FIG. 1

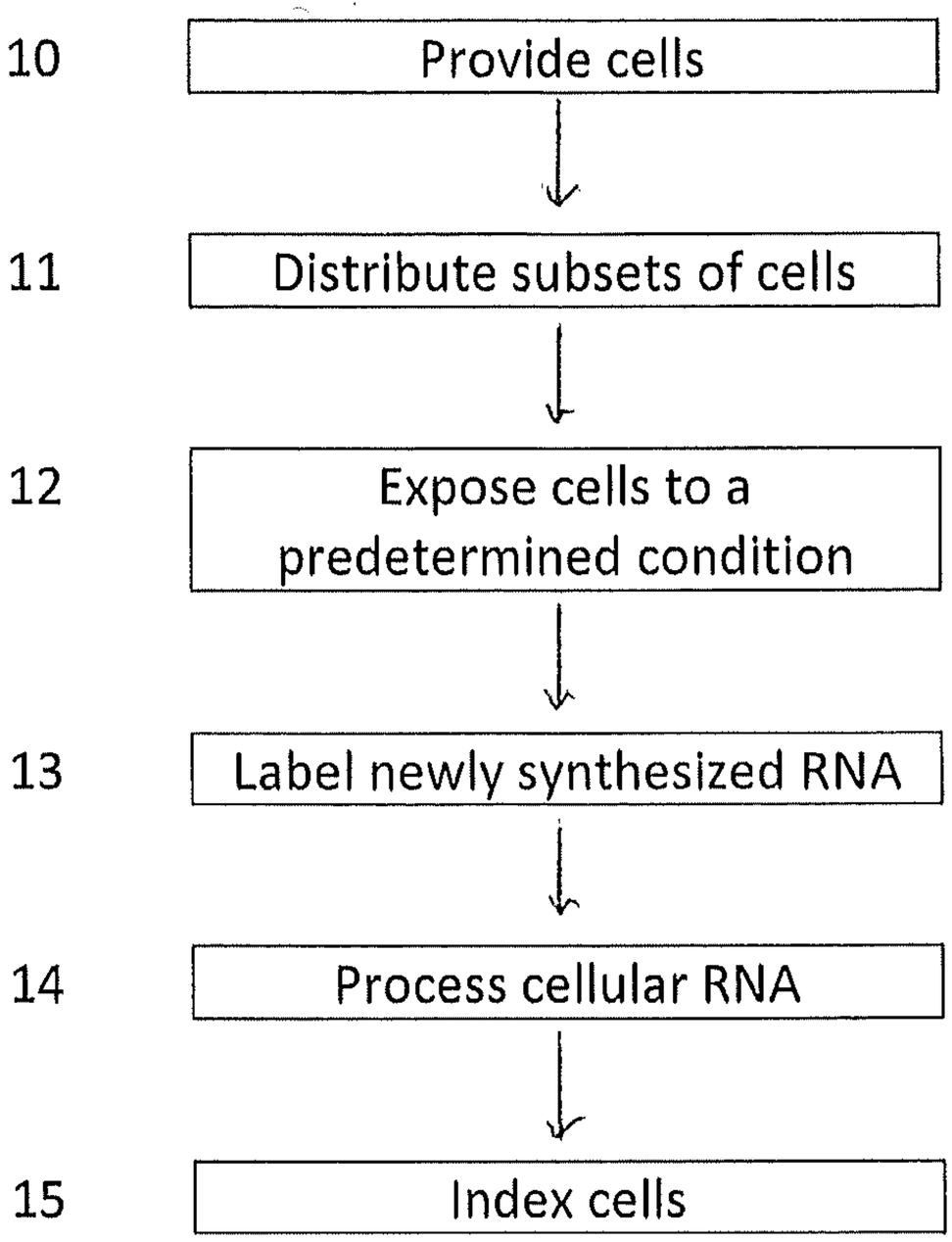

10
Provide cells
11
Distribute subsets of cells
12
Expose cells to a predetermined condition
13
Label newly synthesized RNA
14
Process cellular RNA
15
Index cells

FIG. 2

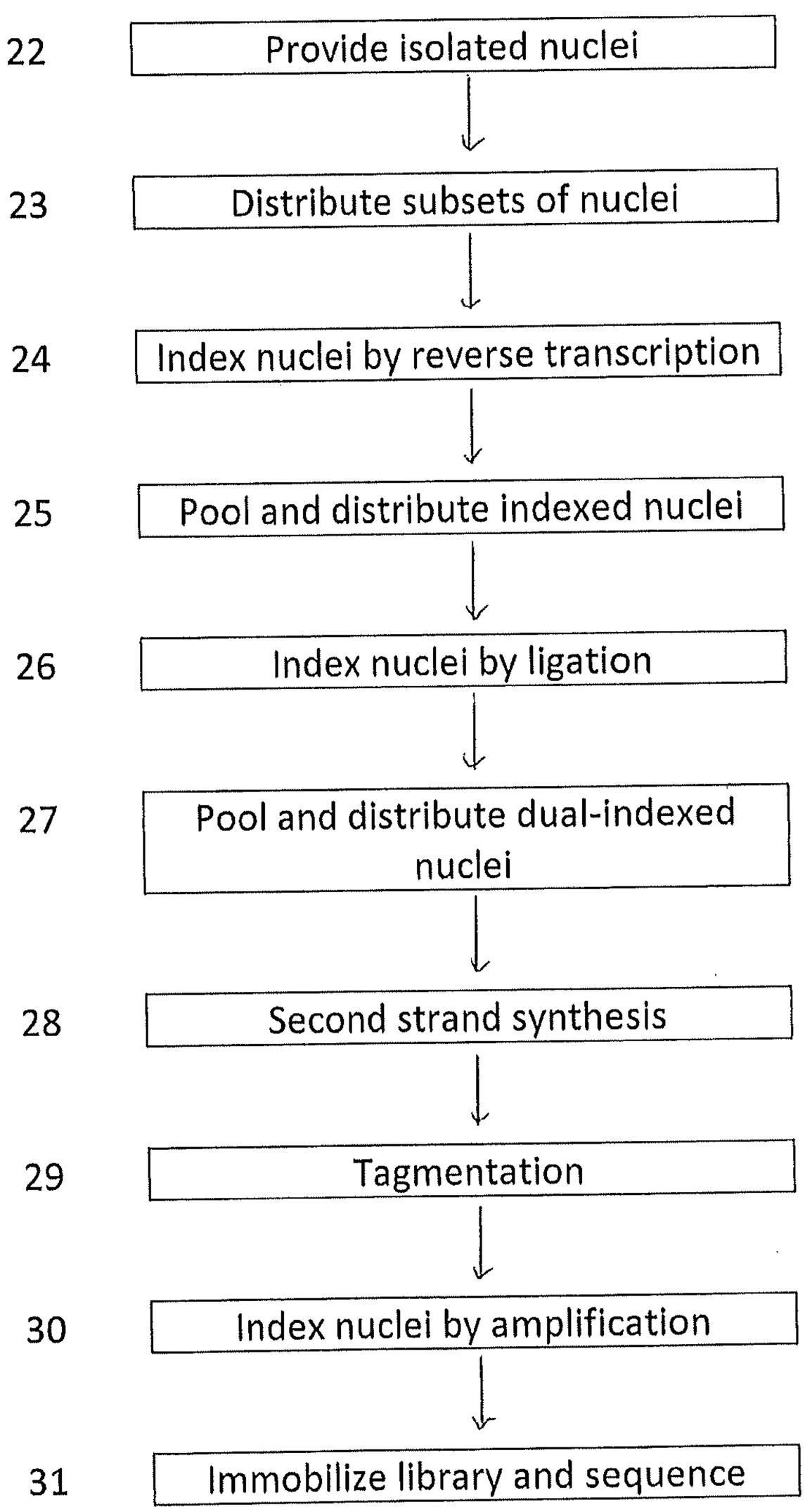
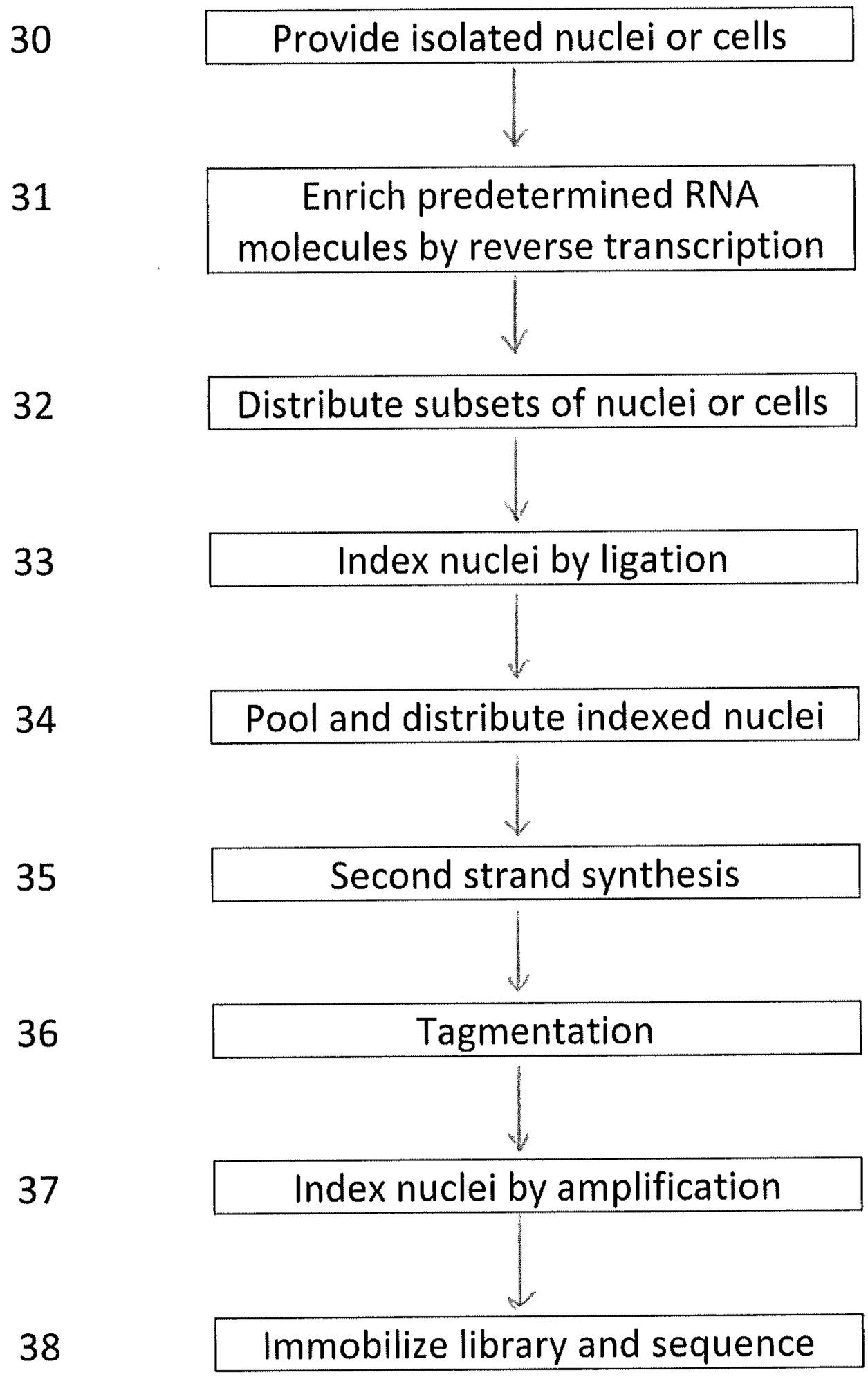
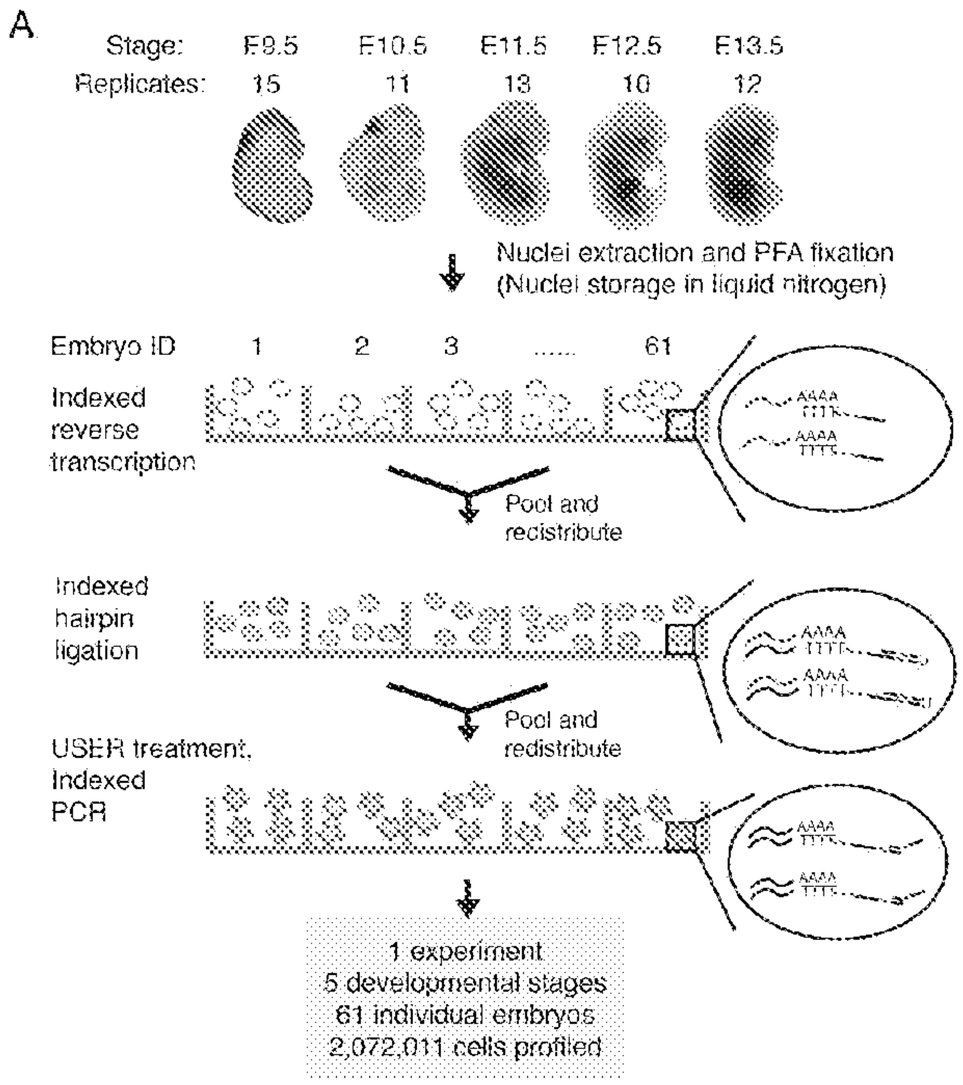
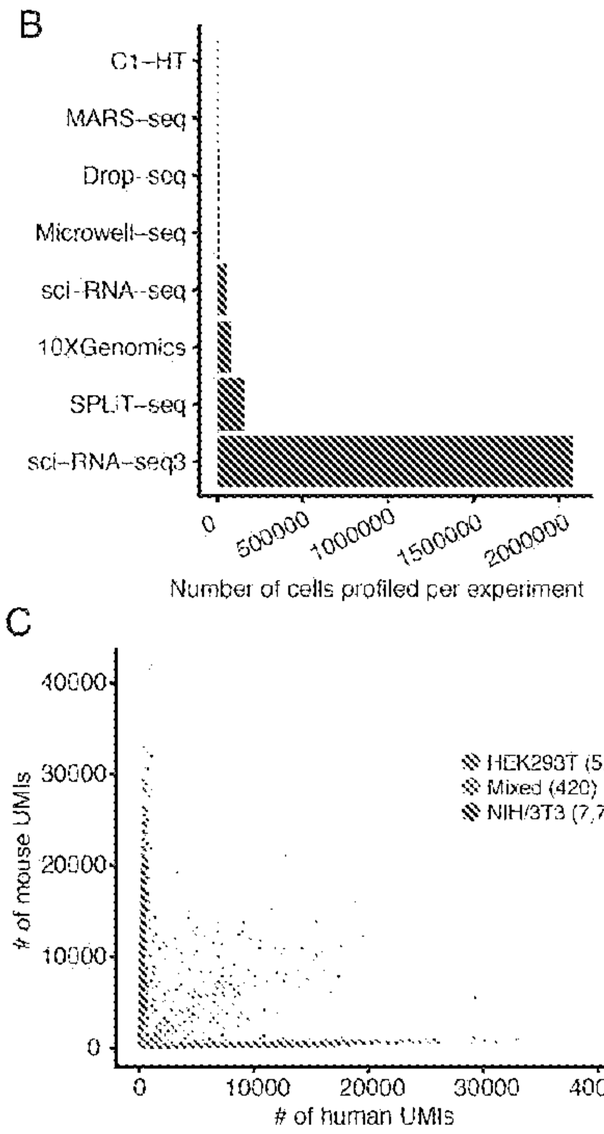
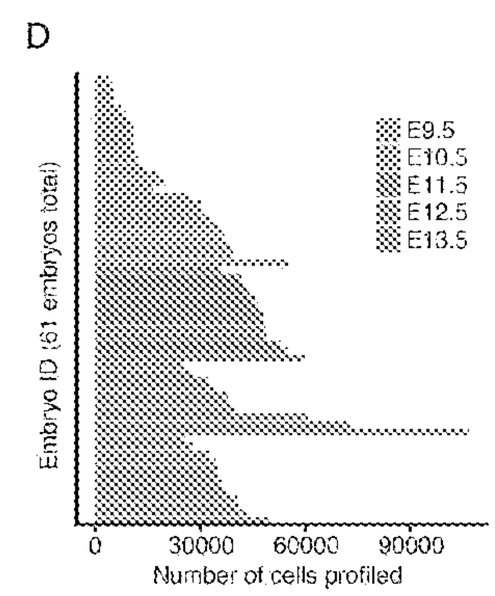

22
Provide isolated nuclei
23
Distribute subsets of nuclei
24
Index nuclei by reverse transcription
25
Pool and distribute indexed nuclei
26
Index nuclei by ligation
27
Pool and distribute dual-indexed nuclei
28
Second strand synthesis
29
Tagmentation
30
Index nuclei by amplification
31
Immobilize library and sequence

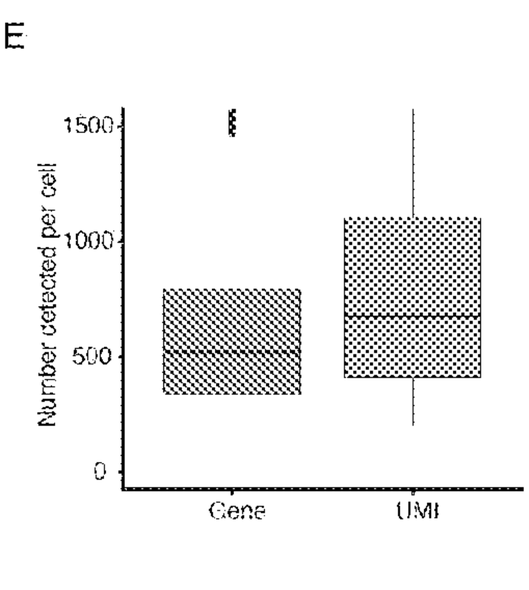
FIG. 3

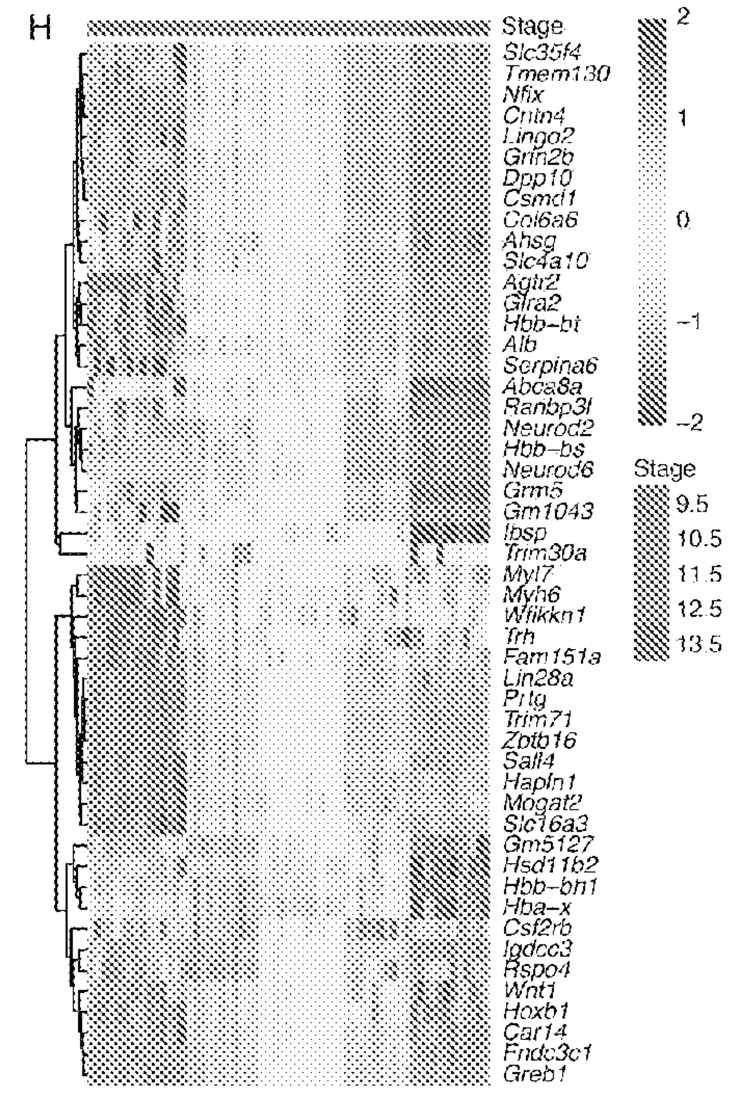

30
Provide isolated nuclei or cells
31
Enrich predetermined RNA molecules by reverse transcription
32
Distribute subsets of nuclei or cells
33
Index nuclei by ligation
34
Pool and distribute indexed nuclei
35
Second strand synthesis
36
Tagmentation
37
Index nuclei by amplification
38
Immobilize library and sequence

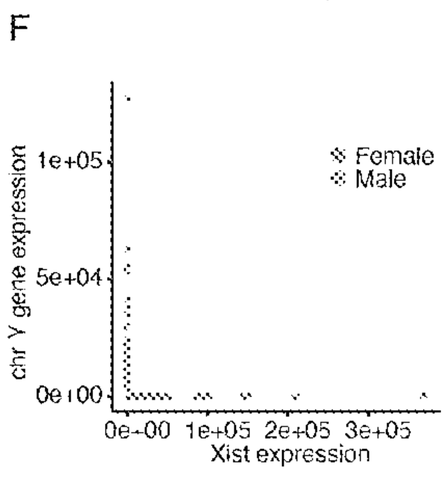

A
Stage: E9.5 E10.5 E11.5 E12.5 E13.5
Replicates: 15 11 13 10 12
Nuclei extraction and PFA fixation
(Nuclei storage in liquid nitrogen)
Embryo ID 1 2 3 61
Indexed reverse transcription
Pool and recistribute
Indexed hairpin ligation
Pool and redistribute
USER treatment, Indexed PCR
1 experiment
5 developmental stages
61 individual embryos
2,072,011 cells profiled


B
C1-HT
MARS-seq
Drop-seq
Microwell-seq
sci-RNA-seq
10XGenomics
SPLiT-seq
sci-RNA-seq3
0
500000
1000000
1500000
2000000
Number of cells profiled per experiment
C
40000
30000
20000
10000
0
# of mouse UMIs
HEK293T (5,284)
Mixed (420)
NIH/3T3 (7,705)
0
10000
20000
30000
40000
# of human UMIs


D
E9.5
E10.5
E11.5
E12.5
E13.5
Embryo ID (61 embryos total)
0
30000
60000
90000
Number of cells profiled


E
1500
1000
500
0
Number detected per cell
Gene
UMI


H
Stage
Slc35f4
Tmem130
Nfix
Cntn4
Lingo2
Grin2b
Dpp10
Csmd1
Col6a6
Ahsg
Slc4a10
Agtr2
Glra2
Hbb-bt
Alb
Serpina6
Abca8a
Ranbp3l
Neurod2
Hbb-bs
Neurod6
Grm5
Gm1043
Ibsp
Trim30a
Myl7
Myh6
Wfikkn1
Trh
Fam151a
Lin28a
Prlg
Trim71
Zbtb16
Sall4
Hapln1
Mogat2
Slc16a3
Gm5127
Hsd11b2
Hbb-bh1
Hba-x
Csf2rb
Igdcc3
Rspo4
Wnt1
Hoxb1
Car14
Fndc3c1
Greb1
2
1
0
−1
−2
Stage
9.5
10.5
11.5
12.5
13.5


F
Female
Male
chr Y gene expression
1e+05
5e+04
0e+00
0e+00 1e+05 2e+05 3e+05
Xist expression

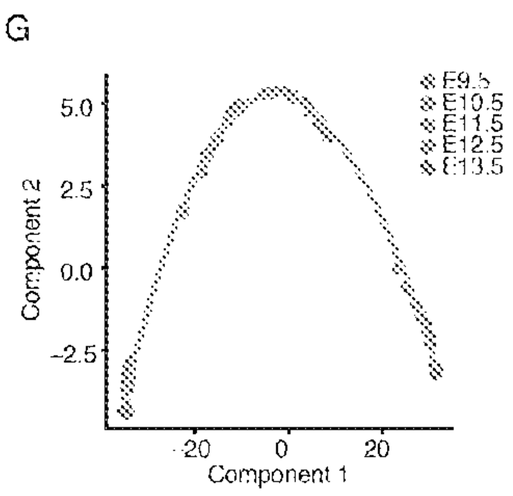

G
E9.5
E10.5
E11.5
E12.5
E13.5
Component 2
5.0
2.5
0.0
−2.5
−20
0
20
Component 1

FIG. 4

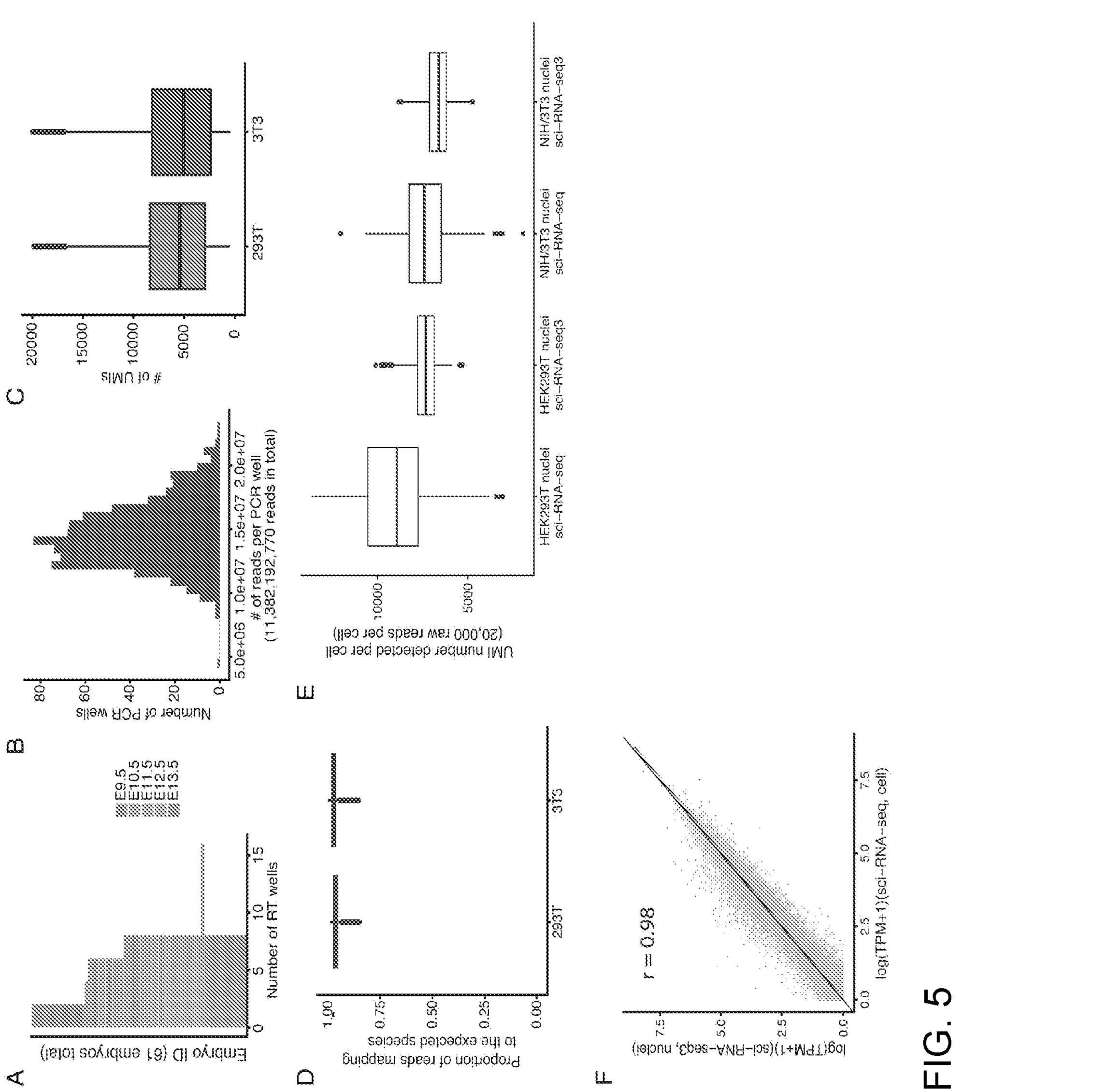

A
Embryo ID (61 embryos total)
Number of RT wells
B
E9.5
E10.5
E11.5
E12.5
E13.5
Number of PCR wells
# of reads per PCR well
(11,382,192,770 reads in total)
C
# of UMIs
293T
3T3
D
Proportion of reads mapping to the expected species
293T
3T3
E
UMI number detected per cell (20,000 raw reads per cell)
HEK293T nuclei sci-RNA-seq
F
r = 0.98
log(TPM+1)(sci-RNA-seq3, nuclei)
log(TPM+1)(sci-RNA-seq, cell)

FIG. 5

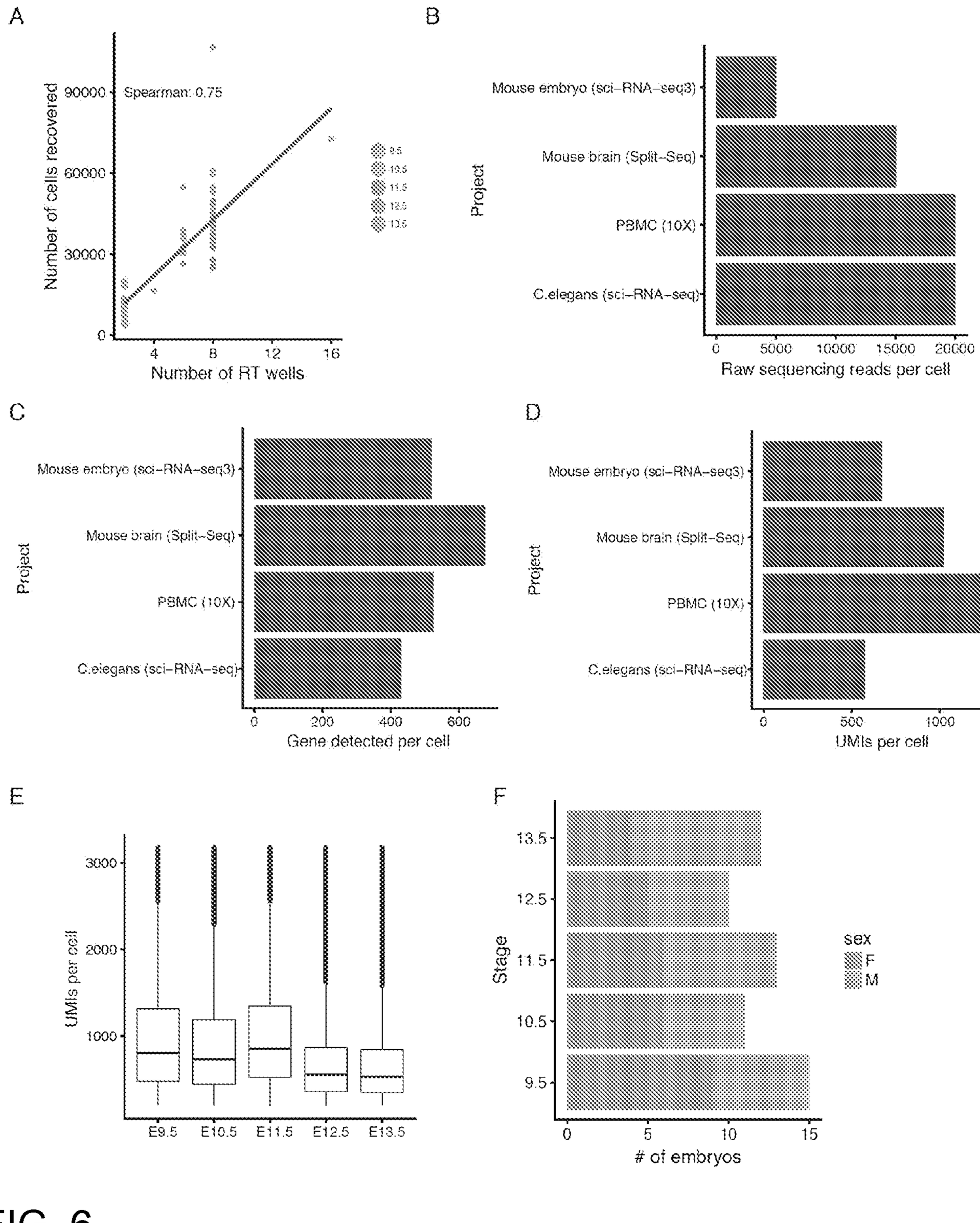

A
Number of cells recovered
90000
60000
30000
0
Spearman: 0.75
9.5
10.5
11.5
12.5
13.5
4
8
12
16
Number of RT wells
B
Project
Mouse embryo (sci-RNA-seq3)
Mouse brain (Split-Seq)
PBMC (10X)
C.elegans (sci-RNA-seq)
0
5000
10000
15000
20000
Raw sequencing reads per cell
C
Project
Mouse embryo (sci-RNA-seq3)
Mouse brain (Split-Seq)
PBMC (10X)
C.elegans (sci-RNA-seq)
0
200
400
600
Gene detected per cell
D
Project
Mouse embryo (sci-RNA-seq3)
Mouse brain (Split-Seq)
PBMC (10X)
C.elegans (sci-RNA-seq)
0
500
1000
UMIs per cell
E
UMIs per cell
3000
2000
1000
E9.5
E10.5
E11.5
E12.5
E13.5
F
Stage
13.5
12.5
11.5
10.5
9.5
0
5
10
15
# of embryos
sex
F
M

FIG. 6

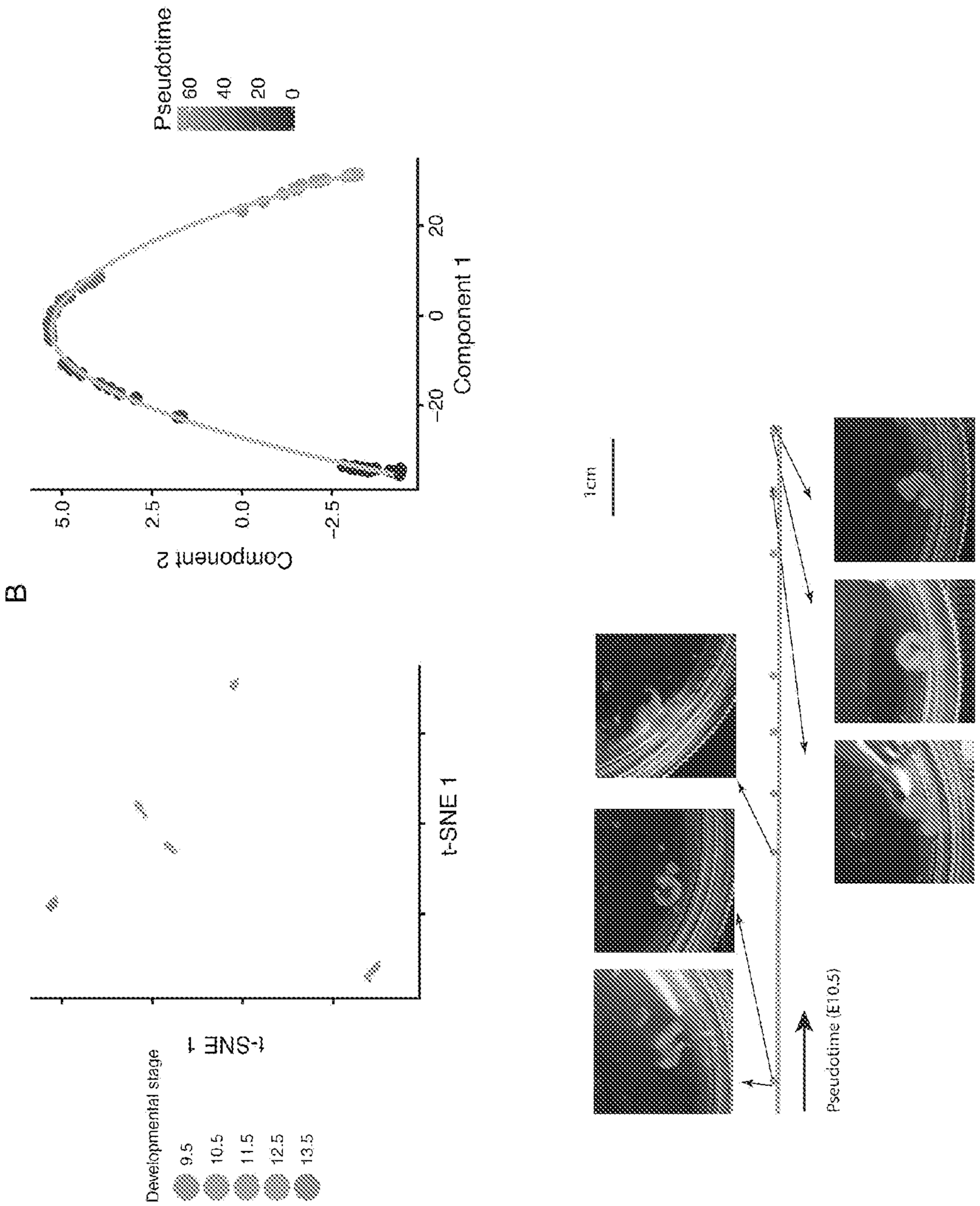

A
Developmental stage
9.5
10.5
11.5
12.5
13.5
B
t-SNE 1
t-SNE 1
C
Component 2
Component 1
Pseudotime
60
40
20
0
5.0
2.5
0.0
−2.5
−20
0
20
1cm
Pseudotime (E10.5)

FIG. 7

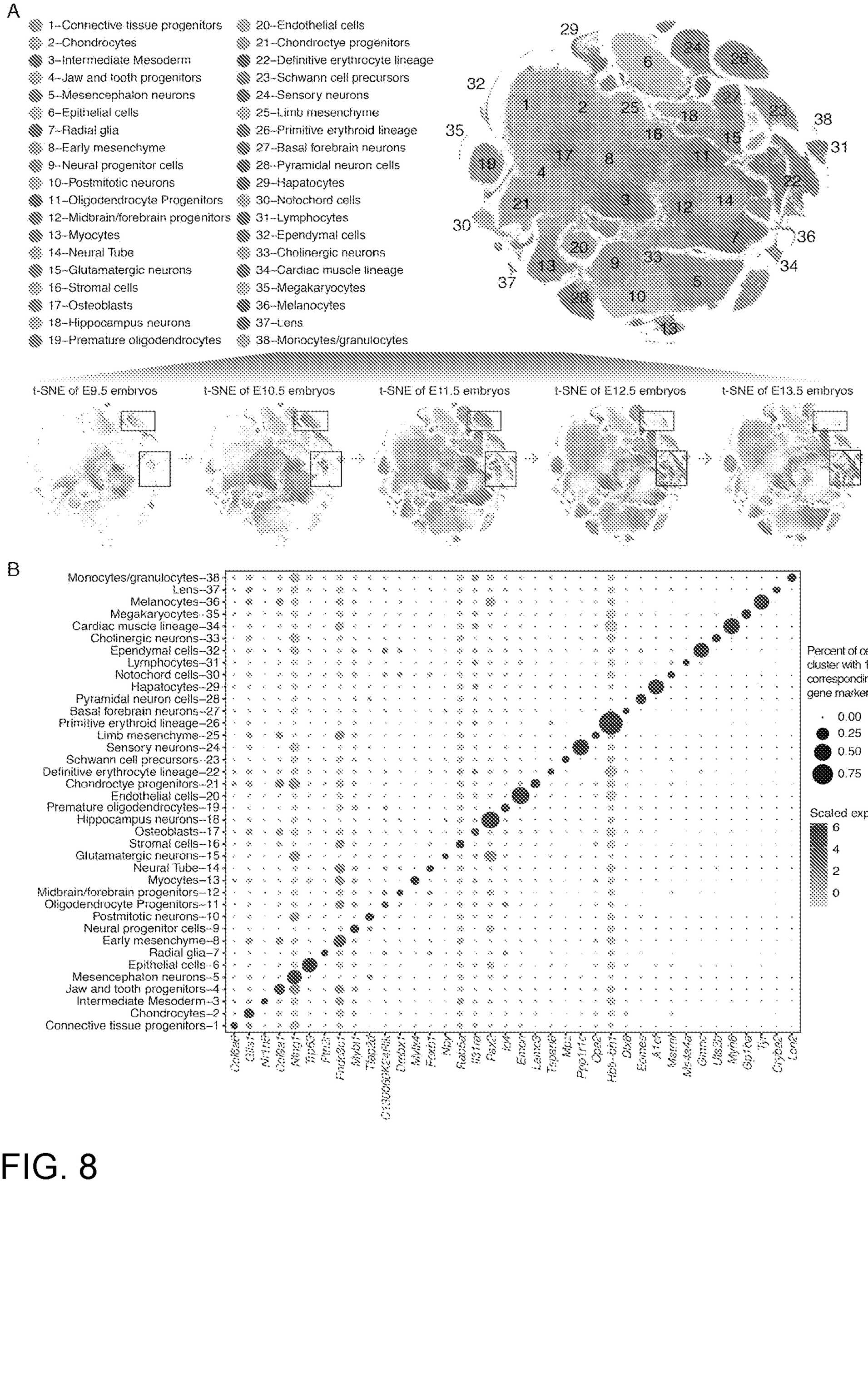
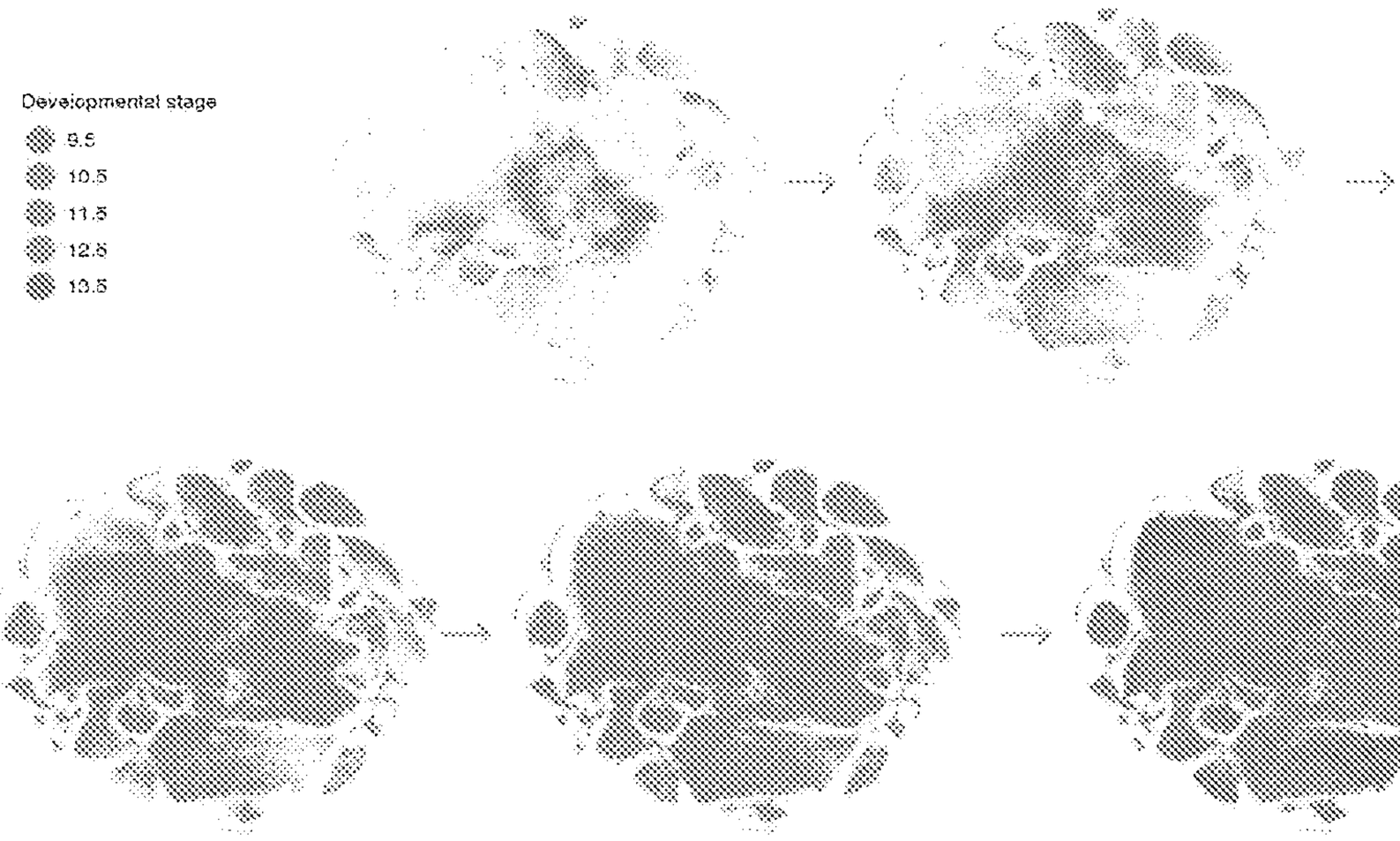
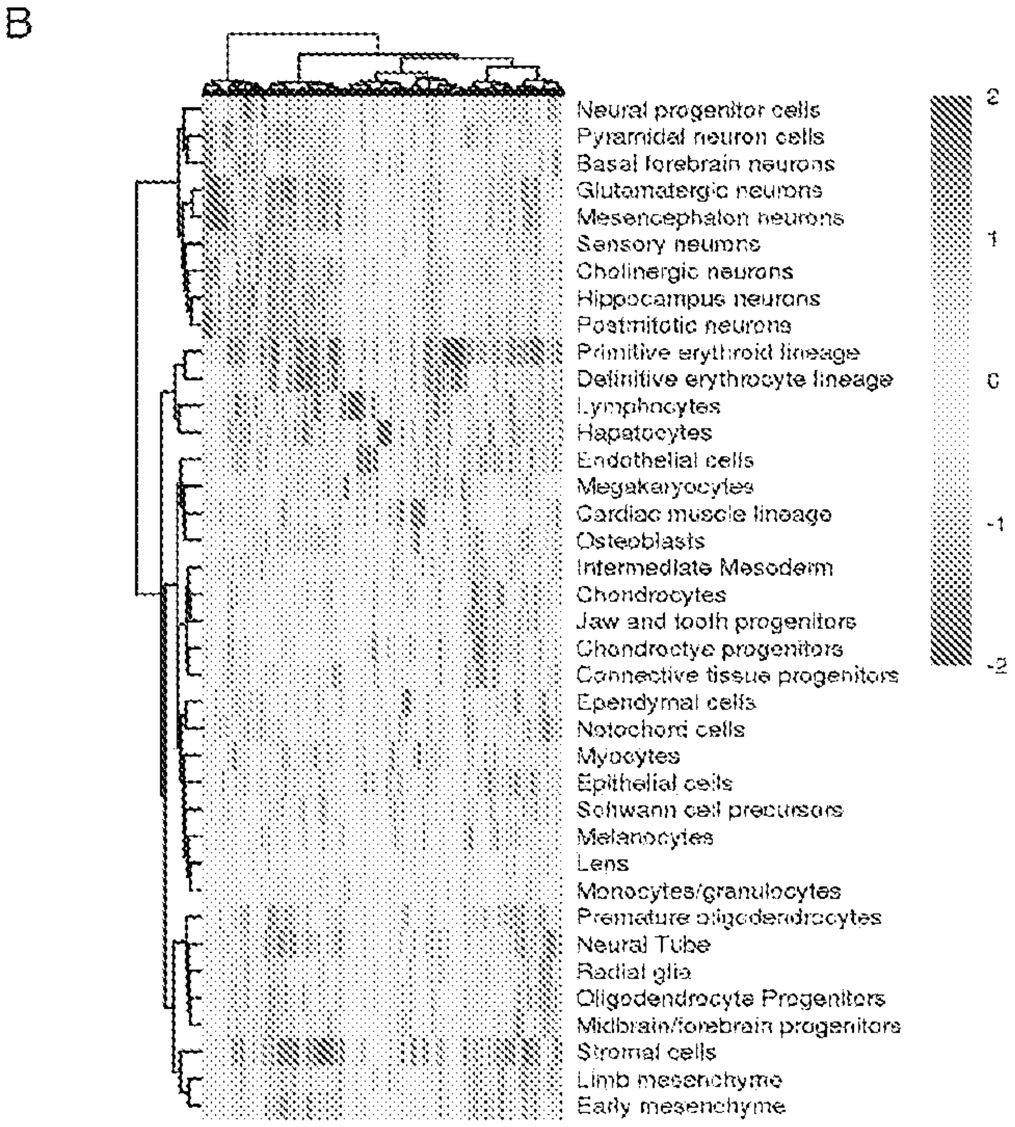

A
1–Connective tissue progenitors
2–Chondrocytes
3–Intermediate Mesoderm
4–Jaw and tooth progenitors
5–Mesencephalon neurons
6–Epithelial cells
7–Radial glia
8–Early mesenchyme
9–Neural progenitor cells
10–Postmitotic neurons
11–Oligodendrocyte Progenitors
12–Midbrain/forebrain progenitors
13–Myocytes
14–Neural Tube
15–Glutamatergic neurons
16–Stromal cells
17–Osteoblasts
18–Hippocampus neurons
19–Premature oligodendrocytes
20–Endothelial cells
21–Chondroctye progenitors
22–Definitive erythrocyte lineage
23–Schwann cell precursors
24–Sensory neurons
25–Limb mesenchyme
26–Primitive erythroid lineage
27–Basal forebrain neurons
28–Pyramidal neuron cells
29–Hapatocytes
30–Notochord cells
31–Lymphocytes
32–Ependymal cells
33–Cholinergic neurons
34–Cardiac muscle lineage
35–Megakaryocytes
36–Melanocytes
37–Lens
38–Monocytes/granulocytes
t-SNE of E9.5 embryos
t-SNE of E10.5 embryos
t-SNE of E11.5 embryos
t-SNE of E12.5 embryos
t-SNE of E13.5 embryos
B
Monocytes/granulocytes–38
Lens–37
Melanocytes–36
Megakaryocytes–35
Cardiac muscle lineage–34
Cholinergic neurons–33
Ependymal cells–32
Lymphocytes–31
Notochord cells–30
Hapatocytes–29
Pyramidal neuron cells–28
Basal forebrain neurons–27
Primitive erythroid lineage–26
Limb mesenchyme–25
Sensory neurons–24
Schwann cell precursors–23
Definitive erythrocyte lineage–22
Chondroctye progenitors–21
Endothelial cells–20
Premature oligodendrocytes–19
Hippocampus neurons–18
Osteoblasts–17
Stromal cells–16
Glutamatergic neurons–15
Neural Tube–14
Myocytes–13
Midbrain/forebrain progenitors–12
Oligodendrocyte Progenitors–11
Postmitotic neurons–10
Neural progenitor cells–9
Early mesenchyme–8
Radial glia–7
Epithelial cells–6
Mesencephalon neurons–5
Jaw and tooth progenitors–4
Intermediate Mesoderm–3
Chondrocytes–2
Connective tissue progenitors–1
Percent of cells in cluster with 1+ read corresponding to gene marker
0.00
0.25
0.50
0.75
Scaled expression
6
4
2
0

FIG. 8

A


Developmental stage
9.5
10.5
11.5
12.5
13.5

B


Neural progenitor cells
Pyramidal neuron cells
Basal forebrain neurons
Glutamatergic neurons
Mesencephalon neurons
Sensory neurons
Cholinergic neurons
Hippocampus neurons
Postmitotic neurons
Primitive erythroid lineage
Definitive erythrocyte lineage
Lymphocytes
Hepatocytes
Endothelial cells
Megakaryocytes
Cardiac muscle lineage
Osteoblasts
Intermediate Mesoderm
Chondrocytes
Jaw and tooth progenitors
Chondrocyte progenitors
Connective tissue progenitors
Ependymal cells
Notochord cells
Myocytes
Epithelial cells
Schwann cell precursors
Melanocytes
Lens
Monocytes/granulocytes
Premature oligodendrocytes
Neural Tube
Radial glia
Oligodendrocyte Progenitors
Midbrain/forebrain progenitors
Stromal cells
Limb mesenchyme
Early mesenchyme
2
1
0
-1
-2

C

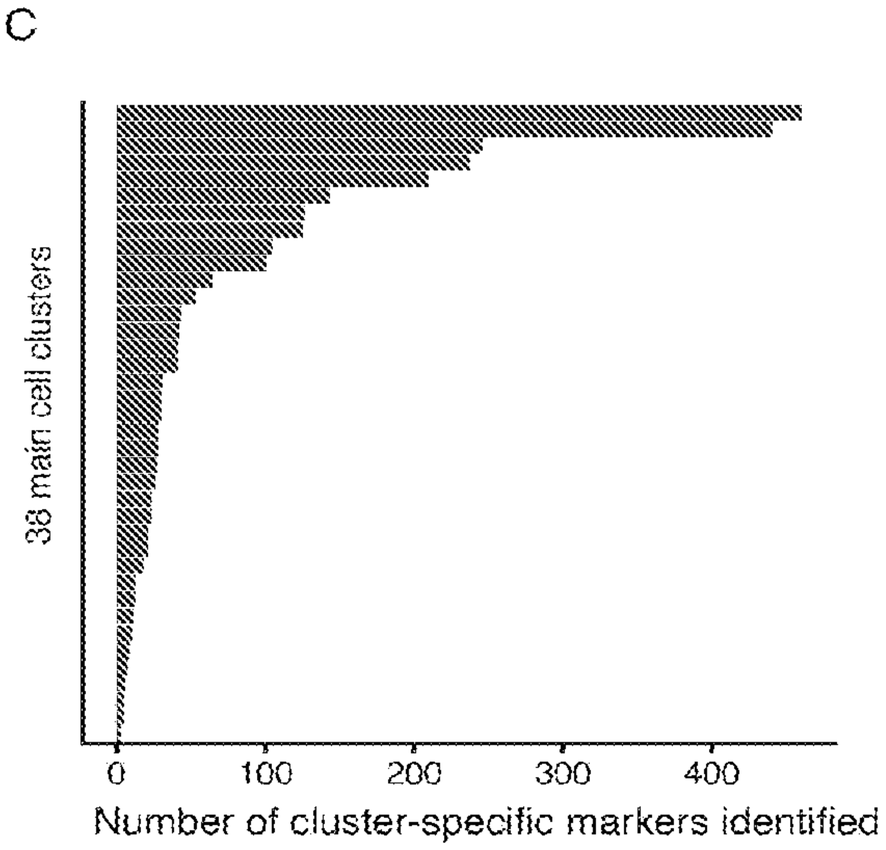

38 main cell clusters
0
100
200
300
400
Number of cluster-specific markers identified

FIG. 9

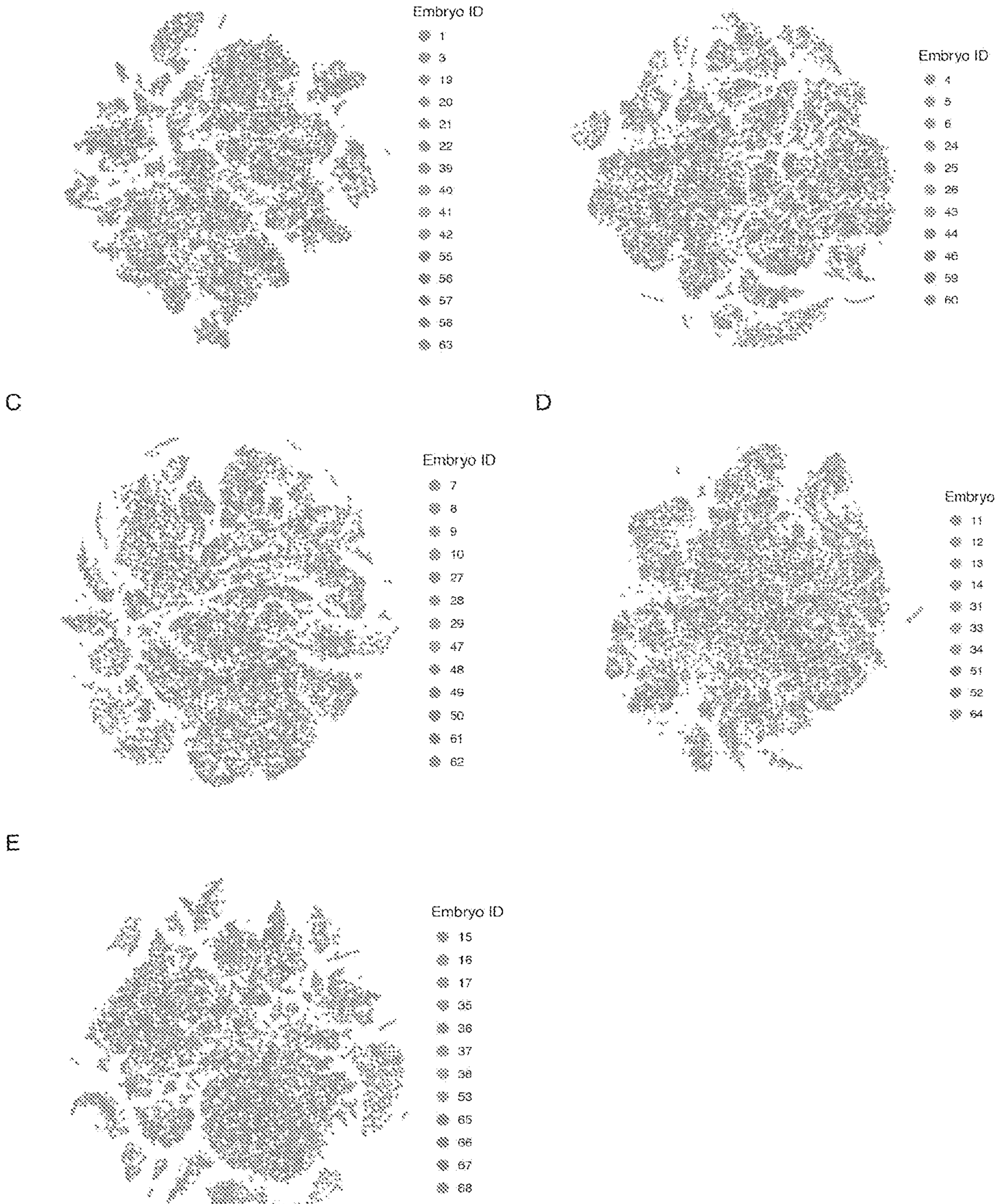
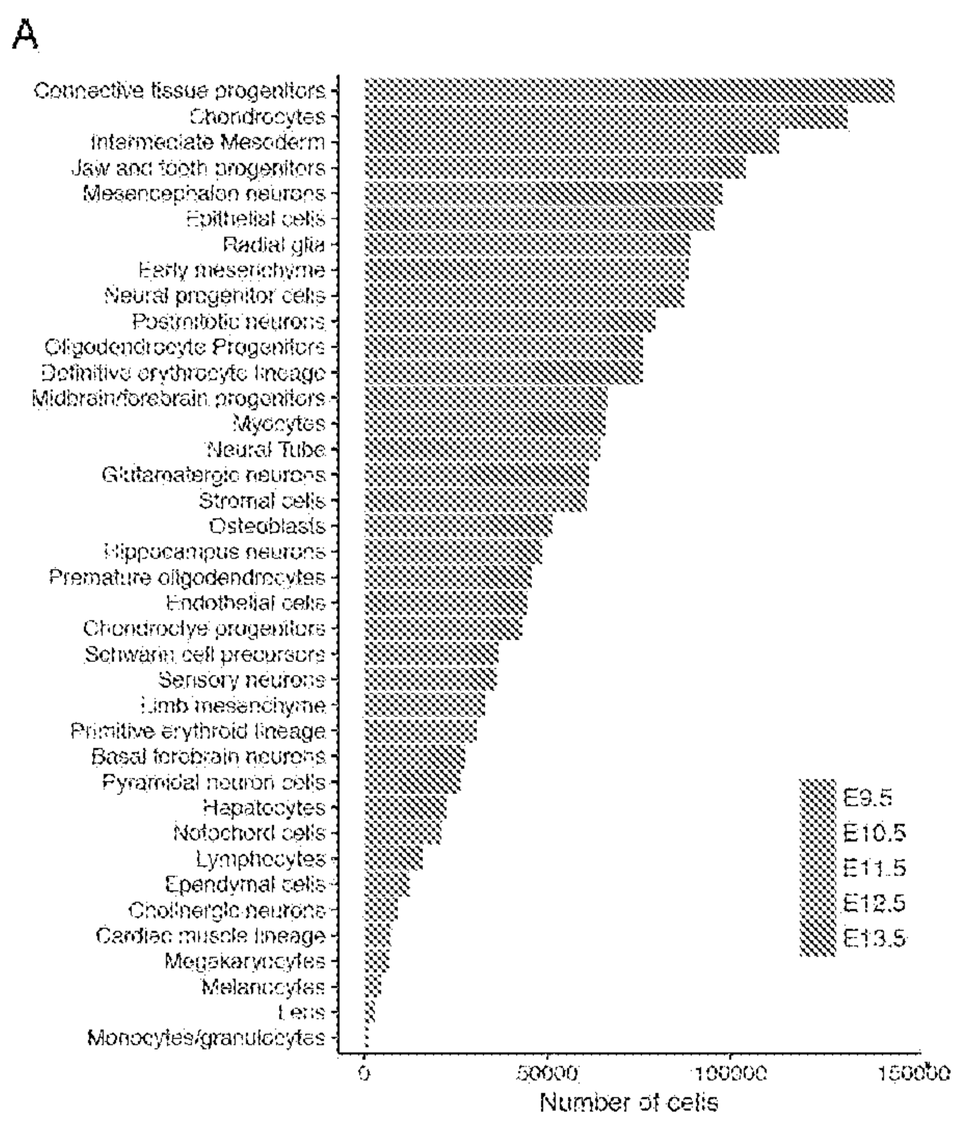
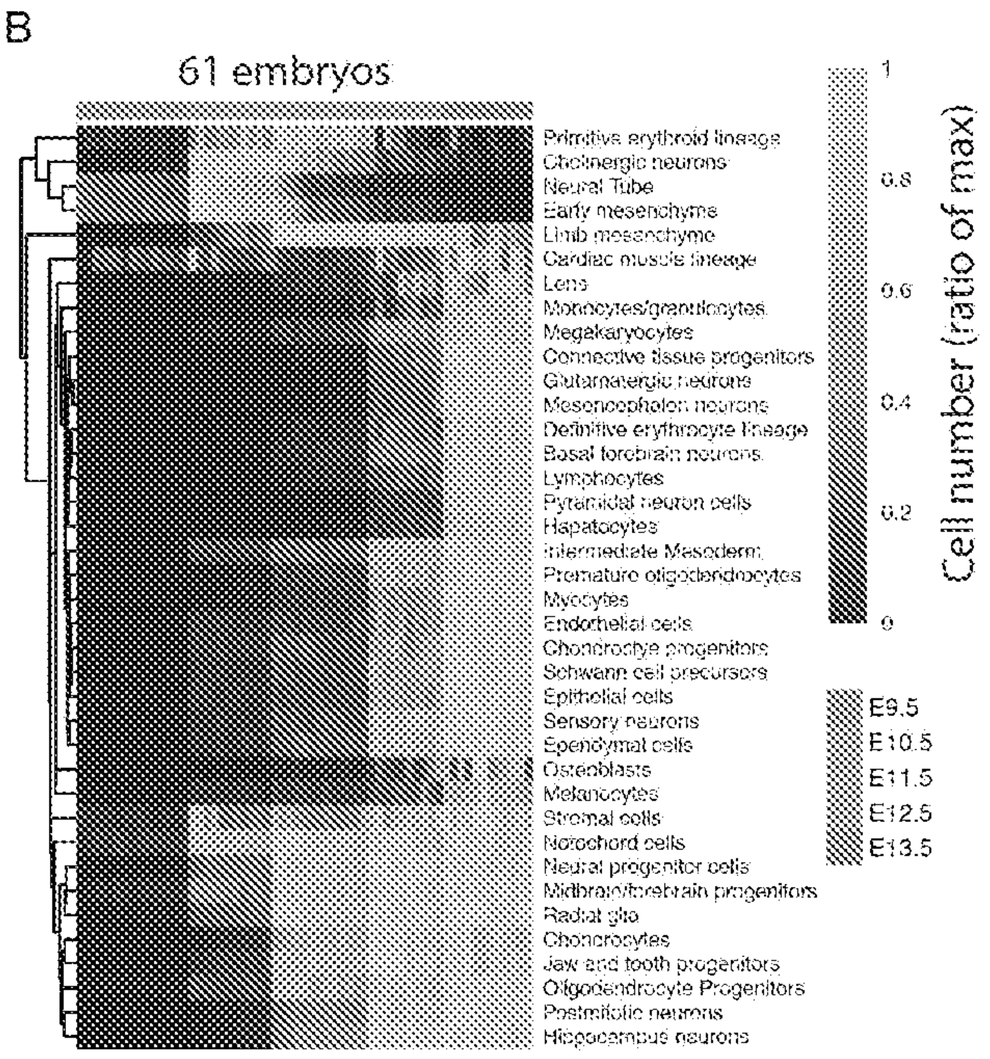

A
Embryo ID
1
3
13
20
21
22
39
40
41
42
55
56
57
58
63
B
Embryo ID
4
5
6
24
25
26
43
44
46
59
60
C
Embryo ID
7
8
9
10
27
28
29
47
48
49
50
61
62
D
Embryo ID
11
12
13
14
31
33
34
51
52
64
E
Embryo ID
15
16
17
35
36
37
38
53
65
66
67
68

FIG. 10


A
Connective tissue progenitors
Chondrocytes
Intermediate Mesoderm
Jaw and tooth progenitors
Mesencephalon neurons
Epithelial cells
Radial glia
Early mesenchyme
Neural progenitor cells
Postmitotic neurons
Oligodendrocyte Progenitors
Definitive erythrocyte lineage
Midbrain/forebrain progenitors
Myocytes
Neural Tube
Glutamatergic neurons
Stromal cells
Osteoblasts
Hippocampus neurons
Premature oligodendrocytes
Endothelial cells
Chondrocyte progenitors
Schwann cell precursors
Sensory neurons
Limb mesenchyme
Primitive erythroid lineage
Basal forebrain neurons
Pyramidal neuron cells
Hepatocytes
Notochord cells
Lymphocytes
Ependymal cells
Cholinergic neurons
Cardiac muscle lineage
Megakaryocytes
Melanocytes
Lens
Monocytes/granulocytes
E9.5
E10.5
E11.5
E12.5
E13.5
0
50000
100000
150000
Number of cells


B
61 embryos
Primitive erythroid lineage
Cholinergic neurons
Neural Tube
Early mesenchyme
Limb mesenchyme
Cardiac muscle lineage
Lens
Monocytes/granulocytes
Megakaryocytes
Connective tissue progenitors
Glutamatergic neurons
Mesencephalon neurons
Definitive erythrocyte lineage
Basal forebrain neurons
Lymphocytes
Pyramidal neuron cells
Hepatocytes
Intermediate Mesoderm
Premature oligodendrocytes
Myocytes
Endothelial cells
Chondrocyte progenitors
Schwann cell precursors
Epithelial cells
Sensory neurons
Ependymal cells
Osteoblasts
Melanocytes
Stromal cells
Notochord cells
Neural progenitor cells
Midbrain/forebrain progenitors
Radial glia
Chondrocytes
Jaw and tooth progenitors
Oligodendrocyte Progenitors
Postmitotic neurons
Hippocampus neurons
1
0.8
0.6
0.4
0.2
0
Cell number (ratio of max)
E9.5
E10.5
E11.5
E12.5
E13.5

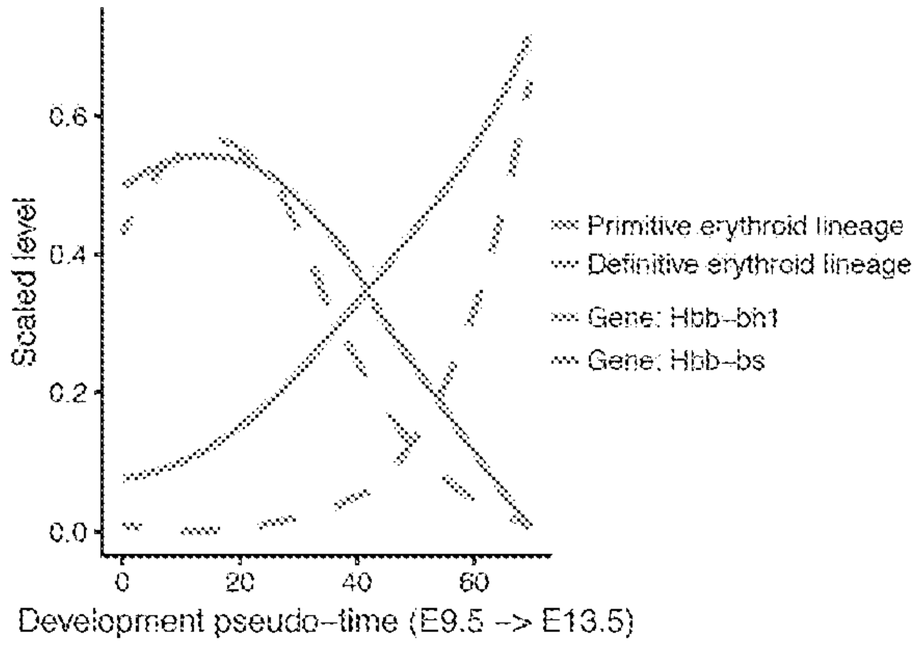

C
0.6
0.4
0.2
0.0
Scaled level
Primitive erythroid lineage
Definitive erythroid lineage
Gene: Hbb-bh1
Gene: Hbb-bs
0
20
40
60
Development pseudo-time (E9.5 -> E13.5)

FIG. 11

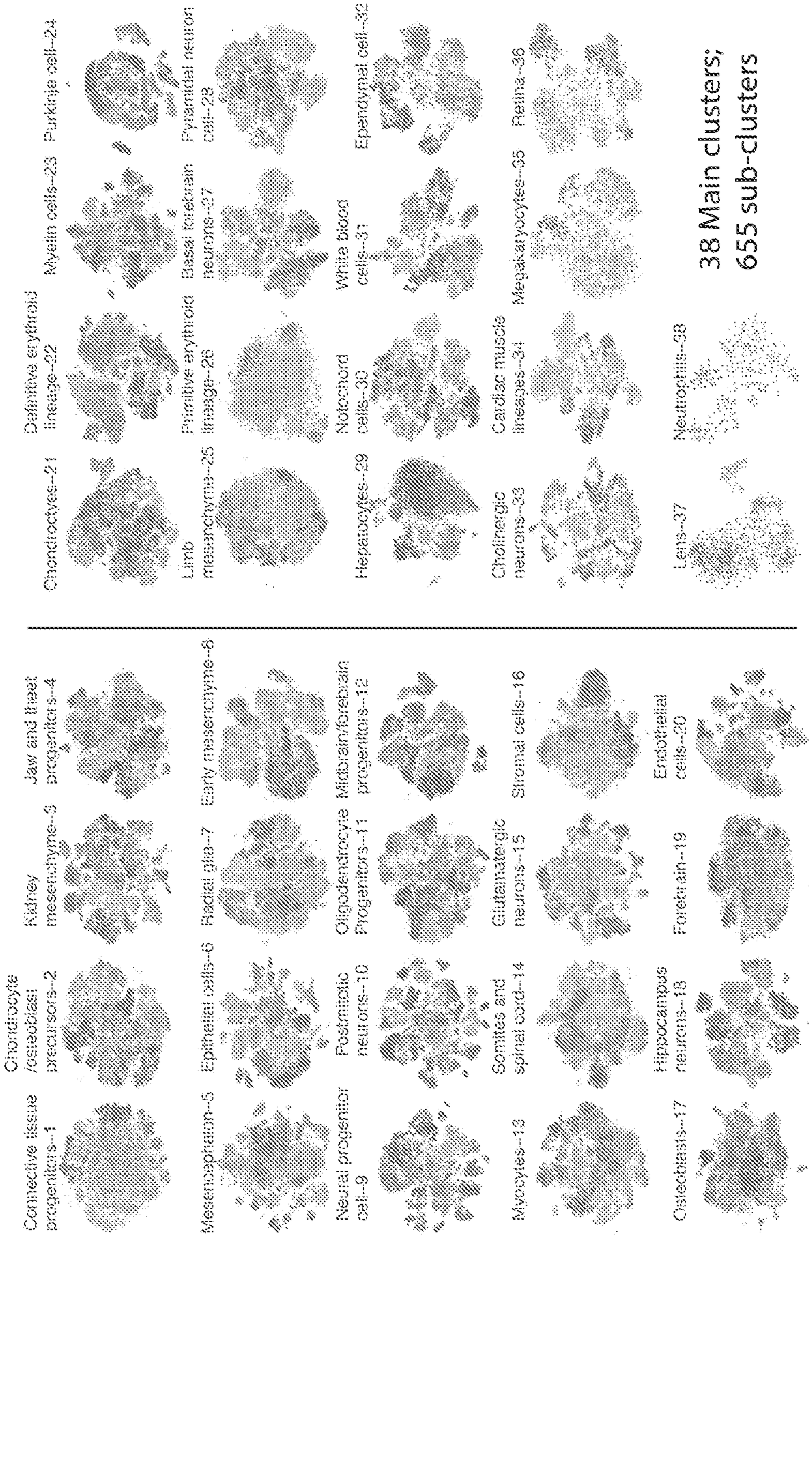

Chondrocytes--21
Definitive erythroid lineage--22
Myelin cells--23
Purkinje cell--24
Limb mesenchyme--25
Primitive erythroid lineage--26
Basal forebrain neurons--27
Pyramidal neuron cell--28
Hepatocytes--29
Notochord cells--30
White blood cells--31
Ependymal cell--32
Cholinergic neurons--33
Cardiac muscle lineages--34
Megakaryocytes--35
Retina--36
Lens--37
Neutrophils--38
38 Main clusters;
655 sub-clusters
Kidney mesenchyme--3
Jaw and tract progenitors--4
6
Radial glia--7
Early mesenchyme--8
Oligodendrocyte Progenitors--11
Midbrain/forebrain progenitors--12
Glutamatergic neurons--15
Stromal cells--16
Endothelial cells--20
Forebrain--19
Chondrocyte /osteoblast precursors--2
Connective tissue progenitors--1
Mesencephalon--5
Epithelial cells--
Neural progenitor cell--9
Postmitotic neurons--10
Somites and spinal cord--14
Myocytes--13
Hippocampus neurons--18
Osteoclasts--17

FIG. 12

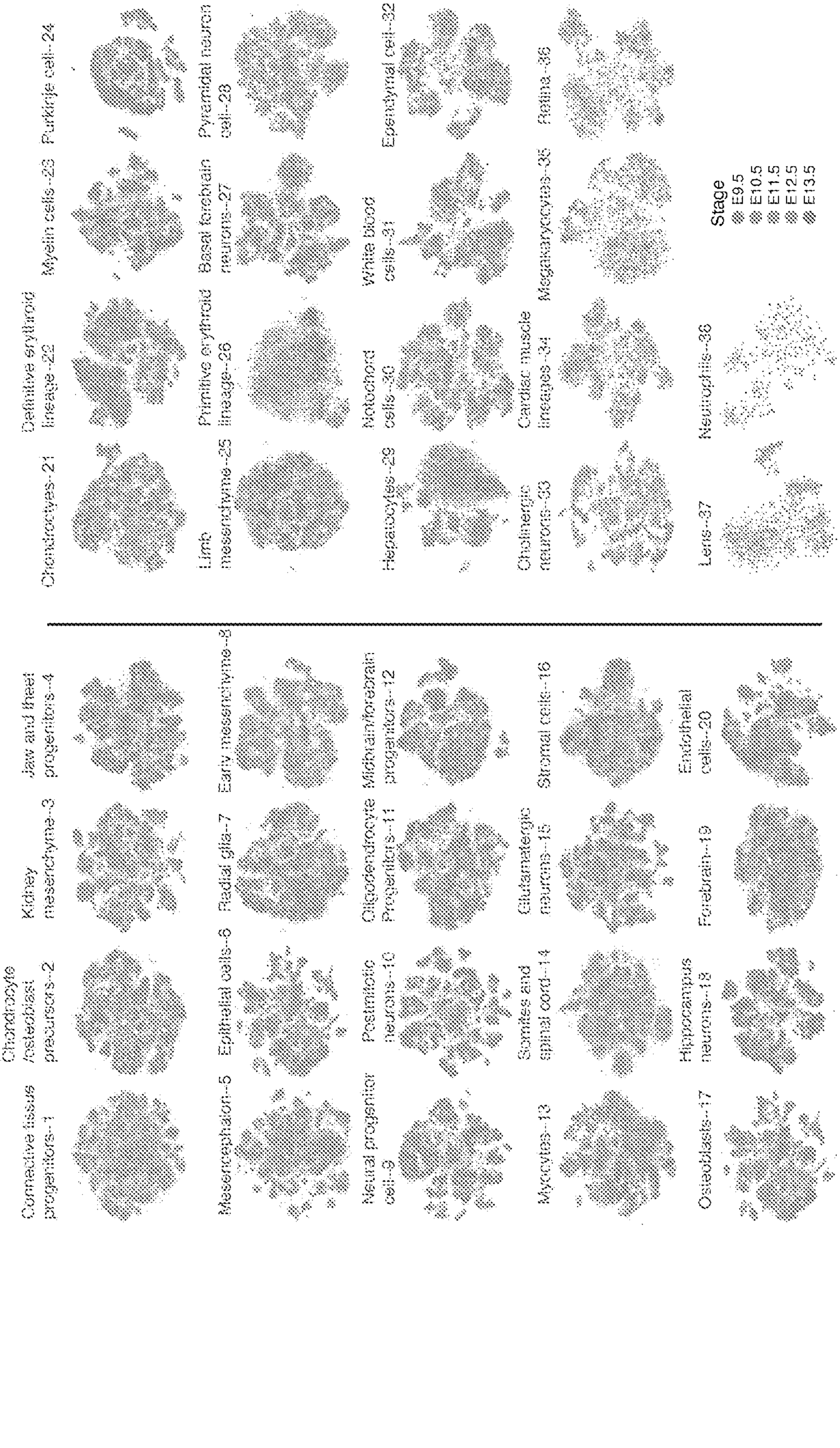
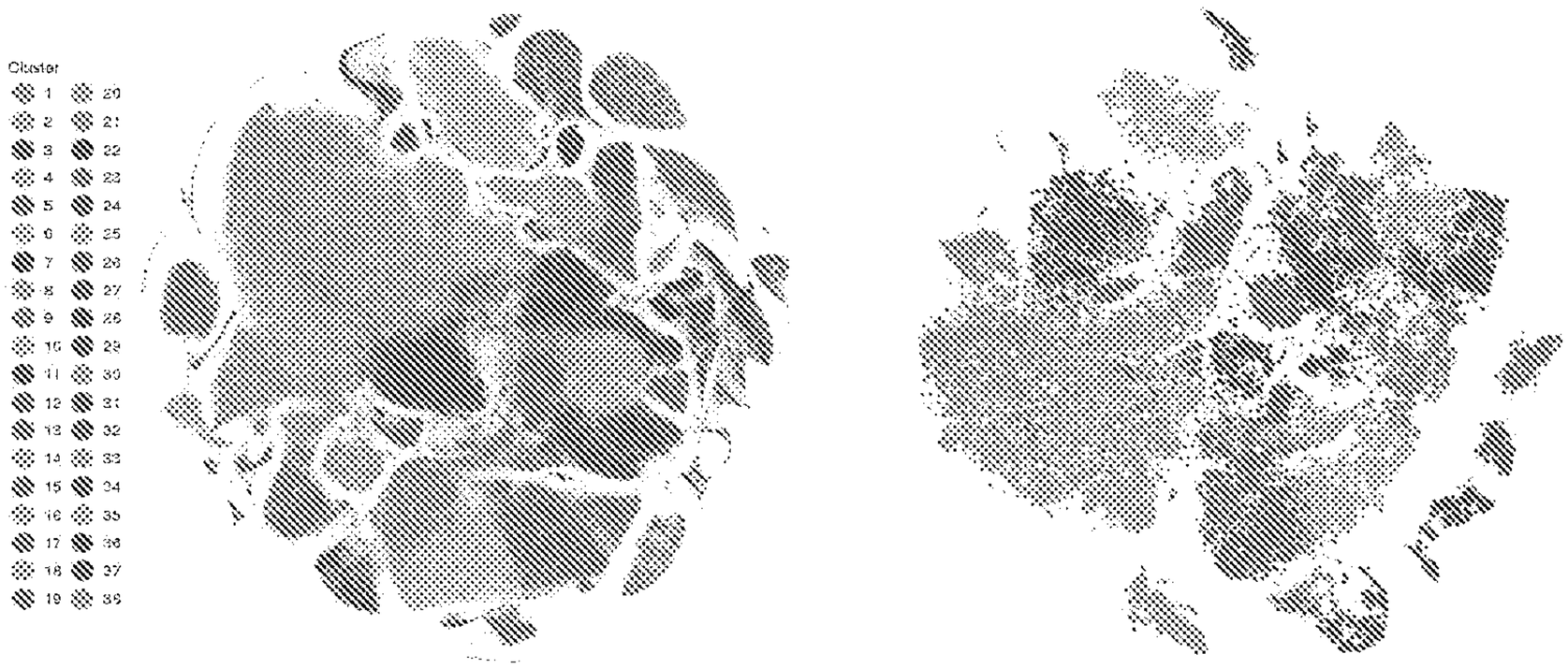

Definitive erythroid lineage--22
Myelin cells--23
Purkinje cell--24
Chondrocytes--21
Limb mesenchyme--25
Primitive erythroid lineage--26
Basal forebrain neurons--27
Pyramidal neuron cell--28
Hepatocytes--29
Notochord cells--30
White blood cells--31
Ependymal cell--32
Cholinergic neurons--33
Cardiac muscle lineages--34
Megakaryocytes--35
Retina--36
Lens--37
Neutrophils--36
Stage
E9.5
E10.5
E11.5
E12.5
E13.5
Kidney mesenchyme--3
Jaw and theet progenitors--4
Radial glia--7
Early mesenchyme--8
Oligodendrocyte Progenitors--11
Midbrain/forebrain progenitors--12
Glutamatergic neurons--15
Stromal cells--16
Forebrain--19
Endothelial cells--20
Connective tissue progenitors--1
Chondrocyte /osteoblast precursors--2
Mesencephalon--5
Epithelial cells--6
Neural progenitor cell--9
Postmitotic neurons--10
Somites and spinal cord--14
Myocytes--13
Hippocampus neurons--18
Osteoblasts--17

FIG. 13

A


Cluster
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38

B


Cluster
1
10
11
12
13
14
15
16
2
3
4
5
6
7
8
9

C

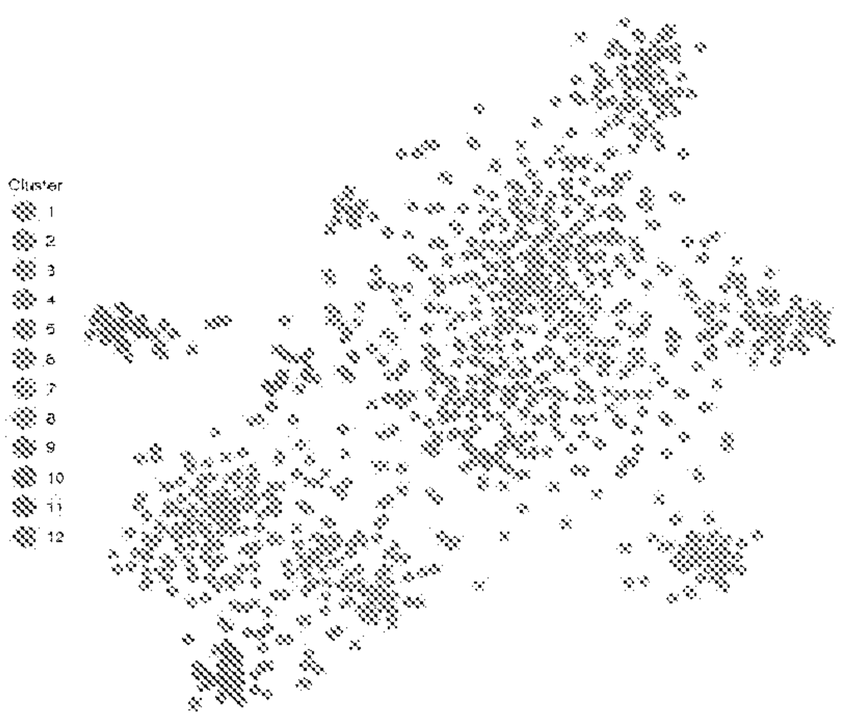

Cluster
1
2
3
4
5
6
7
8
9
10
11
12

FIG. 14

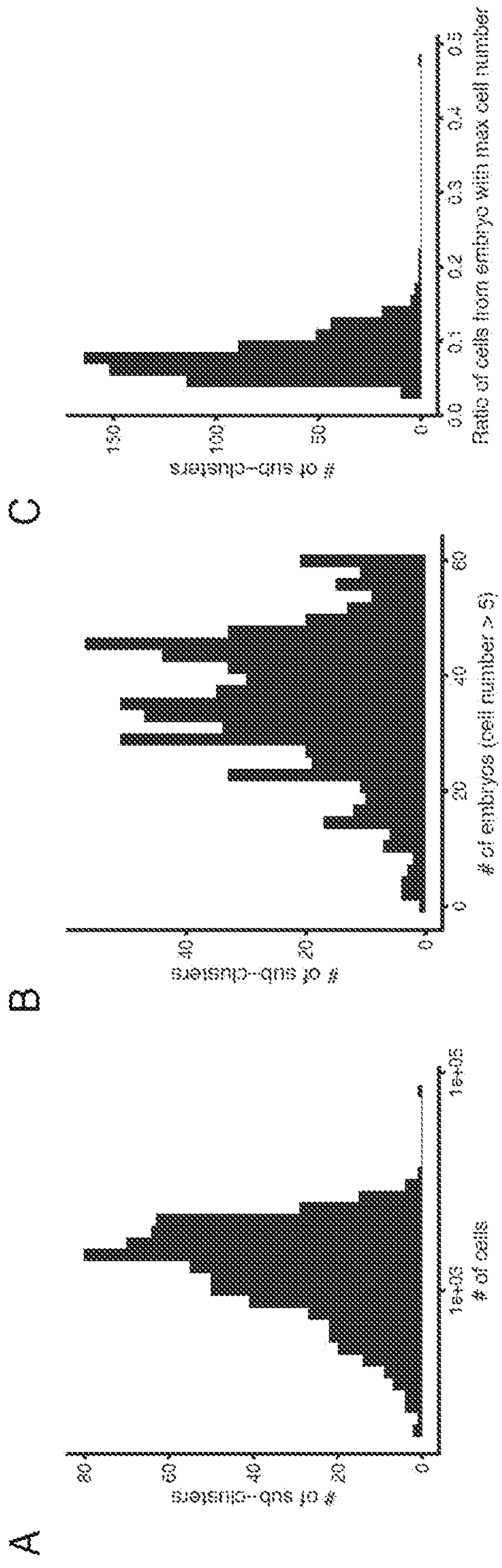

A
# of sub-clusters
# of cells
B
# of sub-clusters
# of embryos (cell number > 5)
C
# of sub-clusters
Ratio of cells from embryo with max cell number
D
Count of sub-clusters
# of gene markers

FIG. 15

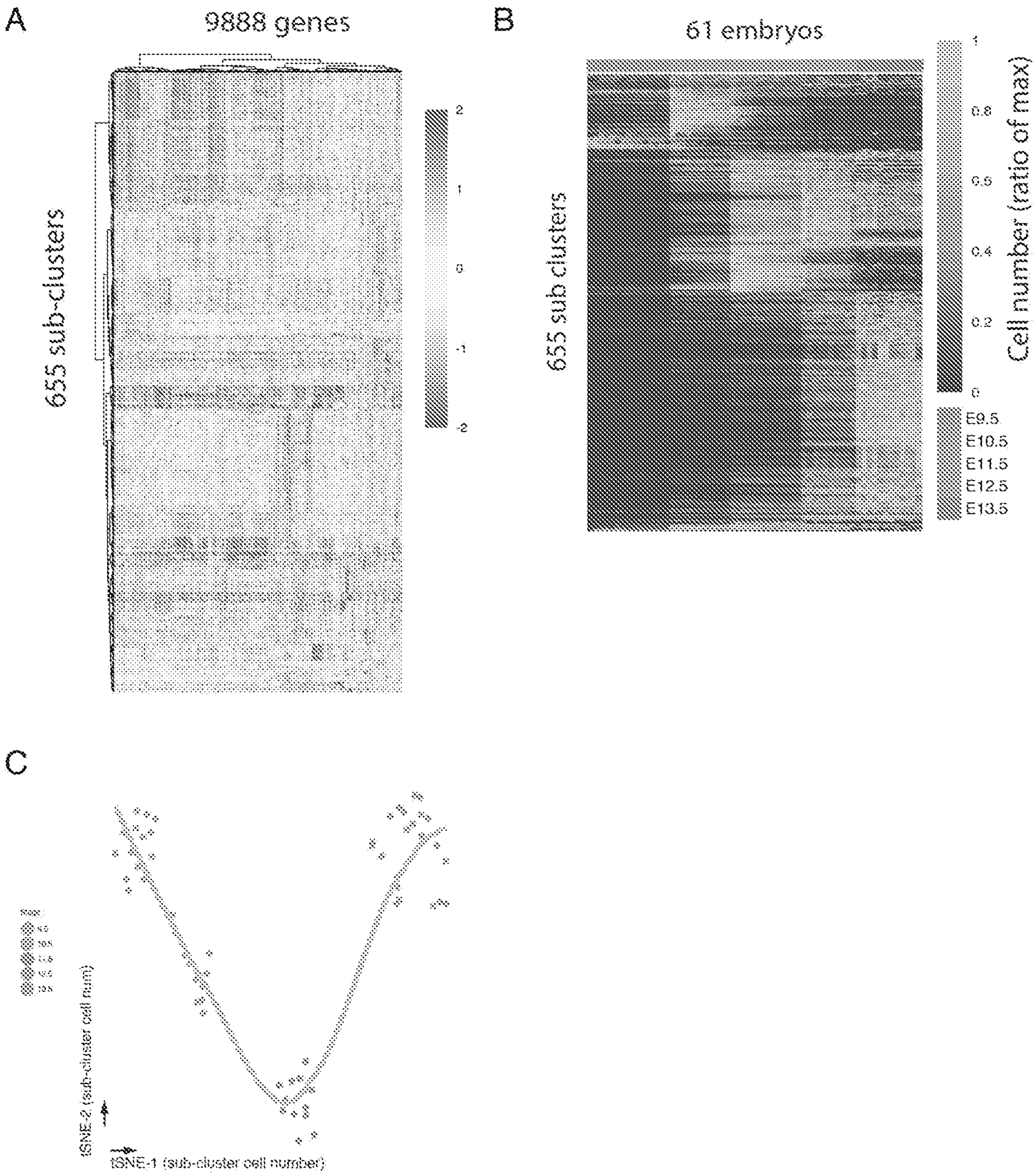

A
9888 genes
655 sub-clusters
2
1
0
-1
-2
B
61 embryos
655 sub clusters
1
0.8
0.6
0.4
0.2
0
Cell number (ratio of max)
E9.5
E10.5
E11.5
E12.5
E13.5
C
tSNE-2 (sub-cluster cell num)
tSNE-1 (sub-cluster cell number)

FIG. 16

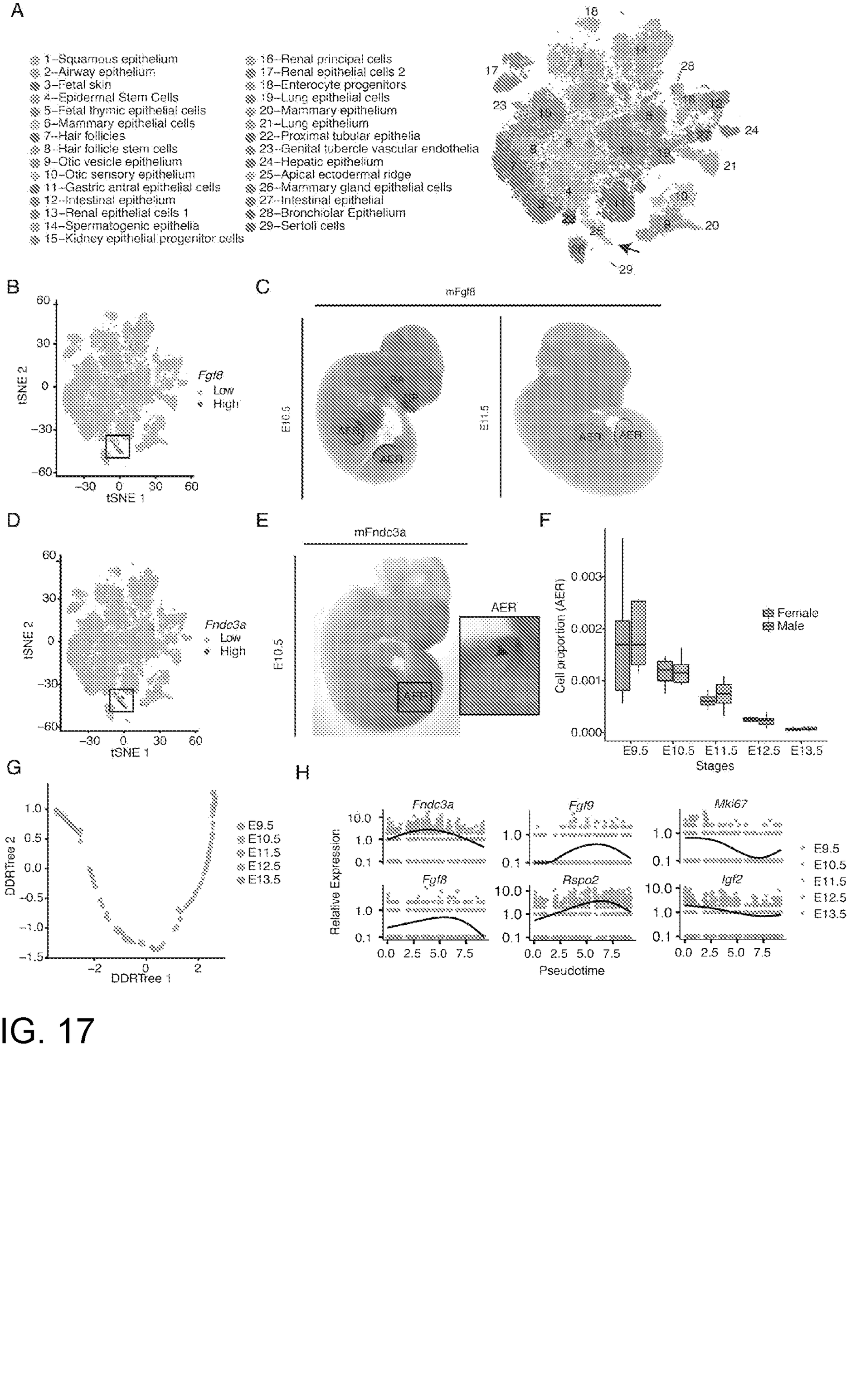

A
1–Squamous epithelium
2–Airway epithelium
3–Fetal skin
4–Epidermal Stem Cells
5–Fetal thymic epithelial cells
6–Mammary epithelial cells
7–Hair follicles
8–Hair follicle stem cells
9–Otic vesicle epithelium
10–Otic sensory epithelium
11–Gastric antral epithelial cells
12–Intestinal epithelium
13–Renal epithelial cells 1
14–Spermatogenic epithelia
15–Kidney epithelial progenitor cells
16–Renal principal cells
17–Renal epithelial cells 2
18–Enterocyte progenitors
19–Lung epithelial cells
20–Mammary epithelium
21–Lung epithelium
22–Proximal tubular epithelia
23–Genital tubercle vascular endothelia
24–Hepatic epithelium
25–Apical ectodermal ridge
26–Mammary gland epithelial cells
27–Intestinal epithelial
28–Bronchiolar Epithelium
29–Sertoli cells
B
tSNE 2
tSNE 1
Fgf8
Low
High
C
mFgf8
E10.5
E11.5
AER
D
tSNE 2
tSNE 1
Fndc3a
Low
High
E
mFndc3a
E10.5
AER
F
Cell proportion (AER)
Female
Male
E9.5
E10.5
E11.5
E12.5
E13.5
Stages
G
DDRTree 2
DDRTree 1
E9.5
E10.5
E11.5
E12.5
E13.5
H
Fndc3a
Fgf9
Mki67
Fgf8
Rspo2
Igf2
Relative Expression
Pseudotime
E9.5
E10.5
E11.5
E12.5
E13.5

FIG. 17

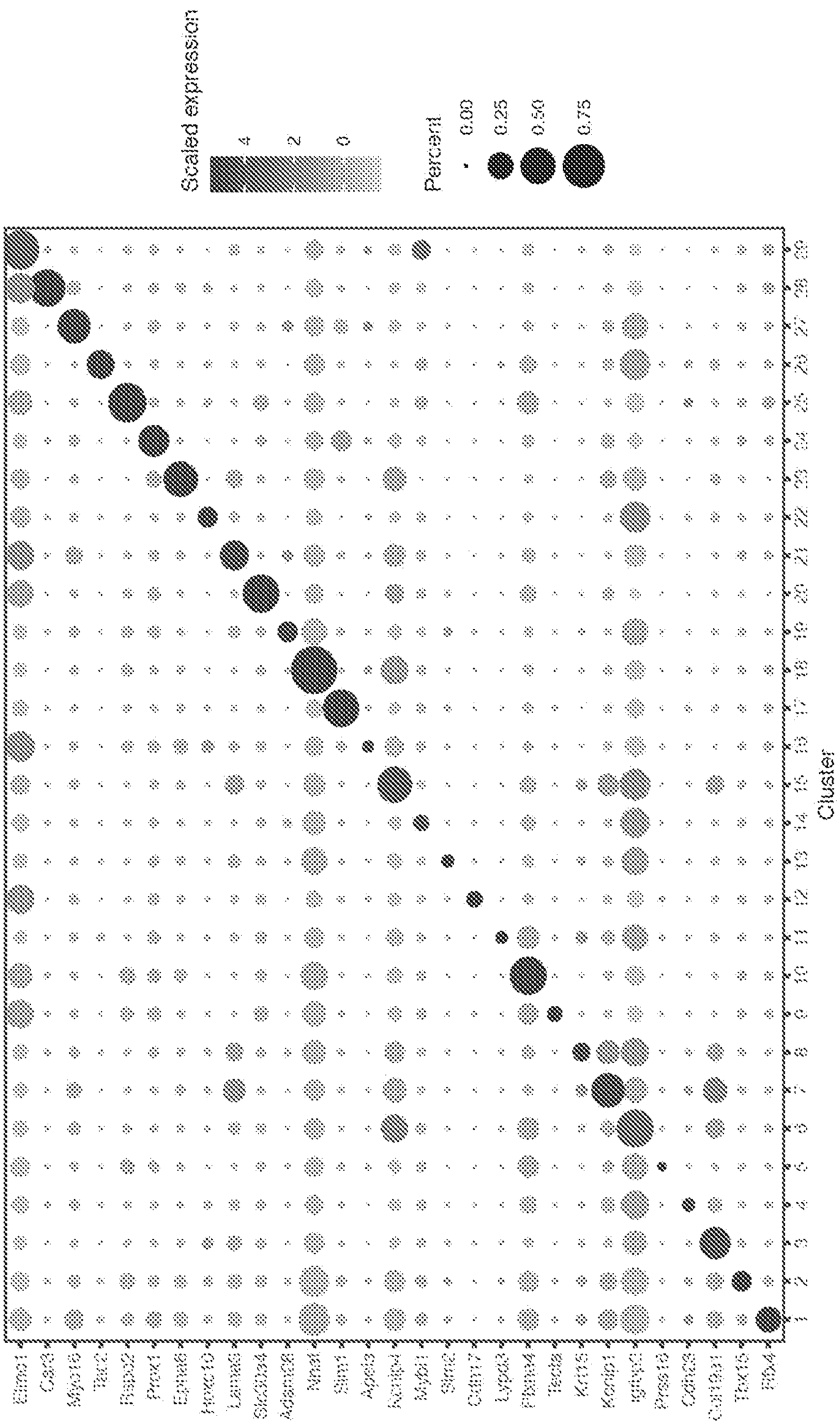

Scaled expression
4
2
0
Percent
0.00
0.25
0.50
0.75
Cluster
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Elmo1
Cers6
Mpeg1
Tbx2
Rasgrp2
Pax1
Edaradd
Hoxb10
Lamb3
Slc22a4
Adamts8
Mmp7
Sim1
Apob
Arhgap4
Myh11
Slco2
Cdh17
Lypd3
Pbsn4
Tecta
Krt15
Kcnip1
Ighg
Prss16
Cdh3
Cartpt
Tex15
Ttr

FIG. 18

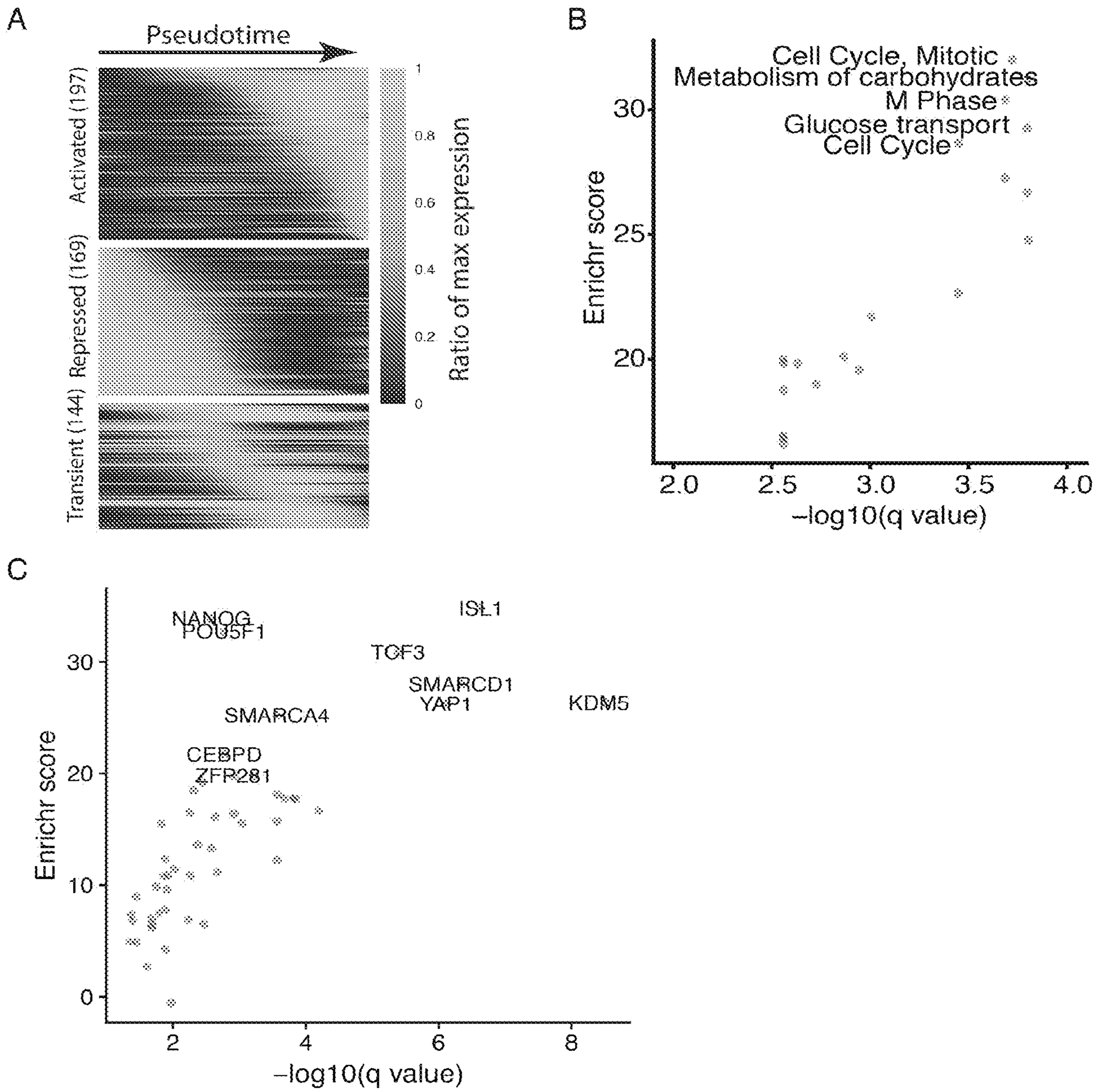

A
Pseudotime
Activated (197)
Repressed (169)
Transient (144)
Ratio of max expression
1
0.8
0.6
0.4
0.2
0
B
Cell Cycle, Mitotic
Metabolism of carbohydrates
M Phase
Glucose transport
Cell Cycle
Enrichr score
30
25
20
2.0
2.5
3.0
3.5
4.0
−log10(q value)
C
NANOG
POU5F1
ISL1
TCF3
SMARCD1
YAP1
KDM5
SMARCA4
CEBPD
ZFP281
Enrichr score
30
20
10
0
2
4
6
8
−log10(q value)

FIG. 19

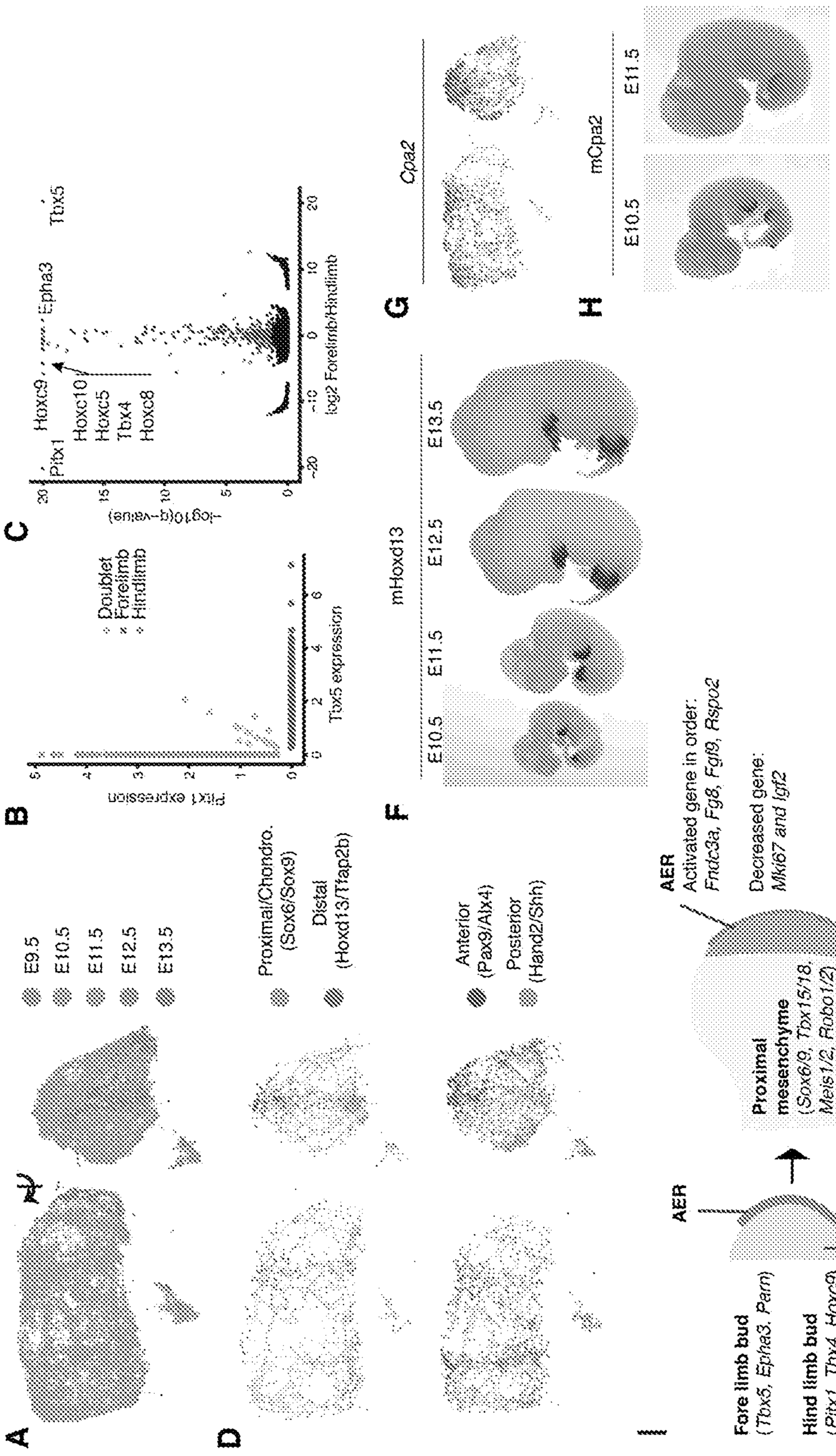

A
B
C
D
E
F
G
H
I
E9.5
E10.5
E11.5
E12.5
E13.5
Tbx1 expression
Tbx5 expression
-log10(q-value)
log2 Forelimb/Hindlimb
Pitx1
Hoxc9
Hoxc10
Hoxc5
Tbx4
Hoxc8
Epha3
Tbx5
Doublet
Forelimb
Hindlimb
Proximal/Chondro.
(Sox6/Sox9)
Distal
(Hoxd13/Tfap2b)
Anterior
(Pax9/Alx4)
Posterior
(Hand2/Shh)
mHoxd13
Cpa2
mCpa2
E10.5
E11.5
E12.5
E13.5
AER
Activated gene in order:
Fndc3a, Fgf8, Fgf9, Rspo2
Decreased gene:
Mki67 and Igf2
Proximal
mesenchyme
(Sox6/9, Tbx15/18,
Meis1/2, Robo1/2)
Distal mesenchyme
(Slit3, Fgf10, Wnt5a, Tfap2b)
Fore limb bud
(Tbx5, Epha3, Parm)
Hind limb bud
(Pitx1, Tbx4, Hoxc9)
Limb mesenchyme

FIG. 20

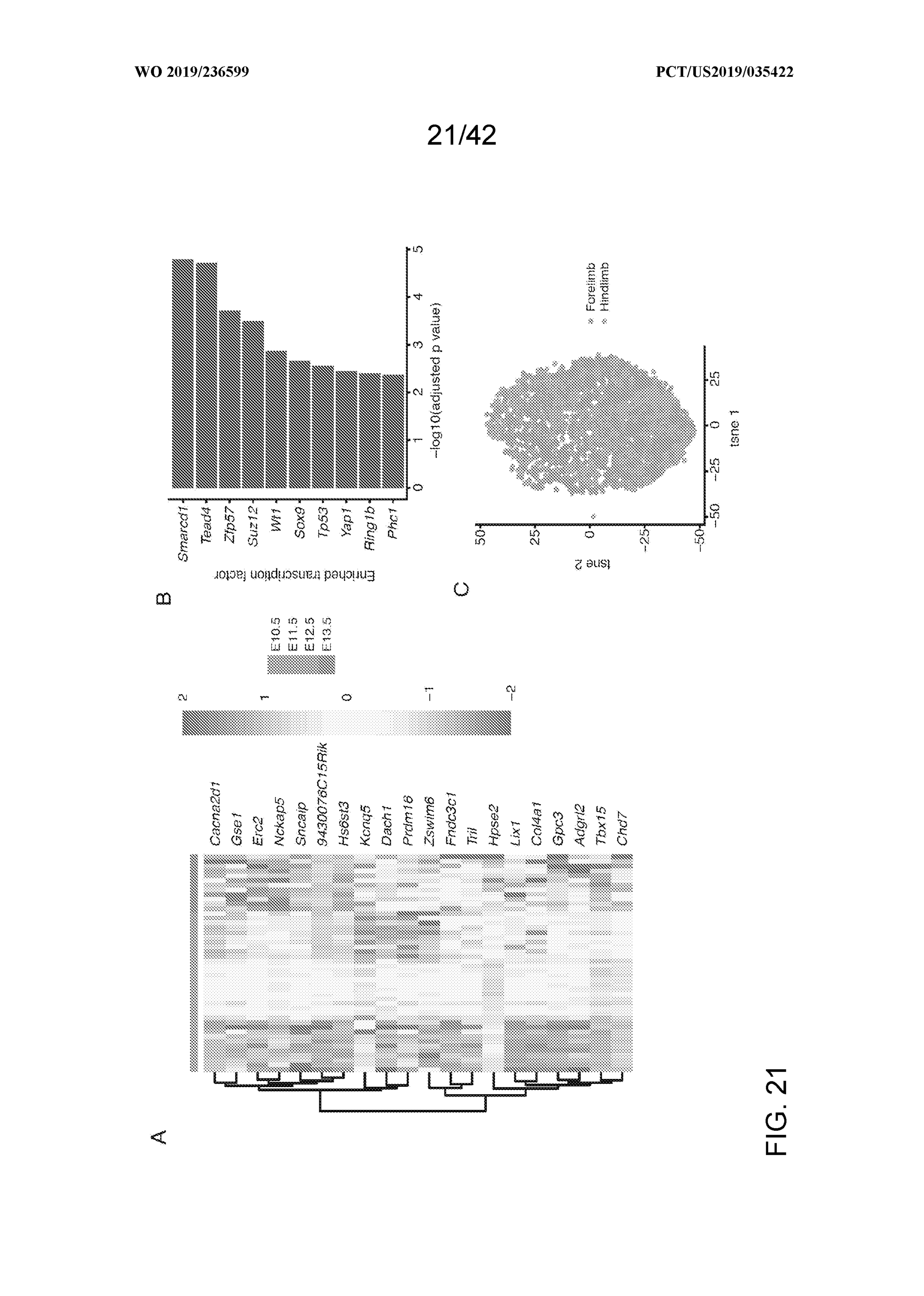

A
2
1
0
−1
−2
Cacna2d1
Gse1
Erc2
Nckap5
Sncaip
9430076C15Rik
Hs6st3
Kcnq5
Dach1
Prdm16
Zswim6
Fndc3c1
Tril
Hpse2
Lix1
Col4a1
Gpc3
Adgrl2
Tbx15
Chd7
B
Smarcd1
Tead4
Zfp57
Suz12
Wt1
Sox9
Tp53
Yap1
Ring1b
Phc1
Enriched transcription factor
0
1
2
3
4
5
−log10(adjusted p value)
E10.5
E11.5
E12.5
E13.5
C
tsne 2
50
25
0
−25
−50
−50
−25
0
25
tsne 1
Forelimb
Hindlimb

FIG. 21

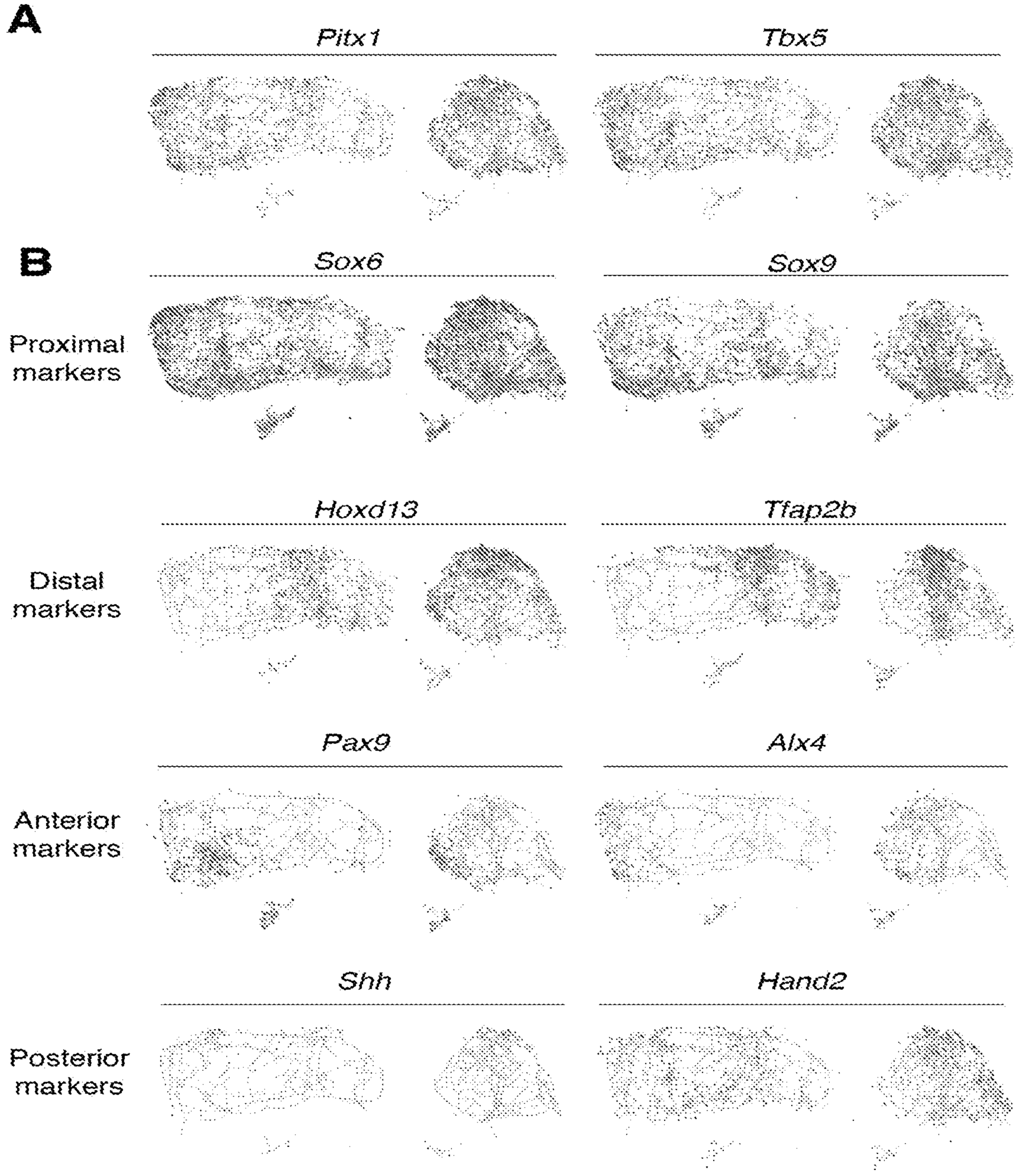

A
Pitx1
Tbx5
B
Sox6
Sox9
Proximal markers
Hoxd13
Tfap2b
Distal markers
Pax9
Alx4
Anterior markers
Shh
Hand2
Posterior markers

FIG. 22

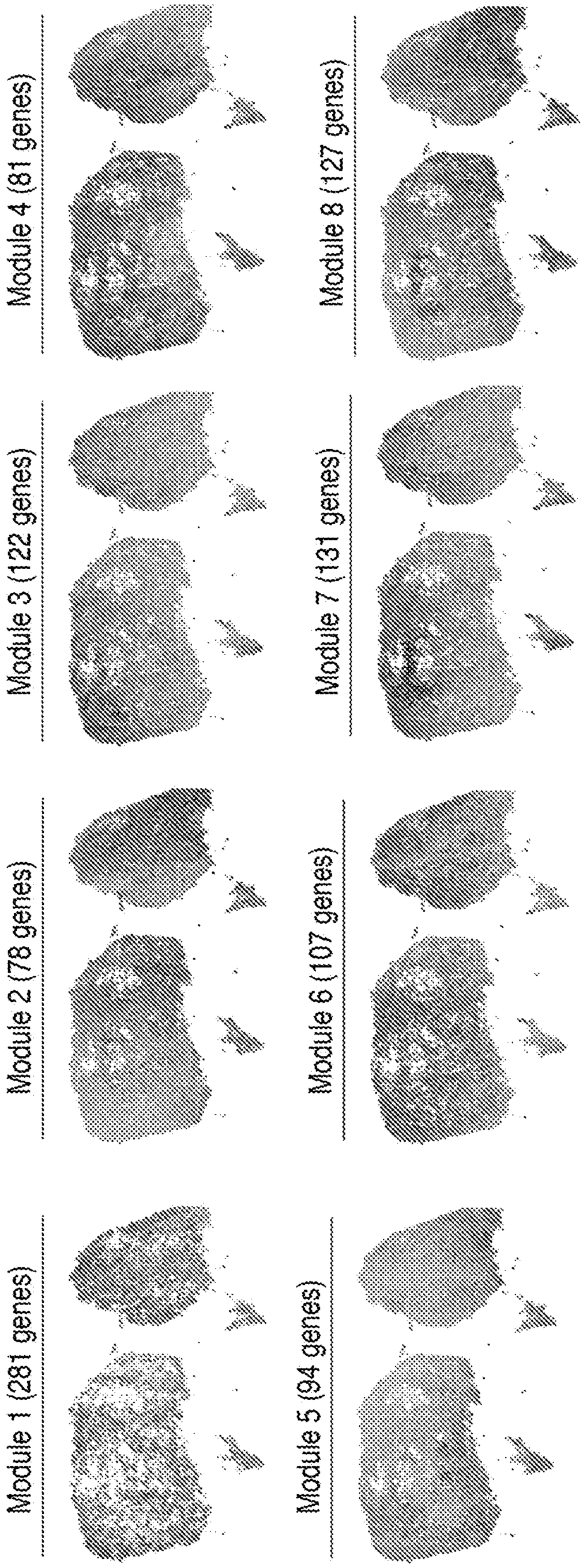

Module 1 (281 genes)
Module 2 (78 genes)
Module 3 (122 genes)
Module 4 (81 genes)
Module 5 (94 genes)
Module 6 (107 genes)
Module 7 (131 genes)
Module 8 (127 genes)

FIG. 23

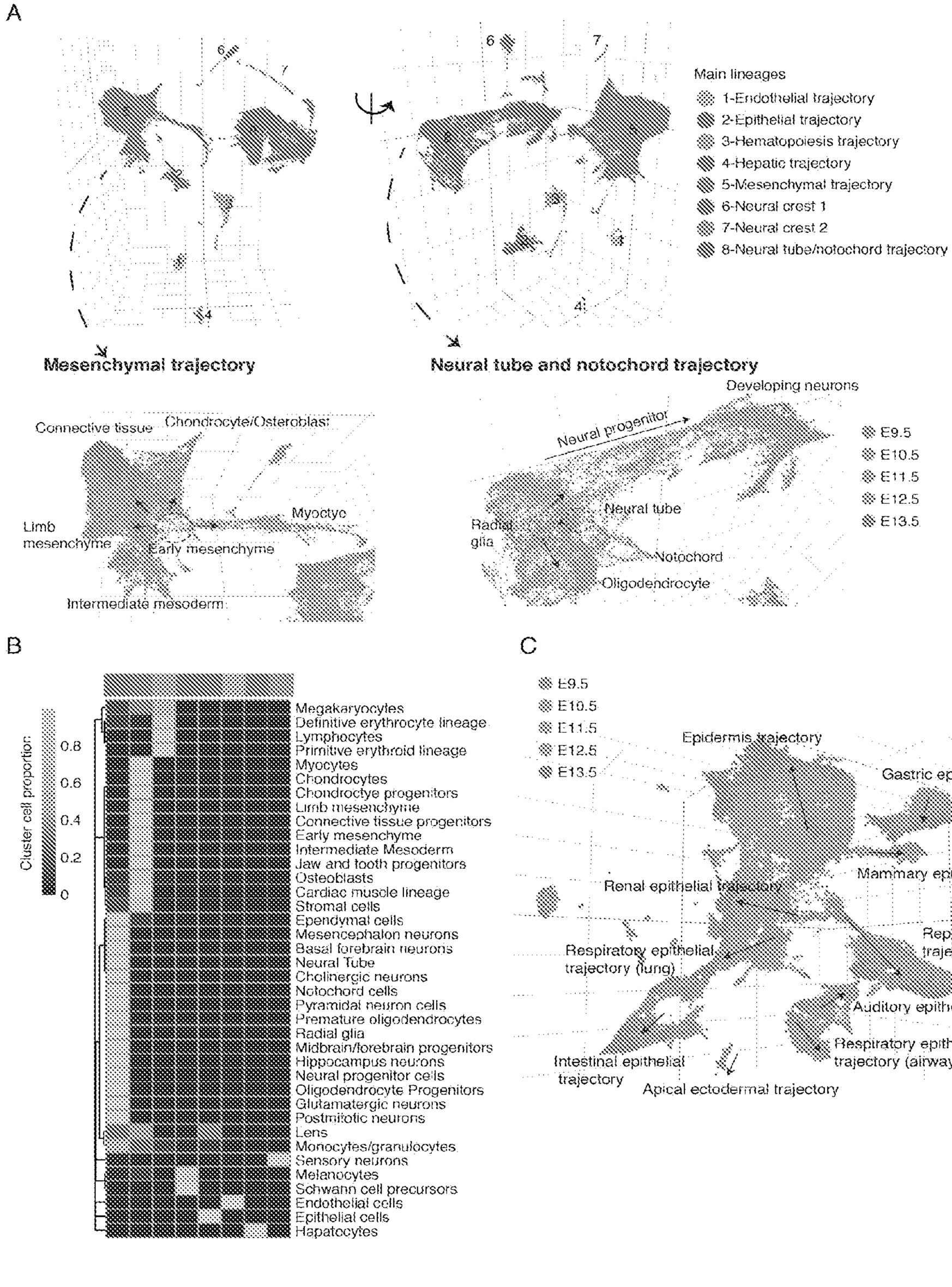

A
6
7
4
Main lineages
1-Endothelial trajectory
2-Epithelial trajectory
3-Hematopoiesis trajectory
4-Hepatic trajectory
5-Mesenchymal trajectory
6-Neural crest 1
7-Neural crest 2
8-Neural tube/notochord trajectory
Mesenchymal trajectory
Neural tube and notochord trajectory
Connective tissue
Chondrocyte/Osteroblast
Myoctye
Limb mesenchyme
Early mesenchyme
Intermediate mesoderm
Developing neurons
Neural progenitor
Neural tube
Radial glia
Notochord
Oligodendrocyte
E9.5
E10.5
E11.5
E12.5
E13.5
B
Cluster cell proportion
0.8
0.6
0.4
0.2
0
Megakaryocytes
Definitive erythrocyte lineage
Lymphocytes
Primitive erythroid lineage
Myocytes
Chondrocytes
Chondroctye progenitors
Limb mesenchyme
Connective tissue progenitors
Early mesenchyme
Intermediate Mesoderm
Jaw and tooth progenitors
Osteoblasts
Cardiac muscle lineage
Stromal cells
Ependymal cells
Mesencephalon neurons
Basal forebrain neurons
Neural Tube
Cholinergic neurons
Notochord cells
Pyramidal neuron cells
Premature oligodendrocytes
Radial glia
Midbrain/forebrain progenitors
Hippocampus neurons
Neural progenitor cells
Oligodendrocyte Progenitors
Glutamatergic neurons
Postmitotic neurons
Lens
Monocytes/granulocytes
Sensory neurons
Melanocytes
Schwann cell precursors
Endothelial cells
Epithelial cells
Hapatocytes
C
E9.5
E10.5
E11.5
E12.5
E13.5
Epidermis trajectory
Gastric epithelial trajectory
Mammary epithelial trajectory
Renal epithelial trajectory
Reproductive epithelial trajectory
Respiratory epithelial trajectory (lung)
Auditory epithelial trajectory
Respiratory epithelial trajectory (airway)
Intestinal epithelial trajectory
Apical ectodermal trajectory

FIG. 24

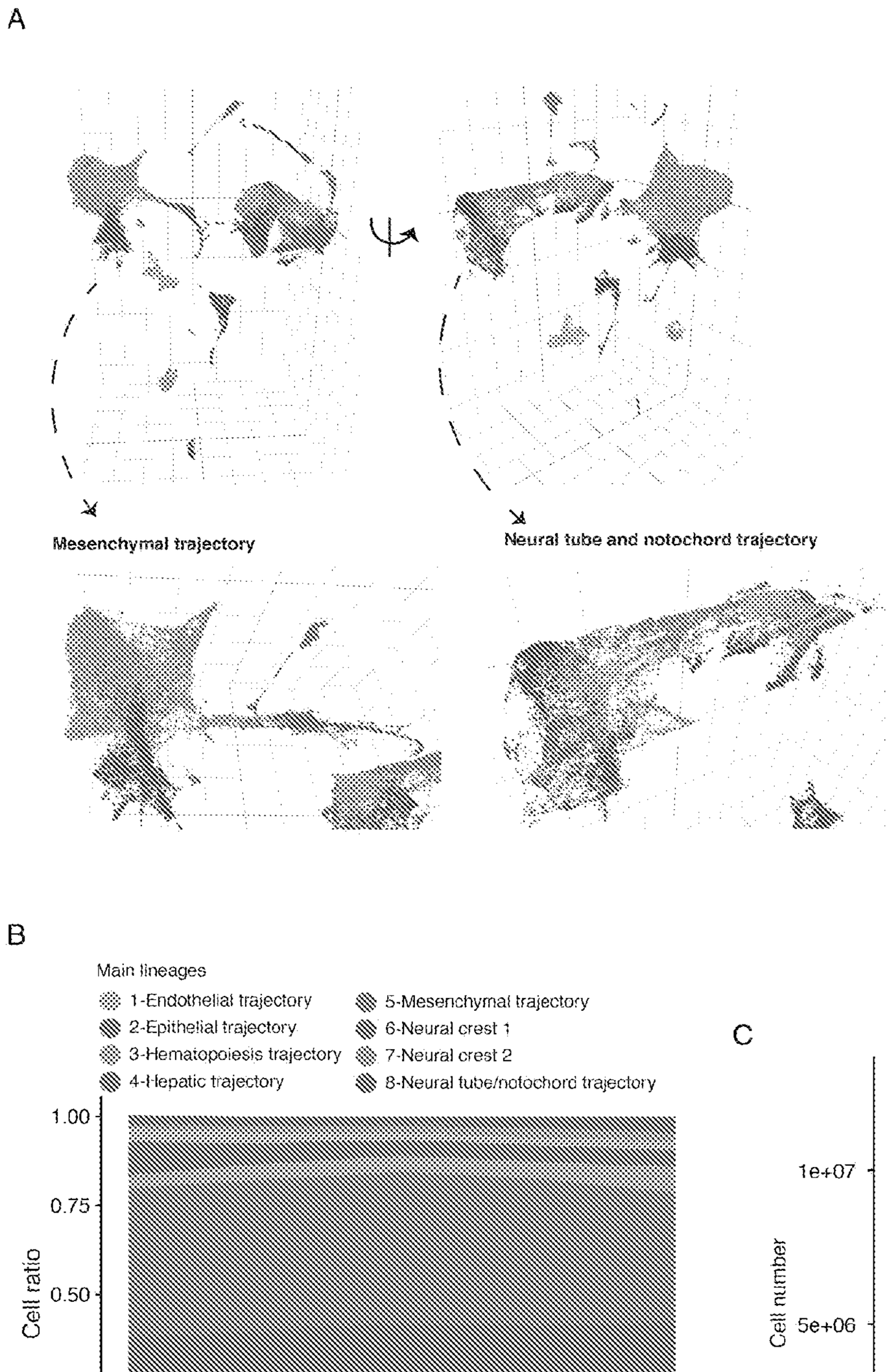

A
Mesenchymal trajectory
Neural tube and notochord trajectory
1--Connective tissue progenitors
2--Chondrocytes
3--Intermediate Mesoderm
4--Jaw and tooth progenitors
5--Mesencephalon neurons
6--Epithelial cells
7--Radial glia
8--Early mesenchyme
9--Neural progenitor cells
10--Postmitotic neurons
11--Oligodendrocyte Progenitors
12--Midbrain/forebrain progenitors
13--Myocytes
14--Neural Tube
15--Glutamatergic neurons
16--Stromal cells
17--Osteoblasts
18--Hippocampus neurons
19--Premature oligodendrocytes
20--Endothelial cells
21--Chondroctye progenitors
22--Definitive erythrocyte lineage
23--Schwann cell precursors
24--Sensory neurons
25--Limb mesenchyme
26--Primitive erythroid lineage
27--Basal forebrain neurons
28--Pyramidal neuron cells
29--Hapatocytes
30--Notochord cells
31--Lymphocytes
32--Ependymal cells
33--Cholinergic neurons
34--Cardiac muscle lineage
35--Megakaryocytes
36--Melanocytes
37--Lens
38--Monocytes/granulocytes
B
Main lineages
1-Endothelial trajectory
2-Epithelial trajectory
3-Hematopoiesis trajectory
4-Hepatic trajectory
5-Mesenchymal trajectory
6-Neural crest 1
7-Neural crest 2
8-Neural tube/notochord trajectory
Cell ratio
1.00
0.75
0.50
0.25
0.00
E9.5
E10.5
E11.5
E12.5
E13.5
Stage
C
Cell number
1e+07
5e+06
0e+00
E9.5
E10.5
E11.5
E12.5
E13.5
Stage

FIG. 25


Epithelial trajectory
Neural tube/notochord trajectory
1–Connective tissue progenitors
2–Chondrocytes
3–Intermediate Mesoderm
4–Jaw and tooth progenitors
5–Mesencephalon neurons
6–Epithelial cells
7–Radial glia
8–Early mesenchyme
9–Neural progenitor cells
10–Postmitotic neurons
11–Oligodendrocyte Progenitors
12–Midbrain/forebrain progenitors
13–Myocytes
14–Neural Tube
15–Glutamatergic neurons
16–Stromal cells
17–Osteoblasts
18–Hippocampus neurons
19–Premature oligodendrocytes
20–Endothelial cells
21–Chondroclye progenitors
22–Definitive erythrocyte lineage
23–Schwann cell precursors
24–Sensory neurons
25–Limb mesenchyme
26–Primitive erythroid lineage
27–Basal forebrain neurons
28–Pyramidal neuron cells
29–Hapatocytes
30–Notochord cells
31–Lymphocytes
32–Ependymal cells
33–Cholinergic neurons
34–Cardiac muscle lineage
35–Megakaryocytes
36–Melanocytes
37–Lens
38–Monocytes/granulocytes
Hematopoiesis trajectory
Mesenchymal trajectory
Neural crest 1
Hepatic trajectory
Endothelial trajectory
Neural crest 2

FIG. 26


Epithelial trajectory
Neural tube/notochord trajectory
E9.5
E10.5
E11.5
E12.5
E13.5
Hematopoiesis trajectory
Mesenchymal trajectory
Neural crest 1
Hepatic trajectory
Endothelial trajectory
Neural crest 2

FIG. 27

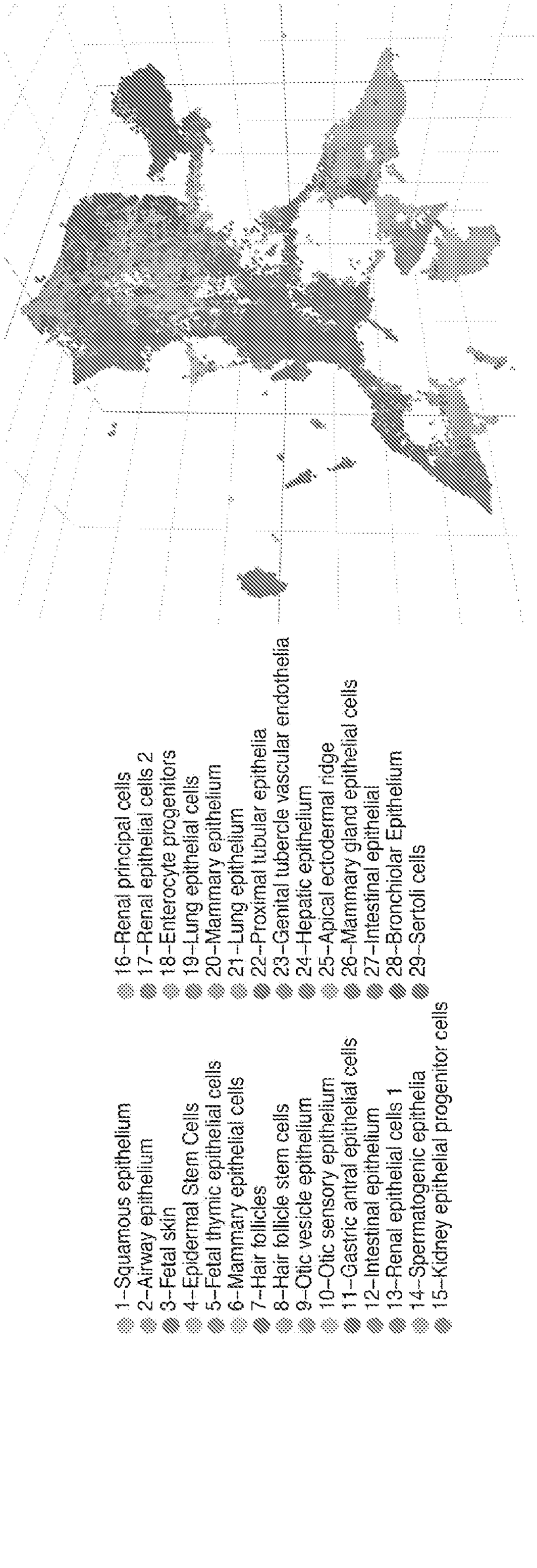

16–Renal principal cells
17–Renal epithelial cells 2
18–Enterocyte progenitors
19–Lung epithelial cells
20–Mammary epithelium
21–Lung epithelium
22–Proximal tubular epithelia
23–Genital tubercle vascular endoth
24–Hepatic epithelium
25–Apical ectodermal ridge
26–Mammary gland epithelial cells
27–Intestinal epithelial
28–Bronchiolar Epithelium
29–Sertoli cells
1–Squamous epithelium
2–Airway epithelium
3–Fetal skin
4–Epidermal Stem Cells
5–Fetal thymic epithelial cells
6–Mammary epithelial cells
7–Hair follicles
8–Hair follicle stem cells
9–Otic vesicle epithelium
10–Otic sensory epithelium
11–Gastric antral epithelial cel
12–Intestinal epithelium
13–Renal epithelial cells 1
14–Spermatogenic epithelia
15–Kidney epithelial progenito

FIG. 28

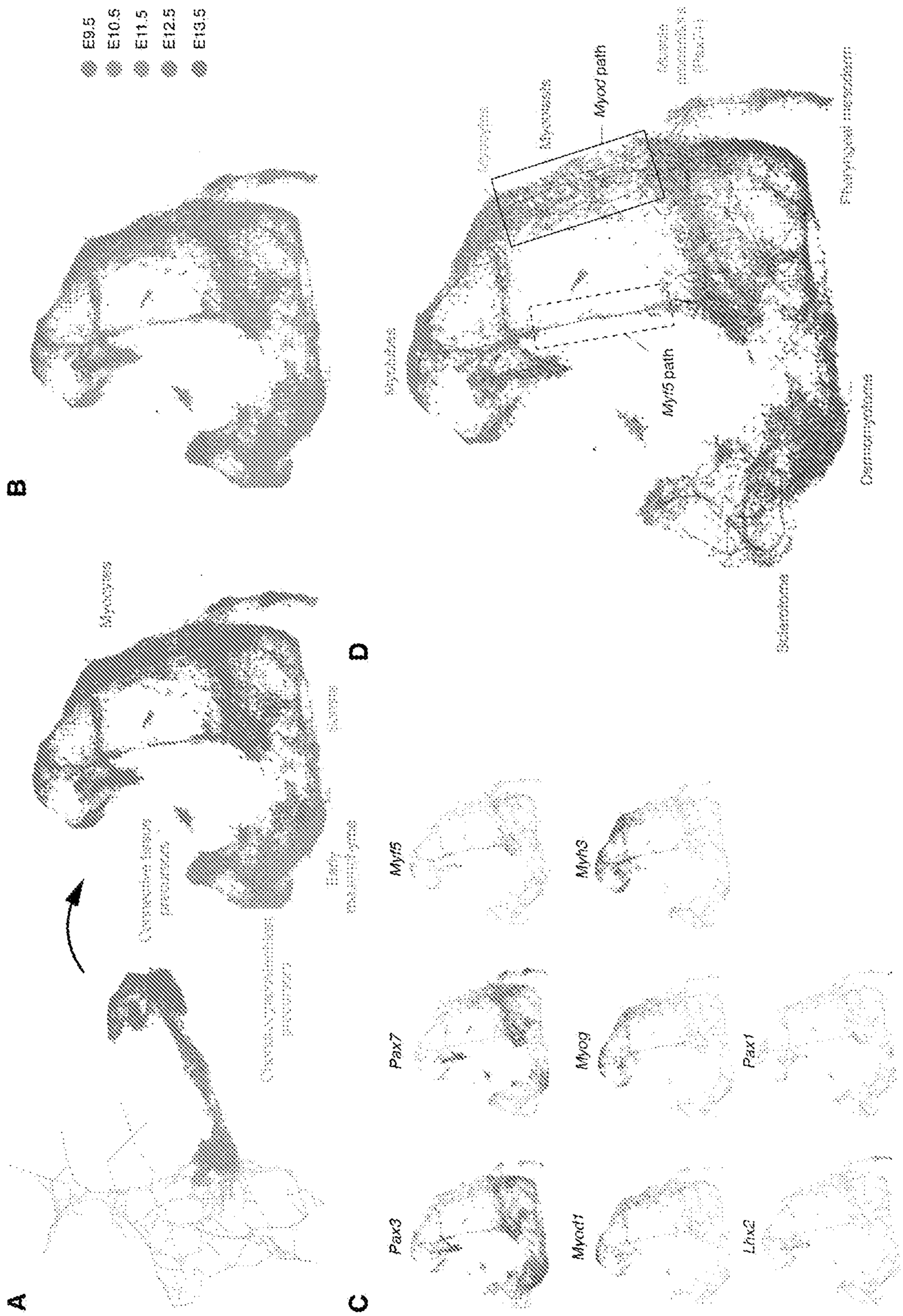

A
B
C
D
Myotome
Dermomyotome
Connective tissue primordia
Limb mesenchyme
Pax7
Myf5
Myog
Myod1
Pax3
Myf5
Myh3
Pax1
Lhx2
Sclerotome
Somite
Myotome
Myod path
Myf5 path
Dermomyotome
Pharyngeal mesoderm

FIG. 29

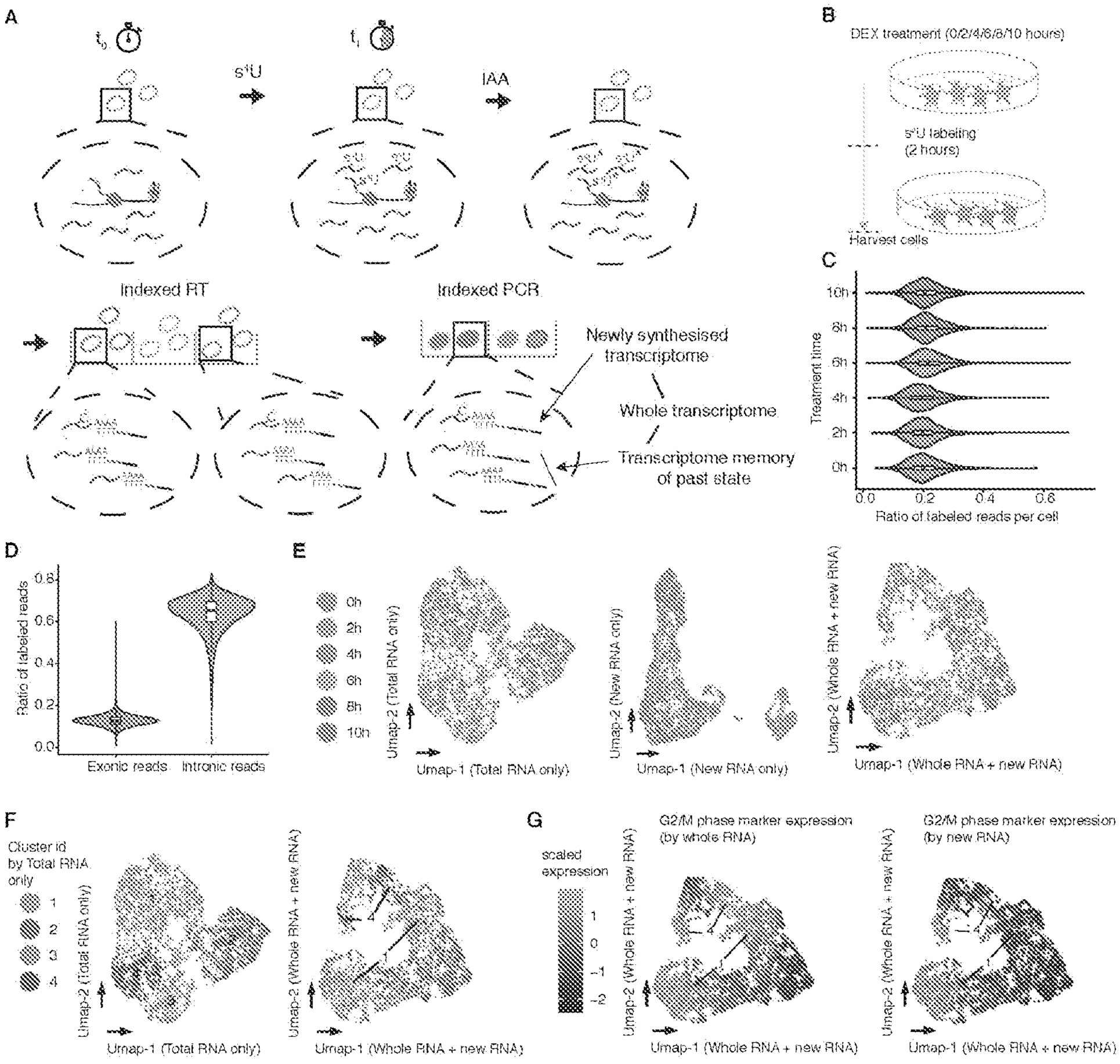

A
t_0
s^4U
t_1
IAA
Indexed RT
Indexed PCR
Newly synthesised transcriptome
Whole transcriptome
Transcriptome memory of past state
B
DEX treatment (0/2/4/6/8/10 hours)
s^4U labeling (2 hours)
Harvest cells
C
Treatment time
10h
8h
6h
4h
2h
0h
0.0
0.2
0.4
0.6
Ratio of labeled reads per cell
D
Ratio of labeled reads
0.0
0.2
0.4
0.6
0.8
Exonic reads
Intronic reads
E
0h
2h
4h
6h
8h
10h
Umap-2 (Total RNA only)
Umap-1 (Total RNA only)
Umap-2 (New RNA only)
Umap-1 (New RNA only)
Umap-2 (Whole RNA + new RNA)
Umap-1 (Whole RNA + new RNA)
F
Cluster id by Total RNA only
1
2
3
4
Umap-2 (Total RNA only)
Umap-1 (Total RNA only)
Umap-2 (Whole RNA + new RNA)
Umap-1 (Whole RNA + new RNA)
G
scaled expression
1
0
−1
−2
G2/M phase marker expression (by whole RNA)
G2/M phase marker expression (by new RNA)
Umap-2 (Whole RNA + new RNA)
Umap-1 (Whole RNA + new RNA)

FIG. 30

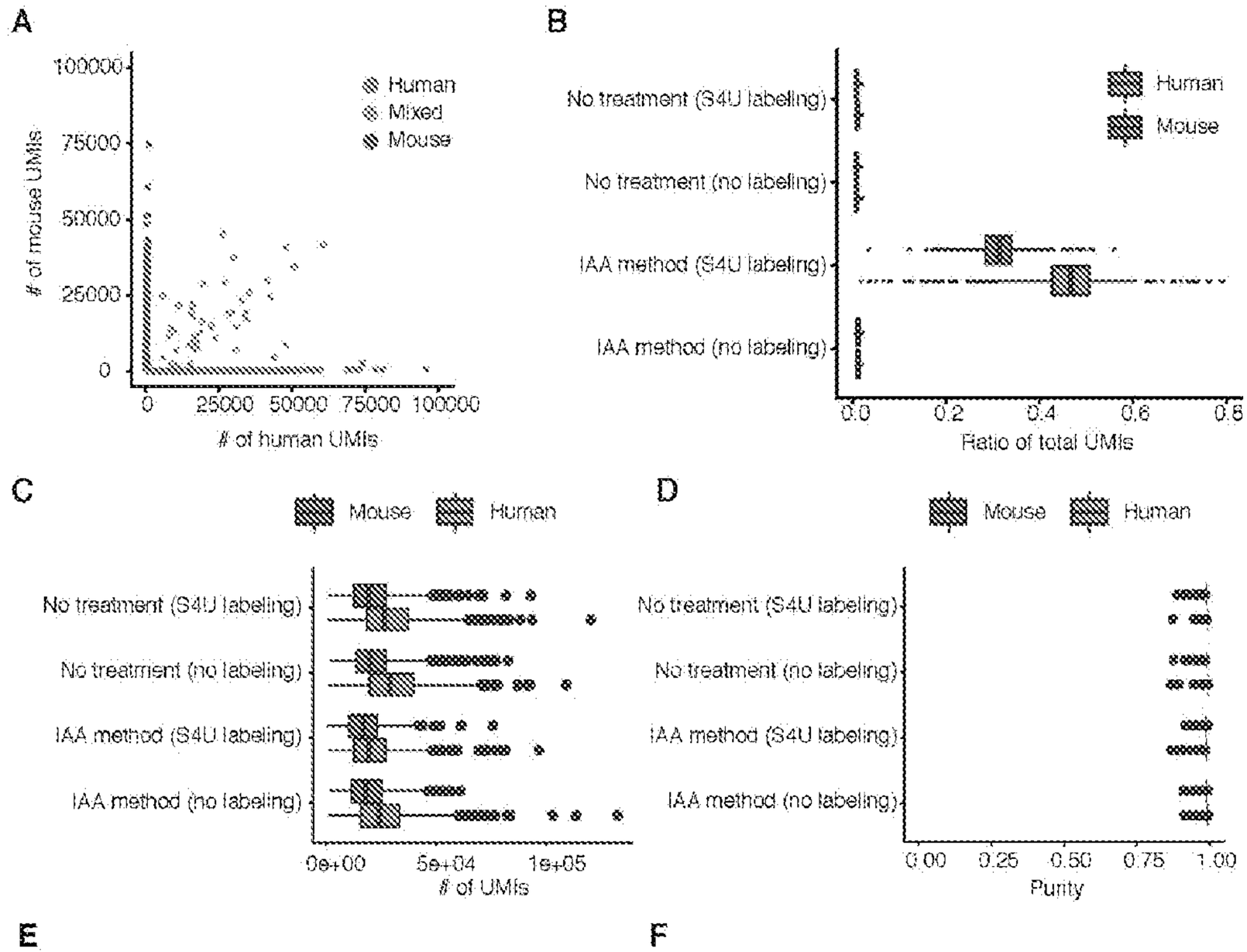

A
100000
75000
50000
25000
0
# of mouse UMIs
Human
Mixed
Mouse
0
25000
50000
75000
100000
# of human UMIs
B
No treatment (S4U labeling)
No treatment (no labeling)
IAA method (S4U labeling)
IAA method (no labeling)
Human
Mouse
0.0
0.2
0.4
0.6
0.8
Ratio of total UMIs
C
Mouse
Human
No treatment (S4U labeling)
No treatment (no labeling)
IAA method (S4U labeling)
IAA method (no labeling)
0e+00
5e+04
1e+05
# of UMIs
D
Mouse
Human
No treatment (S4U labeling)
No treatment (no labeling)
IAA method (S4U labeling)
IAA method (no labeling)
0.00
0.25
0.50
0.75
1.00
Purity
E
log10(TPM + 1), with labeling, human
Spearman r = 0.99
0
1
2
3
4
log10(TPM + 1), no labeling, human
F
log10(TPM + 1), with labeling, mouse
Spearman r = 0.99
0
1
2
3
4
log10(TPM + 1), no labeling, mouse

FIG. 31

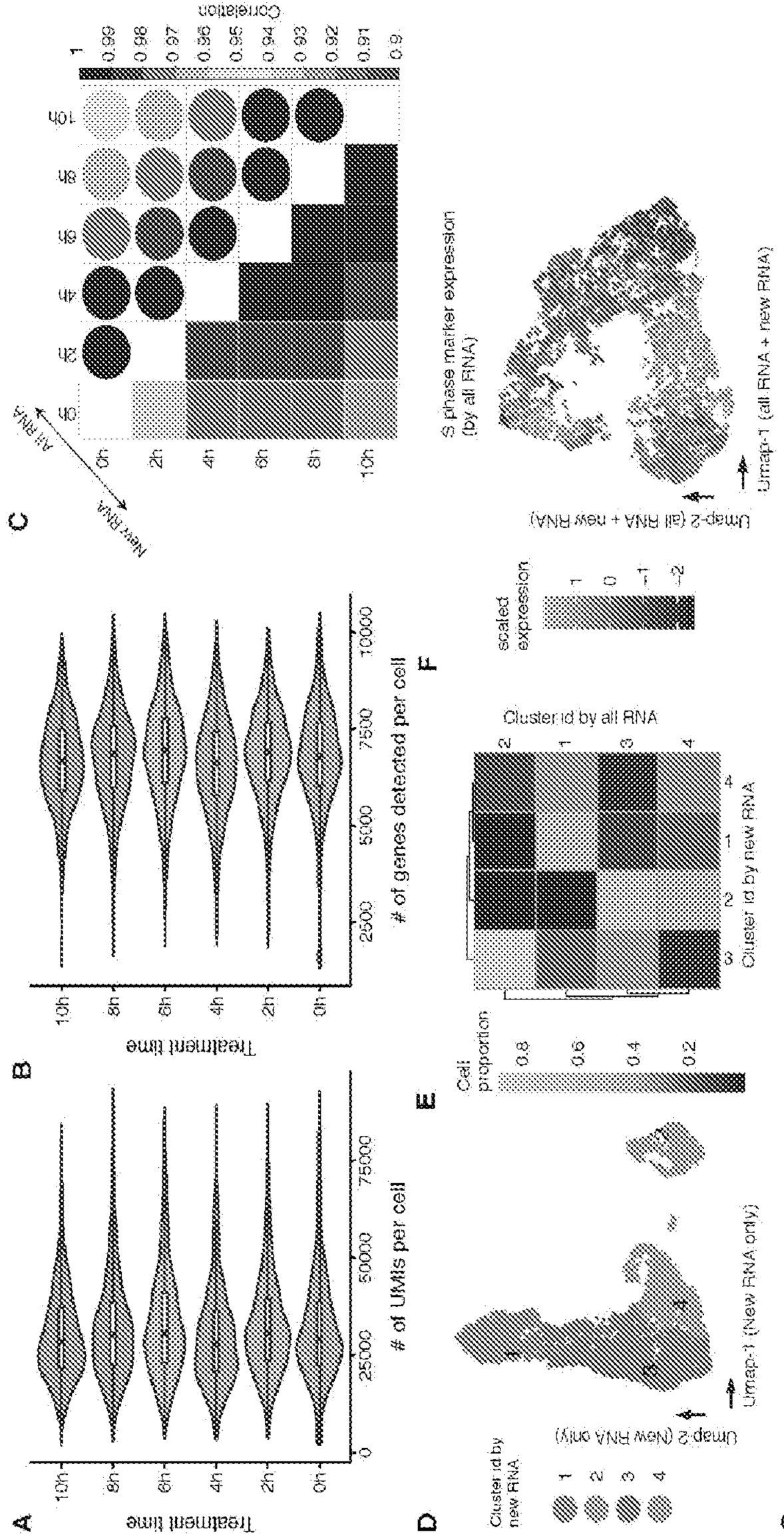

A
Treatment time
10h
8h
6h
4h
2h
0h
0
25000
50000
75000
# of UMIs per cell
B
Treatment time
10h
8h
6h
4h
2h
0h
2500
5000
7500
10000
# of genes detected per cell
C
New RNA
All RNA
0h
2h
4h
6h
8h
10h
Correlation
1
0.99
0.98
0.97
0.96
0.95
0.94
0.93
0.92
0.91
0.9
D
Cluster id by new RNA
1
2
3
4
Umap-2 (New RNA only)
Umap-1 (New RNA only)
E
Cell proportion
0.8
0.6
0.4
0.2
Cluster id by all RNA
Cluster id by new RNA
1
2
3
4
F
scaled expression
1
0
−1
−2
G
S phase marker expression (by new RNA)
Umap-2 (all RNA + new RNA)
Umap-1 (all RNA + new RNA)
S phase marker expression (by all RNA)
Umap-2 (all RNA + new RNA)
Umap-1 (all RNA + new RNA)
scaled expression
1
0
−1
−2

FIG. 32

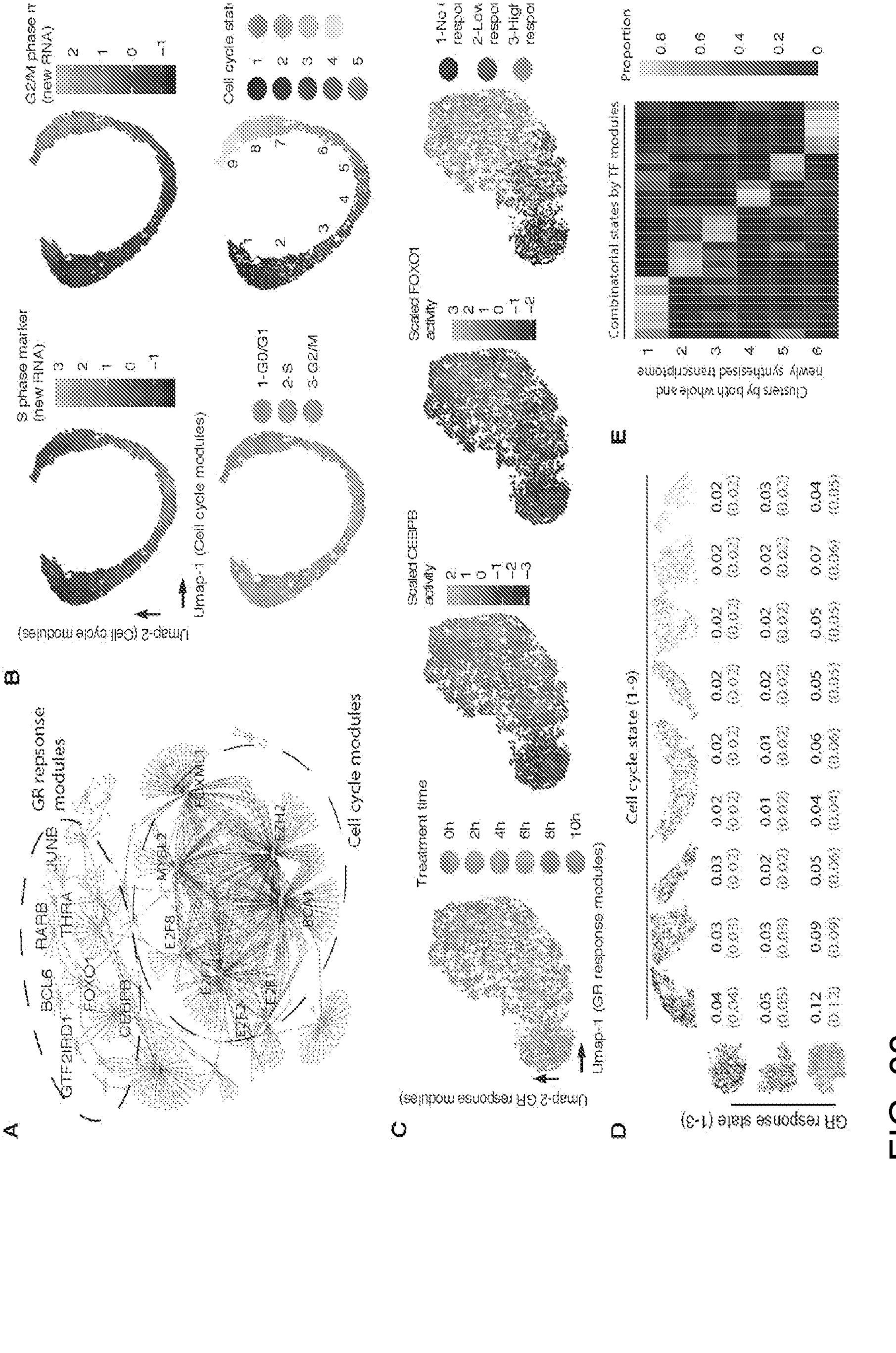

A
B
C
D
E
GR reponse modules
Cell cycle modules
BCL6
RARB
JUNB
GTF2IRD1
THRA
FOXO1
CEBPB
MYBL2
E2F8
FOXM1
EZH2
BCAT1
E2F4
E2F1
Umap-1 (GR response modules)
Umap-2 (Cell cycle modules)
Treatment time
0h
2h
4h
6h
8h
10h
Scaled CEBPB activity
Scaled FOXO1 activity
Cell cycle state (1-9)
GR response state (1-3)
Combinatorial states by TF modules
Clusters by both whole and newly synthesised transcriptome
Proportion
S phase marker (new RNA)
G2/M phase marker (new RNA)
1-G0/G1
2-S
3-G2/M
1-No response
2-Low response
3-High response

FIG. 33

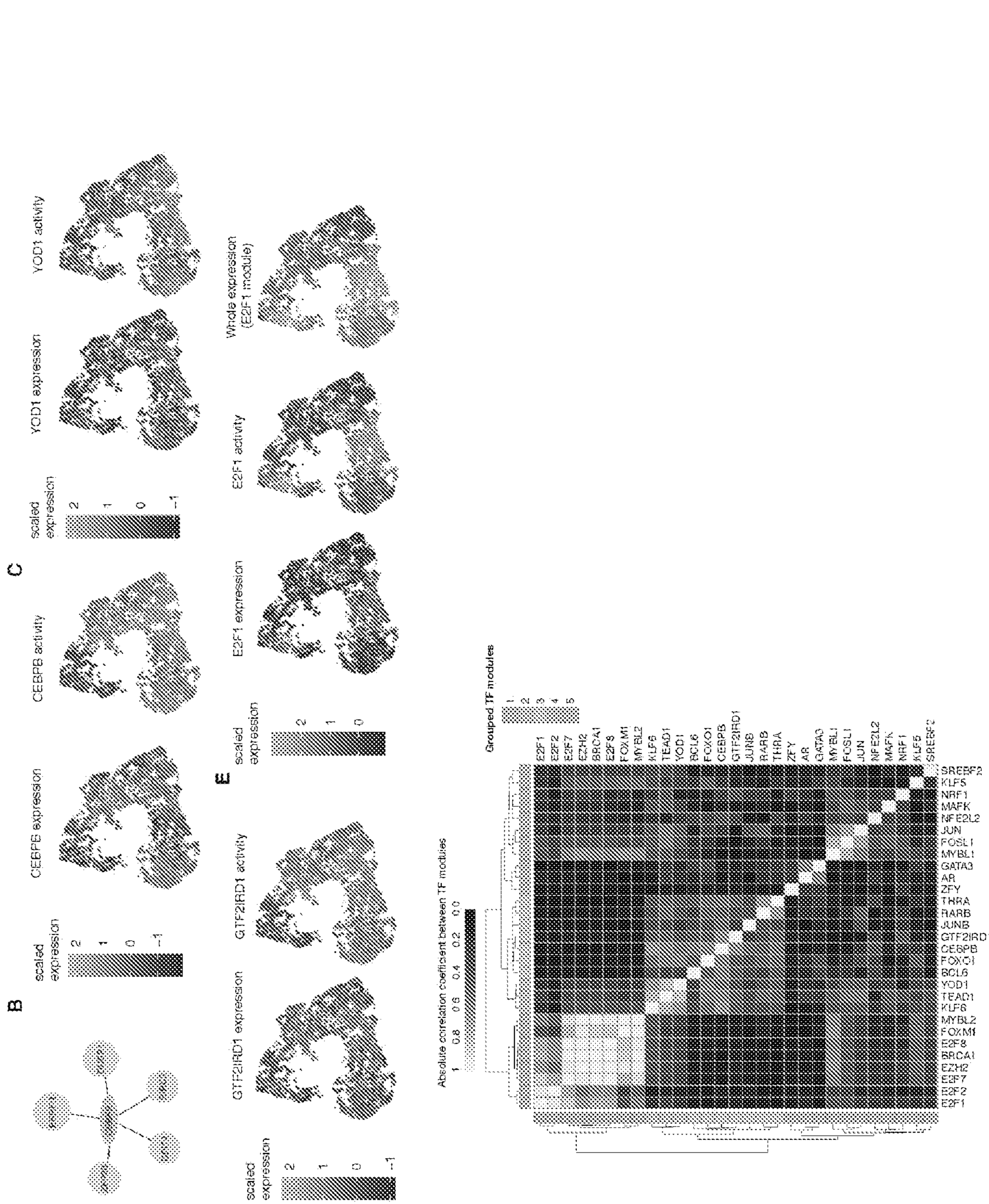
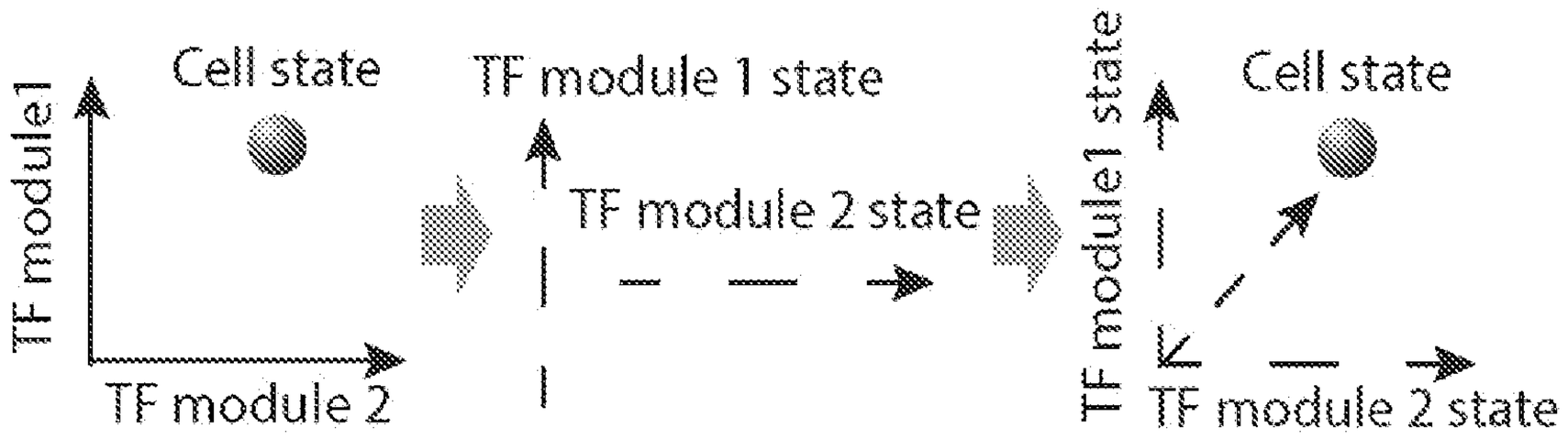

A
B
scaled expression
2
1
0
-1
CEBPB expression
CEBPB activity
C
scaled expression
2
1
0
-1
YOD1 expression
YOD1 activity
D
GTF2IRD1 expression
GTF2IRD1 activity
scaled expression
2
1
0
-1
E
scaled expression
2
1
0
E2F1 activity
Whole expression (E2F1 module)
E2F1 expression
F
Grouped TF modules
1
2
3
4
5
Absolute correlation coefficient between TF modules
1
0.8
0.6
0.4
0.2
0.0
E2F1
E2F2
E2F7
EZH2
BRCA1
E2F8
FOXM1
MYBL2
KLF6
TEAD1
YOD1
BCL6
FOXO1
CEBPB
GTF2IRD1
JUNB
RARB
THRA
ZFY
AR
GATA3
MYBL1
FOSL1
JUN
NFE2L2
MAFK
NRF1
KLF5
SREBF2
SREBF2
KLF5
NRF1
MAFK
NFE2L2
JUN
FOSL1
MYBL1
GATA3
AR
ZFY
THRA
RARB
JUNB
GTF2IRD1
CEBPB
FOXO1
BCL6
YOD1
TEAD1
KLF6
MYBL2
FOXM1
E2F8
BRCA1
EZH2
E2F7
E2F2
E2F1

FIG. 34

A


Cell state
TF module 1 state
TF module 2 state
TF module1
TF module 2
TF module1 state
Cell state
TF module 2 state

B

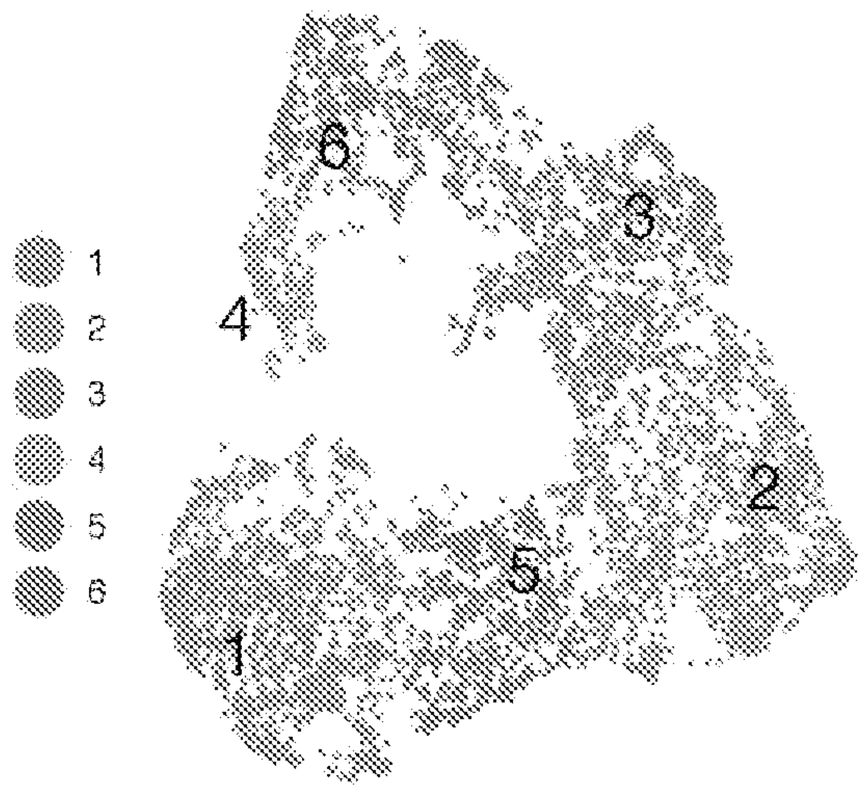
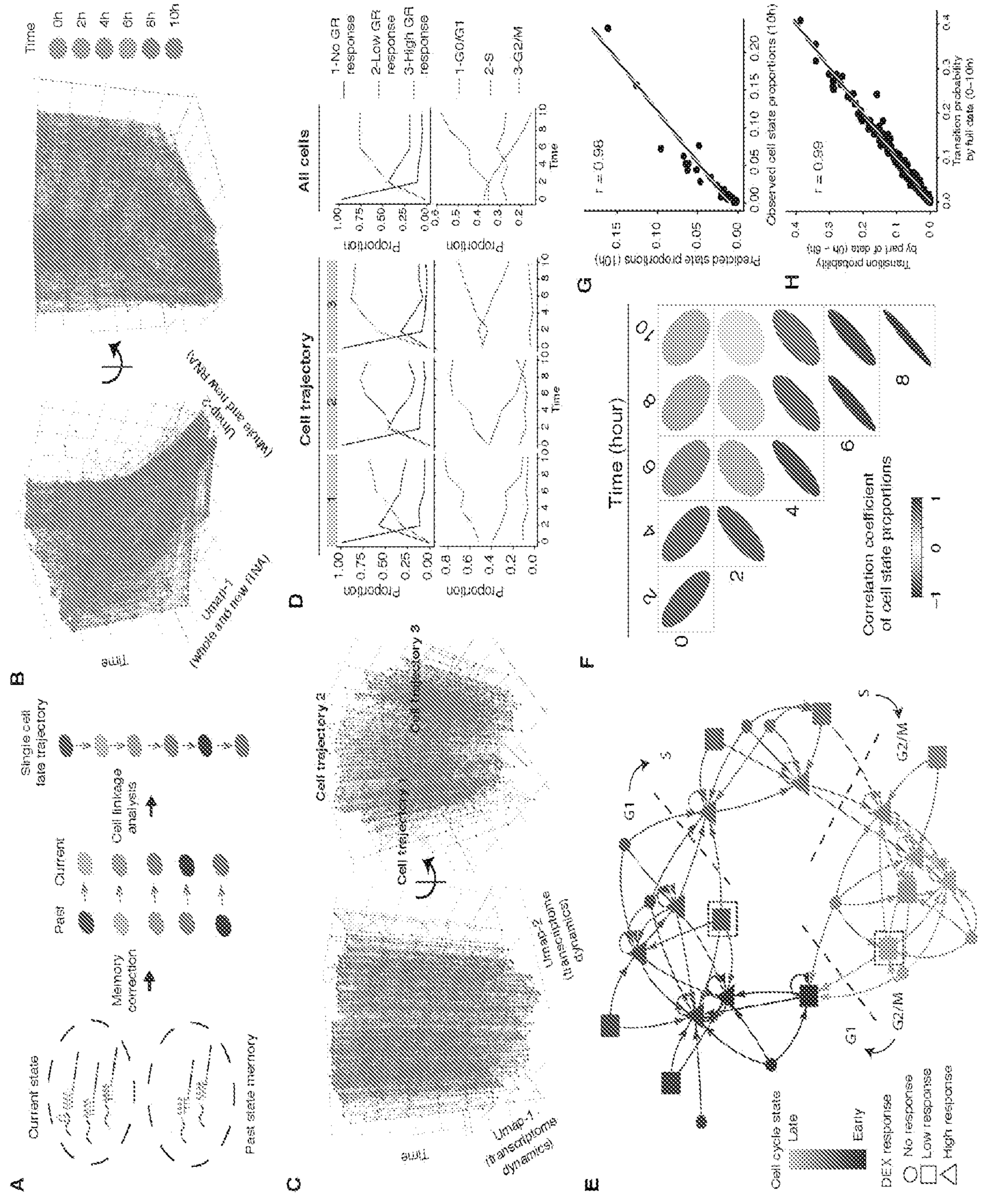

1
2
3
4
5
6
1
2
3
4
5
6

FIG. 35

FIG. 36


A
Current state
Past state memory
Memory correction
B
Single cell fate trajectory
Current
Past
Cell linkage analysis
Time
Umap-1 (whole and new RNA)
Umap-2 (whole and new RNA)
C
Cell trajectory 1
Cell trajectory 2
Cell trajectory 3
Time
Umap-1 (transcriptome dynamics)
Umap-2 (transcriptome dynamics)
D
All cells
1-No GR response
2-Low GR response
3-High GR response
1-G0/G1
2-S
3-G2/M
Proportion
Time
E
G1
S
G2/M
Cell cycle state
Late
Early
DEX response
No response
Low response
High response
F
Time (hour)
Correlation coefficient of cell state proportions
G
r = 0.98
Predicted state proportions (10h)
Observed cell state proportions (10h)
H
r = 0.99
Transition probability by part of data (0h - 6h)
Transition probability by full data (0–10h)

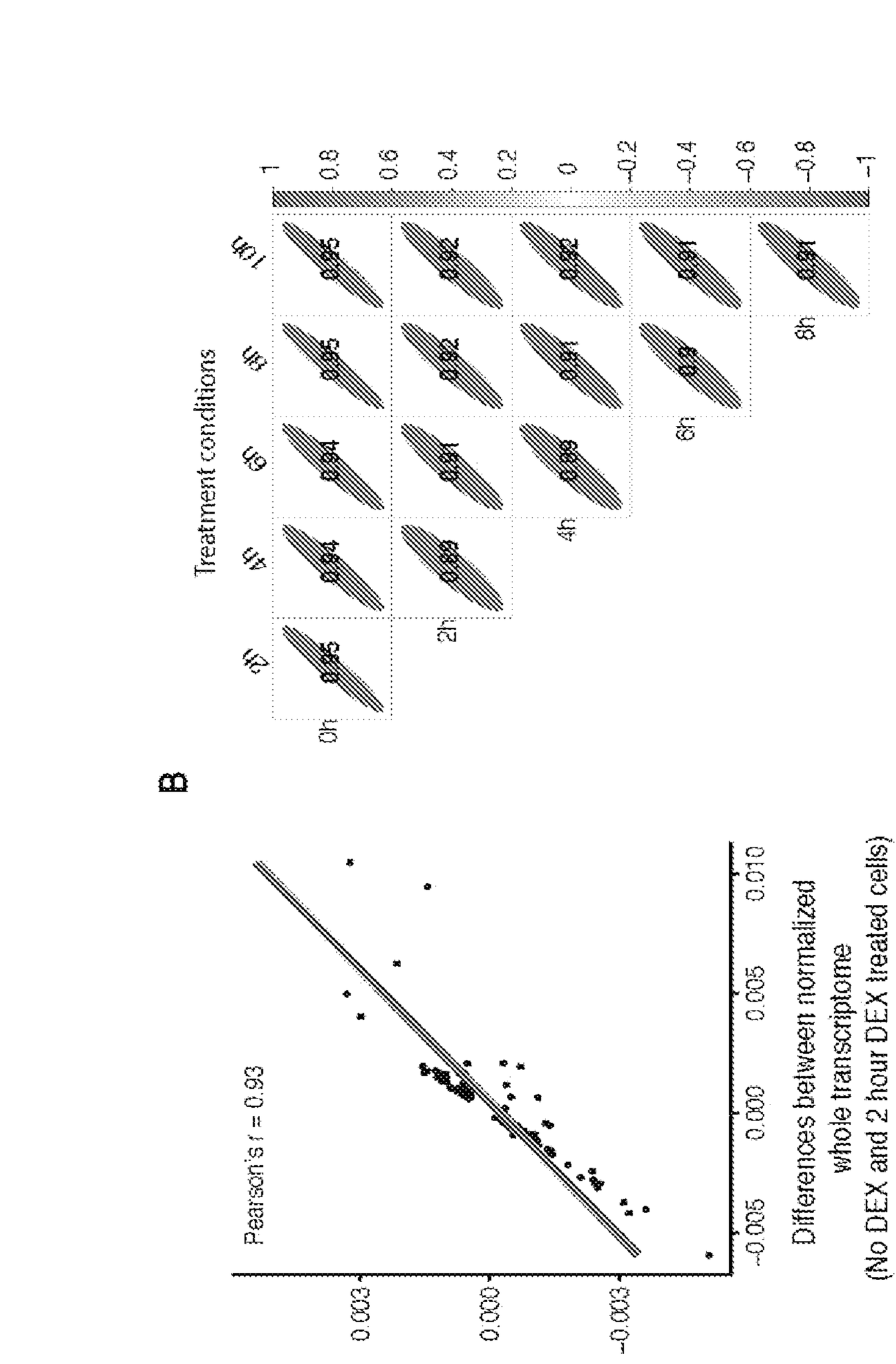

A
Differences between normalized
newly synthesised transcriptome
(No DEX and 2 hour DEX treated cells)
0.003
0.000
−0.003
B
Pearson's r = 0.93
−0.005
0.000
0.005
0.010
Differences between normalized
whole transcriptome
(No DEX and 2 hour DEX treated cells)
0h
2h
4h
6h
8h
Treatment conditions
u01
u3
u9
u4
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1

FIG. 37

A
Predicted ratio of cell state (2h)
r = 0.96
Ratio of cell states (2h)
Predicted ratio of cell state (4h)
r = 0.92
Ratio of cell states (4h)
Predicted ratio of cell state (8h)
r = 0.99
Ratio of cell states (8h)
Predicted ratio of cell state (10h)
r = 0.98
Ratio of cell states (10h)
B
Predicted ratio of cell states (10h)
by part of the data (0h-6h)
r = 0.95
Ratio of cell states (10h)
Predicted ratio of cell state (6h)
r = 0.98
Ratio of cell states (6h)

FIG. 38

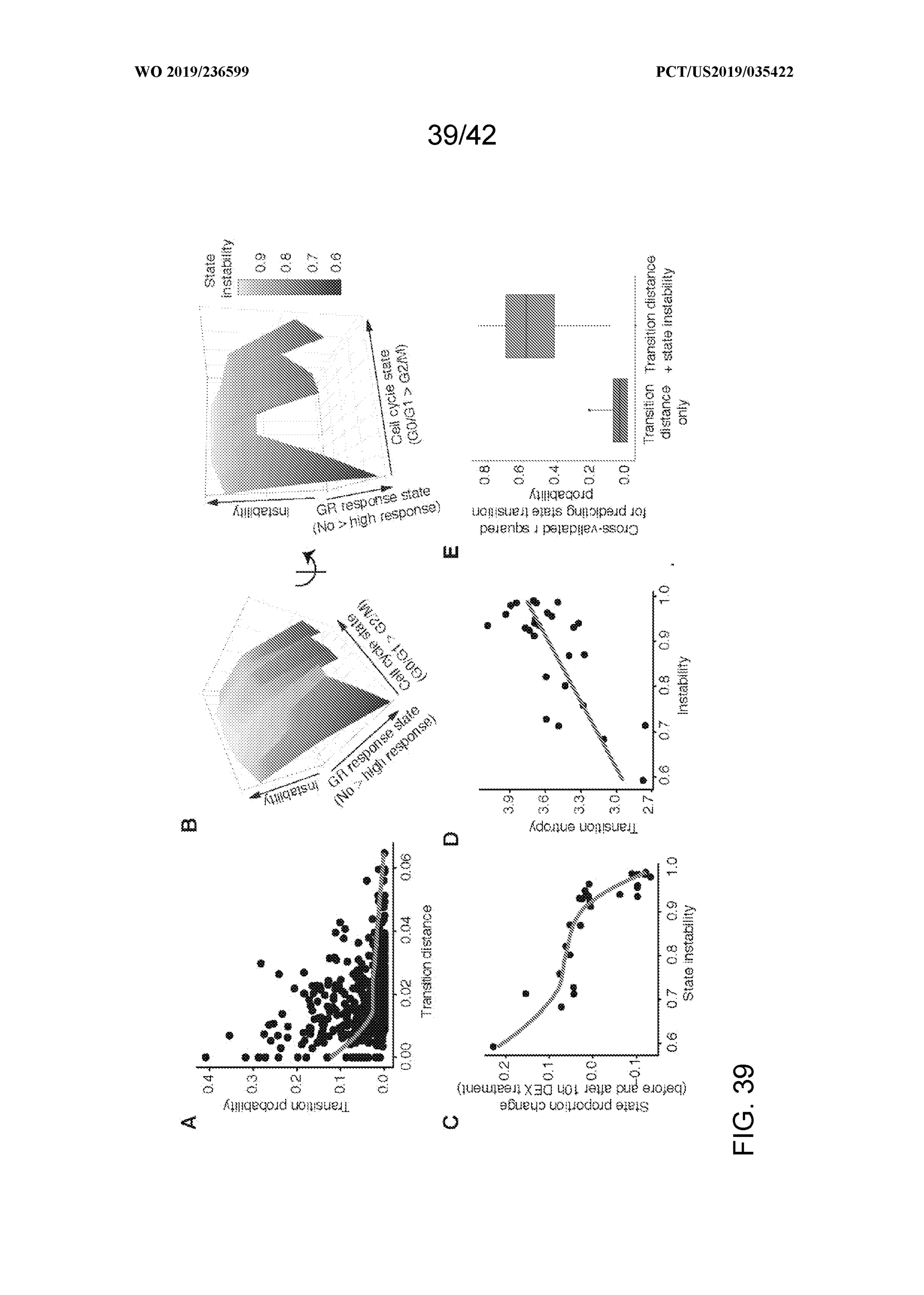

A
Transition probability
0.0
0.1
0.2
0.3
0.4
Transition distance
0.00
0.02
0.04
0.06
B
Instability
GR response state
(No > high response)
Cell cycle state
(G0/G1 > G2/M)
C
State proportion change
(before and after 10n DEX treatment)
0.2
0.1
0.0
−0.1
State instability
0.6
0.7
0.8
0.9
1.0
D
Transition entropy
2.7
3.0
3.3
3.6
3.9
Instability
0.6
0.7
0.8
0.9
1.0
E
Cross-validated r squared
for predicting state transition
probability
0.0
0.2
0.4
0.6
0.8
Transition distance
Transition distance
+ state instability
only

FIG. 39

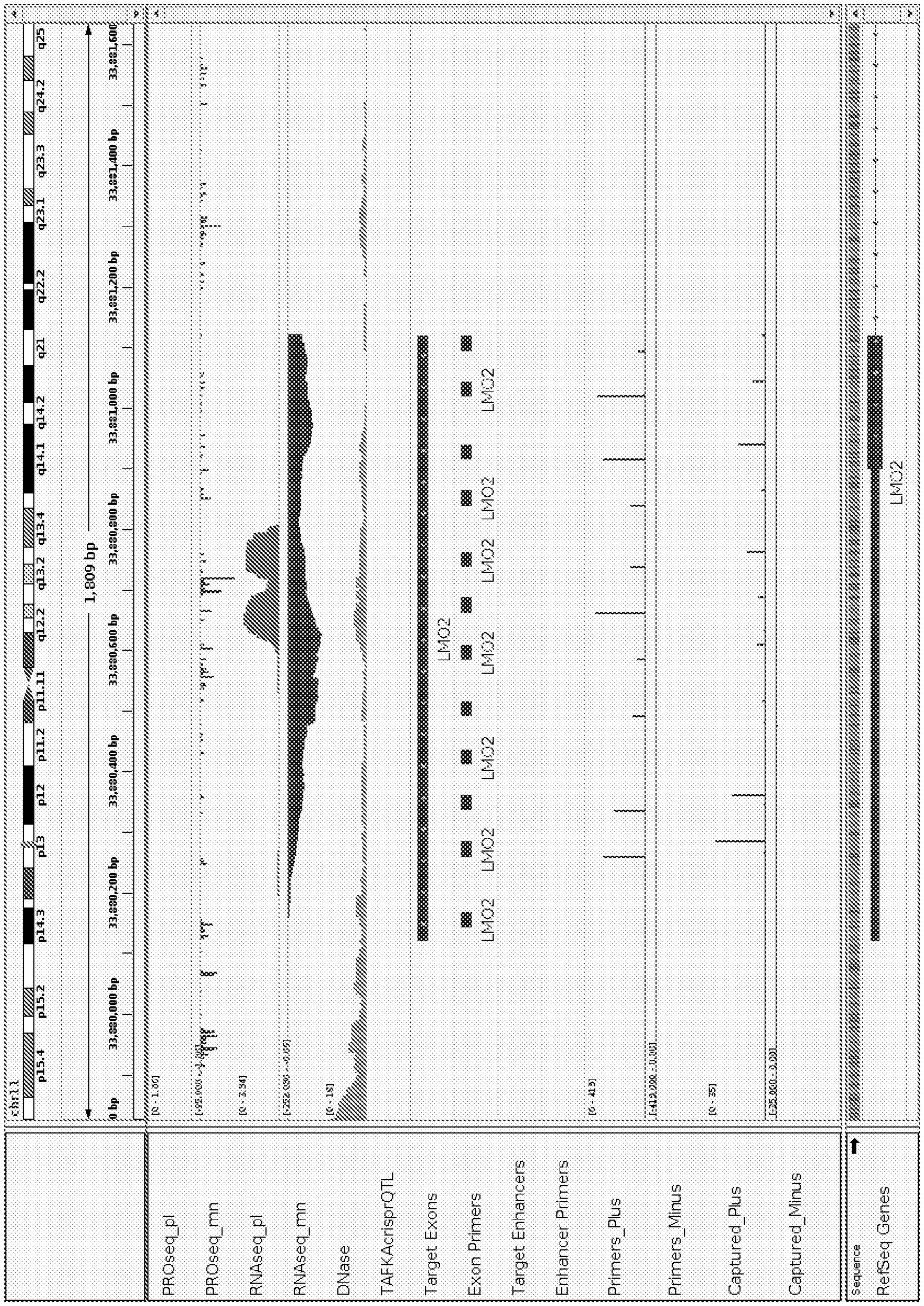

chr11
p15.4
p15.2
0 bp
33,830,000 bp
1,809 bp
PROseq_pl
PROseq_mn
RNAseq_pl
RNAseq_mn
DNase
TAFKAcrisprQTL
Target Exons
Exon Primers
Target Enhancers
Enhancer Primers
Primers_Plus
Primers_Minus
Captured_Plus
Captured_Minus
Sequence
RefSeq Genes
LMO2

FIG. 40

Chr	Start	End	Gene	TotalCounts	ENCODE	Rank	ENCODE
chrM	1672	3230	RNR2	4566	653212	1	1
chr11	34073229	34124157	CAPRIN1	2061	2344	2	451
chr11	33880122	33913836	LMO2	1983	376	3	4627
chrM	649	1603	RNR1	1017	156276	4	4
chr11	34127110	34168458	NAT10	641	1075	5	1489
chr11	33724555	33758025	CD59	420	2503	6	396
chr11	34460471	34493607	CAT	395	263	7	5919
chr11	65265232	65273983	MALAT1	291	443474	8	2
chr11	33278217	33378568	HIPK3	238	505	9	3550
chr22	21061978	21213100	PI4KA	92	4667	10	120
chr11	34937676	35017675	PDHX	90	146	11	7928
chr9	37779710	37785089	EXOSC3	72	356	12	4827

FIG. 41

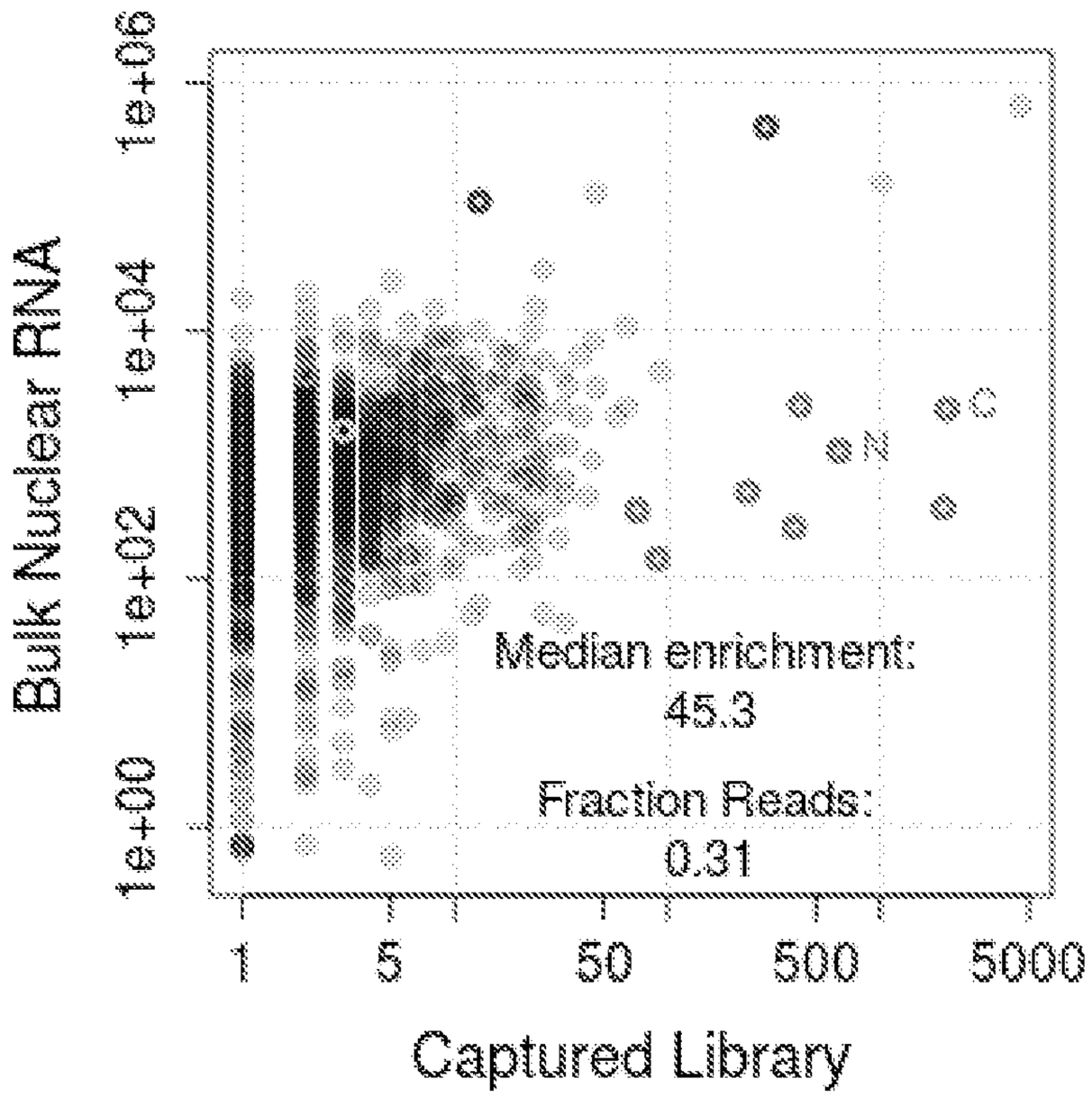

WT4k_S6
Bulk Nuclear RNA
1e+00
1e+02
1e+04
1e+06
1
5
50
500
5000
Captured Library
C
N
Median enrichment:
45.3
Fraction Reads:
0.31

FIG. 42