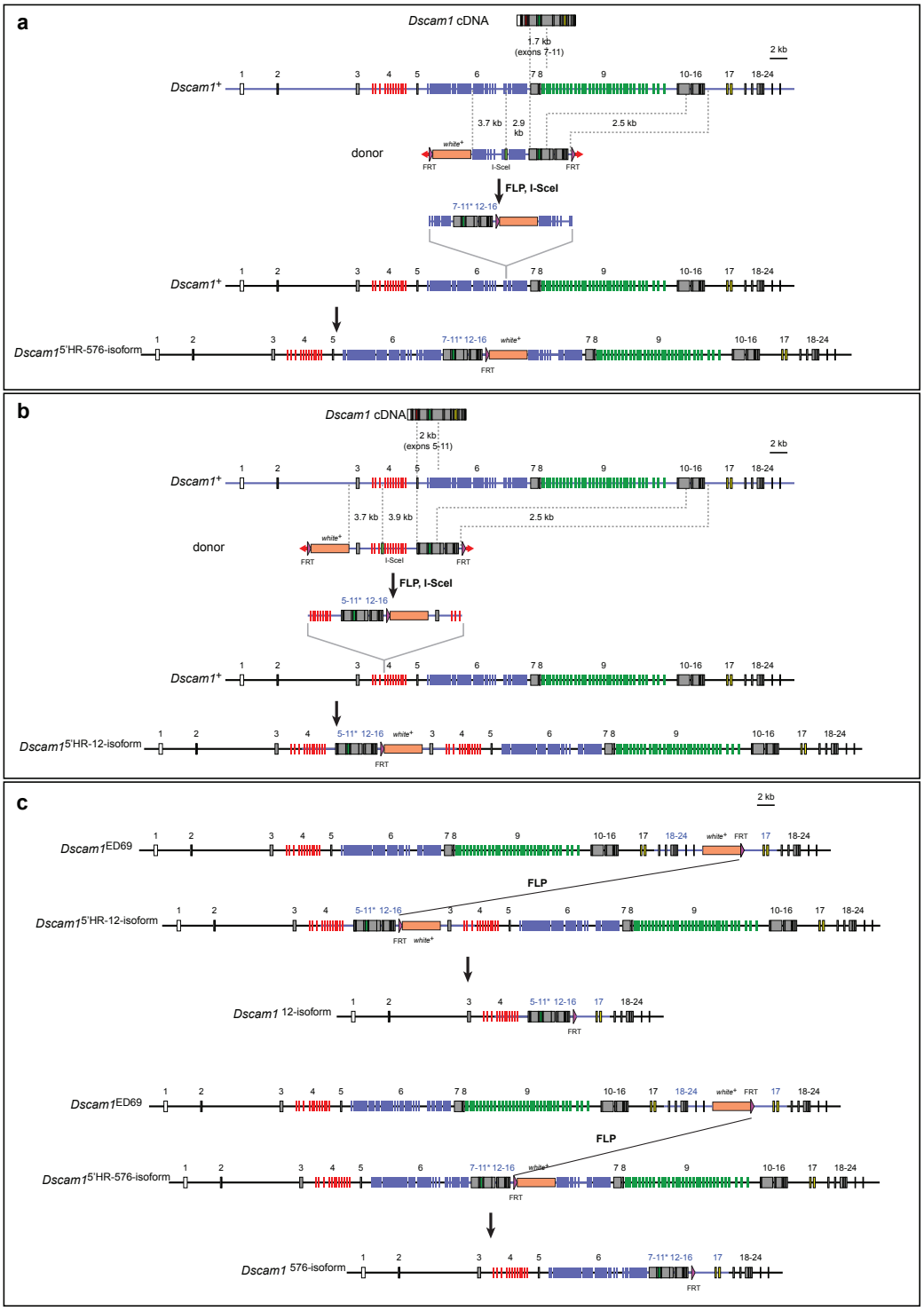
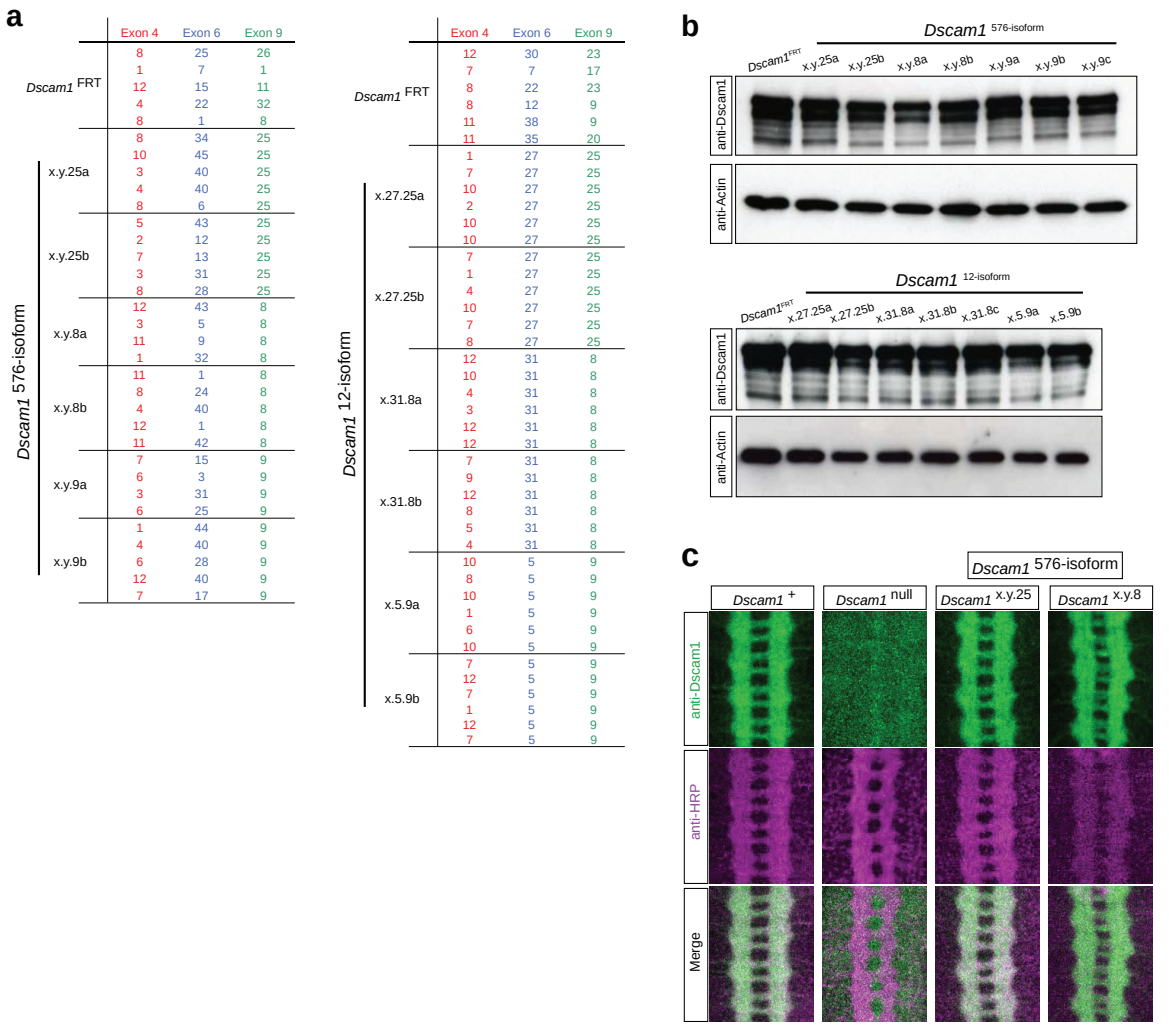
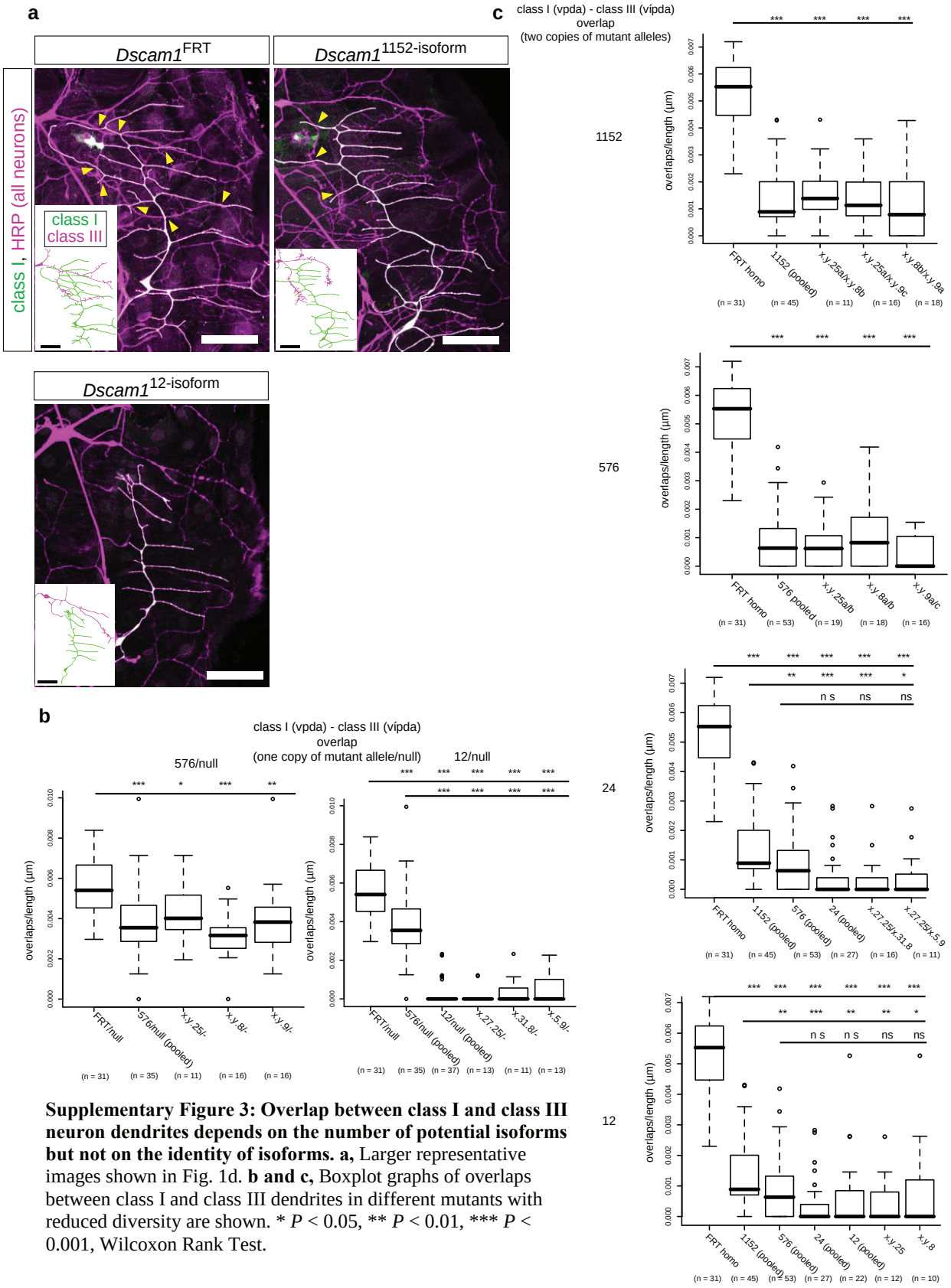
SUPPLEMENTARY INFORMATION



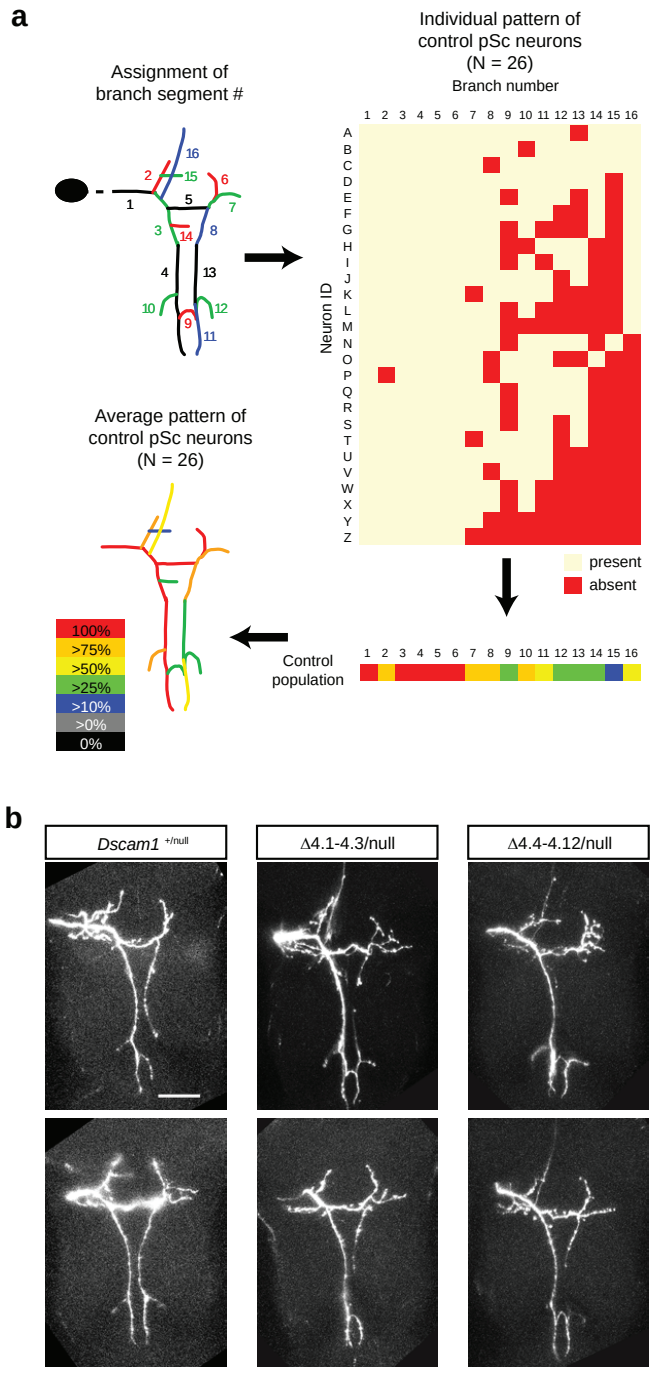
Supplementary Figure 1: A two-step gene targeting strategy to modify the *Dscam1* locus. a and b, Ends-in homologous recombination was utilized to generate *Dscam1*$^{5'\text{HR-576-isoform}}$ and *Dscam1*$^{5'\text{HR-12-isoform}}$ alleles. **c,** Interchromosomal recombination mediated by FLP between *Dscam1*$^{5'\text{HR-576-isoform}}$, or *Dscam1*$^{5'\text{HR-12-isoform}}$, and *Dscam1*^{ED69} alleles[11] results in the resolution of the duplication introduced by ends-in homologous recombination, generating intact *Dscam1*$^{576\text{-isoform}}$ or *Dscam1*$^{12\text{-isoform}}$ alleles. As a consequence of this recombination, an FRT site remains in the intron between exon 16 and 17.



Supplementary Figure 2: Characterization of mutant *Dscam1* alleles with reduced diversity. a, *Dscam1*$^{576\text{-isoform}}$ and *Dscam1*$^{12\text{-isoform}}$ alleles express designated fixed alternative exons in combination with different variants in the remaining alternative clusters. Larval CNS complex was analyzed. Each row in the table represents one cDNA clone derived from the genotype indicated on the left. For example, the top left row shows that a cDNA found in *Dscam1*^{FRT} control animals encodes isoform 8.25.26 (i.e., exons 4.8, 6.25, and 9.26 variants). **b,** *Dscam1*$^{576\text{-isoform}}$ and *Dscam1*$^{12\text{-isoform}}$ alleles express Dscam1 protein at similar levels to wild type control. Dscam1 protein levels in the developing central nervous system were assessed by immunoblotting using anti-Dscam1 antibody raised against the cytoplasmic domain[3]. Immunoblot against actin was used as a loading control. Animals analyzed carried one copy of *Dscam1* mutant allele over one copy of a *Dscam1* protein null allele. **c,** The localization of protein produced from mutant *Dscam1* alleles is similar to that of the control. Stage 17 embryos of indicated genotypes (all homozygous) were stained with anti-Dscam1 antibody raised against a common ectodomain epitope (green). Anti-HRP antibody was used to visualize neuropil (magenta).

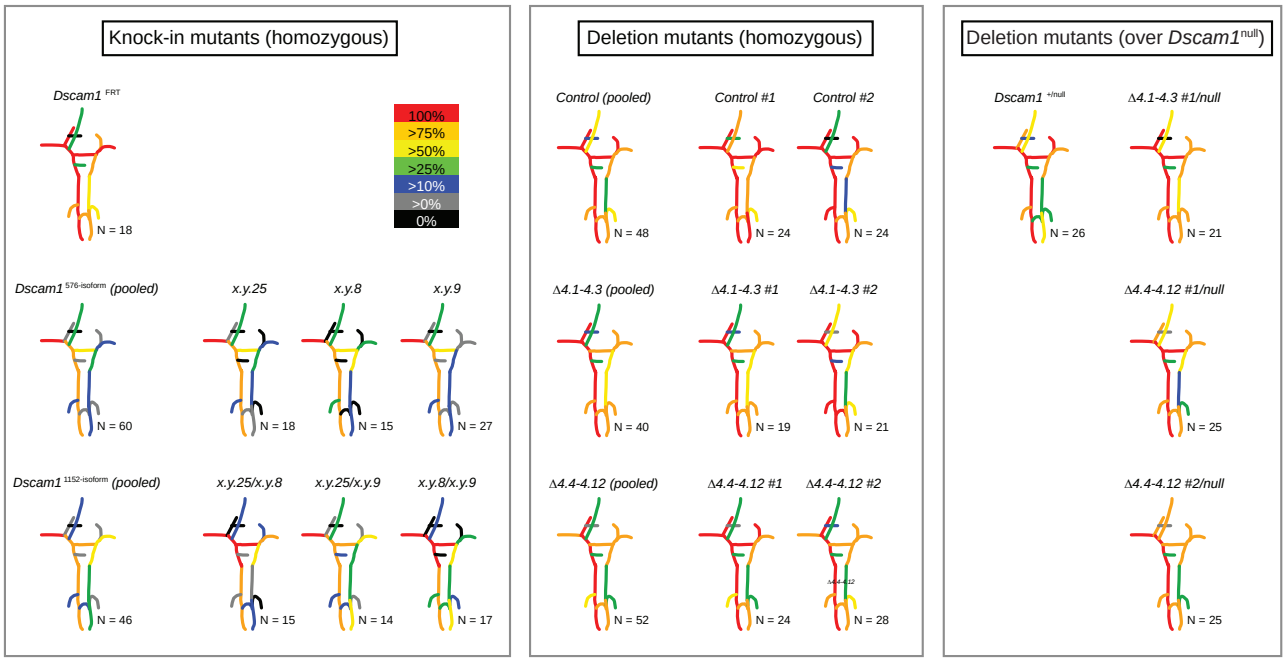


Supplementary Figure 3: Overlap between class I and class III neuron dendrites depends on the number of potential isoforms but not on the identity of isoforms. a, Larger representative images shown in Fig. 1d. **b and c,** Boxplot graphs of overlaps between class I and class III dendrites in different mutants with reduced diversity are shown. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Wilcoxon Rank Test.



Supplementary Figure 4: Analysis of branching patterns of posterior scutellar (pSc) neurons. a, A schematic representation of the scoring method. Each image was examined section by section to score the presence or absence of branch segments. The resulting matrix was averaged to generate an average branching pattern for each genotype. Scoring was done blindly for deletion mutants as well as their controls. **b,** Sample images of posterior scutellar neurons in deletion mutants over *Dscam1*^{null} allele. Scale bar = 50μm.

a Average branching patterns



b

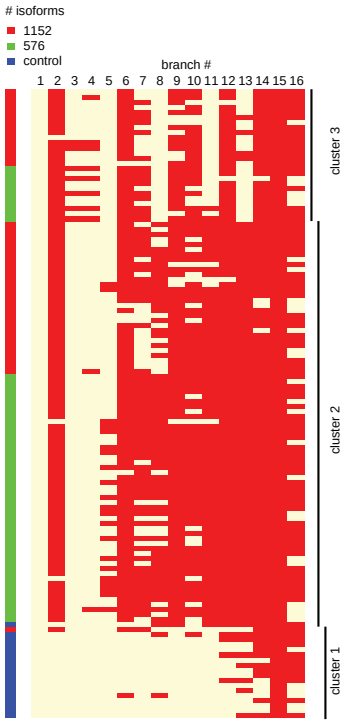
		Branch number															
	N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Dscam1FRT	18	100%	100%	100%	100%	100%	94.4%	100%	83.3%	94.4%	94.4%	94.4%	72.2%	55.6%	38.9%	0%	33.3%
x.y.25	18	100%	5.6%	88.9%	83.3%	61.1%	0%	16.7%	27.8%	5.6%	16.7%	5.6%	0%	16.7%	0%	0%	33.3%
x.y.8	15	100%	0%	93.3%	93.3%	66.7%	0%	40.0%	53.3%	0%	33.3%	20.0%	0%	20.0%	0%	0%	33.3%
x.y.9	27	100%	3.7%	92.6%	92.6%	74.1%	3.7%	7.4%	22.2%	3.7%	14.8%	22.2%	3.7%	18.5%	3.7%	0%	25.9%
x.y.25/x.y.8	15	100%	0%	100%	93.3%	100%	20.0%	80.0%	66.7%	13.3%	6.7%	20.0%	0%	6.7%	6.7%	0%	20.0%
x.y.25/x.y.9	14	100%	7.1%	85.7%	85.7%	85.7%	7.1%	50.0%	35.7%	21.4%	21.4%	50.0%	7.1%	28.6%	14.3%	0%	28.6%
x.y.8/x.y.9	17	100%	0%	100%	94.1%	100%	0%	29.4%	64.7%	29.4%	41.2%	52.9%	11.8%	41.2%	0%	0%	11.8%
576 (pooled)	60	100%	3.3%	91.7%	90.0%	68.3%	1.7%	18.3%	31.7%	3.3%	20.0%	16.7%	1.7%	18.3%	1.7%	0%	30.0%
1152 (pooled)	46	100%	2.2%	95.7%	91.3%	95.7%	8.7%	52.2%	56.5%	21.7%	23.9%	41.3%	6.5%	26.1%	6.5%	0%	19.6%
+/null	26	100%	96.2%	100%	100%	100%	100%	88.5%	76.9%	46.2%	80.8%	69.2%	42.3%	46.2%	30.8%	15.4%	50.0%
Δ4.1-4.3 #1/null	21	100%	100%	100%	100%	100%	95.2%	95.2%	81.0%	90.5%	81.0%	95.2%	76.2%	66.7%	42.9%	0%	57.1%
Δ4.4-4.12 #1/null	25	100%	92.0%	100%	100%	100%	92.0%	96.0%	68.0%	88.0%	88.0%	92.0%	48.0%	20.0%	40.0%	4.0%	60.0%
Δ4.4-4.12 #2/null	25	100%	88.0%	100%	100%	96.0%	96.0%	96.0%	76.0%	88.0%	76.0%	96.0%	36.0%	48.0%	44.0%	4.0%	76.0%
Control #1 (+/+)	24	100%	100%	100%	100%	100%	100%	100%	75.0%	79.2%	95.8%	100%	62.5%	75.0%	54.2%	33.3%	75.0%
Control #2 (+/+)	24	100%	100%	100%	100%	100%	100%	91.7%	83.3%	75.0%	100%	79.2%	50.0%	20.8%	16.7%	0%	29.2%
Δ4.1-4.3 #1	19	100%	100%	100%	100%	94.7%	89.5%	84.2%	63.2%	84.2%	94.7%	89.5%	84.2%	63.2%	31.6%	10.5%	36.8%
Δ4.1-4.3 #2	21	100%	100%	100%	100%	100%	100%	95.2%	71.4%	100%	100%	95.2%	71.4%	42.9%	19.0%	9.5%	52.4%
Δ4.4-4.12 #1	24	100%	100%	100%	100%	100%	100%	91.7%	87.5%	83.3%	54.2%	87.5%	37.5%	45.8%	37.5%	4.2%	37.5%
Δ4.4-4.12 #2	28	96.4%	100%	100%	100%	96.4%	96.4%	85.7%	89.3%	75.0%	78.6%	89.3%	53.6%	39.3%	25.0%	14.3%	32.1%
Control (+/+, pooled)	48	100%	100%	100%	100%	100%	100%	95.8%	79.2%	77.1%	97.9%	89.6%	56.3%	47.9%	35.4%	16.7%	52.1%
Δ4.1-4.3 (pooled)	40	100%	100%	100%	100%	97.5%	95.0%	90.0%	67.5%	92.5%	97.5%	92.5%	77.5%	52.5%	25.0%	10.0%	45.0%
Δ4.4-4.12 (pooled)	52	98.1%	100%	100%	100%	98.1%	98.1%	88.5%	88.5%	78.8%	67.3%	88.5%	46.2%	42.3%	30.8%	9.6%	34.6%

Supplementary Figure 5: Branching patterns of posterior scutellar neurons in different mutants. a, Average branching patterns for individual genotypes are shown. **b,** A table of average branching patterns in each mutant and control.

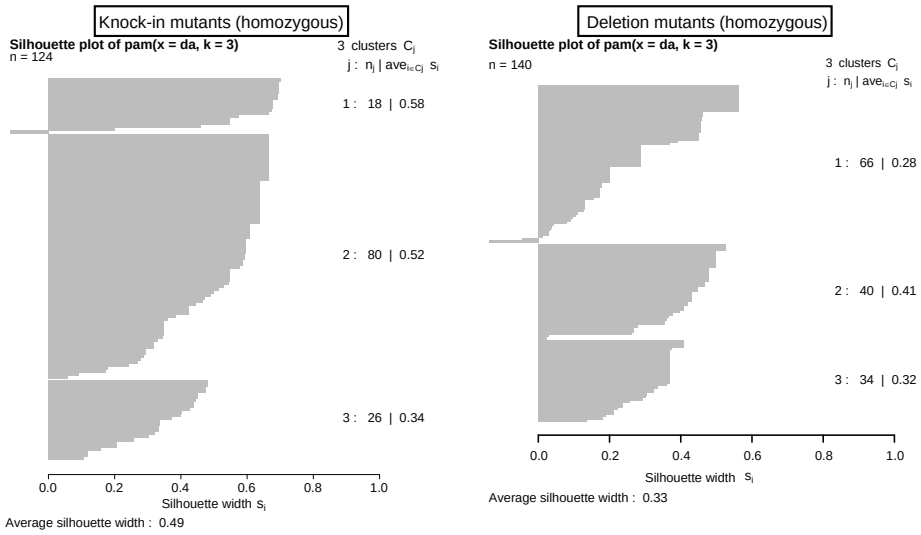
a

Comparison	N1	N2	min P-value	Perm. P-value	Bonferroni adjsted P-values
Dscam1FRT vs 1152 (pooled)	18	46	5.28E-15	0	0
Dscam1FRT vs 576 (pooled)	18	60	8.94E-16	0	0
Dscam1FRT vs x.y.25	18	15	9.64E-10	0	0
Dscam1FRT vs x.y.8	18	14	4.03E-08	0	0
Dscam1FRT vs x.y.9	18	17	2.20E-10	0	0
Dscam1FRT vs x.y.25/x.y.8	18	18	4.19E-09	0	0
Dscam1FRT vs x.y.25/x.y.9	18	15	9.64E-10	0	0
Dscam1FRT vs x.y.8/x.y.9	18	27	1.11E-11	0	0
x.y.25 vs x.y.8	18	15	0.16900	0.585	1
x.y.25 vs x.y.9	18	27	0.21500	0.731	1
x.y.8 vs x.y.9	15	27	0.01640	0.0682	1
x.y.25/x.y.8 vs x.y.25/x.y.9	15	14	0.12800	0.499	1
x.y.25/x.y.8 vs x.y.8/x.y.9	15	17	0.00601	0.0141	0.423
x.y.25/x.y.9 vs x.y.8/x.y.9	14	17	0.15600	0.539	1
pooled 576 vs pooled 1152	60	46	0.00035	0.00135	0.0405
Control #1 (+/+) vs Control #2 (+/+)	24	24	0.00040	0.0005	0.015
Control #1 (+/+) vs Δ4.1-4.3 #1	24	19	0.01556	0.057	1
Control #1 (+/+) vs Δ4.1-4.3 #2	24	21	0.02968	0.1215	1
Control #1 (+/+) vs Δ4.4-4.12 #1	24	24	0.00180	0.0065	0.195
Control #1 (+/+) vs Δ4.4-4.12 #2	24	28	0.00262	0.0112	0.336
Control #2 (+/+) vs Δ4.1-4.3 #1	24	19	0.01091	0.0355	1
Control #2 (+/+) vs Δ4.1-4.3 #2	24	21	0.02319	0.068	1
Control #2 (+/+) vs Δ4.4-4.12 #1	24	24	0.00022	0.0002	0.006
Control #2 (+/+) vs Δ4.4-4.12 #2	24	28	0.02512	0.1111	1
Δ4.1-4.3 #1 vs Δ4.1-4.3 #2	19	21	0.09808	0.351	1
Δ4.1-4.3 #1 vs Δ4.4-4.12 #1	19	24	0.00441	0.0178	0.534
Δ4.1-4.3 #1 vs Δ4.4-4.12 #2	19	28	0.05803	0.2619	1
Δ4.1-4.3 #2 vs Δ4.4-4.12 #1	21	24	0.00028	0.0006	0.018
Δ4.1-4.3 #2 vs Δ4.4-4.12 #2	21	28	0.01514	0.0641	1
Δ4.4-4.12 #1 vs Δ4.4-4.12 #2	24	28	0.07994	0.3284	1

b



c



Supplementary Figure 6: Comparisons of branching patterns of posterior scutellar neurons. a, A table containing raw statistical data. N1 and N2 represent the sample sizes of each genotype. "min P-value" represents the smallest *P* values obtained from Fisher's exact test. Note that these *P* values are not corrected. "Perm. P-value" represents *P* values obtained from Permutation test. 10,000 permutations were used for analysis of deletion mutants and 20,000 permutations were used for analysis of knock-in mutants. "Bonferroni adjusted P-value" represents the final *P* values corrected with Bonferroni correction accounting for 30 different pair-wise comparisons in this study. **b,** A result of partitioning cluster analysis of branching patterns of posterior scutellar neurons in knock-in mutants. **c,** Results of Silhouette analysis of each partitioning clustering. More distinct cluster was observed for knock-in mutants (average silhouette width 0.49) than for deletion mutants (average silhouette width 0.33).

a

Probability that a pair of neurons share *x* or more isoforms out of 30 mRNAs ($Q_{x,i}$)

Number of potential isoforms (*i*)

x	20000	10000	5000	2000	1000	500	200	100
0	1	1	1	1	1	1	1	1
1	4.4E-02	8.6E-02	1.6E-01	3.6E-01	5.9E-01	8.3E-01	9.9E-01	1.0E+00
2	9.2E-04	3.6E-03	1.3E-02	7.1E-02	2.2E-01	5.2E-01	9.3E-01	1.0E+00
3	1.2E-05	9.3E-05	7.0E-04	9.1E-03	5.3E-02	2.4E-01	7.9E-01	9.9E-01
4	1.2E-07	1.7E-06	2.5E-05	8.2E-04	9.5E-03	8.2E-02	5.7E-01	9.6E-01
5	0	2.2E-08	6.7E-07	5.5E-05	1.3E-03	2.2E-02	3.5E-01	8.8E-01
6	0	0	1.0E-08	3.0E-06	1.3E-04	4.5E-03	1.8E-01	7.5E-01
7	0	0	0	1.3E-07	1.1E-05	7.6E-04	7.4E-02	5.7E-01
8	0	0	0	4.0E-09	7.2E-07	1.0E-04	2.6E-02	3.8E-01
9	0	0	0	0	4.0E-08	1.1E-05	7.3E-03	2.2E-01
10	0	0	0	0	1.0E-09	9.7E-07	1.7E-03	1.1E-01
11	0	0	0	0	0	7.4E-08	3.4E-04	4.4E-02
12	0	0	0	0	0	5.0E-09	5.6E-05	1.6E-02
13	0	0	0	0	0	0	7.7E-06	4.6E-03
14	0	0	0	0	0	0	8.6E-07	1.1E-03
15	0	0	0	0	0	0	8.5E-08	2.3E-04
16	0	0	0	0	0	0	3.0E-09	4.0E-05
17	0	0	0	0	0	0	0	5.6E-06
18	0	0	0	0	0	0	0	6.3E-07
19	0	0	0	0	0	0	0	5.3E-08
20	0	0	0	0	0	0	0	5.0E-09
21	0	0	0	0	0	0	0	0

Probability that a pair of neurons share *x* or more isoforms out of 50 mRNAs ($Q_{x,i}$)

Number of potential isoforms (*i*)

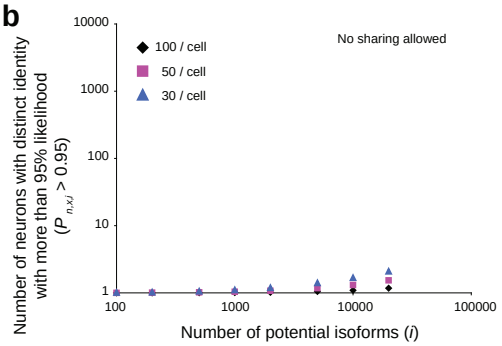
x	20000	10000	5000	2000	1000	500	200	100
0	1	1	1	1	1	1	1	1
1	1.2E-01	2.2E-01	3.9E-01	7.1E-01	9.2E-01	9.9E-01	1.0E+00	1
2	6.9E-03	2.6E-02	8.7E-02	3.5E-01	7.0E-01	9.6E-01	1.0E+00	1
3	2.6E-04	1.9E-03	1.3E-02	1.2E-01	4.3E-01	8.6E-01	1.0E+00	1
4	7.2E-06	1.1E-04	1.4E-03	3.2E-02	2.1E-01	6.9E-01	1.0E+00	1.0E+00
5	1.6E-07	4.6E-06	1.2E-04	6.5E-03	8.4E-02	4.9E-01	9.9E-01	1.0E+00
6	2.0E-09	1.6E-07	7.9E-06	1.1E-03	2.7E-02	3.0E-01	9.6E-01	1.0E+00
7	0	3.0E-09	4.5E-07	1.5E-04	7.5E-03	1.6E-01	9.2E-01	1.0E+00
8	0	0	1.5E-08	1.7E-05	1.7E-03	7.1E-02	8.3E-01	1.0E+00
9	0	0	1.0E-09	1.7E-06	3.5E-04	2.8E-02	7.1E-01	1.0E+00
10	0	0	0	1.5E-07	5.9E-05	9.8E-03	5.6E-01	9.9E-01
11	0	0	0	9.0E-09	8.9E-06	2.9E-03	4.1E-01	9.8E-01
12	0	0	0	0	1.2E-06	7.8E-04	2.7E-01	9.6E-01
13	0	0	0	0	1.2E-07	1.8E-04	1.6E-01	9.2E-01
14	0	0	0	0	1.5E-08	3.8E-05	8.7E-02	8.5E-01
15	0	0	0	0	0	6.9E-06	4.2E-02	7.5E-01
16	0	0	0	0	0	1.1E-06	1.8E-02	6.2E-01
17	0	0	0	0	0	1.7E-07	7.2E-03	4.9E-01
18	0	0	0	0	0	2.1E-08	2.5E-03	3.5E-01
19	0	0	0	0	0	4.0E-09	7.9E-04	2.3E-01
20	0	0	0	0	0	0	2.2E-04	1.4E-01
21	0	0	0	0	0	0	5.6E-05	7.8E-02
22	0	0	0	0	0	0	1.2E-05	3.9E-02
23	0	0	0	0	0	0	2.6E-06	1.8E-02
24	0	0	0	0	0	0	4.7E-07	7.2E-03
25	0	0	0	0	0	0	7.0E-08	2.6E-03
26	0	0	0	0	0	0	1.0E-08	8.6E-04
27	0	0	0	0	0	0	1.0E-09	2.5E-04
28	0	0	0	0	0	0	0	6.4E-05
29	0	0	0	0	0	0	0	1.5E-05
30	0	0	0	0	0	0	0	3.0E-06
31	0	0	0	0	0	0	0	5.2E-07
32	0	0	0	0	0	0	0	8.0E-08
33	0	0	0	0	0	0	0	1.4E-08
34	0	0	0	0	0	0	0	1.0E-09
35	0	0	0	0	0	0	0	0

Probability that a pair of neurons share *x* or more isoforms out of 100 mRNAs ($Q_{x,i}$)

Number of potential isoforms (*i*)

x	20000	10000	5000	2000	1000	500	200	100
0	1	1	1	1	1	1	1	1
1	3.9E-01	6.3E-01	8.6E-01	9.9E-01	1.0E+00	1	1	1
2	8.9E-02	2.6E-01	5.9E-01	9.6E-01	1.0E+00	1	1	1
3	1.4E-02	7.7E-02	3.1E-01	8.7E-01	1.0E+00	1.0E+00	1	1
4	1.6E-03	1.7E-02	1.3E-01	7.1E-01	9.9E-01	1.0E+00	1	1
5	1.4E-04	3.1E-03	4.6E-02	5.2E-01	9.6E-01	1.0E+00	1	1
6	1.1E-05	4.6E-04	1.3E-02	3.4E-01	9.1E-01	1.0E+00	1	1
7	6.7E-07	5.7E-05	3.3E-03	2.0E-01	8.3E-01	1.0E+00	1	1
8	3.4E-08	6.1E-06	7.0E-04	1.0E-01	7.1E-01	1.0E+00	1	1
9	1.0E-09	5.8E-07	1.3E-04	4.5E-02	5.7E-01	9.9E-01	1	1
10	0	4.7E-08	2.1E-05	1.8E-02	4.2E-01	9.8E-01	1	1
11	0	3.0E-09	3.0E-06	6.7E-03	2.9E-01	9.7E-01	1	1
12	0	0	4.1E-07	2.2E-03	1.9E-01	9.4E-01	1	1
13	0	0	3.7E-08	6.7E-04	1.1E-01	8.9E-01	1	1
14	0	0	4.0E-09	1.8E-04	6.0E-02	8.2E-01	1	1
15	0	0	2.0E-09	4.6E-05	3.0E-02	7.3E-01	1.0E+00	1
16	0	0	0	1.1E-05	1.4E-02	6.2E-01	1.0E+00	1
17	0	0	0	2.2E-06	6.0E-03	5.0E-01	1.0E+00	1
18	0	0	0	4.6E-07	2.4E-03	3.9E-01	1.0E+00	1
19	0	0	0	7.4E-08	9.0E-04	2.9E-01	1.0E+00	1
20	0	0	0	1.2E-08	3.1E-04	2.0E-01	1.0E+00	1
21	0	0	0	1.0E-09	1.0E-04	1.3E-01	1.0E+00	1
22	0	0	0	0	3.0E-05	8.0E-02	1.0E+00	1
23	0	0	0	0	8.6E-06	4.6E-02	9.9E-01	1
24	0	0	0	0	2.2E-06	2.5E-02	9.9E-01	1
25	0	0	0	0	5.0E-07	1.3E-02	9.8E-01	1
26	0	0	0	0	1.1E-07	6.4E-03	9.6E-01	1
27	0	0	0	0	2.0E-08	2.9E-03	9.4E-01	1
28	0	0	0	0	4.0E-09	1.3E-03	9.0E-01	1.0E+00
29	0	0	0	0	0	5.1E-04	8.5E-01	1.0E+00
30	0	0	0	0	0	2.0E-04	7.9E-01	1.0E+00
31	0	0	0	0	0	7.2E-05	7.1E-01	1.0E+00
32	0	0	0	0	0	2.5E-05	6.2E-01	1.0E+00
33	0	0	0	0	0	7.9E-06	5.2E-01	1.0E+00
34	0	0	0	0	0	2.4E-06	4.2E-01	1.0E+00
35	0	0	0	0	0	6.8E-07	3.3E-01	1.0E+00
36	0	0	0	0	0	1.8E-07	2.5E-01	1.0E+00
37	0	0	0	0	0	4.4E-08	1.8E-01	1.0E+00
38	0	0	0	0	0	1.5E-08	1.2E-01	9.9E-01
39	0	0	0	0	0	4.0E-09	7.8E-02	9.9E-01
40	0	0	0	0	0	0	4.9E-02	9.8E-01
41	0	0	0	0	0	0	2.9E-02	9.6E-01
42	0	0	0	0	0	0	1.6E-02	9.3E-01
43	0	0	0	0	0	0	8.4E-03	9.0E-01
44	0	0	0	0	0	0	4.2E-03	8.5E-01
45	0	0	0	0	0	0	2.0E-03	7.9E-01
46	0	0	0	0	0	0	9.1E-04	7.1E-01
47	0	0	0	0	0	0	3.9E-04	6.3E-01
48	0	0	0	0	0	0	1.6E-04	5.3E-01
49	0	0	0	0	0	0	6.0E-05	4.4E-01
50	0	0	0	0	0	0	2.1E-05	3.5E-01
51	0	0	0	0	0	0	7.3E-06	2.7E-01
52	0	0	0	0	0	0	2.4E-06	1.9E-01
53	0	0	0	0	0	0	7.3E-07	1.3E-01
54	0	0	0	0	0	0	2.3E-07	8.9E-02
55	0	0	0	0	0	0	6.1E-08	5.6E-02
56	0	0	0	0	0	0	1.2E-08	3.4E-02
57	0	0	0	0	0	0	4.0E-09	1.9E-02
58	0	0	0	0	0	0	1.0E-09	1.0E-02
59	0	0	0	0	0	0	0	5.2E-03
60	0	0	0	0	0	0	0	2.5E-03
61	0	0	0	0	0	0	0	1.1E-03
62	0	0	0	0	0	0	0	4.7E-04
63	0	0	0	0	0	0	0	1.9E-04
64	0	0	0	0	0	0	0	7.0E-05
65	0	0	0	0	0	0	0	2.4E-05
66	0	0	0	0	0	0	0	7.8E-06
67	0	0	0	0	0	0	0	2.4E-06
68	0	0	0	0	0	0	0	7.2E-07
69	0	0	0	0	0	0	0	1.9E-07
70	0	0	0	0	0	0	0	4.6E-08
71	0	0	0	0	0	0	0	1.5E-08
72	0	0	0	0	0	0	0	5.0E-09
73	0	0	0	0	0	0	0	0

b



Supplementary Figure 7: Results of mathematical modeling. a, Tables of $Q_{x,i}$ with different numbers of mRNAs per neuron. **b,** Number of neurons that obtain unique identities at more than 95% likelihood when no sharing of isoforms between neurons is allowed. Both axes are in log scale.