Cell Systems

Analysis of the expression of PIWI-interacting RNAs during cardiac differentiation of human pluripotent stem cells

--Manuscript Draft--

Manuscript Number:	CELL-SYSTEMS-D-19-00275
Full Title:	Analysis of the expression of PIWI-interacting RNAs during cardiac differentiation of human pluripotent stem cells
Article Type:	Research Article
Keywords:	piRNA; cardiac differentiation; pluripotent stem cells; somatic cells; non-coding RNAs
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Abstract:	PIWI-interacting RNAs (piRNAs) are a class of non-coding RNAs initially thought to be restricted exclusively to germline cells. In recent years, accumulating evidence has demonstrated that piRNAs are actually expressed in pluripotent, neural, cardiac and even cancer cells. However, controversy remains around the existence and function of somatic piRNAs. Using small RNA-seq samples from H9 pluripotent cells differentiated to mesoderm progenitors and cardiomyocytes we identified the expression of 447 piRNAs, of which 241 were detected in pluripotency, 218 in mesoderm and 171 in cardiac cells. The majority of them originated from the sense strand of protein coding and lncRNAs genes in all stages of differentiation, though no evidences for secondary piRNAs (ping-pong) were found. Genes hosting piRNAs in cardiac samples were related to critical biological processes in the heart, like contraction and cardiac muscle development. Our results indicate that somatic piRNAs might have a role in fine-tuning the expression of genes involved in differentiation of pluripotent cells to cardiomyocytes.íí
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RE: New submission

June 12, 2019

Analysis of the expression of PIWI-interacting RNAs during cardiac differentiation of human pluripotent stem cells

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Short title: "Expression of piRNAs in cardiac differentiation"

Dear Dr. Editor of Cell Systems,

Hereby we submit the original manuscript entitled "Analysis of the expression of PIWI-interacting RNAs during cardiac differentiation of human pluripotent stem cells" by Alejandro La Greca and collaborators. The manuscript is not under evaluation in any other journal, and it has not been partially or totally published in any peer review form. A preliminary version has been uploaded to the preprint server BiorXiv.org and it is publicly available in the following link http://dx.doi.org/10.1101/639906.

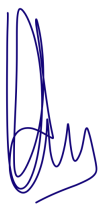
In the present work we describe the identification of piRNA transcripts during differentiation of pluripotent stem cells to cardiomyocytes, using small RNA sequencing samples generated in our laboratory. We analyzed abundance of identified piRNAs and determined differentially expressed transcripts among three stages of differentiation (pluripotent, mesoderm progenitor and cardiomyocytes), suggesting piRNAs are actively regulated during this process. Furthermore, the majority of identified piRNAs originated from protein coding and lncRNA genes in sense orientation. Of note, in cardiomyocytes these genes were related to cardiovascular development and function.

Our evidences indicate that somatic piRNAs participate in fine-tuning expression of key genes involved in the differentiation of pluripotent cells to cardiomyocytes.

Raw sequencing data files used in this work are publicly available for download at GEO (Gene Expression Omnibus) under accession number GSE108021 and custom scripting is available at https://github.com/sgmiriuka/piRNA_custom_scripting.

Authors declare there are no competing financial interests in relation to this investigation and commit to update bioRxiv entry if manuscript is accepted. Additionally, authors would like to submit the manuscript to Stem Cell Reports for joint consideration.

Regards,



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Abstract

PIWI-interacting RNAs (piRNAs) are a class of non-coding RNAs initially thought to be restricted exclusively to germline cells. In recent years, accumulating evidence has demonstrated that piRNAs are actually expressed in pluripotent, neural, cardiac and even cancer cells. However, controversy remains around the existence and function of somatic piRNAs. Using small RNA-seq samples from H9 pluripotent cells differentiated to mesoderm progenitors and cardiomyocytes we identified the expression of 447 piRNAs, of which 241 were detected in pluripotency, 218 in mesoderm and 171 in cardiac cells. The majority of them originated from the sense strand of protein coding and lncRNAs genes in all stages of differentiation, though no evidences for secondary piRNAs (ping-pong) were found. Genes hosting piRNAs in cardiac samples were related to critical biological processes in the heart, like contraction and cardiac muscle development. Our results indicate that somatic piRNAs might have a role in fine-tuning the expression of genes involved in differentiation of pluripotent cells to cardiomyocytes.

Keywords: piRNA, cardiac differentiation, pluripotent stem cells, somatic cells, non-coding RNAs

Introduction

Differentiation of pluripotent stem cells (PSC) to cardiomyocytes (CM) was first reported shortly after the characterization of embryonic stem cells (ESC) (Kehat et al., 2001). Initially, differentiation was non-specific and spontaneously achieved, but in the last 10 years upgraded protocols have been developed significantly improving efficiency and reproducibility in cardiac differentiation (Evseenko et al., 2010; Burridge et al., 2012; Lian et al., 2013). These protocols are based in sequentially adding factors (morphogens) and/or inhibitors that modulate Wnt/β-catenin signaling pathways in pluripotent cells. PSC-based models undergo epithelial-to-mesenchymal transition to an early mesoderm progenitor cell (MPC) (Evseenko et al., 2010; Loh et al., 2016) followed by further committing to cardiac mesoderm and later cardiac progenitor cells (CPC), which may eventually adopt more especialized features. Though this is arguably similar to *in vivo* embryo development, they recapitulate hallmark features of differentiation thus becoming well suited tools for disease modelling, drug screening and potential cell-based therapies.

Like many other developmental processes, changes associated with differentiation to CM are tightly regulated. Only recently, and mostly due to the advent of next generation sequencing technologies, the scientific community is unveiling the complex regulatory networks governing the shifts in gene expression profiles. Non-coding RNAs (ncRNAs) are critical players in these networks, regulating almost all cellular processes including proliferation, differentiation and death (Beermann et al., 2016; Devaux et al., 2015). Although microRNAs (miRNAs) are the most extensively studied in a wide variety of organisms (Ha and Kim, 2014; Li and Gregory, 2008; Espinoza-Lewis and Wang, 2012; Garate et al., 2018), other ncRNAs have been identified such as long non-coding RNAs (lncRNAs), small interfering RNAs (siRNAs), circular RNAs (circRNAs) and PIWI-interacting RNAs (piRNAs). Much has been published about these ncRNAs, though piRNAs is one of the least understood. Thought to be initially confined almost exclusively to germinal cell lines (Girard et al., 2006), piRNAs gained much attention primarily because of an increasing amount of evidence demonstrating that these ncRNAs are not only expressed in somatic cells but they actively participate in gene regulation as well (Ro et al., 2007; Malone et al., 2009; Yan et al., 2011; Ishizu et al., 2012).

Expression of piRNAs was first described as negatively regulating trans-

position of repetitive elements thus protecting genome integrity and favouring self-renewal (Girard et al., 2006; Aravin et al., 2007). Reports in numerous organisms showed that they exert their regulatory function through binding a specific clade of the Argonaute (AGO) family -namely PIWI proteins-, resulting in an association which resembles the well-known AGO/miRNA complex (Lau et al., 2006; Saito et al., 2006; Brennecke et al., 2007; Ha and Kim, 2014). Unlike miRNAs, piRNAs are primarily biosynthesized as single-stranded long precursors which are then clived into the 24-34 nucleotide-long mature forms in a Dicer-independent manner (Girard et al., 2006; Han et al., 2017; Phay et al., 2018). They show a bias for uridine (U) redidues in 5' ends together with a 2'O-methyl modification at their 3' ends. Germ line piRNAs were also found to be synthesized through a secondary pathway named the Ping-Pong amplification loop, which increases levels of primary piRNAs using target mRNAs as intermediary molecules for processing new piRNA precursors (Brennecke et al., 2007; Sato and Siomi, 2013). Of note, these mechanisms seem to be highly conserved across species (Lau et al., 2006; Siomi et al., 2011).

In the last few years, many studies have proposed an active participation of PIWI/piRNAs complexes in diverse and critical pathways such as neural development or body regeneration of lower eukaryotes (Rajasethupathy et al., 2012; Ross et al., 2014). Furthermore, recent work demonstrated a positive correlation between altered piRNA expression profiles and clinically relevant pathologies. The involvement of specific piRNAs in regulating mRNAs levels of genes related to Alzheimer's disease was described in 2017 (Roy et al., 2017), while other groups implicated piRNAs in cardiac function and regeneration through modulation of AKT pathway (Vella et al., 2016; Rajan et al., 2014). However, great controversy still remains around expression, function and biosynthetic pathways of somatic piRNAs. Particularly, the potential role of piRNAs in differentiation of pluripotent stem cells to cardiomyocytes has not been formally addressed. Using small RNAseq data generated in our laboratory (Garate et al., 2018) we characterized the expression profile of small RNAs consistent with piRNAs in three stages of cell differentiation from pluripotency (day 0) to mesoderm (day 3.5) and then contractile cardiocytes (day 21). Results presented here provide evidences supporting the existence of somatic piRNA transcripts and their stage-specific pattern as a mechanism for potentially fine-tuning gene expression during cell differentiation.

Results

Detection and characterization of piRNA

Detection of piRNAs was conducted on small RNAseq samples from three independent experiments consisting of pluripotent stem cells (PSC, day 0), early mesoderm progenitor cells (MPC, day 3.5) and cardiac progenitor cells (CPC, day 21). After aligning reads to human reference genome (hg38), we found that more than half of mapped reads were 20 to 23 nucleotides (nt) long, where the abundant miRNAs were included (Figure 1A, (Garate et al., 2018)). Considering that the average length of piRNAs in mammals ranges between 24 and 34 nt (Girard et al., 2006; Ozata et al., 2018; Phay et al., 2018), mapped reads were filtered by length to accommodate to this restriction. Nearly 50-70% of mapped reads were removed from the samples after this initial processing step (Figure S1A and S1B). Then, employing a similar approach as previously published work (Yan et al., 2011), we filtered out any read that mapped on ncRNAs (DASHR (Leung et al., 2016)) besides piRNAs (Figure S1C) given that previous publications emphasized on the fact that many identified piRNAs were actually fragments of other types of ncRNAs (Tosar et al., 2018). Approximately 5-20% of initial mapped reads remained after this step (Figure S1B). Importantly, all nine aligned samples behaved similarly to both filtering steps (Figure 1B), reflecting consistency among experimental replicates.

To verify the elimination of potential misleading contaminants in fully processed alignments, we analyzed the distribution of mapped reads over two well-characterized miRNAs, pluripotency-associated miR-302b and cardiac-expressed miR-143 (Garate et al., 2018). As expected, expression of miR-302b was evident in unfiltered data of PSC and MPC populations while miR-143 showed appreciable coverage in unfiltered data of CPC (Figure 1C, top panels). No signal was detected for either of the two genes in processed alignments (Figure 1C, bottom panels). However, these samples showed a strong and sharp coverage signal on known piRNA loci (Figure 1D) confirming that the pipeline employed successfully enriched for reads mapping to these known piRNAs. Henceforth, all analyses were performed on fully processed alignments unless explicitly specified otherwise.

Sequence analysis of reads mapping to known piRNA loci showed that all samples but MPC bore a bias for 5' uridine residues as it usually occurs in germline cells (Figure 2A). We corroborated our proceedings by employing the pipeline described above on two small RNA-seq samples from human

testis downloaded from the ENCODE project. Indeed, there was a marked preference for uridine at 5' ends in testis samples (Figure S2A and S2B), which suggest that putative piRNAs in our model are subjected to similar mechanisms of 5' end formation as in germline cells. However, the substantial difference in frequecy of 5'-U residues between our samples and testis samples could be an indicative of unconserved biosynthetic steps (Ross et al., 2014). In addition, no secondary piRNA production was detected in any of the replicates of our samples given that we did not found evidences of the characteristic 10 nt overlap (ping-pong signature) between 5' ends of sense and antisense mapped reads (Figure 2B).

Expression of piRNAs during cardiac differentiation

To study the expression profile of known piRNAs in differentiating PSC we kept those with an average count among replicates higher than or equal to 3. Normalization by library depth showed equivalent distribution of relative quantifications between samples (Figure S3A), enabling confident identification of 447 piRNAs considering the three cell differentiation stages investigated (Table S1). Despite some differences between replicates, each stage of cell differentiation was categorically defined by a specif piRNA expression profile (Figure 3B) which was also reflected in Principal Component Analysis results (Figure S3C). These identifying profiles preferentially aggregated PSC and MPC together indicating a greater resemblance between samples of these two cell populations than with CPC.

Of the 447 identified piRNAs, 241 were expressed in PSC, 218 in MPC and 171 in CPC (Table S1). Differential expression analysis revealed only 30 genes with significant shifts in RNA levels (-1 > log2FC > 1; -log10p-value > 1.30) for the comparison between PSC and MPC, while 137 were differentially expressed (DE) between PSC and CPC and 153 between MPC and CPC (Figure 3A). Of the total 447 piRNAs, 204 were found to be DE with respect to CPC, 86 of which were shared by MPC and PSC (Figure S3D and Table S1). These results were consistent with correlation analysis that showed a higher Pearson coefficient for the MPC-PSC pair (R=0.5, p<2.2e-16) than for CPC-PSC (R=-0.0062, p=0.9) (Figure 3B), suggesting that PSC bear a greater resemblance to MPC than to CPC not only in the identity of expressed piRNAs, but in their abundance as well. Upregulated piRNAs accounted for 14% of total DE piRNAs in CPC (Figure 3C and Table S1), far fewer than the downregulated piRNAs (Figure 3D and Table S1). We validated several piRNA transcripts (piR-1919272, piR-2519215 and

piR-97458) by qPCR in an independent set of samples from H9 pluripotent cells and 14 days after the onset of cardiac differentiation, corroborating our detection pipeline and subsequent DE analysis (Figure S3F).

Differentially expressed piRNAs ranked among the top expressing piRNAs. This is probably due to the fact that highly expressed genes are inherently less sensitive to inter-replicate noise, hence more likely to return a lower p-value for contrasts. Thus, in order to investigate potential patterns underlying expression data which might have been masked from differential expression analysis, we implemented a soft clustering algorithm to data. This approach returned 8 different patterns of piRNA expression (Figure 3E and Table S2), or Expression Clusters (EC), that reflected two dynamically relevant tendencies: downregulation of piRNAs towards cardiac differentiation (cluster 1 to 4 and 7) and upregulation of piRNAs towards cardiac differentiation (cluster 5, 6 and 8). The former, as was previously observed, encompassed the majority of DE piRNAs. Regardless of the condition (up or downregulated) of piRNAs in CPC, it was clear that a fraction of piRNAs sustained early change (PSC to MPC) while others shifted later in the differentiation process (MPC to CPC). Interestingly, expression profile of human *PIWI* genes (*HIWI*, *HILI*, *HIWI2* and *HIWI3*) changed between day 0 and 14 of differentiation (Figure 4a). While no conclusive results were obtained for *HIWI* and *HIWI3*, *HILI* and *HIWI2* were upregulated towards day 14 suggesting that there might be a connection between these *PIWI* genes and cardiac piRNAs. Specific markers of pluripotency and cardiomyocytes were measure at these timepoints to corroborate cell identity (Figure 4b). In addition, analysis of H9 and H1 published RNA-seq data validated upregulation of *HIWI2* with cardiac differentiation (Figure S4, (Liu et al., 2017)).

Genome distribution of expressed piRNAs

Identified piRNAs were distributed rather uniformly throughout the nuclear genome (Figure 5A), except in chromosome Y for which no data was available given the XX karyotype of H9 embryonic stem cells used in this work. Moreover, Expression Clusters did not seem to follow any particular arrangement in these chromosomes as well (Figure 5A, center of circular plot). Inclusion of the mitochondrial chromosome (chrM) in the analysis revealed that 90 of 447 piRNAs originated from the mitochondrial genome (Figure 5B). This was consistent with previous work in human somatic cancer cell lines reporting the synthesis of piRNAs from mitochondrial genome

((Kwon et al., 2014)). In fact, the chrM was the major contributor of expressed piRNAs in our samples and was mostly dominated by three EC: a) cluster 3, with piRNAs highly expressed in PSC; b) cluster 8, with piRNAs highly expressed in CPC; c) cluster 5, with piRNAs highly expressed in both PSC and CPC.

We corroborated this finding by evaluating the distribution of mapped reads over chrM, and thus eliminated the possibility of errors during counting of reads per transcript (Figure S5A). Despite our pipeline for identification of expressed piRNAs filtered out all reads that mapped to ncRNAs -other than piRNAs- using DASHR database, 90% of mitochondrial piRNAs (81 out of 90) mapped directly to tRNA and rRNA annotations (GENCODE v29) (Figure S5B). DASHR database showed only one annotation in chrM (LSU-rRNA) that corresponded to the large ribosomal subunit RNR2 (Figure S5C). This was not the case in the nuclear genome where no piRNAs were found to map on rRNAs and tRNAs annotated in GENCODE database (Figure S5D). Nonetheless, piRNAs identified in length-filtered data (initial step of filtering, Figure 1B) did not map to nuclear rRNA or tRNA annotations from GENCODE to begin with (Figure S5D), suggesting that this step was sufficient enough to remove reads mapping on them.

Regardless of the chromosome distribution, identified piRNAs localized preferentially on gene annotations (Figure 5C). PSC samples showed that 88.5% of piRNAs were generated from gene features, while the percentage was higher in MPC and CPC samples, with 96.2 and 95.5% respectively.

Protein coding and lncRNA genes hosting piRNAs

Further analysis on genomic distribution of identified piRNAs revealed that nearly 65% of those intersected to gene features originated from coding (53%) and long non-coding (12%) annotations (Figure S5D). To test whether these events were random, we shuffled our samples 1500 times (bootstraping) and analyzed intersection to these features in sense and antisense orientation. Once data was collected, we calculated enrichment on genes as "sample piRNAs" over "shuffled piRNAs" and determined that sense-oriented piRNAs occurred non-randomly on protein coding and long non-coding (lnc) genes (Figure 6A). On the contrary, intersection in antisense had poor fold enrichment values suggesting piRNAs were preferentially located elsewhere. We observed similar results for piRNAs identified in all three cell differentiation stages studied in this work, as well as in two samples (isogenic replicates)

downloaded from ENCODE project corresponding to H1-derived neural progenitor cells (NPC). Both neural samples were handled following the same steps and criteria described before (Figure S6A).

Taking into consideration that reads originated from protein coding and lncRNA features might have been the result of ordinary transcript degradation, we investigated the distribution of reads mapped on such piRNA-hosting genes. Results showed that the percentage of bases covered by sense-oriented reads in these genes was low, with a median value of 0.56% in PSC, 1.50% in MPC, 2.08% in CPC and 3.63% in NPC (Figure S6B). Moreover, coverage was localized to a set of specific piRNAs instead of all piRbase annotations described in any single gene (Figure S6C), proving to be inconsistent with random degradation-produced reads. Coverage by antisense-oriented reads was closer to none (Figure S6D) and significantly lower than sense-oriented coverage in all cell population except in MPC (Figure 6B), possibly due to a higher level of dispersion in values of these samples.

The wide majority of piRNA-hosting protein coding and lncRNA genes harboured a single piRNA transcript with a tendency to augment the number of piRNAs per gene throughout the differentiation process (Figure 6C, pie charts). Like MPC and CPC, NPC exhibited a wider spectrum of piRNAs per gene than undifferentiated pluripotent cells (PSC). A more detailed exploration into CPC results revealed that *MALAT1* (lncRNA gene) and *TTN* (protein coding gene) contained the highest number of piRNAs -12 and 6 respectively-, followed by *PLN*, *RPPH1*, *ACTC1* and *AL355075.4* with 4 (Figure 6C, bottom panel). Using RNA-seq data from H9 cells differentiated to CM (Liu et al., 2017) we analyzed the expression profile of these piRNA-containing genes. Transcript abundance of *MALAT1*, *TTN* and *RPPH1* increased from day 0 (PSC) to day 2/4 (MPC) and then dropped between day 4 and day 30 (CPC), which were inversely correlated with the expression dynamics of piRNAs from EC 8 (Figure 6D). *PLN* and *ACTC1* RNA levels increased from day 4 to day 30 practically impervious to piRNA production, though lack of data between day 4 and 30 hindered our analysis for these genes (data not shown). With respect to *AL355075.4* gene, we found no count data available in RNA-seq samples. However, this gene overlaps *RPPH1* in sense orientation and it partially overlaps protein coding gene *PARP2* in antisense orientation, meaning that piRNAs originated from it could be potentially involved in regulating both genes. In fact, *PARP2* expression dynamic showed a steady descent in transcript levels from day 0 to 30, which is also consistent with the fact that the piRNAs originated

from *AL355075.4* were also detected in MPC (Figure S6E). Similar results were found when we studied two genes with high piRNA content (>3) in MPC (Figure 6E) -*APLNR* and *RMRP*- in which almost all piRNAs belong to EC 2. Taken together, these evidences suggest that piRNAs originated from these genes may be implicated in their downregulation or possibly in a moderate fine-tuning as in the case of *PLN* and *ACTC1*.

Functional analysis on piRNA-hosting genes in differentiated cells

The expression profile of piRNAs proved to be sufficient to clearly discriminate CPC samples not only from PSC and MPC populations, but from neural progenitors (NPC) as well. The comparison between CPC and NPC samples revealed that 52 piRNAs were expressed in both types of differentiated cells, but more importantly the majority were not (Figure 7A). Unshared piRNAs constitute a unique repertoire for each cell population which could possibly reflect upon diverse functional processes. To evaluate this notion, we extracted all protein coding genes which were intersected by at least one piRNA and determined their involvement in any biological process (BP). In search for overrepresented terms (BPs with more genes involved than expected), we found that CPC and NPC showed markedly different terms. The BPs associated to CPC were intimately related to heart development and muscle differentiation and contraction (Figure 7B), while overrepresented BPs in NPC showed a clear inclination towards neurogenesis regulation and neural proliferation and development (Figure 7C). The group of genes intersected by piRNAs shared by both CPC and NPC (52 piRNAs in venn diagram) did not participate in any of the statistically significant overrepresented BPs, meaning that enriched categories for each population are mostly based in their unique collection of piRNAs.

Discussion

Since the first mechanistic evidences of piRNA biogenesis in ovarian follicle cells of *D. melanogaster* were published (Malone et al., 2009), much information has emerged on the somatic expression and function of this type of regulatory small RNAs. Several publications demonstrated that piRNAs (or piRNA-like RNAs) originate from discrete genomic regions of somatic cells in a wide diversity of species and tissues (Cichocki et al., 2010; Yan et al., 2011; Vella et al., 2016; Ng et al., 2016; Roy et al., 2017). In agreement with this line of evidence, we report the expression of 447 small RNAs

consistent with piRNAs among three stages of differentiation of pluripotent stem cells to cardiomyocytes using a database-driven approach.

The pipeline leading to the identification and quantification of piRNAs involved two filtering steps that were implemented to avoid innacurate interpretation of results. Firstly, aligned reads shorter than 24nt and longer than 34nt were discarded from further analyses. However, our results showed a higher-than-basal frequency of 36-37nt-long reads, which prompts the issue if these reads should have been kept for further investigation as potential longer piRNAs or perhaps remnants of piRNA precursors. Length restriction answers to one of the hallmark attributes of mature piRNA transcripts, albeit the range seems to vary across species. In fact, in *C. elegans* 21nt-long piRNAs are produced from transcript precursors of 25-27nt in length (Ruby et al., 2006) whose processing machinery is partially unknown. The reason for the range diversity has been convincingly connected to the activity of the proteins involved in their biosynthetic pathway (biogenesis). It is possible that they also contribute to explaining the differences between germline and somatic piRNAs considering that diverse sets of enzimes have been reported to be engaged in piRNA synthesis in these cell lineages (Ruby et al., 2006; Zamore, 2010). Moreover, the differential expression profile of *HIWI* genes observed in our data -and in H9 and H1 external RNA-seq data- points to cell-type specific functions for these proteins and consequently for their piRNA partners.

In a second step, length-filtered reads mapping to small ncRNAs other than piRNAs were removed from samples. The fact that remaining reads in PSC and CPC samples showed a moderate 5' U bias, whereas MPC samples did not could probably be related to the transitional nature of this cell population. However, none of the samples exhibited the characteristic 10nt-overlap signature of secondary piRNAs, which is a generally accepted feature in germline cells of most animals where piRNAs are synthesized through both primary and secondary (ping-pong loop) pathways (Yan et al., 2011; Ross et al., 2014). Despite synthesis in somatic cells has been proposed to produce only primary piRNAs, many of the mechanisms underlying piRNA biogenesis -specially in non-gonadal tisues- are not yet fully understood. Conceivably, the filtering steps eliminated potential piRNAs from the three stages, though it has been argued that a considerable amount of annotated piRNAs are actually ncRNA fragments derived from rRNAs, tRNAs and even miRNAs (Tosar et al., 2018). On the matter, an insightful disscussion by Tosar and collaborators (Tosar et al., 2018), advocating for gonadal piRNAs, suggested

that somatic piRNAs mapping to ncRNA fragments are not unquestionably wrong, still further biochemical evidence is needed to include them as such.

An important aspect of this work lies on the identification of a piRNA expression profile associated to each of the cell populations under study. These expression profiles parallel the embryological connection between the stages, where PSC is more closely related to MPC than to CPC (Evseenko et al., 2010; Sha et al., 2019). Upon this premise, the piRNAs identified as early-changing could potentially be involved in maintaining pluripotency or in the commitment of pluripotent cells to mesoderm progenitors, which might eventually differentiate to multiple lineages. Late-changing piRNAs, on the contrary, would influence further commitment of mesoderm cells to cardiac progenitors. The fact that six times more piRNAs were downregulated rather than upregulated during differentiation to CPC suggests that piRNA pathways become less relevant in differentiated cells. It is possible that mechanisms evolutionarily linked to the regulation of transposable elements are not as critically conserved in differentiated cells as they do in cells with high proliferation rates or reproductive functions, such as pluripotent cells and germ line cells. For example, it has been proposed that cancer cells might promote piRNA biosynthetic pathways as a mechanism to reduce genome instability caused by increased mitotic and transcriptional activities (Liu et al., 2018).

The genomic localization of piRNAs included in anyone particular EC was not the same. In fact, piRNA expression appeared to be uniformly scattered across the genome except for the mitochondrial chromosome. The majority of piRNAs identified in the mitochondrial genome mapped to rRNAs or tRNAs and though we have not definitively proved they are truly piRNAs, previous work established a link between tRNA-/rRNA-derived piRNAs, *HIWI2* expression and regulation of metabolic processes in somatic cells (Keam et al., 2014). Analogously, the increased levels of piRNAs from mitochondrial tRNAs/rRNAs and the significant upregulation of *HIWI2* (day 14 v. day 0, and external H9 RNA-seq data) in CPC could seemingly be connected to the large-scale modifications in CM metabolism (Tohyama et al., 2013).

Even though identified piRNAs were dispersed throughout the genome, it was clear that the vast majority of them originated from gene loci. However, it is not yet clear the reason why these piRNAs are generated from the sense strand of their hosting genes. One possibility relies on the capacity of PIWI/piRNA complexes to direct recruitment of DNA and histone methyltransferases, modifying accesibility of transcriptional machinery to chromatin

(Rajasethupathy et al., 2012; Pezic et al., 2014). Available data of DNA or histone methylation status in the three stages of cardiac differentiation is scarse or dissimilar, so preliminary correlation analysis were not conclusive at this point (data not shown). Nevertheless, further experiments on promoter methylation and H3K9me3 mark deposition ought to be performed to pursuit this possibility. Also, considering that antisense transcripts have been described to positively regulate stability of sense RNA (Zong et al., 2016), it is possible that sense-originated piRNAs regulate antisense transcript levels in a miRNA-like mechanism. For instance, *TALAM1* -an antisense transcript at the *MALAT1* gene locus- promotes stability and maturation of *MALAT1* RNA by facilitating enzymatic cleavage of its 3' end (Zong et al., 2016), thus a potential piRNA-mediated downregulation of *TALAM1* would redound to diminished *MALAT1* levels.

In sum, the evidences presented here contribute to understanding the dynamic expression of piRNAs during differentiation of pluripotent stem cells to cardiomyocytes and further explore their potential function as post-transcriptional modulators in somatic cells. Together with miRNAs, piRNAs seem to participate in the fine-tuning of transcript levels, adding yet another layer to the complex and intrincated networks governing gene expression.

Acknowledgments

We would like to thank Fundación FLENI and Fundacin Pérez Companc for their continuous support.

Author Contributions

A.L.G. and S.G.M. design experiments and wrote manuscript. MAS, M.C.H.C., N.P., S.C., C.C., A.M.M., N.L.S.V. and C.A. performed global bioinformatic analysis. X.G., A.W., L.M., G.S., C.L. and S.G.M. discussed and revised analyses and manuscript. G.S. and S.G.M. provided fundings for this paper.

Conflict of interest statement

None declared.

Funding

This work was supported by FONCYT [PICT-2011-1927,PICT-2015-1469, PID-2014-0052]; and CONICET [PIP2015-2017].

Data Availability

Small RNA sequencing data can be found in GEO under accession number GSE108021.

Figure Legends

Figure 1. Detection of piRNAs in small RNAseq samples. A) Length frequency of unfiltered mapped reads in three samples from pluripotent cells (PSC), three from mesoderm progenitor cells (MPC) and three from cardiac cells (CPC). Grey areas denote the size range for both microRNAs (miRNA) and putative piwi-interacting RNAs (piRNA). The color key used is indicated in top right corner of the plot. B) Number of mapped reads for all nine samples before processing (unfiltered, grey box), after filtering by read length ($23 < \text{RL} < 35$, coral box) and removing non coding RNA other than piRNAs (-ncRNA, green box). C) Distribution of mapped reads as a function of density on a fragment of chromosome 4 (chr4:100,000,000-120,000,000; $-$) which includes pluripotency miR-302b and a fragment of chromosome 5 (chr5:149,300,000-149,600,000, $+$) including cardiac miR-143 for unprocessed alignments (top panels, unfiltered data) and fully processed samples (bottom panels, length+ncRNA filtered). Alignment files from each experimental replicate were merged into one. Color key for the density curves is shown in the graph. D) Analysis of coverage on all piRNA loci available in piRbase for fully processed normalized (counts per million) samples.

Figure 2. Characterization of reads mapped to known piRNAs. A) Frequency of bases per position in reads mapped to known piRNAs after fully processing alignments from PSC, MPC and CPC samples (merged replicates). Position 1 is marked by a vertical grey bar. B) Frequency profile showing overlap between reads mapped to known piRNA loci in sense orientation and complementary reads using an independent approach (ssviz package).

Figure 3. piRNA expression profile in differentiating pluripotent stem cells. A) Differential expression analysis performed on raw

counts with DESeq2 package. Significantly different expression values (-1 >= log2FC >= 1; -log10p-value > 1.30)) are represented as orange dots in the volcano plots for the three possible comparisons: MPC vs. PSC (left), CPC vs. PSC (center) and CPC vs. MPC (right). B) Scatter plot of normalized expression data showing Pearson correlation analysis on MPC (green dots) and CPC (red dots) versus PSC. Marginal density plots to the right denote areas of highly abundant data and black arrows mark the positions of the most DE piRNA genes. C) Heatmap shows normalized counts of upregulated piRNAs genes in CPC considering the three cell populations (PSC, MPC, CPC). Dendrograms resulted from running hard unsupervised clustering algorithms on piRNA genes (left) and samples (top). D) Heatmap as in c showing downregulated piRNA genes in CPC. E) Implementation of soft clustering algorithms (R package MFuzz) produces eight distinct patterns (clusters 1 to 8) of piRNA expression.

Figure 4. Expression of human *PIWI* genes in cardiac differentiation. A) Transcript levels of *HILI* (PIWIL2) and *HIWI2* (PIWIL4) were measure by qPCR in H9 pluripotent cells (D0) and H9-derived cardiac progenitors (D14). B) Stage-specific markers were evaluated by qPCR in samples from a. Pluripotency genes are shown in the top row and genes for cardiac lineage in the bottom. All results were expressed as mean±se of three independent experiments after normalization by the geometric mean of *RPL7* and *HPRT1* housekeeping genes. Statistical significance was evaluated by Student's T test (day14 v. day0) and results are indicated as p-values on the bar plots.

Figure 5. Distribution of piRNAs and Expresion Clusters in the genome. A) Normalized expression of piRNAs from averaged samples of PSC (outer track), MPC (middle track) and CPC (inner track) displayed lengthwise in a circular representation of human autosomal chromosomes (1 to 22) and chromosome X. Level of expression is depicted as heatmaps inside the tracks and follow a red-green scale for high expression and low expression respectively. The center of the plot shows links between piRNAs that belong to the same Expression Cluster (EC); color key for the links appears at the bottom left corner of the plot. B) Bars represent number of piRNAs expressed in all samples per chromosome. EC membership of piRNAs is indicated with color key as in a (top left corner of barplot). C) Percentage of piRNAs in PSC, MPC and CPC samples that intersected or

not with genes from GENCODE database (v29).

Figure 6. Proteing coding and lncRNA-originated piRNAs. A) Fold enrichment was calculated as number of piRNAs intersected to protein coding and lncRNA genes in sense (purple) and antisense (green) orientation over 1500 different random distributions (bootstraping). Red dashed line marks the point of no enrichment (log2 1=0). NPC: human neural progenitor cells. B) Distribution of percent coverage values on genes in sense (purple) and antisense orientation (green). P-value (p) for statistical analysis is shown in the plots (ANOVA). ns: not significant. C) Pie charts show the proportion of genes containing piRNAs. The number of sense piRNAs per gene in each category is indicated in the charts. Plot at the bottom provides detail on pie chart for CPC labeling genes with two or more piRNAs. Mitochondrial genes are not included in the analysis. D) Normalized counts (FPKM) for three genes from c during differentiation of H9 cells (day 0, day 2, day 4 and day 30) to CM. Data was extracted from previously published total RNA-seq experiments. E) Normalized counts (FPKM) for two genes containg piRNAs in MPC.

Figure 7. Functional exploration of piRNAs in differentiated cells. A) Expression profiles of piRNAs (log2 normalized counts) in CPC (cpc1, cpc2 and cpc3) and NPC (neural1 and neural2). Color key for sample clustering is displayed at the top right corner of the heatmap. B) Over-represented biological processes (BPs) on proteing coding genes harbouring piRNAs from CPC samples. C) Overrepresented BPs on proteing coding genes harbouring piRNAs from NPC samples.

Experimental Procedures

Small RNAseq data

Data samples used in this work (PSC: H9 human embryonic stem cells, MPC: early mesoderm progenitor and CPC:cardiomyocytes) were generated in our laboratory following previously described protocols and are available under accession code GSE108021. Briefly, H9 cells (H9-hTnnTZ-pGZ-D2 obtained from WiCell) were routinely maintained in co-culture with irradiated primary mouse embryonic fibroblasts. Mesoderm induction (MPC population) was performed by initially seeding cells with mTeSR (StemCells Technologies) on plates coated with Geltrex (Thermo Fisher Scientific) and

then switching to (Evseenko et al., 2010) StemPro-34 SFM (Thermo Fisher Scientific) supplemented with Activin A only at the first day, BMP4, VEGF and bFGF (Thermo Fisher Scientific) for 3.5 days. At this point, mesoderm progenitors were isolated by FACS with anti-CD326 and anti-CD56 (Biolegend). CPC population was obtained by formation of embryoid bodies with H9 cells using BMP4, bFGF and Activin A in StemPro-34 followed by addition of VEGF and Wnt inhibitor, IWR-1. Libraries for small RNA sequencing were prepared with 200 ng of RNA using NEBNext Small RNA Library Prep Set with modified adaptors and primers compatible for Illumina (New England Biolabs). Single end sequencing was carried out at the TCGB Resources (UCLA Path and Lab Med) using an Illumina HiSeq 2500. Culture conditions and sequencing of small RNAs for these samples are more extensively described in (Garate et al., 2018).

External data

Human testis small RNA-seq samples from two men of 54 and 37 years old (GSE88414 and GSE88124, respectively) and H1-derived neural progenitor cells (GSM1296459 and GSM1296460) were downloaded from ENCODE (encodeproject.org). RNA-seq data (counts per transcript) from H1 and H9 cardiac differentiation protocols can be found under GEO accesion GSE85331.

Data processing and analyses

Adapters were removed from raw sequencing reads with cutadapt (v1.9.1) keeping reads with a minimum of 20 and up to 50 nt in length. Quality checked (FastQC) processed reads were mapped to human reference genome (GRCh38/hg38) using STAR aligner (v2.5.3a (Dobin et al., 2013)) under mostly default parameters. Mapped reads in output SAM/BAM files were filtered by read length ($23 < \mathrm{RL} < 35$) with samtools and custom awk scripting. Resulting reads were intersected (bedtools v2.27.1 (Quinlan and Hall, 2010)) to ncRNAs in a strand specific manner (DASHR (Leung et al., 2016)) to remove potential misleading alignments. Raw counts on piRNAs were determined with htseq-count matching mapped reads to piRNA coordinates downloaded from piRBase (Wang et al., 2018). Counts were then fed into DESeq2 for differential expression analysis ($p<0.05$ and $fdr<0.1$). Soft clustering methods were implemented with R package Mfuzz (v2.42.0 (Futschik and Carlisle, 2005)) using parameter $m=1.15$. In parallel to our customized pipeline, tools for ping-pong signature detection like ssviz R package and PingPongPro (Uhrig and Klein, 2018) were run following recommendations

from authors. Graphics and statistical analyses were performed in R software and deepTools (Ramírez et al., 2016). Further details on custom code is available at https://github.com/sgmiriuka/piRNA_custom_scripting.

Reverse Transcription of piRNA

To obtain cDNA from piRNA transcripts we adapted a previously described methodology employed for miRNA detection and amplification (Chen et al., 2005). Briefly, stem-loop retrotranscription (RT) primers were generated using 6-8nt from the 3' end of every piRNA of interest. Each RT reaction was performed with a maximum of 10 different stem-loop primers including one for RNU6B and hsa-miR-302b as controls. SuperScriptIII retrotranscriptase (Thermo Fisher Scientific) was used for RT reactions following guidelines from manufacturer. Detection by qPCR involved forward primers matching the sequence of target piRNAs and a reverse universal primer complementary to the stem-loop RT primer.

Real time PCR

Total RNA was prepared with TRI-Reagent (Sigma Aldrich) following manufacturer's instructions and then reverse transcribed using MMLV reverse transcriptase (Promega) and random primers for detection of polyadenilated transcripts. Quantitative real time PCR (qPCR) was performed in a StepOne Real Time PCR system (Applied Biosystems). Expression was normalized to the geometrical mean of HPRT1 and RPL7 housekeeping genes and log2 transformed. Statistical significance for qPCR results was analyzed by t test (day14 v. day0). Primers sequences are available on request.

Table S2. Related to Figure 3 and 5. piRNAs included in Expression Clusters.

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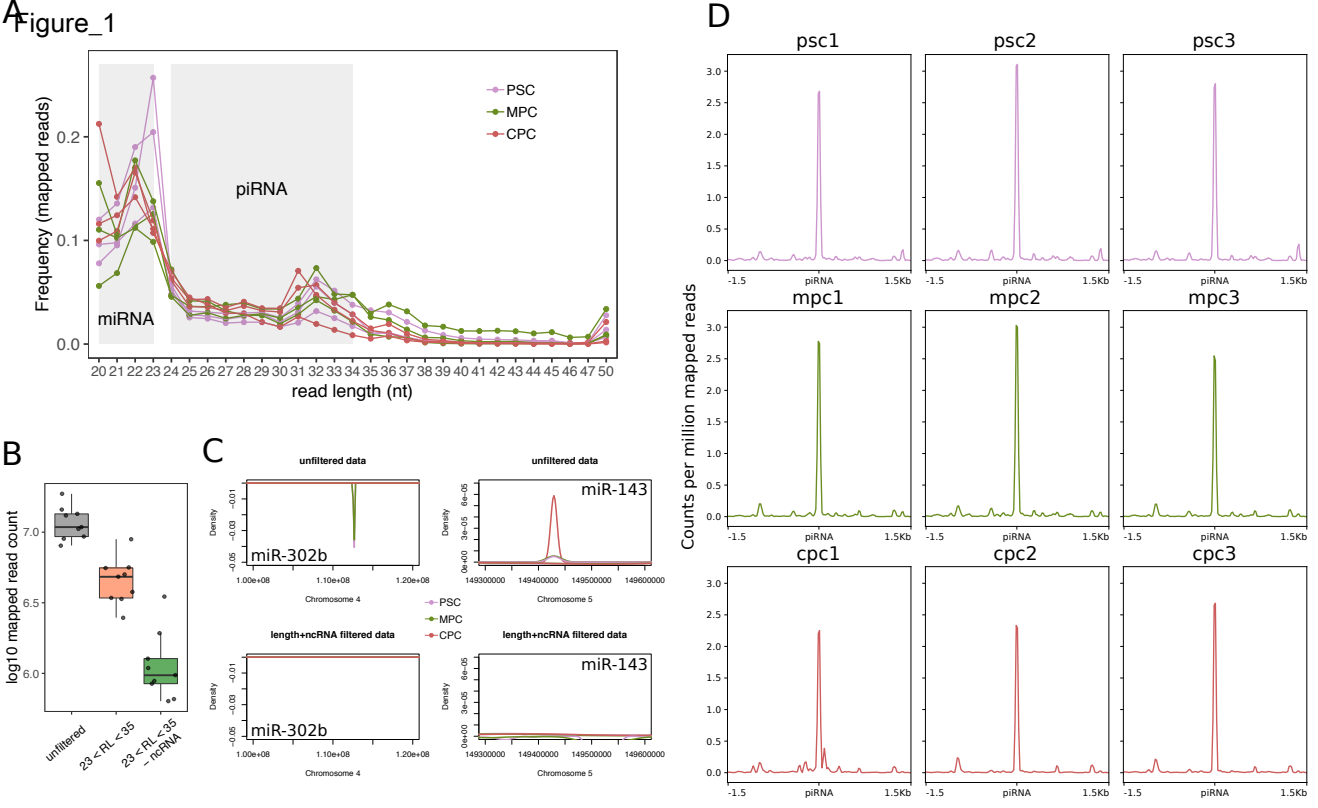
Figure_1


A
Frequency (mapped reads)
0.0
0.1
0.2
PSC
MPC
CPC
piRNA
miRNA
20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 50
read length (nt)
B
log10 mapped read count
6.0
6.5
7.0
unfiltered
23 < RL < 35
23 < RL < 35 _ ncRNA
C
unfiltered data
miR-302b
miR-143
Density
Chromosome 4
Chromosome 5
PSC
MPC
CPC
length+ncRNA filtered data
D
psc1
psc2
psc3
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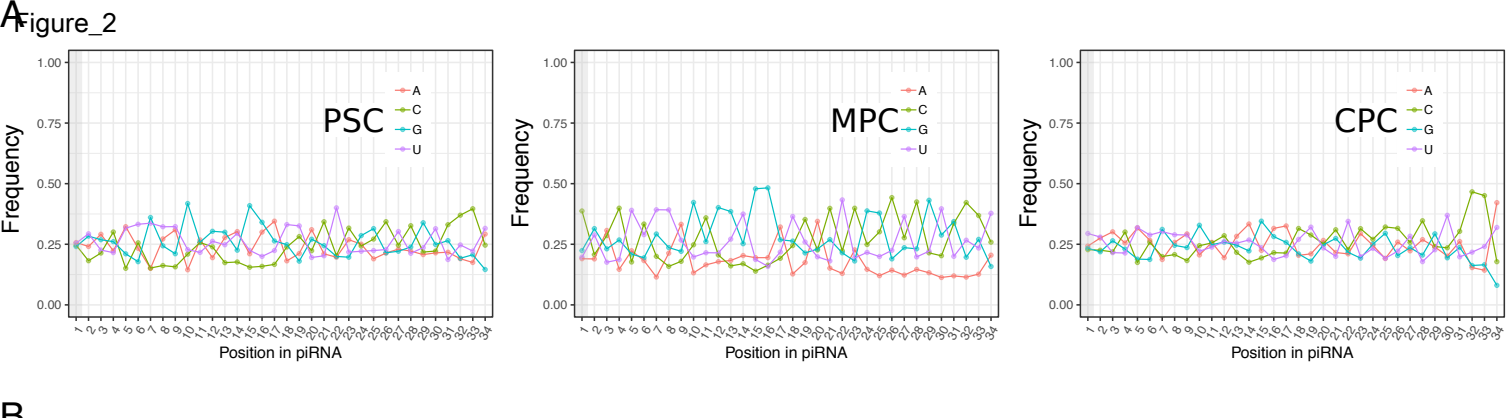
cpc2
cpc3
Counts per million mapped reads
-1.5
piRNA
1.5Kb

Figure_2


A
PSC
MPC
CPC
A
C
G
U
Frequency
Position in piRNA

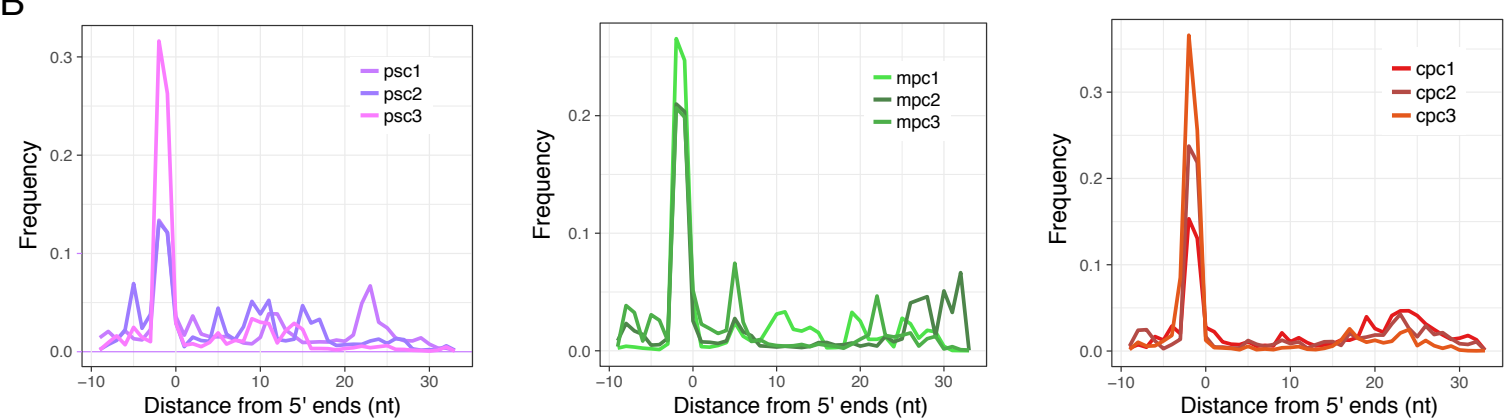
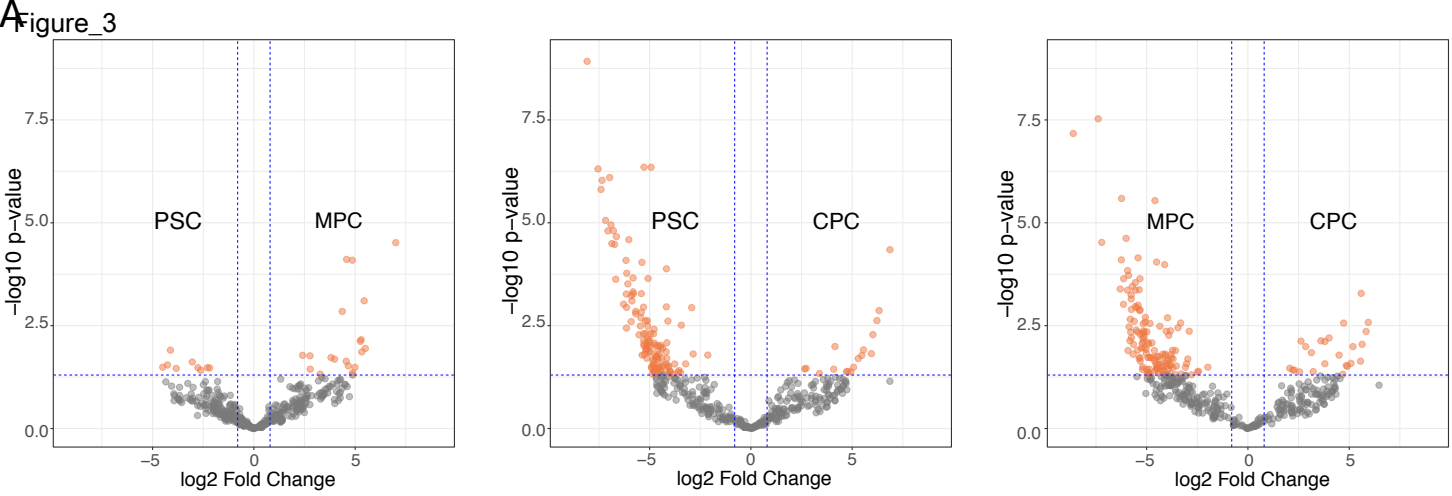
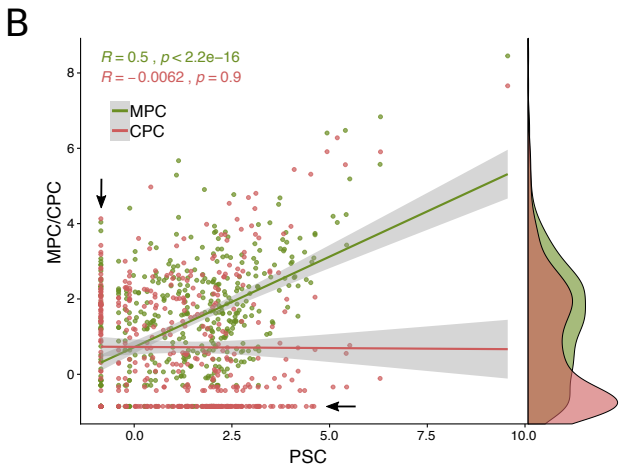
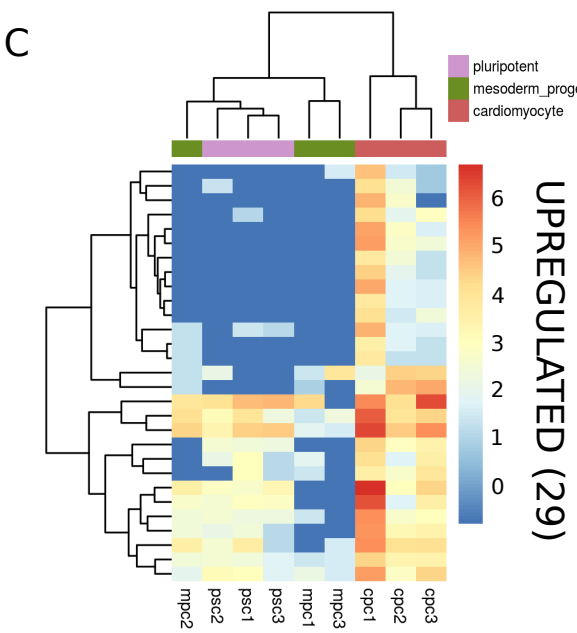
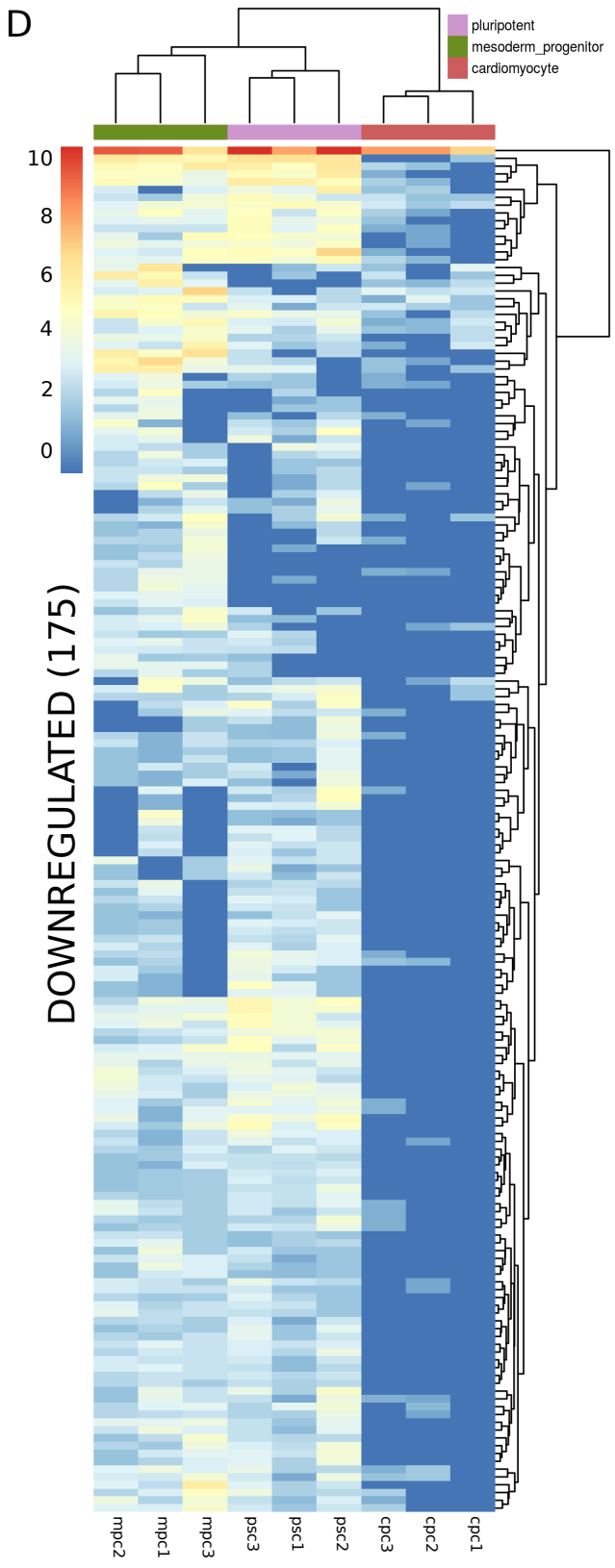

B
psc1
psc2
psc3
mpc1
mpc2
mpc3
cpc1
cpc2
cpc3
Frequency
Distance from 5' ends (nt)

Figure_3


A
PSC
MPC
CPC
−log10 p−value
log2 Fold Change


B
R = 0.5 , p < 2.2e−16
R = −0.0062 , p = 0.9
MPC
CPC
MPC/CPC
PSC


C
pluripotent
mesoderm_progenitor
cardiomyocyte
UPREGULATED (29)
mpc2
psc2
psc1
psc3
mpc1
mpc3
cpc1
cpc2
cpc3


D
pluripotent
mesoderm_progenitor
cardiomyocyte
DOWNREGULATED (175)
mpc2
mpc1
mpc3
psc3
psc1
psc2
cpc3
cpc2
cpc1

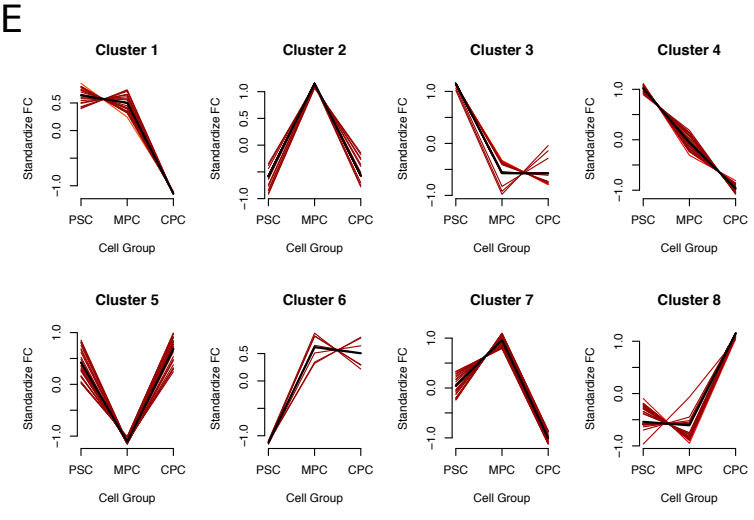
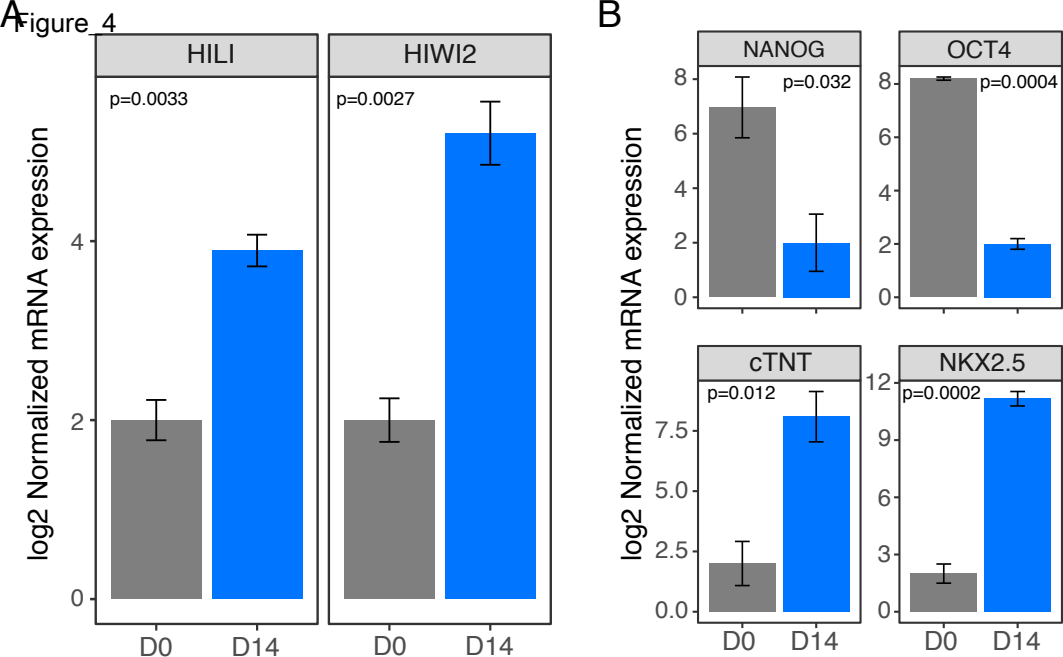

E
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Cluster 7
Cluster 8
Standardize FC
PSC
MPC
CPC
Cell Group

Figure_4


A
HILI
HIWI2
p=0.0033
p=0.0027
log2 Normalized mRNA expression
0
2
4
D0
D14
D0
D14
B
NANOG
OCT4
cTNT
NKX2.5
p=0.032
p=0.0004
p=0.012
p=0.0002
log2 Normalized mRNA expression
0
2
4
6
8
0.0
2.5
5.0
7.5
0
3
6
9
12
D0
D14

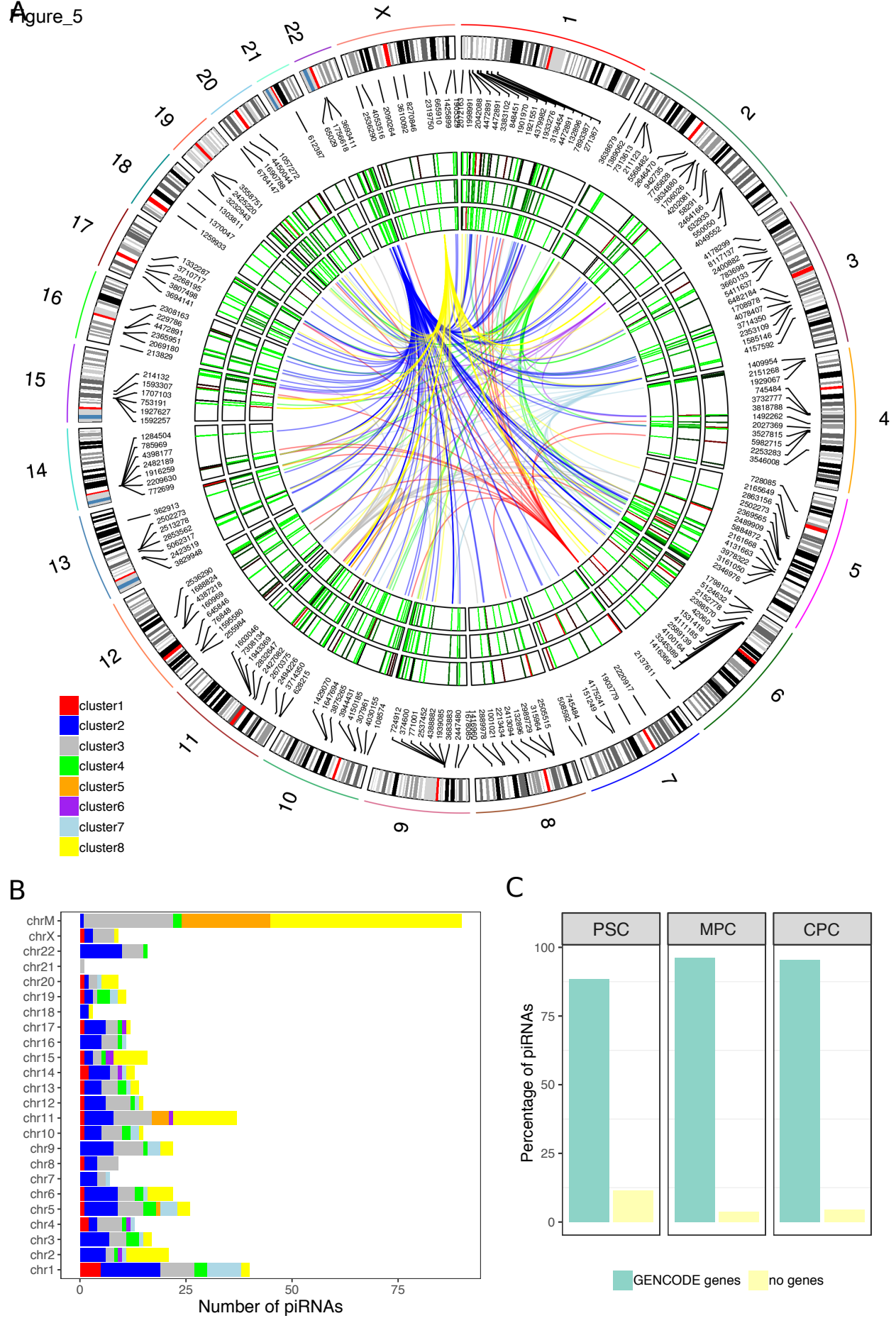

A
Figure_5
cluster1
cluster2
cluster3
cluster4
cluster5
cluster6
cluster7
cluster8
B
chrM
chrX
chr22
chr21
chr20
chr19
chr18
chr17
chr16
chr15
chr14
chr13
chr12
chr11
chr10
chr9
chr8
chr7
chr6
chr5
chr4
chr3
chr2
chr1
0
25
50
75
Number of piRNAs
C
PSC
MPC
CPC
Percentage of piRNAs
100
75
50
25
0
GENCODE genes
no genes

Figure_6

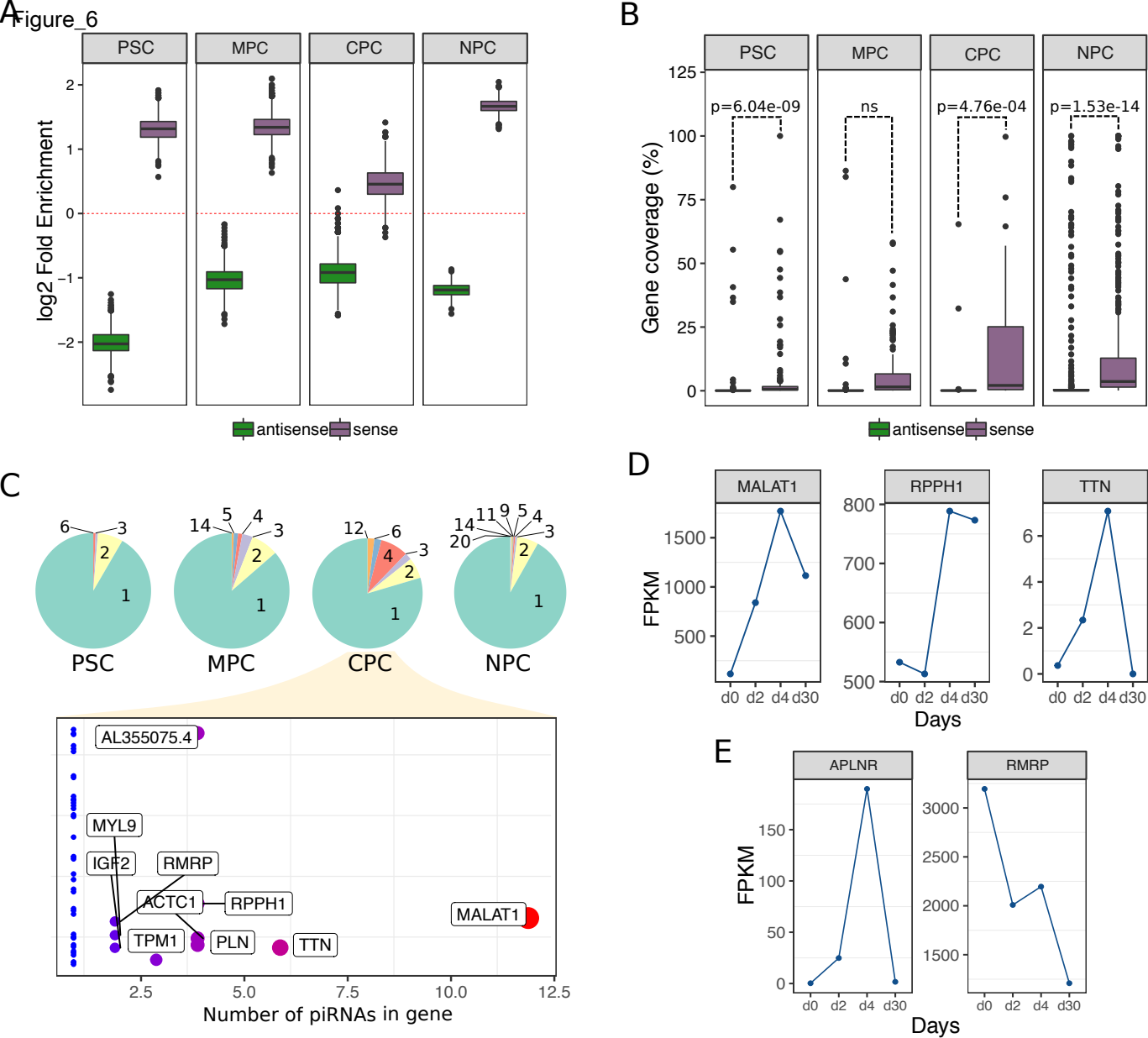
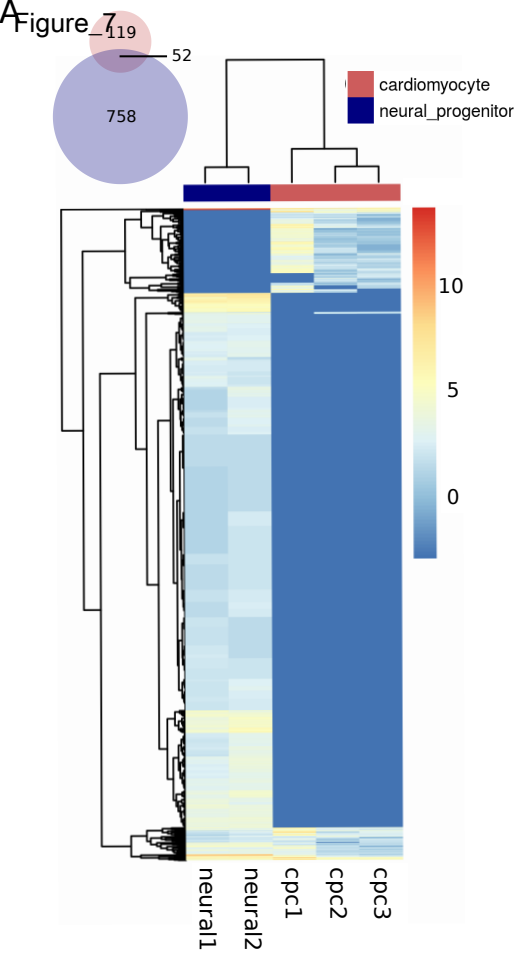
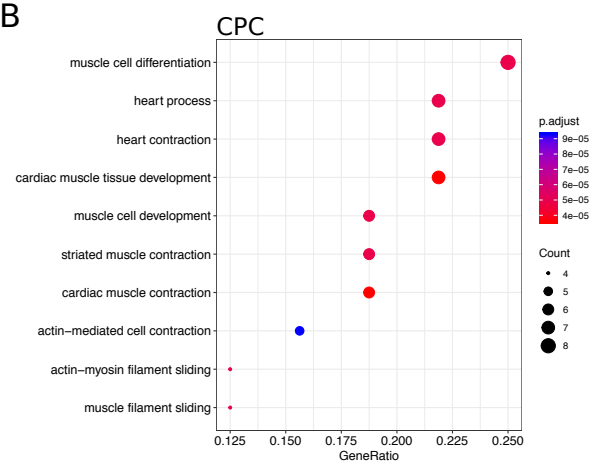
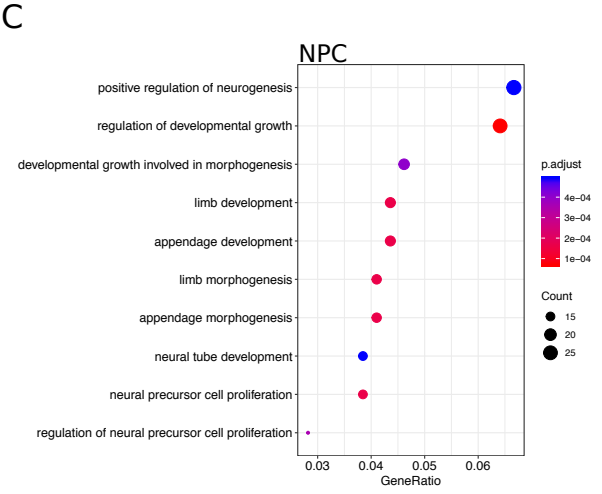
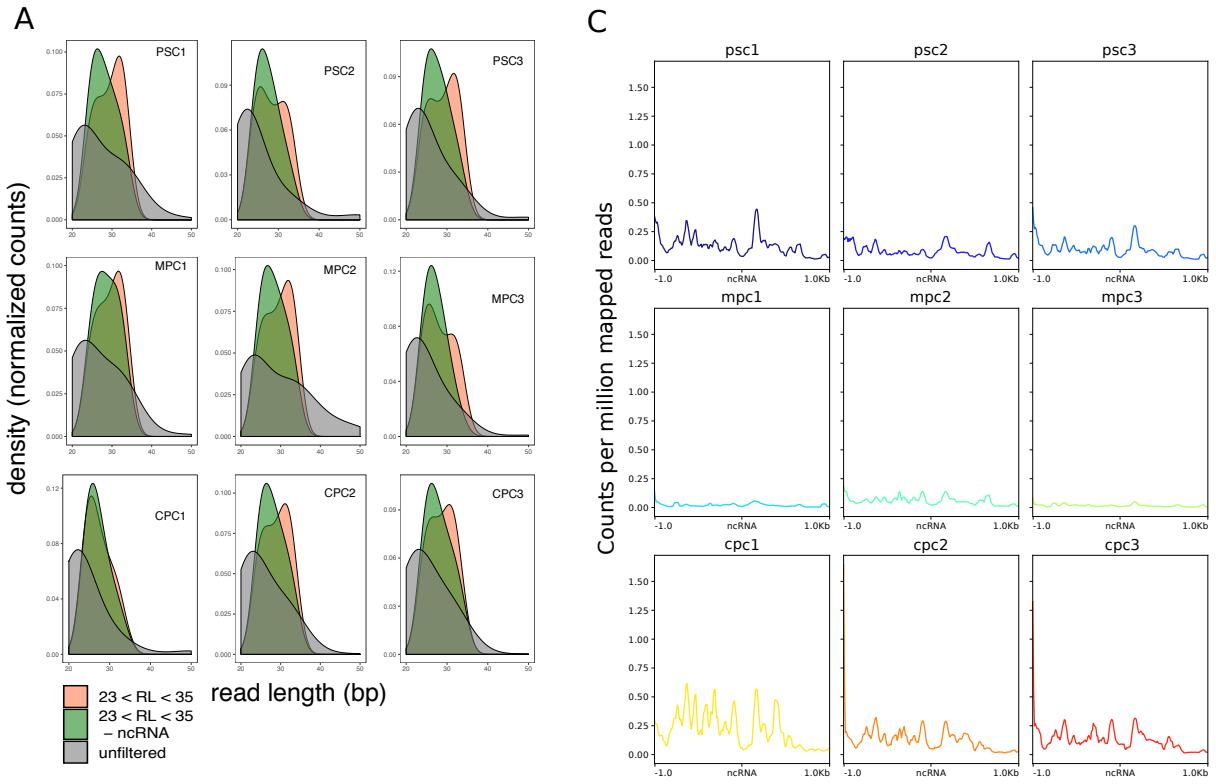

A
PSC
MPC
CPC
NPC
log2 Fold Enrichment
antisense
sense
B
Gene coverage (%)
p=6.04e-09
ns
p=4.76e-04
p=1.53e-14
C
PSC
MPC
CPC
NPC
AL355075.4
MYL9
IGF2
RMRP
ACTC1
RPPH1
MALAT1
TPM1
PLN
TTN
Number of piRNAs in gene
D
MALAT1
RPPH1
TTN
FPKM
Days
E
APLNR
RMRP
FPKM
Days

Figure_7


A
119
52
758
cardiomyocyte
neural_progenitor
10
5
0
neural1
neural2
cpc1
cpc2
cpc3


B
CPC
muscle cell differentiation
heart process
heart contraction
cardiac muscle tissue development
muscle cell development
striated muscle contraction
cardiac muscle contraction
actin−mediated cell contraction
actin−myosin filament sliding
muscle filament sliding
0.125
0.150
0.175
0.200
0.225
0.250
GeneRatio
p.adjust
9e−05
8e−05
7e−05
6e−05
5e−05
4e−05
Count
4
5
6
7
8


C
NPC
positive regulation of neurogenesis
regulation of developmental growth
developmental growth involved in morphogenesis
limb development
appendage development
limb morphogenesis
appendage morphogenesis
neural tube development
neural precursor cell proliferation
regulation of neural precursor cell proliferation
0.03
0.04
0.05
0.06
GeneRatio
p.adjust
4e−04
3e−04
2e−04
1e−04
Count
15
20
25



B

sample	unfiltered (1)	length filtered (2)	length+ncRNA filtered (3)	2/1 (%)	3/1 (%)
psc1	13,475,096	5,622,650	1,272,076	41.73	9.44
psc2	8,979,026	2,481,733	656,781	27.64	7.31
psc3	9,306,433	3,418,745	844,994	36.742	9.08
mpc1	18,690,310	8,922,435	3,494,534	47.74	18.705
mpc2	13,137,228	5,017,796	1,090,201	38.20	8.30
mpc3	14,426,323	5,582,968	1,921,400	38.70	13.32
cpc1	10,550,374	3,362,057	635,333	31.87	6.02
cpc2	10,863,324	4,833,395	882,061	44.49	8.12
cpc3	8,045,810	3,770,766	969,896	46.87	12.05

FIGURE S1: **Read length control in filtered samples and mapping to other non coding RNAs. Related to Figure 1.** A) Read length expressed as a function of density for pluripotent (PSC1, PSC2 and PSC3), mesoderm progenitor (MPC1, MPC2 and MPC3) and cardiomyocytes (CPC1, CPC2 and CPC3) samples before filtering (unfiltered) and after filtering ($23 < RL < 35$ and -ncRNA). Color key is indicated in the plot. B) Number of mapped reads. Reads were counted before processing (1) and after being filtered by length (2) and other ncRNAs (3). Remaining reads after processing are expressed as percentage(%) of unfiltered reads (2/1 and 3/1). C) Analysis of coverage on non coding RNAs loci from DASHR database for fully processed normalized (counts per million) samples.

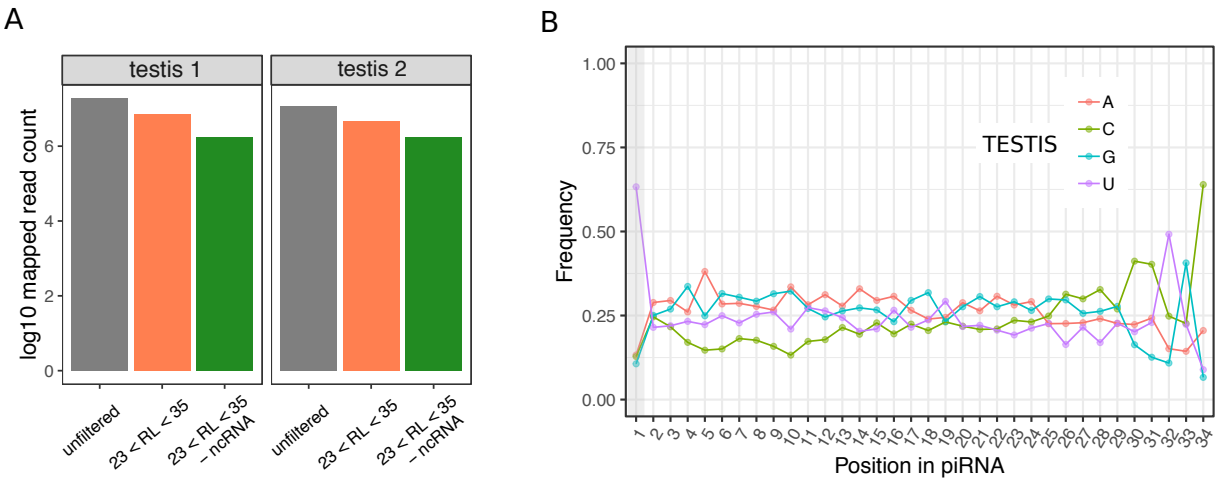


FIGURE S2: **Processing of testis samples. Related to Figure 2.**. a) Number of mapped reads after employing the pipeline described in Figure 1 in human testis samples downloaded from ENCODE (merged replicates). b) Frequency of bases per position in processed mapped reads.

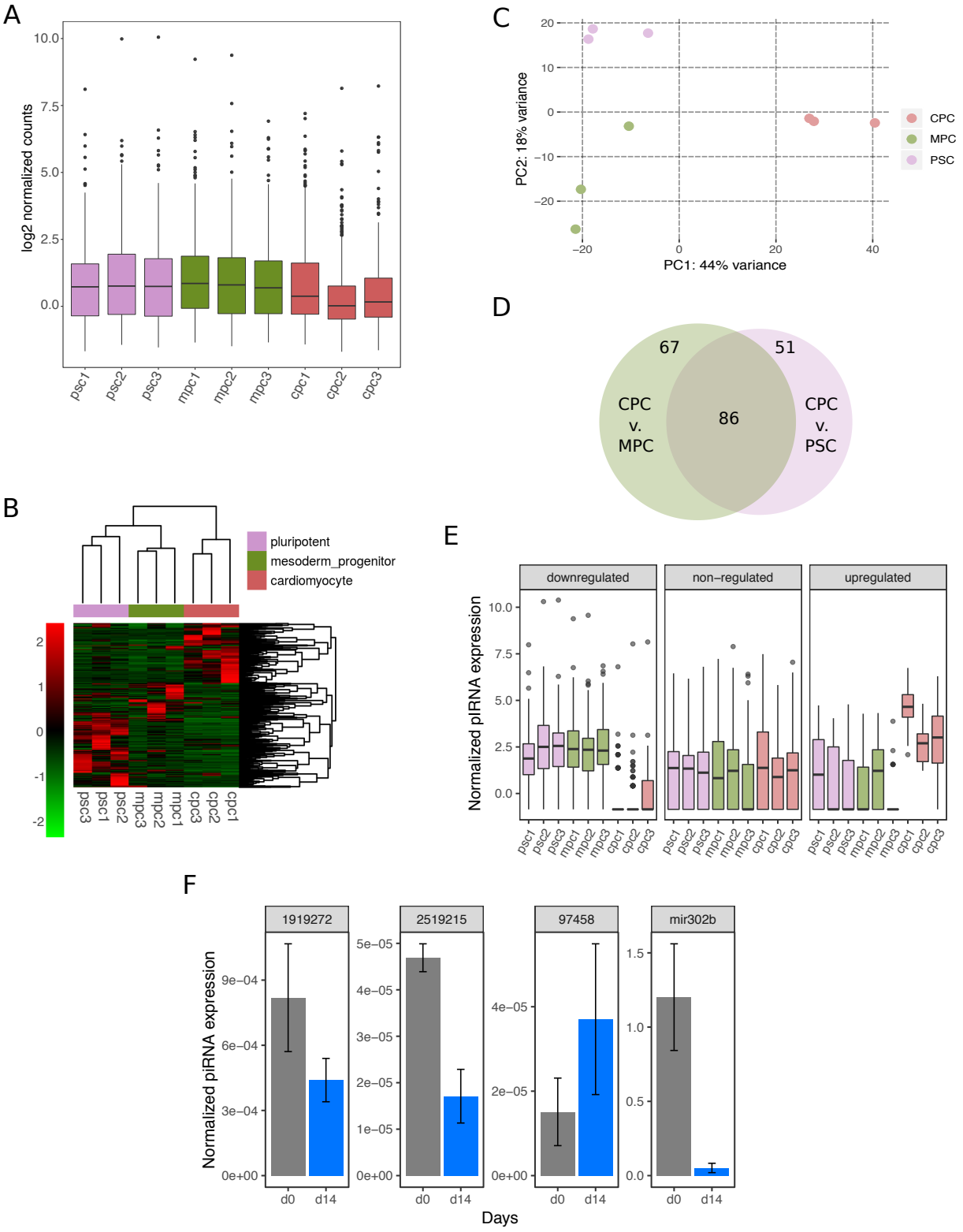


FIGURE S3: **Analysis of expression data and DE results. Related to Figure 3.** A) Boxplot showing reads for the nine samples normalized by library depth and expressed as log2 counts per million (CPM). B) Heatmap in log2 CPM of piRNAs from a. Hard unsupervised clustering was performed on rows (piRNA ID) and columns (sample ID), and is shown as dendrograms. Color keys for heatmap and phenotype are indicated to left and in the top right corner of the graph, respectively. C) Principal Component Analysis performed on DESeq2 normalized counts. The color key is indicated to the right of the plot. D) Overlap of differentially expressed piRNAs in CPC versus MPC (153; green circle) and PSC (137; purple circle). E) Normalized expression of piRNAs upregulated, dowregulated and non-regulated with respect to CPC. F) Three piRNA transcripts (piR-1919272, piR-2519215 and piR-97458) were evaluated by qPCR using a specific retrotranscription protocol designed for small RNAs in day 0 and 14 of cardiac differentiation. Expression of mir302b -marker of pluripotency- was analyzed to assess protocol success. All results were expressed as mean±se of two independent experiments after normalization by small RNA RNU6B.

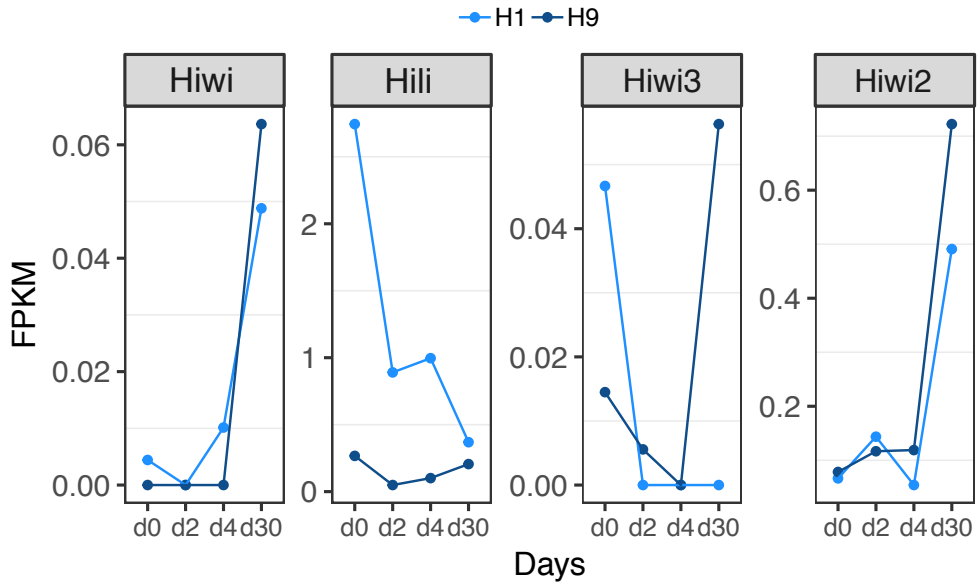


FIGURE S4: **Expression profile of human Piwi genes in H1 and H9 embryonic stem cell lines. Related to Figure 4.** Normalized RNA-seq counts (FPKM) from H1 and H9 cell lines were downloaded from GEO.

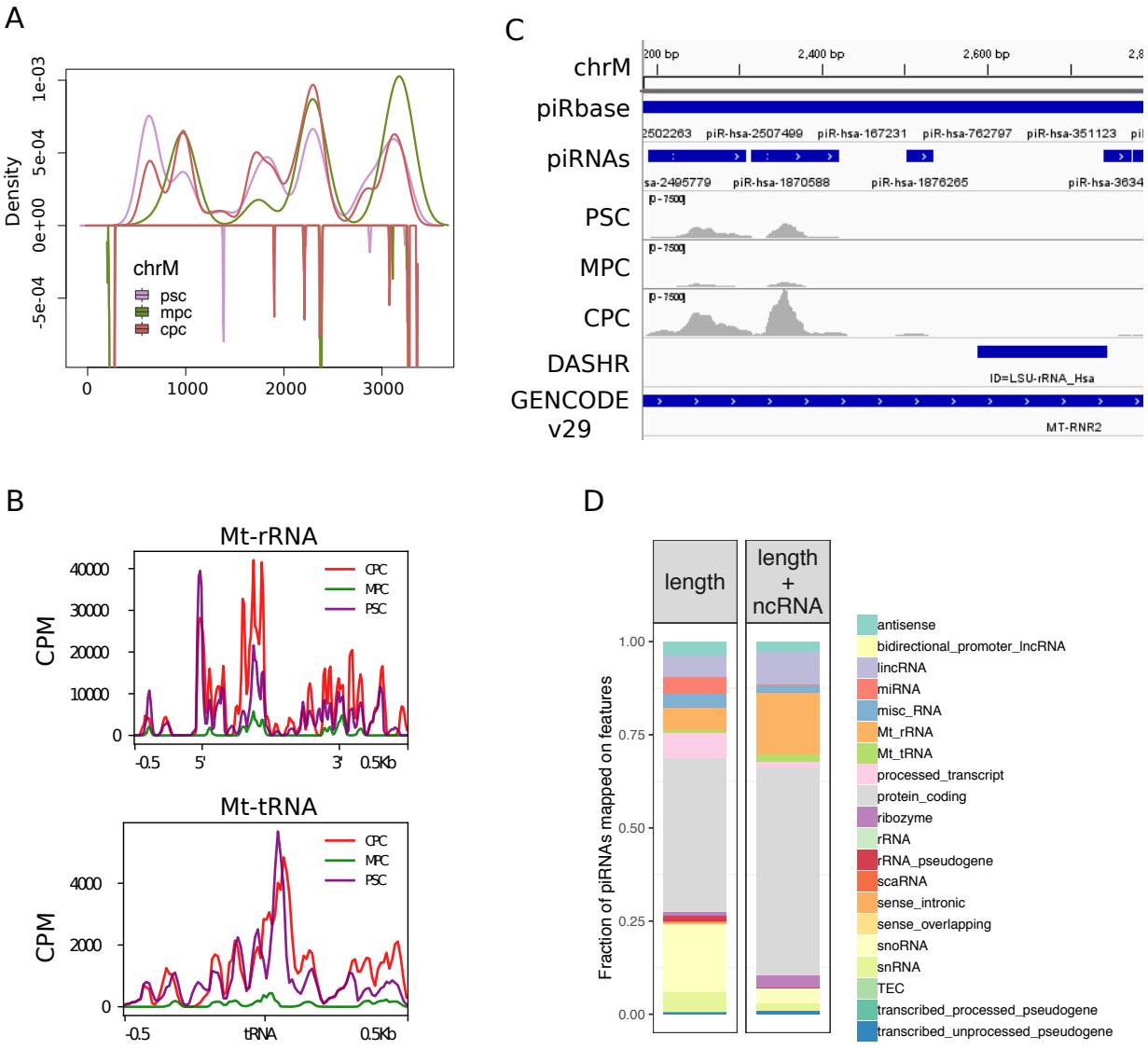


FIGURE S5: **Expression of piRNAs in mitochondrial chromosome. Related to Figure 5.** a) Distribution of PSC, MPC and CPC mapped reads (merged replicates) as a function of density over a fraction of mitochondrial chromosome (chrM:1-4000). Profiles above zero correspond to plus strand and below zero to the minus strand. Color key is located at the bottom left corner of the plot. b) Coverage profiles in counts per million mapped reads (CPM) on the entire mitochondrial rRNA extension (MT-rRNA) and center of tRNA (MT-tRNA). Direction of rRNA genes are indicated by 5‘ and 3‘. c) Image captured from IGV software over a portion of the human mitochondrial chromosome (chrM:2,184-2,780). The tracks from top to bottom are: piRbase annotated piRNAs (piRbase), piRNAs identified in our samples (piRNAs), coverage profiles of PSC, MPC and CPC, DASHR database ncRNA annotations (DASHR) and GENCODE v29 gene annotations (GENCODE v29). d) Fraction of piRNAs mapped to genomic features annotated in GENCODE v29 database in length-filtered samples (length) compared to length+ncRNA-filtered (length+ncRNA) samples. Color key for features is indicated to the rigth of the bars.

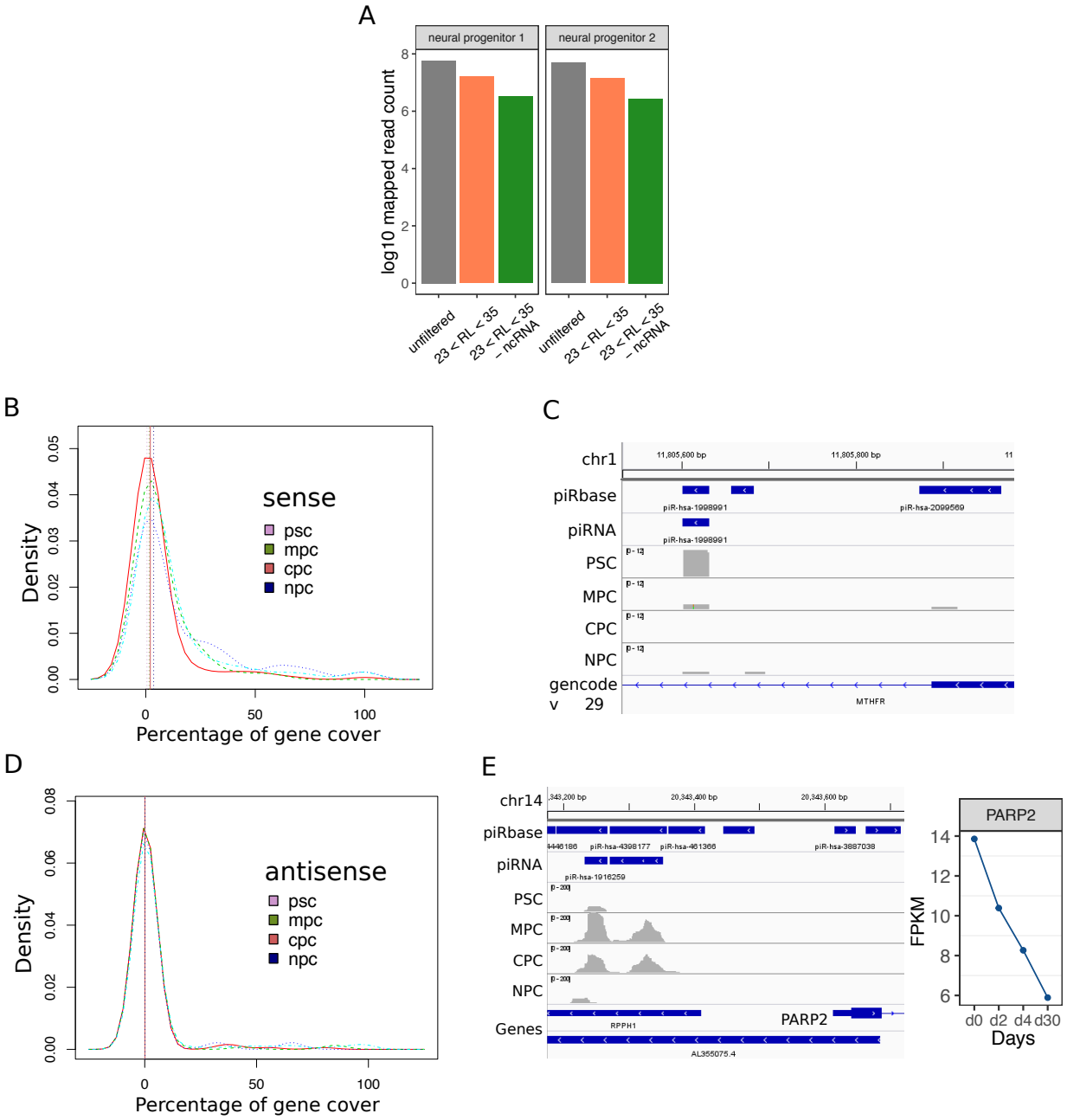


FIGURE S6: **Coverage on protein coding and lncRNA genes. Related to Figure 6 and 7.** A) NPC sample processing. Number of mapped reads after employing the pipeline described in Figure 1 in neural progenitor samples downloaded from ENCODE project. B) Desity estimation of percent (%) coverage on protein coding genes intersected by piRNAs in sense orientation. Vertical lines indicate medians of each curve. C) Image captured from IGV software portraying mapped reads of PSC, MPC, CPC and NPC samples on identified piRNAs. Tracks for piRNA annotation database (piRbase) and gene features (GENCODE v29) are shown. D) Density estimation as in a, in antisense orientation. E) Left panel shows IGV capture depicting piRNAs in PARP2 vecinity. Expression dynamic of PARP2 gene in RNA-seq samples from H9 cells differentiated to CM is shown to the right.

	piRNA	psc1	psc2	psc3	mpc1	mpc2	mpc3	cpc1	cpc2	cpc3
1	piR-hsa-1389062	252.26	1263.26	1335.60	666.72	757.88	83.67	110.68	260.73	280.30
2	piR-hsa-4381848_2	86.38	70.78	77.08	78.63	235.99	77.84	81.81	31.18	79.49
3	piR-hsa-3732777	17.49	35.39	110.80	105.40	106.53	60.32	24.06	55.58	73.21
4	piR-hsa-1904126	12.72	40.01	49.38	96.20	72.82	83.67	57.75	39.76	89.25
5	piR-hsa-1919272	50.88	26.16	33.72	10.87	72.82	3.89	178.05	41.12	60.66
6	piR-hsa-3660133	88.50	67.70	77.08	44.34	64.73	35.03	1.60	0.00	0.00
7	piR-hsa-3817390	25.97	69.24	49.38	23.42	31.02	60.32	0.00	1.36	2.79
8	piR-hsa-151466	25.44	15.39	26.50	18.40	13.49	0.00	44.91	16.27	77.40
9	piR-hsa-2539762	20.14	9.23	21.68	2.51	18.88	1.95	81.81	22.14	41.14
10	piR-hsa-4091280	22.79	112.32	28.90	5.02	6.74	25.30	0.00	0.00	0.70
11	piR-hsa-1939085	0.00	0.00	0.00	148.90	2.70	3.89	33.69	6.78	2.09
12	piR-hsa-169217	49.29	26.16	56.60	19.24	29.67	9.73	0.00	0.90	2.09
13	piR-hsa-97458	0.00	3.08	3.61	22.59	57.99	93.40	0.00	0.00	0.00
14	piR-hsa-665910	1.06	0.00	1.20	2.51	0.00	7.78	14.44	14.01	131.08
15	piR-hsa-2346976	19.61	60.01	30.11	22.59	22.93	9.73	0.00	0.00	0.70
16	piR-hsa-1933276	2.65	0.00	3.61	107.08	36.41	13.62	0.00	0.45	1.39
17	piR-hsa-3634065	5.30	6.15	8.43	0.00	10.79	0.00	105.87	6.78	19.52
18	piR-hsa-2489909	0.00	3.08	3.61	6.69	5.39	114.81	4.81	3.61	7.67
19	piR-hsa-3658275	14.84	7.69	3.61	1.67	16.18	3.89	67.37	14.91	18.83
20	piR-hsa-2832647	7.42	46.16	6.02	7.53	2.70	27.24	20.85	14.91	5.58
21	piR-hsa-3352181	12.72	16.93	10.84	89.51	2.70	1.95	0.00	0.00	2.09
22	piR-hsa-151249	7.95	10.77	19.27	40.15	16.18	15.57	1.60	18.98	4.18
23	piR-hsa-316012	4.77	13.85	6.02	2.51	10.79	31.13	20.85	14.46	26.50
24	piR-hsa-2479371	21.73	10.77	14.45	5.02	24.27	1.95	30.48	8.59	10.46
25	piR-hsa-611204	6.89	0.00	8.43	51.87	45.85	9.73	1.60	0.00	1.39
26	piR-hsa-2513278	10.07	9.23	19.27	27.61	39.11	13.62	3.21	0.00	1.39
27	piR-hsa-3974794	32.86	18.46	21.68	15.89	6.74	15.57	3.21	1.81	4.88
28	piR-hsa-2780538	15.37	35.39	24.09	7.53	9.44	25.30	0.00	0.90	2.79
29	piR-hsa-1872085_4	4.24	26.16	8.43	25.10	4.05	31.13	3.21	5.87	8.37
30	piR-hsa-2526525	6.36	4.62	6.02	0.00	5.39	0.00	73.79	2.26	11.16
31	piR-hsa-745484	2.12	3.08	0.00	62.74	2.70	0.00	33.69	3.61	0.70
32	piR-hsa-147461	5.83	3.08	6.02	32.62	18.88	29.19	1.60	7.68	0.70
33	piR-hsa-4460706	11.66	4.62	1.20	0.00	10.79	1.95	41.71	15.36	16.73
34	piR-hsa-1927965	11.13	12.31	3.61	0.00	10.79	0.00	51.33	2.26	9.76
35	piR-hsa-4303719	13.78	24.62	36.13	14.22	2.70	9.73	0.00	0.00	0.00
36	piR-hsa-114666	18.02	15.39	26.50	1.67	9.44	29.19	0.00	0.45	0.00
37	piR-hsa-1870588	18.02	1.54	9.63	1.67	16.18	0.00	35.29	9.49	8.37
38	piR-hsa-7760463	1.59	0.00	0.00	33.46	55.29	3.89	1.60	0.00	4.18
39	piR-hsa-1706026	1.06	4.62	0.00	74.45	9.44	0.00	8.02	0.00	1.39
40	piR-hsa-2856604	4.24	23.08	24.09	25.93	9.44	7.78	0.00	0.45	2.09
41	piR-hsa-1233052	17.49	3.08	7.23	0.00	12.14	0.00	41.71	9.04	4.18
42	piR-hsa-2482189	2.12	10.77	0.00	49.36	6.74	3.89	14.44	3.16	3.49
43	piR-hsa-2519215	15.90	18.46	37.33	7.53	5.39	7.78	0.00	0.00	0.00
44	piR-hsa-2863156	5.30	15.39	6.02	20.08	4.05	35.03	4.81	0.90	0.70
45	piR-hsa-151136	8.48	21.54	14.45	16.73	5.39	11.68	3.21	8.13	0.70
46	piR-hsa-214132	12.72	23.08	19.27	8.37	12.14	13.62	0.00	0.45	0.00
47	piR-hsa-1332287	6.89	52.32	13.25	0.00	5.39	5.84	0.00	2.26	1.39
48	piR-hsa-1923208	6.89	7.69	2.41	3.35	2.70	1.95	35.29	5.87	19.52
49	piR-hsa-1463989	2.65	21.54	27.70	6.69	5.39	19.46	0.00	0.00	0.00
50	piR-hsa-2413094	12.72	30.77	20.47	0.84	10.79	7.78	0.00	0.00	0.00
51	piR-hsa-2299252	14.84	4.62	28.90	10.04	8.09	15.57	0.00	0.00	0.00
52	piR-hsa-2831593	6.89	1.54	27.70	11.71	14.83	1.95	0.00	11.75	5.58
53	piR-hsa-1429070	2.12	4.62	7.23	3.35	6.74	56.43	0.00	0.00	0.00
54	piR-hsa-2268195	14.84	4.62	32.52	1.67	4.05	13.62	1.60	1.36	4.18
55	piR-hsa-4020841	5.83	23.08	30.11	1.67	5.39	11.68	0.00	0.00	0.00
56	piR-hsa-1647694	9.54	12.31	30.11	8.37	6.74	7.78	0.00	0.00	1.39
57	piR-hsa-359160_2	2.12	7.69	3.61	25.10	8.09	9.73	1.60	8.59	9.06
58	piR-hsa-1001021	4.77	4.62	1.20	23.42	6.74	17.51	11.23	0.00	5.58
59	piR-hsa-772699	0.00	0.00	0.00	53.54	8.09	7.78	3.21	0.90	1.39

	piRNA	psc1	psc2	psc3	mpc1	mpc2	mpc3	cpc1	cpc2	cpc3
60	piR-hsa-3916570	15.90	7.69	28.90	11.71	6.74	3.89	0.00	0.00	0.00
61	piR-hsa-1916259	3.18	3.08	1.20	23.42	8.09	3.89	25.67	4.52	0.70
62	piR-hsa-4322932	6.89	24.62	28.90	4.18	4.05	3.89	0.00	0.45	0.70
63	piR-hsa-2521457	3.71	4.62	3.61	1.67	4.05	0.00	41.71	5.42	6.97
64	piR-hsa-1903779	1.59	3.08	7.23	4.18	6.74	42.81	4.81	0.00	0.70
65	piR-hsa-2646470	0.53	4.62	8.43	22.59	21.58	3.89	1.60	2.26	4.88
66	piR-hsa-2497478	24.38	4.62	8.43	1.67	5.39	0.00	19.25	2.26	3.49
67	piR-hsa-1875212	4.24	3.08	1.20	0.00	4.05	0.00	38.50	8.13	9.76
68	piR-hsa-4387218	0.00	0.00	0.00	0.84	1.35	0.00	4.81	27.11	31.38
69	piR-hsa-6913457	10.60	16.93	7.23	20.91	5.39	1.95	1.60	0.00	0.00
70	piR-hsa-1756618	2.65	0.00	3.61	19.24	21.58	3.89	9.62	0.45	3.49
71	piR-hsa-2592846	0.00	0.00	0.00	0.00	0.00	0.00	60.96	0.90	2.09
72	piR-hsa-1908839	6.36	0.00	2.41	1.67	17.53	0.00	16.04	10.84	7.67
73	piR-hsa-1576285	0.00	3.08	0.00	1.67	1.35	13.62	3.21	20.33	18.83
74	piR-hsa-1862211	0.00	7.69	0.00	12.55	0.00	21.40	11.23	3.61	2.79
75	piR-hsa-3658742	6.89	1.54	4.82	0.00	24.27	0.00	16.04	2.26	2.79
76	piR-hsa-1872235	6.89	3.08	9.63	0.00	8.09	1.95	16.04	7.23	4.88
77	piR-hsa-1277994	3.71	0.00	8.43	2.51	29.67	1.95	4.81	0.00	6.28
78	piR-hsa-343382	4.24	4.62	2.41	1.67	4.05	1.95	19.25	9.04	9.76
79	piR-hsa-2027369	14.84	16.93	12.04	4.18	2.70	5.84	0.00	0.00	0.00
80	piR-hsa-1399886	2.12	24.62	4.82	15.89	8.09	0.00	0.00	0.00	0.00
81	piR-hsa-1798104	7.42	15.39	24.09	2.51	1.35	3.89	0.00	0.00	0.00
82	piR-hsa-1875155	2.12	21.54	21.68	3.35	0.00	5.84	0.00	0.00	0.00
83	piR-hsa-1548068	1.59	6.15	13.25	4.18	10.79	0.00	1.60	8.13	8.37
84	piR-hsa-721859_9	2.12	9.23	8.43	6.69	4.05	17.51	3.21	0.90	1.39
85	piR-hsa-3732088	16.43	3.08	20.47	1.67	1.35	1.95	0.00	3.16	4.88
86	piR-hsa-1303811	1.59	4.62	0.00	31.79	1.35	3.89	9.62	0.00	0.00
87	piR-hsa-2481097	6.89	3.08	6.02	0.00	12.14	0.00	19.25	4.07	1.39
88	piR-hsa-4028152	2.12	15.39	0.00	5.02	2.70	25.30	1.60	0.00	0.70
89	piR-hsa-389007	2.65	4.62	2.41	9.20	10.79	19.46	3.21	0.00	0.00
90	piR-hsa-2477264	5.30	6.15	6.02	0.00	10.79	0.00	17.64	4.07	2.09
91	piR-hsa-255984	2.12	3.08	12.04	5.86	2.70	23.35	0.00	0.00	2.09
92	piR-hsa-137136	7.42	7.69	9.63	8.37	8.09	9.73	0.00	0.00	0.00
93	piR-hsa-3807498	7.95	13.85	15.66	1.67	6.74	3.89	0.00	0.00	0.70
94	piR-hsa-783698	15.37	7.69	6.02	5.02	13.49	1.95	0.00	0.00	0.00
95	piR-hsa-1988800	7.95	4.62	12.04	3.35	14.83	5.84	0.00	0.00	0.00
96	piR-hsa-1941780	3.71	6.15	3.61	1.67	2.70	1.95	19.25	1.36	7.67
97	piR-hsa-3875265	10.60	9.23	10.84	5.86	9.44	1.95	0.00	0.00	0.00
98	piR-hsa-1876265	4.24	4.62	3.61	0.00	0.00	0.00	19.25	6.33	9.76
99	piR-hsa-368987	6.36	4.62	4.82	5.02	4.05	3.89	9.62	5.87	3.49
100	piR-hsa-1890632	6.36	4.62	7.23	0.00	5.39	0.00	11.23	4.52	8.37
101	piR-hsa-4078407	2.12	20.00	9.63	10.04	1.35	3.89	0.00	0.00	0.00
102	piR-hsa-76848	4.24	4.62	1.20	1.67	1.35	25.30	1.60	1.36	5.58
103	piR-hsa-2565910	0.53	12.31	4.82	5.02	5.39	15.57	0.00	1.81	1.39
104	piR-hsa-1205256	3.18	4.62	1.20	21.75	0.00	11.68	3.21	0.45	0.00
105	piR-hsa-2840936	10.60	0.00	1.20	0.00	6.74	0.00	19.25	4.97	2.79
106	piR-hsa-20628	3.71	9.23	12.04	2.51	8.09	9.73	0.00	0.00	0.00
107	piR-hsa-753191	0.00	0.00	0.00	0.00	0.00	0.00	35.29	5.42	4.18
108	piR-hsa-3683883	2.12	3.08	6.02	20.08	1.35	1.95	6.42	0.00	2.79
109	piR-hsa-1690788	1.59	4.62	4.82	5.86	6.74	17.51	0.00	0.00	2.09
110	piR-hsa-2530015	6.89	3.08	1.20	2.51	0.00	0.00	16.04	2.26	11.16
111	piR-hsa-346271	3.71	3.08	15.66	1.67	2.70	0.00	3.21	1.81	10.46
112	piR-hsa-3546008	6.89	3.08	8.43	5.02	14.83	3.89	0.00	0.00	0.00
113	piR-hsa-5982715	7.42	13.85	2.41	6.69	2.70	7.78	0.00	0.90	0.00
114	piR-hsa-2398570	1.06	6.15	3.61	2.51	12.14	15.57	0.00	0.00	0.70
115	piR-hsa-1883972	6.36	0.00	0.00	0.00	2.70	0.00	19.25	3.61	9.76
116	piR-hsa-3718263_3	0.00	0.00	0.00	0.00	0.00	0.00	33.69	5.87	2.09
117	piR-hsa-4472891	2.12	3.08	9.63	11.71	6.74	5.84	0.00	1.81	0.70
118	piR-hsa-2208850	4.24	15.39	7.23	5.02	1.35	7.78	0.00	0.00	0.00

	piRNA	psc1	psc2	psc3	mpc1	mpc2	mpc3	cpc1	cpc2	cpc3
119	piR-hsa-785969	2.12	3.08	0.00	6.69	0.00	1.95	24.06	1.36	1.39
120	piR-hsa-163695	6.36	10.77	7.23	0.84	6.74	7.78	0.00	0.00	0.70
121	piR-hsa-2490897	6.89	0.00	1.20	1.67	0.00	0.00	11.23	5.42	13.95
122	piR-hsa-2151268	1.59	7.69	3.61	7.53	8.09	11.68	0.00	0.00	0.00
123	piR-hsa-771714_3	3.71	4.62	2.41	0.00	0.00	3.89	17.64	0.90	6.97
124	piR-hsa-1920687	4.24	4.62	1.20	1.67	4.05	0.00	12.83	5.42	5.58
125	piR-hsa-2351941	5.30	23.08	2.41	2.51	2.70	1.95	1.60	0.00	0.00
126	piR-hsa-3265318	3.18	13.85	6.02	1.67	2.70	11.68	0.00	0.00	0.00
127	piR-hsa-2426792	2.12	24.62	4.82	6.69	0.00	0.00	0.00	0.00	0.70
128	piR-hsa-2503702	13.25	0.00	8.43	0.00	4.05	0.00	6.42	3.16	2.79
129	piR-hsa-161264_3	1.06	0.00	1.20	8.37	5.39	21.40	0.00	0.00	0.00
130	piR-hsa-1912443	1.59	0.00	1.20	0.00	1.35	0.00	28.87	2.26	2.09
131	piR-hsa-2230204	8.48	6.15	15.66	3.35	2.70	0.00	0.00	0.00	0.70
132	piR-hsa-2090264	7.42	1.54	25.29	0.84	1.35	0.00	0.00	0.00	0.00
133	piR-hsa-1242358	0.00	0.00	0.00	0.00	0.00	0.00	32.08	2.26	2.09
134	piR-hsa-724912	2.65	1.54	3.61	22.59	1.35	0.00	3.21	0.45	0.70
135	piR-hsa-7765828	1.06	7.69	3.61	13.38	4.05	5.84	0.00	0.00	0.00
136	piR-hsa-2172087	1.59	10.77	3.61	5.02	2.70	7.78	3.21	0.90	0.00
137	piR-hsa-1425899	0.00	4.62	1.20	6.69	2.70	17.51	0.00	1.36	1.39
138	piR-hsa-1921188	1.59	3.08	1.20	0.00	10.79	0.00	14.44	3.61	0.70
139	piR-hsa-1917139	2.65	1.54	1.20	0.00	4.05	0.00	20.85	1.36	3.49
140	piR-hsa-3829948	0.53	3.08	0.00	13.38	4.05	13.62	0.00	0.00	0.00
141	piR-hsa-3735787	7.42	3.08	2.41	0.00	2.70	0.00	12.83	4.07	2.09
142	piR-hsa-5996985	0.00	0.00	0.00	0.00	0.00	0.00	28.87	5.42	0.00
143	piR-hsa-2989729	6.36	6.15	13.25	0.84	2.70	3.89	0.00	0.00	0.00
144	piR-hsa-21839	0.53	3.08	0.00	24.26	2.70	1.95	0.00	0.45	0.00
145	piR-hsa-1872463	0.00	4.62	1.20	1.67	0.00	19.46	0.00	3.16	2.79
146	piR-hsa-508592	2.12	13.85	7.23	2.51	1.35	5.84	0.00	0.00	0.00
147	piR-hsa-2252211	1.59	6.15	3.61	0.84	20.23	0.00	0.00	0.45	0.00
148	piR-hsa-4110708	2.65	27.70	1.20	0.84	0.00	0.00	0.00	0.00	0.00
149	piR-hsa-298158	4.24	6.15	6.02	7.53	4.05	3.89	0.00	0.00	0.00
150	piR-hsa-7892960	6.36	1.54	3.61	4.18	2.70	1.95	3.21	4.52	3.49
151	piR-hsa-2395910	2.65	3.08	3.61	3.35	1.35	17.51	0.00	0.00	0.00
152	piR-hsa-2536290	0.53	12.31	3.61	8.37	1.35	3.89	0.00	0.45	0.70
153	piR-hsa-2152778	2.65	10.77	6.02	5.02	2.70	3.89	0.00	0.00	0.00
154	piR-hsa-5077723	0.53	4.62	12.04	8.37	5.39	0.00	0.00	0.00	0.00
155	piR-hsa-1291516	7.42	6.15	10.84	2.51	1.35	0.00	0.00	0.90	1.39
156	piR-hsa-1901970	12.19	7.69	0.00	3.35	5.39	1.95	0.00	0.00	0.00
157	piR-hsa-2450089_2	0.00	1.54	4.82	3.35	1.35	19.46	0.00	0.00	0.00
158	piR-hsa-108574	3.71	7.69	4.82	3.35	8.09	1.95	0.00	0.00	0.70
159	piR-hsa-1259653	2.65	1.54	2.41	0.84	1.35	1.95	16.04	1.81	0.70
160	piR-hsa-3136454	2.65	16.93	2.41	1.67	2.70	1.95	0.00	0.00	0.70
161	piR-hsa-2286229	4.24	3.08	9.63	4.18	5.39	1.95	0.00	0.45	0.00
162	piR-hsa-2209630	2.65	0.00	0.00	23.42	2.70	0.00	0.00	0.00	0.00
163	piR-hsa-1696540	0.00	0.00	0.00	0.00	0.00	1.95	24.06	1.81	0.70
164	piR-hsa-6245615	3.71	4.62	7.23	0.00	10.79	1.95	0.00	0.00	0.00
165	piR-hsa-2072163	1.06	4.62	2.41	1.67	2.70	5.84	8.02	1.81	0.00
166	piR-hsa-1557538	0.00	0.00	2.41	8.37	9.44	7.78	0.00	0.00	0.00
167	piR-hsa-2844156	7.42	3.08	4.82	5.86	0.00	3.89	0.00	1.36	1.39
168	piR-hsa-6744266	0.00	1.54	1.20	5.86	0.00	5.84	9.62	2.26	1.39
169	piR-hsa-1882039	1.06	0.00	0.00	0.00	0.00	0.00	17.64	2.71	6.28
170	piR-hsa-2137611	5.30	15.39	6.02	0.84	0.00	0.00	0.00	0.00	0.00
171	piR-hsa-58291	3.71	6.15	8.43	0.84	4.05	3.89	0.00	0.45	0.00
172	piR-hsa-4408495	0.00	0.00	2.41	5.86	5.39	11.68	1.60	0.45	0.00
173	piR-hsa-374600	1.06	0.00	1.20	10.87	1.35	0.00	12.83	0.00	0.00
174	piR-hsa-2829712	3.71	0.00	1.20	0.00	5.39	0.00	9.62	3.16	4.18
175	piR-hsa-3177742	3.18	4.62	7.23	1.67	0.00	9.73	0.00	0.00	0.70
176	piR-hsa-1585146	2.12	0.00	4.82	9.20	6.74	3.89	0.00	0.00	0.00
177	piR-hsa-4403577	2.65	4.62	7.23	0.84	5.39	3.89	0.00	1.36	0.70

	piRNA	psc1	psc2	psc3	mpc1	mpc2	mpc3	cpc1	cpc2	cpc3
178	piR-hsa-1528884	4.24	10.77	4.82	2.51	1.35	1.95	0.00	0.00	0.70
179	piR-hsa-2494226	4.77	3.08	0.00	0.00	2.70	0.00	8.02	2.71	4.88
180	piR-hsa-229786	0.00	4.62	0.00	2.51	2.70	15.57	0.00	0.00	0.70
181	piR-hsa-1492262	3.71	4.62	1.20	0.84	12.14	0.00	1.60	0.45	1.39
182	piR-hsa-132896	1.59	4.62	4.82	4.18	8.09	1.95	0.00	0.00	0.70
183	piR-hsa-1376916	2.12	10.77	2.41	3.35	2.70	3.89	0.00	0.45	0.00
184	piR-hsa-160969	0.53	0.00	6.02	3.35	2.70	11.68	0.00	0.00	1.39
185	piR-hsa-768321	8.48	3.08	1.20	0.84	0.00	0.00	4.81	0.90	6.28
186	piR-hsa-3842249	1.59	0.00	2.41	10.04	1.35	5.84	3.21	0.45	0.70
187	piR-hsa-362913	1.06	1.54	1.20	21.75	0.00	0.00	0.00	0.00	0.00
188	piR-hsa-1870459	3.18	0.00	4.82	0.84	6.74	0.00	4.81	2.71	2.09
189	piR-hsa-3634880	2.12	0.00	0.00	5.02	6.74	3.89	3.21	0.00	4.18
190	piR-hsa-1941637	2.65	0.00	0.00	16.73	1.35	0.00	3.21	0.45	0.70
191	piR-hsa-834074	0.00	0.00	0.00	0.00	0.00	0.00	20.85	2.71	1.39
192	piR-hsa-1900529	5.83	3.08	3.61	0.00	4.05	1.95	0.00	2.26	4.18
193	piR-hsa-2220917	0.00	3.08	1.20	11.71	8.09	0.00	0.00	0.00	0.70
194	piR-hsa-3739406	3.18	1.54	2.41	5.86	2.70	0.00	1.60	0.45	6.97
195	piR-hsa-7308134	0.00	0.00	0.00	14.22	2.70	7.78	0.00	0.00	0.00
196	piR-hsa-1708978	0.53	0.00	0.00	11.71	2.70	9.73	0.00	0.00	0.00
197	piR-hsa-3231825	3.71	12.31	3.61	1.67	1.35	1.95	0.00	0.00	0.00
198	piR-hsa-2398119	4.77	3.08	0.00	1.67	5.39	0.00	9.62	0.00	0.00
199	piR-hsa-2882083	3.71	4.62	0.00	5.86	4.05	1.95	1.60	1.36	1.39
200	piR-hsa-315964	4.77	1.54	10.84	1.67	5.39	0.00	0.00	0.00	0.00
201	piR-hsa-4030155	6.89	4.62	2.41	1.67	2.70	5.84	0.00	0.00	0.00
202	piR-hsa-2615134	3.71	3.08	2.41	10.87	4.05	0.00	0.00	0.00	0.00
203	piR-hsa-7544198	1.59	0.00	2.41	12.55	6.74	0.00	0.00	0.00	0.70
204	piR-hsa-1843231	1.06	4.62	4.82	4.18	5.39	3.89	0.00	0.00	0.00
205	piR-hsa-2670375	1.06	1.54	2.41	11.71	1.35	5.84	0.00	0.00	0.00
206	piR-hsa-2464166	1.06	3.08	0.00	0.84	1.35	17.51	0.00	0.00	0.00
207	piR-hsa-2589139	1.59	1.54	4.82	12.55	1.35	1.95	0.00	0.00	0.00
208	piR-hsa-3119265	4.24	1.54	4.82	2.51	6.74	3.89	0.00	0.00	0.00
209	piR-hsa-1531418	1.06	3.08	3.61	6.69	5.39	3.89	0.00	0.00	0.00
210	piR-hsa-2253283	2.65	4.62	0.00	11.71	2.70	1.95	0.00	0.00	0.00
211	piR-hsa-2148238	1.59	7.69	8.43	2.51	1.35	1.95	0.00	0.00	0.00
212	piR-hsa-1595580	2.12	6.15	7.23	2.51	5.39	0.00	0.00	0.00	0.00
213	piR-hsa-3978322	0.00	0.00	0.00	10.04	6.74	3.89	1.60	0.90	0.00
214	piR-hsa-1773241	2.65	1.54	3.61	3.35	8.09	3.89	0.00	0.00	0.00
215	piR-hsa-1434629	0.53	1.54	3.61	7.53	4.05	5.84	0.00	0.00	0.00
216	piR-hsa-1340768	0.53	1.54	1.20	3.35	6.74	7.78	0.00	0.45	1.39
217	piR-hsa-333507	1.59	4.62	8.43	1.67	2.70	3.89	0.00	0.00	0.00
218	piR-hsa-118348	3.18	6.15	3.61	3.35	4.05	0.00	0.00	0.45	2.09
219	piR-hsa-3623001	4.24	1.54	1.20	0.00	9.44	0.00	1.60	1.36	3.49
220	piR-hsa-2090890	2.65	4.62	4.82	4.18	2.70	3.89	0.00	0.00	0.00
221	piR-hsa-1929067	2.12	9.23	1.20	2.51	0.00	7.78	0.00	0.00	0.00
222	piR-hsa-2827579	1.06	0.00	0.00	0.00	1.35	1.95	6.42	0.90	11.16
223	piR-hsa-1707103	0.00	1.54	0.00	0.00	0.00	0.00	16.04	4.52	0.70
224	piR-hsa-1256360	0.00	0.00	0.00	9.20	2.70	9.73	0.00	0.45	0.70
225	piR-hsa-1919455	6.89	1.54	2.41	0.00	1.35	0.00	4.81	3.61	2.09
226	piR-hsa-1607096	1.06	12.31	1.20	0.84	2.70	3.89	0.00	0.00	0.70
227	piR-hsa-2515454	5.30	3.08	0.00	0.00	5.39	0.00	6.42	1.81	0.70
228	piR-hsa-368381	0.53	1.54	1.20	2.51	0.00	1.95	4.81	3.16	6.97
229	piR-hsa-4379982	4.24	4.62	1.20	0.84	9.44	0.00	1.60	0.00	0.70
230	piR-hsa-2240007	3.71	7.69	4.82	1.67	2.70	1.95	0.00	0.00	0.00
231	piR-hsa-4416099_9	0.00	0.00	1.20	0.84	0.00	19.46	0.00	0.90	0.00
232	piR-hsa-7106256	1.59	0.00	1.20	10.87	5.39	1.95	0.00	0.45	0.70
233	piR-hsa-1481120	2.12	3.08	1.20	8.37	5.39	1.95	0.00	0.00	0.00
234	piR-hsa-1284504	0.53	1.54	0.00	4.18	0.00	3.89	6.42	2.71	2.79
235	piR-hsa-2505515	0.00	0.00	0.00	7.53	6.74	7.78	0.00	0.00	0.00
236	piR-hsa-1927627	0.00	0.00	0.00	0.00	0.00	0.00	16.04	1.81	4.18

	piRNA	psc1	psc2	psc3	mpc1	mpc2	mpc3	cpc1	cpc2	cpc3
237	piR-hsa-1921551	2.12	1.54	1.20	12.55	2.70	0.00	0.00	0.45	1.39
238	piR-hsa-163499	4.77	3.08	3.61	1.67	2.70	1.95	1.60	0.45	2.09
239	piR-hsa-4202081	1.59	1.54	4.82	4.18	5.39	3.89	0.00	0.45	0.00
240	piR-hsa-147696	1.59	0.00	0.00	0.84	1.35	0.00	9.62	4.07	4.18
241	piR-hsa-2353109	0.00	12.31	1.20	0.84	1.35	5.84	0.00	0.00	0.00
242	piR-hsa-8270846	3.71	4.62	7.23	5.86	0.00	0.00	0.00	0.00	0.00
243	piR-hsa-1905680	0.53	1.54	3.61	0.84	0.00	0.00	8.02	4.07	2.79
244	piR-hsa-307961	2.12	6.15	0.00	3.35	0.00	9.73	0.00	0.00	0.00
245	piR-hsa-2333057	4.77	0.00	3.61	0.84	9.44	1.95	0.00	0.00	0.70
246	piR-hsa-1938524	0.53	0.00	1.20	0.84	1.35	1.95	11.23	1.36	2.79
247	piR-hsa-3232943	3.18	0.00	3.61	5.02	5.39	3.89	0.00	0.00	0.00
248	piR-hsa-3513154	5.30	1.54	7.23	4.18	2.70	0.00	0.00	0.00	0.00
249	piR-hsa-3674332	14.31	0.00	0.00	0.00	2.70	0.00	1.60	0.90	1.39
250	piR-hsa-2213434	6.36	3.08	7.23	4.18	0.00	0.00	0.00	0.00	0.00
251	piR-hsa-2308163	2.65	7.69	3.61	4.18	2.70	0.00	0.00	0.00	0.00
252	piR-hsa-1748898	0.00	7.69	4.82	5.02	1.35	1.95	0.00	0.00	0.00
253	piR-hsa-2525461	0.00	3.08	3.61	6.69	2.70	1.95	0.00	0.00	2.79
254	piR-hsa-1296118	3.18	0.00	2.41	0.00	2.70	0.00	8.02	3.61	0.70
255	piR-hsa-1632961	0.53	12.31	3.61	0.84	1.35	1.95	0.00	0.00	0.00
256	piR-hsa-728085	0.53	1.54	0.00	0.84	0.00	0.00	17.64	0.00	0.00
257	piR-hsa-623353	0.00	1.54	0.00	0.84	0.00	15.57	0.00	0.45	2.09
258	piR-hsa-3527815	1.06	13.85	3.61	0.00	0.00	1.95	0.00	0.00	0.00
259	piR-hsa-2478880_2	0.00	1.54	0.00	0.00	2.70	0.00	8.02	3.16	4.88
260	piR-hsa-3558751	4.24	6.15	4.82	1.67	1.35	1.95	0.00	0.00	0.00
261	piR-hsa-4178299	5.30	4.62	7.23	1.67	1.35	0.00	0.00	0.00	0.00
262	piR-hsa-5411637	2.65	6.15	2.41	0.84	4.05	3.89	0.00	0.00	0.00
263	piR-hsa-1883893	0.00	7.69	0.00	5.86	0.00	3.89	0.00	1.81	0.70
264	piR-hsa-2742244	1.59	0.00	0.00	0.00	4.05	0.00	9.62	1.81	2.79
265	piR-hsa-669874	0.53	0.00	0.00	19.24	0.00	0.00	0.00	0.00	0.00
266	piR-hsa-645846	3.18	4.62	3.61	0.84	1.35	5.84	0.00	0.00	0.00
267	piR-hsa-3776081	0.53	1.54	0.00	9.20	8.09	0.00	0.00	0.00	0.00
268	piR-hsa-211123	2.12	0.00	1.20	5.02	5.39	3.89	0.00	0.90	0.70
269	piR-hsa-2248086	0.53	4.62	6.02	3.35	2.70	1.95	0.00	0.00	0.00
270	piR-hsa-1909905	0.53	0.00	0.00	1.67	1.35	15.57	0.00	0.00	0.00
271	piR-hsa-1593307	3.18	4.62	6.02	1.67	1.35	1.95	0.00	0.00	0.00
272	piR-hsa-2042088	0.00	0.00	0.00	0.00	0.00	0.00	12.83	4.52	1.39
273	piR-hsa-2490509	0.00	1.54	0.00	0.00	1.35	0.00	12.83	0.90	2.09
274	piR-hsa-144277_2	0.00	0.00	0.00	0.00	5.39	0.00	11.23	1.36	0.70
275	piR-hsa-4131663	0.00	0.00	0.00	4.18	4.05	1.95	8.02	0.45	0.00
276	piR-hsa-2542835	1.59	0.00	1.20	0.00	2.70	0.00	11.23	0.45	1.39
277	piR-hsa-1229611	2.12	1.54	1.20	4.18	2.70	5.84	0.00	0.90	0.00
278	piR-hsa-2423519	1.06	0.00	0.00	0.00	4.05	11.68	1.60	0.00	0.00
279	piR-hsa-3021684	4.24	1.54	3.61	7.53	1.35	0.00	0.00	0.00	0.00
280	piR-hsa-2490287	0.00	1.54	0.00	3.35	1.35	1.95	6.42	2.26	1.39
281	piR-hsa-1259933	1.06	1.54	0.00	8.37	2.70	0.00	1.60	2.26	0.70
282	piR-hsa-1302552	0.53	0.00	0.00	5.86	6.74	0.00	0.00	0.00	4.88
283	piR-hsa-67957	1.06	13.85	0.00	0.00	0.00	0.00	0.00	0.90	2.09
284	piR-hsa-1726249	6.89	1.54	7.23	0.84	1.35	0.00	0.00	0.00	0.00
285	piR-hsa-1409954	1.59	1.54	8.43	0.84	5.39	0.00	0.00	0.00	0.00
286	piR-hsa-4053516	2.65	1.54	7.23	1.67	2.70	1.95	0.00	0.00	0.00
287	piR-hsa-2425220	0.00	1.54	1.20	5.02	0.00	0.00	3.21	1.81	4.88
288	piR-hsa-1686806	2.12	3.08	3.61	4.18	2.70	1.95	0.00	0.00	0.00
289	piR-hsa-2319750	2.65	0.00	7.23	1.67	4.05	1.95	0.00	0.00	0.00
290	piR-hsa-4397384	0.00	0.00	3.61	4.18	0.00	9.73	0.00	0.00	0.00
291	piR-hsa-2829413	1.06	0.00	1.20	0.00	1.35	0.00	8.02	0.90	4.88
292	piR-hsa-3839126	0.00	0.00	0.00	7.53	4.05	5.84	0.00	0.00	0.00
293	piR-hsa-3944431	3.71	3.08	3.61	1.67	1.35	3.89	0.00	0.00	0.00
294	piR-hsa-363100_2	2.65	1.54	0.00	1.67	4.05	0.00	3.21	1.36	2.79
295	piR-hsa-6482184	0.53	10.77	1.20	0.84	0.00	3.89	0.00	0.00	0.00

	piRNA	psc1	psc2	psc3	mpc1	mpc2	mpc3	cpc1	cpc2	cpc3
296	piR-hsa-642866	0.00	0.00	0.00	0.00	0.00	0.00	12.83	2.26	2.09
297	piR-hsa-1922210	0.53	0.00	1.20	9.20	0.00	3.89	0.00	0.90	1.39
298	piR-hsa-2436454	3.18	3.08	4.82	1.67	0.00	3.89	0.00	0.45	0.00
299	piR-hsa-7821967_3	0.00	0.00	1.20	0.00	0.00	0.00	11.23	3.16	1.39
300	piR-hsa-4424378	0.00	0.00	0.00	0.00	1.35	0.00	12.83	1.36	1.39
301	piR-hsa-237221	1.59	1.54	0.00	10.87	2.70	0.00	0.00	0.00	0.00
302	piR-hsa-7833890	1.59	1.54	0.00	5.02	2.70	5.84	0.00	0.00	0.00
303	piR-hsa-1905329	0.00	3.08	0.00	2.51	1.35	9.73	0.00	0.00	0.00
304	piR-hsa-4450044	0.53	1.54	1.20	13.38	0.00	0.00	0.00	0.00	0.00
305	piR-hsa-3741185	1.59	0.00	0.00	0.00	1.35	1.95	8.02	0.90	2.79
306	piR-hsa-4144265	1.59	3.08	6.02	0.84	0.00	3.89	0.00	0.45	0.70
307	piR-hsa-4198101	1.59	7.69	1.20	0.84	1.35	3.89	0.00	0.00	0.00
308	piR-hsa-8117137	0.00	0.00	2.41	0.84	0.00	1.95	0.00	0.90	10.46
309	piR-hsa-4100164	1.06	4.62	4.82	1.67	2.70	0.00	1.60	0.00	0.00
310	piR-hsa-3280518	6.36	3.08	3.61	3.35	0.00	0.00	0.00	0.00	0.00
311	piR-hsa-3710717	0.00	0.00	0.00	3.35	1.35	11.68	0.00	0.00	0.00
312	piR-hsa-848451	0.00	0.00	0.00	2.51	4.05	9.73	0.00	0.00	0.00
313	piR-hsa-1057272	0.00	0.00	0.00	0.00	1.35	0.00	11.23	2.26	1.39
314	piR-hsa-343616	0.00	0.00	0.00	2.51	0.00	5.84	1.60	1.36	4.88
315	piR-hsa-2832439	1.59	1.54	1.20	0.84	0.00	1.95	4.81	1.36	2.79
316	piR-hsa-2889978	1.06	1.54	1.20	5.02	1.35	5.84	0.00	0.00	0.00
317	piR-hsa-2529368	3.18	1.54	1.20	0.00	0.00	0.00	1.60	1.36	6.97
318	piR-hsa-3161050	0.00	0.00	0.00	5.86	4.05	3.89	1.60	0.45	0.00
319	piR-hsa-1688824	2.12	1.54	1.20	5.86	2.70	1.95	0.00	0.45	0.00
320	piR-hsa-3638679	4.24	4.62	0.00	1.67	1.35	3.89	0.00	0.00	0.00
321	piR-hsa-2281305	0.00	0.00	1.20	0.00	0.00	0.00	9.62	1.36	3.49
322	piR-hsa-2427082	3.18	4.62	4.82	1.67	1.35	0.00	0.00	0.00	0.00
323	piR-hsa-5062317	0.00	4.62	0.00	0.84	0.00	0.00	0.00	1.81	8.37
324	piR-hsa-1555218	1.59	4.62	6.02	3.35	0.00	0.00	0.00	0.00	0.00
325	piR-hsa-4391981_9	0.00	0.00	0.00	3.35	0.00	3.89	1.60	3.16	3.49
326	piR-hsa-4403628	4.77	3.08	0.00	0.84	0.00	0.00	4.81	0.45	1.39
327	piR-hsa-153942	1.06	1.54	1.20	0.00	0.00	0.00	8.02	1.36	2.09
328	piR-hsa-2856544	4.77	1.54	2.41	1.67	0.00	0.00	3.21	0.90	0.70
329	piR-hsa-1894768	1.59	1.54	1.20	0.00	0.00	1.95	3.21	3.61	2.09
330	piR-hsa-2495779	2.65	1.54	1.20	0.00	4.05	0.00	1.60	2.71	1.39
331	piR-hsa-3706918	0.00	0.00	0.00	3.35	0.00	0.00	6.42	1.81	3.49
332	piR-hsa-4157592	2.12	1.54	1.20	4.18	4.05	1.95	0.00	0.00	0.00
333	piR-hsa-4175186	0.53	1.54	9.63	0.00	1.35	1.95	0.00	0.00	0.00
334	piR-hsa-3714350	0.53	0.00	0.00	9.20	1.35	0.00	3.21	0.00	0.70
335	piR-hsa-354004	1.06	10.77	1.20	0.00	0.00	1.95	0.00	0.00	0.00
336	piR-hsa-3694141	0.00	1.54	1.20	10.87	1.35	0.00	0.00	0.00	0.00
337	piR-hsa-4403262	0.00	0.00	0.00	0.00	0.00	0.00	12.83	0.00	2.09
338	piR-hsa-3383102	1.59	3.08	0.00	4.18	4.05	1.95	0.00	0.00	0.00
339	piR-hsa-3357365	3.71	7.69	1.20	0.84	1.35	0.00	0.00	0.00	0.00
340	piR-hsa-213829	0.53	4.62	1.20	8.37	0.00	0.00	0.00	0.00	0.00
341	piR-hsa-7893387	0.53	0.00	0.00	2.51	8.09	1.95	1.60	0.00	0.00
342	piR-hsa-235205	0.00	0.00	0.00	0.00	0.00	0.00	12.83	0.45	1.39
343	piR-hsa-1416366	0.00	0.00	0.00	11.71	1.35	0.00	1.60	0.00	0.00
344	piR-hsa-1998991	2.12	9.23	0.00	0.00	1.35	1.95	0.00	0.00	0.00
345	piR-hsa-5124632	1.59	7.69	1.20	0.84	1.35	1.95	0.00	0.00	0.00
346	piR-hsa-3365644	0.00	0.00	2.41	7.53	2.70	1.95	0.00	0.00	0.00
347	piR-hsa-1886018	0.53	0.00	0.00	0.00	0.00	0.00	11.23	1.36	1.39
348	piR-hsa-3657078	6.89	0.00	2.41	0.84	1.35	0.00	0.00	0.90	2.09
349	piR-hsa-3104125	4.24	1.54	4.82	2.51	1.35	0.00	0.00	0.00	0.00
350	piR-hsa-4378137_4	0.53	1.54	0.00	4.18	2.70	3.89	0.00	0.90	0.70
351	piR-hsa-1592257	0.00	0.00	0.00	0.00	0.00	0.00	11.23	1.81	1.39
352	piR-hsa-3818788	4.24	0.00	6.02	0.00	4.05	0.00	0.00	0.00	0.00
353	piR-hsa-2839864	1.06	0.00	0.00	0.84	0.00	0.00	6.42	3.16	2.79
354	piR-hsa-1678085	0.00	0.00	1.20	1.67	9.44	1.95	0.00	0.00	0.00

	piRNA	psc1	psc2	psc3	mpc1	mpc2	mpc3	cpc1	cpc2	cpc3
355	piR-hsa-4193743	0.00	0.00	0.00	0.00	0.00	0.00	11.23	0.90	2.09
356	piR-hsa-2537452	0.00	0.00	0.00	14.22	0.00	0.00	0.00	0.00	0.00
357	piR-hsa-4507261	0.00	1.54	0.00	0.00	0.00	0.00	0.00	2.71	9.76
358	piR-hsa-1263612	6.89	0.00	4.82	0.00	0.00	0.00	0.00	0.90	1.39
359	piR-hsa-778924	0.53	1.54	0.00	7.53	2.70	0.00	1.60	0.00	0.00
360	piR-hsa-2165649	1.06	4.62	4.82	0.00	1.35	1.95	0.00	0.00	0.00
361	piR-hsa-1274138	1.06	0.00	0.00	0.00	1.35	1.95	6.42	0.90	2.09
362	piR-hsa-4111185	1.06	3.08	1.20	7.53	0.00	0.00	0.00	0.00	0.70
363	piR-hsa-3693411	0.00	0.00	0.00	5.86	2.70	1.95	1.60	0.00	1.39
364	piR-hsa-1917013	0.53	3.08	0.00	0.00	0.00	0.00	6.42	2.71	0.70
365	piR-hsa-4467055_8	0.00	0.00	2.41	5.86	0.00	3.89	0.00	0.45	0.70
366	piR-hsa-4402141	0.00	0.00	0.00	0.00	0.00	0.00	11.23	1.36	0.70
367	piR-hsa-942735	0.00	0.00	0.00	0.00	0.00	0.00	11.23	1.36	0.70
368	piR-hsa-1574545	0.00	0.00	0.00	0.84	0.00	0.00	9.62	0.00	2.79
369	piR-hsa-2161668	0.00	0.00	1.20	4.18	5.39	1.95	0.00	0.45	0.00
370	piR-hsa-771001	0.53	0.00	0.00	9.20	1.35	0.00	1.60	0.45	0.00
371	piR-hsa-2502273	3.71	0.00	4.82	2.51	0.00	1.95	0.00	0.00	0.00
372	piR-hsa-77303	2.65	1.54	6.02	0.00	1.35	0.00	0.00	0.00	1.39
373	piR-hsa-2853562	0.00	0.00	1.20	8.37	1.35	1.95	0.00	0.00	0.00
374	piR-hsa-2851130	1.59	0.00	0.00	0.00	0.00	0.00	4.81	2.26	4.18
375	piR-hsa-2538984	7.42	0.00	0.00	0.84	1.35	0.00	1.60	0.90	0.70
376	piR-hsa-748358_5	0.00	0.00	0.00	0.00	0.00	0.00	9.62	3.16	0.00
377	piR-hsa-2400882	0.53	0.00	0.00	2.51	5.39	3.89	0.00	0.45	0.00
378	piR-hsa-3610092	5.30	1.54	2.41	0.00	1.35	1.95	0.00	0.00	0.00
379	piR-hsa-727158	5.83	1.54	3.61	0.84	0.00	0.00	0.00	0.00	0.70
380	piR-hsa-1530800	0.00	0.00	0.00	5.86	2.70	3.89	0.00	0.00	0.00
381	piR-hsa-1603046	0.00	0.00	0.00	2.51	4.05	5.84	0.00	0.00	0.00
382	piR-hsa-2518229	0.00	1.54	0.00	0.00	1.35	0.00	3.21	1.81	4.18
383	piR-hsa-3642754	4.77	1.54	4.82	0.84	0.00	0.00	0.00	0.00	0.00
384	piR-hsa-5039329_2	0.00	0.00	0.00	0.00	0.00	0.00	9.62	0.90	1.39
385	piR-hsa-4120912	0.53	0.00	0.00	3.35	5.39	1.95	0.00	0.00	0.70
386	piR-hsa-3263398	5.83	3.08	0.00	0.00	0.00	0.00	0.00	2.26	0.70
387	piR-hsa-2484024	0.00	0.00	0.00	0.84	0.00	0.00	8.02	0.90	2.09
388	piR-hsa-4388882	0.53	1.54	0.00	8.37	1.35	0.00	0.00	0.00	0.00
389	piR-hsa-2499630	0.53	0.00	0.00	0.00	0.00	0.00	8.02	0.90	2.09
390	piR-hsa-2515298	4.24	1.54	0.00	0.00	0.00	0.00	3.21	0.45	2.09
391	piR-hsa-65029	2.12	1.54	4.82	1.67	1.35	0.00	0.00	0.00	0.00
392	piR-hsa-271367	3.71	3.08	2.41	0.84	1.35	0.00	0.00	0.00	0.00
393	piR-hsa-2501382	1.06	0.00	1.20	0.84	0.00	0.00	3.21	2.26	2.79
394	piR-hsa-3345389	0.00	0.00	0.00	3.35	4.05	3.89	0.00	0.00	0.00
395	piR-hsa-1881756	0.00	0.00	0.00	0.00	1.35	0.00	6.42	0.00	3.49
396	piR-hsa-1593686	3.71	3.08	1.20	0.84	0.00	1.95	0.00	0.45	0.00
397	piR-hsa-4359698	2.12	4.62	2.41	0.00	0.00	1.95	0.00	0.00	0.00
398	piR-hsa-1866304	3.18	3.08	4.82	0.00	0.00	0.00	0.00	0.00	0.00
399	piR-hsa-1550295	2.12	7.69	1.20	0.00	0.00	0.00	0.00	0.00	0.00
400	piR-hsa-550050	0.00	0.00	0.00	0.00	0.00	0.00	6.42	3.16	1.39
401	piR-hsa-76694	3.71	0.00	7.23	0.00	0.00	0.00	0.00	0.00	0.00
402	piR-hsa-2491463	4.24	3.08	0.00	0.00	0.00	1.95	0.00	0.90	0.70
403	piR-hsa-2534860	1.06	0.00	0.00	0.00	1.35	3.89	0.00	3.16	1.39
404	piR-hsa-2715002	0.00	0.00	0.00	0.00	0.00	0.00	8.02	1.36	1.39
405	piR-hsa-2511205	0.53	0.00	0.00	0.84	0.00	0.00	3.21	4.07	2.09
406	piR-hsa-4271500	0.53	1.54	0.00	6.69	0.00	1.95	0.00	0.00	0.00
407	piR-hsa-4150185	1.06	0.00	1.20	8.37	0.00	0.00	0.00	0.00	0.00
408	piR-hsa-1943369	0.00	0.00	0.00	5.02	5.39	0.00	0.00	0.00	0.00
409	piR-hsa-2447480	1.59	3.08	4.82	0.84	0.00	0.00	0.00	0.00	0.00
410	piR-hsa-1416960	2.65	4.62	1.20	1.67	0.00	0.00	0.00	0.00	0.00
411	piR-hsa-699024	0.00	0.00	1.20	0.00	0.00	0.00	6.42	1.81	0.70
412	piR-hsa-6764147	0.00	0.00	1.20	0.84	0.00	0.00	0.00	1.81	6.28
413	piR-hsa-644441_3	0.53	0.00	0.00	0.00	0.00	0.00	6.42	3.16	0.00

	piRNA	psc1	psc2	psc3	mpc1	mpc2	mpc3	cpc1	cpc2	cpc3
414	piR-hsa-2500917	0.53	0.00	1.20	0.00	0.00	0.00	4.81	1.36	2.09
415	piR-hsa-1160813	0.00	0.00	0.00	6.69	1.35	1.95	0.00	0.00	0.00
416	piR-hsa-628215	0.00	0.00	0.00	0.00	0.00	0.00	6.42	0.00	3.49
417	piR-hsa-5313694	0.00	0.00	0.00	0.00	0.00	0.00	4.81	4.97	0.00
418	piR-hsa-778446	0.53	0.00	0.00	0.00	0.00	0.00	1.60	1.36	6.28
419	piR-hsa-313568	0.00	0.00	0.00	8.37	1.35	0.00	0.00	0.00	0.00
420	piR-hsa-3153428	2.65	4.62	2.41	0.00	0.00	0.00	0.00	0.00	0.00
421	piR-hsa-2365951	0.00	0.00	0.00	5.86	1.35	1.95	0.00	0.45	0.00
422	piR-hsa-4398177	0.00	0.00	0.00	8.37	0.00	0.00	0.00	0.45	0.70
423	piR-hsa-5884872	1.59	3.08	4.82	0.00	0.00	0.00	0.00	0.00	0.00
424	piR-hsa-851842	3.71	0.00	3.61	0.00	0.00	1.95	0.00	0.00	0.00
425	piR-hsa-4175241	0.53	0.00	1.20	7.53	0.00	0.00	0.00	0.00	0.00
426	piR-hsa-612387	3.71	3.08	2.41	0.00	0.00	0.00	0.00	0.00	0.00
427	piR-hsa-1551388	0.00	0.00	0.00	0.00	0.00	0.00	4.81	2.26	2.09
428	piR-hsa-4049552	0.00	0.00	0.00	0.00	1.35	0.00	3.21	1.81	2.79
429	piR-hsa-42060	1.06	0.00	0.00	6.69	1.35	0.00	0.00	0.00	0.00
430	piR-hsa-338024	3.18	1.54	2.41	0.00	0.00	0.00	0.00	0.45	1.39
431	piR-hsa-2030201	3.71	1.54	3.61	0.00	0.00	0.00	0.00	0.00	0.00
432	piR-hsa-4135859	0.00	0.00	0.00	0.84	0.00	0.00	3.21	4.07	0.70
433	piR-hsa-1692624	0.00	0.00	0.00	6.69	0.00	1.95	0.00	0.00	0.00
434	piR-hsa-1534285	2.12	1.54	4.82	0.00	0.00	0.00	0.00	0.00	0.00
435	piR-hsa-2832794	0.00	0.00	0.00	0.00	0.00	0.00	4.81	2.26	1.39
436	piR-hsa-1370047	0.00	0.00	0.00	0.84	0.00	0.00	0.00	5.87	1.39
437	piR-hsa-632933	0.00	0.00	0.00	0.00	1.35	0.00	1.60	2.26	2.79
438	piR-hsa-2069180	4.24	0.00	1.20	0.84	1.35	0.00	0.00	0.00	0.00
439	piR-hsa-1524373	0.00	0.00	0.00	0.00	0.00	0.00	1.60	0.90	4.88
440	piR-hsa-2487038_7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.81	5.58
441	piR-hsa-5568482	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.61	3.49
442	piR-hsa-4346643	0.00	0.00	0.00	0.84	0.00	0.00	0.00	4.07	2.09
443	piR-hsa-7313613	0.53	1.54	0.00	0.00	0.00	0.00	0.00	3.61	0.70
444	piR-hsa-4398796	4.77	0.00	0.00	0.00	0.00	0.00	0.00	1.36	0.00
445	piR-hsa-2369565	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.90	4.88
446	piR-hsa-7098338	0.00	0.00	0.00	0.00	0.00	0.00	1.60	3.16	0.70
447	piR-hsa-7670531_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.61	1.39

Table S1: Related to Figure 3. Normalized counts for the 447 identified piRNAs arranged in descending order expression (row mean). Differentially regulated piRNAs in CPC are indicated in light blue shading (downregulated) and light red shading (upregulated).

clusters.field1

cluster1	cluster2	cluster3	cluster4	cluster5
piR-hsa-108574	piR-hsa-1001021	piR-hsa-118348	piR-hsa-114666	piR-hsa-1233052
piR-hsa-132896	piR-hsa-1277994	piR-hsa-1263612	piR-hsa-1389062	piR-hsa-1870459
piR-hsa-137136	piR-hsa-147461	piR-hsa-1291516	piR-hsa-1463989	piR-hsa-1870588
piR-hsa-1399886	piR-hsa-151249	piR-hsa-1332287	piR-hsa-151136	piR-hsa-1872235
piR-hsa-1492262	piR-hsa-1872085_4	piR-hsa-1376916	piR-hsa-163695	piR-hsa-1890632
piR-hsa-1843231	piR-hsa-2482189	piR-hsa-1409954	piR-hsa-1647694	piR-hsa-1900529
piR-hsa-2090890	piR-hsa-2646470	piR-hsa-1416960	piR-hsa-169217	piR-hsa-1919455
piR-hsa-2172087	piR-hsa-611204	piR-hsa-1528884	piR-hsa-1901970	piR-hsa-1927965
piR-hsa-2333057	piR-hsa-76848	piR-hsa-1534285	piR-hsa-1988800	piR-hsa-1941780
piR-hsa-2536290	piR-hsa-1160813	piR-hsa-1550295	piR-hsa-20628	piR-hsa-2477264
piR-hsa-2856604	piR-hsa-1229611	piR-hsa-1555218	piR-hsa-214132	piR-hsa-2479371
piR-hsa-2882083	piR-hsa-1256360	piR-hsa-1593307	piR-hsa-2152778	piR-hsa-2481097
piR-hsa-298158	piR-hsa-1259933	piR-hsa-1593686	piR-hsa-2208850	piR-hsa-2494226
piR-hsa-3021684	piR-hsa-1302552	piR-hsa-1595580	piR-hsa-2286229	piR-hsa-2515454
piR-hsa-3119265	piR-hsa-1303811	piR-hsa-1607096	piR-hsa-2299252	piR-hsa-2530015
piR-hsa-3546008	piR-hsa-1340768	piR-hsa-1632961	piR-hsa-2346976	piR-hsa-2831593
piR-hsa-5077723	piR-hsa-1416366	piR-hsa-163499	piR-hsa-2780538	piR-hsa-2832647
piR-hsa-6913457	piR-hsa-1425899	piR-hsa-1686806	piR-hsa-2844156	piR-hsa-2840936
piR-hsa-721859_9	piR-hsa-1434629	piR-hsa-1726249	piR-hsa-3265318	piR-hsa-346271
	piR-hsa-1481120	piR-hsa-1748898	piR-hsa-3660133	piR-hsa-3623001
	piR-hsa-1530800	piR-hsa-1798104	piR-hsa-3817390	piR-hsa-368987
	piR-hsa-1531418	piR-hsa-1866304	piR-hsa-3875265	piR-hsa-3735787
	piR-hsa-1557538	piR-hsa-1875155	piR-hsa-3916570	piR-hsa-768321
	piR-hsa-1585146	piR-hsa-1929067	piR-hsa-4078407	piR-hsa-771714_3
	piR-hsa-1603046	piR-hsa-1998991	piR-hsa-4303719	piR-hsa-7892960
	piR-hsa-160969	piR-hsa-2027369	piR-hsa-5982715	
	piR-hsa-161264_3	piR-hsa-2030201	piR-hsa-6245615	
	piR-hsa-1678085	piR-hsa-2069180	piR-hsa-783698	
	piR-hsa-1688824	piR-hsa-2090264		
	piR-hsa-1692624	piR-hsa-2137611		
	piR-hsa-1706026	piR-hsa-2148238		
	piR-hsa-1708978	piR-hsa-2165649		
	piR-hsa-1756618	piR-hsa-2213434		
	piR-hsa-1883893	piR-hsa-2230204		
	piR-hsa-1905329	piR-hsa-2240007		
	piR-hsa-1909905	piR-hsa-2248086		
	piR-hsa-1921551	piR-hsa-2268195		
	piR-hsa-1922210	piR-hsa-2308163		
	piR-hsa-1933276	piR-hsa-2319750		
	piR-hsa-1939085	piR-hsa-2351941		
	piR-hsa-1941637	piR-hsa-2353109		
	piR-hsa-1943369	piR-hsa-2398119		
	piR-hsa-211123	piR-hsa-2413094		
	piR-hsa-213829	piR-hsa-2426792		
	piR-hsa-2161668	piR-hsa-2427082		
	piR-hsa-21839	piR-hsa-2436454		
	piR-hsa-2209630	piR-hsa-2447480		
	piR-hsa-2220917	piR-hsa-2491463		
	piR-hsa-2253283	piR-hsa-2497478		
	piR-hsa-229786	piR-hsa-2502273		

piR-hsa-2365951	piR-hsa-2503702
piR-hsa-237221	piR-hsa-2515298
piR-hsa-2400882	piR-hsa-2519215
piR-hsa-2423519	piR-hsa-2538984
piR-hsa-2450089_2	piR-hsa-271367
piR-hsa-2464166	piR-hsa-2856544
piR-hsa-2489909	piR-hsa-2989729
piR-hsa-2505515	piR-hsa-3104125
piR-hsa-2525461	piR-hsa-3136454
piR-hsa-2537452	piR-hsa-3153428
piR-hsa-2589139	piR-hsa-315964
piR-hsa-2670375	piR-hsa-3177742
piR-hsa-2853562	piR-hsa-3231825
piR-hsa-2889978	piR-hsa-3263398
piR-hsa-307961	piR-hsa-3280518
piR-hsa-313568	piR-hsa-333507
piR-hsa-3161050	piR-hsa-3357365
piR-hsa-3345389	piR-hsa-338024
piR-hsa-3365644	piR-hsa-3513154
piR-hsa-3383102	piR-hsa-3527815
piR-hsa-362913	piR-hsa-354004
piR-hsa-3634880	piR-hsa-3558751
piR-hsa-3693411	piR-hsa-3610092
piR-hsa-3694141	piR-hsa-3638679
piR-hsa-3710717	piR-hsa-3642754
piR-hsa-3714350	piR-hsa-3657078
piR-hsa-374600	piR-hsa-3674332
piR-hsa-3776081	piR-hsa-3732088
piR-hsa-3829948	piR-hsa-3807498
piR-hsa-3839126	piR-hsa-3818788
piR-hsa-3842249	piR-hsa-3944431
piR-hsa-3978322	piR-hsa-3974794
piR-hsa-4111185	piR-hsa-4020841
piR-hsa-4120912	piR-hsa-4030155
piR-hsa-4131663	piR-hsa-4053516
piR-hsa-4150185	piR-hsa-4091280
piR-hsa-4157592	piR-hsa-4100164
piR-hsa-4175241	piR-hsa-4110708
piR-hsa-4202081	piR-hsa-4144265
piR-hsa-42060	piR-hsa-4175186
piR-hsa-4271500	piR-hsa-4178299
piR-hsa-4378137_4	piR-hsa-4198101
piR-hsa-4388882	piR-hsa-4322932
piR-hsa-4397384	piR-hsa-4359698
piR-hsa-4398177	piR-hsa-4379982
piR-hsa-4408495	piR-hsa-4398796
piR-hsa-4416099_9	piR-hsa-4403577
piR-hsa-4450044	piR-hsa-4403628
piR-hsa-4467055_8	piR-hsa-508592
piR-hsa-623353	piR-hsa-5124632
piR-hsa-669874	piR-hsa-5411637

piR-hsa-7106256	piR-hsa-58291
piR-hsa-7308134	piR-hsa-5884872
piR-hsa-745484	piR-hsa-612387
piR-hsa-7544198	piR-hsa-645846
piR-hsa-771001	piR-hsa-6482184
piR-hsa-772699	piR-hsa-65029
piR-hsa-7760463	piR-hsa-67957
piR-hsa-778924	piR-hsa-727158
piR-hsa-7833890	piR-hsa-76694
piR-hsa-7893387	piR-hsa-77303
piR-hsa-848451	piR-hsa-8270846
piR-hsa-97458	piR-hsa-851842

cluster6	cluster7	cluster8
piR-hsa-1904126	piR-hsa-1205256	piR-hsa-151466
piR-hsa-1916259	piR-hsa-1429070	piR-hsa-1548068
piR-hsa-359160_2	piR-hsa-1690788	piR-hsa-1875212
piR-hsa-3732777	piR-hsa-1773241	piR-hsa-1876265
piR-hsa-1862211	piR-hsa-1903779	piR-hsa-1883972
piR-hsa-1872463	piR-hsa-2151268	piR-hsa-1908839
piR-hsa-6744266	piR-hsa-2252211	piR-hsa-1919272
	piR-hsa-2395910	piR-hsa-1920687
	piR-hsa-2398570	piR-hsa-1923208
	piR-hsa-2513278	piR-hsa-2490897
	piR-hsa-255984	piR-hsa-2521457
	piR-hsa-2565910	piR-hsa-2526525
	piR-hsa-2615134	piR-hsa-2539762
	piR-hsa-2863156	piR-hsa-316012
	piR-hsa-3232943	piR-hsa-343382
	piR-hsa-3352181	piR-hsa-3634065
	piR-hsa-3683883	piR-hsa-3658275
	piR-hsa-389007	piR-hsa-3658742
	piR-hsa-4028152	piR-hsa-3739406
	piR-hsa-4381848_2	piR-hsa-4460706
	piR-hsa-4472891	piR-hsa-1576285
	piR-hsa-724912	piR-hsa-785969
	piR-hsa-7765828	piR-hsa-1057272
		piR-hsa-1242358
		piR-hsa-1259653
		piR-hsa-1274138
		piR-hsa-1284504
		piR-hsa-1296118
		piR-hsa-1370047
		piR-hsa-144277_2
		piR-hsa-147696
		piR-hsa-1524373
		piR-hsa-153942
		piR-hsa-1551388
		piR-hsa-1574545
		piR-hsa-1592257
		piR-hsa-1696540
		piR-hsa-1707103
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		piR-hsa-1882039
		piR-hsa-1886018
		piR-hsa-1894768
		piR-hsa-1905680
		piR-hsa-1912443
		piR-hsa-1917013
		piR-hsa-1917139
		piR-hsa-1921188
		piR-hsa-1927627
		piR-hsa-1938524
		piR-hsa-2042088

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piR-hsa-4346643
piR-hsa-4387218
piR-hsa-4391981_9
piR-hsa-4402141
piR-hsa-4403262
piR-hsa-4424378
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piR-hsa-5039329_2
piR-hsa-5062317
piR-hsa-5313694
piR-hsa-550050
piR-hsa-5568482
piR-hsa-5996985

clusters.field1

piR-hsa-628215
piR-hsa-632933
piR-hsa-642866
piR-hsa-644441_3
piR-hsa-665910
piR-hsa-6764147
piR-hsa-699024
piR-hsa-7098338
piR-hsa-728085
piR-hsa-7313613
piR-hsa-748358_5
piR-hsa-753191
piR-hsa-7670531_2
piR-hsa-778446
piR-hsa-7821967_3
piR-hsa-8117137
piR-hsa-834074
piR-hsa-942735