

Figure S1. Expression profiles of reference cell cycle genes. Related to Figure 3 and Table S6. **(A)** Expression profiles of Cyclin A2, B1, B2, and E2 on protein (inner circle) and mRNA (outer circle) level, obtained from Cyclebase 3.0. **(B)** Expression of *CCNA2*, *CCNB1*, *CCNB2* and *CCNE2* according to the cell cycle pseudo-time, with smoothing splines fit to mark the trend. The dotted line represents the subdivision into “early” and “late” G1, hypothesized to approximate the restriction point.

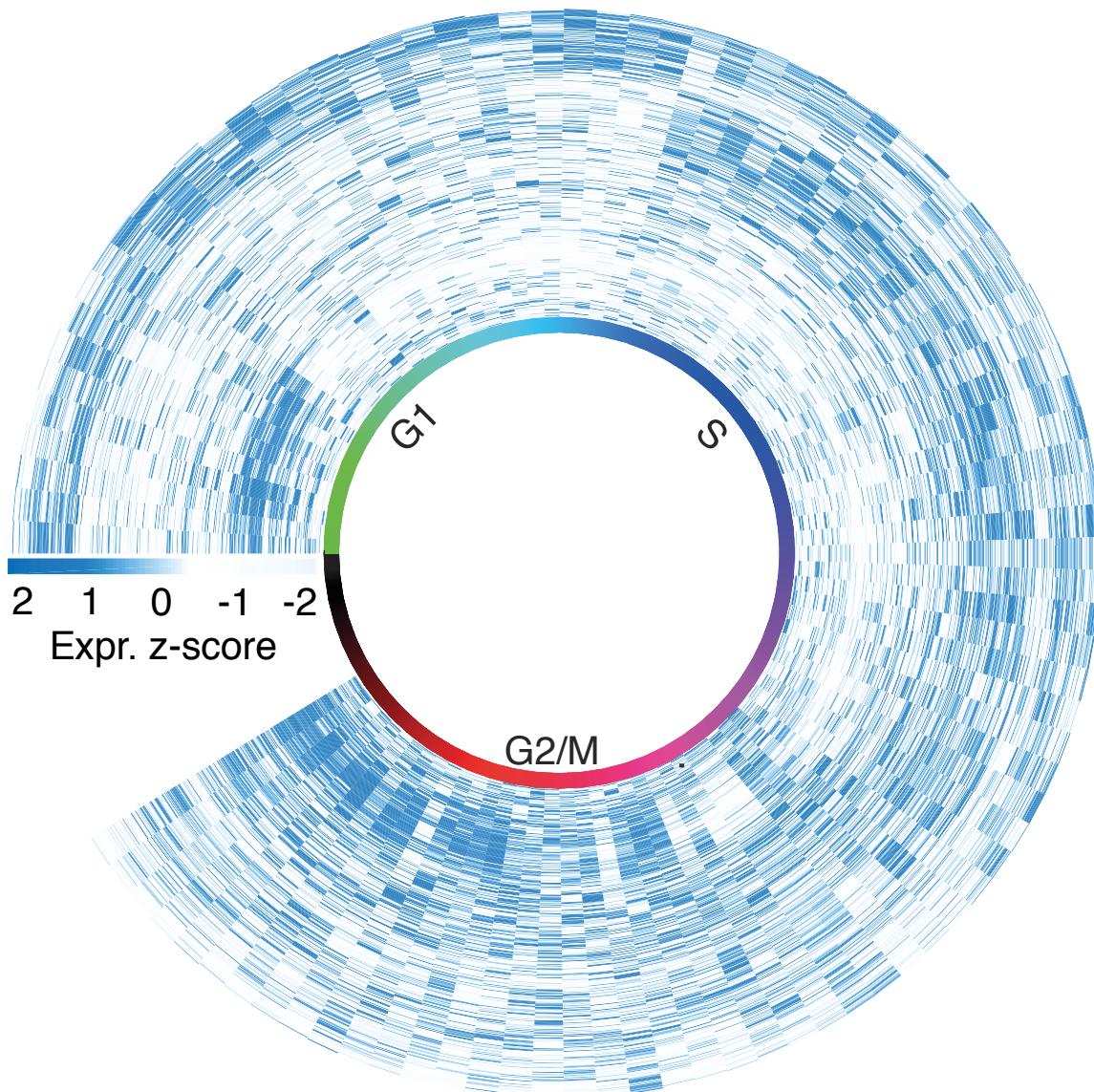


Figure S2. Expression profiles of differentially expressed genes. Related to Figure 2, Table S1 and Table S6. 472 genes detected as differentially expressed between either G1 and S, G1 and G2/M, S and G2/M, G1 compared to S and G2/M together, S compared to G1 and G2/M together or G2/M compared to G1 and S together. Cells have been ordered according to a cell cycle pseudo-timeline. Each row represents one gene and each column represents one cell. Expression values are represented as standardized (z-score) $\log_2(\text{RPM})$.

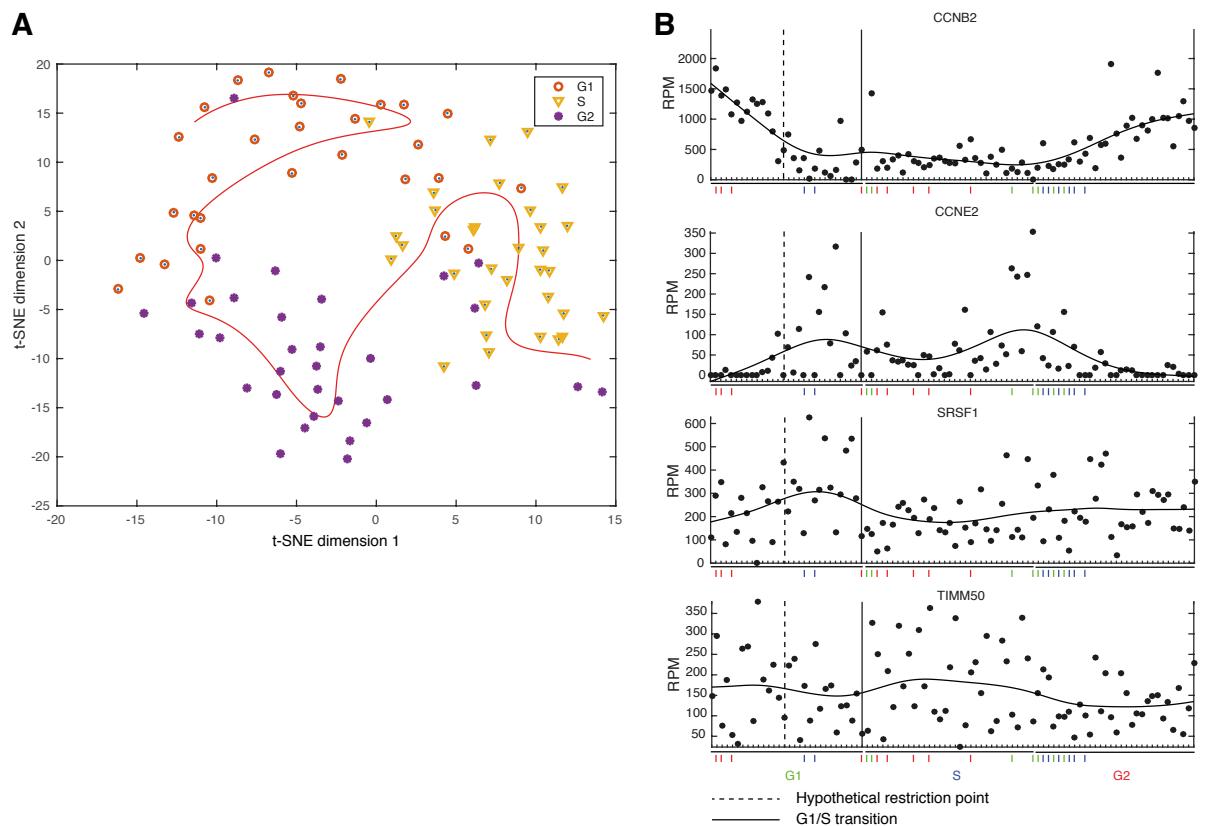


Figure S3. Alternative pseudo-time inference through the fitting of a principal curve to cell locations in t-SNE space. Related to Figures 1- 3 and Table S6. **(A)** Two-dimensional t-SNE of all coding genes expressed in more than 15 cells, with a principal curve fit in order to deduce relative cell-to-cell ordering across the cell cycle. **(B)** Expression of *CCNB2*, *CCNE2*, *SRSF1* and *TIMM50* according to the cell cycle pseudo-time, with smoothing splines fit to mark the trend. The dotted line represents the subdivision into “early” and “late” G1, hypothesized to approximate the restriction point. Colored vertical lines along the x-axes indicate cells whose inferred cell cycle phases disagree with the FACS determined labels.

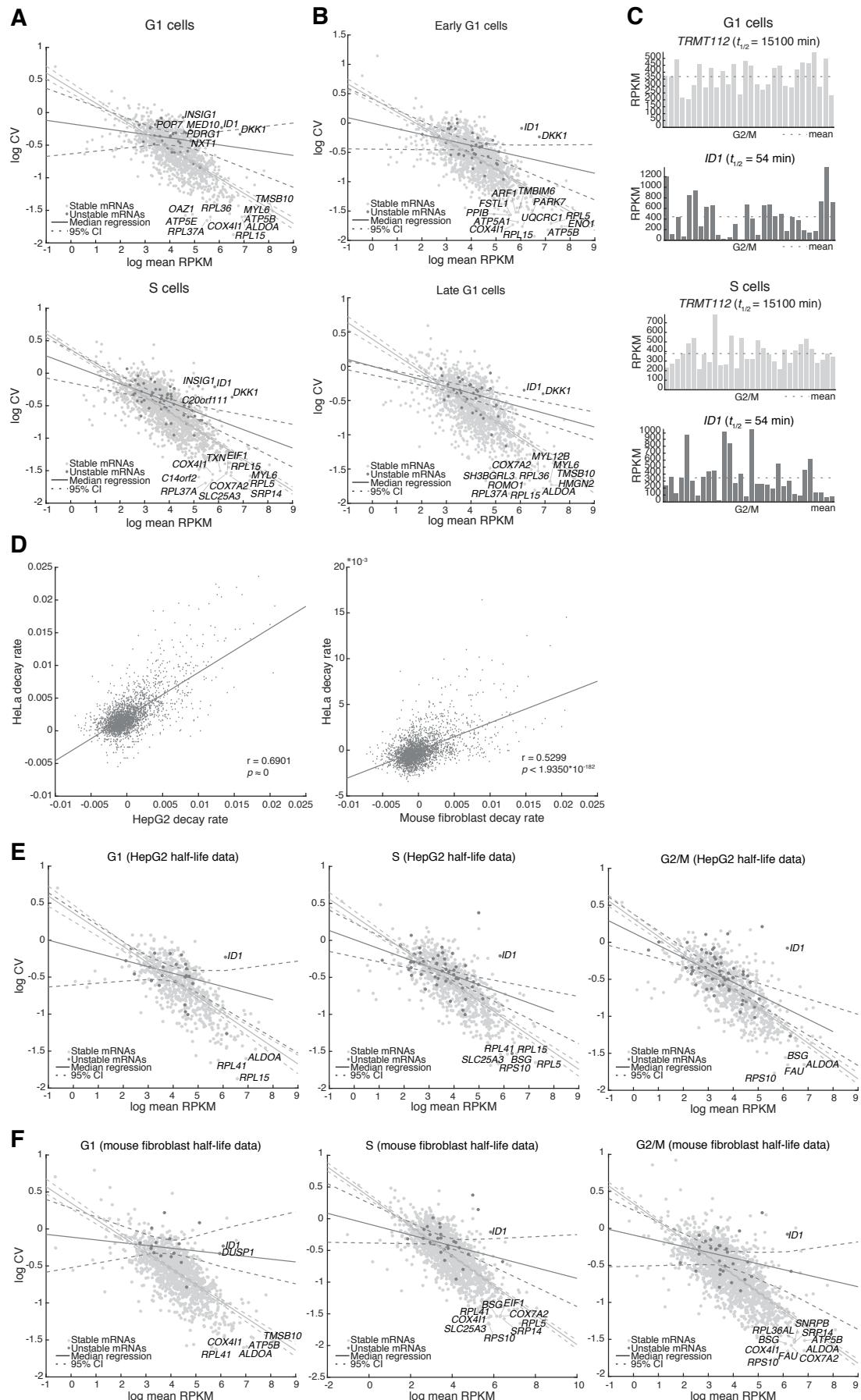


Figure S4. The relationship between gene expression variability and mRNA decay rate for stable and unstable mRNAs. Related to Figure 4. For cells in **(A)** G1 and S phase, **(B)** early and late G1, respectively, only including genes with expression in at least 90% of the cells. Unstable mRNAs were considered to be those with half-lives less than 200 minutes. Solid lines indicate median regression and dotted lines show 95% confidence intervals. CV: coefficient of variation, defined as the standard deviation divided by the mean of RPKM expression values for a given gene across all cells in the phase) **(C)** Pseudotime ordered expression profiles of two genes with very different half-lives (ID1: 54 min, TRMT112: 15100 min), in G1 and S respectively. **(D)** Relationships between decay rates determined in HeLa, HepG2 cells and mouse fibroblasts, assessed for significance using a Pearson correlation test. “ $p \approx 0$ ” indicates a p -value too low for accurate quantification (less than 1.9350×10^{-182}). **(E)** Similar to (A), but using decay rates from Hep G2 cells. **(F)** Similar to (A), but using decay rates from mouse fibroblasts.

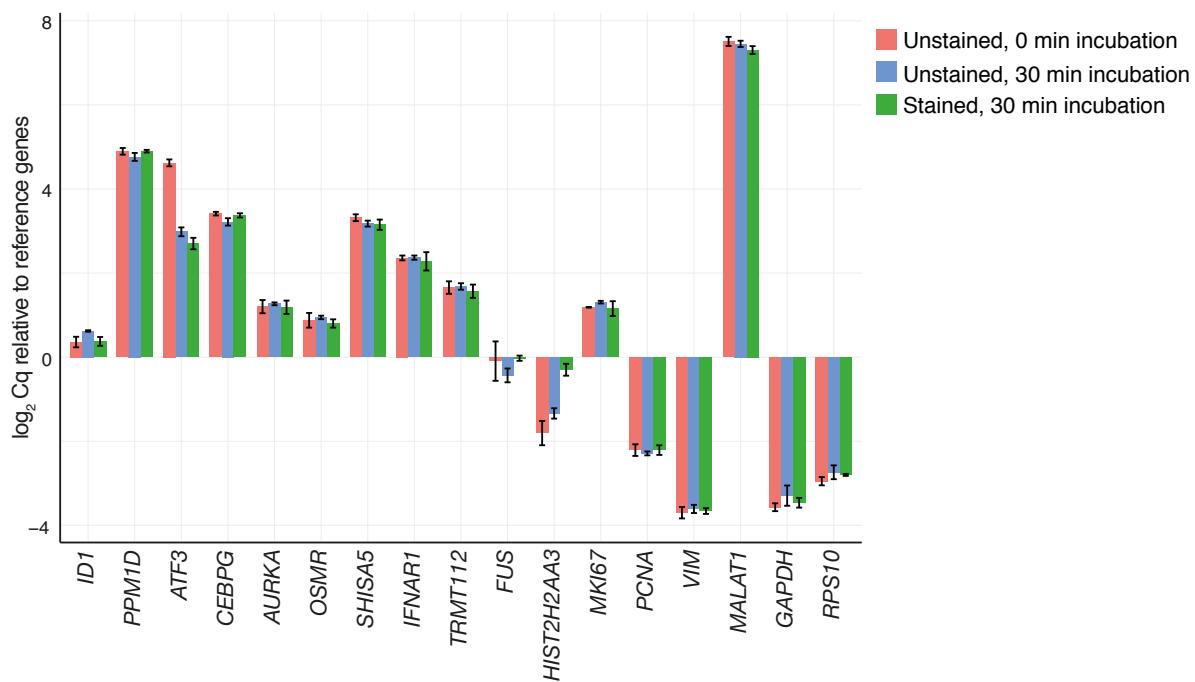


Figure S5. Influence of DNA staining and incubation on the expression of selected genes.

RT-qPCR analysis of gene expression following the addition of DNA stain and 30 min incubation, relative to controls without added stain, with or without incubation. Genes were selected to represent different mRNA half-lives (ID1 to TRMT112, ordered based on half-lives; ~50 min, ~60 min, ~90 min, ~130 min, ~170 min, ~490 min, ~830 min, ~1390 min, ~1830 min, ~15100 min) and biological processes. Cq-values were normalized to the reference genes *CCNG1*, *RPL7* and *CDT1* and log₂-transformed.

Table S8. Primer sequences for RT-qPCR analysis of gene expression changes in relation to DNA staining and incubation time.

Gene	Forward primer	Reverse primer
<i>ID1</i>	CTGAGGGAGAACAAAGACCGAT	CCCCCTAAAGTCTCTGGTGA
<i>PPM1D</i>	AAGGGTTCACCTCGTCCG	GCCATTCCGCCAGTTCTTC
<i>ATF3</i>	TCGGGGTGTCCATCACAAA	TTCTCGTCGCCTTTTCC
<i>CEBPG</i>	TGTGTGTGACCTGTGAACCA	CAAACACCAGCCAAGAGCAC
<i>AURKA</i>	CGGCACCTGAAAATAATCCTGA	TTCCCTTACCCAGAGGGCGA
<i>OSMR</i>	TGTCATCTGGTGGGAAT	CTCAGGGAACTTGGCATCGT
<i>SHISA5</i>	GTGGTGAGGTGTGTATGGCTT	AGGTGCGCTCCGAACCTGA
<i>IFNAR1</i>	GTGAGAAAACAAAACCAGGAAATAC	TGACAAACGGGAGAGCAAAT
<i>TRMT112</i>	GCCGATAACTTGCCTCTGAT	GGGTGCCCTCTATCACTTCC
<i>FUS</i>	TGGAAGTGTCTTAATCCCACC	TCATAGCCTCCTCTGCCACC
<i>HIST2H2AA3</i>	GGCGTCTTGCCTAACATCCA	TCAGCCTCACTTGCCCTTTG
<i>MK167</i>	TGGGTCTGTTATTGATGAGCC	CATCAGGGTCAGAAGAGAAGC
<i>PCNA</i>	GTGGAGAACTTGGAAATGGAA	ACCGTTGAAGAGAGTGGAGTG
<i>VIM</i>	CAGATGCGTGAAATGGAAGA	TGGAAGAGGCAGAGAAATCC
<i>MALAT1</i>	CGACCGAGTTGTGCTGCTATC	TCCTCCAAACCCAAGACCA
<i>GAPDH</i>	CCCACTCCTCACCTTGAC	GCCAAATTGTTGTCTACCAAGG
<i>RPS10</i>	AGCCGCAGAGATGTTGATG	CCTCGGGACTTGAGAGACTG
<i>CCNG1</i>	CCTTGGGTGTGTTGGACTGA	GCTATTCTCCTTCAAGTGGCA
<i>RPL7</i>	GGAGGGTGTAGAAGAGAAGAAGA	TCCTTGTGATAGTGCCTTGCT
<i>CDT1</i>	GAETCGTGCTGCCCTACAA	ACTCCTCAAAACGCCTACG