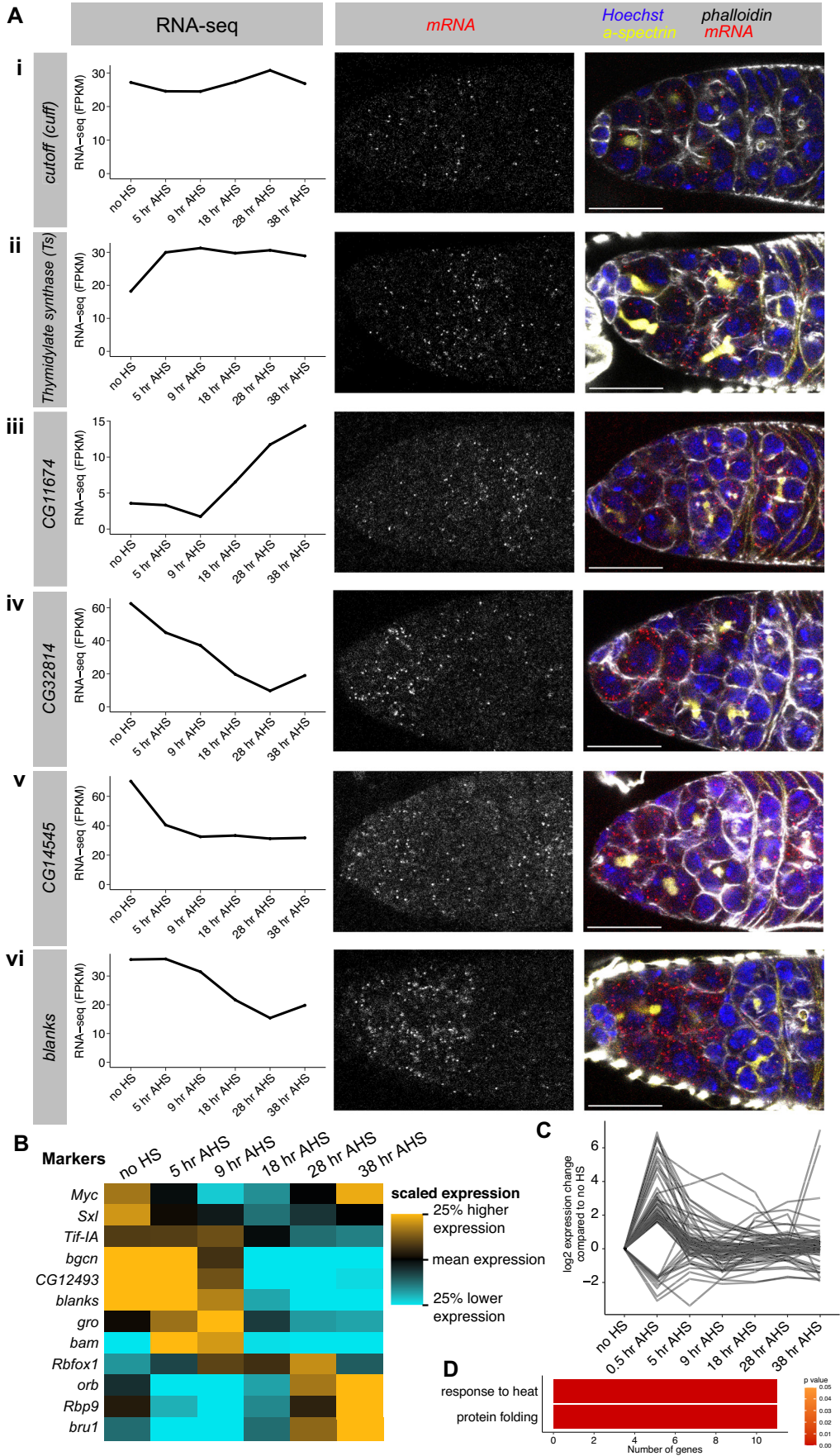


Expanded View Figures

Figure EV1. RNA-seq in synchronised differentiating GSCs recapitulates changes in gene expression during normal differentiation.

(A) As in Fig. 2A, smFISH in *wild type* germaria (right) was used to validate the RNA-seq results (left). Staining for DNA (Hoechst, blue), actin (phalloidin, gray), fusome (α -spectrin, yellow), and mRNA transcript of interest (smFISH, red and grayscale): (i) *cuff*, (ii) *thymidylate synthase*, (iii) *CG11674*, (iv) *CG32814* (*eggplant*), (v) *CG14545*, (vi) *blanks*. Scale bars are 15 μ m. (B) Heatmap illustrating RNA-seq expression level across our time course, of marker genes identified by scRNA-seq pseudotime analysis by Rust et al, 2020 (illustrated in Fig. 2m in that paper, and approximate expression domain labelled here left) for each time point from GSC to 16cc. Each row represents one gene and expression level is scaled per gene: black— mean expression across the time course, gold—25% higher expression than the mean, cyan—25% lower expression than the mean. (C) The 0.5 h AHS sample was used to exclude 67 genes which showed a significant change of >threefold between the 0.5 AHS and the no HS samples (with expression >10 FPKM in one of these samples). The expression of these 67 genes is shown as log2foldchange at each time point relative to no HS. (D) Gene ontology enrichment analysis of the excluded 67 genes shown in (C) found a significant enrichment in terms associated with a heat shock response. Colour of the bar indicates *P* value of the enrichment. Background is the whole genome. GO enrichment analysis was performed using FlyMine (Lyne et al, 2007) with Holm-Bonferroni correction.



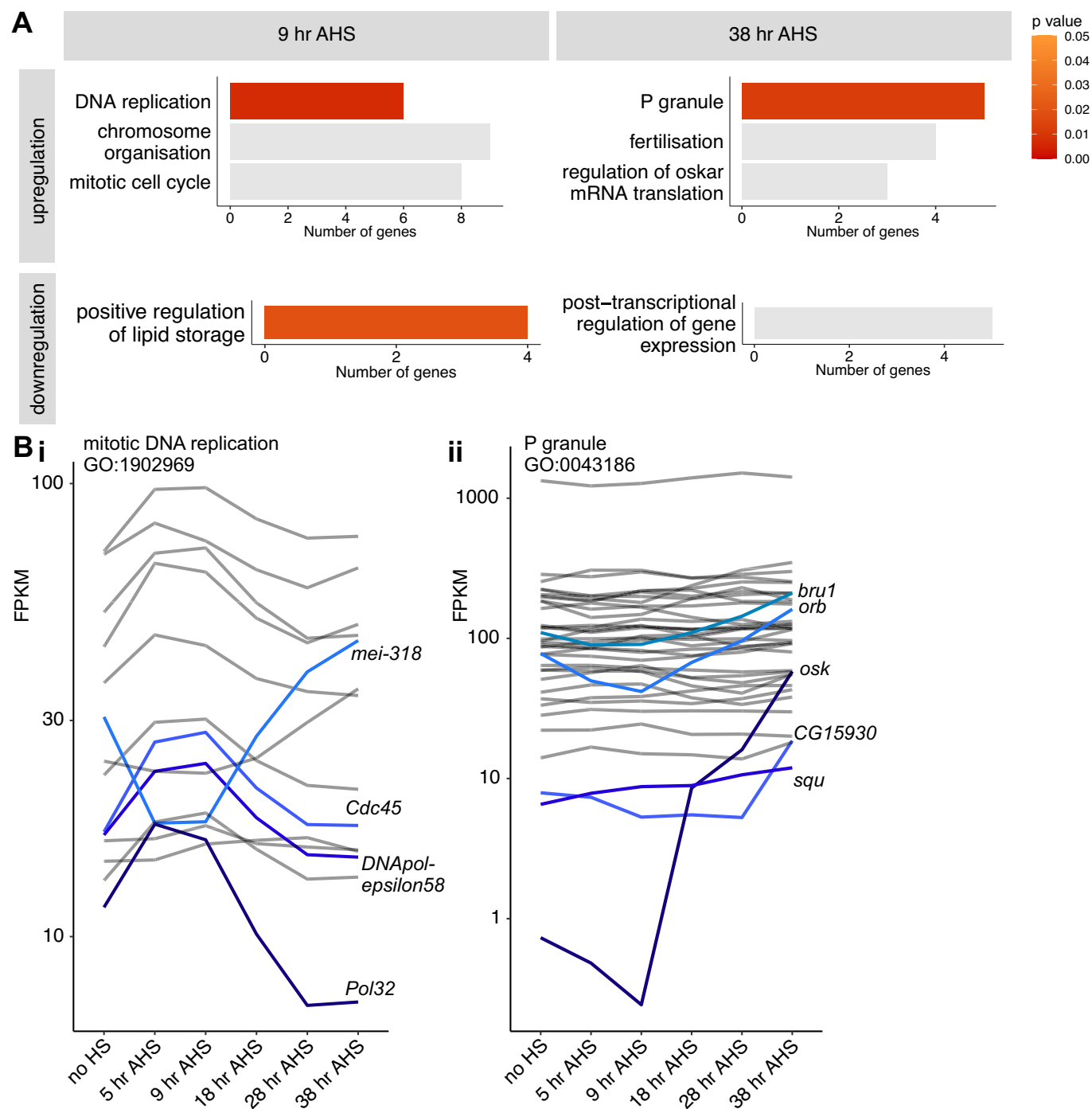


Figure EV2. RNA-seq reveals changes in mRNA level during differentiation.

(A) Gene ontology enrichment analysis of genes upregulated or downregulated in the RNA-seq at 9 h AHS or 38 h AHS compared to 'no HS'. Colour of the bar indicates p value of the enrichment, grey = $P > 0.05$. Background is all genes expressed during the time course. GO enrichment analysis was performed using FlyMine (Lyne et al, 2007) with Holm-Bonferroni correction. (B) RNA-seq FPKM for genes in two gene ontology groups: mitotic DNA replication (i) and P granule (ii). Genes with a >1.6-fold change in gene expression are highlighted.

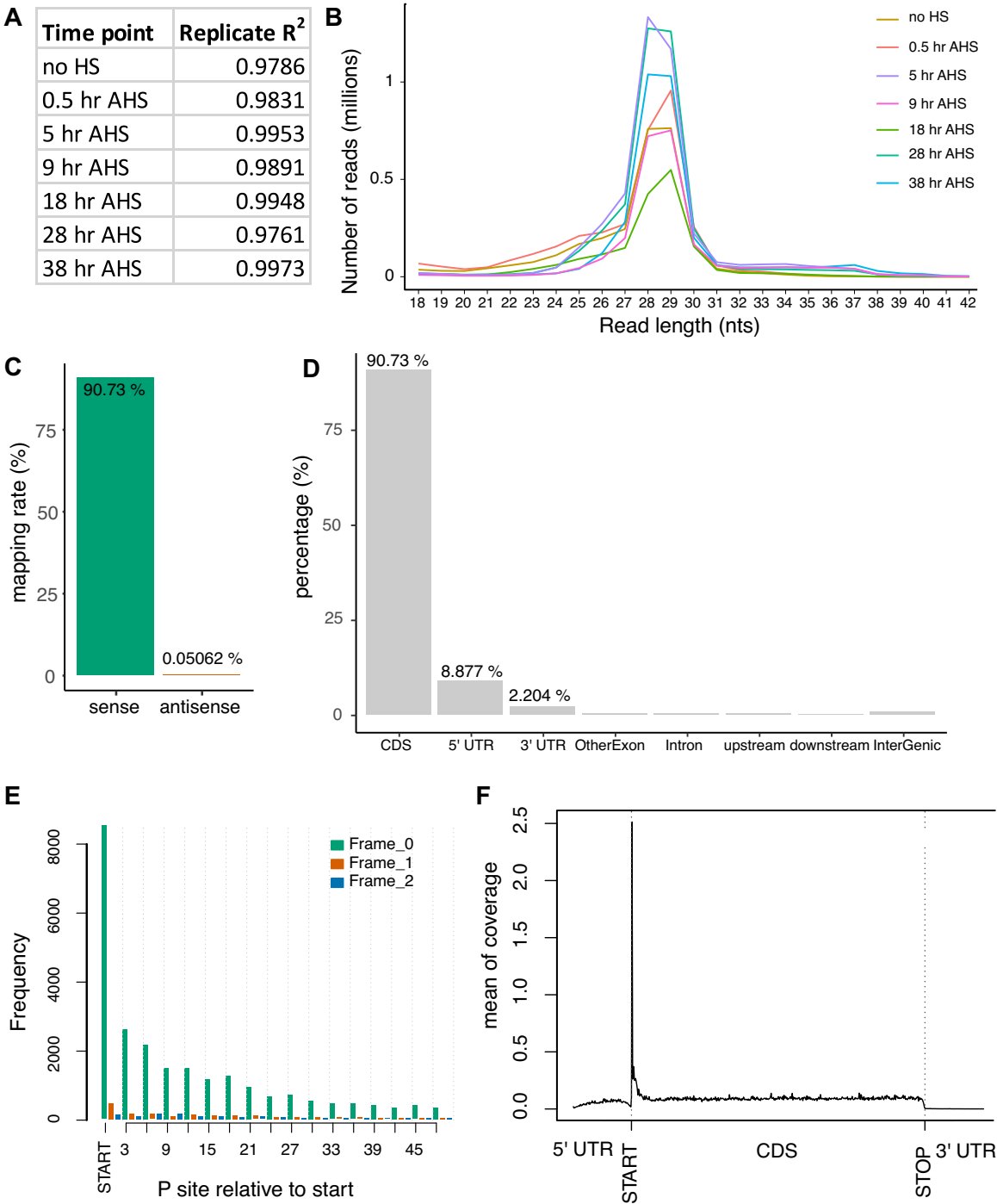


Figure EV3. Ribo-seq quality control.

(A) R^2 show good correlation between Ribo-seq replicate samples at each time point. (B) Distribution of read length in nucleotides for Ribo-seq libraries at each time point. (C-F) Ribo-seq quality control for the 'no HS' sample only for illustration. (C) 90.73% of reads mapped to the CDS on the sense strand of genes compared to 0.05% mapping to the CDS on the antisense strand. (D) 8.88% of reads map to the 5' UTR, 2.20% map to the 3' UTR, and mapping to introns and intergenic regions is negligible. (E) P-site mapping shows a strong three nucleotide periodicity, with highest frequency at the start codon. (F) Metagene analysis plot showing read distribution in 5' UTR, CDS and 3' UTR regions, shows consistent coverage across the CDS with the expected bias at the start codon.

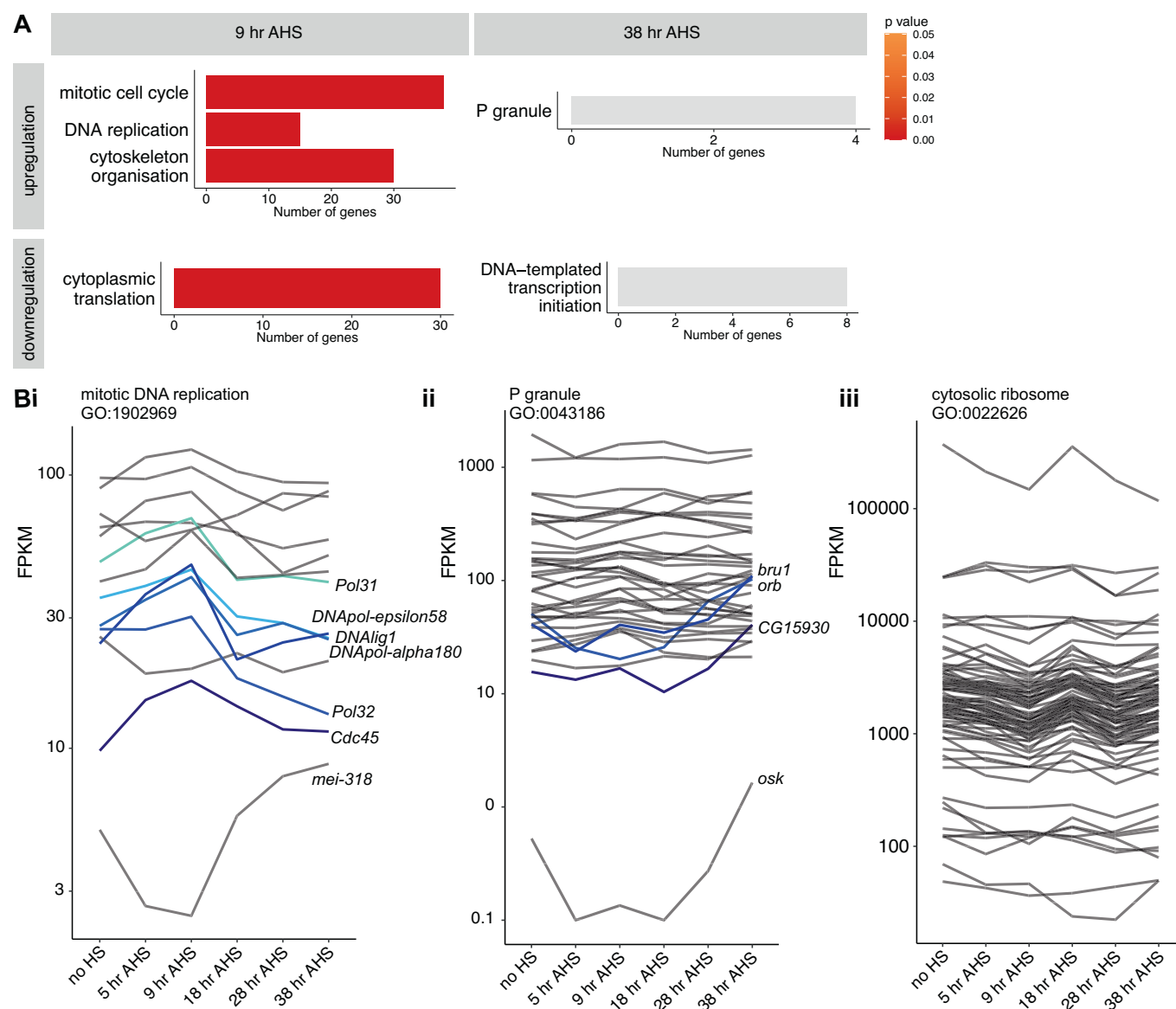
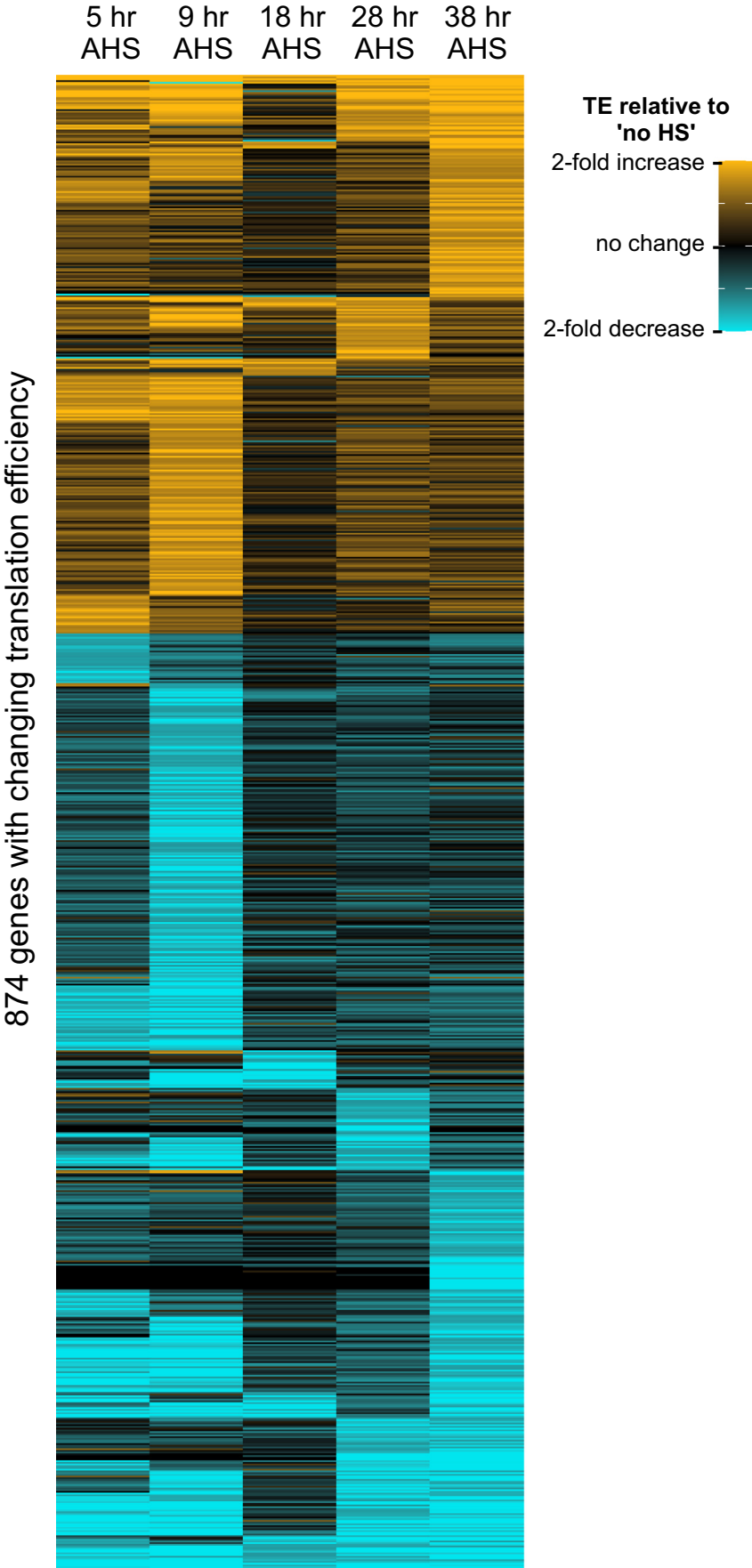


Figure EV4. Ribo-seq reveals changes in translation during GSC differentiation.

(A) Gene ontology enrichment analysis of genes upregulated or downregulated in the Ribo-seq at 9 h AHS or 38 h AHS compared to 'no HS'. Colour of the bar indicates p value of the enrichment, grey = $P > 0.05$. Background is all genes expressed during the time course. GO enrichment analysis was performed using FlyMine (Lyne et al, 2007) with Holm-Bonferroni correction. (B) Ribo-seq FPKM for genes in three gene ontology groups: mitotic DNA replication (i), P granule (ii) and cytosolic ribosome (iii). Genes with a >1.6 -fold change in gene expression are highlighted (except in iii due to too many genes).



**Figure EV5. Many genes are regulated by TE.**

Heatmap showing fold change in TE, compared to the 'no HS' time point, for the 874 genes which exhibit a significant 1.6-fold change in translation between a given time point and 'no HS'. cyan = twofold decrease compared to 'no HS', black = no change, gold = twofold increase.