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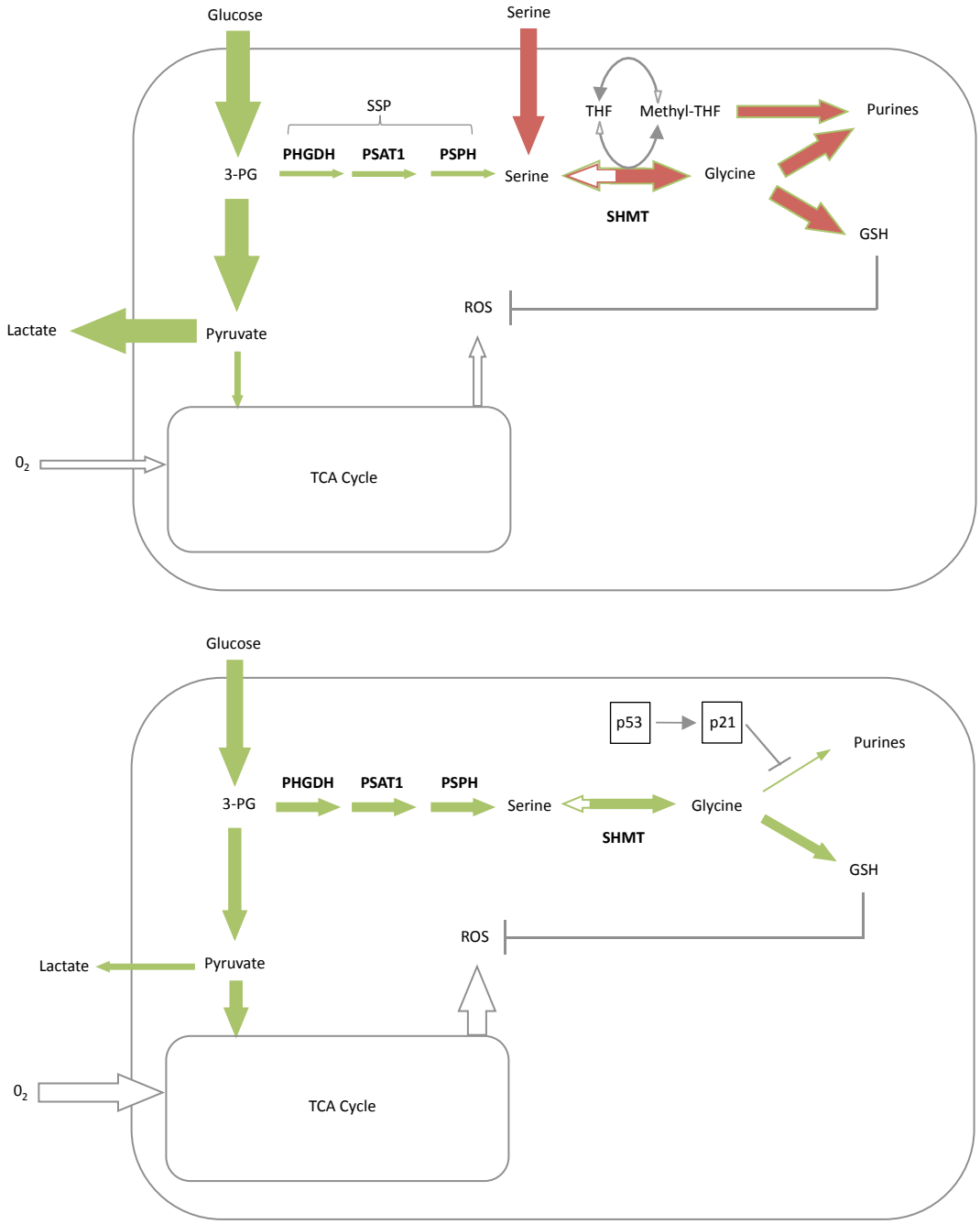
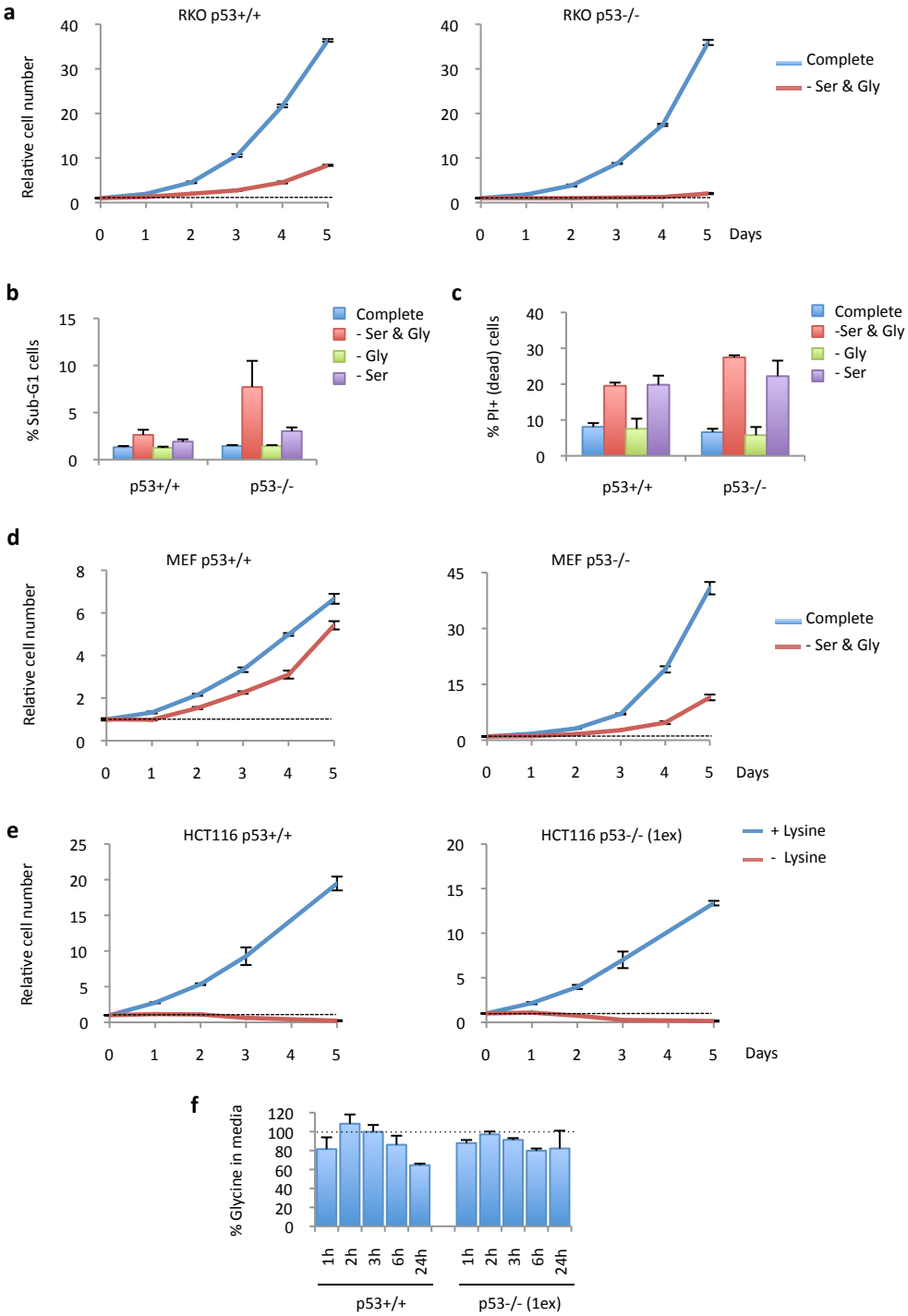
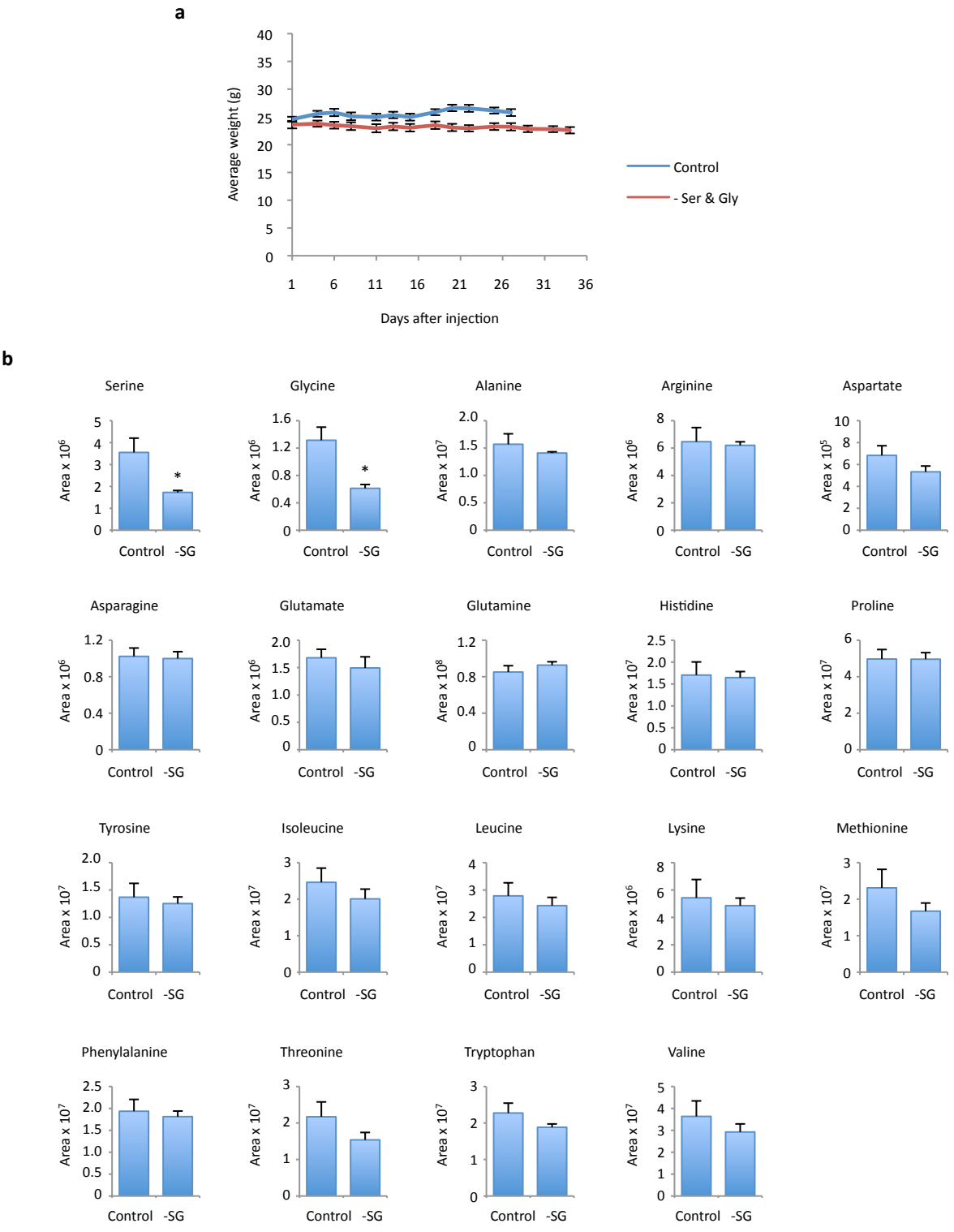


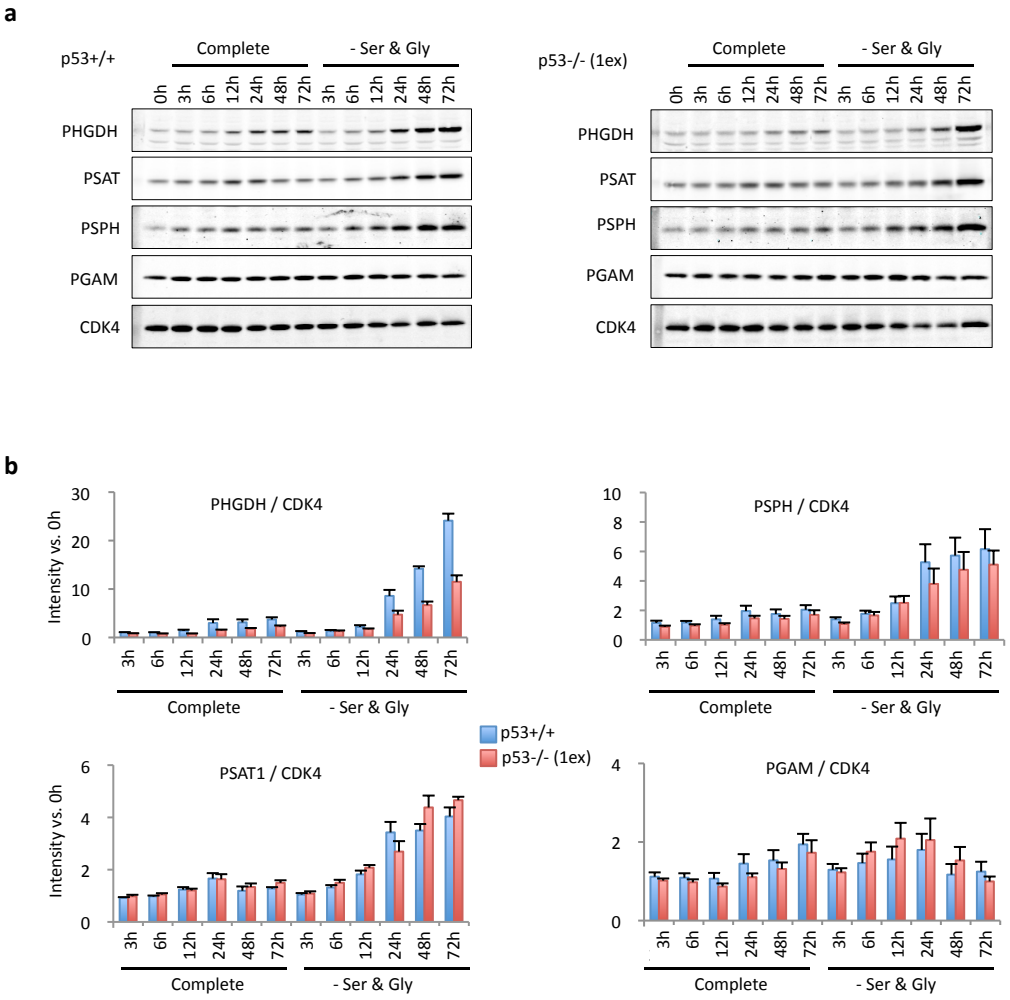

**Supplementary Figure 1. Removal of exogenous serine causes energetic stress and p53-dependent metabolic remodeling in cancer cells.** Cancer cells rapidly utilise exogenous serine, which is converted within cells to glycine, used to synthesise purine nucleotides, GSH and other metabolites. Conversion of serine to glycine also provides methyl-THF, an important intermediate in the synthesis of purine and pyrimidine nucleotides, whereas the reverse (conversion of glycine to serine) consumes methyl-THF. The serine synthesis pathway (SSP) utilises the glycolytic intermediate 3-PG, which is converted by PHGDH, PSAT1 and PSPH into serine. Removal of exogenous serine causes activation of the SSP, accompanied by decreased flux to lactate, resulting in ATP depletion. To compensate, more pyruvate is transferred to the TCA cycle for elevated OXPHOS. p53-dependent activation of p21 induces transient cell cycle arrest, blocking flux to purines, thus maintaining GSH synthesis. Enzymes shown in bold typeface, metabolites in normal typeface. 3-PG; 3-phosphoglycerate, GSH; glutathione, ROS; reactive oxygen species, THF; tetrahydrofolate.



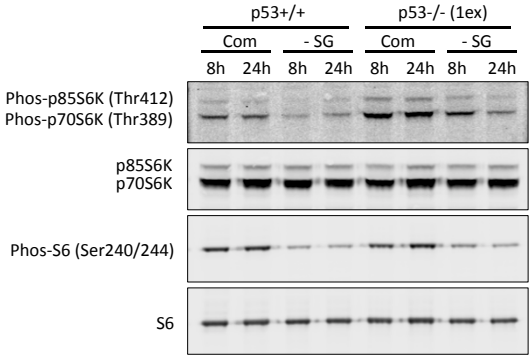

**Supplementary Figure 2. p53 supports proliferation of RKO cells and MEFs during serine starvation. a**, RKO cells were grown in complete media or media deficient in serine and glycine (-SG). **b**, cell death in RKO cells was assessed by PI staining of fixed cells (n=5) and **c**, PI exclusion by live cells (n=3), both quantified by flow cytometry. **d**, MEFs were grown in complete media or media deficient in serine and glycine (-SG). **e**, HCT116 cells were grown in cell culture medias formulated with individual nutrient components to approximately match DMEM, either lacking (-) or containing (+) the essential amino acid lysine. **f**, HCT116 cells were grown in complete media, glycine levels present in the spent media were quantified by LC-MS (averages of triplicate wells vs. fresh media). Cell proliferation curves are averages of triplicate wells. All error bars are SEM.



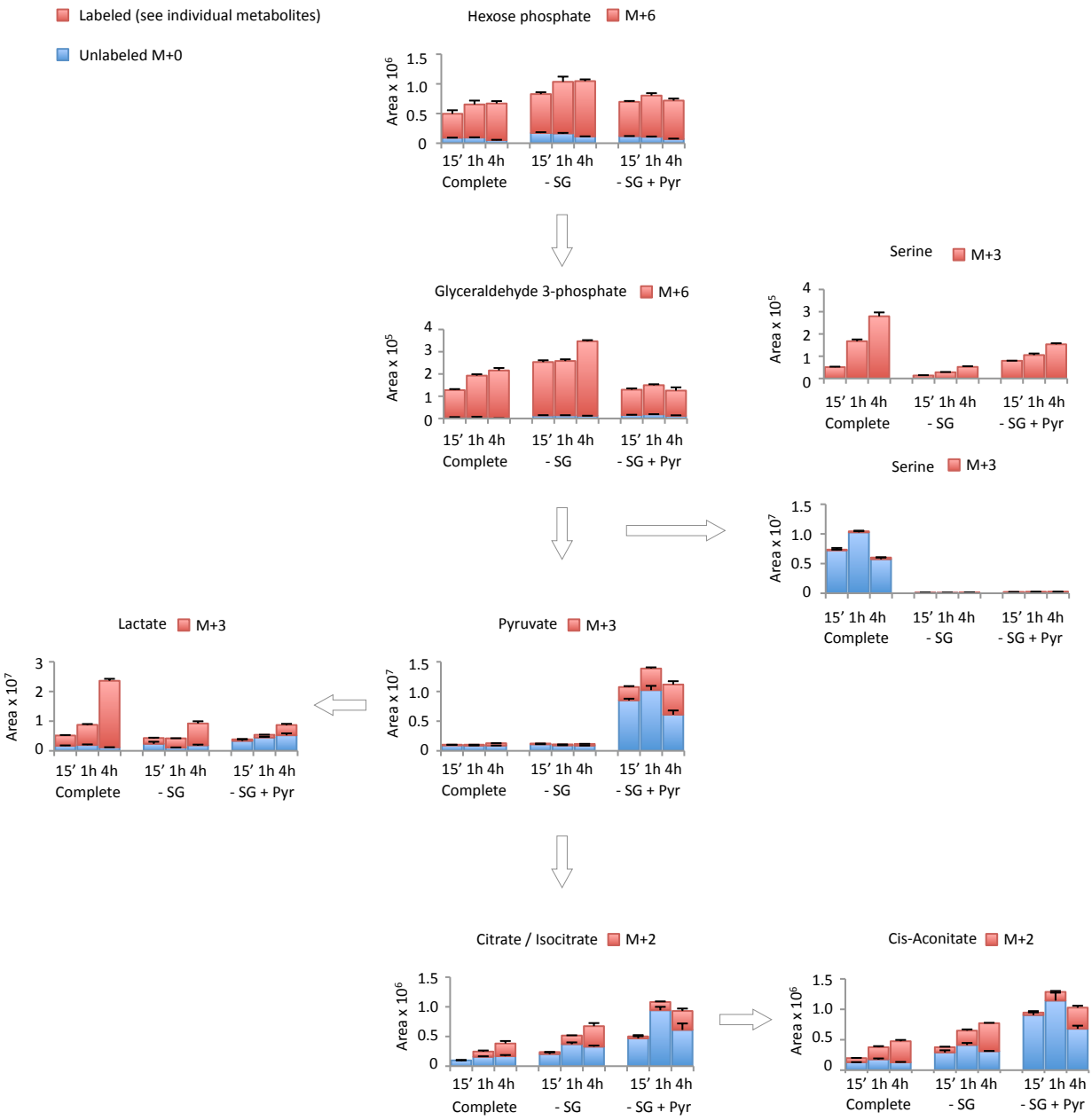

**Supplementary Figure 3. The effects of a serine and glycine deficient diet *in vivo*. a**, The weights of mice used in the xenograft experiment were recorded at regular intervals. Wastage of food in some cages (Control and –Ser & Gly diets) made it difficult to accurately record diet consumption. In a cage where wastage did not occur, consumption of –Ser & Gly diet was 6g/day/mouse, comparable to the predicted normal intake of 5g/day/mouse. **b**, LC-MS was performed on serum samples taken from the mice at time of sacrifice to evaluate amino acid content (*p<0.05). Control diet n=10, -SG diet n=8, all error bars are SEM.



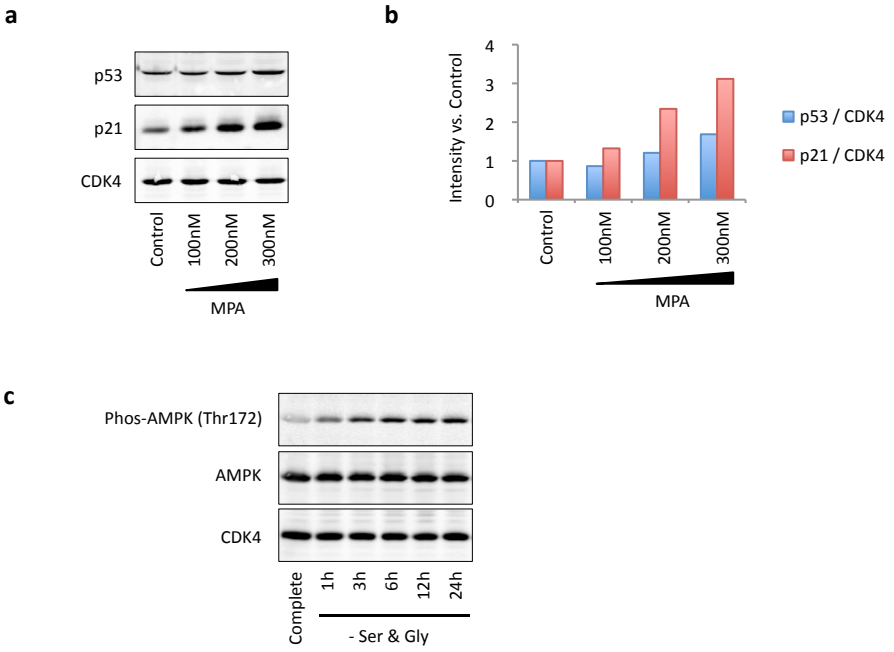

**Supplementary Figure 4. Serine and glycine starvation results in up-regulation of SSP enzymes.** **a**, Representative western blots for PHGDH, PSAT1, PSPH and PGAM protein expression in HCT116 cells either fed complete or serine and glycine deficient (-Ser & Gly) media. **b**, Protein expression was quantified via infrared tagged secondary antibodies on a LiCor infrared scanner. Expression was normalised to CDK4 expression. Data is the average of three independent experiments, error bars are SEM.



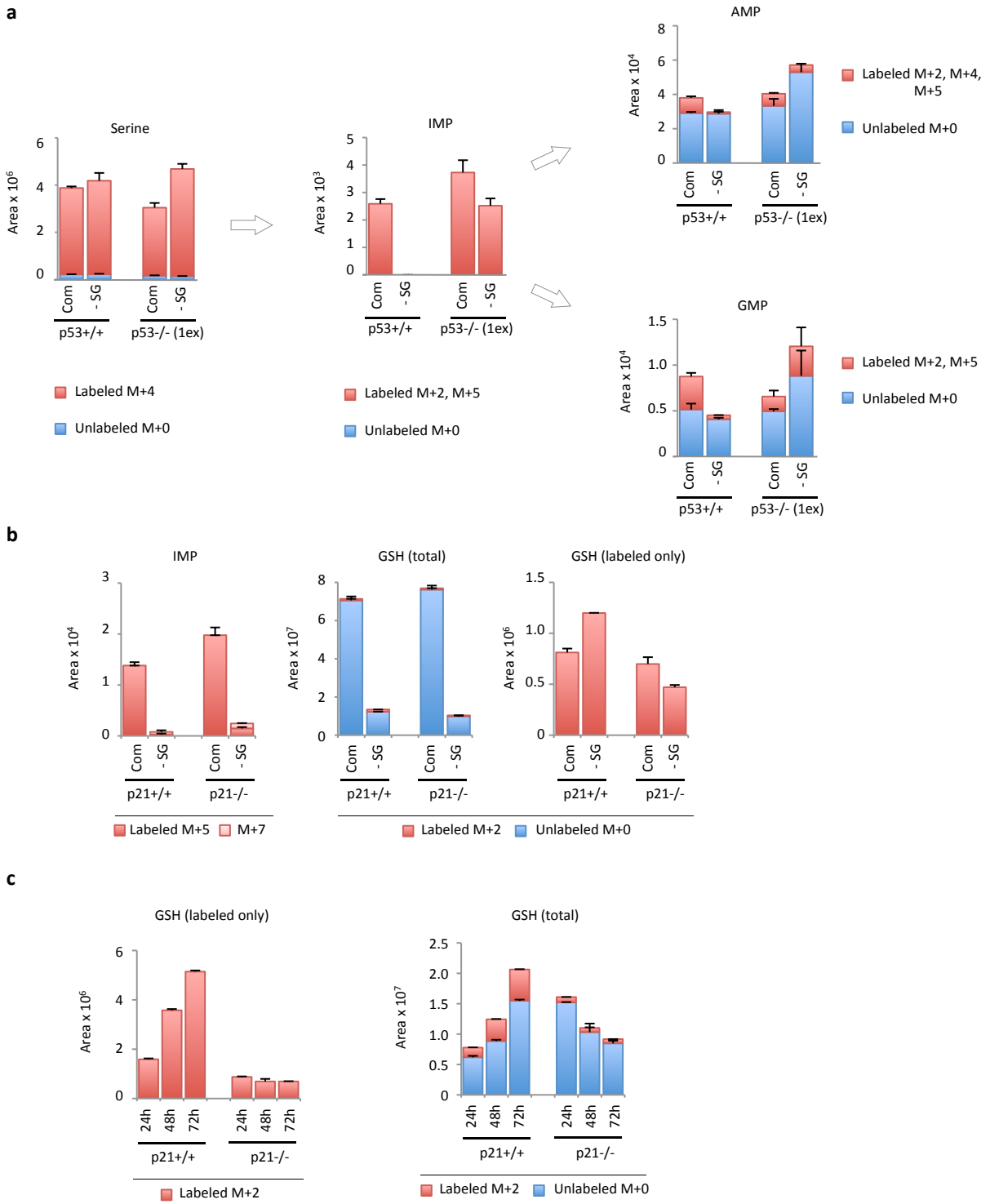

**Supplementary Figure 5. mTORC1 activity is modulated in a p53-independent manner during serine starvation.** HCT116 cells were grown at low density in complete (Com) or serine and glycine deficient (-SG) media. A western blot was probed for markers of mTORC1 activity – p70S6K and S6 are downstream of mTORC1 and are activated by phosphorylation when mTORC1 is active in response to growth promoting signals.



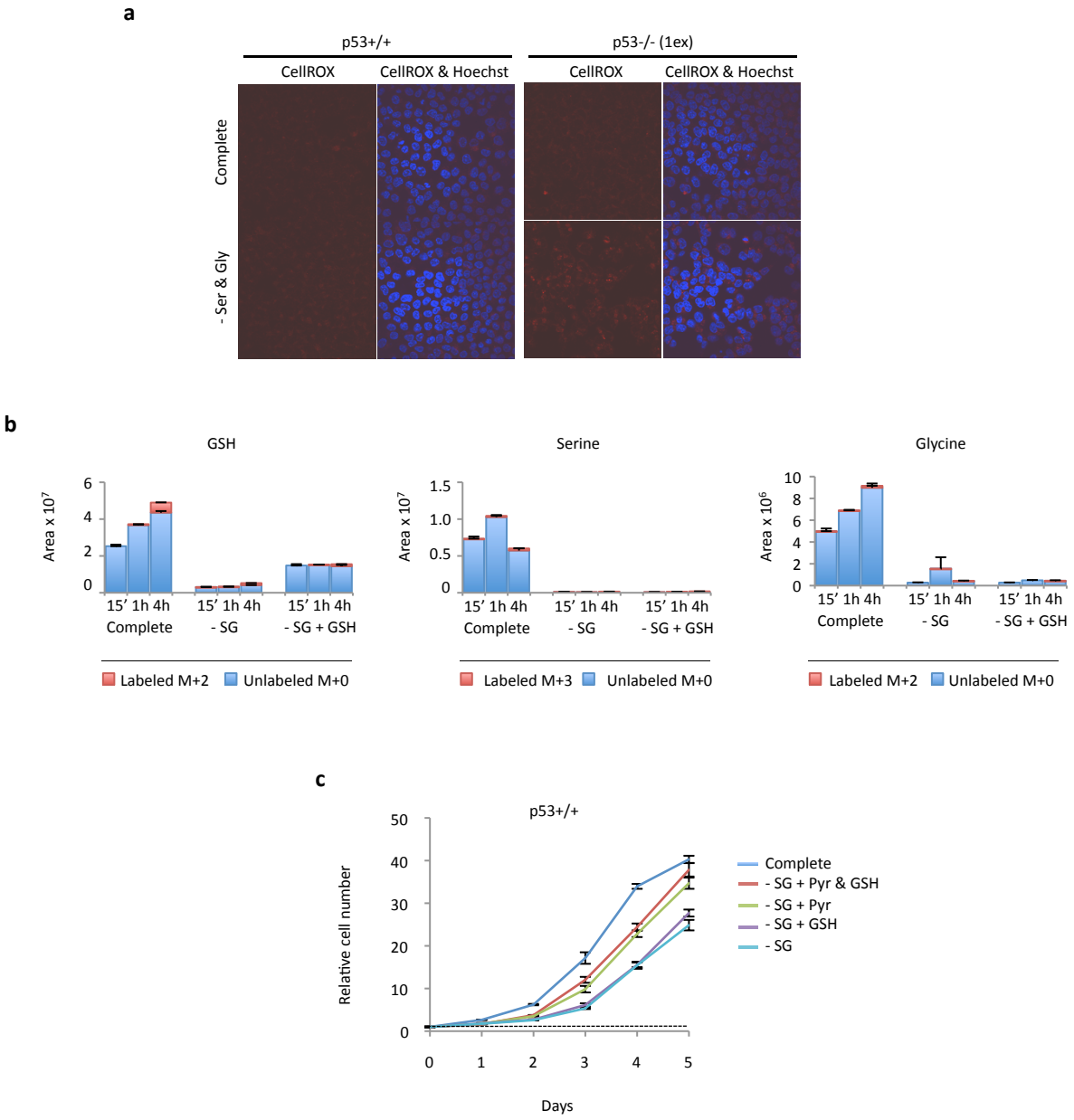

**Supplementary Figure 6. Pyruvate partially rescues to growth of p53-/- cells during serine and glycine starvation by augmenting TCA cycle flux.** HCT116 p53-/- (1ex) cells were grown in complete media or serine and glycine deficient media (-SG), or serine and glycine deficient media supplemented with 5mM pyruvate (-SG + Pyr). After 24h, media was replaced with equivalent media containing U-$^{13}$C-labeled D-Glucose for the indicated times. The relative quantities of intracellular metabolites were analysed by LC-MS. Data is average of triplicate wells, error bars are SEM.




**Supplementary Figure 7. Inhibition of GMP synthesis replicates the p53-p21 response induced by serine starvation. a**, HCT116 cells were treated with mycophenolic acid (MPA) for 24 hours. A western blot was probed for p53 and p21 expression. **b**, Protein expression was quantified via infrared tagged secondary antibodies on a LiCor infrared scanner and normalised to CDK4 expression. **c**, HCT116 cells were grown in complete media or serine and glycine deficient media (-Ser & Gly). A western blot was probed for AMPK activity.




**Supplementary Figure 8. p53+/+ cells inhibit flux of serine to purine synthesis after 24h serine and glycine starvation**. **a**, HCT116 cells were either fed complete media or media deficient in serine and glycine (-SG) for 24h, followed by complete media with U-$^{13}$C,$^{15}$N-labelled L-Serine for 3h. **b**, HCT116 cells were fed complete media, or serine and glycine deficient media for 24h, followed by U-$^{13}$C-Glucose for 1 hour. **c**, HCT116 cells were fed complete media, or serine and glycine deficient media for 24 – 72 hours, followed by U-$^{13}$-C-Glucose for 3 hours. LC-MS was used to detect relative quantities of intracellular metabolites. HCT116 cells (wild-type/parental line) are denoted as p53+/+ and p21+/+ in this figure. Data are averages of triplicate wells, all error bars are SEM.




**Supplementary Figure 9. Serine and glycine starvation elevates ROS in p53-/- cells. a,** HCT116 cells were either fed complete media or media lacking serine and glycine (-Ser & Gly) for 48h. An oxidation-activated fluorescent dye (red) and Hoechst nuclear counter-stain (blue) were added to the media for 30min prior to imaging. **b**, HCT116 p53-/- (1ex) cells were either fed complete media, or media lacking serine and glycine (-SG), or media lacking serine and glycine supplemented with 5mM glutathione (-SG +GSH) for 24h, after which equivalent media containing U-$^{13}$C-labeled D-Glucose was added for the stated times. The relative quantities of intracellular metabolites were analysed by LC-MS. **c**, HCT116 cells were fed complete media, or media deficient in serine and glycine supplemented with pyruvate 5mM and / or GSH 5mM. Data are averages of triplicate wells, all error bars are SEM.