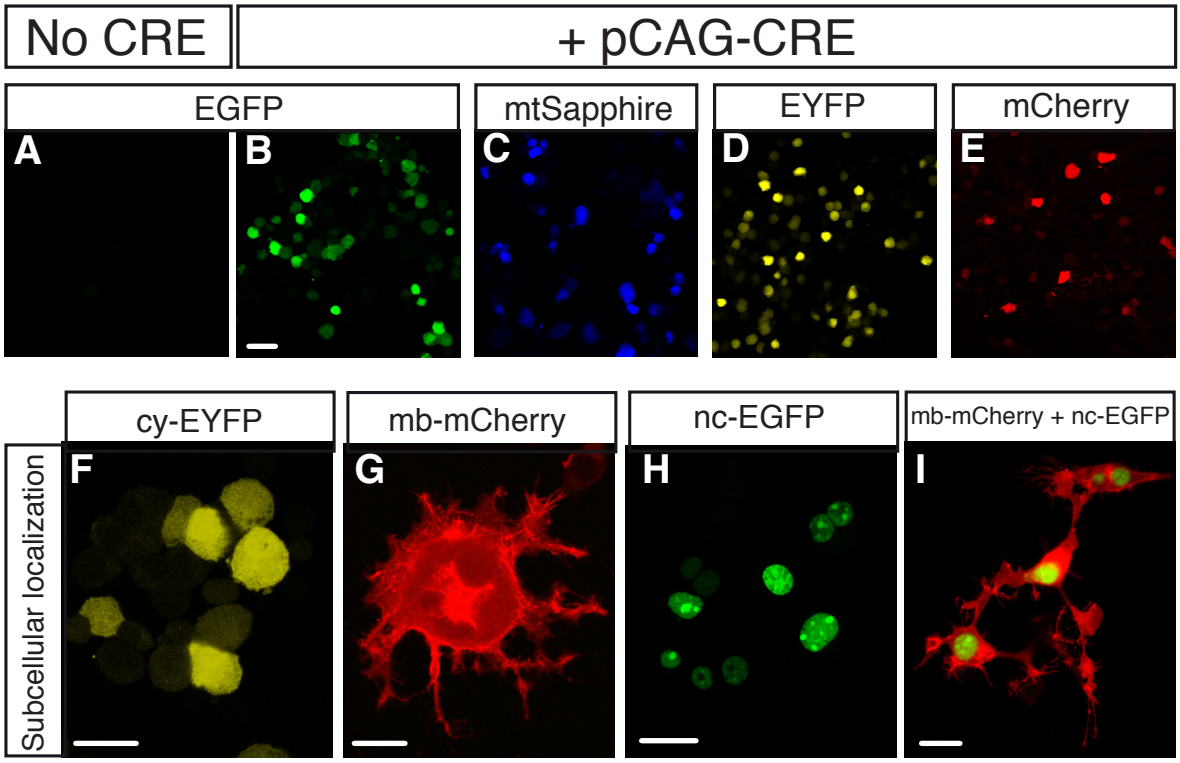
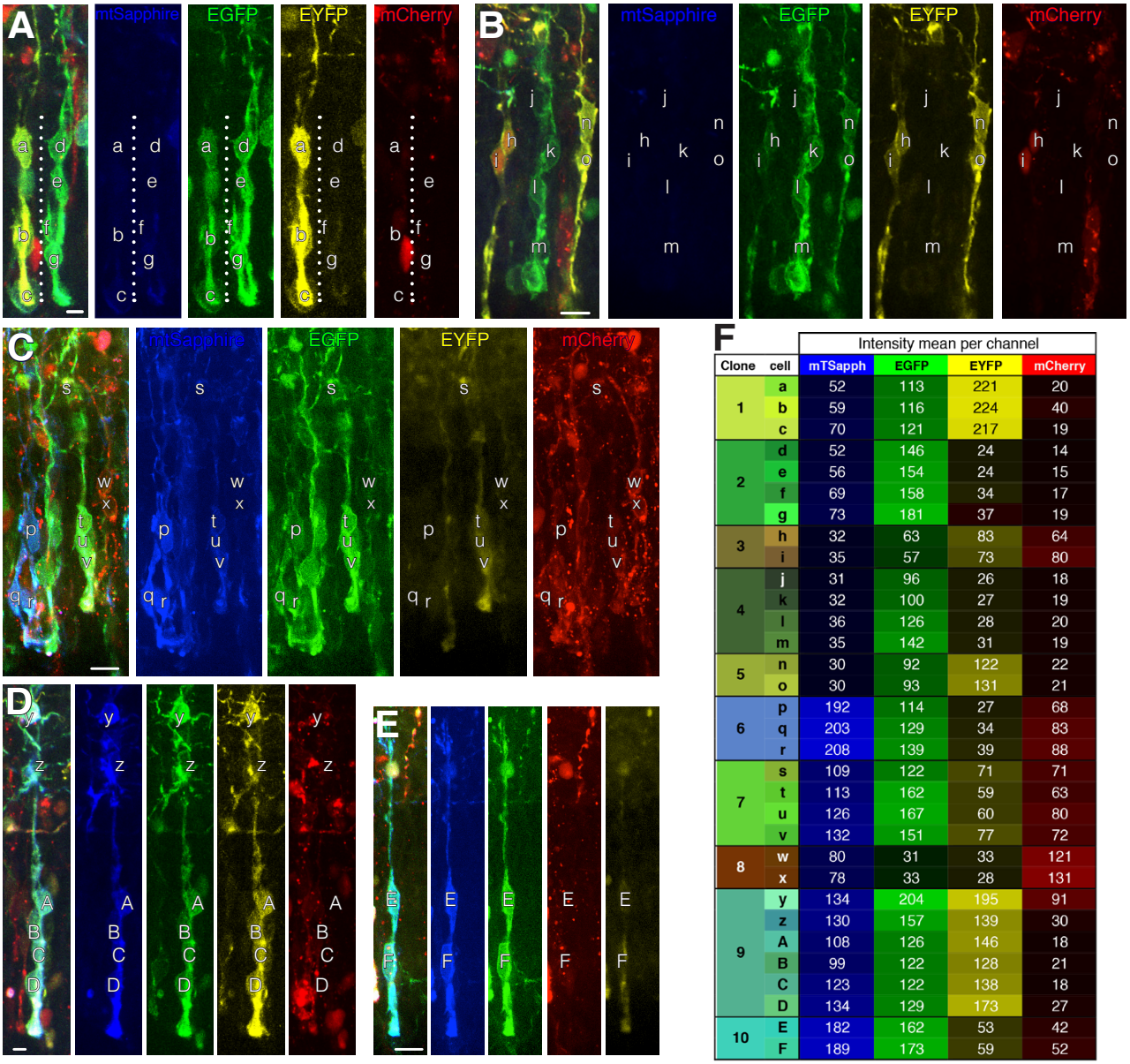


Figure S1. In vitro validation of arrested labelling vectors and the subcellular localization of the fluoroproteins. All newly generated plasmid vectors were validated in vitro. (A) Cultured N2A cells were transfected by FuGENE transfection reagent, but no fluorescence was detected in any of these transfections, showing that the SV40 stop signal efficiently arrested the expression of fluorescent proteins. (B-E) N2A cells transfected along with pCAG-CRE became then fluorescent with each of the different cytoplasmic fluorophores. (F-I) Subcellular localization of arrested tagged fluoroproteins in cultured N2A cells after cotransfection with pCAG-CRE. (F) cytoplasmic EYFP labels the cytoplasm of the cells. (G) mb-mCherry tags the red fluorophore to the cellular membrane. (H) nc-EGFP labels the nuclei in transfected cells. (I) Both membrane-tagged mCherry and nuclear-tagged EGFP fluorophores are complementary in the same cell. Scale bars 50 μm (bar in B applies to B-E).



Clone	cell	Intensity mean per channel mTSapph	EGFP	EYFP	mCherry
1	a	52	113	221	20
	b	59	116	224	40
	c	70	121	217	19
2	d	52	146	24	14
	e	56	154	24	15
	f	69	158	34	17
	g	73	181	37	19
3	h	32	63	83	64
	i	35	57	73	80
4	j	31	96	26	18
	k	32	100	27	19
	l	36	126	28	20
	m	35	142	31	19
5	n	30	92	122	22
	o	30	93	131	21
6	p	192	114	27	68
	q	203	129	34	83
	r	208	139	39	88
7	s	109	122	71	71
	t	113	162	59	63
	u	126	167	60	80
	v	132	151	77	72
8	w	80	31	33	121
	x	78	33	28	131
9	y	134	204	195	91
	z	130	157	139	30
	A	108	126	146	18
	B	99	122	128	21
	C	123	122	138	18
	D	134	129	173	27
10	E	182	162	53	42
	F	189	173	59	52

Figure S2. Fluorescence analysis of chick clones in the germinative zone. (A-D) Thirty two cells (named a-z and A-F) from Fig. 3E were analysed. The expression of each of the fluoroproteins was studied and shown. Notice the conservation of the fluorescent pattern in membrane, nucleus and cytoplasm across all the members of a given clone. (E) Detailed intensity mean of every fluorophore (0 minimum, 255 maximum) represented by the numerical value. Background colour represents the given colour intensity in each of the fluorescent channels and the average in each cell and clone. Whereas siblings cells do not show identical brightness values for each of the fluorophores, we consider that two cells showing complex patterns that only differ slightly in the brightness levels are considered siblings when the number of labelled of cells is reduced (discussed in the manuscript). Scale Bars 10 µm.

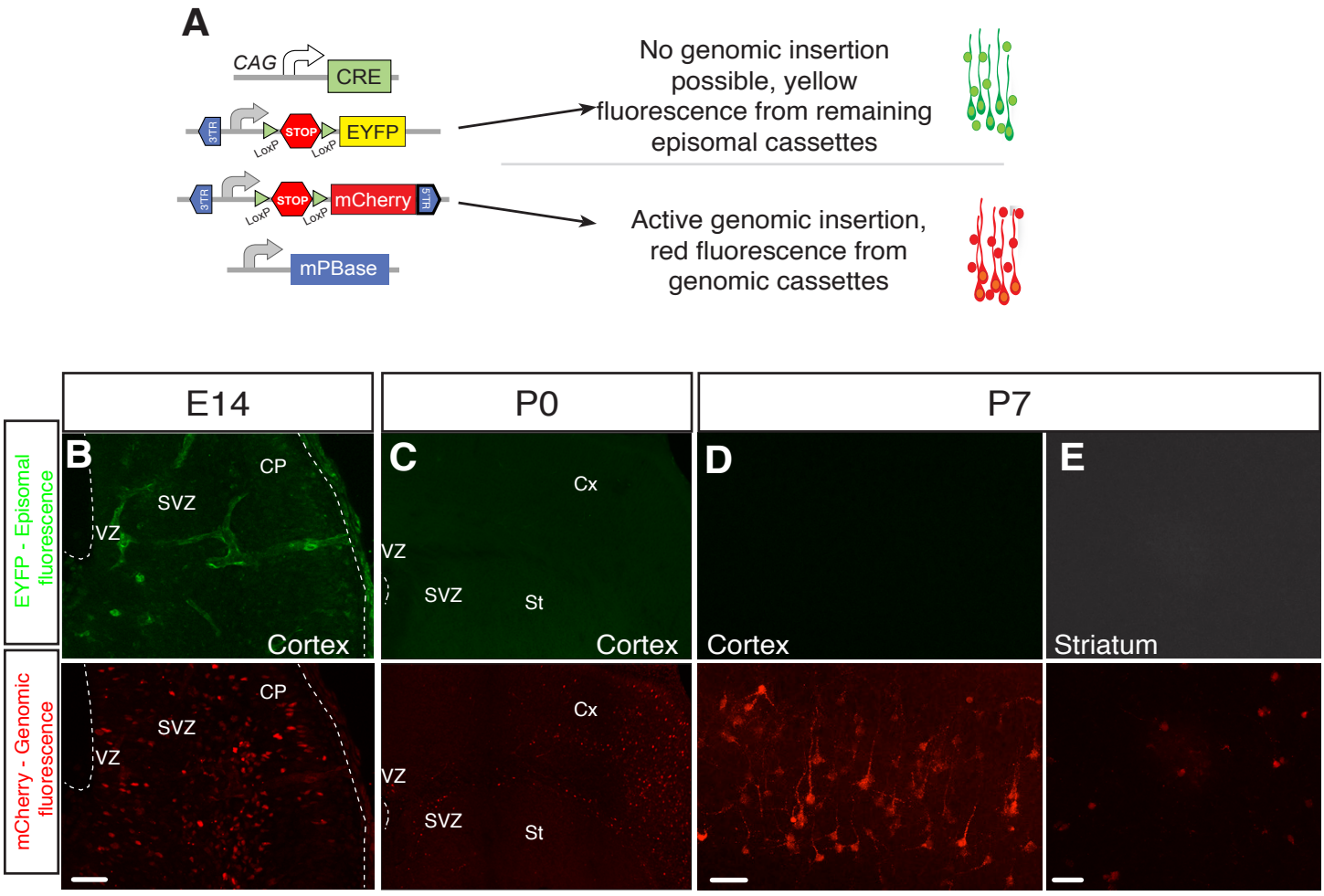


Figure S3. Transposition is necessary to detect fluorescence, episomal fluorescence is neither detected in short or nor long-term experiments. (A) Two different labelling vectors were cotransfected on E12 mouse forebrains, along with pCAG-CRE and mPB and at usual *CLoNe* low DNA concentrations: a pPB-CAG-STOP-mCherry vector and a truncated form of the EYFP labelling vector without the 3' *piggybac* terminal repetitions . Accordingly, only the mCherry expressing vector can be transposed to the genome of the progenitors, whereas the EYFP vector copies remain episomally. (A-B) Examples of detected fluorescence at E14 (A, only two days after electroporation; n=4) and P0 (B; n=2) at the developing neocortex. Only autofluorescence from blood vessels was detected in the yellow channel. As expected by the genomic transposition, fluorescent mCherry-expressing cells can be detected in differentiating postmitotic neurons in the cortical plate (CP) and postnatal cortex (Cx) and in mitotic cells of the germinative ventricular and subventricular zones (VZ and SVZ). (D-E) Examples of detected fluorescence at P7 at the neocortex and striatum (n=2). In all cases only red fluorescence was found, suggesting that episomally-originated fluorescence is diluted and silenced as brief as 2 days after electroporation. St: striatum. Scale Bars (B,D-E) 50 µm.

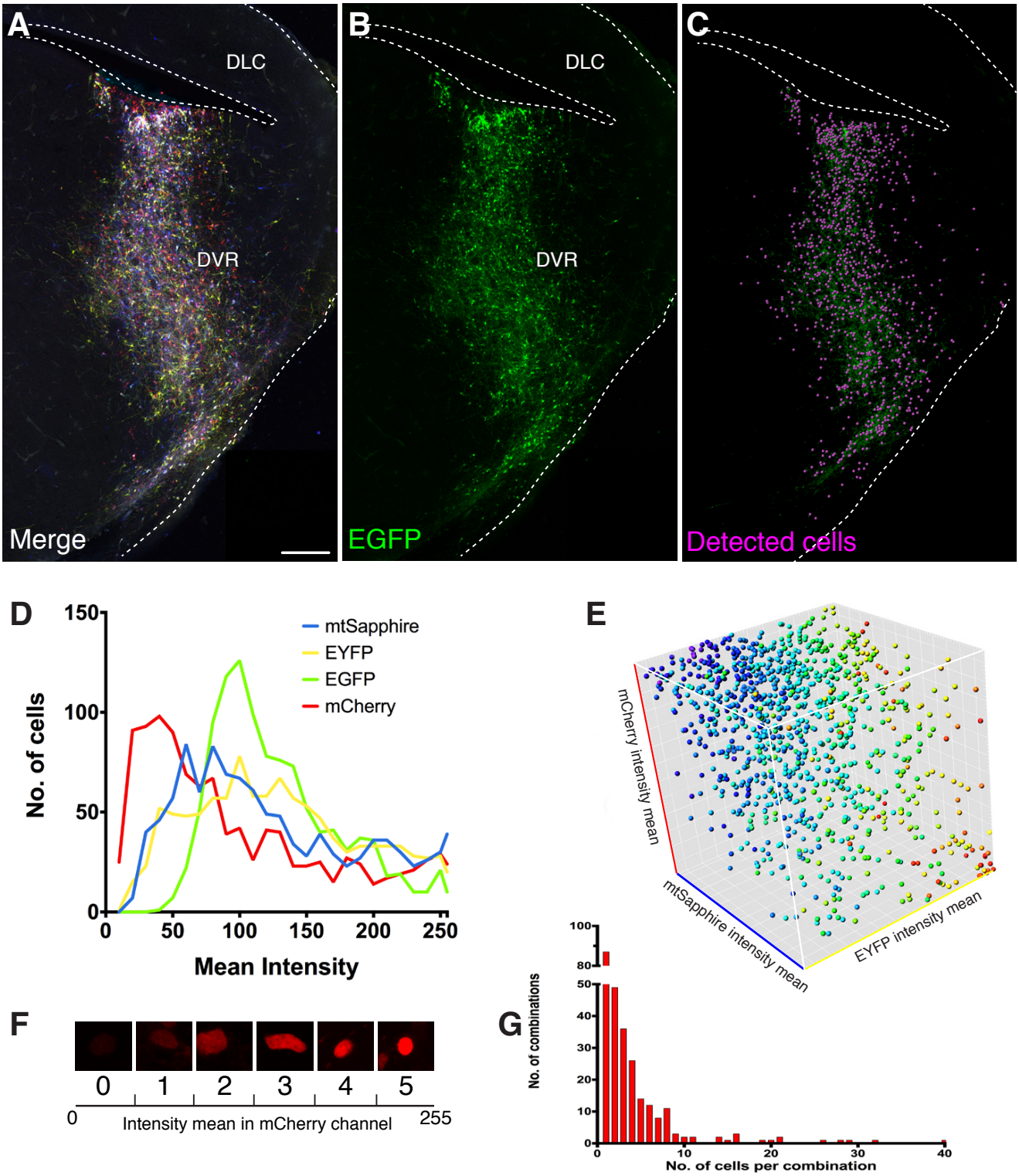


Figure S4. Discrimination of multiple fluorescent combinations. (A) Coronal section of a chick forebrain electroporated at E5 with pCAG-CRE enabled *CLoNe* labeling and imaged at E10. (B) EGFP-expressing cells only. (C) Spot detection of cells by IMARIS. 1072 cells were detected and analysed. All data is presented in Supplementary dataset 1. (D) Distribution of mean intensities across the different fluorophores of the EGFP cells. Intensity means were distributed across the entire brightness spectra. EGFP histogram does not represent EGFP signal below the threshold employed for the spot detection. (E) Distribution of mean intensities of EGFP-expressing cells. The graph shows no preference for any given combination of colors evidencing the independence of the expression of the different fluorophores. (F) Examples of nc-mCherry brightness for each of the thresholded levels. (G) Number of detected combinations respresented in a given number of cells. A maximum of 86 independent combinations were represented in a single cell. The number of combinations represented in a given number cells decayed as the number of cells increased. Scale bars 200 µm (A, B).

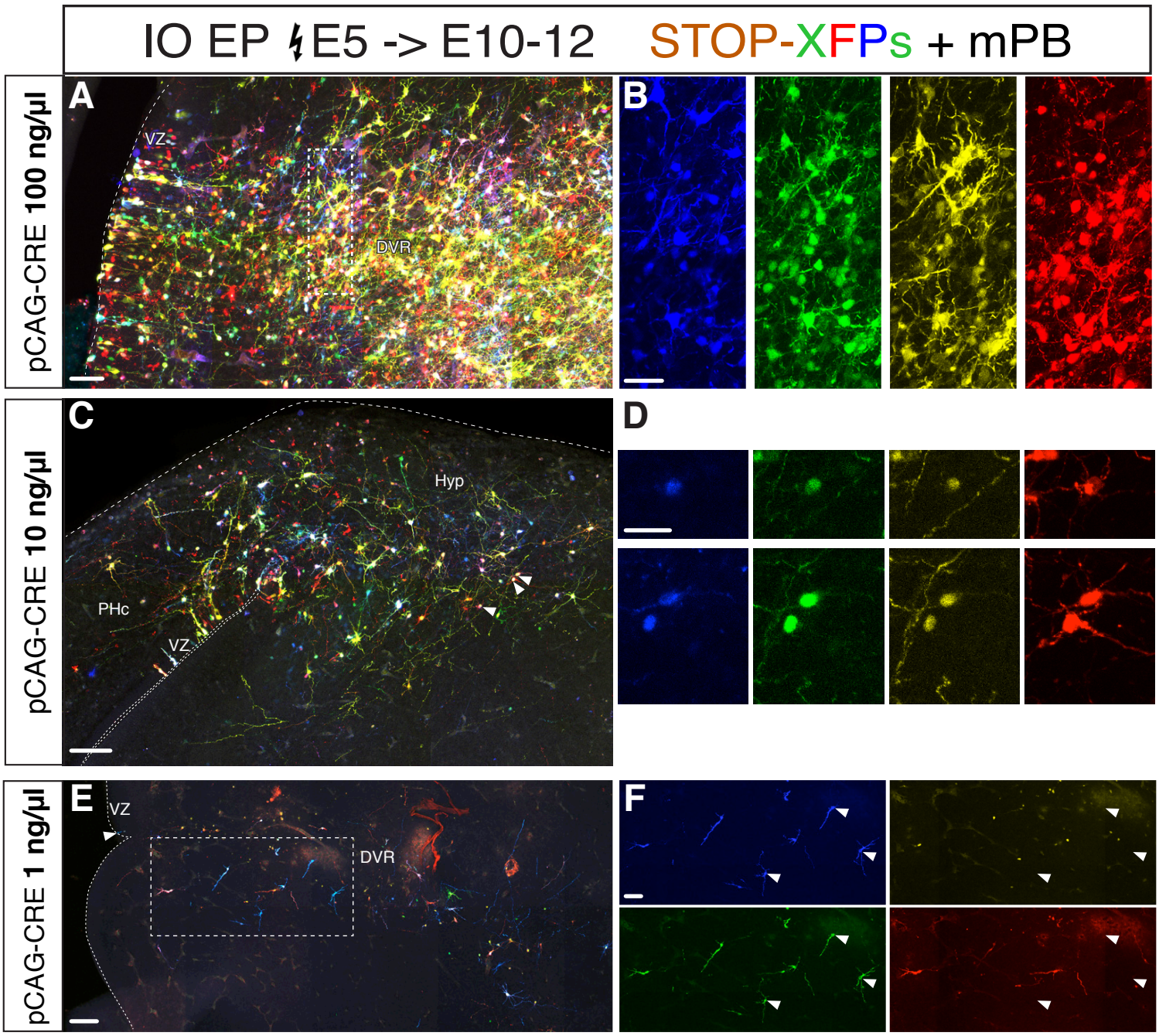


Figure S5. Dilution of targeting vector scatters progenitor labelling. Examples of chick forebrains electroporated with *CLoNe* vectors triggered by decreasing concentrations of pCAG-CRE. Brains were transfected at E5 and collected at E10 or E12. (A) pCAG-CRE at 100 ng/µl labelled a vast number of progenitors. (B) Magnification from rectangle in A. Clonal analysis is difficult some progenitors could acquire the same random combinatorial hue. (C) Most efficient labelling was perfomed at 10 ng/µl, an optimal number of cells was stained and shared combinations can be found. (D) examples of three cells expressing the same palette of colors. (E) At 1 ng/µl, pCAG-CRE labels very few progenitors. (F) Magnification from rectangle in E. Clonal relationships can be described among labelled cells but the cells greatly scattered made the imaging difficult and time-consuming. In addition, the decrease in number of progenitors labelled also affected the number of useful complex combinations. Scale bars 100 µm (A, C, E) and 20 µm (B, D, F).

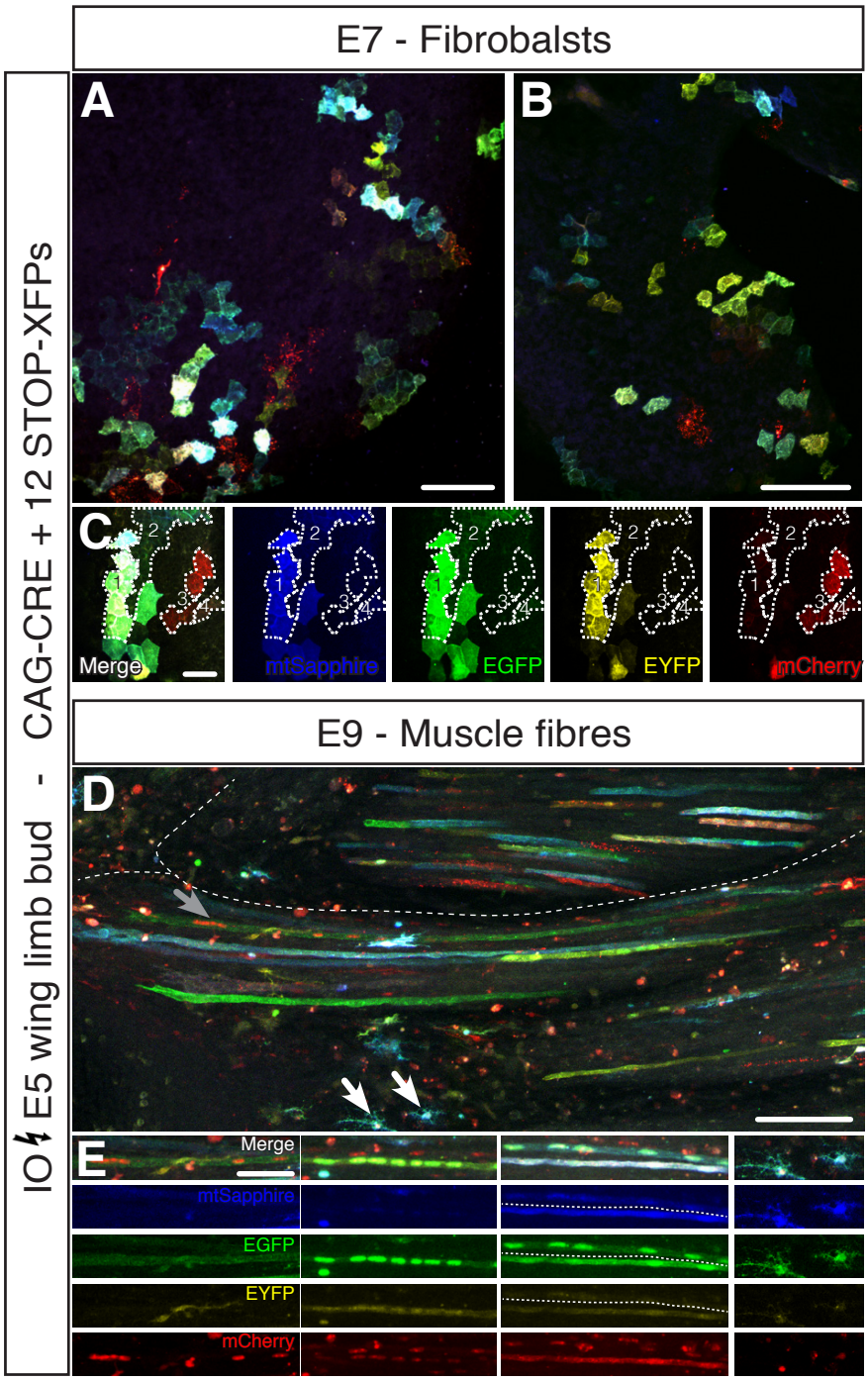


Figure S6. *CLoNe* reveals fibroblast and myocyte lineage. Chick E5 embryos were electroporated at wing limb bud with *CLoNe* vectors triggered by pCAG-CRE. Tissue was processed at either E7 (A-C) or E9 (D-E). (A-C) Clonal labelling at wing fibroblasts. Several small groups or pairs of fibroblasts display the same combination of fluorophores. (C) Magnification of a group of four clones comprising a total of 28 fibroblasts depicted by dashed white lines. (D-E) Clonal labelling with membrane fluorophores at wing muscle cells to reveal the arrangements of the clonally related myocytes. (D) Individual myoblasts were labelled and the clonally related myocytes followed as they line up and fuse into multi-nucleated fibers called myotubes. *CLoNe* labelling revealed that members of the same clone arrange into myotube to form cellular syncytia. White arrows point to two sibling cells and grey arrow indicates a clonal syncytium, all of them magnified in E. (E) Magnification and separate fluorescence expression in muscle fibres. Nuclei inside a syncytium shared the same combinatorial hue. Scale bars 100 µm (A,B,D) and 50 µm (C,E).

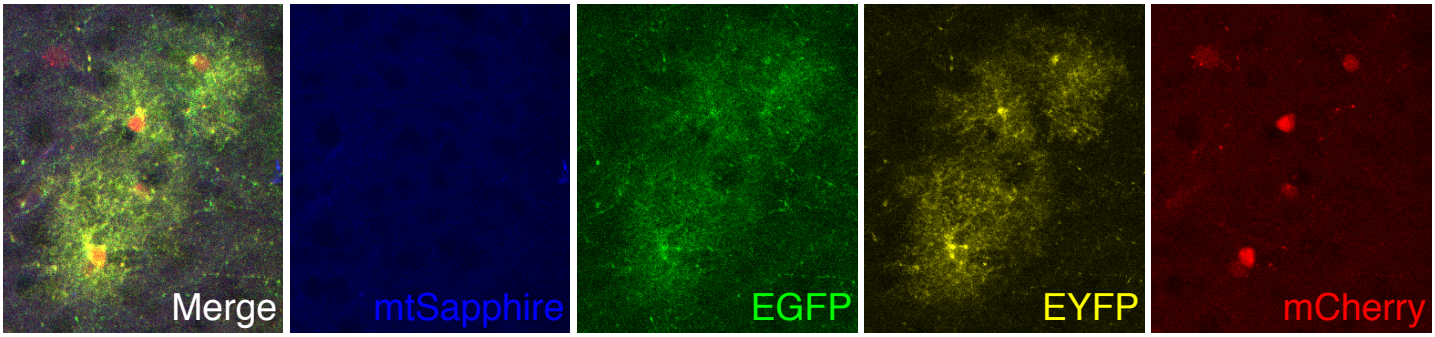
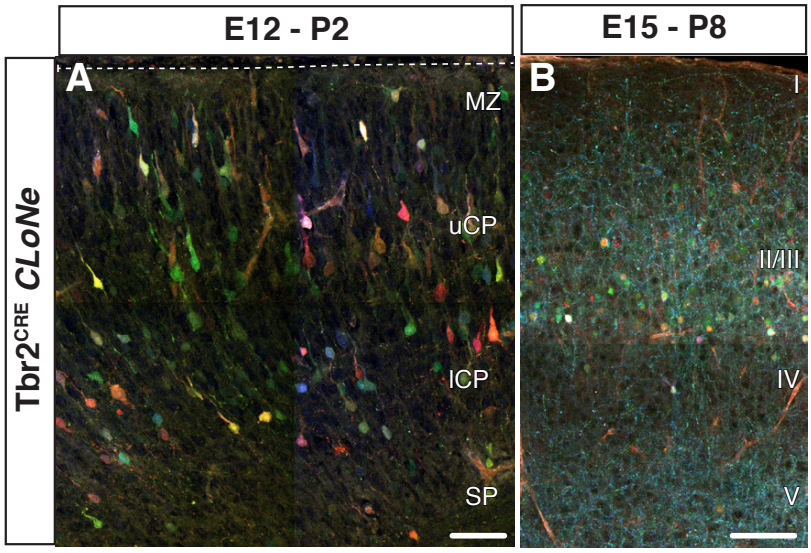


Figure S7. Conserved pattern of nuclear and membrane fluorescence in clonally related astrocytes. Expression of the four different fluorophores by the clone of astrocytes 2 from Fig. 5. Membrane (green and yellow) and nuclear labelling (red) are uniform amongst the 4 clonally related cells. Scale bar 25 µm.



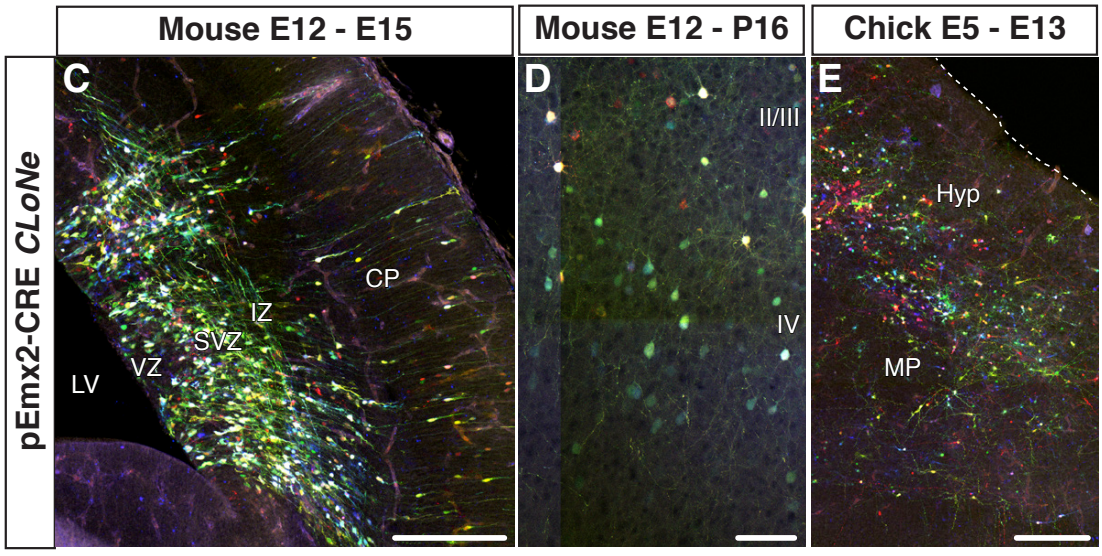


Figure S8 – Multifluorescence neuronal labelling of targeted progenitors. Mouse and chick embryos were labelled at different timepoints through selective *CLoNe*. (A-B) Coronal sections of the cerebral cortices of Tbr2^{CRE} mice electroporated at E12 or E15 and analysed at P0 or P8 respectively. Early electroporations labelled all cortical layers whereas transfection at mid-neurogenesis only labelled the upper cortical layers. (See N.A.V., F.G.-M., Siddharth Arora, Amanda F. P Cheung, Sebastian J. Arnold, Elizabeth J. Robertson and Z.M., unpublished) (C-E) Coronal sections showing multifluorescent labelled neurons by pEmx2-CRE driven *CLoNe* in mouse (C-D) and chick (E). Mouse were electroporated at E12 and analysed at either E15 (C) or P16 (D) and chick electroporations were performed at E5 and studied at E13. Scale bars 200 µm (C,E), 100 µm (B,D) and 50 µm (A).

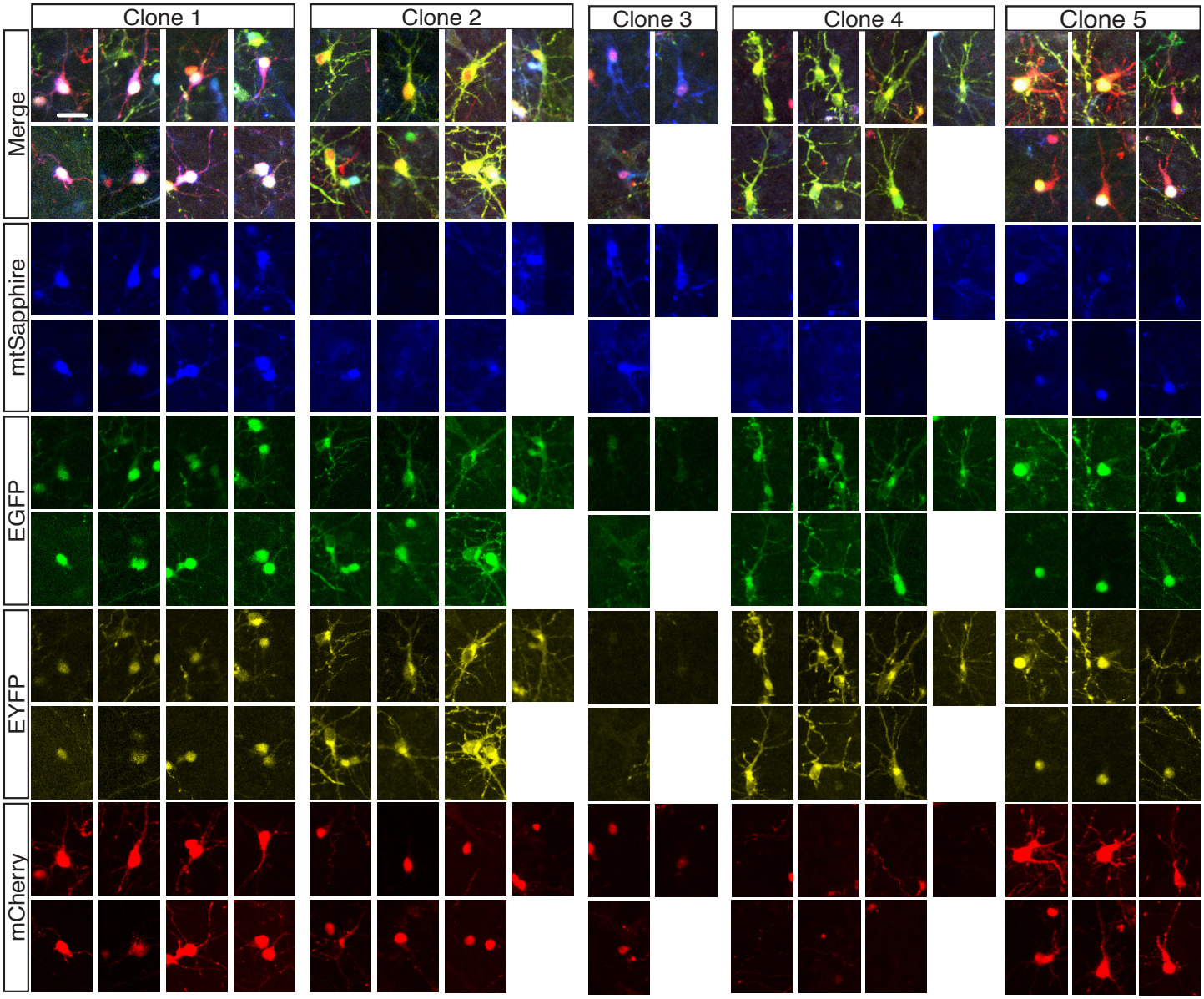


Figure S9. Fluorescence analysis of chick neuronal clones. The expression of each of the fluoroproteins was analyzed in the neurons of the clones 1 to 5 from Fig. 6. Notice the conservation of the fluorescent pattern in membrane, nucleus and cytoplasm across all the members of a given clone. Scale bar 25 μm.

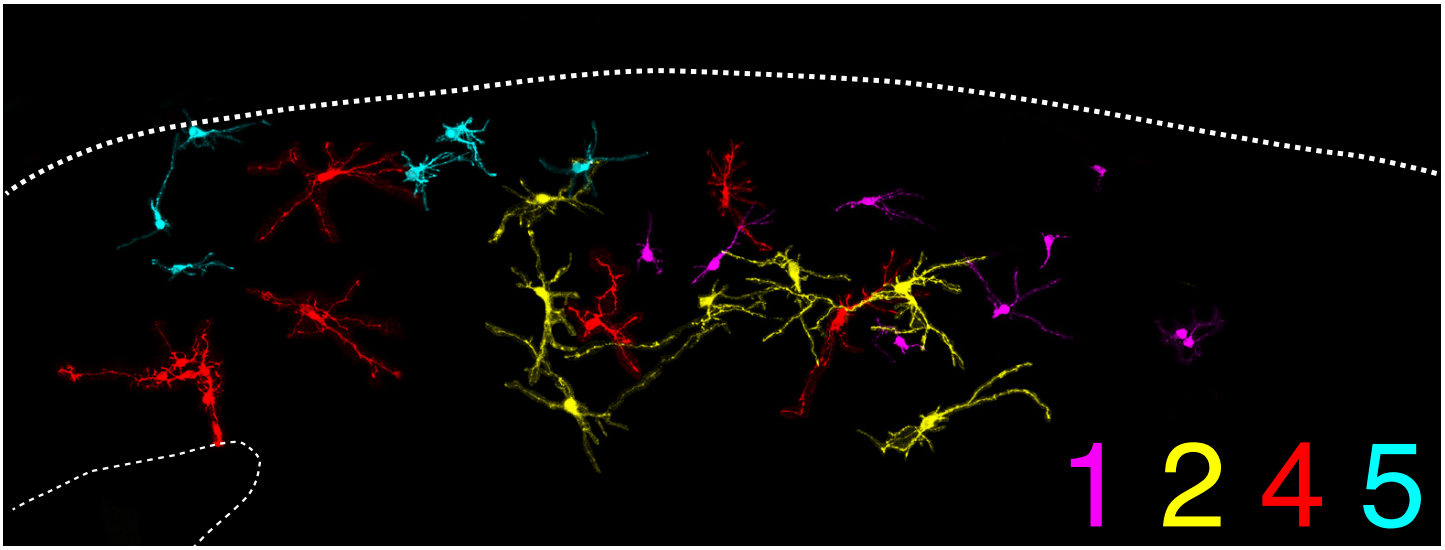


Figure S10. Distribution of 4 clones in the hyperpallium of chick labelled by *CLoNe*. Clones were isolated from original images, collapsed in a single section and pseudo-coloured to enhance distinction of clones.

Table S1. List of fluorophores and their light properties

	Protein	Absorption maximum (nm)	Laser (nm)	Emission maximum (nm)	Wavelentgh employed (nm)	Relative Brightness (% of EGFP)	Observations
Employed	**mtSapphire**	399	405	511	470-545	79	Large stoke shift. Bright, useful blue fluorophore
	EGFP	484	488	507	490-530	100	Bright and independent, no aggregation in cell detected
	EYFP	514	514	527	515-580	151	Bright and independent, slight overlap to EGFP, no aggregation in cell detected
	mCherry	587	561	610	580-695	47	Bright and independent, no aggregation in cell detected
Discarded	**mTFP1**	462	458	492		162	Very bright, overlaps with mtSapphire and EGFP
	mKeima	440	458	620		10	Fluorescence not bright enough to be comparable to the other fluorophores
	mCerulean	433	458	475		79	Fluorescence not bright enough to be comparable to the other fluorophores
	mKO	548	561	559		92	Bright, overlaps with mCherry

The list describes the features of the eight fluoroproteins tested. Accordingly, only four of them were employed in CLoNe. Part of the information was extracted from http://www.olympusconfocal.com/applications/fpcolorpalette.html.