

SUPPLEMENTARY DATA

NADPH Oxidase 4 Induces Cardiac Arrhythmic Phenotype in Zebrafish*

Yixuan Zhang¹, Hirohito Shimizu², Kin Lung Siu¹, Aman Mahajan¹, Jau-Nian

Chen², and Hua Cai¹

From the ¹Divisions of Molecular Medicine and Cardiology, Departments of Anesthesiology and Medicine, Cardiovascular Research Laboratories, David Geffen School of Medicine at University of California Los Angeles (UCLA); and ²Department of Molecular, Cell and Developmental Biology, UCLA, Los Angeles, California, 90095

SUPPLEMENTAL FIGURE LEGENDS

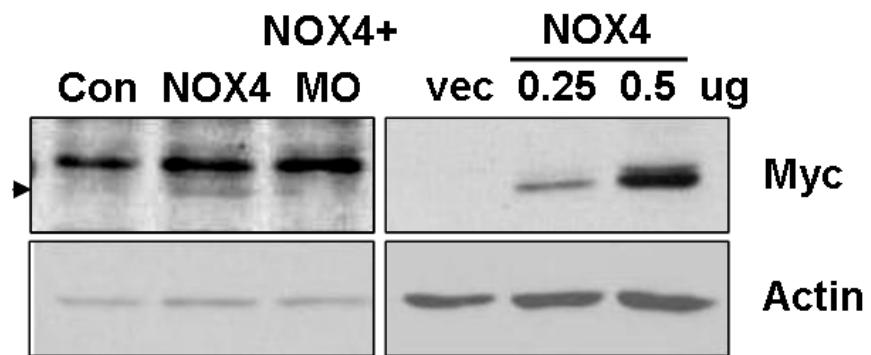
Suppl. Fig. I: NOX4 overexpression induces cardiac arrhythmia in zebrafish embryos: direct video inspection. Shown are representative videos from control and NOX4 RNA injected zebrafish embryos at 24-30 hpf. Zebrafish heart expressing GFP transgene was videotaped under UV illumination. Note the NOX4 embryo has faster or slower heart beats at 11-12th, 15-17th and 31-32nd beats.

Suppl. Fig. II: Efficacies of NOX4 overexpression and MO knockdown in zebrafish embryos at 31 hpf. Left panel: Zebrafish embryos were injected with Myc-tagged NOX4 RNA with or without MO. Embryos from indicated groups were harvested at 31 hpf. Arrowhead: Myc-tagged NOX4 in zebrafish embryos. Right panel: Myc-tagged NOX4 overexpression in HEK293T cells. vec: empty vector.

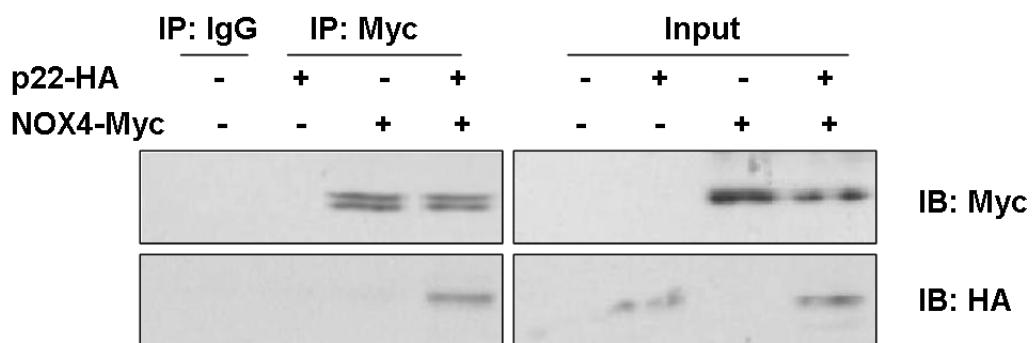
Suppl. Fig. III: Interaction between human NOX4 and zebrafish p22phox: Coimmunoprecipitation of human NOX4 (NOX4-Myc) and zebrafish p22phox (p22-HA). HEK293 cells were transfected with indicated plasmids. Cell lysates were harvested for immunoprecipitation with Myc antibody, followed by immunoblotting with anti-HA.

Suppl. Fig. IV: CaMKII oxidation (Met281/282) is not changed by NOX4 overexpression. Representative western blot and grouped data of CaMKII oxidation in control and NOX4 RNA injected embryos at 31 hpf. n=4.

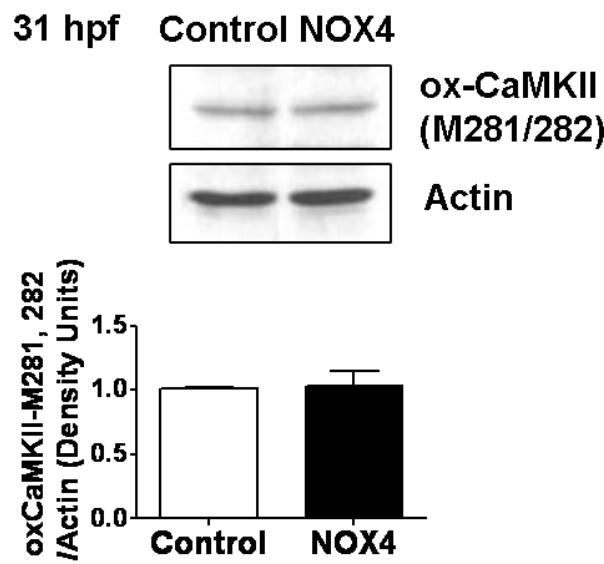
Representative video of Control embryo;
Representative video of NOX4-injected embryo.



Zhang *et. al.* Suppl. Fig. II



Zhang *et. al.* Suppl. Fig. III



Zhang *et. al.* Suppl. Fig. IV