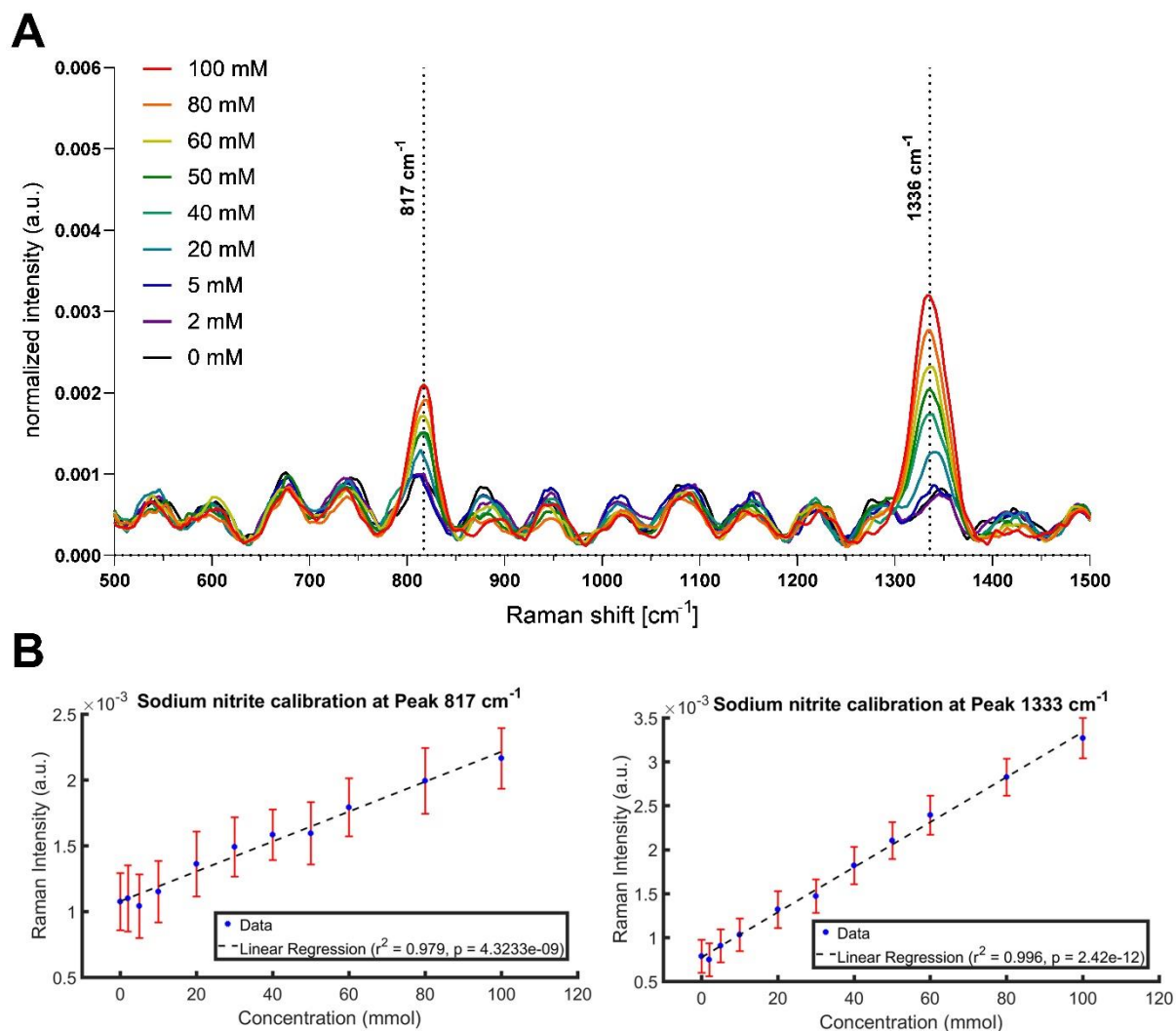
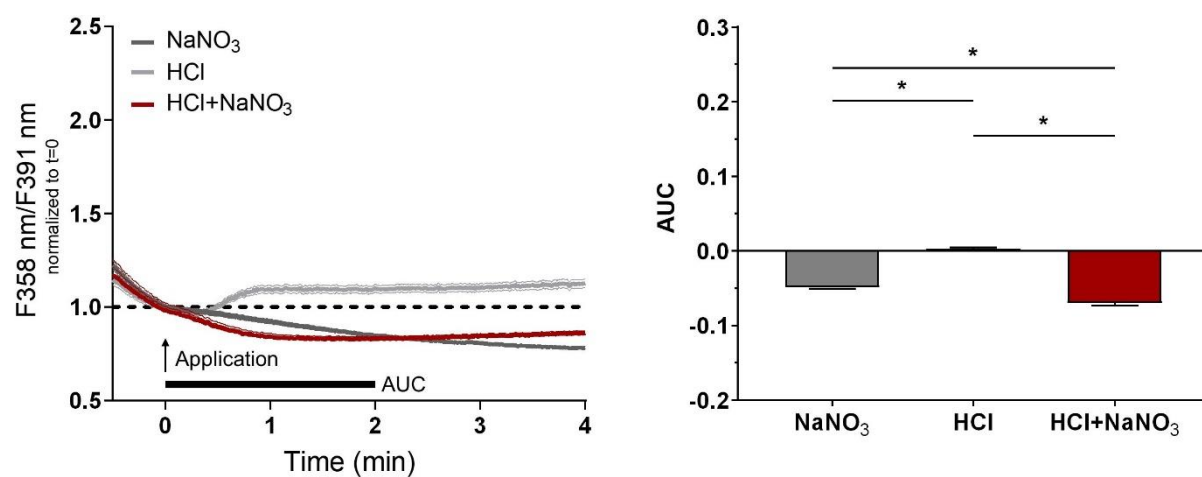


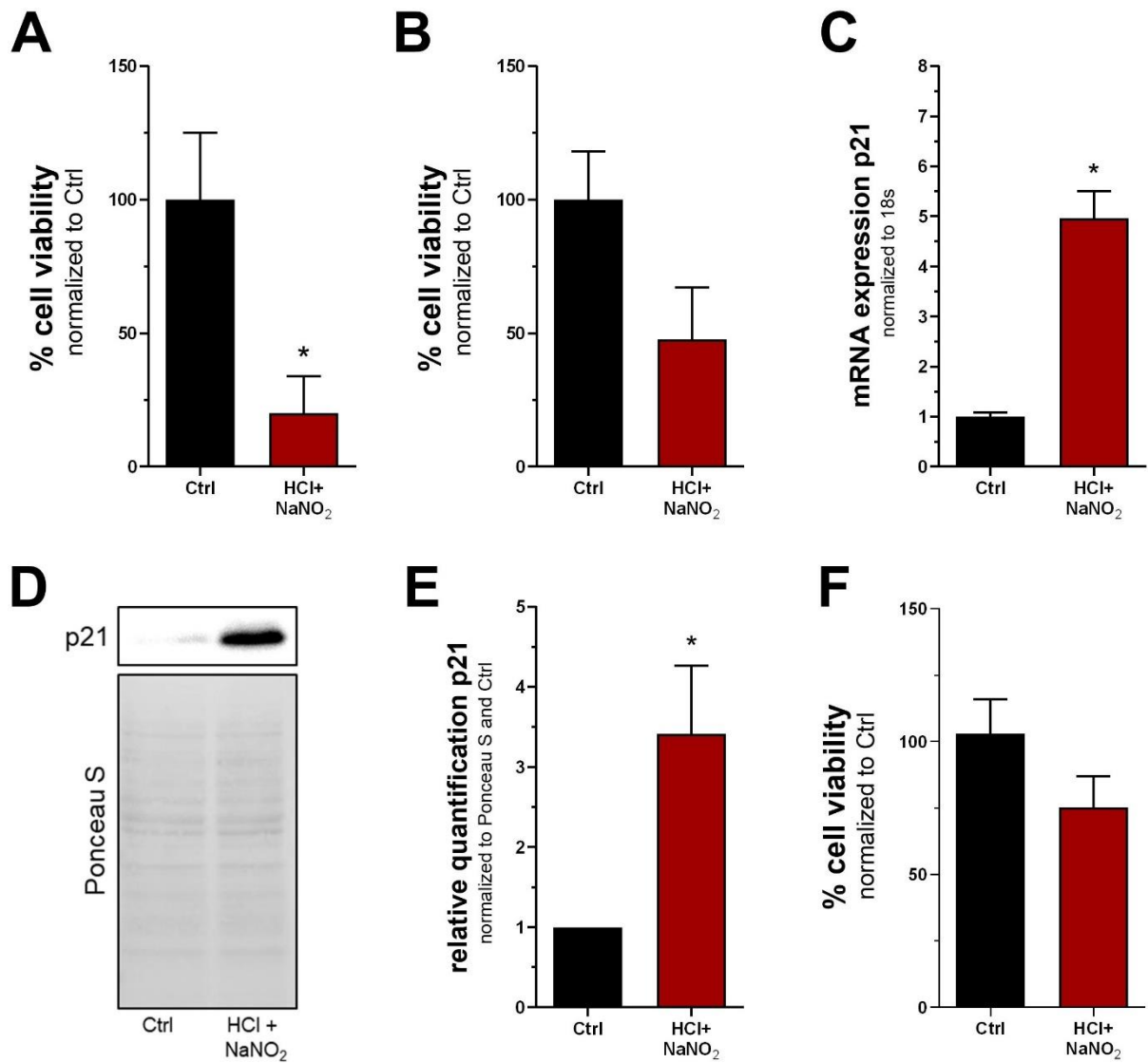
**Figure S1.** Calibration of Raman spectroscopy to potassium nitrate solutions of known concentration. **(A)** Average Raman spectra of the different concentrations. **(B)** Raman intensity of these solutions at  $1048 \text{ cm}^{-1}$ . Data are shown as mean with standard deviation error bars ( $n=100$ ). A linear regression model is shown as dotted line and Anova with subsequent F-test were performed to determine  $r^2$  and  $p$  value.



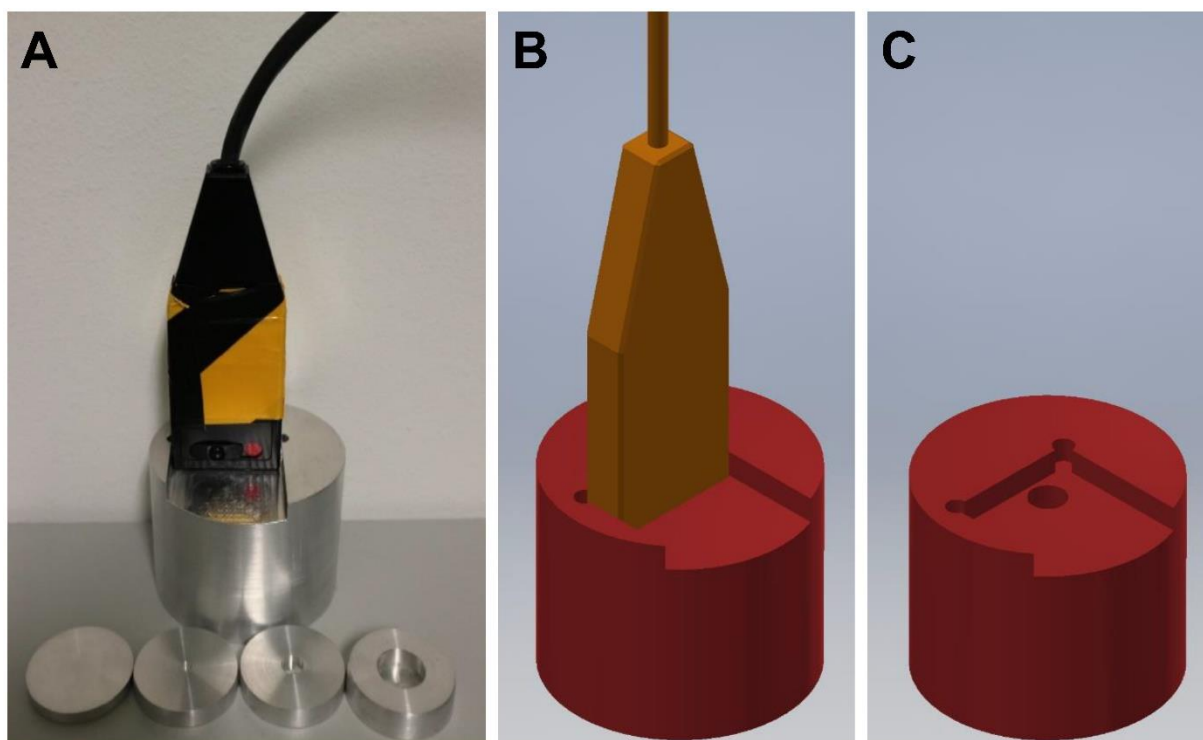
**Figure S2.** Calibration of Raman spectroscopy to sodium nitrite solutions of known concentration. **(A)** Average Raman spectra of the different concentrations. **(B)** Raman intensity of these solutions at 817  $\text{cm}^{-1}$  and 1336  $\text{cm}^{-1}$ . Data are shown as mean with standard deviation error bars ( $n=100$ ). A linear regression model is shown as dotted line and Anova with subsequent F-test were performed to determine  $r^2$  and  $p$  value.



**Figure S3.** Acidic nitrate solution does not induce a cytoplasmic  $\text{Ca}^{2+}$  release in melanoma cells. Time course of cytoplasmic  $\text{Ca}^{2+}$  levels due to a 4-minute treatment with acidic ECS ( $\text{HCl}$ ), nitrate solution ( $\text{NaNO}_3$ ), or a combination of both. Traces are mean with 95% confidence interval, bars are mean  $\pm$  SEM (ANOVA followed by Tukey's HSD post-hoc test,  $F_{(2,863)} = 242.4$ ,  $p < 0,0001$ ,  $n = 286 - 293$ , \*:  $p < 0.05$ ).



**Figure S4.** Effects of acidic nitrite solution in melanoma cell line Mel Im (A-E) and normal human fibroblasts (F). (A) Cell viability analysis 24 h after a 5 min treatment with physiological ECS (Ctrl) or a combination of acidic ECS and nitrite. (B) Cell viability as described in (A), but with HEPES-free pbECS. Every other experiment in this figure was done using HEPES-buffered ECS. Expression (C) as well as Western blot (D,E) analysis of p21 24 h after 5 min treatment with acidic nitrite solution. (F) Cell viability analysis of normal human fibroblasts 24 h after 5 min treatment with physiological ECS or a combination of acidic ECS and nitrite. Bars are mean  $\pm$  SEM (Student's t-test,  $n=3$ , \*,  $p < 0.05$ ).



**Figure S5.** Custom designed experimental chamber for Raman spectroscopy. (A) The Raman probe inside the aluminum chamber with several carriers of different sample volume. (B,C) CAD model of the chamber with and without the probe.