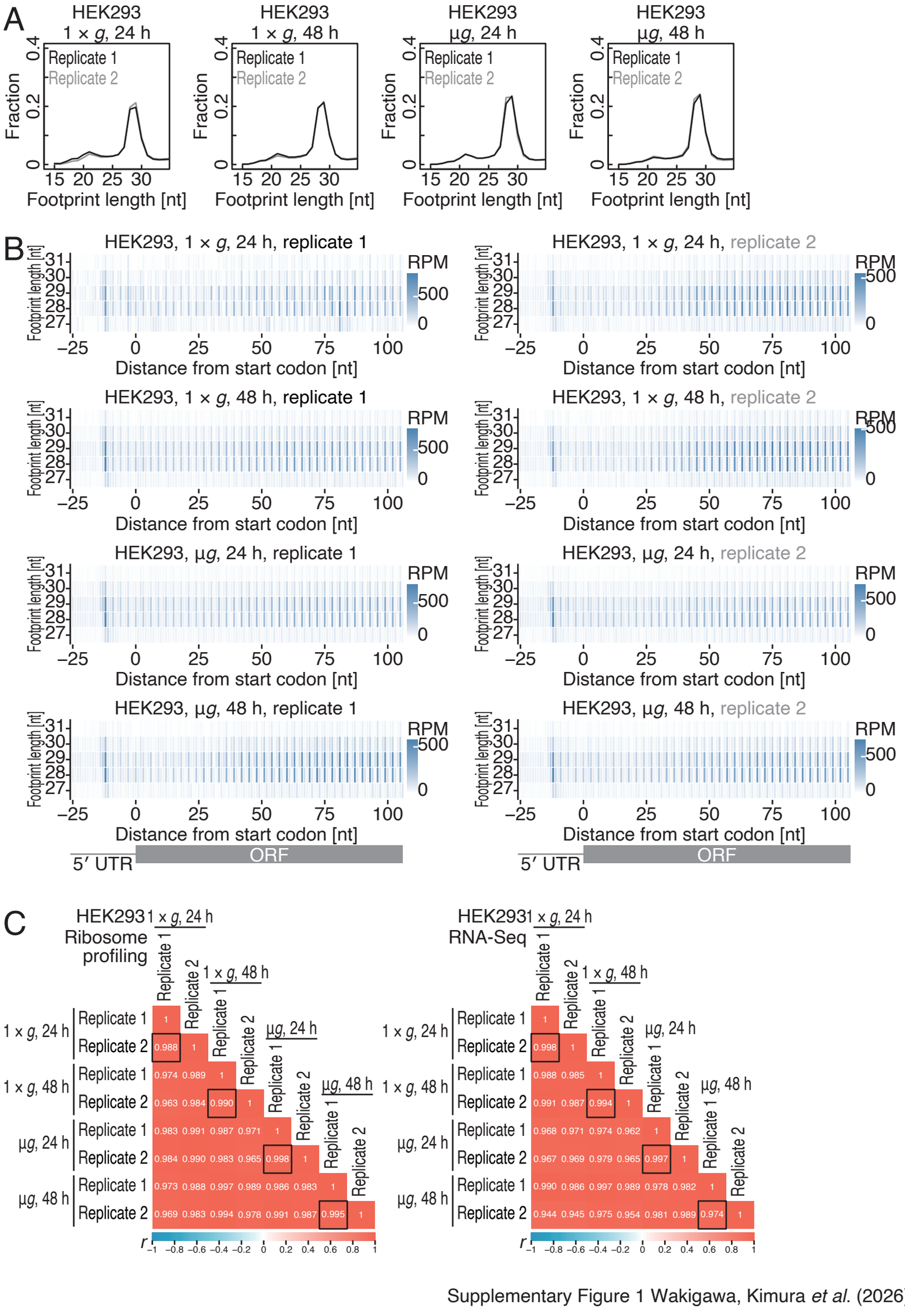
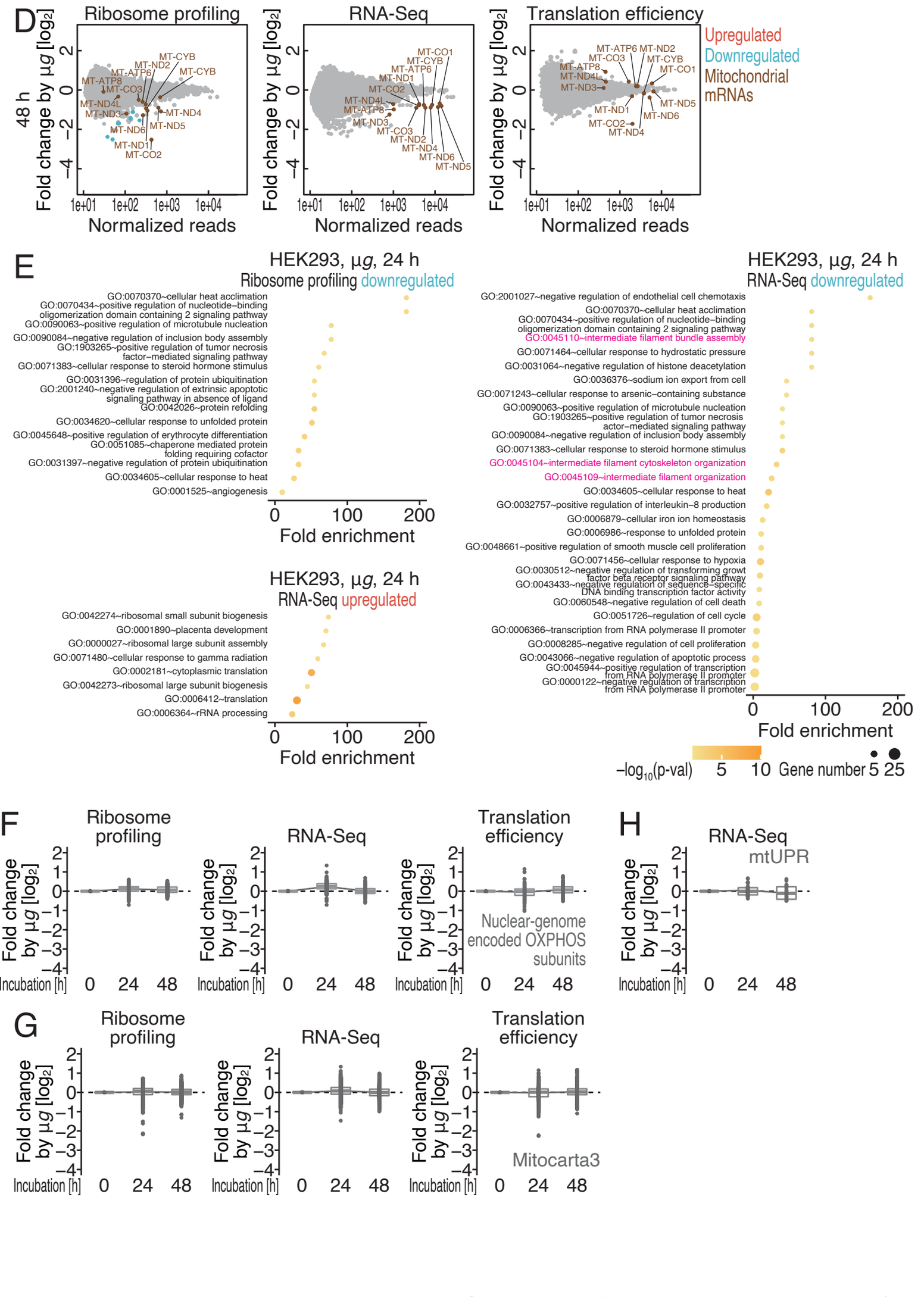
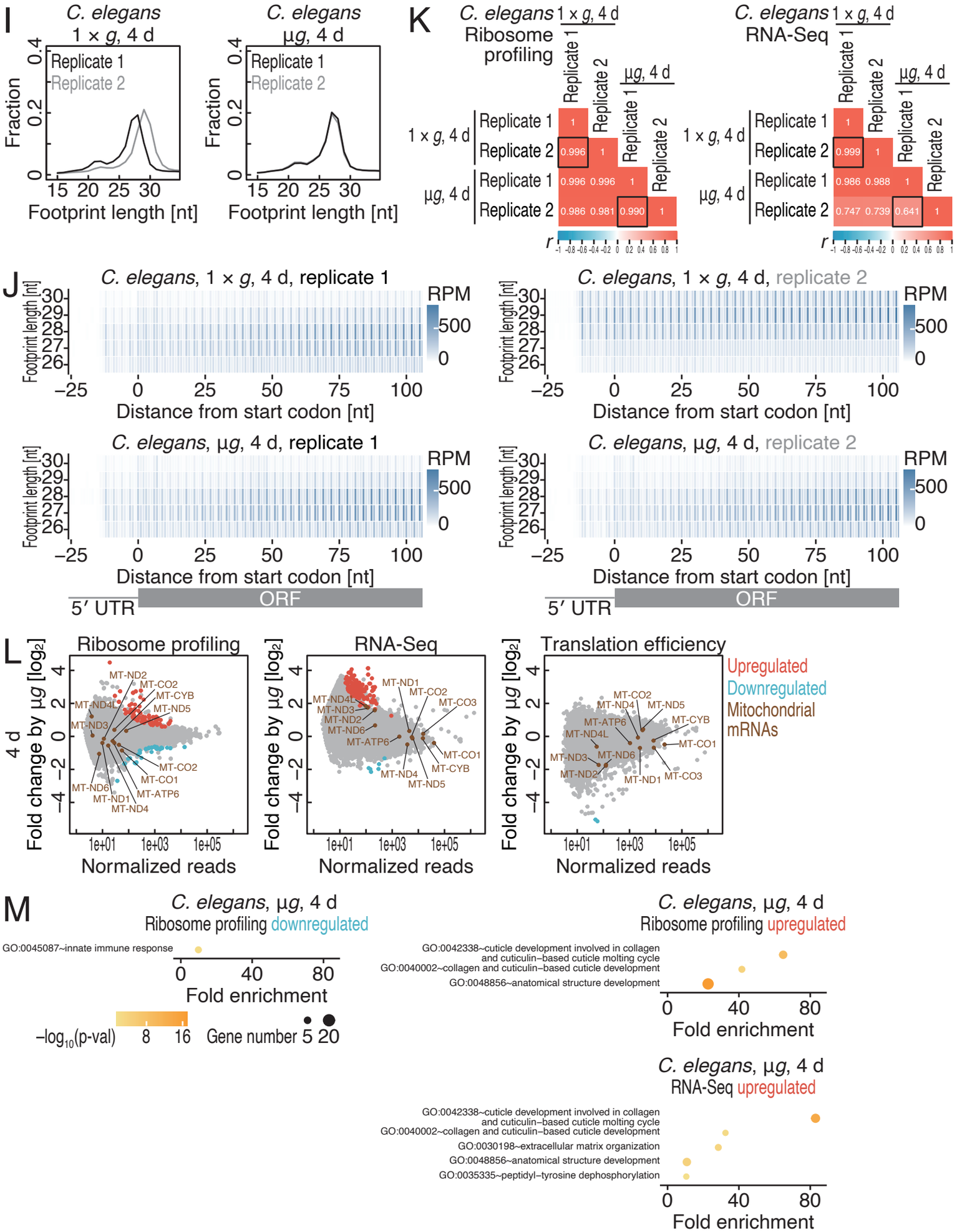


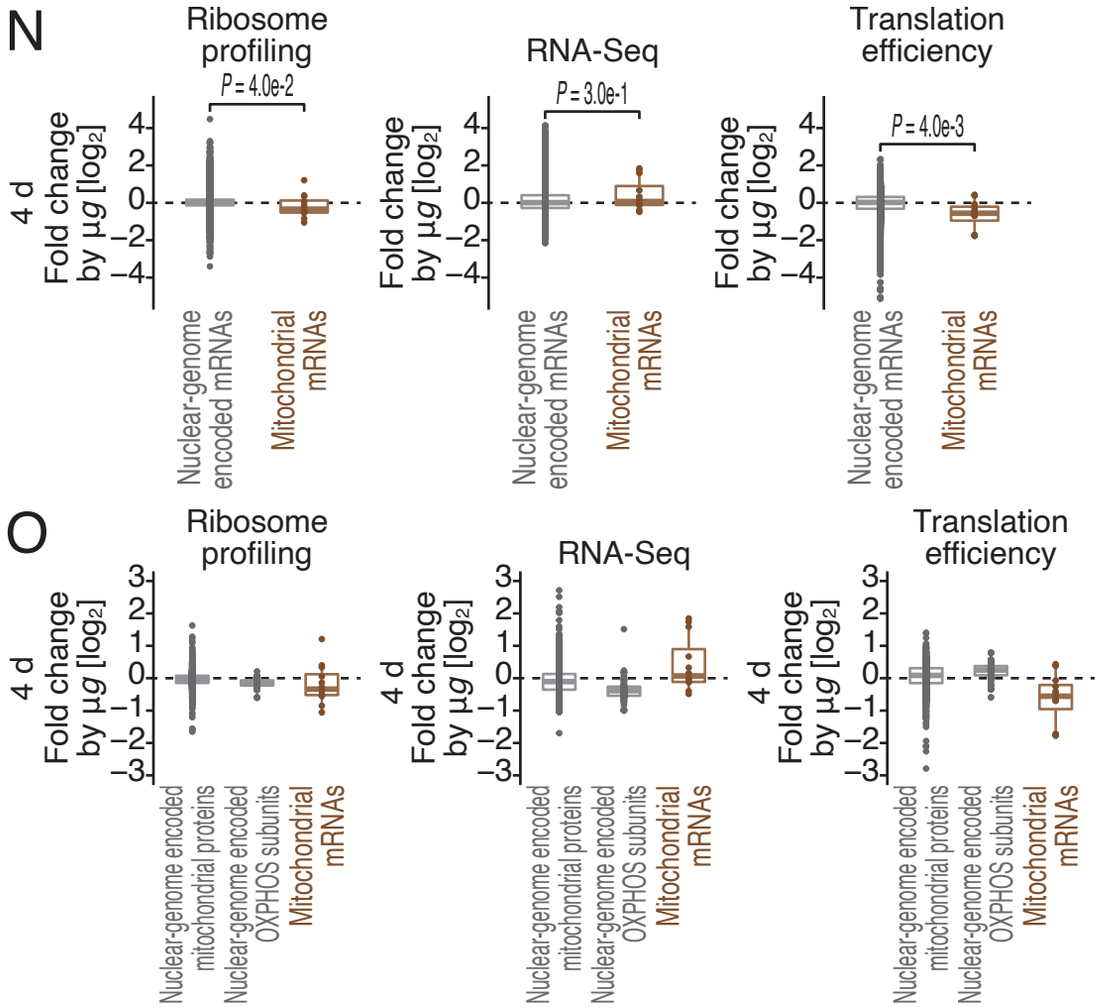
Supplementary Figure 1 Wakigawa, Kimura *et al.* (2026)



Supplementary Figure 1 Wakigawa, Kimura *et al.* (2026)



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Supplementary Figure 1. Characterization of ribosome profiling data in spaceflight samples. Related to Figure 1.

(A and I) The lengths of ribosome footprints in the indicated samples.

(B and J) Metagene plots of ribosome footprints relative to the start codon across the footprint length for the indicated samples. The 5′ end positions of ribosome footprints are depicted. The color indicates the read abundance. RPM, reads per million reads.

(C and K) Pearson's correlation coefficient (*r*, represented in the color as well) among the indicated samples in ribosome profiling and RNA-Seq. The black squares indicate the correlations of the biological replicates.

(D and L) MA (M, log ratio; A, mean average) plots for ribosome footprint change (left), RNA abundance change (middle), and translation efficiency change (right) in HEK293 cells after 48-h microgravity (μ*g*) culture (D) and in *C. elegans* after 4-d microgravity culture (L). Significantly altered transcripts (false discovery rate [FDR] < 0.05) and mitochondrial genome-encoded mRNAs are highlighted.

(E and M) Gene Ontology (GO) analysis by DAVID [113,114] for genes downregulated or upregulated by microgravity in the indicated samples. The color represents statistical significance, and the circle size indicates the number of genes found in the GO category. The p values were calculated by a modified Fisher's exact test (EASE score).

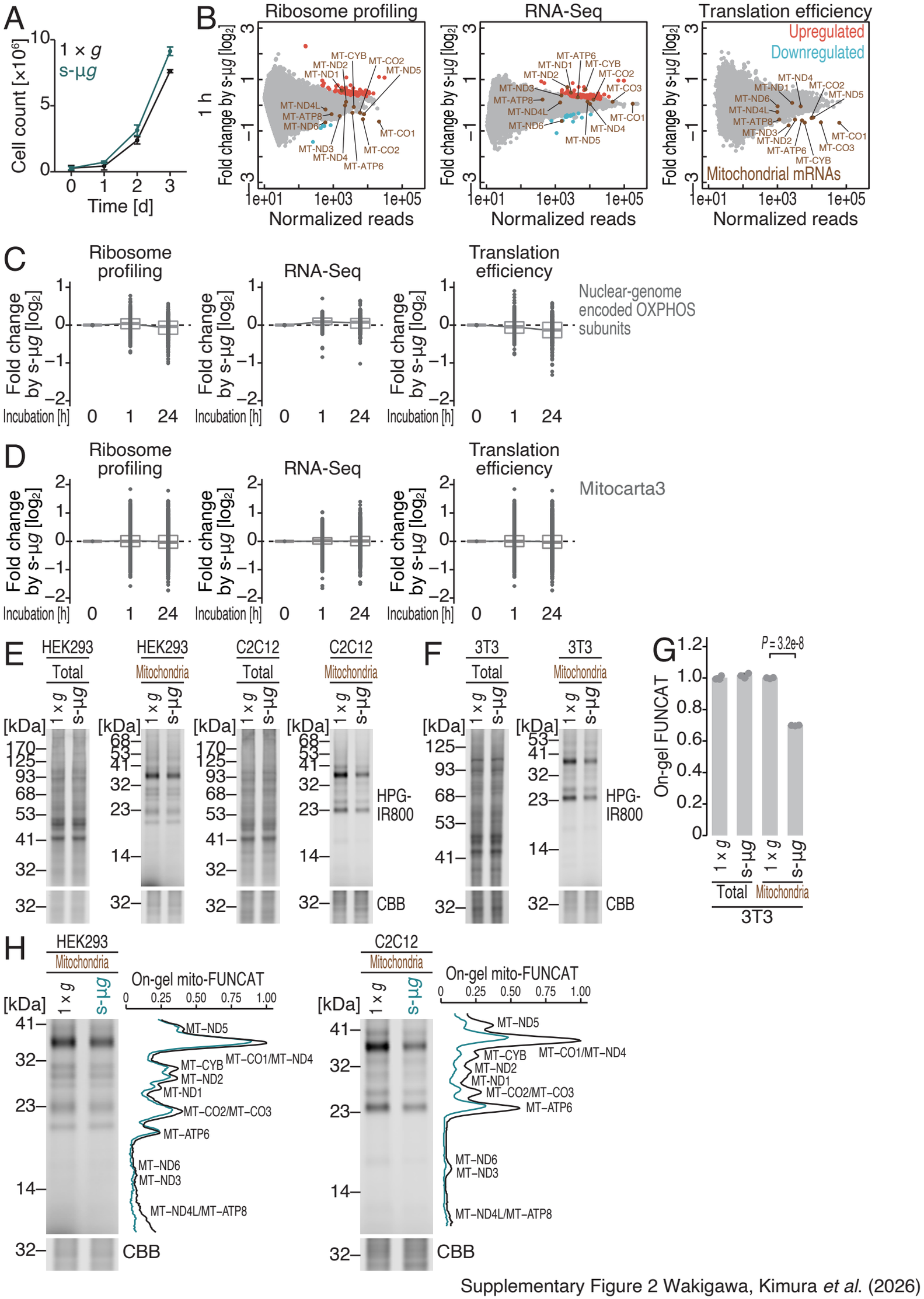
(F and G) Box plots of ribosome footprint change (left), RNA abundance change (middle), and translation efficiency change (right) in nuclear genome-encoded OXPHOS subunits (F) and mitochondria-localized proteins (G) (defined by Mitocarta3 [115]) over the course of microgravity culture in HEK293 cells.

(H) Box plots of the RNA abundance change (middle) of mtUPR target genes (defined by [39]) over the course of microgravity culture in HEK293 cells.

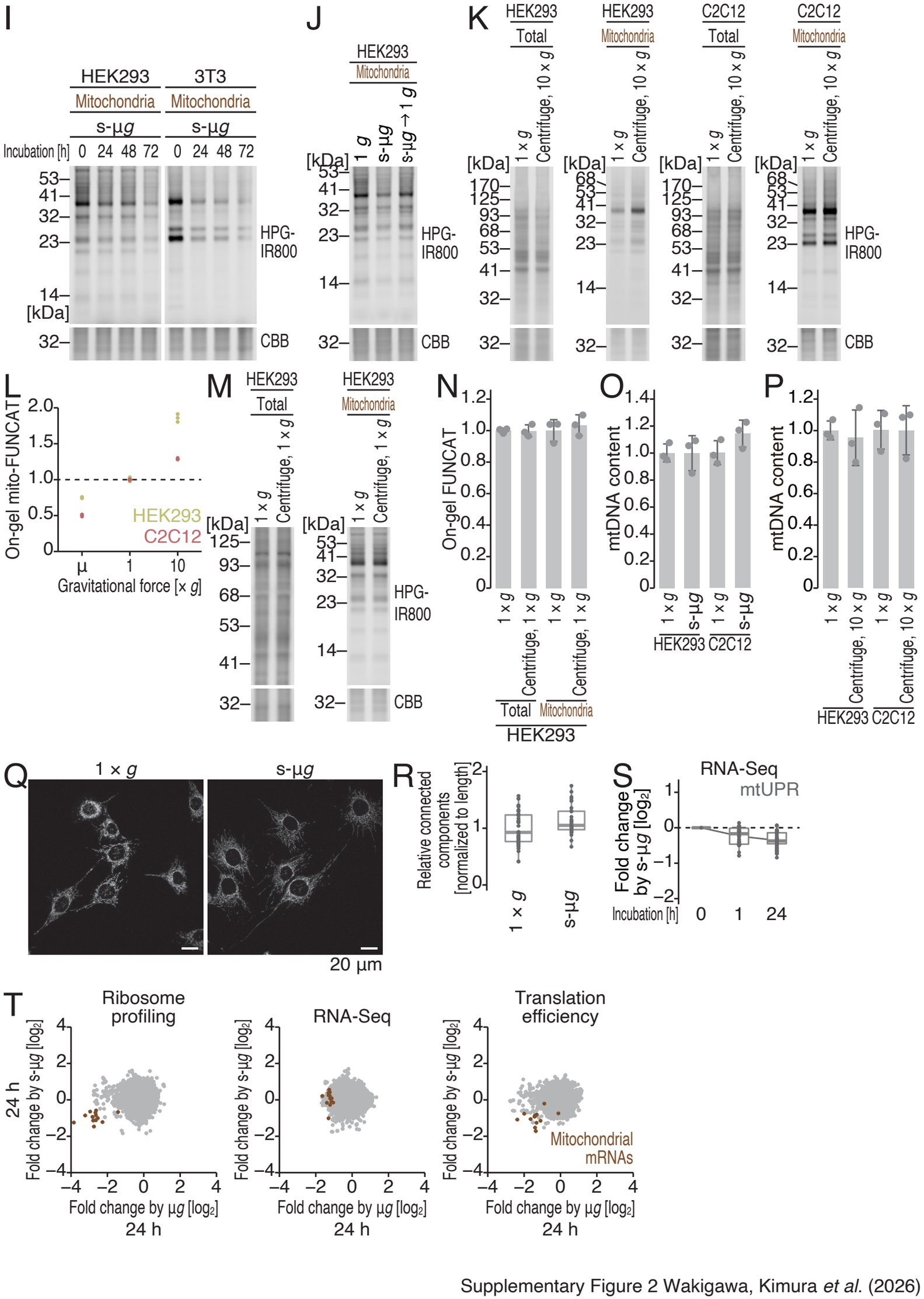
(N) Box plots of ribosome footprint change (left), RNA abundance change (middle), and translation efficiency change (right) in nuclear genome-encoded mRNAs and mitochondria genome-encoded mRNAs, under microgravity culture in *C. elegans*.

(O) Box plots of ribosome footprint change (left), RNA abundance change (middle), and translation efficiency change (right) in nuclear genome-encoded mitochondria-localized proteins, nuclear genome-encoded OXPHOS subunits (defined by [124]), and mitochondrial mRNAs, under microgravity culture in *C. elegans*.

In box plots (F, G, H, and N-O), the median (centerline), upper/lower quartiles (box limits), and 1.5× interquartile range (whiskers) are shown.



Supplementary Figure 2 Wakigawa, Kimura *et al.* (2026)



Supplementary Figure 2 Wakigawa, Kimura *et al.* (2026)

Supplementary Figure 2. Characterization of ribosome profiling data in HEK293 cells in simulated microgravity. Related to Figure 2.

(A) The cell growth (HEK293) over the course of simulated microgravity culture.

(B) MA plots of ribosome footprint change (left), RNA abundance change (middle), and translation efficiency change (right) after 1-h simulated microgravity culture in HEK293 cells. Significantly altered transcripts (false discovery rate [FDR] < 0.05) and mitochondrial genome-encoded mRNAs are highlighted.

(C and D) Box plots of ribosome footprint change (left), RNA abundance change (middle), and translation efficiency change (right) in nuclear-genome-encoded OXPHOS subunits (C) and mitochondria-localized proteins (D) (defined by Mitocarta3 [115]) over the course of simulated microgravity culture in HEK293 cells.

(E, F, H-K, and M) The representative results of on-gel FUNCAT experiments to monitor total and mitochondrial translation for the indicated cell lines and conditions. Coomassie Brilliant Blue (CBB) staining of the total proteins is shown as a loading control. For H, the signal intensities along the lanes are shown on the right.

(G and N) Quantification of on-gel FUNCAT experiments to monitor total and mitochondrial translation in the indicated cell lines and conditions.

(L) Relationships among gravitational force and mitochondrial translation activity for the indicated cell lines. The mean value at 1 × *g* was set to "1".

(O and P) Relative mitochondrial DNA (mtDNA) content in the indicated cell lines and conditions. The cells were cultured under s-μ*g* or 10 × *g* for 24 h before cell harvesting.

(Q and R) Mitochondria in C2C12 cells were immunostained with an AF647-labeled anti-TOMM20 antibody under the indicated culture conditions. Representative images of immunostaining (Q) and quantification of the connected components (R) are shown. Scale bar, 20 μm.

(S) Box plots of the RNA abundance change (middle) of mtUPR target genes (defined by [39]) over the course of simulated microgravity culture in HEK293 cells.

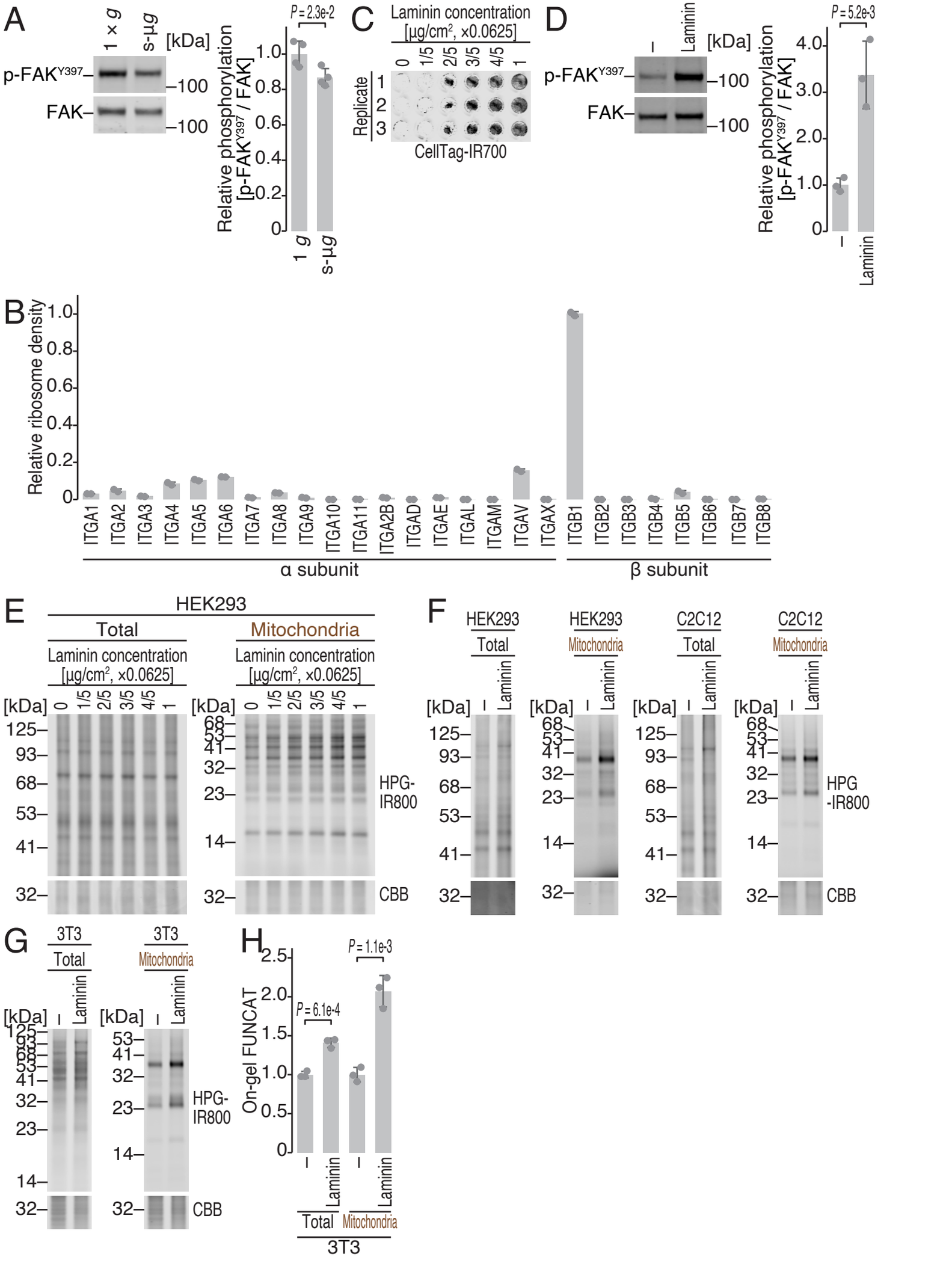
(T) The comparison of ribosome footprint change (left), RNA abundance change (middle), and translation efficiency change (right) after 24-h microgravity and 24-h simulated microgravity culture in HEK293 cells.

For A, the data from three replicates (points) and the mean values (bars) are shown.

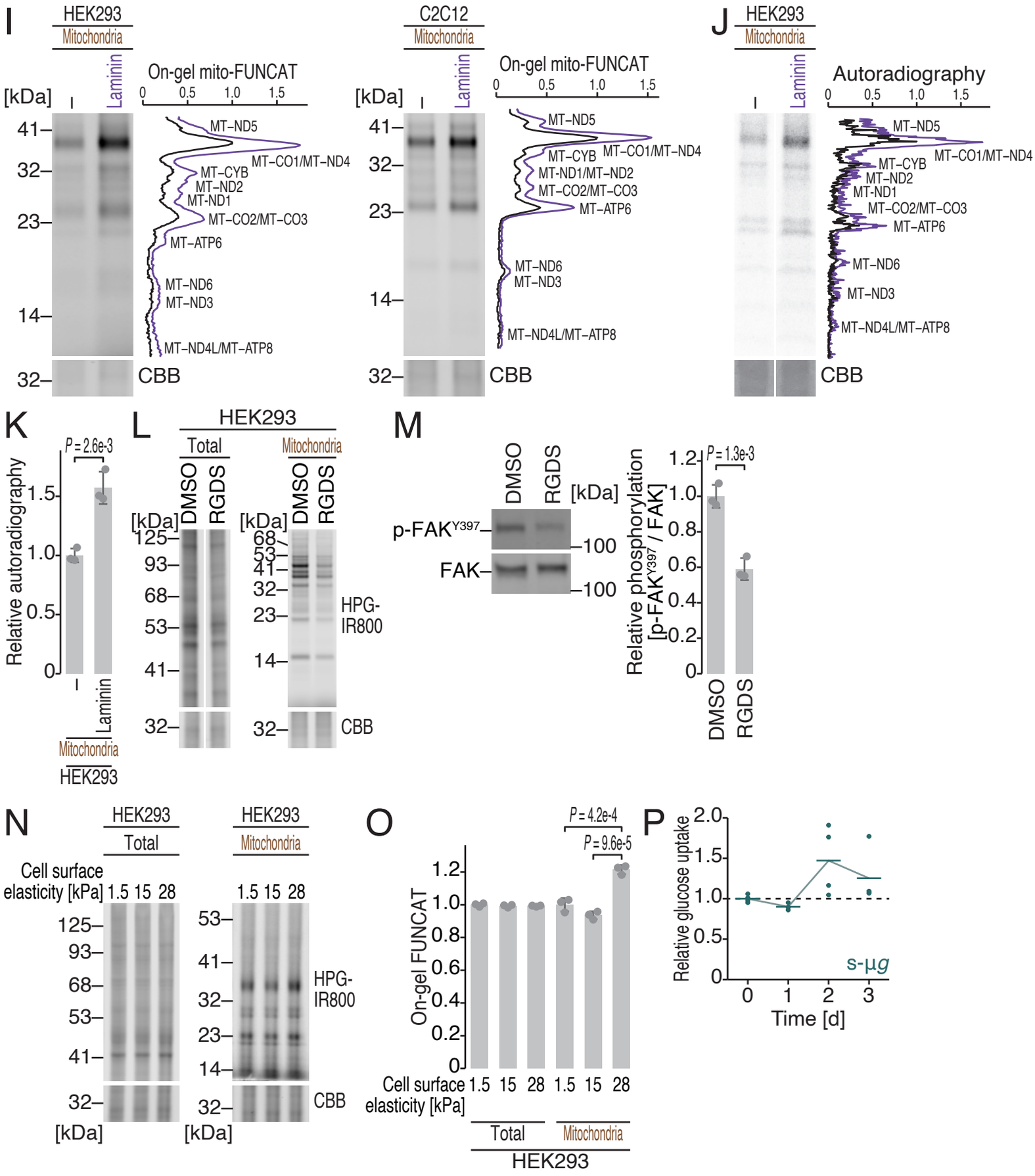
For G and N-P, the data from replicates (points, n = 3), the mean values (bars), and the s.d.s (errors) are shown. The p values were calculated by Student's t test (two-tailed) (G) and by the Tukey–Kramer test (two-tailed) (R).

In box plots (C, D, and R-S), the median (centerline), upper/lower quartiles (box limits), and 1.5× interquartile range (whiskers) are shown.

For E-H, the cells were cultured under s-μ*g* for 24 h before cell harvesting. For the recovery experiments in I, the cells were incubated under s-μ*g* for 24 h and then at 1 × *g* for 24 h. For K, M-P, the cells were cultured at 10 × *g* or 1 × *g* centrifugation for 24 h before cell harvesting.



Supplementary Figure 3 Wakigawa, Kimura *et al.* (2026)



Supplementary Figure 3 Wakigawa, Kimura *et al.* (2026)

Supplementary Figure 3. Characterization of the phosphorylation status of FAK. Related to Figure 3.

(A, D, and M) Western blotting of total FAK protein and the phosphorylated form (at Y397). The quantified relative amount of phosphorylated FAK is shown.

(B) Relative ribosome footprint density (normalized to the average of ITGB1) of the indicated integrin subunit genes. Ribosome profiling data from HEK293 cells cultured at 1 × *g* (Figure 2A-C) were used.

(C) Cell staining on the laminin-treated culture dish via CellTag reagent.

(E, F, G, I, L, and N) The representative results of on-gel FUNCAT experiments to monitor total and mitochondrial translation for the indicated cell lines and conditions. CBB staining of the total proteins is shown as a loading control.

(H and L) Quantification of on-gel FUNCAT experiments to monitor total and mitochondrial translation in the indicated cell lines and conditions.

(J) The representative results of ^{35}S Met-mediated metabolic labeling of proteins synthesized in mitochondria for the indicated cell lines and conditions. CBB staining of the total proteins is shown as a loading control. The signal intensities along the lanes are shown on the right.

(K) Quantification of ^{35}S Met-mediated metabolic labeling of proteins synthesized in mitochondria in the indicated cell lines and conditions.

(P) The relative glucose uptake was measured over the course of simulated microgravity culture.

(Q) Heatmap of the changes in the metabolites related to the TCA cycle. The cells were incubated under s-μg for 24 h. The color scales for the $\log_2$ fold change by the simulated microgravity are shown.

For A and D, H, K, M, and O, the data from replicates (points, n = 4 for A; n = 3 for D, H, K, M, and O), the mean values (bars), and the s.d.s (errors) are shown. The p values were calculated by Student's t test (two-tailed) (A, D, H, K, and M) and by the Tukey–Kramer test (two-tailed) (O).

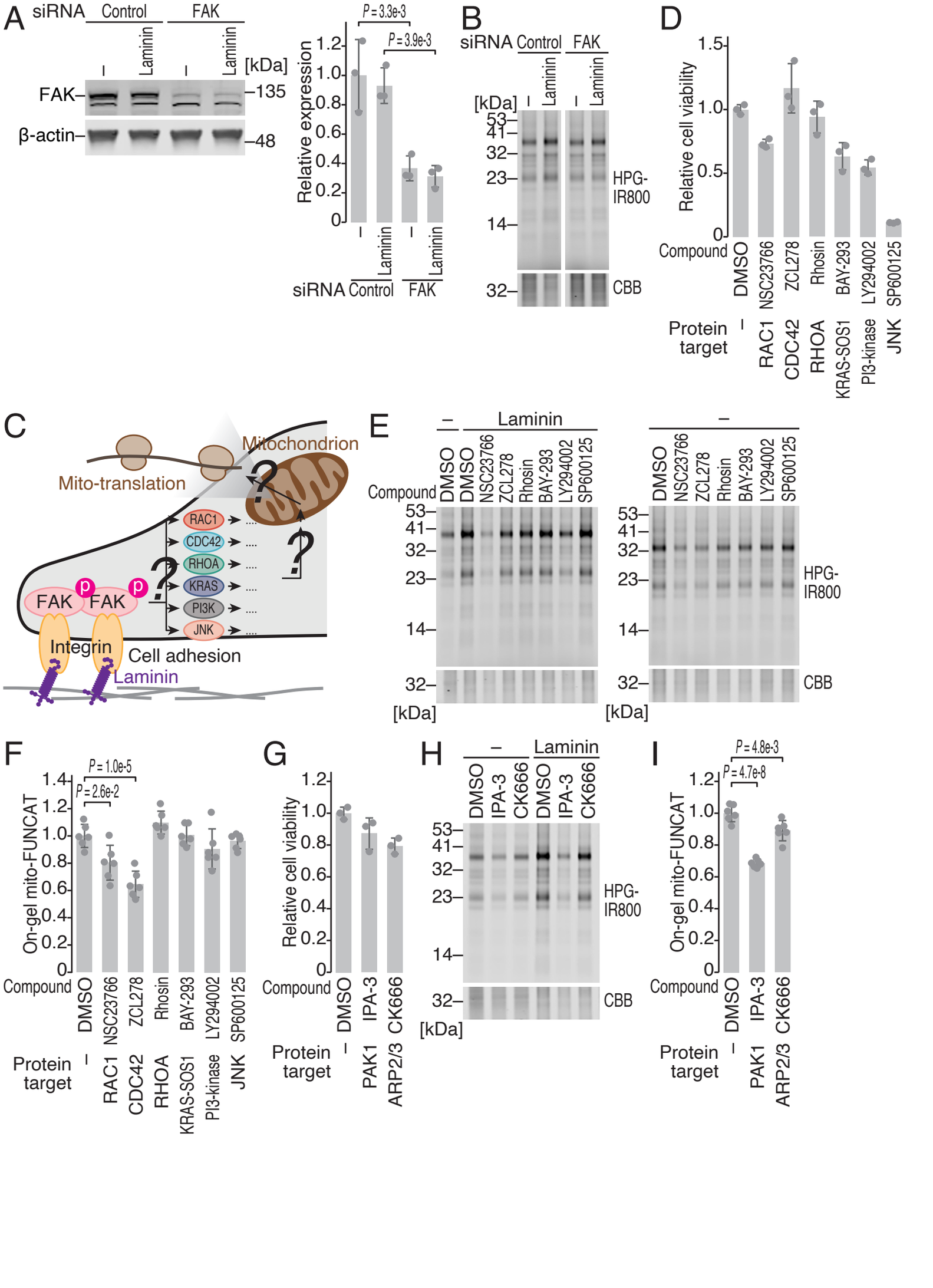
For B, the data from two replicates (points) and the mean values (bars) are shown.

For P, the data from four replicates (points) and the mean values (bars) are shown.

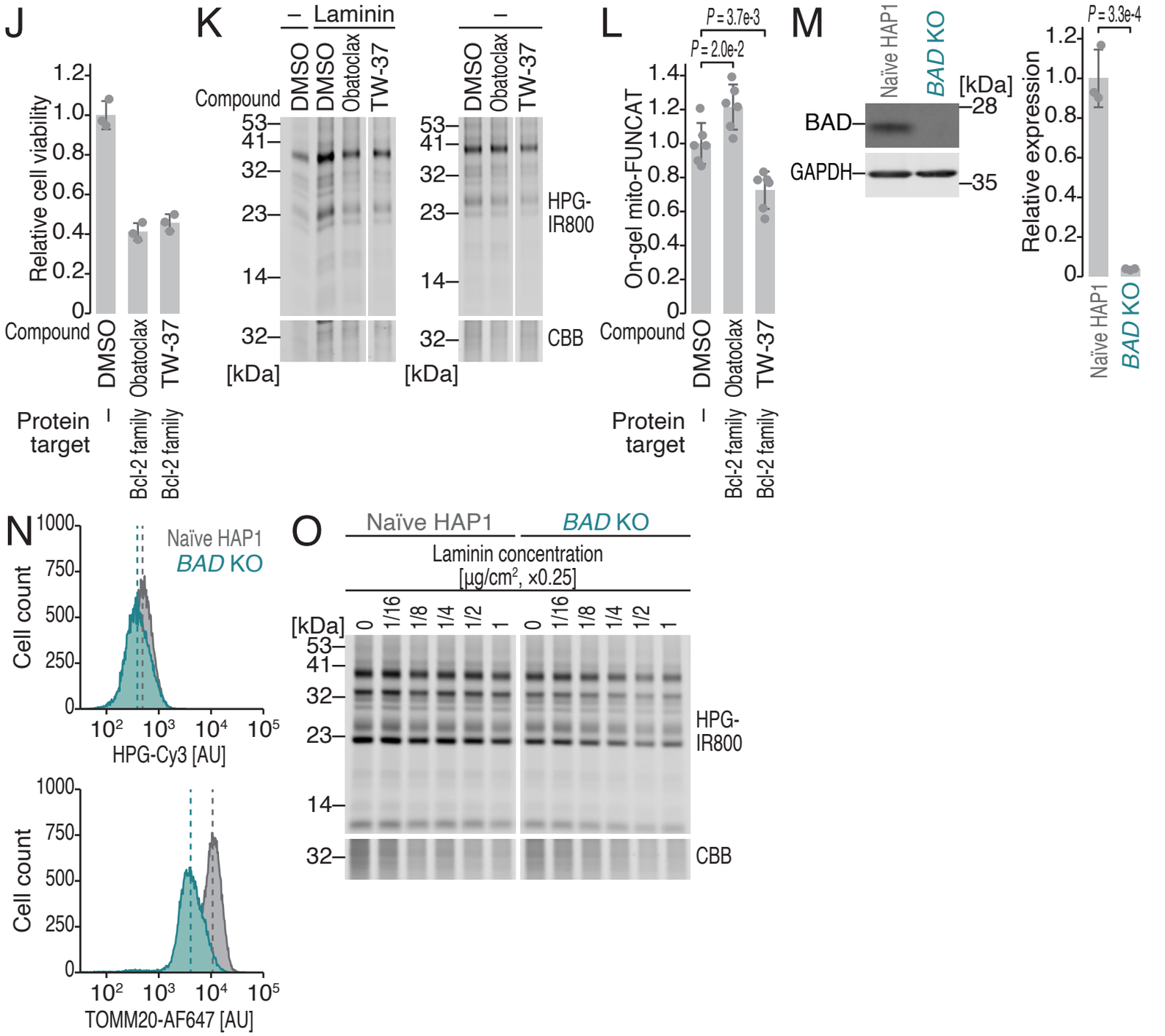
For Q, the average of triplicates is shown.

For L and M, the cells were treated with 10 μg/ml RGDS peptide

The experiments were conducted in HEK293 cells unless otherwise noted.



Supplementary Figure 4 Wakigawa, Kimura *et al.* (2026)



Supplementary Figure 4 Wakigawa, Kimura *et al.* (2026)

Supplementary Figure 4. Characterization of chemical treatment for mitochondrial translation. Related to Figure 4.

(A) Western blotting of FAK protein after siRNA-mediated knockdown. β-Actin was used as the loading control. The quantified relative amount of FAK is shown.

(B, E, H, K, and O) The representative results of on-gel FUNCAT experiments for mitochondrial translation for the indicated cell lines and conditions. CBB staining of the total proteins is shown as a loading control.

(C) Schematic representation of the downstream signal transduction pathway from FAK to mitochondrial translation.

(D, G, and J) Relative cell viability under treatment with the indicated compounds. The reduction in cell viability under compound treatment showed that the corresponding target proteins were efficiently blocked.

(F, I, and L) Quantification of on-gel FUNCAT experiments to monitor mitochondrial translation in the indicated conditions. Cells were cultured in the absence of laminin precoating under treatment with the indicated compounds. We note that the DMSO data in I are the same as the data for DMSO without laminin precoating in Figure 4D.

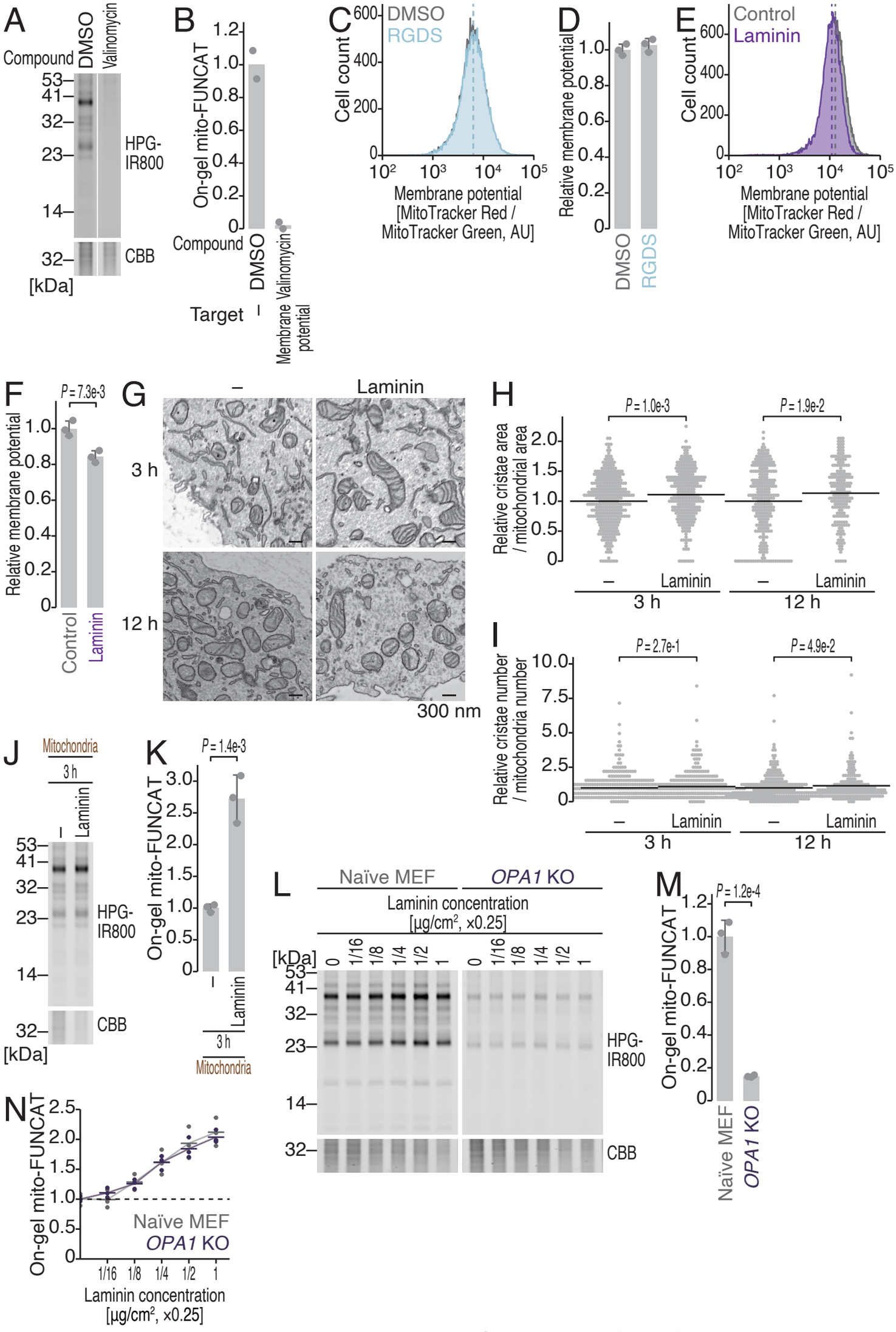
(M) Western blotting of BAD protein in KO HAP1 cells. GAPDH was used as the loading control. The quantification of the relative amount of BAD is shown.

(N) Representative distribution of Cy3-conjugated HPG signals (top) and AF647-labeled TOMM20 signals (bottom) in the indicated cell lines. The dashed line represents the mean of the distribution.

For A and D, F, G, I, J, L, and M, the data from replicates (points, n = 3 for A, D, G, J, and M; n = 6 for F, I, and L), the mean values (bars), and the s.d.s (errors) are shown. The p values were calculated by Student's t test (two-tailed) (M) and by the Tukey–Kramer test (two-tailed) (A, F, I, and L).

For E-L, the cells were treated with the compounds at the following concentrations: NSC23766, 200 μM; ZCL278, 100 μM; Rhosin, 50 μM; BAY-293, 1 μM; LY294002, 10 μM; SP600125, 20 μM; IPA-3, 5 μM; CK666, 25 μM; obatoclax, 2 μM; and TW-37, 10 μM.

The experiments were conducted in HEK293 cells unless otherwise noted.



Supplementary Figure 5 Wakigawa, Kimura *et al.* (2026)

Supplementary Figure 5. Characterization of chemical treatment for mitochondrial translation. Related to Figure 5.

(A, J, and L) The representative results of on-gel FUNCAT experiments for mitochondrial translation for the indicated cell lines and conditions. CBB staining of the total proteins is shown as a loading control.

(B, K, M, and N) Quantification of on-gel FUNCAT experiments to monitor mitochondrial translation for the indicated cell lines and conditions.

(C and E) Representative distribution of MitoTracker Green-normalized MitoTracker Red values under the indicated conditions. Given that MitoTracker Red staining depends on the membrane potential, the values were normalized to MitoTracker Green, which standardizes the mitochondrial volume in cells, representing relative membrane potential. The dashed line represents the mean of the distribution. HEK293 cells were treated with RGDS peptide for 24 h. AU, arbitrary unit.

(D and F) Quantification of the relative membrane potential for the indicated conditions.

(G) Representative electron microscopy image of mitochondria under the indicated conditions. Scale bar, 300 nm.

(H and I) Relative cristae area and relative cristae numbers per mitochondrion in cells precoated with laminin. The data were normalized to those of the control condition.

For B, the data from replicates (points, n = 2) and the mean values (bars) are shown.

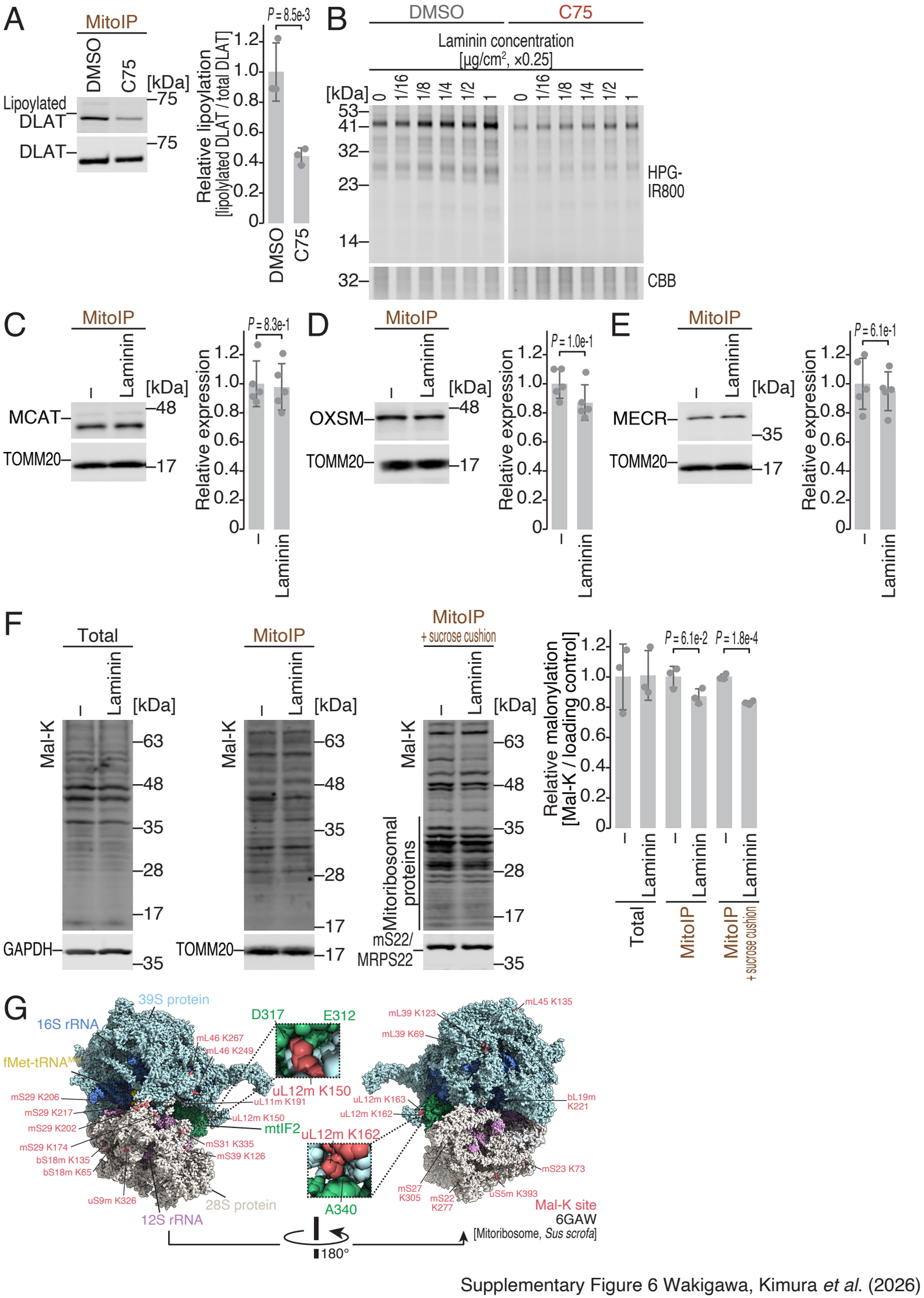
For D, F, K, and M, the data from replicates (points, n = 3), the mean values (bars), and the s.d.s (errors) are shown. The p values were calculated by Student's t test (two-tailed) (F, K, and M).

For H and I, the p values were calculated by the Mann–Whitney *U* test (two-tailed).

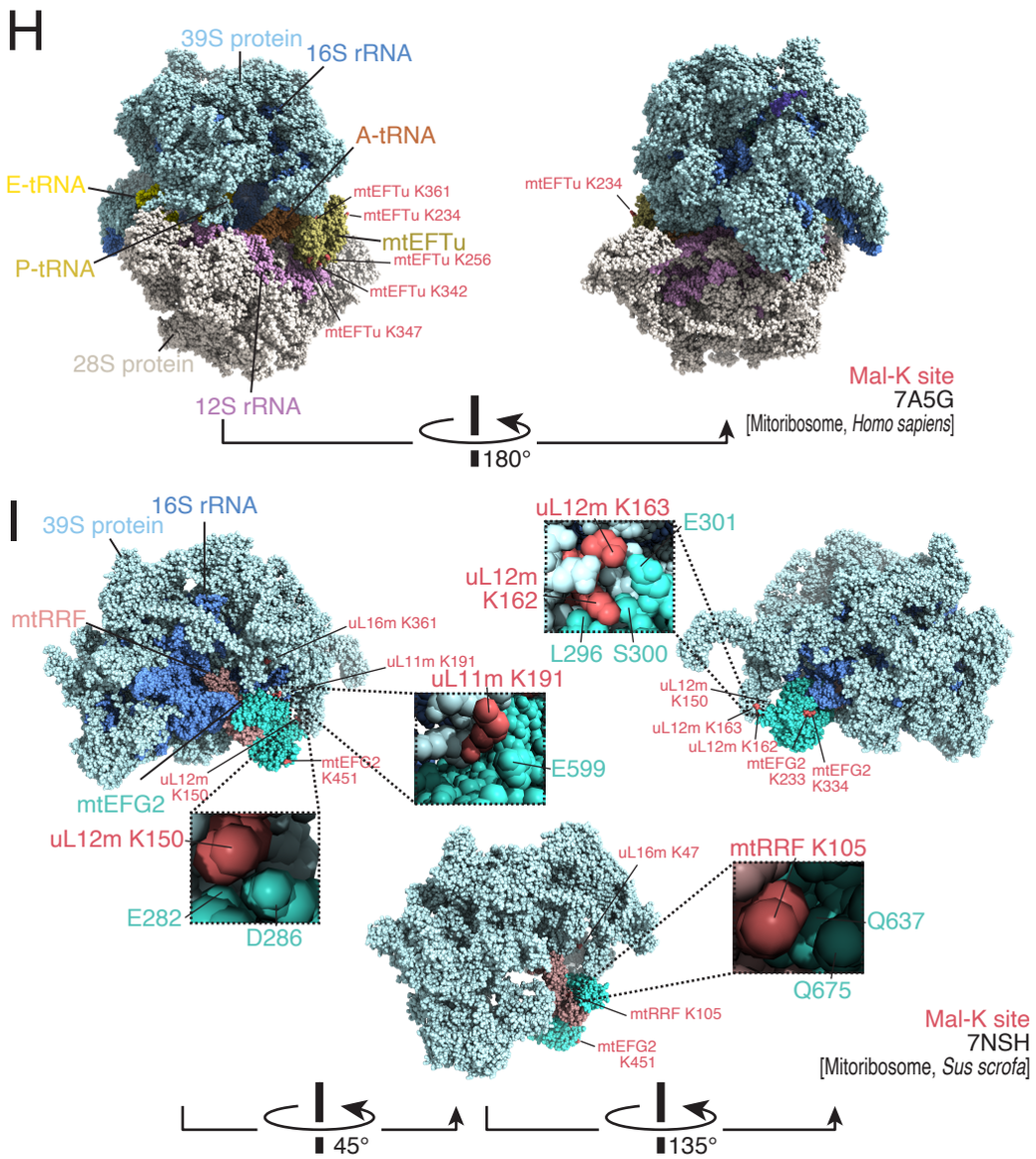
For N, the data from three replicates (points) and the mean values (bars) are shown.

For A-D, the cells were treated with the compounds at the following concentrations: valinomycin, 2 μM; RGDS peptide, 10 μg/ml.

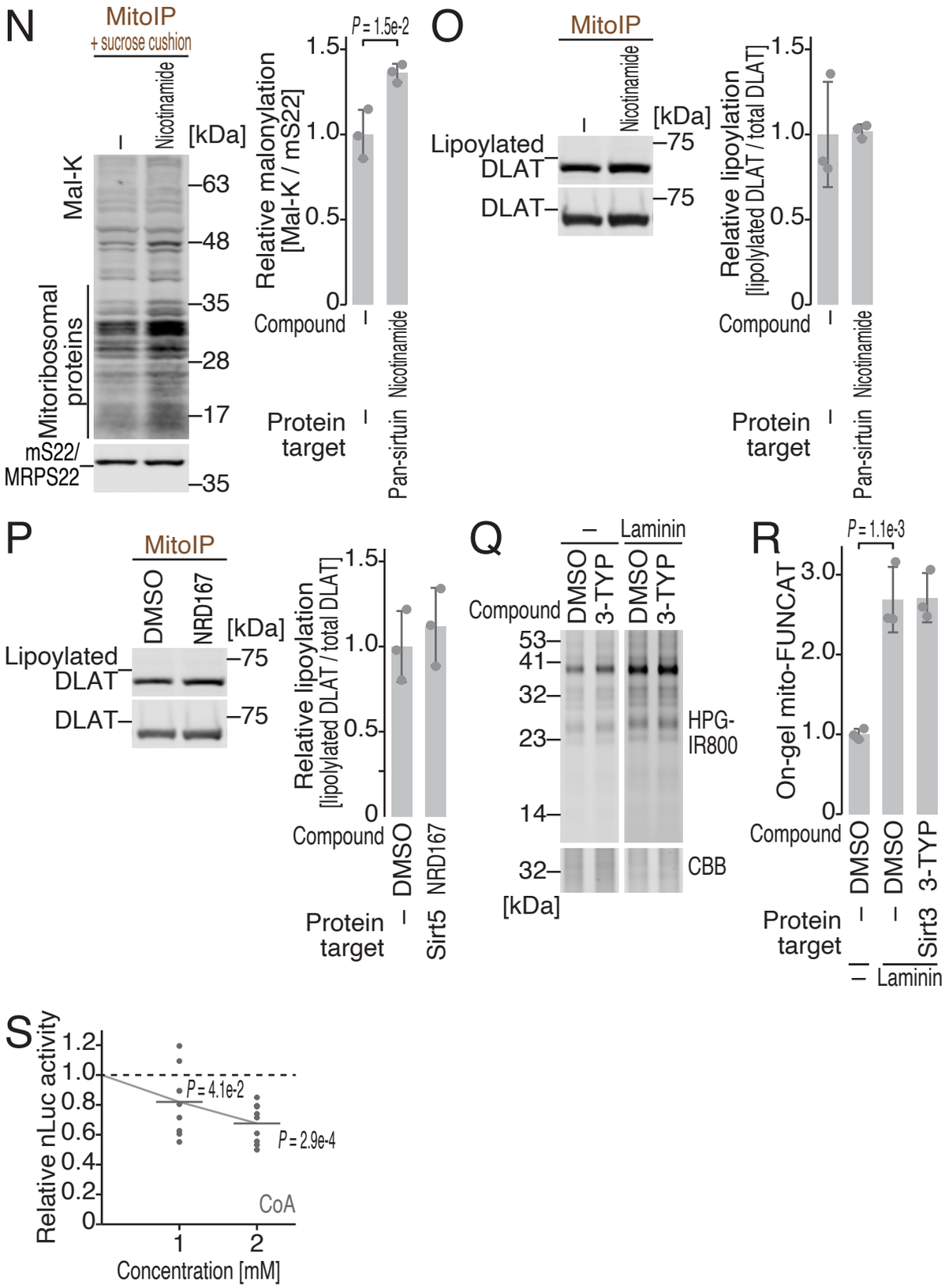
The experiments were conducted in HEK293 cells unless otherwise noted.



Supplementary Figure 6 Wakigawa, Kimura *et al.* (2026)



Supplementary Figure 6 Wakigawa, Kimura *et al.* (2026)



Supplementary Figure 6 Wakigawa, Kimura *et al.* (2026)

Supplementary Figure 6. Characterization of mtFAS-coupled mitochondrial translation activation. Related to Figure 5.

(A, O, and P) Western blotting of DLAT and lipoylated DLAT proteins under the indicated conditions. The quantified relative amount of lipoylated DLAT is shown.

(B, J, L, and Q) The representative results of on-gel FUNCAT experiments for mitochondrial translation for the indicated cell lines and conditions. CBB staining of the total proteins is shown as a loading control.

(C-E) Western blotting of MCAT (C), OXSM (D), and MECR (E) proteins under the indicated conditions. TOMM20 was used as a loading control. The quantified relative amounts of MCAT, OXSM, and MECR are shown.

(F) Western blotting of proteins with malonylated lysine (Mal-K) in total lysate and the mitochondrial immunoprecipitation (MitoIP) fraction with an anti-TOMM22 antibody with or without sucrose cushion ultracentrifugation. GAPDH, TOMM20, and MRPS22 (mS22) were used as loading controls. The cells were cultured under the indicated conditions. The quantified relative amount of Mal-K signal is shown.

(G-I) Malonylated lysine residues (Mal-K) found in the compendium of protein lysine modifications (CPLM) database [76] were mapped on the mitoribosome complex with mtIF2 (6GAW, G) [125], mtEFtu (7A5G, H) [126], and mtRRF (7NSH, I) [127], using UCSF ChimeraX [123].

(K, M, and R) Quantification of on-gel FUNCAT experiments to monitor mitochondrial translation under treatment with the indicated compounds with or without laminin precoating.

(N) Western blotting of proteins with malonylated lysine (Mal-K) in the mitochondrial IP (MitoIP) fraction with an anti-TOMM22 antibody, and then the pellet of the sucrose cushion ultracentrifugation fraction. MRPS22 (mS22) was used as the loading control. The cells were cultured under the indicated conditions. The quantified relative amount of Mal-K signal is shown.

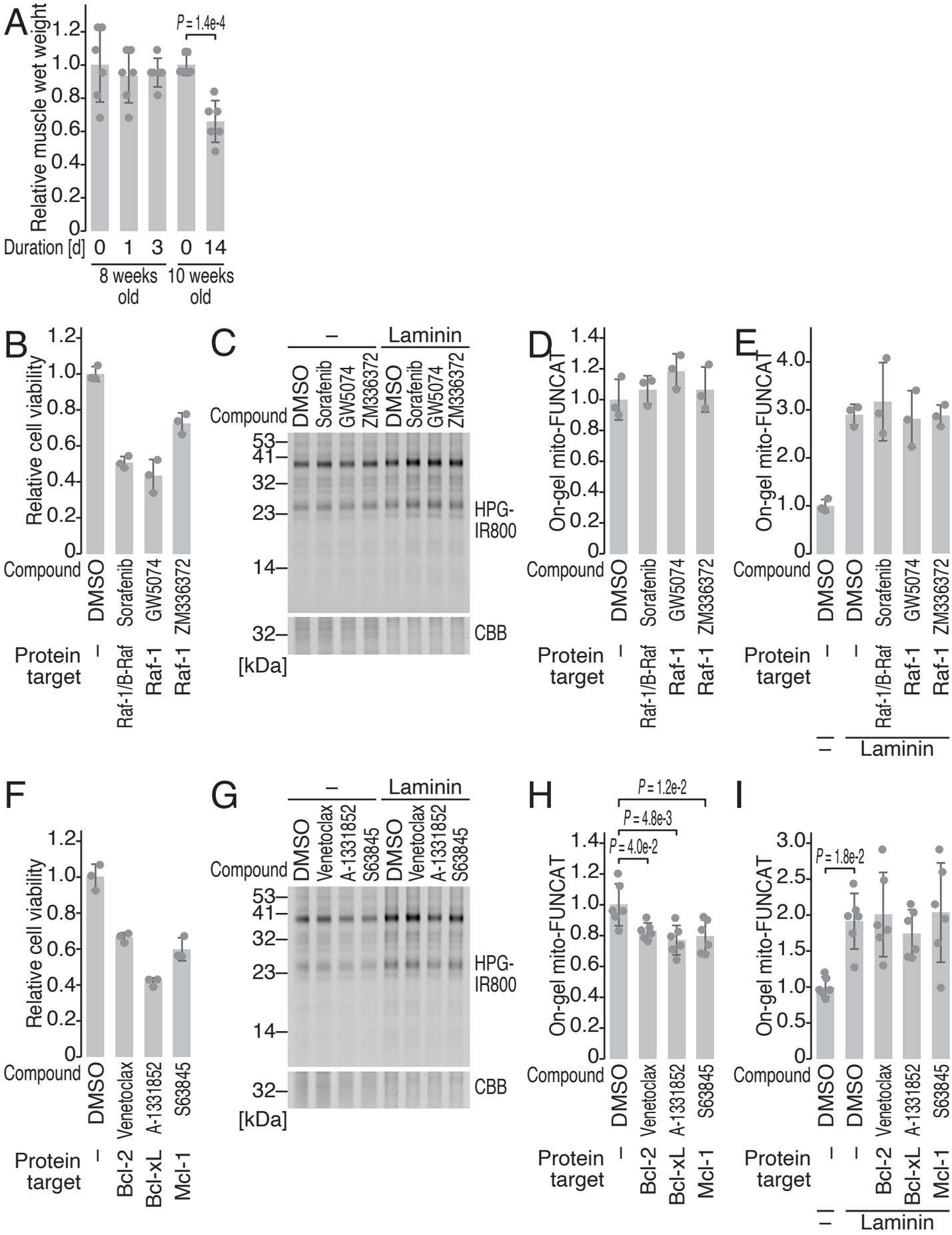
(S) *In vitro* translation of mammalian mitochondria reconstituted with recombinant factors. The effect of CoA is shown.

For A, C-F, K, M, N-P, and R, the data from replicates (points, n = 3 for A, F, K, M, N-P, and R; n = 5 for C-E), the mean values (bars), and the s.d.s (errors) are shown. The p values were calculated by Student's t test (two-tailed) (A, C-E, F, and N) and by the Tukey–Kramer test (two-tailed) (K, M, and R).

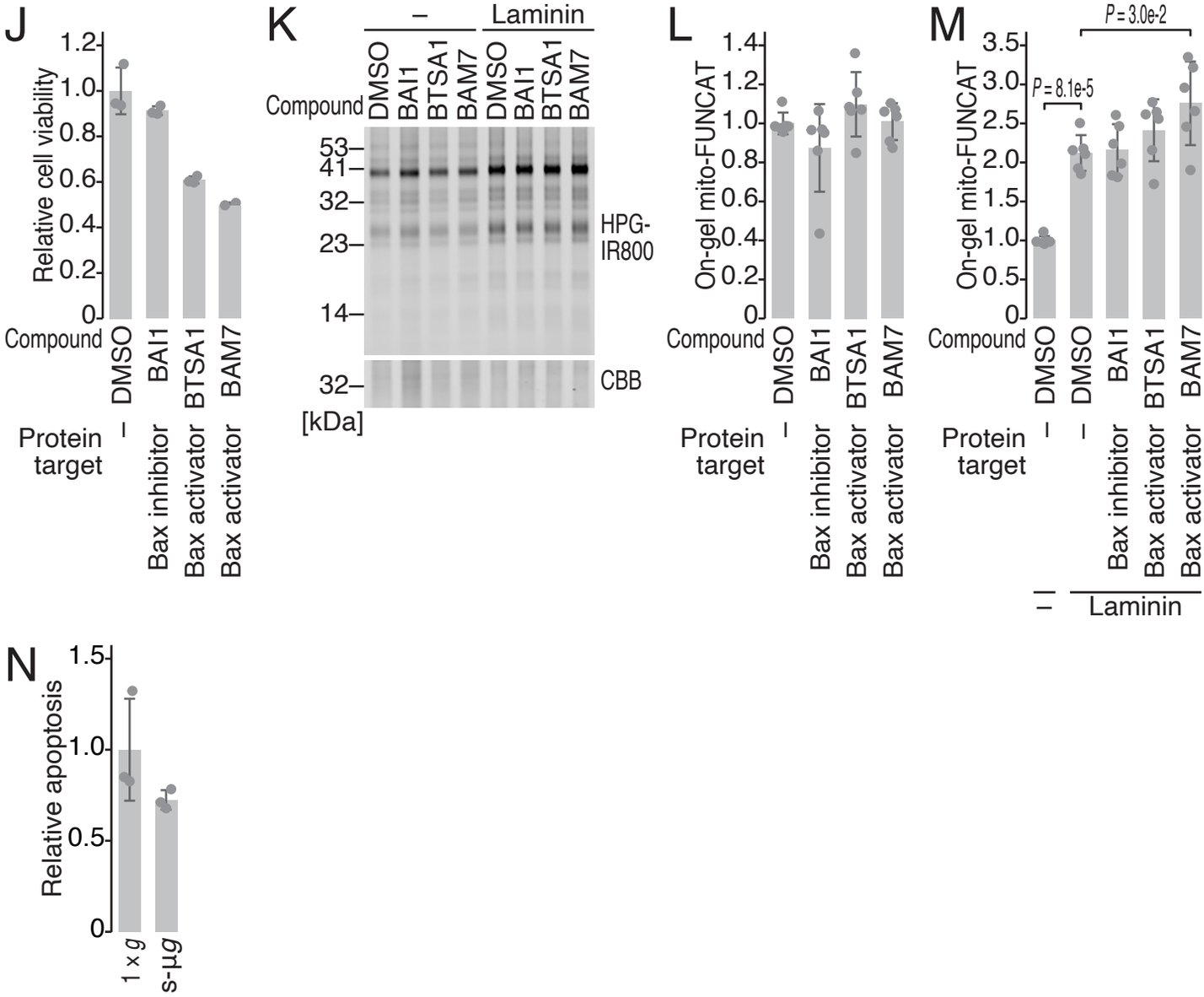
For S, the data from nine replicates (points) and the mean values (bars) are shown. The p values were calculated by the Tukey–Kramer test (two-tailed).

For A and J-R, the cells were treated with the compounds at the following concentrations: C75, 50 μM; nicotinamide, 10 mM; NRD167, 10 μM; and 3-TYP, 10 μM.

The experiments in A-F and J-R were conducted in HEK293 cells.



Supplementary Figure 7 Wakigawa, Kimura *et al*. (2026)



Supplementary Figure 7 Wakigawa, Kimura *et al*. (2026)

Supplementary Figure 7. Characterization of mice under a minimal mechanical stress model. Related to Figure 6.

(A) Relative soleus muscle wet weight under the indicated conditions. The data were normalized to the mean value of age-matched mice without unloading treatment. The right and left knees served as different samples.

(B, F, and J) Relative cell viability under treatment with the indicated compounds. The reduction in cell viability by compound treatment showed that the corresponding target proteins were efficiently blocked.

(C, G, and K) The representative results of on-gel FUNCAT experiments for mitochondrial translation for the indicated cell lines and conditions. CBB staining of the total proteins is shown as a loading control.

(D, E, H, I, L, and M) Quantification of on-gel FUNCAT experiments to monitor mitochondrial translation under treatment with the indicated compounds. We note that the DMSO samples in D, H, and L were the same data for DMSO without laminin precoating in E, I, and M, respectively.

(N) The relative level of apoptosis probed by Annexin V binding to cells at the indicated conditions. The cells were cultured under s-μ*g* for 24 h before cell harvesting.

For A-B, D-F, H-J, and L-N, the data from replicates (points, n = 3 for B, D, E, F, and J; n = 6 for A, H, I, L, and M), the mean values (bars), and s.d.s (errors) are shown. The p values were calculated by Student's t test (two-tailed) (A) and by the Tukey–Kramer test (two-tailed) (H, I, and M).

For B-M, the cells were treated with the compounds at the following concentrations: sorafenib, 1 μM; GW5074, 5 μM; ZM336372, 10 μM; venetoclax, 10 μM; A-1331852, 10 μM; S63845, 5 μM; 10 μM; BAI1, 1 μM; BTSA1, 20 μM; and BAM7, 20 μM.

The experiments, except for A, were conducted in HEK293 cells.