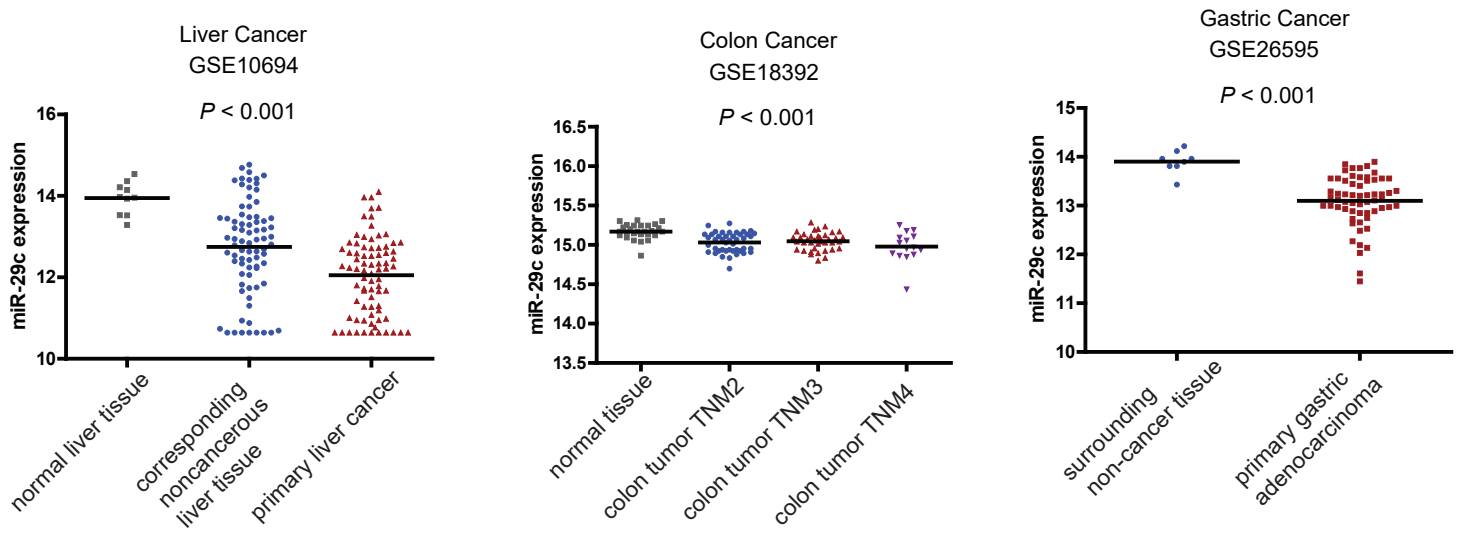
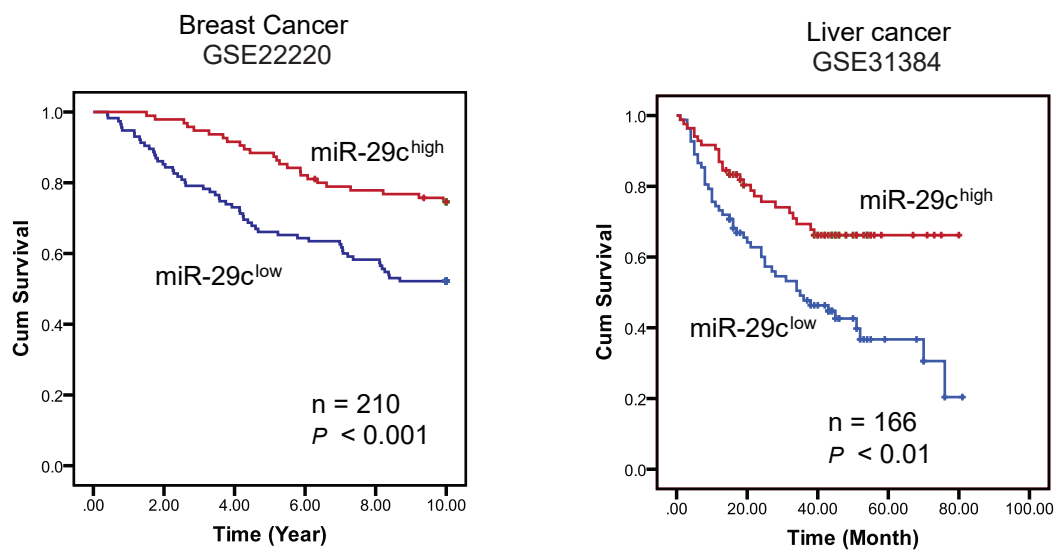
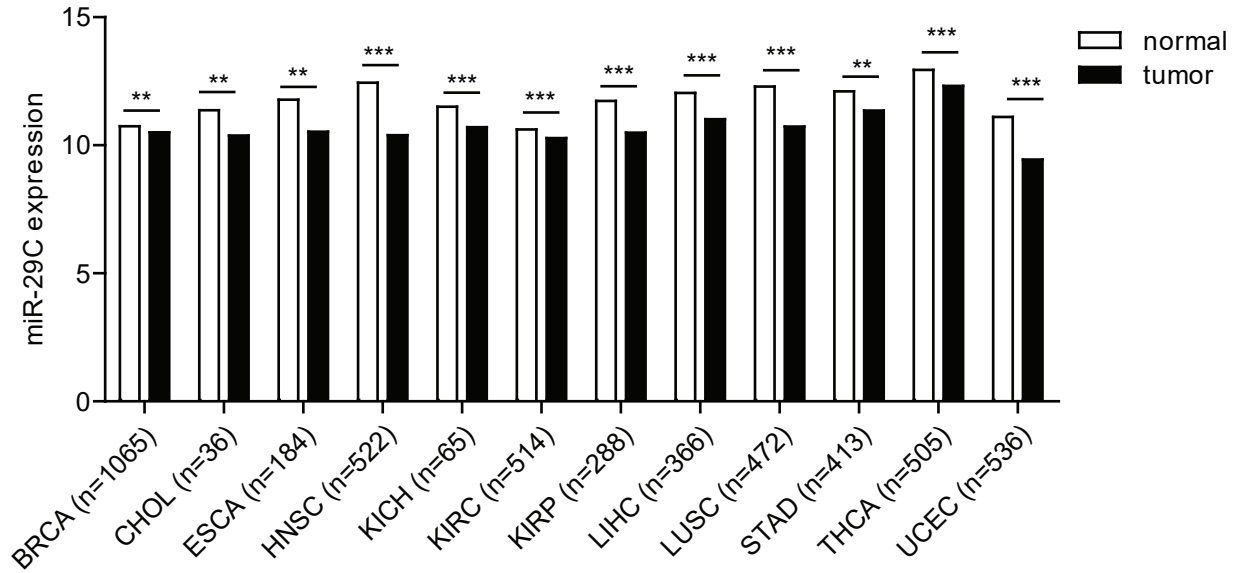
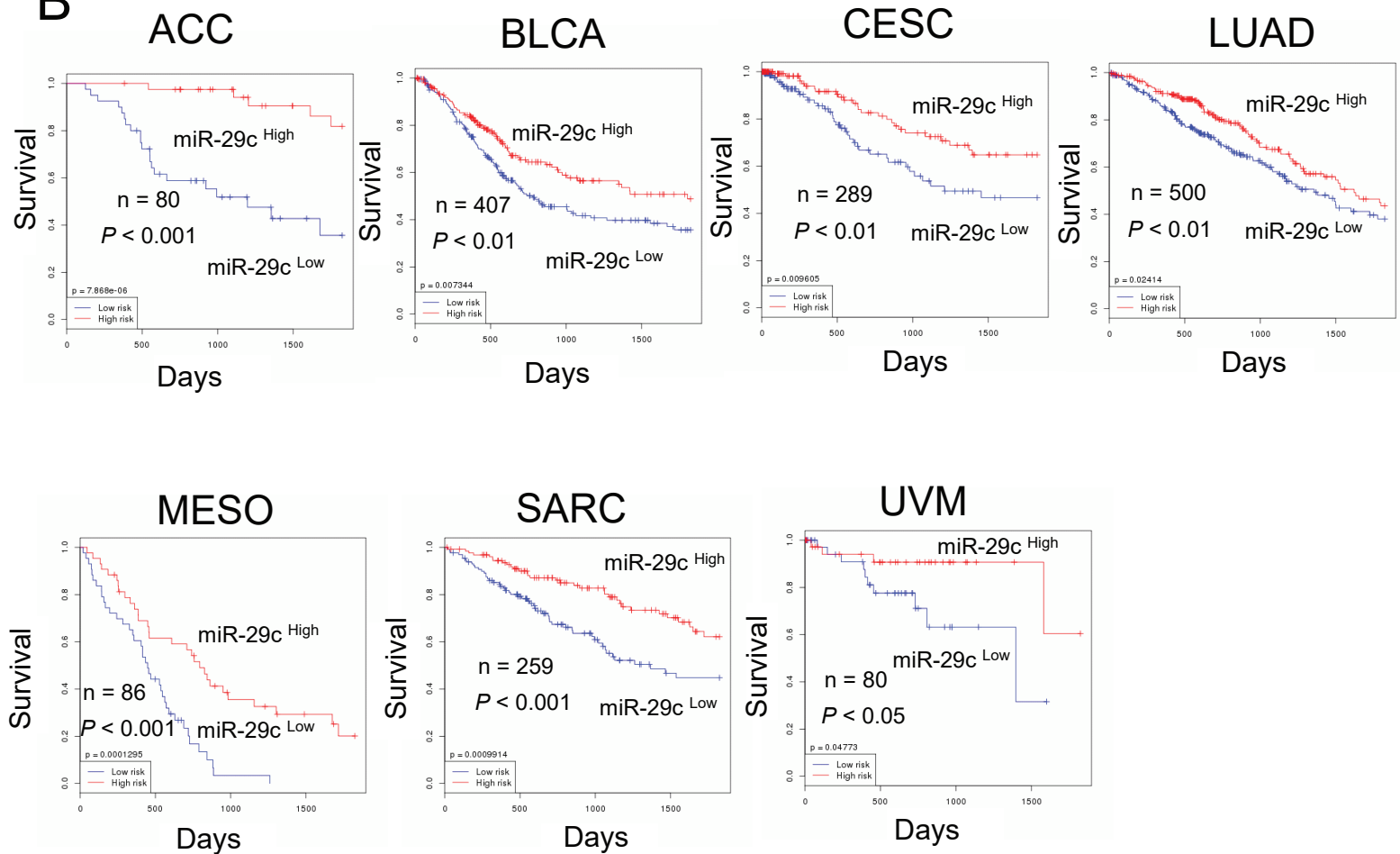


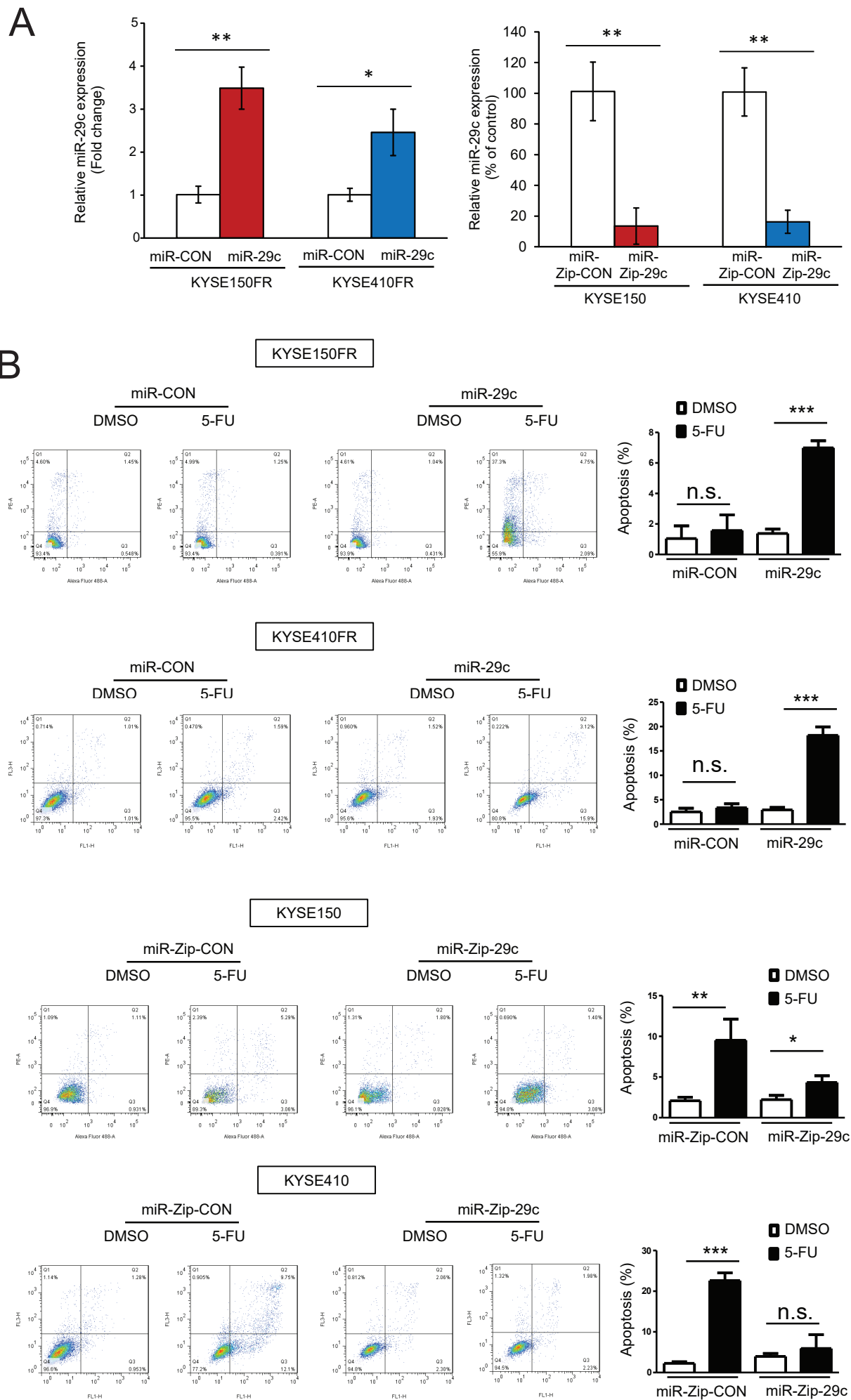
**Figure S1.** Downregulation of miR-29c in 5-FU-chemoresistant ESCC cell lines. **(A)** Diagram showing the approach used to screen and identify potential miRNAs related to 5-FU chemoresistance. **(B)** SYBR green RT-PCR analysis of 12 shortlisted miRNAs in two 5-FU-chemoresistant (FR) cell lines in comparison with the parental cell lines. **(C)** Expression level of miR-29c was robustly downregulated in both FR cell lines as determined by Taqman RT-PCR. Bars, SD; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$  compared with parental cells.

**A****B**

**Figure S2. (A)** Frequent downregulation of miR-29c in tumor tissues, compared with non-tumor tissues, was found in multiple cancer types in Gene Expression Omnibus (GEO) databases, including GSE10694, GSE18392 and GSE26595. **(B)** Kaplan-Meier analysis showing that miR-29c expression is associated with overall survival in breast and liver cancers.

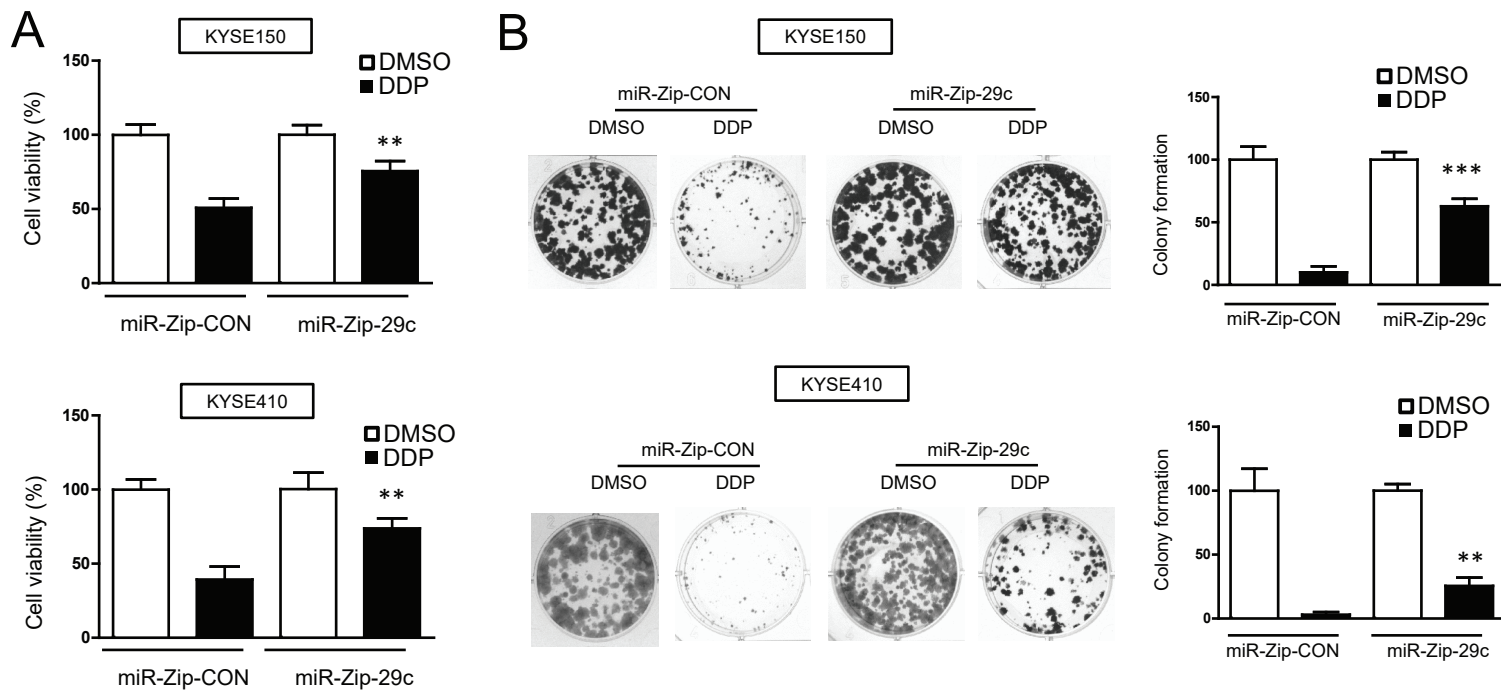
**A****B**

**Figure S3. (A)** The miR-29c expression in different cancer types in TCGA databases is downregulation in tumor tissues compared with normal tissues. BRCA: breast invasive carcinoma; CHOL: cholangiocarcinoma; ESCA: esophageal carcinoma; HNSC: head and neck squamous cell carcinoma; KICH: kidney chromophobe; KIRC: kidney renal clear cell carcinoma; KIRP: kidney renal papillary cell carcinoma; LIHC: liver hepatocellular carcinoma; LUSC: lung squamous cell carcinoma; STAD: stomach adenocarcinoma; THCA: thyroid carcinoma; UCEC: uterine corpus endometrial carcinoma. \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ . **(B)** Kaplan-Meier analysis showing that miR-29c is significantly associated with patient survival in different cancer types in TCGA database. ACC: adrenocortical carcinoma; BLCA: bladder urothelial carcinoma; CESC: cervical squamous cell carcinoma and endocervical adenocarcinoma; LUAD: lung adenocarcinoma; MESO: mesothelioma; SARC: sarcoma; UVM: uveal melanoma

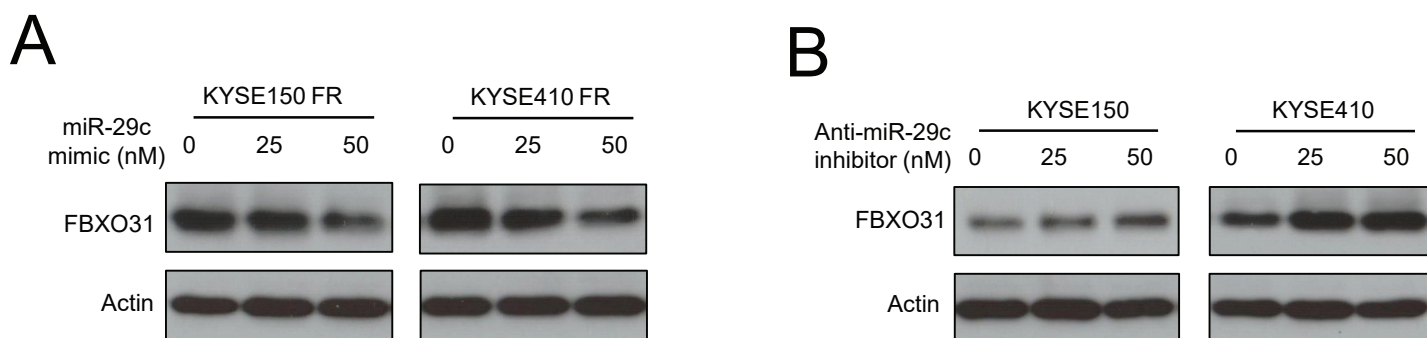


**Figure S4.** (A) KYSE150FR and KYSE410FR cells with stable overexpression of miR-29c, co-overexpression of miR-29c and FBXO31, or vector control, were treated with 5FU (20  $\mu$ M) or DMSO for 48 hours and then subjected to MTT assay. (B) Effect of manipulation of miR-29c on cell apoptosis. Bars, SD; n.s., no significance; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

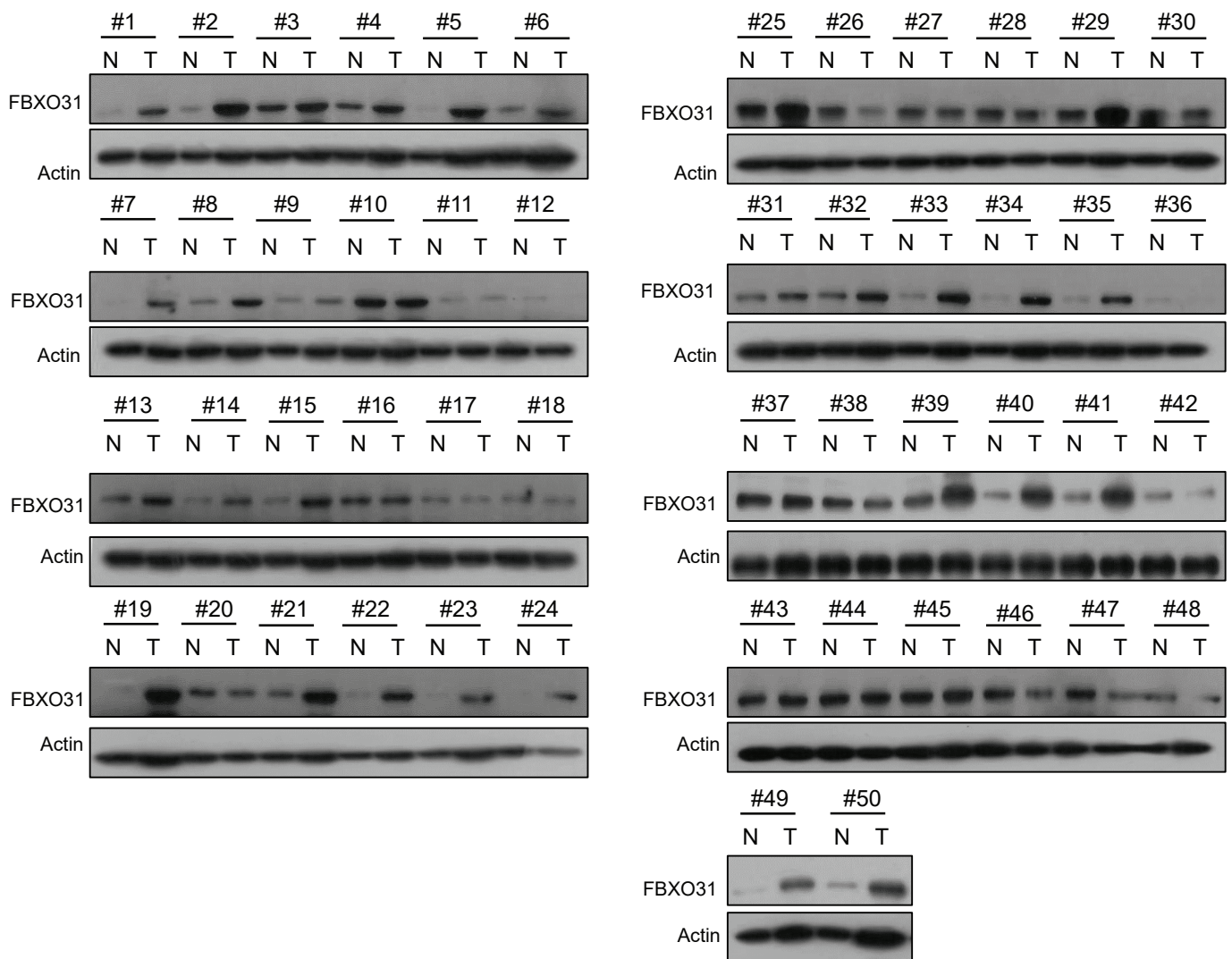




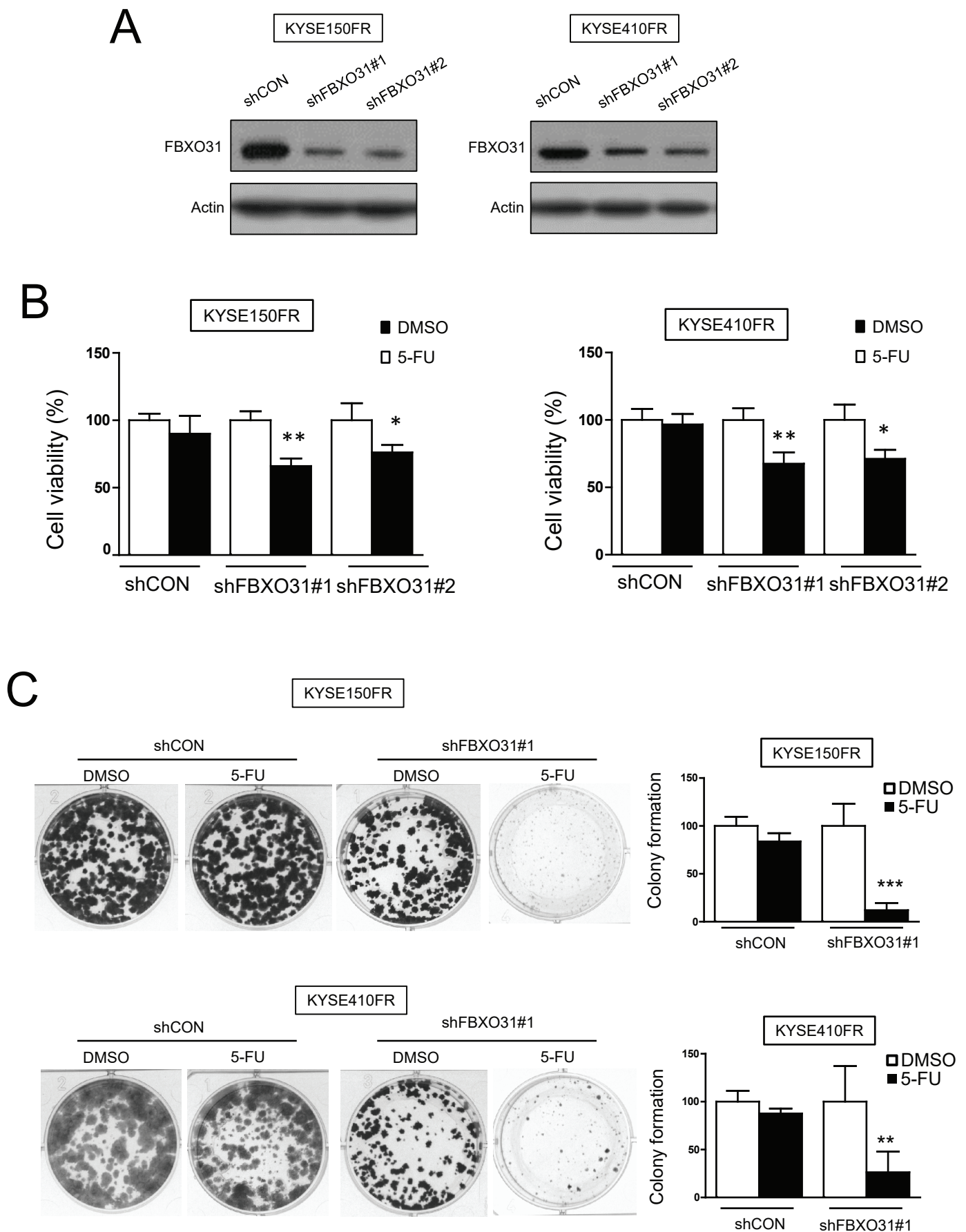
**Figure S5.** miR-29c expression sensitizes ESCC cells to DDP treatment. **(A)** ESCC cells with miR-29c-knockdown and the control cells expressing miR-zip-CON were exposed to DDP for 48 hours, and cell viability was determined by MTT assay. **(B)** Colony formation assay showing the effect of miR-29c knockdown on the sensitivity of ESCC cells to DDP. Bars, SD; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ , compared with corresponding DDP-treated control cells.



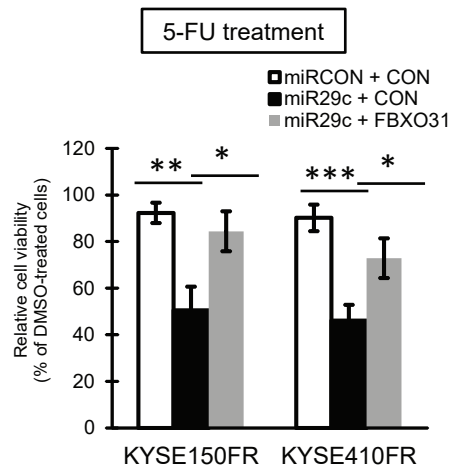
**Figure S6.** Negative regulation of FBXO31 by miR-29c in ESCC cells. **(A)** KYSE150FR and KYSE410FR cells were transfected with different doses of miR-29c mimic (up to 50 nM) for 48 h. Western blot showed that transient transfection of miR-29c resulted in decrease of FBXO31. **(B)** Western blot analyses of FBXO31 expression in ESCC cells transfected with different doses of miR-29c inhibitor (up to 50 nM) for 48 h.



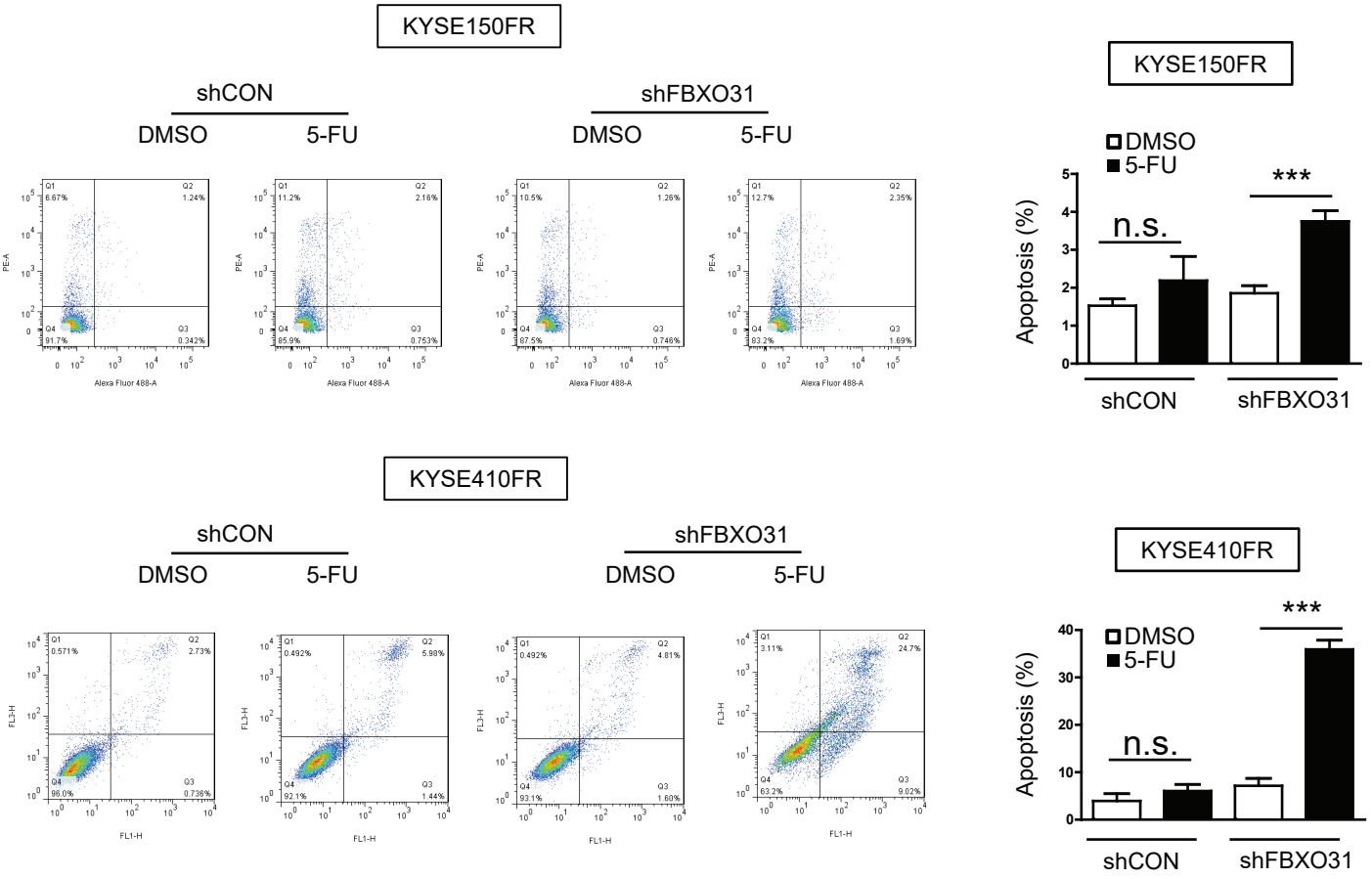
**Figure S7.** Western blot analysis of FBXO31 expression in 50 pairs of ESCC (T) and matched non-tumor (N) tissues.



**Figure S8.** (A) The efficacy of FBXO31 knockdown and overexpression in FR cell lines. (B) MTT assay showing the effect of knockdown of FBXO31 on cell viability. (C) Colony formation assay showing the effect of knockdown of FBXO31 on 5-FU chemoresistance. Bars, SD; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

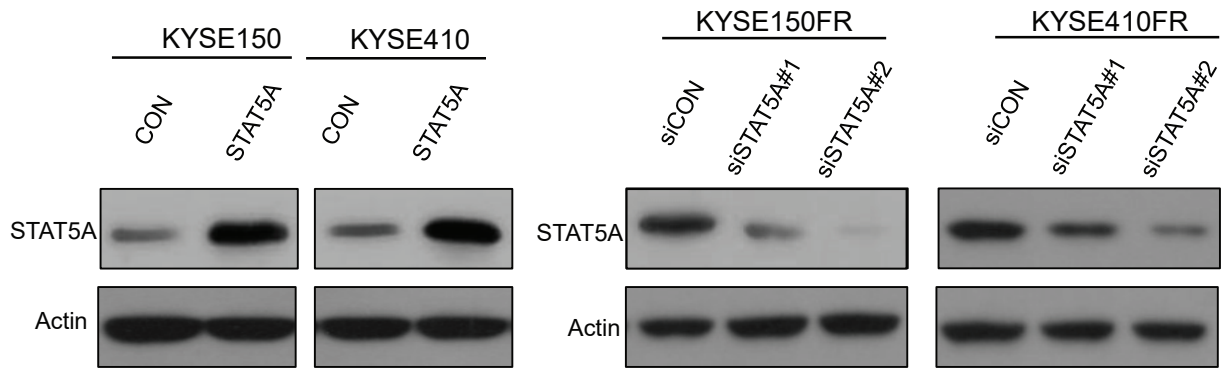


**Figure S9.** KYSE150FR and KYSE410FR cells with stable overexpression of miR-29c, co-overexpression of miR-29c and FBXO31, or control plasmids, were treated with 5-FU (20  $\mu$ M) or DMSO for 48 hours and then subjected to MTT assay.

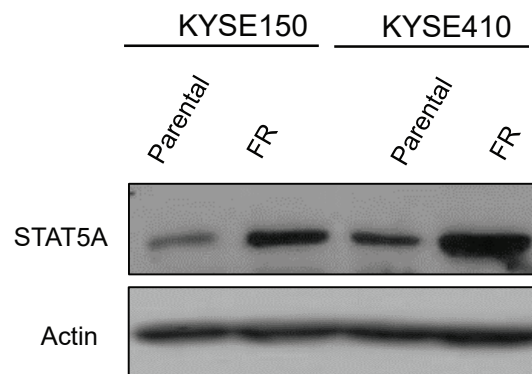


**Figure S10.** Flow cytometry analysis of effect of FBXO31-knockdown on 5-FU-induced apoptosis. Bars, SD; n.s., no significance; \*\*\*,  $P < 0.001$ .

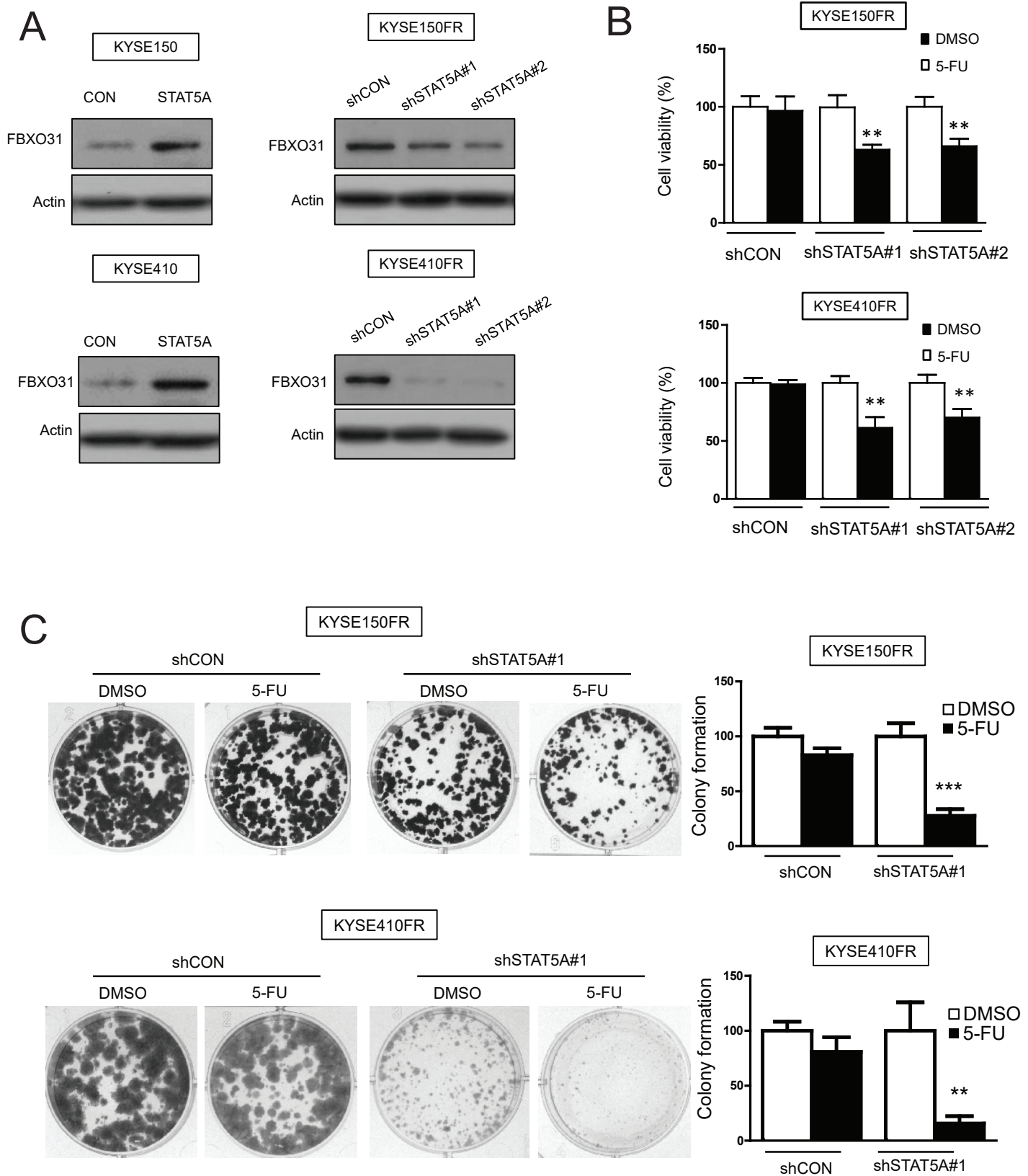
A



B

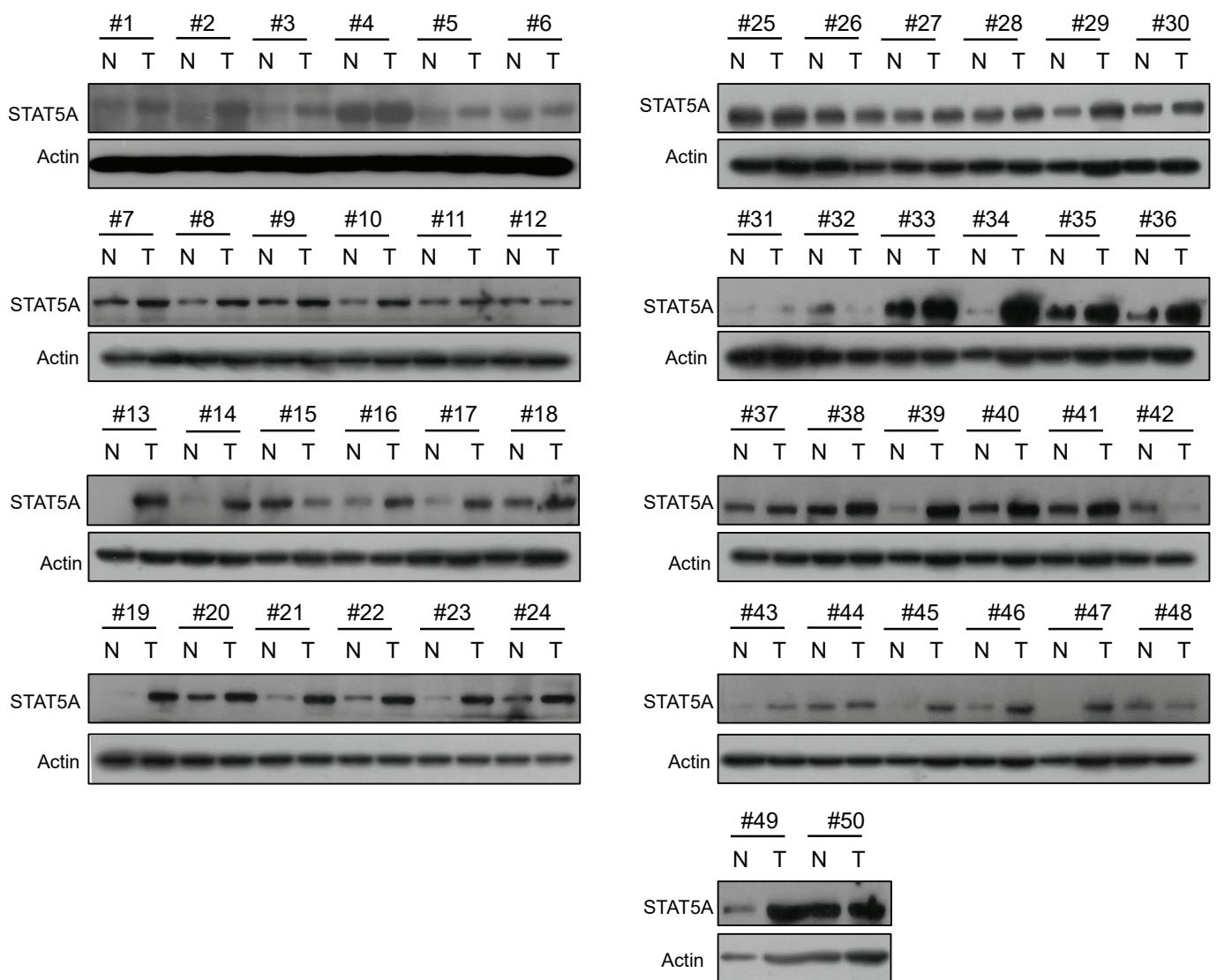


**Figure S11. (A)** Western blot showing the manipulation of STAT5A expression in ESCC cells. **(B)** The expression of STAT5A in parental cells and in 5-FU-chemoresistant cell lines.

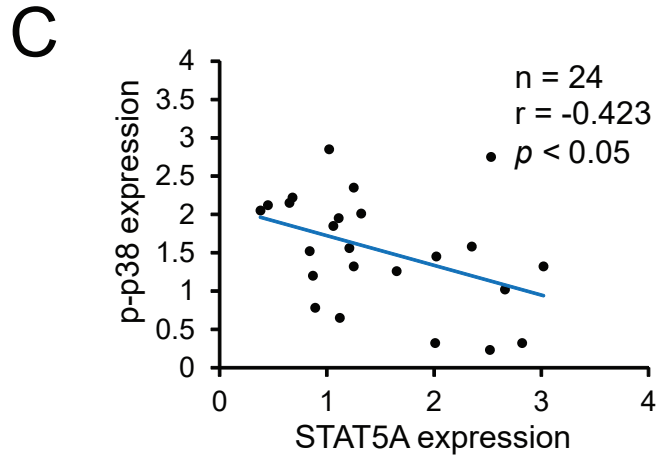
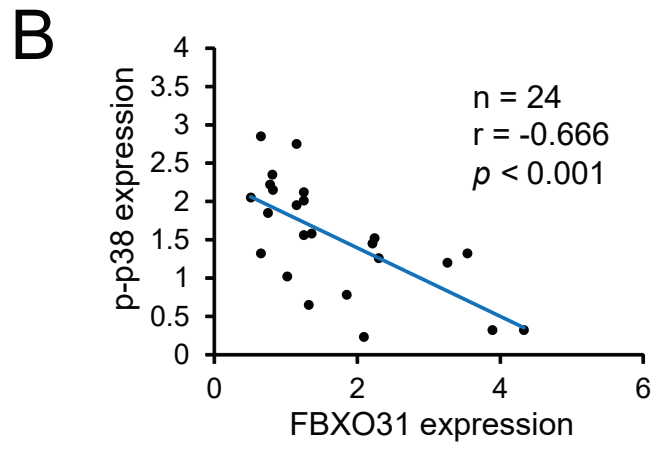
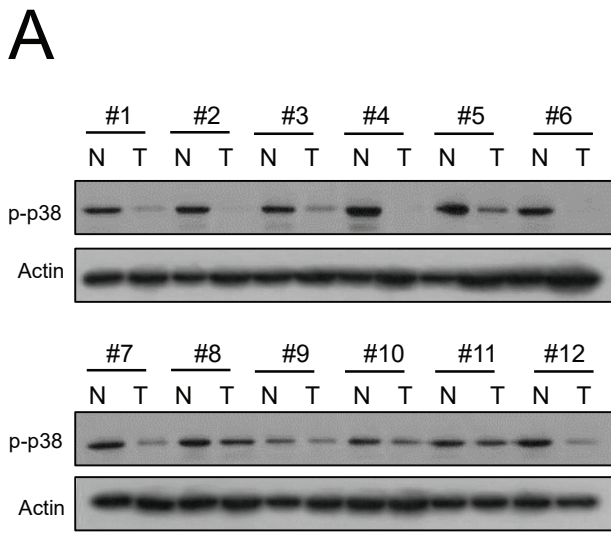


**Figure S12.** (A) Western blot showing the effect of manipulation of STAT5A expression on FBXO31. (B) Effect of knockdown of STAT5A on cell viability. (C) Effect of knockdown of STAT5A on 5-FU chemoresistance. Bars, SD; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .





**Figure S13.** Expression level of STAT5A was determined in 50 pairs of ESCC (T) and matched non-tumor (N) tissues by Western blot.



**Figure S14. (A)** Expression level of p-p38 was determined in 12 pairs of ESCC (T) and matched non-tumor (N) tissues by Western blot. **(B)** Inverse correlation between p-p38 and FBXO31 in clinical ESCC tissues. **(C)** Inverse correlation between p-p38 and STAT5A in clinical ESCC tissues.

Table S1. Forward primers for the 12 shortlisted miRNAs.

	miRNA	Sequence
1	miR-30b	5'-CGCTGTAAACATCCTACACTCAGCTA-3'
2	miR-526b	5'-GCTCTTGAGGGAAGCACTTTCTGT-3'
3	miR-339-5p	5'-CTGTCCTCCAGGAGCTCACGA-3'
4	miR-532-5p	5'-CATGCCTTGAGTGTAGGACCGTA-3'
5	miR-26b	5'-GCGCTTCAAGTAATTCAGGATAGGT-3'
6	miR-339-3p	5'-CCTCGACGACAGAGCCGAAA-3'
7	miR-374b	5'-CGCGCATATAATAACAACCTGCTAAGTG-3'
8	miR-708	5'-GAAGGAGCTTACAATCTAGCTGGGAA-3'
9	miR-29c	5'-GCGTAGCACCATTTGAAATCGGTTA-3'
10	miR-519a	5'-GCGAAAGTGCATCCTTTTAGAGTGT-3'
11	miR-139-5p	5'-TCTACAGTGCACGTGTCTCCAGT-3'
12	miR-331-3p	5'-CCCCTGGGCCTATCCTAGAA-3'

**Table S2.** Primers for site-directed mutagenesis of FBXO31 3'UTR and miR-29c promoter.

	Primers	Sequence
<b>FBXO31 3'UTR</b>	F	5'-GCAGGACCCTCCTGACTCGAGCTCTTCCTG-3'
	R	5'-CAGGAAGAGCTCGAGTCAGGAGGGTCCTGC-3'
<b>miR-29c promoter</b>		
Binding site 3-1	F	5'-CCCTCTGTCTCTGTACACGAAATGCCTCTTCCTTCTTTCT-3'
	R	5'-AGAAAGAAGGAAGAGGCATTCGTGTACAGAGACAGAGGG-3'
Binding site 3-2	F	5'-CCTCTGTCTCTGTACAGGTATAGCCTCTTCCTTCTTTCTTG-3'
	R	5'-CAAGAAAGAAGGAAGAGGCTATACCTGTACAGAGACAGAGG-3'
Binding site 3-3	F	5'-CCCTCTGTCTCTGTACAGCATAAGCCTCTTCCTTCTTTCT-3'
	R	5'-AGAAAGAAGGAAGAGGCTTATGCTGTACAGAGACAGAGGG-3'

**Table S3.** The top 100 downregulated miRNAs in KYSE150FR cells. <sup>^</sup>#

	Name	Fold change	PMID of citations (Relevance based on literature search)	
			miRNA in cancer	miRNA in chemoresistance
1	hsa-miR-106a	0.001	23121918, 23133552, 23178825	
2	hsa-miR-19b	0.001	20634878, 22731656	
3	hsa-miR-20a	0.001	22731656, 22959509, 22904162	
4	hsa-miR-21	0.001	23133552, 20628822	
5	hsa-miR-31	0.001	23121918, 23389368, 23230184	
6	hsa-miR-16	0.002	12434020, 22959509	
7	hsa-miR-191	0.002	20634878, 22898264, 21956418	
8	hsa-miR-19a	0.002	22731656, 22677902	
9	hsa-miR-93	0.002	23111389, 22964854	
10	<b>hsa-miR-30b</b>	0.003	20634878, 20508945	
11	hsa-miR-30c	0.003	20634878, 22042419	18823650
12	hsa-miR-320	0.003	23188675, 20508945, 19462682	23188675
13	<b>hsa-miR-526b</b>	0.003		
14	RNU48	0.004	22745731, 22643910	
15	hsa-miR-126	0.004	23133552, 23389368	22934011
16	hsa-miR-200b	0.004	22904162, 23272653, 22964023	22139708, 22795796
17	hsa-miR-99a	0.004	20508945, 23173671, 22299047	
18	hsa-let-7e	0.005	20634878, 22970210	23167930, 18823650
19	hsa-miR-205	0.005	22964023, 22618509	
20	hsa-miR-221	0.005	16039986, 22964023, 22904162	
21	hsa-let-7g	0.006	20309945, 22862169, 22964023	
22	hsa-miR-26a	0.006	23226446, 23026055, 22885155	
23	hsa-let-7b	0.007	23029111, 23144930	19654291, 20881268
24	hsa-miR-106b	0.007	23133552, 23028803	
25	hsa-miR-135b	0.007	23328512, 23266581, 22868372	
26	hsa-miR-149	0.007	23144691, 21873783	22682234
27	hsa-miR-15b	0.007	22904162, 22908280, 22641662	
28	hsa-miR-27a	0.007	20634878, 23240057	
29	hsa-miR-92a	0.007	23121918, 20634878, 22731656, 23052693, 23272653	
30	hsa-miR-342-3p	0.011	23174013	22112324
31	hsa-miR-429	0.012	23552699, 21897839	
32	hsa-miR-454	0.012	20634878, 19022373	
33	hsa-miR-186	0.013	20979053, 20878113, 20677554	

34	hsa-miR-492	0.013	22850566, 21319197, 18818933,	
35	hsa-miR-125b	0.014	20634878, 22898264	
36	hsa-miR-22	0.014	20508945, 23140295	
37	hsa-miR-29a	0.014	23133552, 20508945	
38	<b>hsa-miR-339-5p</b>	0.014	20932331	
39	<b>hsa-miR-532-5p</b>	0.015	22925189	
40	hsa-miR-99b	0.015	22299047, 22969861	
41	hsa-let-7a	0.016	16530703, 22972404, 22970210	22129484, 21292542
42	hsa-miR-200a	0.016	23272653, 17875710, 22908280	
43	hsa-miR-28-3p	0.017	22686440	
44	hsa-miR-590-5p	0.017	22684895	
45	hsa-miR-574-3p	0.018	22179486, 20815808	
46	hsa-miR-141	0.019	22964023, 23266581	
47	hsa-miR-222	0.019	22904162, 23389368, 22641662	
48	hsa-let-7d	0.021	22929886, 22862169	
49	hsa-miR-103	0.021	23266581, 22641662, 22908280	
50	hsa-miR-140-5p	0.021	23098991, 21063788	
51	<b>hsa-miR-26b</b>	0.021	22964023	
52	hsa-miR-365	0.023	22072615, 21737779, 18812439	
53	hsa-miR-203	0.025	22952344, 22940702	21553120, 21354697
54	hsa-miR-218	0.026	23159910, 22893786, 22860003	23443110
55	hsa-miR-27b	0.026	23300597, 22902387	21899346,21899346
56	hsa-miR-660	0.026	22610076, 19022373	
57	hsa-miR-132	0.028	22929615, 22506400, 21329664	
58	hsa-miR-25	0.028	23205084, 22450326, 22387599	
59	<b>hsa-miR-339-3p</b>	0.028		
60	hsa-miR-345	0.028	22307176	20099276
61	<b>hsa-miR-374b</b>	0.028		
62	hsa-miR-130a	0.029	22540308, 22455133, 20508945	
63	hsa-miR-23a	0.029	23236401, 22628407	
64	hsa-miR-210	0.039	23139634, 23148210	
65	hsa-miR-18a	0.046	22865399, 22540308	
66	hsa-miR-744	0.049	22432036, 21637912	
67	hsa-miR-18b	0.052	23205106, 22964854, 22821209	
68	hsa-miR-130b	0.053	22425712, 22187671	
69	hsa-miR-598	0.053	22429812	
70	hsa-miR-146b-5p	0.054	23363792, 22719913, 22241525	
71	hsa-miR-197	0.054	22027761, 21904633	21998738, 21560177
72	hsa-miR-487a	0.054	20806854	
73	hsa-miR-425	0.055	18765229	
74	hsa-miR-532-3p	0.055	21535956	
75	hsa-miR-224	0.056	23095110, 22747440, 21864507	
76	hsa-miR-196b	0.057	23133552, 22976466,21255435	

77	hsa-miR-28-5p	0.057	22240480, 21199010	
78	hsa-miR-324-5p	0.057	22202009, 21747943	
79	hsa-miR-181a	0.058	23226446, 22971574	
80	hsa-miR-148a	0.063	23056401, 22562546, 22496917	21796641
81	hsa-miR-374a	0.067	23133552, 21563230, 21274007	
82	hsa-miR-652	0.072	21789031, 21035526, 20508945	
83	<b>hsa-miR-708</b>	0.073	23082062	
84	hsa-miR-484	0.083	22510686, 19849700	
85	hsa-miR-100	0.087	23173870, 23151088, 22937028	22120675
86	hsa-miR-29b	0.087	23000157, 22776000	17652184, 20564213
87	hsa-miR-15a	0.09	23104180, 22629365	20839343
88	hsa-miR-34a	0.098	23032974, 23085450	23085450, 18834855
89	<b>hsa-miR-29c</b>	0.101	22664488	
90	hsa-miR-193b	0.106	22491710, 21803762, 20304954	
91	hsa-miR-324-3p	0.107	22745731, 22298638	
92	hsa-miR-125a-5p	0.11	22690848, 22540308	21399894
93	hsa-let-7f	0.111	22929886, 22110251	
94	hsa-miR-494	0.111	22544933, 22110251, 21809359	
95	<b>hsa-miR-519a</b>	0.113	22672989	
96	<b>hsa-miR-139-5p</b>	0.115	21304530	
97	<b>hsa-miR-331-3p</b>	0.126	20510161, 19584056	
98	hsa-miR-362-5p	0.145	21543894	
99	hsa-miR-23b	0.164	23300597, 22649212	21899346
100	hsa-miR-515-3p	0.168	21244772, 22420006	

^ The 66 candidates removed in the 1<sup>st</sup> round selection are shaded in grey.

# The 12 shortlisted novel candidates are highlighted in blue.