

SUPPLEMENTARY ONLINE DATA

The NADPH oxidase NOX4 restricts the replicative lifespan of human endothelial cells

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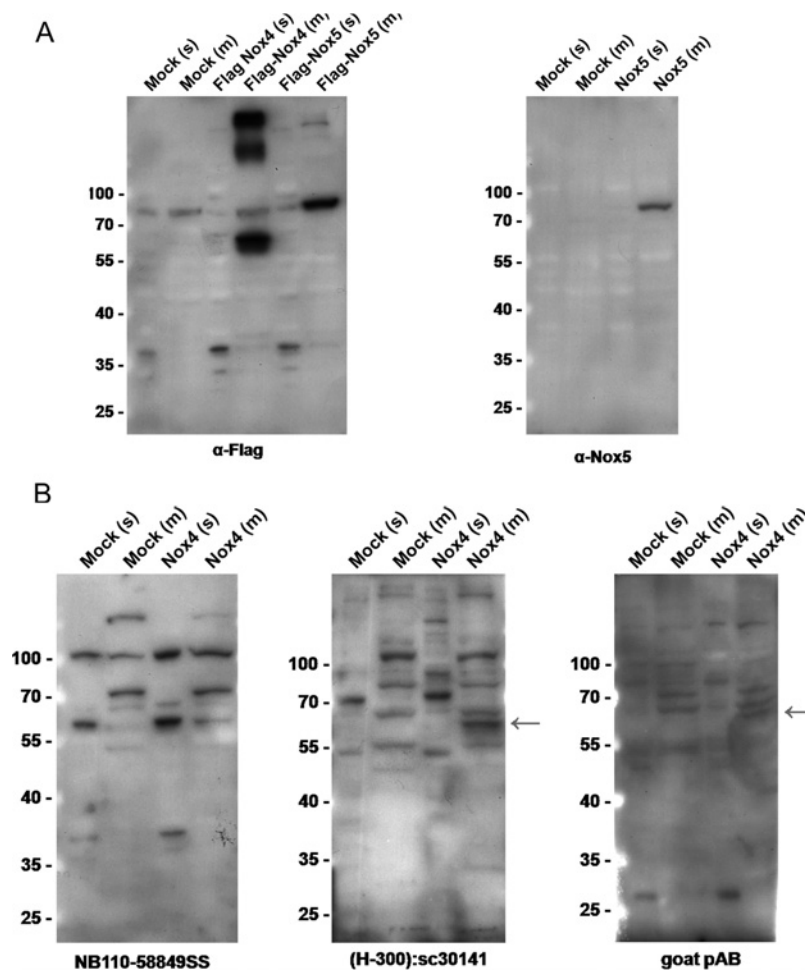


Figure S1 Extraction and expression of Nox4 in U-2OS cells

(A) Validation of lysis protocol. U-2OS osteosarcoma cells were transfected with expression vectors encoding Nox4, Nox5, FLAG–Nox4 and FLAG–Nox5, as indicated. Cells were lysed and separated in membrane (m) and soluble (s) protein fractions. Upon transfection of U-2OS cells with plasmids encoding either FLAG–Nox4 or FLAG–Nox5, specific bands of the expected size were detected in the membrane fraction using anti-FLAG antibodies ( $\alpha$ -Flag, left panel). In case of FLAG–Nox4, one specific band corresponding to approx. 60 kDa was detected, which most likely refers to monomeric FLAG–Nox4. Two additional specific bands of higher molecular mass were present, probably representing FLAG–Nox4 protein that was only partially solubilized under the lysis conditions. In the case of FLAG–Nox5, a single band corresponding to approx. 70 kDa was specifically recognized by anti-FLAG antibodies, a result that could be reproduced by the application of Nox5 antibodies ( $\alpha$ -Nox5, right panel). (B) Analysis of Nox4 overexpression by Western blot. U-2OS cells were transfected with Nox4 expression vector (Nox4) and empty vector (Mock), as indicated. Cells were treated as described for (A) and probed with three different antibodies to Nox4, as indicated. In addition to two commercially available antibodies, we used an affinity-purified goat antiserum that had been obtained against the C-terminal part of Nox4 after expression in *E. coli* and follow-up purification to homogeneity (anti-Nox4 NB110-58849SS; H. Pircher, unpublished work). Specific bands corresponding to overexpressed Nox4 (marked by an arrow) could be detected in the membrane fraction by anti-Nox4 (H-300):sc30141 and the affinity-purified goat antibody, whereas no specific band was detected by anti-Nox4 NB110-58849SS. Molecular mass in kDa is indicated to the left of each gel.

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