Figure S1. Transmission electron microscopy analysis. Representative micrographs showing that SUR cells presented nuclei morphology alterations and mitochondria fragmentation. (A) MEL-XY3 cells. (B) MEL-XY3 SUR cells. (C) MEL-XY3 SUR cells 2 days after PLX4032 removal. (D) MEL-XY3 SUR cells 7 days after PLX4032 removal. Original magnification, x7,000 (panels A, B and D) or x4,400 (panel C). Squares indicate mitochondrial, whereas circles highlight membrane blebbing. N, nucleus; SUR, surviving cells following long-term PLX4032 treatment.

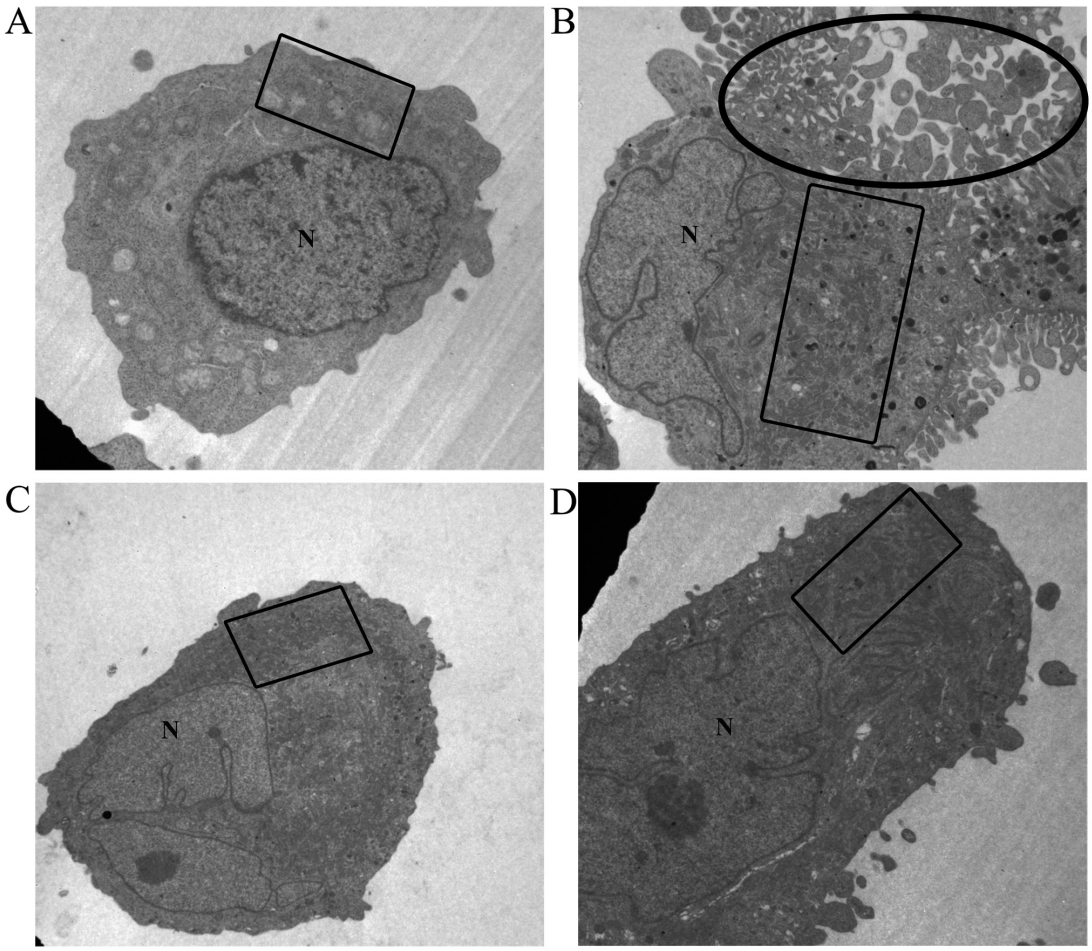

Figure S2. Reverse phase protein array heatmap representing the levels of proteins in MEL-XY3 cells treated for 7 days with PLX4032, SUR cells and SUR cells 7 days after drug removal; results were normalized to MEL-XY3 parental cell expression levels. SUR, surviving cells following long-term PLX4032 treatment.

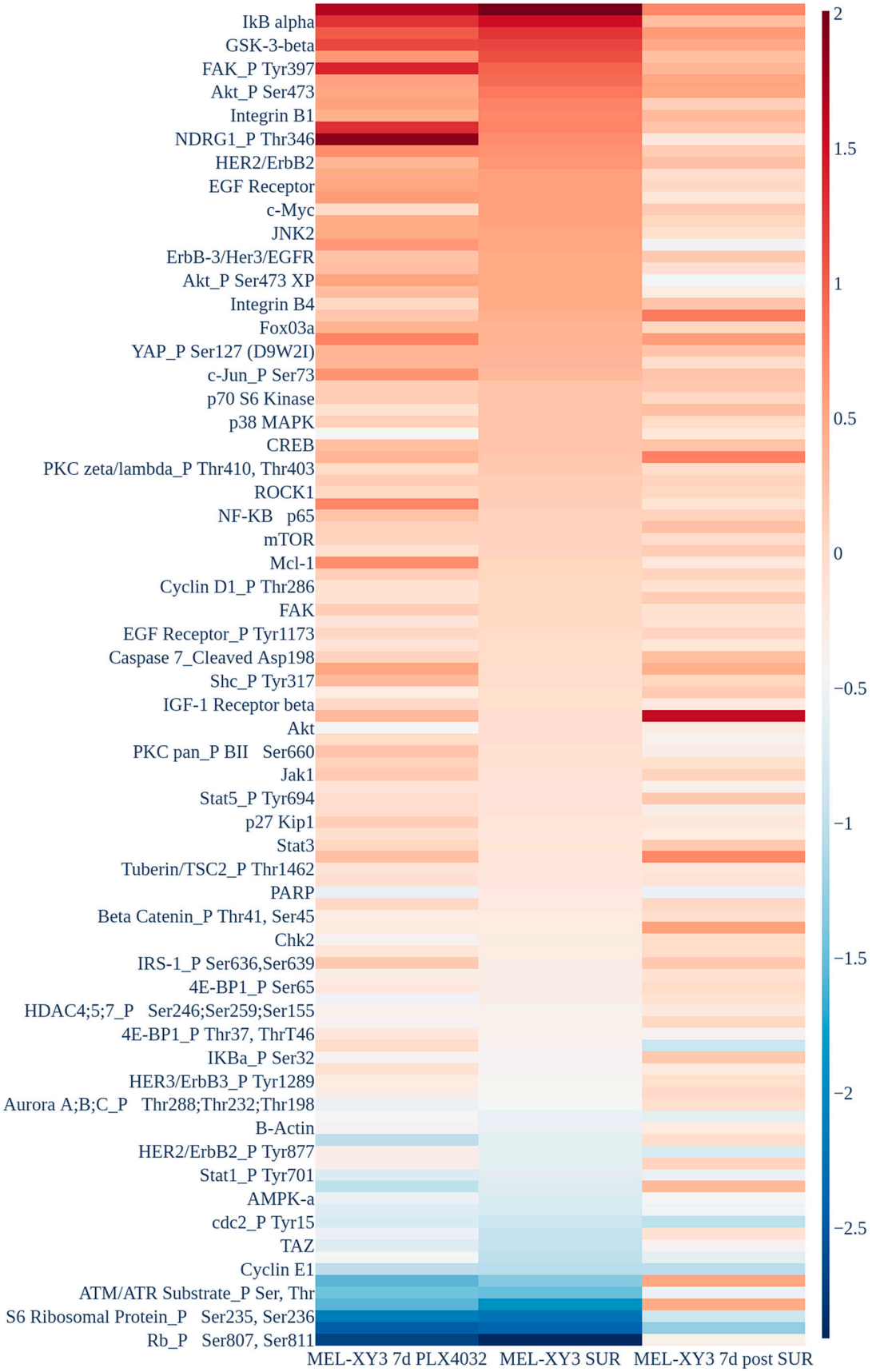

Figure S3. HDAC expression in melanoma samples. HDAC3, 5 and 6 expression values were obtained from GSE50509 dataset (n=19). Statistical analysis was performed by paired t-test. *P<0.05. HDAC, histone deacetylase; Pre, pre-treatment; Prog, progressive treatment with B-Raf inhibitor.

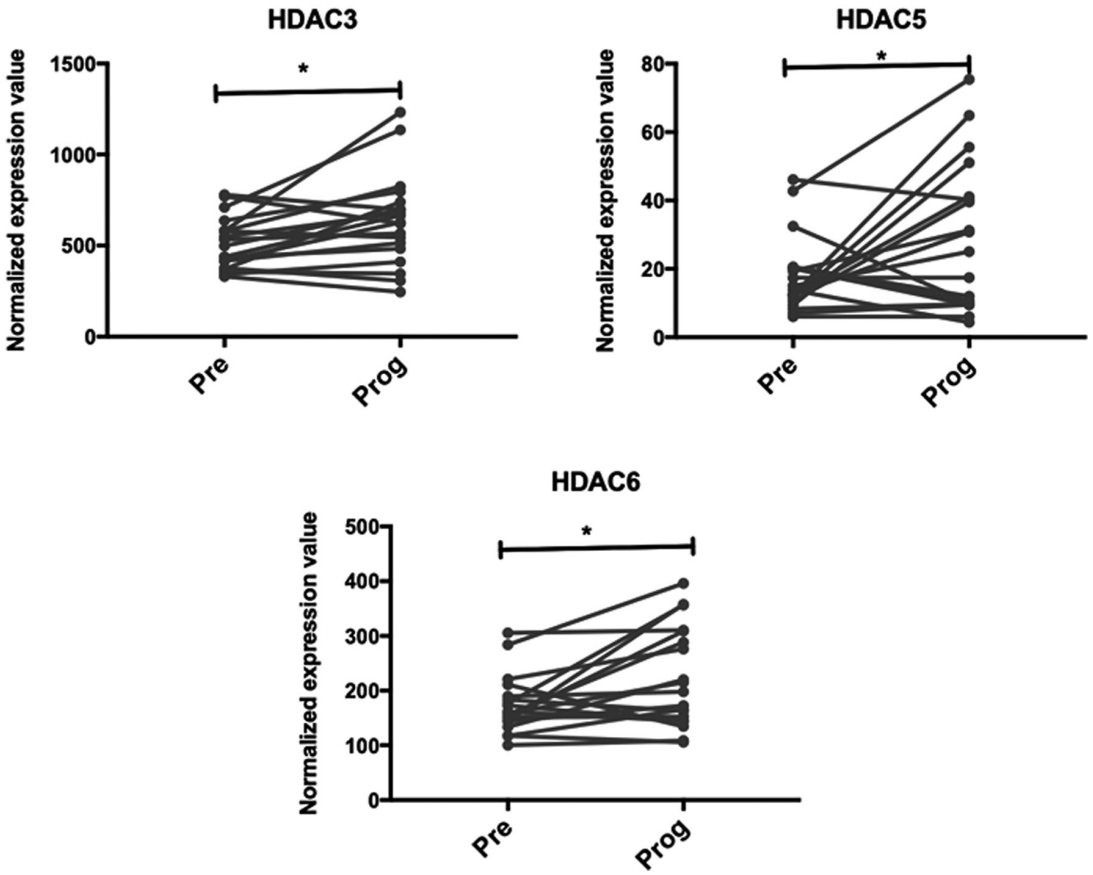

Figure S4. Effect of histone deacetylase and cyclin-dependent kinase 9 inhibitor treatment on apoptosis and cell cycle distribution. (A) Representative dot plots of apoptosis analysis measured by Annexin-V/PI staining; parental and SUR cells were treated for 72 h with DMSO, PLX4032, MGCD0103 or PLX4032 + MGCD0103. (B) Representative histograms of cell cycle analysis after 72 h treatment with DMSO, PLX4032, SAHA or PLX4032 + SAHA. (C) Representative dot plots of apoptosis analysis measured by Annexin-V/PI staining; MEL-XY3 RES cells were treated for 72 h with DMSO, PLX4032, MGCD0103 or PLX4032 + MGCD0103. (D) Representative dot plots of disaggregated spheroids stained by Annexin V/PI. MEL-XY3 SUR spheroids were treated for 72 h with DMSO, PLX4032, MGCD0103 or PLX4032 + MGCD0103. P + M, PLX4032 + MGCD0103; P + S, PLX4032 + SAHA; PI, propidium iodide; RES, resistant to PLX4032; SUR, surviving cells following long-term PLX4032 treatment.