

Manuscript Title: Characterisation of mouse epididymosomes reveals a complex profile of microRNAs and a potential mechanism for modification of the sperm epigenome

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SUPPLEMENTARY INFORMATION FILES

Supplementary Figure S1: Assessment of the specificity of epididymosome binding to aldehyde/sulphate latex beads

Supplementary Figure S2: Identification of the presence of consensus exomotifs among epididymosome miRNAs

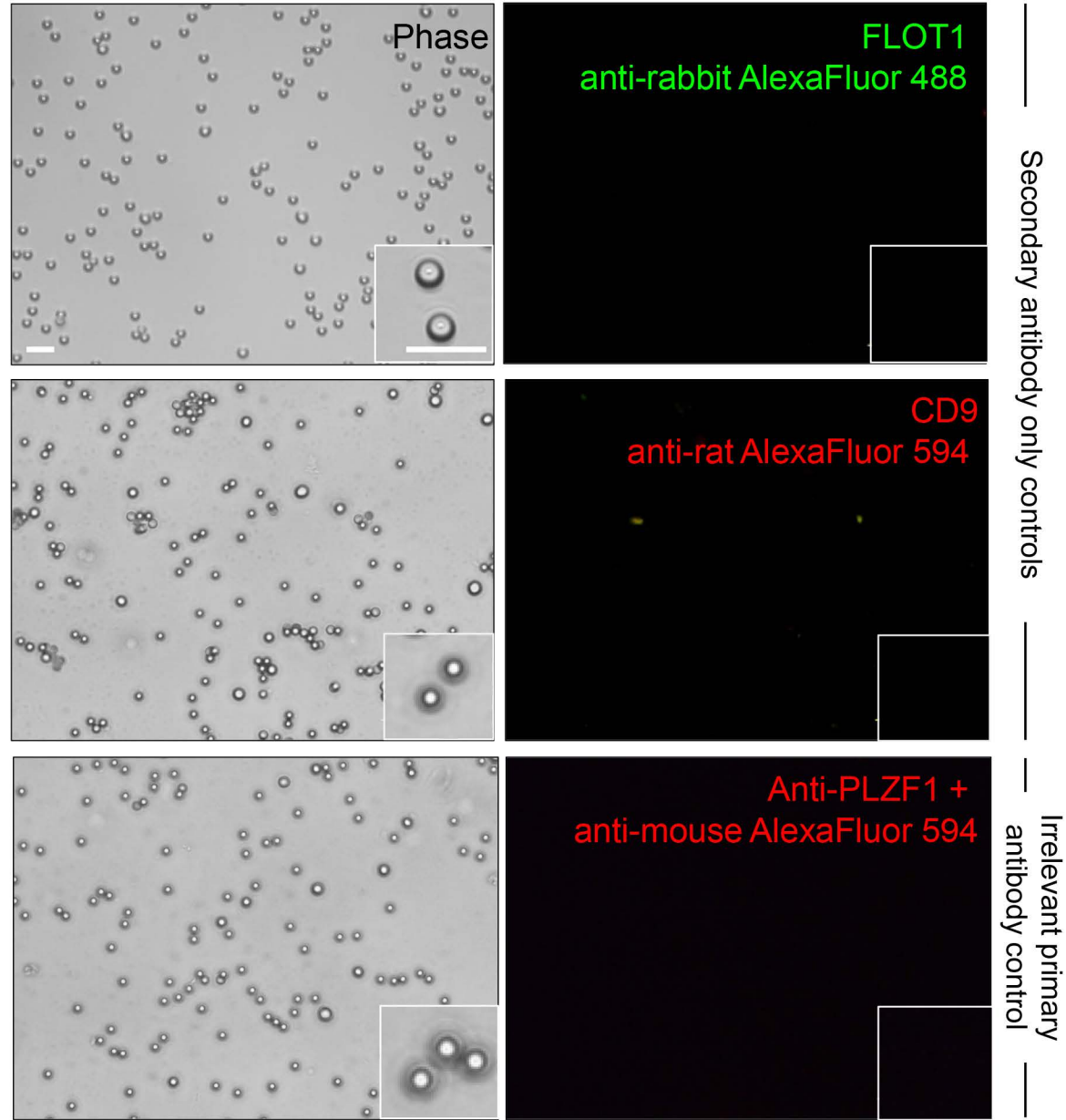
Table S1: Normalised abundance reads of miRNAs identified by next generation sequencing within mouse epididymosomes

Table S2: Fold changes of epididymosome miRNA abundance between epididymal segments

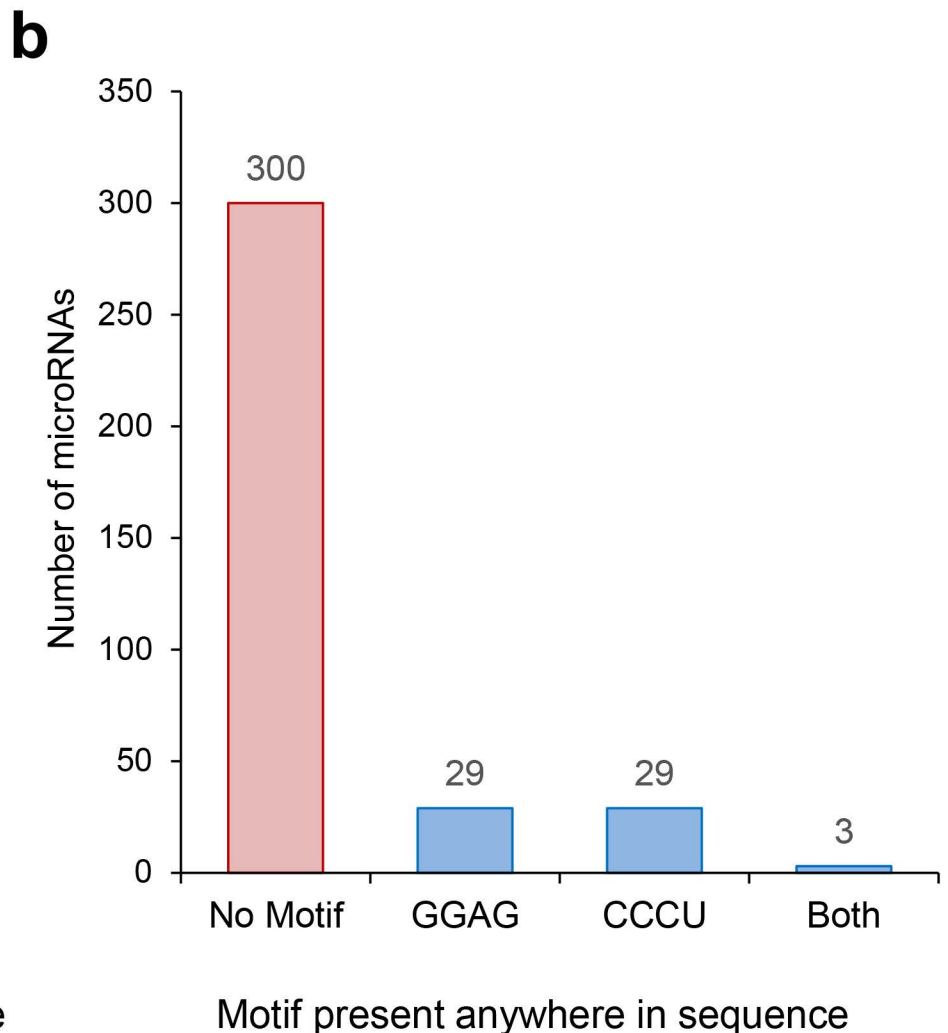
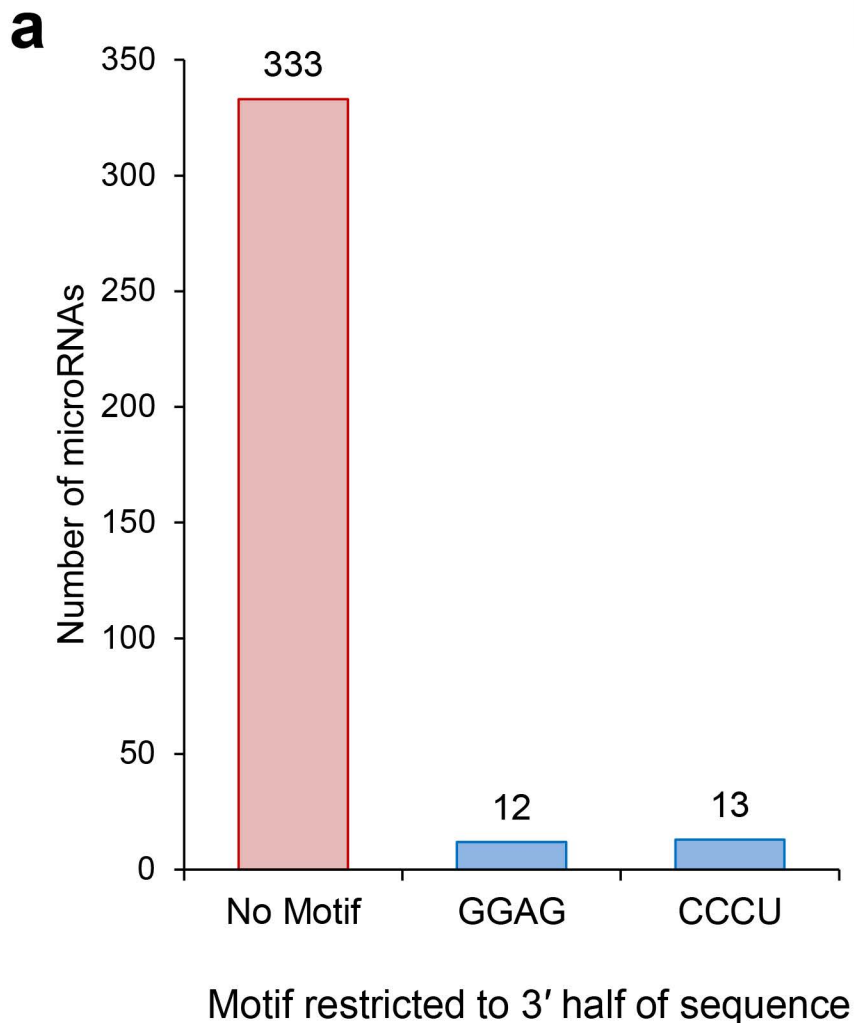
Table S3: Comparison of miRNAs identified in mouse epididymal spermatozoa, epithelial cells and epididymosomes

Table S4: Comparison of mouse epididymal miRNA profile reported by Nixon et al., 2015a,b and Reilly et al., 2016 versus Sharma et al., 2016

Table S5: Comparison of miRNAs identified in the epididymosomes of mice, humans and bulls



Supplementary Figure S1: Assessment of the specificity of epididymosome binding to aldehyde/sulphate latex beads. Epididymosomes were visualised via binding to 4 μm aldehyde/sulphate latex beads and fluorescent labelling of the recognised extracellular vesicle surface markers, CD9 and FLOT1 (see Fig. 1d). The specificity of labelling was established by incubation of epididymosome bound beads with either anti-rabbit AlexaFluor 488 alone (secondary antibody control for FLOT1), anti-rat AlexaFluor 594 alone (secondary antibody control for CD9), or a combination of anti-PLZF1 (irrelevant primary antibody control) and anti-mouse AlexaFluor 594.



Supplementary Figure S2: Identification of the presence of consensus exomotifs among epididymosome miRNAs. The list of 358 unique miRNAs identified in this study were screened for the presence of the consensus exomotifs 'GGAG' and 'CCCU' reported by Villarroya-Beltri et al.³⁷ as playing a key role in the sorting of miRNAs into exosomes. (a) This analysis revealed that only 7% (25/358) of the miRNAs possessed either exomotif in the 3' half of their sequence. (b) Upon relaxation of the criterion for the exomotif to reside in the 3' half of the miRNA sequence, the number of miRNAs possessing either motif extended to 17% (61/358).

Table S1 : Normalised abundance reads of miRNAs identified by next generation sequencing within mouse epididymosomes

Table with 13 columns: miRNA Family (mmu), Mature miRNA, Caput (Replicate 1, 2, 3), Corpus (Replicate 1, 2, 3), Cauda (Replicate 1, 2, 3), and Average Normalised Reads (Caput, Corpus, Cauda). Rows list miRNAs from let-7 to miR-148.

Total miRNAs identified in mouse epididymosomes = 358

	<i>miR-3473e</i>	35.84598	198.1117	150.2287	2.066152859	-0.440317669	1.496756692	0.028180986	0.578160026	0.037893554
	<i>miR-3473f</i>	4.635255	33.71874	22.71387	2.360265643	-0.221326702	1.947355624	0.146734161	0.895155059	0.124901375
<i>miR-3535</i>	<i>miR-3535</i>	28.98144	349.291	66.84868	2.740884499	-2.231954215	0.374767821	0.009651841	0.013093356	0.597324188
<i>miR-5099</i>	<i>miR-5099</i>	143.0932	98.4456	161.5296	-0.826398245	0.395656329	-0.552132718	0.100224923	0.542800189	0.256825235
<i>miR-5100</i>	<i>miR-5100</i>	11.93236	8.339649	40.67933	0.075924667	1.521306572	1.475885424	0.973345036	0.32041671	0.199262978
<i>miR-5119</i>	<i>miR-5119</i>	1.957666	1.360474	26.07558	-0.091209467	2.758751252	2.557904693	0.973345036	0.232503943	0.120856706
<i>miR-5121</i>	<i>miR-5121</i>	37.10512	192.5418	444.534	2.007342541	0.880560582	2.775082265	0.003635076	0.156392554	0.000256703
<i>miR-5126</i>	<i>miR-5126</i>	12.46791	10.74857	6.082526	-0.261478224	-0.654504419	-1.055419228	0.900566621	0.637978879	0.317586175
<i>miR-5128</i>	<i>miR-5128</i>	4.607789	18.58744	13.75491	1.657146721	-0.474383907	1.052286568	0.059245804	0.55389649	0.118316853
<i>miR-5621</i>	<i>miR-5621-5p</i>	3.393188	26.97842	8.131531	1.776804752	-1.23645036	0.341302224	0.397269715	0.564379148	0.85478239
<i>miR-6238</i>	<i>miR-6238</i>	6.439843	68.59899	108.9833	2.872209478	-0.085780454	2.655930352	0.002875447	0.940022491	0.013255487
<i>miR-6240</i>	<i>miR-6240</i>	4120.893	3236.247	1528.887	-0.352840672	-1.209176477	-1.637448131	0.877236949	0.449030635	0.176684376
<i>miR-6412</i>	<i>miR-6412</i>	0.770444	4.151088	14.67231	2.531250418	1.112858085	3.439711994	0.061030138	0.452200482	0.022574041
<i>miR-6538</i>	<i>miR-6538</i>	26.17419	29.68917	11.64147	0.231428507	-1.071497142	-0.961845849	0.925309054	0.524496478	0.467775709
<i>miR-6937</i>	<i>miR-6937-5p</i>	17.28693	11.72446	28.12969	-1.112950805	1.582142177	0.207510628	0.516540828	0.399004793	0.875877556
<i>miR-7033</i>	<i>miR-7033-5p</i>	24.64218	389.7688	61.82609	3.446815876	-2.696377083	0.613102857	0.008185607	0.024798604	0.530653892
<i>miR-7087</i>	<i>miR-7087-3p</i>	0.040285	0.261091	12.3902	0	4.554384766	5.600440643	0	0.084301339	0.022574041

<i>mir-434</i>	<i>mir-434-5p</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-450</i>	<i>mir-450b-5p</i>	-	-	*	-	-	-	-	-	-	-	+	-	-
<i>mir-451</i>	<i>mir-451a</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-455</i>	<i>mir-455-3p</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-466</i>	<i>mir-466i-5p</i>	-	-	*	-	-	-	-	-	-	-	-	+	*
<i>mir-467</i>	<i>mir-467a-3p</i>	-	-	*	-	-	-	-	-	-	-	+	-	-
<i>mir-486</i>	<i>mir-486a-3p</i>	-	-	*	-	-	-	-	-	-	-	+	-	*
	<i>mir-486a-5p</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
	<i>mir-486b-5p</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-496</i>	<i>mir-496a-3p</i>	-	-	*	-	-	-	-	-	-	-	-	-	*
<i>mir-497</i>	<i>mir-497a-5p</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-542</i>	<i>mir-542-3p</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-664</i>	<i>mir-664-3p</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-677</i>	<i>mir-677-3p</i>	-	-	*	-	-	-	-	-	-	-	-	+	*
	<i>mir-677-5p</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-690</i>	<i>mir-690</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-708</i>	<i>mir-708-3p</i>	-	-	*	-	-	-	-	-	-	-	-	+	*
	<i>mir-708-5p</i>	-	-	*	-	-	-	-	-	-	-	-	+	*
<i>mir-709</i>	<i>mir-709</i>	-	-	*	-	-	-	-	-	-	-	-	+	*
<i>mir-714</i>	<i>mir-714</i>	-	-	*	-	-	-	-	-	-	-	-	+	*
<i>mir-879</i>	<i>mir-879-5p</i>	-	-	*	-	-	-	-	-	-	-	+	-	-
<i>mir-1195</i>	<i>mir-1195</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-1247</i>	<i>mir-1247-5p</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-1291</i>	<i>mir-1291</i>	-	-	*	-	-	-	-	-	-	-	-	-	*
<i>mir-1839</i>	<i>mir-1839-3p</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-1843</i>	<i>mir-1843b-5p</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
	<i>mir-1843b-3p</i>	-	-	*	-	-	-	-	-	-	-	+	-	-
<i>mir-1957</i>	<i>mir-1957a</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-1981</i>	<i>mir-1981-3p</i>	-	-	*	-	-	-	-	-	-	-	-	+	*
	<i>mir-1981-5p</i>	-	-	*	-	-	-	-	-	-	-	-	+	*
<i>mir-3470</i>	<i>mir-3470a</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
	<i>mir-3470b</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-3473</i>	<i>mir-3473a</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
	<i>mir-3473c</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
	<i>mir-3473f</i>	-	-	*	-	-	-	-	-	-	-	-	+	*
<i>mir-3535</i>	<i>mir-3535</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-5099</i>	<i>mir-5099</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-5100</i>	<i>mir-5100</i>	-	-	*	-	-	-	-	-	-	-	+	-	*
<i>mir-5119</i>	<i>mir-5119</i>	-	-	*	-	-	-	-	-	-	-	-	-	*
<i>mir-5121</i>	<i>mir-5121</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-5126</i>	<i>mir-5126</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-5128</i>	<i>mir-5128</i>	-	-	*	-	-	-	-	-	-	-	-	+	*
<i>mir-5621</i>	<i>mir-5621-5p</i>	-	-	*	-	-	-	-	-	-	-	-	+	*
<i>mir-6238</i>	<i>mir-6238</i>	-	-	*	-	-	-	-	-	-	-	-	+	*
<i>mir-6240</i>	<i>mir-6240</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-6412</i>	<i>mir-6412</i>	-	-	*	-	-	-	-	-	-	-	-	-	*
<i>mir-6538</i>	<i>mir-6538</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-6937</i>	<i>mir-6937-5p</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-7033</i>	<i>mir-7033-5p</i>	-	-	*	-	-	-	-	-	-	-	+	+	*
<i>mir-7087</i>	<i>mir-7087-3p</i>	-	-	*	-	-	-	-	-	-	-	-	-	*

¹ Nixon, B., Stanger, S. J., Mihalas, B. P., Reilly, J. N., Anderson, A. L., Dun, M. D., Tyagi, S., Holt, J. E., and McLaughlin, E. A. (2015) Next Generation Sequencing Analysis Reveals Segmental Patterns of microRNA Expression in Mouse Epididymal Epithelial Cells. PLoS ONE 10, e0135605

² Nixon, B., Stanger, S. J., Mihalas, B. P., Reilly, J. N., Anderson, A. L., Tyagi, S., Holt, J. E., and McLaughlin, E. A. (2015) The MicroRNA Signature of Mouse Spermatozoa Is Substantially Modified During Epididymal Maturation. Biol Reprod 93, 91

	miR-3473b	*	-	-	*	*	-	-
	miR-3473e	*	-	-	*	*	-	-
	miR-3473f	*	-	-	*	*	-	-
miR-3535	miR-3535	*	-	-	*	*	-	-
miR-5099	miR-5099	*	-	-	*	*	-	-
miR-5100	miR-5100	*	-	-	*	*	-	-
miR-5119	miR-5119	*	-	-	*	*	-	-
miR-5121	miR-5121	*	-	-	*	*	-	-
miR-5126	miR-5126	*	-	-	*	*	-	-
miR-5128	miR-5128	*	-	-	*	*	-	-
miR-6238	miR-6238	*	-	-	*	*	-	-
miR-6240	miR-6240	*	-	-	*	*	-	-
miR-6412	miR-6412	*	-	-	*	*	-	-
miR-6538	miR-6538	*	-	-	*	*	-	-
miR-6937	miR-6937	*	-	-	*	*	-	-
miR-7033	miR-7033	*	-	-	*	*	-	-
miR-7087	miR-7087	*	-	-	*	*	-	-
miRNA Family	Mature miRNA	Total Epididymosomes			Mouse		Bovine	
		Mouse	Bovine ¹	Human ²	Caput	Cauda	Caput	Cauda
miRNAs represented only in bovine epididymosome datasets								
miR-92	miR-92	-	+	-	-	-	+	+
miR-145	miR-145	-	+	-	-	-	+	+
miR-106	miR-106	-	+	-	-	-	+	+
miRNA Family	Mature miRNA	Total Epididymosomes			Mouse		Bovine	
		Mouse	Bovine ¹	Human ²	Caput	Cauda	Caput	Cauda
miRNAs represented only in human epididymosome datasets								
miR-18	miR-18b	-	-	+	-	-	-	-
miR-28	miR-28	-	-	+	-	-	-	-
miR-106	miR-106a	-	-	+	-	-	-	-
miR-197	miR-197	-	-	+	-	-	-	-
miR-198	miR-198	-	-	+	-	-	-	-
miR-202	miR-202	-	-	+	-	-	-	-
miR-297	miR-297	-	-	+	-	-	-	-
miR-320	miR-320a	-	-	+	-	-	-	-
	miR-320b	-	-	+	-	-	-	-
	miR-320c	-	-	+	-	-	-	-
	miR-320d	-	-	+	-	-	-	-
	miR-320e	-	-	+	-	-	-	-
miR-346	miR-346	-	-	+	-	-	-	-
miR-363	miR-363	-	-	+	-	-	-	-
miR-378	miR-378	-	-	+	-	-	-	-
miR-382	miR-382	-	-	+	-	-	-	-
miR-432	miR-432	-	-	+	-	-	-	-
miR-449	miR-449b	-	-	+	-	-	-	-
miR-483	miR-483	-	-	+	-	-	-	-
miR-487	miR-487b	-	-	+	-	-	-	-
miR-491	miR-491	-	-	+	-	-	-	-
miR-494	miR-494	-	-	+	-	-	-	-
miR-502	miR-502	-	-	+	-	-	-	-
miR-505	miR-505	-	-	+	-	-	-	-
miR-508	miR-508	-	-	+	-	-	-	-
miR-509	miR-509	-	-	+	-	-	-	-
miR-510	miR-510	-	-	+	-	-	-	-
miR-513	miR-513a	-	-	+	-	-	-	-
miR-514	miR-514b	-	-	+	-	-	-	-
miR-550	miR-550	-	-	+	-	-	-	-
miR-551	miR-551b	-	-	+	-	-	-	-
miR-572	miR-572	-	-	+	-	-	-	-
miR-575	miR-575	-	-	+	-	-	-	-
miR-595	miR-595	-	-	+	-	-	-	-
miR-602	miR-602	-	-	+	-	-	-	-
miR-625	miR-625	-	-	+	-	-	-	-
miR-628	miR-628	-	-	+	-	-	-	-
miR-629	miR-629	-	-	+	-	-	-	-
miR-638	miR-638	-	-	+	-	-	-	-
miR-639	miR-639	-	-	+	-	-	-	-
miR-658	miR-658	-	-	+	-	-	-	-
miR-659	miR-659	-	-	+	-	-	-	-
miR-663	miR-663	-	-	+	-	-	-	-
miR-665	miR-665	-	-	+	-	-	-	-
miR-675	miR-675	-	-	+	-	-	-	-
miR-720	miR-720	-	-	+	-	-	-	-
miR-762	miR-762	-	-	+	-	-	-	-
miR-769	miR-769	-	-	+	-	-	-	-
miR-877	miR-877	-	-	+	-	-	-	-
miR-885	miR-885	-	-	+	-	-	-	-
miR-886	miR-886	-	-	+	-	-	-	-
miR-888	miR-888	-	-	+	-	-	-	-
miR-890	miR-890	-	-	+	-	-	-	-
miR-891	miR-891a	-	-	+	-	-	-	-
miR-891	miR-891b	-	-	+	-	-	-	-
miR-892	miR-892a	-	-	+	-	-	-	-
	miR-892b	-	-	+	-	-	-	-
miR-933	miR-933	-	-	+	-	-	-	-
miR-939	miR-939	-	-	+	-	-	-	-
miR-940	miR-940	-	-	+	-	-	-	-
miR-1180	miR-1180	-	-	+	-	-	-	-
miR-1202	miR-1202	-	-	+	-	-	-	-
miR-1207	miR-1207	-	-	+	-	-	-	-
miR-1224	miR-1224	-	-	+	-	-	-	-
miR-1225	miR-1225	-	-	+	-	-	-	-
miR-1226	miR-1226	-	-	+	-	-	-	-
miR-1228	miR-1228	-	-	+	-	-	-	-
miR-1231	miR-1231	-	-	+	-	-	-	-
miR-1246	miR-1246	-	-	+	-	-	-	-
miR-1254	miR-1254	-	-	+	-	-	-	-
miR-1260	miR-1260	-	-	+	-	-	-	-
	miR-1260b	-	-	+	-	-	-	-
miR-1268	miR-1268	-	-	+	-	-	-	-
miR-1274	miR-1274b	-	-	+	-	-	-	-
miR-1275	miR-1275	-	-	+	-	-	-	-
miR-1280	miR-1280	-	-	+	-	-	-	-
miR-1281	miR-1281	-	-	+	-	-	-	-
miR-1292	miR-1292	-	-	+	-	-	-	-
miR-1299	miR-1299	-	-	+	-	-	-	-
miR-1301	miR-1301	-	-	+	-	-	-	-
miR-1307	miR-1307	-	-	+	-	-	-	-
miR-1308	miR-1308	-	-	+	-	-	-	-
miR-1469	miR-1469	-	-	+	-	-	-	-
miR-1825	miR-1825	-	-	+	-	-	-	-
miR-1826	miR-1826	-	-	+	-	-	-	-
miR-1908	miR-1908	-	-	+	-	-	-	-
miR-1909	miR-1909	-	-	+	-	-	-	-
miR-1910	miR-1910	-	-	+	-	-	-	-
miR-1914	miR-1914	-	-	+	-	-	-	-
miR-1915	miR-1915	-	-	+	-	-	-	-
miR-1975	miR-1975	-	-	+	-	-	-	-
miR-1979	miR-1979	-	-	+	-	-	-	-
miR-2110	miR-2110	-	-	+	-	-	-	-
miR-2277	miR-2277	-	-	+	-	-	-	-
miR-2861	miR-2861	-	-	+	-	-	-	-
miR-3065	miR-3065	-	-	+	-	-	-	-
miR-3124	miR-3124	-	-	+	-	-	-	-
miR-3128	miR-3128	-	-	+	-	-	-	-
miR-3131	miR-3131	-	-	+	-	-	-	-
miR-3141	miR-3141	-	-	+	-	-	-	-
miR-3147	miR-3147	-	-	+	-	-	-	-
miR-3148	miR-3148	-	-	+	-	-	-	-
miR-3149	miR-3149	-	-	+	-	-	-	-
miR-3162	miR-3162	-	-	+	-	-	-	-
miR-3175	miR-3175	-	-	+	-	-	-	-
miR-3178	miR-3178	-	-	+	-	-	-	-
miR-3180	miR-3180	-	-	+	-	-	-	-
miR-3185	miR-3185	-	-	+	-	-	-	-
miR-3187	miR-3187	-	-	+	-	-	-	-

miR-3188	miR-3188	-	-	+	-	-	-	-
miR-3191	miR-3191	-	-	+	-	-	-	-
miR-3194	miR-3194	-	-	+	-	-	-	-
miR-3195	miR-3195	-	-	+	-	-	-	-
miR-3196	miR-3196	-	-	+	-	-	-	-
miR-3197	miR-3197	-	-	+	-	-	-	-
miR-3201	miR-3201	-	-	+	-	-	-	-
miR-4253	miR-4253	-	-	+	-	-	-	-
miR-4259	miR-4259	-	-	+	-	-	-	-
miR-4270	miR-4270	-	-	+	-	-	-	-
miR-4271	miR-4271	-	-	+	-	-	-	-
miR-4281	miR-4281	-	-	+	-	-	-	-
miR-4286	miR-4286	-	-	+	-	-	-	-
miR-4298	miR-4298	-	-	+	-	-	-	-
miR-4299	miR-4299	-	-	+	-	-	-	-
miR-4306	miR-4306	-	-	+	-	-	-	-
miR-4322	miR-4322	-	-	+	-	-	-	-

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