

**1 RESEARCH**

**2 Modelling the cell-type specific mesoscale murine connectome with  
3 anterograde tracing experiments**

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**ABSTRACT**

**9** The Allen Brain Connectivity Atlas consists of anterograde tracing experiments targeting diverse  
**10** structures and classes of projecting neurons. Beyond anterograde tracing done in C57BL6 wildtype  
**11** mice, a large fraction of these experiments are performed using transgenic cre lines. This allows access  
**12** to cell-class specific connectivity information, with class defined by the transgenic lines. However,  
**13** even though the number of experiments is large, it does not come close to covering all existing cell  
**14** classes in every area where they exist. We study how much we can fill in these gaps and construct a  
**15** voxel-based connectivity given the observations that nearby voxels have similar connections when  
**16** they are in the same area, that connections can change dramatically at area boundaries, and that  
**17** particular cell classes can have similar connections, but that this similarity is region-dependent.

**18** This paper describes the conversion of these experiments into class-specific connectivity matrices  
**19** representing the connection between source and target structures. We introduce and validate a novel  
**20** statistical model for creation of connectivity matrices. We expand a Nadaraya-Watson kernel learning

21 method which we previous used to fill in spatial gaps, to also fill in gaps in cell class connectivity  
22 information. To do this, we construct a "cell-class space" and combine smoothing in 3D spatial as well  
23 as in this abstract space to share information between similar neuron classes. Using this method we  
24 construct a set of connectivity matrices using multiple levels of resolution at which discontinuities in  
25 connectivity are assumed. We show that the connectivities obtained from this model display expected  
26 cell-type and structure specific connectivities. Inspired by how this complexity arises from a much  
27 smaller set of genetic information during development, we also show that the wild-type connectivity  
28 matrix can be factored using a small set of factors, and we uncover the underlying latent structure.

## AUTHOR SUMMARY

29 Large scale studies have described the connections between areas multiple mammalian models in  
30 ever expanding detail. Standard connectivity studies focus on the connection strength between areas.  
31 However, when describing functions at a local circuit level, there is an increasing focus on cell types.  
32 We have recently described the importance of connection types in the cortico-thalamic system, which  
33 allows an unsupervised discovery of its hierarchical organization. In this study we focus on adding a  
34 dimension of connection type for a brain-wide mesoscopic connectivity model. Even with our  
35 massive dataset, the data in the type direction for the connectivity is quite sparse, and we had to  
36 develop methods to more reliably extrapolate in such directions, and to estimate when such  
37 extrapolations are impossible. This allows us to fill in such a connection type specific inter-areal  
38 connectivity matrix to the extent our data allows us to. While analysing this complex connectivity, we  
39 observed that it can be described via a small set of factors. While not complete, this connectivity  
40 matrix represents a large leap forward in mouse connectivity models.

## 1 INTRODUCTION

41 The mammalian nervous system enables an extraordinary range of natural behaviors, and has  
42 inspired much of modern artificial intelligence. Neural connections from one region to another form  
43 the architecture underlying this capability. These connectivities vary by neuron type, as well as source  
44 and target structure. Thus, characterization of the relationship between neuron type and source and  
45 target structure is an important for understanding the overall nervous system.

46 Viral tracing experiments - in which a viral vector expressing GFP is transduced into neural cells  
47 through stereotaxic injection - are a useful tool for mapping these connections on the mesoscale  
48 (Chamberlin, Du, de Lacalle, & Saper, 1998; Daigle et al., 2018; J. A. Harris, Oh, & Zeng, 2012). The GFP  
49 protein moves into the axon of the projecting neurons. The long range connections between different  
50 areas are generally formed by axons which travel from one region to another. Two-photon  
51 tomography imaging can then determine the location and strength of the fluorescent signals in  
52 two-dimensional slices. These locations can then be mapped back into three-dimensional space. The  
53 signal is integrated over area into cubic voxels.

54 Several statistical models for the conversion of such experiment-specific signals into estimates of  
55 connectivity strength have been proposed (K. D. Harris, Mihalas, & Shea-Brown, 2016; Knox et al.,  
56 2019; Oh et al., 2014). Of these, Oh et al. (2014) and Knox et al. (2019) model **structural connectivities**,  
57 which are voxel connectivities integrated by structure. The value of these models is that they provide  
58 some improvement over simply averaging the projection signals of injections in a given region.  
59 However, these previous works only model connectivities observed in wild-type mice transduced with  
60 constitutive promoters, and so are poorly suited for extension to recently developed tracing  
61 experiments that induce cell-type specific fluorescence (J. A. Harris et al., 2019). In particular, GFP  
62 promotion is induced by Cre-recombinase expression in cell-types specified by transgenic strain.  
63 Thus, this paper introduces a **cell class**-specific statistical model to deal with the diverse set of  
64 **cre-lines** described in J. A. Harris et al. (2019).

65 Our model is a to-our-knowledge novel estimator that takes into account both the spatial position  
66 of the labelled source, as well as the categorical cell class. Like the previously state-of-the-art model in  
67 Knox et al. (2019), this model predicts structural connectivity as an average over positions within the

68 structure, with nearby experiments given more weight. However, our model weighs class-specific  
69 behavior in a particular structure against spatial position, so a nearby experiment targeting a similar  
70 cell class would be relatively upweighted, while a nearby experiment targeting a dissimilar class would  
71 be downweighted. This model outperforms the model of Knox et al. (2019) based off of their ability to  
72 predict held-out experiments in leave-one-out cross-validation. We then establish a lower-limit of  
73 detection, and use the trained model to estimate overall connectivity matrices for assayed each cell  
74 class.

75 The resulting cell-type specific connectivity is a directed weighted multigraph which can be  
76 represented as a tensor. We do not attempt an exhaustive analysis of this data, but do manually verify  
77 several cell-type specific connectivity patterns found elsewhere in the literature, and show that these  
78 cell-type specific signals are behaving in expected ways. Finally, we decompose the wild-type  
79 connectivity matrix into factors representing archetypal connective patterns using non-negative  
80 matrix factorization. These components are themselves novel and of some independent interest.

81 Section 2 gives information on the data and statistical methodology, and Section 3 presents our  
82 results. These include connectivities, assessments of model fit, and subsequent analyses. Additional  
83 information on our dataset, methods, and results are given in Supplemental Sections 5, 6, and 7,  
84 respectively.

## 2 METHODS

85 We create and analyze cell class-specific connectivity matrices using models trained on murine  
86 viral-tracing experiments. This section describes the data used to generate the model, the model  
87 itself, the evaluation of the model, and the use of the model in creation of the connectivity matrices. It  
88 also includes background on the non-negative matrix factorization method used for decomposing the  
89 wild-type connectivity matrix into latent structures. Additional information on our data is given in  
90 Supplemental Section 5 methods is given in Supplemental Section 6.

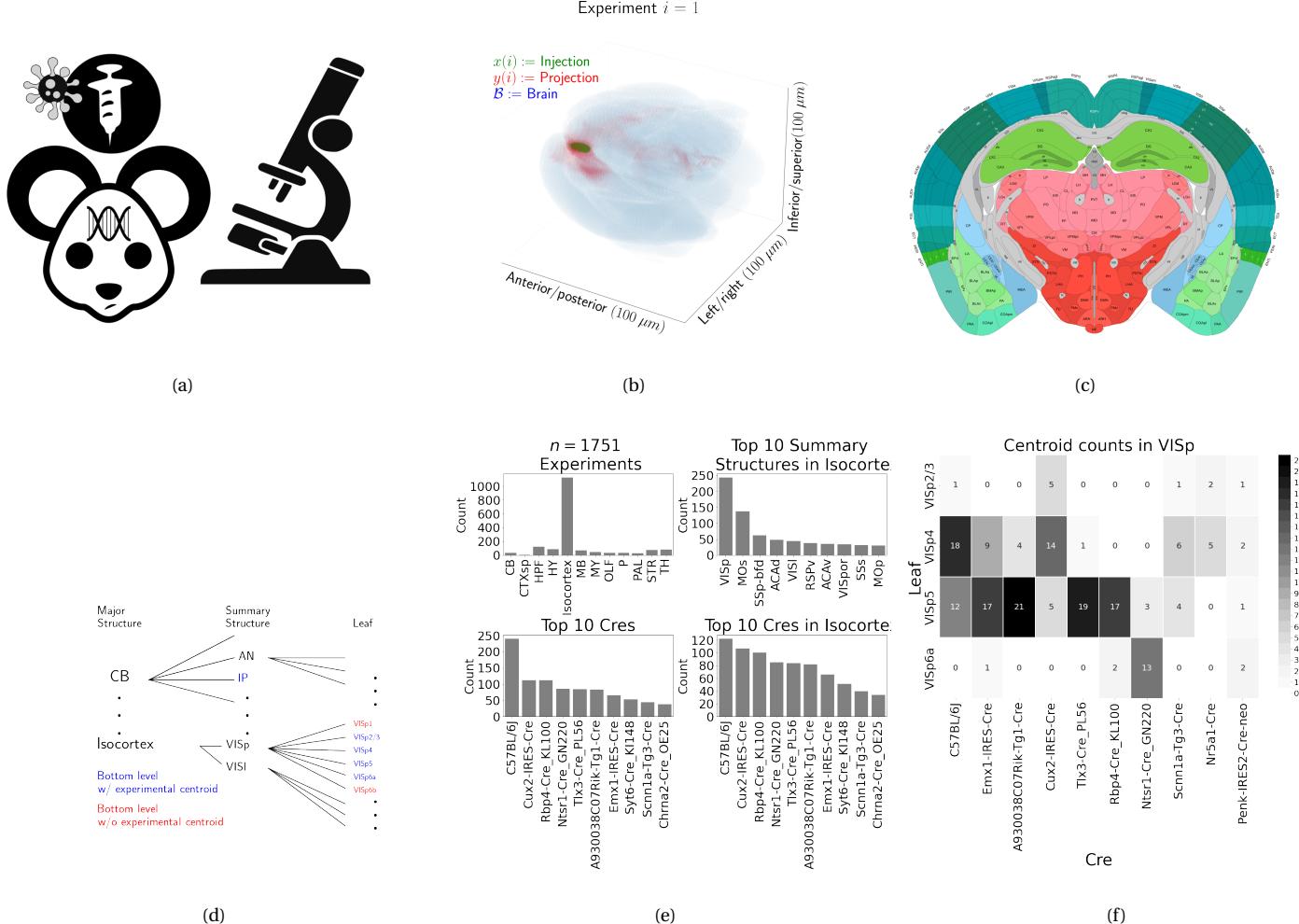


Figure 1: Experimental setting. 1a For each experiment, a potentially Cre-recombinase promoted GFP-expressing transgene cassette is transduced after stereotaxic injection into a Cre-driver mouse, followed by two-photon tomography imaging. 1b An example of the segmentation of projection and injection for a single experiment. Within each assayed brain (blue), injection (green) and projection (red) areas are determined via histological analysis and alignment to the Allen Common Coordinate Framework (CCF). 1c Example of structural segmentation within a horizontal plane. 1d Explanation of nested structural ontology highlighting various levels of structural ontology. Lowest-level (leaf) structures are colored in blue, and structures containing an injection centroid are colored in red. 1e Abundances of Cre-lines and structural injections. 1f Co-occurrence of layer-specific centroids and Cre-line within VIsP

91 **Data**

92 Our dataset  $\mathcal{D}$  consists of  $n = 1751$  publicly available murine viral-tracing experiments from the Allen  
93 Brain Connectivity Atlas. Figure 1a summarizes the multistage experimental process used to generate  
94 this data. In each experiment, a GFP-labelled transgene cassette with a potentially Cre-inducible  
95 promoter is injected into a particular location in a Cre-driver mouse. This causes fluorescence that  
96 depends on the localization of Cre-recombinase expression within the mouse. While frequently this  
97 localization corresponds to a specific cell-type, it can also correspond to a combination of cell-types.  
98 In wild-type mice injected with non-Cre specific promoters, fluorescence is observed in all areas  
99 projected to from the injection site, regardless of cell-type. Thus, we use the term cell class to describe  
100 neurons expressing cre in a specific mouse line. This is the notion of cell-type specificity that we  
101 model.

102 The fluorescent signal imaged after injection is aligned into the Allen Common Coordinate  
103 Framework (CCF) v3, a three-dimensional idealized model of the brain that is consistent between  
104 animals. This imaging and alignment procedure (described in detail in (J. A. Harris et al., 2019))  
105 records fluorescent intensity discretized at the  $100 \mu\text{m}$  voxel level. Given an experiment, this image is  
106 histologically segmented into *injection* and *projection* areas corresponding to areas containing somas,  
107 dendrites and axons or exclusively axons of the transfected neurons. An example for a single  
108 experiment is given in Figure 1b.

109 Our goal is the estimation of **structural connectivity** from one structure to another. A visual  
110 depiction of this structural regionalization for a slice of the brain is given in Figure 1c. For different  
111 areas of the brain, the Allen Brain Atlas contains different depths of regionalization. We denote these  
112 levels as Major Structures, Summary Structures, and Leafs. As indicated in Figure 1d, the dataset used  
113 to generate the connectivity model reported in this paper contains certain combinations of structure  
114 and cell class  $(v, s)$  frequently, and others not at all. A summary of the most frequently assayed cell  
115 classes and structures is given in Figures 1e and 1f. Since users of the connectivity matrices may be  
116 interested in particular combinations, or interested in the amount of data used to generate a  
117 particular connectivity estimate, we present this information about all experiments in Supplemental  
118 Section 5.

119 A cell-class specific neural connectivity is a function  $f: \mathcal{V} \times \mathbb{R}^3 \times \mathbb{R}^3 \rightarrow \mathbb{R}_{\geq 0}$  giving the directed  
 120 connection of a particular cell class from a one position in the brain to another. However, what we will  
 121 actually estimate are structural connectivities defined with respect to a set of  $S$  source regions  
 122  $\mathcal{S} := \{s\}$ ,  $T$  target regions  $\mathcal{T} := \{t\}$ , and  $V$  cell classes  $\mathcal{V} := \{v\}$ . In contrast to Knox et al. (2019), which  
 123 only uses wild type C57BL/6J mice, these experiments utilize  $V = 114$  different Cre-lines. We generally  
 124 consider  $S = 564$  leaf sources and  $T = 1123$  leaf targets, where 559 are contralateral and 5 are  
 125 mediolateral, but other structuralizations could be used.

126 We preprocess our data in several ways. We discretize fluorescent signals like injections and  
 127 projections into  $100\mu m^3$  **voxels**. Given an experiment  $i$ , we represent injections and projections as  
 128 maps  $x(i), y(i) : \mathcal{B} \rightarrow \mathbb{R}_{\geq 0}$ , where  $\mathcal{B} \subset [1 : 132] \times [1 : 80] \times [1 : 104]$  corresponds to the subset of the  
 129  $(1.32 \times 0.8 \times 1.04)$  cm rectangular space occupied by the standard mouse brain. As an abuse of  
 130 notation, a structure  $s$  then contains  $|s|$  voxels at locations  $\{l_{s_j} \in \mathbb{R}^3\}$ , and similarly for targets. We  
 131 calculate injection centroids  $c(i) \in \mathbb{R}^3$  and regionalized projections  $y_{\mathcal{T}}(i) \in \mathbb{R}^T$  giving the sum of  $y(i)$   
 132 in each region. In contrast to Knox et al. (2019), we generally  $L1$  normalize the projection vectors. This  
 133 accounts for differences in the cre-driven expression of eGFP via the various transgene promoters.  
 134 However, we also for completeness include models of projections normalized by injection signal. A  
 135 detailed mathematical description of these steps, including data quality control, is given in  
 136 Supplemental Section 6.

137 ***Modeling Structural Connectivity***

We define

*structural connectivity strength*  $\mathcal{C} : \mathcal{V} \times \mathcal{S} \times \mathcal{T} \rightarrow \mathbb{R}_{\geq 0}$  with  $\mathcal{C}(v, s, t) = \sum_{l_{sj} \in s} \sum_{l_{j'} \in t} f(v, l_j, l_{j'})$ ,

*normalized structural connectivity strength*  $\mathcal{C}^N : \mathcal{V} \times \mathcal{S} \times \mathcal{T} \rightarrow \mathbb{R}_{\geq 0}$  with  $\mathcal{C}^N(v, s, t) = \frac{1}{|s|} \mathcal{C}(v, l_j, l_{j'})$ ,

*normalized structural projection density*  $\mathcal{C}^D : \mathcal{V} \times \mathcal{S} \times \mathcal{T} \rightarrow \mathbb{R}_{\geq 0}$  with  $\mathcal{C}^D(v, s, t) = \frac{1}{|s||t|} \mathcal{C}(v, l_j, l_{j'})$ .

- 138 Since the normalized strength and densities are computable from the strength via a fixed  
 139 normalization, our main statistical goal is to estimate  $\mathcal{C}(v, s, t)$  for all  $v, s$  and  $t$ . In other words, we  
 140 want to estimate matrices  $\mathcal{C}_v \in \mathbb{R}_{\geq 0}^{S \times T}$ . We call this estimator  $\widehat{\mathcal{C}}$ .

Construction of such an estimator raises the questions of what data to use for estimating which connectivity, how to featurize the dataset, what statistical estimator to use, and how to reconstruct the connectivity using the chosen estimator. Mathematically, we represent these considerations as

$$\widehat{\mathcal{C}}(v, s, t) = f^*(\widehat{f}(f_*(\mathcal{D}(v, s, t))). \quad (1)$$

- 141 This makes explicit the data featurization  $f_*$ , statistical estimator  $\widehat{f}$ , and any potential subsequent  
 142 transformation  $f^*$  such as summing over the source and target regions. Denoting  $\mathcal{D}$  as a function of  
 143  $v, s$ , and  $t$  reflects that different data may be used to estimate different connectivities. Table 1 reviews  
 144 estimators used for this data-type used in previous work, as well as our two main extensions: the  
 145 Cre-NW and **Expected Loss** (EL) models. Additional information on these estimators is given in  
 146 Supplemental Section 6.

Name	$f^*$	$\hat{f}$	$f_*$	$\mathcal{D}(v, s)$
NNLS (Oh et al., 2014)	$\hat{f}(S)$	NNLS(X,Y)	$X = x_{\mathcal{S}}, Y = y_{\mathcal{T}}$	$I_m / I_m$
NW (Knox et al., 2019)	$\sum_{l_s \in s} \hat{f}(l_s)$	NW(X,Y)	$X = c, Y = y_{\mathcal{T}}$	$I_m / I_m$
Cre-NW	$\sum_{l_s \in s} \hat{f}(l_s)$	NW(X,Y)	$X = c, Y = y_{\mathcal{T}}$	$(I_l \cap I_v) / I_m$
Expected Loss (EL)	$\sum_{l_s \in s} \hat{f}(s)$	EL( $X, Y, v$ )	$X = c, Y = y_{\mathcal{T}}, v$	$I_l / I_m$

Table 1: Estimation of  $\mathcal{C}$  using connectivity data. The regionalization, estimation, and featurization steps are denoted by  $f^*$ ,  $\hat{f}$ , and  $f_*$ , respectively. The training data used to fit the model is given by  $I$ . We denote experiments with centroids in particular major brain divisions and leafs as  $I_m$  and  $I_l$ , respectively. Data  $I_l / I_m$  means that, given a location  $l_s \in s \in m$ , the model  $\hat{f}$  is trained on all of  $I_m$ , but only uses  $I_l$  for prediction. The non-negative least squares estimator (NNLS) fits a linear model that predicts structural projection signal as a function of structural injection signal. It generates estimated connectivities for individual structures. The Nadaraya-Watson model (NW) is a local smoothing model that generates a prediction for each voxel within a structure. These predictions are averaged to create estimate the structure-specific connectivity.

147 Our contributions have several differences from the previous methods. In contrast to the  
 148 non-negative least squares (Oh et al., 2014) and Nadaraya-Watson (Knox et al., 2019) estimators that  
 149 take into account  $s$  and  $t$ , but not  $v$ , our new estimators specifically account for cell class. The  
 150 Cre-NW estimator only uses experiments from a particular class to predict connectivity for that class,  
 151 while the EL estimator shares information between classes within a structure. A detailed  
 152 mathematical description of our new estimator is given in Supplemental Section 6. This estimator  
 153 takes into account two types of covariate information about each experiment: the centroid of the  
 154 injection, and the Cre-line. Like the NW and Cre-NW estimator, the EL estimator generates  
 155 predictions for each voxel in a structure, and then sums them together to get the overall connectivity.  
 156 However, in contrast to these alternative approaches, when predicting the projection pattern of a  
 157 certain cell-class at a particular location, the EL estimator weights the average behavior of the class in  
 158 the structure containing the location in question against the locations of the various proximal

159 experiments. Thus, nearby experiments with similar Cre-lines can help generate the prediction, even  
160 when there are few nearby experiments of the cell-class in question.

161 ***Model evaluation***

162 We select optimum functions from within and between our estimator classes using **leave-one-out**  
 163 **cross validation**, in which the accuracy of the model is assessed by its ability to predict experiments  
 164 excluded from the training data. Equation 1 includes a deterministic step  $f^*$  included without input  
 165 by the data. The performance of  $\widehat{\mathcal{C}}(v, s, t)$  is thus determined by performance of  $\widehat{f}(f_*(\mathcal{D}(v, s)))$ .  
 166 Furthermore, we can represent  $f$  as  $f_{\mathcal{T}} : \mathbb{R}^3 \rightarrow \mathbb{R}_{\geq 0}^T$  giving the structural connection strength at a given  
 167 location. This is the predictand we evaluate.

168 Another question is what combinations of  $v$ ,  $s$ , and  $t$  to generate a prediction for. Our EL and  
 169 Cre-NW models are leaf specific. They only generate predictions for cell classes in leafs where at least  
 170 one experiment with a Cre-line targeting that class has a centroid. To compare our new estimators  
 171 accurately with less-restrictive models such as used in Knox et al. (2019), we therefore restrict  
 172 to the smallest set of evaluation experiments suggested by any of our models: virus-leaf combinations  
 173 that are present at least twice. The sizes of these evaluation sets are given in Supplemental Section 5.

We use weighted  $l_2$ -loss to evaluate these predictions.

$$\text{l2-loss } \ell(y_{\mathcal{T}}(i)), \widehat{y_{\mathcal{T}}(i)}) := \|y_{\mathcal{T}}(i)) - \widehat{y_{\mathcal{T}}(i)}\|_2^2.$$

$$\text{weighted l2-loss } \mathcal{L}(\widehat{f}(f_*)) := \frac{1}{|\{s, v\}|} \sum_{s, v \in \{\mathcal{S}, \mathcal{V}\}} \frac{1}{|I_s \cap I_v|} \sum_{i \in (I_s \cap I_v)} \ell(y_{\mathcal{T}}(i)), \widehat{f}_{\mathcal{T}}(f_*(\mathcal{D}(v, s) \setminus i)).$$

174 This is a somewhat different loss from Knox et al. (2019), both because of the normalization of  
 175 projection, and because of the increased weighting of rarer combinations of  $s$  and  $v$  implicit in the  
 176 loss. Since the number of parameters fit is very low (at least two orders of magnitude) relative to the  
 177 size of the evaluation set, we do not make use of a formal validation-test split. As a final modeling  
 178 step, we establish a lower limit of detection. The EL model also contains a separate cross-validation  
 179 step. These approaches are covered in Supplemental Section 6

180 ***Connectivity analyses***

181 We show neuronal processes underlying our estimated connectome using two types of unsupervised  
 182 learning. Our use of hierarchical clustering is standard, and so we do not review it here. However, our  
 183 application of non-negative matrix factorization (NMF) to decompose the estimated long-range  
 184 connectivity into **connectivity archetypes** that linearly combine to reproduce the observed  
 185 connectivity is novel and technically of some independent interest. Non-negative matrix factorization  
 186 refers to a collection of **dictionary-learning** algorithms for decomposing a non-negatively-valued  
 187 matrix such as  $\mathcal{C}$  into positively-valued matrices called, by convention, weights  $W \in \mathbb{R}_{\geq 0}^{S \times q}$  and hidden  
 188 units  $H \in \mathbb{R}_{\geq 0}^{q \times T}$ . Unlike PCA, NMF specifically accounts for the fact that data are all in the positive  
 189 orthant. This  $H$  is typically used to identify latent structures with interpretable biological meaning,  
 190 and the choice of matrix factorization method reflects particular scientific subquestions and  
 191 probabilistic interpretations.

192 Our algorithm is

$$\text{NMF}(\mathcal{C}, \lambda, q) := \arg \min_{W \in \mathbb{R}_{\geq 0}^{S \times q}, H \in \mathbb{R}_{\geq 0}^{q \times T}} \frac{1}{2} \| \mathbf{1}_{d(s,t) > 1500\mu m} \odot \mathcal{C} - WH \|_2^2 + \lambda (\| H \|_1 + \| W \|_1).$$

193 For this decomposition we ignore connections between source and target regions less than  
 194  $1500\mu m$  apart. This is because short-range projections resulting from diffusion dominate the  
 195 matrices  $\hat{\mathcal{C}}$ , and represent a less-interesting type of biological structure. We explored different values  
 196 and set  $\lambda = 0.002$  to encourage sparser and therefore more interpretable components. We use  
 197 unsupervised cross-validation to determine an optimum  $q$ , and show the top 15 stable components.  
 198 Stability analysis accounts for the difficult-to-optimize NMF program by clustering the resultant  $H$   
 199 from multiple replicates. The medians of the component clusters appearing frequently across NMF  
 200 replicates are selected as **connectivity archetypes**. Details of these approaches are given in  
 201 Supplementary Sections 6 and 7.

### 3 RESULTS

<sup>202</sup> We provide several types of results. First, we show that the novel expected-loss (EL) estimator  
<sup>203</sup> performs best in our validation assays. Second, qualitative exploratory analysis confirms that the  
<sup>204</sup> Cre-specific connectivity matrices generated using this model are consistent with known biology.  
<sup>205</sup> Third, statistical decomposition of the wild-type connectivity matrix using unsupervised learning  
<sup>206</sup> shows how archetypal components can combine to produce observed signals.

<sup>207</sup> ***Model evaluation***

<sup>208</sup> Our EL model generally performs better than the other estimators that we consider. Table 5 contains  
<sup>209</sup> weighted losses from leave-one-out cross-validation of candidate models, such as the NW Major-WT  
<sup>210</sup> model from Knox et al. (2019). The EL model combines the good performance of class-specific  
<sup>211</sup> models like NW Leaf-Cre in regions like Isocortex with the good performance of class-agnostic models  
<sup>212</sup> in regions like Thalamus. Additional information on model evaluation, including class and structure-  
<sup>213</sup> specific performance, is given in Appendix 5. In particular, Supplementary Table 4 contains the sizes  
<sup>214</sup> of these evaluation sets in each major structure, and Supplementary Section 7 contains the structure-  
<sup>215</sup> and class specific losses.

$\hat{f}$	Mean Leaf-Cre	NW Major-Cre	NW Leaf-Cre	NW Leaf	NW Major-WT	NW Major	EL
$\mathcal{D}$	Mean	NW					EL
	$I_c \cap I_L$	$I_c \cap I_M$	$I_c \cap I_L$	$I_L$	$I_{wt} \cap I_M$	$I_M$	$I_L$
Isocortex	0.239	0.252	0.234	0.279	0.274	0.274	<b>0.228</b>
OLF	0.193	0.233	0.191	<b>0.135</b>	0.179	0.179	0.138
HPF	0.175	0.332	0.170	0.205	0.228	0.228	<b>0.153</b>
CTXsp	<b>0.621</b>	<b>0.621</b>	<b>0.621</b>	<b>0.621</b>	<b>0.621</b>	<b>0.621</b>	<b>0.621</b>
STR	0.131	<b>0.121</b>	0.128	0.169	0.232	0.232	0.124
PAL	0.203	0.205	0.203	0.295	0.291	0.291	<b>0.188</b>
TH	0.673	0.664	0.673	<b>0.358</b>	0.379	0.379	0.369
HY	0.360	0.382	0.353	0.337	0.317	0.317	<b>0.311</b>
MB	0.168	0.191	0.160	0.199	0.202	0.202	<b>0.159</b>
P	0.292	0.292	0.292	0.299	0.299	0.299	<b>0.287</b>
MY	0.268	0.347	0.268	0.190	<b>0.189</b>	<b>0.189</b>	0.204
CB	<b>0.062</b>	<b>0.062</b>	<b>0.062</b>	0.068	0.112	0.112	0.068

Table 2: Losses from leave-one-out cross-validation of candidate models. **Bold** numbers are best for their major structure.

216 ***Connectivities***

217 Our main result is the estimation of matrices  $\hat{\mathcal{C}}_v \in \mathbb{R}_{\geq 0}^{S \times T}$  representing connections of source structures  
 218 to target structures for particular cre-lines  $v$ . We confirm the detection of several well-established  
 219 connectivities within our tensor, although it is our expectation that additional interesting biological  
 220 processes are also manifest. The connectivity tensor and code to reproduce it are available at  
 221 [https://github.com/AllenInstitute/mouse\\_connectivity\\_models/tree/2020](https://github.com/AllenInstitute/mouse_connectivity_models/tree/2020).

222 *Overall connectivity* Several expected biological processes are evident in the wild-type connectivity  
 223 matrix  $\mathcal{C}_{wt}$  from leaf sources to leaf targets shown in Figure 2a. Intraareal connectivities are clear, as  
 224 are ipsilateral connections between cortex and thalamus. The clear intrastructural and intraareal  
 225 connectivities mirror previous estimates in Oh et al. (2014) and Knox et al. (2019) and descriptive  
 226 depictions of individual experiments in J. A. Harris et al. (2019). These short-range connectivities  
 227 define a

228 Our estimated wild-type connectivities appear more variable than those in Knox et al. (2019), which  
 229 used the NW Major-WT model whose accuracy is evaluated in Table 5. This is plausibly because of  
 230 both the layer-specific targeting of the different cre-lines, and also the layer-specificity of the selected  
 231 model. Although layer-specificity is a major advantage of including distinct cre-lines, for comparison,  
 232 we also plot coarser projections between summary-structure sources and targets in the cortex in  
 233 Figure 2b. These are averages over component layers weighted by layer size. Grossly congruent with  
 234 the previous work, these results also exhibit a larger range of connectivities than those in Knox et al.  
 235 (2019). Importantly, as shown in Table 5 this finer spatial resolution corresponds to the increased  
 236 accuracy of our EL model over the NW Major-WT model.

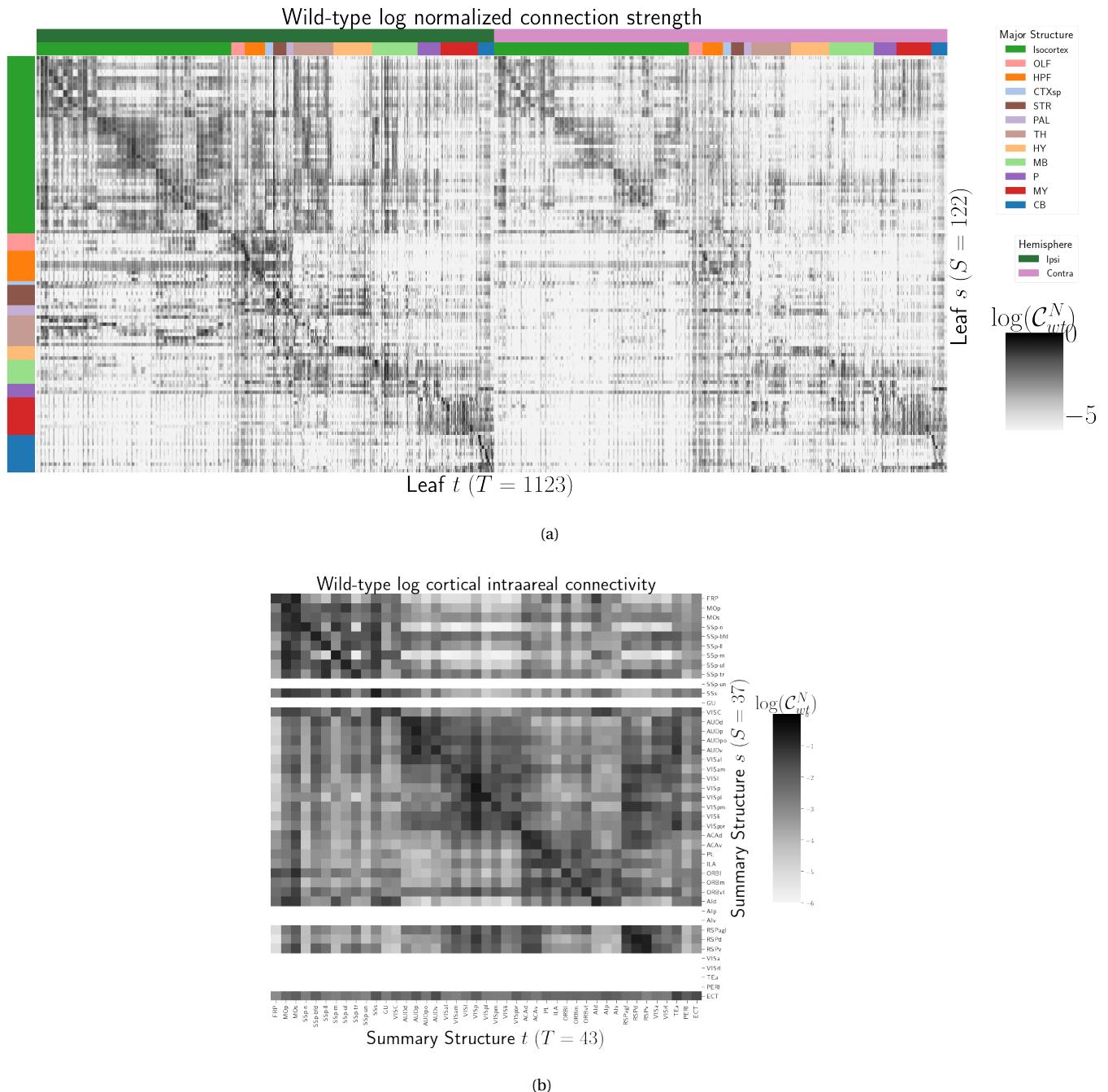
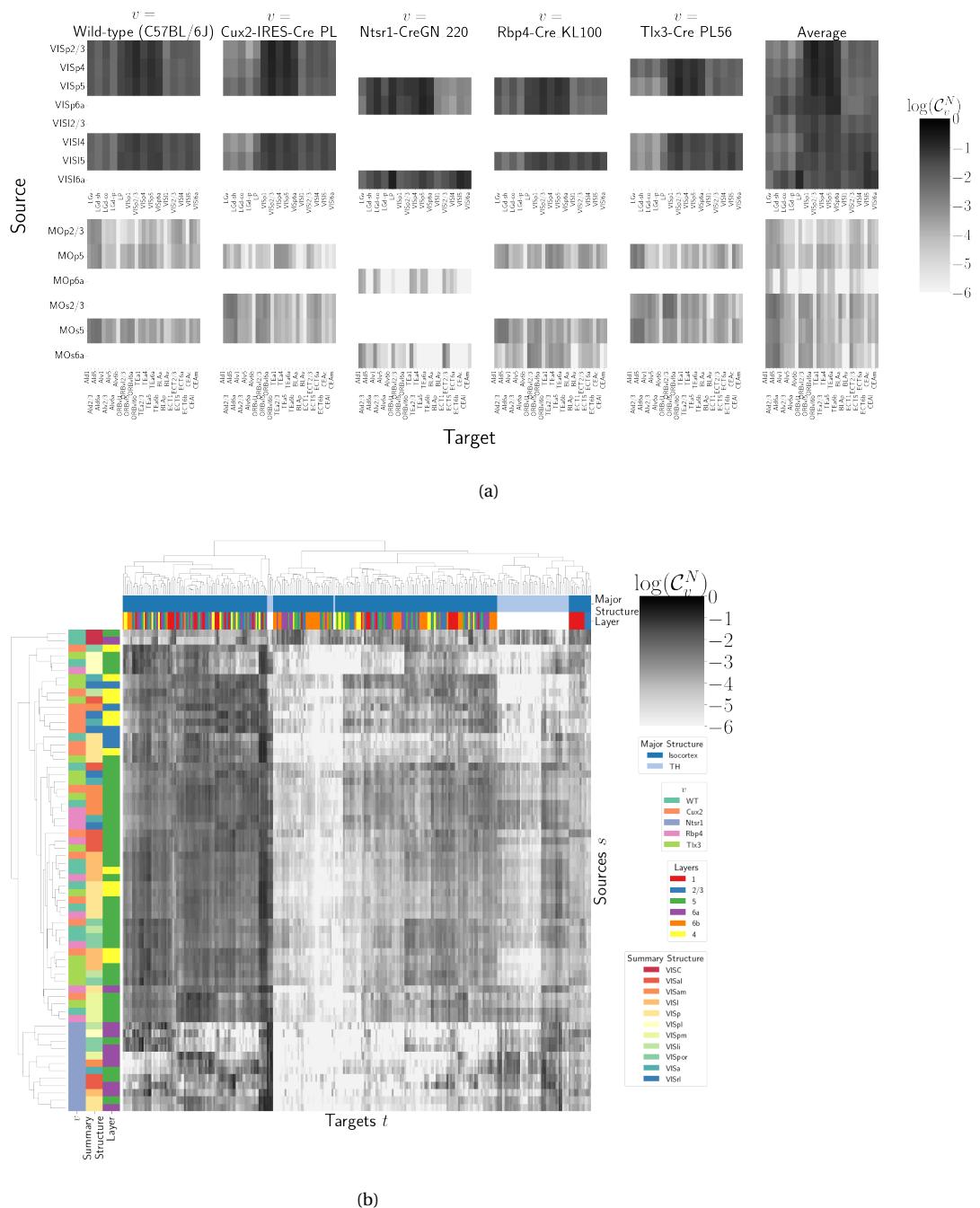


Figure 2: Wild-type connectivities. 2a Log wild-type connectivity matrix  $\log \mathcal{C}(s, t, v_{wt})$ . 2b Log wild-type intracortical connectivity matrix at the summary structure level.

237 *Class-specific connectivities* Source and cell-type combinations which project similarly indicate the  
238 network structure underpinning cognition. Our estimates of these class-specific connectivities exhibit  
239 certain known behaviors. In Figure 3, we display results for the VISp and MO cortical areas. These are  
240 ideal testbeds for our connectivities because they have well-established layer-specific projection  
241 patterns that can be detected with our layer-specific cre-line based targeting Jeong et al. (2016), and  
242 are also well-represented in our dataset.

243 Our results are consistent with anterograde tracing experiments outside our dataset Jeong et al.  
244 (2016). Figure 3a shows that in VISp, the Ntsr1-Cre line strongly targets the thalamic LP nuclei, and in  
245 MO, layer 5 projects to anterior basolateral amygdala (BLA) and capsular central amygdala (CEA),  
246 while layer 6 does not. Recall that we display connectivity estimates for structures with at least one  
247 injection centroid in the structure. Thus, the position of non-zero rows in Figure 3a shows the  
248 localization of Rbp4-Cre and Ntsr1-Cre injection centroids to layers 5 and 6 respectively (this is  
249 further examined in Supplemental Section ??). Thus, as a heuristic alternative model, to also  
250 synthesize information about leafs targeted by different cre-lines, we also generate an average  
251 connectivity matrix over all cre-lines. This model is not evaluated in our testing, and is only a general  
252 stand-in for overall behavior, but provides a useful summary of results.

253 Cell-class, while often correlated with cortical layer, is often a stronger driver of connectivity than  
254 summary structure. Figure 3b shows a collection of connectivity strengths generated using  
255 cre-specific models for wild-type, Cux2, Ntsr1, Rbp4, and Tlx3 cre-lines from visual signal processing  
256 leafs in the cortex to cortical and thalamic nucleii. We use hierarchical clustering to sort source  
257 structure/cell-class combinations by the similarity of their structural projections, and sort target  
258 structures by the structures from which they receive projections. Examining the former, we can see  
259 that the Ntsr1 Cre-line distinctly projects to thalamic nucleii, regardless of summary structure. This  
260 contrasts with the tendency of other cell-classes to project intracortically in a manner determined by  
261 the source structure. Similarly, layer 6 targets are not strongly projected to by any of the displayed  
262 Cre-lines. There are too many targeted summary structures to plot here, but we expect that the source  
263 profile of each target clusters by structure.



**Figure 3: Cell-class specificity.** 3a Selected cell-class and layer specific connectivities from VISp and MO. Sources without a injection of that Cre-type are not estimated due to lack of data for that Cre-line in that structure. 3b Heirarchical clustering of connectivity strengths from visual signal processing cell-types to cortical and thalamic targets. Cre-line, summary structure, and layer are labelled on the sources. Major brain division and layer are labelled on the targets.

**264 *Connectivity Analyses***

265 Each structural connectivity matrix is a high-dimensional realization of relatively few biological  
266 processes, and decomposition of neural signals to recover these processes is a fundamental goal in  
267 neuroscience. In this section, we apply non-negative matrix factorization to decompose the  
268 long-range wild-type connectivities into linear combinations of archetypal connectivities. This  
269 decomposes the remaining censored connectivity matrix into a linear model based off a relatively  
270 small number of distinct signals. This model is able to capture a large amount of the observed  
271 variability, and recovers structure-specific archetypal signals.

272 These signals are plotted in Figure 4, and technical details and intermediate results are given in  
273 Supplemental Sections 6 and 7, respectively. These details include a cross-validation based method  
274 for selecting the number of components, a masking method for focusing only on long range  
275 connections, and a stability method for ensuring that the decomposition is reliable across  
276 computational replicates. The plotted decomposition shows that these underlying connectivity  
277 archetypes correspond strongly to major brain division. However, certain components that  
278 predominantly represent connectivity from a given major brain division may also be accessed from  
279 other areas. For example, the IP and FN regions of CB are strongly associated in 4b with the  
280 component projecting to MY in 4a.

281 Inspection of the reconstructed distal normalized connection strength using the top 15  
282 components shows qualitatively shows that this relatively sparse decomposition is able to capture  
283 much of the observed variability. Layer-specific targeting is evident, indicating that the factorization  
284 method is detecting cell-type specific signals, even though it is trained only on the wild-type  
285 connectivity. Other connectivity patterns like cortical-cortical and cortical-thalamic are also detected.

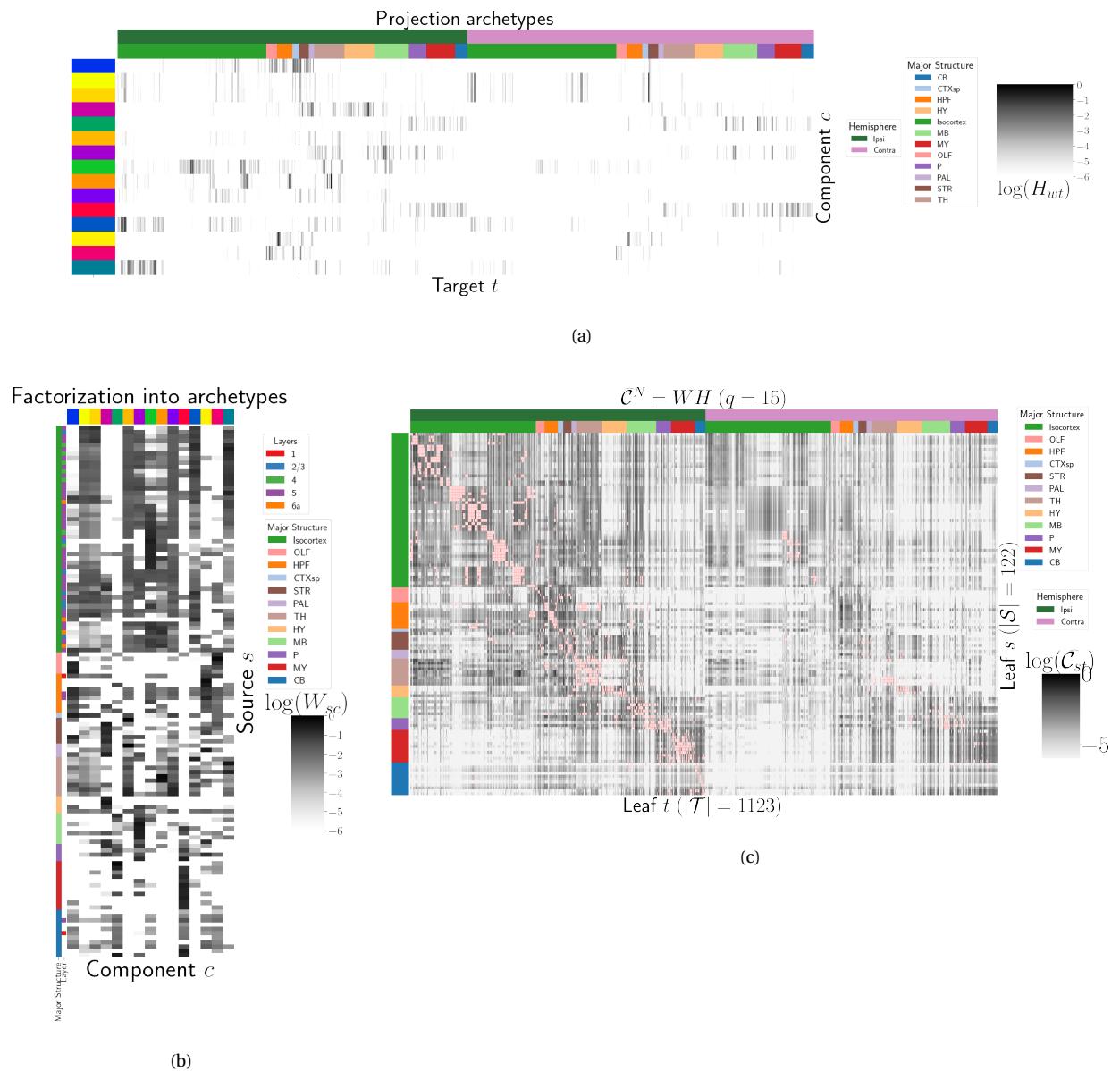


Figure 4: Non-negative matrix factorization results  $\mathcal{C}_w^N = W H$  for  $q = 15$  components. 4a Latent space coordinates  $H$  of  $\mathcal{C}$ . Target major structure and hemisphere are plotted. 4b Loading matrix  $W$ . Source major structure and layer are plotted. 4c Reconstruction of the normalized distal connectivity strength using the top 15 archetypes. Areas less than  $1500 \mu m$  apart are not modeled, and therefore shown in red.

## 4 DISCUSSION

<sup>286</sup> The model presented here is a milestone in characterization of connectomes. It is the first cell-type  
<sup>287</sup> specific whole brain projectomme for a mammalian species, and it opens the door for a large number  
<sup>288</sup> of models linking brain structure to computational architectures.

<sup>289</sup> The Nadaraya-Watson estimator presented here is novel. Beyond using a Nadaraya-Watson kernel  
<sup>290</sup> regression defined in physical space, we define a cell-type space based on similarities of projections,  
<sup>291</sup> and theoretically justify the use of an intermediate shape-constrained estimator. While methods like  
<sup>292</sup> non-negative least squares can also account for covariates, the centroid method from Knox et al.  
<sup>293</sup> (2019) was shown that the more precise notion of injection location than the non-negative least  
<sup>294</sup> squares in Oh et al. (2014). Furthermore, our sample size seems too low to utilize a fixed or mixed  
<sup>295</sup> effect, particularly since the impact of the virus depend on the particular injection region. In a sense  
<sup>296</sup> both the NNLS and NW models can be thought of as improvements over the structure-specific  
<sup>297</sup> average, and so is also possible that a yet undeveloped residual-based data-driven blend of these  
<sup>298</sup> models could provide improved performance.

<sup>299</sup> We see several other opportunities for improving on our model. Ours is certainly not the first  
<sup>300</sup> cross-validation based model averaging method Gao, Zhang, Wang, and Zou (2016). However, our use  
<sup>301</sup> of shape-constrained estimator in target-encoded feature space is novel and fundamentally different  
<sup>302</sup> from Nadaraya-Watson estimators that use an optimization method for selecting the weights (Saul &  
<sup>303</sup> Roweis, 2003). The properties of this estimator, as well as its relation to estimators fit using an  
<sup>304</sup> optimization algorithm, are a possible future avenue of research. A deep model such as Lotfollahi,  
<sup>305</sup> Naghipourfar, Theis, and Alexander Wolf (2019) could be appropriate, provided enough data was  
<sup>306</sup> available. Finally, a Wasserstein-based measure of injection similarity per structure would combine  
<sup>307</sup> both the physical simplicity of the centroid model while also incorporating the full distribution of the  
<sup>308</sup> injection signal.

<sup>309</sup> The factorization of the connectivity matrix could also be improved and better utilized. From a  
<sup>310</sup> statistical perspective, stability-based method for establishing archetypal connectivities in NMF is  
<sup>311</sup> similar to those applied to genomic data Kotliar et al. (2019); Wu et al. (2016). However, non-linear  
<sup>312</sup> data transformations or matrix decompositions, or tensor factorizations that account for correlations

<sup>313</sup> between cell-types could better capture the true nature of archetypal neural connections. It would  
<sup>314</sup> also be of great interest to associate the archetypal signals detected from connectivity analysis with  
<sup>315</sup> undergirding gene expression patterns or functional information.

## ACKNOWLEDGMENTS

- <sup>316</sup> The Funder and award ID information you input at submission will be introduced by the publisher  
<sup>317</sup> under a Funding Information head during production. Please use this space for any additional  
<sup>318</sup> acknowledgements and verbiage required by your funders.

319 This supplement is divided into information about our dataset, supplemental methods, and  
320 supplemental results. However, certain topics are revisited between sections. Thus, if a reader is  
321 interested in, say, non-negative matrix factorization, they may find relevant information in both  
322 methods and results.

## 5 SUPPLEMENTAL INFORMATION

323 Our supplementary information consists of abundances of leaf/Cre-line combinations, information  
324 about distances between structures, and the size of our restricted evaluation dataset.

### 325 *Cre/structure combinations in $\mathcal{D}$*

326 This section describes the abundances of leaf and Cre-line combinations in our dataset. Users of the  
327 connectivity matrices who are interested in a particular Cre-line or structure can see the quantity and  
328 type of data used to compute and evaluate that connectivity.

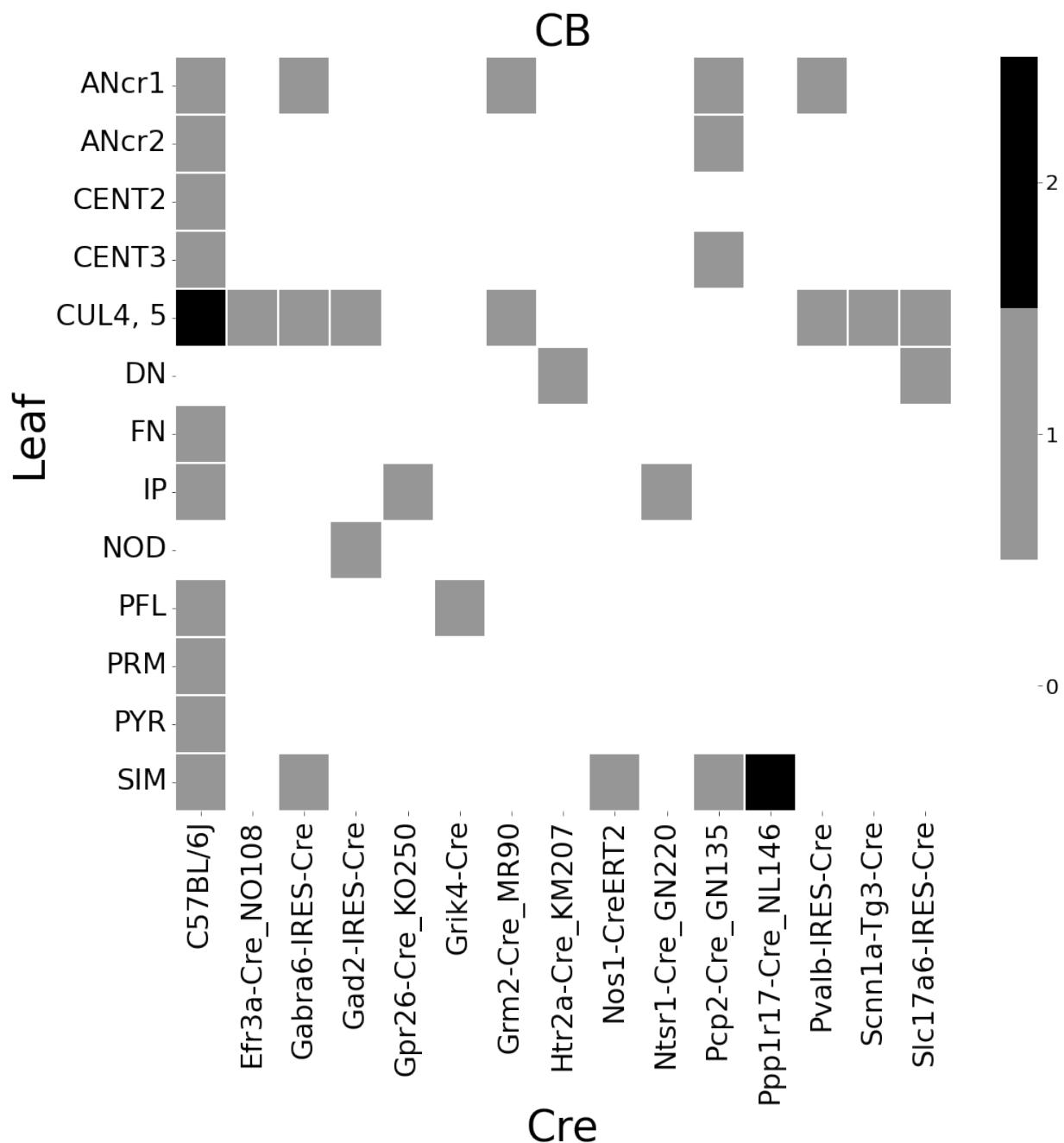


Figure 5: Abundances of cre-line and leaf-centroid combinations.

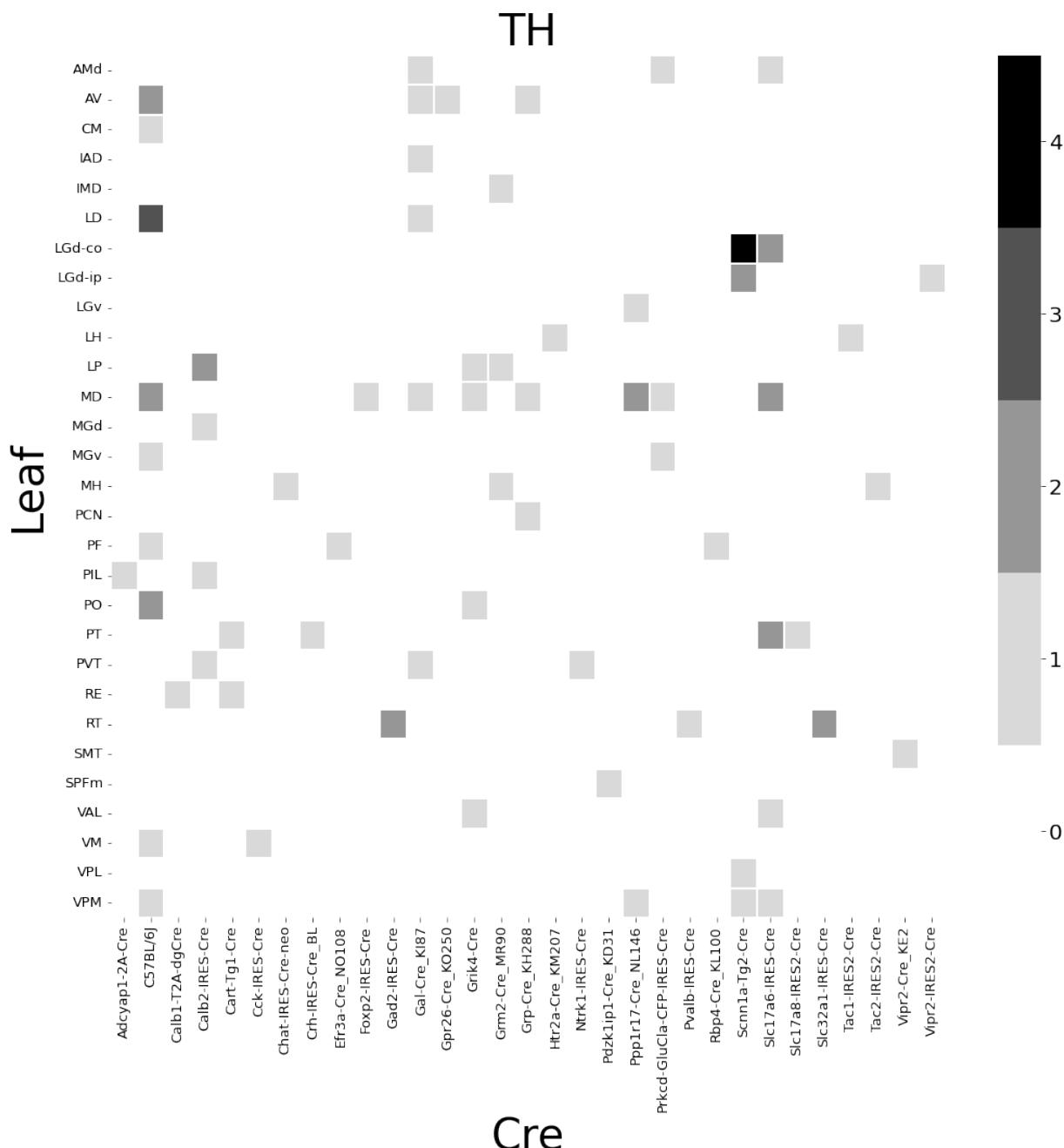


Figure 6: Abundances of cre-line and leaf-centroid combinations.

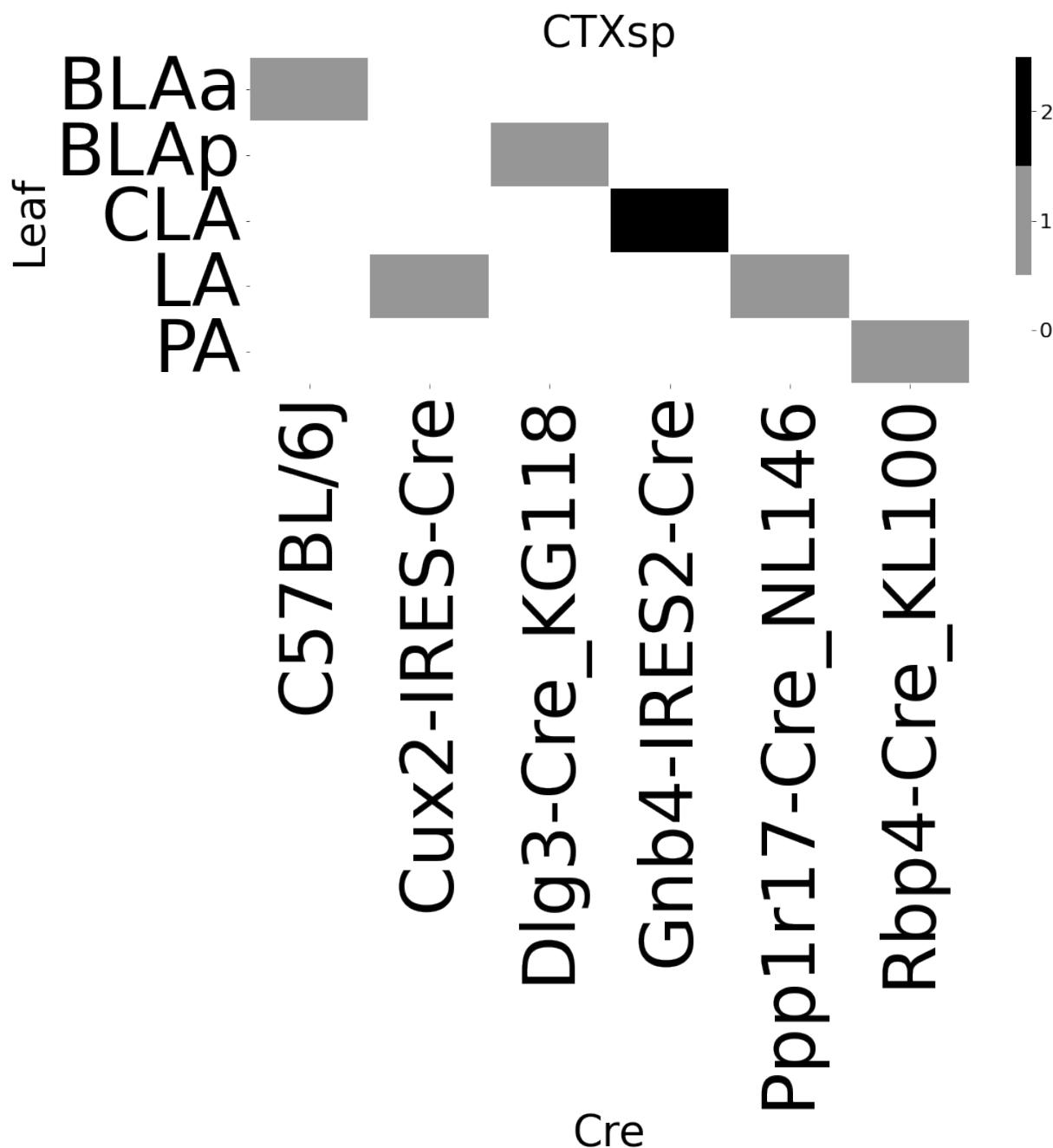


Figure 7: Abundances of cre-line and leaf-centroid combinations.

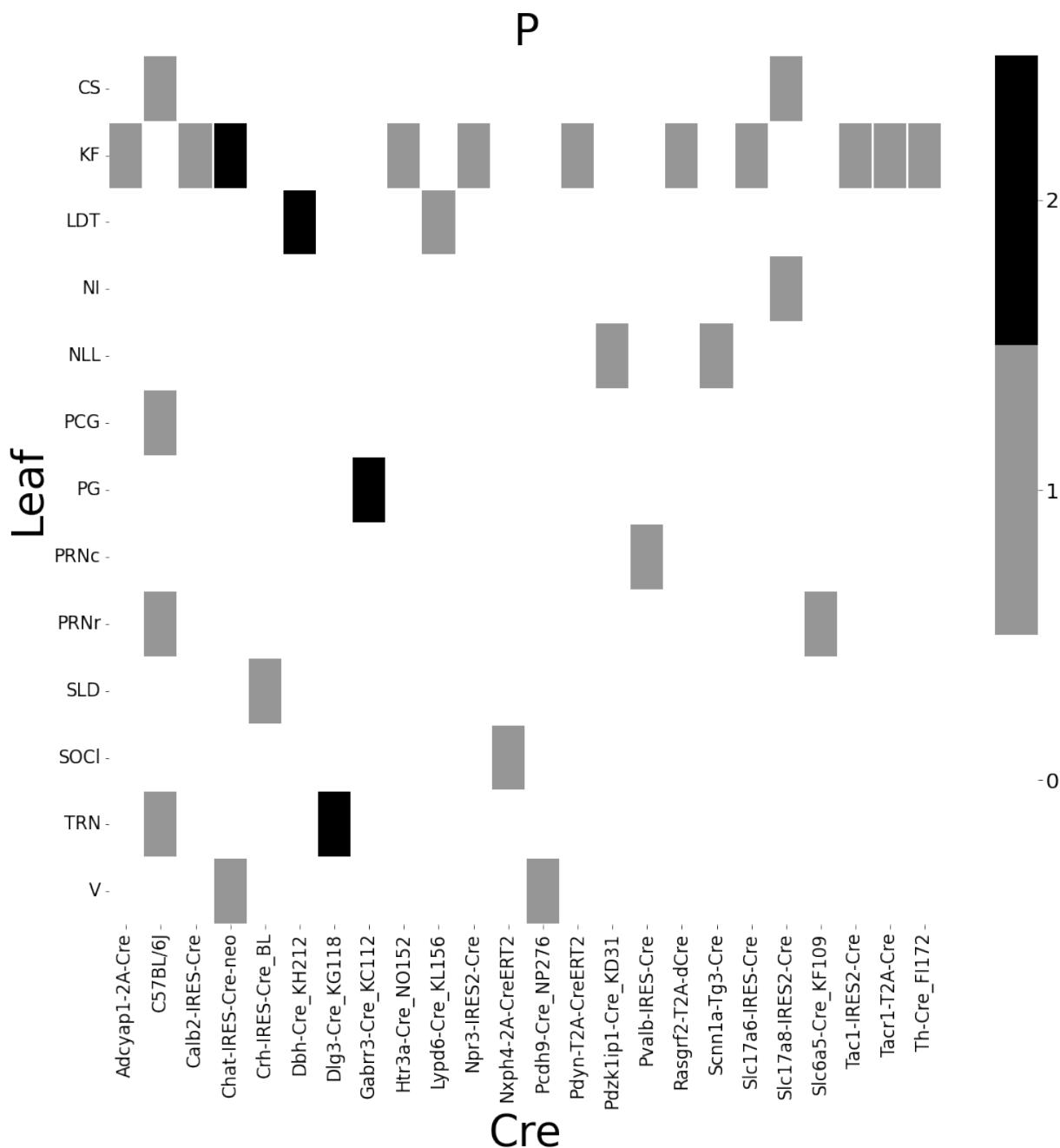


Figure 8: Abundances of cre-line and leaf-centroid combinations.

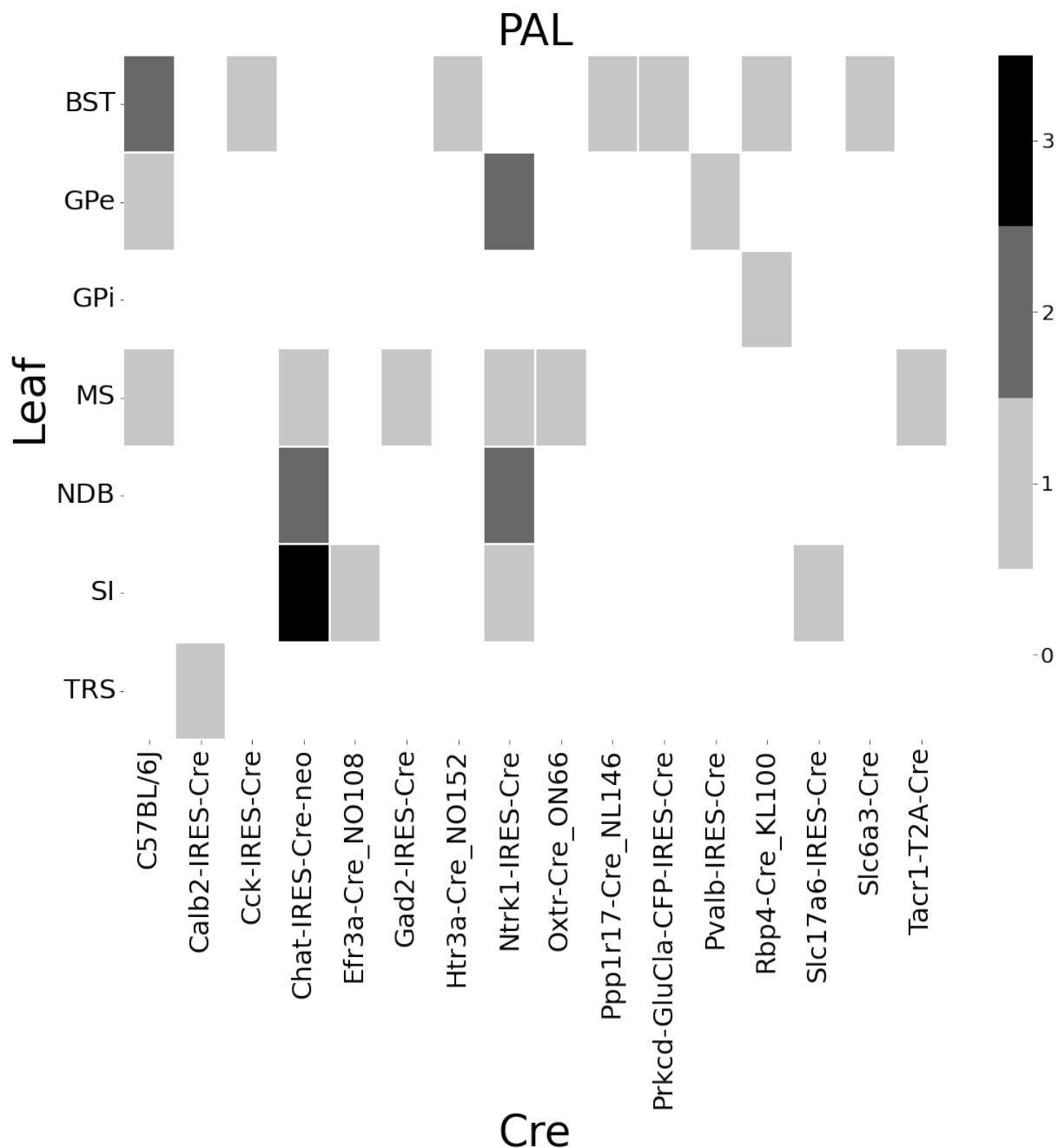


Figure 9: Abundances of cre-line and leaf-centroid combinations.

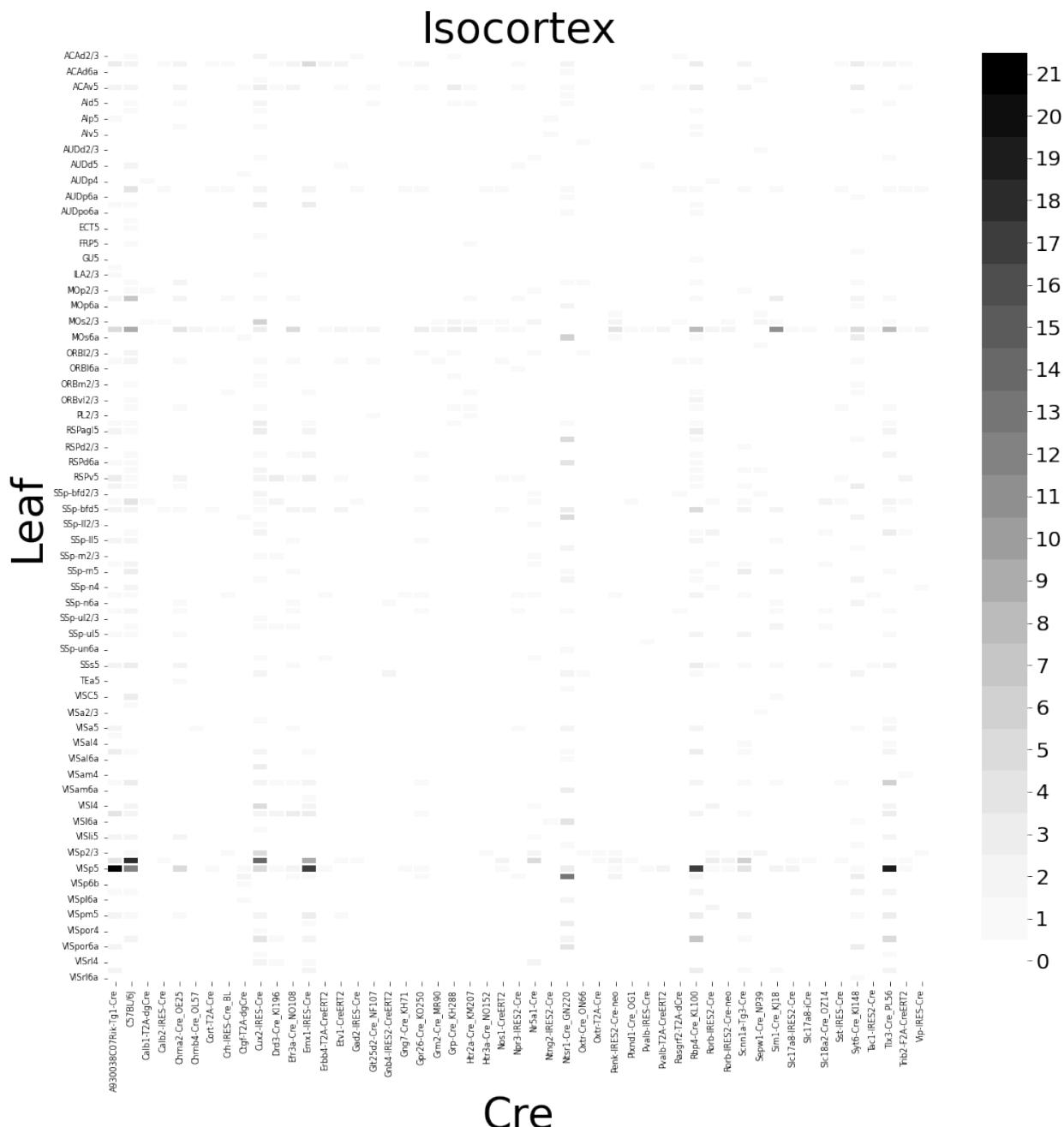


Figure 10: Abundances of cre-line and leaf-centroid combinations.

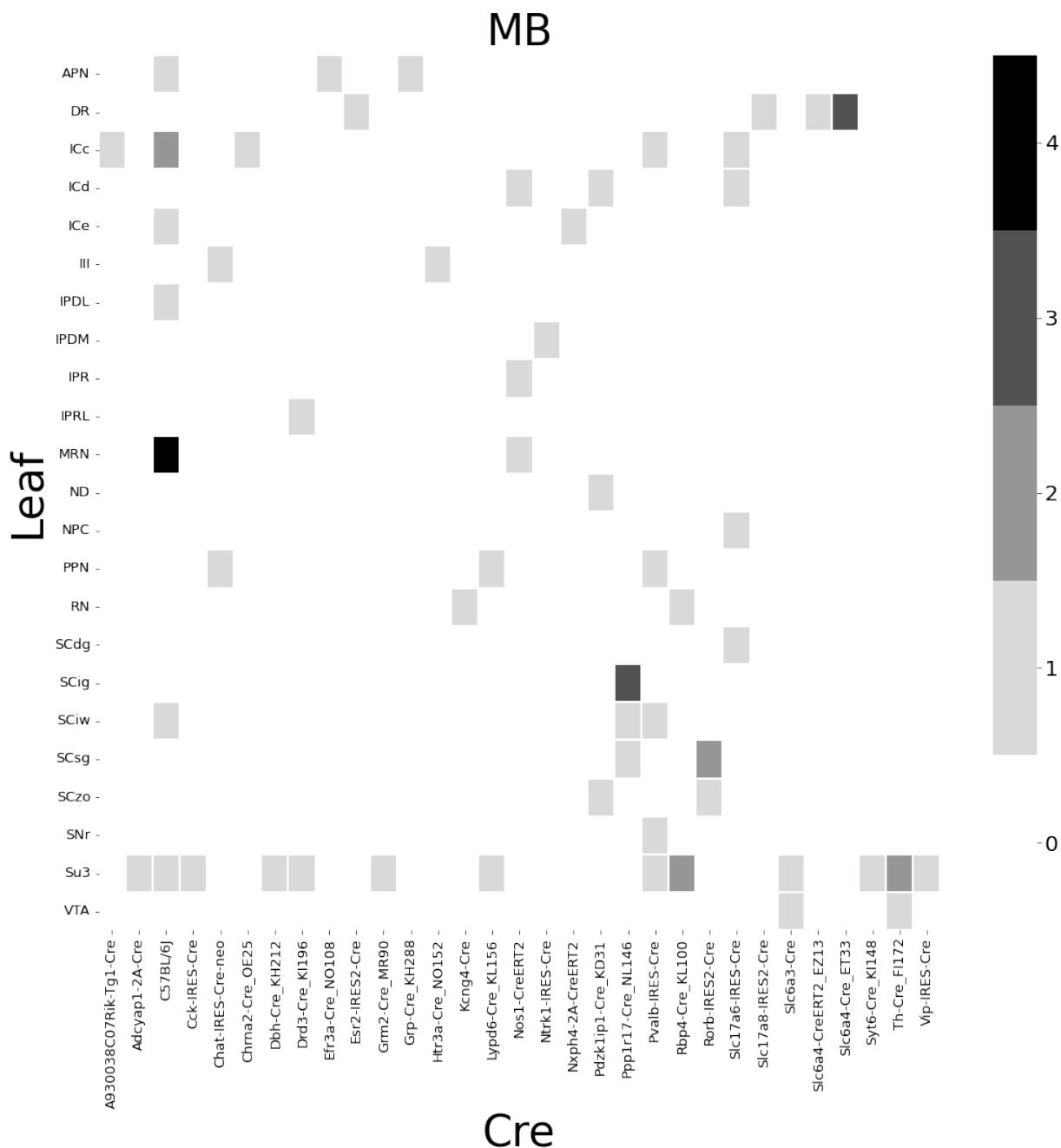


Figure 11: Abundances of cre-line and leaf-centroid combinations.

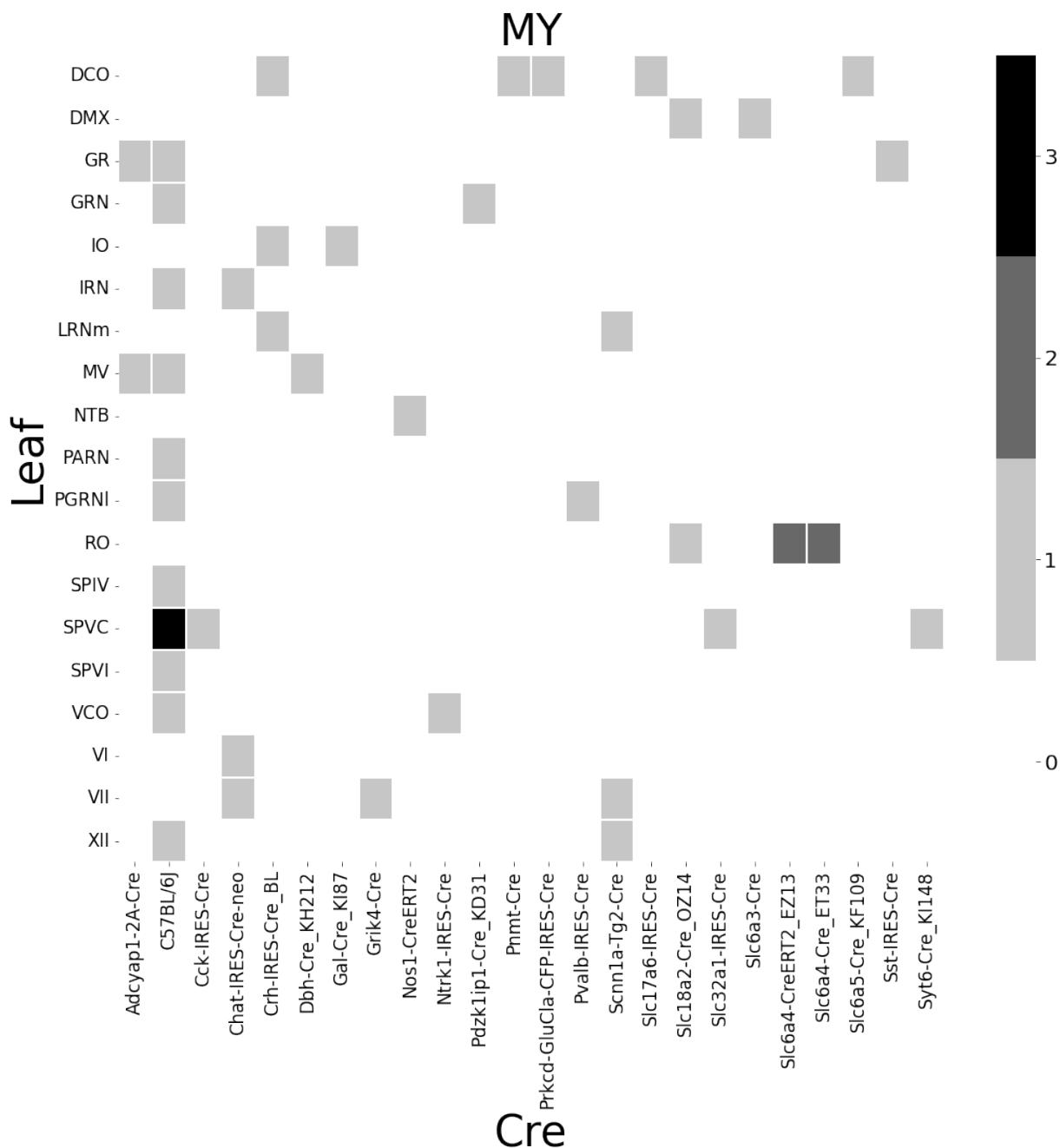


Figure 12: Abundances of cre-line and leaf-centroid combinations.

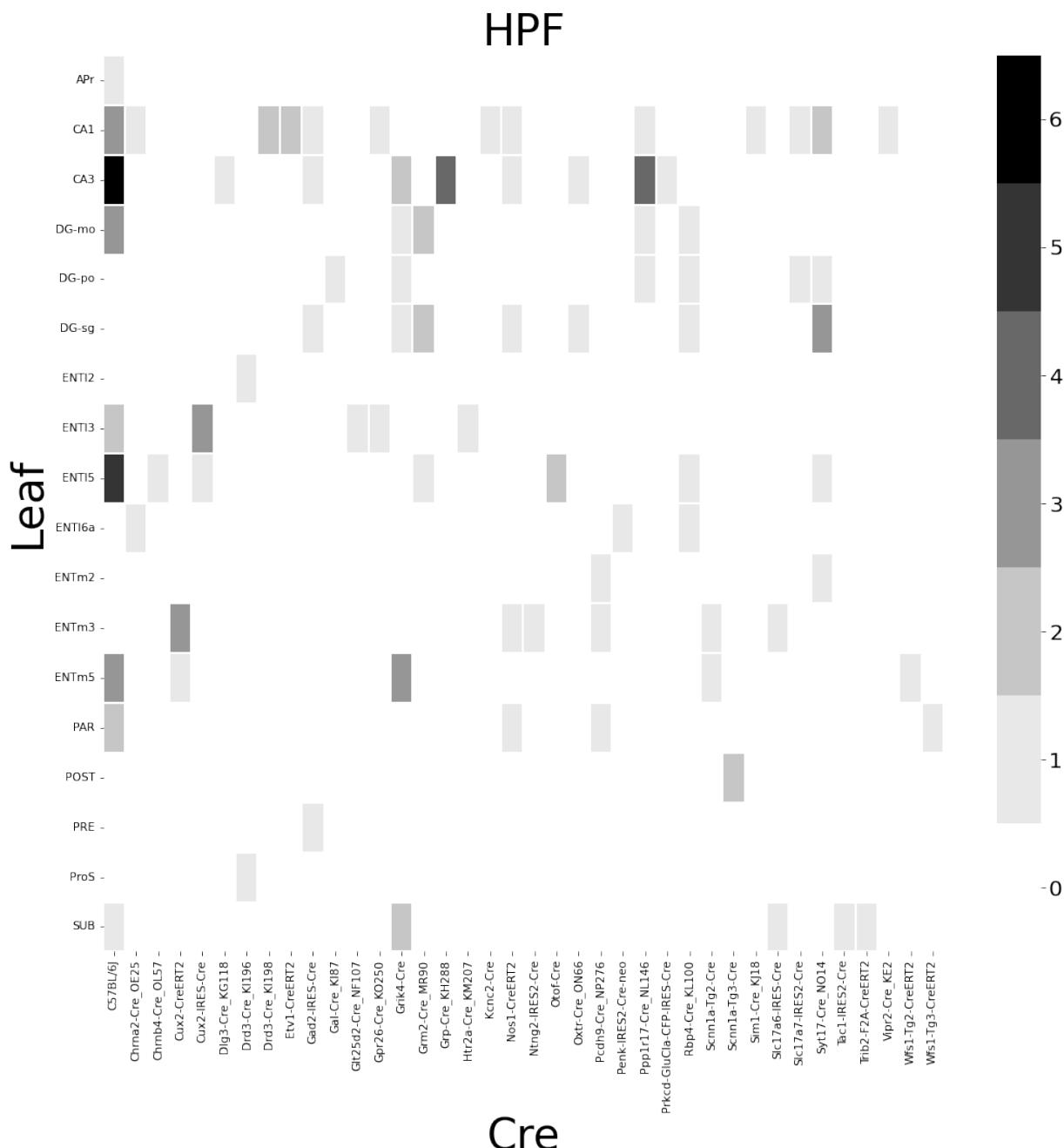


Figure 13: Abundances of cre-line and leaf-centroid combinations.

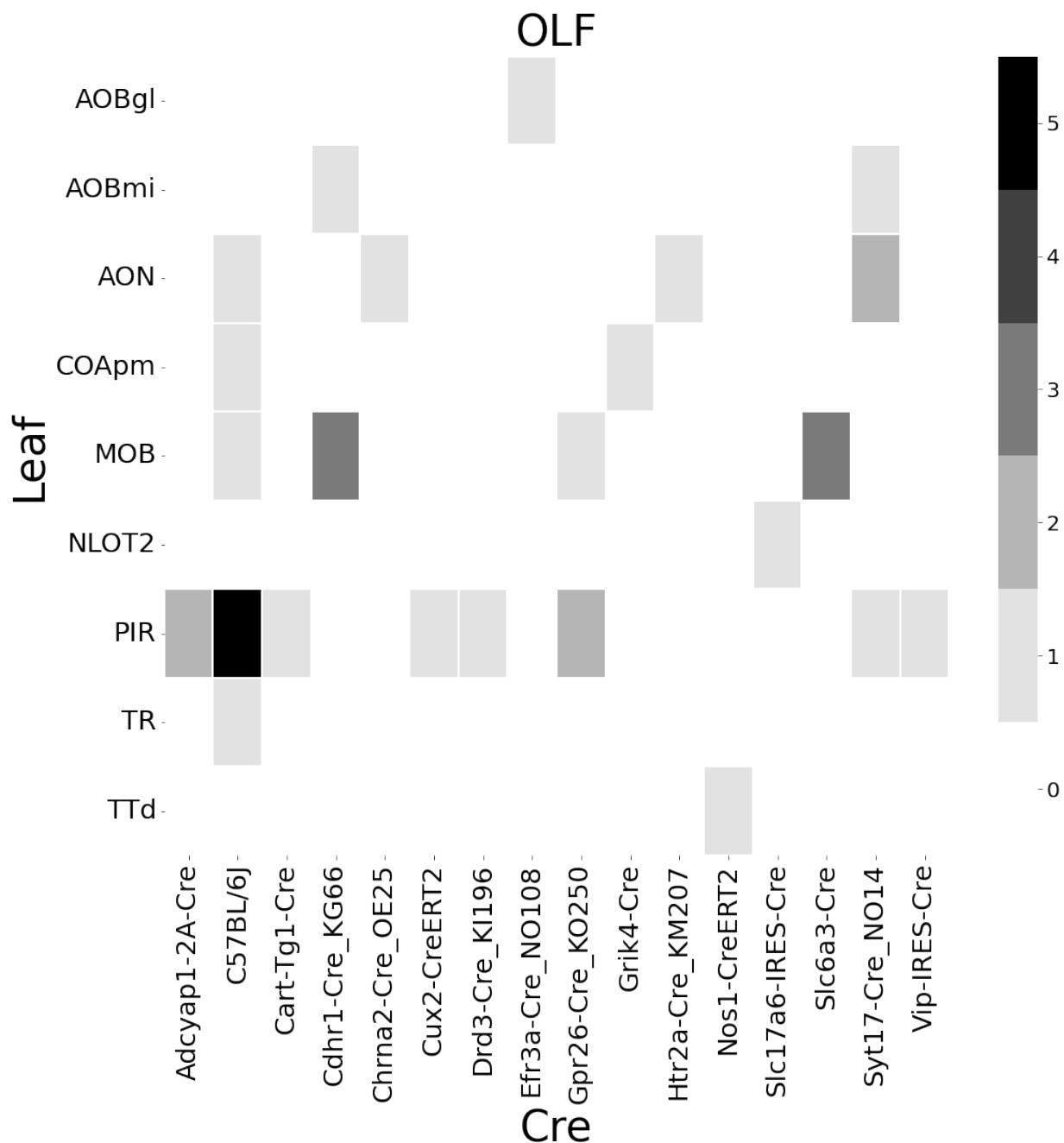


Figure 14: Abundances of cre-line and leaf-centroid combinations.

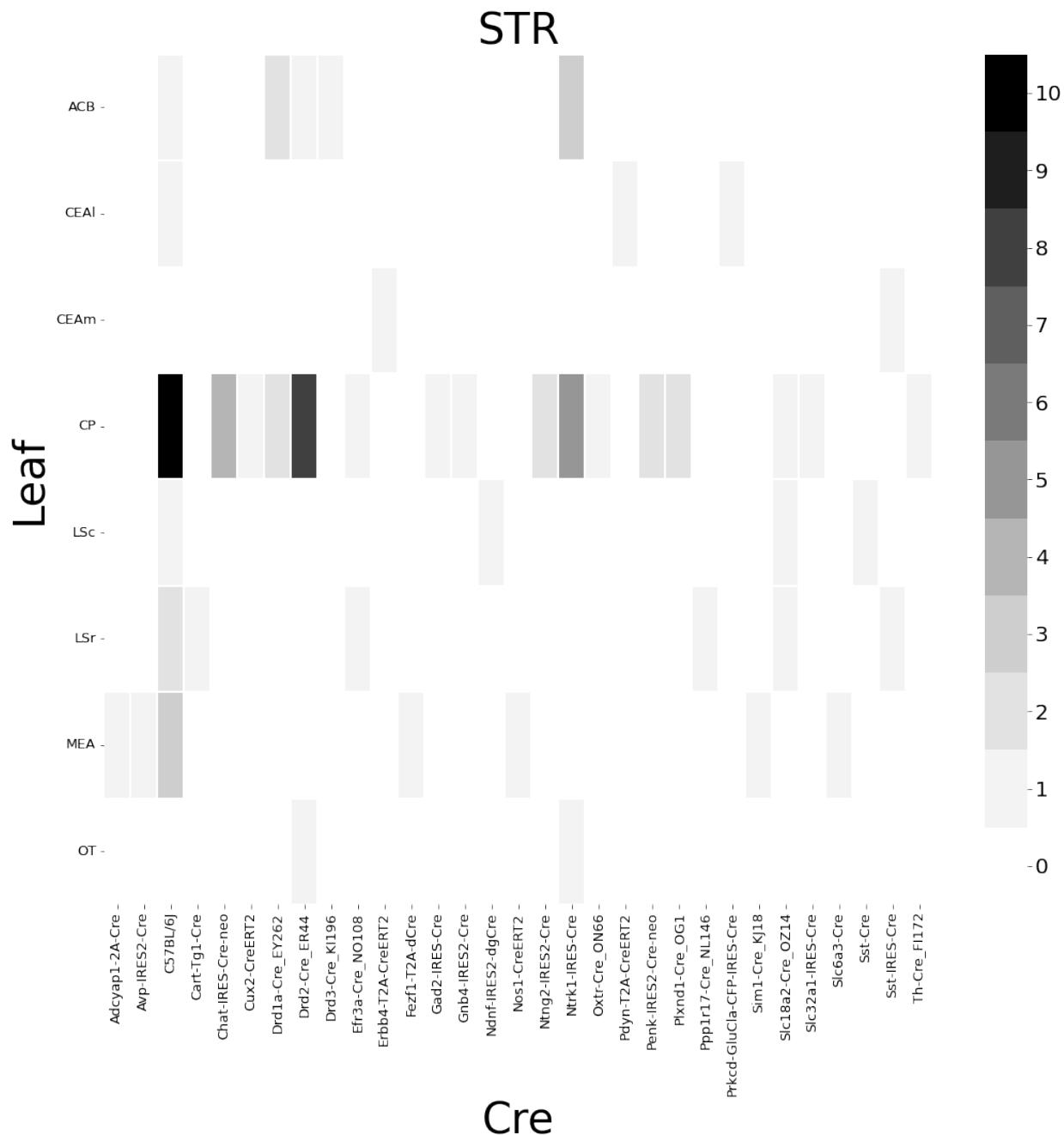


Figure 15: Abundances of cre-line and leaf-centroid combinations.

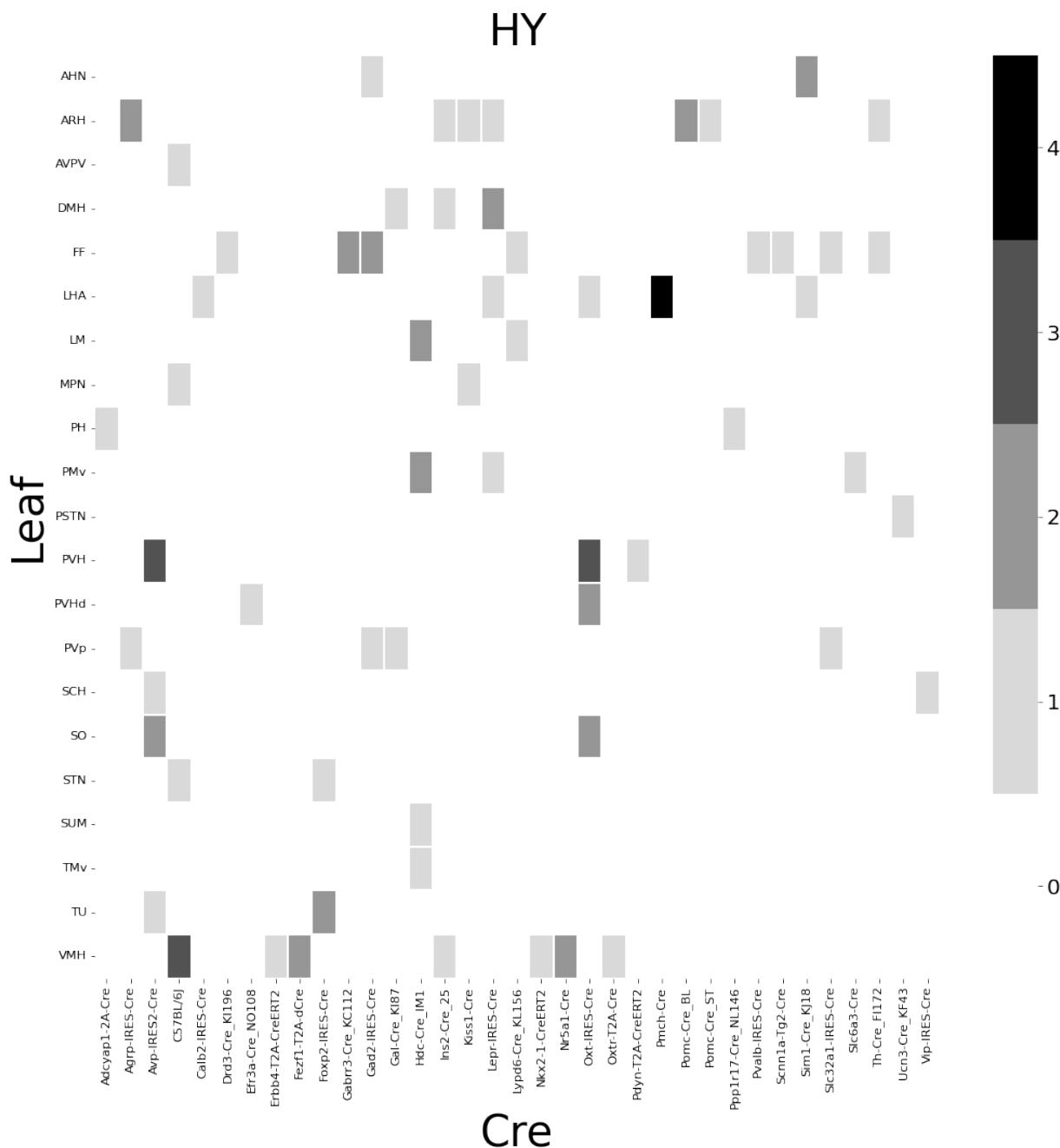


Figure 16: Abundances of cre-line and leaf-centroid combinations.

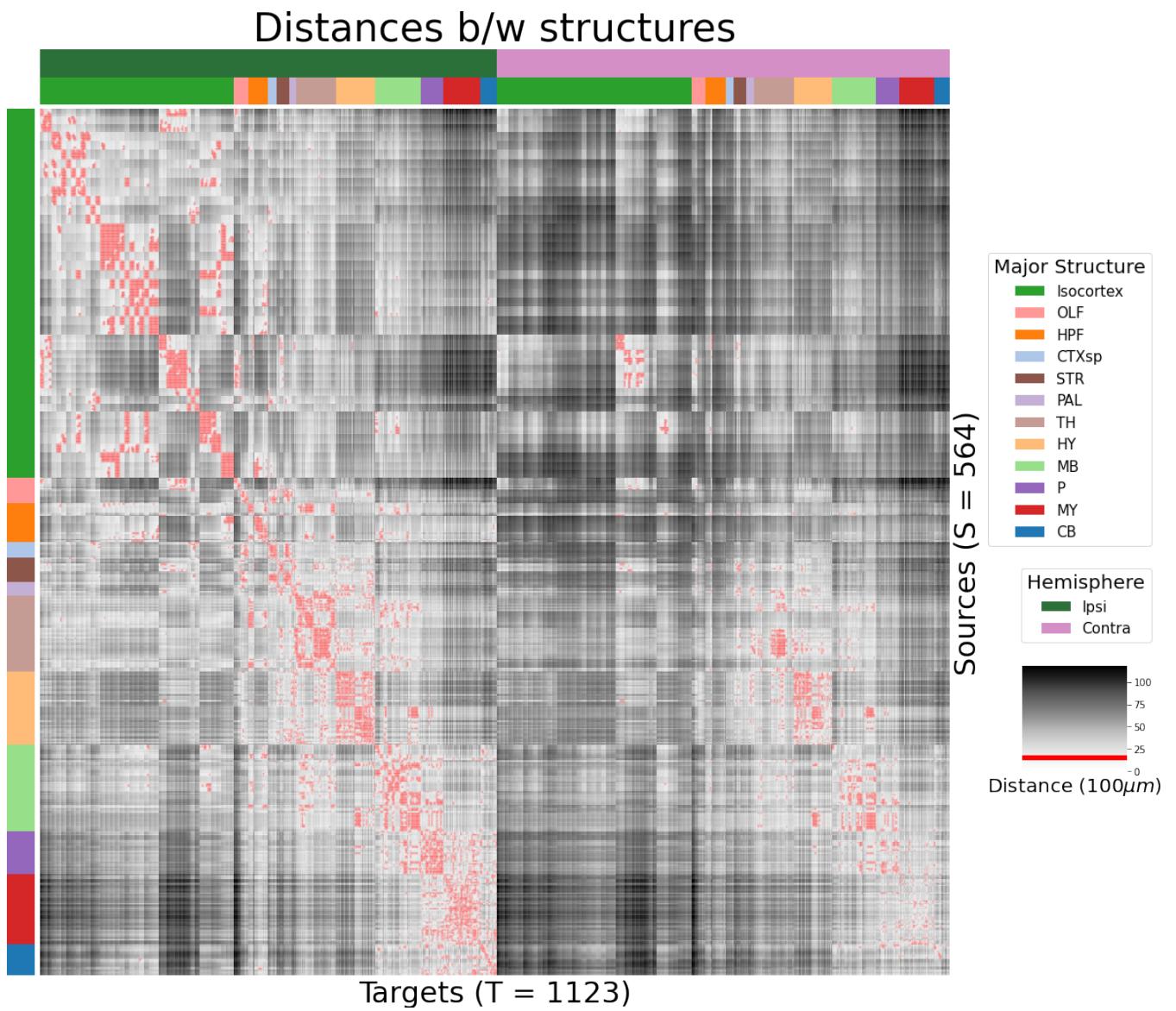
329 ***Distances between structures***

Figure 17: Distance between structures. Short-range connections are masked in red

**330 Model evaluation**

**331** We give information on the quality of our models. This includes the sizes of our evaluation sets in  
**332** leave-one-out cross-validation and additional losses in the injection-normalized case.

**333** NUMBER OF EXPERIMENTS IN EVALUATION SETS In order to compare between methods, we therefore  
**334** restrict to the smallest set of evaluation indices, which is to say, virus-leaf combinations that are  
**335** present at least twice. This means that our evaluation set in size is smaller than our evaluation set is  
**336** smaller in size than our overall list of experiments.

	Total	Cre-Leaf	Cre-leaf over threshold
Isocortex	36	4	4
OLF	7	2	2
HPF	122	62	59
CTXsp	85	41	38
STR	1128	732	7
PAL	68	18	17
TH	46	7	7
HY	35	17	17
MB	33	8	8
P	30	11	11
MY	78	45	44
CB	83	29	29

Table 3: Number of experiments available to evaluate models in leave-one-out cross validation. Models that rely on a finer granularity of modeling have less data available to validate with.

337 INJECTION-NORMALIZED LOSSES To compare with the injection-normalization procedure from Knox  
 338 et al. (2019), we also remove experiments with small injection, and here give results for this slightly  
 339 reduced set using injection-normalization. That is, instead of dividing the projection signal of each  
 340 experiment by its  $l_1$  norm, we divide by the  $l_1$  norm of the corresponding injection signal. We find  
 341 that setting a summed injection-signal of threshold of 1 is sufficient for evading pathological edge  
 342 cases in this normalization, while still retaining a large evaluation set.

$\hat{f}$	Mean	NW					EL
$\mathcal{D}$	$I_c \cap I_L$	$I_c \cap I_M$	$I_c \cap I_L$	$I_L$	$I_{wt} \cap I_M$	$I_M$	$I_L$
Isocortex	0.413	0.453	0.408	0.538	0.528	0.528	<b>0.396</b>
OLF	0.499	0.504	0.494	0.441	0.543	0.543	<b>0.437</b>
HPF	0.336	0.483	0.332	0.444	0.501	0.501	<b>0.321</b>
CTXsp	0.497	0.497	0.497	0.497	0.497	0.497	0.497
STR	0.359	0.386	0.359	0.364	0.433	0.433	<b>0.322</b>
PAL	0.519	0.497	0.519	0.436	0.459	0.459	<b>0.434</b>
TH	0.769	0.767	0.769	<b>0.514</b>	0.539	0.539	0.556
HY	0.414	0.439	0.414	0.441	0.452	0.452	<b>0.399</b>
MB	0.459	0.396	0.397	0.358	<b>0.324</b>	<b>0.324</b>	0.403
P	<b>0.562</b>	<b>0.562</b>	<b>0.562</b>	0.758	0.764	0.764	<b>0.562</b>
MY	0.699	0.552	0.621	<b>0.439</b>	0.578	0.578	<b>0.439</b>
CB	0.849	0.689	0.849	0.500	0.615	0.615	<b>0.495</b>

Table 4: Losses from leave-one-out cross-validation of candidate for injection-normalized structural connectivity on injection-thresholded evaluation set. **Bold** numbers are best for their major structure.

<sup>343</sup> PROJECTION-NORMALIZED LOSSES ON THRESHOLDED SET We also give results for the  
<sup>344</sup> projection-normalization procedure from the main text on this reduced subset.

$\hat{f}$	Mean	NW		EL				
	$\mathcal{D}$	$I_c \cap I_L$	$I_c \cap I_M$	$I_c \cap I_L$	$I_L$	$I_{wt} \cap I_M$	$I_M$	$I_L$
Isocortex	0.229	0.248	0.224	0.274	0.269	0.269	<b>0.217</b>	
OLF	0.193	0.233	0.191	<b>0.135</b>	0.179	0.179	0.138	
HPF	0.178	0.342	<b>0.172</b>	0.212	0.235	0.235	<b>0.172</b>	
CTXsp	<b>0.621</b>	<b>0.621</b>	<b>0.621</b>	<b>0.621</b>	<b>0.621</b>	<b>0.621</b>	<b>0.621</b>	
STR	0.128	<b>0.117</b>	0.124	0.171	0.234	0.234	0.125	
PAL	0.203	0.205	0.203	0.295	0.291	0.291	<b>0.188</b>	
TH	0.673	0.664	0.673	<b>0.358</b>	0.379	0.379	0.417	
HY	0.358	0.378	0.351	0.331	0.312	<b>0.312</b>	0.314	
MB	0.168	0.191	<b>0.160</b>	0.199	0.202	0.202	<b>0.160</b>	
P	0.292	0.292	0.292	0.299	0.299	0.299	<b>0.287</b>	
MY	0.268	0.347	0.268	<b>0.167</b>	0.189	0.189	0.196	
CB	0.062	0.062	0.062	0.068	0.108	0.108	<b>0.061</b>	

Table 5: Losses from leave-one-out cross-validation of candidate for normalized structural connectivity on injection-thresholded evaluation set. **Bold** numbers are best for their major structure.

## 6 SUPPLEMENTAL METHODS

<sup>345</sup> This section consists of additional information on preprocessing of the neural connectivity data,  
<sup>346</sup> estimation of connectivity, and matrix factorization.

<sup>347</sup> ***Data preprocessing***

<sup>348</sup> Several data preprocessing steps take place prior to evaluations of the connectivity matrices. These  
<sup>349</sup> steps are described in Algorithm PREPROCESS. The arguments of this normalization process - injection  
<sup>350</sup> signals  $x(i)$ , projection signals  $y(i)$ , injection fraction  $F(i)$ , and data quality mask  $q(i)$  - were  
<sup>351</sup> downloaded using the Allen SDK. The injections and projection signals  $\mathcal{B} \rightarrow [0, 1]$  were segmented  
<sup>352</sup> manually in histological analysis. The projection signal gives the proportion of pixels within the voxel  
<sup>353</sup> displaying fluorescence, and the injection signal gives the proportion of pixels within the  
<sup>354</sup> histologically-selected injection subset displaying fluorescence. The injection fraction  $\mathcal{B} \rightarrow [0, 1]$  gives  
<sup>355</sup> the proportion of pixels within each voxel in the injection subset. Finally, the data quality mask  
<sup>356</sup>  $\mathcal{B} \rightarrow \{0, 1\}$  gives the voxels that have valid data.

<sup>357</sup> Our preprocessing makes use of the above ingredients, as well as several other essential steps. First,  
<sup>358</sup> we compute the weighted injection centroid

$$c(i) = \sum_{l \in \mathcal{B}} x(i)(l) l$$

<sup>359</sup> where  $x(i)(l)$  is the injection density at location  $l \in \mathbb{R}^3$ . Given a regionalization  $\mathcal{R}$  from the Allen SDK,  
<sup>360</sup> we can also access regionalization map  $R: \mathcal{B} \rightarrow \mathcal{R}$ . This induces a functional of connectivities from  
<sup>361</sup> the space of maps  $\{\mathcal{X} = x: \mathcal{B} \rightarrow [0, 1]$

$$\begin{aligned} 1_{\mathcal{R}}: \mathcal{X} &\rightarrow \mathcal{R} \times \mathbb{R}_{\geq 0} \\ x &\mapsto \sum_{l \in r} x(l) \text{ for } r \in \mathcal{R}. \end{aligned}$$

<sup>362</sup> We also can restrict a signal to a individual structure as

$$\begin{aligned} 1|_s: \mathcal{X} &\rightarrow \mathcal{X} \\ x(l) &= \begin{cases} x(l) & \text{if } l \in S \\ 0 & \text{otherwise.} \end{cases} \end{aligned}$$

<sup>363</sup> Finally, given a vector or array  $a \in \mathbb{R}^T$ , we have the  $L1$  normalization map

$$n: a \mapsto \frac{a}{\sum_{j=1}^T a_j}.$$

<sup>364</sup> We define these objects as functions and functionals, but this is for notational convenience and  
<sup>365</sup> non-essential. A function  $x(i) : \mathcal{B} \rightarrow [0, 1]$  is mathematically equivalent to the graph  
<sup>366</sup>  $\mathcal{G}(x(i)) \in \mathcal{B} \times [0, 1]$ . As an abuse of notation, we define  $x \odot x' := z$  such that  $z(l) = x(l)x'(l)$  for all  $l \in \mathcal{B}$ .  
<sup>367</sup> Also, denote  $m(i)$  as the major structure containing experiment  $i$ . We then can write the  
<sup>368</sup> preprocessing algorithm.

---

**PREPROCESS 1 Input** Injection  $x(i)$ , Projection  $y(i)$ , Injection centroid  $c(i) \in \mathbb{R}^3$ , injection fraction  $F(i)$ ,  
 data quality mask  $q(i)$

---

Injection fraction  $x_F(i) \leftarrow x(i) \odot F(i)$

Data-quality censor  $y_q(i) \leftarrow \odot y(i) \odot q(i), x_q(i) \leftarrow x_F(i) \odot q(i)$

Restrict injection  $x_m(i) = 1|_{m(i)} x_q(i)$ .

Compute centroid  $c(i)$  from  $x_m(i)$

Regionalize  $\tilde{y}_{\mathcal{T}}(i) \leftarrow 1_{\mathcal{T}}(y_q(i))$

Normalize  $y_{\mathcal{T}}(i) \leftarrow n(\tilde{y}_{\mathcal{T}}(i))$

---

**Output**  $\tilde{y}_{\mathcal{T}}(i), c(i)$

---

369 **Estimators**

370 As mentioned previously, we can consider our estimators as modeling a connectivity vector  
 371  $f_{\mathcal{T}}(\nu, s) \in \mathbb{R}_{\geq 0}^T$ . Thus, for the remainder of this section, we will discuss only  $f(\nu, s)$ . We review the  
 372 Nadaraya-Watson estimator from Knox et al. (2019), and describe its conversion into our cell-class  
 373 specific Expected Loss estimator.

374 *Centroid-based Nadaraya-Watson* In the Nadaraya-Watson approach of Knox et al. (2019), the injection  
 375 is considered only through its centroid  $c(i)$ , and the projection is considered regionalized. That is,

$$f_*(i) = \{c(i), y_{\mathcal{T}}(i)\}.$$

376 Since the injection is considered only by its centroid, this model only generates predictions for  
 377 particular locations  $l$ , and the prediction for a structure  $s$  is given by integrating over locations within  
 378 the structure

$$f^*(\hat{f}(f_*(\mathcal{D})))(\nu, s) = \sum_{l \in s} \hat{f}(f_*(\mathcal{D}(I)))(\nu, l).$$

379 Here,  $I$  is the training data, and  $\hat{f}$  is the Nadaraya-Watson estimator

$$\hat{f}_{NW}(c(I), y_{\mathcal{T}}(I))(l) := \sum_{i \in I} \frac{\omega_{il}}{\sum_{i \in I} \omega_{il}} y_{\mathcal{T}}(i)$$

380 where  $\omega_{il} := \exp(-\gamma d(l, c(i))^2)$  and  $d$  is the Euclidean distance between centroid  $c(i)$  and voxel with  
 381 position  $l$ .

382 Several facets of the estimator are visible here. A smaller  $\gamma$  corresponds to a greater amount of  
 383 smoothing, and the index set  $I \subseteq \{1 : n\}$  generally depends on  $s$  and  $\nu$ . Fitting  $\gamma$  via empirical risk  
 384 minimization therefore bridges between 1-nearest neighbor prediction and averaging of all  
 385 experiments in  $I$ . In Knox et al. (2019),  $I$  consisted of experiments sharing the same brain division, i.e.  
 386  $I = I_m$ , while restricting of index set to only include experiments with the same cell class gives the  
 387 class-specific Cre-NW model. Despite this restriction, we fit  $\gamma$  for each  $m$  rather than a smaller subset  
 388 like  $s$  or  $\nu$ . That is,

$$\hat{\gamma}_m = \arg \min_{\gamma \in \mathbb{R}_{\geq 0}} \frac{1}{|\{s, \nu\}|} \sum_{s, \nu \in \{m, \mathcal{V}\}} \frac{1}{|I_s \cap I_\nu|} \sum_{i \in (I_s \cap I_\nu)} \ell(y_{\mathcal{T}}(i)), \hat{f}_{\mathcal{T}}(f_*(\mathcal{D}(\nu, s) \setminus i)). \quad (2)$$

<sup>389</sup> *The Expected-Loss estimator* Besides the injection location, the targeted cell class also influences  
<sup>390</sup> projection. Since Cre-lines that target similar classes are induce similar projections, and including  
<sup>391</sup> similar Cre-lines in the Nadaraya-Watson estimator increases effective sample size, we introduce an  
<sup>392</sup> estimator that assigns a predictive weight to each training point that depends both on its  
<sup>393</sup> centroid-distance and Cre-line. This weight is determined by the expected prediction error of each of  
<sup>394</sup> the two feature types, as determined by cross-validation. For this reason, we call this the Expected  
<sup>395</sup> Loss Estimator. The resulting weights are then utilized in a Nadaraya-Watson estimator in a final  
<sup>396</sup> prediction step.

<sup>397</sup> We formalize Cre-line behavior as the average regionalized projection of a Cre-line in a given  
<sup>398</sup> structure (i.e. leaf). This vectorization of categorical information is known as **target encoding**, and we  
<sup>399</sup> define this as  $\bar{y}_{\mathcal{T},s,v} := \frac{1}{|I_s \cap I_v|} \sum_{i \in (I_s \cap I_v)} y_{\mathcal{T}}(i)$ . We define a **Cre-distance** in a leaf to be the distance  
<sup>400</sup> between the target-encoded projections of two Cre-lines. The relative predictive accuracy of  
<sup>401</sup> Cre-distance and centroid distance is determined by fitting a surface of projection distance as a  
<sup>402</sup> function of Cre-distance and centroid distance.

<sup>403</sup> In mathematical terms, our full feature set consists of the centroid coordinates and the  
<sup>404</sup> target-encoded means of the combinations of virus type and injection-centroid structure. That is,

$$f_*(\mathcal{D}_i) = \{c(i), \{\bar{y}_{\mathcal{T},s,v} \forall v\}, y_{\mathcal{T}}(i)\}.$$

<sup>405</sup>  $f^*$  is defined as in (2). The expected loss estimator is then

$$\hat{f}_{EL}(c(I), y_{\mathcal{T}}(I))(l, v) := \sum_{i \in I} \frac{v_{ilv}}{\sum_{i \in I} v_{ilv}} y_{\mathcal{T}}(i)$$

<sup>406</sup> where

$$v_{ilv} := \exp(-\gamma g(d(l, c(i))^2, d(\bar{y}_{\mathcal{T},s,v}, \bar{y}_{\mathcal{T},s,v(i)})^2))$$

<sup>407</sup> and  $s$  is the structure containing  $l$ .

<sup>408</sup> The key step therefore is finding a suitable  $g$  with which to weight the positional and Cre  
<sup>409</sup> information. Note that  $g$  must be a concave, non-decreasing function of its arguments with with  
<sup>410</sup>  $g(0, 0) = 0$ , then  $g$  defines a metric on the product of the metric spaces defined by experiment centroid  
<sup>411</sup> and target-encoded cre-line, and  $\hat{f}_{EL}$  is a Nadaraya-Watson estimator. A derivation of this fact is given

<sup>412</sup> later in this section, and we therefore use shape-constrained B-splines to estimate  $g$ . Similarly to the  
<sup>413</sup> Nadaraya-Watson model, we make the decision to fit a  $g$  separately for each major brain division. We  
<sup>414</sup> can then select  $\hat{\gamma}$  as in 2.

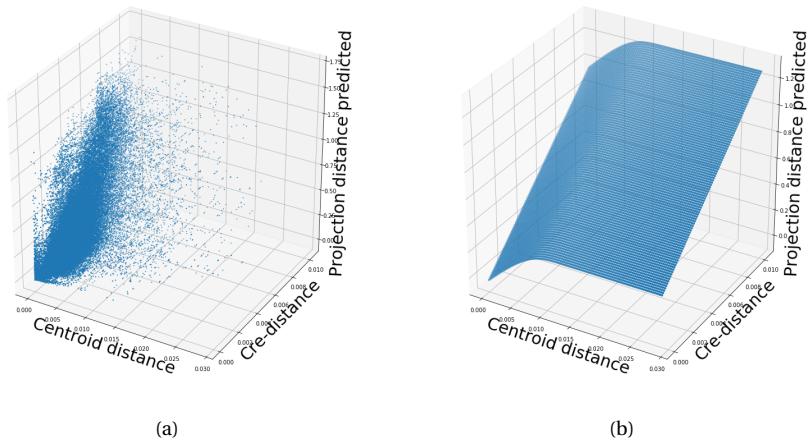


Figure 18: Fitting  $g$ . 18a Distribution of projection errors against centroid distance and cre-distance in Isocortex. 18b  $\hat{g}$  found using B-splines.

415 JUSTIFICATION OF SHAPE CONSTRAINT     The shape-constrained expected-loss estimator introduced  
 416 in this paper is, to our knowledge, novel. It should be considered an alternative method to the classic  
 417 weighted kernel method. While we do not attempt a detailed theoretical study of this estimator, we do  
 418 establish the need for the shape constraint in our spline estimator. Though this fact is probably well  
 419 known, we prove a (slightly stronger) version here for completeness.

420 **Proposition 1.** *Given a collection of metric spaces  $X_1, \dots, X_n$  with metrics  $d_1, \dots, d_n$  (e.g.  $d_{centroid}, d_{cre}$ ),  
 421 and a function  $f : (X_1 \times X_1) \dots \times (X_n \times X_n) = g(d_1(X_1 \times X_1), \dots, d_n(X_n \times X_n))$ , then then  $f$  is a metric iff  $g$  is  
 422 concave, non-decreasing and  $g(d) = 0 \iff d = 0$ .*

423 *Proof.* We first show  $g$  satisfying the above properties implies that  $f$  is a metric.

- 424    ▪ The first property of a metric is that  $f(x, x') = 0 \iff x = x'$ . The left implication:  
 425        $x = x' \implies f(x_1, x'_1, \dots, x_n, x'_n) = g(0, \dots, 0)$ , since  $d$  are metrics. Then, since  $g(0) = 0$ , we have that  
 426        $f(x, x') = 0$ . The right implication:  $f(x, x') = 0 \implies d = 0 \implies x = x'$  since  $d$  are metrics.
- 427    ▪ The second property of a metric is that  $f(x, x') = f(x', x)$ . This follows immediately from the  
 428       symmetry of the  $d_i$ , i.e.  $f(x, x') = f(x_1, x'_1, \dots, x_n, x'_n) = g(d_1(x_1, x'_1), \dots, d_n(x_n, x'_n)) =$   
 429        $g(d_1(x'_1, x_1), \dots, d_n(x'_n, x_n)) = f(x'_1, x_1, \dots, x'_n, x_n) = f(x', x)$ .
- 430    ▪ The third property of a metric is the triangle inequality:  $f(x, x') \leq f(x, x^*) + f(x^*, x')$ . To show this  
 431       is satisfied for such a  $g$ , we first note that  $f(x, x') = g(d(x, x')) \leq g(d(x, x^*) + d(x^*, x'))$  since  $g$  is  
 432       non-decreasing and by the triangle inequality of  $d$ . Then, since  $g$  is concave,  
 433        $g(d(x, x^*) + d(x^*, x')) \leq g(d(x, x^*)) + g(d(x^*, x')) = f(x, x^*) + f(x^*, x')$ .

434 We then show that  $f$  being a metric implies that  $g$  satisfies the above properties.

- 435    ▪ The first property is that  $g(d) = 0 \iff d = 0$ . We first show the right implication:  $g(d) = 0$ , and  
 436        $g(d) = f(x, x')$ , so  $x = x'$  (since  $f$  is a metric), so  $d = 0$ . We then show the left implication:  
 437        $d = 0 \implies x = x'$ , since  $d$  is a metric, so  $f(x, x') = 0$ , since  $f$  is a metric, and thus  $g(d) = 0$ .
- 438    ▪ The second property is that  $g$  is non-decreasing. We proceed by contradiction. Suppose  $g$  is  
 439       decreasing in argument  $d_1$  in some region  $[l, u]$  with  $0 < l < u$ . Then  
 440        $g(d_1(0, l), 0) \geq g(d_1(0, 0), 0) + g(d_1(0, u), 0) = g(d_1(0, u), 0)$ , which violates the triangle inequality on  
 441        $f$ . Thus, decreasing  $g$  means that  $f$  is not a metric, so  $f$  a metric implies non-decreasing  $g$ .

- 442     ▪ The final property is that  $g$  is concave. We proceed by contradiction. Suppose  $g$  is strictly convex.  
443         Then there exist vectors  $d, d'$  such that  $g(d + d') < g(d) + g(d')$ . Assume that  $d$  and  $d'$  only are  
444         non-zero in the first position, and  $d = d(0, x), d' = d(0, x')$ . Then,  $f(0, x) + f(0, x') < f(0, x + x')$ ,  
445         which violates the triangle inequality on  $f$ . Therefore,  $g$  must be concave.

446



***447 Establishing a lower detection limit***

448 The lower detection limit of our approach is a complicated consequence of our experimental and  
 449 analytical protocols. For example, the Nadaraya-Watson estimator is likely to generate many small  
 450 false positive connections, since the projection of even a single experiment within the source region  
 451 to a target will cause a non-zero connectivity in the Nadaraya-Watson weighted average. On the other  
 452 hand, the complexities of the experimental protocol itself and the image analysis and alignment can  
 453 also cause spurious signals. Therefore, it is of interest to establish a lower-detection threshold below  
 454 which we have very little power-to-predict, and set estimated connectivities below this threshold to  
 455 zero. This should make our estimated connectivities more accurate, especially in the  
 456 biologically-important sense of sparsity.

457 We establish this limit with respect to the sum of Type 1 and Type 2 errors

$$\iota = \sum_{i \in \mathcal{E}} 1_{y_{\mathcal{T}}(i)=0}^T 1_{\hat{f}_{\mathcal{T}}(v(i), c(i)) > \tau} + 1_{y_{\mathcal{T}}(i) > 0}^T 1_{\hat{f}_{\mathcal{T}}(v(i), c(i)) < \tau}.$$

458 We then select the  $\tau$  that minimizes  $\iota$ . Results for this approach are given in Supplemental Section 7.

459 ***Decomposing the connectivity matrix***

460 We utilize non-negative matrix factorization (NMF) to analyze the principal signals in our  
 461 connectivity matrix. Here, we review this approach as applied to decomposition of the distal elements  
 462 of the estimated connectivity matrix  $\hat{\mathcal{C}}$  to identify  $q$  connectivity archetypes. Aside from the NMF  
 463 program itself, the key elements are selection of the number of archetypes  $q$  and stabilization of the  
 464 tendency of NMF to give random results over different initializations.

465 *Non-negative matrix factorization* As discussed in Knox et al. (2019), one of the most basic processes  
 466 underlying the observed connectivity is the tendency of each source region to predominantly project  
 467 to proximal regions. For example, the heatmap in Supplemental Figure 17 shows infrastructure  
 468 distances contains a diagonal pattern resembling the connectivity matrix in 2. These connections are  
 469 biologically meaningful, but also unsurprising, and their relative strength biases learned latent  
 470 coordinate representations away from long-range structures. For this reason, we establish a  $1500\mu m$   
 471 'distal' threshold within which to exclude connections for our analysis.

472 Given a matrix  $X \in \mathbb{R}_{\geq 0}^{a \times b}$  and a desired latent space dimension  $q$ , the non-negative matrix  
 473 factorization is thus

$$\text{NMF}(\mathcal{C}, \lambda, q, \mathbf{1}_M) = \arg \min_{W \in \mathbb{R}_{\geq 0}^{S \times q}, H \in \mathbb{R}_{\geq 0}^{q \times T}} \frac{1}{2} \|\mathbf{1}_M \odot \mathcal{C} - WH\|_2^2 + \lambda(\|H\|_1 + \|W\|_1).$$

474 We note the existence of NMF with alternative norms for certain marginal distributions, but leave  
 475 utilization of this approach for future work (Brunet, Tamayo, Golub, & Mesirov, 2004).

476 The mask  $\mathbf{1}_M \in \{0, 1\}^{S \times T}$  serves two purposes. First, it enables computation of the NMF objective  
 477 while excluding self and nearby connections. These connections are both strong and linearly  
 478 independent, and so would unduly influence the *NMF* reconstruction error over more biologically  
 479 interesting or cell-type dependent long-range connections. Second, it enables cross-validation based  
 480 selection of the number of retained components.

481 *Cross-validating NMF* Cross-validation for NMF is somewhat standard but not entirely well-known,  
 482 and so we review it here. In summary, a NMF model is first fit on a reduced data set, and an evaluation

483 set is held out. After random masking of the evaluation set, the loss of the learned model is then  
 484 evaluated on the basis of successful reconstruction of the held-out values. This procedure is  
 485 performed repeatedly, with replicates of random masks at each tested dimensionality  $q$ . This  
 486 determines the point past which additional hidden units provide no reconstructive value.

487 The differentiating feature of cross-validation for NMF compared with supervised learning is the  
 488 randomness of the masking matrix  $1_M$ . Cross-validation for supervised learning generally leaves out  
 489 entire observations, but this is insufficient for our situation. This is because, given  $W$ , our  $H$  is the  
 490 solution of a regularized non-negative least squares optimization problem

$$H := \hat{e}_W(1_M \odot \mathcal{C}) = \arg \min_{\beta \in \mathbb{R}_{\geq 0}^{q \times T}} \|1_M \odot \mathcal{C} - W\beta\|_2^2 + \|\beta\|_1. \quad (3)$$

491 The negative effects of an overfit model can therefore be optimized away from on the evaluation set.

A standard solution is to generate uniformly random masks  $1_{M(p)} \in \mathbb{R}^{S \times T}$  where

$$1_{M(p)}(s, t) \sim \text{Bernoulli}(p).$$

NMF is then performed using the mask  $1_{M(p)}$  to get  $W$ . The cross-validation error is then

$$\epsilon_q = \frac{1}{R} \sum_{r=1}^R (\|1_{M(p)_r^C} \odot X - W(\hat{e}_W(1_{M(p)_r^C} \odot X))\|_2^2$$

where  $1_{M(p)_r^C}$  is the binary complement of  $1_{M(p)_r}$  and  $R$  is a number of replicates. Theoretically, the optimum number of components is then

$$\hat{q} = \arg \min_q \epsilon_q.$$

492 *Stabilizing NMF* The NMF program is non-convex, and, empirically, individual replicates will not  
 493 converge to the same optima. One solution therefore is to run multiple replicates of the NMF  
 494 algorithm and cluster the resulting vectors. This approach raises the questions of how many clusters  
 495 to use, and how to deal with stochasticity in the clustering algorithm itself. We address this issue  
 496 through the notion of clustering stability (von Luxburg, 2010a).

The clustering stability approach is to generate  $L$  replicas of k-cluster partitions  $\{C_{kl} : l \in 1 \dots L\}$  and then compute the average dissimilarity between clusterings

$$\xi_k = \frac{2}{L(L-1)} \sum_{l=1}^L \sum_{l'=1}^l d(C_{kl}, C_{kl'}).$$

Then, the optimum number of clusters is

$$\hat{k} = \arg \min_k \xi_k.$$

<sup>497</sup> A review of this approach is found in von Luxburg (2010b). Intuitively, archetype vectors that cluster  
<sup>498</sup> together frequently over clustering replicates indicate the presence of a stable clustering. For  $d$ , we  
<sup>499</sup> utilize the adjusted Rand Index - a simple dissimilarity measure between clusterings. Note that we  
<sup>500</sup> expect to select slightly more than the  $q$  components suggested by cross-validation, since archetype  
<sup>501</sup> vectors which appear in one NMF replicate generally should appear in others. We then select the  $q$   
<sup>502</sup> clusters with the most archetype vectors - the most stable NMF results - and take the median of each  
<sup>503</sup> cluster to create a sparse representative archetype Kotliar et al. (2019); Wu et al. (2016). We then find  
<sup>504</sup> the according  $H$  using Program 3. Experimental results for these cross-validation and stability  
<sup>505</sup> selection approaches are given in Supplemental Section 7.

## 7 SUPPLEMENTAL EXPERIMENTS

### 506 *Establishing a lower limit of detection*

507 We give results on the false detection rate at different limits of detection. These conclusively show that  
 508  $10^{-6}$  is the good threshold for our normalized data.

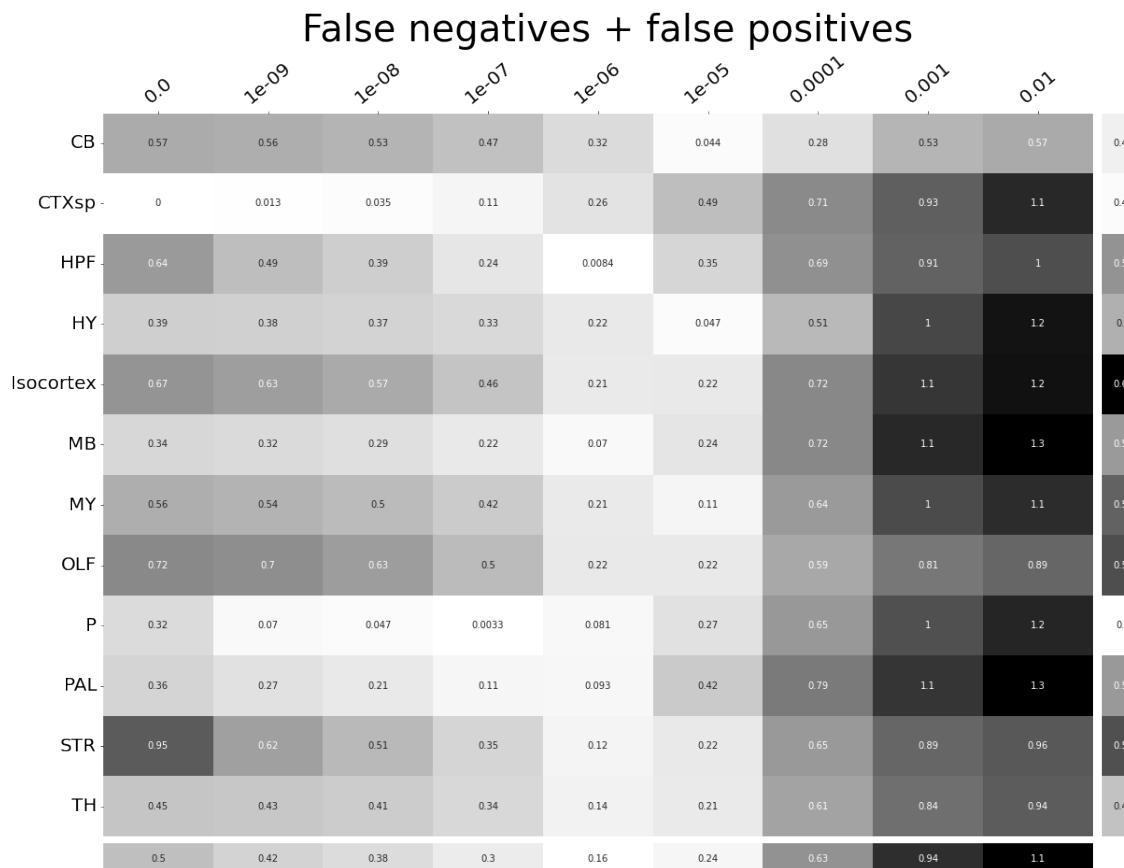


Figure 19:  $\tau$  at different limits of detection in different major structures.  $10^{-6}$  is the optimal detection threshold.

509 ***Loss subsets***

510 We report model accuracies for our *EL* model by neuron class and structure. These expand upon the  
 511 results in Table 5 and give more specific information about the quality of our estimates. CTXsp is  
 512 omitted due to the small nature of the evaluation set.



Figure 20: Weighted loss for cre-leaf combinations in CB. Missing values are omitted. Row and column averages are also plotted.

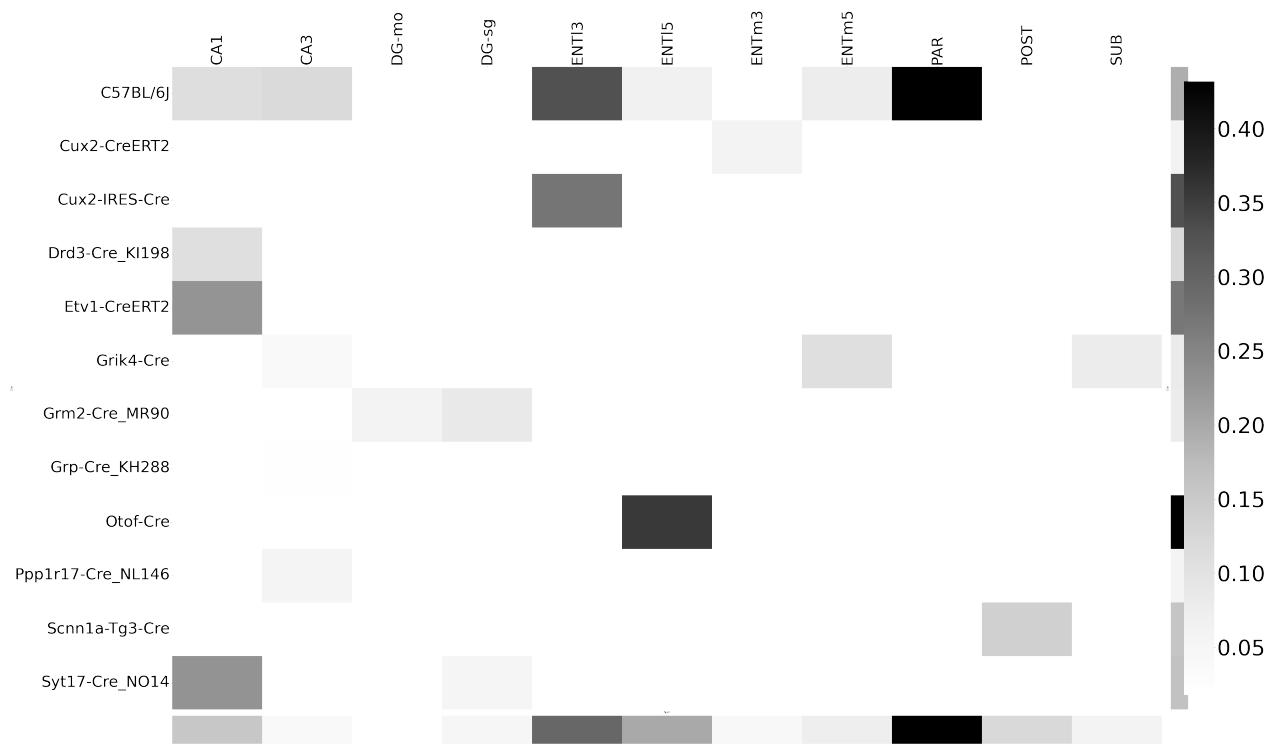


Figure 21: Weighted loss for cre-leaf combinations in HPF. Missing values are omitted. Row and column averages are also plotted.

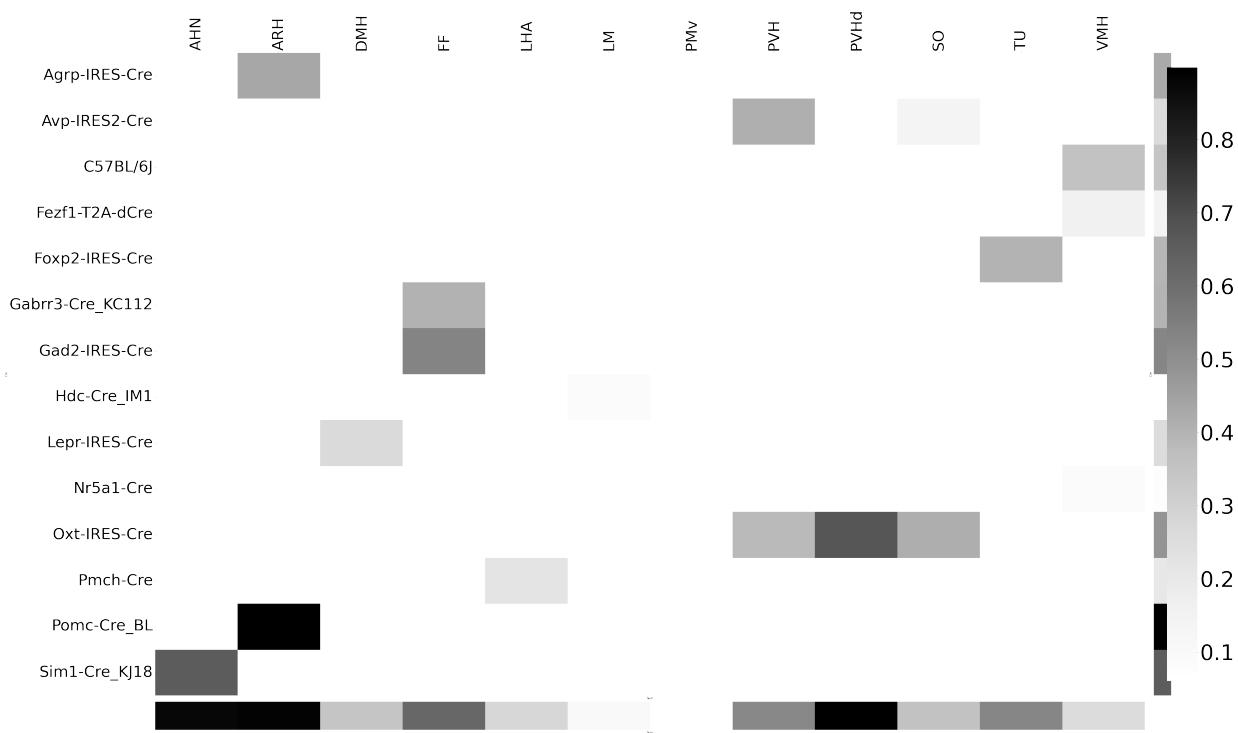


Figure 22: Weighted loss for cre-leaf combinations in HY. Missing values are omitted. Row and column averages are also plotted.

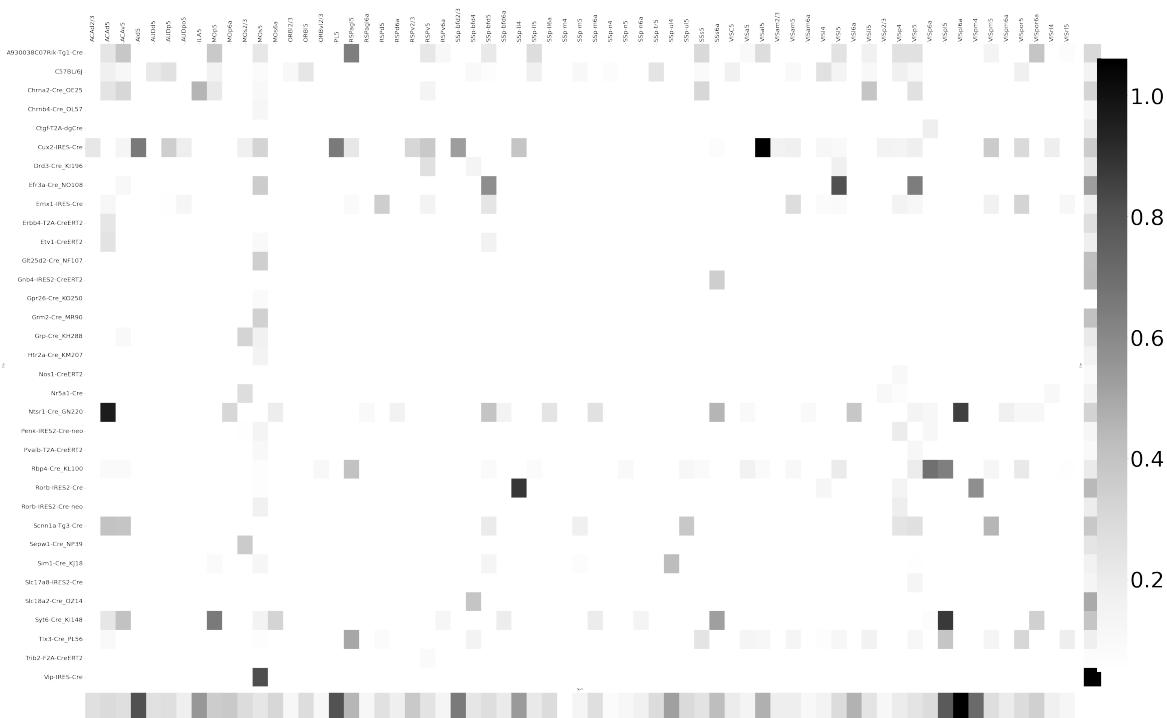


Figure 23: Weighted loss for cre-leaf combinations in Isocortex. Missing values are omitted. Row and column averages are also plotted.



Figure 24: Weighted loss for cre-leaf combinations in MB. Missing values are omitted. Row and column averages are also plotted.



Figure 25: Weighted loss for cre-leaf combinations in MY. Missing values are omitted. Row and column averages are also plotted.



Figure 26: Weighted loss for cre-leaf combinations in OLF. Missing values are omitted. Row and column averages are also plotted.

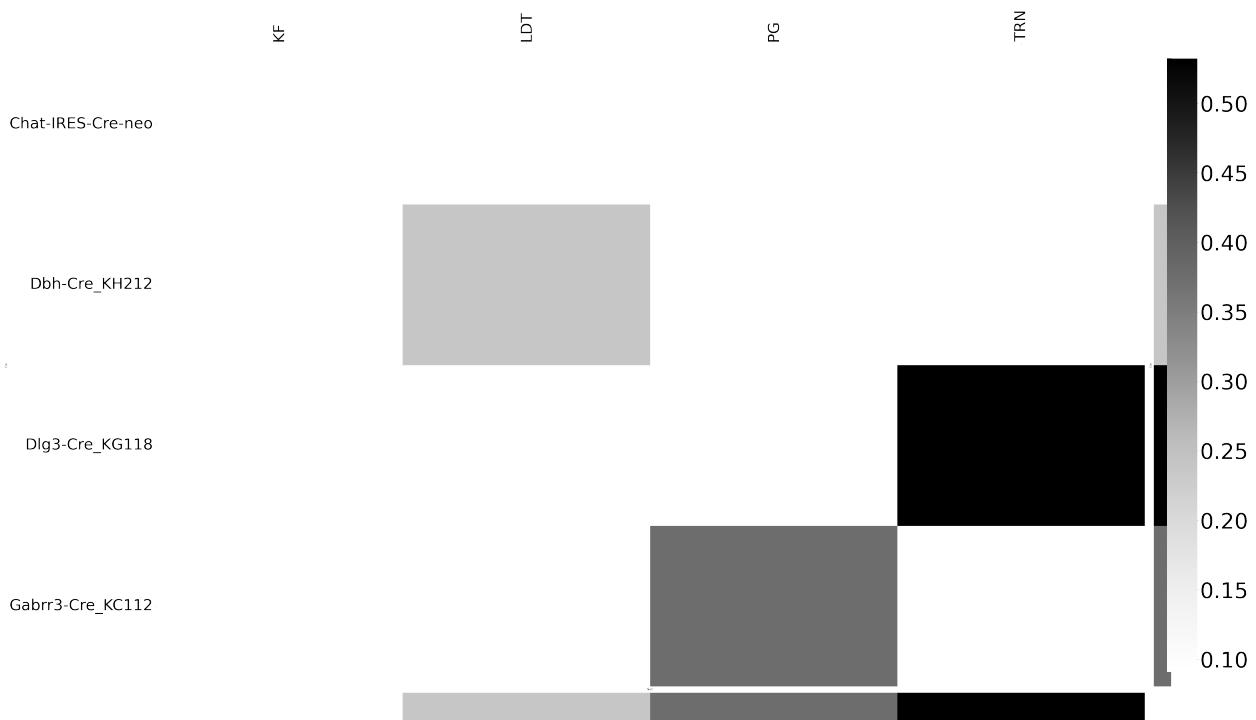


Figure 27: Weighted loss for cre-leaf combinations in P. Missing values are omitted. Row and column averages are also plotted.

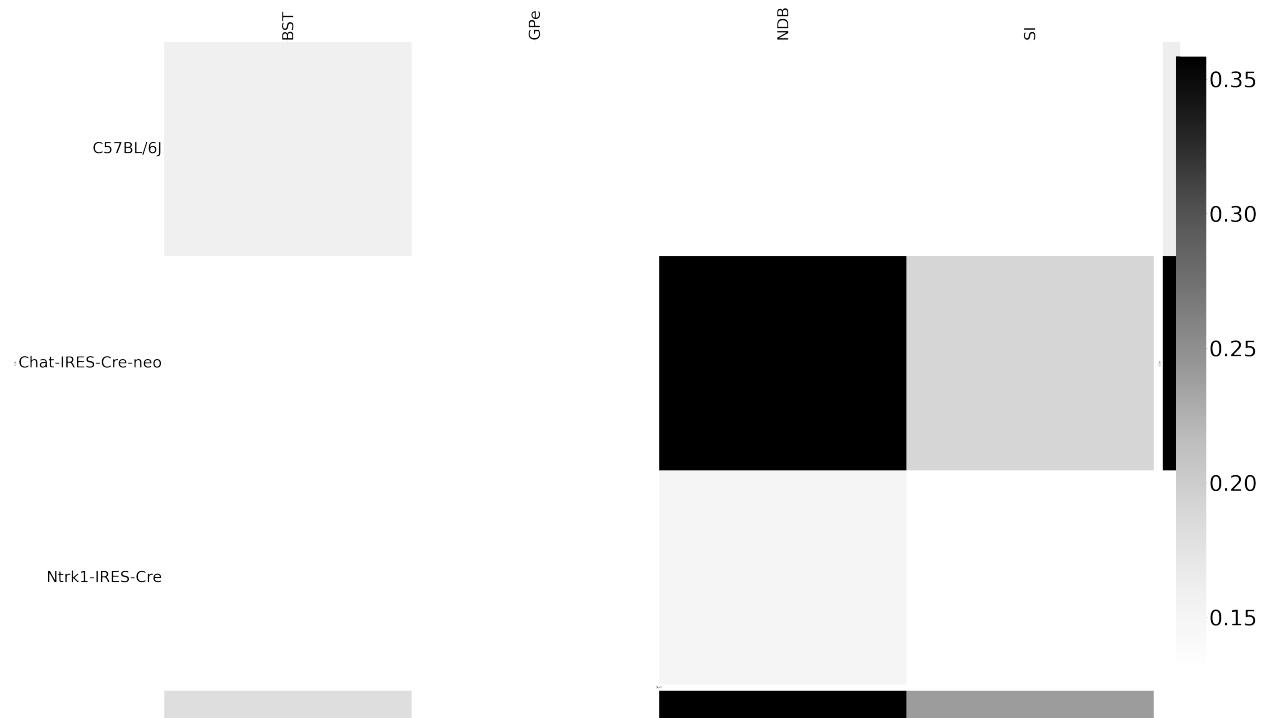


Figure 28: Weighted loss for cre-leaf combinations in PAL. Missing values are omitted. Row and column averages are also plotted.

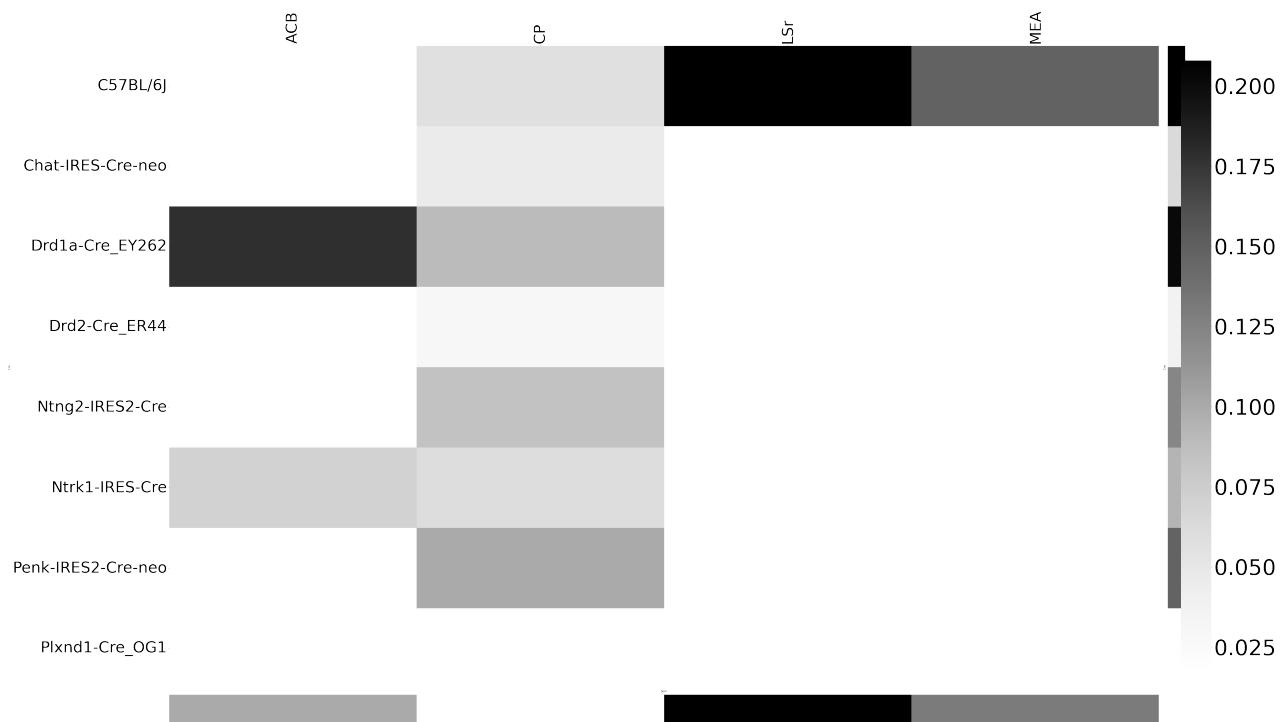


Figure 29: Weighted loss for cre-leaf combinations in STR. Missing values are omitted. Row and column averages are also plotted.

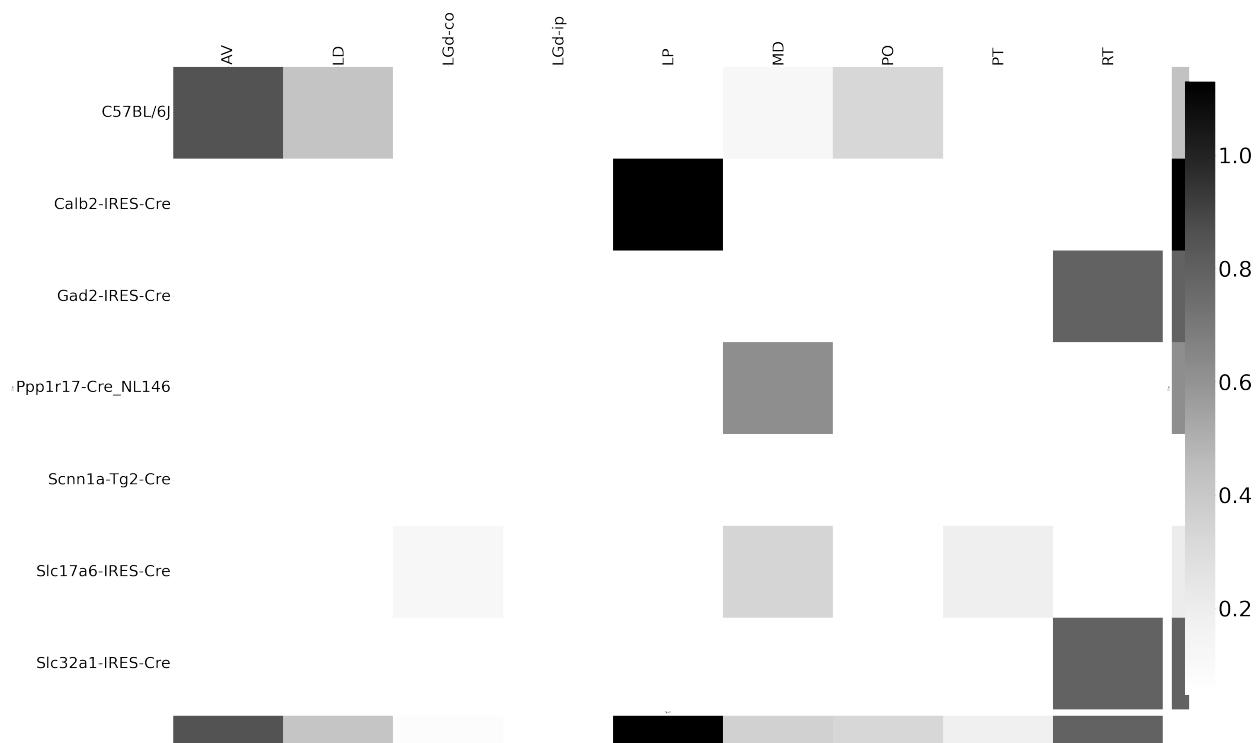


Figure 30: Weighted loss for cre-leaf combinations in TH. Missing values are omitted. Row and column averages are also plotted.

513 **Matrix Factorization**

514 We give additional results on the generation of the archetypal connectome patterns. These consist of  
 515 cross-validation selection of  $q$ , the number of latent components, stability analysis, and visualization  
 516 of the reconstructed wild-type connectivity.

517 *Cross-validation* We set  $\alpha = 0.002$  and run Program 2 on  $\mathcal{C}_{wt}$ . We use a random mask with  $p = .3$  to  
 518 evaluate prediction accuracy of models trained on the unmasked data on the masked data. To  
 519 account for stochasticity in the NMF algorithm, we run  $R = 8$  replicates at each potential dimension  $q$ .  
 520 This selects  $\hat{q} = 60$ .

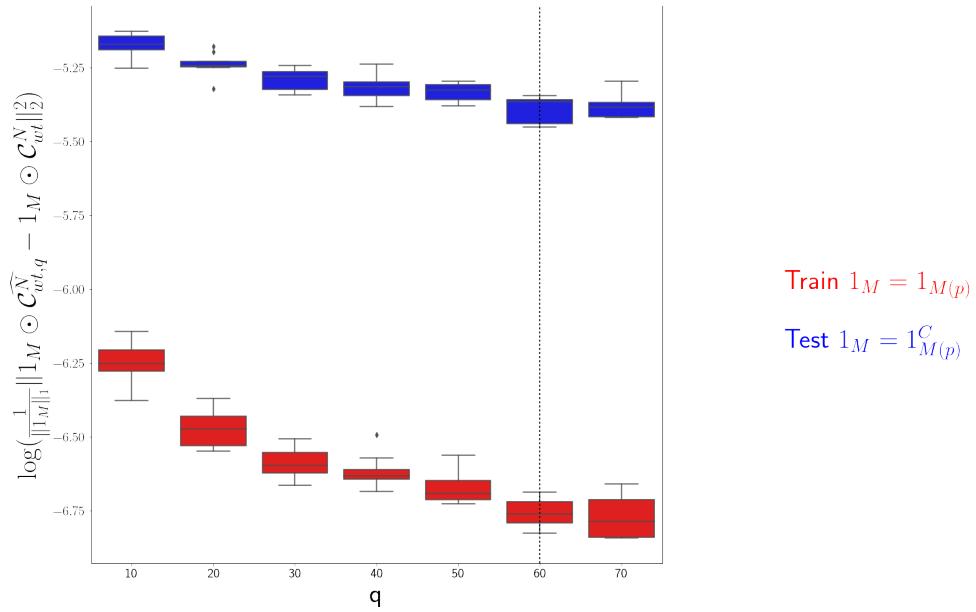


Figure 31: Train and test error using NMF decomposition.

521 *Stability* For the purposes of visualization and interpretability, we restrict to a  $q = 15$  component  
 522 model. To address the instability of the NMF algorithm in identifying components, we  $k - means$   
 523 cluster components over  $R = 10$  replicates with  $k \in \{10, 15, 20, 25, 30\}$ . Since the clustering is itself  
 524 unstable, we repeat the clustering 25 times and select the  $k$  with the largest Rand index.

525 q	10.000000	20.000000	30.000000	40.000000	50.000000
Rand index	0.772544	0.844981	<b>0.932957</b>	0.929827	0.885862

526 Since  $k$ -means is most stable at  $k = 30$ , we cluster the  $qR = 150$  components into 30 clusters and  
 527 select the 15 clusters appearing in the most replicates.

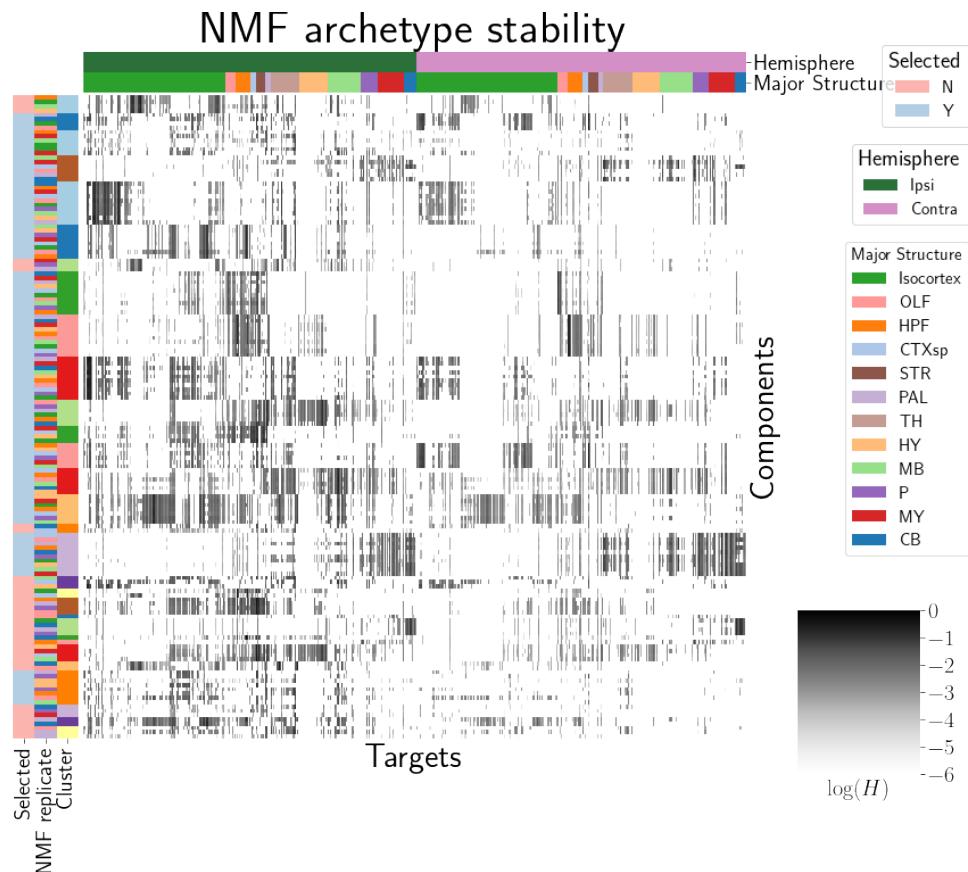


Figure 32: Stability of NMF results across replicates. Replicate and NMF component are shown on rows. Components that are in the top 15 are also indicated.

528 These are the components whose medians are plotted in Figure 4a.

## 8 COMPETING INTERESTS

529 This is an optional section. If you declared a conflict of interest when you submitted your manuscript,  
530 please use this space to provide details about this conflict.

531

532

533

## REFERENCES

- 534 Brunet, J.-P., Tamayo, P., Golub, T. R., & Mesirov, J. P. (2004). Metagenes and molecular pattern discovery using matrix  
535 factorization. *Proc. Natl. Acad. Sci. U. S. A.*, 101(12), 4164–4169.
- 536 Chamberlin, N. L., Du, B., de Lacalle, S., & Saper, C. B. (1998). Recombinant adeno-associated virus vector: use for  
537 transgene expression and anterograde tract tracing in the CNS. *Brain Res.*, 793(1-2), 169–175.
- 538 Daigle, T. L., Madisen, L., Hage, T. A., Valley, M. T., Knoblich, U., Larsen, R. S., ... Zeng, H. (2018). A suite of transgenic  
539 driver and reporter mouse lines with enhanced Brain-Cell-Type targeting and functionality. *Cell*, 174(2), 465–480.e22.
- 540 Gao, Y., Zhang, X., Wang, S., & Zou, G. (2016). Model averaging based on leave-subject-out cross-validation. *J. Econom.*,  
541 192(1), 139–151.
- 542 Harris, J. A., Mihalas, S., Hirokawa, K. E., Whitesell, J. D., Choi, H., Bernard, A., ... Zeng, H. (2019). Hierarchical  
543 organization of cortical and thalamic connectivity. *Nature*, 575(7781), 195–202.
- 544 Harris, J. A., Oh, S. W., & Zeng, H. (2012). Adeno-associated viral vectors for anterograde axonal tracing with fluorescent  
545 proteins in nontransgenic and cre driver mice. *Curr. Protoc. Neurosci., Chapter 1, Unit 1.20.1–18*.
- 546 Harris, K. D., Mihalas, S., & Shea-Brown, E. (2016). Nonnegative spline regression of incomplete tracing data reveals high  
547 resolution neural connectivity.
- 548 Jeong, M., Kim, Y., Kim, J., Ferrante, D. D., Mitra, P. P., Osten, P., & Kim, D. (2016). Comparative three-dimensional  
549 connectome map of motor cortical projections in the mouse brain. *Sci. Rep.*, 6, 20072.
- 550 Knox, J. E., Harris, K. D., Graddis, N., Whitesell, J. D., Zeng, H., Harris, J. A., ... Mihalas, S. (2019). High-resolution  
551 data-driven model of the mouse connectome. *Netw Neurosci*, 3(1), 217–236.
- 552 Kotliar, D., Veres, A., Nagy, M. A., Tabrizi, S., Hodis, E., Melton, D. A., & Sabeti, P. C. (2019). Identifying gene expression  
553 programs of cell-type identity and cellular activity with single-cell RNA-Seq. *Elife*, 8.
- 554 Lotfollahi, M., Naghipourfar, M., Theis, F. J., & Alexander Wolf, F. (2019). Conditional out-of-sample generation for  
555 unpaired data using trVAE.

- 556 Oh, S. W., Harris, J. A., Ng, L., Winslow, B., Cain, N., Mihalas, S., ... Zeng, H. (2014). A mesoscale connectome of the  
557 mouse brain. *Nature*, 508(7495), 207–214.
- 558 Saul, L. K., & Roweis, S. T. (2003). Think globally, fit locally: Unsupervised learning of low dimensional manifolds. *J.  
559 Mach. Learn. Res.*, 4(Jun), 119–155.
- 560 von Luxburg, U. (2010a). Clustering stability: An overview.
- 561 von Luxburg, U. (2010b). Clustering stability: An overview.
- 562 Wu, S., Joseph, A., Hammonds, A. S., Celtniker, S. E., Yu, B., & Frise, E. (2016). Stability-driven nonnegative matrix  
563 factorization to interpret spatial gene expression and build local gene networks. *Proc. Natl. Acad. Sci. U. S. A.*, 113(16),  
564 4290–4295.

## 9 TECHNICAL TERMS

565 **Technical Term** a key term that is mentioned in an NETN article and whose usage and definition may  
566 not be familiar across the broad readership of the journal.

567 **Cre-line** Refers to the combination of cre-recombinase expression in transgenic mouse and  
568 cre-induced promotion in the vector that induces labelling of cell-class specific projection.

569 **Cell class** The projecting neurons targeted by a particular cre-line

570 **Structural connectivities** connectivity between structures

571 **Voxel** A  $100\mu m$  cube of brain.

572 **Structural connection tensor** Connectivities between structures given a neuron class

573 **dictionary-learning** A family of algorithms for finding low-dimensional data representations.

574 **Shape constrained estimator** A statistical estimator that fits a function of a particular shape (e.g.  
575 monotonic increasing, convex).

576 **Nadaraya-Watson** A simple smoothing estimator.

577 **Connectivity archetypes** Typical connectivity patterns

578 **Expected loss** Our new estimator that weights different features by their estimated predictive  
579 power.