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Supplemental information

Cells use multiple mechanisms for cell-cycle arrest upon withdrawal of individual amino acids

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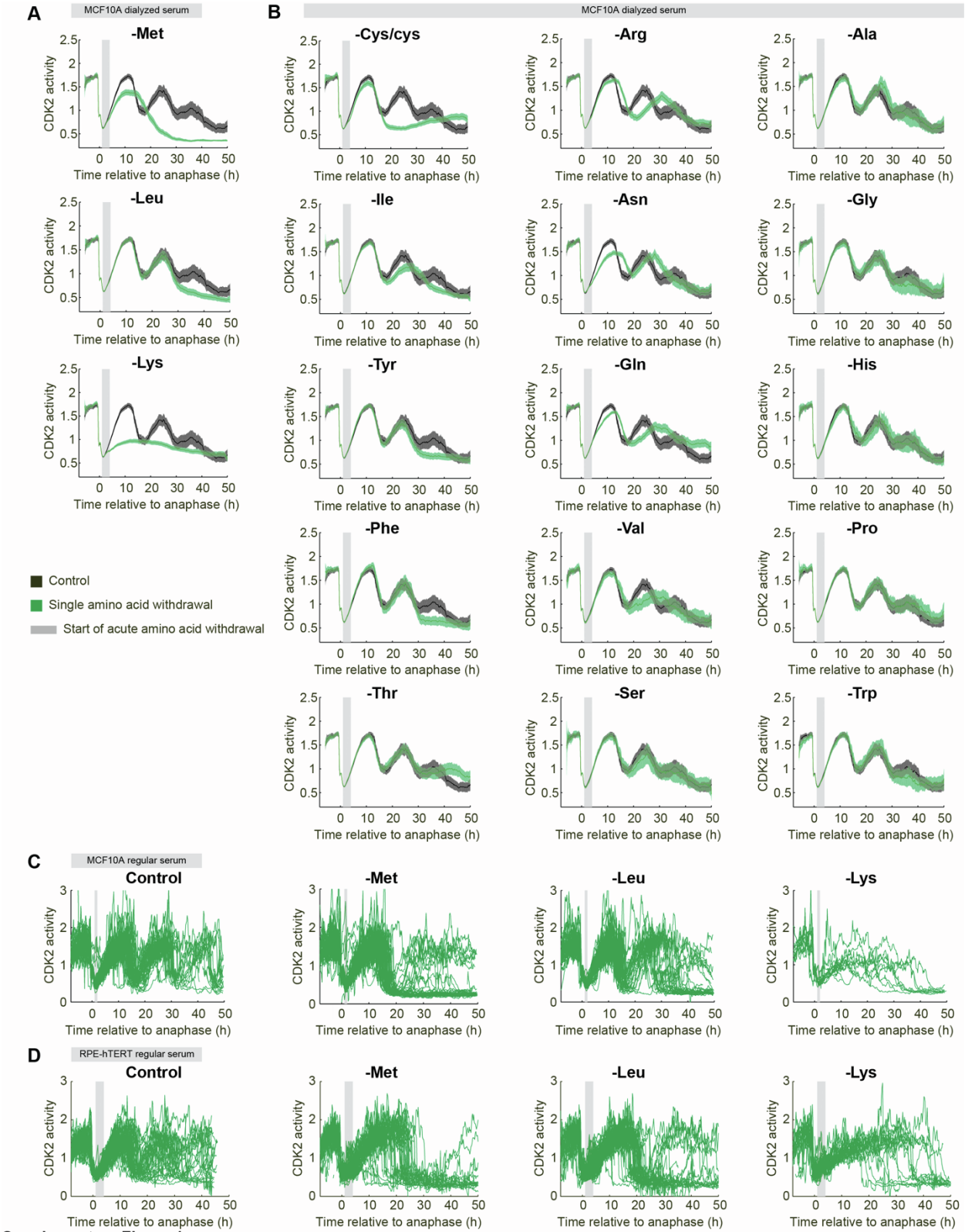
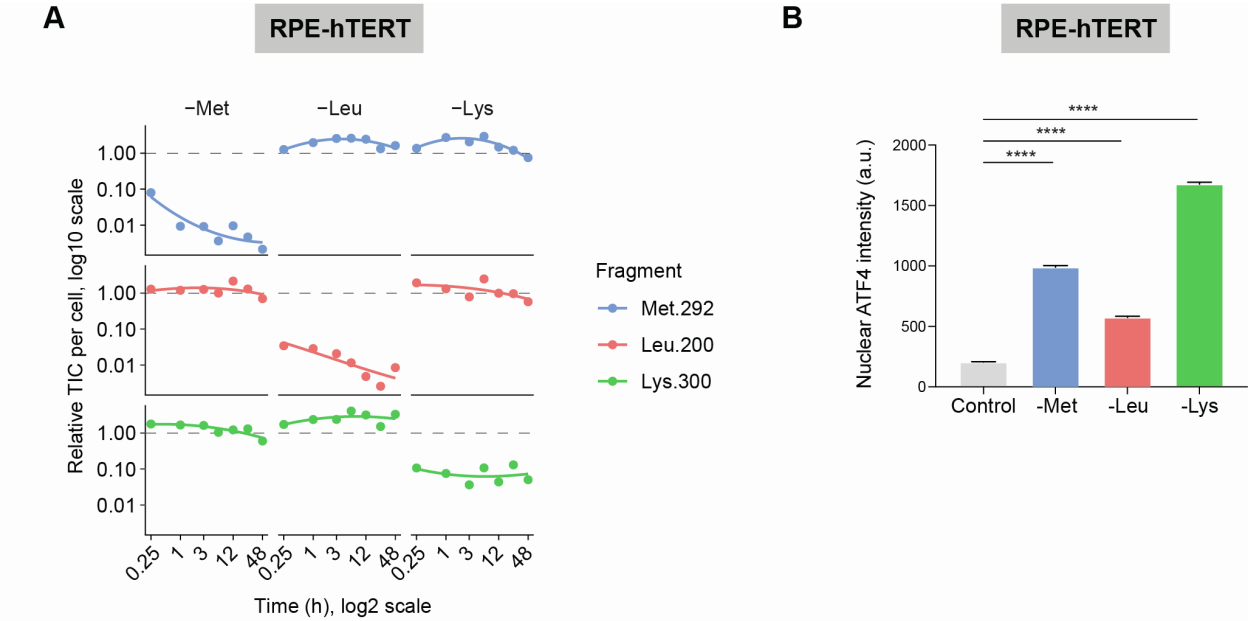
Supplemental Information



Supplementary Figure 1

Figure S1: Acute withdrawal of different amino acids leads to differing cell-cycle effects, related to Figure 2
A-B. Population average and 95% confidence interval of CDK2 activity in MCF10A cells with 5% dialyzed horse serum. Cells were first imaged in full-growth medium for 16 h. The movie was then paused and the indicated amino acid was acutely withdrawn (gray bar) before restarting the movie. Cells were selected for plotting if they completed anaphase 1-4 h before amino acid withdrawal (a G1 phase withdrawal). All plots contain at least 60 cells per condition.
C. Single-cell traces of CDK2 activity aligned to anaphase for MCF10A cells grown in regular horse serum. Cells were selected for plotting if they completed anaphase 1-2 h before amino acid withdrawal (gray bar). All plots contain at least 25 cells per condition.
D. Single-cell traces of CDK2 activity aligned to anaphase for RPE-hTERT cells grown in regular FBS. Cells were selected for plotting if they completed anaphase 1-4 h before amino acid withdrawal (gray bar). Note that in contrast to MCF10A cells, the majority of RPE-hTERT cells complete only one cell cycle after leucine withdrawal before entering a $CDK2^{low}$ quiescence. All plots contain at least 50 cells per condition.

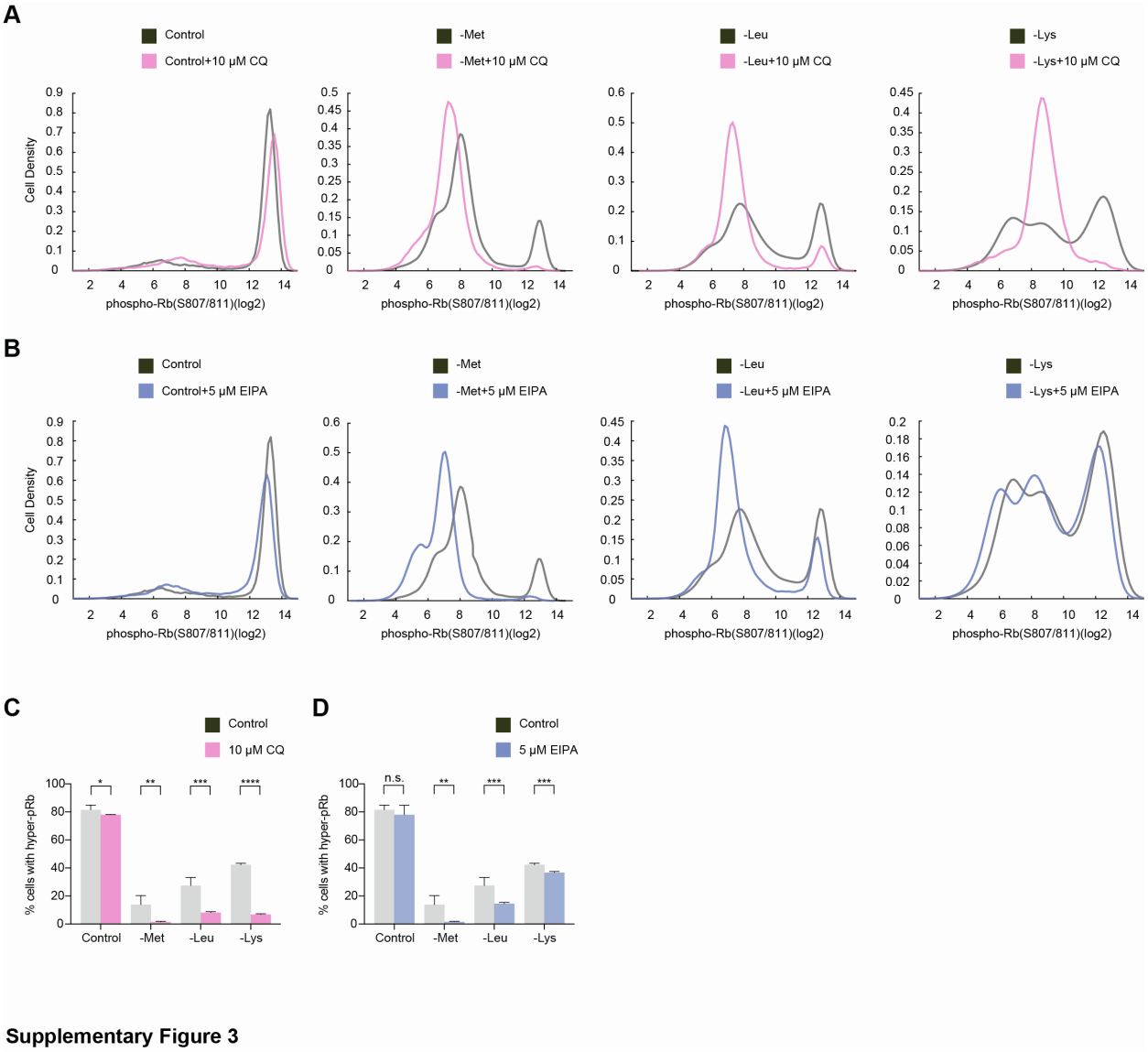


Supplementary Figure 2

Figure S2: Measurement of amino acid concentrations and amino acid sensing in RPE-hTERT cells, related to Figure 3

A. GC-MS quantification of intracellular single amino acid concentration in RPE-hTERT cells grown in regular FBS after acute amino acid withdrawal for 0.25, 1, 3, 6, 12, 24, 48 h.

B. Quantification of ATF4 protein in RPE-hTERT cells grown in regular FBS after 3 h of single amino acid withdrawal. Statistical analyses were performed using permutation test: **** indicates $p < 0.0001$. All plots contain at least 20000 cells per condition.

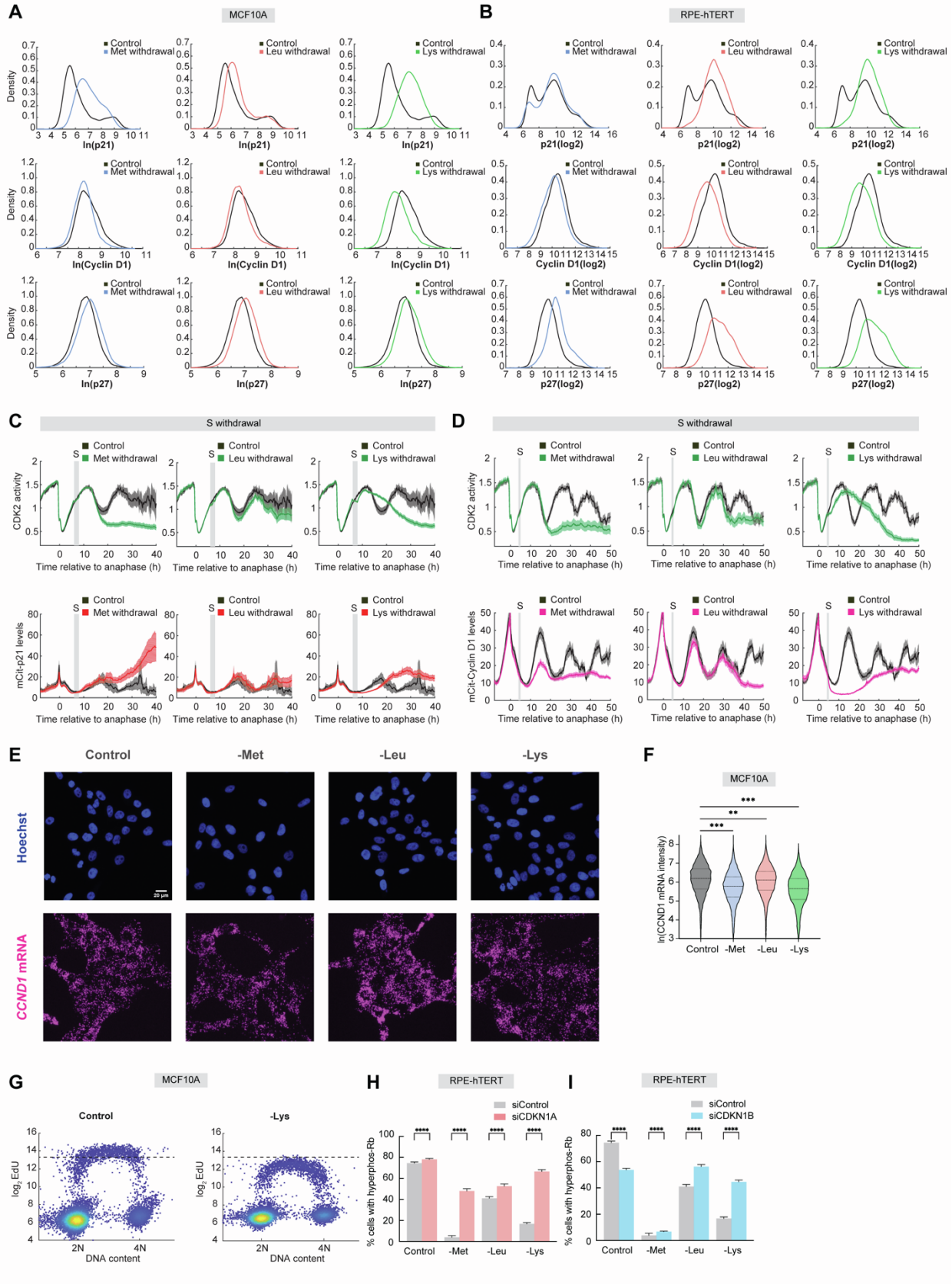


Supplementary Figure 3

Figure S3: Investigating the role of amino acid recycling and scavenging in MCF10A cells upon amino acid starvation, related to Figure 3

A-B. Probability density of phospho-Rb (Ser807/811) intensity measured by immunofluorescence in MCF10A cells after the indicated single amino acid withdrawal with or without 10 μM CQ (A) or 5 μM EIPA (B) treatment for 48 h. Cells were grown in regular horse serum. All plots contain at least 10000 cells per condition.

C-D. Quantification of percentage of MCF10A cells with hyper-phosphorylated Rb (Ser807/811) after methionine, leucine and lysine withdrawal with or without 10 μM CQ (C) or 5 μM EIPA (D) treatment for 48 h. Cells were delineated as having hyper-phosphorylated Rb if they had an x-axis value of ≥ 10.5 in panels A and B. Cells were grown in regular horse serum; the indicated amino acid was withdrawn for 48 h. Error bars indicate 95% confidence intervals; stars indicate significance based on t-test.



Supplementary Figure 4

Figure S4: Further analysis of p21, p27, and Cyclin D1 protein abundance in MCF10A and RPE-hTERT cells, related to Figure 4

A-B. Probability density of p21 (top), Cyclin D1 (middle) and p27 (bottom) intensity measured by immunofluorescence in MCF10A cells (A) or RPE-hTERT cells (B) grown in regular serum after withdrawal of the indicated amino acid for 48 h. All plots contain at least 12000 cells per condition.

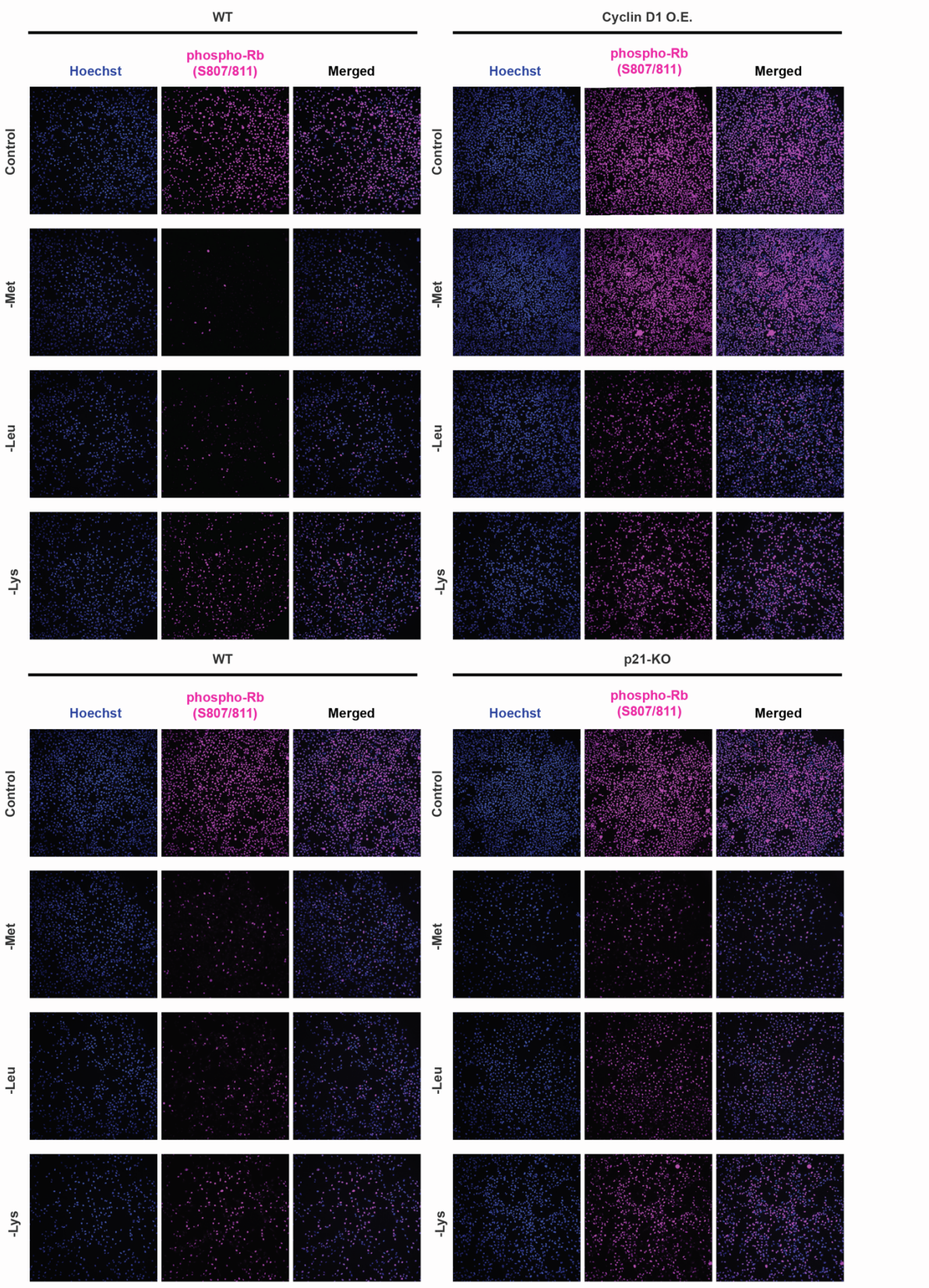
C-D. Population average and 95% confidence interval of CDK2 activity and endogenous p21 (C) or endogenous Cyclin D1 (D) in MCF10A cells grown in regular horse serum. Cells were first imaged in full-growth media for 16 h before the indicated amino acid was acutely withdrawn. Cells were selected for plotting if they completed anaphase 6-8 h (C) or 4-5 h (D) before the time of amino acid withdrawal, marked by the gray bar. All plots contain at least 80 cells per condition.

E. Representative RNA-FISH images for *CCND1* with Hoechst staining in MCF10A cells grown in regular horse serum after 3 h of single amino acid withdrawal. Scale bars = 20 μm.

F. Quantification of Cyclin D1 (*CCND1*) mRNA amount in MCF10A cells grown in regular horse serum after withdrawal of the indicated amino acid for 3 h. Statistical analyses were performed using permutation test. Significance levels are reported as p values < 0.05 (*), 0.01 (**), 0.001 (***) and 0.0001 (****) with corresponding star notations. All plots contain at least 15000 cells per condition.

G. Density scatter plot of EdU versus DNA content in MCF10A cells grown in regular serum after lysine withdrawal for 48 h. Dashed line marks the bottom range of the EdU-high population in untreated cells. All plots contain at least 5000 cells per condition.

H-I. Quantification of percentage of RPE-hTERT cells with hyper-phosphorylated Rb (Ser807/811) in WT, p21 siRNA knockdown (H), or p27 siRNA knockdown (I), grown in regular FBS after withdrawal of the indicated amino acid for 48 h. Statistical analyses were performed using permutation test: **** indicates $p < 0.0001$. All plots contain at least 20000 cells per condition.



Supplementary Figure 5

Figure S5: Representative images of the effect of Cyclin D1 overexpression or p21 knockout after amino acid withdrawal, related to Figure 4
Immunofluorescence images of phospho-Rb (Ser807/811) in wild-type (WT) MCF10A and MCF10A overexpressing Cyclin D1 (top) or MCF10A with p21 knocked out (bottom) after withdrawal of the indicated amino acid for 48 h. Blue, Hoechst; magenta, phospho-Rb (Ser807/811). Cells were gown in regular horse serum.

Video S1. Time-lapse imaging of CDK2 activity upon acute withdrawal of different amino acids in MCF10A cells, related to Figure 2. Asynchronously cycling MCF10A cells expressing DHB-mCherry were first imaged in full-growth medium every 12 min for 16 h with a 10× objective. The movie was then paused and cells were subjected to lysine (top right), methionine (bottom left) or leucine (bottom right) withdrawal for 48 h.

Video S2. CDK2 activity in untreated and Mek inhibitor treated MCF10A cells, related to Figure 2.
Asynchronously cycling MCF10A cells expressing DHB-mCherry were first imaged in full-growth medium every 12 min with a 10× objective. The movie was then paused and cells were either maintained in full-growth medium (left) or treated with 100 nM Mek inhibitor (right) for 48 h. The red and green arrows point to cells whose CDK2 activity is plotted below. In response to Mek inhibition, cycling cells complete the current cell cycle and then enter a CDK2-low quiescence after the subsequent mitosis.