## Supplementary Figures

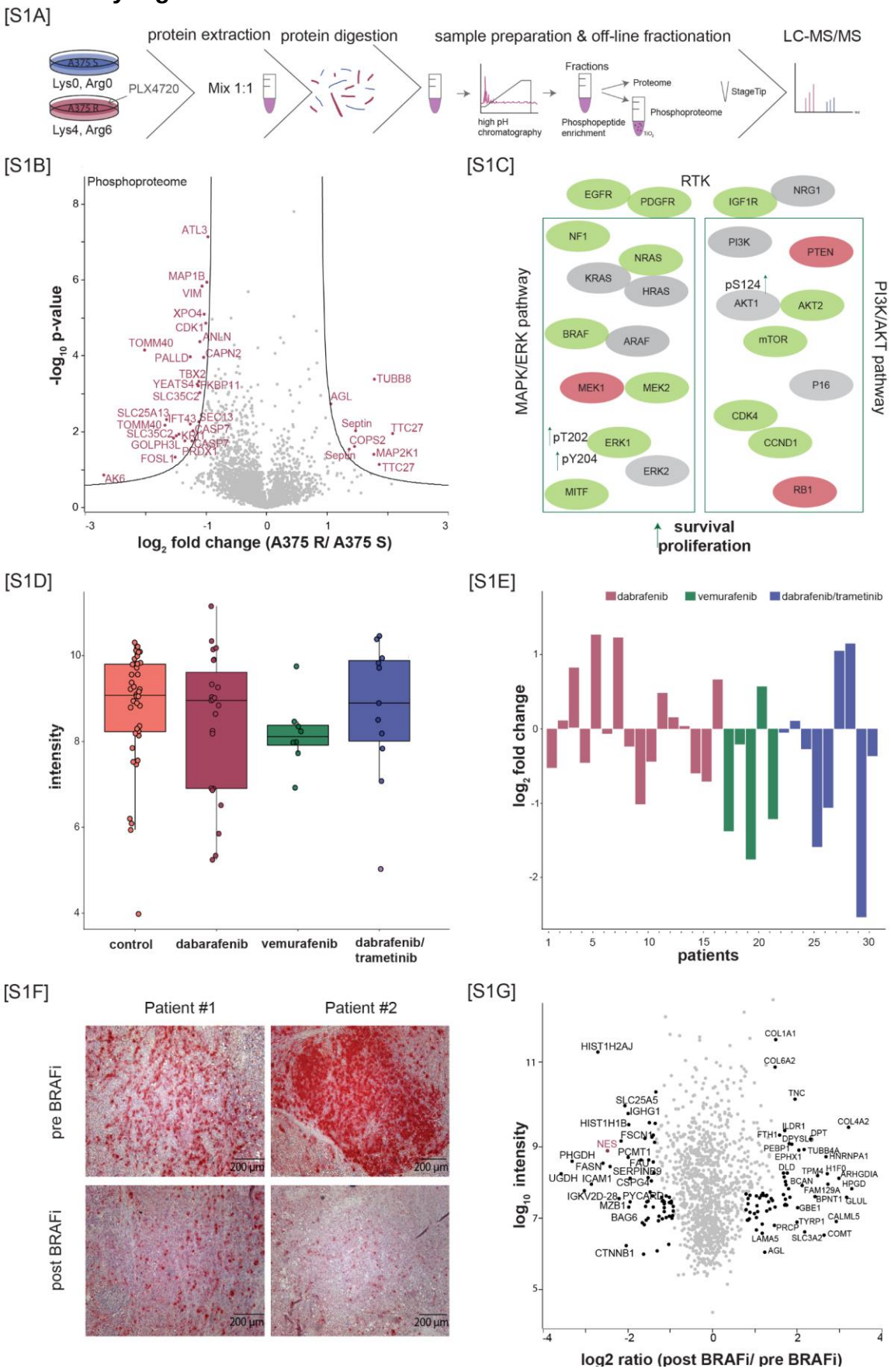


**Figure S1: Quantitative proteome and phosphoproteome analysis of human melanoma cells identifies down-regulation of nestin.** [A] Schematic of sample preparation workflow. Vemurafenib resistant A375 (A375 R) and A375 sensitive cells (A375 S) were 'light' (Lys0/Arg0) and 'medium' (Lys4/Arg6) SILAC labelled. After cell lysis, crude protein extracts were mixed 1:1, reduced, alkylated and trypsin digested. The resulting peptides mixture was fractionated using an off-line HPLC operated with high pH buffers. Fractions were pooled and measured directly (proteome) or applied to phosphopeptide enrichment using titanium dioxide ($TiO_2$) prior to liquid chromatography mass spectrometry analysis (LC-MS/MS). [B] Volcano plot of vemurafenib-resistant and -sensitive A375 proteomes for phosphoproteome. t-test difference of SILAC ratios between A375 R and A375 S ($log_2$) are plotted against p-value ($-log_{10}$) (n=3). Black lines indicate the significance threshold (FDR < 0.01; $s_0 = 1$). Significantly up- and down-regulated proteins are highlighted in magenta. [C] Identified key molecules and phosphorylation sites of the MAPK/ERK and PI3K/AKT signaling pathway. Green: up-regulated in A375 R vs. A375 S cells; red: down-regulated in A375 R vs. A375 S cells; grey: identified, but not quantified; arrows:

up-regulated phosphorylation sites in A375 R vs. A375 S. [D] NES expression profile in human patients with melanoma metastases in vemurafenib, dabrafenib and dabrafenib plus trametinib treated tumors and pre-treatment control tumors (FDR ≤ 0.1). [E] mRNA expression levels of nestin protein in thirty patients with melanoma metastases after BRAF inhibitor therapy compared to control tumors. [F] Immunohistochemical staining for nestin of melanoma metastases obtained before treatment with a BRAF inhibitor vermurafenib and after resistance acquisition for two patients. Nestin levels are shown in red (Fast red substrate). [G] Proteomics of FFPE specimens pre-and post-BRAF inhibitor therapy using quantitative proteomics based dimethyl-labelling. Ratios ($log_2$) of post-BRAF vs. pre-BRAF inhibitor therapy are plotted against intensity ($log_{10}$) (p-value <0.05). Nestin is highlighted in magenta.

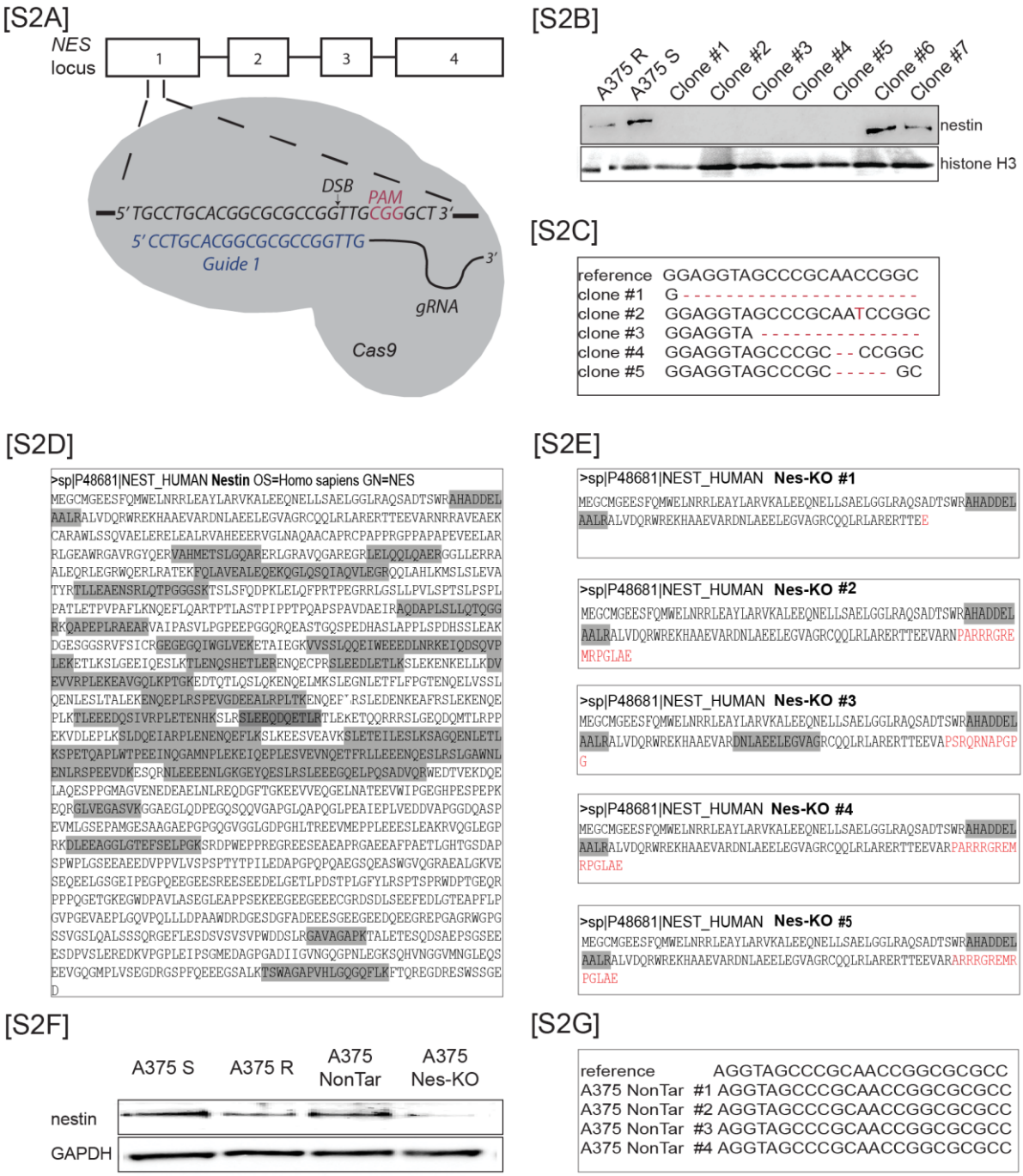


**Figure S2: Nestin expression correlates with invasive properties in melanoma cell lines.** [A] Schematic overview of the establishment of NES knockout cells using CRISPR/Cas9 genome editing system. Blue: guide sequence targeting Exon1 in the genomic sequence of NES; red: protospacer adjacent sequence (PAM) sequence; DSB: double strand break. [B] Western blot analysis of A375 S, A375 R and CRISPR/Cas9 genome edited cell clones Nes-KO #1-7. [C] Sanger sequencing result of reference DNA (A375 S and R) and CRISPR/Cas9 genome-edited cell clones Nes-KO #1 - 5. [D] Amino acid sequence of human nestin from Uniprot database. Grey: peptide sequences identified by LC-MS/MS. [E] Amino acid sequence of CRISPR/Cas9 genome edited cell clones Nes-KO #1-5. Grey: peptide sequences identified by LC-MS/MS. Red: truncated amino acid sequence compared to A375 S. [F] Western blot analysis of nestin and GAPDH protein in A375 S, A375 R, A375 NonTar and A375 Nes-KO cells. [G] Sanger sequencing results of reference DNA (A375 S and R) and CRISPR/Cas9 genome-edited control cell clones A375 NonTar #1 – 4.

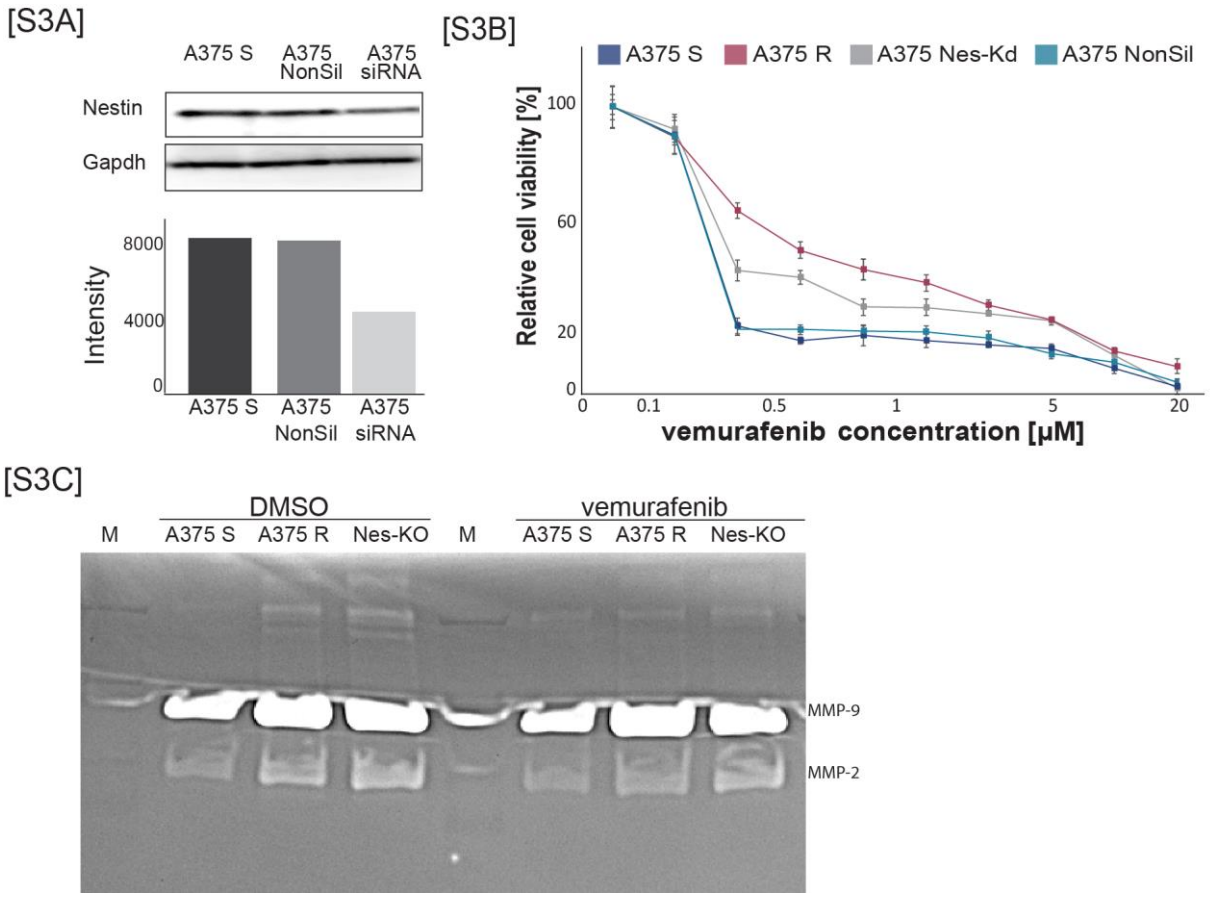


**Figure S3: Depletion of nestin affects cell proliferation and colony formation upon treatment with signaling pathway inhibitors.** [A] Western blot analysis of A375 S, A375 NonSil and A375 siRNA against nestin and quantification of bands intensities using ImageJ software. Nestin was down-regulated in A375 S cells by transfection of a pool of four siRNA oligos (siRNA) against human nestin. Untreated A375 S and NonSilencing siRNA (NonSil) treated A375 S cells were included as control. Cells were harvested 48 h post-transfection. [B] A375 S, A375 R, A375 NonSil and A375 Nes-Kd were cultured for 24 h, and then treated with PLX4720 at the indicated concentrations (0, 0.1, 0.25, 0.5, 1, 2.5, 5, 10 and 20 µM) or DMSO as control, respectively. Cell viability was determined by MTS assay 96 h later. Results expressed as % control represent the mean of three biological experiments (n=24). Error bar represents standard deviations of three biological replicates [C] Gelatine zymography of supernatants of A375 S, A375 R and A375 Nes-KO cell lines treated with DMSO or PLX4720 for 24 h. Image is a representative of three independent experiments.

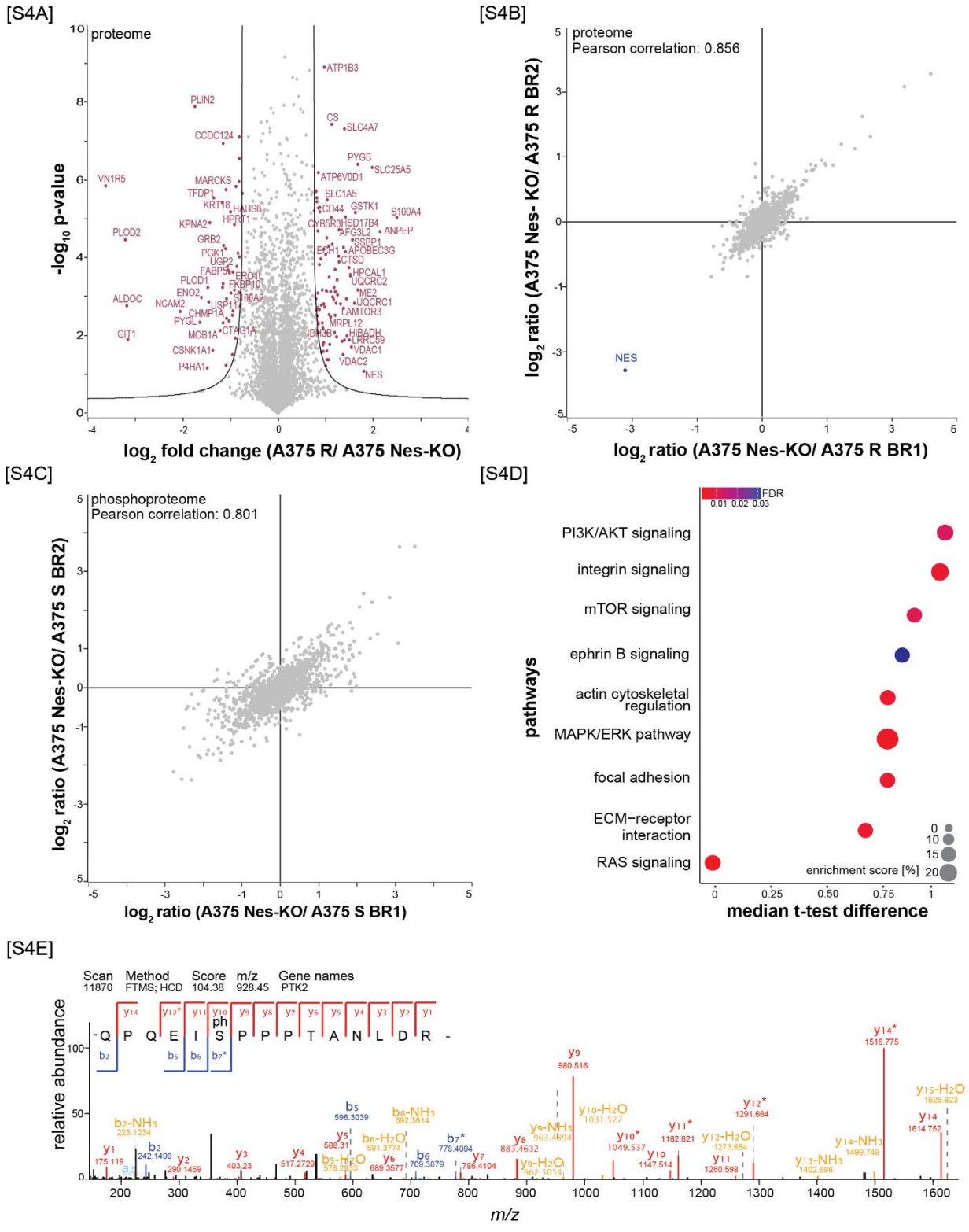


**Figure S4: Quantitative proteomics comparison between nestin knockout and BRAF inhibitor sensitive and resistant cell lines.** [A] Volcano plot of A375 Nes-KO and A375 R proteomes. t-test difference of SILAC Ratios between A375 Nes-KO and A375 R ($log_2$) are plotted against p-value ($-log_{10}$) (n=3). Black lines indicate the significance threshold (FDR < 0.01; $s_0$ = 1). Significantly up- and downregulated proteins are highlighted in magenta. [B] Proteome correlation of A375 Nes-KO relative to A375 R in biological replicate 1 and 2. [C] Phosphoproteome correlation of A375 Nes-KO relative to A375 R in biological replicate 1 and 2. [D] Over-representation of selected signaling KEGG pathways of A375 Nes-KO compared to A375 S cells using String database analysis. The t-test difference of SILAC ratios between A375 Nes-KO and A375 S ($log_2$) were plotted for each pathway (t-test, FDR < 0.1; $s_0$ =1). Enrichment score [%] identified significantly changing proteins mapped to the pathway by the total protein count involved in that pathway. Colour of the dots represents the FDR. [E] Annotated spectra of phosphorylated peptide LQPQEIpSPPPTANLDR containing a phosphosite at S910 on focal adhesion kinase FAK.