

Metastatic melanoma progression is associated with endothelial nitric oxide synthase uncoupling induced by loss of eNOS:BH4 stoichiometry

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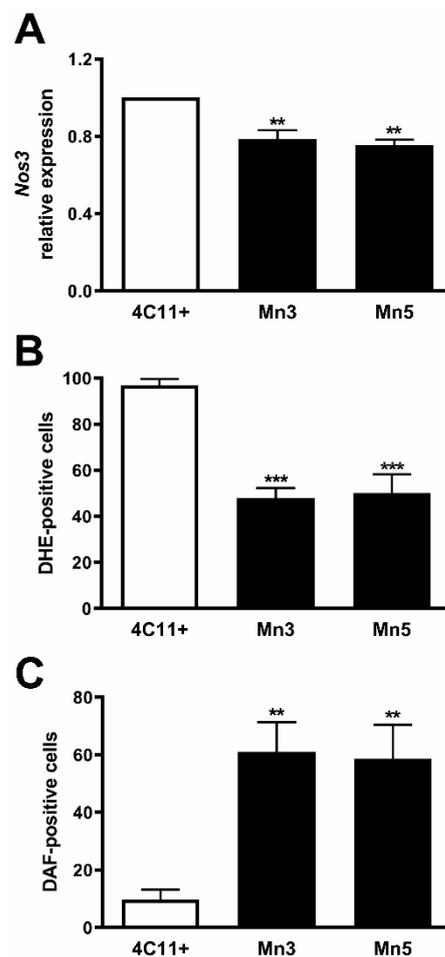


Figure S1. Superoxide depletion decreased *Nos3* expression and restored its activity. **(A)** Relative endothelial nitric oxide synthase mRNA expression was analyzed in 4C11+ metastatic melanoma, Mn3, and Mn5 derived-melan-a cells by real-time qPCR using specific primers. **(B)** Superoxide anion levels were analyzed using DHE and **(C)** nitric oxide amount were evaluated using DAF by flow cytometry. 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 the One-Way ANOVA test followed by Bonferroni post-test * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$.

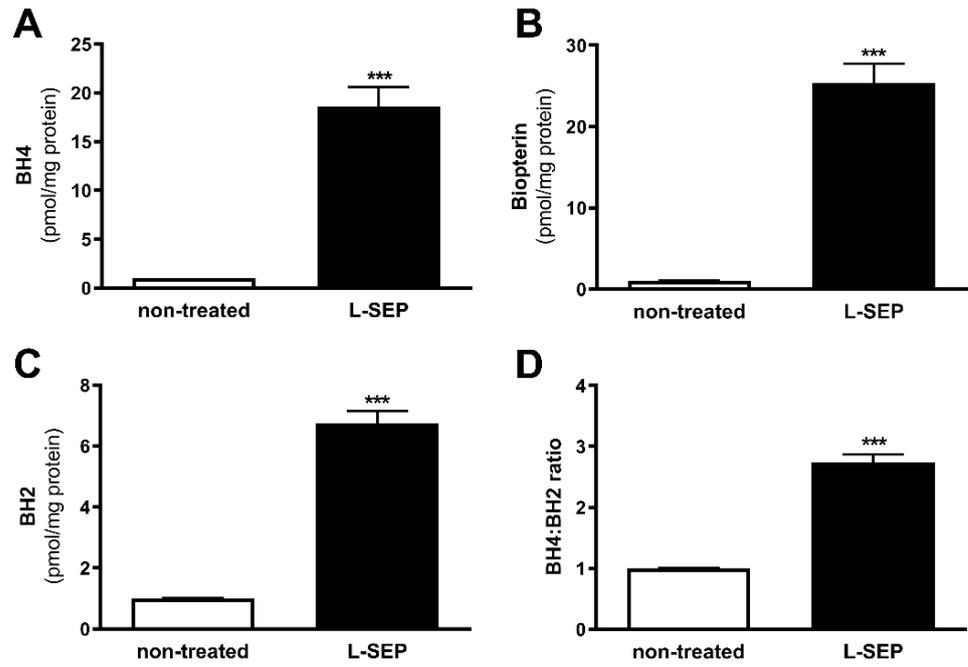


Figure S2. L-sepiapterin treatment increased tetrahydrobiopterin concentration in metastatic melanoma cells. **(A)** The amount of BH4; **(B)** BH2, **(C)** BH4:BH2 ratio and **(D)** total biopterin of 4C11+ metastatic melanoma cells treated or not (non-treated) with 40 μ M L-sepiapterin (L-SEP) were determined by HPLC. 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 Students' t-test ***p<0.001.

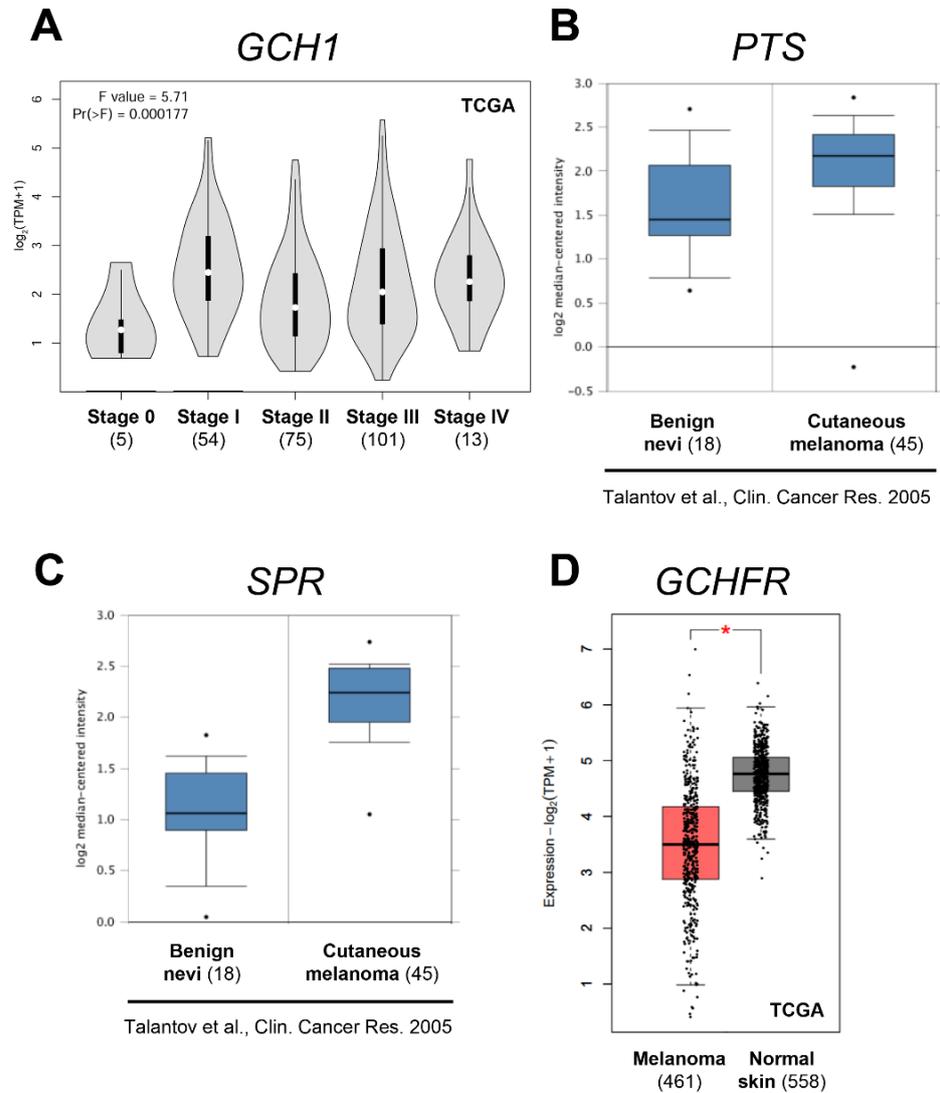


Figure S3. Expression of enzymes from BH₄ *de novo* synthesis pathway in human melanoma compared with benign nevi and normal skin. **(A)** Increased expression of *GCH1* along with melanoma progression (TCGA) (n = 248). **(B)** Cutaneous melanoma (n = 45) express higher levels of *PTS* and **(C)** *SPR* compared to benign nevi (n = 18) (GSE3189). Boxes represent the interquartile range (25th–75th percentile). Microarray data by the Talantov study was obtained from the Oncomine database as indicated. The bars denote the median. $P < 1E5$. **(D)** Melanoma (n = 461) express lower levels of *GCHFR* compared to normal skin (n = 558). Data regarding melanoma progression was obtained from gepia2 (<http://www.gepia2.cancer-pku.cn/#analysis>).