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Supplemental Information

Life-Long Neurogenic Activity of Individual Neural Stem Cells and Continuous Growth Establish an Outside-In Architecture in the Teleost Pallium

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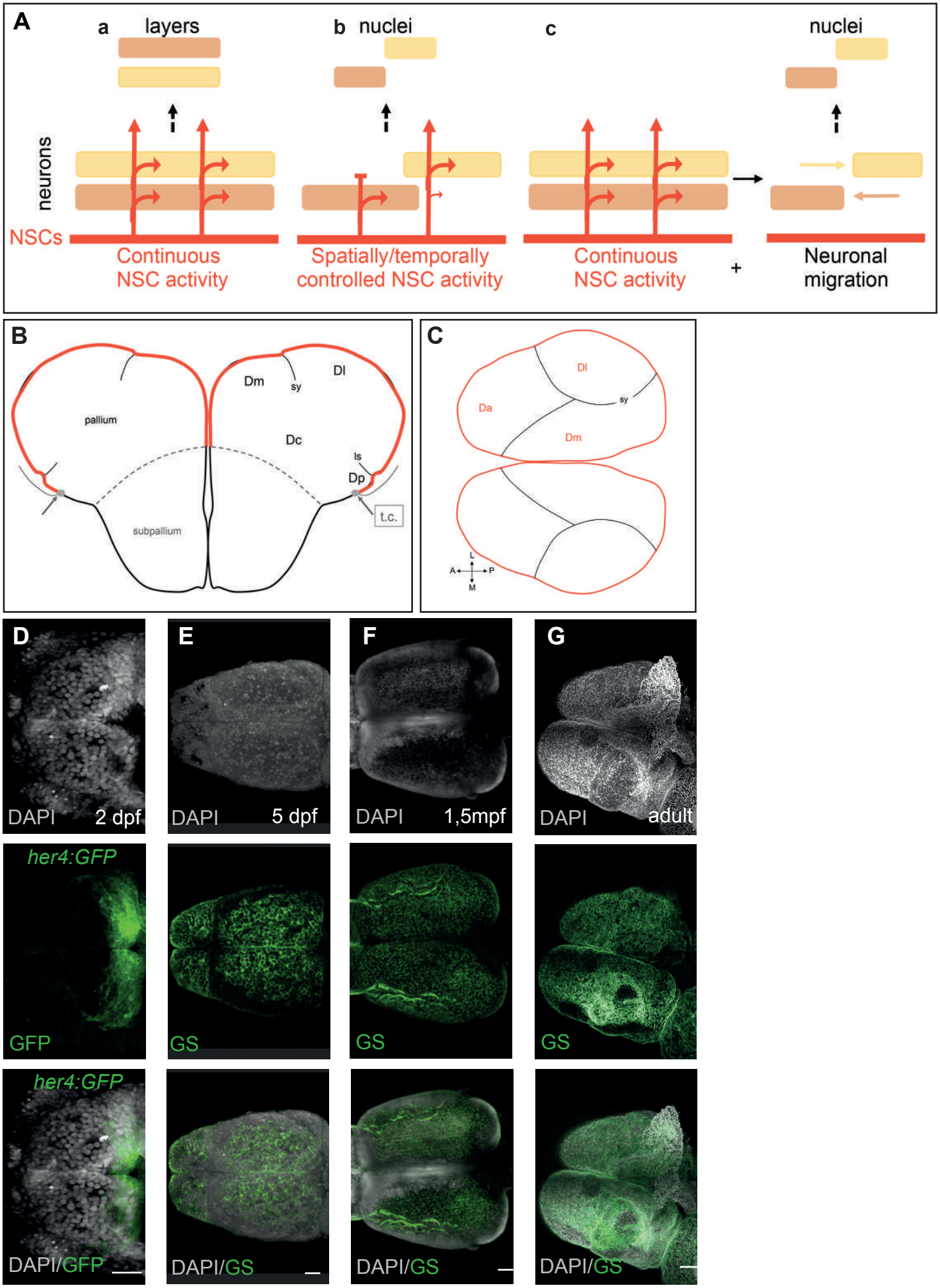
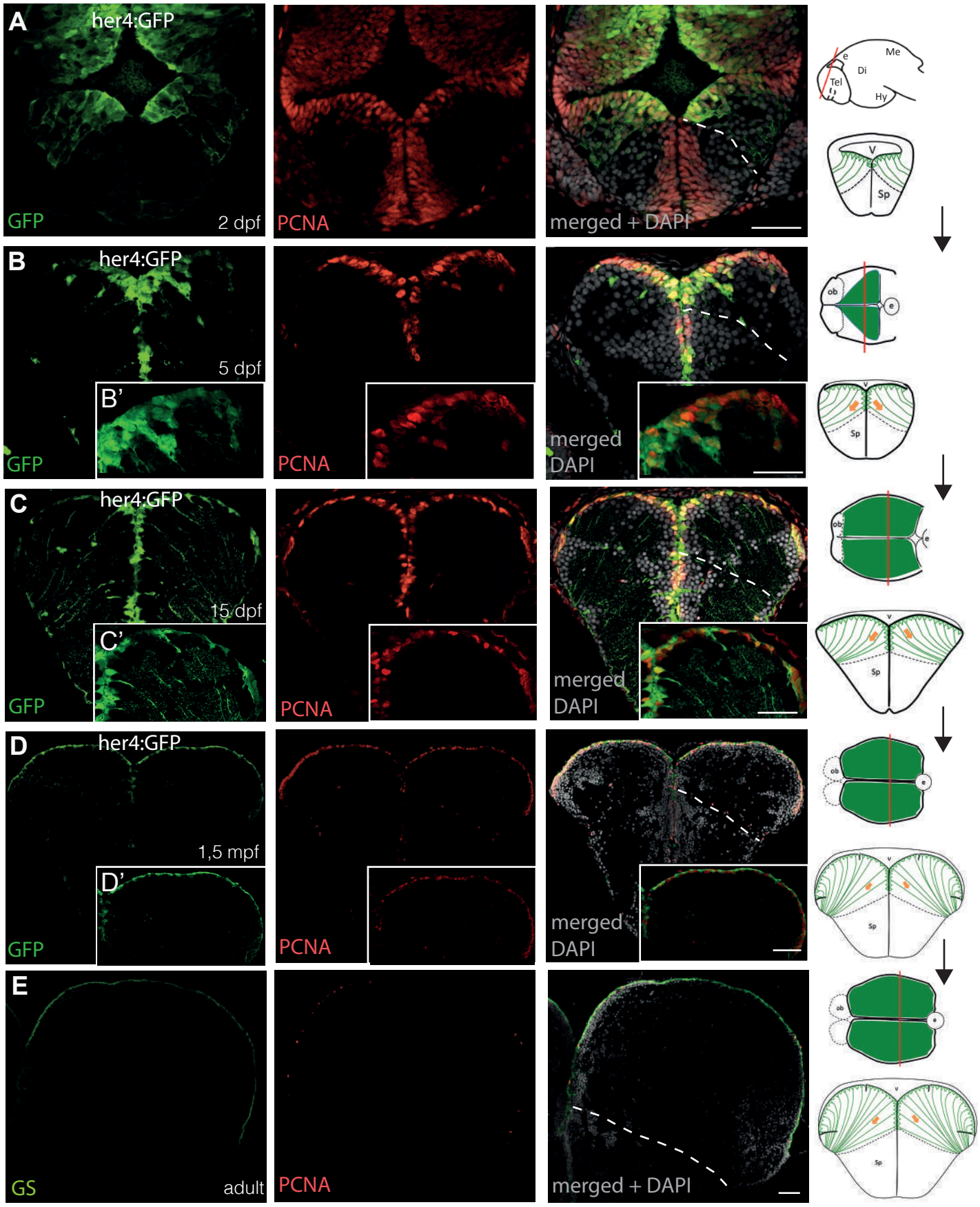

A
a layers
b nuclei
c
nuclei
neurons
NSCs
Continuous NSC activity
Spatially/temporally controlled NSC activity
Continuous NSC activity
+
Neuronal migration
B
pallium
Dm
sy
Dl
Dc
ls
Dp
t.c.
subpallium
C
Dl
Da
sy
Dm
L
A
P
M
D
DAPI
2 dpf
E
DAPI
5 dpf
F
DAPI
1,5mpf
G
DAPI
adult
her4:GFP
GFP
GS
GS
GS
her4:GFP
DAPI/GFP
DAPI/GS
DAPI/GS
DAPI/GS

Figure S1


A
her4:GFP
GFP
2 dpf
PCNA
merged + DAPI
e
Me
Di
Tel
Hy
V
Sp
B
her4:GFP
5 dpf
B'
GFP
PCNA
merged
DAPI
ob
e
C
her4:GFP
15 dpf
C'
GFP
PCNA
merged
DAPI
D
her4:GFP
1,5 mpf
D'
GFP
PCNA
merged
DAPI
E
GS
adult
PCNA
merged + DAPI

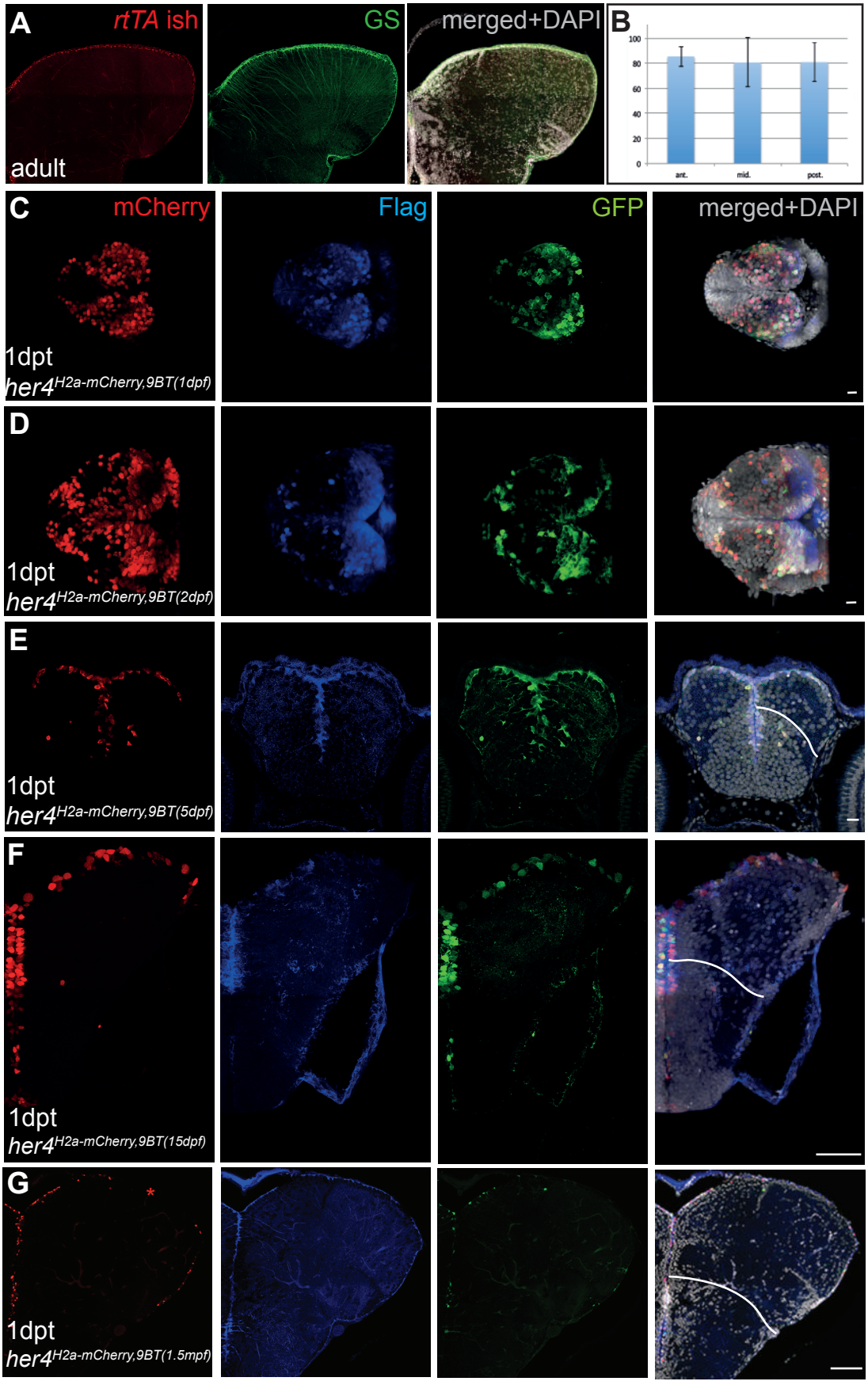

A
rtTA ish
adult
GS
merged+DAPI
B
100
80
60
40
20
0
ant.
mid.
post.
C
mCherry
Flag
GFP
merged+DAPI
1dpt
her4H2a-mCherry,9BT(1dpf)
D
1dpt
her4H2a-mCherry,9BT(2dpf)
E
1dpt
her4H2a-mCherry,9BT(5dpf)
F
1dpt
her4H2a-mCherry,9BT(15dpf)
G
1dpt
her4H2a-mCherry,9BT(1.5mpf)

Figure S3

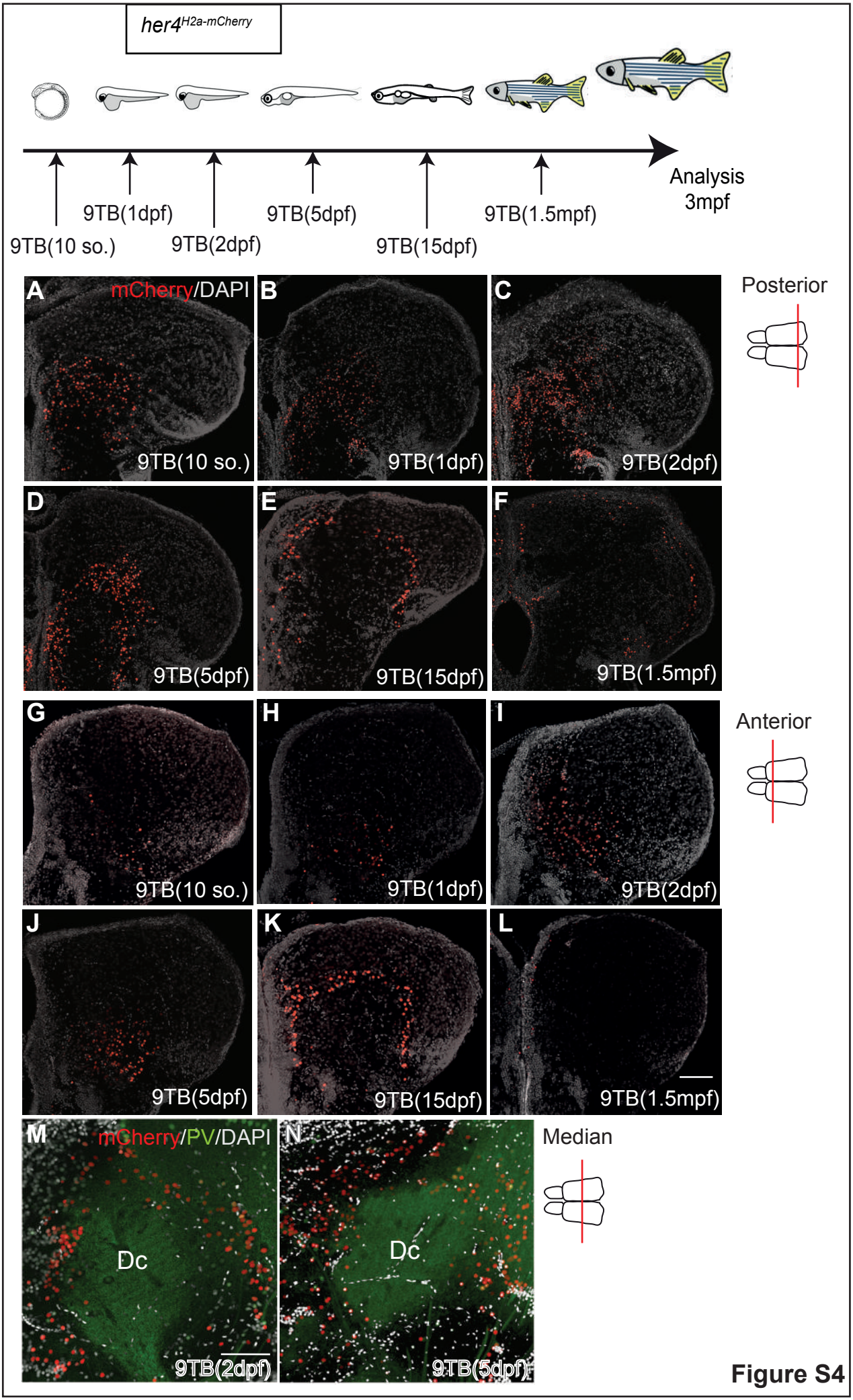

her4H2a-mCherry
Analysis
3mpf
9TB(10 so.)
9TB(1dpf)
9TB(2dpf)
9TB(5dpf)
9TB(15dpf)
9TB(1.5mpf)
A
mCherry/DAPI
9TB(10 so.)
B
9TB(1dpf)
C
9TB(2dpf)
Posterior
D
9TB(5dpf)
E
9TB(15dpf)
F
9TB(1.5mpf)
G
9TB(10 so.)
H
9TB(1dpf)
I
9TB(2dpf)
Anterior
J
9TB(5dpf)
K
9TB(15dpf)
L
9TB(1.5mpf)
M
mCherry/PV/DAPI
Dc
9TB(2dpf)
N
Dc
9TB(5dpf)
Median

Figure S4

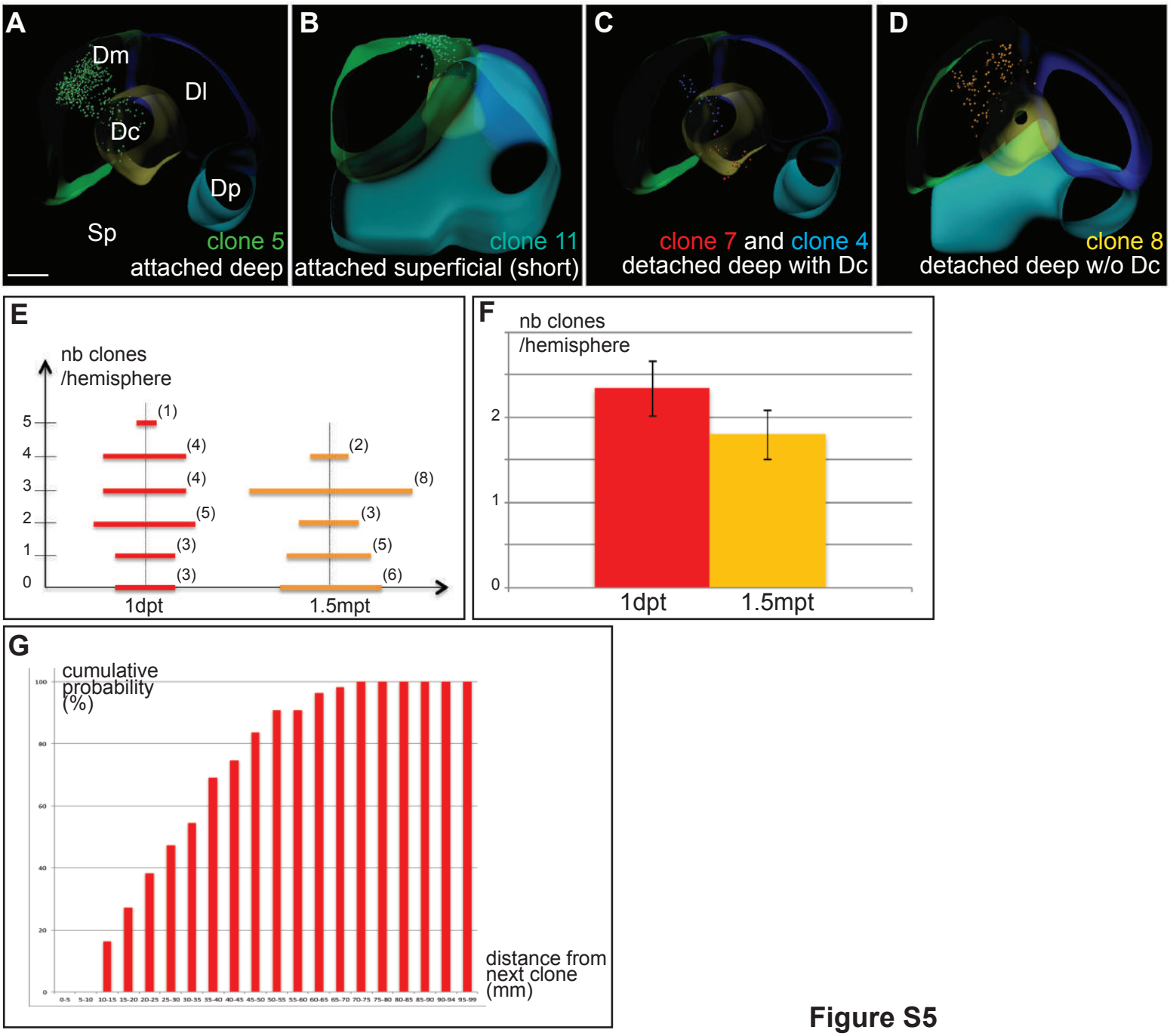

A
Dm
Dl
Dc
Dp
Sp
clone 5
attached deep
B
clone 11
attached superficial (short)
C
clone 7 and clone 4
detached deep with Dc
D
clone 8
detached deep w/o Dc
E
nb clones
/hemisphere
5
4
3
2
1
0
(1)
(4)
(4)
(5)
(3)
(3)
(2)
(8)
(3)
(5)
(6)
1dpt
1.5mpt
F
nb clones
/hemisphere
2
1
0
1dpt
1.5mpt
G
cumulative
probability
(%)
100
80
60
40
20
0
0-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
45-50
50-55
55-60
60-65
65-70
70-75
75-80
80-85
85-90
90-94
95-99
distance from
next clone
(mm)

Figure S5

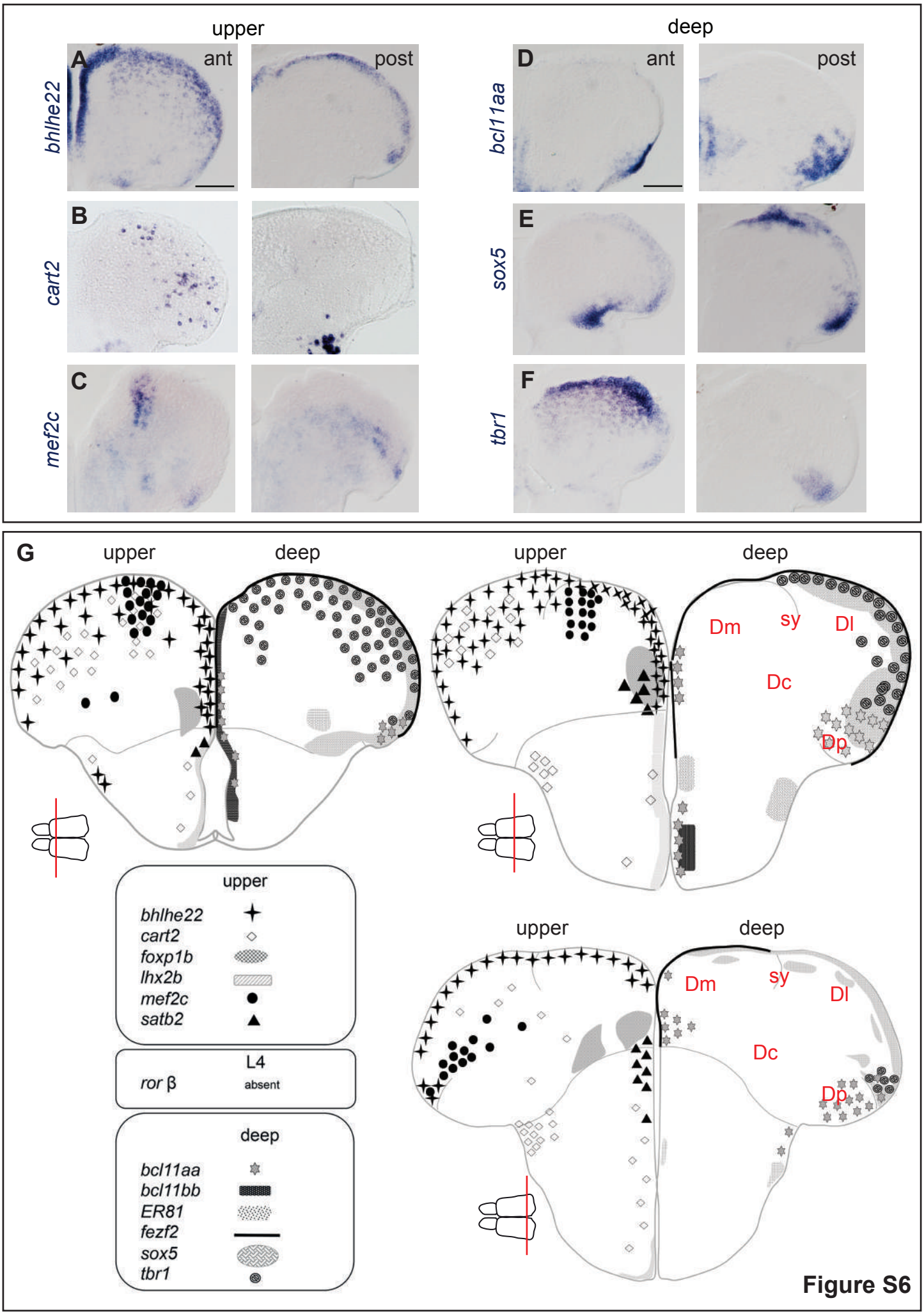

upper
deep
A
ant
post
bhlhe22
D
ant
post
bcl11aa
B
cart2
E
sox5
C
mef2c
F
tbr1
G
upper
deep
upper
deep
Dm
sy
Dl
Dc
Dp
upper
bhlhe22
cart2
foxp1b
lhx2b
mef2c
satb2
L4
ror β
absent
deep
bcl11aa
bcl11bb
ER81
fezf2
sox5
tbr1
upper
deep
Dm
sy
Dl
Dc
Dp

Figure S6

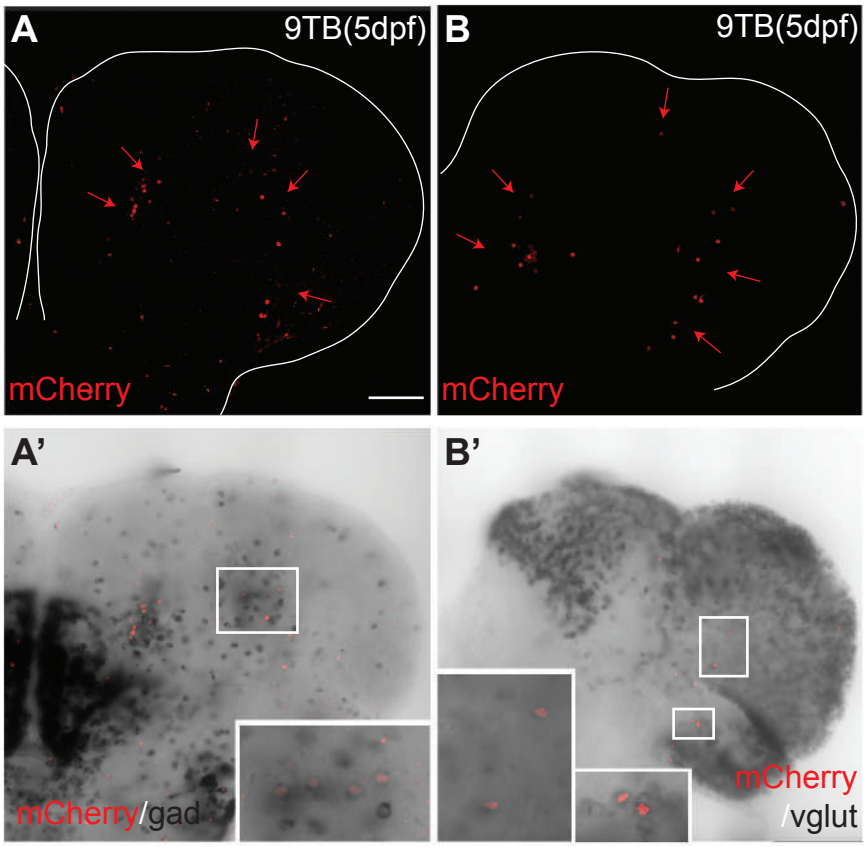

A
9TB(5dpf)
mCherry
B
9TB(5dpf)
mCherry
A'
mCherry/gad
B'
mCherry
/vglut

Figure S7

Figure S1 : **Eversion exposes the germinative RG layer in the adult zebrafish pallium,** related to Figure 1**. A.** Schematic representations of the neural stem cell (NSC)-dependent (**a, b**) and -independent (**c**) strategies shaping brain cytoarchitecture. The germinal NSC pool and its neurogenic activity are indicated in red, and neuronal identities issued from this pool are color-coded. The output structure is depicted at the top of each panel. (**a**) Continuous NSC activity generates age-related layers in the mammalian neocortex, in an inside-out fashion following radial neuronal migration. (**b, c**) Spatially controlled NSC activity and neuronal migration events organize functional nuclei in the bird and reptile neocortex (from [S4][S5]) **B.** Simplified schematized cross section of the zebrafish adult pallium at medial antero-posterior levels indicating the parenchymal subdivisions and sulci referred to in this study (from [S1]). The attachment point of the tela choroida, positioning the limit of the eversion process, is indicated (gray dots and arrows) (from [S2]). The pallial germinative zone (ventricular zone, red) extends from this point to the pallial-subpallial boundary (gray dashed line). **C.** Simplified schematized whole-mount view of the zebrafish adult pallium (viewed from top, anterior left) indicating the germinal zone areas visible from a dorsal view, as identified in [S3]. **D-G.** Whole-mount dorsal views of the pallial germinal zone in transgenic *her4:eGFP* telencephali, identified by the expression of GFP or GS (as indicated) at 2dpf (D), 5dpf (E), 1.5mpf (F) and 3mpf (adult, G). Whole-mount preparations counterstained with DAPI. Abbreviations: Da: anterior pallium; Dc: central pallium; Dl: lateral pallium domain; Dm: medial pallium domain; Dp: posterior pallium domain; ls: lateral sulcus; t.c.: tela choroida; sy: sulcus ypsiloniformis. Scale bars: D, E : 20μm; F : 50μm; G : 100μm.

Figure S2 : **Continuous and direct neurogenesis from radial glial (RG) cells in the zebrafish pallium from embryo to adulthood**, related to Fig.1. **A-E**. Cross sections of transgenic *her4:GFP* telencephali at 2dpf (**A**), 5dpf (**B**), 15dpf (**C**), 1.5mpf (**D**) and adult stage (**E**) at medial antero-posterior levels (see schemes on the right), immunostained for GFP or the RG marker Glutamine Synthase (green, left panel, as indicated), the proliferation marker PCNA (red, middle panel) and counterstained with DAPI (right panel). High magnifications in insets. White dashed line: position of the pallium/subpallium boudary. Scale bars: A, B inset, C inset : 20μm; D inset, E: 50μm. Abbreviations: Di: diencephalon; e: epiphysis; Hy: hypothalamus; Me: mesencephalon; ob: olfactory bulb; Sp: subpallium; Tel: telencephalon, V: ventricle.

Figure S3 : **Validation of the Tet-on strategy**, related to Fig.2. **A.** Compared expression of rtTA (*in situ hybridization*) and GS (immunocytochemistry) (color-coded, as indicated) on cross sections of the adult pallium in a *Tg(her4:rtTA)* fish (one hemisphere is shown, observed in

confocal microscopy). Note that *rtTA* and GS are expressed in the same set of ventricular RG. **B.** Proportion of mCherry-positive cells among ventricular cells at three different anteroposterior levels in *her4*$^{\text{H2a-mCherry, 9TB(5dpf)}}$ fish analyzed at 1 day-post-treatment (1dpt) (n=3 brains, T-test (α=0.05, not significant). **C.** Compared expression of the three reporters mCherry, Flag and GFP (immunocytochemistry, color-coded as indicated) observed in whole-mount pallia (C, D) or pallial cross sections at mid-anteroposterior levels (E-G) in double transgenic *Tg(her4:rtTA)*;*Tg(GFP:biTRE:H2amCHERRY*) fish induced with 9TB at 1dpf (C), 2dpf (D), 5dpf (E), 15dpf (F) and 1.5mpf (G) and sacrificed 1 day post-treatment (1dpt). Note the largely coincident expression of the three markers, the broader expression of mCherry being explained by the stability of the H2a-mCherry protein. Note also that, while *her4*-driven expression is limited to the pallial VZ until 5dpf (C-E), it extends to the subpallium at later stages (F, G). The red asterisk in panel G underlies a territory of the Dl VZ with low induction response at late stages (1.5mpf). The white line in E-G, right panels, indicates the pallial-subpallial boundary. Scale bars: C-E: 10µm; F, G: 50µm.

Figure S4 : **Zebrafish pallial neurogenesis follows a sequential stacking process : anterior and posterior analysis,** related to Fig.2. **A-L. Localization at posterior and anterior adult pallial levels of the mCherry-positive neurons born from *her4*-positive RG.** Position of mCherry-positive neurons on posterior (A-F) and anterior (G-L) 3mpf *her4*$^{\text{H2a-mCherry}}$ adult pallium cross sections observed in confocal microscopy (with DAPI counterstain) following 9TB induction at the stages indicated. **M, N. Localization, at median pallial levels, of the neurons born at 2 and 5dpf relative to Dc.** High magnification views of the confocal images shown in Fig.2C' and D', respectively, stained for mCherry and PV and counterstained with DAPI. Scale bars: 50µm.

Figure S5 : **Superimposed individual brainbow clones and neuroanatomical domains**, related to Figs.5 and 6. **A-D.** 5 clones are shown on thick cross sections of the brain shown in Fig.5A. Clone categories, as defined in Fig.5 C-G and Table S1, are indicated at the bottom right of each panel, and the clones illustrated are color-coded as in Fig.5C-I and Table S1. Note that clone n°5 and 7, shown in A and C respectively, reach into Dc. Scale bar: 80µm. **E-G. Validation of clonality in the analysis of *her4*$^{\textit{actswitch,T(5dpf)}}$ larvae**. **E.** Compared distribution of the number of induced progenitors at 1 day-post-9TB (1dpt) and the number of clones at 1.5mpf (1.5 months post 9TB –mpt-). n = 20 pallial hemispheres at 1dpt and 24 pallial hemispheres at 1.5mpt. The numbers in bracket indicate the number of hemispheres concerned for each number of induced cells/clone. **F.** Average number of induced progenitors per hemisphere at 1dpt or induced clones per hemisphere at 1.5mpt (averaged from panel E). sem: 0.32 at 1dpt, 0.28 at 1.5mpt. Difference

is not significant (P<0.25). **G.** Cumulative distribution of the nearest neighbors distances separating labeled progenitors in the pallium of a *her4*$^{H2a\text{-}mCherry,9TB(5dpf)}$ animal analyzed at 1dpt (Fig.6A, A'). 84% of induced clones are located at least 15μm (approx. 4 cell diameters) away from their nearest neighbor clone.

Figure S6 : **Expression in the adult zebrafish pallium of genes identifying neocortical layers in mammals and/or birds: other anteroposterior levels and summary schemes,** related to Fig.7 **A-F.** Anterior (left panels) and posterior (right panels) pallial cross sections from the specimen shown in Fig.7 (*in situ hybridization* for the indicated probes, blue signal), ordered here for gene probe orthology with markers of deep (left) or upper (right) layer neurons in other vertebrates (see Table S4). **G.** Schematic summary of the expression patterns, on cross sections of the adult zebrafish pallium at anterior, median and posterior levels (equivalent to Fig.7 and panels A-F), of all the genes tested in this study (Table S4). Scale bars: 100μm.

Figure S7 : **Compared localization of GABA and Glutamatergic neurons with the position of neurons born at 5dpf in the adult pallium,** related to Fig.7. **A-B'**: Cross-sections at median pallial levels of *her4*$^{H2a\text{-}mCherry,9TB(5dpf)}$ brains observed at 3mpf (confocal microscopy, single 70μm stacks), immuno-processed for mCherry and stained by *in situ hybridization* with the following probes: *gad65/67a/67b* (*gad*, A') and *vglut1/2.1/2.2* (*vglut*, B'). **A, B**: fluorescence channels; **A', B'**: superimposed bright field and fluorescence channels; red arrows in A, B indicate the shape of the mCherry-positive domains and white dotted lines line the pallial contour. Insets are high magnifications of the boxed areas showing mCherry in the nucleus of *gad*- or *vglut*-positive neurons. Scale bar: 70 μm.

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Clone #	Nb cells	Nb neurons	Nb ventricular cells	Type	Clone length in depth (µm)	Clone width (µm)	Distance of clone from VZ (µm)	Neuroanatomical location (VZ)	Neuroanatomical location (depth)	A/P location	clone deepest z on cross section (µm)	pallium deepest z on cross section (µm)	deepest z / pallial z ratio in %	"deep" limit (table S2)	"Dc" limit (table S2)
5	654	415	238	Attached/Deep	411	238		Dm	Dc	middle	413	450	92	39	71
6	646	568	78	Attached/Deep	384	284		Dm	Dc	posterior	381	450	85	76	82
9	29	22	7	Attached/Deep	406	221		Dm	Dc	middle	458	554	83	39	71
10	398	307	91	Attached/Deep	394	256		Dm	Dc	posterior	394	450	88	76	82
13	120	87	33	Attached/Deep	528	102		Da	Dc	anterior	418	450	93	86	92
14	206	143	63	Attached/Deep	324	164		Dm	Dc	middle	445	554	80	39	71
15	606	380	226	Attached/Deep	512	255		Dm	Dc	posterior	393	450	87	76	82
16	367	265	102	Attached/Deep	491	105		Dm	Dc	middle	420	554	76	39	71
18	560	487	73	Attached/Deep	419	101		Da	Dc	anterior	548	583	94	86	92
20	215	190	25	Attached/Deep	348	85.5		Dm		middle	403	644	63	39	71
26	67	53	14	Attached/Deep	404	110		Dm		middle	520	805	65	39	71
29	192	158	34	Attached/Deep	391	90.7		Da		anterior	592	700	85	86	92
31	42	32	10	Attached/Deep	409	103		Dm		middle	532	805	66	39	71
32	108	70	38	Attached/Deep	325	101		Dm		middle	494	805	61	39	71
33	118	90	28	Attached/Deep	325	129		Dm		middle	503	805	62	39	71
34	133	81	52	Attached/Deep	387	97.5		Dm		middle	511	805	63	39	71
2	162	109	53	Attached/Superficial	394	220 (short)		Dm		posterior	278	554	50	76	82
3	68	52	16	Attached/Superficial	291	252 (short)		Dm		posterior	201	450	45	76	82
11	137	72	65	Attached/Superficial	243	282 (short)		Dm		posterior	171	450	38	76	82
17	304	220	84	Attached/Superficial	214	304 (flat)		Dm		middle	145	644	23	39	71
21	404	269	135	Attached/Superficial	283	436 (flat)		Dm		middle	195	644	30	39	71
22	67	110	21	Attached/Superficial	374	122 (short)		Da		anterior	454	583	78	86	92
23	501	371	130	Attached/Superficial	550	191 (short)		Dm		anterior	550	805	68	86	92
28	139	96	43	Attached/Superficial	498	127 (short)		Dm		posterior	489	805	61	76	82
30	38	28	10	Attached/Superficial	186	85.4 (short)		Dm		middle	236	805	29	39	71
25	144	99	45	Attached	230	79.3 (short)		Dl		middle	230	805	29	39	71
27	247	187	60	Attached	384	117		Dl		anterior	489	700	70	86	92
4	28	28	0	Detached/Deep			160	Dm	Dc	middle	433	450	96	39	71
7	21	21	0	Detached/Deep			425	Dm	Dc	middle	545	554	98	39	71
19	473	473	0	Detached/Deep			254	Dm	Dc	middle	468	644	73	39	71
1	25	25	0	Detached/Deep			123	Dm		middle	339	554	61	39	71
8	119	119	0	Detached/Deep			61	Dm		middle	358	554	64	39	71
12	89	89	0	Detached/Deep			217	Dm		middle	385	554	69	39	71
24	82	82	0	Detached/Deep			218	Dm		middle	391	805	49	39	71

Total clone nb		34			
Attached	27 (80%)	Deep	16 (64%)	Including Dc	9
		Superficial	9 (36%)	Short	7
				Flat	2
		ND	2		
Detached	7 (20%)	Deep	7 (100%)	Including Dc	3
		Superficial	0		

Table S1

A/P section level	anterior			middle			posterior		
9-TB timing	deepest z on cross section (µm)	pallial z on cross section (µm)	deepest z / pallial Z ratio in %	deepest z on cross section (b, in µm)	pallial z on cross section (a, in µm)	deepest z / pallial Z ratio in %	deepest z on cross section (µm)	pallial z on cross section (µm)	deepest z / pallial Z ratio in %
Figure 4, Table S1									
24hpf	500	510	98	355	395	90	380	410	93
48hpf	510	550	**92**	250	350	**71**	329	402	**82**
5dpf	479	559	**86**	136	345	**39**	301	396	**76**
15dpf	280	507	55	67	360	18	244	398	61
1.5mpf	20	461	4	17	370	5	34	410	8
"deep" limit			**86**			**39**			**76**
Dc limit			**92**			**71**			**82**

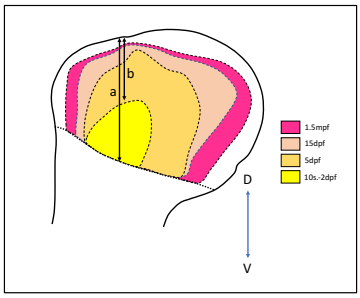


Table S2

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Clone #	Nb cells	Nb neurons	Nb RG (GS+)	Nb proliferating RG (GS+, PCNA+)	Nb proliferating precursors (GS-, PCNA+)	**Type**	Clone length in depth (µm)	Long axis of VZ area (µm)	Short axis of VZ area (µm)	Distance of clone from VZ (µm)	**Neuroanatomical location (VZ)**	**A/P location**	clone deepest z on cross section (µm)	pallium deepest z on cross section (µm)	deepest z / pallial Z ratio (in %)
1	37	33	0	4	0	**Attached**	122	19	10	0	**Da**	**middle**	118	130	91
2	47	38	0	3	6	**Attached**	119	15	1 row	0	**Da**	**anterior**	82	130	63
3	49	44	5	0	0	**Attached**	53	20	12	0	**Dl**	**middle**	52.5	130	40
5	56	53	3	0	0	**Attached**	92	21	1 row	0	**Dp**	**posterior**	46.5	130	36
6	11	6	4	1	0	**Attached**	12	22,1	1 row	0	**Da**	**anterior**	16.8	130	13
7	99	90	9	0	0	**Attached**	66	52	20	0	**Dl**	**posterior**	62.4	120	52
8	176	148	28	0	0	**Attached**	45	57	26	0	**Da**	**anterior**	35.3	120	30
9	39	38	1	0	0	**Attached**	45	1 GS cell only	NR	0	**Dm**	**middle**	45.3	120	38
10	31	27	3	1	0	**Attached**	17	7	1 row	0	**Dm**	**middle**	53.6	120	45
11	48	42	6	0	0	**Attached**	34	31	21	0	**Dm**	**middle**	17.9	90	20
14	48	41	0	4	3	**Attached**	31	28	12	0	**Dm**	**middle**	33.7	112	30
15	28	26	2	0	0	**Attached**	60	17	1 row	0	**Dl**	**posterior**	49.2	112	44
16	42	38	4	0	0	**Attached**	38	23	4	0	**Dp**	**posterior**	45.6	112	41
17	71	62	9	0	0	**Attached**	80	32	17	0	**Da**	**anterior**	69.8	112	62
19	104	94	10	0	0	**Attached**	252	31	18	0	**Da**	**anterior**	94.8	95,8	99
20	76	ND	ND	ND	ND	**Attached**	148	ND	ND	0	**Dm**	**middle**	61.5	95,8	64
21	176	142	34	0	0	**Attached**	100	82	30	0	**Da**	**middle**	76.6	95,8	80
22	201	160	7	6	28	**Attached**	86	22	8	0	**Dl**	**posterior**	80.7	95,8	84
23	16	ND	ND	ND	ND	**Attached**	41	ND	ND	0	**Dm**	**middle**	74.9	90	83
24	99	ND	ND	ND	ND	**Attached**	127	ND	ND	0	**Da**	**anterior**	126	130	97
25	134	ND	ND	ND	ND	**Attached**	97	ND	ND	0	**Dp**	**posterior**	48.2	130	37
26	79	33	6	2	38	**Attached**	133	36	14	0	**Dp**	**posterior**	79	130	61
27	27	23	1	2	1	**Attached**	43	16	1 row	0	**Dp**	**posterior**	47	110	43
28	26	23	0	3	0	**Attached**	69	10	1 row	0	**Dm**	**middle**	37	110	34
29	49	46	2	1	0	**Attached**	61	14	1 row	0	**Dp**	**posterior**	43.8	110	40
30	41	31	1	2	7	**Attached**	31	16	1 row	0	**Dm**	**middle**	17.3	110	16
31	24	17	5	2	0	**Attached**	31	17	11	0	**Dm**	**middle**	45.1	110	41
32	37	36	1	0	0	**Attached**	65	1 GS cell only	NR	0	**Dp**	**posterior**	57.7	106	54
33	41	36	3	2	0	**Attached**	42	19	1 row	0	**Dm**	**middle**	22.4	106	21
34	53	34	18	0	1	**Attached**	48	51	19	0	**Dm**	**middle**	38.4	106	36
35	69	ND	ND	ND	ND	**Attached**	113	ND	ND	0	**Dp**	**posterior**	23.8	104	23
36	47	42	5	0	0	**Attached**	88	23	1 row	0	**Dm**	**middle**	25.5	70,4	36
37	38	35	3	0	0	**Attached**	39	15	8	0	**Dm**	**middle**	42	105	40
38	54	44	9	1	0	**Attached**	33	35	18	0	**Dm**	**middle**	33.6	105	32
39	23	11	12	0	0	**Attached**	42	25	12	0	**Da**	**anterior**	52	105	50
40	71	60	9	1	1	**Attached**	86	29	15	0	**Da**	**anterior**	76.6	105	73
4	33	33	0	0	0	**Detached**	74	NR	NR	10	**Dm**	**middle**	67.9	130	52
12	8	8	0	0	0	**Detached**	35	NR	NR	9	**Dm**	**middle**	26.8	90	30
13	19	19	0	0	0	**Detached**	71	NR	NR	22	**Dl**	**posterior**	74.8	90	83
18	5	5	0	0	0	**Detached**	75	NR	NR	37	**Dm**	**posterior**	107	130	82

Total clone nb	**40**
Attached	**36 (90%)**
Detached	**4 (10%)**

Table S3

Gene	Rodents	Human	Birds	Zebrafish ortholog(s)	Adult zebrafish pallium (>2mpf)	refs
bhlhe22(bhlhb5)	L2-L5			*bhlhe22(bhlhb5)**	active / recent neurogenesis zones	[S7]
Cux2	L2-L3	L2 (and L4)	mesopallium	*cux2a*, cux2b*	no expression	[S4], [S5]
Cart	L5	L2-3		*cart1, cart2*, cart3, cart4*	salt and pepper	[S15]
FoxP1	L3-L6 in embryos, L4-L5 in adult	L3-L6	mesopallium	*foxp1a, foxp1b**	stripe, ventral Dm (neurons)	[S4], [S8]
Lhx2	L3-L4			*lhx2a, lhx2b**	neurogenesis zone in ventral Dm	[S3]
Mef2c	L2 in embryos, L2-6 in adult	L2-L3-L4	mesopallium, nidopallium	*mef2c**	sub-domain in Dm	[S4], [S5], [S13]
Satb2	L2 in embryos, L2-6 in adult	L5	mesopallium	*satb2**	almost no expression (subpallium only)	[S4], [S5], [S8]
Ror β	L4	L4	thal. recipient (entopallium, field L)	*ror β**	no expression (midbrain only)	[S4]
Eag2/Kcnh5	L4		thal. recipient, entopallium	*kcnh5a, kch5b*		[S4]
*CTIP1**	L1 in embryos, all layers at postnatal stages			*bcl11aa**	sub-domain in Dl/Dp, active neurogenesis zone in ventral Dm	[S10]
CTIP2	L5 in embryos, L4-L6 in adult	L5b	medial domains chick pallium, parahippocampal area	*blc11bb**	subpallium	[S8], [S9]
ER81	L5	L5	arcopallium, parahippocampal area	*ER81**	no expression (subpallium only)	[S4], [S5]
Fezf2	L5	L5b	parahippocampal area	*fezf2**	active neurogenesis zone	[S4], [S11]
Sox5	L5-L6	L6		*sox5**	active / recent neurogenesis zones	[S12]
Sulf2	L5			*sulf2a,b*		[S4]
Tbr1	L6	L5-L6		*tbr1**	active / recent neurogenesis zones	[S14]

Table S4

Table S1. Quantitative characteristics of the clones segmented in *her4*$^{Zebrabow,T(1dpf)}$ adult pallia. **Column A** : clone numbers, see Fig.5 and Fig.S5 for illustrations of the clones highlighted in color. **Columns B-D** : total number of cells per clone (B), as the sum of the number of neurons (C) and ventricular cells (D). **Column E** : clone type. "Attached" clones are defined as possessing at least one ventricular (progenitor) cell, while "detached" clones have no ventricular cells. The clones are further classified as "deep" if they contain neurons born at or before 5dpf, and as "superficial" otherwise. The z positions of the 5dpf ("deep") and Dc (48hpf) limits (**columns O and P**) were measured on standard anterior, middle or posterior sections as in Figs.2 and S4, and normalized by pallial depth at these antero-posterior positions (Table S2). These limits are here compared to the ratio of the "z position of the deepest clone cell" to "pallial depth" for each clone (**columns L-N**) to estimate the age of the first-born neuron of each clone. Due to the curvature of the age-related neuronal sheets (eg. see Fig.2G), the age of clones situated laterally could not be ascertained and these clones are only referred to as "attached" (clones 25 and 27). **Columns F-G** : total length (distance from the most superficial to the deepest cell, F) and width (largest measure parallel to the VZ plane, G) of each clone; attached clones are referred to as "flat" if their width is superior to their length; they are referred to as "short" otherwise. **Column H** : in the case of "detached" clones, distance of the most superficial cell to the ventricular surface. **Columns I-J** : neuroanatomical location of the clone at the ventricular zone (I, Da, Dm, Dl or Dp) and in depth (J, clone overlapping – green- or not Dc). **Column K** : antero-posterior location of the clone along the pallium. "Anterior", "middle" and "posterior" levels are those illustrated in Figs.2 and S4 and measured in Table S2. **Bottom table**: total count of the different clone categories, as defined above.

Table S2. Quantitative positioning of neurons born at the indicated ages on anterior, middle and posterior cross sections of the pallium at 3mpf. The position of sections is as shown in Figs.2 and S4. The diagnostic "age ratio" (deepest z/pallial at anterior, middle and posterior level) is obtained by normalizing the z position of the deepest H2a-mCherry-positive neurons (value "b" on the scheme) by the deepest z position of the pallium (value "a" on the scheme). Values in bold are those reported into Table S1.

Table S3. Quantitative characteristics of the clones segmented in *her4*$^{actswitch,T(5dpf)}$ 1.5 mpf pallia. See Fig.6 for illustrations. **Column A :** clone numbers (see Fig.6 for illustrations). **Columns B-F :** number of cells of each type within clones (number of neurons (column C), number of quiescent radial glia (GS-positive, PCNA-negative) (column D), number of activated radial glia (GS-positive, PCNA-positive) (column E) and number of proliferating non-glial precursor (GS-negative, PCNA-positive) (column F)). **Column G :** clone types (only classified as "attached" or "detached" in this

experiment). **Column H :** Clone length (length of longest axis in the parenchyme). **Columns I, J:** Size (length, width) of the VZ component (RGs, aNPs) of each clone along the ventricular plane (see Fig.6E for the schematic representation of each category –the short axis can be composed of several cell rows, one cell row, or no cells if the VZ component only consists in 1 cell-). **Column K:** depth of the clone within the parenchyme (for detached clones). **Column L** : neuroanatomical location of the clone at the ventricular zone (I, Da, Dm, Dl or Dp). **Column M** : antero-posterior location of the clone along the pallium. Columns N-P: Deepest positions of each clone and of the pallium on cross-sections, and corresponding ratios. **Bottom table** : total count of the 2 clone categories defined above. ND: not determined; NR: not relevant.

Table S4. List of the genes identifying neocortical layers in mouse, human and chick used in this study. The zebrafish orthologs are identified, and expression profiles in each case are reported. In case expression differs between embryonic and adult stage in rodents, this is also indicated. Zebrafish genes selected for illustrations in Figs.7 and S6 are indicated with an asterisk. After references [S5][S6][S4][S7][S8][S9][S10][S11][S12][S13][S14][S15].

Abbreviations list

Da anterior subdivision of the pallial ventricular zone

Dc central subdivision of the pallium

Dl lateral subdivision of the pallium

Dm medial subdivision of the pallium

Dp posterior subdivision of the pallium

NPC neural progenitor cell

NSC neural stem cell

PV Parvalbumin

RG radial glia

sy sulcus ypsiloniformis

VZ ventricular zone

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