Region-specific microRNA signatures in the human epididymis

James A Browne1,2,3, Shih-Hsing Leir1,2,3, Scott E Eggener4, Ann Harris1,2,3

The epithelium of the human epididymis maintains an appropriate luminal environment for sperm maturation that is essential for male fertility. Regional expression of small noncoding RNAs such as microRNAs contributes to segment-specific gene expression and differentiated functions. MicroRNA profiles were reported in human epididymal tissues but not specifically in the epithelial cells derived from those regions. Here, we reveal miRNA signatures of primary cultures of caput, corpus, and cauda epididymis epithelial cells and of the tissues from which they were derived. We identify 324 epithelial cell-derived miRNAs and 259 tissue-derived microRNAs in the epididymis, some of which displayed regionalized expression patterns in cells and/or tissues. Caput cell-enriched miRNAs included miR-573 and miR-155. Cauda cell-enriched miRNAs included miR-1204 and miR-770. Next, we determined the gene ontology pathways associated with in silico predicted target genes of the differentially expressed miRNAs. The effect of androgen receptor stimulation on miRNA expression was also investigated. These data show novel epithelial cell-derived miRNAs that may regulate the expression of important gene networks that are responsible for the regionalized gene expression and function of the epididymis.

Asian Journal of Andrology (2018) 20, 539–544; doi: 10.4103/aja.aja_40_18; published online: 24 July 2018

Keywords: caput; cauda; corpus; human epididymis; microRNA

INTRODUCTION

The epididymis epithelium supports a luminal environment that promotes sperm maturation, and each region of the duct (caput, corpus, and cauda) has a unique role in the process. These region-specific functions are maintained by distinct gene expression signatures,1–3 which are coordinated by a network of transcription factors and also by noncoding RNAs. MicroRNAs (miRNAs) are one family of small noncoding RNAs that regulate gene expression posttranscriptionally, generally by binding to specific motifs in the 3′-untranslated regions (3′UTRs) of target genes (reviewed by Bartel).4 miRNAs coordinate diverse biological processes including stem cell maintenance,5 development, metabolism,6 proliferation,7 differentiation,8 and apoptosis.9 In the epididymis, miRNAs may have tissue-specific roles and also be released from the epididymis epithelium in exosomes, which can be taken up by transiting sperm.10,11 Conditional deletion of Dicer, a critical component of the mature miRNA processing pathway, from epididymal principal cells of the mouse has a dramatic effect on the epididymis and impairs male fertility.12,13 Androgens are required for normal epididymal structure and function (reviewed by Robaire and Hamzeh14) and have been shown to regulate miRNA expression in rodents.15,16 Region-specific miRNA profiles were characterized in rodents17,18 and in whole human epididymis tissue by microarray analysis19 and RNA-seq.20 We recently described the transcriptome of human caput, corpus, and cauda epididymis tissues and primary epithelial cell cultures derived from each region,21 though miRNAs were not discussed. Here, we describe the region-specific expression of microRNAs in the caput, corpus, and cauda epididymal cells and tissues. Further, we use in silico prediction methods to identify candidate targets of several abundant miRNAs, which may directly impact regional functions of the epididymal epithelium.

PATIENTS AND METHODS

Preparation of primary cultures

Human epididymal tissue was obtained with the institutional review board permission from institutions listed in the author affiliations, and with informed consent from patients undergoing inguinal radical orchectomy for a clinical diagnosis of testicular cancer. None of the epididymides had extension of the testicular cancer, and no donors were receiving hormone or drug treatments before surgery. Efferent ducts were removed and the three anatomical regions of the epididymis: caput, corpus, and cauda, were separated and segments of each were either snap frozen in liquid nitrogen or epithelial cells were isolated and established in culture as described previously.22 For the experiments to test androgen receptor (AR) function, cells were cultured in phenol-red-free CMRL-1066 medium containing 10% fetal bovine serum (FBS), hormone depleted with dextran coated-charcoal (C6241; Sigma, St. Louis, MO, USA), for 72 h before stimulation with vehicle or the synthetic androgen R1881 (1 nmol l−1, methyltrienolone, NL005005MG; PerkinElmer, Waltham, MA, USA) for a further 16 h.
Human epididymis microRNA signatures

JA Browne et al

RNA sequencing
RNA was extracted using TRizol (Life Technologies, Carlsbad, CA, USA) as per the manufacturer’s protocol. RNA quality was confirmed by NanoDrop (NanoDrop™ One, Thermo Fisher Scientific, Waltham, MA, USA) measurement of OD 260/280 and 260/230 ratios, and the RNA was stored at ~80°C under ethanol. RNA integrity was verified by the Bioanalyzer (Agilent, Santa Clara, CA, USA), and RNA-seq libraries were prepared using the TruSeq RNA Sample Preparation Kit v2 as per the manufacturer’s Low-throughput protocol (Illumina, San Diego, CA, USA). The libraries were sequenced on Illumina HiSeq2500 machines and generated $1.9 \times 10^7$ – $3.9 \times 10^7$ reads per library from the cultured cells (95%–99% mapping to the genome) and $1.4 \times 10^7$ – $3.9 \times 10^7$ reads per library from tissues (84%–99% mapping to the genome). Data were analyzed using TopHat and Cufflinks. All data are deposited at GEO (http://www.ncbi.nlm.nih.gov/geo/GSE72986).

In silico analysis of miRNAs and target prediction
Putative miRNA targets of differentially expressed miRNAs were predicted using TargetScan 7.0 (http://www.targetscan.org/) and miRecords. Only data generated with TargetScan are presented here, as the miRecord database was incomplete with respect to miRNAs of interest. Gene ontology process enrichment analysis was performed to identify statistically significant biological processes associated with the miRNA targets (as shown by both P value and false discovery rate [FDR]).

RESULTS
Regional miRNA expression in the human epididymis
To identify the regional microRNA signature of the epididymis epithelium, we examined RNA-seq data from both cultured human epididymal epithelial (HEE) cells and tissues from the caput, corpus, and cauda segments. The majority of the 324 miRNAs identified in HEE cells (Supplementary Table 1) were expressed in more than one region, as were most of the 259 tissue miRNAs (Supplementary Table 2). Fifty-seven percent (185/324) of the HEE cell miRNAs were also present in the tissues they were derived from. Regionally restricted miRNAs were defined by differential expression of at least 2.5-fold change between caput, corpus, and cauda HEE cells and minimum gene expression levels of $0.3$ fragments per kilobase of transcript per million mapped reads (FPKM; Table 1). The same parameters were used in a comparison of differential gene expression for the miRNAs of the caput, corpus, and cauda tissues (Table 2).

Caput-enriched miRNAs of the cells and tissues
Two microRNAs (miR-573 and miR-155) were significantly more abundant in caput cells than in corpus or cauda HEE cells (A and B in Table 1). miR-30c2 was also enriched in the caput compared to corpus cells, and miR-196A1 was enhanced in the caput over the cauda cells. The most significant differentially expressed miRNAs observed in intact tissues were distinct from those seen in HEE cells, with miR-1247 more abundant in the caput tissue compared to both the corpus and cauda (A and B in Table 2). Likewise, miR-4461 was enriched in the caput in comparison to the corpus and cauda tissues though it was abundant in all tissue regions (A and B in Table 2). Sixteen other miRNAs were more highly expressed in the caput than the cauda tissue (B in Table 2).

Corpus-enriched miRNAs of the cells and tissues
Although our analysis did not identify any corpus-specific cell-derived miRNAs, four miRNAs (miR-4730, miR-196A1, miR-let7d, and miR-3916) were enriched in the corpus over the cauda cells (C in Table 1). In the tissues, miR-662 and miR-39S6 were more abundant in the corpus than either the caput or cauda.

| miRNA    | Caput | Corpus | Log2 fold change | Actual fold change | Differential |
|----------|-------|--------|------------------|--------------------|--------------|
| miR-573  | 10.28 | 0.40   | -4.68            | 25.7               | Cap>Corp     |
| miR-155  | 3.11  | 0.61   | -2.36            | 5.1                | Cap>Corp     |
| miR-30c2 | 3.92  | 1.10   | -1.83            | 3.6                | Cap>Corp     |

Three other miRNAs (miR-33b, miR-135b, and miR-3074) were enriched in the corpus over the caput tissues (A in Table 2). Multiple other tissue-derived miRNAs were enriched in the corpus over the cauda tissues, including the highly expressed miR-295, miR-141, and miR-348-let-675 (C in Table 2).

Cauda-enriched miRNAs of the cells and tissues
In HEE cells, miR-1204 showed enhanced expression in the cauda compared to both the caput and corpus cells (Table 1). H19-miR-675 was also enriched in the cauda over the corpus cells, miR-770 and miR-let7i were higher in the cauda than the caput cells (B in Table 1). In the tissues, miR-146a was more abundant in the cauda compared to the caput and corpus (B in Table 2).

Also of note, but solely in the tissues, miR-135b and miR-3074 were more abundant in both the corpus and the cauda (A in Table 2) than in the caput (B in Table 2).

In silico analysis of microRNA-regulated processes of human epididymal epithelial cells
Putative target genes of the differentially expressed miRNAs were predicted using TargetScan 7.0 (http://www.targetscan.org/). We chose this in silico prediction tool since it was one of the few such resources that were recently updated and included all the differentially expressed miRNAs in the HEE cells. Moreover, it was a robust predictive tool in our recent work on other miRNAs. Target genes ($<-0.2$ total context score) were then subjected to gene ontology process enrichment analysis using the Database for Annotation, Visualization, and Integrated Discovery (DAVID). The top 10 predicted targets and their associated processes are shown in Table 3 and 4.

| miRNA       | FPKM comparison | Log2 fold change | Actual fold change | Differential |
|-------------|-----------------|------------------|--------------------|--------------|
| miR-4730    | 2.14            | 0.00             | infinity           | infinity     | Corp>Caud   |
| miR-196A1   | 72.23           | 0.44             | -7.37              | 165.2        | Corp>Caud   |
| miR-let7d   | 12.80           | 1.64             | -2.97              | 7.8          | Corp>Caud   |
| miR-3916    | 34.98           | 9.98             | -1.81              | 3.5          | Corp>Caud   |
| miR-1204    | 7.84            | 89.13            | 3.51               | 11.4         | Corp>Caud   |
| miR-675     | 0.42            | 2.90             | 2.79               | 6.9          | Corp>Caud   |

A: caput vs corpus; B: caput vs cauda; C: corpus vs cauda; Corp: caput; Corpus: corpus; Caud: cauda; FPKM: fragments per kilobase of transcript per million mapped reads.
Human epididymis microRNA signatures

JA Browne et al

Table 2: Differentially expressed miRNAs comparing the caput, corpus and cauda epididymis tissues

| miRNA     | FPKM comparison | Log2 fold change | Actual fold change | Differential change |
|-----------|-----------------|------------------|--------------------|---------------------|
| miR-1247  | Caput 0.47       | 67.97            | infinity           | infinity            |
| miR-4461  | 5.26E+5         | -2.77            | 6.81               | Cap>Corp            |
| miR-33b   | 0.00            | 10.82            | infinity           | infinity            |
| miR-3074  | 4.43            | 21.54            | Corp>Cap           |
| miR-135b  | 4.14            | 17.61            | Corp>Cap           |
| miR-3936  | 2.72            | 6.60             | Corp>Cap           |
| miR-662   | 1.73            | 3.32             | Corp>Cap           |

A. Caput vs corpus; B: caput vs cauda; C: corpus vs cauda. miRNA FPKM: fragments per kilobase of transcript per million mapped reads

Table 3

MiRNA-regulated processes in the corpus and cauda

Although no corpus-specific HEE cell miRNAs were identified here, processes associated with the predicted targets of cauda cell-enriched miRNAs (miR-1204, miR-770, and miR-let7i) are shown in Table 4. miR-1204-associated processes include “small GTPase-mediated intracellular signaling” and “regulation of cell motion” (A in Table 4). The diverse processes associated with miR-770 included those relevant to metal ion binding (including zinc), transcription, and DNA (B in Table 4). Transcriptional regulation was also enriched in the miR-let7i-associated processes, together with multiple processes of less obvious relevance to HEE cell function including “regulation of neuron differentiation” and “blood vessel development” (C in Table 4).

Androgen-regulated miRNAs of the epididymis

To investigate whether any miRNAs were regulated by androgens in the human epididymis epithelium, caput HEE cells were treated with vehicle or 1R1881 (1 nmol L⁻¹) for 18 h and gene expression was analyzed by RNA-seq (Yang et al., manuscript in review). Eight miRNAs were differentially expressed following R1881 treatment. Of these, four miRNAs were downregulated (miR-137, miR-3074, miR-3190, and miR-3916) and four were upregulated (miR-4740, miR-506, miR-573, and miR-let7d; P = 5 × 10⁻³, q = 0.007, Table 5).

DISCUSSION

Mechanisms that control gene expression along the human epididymis epithelium are pivotal to coordinating its role in sperm maturation and male fertility. One aspect of this coordination likely involves noncoding RNAs including microRNAs. miRNAs were profiled previously in tissue samples from the human epididymis that contained many cell types. In order to focus on the specific functions of cells within the epithelial layer lining the epididymis, we established the HEE cell culture model. Here, we use RNA-seq analysis to reveal the miRNA signatures of caput, corpus, and cauda HEE cells and the tissues from which they were derived.

Tissue-derived miRNAs

Among the epididymis tissue-derived miRNAs identified in this study, we detected approximately 22.0% (116/527) and approximately 24.1% (81/336), respectively of the tissue-derived miRNAs previously reported by others. As previously noted by Bellezza et al., most of the tissue miRNAs were present in all three regions, with similar observations of HEE cell-derived miRNAs. Furthermore, most of the HEE cell-derived miRNAs were also evident in the tissues.

MicroRNA-regulated processes of the caput cells

miR-573 and miR-155, which are differentially expressed in caput HEE cells compared to corpus or cauda cells, were investigated previously in other contexts. miR-573 is downregulated by the inflammatory cytokine tumor necrosis factor (TNF)-alpha in primary human airway epithelial cells. More relevant to the male reproductive tract, miR-573 is downregulated in testicular tissue from patients with nonobstructive azoospermia. It is perhaps of relevance that we observed miR-573 among miRNAs that were upregulated in androgen-stimulated caput HEE cells. Gene ontology process enrichment analysis on the miRNA targets of miR-573 identified processes related to protein modification by small protein conjugation or removal and modification-dependent protein catabolic process, both of which might be relevant to maintenance of optimal luminal environment in the epididymis. The role of miR-155 in the male reproductive tract has yet to be explored, but it is well characterized as a pleiotropic regulator of both immunity and cancer.

some relevant to modification-dependent protein catabolic processes and as for miR-155, to the lumens of nuclei and intracellular organelles including the mitochondrion (C in Table 3).
Table 3: Predicted target genes of caput-enriched microRNAs (left) and their enriched processes (right)

| Top 10 target genes | TCS | Top 10 enriched GO terms | P   | FDR |
|---------------------|-----|--------------------------|-----|-----|
| A. miR-155-3p       |     |                          |     |     |
| 1. Zinc finger protein 140 (ZNF140) | -1.7 | 1. GO:00031974–membrane-enclosed lumen | 2.4E-09 | 3.4E-06 |
| 2. Zinc finger protein 334 (ZNF334) | -1.3 | 2. GO:0043233–organellar lumen | 2.5E-09 | 3.6E-06 |
| 3. ELL associated factor 1 (EAF1) | -1.3 | 3. GO:0006350–transcription | 1.1E-08 | 1.9E-05 |
| 4. NADH dehydrogenase ubiquinone flavoprotein 3, 10kDa (NDUFV3) | -1.0 | 4. GO:0070013–intracellular organelle lumen | 1.1E-08 | 1.6E-05 |
| 5. ZFP14 zinc finger protein (ZFP14) | -1.0 | 5. GO:0031981–nuclear lumen | 1.2E-08 | 1.7E-05 |
| 6. Mitochondrial ribosomal protein L19 (MRPL19) | -0.9 | 6. GO:0051252–regulation of RNA metabolic process | 2.6E-07 | 4.8E-04 |
| 7. Argonaute RISC catalytic component 2 (AGO2) | -0.9 | 7. GO:0045449–regulation of transcription | 3.6E-07 | 6.5E-04 |
| 8. Chromosome 12 open reading frame 49 (C12orf49) | -0.8 | 8. GO:0045449–regulation of transcription | 5.9E-07 | 1.1E-03 |
| 9. Tropomyosin 1 alpha 1 (TPM1) | -0.8 | 9. GO:0005654–nucleolus | 1.3E-06 | 1.9E-03 |
| 10. Molybdenum cofactor synthesis 2 (MOCOS2) | -0.8 | 10. GO:0005730–nucleolus | 8.7E-06 | 1.2E-02 |

| B. miR-155-5p |     |                          |     |     |
| 1. Zinc finger protein 385D (ZNF385D) | -0.8 | 1. GO:0045449–regulation of transcription | 1.5E-11 | 2.6E-08 |
| 2. ADP-ribosylation factor-like 5B (ARL5B) | -0.8 | 2. GO:0030528–transcription regulator activity | 2.2E-10 | 3.6E-06 |
| 3. Zinc finger protein 652 (ZNF652) | -0.7 | 3. GO:0046350–transcription factor activity | 4.4E-08 | 6.1E-05 |
| 4. Casein kinase 1, alpha 1 (CSNK1A1) | -0.7 | 4. GO:0046350–transcription | 6.5E-08 | 1.1E-04 |
| 5. Nedd4 family interacting protein 1 (NDF1P1) | -0.7 | 5. GO:0046357–regulation of transcription from RNA polymerase II promoter | 1.2E-07 | 2.1E-04 |
| 6. Sphingosine-1-phosphate receptor 1 (S1PR1) | -0.7 | 6. GO:0031981–nuclear lumen | 1.3E-07 | 1.7E-04 |
| 7. Cell division cycle 73 (CDC73) | -0.6 | 7. GO:0045873–positive regulation of transcription, DNA-dependent | 1.7E-07 | 2.8E-04 |
| 8. Vav 3 guanine nucleotide exchange factor (VAV3) | -0.6 | 8. GO:0051252–regulation of RNA metabolic process | 1.8E-07 | 3.0E-04 |
| 9. Teashirt zinc finger homeobox 3 (TSHZ3) | -0.6 | 9. GO:0046355–regulation of transcription, DNA-dependent | 1.8E-07 | 3.1E-04 |
| 10. V-ets avian erythroblastosis virus E26 oncogene homolog 1 (ETS1) | -0.6 | 10. GO:0051254–positive regulation of RNA metabolic process | 2.0E-07 | 3.4E-04 |

| C. miR-573 |     |                          |     |     |
| 1. Dynactin associated protein (DYNAP) | -2.4 | 1. GO:0070647–protein modification by small protein conjugation or removal | 2.4E-09 | 3.4E-06 |
| 2. Argonaute RISC catalytic component 2 (AGO2) | -2.4 | 2. GO:0032246–protein modification by small protein conjugation | 2.5E-09 | 3.6E-06 |
| 3. Solute carrier family 25, member 26 (SLC25A26) | -1.0 | 3. GO:0031974–membrane-enclosed lumen | 1.1E-08 | 1.9E-05 |
| 4. RNA binding motif protein 23 (RBM23) | -0.9 | 4. GO:0043233–organellar lumen | 1.1E-08 | 1.6E-05 |
| 5. Fanconi anemia, complementation group M (FANCM) | -0.9 | 5. GO:0019941–modification-dependent protein catabolic process | 1.2E-08 | 1.7E-05 |
| 6. Melan-A (MLANA) | -0.8 | 6. GO:0043632–modification-dependent macromolecule catabolic process | 2.6E-07 | 4.8E-04 |
| 7. Enhancer of yellow 2 homolog Drosofila (ENY2) | -0.8 | 7. GO:0019001–guanylate nucleotide binding | 3.6E-07 | 6.5E-04 |
| 8. Family with sequence similarity 104, member B (FAM104B) | -0.8 | 8. GO:0035261–guanylate ribonucleotide binding | 5.9E-07 | 1.1E-03 |
| 9. BRX1, biogenesis of ribosomes, homolog (S. cerevisiae) (BRX1) | -0.7 | 9. GO:0051603–proteolysis involved in cellular protein catabolic process | 1.3E-06 | 1.9E-03 |
| 10. Killer cell lectin-like receptor subfamily F, member 1 (KLRF1) | -0.7 | 10. GO:0005525–GTP binding | 8.7E-06 | 1.2E-02 |

TCS: total context score; FDR: false discovery rate; GO: gene ontology

MicroRNA-regulated processes of the corpus/cauda cells
The role of miR-1204 was investigated in human carcinoma cell lines, though to date there is no information on its involvement in processes relevant to the male reproductive tract. miR-770 is downregulated in testicular tissue from patients with nonobstructive azoospermia. miR-let7i negatively regulates cardiac inflammation and fibrosis and is downregulated in the serum from ovarian cancer patients. However, deciphering its role in the epididymis will require further study.

Androgen-regulated miRNAs in the caput epididymis
Androgens are important for epididymal epithelial structure and function (reviewed by Robaire and Hamzeh) and regulate their effects via the AR. We previously showed enrichment of AR protein in caput HEE cells and its nuclear accumulation in these cells in response to R1881. Hence, we also examined the effect of R1881 on miRNA expression in caput HEE cells. Among the miRNAs that were differentially regulated in response to R1881, miR-137 is of interest since this miRNA is differentially expressed after androgen treatment in the prostate adenocarcinoma cell line, LnCaP. Of note, in LnCaP cells, androgen treatment increases miR-137 in contrast to the repression observed here in HEE cells. However, the overamplification of AR in LnCaP cells compared to normal prostate epithelium and the strong context-dependence of AR cofactors could account for these differences. Another androgen-regulated miRNA in the caput epididymis is miR-506, which plays a complex role in cancer. It is oncogenic in melanomas, a tumor suppressor in ovarian cancer, and confers chemoresistance in colon cancer.

In conclusion, our data suggest that further investigation of regionalized miRNA expression along the epididymis may contribute to the understanding of mechanisms controlling segment-specific epididymal epithelial function.
### Table 4: Predicted target genes of cauda-enriched microRNAs (left) and their enriched processes (right)

| Top 10 target genes | TCS | Top 10 enriched GO terms | P     | FDR  |
|---------------------|-----|-------------------------|-------|------|
| **A. miR-1204**     |     |                         |       |      |
| Von Willebrand factor A domain containing 1 (VWA1) | −1.4 | 1. GO:0007242–intracellular signaling cascade | 3.2E-04 | 5.7E-01 |
| N-acetyltransferase 8-like (GCN5-related, putative) (NATBL) | −1.2 | 2. GO:0051270–regulation of cell motion | 1.2E-03 | 2.1E-00 |
| Solute carrier family 25 (mitochondrial carrier, dicarboxyrate transporter), member 10 (SLC25A10) | −1.2 | 3. GO:0030036–actin cytoskeleton organization | 1.2E-03 | 2.2E-00 |
| Mitochondrial dicarboxylate carrier, Uncharacterized protein; cDNA FLJ60124 (ENSG00000262660) | −1.1 | 4. GO:0051056–regulation of small GTPase signal transduction | 2.0E-03 | 3.5E+00 |
| Leucine-rich repeat containing 56 (LRRC56) | −1.1 | 5. GO:0030030–cell projection organization | −0.8 | 0.76 |
| Zinc finger, AN1-type domain 3 (ZFAND3) | −1.0 | 6. GO:0051129–negative regulation of cellular component organization | 2.4E-03 | 4.1E+00 |
| Coiled-coil domain containing 127 (CCDC127) | −1.0 | 7. GO:0030029–actin filament-based process | −2.9 | −6.17 |
| Matrix metallopeptidase 11 (stromelysin 3) (MMP11) | −0.9 | 8. GO:0030036–actin cytoskeleton organization | −3.5E-03 | 10.3 |
| Protein ITFG3; Uncharacterized protein; cDNA FLJ60496 | −0.9 | 9. GO:0005794–Golgi apparatus | 2.8E-03 | 4.1E+00 |
| Transmembrane protein 141 (TMEM141) | −0.8 | 10. GO:0005089–Rho guanyl-nucleotide exchange factor activity | 2.9E-03 | 4.3E+00 |
| **B. miR-770-5p**   |     |                         |       |      |
| Zinc finger, DHHC-type containing 11 (ZDHHC11) | −2.9 | 1. GO:0008270–zinc ion binding | 3.6E-09 | 5.6E-06 |
| Zinc finger protein 138 (ZNF138) | −1.5 | 2. GO:0046914–transition metal ion binding | 2.23E-08 | 3.4E-05 |
| Zinc finger protein 83 (ZNF83) | −1.4 | 3. GO:0006355–regulation of transcription, DNA-dependent | 6.03E-07 | 1.1E-03 |
| Glia maturation factor, beta (GMFB) | −1.1 | 4. GO:0006350–transcription, DNA-dependent | 6.15E-07 | 1.1E-03 |
| Zinc finger protein 480 (ZNF480) | −1.0 | 5. GO:0003677–DNA binding | 1.24E-06 | 1.9E-03 |
| Zinc finger protein 140 (ZNF140) | −0.9 | 6. GO:0051252–regulation of RNA metabolic process | 2.00E-06 | 3.5E-03 |
| Zinc finger protein 107 (ZNF107) | −0.9 | 7. GO:0045449–regulation of transcription | 3.02E-06 | 5.4E-03 |
| Motor neuron and pancreas homeobox 1 (MNX1) | −0.8 | 8. GO:0005794–Golgi apparatus | 8.52E-05 | 1.2E-01 |
| Zinc finger protein 616 (ZNF616) | −0.8 | 9. GO:0070013–intracellular organelle lumen | 2.46E-04 | 3.5E-01 |
| Zinc finger protein 676 (ZNF676) | −0.8 | 10. GO:0043233–organelle lumen | 2.49E-04 | 3.5E-01 |
| **C. miR-let-7i-3p** |     |                         |       |      |
| Histone cluster 3, H3 (HIST3H3) | −0.9 | 1. GO:0001568–blood vessel development | 4.7E-04 | 7.8E-01 |
| Calcium/calmodulin-dependent protein kinase II inhibitor 1 (CAMK2I1) | −0.9 | 2. GO:0048514–blood vessel morphogenesis | 4.8E-04 | 8.0E-01 |
| Peptidylprolyl isomerase (cyclophilin)-like 4 (PPI4L4) | −0.8 | 3. GO:0001944–vasculature development | 5.9E-04 | 9.8E-01 |
| IQ motif containing B1 (IQCB1) | −0.8 | 4. GO:0045893–positive regulation of transcription, DNA-dependent | 1.3E-03 | 2.2E+00 |
| Coiled-coil domain containing 151 (CCDC151) | −0.8 | 5. GO:0007411–axon guidance | 1.4E-03 | 2.3E+00 |
| Ribonuclease, RNase A family, 7 (RNASE7) | −0.8 | 6. GO:0051254–positive regulation of RNA metabolic process | 1.5E-03 | 2.4E+00 |
| Rab, member of RAS oncogene family-like 3 (RABL3) | −0.7 | 7. GO:0045446–endothelial cell differentiation | 1.8E-03 | 3.0E+00 |
| Paraspeckle component 1 (PSPC1) | −0.7 | 8. GO:0030030–cell projection organization | 1.9E-03 | 3.2E+00 |
| X-prolyl aminopeptidase (aminopeptidase P) 3, putative (XPNPPEP3) | −0.7 | 9. GO:0031175–neuron projection development | 2.3E-03 | 3.8E+00 |
| Fatty acyl CoA reductase 1 (FAR1) | −0.7 | 10. GO:0043065–positive regulation of apoptosis | 3.1E-03 | 5.0E+00 |

**Table 5: Differentially expressed microRNAs in caput human epididymal epithelial cells after R1881 treatment compared to vehicle**

| miRNAs | Vehicle | R1881 | Log2 fold change | Actual fold change |
|--------|---------|-------|------------------|-------------------|
| miR-137 | 4.55    | 0.06  | −6.17            | 72.2              |
| miR-4740 | 24.04 | 1.55  | −3.95            | 15.5              |
| miR-let7d | 22.58 | 1.96  | −3.53            | 11.5              |
| miR-125a | 3.85  | 23.98 | 2.64             | 6.2               |
| miR-573 | 0.76   | 7.81  | 3.36             | 10.3              |
| miR-3177 | 1.52  | 18.16 | 3.58             | 11.9              |

Average of 4 RNA-seq replicates. HEE: human epididymal epithelial; miRNAs: microRNAs.

**AUTHOR CONTRIBUTIONS**

JAB, SHL, SEE, and AH acquired, analyzed, and interpreted data. JAB and AH wrote the manuscript. All authors read and approved the final manuscript.

**COMPETING INTERESTS**

All authors declared no competing interests.

**ACKNOWLEDGMENTS**

This work was supported by the NIH grant R01HD068901 (PI: AH). We are grateful to the anonymous men with testicular cancer who consented to provide epididymis tissue for these studies. We thank Dr. Rui Yang for bioinformatic analysis.

Supplementary Information is linked to the online version of the paper on the Asian Journal of Andrology website.
REFERENCES

1. Jiang Z, Liu Q, Li YM, Hall SH, French FS, et al. Genome-wide profiling of segmental-regulated transcripts in human epididymis using oligo microarray. Mol Cell Endocrinol 2006; 250: 169–77.

2. Thimon X, Koukoul G, Calvo E, Sullivan R. Region-specific gene expression profiling along the human epididymis. Mol Hum Reprod 2007; 13: 691–704.

3. Browe JA, Yang R, Leir SH, Eggener SE, Harris A. Expression profiles of human epididymis epithelial cells reveal the functional diversity of caput, corpus and cauda regions. Mol Hum Reprod 2016; 22: 69–82.

4. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136: 215–33.

5. Ganganuru VK, Lin H. MicroRNAs: key regulators of stem cells. Nat Rev Mol Cell Biol 2009; 10: 116–25.

6. Rottiers V, Naar AM. MicroRNAs in metabolism and metabolic disorders. Nat Rev Mol Cell Biol 2012; 13: 239–50.

7. Hwang HW, Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer 2006; 94: 776–80.

8. Zeng ZL, Lin XL, Tan LL, Liu YM, Qu K, et al. MicroRNAs: important regulators of induced pluripotent stem cell generation and differentiation. Stem Cell Rev 2018; 14: 71–81.

9. Su Z, Yang Z, Xu Y, Chen Y, Yu Q. MicroRNAs in apoptosis, autophagy and necrosis. Oncotarget 2015; 6: 8474–90.

10. Sullivan R. Epididymosomes: role of extracellular microvesicles in sperm maturation. Front Biosci (Schol Ed) 2016; 8: 106–14.

11. Belleaune C. Extracellular microRNAs from the epididymis as potential mediators of cell-to-cell communication. Asian J Androl 2015; 17: 730–6.

12. Bjorkgren I, Saastamoinen L, Krukkhi A, Huhtaniemi I, Poutanen M, et al. Dicer1 ablation in the mouse epididymis causes de-differentiation of the epithelium and imbalance in sex steroid signaling. PLOS One 2012; 7: e38457.

13. Bjorkgren I, Gylling H, Turenne H, Huhtaniemi I, Strauss L, et al. Imbalanced lipid homeostasis in the conditional Dicer1 knockout mouse epididymis causes instability of the sperm membrane. FASEB J 2015; 29: 433–42.

14. Robaire B, Hamzeh M. Androgen action in the epididymis. J Androl 2011; 32: 992–2.

15. Wang G, Wang Y, Feng W, Wang X, Yang JY, et al. Transcription factor and microRNA regulation in androgen-dependent and -independent prostate cancer cells. BMC Genomics 2008; 9 Suppl 2: S22.

16. Shi XB, Xue L, Yang J, Ma AH, Zhao J, et al. An androgen-regulated miRNA suppresses Bax1 expression and induces androgen-independent growth of prostate cancer cells. Proc Natl Acad Sci U S A 2007; 104: 1998–83.

17. Nixon B, Stanger SJ, Mihalas BP, Reilly JN, Anderson AL, et al. Next generation sequencing analysis reveals segmental patterns of microRNA expression in mouse epididymal epithelial cells. PLOS One 2015; 10: e0135605.

18. Chu C, Zheng G, Hu S, Zhang J, Xie S, et al. Epididymal region-specific microRNA expression and DNA methylation and their roles in controlling gene expression in rats. PLOS One 2015; 10: e0124450.

19. Belleaune C, Calvo E, Thimon V, Cyt DG, Legare C, et al. Role of microRNAs in controlling gene expression in different segments of the human epididymis. PLOS one 2012; 7: e34996.

20. Li Y, Wang HY, Wan FC, Liu FJ, Liu J, et al. Deep sequencing analysis of small non-coding RNAs reveals the diversity of microRNAs and piRNAs in the human epididymis. Gene 2012; 497: 330–5.

21. Leir SH, Browe JA, Eggener SE, Harris A. Characterization of primary cultures of adult human epididymis epithelial cells. Fertil Steril 2015; 103: 647–54.e1.

22. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, et al. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and cufflinks. Nat Protoc 2012; 7: 562–78.

23. Agarwal V, Bell GW, Nam JW, Bartel DP. Predictive effective microRNA target sites in mammalian miRNAs. Elife 2015; 4: e05005.

24. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005; 120: 15–20.

25. Xiao F, Zuo Z, Cai G, Kang S, Gao X, et al. miRecords: an integrated resource for microRNA-target interactions. Nucleic Acids Res 2009; 37: D105–10.

26. Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009a; 37: 1–13.

27. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009b; 4: 44–57.

28. Gillen AE, Gosalia N, Leir SH, Harris A. MicroRNA regulation of expression of the cystic fibrosis transmembrane conductance regulator gene. Biochem J 2011; 438: 25–32.

29. Stolzenburg LR, Wachtel S, Dang H, Harris A. miR-1343 attenuates pathways of fibrosis by targeting the TGF-beta receptors. Biochem J 2016; 473: 245–56.

30. Stolzenburg LR, Harris A. Microvesicle-mediated delivery of miR-1343: impact on markers of fibrosis. Cell Tissue Res 2018; 371: 325–38.

31. Zhang J, Liu Q, Zhang W, Li J, Li Z, et al. Comparative profiling of genes and miRNAs expressed in the newborn, young adult, and aged human epididymes. Acta Biochim Biophys Sin (Shanghai) 2010; 42: 145–53.

32. Huang Y, Crawford M, Higuíta-Castro N, Nana-Sinkam P, Ghadiali SN. miR-146a regulates mecanotransduction and pressure-induced inflammation in small airway epithelium. FASEB J 2012; 26: 3351–64.

33. Lian J, Zhang X, Tian H, Liang N, Wang Y, et al. Altered microRNA expression in patients with non-obstructive azoospermia. Reprod Biol Endocrinol 2009; 7: 13.

34. Klüwer J, Poppema S, de Jong D, Blokzijl T, Harms G, et al. BIC and miR-155 are highly expressed in Hodgkin, primary mediastinal and diffuse large B cell lymphomas. J Pathol 2005; 207: 243–9.

35. O’Connor RM, Rao DS, Chaudhuri AA, Baldin MP, Tagadan KV, et al. Sustained expression of microRNA-155 in hematopoietic stem cells causes a myeloproliferative disorder. J Exp Med 2008; 205: 585–94.

36. Yamanaka Y, Tagawa H, Takahashi N, Watanabe A, Guo YM, et al. aberrant overexpression of microRNAs activate AKT signaling via down-regulation of tumor suppressors in natural killer-cell lymphoma/leukemia. Blood 2009; 114: 3265–75.

37. Peng X, Cao P, Li J, He D, Han S, et al. MiR-1204 sensitizes nasopharyngeal carcinoma cells to paclitaxel both in vitro and in vivo. Cancer Biol Ther 2015; 16: 261–7.

38. Barsotti AM, Beckerman R, Laptenko O, Huppi K, Caplen NJ, et al. p53-dependent induction of PTN1 and miR-1204. J Biol Chem 2012; 287: 2599–19.

39. Wang X, Wang HX, Li YL, Zhang CC, Zhou CY, et al. MicroRNA Let-7i negatively regulates cardiac inflammation and fibrosis. Hypertension 2015; 66: 776–85.

40. Langhe R, Norris L, Saadeh FA, Blackshields G, Varley R, et al. A novel serum microRNA panel to discriminate benign from malignant ovarian disease. Br J Cancer 2009; 101: 116–25.

41. Nisson EM, Laurosen KB, Whitchurch J, McWilliam A, Oudem N, et al. MiR-137 is an androgen regulated repressor of an extended network of transcriptional coregulators. Oncotarget 2015; 6: 35710–25.

42. Streicher KL, Zhu W, Lehmann KP, Georgantas RW, Morehouse CA, et al. A novel oncogenic role for the miRNA-506-514 cluster in initiating melanocyte transformation and promoting melanoma growth. Oncogene 2012; 31: 1558–70.

43. Yang D, Sun Y, Hu L, Zheng H, Ji P, et al. Integrated analyses identify a master microRNA regulatory network for the mesenchymal subtype in serous ovarian cancer. Cancer cell 2013; 23: 186–99.

44. Tong JL, Zhang CP, Nie F, Xu XT, Zhu MM, et al. MicroRNA 506 regulates expression of PPARG alpha in hydroxycamptothecin-resistant human colon cancer cells. FEBS Lett 2011; 585: 3560–8.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.
Supplementary Table 1: List of microRNAs in primary human epididymal epithelial cells (fragments per kilobase of transcript per million mapped reads [FPKM] >0.1)

| miRNAs                              | Caput cell | Corpus cell | Cauda cell |
|-------------------------------------|------------|-------------|------------|
| miR-4461                            | 1.7E+05    | 1.8E+05     | 1.6E+05    |
| miR-548A2                           | 1966.26    | 0.00        | 0.00       |
| miR-3648, miR-3687                   | 1671.15    | 957.40      | 1148.85    |
| miR-3661                            | 1102.61    | 1940.27     | 1845.95    |
| miR-1281                            | 1101.70    | 387.63      | 1734.55    |
| miR-1282                            | 1014.31    | 924.35      | 847.09     |
| miR-4426, RPS27A                     | 777.37     | 652.50      | 691.22     |
| miR-1244-3, PTMA                     | 711.12     | 298.98      | 326.28     |
| miR-1279                            | 573.42     | 272.52      | 146.82     |
| miR-1304, SNORA1, SNORA18, SNORA25, SNORA32, SNORA40, SNORA8, SNORD5, SNORD6, TAF1D | 526.49 | 447.01 | 460.93 |
| miR-3918                            | 444.70     | 89.37       | 33.48      |
| miR-612, NEAT1                       | 395.09     | 323.26      | 298.88     |
| miR-762                             | 281.14     | 261.52      | 474.86     |
| miR-611, TMEM258                     | 274.16     | 161.24      | 219.19     |
| miR-3658, UCK2                       | 274.04     | 11.72       | 11.40      |
| miR-663A                            | 261.77     | 474.04      | 606.03     |
| miR-4750                            | 247.77     | 0.00        | 0.00       |
| miR-1307, USMG5                      | 237.72     | 299.19      | 290.34     |
| miR-4722                            | 201.60     | 250.98      | 0.00       |
| miR-3650                            | 201.11     | 0.00        | 0.00       |
| miR-4489                            | 182.15     | 0.00        | 0.00       |
| miR-4635                            | 168.09     | 0.00        | 68.57      |
| miR-4728                            | 164.83     | 106.89      | 0.00       |
| miR-497HG                           | 164.77     | 86.98       | 106.67     |
| miR-1914, miR-647, UCKL1             | 163.52     | 76.81       | 99.04      |
| miR-4709, NPC2                       | 147.87     | 96.90       | 106.94     |
| miR-4273                            | 144.19     | 125.18      | 42.10      |
| miR-1229                            | 134.65     | 0.00        | 131.72     |
| miR-4517, NFATC2IP                   | 128.76     | 106.77      | 15.63      |
| miR-3605, PHC2                       | 124.66     | 91.17       | 99.54      |
| miR-4746                            | 124.34     | 31.59       | 32.04      |
| miR-4721, TUFM                       | 115.52     | 92.37       | 89.91      |
| miR-1292, NOP56, SNORD86             | 112.86     | 179.88      | 75.91      |
| miR-4449                            | 111.43     | 51.91       | 52.65      |
| miR-4784                            | 108.83     | 38.87       | 57.11      |
| miR-25                              | 107.56     | 12.16       | 54.35      |
| miR-4700, UNC119B                    | 105.79     | 20.00       | 41.59      |
| miR-4263                            | 101.36     | 155.52      | 60.15      |
| miR-4292                            | 100.02     | 121.06      | 336.41     |
| miR-4516                            | 99.53      | 108.04      | 150.05     |
| miR-4785                            | 94.02      | 170.49      | 153.80     |
| miR-637                             | 93.21      | 36.40       | 72.98      |
| miR-103B2                           | 91.08      | 0.00        | 128.09     |
| miR-639, TECR                        | 90.46      | 78.54       | 85.37      |
| miR-631                             | 88.55      | 0.00        | 0.00       |
| miR-21, VMP1                         | 86.68      | 60.45       | 63.40      |
| miR-3684                            | 86.61      | 0.00        | 0.00       |
| miR-1260B                           | 86.36      | 29.21       | 62.99      |
| miR-636, SRSF2                       | 82.81      | 63.90       | 89.84      |
| miR-632, ZNF207                      | 82.38      | 122.83      | 112.16     |
| miR-4492                            | 82.05      | 53.39       | 98.15      |
| miR-1226                            | 80.00      | 0.00        | 22.94      |
| miR-3917, STMN1                      | 77.23      | 47.28       | 46.43      |
| miR-1968                            | 73.74      | 76.55       | 32.98      |
| miR-4440                            | 67.99      | 5.78        | 10.01      |

Contd...
Supplementary Table 1: Contd...

| miRNAs                      | Caput cell | Corpus cell | Cauda cell |
|-----------------------------|------------|-------------|------------|
| miR-135A1                   | 64.30      | 0.00        | 23.67      |
| miR-1248, SNORA81           | 62.48      | 33.60       | 16.85      |
| miR-210                     | 61.67      | 13.90       | 14.34      |
| miR-1251                    | 61.23      | 0.00        | 0.00       |
| miR-4680, PDCD4             | 61.21      | 14.30       | 14.34      |
| miR-641                     | 60.96      | 17.72       | 16.81      |
| miR-3615, SLC9A3R1          | 57.49      | 21.56       | 21.93      |
| miR-4441                    | 54.35      | 33.48       | 5.35       |
| miR-3607                    | 54.25      | 0.00        | 17.09      |
| miR-4647, SLC35B2           | 52.83      | 40.44       | 40.56      |
| miR-661                     | 51.10      | 0.00        | 0.00       |
| miR-3665                    | 50.96      | 0.00        | 0.00       |
| miR-937, SCRIB              | 50.60      | 52.30       | 18.96      |
| miR-3191                    | 50.36      | 57.65       | 70.64      |
| miR-24-1                    | 49.35      | 42.02       | 0.00       |
| miR-553                     | 45.10      | 42.02       | 42.62      |
| miR-4253                    | 45.10      | 0.00        | 0.00       |
| miR-200C                    | 45.10      | 0.00        | 0.00       |
| miR-4312                    | 44.95      | 20.94       | 53.39      |
| miR-943, NELFA              | 44.12      | 19.36       | 20.06      |
| miR-4678                    | 43.31      | 0.00        | 0.00       |
| miR-324                     | 42.63      | 29.48       | 56.10      |
| miR-4741, RBBP8             | 42.26      | 42.51       | 36.76      |
| miR-4754                    | 41.99      | 0.00        | 63.03      |
| miR-1180                    | 40.85      | 84.02       | 0.00       |
| miR-302A                    | 40.85      | 0.00        | 0.00       |
| miR-621, SLC25A15           | 40.14      | 28.67       | 29.08      |
| miR-564, TMEM42             | 39.32      | 68.50       | 12.89      |
| miR-3074                    | 38.91      | 1.36        | 1.66       |
| miR-29C                     | 36.62      | 40.50       | 47.39      |
| miR-3610, RAD21             | 36.50      | 31.86       | 31.41      |
| miR-93                      | 35.95      | 0.00        | 15.95      |
| miR-761, NRD1               | 33.99      | 43.41       | 43.15      |
| miR-4742                    | 33.24      | 36.73       | 0.00       |
| miR-6638                    | 30.71      | 20.74       | 37.43      |
| miR-3177                    | 29.60      | 0.00        | 13.99      |
| miR-3189                    | 28.53      | 164.97      | 118.92     |
| miR-5690                    | 28.53      | 0.00        | 0.00       |
| miR-4688                    | 28.52      | 12.94       | 22.39      |
| miR-604                     | 28.42      | 0.00        | 0.00       |
| miR-568                     | 28.32      | 6.93        | 15.56      |
| miR-3614, TRIM25            | 28.24      | 33.82       | 31.11      |
| miR-LET7G                   | 27.88      | 0.00        | 0.00       |
| miR-4668, UGCG              | 27.78      | 28.78       | 28.47      |
| miR-1227                    | 27.38      | 9.63        | 23.97      |
| miR-4687, STIM1             | 27.24      | 298.95      | 247.37     |
| miR-3916                    | 26.91      | 34.98       | 9.98       |
| miR-1915                    | 26.78      | 0.00        | 0.00       |
| miR-2982                    | 26.03      | 0.00        | 14.93      |
| miR-1278                    | 26.03      | 0.00        | 0.00       |
| miR-2467                    | 26.03      | 0.00        | 0.00       |
| miR-4497                    | 25.88      | 0.00        | 0.00       |
| miR-938                     | 24.70      | 0.00        | 0.00       |
| miR-320A                    | 24.39      | 0.00        | 0.00       |
| miR-4800, MXD4              | 24.32      | 26.93       | 33.59      |
| miR-27B                     | 23.89      | 14.56       | 10.49      |

Contd...
| miRNAs                      | Caput cell | Corpus cell | Cauda cell |
|-----------------------------|------------|-------------|------------|
| miR-4258                    | 23.30      | 8.18        | 16.59      |
| miR-186                     | 23.17      | 10.79       | 18.67      |
| miR-4442, TOP2B             | 22.66      | 65.67       | 99.63      |
| miR-4730                    | 22.66      | 21.14       | 0.00       |
| miR-26A2                    | 22.19      | 0.00        | 28.25      |
| miR-4691                    | 22.02      | 32.76       | 13.94      |
| miR-3192                    | 20.86      | 42.90       | 0.00       |
| miR-940                     | 20.74      | 15.48       | 21.34      |
| miR-1203                    | 20.25      | 0.00        | 0.00       |
| miR-5193, UBA7              | 19.14      | 28.36       | 26.92      |
| miR-2117                    | 19.14      | 23.51       | 27.37      |
| miR-140                     | 18.66      | 0.00        | 0.00       |
| miR-1068                    | 18.54      | 0.00        | 27.97      |
| miR-4640                    | 18.52      | 27.58       | 56.09      |
| miR-22, miR-22HG            | 18.24      | 21.85       | 24.39      |
| miR-3134, PTBP3             | 18.13      | 18.67       | 17.58      |
| miR-5685                    | 18.11      | 0.00        | 0.00       |
| miR-3620                    | 18.08      | 16.85       | 29.15      |
| miR-635                     | 17.89      | 56.60       | 25.88      |
| miR-4420                    | 17.66      | 0.00        | 0.00       |
| miR-193A                    | 17.03      | 21.25       | 0.00       |
| miR-298                     | 17.03      | 0.00        | 0.00       |
| miR-130A                    | 16.11      | 0.00        | 0.00       |
| miR-181B2                   | 16.11      | 0.00        | 0.00       |
| miR-192                     | 16.02      | 0.00        | 0.00       |
| miR-3120                    | 15.80      | 0.00        | 0.00       |
| miR-4308                    | 15.79      | 0.00        | 14.93      |
| miR-4723                    | 15.79      | 0.00        | 0.00       |
| miR-941-2, miR-941-3, miR-941-4 | 15.50 | 19.85 | 10.06 |
| miR-3909                    | 15.46      | 8.00        | 0.00       |
| miR-181B1                   | 15.35      | 0.00        | 0.00       |
| miR-153-1                   | 15.26      | 0.00        | 0.00       |
| miR-100HG                   | 15.15      | 25.61       | 51.07      |
| miR-196A1                   | 15.03      | 72.23       | 0.44       |
| miR-638                     | 15.00      | 5.28        | 0.00       |
| miR-7-1                     | 14.92      | 10.42       | 21.14      |
| miR-548A1                   | 14.82      | 0.00        | 0.00       |
| miR-4748                    | 14.80      | 0.00        | 0.00       |
| miR-4999                    | 14.47      | 0.00        | 0.00       |
| miR-662                     | 14.33      | 119.82      | 27.08      |
| miR-4484                    | 13.89      | 0.00        | 0.00       |
| miR-1236                    | 13.75      | 40.74       | 43.23      |
| miR-30E                     | 13.74      | 0.00        | 0.00       |
| miR-378D2                   | 12.42      | 5.78        | 10.01      |
| miR-339                     | 12.40      | 0.00        | 0.00       |
| miR-135B                    | 12.35      | 0.00        | 0.00       |
| miR-3190                    | 12.29      | 12.37       | 25.38      |
| miR-1909, REXO1             | 11.84      | 58.58       | 81.37      |
| miR-559                     | 11.25      | 0.00        | 0.00       |
| miR-219-1                   | 11.19      | 12.78       | 0.00       |
| miR-5006, VWA8              | 11.16      | 9.64        | 9.06       |
| miR-3176                    | 10.93      | 1.59        | 1.23       |
| miR-3613                    | 10.93      | 0.00        | 0.00       |
| miR-598                     | 10.72      | 0.00        | 0.00       |
| miR-4523, TAOK1             | 10.42      | 13.25       | 11.83      |
| miR-708                     | 10.33      | 0.00        | 0.00       |

Contd...
| miRNAs                                      | Caput cell | Corpus cell | Cauda cell |
|---------------------------------------------|------------|-------------|------------|
| miR-4296                                    | 10.33      | 0.00        | 0.00       |
| miR-573                                     | 10.28      | 0.40        | 0.58       |
| miR-744                                     | 10.23      | 0.00        | 0.00       |
| miR-148B                                    | 9.80       | 0.00        | 0.00       |
| miR-10A                                     | 9.71       | 2.33        | 9.67       |
| miR-146A                                    | 9.40       | 1.11        | 1.10       |
| miR-4315-2, PLEKHM1                         | 9.39       | 11.30       | 10.37      |
| miR-1225                                    | 9.26       | 0.00        | 0.00       |
| miR-205, miR-205HG                          | 8.99       | 104.08      | 149.66     |
| miR-1204, PVT1                              | 8.54       | 7.84        | 89.13      |
| miR-210HG                                   | 8.15       | 4.65        | 4.91       |
| miR-941-1, miR-941-3, miR-941-4             | 7.61       | 15.41       | 28.02      |
| miR-3939                                    | 7.21       | 0.00        | 4.14       |
| miR-200A, miR-200B, miR-429                 | 7.19       | 24.14       | 8.31       |
| miR-4469, RNF170                            | 6.85       | 10.56       | 26.39      |
| miR-3653                                    | 6.83       | 73.59       | 39.59      |
| miR-1287, PYROXD2                           | 6.72       | 4.46        | 7.00       |
| miR-23B                                     | 6.51       | 12.12       | 0.00       |
| miR-590                                     | 6.51       | 46.23       | 30.39      |
| miR-499A                                    | 6.40       | 0.00        | 7.88       |
| miR-484, NDE1                               | 6.26       | 7.56        | 8.98       |
| miR-3651, SNORAB4                           | 6.22       | 20.52       | 18.15      |
| miR-567                                     | 6.21       | 0.00        | 6.91       |
| miR-345                                     | 6.21       | 0.00        | 0.00       |
| miR-4763, miR-LET7A3, miR-LET7B, miR-LET7BHG | 5.85       | 39.49       | 41.06      |
| miR-141                                     | 5.47       | 16.11       | 11.75      |
| miR-570, SDHAP2                             | 5.14       | 6.57        | 11.76      |
| miR-3941                                    | 4.97       | 4.63        | 4.69       |
| miR-221, miR-222                            | 4.74       | 5.23        | 5.91       |
| miR-4767, STS                               | 4.64       | 13.79       | 11.98      |
| miR-769                                     | 4.57       | 0.00        | 0.00       |
| miR-311HG                                   | 4.54       | 3.76        | 4.15       |
| miR-5187, TOMM40L                           | 4.07       | 5.32        | 22.80      |
| miR-30C2                                    | 3.92       | 1.10        | 0.98       |
| miR-5004, SYNGAP1                           | 3.86       | 11.00       | 6.16       |
| miR-5587                                    | 3.75       | 1.79        | 1.25       |
| miR-34A                                     | 3.63       | 2.73        | 2.76       |
| miR-135A2                                   | 3.54       | 0.95        | 1.09       |
| miR-LET7DHG                                 | 3.21       | 2.68        | 2.83       |
| miR-765                                     | 3.20       | 0.00        | 0.00       |
| miR-155, miR-155HG                          | 3.11       | 0.61        | 0.45       |
| miR-508                                     | 3.09       | 0.00        | 0.00       |
| miR-4724, RAB11FIP4                         | 2.75       | 3.95        | 25.66      |
| miR-5194                                    | 2.59       | 0.00        | 0.00       |
| miR-218-1                                   | 2.43       | 0.00        | 0.00       |
| miR-5001, TIGD1                             | 2.00       | 2.30        | 2.36       |
| miR-LET7D                                   | 1.96       | 12.80       | 1.64       |
| miR-331, miR-3685                           | 1.77       | 1.74        | 1.91       |
| miR-181D                                    | 1.72       | 1.83        | 0.00       |
| miR-3714, PLCL2                              | 1.66       | 0.17        | 0.23       |
| miR-3180-4                                  | 1.62       | 0.00        | 0.00       |
| miR-1256, SLC25A53                          | 1.58       | 1.26        | 1.36       |
| miR-1291, SNORA34                           | 1.42       | 5.94        | 3.50       |
| miR-181A2HG                                 | 1.29       | 0.63        | 0.88       |
| miR-17, miR-17HG, miR-18A, miR-19A, miR-19B1, miR-20A, miR-92A1 | 1.10 | 0.73 | 0.84 |
| miRAs                  | Caput cell | Corpus cell | Cauda cell |
|-----------------------|------------|-------------|------------|
| miR-548I2             | 1.09       | 0.00        | 0.00       |
| miR-4720              | 0.97       | 1.25        | 33.19      |
| miR-548AA1, PALMD     | 0.91       | 0.77        | 0.69       |
| miR-600HG             | 0.81       | 0.45        | 0.39       |
| miR-3619              | 0.76       | 0.63        | 22.01      |
| miR-3677              | 0.73       | 0.84        | 0.40       |
| miR-LET7I             | 0.72       | 10.14       | 8.67       |
| miR-4632, TNFRSF1B    | 0.72       | 0.22        | 0.25       |
| miR-320B2             | 0.70       | 0.00        | 1.39       |
| miR-4324, SLC6A16     | 0.64       | 0.12        | 0.10       |
| miR-548N, OSBPL6      | 0.50       | 2.68        | 2.77       |
| miR-374C              | 0.48       | 0.68        | 3.19       |
| miR-374B, miR-421     | 0.43       | 3.57        | 8.80       |
| miR-143, miR-143HG, miR-145 | 0.40 | 0.64 | 9.23 |
| miR-137, miR-137HG    | 0.07       | 0.16        | 0.15       |
| miR-4690              | 0.00       | 251.25      | 412.35     |
| miR-4749              | 0.00       | 97.76       | 0.00       |
| miR-4669              | 0.00       | 84.86       | 0.00       |
| miR-199A1             | 0.00       | 83.60       | 0.00       |
| miR-149               | 0.00       | 67.76       | 35.79      |
| miR-4665              | 0.00       | 67.39       | 63.32      |
| miR-4651              | 0.00       | 58.67       | 0.00       |
| miR-3136              | 0.00       | 39.90       | 31.28      |
| miR-1972-2            | 0.00       | 38.87       | 0.00       |
| miR-4685              | 0.00       | 38.06       | 0.00       |
| miR-4782              | 0.00       | 37.19       | 29.15      |
| miR-3125              | 0.00       | 36.15       | 0.00       |
| miR-2116              | 0.00       | 34.73       | 0.00       |
| miR-5087              | 0.00       | 34.62       | 21.24      |
| miR-548D2             | 0.00       | 28.57       | 0.00       |
| miR-933               | 0.00       | 27.47       | 0.00       |
| miR-1266              | 0.00       | 26.91       | 0.00       |
| miR-3682              | 0.00       | 26.85       | 0.00       |
| miR-593               | 0.00       | 26.73       | 0.00       |
| miR-4505              | 0.00       | 26.58       | 0.00       |
| miR-4656              | 0.00       | 22.65       | 62.11      |
| miR-3184              | 0.00       | 22.61       | 0.00       |
| miR-622               | 0.00       | 22.17       | 22.00      |
| miR-3132              | 0.00       | 21.19       | 0.00       |
| miR-LET7F2            | 0.00       | 19.84       | 0.00       |
| miR-3138              | 0.00       | 19.48       | 0.00       |
| miR-4653              | 0.00       | 16.30       | 54.72      |
| miR-4786              | 0.00       | 15.73       | 15.95      |
| miR-5192              | 0.00       | 15.53       | 0.00       |
| miR-572               | 0.00       | 14.73       | 27.08      |
| miR-3153              | 0.00       | 13.79       | 13.99      |
| miR-624               | 0.00       | 13.38       | 0.00       |
| miR-5088              | 0.00       | 12.18       | 0.00       |
| miR-4787              | 0.00       | 12.16       | 0.00       |
| miR-877               | 0.00       | 10.79       | 0.00       |
| miR-142               | 0.00       | 10.19       | 0.00       |
| miR-1207              | 0.00       | 10.19       | 0.00       |
| miR-152               | 0.00       | 10.19       | 0.00       |
| miR-3944              | 0.00       | 8.30        | 10.32      |
| miR-5188              | 0.00       | 8.00        | 0.00       |
| miR-4314              | 0.00       | 7.76        | 0.00       |

Contd...
## Supplementary Table 1: Contd...

| miRNAs          | Caput cell | Corpus cell | Cauda cell |
|-----------------|------------|-------------|------------|
| miR-5008        | 0.00       | 7.01        | 0.00       |
| miR-645         | 0.00       | 7.01        | 0.00       |
| miR-3662        | 0.00       | 6.69        | 0.00       |
| miR-615         | 0.00       | 6.54        | 0.00       |
| miR-1324        | 0.00       | 6.36        | 0.00       |
| miR-597         | 0.00       | 6.09        | 0.00       |
| miR-218-2       | 0.00       | 4.87        | 3.52       |
| miR-3973        | 0.00       | 3.91        | 0.00       |
| miR-548F4       | 0.00       | 3.90        | 0.00       |
| miR-98          | 0.00       | 3.83        | 0.00       |
| miR-181A2       | 0.00       | 3.47        | 0.00       |
| miR-365B        | 0.00       | 3.34        | 0.00       |
| miR-548K        | 0.00       | 2.78        | 2.82       |
| miR-184C        | 0.00       | 2.55        | 0.00       |
| miR-3180-5      | 0.00       | 2.21        | 2.52       |
| miR-4519        | 0.00       | 0.00        | 851.22     |
| miR-1249        | 0.00       | 0.00        | 180.16     |
| miR-4701        | 0.00       | 0.00        | 128.52     |
| miR-3581        | 0.00       | 0.00        | 115.31     |
| miR-4520A       | 0.00       | 0.00        | 59.89      |
| miR-4271        | 0.00       | 0.00        | 42.62      |
| miR-302C        | 0.00       | 0.00        | 42.62      |
| miR-2909        | 0.00       | 0.00        | 38.60      |
| miR-4524A       | 0.00       | 0.00        | 38.60      |
| miR-4479        | 0.00       | 0.00        | 32.12      |
| miR-4284        | 0.00       | 0.00        | 29.85      |
| miR-3157        | 0.00       | 0.00        | 28.94      |
| miR-328         | 0.00       | 0.00        | 22.94      |
| miR-363         | 0.00       | 0.00        | 22.94      |
| miR-1-2         | 0.00       | 0.00        | 17.04      |
| miR-LET7A1      | 0.00       | 0.00        | 15.95      |
| miR-3162        | 0.00       | 0.00        | 13.99      |
| miR-928         | 0.00       | 0.00        | 13.90      |
| miR-554         | 0.00       | 0.00        | 12.90      |
| miR-4644        | 0.00       | 0.00        | 12.34      |
| miR-659         | 0.00       | 0.00        | 12.30      |
| miR-330         | 0.00       | 0.00        | 12.14      |
| miR-5094        | 0.00       | 0.00        | 11.61      |
| miR-644A        | 0.00       | 0.00        | 9.40       |
| miR-4306        | 0.00       | 0.00        | 8.29       |
| miR-648         | 0.00       | 0.00        | 7.12       |
| miR-4313        | 0.00       | 0.00        | 5.12       |
| miR-1250        | 0.00       | 0.00        | 3.15       |
| miR-64, SNORA36B| 0.00       | 0.00        | 2.94       |
| miR-3689B       | 0.00       | 0.00        | 1.06       |

miRNAs: microRNAs
**Supplementary Table 2: List of microRNAs in primary human epididymal tissues (fragments per kilobase of transcript per million mapped reads [FPKM] >0.1)**

| miRNAs                                      | Caput tissue | Corpus tissue | Cauda tissue |
|---------------------------------------------|--------------|---------------|--------------|
| miR-4461                                    | 3.6E+06      | 5.3E+05       | 4.1E+05      |
| miR-4709, NPC2                              | 41160.70     | 121350.00     | 188.55       |
| miR-3648, miR-3687                          | 12117.00     | 17315.50      | 1614.22      |
| miR-1282                                    | 1565.40      | 1400.44       | 1605.93      |
| miR-663A                                    | 1438.89      | 1222.82       | 47.05        |
| miR-1244-3, PTMA                           | 862.17       | 797.60        | 799.30       |
| miR-3650                                    | 833.36       | 4.67          | 0.23         |
| miR-4426, RPS27A                            | 733.05       | 390.24        | 954.78       |
| miR-611, TMEM258                            | 514.21       | 652.57        | 248.28       |
| miR-29C                                     | 501.88       | 1105.79       | 18.75        |
| miR-4647, SLC35B2                           | 498.77       | 732.85        | 77.57        |
| miR-205, miR-205HG                          | 466.77       | 1770.51       | 5.06         |
| miR-1279                                    | 465.71       | 144.46        | 0.00         |
| miR-1538                                    | 455.79       | 7.06          | 0.00         |
| miR-1307, USMG5                             | 426.02       | 512.42        | 389.86       |
| miR-3661                                    | 413.86       | 78.74         | 271.26       |
| miR-3918                                    | 366.64       | 1374.74       | 255.72       |
| miR-762                                     | 347.98       | 705.28        | 50.41        |
| miR-141                                     | 337.14       | 487.46        | 6.92         |
| miR-4668                                    | 326.47       | 0.00          | 129.06       |
| miR-4690                                    | 312.12       | 0.00          | 0.00         |
| miR-1304, SNORA1, SNORA18, SNORA25, SNORA32, SNORA40, SNORA8, SNORD6, TAF1D | 305.77       | 271.49        | 204.53       |
| miR-155, miR-155HG                          | 298.20       | 8.98          | 1.85         |
| miR-4728                                    | 255.72       | 79.32         | 147.62       |
| miR-5193, UBA7                              | 203.28       | 251.23        | 14.82        |
| miR-135A2, RMST                             | 201.75       | 198.86        | 2.41         |
| miR-639, TECR                               | 161.93       | 332.88        | 121.02       |
| miR-596                                     | 159.87       | 66.17         | 0.00         |
| miR-6638                                    | 151.60       | 73.48         | 0.00         |
| miR-4667                                    | 142.45       | 0.00          | 0.00         |
| miR-1226                                    | 124.20       | 429.02        | 0.00         |
| miR-3120                                    | 121.15       | 241.20        | 0.00         |
| miR-636, SRSF2                              | 120.24       | 116.10        | 125.52       |
| miR-5691                                    | 115.46       | 0.00          | 0.00         |
| miR-1909, REXO1                             | 104.45       | 195.44        | 81.03        |
| miR-99B                                     | 94.99        | 0.00          | 0.00         |
| miR-3607                                    | 92.46        | 57.36         | 0.00         |
| miR-188                                     | 86.70        | 0.00          | 0.00         |
| miR-29B2                                    | 80.76        | 75.16         | 0.00         |
| miR-662                                     | 76.83        | 53.55         | 0.00         |
| miR-4785                                    | 72.93        | 0.00          | 0.00         |
| miR-5093                                    | 72.36        | 53.92         | 0.00         |
| miR-548D2                                   | 71.03        | 0.00          | 0.00         |
| miR-1247                                    | 67.97        | 0.00          | 0.00         |
| miR-3682                                    | 66.76        | 174.39        | 18.63        |
| miR-4674                                    | 62.96        | 48.73         | 0.80         |
| miR-4691                                    | 62.84        | 19.49         | 132.11       |
| miR-363                                     | 62.06        | 38.50         | 0.00         |
| miR-92A2                                    | 62.06        | 0.00          | 0.00         |
| miR-4516                                    | 59.23        | 92.82         | 16.63        |
| miR-34C                                     | 53.33        | 0.00          | 0.00         |
| miR-3912                                    | 52.42        | 0.00          | 0.00         |
| miR-21, VMP1                                 | 49.30        | 52.76         | 146.28       |
| miR-1225                                    | 47.34        | 14.68         | 0.00         |
| miR-1248, SNORAB1                           | 46.51        | 29.13         | 68.95        |
| miR-3620                                    | 46.23        | 0.00          | 98.43        |

*Contd...*
| miRNAs                  | Caput tissue | Corpus tissue | Cauda tissue |
|------------------------|--------------|---------------|--------------|
| miR-1914, miR-647, UCKL1 | 45.88        | 32.86         | 53.33        |
| miR-100HG              | 44.27        | 27.17         | 3.18         |
| miR-4492               | 43.19        | 0.00          | 171.29       |
| miR-760                | 43.17        | 0.00          | 0.00         |
| miR-2117               | 43.17        | 0.00          | 0.00         |
| miR-4672               | 40.38        | 0.00          | 0.00         |
| miR-761, NRD1          | 39.38        | 41.00         | 38.81        |
| miR-564, TMEM42         | 36.73        | 102.04        | 14.21        |
| miR-4721, TUFM          | 35.57        | 40.49         | 101.44       |
| miR-1238               | 35.51        | 44.06         | 25.20        |
| miR-4750, TBC1D17      | 33.73        | 982.62        | 15.97        |
| miR-505                | 33.38        | 0.00          | 0.00         |
| miR-568                | 32.26        | 31.31         | 15.49        |
| miR-37802              | 31.75        | 19.70         | 0.00         |
| miR-632, ZNF207        | 31.60        | 34.27         | 99.60        |
| miR-3621               | 31.42        | 0.00          | 0.00         |
| miR-3610, RAD21        | 31.30        | 34.26         | 49.89        |
| miR-3615, SLC9A3R1     | 30.40        | 50.69         | 76.26        |
| miR-22, miR-22HG       | 30.06        | 36.51         | 29.73        |
| miR-3942               | 29.75        | 18.46         | 7.04         |
| miR-3180-4             | 29.53        | 16.72         | 1.39         |
| miR-3917, STMN1        | 28.96        | 36.46         | 168.86       |
| miR-221, miR-222       | 28.16        | 28.33         | 8.73         |
| miR-3656, TRAPPC4      | 27.47        | 30.43         | 113.30       |
| miR-3671               | 26.41        | 49.16         | 0.00         |
| miR-1287, PYROXD2      | 25.93        | 28.19         | 13.40        |
| miR-4687, STIM1        | 25.58        | 7.82          | 239.96       |
| miR-5006, WUA8         | 23.76        | 8.57          | 5.45         |
| miR-135A1              | 23.67        | 14.68         | 31.42        |
| miR-1292, NOP56, SNORD86 | 22.34    | 19.47         | 89.01        |
| miR-3605, PHC2         | 22.13        | 26.77         | 140.39       |
| miR-191                | 21.30        | 0.00          | 0.00         |
| miR-4800, MXD4         | 20.46        | 21.64         | 119.53       |
| miR-645                | 19.24        | 0.00          | 13.66        |
| miR-374C               | 19.10        | 12.58         | 1.22         |
| miR-4680, PDCD4        | 18.54        | 21.47         | 551.67       |
| miR-LET7D              | 17.86        | 15.18         | 2.37         |
| miR-943, NELFA         | 17.65        | 22.35         | 26.01        |
| miR-4741, RBPP8        | 17.23        | 11.70         | 26.43        |
| miR-635                | 15.87        | 153.56        | 0.00         |
| miR-200A, miR-200B, miR-429 | 14.24   | 9.23          | 12.86        |
| miR-210, miR-210HG     | 13.41        | 55.42         | 39.68        |
| miR-3134, PTBP3        | 13.08        | 11.02         | 40.31        |
| miR-497HG              | 10.12        | 16.11         | 7.07         |
| miR-937, SCRIB         | 9.86         | 14.53         | 98.86        |
| miR-4724, RAB11FIP4    | 9.58         | 11.29         | 22.94        |
| miR-3677, miR-940      | 9.06         | 35.43         | 0.64         |
| miR-4517, NFATC2IP     | 8.92         | 139.88        | 18.73        |
| miR-570, SDHAP2        | 8.24         | 11.09         | 7.02         |
| miR-659                | 8.13         | 2.47          | 0.57         |
| miR-4700, UNC119B      | 8.00         | 4.02          | 222.21       |
| miR-4469, RNF170       | 7.38         | 9.17          | 9.75         |
| miR-LET7DHG            | 7.33         | 4.88          | 5.47         |
| miR-3176, SOLH         | 7.00         | 7.68          | 15.60        |
| miR-4763, miR-LET7A3, miR-LET7B, miR-LET7BHG | 6.58     | 17.98         | 6.43         |
| miR-181A2HG            | 5.58         | 3.90          | 1.34         |
**Supplementary Table 2: Contd...**

| miRNAs                        | Caput tissue | Corpus tissue | Cauda tissue |
|-------------------------------|--------------|---------------|--------------|
| miR-3685                      | 5.06         | 5.17          | 5.23         |
| miR-664, SNORA36B             | 4.63         | 2.87          | 0.00         |
| miR-3614, TRIM25              | 4.62         | 4.94          | 25.36        |
| miR-5001, TIGD1               | 4.58         | 4.34          | 3.49         |
| miR-132                       | 4.51         | 9.33          | 0.12         |
| miR-5004, SYNGAP1             | 4.26         | 2.49          | 2.63         |
| miR-3911                      | 4.04         | 10.32         | 8.18         |
| miR-1291, SNORA34             | 3.87         | 2.41          | 7.39         |
| miR-612                       | 3.54         | 38.31         | 7.24         |
| miR-3658, UCK2                | 3.24         | 1016.70       | 7.48         |
| miR-3714, PLCL2               | 3.18         | 2.84          | 2.26         |
| miR-31HG                      | 3.06         | 4.16          | 6.69         |
| miR-1256, SLC25A53            | 2.89         | 3.00          | 2.67         |
| miR-4315-2, PLEKHM1           | 2.77         | 2.80          | 9.67         |
| miR-548AA1, PALMD             | 2.72         | 2.85          | 0.28         |
| miR-621, SLC25A15             | 2.53         | 1.98          | 21.57        |
| miR-143, miR-143HG, miR-145   | 2.39         | 2.72          | 0.48         |
| miR-4740                      | 2.25         | 1.55          | 0.88         |
| miR-31                        | 2.16         | 3.20          | 6.27         |
| miR-3074                      | 2.08         | 44.72         | 54.46        |
| miR-17, miR-17HG, miR-18A, miR-19A, miR-19B1, miR-20A, miR-92A1 | 2.01 | 1.81 | 1.29 |
| miR-320E                      | 1.71         | 1.61          | 0.60         |
| miR-5187, TOMM40L             | 1.55         | 1.82          | 5.64         |
| miR-146B                      | 1.46         | 2.19          | 0.01         |
| miR-600HG                     | 1.46         | 1.17          | 1.50         |
| miR-548N, OSBPL6              | 1.27         | 1.24          | 0.27         |
| miR-LET7I                    | 1.14         | 1.41          | 1.83         |
| miR-4632, TNFRSF1B            | 1.14         | 1.49          | 0.40         |
| miR-1972-2                    | 1.13         | 0.94          | 0.09         |
| miR-484, NDE1                 | 0.90         | 1.32          | 4.41         |
| miR-146A                      | 0.82         | 1.00          | 6.83         |
| miR-3916                      | 0.81         | 1.21          | 28.96        |
| miR-4712                      | 0.71         | 1.06          | 1.92         |
| miR-3688-2                    | 0.65         | 0.87          | 11.27        |
| miR-4720                      | 0.51         | 0.78          | 1.70         |
| miR-30C2                      | 0.34         | 0.11          | 10.52        |
| miR-4491                      | 0.28         | 6.22          | 0.00         |
| miR-7-3HG                     | 0.26         | 0.22          | 0.00         |
| miR-573                       | 0.25         | 0.09          | 47.53        |
| miR-196A1                     | 0.17         | 0.15          | 139.54       |
| miR-135B                      | 0.15         | 2.59          | 4.85         |
| miR-5587                      | 0.06         | 0.28          | 2.58         |
| miR-631                       | 0.00         | 230.99        | 0.00         |
| miR-4489                      | 0.00         | 144.46        | 165.26       |
| miR-4714                      | 0.00         | 137.92        | 0.00         |
| miR-4467                      | 0.00         | 126.45        | 0.00         |
| miR-4256                      | 0.00         | 111.51        | 0.00         |
| miR-4449                      | 0.00         | 88.37         | 0.00         |
| miR-4292                      | 0.00         | 79.32         | 271.37       |
| miR-328                       | 0.00         | 77.00         | 0.00         |
| miR-200C                      | 0.00         | 71.54         | 0.00         |
| miR-20B                       | 0.00         | 64.80         | 0.00         |
| miR-4306                      | 0.00         | 58.24         | 0.00         |
| miR-4746                      | 0.00         | 53.78         | 61.53        |
| miR-4725                      | 0.00         | 52.49         | 0.00         |
| miR-330                       | 0.00         | 49.30         | 0.00         |

*Contd...*
### Supplementary Table 2: Contd...

| miRNAs                        | Caput tissue | Corpus tissue | Cauda tissue |
|-------------------------------|--------------|---------------|--------------|
| miR-572                       | 0.00         | 48.06         | 0.00         |
| miR-339                       | 0.00         | 47.46         | 0.00         |
| miR-4804                      | 0.00         | 45.24         | 0.00         |
| miR-5690                      | 0.00         | 44.08         | 176.20       |
| miR-4768                      | 0.00         | 41.68         | 0.00         |
| miR-641                       | 0.00         | 39.91         | 79.97        |
| miR-4784                      | 0.00         | 33.09         | 168.92       |
| miR-3125                      | 0.00         | 30.78         | 0.00         |
| miR-LET7A1                    | 0.00         | 26.78         | 0.00         |
| miR-1236                      | 0.00         | 23.96         | 21.25        |
| miR-4642                      | 0.00         | 23.47         | 31.43        |
| miR-5188                      | 0.00         | 20.90         | 3.96         |
| miR-1968                      | 0.00         | 20.74         | 71.06        |
| miR-4513                      | 0.00         | 18.37         | 0.00         |
| miR-1227                      | 0.00         | 16.39         | 52.50        |
| miR-133A1                     | 0.00         | 16.39         | 0.00         |
| miR-604                       | 0.00         | 11.94         | 28.32        |
| miR-331                       | 0.00         | 11.94         | 0.00         |
| miR-33B                       | 0.00         | 10.82         | 16.51        |
| miR-1324                      | 0.00         | 10.82         | 0.00         |
| miR-5002                      | 0.00         | 10.32         | 0.00         |
| miR-561                       | 0.00         | 10.32         | 0.00         |
| miR-27B                       | 0.00         | 10.32         | 0.00         |
| miR-4440                      | 0.00         | 9.85          | 25.01        |
| miR-345                       | 0.00         | 9.85          | 0.00         |
| miR-4441                      | 0.00         | 8.99          | 25.83        |
| miR-593                       | 0.00         | 8.99          | 10.28        |
| miR-5000                      | 0.00         | 7.88          | 0.00         |
| miR-765                       | 0.00         | 5.10          | 0.00         |
| miR-941-1, miR-941-3, miR-941-4 | 0.00         | 4.56          | 15.64        |
| miR-3651, SNORAB4             | 0.00         | 2.71          | 0.00         |
| miR-32082                     | 0.00         | 2.34          | 3.73         |
| miR-548I1                     | 0.00         | 1.73          | 0.00         |
| miR-548I2                     | 0.00         | 1.73          | 0.00         |
| miR-3907                      | 0.00         | 1.64          | 0.00         |
| miR-137, miR-137HG            | 0.00         | 0.07          | 0.14         |
| miR-1260B                     | 0.00         | 0.00          | 450.62       |
| miR-4701                      | 0.00         | 0.00          | 261.36       |
| miR-4273                      | 0.00         | 0.00          | 199.26       |
| miR-553                       | 0.00         | 0.00          | 163.23       |
| miR-25                        | 0.00         | 0.00          | 145.65       |
| miR-3189                      | 0.00         | 0.00          | 96.63        |
| miR-4253                      | 0.00         | 0.00          | 93.04        |
| miR-302C                      | 0.00         | 0.00          | 81.84        |
| miR-4284                      | 0.00         | 0.00          | 74.31        |
| miR-4685                      | 0.00         | 0.00          | 74.13        |
| miR-1229                      | 0.00         | 0.00          | 74.13        |
| miR-4482-1                    | 0.00         | 0.00          | 67.41        |
| miR-27A                       | 0.00         | 0.00          | 65.58        |
| miR-590                       | 0.00         | 0.00          | 52.91        |
| miR-23A                       | 0.00         | 0.00          | 48.97        |
| miR-4640                      | 0.00         | 0.00          | 47.57        |
| miR-3748                      | 0.00         | 0.00          | 42.76        |
| miR-4766                      | 0.00         | 0.00          | 40.78        |
| miR-3191                      | 0.00         | 0.00          | 39.63        |
### Supplementary Table 2: Contd...

| miRNAs | Caput tissue | Corpus tissue | Cauda tissue |
|--------|--------------|---------------|--------------|
| miR-5687 | 0.00         | 0.00          | 37.85        |
| miR-3117 | 0.00         | 0.00          | 35.21        |
| miR-4742 | 0.00         | 0.00          | 33.32        |
| miR-3153 | 0.00         | 0.00          | 29.95        |
| miR-5087 | 0.00         | 0.00          | 26.92        |
| miR-3138 | 0.00         | 0.00          | 26.86        |
| miR-3662 | 0.00         | 0.00          | 26.00        |
| miR-4263 | 0.00         | 0.00          | 25.28        |
| miR-4653 | 0.00         | 0.00          | 25.20        |
| miR-938  | 0.00         | 0.00          | 24.53        |
| miR-3188 | 0.00         | 0.00          | 22.30        |
| miR-186  | 0.00         | 0.00          | 21.02        |
| miR-149  | 0.00         | 0.00          | 19.32        |
| miR-193A | 0.00         | 0.00          | 18.75        |
| miR-661  | 0.00         | 0.00          | 18.39        |
| miR-1231 | 0.00         | 0.00          | 16.16        |
| miR-LET7G| 0.00         | 0.00          | 14.62        |
| miR-421  | 0.00         | 0.00          | 14.11        |
| miR-507  | 0.00         | 0.00          | 13.66        |
| miR-219-1| 0.00         | 0.00          | 13.53        |
| miR-941-2, miR-941-3, miR-941-4 | 0.00 | 0.00 | 13.16 |
| miR-1998 | 0.00         | 0.00          | 12.15        |
| miR-634  | 0.00         | 0.00          | 11.81        |
| miR-455  | 0.00         | 0.00          | 11.27        |
| miR-7-1  | 0.00         | 0.00          | 11.17        |
| miR-423  | 0.00         | 0.00          | 11.14        |
| miR-622  | 0.00         | 0.00          | 10.70        |
| miR-140  | 0.00         | 0.00          | 10.28        |
| miR-3622B| 0.00         | 0.00          | 9.14         |
| miR-4311 | 0.00         | 0.00          | 8.35         |
| miR-181A2| 0.00         | 0.00          | 5.53         |
| miR-769  | 0.00         | 0.00          | 5.03         |
| miR-3909 | 0.00         | 0.00          | 4.86         |
| miR-499A | 0.00         | 0.00          | 4.47         |
| miR-4524B| 0.00         | 0.00          | 4.40         |

miRNAs: microRNAs