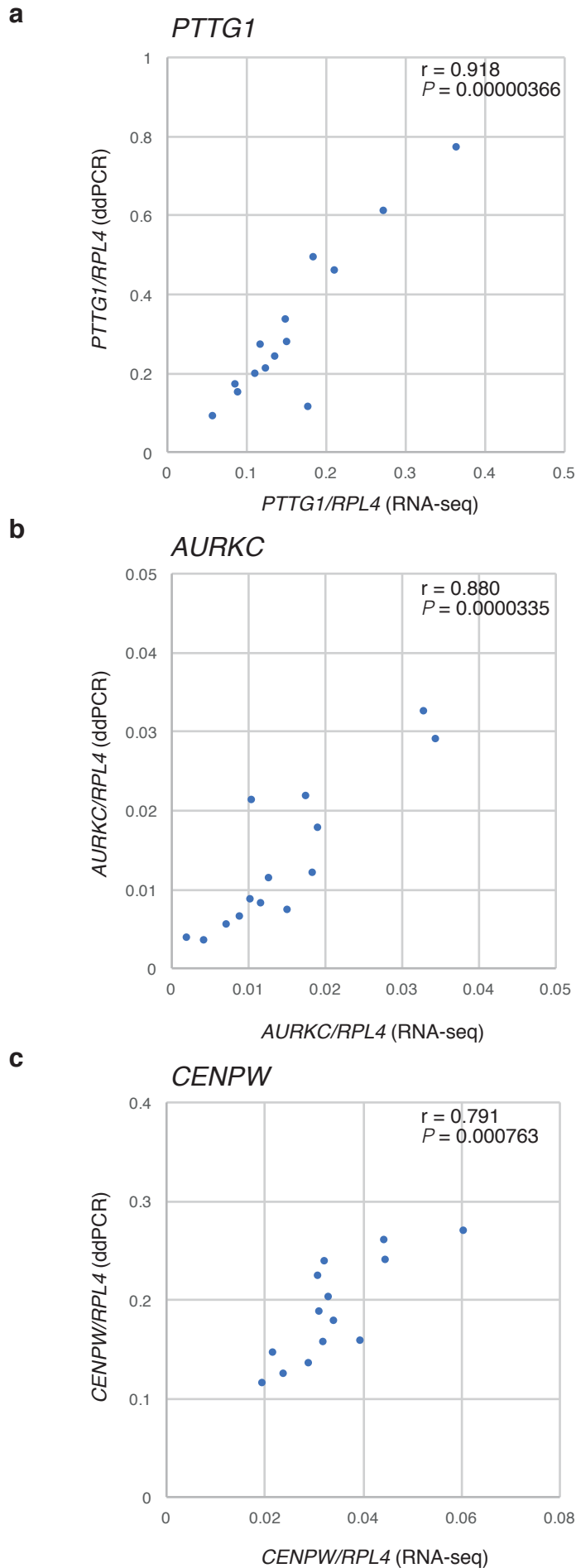


## **Parental age and gene expression profiles in individual human blastocysts**

Kiyotaka Kawai, Tatsuya Harada, Tomonori Ishikawa, Rikikazu Sugiyama, Toshihiro Kawamura, Atsumi Yoshida, Osamu Tsutsumi, Fumitoshi Ishino, Toshiro Kubota and Takashi Kohda.

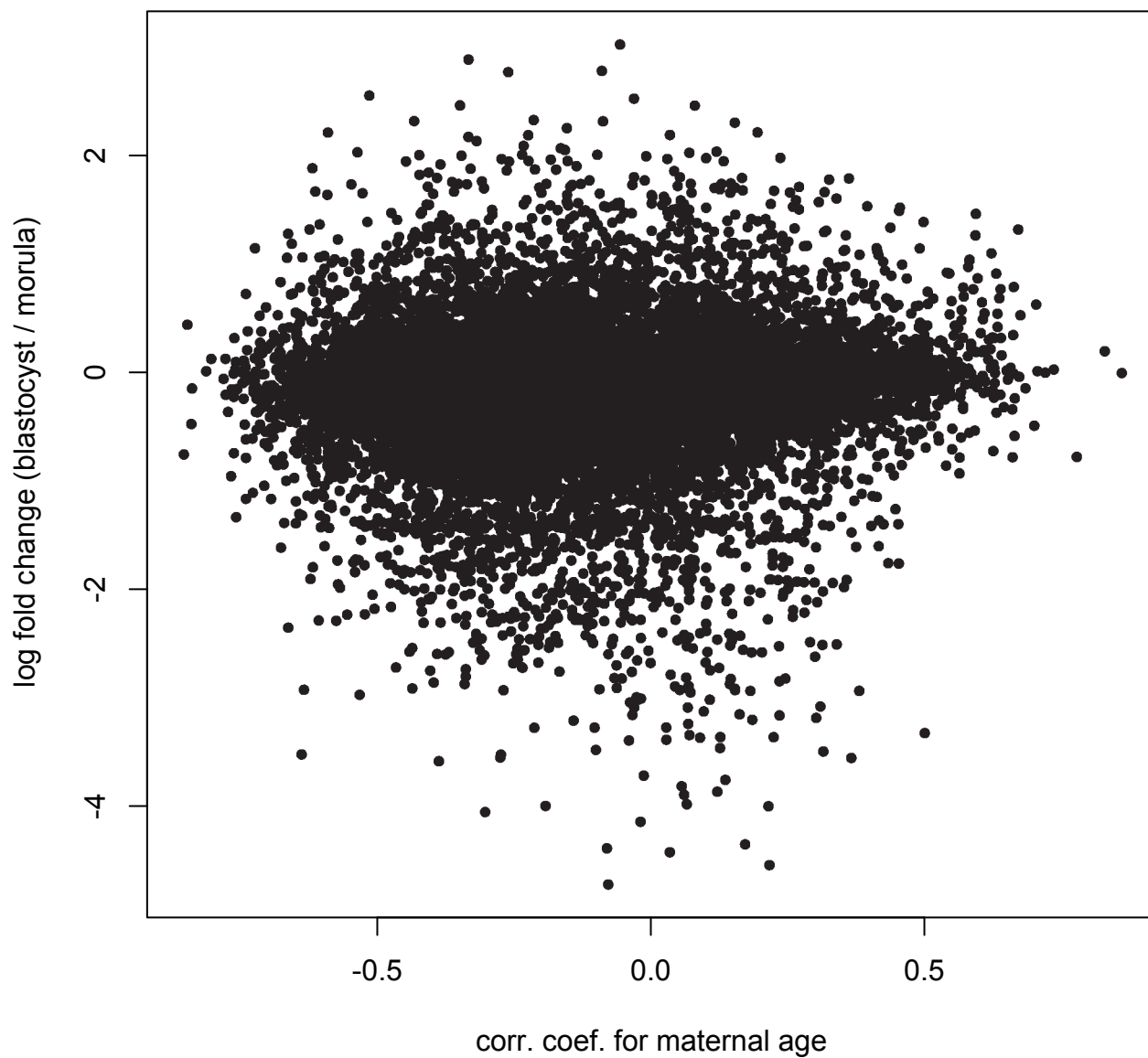
Supplementary Figure 1



**Supplementary Figure 1.** Validation of RNA-seq expression data by ddPCR.

Normalized gene expression level for *PTTG1* (a), *AURKC* (b) and *CENPW* (c) calculated from RT-ddPCR (y-axis) was plotted against that estimated from RNA-seq data (x-axis). Pearson's correlation coefficients  $r$  and  $P$  values are also shown in each panel. *RPL4* expression level was used as a control for normalization.

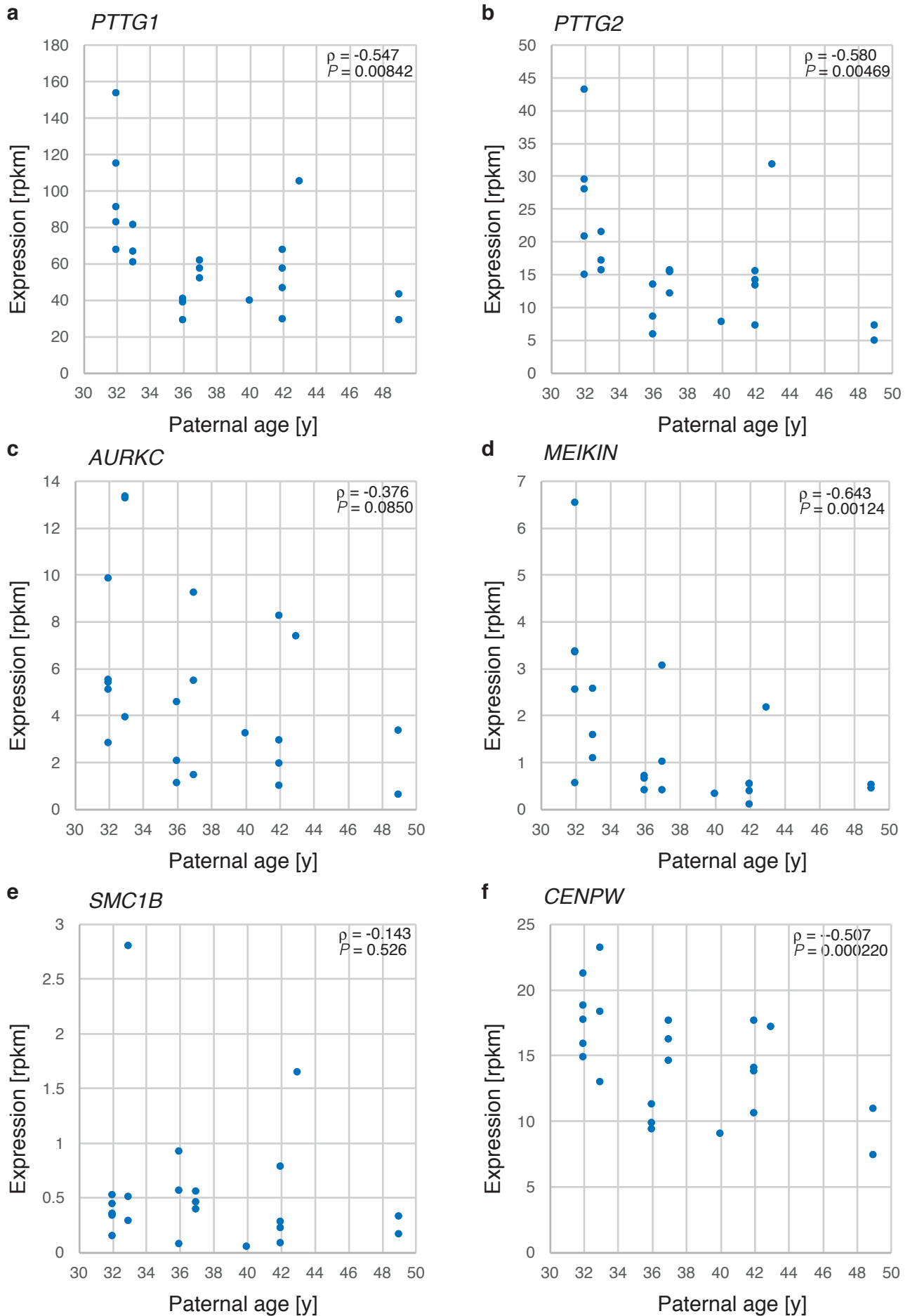
Supplementary Figure 2



**Supplementary Figure 2.** Gene expression changes between morulae and blastocysts compared to gene expression of blastocyst-stage embryo correlation coefficients with maternal age.

The x-axis represents the correlation coefficient between each rpk value of this study and maternal age. The y-axis represents the log fold change between the mean rpk value of morulae and blastocysts for the data reported by Hendrickson *et al.*

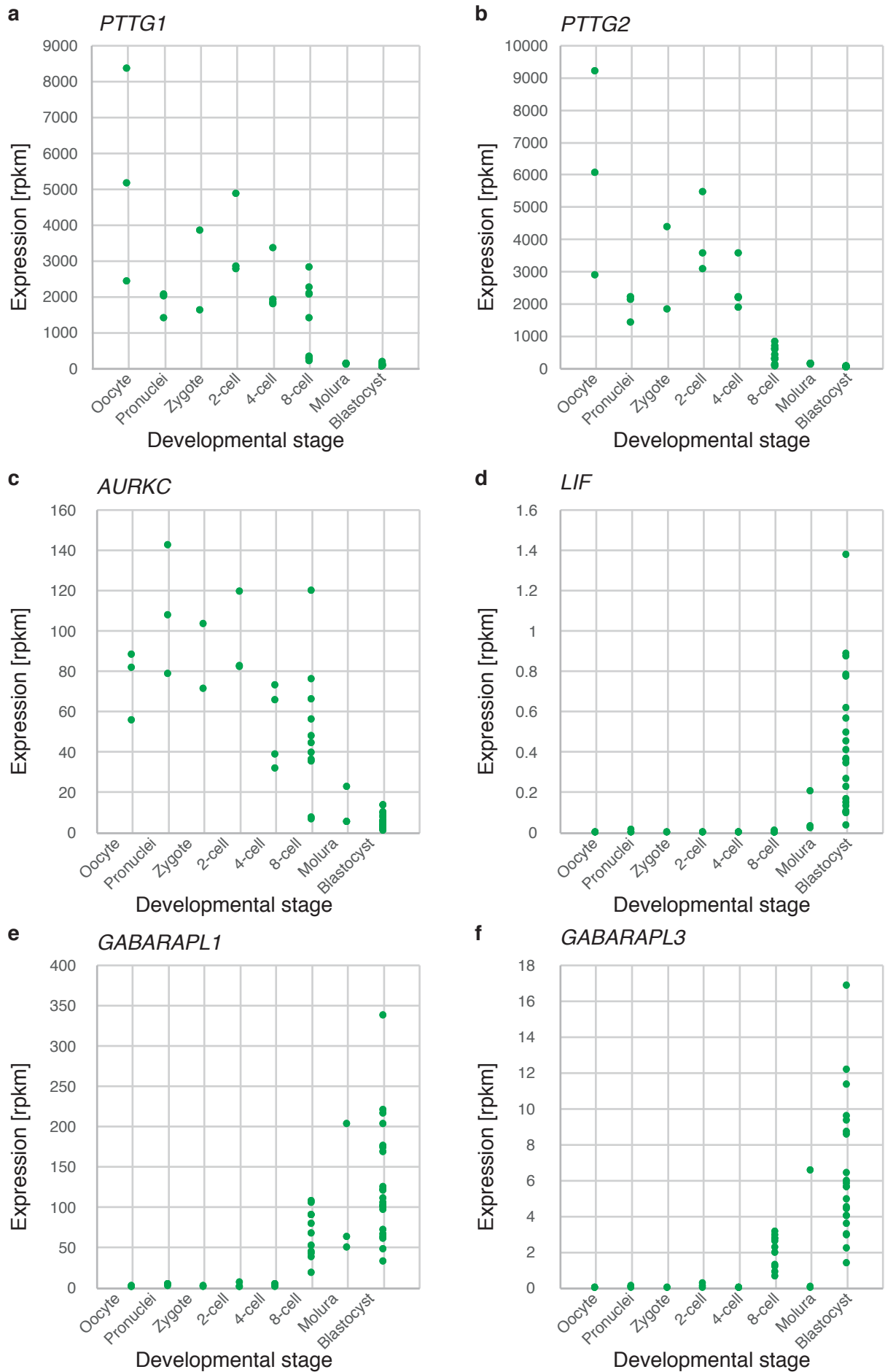
Supplementary Figure 3



**Supplementary Figure 3.** Genes down-regulated with increased maternal age.

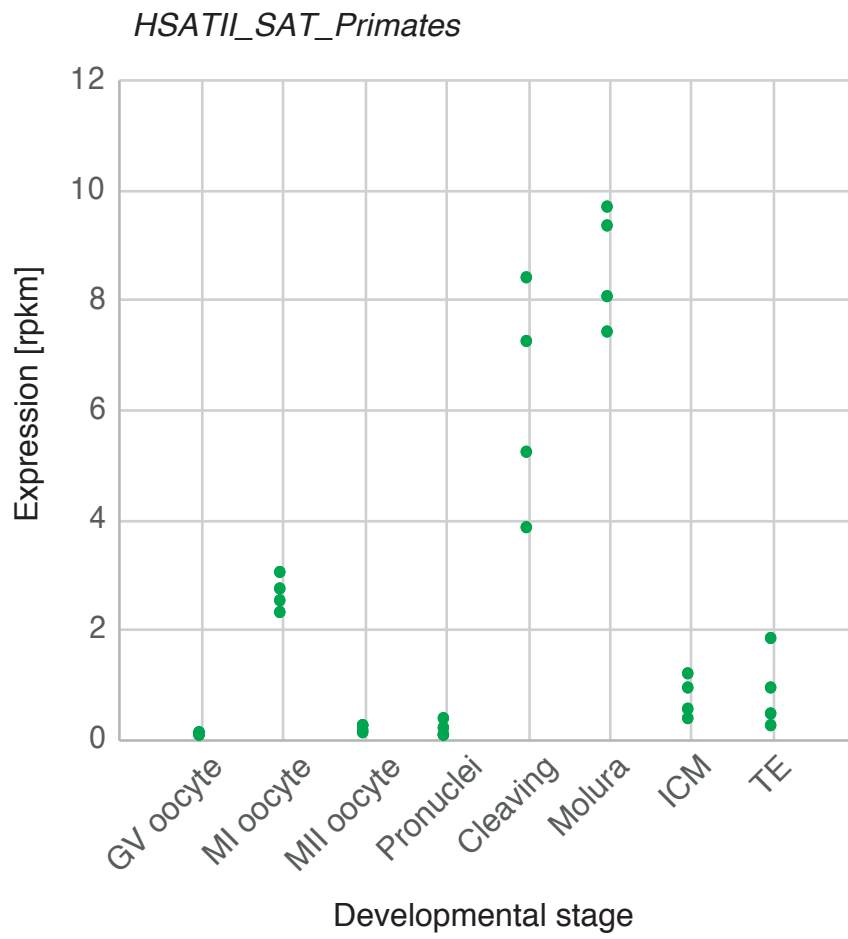
Expression levels in rpkm were plotted against the paternal age for *PTTG1* (a), *PTTG2* (b), *AURKC* (c), *CENPW* (d), *MEIKIN* (e) and *SMC1B* (f). Spearman's rank order correlation coefficients and *P* values are also shown in each panel.

Supplementary Figure 4



**Supplementary Figure 4.** Gene expression patterns at the pre-implantation stage for the genes down-regulated with increased parental age. Expression levels, in rpkm, were plotted during development from the oocyte to the blastocyst stage by Xue *et al.* for *PTTG1* (a), *PTTG2* (b), *AURKC* (c), *LIF* (d), *GABARAPL1* (e) and *GABARAPL3* (f).

Supplementary Figure 5



**Supplementary Figure 5.** Expression patterns at the pre-implantation stage for the transcripts from the major satellite. Expression levels in ppm were plotted during development from the oocyte to the blastocyst stage by Hendrickson *et al.* for *HSATII*.