Cytoskeletal remodeling defines nucleolar architecture during adipogenesis

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Supplementary information

Supplementary Figures

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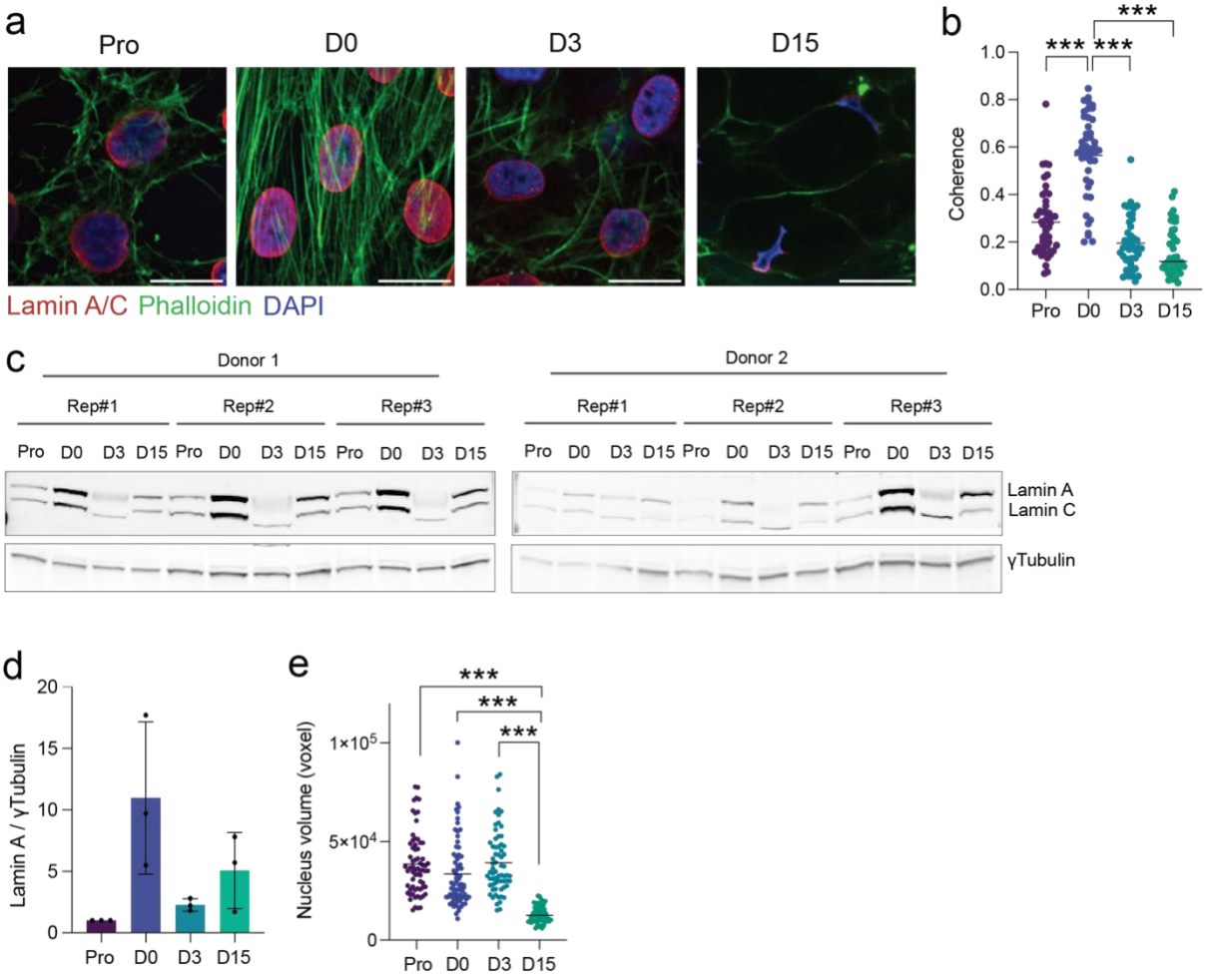
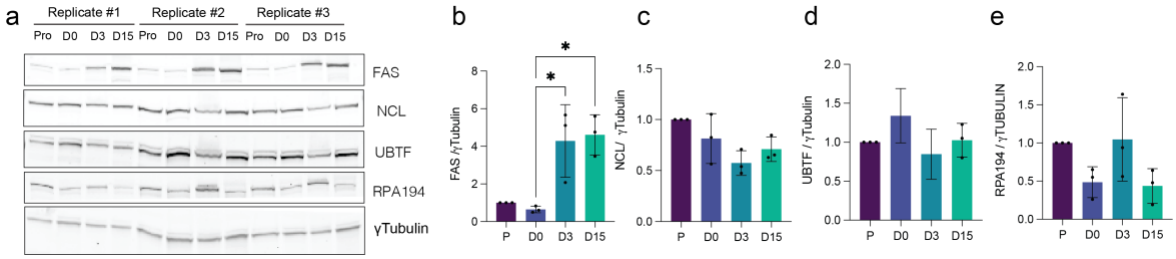
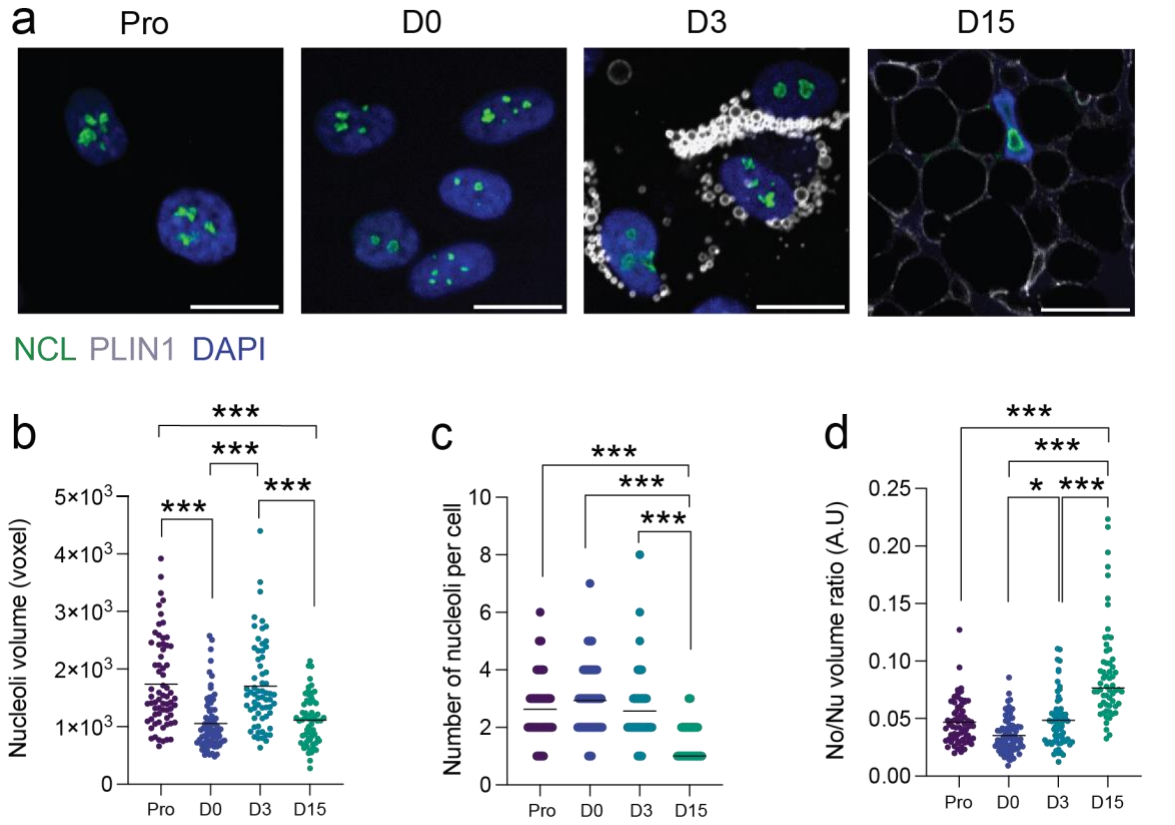
Supplementary Figures



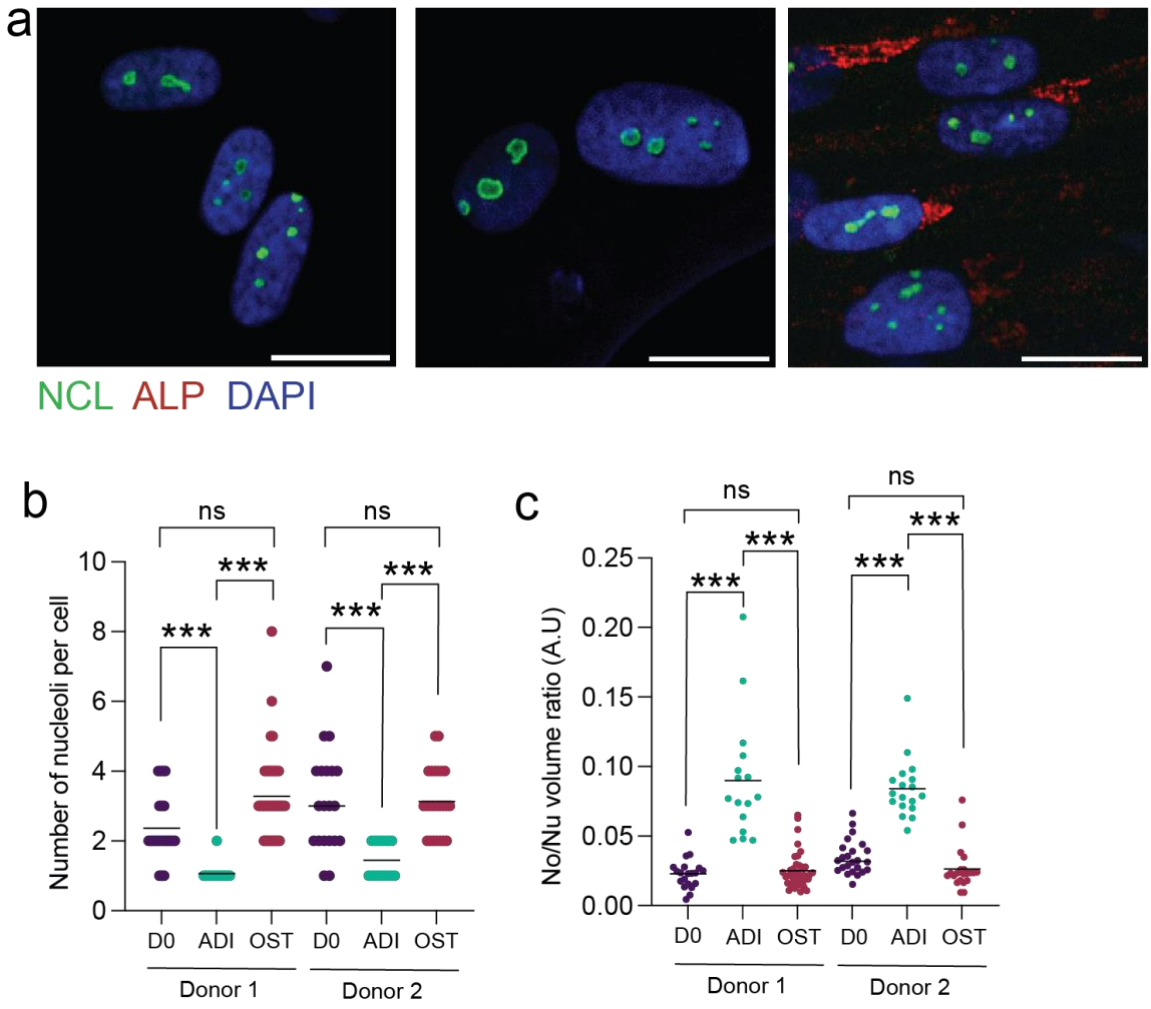
Supplementary Fig. 1. Nuclear remodeling during adipogenesis. a Immunofluorescence of lamin A/C, Phalloidin and DAPI stainings in differentiating ASCs from a second independent donor (donor 2; scale bar: 20 µm). **b** Relative cytoskeleton coherence measured from phalloidin signal (donor 2; ***p < 0.00001 vs D0, two-way ANOVA with Dunnett's multiple comparison test; n ≥ 5 fields of 2500 µm^2). **c** Western blot analysis of Lamin A and Lamin C expression in differentiating ASCs from Donor 1 (left panel) and Donor 2 (right panel). γTubulin is shown as a loading control. **d** Lamin A signals normalized to γTubulin signals quantified from Western blots (Donor 2; non-significant, one-way ANOVA with Holm-Šídák's multiple comparisons test, n = 3 independent experiments). **e** Nuclear volumes (voxel) measured from DAPI signal (Donor2; ***p < 0.0001 vs D0, one-way ANOVA with Tukey's multiple comparison; n ≥ 30 cells per time-point from independent 3 experiments).



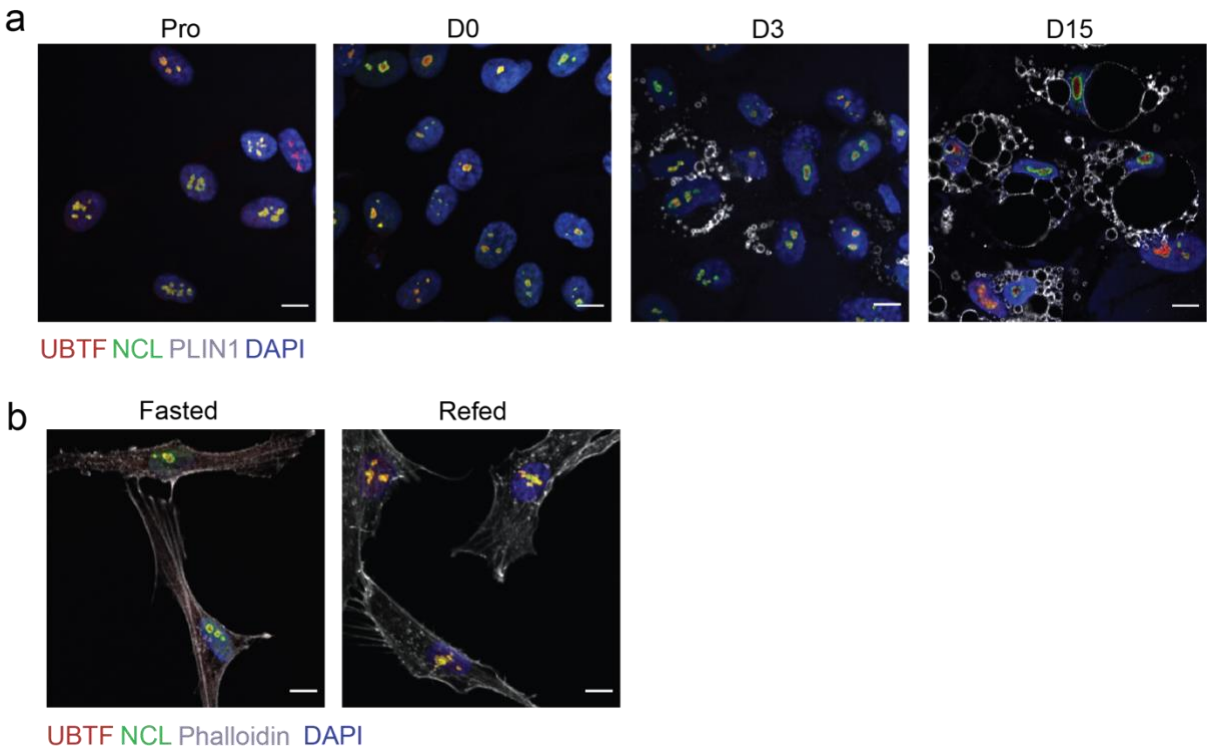
Supplementary Fig. 2. Protein expression of nuclear and nucleolar markers. a Western blot analysis of fatty acid synthase (FAS), nucleolin (NCL), UBTF and RNA Pol1 (RPA194) expression in differentiating ASCs (Donor 1). γTubulin is shown as a loading control, and FAS as a differentiation control. **b**,**c,d,e** Quantification of FAS, NCL UBTF and RPA194 protein levels normalized to γTubulin in differentiating ASCs (*p < 0.05, One-way ANOVA with Holm-Šídák's multiple comparisons test; n = 3 experiments)



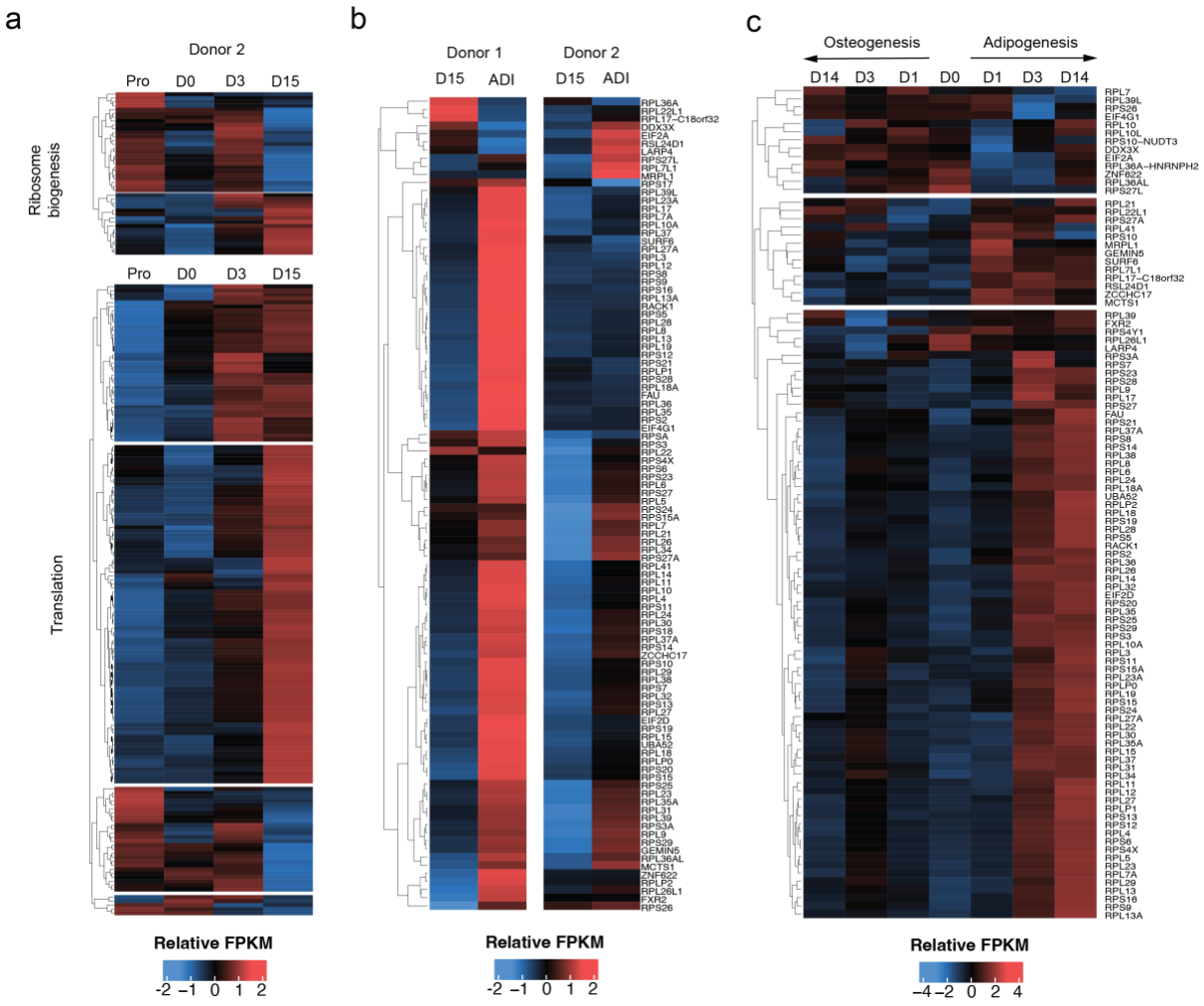
Supplementary Fig. 3. Nucleolar remodeling during adipogenesis. a Immunofluorescence of nucleolin (NCL), perilipin1(PLIN1) and DAPI staining in differentiating ASCs (Donor 2; scale bar: 20 µm). **b** Scatter plot of nucleolar volume measured from nucleolin immunostaining (***p < 0.0001, two-way ANOVA with Tuckey's multiple comparison; n ≥ 60 cells per time-point from 3 experiments). **c** Scatter plot of the number of nucleoli per cell (***p < 0.0001, two-way ANOVA with Tukey's multiple comparison test; n ≥ 60 cells per condition from 3 experiments). **d** Scatter plot of nucleolus-to-nucleus volume (No/Nu) ratio (*p < 0.05 ***p < 0.0001 two-way ANOVA with Tukey's multiple comparison; n ≥ 60 cells per condition from 3 experiments; ns, non-significant).



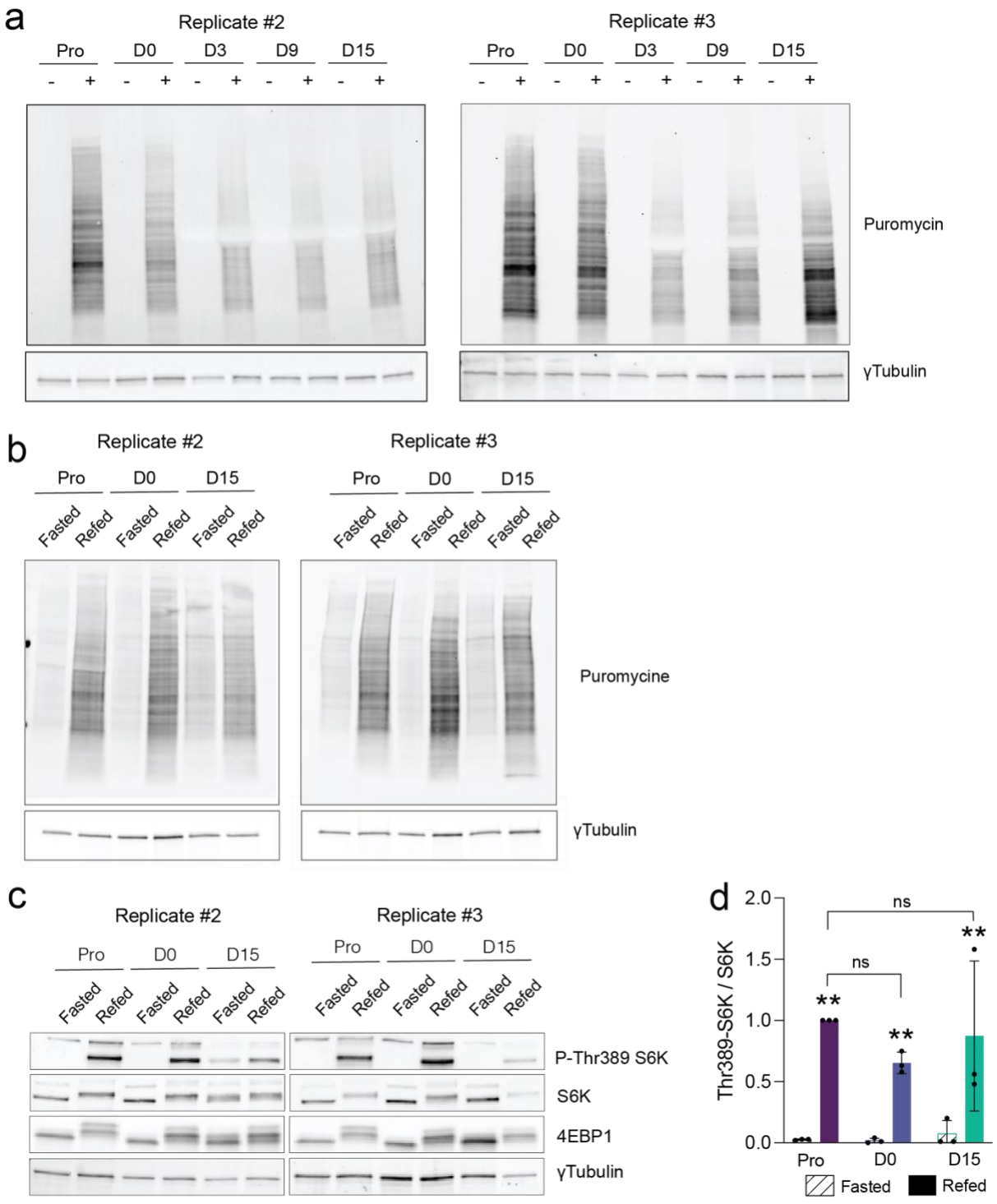
Supplementary Fig. 4. Lineage specificity of nucleolar remodeling. a Immunofluorescence of nucleolin (NCL) and alkaline phosphatase (ALP) and DAPI staining in D0 ASCs, and after 15 days in basal medium (non induced) or osteogenic differentiation medium (Donor 1; scale bar: 20 µm) **b** Number of nucleoli and **c** nucleolus-to-nucleus (No/Nu) volume ratio measured from nucleolin and DAPI staining, respectively, in D0 ASCs and on D15 of adipogenic (ADI) or osteogenic (OST) differentiation (***$p < 0.0001$, two-way ANOVA with Tukey's multiple comparison test; $n \geq 15$ cells per condition from 3 independent experiments).



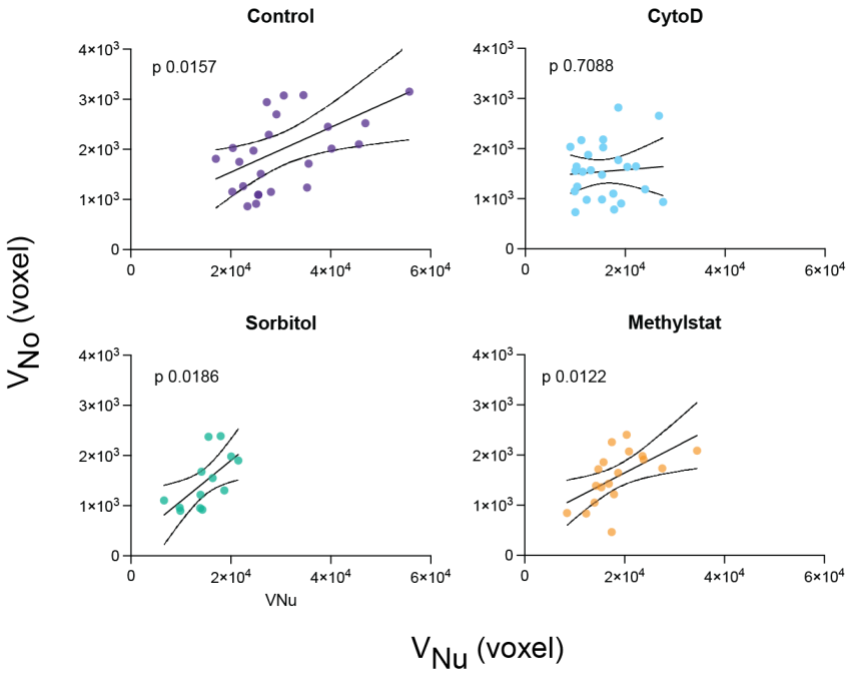
Supplementary Fig. 5. Nucleolus morphology during adipogenesis and upon ASC fasting and refeeding. a Immunofluorescence analysis of UBTF, nucleolin (NCL), perilipin1(PLIN1), and DAPI staining in differentiating ASCs (scale bar: 10 µm). **b** Immunofluorescence analysis of UBTF, nucleolin (NCL) and DAPI staining in proliferating ASCs after 24 h of fasting (fasted) followed by a 24-h culture in Pro medium (scale bar: 10 µm).



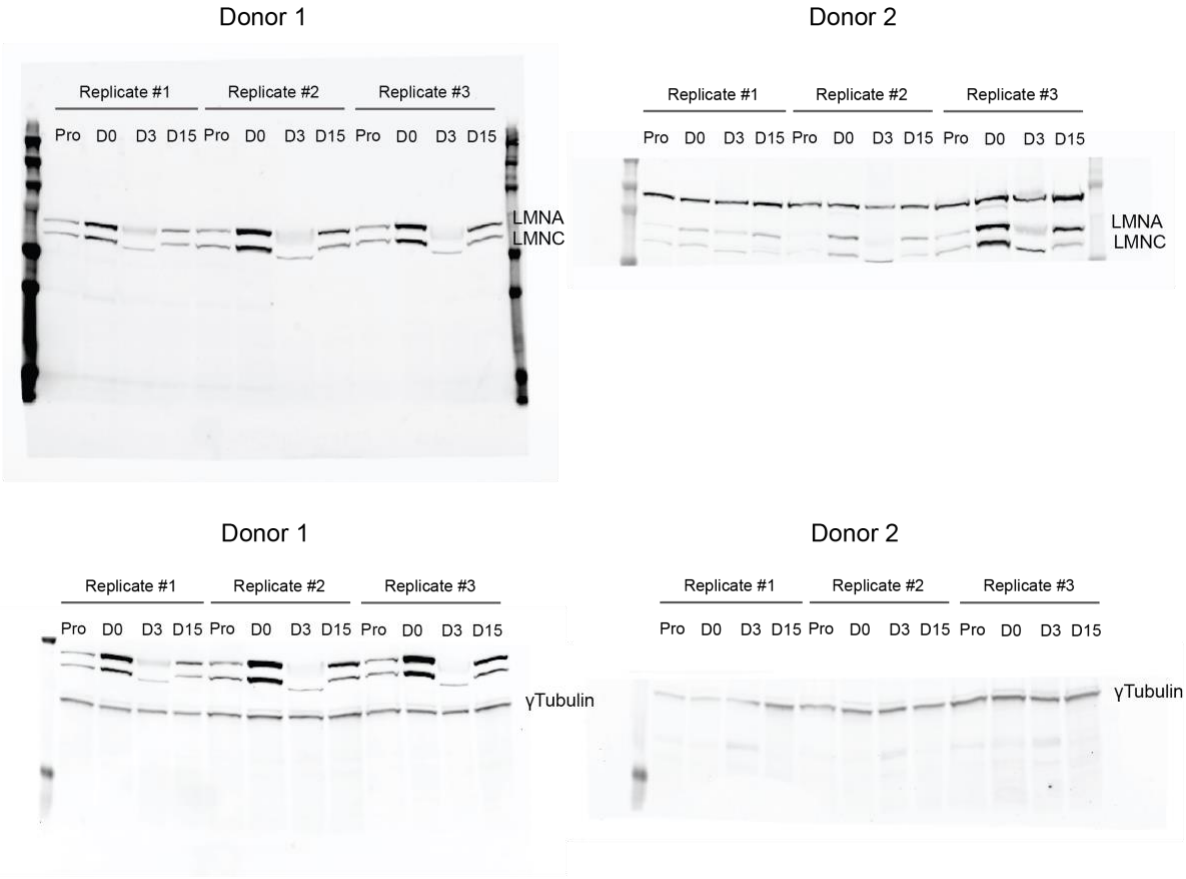
Supplementary Fig. 6. Upregulation of ribosomal protein gene expression during adipogenesis. **a** Heatmap representation of relative gene expression (log2 FPKM) for genes pertaining to the ribosome biogenesis (GO:0042254) and translation (GO:0006412) gene ontology during adipogenesis (Donor 2; $p < 0.01$, eBayes method, limma package). **b** Heatmap representation of relative gene expression (log2 FPKM) for genes pertaining to the Cytosolic ribosome (GO:0022626) gene ontology in D15 cell population vs isolated mature adipocytes (ADI) ($p < 0.01$, eBayes method, limma package). **c** Heatmap representation of relative gene expression (log2 FPKM) for genes pertaining to the Cytosolic ribosome (GO:0022626) gene ontology during adipogenic and osteogenic differentiation of ASCs using an independent RNAseq dataset (Rauch et al. 2019).



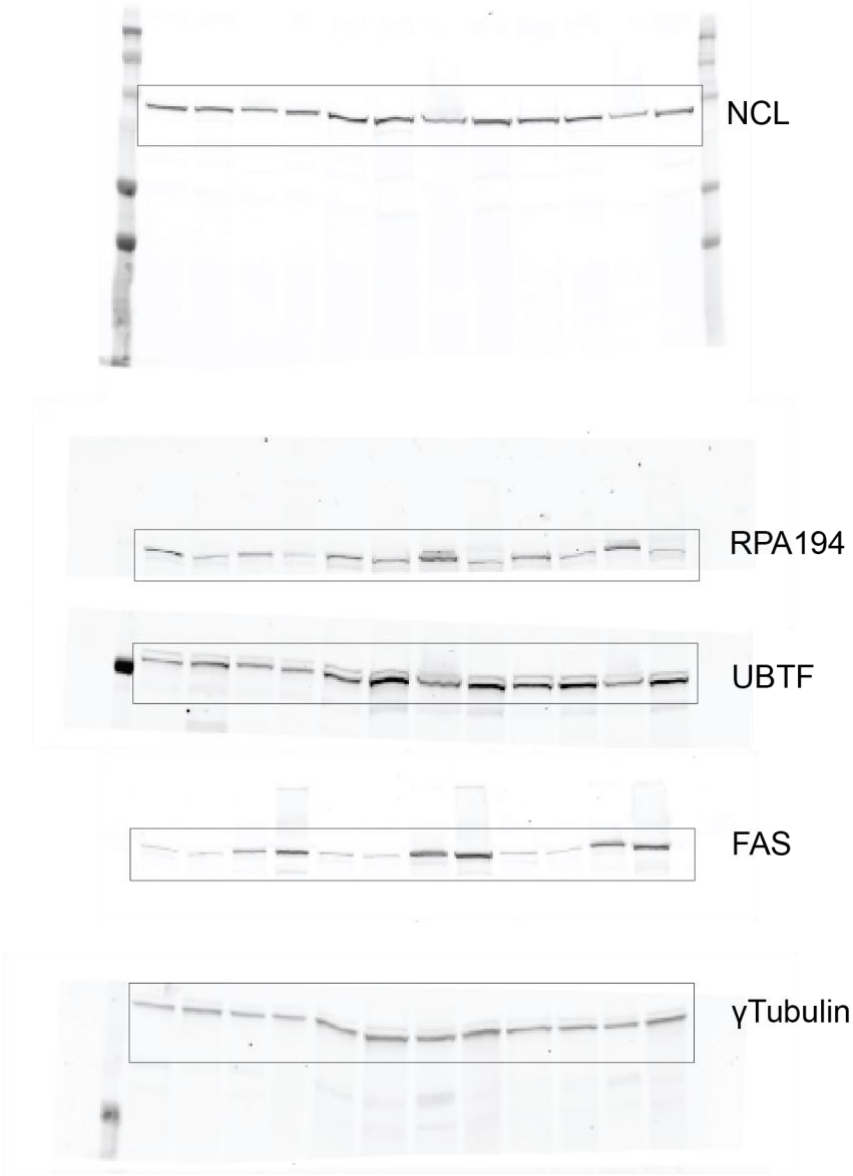
Supplementary Fig. 7. Decrease in translation efficiency following adipogenesis. a SUnSET analysis of protein synthesis rates during adipogenesis with (+) or without (-) 1µM Puromycin (Donor 1; Replicates #2 and #3). γTubulin is shown as a loading control. **b** SUnSET analysis of protein synthesis rates in fasted and refed conditions during adipogenesis (Donor 1; Replicates #2 and #3). γTubulin is shown as a loading control. **c** Western blot analysis of P-Thr389 S6K, total S6K and 4EBP1 in fasted and refed conditions during adipogenesis (Donor 1; Replicates #2 and #3). γTubulin is shown as a loading control. **d** P-Thr389 S6K signals normalized to total S6K signals, quantified from Western blots (Donor 1; **p < 0.01, two-way ANOVA with Sidák's multiple comparisons test; n = 3).



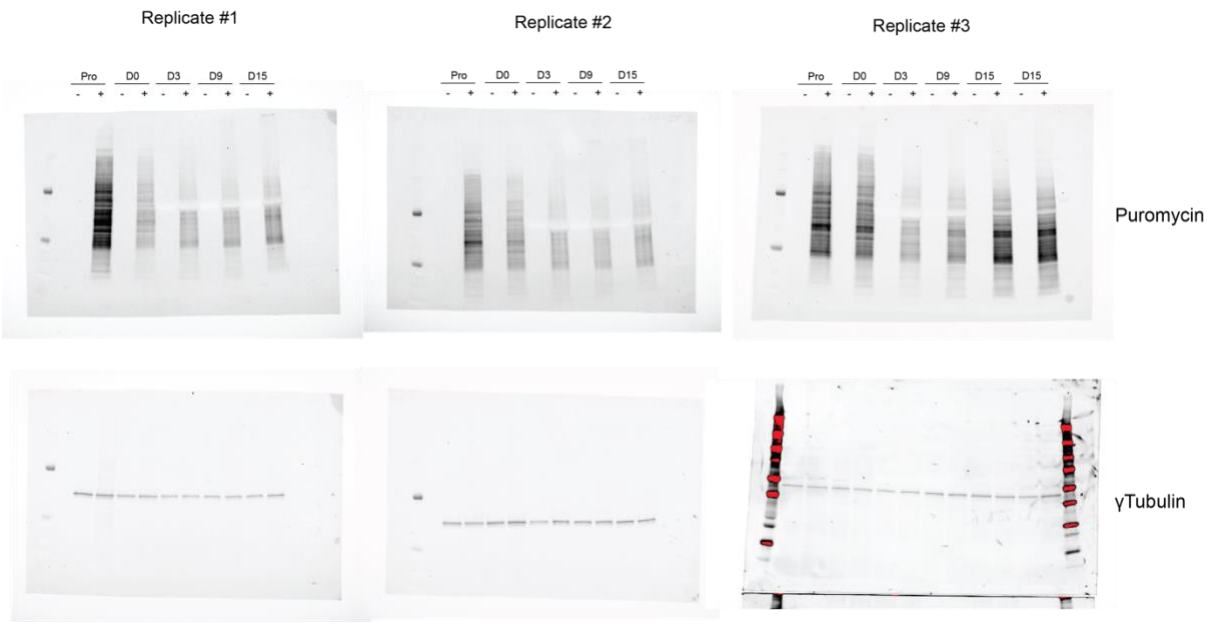
Supplementary Fig. 8. Nucleolus to nucleus volume correlations. Scatter plots of nucleolar volume (V_{No}) vs. nuclear volume (V_{Nu}) and linear correlation with 95% confidence intervals bands for control ASCs (Donor 1) and after cytochalasin D (CytoD), Sorbitol or Methylstat treatments (simple linear regression; p-values are shown on the graphs).



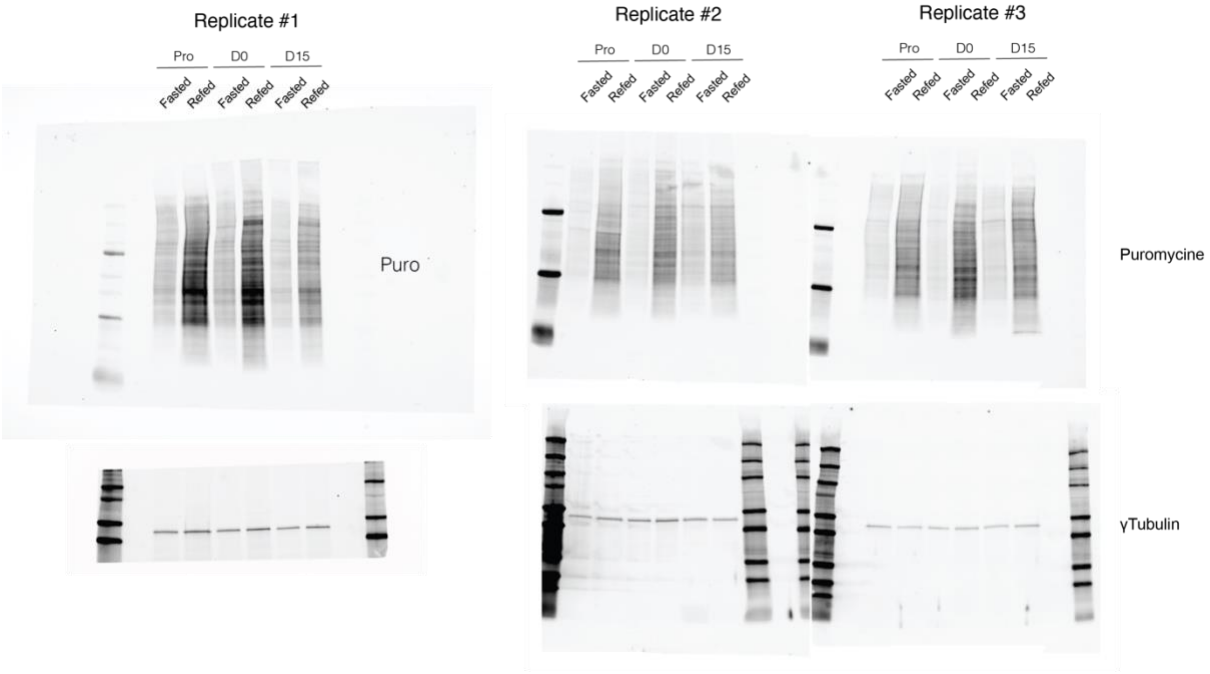
Supplementary Fig. 9. Uncropped membranes for Supplementary Fig. 1



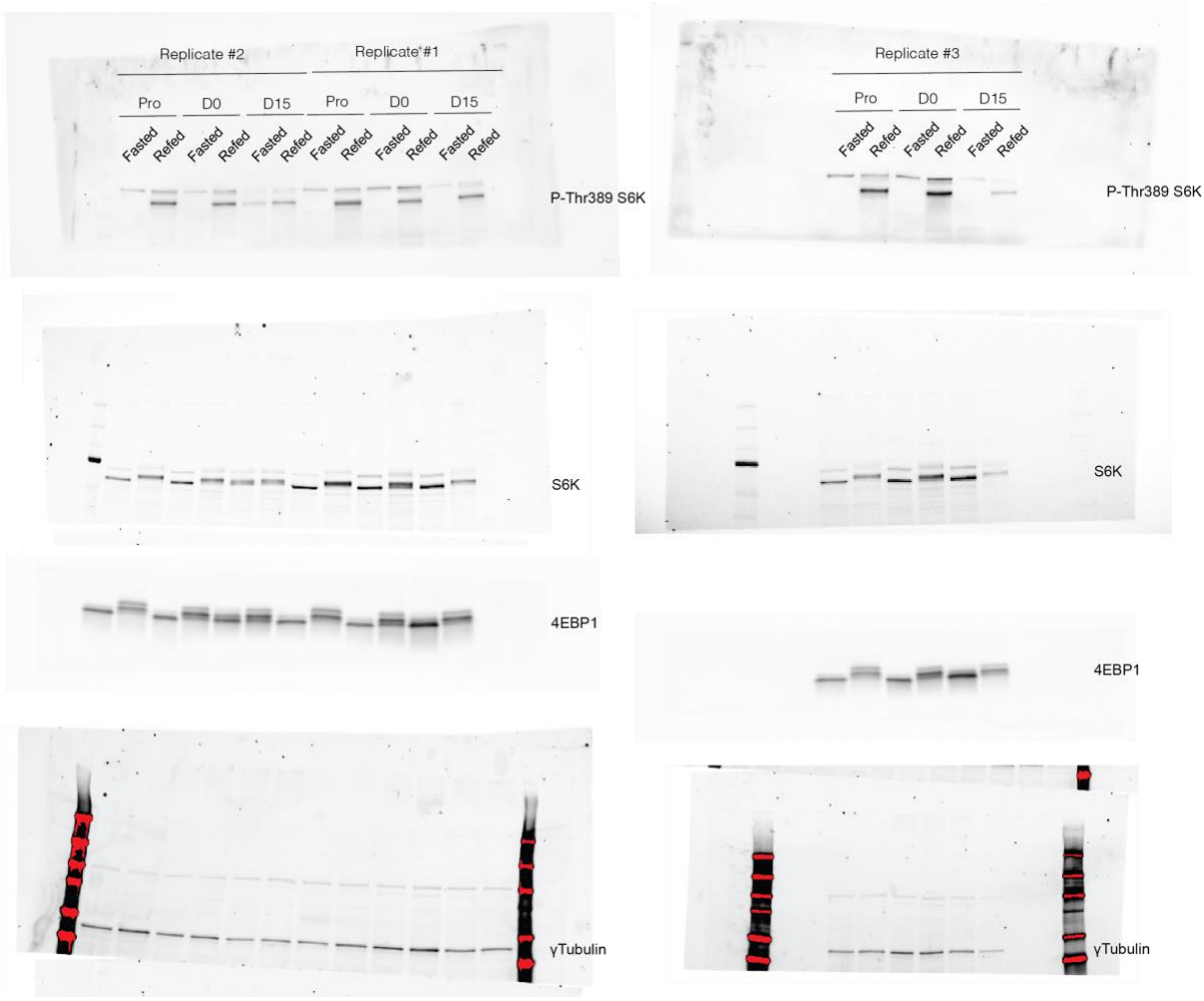
Supplementary Fig. 10. Uncropped membranes for Supplementary Fig. 2



Supplementary Fig. 11. Uncropped membranes for Fig. 3b and Supplementary Fig. 7a



Supplementary Fig. 12. Uncropped membranes for Fig. 3c and Supplementary Fig. 7b



Supplementary Fig.13 Uncropped membranes for Fig. 3e and Supplementary Fig. 7c

Supplementary Table

	Reference	Western Blot	Immunofluorescence
Lamin A/C	Santa Cruz, sc-7292	1/500	-
Nucleolin	Abcam, ab22758	1/1000	1/1000
Perilipin1	Progen, GP29	1/1000	1/500
Nucleophosmin	Santa Cruz, sc-32256	-	1/500
RPA194	Santa Cruz, sc-48385	1/1000	1/500
UBTF	Santa Cruz, sc-13125	1/500	1/200
FAS	Santa Cruz, sc-48357	1/1000	-
Puromycin	Millipore, MABE343	1/10000	-
P-Thr389 P70S6K	Cell signaling, #9234	1/1000	-
P70S6K	Cell signaling, #9202	1/1000	-
4EBP1	Cell signaling, #9452S	1/1000	-
Alkaline Phosphatase	Abcam, ab17272	-	1/250
γTubulin	Sigma, T5326	1/10000	-

Table S1. Antibodies and dilutions.