

1 **Title:**

2 Spatiotemporal dissection of the cell cycle regulated human proteome

3

4 **Authors:**

5 Diana Mahdessian<sup>1</sup>, Devin Sullivan<sup>1</sup>, Frida Danielsson<sup>1</sup>, Muhammad Arif<sup>1</sup>, Cheng  
6 Zhang<sup>1</sup>, Lovisa Åkesson<sup>1</sup>, Christian Gnann<sup>1</sup>, Rutger Shutten<sup>1</sup>, Peter Thul<sup>1</sup>, Oana  
7 Carja<sup>3,4</sup>, Burcu Ayoglu<sup>1</sup>, Adil Mardinoglu<sup>1,2</sup>, Fredrik Pontén<sup>5</sup>, Mathias Uhlén<sup>1</sup>, Cecilia  
8 Lindskog<sup>5</sup>, Emma Lundberg<sup>1,3,4\*†</sup>

9

10 <sup>1</sup> Science for Life Laboratory, School of Engineering Sciences in Chemistry,  
11 Biotechnology and Health, KTH - Royal Institute of Technology, Stockholm, 17121,  
12 Sweden.

13 <sup>2</sup> Centre for Host–Microbiome Interactions, Faculty of Dentistry, Oral & Craniofacial  
14 Sciences, King's College London, London, SE1 9RT, United Kingdom

15 <sup>3</sup> Department of Genetics, Stanford University, Stanford, CA 94305, USA. ‡

16 <sup>4</sup> Chan Zuckerberg Biohub, San Francisco, San Francisco, CA 94158, USA.

17 <sup>5</sup> Department of Immunology, Genetics and Pathology, Science for Life Laboratory,  
18 Uppsala University, SE-751 85 Uppsala, Sweden.

19 \*Correspondence to: [emma.lundberg@scilifelab.se](mailto:emma.lundberg@scilifelab.se)

20 † Visiting appointment, current address.

21

22 **Abstract**

23

24 Here we present a spatiotemporal dissection of proteome single cell heterogeneity in  
25 human cells, performed with subcellular resolution over the course of a cell cycle. We  
26 identify 17% of the human proteome to display cell-to-cell variability, of which we could  
27 attribute 25% as correlated to cell cycle progression, and present the first evidence of  
28 cell cycle association for 258 proteins. A key finding is that the variance, of many of  
29 the cell cycle associated proteins, is only partially explained by the cell cycle, which  
30 hints at cross-talk between the cell cycle and other signaling pathways. We also  
31 demonstrate that several of the identified cell cycle regulated proteins may be clinically  
32 significant in proliferative disorders. This spatially resolved proteome map of the cell  
33 cycle, integrated into the Human Protein Atlas, serves as a valuable resource to  
34 accelerate the molecular knowledge of the cell cycle and opens up novel avenues for  
35 the understanding of cell proliferation.

36

## 37 **Introduction**

38 Cellular processes are, to a great extent, driven by the presence and activity of specific  
39 proteins. Essential processes, such as the cell division cycle, require precise  
40 coordination of the expression of hundreds of genes and the activity of their  
41 corresponding proteins in both time and space. The cell division cycle is tightly  
42 controlled at specific checkpoints<sup>1,2</sup> by regulated transcription<sup>3-7</sup>, intricate feed-  
43 forward and feedback loops of protein post-translational modifications, and protein  
44 degradation<sup>8-12</sup>. Its dysregulation has devastating consequences, such as  
45 uncontrolled cell proliferation, genomic instability<sup>13</sup>, and cancer<sup>14,15</sup>.

46  
47 Given the fundamental role of the cell cycle, its regulation with cyclins and cyclin  
48 dependent kinases (CDKs) has been extensively studied<sup>16</sup>. Recent efforts have  
49 focused on the investigation of genome-wide effects of cell cycle progression.  
50 Transcriptomics studies have revealed 400-1,200 human genes<sup>17-20</sup>, and mass  
51 spectrometry-based proteomics studies have revealed 300-700 human proteins that  
52 show variation in abundance over the cell cycle<sup>21-24</sup>. These studies have commonly  
53 been performed in bulk, with cells sorted into synchronized populations<sup>17,19,25-28</sup>. This  
54 is a disruptive procedure, shown to alter gene expression<sup>29</sup>, and perturb cellular  
55 morphology<sup>30-32</sup> as well as metabolism<sup>33</sup>. In addition, the achieved synchrony could  
56 be contaminated with cells from other phases<sup>33-36</sup>.

57  
58 Single-cell sequencing now allow the analysis of transcriptional changes without the  
59 need for synchronized cells. Recent single-cell transcriptomic studies presented the  
60 first efforts to update the decade old catalogues of periodic gene expression patterns  
61 that were based on bulk analysis<sup>37-39</sup>. For instance, in a study using human myxoid  
62 sarcoma cell line (MSL) cells, 472 genes with periodic expression were identified<sup>37</sup>, of  
63 which 269 had no prior association to the cell cycle, indicating the potential of single-  
64 cell level studies to deepen our knowledge of the cell cycle.

65  
66 Microscopy offers an attractive approach to study cell cycle dynamics in asynchronous  
67 cells at a single-cell level. The readout of such studies has so far been focused only  
68 on cellular growth phenotypes, as conferred by genetically encoded fluorescent  
69 indicators<sup>40-43</sup>. Due to technological limitations, studies of single cell variations at the  
70 proteome level have not yet been feasible. The few studies that exist<sup>44,45</sup> have been  
71 limited to a low number of proteins and none provides a complete view of temporal cell  
72 cycle dynamics of the human proteome with single cell resolution.

73 Here we report on a systematic characterization of temporal protein expression  
74 patterns with single-cell resolution in unsynchronized human cells, and present the first  
75 spatially resolved map of human proteome dynamics during the cell cycle. By  
76 leveraging the Human Protein Atlas (HPA) antibody resource <sup>46</sup> and the high-resolution  
77 image collection within its Cell Atlas <sup>47</sup>, we provide a catalogue of human proteins with  
78 temporal and spatial variation correlating to cell cycle progression. This spatially  
79 resolved proteome map of the cell cycle, integrated into the HPA database, is a  
80 complement to the existing human cell cycle gene expression resources. Altogether  
81 this study has important implications for mechanistic insights into cellular proliferation  
82 as well as the contribution of its miss-regulation to tumorigenesis and disease.

## 83 **Results**

84

### 85 **Single-cell variations of the human proteome**

86 The HPA Cell Atlas aims to localize all human proteins at a subcellular level using  
87 immunofluorescence and confocal microscopy (45). To date, 12,390 (v.19) proteins  
88 have been localized to 33 subcellular structures. This high-resolution image collection  
89 contains protein expression in a variety of human cell lines, always non-synchronized  
90 and in log-phase growth, and provides an unprecedented resource to explore protein  
91 expression variation at single-cell level. Out of these 12,390 proteins mapped in the  
92 HPA Cell Atlas, 2,195 (17%, **Supplementary Table 1**) showed cell-to-cell variations  
93 based on visual inspection, either in terms of variation in protein expression level or  
94 variation in spatial distribution. As exemplified in **Figure 1A**, CCNB1, an important cell  
95 cycle regulator<sup>48</sup> localized to the cytosol, shows variation in abundance, whereas  
96 MRT04, a protein with unknown function, shows spatial variation in its expression  
97 between the nucleus and nucleoli. Out of these 2,195 proteins, 69% showed similar  
98 cell-to-cell variations in more than one human cell line (**Supplementary Table 2**), as  
99 exemplified for RACGAP1 in three different cell lines (**Figure 1B**). This suggests that  
100 these proteome variations might be to a large extent controlled by preserved regulatory  
101 mechanisms. We investigate to what extent these observed protein variations  
102 represent temporally controlled expression patterns correlating to cell cycle  
103 progression.

104

### 105 **Proteins spatiotemporally restricted to mitotic cellular structures**

106 The cell cycle dependency of a protein can be inferred directly, if it localizes to a mitotic  
107 structure (*i.e.* kinetochores, mitotic spindle, midbody, midbody ring, cleavage furrow,  
108 or cytokinetic bridge). For example, the mitotic regulators INCENP<sup>49</sup> and SGO1<sup>50</sup>  
109 appear at the kinetochores during mitosis; KIF20A<sup>51</sup> localizes to the cleavage furrow;  
110 and TACC3<sup>52</sup> to the mitotic spindles (**Figure 1C**). Of the 2,195 proteins identified to  
111 show cell-to-cell variability, a total of 166 mapped to one or several of the mitotic  
112 structures (99 to cytokinetic bridge, 45 to mitotic spindle, 40 to midbody, 17 to midbody  
113 ring, 5 to kinetochores, and 3 to cleavage furrow). Among these proteins, 99 were not  
114 previously annotated to have an association with the cell cycle by a biological process  
115 (BP) term in Gene Ontology (GO)<sup>53</sup> or Reactome<sup>2</sup>, nor did they have any cell cycle  
116 phenotype registered in Cyclebase<sup>54</sup> (**Supplementary Table 3**). Among the proteins  
117 spatiotemporally restricted to mitotic substructures were *e.g.* BIRC5, a well  
118 characterized protein essential for chromosome alignment<sup>55</sup>, which localizes to the  
119 cytokinetic bridge as well as two other uncharacterized proteins, GLI4 and C12orf66

120 (**Figure 1D**). C12orf66 localizes to the lysosomes during interphase<sup>56</sup>. DVL3, a Wnt  
121 signaling component known to be involved in cell proliferation<sup>57</sup>, localized to the  
122 midbody ring, which is the final bridge between dividing cells (**Figure 1D**). It is plausible  
123 to hypothesize that the proteins which localized to the mitotic spindle are involved in  
124 the process of chromosome segregation; these include KIF11 and KNSTRN, both of  
125 which are well-studied components of the mitotic spindle<sup>58,59</sup>. We also identified novel  
126 proteins localizing to the mitotic spindle, such as MGAT5B, a glycosyltransferase for  
127 which downregulation has been shown to inhibit cell proliferation<sup>60</sup>; and FKBPL, a  
128 crucial protein for response to high dose radiation stress<sup>61</sup> (**Figure 1D**). Altogether,  
129 these 166 proteins serve as potentially interesting targets for development of novel  
130 antimitotic drugs for cancer therapy.

131

### 132 **Proteins with temporal expression variation correlated to cell cycle interphase** 133 **progression**

134 To determine if the observed cell-to-cell variations correlate to interphase progression,  
135 the FUCCI cell cycle marker system was used (**Figure 1E**)<sup>42,62</sup>. Of the 2,195 proteins  
136 identified to show cell-to-cell variability, 1,188 proteins that were expressed and  
137 exhibited variations in the U-2 OS cell line were selected for further analysis with the  
138 FUCCI system (**Supplementary Table 4**). The expression of each protein was  
139 quantified across the cell cycle by immunostaining in U-2 OS FUCCI cells. Gaussian  
140 mixture modelling was used to define three clusters representing G1, the S-transition  
141 (denoted G1/S) and the remaining S and G2 phases (denoted S/G2), and the  
142 subsequent assignment of cells to each cluster. A polar coordinate system was used  
143 to transfer the FUCCI marker information into a linear model of interphase pseudo-  
144 time (**Figure 1E**). Examples of this analysis are given in **Figure 1F**: ANLN, a well-  
145 characterized cell cycle regulator<sup>63</sup>, showed a significant (Kruskal Wallis  $p < 0.01$  &  
146  $FDR < 0.05$ ) increase in abundance during cell cycle progression in the nucleus. On the  
147 other hand, FAM71F, an uncharacterized protein localized to the cytosol, revealed  
148 variation that did not correlate to the cell cycle, meaning that both high and low  
149 expressing cells are present in all phases of the cell cycle. Expression of DUSP18, a  
150 member of the DUSP family<sup>64</sup> with no prior association to the cell cycle, was found to  
151 strongly correlate to cell cycle progression. In this analysis, staining of microtubules  
152 with alpha-tubulin in all samples served as a negative control, with no significant  
153 variation of expression during cell cycle progression.

154

155 Based on this analysis, at an FDR of 5%, we identified 298 out of 1,188 proteins (25%)  
156 to have variance in expression levels temporally correlated to cell cycle progression,

157 and for which the cell-cycle explained more than 10% of the variance in expression.  
158 (**Supplementary Table 5 and Supplementary Figure S1**). This cutoff was set as  
159 being significantly above the negative control. It is noteworthy that the majority of the  
160 proteins analyzed (75%) showed cell-to-cell variations that were largely unexplained  
161 by cell cycle progression. Enrichment analysis of GO BP terms was performed for the  
162 genes encoding cell cycle dependent and independent proteins. The set of genes  
163 identified as cell cycle regulated was highly enriched for functions related to  
164 chromosome organization and segregation, regulation of cell cycle processes,  
165 cytoskeleton organization, cell division and cytokinesis (**Figure 2A**). Interestingly, the  
166 set of genes, with variations not correlating to the cell cycle, was not enriched for any  
167 GO BP terms at all. This shows that the identified proteins are indeed involved in cell  
168 cycle processes whereas the proteins not correlated to cell cycle are likely involved in  
169 a variety of different biological processes.

170

#### 171 **Population distribution and fraction of variance explained by the cell cycle**

172 To investigate the pattern of variability for these 1,188 proteins, k-means clustering  
173 was performed using the kurtosis and skewness features of the distribution of the  
174 mean intensity per cell for each protein. The mean fold-change between high and low  
175 expressing cells per protein were 7.97. Three clusters were found to represent distinct  
176 variation patterns (**Figure 2B**): Cluster 1, the largest cluster (n=1,018), contained most  
177 cell cycle dependent and independent proteins, 92% and 83%, respectively. The lower  
178 segment of Cluster 1 contained some proteins with a bimodal distribution (**Figure 2B**,  
179 exemplified by GATA6), but the majority of the proteins in this cluster had a unimodal  
180 normal distribution (**Figure 2B**, exemplified by CCNB1). Cluster 2, the second largest  
181 cluster (n=153), contained proteins with slightly skewed distribution profiles with a  
182 sharp peak distribution, as exemplified by DEF6. Cluster 3 (n=17) mostly contained  
183 proteins not correlated to the cell cycle, where the variation was highly skewed and  
184 tailed with few cells expressing the protein. These results show that cell cycle  
185 dependent variations are mostly unimodal with a normal distribution across a log-  
186 phase growing population of cells.

187

188 In addition to identifying the proteins that are regulated by the cell cycle, the single-cell  
189 resolution of our dataset allowed us to also calculate the fraction of variance that is  
190 determined by the cell cycle. To our knowledge, such analysis has been done neither  
191 at transcriptome, nor at proteome level previously. Here, the Gini index<sup>65</sup> was  
192 calculated and used as a metric for the variance of these 1,188 proteins (**Figure 2C**).  
193 All the proteins analyzed had a Gini index significantly higher than the negative control

194 (alpha tubulin) used, which serve as yet another check that we are indeed analyzing  
195 proteins with heterogeneous expression. The percentage of variance explained by the  
196 cell cycle ranged between 10%-91% (the FUCCI markers themselves were controlled  
197 at green: 80% and red: 65%) and two distinct populations were identified (**Figure 2C**):  
198 one where the variance was determined by the cell cycle (CCD), and one where the  
199 variance was independent of the cell cycle (Non-CCD). Interestingly, the majority of  
200 the observed cell cycle regulated variations appeared to be controlled by the cell cycle  
201 at a low degree (on average 21%). We hypothesize that these cell cycle regulated  
202 proteins, where the percentage of variance explained by the cell cycle is low, are  
203 important for the cross-talk between the cell cycle and other signaling processes.

204

### 205 **Organelle specific differences in temporal cell cycle protein variations**

206 The high subcellular resolution of our analysis allows us to study the role of subcellular  
207 localization in cell cycle regulation. We found significant differences in the localization  
208 of proteins that show cell cycle dependent or independent expression (**Figure 2D**).  
209 Proteins with variations independent of the cell cycle were significantly enriched for  
210 localization to the intermediate filaments, nucleoli, nuclear bodies, and mitochondria  
211 (binomial one sided test,  $p < 0.01$ , mapped proteome as background), whereas proteins  
212 with cell cycle dependent variation were significantly enriched for localization to  
213 nucleoli, nuclear bodies and mitotic structures, constituting 33% of the cell cycle  
214 dependent proteins (binomial one sided test,  $p < 0.01$ , mapped proteome as  
215 background). Half (50%) of the cell cycle dependent proteins resided in the nuclear  
216 compartment (2% nuclear speckles, 11% nuclear bodies, 24% nucleoli and 63%  
217 nucleus), not surprisingly given that one of the main functions of the nucleus is to  
218 perform and control the replication of DNA during the cell cycle.

219

220 In our analysis, we find many functionally uncharacterized proteins that share the same  
221 subcellular localization as some previously well characterized cell cycle dependent  
222 proteins (**Figure 2E**). It is plausible to assume that proteins expressed in the same  
223 organelle with similar temporal profiles may be involved in similar cell cycle processes.  
224 For example, two mitochondrial proteins with known association to cell proliferation -  
225 Pyruvate Carboxylase (PC), involved in gluconeogenesis and shown to be upregulated  
226 in several types of cancer<sup>66-68</sup>, and XAF1, whose inhibition is known to prevent cell  
227 cycle progression<sup>69</sup> were both shown to peak in the S/G2 phase (0.78 and 0.80 in  
228 pseudotime, respectively). We could also identify two proteins without a prior  
229 association to the cell cycle. PC and XAF1 shared the same subcellular location and  
230 temporal expression profile as TTC21B (0.8 pseudotime) and SLIRP (0.8 pseudotime),



231 both with no previously described association to the cell cycle or cell proliferation. In  
232 this manner, we could associate novel and known cell cycle associated proteins with  
233 similar temporal profiles in organelles such as the cytosol, nucleus, nucleoli and the  
234 Golgi apparatus (**Figure 2E**).

235

### 236 **Temporal protein expression patterns through interphase**

237 We next sorted the proteins based on the time of peak expression in order to study the  
238 temporal dynamics of the cell cycle dependent proteome (**Figure 3A**). Despite G1  
239 being the longest period of the cell cycle (G1 10.8h; G1/S 2.6h; S&G2 together 11.9h  
240 in U-2 OS FUCCI cells), the majority (85%) of the proteins peaked towards the end of  
241 the cell cycle corresponding to the S&G2 phases. This analysis enabled identification  
242 of proteins which share a highly similar temporal pattern to well-known cell cycle  
243 regulators, but with no prior association to the cell cycle. For instance, in the G1 group,  
244 well-known cell cycle dependent proteins such as ORC6 (**Figure 3B**), required for the  
245 cell entry into S phase <sup>70</sup>, and MCM10, required for DNA replication <sup>71</sup>, were identified  
246 to have similar patterns as those with no prior association to the cell cycle, such as  
247 ZNF32. Recently, overexpression of ZNF32 was associated with a shorter survival  
248 time in lung adenocarcinoma cells <sup>72,73</sup>. The group peaking in the end of G1 contained  
249 proteins such as JUN, required for progression through the G1 phase of cell cycle <sup>74</sup>;  
250 the G1/S specific cyclin CCNE1<sup>75</sup>; and DUSP19 (**Figure 3B**), a phosphatase whose  
251 depletion results in increased mitotic defects <sup>76</sup>. In the SG2 group, several known cell  
252 cycle dependent proteins were identified: CCNB1, a G2/M specific cyclin <sup>48</sup>, AURKB,  
253 a protein involved in the regulation of alignment and segregation of the chromosomes,  
254 and BUB1B (**Figure 3B**), a mitotic checkpoint kinase <sup>77</sup>. This group also contained  
255 proteins such as PAPSS1, an estrogen sulfating enzyme with no previously described  
256 association to the cell cycle, although its overexpression was reported to affect  
257 proliferation <sup>78</sup>. Other proteins in the SG2 group were N6AMT1, a methyltransferase  
258 <sup>79</sup>; PHLDB1, an uncharacterized protein; DPH2 (**Figure 3B**), required for the synthesis  
259 of diphthamide; and FLI1, a transcription factor associated to Ewing sarcoma <sup>80</sup>  
260 (**Figure 3B**).

261

262 Several of the proteins identified as cell cycle dependent, such as ORC6, RBL2,  
263 BUB1B, CCNA2 and HORMAD1 have been reported to be involved in cell cycle  
264 processes, yet their temporal expression profile across the interphase, which can  
265 provide insight into their functionality, has so far remained uncharacterized  
266 (**Supplementary Figure S2**). In addition, knowledge about the temporal expression

267 patterns and the timing of peak expression relative to other proteins is valuable for a  
268 deeper causal understanding of the molecular effects of cell cycle progression.

269

### 270 **An extended network of cell cycle genes**

271 Of the 464 proteins (298 in interphase and 166 in mitotic structures) identified to  
272 correlate to cell cycle progression, 206 (44%) had a known association to the cell cycle  
273 as determined either by a GO BP term related to cell cycle processes<sup>53</sup> or Reactome  
274 <sup>2</sup>, or a cell cycle phenotype registered in Cyclebase<sup>54</sup>. The remaining 258 proteins  
275 (56%), had no previous association to the cell cycle (**Supplementary Table 6**). To  
276 investigate whether the proteins, identified to be cell cycle regulated in this study, are  
277 connected to proteins previously known to be cell cycle regulated, we analyzed  
278 protein-protein interactions using the STRING database<sup>81</sup>. This analysis revealed  
279 significantly more interactions than expected for a random set of proteins of similar  
280 size (Lambda calculations PPI enrichment p-value <1e-16; 1855 interactions; 649  
281 expected number of edges), indicating that the proteins are likely involved in similar  
282 biological processes. The known cell cycle dependent proteins were tightly clustered  
283 together and made up the core of the network, whereas the newly identified cell cycle  
284 regulated proteins formed an extended network (**Figure 3C**). For instance, KIF23 is an  
285 essential protein for the microtubule bundling during cytokinesis via its interaction with  
286 RACGAP1<sup>82</sup> and it is known to oscillate temporally in the nucleus during the cell cycle  
287 <sup>83</sup>. In our interaction analysis (**Figure 3C**), KIF23 showed a number of interactions with  
288 known cell cycle regulators, but also with proteins with no prior association to the cell  
289 cycle such as DRG1; MICAL3, which further interacts with the known NINL protein  
290 required for cytokinesis<sup>84</sup>; and RAD51AP1, which further interacts with RACGAP1 and  
291 KIF20A required for cytokinesis<sup>85</sup>. This implies that these three proteins with unknown  
292 function, DRG1, MICAL3, and RAD51AP1, are involved in the same process as their  
293 known interaction partners, in this case cytokinesis.

294

### 295 **Poor overlap between the cell cycle dependent proteome and transcriptome**

296 We performed a comparative analysis between the cell cycle regulated proteome  
297 identified in our study and the cell cycle transcriptome of U-2 OS osteosarcoma cells  
298 obtained by bulk RNA-sequencing of synchronized cells (26), as well as the  
299 transcriptome of another type of sarcoma cells (myxoid sarcoma cells) obtained by  
300 single-cell RNA-sequencing of non-synchronized cells (36). Both comparisons  
301 revealed a poor overlap of 19% and 10%, respectively (**Supplementary Table 7**). This  
302 indicates that the temporal dynamics of proteome regulation may be to a large extent  
303 maintained at a translational or post-translational level.

304

305 **Gene expression patterns across tissues and cancers results in clusters**  
306 **reflecting proliferative activity**

307 To further understand whether the identified proteins are functionally important for cell  
308 proliferation in a more native context than cell lines, we investigated the mRNA  
309 expression across cohorts of normal and cancer tissue. Hierarchical clustering of the  
310 transcript data from bulk RNA-sequencing of normal and cancer tissues from HPA  
311 (**Figure 4A**) resulted in four major clusters. The first cluster contained normal tissues  
312 with low proliferative activity, such as heart muscle, skeletal muscle and pancreas. The  
313 different cerebral tissues formed the second cluster, together with testis, which  
314 appeared as an outlier, most likely due to being the only sample with meiotic activity.  
315 The third cluster contained mostly normal tissues, such as kidney and breast, and  
316 showed mid-range expression level of the proliferation markers Ki67, MCM2, PCNA,  
317 CDK1 and MCM6. The fourth cluster contained mostly cancer tissues, such as skin  
318 and breast cancer, but also normal tissues with high proliferative activity, such as bone  
319 marrow, tonsil and fetal lung. The tissues in this cluster showed high expression of the  
320 abovementioned proliferation markers. Most importantly, gene expression levels were  
321 significantly higher in the proliferative tissues than the non-proliferative tissues  
322 (Kruskal Wallis test p-value  $<2e^{-16}$ ) (**Figure 4B**).

323

324 To further strengthen the conclusion that the novel cell cycle regulated proteins are  
325 important for cellular proliferation, we used the RNA-sequencing data from The Cancer  
326 Genome Atlas (TCGA) <sup>86</sup> to create genome wide co-expression networks downloaded  
327 from TCSBN <sup>87</sup>, in which the shortest path between the novel cell cycle regulated  
328 genes identified in our study and known cell cycle genes were measured and  
329 compared to a randomly sampled set of genes. The novel genes indeed had a  
330 significantly (Kolmogorov-Smirnov one-sided test, FDR  $< 0.05$ ) shorter path to the  
331 known cell cycle genes in all cancer tissues and the normal proliferative tissues such  
332 as skin, spleen and colon (**Figure 5A**), whereas there was no significant difference  
333 (Kolmogorov-Smirnov one-sided test, FDR  $< 0.05$ ) of the path length in low- or non-  
334 proliferating tissues such as adipose, brain, heart and muscle tissues. This shows that  
335 even though most of these proteins are not temporally regulated at the gene  
336 expression level, their overall gene expression level is still of importance for cellular  
337 proliferation.

338

339 **Genes encoding cell cycle regulated proteins often have an expression**  
340 **correlating to patient survival in cancer**

341 To further test if the level of expression of genes encoding cell cycle regulated proteins  
342 is associated to cancer patient outcome, the TCGA data incorporated in the cancer  
343 pathology atlas of HPA was used <sup>88</sup>, where genes with a statistically significant  
344 differential expression between patient populations with long and short survival were  
345 identified <sup>86</sup>. Genes with expression levels correlated with long survival time were  
346 denoted as favorable, and with shorter survival time were denoted as unfavorable.  
347 Globally, over half of all human genes (54%) were shown to have a prognostic  
348 association in this manner, as previously described <sup>88</sup>. Interestingly, prognostic genes  
349 were significantly overrepresented among the cell cycle regulated proteins identified  
350 in our study (67% prognostic) and the majority of these genes (61%) were associated  
351 with an unfavorable outcome, further supporting the hypothesis of an important role of  
352 these genes in cellular proliferation.

353

354 We next incorporated this classification into the generated co-expression networks for  
355 different human cancer tissue types. In these networks, an enrichment analysis was  
356 further subjected for each genetic community: communities were denoted as  
357 favorable, unfavorable or not enriched. All communities contained a mixture of known  
358 and novel cell cycle proteins, further strengthening their functional associations.  
359 Strikingly, these networks revealed that the association into clusters were highly  
360 different for different tumors (**Figure 5B** and **Supplementary Figure S3**), with proteins  
361 being in a favorable community in one cancer type while being in an unfavorable  
362 community in another cancer type, emphasizing the complexity of cell cycle regulation  
363 from a systems perspective.

364

365 Many of the proteins identified here as cell cycle regulated are interesting candidates  
366 for in-depth studies of their roles in tumorigenesis, and for potential use as biomarkers.  
367 For instance, the gene RACGAP1, known to regulate cytokinesis, and DLGAP5, which  
368 has been reported to have a role in carcinogenesis <sup>89-91</sup>. In the co-expression network  
369 analysis, these genes showed interactions with known cell cycle related genes and  
370 were enriched in an unfavorable prognostic cluster in breast cancer and pancreatic  
371 cancer, respectively (**Figure 6A**). Immunohistochemical (IHC) analysis showed that  
372 these proteins are expressed at low levels in normal tissues (**Figure 6B**) and high  
373 levels in corresponding tumor tissues (**Figure 6C**). Their expression profile is shown  
374 in **Figure 6D**. To gain an insight into their potential pathway involvement, STRING  
375 analysis was performed (**Figure 6E**). RACGAP1 showed physical interaction with  
376 several members of the mitotic kinesin family required for cytokinesis <sup>92</sup>, whereas

377 DLGAP5 showed direct interaction with AURKA, a protein involved in several mitotic  
378 events <sup>93</sup>.

379

380 A portion of the genes encoding proteins identified in our study (39%) were associated  
381 with a favorable outcome, such as SYNE2 and FAM50B (**Figure 6A**). Comparison of  
382 IHC staining of these two proteins revealed high expression in normal tissue (**Figure**  
383 **6B**), and low expression in the respective cancers (**Figure 6C**). This suggests that  
384 these proteins might function in anti-tumor activities. For example, SYNE2 is a nuclear  
385 membrane protein <sup>94</sup>, for which we demonstrated temporal expression variation  
386 peaking in G2. FAM50B is expressed in the nucleus in interphase and translocates to  
387 the cytokinetic bridge in mitosis (**Figure 6D**). SYNE2 shows interaction with genes  
388 enriched in cell cycle processes, such as STAG1, SUN2, TERF1 and TERF2 and  
389 FAM50B shows a physical interaction with HDAC2 (**Figure 6E**), which is involved in  
390 the regulation of cell cycle progression <sup>95</sup>.

391

392 We conclude that these novel proteins identified to be cell cycle regulated have the  
393 potential of serving as novel diagnostic or therapeutic targets for a variety of human  
394 cancers.

395 **Discussion**

396 In this study, we find that a large extent (17%) of the human proteome displays cell-to-  
397 cell heterogeneity in terms of level of expression. We present the first temporal analysis  
398 of the cell cycle regulated human proteome in unsynchronized cells, mapped at a  
399 single cell level with subcellular resolution. Surprisingly, the majority of the variations  
400 were not correlated to the cell cycle, which opens up intriguing avenues for further  
401 exploration of the deterministic factors that might control these stochastic variations in  
402 expression.

403

404 We present 258 novel cell cycle regulated proteins, and show that despite a poor  
405 overlap with cell cycle transcriptome studies, these genes are expressed significantly  
406 higher in proliferating tissues and tumors. The poor overlap to prior transcriptome-  
407 based studies of the human cell cycle points towards massive regulation of protein  
408 levels at a translational or post-translational level. Another key finding of this study is  
409 that the variance of many cell cycle regulated proteins, in particular the newly identified  
410 proteins, are only partially explained by the cell cycle. We hypothesize that these  
411 proteins are deterministically controlled by other cellular mechanisms which open the  
412 door to further follow up work on the role of various signaling pathways in cell cycle  
413 regulation.

414

415 Finally, we demonstrate that several of the newly identified cell cycle regulated proteins  
416 may be clinically significant and have oncogenic or anti-oncogenic functions. We  
417 believe that this comprehensive dissection of the cell cycle regulated human proteome,  
418 now integrated into the HPA database, will serve as a valuable resource to accelerate  
419 studies towards a greater functional understanding of the human cell cycle, the role of  
420 these proteins in tumorigenesis and identification of novel clinical markers for cellular  
421 proliferation.

## 422 **Material and Methods**

423

### 424 **Initial identification of proteins with cell-to-cell heterogeneity**

425 Protein cell-to-cell heterogeneity was identified in the images from the Cell Atlas of the  
426 Human Protein Atlas <sup>46</sup> either in terms of variation in abundance, defined as the change  
427 of protein expression levels between single cells within the same field of view, or  
428 variations in spatial distribution, defined as translocation of the protein between  
429 different subcellular compartments or independent regulation of the protein in two  
430 different compartments.

431

### 432 **Cell cultivation**

433 U2- OS FUCCI cells were developed and kindly provided by Dr. Miyawaki <sup>42</sup>. These  
434 cells are endogenously tagged with two fluorescent proteins fused to cell cycle  
435 regulators to allow cell cycle monitoring; CDT1 (mKO2-hCdt1<sup>+</sup>) accumulates in G1  
436 phase, while Geminin (mAG-hGem<sup>+</sup>) accumulates in S and G2 phases. Cells  
437 expressing FUCCI probes are divided into red mKO2(+)/mAG(-), yellow  
438 mKO2(+)/mAG(+), and green mKO2(-)/mAG(+) emitting populations. The cells were  
439 cultivated in Petri dishes at 37 °C in a 5.0 % CO<sub>2</sub> humidified environment in McCoy's  
440 5A (modified) medium GlutaMAX supplement, (ThermoFisher, 36600021, MA, USA)  
441 supplemented with 10% fetal bovine serum (FBS, VWR, Radnor, PA, USA). The cells  
442 were maintained sub-confluent and harvested by trypsinization at log-phase growth  
443 (60% confluency) for subsequent analysis.

444

### 445 **Live cell imaging**

446 U-2 OS FUCCI cells were grown on a 96-well glass bottom plates (Whatman, Cat#  
447 7716-2370, GE Healthcare, UK, and Greiner Sensoplate Plus, Cat# 655892, Greiner  
448 Bio-One, Germany). Approximately 6,000 cells were seeded in the wells and subjected  
449 to long-term time-lapse imaging using the molecular device instrument ImageXpress  
450 Micro XL (Molecular Device) high content screening equipped with a 20 x Plan Apo  
451 objective and supported with the MetaXpress software. Three Wavelengths were  
452 acquired; W1 transmitted light, W2 FITC-3540C filter, W3 CY3-4040C filter. Images  
453 were collected every 30 minutes over a course of 72h.

454

### 455 **Antibodies**

456 The rabbit polyclonal antibodies used in this study (**Supplementary Table 8**) were  
457 generated within the HPA project. The antibodies were designed to target as many  
458 different isoforms of the target protein as possible and were affinity purified using



459 antigen fragments<sup>96</sup>. Furthermore, the antibodies were validated and quality assured  
460 for sensitivity and lack of cross-reactivity using the HPA standard quality assurance  
461 including microarray analyses.

462

### 463 **Immunostaining**

464 Immunostaining of the cells<sup>97</sup> was performed in 96-well glass bottom plates (Whatman,  
465 GE Healthcare, UK, and Greiner Sensoplate Plus, Greiner Bio-One, Germany) coated  
466 with 50 µl of 12.5 µg/ml human fibronectin (Sigma Aldrich, Darmstadt, Germany).  
467 Approximately 8,000 cells were seeded in each well and incubated at 37 °C for 24  
468 hours. After washing with Phosphatase Buffered Saline (PBS, PH=7), cells were fixed  
469 with 40 µl 4% ice cold PFA (Sigma Aldrich, Darmstadt, Germany) dissolved in growth  
470 medium supplemented with 10 % serum for 15 minutes and permeabilized with 40 µl  
471 0.1% Triton X-100 (Sigma Aldrich) in PBS for 3x5 minutes. Rabbit polyclonal HPA  
472 antibodies targeting the proteins of interest were dissolved to 2-4 µg/ml in blocking  
473 buffer (PBS + 4% FBS) containing 1 µg/ml mouse anti-tubulin (Abcam, ab7291,  
474 Cambridge, UK). After washing with PBS, the diluted primary antibodies were added  
475 (40 µl/well) and the plates were incubated over night at 4 °C. After overnight incubation,  
476 wells were washed with PBS for 3x10 minutes. Secondary antibodies, goat anti-mouse  
477 Alexa405 (A31553, ThermoFisher) and goat anti-rabbit Alexa647 (A21245,  
478 ThermoFisher) diluted to 2,5 µg /ml in blocking buffer were added and the plates were  
479 incubated for 90 minutes at room temperature. After washing with PBS, all wells were  
480 mounted with PBS containing 78 % glycerol before sealed.

481

### 482 **Image acquisition**

483 Image acquisition was performed using ImageXpress Micro XL (Molecular Device)  
484 high content screening equipped with a 40 x Plan Apo objective and supported with  
485 the MetaXpress software for automated acquisition. Images of the four channels were  
486 acquired at room temperature from six positions per sample. Four wavelengths were  
487 acquired; W1 for the microtubules DAPI-5060C filter, W2 FITC-3540C filter, W3 CY3-  
488 4040C filter and W4 CY5-4040C for the protein of interest. The images were unbinned  
489 with a pixel size of 0.1625x0.1625 µm.

490

### 491 **Image processing and analysis**

492 The segmentation of each cell was performed using the Cell Profiler software<sup>98</sup>, where  
493 the overlay of the FUCCI tags were used for the nuclei identification and the  
494 microtubule staining was used for identification of the cell outline. Size exclusion was  
495 used to prune image mitotic cells from the population.



496 For each cell, the green and red tag mean intensity value was used and the cells were  
497 clustered in one of the cell cycle clusters using the Gaussian Clustering. The mean  
498 intensity of the target protein was measured in one of the three main compartments;  
499 nucleus, cytosol or cell, based on the a priori-known subcellular localization of the  
500 target protein from the HPA Cell Atlas.

501

502 Statistical analysis was performed using Kruskal-Wallis statistical test to determine the  
503 p-values that significantly differed between the three cell cycle groups. An arbitrary cut-  
504 off, based on a negative control,  $p < 0.01$  was chosen. FDR was calculated to adjust for  
505 multiple comparisons<sup>99</sup>. The plots were generated using R studio v1.1.423<sup>100</sup>. The  
506 image montages were created using Image J and FIJI<sup>101</sup>. k-means clustering was  
507 performed using the features kurtosis and skewness, where each gene was assigned  
508 to a specific K-cluster. The optimal number of clusters was chosen using the Elbow  
509 method, where it looks at the percentage of variance explained as a function of the  
510 number of clusters. The bimodal distribution of the protein expression was indicated  
511 by Hartigan's dip test.

512

### 513 **Polar-coordinate pseudo time model**

514 In this work we utilized the FUCCI system to model cell cycle position. To generate a  
515 continuous representation of cell cycle position we utilized a polar regression based  
516 on a log-scale scatter plot of GMNN (FUCCI-green) and CDT1 (FUCCI-red) where  
517 each point represents a single cell (**Supplementary Figure S4**). This data was shifted  
518 such that the origin point lay at the center of mass. This allowed us to use the fractional  
519 radius of the circle could be used to estimate time for each cell as traced by a ray from  
520 the origin generating a polar regression representing continuous cell cycle position.  
521 The cell-division point was selected by using the area of lowest cell density on the  
522 polar ray from the origin. This is justified by the knowledge that M phase (where cells  
523 express neither GMNN nor CDT1 highly) is much shorter than all other phases. The  
524 selected point was validated via visual inspection of nearby cells. This allowed us to  
525 linearize the progression of time from 0 to 1 representing the fractional distance along  
526 this polar axis from 0 to 360 degrees. This fit was done on a per-plate basis to account  
527 for batch-variance observed in the data.

528

### 529 **Moving average model**

530 Cell-cycle correlation was measured using a moving-average model within the  
531 linearized time from the polar fit described above. A range of window sizes were tested  
532 from 5-30. The analysis proved robust to this range of window size, and results

533 reported are for a window size of 20 cells which was chosen to balance the robustness  
534 to outliers with potentially destroying signal.

535

### 536 **Percent explained variance**

537 We used the metric percent explained variance to describe the goodness of our model  
538 fit. This metric is appealing as it is scale-invariant. That is, unlike a p-value significance  
539 metric which becomes more significant as sample size increases, the percent-variance  
540 converges to a stable solution as more cells are sampled. The percent explained  
541 variance is calculated as:

$$542 \quad (1) \quad \% \sigma_{prot} = 1 - \frac{\sigma_{residual}}{\sigma_{total}}$$

543 Here,  $\sigma_{polar}$  represents the variance of the protein of interest for an experiment and  
544  $\sigma_{residual}$  represents the variance remaining calculated from the moving average line  
545 along the pseudo-time axis.

546

### 547 **Periodic regression model**

548 To model protein response over time, a novel continuous-time periodic regression  
549 model was developed. This model made the following assumptions.

- 550 1. Protein expression is smoothly differentiable
- 551 2. Protein expression in continuously dividing cells must be periodic
- 552 3. Cell cycle-dependent protein expression shows a single peak as is commonly  
553 assumed for gene expression<sup>102,103</sup>.

554 To model the asymmetric nature of protein accumulation and depletion over the cell  
555 cycle we developed a sin-based equation of fit describing the expression of protein  $\chi$   
556 over the cell cycle as seen in equation (2) below.

557

$$558 \quad (2) \quad f(x) = b \cdot \sin(\pi \cdot x^\alpha)^\gamma + C$$

559 Where  $b$  describes the magnitude and sign of response,  $\alpha$  describes the position of  
560 extremeum,  $\gamma$  defines the steepness of response, and  $C$  defines the y-intercept. Here  
561 we use  $\pi$  to define the single-extremum period 0-1 as represented by the normalized  
562 relative time since division. This function is fit to the normalized protein expression in  
563 the relevant meta-compartment where protein expression is observed (nucleus,  
564 cytoplasm, or both). Parameters of these functions are bounded to ensure reasonable  
565 differentiability as follows.

566

$$0 < b \leq 1$$

567 
$$\frac{1}{6} < \alpha \leq 100$$

568 
$$\frac{1}{2} < \gamma \leq 100$$

569 
$$0 \leq C \leq 1$$

570 It is worth noting that these functions do not have a stable period and may behave  
571 erratically outside the defined 0-1 interval, however they are not designed to be  
572 evaluated outside this interval.

573

#### 574 **Gene set enrichment and interaction analysis**

575 Functional enrichment analysis for the GO domain biological process was performed  
576 using the Database for Annotation, Visualization and Integrative Discovery (DAVID)  
577 tool<sup>104</sup> and Cytoscape v3.6.1<sup>105</sup> was used for the network visualization. Enrichment  
578 map plugin was used to visualize the results of the highly significant gene-set  
579 enrichment as a network<sup>106</sup>.

580

581 The interaction analysis was done using the Search Tool for the Retrieval of Interacting  
582 Genes/Proteins (STRING) database v10.5<sup>81</sup>, where a medium confidence (0.4) score  
583 was used to highlight the protein-protein interaction edges.

584

585 The open sources Cyclebase v3.0<sup>54</sup>; Reactome and QuickGO<sup>107</sup> were used for  
586 downloading the previously characterized cell cycle regulators.

587

#### 588 **RNA extraction and RNA sequencing**

589 The RNA extraction and sequencing were performed as previously reported<sup>46,47,88</sup>.  
590 Briefly, for cell lines early-split samples and duplicates were used for total RNA  
591 extraction. Tissue samples were embedded in Optimal Cutting Temperature  
592 compound and stored at  $-80^{\circ}\text{C}$ . HE-stained frozen sections (4  $\mu\text{m}$ ) were prepared  
593 from each sample using a cryostat and the CryoJane® Tape-Transfer System  
594 (Instrumedics, St. Louis, MO, USA). Three sections (10  $\mu\text{m}$ ) were cut from each frozen  
595 tissue block and collected in a tube for subsequent RNA extraction<sup>108</sup>. Total RNA was  
596 extracted from the cell lines and tissue samples using the RNeasy Mini Kit (Qiagen,  
597 Hilden, Germany) according to the manufacturer's instructions. Only samples of high-  
598 quality RNA (RNA Integrity Number  $\geq 7.5$ ) were used in the following mRNA sample  
599 preparation for sequencing.

600

601 A total of 172 samples from 37 tissues and organs was sequenced using Illumina  
602 Hiseq2000 and Hiseq2500, and the standard Illumina RNAseq protocol with a read  
603 length of 2x100 bases. Briefly, the reads were mapped to the human genome  
604 (GRCh37) using Tophat v2.0.8b<sup>109</sup>. Transcript abundance estimation was performed  
605 using Kallisto v0.42.4<sup>110</sup>. For each gene, the abundance was reported in 'Transcript  
606 Per Million' (TPM) as the sum of the TPM values of all its protein-coding transcripts.  
607 For each cell line and tissue type, the average TPM value for replicate samples was  
608 used as abundance score. The threshold level to detect presence of a transcript for a  
609 particular gene was set to  $\geq 1$  TPM.

610

### 611 **Co-Expression Network Analysis**

612 The co-expression networks for different tissues and cancer were downloaded from  
613 TCSBN website<sup>87</sup>. The nodes (genes) in the networks were classified into three  
614 categories: i) candidate cell-cycle genes (T1), ii) known cell-cycle genes (T2) and iii)  
615 other genes (T3). Following that, the shortest path in the co-expression network was  
616 compared between each category by using simple Breadth-First Search (BFS)  
617 method. The distribution between shortest path of T1-T2 was compared with T3-T2 by  
618 FDR-Adjusted Kolmogorov-Smirnov one-sided test (FDR < 0.05).

619

620 For the next step, we then incorporated the cancer pathology data from the HPA<sup>88</sup> into  
621 the cancer co-expression networks. The significant prognostic property ("favorable" or  
622 "unfavorable") was mapped into the nodes of the networks. We then employed Louvain  
623 community detection algorithm<sup>111</sup> to identify the communities in the network, to  
624 maximize the modularity score. For each community, we calculated hypergeometric  
625 test to understand further the behavior of each community. A community was  
626 considered as showing specific behavior if it fulfilled p-value < 0.01. Each community  
627 was mapped into one of the four categories: i) Favorable, ii) Unfavorable, iii) Both, iv)  
628 Not significant.

629

630 The aforementioned analyses were performed with in-house Python script, with Scipy  
631 module<sup>112</sup> for the statistical analysis and Igraph<sup>113</sup> for the network analysis and  
632 manipulation.

633

### 634 **Immunohistochemical staining**

635 Immunohistochemical (IHC) staining of tissue microarray (TMA) sections and slide  
636 scanning were performed essentially as previously described<sup>114</sup>. In brief, normal and  
637 cancer tissues were derived from surgical material obtained from the Department of

638 Pathology, Uppsala University Hospital, Uppsala, Sweden as part of the sample  
639 collection governed by the Uppsala Biobank (<http://www.uppsalabiobank.uu.se/en/>).  
640 All human tissue samples used in the present study were anonymized in accordance  
641 with approval and advisory report from the Uppsala Ethical Review Board (Reference  
642 # 2002-577, 2005-338 and 2007-159). Representative tissue cores (1 mm diameter)  
643 were sampled from formalin fixed and paraffin embedded (FFPE) blocks and  
644 assembled into six TMAs, containing normal tissue samples from 144 individuals, as  
645 well as cancer tissue samples from 216 individuals. TMA blocks were cut in 4  $\mu$ m thick  
646 sections using waterfall microtomes (Microm HM 355S, Thermo Fisher Scientific,  
647 Fremont, CA, USA), dried in RT overnight and baked in 50°C for 12-24 hours prior to  
648 IHC staining. Automated immunohistochemistry was performed using Autostainer  
649 480® instruments (Lab Vision, Fremont, CA, USA), followed by slide scanning using  
650 Aperio AT2 (Leica Biosystems, Wetzlar, Germany). The high-resolution images of IHC  
651 stained TMA sections were evaluated and annotated by certified pathologists (Lab  
652 SurgPath, Mumbai, India).

653 **References**

- 654 1 Barnum, K. J. & O'Connell, M. J. in *Cell Cycle Control* 29-40 (Springer, 2014).
- 655 2 Croft, D. *et al.* Reactome: a database of reactions, pathways and biological  
656 processes. *Nucleic acids research* **39**, D691-D697 (2010).
- 657 3 Crosby, M. E. Cell cycle: principles of control. *The Yale journal of biology and*  
658 *medicine* **80**, 141 (2007).
- 659 4 Weinberg, R. A. The retinoblastoma protein and cell cycle control. *Cell* **81**, 323-  
660 330 (1995).
- 661 5 Morgan, D. O. Principles of CDK regulation. *Nature* **374**, 131 (1995).
- 662 6 Nurse, P. A long twentieth century of the cell cycle and beyond. *Cell* **100**, 71-  
663 78 (2000).
- 664 7 Ly, T., Endo, A. & Lamond, A. I. Proteomic analysis of the response to cell  
665 cycle arrests in human myeloid leukemia cells. *eLife* **4**, e04534,  
666 doi:10.7554/eLife.04534 (2015).
- 667 8 Teixeira, L. K. & Reed, S. I. Ubiquitin ligases and cell cycle control. *Annual*  
668 *review of biochemistry* **82**, 387-414 (2013).
- 669 9 Eifler, K. & Vertegaal, A. C. SUMOylation-mediated regulation of cell cycle  
670 progression and cancer. *Trends in biochemical sciences* **40**, 779-793 (2015).
- 671 10 King, R. W., Deshaies, R. J., Peters, J.-M. & Kirschner, M. W. How proteolysis  
672 drives the cell cycle. *Science* **274**, 1652-1659 (1996).
- 673 11 Skaar, J. R. & Pagano, M. Control of cell growth by the SCF and APC/C  
674 ubiquitin ligases. *Current opinion in cell biology* **21**, 816-824 (2009).
- 675 12 Reed, S. I. in *Cell Cycle Regulation* 147-181 (Springer, 2006).
- 676 13 Malumbres, M. & Barbacid, M. Cell cycle, CDKs and cancer: a changing  
677 paradigm. *Nature reviews cancer* **9**, 153 (2009).
- 678 14 Massagué, J. G1 cell-cycle control and cancer. *Nature* **432**, 298 (2004).
- 679 15 Hartwell, L. H. & Kastan, M. B. Cell cycle control and cancer. *Science* **266**,  
680 1821-1828 (1994).
- 681 16 Orlando, D. A. *et al.* Global control of cell-cycle transcription by coupled CDK  
682 and network oscillators. *Nature* **453**, 944, doi:10.1038/nature06955  
683 <https://www.nature.com/articles/nature06955#supplementary-information> (2008).
- 684 17 Cho, R. J. *et al.* Transcriptional regulation and function during the human cell  
685 cycle. *Nature Genetics* **27**, 48, doi:10.1038/83751 (2001).
- 686 18 Rustici, G. *et al.* Periodic gene expression program of the fission yeast cell  
687 cycle. *Nature genetics* **36**, 809 (2004).

- 688 19 Whitfield, M. L. *et al.* Identification of Genes Periodically Expressed in the  
689 Human Cell Cycle and Their Expression in Tumors. *Molecular Biology of the*  
690 *Cell* **13**, 1977-2000, doi:10.1091/mbc.02-02-0030 (2002).
- 691 20 Boström, J. *et al.* Comparative cell cycle transcriptomics reveals  
692 synchronization of developmental transcription factor networks in cancer cells.  
693 *PloS one* **12**, e0188772 (2017).
- 694 21 Lane, K. R. *et al.* Cell cycle-regulated protein abundance changes in  
695 synchronously proliferating HeLa cells include regulation of pre-mRNA splicing  
696 proteins. *PloS one* **8**, e58456 (2013).
- 697 22 Ohta, S. *et al.* The protein composition of mitotic chromosomes determined  
698 using multiclassifier combinatorial proteomics. *Cell* **142**, 810-821 (2010).
- 699 23 Ly, T. *et al.* A proteomic chronology of gene expression through the cell cycle  
700 in human myeloid leukemia cells. *Elife* **3** (2014).
- 701 24 Pagliuca, F. W. *et al.* Quantitative proteomics reveals the basis for the  
702 biochemical specificity of the cell-cycle machinery. *Molecular cell* **43**, 406-417  
703 (2011).
- 704 25 Bar-Joseph, Z. *et al.* Genome-wide transcriptional analysis of the human cell  
705 cycle identifies genes differentially regulated in normal and cancer cells.  
706 *Proceedings of the National Academy of Sciences* **105**, 955 (2008).
- 707 26 Dominguez, D. *et al.* A high-resolution transcriptome map of cell cycle reveals  
708 novel connections between periodic genes and cancer. *Cell Research* **26**, 946,  
709 doi:10.1038/cr.2016.84  
710 <https://www.nature.com/articles/cr201684#supplementary-information> (2016).
- 711 27 Grant, G. D. *et al.* Identification of cell cycle-regulated genes periodically  
712 expressed in U2OS cells and their regulation by FOXM1 and E2F transcription  
713 factors. *Mol Biol Cell* **24**, 3634-3650, doi:10.1091/mbc.E13-05-0264 (2013).
- 714 28 Peña-Díaz, J. *et al.* Transcription profiling during the cell cycle shows that a  
715 subset of Polycomb-targeted genes is upregulated during DNA replication.  
716 *Nucleic Acids Research* **41**, 2846-2856, doi:10.1093/nar/gks1336 (2013).
- 717 29 Cooper, S. *et al.* Membrane-elution analysis of content of cyclins A, B1, and E  
718 during the unperturbed mammalian cell cycle. *Cell Division* **2**, 28,  
719 doi:10.1186/1747-1028-2-28 (2007).
- 720 30 Mgbonyebi, O. P., Russo, J. & Russo, I. H. Roscovitine induces cell death and  
721 morphological changes indicative of apoptosis in MDA-MB-231 breast cancer  
722 cells. *Cancer research* **59**, 1903-1910 (1999).



- 723 31 Wright, J. Morphology and growth rate changes in Chinese hamster cells  
724 cultured in presence of sodium butyrate. *Experimental cell research* **78**, 456-  
725 460 (1973).
- 726 32 Maltese, W. A. & Sheridan, K. M. Isoprenylated proteins in cultured cells:  
727 subcellular distribution and changes related to altered morphology and growth  
728 arrest induced by mevalonate deprivation. *Journal of cellular physiology* **133**,  
729 471-481 (1987).
- 730 33 Davis, P. K., Ho, A. & Dowdy, S. F. Biological methods for cell-cycle  
731 synchronization of mammalian cells. *Biotechniques* **30**, 1322-1331 (2001).
- 732 34 Cooper, S. Rethinking synchronization of mammalian cells for cell cycle  
733 analysis. *Cellular and Molecular Life Sciences CMLS* **60**, 1099-1106,  
734 doi:10.1007/s00018-003-2253-2 (2003).
- 735 35 Cooper, S., Iyer, G., Tarquini, M. & Bissett, P. Nocodazole does not  
736 synchronize cells: implications for cell-cycle control and whole-culture  
737 synchronization. *Cell and Tissue Research* **324**, 237-242, doi:10.1007/s00441-  
738 005-0118-8 (2006).
- 739 36 Cooper, S. & Shedden, K. Microarray analysis of gene expression during the  
740 cell cycle. *Cell & Chromosome* **2**, 1, doi:10.1186/1475-9268-2-1 (2003).
- 741 37 Karlsson, J., Kroneis, T., Jonasson, E., Larsson, E. & Ståhlberg, A.  
742 Transcriptomic Characterization of the Human Cell Cycle in Individual  
743 Unsynchronized Cells. *Journal of molecular biology* **429**, 3909-3924 (2017).
- 744 38 Liu, Z. *et al.* Reconstructing cell cycle pseudo time-series via single-cell  
745 transcriptome data. *Nature communications* **8**, 22 (2017).
- 746 39 Scialdone, A. *et al.* Computational assignment of cell-cycle stage from single-  
747 cell transcriptome data. *Methods* **85**, 54-61 (2015).
- 748 40 Sigal, A. *et al.* Dynamic proteomics in individual human cells uncovers  
749 widespread cell-cycle dependence of nuclear proteins. *Nature Methods* **3**, 525  
750 (2006).
- 751 41 Neumann, B. *et al.* Phenotypic profiling of the human genome by time-lapse  
752 microscopy reveals cell division genes. *Nature* **464**, 721 (2010).
- 753 42 Sakaue-Sawano, A. *et al.* Visualizing spatiotemporal dynamics of multicellular  
754 cell-cycle progression. *Cell* **132**, 487-498 (2008).
- 755 43 Mukherji, M. *et al.* Genome-wide functional analysis of human cell-cycle  
756 regulators. *Proceedings of the National Academy of Sciences* **103**, 14819-  
757 14824 (2006).



- 758 44 Gut, G., Tadmor, M. D., Pe'er, D., Pelkmans, L. & Liberali, P. Trajectories of  
759 cell-cycle progression from fixed cell populations. *Nature methods* **12**, 951  
760 (2015).
- 761 45 Gookin, S. *et al.* A map of protein dynamics during cell-cycle progression and  
762 cell-cycle exit. *PLoS biology* **15**, e2003268 (2017).
- 763 46 Thul, P. J. *et al.* A subcellular map of the human proteome. *Science* **356**,  
764 eaal3321 (2017).
- 765 47 Uhlen, M. *et al.* Towards a knowledge-based human protein atlas. *Nature*  
766 *biotechnology* **28**, 1248 (2010).
- 767 48 Brown, N. R. *et al.* Cyclin B and cyclin A confer different substrate recognition  
768 properties on CDK2. *Cell Cycle* **6**, 1350-1359 (2007).
- 769 49 Li, X. *et al.* Direct association with inner centromere protein (INCENP) activates  
770 the novel chromosomal passenger protein, Aurora-C. *Journal of Biological*  
771 *Chemistry* **279**, 47201-47211 (2004).
- 772 50 Wang, X., Yang, Y. & Dai, W. Differential subcellular localizations of two human  
773 Sgo1 isoforms: implications in regulation of sister chromatid cohesion and  
774 microtubule dynamics. *Cell Cycle* **5**, 636-641 (2006).
- 775 51 Neef, R. *et al.* Phosphorylation of mitotic kinesin-like protein 2 by polo-like  
776 kinase 1 is required for cytokinesis. *The Journal of cell biology* **162**, 863-876  
777 (2003).
- 778 52 Gangisetty, O., Lauffart, B., Sondarva, G. V., Chelsea, D. M. & Still, I. H. The  
779 transforming acidic coiled coil proteins interact with nuclear histone  
780 acetyltransferases. *Oncogene* **23**, 2559 (2004).
- 781 53 Botstein, D. *et al.* Gene Ontology: tool for the unification of biology. *Nat genet*  
782 **25**, 25-29 (2000).
- 783 54 Santos, A., Wernersson, R. & Jensen, L. J. Cyclebase 3.0: a multi-organism  
784 database on cell-cycle regulation and phenotypes. *Nucleic acids research* **43**,  
785 D1140-D1144 (2014).
- 786 55 Vong, Q. P., Cao, K., Li, H. Y., Iglesias, P. A. & Zheng, Y. Chromosome  
787 alignment and segregation regulated by ubiquitination of survivin. *Science* **310**,  
788 1499-1504 (2005).
- 789 56 Wolfson, R. L. *et al.* KICSTOR recruits GATOR1 to the lysosome and is  
790 necessary for nutrients to regulate mTORC1. *Nature* **543**, 438 (2017).
- 791 57 Kikuchi, K., Niikura, Y., Kitagawa, K. & Kikuchi, A. Dishevelled, a Wnt signalling  
792 component, is involved in mitotic progression in cooperation with Plk1. *The*  
793 *EMBO journal* **29**, 3470-3483 (2010).

- 794 58 Fang, L., Seki, A. & Fang, G. SKAP associates with kinetochores and promotes  
795 the metaphase-to-anaphase transition. *Cell cycle* **8**, 2819-2827 (2009).
- 796 59 Rapley, J. *et al.* The NIMA-family kinase Nek6 phosphorylates the kinesin Eg5  
797 at a novel site necessary for mitotic spindle formation. *Journal of cell science*  
798 **121**, 3912-3921 (2008).
- 799 60 Demetriou, M., Granovsky, M., Quaggin, S. & Dennis, J. W. Negative regulation  
800 of T-cell activation and autoimmunity by Mgat5N-glycosylation. *Nature* **409**,  
801 733 (2001).
- 802 61 Robson, T. & James, I. F. The therapeutic and diagnostic potential of FKBPL;  
803 a novel anticancer protein. *Drug discovery today* **17**, 544-548 (2012).
- 804 62 Zielke, N. & Edgar, B. FUCCI sensors: powerful new tools for analysis of cell  
805 proliferation. *Wiley Interdisciplinary Reviews: Developmental Biology* **4**, 469-  
806 487 (2015).
- 807 63 Suzuki, C. *et al.* ANLN plays a critical role in human lung carcinogenesis  
808 through the activation of RHOA and by involvement in the phosphoinositide 3-  
809 kinase/AKT pathway. *Cancer research* **65**, 11314-11325 (2005).
- 810 64 Patterson, K. I., Brummer, T., O'brien, P. M. & Daly, R. J. Dual-specificity  
811 phosphatases: critical regulators with diverse cellular targets. *Biochemical*  
812 *Journal* **418**, 475-489 (2009).
- 813 65 O'Hagan, S., Muelas, M. W., Day, P. J., Lundberg, E. & Kell, D. B. GeneGini:  
814 Assessment via the Gini Coefficient of Reference "Housekeeping" Genes and  
815 Diverse Human Transporter Expression Profiles. *Cell systems* **6**, 230-244.  
816 e231 (2018).
- 817 66 Phannasil, P. *et al.* Pyruvate carboxylase is up-regulated in breast cancer and  
818 essential to support growth and invasion of MDA-MB-231 cells. *PloS one* **10**,  
819 e0129848 (2015).
- 820 67 Sellers, K. *et al.* Pyruvate carboxylase is critical for non-small-cell lung cancer  
821 proliferation. *The Journal of clinical investigation* **125**, 687-698 (2015).
- 822 68 Christen, S. *et al.* Breast cancer-derived lung metastases show increased  
823 pyruvate carboxylase-dependent anaplerosis. *Cell reports* **17**, 837-848 (2016).
- 824 69 Wang, J. *et al.* Identification of XAF1 as a novel cell cycle regulator through  
825 modulating G 2/M checkpoint and interaction with checkpoint kinase 1 in  
826 gastrointestinal cancer. *Carcinogenesis* **30**, 1507-1516 (2009).
- 827 70 Semple, J. W. *et al.* An essential role for Orc6 in DNA replication through  
828 maintenance of pre-replicative complexes. *The EMBO journal* **25**, 5150-5158  
829 (2006).

- 830 71 Izumi, M. *et al.* The Mcm2-7-interacting domain of human mini-chromosome  
831 maintenance 10 (Mcm10) protein is important for stable chromatin association  
832 and origin firing. *Journal of Biological Chemistry*, jbc. M117. 779371 (2017).
- 833 72 Li, Y. *et al.* ZNF32 inhibits autophagy through the mTOR pathway and protects  
834 MCF-7 cells from stimulus-induced cell death. *Scientific reports* **5**, 9288 (2015).
- 835 73 Li, J. *et al.* ZNF32 contributes to the induction of multidrug resistance by  
836 regulating TGF- $\beta$  receptor 2 signaling in lung adenocarcinoma. *Cell death &*  
837 *disease* **7**, e2428 (2016).
- 838 74 Wisdom, R., Johnson, R. S. & Moore, C. c-Jun regulates cell cycle progression  
839 and apoptosis by distinct mechanisms. *The EMBO journal* **18**, 188-197 (1999).
- 840 75 Pils, D. *et al.* Cyclin E1 (CCNE1) as independent positive prognostic factor in  
841 advanced stage serous ovarian cancer patients—A study of the OVCAD  
842 consortium. *European journal of cancer* **50**, 99-110 (2014).
- 843 76 St-Denis, N. *et al.* Phenotypic and interaction profiling of the human  
844 phosphatases identifies diverse mitotic regulators. *Cell reports* **17**, 2488-2501  
845 (2016).
- 846 77 Shin, H.-J. *et al.* Dual roles of human BubR1, a mitotic checkpoint kinase, in  
847 the monitoring of chromosomal instability. *Cancer cell* **4**, 483-497 (2003).
- 848 78 Xu, Y. *et al.* Effect of estrogen sulfation by SULT 1 E 1 and PAPSS on the  
849 development of estrogen-dependent cancers. *Cancer science* **103**, 1000-1009  
850 (2012).
- 851 79 Figaro, S., Scrima, N., Buckingham, R. H. & Heurgué-Hamard, V. HemK2  
852 protein, encoded on human chromosome 21, methylates translation  
853 termination factor eRF1. *FEBS letters* **582**, 2352-2356 (2008).
- 854 80 Javaheri, T. *et al.* Increased survival and cell cycle progression pathways are  
855 required for EWS/FLI1-induced malignant transformation. *Cell death & disease*  
856 **7**, e2419 (2016).
- 857 81 Szklarczyk, D. *et al.* STRING v10: protein–protein interaction networks,  
858 integrated over the tree of life. *Nucleic acids research* **43**, D447-D452 (2014).
- 859 82 Hutterer, A., Glotzer, M. & Mishima, M. Clustering of centralspindlin is essential  
860 for its accumulation to the central spindle and the midbody. *Current biology* **19**,  
861 2043-2049 (2009).
- 862 83 Fischer, M. *et al.* p53 and cell cycle dependent transcription of kinesin family  
863 member 23 (KIF23) is controlled via a CHR promoter element bound by  
864 DREAM and MMB complexes. *PloS one* **8**, e63187 (2013).

- 865 84 Wang, Y. & Zhan, Q. Cell cycle-dependent expression of centrosomal ninein-  
866 like protein in human cells is regulated by the anaphase-promoting complex.  
867 *Journal of Biological Chemistry* **282**, 17712-17719 (2007).
- 868 85 Morita, H. *et al.* KIF20A, highly expressed in immature hematopoietic cells,  
869 supports the growth of HL60 cell line. *International journal of hematology* **108**,  
870 607-614 (2018).
- 871 86 Weinstein, J. N. *et al.* The cancer genome atlas pan-cancer analysis project.  
872 *Nature genetics* **45**, 1113 (2013).
- 873 87 Lee, S. *et al.* TCSBN: a database of tissue and cancer specific biological  
874 networks. *Nucleic acids research* **46**, D595-D600 (2017).
- 875 88 Uhlen, M. *et al.* A pathology atlas of the human cancer transcriptome. *Science*  
876 **357**, eaan2507 (2017).
- 877 89 Yang, X.-M. *et al.* Overexpression of Rac GTPase activating protein 1  
878 contributes to proliferation of cancer cells by reducing hippo signaling to  
879 promote cytokinesis. *Gastroenterology* **155**, 1233-1249. e1222 (2018).
- 880 90 Hirose, K., Kawashima, T., Iwamoto, I., Nosaka, T. & Kitamura, T. MgcRacGAP  
881 is involved in cytokinesis through associating with mitotic spindle and midbody.  
882 *Journal of Biological Chemistry* **276**, 5821-5828 (2001).
- 883 91 Schneider, M. A. *et al.* AURKA, DLGAP5, TPX2, KIF11 and CKAP5: Five  
884 specific mitosis-associated genes correlate with poor prognosis for non-small  
885 cell lung cancer patients. *International journal of oncology* **50**, 365-372 (2017).
- 886 92 Vanneste, D., Ferreira, V. & Vernos, I. (Portland Press Limited, 2011).
- 887 93 Mosquera, J. M. *et al.* Concurrent AURKA and MYCN gene amplifications are  
888 harbingers of lethal treatment-related neuroendocrine prostate cancer.  
889 *Neoplasia* **15**, IN1-IN4 (2013).
- 890 94 Zhen, Y.-Y., Libotte, T., Munck, M., Noegel, A. A. & Korenbaum, E. NUANCE,  
891 a giant protein connecting the nucleus and actin cytoskeleton. *J Cell Sci* **115**,  
892 3207-3222 (2002).
- 893 95 Li, S. *et al.* HDAC2 regulates cell proliferation, cell cycle progression and cell  
894 apoptosis in esophageal squamous cell carcinoma EC9706 cells. *Oncology*  
895 *letters* **13**, 403-409 (2017).
- 896 96 Nilsson, P. *et al.* Towards a human proteome atlas: high-throughput generation  
897 of mono-specific antibodies for tissue profiling. *Proteomics* **5**, 4327-4337  
898 (2005).
- 899 97 Stadler, C., Skogs, M., Brismar, H., Uhlén, M. & Lundberg, E. A single fixation  
900 protocol for proteome-wide immunofluorescence localization studies. *Journal*  
901 *of proteomics* **73**, 1067-1078 (2010).

- 902 98 Carpenter, A. E. *et al.* CellProfiler: image analysis software for identifying and  
903 quantifying cell phenotypes. *Genome biology* **7**, R100 (2006).
- 904 99 Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical  
905 and powerful approach to multiple testing. *Journal of the royal statistical*  
906 *society. Series B (Methodological)*, 289-300 (1995).
- 907 100 Team, R. C. R: A language and environment for statistical computing. (2013).
- 908 101 Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25  
909 years of image analysis. *Nature methods* **9**, 671 (2012).
- 910 102 de Lichtenberg, U., Jensen, L. J., Brunak, S. & Bork, P. Dynamic complex  
911 formation during the yeast cell cycle. *science* **307**, 724-727 (2005).
- 912 103 Spellman, P. T. *et al.* Comprehensive identification of cell cycle-regulated  
913 genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization.  
914 *Molecular biology of the cell* **9**, 3273-3297 (1998).
- 915 104 Sartor, M. A. *et al.* ConceptGen: a gene set enrichment and gene set relation  
916 mapping tool. *Bioinformatics* **26**, 456-463 (2009).
- 917 105 Shannon, P. *et al.* Cytoscape: a software environment for integrated models of  
918 biomolecular interaction networks. *Genome research* **13**, 2498-2504 (2003).
- 919 106 Merico, D., Isserlin, R., Stueker, O., Emili, A. & Bader, G. D. Enrichment map:  
920 a network-based method for gene-set enrichment visualization and  
921 interpretation. *PloS one* **5**, e13984 (2010).
- 922 107 Binns, D. *et al.* QuickGO: a web-based tool for Gene Ontology searching.  
923 *Bioinformatics* **25**, 3045-3046 (2009).
- 924 108 Uhlén, M. *et al.* Tissue-based map of the human proteome. *Science* **347**,  
925 1260419 (2015).
- 926 109 Trapnell, C., Pachter, L. & Salzberg, S. L. TopHat: discovering splice junctions  
927 with RNA-Seq. *Bioinformatics* **25**, 1105-1111 (2009).
- 928 110 Bray, N. L., Pimentel, H., Melsted, P. & Pachter, L. Near-optimal probabilistic  
929 RNA-seq quantification. *Nature biotechnology* **34**, 525 (2016).
- 930 111 Blondel, V. D., Guillaume, J.-L., Lambiotte, R. & Lefebvre, E. Fast unfolding of  
931 communities in large networks. *Journal of statistical mechanics: theory and*  
932 *experiment* **2008**, P10008 (2008).
- 933 112 Jones, E., Oliphant, T. & Peterson, P. {SciPy}: open source scientific tools for  
934 {Python}. (2014).
- 935 113 Csardi, G. & Nepusz, T. The igraph software package for complex network  
936 research. *InterJournal, Complex Systems* **1695**, 1-9 (2006).

937 114 Kampf, C., Olsson, I., Ryberg, U., Sjöstedt, E. & Pontén, F. Production of tissue  
938 microarrays, immunohistochemistry staining and digitalization within the  
939 human protein atlas. *Journal of visualized experiments: JoVE* (2012).  
940

941 **Acknowledgements**

942 We acknowledge the entire staff of the Human Protein Atlas program. We  
943 acknowledge Dr. Hisao Masai (Tokyo Metropolitan Institute of Medical Science) for  
944 providing the stable U-2 OS FUCCI cell line. We also acknowledge Dr. Magdalena  
945 Otrocka from the National Laboratories for Chemical Biology at Karolinska Institutet  
946 (LCBKI) for access to imaging infrastructure; Dr. Petter Ranefall and Dr. Carolina  
947 Wählby for providing support for establishing the Cell Profiler pipeline. Funding was  
948 provided by the Knut and Alice Wallenberg Foundation (2016.0204) and Swedish  
949 Research Council (2017-05327) to E.L. The data is available for download in the  
950 supplementary material. Images and Cell Atlas transcriptome and proteome data is  
951 available in the Human Protein Atlas ([www.proteinatlas.org/humancell](http://www.proteinatlas.org/humancell)).

952

953 **Life Sciences Reporting Summary.**

954 Further information on experimental design is available in the Nature  
955 Research Reporting Summary linked to this article.

956 **Data availability statement.**

957 The images from the Human Protein Atlas are available at:  
958 <https://www.proteinatlas.org>. The images from the FUCCI screening is available  
959 upon request, and will be made publicly available in the Human Protein Atlas  
960 database in the release of version 19. The RNA-sequencing data is available at  
961 [www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-2836/](http://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-2836/)

962 **Code availability statement.**

963 The cell profiler pipeline for image analysis and the code for generating the polar-  
964 coordinate pseudotime model and the periodic regression model are available at:  
965 [https://github.com/CellProfiling/fucci\\_screen](https://github.com/CellProfiling/fucci_screen)

966

967 **Author contributions**

968 E.L. conceived the study. D.M., D.P.S. and E.L. developed the methodology for the  
969 study. D.M., L.Å., R.S., C.G, and P.T. carried out the experimental work and  
970 contributed to the cell atlas implementation. D.M., D.P.S. and E.L. carried out data  
971 analysis and investigation. F.D. analyzed the RNA-seq data, M.A., C.Z. and A.M.  
972 carried out analysis for the co-expression network analysis and generated the  
973 corresponding figures. C.L. and F.P. provided the tissue data. D.M. and E.L. wrote the  
974 manuscript. B.A., D.P.S, O.C and P.T revised the manuscript. D.M and D.P.S. created



975 the figures. M.U. initiated the HPA project and provided antibodies. E.L. supervised  
976 and administered the project and acquired funding. All authors reviewed and approved  
977 the final manuscript.

978

979 **Competing interests**

980 The authors declare that they have no conflict of interest.

981

982 **Corresponding author**

983 Correspondence and requests for materials should be addressed to E.L.



984 **Figure legends**

985

986 **Figure 1: Temporal dissection of cell-to-cell heterogeneity of the human**  
987 **proteome**

988 In A-D the target protein is shown in green, microtubules in red and the nucleus in  
989 blue. The scalebars in A-F represents 10 $\mu$ m.

990 A: Example images of proteins with observed cell-to-cell heterogeneity in  
991 immunostained U-2 OS cells in terms of variation in protein abundance (CCNB1) and  
992 in spatial distribution (MRTO4) respectively.

993 B: The RACGAP1 protein shows the same type of cell-to-cell heterogeneity in several  
994 different cell types (U-2 OS, A-431 and MCF7).

995 C: Example images of proteins localized to one of the mitotic substructures  
996 (Kinetochores, Cytokinetic bridge, Cleavage furrow, Mitotic spindle, Midbody ring and  
997 Midbody). INCENP localized to kinetochores in MCF-7 cells, SGO1, KIF20A and  
998 TACC3 localized to the kinetochores, the cleavage furrow and the mitotic spindle in U-  
999 2 OS cells, respectively.

1000 D: Proteins localized to the cytokinetic bridge (BIRC5, GLI4, C12orf66) midbody ring  
1001 (DVL3), and mitotic spindle (KIF11, KNSTRN, MGAT5B and FKBPL) in U-2 OS cells.

1002 E: U-2 OS FUCCI cells allow monitoring the cell cycle by expressing two fluorescently-  
1003 tagged cell cycle markers, CDT1 expressed during G1 phase (red) and Geminin  
1004 expressed during S and G2 phases (green) and their co-expression during G1/S  
1005 transition (yellow). Intensity map of the FUCCI cells defined in three clusters  
1006 representing G1, G1/S and SG2 phases by Gaussian clustering. The polar coordinate  
1007 model transfers the FUCCI marker information into a linear model of pseudo-time.

1008 F: Examples images of the analyzed proteins ANLN, FAM171F1, DUSP18 and alpha-  
1009 tubulin (MT) as negative control combined with their respective boxplot, intensity plot  
1010 and expression profile. In the boxplots the cells expressing the different markers (G1,  
1011 G1S and SG2) are grouped and the mean intensity of the target protein is plotted.  
1012 Kruskal- Wallis statistical test was used to check the significance variation across the  
1013 different groups. In the intensity plot, the cells corresponding to the specific target  
1014 protein is highlighted using a gradient color code of the mean intensity of the target.

1015

1016 **Figure 2: Variation distribution and organelle proteomes**

1017 A: Gene ontology (BP) based enrichment analysis for cell cycle regulated proteins  
1018 showing significantly enriched terms for the domain biological process. Each node  
1019 represents a GO term and edge size corresponds to the number of genes that overlap  
1020 between the two connected gene sets.

1021 B: Scatterplot showing the three different clusters generated by K mean clustering  
1022 based on Kurtosis and skewness as features for the cell cycle regulated proteins (dark  
1023 blue) and the ones not correlated to cell cycle (grey).  
1024 Violin-plots and histograms showing the distinct distributions of the normalized mean  
1025 intensity of each cell per protein of selected examples (GTA6; CCNB1 and DEF6).  
1026 C: Scatterplot of percentage explained variance and Gini index for each investigated  
1027 protein color coded by  $-\log_{10}(\text{FDR})$ .  
1028 D: Bar plot showing the distribution of the cell cycle regulated proteins (dark blue) and  
1029 the ones not correlated to cell cycle (grey) proteins to the different subcellular  
1030 compartments. Asterisk marks statistically significant deviations from the mapped  
1031 human proteome ( $p < 0.01$ ) based on a binomial test.  
1032 E: Examples of cell cycle correlated proteins localized to the different subcellular  
1033 structures respectively: Cytosol, Mitochondria, Nucleus, Nucleoli, Nuclear sub-  
1034 compartments and Secretory pathway. The scalebar represents  $10\mu\text{m}$ . The target  
1035 protein is shown in green and microtubules in red.

1036

### 1037 **Figure 3: Temporal profiles of the cell cycle regulated human proteome**

1038 A: Heat map of the cell cycle regulated proteins showing the relative expression levels  
1039 of the protein across the cell cycle. Yellow represents high expression level and blue  
1040 represents low expression levels. The heatmap is sorted by the timepoint of their peak  
1041 of expression.  
1042 B: Examples of selected cell cycle regulated proteins peaking in different phases of  
1043 the cell cycle. ORC6 peaking in G1, DUSP19 peaking end of G1, BUB1B, DPH2 and  
1044 FLI1 peaking in S&G2 phases.  
1045 C: Protein-Protein interactions network plot of the 464 CCD proteins using the STRING  
1046 database. The proteins with a known association to the cell cycle (GO BP terms) are  
1047 shown as squares.

1048

### 1049 **Figure 4: Gene expression across normal and cancer tissues**

1050 A: Hierarchical clustering of transcript levels (TPM values) for the cell cycle regulated  
1051 proteins derived from bulk RNA sequencing of various normal and cancer tissue types.  
1052 The expression level of the proliferation markers MCM6, CDK1, PCNA, MCM2 and  
1053 KI67 is highlighted on top, as a general measure of the proliferative activity of the  
1054 tissues. Four clusters are identified; Cluster 1 contains normal tissues with low  
1055 proliferative activity, 2 contains cerebral tissues with testis, 3 contains mostly normal  
1056 tissues with midrange expression level of the proliferation markers and 4 contains  
1057 tissues with high expression of the proliferation markers, including tumors.

1058 B: Box plots of the average transcript level corresponding to the cell cycle regulated  
1059 proteins for the four different clusters from A.

1060

1061 **Figure 5: Co-expression networks of the cell cycle regulated proteome**

1062 A: Bar plot showing the path distance from gene co-expression networks between  
1063 novel cell cycle proteins and previously known cell cycle proteins in different normal  
1064 and cancer tissues.

1065 B: Co-expression network analysis of the cell cycle regulated proteins in pancreatic,  
1066 breast and colorectal cancer. The network is clustered into communities using  
1067 mathematical models. Each community has been classified as favorable (green),  
1068 unfavorable (red) or both based on an enrichment / hypergeometric analysis.

1069

1070 **Figure 6: Novel cell cycle regulated proteins as potential clinical biomarkers**

1071 A: Kaplan-Meier plots showing the correlation between survival and gene expression  
1072 (FPKM) for four cell cycle regulated proteins. For RACGAP1 and DLGAP5 a high  
1073 expression was associated to a shorter survival (unfavorable), whereas for SYNE2 and  
1074 FAM50B a high expression was associated to a longer survival (favorable). Purple and  
1075 blue lines show high and low expression, respectively.

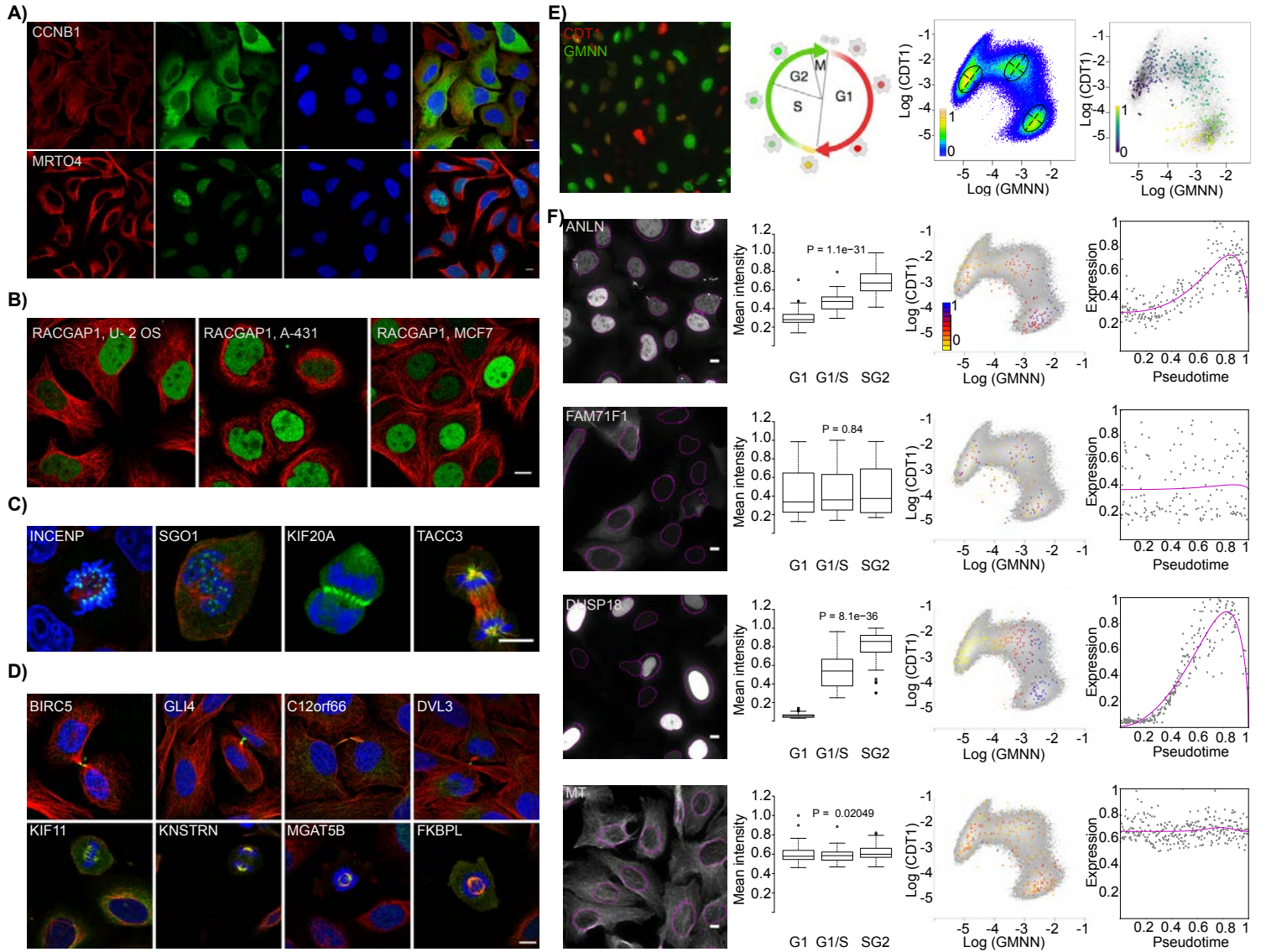
1076 B: Images of immunohistochemically stained proteins in normal tissue. RACGAP1 in  
1077 breast, DLGAP5 in pancreas, SYNE2 and FAM50B in kidney. The target protein is  
1078 shown in brown and the nuclei in blue.

1079 C: Images of immunohistochemically stained proteins in the corresponding tumor  
1080 tissue as to in B. RACGAP1 in breast cancer, DLGAP5 in pancreatic cancer, SYNE2  
1081 and FAM50B in renal cancer. The target protein is shown in brown and the nuclei in  
1082 blue.

1083 D: Temporal interphase expression profile of RACGAP1, DLGAP5, SYNE2 and the  
1084 localization of FAM50B to the Cytokinetic bridge during mitosis.

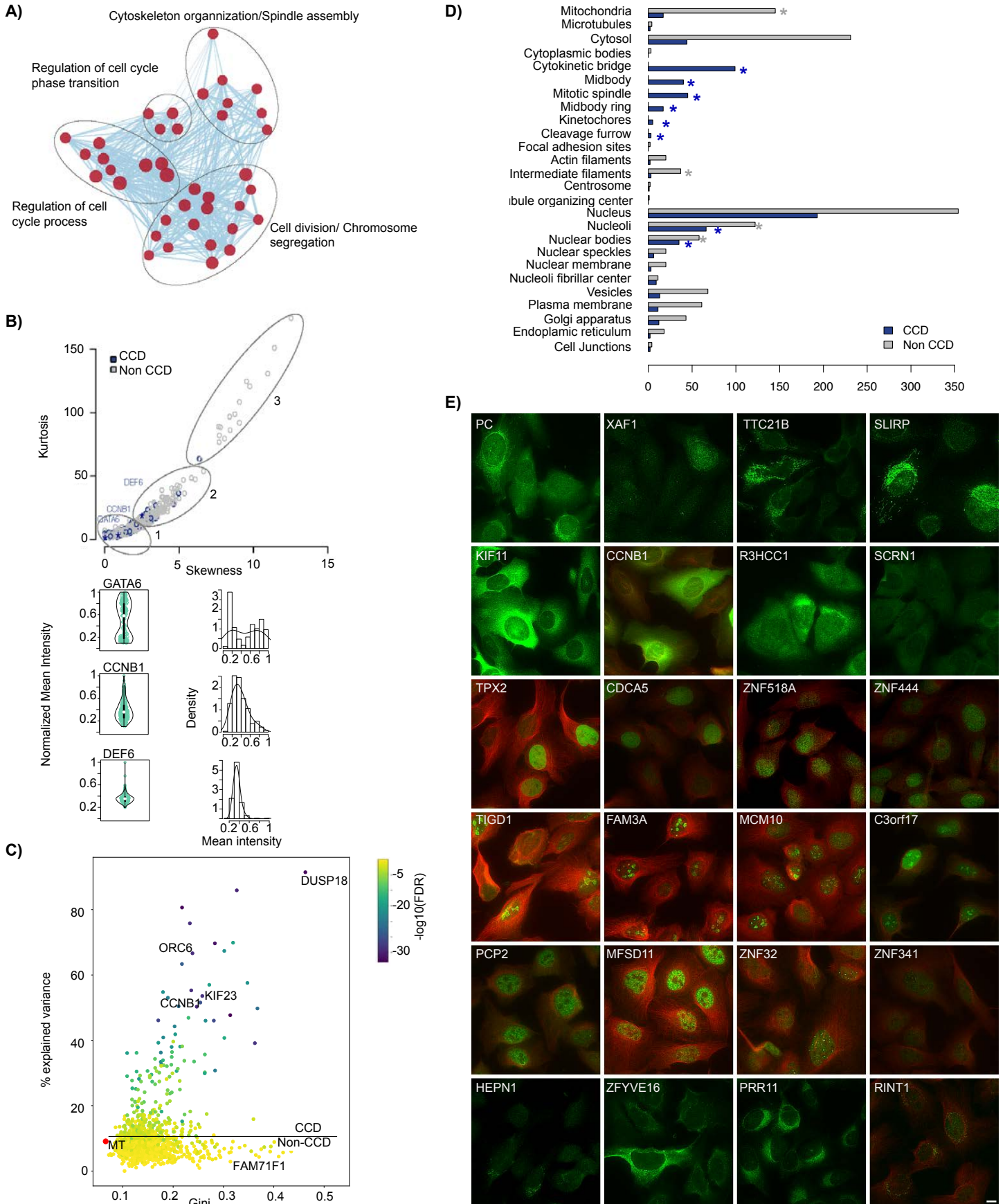
1085 E: Interaction networks for each of the proteins, using a medium confidence score with  
1086 a minimum interaction score of 0.4 and showing not more than 10 interactors.

**Figure 1**





**Figure 2**



**Figure 3**

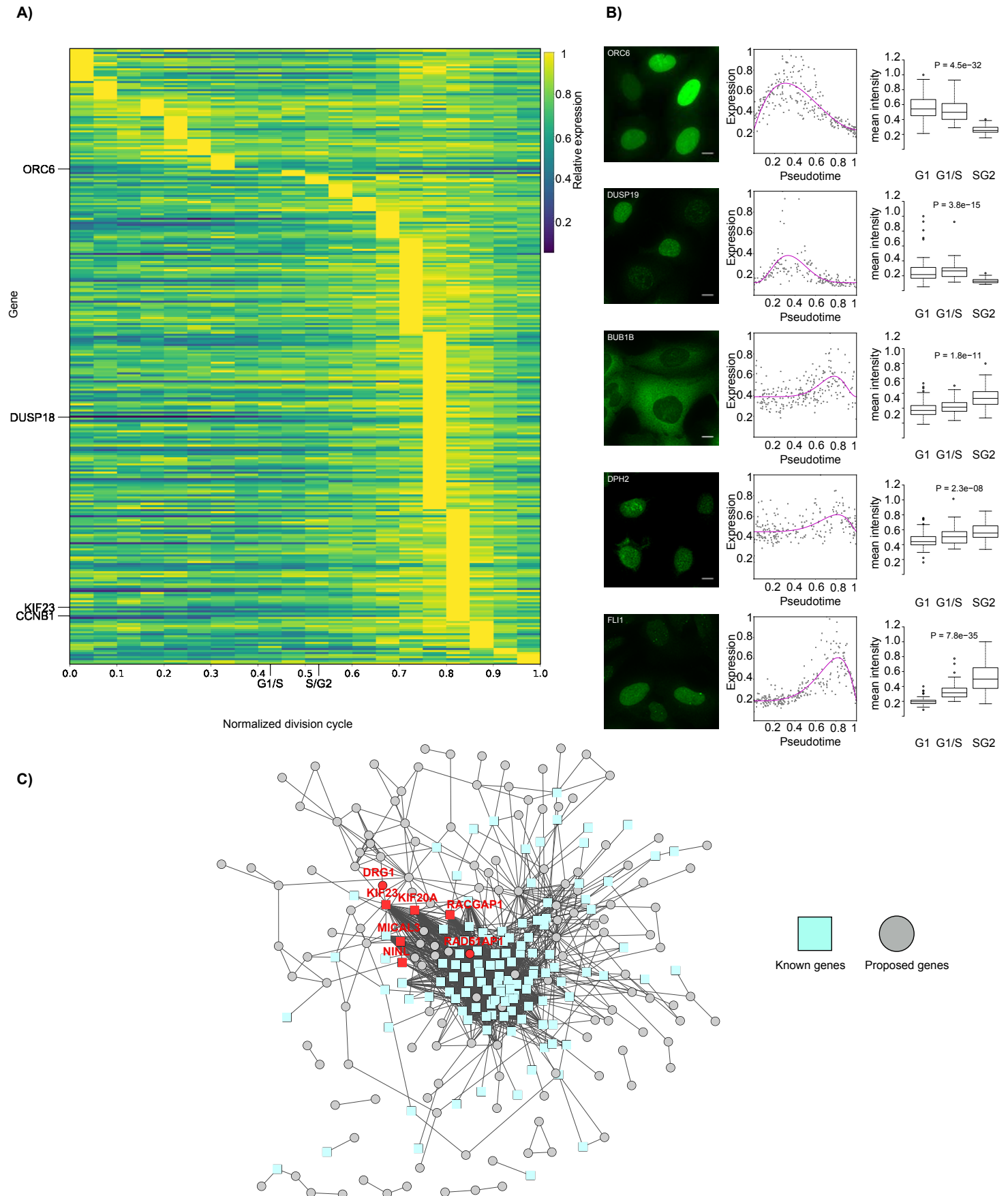
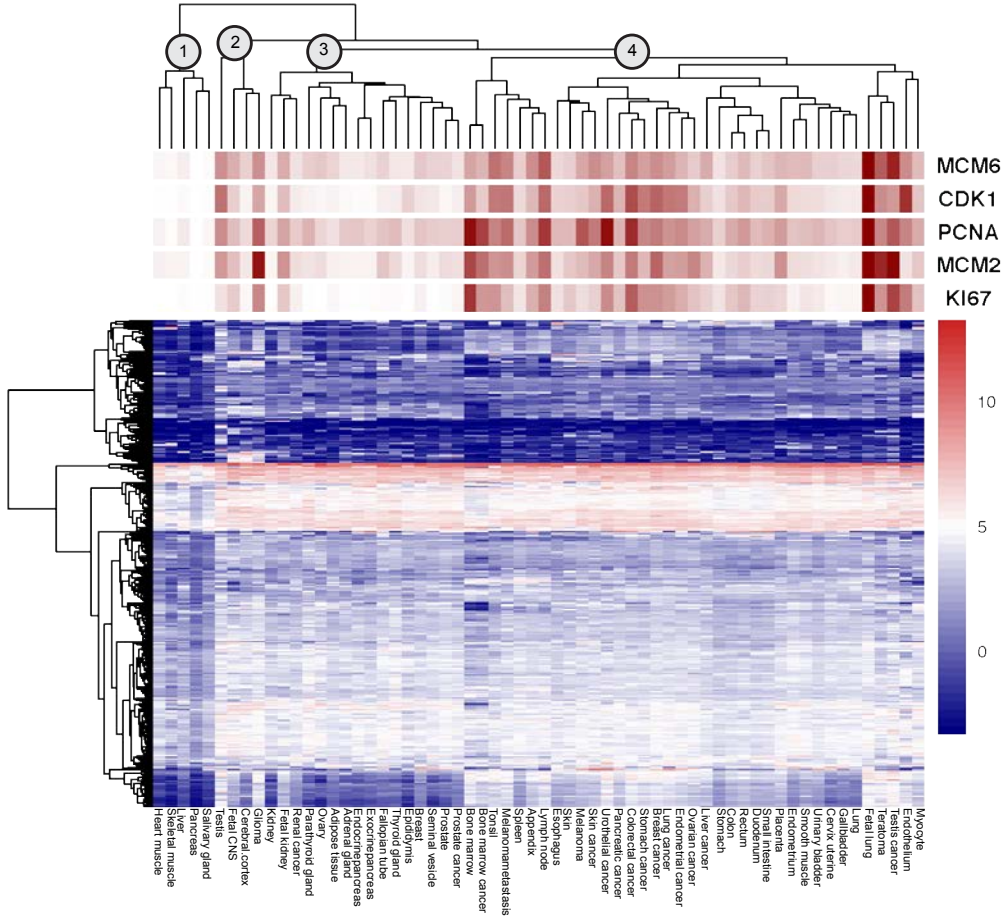
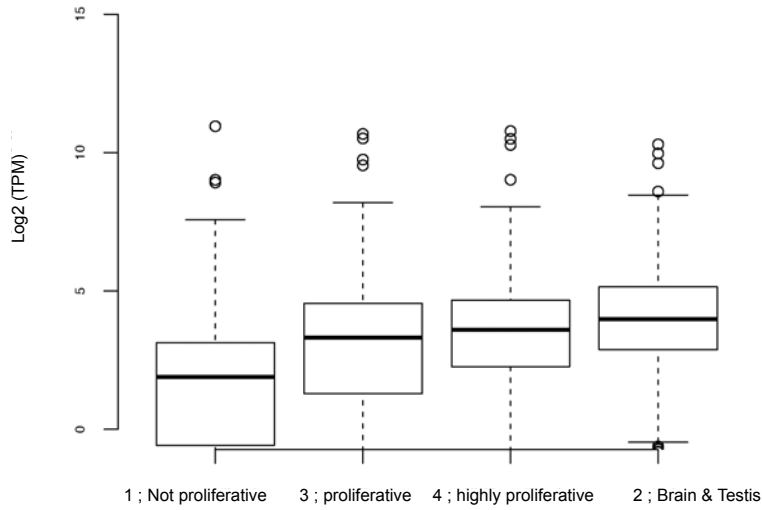


Figure 4

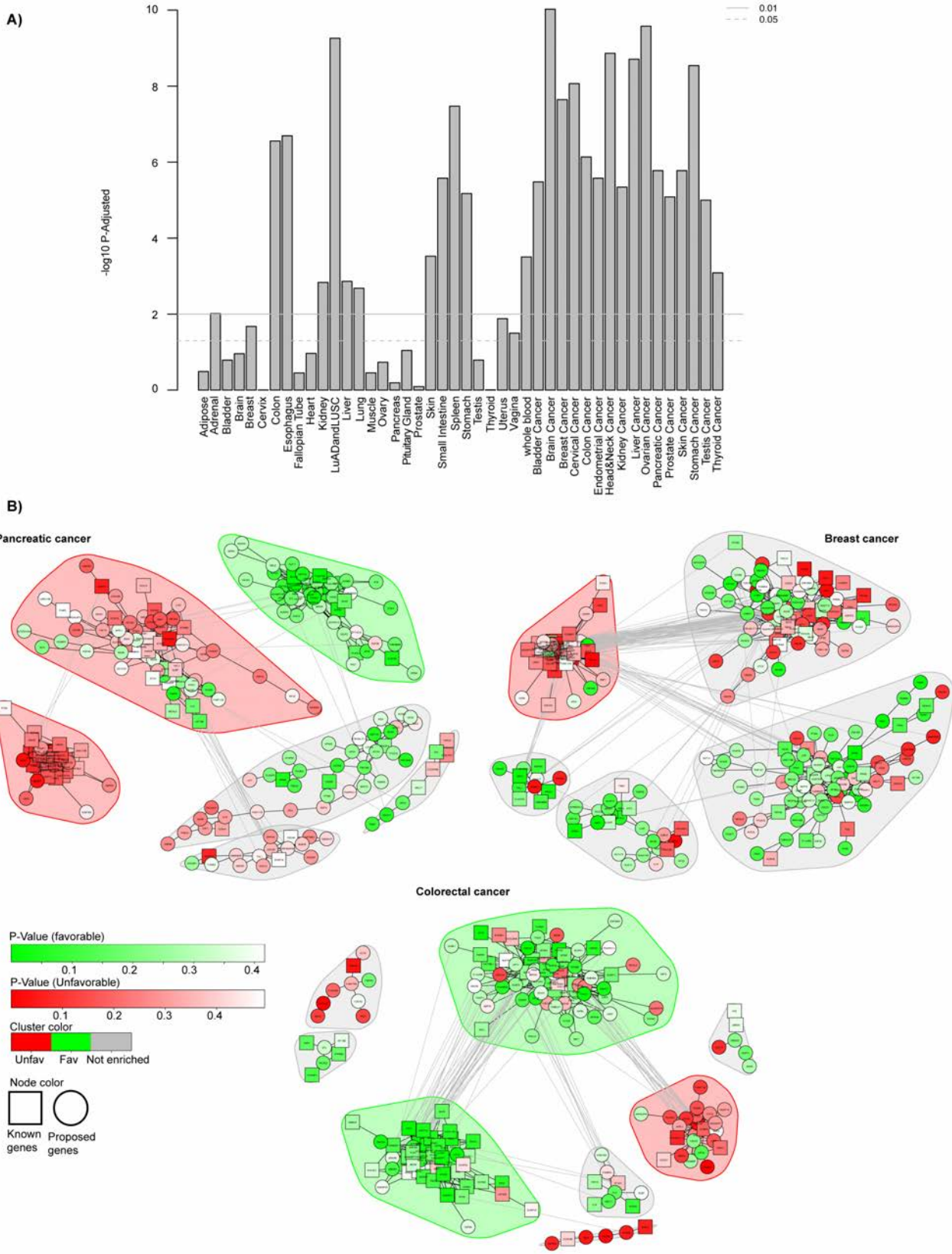
A)



B)



**Figure 5**





**Figure 6**

