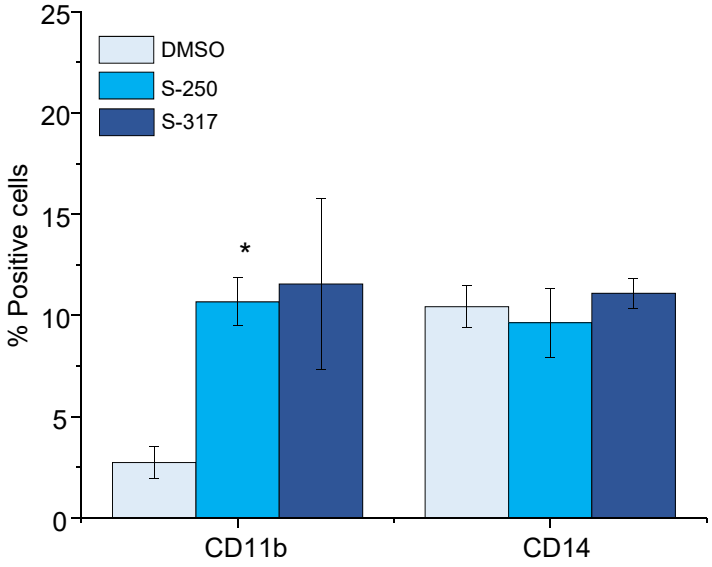
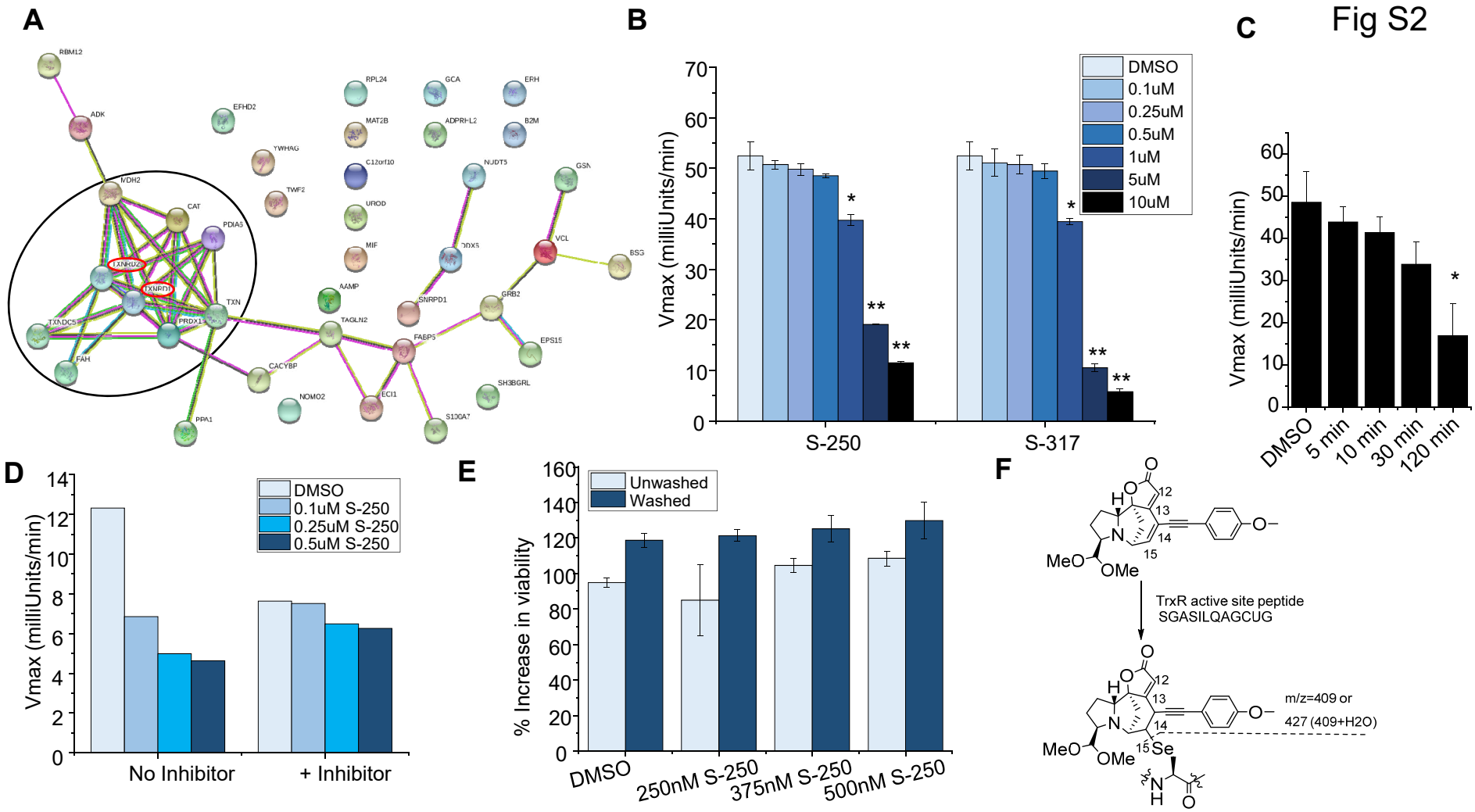
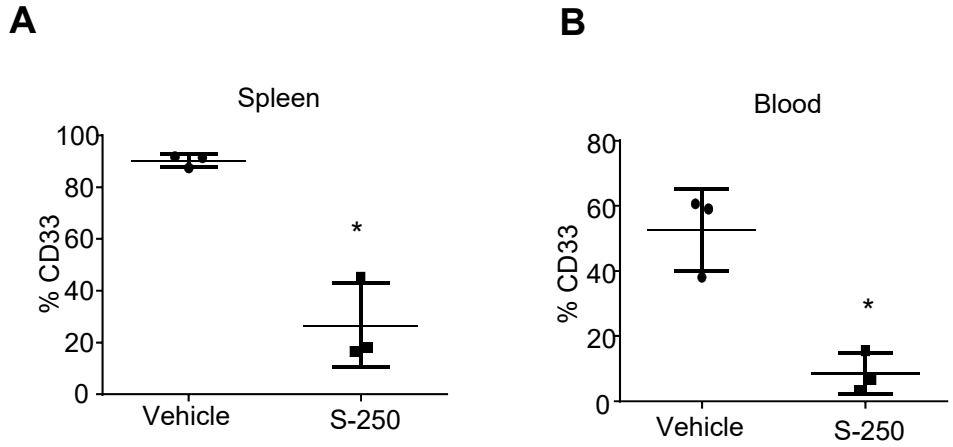
Fig S1



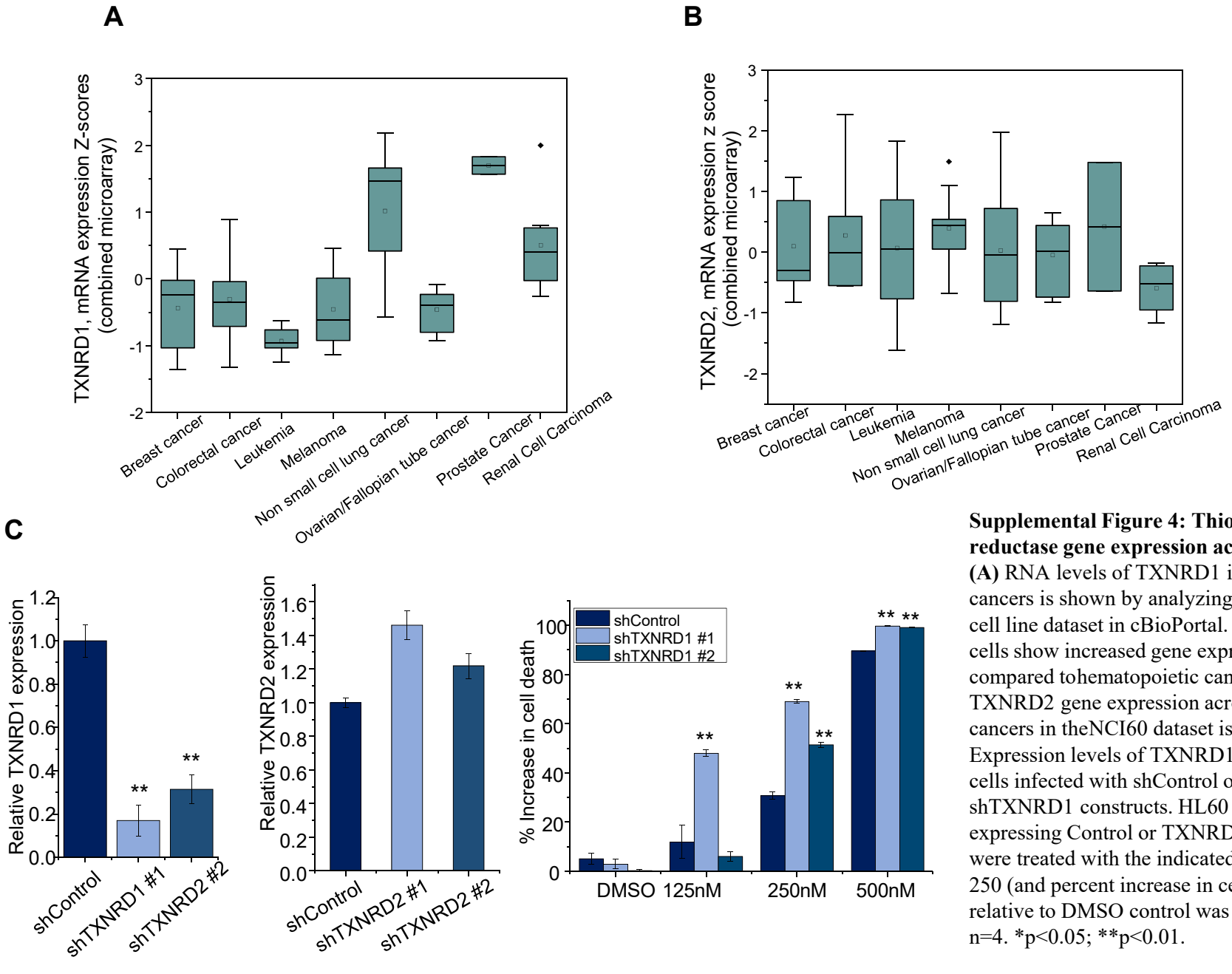
Supplemental Figure 1: Securinine derivatives impact AML differentiation. HL60 cells were treated with DMSO, 375nM S-250 or 750nM S-317 for 6 days. Cells were then stained with CD11b-FITC and CD14-PE and analyzed by flow cytometry. Percentage of positive cells for each antibody isplotted. The average of 3 independent experiments are shown.



Supplemental Figure 2: S-250 inhibits TrxR1 *in vitro*. (A) Interaction network of proteins identified by DARTS screen as potential targets of S-250 as obtained from STRINGanalysis. A cluster of related proteins is circled in black. TrxR1 and TrxR2 were extrapolated into the analysis (circled in red). Light blue and pink connectors are known interactions. Deep blue, red and green connectors are predicted interactions. Black and olive green connectors are interactions based on co-expression or text mining. **(B)** Pre- reduced rat TrxR1 (85nM) was incubated with the indicated doses of S-250 or S-317 for 2h. The DTNB reduction assay was performed and the maximum Velocity (Vmax) of TrxR1 in each condition is shown. n=3. **(C)** Timecourse of TrxR1 inhibition by S-250 (5mM). The assay was performed as described in B. (n=3). **(D)** HL60 cells were incubated with indicated doses of DMSO or S-250 for 2h. The DTNB reduction assay was used to assay TrxR1 activity in cell lysates with or without treatment with sodium authiomalate.
(E) HepG2 cells were treated with indicated doses of S-250 for 4h. Cells were either washed to remove the drug or seeded without washing and cell viability was measured using Prestoblue reagent. n=4. **(F)** Proposed mechanism for the Michael addition reaction of S-250 with TrxR active site peptide. *<0.05; **p<0.01.

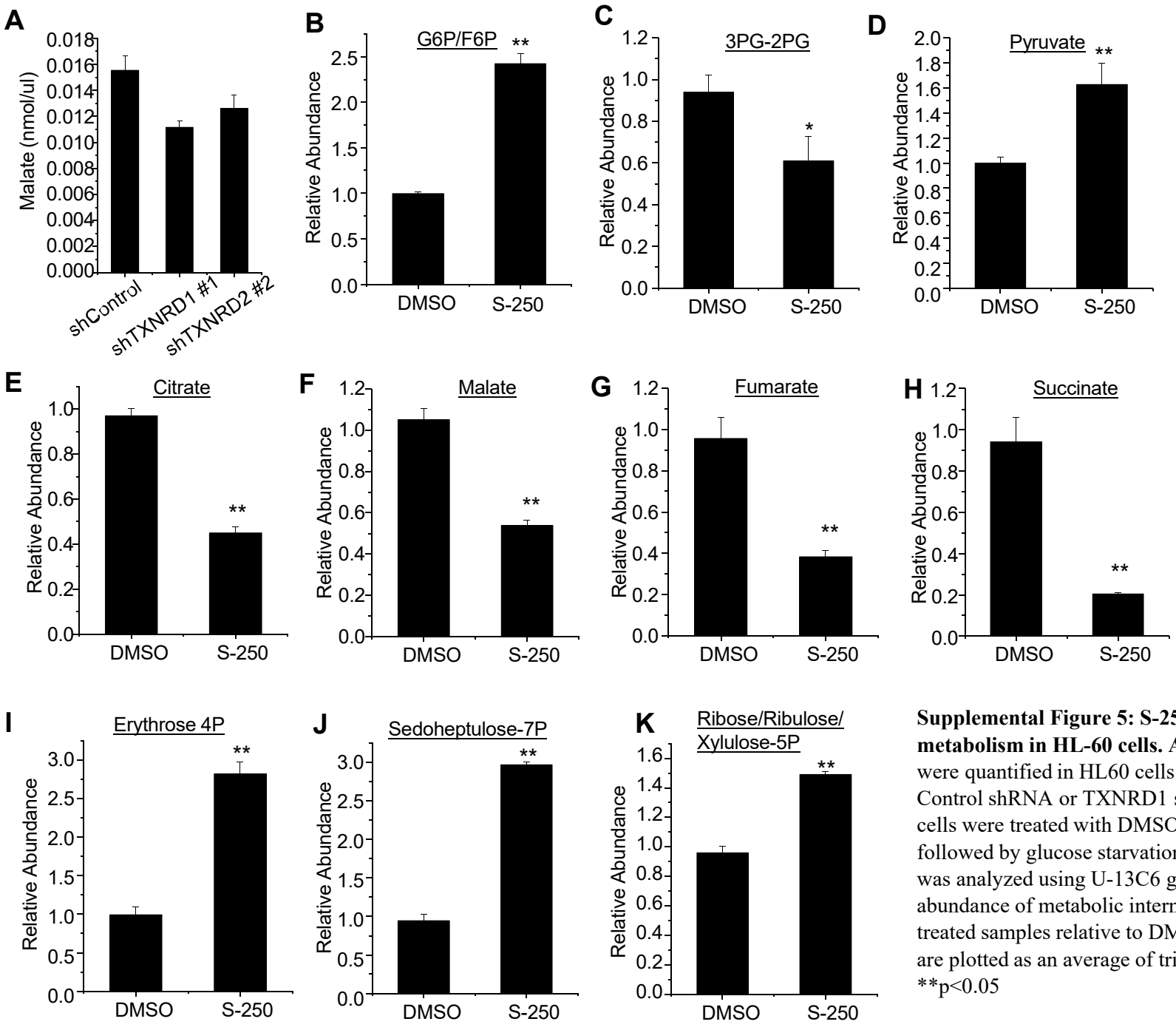


Supplemental Figure 3: Validation engrafted primary human cells represent AML cells. (A-B) Spleen cells (A) and blood cells (B) from NSG mice injected with patient derived leukemic cells described in figure 3 were assessed for CD33 expression by flow cytometry to confirm that the human CD45+ engrafted cells represent AML cells.



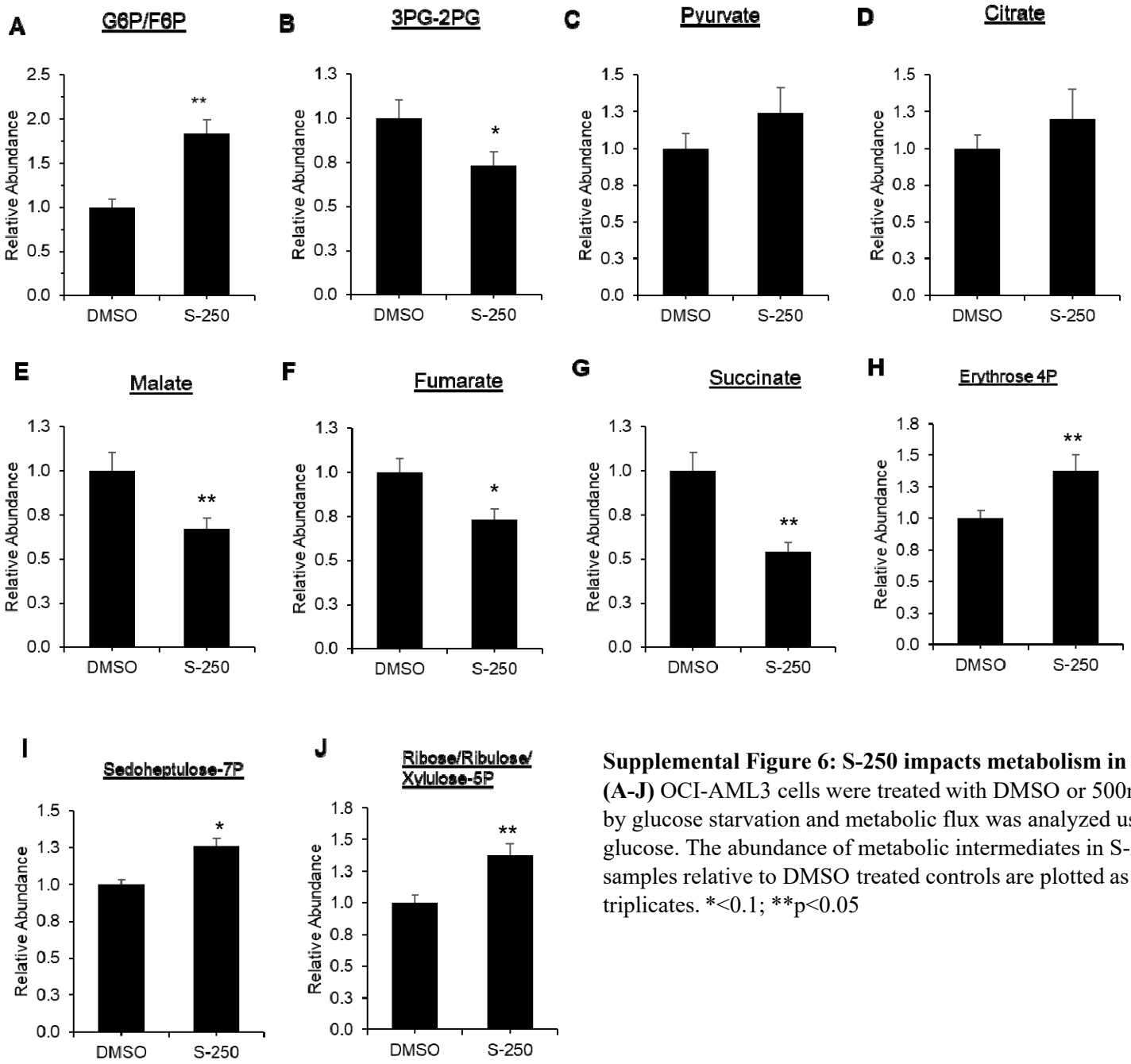
Supplemental Figure 4: Thioredoxin reductase gene expression across cancers **(A)** RNA levels of TXNRD1 in various cancers is shown by analyzing the NCI60 cell line dataset in cBioPortal. Solid cancer cells show increased gene expression as compared tohematopoietic cancer cells. **(B)** TXNRD2 gene expression across different cancers in theNCI60 dataset is shown. **(C)** Expression levels of TXNRD1/2 in HL60 cells infected with shControl or two shTXNRD1 constructs. HL60 cells stably expressing Control or TXNRD1 shRNAs were treated with the indicated doses of S-250 (and percent increase in cell death relative to DMSO control was assessed. n=4. *p<0.05; **p<0.01.

Fig S5



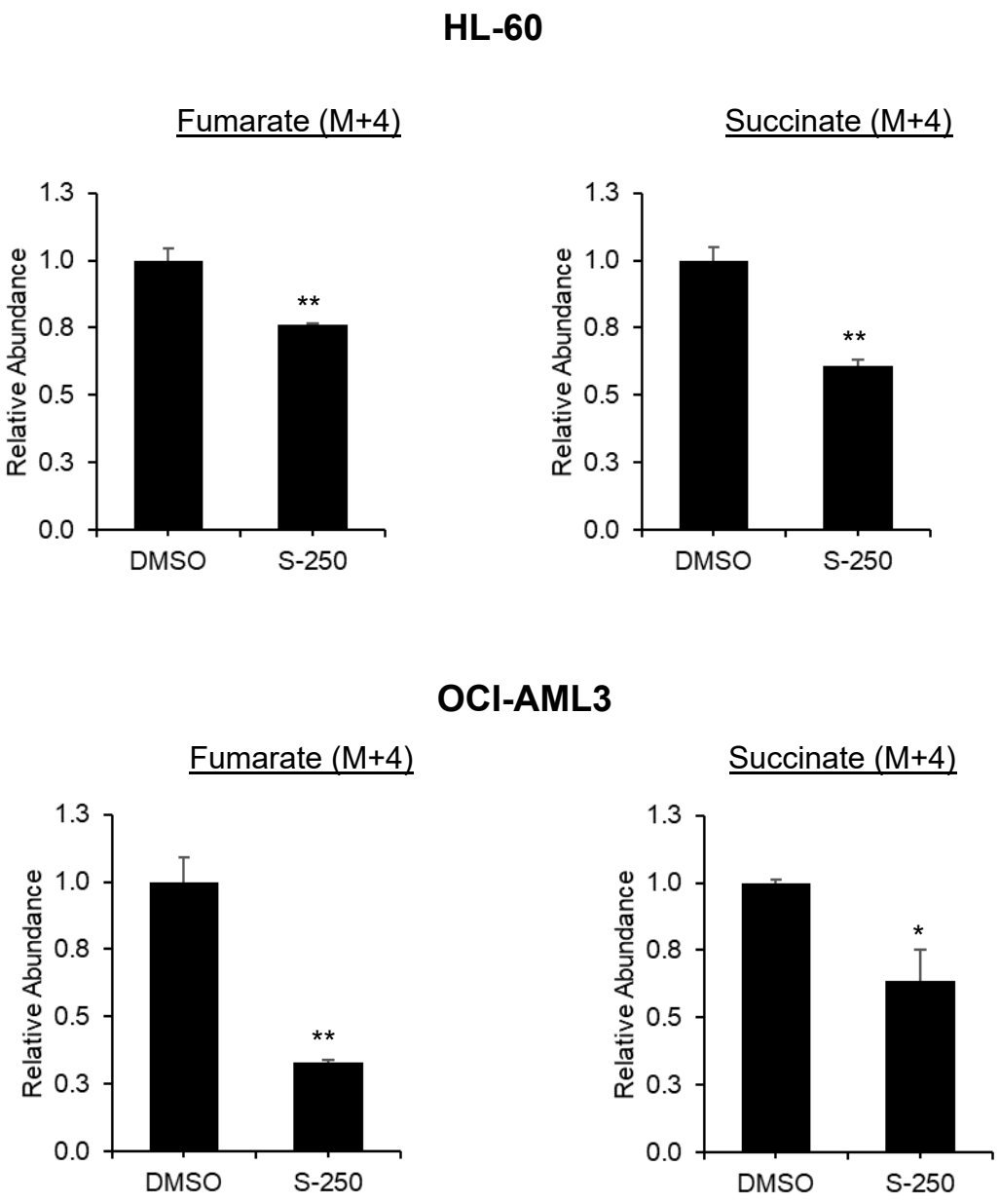
Supplemental Figure 5: S-250 impacts metabolism in HL-60 cells. A) Malate levels were quantified in HL60 cells stably expressing Control shRNA or TXNRD1 shRNA. **B-K)** HL60 cells were treated with DMSO or 500nM S-250 followed by glucose starvation and metabolic flux was analyzed using U-13C6 glucose. The abundance of metabolic intermediates in S-250 treated samples relative to DMSO treated controls are plotted as an average of triplicates. *<0.1; **p<0.05

Fig S6



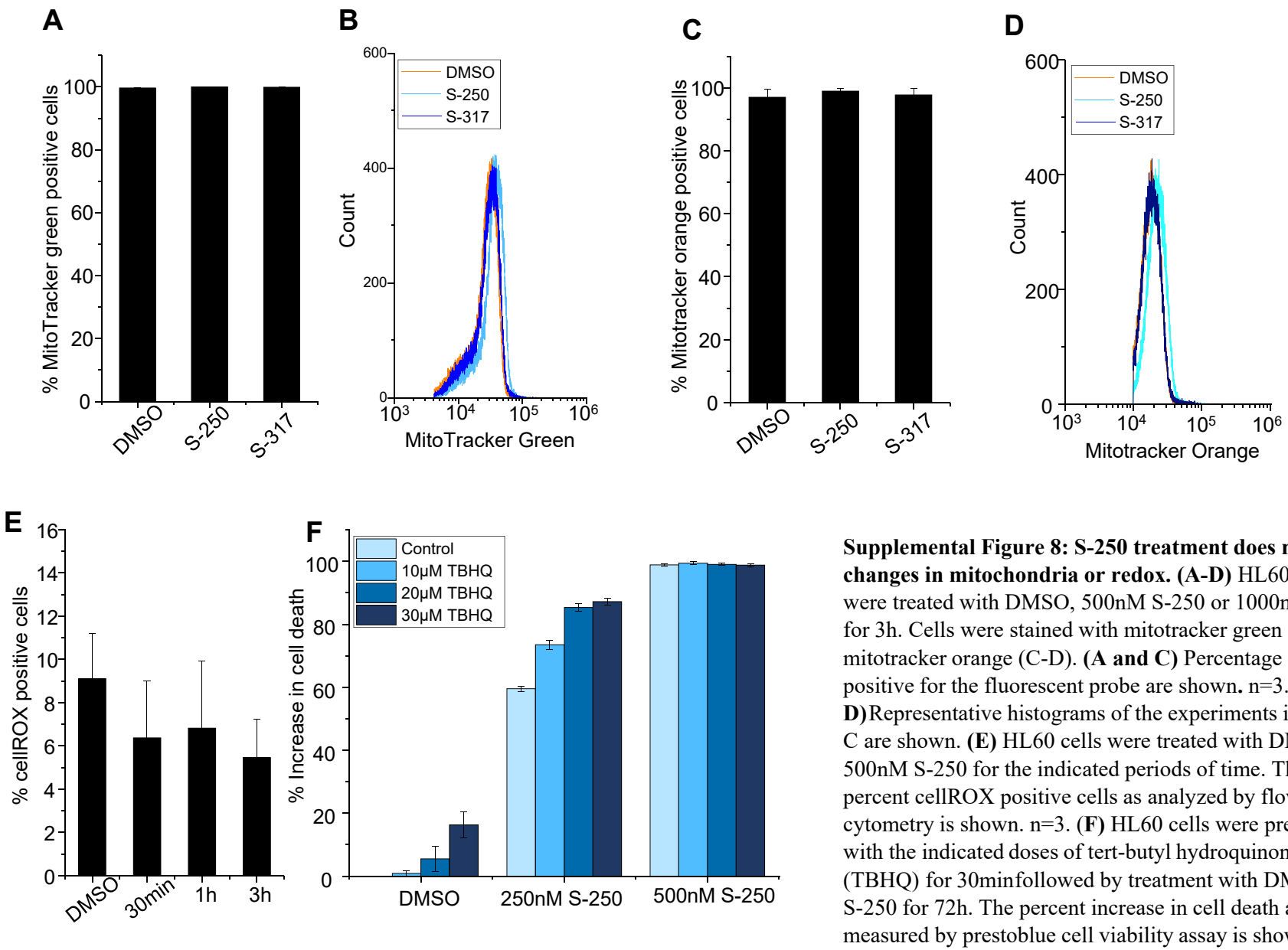
Supplemental Figure 6: S-250 impacts metabolism in OCI-AML3 cells. (A-J) OCI-AML3 cells were treated with DMSO or 500nM S-250 followed by glucose starvation and metabolic flux was analyzed using U-13C6 glucose. The abundance of metabolic intermediates in S-250 treated samples relative to DMSO treated controls are plotted as an average of triplicates. *<0.1; **p<0.05

Fig S7



Supplemental Figure 7: S-250 impacts glutamine metabolism in AML cells. (A-J) OCI-AML3 cells were treated with DMSO or 500nM S-250 followed by metabolic flux analyzed using L-$^{13}C_5$ glutamine. The abundance of select metabolic intermediates in S-250 treated samples relative to DMSO treated controls are plotted as an average of triplicates. *<0.1; **p<0.05

Fig S8



Supplemental Figure 8: S-250 treatment does not cause changes in mitochondria or redox. (A-D) HL60 cells were treated with DMSO, 500nM S-250 or 1000nM S-317 for 3h. Cells were stained with mitotracker green (A-B) or mitotracker orange (C-D). **(A and C)** Percentage of cells positive for the fluorescent probe are shown**.** n=3. **(B and D)**Representative histograms of the experiments in A and C are shown. **(E)** HL60 cells were treated with DMSO or 500nM S-250 for the indicated periods of time. The percent cellROX positive cells as analyzed by flow cytometry is shown. n=3. **(F)** HL60 cells were pre-treated with the indicated doses of tert-butyl hydroquinone (TBHQ) for 30minfollowed by treatment with DMSO or S-250 for 72h. The percent increase in cell death as measured by prestoblue cell viability assay is shown. n=4.