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*Supplemented data*

# Nitro-oleic acid inhibits stemness maintenance and enhances neural differentiation of mouse embryonic stem cells via STAT3 signaling

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## 1. Methods

*1 .1. Cytotoxicity assay*

To determine the cytotoxicity of nitro oleic acid ($NO_2$-OA) on mouse embryonic stem cells (mESC), the cells were cultured in complete medium with or without leukemia inhibitory factor on gelatin-coated 24-well plates. Duplicate wells were exposed to $NO_2$-OA at concentration of 0, 1, and 10 μM. After 72 h, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) assay was performed. MTT was added to cells at 2.5 mg/ml. After 2 h, the medium was aspired and 10 % Triton X-100 was added. The quantity of formazan was measured after 5 min incubation by the absorbance at 570 nm using a plate reading spectrophotometer.

## 2. Figures

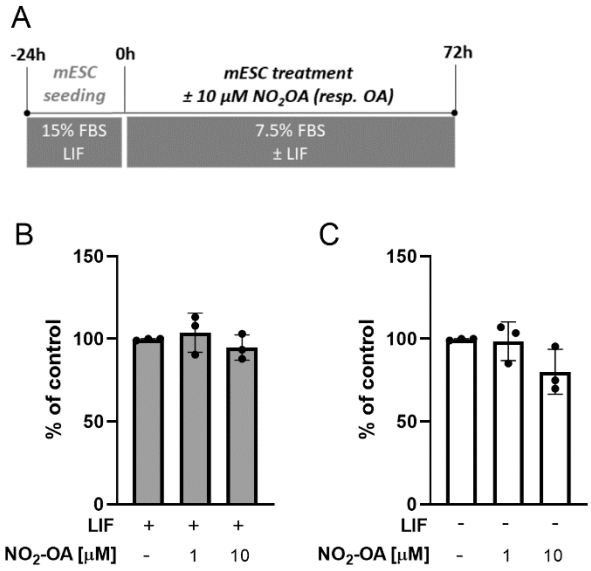


**Figure S1: Cytotoxic effects of $NO_2$-OA treatment on mouse embryonic stem cells (mESC).** Cytotoxicity of $NO_2$-OA was analyzed after 72h of incubation at different concentration (0, 1, and 10 μM), with (B) or without (C) leukemia inhibitory factor (LIF). Data passed Shapiro-Wilk test for normal distribution. Results are expressed as means and standard deviation from three independent experiments. Statistical significance was determined by Unpaired Student's t-test; statistical differences compare to non-treated control were analyzed.

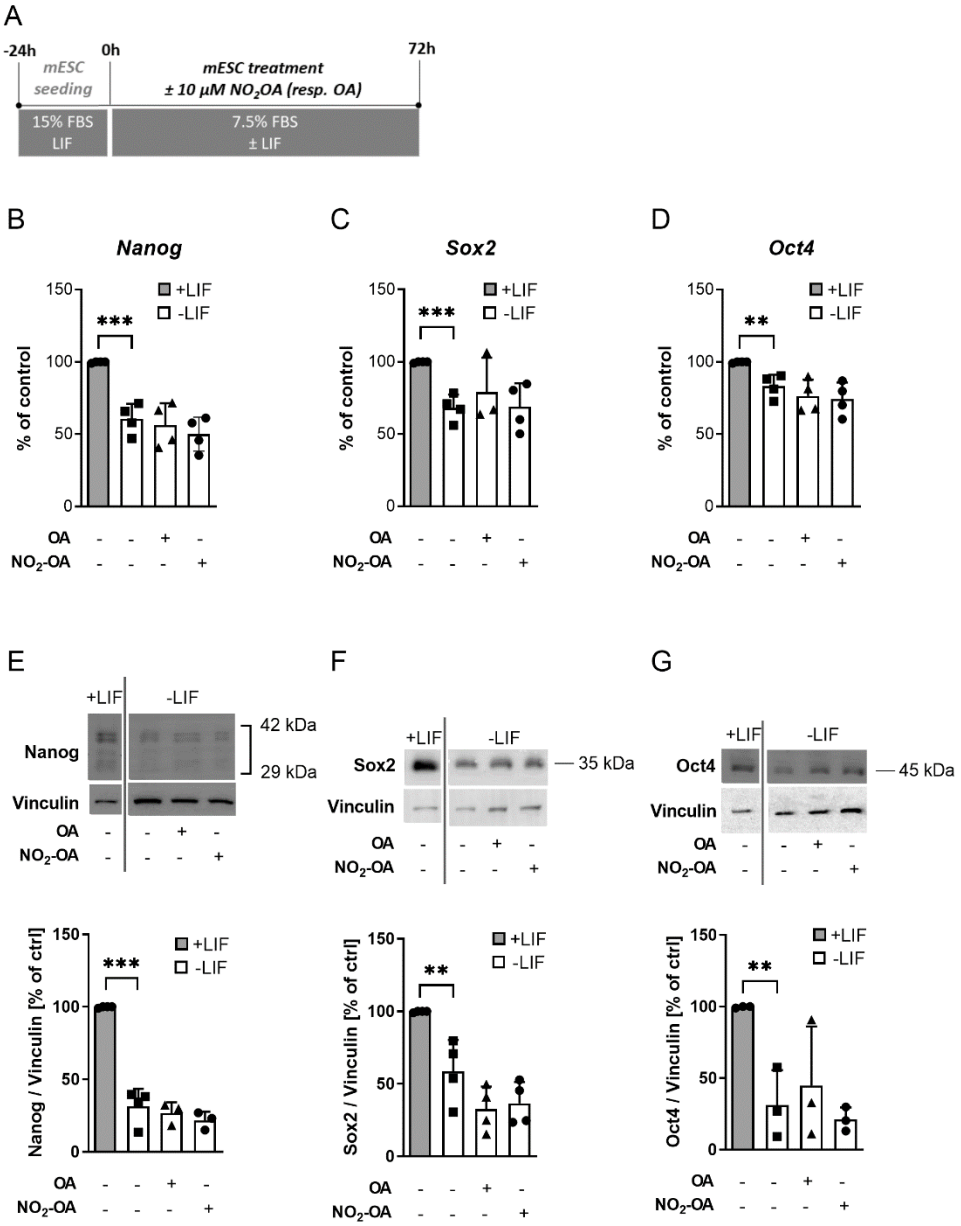


**Figure S2: $NO_2$-OA does not affect the expression of pluripotency markers without the presence of leukemia inhibitory factor (LIF) in mouse embryonic stem cells (mESC).** (A) Time-scheme of the mESC treatment used in this experiment. (B-G) The levels of selected markers of pluripotency; (B) Nanog, (C) Sex-determining region Y-box 1 transcription factor (Sox2), and (D) octamer-binding transcription factor 4 (Oct4) were evaluated by real-time quantitative PCR (B-D) and western blot (E-G) in mESC incubated for 72 h in experimental medium ± leukemia inhibitory factor + 7.5% FBS and treated by NO2-OA or OA (10 μM). Data passed Shapiro-Wilk test for normal distribution. Results are expressed as means and standard deviation from at least three independent experiments. Statistical significance was determined by Unpaired Student's t-test; statistical differences compare to corresponding non-treated control were analyzed, **p<0.01, ***p<0.001.

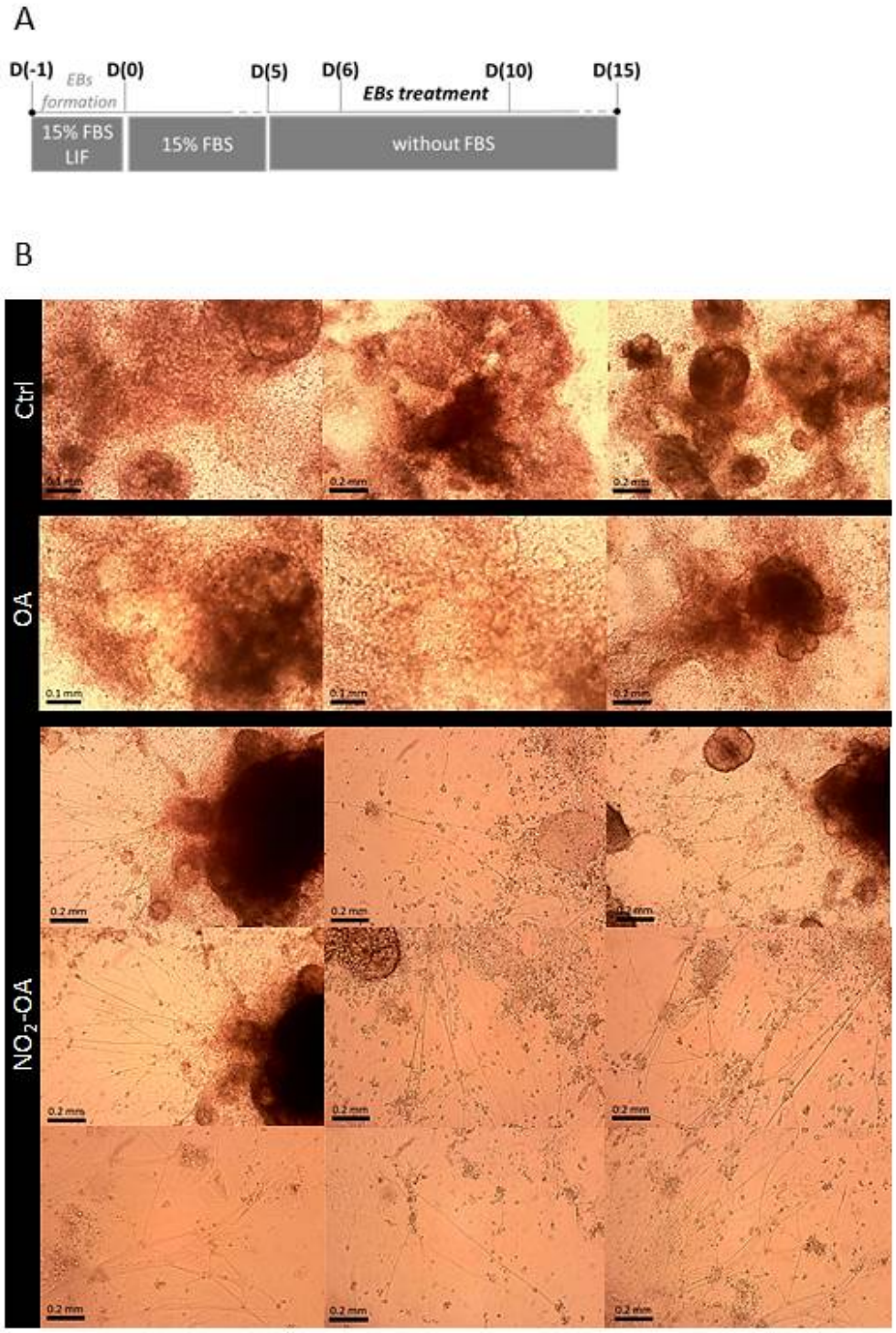


**Figure S5: $NO_2$-OA enhances neural differentiation in mESC-derived EBs.** The morphological study was performed using light microscope. (A) Time-scheme of the mESC treatment used in this experiment. (B) Cells in control (Ctrl), OA and $NO_2$-OA groups were analyzed at day 14 of EBs differentiation.