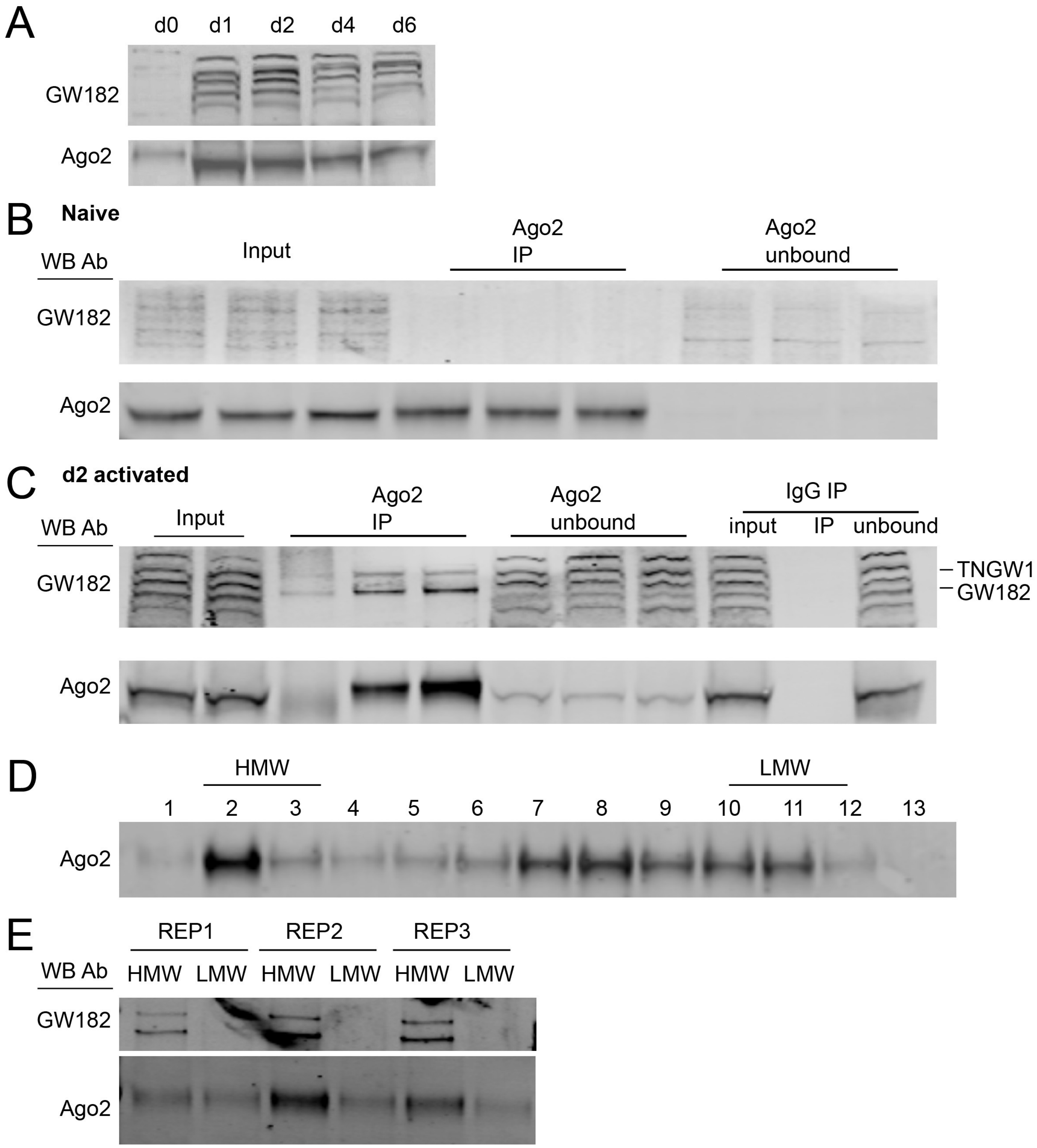
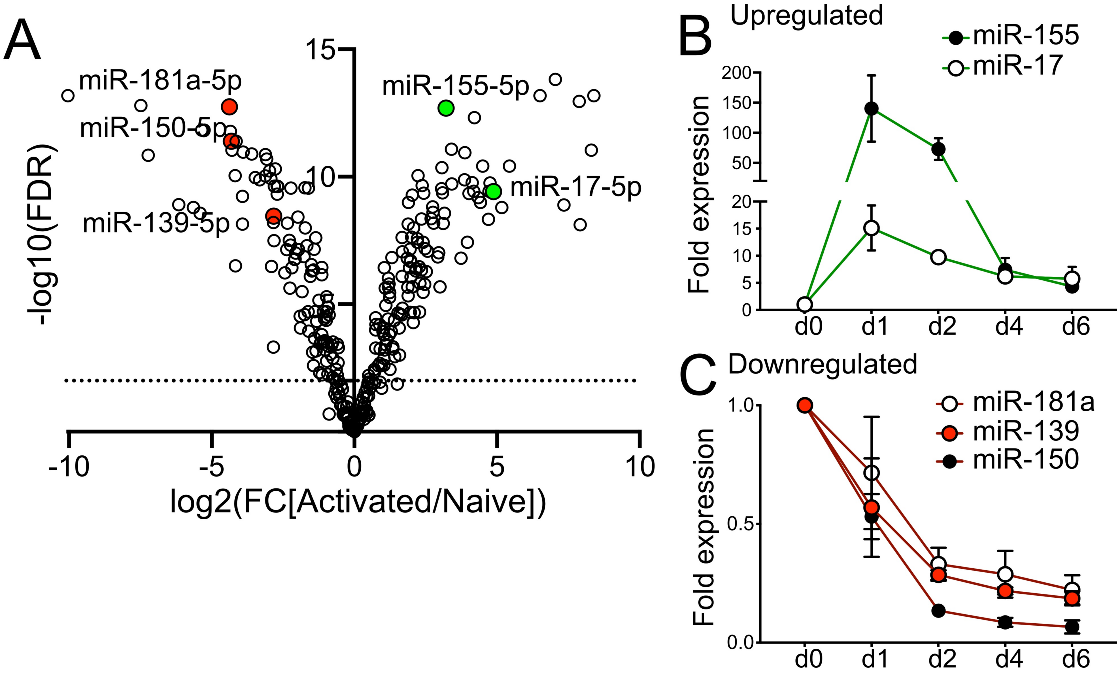


Supplementary Figure 1. Activated cell inputs for Ago IP and for preparation of small RNA libraries from HMW and LMW RISC

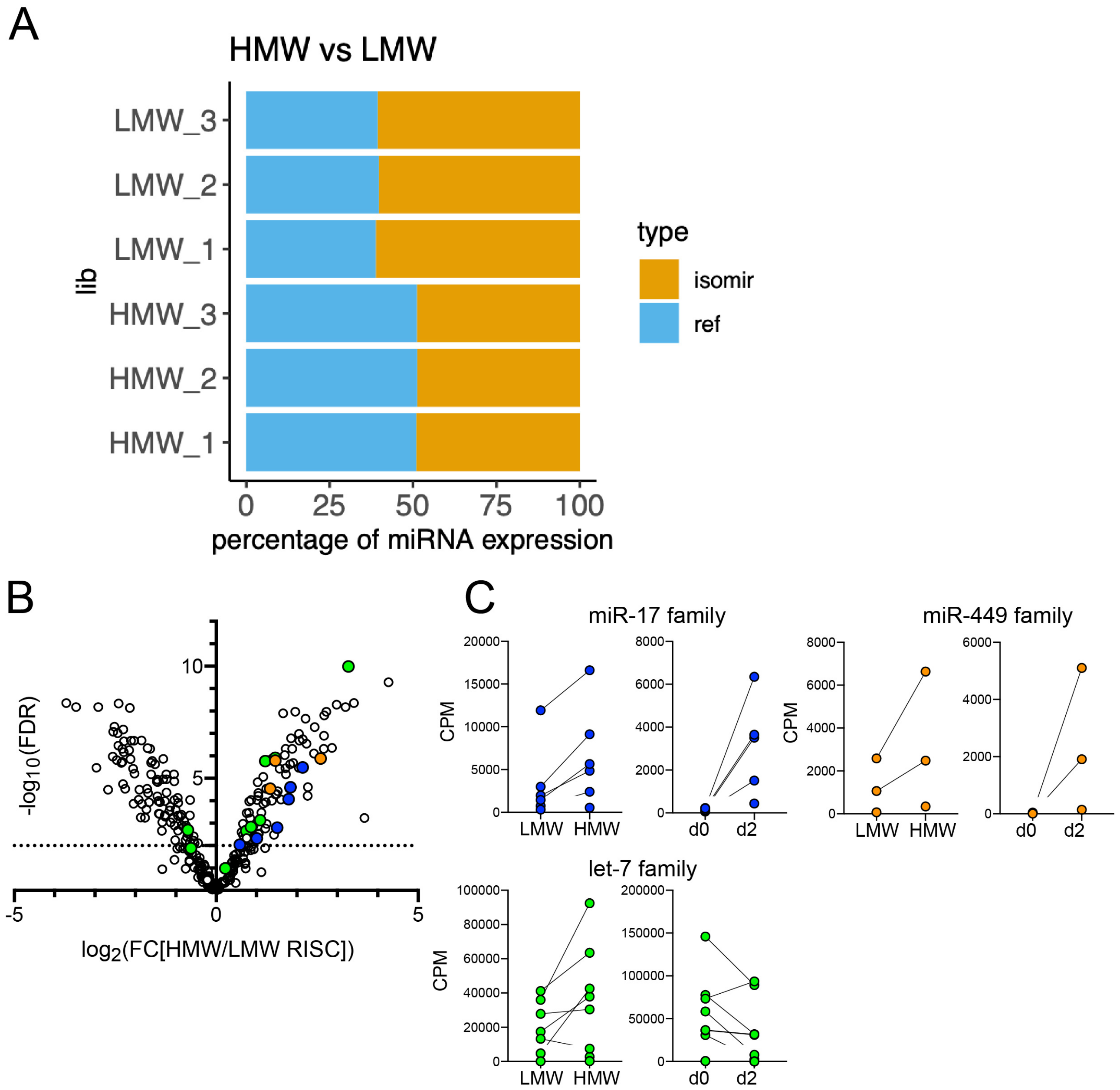
(A) Western blot of total lysates used for time course of Ago2 immunoprecipitates in Fig 1. (B-C) Western blots from input, Ago2 IP (and control IgG IP) and unbound fractions from naive (B) and d2 activated (C) OT-I T cells. Blots are probed with Ago2 and GW182 antibodies. The 2 isoforms TNGW1 and GW182 recognised by the anti-GW182 sera are indicated. Three biological replicates are shown for naive and activated cells. (D) Western blot from protein fractions eluted by size exclusion chromatography, probed with Ago2 antibody. One replicate is shown as representative of three. (E) Western blot of Ago2 IP from pooled HMW (lanes 2-3 in D) and LMW (lanes 10-12 in D) fractions, probe with Ago2 and GW182 antibodies. Three biological replicates are shown.



Supplementary Figure 2 miRNA expression changes dynamically upon CD8^{+} T cell activation

(A) Differential expression of Ago2 bound miRNAs between naïve and d2 activated OT-I T cells shown in a volcano plot of $\log_2$ fold change expression in naïve vs activated cells and $-\log_{10}$ of false discovery rate. Significant differences (FDR<0.01) above dashed line. Data are from three biological replicates in one experiment.

(B-C) miRNA expression measured by qPCR shown as fold expression relative to naïve cells. Mean and SD from 3-4 independent experiments.

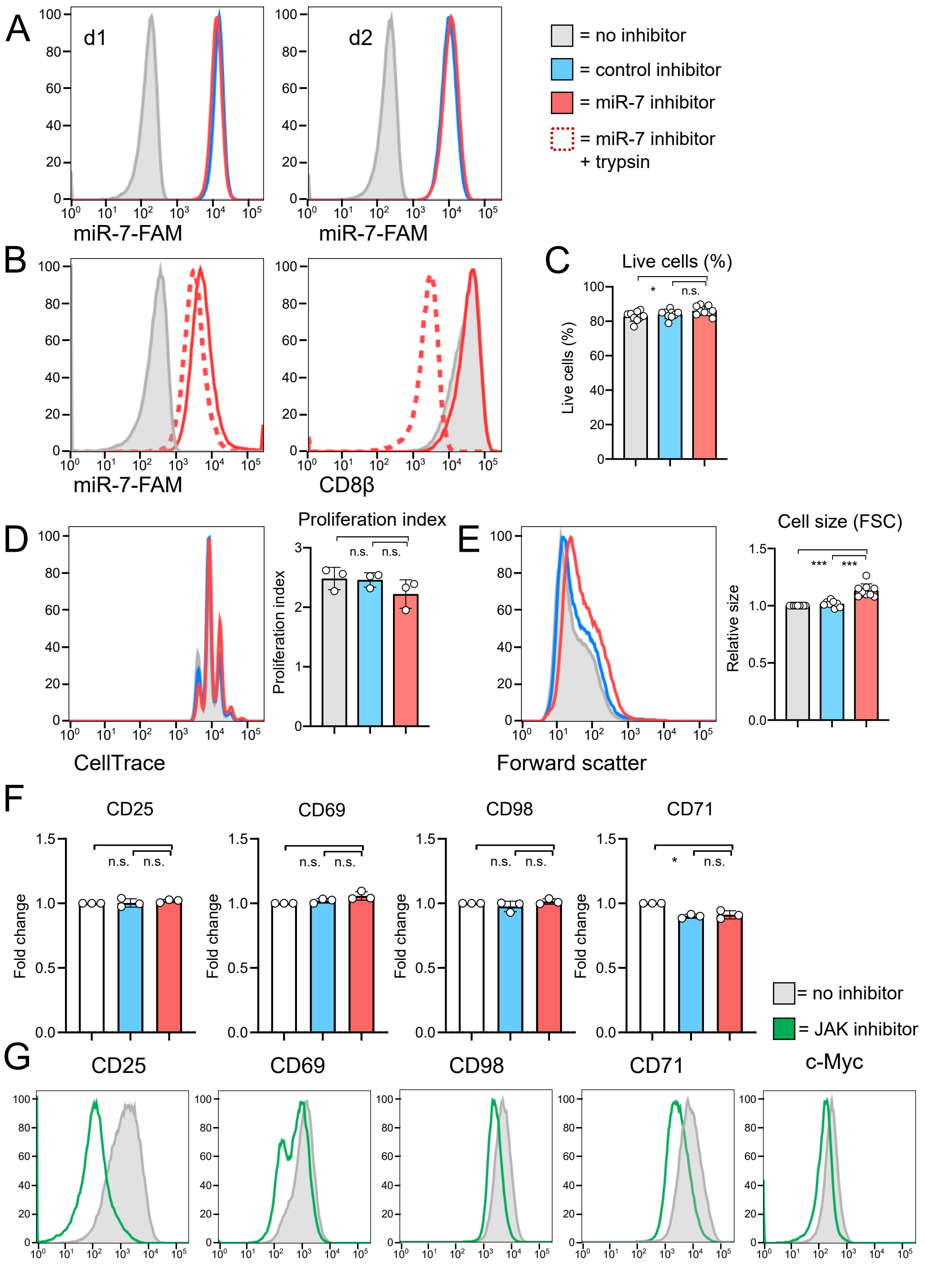


Supplementary Figure 3 Reference miRNAs are more abundant in HMW compared to LMW RISC and partitioning is consistent across family members

A) Reference miRNAs are more abundant than isomiRs in HMW compared to LMW libraries. Bars represent the percentage of all miRNA-related reads after processing with the isomiRs R package v1.26.0. Reference miRNAs are those defined by miRBase release 22.1, isomiRs include 5' or 3' extensions or reductions, non-template additions as well as single nucleotide variations. Statistical significance was calculated with a Welch two sample t-test comparing the percentage of isomiRs in HMW vs LMW.

(B) Differential expression of miRNAs between HMW and LMW RISC shown in a volcano plot. The x axis corresponds to the $\log_2$ (Fold-change) of miRNAs in HMW vs LMW RISC and the y axis corresponds to the $-\log_{10}$ of the False Discovery Rate. Significant differences ($FDR<0.01$) are above the dashed line. Members of the miR-17 family (in blue: miR-17-5p, miR-20a-5p, miR-20b-5p, miR-106a-5p, miR-106b-5p and miR-93-5p), let-7 family (in green: let-7a-5p, let-7b-5p, let-7c-5p, let-7d-5p, let-7e-5p, let-7f-5p, let-7g-5p, let-7i-5p, let-7j) and miR-449 family (in orange: miR-449a-5p, miR-449c-5p, miR-34a-5p) are highlighted.

(C) Absolute expression (in CPM) of miRNAs belonging to miR-17, miR-449 and let-7 families in HMW vs LMW RISC (left) and in Ago2 IP from naïve d0 vs activated d2 cells (right). Each data point is the average from three biological replicates in one experiment.



Supplementary Figure 4 Uptake of the miR-7 inhibitor does not affect cell viability, proliferation and size

(A-B) Cells were activated with SIINFEKL peptide in the presence of a fluorescently tagged miR-7 inhibitor (miR-7-FAM). Uptake was measured by flow cytometry on d1 and d2 (A), with or without a pre-incubation with trypsin, which removes CD8β from the cell surface, but not the inhibitor (B).

(C) Percentage of live cells on d2 measured using a live/dead cell stain.

(D) Cell proliferation measured by dilution of a CellTrace tracker dye on d2. Representative flow cytometry histogram is shown alongside proliferation index measured from three independent experiments. Statistical analysis for (C) and (D) and was performed using a one-way ANOVA with Tukey's multiple comparisons test.

(E) Cell size measured by the increase in forward scatter in flow cytometry, is shown relative to 'no inhibitor' control. Graph is showing individual biological replicates from 4-6 independent experiments, with fold expression shown relative to 'no inhibitor' control.

(F) Cells were activated with SIINFEKL peptide in the presence of miR-7 inhibitor and expression of surface markers was measured on d1. Graphs show individual biological replicates pooled from three independent experiments, with fold expression shown relative to 'no inhibitor' control. Statistical analysis for (E) and (F) was performed using a one-sample t-test to compare 'miR-7 inhibitor' to the hypothetical mean of 1 and a two-tailed unpaired student's t-test to compare 'control inhibitor' and 'miR-7 inhibitor' conditions.

(G) Cells were activated with SIINFEKL peptide on d0 and JAK inhibitor was added on d1. Expression of surface markers and transcription factors was measured on d2. Representative flow cytometry histograms (from four independent experiments) show effect of JAK inhibitor compared to no inhibitor control.

Supplementary Table 1: HMW enriched miRNAs as determined by differential expression analysis in Degust.

miRNA	**HMW_IP_FC**	**FDR**	**AveExpr**
mmu-let-7g-5p	3.265543	0.0000000017	14.463161
mmu-miR-671-5p	4.257203	0.0000000017	4.159988
mmu-miR-669b-5p	2.968304	0.0000000052	5.644416
mmu-miR-378c	3.402537	0.0000000057	6.362360
mmu-miR-669f-5p	3.191821	0.0000000057	4.172738
mmu-miR-466h-5p	2.719844	0.0000000110	4.064149
mmu-miR-28a-5p	2.200200	0.0000000220	7.884315
mmu-miR-6240	2.662722	0.0000000443	4.041073
mmu-miR-5104	2.055297	0.0000000800	3.537787
mmu-miR-363-5p	2.128488	0.0000000959	5.807895
mmu-miR-7a-5p	2.428759	0.0000001023	13.318687
mmu-miR-185-5p	2.039133	0.0000001292	6.463453
mmu-miR-210-3p	2.659067	0.0000002839	10.574171
mmu-miR-140-5p	2.221178	0.0000003572	7.278775
mmu-miR-147-3p	2.650229	0.0000006254	3.899422
mmu-miR-466a-5p	2.409294	0.0000007571	4.207382
mmu-miR-466f	2.859220	0.0000008070	2.309574
mmu-miR-497a-5p	2.038513	0.0000016288	2.913326
mmu-miR-34a-5p	2.571077	0.0000029010	6.617295
mmu-miR-20b-5p	2.129293	0.0000065721	10.711039
mmu-miR-18a-5p	2.259623	0.0000371934	7.049924
mmu-miR-700-5p	2.252895	0.0000863115	3.540629
mmu-miR-5099	3.660892	0.0012974885	4.523326

Supplementary Table 2: LMW enriched miRNAs as determined by differential expression analysis in Degust.

miRNA	**HMW_IP_FC**	**FDR**	**AveExpr**
mmu-miR-181a-1-3p	-2.427871	0.0000000067	6.137587
mmu-miR-126a-5p	-2.151761	0.0000000110	6.318017
mmu-miR-3968	-3.729721	0.0000000110	2.858545
mmu-miR-211-5p	-2.929165	0.0000000149	6.018648
mmu-miR-664-5p	-3.482775	0.0000000208	4.851836
mmu-miR-501-3p	-2.314122	0.0000000447	3.227106
mmu-miR-181a-2-3p	-2.532804	0.0000000800	4.501193
mmu-miR-3061-3p	-2.558268	0.0000001292	3.616119
mmu-miR-150-5p	-2.453002	0.0000003520	11.579011
mmu-miR-192-5p	-2.049889	0.0000003520	9.323928
mmu-miR-155-3p	-2.337968	0.0000004242	2.103109
mmu-miR-342-3p	-2.122185	0.0000005112	8.585091
mmu-miR-1843a-3p	-2.173628	0.0000007389	4.915914
mmu-miR-142a-5p	-2.293832	0.0000008091	13.121335
mmu-miR-148a-5p	-2.305780	0.0000011546	4.068077
mmu-miR-320-3p	-2.434338	0.0000011546	10.072751
mmu-miR-151-3p	-2.129760	0.0000016288	8.535898
mmu-miR-8103	-2.569792	0.0000024193	4.686928
mmu-miR-29b-1-5p	-2.480189	0.0000028916	4.950172
mmu-miR-10a-5p	-2.365016	0.0000041999	4.359784
mmu-miR-664-3p	-2.070453	0.0000041999	2.130888
mmu-miR-210-5p	-2.337021	0.0000052076	7.058187
mmu-miR-1943-5p	-2.990414	0.0000082881	3.894934
mmu-miR-146b-5p	-2.039802	0.0000087561	9.891156
mmu-miR-98-3p	-2.311713	0.0000239559	2.289731
mmu-miR-139-3p	-2.265054	0.0000417432	4.798624
mmu-miR-486b-5p	-2.691938	0.0000541525	8.196442
mmu-miR-128-1-5p	-2.060506	0.0001768377	4.227409