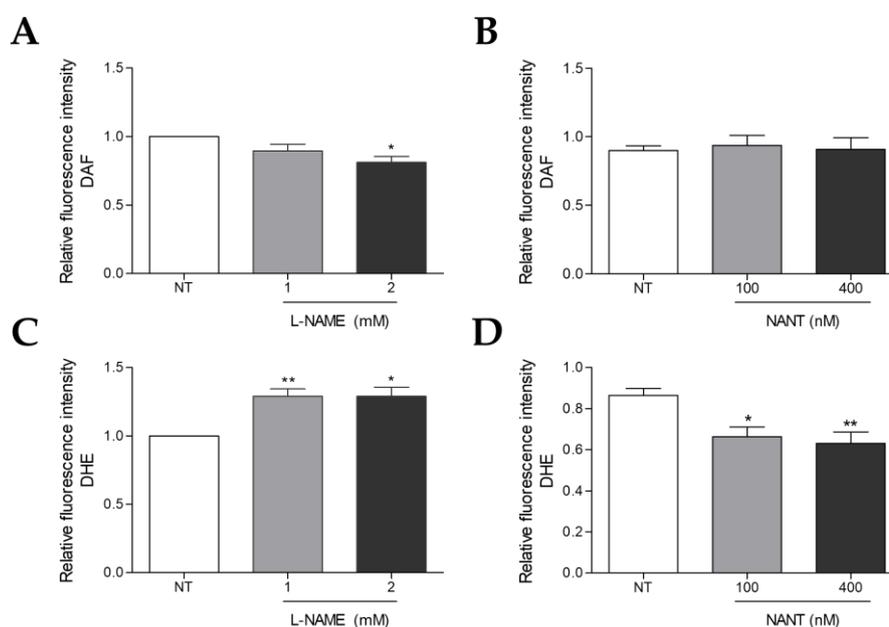


## The differential contribution of tetrahydrobiopterin along melanoma progression

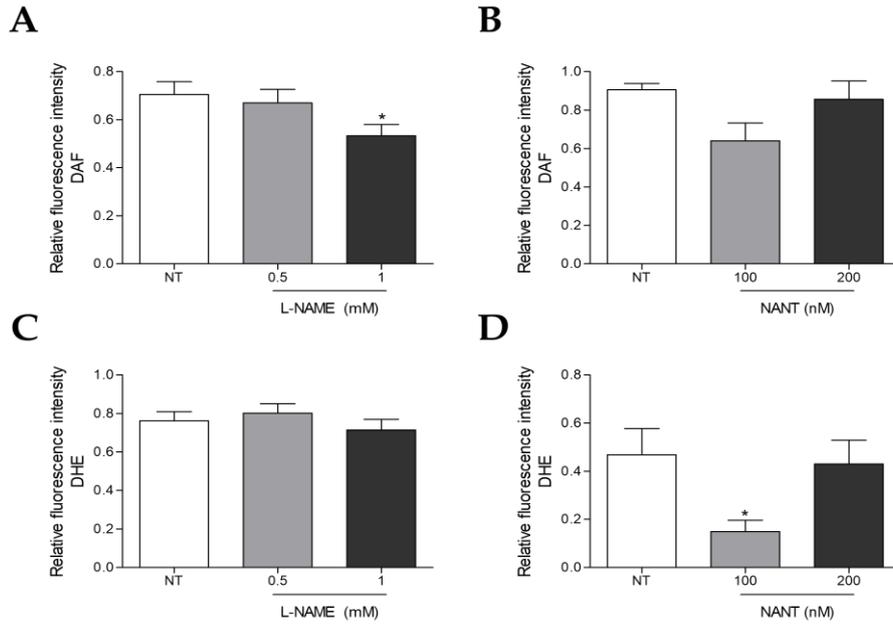
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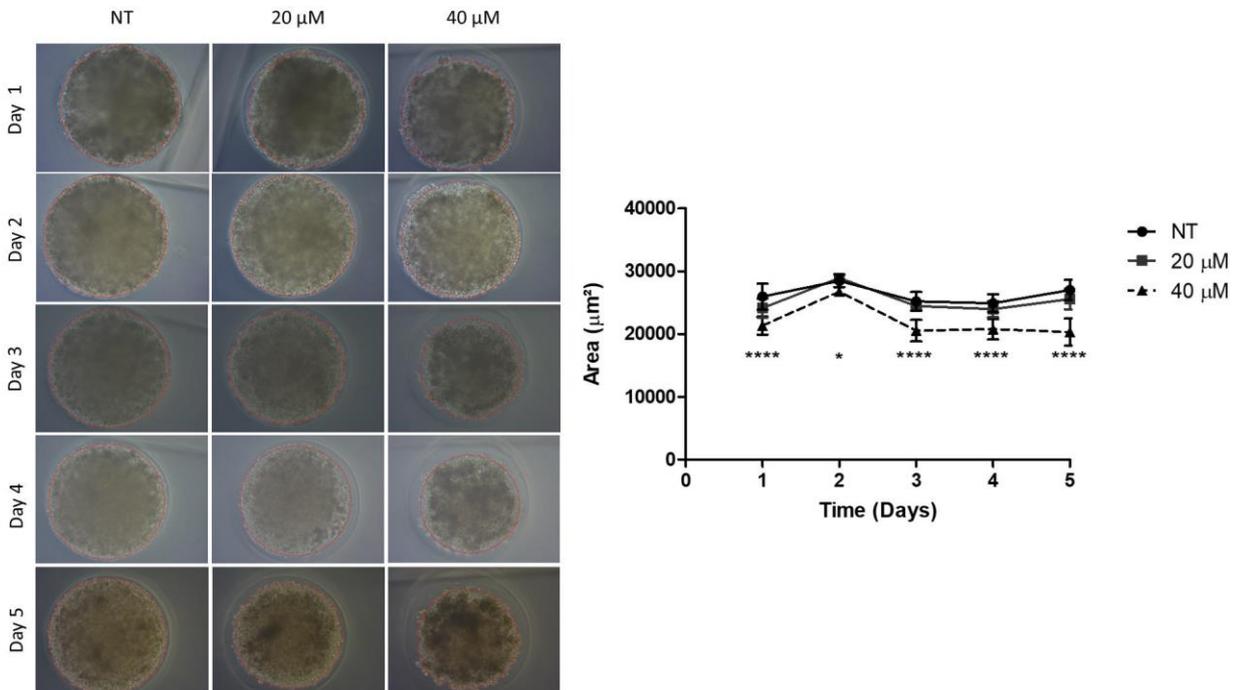
† These authors contributed equally to this work and share first authorship



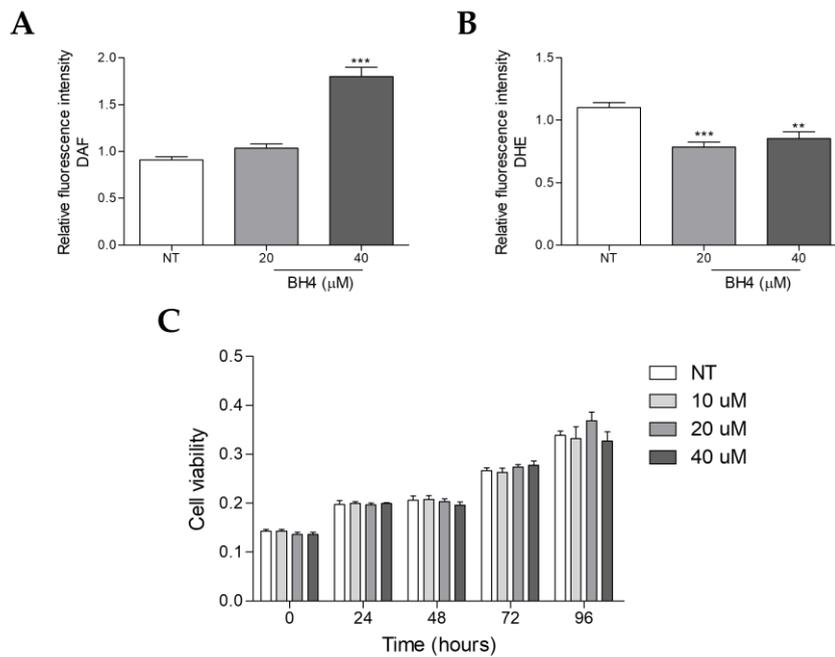
**Figure S1.** Neuronal nitric oxide synthase is uncoupled in radial growth phase melanoma. WM1552C cells were treated or not for 45 min with 1 and 2 mM L-NAME, an eNOS inhibitor (A, C) or 100 and 400 nM NANT, a nNOS inhibitor (B, D). NO amount was evaluated by flow cytometry using DAF (A,B) and  $O_2^{\cdot-}$  levels using DHE (C,D). Values are reported in the bar graphs and expressed as the means  $\pm$  S.D. The experiments were performed in triplicate and p values were based on One-Way ANOVA test followed by Bonferroni's post-test \* $p < 0.05$ , \*\* $p < 0.01$ .



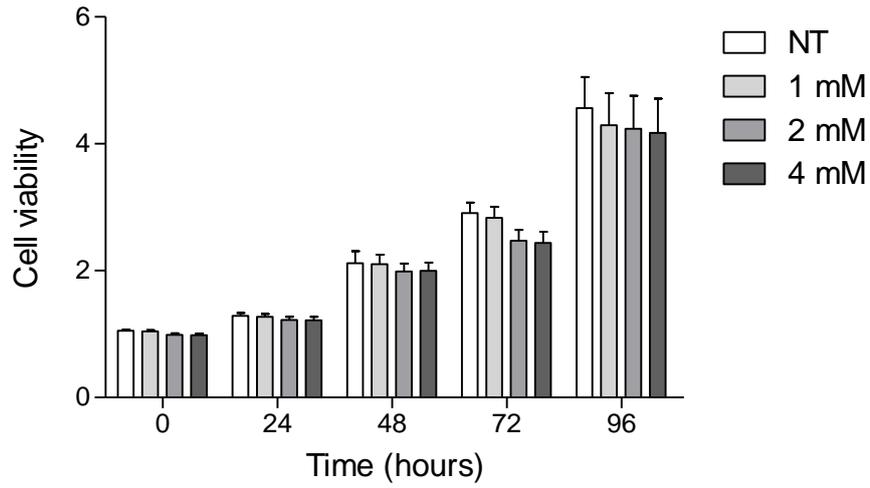
**Figure S2.** Neuronal nitric oxide synthase is uncoupled in metastatic melanoma. WM983B cells were treated or not for 45 min with 0,5 and 1 mM L-NAME, an eNOS inhibitor (A, C) or 100 and 200 nM NANT, a nNOS inhibitor (B, D). NO amount was evaluated by flow cytometry using DAF (A,B) and  $O_2^{\cdot-}$  levels using DHE (C,D). Values are reported in the bar graphs and expressed as the means  $\pm$  S.D. The experiments were performed in triplicate and p values were based on One-Way ANOVA test followed by Bonferroni's post-test \* $p < 0.05$ .



**Figure S3.** Tumorsphere formation reduction in the presence of BH4. WM1552C RGP melanoma cells was treated or not with 20 and 40  $\mu\text{M}$  with BH4 and tumorsphere area was calculated over five days. Values are reported in the bar graphs and expressed as the means  $\pm$  S.D and p values were based on Two-Way ANOVA test followed by Bonferroni's post-test , \* $p < 0.05$ , \*\*\* $p < 0.001$ .



**Figure S4.** The viability of WM983B melanoma cells was not impaired by tetrahydrobiopterin. WM983B was treated or not with 20 and 40  $\mu\text{M}$  with BH4 and NO amount was evaluated by flow cytometry using DAF (**A**) and  $\text{O}_2^{\cdot-}$  levels using DHE (**B**) and with 10, 20 and 40  $\mu\text{M}$  BH4 for 24, 48, 72 and 96 hours and cell viability was evaluated by MTT (**C**). Values are reported in the bar graphs and expressed as the means  $\pm$  S.D. The experiments (**A,B**) were performed in triplicate and p values were based on One-Way ANOVA test followed by Bonferroni's post-test , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . MTT analyses were performed in triplicate and p values were based on Two-Way ANOVA test followed by Bonferroni's post-test \* $p < 0.05$ .



**Figure S5.** Melanocytes viability is not affected by DAHP treatment. NGM melanocytes were treated or not with 1, 2 e 3 mM DAHP, the GTPCHI inhibitor, for 24, 48, 72 and 96 hours and cell viability was evaluated by MTT. The experiments were performed in triplicate and p values were based on One-Way ANOVA test followed by Bonferroni's post-test \* $p < 0.05$ .