

**Supplementary Figure 1: Analysis of MRE activity in** *Drosophila***S2R+ cells in culture.** (a) Two predicted targets of miR-184, PCK and CG13088 were tested in this system. Sequences deleted by HDR are shown in bold; the miR-184 seed sequence is in red. (b) The ratio of T7 signal to T3 signal is displayed on the y-axis, and results from gDNA are shown in blue and cDNA in red. Both transcripts appear to be upregulated upon MRE deletion in this cell type (p<0.05, one-tailed T-test, n=3).

	WT	
	microRNA	Seed
	hsa-miR-92b	8mer
	hsa-miR-92a	8mer
	hsa-miR-513a-3p	8mer
	hsa-miR-367	8mer
	hsa-miR-25	8mer
	hsa-miR-1290	8mer
	hsa-miR-944	7mer
	hsa-miR-643	7mer
	hsa-miR-363	7mer
	hsa-miR-32	7mer

PCMTD1

T7 MRE <sup>mut</sup>	
microRNA	Seed
hsa-miR-513a-3p	8mer
hsa-miR-1290	8mer
hsa-miR-944	7mer
hsa-miR-643	7mer
hsa-miR-921	7mer

T3 MRE <sup>WT</sup>	
microRNA	Seed
hsa-miR-92b	8mer
hsa-miR-92a	8mer
hsa-miR-513a-3p	8mer
hsa-miR-367	8mer
hsa-miR-25	8mer
hsa-miR-1290	8mer
hsa-miR-944	7mer
hsa-miR-643	7mer
hsa-miR-363	7mer
hsa-miR-32	7mer
hsa-miR-944	8mer
hsa-miR-921	8mer
hsa-miR-211	7mer
hsa-miR-204	7mer

	hsa-miR-596	8mer
	hsa-miR-1226	8mer
C9orf7	hsa-miR-483-3p	7mer
C96		

hsa-miR-596	8mer
hsa-miR-1226	8mer
hsa-miR-483-3p	7mer
hsa-miR-921	7mer

MAPRE1	hsa-miR-548l	8mer
	hsa-miR-211	8mer
	hsa-miR-204	8mer
	hsa-miR-922	7mer
	hsa-miR-570	7mer
2		

hsa-miR-548l	8mer
hsa-miR-211	8mer
hsa-miR-204	8mer
hsa-miR-922	7mer
hsa-miR-570	7mer
hsa-miR-921	7mer

hsa-miR-596	8mer
hsa-miR-1226	8mer
hsa-miR-483-3p	7mer
hsa-miR-921	8mer
hsa-miR-211	7mer
hsa-miR-204	7mer

hsa-miR-548l	8mer
hsa-miR-211	8mer
hsa-miR-204	8mer
hsa-miR-922	7mer
hsa-miR-570	7mer
hsa-miR-921	8mer
hsa-miR-211	7mer
hsa-miR-204	7mer

**Supplementary Table 1: Comparative miRNA target predictions before and after T7 or T3 barcode integration.** MRE prediction was performed using the PITA algorithm for WT sequences and insertions of the T7 MRE<sup>mut</sup> and T3 MRE<sup>WT</sup> HDR oligonucleotides. For T7 and T3 integrant sequences, MREs added to the WT sequence are highlighted in grey. The miR-944 MRE differentially generated by the T3 MRE<sup>WT</sup> in PCMDT1 is unlikely to be functional since miR-944 is not expressed to any degree in HEK-293 cells.