Supplemental Material

Supplemental Figure Legends

Supplemental Fig I. Wnt3a conditioned medium (Wnt3a-CM) increases total as well as active (dephosphorylated) β-catenin in BAEC. Data is representative of 3 experiments.

Supplemental Fig II. (a) Wnt3a-CM increases active β-catenin in HUVEC. (b & c) Recombinant Wnt3a increases β-catenin in HUVEC in a dose and time-dependent manner. (d) Wnt3a (200 ng/ml) stimulates rapid phosphorylation of p66shc in HUVEC. Data is representative of 3 experiments.

Supplemental Fig III. Wnt3a or non-phsophorylatable active β-catenin (S37A) do not inhibit **s**odium-nitroprusside (SNP)-stimulated endothelium-independent vasorelaxation of mouse aortas. There was no significant difference between the groups. n = 3-5. (a) Ref # Fig. 4a (b) Ref # Fig. 4c (c) Ref # Fig. 4e. All values are shown as Mean ± SEM.

Supplemental Fig IV. High-fat diet (HFD)-feeding leads to (a) impaired endothelium-dependent vasorelaxation, and (b) decreased NO bioavailability. (c) HFD-feeding has no effect on SNP-stimulated endothelium-independent vasorelaxation ND = normal diet. All values are shown as Mean ± SEM. * $P < 0.05$, *** $P < 0.001$ *vs.* ND. N=3-12.

Supplemental Fig V. Decreased HFD-induced aortic fatty streak formation (Oil-Red-O staining) in mice transgenic for $p66^{Shc}$ shRNA ($p66^{Shc}$RNAi). N = 3-4. All values are shown as Mean ± SEM. * $P < 0.05$ *vs.* WT-ND.

Supplemental Fig VI. Schematic showing proposed model of canonical Wnt signaling in the endothelium and vasculature, and the role of p66shc and ROS in this model. LRP: low-density lipoprotein receptor-related protein; Fz: Frizzled receptor. In addition to TNFα, other target genes of β-catenin in the endothelium that could potentially contribute to vascular oxidative stress and endothelial dysfunction are not shown.

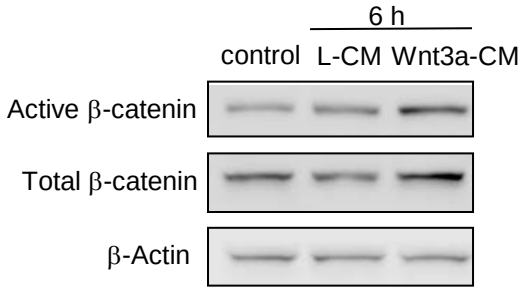
6 h
control
L-CM
Wnt3a-CM
Active β-catenin
Total β-catenin
β-Actin

Figure I

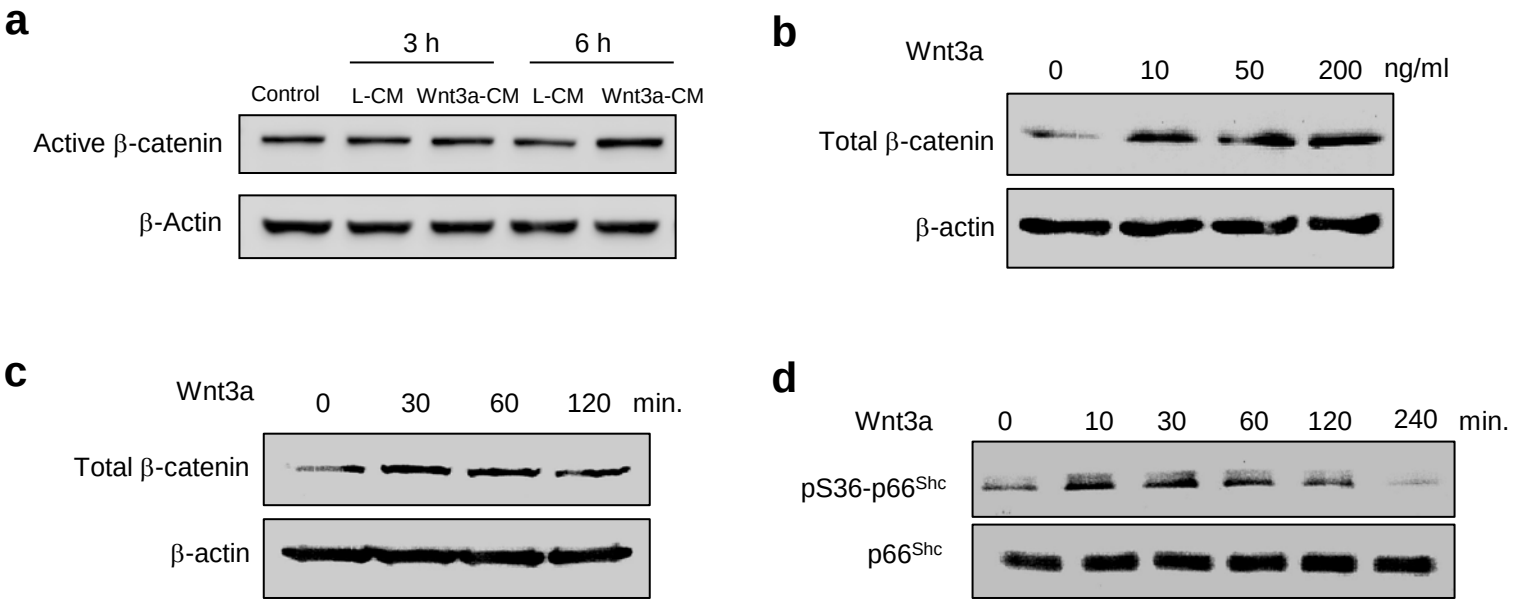
a
3 h
6 h
Control
L-CM
Wnt3a-CM
L-CM
Wnt3a-CM
Active β-catenin
β-Actin
b
Wnt3a
0
10
50
200
ng/ml
Total β-catenin
β-actin
c
Wnt3a
0
30
60
120
min.
Total β-catenin
β-actin
d
Wnt3a
0
10
30
60
120
240
min.
pS36-p66Shc
p66Shc

Figure II

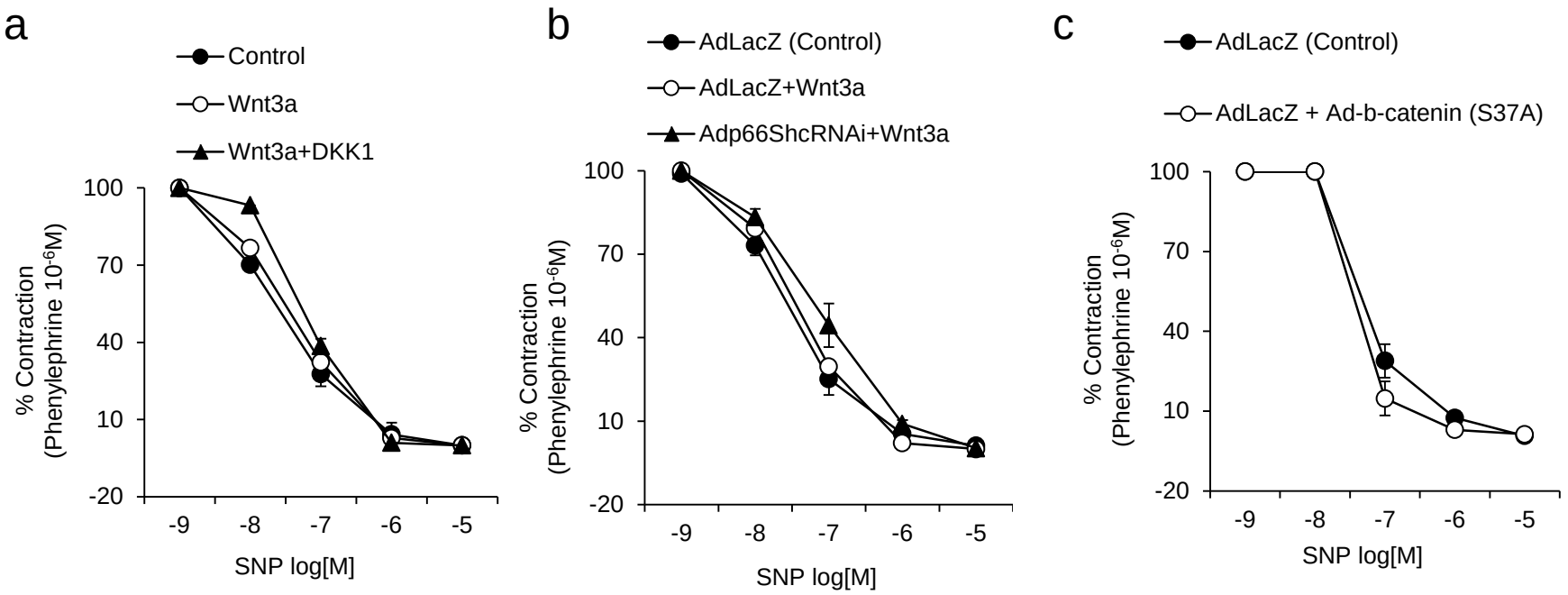
a
Control
Wnt3a
Wnt3a+DKK1
% Contraction (Phenylephrine 10-6M)
100
70
40
10
-20
-9
-8
-7
-6
-5
SNP log[M]
b
AdLacZ (Control)
AdLacZ+Wnt3a
Adp66ShcRNAi+Wnt3a
% Contraction (Phenylephrine 10-6M)
100
70
40
10
-20
-9
-8
-7
-6
-5
SNP log[M]
c
AdLacZ (Control)
AdLacZ + Ad-b-catenin (S37A)
% Contraction (Phenylephrine 10-6M)
100
70
40
10
-20
-9
-8
-7
-6
-5
SNP log[M]

Figure III

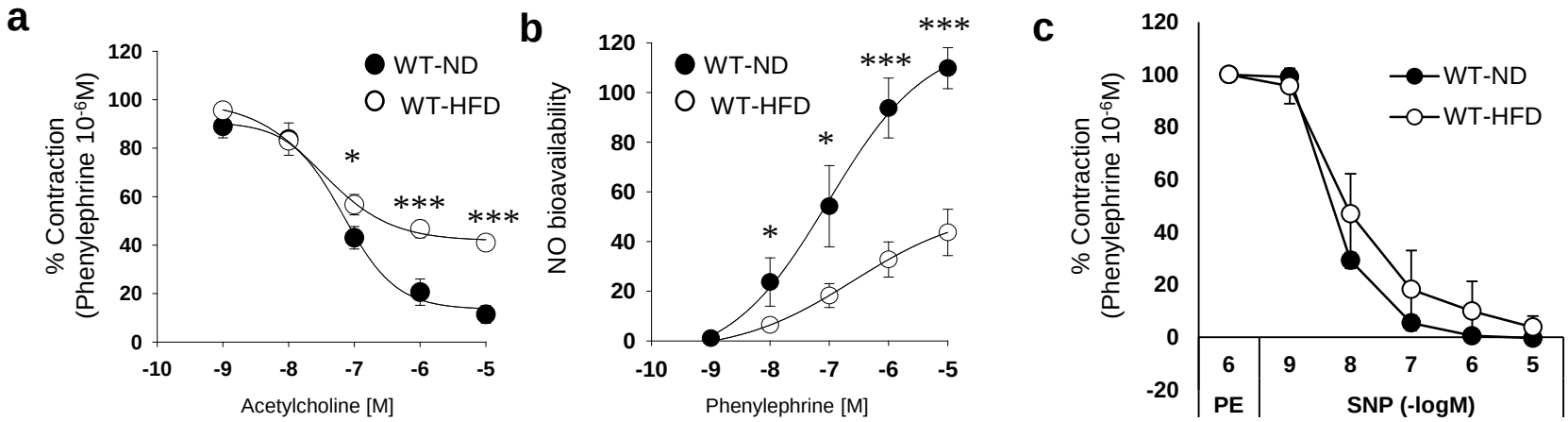
a
% Contraction (Phenylephrine 10-6M)
WT-ND
WT-HFD
Acetylcholine [M]
b
NO bioavailability
WT-ND
WT-HFD
Phenylephrine [M]
c
% Contraction (Phenylephrine 10-6M)
WT-ND
WT-HFD
PE
SNP (-logM)

Figure IV

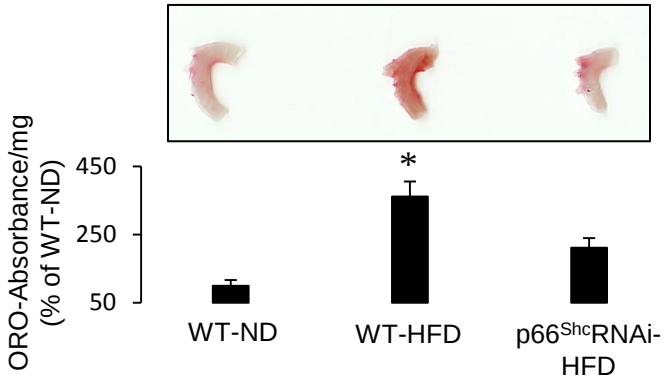
ORO-Absorbance/mg
(% of WT-ND)
450
250
50
*
WT-ND
WT-HFD
p66ShcRNAi-
HFD

Figure V

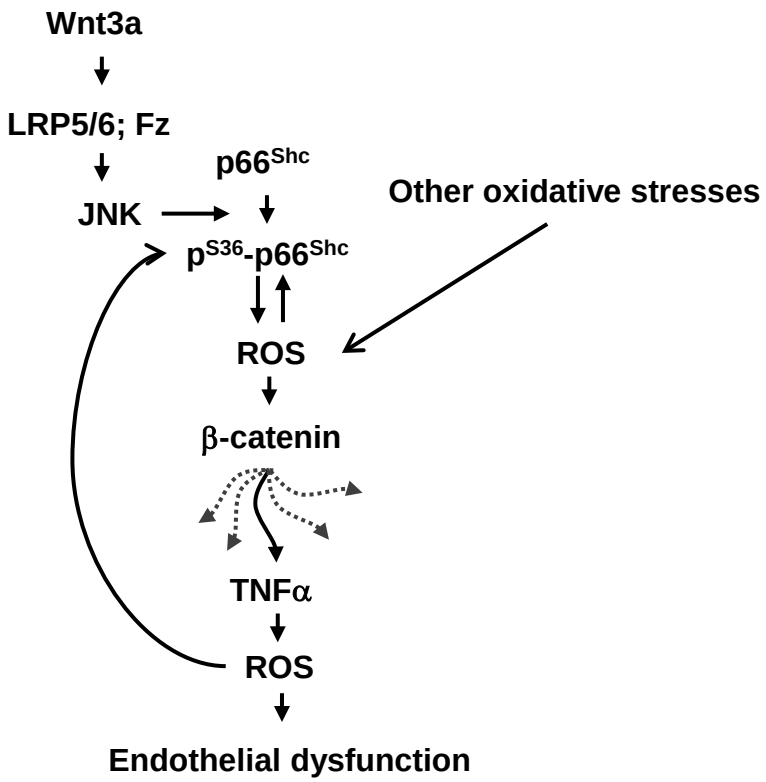
Wnt3a
LRP5/6; Fz
p66Shc
JNK
Other oxidative stresses
pS36-p66Shc
ROS
β-catenin
TNFα
ROS
Endothelial dysfunction

Figure VI