Supplementary Fig 1. Spatial transcriptomics of LPC lesions show that fibroblasts are distinct from cells expressing *Rgs5* or *Tagln* genes; Ligand – receptor (L-R) interactions between cells in day 14 LPC lesions. a, b: Spatial transcriptomic analyses reveal that the fibroblast cluster contains minimally *Rgs5* which is enriched in pericytes, or *Tagln.* c, e. Number of significant ligand-receptor interactions between any pair of two cell populations shown as (c) circle plot and (e) heatmap. d, f. Interaction strength between ligand-receptor of any pair of two cell populations shown as (d) circle plot and (f) heatmap. In the circle plots, the circle sizes are proportional to the number of cells in each group; edge weights are proportional to the interaction strength. g. Scatter plot of incoming and outgoing interaction strength showing fibroblasts as major outgoing signaling source. Dot size is proportional to the number of inferred links (outgoing and incoming) associated with each cell group. h, i. Chord plot showing L-R interactions with fibroblasts as source (h) or receiver (i) of signal.

Supplementary Fig 2. Incoming and outgoing fibroblast L-R interactions in day 14 LPC lesions. a, b. Bubble plot delineating L-R interactions from one cell group (i.e. sender) to another (i.e. target) as indicated in the plot.

Supplementary Fig 3. Cell contributions to communication networks. a, b. Dot plots indicating cell type contribution to (a) outgoing and (b) incoming communication patterns.

Supplementary Fig 4. Inferred Incoming and outgoing signaling patterns between fibroblasts, OPCs and macrophages in day 14 LPC lesions. Heatmap of (a) inferred outgoing and (b) incoming signaling pathways as assessed for the 3 cell groups. Color bar in the heatmap represents the relative signaling strength of a pathway across all cell groups (values are row-scaled). Top colored bar plot shows the total signaling strength of a cell group by summarizing all pathways displayed in the heatmap. The right grey bar plot shows the total signaling strength of a pathway by summarizing all cell groups displayed in the heatmap. Heatmap of (c) outgoing and (e) incoming signaling pathways that contribute to each communication pattern. River plots showing the cell source for (d) outgoing and (f) incoming communication patterns and the pathways associated with them. The thickness of the flow indicates contribution of cell group or signaling pathway to each pattern.

Supplementary Fig 5. Axon densities are unchanged in fibroblast occupied regions. a. Representative image of a day 21 LPC lesion stained for DAPI, NFH, and PDGFRβ. b. Quantification comparing NFH positive axon density in fibroblast occupied areas with the rest of the LPC lesion. Sample sizes n = 7 across two experiments. Two-tailed, unpaired t-test comparing groups. Data are the mean ± s.d.

Supplementary Fig 6. Main cell types as assessed in MS lesions. a. Heatmap of top 5 differentially expressed genes across annotated cell groups.

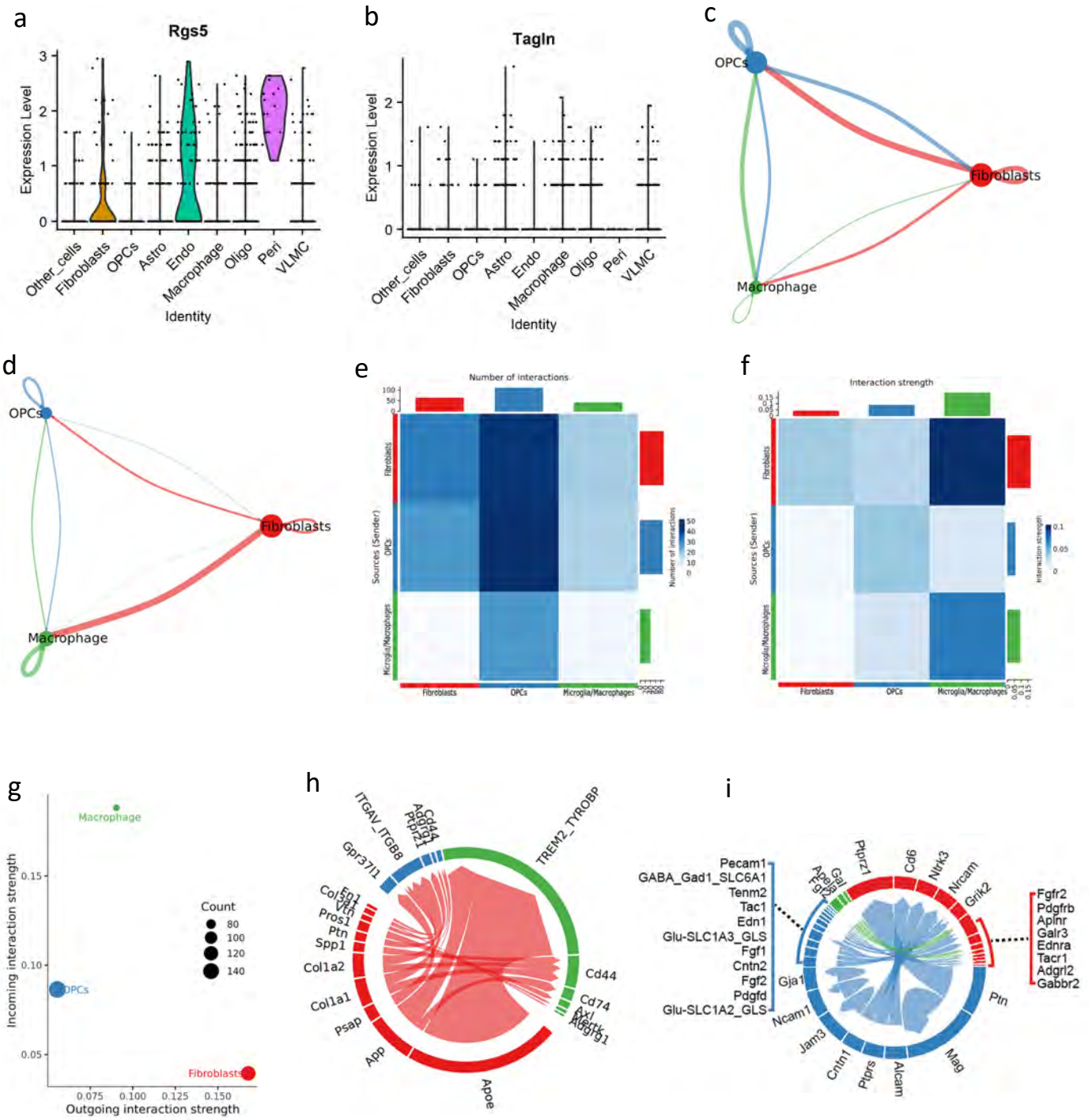
Supplementary Fig 7. Non-fibroblast related marker expression across cell clusters assessed in MS lesions. a. Violin plots of TAGLN and RGS5 normalised gene expression in snRNAseq dataset. b, c. String-db plot of DEG between fibroblast (b) subcluster 0, and (c) subclusters 1.

Supplementary Fig 8. L-R interactions between fibroblasts, M/M and OPCs in MS lesions. a, c. Number of significant ligand-receptor between any pair of two cell populations shown as (a) circle plot and (c) heatmap. b, d. Interaction strength between ligand-receptor any pair of two cell populations shown as (b) circle plot and (d) heatmap. In the circle plots, the circle sizes are proportional to the number of cells in each group; edge weights are proportional to the interaction strength. e. Schematic indicating inferred outgoing and incoming fibroblast signals and their distribution across signaling databases. f, g. Chord plot showing L-R interactions with fibroblasts as (f) source of signals, and (g) receiver of signals.

Supplementary Fig 9. Inferred Fibroblast communication patterns in MS. a, b. Bubble plots delineating L-R interactions from one cell group (i.e. sender) to another (i.e. target) as assessed with different signaling databases indicated in the plot (a) ECM-receptor and (b) secreted signaling databases. c, d. Dot plots of (c) outgoing and (d) incoming communication patterns.

Supplementary Fig 10. Fibroblast interactions in MS lesions. a, b. Heatmap and river plots indicating cell and signaling pathways associated with outgoing and incoming communication patterns in MS lesions. The thickness of the flow indicates contribution of cell group or signaling pathway to each pattern. c, d. Venn diagrams of (l) outgoing and (m) incoming signaling pathways comparing MS and LPC lesions.

Supplementary Fig 11. Inferred Communication pattern contributions in MS lesions. a, b. Heatmap of (a) inferred outgoing and (b) incoming signaling pathways as assessed for the 3 cell groups. Colour bar in the heatmap represents the relative signaling strength of a pathway across all cell groups (values are row-scaled). Top colored bar plot shows the total signaling strength of a cell group by summarizing all pathways displayed in the heatmap. The right grey bar plot shows the total signaling strength of a pathway by summarizing all cell groups displayed in the heatmap. c, d. Venn-diagrams comparing LPC (CosMx) and MS (snRNAseq) (c) outgoing and (d) incoming fibroblast L-R interactions.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Rgs5
Expression Level
Other_cells
Fibroblasts
OPCs
Astro
Endo
Macrophage
Oligo
Peri
VLMC
Identity
b
Tagln
Expression Level
Identity
c
OPCs
Fibroblasts
Macrophage
d
OPCs
Fibroblasts
Macrophage
e
Number of interactions
Sources (Sender)
Fibroblasts
OPCs
Microglia/Macrophages
f
Interaction strength
g
Macrophage
OPCs
Fibroblasts
Count
80
100
120
140
Incoming interaction strength
Outgoing interaction strength
h
TREM2_TYROBP
ITGAV_ITGB8
Gpr37l1
Col1a2
Col1a1
Psap
App
Apoe
Cd44
Cd74
i
Pecam1
GABA_Gad1_SLC6A1
Tenm2
Tac1
Edn1
Glu-SLC1A3_GLS
Fgf1
Cntn2
Fgf2
Pdgfd
Glu-SLC1A2_GLS
Gja1
Ncam1
Jam3
Cntn1
Ptprs
Alcam
Mag
Ptn
Ptprz1
Cd9
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Aplnr
Galr3
Ednra
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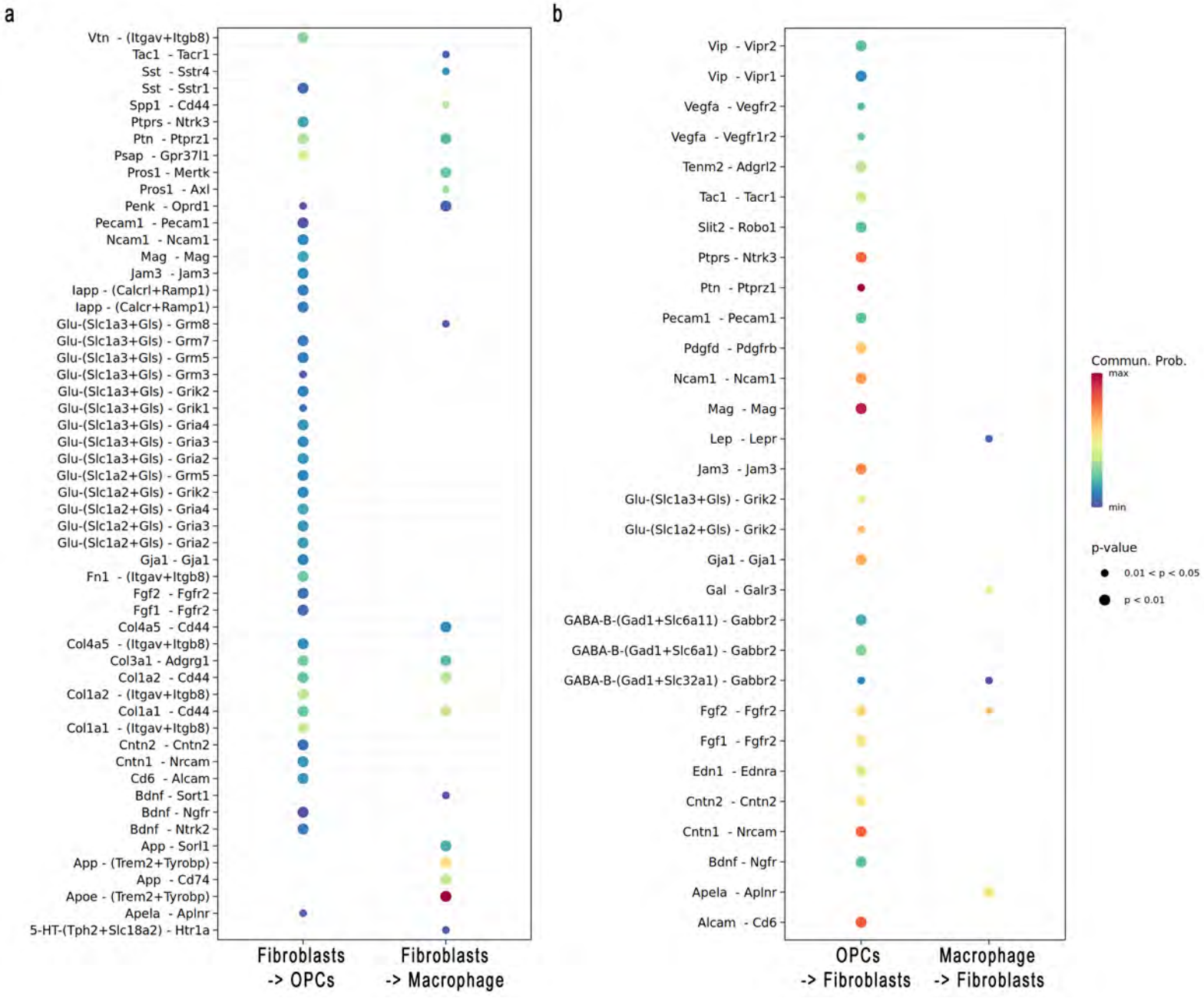
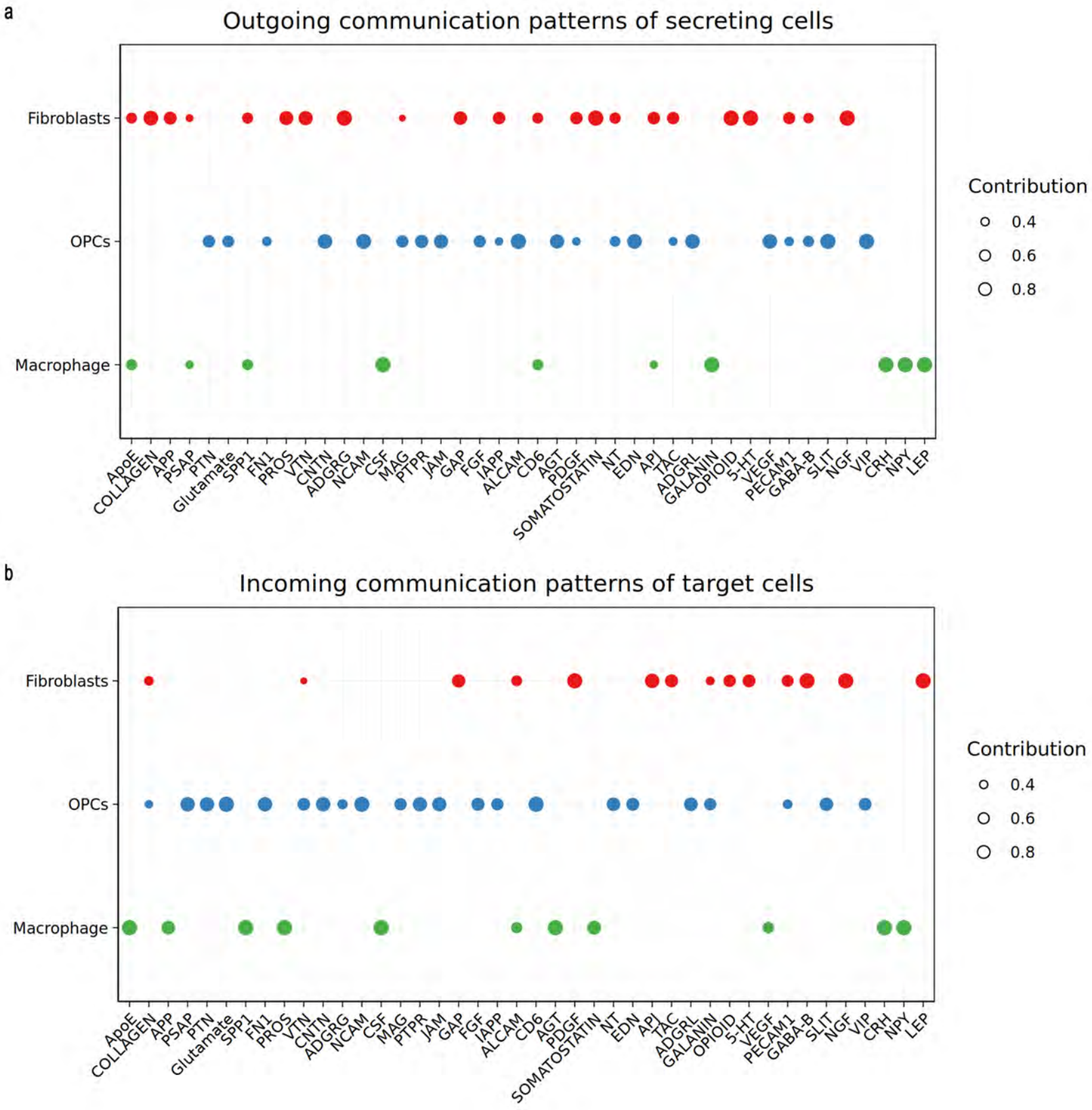
Gabbr2


a
Vtn - (Itgav+Itgb8)
Tac1 - Tacr1
Sst - Sstr4
Sst - Sstr1
Spp1 - Cd44
Ptprs - Ntrk3
Ptn - Ptprz1
Psap - Gpr37l1
Pros1 - Mertk
Pros1 - Axl
Penk - Oprd1
Pecam1 - Pecam1
Ncam1 - Ncam1
Mag - Mag
Jam3 - Jam3
Iapp - (Calcrl+Ramp1)
Iapp - (Calcr+Ramp1)
Glu-(Slc1a3+Gls) - Grm8
Glu-(Slc1a3+Gls) - Grm7
Glu-(Slc1a3+Gls) - Grm5
Glu-(Slc1a3+Gls) - Grm3
Glu-(Slc1a3+Gls) - Grik2
Glu-(Slc1a3+Gls) - Grik1
Glu-(Slc1a3+Gls) - Gria4
Glu-(Slc1a3+Gls) - Gria3
Glu-(Slc1a3+Gls) - Gria2
Glu-(Slc1a2+Gls) - Grm5
Glu-(Slc1a2+Gls) - Grik2
Glu-(Slc1a2+Gls) - Gria4
Glu-(Slc1a2+Gls) - Gria3
Glu-(Slc1a2+Gls) - Gria2
Gja1 - Gja1
Fn1 - (Itgav+Itgb8)
Fgf2 - Fgfr2
Fgf1 - Fgfr2
Col4a5 - Cd44
Col4a5 - (Itgav+Itgb8)
Col3a1 - Adgrg1
Col1a2 - Cd44
Col1a2 - (Itgav+Itgb8)
Col1a1 - Cd44
Col1a1 - (Itgav+Itgb8)
Cntn2 - Cntn2
Cntn1 - Nrcam
Cd6 - Alcam
Bdnf - Sort1
Bdnf - Ngfr
Bdnf - Ntrk2
App - Sorl1
App - (Trem2+Tyrobp)
App - Cd74
Apoe - (Trem2+Tyrobp)
Apela - Aplnr
5-HT-(Tph2+Slc18a2) - Htr1a
Fibroblasts -> OPCs
Fibroblasts -> Macrophage
b
Vip - Vipr2
Vip - Vipr1
Vegfa - Vegfr2
Vegfa - Vegfr1r2
Tenm2 - Adgrl2
Tac1 - Tacr1
Slit2 - Robo1
Ptprs - Ntrk3
Ptn - Ptprz1
Pecam1 - Pecam1
Pdgfd - Pdgfrb
Ncam1 - Ncam1
Mag - Mag
Lep - Lepr
Jam3 - Jam3
Glu-(Slc1a3+Gls) - Grik2
Glu-(Slc1a2+Gls) - Grik2
Gja1 - Gja1
Gal - Galr3
GABA-B-(Gad1+Slc6a11) - Gabbr2
GABA-B-(Gad1+Slc6a1) - Gabbr2
GABA-B-(Gad1+Slc32a1) - Gabbr2
Fgf2 - Fgfr2
Fgf1 - Fgfr2
Edn1 - Ednra
Cntn2 - Cntn2
Cntn1 - Nrcam
Bdnf - Ngfr
Apela - Aplnr
Alcam - Cd6
OPCs -> Fibroblasts
Macrophage -> Fibroblasts
Commun. Prob.
max
min
p-value
0.01 < p < 0.05
p < 0.01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Outgoing communication patterns of secreting cells
Fibroblasts
OPCs
Macrophage
ApoE
COLLAGEN
APP
PSAP
PTN
Glutamate
SPP1
FN1
PROS
VTN
CNTN
ADGRG
NCAM
CSF
MAG
PTPR
JAM
GAP
FGF
IAPP
ALCAM
CD6
AGT
PDGF
SOMATOSTATIN
NT
EDN
APJ
TAC
ADGRL
GALANIN
OPIOID
5-HT
VEGF
PECAM1
GABA-B
SLIT
NGF
VIP
CRH
NPY
LEP
Contribution
0.4
0.6
0.8
b
Incoming communication patterns of target cells
Fibroblasts
OPCs
Macrophage
ApoE
COLLAGEN
APP
PSAP
PTN
Glutamate
SPP1
FN1
PROS
VTN
CNTN
ADGRG
NCAM
CSF
MAG
PTPR
JAM
GAP
FGF
IAPP
ALCAM
CD6
AGT
PDGF
SOMATOSTATIN
NT
EDN
APJ
TAC
ADGRL
GALANIN
OPIOID
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VEGF
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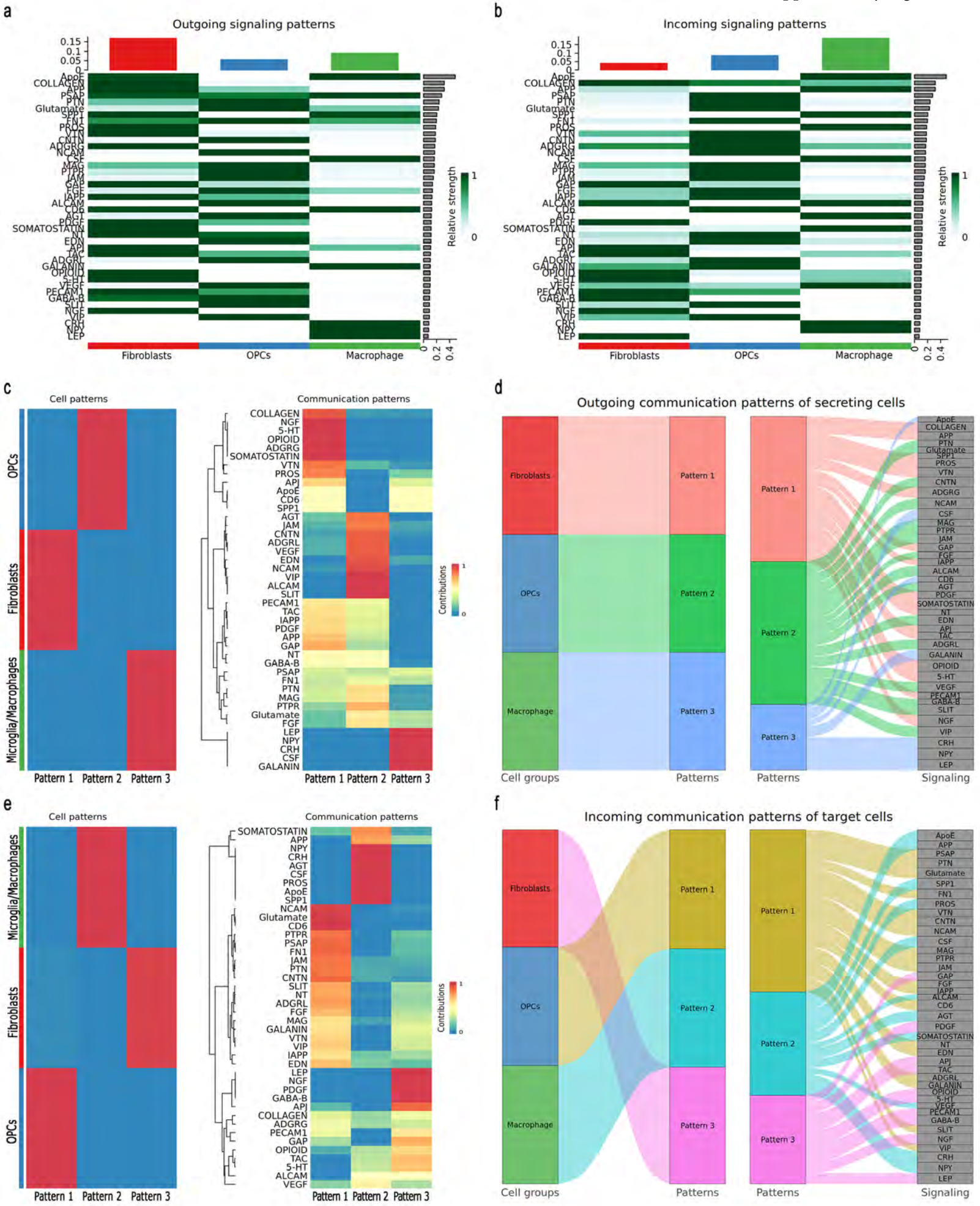
NGF
VIP
CRH
NPY
LEP
Contribution
0.4
0.6
0.8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Relative strength
Fibroblasts
OPCs
Macrophage
b
Incoming signaling patterns
Relative strength
Fibroblasts
OPCs
Macrophage
c
Cell patterns
Communication patterns
OPCs
Fibroblasts
Microglia/Macrophages
Pattern 1 Pattern 2 Pattern 3
Contributions
d
Outgoing communication patterns of secreting cells
Fibroblasts
OPCs
Macrophage
Pattern 1
Pattern 2
Pattern 3
Cell groups
Patterns
Signaling
e
Cell patterns
Communication patterns
Microglia/Macrophages
Fibroblasts
OPCs
Pattern 1 Pattern 2 Pattern 3
Contributions
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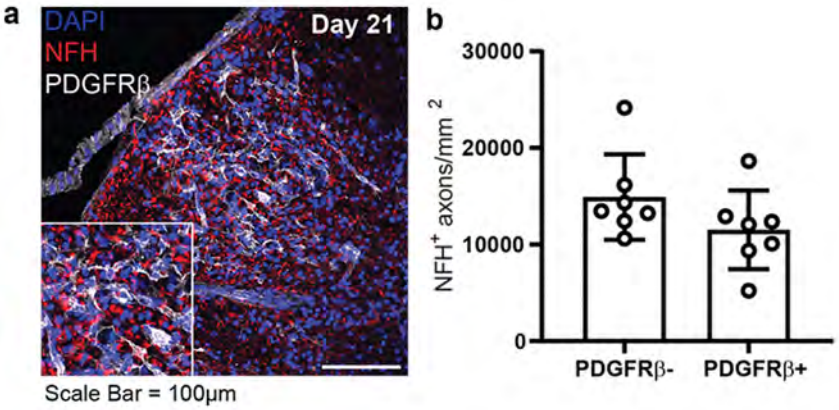
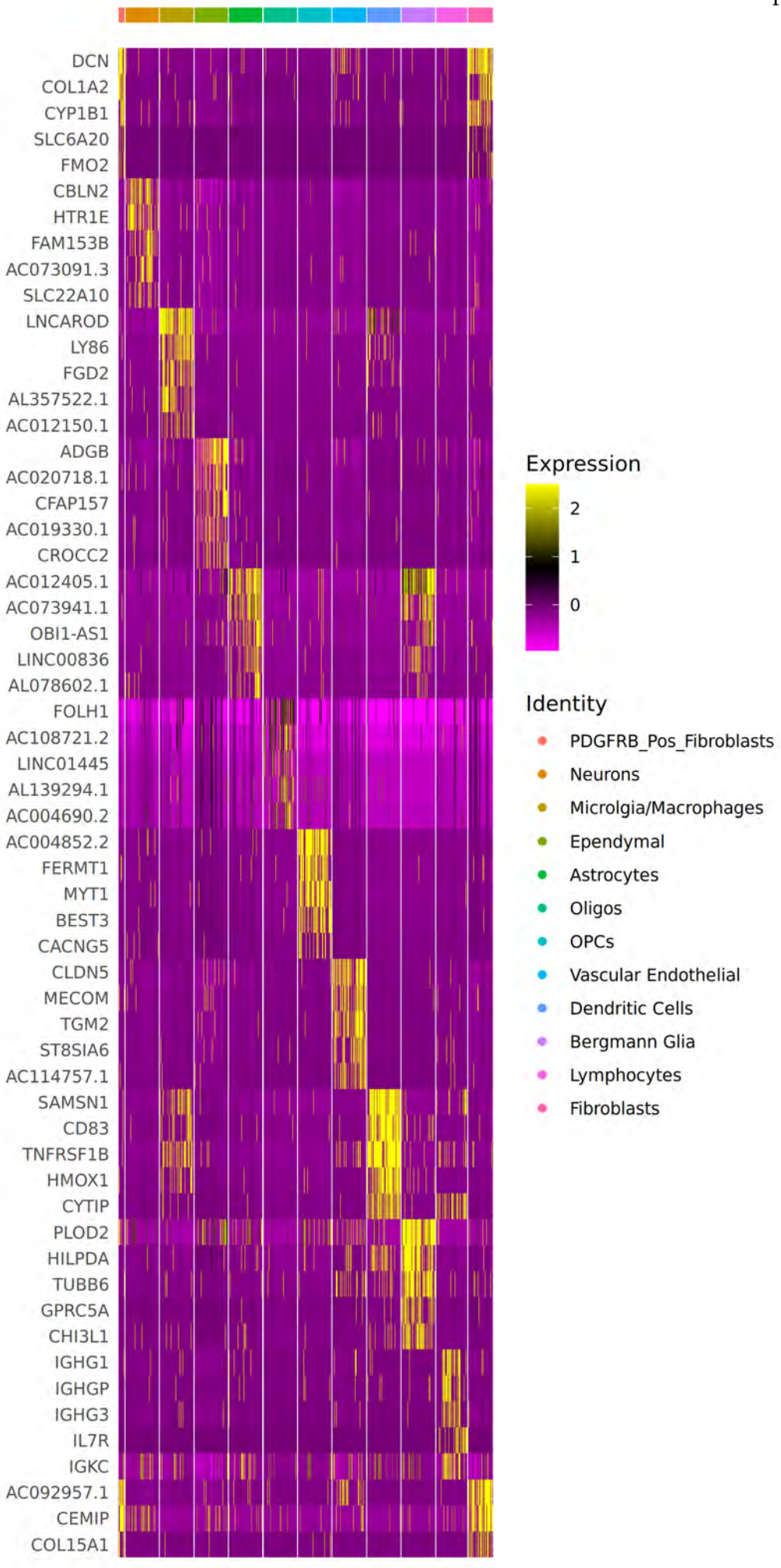
Incoming communication patterns of target cells
Fibroblasts
OPCs
Macrophage
Pattern 1
Pattern 2
Pattern 3
Cell groups
Patterns
Signaling


a
DAPI
NFH
PDGFRβ
Day 21
Scale Bar = 100μm
b
30000
20000
10000
0
NFH+ axons/mm 2
PDGFRβ-
PDGFRβ+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LY86
FGD2
AL357522.1
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CROCC2
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LINC01445
AL139294.1
AC004690.2
AC004852.2
FERMT1
MYT1
BEST3
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MECOM
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CD83
TNFRSF1B
HMOX1
CYTIP
PLOD2
HILPDA
TUBB6
GPRC5A
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IGHG1
IGHGP
IGHG3
IL7R
IGKC
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1
0
Identity
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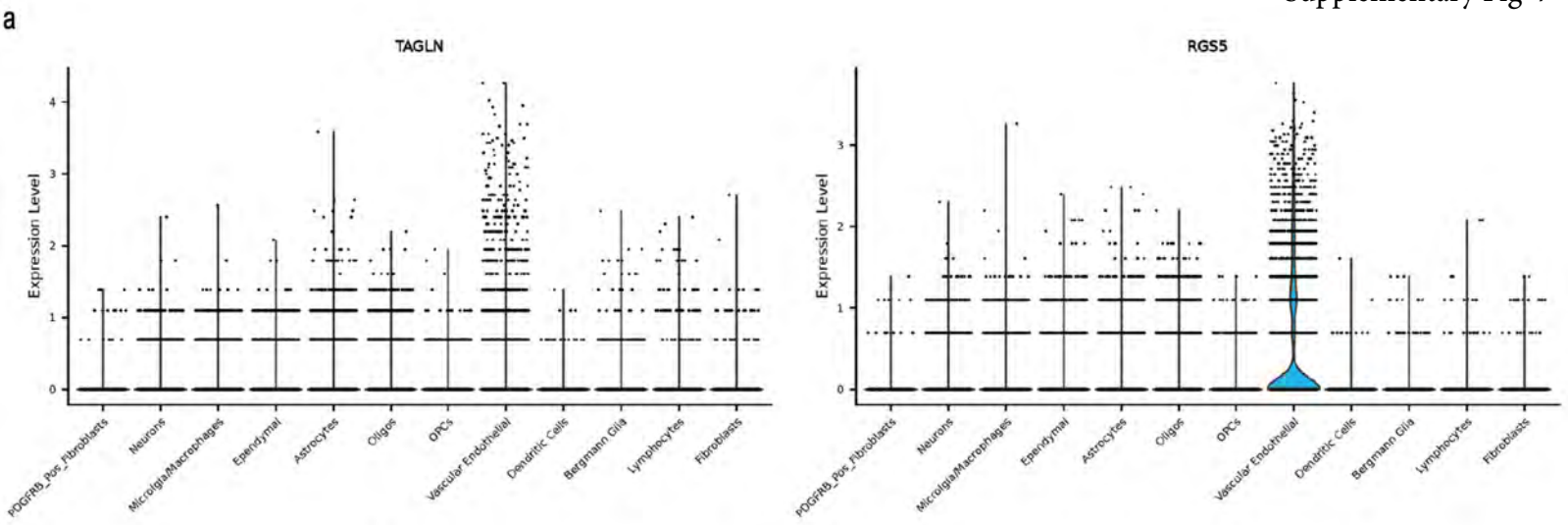
Microlgia/Macrophages
Ependymal
Astrocytes
Oligos
OPCs
Vascular Endothelial
Dendritic Cells
Bergmann Glia
Lymphocytes
Fibroblasts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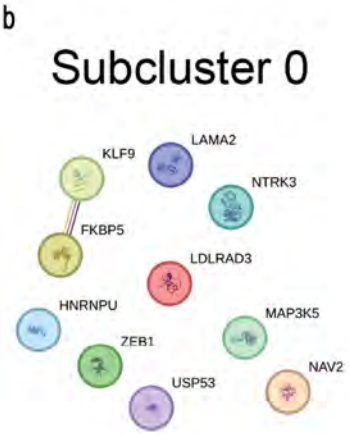
RGS5
Expression Level
PDGFRB_Pos_Fibroblasts
Neurons
Microglia/Macrophages
Ependymal
Astrocytes
Oligos
OPCs
Vascular Endothelial
Dendritic Cells
Bergmann Glia
Lymphocytes
Fibroblasts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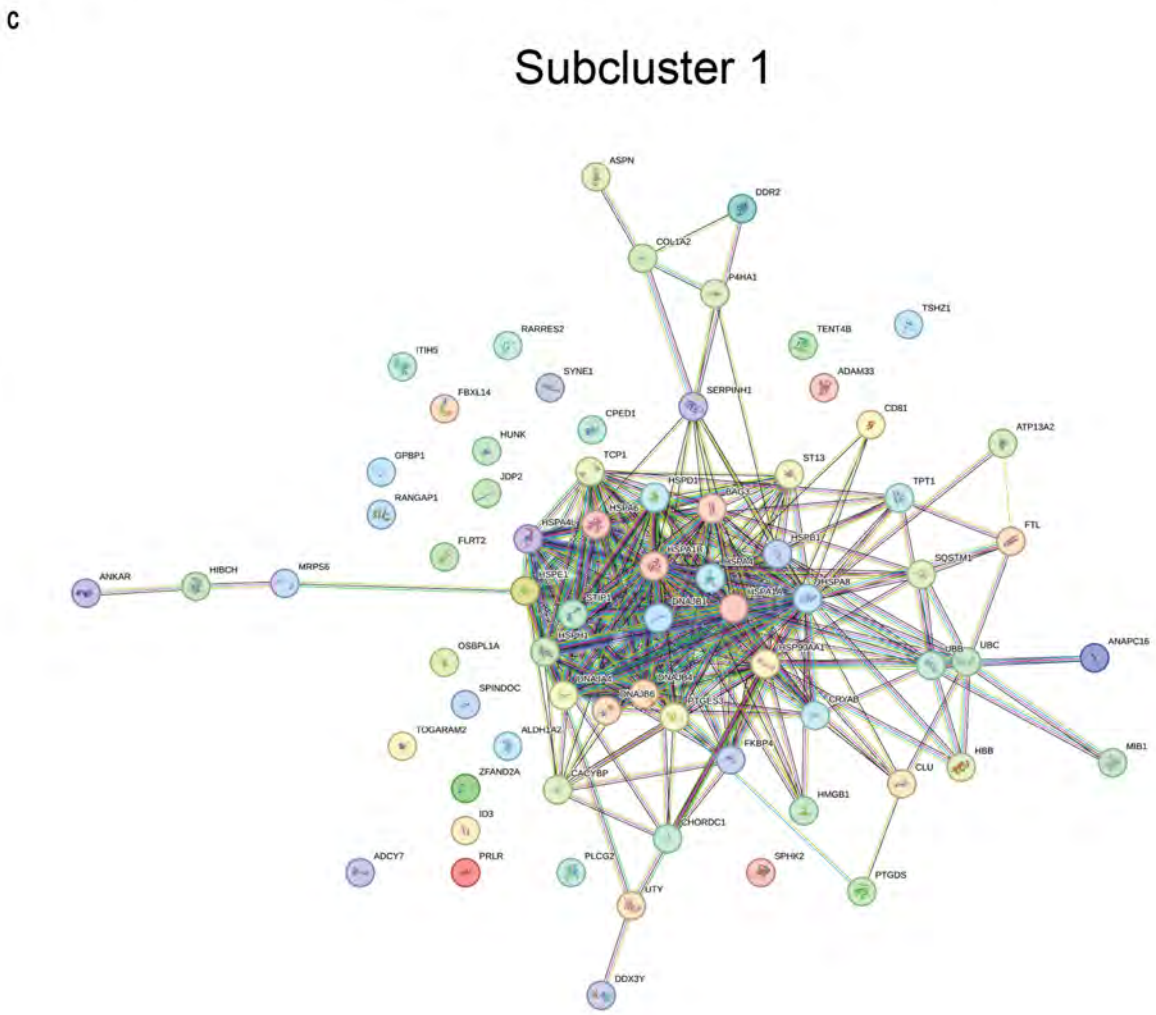
NTRK3
FKBP5
LDLRAD3
HNRNPU
MAP3K5
ZEB1
NAV2
USP53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DDR2
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FBXL14
SERPINH1
CD81
CPED1
ATP13A2
HUNK
TCP1
GPBP1
ST13
JDP2
TPT1
RANGAP1
FTL
FLRT2
SQSTM1
ANKAR
HIBCH
MRPS6
OSBPL1A
UBB
UBC
ANAPC16
SPINDOC
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TOGARAM2
ALDH1A2
FKBP4
HBB
MIB1
ZFAND2A
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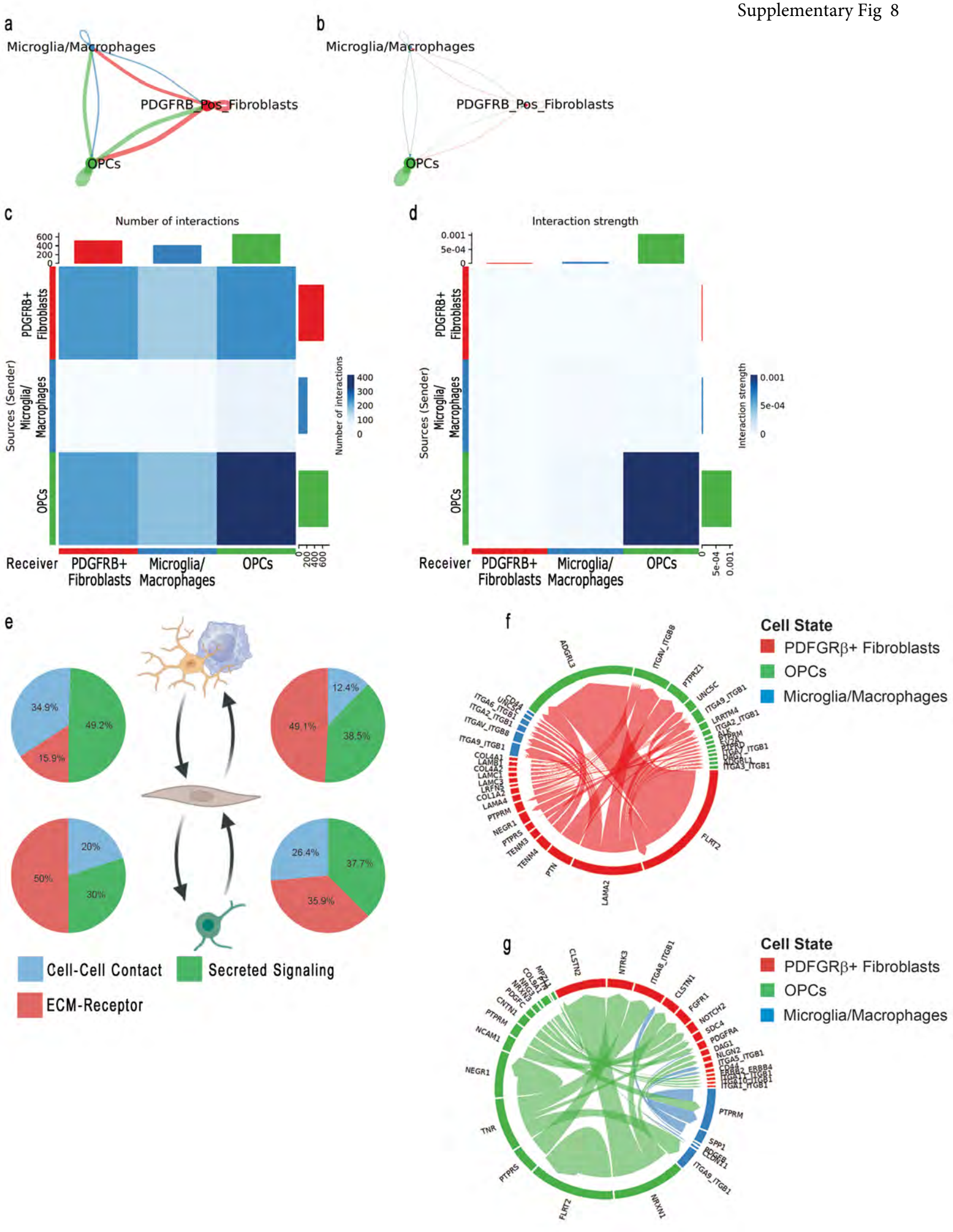
ADCY7
PRLR
PLCG2
SPHK2
PTGDS
UTY
DDX3Y


a
Microglia/Macrophages
PDGFRB_Pos_Fibroblasts
OPCs
b
Microglia/Macrophages
PDGFRB_Pos_Fibroblasts
OPCs
c
Number of interactions
Sources (Sender)
PDGFRB+ Fibroblasts
Microglia/ Macrophages
OPCs
Receiver
Number of interactions
d
Interaction strength
Sources (Sender)
Receiver
Interaction strength
e
34.9%
49.2%
15.9%
12.4%
49.1%
38.5%
20%
50%
30%
26.4%
37.7%
35.9%
Cell-Cell Contact
Secreted Signaling
ECM-Receptor
f
Cell State
PDFGRβ+ Fibroblasts
OPCs
Microglia/Macrophages
ADGRL3
ITGAV_ITGB8
PTPRZ1
UNC5C
ITGA9_ITGB1
LRRTM4
ITGA2_ITGB1
FLRT2
LAMA2
PTN
TENM4
TENM3
PTPRS
NEGR1
PTPRM
g
Cell State
PDFGRβ+ Fibroblasts
OPCs
Microglia/Macrophages
CLSTN2
NTRK3
ITGA8_ITGB1
CLSTN1
FGFR1
NOTCH2
SDC4
PDGFRA
DAG1
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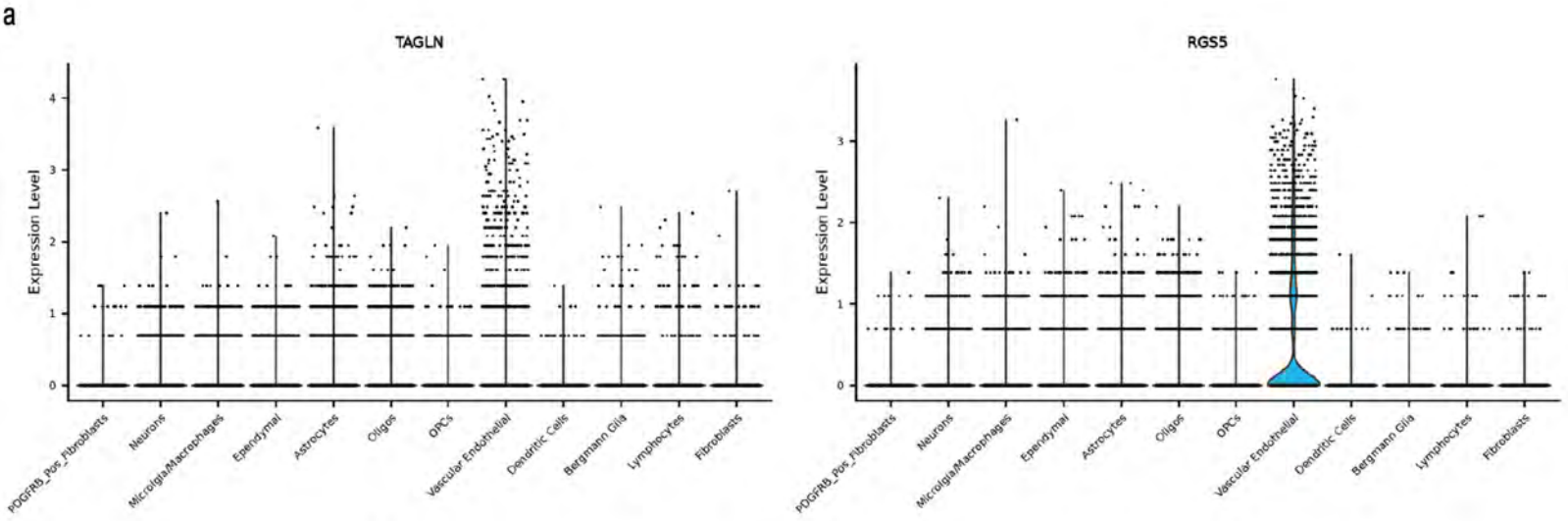
SPP1
PTPRM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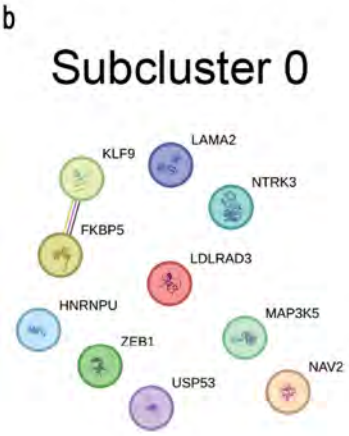
Expression Level
PDGFRB_Pos_Fibroblasts
Neurons
Microglia/Macrophages
Ependymal
Astrocytes
Oligos
OPCs
Vascular Endothelial
Dendritic Cells
Bergmann Glia
Lymphocytes
Fibroblasts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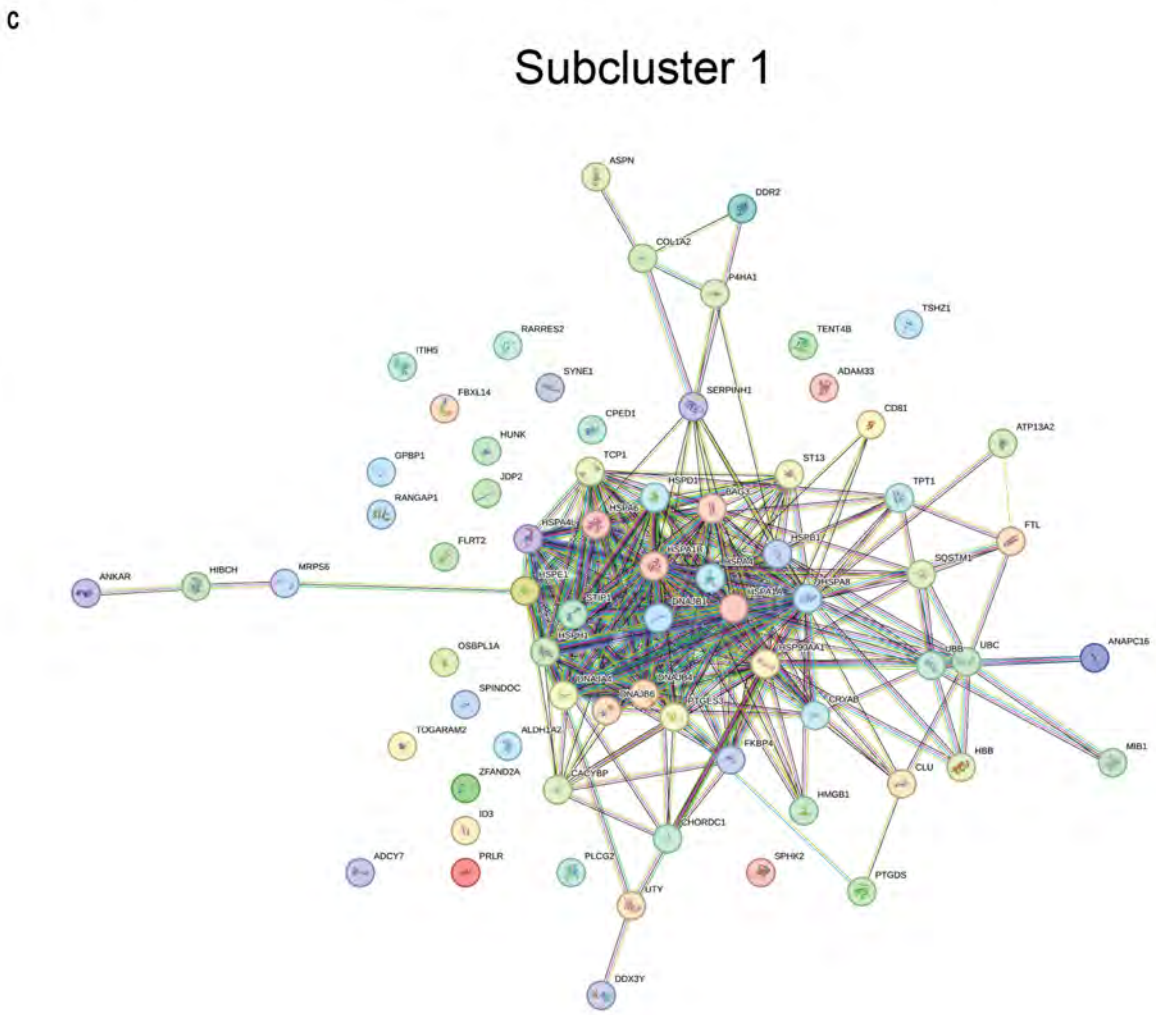
USP53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ITIH5
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FBXL14
SERPINH1
CD81
CPED1
ATP13A2
HUNK
TCP1
GPBP1
ST13
JDP2
TPT1
RANGAP1
FTL
FLRT2
SQSTM1
ANKAR
HIBCH
MRPS6
OSBPL1A
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HBB
MIB1
ZFAND2A
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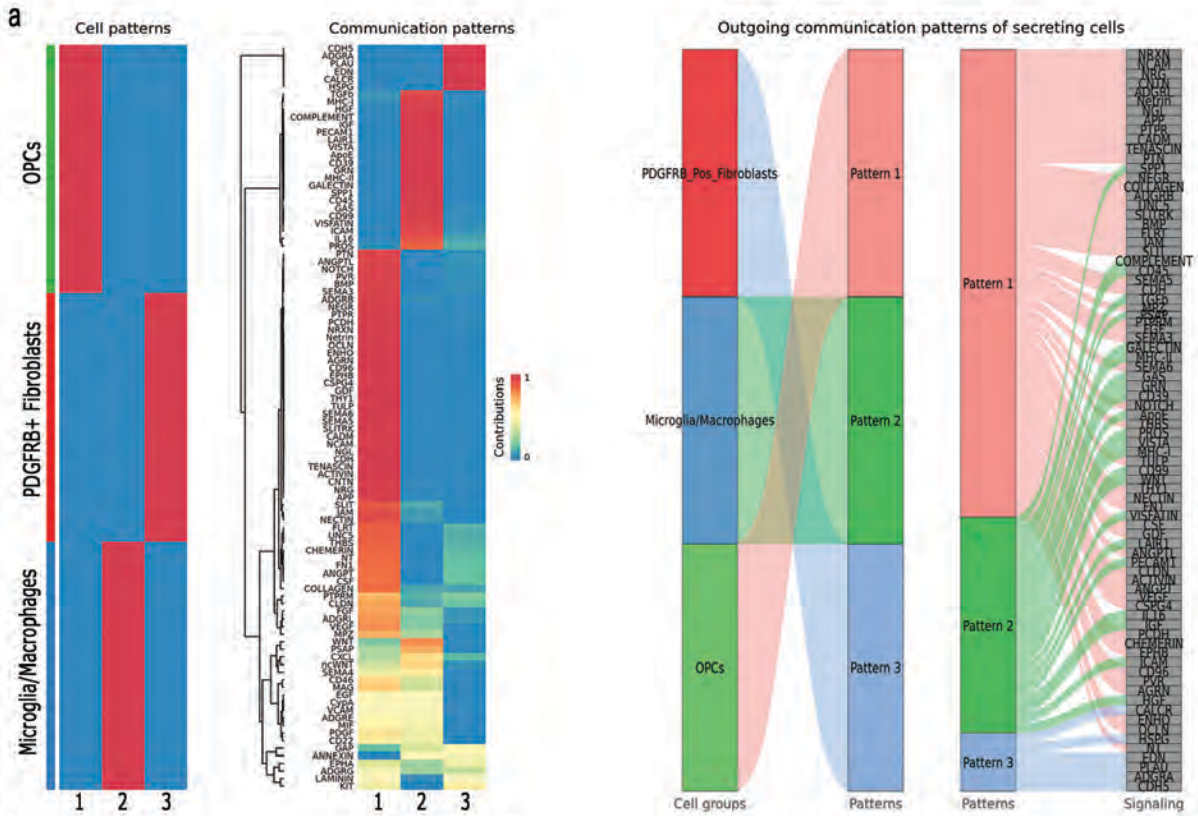
DDX3Y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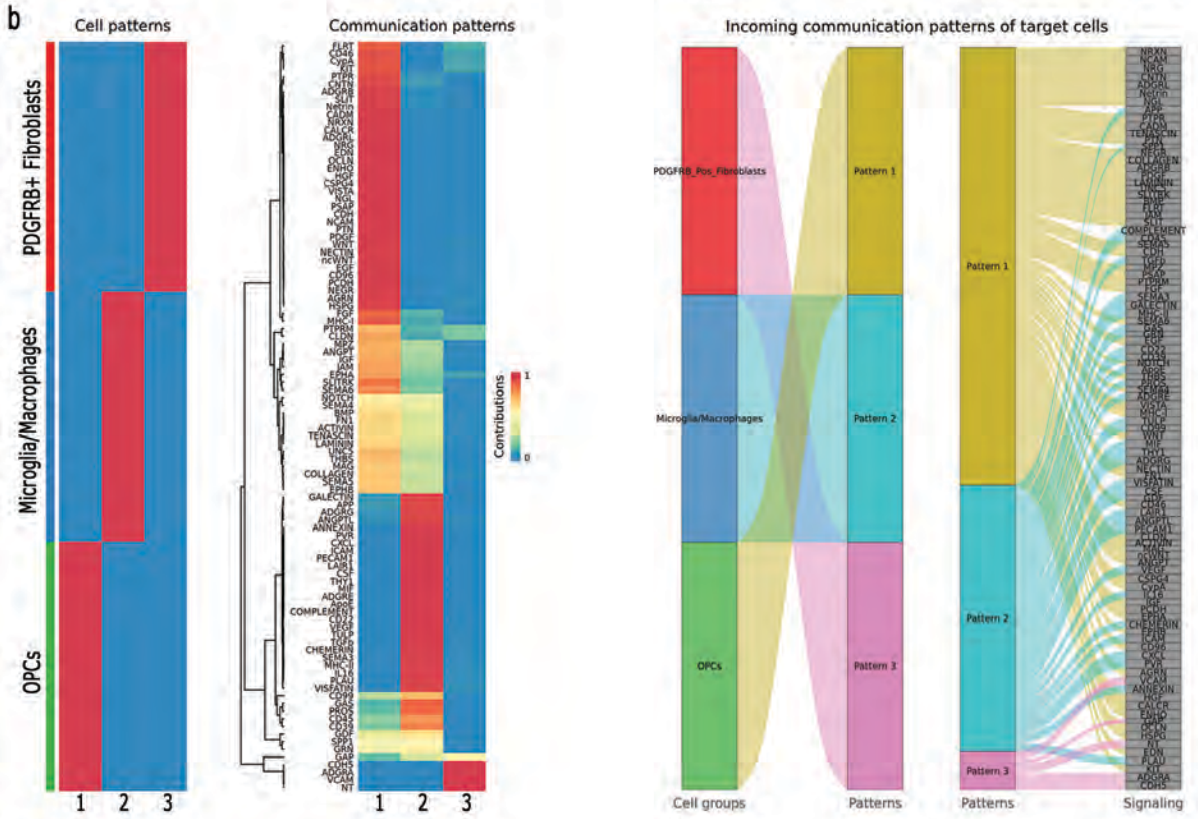
Cell patterns
Communication patterns
Outgoing communication patterns of secreting cells
OPCs
PDGFRB+ Fibroblasts
Microglia/Macrophages
Contributions
1
2
3
PDGFRB_Pos_Fibroblasts
Microglia/Macrophages
OPCs
Pattern 1
Pattern 2
Pattern 3
Cell groups
Patterns
Signaling


b
Cell patterns
Communication patterns
Incoming communication patterns of target cells
PDGFRB+ Fibroblasts
Microglia/Macrophages
OPCs
Contributions
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2
3
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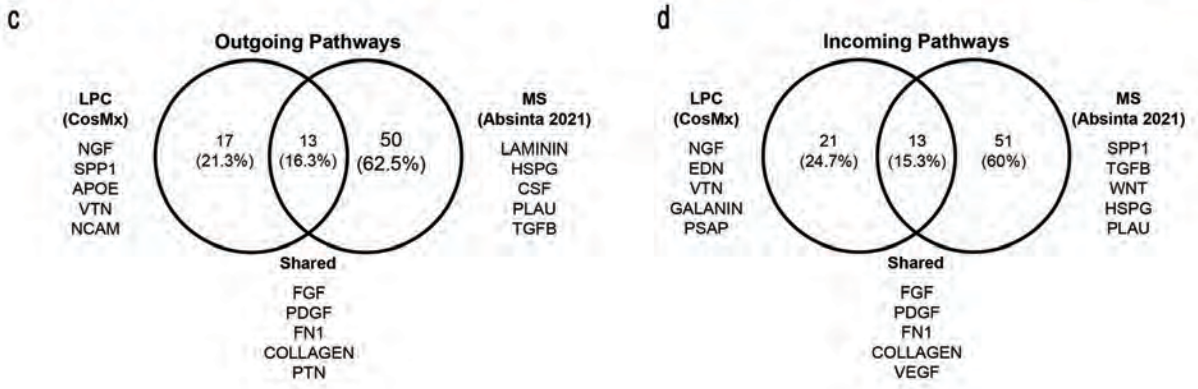
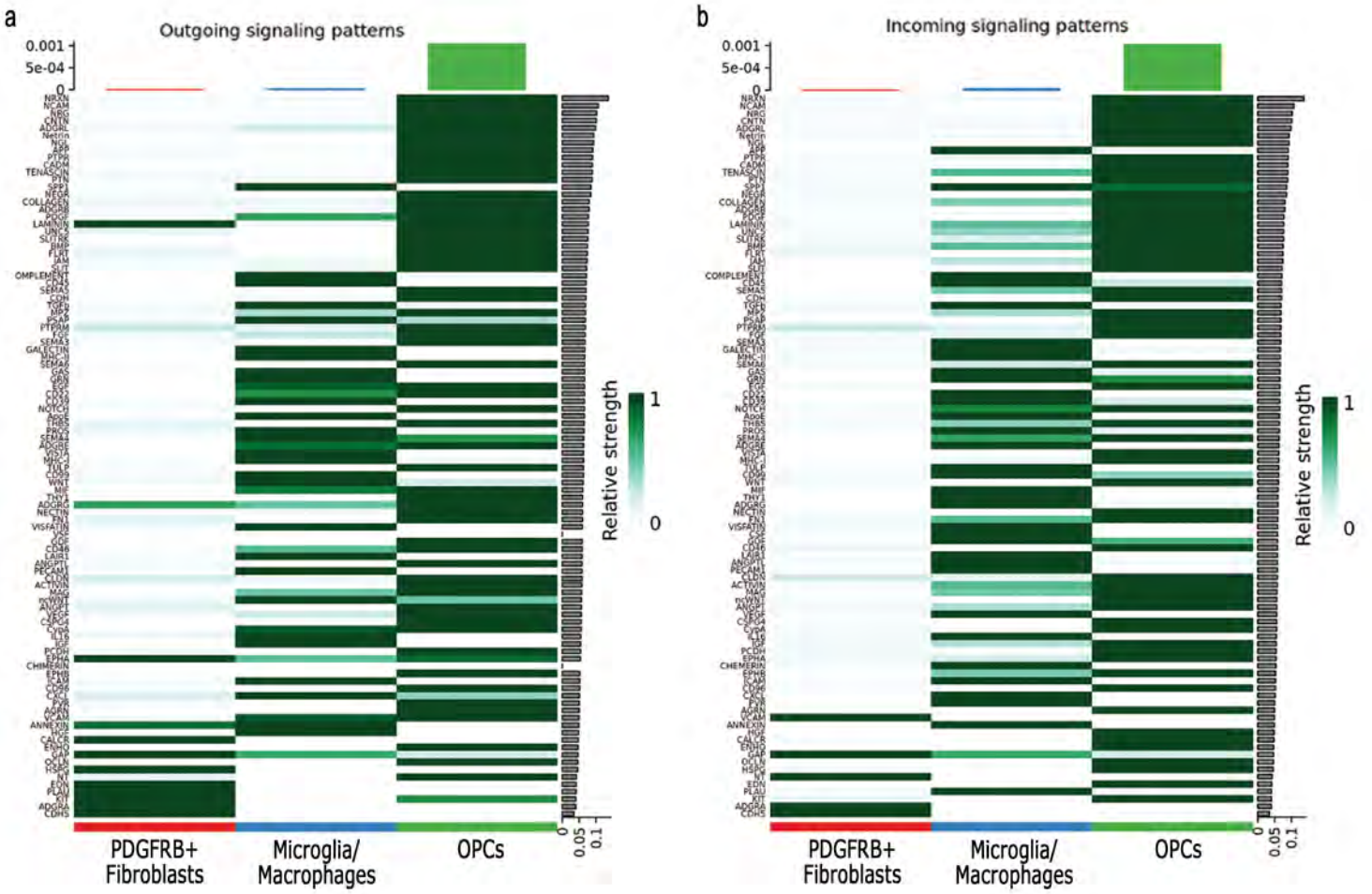
Microglia/Macrophages
OPCs
Pattern 1
Pattern 2
Pattern 3
Cell groups
Patterns
Signaling


c
Outgoing Pathways
LPC (CosMx)
NGF
SPP1
APOE
VTN
NCAM
17 (21.3%)
13 (16.3%)
50 (62.5%)
MS (Absinta 2021)
LAMININ
HSPG
CSF
PLAU
TGFB
Shared
FGF
PDGF
FN1
COLLAGEN
PTN
d
Incoming Pathways
LPC (CosMx)
NGF
EDN
VTN
GALANIN
PSAP
21 (24.7%)
13 (15.3%)
51 (60%)
MS (Absinta 2021)
SPP1
TGFB
WNT
HSPG
PLAU
Shared
FGF
PDGF
FN1
COLLAGEN
VEGF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Outgoing signaling patterns
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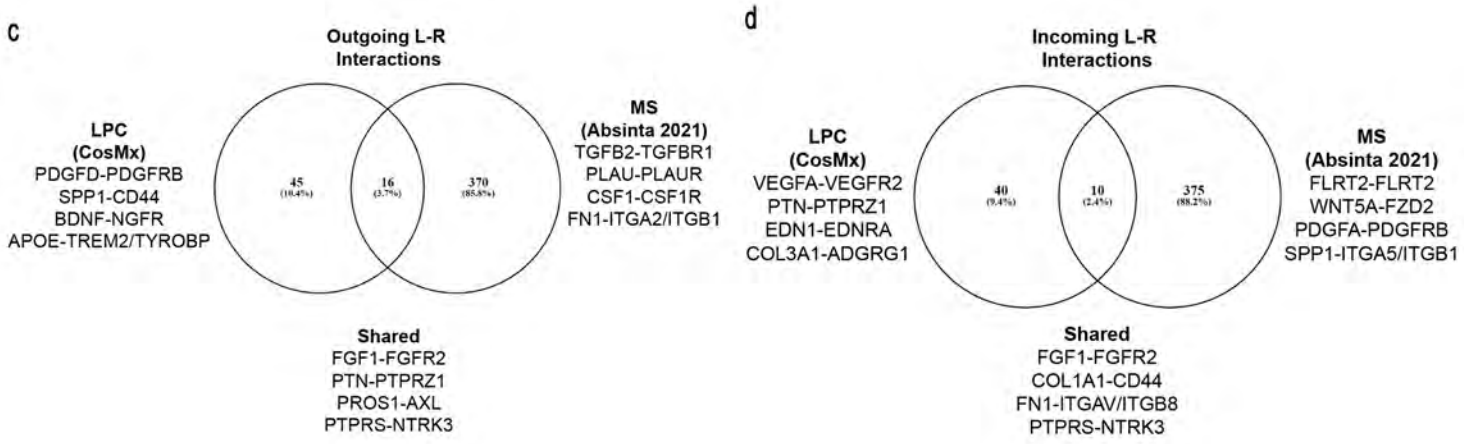
5e-04
0
Relative strength
1
0
PDGFRB+ Fibroblasts
Microglia/ Macrophages
OPCs
0
0.05
0.1
b
Incoming signaling patterns
0.001
5e-04
0
Relative strength
1
0
PDGFRB+ Fibroblasts
Microglia/ Macrophages
OPCs
0
0.05
0.1


c
Outgoing L-R Interactions
LPC (CosMx)
PDGFD-PDGFRB
SPP1-CD44
BDNF-NGFR
APOE-TREM2/TYROBP
45 (10.4%)
16 (3.7%)
370 (85.8%)
MS (Absinta 2021)
TGFB2-TGFBR1
PLAU-PLAUR
CSF1-CSF1R
FN1-ITGA2/ITGB1
Shared
FGF1-FGFR2
PTN-PTPRZ1
PROS1-AXL
PTPRS-NTRK3
d
Incoming L-R Interactions
LPC (CosMx)
VEGFA-VEGFR2
PTN-PTPRZ1
EDN1-EDNRA
COL3A1-ADGRG1
40 (9.4%)
10 (2.4%)
375 (88.2%)
MS (Absinta 2021)
FLRT2-FLRT2
WNT5A-FZD2
PDGFA-PDGFRB
SPP1-ITGA5/ITGB1
Shared
FGF1-FGFR2
COL1A1-CD44
FN1-ITGAV/ITGB8
PTPRS-NTRK3