A population of Insula neurons encodes for social preference only after acute social isolation in mice

Methods

Supplementary Table 1

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
a mouse anti-Satb2 primary antibody	Abcam	Cat#ab51502; RRID: AB_882455
a rat anti-Ctip2 primary antibody	Abcam	Cat#ab18465; RRID: AB_2064130
a rat anti-MBP primary antibody	Merckmillipore	Cat#MAB386; RRID: AB_94975
a rabbit anti-GFP primary antibody	Millipore	Cat#ab3080; RRID: AB_91337
a guinea pig anti-PV primary antibody	Synaptic system Merckmillipore	Cat #195004; RRID: AB_2156476
a mouse anti-GAD67 primary antibody	Life technologies	Cat#MAB5406; RRID:AB_2278725
a guinea pig anti-Neun/Fox3	Synaptic System	Cat#266004; RRID:AB_2619988
a goat anti-CTb	List Labs	#703;RRID:AB_10013220
a donkey anti-mouse secondary antibody alexa 647	Life technologies	Cat#A31571; RRID: AB_162542
a donkey anti-rat secondary antibody alexa 488	Invitrogene	Cat# A21208; RRID: AB_141709
a goat anti-guinea pig secondary antibody alexa 488	Invitrogen	Cat#A11073;
a goat anti-rabbit secondary antibody conjugated with gold particle	Nanoprobe	1.4 nm
a donkey anti-rabbit secondary antibody alexa 488	life technologies	Cat#A21206;
streptavidin alexa 557	R&D system	Cat#NL999; RRID: AB_10175722
a donkey anti-goat alexa647	Jackson Immunoresearch	Cat#705-605-147;RRID: AB_2340437
Bacterial and viral strains		
rAAV2-retro-CAG-Cre	UNC vector	Ed Boyden
AAV2.2-eif1a-DIO-eYFP	Addgene	Karl Deisseroth cat#27056-AAV2
AAV2.2-hSyn-eYFP	Addgene	Bryan Roth cat#50465-AAV2
AAV2.5-eif1a-DIO-eYFP	Addgene	Cat#27056-AAV5 RRID:Addgene_27056
AAV2.2-hSyn-ChR2(H134R)-eYFP	UNC vector core	Cat# 45580-AAV5;
AAV5-flex-taCasp3-TEVp	Addgene	RRID: Addgene_45580
AAV1-phSyn1(S)-FLEX_tdTomato-T2A-SypEGFP-WPRE	Addgene	Cat# 51509_AAV1; RRID:Addgene_51509
rAAV2-retro-CAG-eGFP	UNC GTC Vector Core	Lot#AV7493C
rAAV2-retro-CAG-tdTomato	UNC GTC Vector Core	Lot#AV7494C
pAAV9-syn-jGCaMp8f-WPRE	Addgene	RRID: Addgene 162379_
AAV9-pCAG-FLEX-EGFP-WPRE	Addgene	Cat# 51502-AAV9
AAV8-hSyn-DIO-HA-hM4D(GI)-IRES-mCitrine	Addgene	Cat# 50455-AAV5
AAV8-hSyn-DIO-hlM3D(Gq)-mCherry	Addgene	Cat# 4361-AAV5
Chemicals		
Isoflurane	virbac	
Lurocaine	centravet	
Buprenorphine	virbac	

Rimadyl	centravet	
Exagon	centravet	
Normal Donkey Serum	Sigma-aldrich	Cat#D9663;RRID: AB_2810235
Normal Goat Serum	Sigma-aldrich	Cat#G9023
Fluoromont-G	Southern Biotechn	Cat#0100-01
Skye blue pontamine	Sigma-aldrich	Cat#C8679-25g
CNO	Bio-techne	Cat#4936/50
TTX	ABCAM	Cat#ab120055
4AP	Ascent scientific	Cat#ASC-122-100mg
Experimental models:		
Organisms/strains		
C57BL/6JRj mice	Janvier-Labs	
Ai9 tdTomato; Gt(Rosa)26Sortm6(CAG-tdTomato)Hze	Jackson	Cat#007909
Software and algorithms		
Prism 9	GraphPad	RRID: SCR_002798
NDP.view2	Hamamatsu	
Fiji software	Schindelin et al 2012	https://imagej.net/software/fiji/
Ethovision XT 16		RRID:SCR_000441
Spike2		RRID:SCR_000903
PClamp		RRID:SCR_011323
Zotero		RRID:SCR_013784
Inskape		RRID : SCR_013784
Deep lab Cut		
QuPath		
Other		
Epifluorescent microscope	Olympus BX63	
Confocal microscope	Leica TCS SP5	
Slide scanner	Nanozomeer 2.0HT	

Resource availability

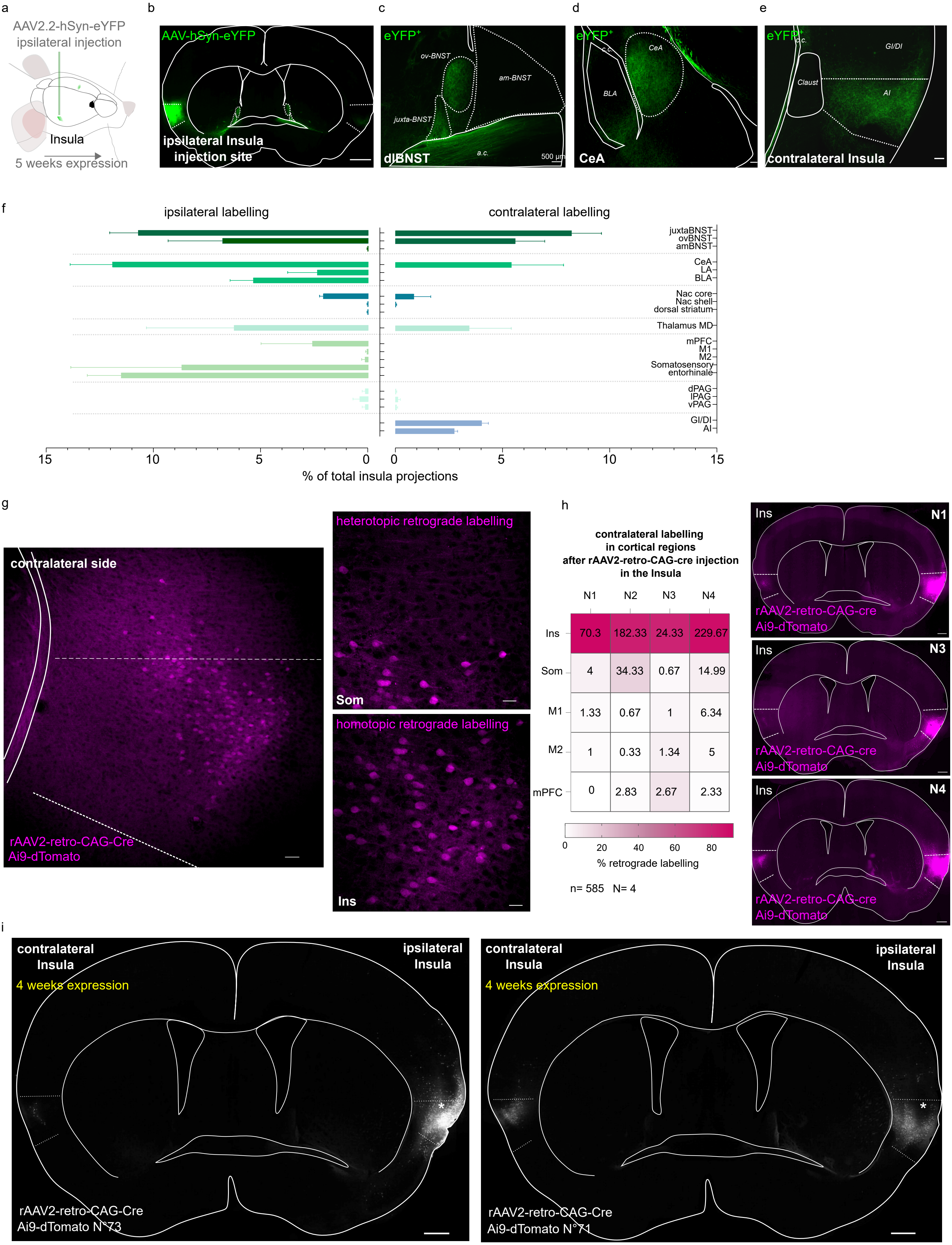
Lead contact

Further information and requests for resources and reagents should be directed and will be fulfilled by the lead contact, François Georges: francois.georges@u-bordeaux.fr

Materials availability

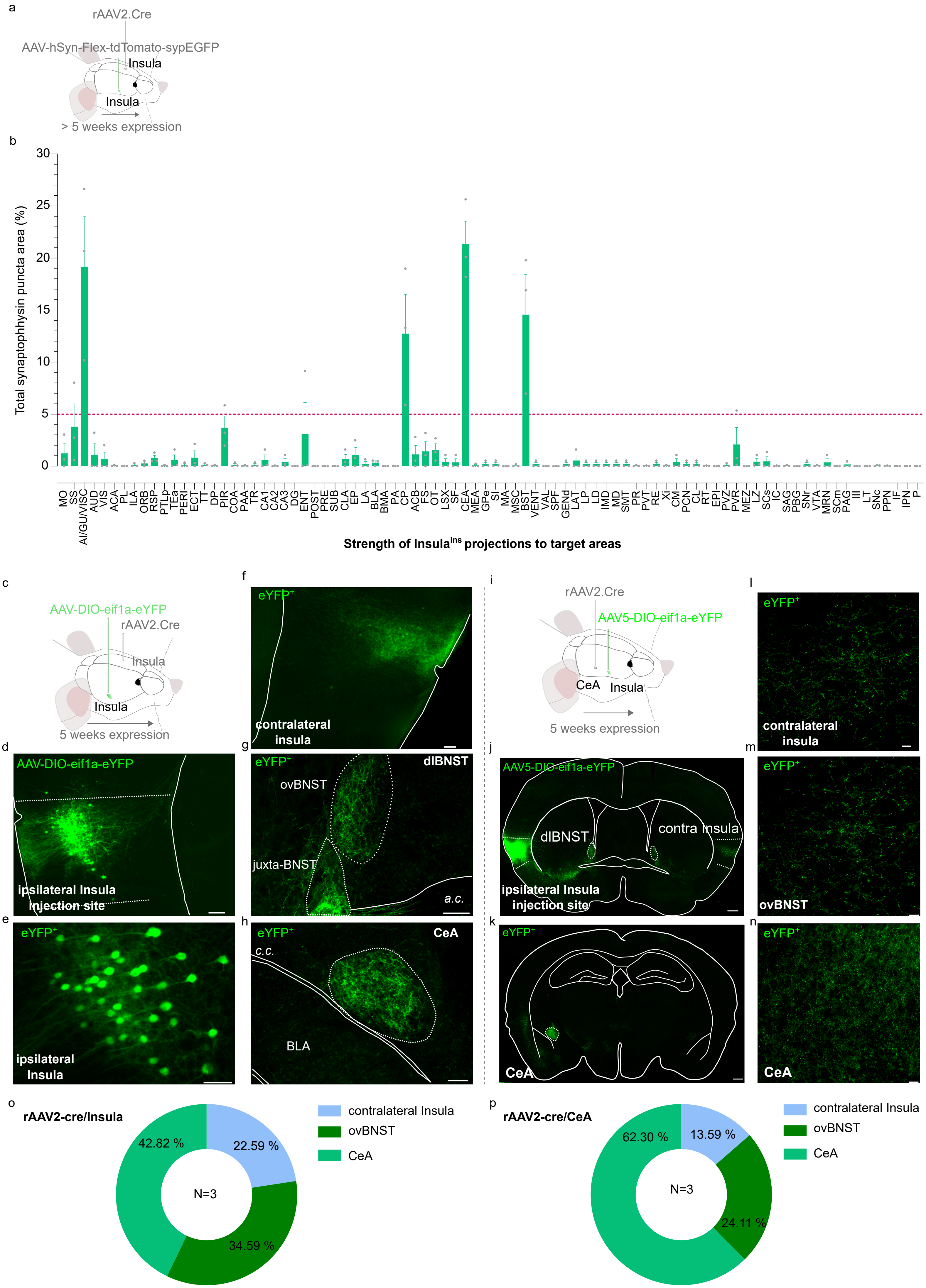
This study did not generate new unique reagents.

Supp Figure 1



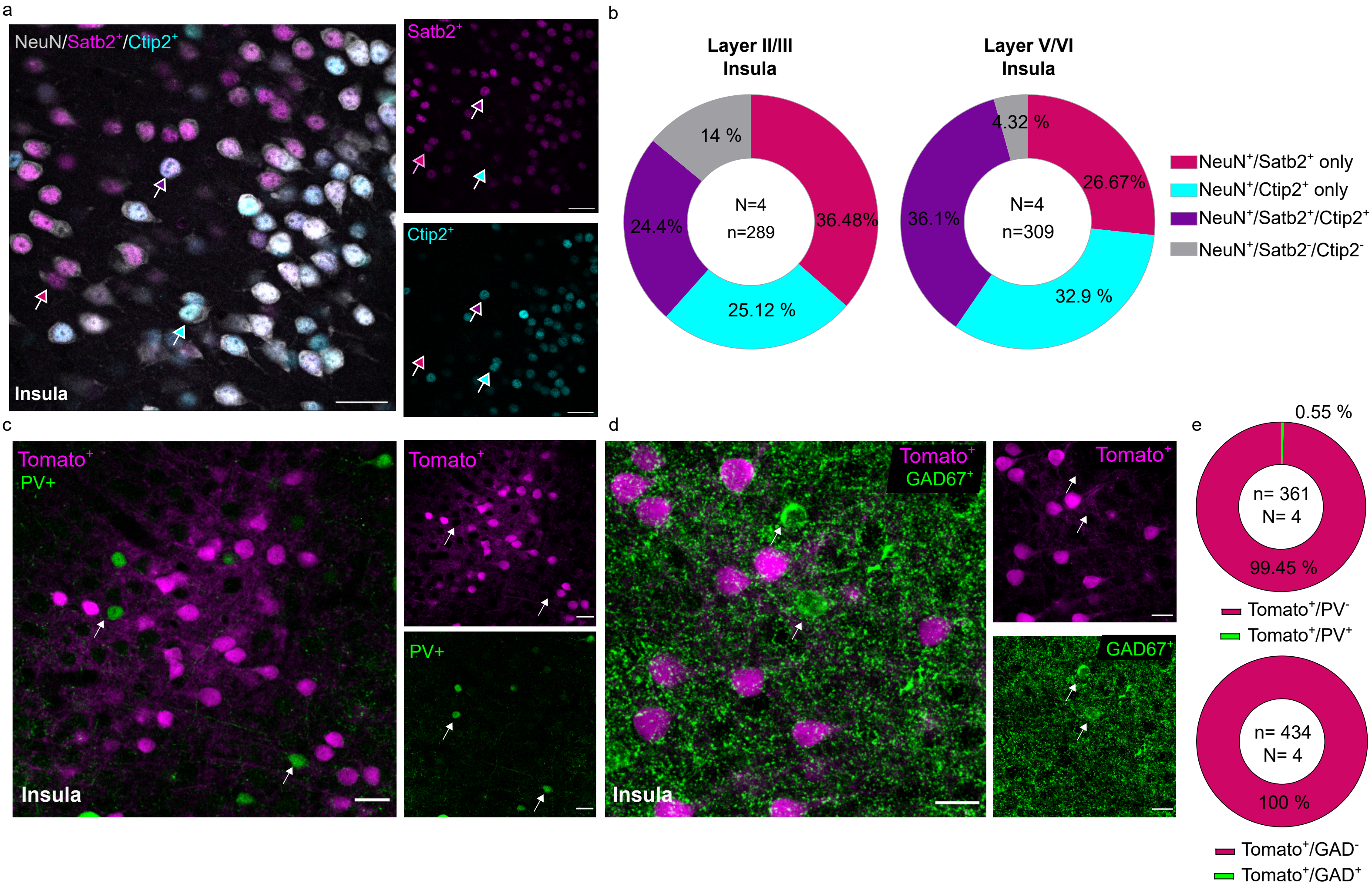
Supplementary Figure 1. Anterograde and retrograde labelling of Insula interhemispheric neurons. a. Experimental design. **b-e.** Representative epifluorescent image of a coronal slice of brain injected with an AAV2-hSyn-eYFP anterograde virus in the insula showing the injection site in the Insula (b) and projections to the dlBNST (c), the CeA (d), the contralateral insula (e). Scale bar: 1 mm for (b) and 500 µm for (c-e). **f.** Quantification of the Insula bilateral projections (N= 3 mice)**. g.** Confocal image of a coronal slice of brain injected with a rAAV2-retro-CAG-Cre retrograde virus in the Insula in Ai9-dTomato. Right, high magnification of heterotopic labelling in the somatosensory cortex (top) and homotopic labelling in the Insula (bottom). **h.** Left, quantification of cortical homotopic and heterotopic labelling (values in the heatmap represent the number of neurons), with on the right, the associated epifluorescent images of the Insula injection sites (N1, N3, N4). **i.** Example of rAAV2-retro-CAG-cre virus expression 4 weeks after injection in the Insula in two mice (AI9-dTomato N°73 and N°71).

Supp Figure 2



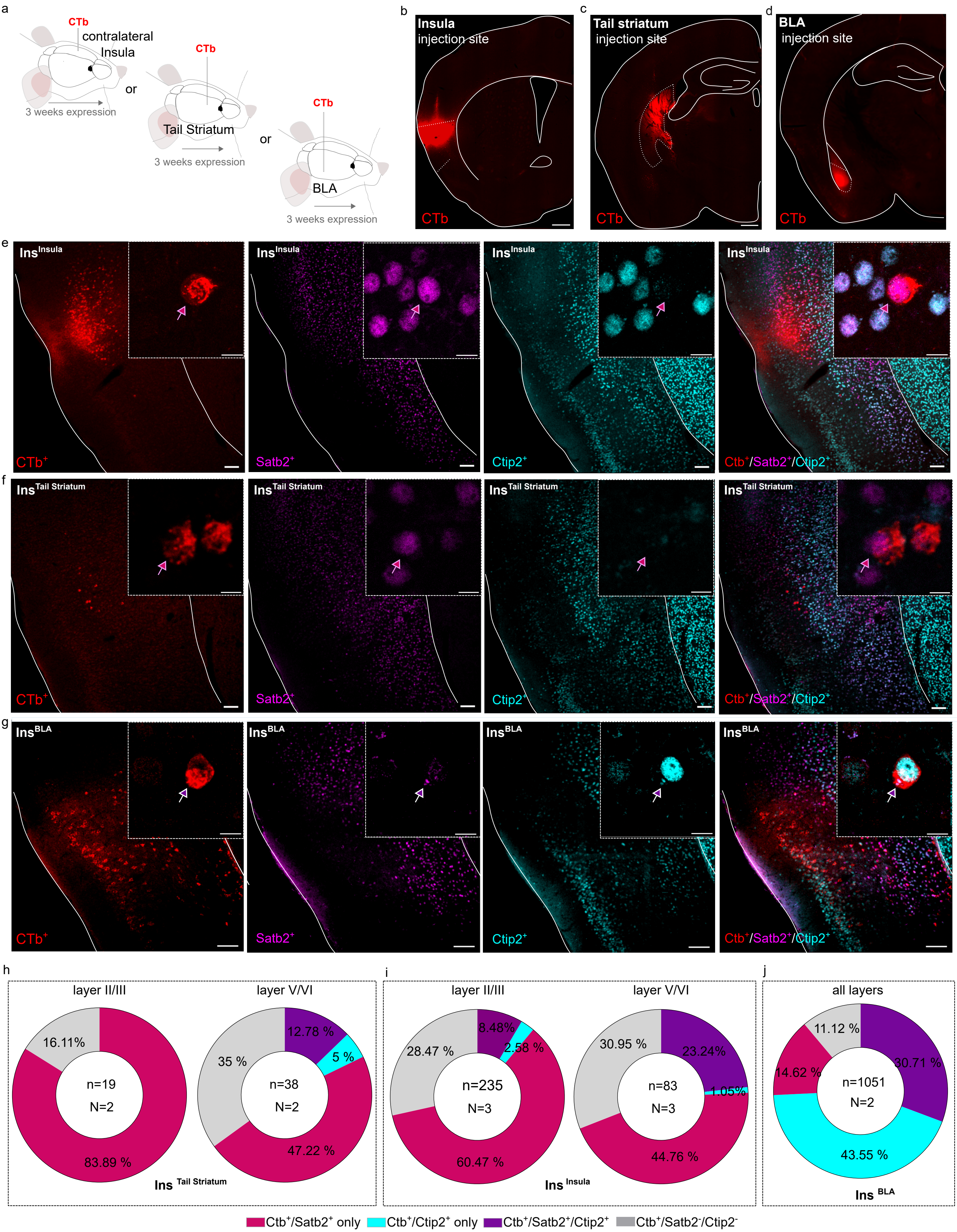
Supplementary Figure 2. Projections of Insula interhemispheric neurons. a.,c., i. Experimental design. **b**. Total synaptophysin eGFP fluorescence labelling for regions targeted by Insula^{Ins} neurons. **d-h,j-n.** Representative epifluorescent image of a coronal slice of brain injected with an AAV2-DIO-eif1a-eYFP anterograde virus in the Insula ipsilateral coupled with a rAAV2-retro-CAG-Cre in the contralateral Insula (d,e) or the ipsilateral CeA (j) showing projections to the contralateral insula (f,l), the dlBNST (g,m), the CeA (h,n). Scale 100 µm for d,f,g,h;50 µm for e.; 500 µm (j, k) and 25 µm (l-n). **o.,p.** Quantification of the Insula^{Ins} projections in bilateral ovBNST, CeA, and contralateral Insula targeted with rAAV2-retro-Cre virus injection in the contralateral Insula (o) or CeA (p). N: number of mice.

Supp Figure 3



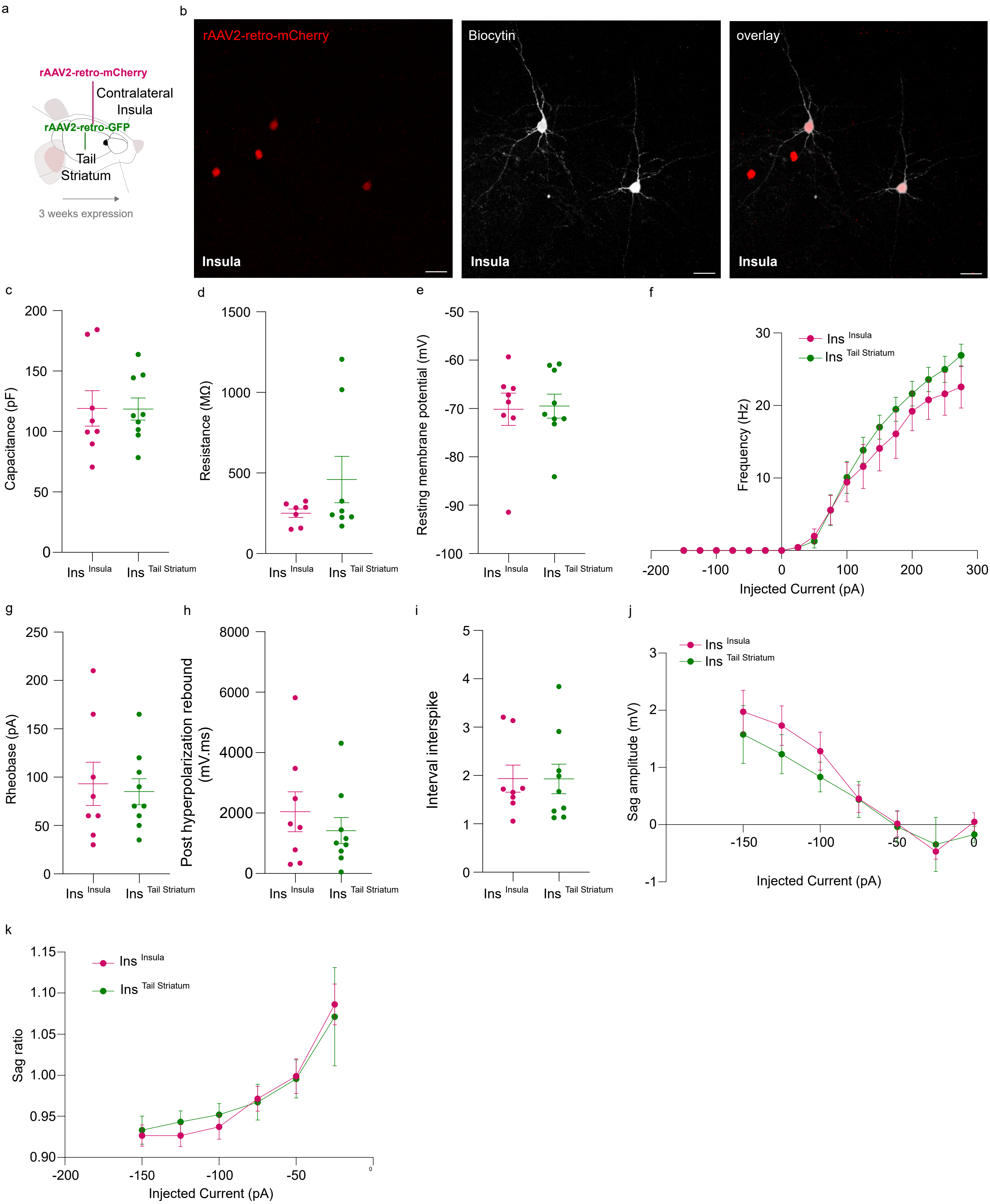
Supplementary Figure 3. Quantification of the expression of Insula molecular marker. a.,b. Representative confocal images showing co-localization between NeuN (grey) and $Satb2^+$ (magenta) and $Ctip2^+$(cyan) immunofluorescence staining in Insula with its pie-chart quantifications. n: number of neurons. N: number of mice. **c.,d.** Representative confocal images showing an absence of co-localization between PV (c, green) or GAD67 (d, green) immunofluorescence staining and $Insula^{Ins}$ neurons (tomato labelling and its quantification (e). scale bar: 25 μm. N: number of mice

Supp Figure 4



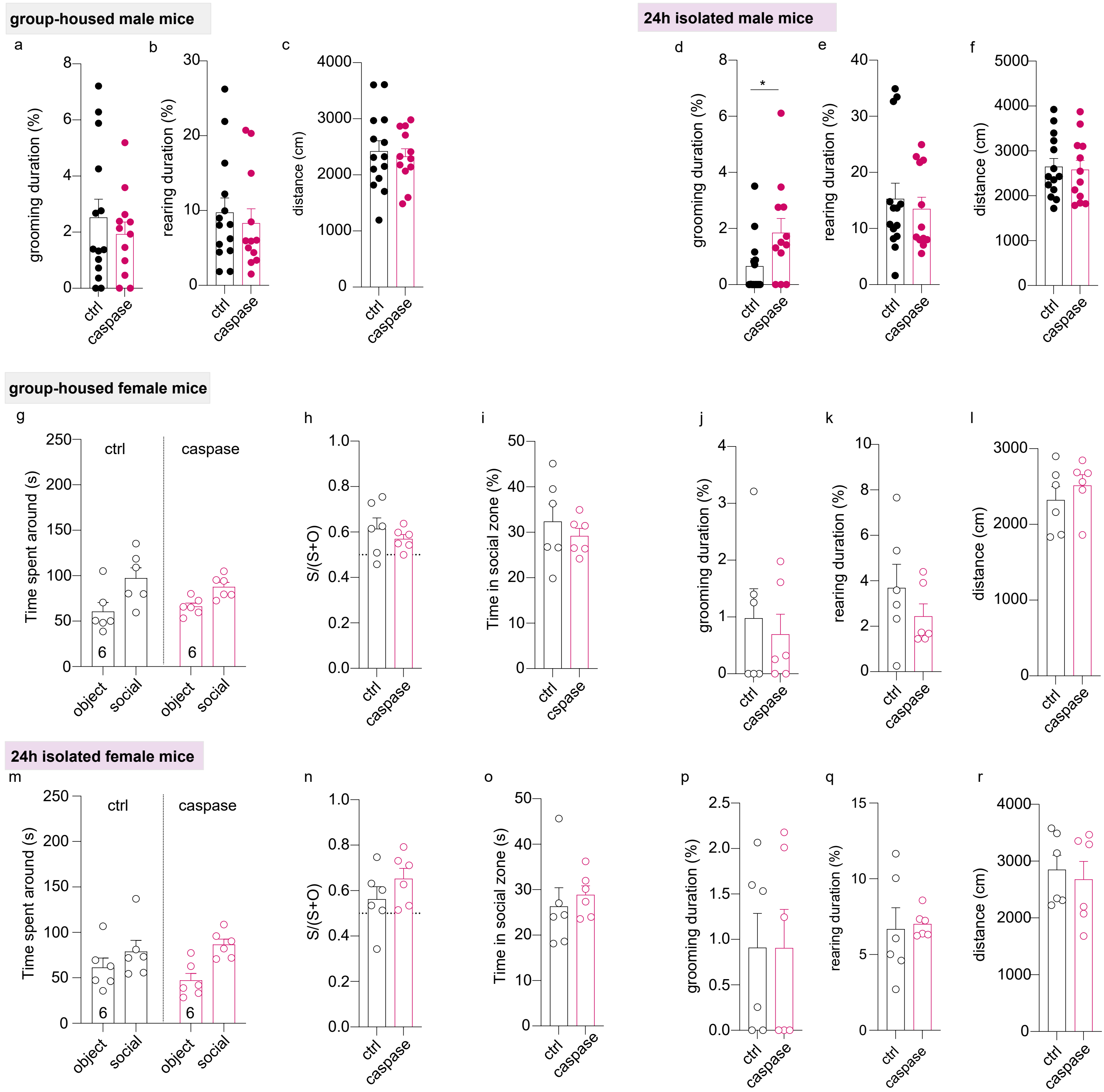
Supplementary Figure 4. Molecular characterization of Insula^{Ins}, Insula$^{Tail\ Striatum}$ and Insula^{BLA} subpopulations. a. Experimental design. **b-d.** Epifluorescent image of CTb injection site in the Insula (b), in the tail of the striatum (c), and in the BLA (d) (scale 500 µm). e-g Immunofluorescence confocal images showing Insula^{Ins} neurons (e) or Insula $^{Tail\ Striatum}$ (f) or Insula^{BLA} (g) (tomato labelling), insula Satb2 staining (magenta labelling), insula Ctip2 staining (cyan labelling), and the overlay at low (top, scale 100 µm) and high magnification (bottom, scale 10 µm). Magenta arrows show examples of Tomato and Satb2 colocalizations and purple arrows show triple colocalizations. **h-j.** Quantification of Tomato, Satb2, and Ctip2 colocalization in Insula$^{Tail\ Striatum}$ (h), Insula^{Insula} (i), Insula^{BLA} (j). n: number of neurons. N: number of mice.

Supp Figure 5



Supplementary Figure 5. *Ex vivo* electrophysiological characterization of Insula^{Ins} and Insula$^{Tail\ Striatum}$ subpopulations. a. Experimental design. **b.** Representative example of a histological control showing Insula^{Ins} neurons (tomato labelling) and insula recorded neurons filled with biocytin (grey labelling) and the overlay. **c-e.** Group mean of the capacitance (c), resistance (d), and resting membrane potential (e) of Insula^{Insula} (n= 8 neurons) and Insula$^{Tail\ Striatum}$ (n= 9 neurons) neurons. **f.** Mean frequency of current-evoked APs in response to different step currents in Insula^{Insula} and Insula$^{Tail\ Striatum}$ pyramidal neurons. **g-i.** Group mean of the rheobase (g), post-hyperpolarization rebound (h), and interval interspike (i) in both groups. **j.k.** Group mean of Sag amplitude and Sag ratio evoked by different step currents in Insula^{Insula} and Insula$^{Tail\ Striatum}$ pyramidal neurons.

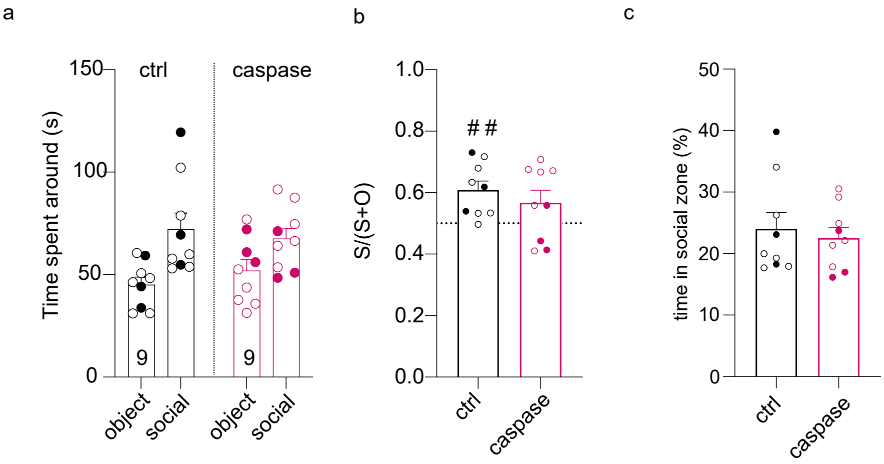
Supp Figure 6



Supplementary Figure 6. Behavioral characterization of control and caspase Insula mice during the three-chamber social interaction test. a-f. Group mean of the time spent in grooming (a,d, Mann Whitney test, U=45.50, p=0.04), rearing (b,e), and distance travelled (c,f) during the habituation in the three-chamber social interaction test in control and caspase Insula mice. **g.,m.** Quantification of the time spent around the object and social enclosures in the three-chamber test in group-housed condition (g) or after 24 hours of social isolation (m) in Insula control and Insula caspase female groups (N=6 mice per group). **h,n.** Social preference ratio in the control group and caspase group in group-housed mice (h) or isolated female mice (n, N=6 mice per group). **i,o.** Time spent in the social zone between control and caspase mice in group-housed (i) or in 24-hour isolated conditions (o). **j,k,p,q.** Time spent in grooming and rearing in group-housed (j, k) or after 24 hours of social isolation (p,q). **l.,r.** Total distance travelled during social interaction test in both female groups.

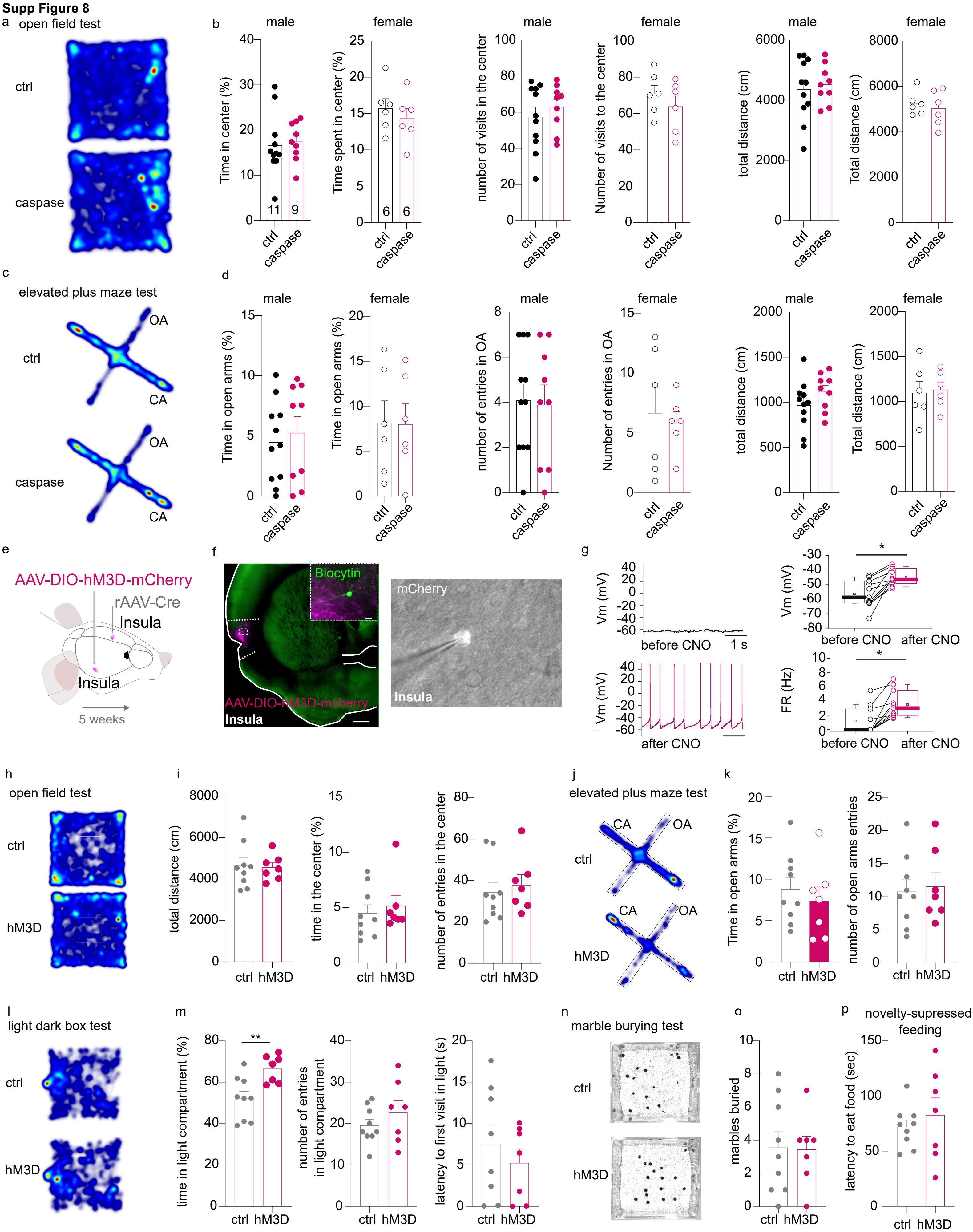
Supp Figure 7

2 weeks isolated mice



Supplementary Figure 7. Impact of two weeks of social isolation on the three-chamber social interaction test in control and caspase Insula mice. **a.** Quantification of the time spent around the object and social enclosures in the three-chamber test in 2 weeks isolated male (black and pink circle) and female (white circles) Insula control (N= 9 mice) and insula caspase mice (N=9 mice). **b.** Social preference ratio in the two weeks isolated mice. One sample Wilcoxon test for ctrl: W=43, $p<0.05$, and caspase: W=25, $p>0.05$; **c.** Time spent in the social zone between control and caspase mice after two weeks of isolation. Numbers in the histogram bars indicate the number of mice. ## *is used to mention a difference with a social preference ratio equal to 0.5.*

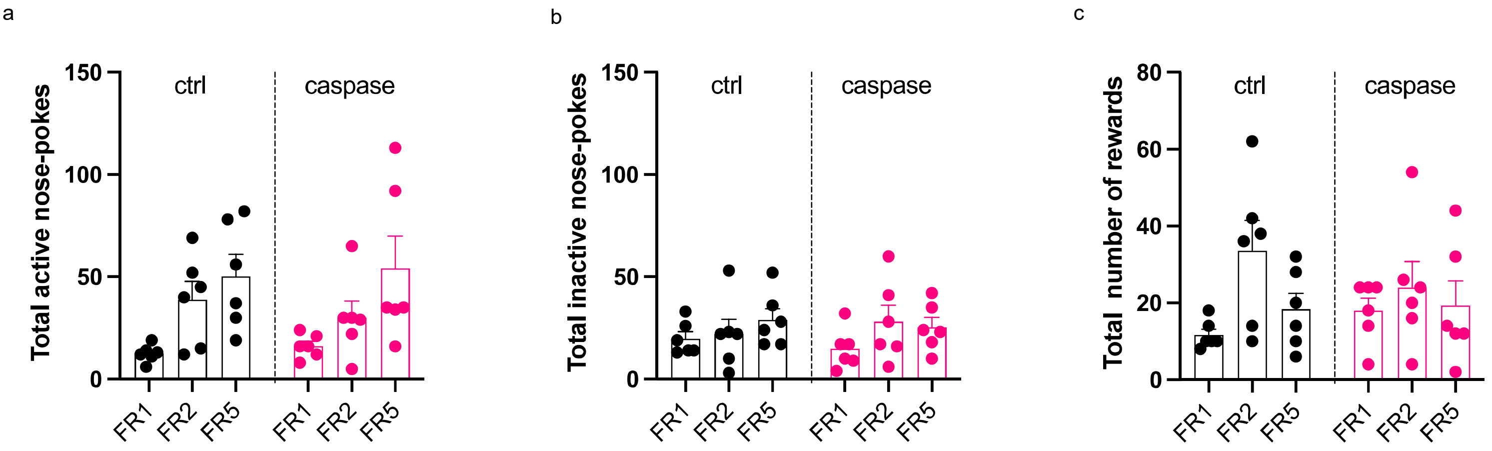
Supp Figure 8



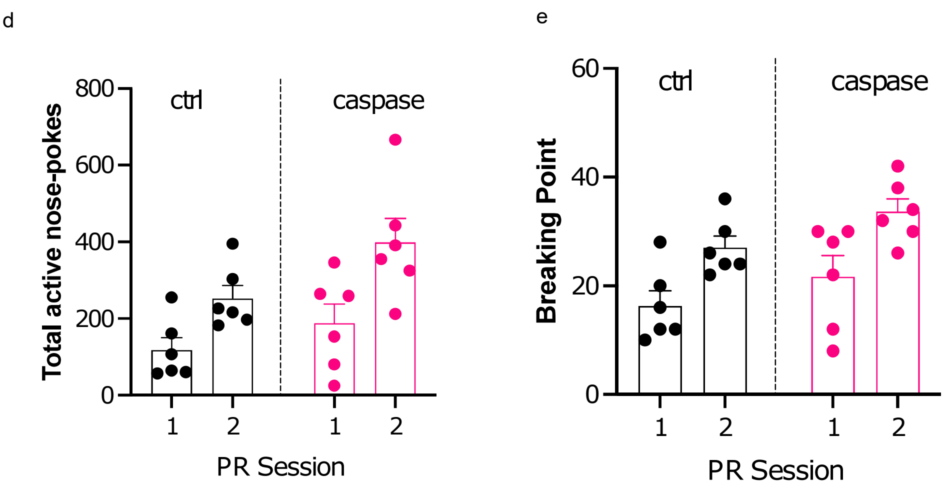
Supplementary Figure 8. General locomotion and anxiety profile of control, caspase Insula and hM3D Insula mice. a., c. Example heatmaps of insula control and caspase mice performance in the open field test (a) or in the elevated plus maze (c). **b.** Quantification of the time spent in the center of the open field, the number of visits to the center, and the total distance travelled in control and caspase male and female mice. The number of mice used is indicated in the bar graph. **d.** Quantification of the time spent, the number of entries in the open arms of the elevated plus maze, and the total distance travelled in both groups. **e.,q.** Experimental design. **f.** Histological control of record biocytin Insula neuron **g.** *Ex vivo* validation of CNO effect in $Insula^{Ins}$ neurons expressing excitatory Dreadd (n= 10 neurons). **h,j,l.** Example heatmaps of Insula control and hM3D mice performance in the open field test (h) the elevated plus maze (j) the light-dark box test (l). **i.** Quantification of the time spent in the center of the open field, the number of visits to the center, and the total distance travelled in control (N= 9 mice) and hM3D male mice (N= 7 mice). **k.** Quantification of the time spent, the number of entries in the open arms of the elevated plus maze, and the total distance travelled in both groups. **m.** Quantification of the time spent, the number of entries, and the latency to the first visit in the light compartment in both groups (two-tailed unpaired t-test, $t(14)=3.165$, $p=0.0069$). **n.** Representative examples of the marble burying test arena following the test. **o.** Group mean of the marble buried in ctrl and hM3D group. **p.** Group mean of the latency to eat food in novelty-suppressed feeding behaviour in ctrl and hM3D group. *Ctrl: control; OA: open arms; CA: closed arms.*

Supp Figure 9

Operant training

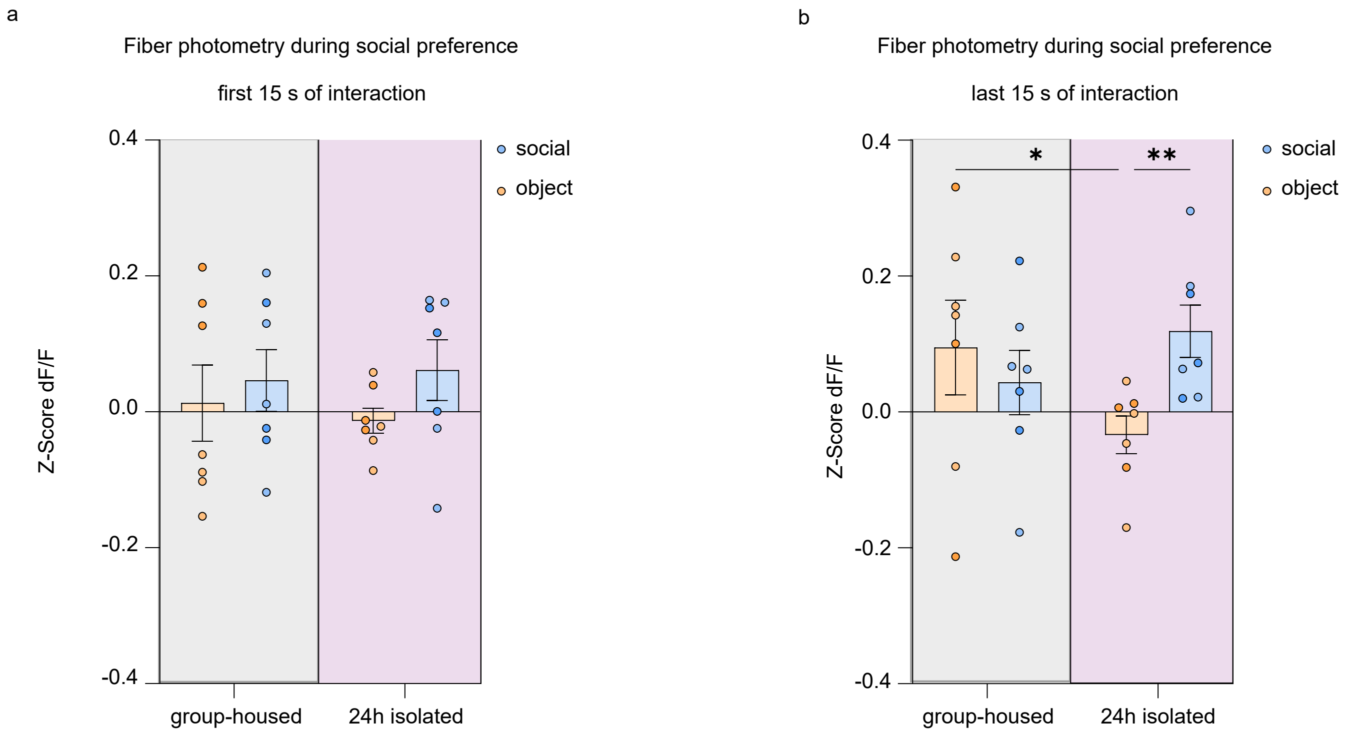


Motivation for food reward



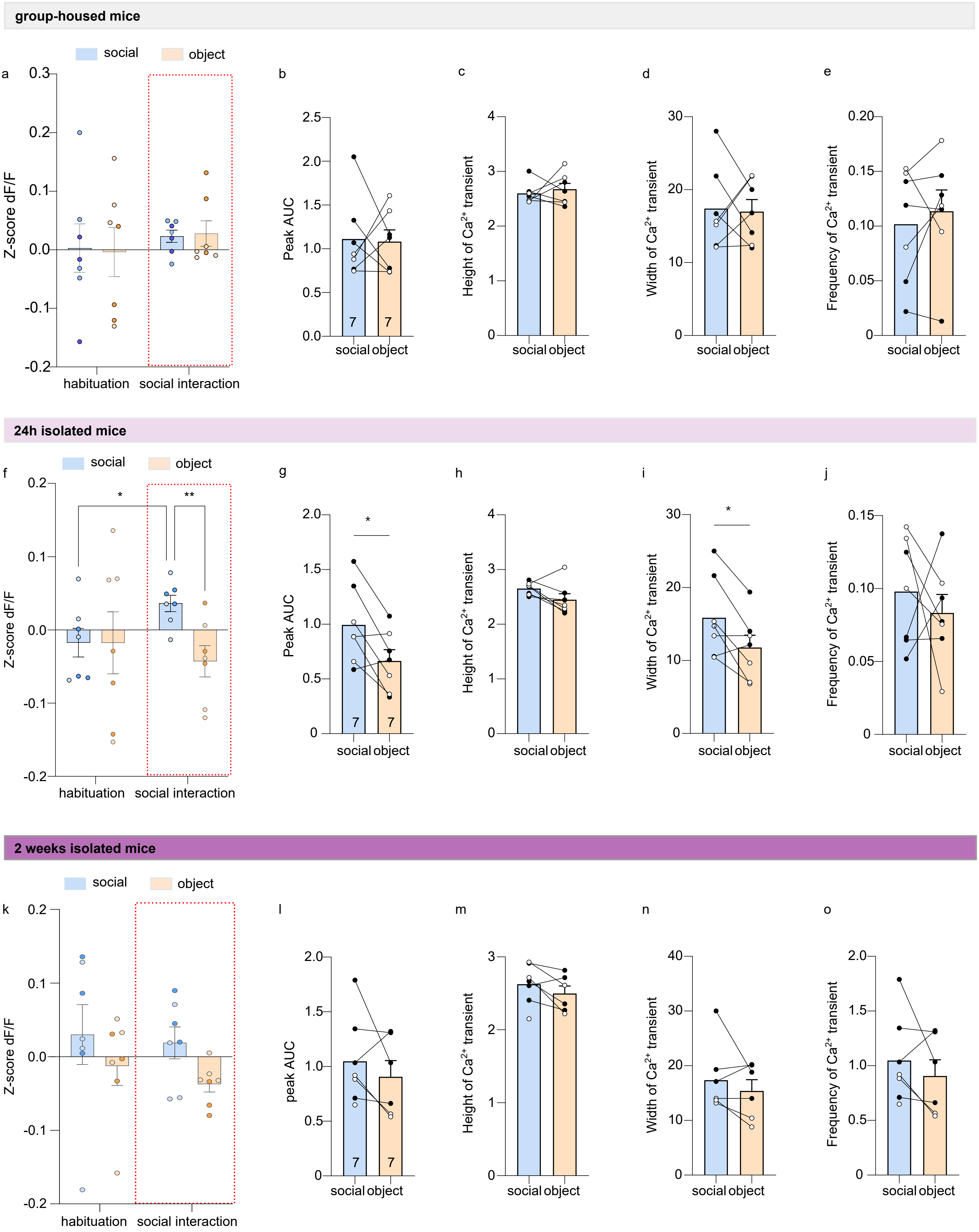
Supplementary Figure 9. Motivation for food reward in control and caspase Insula mice. Quantification of operant responding for sweet condensed milk (scm) on the last day of each schedule of reinforcement from fixed ratio 1 to fixed ratio 5 (FR1, FR2, FR5) such as **a.** total number of active nose-pokes, **b**. total number of inactive nose-pokes and **c.** total number of rewards obtained during training. Quantification of the motivation for scm on 2 sessions of progressive ratio (PR) such as **d.** total number of active nose-pokes and **e.** the breaking point value (last ratio completed) with N= 6 mice per group.

Supp Figure 10



Supplementary Figure 10. Specific Ca^{2+} activity in Insula^{Ins} neurons during the three-chamber social interaction test after 24 hours of social isolation. **a.,b.** Quantification of the ΔF/F mean fluorescence z-score of Insula^{Ins} neurons around the social or object enclosure in the three-chamber social interaction test in group-housed (N= 7 mice) and 24-hour isolated mice (N=7 mice) during the first 15 seconds of interaction (a) or the last 15 seconds of interaction (b, two way repeated measure Anova, Interaction $F(1,6)=14.44, p=0.009$, no main effect of housing $F(1,6)=0.1887$, $p>0.05$, no main effect of zone $F(1,6)=1.550$, $p>0.05$; followed by an uncorrected Fisher's LSD post hoc, group-housed object vs 24 h isolated object, $p=0.0148$, object vs social in 24 h isolated condition, $p=0.007$. dark blue/orange color circle: female mice, light blue/orange color: male mice.

Supp Figure 11



Supplementary Figure 11. Specific Ca^{2+} activity in Insula^{Ins} neurons during the habituation and the social interaction phase in the three-chamber social interaction test after 24 hours of social isolation. **a.,f.,k.** Quantification of ΔF/F mean fluorescence z-score of Insula^{Ins} neurons when the mouse is around social or object enclosure during habituation and social interaction phase in the three-chamber social interaction test in group-housed (a, (N= 7 mice per group), 24 hours isolated (f, (N= 7 mice per group; Two Way repeated measure Anova, Interaction $F(1,6)=7.026, p=0.038$, no main effect of time $F(1,6)=0.1613$, $p>0.05$, no main effect of zone $F(1,6)=0.1283$, $p>0.05$, followed with Uncorrected Fisher's LSD, during habituation social vs object $p>0.05$, during social interaction time social vs object $p=0.0094$, habituation social vs social interaction time social $p=0.04$, habituation object vs social interaction time object $p>0.05$) and 2 weeks isolated condition (k, N= 7 mice). **b.,g.,l.** Group mean of the peak of the area under the curve (AUC) of calcium transient in Insula^{Ins} neurons when the mouse is around the social or the object enclosure in group-housed condition (b) or after 24 h of social isolation (g, Paired t-test, $t(6)=3.106$, $p=0.021$) and after two weeks of social isolation (l). **c.,h.,m.** Group mean of the height of calcium transient in Insula^{Ins} neurons when the mouse is around the social or the object enclosure in the three-chamber social interaction test in group-housed condition (c) or after 24 hours of social isolation (h) and after two weeks of social isolation (m). **d.,i.,n.** Group mean of the width of calcium transient in Insula^{Ins} neurons when the mouse is around the social or the object enclosure in the three-chamber social interaction test in group-housed condition (d) or after 24 hours of social isolation (i, Paired t-test, $t(6)=2.940$, $p=0.0259$) and after two weeks of social isolation (n)**, e.,j.,o.** Group mean of the frequency of calcium transients in Insula^{Ins} neurons when the mouse is around the social or the object enclosure in group-housed condition (e) or after 24 hours of social isolation (j) and after two weeks of social isolation (o). Numbers in histogram bars indicate the number of mice.