

Additional File 2

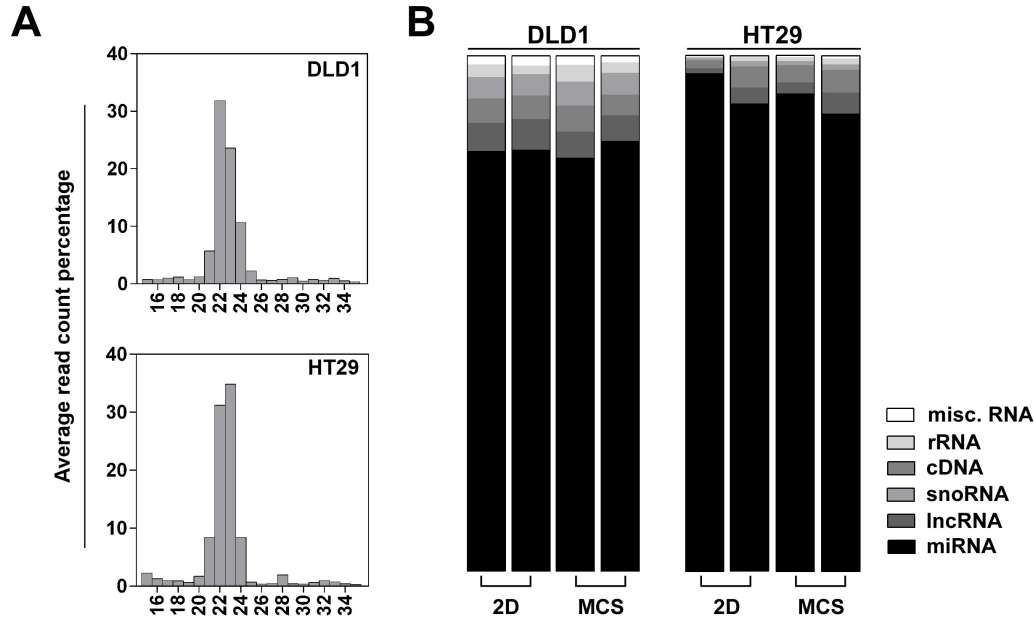


Figure S1. miR-Seq read data quality control. (A) Proportional distribution of read lengths in small RNA libraries, the total amount of sequenced reads was set to 100%. (B) Distribution of mapped reads, considering the total number reads mapped to genome as 100%.

Table S1. High-throughput miRNA sequencing read count summary.

	Total read count	Total reads after QC	Reads mapped to human genome	Reads mapped to human miRNA
DLD1_2D1	23 498 569	21 593039 (92%)	21 252 619 (98%)	15 814 475 (74%)
DLD1_2D2	36 069 614	34 124874 (95%)	33 579 814 (98%)	25 477 315 (76%)
DLD1_SF1	29 624 600	26 570728 (90%)	26 171 877 (98%)	18 069 336 (69%)
DLD1_SF2	29 228 303	28 150644 (96%)	27 745 559 (99%)	20 463 716 (74%)
HT29_2D1	11 544 757	11 359998 (98%)	11 185 452 (98%)	10 495 634 (94%)
HT29_2D2	33 207 481	32 377468 (98%)	30 934 141 (96%)	26 211 524 (85%)
HT29_SF1	12 732 347	12 353231 (97%)	12 026 782 (97%)	10 745 232 (89%)
HT29_SF2	23 718 678	22 652552 (96%)	21 584 426 (95%)	17 182 624 (80%)

Note: sample name abbreviations correspond to cell line used for experiment (DLD1 or HT29) and cell culture of either monolayer (2D) or multicellular spheroids (SF). Last numeral in sample name denominates the number of biological replicate.

Table S2. Top 10 up-regulated and down-regulated miRNAs in DLD1 and HT29 cells cultivated in 3D multicellular spheroids compared to miRNA levels in cells grown in 2D.

DLD1			HT29		
miRNA	Fold Change	p value	miRNA	Fold Change	p value
miR-1246	10.82	8.92e-14	miR-210-3p*	6.27	1.40e-05
miR-9-3p	8.55	2.02e-08	miR-215-5p	4.73	1.95e-04
miR-9-5p	8.11	2.01e-13	miR-375*	3.53	2.37e-04
miR-95-3p	7.80	7.26e-15	miR-142-5p*	3.53	3.96e-10
miR-10b-5p	5.23	1.85e-26	miR-194-5p*	2.85	3.59e-02
miR-192-5p	5.07	4.14e-26	miR-378c*	2.11	1.65e-05
miR-142-5p*	4.54	2.87e-18	miR-455-3p	2.10	2.46e-02

miR-194-5p*	4.37	6.06e-25	miR-29b-3p*	2.02	1.39e-03
miR-210-3p*	4.32	1.06e-27	miR-27a-3p	1.87	1.53e-19
miR-146a-5p	4.32	1.79e-14	miR-141-3p*	1.87	7.82e-07
miR-371b-3p	-7.43	2.74e-18	miR-934	-3.24	1.33e-06
miR-30a-5p	-5.67	5.11e-25	miR-205-5p	-2.47	2.56e-02
miR-5100	-4.22	1.50e-34	miR-3677-3p	-2.12	9.75e-09
miR-372-3p	-3.93	1.89e-10	miR-452-5p	-2.07	5.48e-07
miR-3187-3p	-3.40	8.91e-16	miR-7974	-2.02	2.82e-03
miR-139-5p	-3.34	2.89e-17	miR-301a-5p*	-1.80	1.75e-05
miR-7-5p	-3.11	2.22e-06	miR-874-3p	-1.75	2.77e-02
miR-760	-2.93	5.05e-14	miR-589-5p*	-1.67	1.47e-10
miR-3529-3p	-2.91	1.76e-06	miR-16-2-3p	-1.49	1.60e-02
miR-500a-5p	-2.90	7.97e-07	miR-31-3p	-1.48	1.57e-02

Note: * indicates that significant expression change was observed in both cell lines cultivated in multicellular spheroids.