# Additional Information For

# Cortical somatostatin interneuron subtypes form cell type-specific circuits

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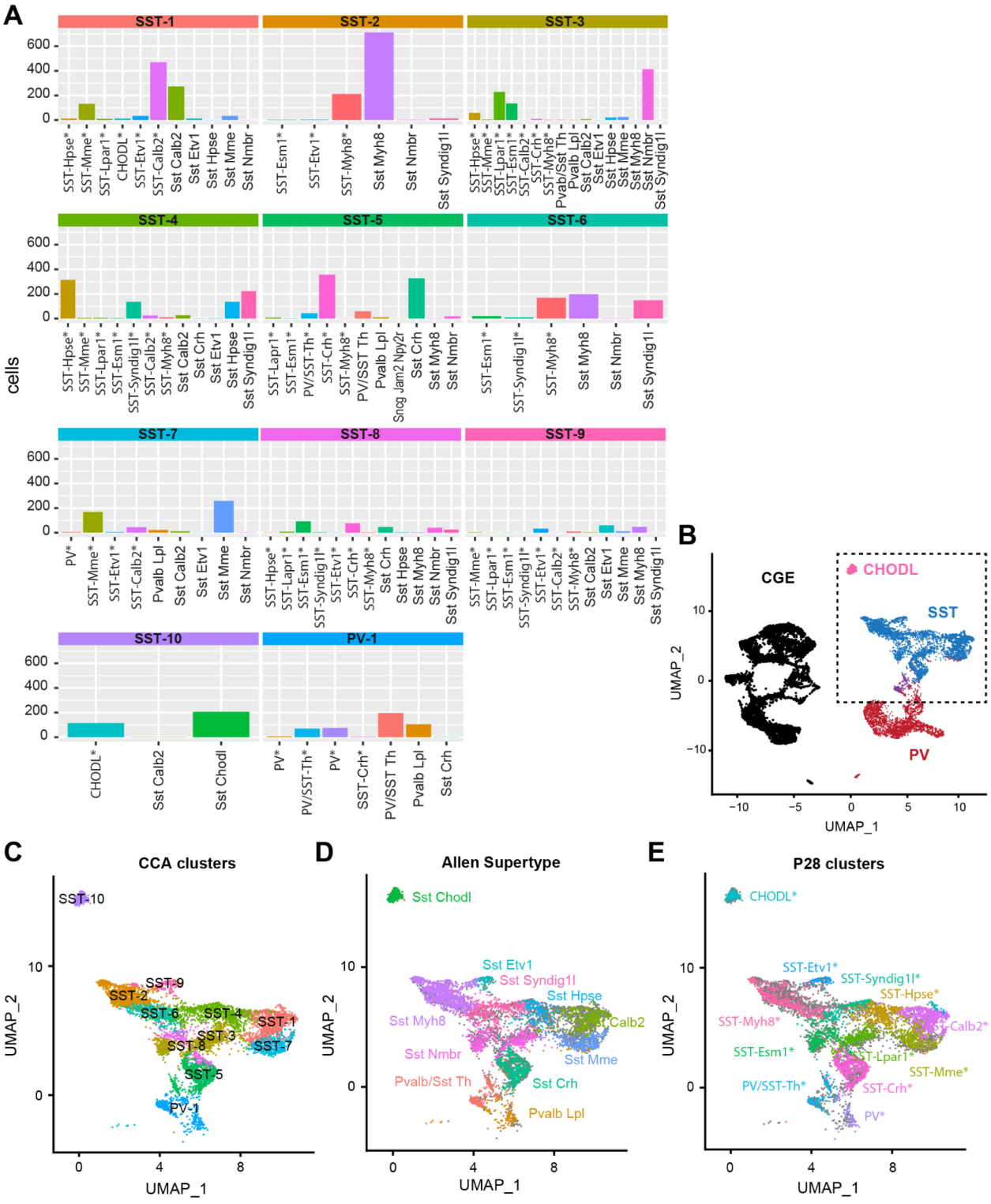


**Figure A1. Label transfer from supertypes defined in Yao *et al.*, 2021 to P28 cortical SST interneuron clusters.**

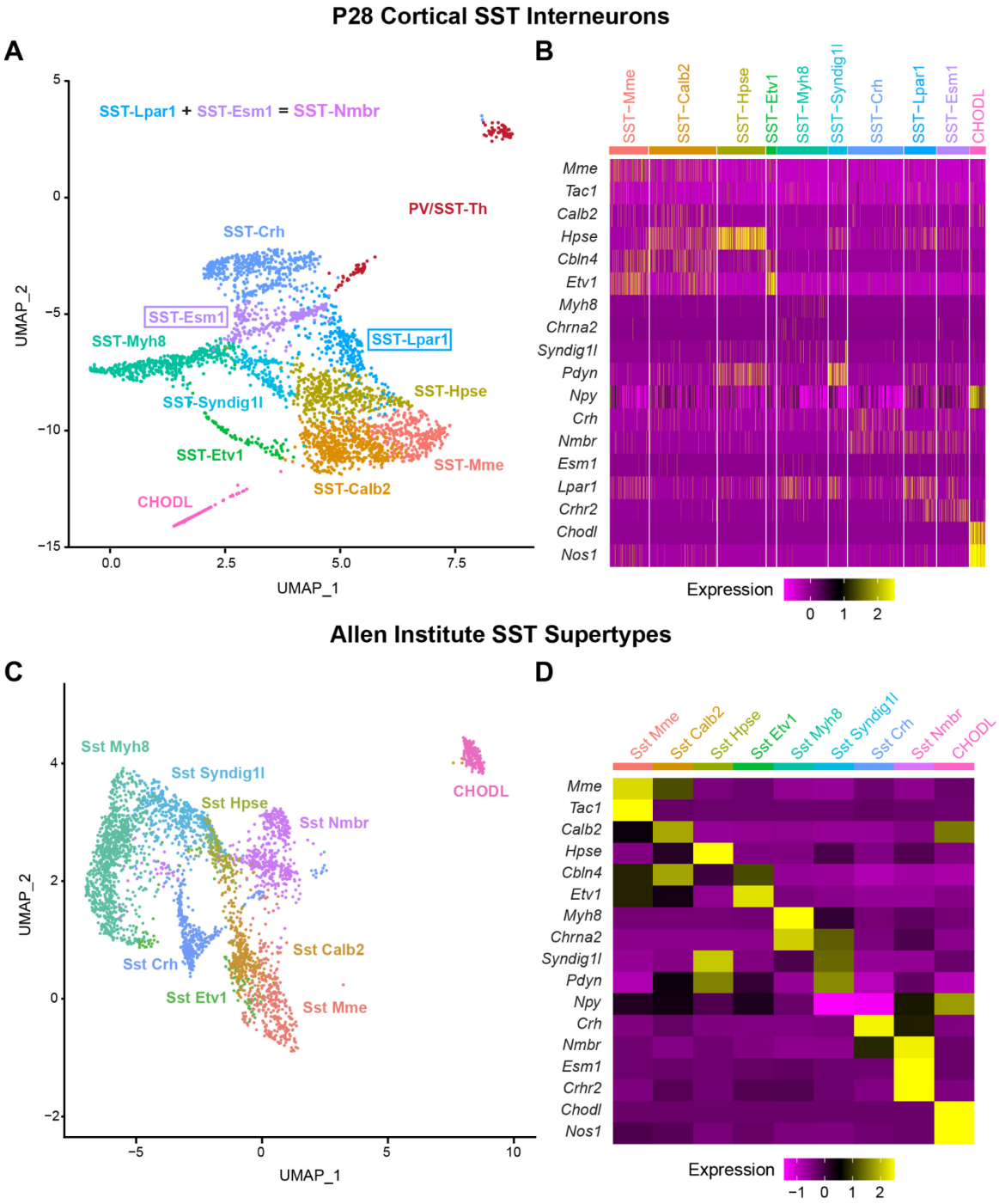


**Figure A2. Marker gene expression of different SST subtypes in two datasets.**

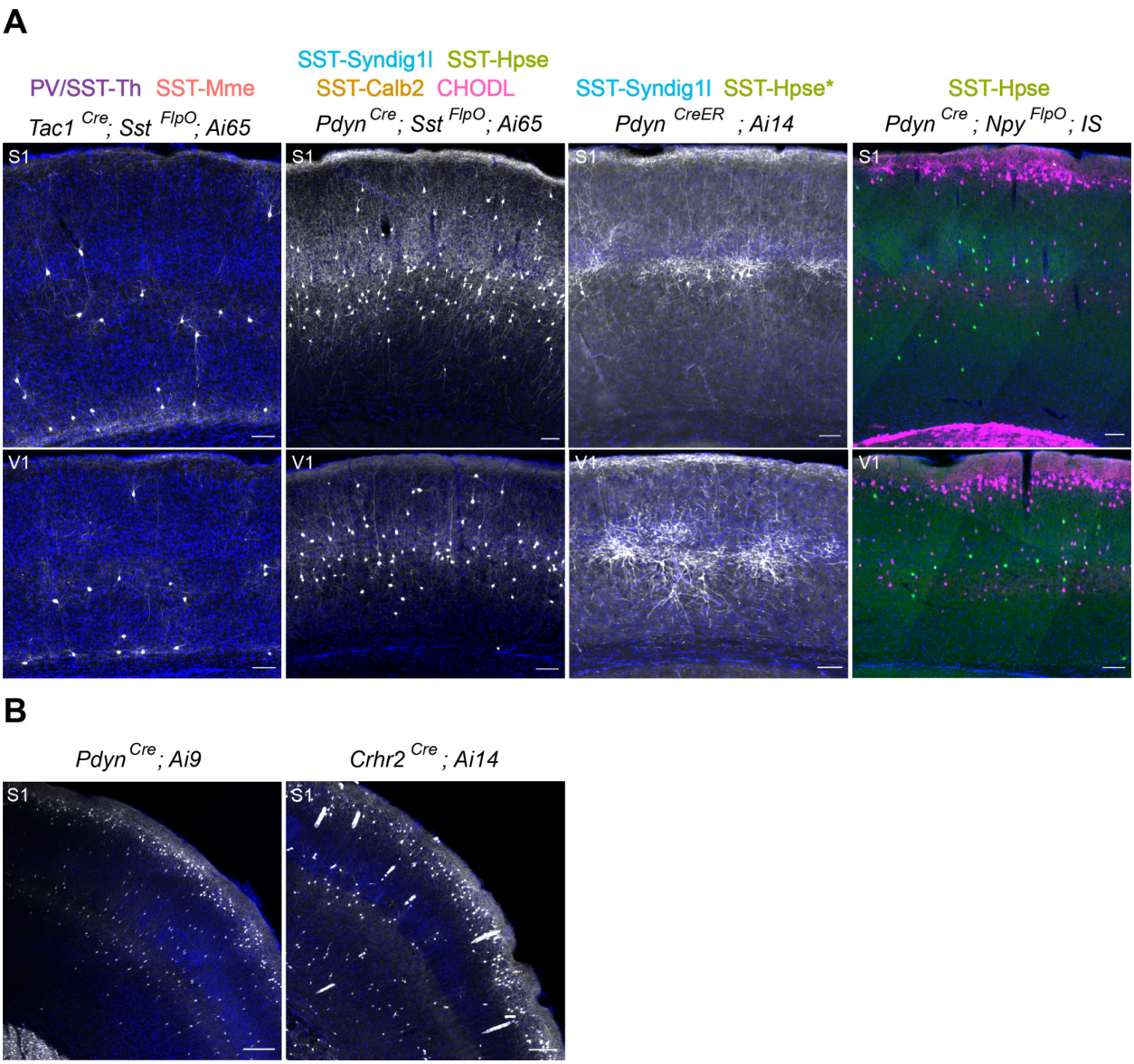


**Figure A3. Labeling patterns of additional genetic strategies in S1 and V1.**

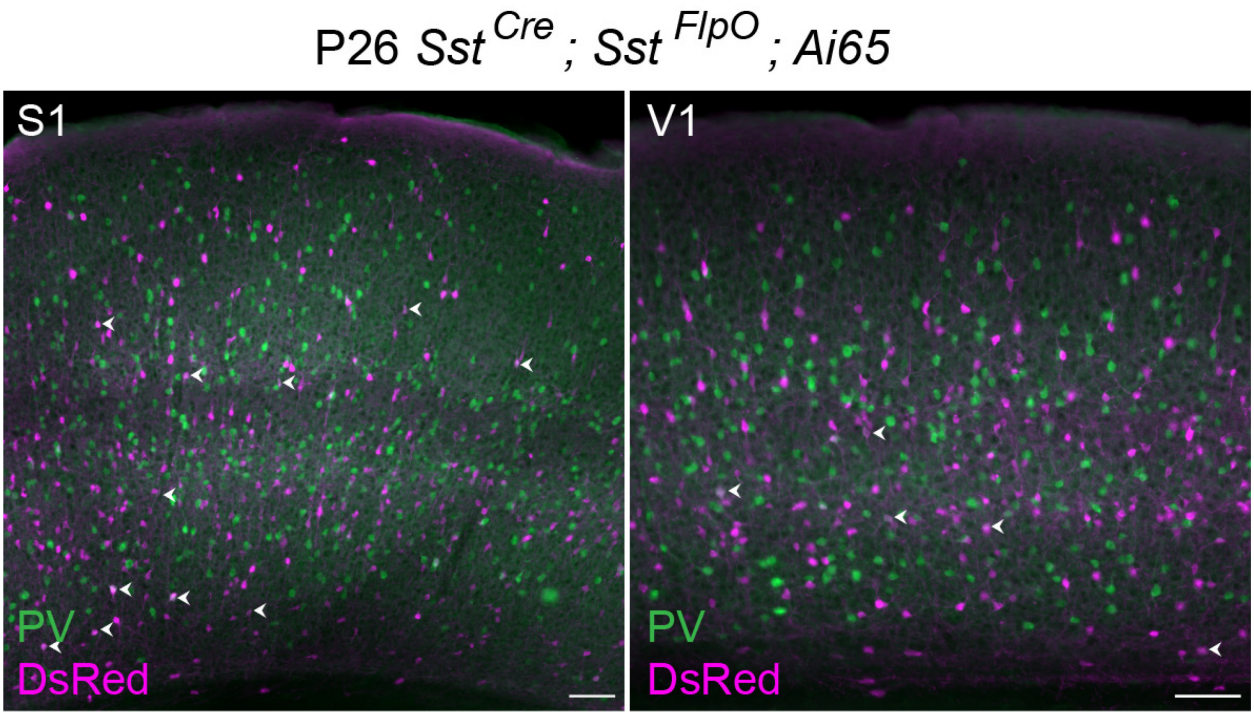


**Figure A4. Intersectional genetic strategy Sst-Cre; Sst-FlpO; Ai65 does not label many PV+ interneurons.**

3.25% of genetically labeled SST neurons were positive for PV in S1 (29/892 neurons), and 1.95% of genetically labeled SST neurons were positive for PV in V1 (17/873 neurons).

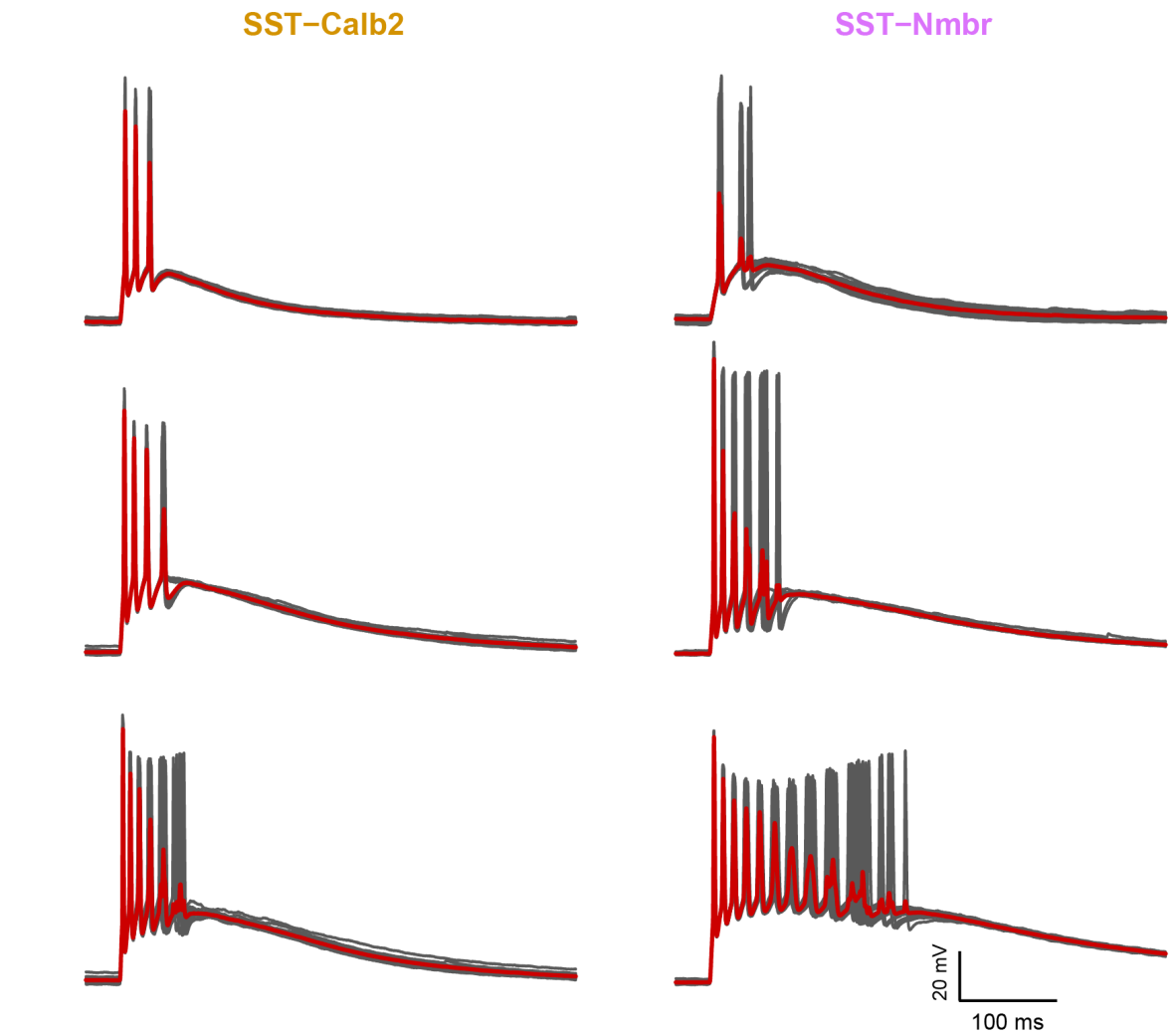


**Figure A5. Optogenetic stimulation induces a variable number of spikes in SST interneurons.**

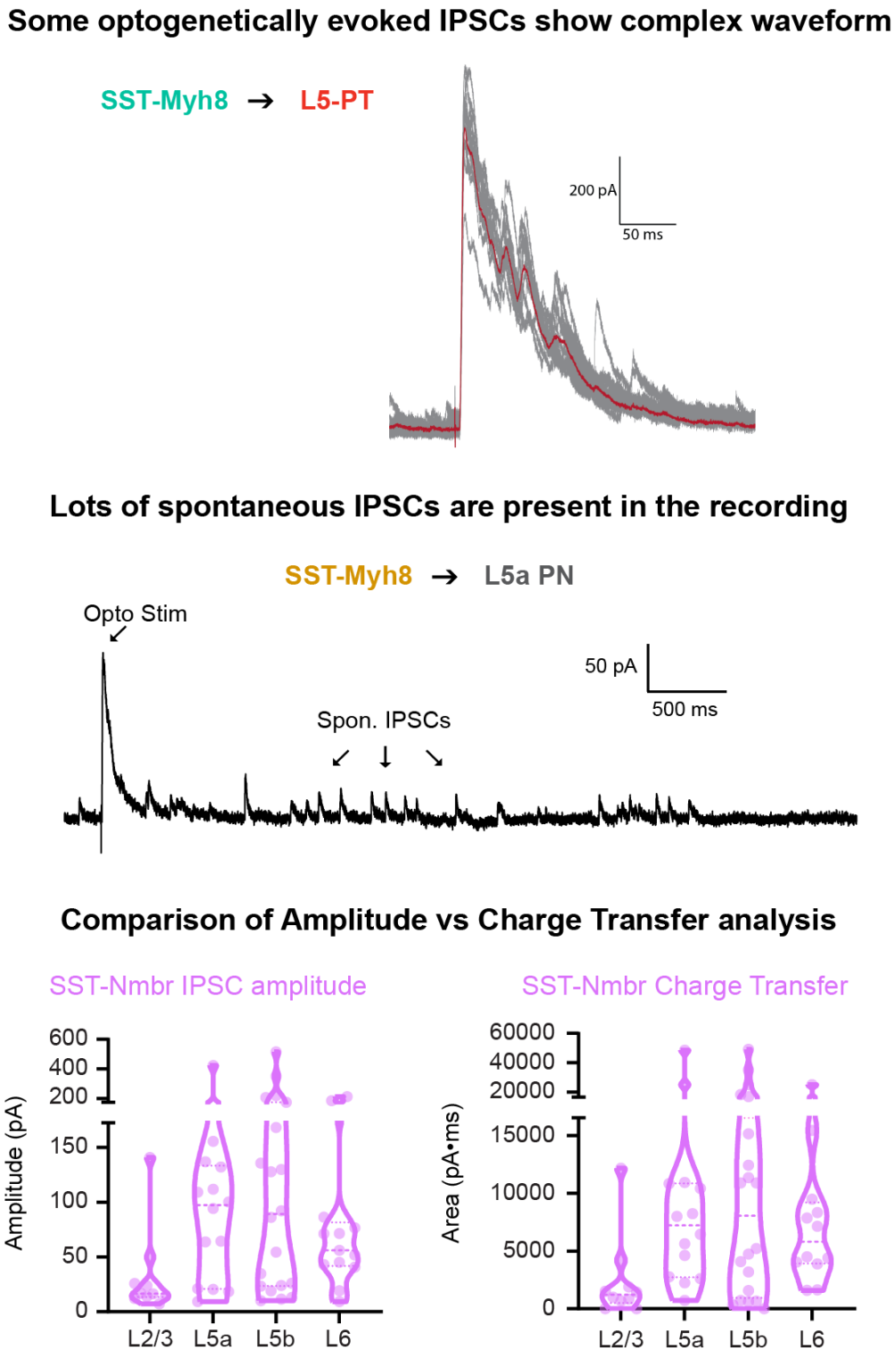


**Figure A6. Some optogenetically evoked IPSCs show complex waveforms, making charge transfer analysis more variable than amplitude.**

## Example of membrane potential change in respond to optogenetic stimulation of SST-Calb2

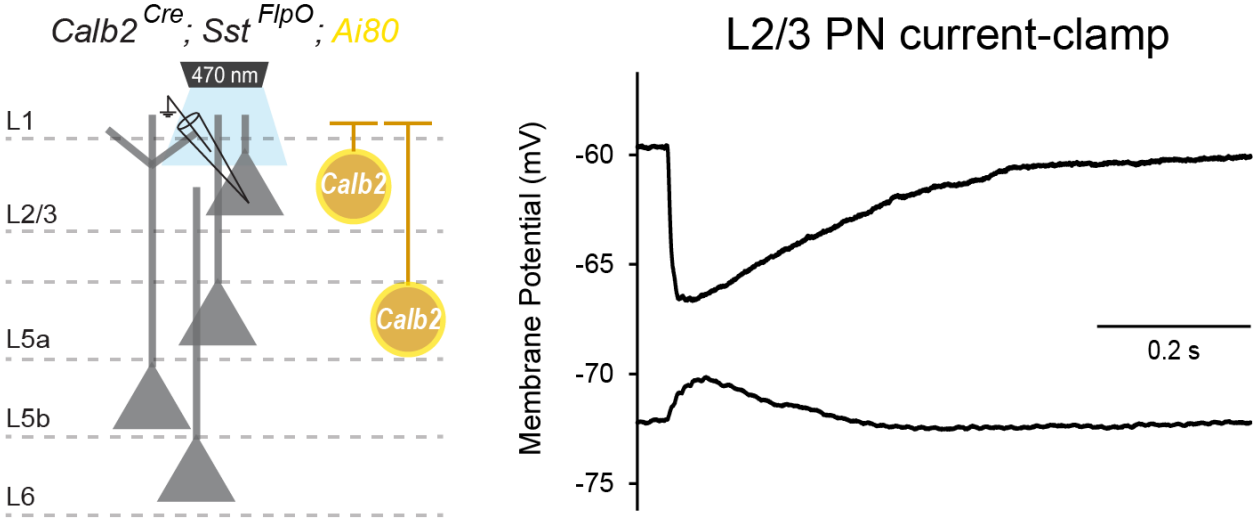


**Figure A7. Optogenetic stimulation of SST-Calb2 interneurons drives the membrane potential of L2/3 pyramidal neuron towards chloride equilibrium potential.**

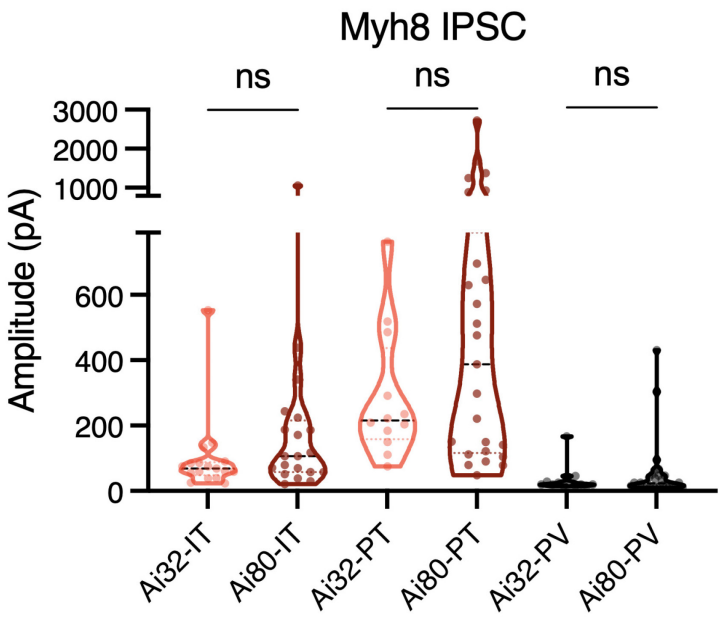


**Figure A8. Two different channelrhodopsin reporter lines showed comparable results.**

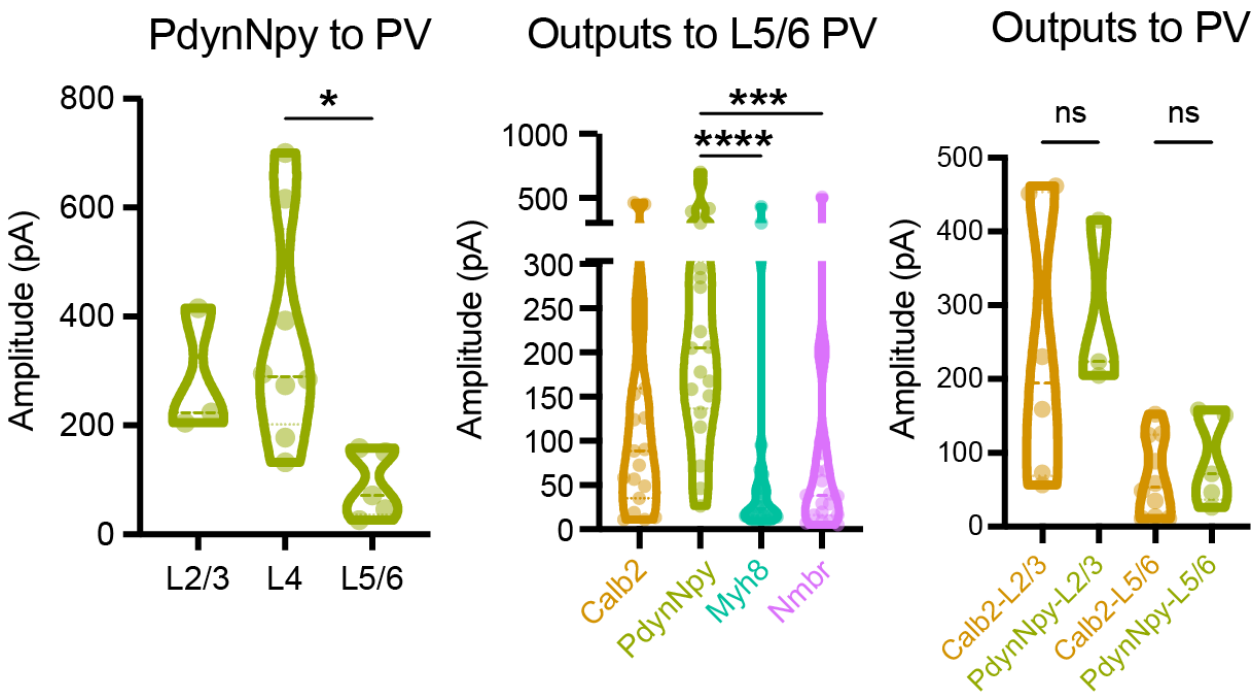


**Figure A9. Pdyn;Npy intersectional strategy labeled SST interneurons innervate L4 PV interneurons.**

A. Violin plot of evoked IPSC upon stimulation of cells labeled with Pdyn-Cre;Npy-FlpO. IPSC in L4 PV interneurons was significantly greater than L5/6 PV interneurons ($p = .01$) by Kruskall-Wallis test with Dunn's correction.

B. Evoked IPSCs from all four SST types. SST-PdynNpy responses were significantly greater than SST-Chrna2 ($p < .0001$) and SST-Crhr2 ($p = .0004$).

C. In L2/3 and L5/6, evoked IPSC from SST-Calb2 and SST-PdynNpy were not significantly different ($p > .999$).

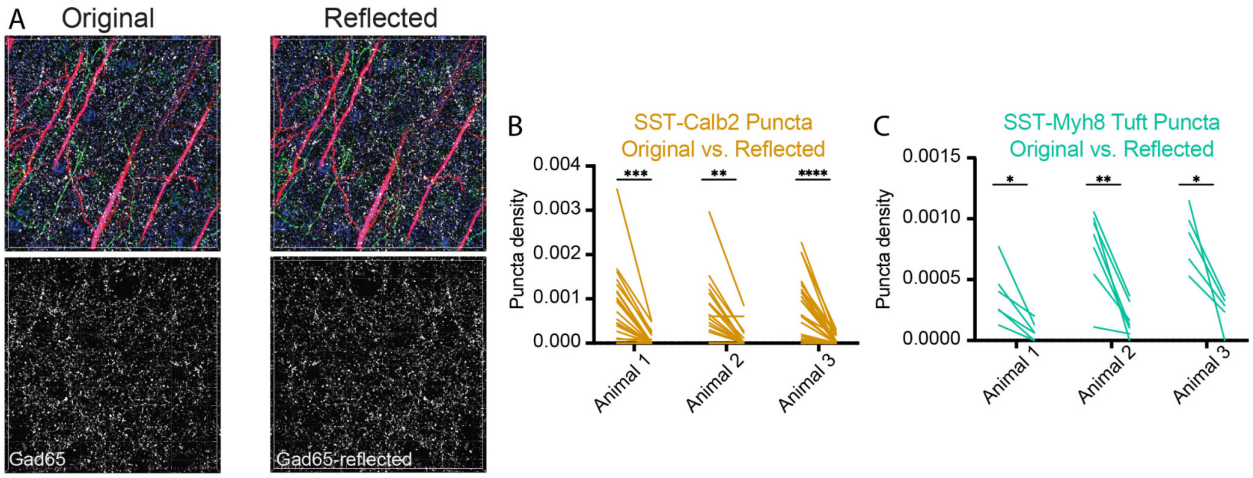


**Figure A10. Synaptic puncta are detectable above noise levels.**

A. Example images before (left) and after (right) reflecting the Gad65 channel.

B. Puncta density from Calb2 interneurons across all dendritic compartments before and after reflecting the Gad65 channel. Columns represent individual animals. Puncta density was significantly higher in the original images by multiple paired t-tests with Bonferroni-Dunn correction. CR-PT-1 $p = .0001$, CR-PT-3 $p = .0005$, CR-PT-6 $p < .0001$.

C. Same as in B but for Chrna2 puncta on tuft dendrites only. A-PT-7 $p = .035$, A-PT-8 $p = .006$, A-PT-9 $p = .047$.

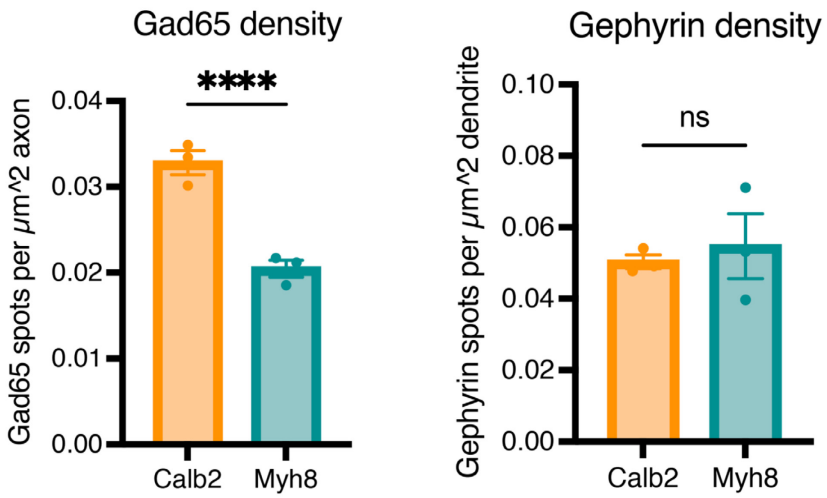


**Figure A11. The density of Gad65 and Gephyrin immunolabeling for SST-Calb2 and SST-Myh8 on L5-PT neurons.**

## Response to other questions we received through emails

*"… have you tried other probes with RNAscope besides the 6 mentioned in the supplement, especially any that may correlate with the SST-Myh8 subclass?"*
*"… (Are there additional) post verification that the Chrna-Cre mouse labels specifically and exclusively SST-Myh8 neurons"*

We note that various markers for SST-Myh8 subtypes decrease their expression 2-3 weeks postnatally (Figure A12 A). While all our experiments assessing the coverage and specificity of genetic strategies were performed on young adult mice. Therefore, markers we have attempted that are specific for SST-Myh8 subtype showed very sparse labeling patterns and are not suited for quantification (Figure A12 C).

A summary of evidence that *Chrna2-Cre* selectively label SST-Myh8 population.

- *Chrna2* is a specific marker for SST-Myh8 cluster (Figure 1B).
- *Chrna2-Cre* expression is directed by *Chrna2* gene promoter region through BAC insertion.
- *Chrna2-Cre* labeled interneurons all express *Sst* mRNA by in situ hybridization experiments.
- *Chrna2-Cre* labeled SST interneurons do not overlap with various markers for other SST subtypes (Figure A12 B)
- *Chrna2-Cre* labeled SST are positive for markers expressed in SST-Myh8, such as *Pld5*, *Plpp4* (*Ppapdc1a*), and *Nr2f2* (also labels some SST-Etv1 and SST-Nmbr interneurons). Please note that *Nr2f2* and *Pld5* are also present in CGE interneurons. (Figure A12 C)
- *Chrna2-Cre* labeled SST interneurons reside in L5b which is consistent with the laminar location predicted by Slide-Seq V2 experiments.
- The proportion of Chrna2-Cre labeled SST interneurons out of total SST interneurons matches with what is expected from snRNA-seq and Slide-seq V2 experiments.
- The *Chrna2-Cre OE25Gsat/Mmucd* line labels 90% of SST-Myh8 population, despite some off-targeting of VIP interneurons (Yao *et al.*, 2021)

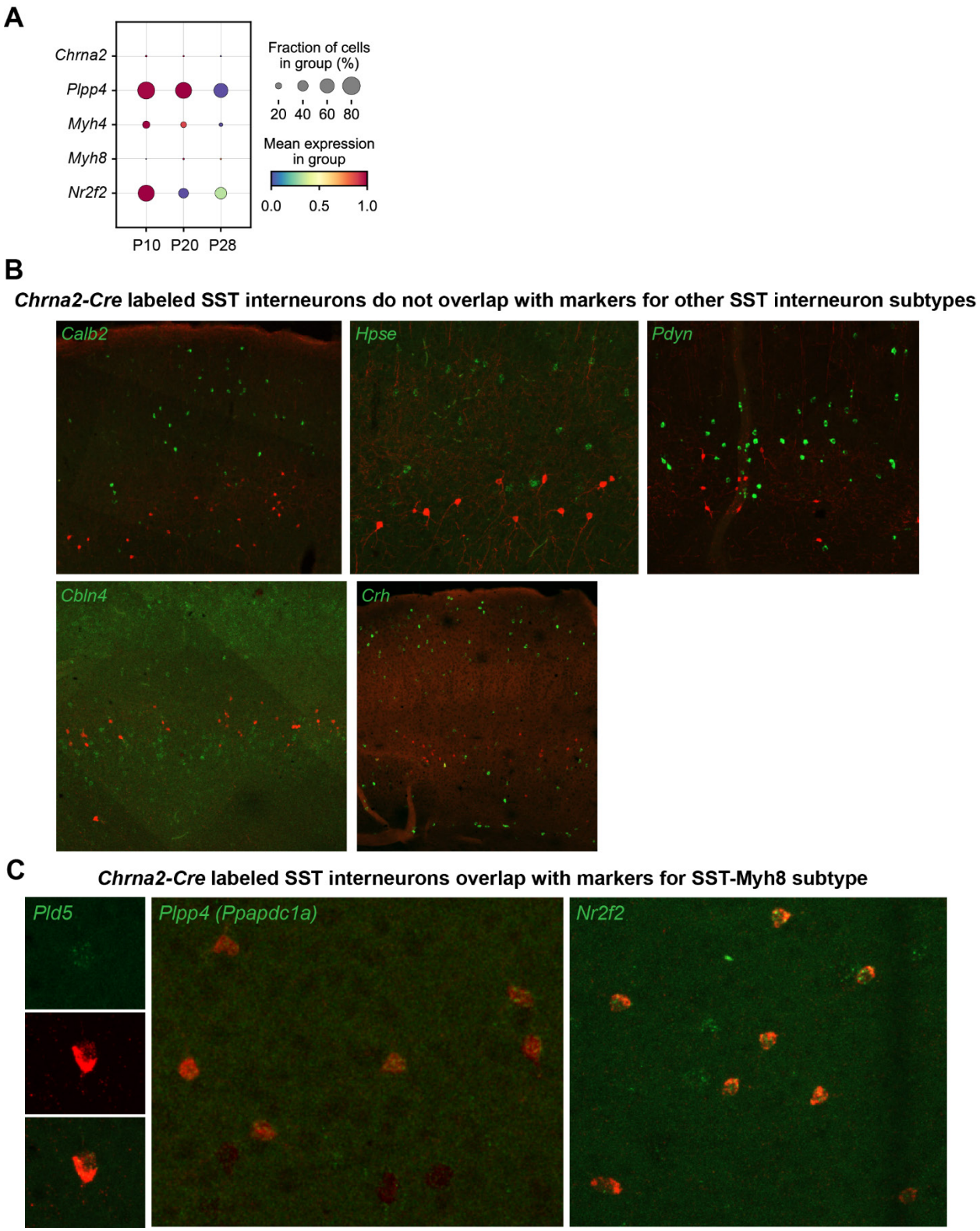


**Figure A12. Additional results supporting the specific targeting of SST-Myh8 interneurons using *Chrna2-Cre* mouse line.**

A. Scaled expression of marker genes for SST-Myh8 subtype across different ages based on snRNA-seq experiments (data to be published).
B, C. Representative images of in situ hybridization experiments against various SST interneuron subtype markers on *Chrna2-Cre; Ai14* mouse brain slices.