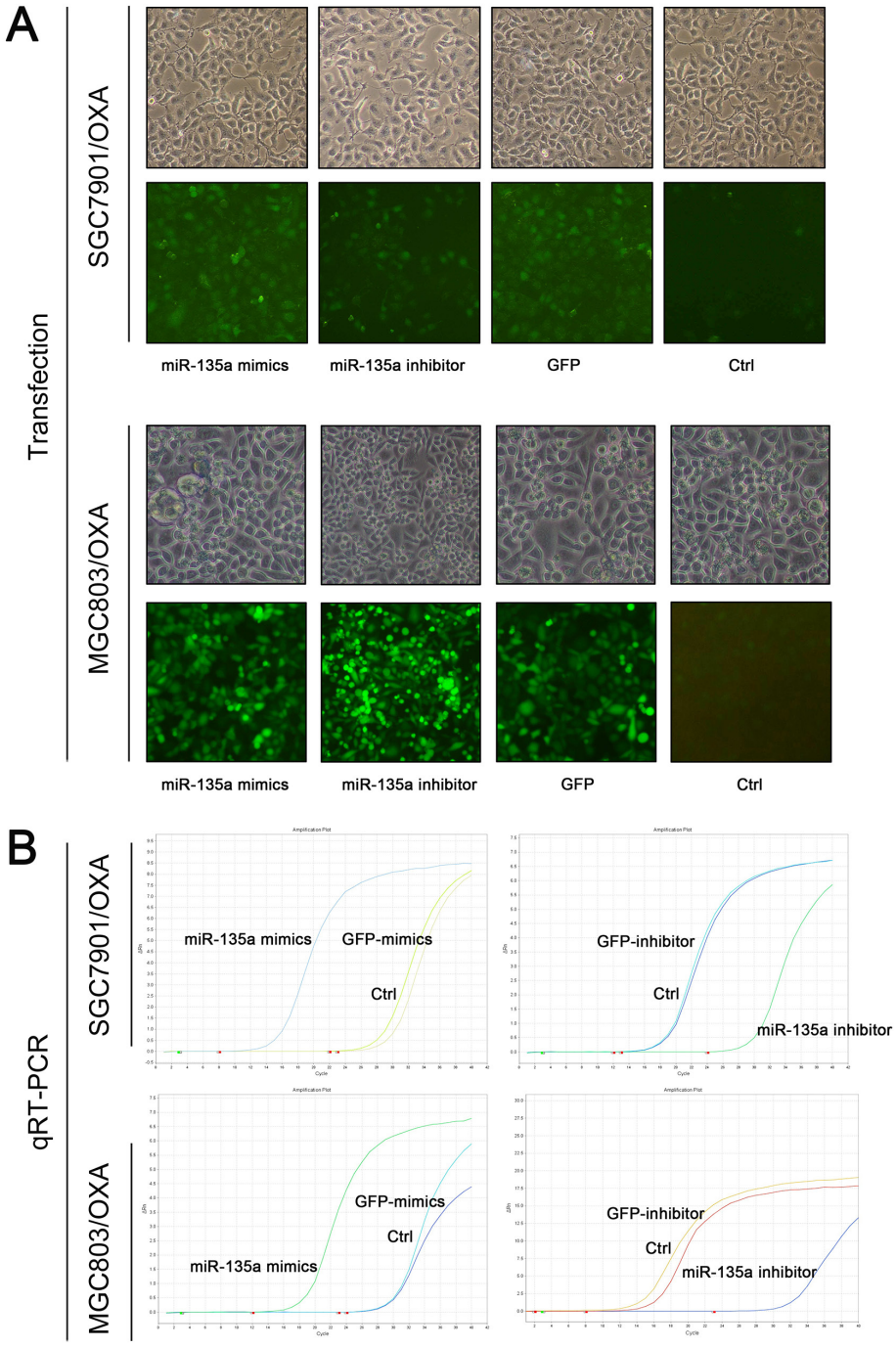
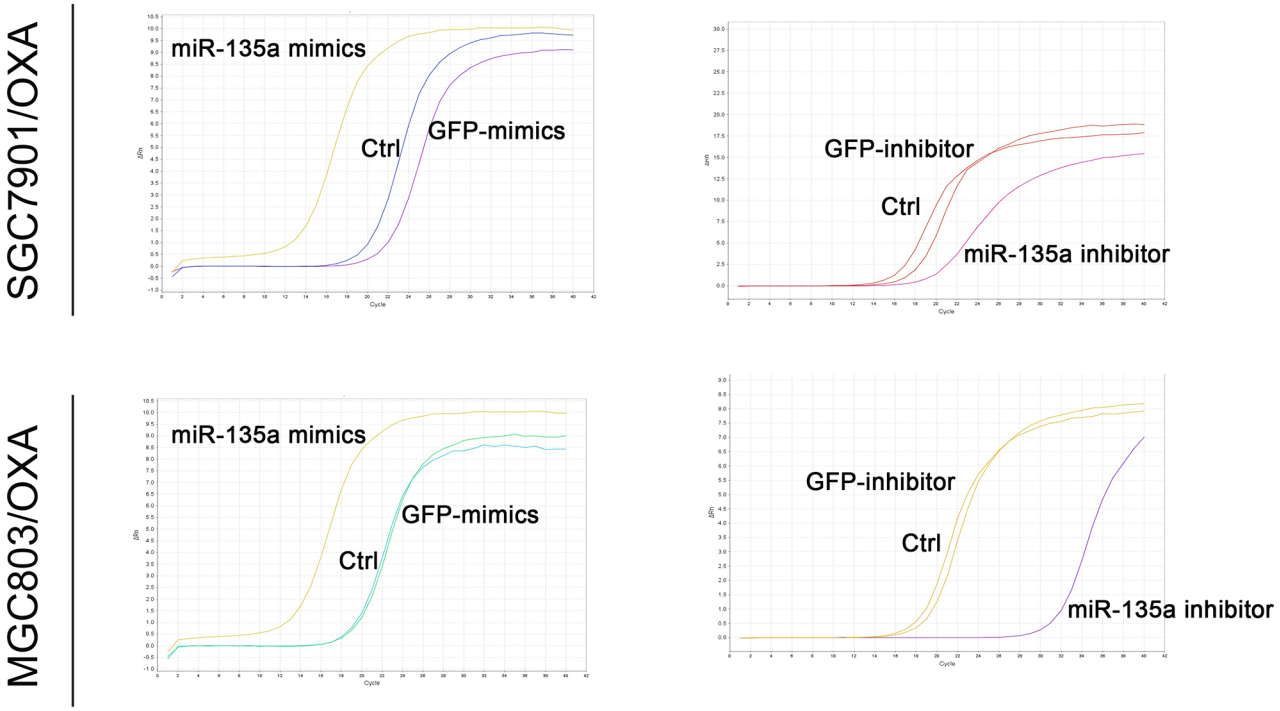
miR-135a promotes gastric cancer progression and resistance to oxaliplatin

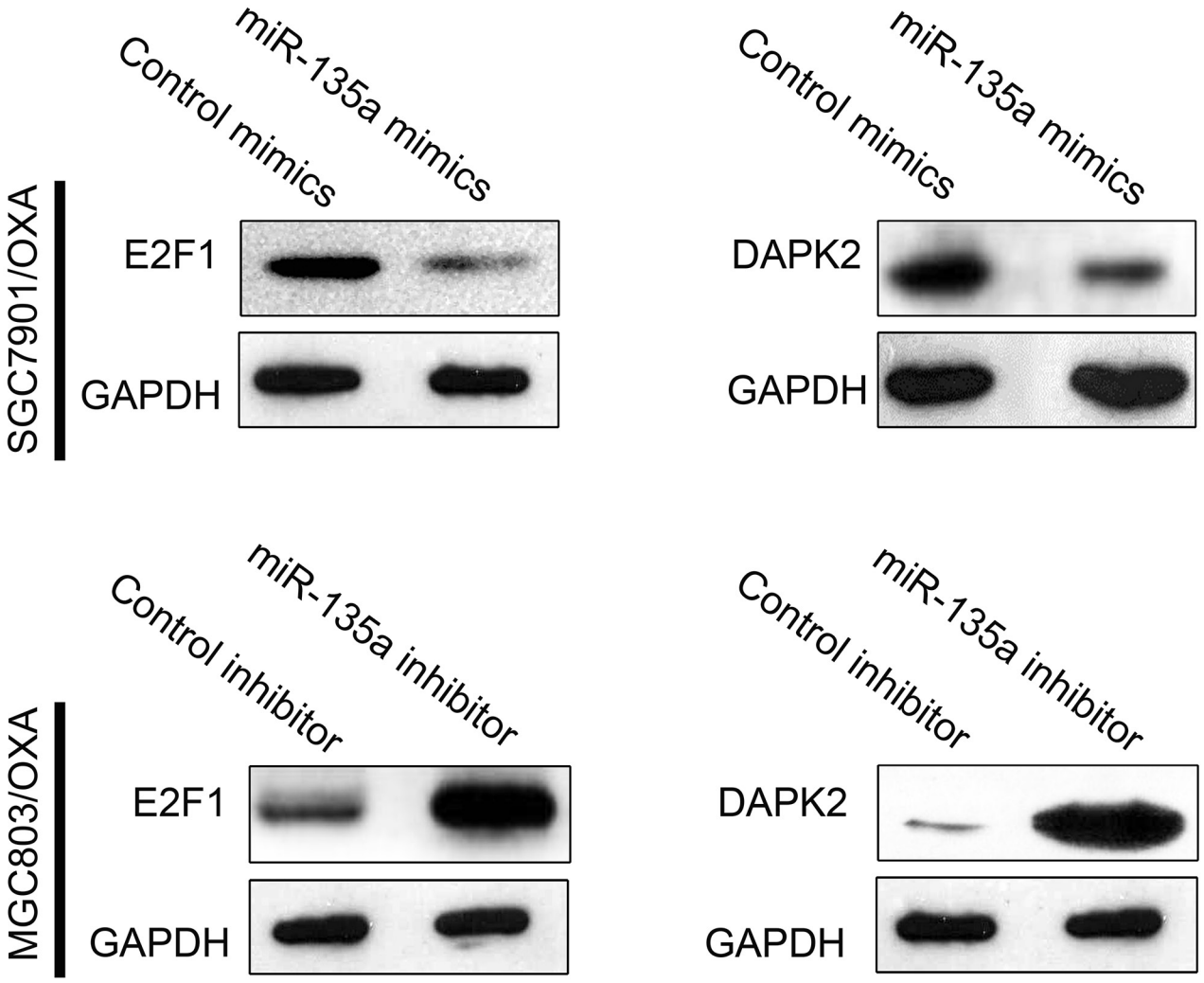
SUPPLEMENTARY FIGURES AND TABLES



Supplementary Figure S1: Effect of the *miR-135a* inhibitor (or mimic) on *miR-135a* expression *in vitro*. A. Transfection efficiency after screening by G418 and evaluation by fluorescence microscopy. Under light microscope (×100) and fluorescence microscope (×100). **B.** qRT-PCR of *miR-135a* expression. The expression of *miR-135a* was stably downregulated by a *miR-135a* inhibitor and upregulated by a *miR-135a* mimic in SGC7901/OXA and MGC803/OXA cells.



Supplementary Figure S2: Effect of the *miR-135a* inhibitor (or mimic) on *miR-135a* expression *in vivo*. The miRNA level of *miR-135a* was determined by a semi-quantitative reverse-transcriptase polymerase chain reaction. *miR-135a* expression was stably downregulated (with an inhibitor) or upregulated (with a mimic) in SGC7901/OXA and MGC803/OXA cells, and the cells were inoculated subcutaneously into the flanks of nude mice.



Supplementary Figure S3: Effect of the *miR-135a* inhibitor (or mimic) on E2F1 and DAPK2 expression *in vivo*. Protein levels of E2F1 and DAPK2 were determined by Western blotting. *miR-135a* significantly reduced the protein levels of E2F1 and DAPK2 in the tumor tissues of nude mice.

Supplementary Table S1: Distribution of patients' characteristics and prognosis analysis

Characteristics	Total, N = 280	Recurrence, N =198	Crude HR (95% CI)s	Adjusted HR, (95% CI)
Age				
< 50 years	132	92	-	-
≥ 50 years	148	106	1.56 (0.79–2.51)	1.76 (1.12–3.08)
Sex				
Female	123	87	-	-
Male	157	111	0.67 (0.51–1.13)	0.58 (0.37–1.02)
Gastrohelcosis				
No	108	67	-	-
Yes	172	131	1.25 (0.56–1.88)	1.66 (0.67–1.95)
Smoking				
No	131	92	-	-
Yes	149	106	0.77(0.40–1.15)	0.70 (0.40–1.22)
Drinking				
No	158	124	-	-
Yes	122	74	0.69 (0.40–1.32)	0.82 (0.44–1.53)
CEA				
≤ 20 μg/L	91	66	-	-
> 20 μg/L	189	132	1.26 (0.75–2.12)	2.10 (1.20–3.67)
CA19-9				
≤ 40 U/L	95	76	-	-
> 40 U/L	185	122	1.54 (0.94–2.56)	1.86 (1.13–3.29)
HP infection				
Negative	97	63	-	-
Positive	183	135	1.35 (0.72–2.51)	1.94 (0.89–2.80)
Tumor size				
< 3 cm	79	48	-	-
≥ 3 cm	201	150	2.65 (1.50–4.67)	3.68 (2.02–6.71)
Tumor number				
Single	188	121	-	-
Multiple	92	77	1.66 (0.85–3.23)	1.34 (0.75–3.12)
TNM staging				
I+II	79	28	-	-
III+IV	201	170	1.87 (0.64–2.84)	1.82 (0.46–2.44)
Microvascular invasion				
No	109	58	-	-
Yes	171	140	1.66 (0.85–3.23)	1.30 (0.65–2.60)
Lymphatic metastasis				
No	99	68	-	-
Yes	181	130	1.13 (0.65–2.36)	0.91 (0.42–1.95)

Supplementary Table S2: Correlation of Clinicopathologic Characteristics with miR-135a Expression level

Characteristics	Total, N = 280	miR-135a level		*P* value
		Low	High	
Age				0.027
< 50 years	132	86	46	
≥ 50 years	148	56	92	
Sex				0.443
Female	123	68	55	
Male	157	79	78	
Gastrohelcosis				0.104
No	108	57	51	
Yes	172	99	73	
Smoking				0.221
No	131	79	52	
Yes	149	87	62	
Drinking				0.183
No	158	88	70	
Yes	122	45	77	
CEA				<.001
≤ 20 μg/L	91	25	66	
> 20 μg/L	189	85	104	
CA19-9				0.035
≤ 40 U/L	95	56	39	
> 40 U/L	185	98	87	
HP infection				<.001
Negative	97	49	48	
Positive	183	56	127	
Tumor size				0.368
< 3 cm	79	53	26	
≥ 3 cm	201	83	118	
Tumor number				0.013
Single	188	64	124	
Multiple	92	56	36	
TNM staging				<.001
I+II	79	29	50	
III+IV	201	62	139	
Microvascular invasion				<.001
No	109	33	76	
Yes	171	68	103	
Lymphatic metastasis				<.001
No	99	43	56	
Yes	181	53	128	

Supplementary Table S3: The base sequence of primers for RT-PCR

Gene	Primer	Base sequence
miR-135a	Forward	5'-AACCCTGCTCGCAGTATTTGA G-3'
	Reverse	5'-GCGGCAGTATGGCTTTTTATTCC-3'
miR-135b	Forward	5'-TATGGCTTTTCATTCCTATGTGA-3'
	Reverse	5'-ATGGCTTTTTATTCCTATGTGA-3'
E2F1	Forward	5'-CCCAACTCCCTCTACCCT-3'
	Reverse	5'-AAGCGAATCAATGGACTC-3'
DAPK2	Forward	5'-TCTGGGAGGA GAGGACTGCA-3'
	Reverse	5'-TACTCACGGCGGGAGGCTGA-3'
P-gp	Forward	5'-ACCAAGCGGCTCCGATAC A-3'
	Reverse	5'-TCATTGGCGAGCCTGGTAGTC-3'
c-MYC	Forward	5'-TTCTCTCCGTCCTCGGATTC-3'
	Reverse	5'-GTAGTTGTGCTGATGTGTGG-3'
GAPDH (1)	Forward	5'-ACAGCAACAGGGTGGTGGAC-3'
	Reverse	5'-TTTGAGGGTGCAGCGAACTT-3'
GAPDH (2)	Forward	5'-ACCACAGTCCATGCCATCAC-3'
	Reverse	5'-TCACCACCCTGTTGCTGTA-3'