

# Regulatory dynamics of cell differentiation revealed by true time series from multinucleate single cells

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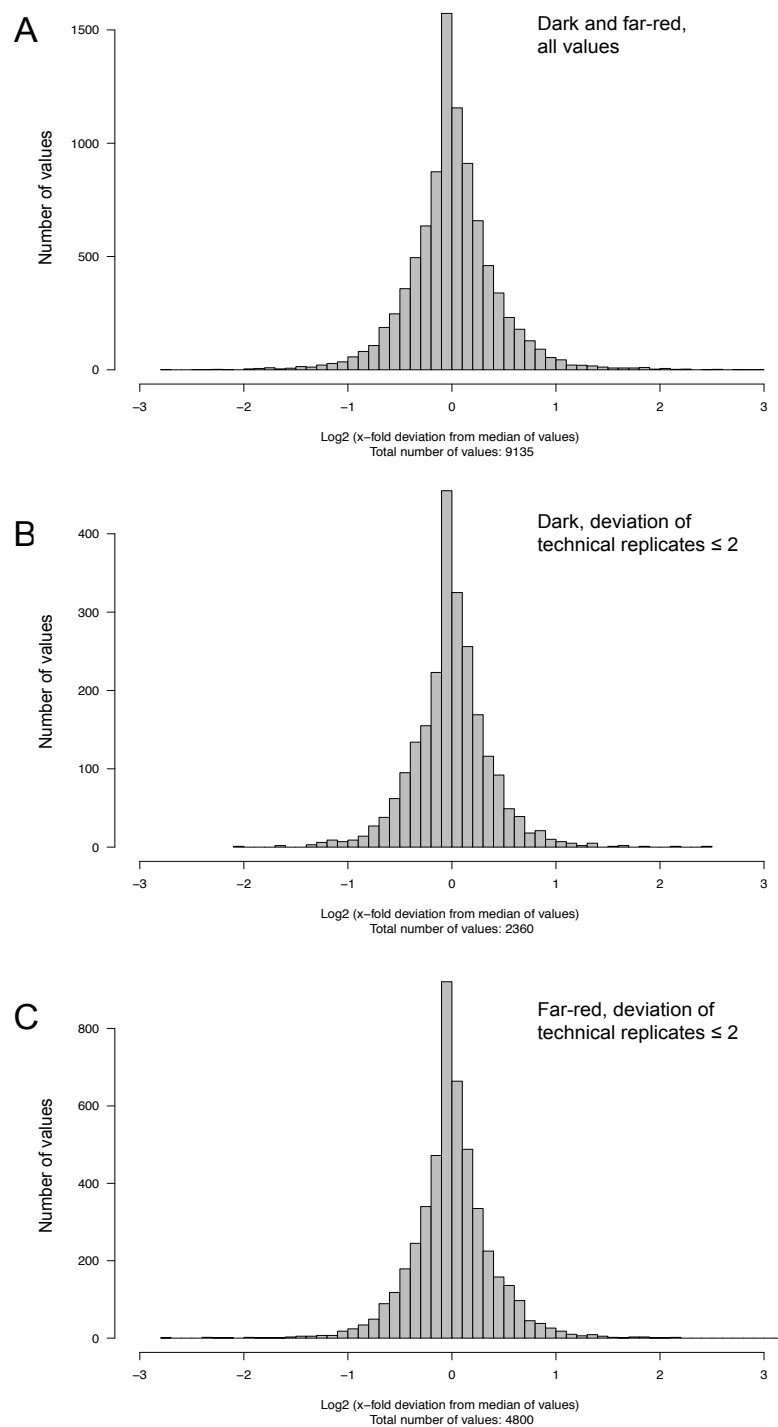
## Supplementary Tables and Figures

**SI Table 1.** Quantil distributions of the x-fold deviation (x) of a value from the median of values measured in dark controls or far-red stimulated cells. The table quantitatively characterizes the frequency distributions shown in SI Fig. 1B,C.

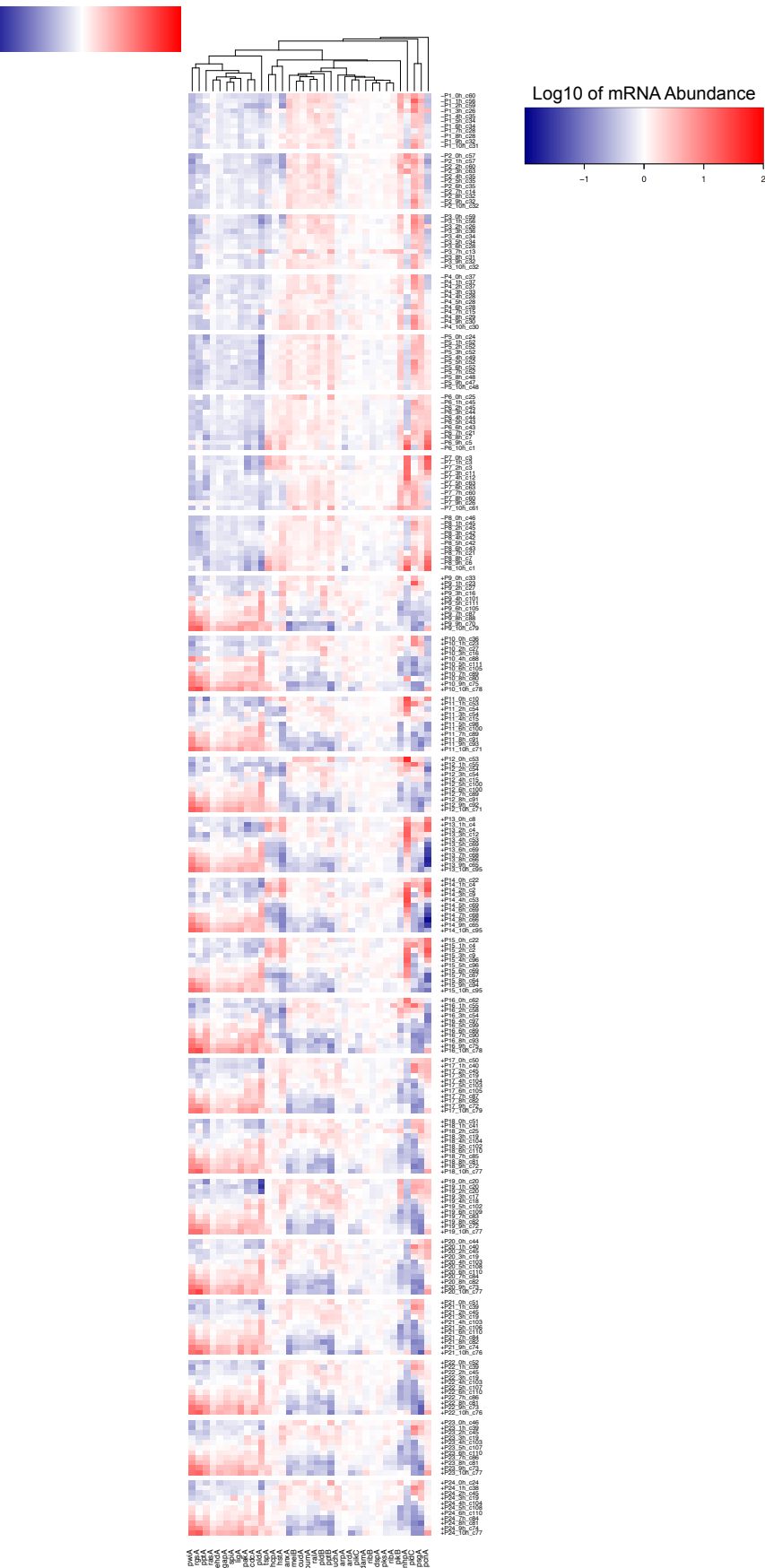
| Percent of values | Dark               |              | Far-red            |              |
|-------------------|--------------------|--------------|--------------------|--------------|
|                   | Quantile (Log2(x)) | Quantile (x) | Quantile (Log2(x)) | Quantile (x) |
| 1%                | -1.055             | 0.481        | -1.040             | 0.486        |
| 5%                | -0.593             | 0.663        | -0.609             | 0.655        |
| 25%               | -0.186             | 0.879        | -0.185             | 0.880        |
| 50%               | 0.000              | 1.000        | 0.000              | 1.000        |
| 75%               | 0.181              | 1.134        | 0.180              | 1.133        |
| 95%               | 0.584              | 1.499        | 0.622              | 1.539        |
| 99%               | 1.017              | 2.024        | 1.073              | 2.103        |

**SI Table 2.** Genes analysed.

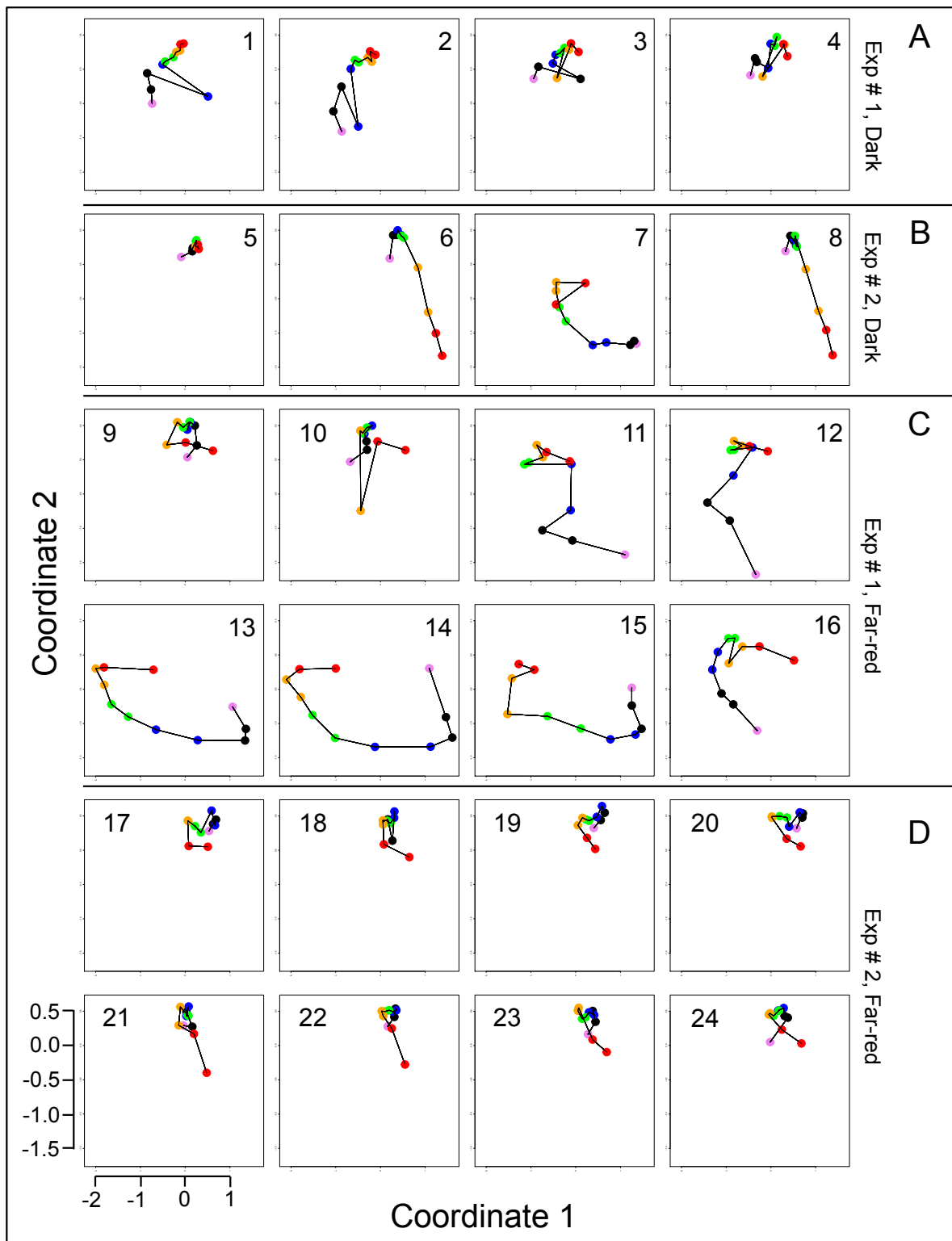
| Transcript ID            | Gene        | Similarity                                           | Organism                           | UniProtKB  | % Query Coverage | E-value   |
|--------------------------|-------------|------------------------------------------------------|------------------------------------|------------|------------------|-----------|
| Phypoly_transcript_07700 | <i>anxA</i> | Annexin VII                                          | <i>Mus musculus</i>                | Q07076     | 39.157           | 1.04E-32  |
| Phypoly_transcript_06541 | <i>ardA</i> | Actin, plasmodial isoform                            | <i>Physarum polycephalum</i>       | P02576     | 100              | 0         |
| Phypoly_transcript_11686 | <i>arpA</i> | Probable basic-leucine zipper transcription factor G | <i>Dictyostelium discoideum</i>    | Q54RZ9     | 44.068           | 1.15E-22  |
| Phypoly_transcript_05813 | <i>cdcA</i> | Caltractin isoform 1                                 | <i>Homo sapiens</i>                | P41208     | 59.756           | 1.26E-32  |
| Phypoly_transcript_06284 | <i>cudA</i> | Putative transcriptional regulator cudA              | <i>Dictyostelium discoideum</i>    | O00841     | 43.478           | 3.96E-28  |
| Phypoly_transcript_01287 | <i>dama</i> | DNA damage-binding protein 1                         | <i>Oryza sativa Japonica Group</i> | Q6L4S0     | 61.892           | 0         |
| Phypoly_transcript_03245 | <i>dspA</i> | DNase TatD                                           | <i>Pantoea vagans</i>              | E1SKR8     | 28.986           | 9.63E-31  |
| Phypoly_transcript_06735 | <i>ehdA</i> | EH domain-containing protein 1                       | <i>Bos taurus</i>                  | Q5E9R3     | 41.315           | 9.57E-130 |
| Phypoly_transcript_16748 | <i>gapA</i> | ADP-ribosylation factor GTPase-activating protein    | <i>Arabidopsis thaliana</i>        | O82171     | 35.22            | 2.61E-24  |
| Phypoly_transcript_17984 | <i>hcpA</i> | Anti-silencing function protein 1 homolog A          | <i>Bos taurus</i>                  | Q2KIG1     | 52.88            | 8.27E-64  |
| Phypoly_transcript_18509 | <i>hstA</i> | Probable histone H2B 4                               | <i>Caenorhabditis elegans</i>      | Q27876     | 59.559           | 2.41E-37  |
| Phypoly_transcript_16372 | <i>ligA</i> | Checkpoint protein hus1 homolog                      | <i>Dictyostelium discoideum</i>    | Q54NC0     | 41.241           | 7.24E-61  |
| Phypoly_transcript_00697 | <i>meiB</i> | Protein MEI2-like 5                                  | <i>Arabidopsis thaliana</i>        | Q8VWF5     | 73.545           | 6.34E-80  |
| Phypoly_transcript_18969 | <i>nhpA</i> | Non-histone chromosomal protein 6                    | <i>Debaryomyces hansenii</i>       | Q6BRB4     | 55.224           | 4.57E-15  |
| Phypoly_transcript_00387 | <i>pakA</i> | Serine/threonine-protein kinase <i>pakA</i>          | <i>Dictyostelium discoideum</i>    | Q55D99     | 41.085           | 1.96E-87  |
| Phypoly_transcript_13303 | <i>pcnA</i> | Proliferating cell nuclear antigen                   | <i>Brassica napus</i>              | Q43124     | 55.484           | 1.27E-81  |
| Phypoly_transcript_00857 | <i>pikB</i> | Phosphatidylinositol 3-kinase 2                      | <i>Dictyostelium discoideum</i>    | P54674     | 68.809           | 0         |
| Phypoly_transcript_01882 | <i>pikC</i> | Phosphatidylinositol 4-kinase beta                   | <i>Sorex araneus</i>               | B3EX61     | 45.667           | 6.23E-76  |
| Phypoly_transcript_02833 | <i>pkSA</i> | Serine/threonine-protein kinase phg2                 | <i>Dictyostelium discoideum</i>    | Q54QQ1     | 50.68            | 1.34E-93  |
| Phypoly_transcript_02552 | <i>pldA</i> | Phosphatidylinositol-glycan-specific phospholipase D | <i>Rattus norvegicus</i>           | Q8R2H5     | 32.237           | 2.26E-71  |
| Phypoly_transcript_04506 | <i>pldB</i> | Phosphatidylinositol-glycan-specific phospholipase D | <i>Homo sapiens</i>                | P80108     | 28.774           | 4.91E-49  |
| Phypoly_transcript_02197 | <i>pldC</i> | Phosphatidylcholine-hydrolyzing phospholipase D1     | <i>Schizosaccharomyces pombe</i>   | Q09706     | 31.068           | 7.83E-55  |
| Phypoly_transcript_23026 | <i>pptA</i> | Probable inactive purple acid phosphatase 29         | <i>Arabidopsis thaliana</i>        | Q9FMK9     | 46.269           | 1.10E-09  |
| Phypoly_transcript_08298 | <i>pptB</i> | Probable protein phosphatase 2C 34                   | <i>Oryza sativa Japonica Group</i> | Q94H98     | 30.208           | 1.11E-28  |
| Phypoly_transcript_16094 | <i>psgA</i> | <i>Physarum</i> -specific gene A                     | <i>Physarum polycephalum</i>       |            |                  |           |
| Phypoly_transcript_00670 | <i>pumA</i> | Pumilio homolog 1                                    | <i>Arabidopsis thaliana</i>        | Q9ZW07     | 61.281           | 1.91E-138 |
| Phypoly_transcript_11692 | <i>piwA</i> | Piwi-like protein Ago3                               | <i>Bombyx mori</i>                 | A9ZS22     | 30.672           | 8.11E-37  |
| Phypoly_transcript_17606 | <i>ralA</i> | Circularly permuted Ras protein 1                    | <i>Dictyostelium discoideum</i>    | Q75J93     | 39.161           | 7.24E-21  |
| Phypoly_transcript_09675 | <i>rasA</i> | Ras-like GTP-binding protein YPT1                    | <i>Phytophthora infestans</i>      | Q01890     | 43.931           | 3.66E-43  |
| Phypoly_transcript_12613 | <i>rgsA</i> | Regulator of G-protein signaling 2                   | <i>Mus musculus</i>                | O08849     | 41.176           | 0.048     |
| Phypoly_transcript_02603 | <i>ribA</i> | Poly(ADP-ribose) glycohydrolase                      | <i>Drosophila melanogaster</i>     | Q46043     | 42.67            | 4.66E-95  |
| Phypoly_transcript_01309 | <i>ribB</i> | Poly(ADP-ribose) glycohydrolase                      | <i>Oryza sativa Japonica Group</i> | Q9AV81     | 50.313           | 6.02E-146 |
| Phypoly_transcript_06597 | <i>spiA</i> | Polyribonucleotide nucleotidyltransferase            | <i>Staphylococcus haemolyticus</i> | Q4L5X7     | 23.932           | 0.13      |
| Phypoly_transcript_02655 | <i>tspA</i> | Tumor suppressor p53-binding protein 1 homolog       | <i>Caenorhabditis elegans</i>      | Q7JKP6     | 33.803           | 1.16E-05  |
| Phypoly_transcript_03260 | <i>uchA</i> | Secretory immunoglobulin A-binding protein EsIB      | <i>Escherichia coli</i>            | A0A0H2VDN9 | 33.562           | 9.47E-14  |



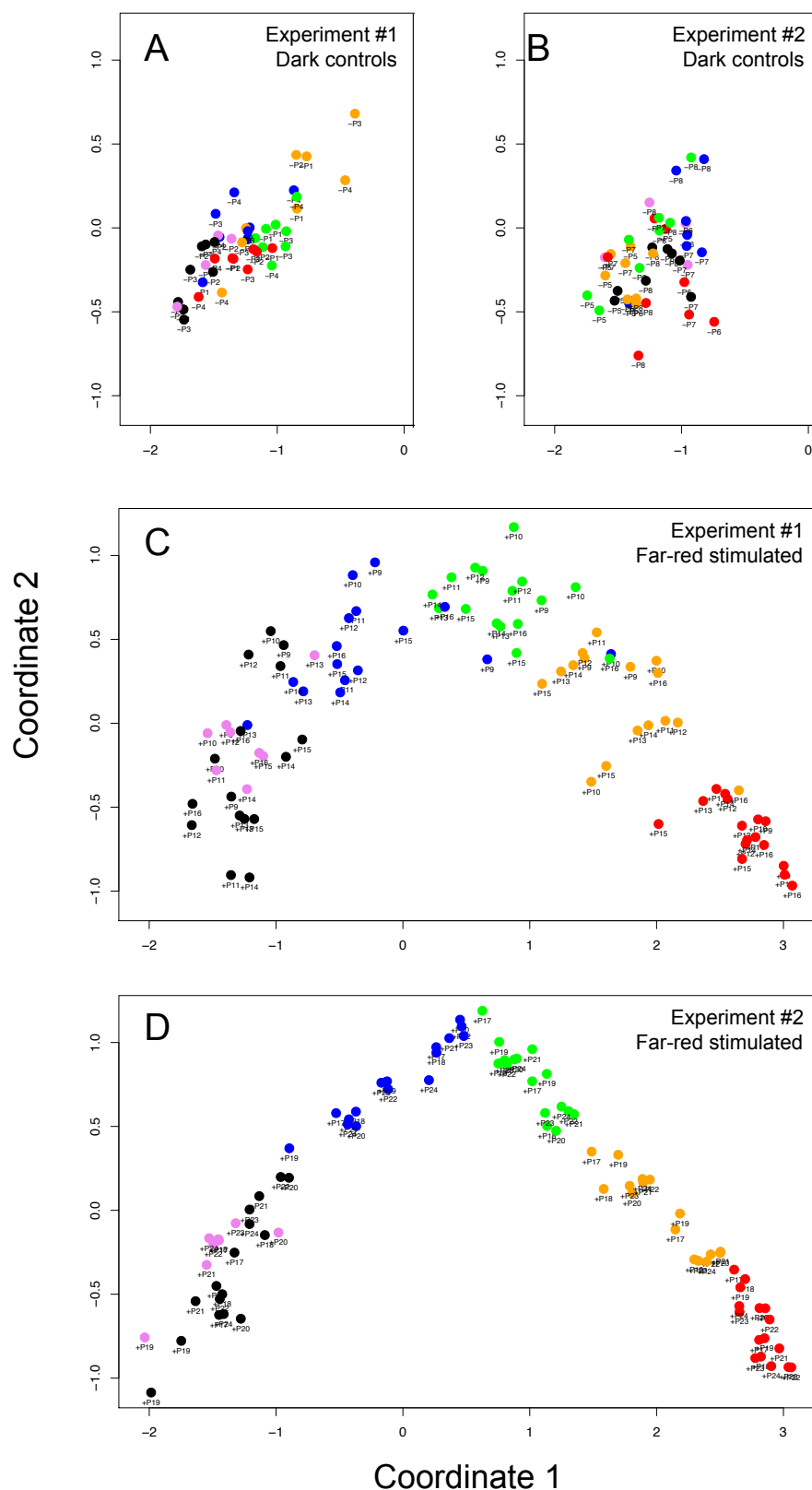
**SI Figure 1.** Homogeneity in gene expression, assayed by multiple sampling of individual plasmodial cells. In total, 43 plasmodia (15 dark controls and 28 far-red stimulated plasmodia, analysed at 6h after the stimulus pulse) were evaluated. Nine (3 x 3) or sixteen (4 x 4) samples were simultaneously taken from the same plasmodium and the concentration of the mRNAs of the set of 35 genes (SI Table 2) was determined twice by RT-PCR for each RNA sample. The frequency distributions display the Log2 of the x-fold deviation of each expression value from the median of all values for each gene. Panel (A) displays all values, measured in far-red stimulated cells and dark controls. Panels (B) and (C) display only those measurements where the two values of a RNA sample obtained by technical replication of the RT-PCR differed at maximum by a factor of two. Dark controls (B) and far-red stimulated cells (C) were evaluated separately



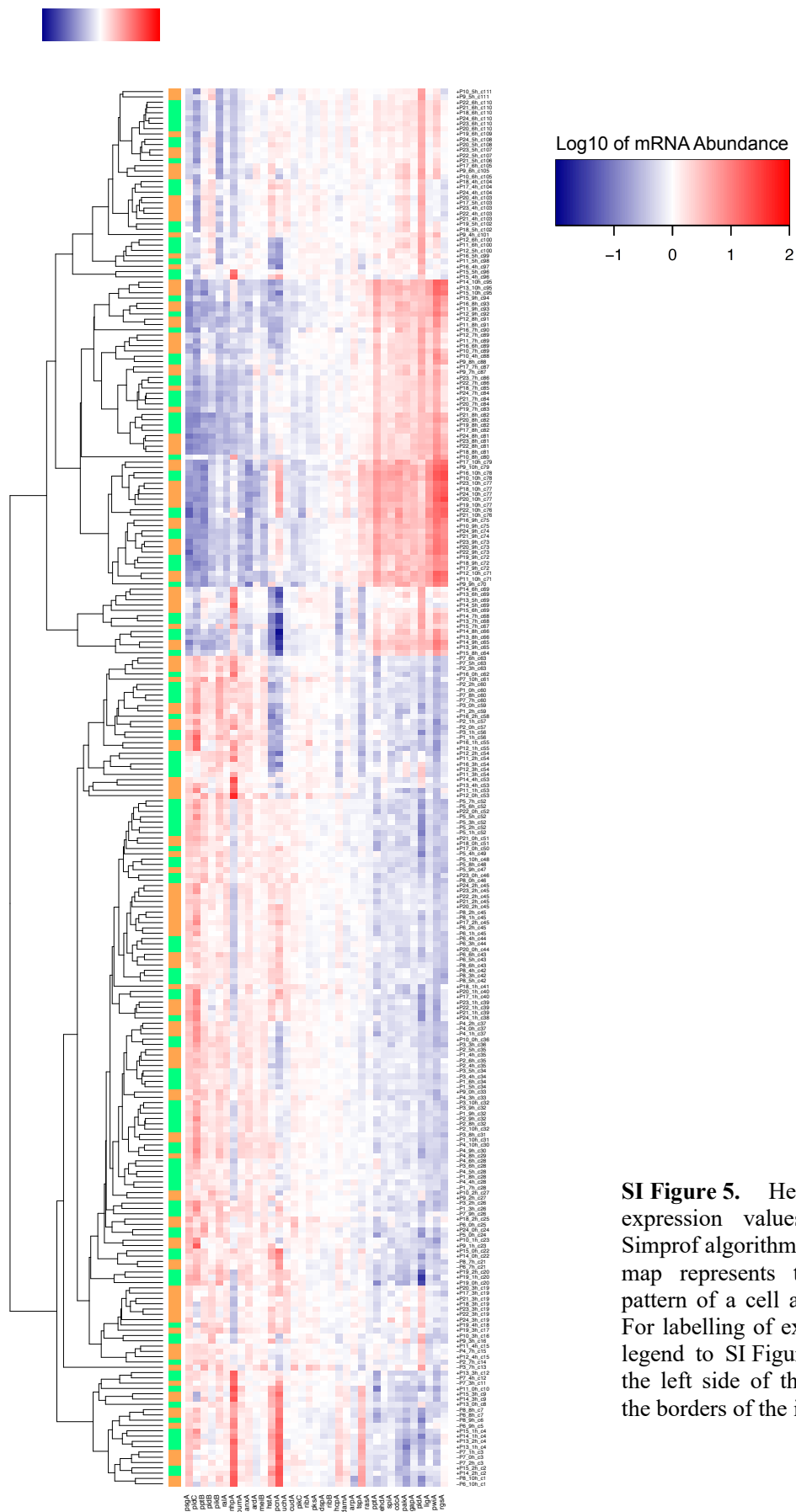
**SI Figure 2.** Heat map of time series of gene expression measured in individual plasmodial cells. Plasmodia are numbered according to Table 2. Each line of the heat map represents the gene expression pattern of a cell at the given time point. The label +P12\_1h\_c55, for example, indicates that the expression pattern of plasmodium number 12 as measured at 1h after the start of the experiment (corresponding to the onset of the far-red light stimulus in light-stimulated cells) was assigned to Simprof cluster number 55 and that the plasmodium had sporulated (+) in response to the stimulus (+, sporulated; -, not sporulated).



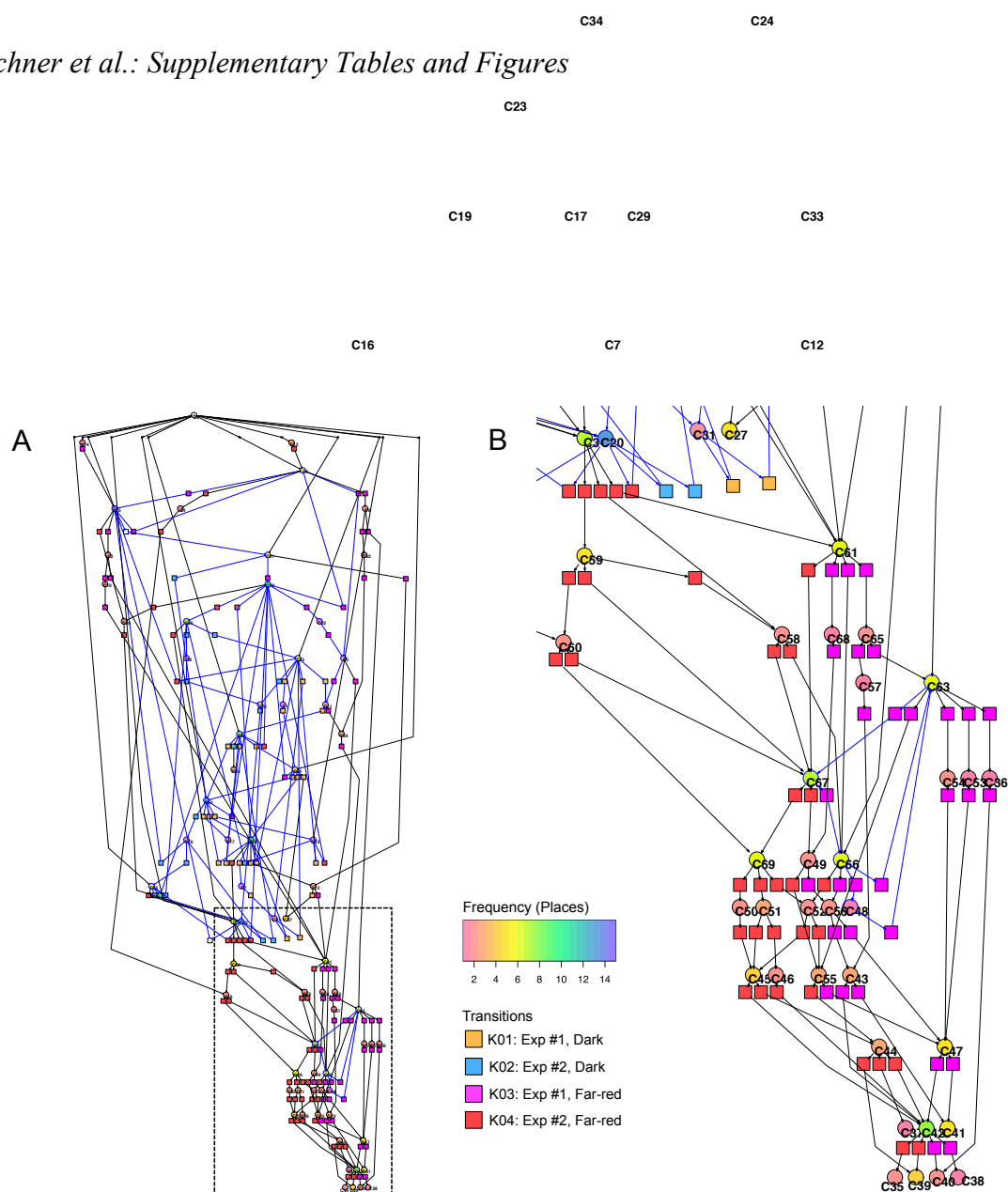
**SI Figure 3.** Single cell trajectories of gene expression displayed after multidimensional scaling (MDS) of the expression patterns of the *pcnA* group of genes (*hstA*, *nhpA*, *pcnA*, *uchA*). For further details see legend to Figure 3.



**SI Figure 4.** Multidimensional scaling of expression patterns of genes that were obviously up- (*cdcA*, *ehdA*, *gapA*, *ligA*, *pakA*, *pldA*, *pptA*, *pwiA*, *rgsA*, *spiA*) or down-regulated (*anxA*, *cudA*, *meiB*, *pikB*, *pldB*, *pldC*, *pptB*, *psgA*, *pumA*, *ralA*). Time is encoded by color (0h, pink; 1h, 2h, black; 3h, 4h, blue; 5h, 6h, green; 7h, 8h, ocher; 9h, 10h, red).

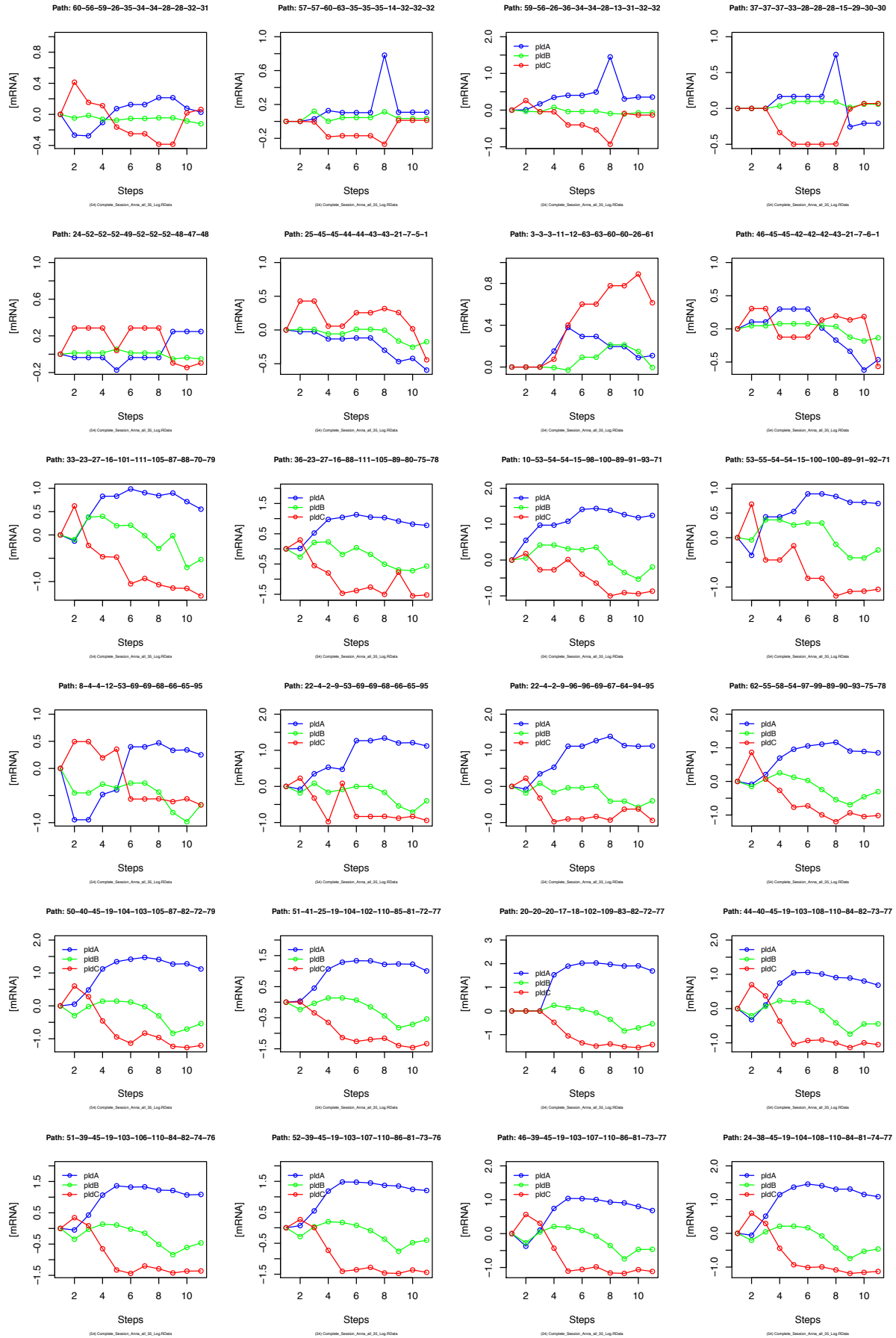


**SI Figure 5.** Heat map of gene expression values clustered by the Simprof algorithm. Each line of the heat map represents the gene expression pattern of a cell at a given time point. For labelling of expression patterns see legend to SI Figure 2. The side bar at the left side of the heat map indicates the borders of the individual clusters.



**SI Figure 6.** Petri net constructed for the set of up- or down-regulated genes, as listed in the legend to SI Figure 4. (A) Complete Petri net with a high number of T-invariants (indicated by arcs in blue) seen in the upper part of the net. (B) Magnified lower part of the Petri net in panel (A), marked by the rectangle of dashed lines. The part shown in (B) contains mainly transitions corresponding to far-red stimulated cells. Color coding of places indicates the relative frequencies of states of gene expression. Color coding of transitions indicates the group of cells (Table 2) in which the transits occurred.





**SI Figure 7.** Gene expression kinetics as derived from single cell trajectories. For details see legend to Figure 9.

