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Supplemental information

A spatial vascular transcriptomic, proteomic, and phosphoproteomic atlas unveils an angiocrine Tie–Wnt signaling axis in the liver

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Figure S1

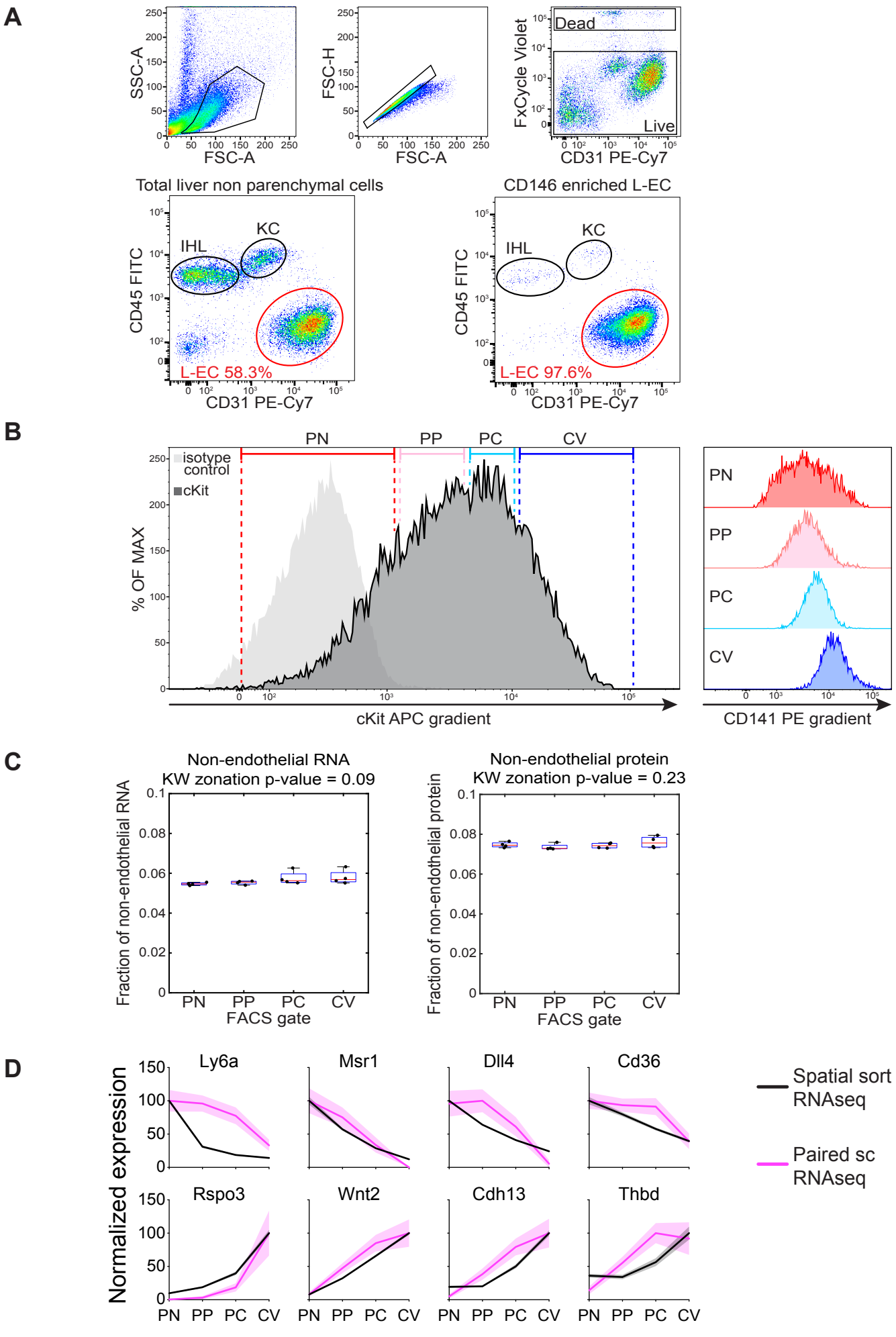

A
SSC-A
FSC-A
FSC-H
FSC-A
FxCycle Violet
CD31 PE-Cy7
Dead
Live
Total liver non parenchymal cells
CD146 enriched L-EC
CD45 FITC
IHL
KC
L-EC 58.3%
L-EC 97.6%
B
PN
PP
PC
CV
isotype control
cKit
% OF MAX
cKit APC gradient
CD141 PE gradient
C
Non-endothelial RNA
KW zonation p-value = 0.09
Non-endothelial protein
KW zonation p-value = 0.23
Fraction of non-endothelial RNA
Fraction of non-endothelial protein
FACS gate
D
Ly6a
Msr1
Dll4
Cd36
Rspo3
Wnt2
Cdh13
Thbd
Normalized expression
Spatial sort RNAseq
Paired sc RNAseq

Figure S1. FACS gating strategy and L-EC purity. Related to Figure 1.

(**A** and **B**) FACS gating strategy for L-EC isolation (**A**) and for L-EC spatial sort (**B**). (**A**) Nested gating strategy for live singlets (upper panel) and comparison of liver non parenchymal cell (NPC) populations before and after CD146 magnet beads enrichment (lower panel). L-EC: liver endothelial cell; IHL: intrahepatic lymphocyte; KC: Kupffer cell. (**B**) Gating strategy to sort four consecutive L-EC subpopulations depending on their cKit staining gradient (left), which was controlled by the CD141 staining gradient (right). PN: portal node; PP: peri-portal; PC: peri-central; CV: central vein.

(**C**) Quantification of non-endothelial RNA and protein represented as fraction of total RNA (left panel) and protein (right panel) for each of the sorted fractions (Methods). Individual replicates are represented as black dots. Kruskal-Wallis (KW) test was performed to compare the non-endothelial fraction in the four different FACS gates. Red lines mark the median, blue boxes mark the interquartile range (IQR) and whiskers extend to the most extreme data points.

(**D**) Correlation of spatial sort RNAseq to scRNAseq. Expression profiles of representative portal (top row) and central (bottom row) zonated mRNA from scRNAseq (magenta) and spatial RNAseq (black). Gene expression is represented by percentage of maximum; patches represent SEM.

Figure S2

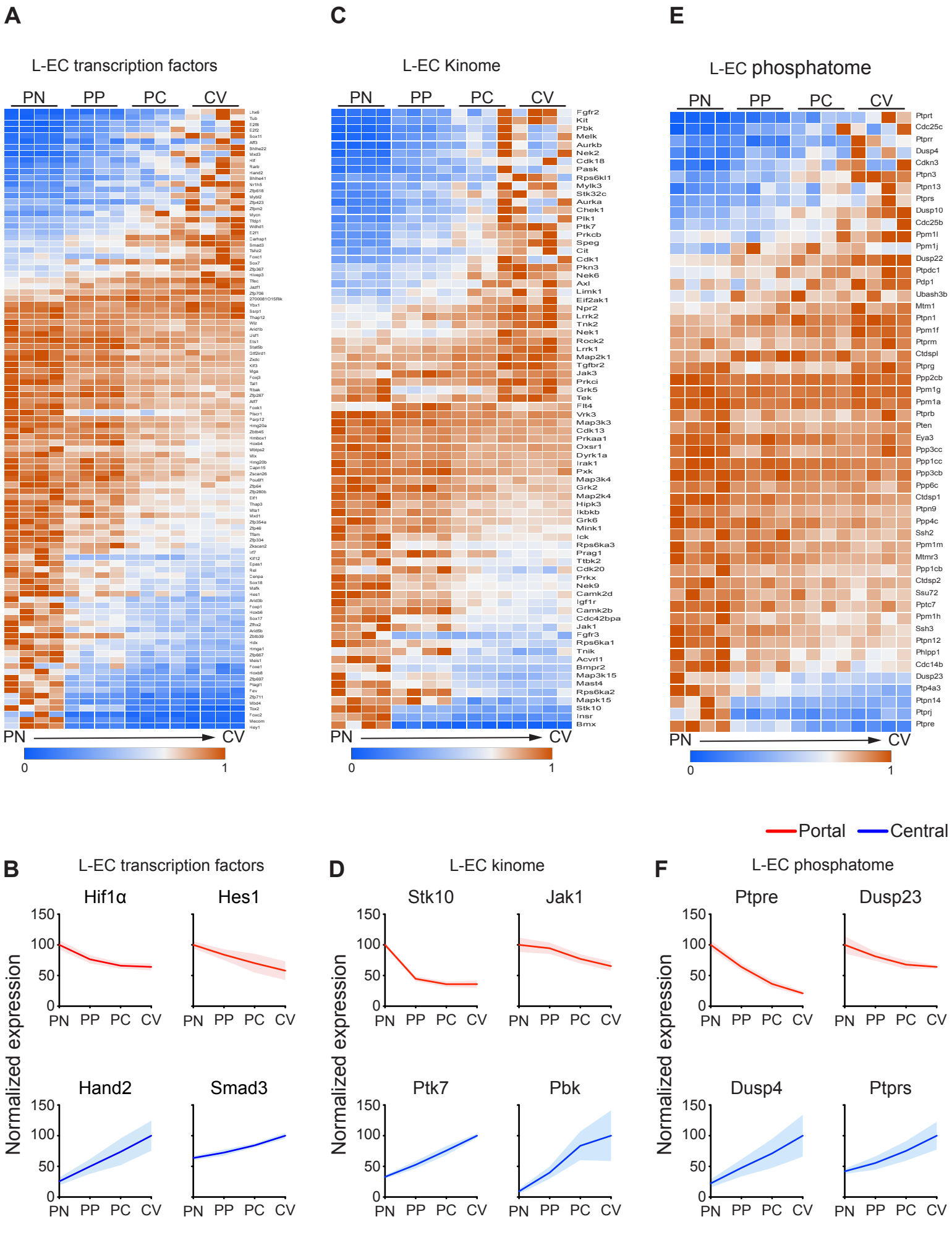

A
L-EC transcription factors
PN
PP
PC
CV
PN
CV
0
1
C
L-EC Kinome
PN
PP
PC
CV
Fgfr2
Kit
Pbk
Melk
Aurkb
Nek2
Cdk18
Pask
Rps6kl1
Mylk3
Stk32c
Aurka
Chek1
Plk1
Ptk7
Prkcb
Speg
Cit
Cdk1
Pkn3
Nek6
Axl
Limk1
Eif2ak1
Npr2
Lrrk2
Tnk2
Nek1
Rock2
Lrrk1
Map2k1
Tgfbr2
Jak3
Prkci
Grk5
Tek
Flt4
Vrk3
Map3k3
Cdk13
Prkaa1
Oxsr1
Dyrk1a
Irak1
Pxk
Map3k4
Grk2
Map2k4
Hipk3
Ikbkb
Grk6
Mink1
Ick
Rps6ka3
Prag1
Ttbk2
Cdk20
Prkx
Nek9
Camk2d
Igf1r
Camk2b
Cdc42bpa
Jak1
Fgfr3
Rps6ka1
Tnik
Acvrl1
Bmpr2
Map3k15
Mast4
Rps6ka2
Mapk15
Stk10
Insr
Bmx
PN
CV
0
1
E
L-EC phosphatome
PN
PP
PC
CV
Ptprt
Cdc25c
Ptprr
Dusp4
Cdkn3
Ptpn3
Ptpn13
Ptprs
Dusp10
Cdc25b
Ppm1l
Ppm1j
Dusp22
Ptpdc1
Pdp1
Ubash3b
Mtm1
Ptpn1
Ppm1f
Ptprm
Ctdspl
Ptprg
Ppp2cb
Ppm1g
Ppm1a
Ptprb
Pten
Eya3
Ppp3cc
Ppp1cc
Ppp3cb
Ppp6c
Ctdsp1
Ptpn9
Ppp4c
Ssh2
Ppm1m
Mtmr3
Ppp1cb
Ctdsp2
Ssu72
Pptc7
Ppm1h
Ssh3
Ptpn12
Phlpp1
Cdc14b
Dusp23
Ptp4a3
Ptpn14
Ptprj
Ptpre
PN
CV
0
1
Portal
Central
B
L-EC transcription factors
Hif1α
Hes1
Hand2
Smad3
Normalized expression
150
100
50
0
PN PP PC CV
D
L-EC kinome
Stk10
Jak1
Ptk7
Pbk
Normalized expression
150
100
50
0
PN PP PC CV
F
L-EC phosphatome
Ptpre
Dusp23
Dusp4
Ptprs
Normalized expression
150
100
50
0
PN PP PC CV

Figure S2. Zonation of L-EC transcription factors, kinases and phosphatases. Related to Figure 2.

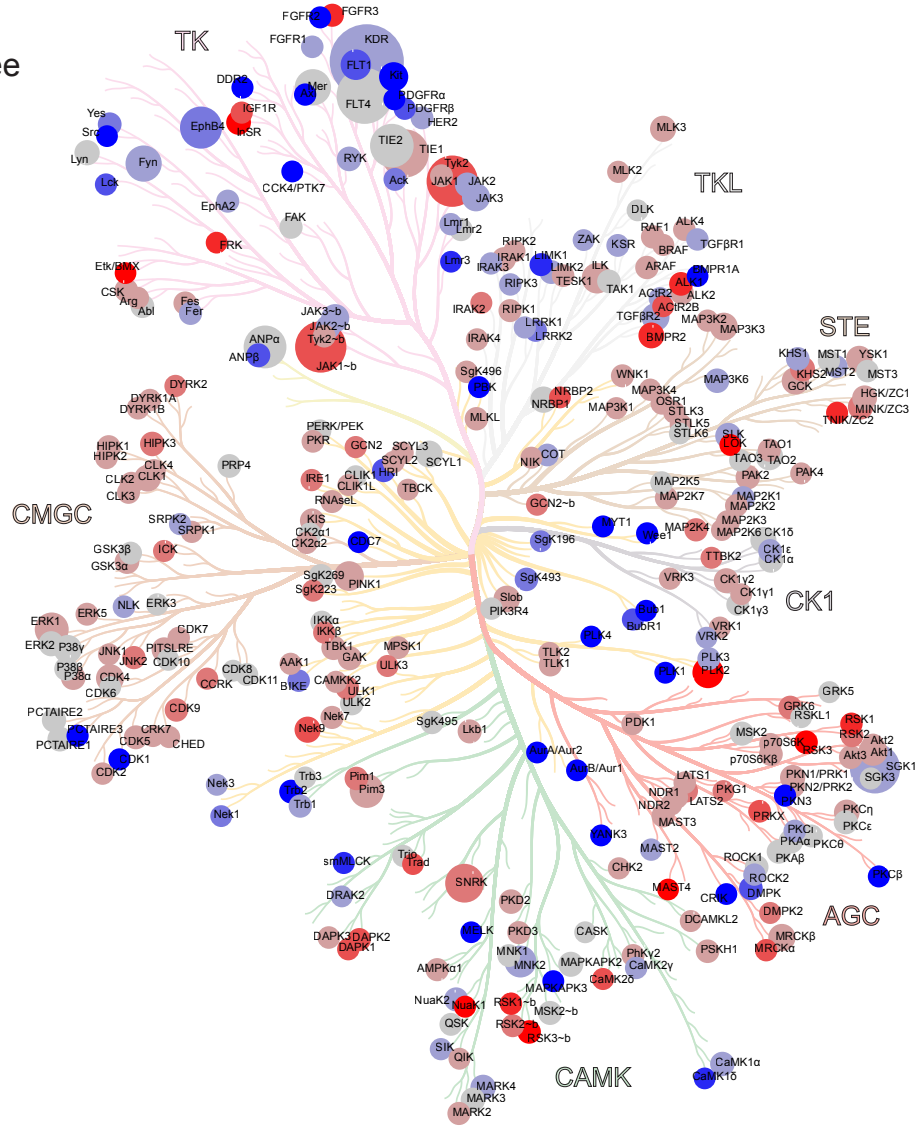
(**A, C** and **E**) Heat map representation of the expression profiles of 103 transcription factors (**A**), 76 kinases (**C**) and 52 phosphatases (**E**), with significantly zonated expression. Genes are normalized to their maximum expression and sorted by their Center-of-Mass (n=4).

(**B, D** and **F**) Representative expression profiles of portal (red) and central (blue) zonated transcription factors (**B**), kinases (**D**) and phosphatases (**F**). Gene expression is represented by percentage of maximum; patches represent SD (n=4).

Figure S3

A

Kinome phylogenetic tree



B

Phosphatase phylogenetic tree

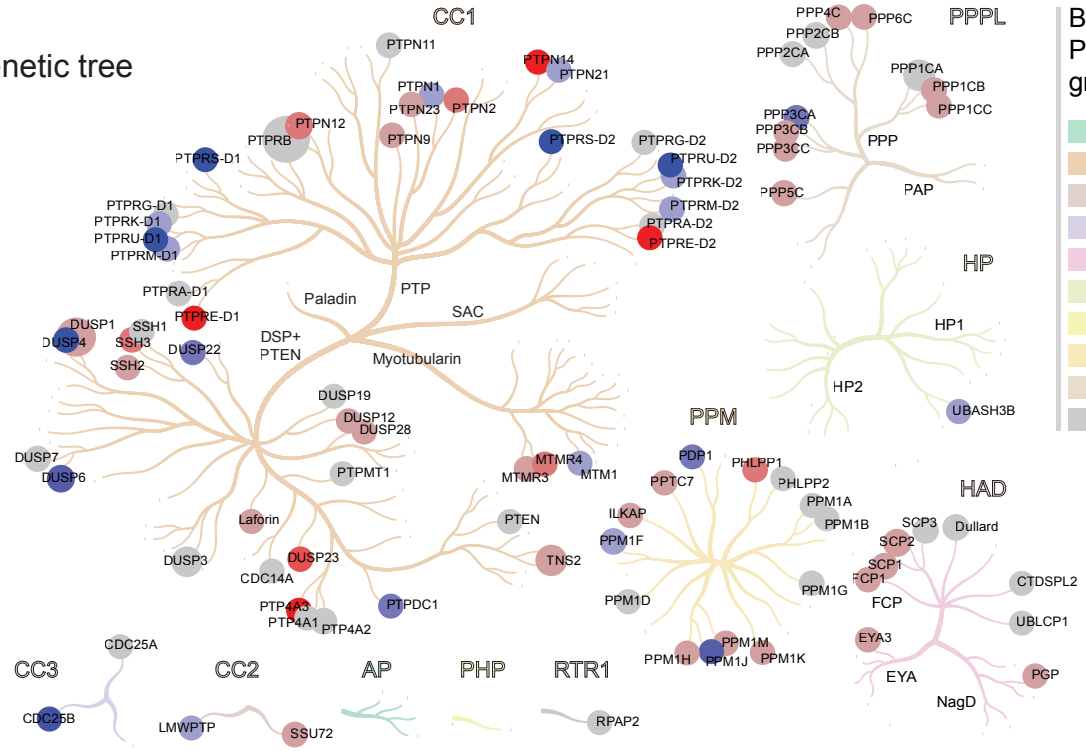


Figure S3. Phylogenetic tree analysis of L-EC kinases and phosphatases. Related to Figure 2.
Each gene is represented by a circle and grouped by the kinases (**A**) or phosphatase (**B**) family. The circle size is proportional to the mean TPM across zones. The color represents expression zonation from portal (red) to central (blue).

Figure S4

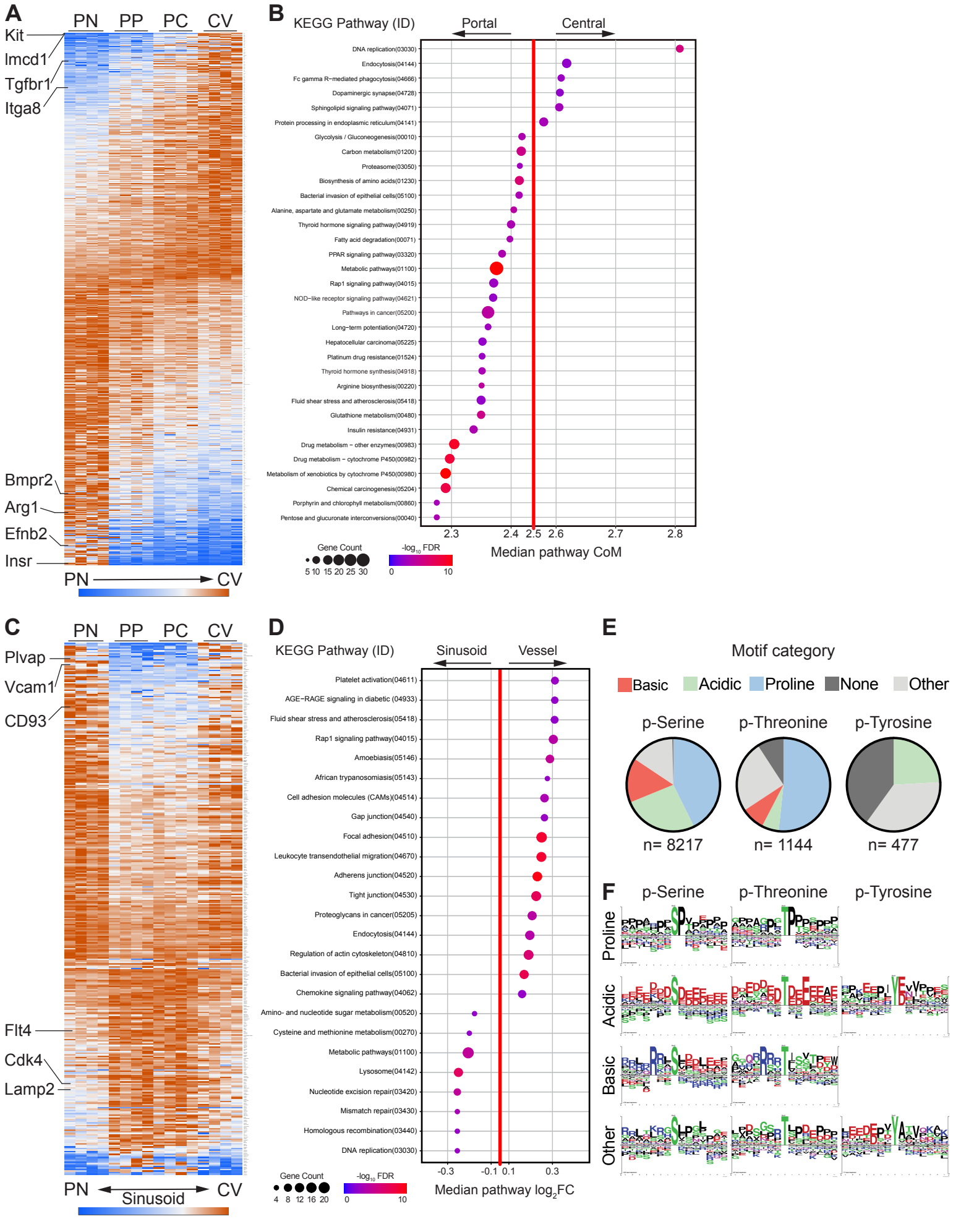

A
PN
PP
PC
CV
Kit
Imcd1
Tgfbr1
Itga8
Bmpr2
Arg1
Efnb2
Insr
PN
CV
B
KEGG Pathway (ID)
Portal
Central
DNA replication(03030)
Endocytosis(04144)
Fc gamma R−mediated phagocytosis(04666)
Dopaminergic synapse(04728)
Sphingolipid signaling pathway(04071)
Protein processing in endoplasmic reticulum(04141)
Glycolysis / Gluconeogenesis(00010)
Carbon metabolism(01200)
Proteasome(03050)
Biosynthesis of amino acids(01230)
Bacterial invasion of epithelial cells(05100)
Alanine, aspartate and glutamate metabolism(00250)
Thyroid hormone signaling pathway(04919)
Fatty acid degradation(00071)
PPAR signaling pathway(03320)
Metabolic pathways(01100)
Rap1 signaling pathway(04015)
NOD−like receptor signaling pathway(04621)
Pathways in cancer(05200)
Long−term potentiation(04720)
Hepatocellular carcinoma(05225)
Platinum drug resistance(01524)
Thyroid hormone synthesis(04918)
Arginine biosynthesis(00220)
Fluid shear stress and atherosclerosis(05418)
Glutathione metabolism(00480)
Insulin resistance(04931)
Drug metabolism − other enzymes(00983)
Drug metabolism − cytochrome P450(00982)
Metabolism of xenobiotics by cytochrome P450(00980)
Chemical carcinogenesis(05204)
Porphyrin and chlorophyll metabolism(00860)
Pentose and glucuronate interconversions(00040)
2.3
2.4
2.5
2.6
2.7
2.8
Median pathway CoM
Gene Count
5 10 15 20 25 30
-log10 FDR
0
10
C
PN
PP
PC
CV
Plvap
Vcam1
CD93
Flt4
Cdk4
Lamp2
PN
Sinusoid
CV
D
KEGG Pathway (ID)
Sinusoid
Vessel
Platelet activation(04611)
AGE−RAGE signaling in diabetic (04933)
Fluid shear stress and atherosclerosis(05418)
Rap1 signaling pathway(04015)
Amoebiasis(05146)
African trypanosomiasis(05143)
Cell adhesion molecules (CAMs)(04514)
Gap junction(04540)
Focal adhesion(04510)
Leukocyte transendothelial migration(04670)
Adherens junction(04520)
Tight junction(04530)
Proteoglycans in cancer(05205)
Endocytosis(04144)
Regulation of actin cytoskeleton(04810)
Bacterial invasion of epithelial cells(05100)
Chemokine signaling pathway(04062)
Amino- and nucleotide sugar metabolism(00520)
Cysteine and methionine metabolism(00270)
Metabolic pathways(01100)
Lysosome(04142)
Nucleotide excision repair(03420)
Mismatch repair(03430)
Homologous recombination(03440)
DNA replication(03030)
-0.3
-0.1
0.1
0.3
Median pathway log2FC
Gene Count
4 8 12 16 20
-log10 FDR
0
10
E
Motif category
Basic
Acidic
Proline
None
Other
p-Serine
p-Threonine
p-Tyrosine
n= 8217
n= 1144
n= 477
F
p-Serine
p-Threonine
p-Tyrosine
Proline
Acidic
Basic
Other

Figure S4. Zonated pathways of proteome and phosphorylation motif analysis. Related to Figure 4.

(**A**) Heat map representation of the expression profiles of 688 proteins significantly zonated on portal or central side. Proteins are normalized to their maximum LFQ value and sorted by their Center-of-Mass (n=4).

(**B**) Dot plot of the KEGG pathways significantly enriched in portal or central. Pathways (Y axis) are ordered from portal to central by increasing median Center-of-Mass (X-axis) of the proteins enriched in the pathway. Dot size and color indicate gene count and $-\log_{10}$ FDR, respectively.

(**C**) Heat map representation of the expression profiles of 352 proteins significantly zonated on vessels or sinusoids. Proteins are normalized to their maximum LFQ value and sorted by their vessel to sinusoid $\log_2$ fold change (n=4).

(**D**) Dot plot of the KEGG pathways significantly enriched on vessels or sinusoids. Pathways (Y axis) are ordered from sinusoids to vessels by increasing median $\log_2$ fold change (X-axis) of the proteins enriched in the pathway. Dot size and color scale indicate gene count and $-\log_{10}$ FDR, respectively.

(**E-F**) Phosphorylation motif analysis of the p-S, p-T and p-Y sites. (**E**) Pie charts showing the proportion of proline-directed, acidic, basic and other motif categories for phosphorylated Serine, Threonine and Tyrosine residues. Only class I P-sites (localization score >0.75) were considered. “None” indicates sequences not associated with any motif. (**F**) Motif logos of consensus sequences surrounding p- Serine, p-Threonine and p-Tyrosine for proline-directed, acidic, basic and other motifs classified in (**E**).

Figure S5

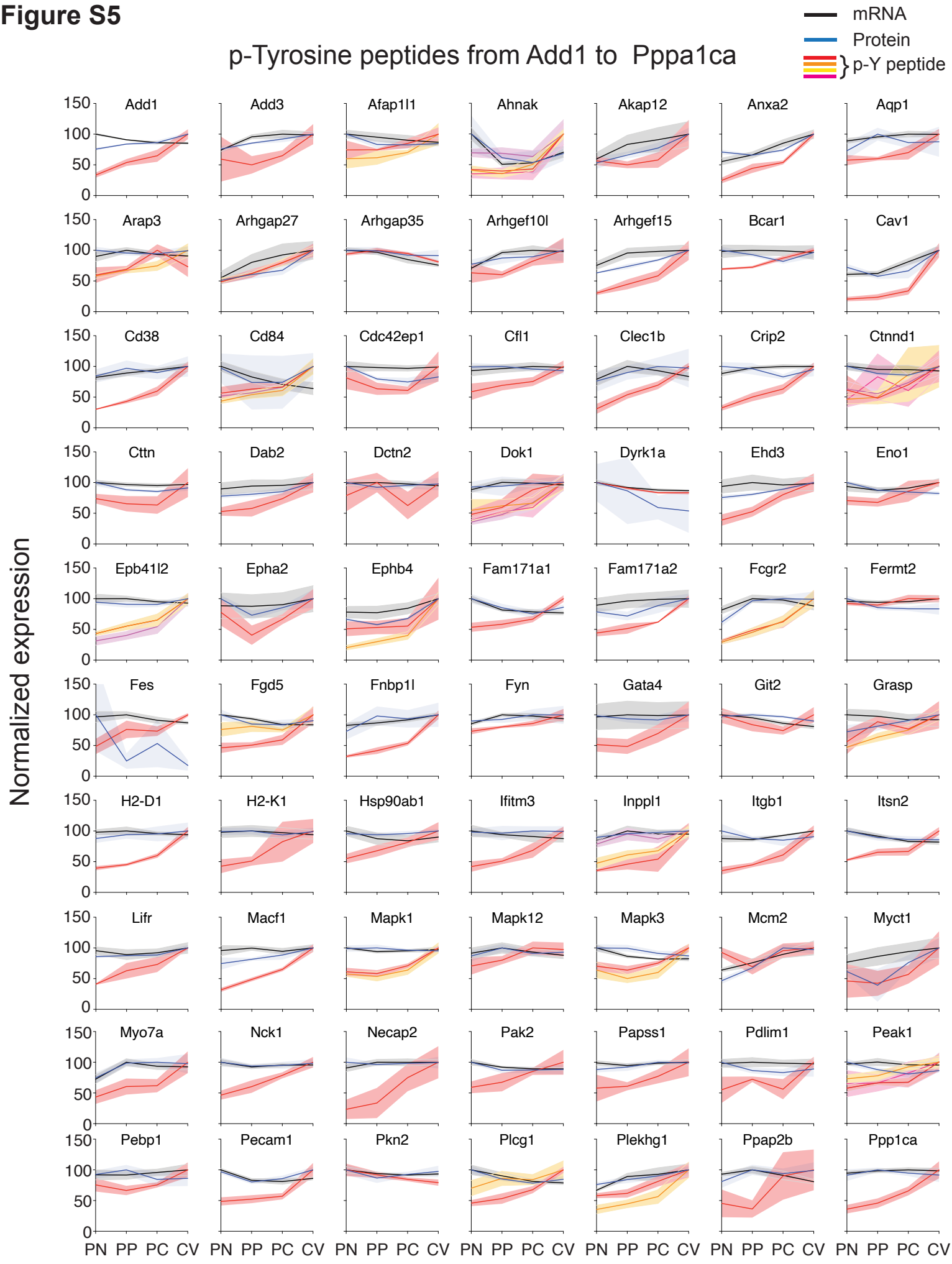
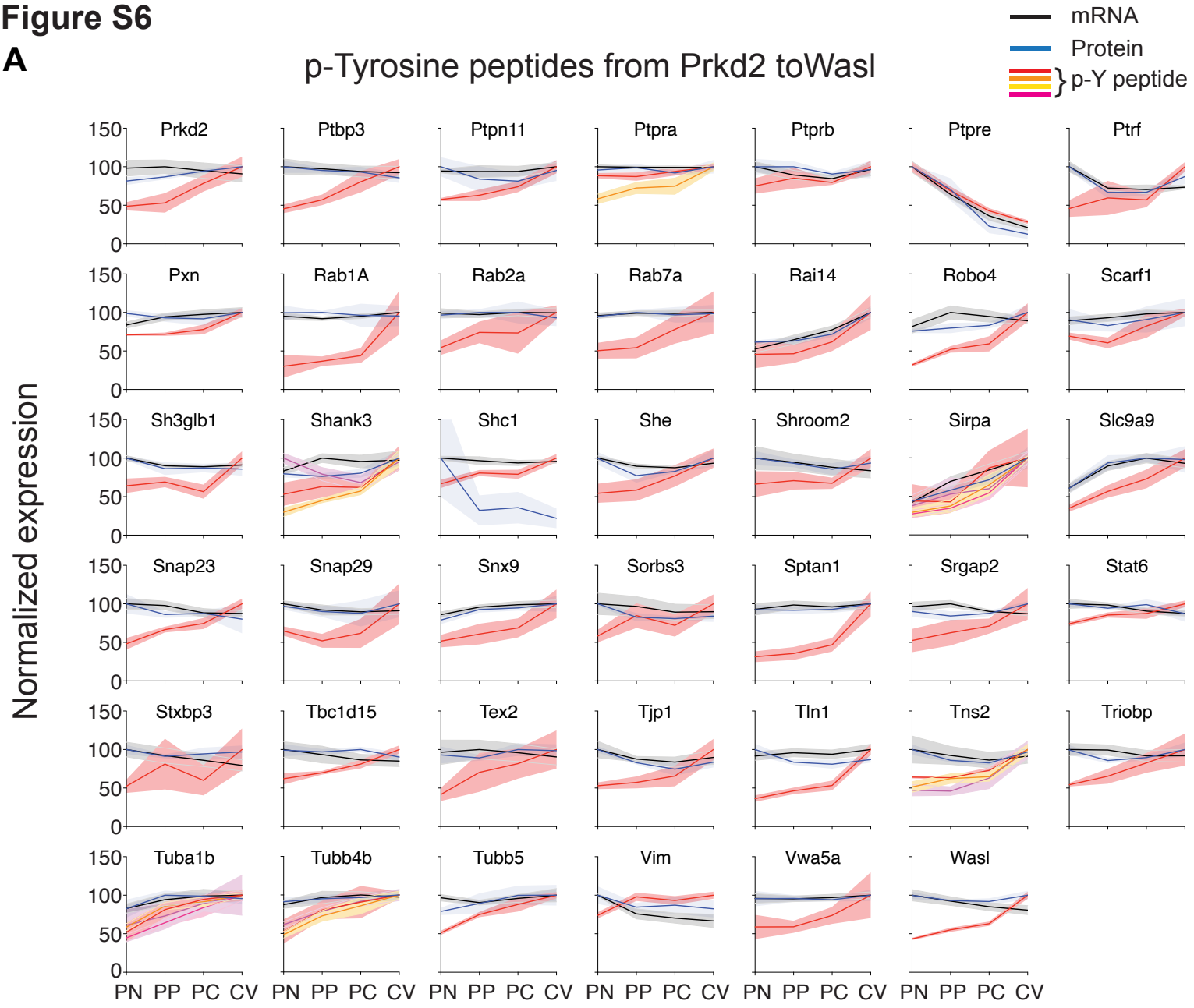

p-Tyrosine peptides from Add1 to Pppa1ca
mRNA
Protein
p-Y peptide
Normalized expression
Add1
Add3
Afap1l1
Ahnak
Akap12
Anxa2
Aqp1
Arap3
Arhgap27
Arhgap35
Arhgef10l
Arhgef15
Bcar1
Cav1
Cd38
Cd84
Cdc42ep1
Cfl1
Clec1b
Crip2
Ctnnd1
Cttn
Dab2
Dctn2
Dok1
Dyrk1a
Ehd3
Eno1
Epb41l2
Epha2
Ephb4
Fam171a1
Fam171a2
Fcgr2
Fermt2
Fes
Fgd5
Fnbp1l
Fyn
Gata4
Git2
Grasp
H2-D1
H2-K1
Hsp90ab1
Ifitm3
Inppl1
Itgb1
Itsn2
Lifr
Macf1
Mapk1
Mapk12
Mapk3
Mcm2
Myct1
Myo7a
Nck1
Necap2
Pak2
Papss1
Pdlim1
Peak1
Pebp1
Pecam1
Pkn2
Plcg1
Plekhg1
Ppap2b
Ppp1ca
150
100
50
0
PN PP PC CV

Figure S5. Expression profiles of zonated p-Y peptides and their corresponding RNA and protein. Related to Figure 5. (continued in Figure S6A)

Plots of the Expression profiles of 154 significantly zonated p-Y peptides matched to 111 proteins. Plots are arranged in alphabetical order of the respective gene names from *Add1* to *Ppp1ca* (see Figure S6A from *Prkd2* to *Wasl*). Expression values are expressed as percentage of maximum; patches represent SD (n=4).

Figure S6

A


p-Tyrosine peptides from Prkd2 toWasl
mRNA
Protein
p-Y peptide
Normalized expression
Prkd2
Ptbp3
Ptpn11
Ptpra
Ptprb
Ptpre
Ptrf
Pxn
Rab1A
Rab2a
Rab7a
Rai14
Robo4
Scarf1
Sh3glb1
Shank3
Shc1
She
Shroom2
Sirpa
Slc9a9
Snap23
Snap29
Snx9
Sorbs3
Sptan1
Srgap2
Stat6
Stxbp3
Tbc1d15
Tex2
Tjp1
Tln1
Tns2
Triobp
Tuba1b
Tubb4b
Tubb5
Vim
Vwa5a
Wasl
150
100
50
0
PN PP PC CV

B

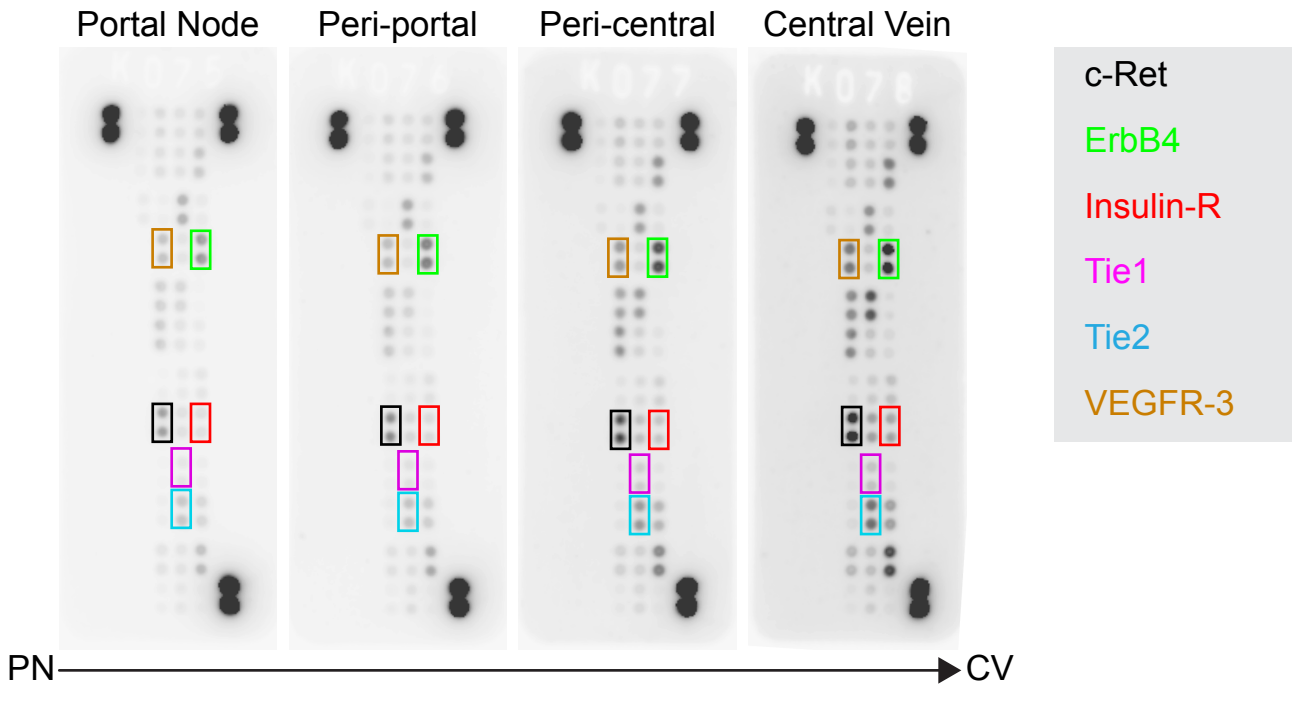

Portal Node
Peri-portal
Peri-central
Central Vein
c-Ret
ErbB4
Insulin-R
Tie1
Tie2
VEGFR-3
PN
CV

Figure S6. Centrally zonated Phospho-RTK on spatially sorted L-EC. Related to Figure 5.

(**A**) Plots of the Expression profiles of 154 significantly zonated p-Y peptides matched to 111 proteins (Continued from figure S5). Plots are arranged in alphabetical order of the respective gene names from *Prkd2* to *Wasl*. Expression values are expressed as percentage of maximum; patches represent SD (n=4).

(**B**) Dot-blot phospho-RTK array analysis of L-EC spatially sorted from the indicated zones. Selected pRTKs are indicated by color matched boxes.

Figure S7

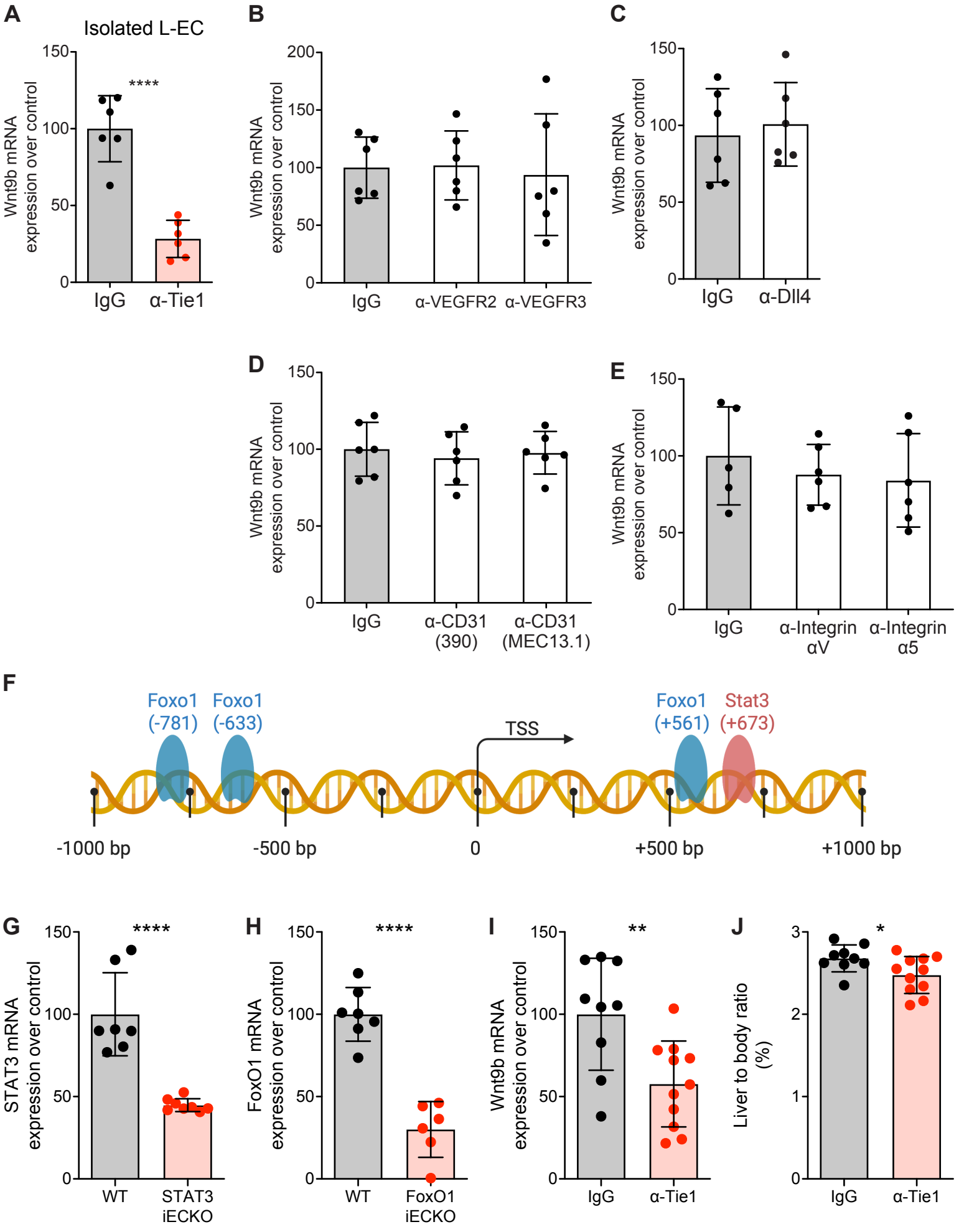

A
Isolated L-EC
Wnt9b mRNA expression over control
****
IgG
α-Tie1
B
Wnt9b mRNA expression over control
IgG
α-VEGFR2
α-VEGFR3
C
Wnt9b mRNA expression over control
IgG
α-Dll4
D
Wnt9b mRNA expression over control
IgG
α-CD31 (390)
α-CD31 (MEC13.1)
E
Wnt9b mRNA expression over control
IgG
α-Integrin αV
α-Integrin α5
F
Foxo1 (-781)
Foxo1 (-633)
TSS
Foxo1 (+561)
Stat3 (+673)
-1000 bp
-500 bp
0
+500 bp
+1000 bp
G
STAT3 mRNA expression over control
****
WT
STAT3 iECKO
H
FoxO1 mRNA expression over control
****
WT
FoxO1 iECKO
I
Wnt9b mRNA expression over control
**
IgG
α-Tie1
J
Liver to body ratio (%)
*
IgG
α-Tie1

Figure S7. The Tie1-Wnt9b signaling circuits. Related to Figure 6 and Figure 7.

(A) qRT-PCR analysis of *Wnt9b* mRNA expression from freshly isolated L-EC 2 hours after IgG (grey bar) or anti-Tie1 (red bar) administration in WT C57/B6 mice.

(**B-E**) qRT-PCR analysis of *Wnt9b* mRNA measured in whole liver lysates 2 hours after administration of the indicated blocking antibody against VEGFRs (**B**), delta-like ligand 4 (Dll4) (**C**), PECAM1/CD31 (**D**), and alpha-chain integrins (**E**) in WT C57/B6 mice.

(**F**) Representation of Wnt9b promoter region. Putative binding sites predicted by JASPAR for FoxO1 and STAT3 are indicated in blue and red respectively.

(G and **H**) qRT-PCR analysis of *Stat3* (**G**) and *Foxo1* (**H**) mRNA expression from freshly isolated L-EC after tamoxifen treatment of $Stat3^{iECKO}$ and $Foxo1^{iECKO}$ mice (red bar) and relative control mice (grey bar).

(I) Whole liver *Wnt9b* mRNA expression measured in WT C57/B6 mice 2 days after 2/3 PHx, treated with anti-Tie1 blocking antibody Tie1-39 (red bar) or IgG control (grey bar) at day 0.

(**J**) Liver to body ratio measured in WT C57/B6 mice 2 days after 2/3 PHx, treated with anti-Tie1 blocking antibody Tie1-39 (red bar) or IgG control (grey bar) at day 0.

(**A-E** and **G-I**) RNA expression was determined by qRT-PCR and normalized to *Actb*. Data are expressed as percentage normalized to the corresponding controls. Each data point represents one animal. Bars indicate group mean $\pm$ SD. Unpaired Student's t-test was used to determine the difference between experimental groups. *, $p< 0.05$; **, $p< 0.01$; ****, $p< 0.0001$.