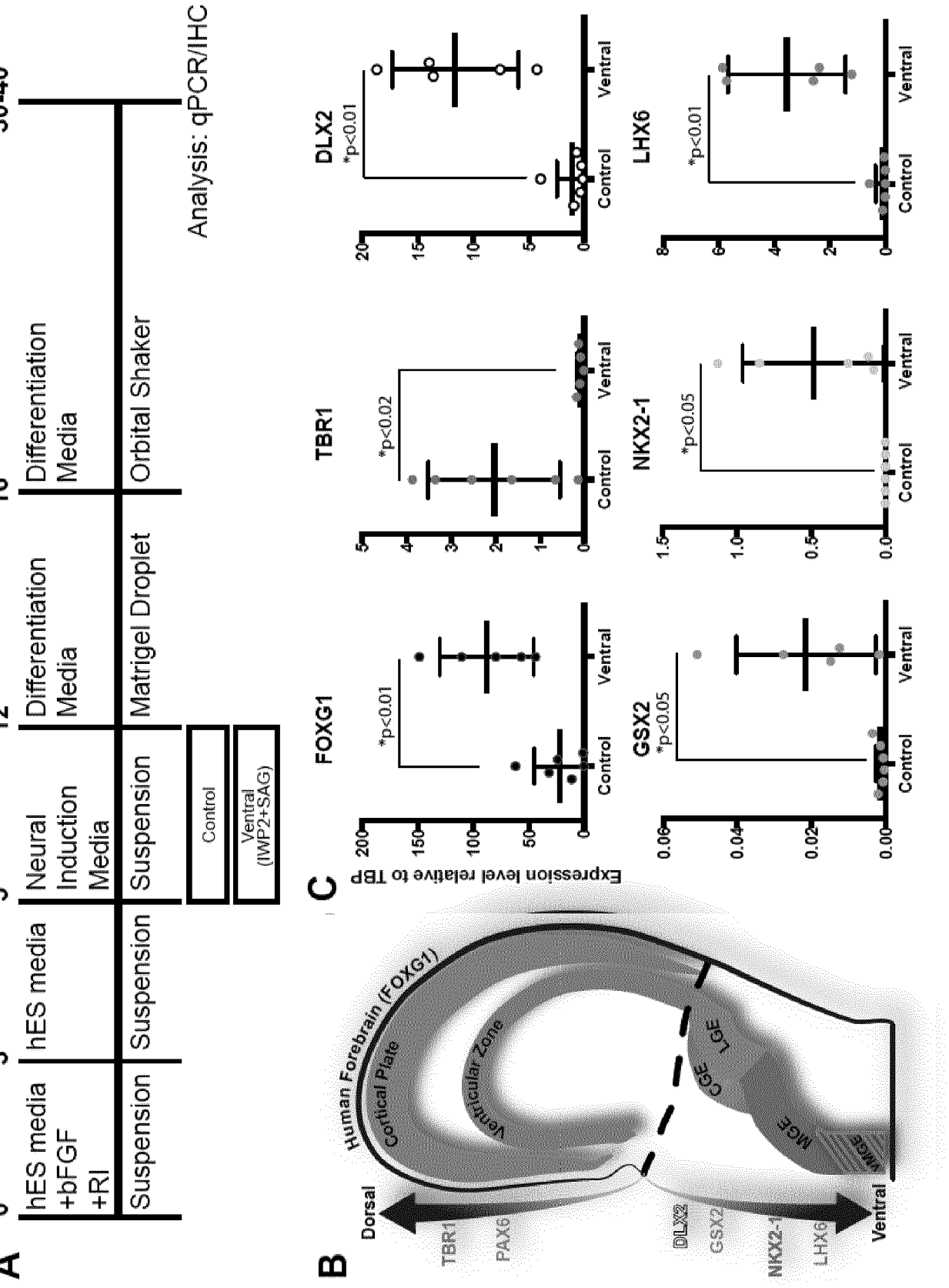
Fig. 1


A
0
3
5
12
16
30-40
hES media +bFGF +RI
hES media
Neural Induction Media
Differentiation Media
Differentiation Media
Suspension
Suspension
Suspension
Matrigel Droplet
Orbital Shaker
Control
Ventral (IWP2+SAG)
Analysis: qPCR/IHC
B
Dorsal
Ventral
Human Forebrain (FOXG1)
Cortical Plate
Ventricular Zone
CGE
LGE
MGE
vMGE
TBR1
PAX6
DLX2
GSX2
NKX2-1
LHX6
C
FOXG1
TBR1
DLX2
GSX2
NKX2-1
LHX6
Expression level relative to TBP
*p<0.01
*p<0.02
*p<0.01
*p<0.05
*p<0.05
*p<0.01
Control
Ventral

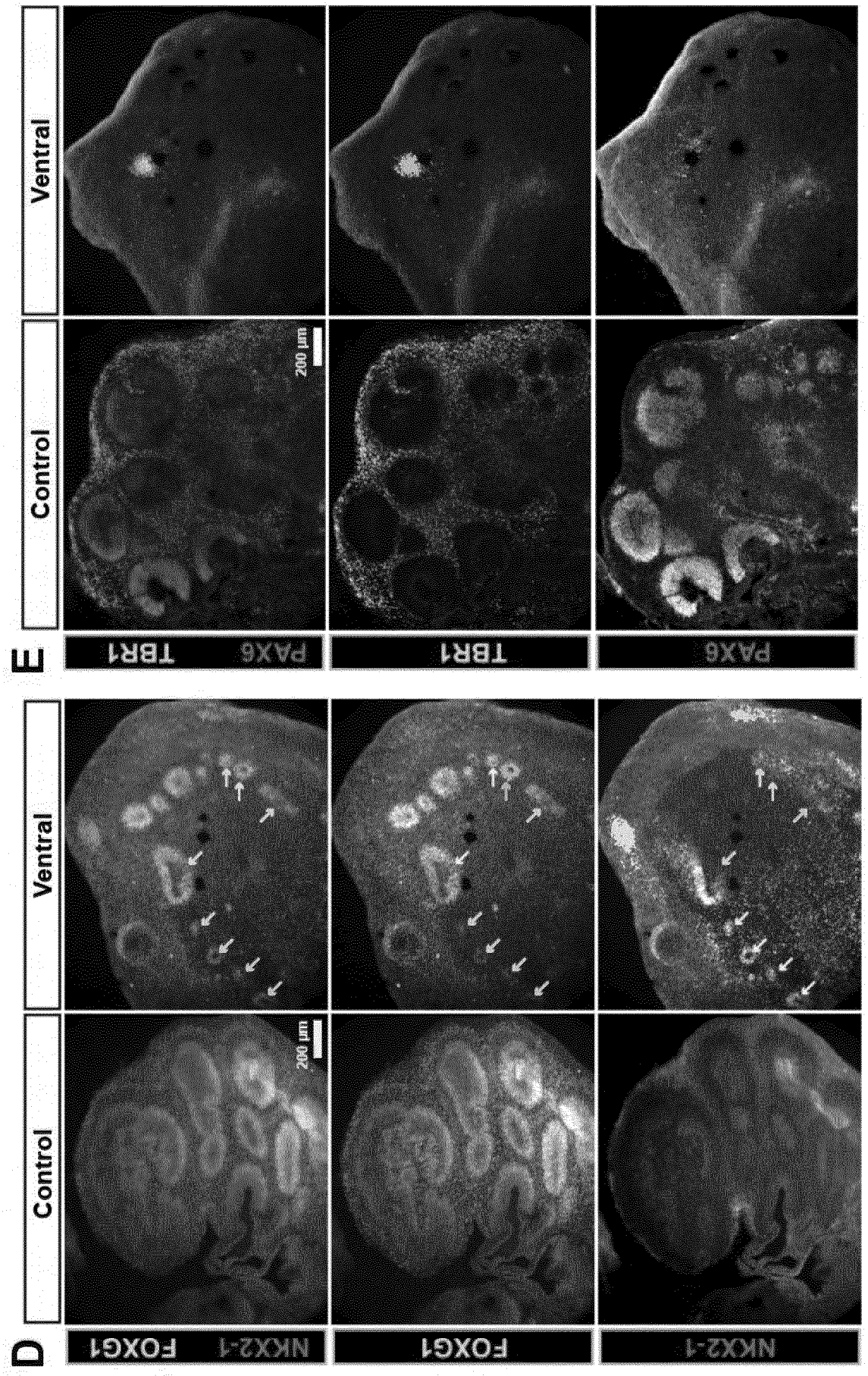

D
Control
Ventral
NKX2-1 FOXG1
FOXG1
NKX2-1
200 µm
E
Control
Ventral
PAX6 TBR1
TBR1
PAX6
200 µm

Fig. 1

Fig. 2

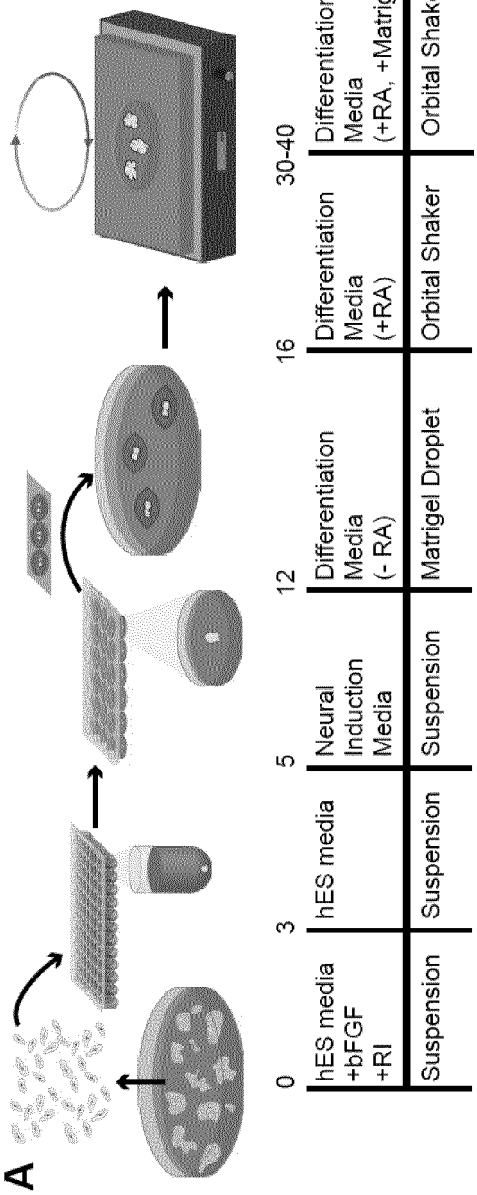

A
0
3
5
12
16
30-40
60-100
hES media +bFGF +RI
hES media
Neural Induction Media
Differentiation Media (- RA)
Differentiation Media (+RA)
Differentiation Media (+RA, +Matrigel)
Suspension
Suspension
Suspension
Matrigel Droplet
Orbital Shaker
Orbital Shaker

Fig. 2

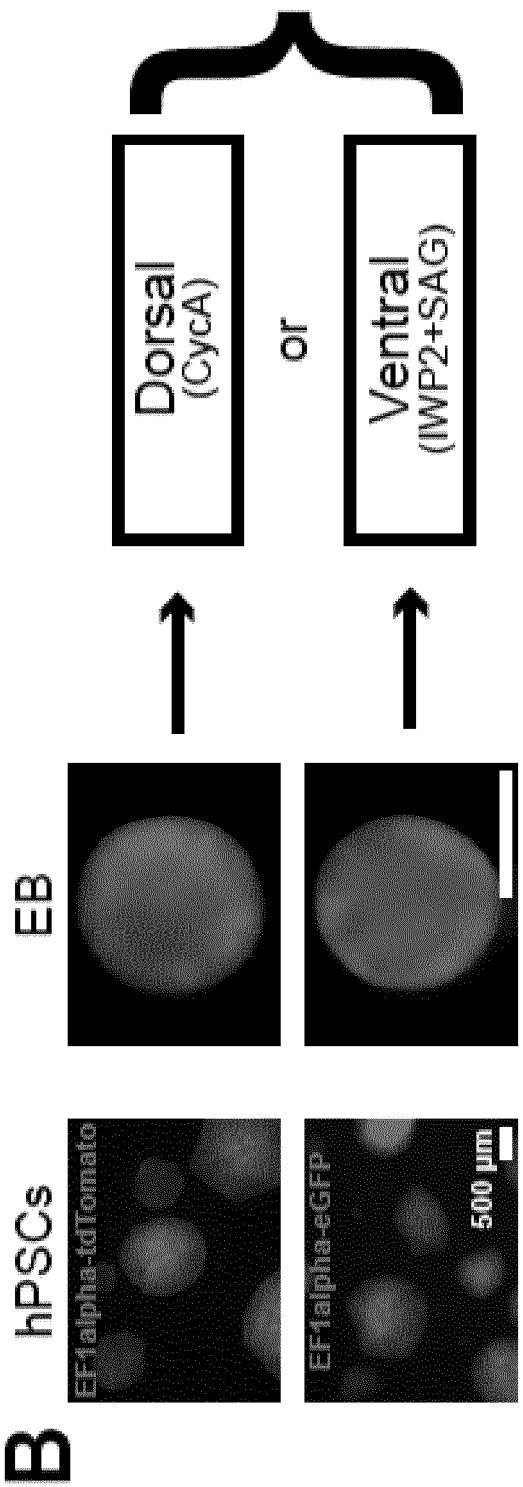


Fig. 2B
Continuation

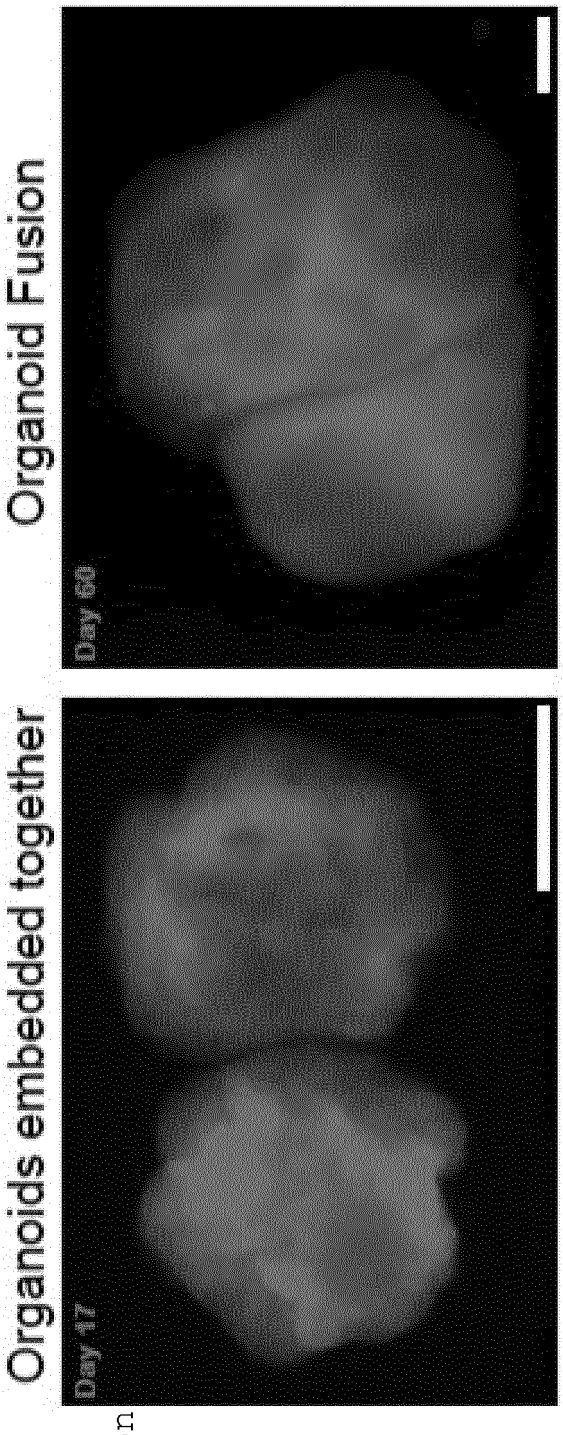
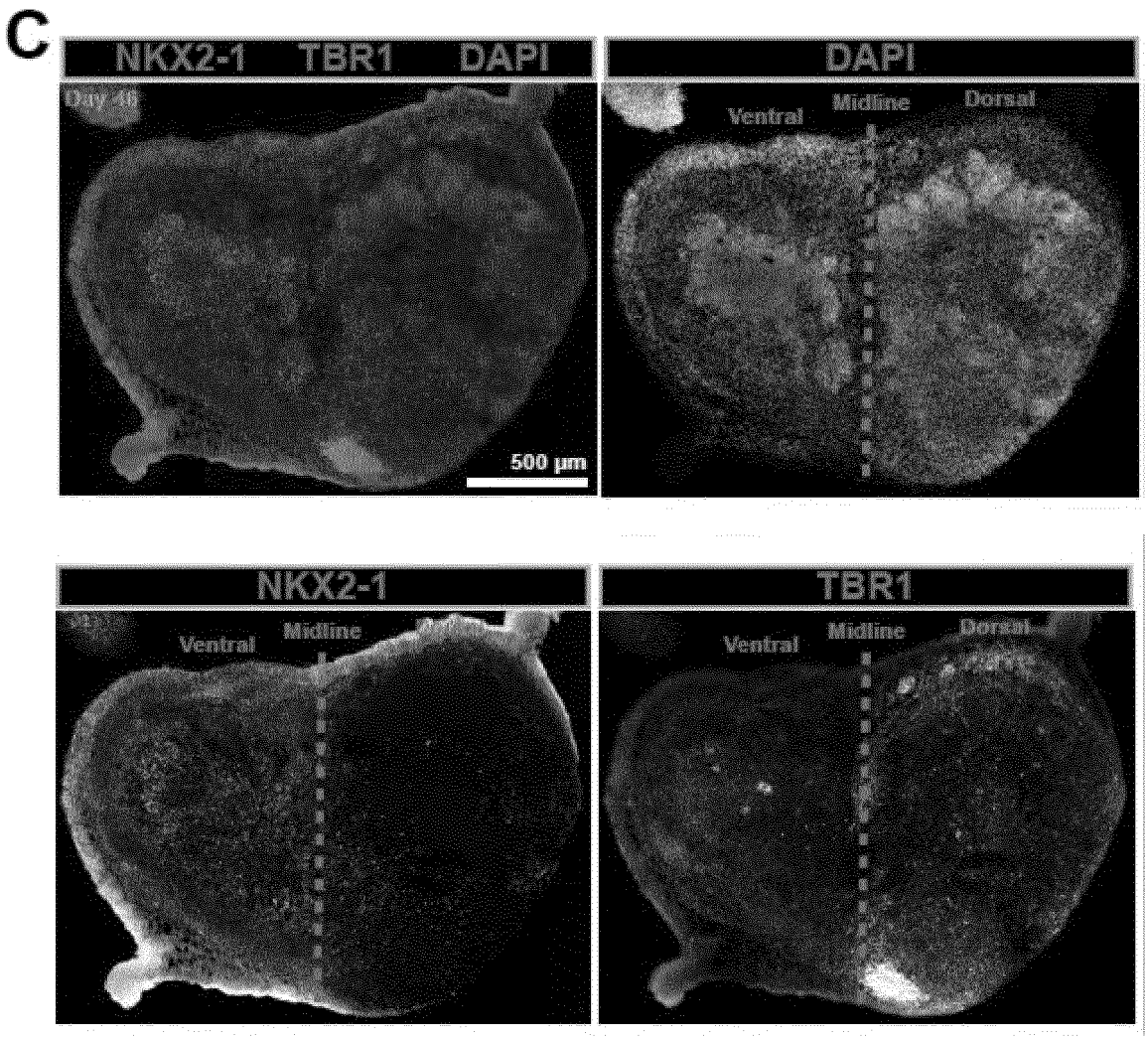
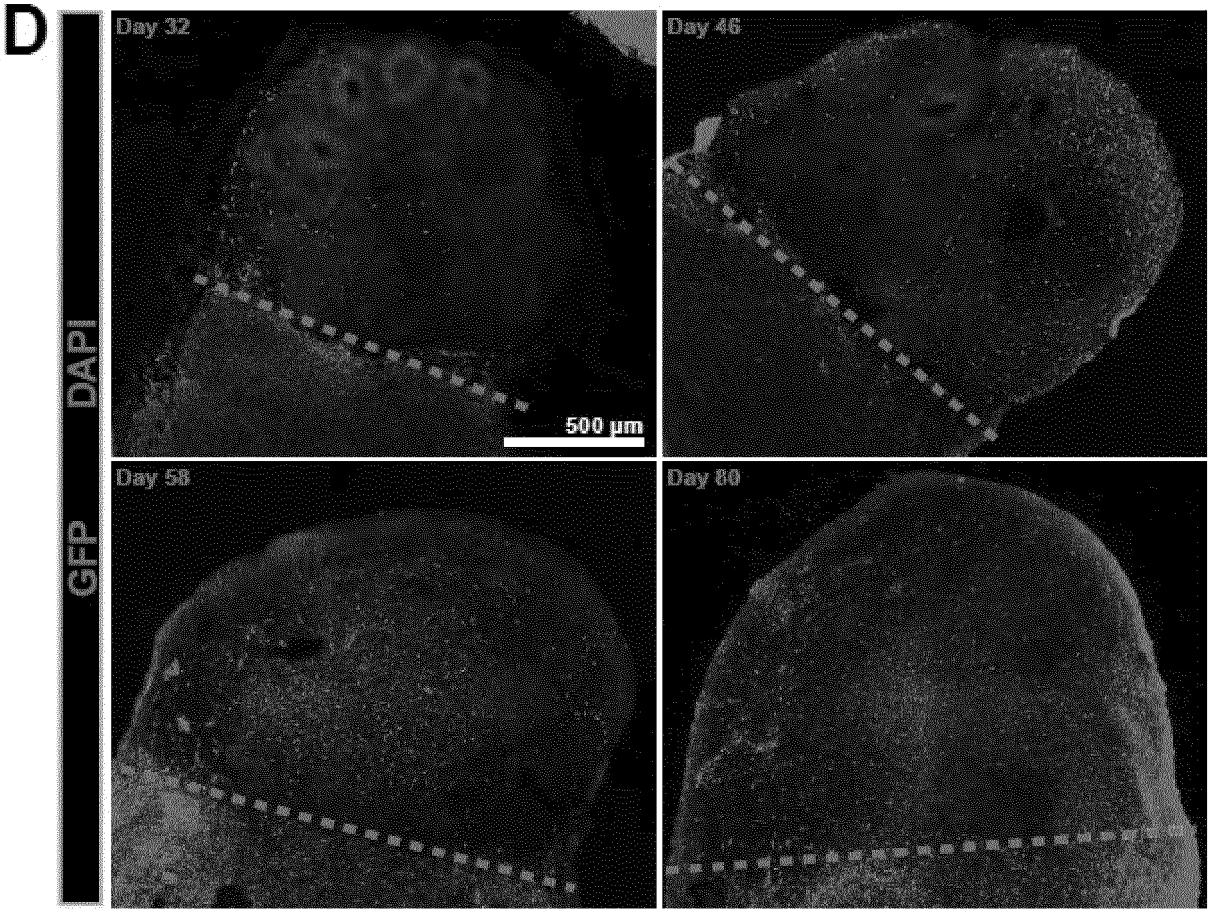


Fig. 2


C
NKX2-1 TBR1 DAPI
Day 46
500 µm
DAPI
Ventral
Midline
Dorsal
NKX2-1
Ventral
Midline
TBR1
Ventral
Midline
Dorsal


D
DAPI
GFP
Day 32
Day 46
Day 58
Day 80
500 µm

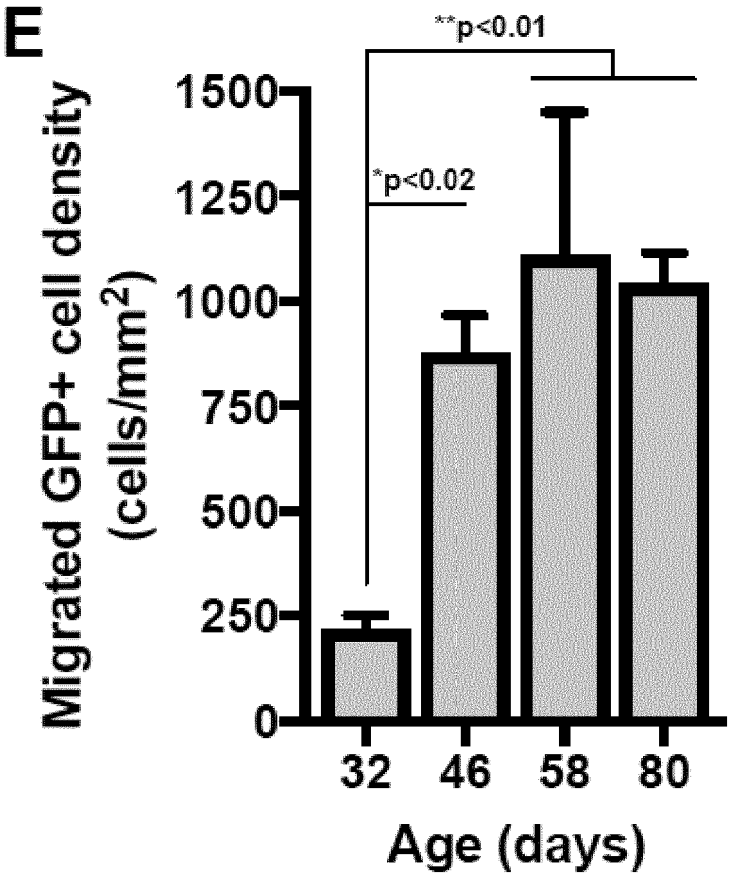

E
**p<0.01
*p<0.02
Migrated GFP+ cell density
(cells/mm2)
1500
1250
1000
750
500
250
0
32
46
58
80
Age (days)

Fig. 2

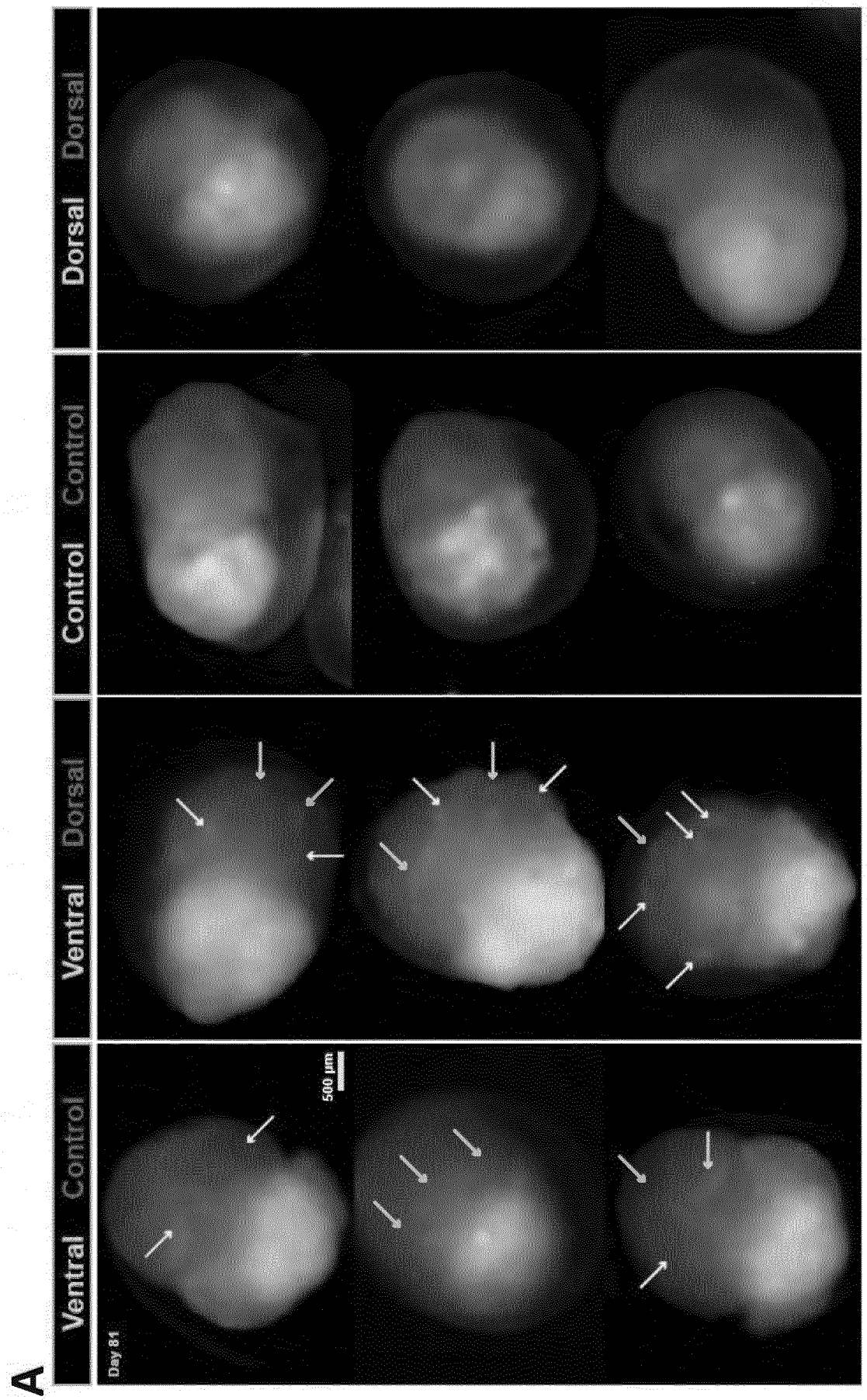
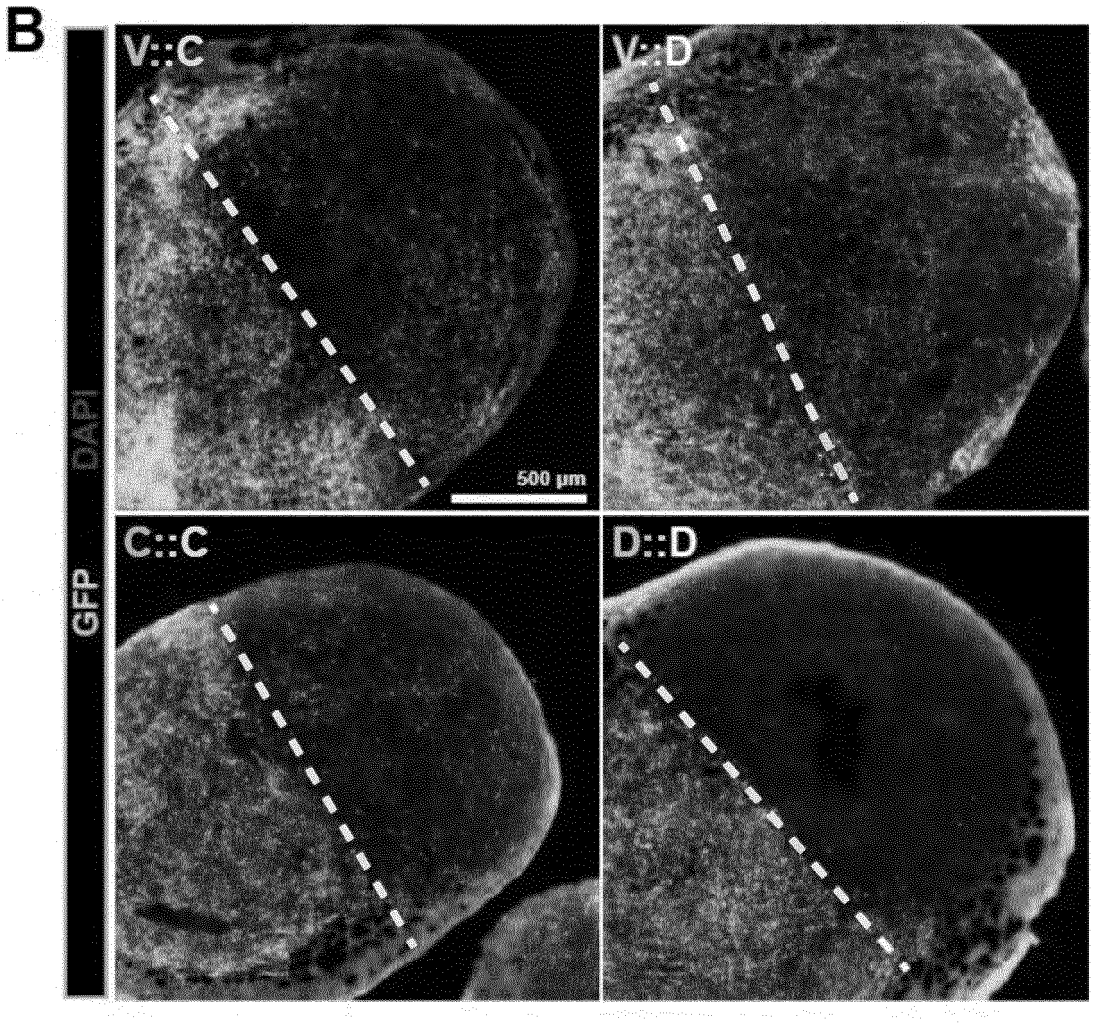

A
Ventral Control
Ventral Dorsal
Control Control
Dorsal Dorsal
Day 81
500 µm

Fig. 3


B
V::C
V::D
C::C
D::D
DAPI
GFP
500 µm

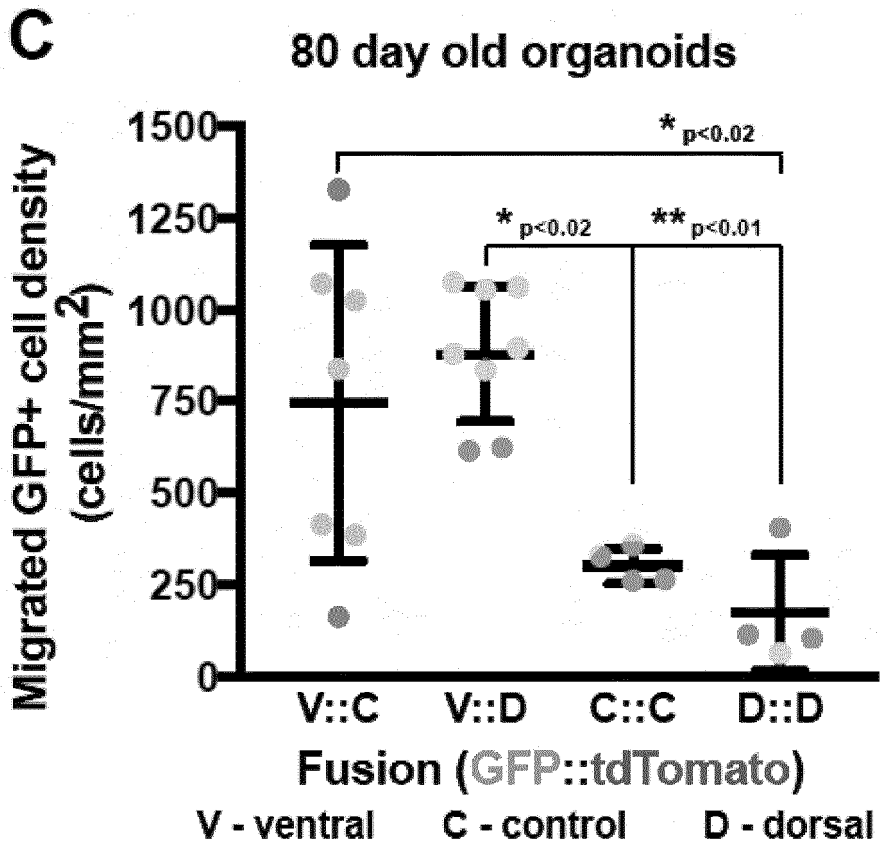

C
80 day old organoids
Migrated GFP+ cell density (cells/mm2)
1500
1250
1000
750
500
250
0
* p<0.02
* p<0.02
** p<0.01
V::C
V::D
C::C
D::D
Fusion (GFP::tdTomato)
V - ventral
C - control
D - dorsal

Fig. 3

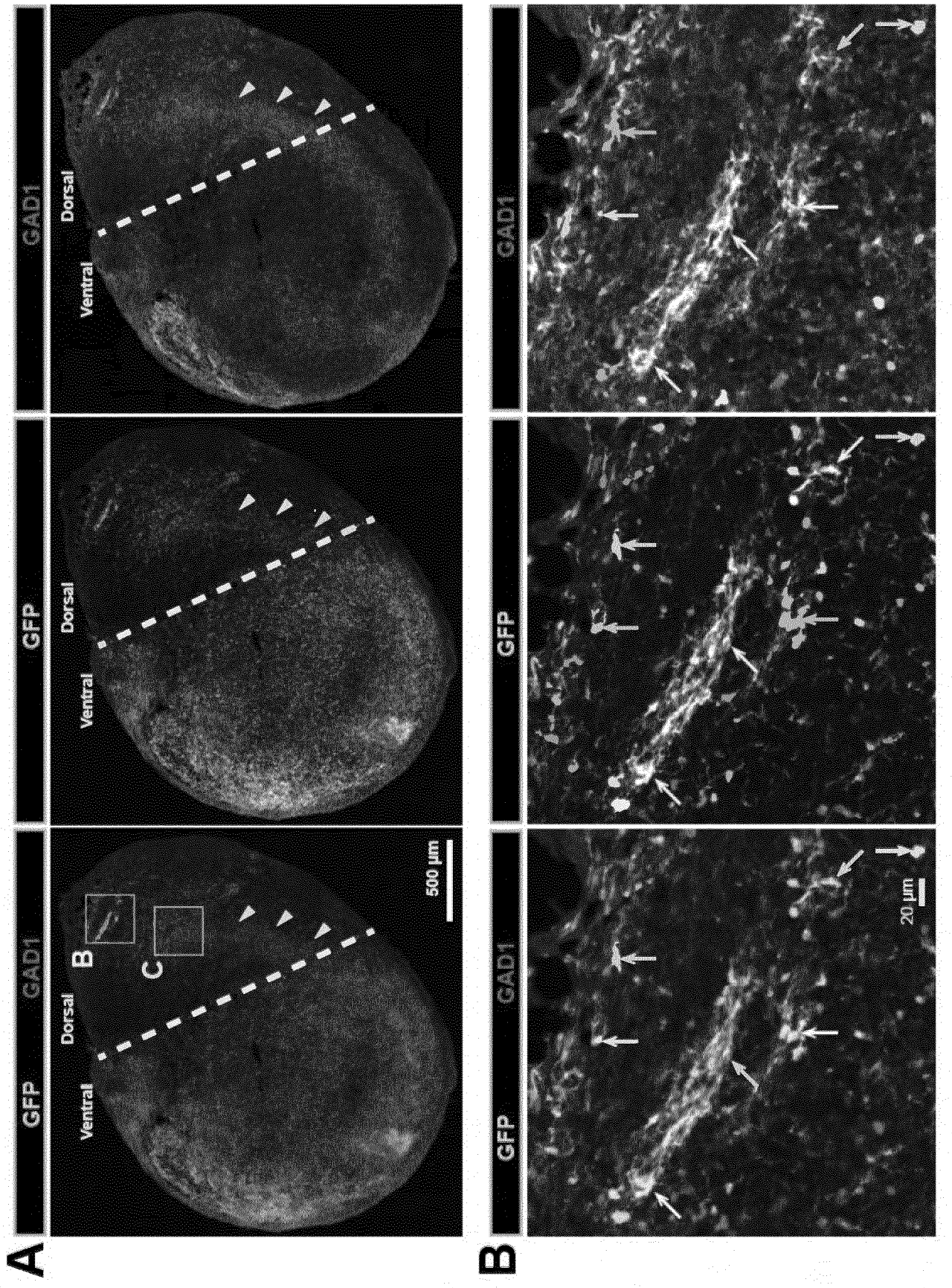


Fig. 4

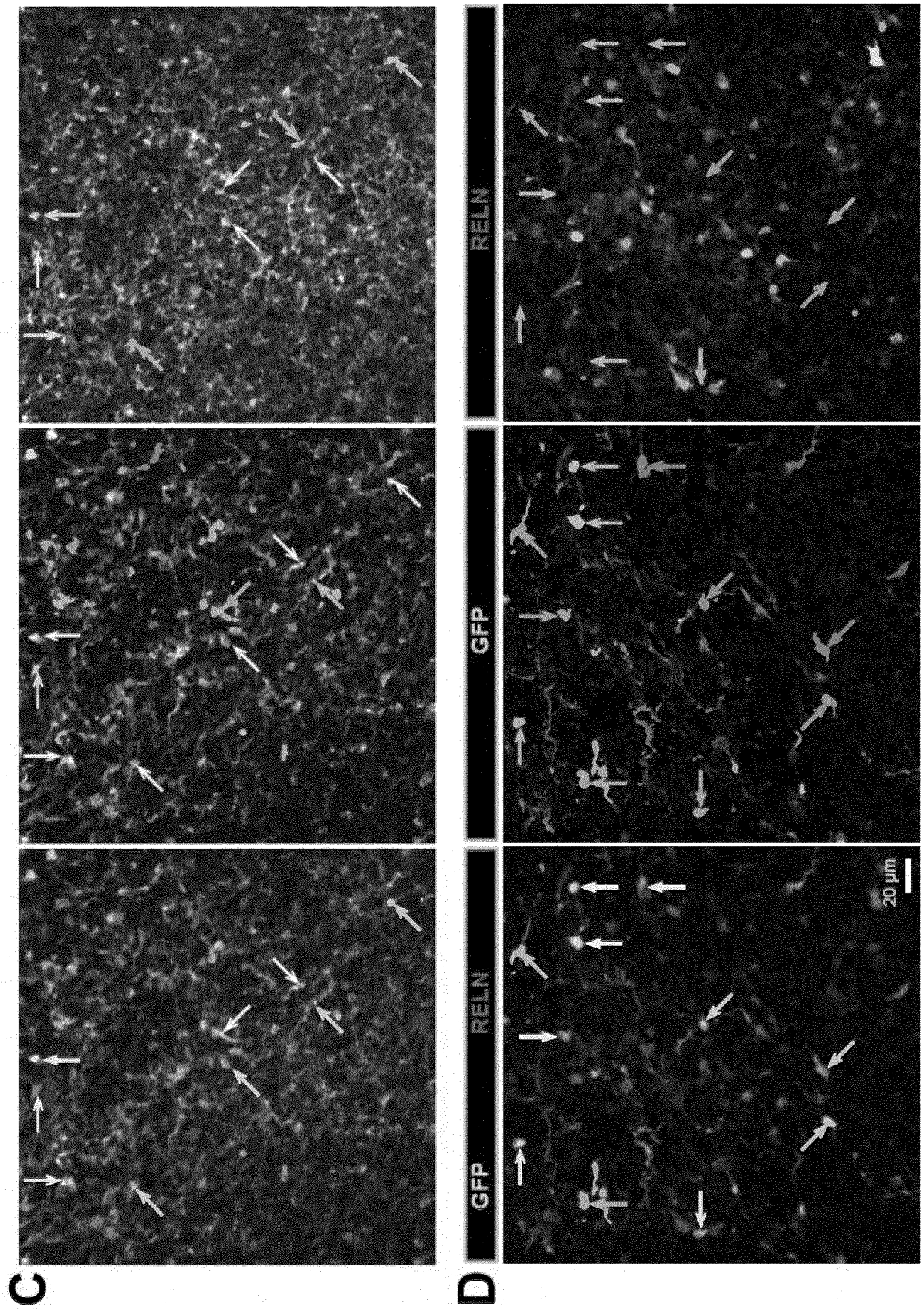


Fig. 4

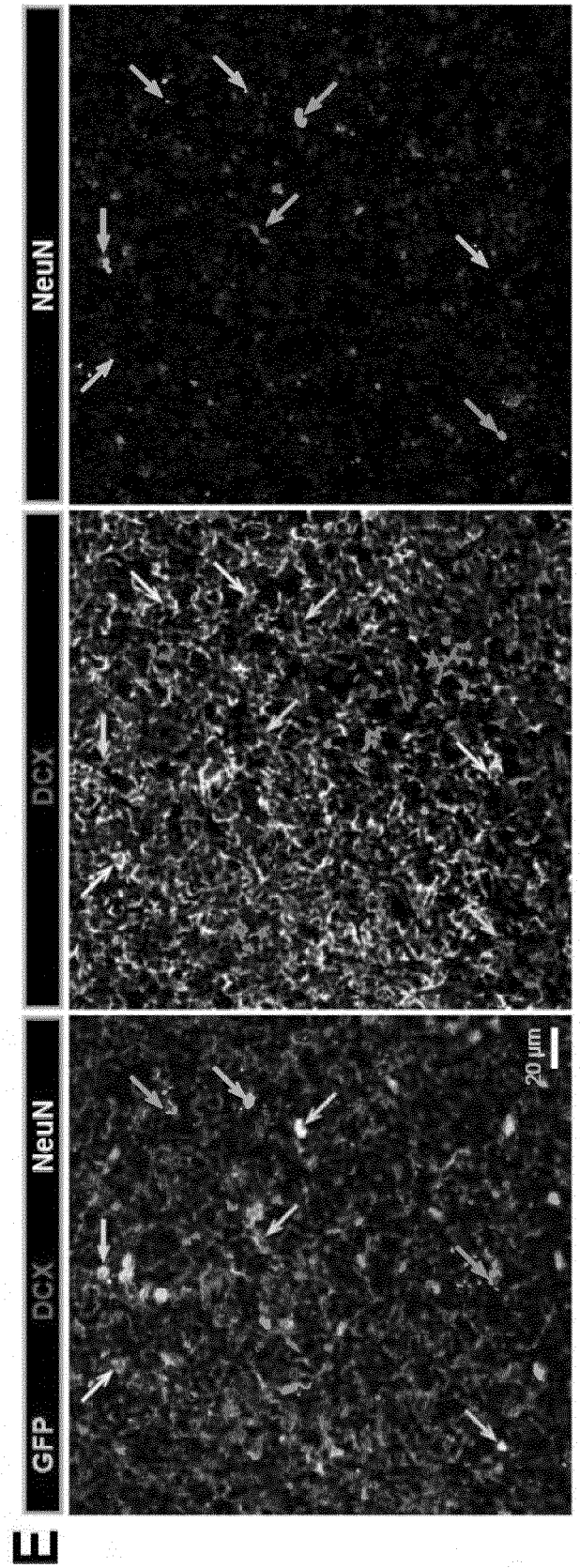


Fig. 4

Fig. 5

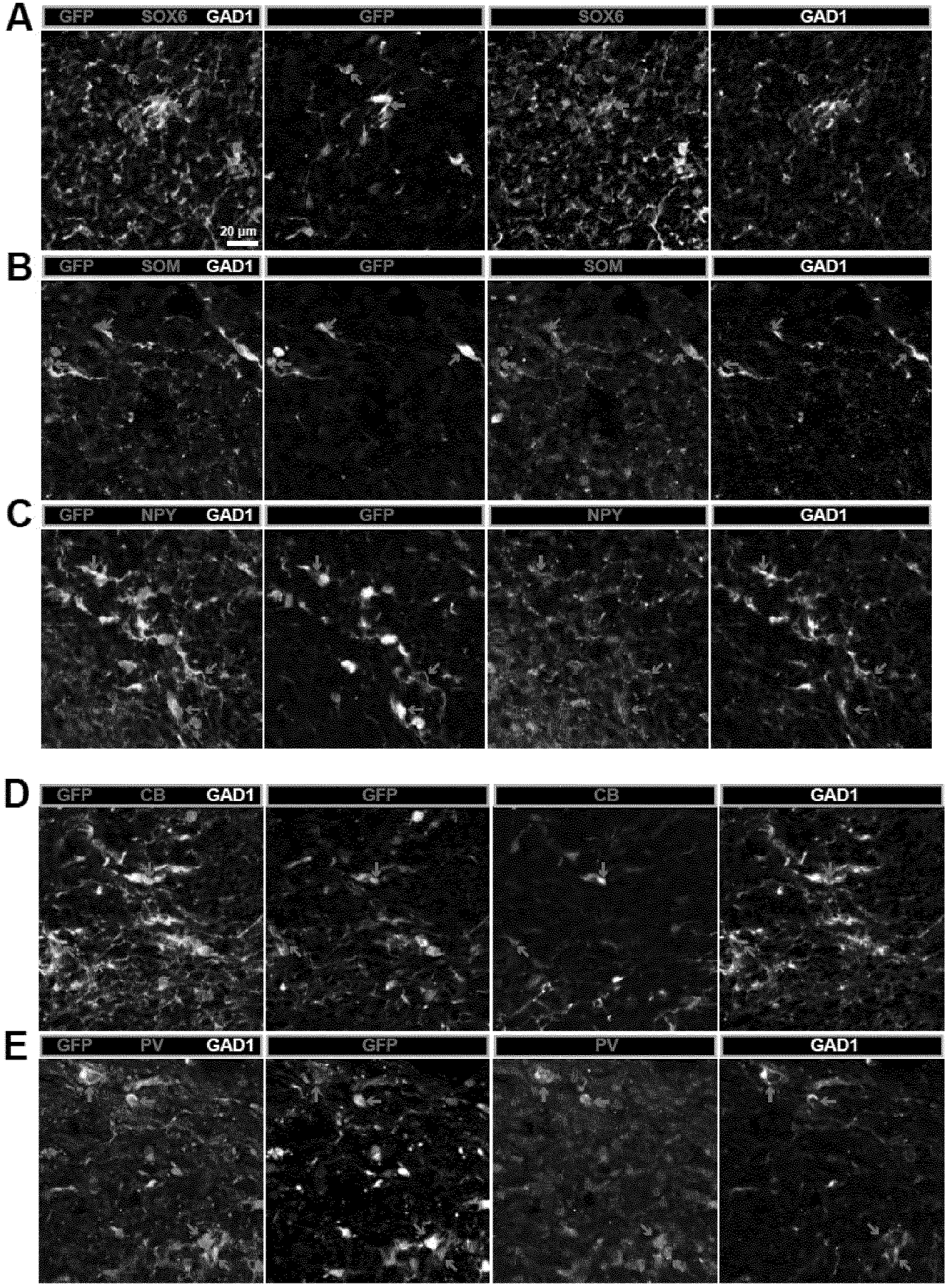
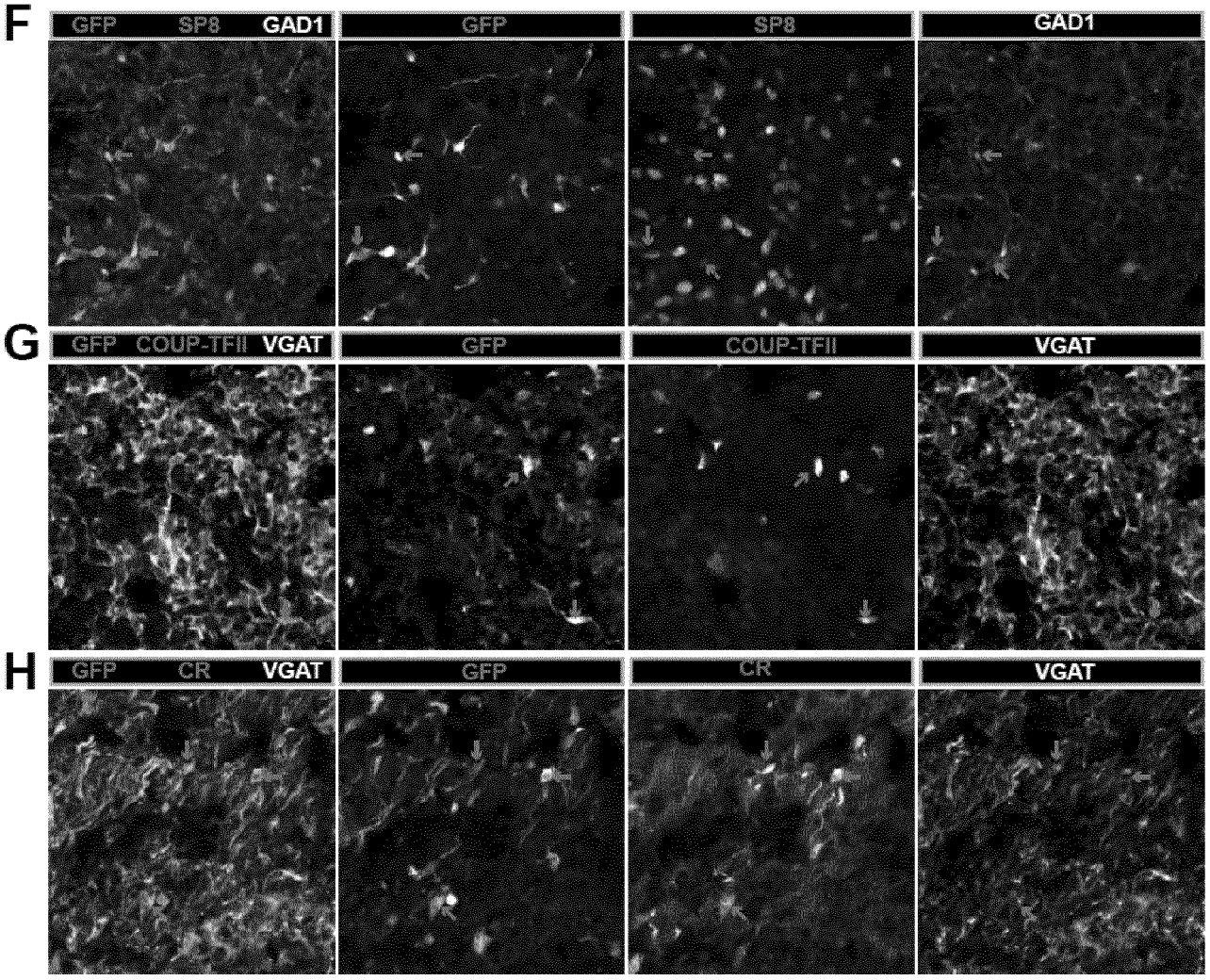

A
GFP SOX6 GAD1
GFP
SOX6
GAD1
20 µm
B
GFP SOM GAD1
GFP
SOM
GAD1
C
GFP NPY GAD1
GFP
NPY
GAD1
D
GFP CB GAD1
GFP
CB
GAD1
E
GFP PV GAD1
GFP
PV
GAD1

Fig. 5


F
GFP SP8 GAD1
GFP
SP8
GAD1
G
GFP COUP-TFII VGAT
GFP
COUP-TFII
VGAT
H
GFP CR VGAT
GFP
CR
VGAT

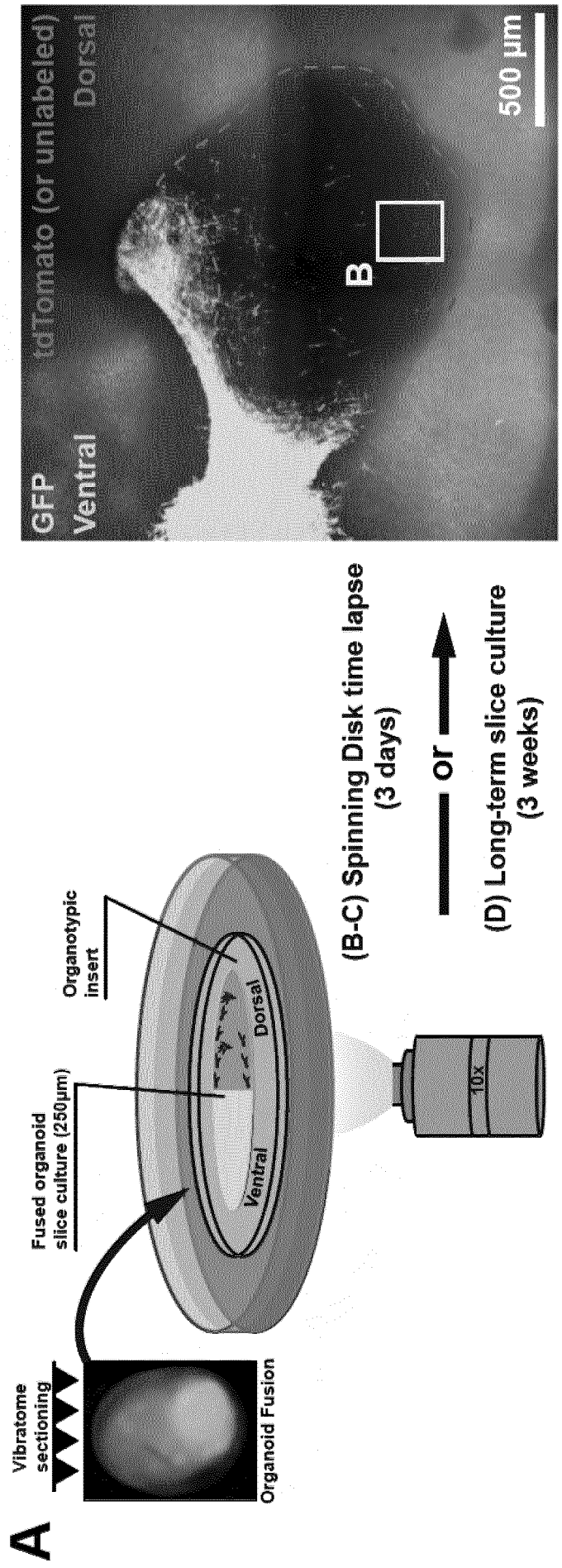

A
Vibratome sectioning
Organoid Fusion
Fused organoid slice culture (250µm)
Organotypic insert
Dorsal
Ventral
10x
(B-C) Spinning Disk time lapse (3 days)
or
(D) Long-term slice culture (3 weeks)
GFP
(dTomato (or unlabeled)
Ventral
Dorsal
B
500 µm

Fig. 6

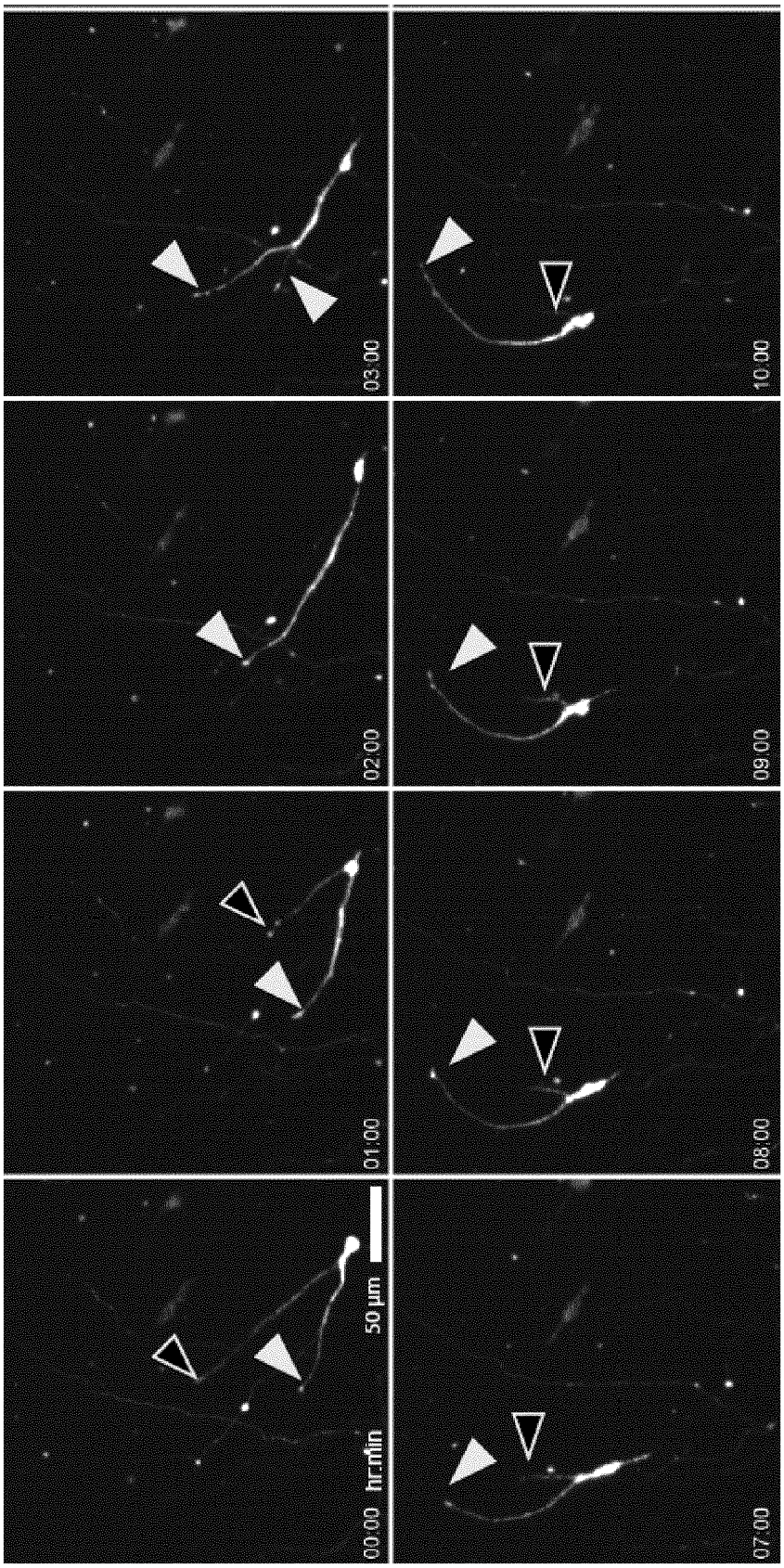


Fig. 6

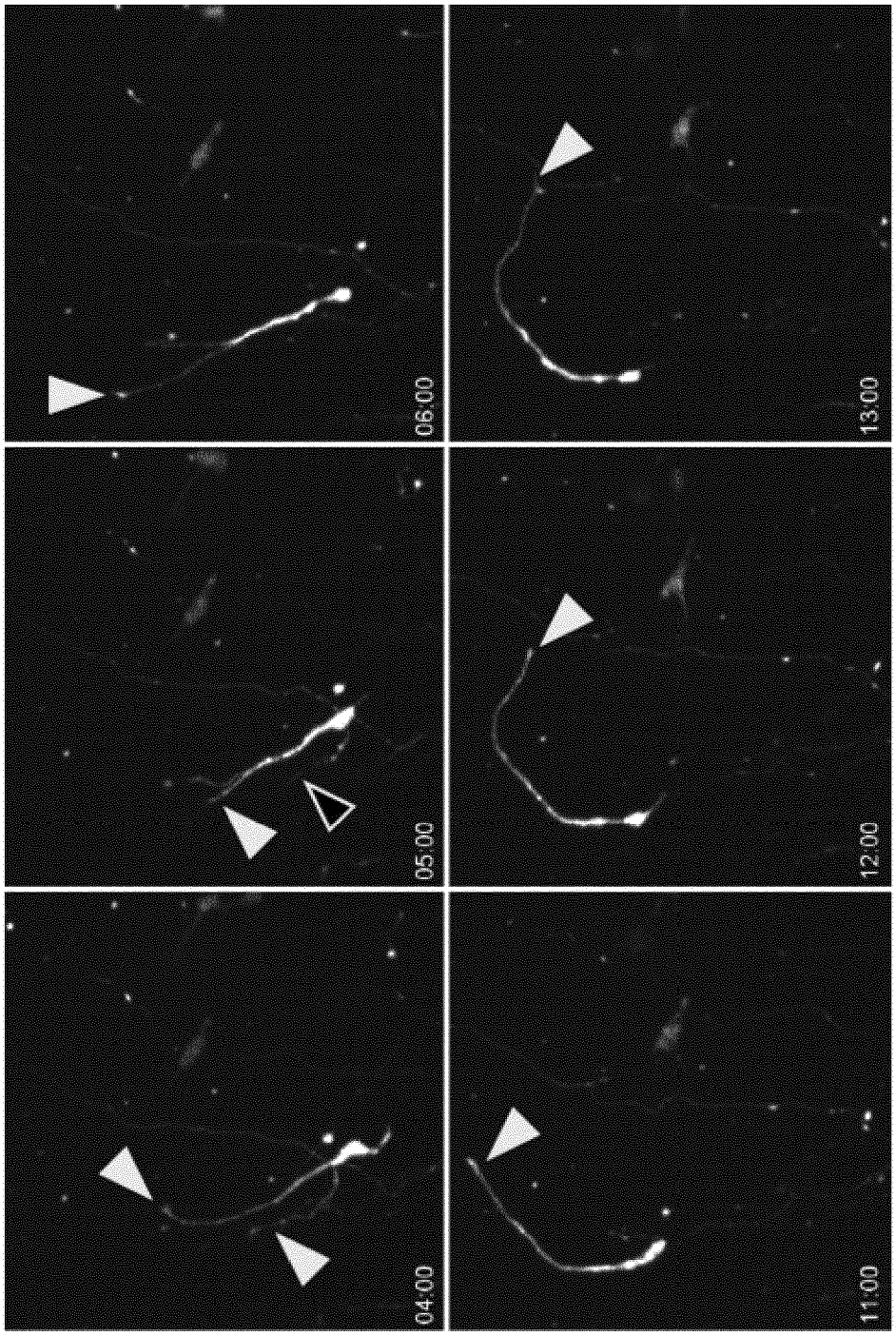


Fig. 6B Continuation

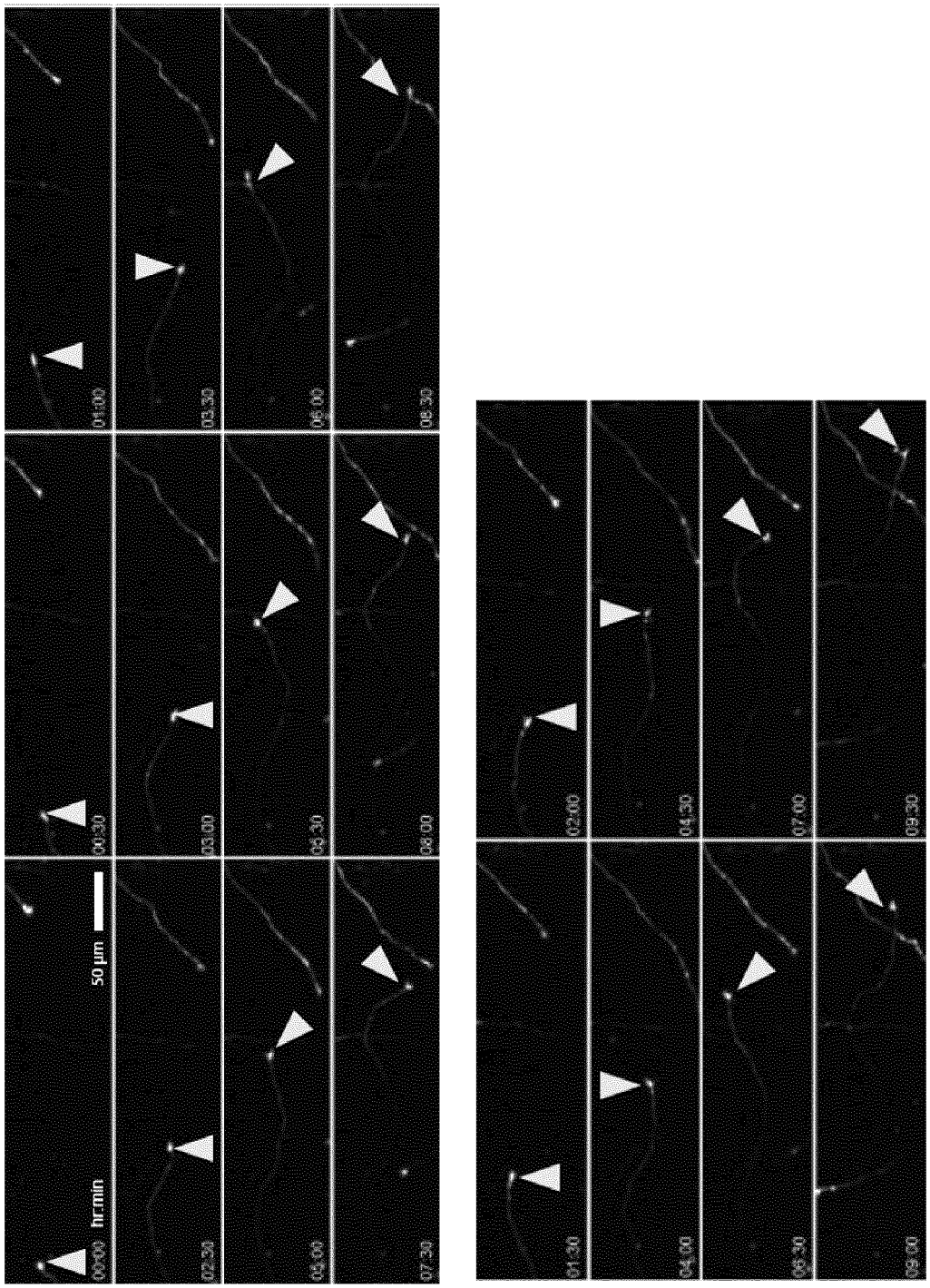
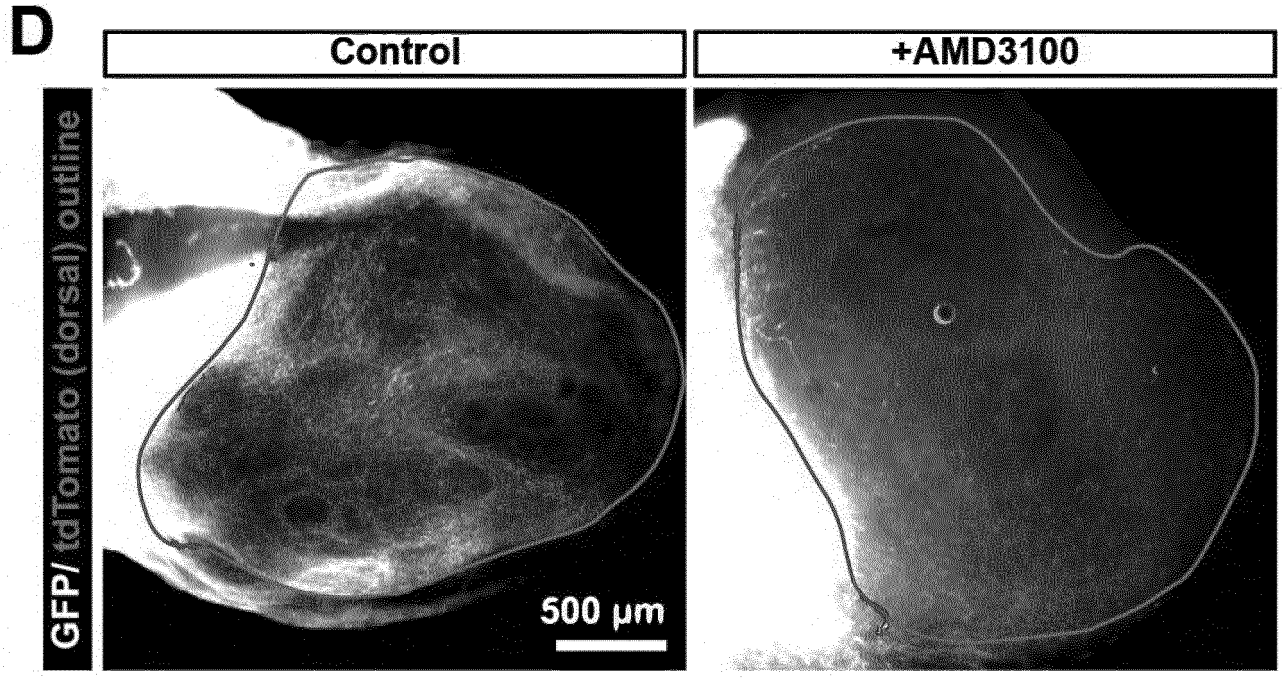


Fig.6C


D
Control
+AMD3100
GFP/ tdTomato (dorsal) outline
500 µm

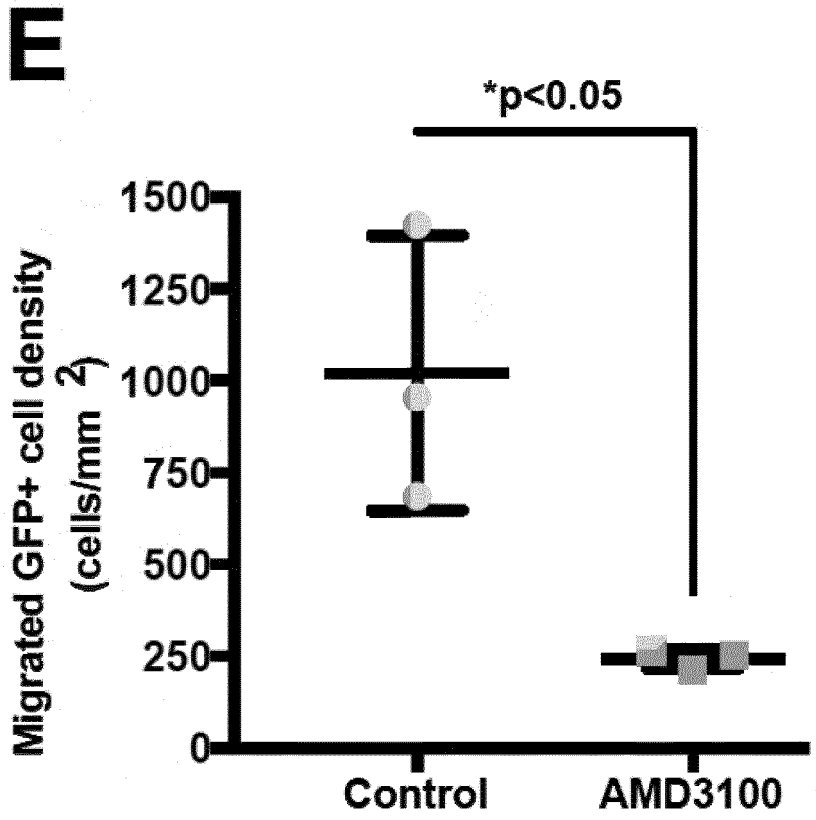

E
*p<0.05
Migrated GFP+ cell density (cells/mm 2)
1500
1250
1000
750
500
250
0
Control
AMD3100

Fig. 6