

Supporting Information

The pyrazolo[3,4-*d*]pyrimidine-based kinase inhibitor NVP-BHG712: Effects of regioisomers on tumor growth, perfusion, and hypoxia in EphB4-positive A375 melanoma xenografts

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Received: date; Accepted: date; Published: date

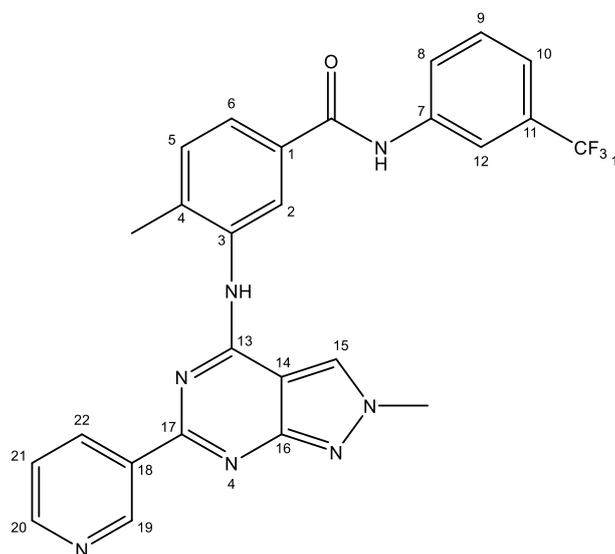


Figure S1. Structure of NVPiso. Arbitrary atom numbering as suggested by Tröster et al. [1].

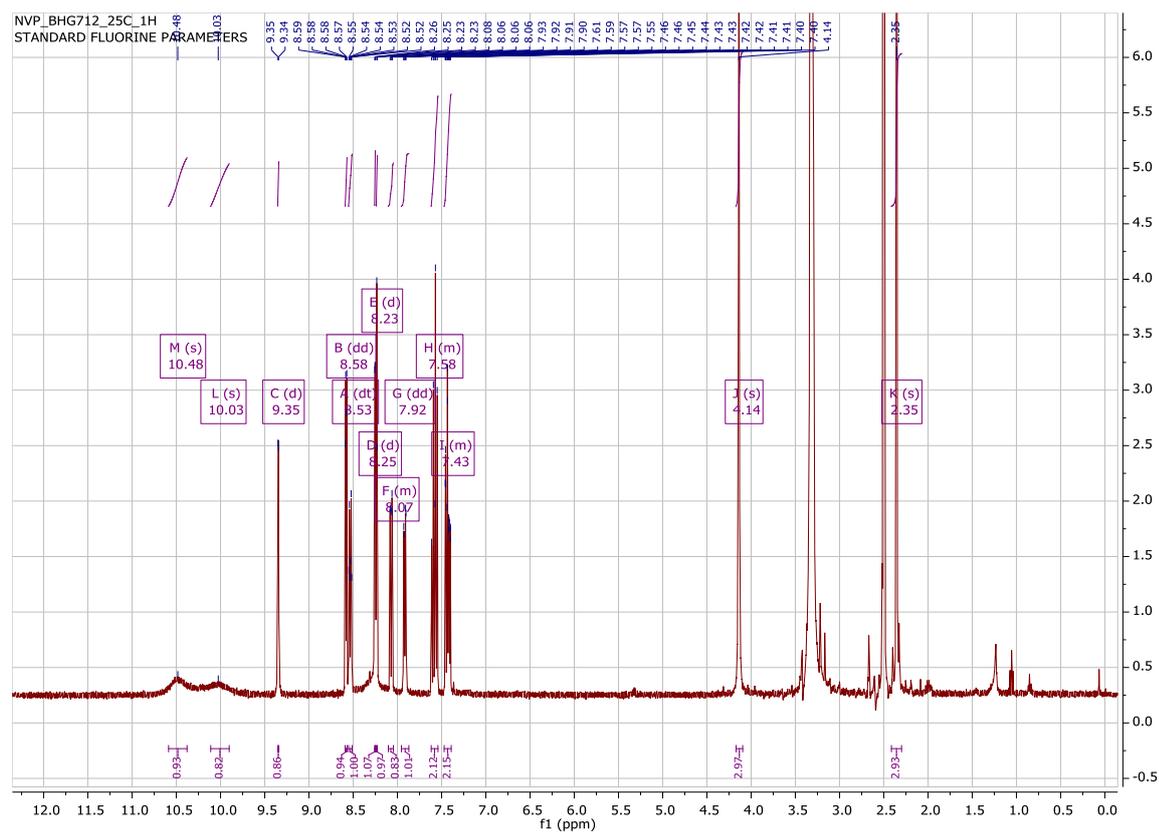


Figure S2. ^1H NMR spectrum of commercially acquired 'NVP' at 298 K.

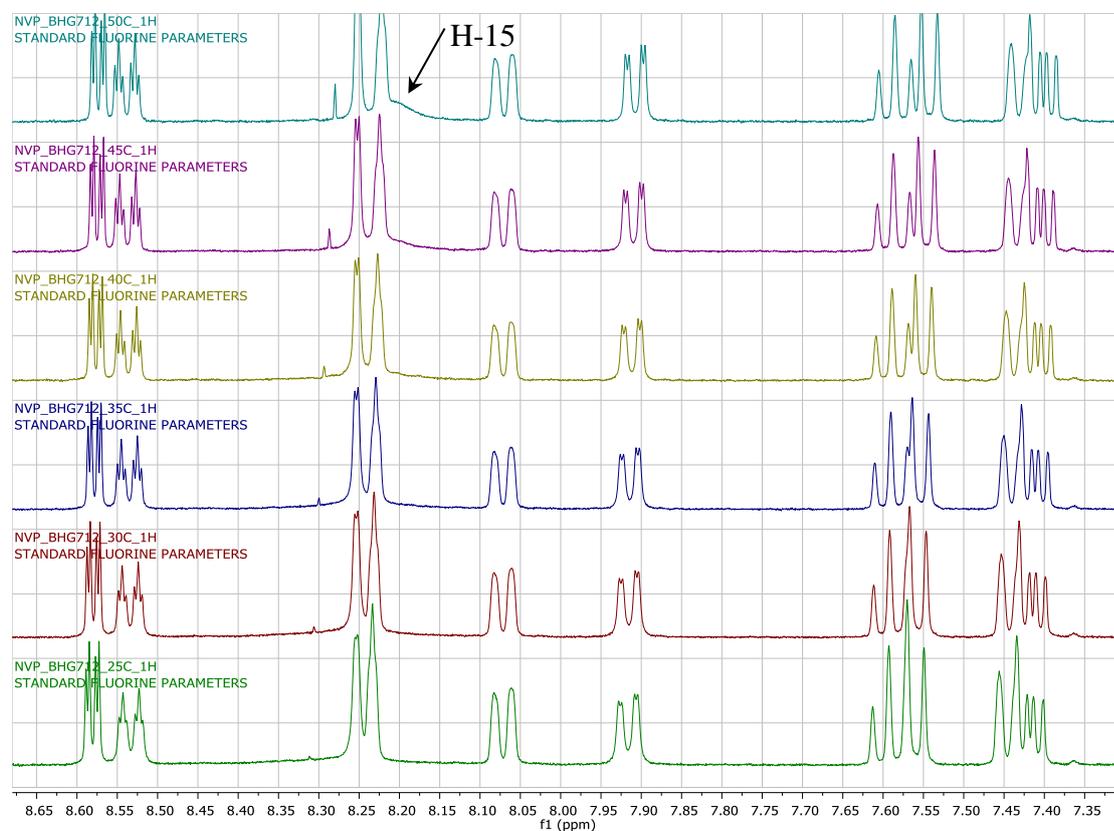


Figure S3. Temperature dependence of ^1H NMR spectra of commercially acquired 'NVP'. Shown is the aromatic region (signal of H-19 lies out) at 298, 303, 308, 313, 318 and 323 K to demonstrate that H-15 at

the pyrazole ring is only visible as broad singlet at temperatures above 318 K as reported by Tröster et al. [1].

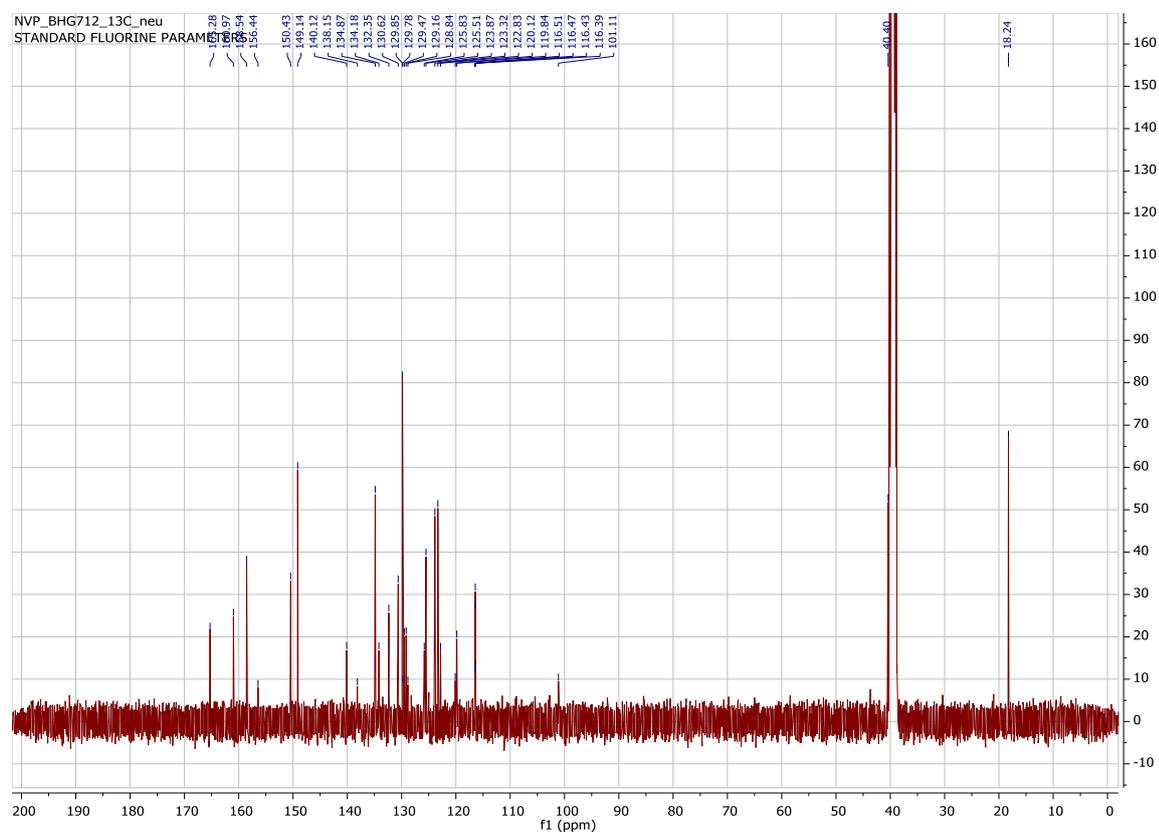


Figure S4. ^{13}C NMR spectrum of commercially acquired 'NVP' at 298 K.

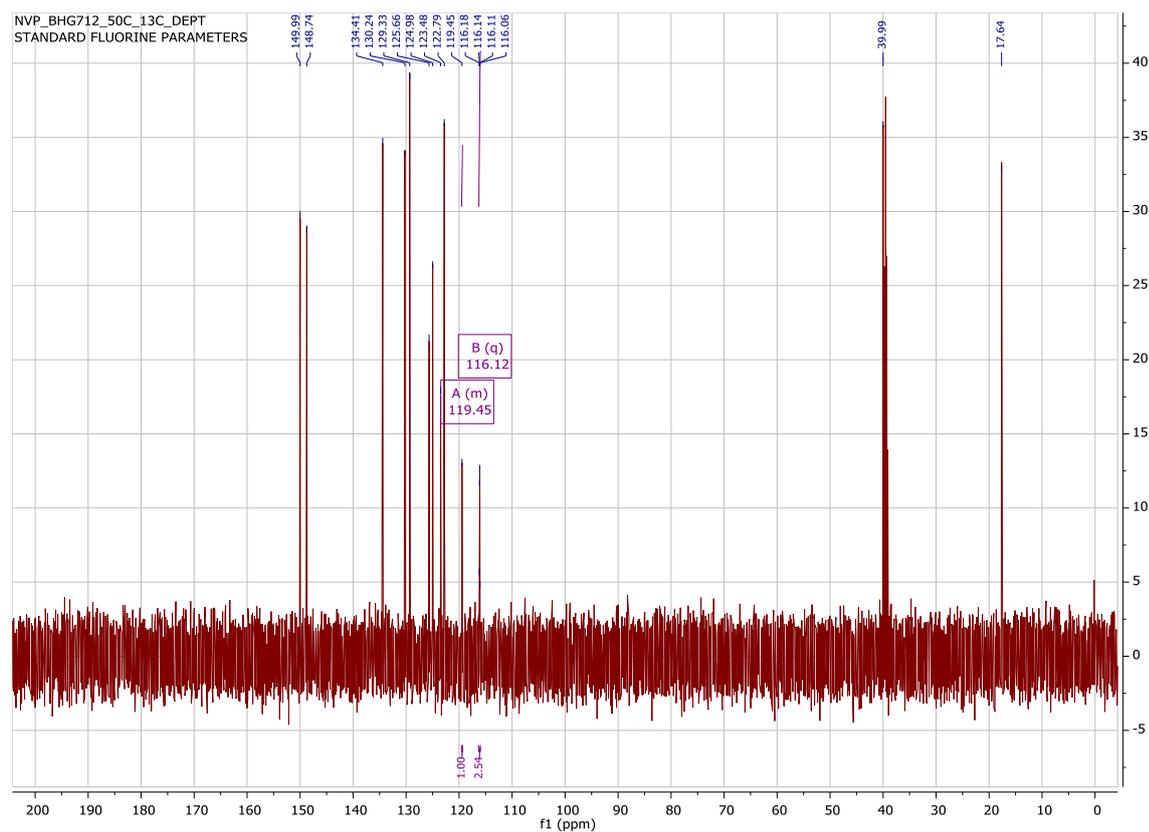


Figure S5. ^{13}C (DEPT) NMR spectrum at 323 K of commercially acquired 'NVP'.

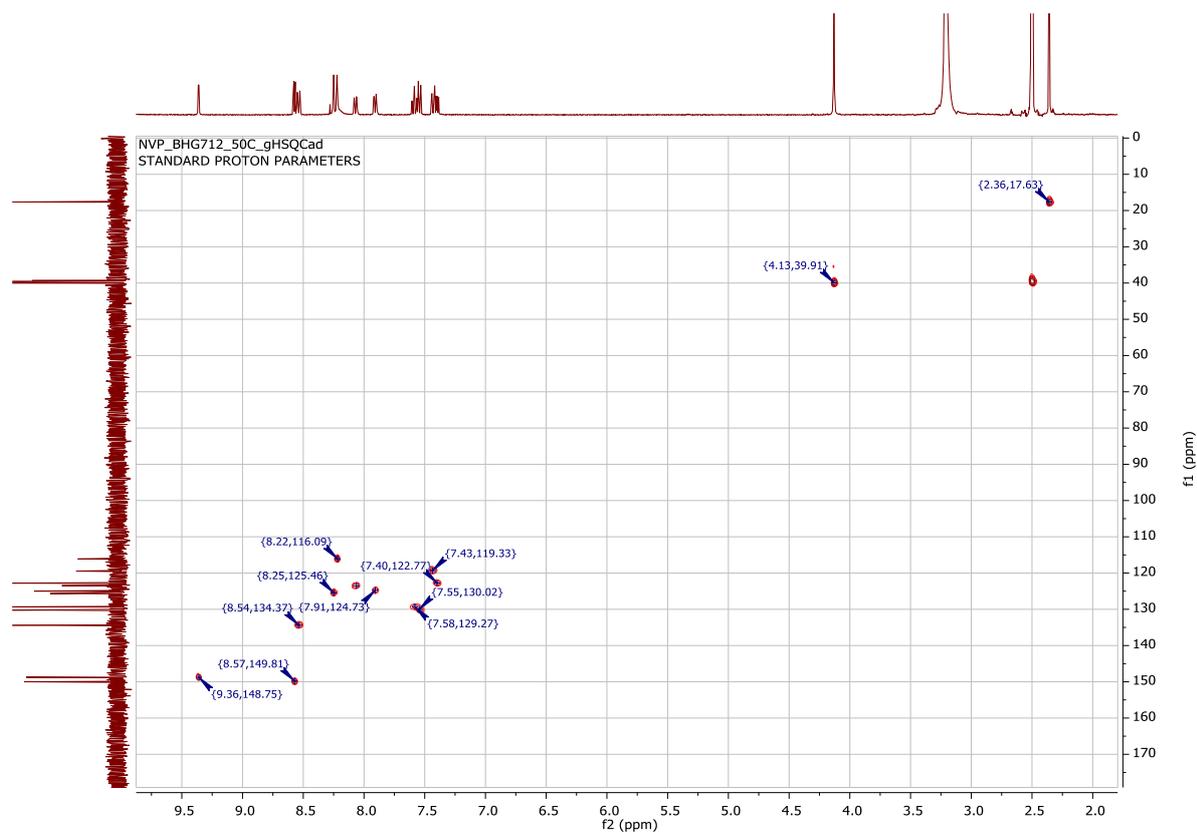


Figure S6. ^1H , ^{13}C HSQC spectrum of commercially acquired 'NVP' at 323 K. The DEPT spectrum is shown on the ^{13}C edge.

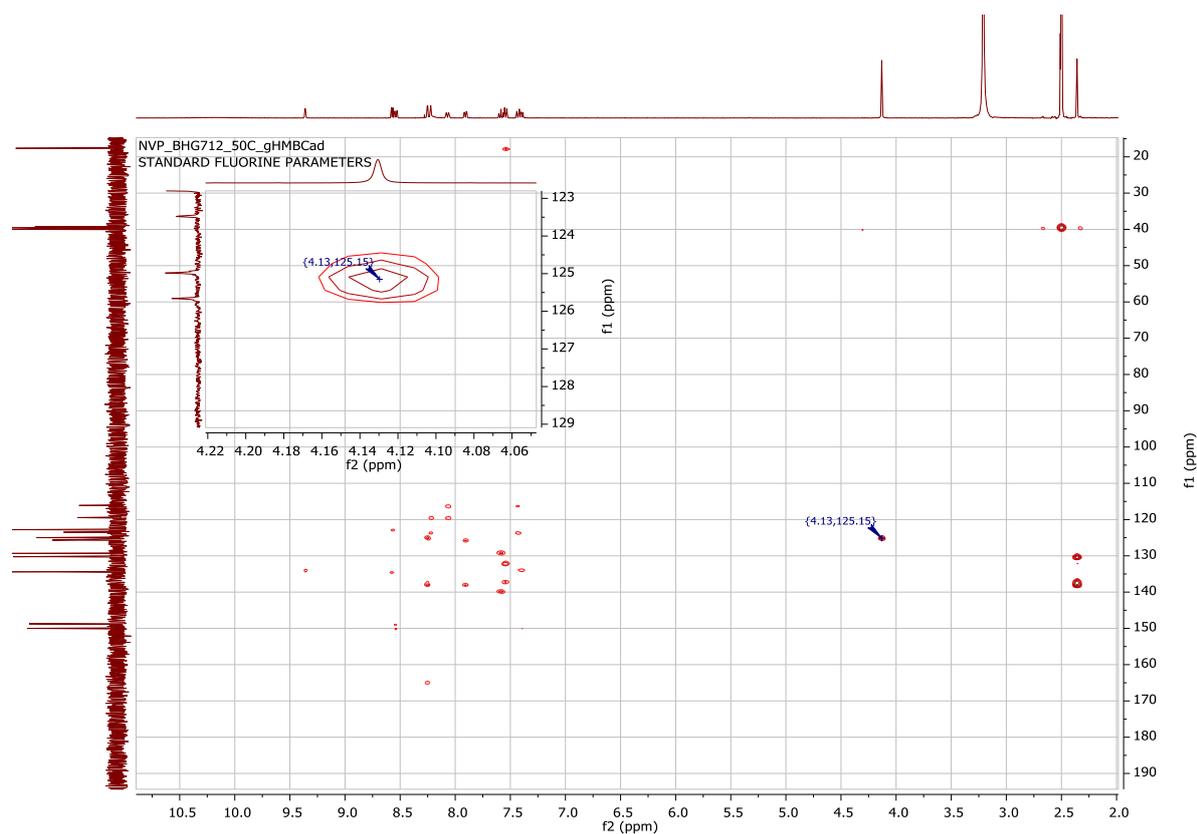


Figure S7. $^1\text{H},^{13}\text{C}$ HMBC spectrum of commercially acquired 'NVP' at 323 K. The inset highlights the $^3J\text{-H,C}$ correlation of the protons attached to the 2-methyl group to the C15 methine carbon in the pyrazole ring at 323 K. The DEPT spectrum is shown on the ^{13}C edge.

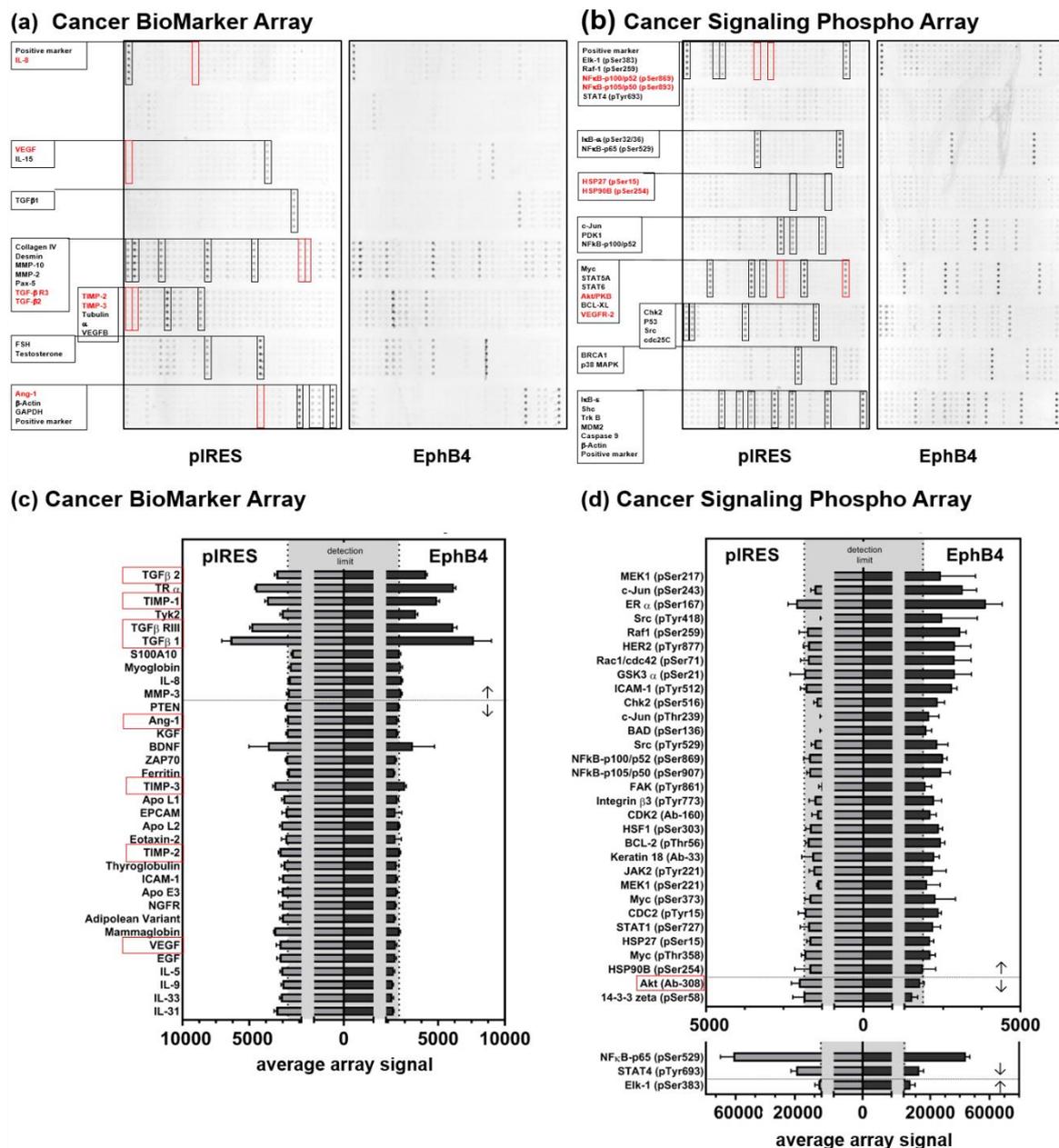


Figure S8. Differential expression and phosphorylation of cancer-related proteins in A375-EphB4 cells in comparison to A375-pIRES cells. (a/c) 34 out of 247 analyzed, cancer-relevant proteins differed between A375-pIRES and A375-EphB4 cells including angiogenesis-related proteins (marked in red). (b/d) 31 out of 136 analyzed phosphorylation variants differed between A375-pIRES and A375-EphB4 cells including angiogenesis-related proteins. (c/d) Detection level of the respective array was marked in grey since often one sample (A375-pIRES or A375-EphB4) was beneath detection level. Bars represent means of average array signals from 6 independent spots + SD for A375-pIRES (left panel) and A375-EphB4 cells (right panel).

References

1. Tröster, A.; Heinzlmeir, S.; Berger, B. T.; Gande, S. L.; Saxena, K.; Sreeramulu, S.; Linhard, V.; Nasiri, A. H.; Bolte, M.; Müller, S.; Kuster, B.; Medard, G.; Kudlinzki, D.; Schwalbe, H., NVP-BHG712: Effects of Regioisomers on the Affinity and Selectivity toward the Ephrin Family. *ChemMedChem* **2018**, *13*, (16), 1629-1633.