Supplementary figure legends

SUPPLEMENTARY FIGURE S1. **Lentiviral construct.** Schematic representation of the PCR fragment encompassing the genomic locus of miR-33a that was introduced in the lentiviral construct.

SUPPLEMENTARY FIGURE S2. **Reporter gene constructs.** Schematic representation of the PCR product inserted in the reporter constructs for *A*. human ABCA1, *B*. human CROT, *C*. human HADHB, *D*. human CPT1A, *E*. Drosophila melanogaster CPTI, *F*. human ATP8B1, *G*. NPC1 and *H*. SLC25A25. Numbers denote the nucleotide position in GRCh37/hg19 human and BDGP R5/dm3 drosophila genome assembly. Feathered lines denote introns, and black boxes the PCR product, the miR-33 binding site or the open reading frame (ORF) as indicated.

SUPPLEMENTARY FIGURE S3. **miR-33b is localized in the SREBP1 gene.** Schematic representation of the human SREBP1 locus with embedded miR-33b sequence including evolutionary conservation of the latter.

SUPPLEMENTARY FIGURE S4. **The binding site for miR-33 in ABCA1 is highly conserved and consists of tandem repetitions of seed-binding sites.** *A.* Evolutionary conservation of the predicted miR-33 binding site in the 3'UTR of ABCA1. Light grey indicates possibility of classical Watson-Crick pairing with miR-33 and dark grey indicates GU pairs. *B.* The predicted miR-33 binding site in the 3'UTR of ABCA1 contains three tandem repeats of a sequence perfectly complementary to miR-33 (circled). The most favorable annealing is depicted. Since mutagenesis of one, two or three of these repeats might lead to alternative site usage in a potentially suboptimal annealing, which does not allow any conclusions about normal seed binding, we decided to mutagenize all three seed sequences in parallel.

SUPPLEMENTARY FIGURE S5. **miR-33 targets ABCA1 and HADHB in mouse adrenocortical cancer cell line Y1.** Western Blot analysis of Y1 mouse adrenocortical cancer cell lines engineered to overexpress miR-33a. Note that the HADHB antibody crossreacts with a band around 45kDa in mouse samples. The band that corresponds to the size of human HADHB is marked with an arrow.

SUPPLEMENTARY FIGURE S6. **Conservation of miR-33 binding site in HADHB 3'UTR.** Evolutionary conservation of the predicted miR-33 binding site in the 3'UTR of HADHB. Light grey shading indicates a residue that can undergo a classical Watson-Crick pair with miR-33 while dark grey shading indicates GU pairs.

SUPPLEMENTARY FIGURE S7. **Conservation of miR-33 binding site in CPT1A 3'UTR.**
A. and *B.* Predicted binding of miR-33a and miR-33b to the (A) unconserved and (B) conserved miR-33 binding site in the CPT1A 3' UTR. *C.* Alignment of the conserved miR-33 binding site in *Drosophila* and *Ciona intestinalis* CPT1 gene. *D.* Predicted seed binding region in the 3'UTR of Xenopus tropicalis CPT1A. Shading represents the same as in Figure 5

SUPPLEMENTARY FIGURE S8. **Conservation of miR-33 binding site in CROT 3' UTR**
A. Evolutionary conservation of the predicted miR-33 binding site in the 3'UTR of CROT. *B.* and *C.* Predicted binding of miR-33b (B) shows two additional Watson-Crick pairs than binding to miR-33a (C). *D.* Evolutionary conservation of second miR-33 binding site in CROT 3' UTR. Shading represents the same as in Figure 5.

SUPPLEMENTARY FIGURE S9. **The splice variant carrying the predicted miR-33 binding site is dominant.** *A.* Schematic representation of the CPT1 alternative transcripts affecting the 3'UTR *B.*

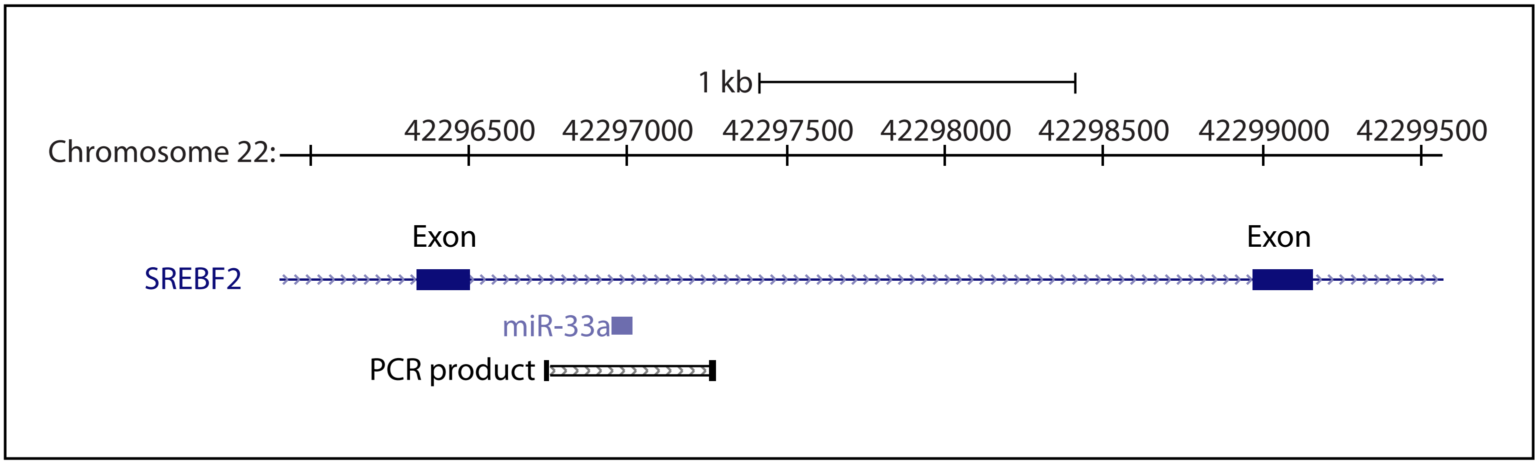
Absolute Ct values of quantitative RT PCR for transcripts with or without the miR-33 binding site in different human organs

SUPPLEMENTARY FIGURE S10. **miR-33 potentially targets additional genes involved in lipid metabolism.** *A. – D.* Evolutionary conservation of predicted binding sites in the 3'UTR of NPC1 (A and B), ATP8B1 (C) and SLC25A25 (D). Formating is identical to Figure 7. *E.* and *F.* Reporter assays to assess influence of premiR-33 on the indicated 3'UTRs cloned downstream of a constitutively active firefly luciferase cassette in the pGL3-control plasmid. Experimental setup and presentation is identical to Figure 2B.

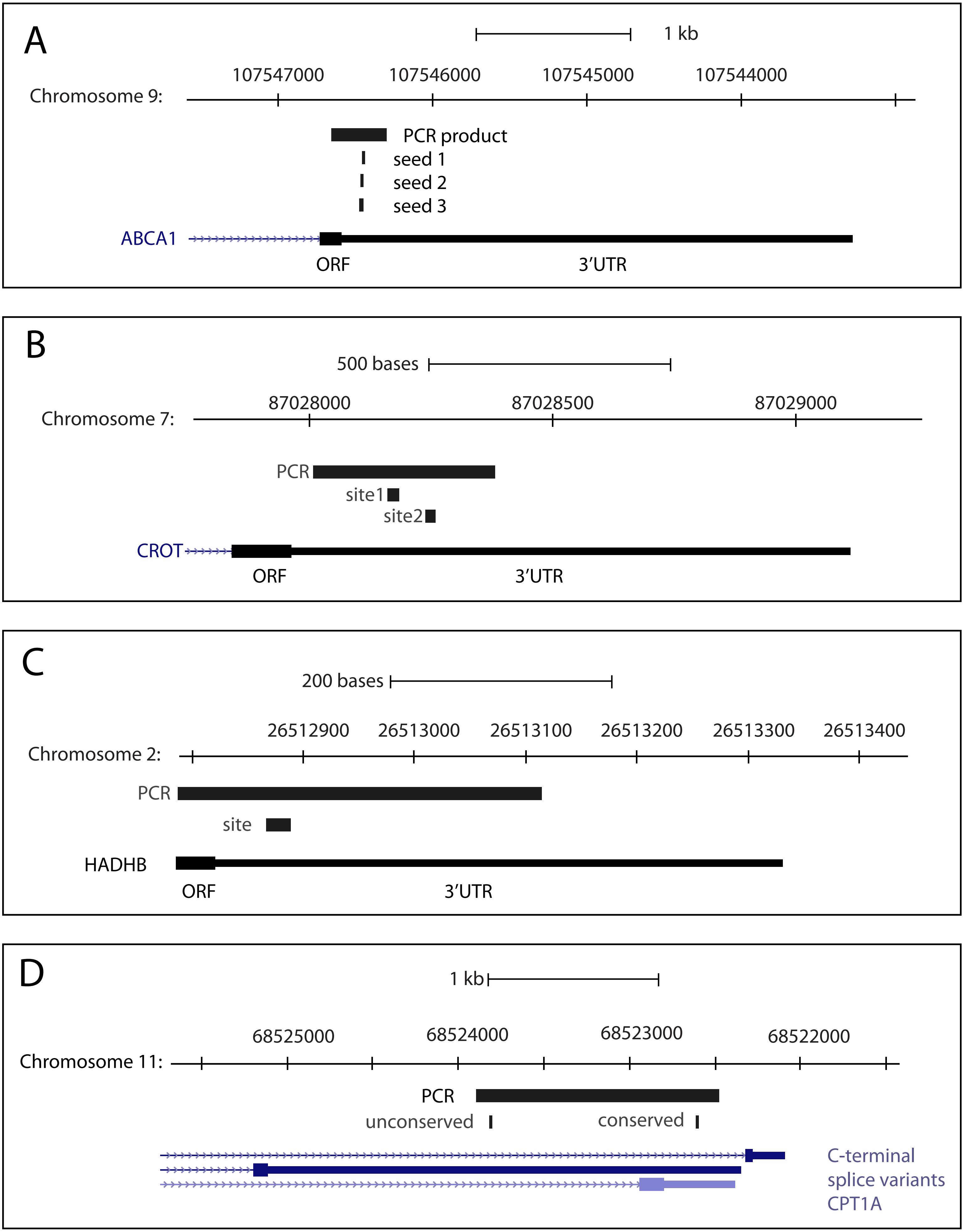
SUPPLEMENTARY FIGURE S11. **TLC analysis of cellular lipids.** Representative Thin layer chromatography scan used to quantify cellular lipids after staining with Primulin and scanning using a fluorescence scanner.

SUPPLEMENTARY FIGURE S12. **Influence of culture conditions and mouse diet on miR-33 levels.** *A.* miR-33 levels are increased upon culture at high cell density and in medium containing lipid free FCS. *B.* Northern Blot analysis of mice fed with high fat diet or control chow for 16 weeks starting at age 4 weeks. As a positive control, HepG2 overexpressing miR-33a were used. Hybridization to miR-33a LNA probe and for U6 RNA are shown. *C.* Northern Blot analysis of mice fed with high fat diet or control chow for 16 weeks starting at age 8 weeks as in (B) *D.* Northern Blot analysis of mice on chow diet, after 16h and 39h of starvation or refeeding for 12h.

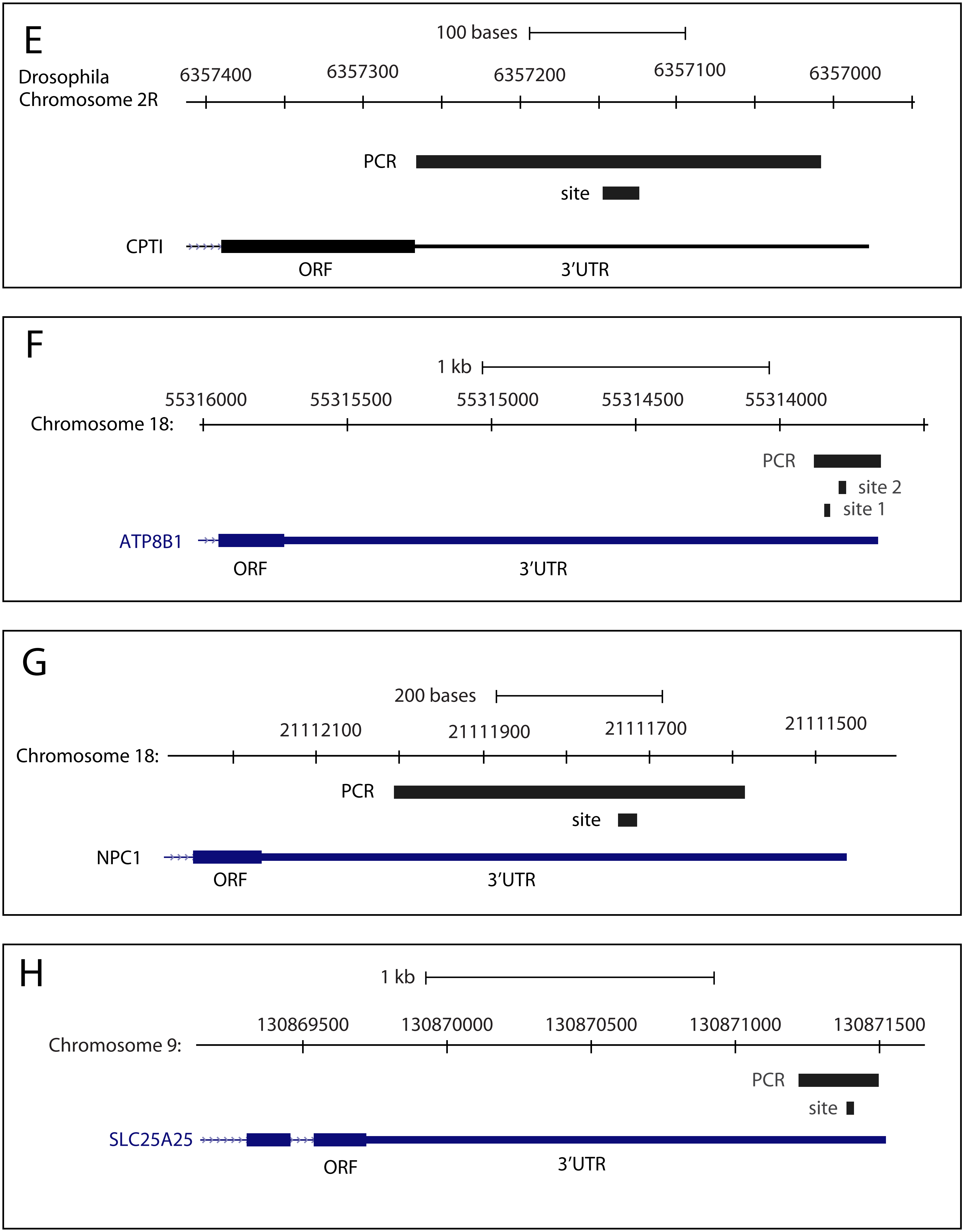
SUPPLEMENTARY FIGURE S13. **Tissue distribution of miR-33a**. Equal amounts (10μg) of mouse tissue total RNA were analyzed by northern blot for expression of miR-33a and U6. Position of mature miR-33a and premiR-33a are indicated. Ethidium Bromide staining of polyacrylamide gels is shown to demonstrate equal loading. HepG2 cell transduced with a control lentivirus (HepG2 GIPZ) and a lentivirus driving miR-33a (HepG2 miR-33a) were loaded to show the expression level of miR-33a in overexpression experiments. Please note that the HepG2 miR-33a sample on the second gel is from the same gel and exposure as the remainder of this northern blot.



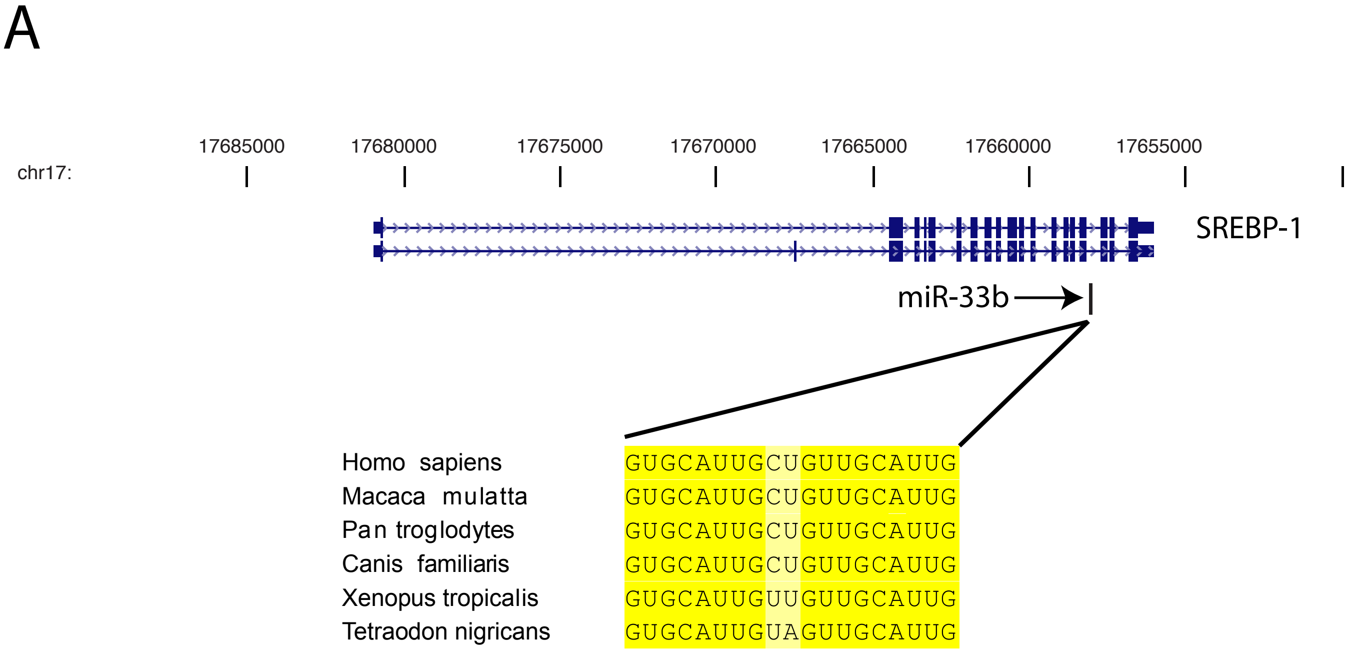
Supplemental Figure S1



Supplemental Figure S2 A-D



Supplemental Figure S2 E-H

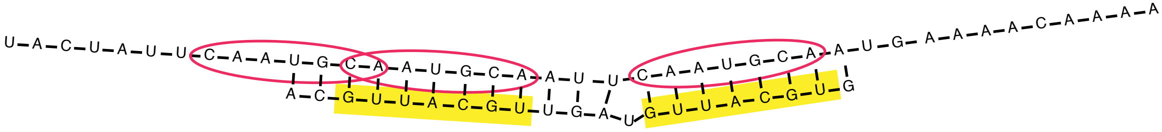


Supplemental Figure S3

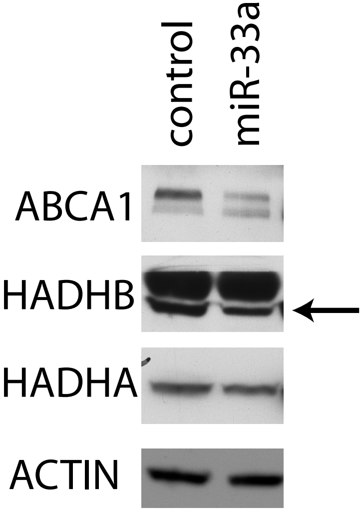
A

```
   3’ CGUUACGUUGUCGUUACGUG 5’ miR-33b
   3’ACGUUACGUUGAUGUUACGUG 5’ miR-33a
     ||||||||||:|_|||||||
UUCAAUGCAAUGCAAUU-CAAUGCAA    Homo sapiens              (ABCA1 3’UTR)
UUCAAUGCAAUGCACUU-CAAUGCAA    Mus musculus              (ABCA1 3’UTR)
UUCAAUGCAAUGCACUU-CAAUGCAA    Rattus norvegicus         (ABCA1 3’UTR)
UUCAAUGCAAUGCAAUU-CAAUGCAA    Canis familiaris          (ABCA1 3’UTR)
CUCAAUGCAAUGCAAAU-CAAUGCAA    Gallus gallus             (ABCA1 3’UTR)
AACAAACCAAUGCAAAU-CAAUGCAA    Danio rerio               (ABCA1 3’UTR)
GACAAUUCAAUGCAGA-GCAAUGCAA    Tetraodon nigroviridis    (ABCA1 3’UTR)
CUCAAUGCAAUGCAAAU-CAAUGCAA    Xenopus tropicalis        (ABCA1 3’UTR)
```

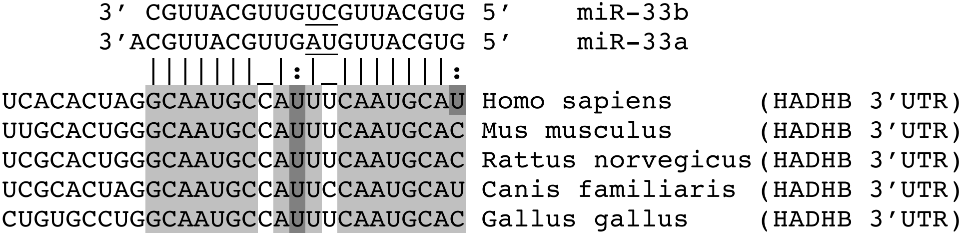
B



Supplementary Figure S4

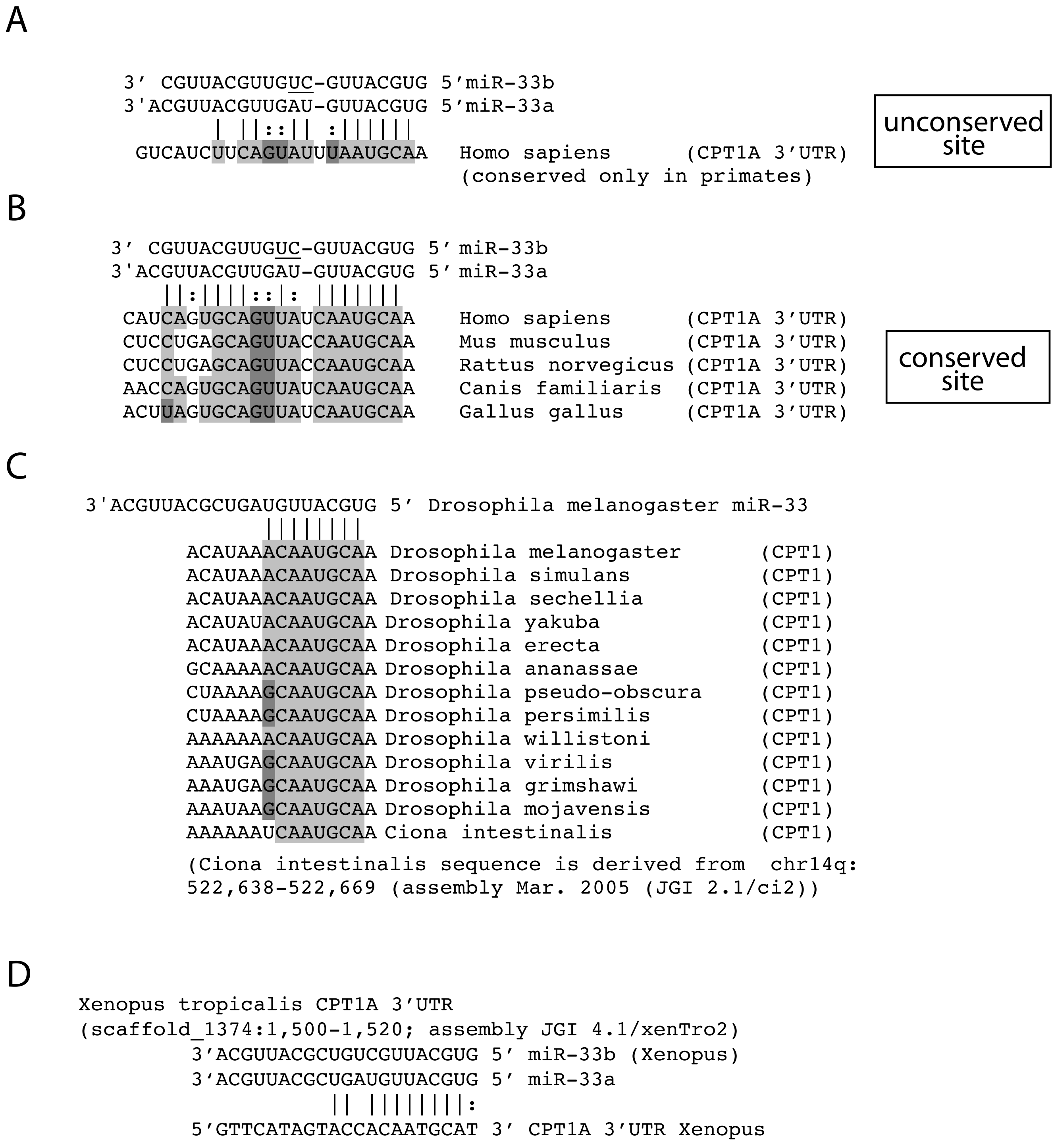


Supplemental Figure S5



```
      3’ CGUUACGUUGUCGUUACGUG 5’    miR-33b
      3’ACGUUACGUUGAUGUUACGUG 5’    miR-33a
         |||||||_|:|_|||||||:
UCACACUAGGCAAUGCCAUUUCAAUGCAU Homo sapiens     (HADHB 3’UTR)
UUGCACUGGGCAAUGCCAUUUCAAUGCAC Mus musculus     (HADHB 3’UTR)
UCGCACUGGGCAAUGCCAUUUCAAUGCAC Rattus norvegicus(HADHB 3’UTR)
UCGCACUAGGCAAUGCCAUUCCAAUGCAU Canis familiaris (HADHB 3’UTR)
CUGUGCCUGGCAAUGCCAUUUCAAUGCAC Gallus gallus    (HADHB 3’UTR)
```

Supplemental Figure S6



A

```
 3’ CGUUACGUUGUC-GUUACGUG 5’miR-33b
 3'ACGUUACGUUGAU-GUUACGUG 5’miR-33a
       | ||::|| :||||||
 GUCAUCUUCAGUAUUUAAUGCAA   Homo sapiens     (CPT1A 3’UTR)
                           (conserved only in primates)
```

B

```
3’ CGUUACGUUGUC-GUUACGUG 5’ miR-33b
3'ACGUUACGUUGAU-GUUACGUG 5’ miR-33a
   ||:||||::|: |||||||
CAUCAGUGCAGUUAUCAAUGCAA   Homo sapiens       (CPT1A 3’UTR)
CUCCUGAGCAGUUACCAAUGCAA   Mus musculus       (CPT1A 3’UTR)
CUCCUGAGCAGUUACCAAUGCAA   Rattus norvegicus  (CPT1A 3’UTR)
AACCAGUGCAGUUAUCAAUGCAA   Canis familiaris   (CPT1A 3’UTR)
ACUUAGUGCAGUUAUCAAUGCAA   Gallus gallus      (CPT1A 3’UTR)
```

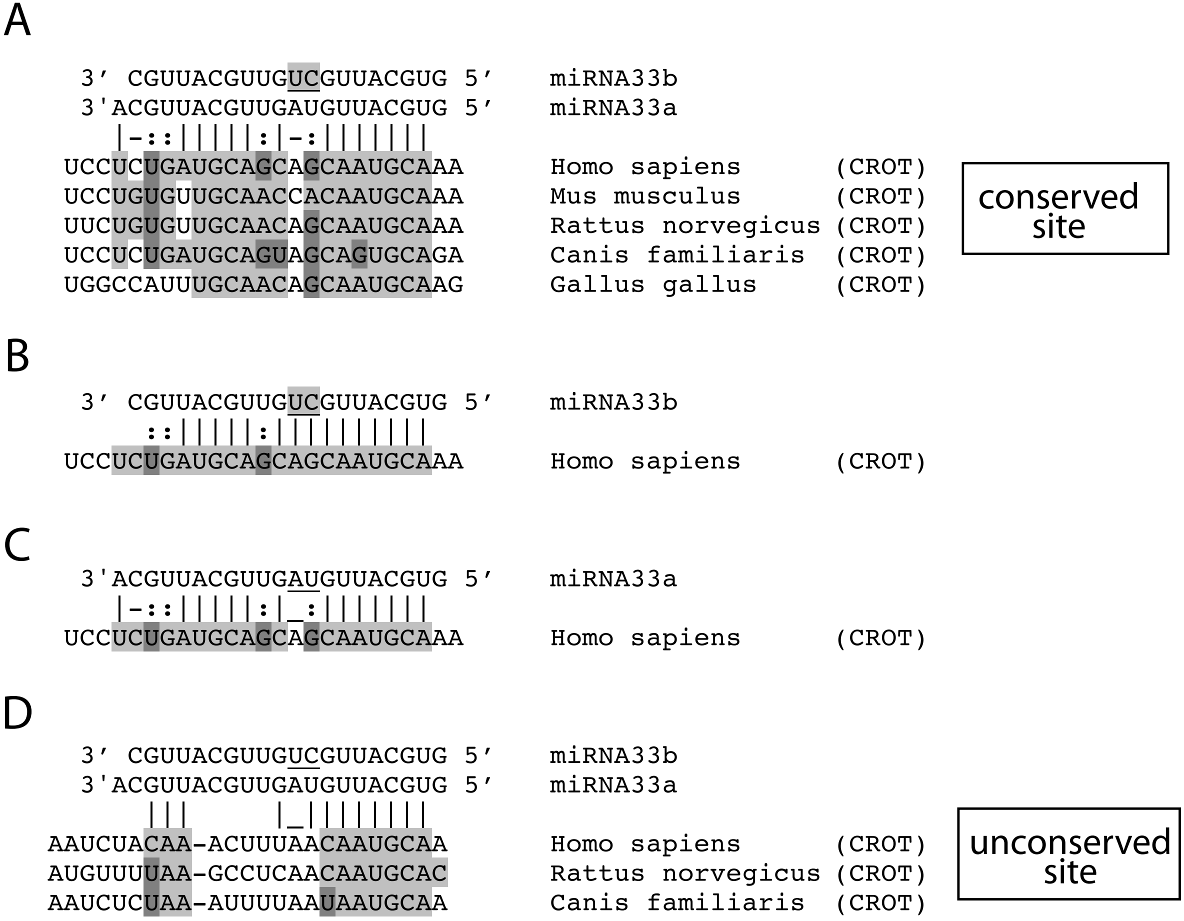
C

```
3'ACGUUACGCUGAUGUUACGUG 5’ Drosophila melanogaster miR-33
              ||||||||
        ACAUAAACAAUGCAA Drosophila melanogaster      (CPT1)
        ACAUAAACAAUGCAA Drosophila simulans          (CPT1)
        ACAUAAACAAUGCAA Drosophila sechellia         (CPT1)
        ACAUAUACAAUGCAA Drosophila yakuba            (CPT1)
        ACAUAAACAAUGCAA Drosophila erecta            (CPT1)
        GCAAAAACAAUGCAA Drosophila ananassae         (CPT1)
        CUAAAAGCAAUGCAA Drosophila pseudo-obscura    (CPT1)
        CUAAAAGCAAUGCAA Drosophila persimilis        (CPT1)
        AAAAAAACAAUGCAA Drosophila willistoni        (CPT1)
        AAAUGAGCAAUGCAA Drosophila virilis           (CPT1)
        AAAUGAGCAAUGCAA Drosophila grimshawi         (CPT1)
        AAAUAAGCAAUGCAA Drosophila mojavensis        (CPT1)
        AAAAAAUCAAUGCAA Ciona intestinalis           (CPT1)
        (Ciona intestinalis sequence is derived from  chr14q:
        522,638-522,669 (assembly Mar. 2005 (JGI 2.1/ci2))
```

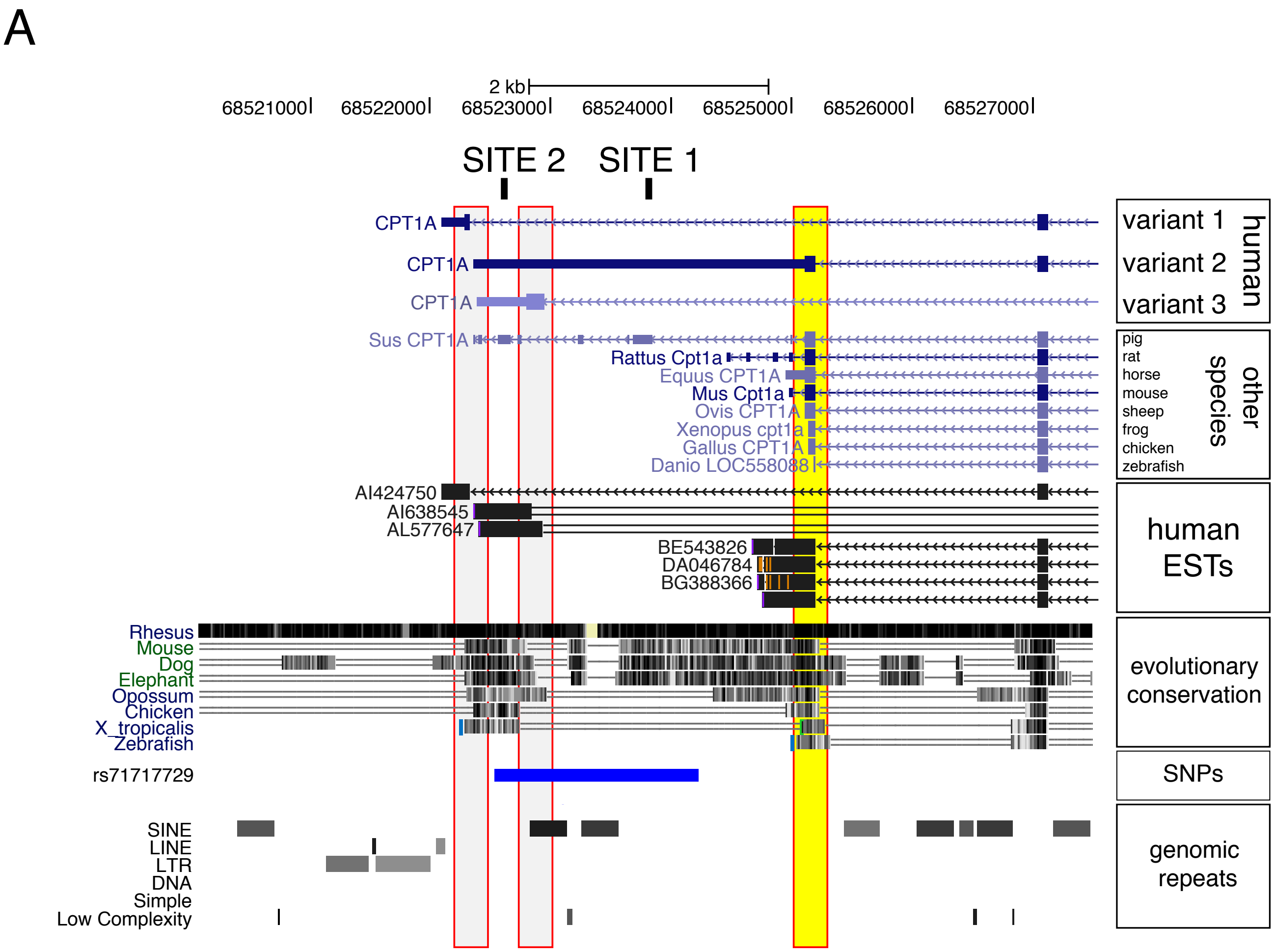
D

```
Xenopus tropicalis CPT1A 3’UTR
(scaffold_1374:1,500-1,520; assembly JGI 4.1/xenTro2)
         3’ACGUUACGCUGUCGUUACGUG 5’ miR-33b (Xenopus)
         3‘ACGUUACGCUGAUGUUACGUG 5’ miR-33a
                    || ||||||||:
         5’GTTCATAGTACCACAATGCAT 3’ CPT1A 3’UTR Xenopus
```

Supplemental Figure S7



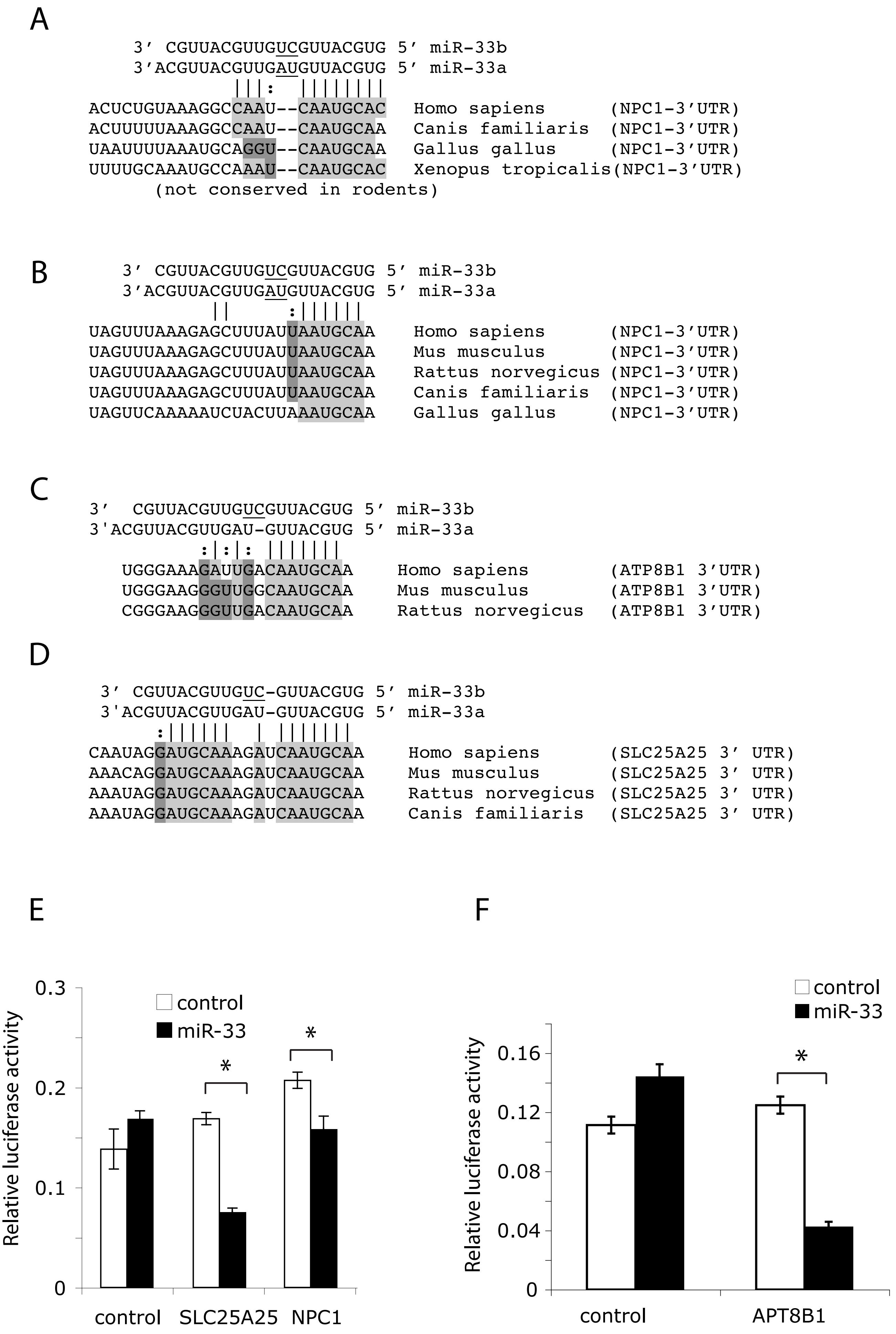
Supplemental Figure S8



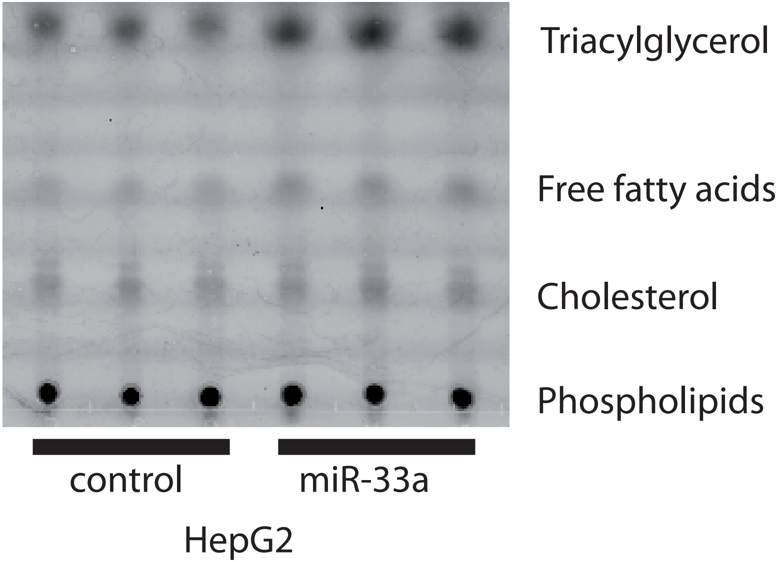
B

	variant 2	variant 1	difference in Ct
Brain	26.56	33.12	6.57
Colon	23.55	30.79	7.24
Heart	22.68	31.54	8.86
Kidney	22.57	29.92	7.36
Liver	23.19	30.94	7.75
Lung	25.56	34.21	8.66
Spleen	24.15	31.66	7.51
Testis	23.55	33.04	9.49
Thymus	24.25	32.83	8.58

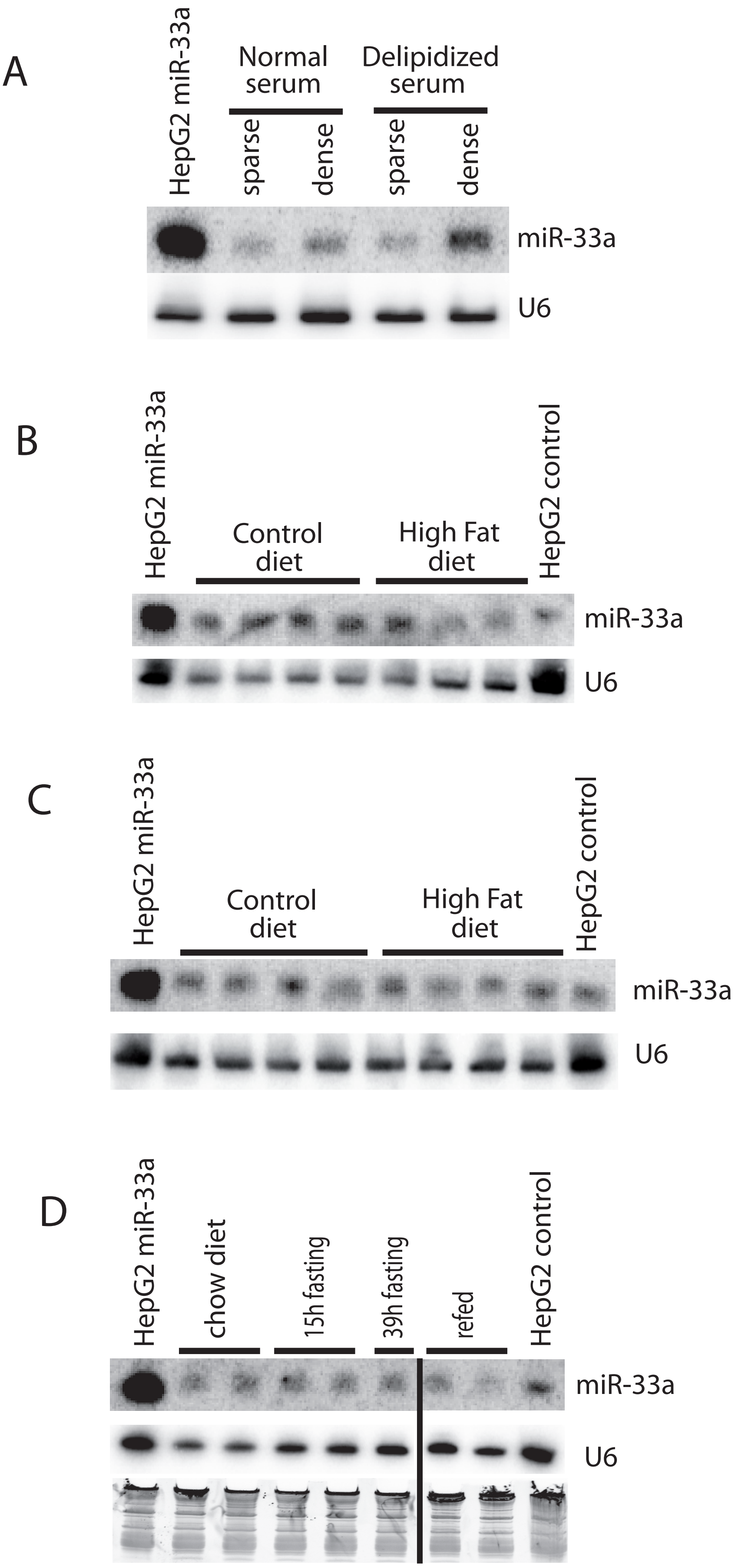
Supplemental Figure S9



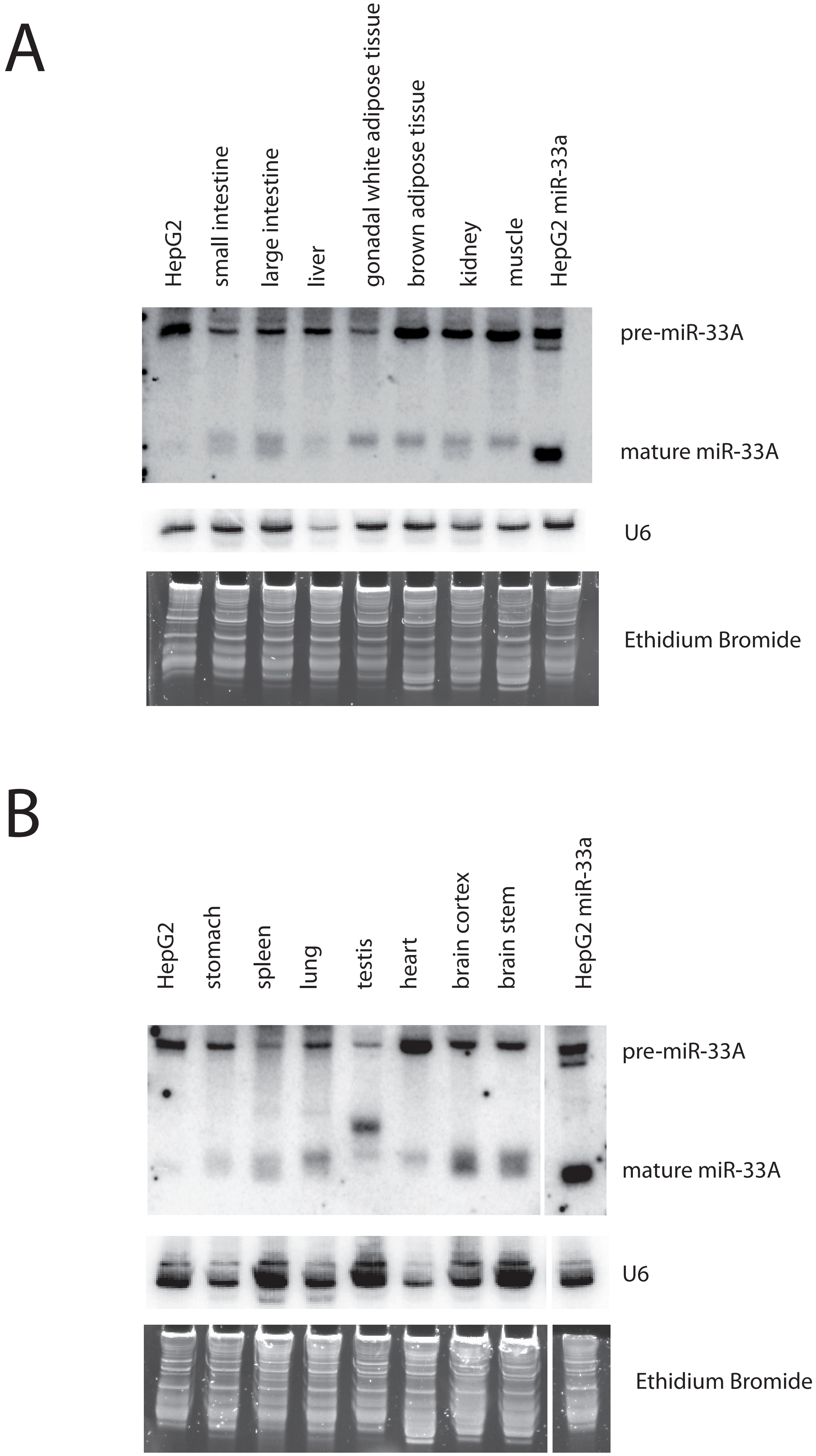
Supplemental Figure S10



Supplemental Figure S11



Supplemental figure S12



Supplemental Figure S13