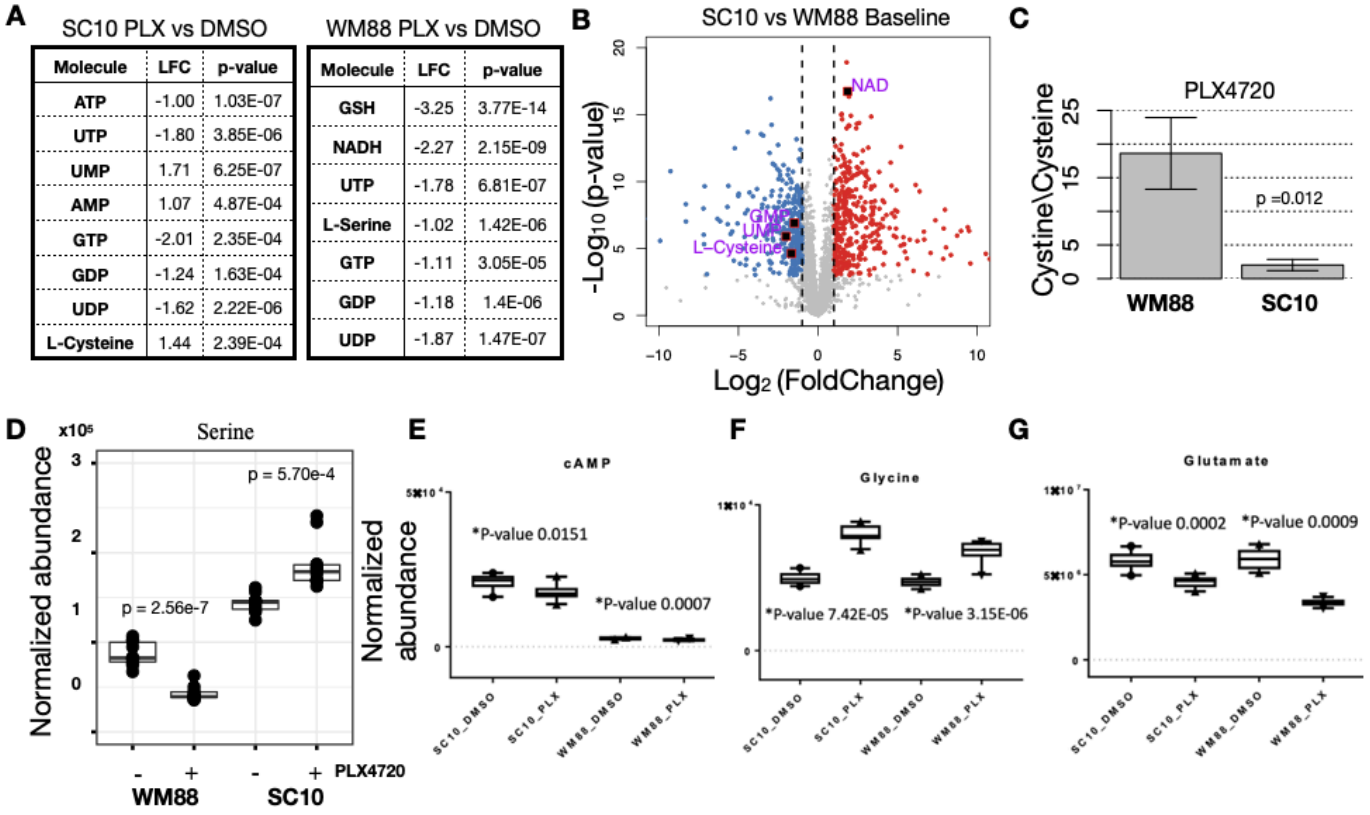
A

SC10 PLX vs DMSO

Molecule	LFC	p-value
ATP	-1.00	1.03E-07
UTP	-1.80	3.85E-06
UMP	1.71	6.25E-07
AMP	1.07	4.87E-04
GTP	-2.01	2.35E-04
GDP	-1.24	1.63E-04
UDP	-1.62	2.22E-06
L-Cysteine	1.44	2.39E-04

WM88 PLX vs DMSO

Molecule	LFC	p-value
GSH	-3.25	3.77E-14
NADH	-2.27	2.15E-09
UTP	-1.78	6.81E-07
L-Serine	-1.02	1.42E-06
GTP	-1.11	3.05E-05
GDP	-1.18	1.4E-06
UDP	-1.87	1.47E-07



Paudel et al. Fig. S1

Figure S1: Redox-related metabolites are altered in *BRAF*-mutated melanoma cells upon BRAF-inhibition. (A) Significantly altered redox-related metabolites in drug-insensitive (SC10), and drug-sensitive (WM88) *BRAF*-mutated melanoma cells in PLX4720 compared to DMSO control. (B) Volcano plots comparing baseline differences between drug-insensitive (SC10), and drug-sensitive (WM88) cells. Red dots are up-regulated metabolites in SC10, while blue dots are upregulated in WM88. Significantly different redox-related metabolites are highlighted. (C) Barplot of ratio of Cystine, and Cysteine for WM88, and SC10 cells upon 8μM PLX4720 treatment. Box plots showing normalized abundances of: (D) Serine, (E) cAMP, (F) Glycine, (G) Glutamate in indicated melanoma cells treated with either DMSO or 8μM PLX4720 for 24 h. For box plots, the solid line is the median, the box spans the first, and third quartiles, the whiskers extend to 1.5x the interquartile range--total of 10 replicates shown. In all figures, error bars on bar plots represent mean+-SEM, unless otherwise indicated. p-values indicated in the figure are estimated by Student's two-sample t-test.

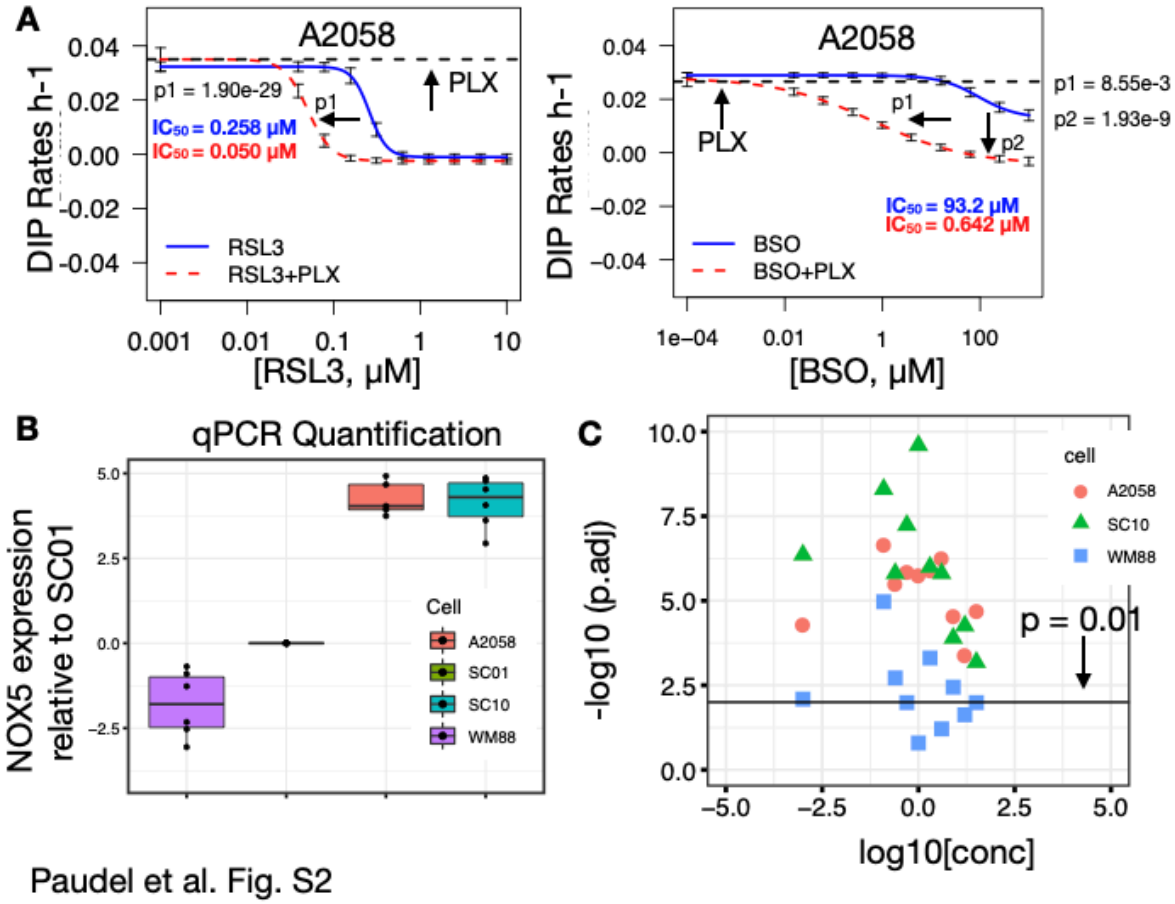
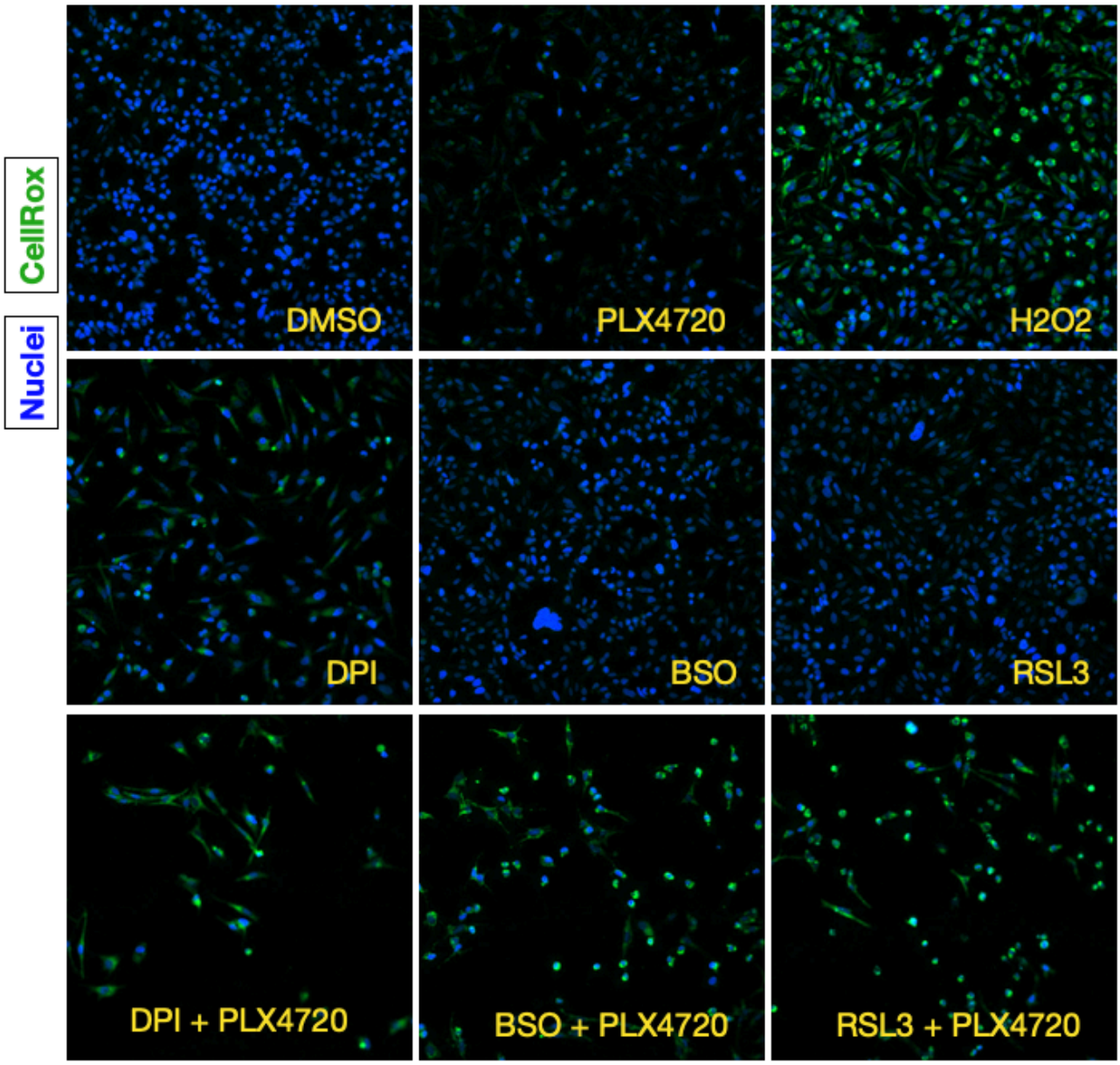
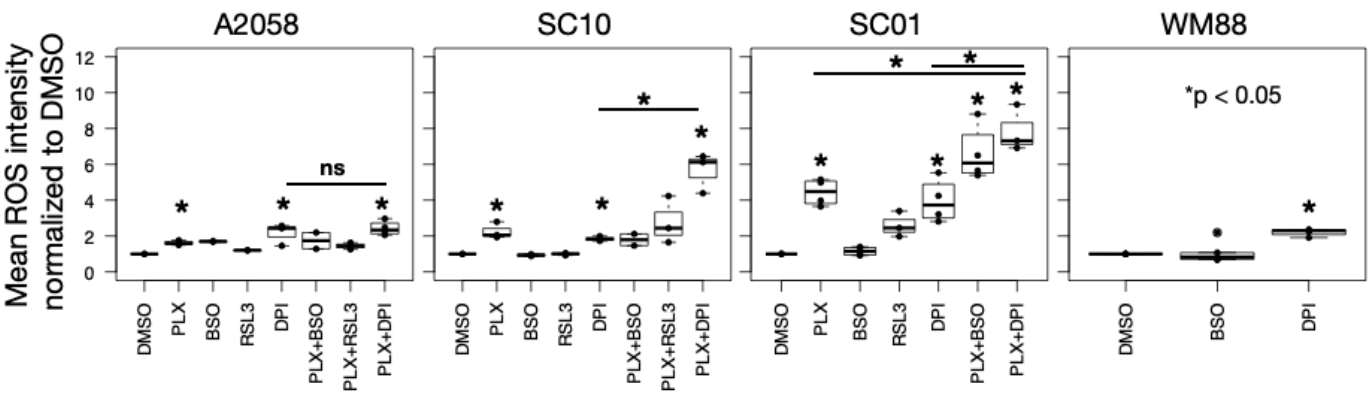


Figure S2: Inhibition of redox enzymes enhances the effects of BRAFi in BRAF-mutated melanoma cells. (A) Drug-dose response curves of A2058 either in single agents or in combination with 8µM PLX4720. Black dotted lines represent the DIP rates in 8µM PLX4720; colored solid lines denote the DIP rates in increasing concentrations of single agents (BSO: GCLC inhibitor, potent depletion of GSH; RSL3: GPX4 inhibitor); while the colored dotted lines denote the drug responses of combination of 8µM PLX4720 and specific single agents corresponding to the colored solid curve. p1 and p2 represent p-values from fitted dose-response curves as described in the Methods. (B) Expression of NOX5 in BRAF-mutated melanoma cells relative to its expression in SC01, estimated by quantitative PCR (qPCR). (C) Negative logarithms (log10) of adjusted p-valued plotted against log10 concentrations of PLX4720 for comparison of scrambled control and NOX5-siRNA, solid-dark lines indicates p-value of 0.01. p-values are adjusted for multiple testing by Benjamini-Hochberg (BH) procedure.



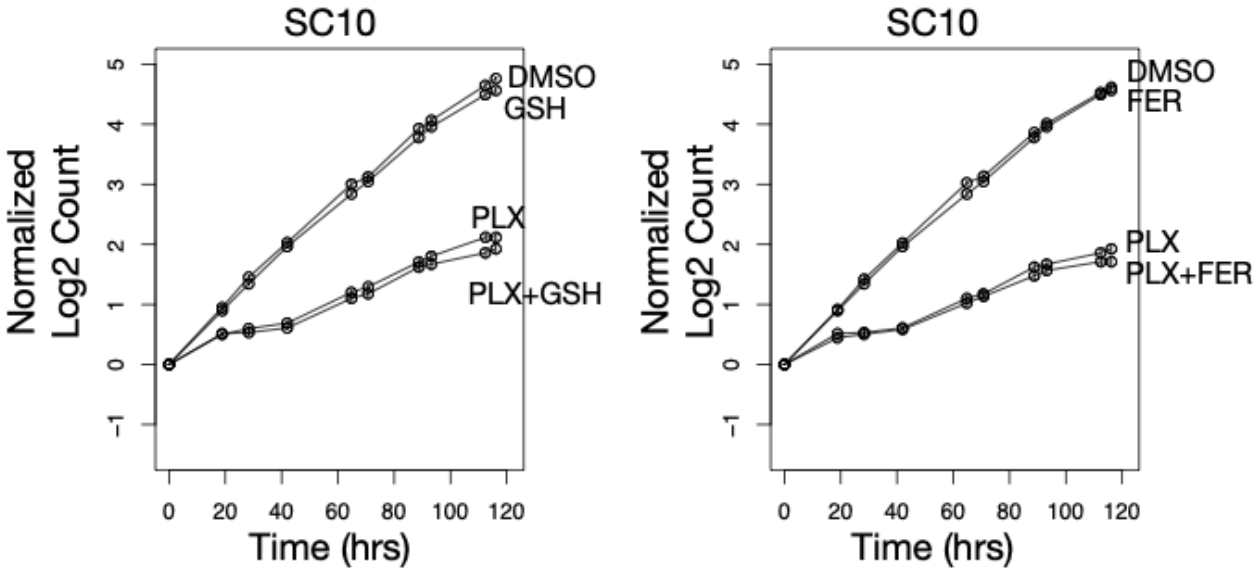
Paudel et al. Fig. S3A

Figure S3A: Co-treatment of redox inhibitors and BRAFi induces oxidative stress by elevating total cellular ROS levels. Representative images for BRAF-mutated melanoma cell, SKMEL5 fluorescently tagged with GFP-H2B, co-treated with indicated drug combinations stained with CellROX® Deep Red Reagent. Cells were treated with DMSO control, 8μM PLX4720, BSO (500μM), RSL3 (78.125nM), and DPI (1.25μM), and the combinations of PLX4720 and respective single-agents at concentrations indicated. H_2O_2 (1mM incubated for an hour) is used as positive control. Blue denotes nuclei, green denotes total ROS intensity.



Paudel et al. Fig. S3B

Figure S3B: Co-treatment of redox inhibitors and BRAFi induces oxidative stress by elevating total cellular ROS levels. Box plots showing total cellular ROS mean intensity per cell for either single agents or in combinations normalized to intensity in DMSO control for indicated melanoma cells stained with CellROX® Deep Red Reagent. Cells were treated with DMSO control, 8μM PLX4720, BSO (500μM), RSL3 (78.125nM), and DPI (1.25μM); in box plot , the solid line is the median, the box spans the first, and third quartiles, the whiskers extend to 1.5x the interquartile range. Significance denotes comparison between the group indicated with DMSO control, or between the two groups at the start and the end of the line. * $p < 0.05$ by Student's two-sample t-test.



Paudel et al. Fig. S4

Figure S4: The effects of PLX4720 alone cannot be rescued by the known antioxidants. Population growth curves for SC10 treated with: (*left*) 8μM PLX4720, 5mM reduced GSH, and the 1:1 combination; (*right*) 8μM PLX4720, 5μM Ferrostatin-1 (FER), and the 1:1 combination. Both single or double-agents are added at day 0, and replenished at 72 hrs along with growth medium.

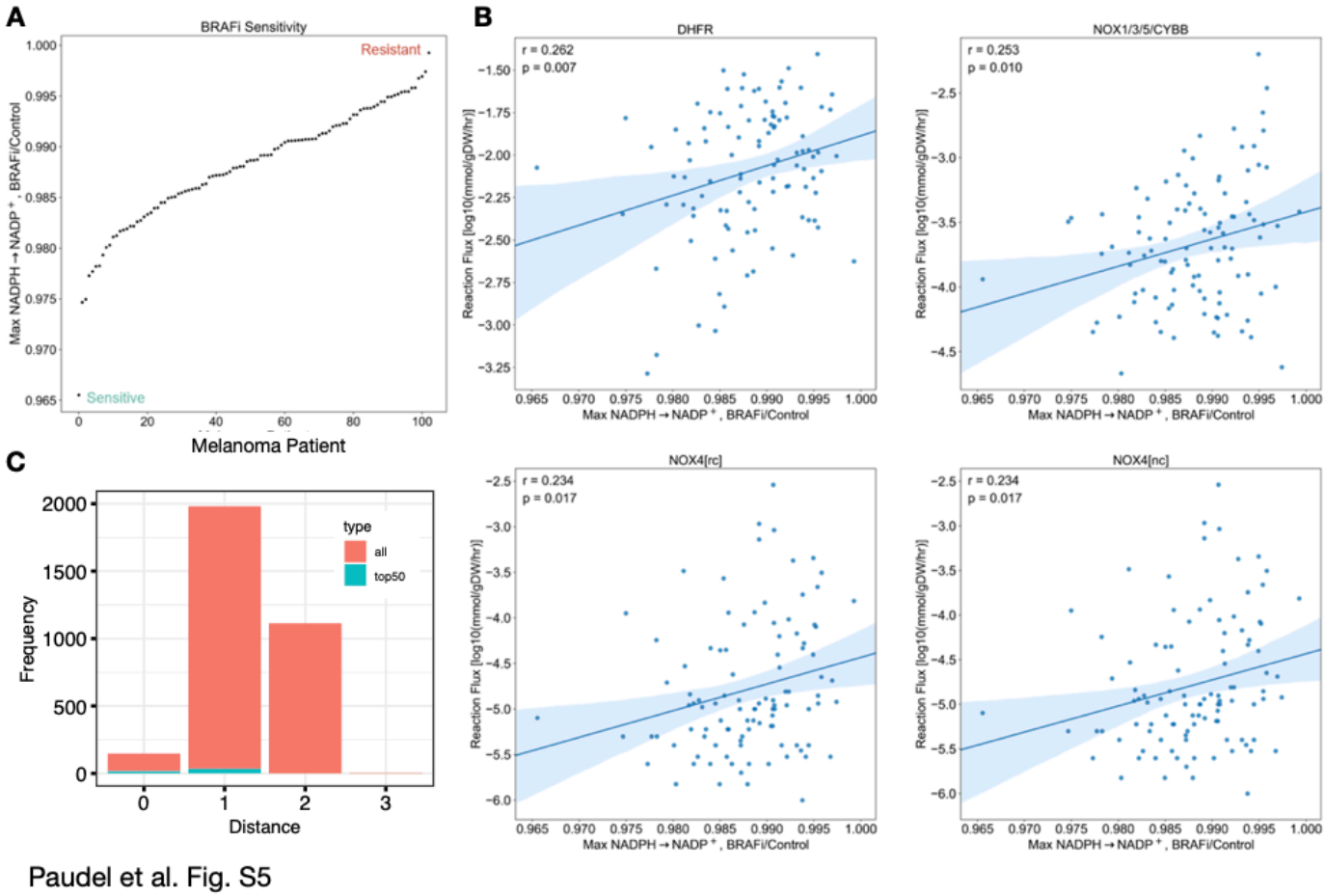
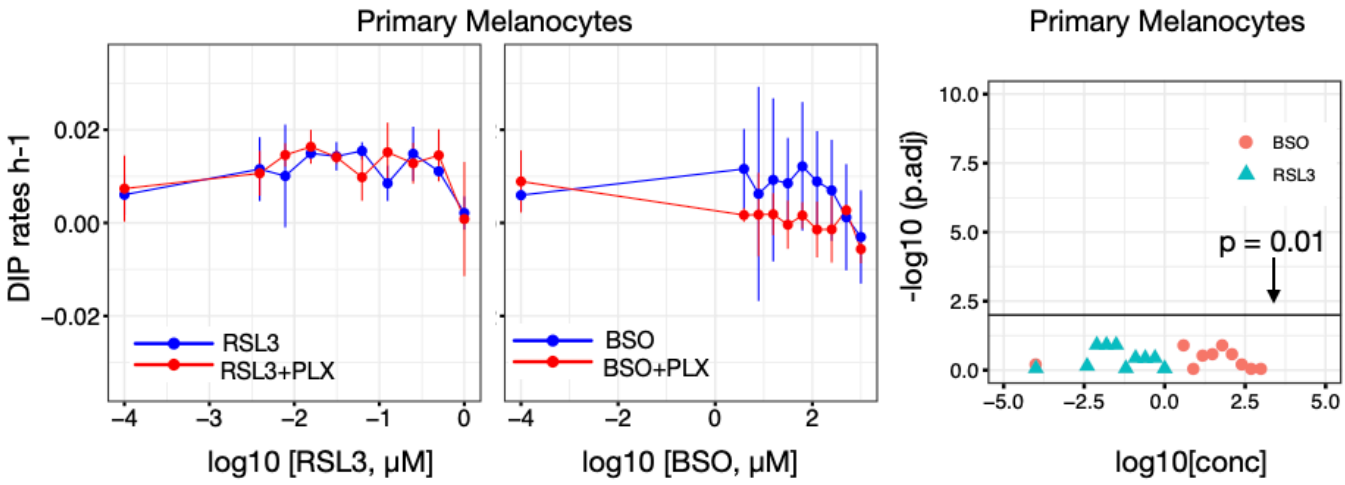
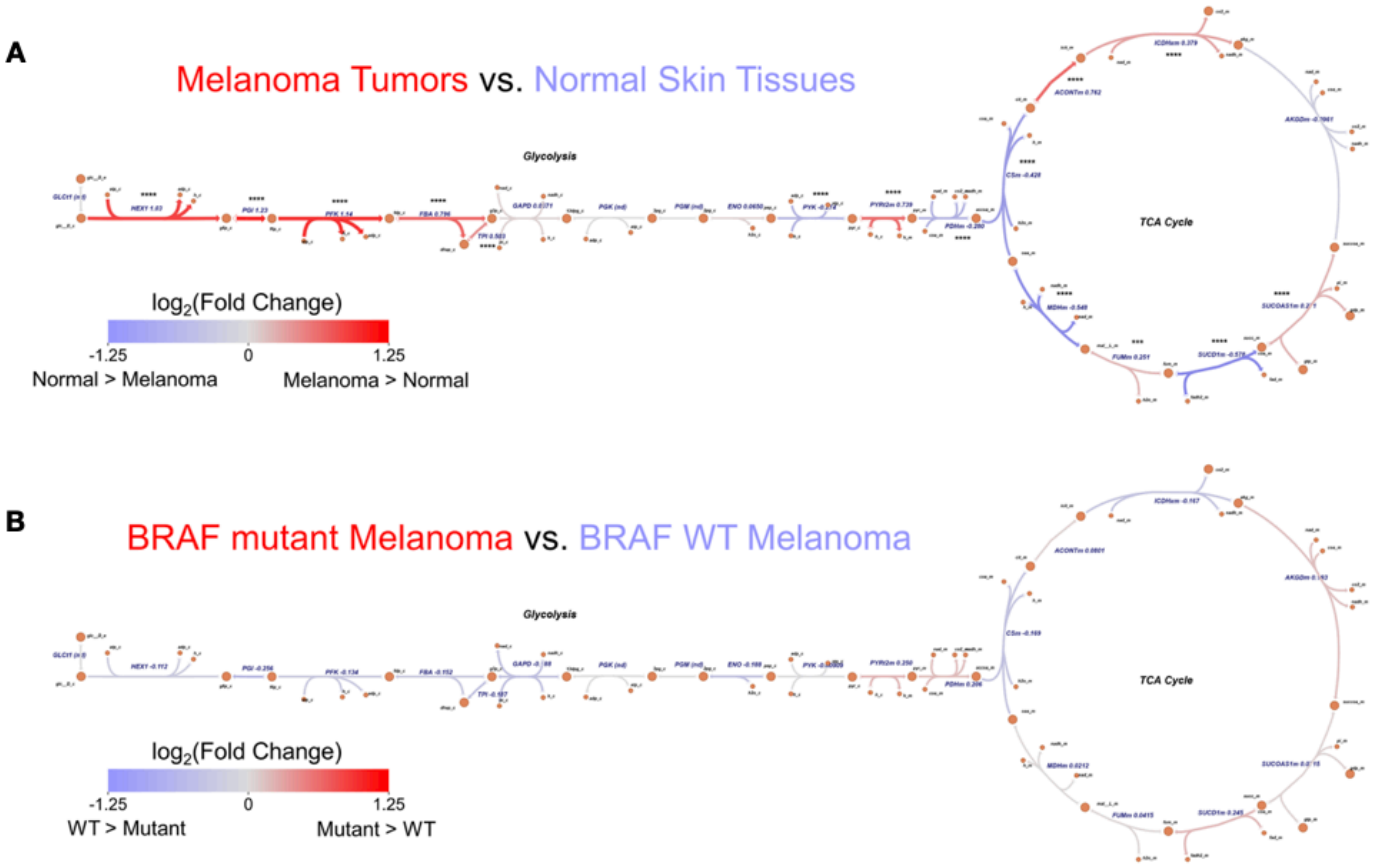


Figure S5: The Flux Balance Analysis model can be used to predict BRAFi sensitivity in melanoma patients. (A) Stratification of FBA model-predicted BRAFi sensitivity in melanoma TCGA tumors, y-axis represents maximum flux through NADPH—> NADP+ in BRAFi conditions normalized to control, and x-axis represents melanoma patients in TCGA cohort. (B) Correlation of model-predicted fluxes through major NADPH-oxidizing reactions and BRAFi sensitivity. Fluxes via DHFR, NOX1/3/5 and NOX4 correlate positively with BRAFi sensitivity. (C) Bar plot of minimum distance values for reactions involving all enzymes in Recon3D and NADPH-reducing reactions (number of reactions separating the two), overlaid with distance values for reactions of enzymes in top 50 indicated in Fig. 5B, gene distances had a mean value of 1.31 with maximum value of 3.



Paudel et al. Fig. S6

Figure S6: Primary melanocytes are insensitive to redox inhibitors and combination with BRAFi. Drug-dose response curves (measured in Drug-induced proliferation rates) of Primary human epidermal melanocytes (PC2-200-013) either in single agents or in combination with 8uM PLX4720. (right) red lines denote the DIP rates in increasing concentrations of RSL3 (GPX4 inhibitor), and blue lines denote the drug responses of combination of 8uM PLX4720 and specific agents corresponding to the blue lines, (middle) red lines denote the DIP rates in increasing concentrations of BSO (GCLC inhibitor), and blue lines denote the drug responses of combination of 8uM PLX4720 and specific agents corresponding to the blue lines, (right) Negative logarithms (log10) of adjusted p-valued plotted against log10 concentrations of indicated drugs for comparison of single-agents and combination, solid-dark lines indicates p-value of 0.01. p-values are adjusted for multiple testing by Benjamini-Hochberg (BH) procedure, primary melanocytes grow slowly and their responses in either single agents or in combination are statistically insignificant. Figure denote 3+ replicates.



Paudel et al. Fig. S7

Figure S7: FBA predicted Central carbon metabolic fluxes show significant differences between TCGA melanoma tumors vs normal skin tissues. Central carbon metabolic fluxes between for: (A) TCGA melanoma tumors vs GTEx skin tissues. (B) TCGA BRAF-mutated melanomas vs TCGA non-BRAF-mutated melanomas. Log2 (tumor/normal), and log2 (BRAF/ NoBRAF) calculated for each reaction flux. Red means log2(ratio) > 0, blue means log2(ratio) < 0.