

The differential contribution of tetrahydrobiopterin along melanoma progression

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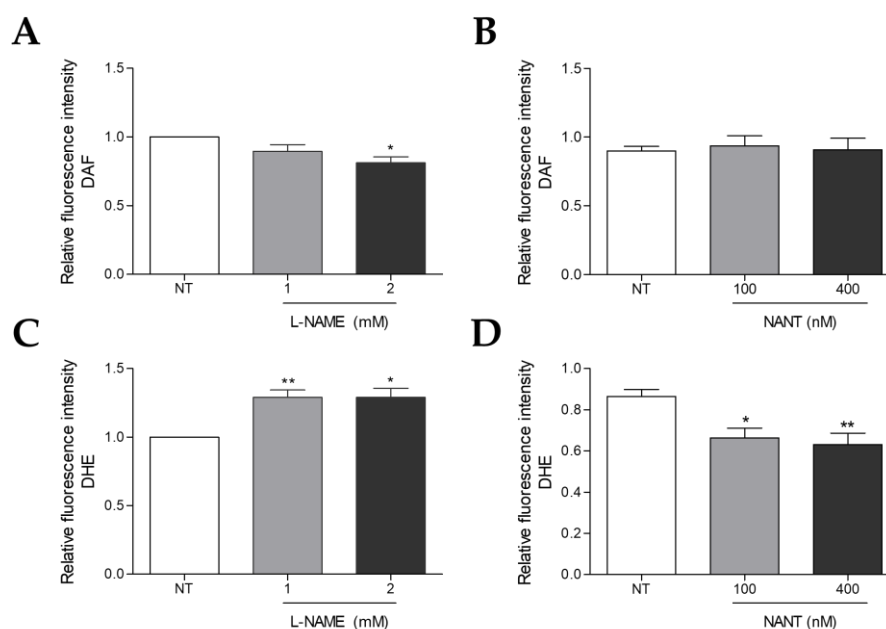


Figure S1. Neuronal nitric oxide synthase is uncoupled in radial growth phase melanoma. WM1552C cells were treated or not for 45 min with 1 and 2 mM L-NAME, an eNOS inhibitor (**A**, **C**) or 100 and 400 nM NANT, a nNOS inhibitor (**B**, **D**). NO amount was evaluated by flow cytometry using DAF (**A**, **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 * $p < 0.05$, ** $p < 0.01$.

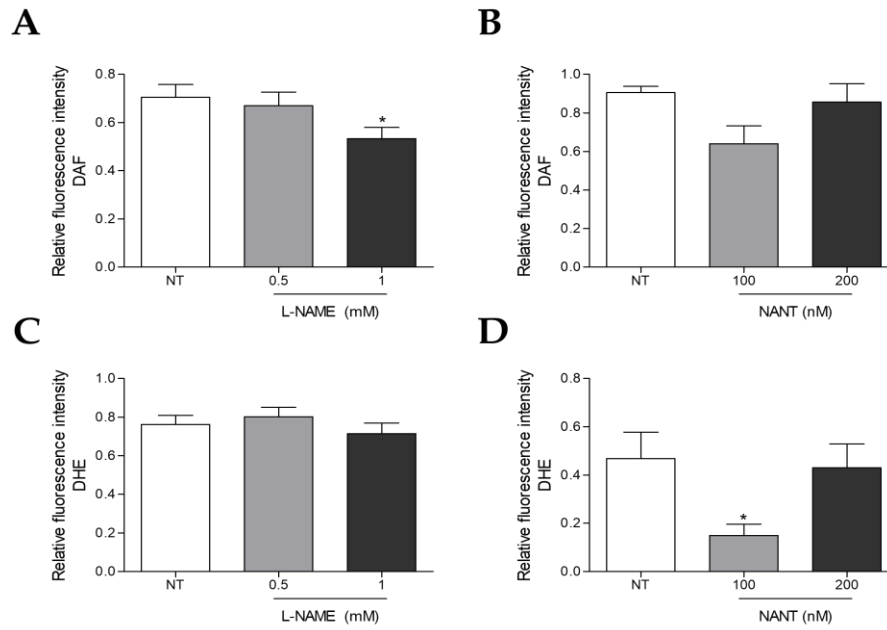


Figure S2. Neuronal nitric oxide synthase is uncoupled in metastatic melanoma. WM983B cells were treated or not for 45 min with 0,5 and 1 mM L-NAME, an eNOS inhibitor (**A, C**) or 100 and 200 nM NANT, a nNOS inhibitor (**B, D**). NO amount was evaluated by flow cytometry using DAF (**A,B**) and $O_2^{\bullet-}$ 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 * $p < 0.05$.

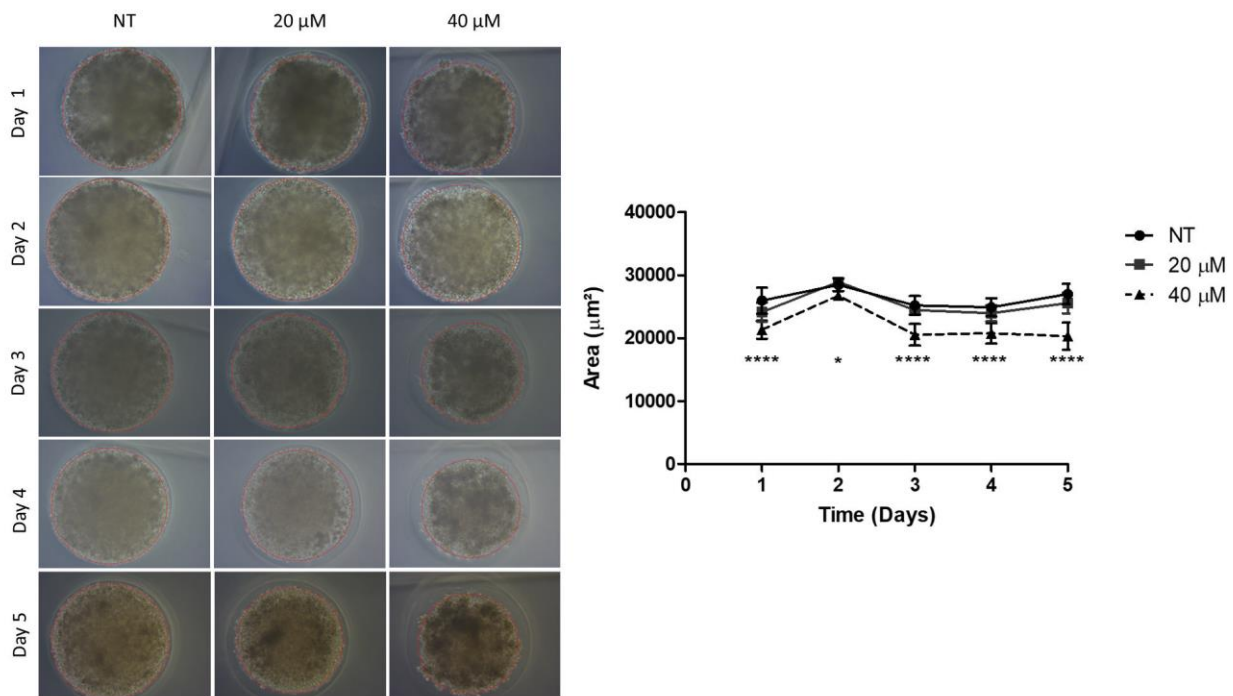


Figure S3. Tumorsphere formation reduction in the presence of BH4. WM1552C RGP melanoma cells was treated or not with 20 and 40 μ M with BH4 and tumorsphere area was calculated over five days. Values are reported in the bar graphs and expressed as the means \pm S.D and p values were based on Two-Way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

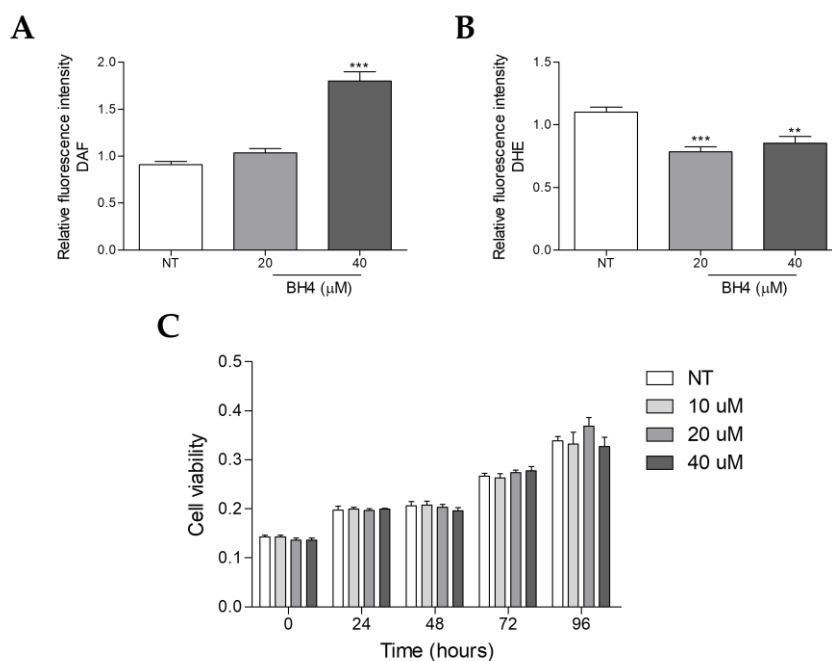


Figure S4. The viability of WM983B melanoma cells was not impaired by tetrahydrobiopterin. WM983B was treated or not with 20 and 40 μ M with BH4 and NO amount was evaluated by flow cytometry using DAF (**A**) and $O_2^{\bullet-}$ levels using DHE (**B**) and with 10, 20 and 40 μ M BH4 for 24, 48, 72 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 * $p < 0.05$.

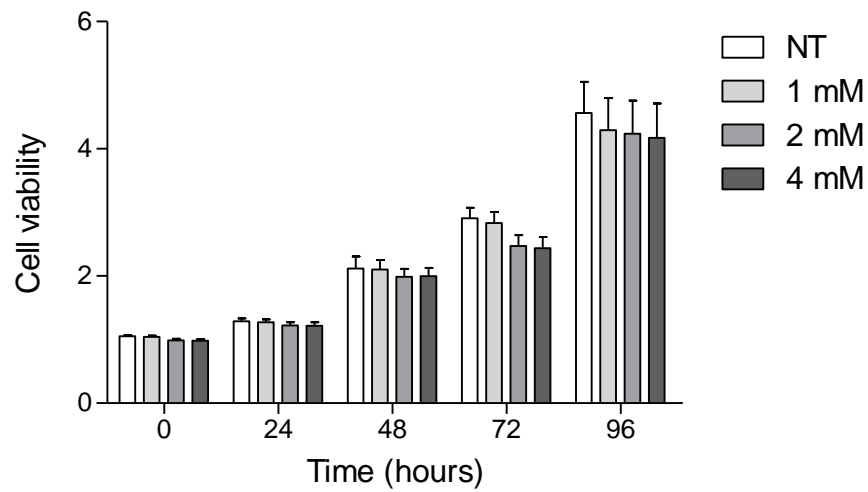


Figure S5. Melanocytes viability is not affected by DAHP treatment. NGM melanocytes were treated or not with 1, 2 e 3 mM DAHP, the GTPCHI inhibitor, for 24, 48, 72 and 96 hours and cell viability was evaluated by MTT. The experiments were performed in triplicate and p values were based on One-Way ANOVA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