

Greystoke et al Supplementary Material

Supplementary Material Table 1

Sequences of mature miRNAs-16 and -21 in Mouse and Human. Downloaded from miRBase Version 21 accessed 20/7/15
(www.mirbase.org)

	miR-16	miR-21
Mouse	UAGCAGCACGUAAAUAUUGGCG	UAGCUUAUCAGACUGAUGUUGA
Human	UAGCAGCACGUAAAUAUUGGCG	UAGCUUAUCAGACUGAUGUUG

Supplementary Material Table 2

Expression of 65 miRNAs significantly elevated in pooled plasma from 10 SCID-Beige mice bearing s.c. H526 xenografts was compared to plasma pooled from 5 non-tumour bearing animals and analysed in triplicated using Taqman Low Density Arrays Human Pools A and B. Expression in non-tumour bearing NSG mice (n=3 individual mice) also shown. (Levels given as 40-Ct)

	Non Tumour Bearing (SCID)	Non Tumour Bearing (NSG)	H526 Xenografts (SCID)
hsa-miR-9#	2.1±2	2.3±2.2	18.1±0.2
hsa-miR-22#	0.1±0	1±0.9	8.3±0.7
hsa-miR-95	0.1±0	0.1±0	15.1±0.3
hsa-miR-182	1.4±1.3	0.1±0	10.8±0.3
hsa-miR-183	6±1.2	1.8±1.7	13.8±0.2
hsa-miR-183#	6.9±1.2	1.1±1	16.8±0.3
hsa-miR-193b	12±0.5	10.3±1.3	17.7±0.2
hsa-miR-197	8.8±1	0.1±0	18.3±0.3
hsa-miR-345	2.1±2	0.1±0	18.1±0.2
hsa-miR-429	1.2±1.1	0.1±0	17±0.1
hsa-miR-660	3.3±3.2	0.1±0	16±0.5
hsa-miR-1224-3P	0.1±0	0.6±0.5	12±1
hsa-miR-18a#	0.1±0	1.1±1	15±0.2
hsa-miR-106b#	4.2±2.6	2.8±2	13.7±0.7
hsa-miR-335#	0.1±0	0.1±0	8.5±0.3
hsa-miR-577	0.1±0	0.1±0	13.9±0.2
hsa-miR-590-5p	3±1.6	1.9±1.8	16±0.5
hsa-miR-766	0.1±0	0.1±0	12.5±0.3
hsa-miR-15a#	0.1±0	1.1±1	10.2±0.1

hsa-miR-25#	0.1±0	3±1.8	12±0.6
hsa-miR-28-3p	2.6±2.5	0.1±0	12.1±0.3
hsa-miR-136#	0.1±0	1.9±1.8	6.9±0.6
hsa-miR-502-3p	0.1±0	0.1±0	11.1±0.8
hsa-miR-509	1±0.9	6.4±3.2	10.7±0.5
hsa-miR-598	4.7±4.6	11.1±3	17.7±0.3
hsa-miR-636	2.1±2	2.2±2.1	12.3±0.5
hsa-miR-663B	0.1±0	0.1±0	11.6±0.4
hsa-miR-769-5p	0.1±0	0.1±0	8.4±0.4
hsa-miR-885-5p	0.1±0	0.1±0	16.6±0.2
hsa-miR-1227	0.1±0	5±2.9	14.3±0.4
hsa-miR-1271	0.1±0	0.1±0	13±0.4
hsa-miR-16-1#	0.1±0	0.1±0	9.8±0.5
hsa-miR-135a	0.1±0	0.1±0	7±0.3
hsa-miR-191#	0.1±0	2±1.4	9.2±0.4
hsa-miR-216b	2.8±2.7	0.1±0	11.8±0.1
hsa-miR-301b	7.4±0.4	6.1±3.1	15.5±0.2
hsa-miR-330	0.1±0	2.5±2.4	8.6±1.1
hsa-miR-362-3p	0.1±0	2.7±2.6	11.3±0.6
hsa-miR-625#	4.7±1	9±1.6	15.2±0.1
hsa-miR-628-3p	0.1±0	4.7±4.6	11.6±0.4
hsa-miR-1180	0.1±0	5.5±2.7	13±1.9
hsa-miR-1236	0.1±0	0.8±0.7	10.4±1.1
hsa-miR-1288	0.1±0	3.6±3.5	13±1.8
hsa-miR-33b	6.9±0.8	2.8±2.7	14.1±0.3
hsa-miR-501-3p	0.1±0	0.1±0	9.8±0.4

hsa-miR-941	0.1±0	0.1±0	7.8±0.4
hsa-miR-1226#	0.1±0	2.2±1.1	10.1±1.4
hsa-miR-1249	1.5±1.4	7.8±4.1	12.6±4.8
hsa-miR-1254	0.1±0	0.1±0	9.5±0.7
hsa-miR-1269	0.1±0	1.6±0.8	10.6±0.5
hsa-miR-7-2#	0.1±0	1.1±1	9.8±0.3
hsa-miR-20a#	0.1±0	1.2±1.1	8.8±0.5
hsa-miR-217	0.1±0	3.7±3.6	7.5±0.2
hsa-miR-454#	0.1±0	0.1±0	9.3±0.2
hsa-miR-507	0.1±0	0.1±0	7.2±0.1
hsa-miR-550	0.1±0	0.1±0	5.3±0.5
hsa-miR-576-3p	1.1±1	1.9±1.8	12±1.1
hsa-miR-579	1.5±1.4	0.1±0	11.5±0.6
hsa-miR-1233	1.1±1	2.9±2.8	9.5±0.2
hsa-miR-1278	0.1±0	8.2±4.9	12.2±6.1
hsa-miR-570	0.1±0	2.5±2.4	7.8±0.6
hsa-miR-767-5p	0.1±0	0.1±0	9.5±0.4
hsa-miR-1248	2.2±2.1	7.4±2.1	12.6±2.5
hsa-miR-1252	0.1±0	12±2.5	18.1±2.8
hsa-miR-1292	0.1±0	0.7±0.6	8.2±1.7

Supplementary Material Table 3. Summary of literature reporting deregulated miRNAs in SCLC. Papers identified from PubMed using search criteria of SCLC and miRNA on 1/09/13.

1st Author	Pubmed ID	Model	Number of samples assessed	Number of MiRNAs evaluated	Up-regulated	Down-regulated
Du	20624269	Cell Lines	9 (cf to 7 NSCLC and 3 immortalized cell lines)	136	miR-338 miR-101 miR-98 miR-106b miR-17-5p miR-96 miR-15a miR-92 miR-326 miR-1 miR-15b miR-195 miR-103 miR-135 miR-301 miR-328 miR-93 miR-16 miR-324-5p miR-107 miR-149 miR-181c miR-148b miR-142-3p miR-30c	miR-199a* miR-27a miR-23b miR-222 miR-221 miR-99a miR-24 miR-29c miR-23a miR-205 miR-29b miR-29a miR-22 miR-21 miR-31
Lee	21731696	Human Tumours	31	7 (21,29b,34a, 34c,155, let-7a)		

		Cell Lines	14 (cf to 26 NSCLC)			
Voortman	20615970	Human Tumours	33	altered copy number across spectrum		
		Cell Lines	13			
Miko	19895320	Cell Lines	3	319	miR-200b miR-200a miR-429 miR-10b miR-128a miR-375 miR-128b miR-95 miR-206 miR-25 miR-93 miR-106b miR-182 miR-96 miR-183 miR-335 miR-200c miR-141 miR-17-5p miR-18a miR-19a miR-20a miR-19b-1,2 miR-92-1,2 miR-494 miR-301 miR-181c miR-181d	miR-34a miR-214 miR-199a2 miR-135b miR-29c miR-29b-2,1 miR-302c miR-143 miR-145 miR-146a miR-133b miR-490 miR-29a miR-29b-1,2 miR-32 miR-199b miR-126 miR-511-1,2 miR-125b miR-100 miR-190 miR-497 miR-451 miR-142-3,p miR-142-5,p miR-338 miR-150 miR-512/373 (30)

				miR-130b	different ones)
				miR-374	miR-296
				miR-363	miR-155
				miR-92-2,1	miR-33
				miR-19b-2,1	miR-502
				miR-20b	miR-223
				miR-18b	miR-222
				miR-106a	miR-325
				miR-9-1,2	miR-506
				miR-7-1,2,3	miR-514-1,2,3
					miR-224
Human Tumours	6 Pooled)	62		miR-17-5p	miR-29a
				miR-301	miR-125a
				miR-98	miR-199a
				miR-106a	miR-214
				miR-335	miR-223
				miR-95	miR-126
				miR-19a	miR-150
				miR-374	miR-27a
				miR-96	
				miR-200a	
				miR-182	
				miR-25	
				miR-210	
				miR-183	
				miR-105	
				miR-200c	
Human Tumours	17 (individual)	15		miR-301	miR-126
				miR-183	miR-150
				miR-106a	miR-222

					miR-25 miR-95	miR-223
Hayashita	16266980	Cell lines	19 (mix of SCLC and NSCLC)	21	miR-17-92 cluster miR-19a miR-20 miR-18 miR-17-3p	
Miao	23117485	Cell Lines	3 (sphere forming colonies vs. not)	1212		miR-27-a
Tanaka	22047961	Cell Lines	11		methylation of miR-34b/c	
		Human Tumours	27		methylation of miR-34b/c	
Zhao	22172490	Cell Lines	1		miR-375	
Guo	20371173	Cell Lines	1 (paired drug resistant and parental)	856	miR-224 miR-143 miR-145 miR-100 miR-99a miR-214 miR-29a	miR-375 miR-200b miR-335 miR-217 miR-379 miR-495 miR-134
Ranade	20548249	Human Tumours	34	880	miR-92a-2* miR-147 miR-574-5p	
Roth	22675530	Serum	8 (mixed in with 19 NSCLC; separate results not reported)	4	miR-10b miR-34a miR-141 miR-155	
Salim	22929890	Cell Lines	5 (radio resistant vs	7815	miR-324-5p	

radiosensitive)

miR-1227
miR-625
miR-423-3p
miR-1249

Supplementary Material Table 4. *Individual levels of the 10 circulating miRNAs used in Figure 4.*

Individual levels of the 10 circulating miRNAs of interest in 200 µl plasma across different cancer sub-types; patients prior to therapy with SCLC (n=12), NSCLC (n=15), by treatment state in CRC; patients following resection for stage 3 cancer (n=7) compared to patients with colorectal cancer prior to and following 6 weeks of cytotoxic chemotherapy (n=24), pancreas cancer (n=26) compared to healthy volunteers (n=11).

	Healthy Volunteers	SCLC (Limited Stage)	SCLC (Extensive Stage)	CRC (early stage)	CRC (pre chemotherapy)	CRC (post chemotherapy)	Pancreatic cancer
miR-200a	0.4±0.3	3.45±1.94	9.66±1.33	0.94±0.65	7.43±0.67	4.75±0.65	8.3±0.66
miR-200b	1.81±0.81	2.97±1.87	10.56±1.02	7.59±0.66	11.42±0.44	10.22±0.42	8.31±0.63
miR-200c	3.51±1.22	6.83±2.24	11.11±0.65	10.91±0.48	12.36±0.33	11.58±0.33	9.71±0.59
miR-141	0.1±0	4.59±2.61	8.25±1.18	6±0.67	7.62±0.72	6.23±0.66	8.36±0.64
miR-429	2.21±1.09	2.48±2.38	8.65±1.31	4.46±1.18	5.97±0.71	3.82±0.66	6.24±0.68
miR-95	4.01±1.37	2.4±2.3	9.11±1.14	8.83±0.69	9.11±0.26	9.24±0.24	5.78±0.39
miR-195	8.49±0.69	12.1±1.16	12.53±0.52	11.27±0.56	16.4±0.25	16.11±0.15	16.09±0.44

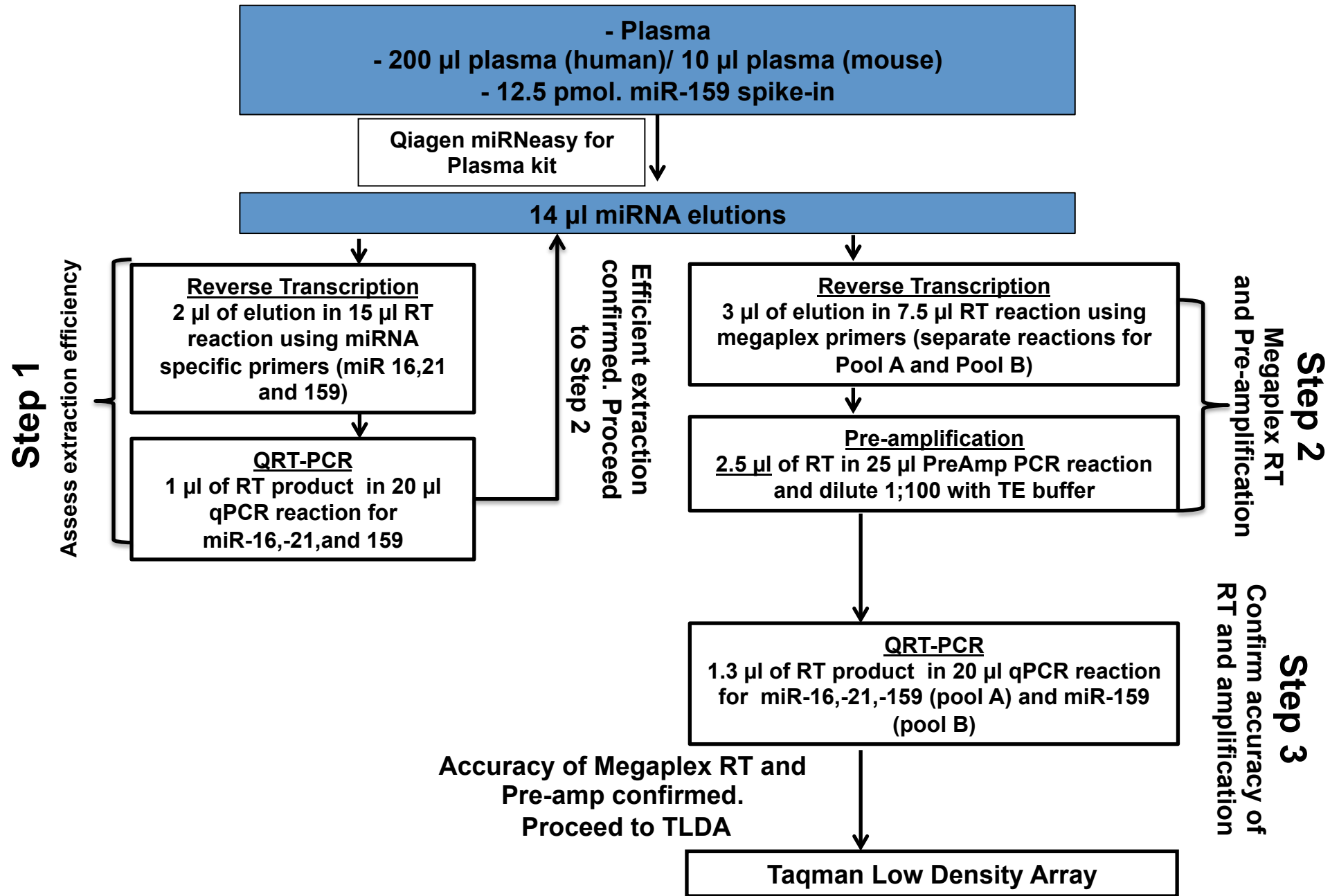
miR-210	3.82±1.12	10.13±1.79	12.41±0.55	11.71±0.22	12.27±0.32	11.36±0.23	11.45±0.49
miR-335	0.65±0.39	1.83±1.73	6.59±0.78	10.76±0.33	8.51±0.54	7.17±0.7	10.17±0.5
miR-375	10.46±1.15	15.54±1.04	17.56±0.53	13.01±0.39	14.38±0.29	13.64±0.19	8.59±0.33

Supplementary Material Figure Titles and Legends

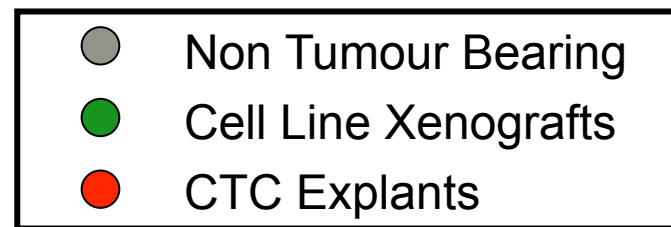
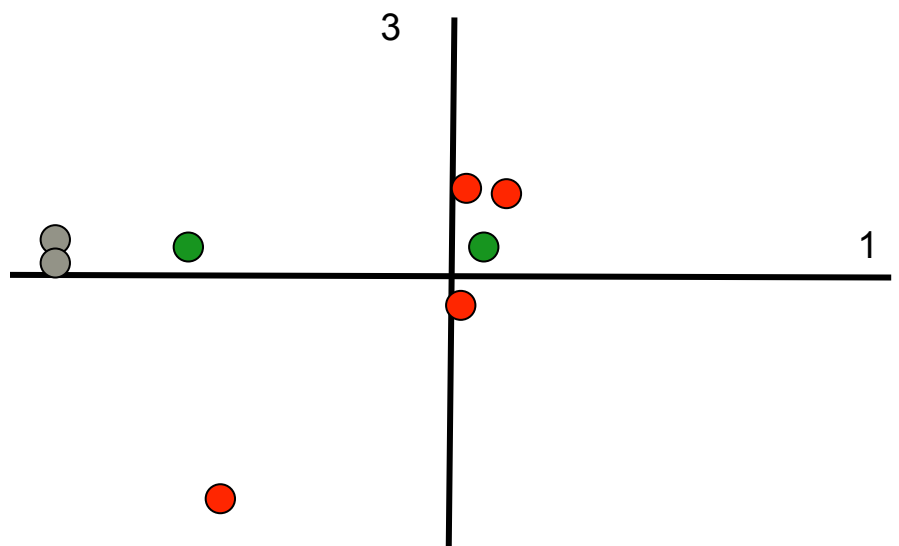
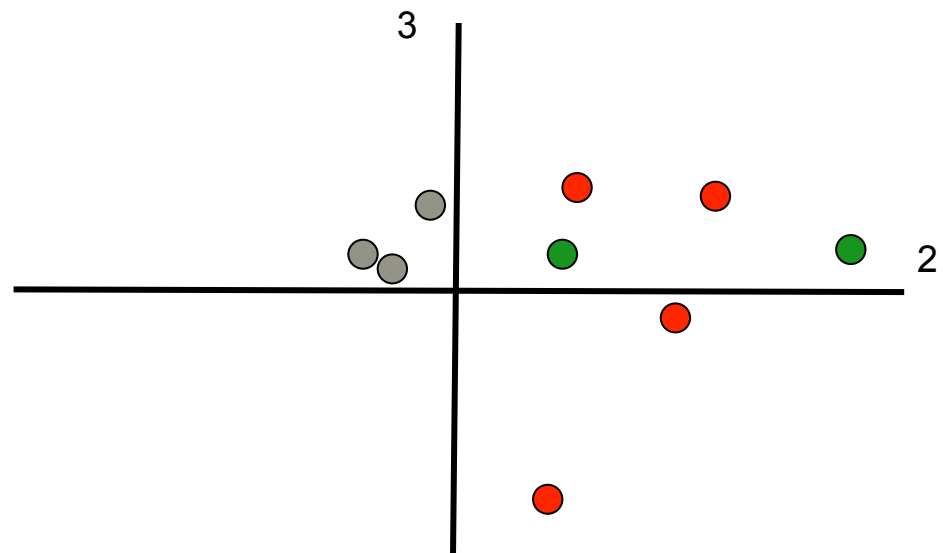
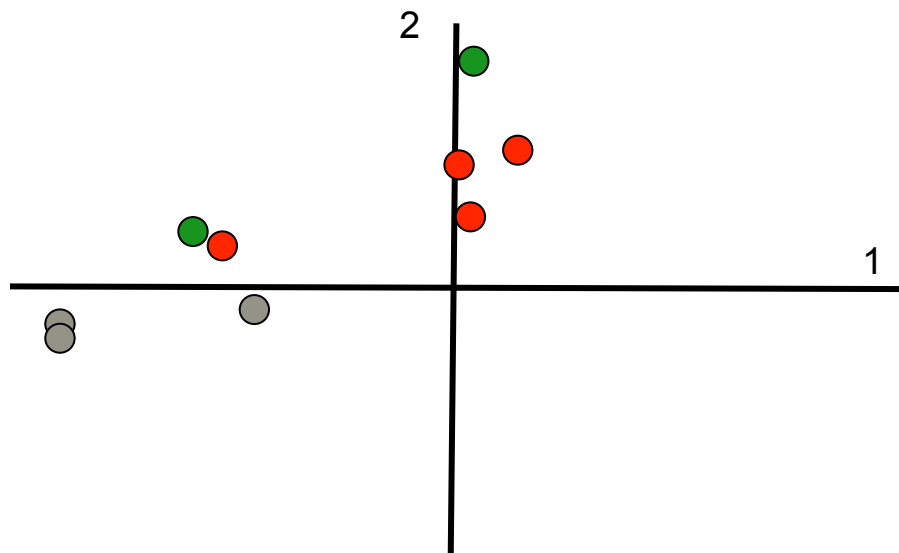
Supplemental Figure 1. *Final workflow for Taqman Low Density Arrays analysis of clinical samples*

Supplemental Figure 2. *Principle component analysis of mouse plasma miRNAs.* The circulating levels of 754 miRNAs in 10 μ l of plasma comparing non tumour bearing NOD scid gamma (NSG) mice (n=3) compared to NSG mice bearing cell line xenografts (n=2) or CTC explants (n=4). miRNA levels assessed using Taqman Low Density Arrays (Human Pool A and B) and normalised to global expression of miRNA.

Supplemental Figure 3. *Rationale for choosing the 10 miRNAs for inclusion in qRT-PCR panel.* miRNAs of interest were identified in clinical and pre-clinical models and previously reported as up-regulated in SCLC tumours or cell-lines.



Supplemental Figure 1



Supplemental
Figure 2

Circulating miRNAs that correlated with Stage in Patients with SCLC (i.e. ED>LD>HNV)

miR-9#
miR-26b#
miR-29c
miR-34b
miR-141
miR-182#
miR-195
miR-200a
miR-200b
miR-200c
miR-210
miR-362-3p
miR-410
miR-431#
miR-453
miR-486-3p
miR-518d
miR-519a
miR-542- 5p
miR-592
miR-625#
miR-874
miR-1244
miR-1274

Circulating miRNAs elevated in CTC Derived Explants vs. Non- Tumour Bearing animal

miR-802
miR-28
miR-1224-3p
miR-96
miR-95
miR-429
miR-182
miR-345
miR-660

miR-183#
miR-335#
miR-197
miR-375
miR-1290

Previously reported as elevated in SCLC tumours or cell lines

More predominant miRNA from opposite arm of precursor reported as elevated in SCLC tumours or cell lines

Underlined miRNAs were chosen for SCLC tumour miRNA 10-plex

Supplemental
Figure 3