Figure S1

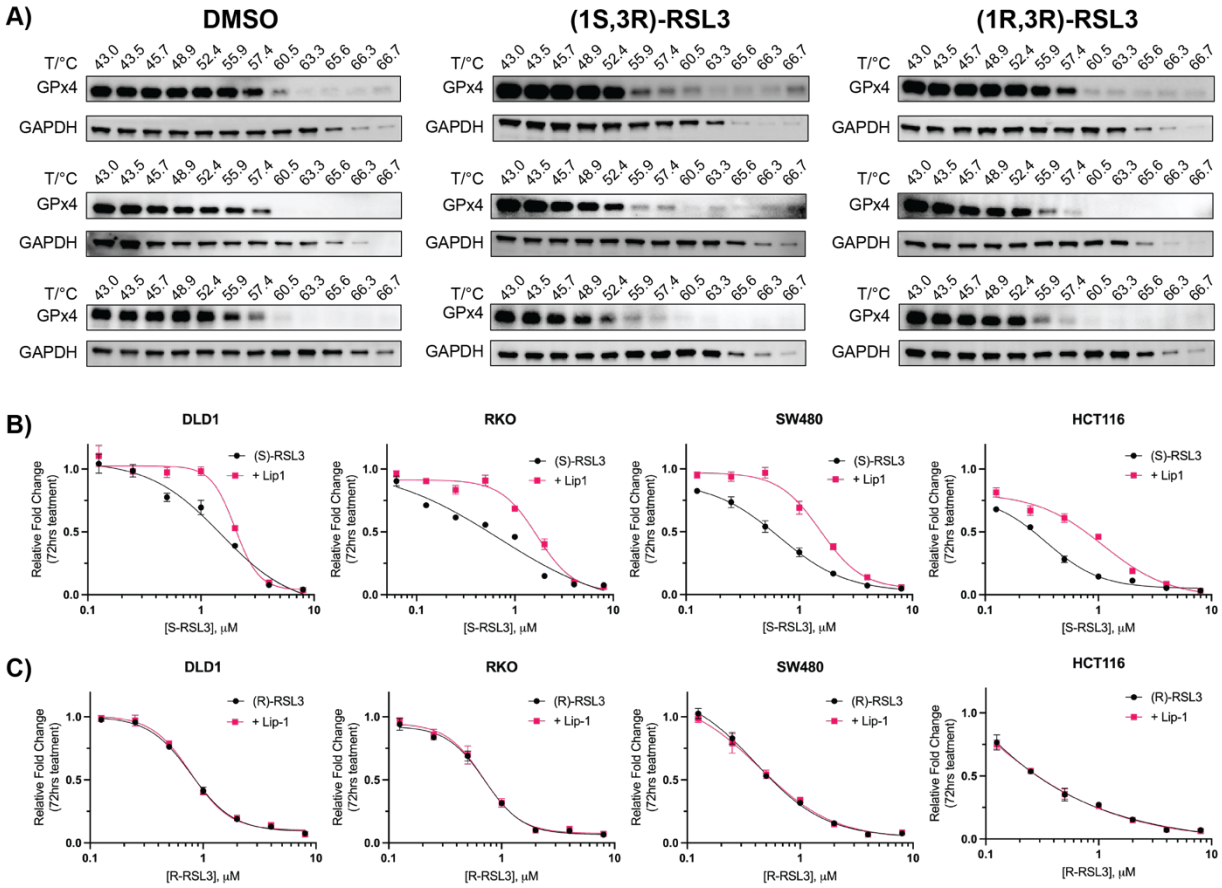


Figure S1

A) Western blots of GPx4 CETSA experiments used for generation of Figure 1C. 293T cells were treated with compound as indicated (10μM, 1hr) and lysates were heated to the indicated temperature before pelleting precipitated proteins **B and C)** Cell growth normalized to untreated control at 72 hr following (S) or (R) RSL3 dose response +/- Liproxstatin-1 (1μM) co-treatment in CRC cell lines.

Figure S2

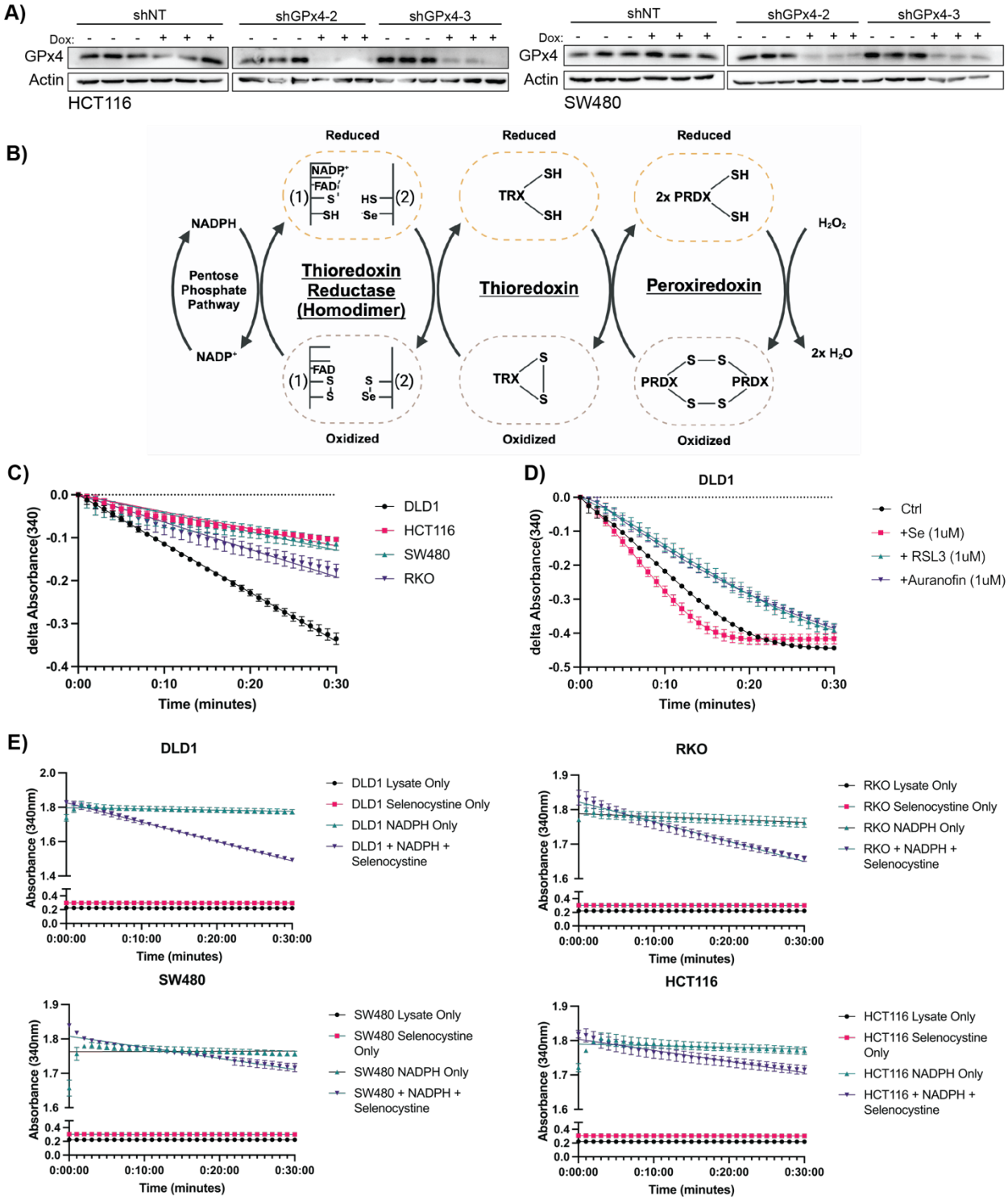


Figure S2

A) Western Blots of GPx4 levels in HCT116 and SW480 shNT, shGPx4-2, shGPx4-3 cell lines following 72hr treatment with doxycycline (250 ng/mL) **B)** Schematic of the mechanism of the Thioredoxin Reductase-Peroxiredoxin system, where NADPH from the Pentose Phosphate Pathway is utilized in the selenoprotein Thioredoxin Reductase to recycle oxidized 2-Cys Peroxiredoxins after the redox mediated detoxification of hydrogen peroxide, forming a Peroxiredoxin dimer. The peroxiredoxin dimer is reduced through oxidation of thioredoxin, and oxidized thioredoxin is reduced by TxnRD1 **C)** Thioredoxin Reductase Kinetic Activity Assay measuring NADPH consumption through reduction of NADPH absorbance at 340 nm upon addition of NADPH and Selenocystine to cell lysates of indicated CRC cell lines **D)** Modulation of the thioredoxin reductase activity assay after 24hr treatment of DLD1 cells as indicated. **E)** Internal controls utilized for the thioredoxin reductase kinetic activity assay. Absorbance at 340 nm was measured every 60 s for 30 min on a Cytation 5 plate reader. Wells contained either lysate only, lysate + selenocystine, lysate + NADPH, or lysate + selenocystine & NADPH as indicated. 3 technical replicates were averaged for each point and plotted as mean +/- SD

Figure S3

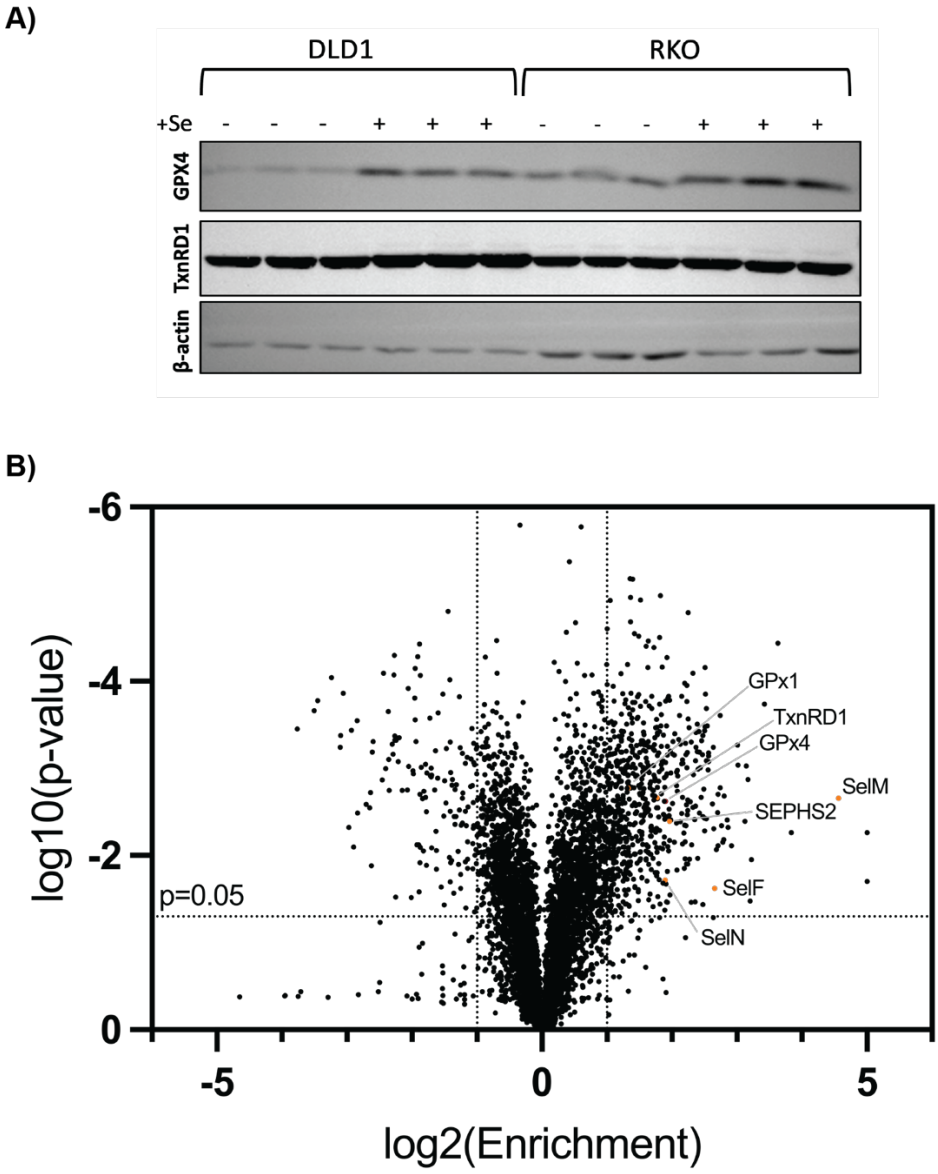


Figure S3

A) Western blot analysis of GPx4 and TxnRD1 protein levels in DLD1 and RKO cell lines following 24 hr supplemention with 1 μM Sodium Selenite. **B)** AP-MS results of Biotin-Cpd24 pulldown compared to Cpd24 mock pulldown control. Significant selenoproteins hits are identified

Figure S4

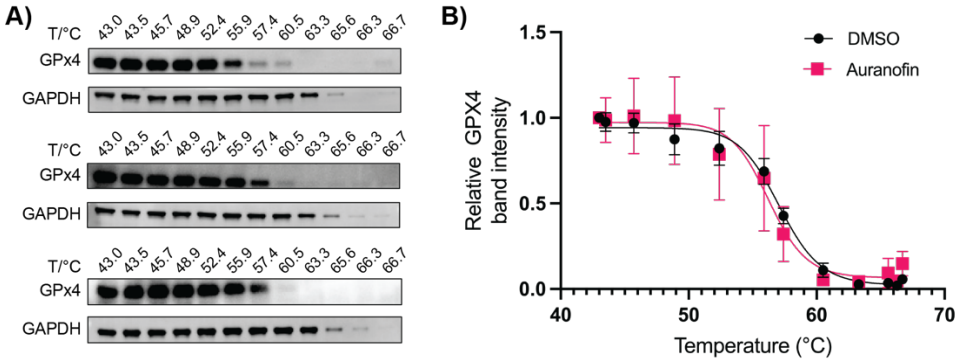


Figure S4

A) CETSA analysis of GPx4 protein thermal stability following auranofin treatment (10 μM, 1 hr) in 293T cells. DMSO controls are included as Figure S1A. **B)** Results of CETSA analysis from A calculating deviation of GPx4 band intensity between DMSO treated and auranofin treated cells (DMSO controls shown as Fig S1A)

Figure S5

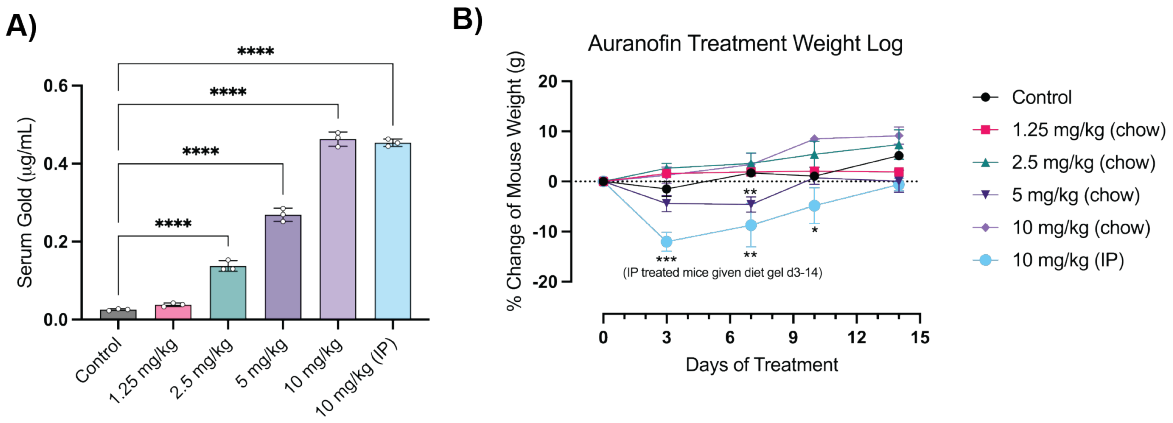
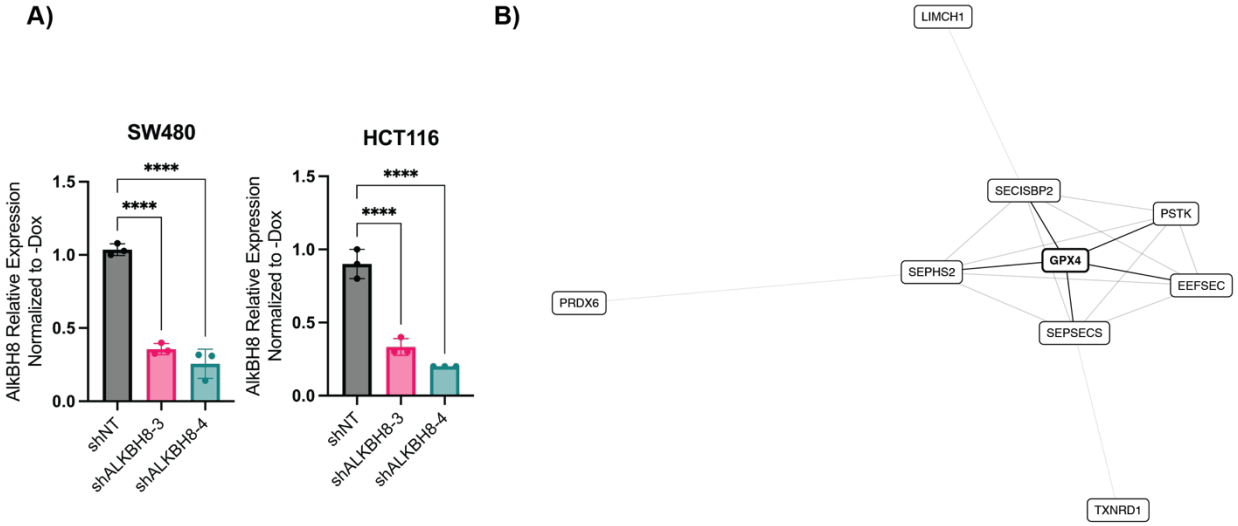
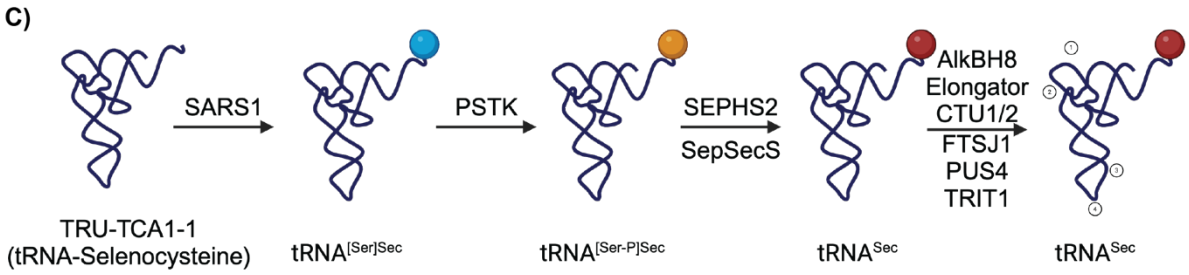


Figure S5

A) Terminal serum gold measurements by ICPMS of non tumor bearing control mice (C57Bl/6J) treated with indicated dosing of auranofin via chow or IP as indicated for two weeks. **B)** Weight log of non tumor bearing control mice treated with auranofin via chow or IP as indicated. IP treated mice were administered supplemental diet gel beginning d3 following rapid initial weight loss

Figure S6





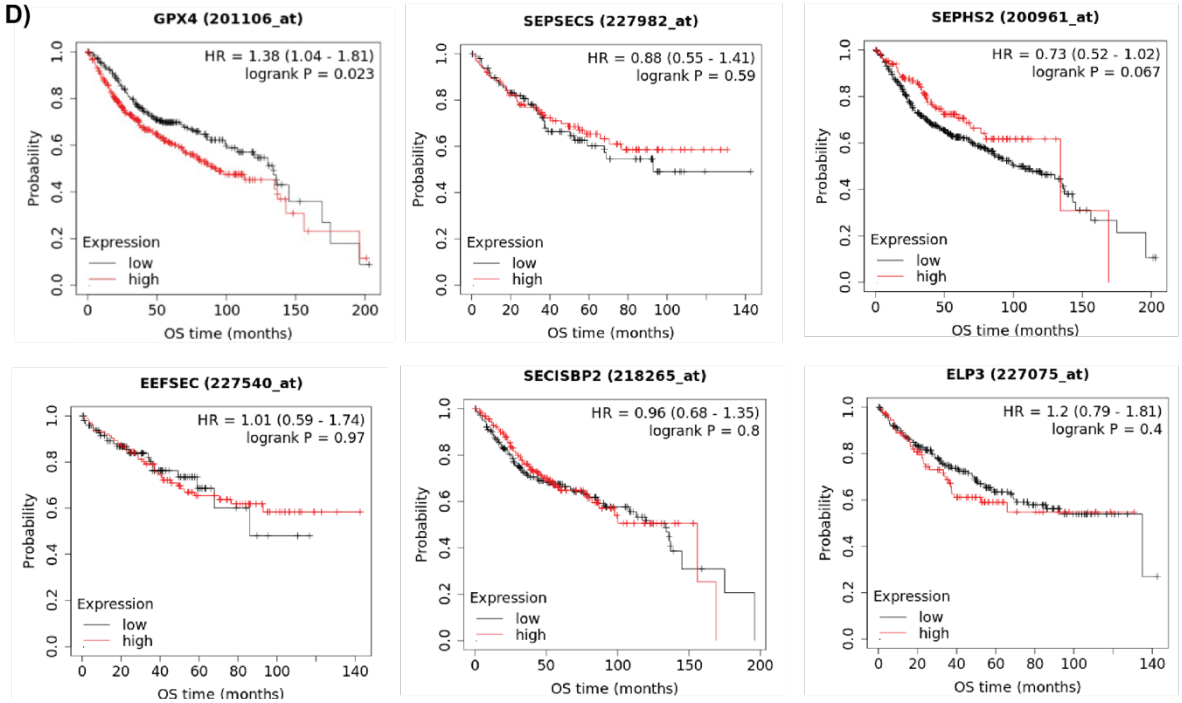


Figure S6

A) qPCR analysis of shNT or shAlkBH8 HCT116/SW480 cell lines measuring AlkBH8 mRNA expression following 72 hr dox treatment normalized to -dox control. **B)** CRISPR co-essentiality network map of GPx4 co-dependencies with distances indicating degree of co-essentiality. **C)** Biosynthetic pathway of tRNA-Selenocysteine where following transcription of the TRU-TCA1-1 gene, the tRNA-Sec mRNA undergoes on-tRNA biosynthesis of selenocysteine and post-transcriptional modification. **D)** Km plots of select genes in the tRNA-sec pathway with sufficient patient samples to perform a high-powered analysis