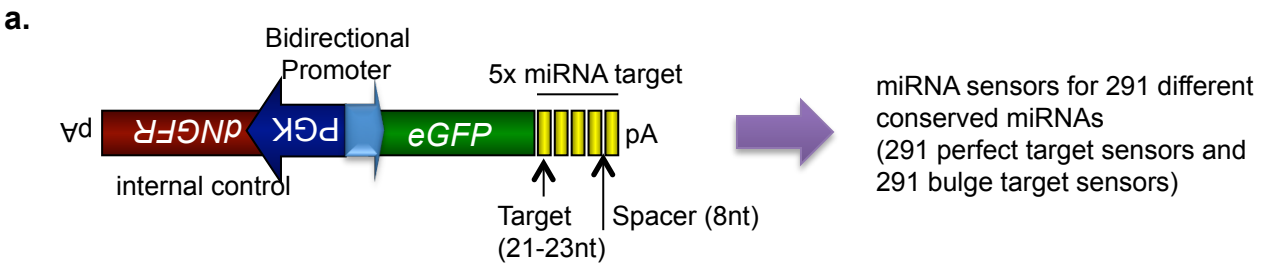
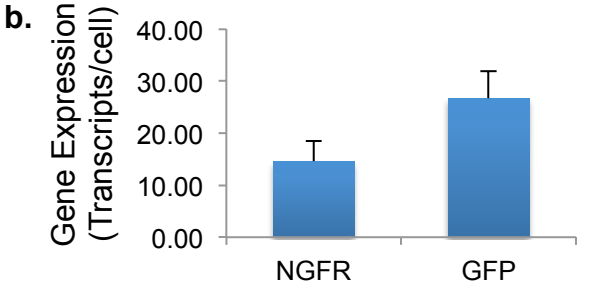
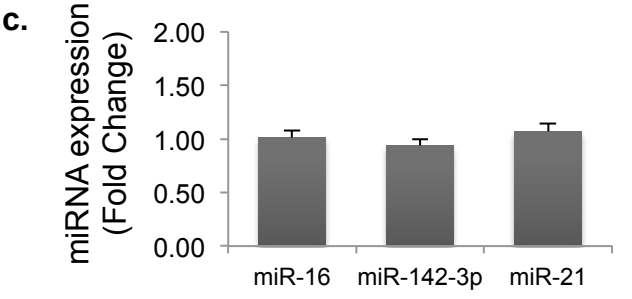
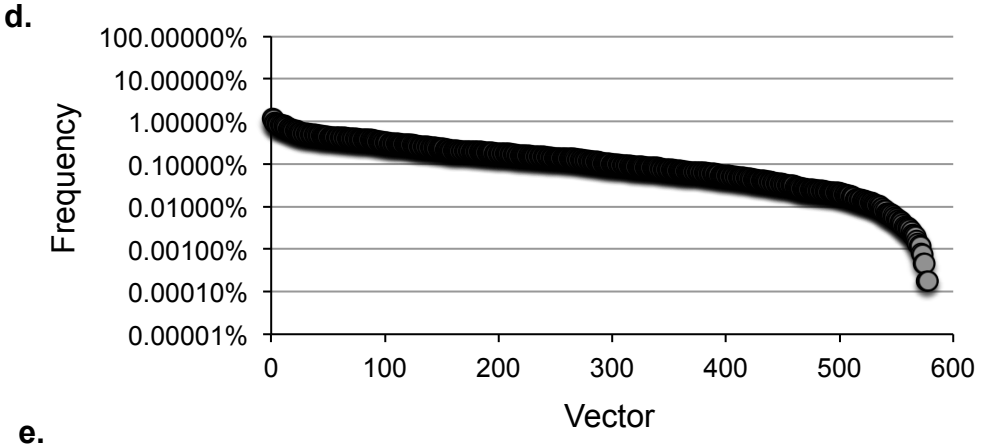
Supplementary Figure 1. BdLV-based miRNA Sensor Library.









e.

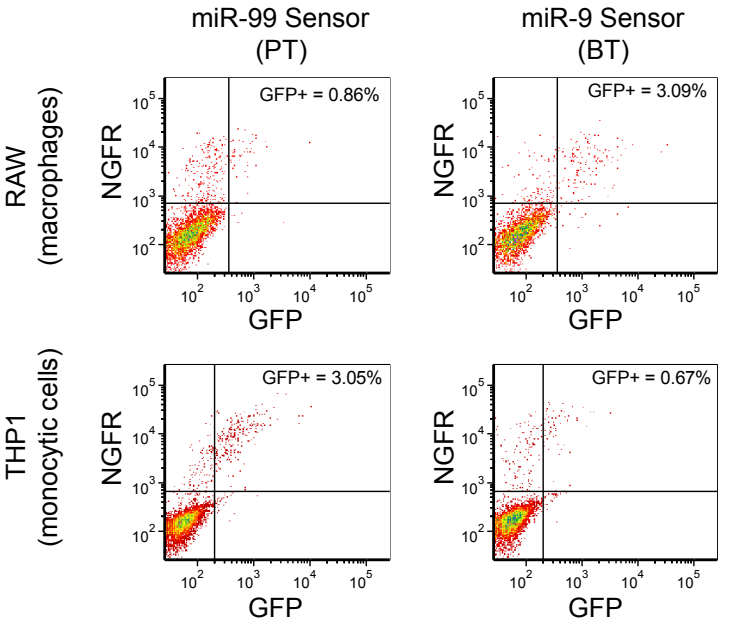
Pearson's correlation matrix between all bins/replicates from Sensor-seq of monocytic cells

Min Median Max

	bin0_1	bin0_2	bin0_3	bin1_1	bin1_2	bin1_3	bin2_1	bin2_2	bin2_3	bin3_1	bin3_2	bin3_3	bin4_1	bin4_2	bin4_3
bin0_1	1.00														
bin0_2	0.94	1.00													
bin0_3	0.84	0.83	1.00												
bin1_1	0.36	0.37	0.36	1.00											
bin1_2	0.34	0.33	0.32	0.98	1.00										
bin1_3	0.37	0.39	0.42	0.93	0.89	1.00									
bin2_1	0.59	0.62	0.48	0.35	0.32	0.39	1.00								
bin2_2	0.58	0.60	0.45	0.34	0.31	0.38	0.98	1.00							
bin2_3	0.54	0.57	0.53	0.32	0.29	0.40	0.91	0.88	1.00						
bin3_1	0.73	0.70	0.64	-0.04	-0.05	-0.02	0.15	0.13	0.15	1.00					
bin3_2	0.74	0.71	0.66	-0.04	-0.06	-0.02	0.20	0.18	0.18	0.95	1.00				
bin3_3	0.68	0.65	0.75	0.00	-0.02	0.03	0.16	0.15	0.18	0.86	0.88	1.00			
bin4_1	0.59	0.58	0.57	-0.06	-0.07	-0.03	0.01	0.00	0.04	0.44	0.43	0.36	1.00		
bin4_2	0.59	0.58	0.55	-0.05	-0.07	-0.03	0.01	0.00	0.03	0.44	0.42	0.36	0.97	1.00	
bin4_3	0.55	0.52	0.59	-0.03	-0.05	-0.02	-0.01	-0.01	0.03	0.38	0.36	0.35	0.93	0.92	1.00

Supplementary Figure 1. (**a**) The tandem target sites used for the sensor vector were cloned into a bidirectional lentiviral (BdLV) vector. (**b**) THP1 cells transduced with the BdLV were bead sorted to enrich for NGFR-positive cells. Total RNA was extracted and NGFR and GFP were measured by qPCR. Absolute mRNA levels were determined by extrapolation from a standard curve. Shown is the mean±s.d. (*n* = 4). (**c**) THP1 cells were transduced with the miR-16, miR-142-3p, or miR-21 sensors, bead sorted to enrich for NGFR-positive cells, and miR-16, miR-142-3p, or miR-21 expression was measured by qPCR. Shown are the fold difference in expression between transduced and untransduced cells. (**d**) After vector production, the RNA genome of the vector was isolated, reverse transcribed, and the target sites were amplified and prepared for sequencing. The relative frequency of each vector in the Sensor library was plotted. (**e**) Pearson correlation coefficient matrix between normalized read counts from THP1 Sensor-seq data.

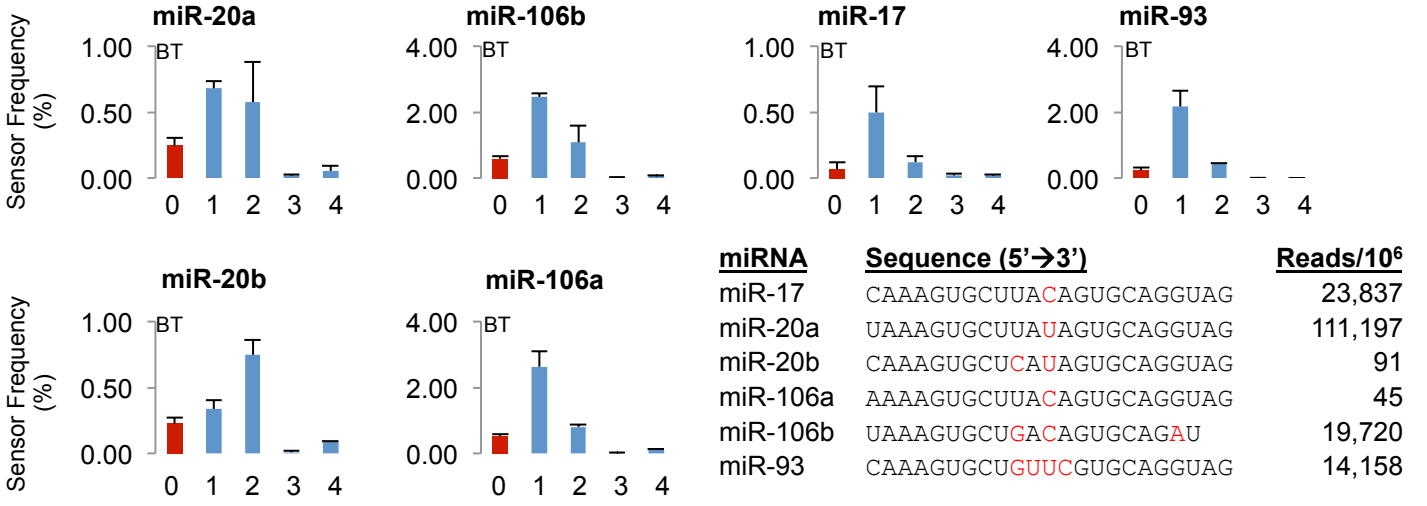
Supplementary Figure 2. Comparison of miR-9 and miR-99 sensors.



Supplementary Figure 2. Expression pattern of individual miR-99a perfect target (PT) sensor and miR-9 bulge target (BT) sensor in THP1 and RAW cells. Note that the BT sensor was used for miR-9 because of availability.

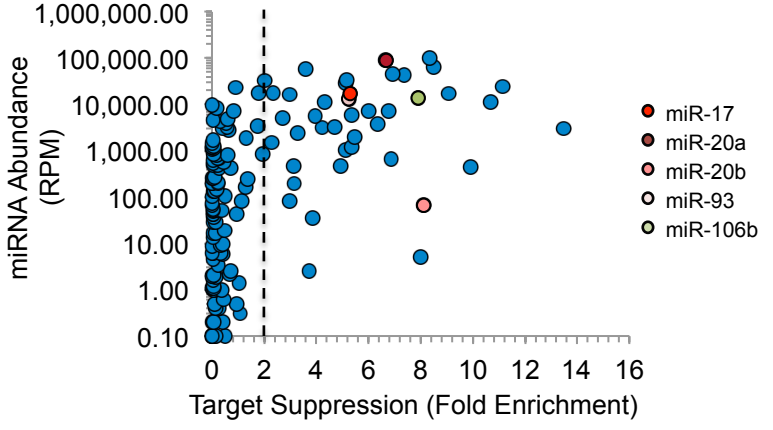
Supplementary Figure 3. Assessing the specificity of Sensor-seq.

a.

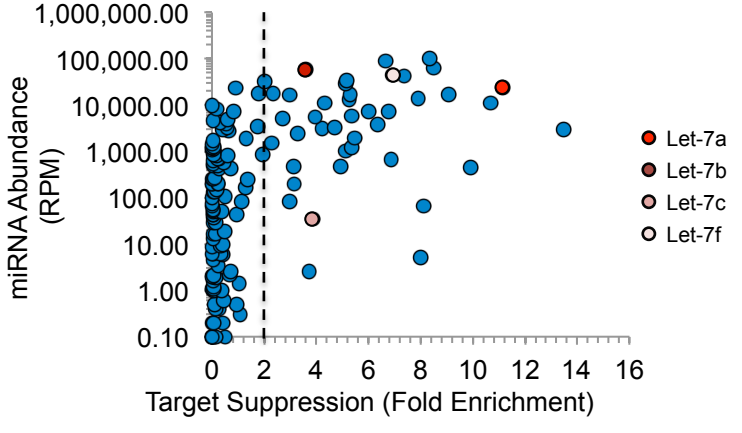


miRNA	Sequence (5'→3')	Reads/10^6
miR-17	CAAAGUGCUUACAGUGCAGGUAG	23,837
miR-20a	UAAAGUGCUUAUAGUGCAGGUAG	111,197
miR-20b	CAAAGUGCUCAUAGUGCAGGUAG	91
miR-106a	AAAAGUGCUUACAGUGCAGGUAG	45
miR-106b	UAAAGUGCUGACAGUGCAGAU	19,720
miR-93	CAAAGUGCUGUUCGUGCAGGUAG	14,158

b.



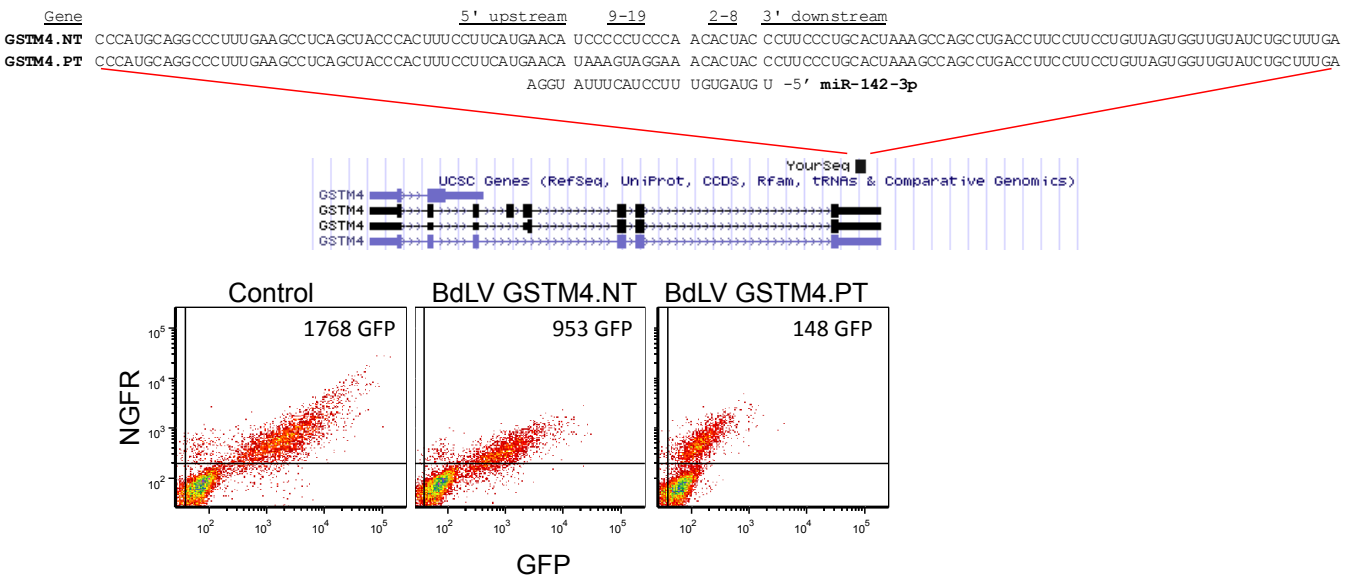
c.



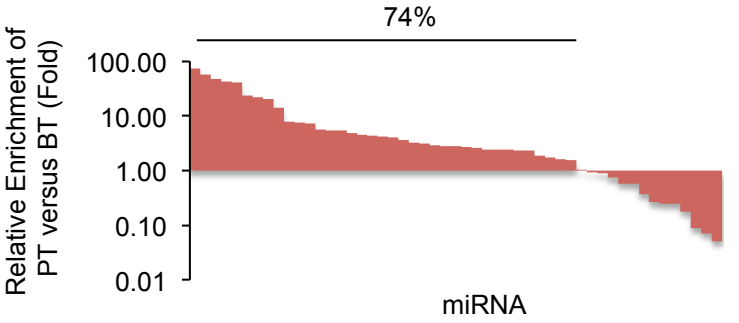
Supplementary Figure 3. Assessing the specificity of Sensor-seq. (**a**) Expression pattern of miR-17 family sensors as determined by Sensor-seq. The mean±s.d. (*n* = 3 replicates) of the indicated sensor frequency (sensor reads/total reads) in a given Bin was plotted. BT, bulge target sensor. A comparison of the sequences of each family member is shown along with the number of RPM of the microRNA in the monocytic cells. Highlighted in red are nucleotide differences between the miRNAs. (**b-c**) Analysis of correlation between target repression and miRNA abundance for (b) miR-17 family and (c) let-7 family. Data for the PT sensors is shown.

Supplementary Figure 4. Assessing the sensitivity of the BdLV sensors.

a.



b.



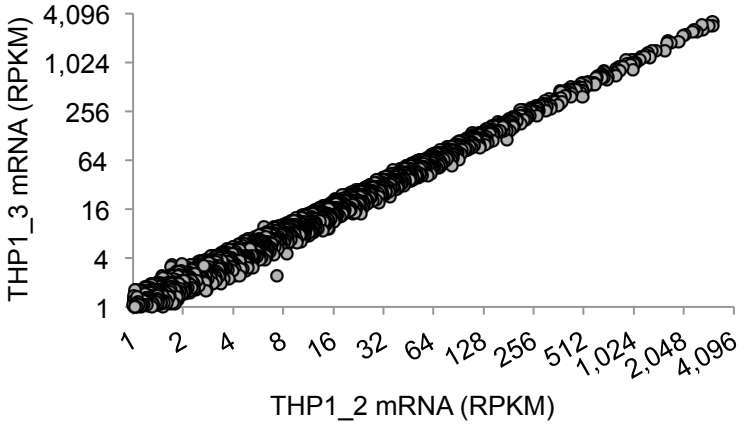
Supplementary Figure 4. (**a**) A region from the GSTM4 3'UTR containing a binding site for miR-142-3p, along with 49 nucleotides of upstream sequence and 60 nucleotides of downstream sequence, was cloned into the 3'UTR of the GFP expression cassette of the BdLV vector (BdLV.GSTM4.NT). An additional vector was made in which we modified the miR-142-3p target site in GSTM4 so that it paired with perfect complementarity to nucleotides 2 – 19 of miR-142-3p (BdLV.GSTM4.PT). THP1 cells were transduced in triplicate and the expression of the sensors was measured by FACS analysis. Representative FACS plots are shown. (**b**) Comparison of perfect versus bulge target suppression. For each miRNA whose sensor was suppressed, based on Sensor-seq analysis, the fold suppression between the perfect and bulge sensor was compared.

Supplementary Figure 5. THP1 monocytic cell transcriptome abundance.

a.

	THP1_1	THP1_2	THP1_3
Exons	23,534,736	22,923,714	22,398,326
None	7,163,862	7,269,057	6,863,161
Repeat	5,261,740	5,213,445	5,068,638
Introns	3,685,341	3,682,685	3,769,134
miRNA	40,591	42,771	41,257
non-coding RNA	5,304	5,674	4,709

b.

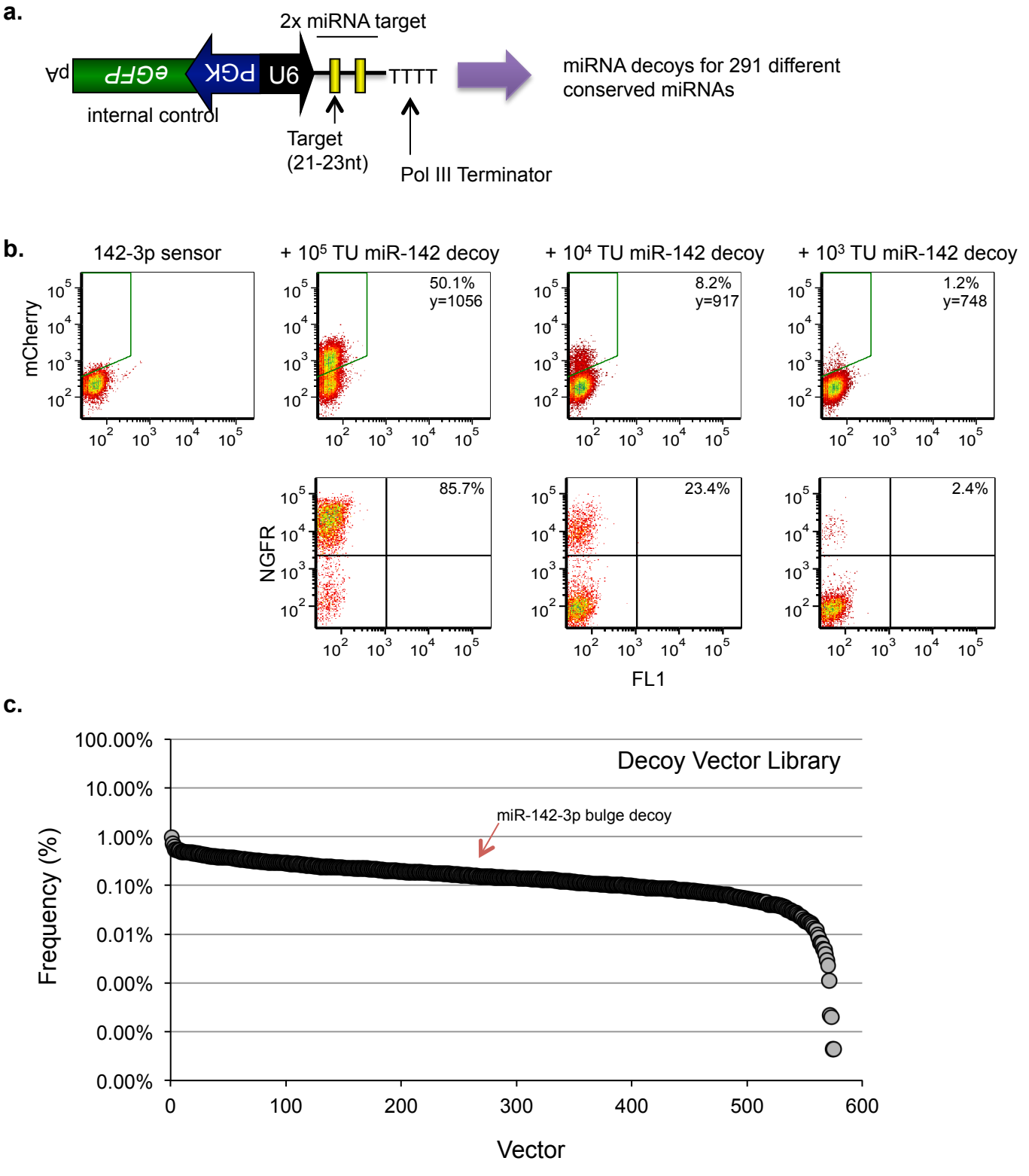


c.

microRNA	Sequence	Length	%
hsa-miR-16	UAGCAGCACGUAAAUAUUGGC	21	3.24%
hsa-miR-16	UAGCAGCACGUAAAUAUUGGCA	22	0.43%
hsa-miR-16	UAGCAGCACGUAAAUAUUGGCG	22	59.74%
hsa-miR-16	UAGCAGCACGUAAAUAUUGGCGA	23	9.83%
hsa-miR-16	UAGCAGCACGUAAAUAUUGGCGAA	24	0.29%
hsa-miR-16	UAGCAGCACGUAAAUAUUGGCGU	23	23.02%
hsa-miR-16	UAGCAGCACGUAAAUAUUGGCGUA	24	0.75%
hsa-miR-21	UAGCUUAUCAGACUGAUGUUG	21	6.60%
hsa-miR-21	UAGCUUAUCAGACUGAUGUUGA	22	50.30%
hsa-miR-21	UAGCUUAUCAGACUGAUGUUGAA	23	0.43%
hsa-miR-21	UAGCUUAUCAGACUGAUGUUGAC	23	36.49%
hsa-miR-21	UAGCUUAUCAGACUGAUGUUGACA	24	2.55%
hsa-miR-21	UAGCUUAUCAGACUGAUGUUGACG	24	0.24%
hsa-miR-21	UAGCUUAUCAGACUGAUGUUGACU	24	1.97%

Supplementary Figure 5. (**a**) Summary of mRNA-seq coverage depth of THP1 monocytic cells. The values for each of the 3 replicates are provided. (**b**) Dynamic range of mRNA transcript abundance in THP1 cells. Shown is a scatterplot comparison of the reads per kilobase of exon per million mapped sequence reads (RPKM) from two of three biological replicates. (**c**) Analysis of miR-16 and miR-21 isoforms in THP1 monocytic cells. The percent of sequence reads for each isoform was calculated by comparing the number of reads of the isform to the total number of reads for the miRNA. Highlighted in red are non-templated nucleotides.

Supplementary Figure 6. LV-based microRNA Decoy Library.



Supplementary Figure 6. (**a**) Schematic of the lentiviral vector-based miRNA decoy vector. miRNA tough decoy target sites were cloned into a lentiviral vector downstream of the U6 promoter. This vector also encodes GFP or NGFR as a reporter from a separate promoter. (**b**) Dose assessment of miR-142-3p decoy vector. miR-142-3p sensor cells were transduced with serial dilutions of a lentiviral vector expressing a decoy for miR-142-3p. Cell transduction was marked by NGFR (below). The experiment was done in triplicate and representative dotplots are shown. (**c**) After vector production the RNA genome of the vector was isolated, reverse transcribed to cDNA, and the target sites were amplified and prepared for sequencing. The relative frequency of each vector in the decoy library is plotted. Highlighted is the frequency of the miR-142-3p decoy.