Supplementary Material

# Unsupervised automated high throughput phenotyping of RNAi time-lapse movies

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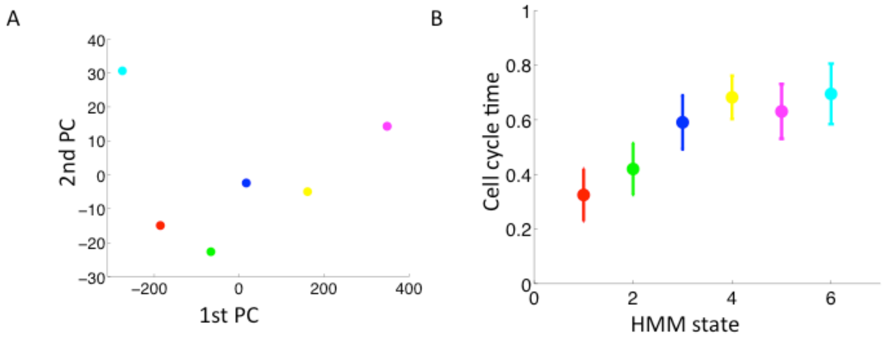
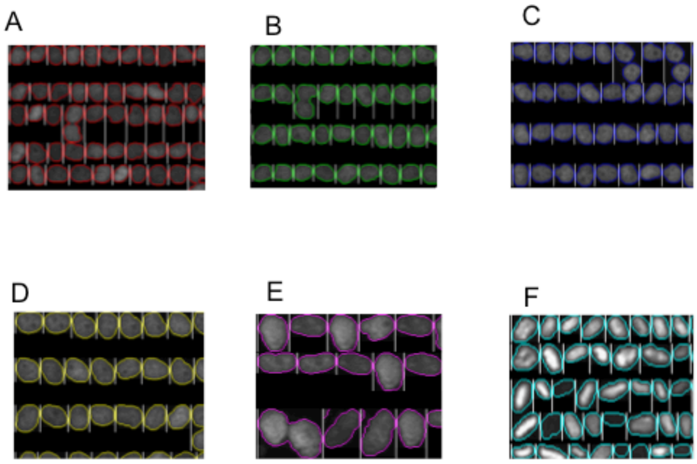
## Figure S1



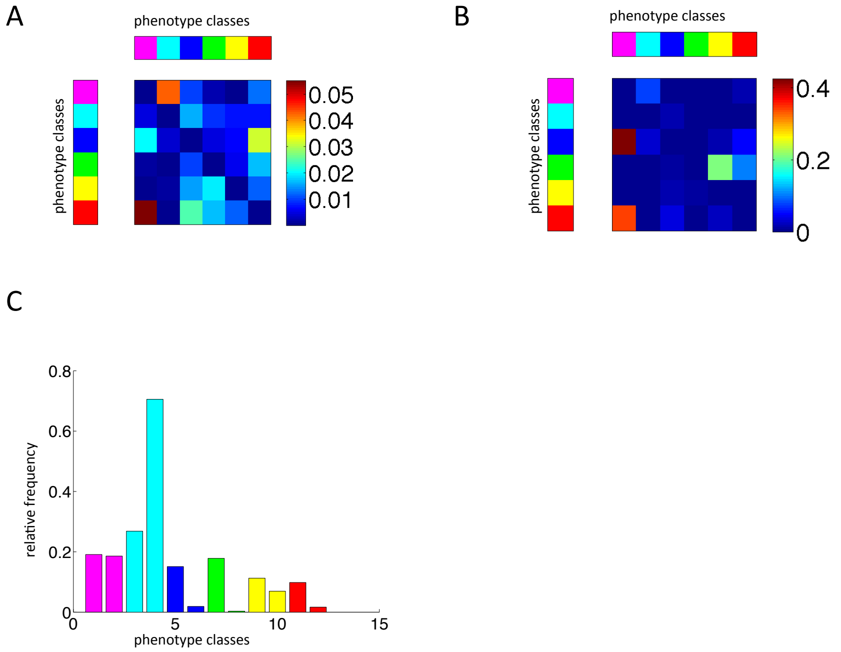
Principal Component Analysis (A) and cell cycle time distribution of phenotype classes (B). The first two principal components of the Gaussian mixture means of phenotype classes are showed in A. Mean cell cycle time and standard deviation from the mean are showed in B. The mitosis class (turquoise) is the morphologically most distant class and has the latest mean value in cell cycle time.

## Figure S2



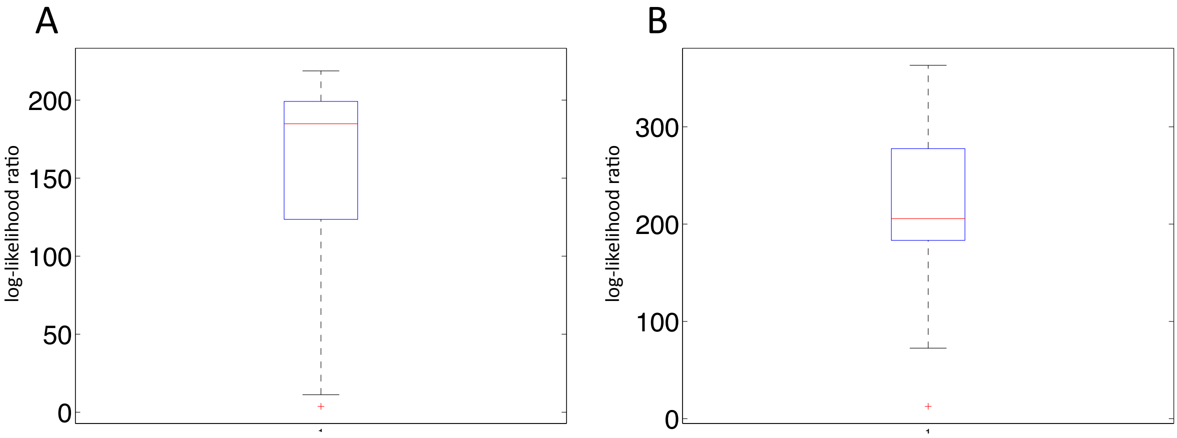
Morphologies from wild type phenotype classes that characterize different cell cycle stages.

## Figure S3



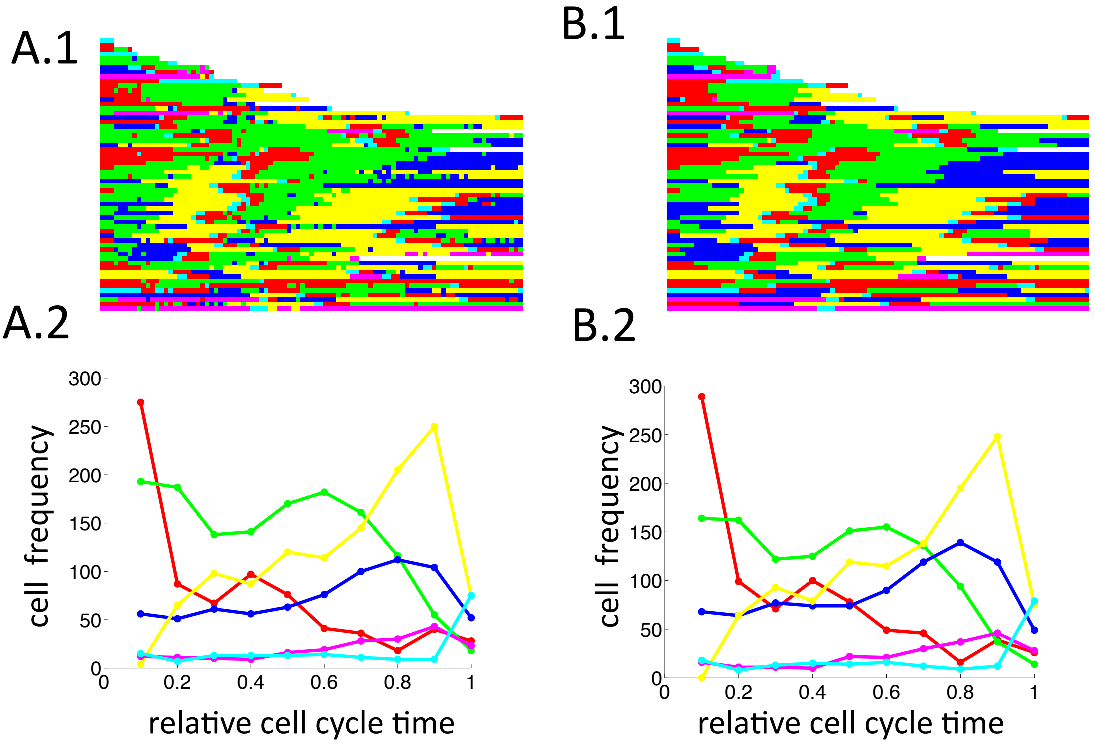
Visualisations of phenotype class transition probabilities and frequencies. Transition probability matrix of the PK1 knockdown (A) and the negative control (B). Relative phenotype class frequencies in PLK1 knockdown (left bars) and negative control (right bars).

## Figure S4



Log-likelihood rations of the HMM compared with a GMM. Covariance and means for the GMM were used from the HMM, mixture proportions were learned. Log-likelihood ratios were calculated for ten previously unseen trajectories from a PLK1 knockdown (A) and a wildtype movie (B).

Figure S5



Cluster annotations by a GMM (A.1) and Viterbi paths by an HMM (B.1). Cell cycle time histograms for GMM cluster annotations (A.2) and HMM Viterbi annotations (B.2).

## Table S1

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A2M | MN1 | CDKN2B | REN | BIN1 | PLK2 | FCAR | SMAD4 | M6PR |
| ABHD2 | MRPL15 | CDKN2D | RET | BRCA2 | PNISR | FCGBP | SMAD9 | MAF |
| ABR | MSH2 | CDS1 | RGS10 | BUB1B | PNN | FGD4 | SMARCB1 | MAFA |
| AC007040.10 | MSN | CENPF | RGS12 | C19orf56 | PPIG | FGD5 | SMS | MAPKAPK5 |
| ACAP2 | MTA2 | CHEK2 | RGS14 | C1orf177 | PPP2R3A | FGF6 | SP1 | MCM10 |
| ACAP3 | MTL5 | CHN1 | RHOA | C7orf25 | PRC1 | FH | SSH3 | MDM2 |
| ACTN4 | MTSS1 | CHN2 | RHOB | CADM1 | PRDM7 | FHIT | STARD13 | MDM4 |
| AGAP2 | MUTYH | CKS1B | RND1 | CAMKK2 | PRKCD | FKBP14 | STAT3 | MFHAS1 |
| AGBL2 | MYOD1 | CLTC | RNF111 | CAPG | PRKCDBP | FOXM1 | STK11 | MIS12 |
| AHRR | NAPSA | CMKLR1 | RP5 | CAPNS1 | PRKCI | FRG1 | STK4 | MKLN1 |
| APC | NBL1 | COMMD3 | RPN2 | CCNA1 | PRR5 | FRK | SUFU | MLH1 |
| APC2 | NCK1 | CREB1 | RPS6KA1 | CCNB1 | PSMC5 | GHITM | SUMO3 | MMP11 |
| APEH | NDE1 | CREBL2 | RPS6KA2 | CCND1 | PTCH1 | GHRHR | SYNC | |
| ARHGAP10 | NDRG2 | CREBZF | RPS6KA3 | CCND2 | PTEN | GSN | SYNPO2L | |
| ARHGAP20 | NGEF | CYLD | RPS6KA4 | CCND3 | PTPLA | HCK | TBRG1 | |
| ARHGAP26 | NGFR | DARS | RPS6KA5 | CCT7 | PXN | HES1 | TCHP | |
| ARHGAP35 | NIPA2 | DAXX | RRAS | CDC25A | PYCARD | HIPK3 | TEAD1 | |
| ARHGEF1 | NPAS3 | DCC | RTP3 | CDC25B | PYHIN1 | HIST1H2AB | TET2 | |
| ARHGEF11 | NPRL2 | DEC1 | RUNX2 | CDC25C | RAB11FIP2 | HMGB2 | TNIP2 | |
| ARHGEF12 | NRP1 | DEPDC7 | RXRA | CDC37 | RAB35 | HOXC11 | TP53 | |
| ARHGEF16 | O3FAR1 | DIAPH1 | SAP30 | CDC42 | RAP1A | HPGD | TP53INP1 | |
| ARHGEF19 | ORMDL1 | DIAPH3 | SASH1 | CDC73 | RAP1GAP | HSPB3 | TP73 | |
| ARMC1 | OS9 | DLEU1 | SCARA3 | CDK1 | RAPGEF1 | HTATIP2 | TPBG | |
| ARPC1B | PAF1 | DMBT1 | SCEL | CDK2 | RAPGEF6 | IFI27L2 | TRIM22 | |
| ARPC2 | PARK7 | DMP1 | SCN3B | CDK2AP1 | RASL10A | IGF1R | TRIO | |
| ATM | PBRM1 | DMTF1 | SCUBE1 | CDK4 | RASSF2 | IL22RA2 | TSC1 | |
| ATP1A1 | PCDHB12 | DNASE1L1 | SDF4 | CDK7 | RASSF4 | ING1 | TSC2 | |
| ATP1A4 | PDE5A | DYNC1I1 | SDHA | CDKL1 | RASSF5 | ING4 | TSPAN8 | |
| ATP6V0A2 | PHLPP2 | E2F6 | SEPT14 | CDKN1A | RB1CC1 | IRF1 | TTLL5 | |
| ATP6V1A | PIK3C2A | EGFR | SETDB1 | CDKN1B | RBMX | ITGB2 | TUSC2 | |
| ATXN3 | PLEKHA5 | EPHB2 | SETX | CDKN1C | RBPJL | JAK1 | TXNIP | |
| AXIN1 | PLEKHF1 | ERBB2 | SIK1 | CDKN2A | RECK | JAK2 | UBTF | |
| BANP | PLEKHF2 | ERRFI1 | SIVA1 | KCNG1 | UHRF1 | JAK3 | UGT3A2 | |
| BAX | PLEKHG2 | EXT1 | SKP2 | KEAP1 | USF2 | KRT18 | WSB2 | |
| BCL10 | PLEKHO1 | EZH2 | SLC25A11 | KIAA0776 | USP29 | LAMC1 | WT1 | |
| BCR | PLK1 | FBN3 | SLC9A2 | KIF11 | USP33 | LATS1 | WWOX | |
| KLHDC2 | VOPP1 | LIMD1 | ZFAND1 | KIF20B | VHL | LBR | YY1 | |
| KLHL23 | VTA1 | LMNB2 | ZNF449 | KLK10 | WEE1 | | ZFAND1 | |

Gene names of processed genes.

## Table S2

| | All | KNSP | KNSP>=3 replicates | KNSP>=2 siRNA |
|---|---|---|---|---|
| #Genes | 315 | 140 | 83 | 67 |
| #Movies | 1656 | 854 | 471 | 422 |

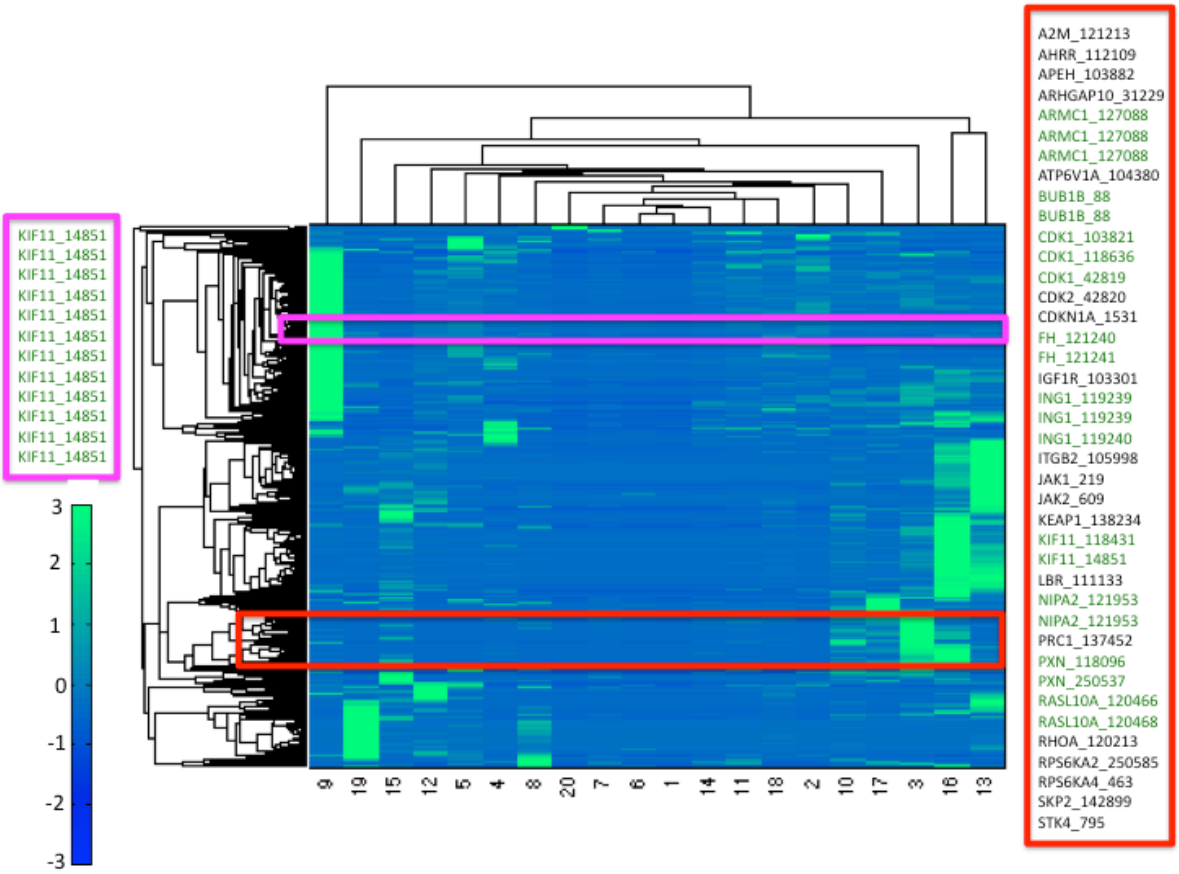
Number of processed genes and replicates. Genes and movies with knockdown-specific phenotype (2nd column). Genes and movies with knockdown-specific phenotypes in at least 3 replicates (3rd column), Genes and movies with at least 2 independent siRNAs below the replicates (4th column).

Table S3

| **Gene** | **Replicate Score** |
|---|---|
| AGAP1 | 0.592374865656471 |
| APC | 0.783755299615026 |
| ARHGAP10 | 1.2005 |
| ARHGAP35 | 0.624253962624991 |
| ATP6V1A | 0.829597033557676 |
| CDK1 | 0.598632039443441 |
| CDK2 | 0.584959722095466 |
| ITGB2 | 0.978303222428244 |
| LBR | 0.615701751072882 |
| M6PR | 0.558892340764823 |
| MDM4 | 0.357997461468261 |
| NGFR | 0.953782131193729 |
| NIPA2 | 0.318785113179696 |
| O3FAR1 | 1.26931573383935 |
| PLK1 | 0.362955612059338 |
| PRC1 | 1.1248 |
| PTPLA | 0.457869352532224 |
| PXN | 0.334467155896423 |
| RASL10A | 0.742949956391633 |
| TET2 | 0.437393195565148 |

Score for the affinity of siRNA replicates to fall in nearer clusters. Scores smaller zero indicate that replicates targeted by the same siRNA tend to have smaller distances. The scores were calculated for genes with at least two replicates for every siRNA only.

Figure S6



Phenotypic fingerprints of replicates. Knockdown- specific phenotypes were clustered to 20 components by a Gaussian Mixture model. The entries in the rows indicate the affiliations to the Gaussian Mixture components. Rows are grouped by hierarchical average linkage clustering, the entries in the heat map are z-score normalized. Genes that fell in a common group with at least two replicates are shown in green.

Table S4

| Gen | Mitocheck phenotype | Gen | Mitochek phenotype |
|---|---|---|---|
| A2M | | MAV | |
| AGAP1 | | MDM4 | |
| AHRR | | NGFR | |
| APC | | NIPA2 | |
| APEH | | O3FAR1 | |
| ARHGAP10 | | PLK1 | Metaphase alignment, cell death, grape, mitotic delay |
| ARHGAP35 | | PRC1 | Nuclear shape, segregation problems, binculear, mitotic delay |
| ARHGEF1 | | PRKCDBP | |
| ARHGEF12 | | PTPLA | Migration |
| ARMC1 | Nuclear shape, segregation, polylobed, large, grape | PXN | |
| ARPC2 | Binuclear, metaphase delay, metaphase alignment problems | PYCARD | |
| ATM | | RAB35 | |
| ATP1A4 | | RAC1 | Cell death |
| ATP6V1A | | RAPGEF1 | Dynamic changes |
| BAX | | RASL10A | |
| BRCA1 | | RET | Polylobed, dynamic changes |
| BUB1B | Nuclear shape, segregation, polylobed | RHOA | Migration |
| CDC25A | | RPS6KA2 | Binculear, nuclear shape, segregation problems |
| CDC42 | Dynamic changes | RPS6KA4 | |
| CDK1 | Nuclear shape, segregation metaphase delay, binuclear | PRAS | |
| CDK2 | | RXRA | |
| CDKN1A | | SEPT14 | |
| CDKN2B | | SKP2 | |
| CMKLR1 | | SLC9A2 | |
| CREB1 | | SMS | |
| DAB2IP | Dynamic changes | STK11 | |
| FH | | STK4 | |
| HCK | Cell death | TET2 | |
| HIPK3 | | | |
| IGF1R | | | |
| ING1 | | | |
| ING5 | | | |
| ITGB2 | Dynamic changes, | | |
| JAK1 | Cell death | | |
| JAK2 | | | |
| JAK3 | Dynamic changes | | |
| KEAP1 | Polylobed, nuclear shape, segregation problems | | |
| LBR | | | |
| M6PR | Nuclear shape, segregation problems, polylobed | | |

Genes with a knockdown-specific phenotype in our analysis and their corresponding Mitocheck phenotype.