SUPPLEMENTAL MATERIAL

Divergent Target Recognition by Co-expressed 5-IsomiRs of miR-142-3p and Selecive Viral Mimicry

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Figs. S1-S4

References

1. Du P, Kibbe WA, Lin SM. 2008. *lumi*: a pipeline for processing Illumina microarray. *Bioinformatics* **24**(13):1547-8.
2. van Dongen S, Abreu-Goodger C, Enright AJ. 2008. Detecting microRNA binding and siRNA off-target effects from expression data. *Nat Methods* **5**:1023-1025.
3. Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP. 2007. MicroRNA targeting specificity in mammals: determinants beyond seed pairing. *Mol Cell* **27**: 91-105.
4. Shin C, Nam J-W, Farh KK-H, Chiang HR, Shkumatava A, Bartel DP. 2010. Expanding the microRNA targeting code: functional sites with centered pairing. *Mol Cell* **36**: 789-802.
5. Chi SW, Hannon GJ, Darnell RB. 2012. An alternative mode of microRNA target recognition. *Nat Struct Mol Biol* **19**:321-27.

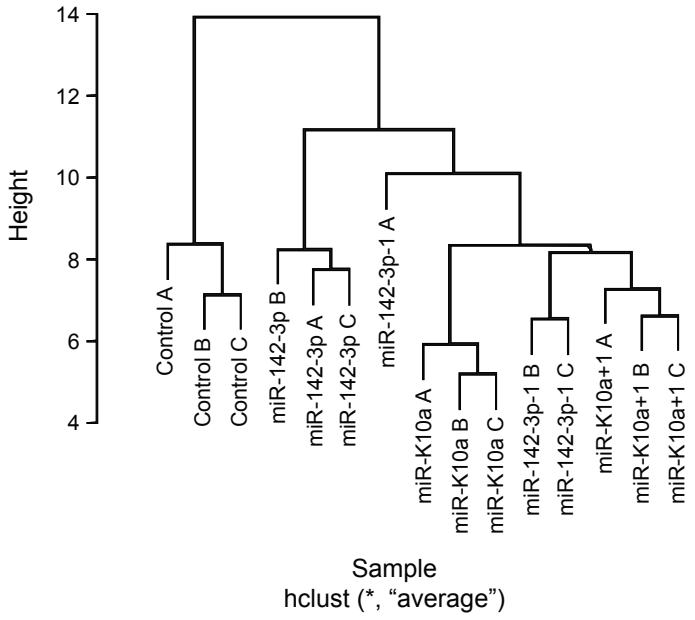

Fig. S1. Hierarchal clustering using Bioconductor *lumi* package (Pan et al., 2008) of Illumina BeadArray analyses of total RNAs from HEK293T cells transfected with control, miR-142-3p, miR-142-3p-1, miR-K10a+1, or miR-K10a miRNA mimics. Sample relations are based on 5446 probes with SD/mean > 0.1.

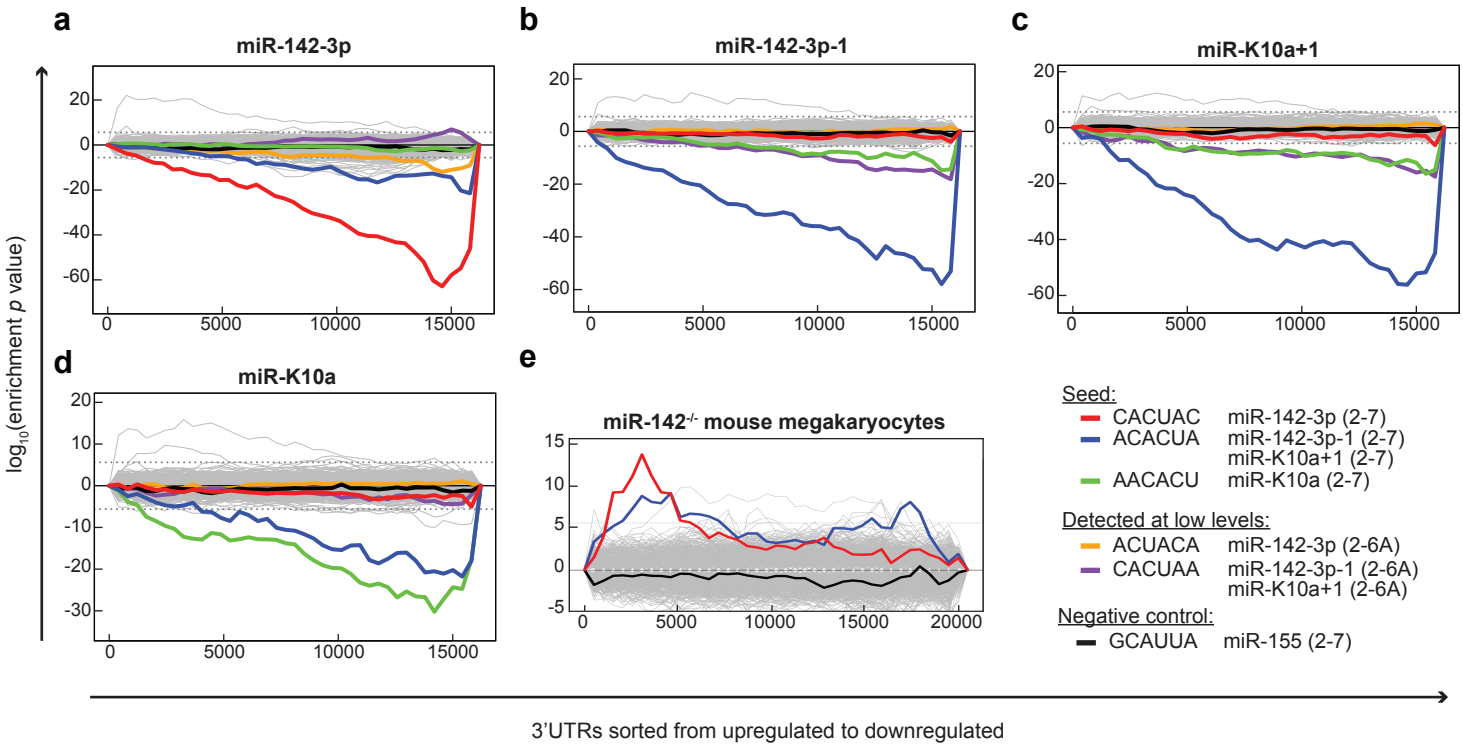

Fig. S2. Sylamer analyses (van Dongen et al. 2008) of 6mer matches to the miR-142-3p and miR-K10a 5'-isomiRs in **a-d**. HEK293T cells transfected with **a.** miR-142-3p, **b.** miR-142-3p-1, **c.** miR-K10a+1, or **d.** miR-K10a miRNA mimics and **e.** miR-142$^{-/-}$ mouse megakaryocytes (GSE52141). x-axis represents the ranked gene list from microarray expression data.

a

Seed

Seed	..CCGGGGGCAAAACAACACUACG..	-3’	
miR-142-3p	AGGUAUUUCAUCCU**UUGUGAUG**U	-5’	2-9
miR-142-3p-1	AGGUAUUUCAUCCU**UUGUGAU**G	-5’	2-8
miR-K10a+1	CGGUGAGCCCCCC**UGUUGUGAU**U	-5’	2-10
miR-K10a	CGGUGAGCCCCCC**UGUUGUGA**U	-5’	2-9A

b

Centered Sites

M4-15	..GGCGCCCGUAGGAAACACUUGG..	-3’	
miR-142-3p	AGGUAUUU**CAUCCUUUGUGA**UGU	-5’	4-15
miR-142-3p-1	AGGUAUUU**CAUCCUUUGUGA**UG	-5’	3-14
miR-K10a+1	CGGUGAGCCCCCCUG**UUGUGA**UU	-5’	3-8
miR-K10a	CGGUGAGCCCCCCUG**UUGUGA**U	-5’	2-7

M5-16	..GGCGCCAGUAGGAAACACAUGG..	-3’	
miR-142-3p	AGGUAUU**UCAUCCUUUGUG**AUGU	-5’	5-16
miR-142-3p-1	AGGUAUU**UCAUCCUUUGUG**AUG	-5’	4-15
miR-K10a+1	CGGUGAGCCCCCCUG**UUGUG**AUU	-5’	4-8
miR-K10a	CGGUGAGCCCCCCUG**UUGUG**AU	-5’	3-7

M6-17	..GGCGCAAGUAGGAAACAGAUGG..	-3’	
miR-142-3p	AGGUAU**UUCAUCCUUUGU**GAUGU	-5’	6-17
miR-142-3p-1	AGGUAU**UUCAUCCUUUGU**GAUG	-5’	5-16
miR-K10a+1	CGGUGAGCCCCCCUG**UUGU**GAUU	-5’	5-8
miR-K10a	CGGUGAGCCCCCCUG**UUGU**GAU	-5’	4-7

c

3’ Supplementary Site

M3’Supp	..ACCGGAAAGUAAACUACACUAGG..	-3’	
miR-142-3p	AGGUA**UUUCAU**CCUU**UGUGAU**GU	-5’	3-8 and 13-18
miR-142-3p-1	AGGUA**UUUCAU**CCUU**UGUGAU**G	-5’	2-7 and 12-17
miR-K10a+1	CGGUGAGCCCCCCUGU**UGUGAU**U	-5’	2-7
miR-K10a	CGGUGAGCCCCCCUGU**UGUGA**U	-5’	2-6A

d

Pivot Sites

PIV-A	..CCGGGGGCAAAACAAC<u>A</u>ACUAGGU..	-3’	
miR-142-3p	AGGUAUUUCAUCCUUUG**UGAU**GU	-5’	3-6
miR-142-3p-1	AGGUAUUUCAUCCUUUG**UGAU**G	-5’	2-5
miR-K10a+1	CGGUGAGCCCCCCUGUUG**UGAU**U	-5’	2-5
miR-K10a	CGGUGAGCCCCCCUGUUG**UGA**U	-5’	2-4

PIV-C	..CCGGGGGCAAAACAAC<u>C</u>ACUAGGU..	-3’	
miR-142-3p	AGGUAUUUCAUCCUUUG**UGAU**GU	-5’	3-7
miR-142-3p-1	AGGUAUUUCAUCCUUUG**UGAU**G	-5’	2-6
miR-K10a+1	CGGUGAGCCCCCCUGUUG**UGAU**U	-5’	2-6
miR-K10a	CGGUGAGCCCCCCUGUUG**UGA**U	-5’	2-5

Pivot →

PIV-C	..CCGGGGGCAAAACAAC<u>C</u>ACUAGGU..	-3’	
miR-142-3p	AGGUAUUUCAUCCU**UUG-UGAU**GU	-5’	3-6^7-9
miR-142-3p-1	AGGUAUUUCAUCCU**UUG-UGAU**G	-5’	2-5^6-8
miR-K10a+1	CGGUGAGCCCCCC**UGUUG-UGAU**U	-5’	2-5^6-10
miR-K10a	CGGUGAGCCCCCC**UGUUG-UGA**U	-5’	2-4^5-9

PIV-G	..CCGGGGGCAAAACAAC<u>G</u>ACUAGGU..	-3’	
miR-142-3p	AGGUAUUUCAUCCUUUG**UGAU**GU	-5’	3-6
miR-142-3p-1	AGGUAUUUCAUCCUUUG**UGAU**G	-5’	2-5
miR-K10a+1	CGGUGAGCCCCCCUGUUG**UGAU**U	-5’	2-5
miR-K10a	CGGUGAGCCCCCCUGUUG**UGA**U	-5’	2-4

PIV-U	..CCGGGGGCAAAACAAC<u>U</u>ACUAGGU..	-3’	
miR-142-3p	AGGUAUUUCAUCCUUUG**UGAU**GU	-5’	3-7
miR-142-3p-1	AGGUAUUUCAUCCUUUG**UGAU**G	-5’	2-6
miR-K10a+1	CGGUGAGCCCCCCUGUUG**UGAU**U	-5’	2-6
miR-K10a	CGGUGAGCCCCCCUGUUG**UGA**U	-5’	2-5

Pivot →

PIV-U	..CCGGGGGCAAAACAAC<u>U</u>ACUAGGU..	-3’	
miR-142-3p	AGGUAUUUCAUCCU**UUG-UGAU**GU	-5’	3-6^7-9
miR-142-3p-1	AGGUAUUUCAUCCU**UUG-UGAU**G	-5’	2-5^6-8
miR-K10a+1	CGGUGAGCCCCCC**UGUUG-UGAU**U	-5’	2-5^6-10
miR-K10a	CGGUGAGCCCCCC**UGUUG-UGA**U	-5’	2-4^5-9

Fig. S3. Sequences of miRNA-binding sites to test non-canonical interactions in Fig. 4. **a**. Seed interaction (positive control); **b.** Centered Sites (Shin et al., 2010); **c.** 3’ Supplementary Sites (Grimson et al., 2007); and **d.** Pivot Sites (Chi et al., 2012) where ^ represents the bulge position in the mRNA. Bases in bold are expected to be paired with the 3‘UTR. Interactions that are predicted to result in regulation are highlighted in red. Underlined bases, bulged nt.

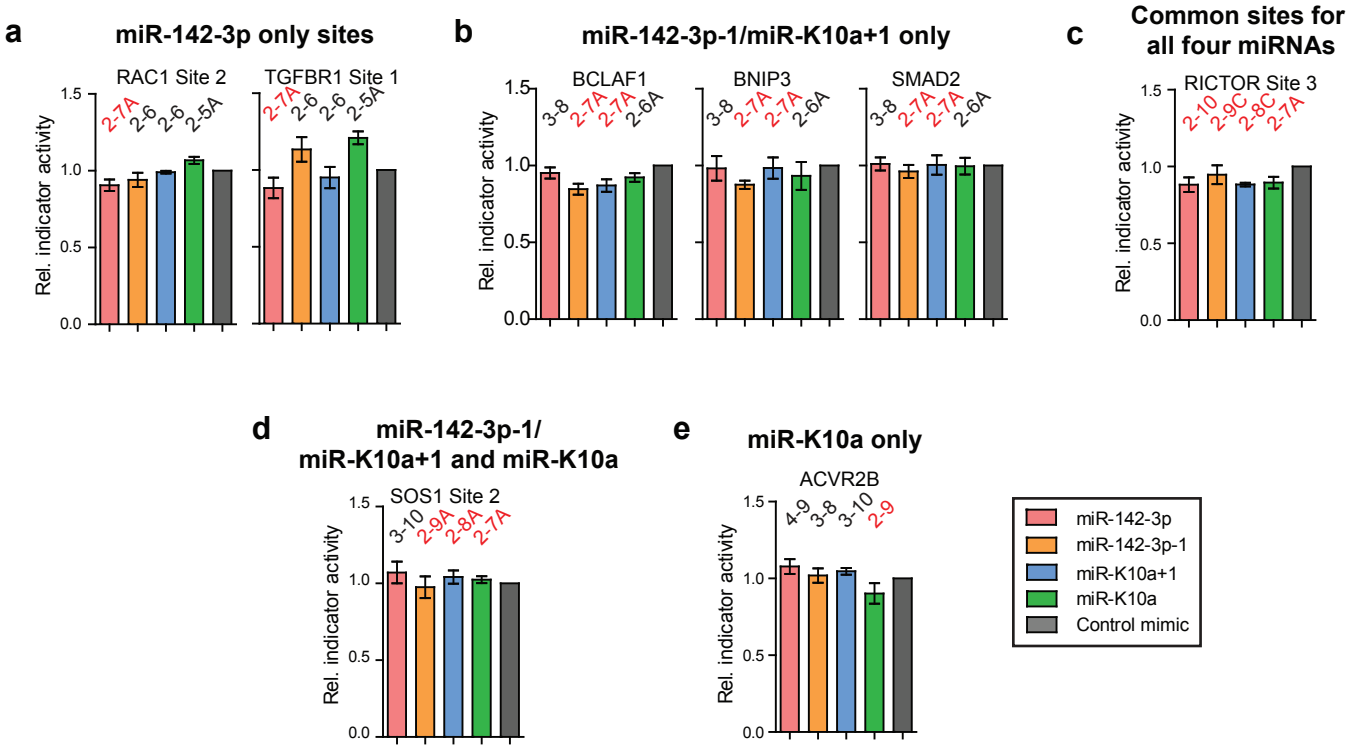

Fig. S4. Additional 3'UTR reporter assays performed and analyzed as in Fig.3. Error bars, s.e.m. (n ≥ 3 biological replicates).