

Figure S5. The original western blots of Figure 1.

Figure 1A The Expression of EGFR and β -actin band in B2B and As-T cells using an immunoblotting assay

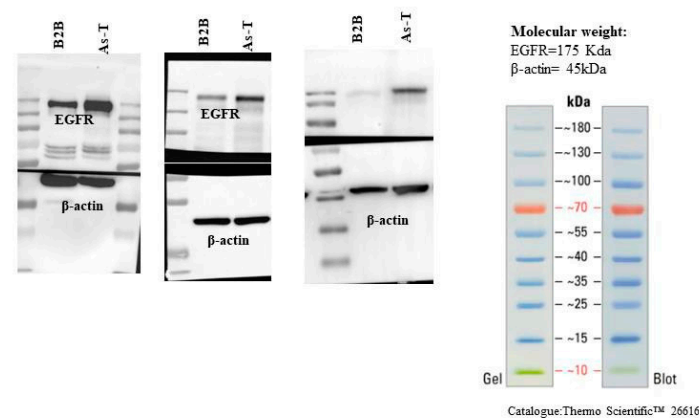


Figure 1A Quantification: The intensity of EGFR/ β -actin band in B2B and As-T cells using an immunoblotting assay

No	B2B	As-T
1	1	1.71978
2	1	1.415135
3	1	1.65353

Figure 1B. The expression levels of phospho-PKM2, PKM2, p-p65, p-65, HIF-1 α , p-ERK (1/2) and β -actin in total protein extracts of control and As-T cells

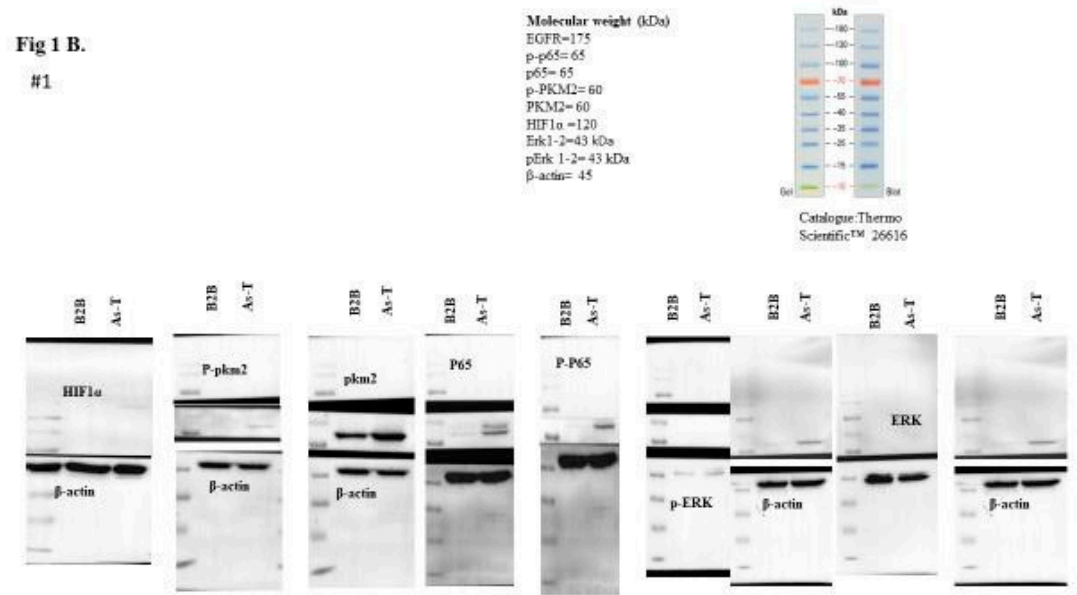


Fig 1 B.

#2-3

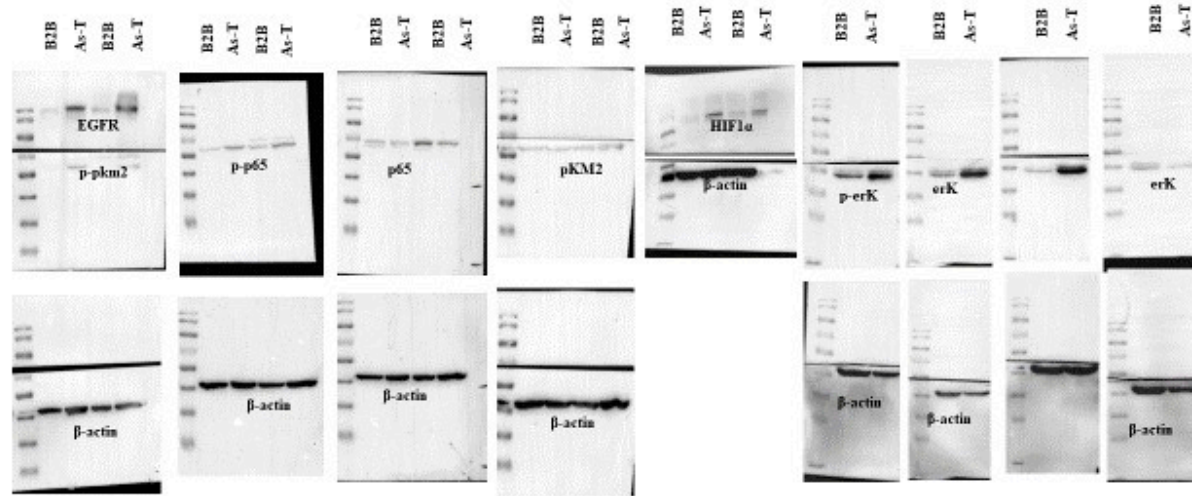


Figure 1B Quantification: The intensity of protein band/ β -actin band in B2B and As-T cells using an immunoblotting assay

		Fold Change		
		#1	#2	#3
HIF1-a	B2B	0	1.038350114	1.003963517
	AsT	7.513476675	7.205567449	3.35526823
p-PKM2	B2B	0	1.03902834	1.016150564
	AsT	10.81973799	6.812428251	5.067618473

PKM2	B2B	1	1.013868787	1.005457659
	AsT	1.68360821	1.172101493	1.709965375
p-P65	B2B	1	1	1.011226315
	AsT	3.841935084	3.441654966	2.190570706
p65	B2B	1	1.01871613	1
	AsT	14.62522025	0.851738119	0.44564078
p-ERK	B2B	1	1.003603744	1.011132791
	AsT	4.593193286	8.462779656	10.06550657
ERK	B2B	1	1.00055142	1.009407112
	AsT	0.757908751	5.387219528	0.262748519

Figure 1C. The expression levels of phospho-PKM2, PKM2, p-p65, p-65, HIF-1 α , and β -actin in total protein extracts in the As-T cells transfected with siRNA against EGFR or scrambled control (siSCR)

Fig 1 C.

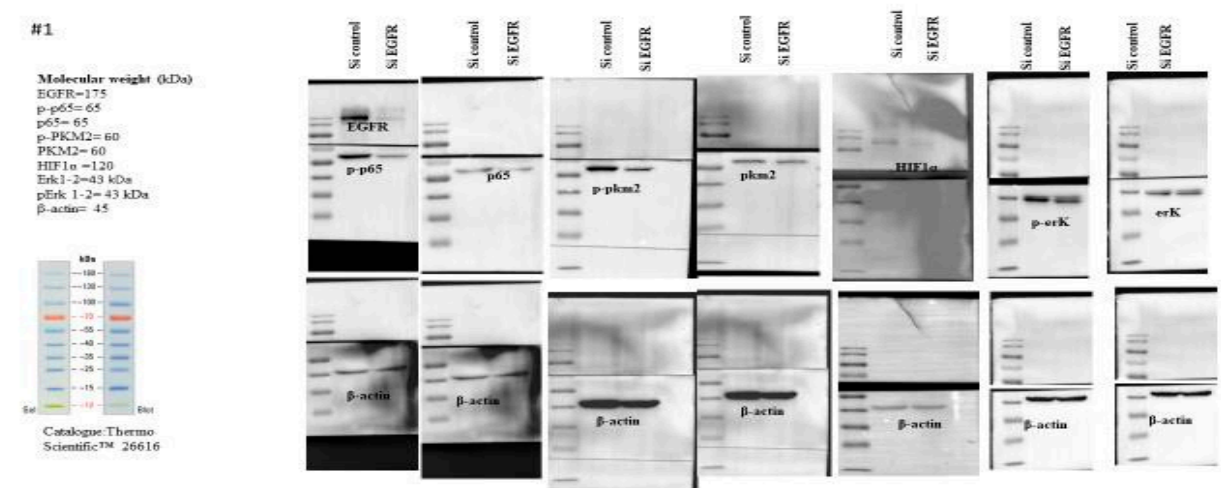


Fig 1 C.

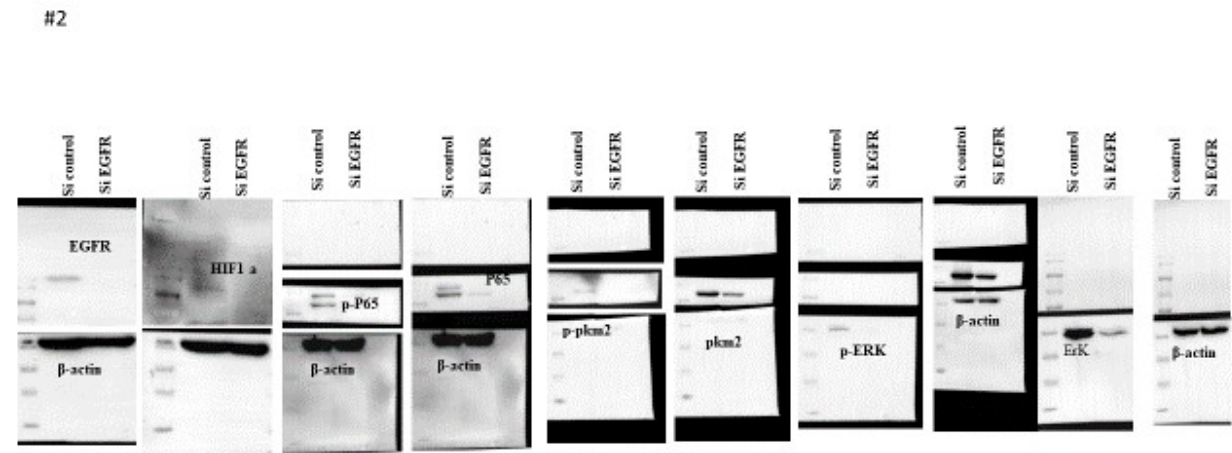


Fig 1 C.

#3

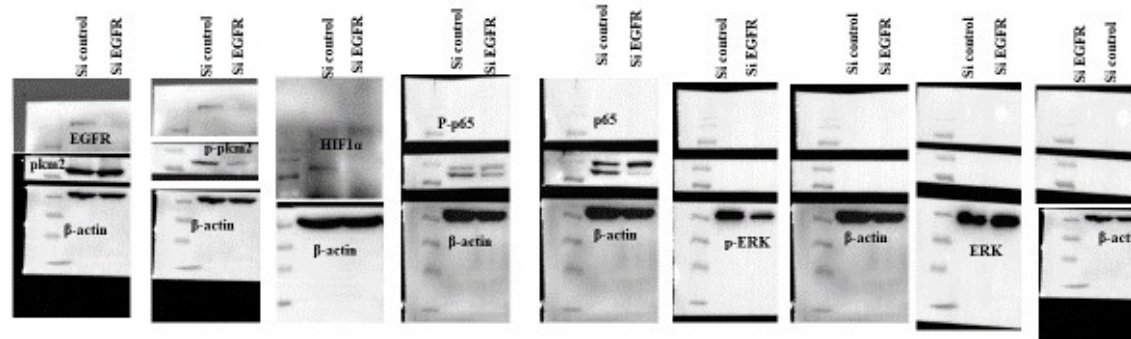


Figure 1C Quantification: The intensity of protein band/ β -actin band in Si-SCR and Si-EGFR cells using an immunoblotting assay

		Fold Change		
		#1	#2	#
EGFR	Si-SCR	1	1.001863026	1.007315
	Si-EGFR	0.079959	0	0.768502
HIF1-a	Si-SCR	1.006041	1.004669016	1.00551
	Si-EGFR	0.325758	0	0.28911
p-PKM2	Si-SCR	1.008048	1.002692505	1.00254
	Si-EGFR	0.565169	0	0.643737
PKM2	Si-SCR	1.00636	1.000443398	1.006012
	Si-EGFR	1.245826	0.34280569	1.524688
p-P65	Si-SCR	0.999127	1.003827943	1.007185
	Si-EGFR	0.112555	0.175697657	0.700818
p65	Si-SCR	1.000451	1.009529456	1.001061
	Si-EGFR	0.600757	0.193288903	0.786167
p-ERK	Si-SCR	1.00003	1.004118301	1.000603
	Si-EGFR	0.659367	0.180814844	0.674251
ERK	Si-SCR	1.008662	1.001599053	1.005293
	Si-EGFR	0.803249	0.153028261	1.0109

Figure 1D Quantification: Expression of miR-218-5p/U6 in B2B and As-T cells using TaqMan real-time RT-PCR assay

No	B2B	As-T
1	1.011184355	0.001658592
2	1.045012323	0.001824573
3	0.977954818	0.001716582
4	0.967674856	0.002017178

5	0.855389236	0.003303905
6	1.093999778	0.003251155
7	1.036316411	0.003777692
8	1.03116131	0.003782759
9	0.870057644	0.005887638
10	0.945756312	0.00692946
11	1.037629442	0.00700525
12	1.171198314	0.006959501

Figure S6. The original western blots of Figure 2.

Figure 2A. Expression of miR-218-5p/U6 in As-T was confirmed by TaqMan real-time RT-PCR

No.	miR-NC	miR-218-5p
1	1.0039648	27.93909769
2	1.0014514	24.1584295
3	1.0424178	26.02776697
4	0.954135	21.50351084
5	0.985496	262.8416648
6	1.0439328	252.5643798
7	0.9922668	256.3014832
8	0.9795895	264.5058568

Figure 2A. Cell proliferation assay was performed by count number of alive cells using cell counter under microscope

Days	control				miR-218-5p			
0	10000	10000	10000	10000	10000	10000	10000	10000
1	13000	16000	13200	15000	11000	13000	10800	15000
2	18000	21000	15600	22500	19000	15000	18000	22000

3	36000	42000	45000	38000	26000	24000	36000	26000
4	55000	56000	59000	49000	36000	39000	44600	33300
5	89000	86000	95000	87000	56000	65000	70000	62000

Figure 2B. Colony formation assay using soft agar

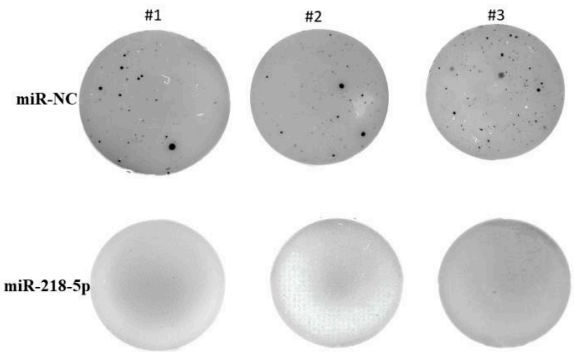


Figure 2B. Quantification: Number of colonies

No.	miR-NC	miR-218-5p
1	44	15
2	58	8
3	67	5

Figure 2C. Wound healing assay

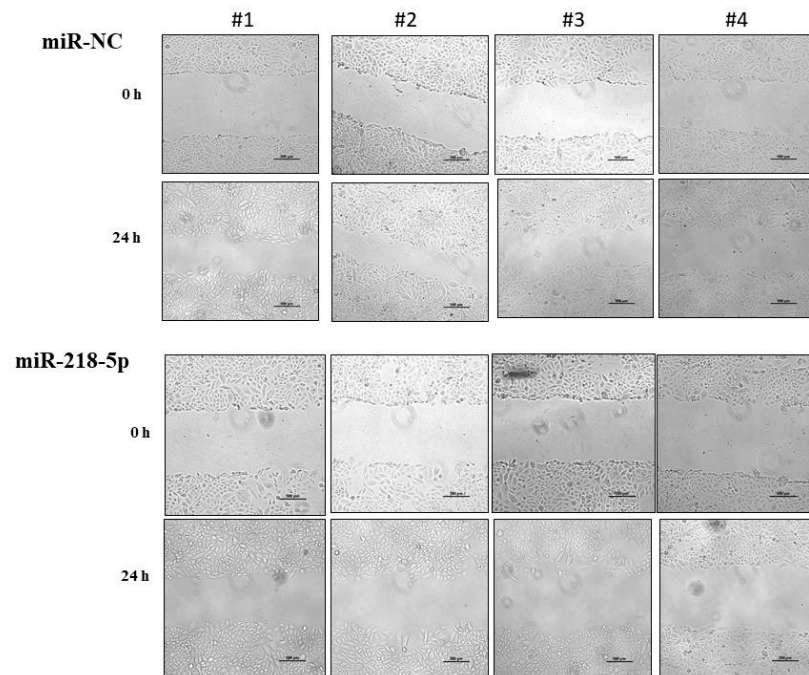
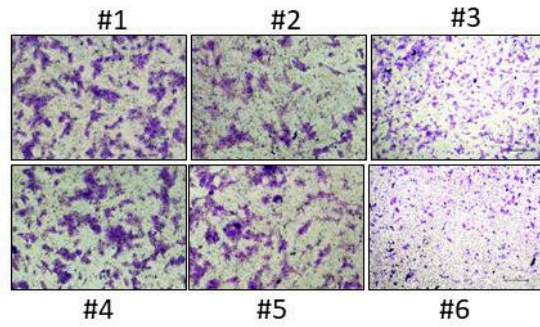


Figure 2C. Quantification: Percentage of wound healing

No	miR-NC	miR-218-5P
1	23.1	1.84
2	40.94	8.42
3	38.94	17.98
4	31	15.64

Figure 2D. Cell migration assay

miR-NC



miR-218-5p

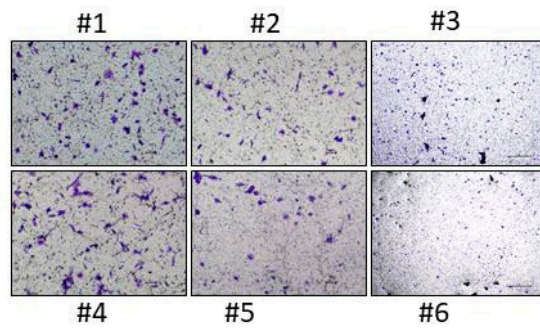


Figure 2D. Quantification: Percentage of cells migrates compare to control

No	miR-NC	miR-218-5p
1	116.1593	30.445
2	115.2225	28.5714
3	57.1429	20.6089
4	79.6253	16.39344
5	139.11	17.3302
6	92.7401	14.5199

Figure 2E. Tube formation assay

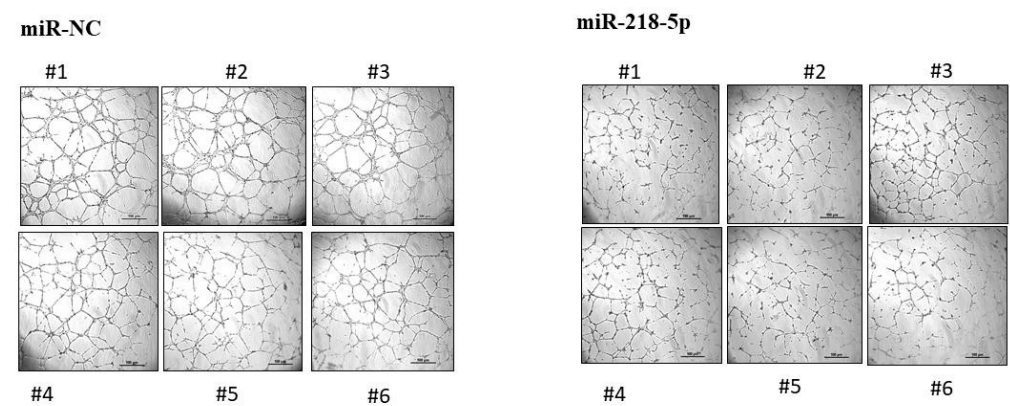


Figure 2E. Quantification: No of meshes

No	miR-NC	miR-218-5p
1	41	19
2	35	9
3	33	13
4	23	7
5	21	8
6	34	30

Figure 2E. Quantification: Total tube length

No	miR-NC	miR-218-5p
1	102.3	75.82
2	107.3	51.63
3	112.2	66.35
4	88.6	51.95

5	85.90	56.36
6	103.4	90.19

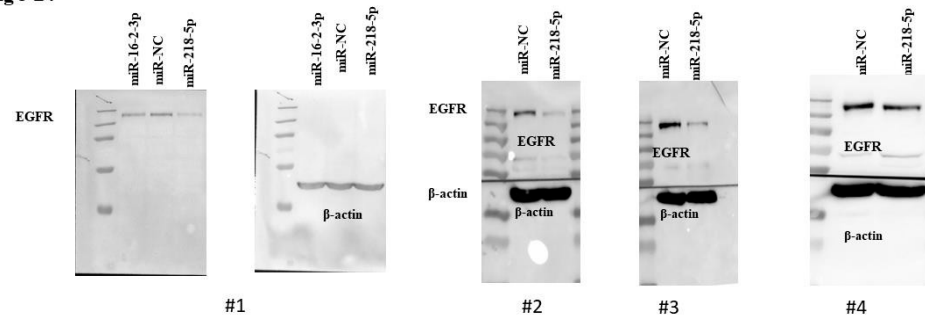
Figure S7. The original western blots of Figure 3.

Figure 3C. Luciferase assay was performed using HEK-293 T cells to detect the relative luciferase activities of WT and MUT EGFR reporters with or without miR-218-5p overexpression. Renilla luciferase vector was used as an internal control. The result is the ratio of Firefly and Renilla signals

Figure 3C		Luciferase ctivity											
Plasmids		miR-NC						miR-218-5p					
EGFR-WT		0.959612	1.00808	1.00162	1.06641	0.92578	1.00781	0.63791	0.76484	0.75299	0.59766	0.64453	0.60938
3' UTR-mutant EGFR		0.952073	1.01454	0.88315	0.79688	1.03125	0.90234	0.93807	0.965	0.84437	0.78516	0.64453	0.69141

Figure 3D. As-T cells, Cr-T cells, A431 cells were transfected with miR-NC and miR-218-5p mimic. After 72 h, the protein levels of EGFR and β -actin were analyzed using immunoblotting assay.

Fig 3 D.



As-T

Fig 3 D.

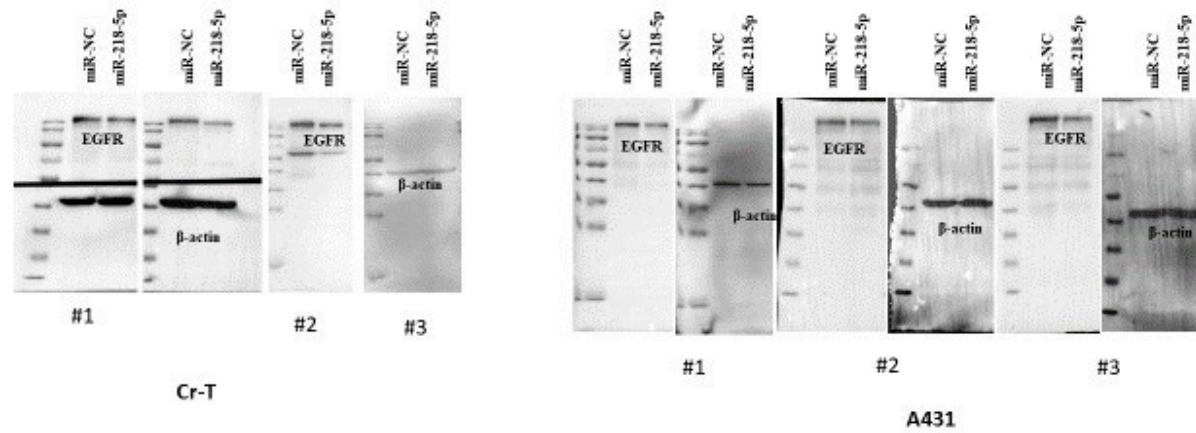


Figure 3D. Quantification: The intensity of EGFR/β-actin band in As-T, Cr-T, and A431 cells using an immunoblotting assay

	As-T		Cr-T		A431	
	miR-NC	miR-218-5p	miR-NC	miR-218-5p	miR-NC	miR-218-5p
1	1.58014	0.419865	0.884018	0.43351	1.127782	0.790391
2	1.80631	0.193689	0.967345	0.310388	0.981732	0.718768
3	1.47298	0.527017	1.148637	0.626529	0.95486	0.436682
4	1.46893	0.531072				

Figure S8. The original western blots of Figure 4.

Figure 4A. The expression of miR-218-5p in miR-218-5p stable cell lines confirmed by TaqMan real-time PCR.

No	miR-NC	miR-218-5p
1	0.95084	1238.63
2	1.03327	1144.25
3	1.00323	1289.74
4	1.014566	1172.8

Figure 4B. Tumor size was measured twice per week when xenografts were visible

Days	miR-NC										miR-218-5p									
8	0.00	0.00	8.79	6.29	33.44	0.00	16.40	0.00	0.00	8.79	1.50	0.00	0.00	12.19	0.00	0.00	0.00	0.00	2.45	0.00
11	33.69	261.12	181.66	233.47	4.67	27.56	18.25	48.23	45.94	463.96	0.00	0.00	0.00	15.00	0.00	19.46	0.00	0.00	3.24	11.26
14	175.14	164.63	932.22	815.89	106.59	31.97	427.00	167.17	776.04	447.18	0.00	0.00	0.00	68.80	63.89	55.81	15.75	0.00	10.98	30.26
17.00	569.53	285.01	1667.19	1493.89	165.19	86.62	950.95	1250.16	589.36	478.22	0.00	0.00	0.00	262.15	128.44	144.87	93.31	0.00	15.75	83.96
21.00	1054.41	944.07	2678.07	3015.07	362.91	2491.54	451.00	1740.78	1374.99	805.00	0.00	224.32	0.00	614.13	233.29	436.39	118.62	0.00	104.28	244.36

Figure 4C. The image showed representative tumors collected from each group



Figure 4D. Tumor weight (g) of xenografts from miR-NC and miR-218-5p groups

Tumors No.	miR-NC	miR-218-5p
1	0.36	0.09
2	1.74	0
3	1.8	0.36
4	1.6	0.22
5	0.22	0.21
6	0.53	0.1
7	0.95	0
8	1.17	0.07
9	0.64	0.11
10	0.54	0

Figure 4E. The expression level of miR-218-5p/U6 in xenografts using TaqMan real-time PCR assay

No.	miR-NC	miR-218-5p
1	0.99782	15.062048
2	0.98272	15.807933
3	1.01546	15.083351
4	1.00428	15.136168
5	0.95606	3.7783799
6	1.01314	3.9941218
7	1.01987	3.802338
8	1.01228	3.8551988
9	0.97378	2.8413763
10	1.02721	2.8004685
11	1.0093	2.908962
12	0.99052	2.9271403

The expression level of KDR/ β -actin in xenografts using real-time PCR assay

No.	miR-NC	miR-218-5p
1	1.286116	0.334264
2	0.765359	0.238138
3	1.015909	0.38

The expression level of FLT4/ β -actin in xenografts using real-time PCR assay

No.	miR-NC	miR-218-5p
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1	0.365263	0.16544
2	3.319248	0.081518
3	0.355716	0.222806
4	2.318731	0.288698
5	0.839968	0.214954
6	1.190522	0.183294

The expression level of FLT1/ β -actin in xenografts using real-time PCR assay

No.	miR-NC	miR-218-5p
1	2.002295	0.05129
2	0.516729	0.135622
3	1.082032	0.088556
4	0.893241	0.087081

Figure 4F. EGFR and β -actin expression in tumor tissues from miR-NC and miR-218-5p groups

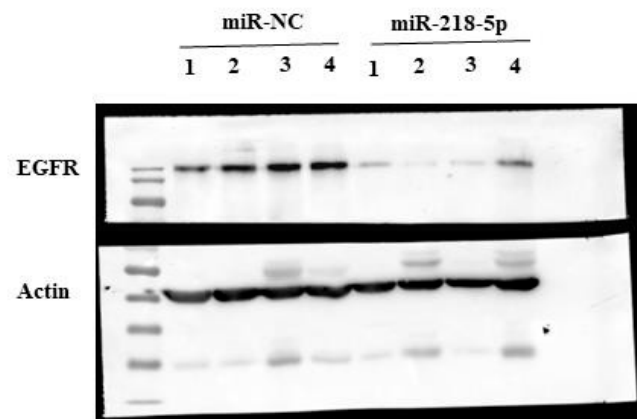


Figure 4F. quantification: the intensity of EGFR/ β -actin expression in tumor tissues from miR-NC and miR-218-5p groups

No.	miR-NC	miR-218-5p
1	0.69787	0.1183
2	0.7483	0.025
3	1.405	0.0509
4	1.148	0.3201

Figure 4G. CD31 positive microvessels by immunohistochemical staining assay

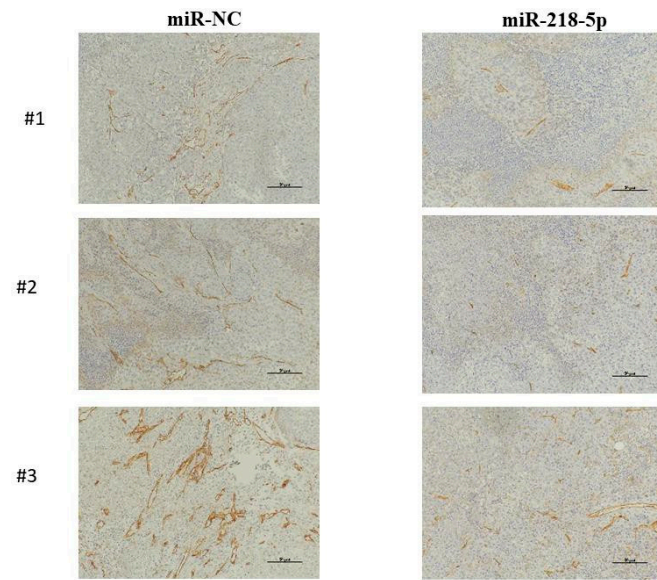
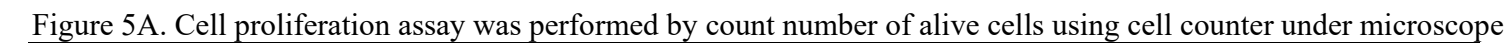


Figure 4G. Quantification: CD31 positive micro vessels by immunohistochemical staining assay

No	miR-NC	miR-218-5p
1	100	20
2	71	22
3	128	60

Figure 5A. The expression of EGFR and β -actin was analyzed 72 h after transfection using immunoblotting assay.



Days	miR-NC+ Vector				miR-218-5p+ Vector				miR-218-5p+EGFR			
0	10000	10000	10000	10000	10000	10000	10000	10000	10000	10000	10000	10000
1	13000	15500	14200	12500	11000	12600	11000	14000	12900	17000	15800	14000
2	18000	17900	22000	17500	17000	15900	14500	18000	19200	17800	20500	18500
3	37000	35600	32000	34000	28000	23500	24500	24400	29000	31000	32600	39000
4	44400	43300	46000	57000	37000	33000	32900	36000	52000	42000	41600	46000
5	81000	89000	96000	100000	50000	49000	53000	60000	93000	82000	89000	85000

Figure 5B. Colony formation assay using soft agar

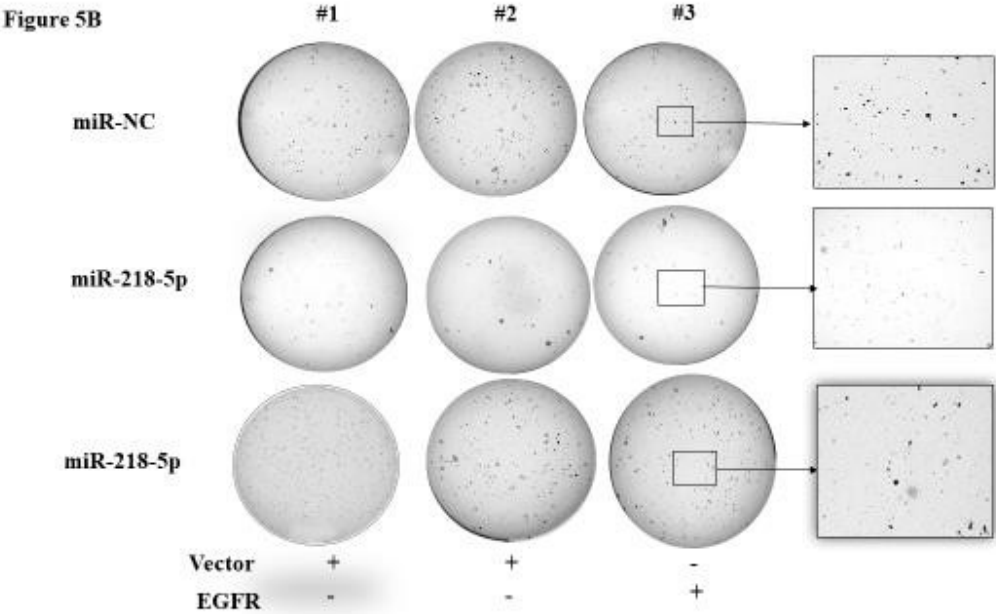


Figure 5B. Quantification: Number of colony in different treatments groups agar

miR-NC	miR218-5P	miR218-5P
119	27	101
114	39	120
98	55	105

Figure 5C. Trans well migration assay

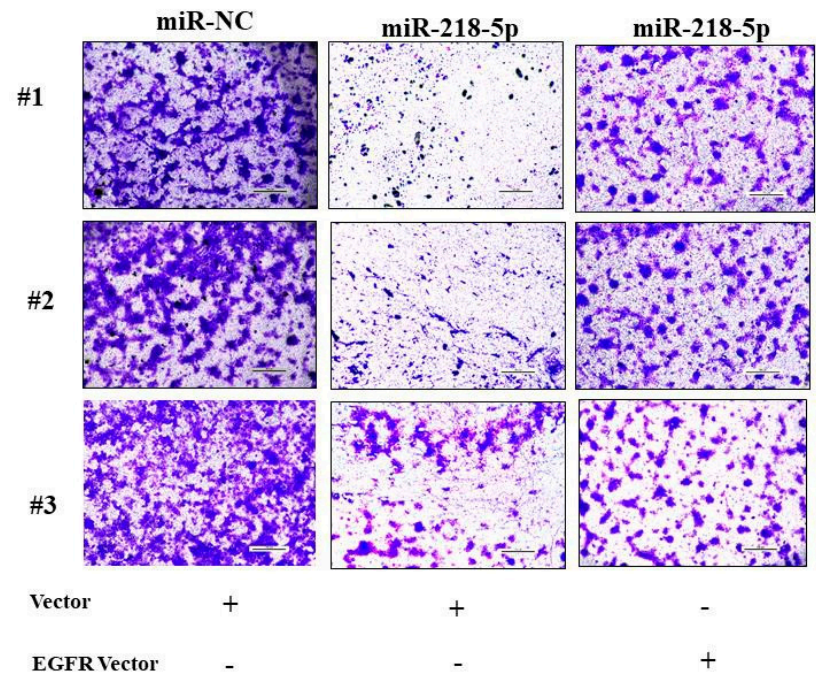


Figure 5C. Quantification: Percentage of cells migrates compare to control

miR-NC	miR-218-5p	miR-218-5p
89.69521	34.83309	74.89115
93.61393	30.91437	81.42235
116.6909	49.63716	77.50363

Figure 5D. Wound healing assay

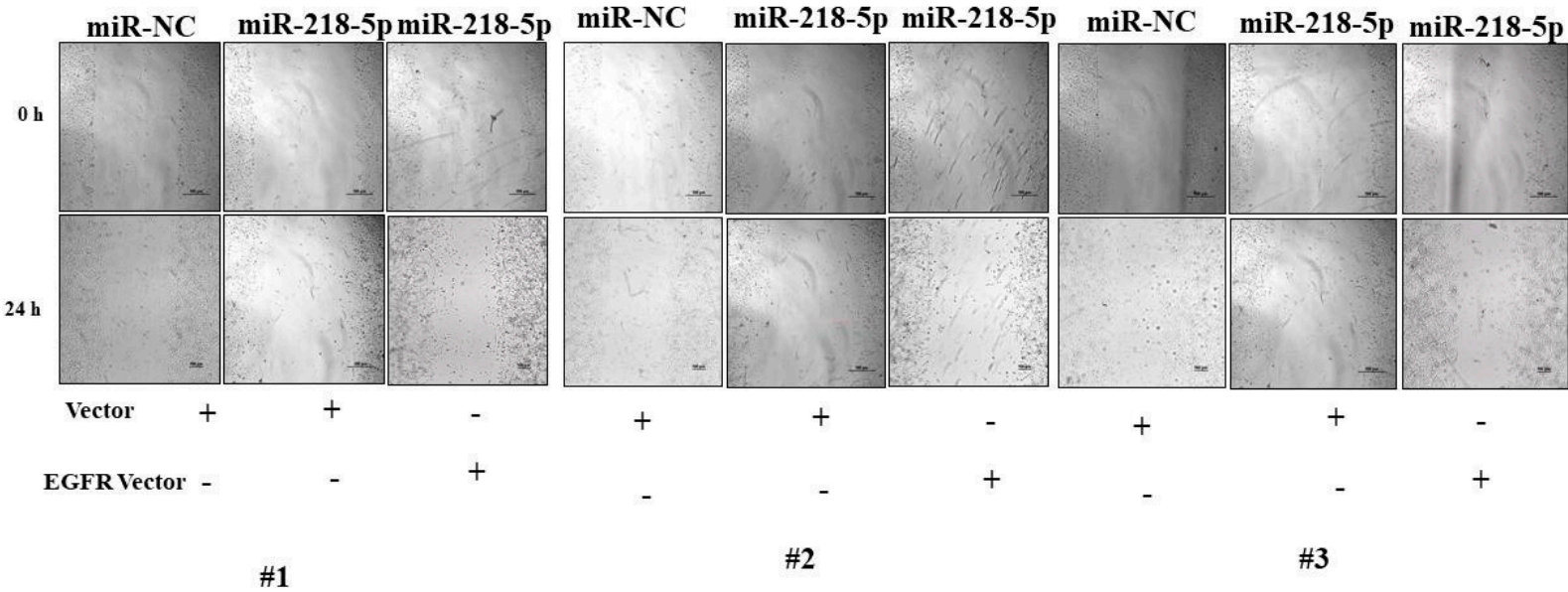


Figure 5D. Quantification: percentage of wound healing

No.	miR-NC	miR-218-5p	miR-218-5P
1	43.21502	6.669697	31.75959
2	27.89	16.50543	49.27507
3	31.82	0.251334	49.99505

Figure 5E. Tube formation assay

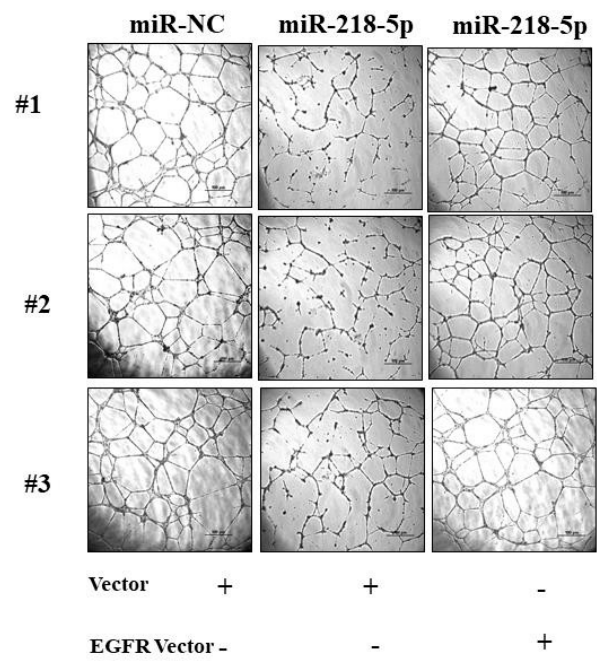


Figure 5E. Quantification: Number of meshes

No	miR-NC	miR-218-5p	miR-218-5p
1	39	12	37
2	30	3	30

3	31	6	31
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Vector + + -
EGFR - - +

Figure 5E. Quantification: Total tube length

No	miR-NC	miR-218-5p	miR-218-5p
1	108.06	59.30	97.31
2	95.21	37.04	96.19
3	96.72	52.96	86.56

Vector + + -
EGFR - - +