ONLINE SUPPLEMETAL MATERIALS TO:

Endothelial-specific expression of mitochondrial thioredoxin improves endothelial cell function and reduces atherosclerotic lesions

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Fig.S1. **Characterization of Trx2 TG expression in heart and lung**. The Trx2 transgene in WT and Trx2 TG (line #3) heart and lung sections were detected by indirect immunofluorescence microscopy with anti-HA antibody followed by Alexa Fluor 488 (green)-conjugated anti-mouse secondary antibody. Trx2 transgene was detected in vascular endothelium of heart (**a**) and lung tissues (**b**).

Fig.S2. Localization of the Trx2 tranegene in mitochondria. Mouse aortic EC (MEC) were isolated from WT and Trx2 TG mice. The localization of Trx2 transgene was determined by indirect immunofluorescence staining with anti-HA antibody followed by Alexa Fluor 594 (red)-conjugated anti-mouse secondary antibody. Cytochrome c (a mitochondrial protein) or Trx1 (a non-mitochondrial protein) was detected with a respective antibody followed by Alexa Fluor 488 (green)-conjugated anti-rabbit secondary antibody. The merged images are shown on the right.

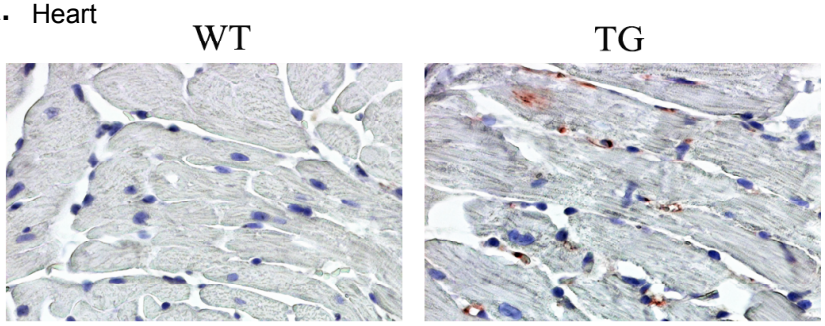
Fig.S3. **Characterization of Trx2 expression and activity in ApoE-deficiency mice**. WT and ApoE-deficiency (ApoE) (n=5 for each group) were fed with Western-type diet for 4 and 8 wks. **a**. Aortas were harvested and paraffin sections were stained with Oil-red. High power images are shown at the bottom. **b-c**. Trx2 expression is reduced in endothelium of ApoE-deficiency mice. Trx2 expression in aortas of WT and ApoE-deficiency mice (8 wks post-diet) was determined by immunohistochemistry (**b**) or Western blot with anti-Trx2 antibody (**c**). The ratio of Trx2/β-tubulin is shown at the bottom of **c**. **d**. Nitrotyrosine and ASK1 activities were dramatically increased in ApoE-deficiency aortas. Trx1, nitrotyrosine (NTY) and ASK1 phosphorylation (pThr845) in aortas of WT and ApoE-deficiency mice (8 wks post-diet) were determined by immunohistochemistry with respective antibodies.

Fig.S4. ApoE-KO mice show reduction of basal NO release in aortic endothelium. WT, Trx2-TG, ApoE-deficiency (ApoE) and ApoE/TG (n=5 for each group) were fed with Western-type diet for 4 wks. **a**. Aortas from ApoE-KO mice show an enhanced response to PE. Aortas were contracted with PE at a full range of doses (10^{-9}-10^{-4} M). **b**. Basal NO is decreased in ApoE-KO mice. Aortic rings were incubated with a NOS inhibitor L-

NAME to remove basal NO synthesis and then contracted with PE as in **a**. **c**. Ratio of EC_{50} in response to PE in the presence of L-NAME to PE in the absence of L-NAME. **d**. Aortas from ApoE-KO mice show an attenuated response to Ach. Aortic rings were precontracted with PE and then relaxed with Ach at a full range of doses (10^{-9}-10^{-4} M). % of relaxation is shown. **e**. ApoE-KO had no effects on vessel constriction in response to KCl. Aortic rings were contracted with 50 mM of KCl. Data presented are mean±SEM, with n=4 animals and eight aortic rings per animal, *, $p<0.05$.

Fig.S1

a. Heart



b. Lung

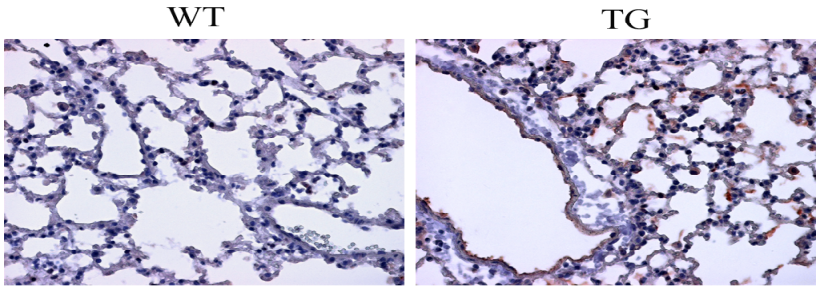


Fig.S2

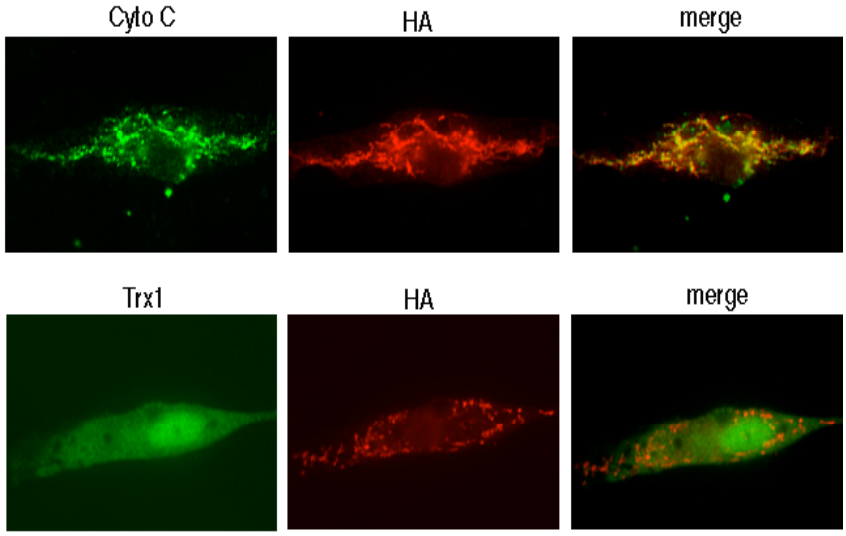


Fig.S3

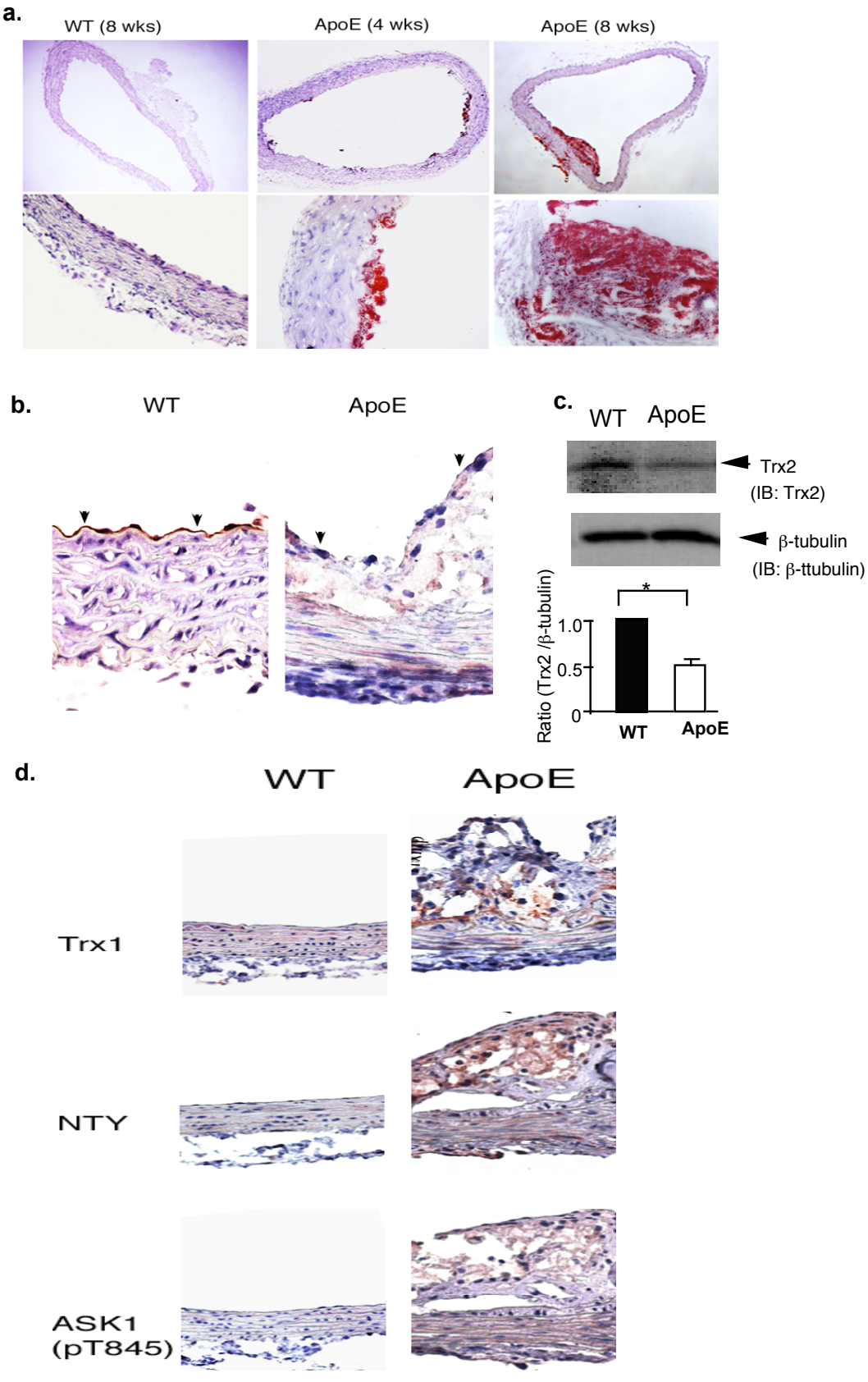
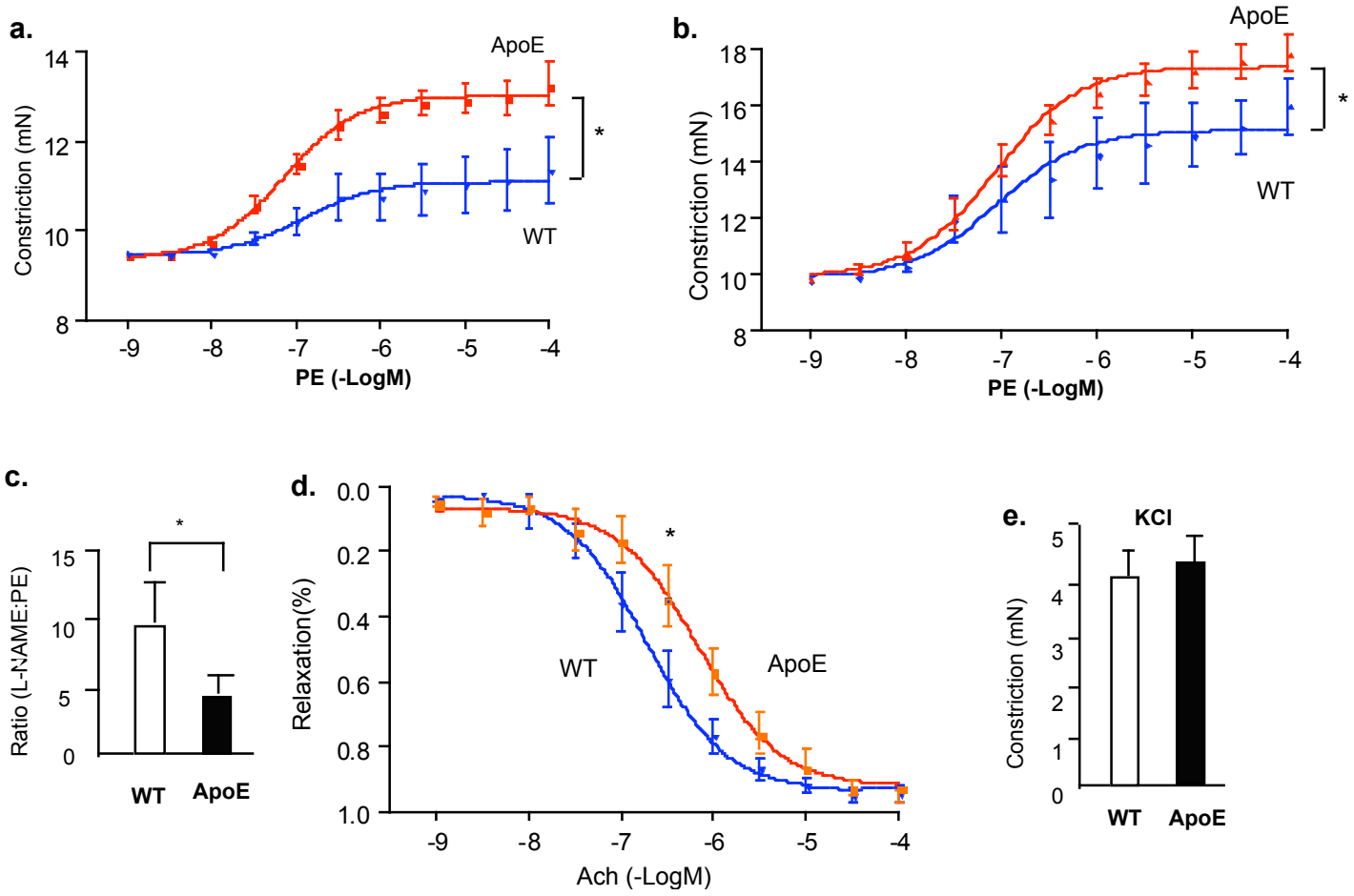

a.
WT (8 wks)
ApoE (4 wks)
ApoE (8 wks)
b.
WT
ApoE
c.
WT
ApoE
Trx2
(IB: Trx2)
β-tubulin
(IB: β-ttubulin)
Ratio (Trx2 /β-tubulin)
1.0
0.5
0
*
WT
ApoE
d.
WT
ApoE
Trx1
NTY
ASK1
(pT845)

Fig.S4


a.
ApoE
WT
Constriction (mN)
PE (-LogM)
*
b.
ApoE
WT
Constriction (mN)
PE (-LogM)
*
c.
Ratio (L-NAME:PE)
WT
ApoE
*
d.
Relaxation(%)
WT
ApoE
Ach (-LogM)
*
e.
KCl
Constriction (mN)
WT
ApoE