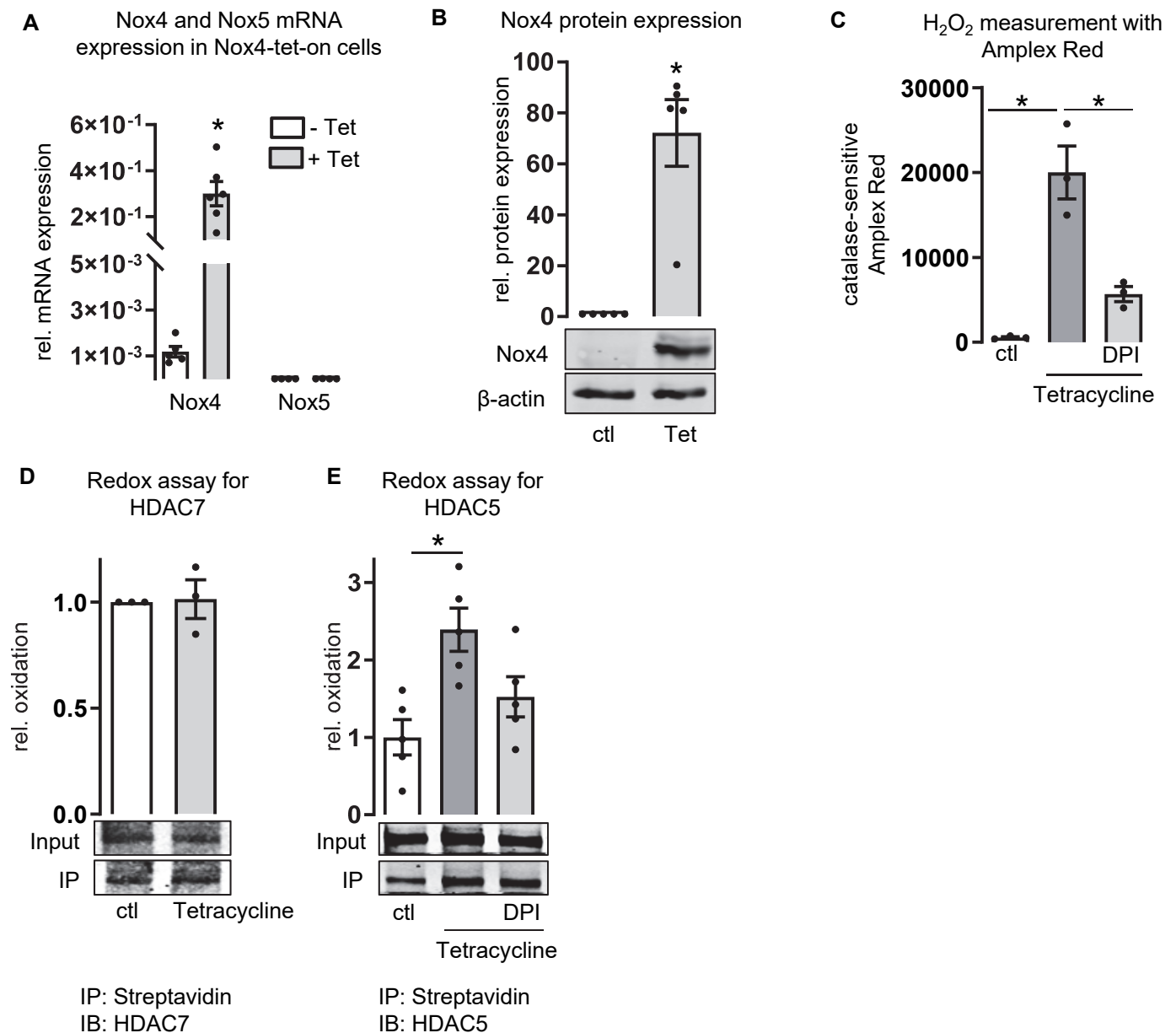
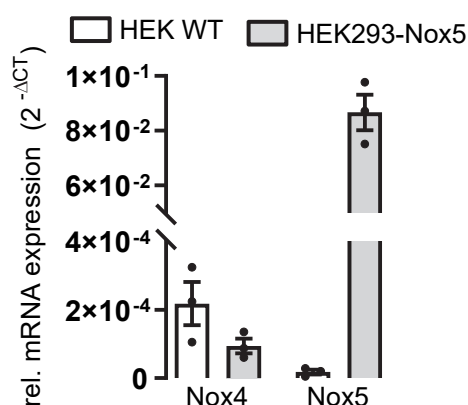


Supplemental figure 1: Relative mRNA expression of class IIa HDACs in HUVECs (n=3) (A), HMECs (n=3) (B), HEK293 (n=1) (C) and HEK293 Nox4-tet-on cells (n=3) (D) analyzed by qPCR. (E) HMECs were treated with H₂O₂ (10/100/1000 μ M, 6 h). Relative mRNA expression of HDAC4 was determined by qPCR (n>5). All bar graphs show means \pm SEM.

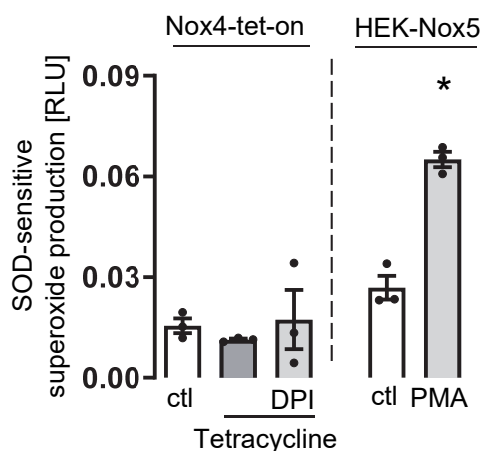


Supplemental figure 2: Characterization of HET-tet-on cells. (A) Analysis of Nox4 and Nox5 mRNA expression (n=6) and (B) Nox4 protein expression in Nox4 tet-on cells with and without tetracycline (Tet, 1 µg/ml, 24 h) induction by qPCR (n=5). mRNA and protein expression were normalized to β-actin (Unpaired t-test; One-sample t-test *p<0.05). (C) Amplex Red assay. Catalase-sensitive H₂O₂ measurement of tetracycline-inducible Nox4 overexpression in Nox4-tet-on cells with and without Tet (1 µg/ml, 24 h) and DPI (3 h, 3 µM). PEG-catalase (500 U/ml) was added before Amplex Red reagent (50 µM) (n=3) (One-way ANOVA with Tukey post-hoc test, *p<0.05). All bar graphs show means ± SEM. (D+E) BIAM switch redox assays for HDAC7 (n=3) (D), HDAC5 (n=5) (E) in Nox4-tet-on cells with or without tetracycline (1 µg/ml, 24 h) and DPI (3 µM, 3 h). One representative Western blot is depicted. (One-way ANOVA with Tukey post-hoc test, *p<0.05).

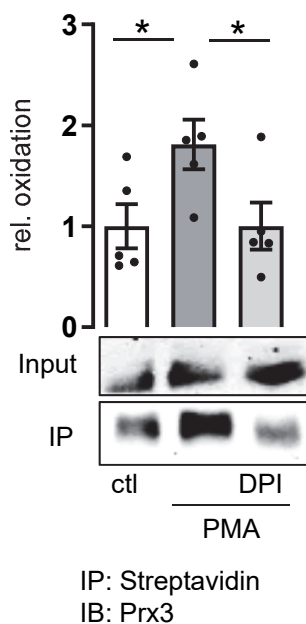
A Nox4 and 5 mRNA expression in HEK293-Nox5 cells



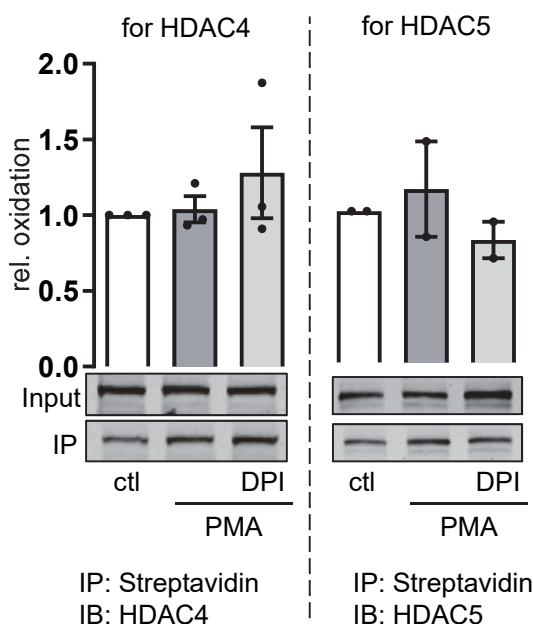
B Superoxide anion measurement with cytochrome c



C Redox assay for Prx3

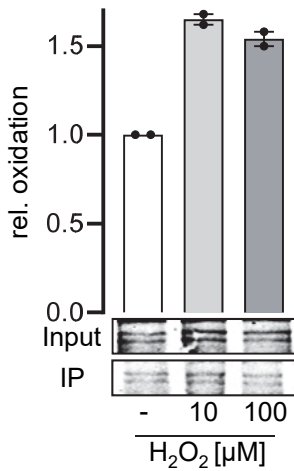


D Redox assays in HEK293-Nox5 cells



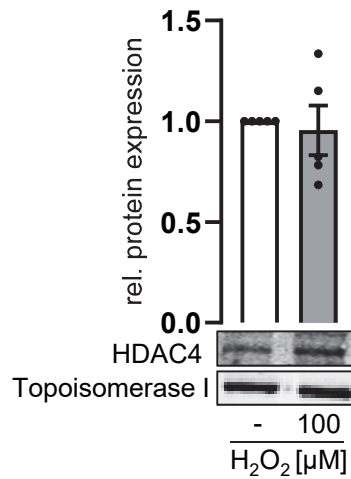
Supplemental figure 3: Characterization of HEK-Nox5 cells. (A) Analysis of Nox4 and Nox5 mRNA expression in HEK293 WT and HEK-Nox5 cells. mRNA levels were normalized to β -actin (n=3). (B) SOD-sensitive superoxide anion measurement in Nox4-tet-on cells or stably overexpressing HEK293-Nox5 cells. Nox4-tet-on cells were treated with or without tetracycline (1 μ g/ml, 24 h) and DPI (3 μ M, 3 h). Nox5 activation and superoxide production was induced by PMA (100 nM, 40 min) and detected with cytochrome c (20 μ M) (n=3) (Unpaired t-test, *p<0.05). (C) BIAM switch assay in Nox5 overexpressing cells with or without PMA (100 nM, 40 min) and DPI (3 μ M, 3 h). Redox modification of peroxiredoxin (Prx) 3 (n=5) (One-way ANOVA with Tukey post-hoc test, *p<0.05). (D+E) BIAM switch assays in Nox5 overexpressing cells with or without PMA (100 nM, 40 min) and DPI (3 μ M, 3 h). Redox modifications of HDAC4 (n=3) (D) and HDAC5 (n=2) (E) were analyzed. Representative Western blots are shown.

A BIAM Switch Redox Assay in HMECs



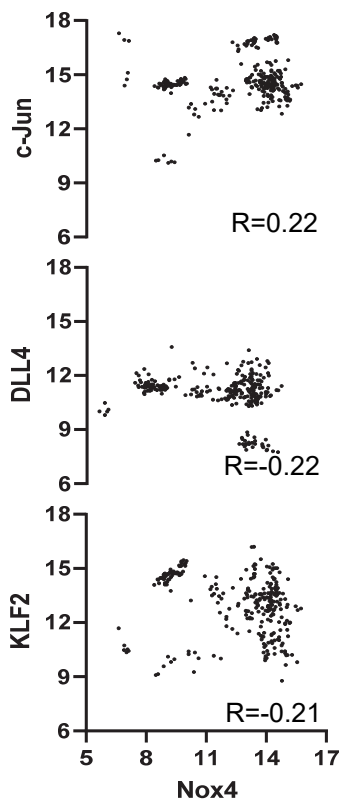
IP: Streptavidin
IB: HDAC4

B HDAC4 expression in HMECs

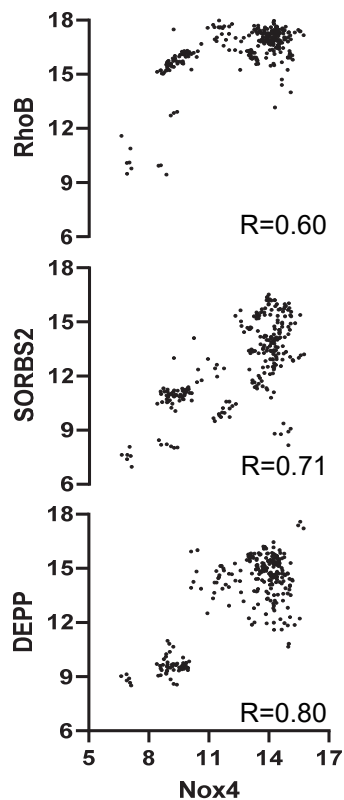


C

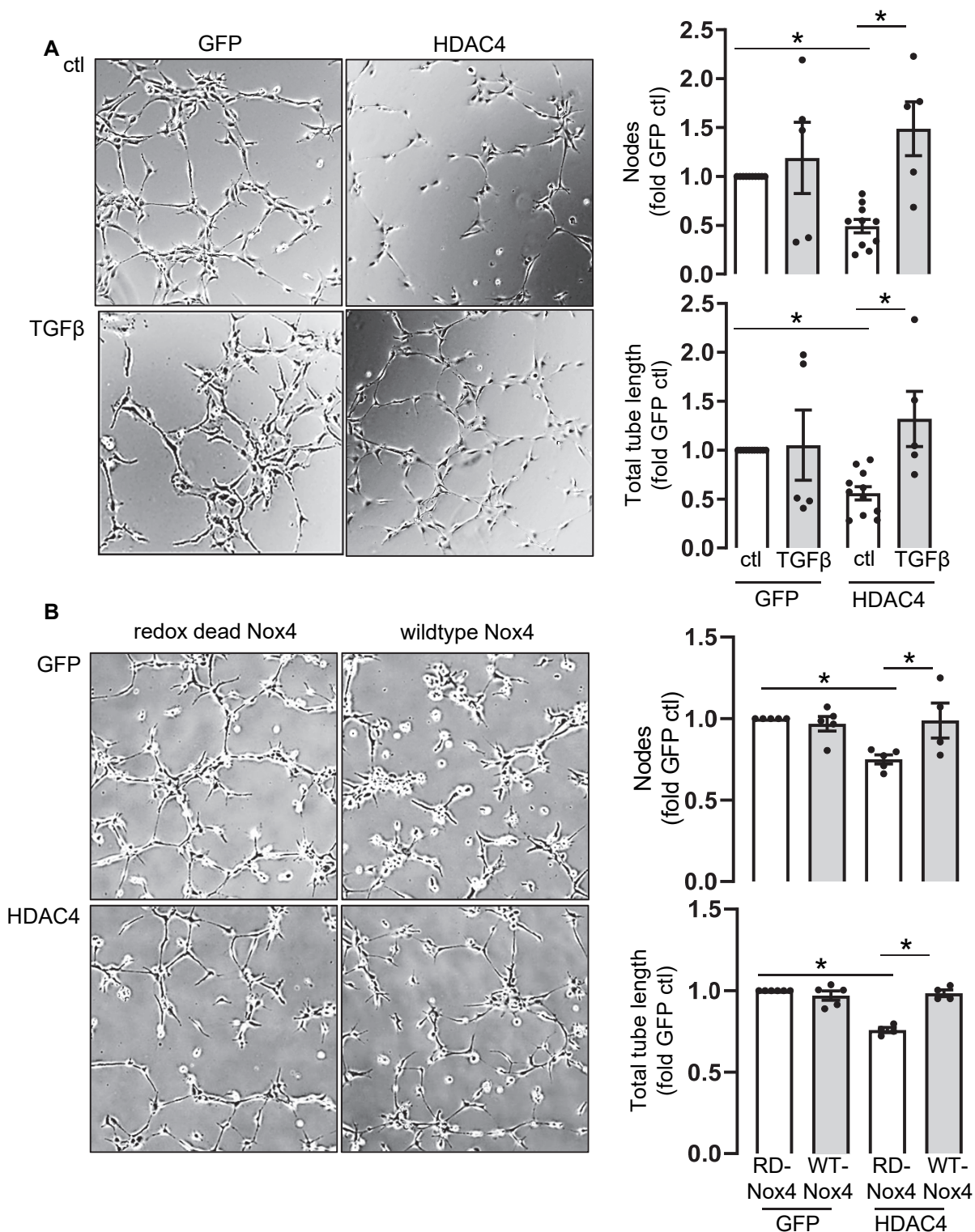
Nox4 vs. c-Jun, DLL4 and KLF2



Nox4 vs. RhoB, SORBS2 and DEPP



Supplemental Figure 4: (A) BIAM switch redox assay in HMECs stained for HDAC4. Cells were treated with or without H₂O₂ (10/100 μM, 30 min) (n=2). (B) HMECs were treated with or without H₂O₂ (100 μM, 6 h) and analyzed by Western blot (n=4). (C) GeneVestigator database was used to correlate Nox4 expression with Mef2A target gene expression in all datasets of endothelial cells which were available. Pearson coefficient (R) is shown for each correlation.



Supplemental figure 4 : Tubeformation with co-overexpression of HDAC4 and Nox4 . Tube formation assay with HUVECs and HMECs. **(A)** HUVECs were transfected with HDAC4 by electroporation. After 24 h cells were treated with or without TGFβ (10 ng/ml) for 24 h and seeded on matrigel. **(B)** HMECs were transfected by lipofection with Lipofectamin3000 with RD- or WT-Nox4 and GFP or HDAC, respectively. Representative images after 4 h are shown. Numbers of nodes were counted and total tube length was measured with an image angiogenesis analyzer (Carpentier, 2012) in ImageJ after 4 h and summarized in the statistics (n≥4). All bar graphs show means ± SEM. (One-way ANOVA with Sidak post-hoc test, *p≤0.05)