Science Advances
AAAS

Supplementary Materials for

Glutaredoxin attenuates glutathione levels via deglutathionylation of Otub1 and subsequent destabilization of system x_C^-

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Sci. Adv. **9**, eadi5192 (2023)
DOI: 10.1126/sciadv.adi5192

This PDF file includes:

Figs. S1 to S5

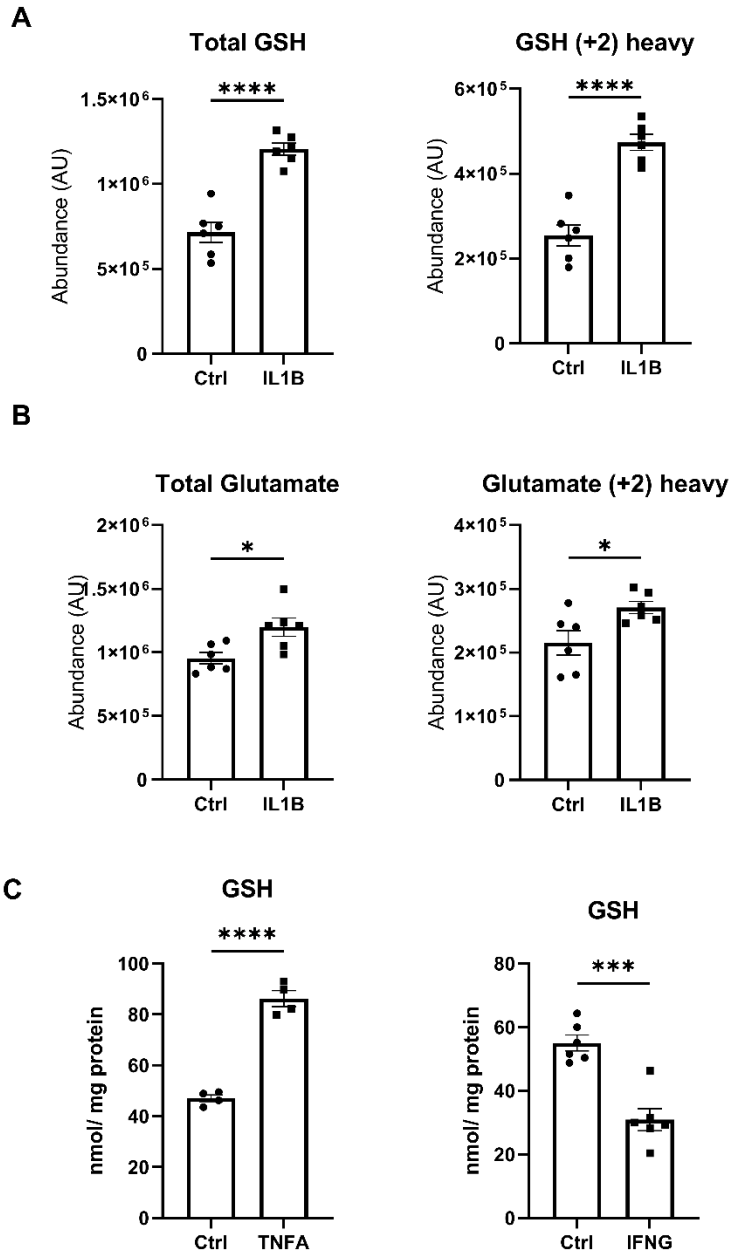

Fig. S1. GSH levels in MTE cells treated with IL1B, TNFA or IFNG

Levels of total and heavy fraction (labeled +2) of GSH (A) or total and heavy fraction of glutamate **(B)** in control and IL1B stimulated cells for 24 hours. *P value* * <0.05, **** <0.0001 **(C)** GSH measurement in MTE cells treated with TNFA (left) or IFNG (right) for 24 hours.

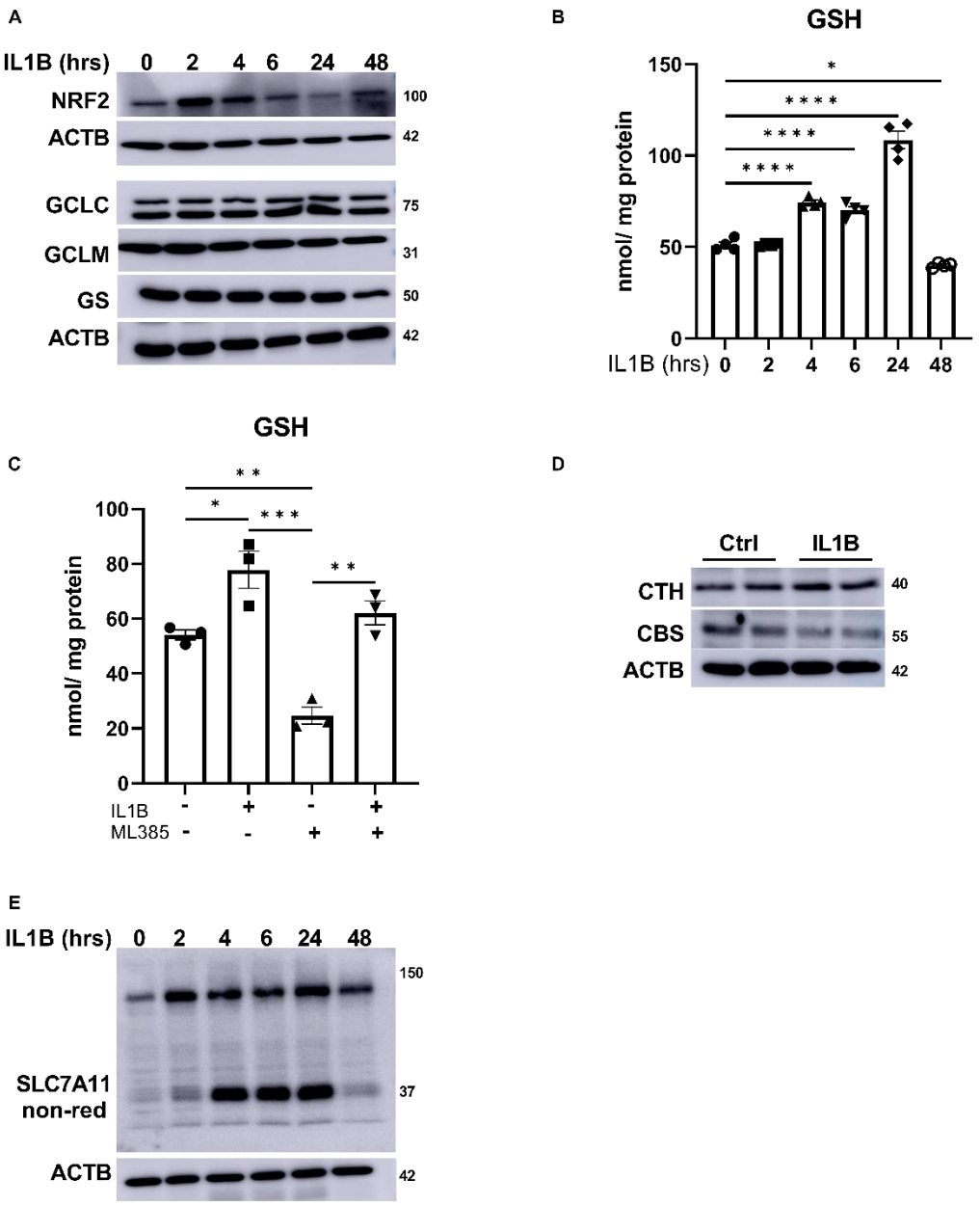

Fig. S2. Impact of IL1B treatment on NRF2, GSH and downstream protein targets

(A) Western blots showing protein levels of NRF2, GCLC, GCLM and GS in MTE cells treated with IL1B for 2, 4, 6, 24 or 48 hours. **(B)** GSH measurement following the same IL1B treatment time course. **(C)** GSH levels in MTE cells pretreated with 10µM ML385 for 1 hour, followed by IL1B for 24 hours. **(D)** Western blot analysis of CTH and CBS in response to 24 hours of IL1B stimulation. **(E)** Non-reducing blot of SLC7A11 following IL1B time course treatment in MTE cells. ACTB: loading control. Numbers on the right refer to the protein MW in kDa. *P value* * <0.05, ** <0.01, *** <0.001, **** <0.0001

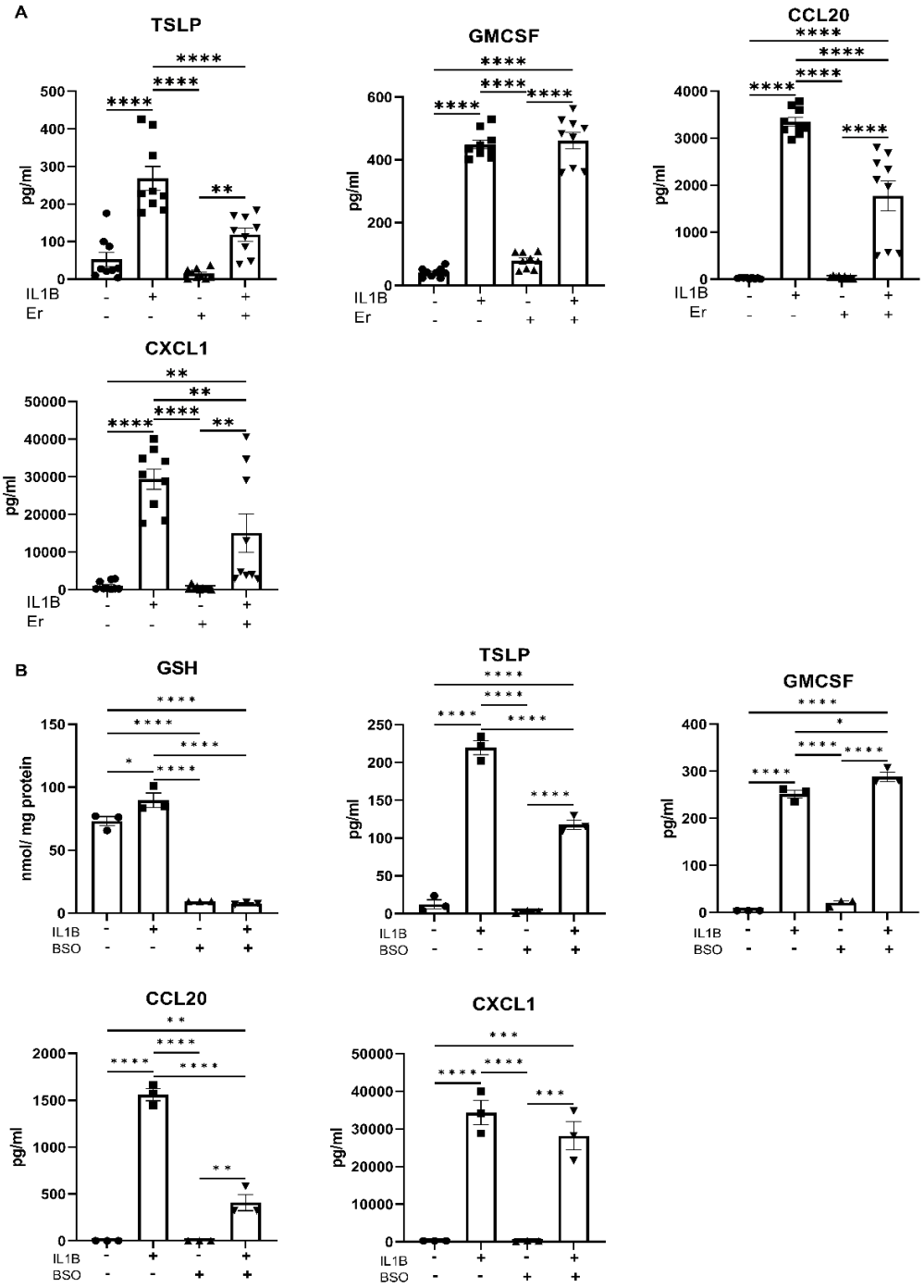

Fig. S3. Evaluation of IL1B- induced cytokines in MTE cells pretreated with erastin or BSO

(A) Evaluation of TSLP, GM-CSF, CCL20 and CXCL1 cytokine levels secreted in cell culture supernatant of MTE cells after 1 hour pretreatment with erastin (0.5µM) followed by 24 hours stimulation with IL1B. **(B)** GSH levels in response to pretreatment of MTE cells with 1mM BSO for 1 hour followed by 24 hours of IL1B (top left). TSLP, GM-CSF, CCL20 and CXCL1 cytokines analysis following the same treatment strategy. *P value* * <0.05, ** <0.01, *** <0.001, **** <0.0001

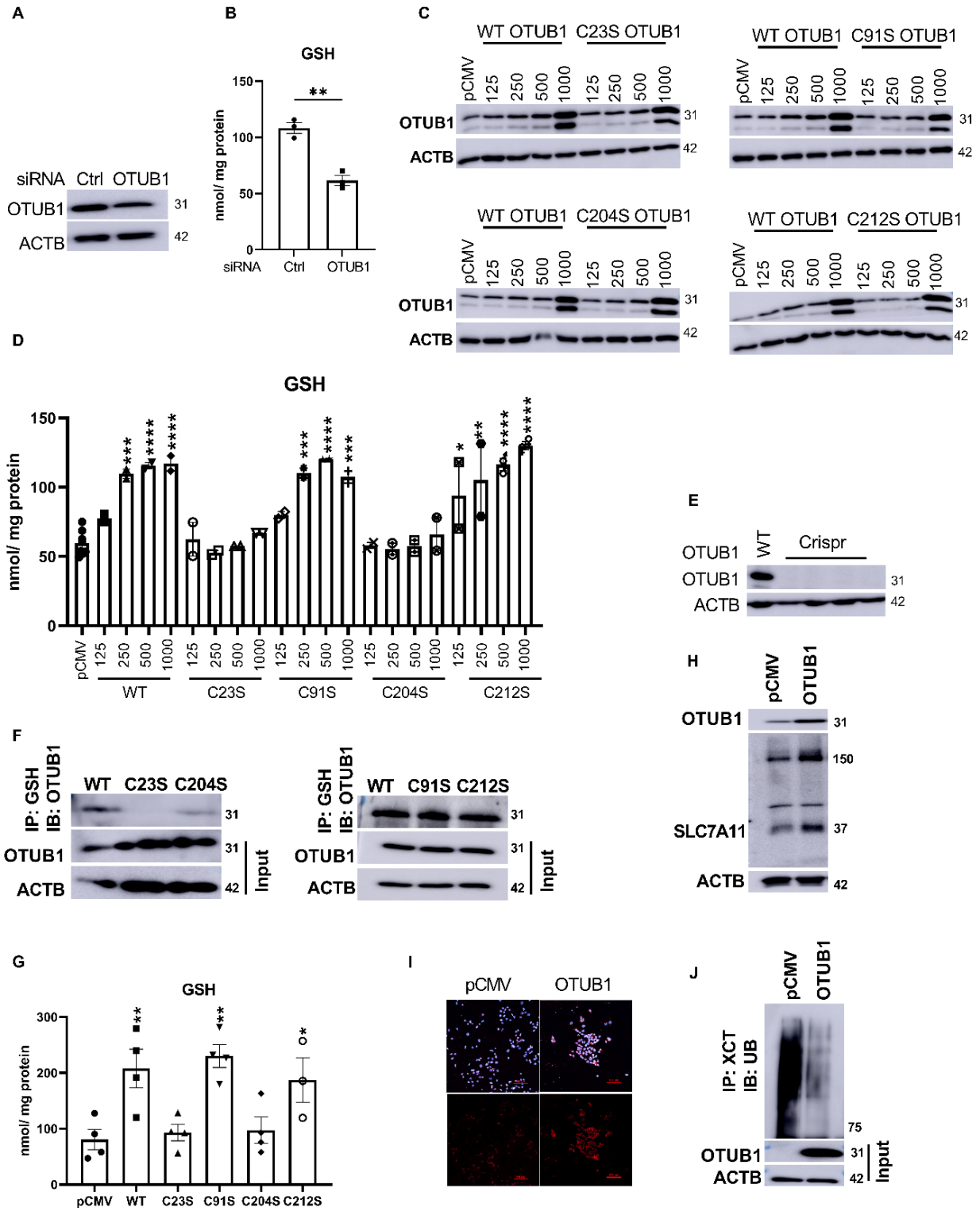

Fig. S4. Evaluating the effect of mutating C23, C91, C204 or C212 of OTUB1 on GSH

(A) Western blot analysis of OTUB1 in MTE cells transfected with control or OTUB1 siRNA. ACTB: loading control. **(B)** Measurement of total GSH levels in MTE cells following control or siRNA-mediated OTUB1 knockdown**. (C)** OTUB1 western blots of H522 cells overexpressing different concentrations (ng) of OTUB1-WT, OTUB1-C23S, OTUB1-C91S, OTUB1-C204S,

OTUB1-C212S or pCMV plasmids. ACTB was used as loading control **(D)** Assessment of GSH levels in H522 cells overexpressing OTUB1-WT, OTUB1-C23S, OTUB1-C91S, OTUB1-C204S and OTUB1-C212S plasmids or pCMV vector control at the indicated plasmid concentrations (ng). **(E)** Western blot analysis of OTUB1 in WT H522 cells or OTUB-1 CRIPSR-edited H522 cells **(F)** Assessment of OTUB1-SSG in OTUB1-CRISPR H522 cells overexpressing OTUB1-WT, OTUB1-C23S or OTUB1-C204S plasmids (left) and OTUB1-WT, OTUB1-S91S or OTUB1-C212S (right). Western blots for OTUB1 and ACTB from whole cell lysates were used as input control. **(G)** GSH measurements in OTUB1-CRISPR H522 cells overexpressing OTUB1-WT, OTUB1-C23S, OTUB1-C91S, OTUB1-C204S or OTUB1-C212S plasmids. **(H)** Non-reducing SLC7A11 western blot following overexpression of OTUB1 in H522 cells. **(I)** Proximity ligation assay of HA and SLC3A2 in OTUB1-CRISPR cells overexpressing SLC7A11-HA and pCMV (left) or SLC7A11-HA and OTUB1 (right). Bottom panel show the PLA signal and top panels show the PLA signal and DAPI as a counter stain **(J)** SLC7A11 ubiquitination measured by immunoprecipitating SLC7A11 and blotting for ubiquitin in H522 cells overexpressing OTUB1 or pCMV empty vector and treated with 40µg of MG-132 for 4 hours. OTUB1 and ACTB from whole cell lysates were used as input. *P value* * <0.05, ** <0.01, *** <0.001, **** <0.0001

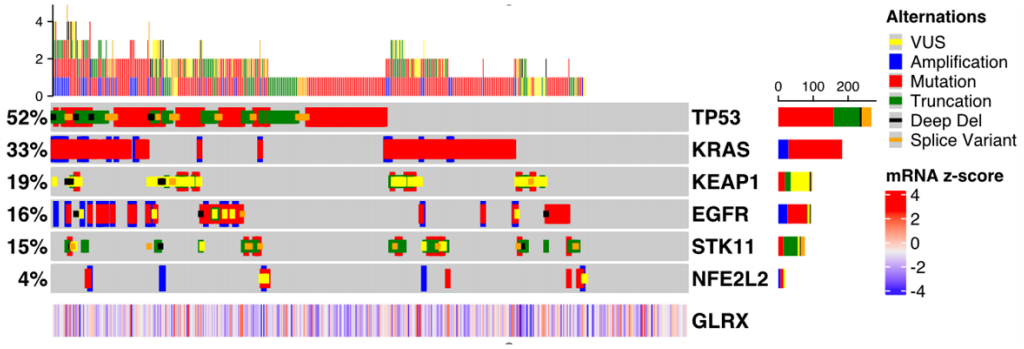

Fig. S5. OncoPrint visualization of *GLRX* expression in LUAD (n=503) as a function of oncogenic drivers.