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Supplemental information

The organization and development of cortical interneuron presynaptic circuits are area specific

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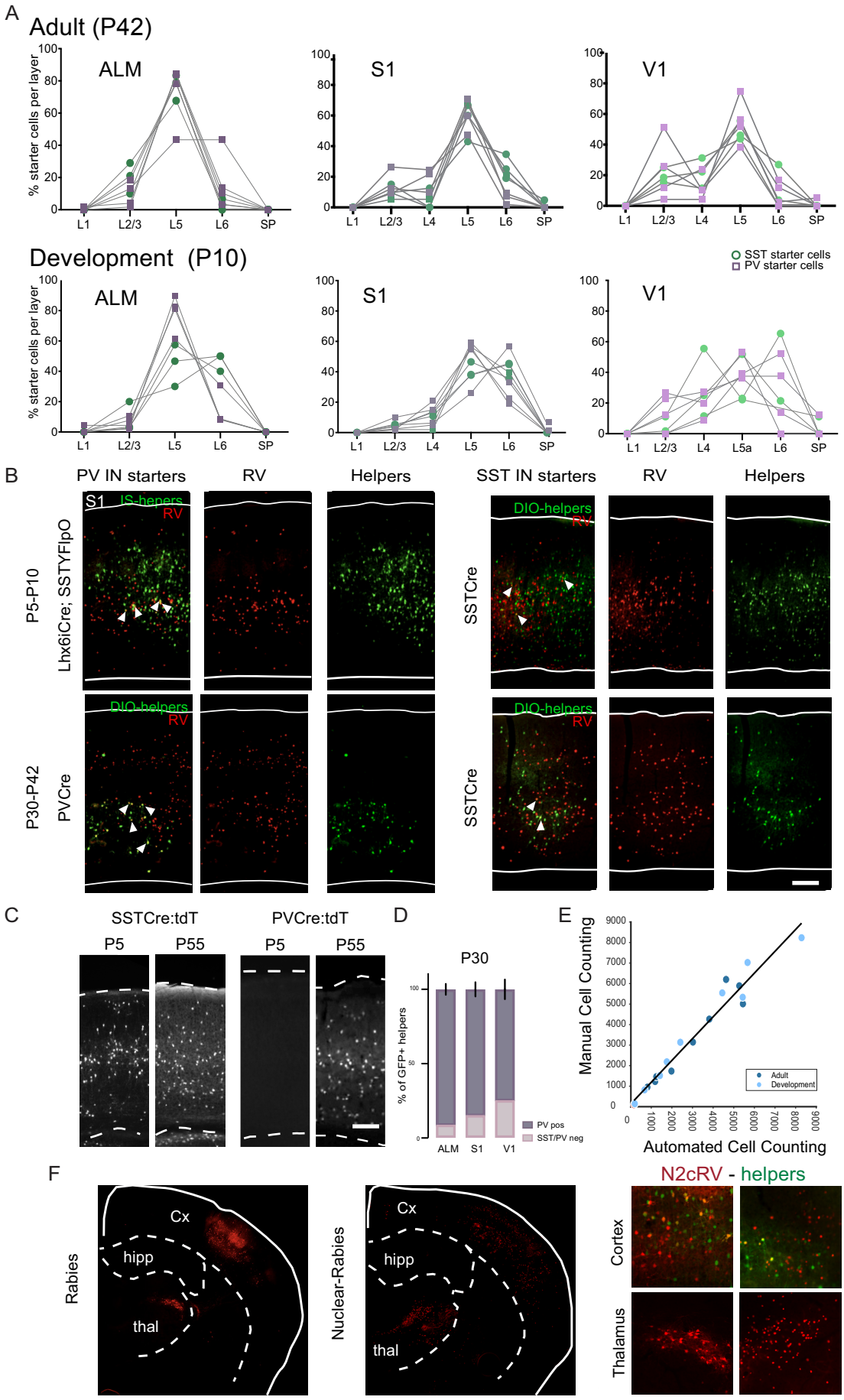

Figure S1. Monosynaptic rabies tracing method: layer distribution, normalization, Cre lines and controls.

Figure S1: Monosynaptic rabies tracing method: layer distribution, normalization, Cre lines and controls. Related to Figure 1

(A) Deep layer cortical distribution of the starter cells for each N, grouped per time point and area. squares = PV starter cells, circles = SST starter cells.

(B) Example of starter cells during development and adults for PV INs (Lhx6-icre; SST-flpO + AAV-IS-helpers (intersectional) and PV-cre + AAV-DIO-helpers- left) and for SST INs (SST-cre + AAV-DIO-helpers, right). Helpers are in green, Rabies (RV) are in red and starter cells are detected from the colocalization of both. (Scale bar: 100μm).

(C) Onset of reporter expression (tdTomato, Ai9 mouse line) upon SST-Cre and PV-cre drivers. (Scale bar: 100μm).

(C) The specificity of the PV cINs targeting using AAV-IS-helpers was verified for parvalbumin colocalization with AAV-IS-helpers at P30.

(E) Prediction of manual vs automated quantification using linear regression model. f(x) = p1*x + p2. R^2=0.9566. p1=1.062; p2=138.5.

(F) Example of retrograde-labeling tracing with mCherry vs nuclear-tdTomato reporter RV at P42 (right) (scale bar: 500μm). Insets: RV (red) colocalization with helpers (green) (scale bar:100μm).

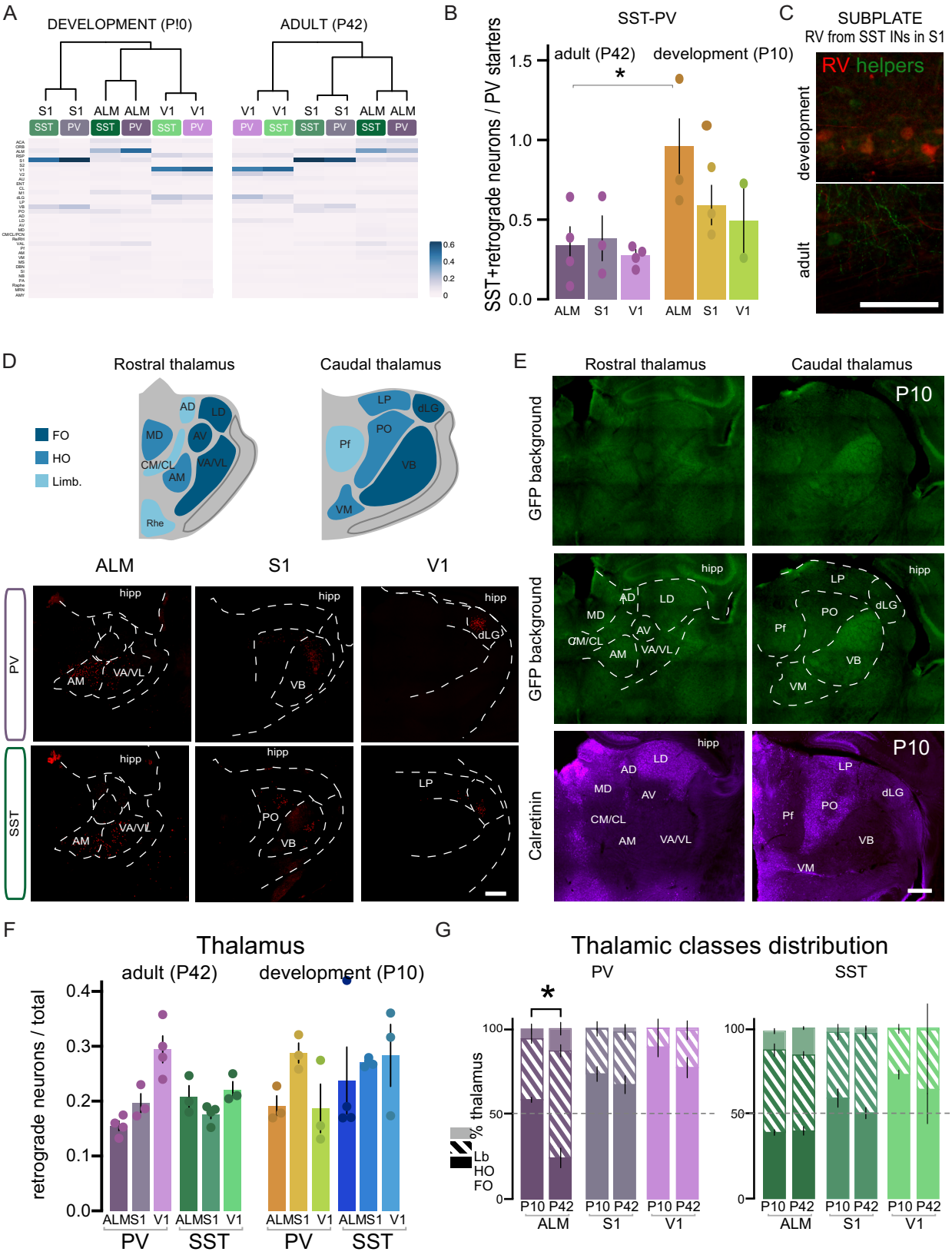

Figure S2: Temporal dynamics of afferents inputs to PV and SST INs during develpoment

Figure S2: Temporal dynamics of afferents inputs to PV and SST cINs during development. Related to Figure 2,3

(A) Heatmap and hierarchical clustering of the degree of connectivity from all afferents during development and in adults.
(B) SST to PV cIN connectivity in all areas normalized per starter cell (PV INs) numbers, in adult and during development. (One-way ANOVA, followed by Tukey's test, adjusted *p=0.0104. Data shown are as mean ± sem.
(C) Rabies (RV) retrograde labeling in the subplate are not starter cells as helpers (green) are not expressed both in adult and during development. The examples are the same than the one shown with CTGF in Figure 3B. (scale bar: 50μm)
(D) FO, HO and Lb classes identification within the thalamus. Representation of thalamic nuclei on rostral and caudal anatomical levels, as previously described. FO=dark blue, HO=blue, Lb=light blue. Example of RV tracing from PV and SST cINs in ALM, S1 and V1 in the whole thalamus in adults (scale bar: 500μm).
(E) Identification of thalamic nuclei in tissue using GFP background (top) and the distinct density of the thalamic nuclei (middle with labels). This was confirmed using Calretinin staining as a marker of distinct thalamic nuclei (purple, bottom). (scale bar: 500μm).
(F) Degree of whole thalamus connectivity to PV and SST cINs in ALM, S1 and V1 during development and in adults
(G) Ratio of FO, HO and Lb thalamocortical neurons connecting to PV and SST cINs (left). Student's t-test between PV cIN at P10 and at P42 *p=0.0247, others are n.s.

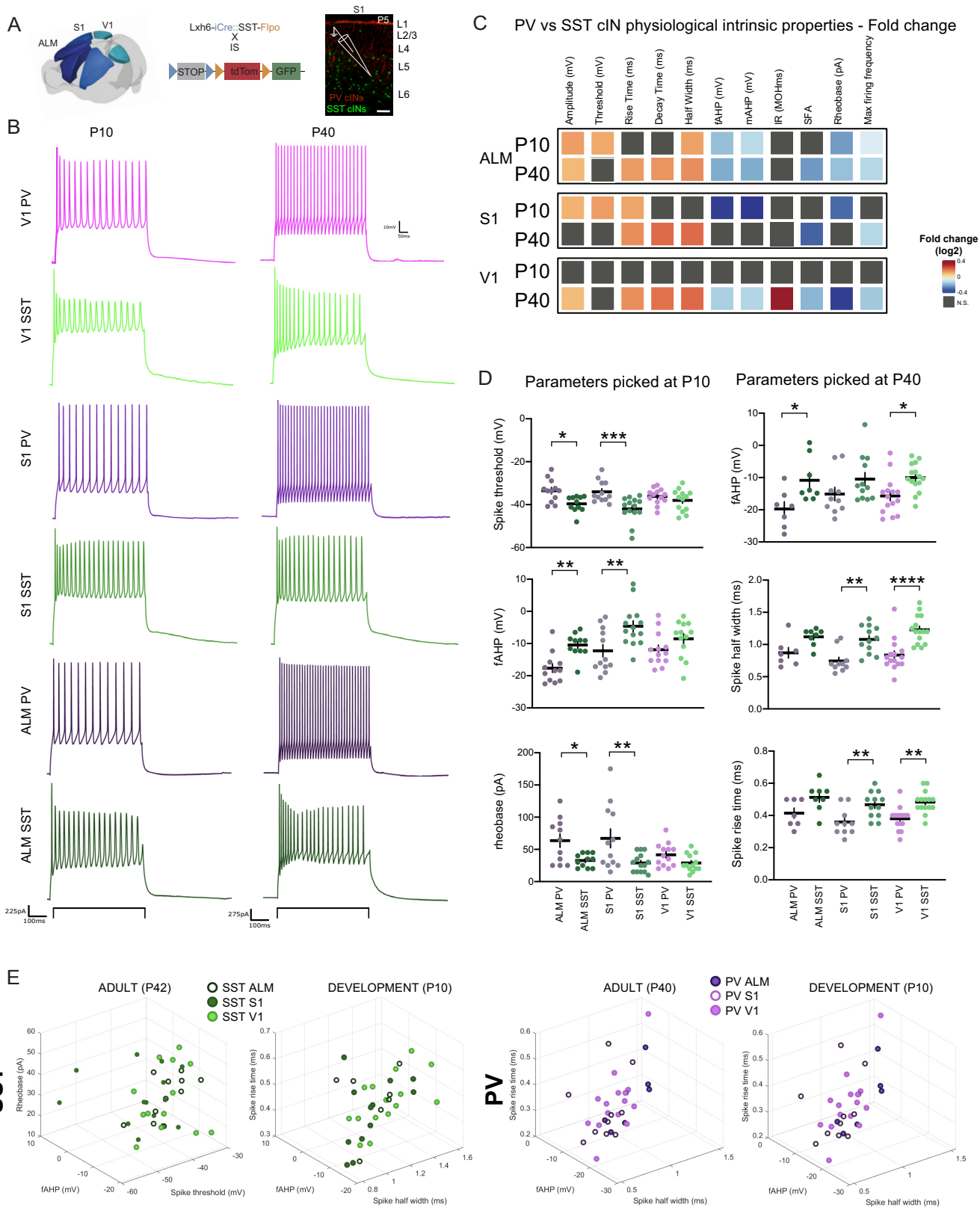

Figure S3: Intrinsic properties of PV and SST cINs during development

Figure S3: Intrinsic properties of PV and SST cINs during development. Related to Figure 4
(A) Strategy design: PV and SST cINs are recorded within ALM, S1 and V1 during development and adult (P10-P40). They are labeled using Lhx6iCre:SSTFlpO driver mice crossed with the IS reporter mouse line
(B) Example of electrophysiological trace of patch clamp recording from PV and SST cINs at P10 and P40 in V1, S1 and ALM.
(C) Heatmap of log2(fold change) from Sidák's multiple comparison test between PV and SST cINs average features within each area at P10 and P40.
(D) Plots of all cells for the parameters selected for the 3D plots I Figure 4. Sidák's test at P10 spike threshold *p=0.019 in ALM, ***p=, 0.0003 in S1, fAHP **p=0.0079 in ALM, **p=0.0022 in S1, rheobase *p=0.0279 in ALM, **p=0.0019 in S1; at P40 fAHP *p=0.0191 in ALM, *p=0.0369 in V1, spike half width **p=0.0015 in S1, ****p<0.0001 in V1, spike rise time **p=0.0062 in S1, **p=0.0016 in V1.
(E) 3D plot of PV versus SST cINs within ALM, S1 and V1 during development and in adults using the most significant features at P10 and P40.

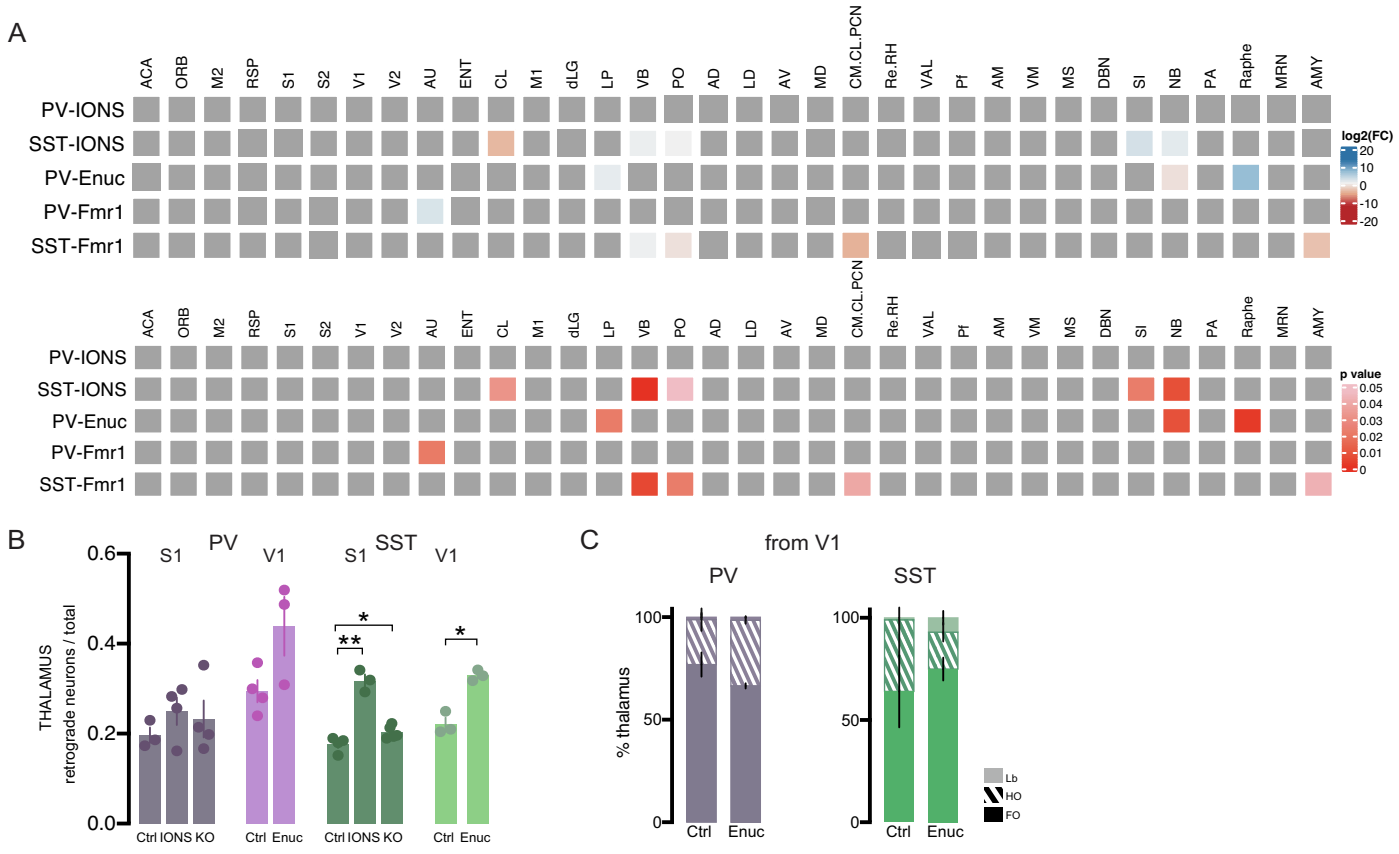

Figure S4: Connectivity from all afferents in Fmr1 KO and in sensory deprived animals

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Related to Figure 5

(A) Heatmap of log2(Fold change) and *p* values from Student's t-test between all afferents from PV and SST cINs in Enuc, IONS or Fmr1 KO animals and their respective controls.
(B) Degree of connectivity of whole thalamus to PV and SST cINs in IONS, Enuc and Fmr1 KO animals compared to S1 controls and in Enuc compared to V1 controls.
(C) Ratio of FO, HO and Lb thalamic within the whole thalamus (100%). Student's t-test with no ratio being significant.

Table S1: All raw numbers of starter cells used in the study. Related to Figure 1

IN	area	N	Ad	out	Total retro	Dev	out	Total retro	IONS /Enuc	out	Total retro	KO	out	Total retro
PV	ALM	N1	49	0	6130	48	10	377	x	x	x	x	x	x
PV	ALM	N2	46	19	5648	13	0	101	x	x	x	x	x	x
PV	ALM	N3	60	0	3710	22	0	342	x	x	x	x	x	x
PV	ALM	N4	90	32	10332	x	x	x	x	x	x	x	x	x
PV	S1	N1	53	0	1865	81	2	2207	152	0	7650	106	0	8254
PV	S1	N2	154	0	6846	80	0	1524	13	0	530	19	0	10760
PV	S1	N3	55	0	4367	53	0	981	20	0	1630	54	0	2183
PV	S1	N4	x	x	x	x	x	x	39	0	315	86	0	4721
PV	V1	N1	39	0	1296	8	0	58	68	5	1227	x	x	x
PV	V1	N2	25	0	874	22	0	827	107	1	2187	x	x	x
PV	V1	N3	24	0	1465	23	2	145	33	0	2302	x	x	x
PV	V1	N4	43	1	2795	x	x	x	x	x	x	x	x	x
SST	ALM	N1	62	0	6952	218	64	14937	x	x	x	x	x	x
SST	ALM	N2	19	0	1587	10	0	750	x	x	x	x	x	x
SST	ALM	N3	36	6	6797	120	20	9347	x	x	x	x	x	x
SST	ALM	N4	x	x	x	40	5	6247	x	x	x	x	x	x
SST	S1	N1	19	0	4425	158	0	8439	127	0	666	103	0	465
SST	S1	N2	20	0	1475	55	4	5738	44	0	252	103	0	3607
SST	S1	N3	21	0	4657	101	3	7239	11	0	980	80	0	2424
SST	S1	N4	72	0	8595	98	3	5798	x	x	x	62	0	628
SST	S1	N5	x	x	x	x	x	x	x	x	x	20	0	311
SST	V1	N1	26	0	2999	9	0	474	89	2	1255	x	x	x
SST	V1	N2	27	0	2609	52	0	3274	185	0	1850	x	x	x
SST	V1	N3	16	0	964	56	0	3124	58	8	1392	x	x	x

Adult = Ad, Development= Dev, out = cell outside of the main area found in neighboring area, KO = Fmr1 KOs, IONS = animals after infraorbital nerve section, Enuc = animals after enucleation. Total retro = total number of retrogradely labeled cells.

Table S2: All intrinsic physiological properties recorded from PV and SST cINs in ALM, S1 and V1 during development (P10) and in adults (P40). Related to Figure 4

	Amplitude (mV)	Threshold (mV)	Rise Time (ms)	Decay Time (ms)	Half width (ms)	fAHP (mV)	mAHP (mV)	IR (MOhms)	SFA	Rheobase (pA)	Max firing frequency
ALM PV P10	71.70 ±4.08	-33.51 ±1.53	0.64 ±0.03	2.84 ±0.11	1.80 ±0.06	-17.64 ±1.35	-18.97 ±1.59	479.09 ±55.86	0.71 ±0.05	63.64 ±10.51	42.36 ±1.98
S1 PV P10	72.22 ±3.20	-34.02 ±1.45	0.59 ±0.03	3.05 ±0.36	2.10 ±0.19	-12.26 ±1.94	-14.51 ±2.09	446.75 ±58.38	0.49 ±0.05	67.08 ±14.58	33.17 ±2.58
V1 PV P10	72.90 ±4.38	-36.16 ±1.01	0.75 ±0.05	3.45 ±0.21	2.29 ±0.10	-11.92 ±1.43	-14.68 ±1.77	622 ±70.08	0.63 ±0.05	41.54 ±4.98	32.46 ±1.90
ALM SST P10	87.52 ±2.63	-39.63 ±1.12	0.70 ±0.03	3.06 ±0.29	2.17 ±0.13	-10.46 ±1.10	-12.85 ±1.23	563.36± 52.37	0.62 ±0.04	32.73 ±3.12	32.91 ±1.36
S1 SST P10	82.57 ±2.52	-41.96 ±1.55	0.71 ±0.03	2.82 ±0.14	2.20 ±0.09	-4,60 ±1.7	-5.34 ±1.60	548.07 ±57.31	0.41 ±0.05	28.93 ±3.82	31.14 ±1.35
V1 SST P10	80.59 ±2.04	-38.03 ±1.42	0.74 ±0.03	3.38 ±0.16	2.45 ±0.09	-8.51 ±1.56	-11.06 ±2.20	734.23 ±91.84	0.55 ±0.05	28.85 ±3.72	33.38 ±3.65
ALM PV P40	77.89 ±1.99	-39.67 ±1.35	0.41 ±0.03	0.99 ±0.09	0.87 ±0.08	-19.74 ±2.24	-13.29 ±1.42	379.57 ±51.77	0.73 ±0.09	78.57 ±10.10	91.67 ±10.02
S1 PV P40	78.74 ±2.97	-40.30 ±2.12	0.36 ±0.03	0.85 ±0.07	0.74 ±0.06	-15.12 ±2.11	-9.67 ±1.84	225.60 ±30.91	0.78 ±0.07	90 ±14.53	120 ±8.25
V1 PV P40	73.55 ±2.98	-41.42 ±1.37	0.38 ±0.02	1.01 ±0.09	0.84 ±0.07	-15.70 ±1.73	-11.48 ±1.30	227.33 ±16.90	0.76 ±0.07	128.85 ±11.96	86.33 ±10.27
ALM SST P40	86.72 ±2.70	-44.08 ±2.67	0.51 ±0.03	1.26 ±0.06	1.12 ±0.05	-10.84 ±2.43	-7.29 ±2.02	475.14 ±95.49	0.37 ±0.05	46.88 ±5.66	61 ±6.79
S1 SST P40	78.21 ±1.95	-42.79 ±1.85	0.46 ±0.03	1.24 ±0.08	1.08 ±0.07	-10.34 ±2.27	-8.36 ±1.61	308.50 ±43.63	0.33 ±0.03	75 ±11.18	80 ±8.07
V1 SST P40	81.22 ±2.39	-41.18 ±1.17	0.48 ±0.02	1.43 ±0.07	1.23 ±0.06	-10 ±1.15	-7.56 ±1.21	471.08 ±41.01	0.43 ±0.04	48.33 ±5.16	53.14 ±7.46

Data represented as mean ± sem