reproduce-fat-exosome-microrna

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## Purpose

To reproduce qPCR analyses, including statements in text and output, from Thomou et al. article.

## Adipose Tissue is a Major Source of Circulating Exosomal miRNAs

## There were 709 miRNAs profiled of which 653 were detected.  
## Min n detectable: 3

qPCR profiling of the serum exosomes for 709 miRNAs revealed 653 detectable miRNAs (Ct<34; see Supplemental Methods). Compared to control, ADicerKO mice exhibited significant alterations in 422 circulating miRNAs. Of these, only 3 miRNAs were significantly increased, while 419 had significant decreases (Figures 1c-d, Extended Data Figure 1d and Supplemental Table 1) with 88% reduced by >4-fold, suggesting that adipose tissue is a major source of circulating exosomal miRNAs.

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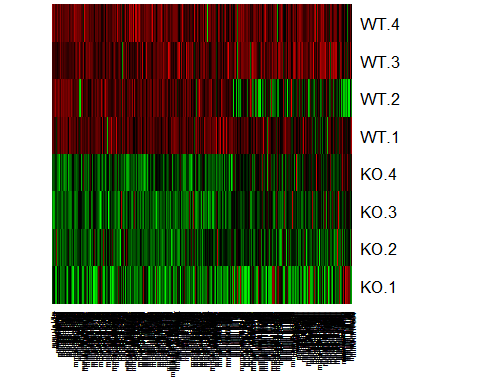
## :-)

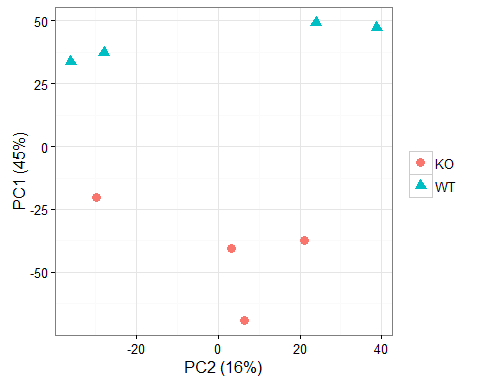
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Heatmap Fig 1c  


PCA in S1d  


Consistent with this, among these most reduced miRNAs (Supplemental Table 1), many have been previously identified as highly expressed in fat, including miR-221, miR-201, miR-222 and miR-16.

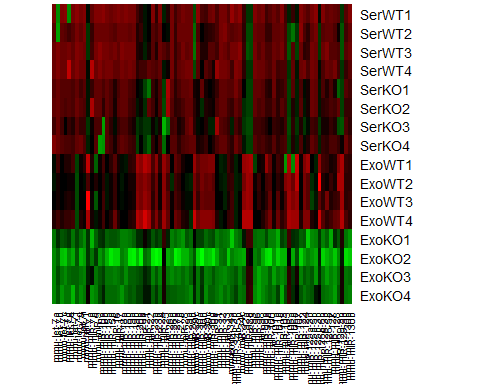
## :-)

Indeed, in sample of 80 miRNAs there was a broad reduction in total serum miRNAs in ADicerKO serum when compared to total serum of WT mice (Extended Data Figure 2a)

## There were 80 miRNAs profiled of which 79 were detected.  
## Min n detectable: 3

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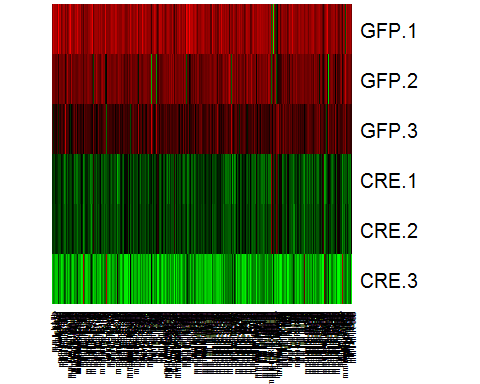
Thus, in brown preadipocytes isolated from Dicer-floxed animals and recombined by transduction with Ad-Cre, there was reductions in >90% of the 359 detectable miRNAs (of 380 miRNAs profiled) released as exosomal miRNAs in the culture supernatants when compared to control Ad-GFP transduced cells (Extended Data Figure 2b).

## There were 380 miRNAs profiled of which 359 were detected.  
## Min n detectable: 2

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4 week old mice

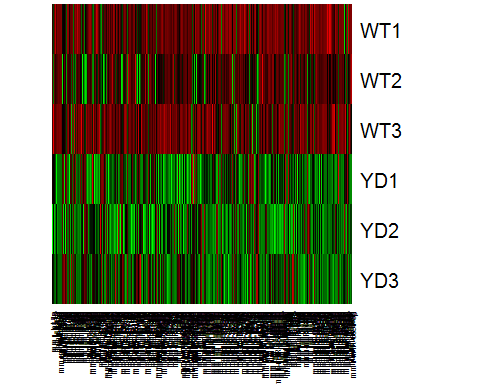
## There were 380 miRNAs profiled of which 373 were detected.  
## Min n detectable: 2

To further dissociate altered metabolism from lipodystrophy as a cause of reduced exosomal miRNAs, we compared serum miRNAs from 4-week-old control and AdicerKO mice, since at this age the metabolic phenotypes of ADicerKO mice have not yet appeared (Extended Data Figure 2c). Again, of the 380 miRNAs profiled, 373 miRNAs were detectable with 202 down-regulated in ADicerKO mice and only 23 miRNAs up-regulated when compared to littermate controls, indicating that reduction in circulating miRNAs reflects primarily the difference in miRNA processing rather than the effect of chronic lipodystrophy.

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Extended Data Figure 2c (wk4 serum)  


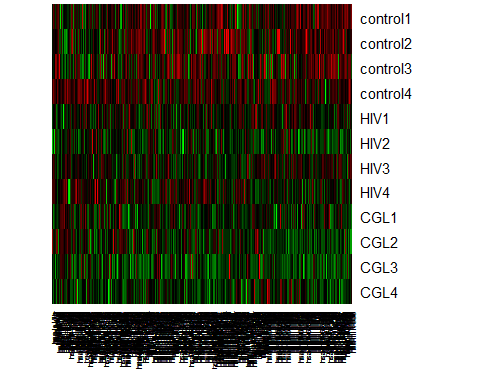
human

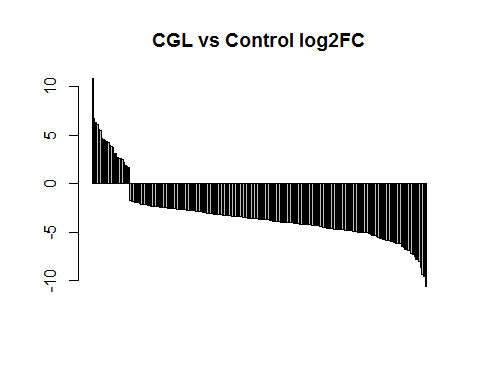
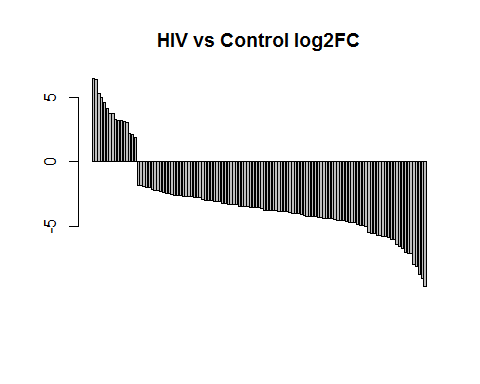
Genome-wide qPCR profiling of 572 miRNAs in exosomes from human sera revealed 119 significantly different between control and HIV lipodystrophy subjects and 213 significantly different between control and CGL subjects (Figures1e-f, Extended Data Figure 3c, Supplemental Tables 2 and 3).

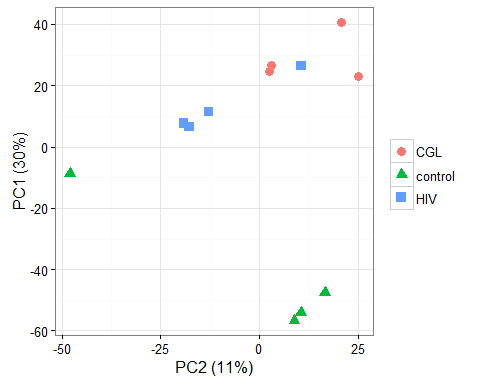
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heatmap 1e  


waterfall plots 1f  


PCA S3c  


write supp tables 2 & 3

Of these, only 5% (29 miRNAs) were upregulated in either HIV or CGL, while 217 (38%) were down-regulated in either CGL or HIV lipodystrophy, with 75 decreased in both groups (Figure 1g, Supplemental Table 4). Again, several of these miRNAs (miR-201, miR-222 and miR-16) have been previously implicated in regulation of adipose tissue.

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Thirty of the miRNAs that were decreased in serum of both patient cohorts were also decreased in the serum of the ADicerKO mice (Supplemental Table 5).

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## Adipose Tissue Transplantation Reconstitutes Circulating miRNAs in Lipodystrophic Mice

## There were 380 miRNAs profiled of which 371 were detected.  
## Min n detectable: 3

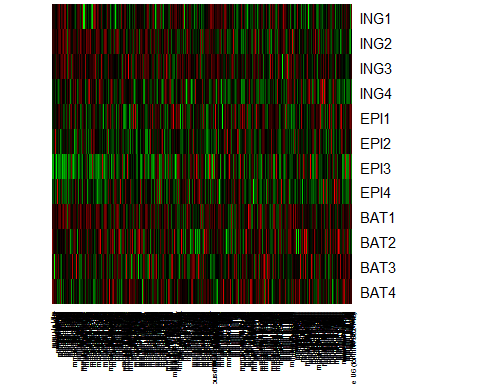
## There were 329 miRNAs profiled of which 321 were detected.  
## Min n detectable: 3

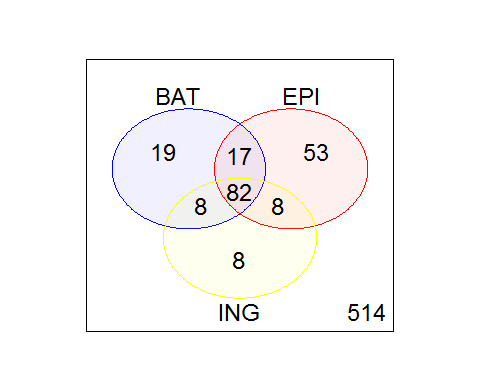
miRNA profiling of subcutaneous inguinal (Ing) WAT, intraabdominal epididymal (Epi) WAT, and interscapular BAT from the normal donor mice taken at the time of transplantation revealed distinct, depot-specific signatures consistent with previous studies (Figure 2b).

Using mean normalization from [Mestdagh et al, 2009](https://genomebiology.biomedcentral.com/articles/10.1186/gb-2009-10-6-r64).

## There were 380 miRNAs profiled of which 371 were detected.  
## Min n detectable: 3

## There were 329 miRNAs profiled of which 321 were detected.  
## Min n detectable: 3



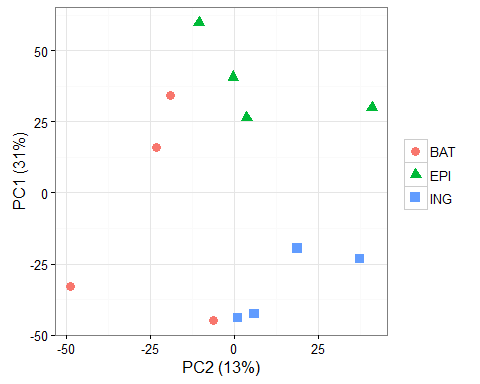
Venn of mirs over u6 (fig 2b), independent of global avg.  


Considering only the miRNAs that were expressed in at least one fat depot, 126 were highly expressed in BAT, 106 in Ing-WAT, and 160 in Epi-WAT, with 82 of these miRNAs expressed in all three depots (Figures 2b, Extended Data Figure 4a; Supplemental Table 6).

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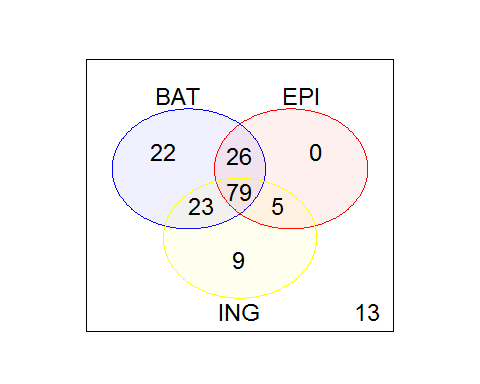
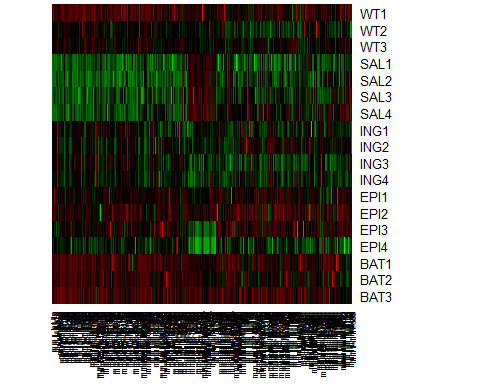
with 82 of these miRNAs expressed in all three depots.

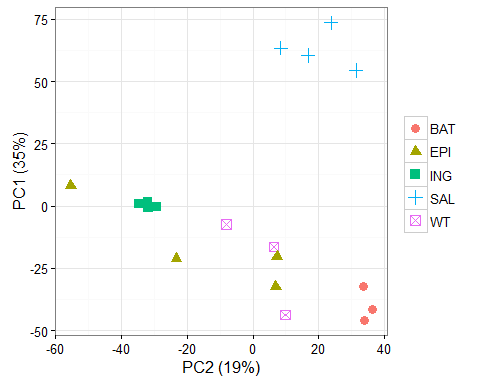
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PCA S4a  


### Transplant data

## There were 375 miRNAs profiled

As in the first cohort, in the sham-operated ADicerKO mice circulating exosomal miRNAs were markedly reduced compared to controls (Figure 2c). By comparison, ADicerKO mice that received fat transplants showed remarkable restoration of circulating exosomal miRNAs (Figures 2c and Extended Data Figure 5a; Supplemental Tables 7 and 8). 

PCA S5a  


Indeed, of the circulating exosomal miRNAs profiled, 375 revealed detectable levels of expression (Ct<34), and most of which were significantly decreased in ADicerKO serum, fat transplantation restored the levels of 150, 110, and 116 at least 50% of the way to normal in mice receiving BAT, Epi-WAT and Ing-WAT transplants, respectively.

Indeed, of the 177 circulating exosomal miRNAs that were detectable in wild-type and significantly decreased in ADicerKO serum, fat transplantation restored the levels of the majority of these at least 50% of the way to normal...

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Four miRNA candidates were identified (miR-99a, miR-99b, miR-100, and miR-466i), and three of these (miR-99a, -99b, and -100) were significantly decreased in the serum of ADicerKO mice compared to controls.

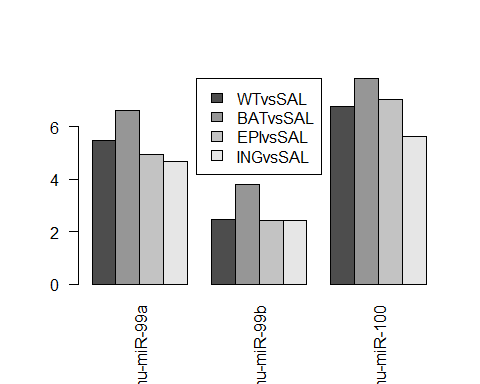
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This was consistent with the fact that these three miRNAs were also highly expressed in both BAT and WAT

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and after BAT or WAT transplantation, there was a >=50% recovery of these miRNAs in the serum of ADicerKO mice (Extended Data Figure 6b).

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Extended Data Figure 6b  


## Session Info

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## Running under: Windows 7 x64 (build 7601) Service Pack 1  
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## [2] LC\_CTYPE=English\_United States.1252   
## [3] LC\_MONETARY=English\_United States.1252  
## [4] LC\_NUMERIC=C   
## [5] LC\_TIME=English\_United States.1252   
##   
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##   
## other attached packages:  
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##   
## loaded via a namespace (and not attached):  
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