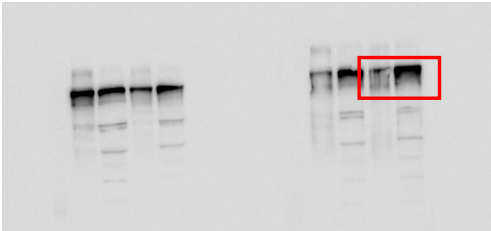
SNAI1



VCAM1



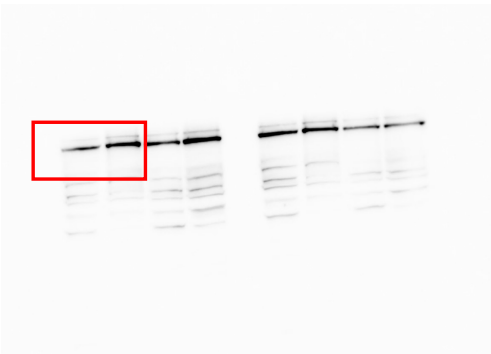
CDH2



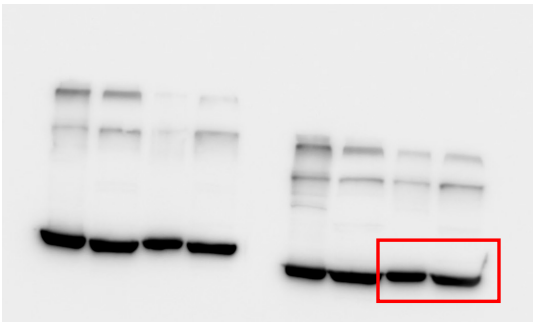
ZNF217 levels



ZEB1

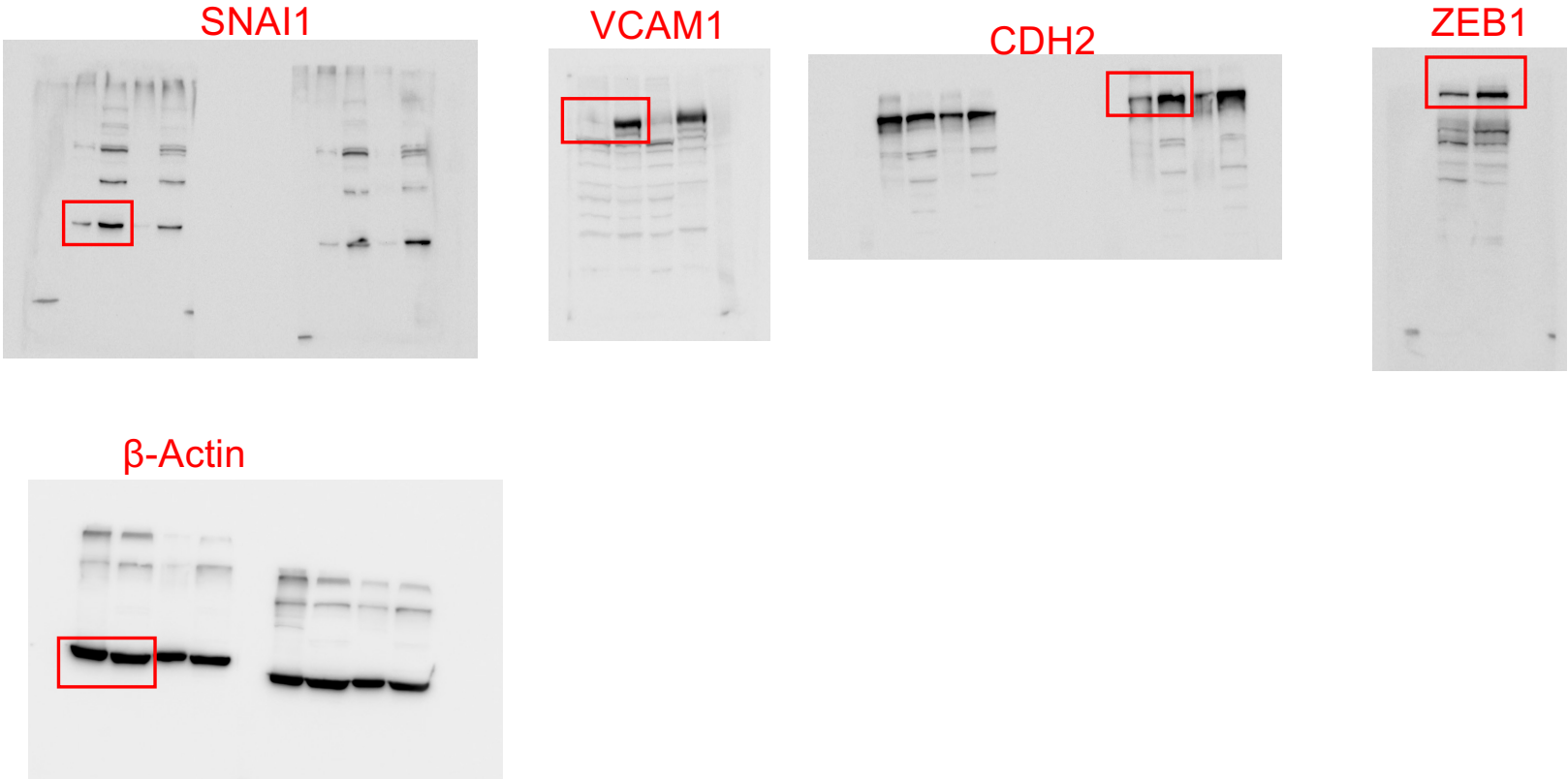


β -Actin



Full Unedited Blots: Figure 6F

FT282



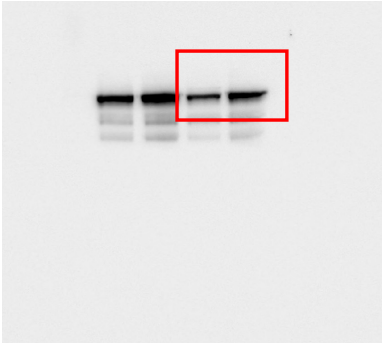
Full Unedited, Annotated Blots
Supplemental Figures

**ZNF217 promotes ovarian cancer progression by impacting multiple pivotal steps
in the metastatic process**

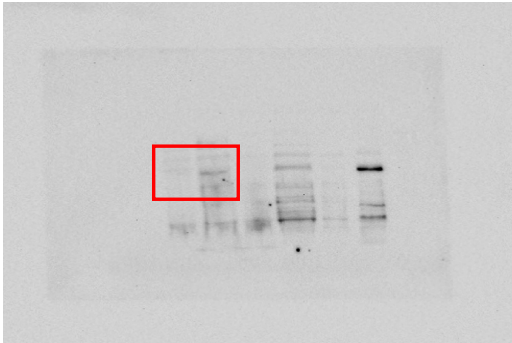
Wardrup KC et al.

Full Unedited Blots: Figure S1C

OVCA420 cells



ZNF217



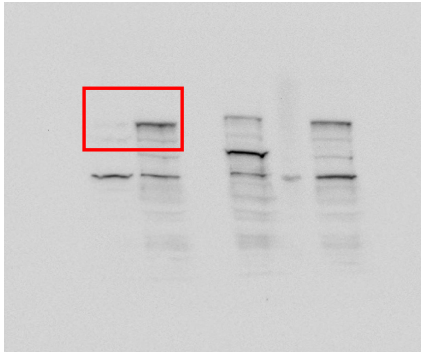
FLAG



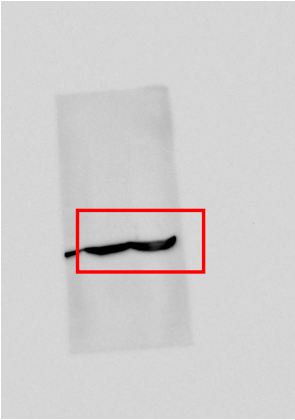
β -Actin

Full Unedited Blots: Figure S1C

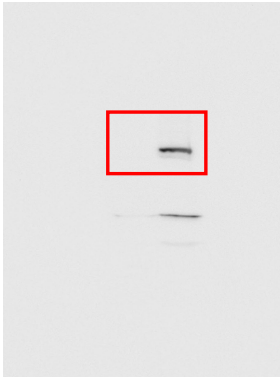
TYK-Nu cells



ZNF217



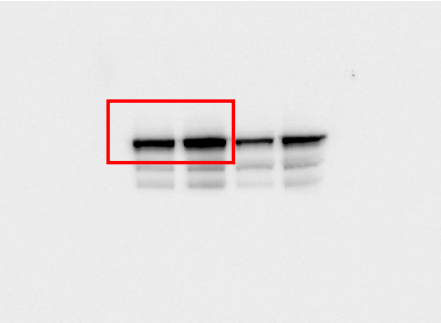
β -Actin



V5

Full Unedited Blots: Figure S1C

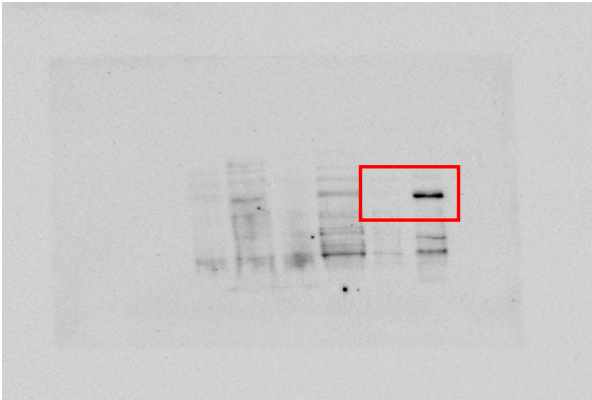
ALST cells



ZNF217



β -Actin



FLAG