Cyclin B/CDK1 and Cyclin A/CDK2 phosphorylate DENR to promote mitotic protein translation and faithful cell division

Katharina Clemm von Hohenberg1,2,3,4, Sandra Müller1,2, Sibylle Schleich1,2, Matthias Meister5, Jonathan Bohlen1,2,3,6,7, Thomas G. Hofmann8 & Aurelio A. Teleman1,2,3

DENR and MCTS1 have been identified as oncogenes in several different tumor entities. The heterodimeric DENR-MCTS1 protein complex promotes translation of mRNAs containing upstream Open Reading Frames (uORFs). We show here that DENR is phosphorylated on Serine 73 by Cyclin B/CDK1 and Cyclin A/CDK2 at the onset of mitosis, and then dephosphorylated as cells exit mitosis. Phosphorylation of Ser73 promotes mitotic stability of DENR protein and prevents its cleavage at Asp26. This leads to enhanced translation of mRNAs involved in mitosis. Indeed, we find that roughly 40% of all mRNAs with elevated translation in mitosis are DENR targets. In the absence of DENR or of Ser73 phosphorylation, cells display elevated levels of aberrant mitoses and cell death. This provides a mechanism how the cell cycle regulates translation of a subset of mitotically relevant mRNAs during mitosis.
The ability of mammalian cells to adjust their gene expression to their environmental and developmental state is vital\(^1\). To this end, one of the most important layers of regulation is mRNA translation\(^2\)-\(^5\). For instance, in response to stress or quiescence, canonical translation is diminished and cellular translation becomes more dependent on noncanonical factors\(^6\)-\(^7\). In proliferating cells, the various phases of the cell cycle likely impose distinct gene expression requirements on the cell, for instance necessitating nucleotide biosynthesis proteins and histones during S-phase or spindle components during mitosis\(^8\)-\(^9\). The molecular mechanisms regulating protein translation at different phases of the cell cycle, such as mitosis, are not well understood.

Mitotic translation has been a topic of interest the last few years\(^10\)-\(^17\). Several studies have investigated the mitotic regulation of translation, thereby identifying mRNAs that are selectively translated during mitosis\(^18\)-\(^23\). Mitosis is a key feature of proliferative cells, such as tumor cells, hence mitotic translation may represent an Achilles’ heel that could be targeted pharmacologically for cancer therapy\(^24\). Specific molecular mechanisms of translation initiation in mitosis, however, have not been identified.

The DENR-MCTS1 heterodimeric protein complex is involved in noncanonical translation initiation\(^25\),\(^26\) and has been linked to cell proliferation and to stress-dependent translation\(^27\)-\(^32\). Biochemically, the DENR-MCTS1 complex promotes recycling of post-termination 40S ribosomes\(^33\),\(^34\) and its related protein elf2d is able to recruit initiator tRNA in an elf2-independent manner on certain viral IRESs\(^35\). We previously showed that the DENR-MCTS1 complex promotes “translation re-initiation” on mRNAs containing upstream Open Reading Frames (uORFs)\(^30\),\(^36\). Translation reinitiation is the process whereby ribosomes initiate a second round of translation after translating a uORF, rather than dissociating from the mRNA\(^37\),\(^38\). This process is relevant after uORFs with a strong Kozak sequence (stuORFs), which causes them to be translated rather than skipped by leaky scanning. In such cases, re-initiation likely involves stabilization of the post-termination 40S on the mRNA, re-recruitment of initiation factors, resumed scanning, and a new round of initiation on the main downstream ORF. By doing so, DENR-MCTS1 promotes translation of mRNAs involved in neurobiology and in cell proliferation\(^30\)-\(^32\). Phenotypically, loss-of-function mutations in DENR are associated with impaired neurocortical migration and brain developmental disorders\(^39\). Overexpression or copy-number gains of DENR and MCTS1, on the other hand, have been described in several tumor entities\(^40\)-\(^42\).

In sum, DENR and MCTS1 act in a pro-proliferative and pro-tumorigenic manner and appear to do so by modulating translation of mRNAs containing uORFs. One important open question is whether and how activity of the DENR-MCTS1 protein complex is regulated. MCTS1 can be phosphorylated by Cdc2 on Ser118 and by MAPK on Thr81, the later of the two leading to stabilization of MCTS1 protein\(^43\). Whether other post-translational modifications affect activity of the complex is not known. We study here regulation of the DENR-MCTS1 complex via phosphorylation. We find that DENR undergoes CDK1- and CDK2-dependent phosphorylation on Ser73 in mitosis to promote translation of specific mitotic target genes that enable timely and faithful cell division and hence mitotic cell survival.

### Results

**DENR is phosphorylated at Serine 73 in mitosis.** To investigate the post-translational regulation of the DENR-MCTS1 complex we undertook three approaches. First, we mutated all sites in the DENR-MCTS1 complex that have been reported on phosphosite.org to be phosphorylated, ubiquitylated or acetylated, and assayed the consequence on DENR-MCTS1 activity using a luciferase translation reporter. Second, we screened all serine/threonine kinases for their ability to phosphorylate DENR-MCTS1 in vitro and further delineated which residues they phosphorylate by mutating DENR and MCTS1. We followed this up by knocking down the kinases in HeLa cells and testing if this affects DENR-MCTS1 activity with the luciferase reporter. All these data are provided to the reader for future reference in the Supplementary Discussion, Supplementary Figs. 8-9, and Supplementary Data 1, 2.

We focus here on the third approach: we aimed to raise phospho-specific antibodies against six phospho-sites that have been detected by mass spectrometry and reported at phosphosite.org (DENR Ser20, Thr69, Ser73, and Ser189 and MCTS1 Thr117 and Ser118). We thereby successfully generated an antibody that specifically detects DENR when phosphorylated on Ser73 (Supplementary Fig. 1a). In agreement with this, we analyzed endogenous DENR-MCTS1 immuno-purified from untreated HeLa cells by mass spectrometry and observed phosphorylation of DENR on Ser73 and Thr69 (Supplementary Data 1), confirming that DENR is phosphorylated at Ser73 in vivo. (Generation of phosphoantibodies against the other five sites was not successful, precluding us from studying them further in vivo.) We screened different stresses (ER stress, apoptosis, DNA damage, amino acid and glucose starvation) and environmental conditions (different densities, different cell cycle phases), and discovered that DENR is phosphorylated at Ser73 in a cell cycle-dependent manner (Fig. 1): Synchronization of U2OS cells in vitro (Fig. 1d). (The pDENR staining at the cytokinetic bridge is unspecific as it does not drop upon DENR knockdown, Supplementary Fig. 1c). Also total levels of the DENR-MCTS1 complex vary somewhat throughout the cell cycle, accumulating from G1 to mitosis and decreasing at mitotic exit (Fig. 1a, c). The increase in Ser73 phosphorylation is visible, however, also when normalized to total DENR protein levels (Fig. 1b).

**CDK1/Cyclin B1 and CDK2/Cyclin A2 phosphorylate DENR on Ser73 in mitosis.** We next aimed to identify the kinase responsible for phosphorylating DENR on Ser73 during mitosis. We noticed that Ser73 is positioned within a CDK target consensus motif\(^44\)-\(^46\) (Supplementary Fig. 2a). We, therefore, tested a panel of CDKs for their ability to phosphorylate DENR Ser73 by in vitro kinase assay and found that both CDK1/Cyclin B1 and CDK2/Cyclin A2 phosphorylate DENR Ser73 in vitro (Fig. 2a, b, Supplementary Data 2). Since both CDK1/Cyclin B1 and CDK2/Cyclin A2 are most active in mitosis or G2/M, these data fit with DENR Ser73 phosphorylation being highest in mitosis (Fig. 1). Interestingly, the differential phosphorylation of DENR-MCTS1 by CDK2 bound to Cyclin A2 (active in G2/M) versus Cyclin E1 (active in G1/S) is a nice example of a cyclin providing substrate specificity to CDK2 (also shown for Cyclin D/CDK4 in\(^47\)). These results fit with the principle that cyclins modulate Cdk specificity, and that as cells progress through the cell cycle towards mitosis Cyclin/CDK complexes become progressively more specific for the consensus CDK phosphorylation motif\(^45\)-\(^47\).

---
To test if CDK1 phosphorylates Ser73 in vivo, we synchronized HeLa cells in G2 (lane 2, Fig. 2c), released them to allow entry into mitosis (lane 3) and then added the CDK1 inhibitor RO3306 (lanes 4–6). This revealed a reduction of DENR Ser73 phosphorylation upon CDK1 inhibition (Fig. 2c, d). A similar effect was observed in U2OS cells (Supplementary Fig. 2b).

We next tested if also CDK2 phosphorylates Ser73 in vivo. DENR Ser73 phosphorylation was reduced in mitotic U2OS cells in a dose-dependent manner in response to the CDK2 inhibitors seliciclib or A-674563 (Fig. 2e, f, Supplementary Fig. 2c), suggesting that CDK2 is also phosphorylating Ser73 in cells. For this experiment, in order to inhibit CDK2/CycA we released cells from a double-thymidine S-phase block, waited for 9 h (Fig. 1a), and then added seliciclib or A-674563. However, to rule out the possibility that the reduction in Ser73 phosphorylation is a secondary consequence of impaired mitotic entry due to reduced CDK1 activation by CDK2/Cyclin A,

To test if CDK1 phosphorylates Ser73 in vivo, we synchronized HeLa cells in G2 (lane 2, Fig. 2c), released them to allow entry into mitosis (lane 3) and then added the CDK1 inhibitor RO3306 (lanes 4–6). This revealed a reduction of DENR Ser73 phosphorylation upon CDK1 inhibition (Fig. 2c, d). A similar effect was observed in U2OS cells (Supplementary Fig. 2b).

We next tested if also CDK2 phosphorylates Ser73 in vivo. DENR Ser73 phosphorylation was reduced in mitotic U2OS cells in a dose-dependent manner in response to the CDK2 inhibitors seliciclib or A-674563 (Fig. 2e, f, Supplementary Fig. 2c), suggesting that CDK2 is also phosphorylating Ser73 in cells. For this experiment, in order to inhibit CDK2/CycA we released cells from a double-thymidine S-phase block, waited for 9 h (Fig. 1a), and then added seliciclib or A-674563. However, to rule out the possibility that the reduction in Ser73 phosphorylation is a secondary consequence of impaired mitotic entry due to reduced CDK1 activation by CDK2/Cyclin A, we treated non-synchronized U2OS cells briefly for one hour with the CDK2 inhibitor seliciclib and then analyzed only the cells that were visibly in mitosis. Representative images from n = 3 independent biological replicates are shown. Scale bars indicate 10 µM.

To test if CDK1 phosphorylates Ser73 in vivo, we synchronized HeLa cells in G2 (lane 2, Fig. 2c), released them to allow entry into mitosis (lane 3) and then added the CDK1 inhibitor RO3306 (lanes 4–6). This revealed a reduction of DENR Ser73 phosphorylation upon CDK1 inhibition (Fig. 2c, d). A similar effect was observed in U2OS cells (Supplementary Fig. 2b).

We next tested if also CDK2 phosphorylates Ser73 in vivo. DENR Ser73 phosphorylation was reduced in mitotic U2OS cells in a dose-dependent manner in response to the CDK2 inhibitors seliciclib or A-674563 (Fig. 2e, f, Supplementary Fig. 2c), suggesting that CDK2 is also phosphorylating Ser73 in cells. For this experiment, in order to inhibit CDK2/CycA we released cells from a double-thymidine S-phase block, waited for 9 h (Fig. 1a), and then added seliciclib or A-674563. However, to rule out the possibility that the reduction in Ser73 phosphorylation is a secondary consequence of impaired mitotic entry due to reduced CDK1 activation by CDK2/Cyclin A, we treated non-synchronized U2OS cells briefly for one hour with the CDK2 inhibitor seliciclib and then analyzed only the cells that were visibly in mitosis. Representative images from n = 3 independent biological replicates are shown. Scale bars indicate 10 µM.
Serine 73 phosphorylation protects DENR from mitotic degradation. To characterize the functional effects of DENR phosphorylation at Ser73, we tested whether a non-phosphorylatable mutant, DENR<sup>S73A</sup>, is impaired in either protein stability or interaction with its binding partner MCTS1<sup>54</sup>. To this end, we expressed FLAG-tagged DENR<sup>WT</sup> or DENR<sup>S73A</sup> in cells and assayed MCTS1 binding by co-immunoprecipitation. In asynchronous cells, the vast majority of which are in interphase, the levels of FLAG-DENR<sup>S73A</sup> protein were similar to those of FLAG-DENR<sup>WT</sup> protein, and both co-immunoprecipitated MCTS1 equally well (Fig. 3a). This is consistent with the fact that Ser73 is not phosphorylated in interphase cells, hence mutation of Ser73 to alanine has little functional consequence in this context. In contrast, in mitotically synchronized cells we observed dramatically reduced levels of DENR<sup>S73A</sup> protein compared to wildtype protein, and correspondingly reduced co-immunoprecipitating MCTS1 (Fig. 3b). A cycloheximide time course in mitotically synchronized cells revealed that DENR<sup>S73A</sup> protein is less stable than wildtype protein (Fig. 3c). Of note, both DENR<sup>WT</sup> and DENR<sup>S73A</sup> proteins are stable in interphase cells (cycloheximide time course in Supplementary Fig. 3a) indicating that Ser73 phosphorylation protects DENR from a degradation mechanism that is most active in mitosis. Since we previously showed that DENR and MCTS1 are interdependent on each other for protein stability, and that DENR mutants that cannot bind MCTS1 are unstable<sup>54</sup>, this means that the S73A mutation could either directly affect DENR stability, or indirectly affect DENR stability by impairing MCTS1 binding. To distinguish these two options, we aimed to test whether proteasomal inhibition with MG132 stabilizes DENR<sup>S73A</sup> protein, which was not the case (not shown). Sequence analysis revealed that DENR contains a caspase cleavage site at a.a. 26 (https://web.expasy.org/peptide_cutter/). Although caspases are well known to be active during apoptosis, caspase activity is also involved
in mitosis of normally proliferating cells.\(^{55}\) Mutation of this putative caspase cleavage site mildly restored the stability of DENR\(^{573A}\) in mitosis (Fig. 3d, Supplementary Fig. 3b) as did the pan-caspase inhibitor Z-VAD-FMK (Supplementary Fig. 3c,d). The fact that stability is not fully rescued suggests that additional cleavage sites or modes of degradation are involved. Nonetheless, the partial recovery of DENR protein stability was accompanied by a corresponding partial recovery of co-immunoprecipitated MCTS1 (Fig. 3d, Supplementary Fig. 3c) suggesting that Ser73 primarily affects DENR protein stability, not MCTS1 binding. Furthermore, a quantification of the amount of MCTS1 binding per DENR shows that DENR\(^{573A}\) and DENR\(^{D26E,S73A}\), if anything, both bind more MCTS1 than DENR\(^{WT}\) (Fig. 3d). Consistent with this, DENR binds to MCTS1 during interphase when it is not phosphorylated (Fig. 3a) and

---

**Fig. 3 Ser73 phosphorylation protects DENR from mitotic degradation.**

**a** Immunoprecipitation of overexpressed FLAG-DENR\(^{WT}\) or FLAG-DENR\(^{573A}\) from asynchronous HeLa cells shows MCTS1 binding to both DENR versions. The Western Blot shown is representative of \(n = 3\) independent biological replicates. **b** Immunoprecipitation of overexpressed FLAG-DENR\(^{WT}\) or FLAG-DENR\(^{573A}\) from mitotically synchronized U2OS cells shows a strong loss of DENR\(^{573A}\) protein. The Western Blot shown is representative of \(n = 3\) independent biological replicates. **c** FLAG-DENR\(^{573A}\) is less stable than FLAG-DENR\(^{WT}\) in mitotic U2OS cells. Cells were transfected to express either version of DENR, then synchronized in mitosis, harvested by shake-off, and finally exposed to cycloheximide for the indicated times and analyzed by Western Blot. Quantification of FLAG signal normalized to CUL1 from \(n = 3\) independent biological replicates. Nonlinear regression lines are shown and Extra sum-of-squares F test of one phase decay constant (K) was performed with **\(p = 0.009\).** **d** Partial restabilization of DENR by the D26E mutation re-establishes binding to MCTS1 in mitotic U2OS cells. This experiment was performed as described in **b** using in addition the non-phosphorylatable, non-cleavable mutant DENR\(^{D26E,S73A}\) and STLC instead of nocodazole as a mitotically synchronizing agent. Right panel: quantification of MCTS1 binding to DENR versions. \(n = 5\) independent biological replicates for DENR\(^{WT}\) and DENR\(^{573A}\) and \(n = 2\) for DENR\(^{D26E,S73A}\). **e** Destabilization of DENR at mitotic exit is partly caspase-dependent. U2OS cells expressing a FLAG-DENR\(^{WT}\) cDNA construct were synchronized in mitosis (red) and then released from mitosis after addition of DMSO (gray) or the pan caspase inhibitor Z-VAD-FMK 40 \(\mu\)M (light-red). Samples were collected at the time of release (before addition of DMSO or Z-VAD-FMK) or 2 h later and analyzed by Western blot. Quantification of total FLAG-DENR signal using ImageJ (v1.50i or 1.53c), normalized to CUL1 and then to the 0 h time point from \(n = 3\) independent biological replicates is shown. Error bars indicate standard deviations and unpaired t-test was performed with *\(p = 0.01\).
recombinant DENR binds strongly to MCTS1 in bacteria, where Ser73 is not phosphorylated.

Ser73 phosphorylation drops when cells exit mitosis (Fig. 1a) starting from anaphase onwards (Fig. 1d), and this drop coincides with a drop in total DENR levels (Fig. 1a, c). We asked if this drop in DENR protein levels during mitotic exit is caspase-mediated. Indeed, the caspase inhibitor Z-VAD-FMK partially rescued the drop in DENR levels as cells exit mitosis (Fig. 3e). In summary, phosphorylation of DENR at Ser73 prevents degradation of DENR during the first stages of mitosis, from prophase to metaphase, while in later mitotic stages and mitotic exit DENR protein is dephosphorylated and cleaved partly in a caspase-dependent manner.

**pDENR (Ser73) induces mitotic translation of DENR target genes.** Since DENR is a translation re-initiation factor, we asked what impact Ser73 phosphorylation has on translation. The data presented above raise the possibility that in mitosis DENR phosphorylation on Ser73 protects it from degradation because DENR is required to drive translation of mitotically relevant mRNAs. We recently performed a RiboSeq analysis of DENRWT and DERNKO HeLa cells to identify DENR target mRNAs that require DENR for optimal translation. Interestingly, amongst the top DENR targets we found many genes with mitotic functions, primarily concerning the mitotic cytoskeleton (e.g., 10 genes in the top 30, Supplementary Data 3). We first studied translation of these target genes by measuring activity of luciferase reporters carrying the 5’UTRs of these mRNAs (Fig. 4a). The advantage of these luciferase reporters is that they control for transcriptional effects (via the FLuc normalization control reporter) as well as for protein stability effects (via the negative control RLuc reporter). These luciferase assays confirmed that the 5’UTRs of these mitotic genes impart DENR-dependent translation, decreasing in DENR knockout cells, and returning to control levels in DENR knockouts transfected to re-express DENR (Supplementary Fig. 4a). We tested if translation of these DENR target reporters increases in mitosis, by comparing their activity in asynchronous cells versus cells synchronized in mitosis. This revealed that indeed translation of most reporters increased in mitotic cells by 1.5 to 2-fold, both for DENR targets with mitotic functions (Fig. 4b) as well as DENR targets with no known link to mitosis, which we had previously validated as bona fide DENR targets (Fig. 4c). Most likely, this assay which is performed on the bulk population of cells underestimates the magnitude of the real mitotic effect due to the fact that available protocols only cause a minor fraction of the cells to synchronize in mitosis. In contrast to reporters carrying the 5’UTRs of endogenous DENR targets, a synthetic reporter carrying a stuORF did not increase translation in mitosis (Fig. 4b), suggesting it lacks an element required for mitotic translation. The increased translation of these reporters in mitosis is due to elevated mitotic DENR activity because it is blunted in DENR knockdown cells (Fig. 4d–g, Supplementary Fig. 4b–d), and it is reversed by CDK2 inhibition with seliciclib (Supplementary Fig. 4e). The mitotic induction of the DUSP4 and DCLK5 reporters was abolished when the ATGs of the uORFs were mutated (Supplementary Fig. 4f), consistent with DENR-MCTS1 promoting translation re-initiation after uORFs.

In sum, these data indicate that DENR activity increases in mitosis compared to interphase.

We previously identified DENR targets by performing RiboSeq on asynchronous HeLa cells. To test whether DENR promotes translation of the same set of target mRNAs in mitotic cells as in interphase cells, or whether phosphorylation on Ser73 might affect the DENR target set, we performed RiboSeq and RNA-seq on mitotic and interphase DENRWT and DERNKO cells (Supplementary Data 4). Agents used to synchronize cells in mitosis such as nocodazole, and to a lesser extent the Eg5 inhibitor STLC, induce cell stress and hence perturb the translatome. We therefore used alternative methods to enrich or deplete mitotic cells from our populations. For the mitotic population, we synchronized HeLa cells using a double thymidine block and collected them for analysis 9 h after the second S phase release. For the interphase sample, we shook off and discarded mitotic cells, which anyways constitutes a small minority (<5%) of an asynchronous population. A Z-vs-Z analysis of the RNA-seq data from wildtype cells identified 1326 transcripts corresponding to 694 genes with elevated mRNA levels in mitosis (Supplementary Fig. 5a, Supplementary Data 5) and none with reduced mRNA levels, probably due to the short duration of mitosis. A comparison of translation efficiency (ribosome footprints normalized to mRNA) between mitotic and interphase cells identified 266 transcripts (181 genes) that were translationally up-regulated in mitotic cells and 1090 transcripts (696 genes) that were translationally down-regulated in mitotic cells (Supplementary Fig. 5b, Supplementary Data 6). A comparison of translation efficiency in DERNKO versus DENRWT cells identified 1108 transcripts (653 genes) and 990 transcripts (576 genes) as DENR targets in interphase and mitotic cells, respectively (Supplementary Fig. 5c, d, Supplementary Data 7). Interestingly, a comparison of the change in translation efficiency upon DENR loss in interphase versus mitotic cells showed a good correlation (Supplementary Fig. 5e) suggesting that in general the mRNAs that are DENR targets in mitosis are also DENR targets in interphase, although to varying degrees. A hand-full of mRNAs appear to be strong DENR targets in interphase cells but not in mitotic cells (Supplementary Fig. 5e), but these genes are amongst the ones with the strongest drop in translation efficiency transcriptome-wide in wildtype mitotic cells compared to interphase cells (e.g., IL11). This suggests that these mRNAs are not well translated in mitosis, and hence are not sensitive to DENR loss. In sum, since the set of DENR target mRNAs does not change during mitosis, this suggests that phosphorylation of DENR on Ser73 in mitosis affects its stability but not another aspect of its function. Interestingly, of the 266 transcripts that are translationally up-regulated during mitosis, 114 are DENR targets. This fraction (~40%) is significantly higher than the 10% of transcripts that are DENR targets in interphase cells (p = 0.0 by binomial distribution). Hence DENR appears to play a particularly important role in mitotic translation.

To investigate if DENR-dependent translation affects the level of its targets during mitosis, we first performed Western Blot analysis of asynchronous and mitotic cells and found that the level of DENR target proteins increases in mitosis (Supplementary Fig. 6a). Interestingly, we observed that the mobility of DENR protein on the SDS-PAGE gel shifts completely upwards to a slower migrating form in mitotically synchronized cells (Supplementary Fig. 6a), suggesting that DENR is highly phosphorylated in mitotic cells. We next asked whether this increase in protein levels is DENR dependent. To this end, we immunostained unsynchronized DENRWT or DERNKO cells for target proteins and quantified protein levels specifically in mitotic cells identified by chromosome morphology. This revealed a significant decrease in target protein levels upon loss of DENR in mitotic cells (Fig. 4h, i, Supplementary Fig. 6b, c). The same could be observed by western blotting lysates of mitotically synchronized cells (Supplementary Fig. 6d). A drop in target protein levels was also present in asynchronous cells, although less dramatic than in mitotic cells (Supplementary Fig. 6e). These drops in target protein levels were rescued by reconstituting the DERNKO cells with a DENRWT expression construct (Supplementary Fig. 6d), confirming they are on-target effects. When,
however, reconstitution was performed with the non-phosphorylatable DENR\textsubscript{S73A} mutant, rescue of target protein levels in mitotic cells was impaired (compare lane 2 to lane 1 in Fig. 4j, k, Supplementary Fig. 6f). Since DENR is phosphorylated at Ser73 only in mitotic cells, this confirms there is DENR-dependent translation ongoing during mitosis. In accordance with our previous findings showing that mutation of the caspase cleavage site partially reconstitutes DENR stability, mitotic target protein levels are also partially rescued when the non-phosphorylatable, non-cleavable version of DENR (DENR\textsubscript{D26E,S73A}) is expressed in DENR\textsuperscript{KO} cells (Fig. 4j, k). In sum, our findings show that there is a set of mitotically relevant mRNAs, such as CDKL5.
mRNA, that are translated in a DENR-dependent manner in mitosis.

**pDENR (Ser73) prevents aberrant mitosis and promotes faithful cell division.** Since the DENR-MCTS1 complex promotes translation of mRNAs with mitotic functions, we asked if DENR-MCTS1 is required for proper mitosis. To this end, we performed two-dimensional flow cytometry of unsynchronized DENRWT or DENRKO cells and observed a four-fold accumulation of DENRKO cells in mitosis (Fig. 5a, Supplementary Fig. 7a), raising the possibility of a defect in progression through mitosis. Indeed, an elevated number of mitotic DENRKO cells undergo apoptosis (Fig. 5b, Supplementary Fig. 7a). This suggests that loss of DENR leads to slower mitotic progression and increased mitotic failure, likely contributing to the reduced proliferation rate of DENRKO cells which we previously reported. We then examined whether there are any mitotic defects in DENRKO cells (Fig. 5c, d). While the fraction of early mitotic phases (prophase, prometaphase, metaphase) is not significantly influenced by the absence of DENR, there is a reduction in the number of cells in the late mitotic phases (anaphase, telophase), and instead an accumulation of atypical mitotic figures and mitotic blebs, representative of mitotic cell death (Fig. 5c, d). Taken together with the increase in the fraction of mitotic cells, these findings indicate that the early phases of mitosis are prolonged in DENRKO cells, and in some DENRKO cells mitotic cell death occurs after anaphase onset. This effect can be rescued by re-expression of DENRWT in DENRKO cells, but not by the non-phosphorylatable DENR573A mutant, and only partially by the non-phosphorylatable, non-cleavable DENRΔ26E,S73A mutant (Fig. 5c, d). We observed a similar phenotype in asynchronous DENR·MCTS1 knockout U2OS cells (Supplementary Fig. 7b), ruling out a cell type-specific defect in HeLa cells. Interestingly, we observed an increased fraction of irregular and multinuclear spindles in DENRKO compared to DENRWT cells (Supplementary Fig. 7c), which could explain in part the mitotic failure in DENR-depleted cells. Since DENRKO cells reconstituted with DENR573A have mitotic defects, and since Ser73 is specifically phosphorylated in mitosis, these data show that the translation occurring during mitosis is important for mitosis to proceed correctly. Furthermore, the DENR/MCTS1 complex appears to be playing a particularly important role during mitosis because 114 of the 226 transcripts which are translationally upregulated during mitosis are DENR targets (i.e., 40%), which is a significantly larger fraction than the 10% of transcripts that are DENR targets in interphase cells (p = 0 by binomial distribution).

In sum, phosphorylation of DENR at Ser73 and stabilization of the DENR-MCTS1 complex in the early phases of mitosis is important to guarantee efficient cell division and to prevent aberrant mitosis. It is likely that the mitotic defects that result from loss of DENR or DENR Ser73 phosphorylation (Fig. 5d) reflect a combined contribution of multiple DENR targets, since multiple DENR target mRNAs are insufficiently translated during mitosis in the absence of DENR activity (Fig. 4, Supplementary Fig. 6), and multiple DENR targets have mitotic functions including spindle dynamics (Supplementary Data 3).

**Discussion** We have identified a signaling pathway promoting mitotic translation of genes that are crucial for faithful and timely cell division (Fig. 5b). At the onset of mitosis CDK1/Cyclin B and CDK2/Cyclin A phosphorylate the non-canonical translation initiation factor DENR at serine 73 and thereby protect DENR from degradation (Figs. 1–3). DENR then acts to promote translation of a set of target genes that are known to be involved in proper cell division (Fig. 4), thereby supporting mitotic progression and preventing aberrant mitosis and mitotic cell death (Figs. 5, 6). Interestingly, almost half of all mRNAs with increased translation in mitosis depend on DENR for their translation, suggesting that DENR activity is particularly important during this phase of the cell cycle.

Previous studies have identified mRNAs whose translation is upregulated during mitosis, however, the functional significance of this up-regulation for mitotic progression was difficult to test, given that there was no intervention known to specifically block mitotic translation. Since DENR phosphorylation on Ser73 is specific for mitosis, this enables such an intervention. The fact that reconstitution of DENRKO cells with DENR573A does not rescue their mitotic defects indicates that the translation occurring during mitosis is indeed important for mitosis itself.

This regulatory mechanism is initiated through DENR phosphorylation by CDK1 and CDK2. Interestingly, we find that Cyclin A plays an important role in enabling CDK2 to phosphorylate DENR, likely via substrate recognition, because CDK2 bound to Cyclin A phosphorylates DENR in vitro more efficiently than CDK2 bound to Cyclin E (Fig. 2a, b). This is consistent with the fact that in vivo DENR Ser73 is phosphorylated in mitosis, when CycA/CDK2 is active, but not during S-phase, when CycE/CDK2 is active (Fig. 1a).

CDK2 and CDK1 are vital in tumors and therefore attractive targets for cancer therapy. Different chemical CDK2 inhibitors have been developed and the most advanced one, seliciclib, is
currently being tested in clinical studies. While its molecular action has only been partially discovered, seliciclib is known to prevent faithful cell division and cause cell death in mitosis, due to a type of uncoordinated cellular division called anaphase catastrophe\(^5\). From the fact that DENR loss of function phenocopies this mitotic failure (Fig. 5, Supplementary Fig. 7), it is possible that part of the effect of seliciclib might be due to inhibition of DENR phosphorylation at Ser73 and accordingly impaired translation of DENR-dependent mitotic target genes that act towards coordinated cell division and cytokinesis.

In line with our observations that DENR·MCTS1 protein complex is essential for faithful cell division, mitotic catastrophe and delayed cytokinesis, there has been evidence in cells depleted of MCTS1\(^6\), however, the mechanism of this MCTS1 effect was unknown. Since DENR and MCTS1 are co-dependent on each other for protein stability and act together as one functional heterodimeric complex, it is likely that reduced translation of the mitotic target genes as we describe here is one contributing factor.

We observed that DENR is stabilized by phosphorylation at Serine 73 in mitosis. When not phosphorylated, DENR is degraded in a manner that is partly caspase-dependent (Fig. 3d, e, Supplementary Fig. 3). Although caspases are well known to be active during apoptosis, there is also increasing evidence of non-apoptotic, cell cycle-related functions for caspases\(^6\). Caspases 2 and 7 have been described to play a role in mitotic exit\(^5\). Consistent with this, we observed caspase-dependent degradation of DENR during the late phases of mitosis (anaphase to telophase). Interphase DENR, however, is very stable independently of its phosphorylation status (Fig. 3a, Supplementary Fig. 3a). This suggests that caspase activity towards DENR increases in mitosis. We noticed that caspases 2 and 3 are described as being nuclear.

Fig. 5 pDENR (S73) prevents aberrant mitosis and promotes faithful cell division. DENR knockout cells have an elevated proportion of cells in mitosis (a) that are apoptotic (b). Unsynchronized HeLa DENRWT (gray) or DENRKO (white) cells were fixed, permeabilized, stained for cleaved caspase 3 (CC3) and pH3 (S10) and then analyzed by flow cytometry. Shown are means, normalized to DENRWT, and standard deviations as error bars from \(n=3\) biological replicates. One sample t-test with \(p=0.01\) for both a and b. DENR knockout cells have a reduced proportion of cells in late mitotic phases and elevated aberrant mitoses and mitotic cell death, both of which are rescued by reconstitution with DENRWT (checkered) but not with DENR\(^S73A\) (finely checkered light) and only partially with DENR\(^D26E\,573A\) (finely checkered dark) protein. Unsynchronized HeLa DENRWT (filled) or DENRKO (empty) cells were transfected with empty expression vector (EV) or one of the indicated FLAG-tagged constructs for 72 h, then fixed and stained with DAPI. c Sample images. Mitotic cells are outlined by white circles. Examples of failed mitoses (multipolar spindles, mitotic blebs/mitotic cell death) are encircled in orange. Scale bars indicate 10\(\mu\)M. d Quantification of mitotic phases and mitotic defects, assessed via DAPI stain. Displayed are means of fractions of all mitotic cells, and standard deviations as error bars from \(n=3\) independent biological replicates. Early mitosis comprises prophase, prometaphase and metaphase, late mitosis comprises anaphase and telophase and failed mitosis comprises aberrant mitosis or mitotic blebs. Two-way ANOVA was performed for Early mitosis (blue) and unpaired t-tests were performed for Late mitosis (green) and Failed mitosis (orange).
mutated all amino acids in DENR or MCTS1 which were by in vitro kinase assay, we knocked down all hits from this DENR·MCTS1 complex. We tested all serine/threonine kinases and comprehensive screens for pathways that might regulate the mitosis, we believe the most likely explanation is that it is not cannot affect them. Therefore, although we do not know why our not translated in mitosis, the presence or absence of DENR transcriptome-wide in wildtype mitotic cells compared to inter-

As of anaphase, DENR phosphorylation on Ser73 decreases, concomitant with a caspase-dependent decrease in DENR protein levels as cells exit mitosis.

https://www.proteinatlas.org/ENSG00000164305-CASP3/cell), so one could speculate that, breakdown of the nuclear envelope renders DENR accessible to otherwise nuclear caspases and therefore allows for a surge of DENR cleavage unless prevented by Ser73 phosphorylation. This interplay could be topic of future study. That said, the rescue of DENR stability either upon mutating the caspase site in DENR or with the Z-VAD-FMK study. That said, the rescue of DENR stability either upon mutating the caspase site in DENR or with the Z-VAD-FMK caspase inhibitor is only partial (Fig. 3d, e), suggesting additional degradation mechanisms may be at play.

We noticed that unlike other reporter constructs carrying the 5′UTRs of endogenous DENR target mRNAs, our synthetic stuORF reporter does not increase in translation during mitosis (Fig. 4b). One possible explanation we explored is that during mitosis DENR Ser73 phosphorylation changes DENR function so that it no longer acts on one set of interphase target mRNAs (represented by the stuORF reporter) and instead it acts on a distinct set of mRNAs. From our ribosome profiling experiment, however, this does not seem to be the case (Supplementary Fig. 5e). There is a good correlation between the mRNAs that are DENR targets in interphase and in mitosis, suggesting there are not two distinct sets of target genes. The few target genes that are strongly DENR dependent in interphase but less so in mitosis (e.g., IL11, ELFN2, and FBXO46) are poorly translated in mitosis. The IL11 mRNA for instance is the most down-translated mRNA transcriptome-wide in wildtype mitotic cells compared to inter-phase cells (Supplementary Data 6). Hence if these mRNAs are not translated in mitosis, the presence or absence of DENR cannot affect them. Therefore, although we do not know why our synthetic stuORF reporter does not increase its translation during mitosis, we believe the most likely explanation is that it is not being translated in mitosis for some technical reason.

We present here in Supplementary Materials a few unbiased and comprehensive screens for pathways that might regulate the DENR-MCTS1 complex. We tested all serine/threonine kinases by in vitro kinase assay, we knocked down all hits from this kinase screen and assayed DENR·MCTS1 activity using the stuORF reporter in HeLa and MCF7 cells, and we systematically mutated all amino acids in DENR or MCTS1 which were reported in public databases to be post-translationally modified. While we focused here on the CDK axis, our data suggest that there might be additional regulatory pathways that could be further explored. One example is the in vitro phosphorylation of MCTS1 at threonines 81 and 179 by STK3 (Supplementary Fig. 9d). Additional examples are the effects of PRKCG or PRKG1 knockdown on DENR-MCTS1/stuORF activity (Supplementary Fig. 9a, b). Furthermore, abrogation of possible posttranslational modifications on MCTS1 lysine 51 showed a small but significant reduction in stuORF activity (Supplementary Fig. 8e), suggesting acetylation or ubiquitylation might provide additional levels of regulation to this translation complex. These might be interesting starting points for future studies on the regulation of the DENR-MCTS1 complex.

Methods
Cloning. Sequences of oligos for cloning were provided in Supplementary Data 4. For expression of MCTS1 or DENR and their mutants (Supplementary Fig. 8b, c) the human MCTS1 or DENR coding sequences were cloned into a pRK vector backbone (pAT1063, Telemen lab collection) via restriction sites EcoRI and Clal. Then four silent mutations within exon 6 were inserted into the MCTS1 coding sequence by site-directed mutagenesis to make it resistant against MCTS1 mRNA. Likewise, all mutations of the DENR and MCTS1 coding sequence were inserted by site-directed mutagenesis (Supplementary Fig. 8b, c, Supplementary Data 1). For negative controls, the empty pRK vector backbone was used. In order to clone FLAG-tagged DENR constructs (Fig. 3, Fig. 4, Fig. 5c, d, Supplementary Fig. 1b, 3, 6, 8b–e), DENR (wildtype, ST73 or D268/E373A mutant) coding sequence was amplified using primers containing a C-terminal FLAG-tag and again cloned into the above mentioned pRK vector backbone using EcoRI and Clal restriction sites or into a pcDNA3.1 vector containing a puromycin resistance (pf9045) using BamHII and EcoRI restriction sites.

In order to obtain mutated constructs for the in vitro kinase assays (Supplementary Fig. 9c, d) site-directed mutagenesis was performed on a plasmid (pET-DUET-1, pSS290)34 coding for both His-tagged DENRWT and His-tagged MCTS1WT, before recloning into the pET-DUET-1 vector using XbaI and NotI (DENR) or Munl/EcoRI and XhoI (MCTS1) as restriction sites. The Lamin B1 5′UTR firefly and renilla luciferase reporters and the Lamin B1 5′UTR stuORF reporter were obtained from a previous project in our lab30. Renilla luciferase reporters with 5′UTRs of various genes (Fig. 4b–g, Supplementary Fig. 4) were cloned by amplifying the 5′UTR of the gene of interest from dena and cloning it into the renilla luciferase reporter plasmid at the HindIII and Bsp119I sites. Cloning of the reporter plasmids for AT4a, -raf, -craf, DROSHA, MAP2K6, and PKD2R was performed likewise35. All sequences of primers used for cloning are detailed in Supplementary Data 8.

Expression and puriﬁcation of the DENR-MCTS1 protein complex. Proteins were expressed using E. coli BL21 (DE3) cells in 2YT media supplemented with Kanamycin (30 μg/ml). Cells were grown to an OD600 of 0.8–1.0 at 37 °C, then shifted to 18 °C. Expression was induced with the addition of 0.4 μM IPTG, and cultures were grown further overnight, harvested by centrifugation, and the cell pellets either used immediately for lysis and purification or frozen with LN2 and stored at −20 °C. All variants of the DENR-MCTS1 complex (Supplementary Fig. 9c, d, Supplementary Data 2) were purified via a C-terminal His6-tag using NNTA and size exclusion chromatography. Cells were resuspended in lysis buffer (30 mM HEPES, 30 mM imidazol, 500 mM NaCl) and lysed with a Microfluidizer (Microfluidics) at 0.55 MPa. The lystate was cleared by centrifugation for 35 min at 35,000 × g for 4 °C, and the resulting supernatant was applied to a 2 ml NiNTA-eluate was applied to a Superdex 200 26/60 column, equilibrated with SEC-buffer 1 (10 mM HEPES pH 7.5, 500 mM NaCl). Peak fractions containing the DENR–MCTS1 complex were pooled, concentrated to 10–13 mg/ml, and either used directly or shock-frozen with LN2 and stored at −80 °C.

Cell culture. HeLa cells (American Type Culture Collection, ATCC #CCL-2), MCF7 cells (ATCC #HTB-22) and U-2 OS cells (ATCC #HTB-96) were cultured in Dulbecco’s modified Eagle’s medium (DMEM), supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. HeLa DENRKO cells were generated in our lab32. For this manuscript clone no. 3.42 was used throughout, only for cell culture

![Fig. 6 Schematic summary of DENR regulation in mitosis.](https://example.com/fig6.png)

During early stages of mitosis, DENR protein is stabilized via phosphorylation of Ser73 which enables it to promote translation of target genes involved in mitosis. As of anaphase, DENR phosphorylation on Ser73 decreases, concomitant with a caspase-dependent decrease in DENR protein levels as cells exit mitosis.

Network of DENR regulation in mitosis.
For reconstitution of DENR mutant versions, DENRKO cells were plated at 2 
plasmid transfection (Supplementary Fig. 3c, d) or of mitotic release (Fig.3g, h).  

NATURE COMMUNICATIONS|          (2022) 13:668 | https://doi.org/10.1038/s41467-022-28265-0 | www.nature.com/naturecommunications 
(Figs. 1a 

drugs were used: Z-VAD-FMK 40 
#S1153), A-674563 1 
2000 (Thermo Fisher Scien
tific). Transient transfections 
assay or Western Blot in puromycin and thymidine-containing medium. 24 h later, 

Antibodies 

M (Sigma, #164739-5 G). Z-VAD-FMK was added at the time of FLAG-DENR 

Translation/luciferase reporter assay. To produce reporter constructs, 5'UTR 
sequences of the indicated genes were PCR-amplified from cDNA or genomic DNA. The constructs were cloned at the 5'UTR of Renilla luciferase using HindIII and Bsp19I.  

For the luciferase reporter assays, MCF7 or HeLaWT cells were seeded at a 
density of 8.000 cells and HeLaDENR-KO cells at a density of 12.000 cells per 96-well 
(Supplementary Figs. 4a, 4b, 4c, 4a, 9b). For luciferase assays with synchronized 
U2OS cells (Fig. 4b-g, Supplementary Fig. 4b-3) cells were seeded at a density of 
15.000 cells per 96-well plates. Cells were released from S phase arrest (thymidine) 
by exposure to the CDK1 inhibitor RO3306 at a concentration of 5 
mM. 15.000 cells in thymidine. For knockdown experiments, U2OS or HeLa cells were 
seeded at a density of 1.5.000 × 10^4 × 10^6 cells per 96-well (all conditions), in thymidine-containing medium if applicable. 16–20 h after (re-)plating, cells were transfected via Lipofectamine 

Western blots. Equal protein amounts were run on SDS-PAGE gels and 
transferred to nitrocellulose membrane with 0.2 µm pore size. After Ponceau staining, 
membranes were incubated in 5% skim milk PBS for 20–60 min, briefly rinsed 
with PBST and then incubated in primary antibody solution (5% BSA PBS or 5% skim milk PBS) overnight at 4 °C. Membranes were then washed three times, 
twice with PBST, incubated in a secondary antibody solution (1:10.000 in 5% 

sIRNAs and DENRKO. sIRNAs were obtained from Dharmacon® (Horizon Dis
covey Ltd.). Catalog numbers and sequences are specified in Supplementary Data 9. For DENR knockdown, a pool of three different sIRNAs (–02,19, 20) was used. sIRNA transfaction was performed using Lipofectamine RNAiMAX®. 

Transfection Reagent (Thermo Fisher Scientific), HeLa DENRKO cells were gen
erated in our laboratory using CRISPR/Cas9-mediated knockout12, Clone 3.42 was 
used throughout this paper, but effects were observed similarly with clone 2.11.
Flow cytometry. Proliferating HeLa[DENR,WT] or HeLa[DENR,KO] cells were trypsinized, collected, washed once with cold PBS, spun down for 5 min at 400 g, taken up in 600 µl of cold PBS and slowly dropped into an epi containing 1.2 ml of ice-cold PBS to arrest cells. Flow cytometry data were done with Guava Soft 3.3.

Data availability
The riboseq and RNA-seq datasets in this study have been deposited at NCBI SRA under accession code PRJNA768478. The riboseq and RNASeq source data generated in this study are available at the Zenodo repository (https://doi.org/10.5281/zenodo.5751288).

Received: 27 January 2021; Accepted: 12 January 2022; Published online: 03 February 2022.

References
1. Pakos-Zebrucka, K. et al. The integrated stress response. EMBO Rep. 17, 1374–1395 (2016).
2. Ron, D. Translational control in the endoplasmic reticulum stress response. J. Clin. Invest. 110, 1383–1388 (2002).
3. Schwanhausser, B., Gossen, M., Dittmar, G. & Selbach, M. Global analysis of cellular protein translation by pulsed SILAC. Proteomics 9, 205–209 (2009).
4. Schwanhausser, B. et al. Global quantification of mammalian gene expression control. Nature 473, 337–342 (2011).
5. Tanenbaum M. E., Stern-Ginossar N., Weissman J. S., Vale R. D. Regulation of mRNA translation during mitosis. eLife 4, e07957 https://doi.org/10.7554/eLife.07957 (2015).
6. Bukhari, S. I. & Vasudevan, S. FXR1-associated microRNA: A driver of specialized non-canonical translation in quiescent conditions. RNA Biol. 14, 137–145 (2017).
7. Seth A., Bohlen J., Telemen A. A. Translation-acrobatic: how cancer cells exploit alternate modes of translational initiation. EMBO Rep. 19, e49547 https://doi.org/10.15252/embr.201849547 (2018).
8. Stumpf, C. R., Moreno, M. V., Olshen, A. B., Taylor, B. S. & Ruggiero, D. The translational landscape of the mammalian cell cycle. Mol. Cell 52, 574–582 (2013).
9. Sudavath, S. & DeCaprio, J. A. The DREAM complex: master coordinator of cell cycle-dependent gene expression. Nat. Rev. Cancer 13, 585–595 (2013).
10. Wilker, E. W. et al. 14-3-3 sigma controls mitotic translation to facilitate cytokinesis. *Nature* **446**, 329–332 (2007).

11. Cockwell, M. J. et al. Phosphorylation of eIF4GII and 4E-BP1 in response to nocodazole treatment: a reappraisal of translation initiation during mitosis. *Cell Cycle* **12**, 3615–3628 (2013).

12. Shuda, M. et al. CDK1 substitutes for mTOR kinase to activate mitotic cap-dependent protein translation. *Proc. Natl Acad. Sci. USA* **112**, 5875–5882 (2015).

13. Wang, Y. et al. Mitotic MELK-eIF4B signaling controls protein synthesis and tumor cell survival. *Proc. Natl Acad. Sci. USA* **113**, 9810–9815 (2016).

14. Stoney V., Boye E., Grallet R. Regulation of global translation during the cell cycle. *J. Cell Sci.* **131**, jcs220372 https://doi.org/10.1242/jcs.jcs220372 (2018).

15. Kronja, I. & Orr-Weaver, T. L. Translational regulation of the cell cycle: when, where and why? *Philos. Trans. R. Soc. Lond. Ser. B, Biol. Sci.* **366**, 3638–3652 (2011).

16. An, S., Kwon, O. S., Yu, J. & Jang, S. K. A cyclin-dependent kinase, CDK11/p38, represses cap-dependent translation during mitosis. *Cell Mol. Life Sci.* **77**, 4693–4708 (2020).

17. Aramayo, R. & Polymenis, M. Ribosome profiling of the ribosomal protein RPL12/uL11 affects translation during mitosis. *Nat. Commun.* **10**, 141–148 (2019).

18. Shi, B., Hsu, H. L., Evens, A. M., Gordon, L. I. & Gartenhaus, R. B. Expression of the candidate MCT-1 oncogene in B- and T-cell lymphoid malignancies. *Blood* **102**, 297–302 (2003).

19. Weng, Y. S. et al. MCT-1/miR-34a/IL-6/IL-6R signaling axis promotes EMT progression, cancer stemness and M2 macrophage polarization in triple-negative breast cancer. *Mol. Cancer* **18**, 42 (2019).

20. Wang, D. et al. High expression of density-regulated re-initiation and release factor drives tumorigenesis and affects clinical outcome. *Oncol. Lett.* **15**, 141–148 (2019).

21. Nandi, S. et al. Phosphorylation of MCT-1 by p44/42 MAPK is required for its stabilization in response to DNA damage. *Oncogene* **26**, 2283–2289 (2007).

22. Stevenson-Lindert, L. M., Fowler, P. & Lew, J. Substrate specificity of CDK2-cyclin A. What is optimal? *J. Biol. Chem.* **278**, 50956–50960 (2003).

23. Koivomagi, M. et al. Dynamics of Cdk1 substrate specificity during cell cycle. *Mol. Cell. Biol.* **42**, 610–623 (2021).

24. Ordi, M. et al. Multisite phosphorylation code of Cdk. *Nat. Struct. Mol. Biol.* **26**, 649–658 (2019).

25. Topacio, B. R. et al. Cyclin D-Cdk6 drives cell-cycle progression via the retinoblastoma protein’s C-terminal helix. *Mol. Cell* **74**, 758–770 e754 (2019).

26. Meijer, L. et al. Biochemical and cellular effects of roscovitine, a potent and selective inhibitor of the cyclin-dependent kinases cdcl2 and cdk5. *Eur. J. Biochem.* **243**, 527–536 (1997).

27. Chorner, P. M. & Moorehead, R. A. A-674563, a putative AKT1 inhibitor that also suppresses CDK2 activity, inhibits human NSCLC cell growth more effectively than the pan-AKT inhibitor, MK-2206. *PloS One* **13**, e0193344 (2018).

28. Mitra, J. & Enders, G. H. Cyclin A/Cdk2 complexes regulate activation of Cdk1 and Cdc25 phosphatases in human cells. *Oncogene* **23**, 3361–3367 (2004).

29. Raje, N. et al. Seliciclib (CYC202 or R-roscovitine), a small-molecule cyclin-dependent kinase inhibitor, mediates activity via down-regulation of Mcl-1 in multiple myeloma. *Blood* **106**, 1042–1047 (2005).

30. Loo, Y. et al. Potent and selective inhibitors of Akt kinases slow the progress of tumors in vivo. *Mol. Cancer Ther.* **4**, 977–986 (2005).

31. Morris, E. J. et al. Discovery of a novel ERK inhibitor with activity in models of acquired resistance to BRAF and MEK inhibitors. *Cancer Discov.* **3**, 742–750 (2013).

32. Ahmed, Y. L. et al. DENR-MCTS1 heterodimerization and tRNA recruitment are required for translation reinitiation. *PloS Biol.* **16**, e2005160 (2018).

33. Hashimoto, T., Kikkawa, U. & Kamada, S. Contribution of caspase(s) to the cell cycle regulation at mitotic phase. *PloS One* **6**, e18449 (2011).

34. Wu, W. et al. S-trityl-L-cysteine, a novel Eg5 inhibitor, is a potent chemotherapeutic strategy in neuroblastoma. *Oncol. Lett.* **16**, 1023–1030 (2018).

35. Signoretto, E. et al. Nocodazole induced suicidal death of human erythrocytes. *Cell Physiol. Biochem.* **38**, 379–392 (2016).

36. Galamberti, F. et al. Targeting the cyclin E-Cdk2 complex represses lung cancer growth by triggering anaphase catastrophe. *Clin. Cancer Res.* **16**, 109–120 (2010).

37. Kawakami, M., Mustachio, L. M., Liu, X. & Dmitrovsky, E. Engaging anaphase catastrophe mechanisms to eradicate aneuploid cancers. *Mol. Cancer Ther.* **17**, 724–731 (2018).

38. Shih, H. J. et al. The involvement of caspase-7 in cell cycle progression at mitosis. *Genes Cells* **33**, 141–145 (2012).

39. Levenson, A. S. et al. MCT-1 oncogene contributes to increased in vivo tumorigenicity of MCF7 cells by promotion of angiogenesis and inhibition of apoptosis. *Cell. Res.* **16**, 1051–1056 (2005).

40. Shih, H. J. et al. Targeting MCT-1 oncogene inhibits She pathway and xenograft tumorigenesis. *Oncotarget* **3**, 1403–1415 (2012).

41. Meakoea, D. et al. Translational and transectome analysis of TMA20 (MCT-1) and TMA64 (eIF2D) knockout yeast strains. *Data Brief.* **23**, 103701 (2019).

42. Schleis, C. et al. DENR-MCT-1 promotes translation re-initiation downstream of sORFs to control tissue growth. *Nature* **512**, 208–212 (2014).

43. Vasandevan , D. et al. Translational induction of AT4F during integrated stress response requires noncanonical initiation factors eIF2D and DENR. *Nat. Commun.* **11**, 4677 (2020)

44. Bohlen, J. et al. DENR promotes translation reinitiation via ribosome recycling to drive expression of oncogenes including AT4F. *Nat. Commun.* **11**, 4676 (2020).

45. Makeeva, D. S. et al. Translational and transcriptome analysis of TMA20 (MCT-1) and TMA64 (eIF2D) knockout yeast strains. *Data Brief.* **23**, 103701 (2019).

46. Schleis, C., Acevedo, J. M., Clemm von Hohenberg, K. & Telean, A. A. Identification of transcripts with short sORFs as targets for DENR- and Identi- dependent translation in human cells. *Sci. Rep.* **7**, 3722 (2017).

47. Gunisova, S., Hronova, V., Mohammad, M. P., Hinnebusch, A. G. & Valasek, L. S. Please do not recycle! Translation reinitiation in microbes and higher eukaryotes. *FEBS Microbiol. Rev.* **42**, 165–192 (2018).

48. Hinnebusch, A. G., Ivanov, I. P. & Sonenberg, N. Translational control by 5'-untranslated regions of eukaryotic mRNAs. *Science* **352**, 1413–1416 (2016).

Acknowledgements

This work was supported by a DFG grant (project number 31695455) to A.A.T., by the DFG SFB 1036 to A.A.T., by a DKEFZ NCT3.0 Integrative Project in Cancer Research (NCT3.0, 2015.54 DsregPT) grant to A.A.T., and by a Cell Networks—Cluster of Excellence (EXC81) grant to K.C.v.H.
Author contributions
K.C.v.H., S.M., S.S., and M.M. performed experiments with help from J.B., K.C.v.H., M.M., T.G.H., and A.A.T. designed the work. K.C.v.H., S.M., S.S., M.M., J.B., T.G.H., and A.A.T. analyzed and interpreted the data. K.C.v.H. and A.A.T. wrote the manuscript.

Funding
Open Access funding enabled and organized by Projekt DEAL.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-022-28265-0.

Correspondence and requests for materials should be addressed to Aurelio A. Teleman.

Peer review information Nature Communications thanks David Gatfield and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.