ATR is essential for preservation of cell mechanics and nuclear integrity during interstitial migration

Gururaj Rao Kidlyoor, Qingsen Li, Giulia Bastianello, Christopher Bruhn, Irene Giovannetti, Adhil Mohamood, Galina V. Beznoussenko, Alexandre Mironov, Matthew Raab, Matthieu Piel, Umberto Restuccia, Vittoria Matafora, Angela Bachi, Sara Barozzi, Dario Parazzoli, Emanuela Frittoli, Andrea Palamidessi, Tito Panciera, Stefano Piccolo, Giorgio Scita, Paolo Maiuri, Kristina M. Havas, Zhong-Wei Zhou, Amit Kumar, Jiri Bartek, Zhao-Qi Wang & Marco Foiani

ATR responds to mechanical stress at the nuclear envelope and mediates envelope-associated repair of aberrant topological DNA states. By combining microscopy, electron microscopic analysis, biophysical and in vivo models, we report that ATR-defective cells exhibit altered nuclear plasticity and YAP delocalization. When subjected to mechanical stress or undergoing interstitial migration, ATR-defective nuclei collapse accumulating nuclear envelope ruptures and perinuclear cGAS, which indicate loss of nuclear envelope integrity, and aberrant perinuclear chromatin status. ATR-defective cells also are defective in neuronal migration during development and in metastatic dissemination from circulating tumor cells. Our findings indicate that ATR ensures mechanical coupling of the cytoskeleton to the nuclear envelope and accompanying regulation of envelope-chromosome association. Thus the repertoire of ATR-regulated biological processes extends well beyond its canonical role in triggering biochemical implementation of the DNA damage response.

https://doi.org/10.1038/s41467-020-18580-9
Mechanical properties of the nucleus and nuclear mechanosensing affects genome integrity, nuclear architecture, gene expression, cell migration, and differentiation. The physical properties of the nucleus are conveniently modulated following the inputs from the cell microenvironment or from chromatin dynamics. The nuclear envelope (NE) plays a critical role in this process by connecting the cytoskeleton and the chromatin.

Ataxia Telangiectasia and Rad3-related protein (ATR) regulates the DNA damage response (DDR) and protects genome integrity by regulating multiple pathways. ATR mutations cause the Seckel syndrome, an autosomal recessive disorder characterized by growth retardation, dwarfism, and microcephaly with mental retardation. We previously reported that ATR directly senses mechanical stress at the NE/chromatin interface and facilitates release of chromatin from the NE. This possibility is supported by the fact that ATR comprises HEAT (huntingtin, elongation factor 3, A subunit of protein phosphatase 2A, and TOR1) repeats, which are elastic connectors, ideal to sense mechanical stimuli.

Here we explore the possibility that ATR-mediated mechanical communication are also important for the state of the NE itself and, having obtained evidence to this effect, explore its functional implications.

Results

ATR is enriched at membranes and actin filaments around the nucleus. We visualized ATR distribution in exponentially growing HeLa cells by electron microscope (EM) and found ATR in the nucleus, cytosol, and other organelles, including endoplasmic reticulum (ER), Golgi, and mitochondria (Supplementary Fig. 1a). ATR (18.8%) was bound to actin filaments, particularly in the proximity of the NE and more than 20% was bound to cellular membranes (Fig. 1a). Membrane fractionation analysis confirmed that 17% of ATR co-fractionated with membranes, also when nucleic acids were degraded by Benzonase treatment (Supplementary Fig. 1b). We used TopBP1, a chromatin-bound protein, tubulin, a cytoplasmic protein, and Nup133, a NE protein, as controls in our fractionation experiments (Supplementary Fig. 1b). The Kyte and Doolittle, and the SOSUI and WoLF PSORT analyses, which recognize hydrophobic and membrane-associated domains, respectively, identified seven putative membrane binding and hydrophobic regions in ATR (Supplementary Fig. 1c).

ATR depletion results in multiple nuclear membrane defects. Short hairpin RNA (shRNA)-mediated ATR depletion in HeLa cells caused 80% reduction of ATR (Supplementary Fig. 1d) and no obvious cell cycle anomalies (Supplementary Fig. 1e). Immunofluorescence (IF) analysis showed that shATR cells have compromised nuclear morphology, characterized by altered nuclear circularity index, invaginations, and micronuclei (Fig. 1b–e). Similar defects were observed in ATR-depleted U2OS cells (Supplementary Fig. 1f), human ATR Seckel fibroblasts, and non-cycling primary neurons isolated from humanized Seckel mice (Supplementary Fig. 1g, h). ATR<sup>lox/−</sup>-HCT116 cells, which have reduced ATR levels (Supplementary Fig. 1i), also displayed compromised nuclear morphology (Supplementary Fig. 1j, k). We transfected ATR<sup>lox/−</sup>-cells with wild-type green fluorescent protein (GFP) tagged ATR (GFP-ATR) or with a kinase inactive version of GFP-ATR. Although wild-type GFP-ATR rescued the nuclear defects, the mutant form did not (Supplementary Fig. 1i, m). We then performed EM analysis of shATR nuclei (Fig. 1f, Supplementary Fig. 1n–s, and Supplementary Video 1). ATR-depleted cells exhibited NE invaginations of type II (outer and inner membranes invaginations) and type I (inner membranes invaginations), associated with condensed chromatin and/or nucleoli (Fig. 1f and Supplementary Fig. 1n–r). NE invaginations also associated with nucleoli forming nucleolus canals that represent intermediates in rRNA export through the NE (Fig. 1f and Supplementary Fig. 1r). We also found, within the nucleus, inner membrane invaginations/fragments attached to chromatin and micronuclei (Supplementary Fig. 1r, s).

ATR depletion alters nuclear mechanical properties. NE abnormalities can affect the mechanical properties of the nucleus. When we measured the elastic modulus of ATR-depleted cells by atomic force microscopy (AFM), we found a reduced elasticity compared to controls (Fig. 2a). As the nucleus is the stiffest organelle in the cell, we performed the same analysis on isolated ATR-defective nuclei and found, again, a reduced elasticity, compared to controls (Fig. 2b). Acute treatment with ATR inhibitors for 4 h did not alter nuclear stiffness (Supplementary Fig. 2a). Hence, the reduced nuclear elasticity results from chronic ATR depletion.

Lipid composition of the NE is altered in ATR-defective cells. Nuclear stiffness is influenced by lamin and nuclear membrane fluidity. We did not find significant alterations in Lamins protein levels or in their relative cellular localization in ATR-depleted cells (Supplementary Fig. 2b). However, when we measured the lipid composition of isolated nuclear membranes from ATR-depleted and control cells, out of 855 lipid species analyzed, we observed significant differences in the phosphatidylcholine (PC)/phosphatidylethanolamine (PE) ratio (Fig. 2c). In particular, we observed a specific altered ratio in the 18-carbon and 17-carbon lipid species that represent the most common lipids in membranes (Fig. 2c). Of note, when we performed a whole cell metabolic analysis, we did not observe specific alterations at the levels of PC/PE ratios.

ATR depletion alters chromatin organization. Chromatin conformation and distribution can also influence mechanical properties of the nucleus. We first performed the DNase I sensitivity assay to analyze the chromatin state of control and ATR-depleted cells (Supplementary Fig. 2c). DNase preferentially cleaves euchromatin, which is more accessible than heterochromatin to its enzymatic activity. We found that at early time points, ATR-defective cells exhibited a higher level of undigested DNA that failed to migrate in the gel and likely resulted from heterochromatin accumulation (Supplementary Fig. 2c). Perinuclear chromatin is generally in the heterochromatic state and is influenced by the levels of H3K9 trimethylation. ATR-defective cells exhibited a 20% increase in K9 trimethylated histone H3 compared to control (Supplementary Fig. 2d), implying that ATR depletion promotes increased heterochromatization. This conclusion is confirmed by the fluorescence energy transfer (FRET)-based fluorescent lifetime imaging microscopy (FLIM) assay utilizing GFP- and mCherry-tagged histone H2B to measure chromatin compaction. We found that, under unperturbed conditions, loss of ATR causes a reduction in the fluorescent lifetime signal, indicating that H2B histones are more compacted (Fig. 2d). However, we did not observe changes at the level of FRET signal or H3K9 trimethylation upon short-term inhibition of ATR using kinase inhibitors (Fig. 2d and Supplementary Fig. 2e), suggesting that the aberrant chromatin state owing to ATR depletion results from long-term effects.

ATR depletion affects LINC-mediated nuclear-cytoskeleton connections. Another parameter affecting the mechanical properties of the nucleus is the connection between the NE and the cytoskeleton, which is mediated by the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex. We measured this parameter in cells with or without ATR, using a FRET sensor of Nesprin 2G.
The LINC-mediated mechanical coupling between the NE and the cytoskeleton also influences the nuclear accumulation of YAP, a key mechanosensing transcriptional activator. We investigated whether ATR depletion affected YAP nuclear accumulation. We found that, although in control cells YAP distribution was mostly nuclear, the YAP cytoplasmic fraction increased significantly in ATR-defective cells. Moreover, following ATR depletion, cells accumulated the cytoplasmic form of YAP, phosphorylated in Serine 127. Following an acute treatment with an ATR inhibitor, essential for YAP nuclear exit under physiological conditions, could counteract the effect of ATR depletion. Treatment of ATR-depleted cells with Leptomycin-B rescued nuclear YAP levels, with concomitant decrease of phosphorylated YAP (Supplementary Fig. 2f, g). Following an acute treatment with an ATR...
inhibitor for 3 h, again, we found enrichment of YAP in the cytoplasm and this effect was abolished by Leptomycin-B treatment (Supplementary Fig. 2h, i).

**ATR-defective nuclei collapse following mechanical compression.** The previous results suggest that ATR is required for the normal response of the nucleus to mechanical forces and, as the responses of the cell are dominated by effects on the nucleus, that ATR may also influence cell mechanoresponsiveness. We therefore analyzed the response of ATR-defective cells to mechanical stress using a microfluidic device able to compress cells with a linear step-wise increment of mechanical forces (see “Methods”) (Fig. 3a). GFP-tagged cGAS, a cytoplasmic DNA-binding protein, was used as an NE damage marker. We compressed cells expressing cGAS-GFP using the microfluidic device by applying linear step-wise increment of mechanical forces (see “Methods”).

**Fig. 2 ATR preserves nuclear mechanics.** a, b Elastic modulus measurements using AFM. a Cellular stiffness (n = 171, 161, and 179 measurements for shCtrl, shATR, and shATR2, respectively) and b stiffness of isolated nuclei from control and ATR-depleted cells (n = 144, 110, and 98 measurements; N = 2 independent experiments). c Membrane phospholipid composition analysis; total cell and nuclear membrane PC/PE ratio, nuclear PC/PE ratio of individual species (from three biological and two technical replicates; two-way ANOVA test; Bonferroni’s multiple comparisons test; N = 2 independent experiments). d FLIM-FRET analysis of cells expressing H2B-GFP and H2B-mCherry; sample images of Fluorescent lifetime (FLIM-FRET index) and overall Lifetime in Hela cells infected with shATR (n = 13) or control (n = 7), or treated with DMSO (n = 10) or ATR inhibitor for 4 h (n = 8). e Examples of FRET signals at the NE as measured by Nesprin-2 FRET sensor in control, shATR, and control cells in the presence of ATR inhibitor (3 h), and quantification of FRET signals (n = 112, 88, and 96 measurements for shCtrl, shATR, and ShATR2 respectively; data pooled from two or three independent experiments). f Immunofluorescent images of YAP cellular distribution in control and shATR cells, (below) quantification of nuclear to cytoplasmic YAP signal ratio (n = 69, 66, and 58 shCtrl, shATR1, and ShATR2, respectively). g Western blotting of ser127-phosphorylated YAP (N = 2 independent experiments. Uncropped images available in Source Data file). Scale bar is 20 μm in all images. Bar graphs presented as mean ± SEM and box plot whiskers and outliers plotted using Tukey’s method in prism7 software. p-values calculated using one-way ANOVA test with Tukey’s multiple comparisons test (for d) or Dunnett’s multiple comparisons test (for a, b, e, f). (***, p < 0.0001; ***, ***, ***, p < 0.001; ***, ***, ***, ***, p < 0.01; *p < 0.05; n.s., not significant).
ten steps of compression with a range of pressure spanning 0 to 50 mBar, with an increment of 5 mBar at each step. ATR-depleted cells accumulated perinuclear cGAS-GFP foci in the range between 15 and 30 mBar, whereas in control cells cGAS-GFP foci started to appear only at 45 mBar (Fig. 3b–d). We also analyzed the recovery from cell compression and found that control cells restored the initial nuclear size following de-compression, but ATR-defective nuclei did not completely revert back to the original size (Fig. 3b, c). We conclude that ATR-defective cells, experiencing compression forces, undergo irreversible nuclear deformation and collapse followed by NE ruptures, thus exposing DNA into the cytoplasm, and accumulating perinuclear cGAS.

**ATR defects impair interstitial migration.** When migrating through tight spaces, nuclei experience tremendous mechanical stress that causes chromatin compaction and nuclear ruptures. Cell survival under these conditions depends on cellular pathways controlling nuclear integrity and nuclear membrane repair. The previous sections suggest that ATR defects should compromise the ability of cells to migrate through narrow pores. To explore this possibility, we analyzed the contribution of ATR on interstitial migration using in vitro experiments. Moreover, as nuclear stress and chromatin compaction are prominent features during neurogenesis and metastasis, we also addressed whether ATR influences neurogenesis and metastasis by in vivo experiments.

We analyzed H2B-mCherry-expressing shATR and control HeLa cells migrating through 4 μm-wide and 15 μm-long microfabricated constrictions (Fig. 4a). The fraction of cells undergoing nuclear collapse and cell death (Fig. 4a, see also Supplementary Movie. 2) while engaging the constrictions were increased in the absence of ATR (Fig. 4b). Similar results were observed in cells treated with ATR inhibitors (Supplementary Fig. 3a). We measured DNA damage occurrence by counting 53BP1 foci of cells in the constrictions and found comparable level of foci numbers between normal and ATR-defective cells (Fig. 4c). Given the well-established links between ATR activity and cell cycle checkpoints, we tested whether the migration defects of ATR-depleted cells were also connected to the cell cycle. Using U2OS-FUCCI cells that mark different phases of cell cycle, we found that cell death in shATR cells was not dependent on the cell cycle phase (Supplementary Fig. 3b). We found comparable range of 53BP1-GFP foci in ATR-inhibited cells and in control cells; moreover, the presence of 53BP1 foci did not correlate with cell death (Fig. 4c). This finding is in agreement with previous reports showing that 53BP1 foci accumulate in a Ataxia-Telangiectasia mutated protein (ATM)-dependent manner. We note that ATM is fully functional in ATR-defective cells. Therefore, nuclear collapse and cell death of ATR-defective cells in constrictions does not correlate with increased DNA damage or cell cycle stage. Using the Nesprin 2G FRET sensor, we found lower FRET intensity at the leading edge of the nucleus and higher intensity at the lagging edge (Fig. 4d, e), suggesting that cells respond to migration-induced mechanical stress by modulating the cytoskeleton-NE connection at the leading edge of the NE. ATR inhibition caused a general increase in FRET signal (Supplementary Fig. 3c, d) and abolished the differential coupling of the cytoskeleton-NE connection in the nuclei engaged in the constrictions (Fig. 4e). As a control, we used a headless form of Nesprin 2G sensor, which cannot bind cytoskeleton, and found that it did not exhibit significant changes in FRET signals in control or ATR-defective cells (Supplementary Fig. 3e).
We then analyzed the cGAS-GFP foci distribution during interstitial migration (Fig. 4f). We found that, compared to normal cells, in ATR-defective cells cGAS-GFP foci appeared much earlier at the leading tip of nuclei engaged in the constrictions, suggesting that ATR defects enhance NE fragility during interstitial migration. To directly analyze the integrity of the NE experiencing interstitial migration, we performed EM analysis of cells migrating across constrictions with or without ATR (Fig. 4g and Supplementary Fig. 3f–i). Control cells approached the constrictions by deforming their nuclei at the leading edge without aberrant NE structures (Fig. 4g and Supplementary Fig. 3f) or, occasionally, with well-organized NE invaginations parallel to the direction of migration (Supplementary Fig. 3g). Conversely, most of ATR-depleted nuclei were deformed, exhibiting several NE invaginations in the front.
ATR influences neurogenesis and metastatic dissemination. During neurogenesis and metastasis, cells migrate through narrow places. The in vitro observations described above suggest that ATR may play a relevant role in these processes. We analyzed the contribution of ATR in neurogenesis by performing a transwell migration assay of neuroepithelial progenitors isolated from ATR-conditioned knockout mouse brain (E13.5 days) (Fig. 5a). ATR depletion impaired migration of neurosphere-derived cells through 3 or 8 μm pore size membranes and, as expected, the defect was more pronounced in the smaller pore size. We next noticed that the leading edge of ATR-defective nuclei exhibited NE portions with a disorganized distribution of outer and inner membranes, likely due to aberrant nuclear membrane remodeling. The NE at the rear part of the nucleus was normal and comparable in control and in ATR-depleted cells (Supplementary Fig. 3i). Hence, as soon as ATR-defective cells engage narrow constrictions, they fail to adequately respond to the mechanical stress arising at the leading edge of the nucleus and undergo NE deformation and extensive nuclear membrane damage, which, in turn, cause nuclear collapse and cell death. We failed to observe NE ruptures in ATR-depleted cells grown under normal conditions, suggesting that they represent a consequence of mechanical compression. Although the intrinsic alteration of the mechanical properties of ATR-defective nuclei may not affect cell viability under normal conditions, the consequences of nuclear collapse following mechanical stress certainly contribute to cell lethality when cells are forced through narrow passages.

We then examined the contribution of ATR in the migration of cancer cells. We injected equal number of shRNA control and ATR-depleted HeLa cells labeled with a vital dye into the tail vein of immunocompromised mice and recovered lung disseminated cells at 2 and 48 h after injection (see scheme in Fig. 5d). We found a significant reduction of fluorescent-positive shATR cells in the lung 48 h after injection compared to controls (Fig. 5e, f), indicating that ATR is essential to allow cells to sustain the harsh mechanical environment imposed by blood flow and extravasation.

ATR interactors known to influence nuclear mechanoresponse. To identify potential ATR interactors and targets contributing to nuclear mechanics and dynamics, we performed high-resolution mass spectrometry screens (IP-liquid chromatography (LC)-MS/MS) in exponentially growing U2OS cells expressing GFP-ATR. Combining data from three SILAC (stable isotopes labeling with amino acids in cell culture) quantitative proteomics (LC)-MS/MS experiments in exponentially growing U2OS cells expressing GFP-ATR. Combining data from three SILAC (stable isotopes labeling with amino acids in cell culture) quantitative proteomics experiments in exponentially growing U2OS cells expressing GFP-ATR. Combining data from three SILAC (stable isotopes labeling with amino acids in cell culture) quantitative proteomics experiments. We identified several ATR interactors for which previous studies have identified roles in the mechanical properties of the nucleus and whose depletion mimics, at least in part, some of the phenotypes observed in ATR-defective cells (Fig. 6a and Supplementary Fig. 4d–f). TOPII, an ATR/ATM phosphorylation target, is involved in modulating DNA topology in S phase and in prophase to deal with the mechanical stress caused by chromosome dynamics. Moreover, genetic evidence suggests that Top2 activity is repressed by Mec1ATR. We also found HADC2 and CHD4, which are members of the Nuclear Remodeling and Deacetylating (NRD) complex, previously identified as ATR interactors. An ATR-mediated regulation of the TOP2 and NRD complex could account for the phenotypes associated with the heterochromatic and condensed chromatin observed in ATR-defective cells. The screen identified four proteins of the nuclear pore complex (Nup50, 107, 133, 160), regulating nuclear transport, centrosome attachment to the NE during mitosis, as well as YAP mechanotransduction. In addition, we identified several transport proteins including Exportin1 (XPO1/CRM1), an ATR/ATM phosphorylation target, involved in rRNA transport, which might be connected to the accumulation of nucleolar canals described in this study. We also

Fig. 4 ATR-defective nuclei are inefficient in migrating through narrow pores. a Snapshots of H2B-mCherry labeled control and shATR nuclei passing through constriction. b Cell death measured as the percentage of engaged cells that burst at the constriction (n = 122 and 88 cells for shCtrl and shATR; data pooled from three independent experiments). c Quantification of 33BP1-GFP foci generated due to constriction in HeLa cells expressing 33BP1-GFP in the presence of DMSO or ATR inhibitor, VE-821 (n = 24 and 17 for DMSO and ATRinhib; pooled from two independent experiments). Cells that undergo cell death in the constriction are highlighted in red (for ARTinhib). d, e FRET signal measurements of cells engaged in constrictions. d Images of FRET signal at various stages of migration through the constriction and measurement of signal ratio between front (leading half of the nucleus) and back (lagging half of the nucleus) of a nuclei at various stages of migration (n = 11, 10, 10, and 6, respectively). e Ratio of front to back FRET signal in migrating cells (inside or outside the constriction) in the presence DMSO or ARTinhib (n = 11, 10, 9, and 10; data from 2 to 3 experiments). f Quantification of nuclear position in the constriction during the first cGAS foci formation (n = 47, 43, and 28; numbers pooled from 3 experiments). g EM images of control and shATR nuclei in constriction (routine 200 nm EM sections). Arrowheads indicate invaginations and NE attached chromatin or nucleoli. h 3D reconstruction of NE at the leading edge from control nucleus in constriction. NE color indicates inner nuclear membrane (INM) and yellow indicates outer nuclear membrane (ONM). i 3D reconstruction of NE section from leading edge of shATR nucleus in constriction. j Quantification of ratio between number of inner nuclear membrane breaks to that of the outer membrane (n = 15 and 13). Scale bar for a, d is 20 μm, for g is 9 μm, and for i, h is 200 nm). Bar graphs presented as mean ± SEM and dot-plot as mean ± SD. P-value calculated using two-tailed Student’s t-test for b, c, j. One-way ANOVA for d, e with Tukey’s or Sidak’s multiple comparisons test, and two-way ANOVA for f (**P < 0.0001, ***P < 0.001, **P < 0.01, and *P < 0.05; n.s., not significant).
identified Nesprin-2. Intriguingly, Nesprin-2-defective cells also exhibit NE invaginations, and chromatin architecture and nuclear mechanics dysfunctions\(^3^9,^4^0\). Moreover, loss of Nesprin-2 leads to defective neuronal migration in developing mice brain\(^4^1\). We confirmed the ATR–Nesprin-2 interaction by immunoprecipitation (IP) followed by western blotting (Fig. 6b) and proximity ligation assay (PLA) (Fig. 6c). PLA showed that the number of ATR–Nesprin-2 foci at the NE increased in cells undergoing chromatin condensation in prophase (Fig. 6d and Supplementary Fig. 4i). This observation, combined with the previous result showing that ATR influences nesprin 2 function (Fig. 2e), suggests that ATR and Nesprin-2 dynamically interact at the NE in response to mechanical stress. ATR depletion did not affect Nesprin-2, protein levels, or intracellular localization (Supplementary Fig. 4g). In sum, the ATR-phosphointeractome reveals a number of ATR targets involved in the response of the NE to mechanical strains. Although certain targets might be directly regulated by ATR to allow cells to properly respond to mechanical stress, a set of ATR interactors might mediate the recruitment of ATR to the NE or even promote ATR activation when nuclei are stressed. Of note, we did not find ATR interactors involved in lipid metabolism.

**Discussion**

Our findings unravel a non-canonical role for ATR in maintaining the normal properties of the NE and in mediating a communication between the cytoplasm and the nuclear interior (chromatin/chromosomes). These observations, together with previous
findings showing that (i) ATR is a giant HEAT repeat protein, therefore ideal for sensing mechanical stress; (ii) ATR relieves the mechanical stress at the NE exerted by the mRNA export machinery; and (iii) ATR relocalizes at the NE following mechanical stress, suggesting that ATR might directly sense and transduce mechanical stress in general and at the NE.

We described a variety of nuclear defects owing to ATR depletion, some of which are evident already in unchallenged cells; others become obvious when cells (and thus their nuclei, which are their primary load-bearing element) experience mechanical deformations. Although some of the nuclear defects likely represent a direct consequence of ATR inactivation/depletion, others may be related to long-term adaptive responses to limiting ATR. We also identified a set of ATR interactors that might be involved in the cellular response to mechanical stress by mediating ATR recruitment at the NE, transducing the mechanical stress signals or in triggering short and long-term response pathways. Finally, we show that ATR is essential for neurogenesis and cancer cell migration, two processes in which cells and their nuclei must squeeze through tight spaces.

**Nuclear defects in unchallenged ATR-depleted cells.** Under normal conditions, ATR-defective cells exhibit NE invaginations tethered with semicondensed chromatin and nucleoli. These nuclear defects appear only upon long-term depletion of ATR. Considering that (i) condensation of perinuclear chromatin and nucleolar canals generates transient NE invaginations; (ii) ATR-defective cells are unable to coordinate NEBD with chromatin condensation, as they exhibit a slow condensation process; and (iii) ATR has been involved in coordinating RNA export through the NE with topological stress, the most likely possibility is that in ATR-defective cells NE remains deformed due to the inability to efficiently separate from condensed chromatin and to complete the export of nucleolar RNA species.

NE invaginations are rare in normal cells, as the NE efficiently recovers its original shape. This process requires the separation of condensed and transcribed chromatin from the NE, and is influenced by NE remodeling activities and by proteins modulating the topological and epigenetic context of chromatin and nuclear transport. ATR regulates type II A and B topoisomerases and condensins, as well as the NRD complex that controls chromatin epigenetics. It is possible that, in ATR-defective cells, deregulated topological and condensation activities may cause nuclear membranes invaginations and ruptures, nuclear fragmentation, and micronuclei formation; the heterochromatic and heavily condensed chromatin could instead result from the deregulation of the NRD complex. Recent observations showed that H3K9 trimethylation marked heterochromatin levels rearrange in response to mechanical stress at the NE and the recovery of the nucleus. In this scenario, our finding that a long-term depletion of ATR accumulates hypercompacted chromatin at the nuclear periphery and elevated levels of H3K9 trimethylation may therefore reflect the inability of ATR-depleted cells to properly recover from nuclear stress. The hypercompacted chromatin at the nuclear periphery and the consequent reduction in nucleosome packaging density in the rest of the nucleoplasm
might contribute to explain the low nuclear stiffness of ATR-defective cells.

Nuclear membrane defects owing to ATR depletion. The lack of coordination between chromatin condensation and NEBD during cell division in ATR-defective cells causes accumulation of semi-condensed chromatin attached to NE fragments. Moreover, our EM analysis showed that ATR-depleted cells accumulate membrane ruptures already under unperturbed conditions and, following nuclear deformation during interstitial migration, they exhibit massive breakage of the outer nuclear membrane and aberrant membrane remodeling. The ESCRTIII complex plays a key role in sealing membrane holes in the reforming NE during mitotic exit and in repairing the NE upon migration-induced rupture. It is possible that the activity of the ESCRTIII complex becomes limiting in ATR-defective cells, due to the massive damage of nuclear membranes. The extensive NE damage and remodeling in ATR-defective cells may also represent the primary cause of the aberrant phospholipid composition of their nuclear membranes. In agreement with this hypothesis, the aberrant phospholipid composition of the NE in ATR-defective cells does not reflect a direct metabolic problem and we failed to identify ATR interactors involved in lipid metabolism.

Altered NE-cytoskeleton coupling upon ATR inactivation. A key finding of this work is that ATR is a component of the cell mechanotransduction machinery by ensuring appropriate mechanical coupling of the cytoskeleton to the NE. The NE is exposed to forces acting in opposite directions: forces deriving from chromatin dynamics (as outlined above) and opposite forces generated by extracellular matrix (ECM) attachment and conveyed to the NE through the LINC complex and the NE-associated cytoskeleton. ATR orchestrates the integration of all these mechanical inputs, by regulating at once NE dynamics, chromatin condensation, and the LINC function as the mechanosensory properties of the entire cell; this is visualized by the here discovered ATR-YAP mechano-signaling axis.

Although abnormalities in nuclear shape and mechanics can impact on genome integrity by generating chromatin fragmentation and fork collapse, under normal conditions, the nuclei remain relatively stable as well as the NE. However, at raising levels of mechanical strain, cells must promptly respond to mechanical stress. Here we show that ATR is critical also for the nuclear response to more severe mechanical challenges. ATR-defective cells fail to properly respond to sub-lethal compression forces and undergo extensive nuclear collapse characterized by NE ruptures. In turn, this imposes an additional stress at the level of nuclear membrane remodeling, as revealed by the presence of mixed portions of the outer and inner membranes at the NE engaged in the constrictions. NE fragmentation under high level of mechanical stress exposes nuclear DNA into the cytoplasm, leading to activation of the cGAS-STING pathway. The functional consequences of the ATR and cGAS connection remain unexplored but may hold relevant pathological implications, particularly in tissues undergoing mechanical stress.

Pathological consequences owing to ATR defects. Our observations indicate that, when the nucleus engages the narrow constrictions, the LINC complexes at the leading edge of the nucleus are tightly bound to the cytoskeleton and the mechanical strain generates extensive ruptures at the outer nuclear membranes. Hence, the nucleus during interstitial migration is polarized at the level of NE. ATR-defective cells fail to maintain the coupling between Nesprin-2 and the cytoskeleton, and to polarize the NE under these conditions and accumulate NE invaginations and extensive ruptures at both nuclear membranes at the leading edge of the nucleus. Moreover, the extensive NE invaginations tethered with semi-condensed chromatin may hinder efficient nuclear squeezing and prevent an efficient repair of the nuclear membranes.

Cancer cell migration through the ECM requires nuclear deformability, particularly when cells must meander through dense and highly crosslinked collagen type I-rich stroma, extravagate, and sustain the harsh conditions of the blood circulation before extravasating, passing through pores as small as 2 µm in diameter. In fact, altered NE morphology is typical of cancer cells and crucial in the tumor grade assessment, and correlates with prognosis. Cancer cells can adapt to metastatic migration by deregulating the expression of Laminins, but a certain degree of NE stiffness is required to allow a productive migration and to prevent massive NE ruptures. Our observations suggest that ATR activity might be therefore beneficial for cancer cell migration, thus implying that ATR might play opposite roles in cancer progression, by preventing genome instability and by promoting metastasis. Along this idea, it is interesting to note that our experiments indicate that ATR depletion impairs three-dimensional (3D) invasion and lung homing of cancer cells.

Our findings describe a variety of nuclear defects and their pathological consequences (Fig. 7). However some of these defects such as the altered mechanical coupling between cytoskeleton and NE, and the YAP cytoplasmic retention occur soon after ATR catalytic inactivation, suggesting that these two phenotypes represent a direct consequence of ATR deregulation. Considering that during development, organogenesis requires that stem/progenitor cells migrate towards destination tissues, our observations may contribute to explain some of the developmental defects of Seckel patients bearing genetic defects in ATR. Our results might also explain the increased cell death in non-proliferating neuroprogenitors and neurons of ATR-knockout mice, which cannot be directly ascribed to the role of ATR in replication stress. Moreover, the delocalization of YAP might also contribute to a variety of pathological outcomes such as loss of stem cells and cardiomyopathies. Intriguingly, ATR conditional knockout mice exhibit a progeroid phenotype that has been related to stem cell loss.

Methods

Plasmids. ATR shRNA1 and control (pLKO1) plasmids were from Dr. O.F. Capetillo (CNIO, Spain); ATR shRNA2 was purchased from Sigma (TRCN0000219647); the GFP-ATR plasmid was from Dr. R.Tibbetts (Wisconsin, USA). GFP-AU1-ATR plasmid was digested with BamH1 to excise out GFP cDNA. The BamH1-digested GFP cDNA insert was cloned into FLAG-ATR-KD plasmid also linearized with BamH1, followed by transformation in Escherichia coli. Positive clones after transformations were screened by PCR and BamH1 restriction digestion, finally sequenced (list of primers used are provided in Supplementary Table 1). TPRIP-CMV-GFP, FLAG-cGAS (Plasmid #86675), Nesprin tension sensor (pcDNA nesprin TS; plasmid #68127), and nesprin headless control (pcDNA nesprin HL, plasmid #68128) were acquired from Addgene plasmid repository.

| Antibody | Source | Catalog number | Dilution |
|----------|--------|----------------|---------|
| 1. ATR | Cell Signal | 2790 | 1:1000 (WB); 1:100 (IF) |
| 2. TopBP1 | Abcam | ab2402 | 1:1000 (WB) |
| 3. Nup133 | Santacruz | sc-27392 | 1:500 (WB) |
| 4. Tubulin | Sigma | T5168 | 1:5000 (WB) |
| 5. Lamin B1 | Abcam | ab16048 | 1:10,000 (WB); 1:1000 (IF) |
| 6. Lamin A/C | Santacruz | sc-7292 | 1:500 (WB); 1:200 (IF) |
| 7. Nesprin 2 | Thermo Scientific | MAS-18075 | 1:500 (WB); 1:500 (IF) |
| 8. Histone H3(tri-met K9) | Abcam | ab8989 | 1:1000 (WB) |
| 9. Total Histone H3 | Abcam | ab1791 | 1:5000 (WB) |
| 10. Total YAP(63.7) | Santacruz | sc-10199 | 1:500 (WB); 1:200 (IF) |
| 11. Phospho YAP (Ser127) | Cell Signal | 4911S | 1:1000 (WB) |

Secondary antibodies were obtained from IFOM imaging facility: Polyclonal Donkey anti-mouse AlexaFluor-488 AB_2340846 (Jackson ImmunoResearch).

10 NATURE COMMUNICATIONS | (2020)11:4828 | https://doi.org/10.1038/s41467-020-18580-9 | www.nature.com/naturecommunications
Cell lines. U2OS cells stably expressing GFP-ATR and HeLa cells stably expressing mCherry-H2B were reported previously36. U2OS cells expressing the Fucci reporter was a kind gift from Libor Macárek33. Human primary fibroblasts derived from Seckel patient were maintained in DMEM supplemented with 15% FBS (not activated, Sigma-Aldrich) and IMR90 were grown in 10% FBS (not activated). HCT116 and ATR\textsuperscript{lox/lox} cells were grown in McCoy’s 5A media. All cells were grown in a humidified incubator at 37 °C and 5% CO\textsubscript{2}.

We used Lipofectamine 2000 (Invitrogen) for transfecting plasmids into cells, using the protocol recommended by the manufacturer. HEK293T cells were transfected with shRNA plasmids and viral packaging plasmids to generate lentiviral particles. Desired cell lines were then infected for 16 h followed by 2 μg/ml puromycin selection for 24 h. Infected cells were cultured in 1 μg/ml puromycin containing media and were utilized for experiments up to 10 days after infection.

Cells were treated with ATR inhibitors (2 μM ETP46464, 10 μM VE-821, or 1 μM AZ-20) 1 h before (unless mentioned otherwise) starting the experiment and were maintained in the media throughout the course of the experiment.

For cell cycle analysis, cells were fixed with ice-cold ethanol, DNA was labeled with propidium iodide, and quantified using FACS calibur (BD bioscience) system.

Membrane fractionation using Mem-Per Plus kit. Membrane fractions were performed following protocol provided by the vendor. Briefly, cells were trypsinized, washed with cell wash solution, resuspended in permeabilization buffer (with or without Benzonase), and incubated for 30 min at 4 °C with constant mixing. Permeabilized cells were then centrifuged for 15 min at 16,000 × g, soluble fraction was collected, and pellet resuspended and incubated in solubilization buffer for 30 min at 4 °C. Samples were then centrifuged for 15 min at 16,000 × g and supernatant was collected as a membrane fraction.

Cell lysis and immunoblotting. Total cell lysates were prepared in lysis buffer (50 mM Tris–HCl pH 8.0, 1 mM MgCl\textsubscript{2}, 200 mM NaCl, 10% Glycerol, 1% NP-40) Protease (Roche) and Phosphatase inhibitors (Sigma) were added at the time of experiment, and Benzonase (50 U/ml) was added if degradation of nucleic acid was needed. Cell lysates boiled with Laemmli buffer were (20–50 μg) resolved using NuPAGE® (Invitrogen) or Mini-PROTEAN® (Biorad) precast gels, transferred to nitrocellulose membrane, and probed as with primary (2 h at roomtemperature (RT) or overnight at 4 °C) and secondary antibodies (1 h at RT), and acquired using ChemiDoc imaging system (Image Lab v3.0). Image intensity measurements were performed using ImageJ.

If assays and quantifications. Briefly, cells were fixed with 4% formaldehyde (15 min), permeabilized with 0.2% Triton X-100 in phosphate-buffered saline (PBS) (15 min), blocked with 1% bovine serum albumin in PBS for 1 h (blocking buffer), incubated with primary antibodies (diluted in blocking buffer) for 1 h in RT, followed by three PBS washes and then incubated in secondary antibodies (1:400 in blocking solution) for 1 h in the dark at RT followed by three PBS washes. Samples were mounted with Vectashield mounting medium containing 4’,6-diamidino-2-phenylindole (DAPI). Image acquisition was performed using Leica TCS SP2 confocal scanning microscope, equipped with x63/1.4 numerical aperture (NA) objective. Single optical sections of the images or maximum projections (step size 0.5 μm) were processed using ImageJ and smoothed to reduce the background noise.

Quantification of nuclear morphology, YAP localization: images from random fields (upto 50) were acquired from coverslips stained with DAPI and Lamin or YAP on a UltraVIEW VoX spinning-disc confocal unit with Velocity software (PerkinElmer). Nuclei from each field were manually binned into normal, mild (blebs, invaginations, wrinkles, micronuclei, and multi-nuclei), and severely deformed (with multiple defects), as well as with or without micronuclei alone (in case if field has <20 cells it is combined with next field), which then are averaged to perform statistical analysis. Circularity index was calculated on central section of Lamin staining using Imagent pixel analysis tool and ABSnake plugin. YAP localization analysis was performed following the method described in Eloegui-Artola et al.56. Ratio was calculated between gross intensity measurements from a circular region of 30 pixel diameter in the nucleus and in the cytoplasm from individual cell.

Electron microscopy. The staff of EM facility at IJOFM performed all the EM analysis. EM examination, Immuno-EM gold labeling based on pre-embedding, EM tomography, and correlative light-electron microscopy (CLEM) were
incubated for 2 h in a 1:1 mixture of 100% ethanol and Epon-EMS at 90% ethanol; 3 × 10 min in 100% ethanol. The samples were subsequently rinsed in 3% potassium ferrocyanide (in 0.2 M sodium cacodylate pH 7.4) and then rinsed with water for 5 min. DNA was then extracted using standard phenol–chloroform technique and was resuspended in 200 μl of 0.1 M Tris, quantified, and were run on 1% agarose gels.

**AFM measurements.** The AFM measurements were performed using a Nanowizard 3 (IPK Instruments, Germany) and a modified silicon nitride cantilever (NovaScan, USA) with a spring constant of 0.03 N/m and a 5 μm diameter polystyrene bead adhered at the tip. Central region of the cell was indented with a loading rate of 1.5 μm/s. The ramp size was 3 μm was used. All the measurements were performed as previously described in ref. 37. Nuclei were isolated by treating cells with 1 ml of 1% IGEPAL CA-630 (a non-ionic detergent, Sigma) and 1% citric acid solution in water for 5 min. Expelled nuclei from the adherent cells were collected, washed with 5 ml PBS, centrifuged at 800 × g for 5 min, resuspended in PBS, and dropped onto coverslip for AFM experiments.

**FRET image acquisition and analysis.** Cells grown on coverslips were injected with the Nespren 2G Ts construct (50 ng/cell) and the following day, imaging a single cell on an Olympus microscope with a ×60 objective and a ×63/1.40 oil-immersion objective. Three images were collected in sequence at each point: Cyan Fluorescent Protein (CFP) (for mTPFP1) (ex: 438/24 nm, em: 470/24 nm), FRET (ex: 438/24 nm, em: 559/38 nm), and Yellow Fluorescent Protein (for mVenus) (ex: 513/17 nm, em: 528/38 nm). A single-plane image was background corrected, reoriented converted into 32 bits, and analyzed using ImageJ in how macro in imaging. The nuclear membrane of each cell was manually selected as a region of interest and average FRET/CFP ratios calculated for the nuclear membrane region. Approximately 30 cells/conditions were analyzed for n = 2 experiments. ATR inhibitor was added (ETP46464 2 μM) 3 h before image acquisition. ATR inhibitor VE-821 could not be used for these measurements, as they exhibited auto fluorescence. HeLa cells stably expressing Nespren 2G sensor or the Headless control sensor were generated by Lipofectamine 2000 transfection, Neomycin (G418) selection, and single-cell fluorescence-activated cells sorting of the mVenus/mTfP1-positive population. These cells were loaded onto the channels in the presence of ATR inhibitor or DMSO. A single stack image acquired for each field of view every 2 h for 10 h duration. Image acquisition parameters and analysis was similar to the above-mentioned experiment.

**FLIM–FRET analysis.** For the acquisition, we used the Leica TCS SP8 confocal microscope with White light laser as excitation source tuned at 488 nm and HC PL APO CS2 ×63/1.40 oil-immersion objective, everything managed by Leica Application Suite X. A 2D/3D single stack image acquired for each field of view every 2 h for 10 h duration. Image acquisition parameters and analysis was similar to the above-mentioned experiment.

**Micro-fabricated cell compression chamber.** A custom-made cell compression device has been invented based on movement of thin membrane attached with a pH sensitive membrane pressure responsive encapsulated cell compression device was designed using Solidworks and device components were 3D-printed using Dental SG resin (Formlabs) for its biocompatibility. All the components were used for a specific experiment as described previously in ref. 37. The minimal set of samples was performed according to ref. 37. Correlation between two variables was calculated using Pearson’s product moment correlation.
printed and then washed with IPA for 20 min, followed by post processing in ultraviolet chamber as suggested by Formlabs. A 20 mm diameter coverslip was stick on the top center of the cell compression device. Silicon membrane was stuck with a piston and then clamped to the bottom of the cell compression device by clamping tools. The assembled cell compression device was then connected to the air pressure regulator. Cells were plated on glass-bottom petri dish and maintained in cell incubator. Before the experiment, a cell compression device was captured cell culture dish was acquired using x40 oil lens (NA = 1.3) in PerkinElmer spinning disc microscope.

Analysis of cell migration in micro-fabricated channels. We followed the protocol established previously26 for PDMS channels preparation. Briefly, Polymer and crosslinking agent (RTV615 kit) (mixed in 1 : 10 ratio) was used to prepare PDMS channels. These are then plasma treated and embedded onto a glass-bottom dish (Thermo Fisher). Then channels were fibronectin-coated and cells were loaded the day before the beginning of time lapse. We chose 15 μm-long, 4 μm-wide constriction for experiments involving HeLa and U2OS cell lines. Time-lapse images were acquired (every 10 or 15 min, with z-stacks) on a UltraVIEW VoX spinning-disc confocal unit with Velocity software (PerkinElmer, eXel). Images were captured using an Eclipse microscope (Nikon) and a C9100-50 electron-multiplying CCD (charge-coupled device) camera (Hama-matsu) or Confocal Scanning Disk microscope (Olympus) equipped with IX83 inverted microscope provided with an IXON 897 Ultra camera (Andor) with OLYMPUS cellSens Dimension software, or on a DeltaVision Elite imaging system using the UltraVIEW VoX camera. 33PI foci were counted and 320× dry objective for a duration of 18–24 h. The images were processed using ImageJ and smoothed to reduce the background noise. All the quantifications were performed manually. Number of cells reaching the constriction within the experimental period was considered as a total cell number. Number of cell death and cell passing were counted and the percentage of cells with no cell migration were discarded from analysis. 33PI foci were counted manually using ImageJ. Difference was calculated by subtracting number of Foci before engaging the constriction from the number of foci present in the constriction.

Lipidomic analysis. (I) Lipid extraction: Nuclei were isolated according to the protocol66. Nuclei and total cell samples were resuspended in 150 mM ammonium bicarbonate and passed through a 26 G syringe needle to fragment nucleic acids. Samples were centrifuged at 10 000 × g for 10 min at 4 °C to eliminate cell debris. Lipids were extracted starting from an sample size equivalent of 50 μg of proteins, using a two-step extraction protocol (Folch method) with methanol and chloroform in different proportions67. Organic phase fractions were then dried out and resuspended in 50 μL of 95% v/v H2O:CH3CN 1 : 10 plus 5% v/v B (IPA: H2O: 90 : 10 : 5 mM NH4CO3 0.1% FA) for subsequent analysis. Before extraction, samples were spiked in 16 internal standards: PC (12 : 0/13 : 0) 40 pmol, PE (12 : 0/13 : 0) 52 pmol, phosphatidylglycerol (PG) (12 : 0/13 : 0) 7.5 pmol, phosphatidylserine (PS) (12 : 0/13 : 0) 43 pmol, phosphatidylinositol (PI) (12 : 0/13 : 0) 54 pmol, Cer (D18 : 1/25 : 0) 100 pmol, cholesterol ester (CE) (19 : 0) 180 pmol, GlcCer (d18 : 1/25 : 0) 50 pmol, LacCer (d18 : 1/25 : 0) 170 pmol, d18 : 1/25 : 0 50 pmol, sphinganine (d17 : 1) 50 pmol, sphingosine-1-P (d17 : 1) 100 pmol, sphingosine (d17 : 1) 50 pmol, GalactosyldiCer (d5) 20 pmol, d5-TG ISTD Mix I 20 pmol, d5-DG ISTD Mix I 20 pmol, and cholesterol (d7) 800 pmol. (II) Protein quantification: Proteins were extracted form 20 μL of ammonium bicarbonate resuspended fractions by adding 5 μL of lysis buffer (10% NP-40, 2% SDS in PBS) and endogenous proteases inhibitor cocktail (Protease and Phosphatase inhibitors) containing 15,000. Target metabolites were identified using Proxeon, Denmark. Peptide mixtures were desalted and concentrated on a homemade c18 desalting tip, then peptides were injected in a nanoHPLC (EasyLC Proxeon, Denmark). Peptides separation occurred on a 25 cm-long column, reverse-phase spraying fused silica capillary column (75 μm i.d.) packed with 3 μm ReproSil-Pur C18-AQ (Dr. Maisch GmbH, Germany). A gradient of eluents A (5% acetonitrile and 0.1% formic acid) and B (IPA : H2O : 90 : 10 : 5 mM NH4CO3 0.1% FA) and B (IPA : H2O : 90 : 10 ; 5 mM NH4CO3 0.1% FA) were used in positive mode. The gradient elution was initially started from 5% B, linearly increased to 100% B in 5 min, maintained for 45 min, then returned to the initial ratio in 2 min, and maintained for 8 min. Acquisition in MS was performed in positive with the following parameters: mass over charge (m/z) range 100–1700, T source 80 °C, Ion Spray Voltage 2000, declustering potential 80, fixed collision energy 40 V (+). For Information Dependent Acquisition analysis (Top 8), range of m/z was set as 200–1800 in positive ion mode; target ions were excluded for 20 s after two occurrences (Analyst TF 1.7.1). (IV) Data processing: Lipidview worked on the .raw file. LipidQuant was used for lipid identities identification and quantification. Lipid identification was based on exact mass, retention time, and MS/MS pattern. Lipid species based on precursor fragment ion pairs were determined using a comprehensive target list in LipidView (Sciex). Lipid species identification was performed using the mass tolerance of 0.05 m/z in MS and 0.02 m/z in MS/ MS. The neutral losses of 184, 190, and 196 were used to assign lipid classes based on stats and downstream analysis were cholesterol ester (CE), sphingomyelin, diacylglycerol, triacylglycerol, ceramide (Cer), PE, PG, PI, P5 and lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysophosphatidylinositol, lysophosphatidylserine, hexosylceramide, dihexosylceramide, trihexosylceramide, rhapalaglycerol, sphingosylphosphorylcholine, and Cer-phosphate, in positive mode. All statistical analysis was performed using Metaboanalyst 4.0 web tool68 (https://www.metaboanalyst.ca/MetaboAnalyst/faces/home.xhtml). Three experimental and two technical replicates were measured for each condition and 854 lipid metabolites were detected in total. Missing metabolite intensities were imputed using the nearest neighbor algorithm. Lipid classes included in the analysis were the median of the 322 technically most reliable metabolites (detectable in ~95% of all samples) and averaged over two technical replicates. The total intensities of PE and PE were calculated as sum of all individual phospholipids with choline and ethanolamine head groups.

Proximity ligation assay. Experiment was performed using rabbit polyclonal ATR antibody (1 : 100) and mouse monoclonal Nesprin-2 antibody (1 : 250), following the protocol from the manufacturer (Sigma, Duolink PLA Technology).

Animals. Wild-type B6.CBAF1 mice used for in utero electroperoration were purchased from The Jackson Laboratory. Mice lines (Atr-CER, 129/Sv, and C37BL/6 mixed background) with inducible deletion of Atr were generated as reported in ref. 51. All animals were maintained in the Specific Pathogen-Free facility and were used in experiments conducted according to National animal welfare legislation. All animals were housed at a temperature 22 ± 2 °C, humidity 30–70%, and 12 h/12 h photoperiod.
dark/light cycle. Animal experiment protocol was approved by the Thüringer Landesamt für Verbraucherschutz, Germany.

Primers used for genotyping were as follows: ATR10 (5′-CTATTTTGGTTGCTGGTTTG-3′), ATR15 (5′-CTTTCAATCTCT-TCGACATGGTTAAAGG-3′), Cret (5′-GGTGTAGGCAAGGGAGTGATG-3′), Cret (5′-CCAGA-GAGCAATCCATGCGC-3′).

Transwell membrane migration assay. Neuroprogenitors were isolated from E13.5 embryonic brain of Atr-inducible deletion (ATR-CER) mouse line and cultured in neurosphere medium (DMEM/F12 supplemented with B-27, penicillin/ streptomycin, 10 ng/ml epidermal growth factor (EGF), and 20 ng/ml basic fibro- blast growth factor (bFGF)) for 1–2 days. Then 4-hydroxytamoxifen (4-OHT) was added to induce Atr deletion. Four days after tamoxifen or 4-OHT treatment, the neurosphere were trypsinized and reseeded in EGF- and bFGF-free medium. Cells were plated into polycarbonate membrane insert (ThinCert, Greiner-Bio One GmbH, Frickenhausen Germany) coated with poly-l-lysine. The cells were allowed to migrate for 20 h at 37 °C in 5% CO2 before fixation with paraformaldehyde and staining with DAPI. All cells in the upper side of membrane were carefully removed with a cotton ball before mounting the membrane to glass slide with coverslip on the top. Cells on the underside of filter membrane were counted under a fluorescence microscope and migration activity was calculated by bivariate number of cells per object (×20) field.

In vivo neuronal migration. The construction of shRNA expression vectors was reported in ref. 66. All oligonucleotides contained the hairpin loop sequence 5′-TTCAAGAGA-3′. The targeting sequences are as follows: shLuc: 5′-GCGTGTCGACAACTTACAC-3′; shATR-4: 5′-GACCTAAATAGCAGGATCT-3′; shATR-6: 5′-GACGTCGTAGCAGGATCAAAAGA-3′. The efficiency of the shRNA was screened with mouse embryonic fibroblasts cells and Neuro 2A cells. In utero electroporation was performed as described in ref. 70. One microgram of plasmid DNA in PBS was injected into the lateral ventricle of E14.5 embryos followed by electroporation. The embryos were isolated 4 days after electroporation (at E18.5) and processed for cryo- section. Images were acquired with Zeiss ApoTome (Carl Zeiss, Germany) after immunostaining with DAPI. GFP-tagged shRNA vectors were electroporated into wild-type embryonic brain ventricles at E14.5. The embryonic brain is analyzed by imaging at E18.5. Brain cortex was equally divided into ten sections. Images were acquired with Zeiss ApoTome (Carl Zeiss, Germany) after immunostaining with DAPI. GFP-taggedshRNA vectors were electroporated into wild-type embryonic brain ventricles at E14.5. The embryonic brain is analyzed by imaging at E18.5. Brain cortex was equally divided into ten sections and percentage of GFP-positive (GFP+) cells from each segmentation were quantified based on 1–2 sections from indicated number of animals for each plasmid.

Short-term lung colonization assay. Control HeLa cells (5 × 10⁵) and shATR HeLa cells (5 × 10⁵) were labeled with E-Fluo 670 (Molecular Probes), mixed in 200 µl PBS, and injected intravenously. Mice were then sacrificed after 2 and 48 h. The lungs were isolated and fixed in 4% phosphate-buffered formalin. Micrometastases were visualized using a confocal microscope and counted. All animal experiments were approved by the OPBA (Organisms for the well-being of the animal) of IFOM and Cogentech. All experiments complied with national guidelines and legislation for animal experimentation. All animal experiments were performed in accordance with national and international laws and policies. Mice were bred and housed under pathogen-free conditions in our animal facilities at Cogentech Consortium at the FIRC Institute of Molecular Oncology Foundation under the authorization from the Italian Ministry of Health (Autorizzazione Numero 604-2016).

Statistics and reproducibility. Statistical calculations and graphs were generated with GraphPad Prism5 and Prism7 or using Microsoft Excel (2011) software. All bar graphs are represented as mean ± s.e.m. Box plot, whiskers, and outliers are plotted in GraphPad Prism7 using Tukey’s method. Each dot on the box plots represents a measurement from a single cell. P-values were calculated by Student’s t-test, one-way or two-way analysis of variance with Sidak’s or Bonferroni multiple comparisons as indicated in the figure legends of the respective figure. All detailed report of statistical analysis for each graph is included in the source data file. All the experiments presented in this manuscript are successfully reproduced at least in two independent experiments. Exact numbers of replicates are included in the figure legend.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium through the PRIDE partner repository with the dataset identifier PXD003262. All other data supporting the findings of this study are available in main text, the Supplementary Material or in Source Data file. Any additional information can be made available personally upon reasonable request to the corresponding author. Source Data are provided with this paper.

Received: 6 June 2019; Accepted: 25 August 2020; Published online: 24 September 2020
31. E. S. Bell, J. Lammerding. Nuclear mechanics in disease. Annu. Rev. Biomed. Eng. 13, 397–428 (2011).
32. P. Isermann, J. Lammerding. Nuclear mechanics and mechanotransduction in health and disease. Curr. Biol. 23, R1113–R1121 (2013).
33. A. M. Nava, M. M. et al. Heterochromatin-driven nuclear softening protects the genome against mechanical stress-induced damage. Cell Res. 527 (2016).
34. J. Basta, J. Rauchman. The nucleosome remodeling and deacetylase. Nucleic Acids Res. 43, D512–D520 (2015).
35. J. V. Olsen et al. Quantitative phosphoproteomics reveals widespread full phosphorylation site occupancy during mitosis. Sci. Signal. 3, ra3 (2010).
36. J. Hashsh, N. Johnson, A. L. Cha, R. S. Topoisomerase II- and condensin-dependent breakage of MECATR-sensitive fragile sites occurs independently of spindle tension, anaphase, or cytokinesis. PLoS Genet. 8, e1002978 (2012).
37. D. R. Schmidt, S. Schreiber. L. Molecular association between ATR and two components of the nucleosome remodeling and deacetylating complex, HDAC2 and CHD4. Biochemistry 38, 14711–14717 (1999).
38. J. Zemp, L. Kutay. U. Nuclear export and cyttoplasmatic maturation of ribosomal subunits. FEBS Lett. 581, 2783–2793 (2007).
39. J. Luke, Y. et al. Nesprin-2 Giant (NUANCE) maintains nuclear envelope architecture and composition in skin. J. Cell Sci. 121, 1887–1898 (2008).
40. J. Banerjee, J. et al. Targeted ablation of nesprin 1 and nesprin 2 from murine myocardiun results in cardiomyopathy, altered nuclear morphology and composition of the mechanical gene response. PLoS Genet. 10, e1004144 (2014).
41. J. Zhang, et al. SUN1/2 and Smy/Neispiel-1 complexes connect centrosome to the nucleus during neuronalgenesis and neuronal migration in mice. Neuron 64, 173–187 (2009).
42. J. Bermejo, R. et al. The replication checkpoint protects fork stability by releasing transcribed genes from nuclear pores. Cell 146, 233–246 (2011).
43. J. Kidiyoor, G. F., A. Foti. A. M. -mediated regulation of nuclear and cellular plasticity. DNA Repair (Amst). "https://doi.org/10.1016/j.dnarep.2016.05.020" (2016).
44. J. Bozler, J. Q., C. C. Bosco, G. Condensins exert force on chromatin-nuclear envelope tethers to mediate nucleoplastic reemuction in Drosophila melanogaster. G3 5, 341–352 (2015).
45. J. Ghaovidel, A. et al. Impaired RNA nuclear export links DNA damage and cell-cycle checkpoint. Cell 131, 915–926 (2007).
46. J. Basta, J. Rauschman. The nesprin complex and deacetylase complex in development and disease. Trans. Res. 165, 36–47 (2015).
47. J. Nava, M. M. et al. Heterochromatin-driven nuclear softening protects the genome against mechanical stress-induced damage. Cell, "https://doi.org/10.1016/j.cell.2010.03.052" (2020).
48. J. Olmos, Y., Hodgson, L., Mantell, J., Verkade, P. & Carlton, J. G. ESCR-III controls nuclear envelope reformation. Nature 522, 236–239 (2015).
49. J. Bakhoun, S. F. & Cantley, L. C. The multifaceted role of chromosomal instability in cancer and its miomeroenvironment. Cell 174, 1347–1360 (2018).
50. J. Bell, E. S. & Lammerding. J. Causes and consequences of nuclear envelope alterations in tumour progression. Eur. J. Cell Biol. 95, 449–464 (2016).
51. J. Zhou, Z., Buhn, C. & Wang, Z. Q. Differential function of NBS1 and ATR in neurogenesis. DNA Repair 11, 210–221 (2012).
52. J. Lee, Y. et al. ATR maintains select progenitors during nervous system development. EMBO J. 31, 1177–1189 (2012).
53. J. Wang, J., Liu, S., Heallen, T. & Martin, J. F. The Hippo pathway in the heart: pivotal roles in development, disease, and regeneration. Nat. Rev. Cardiol. 15, 672–684 (2018).
54. J. Ruzankina, Y. & Deletion of the developmental essential gene ATR in adult mice leads to age-related phenotypes and stem cell loss. Cell Stem Cell 1, 113–126 (2007).
55. J. Tibbetts, R. S. et al. Functional interactions between BRCA1 and the checkpoint kinase ATR during genotoxic stress. Genes Dev. 14, 2989–3002 (2000).
56. J. Tibbetts, R. S. et al. A role for ATR in the DNA damage-induced phosphorylation of p53. Genes Dev. 13, 152–157 (1999).
57. J. Mironov, A. A. et al. Dicumarol, an inhibitor of ADP-ribosylation of CBP/P300, fragments golgi non-compact tubular zones and inhibits intra-golgi transport. Eur. J. Cell Biol. 83, 263–279 (2004).
58. J. Fusella, A., Micaroni, M., Di Giandomenico, D., Mironov, A. A. & Beznoussenko, G. V. Segregation of the Qb-SNAREs GS27 and GS28 into Golgi vesicles regulates intra-Golgi transport. Traffic 14, 568–584 (2013).
59. J. Beznoussenko, G. V. et al. Transport of soluble proteins through the Golgi occurs by diffusion via continuities across cisternae. Elife 3, "https://doi.org/10.7554/eLife.02009" (2014).
60. J. Bonnici, V., W. Magnani-Wilson, A., Wilson, C. & Mironov, A. A. Three-dimensional and immune electron microscopic analysis of the secretory pathway in Saccharomyces cerevisiae. Histochem. Cell Biol. 146, S15–S27 (2016).
