Epigenetically inhibiting CYP3A5 modulates the migration and invasion of esophageal squamous cell carcinoma via ZEB2

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Supporting Information

Supplementary tables

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Supplementary Tables

Table S1. ESCC fresh tissue specimen information

Number	Gender	Age	Subtype	TNM stage
1	Male	59	ESCC	T1N2M0
2	Male	60	ESCC	T3N2M0
3	Male	69	ESCC	T1N0M0
4	Female	56	ESCC	T1N1M0
5	Female	64	ESCC	T1N2M0
6	Male	49	ESCC	T1N0M0
7	Male	57	ESCC	T2N2M0
8	Female	58	ESCC	T1N2M0
9	Female	61	ESCC	T2N0M0
10	Male	66	ESCC	T2N0M0
11	Male	62	ESCC	T3N1M0
12	Female	55	ESCC	T1N0M0
13	Female	51	ESCC	T2N0M0
14	Male	51	ESCC	T3N1M0
15	Female	59	ESCC	T2N0M0
16	Female	68	ESCC	T1N0M0
17	Female	60	ESCC	T2N0M0
18	Female	63	ESCC	T3N0M0
19	Male	50	ESCC	T2N0M0
20	Male	53	ESCC	T1N1M0
21	Male	50	ESCC	T1bN0M0
22	Female	52	ESCC	T1N1M0
23	Female	59	ESCC	T2N0M0
24	Male	71	ESCC	T2N0M0
25	Male	68	ESCC	T1N0M0
26	Male	63	ESCC	T1N0M0
27	Male	62	ESCC	T3N0M0
28	Male	79	ESCC	T3N2M0
29	Male	77	ESCC	T3N0M0
30	Male	65	ESCC	T2N0M0
31	Female	75	ESCC	T3N0M0
32	Male	62	ESCC	T3N1M0
33	Female	62	ESCC	T2N0M0
34	Female	71	ESCC	T3N0M0
35	Male	53	ESCC	T3N1M0
36	Female	68	ESCC	T2N0M0
37	Female	57	ESCC	T1N0M0
38	Male	71	ESCC	T2N0M0
39	Female	65	ESCC	T1N0M0

40	Male	68	ESCC	T2N0M0
41	Male	63	ESCC	T1N0M0
42	Male	62	ESCC	T3N1M0
43	Male	67	ESCC	T3N0M0
44	Male	65	ESCC	T1N0M0
45	Male	67	ESCC	T2N1M0
46	Female	60	ESCC	T3N2M0
47	Male	71	ESCC	T3N1M0
48	Male	72	ESCC	T3N2M0
49	Male	77	ESCC	T3N0M0
50	Male	54	ESCC	T2N1M0
51	Male	73	ESCC	T1N0M0
52	Male	88	ESCC	T3N1M0
53	Male	64	ESCC	T2N1M0
54	Male	67	ESCC	T3N2M0
55	Male	68	ESCC	T2N0M0
56	Female	62	ESCC	T2N0M0
57	Male	85	ESCC	T2N0M0
58	Male	54	ESCC	T1N0M0
59	Male	62	ESCC	T3N1M0
60	Male	65	ESCC	T2N1M0
61	Male	66	ESCC	T2N0M0
62	Female	57	ESCC	T2N0M0
63	Male	57	ESCC	T2N0M0
64	Male	57	ESCC	T2N0M0
65	Male	61	ESCC	T2N0M0
66	Male	63	ESCC	T2N0M0
67	Female	56	ESCC	T2N0M0
68	Female	63	ESCC	T3N1M0
69	Male	79	ESCC	T2N0M0
70	Male	59	ESCC	T1N0M0
71	Male	62	ESCC	T1N0M0
72	Male	61	ESCC	T3N0M0
73	Female	68	ESCC	T2N0M0
74	Male	72	ESCC	T2N0M0
75	Female	57	ESCC	T2N0M0
76	Male	55	ESCC	T2N0M0
77	Female	69	ESCC	T2N0M0
78	Male	74	ESCC	T2N0M0
79	Female	66	ESCC	T1N0M0
80	Female	67	ESCC	T1N0M0
81	Male	63	ESCC	T2N0M0

Table S2. ESCC FFPE tissue specimen information

FFPE_ID	Age	Gender	TNM stage	Subtype	OS_month
126075A	69	Female	T1N0M0	ESCC	8
125183A	67	Male	T3N1M0	ESCC	11
125036A	60	Male	T1N0M0	ESCC	18
125562A	59	Male	T4N0M0	ESCC	6
126516B	62	Male	T3N2M0	ESCC	11
125176A	62	Female	T3N0M0	ESCC	17.5
121689B	47	Female	T1N0M0	ESCC	17.5
122954B	69	Female	T1N1M0	ESCC	26.9
126731A	59	Male	T1N1M0	ESCC	35.6
121187B	60	Male	T4N1M0	ESCC	28
122684A	81	Male	T2N2M0	ESCC	31
124276A	61	Male	T4N1M0	ESCC	24.5
124470A	74	Female	T3N2M0	ESCC	17
122347A	66	Male	T4N1M0	ESCC	25
126436A	57	Male	T1N3M0	ESCC	24
124911A	62	Male	T2N2M0	ESCC	16.5
123406A	70	Female	T1N2M0	ESCC	32
123416A	56	Male	T2N2M0	ESCC	14.5
125770A	76	Male	T3N0M0	ESCC	26.5
123611B	66	Male	T3N0M0	ESCC	25
125113B	69	Male	T3N2M0	ESCC	25
122963A	58	Male	T3N1M0	ESCC	24
126336A	54	Male	T2N2M0	ESCC	22
125268A	77	Male	T4N2M0	ESCC	9
122138B	66	Male	T4N3M0	ESCC	27.8
122492A	50	Female	T3N3M0	ESCC	26
12101A	71	Male	T3N3M0	ESCC	8
122960A	60	Male	T1N3M0	ESCC	25
120426B	66	Male	T4N2M0	ESCC	17
122573A	58	Male	T4N3M0	ESCC	21
122500A	72	Female	T4N2M0	ESCC	23
126321A	60	Male	T3N1M0	ESCC	7
125547A	68	Female	T3N1M0	ESCC	7
122588B	60	Male	T1N1M0	ESCC	8
122961A	20	Female	T2N0M0	ESCC	13
126628A	55	Male	T2N3M0	ESCC	10
123408B	60	Female	T1N3M0	ESCC	12
125277A	64	Male	T4N3M0	ESCC	13
125278A	75	Female	T1N0M0	ESCC	14
125190A	62	Female	T1N1M0	ESCC	12

124727A	71	Male	T1N1M0	ESCC	17.5
125289A	66	Male	T1N0M0	ESCC	18
124820A	63	Male	T3N1M0	ESCC	19.5
124707A	74	Male	T2N0M0	ESCC	16.5
125584A	58	Male	T1N3M0	ESCC	16.5
124467A	61	Male	T3N0M0	ESCC	18
125040B	48	Male	T3N1M0	ESCC	19.6
122143A	68	Male	T3N0M0	ESCC	16.8
120428A	58	Female	T1N0M0	ESCC	18.5
126356A	68	Male	T1N3M0	ESCC	15
122137A	63	Male	T1N0M0	ESCC	15.6
123043A	66	Female	T1N3M0	ESCC	16.5
124446A	69	Male	T3N1M0	ESCC	19
124622A	63	Male	T2N0M0	ESCC	15.5
123700A	71	Female	T3N1M0	ESCC	19.6
123594A	72	Female	T3N3M0	ESCC	18.4
123044B	75	Female	T2N2M0	ESCC	18.5
124562A	69	Female	T3N2M0	ESCC	15
121066A	53	Male	T2N1M0	ESCC	19
122134A	64	Female	T2N2M0	ESCC	14.9
120638B	69	Male	T1N1M0	ESCC	15.8
123324B	57	Male	T3N0M0	ESCC	17.5
124564A	66	Male	T2N0M0	ESCC	16
123415B	68	Male	T1N0M0	ESCC	18
125095A	69	Male	T2N0M0	ESCC	18.5
122354B	72	Male	T4N2M1	ESCC	25
124914A	59	Male	T4N3M0	ESCC	17.5

Table S3. IHC scores of ESCC FFPE tissue specimen

	Normal			Tumor		
FFPE_ID	**Intensity**	**Density**	**Score**	**Intensity**	**Density**	**Score**
126075A	3+	100%	12	2+	5%	2
125183A	0	0	0	3+	20%	3
125036A	3+	100%	12	3+	10%	3
125562A	3+	95%	12	1+	10%	1
126516B	3+	90%	12	2+	15%	2
125176A	3+	100%	12	3+	5%	3
121689B	NA	NA	NA	2+	5%	2
122954B	3+	80%	12	3+	5%	3
126731A	NA	NA	NA	2+	25%	2
121187B	NA	NA	NA	1+	10%	1
122684A	NA	NA	NA	3+	5%	3
124276A	0	0	0	1+	1%	1
124470A	NA	NA	NA	1+	5%	1
122347A	0	0	0	1+	20%	1
126436A	NA	NA	NA	1+	5%	1
124911A	1+	60%	3	3+	85%	12
123406A	NA	NA	NA	3+	10%	3
123416A	NA	NA	NA	3+	50%	6
125770A	NA	NA	NA	3+	10%	3
123611B	NA	NA	NA	2+	5%	1
125113B	3+	90%	12	2+	20%	2
122963A	NA	NA	NA	3+	35%	6
126336A	3+	80%	12	2+	40%	4
125268A	3+	60%	9	0	0	0
122138B	2+	40%	4	0	0	0
122492A	NA	NA	NA	0	0	0
12101A	NA	NA	NA	0	0	0
122960A	1+	20%	1	0	0	0
122354A	3+	80%	12	0	0	0
120426B	0	0	0	0	0	0
122573A	NA	NA	NA	0	0	0
122500A	NA	NA	NA	0	0	0
126321A	0	0	0	0	0	0
125547A	1+	20%	1	0	0	0
122588B	0	0	0	0	0	0
122961A	0	0	0	0	0	0
126628A	NA	NA	NA	0	0	0
123408B	NA	NA	NA	0	0	0
125277A	1+	10%	1	0	0	0
125278A	0	0	0	0	0	0

125190A	NA	NA	NA	0	0	0
124727A	3+	40%	6	0	0	0
125289A	3+	95%	12	0	0	0
124820A	0	0	0	0	0	0
124707A	3+	90%	12	0	0	0
125584A	0	0	0	0	0	0
124467A	2+	75%	6	0	0	0
125040B	1+	15%	1	0	0	0
122143A	0	0	0	0	0	0
120428A	2+	80%	8	0	0	0
126356A	1+	80%	4	0	0	0
122137A	1+	60%	3	0	0	0
123043A	3+	80%	12	0	0	0
124446A	3+	80%	12	0	0	0
124622A	3+	80%	12	0	0	0
123700A	1+	20%	1	0	0	0
123594A	0	0	0	0	0	0
123044B	0	0	0	0	0	0
124562A	0	0	0	0	0	0
121066A	NA	NA	NA	0	0	0
122134A	NA	NA	NA	0	0	0
120638B	NA	NA	NA	0	0	0
123324B	3+	80%	12	0	0	0
124564A	NA	NA	NA	0	0	0
123415B	NA	NA	NA	0	0	0
125095A	NA	NA	NA	0	0	0
124914A	NA	NA	NA	0	0	0

Table S4. Primers used in this study

Application	**Gene**	**Primer (5′–3′)**
RT-qPCR	CYP3A5	GGAGTTCCGCCCTGAAAG
		TCCAGTTCCAAAGGGTGTGT
	GAPDH	AGGTGAAGGTCGGAGTCA
		GGTCATTGATGGCAACAA
	HDAC1	ACGAAGACGACCCTGACAAG
		TCCTCACAGGCAATTCGTTT
	HDAC2	ATAAAGCCACTGCCGAAGAA
		TCCTCCAGCCCAATTAACAG
	HDAC3	ACGTGGGCAACTTCCACTAC
		GACTCTTGGTGAAGCCTTGC
	HDAC4	AAGAACAAGGAGAAGGGCAAG
		TGGAGAACTCTGGTCAAGGGA
	HDAC5	GTCTCGGCTCTGCTCAGTGTAGA
		GGCCACTGCGTTGATGTTG
	HDAC6	AGTCTTATGGATGGCTATTGCATG
		TGGACCAGTTAGAGGCCTTCAGG
	HDAC7	CCATGACGACGGCAACTTCTT
		TGCTGCGTCATGTATCCAAAAC
	HDAC8	AAGAGGGCGATGATGATC
		GTGGCTGGGCAGTCATAACC
	HDAC9	AGTGTGAGACGCAGACGCTTAG
		TTTGCTGTCGCATTTGTTCTTT
	HDAC10	TTACTTCTCCTGGCACCGCTA
		CCACGTAGTCAGCGTTTCCC
	P300	TTCAGCACCATGGACAGTTG
		GTTGCATACGAGGCCCATAG
	CBP	TTTCCGGCAGCCTGTAGAT
		TCCATGGGATTCTTTACGATG
ChIP-qPCR	CYP3A5	ACCCTTGGACTCCCCGATAA
		AAGAATCGCACACACCCCTT

Table S5. siRNAs sequences

Name	**Sense (5′–3′)**
si-NC	UUCUCCGAACGUGUCACGU
siHDAC1	CCGGUCAUGUCCAAAGUAA
siHDAC2	CUACGACGGUGAUAUUGGA
siHDAC3	AAAGCGAUGUGGAGAUUUA
siHDAC4#1	GCAAGAUCCUCAUCGUGGA
siHDAC5	ACACGUUCAUGCUAAAGCA
siHDAC6	CCGUGAGAGUUCCAACUUU
siHDAC7	UCACUGACCUCGCCUUCAA
siHDAC8	GGACGGUACUACAGUGUAA
siHDAC9	GAAAGACACUCCAACUAAU
siHDAC10	GGUGAACAGUGGUAUAGCA
siP300	GCACGAACUAGGAAAGAAA
siCBP	GCAAACAGAGCAUUGGUCAA
siCYP3A5#1	GGUGCUCCUCUAUCUAUAUGG
siCYP3A5#2	GGUGGUGAUUCCAACUUAUGC
siHDAC4#2	GCCAAAGAUGACUUCCCUCUU
siZEB2#1	CCACAACAUAUCCACUCCAUU
siZEB2#2	CCCGAAACGAUACGAGAUGAA

Table S6. Specific primers of *CYP3A5* promoter with progressive deletion constructs

Promoter constructs	CYP3A5 -2386 to +55bp	GCTAGCAGTCTCTTCTGGTGCTGGGA
		CTCGAGCTGAGTGCTGCTGTTTGCTG
	CYP3A5 -1342 to +55bp	GCTAGCTCCAGAAATCCCCATGCTGG
		CTCGAGCTGAGTGCTGCTGTTTGCTG
	CYP3A5 -912 to +55bp	GCTAGCGAGGCACAAACTGGTTGGGT
		CTCGAGCTGAGTGCTGCTGTTTGCTG
	CYP3A5 -292 to +55bp	GCTAGCGTGTGGCTTGTGAGGATGGA
		CTCGAGCTGAGTGCTGCTGTTTGCTG
	CYP3A5 -59 to +55bp	GCTAGCAGCTATAGCCCTGCCTCCTT
		CTCGAGCTGAGTGCTGCTGTTTGCTG

Restriction enzyme sites in construct primers are shown in red.

Table S7. Univariate analysis for overall survival of TCGA ESCC patients

Variables	**n (%)**	**Median OS (months)**	***p* value***
Gender			
Male	54 (88.52)	21.67 (16.55-26.78)	0.091
Female	7 (11.48)	NR	
Age			
≤65	53 (86.89)	25.43 (11.74-39.13)	0.173
>65	8 (13.11)	16.13 (0-38.42)	
T stage			
I-II	24 (39.34)	25.43 (17.95-32.92)	0.887
III-IV	37 (60.66)	28.43 (0-40.24)	
N stage [a]			
0-I	50 (84.75)	28.50 (18.77-38.23)	0.062
II-III	9 (15.25)	NA	
Metastasis			
No	57(93.44)	25.43(15.26-35.60)	0.883
Yes	4(6.56)	NA	
CYP3A5 score [b]			
Low	41 (67.21)	9.30 (0-21.50)	0.031
High	20 (32.79)	28.50 (18.38-38.62)	

Abbreviations: OS, overall survival; NR, not reached; NA, not available

* *p* value was calculated by log-rank test. Because only CYP3A5 score was significant associated with OS, multivariate analysis was not done.

[a] data of 59 patients were available;

[b] Cutoff value of CYP3A5 expression score was 10.

Table S8. The correlations between CYP3A5 expression and the clinicopathological characteristics of TCGA cases.

Variables	n (%)	CYP3A5 [a]		*p* value
		Low group	High group	
Gender				
Male	54 (88.5)	18 (90.0)	36 (87.8)	1.000
Female	7 (11.5)	2 (10.0)	5 (12.2)	
Age (years)				
≤65	53 (86.9)	18 (90.0)	35 (85.4)	0.710
>65	8 (13.1)	2 (10.0)	6 (14.6)	
T stage				
I-II	24 (39.3)	5 (25.0)	19 (46.3)	0.163
III-IV	37 (60.7)	15 (75.0)	22 (53.7)	
N stage [a]				
0-I	50 (84.7)	17 (85.0)	33 (84.6)	1.000
II-III	9 (15.3)	3 (15.0)	6 (15.4)	
Metastasis				
No	57 (93.4)	20 (100.0)	37 (90.2)	0.293
Yes	4 (6.6)	0 (0)	4 (9.8)	

[a] Cutoff value of CYP3A5 expression score was 10;

[b] data of 59 patients were available.

Supplementary Figures

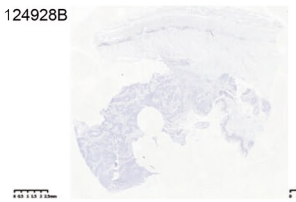
Figure S1. IHC staining of CYP3A5 in 67 pairs ESCC tumor tissues and paracancerous tissues.

Figure S2. HDACs knockdown affects CYP3A5 mRNA expression in ESCC cells. RT-qPCR analysis of *HDACs* mRNA expression in KYSE-150 and TE1 cells. Cells were treated with different siRNAs against HDAC1-10. Data are shown as means ± SD, two-tailed unpaired t-test, $*P < 0.5$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

Figure S3. Luciferase assay in ESCC cells. **(A)** The luciferase activity in KYSE 150 cells with siHDAC4 treatment. Data are shown as means ± SD, two-tailed unpaired t-test, ns, no significance. **(B)** Schematic of luciferase reporters containing the truncated (-292 bp to +55 bp) and GGGAGT-deleting sequences of *CYP3A5* promoter. **(C)** The luciferase activity in KYSE 150 cells with treatment of siRNA against *P300*/*CBP*. Data are shown as means ± SD, two-tailed unpaired t-test, ns, no significance, $**P < 0.01$.

Figure S4. The protein levels of other EMT markers after CYP3A5 overexpression and the effect of ZEB2 knockdown on the migration and invasion of ESCC cells. **(A)** Western blotting assays showed the protein levels of EMT markers after CYP3A5 overexpression in KYSE 150 and TE1 cells. **(B)** Transwell assays showed that ZEB2 knockdown inhibited the migration and invasion in KYSE 150 and TE1 cells.

Figure S5. *CYP3A4* mRNA expression of ESCC in TCGA database by GEPIA. Box plots showed the expression levels of *CYP3A4* in esophageal epithelial tumor tissues (T, orange, n=182) or non-tumor tissues as control (N, grey, n=286) patients. ns, no significance.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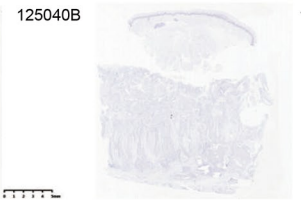
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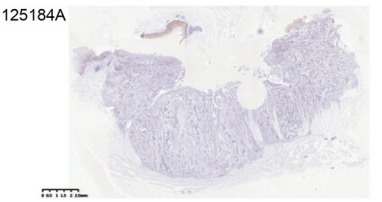
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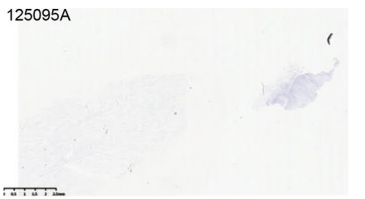
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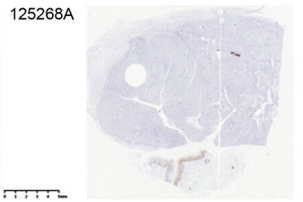
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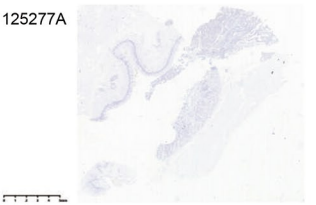
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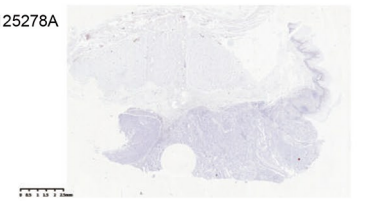
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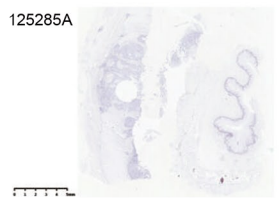
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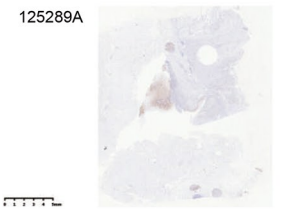
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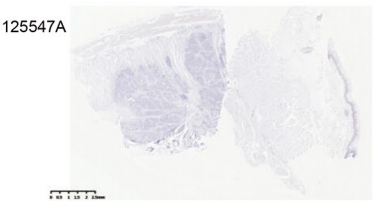
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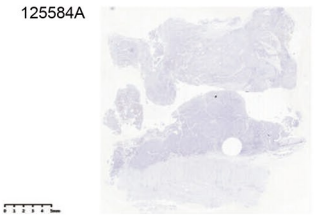
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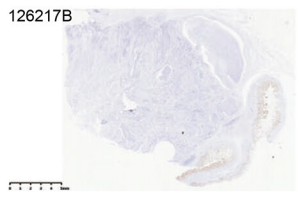
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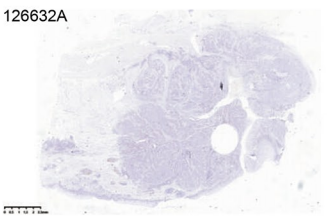
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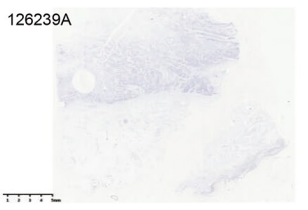
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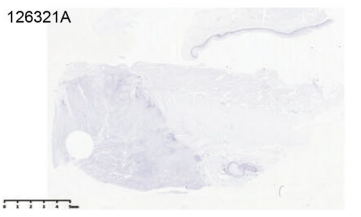
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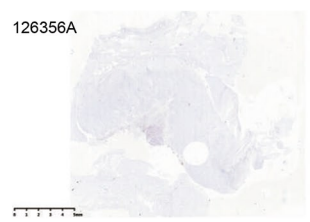
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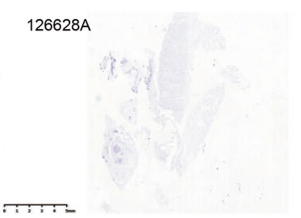
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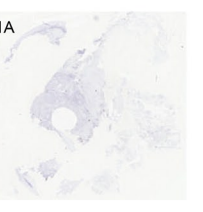
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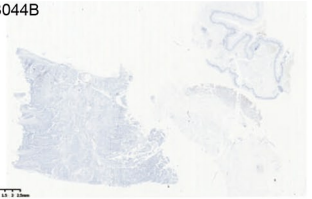

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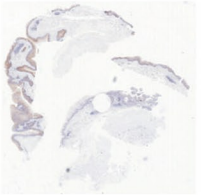

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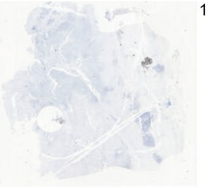

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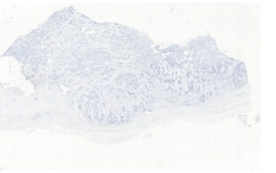

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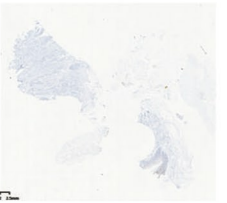

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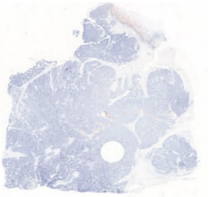

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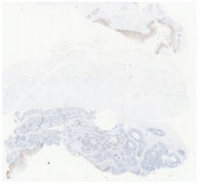

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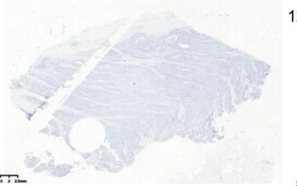

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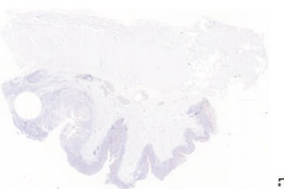

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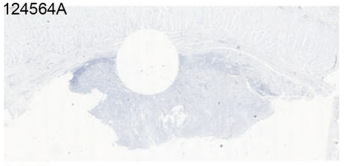

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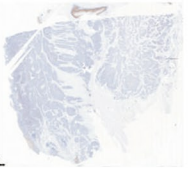

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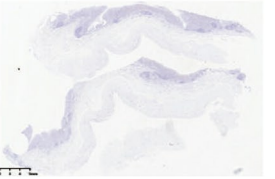

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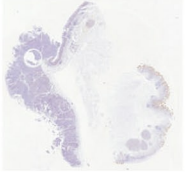

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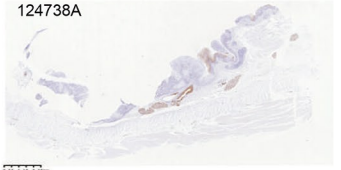

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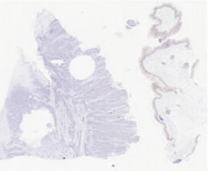

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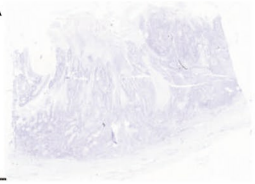

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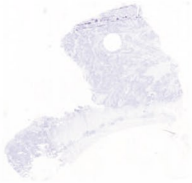

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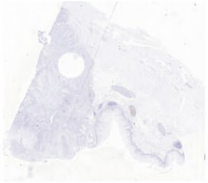

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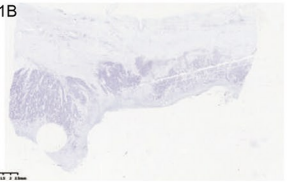

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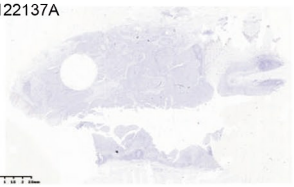

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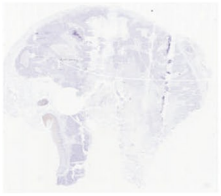

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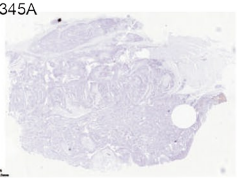

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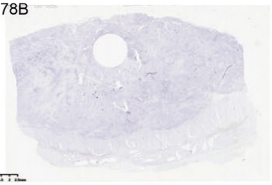

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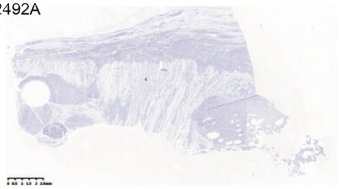

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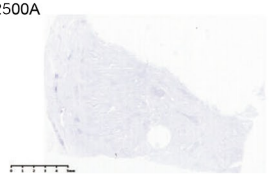

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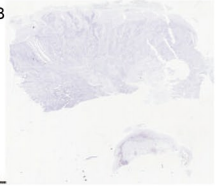

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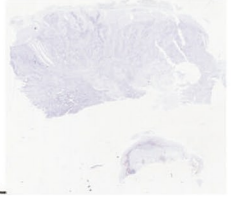

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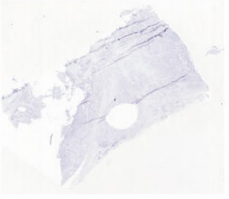

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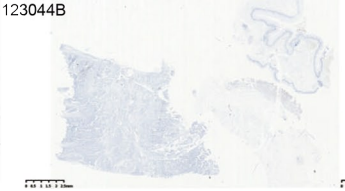

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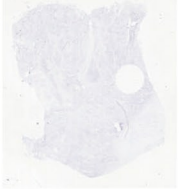

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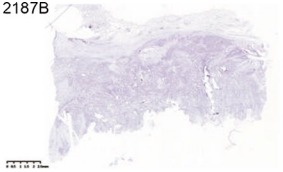

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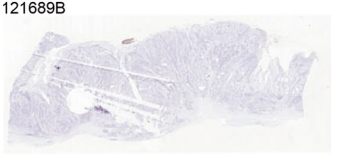

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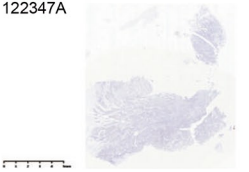

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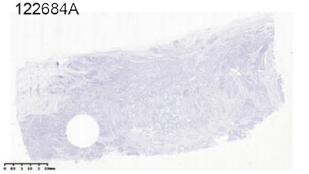

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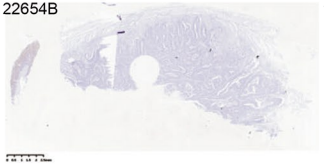

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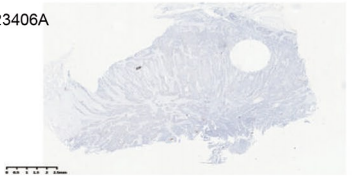

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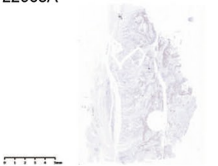

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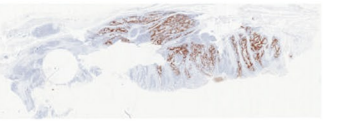

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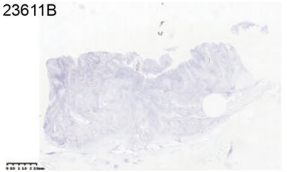

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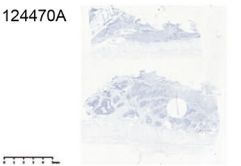

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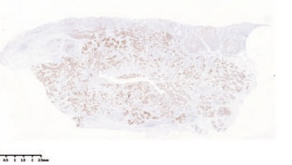

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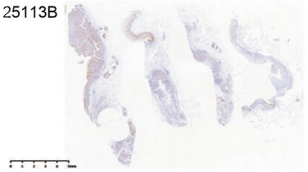

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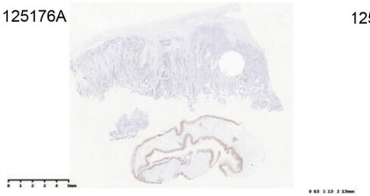

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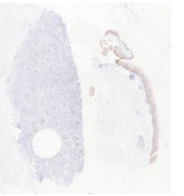

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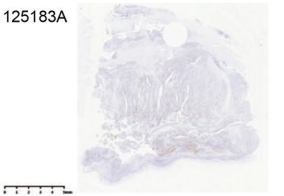

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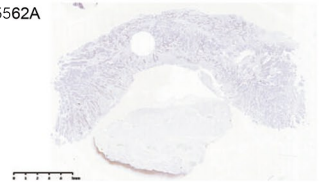

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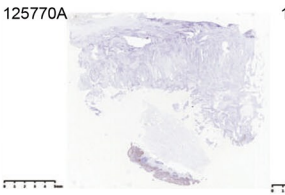

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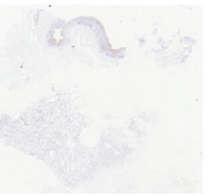

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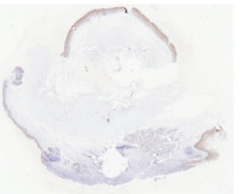
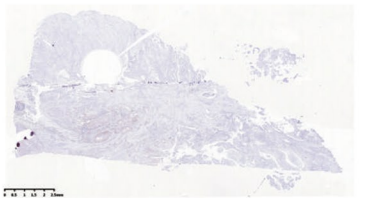
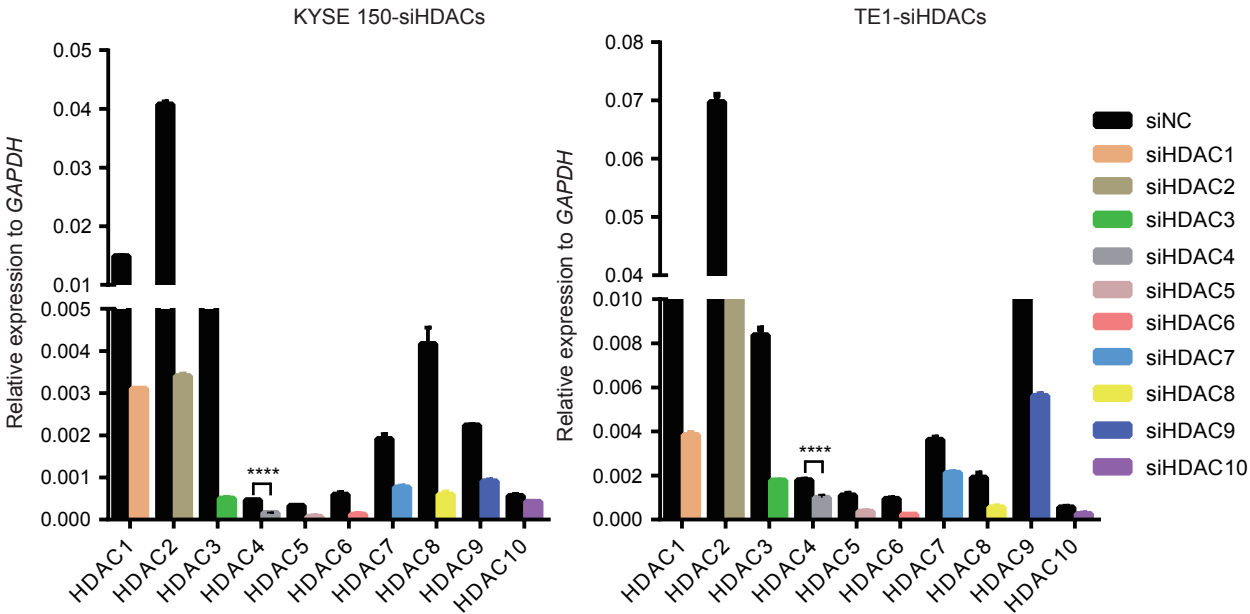


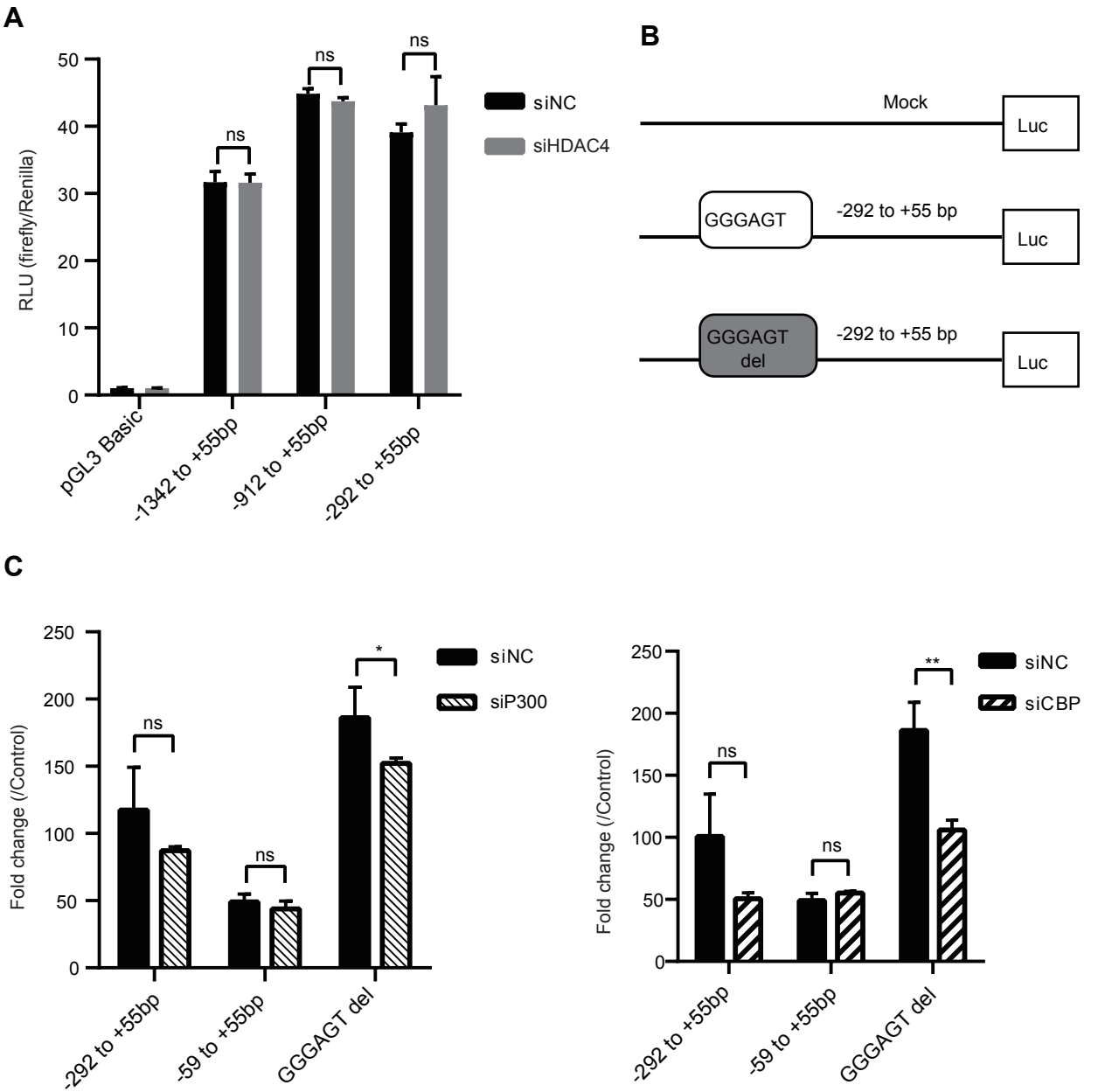
126731A



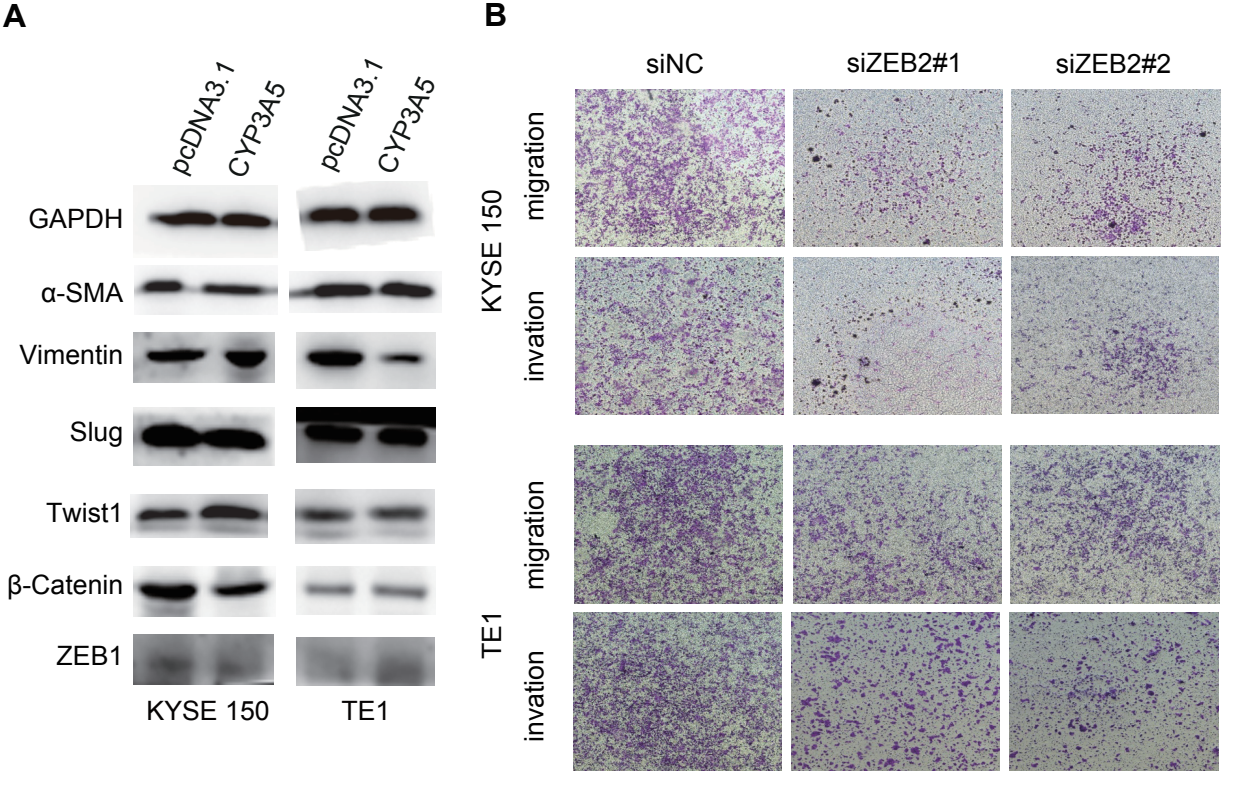
Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5