

Tumor-derived Circulating Exosomal miR-342-5p And miR-574-5p As Novel Promising Diagnostic Biomarkers For Early-stage Lung Adenocarcinoma

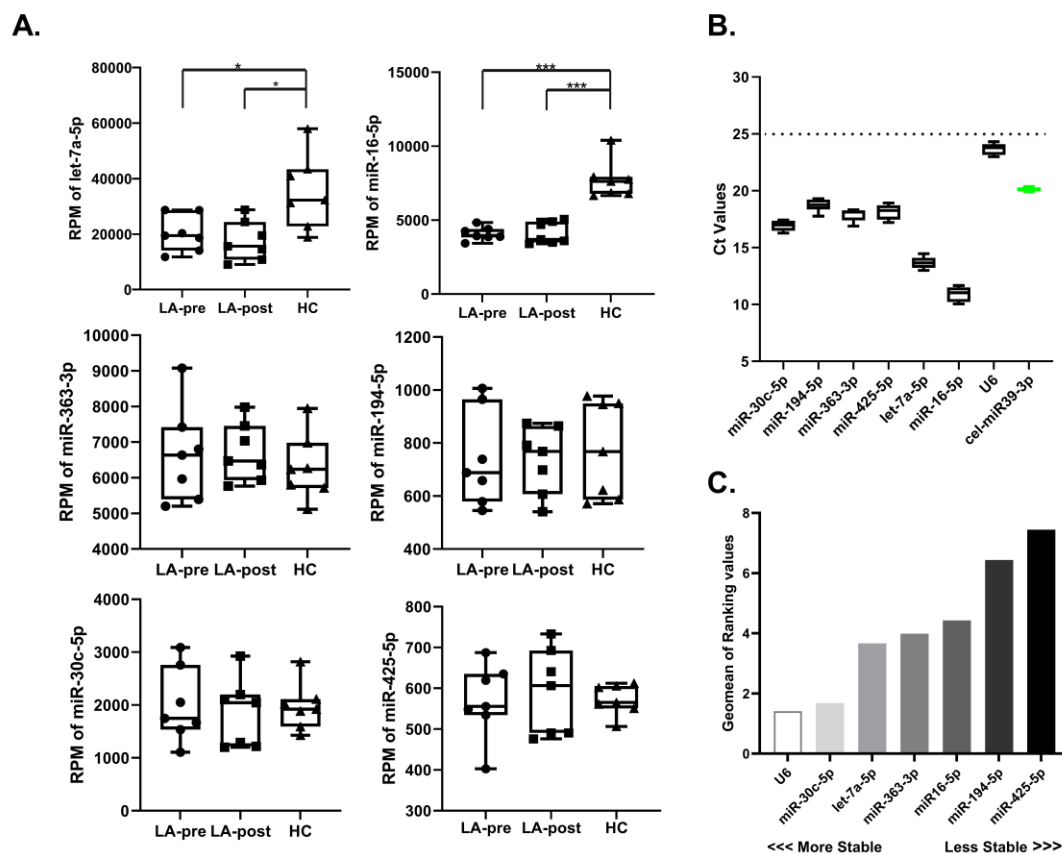


Figure S1. Selection of stable internal reference. (A) The expression level of selected six candidate reference miRNAs in sequencing data (*P < 0.05, ***P < 0.001). (B) Ct values of tested candidate internal references. (C) Histogram showing stability of candidate internal references analyzed by RefFinder. The less the geomean of ranking value is, the more stable the internal reference is.

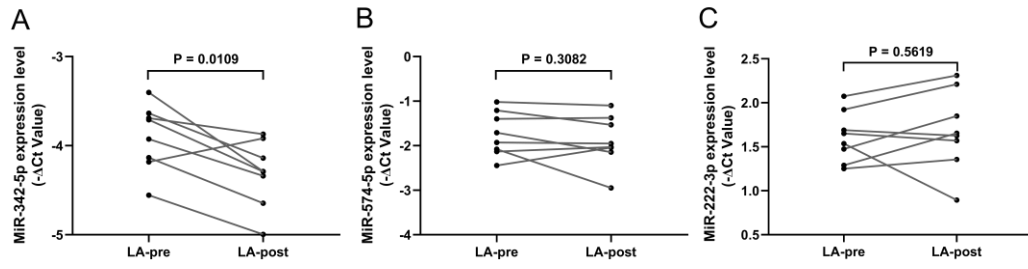


Figure S2. RT-qPCR results of candidate miRNAs in advanced-stage LA patients. Relative expression level of (A) miR-342-5p, (B) miR-574-5p and (C) miR-222-3p in paired LA-pre and LA-post (IIIA stage, n = 8). Statistical significance was analyzed by two-sided paired Student's *t*-test.

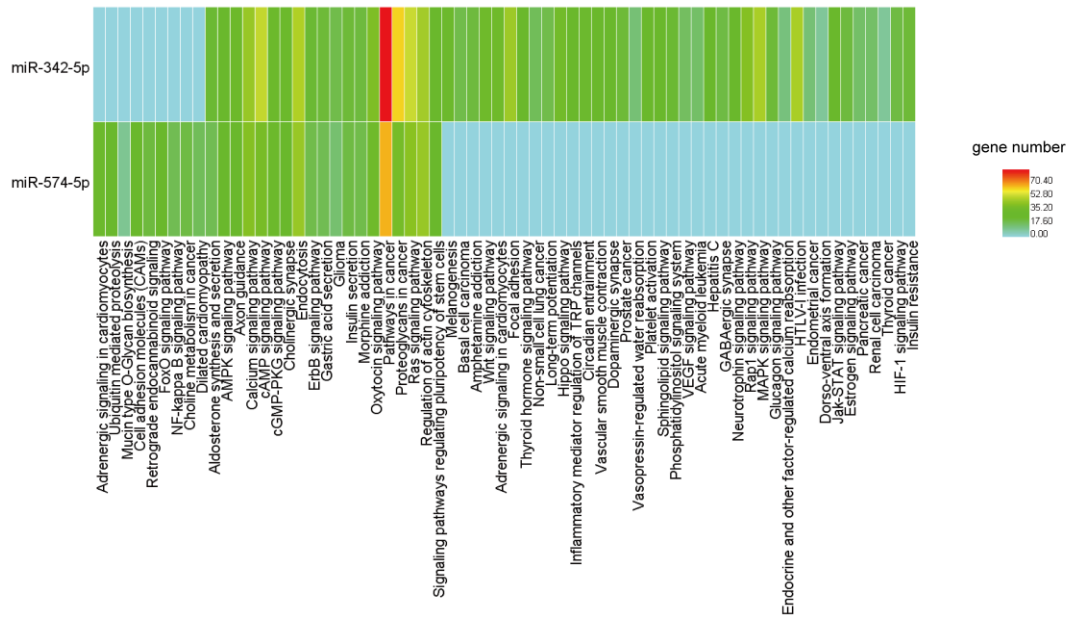


Figure S3. Heat map showing associated KEGG pathways of miR-342-5p and miR-574-5p predicted target genes.

Table S1. Differentially expressed circulating exosomal miRNAs in LA-pre and HC

miRNA	Average RPM of LA-pre	Average RPM of HC	FC	Adjusted P-value
hsa-miR-144-5p	1249	3065	0.4076	<0.0001
hsa-miR-574-5p	65.81	2.43	27.0889	<0.0001
hsa-miR-103a-3p	2300	9163	0.2511	<0.0001
hsa-miR-23a-3p	72.4	202.7	0.3573	<0.0001
hsa-miR-27b-3p	239.4	500.5	0.4783	<0.0001
hsa-miR-3912	2.909	7.915	0.3676	<0.0001
hsa-miR-548k	6.987	12.41	0.5630	0.0002
hsa-miR-221-3p	94.43	256.5	0.3681	0.0003
hsa-miR-185-5p	3144	9832	0.3198	0.0003
hsa-miR-3140-3p	1.085	2.134	0.5083	0.0004
hsa-miR-548av-5p	3.554	8.737	0.4068	0.0004
hsa-miR-451a	582925	386853	1.5068	0.0004
hsa-miR-374b-3p	0.9535	2.872	0.3320	0.0004
hsa-miR-17-5p	803.3	1762	0.4559	0.0004
hsa-miR-16-5p	4082	7711	0.5294	0.0004
hsa-miR-500a-3p	27.82	65.06	0.4276	0.0004
hsa-miR-484	323.9	739.6	0.4380	0.0005
hsa-miR-190a	5.522	20.46	0.2699	0.0006
hsa-let-7g-5p	30374	57165	0.5313	0.0006
hsa-miR-361-5p	12.08	27.61	0.4376	0.0006
hsa-miR-22-3p	1212	3217	0.3767	0.0007
hsa-miR-3158-3p	318.6	161.8	1.9690	0.0008
hsa-miR-548ay-5p	23.6	57.79	0.4084	0.0008
hsa-miR-590-3p	7.487	20.79	0.3601	0.0008
hsa-miR-107	5.178	25.84	0.2004	0.0008
hsa-miR-93-5p	1111	2901	0.3831	0.0008
hsa-miR-502-3p	38.1	117.3	0.3248	0.001
hsa-miR-651	13.49	40.7	0.3314	0.0011
hsa-miR-1180	115	62.77	1.8324	0.0011
hsa-miR-598	12.61	24.78	0.5090	0.0011
hsa-miR-21-5p	4028	9986	0.4033	0.0011
hsa-miR-15a-5p	43.05	111.4	0.3863	0.0013
hsa-miR-150-5p	168.4	304.7	0.5527	0.0013
hsa-miR-1	1.069	10.62	0.1006	0.0014
hsa-miR-181a-5p	422.7	857	0.4932	0.0015
hsa-miR-146b-5p	406.4	729.6	0.5570	0.0016
hsa-miR-190b	2.52	5.516	0.4568	0.0017
hsa-miR-130b-3p	6.463	13.77	0.4694	0.0017
hsa-miR-378g	1.89	4.011	0.4711	0.0017

hsa-miR-548at-5p	0.6231	1.381	0.4511	0.002
hsa-miR-148a-3p	13847	6404	2.1622	0.0021
hsa-miR-339-3p	1.878	4.626	0.4061	0.0021
hsa-miR-4521	1.852	5.017	0.3691	0.0022
hsa-miR-199b-3p	37.41	114.3	0.3272	0.0022
hsa-miR-4306	416.1	898.9	0.4629	0.0022
hsa-miR-148b-3p	966.8	1765	0.5479	0.0023
hsa-miR-30e-5p	766.9	3138	0.2444	0.0025
hsa-miR-659-5p	7.024	3.745	1.8757	0.0025
hsa-miR-340-3p	38.13	16.06	2.3743	0.0025
hsa-miR-221-5p	6.485	14.91	0.4350	0.0027
hsa-miR-421	24.76	43.07	0.5750	0.0027
hsa-miR-20a-5p	1235	2942	0.4198	0.0032
hsa-miR-152	23.08	47.05	0.4904	0.0032
hsa-miR-199b-5p	6.704	18.64	0.3597	0.0032
hsa-miR-185-3p	51.02	104.9	0.4866	0.0033
hsa-miR-20b-5p	275.5	545.2	0.5054	0.0034
hsa-miR-126-5p	100.3	385.9	0.2598	0.0034
hsa-miR-342-3p	15.39	32.09	0.4795	0.0034
hsa-miR-374a-3p	34.29	95.27	0.3599	0.0037
hsa-miR-106b-5p	272.1	818.4	0.3325	0.004
hsa-miR-378a-3p	380.6	715.6	0.5318	0.004
hsa-let-7f-5p	31855	61073	0.5216	0.004
hsa-miR-576-3p	12.1	35.17	0.3442	0.0043
hsa-miR-98-5p	504.5	822.3	0.6135	0.0043
hsa-miR-106a-5p	82.76	174.5	0.4742	0.0045
hsa-miR-362-5p	9.323	13.76	0.6776	0.0051
hsa-miR-330-5p	10.98	6.599	1.6643	0.0051
hsa-miR-374a-5p	362.3	724.6	0.5000	0.0055
hsa-miR-660-5p	65.09	178.3	0.3650	0.0061
hsa-miR-26b-3p	3.851	6.934	0.5554	0.0063
hsa-miR-652-3p	448.6	707.2	0.6343	0.0063
hsa-miR-532-5p	748.5	1230	0.6085	0.0063
hsa-miR-186-5p	1402	2844	0.4928	0.0063
hsa-miR-454-3p	151.7	274	0.5535	0.0063
hsa-miR-200c-3p	7.307	12.72	0.5744	0.0068
hsa-miR-548e	5.097	10.18	0.5009	0.0068
hsa-miR-93-3p	18.12	34.45	0.5260	0.0068
hsa-miR-29b-2-5p	1.906	3.901	0.4886	0.0071
hsa-miR-324-3p	38.48	67.24	0.5722	0.0078
hsa-let-7d-5p	2482	5901	0.4205	0.0094
hsa-miR-340-5p	81.04	322.4	0.2513	0.0096
hsa-miR-210	28.87	58.83	0.4908	0.0097

hsa-miR-101-3p	5838	14426	0.4047	0.0097
hsa-miR-146a-5p	306	707.6	0.4325	0.0097
hsa-miR-26a-5p	12902	23324	0.5532	0.0097
hsa-miR-624-5p	5.307	10.52	0.5045	0.0097
hsa-miR-21-3p	3.81	9.409	0.4049	0.0101
hsa-miR-184	2.856	1.143	2.4996	0.0104
hsa-miR-16-2-3p	41.1	19.93	2.0626	0.0104
hsa-miR-151a-3p	3724	2472	1.5065	0.0104
hsa-miR-342-5p	56.43	29.16	1.9353	0.0104
hsa-miR-18a-5p	23.81	42.06	0.5662	0.0104
hsa-miR-28-3p	36.51	63.39	0.5760	0.0104
hsa-miR-19a-3p	59.26	147.3	0.4024	0.0104
hsa-miR-4446-3p	0.4439	1.055	0.4207	0.0107
hsa-miR-548ah-3p	1.012	2.227	0.4546	0.0109
hsa-miR-450b-5p	4.15	12.84	0.3233	0.0109
hsa-let-7c	75.43	141.9	0.5316	0.0113
hsa-miR-378f	1.126	2.861	0.3937	0.0115
hsa-miR-144-3p	1026	4040	0.2539	0.0119
hsa-miR-766-3p	1.879	3.224	0.5827	0.0122
hsa-miR-425-3p	6.894	13.34	0.5169	0.0122
hsa-miR-130a-3p	24.14	38.51	0.6268	0.0122
hsa-miR-939-5p	2.383	3.521	0.6769	0.0124
hsa-miR-29a-3p	34.09	80.27	0.4247	0.0133
hsa-miR-140-3p	1938	3451	0.5615	0.0133
hsa-miR-4286	2.383	6.872	0.3468	0.0145
hsa-miR-19b-3p	115.4	490.5	0.2352	0.0146
hsa-miR-142-5p	119.6	467.6	0.2557	0.0155
hsa-miR-18b-5p	0.483	1.08	0.4472	0.0155
hsa-miR-99a-5p	149.4	90.92	1.6438	0.0158
hsa-miR-664a-5p	2.099	3.346	0.6273	0.0158
hsa-miR-374b-5p	411.2	712.4	0.5772	0.0163
hsa-miR-3656	7.603	2.503	3.0374	0.0179
hsa-miR-581	5.833	3.962	1.4723	0.0195
hsa-miR-1273c	0.8208	1.921	0.4274	0.0202
hsa-miR-1306-3p	6.353	2.813	2.2582	0.021
hsa-miR-320b	107.1	167.7	0.6388	0.0217
hsa-miR-25-5p	7.745	13.8	0.5613	0.0221
hsa-miR-1976	8.176	12.36	0.6613	0.0227
hsa-miR-222-3p	415.8	237.9	1.7475	0.0229
hsa-let-7e-5p	8.268	18.58	0.4450	0.0237
hsa-miR-3200-5p	3.545	7.401	0.4791	0.0239
hsa-miR-155-5p	51.13	73.21	0.6984	0.0239
hsa-miR-1255b-5p	46.88	29.89	1.5684	0.0256

hsa-let-7i-3p	7.251	12.53	0.5787	0.0259
hsa-miR-106b-3p	237.9	340.8	0.6982	0.0265
hsa-miR-181c-5p	2.889	5.39	0.5360	0.0265
hsa-miR-3173-5p	0.9002	1.996	0.4509	0.027
hsa-miR-3176	1.037	3.032	0.3419	0.027
hsa-miR-224-5p	1.952	5.105	0.3823	0.027
hsa-miR-17-3p	7.03	10.92	0.6437	0.027
hsa-miR-301a-3p	0.7206	2.258	0.3191	0.0289
hsa-miR-532-3p	7.676	13.07	0.5872	0.0295
hsa-miR-5582-3p	2.165	0.8586	2.5216	0.0301
hsa-miR-335-5p	6.095	12.95	0.4708	0.0341
hsa-miR-450a-5p	12.77	24.99	0.5111	0.0352
hsa-miR-181d	1.88	3.236	0.5808	0.0368
hsa-miR-3200-3p	13.64	24.22	0.5629	0.0368
hsa-miR-1288	0.9254	0.4983	1.8571	0.038
hsa-miR-15b-5p	186.8	360.2	0.5185	0.038
hsa-miR-135a-5p	1.727	3.128	0.5521	0.0384
hsa-miR-501-3p	30.44	54.58	0.5577	0.0384
hsa-miR-29c-3p	16.1	30.14	0.5342	0.0384
hsa-miR-514a-3p	1.791	0.8799	2.0356	0.0394
hsa-miR-1307-5p	1.836	10.97	0.1673	0.0398
hsa-let-7i-5p	69571	87935	0.7912	0.0398
hsa-miR-30e-3p	552	739.4	0.7466	0.0405
hsa-miR-24-3p	521.3	413.6	1.2604	0.0405
hsa-miR-548o-3p	8.131	11.58	0.7021	0.0405
hsa-miR-345-5p	22.68	40.31	0.5626	0.0405
hsa-miR-5001-3p	1.642	2.311	0.7104	0.0414
hsa-miR-132-3p	5.75	8.913	0.6452	0.042
hsa-miR-874	0.7498	2.044	0.3668	0.0435
hsa-let-7a-3p	74.96	115.7	0.6477	0.0444
hsa-miR-454-5p	12.79	17.83	0.7170	0.0466
hsa-miR-941	79.2	170.6	0.4643	0.0487
hsa-miR-28-5p	11.33	17.65	0.6421	0.0488
hsa-let-7a-5p	20236	35337	0.5727	0.0489

Table S2. Average Ct values of candidate biomarkers detected in validation samples

miRNA	Mean \pm SD (Ct)		
	LA-pre(n=56)	LA-post(n=51)	HC (n=40)
hsa-miR-342-5p	25.96 \pm 0.90	26.66 \pm 0.97	26.64 \pm 0.98
hsa-miR-574-5p	23.41 \pm 0.89	23.87 \pm 0.92	24.47 \pm 0.87
hsa-miR-222-3p	20.35 \pm 1.16	20.76 \pm 1.36	21.24 \pm 0.99
RNU6	22.23 \pm 1.09	22.52 \pm 1.18	22.47 \pm 1.05