G-Quadriples (G4s) are nucleic acid structures found enriched within gene regulatory sequences. G4s control fundamental cellular processes, including replication, transcription, and translation. Proto-oncogenes are enriched with G4 sequences, while tumor-suppressor genes are depleted, suggesting roles for G4s in cell survival and proliferation. Specialized helicases participate in G4-mediated gene regulation via enzymatic unwinding activity. One such enzyme, DHX36/G4R1, is the major G4-helicase and is a master regulator of G4-DNAs and mRNAs. G4-resolution promotes the expression of proliferative genes; as such, DHX36/G4R1 promotes cell proliferation. Little is known about how DHX36/G4R1 itself is regulated in nondividing cells. We hypothesized that DHX36/G4R1 protein binding partners are altered when a cell transitions from a dividing to a quiescent state. We found that DHX36/G4R1 co-purifies with a distinct set of proteins under quiescent conditions, which may represent a novel complex that regulates DHX36/G4R1 during cell cycle transitions and have implications for development and cancer.
spematogonia differentiation, reduced proliferation, and enhanced apoptosis.\textsuperscript{32} Similarly, a Dlx36 knockout in human erythroid progenitor (ProE) cells decreased proliferation and caused dysregulation of genes with promoters enriched with G4 structures.\textsuperscript{30} Likewise, DHX6 knockout in human cell lines reduces cell proliferation.\textsuperscript{13} Taken together, these data indicate that DHX36/G4R1 is a positive regulator of cell division.

Though much has been revealed about the roles of DHX36/G4R1 in stimulating cell division, little is known of how DHX36/G4R1 is negatively regulated when a cell transitions from a dividing to a quiescent state. Interestingly, DHX36/G4R1 is highly expressed in nondoning adult tissues,\textsuperscript{35–40} suggesting that expression-independent mechanisms may negatively regulate DHX36/G4R1 during quiescence. We hypothesized that DHX36/G4R1 binding partners are altered when a cell switches from a dividing to a quiescent state. To test this, we immunoprecipitated DHX36/G4R1–protein complexes from dividing and quiescent cells and compared interacting proteins by mass spectrometry. We found that DHX36/G4R1 partners with a distinct set of proteins under quiescence. Differential interactomes may represent a novel mechanism to rapidly sequester and release DHX36/G4R1 during cell cycle transitions that has implications for cancer and development.

\section*{RESULTS}

\textbf{Protein Identification and Functional Analysis.} We sought to determine if DHX36/G4R1 protein binding partners change during quiescence. To do this, we immunoprecipitated DHX36/G4R1 protein complexes from two replicates each of actively dividing and quiescent cells. Increasing salt concentrations were used to wash lysate-bound beads and to enrich for the tightest DHX36/G4R1 binders. Following Laemmli buffer elution, a prominent DHX36/G4R1-specific band was observed (Figure 1).

To induce quiescence, MCF-7 cells were subjected to serum starvation, previously shown to synchronize cells in the G0 phase (quiescence).\textsuperscript{41,42} A second set of actively dividing cells were grown in standard serum conditions. In both groups, purified DHX36/G4R1-containing protein complexes were identified via mass spectrometry. To identify nonspecific bead binders, a beads-alone negative control immunoprecipitation reaction was included, which lacked DHX36/G4R1 antibody. A total of 211 different proteins were identified in at least one replicate from 18 687 spectra, after removal of contaminants and three decoys. An additional 18 proteins were members of 6 groups of proteins that could not be discriminated based on the peptides identified in the experiment (Supporting Information, Table 1). In these cases, the member of each group with the highest probability was chosen as a representative for further analysis. Functional annotation of the complete set of DHX36/G4R1 bound proteins using GO-Slim terms indicated significant enrichment for terms including RNA and actin filament binding, regulation of actin filament polymerization and depolymerization, and mRNA splicing and stability, among others (Supporting Information, Table 2).

Based on the values for normalized total spectra, we explored differences in DHX36/G4R1 binding partners between dividing and quiescent cells, considering only those proteins consistently found in both replicates of at least one treatment. More DHX36/G4R1-interacting proteins were identified in serum-fed cells (dividing), as 79 proteins bound to DHX36/G4R1 were identified in both replicates of serum-fed cells, but only 46 in both replicates of serum-starved cells (Supporting Information, Table 1: columns C–F). A total of 35 of these proteins were identified to have nonzero values in all replicates across both treatments representing broadly enriched proteins. Additionally, there were 90 unique proteins found in both replicates in either or both treatments. Of these 90 proteins, 24 were either exclusively identified in purifications from serum-fed cells (17 proteins) or exhibited 3-fold or higher average abundance in the fed relative to the
starved treatment (7 proteins). In serum-starved cells, 13 were exclusive or exhibited 3-fold or greater increase in average abundance relative to the fed treatment (four and nine proteins, respectively). Furthermore, 53 proteins were shared between both treatment groups but were not exclusive to or 3-fold enriched in either treatment group. (Figure 2). Among the 24 proteins preferentially identified in the serum-fed conditions, enriched GO terms included molecular functions (protein and rRNA binding and ubiquitin ligase inhibition), biological processes (xenophagy, translational initiation and co-translational membrane targeting, and nonsense-mediated decay), and cellular components (ribosome, actin cytoskeleton, nucleolus, and extracellular exosome) (Table 1). Among the 13 proteins preferentially identified in serum-starved conditions, the molecular functions included cadherin binding and protein heterodimerization activity, while enriched biological process and cellular components included nuclear envelope organization and reassembly, nuclear lamina and nuclear envelope (Table 2).

**Protein Connectivity.** String database analysis of proteins enriched in serum-starved conditions suggests biologically connectivity, with more observed known or predicted interactions (n = 12) than expected (n = 2) interactions (p-value = 8.09 × 10⁻⁸) (Figure 3). Numerous interactions among this set of proteins occur between laminins (LMNA, LMNB1), lamina-associated proteins (LAP2A), emerin, and BAF, with further linkages to histones (HIST1H2BK and HIST1H3J). Proteins enriched in serum-fed conditions also demonstrated evidence for biological connection, with more known or predicted (n = 21) versus expected (n = 8) interactions (PPP p-value = 1.39 × 10⁻⁵) (Figure 4). The interactome specific to serum-fed conditions is dominated by ribosomal proteins (RPS15A, RPL23, RPL11, RPS11) and translation initiation and elongation factors (EIF4A1, TUFM), with additional interactions visible between corin (CORO1C), filamins (FLNA, FLNB), protein phosphatase subunits (PPP1R12A, PPP1CB), taperin (TPRN), and the tumor-suppressor leucine-zipper domain protein PAWR.

## DISCUSSION

DHX36/G4R1 is a master regulator of G4-containing DNA and RNA molecules and is essential during early mammalian development. Conditional deletion of Dhx36 in a variety of mouse tissues leads to proliferation defects and stark shifts in gene expression. Less is known about how DHX36 is negatively regulated when in nondividing cells. Since DHX36/G4R1 remains ubiquitously expressed even in adult cells, we hypothesized that DHX36 protein binding partners are altered during nonproliferative cellular states. To test this, we simulated dividing and quiescent states by placing cells in serum-fed or serum-starved conditions, respectively. Although many proteins interacted with DHX36/G4R1 in both treatment groups, we found that DHX36/G4R1 engages with a distinct set of proteins in each state, suggesting that dynamic protein-protein interactions (PPI) may regulate DHX36/G4R1 activity during dividing and quiescent cellular states.

Notably, DHX36/G4R1 binds to a subset of proteins linked to the nuclear envelope and lamina during quiescence. Nuclear envelope and nuclear lamina proteins provide the basic structure of the nucleus while also regulating chromatin state, transcription, and recruitment of transcriptional regulators and epigenetic modifiers. The nuclear lamina is comprised of A- and B-type filamentous lamins that provide docking sites for hundreds of proteins. Transcriptionally silent regions of heterochromatin are often found at the periphery of the nucleus, juxtaposed to the nuclear lamina. We found that DHX36/G4R1 partners with a distinct set of nuclear envelope and nuclear lamina proteins in quiescent cells, including key nuclear lamina components LMNA and LMNB1, as well as LAP2, BAF, EMD, and HIST1H2BK/H2B1K (Figure 3). This observation suggests that DHX36/G4R1 may be regulated during quiescence via engagement with the nuclear lamina.

During quiescence, DHX36/G4R1 interacts with most members of the family of nuclear lamina binding proteins, termed LEM domain (LEM-D) proteins. The LEM-D proteins LAP2, emerin, and MAN1 have a protein domain that specifically interacts with barrier-to-autointegration factor (BAF). Together BAF and LEM-D proteins tether interphase chromosomes to the nuclear lamina, contributing to nuclear organization, chromatin structure, and transcription repression. Interestingly, DHX36/G4R1 interacts with two of the three LEM-D proteins (LAP2 and emerin), as well as BAF, during nutrient starvation conditions (Figure 3). Taken together, these data suggest a potential role for DHX36/G4R1 in nuclear structure and transcriptional changes in the nucleus during nutrient stress-induced cell cycle repression. Given that DHX36/G4R1 is a positive regulator of a host of genes that promote cell division (i.e., proto-oncogenes), an intriguing possibility is that the LEM-D/BAF complex may contribute to cell division repression via sequestering DHX36/G4R1 from proto-oncogene promoter sequences. Sequestration of DHX36/G4R1 by the nuclear lamina may also allow for rapid reinitiation of transcription of G4-containing genes once nutrient stress ends (Figure 5).

Serum starvation is a common strategy to cause cells to exit the cell cycle and synchronize in G0/G1. Protracted nutrient deprivation may also induce a stress response. DHX36/G4R1 is recruited to stress granules during a variety of cellular stresses, where it regulates the accumulation of translationally inactive G-quadruplex containing mRNAs. Therefore, differences in protein association may not be exclusively due to a difference in cell proliferation status but
Table 1. Significantly Enriched GO Terms for Fed-Exclusive or Fed 3-Fold Biased Proteins. FDR = False Discovery Rate

| gene ontology (GO) term                                      | FDR      | protein names                                                                 |
|-------------------------------------------------------------|----------|-------------------------------------------------------------------------------|
| biological processes                                        |          |                                                                               |
| xenophagy (GO:0098792)                                      | $2.16 \times 10^{-2}$ | Galactin-8, calcium-binding, and coiled-coil domain-containing protein 2 |
| establishment of protein localization to endoplasmic reticulum (GO:0072599) | $3.44 \times 10^{-3}$ | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, Ras-related protein Rab-10, and 40S ribosomal protein S11 |
| SRP-dependent co-translational protein targeting to membrane (GO:0006614) | $1.30 \times 10^{-2}$ | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, and 40S ribosomal protein S11 |
| co-translational protein targeting to membrane (GO:0006613) | $1.36 \times 10^{-3}$ | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, and 40S ribosomal protein S11 |
| protein targeting to ER (GO:0045047)                       | $1.46 \times 10^{-2}$ | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, and 40S ribosomal protein S11 |
| translational initiation (GO:0046413)                       | $4.30 \times 10^{-3}$ | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, and 40S ribosomal protein S11 |
| viral transcription (GO:0010983)                             | $2.55 \times 10^{-3}$ | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, and 40S ribosomal protein S11 |
| nuclear-transcribed mRNA catabolic process, nonsense-mediated decay (GO:0000184) | $1.67 \times 10^{-2}$ | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, and 40S ribosomal protein S11 |
| viral gene expression (GO:0019080)                           | $2.20 \times 10^{-2}$ | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, and 40S ribosomal protein S11 |
| protein targeting to membrane (GO:0006612)                  | $3.94 \times 10^{-2}$ | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, and 40S ribosomal protein S11 |
| establishment of protein localization to membrane (GO:0090180) | $2.32 \times 10^{-2}$ | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, Ras-related protein Rab-10, and 40S ribosomal protein S11 |
| translation (GO:0006412)                                    | $1.44 \times 10^{-2}$ | eukaryotic initiation factor 4A-I, 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, elongation factor Tu: mitochondrial, and 40S ribosomal protein S11 |
| peptide biosynthetic process (GO:0030403)                   | $1.30 \times 10^{-2}$ | eukaryotic initiation factor 4A-I, 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, elongation factor Tu: mitochondrial, and 40S ribosomal protein S11 |
| protein localization to membrane (GO:0072657)                | $2.39 \times 10^{-2}$ | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, Ras-related protein Rab-10, filamin-A, and 40S ribosomal protein S11 |
| amid biosynthetic process (GO:0043604)                       | $2.46 \times 10^{-2}$ | eukaryotic initiation factor 4A-I, 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, elongation factor Tu: mitochondrial, and 40S ribosomal protein S11 |
| peptide metabolic process (GO:0005818)                       | $2.55 \times 10^{-2}$ | eukaryotic initiation factor 4A-I, 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, elongation factor Tu: mitochondrial, and 40S ribosomal protein S11 |
| establishment of protein localization (GO:0045184)           | $3.72 \times 10^{-2}$ | TOM1-like protein 2, 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, coronavirus-1C, Ras-related protein Rab-10, filamin-A, 40S ribosomal protein S11, and pericentriolar material 1 protein |
| cellular protein localization (GO:0034613)                   | $3.78 \times 10^{-2}$ | TOM1-like protein 2, 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, filamin-B, Ras-related protein Rab-10, filamin-A, 40S ribosomal protein S11, and pericentriolar material 1 protein |
| cellular macromolecule localization (GO:00727)               | $3.76 \times 10^{-2}$ | TOM1-like protein 2, 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, filamin-B, Ras-related protein Rab-10, filamin-A, 40S ribosomal protein S11, and pericentriolar material 1 protein |
| viral process (GO:0016032)                                   | $2.27 \times 10^{-2}$ | eukaryotic initiation factor 4A-I, 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, elongation factor Tu: mitochondrial, and calcium-binding and coiled-coil domain-containing protein 2, 40S ribosomal protein S11 |
| symiotic process (GO:0044403)                                | $3.84 \times 10^{-2}$ | eukaryotic initiation factor 4A-I, 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, elongation factor Tu: mitochondrial, and calcium-binding and coiled-coil domain-containing protein 2, 40S ribosomal protein S11 |
| interspecies interaction between organisms (GO:0044419)       | $2.98 \times 10^{-2}$ | 60S ribosomal protein L11, 60S ribosomal protein L23, Galectin-8, filamin-B, elongation factor Tu: mitochondrial, complement C1s subcomponent, calcium-binding and coiled-coil domain-containing protein 2, Filamin-A, and 40S ribosomal protein S11 |
| molecular function                                           |          |                                                                               |
| ubiquitin ligase inhibitor activity (GO:1990948)             | $5.56 \times 10^{-2}$ | 60S ribosomal protein L11, 60S ribosomal protein L23 |
| ubiquitin-protein transferase inhibitor activity (GO:0055105) | $4.29 \times 10^{-2}$ | 60S ribosomal protein L11, 60S ribosomal protein L23 |
| rRNA binding (GO:0019843)                                    | $4.80 \times 10^{-2}$ | 60S ribosomal protein L11, 60S ribosomal protein L23, and 40S ribosomal protein S11 |
| structural constituent of ribosome (GO:0003735)              | $5.00 \times 10^{-2}$ | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, and 40S ribosomal protein S11 |
| RNA binding (GO:0003723)                                    | $1.91 \times 10^{-2}$ | probable ATP-dependent RNA helicase DDX17, eukaryotic initiation factor 4A-I, 40S ribosomal protein S15a, 40S ribosomal protein L11, 40S ribosomal protein L23, filamin-B, elongation factor Tu: mitochondrial, filamin-A, and 40S ribosomal protein S11 |
| protein binding (GO:0005515)                                 | $1.37 \times 10^{-2}$ | 60S ribosomal protein L11, 60S ribosomal protein L23, and 40S ribosomal protein S11 |

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Table 1. continued

| Molecular Function                                                                 | gene ontology (GO) term | FDR        | protein names                                                                                           |
|-----------------------------------------------------------------------------------|-------------------------|------------|--------------------------------------------------------------------------------------------------------|
| PTW/PP1 phosphatase complex (GO:0072357)                                          | 7.59 × 10⁻³             | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, and 40S ribosomal protein S11 |
| cytosolic ribosome (GO:0022626)                                                   | 1.49 × 10⁻³             | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, and 40S ribosomal protein S11 |
| Z disc (GO:00330018)                                                             | 3.97 × 10⁻²             | 40S ribosomal protein L23, guanine nucleotide-binding protein G(1)/G(S)/G(T) subunit β-2, filamin-B, coronin-1C, Ras-related protein Rab-10, filamin-A, serine/threonine-protein phosphatase PP1-β catalytic subunit, 40S ribosomal protein S11, and protein phosphatase 1 regulatory subunit 12A |
| focal adhesion (GO:0005992)                                                      | 1.09 × 10⁻⁶             | 60S ribosomal protein L23, guanine nucleotide-binding protein G(1)/G(S)/G(T) subunit β-2, filamin-B, coronin-1C, Ras-related protein Rab-10, filamin-A, serine/threonine-protein phosphatase PP1-β catalytic subunit, 40S ribosomal protein S11, and protein phosphatase 1 regulatory subunit 12A |
| cell-substrate junction (GO:0030055)                                              | 6.01 × 10⁻⁷             | 40S ribosomal protein L23, guanine nucleotide-binding protein G(1)/G(S)/G(T) subunit β-2, filamin-B, coronin-1C, Ras-related protein Rab-10, filamin-A, serine/threonine-protein phosphatase PP1-β catalytic subunit, 40S ribosomal protein S11, and protein phosphatase 1 regulatory subunit 12A |
| I band (GO:0031674)                                                              | 4.89 × 10⁻²             | 60S ribosomal protein L23, guanine nucleotide-binding protein G(1)/G(S)/G(T) subunit β-2, filamin-B, coronin-1C, Ras-related protein Rab-10, filamin-A, serine/threonine-protein phosphatase PP1-β catalytic subunit, 40S ribosomal protein S11, and protein phosphatase 1 regulatory subunit 12A |
| ribosomal subunit (GO:0044591)                                                    | 7.84 × 10⁻³             | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, and 40S ribosomal protein S11 |
| sarcomere (GO:0030017)                                                           | 9.54 × 10⁻³             | filamin-B, coronin-1C, filamin-A, and protein phosphatase 1 regulatory subunit 12A |
| myofibril (GO:0030016)                                                            | 1.33 × 10⁻³             | filamin-B, coronin-1C, filamin-A, and protein phosphatase 1 regulatory subunit 12A |
| contractile fiber (GO:0038492)                                                    | 1.51 × 10⁻³             | filamin-B, coronin-1C, filamin-A, and protein phosphatase 1 regulatory subunit 12A |
| ribosome (GO:0030016)                                                             | 1.47 × 10⁻³             | 60S ribosomal protein L11, 60S ribosomal protein L23, and 40S ribosomal protein S11 |
| anchoring junction (GO:0070161)                                                   | 1.36 × 10⁻⁴             | 60S ribosomal protein L23, guanine nucleotide-binding protein G(1)/G(S)/G(T) subunit β-2, filamin-B, coronin-1C, Ras-related protein Rab-10, filamin-A, serine/threonine-protein phosphatase PP1-β catalytic subunit, 40S ribosomal protein S11, and protein phosphatase 1 regulatory subunit 12A |
| cell cortex (GO:0005938)                                                          | 5.97 × 10⁻³             | 40S ribosomal protein L11, 60S ribosomal protein L23, and 40S ribosomal protein S11 |
| actin cytoskeleton (GO:0031629)                                                   | 1.36 × 10⁻⁴             | PRKc apoptosis WTI regulator protein, filamin-B, coronin-1C, filamin-A, and protein phosphatase 1 regulatory subunit 12A |
| nucleus (GO:0005730)                                                              | 8.29 × 10⁻³             | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, and 40S ribosomal protein S11 |
| cell junction (GO:0030054)                                                        | 8.95 × 10⁻³             | 60S ribosomal protein L23, guanine nucleotide-binding protein G(1)/G(S)/G(T) subunit β-2, filamin-B, coronin-1C, elongation factor Tu: mitochondrial, Ras-related protein Rab-10, filamin-A, serine/threonine-protein phosphatase PP1-β catalytic subunit, 40S ribosomal protein S11, and protein phosphatase 1 regulatory subunit 12A |
| extracellular exosome (GO:0070062)                                                | 4.68 × 10⁻⁴             | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, guanine nucleotide-binding protein G(1)/G(S)/G(T) subunit β-2, filamin-B, elongation factor Tu: mitochondrial, Ras-related protein Rab-10, filamin-A, serine/threonine-protein phosphatase PP1-β catalytic subunit, and 40S ribosomal protein S11 |
| extracellular vesicle (GO:1903561)                                                | 4.17 × 10⁻⁴             | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, guanine nucleotide-binding protein G(1)/G(S)/G(T) subunit β-2, filamin-B, elongation factor Tu: mitochondrial, Ras-related protein Rab-10, filamin-A, serine/threonine-protein phosphatase PP1-β catalytic subunit, and 40S ribosomal protein S11 |
| extracellular organelle (GO:0043230)                                              | 3.56 × 10⁻⁴             | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, guanine nucleotide-binding protein G(1)/G(S)/G(T) subunit β-2, filamin-B, elongation factor Tu: mitochondrial, Ras-related protein Rab-10, filamin-A, serine/threonine-protein phosphatase PP1-β catalytic subunit, and 40S ribosomal protein S11 |
| extracellular space (GO:0005615)                                                  | 7.57 × 10⁻⁴             | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, guanine nucleotide-binding protein G(1)/G(S)/G(T) subunit β-2, filamin-B, elongation factor Tu: mitochondrial, Ras-related protein Rab-10, filamin-A, serine/threonine-protein phosphatase PP1-β catalytic subunit, and 40S ribosomal protein S11 |
| vesicle (GO:0031982)                                                              | 7.95 × 10⁻⁴             | 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, guanine nucleotide-binding protein G(1)/G(S)/G(T) subunit β-2, filamin-B, elongation factor Tu: mitochondrial, Ras-related protein Rab-10, filamin-A, serine/threonine-protein phosphatase PP1-β catalytic subunit, and 40S ribosomal protein S11 |
| cytosol (GO:0005829)                                                             | 7.31 × 10⁻⁴             | probable ATP-dependent RNA helicase DDX17, eukaryotic initiation factor 4A-I, TOM1-like protein 2, 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, guanine nucleotide-binding protein G(1)/G(S)/G(T) subunit β-2, filamin-B, coronin-1C, elongation factor Tu: mitochondrial, Ras-related protein Rab-10, filamin-A, serine/threonine-protein phosphatase PP1-β catalytic subunit, 40S ribosomal protein S11, and protein phosphatase 1 regulatory subunit 12A, pericentriolar material 1 protein |
Table 1. continued

| gene ontology (GO) term          | FDR      | protein names                                                                 |
|----------------------------------|----------|-------------------------------------------------------------------------------|
| extracellular region (GO:0005576)| 8.26 × 10^-3| eukaryotic initiation factor 4A-I, TOM1-like protein 2, 40S ribosomal protein S15a, 60S ribosomal protein L11, 60S ribosomal protein L23, guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit β-2, galectin-8, filamin-B, elongation factor Tu: mitochondrial, complement C1s subcomponent, Ras-related protein Rab-10, filamin-A, serine/threonine-protein phosphatase PP1-β catalytic subunit, and 40S ribosomal protein S11 |
| intracellular nonmembrane-bounded organelle (GO:0043228) | 8.28 × 10^-3| ribosomal protein L23, filamin-B, coronin-1C, elongation factor Tu: mitochondrial, calcium-binding and coiled-coil domain-containing protein 2, Ras-related protein Rab-10, filamin-A, serine/threonine-protein phosphatase PP1-β catalytic subunit, 40S ribosomal protein S11, protein phosphatase 1 regulatory subunit 12A, and pericentriolar material 1 protein |

Overall, these data reveal a novel cadre of proteins that interact with DHX36/G4R1 during cellular quiescence induced by serum starvation. As DHX36/G4R1 promotes the expression of many genes, many of the starvation-enriched proteins may contribute to transcriptional repression by sequestering DHX36/G4R1. The nuclear envelope and lamina are the fundamental structural and regulatory elements of the nucleus, and disruption of these elements results in neurodegeneration, muscular dystrophies, lipodystrophies, and premature aging diseases. A notable example of this is mutations of LMNA, which causes Hutchinson–Gilford progeria syndrome (HGPS), a disease characterized by premature aging. The preponderance of nuclear lamina proteins associated with DHX36/G4R1 during serum starvation, including LMNA, suggests the possibility of involvement of DHX36/G4R1 in premature aging and laminopathy. It should be noted that the experiments described herein were completed with replicates in a single breast cancer cell line; whether the DHX36/G4R1-interacting proteins observed in the quiescent cells in these experiments are representative of DHX36/G4R1 binding partners in other may also reflect a cellular stress response. Notably, a subset of the nuclear envelope and lamina proteins that co-purify with DHX36/G4R1 during serum starvation have also been observed in stress granules, including LMNA and BAF, suggesting an overlap between nuclear lamina proteins and stress proteins. Finally, appreciable cell death has been observed in cells synchronized with serum starvation, leaving open the possibility that DHX36/G4R1 might interact with the nuclear lamina to prepare for apoptosis and/or nuclear envelope breakdown. Future work may further validate and explore this observation by reverse IP analysis and assessing whether serum starvation induces DHX36/G4R1 expression changes, respectively.
quiescent cells remain to be determined. However, serum-dependent differences in association are consistent with proliferation state-dependent regulation of DHX36/G4R1 protein interaction.

**METHODS**

**MCF-7 Cell Culture and Harvest.** Immortalized human breast cancer (MCF-7) cells were grown in DMEM supplemented with 10% (v/v) bovine growth serum and 1% (v/v) antibacterial-antimycotic. Serum-starved cells were cultured in phenol red-free RPMI-1640 medium supplemented with 1% (v/v) antibacterial-antimycotic, without bovine growth serum for 24 h. The cells were trypsinized with 1 ml of 0.25% (w/v) trypsin at 37 °C for 4 min. The trypsin was then neutralized with 9 ml of DMEM. For each sample, $7 \times 10^6$ cells were pelleted by centrifugation at 500 × g at 4 °C for 10 min before being washed with ice-cold DBPS and stored at −20 °C.

**Non-denaturing Cell Lysis.** The cells were resuspended in non-denaturing lysis buffer (20 mM Tris-HCl pH 8.0, 137 mM NaCl, 1% (v/v) NP-40, 2 mM EDTA) supplemented with 1% (v/v) protease inhibitor cocktail (Roche, EDTA-free tablets) and mechanically lysed using a plastic homogenizer pestle. The insoluble material was separated by centrifugation at 20 000 g for 5 min at 4 °C. The supernatant was then stored at −80 °C.

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Table 2. Significantly enriched GO terms for starved-exclusive or starved 3-fold biased proteins. FDR = false discovery rate.

| gene ontology (GO) term                      | FDR     | protein names                                                                 |
|---------------------------------------------|---------|-------------------------------------------------------------------------------|
| mitotic nuclear envelope reassembly         | 5.6 × 10^{-2} | barrier-to-autointegration factor (BAF), prelamin-A/C, and emerin          |
| nuclear envelope reassembly (GO:0031468)    | 3.34 × 10^{-2} | barrier-to-autointegration factor, prelamin-A/C, and emerin                  |
| cadherin binding (GO:0045296)               | 4.08 × 10^{-2} | histone H3.1, 14-3-3 protein epsilon, emerin, lamina-associated polypeptide 2, and isoform α |
| protein heterodimerization activity (GO:00469832) | 3.10 × 10^{-4} | histone H3.1, protein disulphide isomerase, 14-3-3 protein epsilon, and histone H2B type 1-K |
| cell adhesion molecule binding (GO:0005839) | 2.70 × 10^{-2} | histone H3.1, protein disulphide isomerase, 14-3-3 protein epsilon, emerin, lamina-associated polypeptide 2, and isoform α |
| protein dimerization activity (GO:0046983)  | 2.36 × 10^{-2} | histone H3.1, protein disulphide isomerase, barrier-to-autointegration factor, 14-3-3 protein epsilon, glutathione S-transferase Mα 3, and histone H2B type 1-K |
| lamin filament                               | 6.38 × 10^{-3} | lamin-B1, prelamin-A/C                                                      |
| nuclear lamina                               | 7.88 × 10^{-3} | lamin-B1, prelamin-A/C                                                      |
| nuclear inner membrane                       | 4.36 × 10^{-3} | lamin-B1, emerin, lamina-associated polypeptide 2, and isoform α            |
| nuclear membrane (GO:0031965)                | 1.08 × 10^{-2} | lamin-B1, prelamin-A/C, emerin, lamina-associated polypeptide 2, and isoform α |
| nuclear envelope (GO:0005635)                | 9.52 × 10^{-3} | lamin-B1, barrier-to-autointegration factor, prelamin-A/C, emerin, lamina-associated polypeptide 2, and isoform α |
| intracellular nonmembrane-bounded organelle (GO:0043232) | 9.45 × 10^{-3} | ATP-dependent DNA/RNA helicase DHX36/G4R1, lamin-B1, histone H3.1, protein disulphide isomerase, barrier-to-autointegration factor, prelamin-A/C, 14-3-3 protein epsilon, emerin, histone H2B type 1-K, lamin-associated polypeptide 2, and isoform α |
| nonmembrane-bounded organelle (GO:0043228)   | 8.01 × 10^{-3} | ATP-dependent DNA/RNA helicase DHX36/G4R1, lamin-B1, histone H3.1, protein disulphide isomerase, barrier-to-autointegration factor, prelamin-A/C, 14-3-3 protein epsilon, emerin, histone H2B type 1-K, lamin-associated polypeptide 2, and isoform α |

Figure 3. Protein interaction network for proteins exclusive to the serum-starved/quiescent treatment or with average abundance 3x biased toward in serum-starved (quiescent) compared serum-fed (dividing) treatments.

Figure 4. Protein interaction network for proteins exclusive to serum-fed/dividing treatment, or with average abundance 3-fold higher in serum-fed (dividing) vs. serum-starved (quiescent) treatments.
DHX36/G4R1 Immunoprecipitation. Protein concentrations from non-denatured lysates were determined using a bicinchoninic acid (BCA) assay. One milligram of total protein was incubated with 50 μl of A/G magnetic beads and 4 μg of mouse anti-DHX36/G4R1 monoclonal antibody (Santa Cruz). In a separate reaction, no DHX36/G4R1 antibody was added to the beads, as a negative control (beads-alone). The samples were incubated for 15 min on ice before incubating overnight at 4 °C while rotating. The beads were then pelleted using a magnetic separator and washed with a low-salt wash buffer (0.1% (w/v) SDS, 0.1% (v/v) Triton X-100, 150 mM NaCl, 2 mM EDTA, 20 mM Tris-HCl pH 8.0), high-salt wash buffer (0.1% (w/v) SDS, 0.1% (v/v) Triton X-100, 500 mM NaCl, 2 mM EDTA, 20 mM Tris-HCl pH 8.0), and LiCl wash buffer (500 mM LiCl, 1% (v/v) NP-40, 1% (v/v) deoxychloric acid, 100 mM Tris-HCl pH 8.0) for 5 min at room temperature while rotating. The proteins were then eluted using 50 μl of 3X Laemmli sample buffer (supplemented with β-mercaptoethanol to a final concentration of 10% (v/v) and with 1% (v/v) protease inhibitor cocktail (Roche, EDTA-free tablets)) and incubated at 70 °C for 10 min. The beads were pelleted using a magnetic separator, and the supernatant was stored at −80 °C. Immunoprecipitation was confirmed using western blot analysis.

Western Blot Analysis. Lysates were prepared with 4X Laemmli buffer (supplemented with β-mercaptoethanol to a final concentration of 10% (v/v)) and loaded onto a 10% denaturing polyacrylamide gel. The gel was run in 1X Tris-glycine running buffer (0.25 M Tris, 1.92 M glycine, 0.1% (w/v) SDS) at 120 V until the loading dye had reached the bottom of the gel. Proteins were then transferred in 1X Tris-glycine transfer buffer (0.25 M Tris, 192 mM glycine, 20% (v/v) methanol) to a poly(vinylidene fluoride) (PVDF) membrane at 40 V for 2 h at 4 °C. The membrane was blocked in 1% (w/v) milk blocking buffer for 1 h while rocking. The membrane was incubated with an anti-DHX36/G4R1 monoclonal antibody conjugated with a 790 Alexa Fluor (1:800) in 1X milk blocking buffer and incubated overnight at 4 °C while rocking. The membranes were washed 3 times in 1X Tris-buffered saline with Tween-20 (TBST, 100 mM Tris-HCl, 1.5 M NaCl, 0.5% (v/v) Tween-20, pH 7.5) for 10 min. The membrane was imaged on a LICOR Odyssey CLx imager.

Protein Mass Spectrometry. DHX36/G4R1 immunoprecipitation elutions were performed in duplicate and were processed by MSBioworks (Ann Arbor, MI) using SDS-PAGE with a 10% bis–tris NuPAGE gel (Invitrogen) with the MES buffer system. The mobility region was excised into 10 equal segments. The gel fragments were washed with 25 mM ammonium bicarbonate followed by acetonitrile. The gel was then reduced with 10 mM dithiothreitol at 60 °C and 50 mM iodoacetamide at room temperature. The gel was digested with sequencing grade trypsin (Promega) at 37 °C for 4 h before being quenched with formic acid. One half of each immunoprecipitation replicate was analyzed by nano LC-MS/MS using a Waters NanoAcquity system interfaced to a ThermoFisher Fusion Lumos mass spectrometer, while the other half was used for western blot analysis. The peptides were loaded on a trapping column and eluted over a 75 μm analytical column at 350 nL/min; both columns were packed with Luna C18 resin (Phenomenex). A 2 h gradient was employed. The spectra were matched to proteins using Mascot v.2.6.0 with the SwissProt Human-010119-2019101 database, and the results were imported into Scaffold v.4.10.0. Proteins were identified using 1% protein and peptide false discovery rate (FDR) and requiring at least two unique peptides per protein, yielding a decoy FDR of 1%. Prior to analysis, common contaminants (keratin, trypsin, myosin, tropomyosin) and decoy sequences were removed, as were proteins identified in a separate bead-only negative control experiment. Analysis of abundance among treatments utilized the normalized total spectra values calculated in Scaffold v.4.10.0, where spectral counts in each replicate are multiplied by the ratio of the average spectrum count across all replicates to individual replicate total spectrum count.

Gene Ontology Analysis. Functional enrichment analysis for either cellular component, molecular function, or biological process was performed with complete GO terms or GO-Slims for sets of proteins via a web interface (http://geneontology.org/docs/go-enrichment-analysis) with the GO Ontology database DOI: 10.5281/zenodo.3727280 Released 2020-03-23 and using the PANTHER classification system, release 20200407 with significance set to an FDR of 5% (q<0.05%).

String-db Analysis. Interaction networks among sets of proteins identified by MS analysis were defined using the STRING database, which includes information on both direct and indirect interactions between proteins using data from multiple sources. For each set, we calculated the significance of the enrichment of protein–protein interactions (PPI) relative to a set of proteins of similar size drawn from the genome.

**Figure 5.** Working model of LEM-D proteins and BAF sequestration of DHX36/G4R1 during serum starvation-induced quiescence.
starved replicates (Table 1); functional annotation of the complete set of DHX36/G4R1 bound proteins using GO-Slim terms with indicated significant enrichment (Table 2) (XLSX)

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Notes
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■ REFERENCES

(1) Mendoza, O.; Bourdoncle, A.; Boule, J. B.; Brosh, R. M., Jr; Mergny, J. L. G-quadruplexes and helicases. Nucleic Acids Res. 2016, 44, 1989–2006.
(2) Maiel, N. G4-associated human diseases. EMBO Rep. 2015, 16, 910–922.
(3) Rhodes, D.; Lipps, H. J. G-quadruplexes and their regulatory roles in biology. Nucleic Acids Res. 2015, 43, 8627–8637.
(4) Biffi, G.; Tannahill, D.; McCafferty, J.; Balasubramanian, S. Quantitative visualization of DNA G-quadruplex structures in human cells. Nat. Chem. 2013, 5, 182–186.
(5) Henderson, A.; Wu, Y.; Huang, Y. C.; Chavez, E. A.; Platt, J.; Johnson, F. B.; Brosh, R. M., Jr; Sen, D.; Lansdorp, P. M. Detection of G-quadruplex DNA in mammalian cells. Nucleic Acids Res. 2014, 42, 860–869.
(6) Laguerre, A.; Wong, J. M. Y.; Monchaud, D. Direct visualization of both DNA and RNA quadruplexes in human cells via an uncommon spectroscopic method. Sci. Rep. 2016, 6, No. 32411.
(7) Zhang, S.; Sun, H.; Wang, L.; Liu, Y.; Chen, H.; Li, Q.; Guan, A.; Liu, M.; Tang, Y. Real-time monitoring of DNA G-quadruplexes in living cells with a small-molecule fluorescent probe. Nucleic Acids Res. 2018, 46, 7522–7532.
(8) Chambers, V. S.; Marsico, G.; Boutell, J. M.; Di Antonio, M.; Smith, G. P.; Balasubramanian, S. High-throughput sequencing of DNA G-quadruplex structures in the human genome. Nat. Biotechnol. 2015, 33, 877–881.
(9) Bedrat, A.; Lacroix, L.; Mergny, J. L. Re-evaluation of G-quadruplex propensity with G4Hunter. Nucleic Acids Res. 2016, 44, 1746–1759.
(10) Marsico, G.; Chambers, V. S.; Sahakyan, A. B.; McCauley, P.; Boutell, J. M.; Antonio, M. D.; Balasubramanian, S. Whole genome experimental maps of DNA G-quadruplexes in multiple species. Nucleic Acids Res. 2019, 47, 3862–3874.
(11) Huppert, J. L.; Balasubramanian, S. Prevalence of quadruplexes in the human genome. Nucleic Acids Res. 2005, 33, 2908–2916.
(12) Huppert, J. L.; Balasubramanian, S. G-quadruplexes in promoters throughout the human genome. Nucleic Acids Res. 2007, 35, 406–413.
(13) Sauer, M.; Jurank, S. A.; Marks, J.; De Magis, A.; Kazemier, H. G.; Hilbig, D.; Benhaley, D.; Wang, X.; Hafner, M.; Paeschke, K. DHX36 prevents the accumulation of translationally inactive mRNAs with G4-structures in untranslated regions. Nat. Commun. 2019, 10, No. 2421.
(14) Creacy, S. D.; Routh, E. D.; Iwamoto, F.; Nagamine, Y.; Akman, S. A.; Vaughan, J. P. G4 resolvease 1 binds both DNA and RNA tetramolecular quadruplex with high affinity and is the major source of tetramolecular quadruplex G4-DNA and G4-RNA resolving activity in HeLa cell lysates. J. Biol. Chem. 2008, 283, 34626–34634.
(15) Vaughan, J. P.; Creacy, S. D.; Routh, E. D.; Joyner-Butt, C.; Jenkins, G. S.; Pauli, S.; Nagamine, Y.; Akman, S. A. The DEXH protein product of the DHX36 gene is the major source of tetramolecular quadruplex G4-DNA resolving activity in HeLa cell lysates. J. Biol. Chem. 2005, 280, 38117–38120.
(16) Tran, H.; Schilling, M.; Wirbelsauer, C.; Hess, D.; Nagamine, Y. Facilitation of mRNA deadenylation and decay by the exosome-bound, DExH protein RHAU. Mol. Cell 2004, 13, 101–111.
(17) Newman, M.; Sfazi, R.; Saha, A.; Monchaud, D.; Teulead-Fichou, M. P.; Vagner, S. The G-Quadruplex-Specific RNA Helicase DHX36 Regulates p53 Pre-mRNA 3’-End Processing Following UV-Induced DNA Damage. J. Mol. Biol. 2017, 429, 3121–3131.
(18) Nakamura, J.; Kawasaki, Y.; Miyamoto, M.; Kamoshida, Y.; Nakamura, J.; Negishi, L.; Suda, S.; Akiyama, T. The novel G-quadruplex-containing long non-coding RNA GSEC antagonizes DHX36 and modulates colon cancer cell migration. Oncogene 2017, 36, 1191–1199.
(19) Chen, M. C.; Tippana, R.; Demeshkina, N. A.; Murat, P.; Balasubramanian, S.; Myong, S.; Ferre-D’Amare, A. R. Structural basis of G-quadruplex unfolding by the DEAH/RHA helicase DHX36. Nature 2018, 558, 465–469.
(20) Chen, W. F.; Rety, S.; Guo, H. L.; Dai, Y. X.; Wu, W.; Qi, L.; Liu, N. N.; Auguin, D.; Liu, Q. W.; Hou, X. M.; Dou, S.; et al. Molecular Mechanistic Insights into Drosophila DHX36-Mediated G-Quad-
ruplex Unfolding: A Structure-Based Model. Structure 2018, 26, 403–415.
(21) Murat, P.; Marsico, G.; Herdy, B.; Ghanbarian, A. T.; Portella, G.; Balasubramanian, S. RNA G-quadruplexes at upstream open reading frames cause DHX36- and DHX9-dependent translation of human mRNAs. Genome Biol. 2018, 19, No. 229.
(22) Yang, P. M.; Bradburn, D. A.; Liu, Z.; Xiao, T. S.; Russell, R. The G-quadruplex (G4) resolvase DHX36 efficiently and specifically disrupts DNA G4s via a translocation-based helicase mechanism. J. Biol. Chem. 2018, 293, 1924–1932.
(23) Tippana, R.; Chen, M. C.; Demeshkina, N. A.; Ferre-D’Amare, A. R.; Myong, S. RNA G-quadruplex is resolved by repetitive and ATP-dependent mechanism of DHX36. Nat. Commun. 2019, 10, No. 1855.
(24) Giri, B.; Smaldino, P. J.; Thys, R. G.; Creacy, S. D.; Routh, E. D.; Hantgan, R. R.; Lattmann, S.; Nagamine, Y.; Akman, S. A.; Vaughn, J. P. G4 resolvase 1 tightly binds and unwinds unimolecular G4-DNA. Nucleic Acids Res. 2011, 39, 7611–7718.
(25) Smaldino, P. J.; Routh, E. D.; Kim, J. H.; Giri, B.; Creacy, S. D.; Hantgan, R. R.; Akman, S. A.; Vaughn, J. P. Mechanistic Detail of DHX36 shows that ATP-dependent G4 resolvase 1. Proc. Natl. Acad. Sci. U.S.A. 2015, 112, 9608–9613.
(26) Huang, W.; Smaldino, P. J.; Zhang, Q.; Miller, L. D.; Cao, P.; Stadelman, K.; Wan, M.; Giri, B.; Lei, M.; Nagamine, Y.; et al. Sui, G.; Yin Yang 1 contains G-quadruplex structures in its promoter and 5'-UTR and its expression is modulated by G4 resolvase 1. Nucleic Acids Res. 2012, 40, 1035–1049.
(27) Heddi, B.; Cheong, V. V.; Martadina, H.; Phan, A. T. Insights into G-quadruplex specific recognition by the DEAH-box helicase RHUA: Solution structure of a peptide-quadruplex complex. Proc. Natl. Acad. Sci. U.S.A. 2015, 112, 9608–9613.
(28) Booy, E. P.; Meier, M.; Okun, N.; Novakovski, S. K.; Xiong, S.; Stetfeld, J.; McKenna, S. A. The RNA helicase RHUA (DHX36) unwinds a G4-quadruplex in human telomerase RNA and promotes the formation of the P1 helix template boundary. Nucleic Acids Res. 2012, 40, 4110–4124.
(29) Guedoudou, N. M.; Mendoza, O.; Gomez, D.; Bourdoncle, A.; Mergny, J. L. G-quadruplexes unfolding by RHUA helicase. Biochim. Biophys. Acta 2017, 1861, 1382–1388.
(30) Lai, J. C.; Ponti, S.; Pan, D.; Kohler, H.; Skoda, R. C.; Matthias, P.; Nagamine, Y. The DEAH-box helicase RHUA is an essential gene and critical for mouse hematopoiesis. Blood 2012, 119, 4291–4300.
(31) Nie, J.; Jiang, M.; Zhang, X.; Tang, H.; Jin, H.; Huang, X.; Yuan, B.; Zhang, C.; Lai, J. J.; Nagamine, Y.; et al. Post-transcriptional Regulation of Nkx2-5 by RHUA in Heart Development. Cell Rep. 2015, 13, 723–732.
(32) Gao, X.; Ma, W.; Nie, J.; Zhang, C.; Zhang, J.; Yao, G.; Han, J.; Xu, J.; Hu, B.; Du, Y.; et al. A G-quadruplex DNA structure resolvase, RHUA, is essential for spermatogenesis differentiation. Cell Death Dis. 2015, 6, No. e1610.
(33) Vester, K.; Eravci, M.; Senikawa, T.; Schutze, T.; Weise, C.; Kurreck, J. RNA-mediated knockdown of the Rhua helicase preferentially depletes proteins with a Guanine-quadruplex motif in the 5'-UTR of their mRNA. Biochim. Biophys. Acta. Res. Commun. 2018, 508, 756–761.
(34) Chalupniková, K.; Lattmann, S.; Selak, N.; Iwamoto, F.; Fujiki, Y.; Nagamine, Y. Recruitment of the RNA helicase RHUA to stress granules via a unique RNA-binding domain. J. Biol. Chem. 2008, 283, 35186–35198.
(35) Yoo, J. S.; Takahasi, K.; Ng, C. S.; Ouda, R.; Onomoto, K.; Yoneyama, M.; Lai, J. J.; Lattmann, S.; Nagamine, Y.; Matsui, T.; et al. DHX36 enhances RIG-I signaling by facilitating PKR-mediated antiviral stress granule formation. PLoS Pathog. 2014, 10, No. e1004012.
(36) Zhang, Z.; Kim, T.; Bao, M.; Facchinetti, V.; Jung, S. Y.; Ghaffari, A. A.; Qin, J.; Cheng, G.; Liu, Y. J. DDX1, DDX21, and DHX36 helicases form a complex with the adaptor molecule TRIF to sense dsRNA in dendritic cells. Immunity 2011, 34, 866–878.
(57) Zheng, R.; Ghirlando, R.; Lee, M. S.; Mizuuchi, K.; Krause, M.; Craigie, R. Barrier-to-autointegration factor (BAF) bridges DNA in a discrete, higher-order nucleoprotein complex. *Proc. Natl. Acad. Sci. U.S.A.* 2000, 97, 8997–9002.

(58) Furukawa, K.; Sugiya, S.; Osouda, S.; Goto, H.; Inagaki, M.; Horigome, T.; Omata, S.; McConnell, M.; Fisher, P. A.; Nishida, Y. Barrier-to-autointegration factor plays crucial roles in cell cycle progression and nuclear organization in Drosophila. *J. Cell Sci.* 2003, 116, 3811–3823.

(59) Reineke, L. C.; Cheema, S. A.; Dubrulle, J.; Neilson, J. R. Chronic starvation induces noncanonical pro-death stress granules. *J. Cell Sci.* 2018, 131, No. jcs220244.

(60) Jain, S.; Wheeler, J. R.; Walters, R. W.; Agrawal, A.; Barsic, A.; Parker, R. ATPase-Modulated Stress Granules Contain a Diverse Proteome and Substructure. *Cell* 2018, 164, 487–498.

(61) Braun, F.; Bertín-Ciftci, J.; Gallouet, A.-S.; Millour, J.; Juin, P. Serum-nutrient starvation induces cell death mediated by Bax and Puma that is counteracted by p21 and unmasked by Bcl-x(L) inhibition. *PLoS One* 2011, 6, e23577.

(62) Booy, E. P.; Howard, R.; Maruschak, O.; Ariyo, E. O.; Meier, M.; Novakowski, S. K.; Deo, S. R.; Dzananovic, E.; Stetefeld, J.; McKenna, S. A. The RNA helicase RHAU (DHX36) suppresses expression of the transcription factor PITX1. *Nucleic Acids Res.* 2014, 42, 3346–3361.

(63) Bicker, S.; Khudayberdiev, S.; Weiss, K.; Zocher, K.; Baumeister, S.; Schratt, G. The DEAH-box helicase DHX36 mediates dendritic localization of the neuronal precursor-microRNA-134. *Genes Dev.* 2013, 27, 991–996.

(64) Zampa, F.; Bicker, S.; Schratt, G. Activity-Dependent Pre-miR-134 Dendritic Localization Is Required for Hippocampal Neuron Dendritogenesis. *Front. Mol. Neurosci.* 2018, 11, 171.

(65) Vidak, S.; Foisner, R. Molecular insights into the premature aging disease progeria. *Histochem. Cell Biol.* 2016, 145, 401–417.

(66) Gonzalo, S.; Kreienkamp, R.; Askjaer, P. Hutchinson-Gilford Progeria Syndrome: A premature aging disease caused by LMNA gene mutations. *Ageing Res. Rev.* 2017, 33, 18–29.