Inhibition of Inhibition in Visual Cortex: The Logic of Connections Between Molecularly Distinct Interneurons

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Supplementary figures and legends 1-9, supplementary table 1

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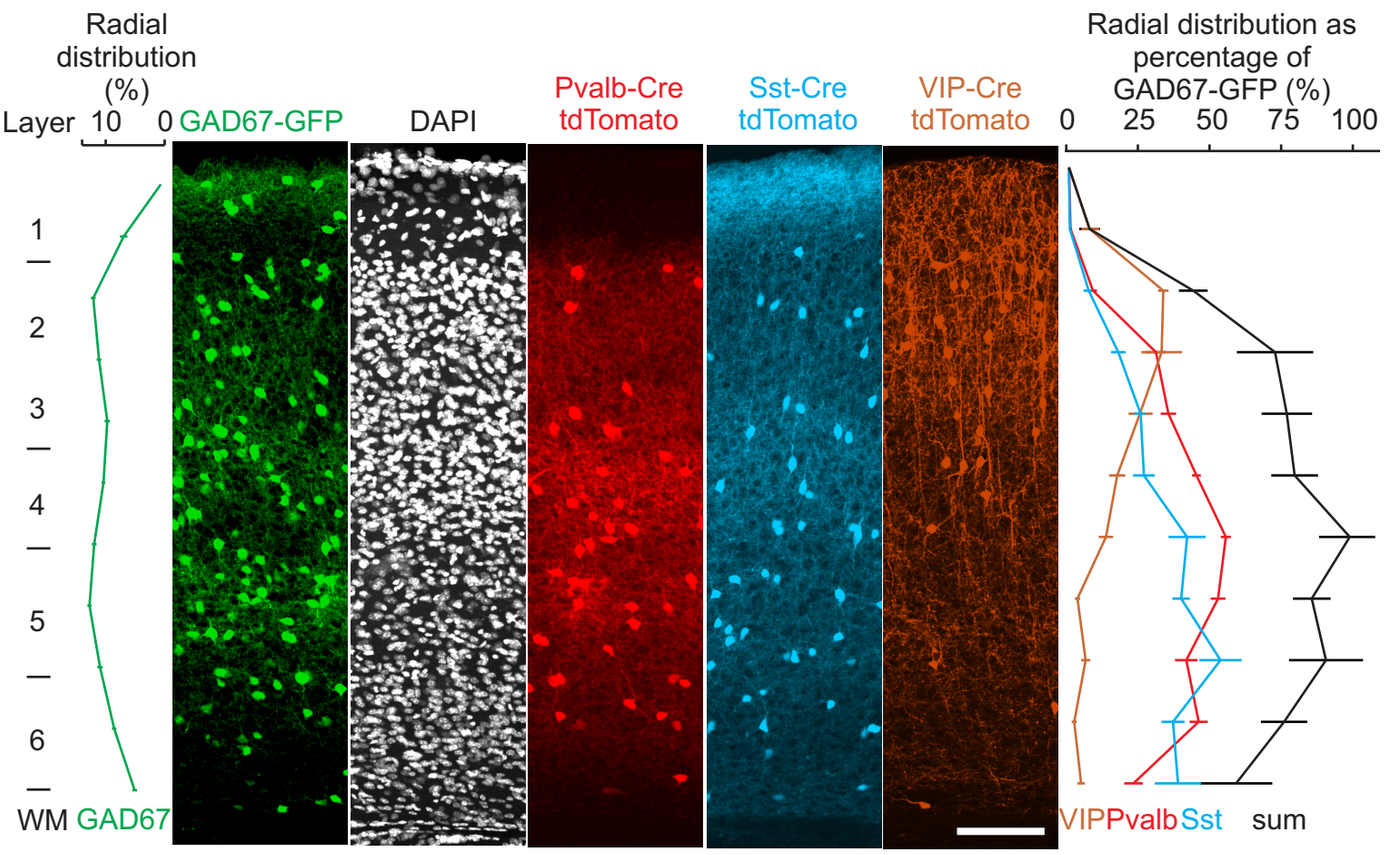
Supplementary Figure 1

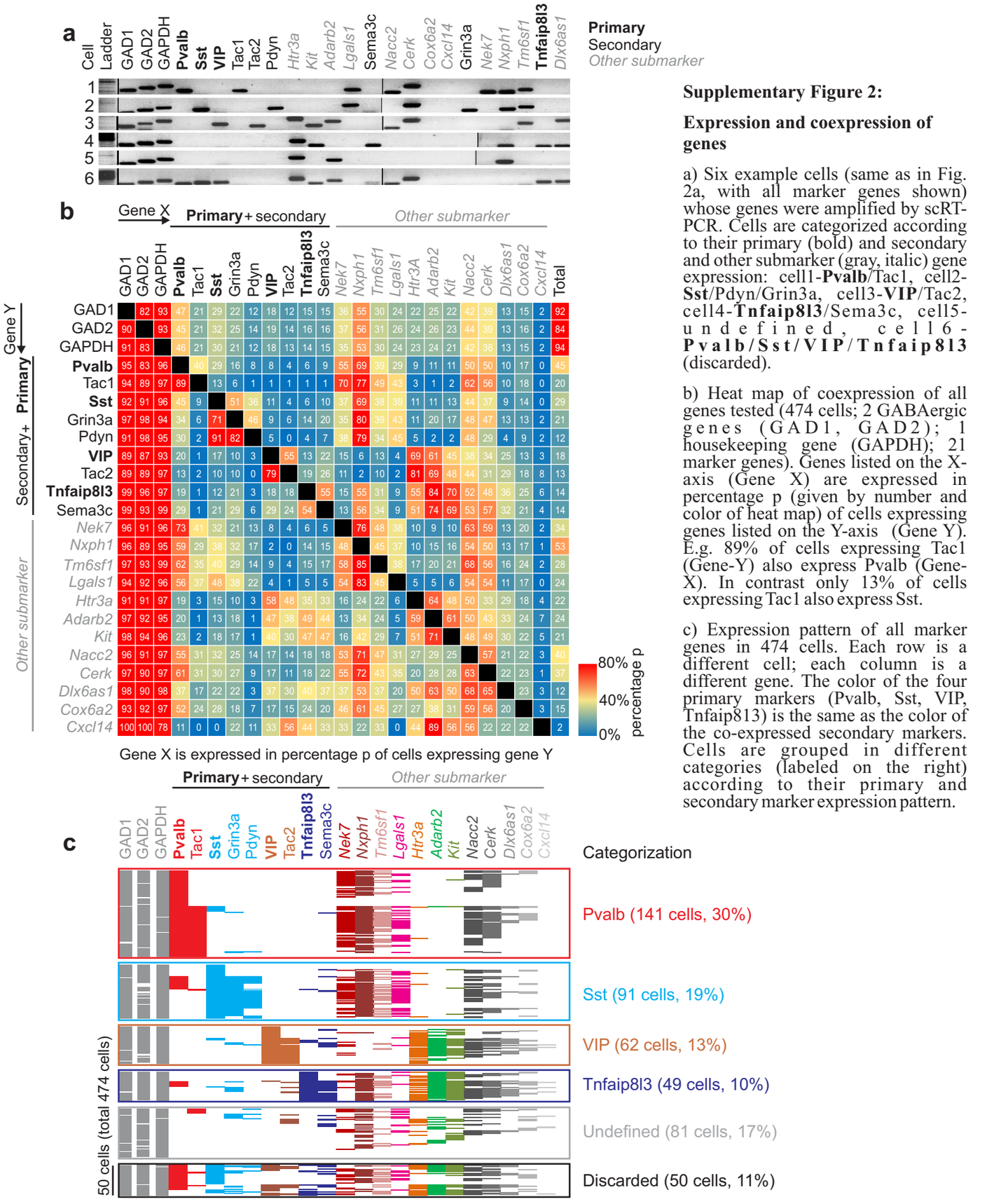


Supplementary Figure 1

Distribution of interneurons in visual cortex

Confocal fluorescence images of coronal sections through V1 of triple transgenic mice in which the three Cre-driver lines were crossed with the ROSA-tdTomato line to report Cre expression and to the GAD67-GFP line to reveal all interneurons (GAD67-GFP: colored green; Pvalb-Cre: colored red; Sst-Cre: colored blue; VIP-Cre: colored brown). The nuclear DAPI staining (white; same section as Pvalb-Cre) is shown for reference. Layer borders are indicated on the left. The radial distribution of GAD67-GFP neurons is shown on the left. The radial distribution of Cre expressing cells as a percentage of GAD67-GFP cells in the corresponding radial bin is shown on the right. For quantification, the radial extent of the cortex (from the pia to the white matter) was divided into 10 bins of equal length. Note that all three interneuron classes sum up to more than 75% in layers 3 - 5 (black distribution on the right). Error bars represent s.e.m (Reporter line (n=cells/sections/mice), GAD67-GFP (5310/14/6), Pvalb (690/4/2), Sst (929/6/2), VIP (286/4/2). Cre expressing cells with no apparent GAD67-GFP label were excluded). White scale bar in the VIP-Cre tdTomato panel: 100µm.

Supplementary Figure 2



Supplementary Figure 2:

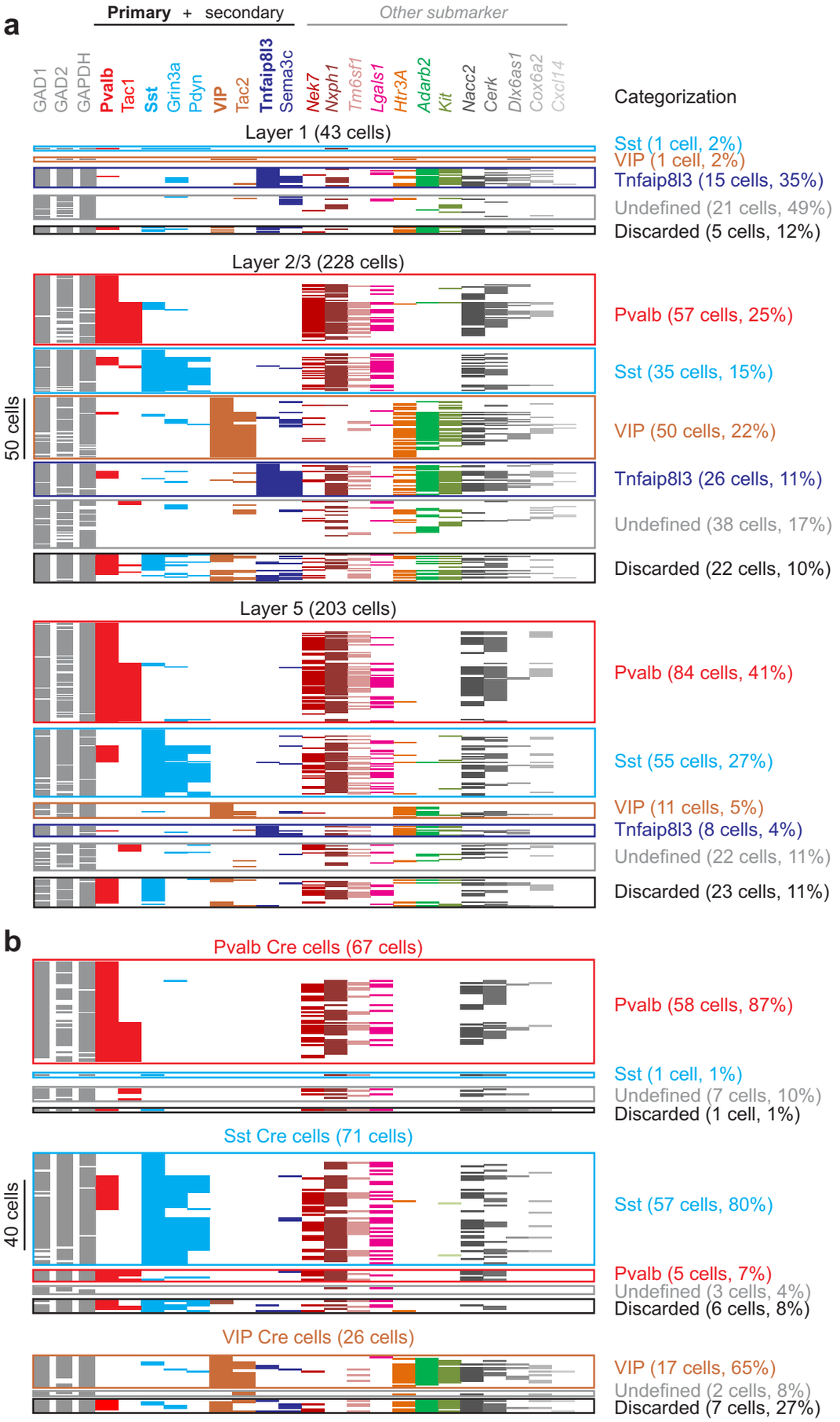
Expression and coexpression of genes

a) Six example cells (same as in Fig. 2a, with all marker genes shown) whose genes were amplified by scRT-PCR. Cells are categorized according to their primary (bold) and secondary and other submarker (gray, italic) gene expression: cell1-**Pvalb**/Tac1, cell2-**Sst**/Pdyn/Grin3a, cell3-**VIP**/Tac2, cell4-**Tnfaip8l3**/Sema3c, cell5-undefined, cell6-**Pvalb/Sst/VIP/Tnfaip8l3** (discarded).

b) Heat map of coexpression of all genes tested (474 cells; 2 GABAergic genes (GAD1, GAD2); 1 housekeeping gene (GAPDH); 21 marker genes). Genes listed on the X-axis (Gene X) are expressed in percentage p (given by number and color of heat map) of cells expressing genes listed on the Y-axis (Gene Y). E.g. 89% of cells expressing Tac1 (Gene-Y) also express Pvalb (Gene-X). In contrast only 13% of cells expressing Tac1 also express Sst.

c) Expression pattern of all marker genes in 474 cells. Each row is a different cell; each column is a different gene. The color of the four primary markers (Pvalb, Sst, VIP, Tnfaip813) is the same as the color of the co-expressed secondary markers. Cells are grouped in different categories (labeled on the right) according to their primary and secondary marker expression pattern.

Supplementary Figure 3



Supplementary Figure 3:

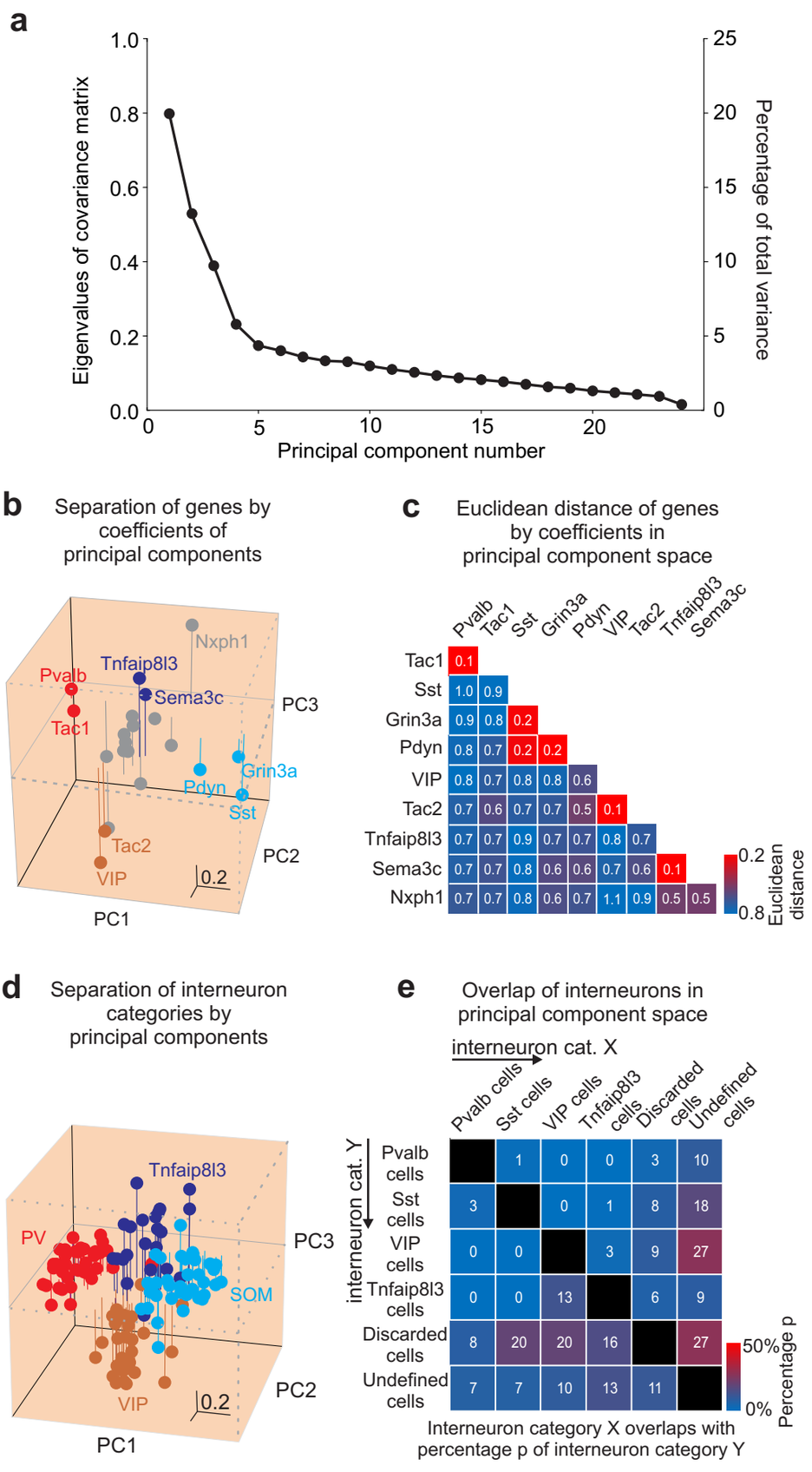
Interneuron categories and their gene expression patterns by layers and Cre-lines.

a) Expression pattern of all marker genes in 474 cells. Each row is a different cell; each column is a different gene. The color of the four primary markers (Pvalb, Sst, VIP, Tnfaip813) is the same as the color of the co-expressed secondary markers. Cells are sorted according to layer and grouped in different categories (labeled on the right) according to their primary and secondary marker expression pattern. The relative abundance of sampled cells across layers does not necessarily represent their natural distribution because of sampling biases occurring when using specific lines for targeting interneurons (e.g. Gin, HTR3a-GFP, Pvalb-Cre).

b) Expression patterns of all marker genes in Pvalb-Cre expressing cells (top), Sst-Cre expressing cells (middle) and VIP-Cre expressing cells (bottom). Each row is a different cell; each column is a different gene. The color of the four primary markers (Pvalb, Sst, VIP, Tnfaip813) is the same as the color of the co-expressed secondary markers. Cells are grouped in different categories (labeled on the right) according to their primary and secondary expression pattern.

Note that the categorization based on the gene expression pattern matches the cell class defined by the Cre-driver line for the vast majority of cells.

Supplementary Figure 4



Supplementary Figure 4

Principal Component Analysis, gene clustering and cell separation

a) Scree plot showing the eigenvalues of the covariance matrix for each principal component obtained from the analysis of the single cell RT-PCR gene expression of all 474 cells. The first 4 principal components account for more than 50% of the total variance.

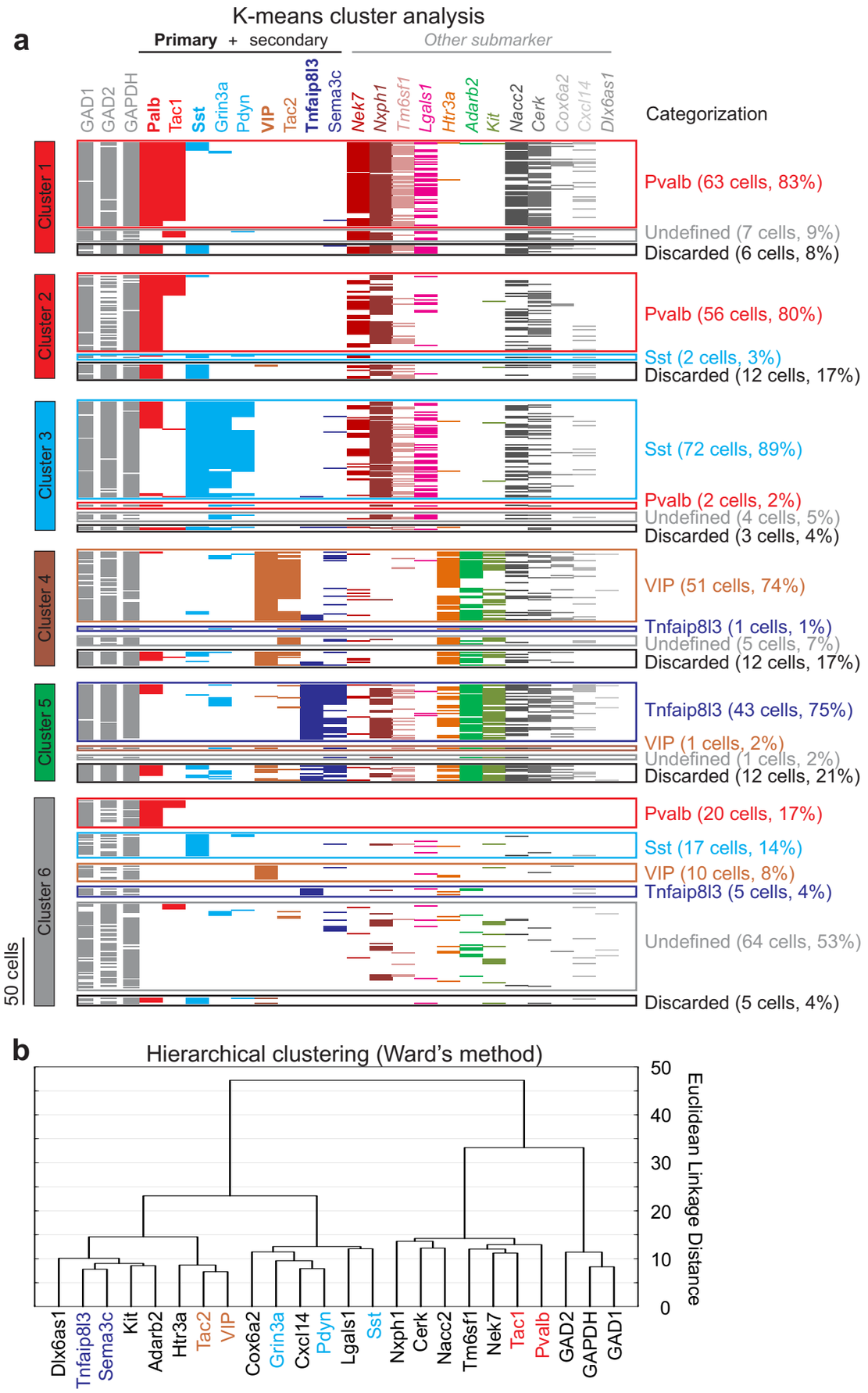
b) 3D representation of the coefficients of the first three principal components (PC1-3) for all genes. Genes which show the largest distance to the mean are color coded. Nearest neighbors have the same color (see methods for details on calculation). Note that nearest neighbors correspond to **primary**/secondary marker pairs or triplets as defined by expression analysis (Fig. 2): **Pvalb**/Tac1, **Sst**/Grin3a/Pdyn, **VIP**/Tac2 and **Tnfaip8l3**/Sema3c. Scale bars represent distance in Euclidean space.

c) Heat map of the Euclidean distance of the coefficients of principal components of selected genes for the principal components 1-4. Note the short distances (red squares) between primary and secondary markers.

d) 3D representation of the first three principal components (PC1-3) for individual interneurons. The individual cells were color coded for the gene clusters (Pvalb-red, Sst-blue, VIP-brown, Tnfaip8l3-green) as determined in (b) (see methods). The data set was reduced randomly by a factor of 2 for clarity. Cells defined as 'discarded' or 'undefined' were omitted. Note the clear separation of the four cell categories as defined by expression analysis (Fig. 2). Scale bars represent distance in Euclidean space.

e) Heat map of the overlap between cell categories in Euclidean space, as defined by the principal components PC1-4 (for details on quantification see methods). Percentage p (given by number and color of heat map) of cells in interneuron categories listed on the X-axis (interneuron cat. X) overlaps with the interneuron category listed on the Y-axis (interneuron cat. Y). E.g. 3% of PV cells overlap with the SOM cell category, whereas 1% of SOM cells overlap with the PV cell category. Note little overlap between cells of defined categories. Undefined cells show the highest overlap with other categories.

Supplementary Figure 5



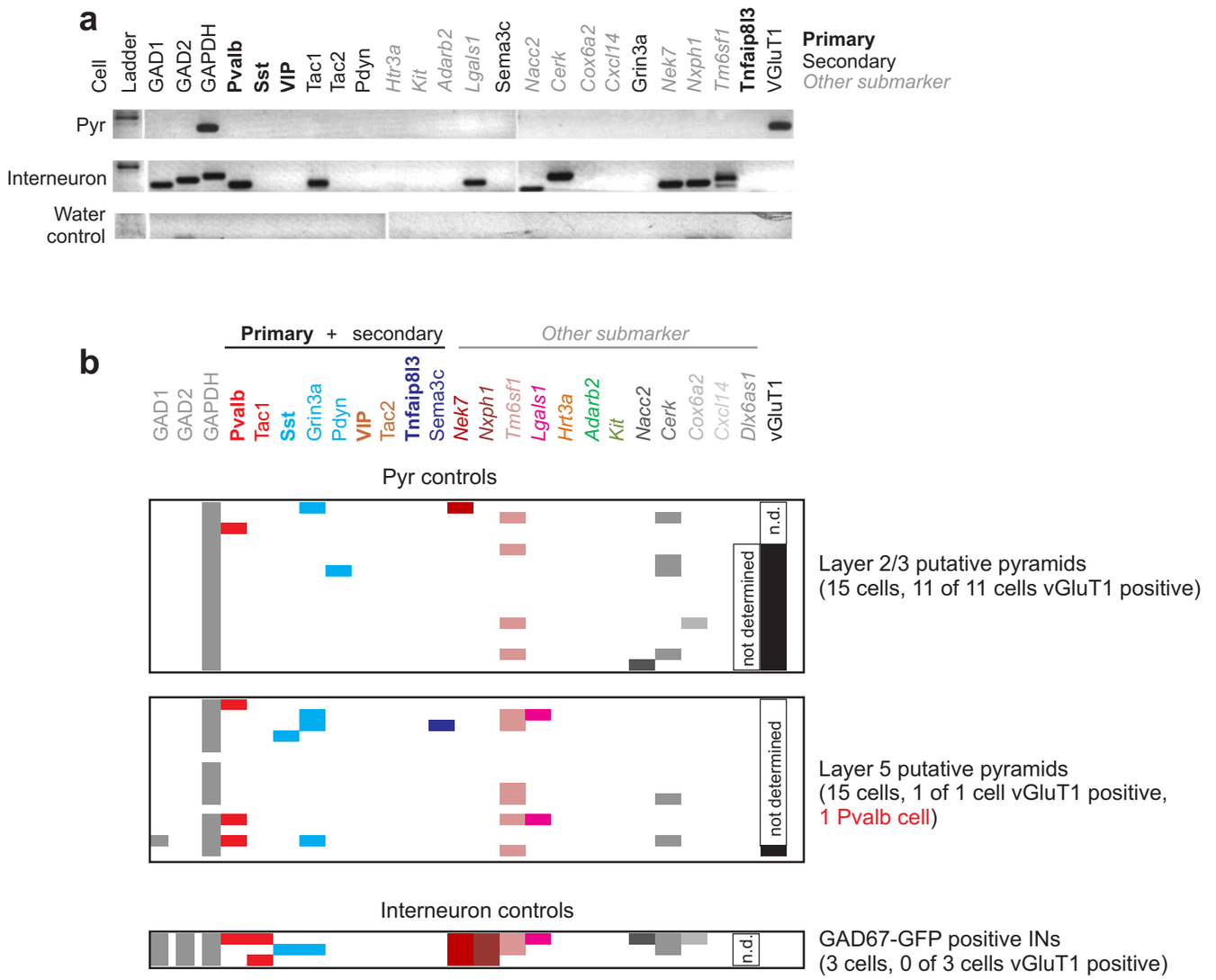
Supplementary Figure 5

K-means clustering and Ward's hierarchical clustering

a) Supervised cluster analysis by the kmeans centroid based algorithm. The initial number of clusters was set as 6. The individual cells are sorted according to each kmeans cluster. Each row is a different cell; each column is a different gene. The color of the four primary markers (Pvalb, Sst, VIP, Tnfaip813) is the same as the color of the co-expressed secondary markers. Cells are grouped in different categories (labeled on the right) according to their primary and secondary expression pattern. Note that the vast majority of cells in each cluster 1-5 can be grouped into one of our four molecularly defined interneuron categories defined by expression analysis (Fig. 2) or principal component analysis (Suppl. Fig. 4). Furthermore, the majority of undefined cells are grouped into cluster 6.

b) Hierarchical tree as defined by Ward's method based on linkage distance between genes. Note that the color coded genes form clusters that match well the cell categories defined by expression analysis (Fig. 2), principal component analysis (Suppl. Fig. 4), or k-means clustering (Suppl. Fig 5a).

Supplementary Figure 6



Supplementary Figure 6

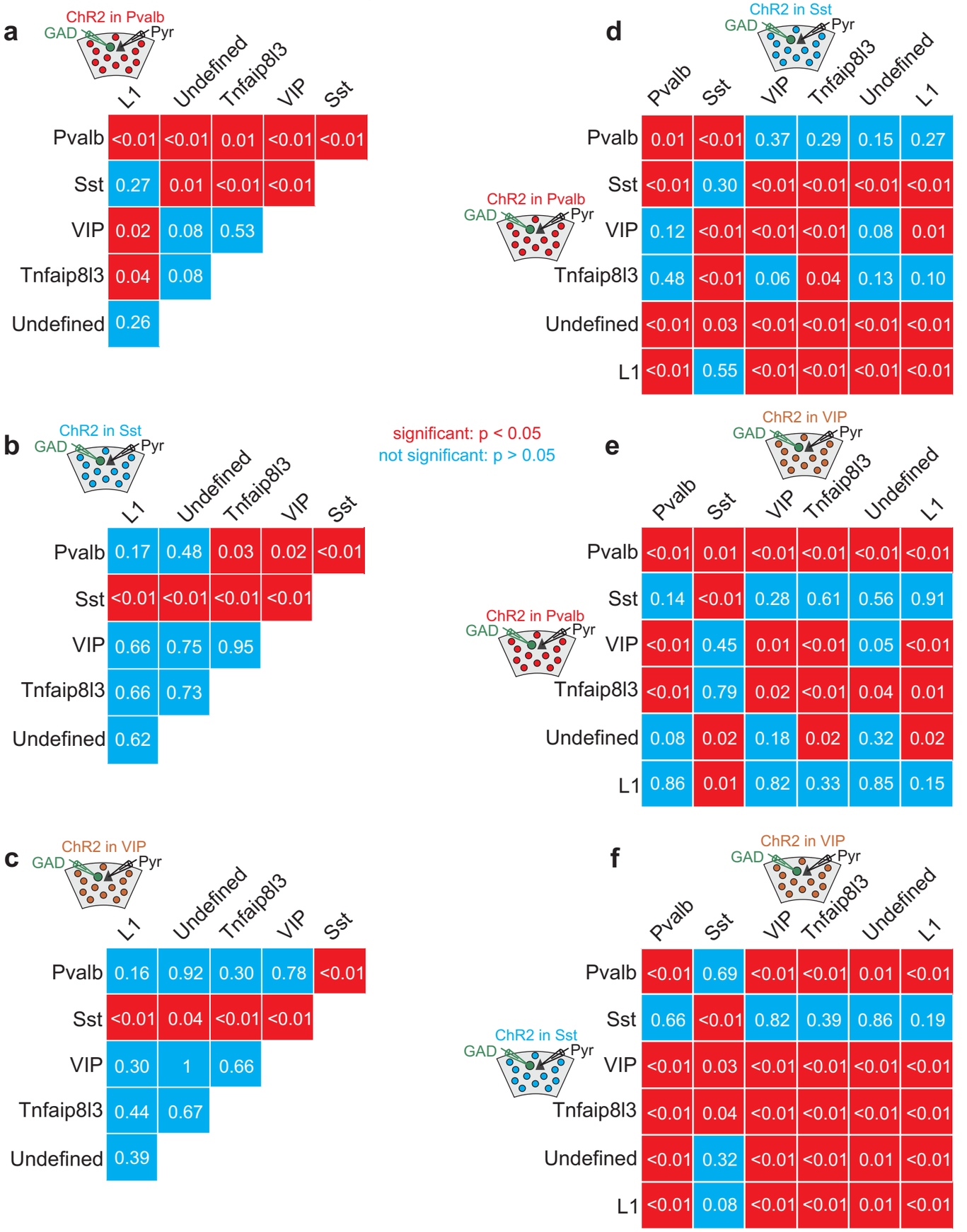
Gene expression controls in single cell RT-PCR

a) Agarose gels stained for DNA illustrating the single cell RT-PCR products from 24 genes (listed above) from a layer 5 pyramidal cell (Pyr), an interneuron and the water control used to determine PCR product contamination. The interneuron expresses GAD1, GAD2, and GAPDH but not VGluT1, whereas the pyramid expressed only GAPDH and VGluT1. The lower, partially cut, faint bands in the water control are the monomers or dimers of unused primer DNA.

b) Expression pattern of marker genes in pyramids of layers 2/3 and 5. Each row is a different cell; each column is a different gene. Primary and secondary marker genes can be found occasionally in pyramids. Note that only one cell out of 30 putative PCs can be classified as a Pvalb-interneuron (second last in layer 5 pyramid frame), indicating that the combination of markers defining interneurons is highly specific for interneurons. Note that 3 layer 5 and 1 layer 2/3 pyramids express Pvalb, which is consistent with published findings (see methods on interneuron overlap). n=30 cell, 10 slices, 3 mice.

Supplementary Figure 7

Pairwise statistical comparison of individual neuronal contribution



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Supplemetary Figure 7

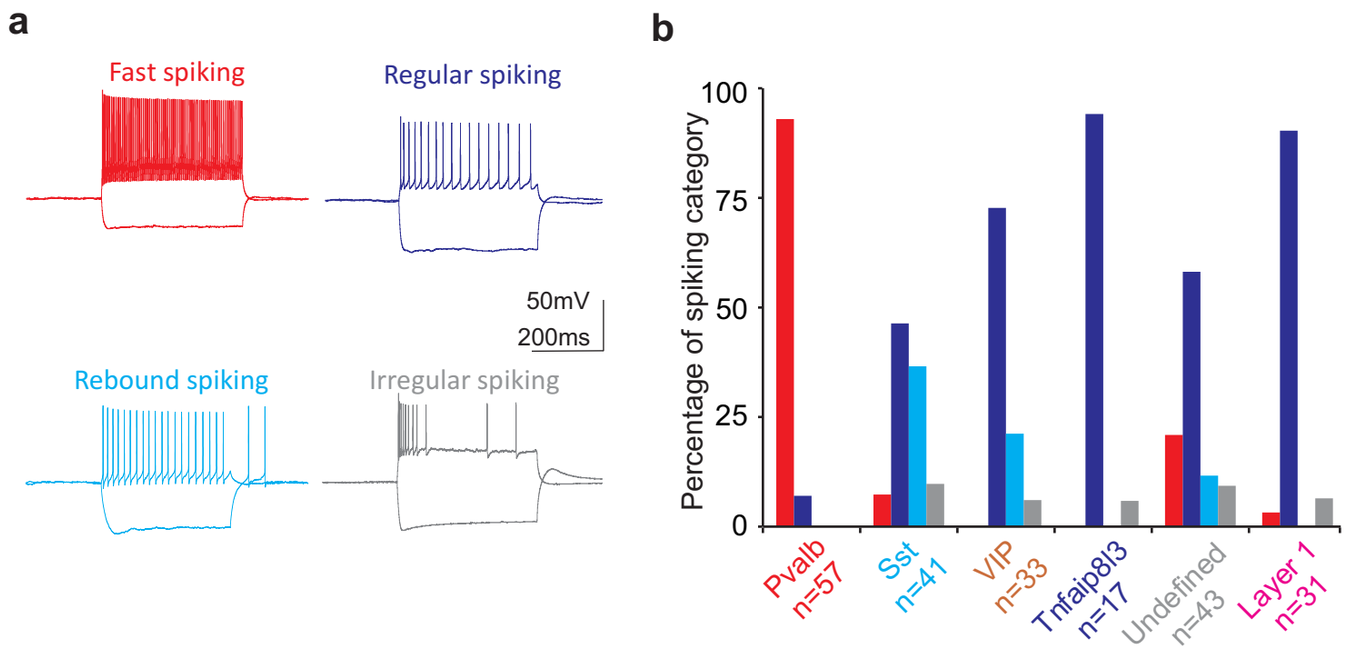
Statistical comparisons of INCs across interneuron categories

a,b,c) P-values (2-sided Mann-Whitney) between INCs of different postsynaptic interneuron categories for inhibition generated by the photoactivation of each of the three presynaptic interneuron classes: Pvalb-Cre (a); Sst-Cre (b); VIP-Cre (c). Schematics of the experimental configuration are shown above.

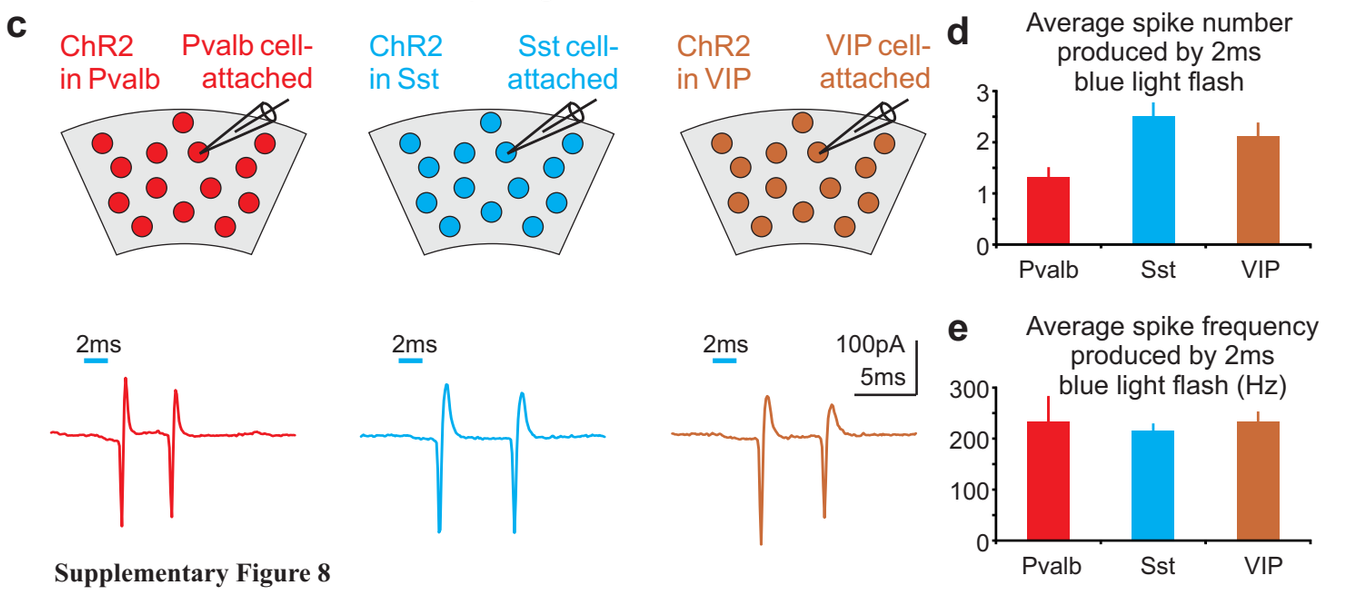
d,e,f) P-values (2-sided Mann-Whitney) between INCs of postsynaptic interneuron categories for inhibition generated by photoactivation of different presynaptic interneuron classes: Pvalb-Cre vs. Sst-Cre (d); Pvalb-Cre vs. VIP-Cre (e); Sst-Cre vs. VIP-Cre (f). Schematics of the experimental configuration are shown to the left and above.

Supplementary Figure 8

Intrinsic spiking properties and postsynaptic interneuron category



Photoactivation induced spiking of interneurons



Supplementary Figure 8

Intrinsic and photoinduced spiking of molecularly defined interneuron categories

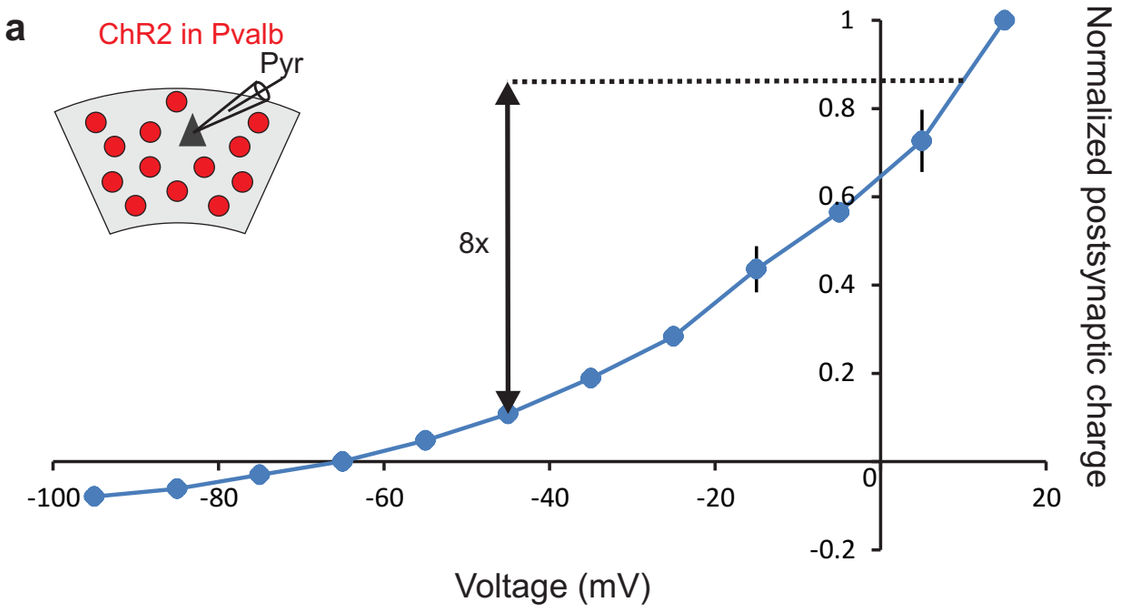
a) The four predominant spiking patterns recorded in interneurons in response to current injection.

b) Distribution of spiking patterns according to interneuron category. (Pvalb: n=57, 20 slices, 8 mice; Sst: 41 cells, 17 slices, 6 mice; VIP: 33 cell, 15 slices, 5 mice; Tnfaip8l3: 17 cells, 15 slices, 6 mice; Undefined: 43 cells, 20 slices, 10 mice; L1: 31 cells, 16 slices, 6 mice)

c) Full field 2ms photo-stimulation elicits multiple spikes in interneurons expressing ChR2 recorded in cell-attached mode. Schematics of recording condition shown above each sample trace from a cell attached recording of molecularly different interneurons (Pvalb – red, Sst – blue, VIP – brown).

d) Histogram of average spike number (Pvalb – red = 1.32±0.17, n=10, 5 slices, 3 mice; Sst – blue = 2.52±0.24, n=19, 6 slices, 3 mice; VIP – brown = 2.13±0.24, n=17, 6 slices, 3 mice; values are given as mean ± s.e.m)

Supplementary Figure 9



Supplementary Figure 9

Charge voltage relationship of inhibitory postsynaptic currents
Charge voltage relationship of the IPSQs recorded in pyramidal cells in response to Pvalb-cell photostimulation (inset: schematic of experimental configuration). The IPSQ value is normalized by the value measured at +15 mV. Inhibition at +10mV is 8x larger than the inhibition measured at -45mV (n=6 cells, 3 slices, 2 mice; error bars show s.e.m)

Supplementary table 1:

gene (multiplex/nested -PCR)	primer sequence 5'>3'
GAD1 sense (multiplex)	cacaggtcaccctcgatttt
GAD1 antisense (multiplex)	tctatgccgctgagtttgtg
GAD1 sense (nested)	tagctggtgaatggctgaca
GAD1 antisense (nested)	cttgtaacgagcagccatga
GAD2 sense (multiplex)	cagccttagggattggaaca
GAD2 antisense (multiplex)	acccagtagtccccttgct
GAD2 sense (nested)	gttcctttcctggtgagtgc
GAD2 antisense (nested)	tgcatcagtccctcctctct
GAPDH sense (multiplex)	actccactcacggcaaattc
GAPDH antisense (multiplex)	cacattgggggtaggaacac
GAPDH sense (nested)	agcttgtcatcaacgggaag
GAPDH antisense (nested)	gtcatgagcccttccacaat
Pvalb sense (multiplex)	ggatgtcgatgacagacgtg
Pvalb antisense (multiplex)	cagccaccagagtggagaat
Pvalb sense (nested)	cagcgctgaggacatcaag
Pvalb antisense (nested)	ctgaggagaagcccttcaga
Sst sense (multiplex)	agatgctgtcctgccgtct
Sst antisense (multiplex)	gggccaggagttaaggaaga
Sst sense (nested)	cccagactccgtcagtttct
Sst antisense (nested)	gaagttcttgcagccagctt
VIP sense (multiplex)	cagaagcaagcctcagttcc
VIP antisense (multiplex)	gcagaatctccctcactgct
VIP sense (nested)	ggtgaccctgaccaagtctc
VIP antisense (nested)	gtgaagacggcatcagagtg
Tac1 sense (multiplex)	cagaggaaatcgatgccaac
Tac1 antisense (multiplex)	gcatcgcgcttctttcat
Tac1 sense (nested)	accagatcaaggaggcaatg
Tac1 antisense (nested)	gcccattagtccaacaaagg
Tac2 sense (multiplex)	ctcagcttggcttggacct
Tac2 antisense (multiplex)	aaagctgggggtgttctctt
Tac2 sense (nested)	agggagggaggctcagtaag
Tac2 antisense (nested)	tctggttggctgttcctctt
Pdyn sense (multiplex)	tcctcgtgatgccctctaat
Pdyn antisense (multiplex)	ccatctcggaactcctcttg
Pdyn sense (nested)	tgcagtgaggattcaggatg
Pdyn antisense (nested)	ggcttttctccagctccttc
Htr3a sense (multiplex)	taccacccagcctgctctac
Htr3a antisense (multiplex)	gacctcacttcttccggtga
Htr3a sense (nested)	gtcagaccacctcctggcta
Htr3a antisense (nested)	ctgcacatcaaaggggaagt
Adarb2 sense (multiplex)	aggttacaggctgcgagaaa
Adarb2 antisense (multiplex)	ctgcttggcctcacagtaca
Adarb2 sense (nested)	tcctgcacagacaagattgc
Adarb2 antisense (nested)	ctcacgccactaaggagagg
Kit sense (multiplex)	gccctaatgtcggaactgaa
Kit antisense (multiplex)	aggagaagagctcccagagg
Kit sense (nested)	acaagaggagatccgcaaga

gene (multiplex/nested -PCR)	primer sequence 5'>3'
Kit antisense (nested)	aaatgctctctggtgccatc
Lgals1 sense (multiplex)	ctctcgggtggagtcttctg
Lgals1 antisense (multiplex)	aggccacgcacttaatcttg
Lgals1 sense (nested)	tcaaacctggggaatgtctc
Lgals1 antisense (nested)	agcttgatggtcaggtcagc
Sema3c sense (multiplex)	gatggcccagagacacattt
Sema3c antisense (multiplex)	tagatatggcagcgatgcag
Sema3c sense (nested)	atatcctcgcccaggaactt
Sema3c antisense (nested)	acgtggtatctcccatcagc
Nacc2 sense (multiplex)	agcatccagcaggtacccta
Nacc2 antisense (multiplex)	cagtttcacagcgttcagga
Nacc2 sense (nested)	atggtggaggagcagtatgg
Nacc2 antisense (nested)	tctccaggatctccctctga
Cerk sense (multiplex)	ggggactcactggcaataga
Cerk antisense (multiplex)	agagggtctgtccttgcaga
Cerk sense (nested)	cggggacttaatcaaggaca
Cerk antisense (nested)	gcacttccggataaggatga
Cox6a2 sense (multiplex)	ctctcgactgggtgaaggag
Cox6a2 antisense (multiplex)	accatgctgttcccaaagag
Cox6a2 sense (nested)	ttcctagcctccctttgaca
Cox6a2 antisense (nested)	ggtggtgatacgggatgaac
Cxcl14 sense (multiplex)	ctccttgcctccctgctc
Cxcl14 antisense (multiplex)	agtctctcaactggcctgga
Cxcl14 sense (nested)	gtgtaagtgttcccggaagg
Cxcl14 antisense (nested)	tccatgatcgtccaccctat
Grin3a sense (multiplex)	ccctaaatgctggactggaa
Grin3a antisense (multiplex)	ttgattcgtggtagcagcag
Grin3a sense (nested)	attggtctccctcccaactc
Grin3a antisense (nested)	gaatcctatgcacagcagca
Nek7 sense (multiplex)	gaaggcattacggccagata
Nek7 antisense (multiplex)	cttgtcgccgtagaaaggac
Nek7 sense (nested)	agcagatgctggtgacctct
Nek7 antisense (nested)	gagctgaaaaaccgaccaag
Nxph1 sense (multiplex)	gcgtgctggtacgttcttct
Nxph1 antisense (multiplex)	ttcccttgaccagtggagtt
Nxph1 sense (nested)	cccaccgtctacttggtcac
Nxph1 antisense (nested)	cagtcccagaggtcttgctc
Tm6sf1 sense (multiplex)	gcctggaaacatcgtaggaa
Tm6sf1 antisense (multilpex)	ggtacaacggtaggccaaga
Tm6sf1 sense (nested)	agtatgggacacggatttgc
Tm6sf1 antisense (nested)	gcagggtcctttaggtaggg
Tnfaip8l3 sense (multiplex)	tcagattctggagagcagagc
Tnfaip8l3 antisense (multiplex)	agtttccatgcggaccatag
Tnfaip8l3 sense (nested)	actgctgcaggtcctcatgt
Tnfaip8l3 antisense (nested)	gccaccttgatagcatcctt
Dlx6as1 sense (multiplex)	agctggaggctttgttcaga
Dlx6as1 antisense (multiplex)	taagaggtcagcgctccaat
Dlx6as1 sense (nested)	agggaacggggatattgaac
Dlx6as1 antisense (nested)	tccagctggttcatctctga

vGluT1 sense (multiplex)	ctggggtccttgtgcagtat
vGluT1 antisense (multiplex)	gaaatgcccatcaagatgct
vGluT1 sense (nested)	ctgaggaggagcgcaaatac
vGluT1 antisense (nested)	agctttcgcacgttggtagt