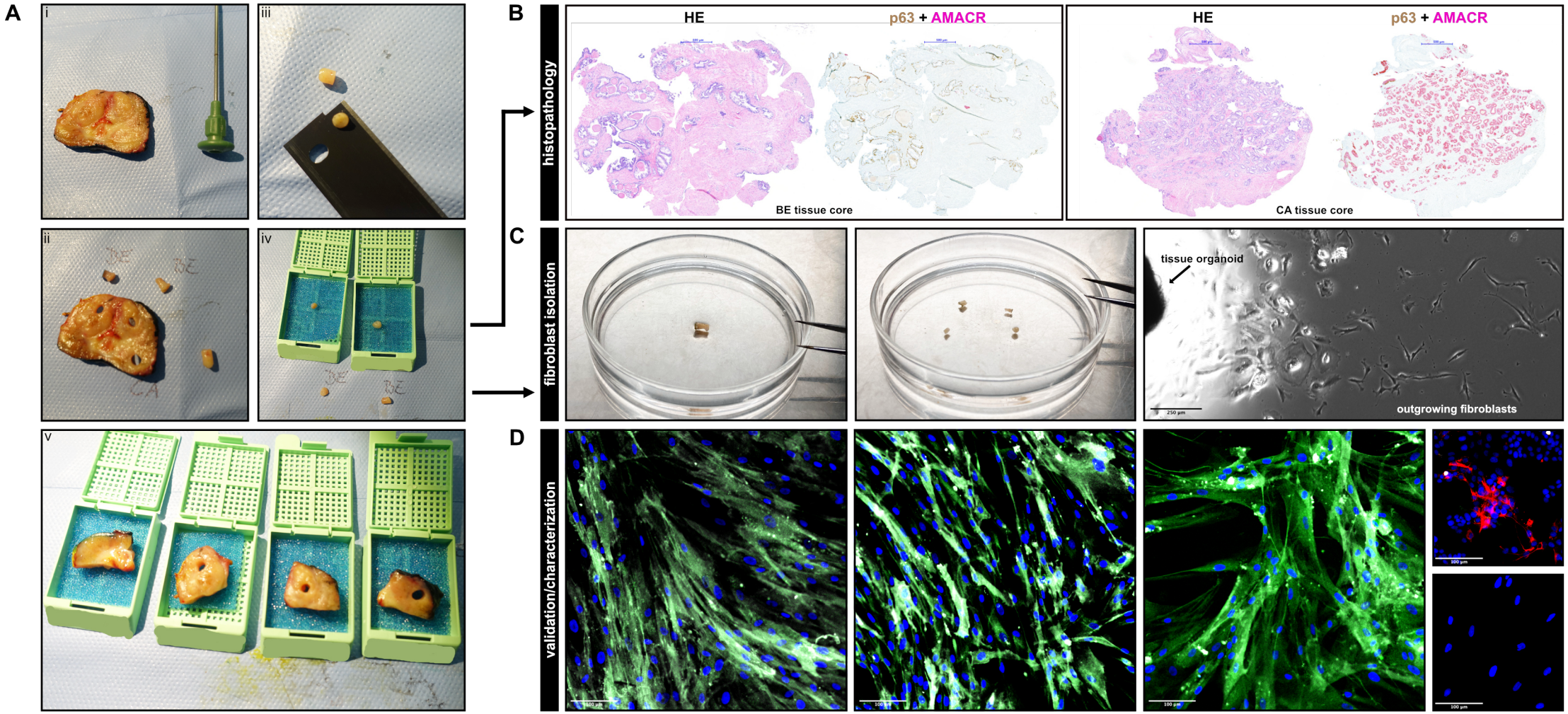
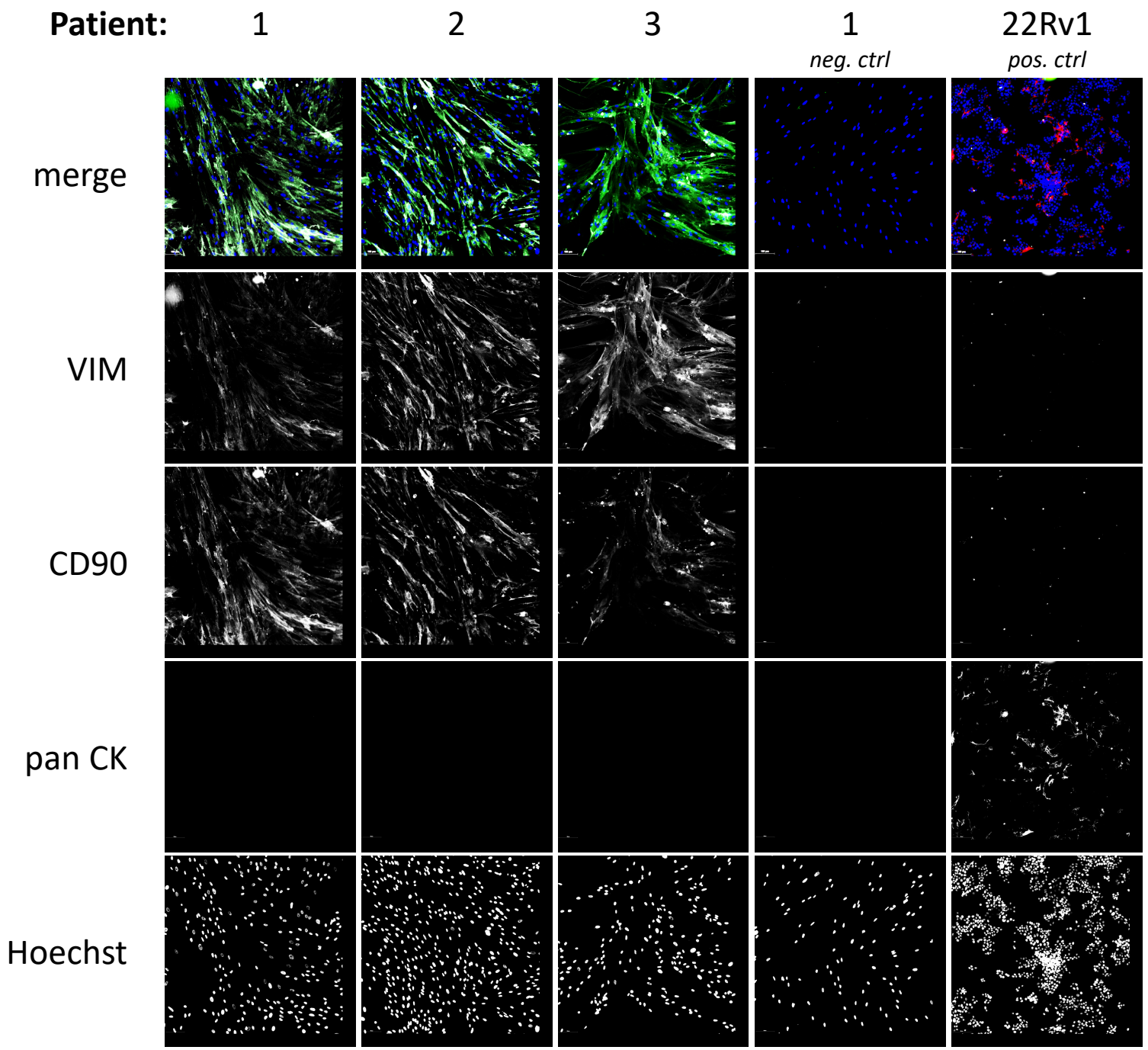
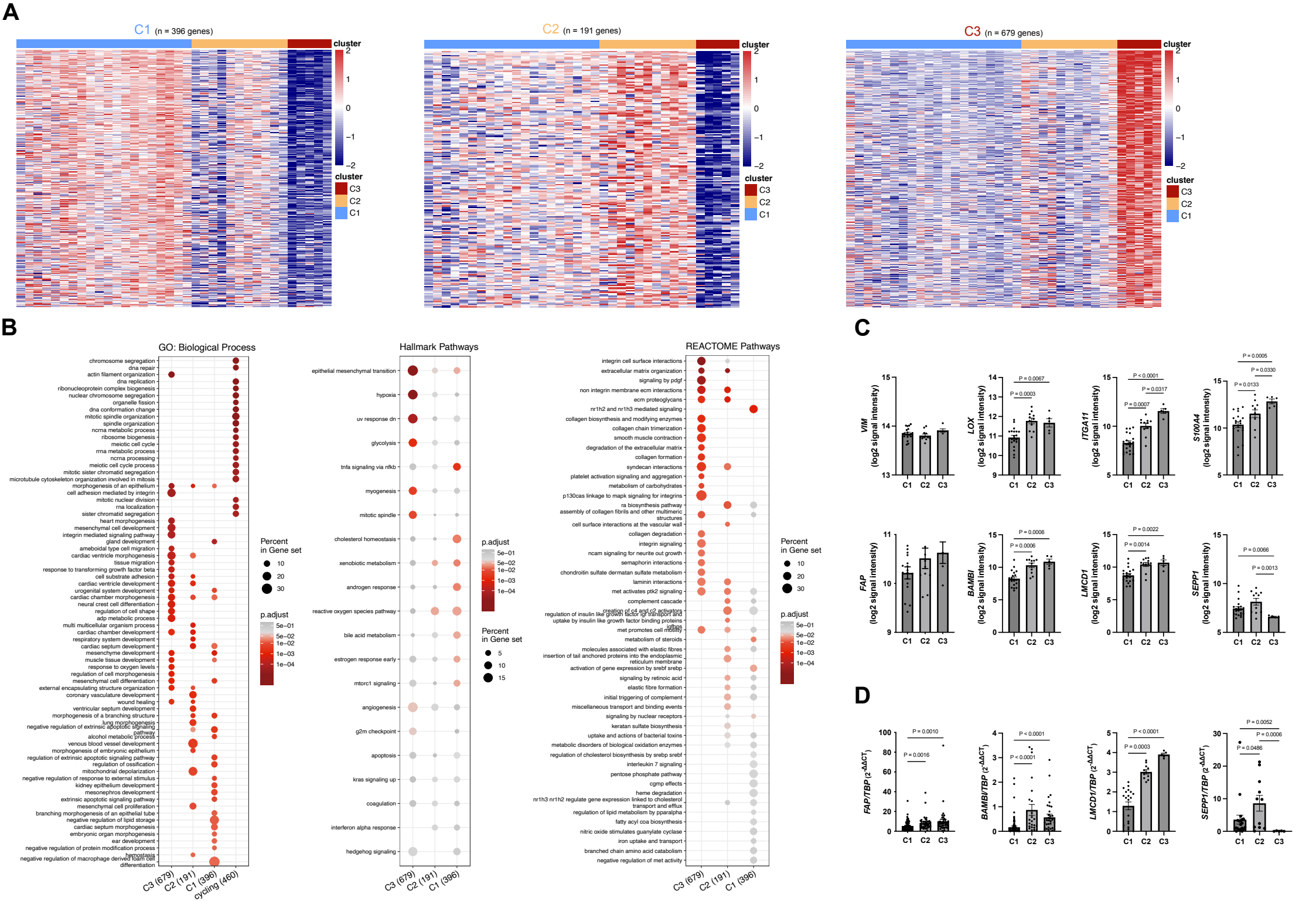

A
i
iii
ii
iv
v
B
histopathology
HE
p63 + AMACR
BE tissue core
HE
p63 + AMACR
CA tissue core
C
fibroblast isolation
tissue organoid
outgrowing fibroblasts
D
validation/characterization

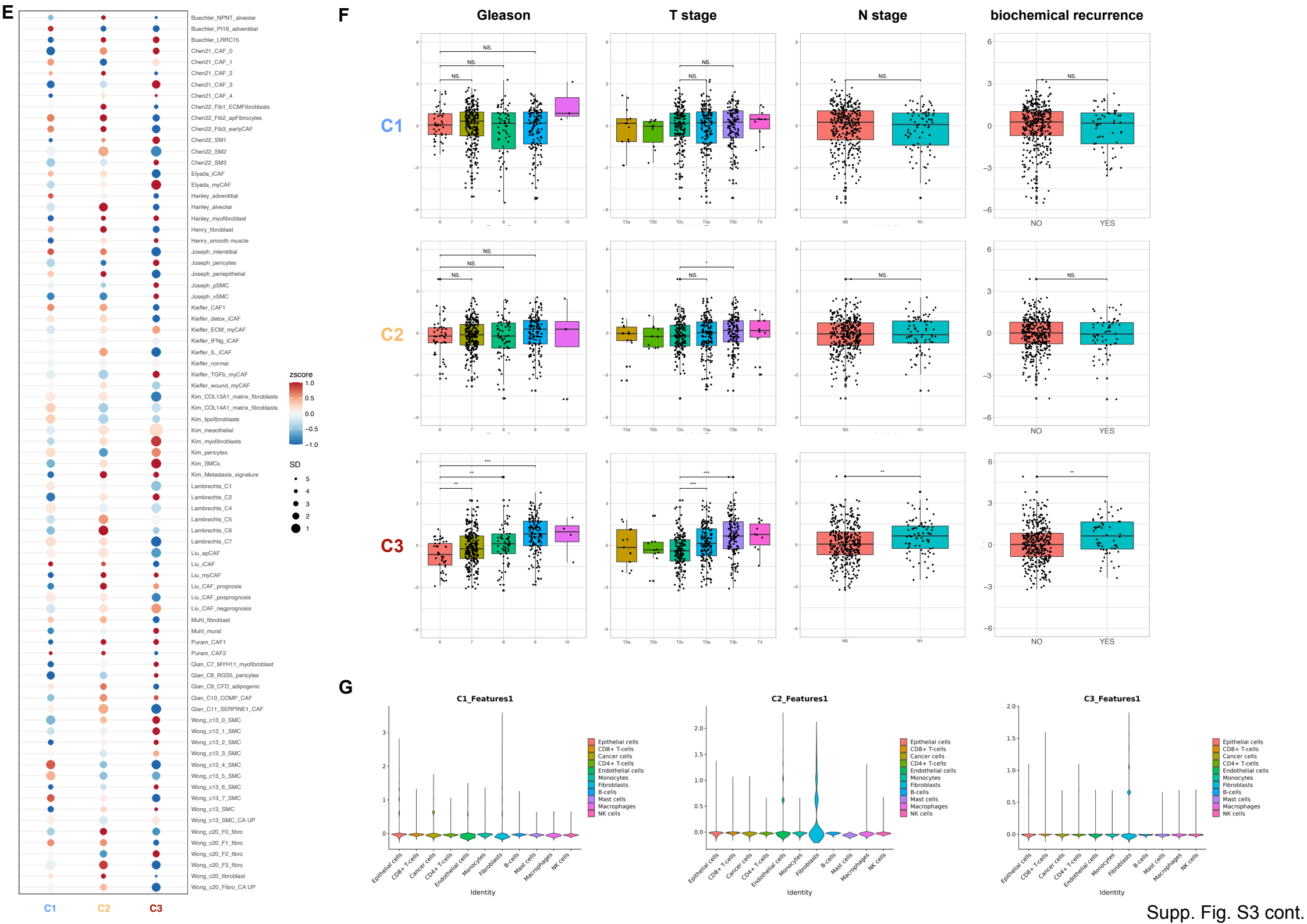
Supp. Fig. S1


Patient:
1
2
3
1
neg. ctrl
22Rv1
pos. ctrl
merge
VIM
CD90
pan CK
Hoechst

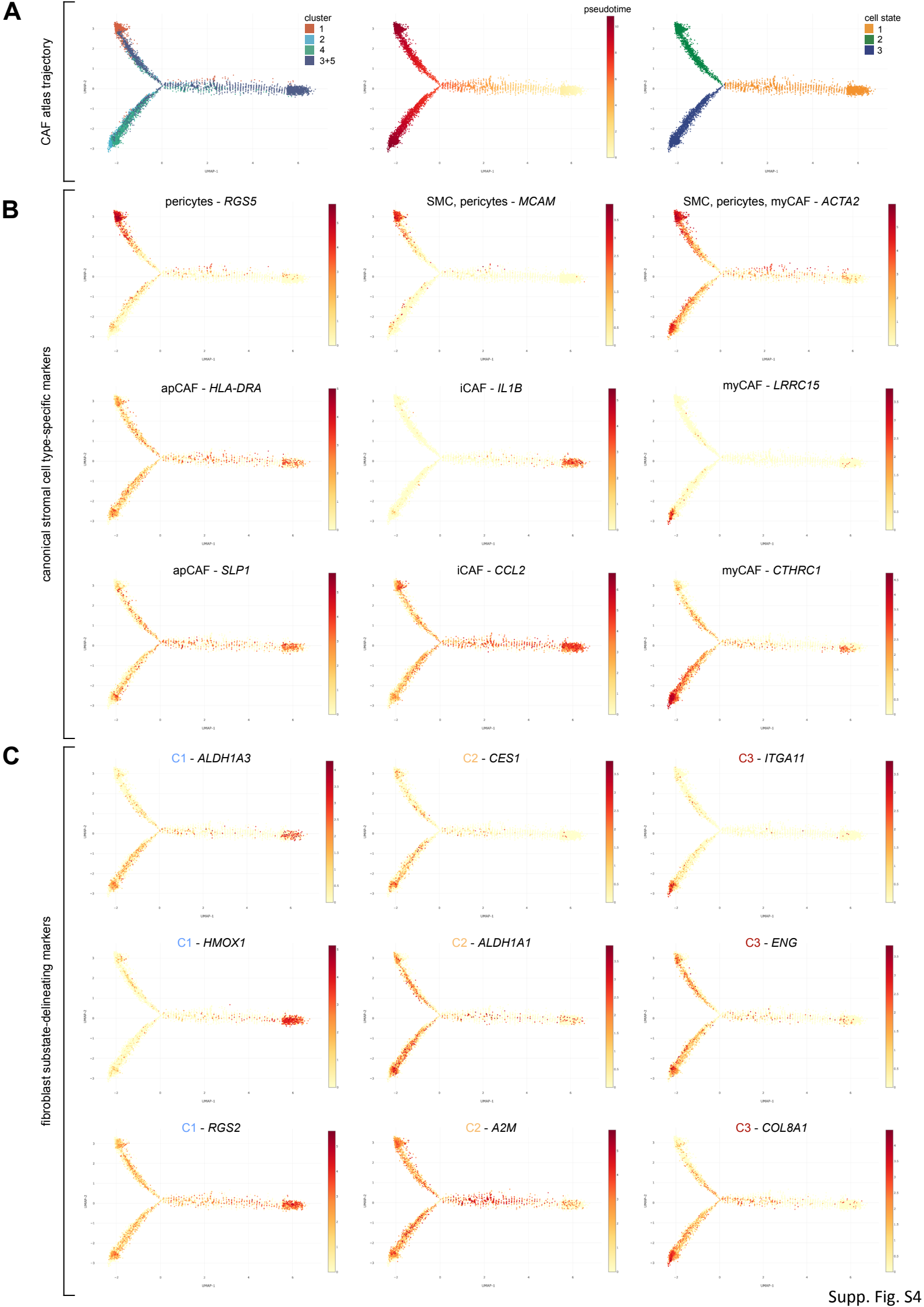
Supp. Fig. S2


A
C1 (n = 396 genes)
C2 (n = 191 genes)
C3 (n = 679 genes)
cluster
2
1
0
−1
−2
cluster
C3
C2
C1
B
GO: Biological Process
chromosome segregation
dna repair
actin filament organization
dna replication
ribonucleoprotein complex biogenesis
nuclear chromosome segregation
organelle fission
dna conformation change
mitotic spindle organization
spindle organization
ncrna metabolic process
ribosome biogenesis
meiotic cell cycle
rrna metabolic process
ncrna processing
meiotic cell cycle process
mitotic sister chromatid segregation
microtubule cytoskeleton organization involved in mitosis
morphogenesis of an epithelium
cell adhesion mediated by integrin
mitotic nuclear division
rna localization
sister chromatid segregation
heart morphogenesis
mesenchymal cell development
integrin mediated signaling pathway
gland development
ameboidal type cell migration
cardiac ventricle morphogenesis
tissue migration
response to transforming growth factor beta
cell substrate adhesion
cardiac ventricle development
urogenital system development
cardiac chamber morphogenesis
neural crest cell differentiation
regulation of cell shape
adp metabolic process
multi multicellular organism process
cardiac chamber development
respiratory system development
cardiac septum development
mesenchyme development
muscle tissue development
response to oxygen levels
regulation of cell morphogenesis
mesenchymal cell differentiation
external encapsulating structure organization
coronary vasculature development
wound healing
ventricular septum development
morphogenesis of a branching structure
lung morphogenesis
negative regulation of extrinsic apoptotic signaling pathway
alcohol metabolic process
venous blood vessel development
morphogenesis of embryonic epithelium
regulation of extrinsic apoptotic signaling pathway
regulation of ossification
mitochondrial depolarization
negative regulation of response to external stimulus
kidney epithelium development
mesonephros development
extrinsic apoptotic signaling pathway
mesenchymal cell proliferation
branching morphogenesis of an epithelial tube
negative regulation of lipid storage
cardiac septum morphogenesis
embryonic organ morphogenesis
ear development
negative regulation of protein modification process
hemostasis
negative regulation of macrophage derived foam cell differentiation
C3 (679)
C2 (191)
C1 (396)
cycling (460)
Percent in Gene set
10
20
30
p.adjust
5e−01
5e−02
1e−02
1e−03
1e−04
Hallmark Pathways
epithelial mesenchymal transition
hypoxia
uv response dn
glycolysis
tnfa signaling via nfkb
myogenesis
mitotic spindle
cholesterol homeostasis
xenobiotic metabolism
androgen response
reactive oxygen species pathway
bile acid metabolism
estrogen response early
mtorc1 signaling
angiogenesis
g2m checkpoint
apoptosis
kras signaling up
coagulation
interferon alpha response
hedgehog signaling
C3 (679)
C2 (191)
C1 (396)
p.adjust
5e−01
5e−02
1e−02
1e−03
1e−04
Percent in Gene set
5
10
15
REACTOME Pathways
integrin cell surface interactions
extracellular matrix organization
signaling by pdgf
non integrin membrane ecm interactions
ecm proteoglycans
nr1h2 and nr1h3 mediated signaling
collagen biosynthesis and modifying enzymes
collagen chain trimerization
smooth muscle contraction
degradation of the extracellular matrix
collagen formation
syndecan interactions
platelet activation signaling and aggregation
metabolism of carbohydrates
p130cas linkage to mapk signaling for integrins
ra biosynthesis pathway
assembly of collagen fibrils and other multimeric structures
cell surface interactions at the vascular wall
collagen degradation
integrin signaling
ncam signaling for neurite out growth
semaphorin interactions
chondroitin sulfate dermatan sulfate metabolism
laminin interactions
met activates ptk2 signaling
complement cascade
creation of c4 and c2 activators
regulation of insulin like growth factor igf transport and uptake by insulin like growth factor binding proteins
met promotes cell motility
metabolism of steroids
molecules associated with elastic fibres
insertion of tail anchored proteins into the endoplasmic reticulum membrane
activation of gene expression by srebf srebp
signaling by retinoic acid
elastic fibre formation
initial triggering of complement
miscellaneous transport and binding events
signaling by nuclear receptors
keratan sulfate biosynthesis
uptake and actions of bacterial toxins
metabolic disorders of biological oxidation enzymes
regulation of cholesterol biosynthesis by srebp srebf
interleukin 7 signaling
pentose phosphate pathway
cgmp effects
heme degradation
nr1h3 nr1h2 regulate gene expression linked to cholesterol transport and efflux
regulation of lipid metabolism by pparalpha
fatty acyl coa biosynthesis
nitric oxide stimulates guanylate cyclase
iron uptake and transport
branched chain amino acid catabolism
negative regulation of met activity
C3 (679)
C2 (191)
C1 (396)
Percent in Gene set
10
20
30
p.adjust
5e−01
5e−02
1e−02
1e−03
1e−04
C
VIM (log2 signal intensity)
LOX (log2 signal intensity)
ITGA11 (log2 signal intensity)
S100A4 (log2 signal intensity)
FAP (log2 signal intensity)
BAMBI (log2 signal intensity)
LMCD1 (log2 signal intensity)
SEPP1 (log2 signal intensity)
C1
C2
C3
D
FAP/TBP ($2^{-\Delta\Delta CT}$)
BAMBI/TBP ($2^{-\Delta\Delta CT}$)
LMCD1/TBP ($2^{-\Delta\Delta CT}$)
SEPP1/TBP ($2^{-\Delta\Delta CT}$)
C1
C2
C3

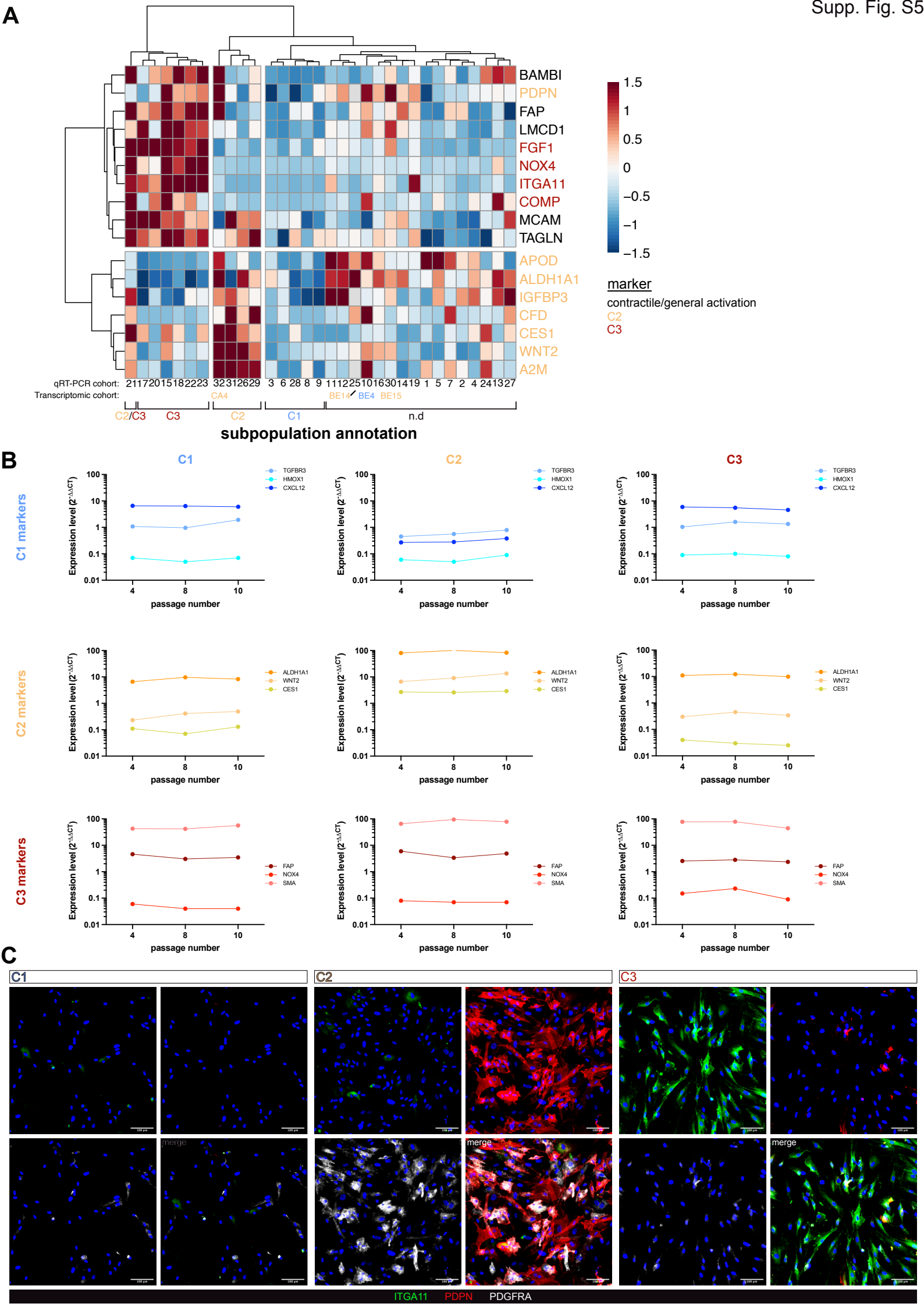
Supp. Fig. S3

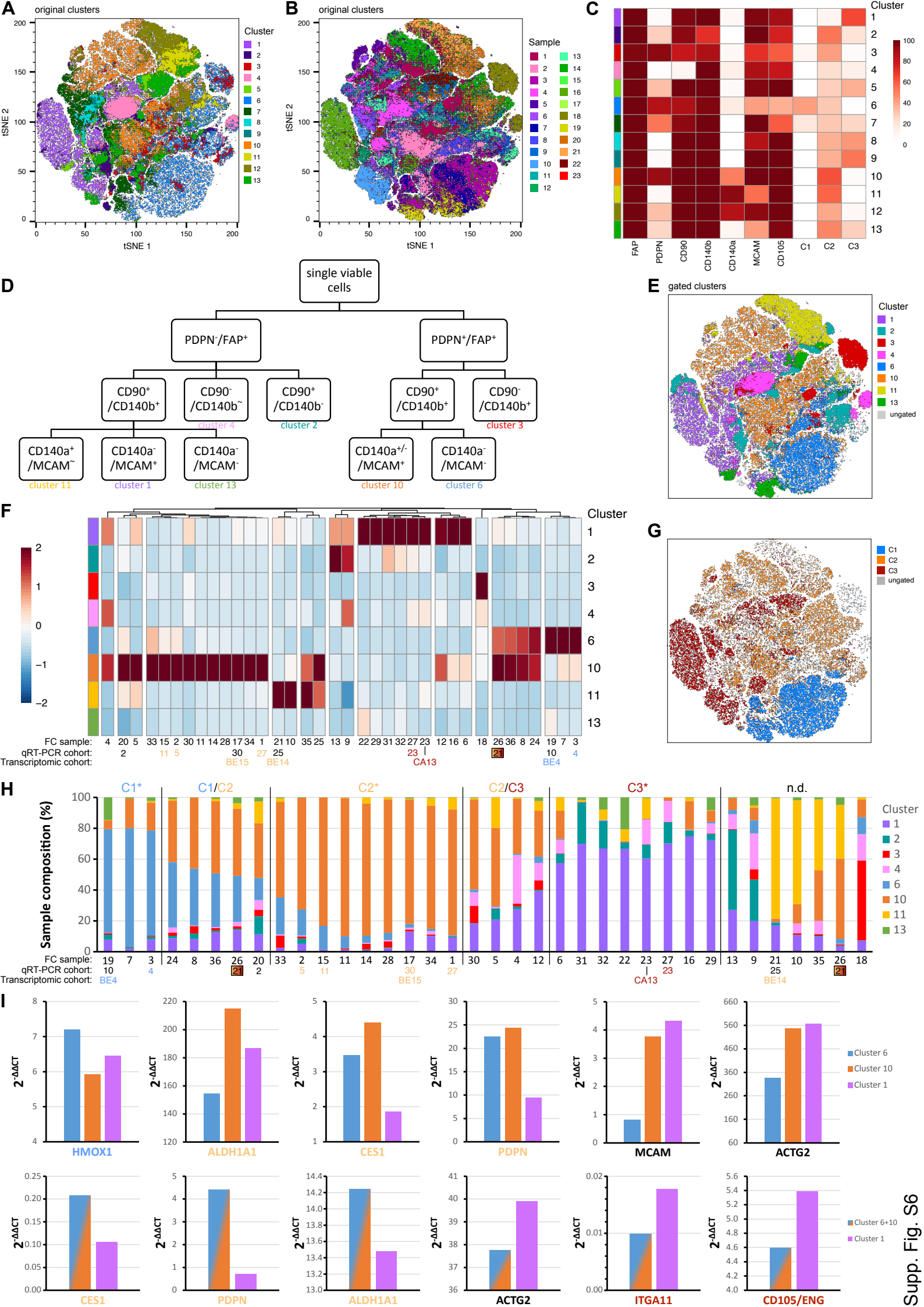


Supp. Fig. S3 cont.


A
CAF atlas trajectory
cluster
1
2
4
3+5
pseudotime
cell state
1
2
3
B
canonical stromal cell type-specific markers
pericytes - RGS5
SMC, pericytes - MCAM
SMC, pericytes, myCAF - ACTA2
apCAF - HLA-DRA
iCAF - IL1B
myCAF - LRRC15
apCAF - SLP1
iCAF - CCL2
myCAF - CTHRC1
C
fibroblast substate-delineating markers
C1 - ALDH1A3
C2 - CES1
C3 - ITGA11
C1 - HMOX1
C2 - ALDH1A1
C3 - ENG
C1 - RGS2
C2 - A2M
C3 - COL8A1

Supp. Fig. S4

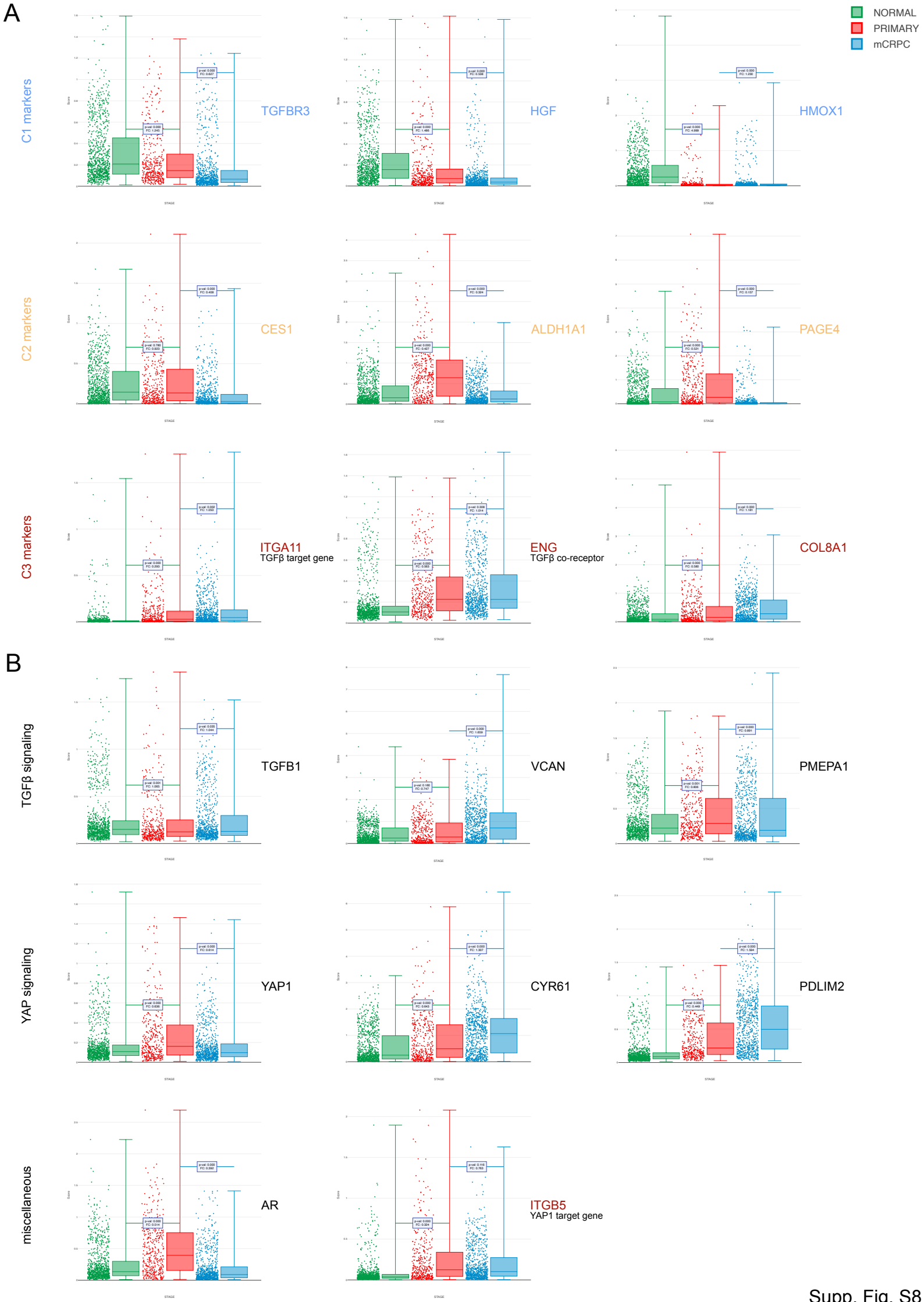

A
BAMBI
PDPN
FAP
LMCD1
FGF1
NOX4
ITGA11
COMP
MCAM
TAGLN
APOD
ALDH1A1
IGFBP3
CFD
CES1
WNT2
A2M
1.5
1
0.5
0
−0.5
−1
−1.5
marker
contractile/general activation
C2
C3
qRT-PCR cohort: 21 17 20 15 18 22 23 32 31 26 29 3 6 28 8 9 11 12 25 10 16 30 14 19 1 5 7 2 4 24 13 27
Transcriptomic cohort: CA4 BE14 BE4 BE15
C2/C3 C3 C2 C1 n.d
subpopulation annotation
B
C1
C2
C3
C1 markers
C2 markers
C3 markers
Expression level (2^-ΔΔCT)
passage number
TGFBR3
HMOX1
CXCL12
ALDH1A1
WNT2
CES1
FAP
NOX4
SMA
C
C1
C2
C3
merge
ITGA11 PDPN PDGFRA


A
original clusters
tSNE 2
tSNE 1
Cluster
B
original clusters
Sample
C
Cluster
FAP
PDPN
CD90
CD140b
CD140a
MCAM
CD105
C1
C2
C3
D
single viable cells
PDPN-/FAP+
PDPN+/FAP+
CD90+ /CD140b+
CD90- /CD140b~
CD90+ /CD140b-
CD90+ /CD140b+
CD90- /CD140b+
CD140a+ /MCAM~
CD140a- /MCAM+
CD140a- /MCAM-
CD140a+/- /MCAM+
CD140a- /MCAM-
cluster 11
cluster 1
cluster 13
cluster 4
cluster 2
cluster 10
cluster 6
cluster 3
E
gated clusters
Cluster
ungated
F
Cluster
FC sample:
qRT-PCR cohort:
Transcriptomic cohort:
BE15
BE14
CA13
BE4
G
C1
C2
C3
ungated
H
C1*
C1/C2
C2*
C2/C3
C3*
n.d.
Sample composition (%)
Cluster
FC sample:
qRT-PCR cohort:
Transcriptomic cohort:
BE4
BE15
CA13
BE14
I
2-ΔΔCT
HMOX1
ALDH1A1
CES1
PDPN
MCAM
ACTG2
Cluster 6
Cluster 10
Cluster 1
CES1
PDPN
ALDH1A1
ACTG2
ITGA11
CD105/ENG
Cluster 6+10
Cluster 1

Supp. Fig. S6


A
all disease stages
primary PCa
NEPC
mCRPC
normal fibroblasts
iCAF
mCAF
SMC
myoCAF
pericytes
B
C1-associated markers
C2-associated markers
C3-associated markers
YAP1/TGFβ-associated markers
AR
ALDH1A3
HMOX1
TGFBR3
CES1
PAGE4
ALDH1A1
PDPN
ITGA11
ENG
FAP
NOX4
YAP1
ITGB5 - YAP target gene
LOX - TGFβ target gene
SERPINE1 - TGFβ target gene
UMAP_1
UMAP_2

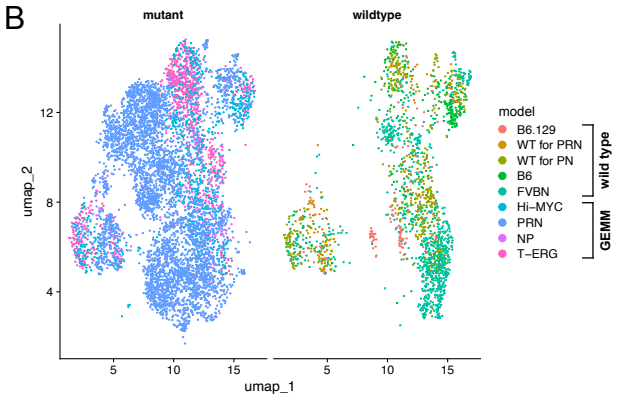
Supp. Fig. 7

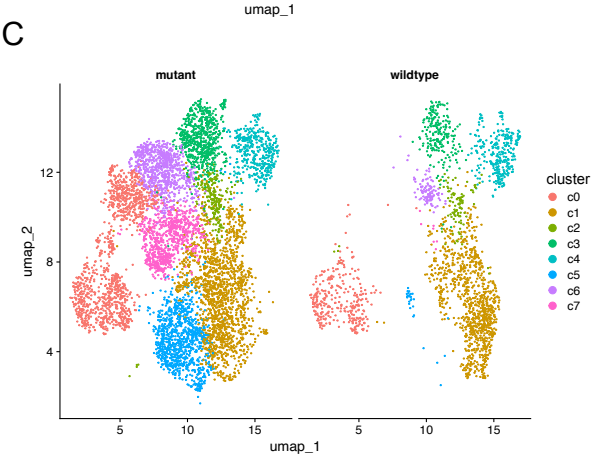

A
NORMAL
PRIMARY
mCRPC
C1 markers
TGFBR3
HGF
HMOX1
C2 markers
CES1
ALDH1A1
PAGE4
C3 markers
ITGA11
TGFβ target gene
ENG
TGFβ co-receptor
COL8A1
B
TGFβ signaling
TGFB1
VCAN
PMEPA1
YAP signaling
YAP1
CYR61
PDLIM2
miscellaneous
AR
ITGB5
YAP1 target gene

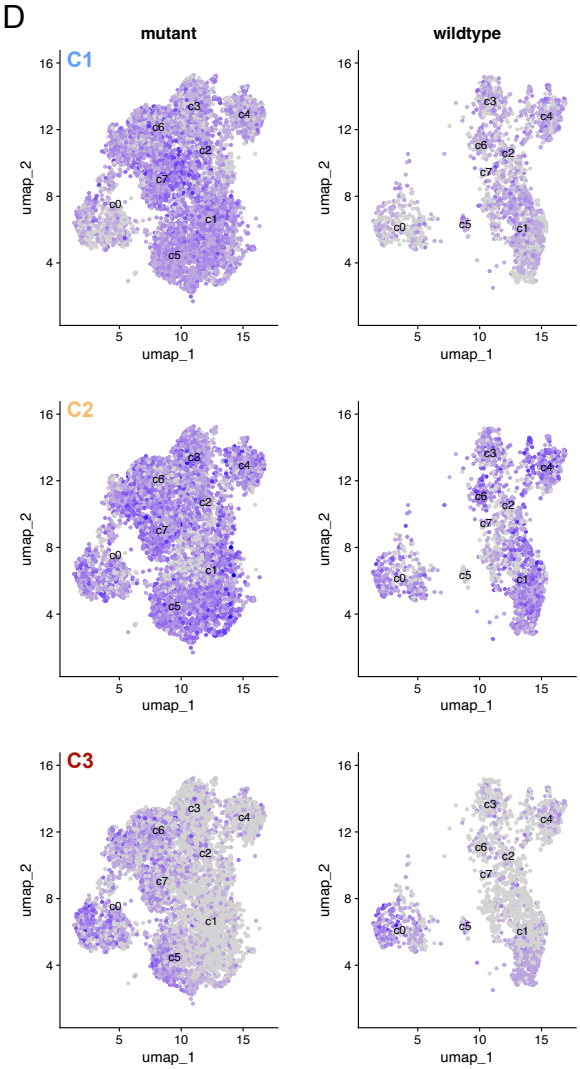
Supp. Fig. S8

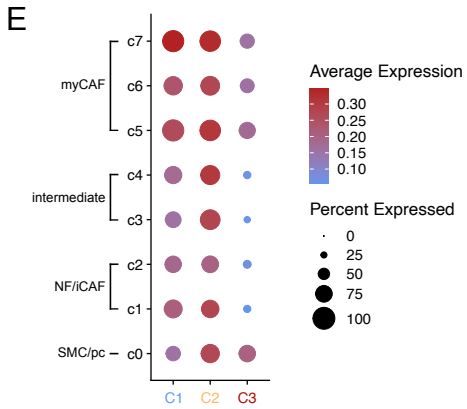
A

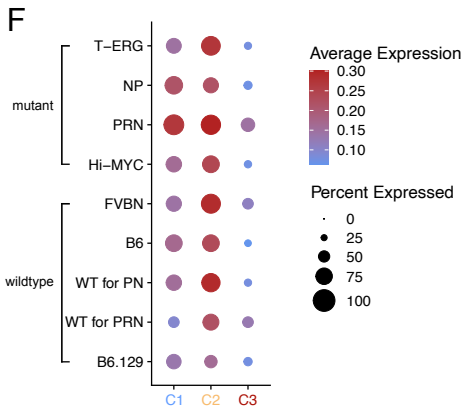
model	mutation	phenotype
T-ERG	*Tmprss2-ERG* knock-in	mild epithelial phenotype, model of PCa initiation
Hi-MYC	*Tg(ARR2/Pbsn-MYC)7Key*	PIN with subsequent invasion
NP	*Nkx3.1creERT2;Pten$^{f/f}$*	
PRN	*Pb-Cre4$^{+/-}$;Pten$^{f/f}$;LSL-MYCN$^{+/+}$*	invasive adenocarcinoma and neuroendocrine PCa

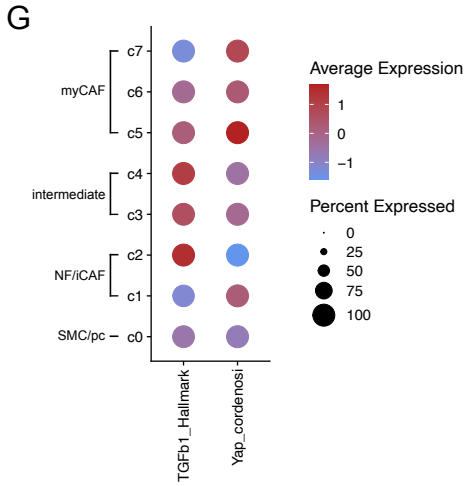


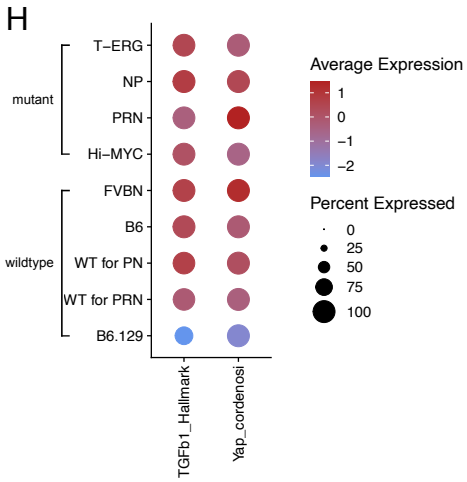


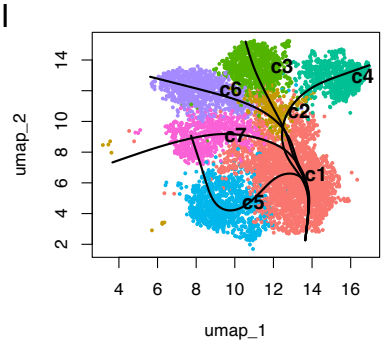


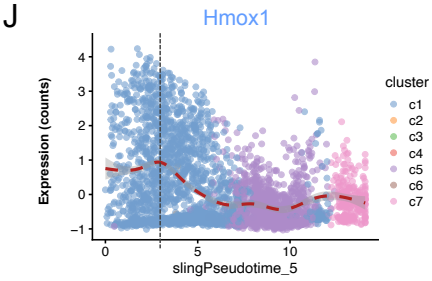


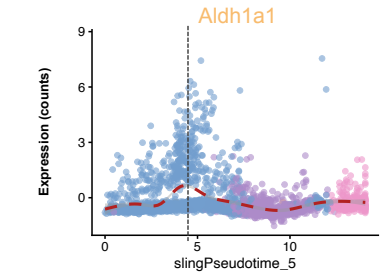


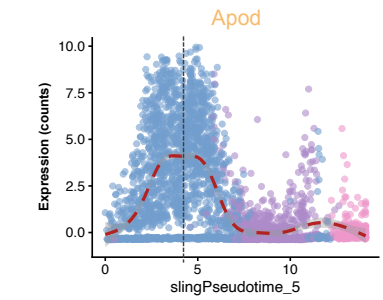


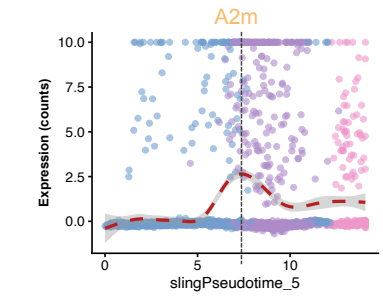


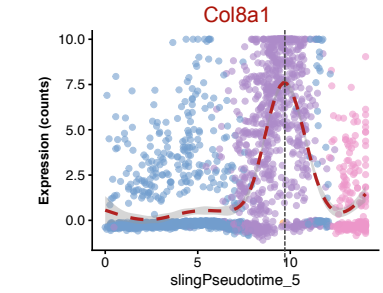




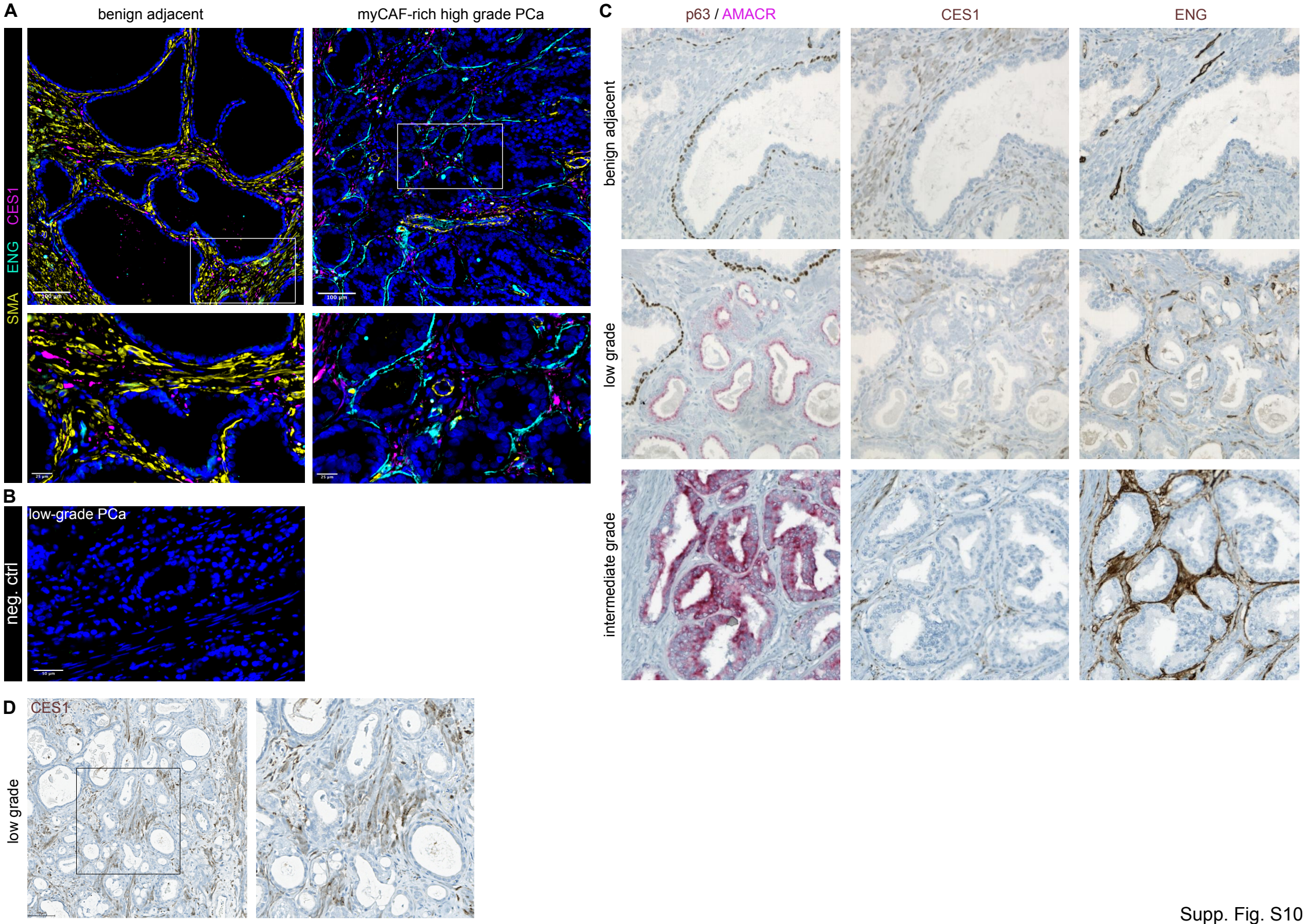




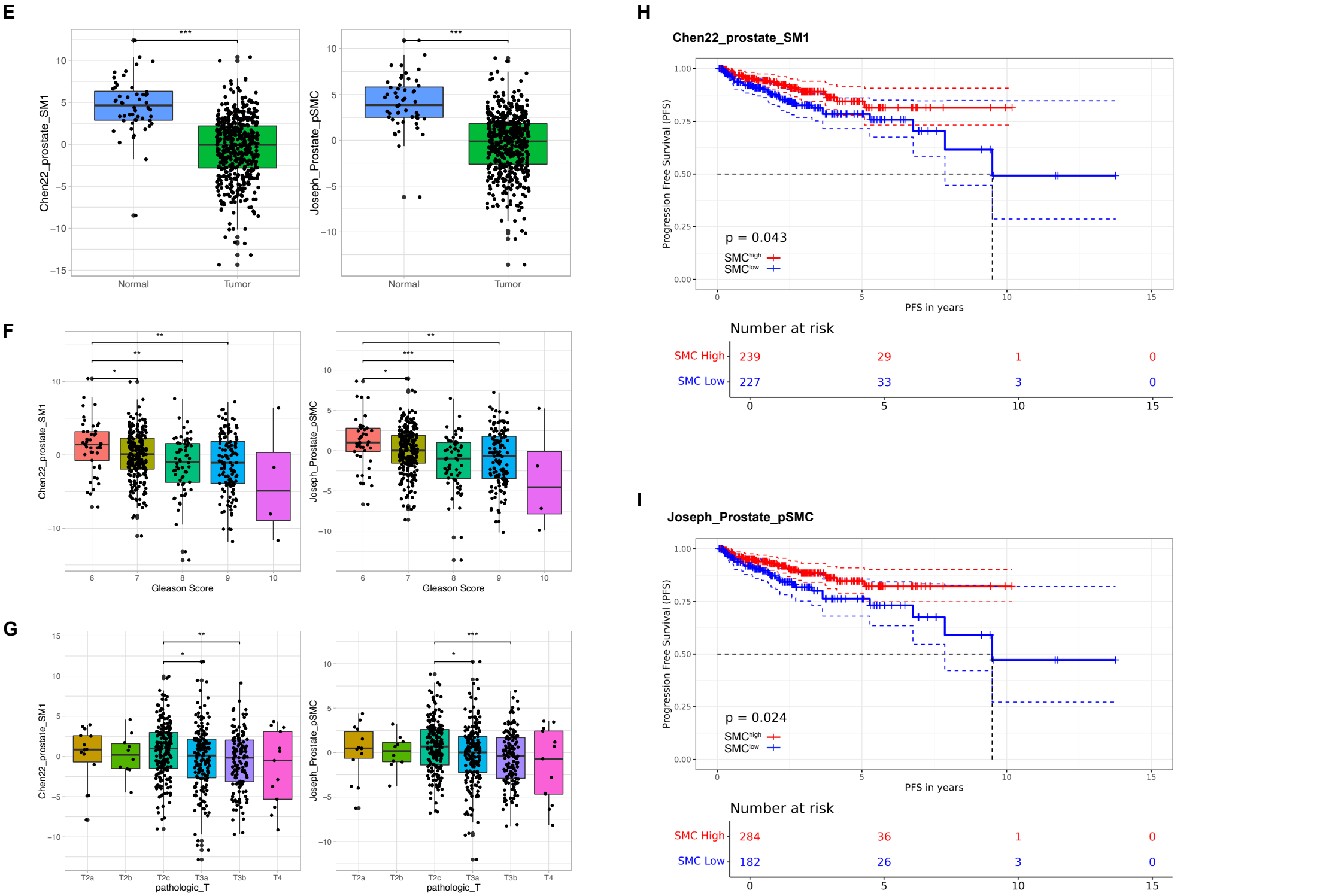




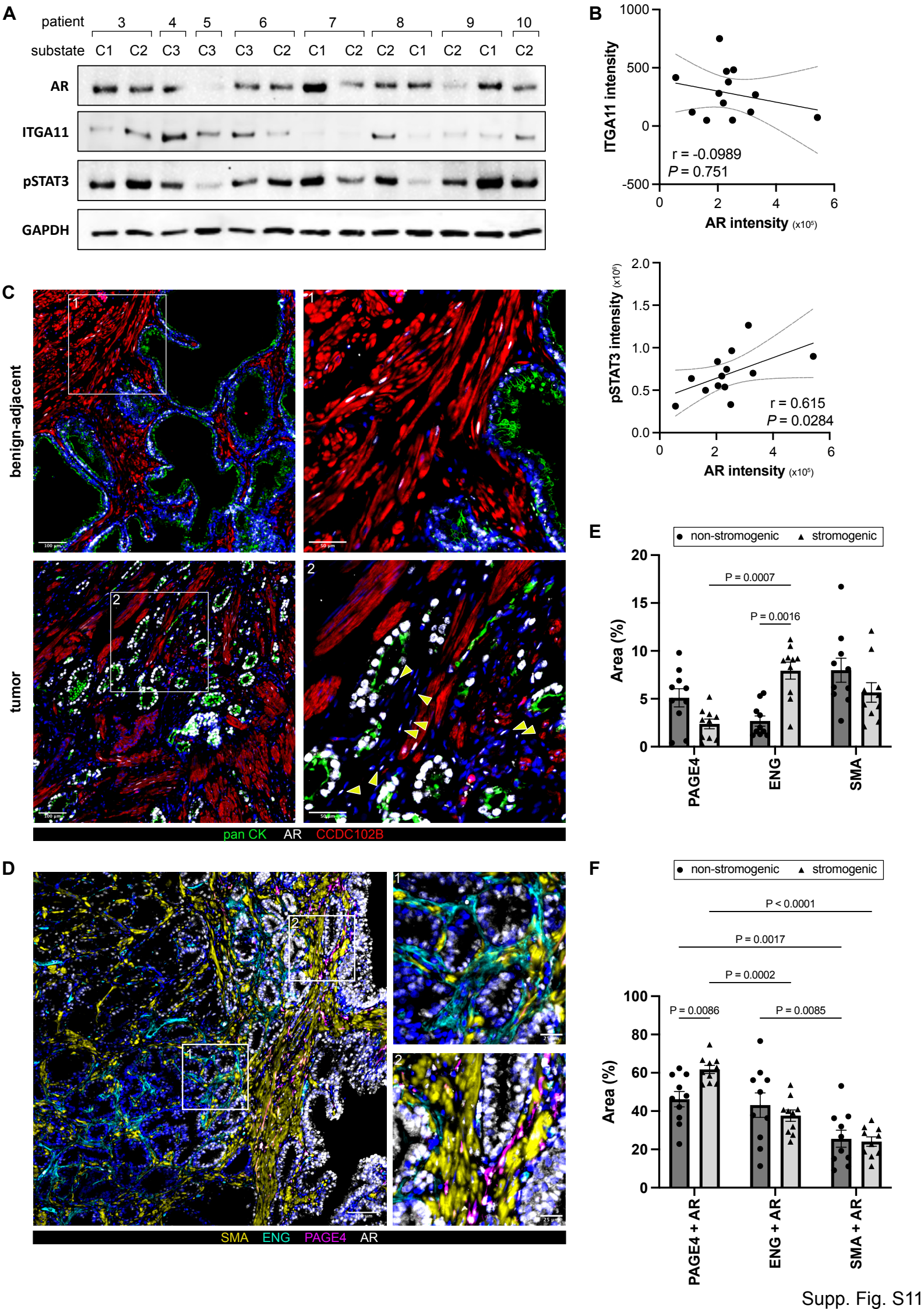
Supp. Fig. S9


A
benign adjacent
myCAF-rich high grade PCa
SMA ENG CES1
100 µm
25 µm
B
neg. ctrl
low-grade PCa
50 µm
C
p63 / AMACR
CES1
ENG
benign adjacent
low grade
intermediate grade
D
low grade
CES1

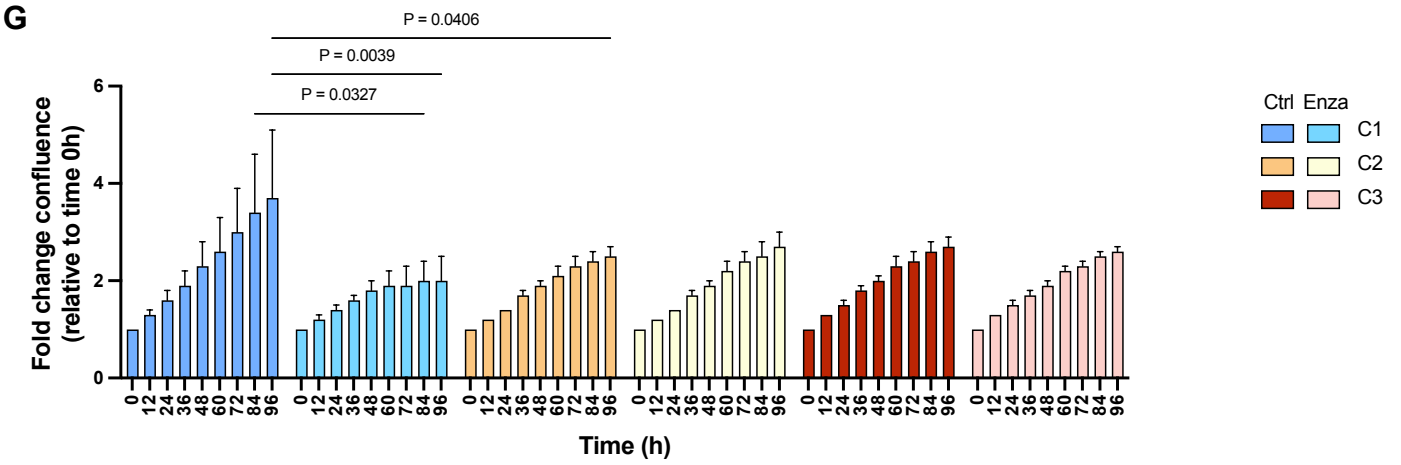
Supp. Fig. S10

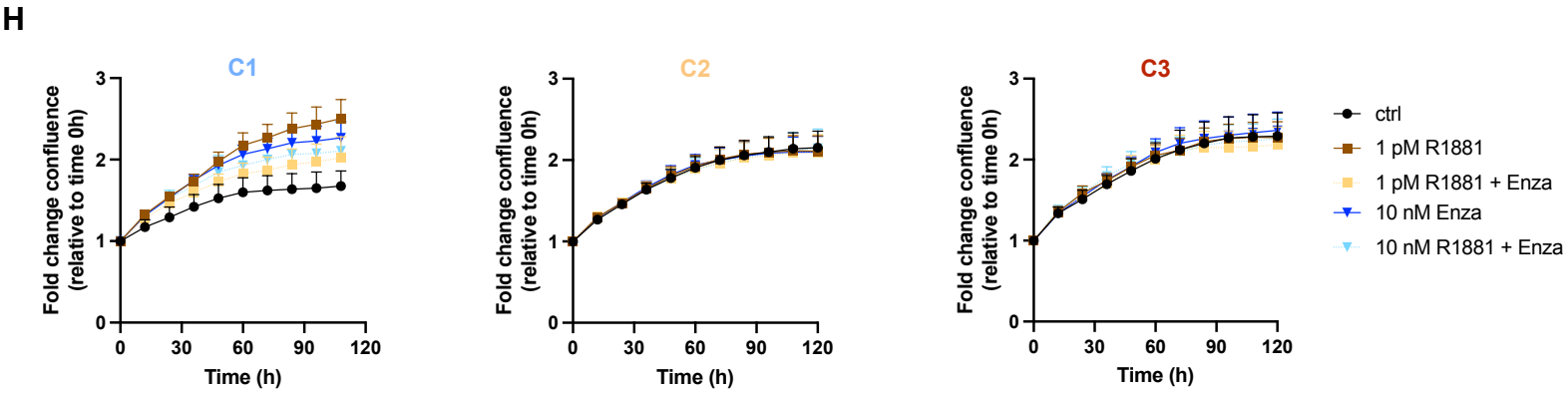


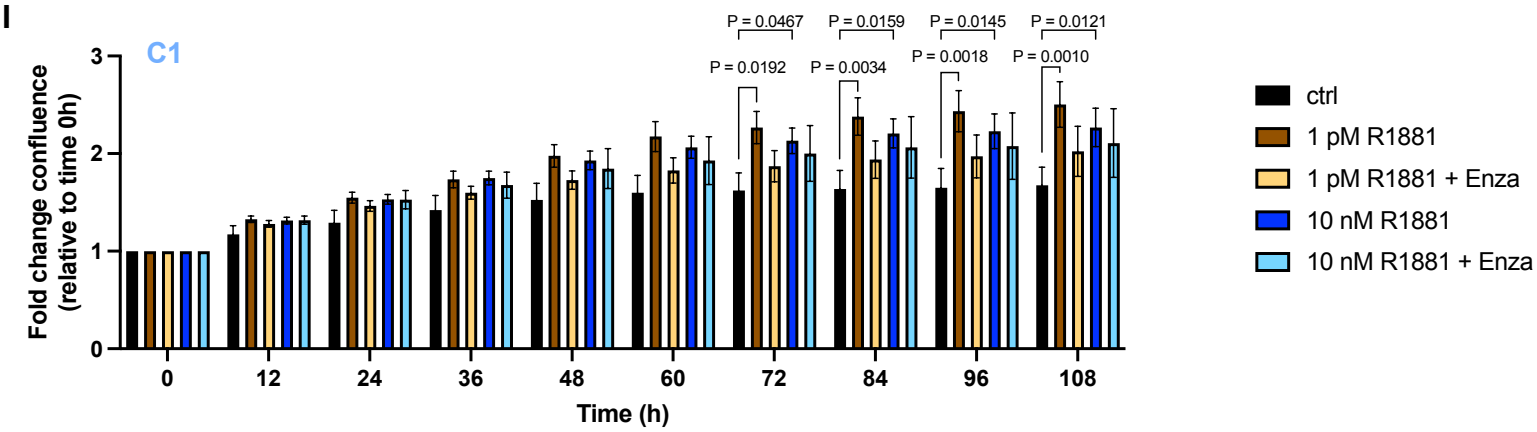
Supp. Fig. S10 cont.

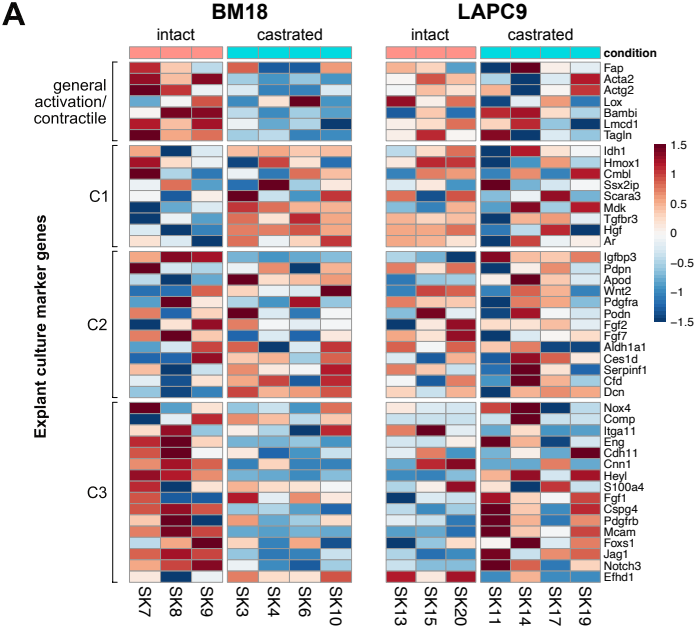

A
patient 3 4 5 6 7 8 9 10
substate C1 C2 C3 C3 C3 C2 C1 C2 C2 C1 C2 C1 C2
AR
ITGA11
pSTAT3
GAPDH
B
ITGA11 intensity
r = -0.0989
P = 0.751
AR intensity (x10⁵)
pSTAT3 intensity (x10⁶)
r = 0.615
P = 0.0284
AR intensity (x10⁵)
C
benign-adjacent
tumor
pan CK AR CCDC102B
D
SMA ENG PAGE4 AR
E
non-stromogenic stromogenic
P = 0.0007
P = 0.0016
Area (%)
PAGE4
ENG
SMA
F
non-stromogenic stromogenic
P < 0.0001
P = 0.0017
P = 0.0002
P = 0.0086
P = 0.0085
Area (%)
PAGE4 + AR
ENG + AR
SMA + AR

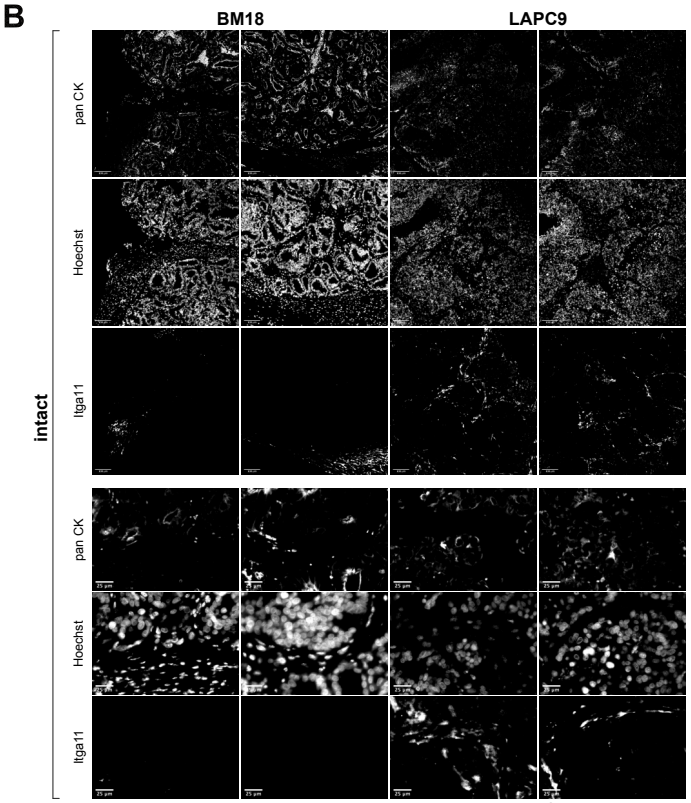
Supp. Fig. S11

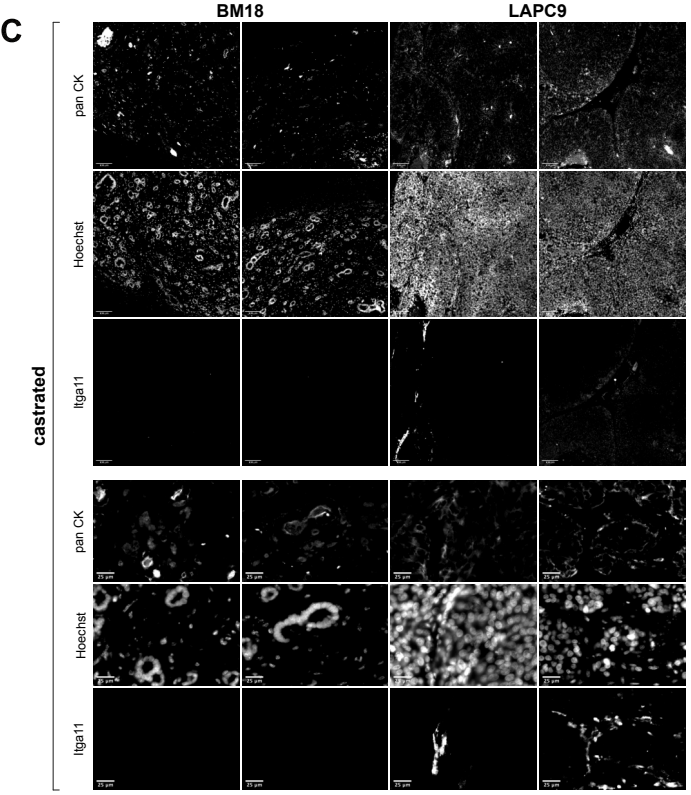

G
P = 0.0406
P = 0.0039
P = 0.0327
Fold change confluence (relative to time 0h)
Time (h)
Ctrl Enza
C1
C2
C3

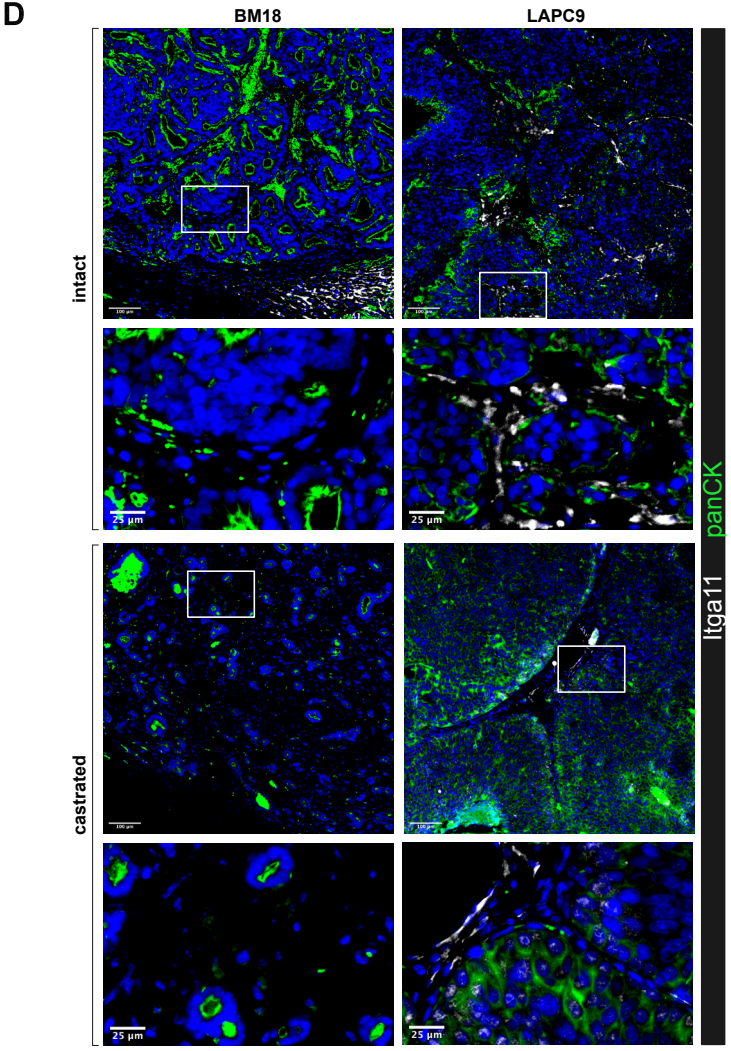

H
C1
C2
C3
Fold change confluence (relative to time 0h)
Time (h)
ctrl
1 pM R1881
1 pM R1881 + Enza
10 nM Enza
10 nM R1881 + Enza

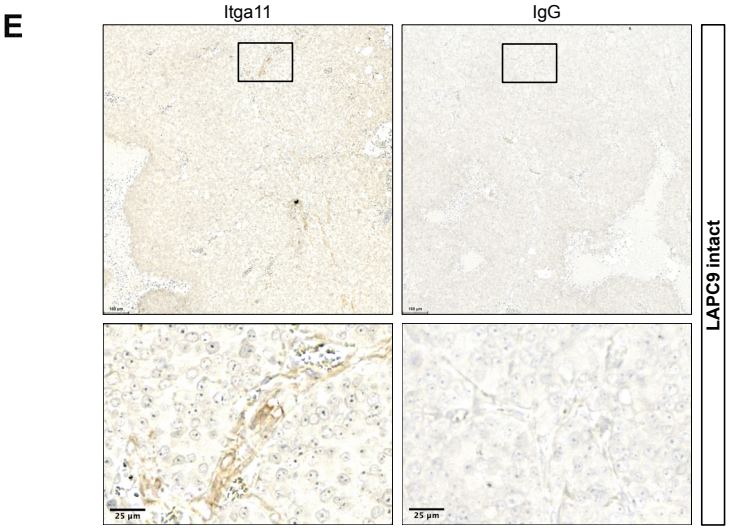

I
C1
P = 0.0467
P = 0.0159
P = 0.0145
P = 0.0121
P = 0.0192
P = 0.0034
P = 0.0018
P = 0.0010
Fold change confluence (relative to time 0h)
Time (h)
ctrl
1 pM R1881
1 pM R1881 + Enza
10 nM R1881
10 nM R1881 + Enza

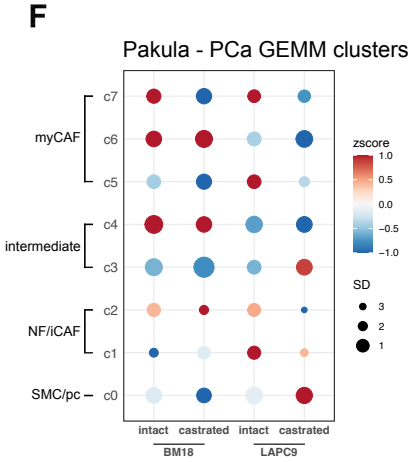

A
BM18
LAPC9
intact
castrated
condition
general activation/ contractile
Explant culture marker genes
C1
C2
C3
Fap
Acta2
Actg2
Lox
Bambi
Lmcd1
Tagln
Idh1
Hmox1
Cmbl
Ssx2ip
Scara3
Mdk
Tgfbr3
Hgf
Ar
Igfbp3
Pdpn
Apod
Wnt2
Pdgfra
Podn
Fgf2
Fgf7
Aldh1a1
Ces1d
Serpinf1
Cfd
Dcn
Nox4
Comp
Itga11
Eng
Cdh11
Cnn1
Heyl
S100a4
Fgf1
Cspg4
Pdgfrb
Mcam
Foxs1
Jag1
Notch3
Efhd1
1.5
1
0.5
0
−0.5
−1
−1.5
SK7
SK8
SK9
SK3
SK4
SK6
SK10
SK13
SK15
SK20
SK11
SK14
SK17
SK19

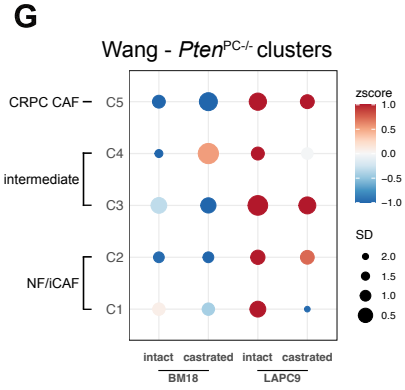

B
BM18
LAPC9
intact
pan CK
Hoechst
Itga11


C
BM18
LAPC9
castrated
pan CK
Hoechst
Itga11

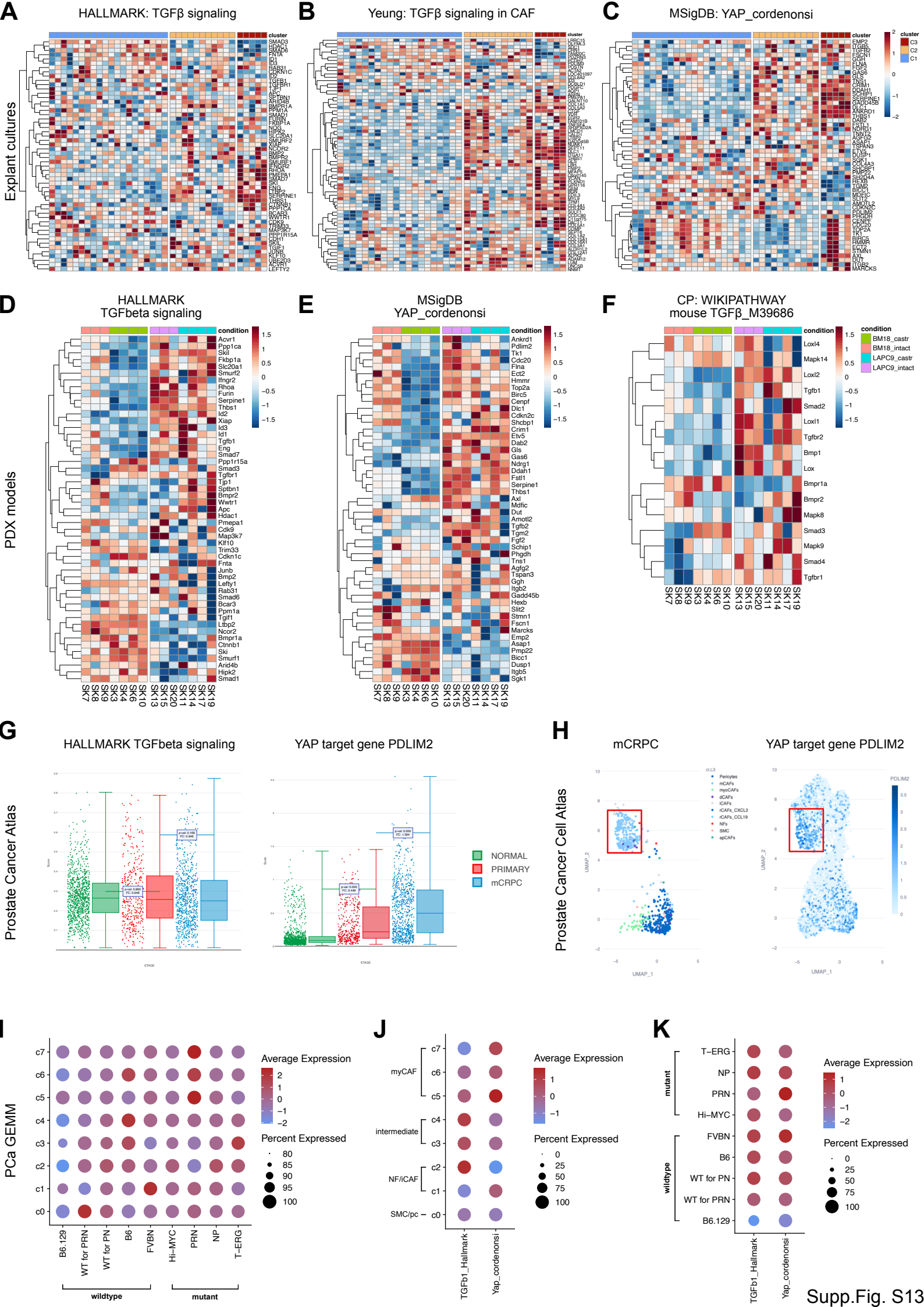

D
BM18
LAPC9
intact
castrated
panCK
Itga11
25 µm


E
Itga11
IgG
LAPC9 intact
25 µm

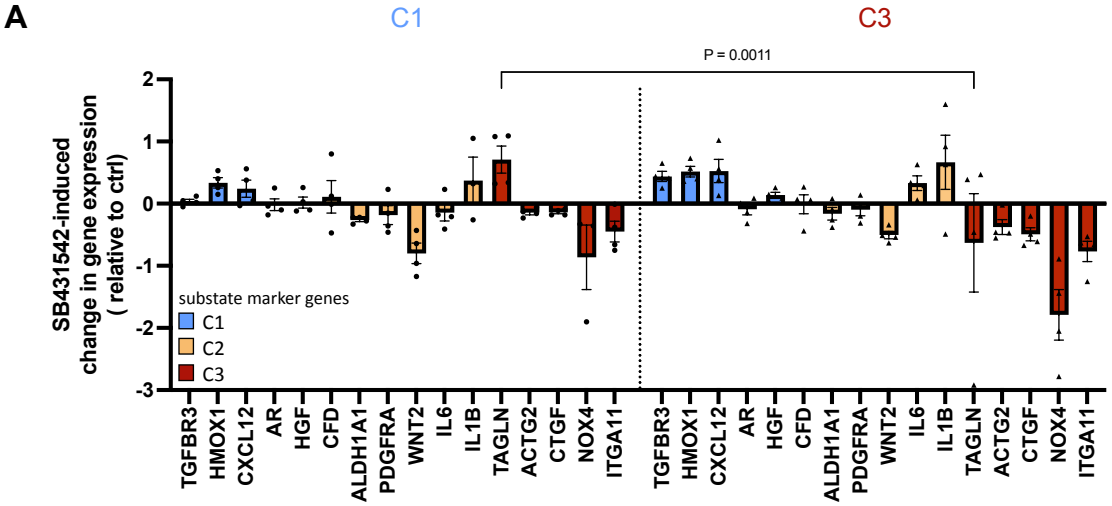

F
Pakula - PCa GEMM clusters
myCAF
intermediate
NF/iCAF
SMC/pc
c7
c6
c5
c4
c3
c2
c1
c0
zscore
1.0
0.5
0.0
−0.5
−1.0
SD
3
2
1
intact
castrated
intact
castrated
BM18
LAPC9

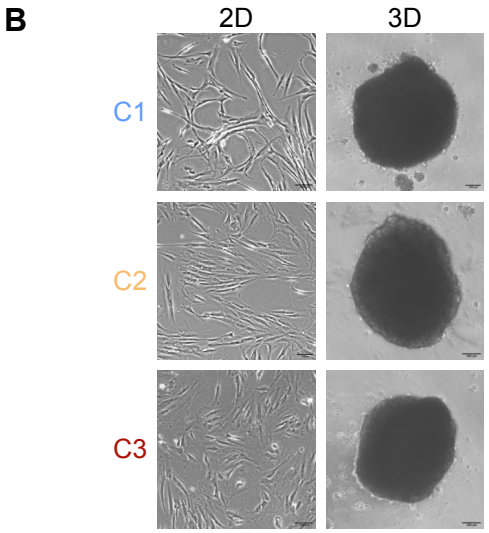

G
Wang - Pten PC-/- clusters
CRPC CAF
intermediate
NF/iCAF
C5
C4
C3
C2
C1
zscore
1.0
0.5
0.0
−0.5
−1.0
SD
2.0
1.5
1.0
0.5
intact
castrated
intact
castrated
BM18
LAPC9

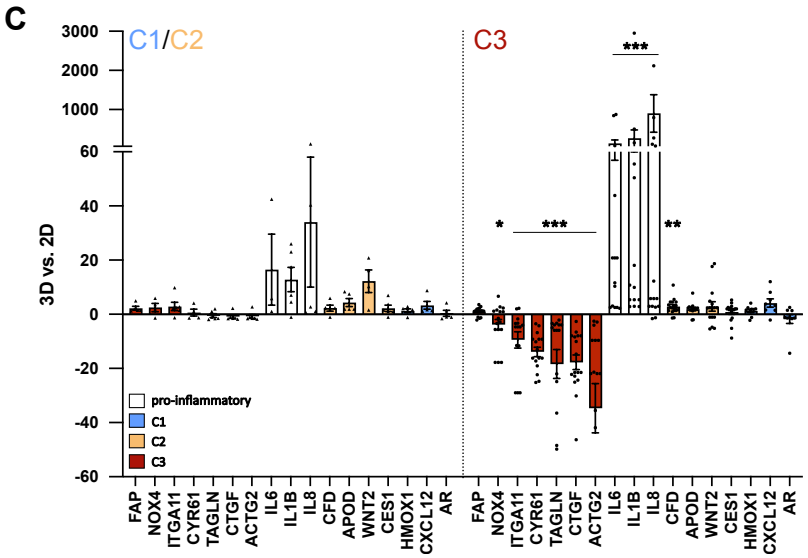
Supp. Fig. S12

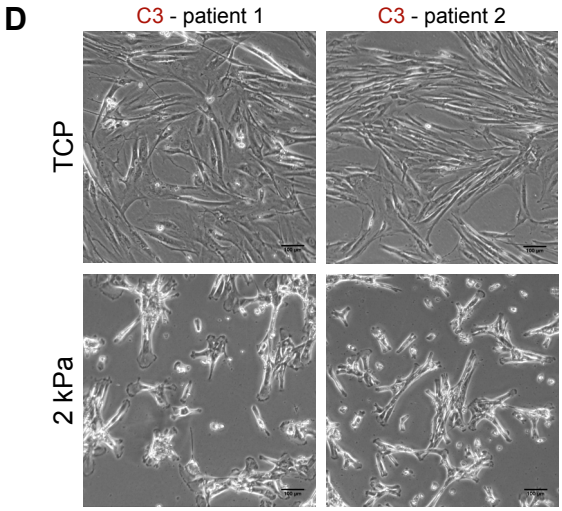

A
HALLMARK: TGFβ signaling
Explant cultures
B
Yeung: TGFβ signaling in CAF
C
MSigDB: YAP_cordenonsi
cluster
C3
C2
C1
D
HALLMARK
TGFbeta signaling
PDX models
condition
E
MSigDB
YAP_cordenonsi
F
CP: WIKIPATHWAY
mouse TGFβ_M39686
BM18_castr
BM18_intact
LAPC9_castr
LAPC9_intact
G
HALLMARK TGFbeta signaling
YAP target gene PDLIM2
Prostate Cancer Atlas
NORMAL
PRIMARY
mCRPC
H
mCRPC
YAP target gene PDLIM2
Prostate Cancer Cell Atlas
I
PCa GEMM
Average Expression
Percent Expressed
B6.129
WT for PRN
WT for PN
B6
FVBN
Hi–MYC
PRN
NP
T–ERG
wildtype
mutant
J
myCAF
intermediate
NF/iCAF
SMC/pc
TGFb1_Hallmark
Yap_cordenonsi
K
mutant
wildtype

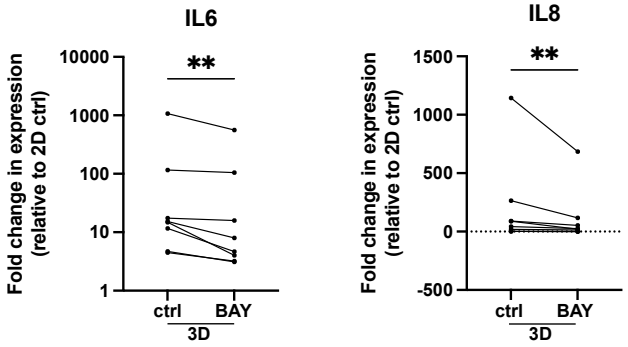
Supp.Fig. S13

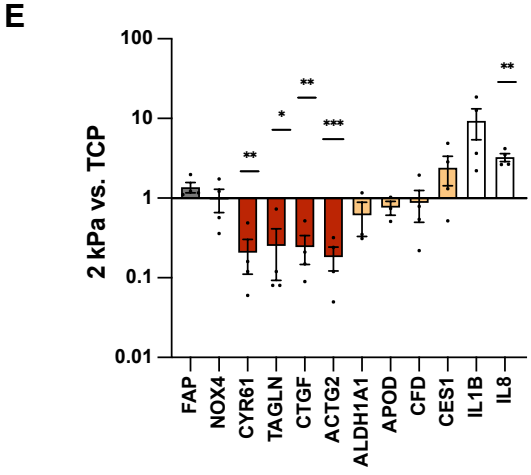

A
C1
C3
P = 0.0011
SB431542-induced change in gene expression (relative to ctrl)
substate marker genes
C1
C2
C3
TGFBR3 HMOX1 CXCL12 AR HGF CFD ALDH1A1 PDGFRA WNT2 IL6 IL1B TAGLN ACTG2 CTGF NOX4 ITGA11


B
2D
3D
C1
C2
C3


C
C1/C2
C3
3D vs. 2D
pro-inflammatory
C1
C2
C3
FAP NOX4 ITGA11 CYR61 TAGLN CTGF ACTG2 IL6 IL1B IL8 CFD APOD WNT2 CES1 HMOX1 CXCL12 AR

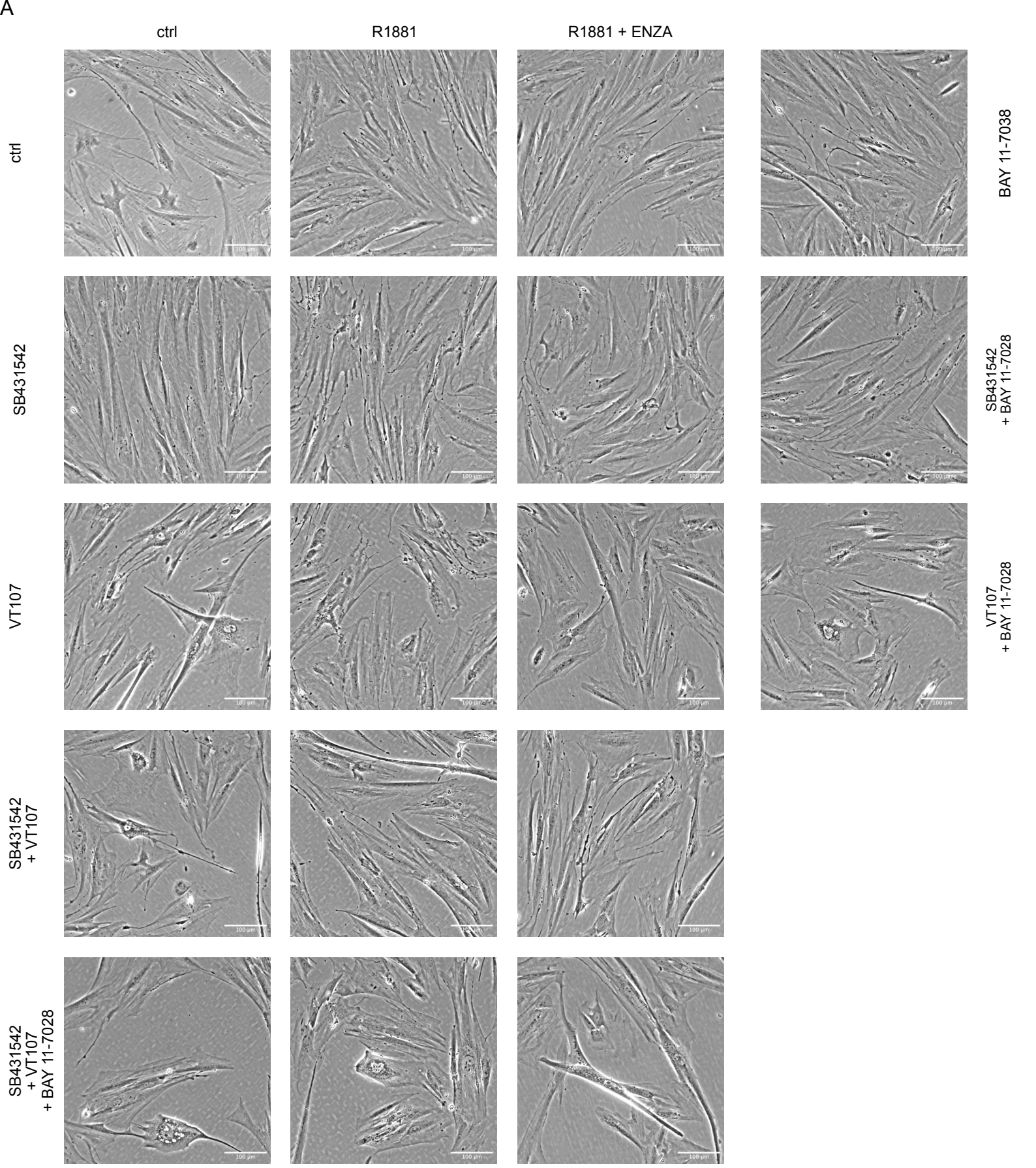

D
C3 - patient 1
C3 - patient 2
TCP
2 kPa


F
IL6
IL8
Fold change in expression (relative to 2D ctrl)
ctrl
BAY
3D


E
2 kPa vs. TCP
FAP NOX4 CYR61 TAGLN CTGF ACTG2 ALDH1A1 APOD CFD CES1 IL1B IL8

Supp.Fig. S14

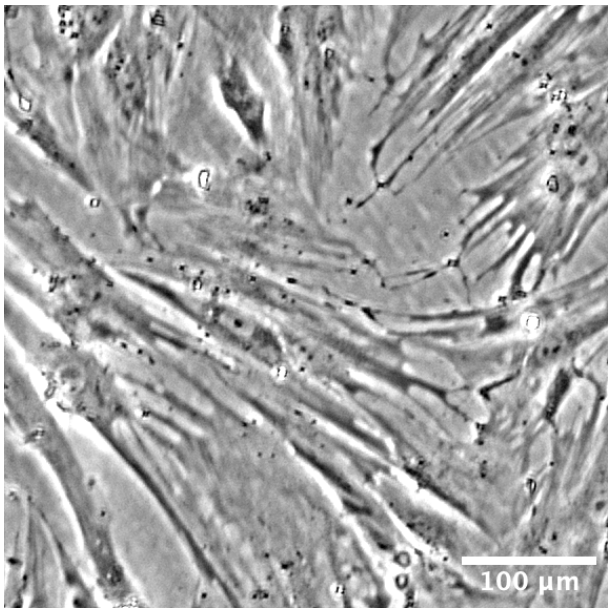
A



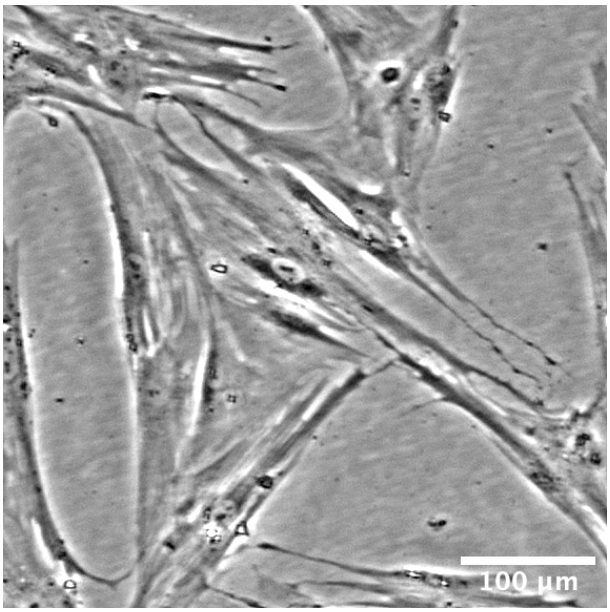
Supp Fig. S15

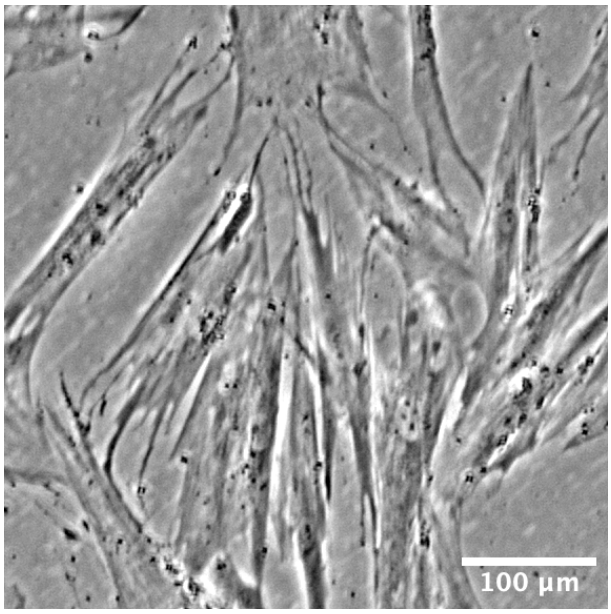
B

scrambled siRNA

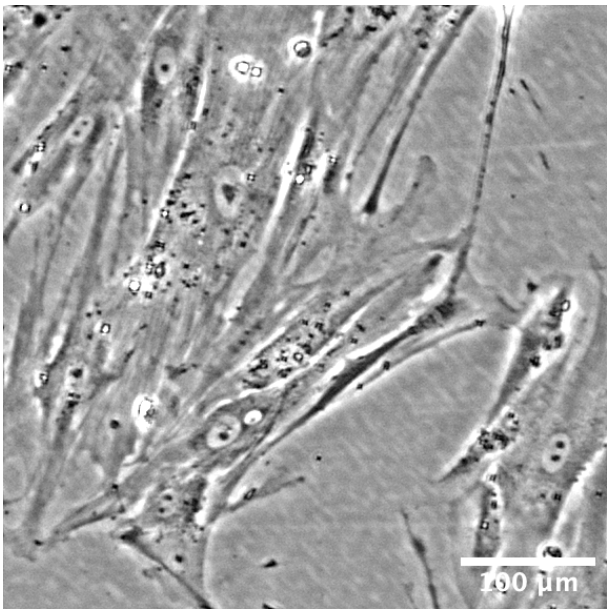


TGFBR1 siRNA





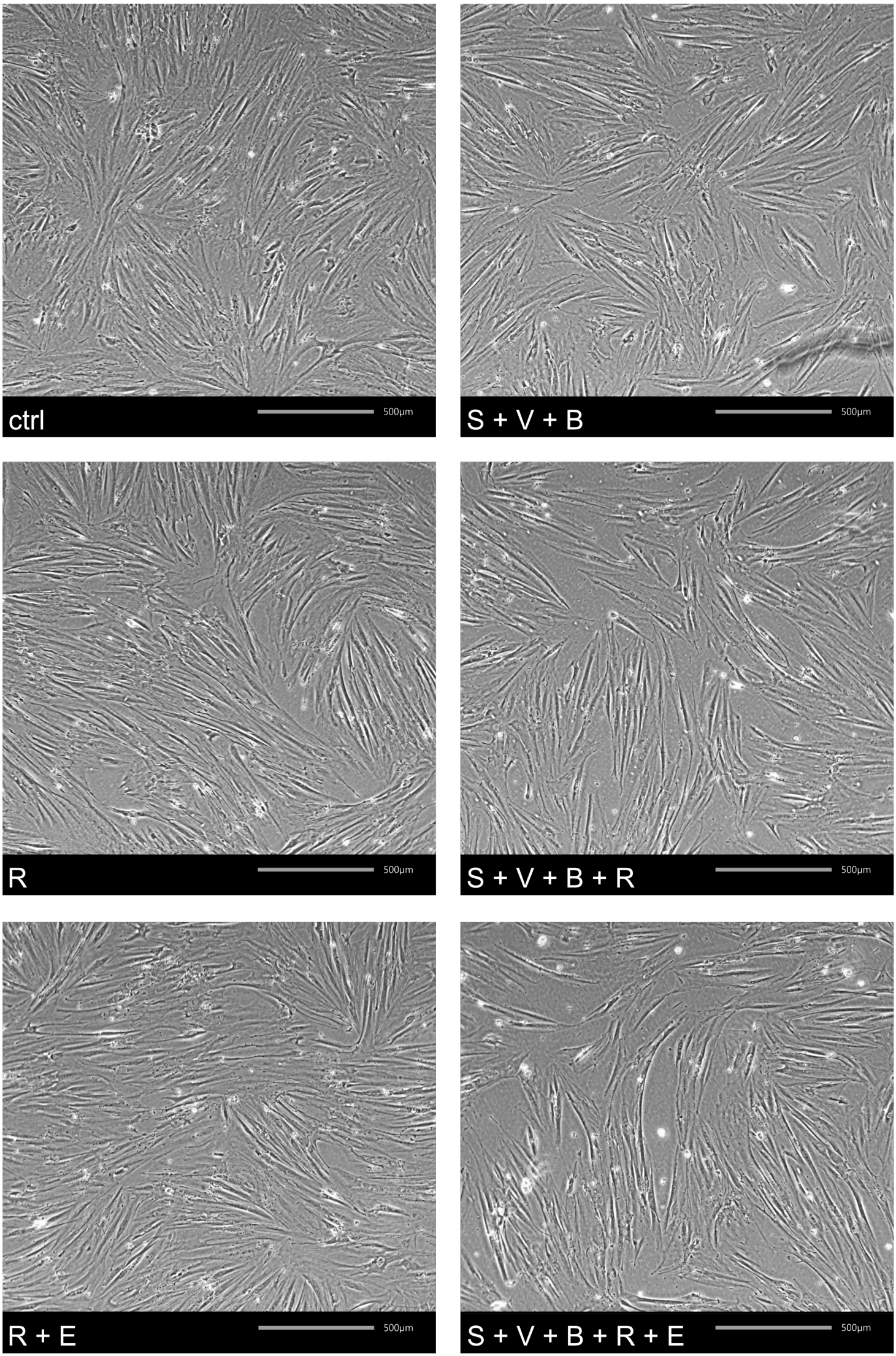
YAP1 siRNA



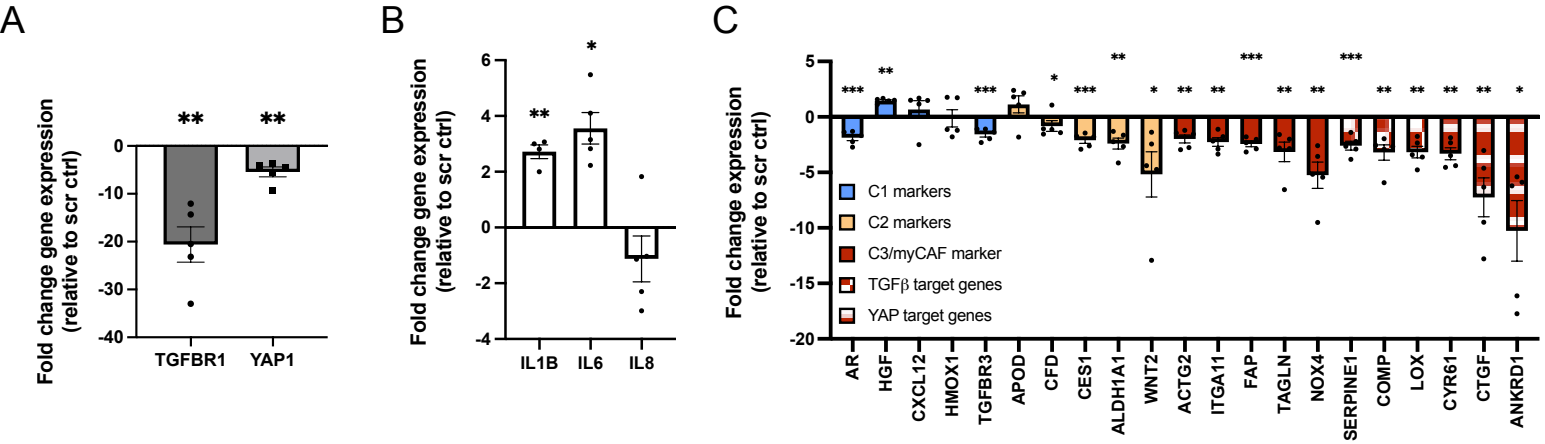
YAP1 + TGFBR1 siRNA

Supp. Fig. S15 cont.

C



Supp. Fig. S15 cont.


A
Fold change gene expression (relative to scr ctrl)
0
-10
-20
-30
-40
**
**
TGFBR1
YAP1
B
Fold change gene expression (relative to scr ctrl)
6
4
2
0
-2
-4
**
*
IL1B
IL6
IL8
C
Fold change gene expression (relative to scr ctrl)
5
0
-5
-10
-15
-20
C1 markers
C2 markers
C3/myCAF marker
TGFβ target genes
YAP target genes
AR
HGF
CXCL12
HMOX1
TGFBR3
APOD
CFD
CES1
ALDH1A1
WNT2
ACTG2
ITGA11
FAP
TAGLN
NOX4
SERPINE1
COMP
LOX
CYR61
CTGF
ANKRD1