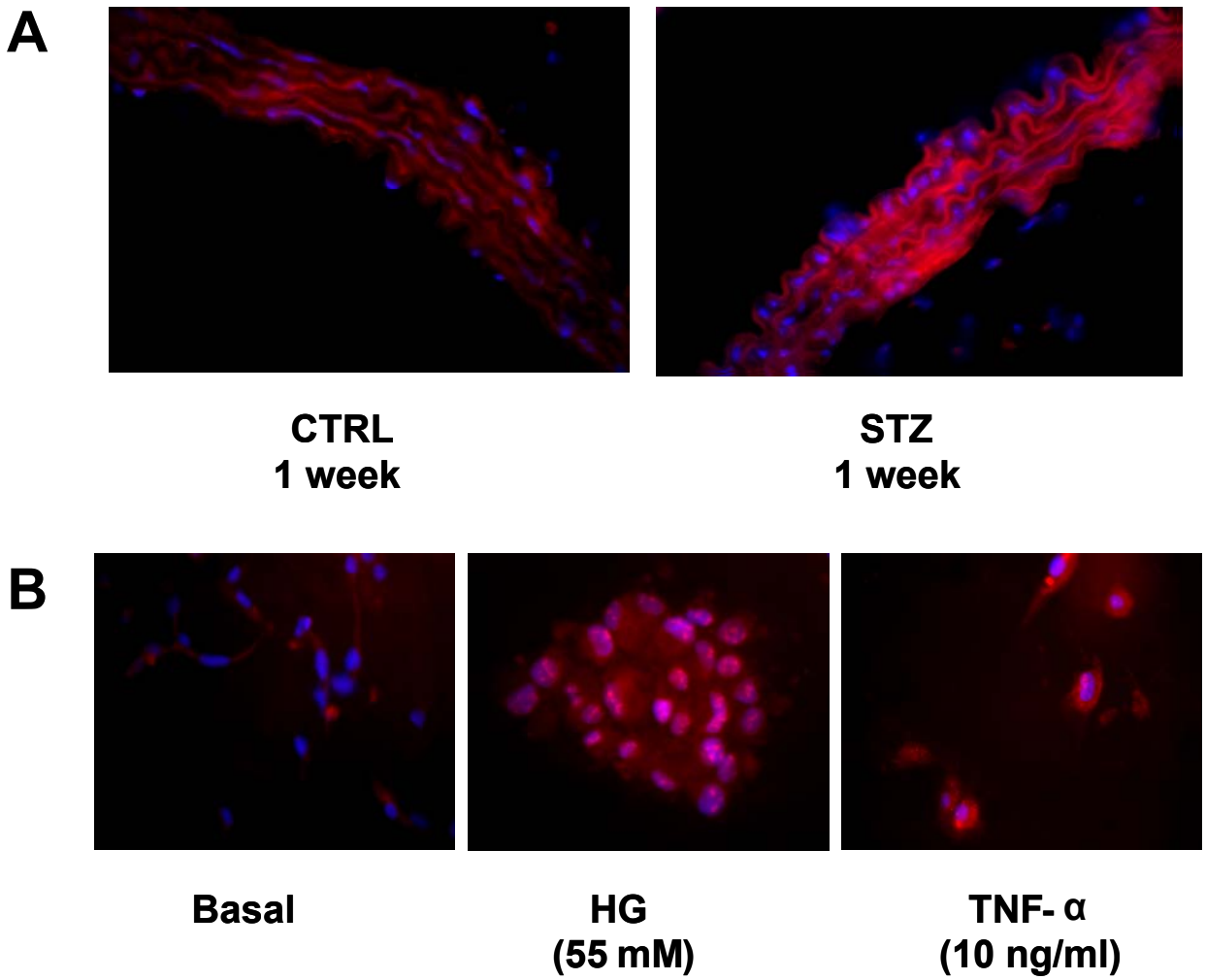
Suppl Fig 3



Suppl. Figure 3 *Exposure to TNF-α and/or high glucose concentrations increase ROS production in vascular tissues.* Dihydroethidium (DHE; Molecular Probes Inc., USA) was used to evaluate superoxide (O2-) production in mice aorta sections and human aortic endothelial cells. DHE freely enters cells where, in the presence of O2-, it is oxidized to ethidium bromide that intercalates within nuclear DNA and emits red fluorescence in proportion to the amount of O2- present (excitation at 488 nm, emission at 610 nm). **A**. Unfixed, frozen aortas from mice treated with CTRL or STZ for 1 week were cut into 10-µm-thick sections with an automated cryostat (Leica, Bensheim, Germany) and placed on a glass slide. Sections were pre-hydrated with PBS (10 min) and then incubated with DHE (2 µM, 30 min, 37 °C) in a dark, humidified chamber. Sections were subsequently washed, stained with 4,6-diamidin-2-phenylindol dichlorohydrate (DAPI, Santa Cruz Biotech, Inc., California, USA) to visualize cell nuclei, and mounted on a coverslip. Results were observed with an epifluorescent Zeiss Axiovert TS100 microscope with appropriate filters. Images were captured with a CCD camera in conjunction with Axiovision Software (Zeiss) using identical imaging settings for each image acquisition. Increased red staining demonstrates enhanced O2- production in aortas from mice treated with STZ for 1 week when compared to aortas from CTRL mice. Representative images are shown from 3 independent experiments. **B.** Superoxide production is enhanced in human aortic endothelial cells (HAEC) exposed to high glucose concentrations (HG, 55 mM) or TNF-α treatment (10 ng/ml) for 24 h. HAEC cells were incubated with DHE under conditions described above. Enhanced red fluorescence was observed in HAEC treated with HG or TNF-α when compared to cells under basal (untreated) conditions. Representative images are shown from experiments that were repeated independently 3 times.