

Supporting Information

Temporal inhibition of mouse mammary gland cancer metastasis by CORM-A1 and DETA/NO combination therapy

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Supplementary Figures

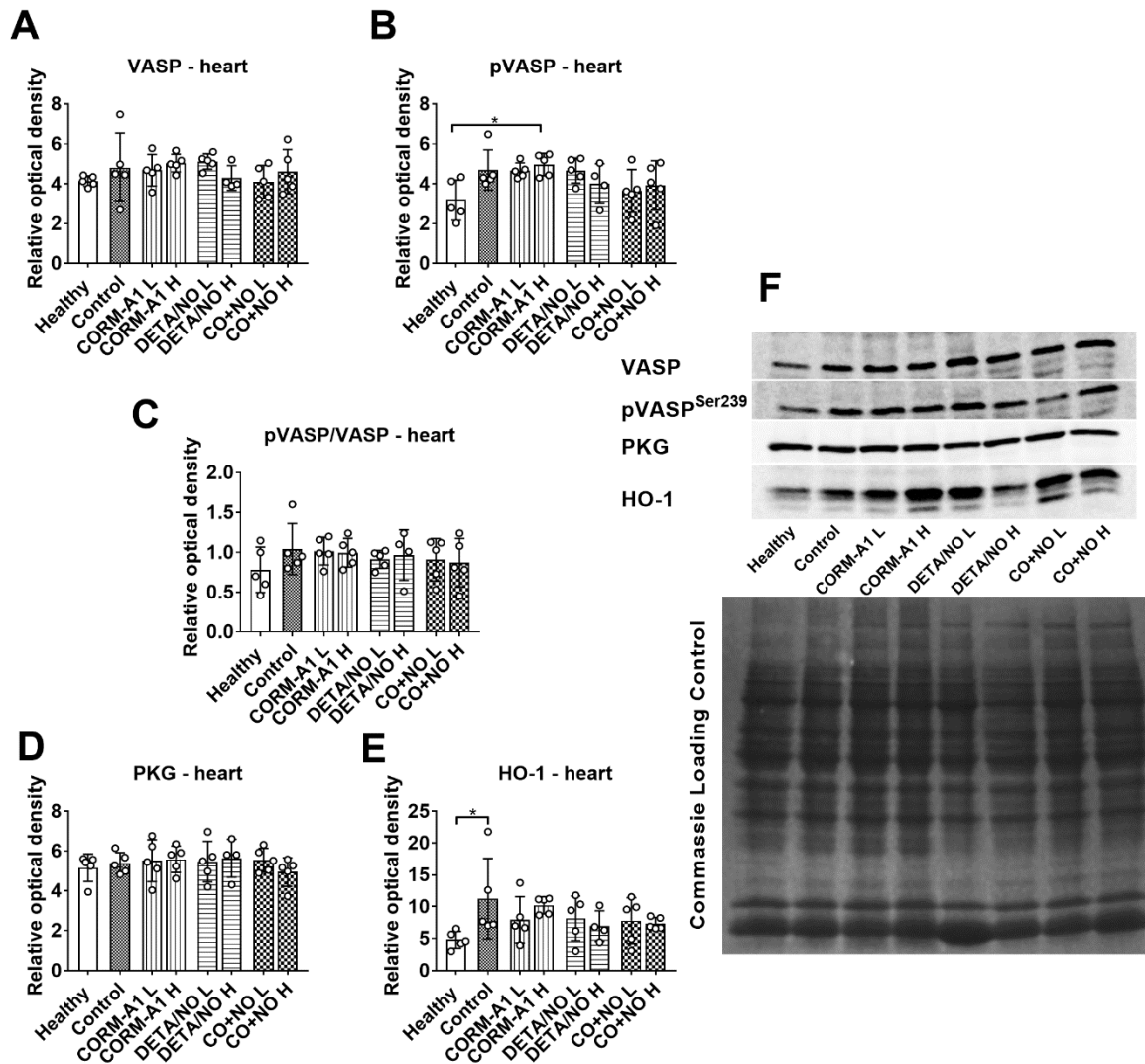


Figure S1. The impact of CORM-A1 and DETA/NO on protein levels in heart tissue in the experimental models of metastasis of 4T1-luc2-tdTomato cells. (A) Total VASP, (B) pVASP^{Ser239}, (D) PKG and (E) HO-1 densitometric analysis (mean optical density of bands of protein tested to Commassie loading control) and representative blots (F). (C) Calculated pVASP^{Ser239}/VASP ratio. “L” means lower dose of CORM-A1 and DETA/NO (0.5 mg/kg/12h and 1.57 mg/kg/24h, respectively; treatment schedule indicated by yellow circle in Scheme 1A and Figure 1A). “H” means higher dose of CORM-A1 and DETA/NO (1.5 mg/kg/12h and 2.358 mg/kg/24h, respectively; treatment schedule indicated by green circle in Scheme 1A and Figure 1A). Data presented as mean with standard deviation with points for individual measurements. Number of mice: 4-5 per group. Healthy mice used as a control: 4-5. Statistical analysis: (B) Sidak’s multiple comparison tests. * $p < 0.05$ as compared to control or as indicated.

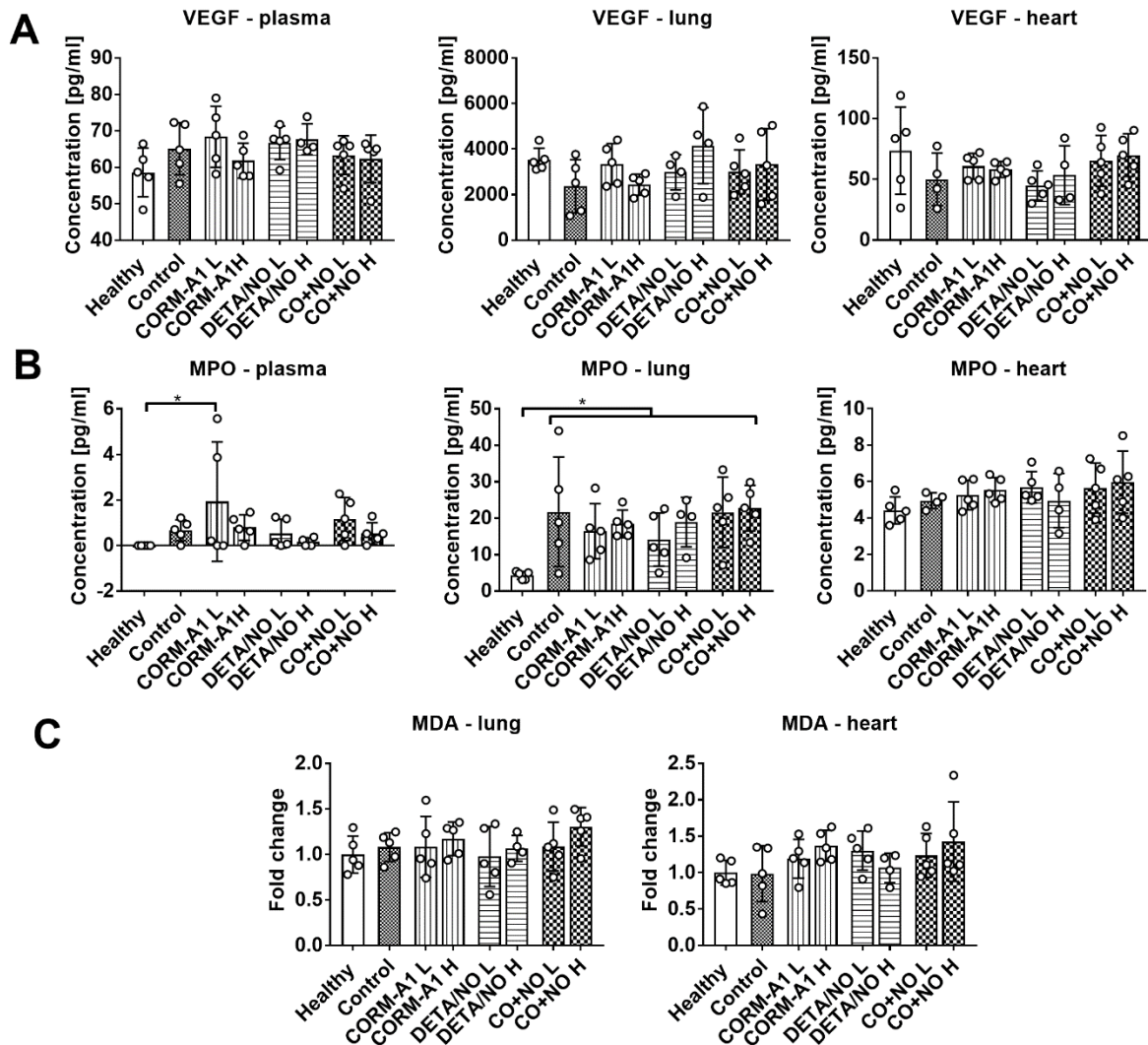


Figure S2. (A) VEGF and (B) myeloperoxidase (MPO) measured in plasma, lung and heart tissue as well as (C) lipids peroxidation (malondialdehyde, MDA level) measured in lung and heart tissue in the experimental models of metastasis of 4T1-luc2-tdTomato cells. “L” means lower dose of CORM-A1 and DETA/NO (0.5 mg/kg/12h and 1.57 mg/kg/24h, respectively; treatment schedule indicated by yellow circle in Scheme 1A and Figure 1A). “H” means higher dose of CORM-A1 and DETA/NO (1.5 mg/kg/12h and 2.358 mg/kg/24h, respectively; treatment schedule indicated by green circle in Scheme 1A and Figure 1A). Data presented as mean with standard deviation with points for individual measurements. Number of mice: 4-5 per group. Healthy mice used as a control: 4-5. Statistical analysis: Dunnett’s multiple comparison tests. * $p < 0.05$ as compared to control or as indicated.

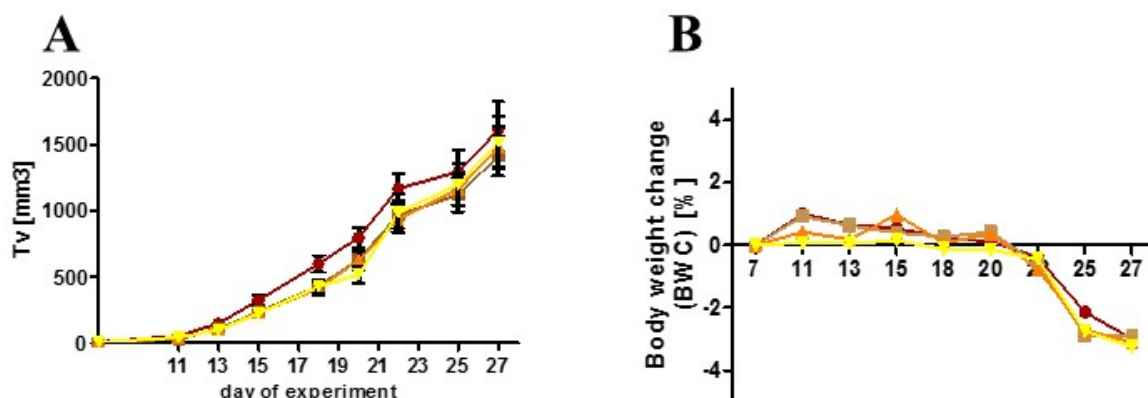


Figure S3. The influence of DETA/NO, CORM-A1 and their combination on the (A) tumor growth kinetics; (B) body weight change of BALB/c mice bearing 4T1 tumors. The treatment started 7 days after tumor cells transplantation. Mice were euthanized, and tissue samples were collected for further analysis on the day 14, 21 and 28 after tumor cells transplantation to investigate the action of substances in the different stages of tumor progression. DETA/NO was administered intraperitoneally (i.p.) 1.6 mg/kg/24h. CORM-A1 was administered intraperitoneally (i.p.) 0.5 mg/kg/12h. The number of mice in the experiment: 8 - 12. The results are presented as a mean values \pm standard deviation (A) or calculated as BWC from the formula:

$$BWC [\%] = \frac{BW_N}{BW_0} \times 100 - 100\%$$

BWC – Body weight change [%]

BW_N – The average body weight of mice in a group on the n-day of the experiment

BW_0 – The average body weight of mice in a group on the day 0 of experiment

Designations:

■ - control; □ - DETA/NO (NO); ▲ - CORM-A1 (CO); ◆ - DETA/NO + CORM-A1 (NO + CO).

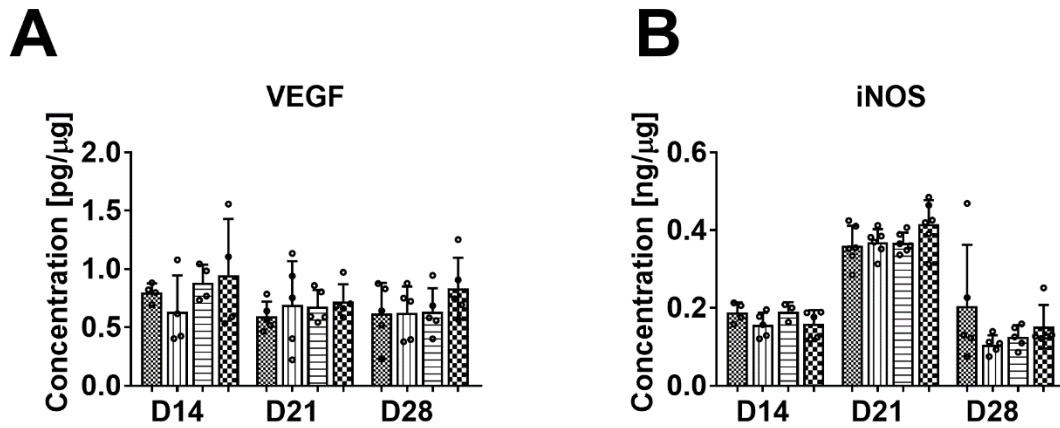


Figure S4. The effect of DETA/NO, CORM-A1 and their combination on the agents of inflammation and vessel formation. The estimation was carried out on mouse plasma using enzyme-linked immunosorbent assay (ELISA) on the 14th (D14), 21th (D21), 28th (D28) day of experiment. **(A)** Vascular endothelial growth factor (VEGF); **(B)** Inducible NO synthase (iNOS). **Statistical analysis:** Mann-Whitney U test; mean \pm SD; * $p < 0.05$ compared to control group. **Designations:** ■ - Control, ▨ - CORM-A1, ▩ - DETA/NO, ▩ - CO + NO

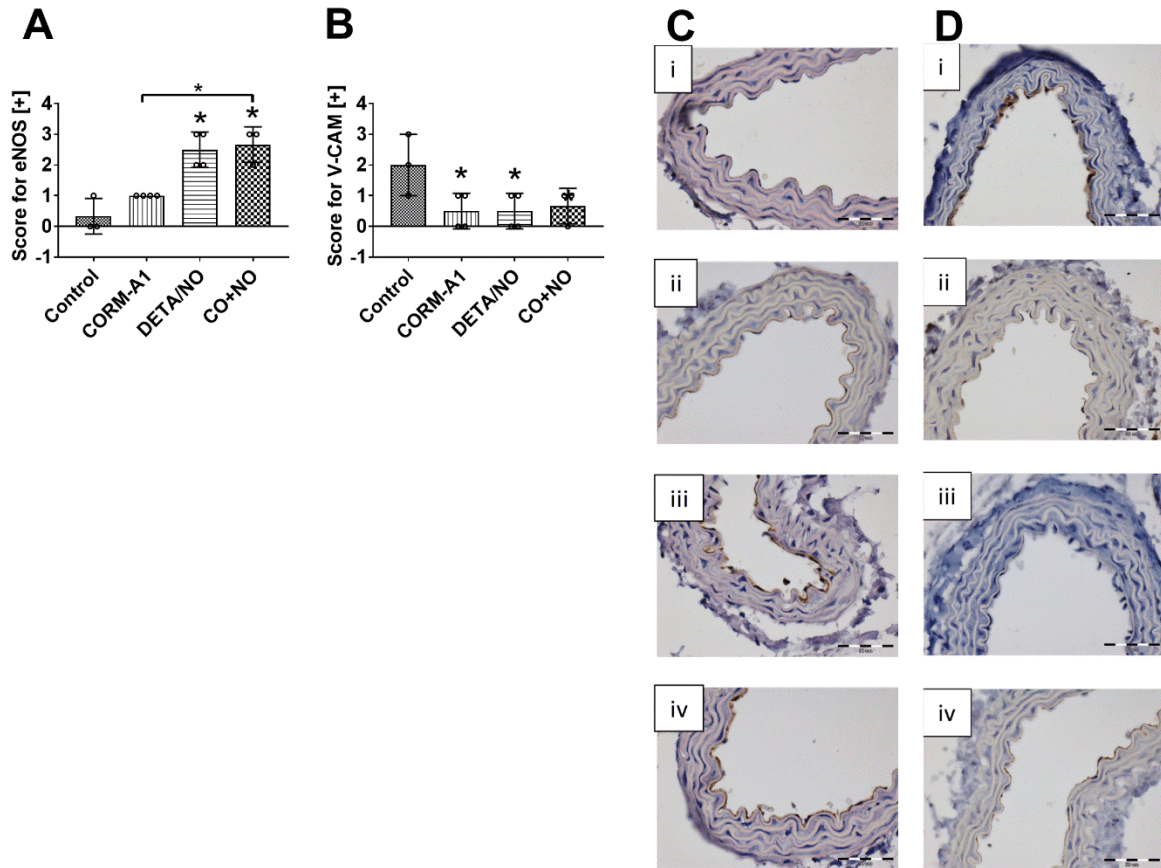


Figure S5. The effect of DETA/NO, CORM-A1 and their combination on the eNOS and V-CAM expression in mice aorta evaluated on day 21. 4% PFA fixed mice aorta were stained using mouse anti-eNOS and mouse anti-VCAM/CD106 antibody. Quantification performed in IRS scale: no reaction (-); $\leq 10\%$ cells with positive reaction and low intensity of color reaction (+), 11%-50% cells with positive reaction and average intensity of color reaction (++); 51%-80% cells with positive reaction and intense color reaction (+++); $>80\%$ cells with positive reaction (++++). **(A)** eNOS expression; **(B)** V-CAM expression. **(C)** Representative microphotographs of aorta sections stained with anti-eNOS antibody and **(D)** anti-VCAM antibody. Scale bar = 50 μm . i – control; ii – CORM-A1; iii – DETA/NO; iv – CO+NO. Aortas from 3-4 mice per group were analyzed. Statistical analysis: Kruskal-Wallis test with Dunn's test for multiple comparisons. * $p < 0.05$ as compared to control tumor-bearing mice.