Electronic Supplementary Material (ESI) for Molecular BioSystems.


## SUPPLEMENTARY DATA

### Supplementary Figures

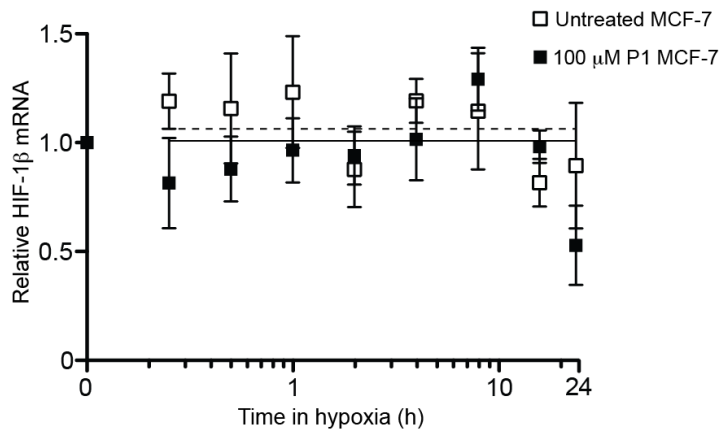



**Figure S1. The effect of P1 on HIF-1β mRNA levels in hypoxia.** The data shows that P1 does not significantly alter HIF-1β expression in hypoxic MCF-7 cells.

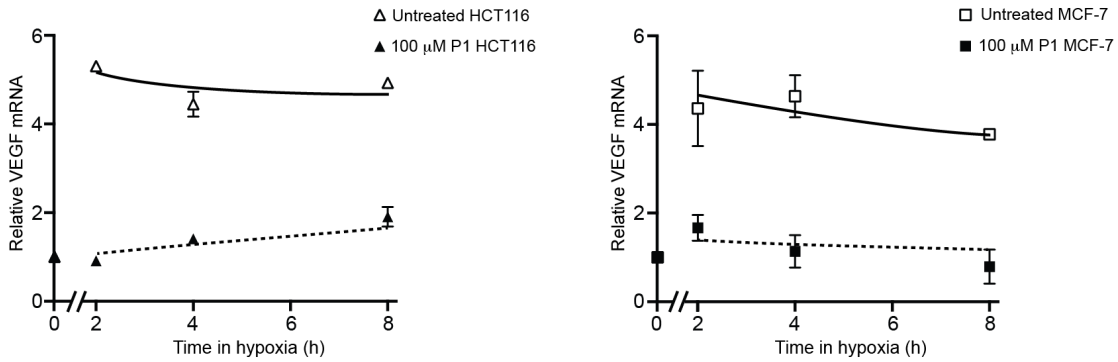
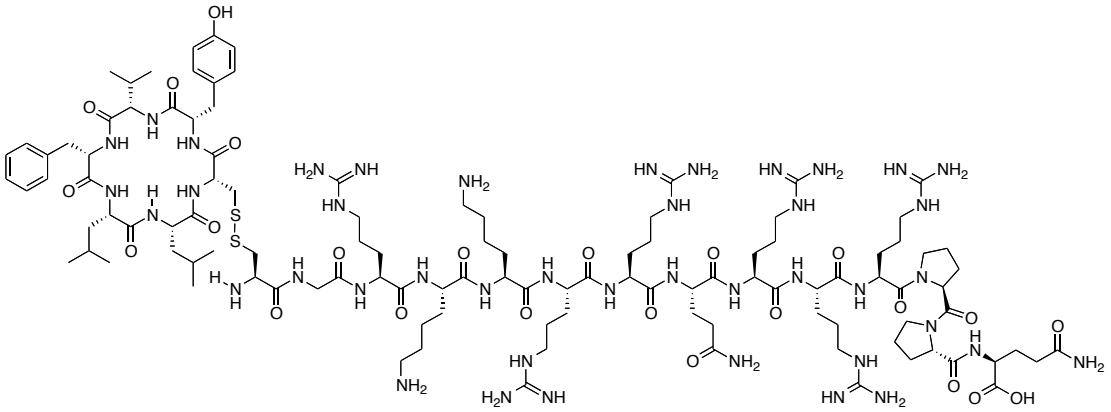


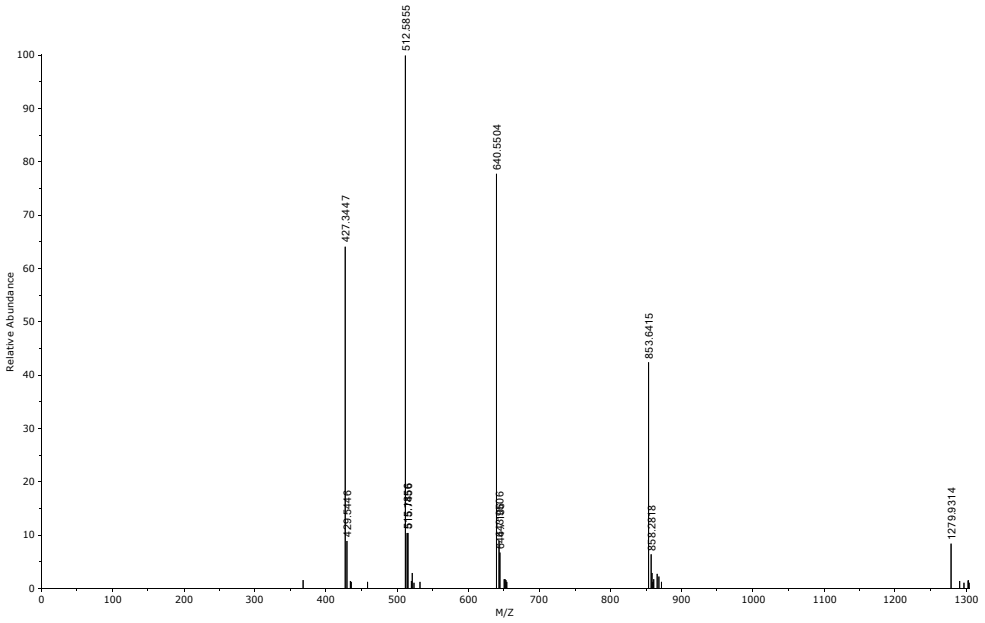

**Figure S2. The effect of P1 on VEGF mRNA as measured by qPCR in hypoxic HCT116 and MCF-7 cells.** The data shows that treatment with P1 leads to reduction of VEGF mRNA to normoxic levels in both cell lines.

## Supplementary Methods

Tat-tagged *cyclo*-CLLFVY (P1) Synthesis



P1 was synthesized by Fmoc solid-phase peptide synthesis as previously detailed. Mass spectrum of the sample used in this study is shown below.[1] $ESI^+$ (m/z) calc. for $C_{111}H_{188}N_{42}O_{24}S_2$ 2557.42, found 1279.9 $[M+2H]^{2+}$, 853.8 $[M+3H]^{3+}$, 640.5 $[M+4H]^{4+}$, 512.5 $[M+5H]^{5+}$, 427.3 $[M+6H]^{6+}$.




Quantitative reverse-transcription polymerase chain reaction (qPCR)

Total RNA was extracted from MCF7, U2OS and HCT116 cells using RNeasy Mini Kit (74104, QIAGEN) and quantified using a Nanodrop ND-1000 spectrophotometer. Complementary DNA was synthesised in a 20µl reaction containing 1µg of total RNA, using qScript™ cDNA SuperMix (95048-100, Quanta Biosciences) according to the manufacturer's instructions. Quantitative real-time PCRs were performed using Universal Taqman PCR master mix (Applied Biosystems) and the Taqman Gene Expression Assay of interest (Applied Biosystems) on a CFX connect Real-Time PCR system (Bio-Rad). Expression

assays used in this study were HIF1α (00936376_m1) and VEGF-A (Hs00173626_m1). All expression values were normalized using the geometric mean of the expression of 18S and TBP.[2]

Western immunoblotting

Cells were washed with ice-cold PBS and lysed by incubation on ice in Radioimmunoprecipitation Assay Buffer (50mM Tris pH 7.4, 150nM NaCl, 1mM EDTA, 1% v/v Triton x-100) and protease inhibitor cocktail (Sigma) for 20 min. Lysates were centrifuged at 14,500 rpm for 15 minutes at 4°C and the protein concentration in the supernatant was quantified by Bradford Assay. Proteins were separated on precast NuPAGE 4% to 12% polyacrylamide gradient Bis–Tris gels (Invitrogen) under denaturing conditions, transferred to PVDF membranes (Invitrogen) and subjected to immunoblot analysis. Mouse monoclonal anti-HIF1α (610958, BD Biosciences) and rabbit anti-β-actin antibody (ab8226, Abcam) antibodies were diluted (1:250 and 1:10000 respectively) in PBS containing 5% non-fat powdered milk and 0.1% Tween-20 and incubated with the membrane overnight at 4 °C. Horseradish peroxidase conjugated secondary antibodies (Cell Signaling) were used. Bound immunocomplexes were detected using ECL prime western blot detection reagent (RPN2232, GE Healthcare).

## References

1. Miranda, E.; Nordgren, I. K.; Male, A. L.; Lawrence, C. E.; Hoakwie, F.; Cuda, F.; Court, W.; Fox, K. R.; Townsend, P. A.; Packham, G. K.; Eccles, S. A.; Tavassoli, A., A cyclic peptide inhibitor of HIF-1 heterodimerization that inhibits hypoxia signaling in cancer cells. *Journal of the American Chemical Society* **2013,** *135* (28), 10418-25.
2. Caradec, J.; Sirab, N.; Keumeugni, C.; Moutereau, S.; Chimingqi, M.; Matar, C.; Revaud, D.; Bah, M.; Manivet, P.; Conti, M.; Loric, S., 'Desperate house genes': the dramatic example of hypoxia. *British journal of cancer* **2010,** *102* (6), 1037-43.