

Supplementary Materials

for

Endogenous but not sensory-driven activity controls migration, morphogenesis and survival of adult-born neurons in the mouse olfactory bulb

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This PDF file includes:

Supplementary Figure Legends

Supplementary Figures S1 to S12

Supplementary Tables S1 to S4

Supplementary Figure S1. Bicistronic lentiviral vectors induced simultaneous expression of Kv1.2/Kir2.1 and Twitch-2B. (A) Sample images showing the transient expression of the lentiviral vectors in HEK-293T cells. (B) Sample MIP immunofluorescence images (20 μm depth) showing the expression of Kv1.2 in abJGCs at 10 dpi in control (upper panel) and Kv1.2 (lower panel) groups. Arrowheads highlight the location of abJGCs. (C) Cumulative distributions of the relative Kv1.2 expression levels in control and Kv1.2-expressing abJGCs. (D) Box plot showing the median fractions (per mouse) of abJGCs with a ring-shape Kv1.2 expression pattern, likely reflecting enhanced somatic targeting of the protein. (C, D) $n=96/6$ and $155/6$ cells/mice for control and Kv1.2 groups, respectively. (E, F) Bar graphs showing the mean relative expression levels of mRNA encoding for the potassium voltage-gated channel subfamily A member 2 (*Kcna2*, encoding Kv1.2. (E)) and the potassium inwardly rectifying channel subfamily J member 2 (*Kcnj2*, encoding Kir2.1. (F)) as determined by qPCR analyses of FACS-sorted adult-born cells (see SI Materials and Methods). Data are shown as median $\pm$ IQR. * $P<0.05$, *** $P<0.001$, ns=not significant.

Supplementary Figure S2. Electrical properties of Kv1.2/Kir2.1-overexpressing abJGCs. (A) Trains of action potentials, evoked by step-like depolarizing injection currents of +20 pA (schematically depicted below the traces) in control (upper panel), Kv1.2- (middle panel) and Kir2.1-overexpressing abJGCs (dpi 20). The double arrow in the middle panel marks the so called distance to baseline, analyzed in F; the boxed area is expanded in the inset to better visualize the afterhyperpolarization, analyzed in G. (B-G) Box plots comparing the input resistance measured in voltage-clamp mode (B; control: $0.9 \pm 1.6 \text{ G}\Omega$, $n=20$ cells; Kv1.2-overexpression: $1.5 \pm 2.5 \text{ G}\Omega$, $n=15$ cells); the AP firing threshold (C; control: $-34.2 \pm 6.0 \text{ mV}$, $n = 9$ cells; Kv1.2-overexpression: $-41.8 \pm 18.4 \text{ mV}$, $n = 7$ cells); the AP amplitude (D; control: $95.5 \pm 45.3 \text{ mV}$, $n = 9$ cells;

Kv1.2-overexpression: 89.5 ± 42.8 mV, $n = 7$ cells); the width of APs measured at half maximum (FWHM) (E; control: 0.49 ± 0.32 ms, $n = 9$ cells; Kv1.2-overexpression: 0.46 ± 0.27 ms, $n = 7$ cells); the median (per cell) distance from a minimum voltage value measured after individual APs (in bursts of 6 APs induced by 100 ms depolarizing pulses) to the baseline voltage (F; control: 15 ± 1.4 mV, $n = 6$ APs in 5 cells; Kv1.2-overexpression: 8.7 ± 1.5 mV, $n = 6$ APs in 5 cells); and the burst afterhyperpolarization, measured as a most negative voltage reached after the burst end relative to the baseline voltage 50-100 ms after the end of current injection (G; control: $+2.3 \pm 3.4$ mV, Kv1.2-overexpression: -2.6 ± 4.1 mV; the same traces as in (F)). $*P < 0.05$, $***P < 0.001$, ns=not significant.

Supplementary Figure S3. Expression of NeuN and DCX in adult-born JGCs. (A) Sample MIP immunofluorescence images (20 μ m depth) showing the adult-born JGCs (Twitch-2B) and NeuN positive cells at 28 dpi. (B) Sample MIP immunofluorescence images (20 μ m depth) showing the adult-born JGCs (Twitch-2B) and DCX positive cells at 28 dpi. (C) Box plot showing the fractions (per mouse) of adult-born JGCs, expressing NeuN. $n=165/5$, $155/5$, and $159/5$ cells/mice for control, Kv1.2 and Kir2.1 respectively. (D) Box plot showing the fractions (per mouse) of adult-born JGCs, expressing DCX. $n=170/5$, $171/5$, and $180/5$ cells/mice for control, Kv1.2 and Kir2.1 respectively. Data are shown as median $\pm$ IQR. ns=not significant.

Supplementary Figure S4. Kv1.2 or Kir2.1 overexpression did not influence the arrival of adult-born JGCs in the glomerular layer. Box plots showing the median (per 3D stack volume) density of abJGCs in the OB glomerular layer (i.e., number of abJGCs divided by the respective 3D stack volume) at 8 dpi (A) and 14 dpi (B) in control, Kv1.2 and Kir2.1 groups. (A) $n = 57/5$, $131/9$, and $65/6$ cells/mice for control,

Kv1.2, and Kir2.1 groups, respectively. (B) $n = 71/5$, $89/7$, and $60/4$ mice/mice for control, Kv1.2, and Kir2.1 groups, respectively. Data are shown as median $\pm$ IQR. ns=not significant.

Supplementary Figure S5. Kv1.2 or Kir2.1 overexpression impaired the migration of adult-born JGCs at 14 dpi. (A) Box plot showing the median (per mouse) fractions of abJGCs migrating during 4-hour-long recordings in control, Kv1.2, and Kir2.1 groups. Here and below $n=30/10$, $5/7$, and $0/4$ migrating cells/mice for control, Kv1.2 and Kir2.1 groups, respectively. In total, we analyzed $216/10$, $86/7$, and $60/4$ cells/mice for control, Kv1.2, and Kir2.1 groups, respectively. (B-D) Box plots showing (per cell) the median migration speed (B), maximum migration speed (C), and the translocation distance in 4 hours (D) of abJGCs in control and Kv1.2 groups. Data are shown as median $\pm$ IQR. $*P<0.05$, ns=not significant.

Supplementary Figure S6. Nostril occlusion blocked odor-evoked responsiveness of adult-born JGCs. (A) Sample odor-evoked Ca^{2+} transients of abJGCs residing in the contralateral control and ipsilateral odor-deprived hemibulbs. (B-D) Box plots illustrating the fractions of odor responding cells per mouse (B) and (per cell) the amplitude (C) and AUC (D) of odor-evoked Ca^{2+} transients. $n=13/4$ and $29/4$ cells/mice for contralateral control and ipsilateral odor-deprived hemibulbs. Data are shown as median $\pm$ IQR. $**P<0.01$.

Supplementary Figure S7. Immunohistochemical analyses confirming the retarded morphology of Kv1.2- and Kir2.1-expressing adult-born JGCs. (A) Sample images of abJGCs (20 dpi) belonging to control, Kv1.2 and Kir2.1 groups, reconstructed from *in vitro* images taken in 50 μm thick fixed slices,

immunofluorescently labeled against Twitch-2B. (B) Box plots showing (per cell) the TDBL of abJGCs. (C) Box plots showing (per cell) the number of dendritic branches of abJGCs. (B) and (C): n=26/5, 25/5, and 25/5 cells/mice for control, Kv1.2, and Kir2.1, respectively. (D) Sholl analysis illustrating the complexity of adult-born JGC's dendritic morphology in fixed brain slices at 20 dpi. n=25/5 cells/mice per group. (B) and (C): Data are shown as median $\pm$ IQR. (D) Data are shown as mean $\pm$ SEM. * P <0.05, ** P <0.01, *** P <0.001, ns=not significant.

Supplementary Figure S8. Expression of Kir2.1 channel with a dominant-negative loss-of-function mutation (Kir2.1mut) did not impair the morphogenesis of adult-born JGCs. (A) Sample reconstructions of *in vivo* abJGCs (20 dpi) expressing Kir2.1mut. (B) Box plots showing (per cell) the TDBL of abJGCs imaged *in vivo* at 20 dpi in control, Kir2.1, and Kir2.1mut groups. (C) Box plots showing (per cell) the number of dendritic branches of abJGCs imaged *in vivo* at 20 dpi in control, Kir2.1, and Kir2.1mut groups. Control and Kir2.1 datasets are the same as in Figure 2. (D) Sholl analysis of Kir2.1- and Kir2.1mut-expressing abJGCs. (E) Sholl analysis of control and Kir2.1mut-expressing abJGCs. (D) and (E): n=33/7, 46/6, and 35/3 cells/mice for control, Kir2.1, and Kir2.1mut groups. (B) and (C): Data are shown as median $\pm$ IQR. (D) and (E): Data are shown as mean $\pm$ SEM. *** P <0.001, ns=not significant. Note that Kir2.1mut was overexpressed using a retrovirus. The lack of functional difference between the retro- and lentivirus-infected neuronal progenitors is documented by the high similarity of their migration properties (compare Fig. 1 of the present study with Fig. 4 in ref. (2)).

Supplementary Figure S9. Neither Kv1.2 or Kir2.1 overexpression nor odor deprivation affected basal and maximum Twitch-2B ratios and AUCs of adult-

born JGCs. Box plots showing the median (per cell) basal and maximum Twitch-2B ratios and AUCs of spontaneous Ca^{2+} transients in abJGCs in control, Kv1.2, and Kir2.1 groups (A-C) as well as contralateral control and ipsilateral odor-deprived groups (D-F). n=60/8, 68/7, 66/5, 34/4, and 47/4 cells/mice for control, Kv1.2, Kir2.1, contralateral control, and ipsilateral odor-deprived groups, respectively. Data are shown as median $\pm$ IQR. ns=not significant.

Supplementary Figure S10. Relative expression levels of transcripts involved in pathways related to neuronal development, differentiation, and cell death. Bar graphs showing mean relative expression levels of mRNA encoding for the calcium/calmodulin-dependent protein kinase II gamma (*Camk2g*; A), doublecortin (*Dcx*; B), microtubule-associated protein tau (*Mapt*; C), neural cell adhesion molecule 1 (*Ncam1*; D), synaptophysin (*Syp*; E), BH3 interacting domain death agonist (*Bid*; F) and ribosomal protein L5 (*Rpl5*; G), as determined by qPCR analyses of FACS-sorted adult-born cells (see SI Materials and Methods). Data are shown as mean $\pm$ SEM. n = 3 biological replicates. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=not significant.

Supplementary Figure S11. Morphology and relative pCREB levels of adult-born granule cells from control, Kv1.2, and Kir2.1 groups. (A) Sample images of adult-born GCs (20 dpi) belonging to control, Kv1.2 and Kir2.1 groups, reconstructed from *in vitro* images taken in 50 μm thick fixed slices, immunofluorescently labeled against Twitch-2B. (B) Box plots showing (per cell) the TDBL (B) and the number of dendritic branches (C) of adult-born GCs. (D) Sholl analysis illustrating the complexity of adult-born GCs' dendritic morphology in fixed brain slices at 20 dpi. (E) Sample MIP images (15 μm depth) showing pCREB-, Twitch-2B- and NeuN-positive cells in the granule cell layer of the OB slices from control, Kv1.2 and Kir2.1 groups at 10 dpi. Twitch-2B labels

the adult-born GCs whereas NeuN labels the mature neurons. Arrowheads highlight the location of abJGCs. (F) Box plots illustrating the median (per mouse) relative pCREB levels of adult-born GCs in control, Kv1.2, and Kir2.1 groups at 10 dpi. n=119/5, 207/4, and 222/5 cells/mice for control, Kv1.2 and Kir2.1, respectively. (B-D): n=177/7, 149/5, and 133/6 cells/mice for control, Kv1.2 and Kir2.1 respectively. (B) (C) and (F): Data are shown as median $\pm$ IQR. (D): Data are shown as mean $\pm$ SEM. * P <0.05, *** P <0.001, ns=not significant.

Supplementary Figure S12. Relative expression levels of transcripts encoding for N-methyl-D-aspartate (NMDA) receptors and members of mitogen-activated protein kinase (MAPK) signaling cascade. Box plots showing mean values of z-scores for four isoforms of the NMDA receptor (A) and 18 members of the MAPK/ERK signaling cascade (B). All transcripts included in the box plots are listed in Table S1. n = 2 biological replicates (12 mice in total, 2 mice per replicate per group). Data are shown as median $\pm$ IQR. ** P <0.01, *** P <0.001, ns=not significant.

Supplementary Table S1. Transcripts included in the box plots shown in Figures 7 and S11.

Figure	Genes
7B	<i>Camk2g, Camk2b, Camk2a, Calm3, Cacna1c, Cacna1d</i>
7C	<i>Vgefa, Nr4a1, Egr1, Negr1, Fra2 (from cFos)</i>
7D	<i>Camk2a, Camk2b, Cck, Dcl1, Dcx, Nr2f1, Fyn, Fzd3, Gja1, Mdk, Mapt, Ntrk2, Ptprz1, Reln, Olfm1, Kirrel3, Fbxo31, Gpm6a, Mrtfb, Adgrl3, Cdkl5, Flrt2</i>
7E	<i>Bdnf, Camk2b, Cdh2, Cntn1, Dcx, Nr2f1, Fyn, Grin1, Id4, Itsn1, Mdk, Meis1, Map1b, Map2, Mapt, Ncoa1, Nedd4, Nefl, Nf1, Ntrk2, Pcp4, Ptn, Ptprs, Ptprz1, Reln, Snap25, Snap91, Plk2, Sox9, Syt1, Dpysl3, Vim, Ywhag, Magi2, Olfm1, Rapgef4, Syne1, Fuom, Grip1, Plppr5, Fbxo31, Kank1, Baiap2, Eif4g1, Gpr37l1, Cpeb3, Adgrb3, Cdkl3, Arhgap44, Acsl6, Arhgap33, Amigo1, Ndr4, Ntm, Fez1, Shank1, Nlgn3, Robo2, Nrcam, Il1rapl1, Cdkl5, Kalrn, Tnik, Acp4</i>
7F	<i>Camk2a, Camk2b, Dcl1, Dcx, Eph5, Fyn, Grin1, Nedd4, Ctnnd2, Ptn, Ptprz1, Reln, Syne1, Fbxo31, Baiap2, Adgrb3, Cdkl3, Arhgap44, Arhgap33, Shank1, Nlgn3, Il1rapl1, Cdkl5, Kalrn, Tnik</i>
7G	<i>Bcl10, Bcl7a, Bad, Casp8, Casp6, Casp1, Casp4, Casp12, Casp7, Casp2, Bcl2a1a, Bax, Bid</i>
7H	<i>Psmc5, Gapdh, Mkrn1, Tuba1b, Dars, Rpl27, Ldha, Rpl5, Actb</i>
S11A	<i>Nmda2b, Nmda2a, Nmda1, Nmda3a</i>
S11B	<i>Mapk1, Mapk2, Mapk3, Mapk6, Mapk7, Mapk8, Mapk9, Mapk10, Mapk14, Lamtor1, Lamtor2, Lamtor3, Lamtor4, Lamtor5, Mapkapk3, Mapkap1, Mapk1ip1, Jkamp</i>

Supplementary Table S2. Differential expression of factors interacting with CREB

Genes	Control vs Kv1.2		Control vs Kir2.1	
	logFC	P value	logFC	P value
<i>Crtc2</i>	4.59	0.1818	6.34	0.1002
<i>Crtc3</i>	0.21	0.9484	0.61	0.8533
<i>Lmo4</i>	-1.65	0.2554	-1.04	0.4539
<i>Dream (Kcnip3)</i>	-0.84	0.5959	-8.61	0.0046
<i>Crest (Ss18l1)</i>	-6.04	0.1098	0.56	0.8377
<i>Smarca4</i>	-1.55	0.5495	-0.91	0.7210
<i>Hdac1</i>	-1.84	0.3210	0.29	0.8663

Supplementary Table S3. Primers used for qPCR

Gene	Accession N°	Primers	Size (bp)
<i>Gapdh</i>	NM_001289726.1	F: TGCACCACCAACTGCTTAG R: GGATGCAGGGATGATGTTC	177
<i>Rpl5</i>	NM_016980.2	F: TGGCAGACTACATGCGCTAC R: ATACATCTCCTCCATCATGTCTG	109
<i>Kcna2</i>	NM_008417.5	F: TGGTACCCATCTGCAAGGGC R: CCTTTGGAAGGAAGGAGGCAA	99
<i>Kcnj2</i>	NM_008425.4	F: GGCAAGCAGTGTCTTGGGAAT R: AGAGAACTTGTCTGTTGCTGG	138
<i>Dcx</i>	NM_001110222.1	F: GAGTGGGGCTTTTCGAGTGAT R: AAAGAAAGCCGTGTGCCTTG	78
<i>Camk2g</i>	NM_178597.5	F: AGTCAGATGGCGGTGTCAAG R: GCTGGGCTTACGAGACTGTT	91
<i>Mapt</i>	NM_001038609.2	F: CGCCCCTAGTGGATGAGAGA R: TCCTTCTGGGATCTCCGTGT	71
<i>Ncam1</i>	NM_001081445.1	F: CAAGTACAGAGCGCTCGCC R: AGGGACTTGAGCATGACGTG	81
<i>Syp</i>	NM_009305.2	F: CTGCGTTAAAGGGGGCACTA R: GGAAGTCCATCATTGGCCCT	175
<i>Bid</i>	NM_007544.4	F: GAGTGTGGCTCCGCAAACC R: AGGGAATCACACGCAGACG	78

mRNA sequence accession numbers were obtained from the Mouse Genome Database accessed at www.ncbi.nlm.nih.gov/genome.

Supplementary Table S4. Exact results of all statistical tests

Figure	Statistical test	<i>Post hoc</i> comparisons
1C	Kruskal-Wallis test: $P=2\times 10^{-4}$	Dunn's multiple comparison test: control vs. Kv1.2: $P=0.04$, control vs. Kir2.1: $P=2\times 10^{-4}$, Kv1.2 vs. Kir2.1: $P=0.22$.

1D	Kruskal-Wallis test: $P=6\times 10^{-5}$	Dunn's multiple comparison test: control vs. Kv1.2: $P=2\times 10^{-4}$, control vs. Kir2.1: $P=1.6\times 10^{-3}$, Kv1.2 vs. Kir2.1: $P=0.36$.
1E	Kruskal-Wallis test: $P=2\times 10^{-4}$	Dunn's multiple comparison test: control vs. Kv1.2: $P=3\times 10^{-4}$, control vs. Kir2.1: $P=6.3\times 10^{-3}$, Kv1.2 vs. Kir2.1: $P=0.67$.
1F	Kruskal-Wallis test: $P=3\times 10^{-4}$	Dunn's multiple comparison test: control vs. Kv1.2: $P=8.9\times 10^{-3}$, control vs. Kir2.1: $P=3\times 10^{-3}$, Kv1.2 vs. Kir2.1: $P=0.55$.
1G	One-way ANOVA: $F_{2,19}=7.982$, $P=3\times 10^{-3}$	Tukey's multiple comparison test: control vs. Kv1.2: $P=0.01$, control vs. Kir2.1: $P=6.6\times 10^{-3}$, Kv1.2 vs. Kir2.1: $P=0.59$.
1I, M	Paired t test, $P>0.05$ for all comparisons	
1J-L	Two-sided unpaired t test, $P>0.05$ for all comparisons.	For the 4 paired olfactory bulbs: Paired t test, $P>0.05$ for all comparisons
2B	Kruskal-Wallis test: $P=2\times 10^{-9}$	Dunn's multiple comparison test: control vs. Kv1.2: $P=3\times 10^{-9}$; control vs. Kir2.1: $P=8\times 10^{-6}$; Kv1.2 vs. Kir2.1: $P=0.21$
2C	Kruskal-Wallis test: $P=5\times 10^{-10}$	Dunn's multiple comparison test: control vs. Kv1.2: $P=2\times 10^{-9}$; control vs. Kir2.1: $P=5\times 10^{-7}$; Kv1.2 vs. Kir2.1: $P=0.58$
2D	Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)	control vs. Kv1.2: $P<0.001$, control vs. Kir2.1: $P<0.001$, Kv1.2 vs. Kir2.1: $P<0.001$
2F, G	Paired t test, $P>0.05$ for both comparisons	
2H	Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)	$P=0.98$
3B	One-way ANOVA: $F_{2,15}=6.747$, $P=8.1\times 10^{-3}$	Tukey's multiple comparison test: control vs. Kv1.2: $P=0.02$; control vs. Kir2.1: $P=9.6\times 10^{-3}$; Kv1.2 vs. Kir2.1: $P=0.69$.
3D	One-way ANOVA: $F_{2,11}=13.84$, $P=1\times 10^{-3}$	Tukey's multiple comparison test: control vs. Kv1.2: $P=0.81$; control vs. Kir2.1: $P=1.2\times 10^{-3}$; Kv1.2 vs. Kir2.1: $P=3.3\times 10^{-3}$.
4A	One-way ANOVA: $F_{2,16}=7.061$, $P=0.0063$	Tukey's multiple comparison test: control vs. Kv1.2: $P=0.54$; control vs. Kir2.1: $P=5.9\times 10^{-3}$; Kv1.2 vs. Kir2.1: $P=0.04$.

4C	Kruskal-Wallis test: $P=2\times 10^{-6}$	Dunn's multiple comparison test: control vs. Kv1.2: $P=4\times 10^{-5}$; control vs. Kir2.1: $P=6\times 10^{-4}$; Kv1.2 vs. Kir2.1: $P>0.99$.
4D	Kruskal-Wallis test: $P=7\times 10^{-9}$	Dunn's multiple comparison test: control vs. Kv1.2: $P=3\times 10^{-6}$; control vs. Kir2.1: $P=4\times 10^{-6}$; Kv1.2 vs. Kir2.1: $P=0.39$.
5B	Chi-square test, $P=3\times 10^{-6}$	
5C	One-way ANOVA: $F_{2,15}=5.468$, $P=0.016$	Tukey's multiple comparison test: control vs. Kv1.2: $P=0.03$; control vs. Kir2.1: $P=6.2\times 10^{-3}$; Kv1.2 vs. Kir2.1: $P=0.57$.
5E	Chi-square test, $P=0.28$	
5F	Paired t test, $P=0.79$	
6B	One-way ANOVA: $F_{2,14}=45.73$, $P=7\times 10^{-7}$	Tukey's multiple comparison test: control vs. Kv1.2: $P=2\times 10^{-6}$; control vs. Kir2.1: $P=4\times 10^{-6}$; Kv1.2 vs. Kir2.1: $P=0.99$.
6C	Paired t -test, $P=0.57$	
7A	One-way ANOVA: $F_{2,10}=14.12$, $P=2.4\times 10^{-3}$	Tukey's multiple comparison test: control vs. Kv1.2: $P=2.9\times 10^{-3}$; control vs. Kir2.1: $P=2.2\times 10^{-3}$; Kv1.2 vs. Kir2.1: $P=0.99$.
7B	One-way ANOVA: $F_{2,14}=18.02$, $P=1.3\times 10^{-4}$	Tukey's multiple comparison test: control vs. Kv1.2: $P=4.3\times 10^{-3}$; control vs. Kir2.1: $P=1.1\times 10^{-4}$; Kv1.2 vs. Kir2.1: $P=0.15$.
7C	One-way ANOVA: $F_{2,11}=9.768$, $P=3.6\times 10^{-3}$	Tukey's multiple comparison test: control vs. Kv1.2: $P=1.5\times 10^{-2}$; control vs. Kir2.1: $P=4.6\times 10^{-3}$; Kv1.2 vs. Kir2.1: $P=0.89$.
7D	Friedman test, $P=1\times 10^{-15}$	Dunn's multiple comparison test: control vs. Kv1.2: $P=1\times 10^{-15}$; control vs. Kir2.1: $P=1\times 10^{-15}$; Kv1.2 vs. Kir2.1: $P=0.75$.
7E	One-way ANOVA: $F_{2,48}=99.8$, $P=1\times 10^{-15}$	Tukey's multiple comparison test: control vs. Kv1.2: $P=1\times 10^{-15}$; control vs. Kir2.1: $P=2\times 10^{-15}$; Kv1.2 vs. Kir2.1: $P=0.84$.
7F	One-way ANOVA: $F_{2,26}=22.02$, $P=2.5\times 10^{-6}$	Tukey's multiple comparison test: control vs. Kv1.2: $P=1.3\times 10^{-5}$; control vs. Kir2.1: $P=1.4\times 10^{-5}$; Kv1.2 vs. Kir2.1: $P=0.99$.
7G	One-way ANOVA: $F_{2,16}=1.27$, $P=0.31$	
S1C	Kolmogorov-Smirnov test, $P=1\times 10^{-8}$, $D=0.399$.	
S1D	Unpaired t test with Welch's correction, $t_5=17.13$, $P=1\times 10^{-5}$.	

S1E	One-way ANOVA: $F_{2,6}=696.8$, $P=7.9\times 10^{-8}$	Tukey's multiple comparison test: control vs. Kv1.2: $P=2.4\times 10^{-7}$; control vs. Kir2.1: $P=0.98$; Kv1.2 vs. Kir2.1: $P=2.5\times 10^{-7}$.
S1F	One-way ANOVA: $F_{2,6}=8.16$, $P=0.02$	Tukey's multiple comparison test: control vs. Kv1.2: $P=0.97$; control vs. Kir2.1: $P=0.03$; Kv1.2 vs. Kir2.1: $P=0.03$.
S2B	Two-sided unpaired t test, $P=0.26$	
S2C	Two-sided unpaired t test, $P=0.26$	
S2D	Two-sided unpaired t test, $P=0.77$	
S2E	Two-sided unpaired t test, $P=0.78$	
S2F	Two-sided paired t test, $P<0.001$	
S2G	Two-sided unpaired t test, $P=0.04$	
S3C	One-way ANOVA: $F_{2,12}=0.943$, $P=0.416$	
S3D	One-way ANOVA: $F_{2,12}=0.95$, $P=0.415$	
S4A	One-way ANOVA: $F_{2,17}=0.05$, $P=0.95$	
S4B	One-way ANOVA: $F_{2,13}=2.18$, $P=0.15$	
S5A	One-way ANOVA: $F_{2,18}=4.01$, $P=0.04$	unpaired t test with Welch's correction for multiple comparisons: control vs. Kv1.2: $P=0.17$; control vs. Kir2.1: $P=1.9\times 10^{-3}$; Kv1.2 vs. Kir2.1: $P=0.04$.
S5B	Two-sided Mann-Whitney test, $P=0.68$	
S5C	Two-sided Mann-Whitney test, $P=0.82$	
S5D	Two-sided Mann-Whitney test, $P=0.02$	
S6B	Unpaired t test with Welch's correction, $t_3=6.31$, $P=8\times 10^{-3}$	
S7B	Kruskal-Wallis test: $P=5\times 10^{-9}$	Dunn's multiple comparison test: control vs. Kv1.2: $P=5\times 10^{-9}$; control vs. Kir2.1: $P=4\times 10^{-6}$; Kv1.2 vs. Kir2.1: $P=0.72$

S7C	Kruskal-Wallis test: $P=6\times 10^{-10}$	Dunn's multiple comparison test: control vs. Kv1.2: $P=1\times 10^{-9}$; control vs. Kir2.1: $P=2\times 10^{-5}$; Kv1.2 vs. Kir2.1: $P=0.27$
S7D	Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)	control vs. Kv1.2: $P=0.004$, control vs. Kir2.1: $P=0.011$, Kv1.2 vs. Kir2.1: $P=0.016$
S8B	Kruskal-Wallis test: $P=1\times 10^{-9}$	Dunn's multiple comparison test: control vs. Kir2.1: $P=1\times 10^{-5}$; control vs. Kir2.1mut: $P=0.7$; Kir2.1 vs. Kir2.1mut: $P=6\times 10^{-9}$
S8C	Kruskal-Wallis test: $P=7\times 10^{-11}$	Dunn's multiple comparison test: control vs. Kir2.1: $P=3\times 10^{-7}$; control vs. Kir2.1mut: $P=0.99$; Kir2.1 vs. Kir2.1mut: $P=3\times 10^{-9}$
S8D	Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)	$P<0.001$
S8E	Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)	$P=0.28$
S9A-C	Kruskal-Wallis test, $P>0.05$ for all tests	
S9D-F	Mann-Whitney test, $P>0.05$ for all tests	
S10A	One-way ANOVA: $F_{2,6}=25.21$, $P=0.0012$	Fisher's multiple comparison test: control vs. Kv1.2: $P=8.2\times 10^{-4}$; control vs. Kir2.1: $P=8.8\times 10^{-4}$; Kv1.2 vs. Kir2.1: $P=0.94$.
S10B	One-way ANOVA: $F_{2,6}=10.77$, $P=0.01$	Fisher's multiple comparison test: control vs. Kv1.2: $P=8.3\times 10^{-3}$; control vs. Kir2.1: $P=6\times 10^{-3}$; Kv1.2 vs. Kir2.1: $P=0.78$.
S10C	One-way ANOVA: $F_{2,6}=27.17$, $P=9.8\times 10^{-4}$	Fisher's multiple comparison test: control vs. Kv1.2: $P=6.5\times 10^{-4}$; control vs. Kir2.1: $P=7.4\times 10^{-4}$; Kv1.2 vs. Kir2.1: $P=0.88$.
S10D	One-way ANOVA: $F_{2,6}=43.04$, $P=2.7\times 10^{-4}$	Fisher's multiple comparison test: control vs. Kv1.2: $P=1.9\times 10^{-4}$; control vs. Kir2.1: $P=2.1\times 10^{-4}$; Kv1.2 vs. Kir2.1: $P=0.91$.
S10E	One-way ANOVA: $F_{2,6}=46.98$, $P=2.2\times 10^{-4}$	Fisher's multiple comparison test: control vs. Kv1.2: $P=1.6\times 10^{-4}$; control vs. Kir2.1: $P=1.5\times 10^{-4}$; Kv1.2 vs. Kir2.1: $P=0.89$.

S10F	One-way ANOVA: $F_{2,6}=9.19$, $P=0.015$	Fisher's multiple comparison test: control vs. Kv1.2: $P=6\times 10^{-3}$; control vs. Kir2.1: $P=0.03$; Kv1.2 vs. Kir2.1: $P=0.24$.
S10G	One-way ANOVA: $F_{2,6}=0.15$, $P=0.86$	
S11B	Kruskal-Wallis test: $P=1\times 10^{-14}$	Dunn's multiple comparison test: control vs. Kv1.2: $P=1\times 10^{-14}$; control vs. Kir2.1: $P=1\times 10^{-14}$; Kv1.2 vs. Kir2.1: $P=0.99$
S11C	Kruskal-Wallis test: $P=1\times 10^{-14}$	Dunn's multiple comparison test: control vs. Kv1.2: $P=1\times 10^{-14}$; control vs. Kir2.1: $P=1\times 10^{-14}$; Kv1.2 vs. Kir2.1: $P=0.99$
S11D	Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)	control vs. Kv1.2: $P<0.001$, control vs. Kir2.1: $P<0.001$, Kv1.2 vs. Kir2.1: $P<0.001$
S11F	One-way ANOVA: $F_{2,11}=6.524$, $P=0.013$	Tukey's multiple comparison test: control vs. Kv1.2: $P=0.02$; control vs. Kir2.1: $P=0.049$; Kv1.2 vs. Kir2.1: $P=0.69$.
S12A	One-way ANOVA: $F_{2,6}=31.74$, $P=6\times 10^{-4}$	Tukey's multiple comparison test: control vs. Kv1.2: $P=8\times 10^{-4}$; control vs. Kir2.1: $P=1.6\times 10^{-3}$; Kv1.2 vs. Kir2.1: $P=0.67$.
S12B	One-way ANOVA: $F_{2,34}=0.18$, $P=0.83$	

Fig. S1

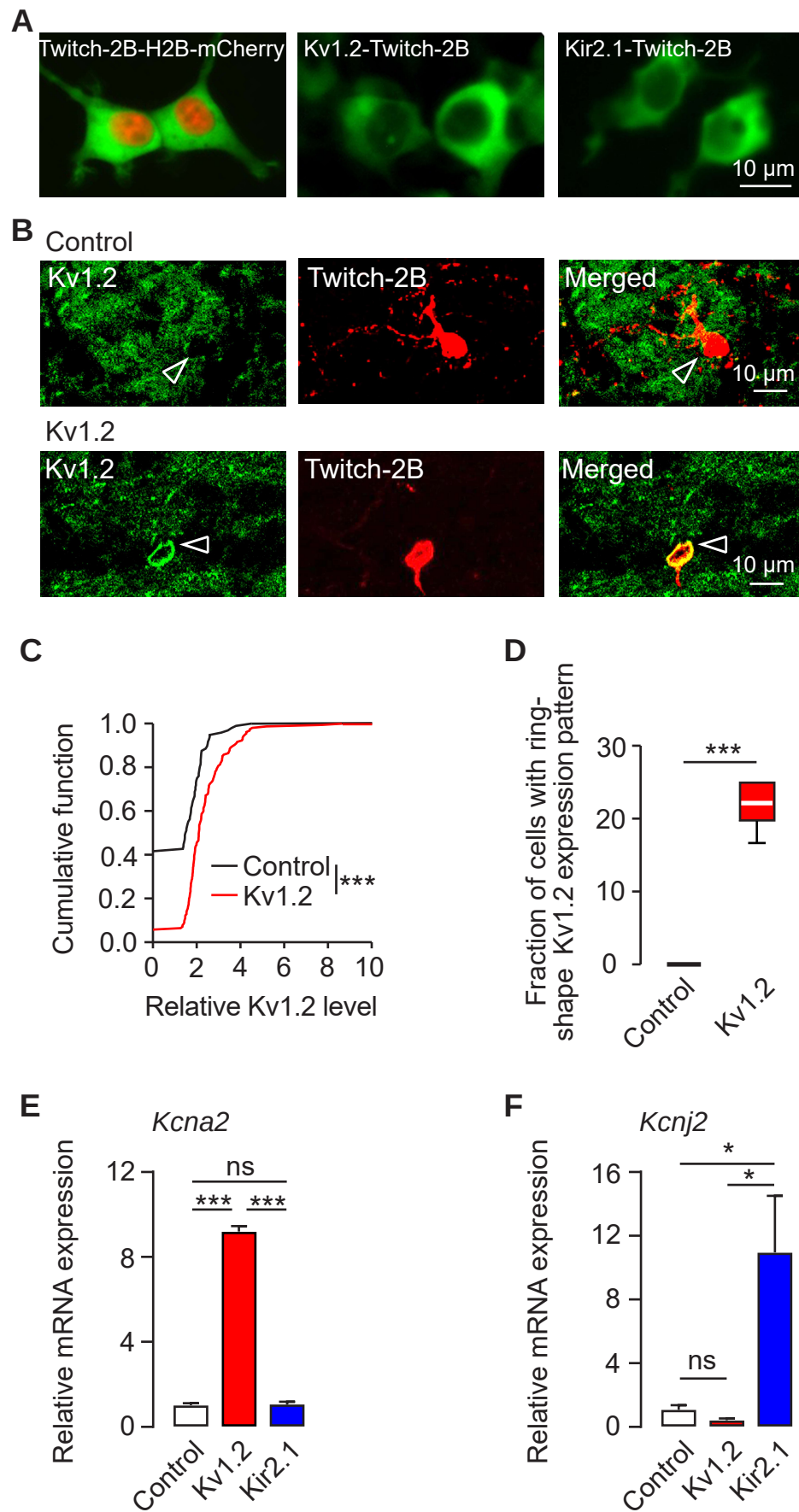


Fig. S2

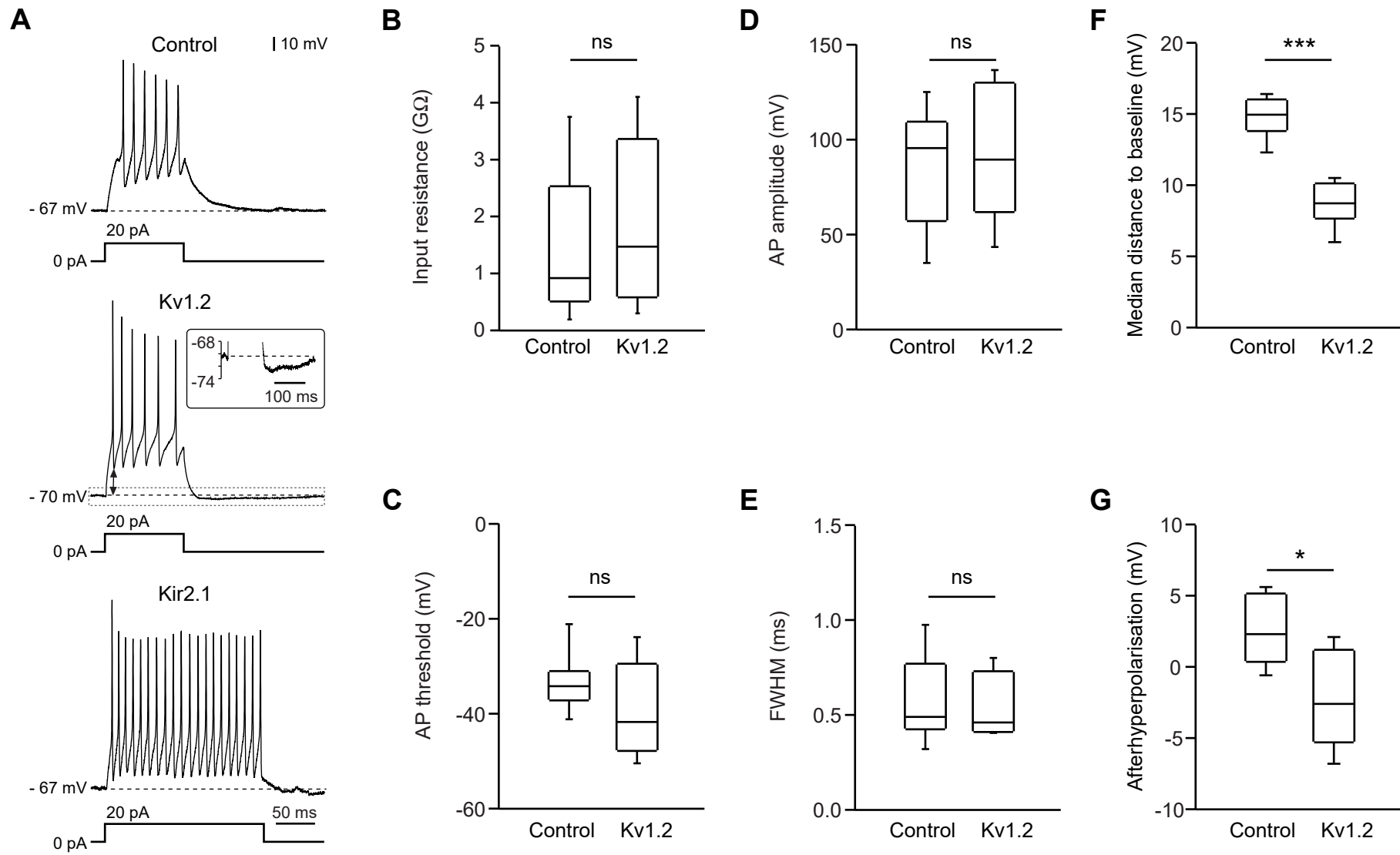


Fig. S3

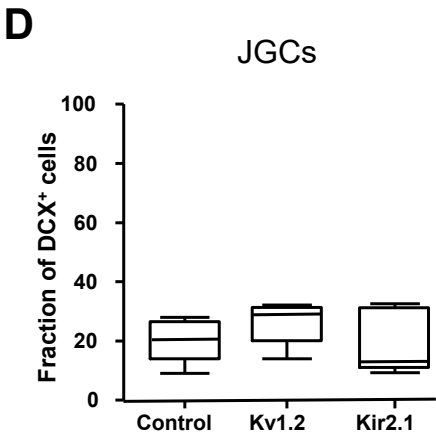
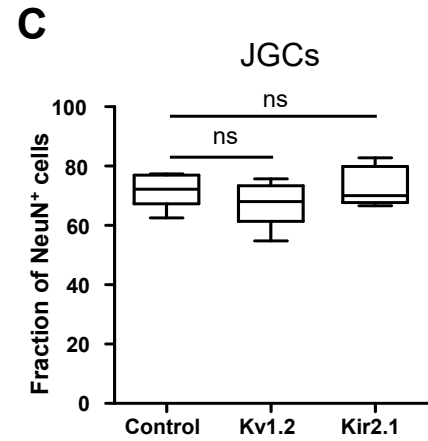
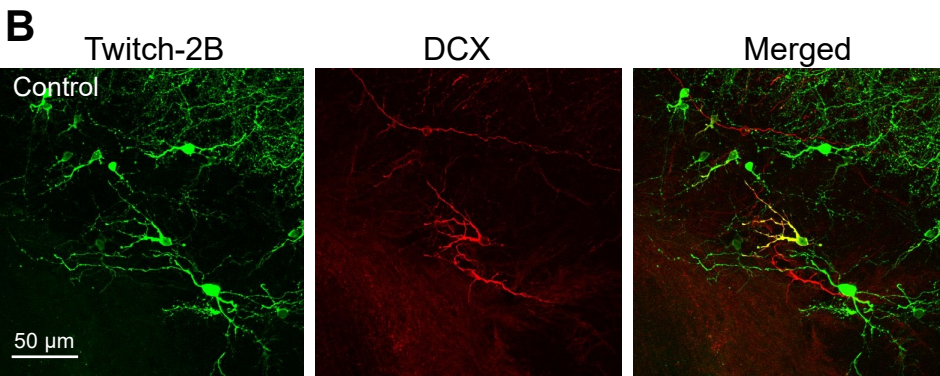
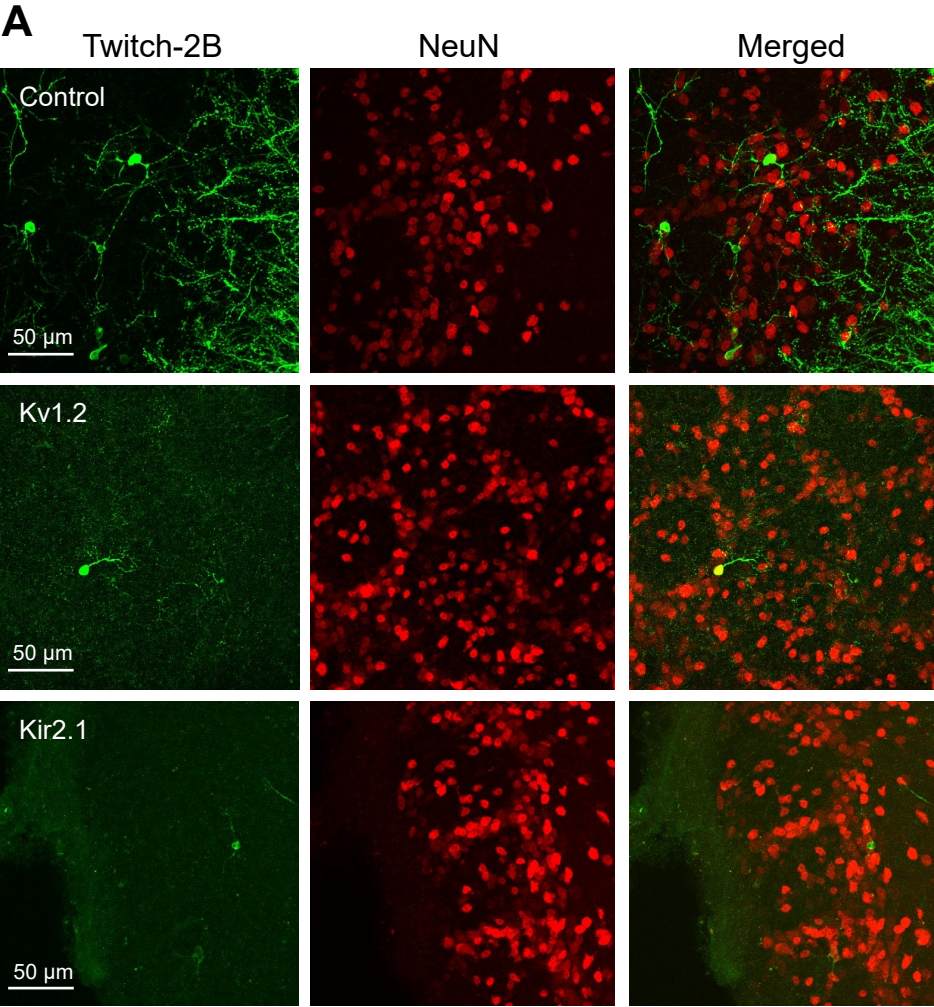
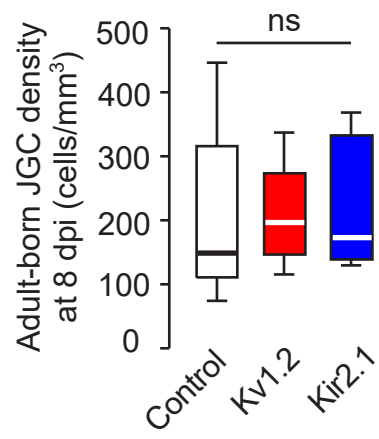


Fig. S4

A



B

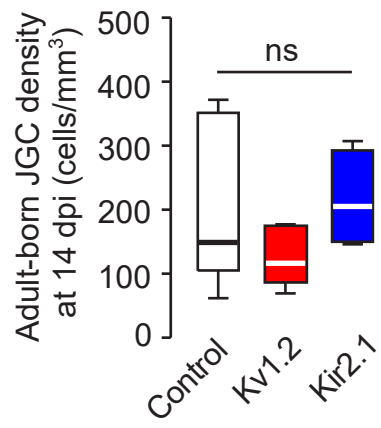


Fig. S5

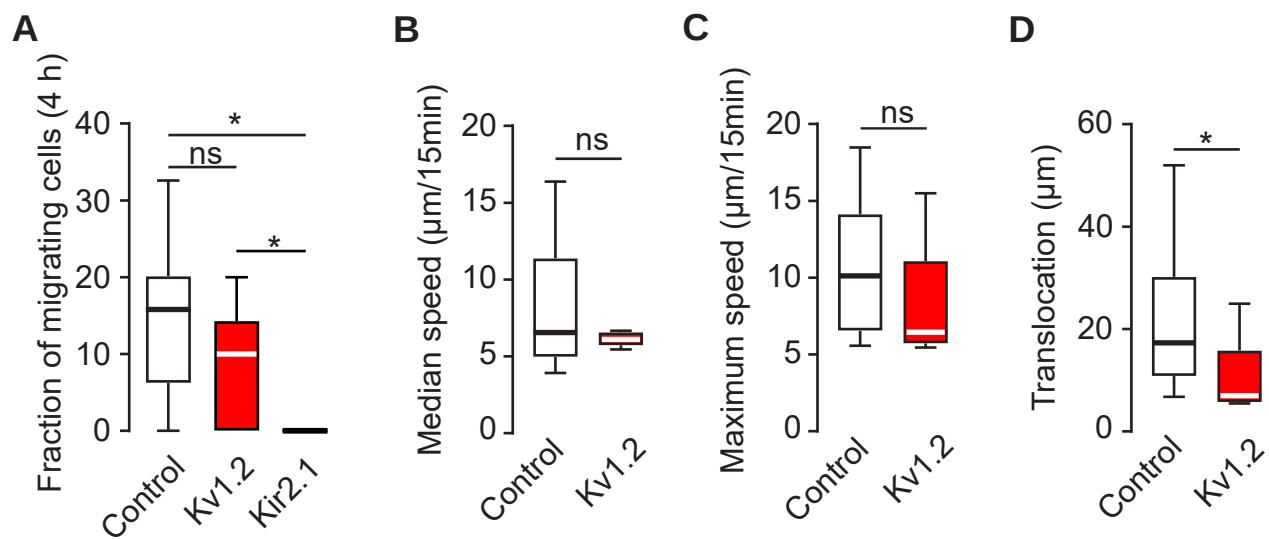


Fig. S6

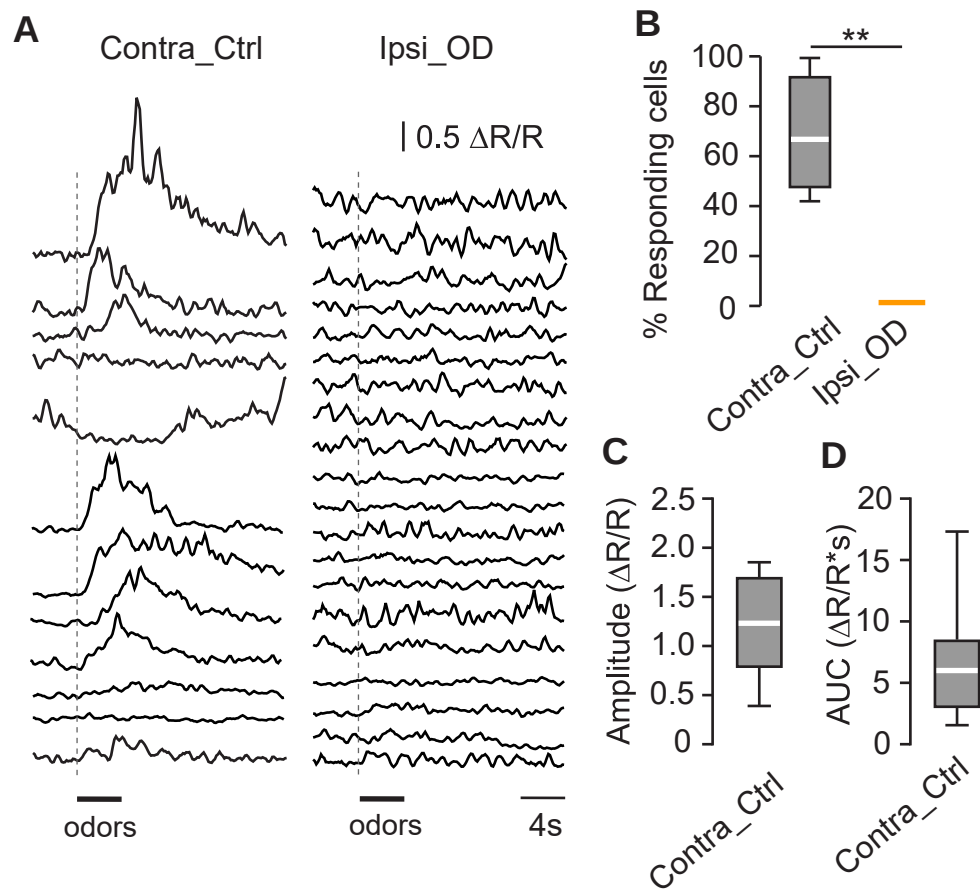


Fig. S7

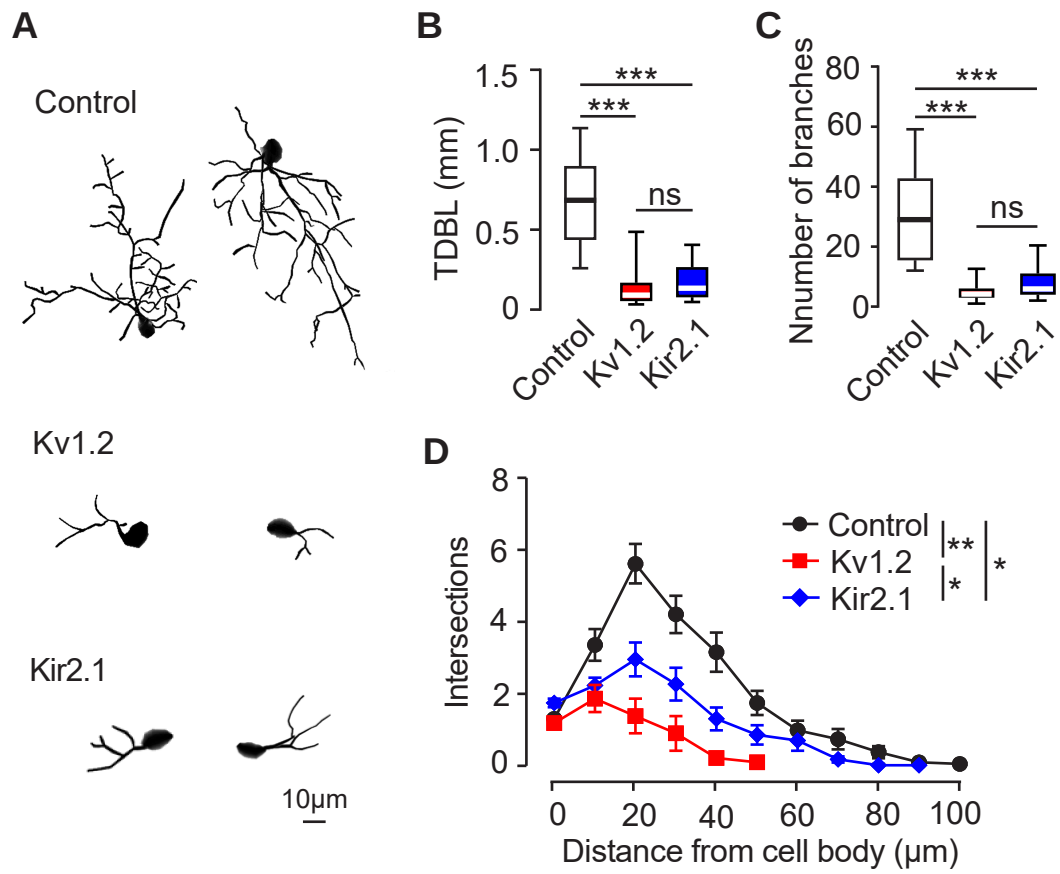


Fig. S8

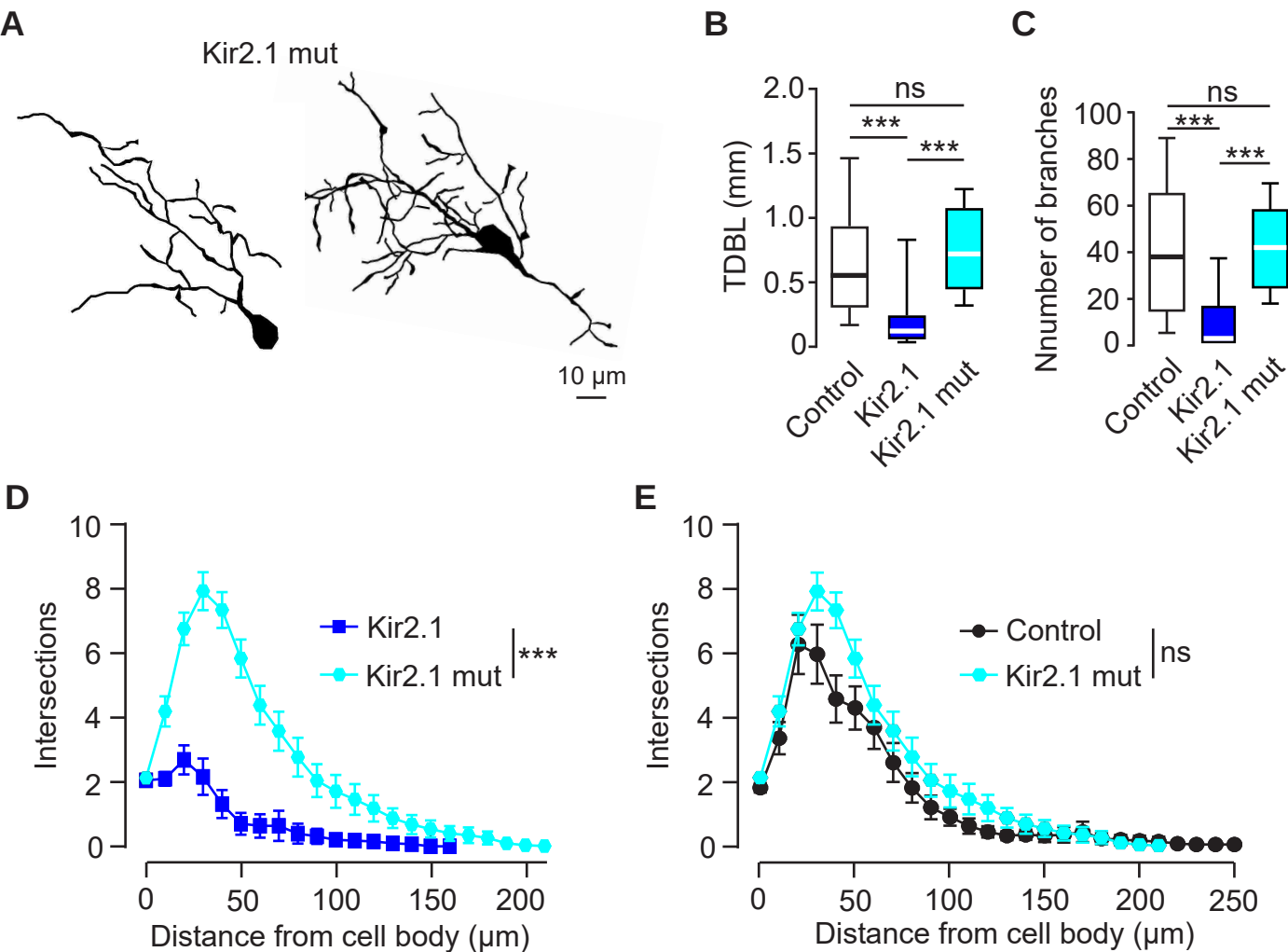


Fig. S9

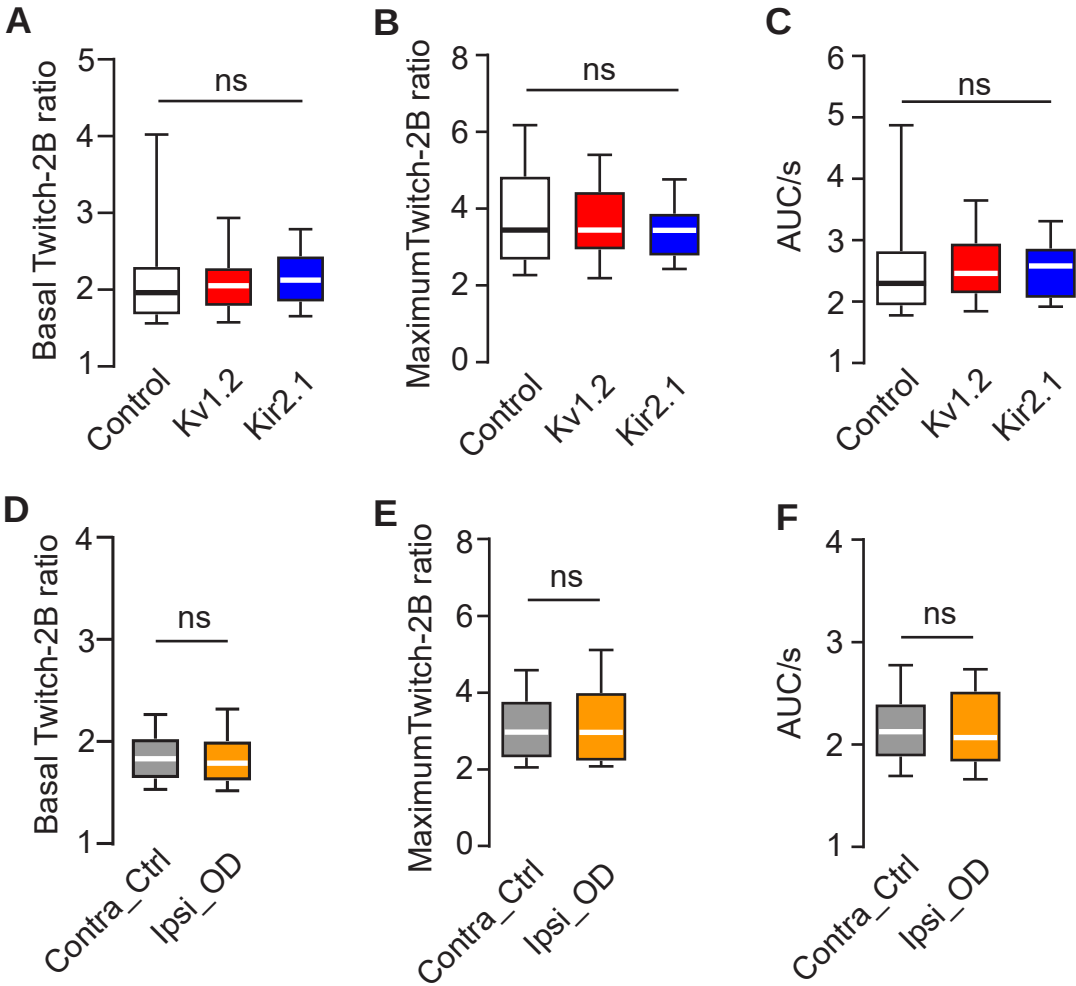


Fig. S10

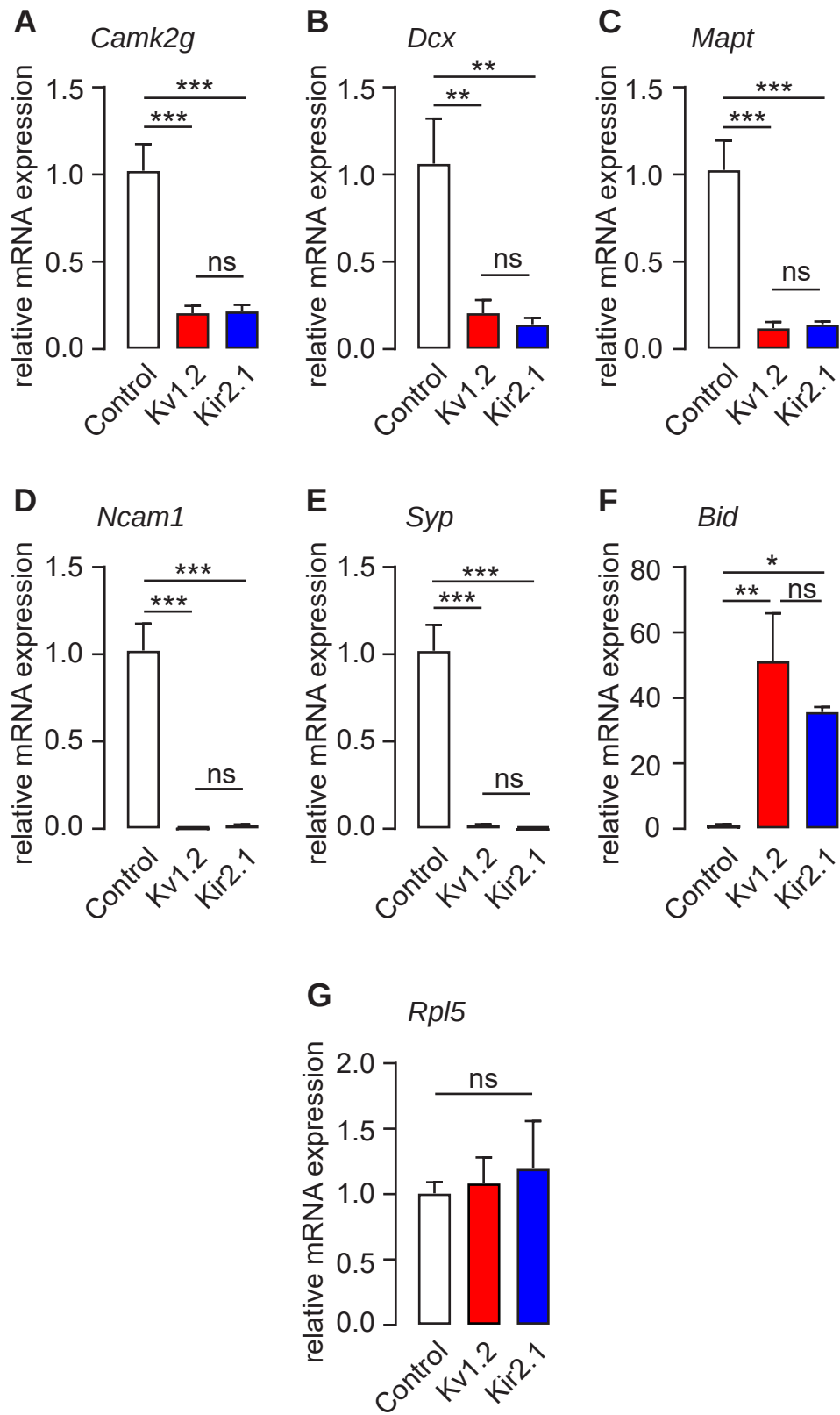


Fig. S11

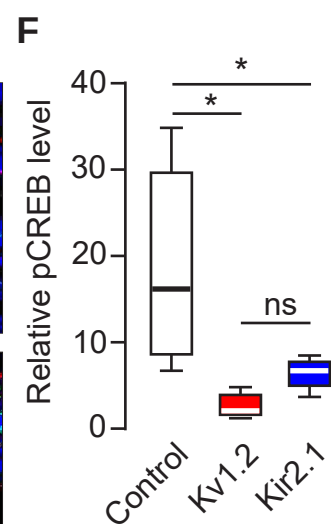
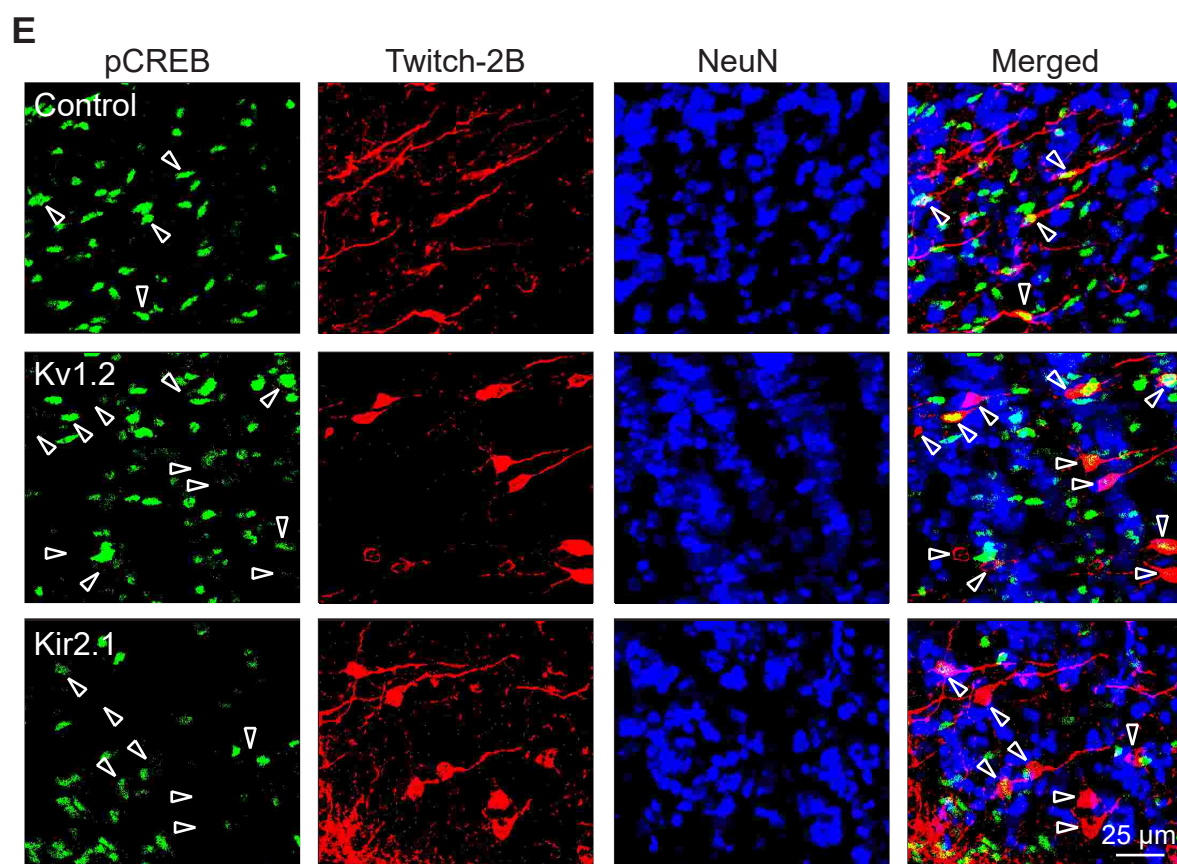
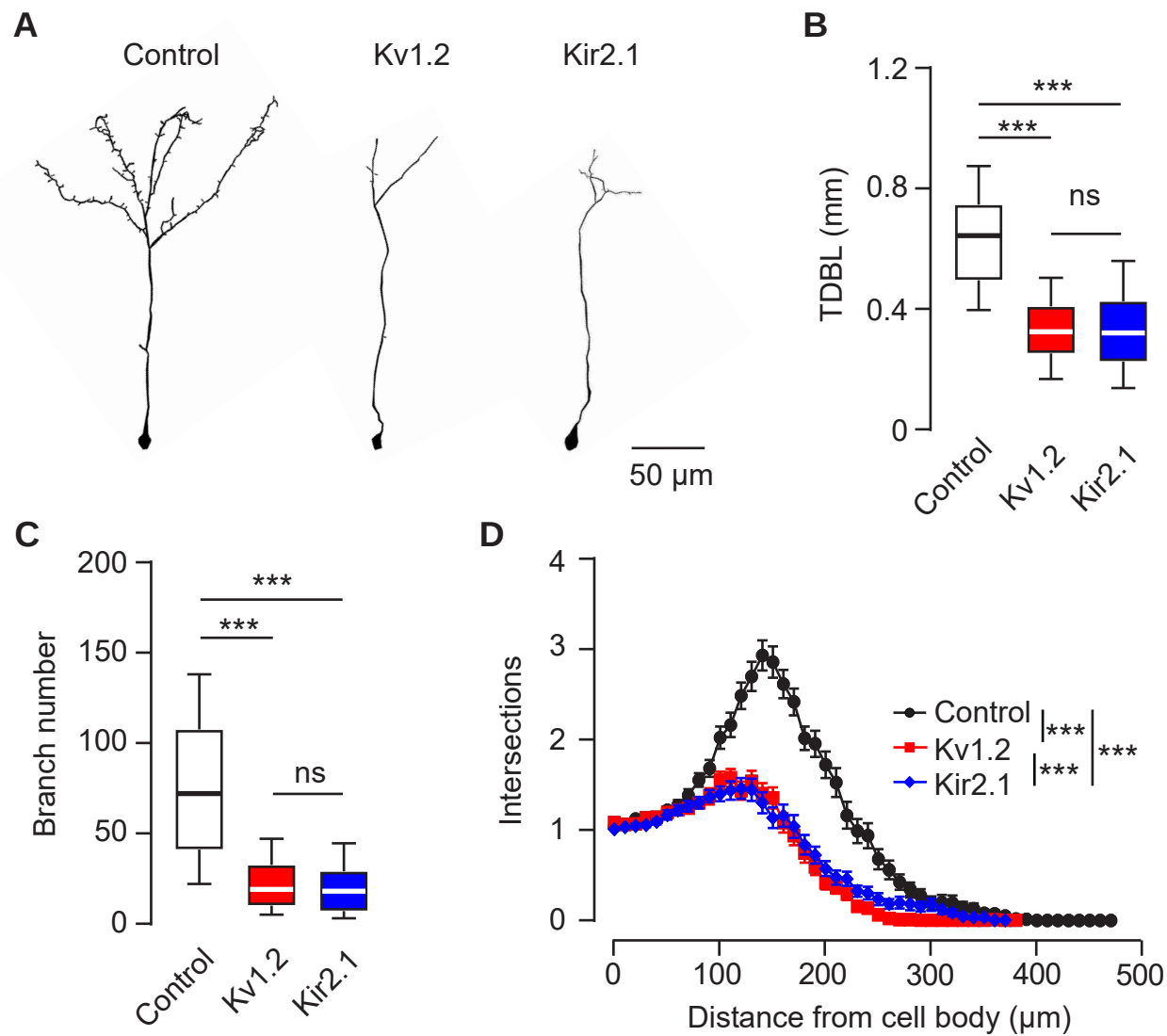


Fig. S12

