uL3 Regulates Redox Metabolism and Ferroptosis Sensitivity of p53-Deleted Colorectal Cancer Cells

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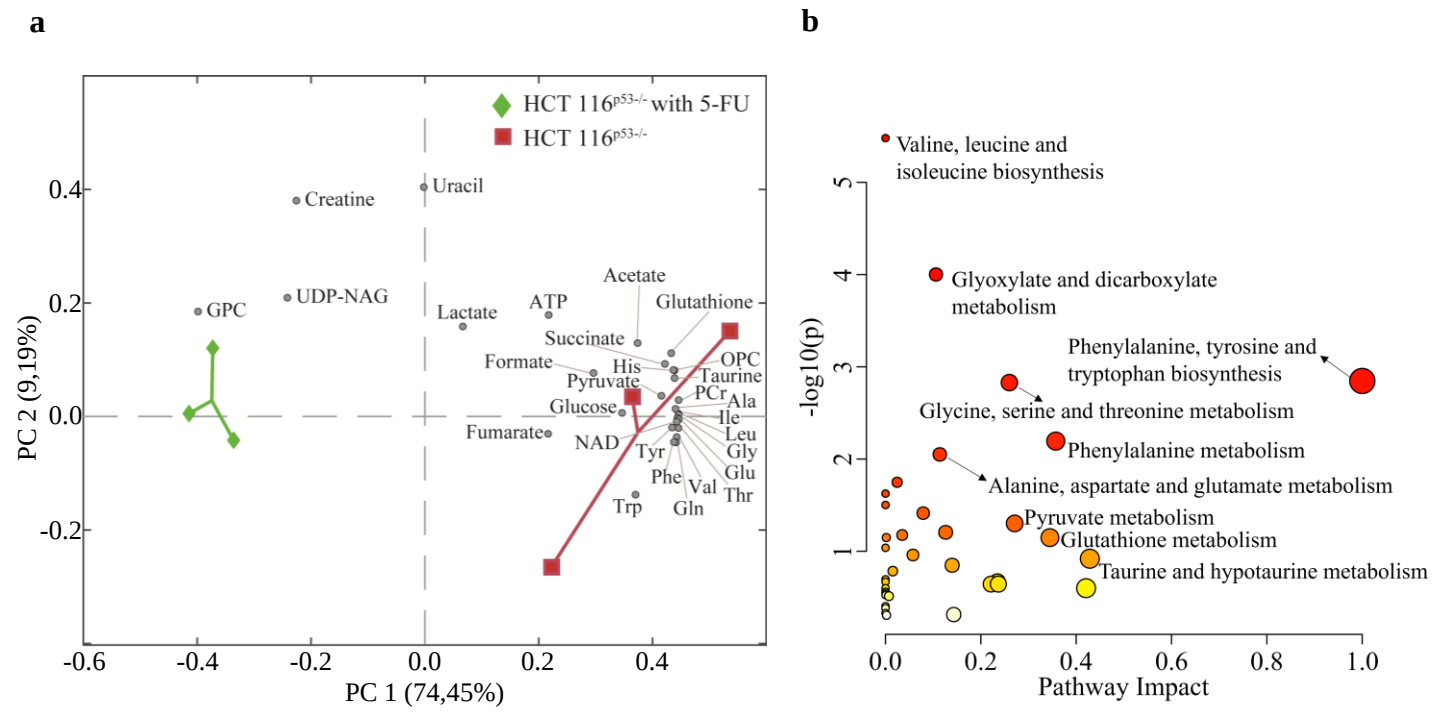

Figure S1. Effect of 5-FU treatment on metabolite profiles of HCT 116$^{p53-/-}$ cells. (**a**) Biplot (scores plot combined with loadings plot) of PCA model performed on the NMR-based metabolomic analyses of untreated HCT 116$^{p53-/-}$ cells vs. 5-FU-treated HCT 116$^{p53-/-}$ cells. (**b**) Relative pathway analyses (NAD, Nicotinamide adenine dinucleotide; CrP, Creatine Phosphate; UDP-NAG, Uridine diphosphate N-acetylglucosamine; OPC, O-phosphocholine; GPC, Glycerophosphocholine).

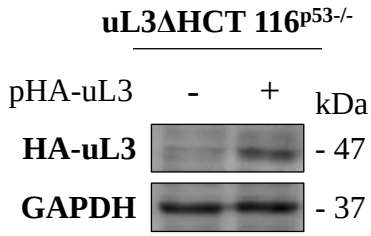

Figure S2. HA-uL3 expression levels in uL3ΔHCT 116$^{p53-/-}$ cells transfected with pHA-uL3 by WB. Cells were transiently transfected with pHA-uL3. 24 h later, protein extracts from the samples were analyzed by WB with antibodies against HA and GAPDH as loading control. Full-length blots are presented in Figure S6.

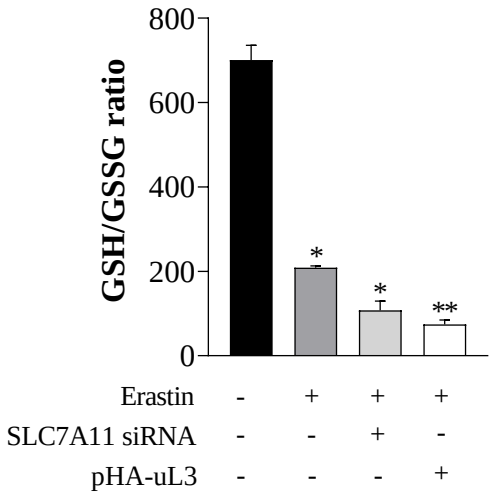

Figure S3. Evaluation of GSH/GSSG ratio. uL3ΔHCT 116$^{p53-/-}$ cells were transfected or not with SLC7A11 siRNA or pHA-uL3 and then, treated with erastin (10 μM). 24 h later, GSH was measured and GSH/GSSG ratio was calculated accordingly. *$p < 0.05$, **$p < 0.01$ vs. control cells.

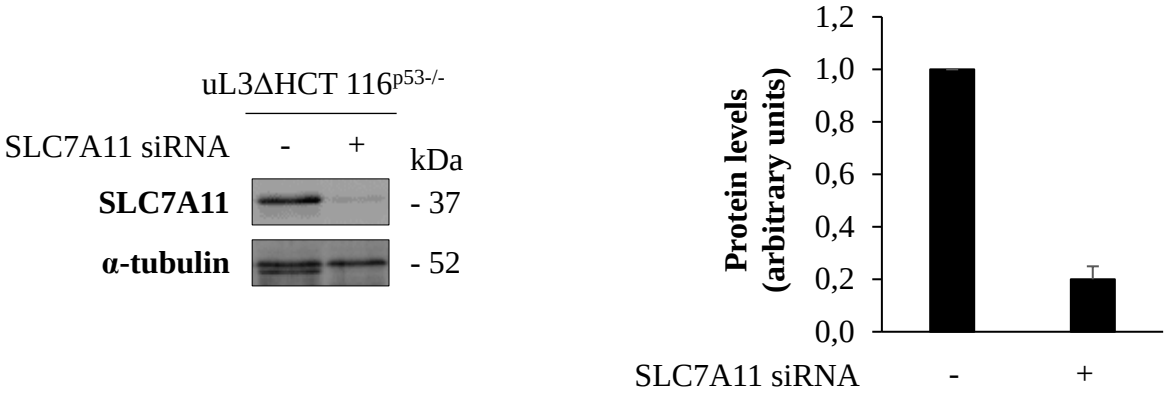

Figure S4. SLC7A11 expression levels in uL3ΔHCT 116$^{p53-/-}$ cells transfected with SLC7A11 siRNA. uL3ΔHCT 116$^{p53-/-}$ cells were transiently transfected SLC7A11 siRNA. 24 h later, protein extracts from amples were analyzed by WB with antibodies against SLC7A11 and α-tubulin as loading control. Full-length blots are presented in Figure S7.

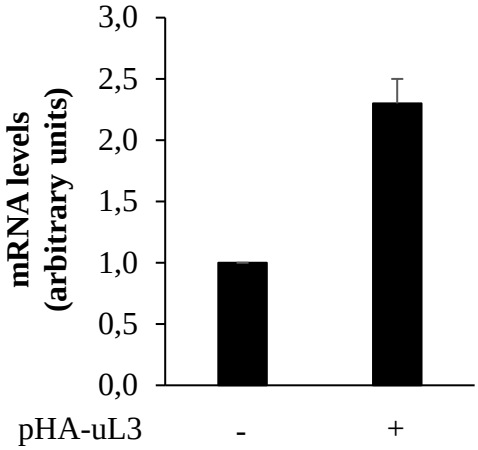

Figure S5. HA-uL3 expression levels in uL3ΔHCT 116$^{p53-/-}$ cells transfected with pHA-uL3 by RT-qPCR. Cells were transiently transfected with pHA-uL3. 12 h later, total RNA from samples were analyzed by RT-qPCR.

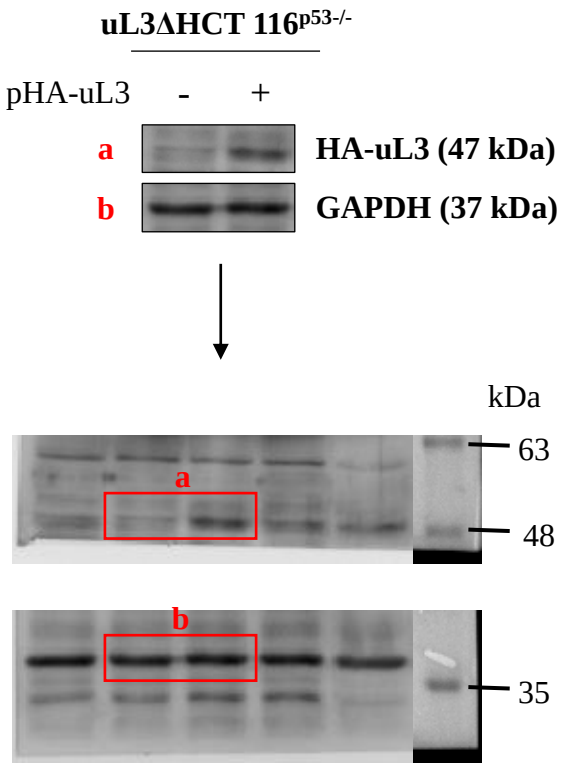

Figure S6. Full-length blots of Figure S2.

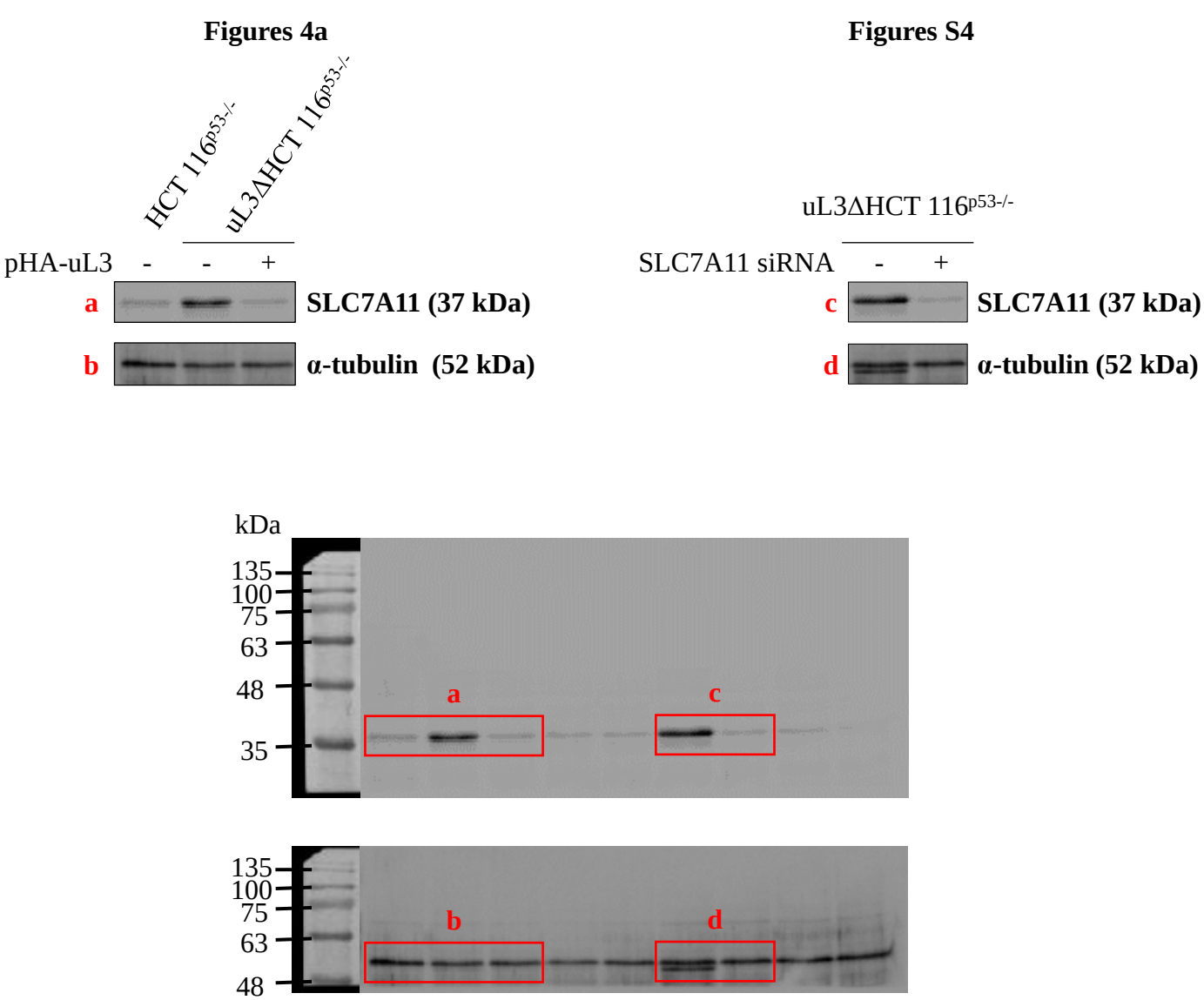

Figure S7. Full-length blots of Figures 4a and S4.

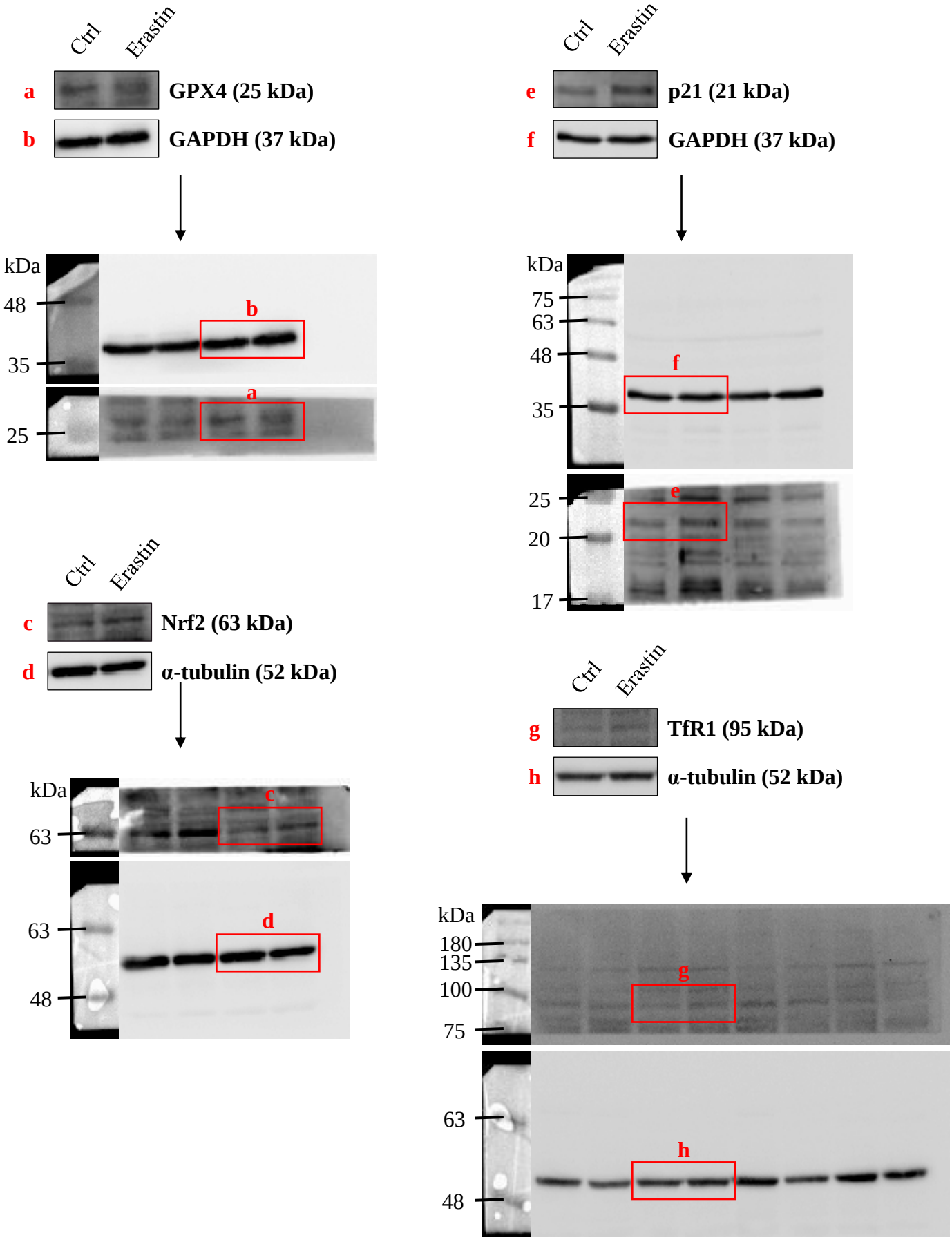

Figure S8. Full-length blots of Figure 6b.