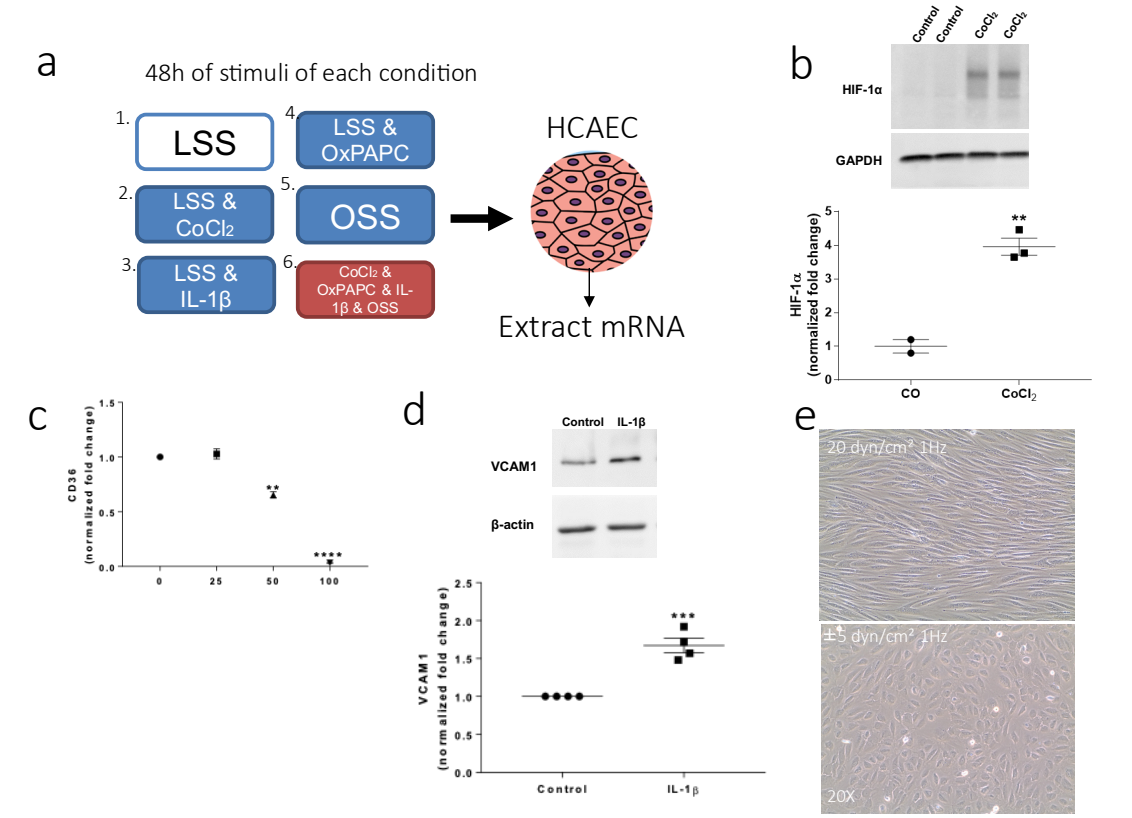
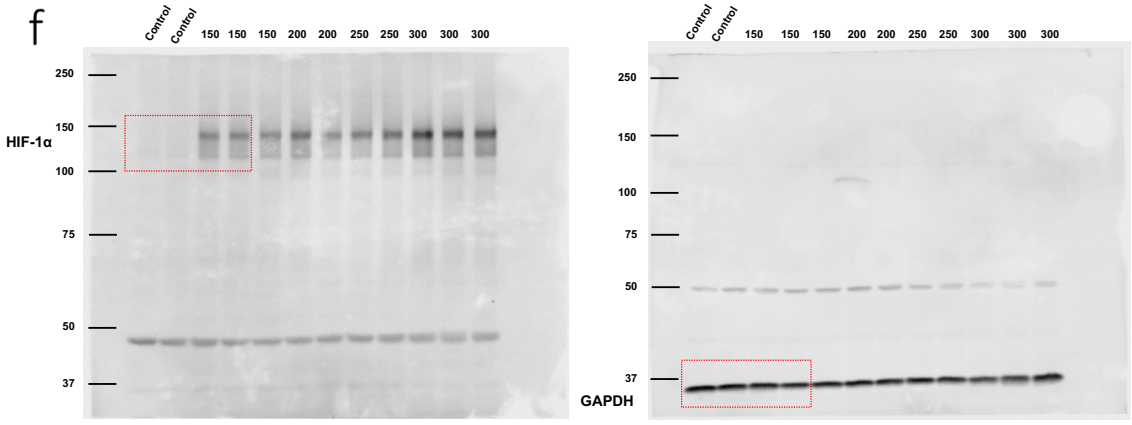
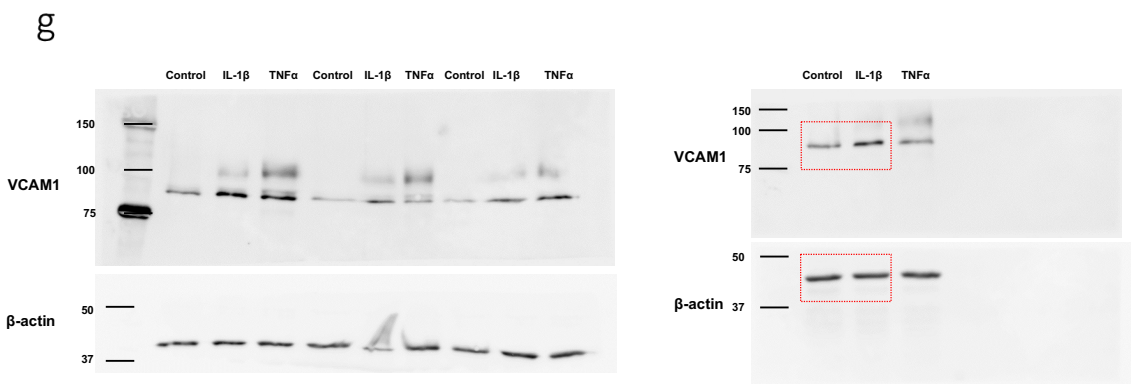
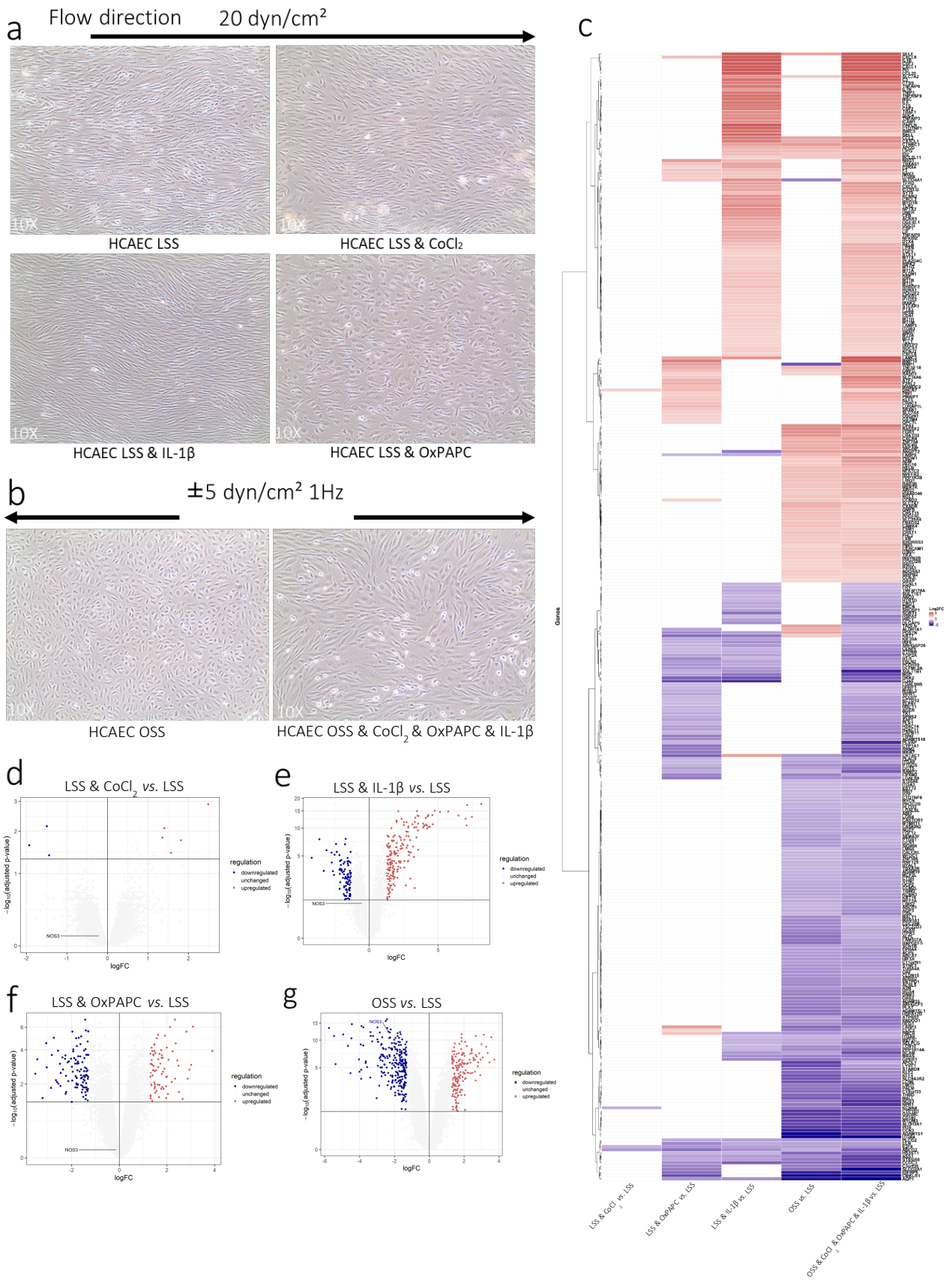

a
48h of stimuli of each condition
1. LSS
2. LSS & CoCl2
3. LSS & IL-1β
4. LSS & OxPAPC
5. OSS
6. CoCl2 & OxPAPC & IL-1β & OSS
HCAEC
Extract mRNA
b
Control
Control
CoCl2
CoCl2
HIF-1α
GAPDH
HIF-1α (normalized fold change)
CO
CoCl2
c
CD36 (normalized fold change)
d
Control
IL-1β
VCAM1
β-actin
VCAM1 (normalized fold change)
e
20 dyn/cm² 1Hz
±5 dyn/cm² 1Hz
20X


f
Control
Control
150 150 150 200 200 250 250 300 300 300
HIF-1α
GAPDH

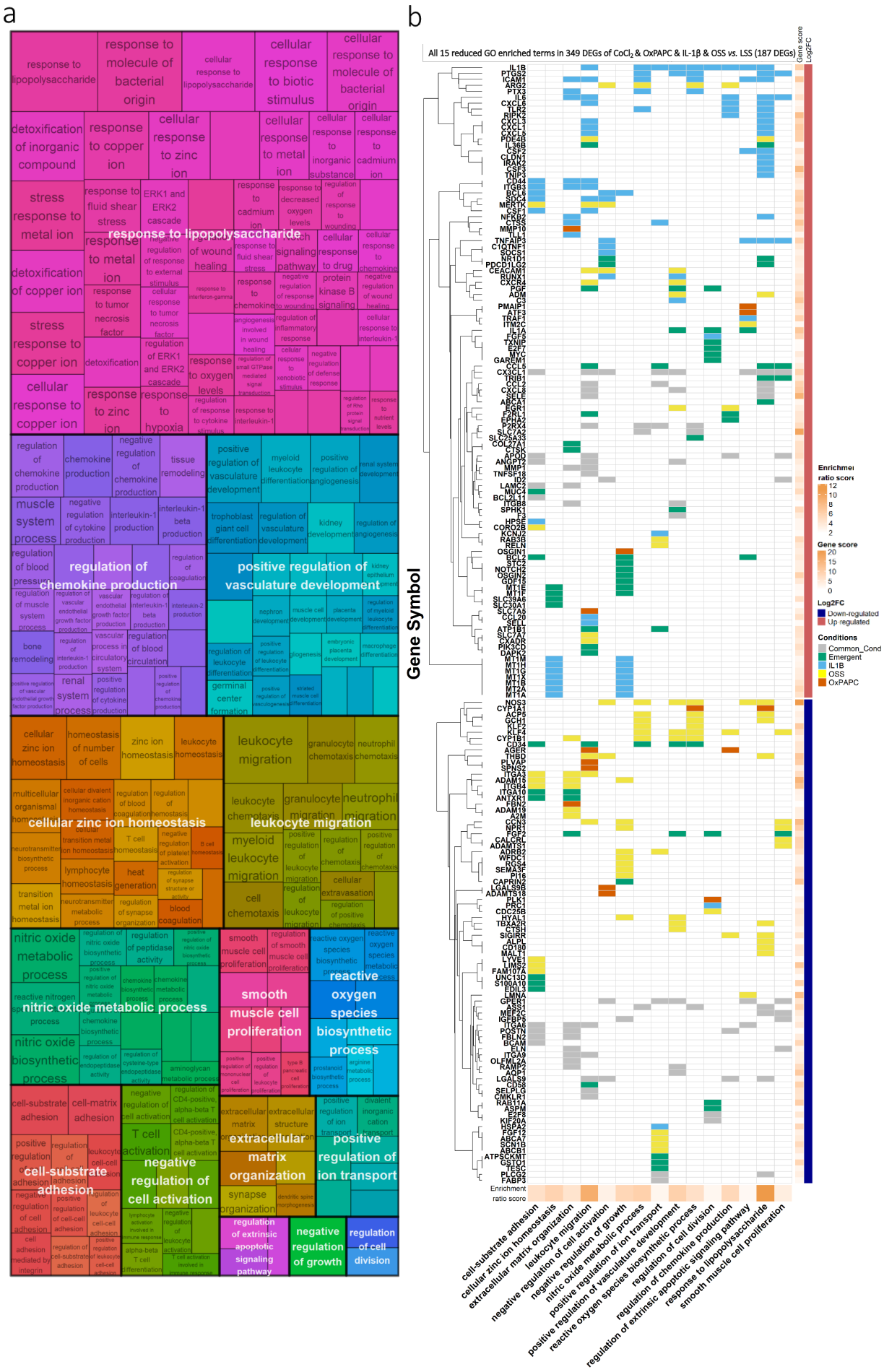

g
Control IL-1β TNFα Control IL-1β TNFα Control IL-1β TNFα
VCAM1
β-actin
Control IL-1β TNFα
VCAM1
β-actin

Supplementary Fig 1. *In vitro* shear stress in human coronary artery endothelial cell (HCAEC) model and further standardization of each stimuli. a Overall design of HCACE submitted to each stimulus, either individual or combined . **b** HCAEC under static condition stimulated with $CoCl_2$ for 48 h, and then HIF-1α protein expression was determined by Western blot (***p*<0.01, *vs.* static control**,** n=3), GAPDH served as a housekeeping protein. **c** HCAEC under static condition simulated by OxPAPC by 48h, RNA collected was reverse transcribed and cDNA was used for real-time PCR quantification of *CD36* and *ATF3* using GAPDH as an internal control (***p*<0.01, ****p* <0.001, *****p*<0.0001 *vs.* static control, n=2). **d** HCAEC under static condition stimulated with IL-1β, and then VCAM1 protein expression was determined by Western blot (****p*<0.001 *vs.* static control, n=4), served as a housekeeping protein β-actin. **e** HCAEC under LSS and OSS for 48h showing an aligned cell pattern in LSS when compared to EC under OSS. Data are presented as mean ± SEM; *p* values were determined by Student's t-test (**c**,**d**) and One way-Anova (**c**). **f** HCAEC under static condition stimulated with CoCl2 (100, 200, 250 or 300μM) for 48 h, and then HIF-1α protein expression was determined by Western blot. GAPDH served as a housekeeping protein. **g** HCAEC under static condition stimulated with IL-1β and TNFα, and then VCAM1 protein expression was determined by Western blot. β-actin served as a housekeeping protein**.** Figure **a** created in the Mind the Graph platform (www.mindthegraph.com).

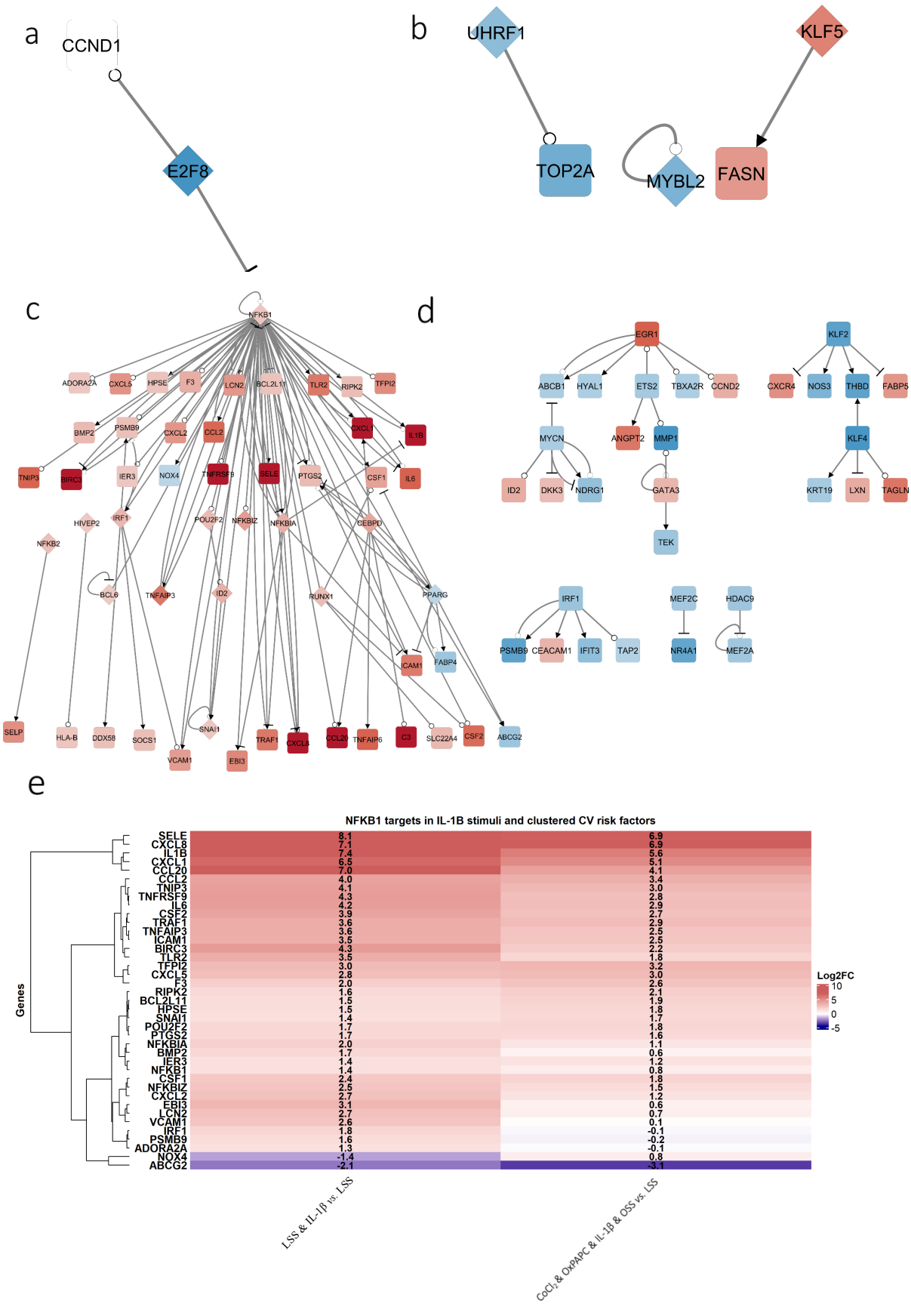


Supplementary Fig 2. Human coronary artery endothelial cell (HCAEC) under the $CoCl_2$, IL-1β, OxPAPC and OSS and their differentially expressed genes (DEGs) when compared to LSS. a HCAEC submitted to unidirectional LSS (20dyn/cm²) alone or combined with $CoCl_2$ (150µM), IL-1β (10ng/ml) and OxPAPC (40µg/ml) by 48h. **b**

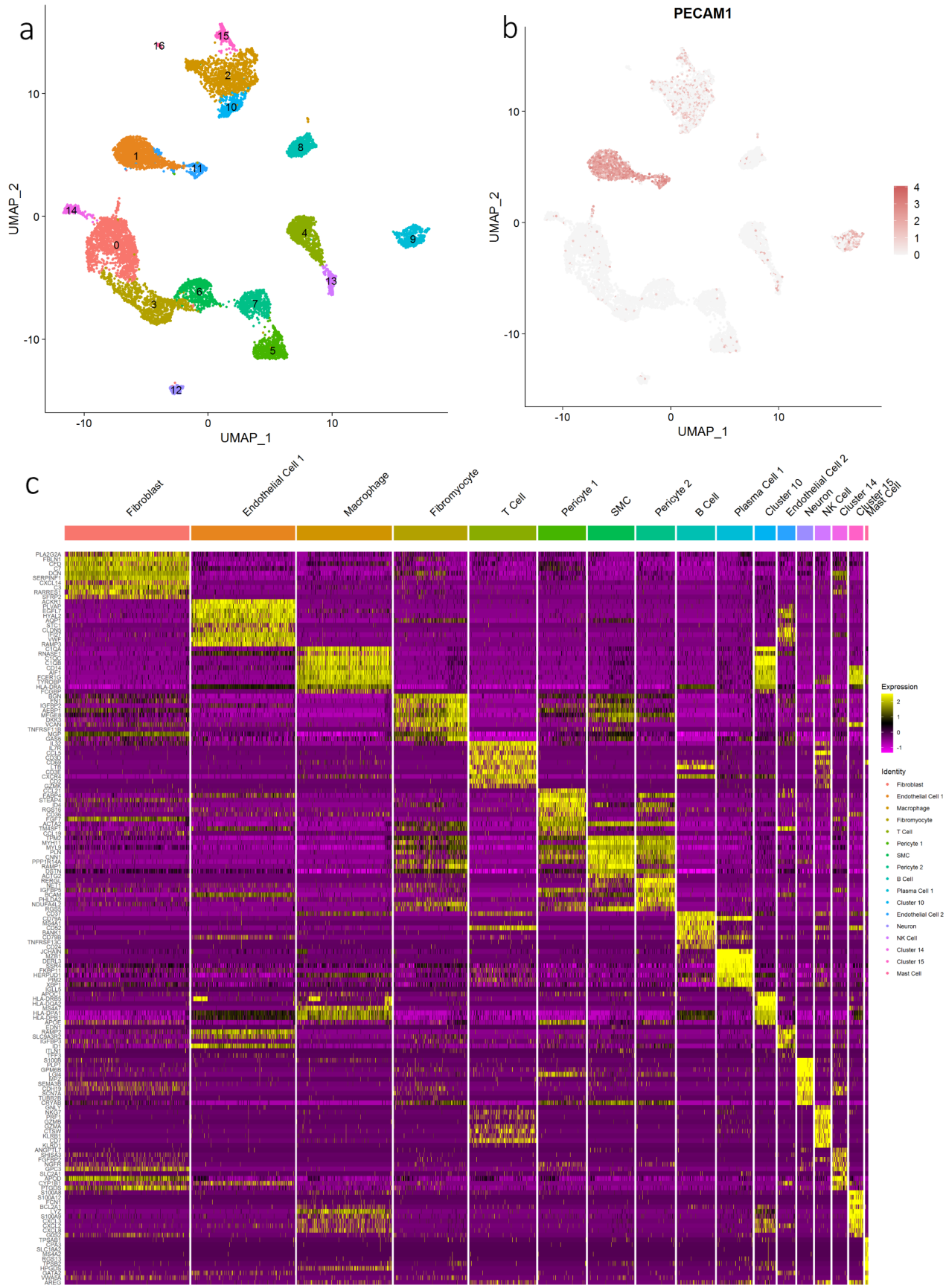
HCAEC submitted to bidirectional OSS (± 5dyn/cm² 1Hz) alone or together with $CoCl_2$, IL-1β, and OxPAPC. **c** Heatmap of 349 DEGs of the clustered risk factors that are differentially expressed in the individual condition. Example of stimuli-dependent genes red arrows. **d-g** Volcano plots showing the DEGs when comparing each stimulus to LSS. The DEGs were considered as adjusted *p*-value < 0.05 and |Log2foldchange| > 1.3 highlighting the expression of *NOS3*.

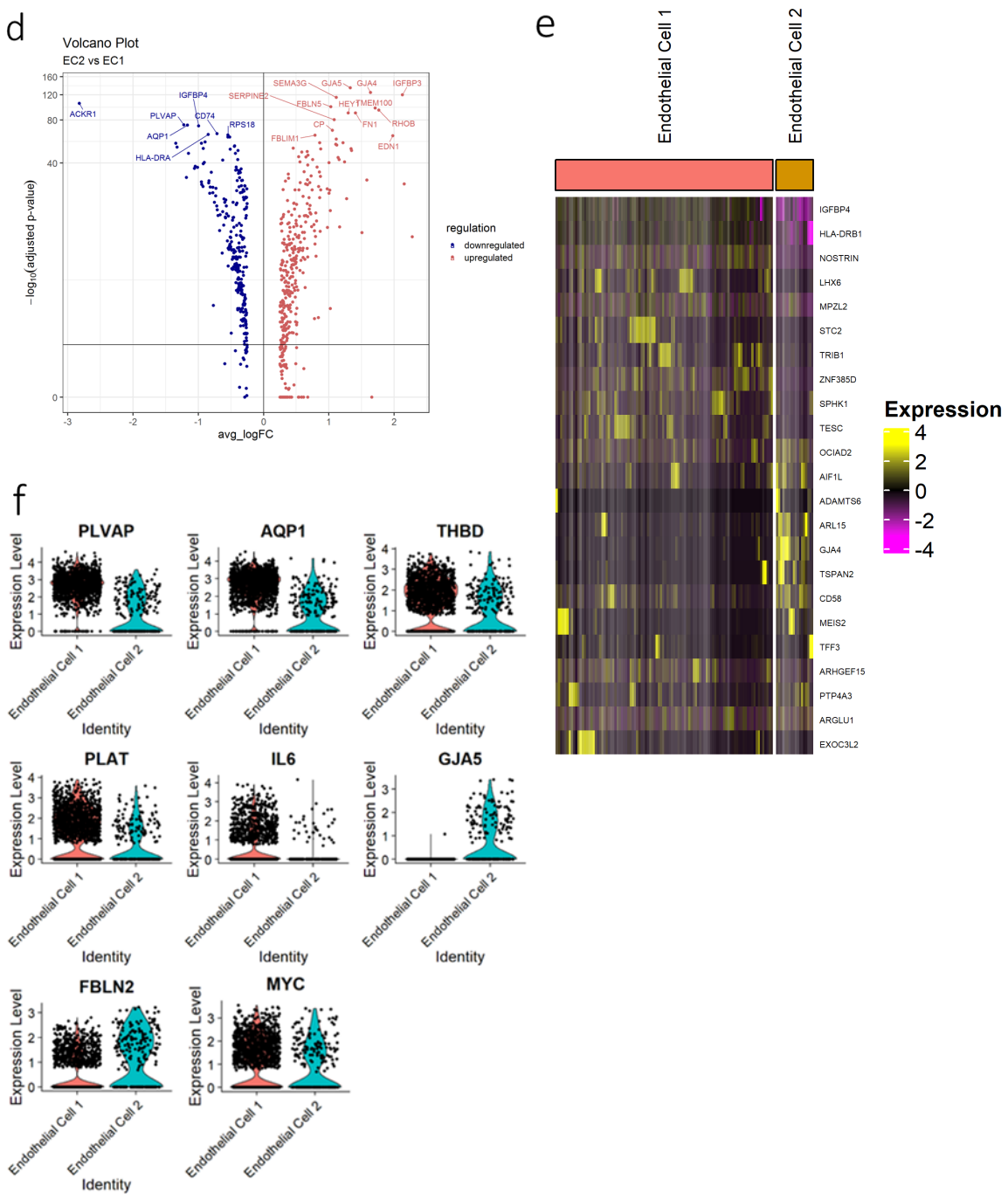

a
response to lipopolysaccharide
response to molecule of bacterial origin
cellular response to lipopolysaccharide
cellular response to biotic stimulus
cellular response to molecule of bacterial origin
detoxification of inorganic compound
response to copper ion
cellular response to zinc ion
cellular response to metal ion
cellular response to inorganic substance
cellular response to cadmium ion
stress response to metal ion
response to fluid shear stress
ERK1 and ERK2 cascade
response to cadmium ion
response to decreased oxygen levels
regulation of response to wounding
response to lipopolysaccharide
detoxification of copper ion
response to metal ion
regulation of wound healing
response to fluid shear stress
signaling pathway
cellular response to drug
cellular response to chemokine
regulation of response to external stimulus
response to chemokine
protein kinase B signaling
negative regulation of wound healing
stress response to copper ion
response to tumor necrosis factor
cellular response to tumor necrosis factor
response to interferon-gamma
angiogenesis involved in wound healing
regulation of inflammatory response
cellular response to interleukin-1
cellular response to copper ion
detoxification
regulation of ERK1 and ERK2 cascade
response to oxygen levels
regulation of small GTPase mediated signal transduction
cellular response to xenobiotic stimulus
negative regulation of defense response
response to zinc ion
response to hypoxia
regulation of response to cytokine stimulus
response to interleukin-1
regulation of Rho protein signal transduction
response to nutrient levels
regulation of chemokine production
chemokine production
negative regulation of chemokine production
tissue remodeling
positive regulation of vasculature development
myeloid leukocyte differentiation
positive regulation of angiogenesis
renal system development
muscle system process
negative regulation of cytokine production
interleukin-1 production
interleukin-1 beta production
trophoblast giant cell differentiation
regulation of vasculature development
kidney development
regulation of angiogenesis
regulation of blood pressure
regulation of coagulation
positive regulation of vasculature development
kidney epithelium development
regulation of muscle system process
regulation of vascular endothelial growth factor production
vascular endothelial growth factor production
regulation of interleukin-1 beta production
interleukin-2 production
nephron development
muscle cell development
placenta development
regulation of myeloid leukocyte differentiation
bone remodeling
regulation of interleukin-1 production
vascular process in circulatory system
regulation of blood circulation
gliogenesis
embryonic placenta development
macrophage differentiation
positive regulation of vascular endothelial growth factor production
renal system process
positive regulation of cytokine production
positive regulation of chemokine production
regulation of leukocyte differentiation
positive regulation of leukocyte differentiation
germinal center formation
positive regulation of vasculogenesis
striated muscle cell differentiation
cellular zinc ion homeostasis
homeostasis of number of cells
zinc ion homeostasis
leukocyte homeostasis
leukocyte migration
granulocyte chemotaxis
neutrophil chemotaxis
multicellular organismal homeostasis
cellular divalent inorganic cation homeostasis
regulation of blood coagulation
regulation of hemostasis
leukocyte chemotaxis
granulocyte migration
neutrophil migration
cellular zinc ion homeostasis
neurotransmitter biosynthetic process
cellular transition metal ion homeostasis
T cell homeostasis
negative regulation of platelet activation
B cell homeostasis
leukocyte migration
myeloid leukocyte migration
positive regulation of leukocyte migration
regulation of chemotaxis
positive regulation of chemotaxis
lymphocyte homeostasis
heat generation
regulation of synapse structure or activity
cellular extravasation
transition metal ion homeostasis
neurotransmitter metabolic process
regulation of synapse organization
blood coagulation
cell chemotaxis
regulation of leukocyte migration
regulation of positive chemotaxis
nitric oxide metabolic process
regulation of nitric oxide biosynthetic process
regulation of peptidase activity
positive regulation of nitric oxide biosynthetic process
smooth muscle cell proliferation
regulation of smooth muscle cell proliferation
reactive oxygen species biosynthetic process
reactive oxygen species metabolic process
reactive nitrogen species metabolic process
positive regulation of nitric oxide metabolic process
chemokine biosynthetic process
chemokine metabolic process
nitric oxide metabolic process
smooth muscle cell proliferation
reactive oxygen species biosynthetic process
nitric oxide biosynthetic process
regulation of endopeptidase activity
regulation of cysteine-type endopeptidase activity
aminoglycan metabolic process
positive regulation of mononuclear cell proliferation
positive regulation of leukocyte proliferation
type B pancreatic cell proliferation
prostanoid biosynthetic process
arginine metabolic process
cell-substrate adhesion
cell-matrix adhesion
negative regulation of cell activation
regulation of CD4-positive, alpha-beta T cell activation
extracellular matrix
extracellular structure
positive regulation of ion transport
divalent inorganic cation transport
T cell activation
CD4-positive, alpha-beta T cell activation
extracellular matrix organization
positive regulation of ion transport
negative regulation of cell activation
cell-substrate adhesion
positive regulation of cell-matrix adhesion
regulation of cell-cell adhesion
leukocyte cell-cell adhesion
synapse organization
dendritic spine morphogenesis
regulation of cell-cell adhesion
leukocyte cell-cell adhesion
lymphocyte activation involved in immune response
negative regulation of leukocyte activation
regulation of extrinsic apoptotic signaling pathway
negative regulation of growth
regulation of cell division
positive regulation of cell-substrate adhesion
cell adhesion mediated by integrin
alpha-beta T cell differentiation
T cell activation involved in immune response
b
All 15 reduced GO enriched terms in 349 DEGs of CoCl2 & OxPAPC & IL-1β & OSS vs. LSS (187 DEGs)
Gene Symbol
Gene score
Log2FC
Enrichment ratio score
Gene score
Log2FC
Down-regulated
Up regulated
Conditions
Common_Cond
Emergent
IL1B
OSS
OxPAPC
Enrichment ratio
cell-substrate adhesion
cellular zinc ion homeostasis
extracellular matrix organization
leukocyte migration
negative regulation of cell activation
negative regulation of growth
nitric oxide metabolic process
positive regulation of ion transport
positive regulation of vasculature development
reactive oxygen species biosynthetic process
regulation of cell division
regulation of chemokine production
regulation of extrinsic apoptotic signaling pathway
response to lipopolysaccharide
smooth muscle cell proliferation

Supplementary Fig 3. Gene Ontologies (GO) reduced enriched terms for the 620 differentially expressed genes (DEGs) of the clustered risk factors *vs.* LSS. a GO enrichment analysis followed by redundancy analysis identified and kept the most representative term from the redundant terms (cutoff = 0.9 and adjusted p-value ≤ 0.05). Gene score and enrichment risk score were calculated by -$\log_{10}$(adjusted *p*-value). The DEGs considered were adjusted as *p*-value ≤ 0.05 and |Log2foldchange| ≥ 1.3. **b** Canonical enriched terms in $CoCl_2$ & IL1B & OxPAPC & OSS *vs.* LSS (121 DEGs) and the source of the stimulus individual (represented by colours light blue, yellow and orange), common (grey), more than one stimulus and if it is emergent, meaning that the gene is only altered if all four stimuli are combined (green). Gene score and enrichment risk score were calculated by -$\log_{10}$(adjusted *p*-value).


a
CCND1
E2F8
b
UHRF1
KLF5
TOP2A
MYBL2
FASN
c
NFKB1
ADORA2A
CXCL5
HPSE
F3
LCN2
BCL2L11
TLR2
RIPK2
TFPI2
BMP2
PSMB9
CXCL2
CCL2
CXCL1
IL1B
TNIP3
BIRC3
IER3
NOX4
TNFRSF9
SELE
PTGS2
CSF1
IL6
HIVEP2
IRF1
POU2F2
NFKBIZ
NFKBIA
CEBPD
NFKB2
BCL6
TNFAIP3
ID2
RUNX1
PPARG
ICAM1
FABP4
SELP
HLA-B
DDX58
SOCS1
SNAI1
VCAM1
EBI3
TRAF1
CXCL8
CCL20
TNFAIP6
C3
SLC22A4
CSF2
ABCG2
d
EGR1
ABCB1
HYAL1
ETS2
TBXA2R
CCND2
MYCN
ANGPT2
MMP1
ID2
DKK3
NDRG1
GATA3
TEK
KLF2
CXCR4
NOS3
THBD
FABP5
KLF4
KRT19
LXN
TAGLN
IRF1
PSMB9
CEACAM1
IFIT3
TAP2
MEF2C
NR4A1
HDAC9
MEF2A
e
NFKB1 targets in IL-1B stimuli and clustered CV risk factors
Genes
SELE 8.1 6.9
CXCL8 7.1 6.9
IL1B 7.4 5.6
CXCL1 6.5 5.1
CCL20 7.0 4.1
CCL2 4.0 3.4
TNIP3 4.1 3.0
TNFRSF9 4.3 2.8
IL6 4.2 2.9
CSF2 3.9 2.7
TRAF1 3.6 2.9
TNFAIP3 3.6 2.5
ICAM1 3.5 2.5
BIRC3 4.3 2.2
TLR2 3.5 1.8
TFPI2 3.0 3.2
CXCL5 2.8 3.0
F3 2.0 2.6
RIPK2 1.6 2.1
BCL2L11 1.5 1.9
HPSE 1.5 1.8
SNAI1 1.4 1.7
POU2F2 1.7 1.8
PTGS2 1.7 1.6
NFKBIA 2.0 1.1
BMP2 1.7 0.6
IER3 1.4 1.2
NFKB1 1.4 0.8
CSF1 2.4 1.8
NFKBIZ 2.5 1.5
CXCL2 2.7 1.2
EBI3 3.1 0.6
LCN2 2.7 0.7
VCAM1 2.6 0.1
IRF1 1.8 -0.1
PSMB9 1.6 -0.2
ADORA2A 1.3 -0.1
NOX4 -1.4 0.8
ABCG2 -2.1 -3.1
Log2FC
10
5
0
-5
LSS & IL-1β vs. LSS
CoCl2 & OxPAPC & IL-1β & OSS vs. LSS

Supplementary Fig 4. Transcription factors (TFs) regulatory network of each stimulus. a $CoCl_2$ & LSS *vs.* LSS regulatory network. **b** OxPAPC & LSS *vs.* LSS regulatory network. **c** IL-1β & LSS *vs.* LSS regulatory network. **d** OSS *vs.* LSS regulatory network. Using the TRRUST database (version 2), the TFs were mapped to their published transcriptional targets. The identified transcriptional network was further filtered based on whether the TF and their targets were a DEG (adjusted *p*-value ≤ 0.05 and |Log2foldchange| ≥ 1.3). The diamond shape is TF and the square is the DEG target. The edges mean arrow (activation), circle (unknow) and bar (repression). Node size according to the degree. Genes in blue are downregulated, genes in red upregulated. Green is a modulated emergent gene; Orange is an OxPAPC modulated gene; Light blue is an IL-1β modulated gene; Yellow is an OSS modulated gene; Gray when the gene is modulated in more than one condition. **e** Heatmap of 38 target DEGs from the TF NFKB1 and their log2foldchange in LSS & IL-1β *vs.* LSS and $CoCl_2$ & OxPAPC & IL1B & OSS *vs.* LSS.


a
b
PECAM1
UMAP_1
UMAP_2
c
Fibroblast
Endothelial Cell 1
Macrophage
Fibromyocyte
T Cell
Pericyte 1
SMC
Pericyte 2
B Cell
Plasma Cell 1
Cluster 10
Endothelial Cell 2
Neuron
NK Cell
Cluster 14
Cluster 15
Mast Cell
Expression
Identity



Supplementary Fig 5. a UMAP visualization of clustering identified 17 cell populations (n = 10,671 cells) using the cluster marker genes found with Seurat's function FindAllMarkers(), which finds differentially expressed genes between a cluster and all remaining cells. DEG were considered as adjusted *p*-value ≤ 0.05 and |Log2foldchange| ≥ 0.25. **b** UMAP visualization of a key endothelial cell (EC) marker *PECAM1* (CD31). **c** Heatmap of the top 10 gene markers expression levels from each cell in the 17 cell clusters. **d** Volcano plots showing the differentially expressed genes (DEGs) when comparing EC2 *vs.* EC1. The DEGs were considered as adjusted *p*-value < 0.05 and |Log2foldchange| > 0.25 highlighting the top 20 DEGs. **e** Heatmap of the emergent DEGs expression levels in which they are differentially expressed in the comparison EC2 *vs.*

EC1. **f** Violin plot of canonical clustered risk factors DEGs which are differentially expressed in EC2 *vs.* EC1.