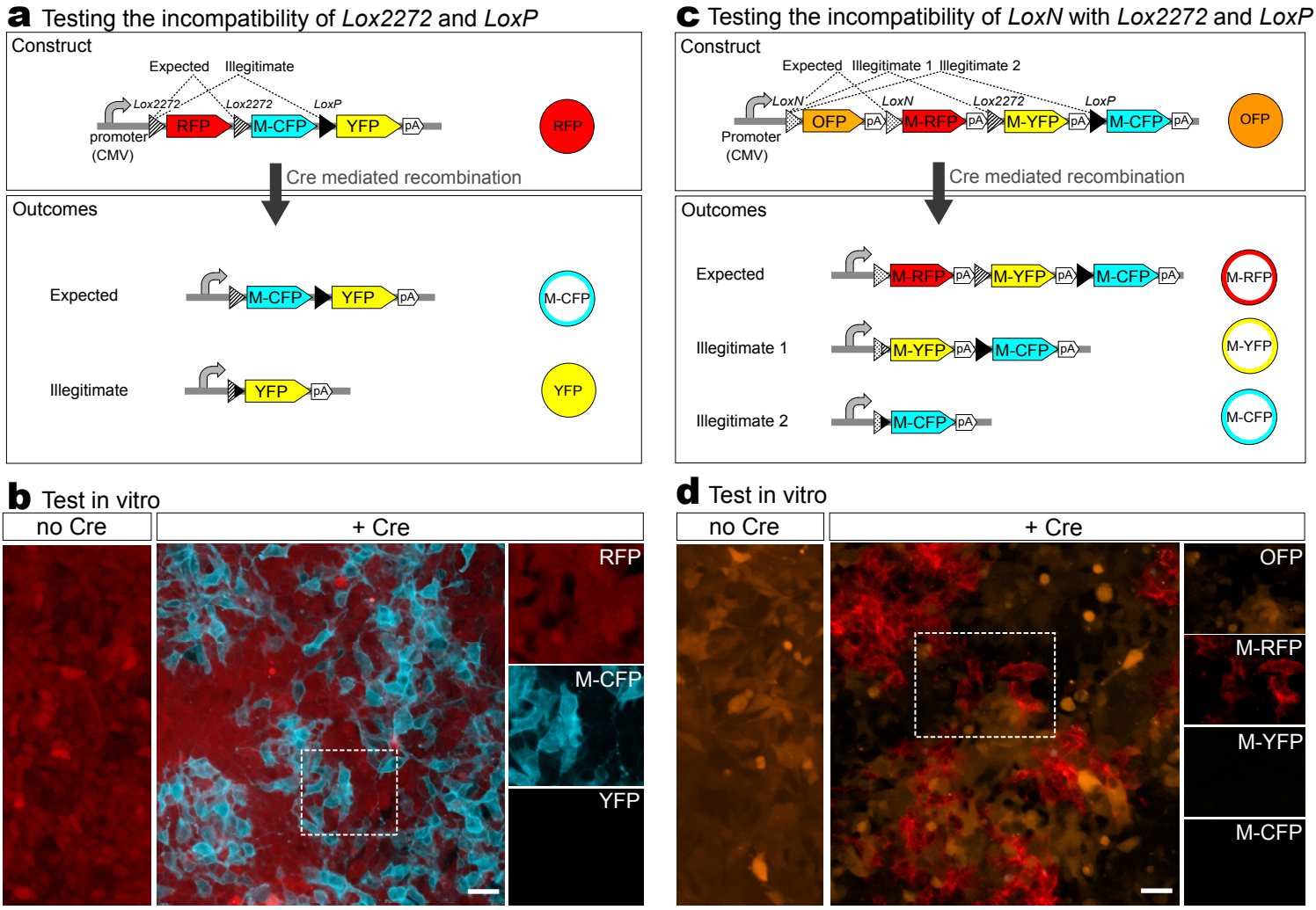
SUPPLEMENTARY INFORMATION



Supplementary Figure 1 | Mutual incompatibility of Lox variants.

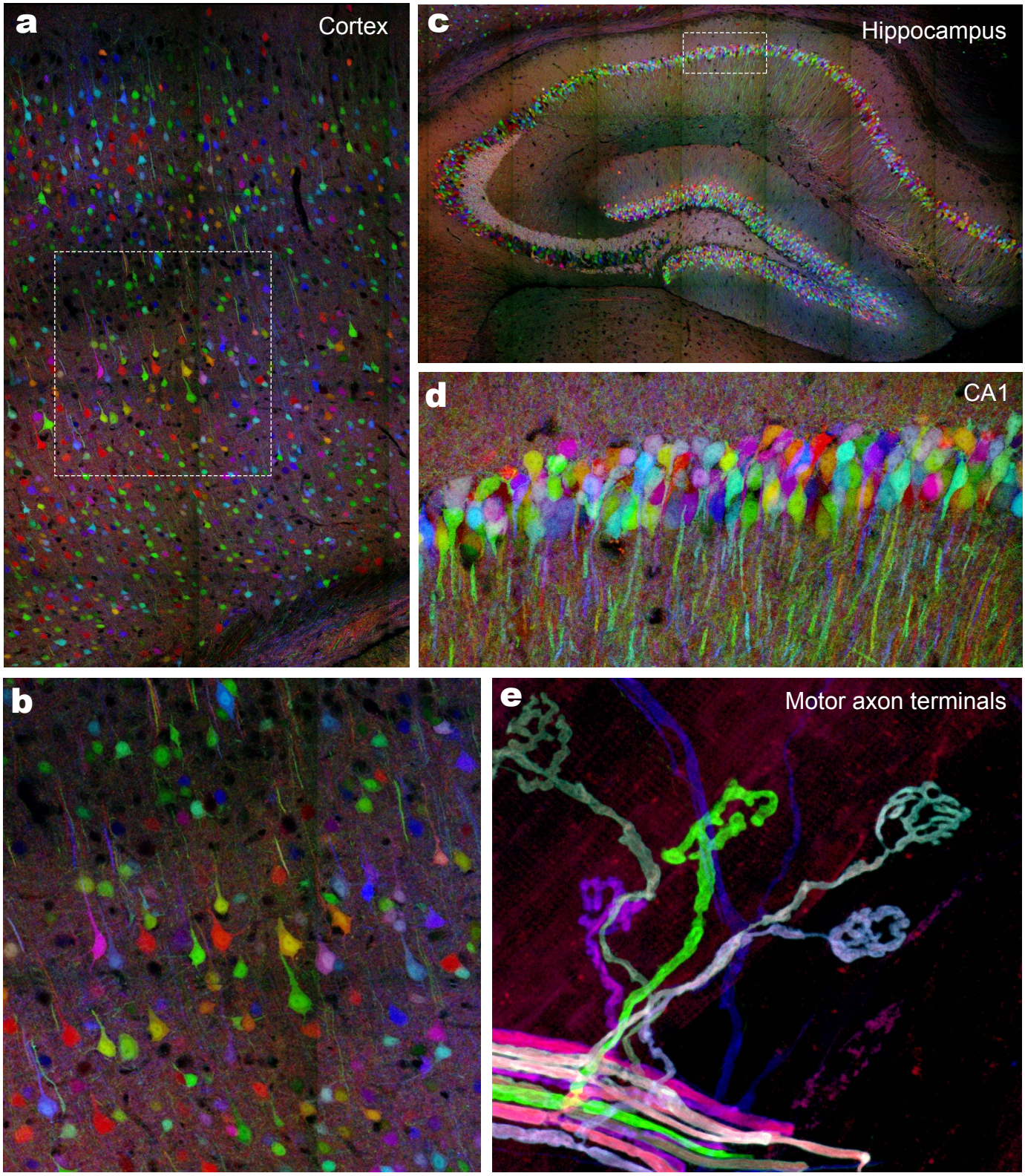
a, A control construct was designed to detect promiscuous recombination of *Lox2272* with the canonical *LoxP* sites. In this construct, recombination between the two *Lox2272* sites triggers M-CFP expression, while illegitimate recombination between *LoxP* and *Lox2272* would switch on YFP.

b, In HEK cell stably expressing this control construct, RFP was expressed in absence of recombination. Cre recombination between the two *Lox2272* led to M-CFP expression. YFP expression, which detects illegitimate recombination with the *LoxP* site, was not observed, indicating that *Lox2272* is incompatible with *LoxP*.

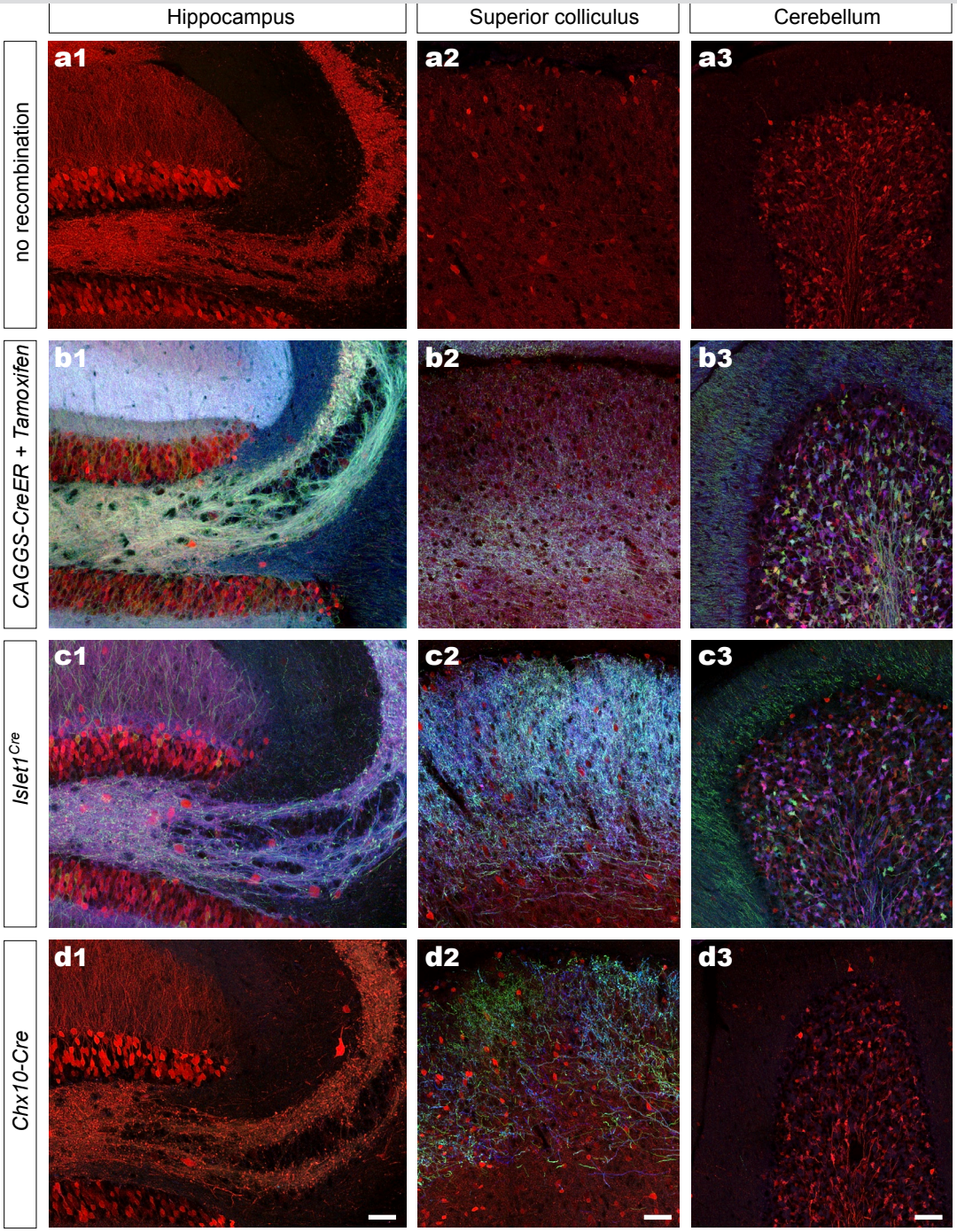
c, A novel *Lox* variant, *LoxN*, was designed. Incompatibility of *LoxN* with both *Lox2272* and *LoxP* was simultaneously tested. In the control construct, recombination between the two *Lox* variants triggers M-RFP expression, while illegitimate recombination between *LoxN* and one of the other *Lox* sites would switch on M-YFP or M-CFP expression.

d, In HEK cells stably expressing this construct, OFP is expressed in absence of recombination. Cre recombination induces recombination between the two *LoxN* variants, leading to M-RFP expression. M-YFP or M-CFP expression, which detects illegitimate recombination with the *LoxP* site, was not observed, indicating incompatibility of *LoxN* with both *Lox2272* and *LoxP*.

Scale bars: 50 μm.



Supplementary Figure 2 | Combinatorial XFP expression in Brainbow mice.
Additional examples of combinatorial expression in *Thy1-Brainbow* animals. Recombination was induced with CreERT2 and perinatal Tamoxifen injection.
a-d, Cortex and hippocampus of *Thy1-Brainbow-1.0* line L. **b** and **d** shows higher magnification of boxed regions in **a** and **c**.
e, Motor axon terminals in a skeletal muscle of *Thy1-Brainbow-1.0* line H.
Scale bars **a-d**, 50 μm; **e**, 10 μm.



Supplementary Figure 3. Restriction of Brainbow-1 expression with specific Cre drivers

Comparison of XFP expression in *Thy1-Brainbow-1.0* mice line H under different recombination conditions

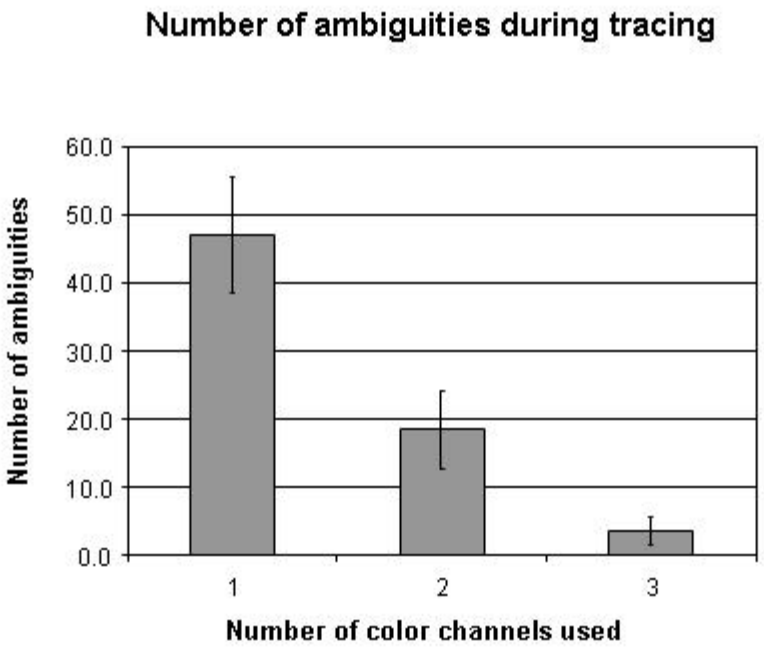
a, In absence of recombination, RFP is visible in granule cells of the dentate gyrus (a1), scattered neurons in the superior colliculus (a2) and in the inner granular layer of the cerebellum (a3). No leaky expression is detected for the other XFP genes present in the Brainbow-1.0 construct (M-YFP or M-CFP).

b, Induction of recombination using CAGGS- CreERT2 mice leads to widespread expression of M-YFP and M-CFP in Tamoxifen injected animals. The membrane-tethered XFPs are primarily detected in neuronal processes, such as hippocampal mossy fibers (b1), axons arborizing in the colliculus (b2), parallel fibers and mossy fibers in the molecular and internal granule cell layer of the cerebellum (b3).

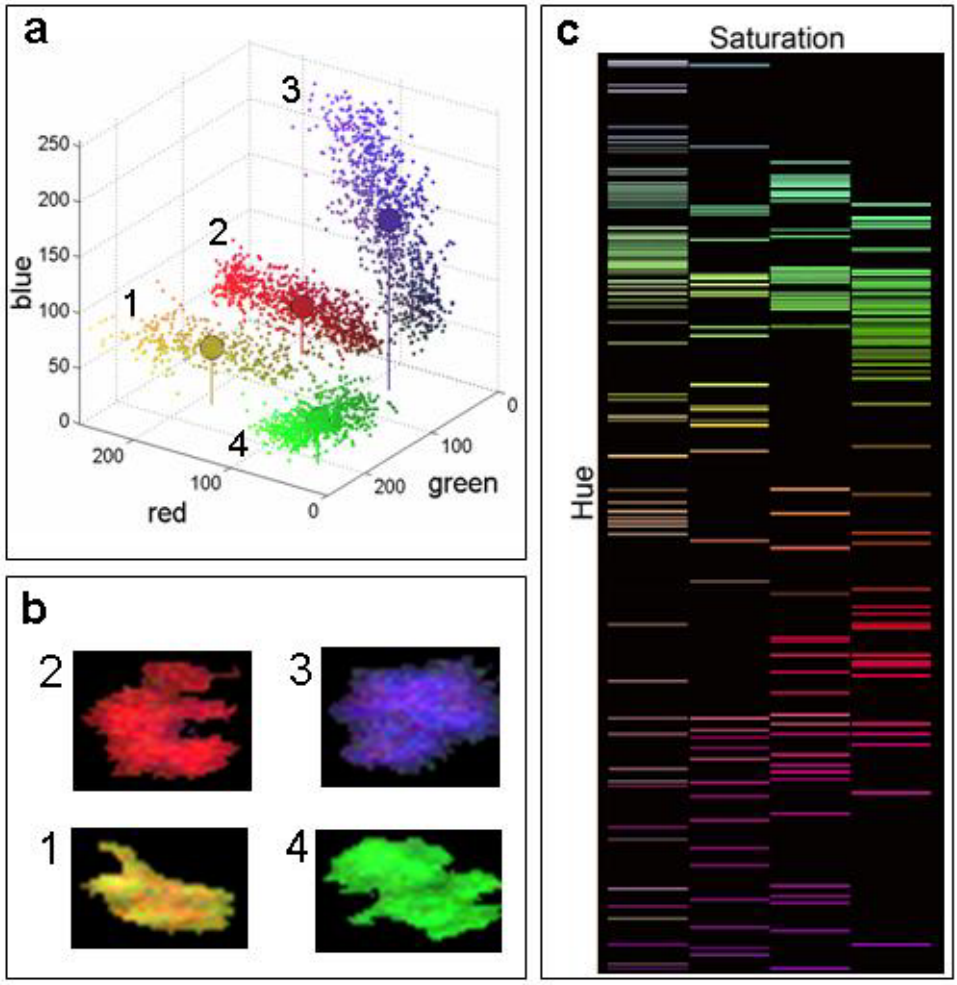
c, Crossing Thy1-Brainbow-1.0 mice with Islet1Cre animals gives rise to a narrower pattern of recombination. In the dentate gyrus, M-YFP and M-CFP expression is restricted to scattered neurons (c1). Many axons aborizing in the superior colliculus show recombination (c2), as well as components of the cerebellar circuitry (c3).

d, With Chx10-Cre driver mice, no or very little recombination is detected in the hippocampus and cerebellum (d1, d3). M-CFP and M-YFP are almost exclusively expressed in retinal ganglion axons, which arborize in the superior colliculus (d2).

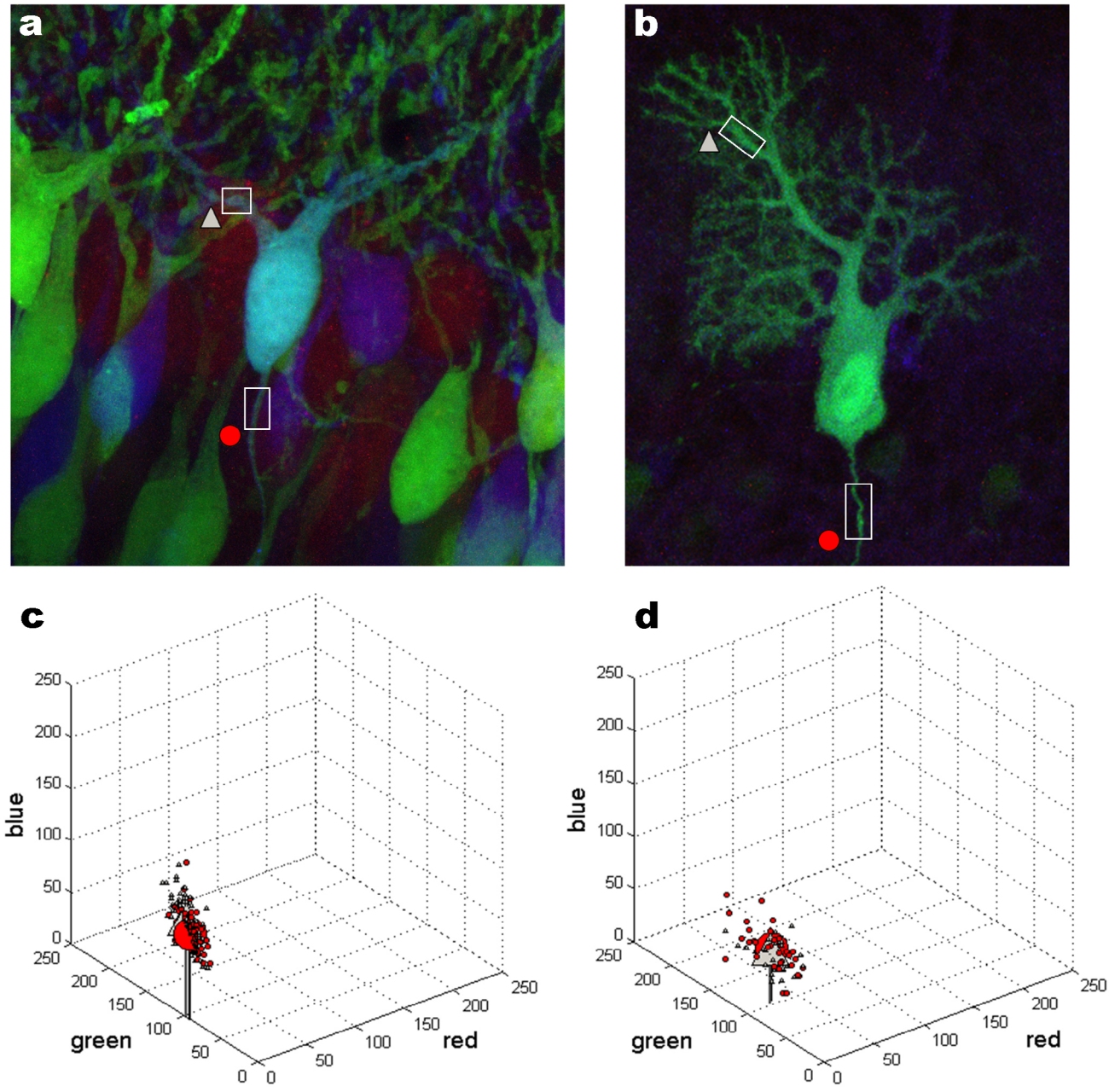
Scale bars: 100 μm.



Supplementary Figure 4 | Use of color to resolve tracing ambiguities.
In the dataset used for reconstruction of cerebellar circuitry (Fig. 5b), axon tracing throughout the volume was compared using one, two or three of the available color channels. The tracing power of the dataset was quantified by counting the number of ambiguities along the length of the axon. (Proximity to another axon of the same color caused ambiguity.) Increasing the number of color channels used for tracing increased color information and reduced the number of ambiguities.



Supplementary Figure 5. Color profiles of individual mossy fiber rosettes.
a, Distribution in RGB space of individual pixels from four separate axons. The centroid of each cluster is represented as a large dot.
b, Actual images from mossy fiber rosettes along each of the four axons represented in a.
c, Axonal colors are represented in hue-saturation coordinates (binned). Each horizontal bar represents the centroid for a given axon's color. 341 axons are shown in total.



Supplementary Figure 6. Color consistency between axons and dendrites of a given neuron.

a, Hippocampal granule neuron in dentate gyrus from *Brainbow-1.0* line L.

b, Cerebellar Purkinje cell from *Brainbow-1.0* line J. Sampled regions from dendrite (triangle) and axon (circle) are indicated in a and b.

c, d, Distributions in RGB space of individual pixels from sampled regions above. Dendritic and axonal distributions are superimposed. The centroid of each cluster is represented as a large triangle (dendrite) or circle (axon).

Supplementary Table 1. Incompatible *Lox* variants used to generate *Brainbow-1* constructs

***Lox* variant**	**Sequence**
LoxP	ATAACTTCGTATA **GCATACAT** TATACGAAGTTAT
Lox2272	ATAACTTCGTATA **GGATACTT** TATACGAAGTTAT
LoxN	ATAACTTCGTATA **GTATACCT** TATACGAAGTTAT

The 8-bp spacer which directs the specificity and the directionality of the recombination[12] is indicated in bold characters. Changes from the original *LoxP* sequence are underlined. *Lox2272*[12], which bears two changes in position 2 and 7 of the spacer, was confirmed to be incompatible with *LoxP* while efficiently mediating recombination with sites of identical sequences (Fig. 1a; Supplementary Fig. 1a). A new variant, *LoxN*, was designed based on the *Lox2272* model, with different changes in position 2 and 7. This new variant was found to be incompatible with both *LoxP* and *Lox2272* (Supplementary Fig.1b), while retaining comparable recombination efficiency (Fig. 1d, e). Sequences are displayed in the orientation used in the constructs, which avoids introducing start codons.

Supplementary Table 2. *Thy1-Brainbow* transgenic mouse lines

# line	XFPs				subset size		type of expression	cell types labeled
	1st	2nd	3rd	4th	basal	after Cre		
Brainbow-1.0	RFP	M-YFP	M-CFP					
A	dsRed2	M-EYFP	M-mCerulean		small	medium	combinatorial	peripheral and central neurons
B	dsRed2	M-EYFP	M-mCerulean		large	large	exclusive	**non-myelinating Schwann cells** (Schwann cells associated with neuromuscular junctions, autonomic ganglia and non-myelinated peripheral axons); irregular scattered motor neurons.
C	dsRed2	M-EYFP	M-mCerulean		small	small	combinatorial	peripheral and central neurons
D	dsRed2	M-EYFP	M-mCerulean		small	medium	exclusive	peripheral and central neurons
E	dsRed2	M-EYFP	M-mCerulean		small	medium	exclusive	peripheral and central neurons
F	dsRed2	M-EYFP	M-mCerulean		large	large	combinatorial	peripheral and central neurons
G	dsRed2	M-EYFP	M-mCerulean		large	large	combinatorial	**Bergmann glia** of the molecular layer of the cerebellum; scattered neurons (few)
$G^{(Flp)}$	dsRed2	M-EYFP	M-mCerulean		large	large	exclusive	ibid.
H	tdimer2	M-EYFP	M-mCerulean		large	large	combinatorial	**peripheral and central neurons** including peripheral sensory neurons, cranial and spinal motor neurons (~75%), retinal ganglion cells, dentate gyrus granule cells, pyramidal neurons of CA1 and some cortical areas, inferior olive neurons and associated mossy fibers (~20%).
$H^{(Flp)}$	tdimer2	M-EYFP	M-mCerulean		large	large	exclusive	ibid.
I	tdimer2	M-EYFP	M-mCerulean		large	large	combinatorial	peripheral and central neurons
Brainbow-1.0	RFP	CFP	YFP					
J	dTomato	mCerulean	EYFP		large	large	combinatorial	peripheral and central neurons
K	dTomato	mCerulean	EYFP		large	large	combinatorial	peripheral and central neurons
L	dTomato	mCerulean	EYFP		large	large	combinatorial	**peripheral and central neurons** (same types labeled as H; a few cerebellar Purkinje neurons)
Brainbow-1.1	OFP	M-RFP	M-YFP	M-CFP				
M	Kusabira	M-mCherry	M-EYFP	M-mCerulean	undetected	large	exclusive	**astrocytes** of all areas of the brain and spinal cord; dentate gyrus granule cells; scattered neurons (few).
Brainbow-2.0	RFP	M-CFP						
N	tdimer2	M-ECFP			large	large	combinatorial	peripheral and central neurons
O	tdimer2	M-ECFP			small	small	combinatorial	peripheral and central neurons
Brainbow-2.1	nuc-GFP	YFP	RFP	M-CFP				
P	hrGFPII-NLS	EYFP	tdimer2	M-mCerulean	small	small	too sparse	peripheral and central neurons
Q	hrGFPII-NLS	EYFP	tdimer2	M-mCerulean	small	medium	combinatorial	some neurons, astrocytes
R	hrGFPII-NLS	EYFP	tdimer2	M-mCerulean	medium	large	combinatorial	**peripheral and central neurons** (same types labeled as H; a few cerebellar Purkinje neurons)
S	hrGFPII-NLS	EYFP	tdimer2	M-mCerulean	medium	large	combinatorial	peripheral and central neurons

A total of 25 *Thy1-Brainbow* transgenic mouse lines were generated. 9 lines not described here had either no detectable XFP expression, or did not transmit the *Brainbow* transgene to their progeny. Two lines (H and I) were initially derived from the same founder and were later segregated based on their distinct expression patterns. $G^{(Flp)}$ and $H^{(Flp)}$ are lines with reduced transgene copy number derived by crossing lines G and H with *βactin-Flp$_e$* mice (Fig. 4d). In line H, percentage of labeled cell types were estimated by counting the number of postsynaptic sites occupied by a labeled presynaptic axon. Recommended lines for the study of particular cell types are indicated in bold characters.

M: membrane targeting signal; mXFP: monomeric variant of XFP; NLS: nuclear localization signal.