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Supplementary Materials for

cSTAR analysis identifies endothelial cell cycle as a key regulator of flow-dependent artery remodeling

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Supplementary Figure Legends

Figure S1. Leukocytes recruitment under LSS and OSS in vivo. (**A**) Heatmap of log2 fold changes in expression of chemokines that preferentially attract macrophages (CCL2) vs neutrophils (CXCL2 and CXCL8) in PSS, LSS and OSS conditions. (**B**) Left common carotid artery (LCA) ligation to generate low shear stress (LSS): external carotid artery (ECA) and superior thyroid artery (STA) were ligated, leaving internal carotid artery (ICA) and occipital artery (OA) open. (**C**) Left common carotid artery (LCA) ligation to generate oscillatory shear stress (OSS): ECA, STA, ICA and OA were ligated. (**D** and **E**) Representative images (D) and quantification (E) of anti-Myeloperoxidase (MPO, neutrophil marker) on RCA and LCA sections from LSS and OSS models. (**F** and **G**) Representative images (F) and quantification (G) of F4/80 (macrophage marker) on RCA and LCA sections from LSS and OSS models, n=6 mice per group. Scale bars, 25 μm. L, lumen. Data are presented as mean ± s.e.m. ***P < 0.001, ns: not significant, calculated by two-way ANOVA with Tukey's multiple comparison.

Figure S2. CDK2 depletion induces early G1 arrest under OSS. FUCCI HUVECs were transfected with control (siCtrl) or CDK2 (siCDK2) siRNA for 3 days, and then were subjected to OSS or Static for 24 hours. (**A**) Cells were fixed and mounted with DAPI (4',6-diamidino-2-phenylindole). Representative images are showed. Scale bars, 100 μm. (**B**) Quantification of cell cycle state, n=5 experiments, data are presented as mean ± s.e.m. ***P < 0.001, ns: not significant, calculated by two-way ANOVA with Tukey's multiple comparison.

Figure S3. Lung histology images. (**A**) H&E (Hematoxylin & Eosin) staining of Ctrl and CDK2 iECKO lung sections. V, vessels. Arrowheads are narrowed and occlusive vessels.

Figure S4. Activation of Smad2/3 in CDK2 ECKO lung tissue. (**A** and **B**) Representative images and quantification of p-Smad2 Ser465/467 (A) and p-Smad3 Ser423/425 (B) in lung sections from Ctrl and CDK2 iECKO mice, n=6 mice per group. Scale bars, 25 μm. Data are presented as mean ± s.e.m. ***P < 0.001, calculated by two-tailed unpaired t tests. (**C**) cSTAR-predicted global responses of the TGFβR

pathway activity to perturbations of all other core network modules, except TGFβR itself. A negative global response of TGFβR to CDK2 implies that CDK2 inhibition will activate the TGFβR pathway.

Figure S5. Body weight and plasma lipid analysis. (**A** and **B**) Body weight, plasma total cholesterol, and triglyceride levels between male (A) and female (B) Ctrl and CDK2 iECKO mice. N=6 mice per group per gender, data are presented as mean ± s.e.m. ns: not significant, calculated by two-tailed unpaired t tests.

Figure S1

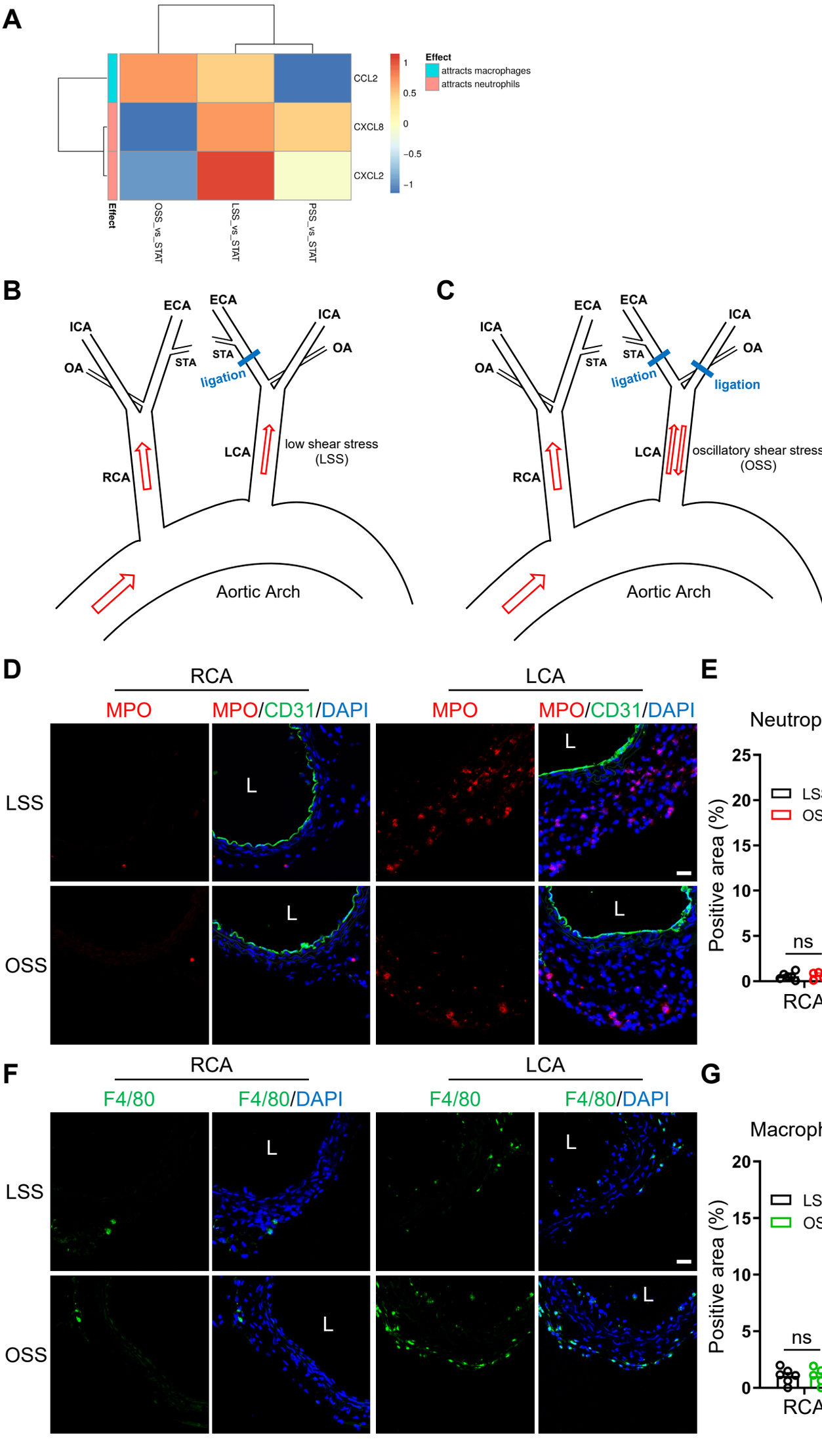
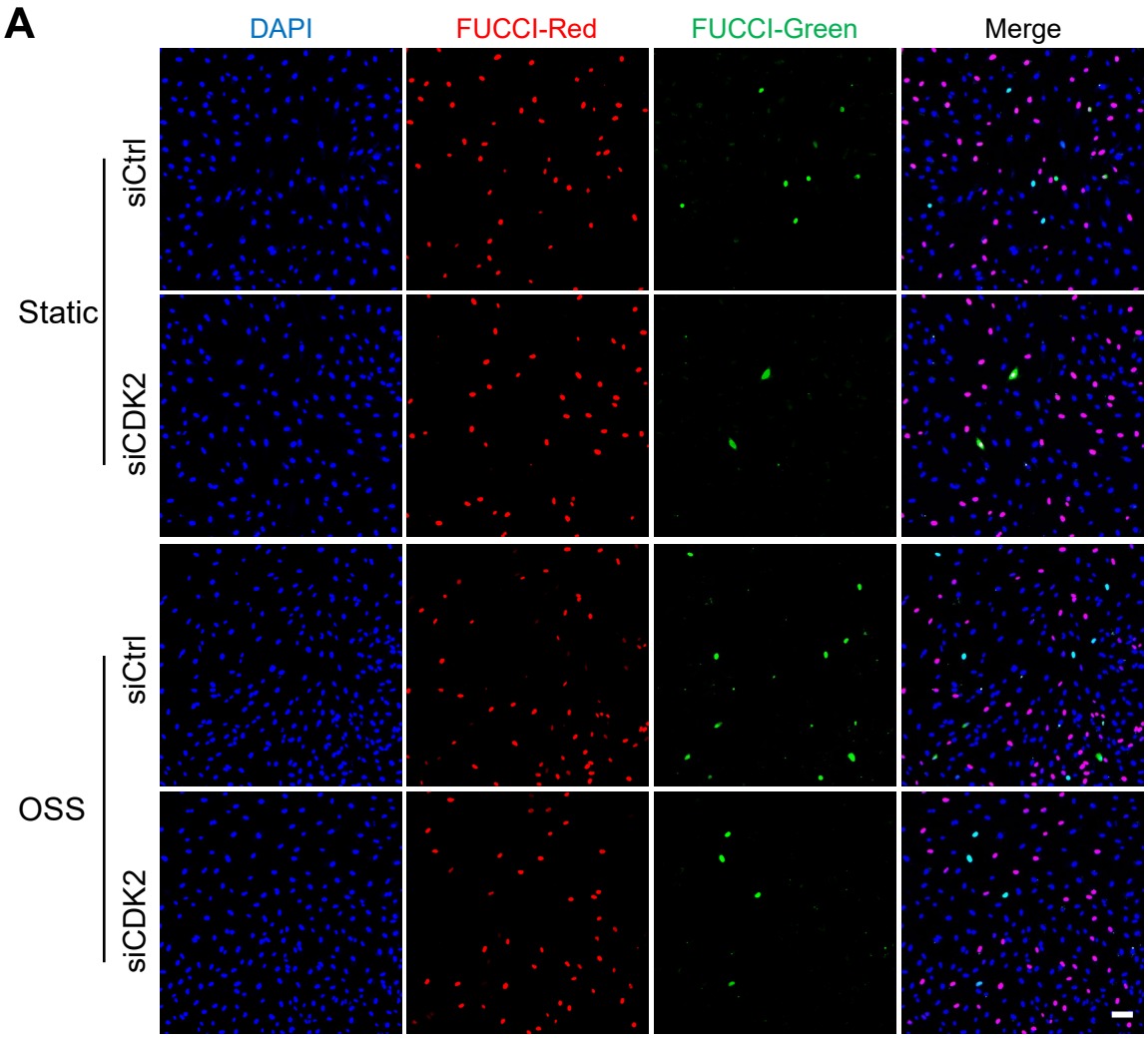

A
Effect
attracts macrophages
attracts neutrophils
CCL2
CXCL8
CXCL2
1
0.5
0
−0.5
−1
Effect
OSS_vs_STAT
LSS_vs_STAT
PSS_vs_STAT
B
ICA
ECA
OA
STA
ligation
ECA
ICA
OA
STA
ligation
LCA
low shear stress
(LSS)
RCA
Aortic Arch
C
ICA
ECA
OA
STA
ligation
ECA
ICA
OA
STA
ligation
LCA
oscillatory shear stress
(OSS)
RCA
Aortic Arch
D
RCA
LCA
MPO
MPO/CD31/DAPI
MPO
MPO/CD31/DAPI
LSS
OSS
L
E
Neutrophil (MPO)
Positive area (%)
25
20
15
10
5
0
LSS
OSS
***
ns
RCA
LCA
F
RCA
LCA
F4/80
F4/80/DAPI
F4/80
F4/80/DAPI
LSS
OSS
L
G
Macrophage (F4/80)
Positive area (%)
20
15
10
5
0
LSS
OSS
***
ns
RCA
LCA

Figure S2


A
DAPI
FUCCI-Red
FUCCI-Green
Merge
Static
siCtrl
siCDK2
OSS
siCtrl
siCDK2

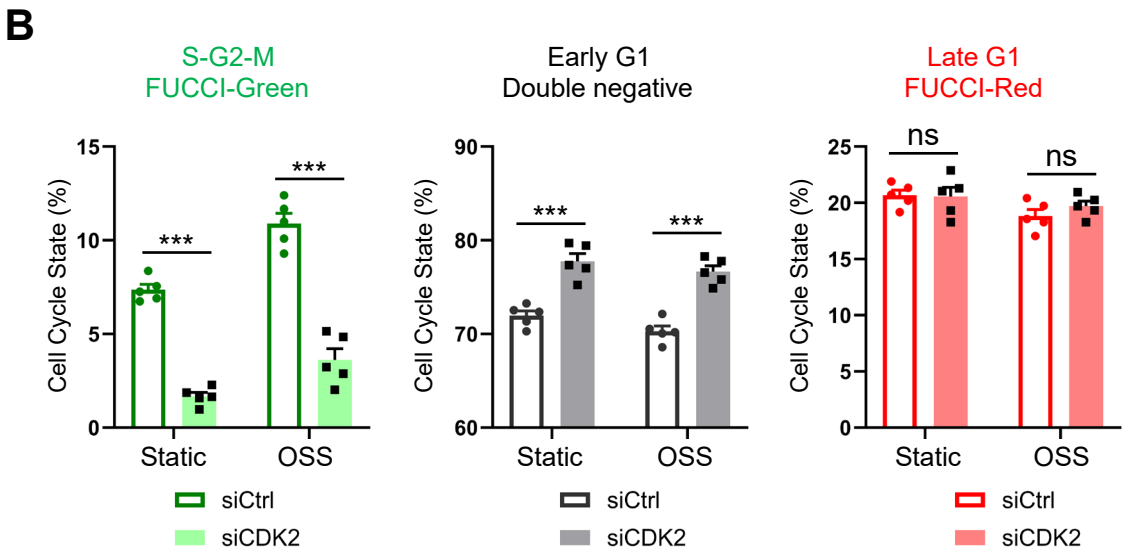

B
S-G2-M
FUCCI-Green
Early G1
Double negative
Late G1
FUCCI-Red
Cell Cycle State (%)
15
10
5
0
***
***
Static
OSS
siCtrl
siCDK2
90
80
70
60
***
***
Static
OSS
siCtrl
siCDK2
25
20
15
10
5
0
ns
ns
Static
OSS
siCtrl
siCDK2

Figure S3

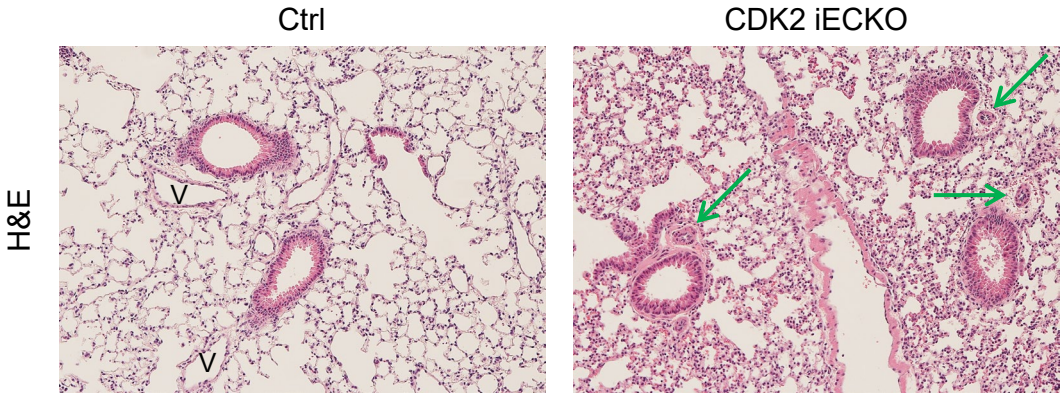
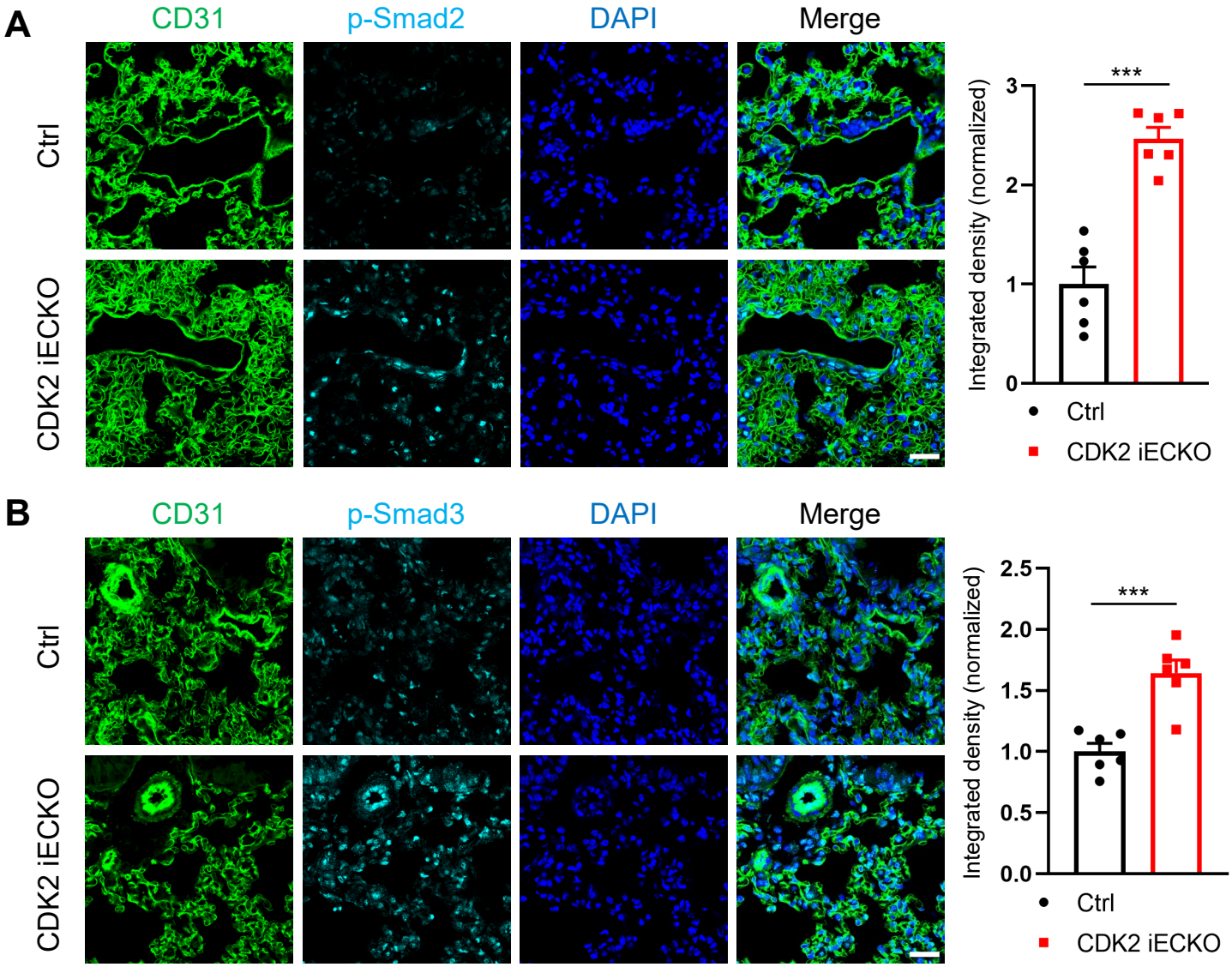


Figure S4


A
CD31
p-Smad2
DAPI
Merge
Ctrl
CDK2 iECKO
Integrated density (normalized)
***
Ctrl
CDK2 iECKO
B
CD31
p-Smad3
DAPI
Merge
Ctrl
CDK2 iECKO
Integrated density (normalized)
***
Ctrl
CDK2 iECKO

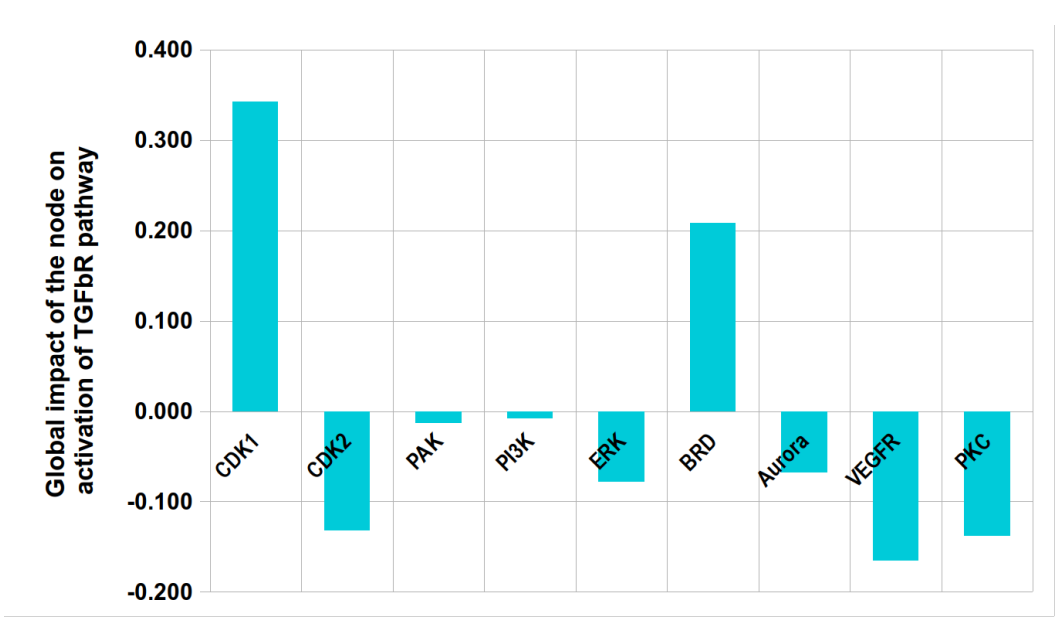
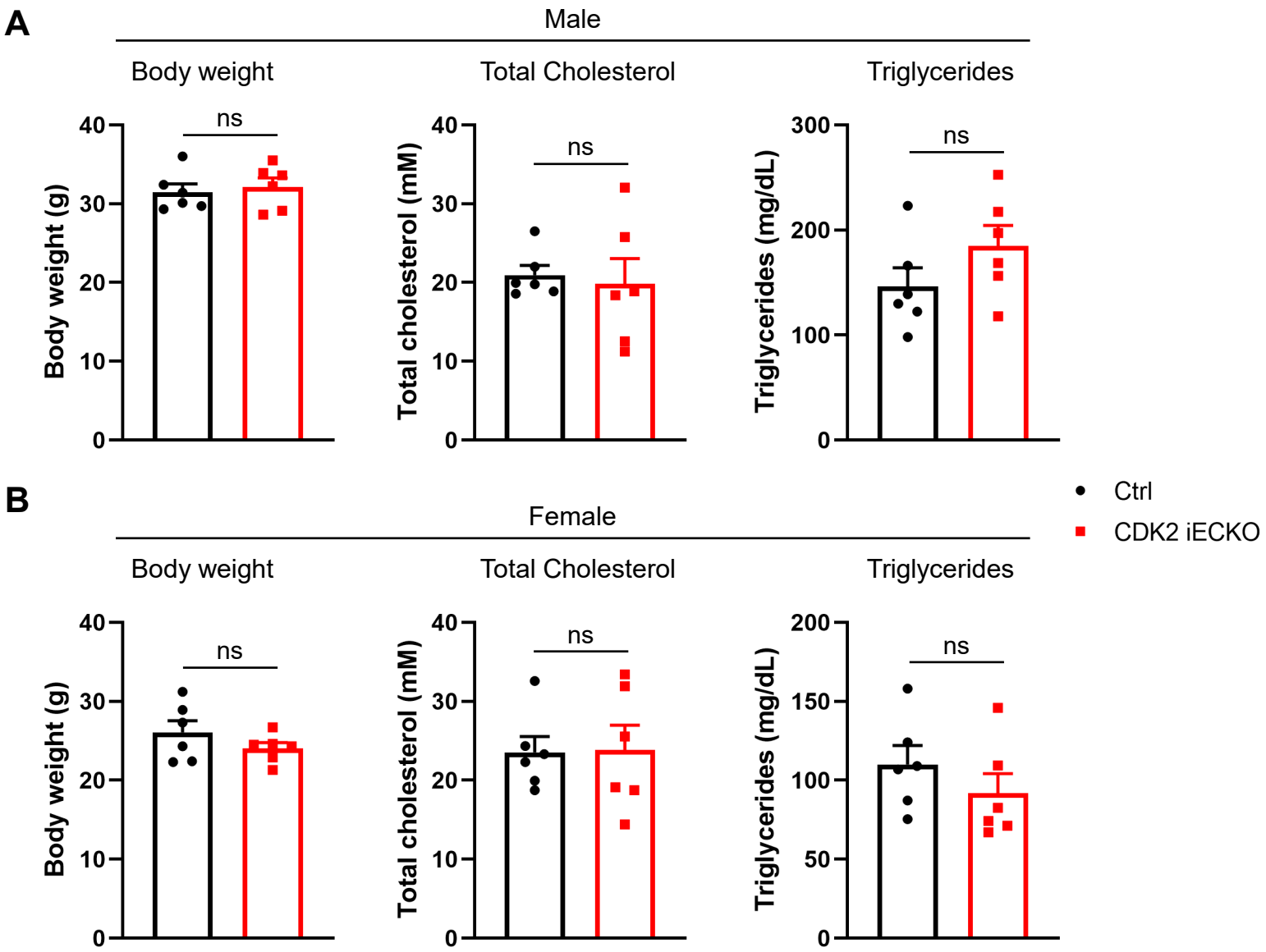

C
Global impact of the node on activation of TGFbR pathway
CDK1
CDK2
PAK
PI3K
ERK
BRD
Aurora
VEGFR
PKC

Figure S5


A
Male
Body weight
Total Cholesterol
Triglycerides
Body weight (g)
Total cholesterol (mM)
Triglycerides (mg/dL)
ns
B
Female
Ctrl
CDK2 iECKO

Supplementary Tables

All supplementary tables are separate Excel files. Below are the table captions.

Table S1. The STV vectors determining phenotypic cell state transitions in original dataspace.

Table S2. The STV vectors determining phenotypic cell state transitions in L1000 dataspace.

Table S3. The cSTAR-inferred local response matrix r: mean values and standard deviations of the BMRA reconstructed local response matrix elements.

Table S4. The global response matrix: cSTAR predicted global response matrix $-r^{-1}$.

Table S5. List of secondary antibodies.