

SUPPLEMENTAL MATERIAL

Table S1. NanoString nCounter™ assay measurements of miRNA copy numbers/cell detected in MCF7 and MCF10A cells at 72 hrs of serum deprivation and in EVs derived from MCF7 and MCF10A cells over 72 hrs of serum deprivation. These miRNAs are the most abundant species shown in **Figure 3**. For MCF7 and MCF10A cellular miRNA, average copy numbers/cell are given with the standard deviation in parentheses (n=4 for MCF7 cells and n=3 for MCF10A cells*). Average copy numbers/cell are given with the range in parentheses for MCF7 EV miRNA (n=2), and copy numbers/cell are given for MCF10A EV miRNA (n=1). “ND” indicates the miRNA was not detected in the assay.

miRNA	miR copy numbers per MCF7 cell	EV miR copy number per MCF7 cell	miR copy numbers per MCF10A cell	EV miR copy number per MCF10A cell
let-7a	1979 (1486)	9.0 (5.7-12.2)	1132 (340)	1.1
let-7f	548 (459)	0.2 (0.0-0.5)	209 (75)	ND
miR-15b	238 (138)	0.4 (0.4-0.5)	110 (57)	ND
miR-16	1209 (898)	2.8 (2.8-2.9)	372 (179)	0.6
miR-19b	161 (130)	0.2 (0.1-0.2)	430 (119)	1.0
miR-21	2987 (3123)	6.1 (5.5-6.7)	196 (60)	0.1
miR-24	467 (365)	2.6 (2.5-2.8)	320 (115)	1.4
miR-26a	1027 (828)	0.4 (0.0-0.9)	152 (38)	ND
miR-29a	318 (195)	1.1 (1.0-1.2)	436 (187)	0.8
miR-92a	318 (176)	6.1 (2.9-9.4)	801 (546)	3.0
miR-100	882 (611)	14.5 (9.4-19.7)	350 (139)	1.7
miR-125b	483 (253)	8.1 (3.3-12.9)	254 (191)	0.5
miR-200c	704 (556)	2.1 (1.9-2.3)	337 (161)	0.3
miR-205	32 (20)	0.1 (0.1-0.1)	1127 (370)	8.2
miR-221	161 (102)	2.1 (1.5-2.6)	257 (82)	2.2
miR-222	50 (22)	0.9 (0.8-1.1)	41 (21)	0.1
miR-423-3p	54 (35)	0.5 (0.4-0.6)	48 (26)	0.2
miR-423-5p	60 (46)	1.1 (0.8-1.3)	37 (15)	0.4
miR-630	ND	7.7 (5.8-9.6)	43 (30)	18.3
miR-1246	ND	0.3 (0.0-0.6)	38 (37)	0.9
miR-1290	ND	0.3 (0.1-0.4)	ND	1.6
miR-720	1878 (1996)	132.7 (73.0-192.4)	1684 (891)	11.9
miR-1274a	137 (123)	19.7 (10.9-28.5)	282 (145)	2.6
miR-1274b	198 (171)	21.3 (11.7-31.0)	247 (143)	1.6
miR-1260	455 (370)	17.6 (8.2-27.1)	394 (51)	1.3
All other miRNAs	(37.2%)	(12.0%)	(34.4%)	(9.6%)

* For the cellular miRNA, 15 million cells were collected for each of the 3 or 4 samples, and the total RNA isolated from each sample. For the EV miRNA, supernatant in the EV isolation from 350 million cells were combined and total RNA isolated for each of the samples, while the total number of cells were estimated for each sample by randomly selecting 2-4 plates and counting cells per plate.

Table S2. qRT-PCR assay measurements of miRNA copy numbers/cell detected in MCF7 and MCF10A cells at 72 hrs of serum deprivation and in EVs secreted from MCF7 and MCF10A cells over 72 hrs of serum deprivation. Numbers in parentheses for *miR-21* are repeated, independent measurements on the same biological sample. Exp-1, Exp-2, ... are independent measurements on different samples as explained in the **Table S1** footnote. “ND” indicates the miRNA was not detected in the assay. Raw qRT-PCR data (Ct values, total cell numbers, total RNA) are given in **Table S3**; raw qRT-PCR data (Ct values and miRNA copy numbers) for the standard curves for synthetic *miR-21* and *miR-720* are given in **Table S4**.

	miR-21	miR-720	miR-205	miR-1274b	let-7a	miR-125b	miR-100
MCF10A EV miRNA copy numbers/cell							
Exp-1	0.8	15.3	ND	ND	1.2	0.7	0.9
Exp-2	2.0 (3.4)	19.0	15.9	ND	2.5	3.6	3.1
Exp-3	4.8 (4.5)	47.9	42.5	ND	4.0	4.7	6.1
AVG	2.5	27.4	29.2	---	2.6	3.0	3.4
STD	2.0	17.8	---	---	1.4	2.1	2.5
MCF7 EV miRNA copy numbers/cell							
Exp-1	132.4 (159.2)	385.5	0.3	184.9	45.6	137.5	130.9
Exp-2	155.9 (77.4)	438.4	0.3	567.6	29.4	86.3	54.6
Exp-3	157.5 (298.2)	455.6	0.4	1622.0	29.5	127.6	120.6
Exp-4	382.6 (314.0)	700.0	0.7	1468.6	84.9	237.3	166.1
Exp-5	68.3	508.9	0.1	2302.9	49.5	168.4	122.2
Exp-6	136.0	548.3	0.1	2217.0	101.0	318.0	215.7
Exp-7	95.1	434.7	0.2	2961.3	37.9	153.7	99.2
AVG	179.7	495.9	0.3	1617.8	54.0	175.6	129.9
STD	104.4	104.5	0.2	984.2	28.0	77.8	50.7

	miR-21	miR-720	miR-205	miR-1274b	let-7a	miR-125b	miR-100
MCF10A cellular miRNA copy numbers/cell							
Exp-1	3299	8682	5256	13 514	1136	808	619
Exp-2	3918	8619	6213	11 809	1378	1006	857
Exp-3	3410	7277	6350	17 962	2330	931	886
AVG	3542	8193	5940	14 429	1615	915	787
STD	330	794	596	3177	631	100	147
MCF7 cellular miRNA copy numbers/cell							
Exp-1	9941	3619	3	17 930	683	960	837
Exp-2	27 419	2975	6	11 925	440	373	337
Exp-3	29 396	3666	5	17 829	1329	1192	954
Exp-4	23 119	6205	7	15 714	529	659	534
Exp-5	48 555	10 621	8	31 813	6296	7342	5053
Exp-6	50 319	11 414	8	17 191	6238	4555	3553
Exp-7	43 431	7816	5	16 808	5233	3663	2605
AVG	33 166	6617	6	18 458	2964	2677	1982
STD	14 856	3455	2	6239	2803	2607	1801

Table S3. Raw data for the miRNA copy numbers/cell reported in **Table S2** derived from qRT-PCR assay measurements of seven miRNAs (*miR-21*, *miR-720*, *miR-205*, *miR-1274b*, *let-7a*, *miR-125b*, and *miR-100*) detected in MCF7 and MCF10A cells at 72 hrs of serum deprivation and in EVs secreted from MCF7 and MCF10A cells over 72 hrs of serum deprivation.

MCF7 EVs									
miR-21									
	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (0.3%)	EV miR/Cell
EXP 1	1.37E+08	671.55	25.48	5.21	162368.78	81184	5.45E+07	1.82E+10	132.4
EXP 2	1.50E+08	716.85	25.21	5.29	195714.51	97857	7.01E+07	2.34E+10	155.9
EXP 3	1.05E+08	451.8	25.05	5.34	220274.95	110137	4.98E+07	1.66E+10	157.5
EXP 4	2.12E+08	1036.8	23.98	5.67	468303.70	234152	2.43E+08	8.09E+10	382.6
EXP 5	1.38E+08	943.84	26.88	4.78	59900.72	29950	2.83E+07	9.42E+09	68.3
EXP 6	2.35E+08	1421.54	25.74	5.13	134704.48	67352	9.57E+07	3.19E+10	136.0
EXP 7	2.88E+08	1450.78	25.99	5.05	113216.36	56608	8.21E+07	2.74E+10	95.1
EXP 1 (REPEAT)	1.37E+08	671.55	25.22	5.29	195252.31	97626	6.56E+07	2.19E+10	159.2
EXP 2 (REPEAT)	1.50E+08	716.85	26.20	4.99	97202.60	48601	3.48E+07	1.16E+10	77.4
EXP 3 (REPEAT)	1.05E+08	451.8	24.15	5.62	417073.26	208537	9.42E+07	3.14E+10	298.2
EXP 4 (REPEAT)	2.12E+08	1036.8	24.26	5.58	384311.72	192156	1.99E+08	6.64E+10	314.0
AVG									179.7
STD DEV									104.4
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miR-720									
	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (0.3%)	EV miR/Cell
EXP 1	1.37E+08	671.55	23.19	5.67	472671.64	236336	1.59E+08	5.29E+10	385.5
EXP 2	1.50E+08	716.85	22.94	5.74	550434.92	275217	1.97E+08	6.58E+10	438.4
EXP 3	1.05E+08	451.8	22.71	5.80	637046.01	318523	1.44E+08	4.80E+10	455.6
EXP 4	2.12E+08	1036.8	22.23	5.93	856817.22	428409	4.44E+08	1.48E+11	700.0
EXP 5	1.38E+08	943.84	23.28	5.65	446431.30	223216	2.11E+08	7.02E+10	508.9
EXP 6	2.35E+08	1421.54	22.97	5.73	543120.03	271560	3.86E+08	1.29E+11	548.3
EXP 7	2.88E+08	1450.78	23.04	5.71	517741.91	258871	3.76E+08	1.25E+11	434.7
AVG									495.9
STD DEV									104.5
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miR-205									
	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (0.3%)	EV miR/Cell
EXP 1	1.37E+08	671.55	34.22	2.52	330.09	165	1.11E+05	3.69E+07	0.27
EXP 2	1.50E+08	716.85	34.03	2.58	377.72	189	1.35E+05	4.51E+07	0.30
EXP 3	1.05E+08	451.8	33.34	2.79	614.02	307	1.39E+05	4.62E+07	0.44
EXP 4	2.12E+08	1036.8	32.81	2.95	897.41	449	4.65E+05	1.55E+08	0.73
EXP 5	1.38E+08	943.84	36.21	1.90	80.04	40	3.78E+04	1.26E+07	0.09
EXP 6	2.35E+08	1421.54	35.49	2.12	133.30	67	9.47E+04	3.16E+07	0.13
EXP 7	2.88E+08	1450.78	34.68	2.38	237.91	119	1.73E+05	5.75E+07	0.20
AVG									0.3
STD DEV									0.2

miR-1274b									
	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (0.3%)	EV miR/Cell
EXP 1	1.37E+08	671.55	24.38	5.36	226698.00	113349	7.61E+07	2.54E+10	185
EXP 2	1.50E+08	716.85	22.53	5.85	712667.17	356334	2.55E+08	8.51E+10	568
EXP 3	1.05E+08	451.8	20.65	6.36	2268240.18	1134120	5.12E+08	1.71E+11	1622
EXP 4	2.12E+08	1036.8	21.03	6.25	1797552.77	898776	9.32E+08	3.11E+11	1469
EXP 5	1.38E+08	943.84	20.84	6.31	2020267.19	1010134	9.53E+08	3.18E+11	2303
EXP 6	2.35E+08	1421.54	20.70	6.34	2195889.98	1097945	1.56E+09	5.20E+11	2217
EXP 7	2.88E+08	1450.78	19.94	6.55	3527153.10	1763577	2.56E+09	8.53E+11	2961
AVG									1617.8
STD DEV									984.2

let-7a									
	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (0.3%)	EV miR/Cell
EXP 1	1.37E+08	671.55	26.98	4.75	55898.09	27949	1.88E+07	6.26E+09	46
EXP 2	1.50E+08	716.85	27.56	4.57	36957.25	18479	1.32E+07	4.42E+09	29
EXP 3	1.05E+08	451.8	27.41	4.62	41301.06	20651	9.33E+06	3.11E+09	30
EXP 4	2.12E+08	1036.8	26.11	5.02	103855.49	51928	5.38E+07	1.79E+10	85
EXP 5	1.38E+08	943.84	27.34	4.64	43454.86	21727	2.05E+07	6.84E+09	50
EXP 6	2.35E+08	1421.54	26.16	5.00	100000.00	50000	7.11E+07	2.37E+10	101
EXP 7	2.88E+08	1450.78	27.28	4.65	45183.64	22592	3.28E+07	1.09E+10	38
AVG									54
STD DEV									28

miR-125b									
	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (0.3%)	EV miR/Cell
EXP 1	1.37E+08	671.55	25.42	5.23	168628.90	84314	5.66E+07	1.89E+10	138
EXP 2	1.50E+08	716.85	26.05	5.03	108370.88	54185	3.88E+07	1.29E+10	86
EXP 3	1.05E+08	451.8	25.34	5.25	178474.51	89237	4.03E+07	1.34E+10	128
EXP 4	2.12E+08	1036.8	24.66	5.46	290472.87	145236	1.51E+08	5.02E+10	237
EXP 5	1.38E+08	943.84	25.61	5.17	147716.45	73858	6.97E+07	2.32E+10	168
EXP 6	2.35E+08	1421.54	24.54	5.50	314974.15	157487	2.24E+08	7.46E+10	318
EXP 7	2.88E+08	1450.78	25.31	5.26	183068.97	91534	1.33E+08	4.43E+10	154
AVG									175.6
STD DEV									77.8

miR-100									
	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (0.3%)	EV miR/Cell
EXP 1	1.37E+08	671.55	25.49	5.21	160460.57	80230	5.39E+07	1.80E+10	131
EXP 2	1.50E+08	716.85	26.69	4.84	68502.33	34251	2.46E+07	8.18E+09	55
EXP 3	1.05E+08	451.8	25.42	5.23	168628.90	84314	3.81E+07	1.27E+10	121
EXP 4	2.12E+08	1036.8	25.16	5.31	203260.27	101630	1.05E+08	3.51E+10	166
EXP 5	1.38E+08	943.84	26.06	5.03	107160.60	53580	5.06E+07	1.69E+10	122
EXP 6	2.35E+08	1421.54	25.09	5.33	213607.34	106804	1.52E+08	5.06E+10	216
EXP 7	2.88E+08	1450.78	25.93	5.07	118138.74	59069	8.57E+07	2.86E+10	99
AVG									129.9
STD DEV									50.7

MCF10A EVs

miR-21

	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (0.3%)	EV miR/Cell
EXP 1	1.12E+08	238.8	31.46	3.37	2329.82	1165	278180	9.27E+07	0.8
EXP 2	1.46E+08	351.3	30.39	3.70	4964.91	2482	872086	2.91E+08	2.0
EXP 3	1.49E+08	327.3	29.03	4.12	13089.29	6545	2142063	7.14E+08	4.8
EXP 2 (Repeat)	1.46E+08	351.3	29.65	3.92	8411.95	4206	1477559	4.93E+08	3.4
EXP 3 (Repeat)	1.49E+08	327.3	29.11	4.09	12308.87	6154	2014347	6.71E+08	4.5
AVG									3.1
STD DEV									1.7

miR-720

	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (0.3%)	EV miR/Cell
EXP 1	1.12E+08	238.8	27.08	4.63	42885.09	21443	5120479	1.71E+09	15.3
EXP 2	1.46E+08	351.3	26.92	4.68	47338.24	23669	8314961	2.77E+09	19.0
EXP 3	1.49E+08	327.3	25.27	5.12	130584.11	65292	21370090	7.12E+09	47.9
AVG									27.4
STD DEV									17.8

miR-205

	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (0.3%)	EV miR/Cell
EXP 1	1.12E+08	238.8							
EXP 2	1.46E+08	351.3	27.47	4.60	39486.74	19743	6935845	2.31E+09	15.9
EXP 3	1.49E+08	327.3	25.95	5.06	116062.26	58031	18993589	6.33E+09	42.5
AVG									29.2

miR-1274b

	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (0.3%)	EV miR/Cell
EXP 1	1.12E+08	238.8							
EXP 2	1.46E+08	351.3	No Ct						
EXP 3	1.49E+08	327.3							
AVG									

let-7a

	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (0.3%)	EV miR/Cell
EXP 1	1.12E+08	238.8	30.98	3.51	3267.08	1634	390090	1.30E+08	1.16
EXP 2	1.46E+08	351.3	30.05	3.80	6319.01	3160	1109934	3.70E+08	2.54
EXP 3	1.49E+08	327.3	29.28	4.04	10962.33	5481	1793986	5.98E+08	4.02
AVG									2.6
STD DEV									1.4

miR-125b

	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (0.3%)	EV miR/Cell
EXP 1	1.12E+08	238.8	31.75	3.28	1901.14	951	226996	7.57E+07	0.68
EXP 2	1.46E+08	351.3	29.56	3.95	8987.70	4494	1578689	5.26E+08	3.61
EXP 3	1.49E+08	327.3	29.07	4.10	12693.09	6347	2077224	6.92E+08	4.65
AVG									3.0
STD DEV									2.1

miR-100

	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (0.3%)	MV miR/Cell
EXP 1	1.12E+08	238.8	31.37	3.39	2477.53	1239	295818	9.86E+07	1
EXP 2	1.46E+08	351.3	29.76	3.89	7798.98	3899	1369891	4.57E+08	3
EXP 3	1.49E+08	327.3	28.69	4.22	16659.20	8330	2726277	9.09E+08	6
AVG									3.4
STD DEV									2.6

MCF7 Cells

miR-21

	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng Copy/ng)	Total Copy #	Eff (75%)	miR/Cell	
EXP 1	3.81E+06	61810	23.03	5.96	919794.65	459897	2.84E+10	3.79E+10	9941
EXP 2	6.00E+06	73348	21.20	6.53	3364445.67	1682223	1.23E+11	1.65E+11	27419
EXP 3	4.05E+06	86024	21.89	6.32	2074552.38	1037276	8.92E+10	1.19E+11	29376
EXP 4	5.88E+06	89714	21.76	6.36	2270916.14	1135458	1.02E+11	1.36E+11	23119
EXP 5	4.31E+06	73698	20.87	6.63	4261845.32	2130923	1.57E+11	2.09E+11	48555
EXP 6	7.33E+06	104982	20.57	6.72	5272461.67	2636231	2.77E+11	3.69E+11	50319
EXP 7	9.00E+06	140202	20.90	6.62	4181989.62	2090995	2.93E+11	3.91E+11	43431
AVG									33166
STD DEV									14856

miR-720

	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng Copy/ng)	Total Copy #	Eff (75%)	miR/Cell	
EXP 1	3.81E+06	61810	23.75	5.52	334839.12	167420	1.03E+10	1.38E+10	3619
EXP 2	6.00E+06	73348	23.61	5.56	365072.20	182536	1.34E+10	1.79E+10	2975
EXP 3	4.05E+06	86024	24.17	5.41	258882.30	129441	1.11E+10	1.48E+10	3666
EXP 4	5.88E+06	89714	22.78	5.78	609470.35	304735	2.73E+10	3.65E+10	6205
EXP 5	4.31E+06	73698	22.09	5.97	932259.65	466130	3.44E+10	4.58E+10	10621
EXP 6	7.33E+06	104982	21.69	6.08	1195901.04	597951	6.28E+10	8.37E+10	11413
EXP 7	9.00E+06	140202	22.44	5.88	752617.53	376309	5.28E+10	7.03E+10	7816
AVG									6617
STD DEV									3455

miR-205

	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng Copy/ng)	Total Copy #	Eff (75%)	miR/Cell	
EXP 1	3.81E+06	61810	34.46	2.44	278.09	139	8.59E+06	1.15E+07	3
EXP 2	6.00E+06	73348	33.14	2.85	705.94	353	2.59E+07	3.45E+07	6
EXP 3	4.05E+06	86024	34.02	2.58	379.96	190	1.63E+07	2.18E+07	5
EXP 4	5.88E+06	89714	33.24	2.82	659.16	330	2.96E+07	3.94E+07	7
EXP 5	4.31E+06	73698	33.16	2.84	695.99	348	2.56E+07	3.42E+07	8
EXP 6	7.33E+06	104982	32.86	2.94	861.04	431	4.52E+07	6.03E+07	8
EXP 7	9.00E+06	140202	33.74	2.66	461.25	231	3.23E+07	4.31E+07	5
AVG									6
STD DEV									2

miR-1274b									
	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng Copy/ng)	Total Copy #	Eff (75%)	miR/Cell	
EXP 1	3.81E+06	61810	21.16	6.22	1658901.84	829451	5.13E+10	6.84E+10	17930
EXP 2	6.00E+06	73348	21.36	6.17	1463169.28	731585	5.37E+10	7.15E+10	11925
EXP 3	4.05E+06	86024	21.60	6.10	1259047.20	629524	5.42E+10	7.22E+10	17829
EXP 4	5.88E+06	89714	21.27	6.19	1543601.58	771801	6.92E+10	9.23E+10	15714
EXP 5	4.31E+06	73698	20.31	6.45	2792350.84	1396175	1.03E+11	1.37E+11	31813
EXP 6	7.33E+06	104982	21.02	6.26	1801256.32	900628	9.45E+10	1.26E+11	17191
EXP 7	9.00E+06	140202	21.20	6.21	1618431.19	809216	1.13E+11	1.51E+11	16808
AVG									18458
STD DEV									6239

let-7a									
	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng Copy/ng)	Total Copy #	Eff (75%)	miR/Cell	
EXP 1	3.81E+06	61810	26.81	4.80	63211.01	31606	1.95E+09	2.60E+09	683
EXP 2	6.00E+06	73348	27.03	4.73	53950.36	26975	1.98E+09	2.64E+09	440
EXP 3	4.05E+06	86024	26.25	4.97	93815.65	46908	4.04E+09	5.38E+09	1328
EXP 4	5.88E+06	89714	27.08	4.72	51947.53	25974	2.33E+09	3.11E+09	529
EXP 5	4.31E+06	73698	23.75	5.74	552594.43	276297	2.04E+10	2.72E+10	6296
EXP 6	7.33E+06	104982	23.51	5.82	653600.33	326800	3.43E+10	4.57E+10	6238
EXP 7	9.00E+06	140202	23.88	5.70	503917.78	251959	3.53E+10	4.71E+10	5233
AVG									2964
STD DEV									2803

miR-125b									
	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng Copy/ng)	Total Copy #	Eff (0.3%)	miR/Cell	
EXP 1	3.81E+06	61810	26.33	4.95	88850.10	44425	2.75E+09	3.66E+09	960
EXP 2	6.00E+06	73348	27.26	4.66	45720.97	22860	1.68E+09	2.24E+09	373
EXP 3	4.05E+06	86024	26.40	4.93	84147.37	42074	3.62E+09	4.83E+09	1192
EXP 4	5.88E+06	89714	26.77	4.81	64723.37	32362	2.90E+09	3.87E+09	659
EXP 5	4.31E+06	73698	23.53	5.81	644393.59	322197	2.37E+10	3.17E+10	7342
EXP 6	7.33E+06	104982	23.96	5.68	477246.03	238623	2.51E+10	3.34E+10	4555
EXP 7	9.00E+06	140202	24.38	5.55	352619.72	176310	2.47E+10	3.30E+10	3662
AVG									2677
STD DEV									2607

miR-100									
	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng Copy/ng)	Total Copy #	Eff (0.3%)	miR/Cell	
EXP 1	3.81E+06	61810	26.52	4.89	77464.21	38732	2.39E+09	3.19E+09	837
EXP 2	6.00E+06	73348	27.40	4.62	41398.83	20699	1.52E+09	2.02E+09	337
EXP 3	4.05E+06	86024	26.72	4.83	67377.90	33689	2.90E+09	3.86E+09	954
EXP 4	5.88E+06	89714	27.07	4.72	52441.15	26221	2.35E+09	3.14E+09	534
EXP 5	4.31E+06	73698	24.06	5.65	443516.96	221758	1.63E+10	2.18E+10	5053
EXP 6	7.33E+06	104982	24.31	5.57	372326.50	186163	1.95E+10	2.61E+10	3553
EXP 7	9.00E+06	140202	24.86	5.40	250865.76	125433	1.76E+10	2.34E+10	2605
AVG									1982
STD DEV									1801

MCF10A Cells

miR-21

	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (75%)	miR/Cell
EXP 1	4.66E+06	93798	24.89	5.39	245583.86	122792	1.15E+10	1.54E+10	3299
EXP 2	4.55E+06	82968	24.51	5.51	322319.52	161160	1.34E+10	1.78E+10	3918
EXP 3	4.65E+06	105196	25.01	5.35	226079.07	113040	1.19E+10	1.59E+10	3410
AVG									3542
STD DEV									330

miR-720

	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (75%)	miR/Cell
EXP 1	4.66E+06	93798	22.68	5.81	646290.68	323145	3.03E+10	4.04E+10	8682
EXP 2	4.55E+06	82968	22.53	5.85	709009.54	354505	2.94E+10	3.92E+10	8619
EXP 3	4.65E+06	105196	23.16	5.68	482501.04	241251	2.54E+10	3.38E+10	7277
AVG									8193
STD DEV									794

miR-205

	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (75%)	miR/Cell
EXP 1	4.66E+06	93798	24.24	5.59	391279.98	195640	1.84E+10	2.45E+10	5256
EXP 2	4.55E+06	82968	23.86	5.71	511117.48	255559	2.12E+10	2.83E+10	6213
EXP 3	4.65E+06	105196	24.13	5.62	421036.47	210518	2.21E+10	2.95E+10	6350
AVG									5940
STD DEV									596

miR-1274b

	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (75%)	miR/Cell
EXP 1	4.66E+06	93798	21.97	6.00	1006028.08	503014	4.72E+10	6.29E+10	13514
EXP 2	4.55E+06	82968	22.02	5.99	971436.24	485718	4.03E+10	5.37E+10	11809
EXP 3	4.65E+06	105196	21.69	6.08	1190988.32	595494	6.26E+10	8.35E+10	17962
AVG									14429
STD DEV									3177

let-7a

	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (75%)	miR/Cell
EXP 1	4.66E+06	93798	26.40	4.93	84546.23	42273	3.97E+09	5.29E+09	1136
EXP 2	4.55E+06	82968	25.98	5.05	113350.28	56675	4.70E+09	6.27E+09	1378
EXP 3	4.65E+06	105196	25.55	5.19	154503.69	77252	8.13E+09	1.08E+10	2330
AVG									1615
STD DEV									631

miR-125b

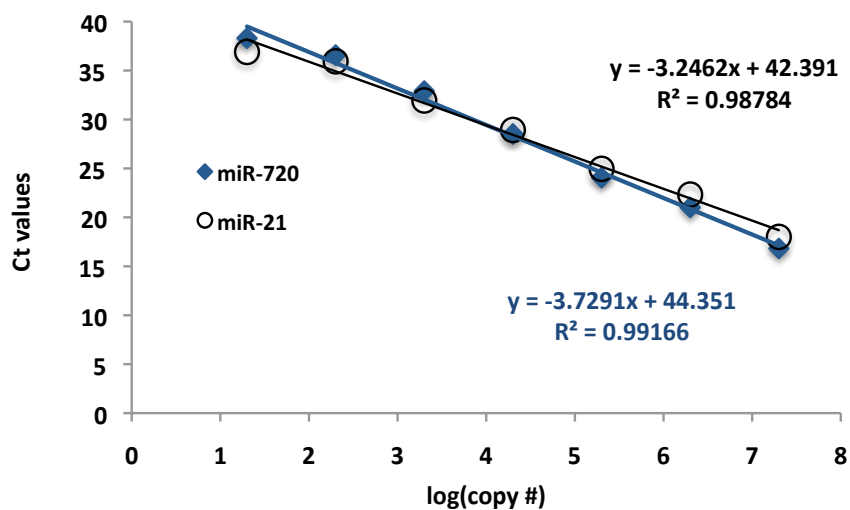
	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (75%)	miR/Cell
EXP 1	4.66E+06	93798	26.88	4.78	60149.09	30075	2.82E+09	3.76E+09	808
EXP 2	4.55E+06	82968	26.43	4.92	82766.13	41383	3.43E+09	4.58E+09	1006
EXP 3	4.65E+06	105196	26.84	4.79	61733.98	30867	3.25E+09	4.33E+09	931
AVG									915
STD DEV									100

miR-100

	Total Cell #	Total RNA (ng)	AVG Ct	log (copy #)	Copy # (2ng PCR)	Copy/ng	Total Copy #	Eff (75%)	miR/Cell
EXP 1	4.66E+06	93798	27.25	4.66	46046.43	23023	2.16E+09	2.88E+09	619
EXP 2	4.55E+06	82968	26.65	4.85	70473.76	35237	2.92E+09	3.90E+09	857
EXP 3	4.65E+06	105196	26.91	4.77	58743.61	29372	3.09E+09	4.12E+09	886
AVG									787
STD DEV									147

Table S4. Raw qRT-PCR data for synthetic *miR-21* and *miR-720* standard curves for converting the measured Ct values to miRNA copy numbers in **Table S3**.

miRNA copy number	Log(copy #)	measured Ct value synthetic <i>miR-21</i>	measured Ct value synthetic <i>miR-720</i>
2×10^1	1.30	36.90	38.30
2×10^2	2.30	35.94	36.58
2×10^3	3.30	31.94	32.90
2×10^4	4.30	28.93	28.52
2×10^5	5.30	24.96	24.05
2×10^6	6.30	22.33	21.01
2×10^7	7.30	18.00	16.82



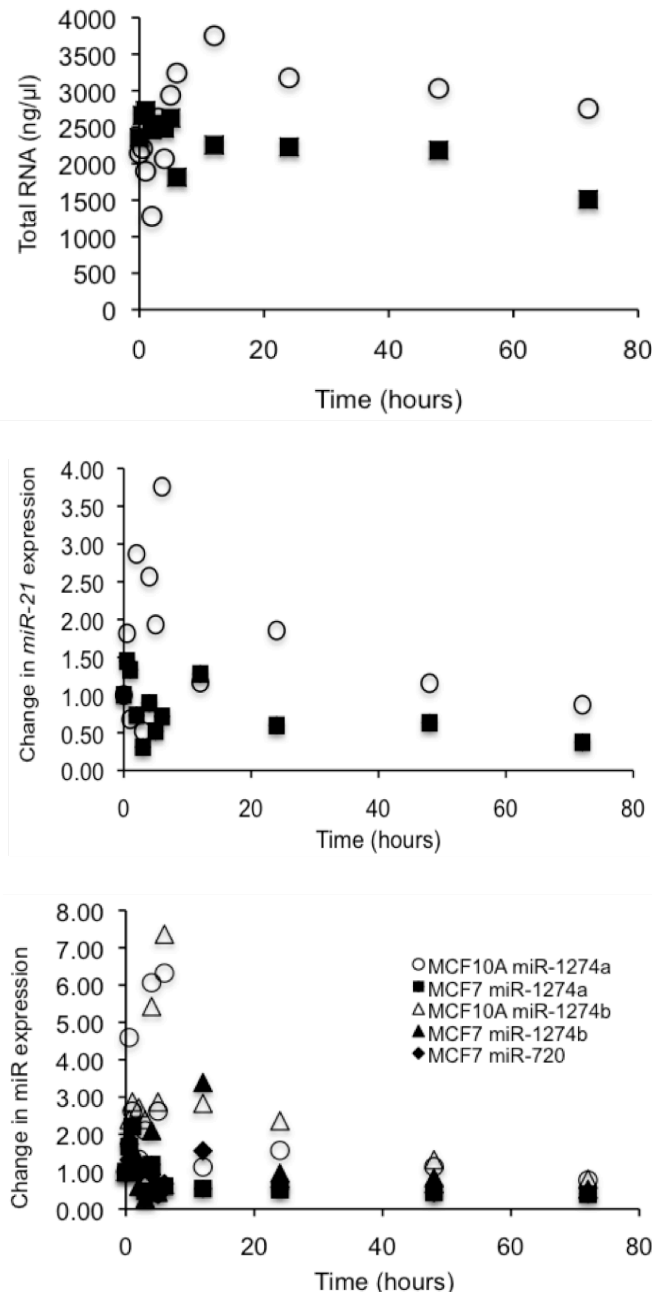


Figure S1. Total cellular RNA concentration (**top panel**) and changes in *miR-21* expression levels (**middle panel**) and changes in *miR-1274a* and *miR-1274b* expression levels (**bottom panel**) for MCF10A cells (open symbols) and MCF7 cells (filled symbols) as a function of time in response to serum deprivation at 0 hrs. Changes in *miR-720* expression levels for MCF7 cells are also shown in the bottom panel. Cells were collected every hour for 6 hrs, and then at 12, 24, 48 and 72 hrs after serum removal from the culture medium. Total RNA concentration was measured using a NanoDrop 2000 spectrophotometer. Changes in cellular miRNA expression levels, $2^{-\Delta Ct}$, were measured by quantitative real-time PCR where ΔCt is the Ct value measured at a certain time after serum deprivation relative to the value measured just prior to removing cells from the complete culture media.

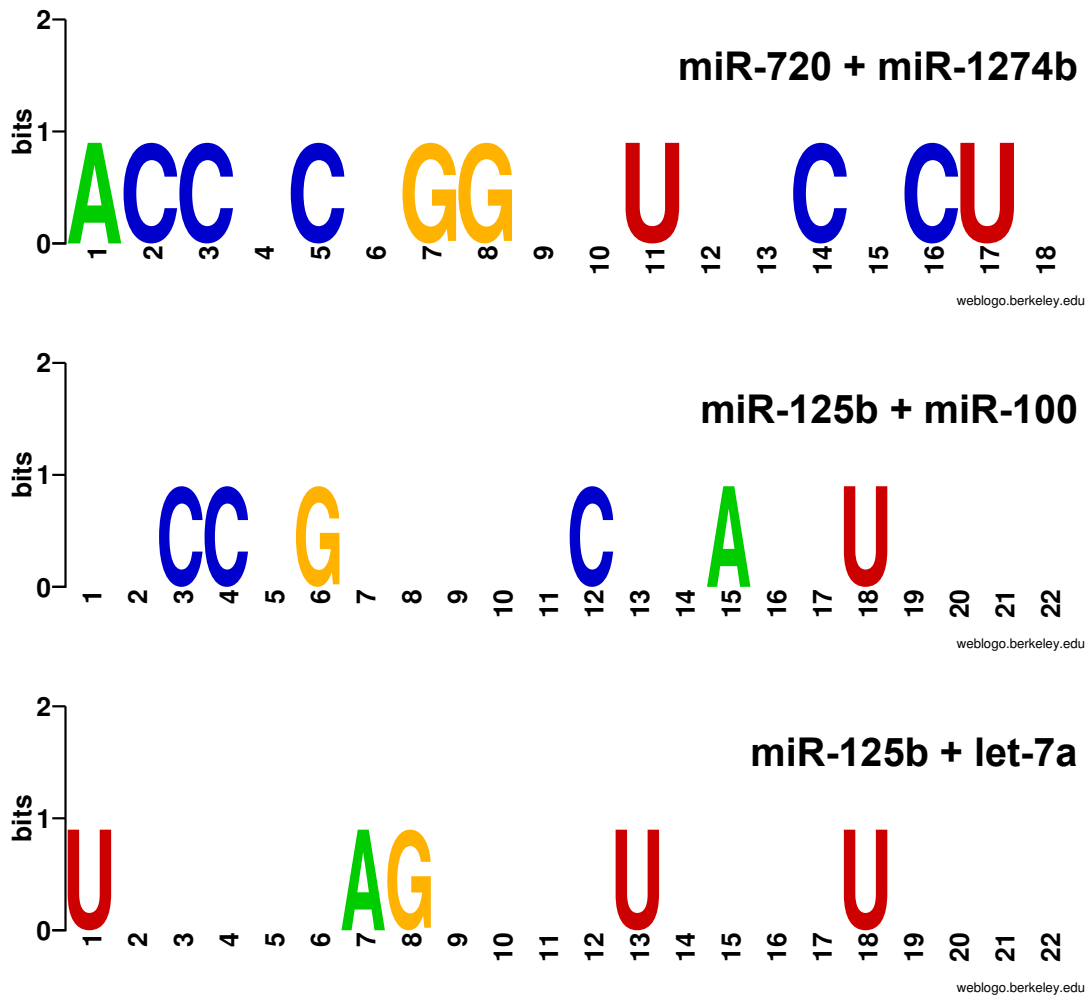


Figure S2. Sequence alignments for miRNA pairs depicting positions that share identical nucleotides. Weblogs created by G. E. Crooks *et al.*, Computational Genomics Research Group, Department of Plant and Microbial Biology, University of California, Berkeley (<http://weblogo.berkeley.edu/>).

Bayesian probability that the population mean of miRNA copy numbers for one cell line/secreted vesicles is greater than that for the other cell line/secreted vesicles.

Following Jaynes [1], we seek the probability that the population mean μ_i of the cellular expression level/vesicular abundance of a miRNA species for $i = \text{MCF7}$ or MCF10A is greater than the population mean of the expression level/abundance of this miRNA for the other cell line/vesicles. That is,

$$p(\mu_1 > \mu_2) = \int_{-\infty}^{\infty} d\mu_2 \int_{\mu_2}^{\infty} d\mu_1 p(\mu_2|x_1, \dots, x_{n_2}) p(\mu_1|x'_1, \dots, x'_{n_1}) , \quad (1)$$

where $p(\mu_i|x_1, \dots, x_{n_i})$ is the posterior probability of μ_i based on a sample of n_i measurements of x_i , the miRNA cellular expression level or vesicular abundance. When the population variance is known, the posterior probability follows a Student's t -distribution [2],

$$p(\mu|x_1, \dots, x_n) = \frac{\Gamma(\frac{n}{2})}{\Gamma(\frac{n-1}{2})} \frac{1}{\sqrt{r^2\pi}} \left[1 + \frac{(\mu - \bar{x})^2}{r^2} \right]^{-\frac{n}{2}} , \quad (2)$$

where $nr^2 = \sum (x_i - \bar{x})^2$ with \bar{x} the sample mean, and μ is the population mean. The double integral in eq. 1 is readily evaluated using quadrature methods within the SciPy module for Python.

References

- [1] E. T. Jaynes, *Probability Theory – The Logic of Science*. Cambridge University Press, New York (2003).
- [2] P. Gregory, *Bayesian Logical Data Analysis for the Physical Sciences*. Cambridge University Press, New York (2005).

Mutual information for characterizing pairwise correlations in miRNA cellular expression/vesicle abundance.

Let $X_i = \{x_{i,1}, x_{i,2}, \dots, x_{i,n}\}$ be the n measured expression levels/vesicle abundances (copy numbers/cell) of $i = \{1, \dots, m\}$ miRNAs; here n represents the n different biological samples. Assuming all the X_i are Gaussian distributed, the correlation coefficient between the expression of miR- i and miR- j is given by

$$\rho_{i,j} = \frac{\langle (X_i - \mu_i)(X_j - \mu_j) \rangle}{\sigma_i \sigma_j}, \quad (1)$$

where μ_i is the mean and σ_i is the standard deviation of the distribution, and $\langle \dots \rangle$ denotes the expectation value over the joint distribution, $P(X_i, X_j)$, which we implicitly assume is a bivariate normal distribution. The mutual information measured in *bits* of $P(X_i, X_j)$ relative to the marginal distributions $P(X_i)$ and $P(X_j)$ is given by

$$\begin{aligned} I(X_i, X_j) &= \left\langle \log_2 \frac{P(X_i, X_j)}{P(X_i)P(X_j)} \right\rangle \\ &= -0.72 \ln(1 - \rho_{i,j}^2). \end{aligned} \quad (2)$$

If X_i and X_j are uncorrelated, then $I(X_i, X_j) = 0$; that is, knowing the expression/abundance of miR- i does not teach us anything about the expression/abundance of miR- j . In other words, knowing the expression/abundance of miR- i contributes nothing to reducing our uncertainty about the expression/abundance of miR- j . Conversely, if X_i and X_j are highly correlated or highly anti-correlated, $I(X_i, X_j) \rightarrow \infty$. In this limit, knowing the expression/abundance of miR- i contributes maximally to reducing our uncertainty about the expression/abundance of miR- j . High mutual information between miRNA cellular expression levels/vesicle abundance is suggestive of functional/physical miRNA interactions.