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Supplementary Figure Legends

Figure S1. Re-expression of Rb-1 in Rb-1$^{-/-}$ or Rb TKO Cells Decreases ASCT2 Expression and Cell Proliferation. a. Contrast and fluorescent microscopy of Rb$^{-/-}$ and Rb TKO cells 48 hours post-transfection. Re-constitution of Rb-1 is confirmed by GFP labeling stemming from expression of a GFP-Rb-1 fusion protein in both cell types. **b.** Western blot analysis of ASCT2 expression in Rb-1$^{-/-}$ or Rb TKO cells 48-hours post transfection of vector alone or plasmid encoding Rb-1. Shown are representative images from three independent cell lysate preparations. **c.** Viable cells were enumerated by trypan blue exclusion in Rb-1$^{-/-}$ or Rb TKO cells 72-hours post-transfection of vector alone or an Rb-1 encoding plasmid.

Figure S2. Genetic Deletion of the Rb-Family Increases the Incorporation of Exogenous Glutamine Into Glutathione. a. Rb WT and Rb TKO cells were labeled with ^{13}C, ^{15}N-glutamine for two cell doublings and the extracted metabolites were subjected to NMR analysis. Shown are representative 1D HSQC NMR spectra of Rb WT or Rb TKO cells. Spectral peaks are labeled for the alpha glutamate carbon, as well as the gamma carbon within glutamine, glutamate, and the glutamate carbon within GSH. **b.** Relative labeling of ^{13}C-GSHEγ from exogenous glutamine in Rb WT or Rb TKO cells. Data is represented as the ratio of the absolute integral of the metabolite as calculated from the 1D HSQC spectra to dry mass.

Figure S3. Suppression of ASCT2 or GLS1 Decreases Glutamine Uptake and Ammonia Secretion in both the WT and Rb TKO MEFs, but Selectively Decreases Cell Proliferation only in the Rb TKO Cells. Western blot analysis of protein expression of ASCT2 (**a**: WT; **h**: TKO) or GLS1 (**d**: WT; **k**: TKO) after siRNA silencing for 48 hours. Shown are representative images from two independent cell lysate preparations. The ratio of expression was determined by densitometry analysis with WT expression set to 1. Viable cell number was determined by trypan blue staining in WT or Rb TKO cells 72 hours after ASCT2 (**b**: WT; **i**: TKO) or GLS1 (**e**: WT; **l**: TKO) siRNA transfection. Data are represented as cell number (mean ± s.d.). *$p < 0.05$.* Glutamine uptake was measured by ^{14}C-glutamine labeling in WT or Rb TKO cells 48-hours after ASCT2 (**c**: WT; **j**: TKO) or GLS1 (**f**: WT; **m**: TKO) siRNA transfection. Data are represented as % Cell Control (mean ± s.d.), which was set to 1. * *$p < 0.05$.* Ammonium levels from WT or Rb TKO MEF culture medium were measured 48-hours after GLS1 (**g**: WT; **n**: TKO) siRNA transfection. Data are represented as % Cell Control (mean ± s.d.), which was set to 1. * *$p < 0.05$.*

Real-time PCR ChIP Primer Sets:

ASCT2:	Forward: 5'- GGC TGA TCT TGA ACT CAC AGA GAT -3'
	Reverse: 5'- TCA CCA CTG GTC AAA AGA ATC TAA -3'
cdc2:	Forward: 5'- GGT AAA GCT CCC GGG ATC CGC CAA T -3'
	Reverse: 5'- GTG GAC TGT CAC TTT GGT GGC TGG C -3'
GAPDH:	Forward: 5'- AGT GCC AGC CTC GTC CCG TAG ACA AAA TG -3'
	Reverse: 5'- AAG TGG GCC CCG GCC TTC TCC AT -3'