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34 **Data Description**

35 **Context**

36 Sensory experience powerfully shapes neural circuits. Changes due to sensory organ deprivation  
37 such as eye closure, digit amputation, and whisker trimming provide powerful means for studying  
38 mechanisms of experience dependent cortical plasticity.

39 In the whisker system experience dependent plasticity is most commonly studied in the  
40 barrel cortex subfield of the primary somatosensory cortex where neural representations of  
41 whiskers change in response to altered patterns of incoming sensory information. As originally  
42 shown in the barrel cortex [1] sensory deprivation induced by transient whisker trimming is  
43 sufficient to perturb neural receptive fields both during development and in adulthood. Previous  
44 work has also shown that the cellular basis of deprivation-induced decreases in whisker evoked  
45 representations are primarily due to a reduction of synaptic strength in monosynaptically  
46 connected feed-forward neuronal networks in behaving animals [2, 3]. Conversely whisker  
47 sparing induced enhancement in whisker representation is mediated at least in part by the long-  
48 term synaptic facilitation expressed along the L4 projections *in vivo* [4]. Identification of the  
49 molecular events that mediate these bidirectional changes in synaptic connectivity will benefit  
50 from systematic analysis of the gene transcription. Therefore, we performed RNA sequencing in  
51 the barrel cortex with or without sensory deprivation across cortical layers 2-4. This database will  
52 assist molecular and cellular neurobiologists in addressing the molecular mechanisms associated  
53 with experience dependent plasticity, and will enable statistical approaches to determine the  
54 dynamics of the coupled changes across molecular pathways as cortical circuits undergo plastic  
55 changes in their organization.

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4 57 **Methods**

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6 58 *Animals*

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8 59 All experiments were performed in accordance with the Animal Ethics Committee of the Radboud  
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10 60 University in Nijmegen, the Netherlands. Pregnant wild type mice (Charles River) were kept at a  
11  
12 61 12-hour light/dark cycle with access to food *ad libitum*. Cages were checked for birth daily. To  
13  
14 62 induce experience-dependent plasticity, pups underwent bilateral plucking of their C-row whiskers  
15  
16 63 under isoflurane anaesthesia at P12 (**Figure 1**). Control animals were not plucked but  
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18 64 anaesthetized and handled similarly. After recovery pups were returned to their home cage. Every  
19  
20 65 other day pups were checked for whisker regrowth, which were plucked if present. At P23-P24,  
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22 66 pups were randomly selected from their litter for slice preparation and tissue collection. For each  
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24 67 experimental condition (i.e. whisker deprived or control), 4 female pups were used, thus each  
25  
26 68 group consisted of 4 independent biological samples (also known as biological replicates).  
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28 69 Samples from cortical layer (L) 4 and L2/3 were treated independently with their own  
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30 70 corresponding groups of control, deprived, 1<sup>st</sup> order spared, 2<sup>nd</sup> order spared columns as detailed  
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32 71 in Figure 1.  
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40 73 *Figure 1 is about here*

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42 74  
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44 75 *Slice preparation and sample collection*

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46 76 Pups were anaesthetized using isoflurane and then perfused with ice-cold carbogenated slicing  
47  
48 77 medium (108 mM ChCl, 3 mM KCl, 26 mM NaHCO<sub>3</sub>, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 25 mM glucose, 1 mM  
49  
50 78 CaCl<sub>2</sub>, 6 mM MgSO<sub>4</sub> and 3 mM Na-pyruvate). Next, pups were decapitated before the brain was  
51  
52 79 quickly dissected out and 400 µm thalamocortical slices from each hemisphere were prepared as  
53  
54 80 described before [2, 3]. Slices were transferred to 37 degrees Celsius carbogenated ACSF (120  
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56 81 mM NaCl, 3.5 mM KCl, 10 mM glucose, 2.5 mM CaCl<sub>2</sub>, 1.3 mM MgSO<sub>4</sub>, 25 mM NaHCO<sub>3</sub> and 1.25  
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4 82 mM NaH<sub>2</sub>PO<sub>4</sub>) where they were kept for 30 minutes and recovered at room temperature for  
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6 83 another 30 minutes until tissue collection.  
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10 85 After incubation, slices were placed under a Nikon Eclipse FN1 microscope. The holding chamber  
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13 86 was continuously perfused with room temperature carbogenated ACSF. Due to the 55 degree  
14  
15 87 cut, slices were obtained in which S1 barrels from specific rows (A-E) could be identified [2]. A  
16  
17 88 thin, long glass pipette was pulled using a Sutter instruments P-2000 pipette puller and was used  
18  
19  
20 89 to make intercolumnar incisions from L1 to the bottom of L4 after which the slice was placed under  
21  
22 90 a binocular dissection microscope where the location of specific barrel columns could now be  
23  
24 91 readily identified by eye. A sterile 32G needle was then used to cut out L2/3 and L4 separately  
25  
26 92 from each column. Tissue from columns A/E and B/D were pooled as they both constitute second  
27  
28 93 and first order spared whiskers, respectively. Immediately after dissection, tissue samples were  
29  
30 94 snap frozen in liquid nitrogen and stored at -80 degrees Celsius until further use. All tools that  
31  
32  
33 95 came into direct contact with brain tissue were treated using RNaseZap in order to minimize  
34  
35 96 RNase contamination.  
36  
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#### 39 40 98 *RNA isolation and quality control*

41  
42 99 Tissue samples originating from the same rows and layers were pooled within each animal. From  
43  
44 100 control animals, only the C column tissues were used (also see *Re-use potential*). Tissue was  
45  
46 101 quickly dissolved in Qiazol (Qiagen #79306), after which RNA was isolated using the miRNeasy  
47  
48 102 Mini kit (Qiagen #217004), DNase treated (Thermo Scientific, #EN0521) and cleaned up using  
49  
50 103 RNeasy MinElute kit (Qiagen #74204), all following the manufacturer's instructions. Samples were  
51  
52  
53 104 then stored at -80 degrees Celsius until further processing.  
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57  
58 106 RNA sample integrity was determined using Agilent TapeStation (High Sensitivity RNA  
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60 107 Screentape). Sample RINs ranged from 7.1 to 8.8. To further assess RNA purity and integrity,  
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108 RNA samples were used in RT-PCR to confirm that cDNA could be produced and that a large  
109 (~1000 bp) amplicon could be obtained. To produce cDNA, SuperScript® II Reverse  
110 Transcriptase (Thermo Scientific #18064014) and random hexamer primers (Roche  
111 #11034731001) were used. The resulting cDNA was then added to a PCR reaction mix which  
112 further consisted of Jumpstart Ready Mix (Sigma P2893) and exon-exon junction spanning  
113 CamKII primers (FW TCCAACATTGTACGCCTCCAT; RV TGTTGGTGCTGTCGGAAGAT).  
114 From all cDNA samples a fragment of the expected size could be amplified, suggesting that the  
115 RNA samples contained pure RNA of sufficient integrity. All RNA samples thus passed our quality  
116 control criteria and were subjected to RNA sequencing.

117

118 *RNA sequencing*

119 RNA sequencing was conducted at the Genomics Core Facility of the EMBL, Heidelberg,  
120 Germany. The cDNA library was generated using the non-stranded NEBNext Ultra RNA Library  
121 Preparation Kit for Illumina (New England Biolabs, catalogue #E7530), which includes oligo-dT  
122 bead selection of mRNA. For library enrichment, 13-14 PCR cycles were performed. Pooled  
123 libraries were sequenced on the Illumina NextSeq 500 instrument in a 75bp paired-end mode  
124 using High output flow cells.

125

126 **Data validation and quality control**

127 Sequencing read quality was assessed using FastQC (Babraham Bioinformatics), the results of  
128 which were merged using MultiQC (<http://multiqc.info>). Results are displayed in **Figure 2**. Per  
129 base quality *phred* scores range from 34.80 to 35.15, indicating base call accuracies of >99.9%  
130 (**Figure 2A**). Overall 91.48-94.03% of reads had a mean *phred* score of 30 or above (**Figure 2B**).  
131 In line with these scores, per base N content (i.e. percentage of bases that could not confidently  
132 called) was very low, with a maximum value 0.053%.

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*Figure 2 is about here*

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136 Reads were then mapped to the mm10 reference genome using STAR [5], which uniquely  
137 mapped between 39,000,000 and 59,000,000 reads, constituting an average 90.15% unique map  
138 rate across samples (**Figure 2D**). Since the library preparation protocol entails a PCR enrichment  
139 step, which can lead to technical duplication, hence an overestimation of observed transcripts,  
140 we used Seqmonk (Babraham Bioinformatics) to plot the read density against the duplication  
141 levels (i.e. the percentage of duplicate reads) for each transcript. The obtained duplication plots  
142 showed a clear positive relation between read density and duplication levels (**Figure 3** and  
143 **Supplemental Figure 1**), suggesting that the origin of read duplication is biological, rather than  
144 technical.

145 Based on the above quality control measures we determined that our RNA-sequencing data was  
146 of sufficient quality to be used in downstream analyses, therefore we continued with gene  
147 expression analysis.

*Figure 3 is about here*

### *Analysis of gene expression*

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152  
153 Using a 2 read cut-off we identified 16,900 to 17,600 transcripts per sample (**Figure 4A**). Raw  
154 gene counts can be found online (see Supporting Data – DOI to appear). Differential gene  
155 expression analyses across groups were performed using EdgeR v3.12.1 [6, 7] using only genes  
156 with a count per million (CPM) >1 in at least 4 samples (**Supplementary Table 1** for details on  
157 the commands used). Since laminar identity is an important feature of our experimental setup, we  
158 assessed the relative expression of known molecular markers for L2/3 (*Cacna1h*, *Id2*, *Igfbp4*,  
159 *Igfn1*, *Mdga1*, *Plcx1*, *Rasgrf2*, *Rgs8*, *Tle3*) and L4 (*Cartpt*, *Cyp39a1*, *Kcnh5*, *Kcnip2*, *Lmo3*,

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160 *Rorb*, *Scnn1a*) [8–10], which showed selective enrichment of the laminar markers in isolated  
161 layers (**Figure 4B**).

162  
163 *Figure 4 is about here*

164  
165 To assess the variance in transcript counts, we calculated the coefficient of variation (CV) for  
166 each transcript with a cut-off of 50 as the minimal read count separately for each group (**Figure**  
167 **4C**). This analysis showed that, on average, 85.93% of transcripts have a CV below 15%,  
168 suggesting low variance across transcript counts for individual genes. Principal component  
169 analysis (PCA) showed that samples cluster based on layer, and the first two components  
170 explained ~88% variance the data (**Figure 4C, Supplemental Figure 2B**).

171  
172 These quality control routines suggest that we have obtained RNA-sequencing data of high read  
173 quality, with individual bases being called confidently throughout the length of reads, which  
174 uniquely map to the mm10 reference genome at high rates (>90% average). The laminar origin  
175 of our samples could be identified through known molecular markers, confirming our samples are  
176 of high anatomical specificity.

177  
178 **Re-use potential**

179 The current RNA-seq dataset might help address the molecular underpinnings of cortical  
180 experience-dependent plasticity. For example, it could be used (1) to identify genes whose  
181 transcription is modulated in an experience-dependent manner, (2) to statistically map the  
182 transcriptional networks at laminar resolution, (3) creating synergy with the single neuron RNA-  
183 seq datasets [11, 12], to address the molecular diversity of the cortical networks, (4) combined  
184 with the proteomic analysis performed under comparable experimental conditions in the  
185 accompanying manuscript (Kole et al, submitted), to systematically study the transcriptional and

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186 translational regulation of the genome upon altered sensory experience, and finally (5) to identify  
187 and quantify splice isoforms, given the sequencing depth of the current dataset. Since splicing  
188 and other posttranscriptional mechanisms govern which proteins are ultimately produced,  
189 combining the current transcriptomic dataset with a proteomics approach would also be of high  
190 importance.

191  
192 The current dataset focuses on isolated cortical columns and layers, which are necessarily  
193 diverse samples containing neuronal and non-neuronal cell classes. In terms of experience  
194 dependent plasticity, although most previous studies focus on excitatory projections, inhibitory  
195 cells and even non-neuronal cells have been implicated in plasticity [13–15]. This heterogeneity  
196 might be particularly important for L2/3, as also shown by the principal component analysis  
197 (**Figure 4D**), given the relative diversity of cellular populations in supragranular layers and their  
198 heterogeneous connectivity patterns [16].

199  
200 Researchers reusing our dataset should be aware that comparisons between control column C  
201 and spared columns (A/E, B/D) may have to be approached with caution, as this would involve  
202 two different columnar identities (whose transcriptomic dissimilarities are currently unknown),  
203 each coming from cortices that have had different sensory experience. However direct  
204 comparisons between the C columns across experimental conditions (i.e control versus deprived)  
205 as well as within-animal across-column comparisons in deprived animals control for these  
206 confounding variables.

207  
208 Taken together we hope that this data will prove useful in discovering novel molecular targets  
209 responsible for cortical plasticity and will lead to targeted control of plasticity in health and disease.

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**271 Availability of the supporting data**

272 Supporting data are available online (<https://goo.gl/tBof51>) and will be distributed via GigaScience  
273 DB.

274 Raw sequence reads were deposited in NCBI GEO.

275 Link: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE90929>

**277 List of abbreviations**

278 EDP Experience dependent plasticity

279 L2/3 Cortical Layer 2/3, also known as supragranular layers

280 L4 Cortical Layer 4, i.e. granular layer

**282 Competing interests**

283 The authors declare no competing interests.

**285 Author contributions**

286 KK performed all experimental manipulations, sample acquisition, biological and bioinformatic  
287 quality controls, and prepared the tables and figures. YK and JaP performed bioinformatic  
288 analysis. JeP performed library prep. VB supervised RNA-seq. PT contributed bioinformatic  
289 analysis and co-supervised the project. TC designed and supervised the project. KK and TC wrote  
290 the manuscript. All authors edited otherwise approved the final version of the manuscript.

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4 **291 Figure Legends**

5 **292**  
6 **293** **Figure 1.** Overview of the experimental design, sample collection and data organization. **(A)** Pups  
7 **294** were bilaterally spared or deprived of off their C-row whiskers between P12 and P23-P24, when  
8 **295** acute slices are made and column- and layer-specific tissues were excised. **(B)** RNA was isolated,  
9 **296** checked for integrity and purity, and subsequently sequenced. **(C)** Organization of the database.  
10 **297** Colour codes denote experimental groups. Same denominations are used in the read counts  
11 **298** matrix file (see **Supplemental Data**).  
12 **299**

13 **300** **Figure 2.** FastQC and STAR output graphs for all samples. **(A-B)** *Phred* scores per base and  
14 **301** per sequence. **(C)** Per sequence GC content. **(D)** STAR output of alignment scores.  
15 **302**

16 **303** **Figure 3.** Overlays of duplication plot contours, showing a positive correlation between read  
17 **304** density and duplication levels. Depicted contours enclose 90% of the data points.  
18 **305**

19 **306** **Figure 4.** Gene expression analyses. **(A)** Histogram of read counts per transcript per sample.  
20 **307** With a cut-off of 2 reads, between 16,900 and 17,600 transcripts could be identified across  
21 **308** samples. **(B)** Relative expression of known molecular markers for cortical laminae. Layer 4  
22 **309** markers are enriched in samples originating from this layer; the same is true for Layer 2/3 marker  
23 **310** expression in Layer 2/3 samples. **(C)** Cumulative plots of the coefficient of variance (CV) of  
24 **311** individual experimental groups. Including only transcripts identified by 50 reads or more, average  
25 **312** CVs of <15% are found in ~85% of transcripts. **(D)** Principal component analysis (PCA) showing  
26 **313** sample clustering by layer, including only transcripts identified by at least 50 reads. Principal  
27 **314** component (PC) 1 and 2 account for 88% of overall variance.  
28 **315**

29 **316** **Supplemental Figure 1.** Duplication plots for all samples, produced using SeqMonk (Babraham  
30 **317** Bioinformatics).  
31 **318**

32 **319** **Supplemental Figure 2.** **(A)** Cumulative plots of the coefficient of variance (CV) of experimental  
33 **320** each group, including transcripts identified by at least one read. Average CVs of <25% are  
34 **321** found in ~85% of transcripts. **(B)** Principal component analysis (PCA) including transcripts  
35 **322** identified by at least one read. The majority (88%) of overall variance is explained by Principal  
36 **323** components (PC) 1 and 2.  
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## 4   1   **Transcriptional mapping of the primary somatosensory cortex upon sensory deprivation**

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### 10           11   **Background (138)**

12           13           14           15           16           17           18           19           20           21           22           23

12   Experience-dependent plasticity (EDP) is essential for anatomical and functional maturation of  
13   sensory circuits during development. Although the principal synaptic and circuit mechanisms of  
14   EDP are [increasingly well studied](#) experimentally and computationally, its molecular mechanisms  
15   remain largely elusive. EDP can be readily studied in the rodent barrel cortex, [where each](#) 'barrel  
16   column' [preferentially represents deflections of its own](#) principal whisker. Depriving select  
17   whiskers while sparing their neighbours introduces competition between barrel columns,  
18   ultimately leading to weakening of intracortical, translaminal (i.e. Cortical Layer (L)4-to-L2/3) feed-  
19   forward excitatory projections in the deprived columns. The same synapses are potentiated in the  
20   neighbouring spared columns. These experience-dependent alterations of synaptic strength are  
21   thought to underlie somatosensory map plasticity. We used RNA sequencing in this model system  
22   to uncover cortical-column and -layer specific changes on the transcriptome level that are induced  
23   by altered sensory experience.

### 24           25   **Findings (66)**

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25   [Column-](#) and layer-specific barrel cortical tissues [were collected from juvenile mice with all](#)  
26   [whiskers intact and mice that received 11-12 days long whisker \(C-row\) deprivation before](#) high  
27   quality RNA was purified and sequenced. The current dataset entails an average of 50 million  
28   paired-end reads per sample, 75 base pairs in length. On average, 90.15% of reads could be  
29   uniquely mapped to the mm10 reference mouse genome.

### 30           31   **Conclusions (32) – Word total for the abstract: 246 out of 250**

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31   The current data reveal the transcriptional changes in gene expression in the barrel cortex upon  
32   altered sensory experience in juvenile [mice](#) and will help to molecularly map the mechanisms of  
33   cortical plasticity.

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34 **Data Description**

35 Context

36 Sensory experience powerfully shapes neural circuits. Changes due to sensory organ deprivation  
37 such as eye closure, digit amputation, and whisker trimming provide powerful means for studying  
38 mechanisms of experience dependent cortical plasticity.

39 In the whisker system experience dependent plasticity is most commonly studied in the  
40 barrel cortex subfield of the primary somatosensory cortex where neural representations of  
41 whiskers change in response to altered patterns of incoming sensory information. [As originally](#)  
42 [shown in the barrel cortex \[1\]](#) sensory deprivation induced by transient whisker trimming is  
43 sufficient to perturb neural receptive fields both during development and in adulthood. Previous  
44 work has also shown that the cellular basis of deprivation-induced decreases in whisker evoked  
45 representations are primarily due to a reduction of synaptic strength in monosynaptically  
46 connected feed-forward neuronal networks in behaving animals [\[2, 3\]](#). Conversely whisker  
47 sparing induced enhancement in whisker representation is mediated at least in part by the long-  
48 term synaptic facilitation expressed along the L4 projections *in vivo* [\[4\]](#). Identification of the  
49 molecular events that mediate these bidirectional changes in synaptic connectivity will benefit  
50 from systematic analysis of the gene transcription. Therefore, we performed RNA sequencing in  
51 the barrel cortex with or without sensory deprivation across cortical layers 2-4. This database will  
52 assist molecular and cellular neurobiologists in addressing the molecular mechanisms associated  
53 with experience dependent plasticity, and will enable statistical approaches to determine the  
54 dynamics of the coupled changes across molecular pathways as cortical circuits undergo plastic  
55 changes in their organization.

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## 58 [Methods](#)

### 59 *Animals*

60 All experiments were performed in accordance with the Animal Ethics Committee of the Radboud  
61 University in Nijmegen, the Netherlands. Pregnant wild type mice (Charles River) were kept at a  
62 12-hour light/dark cycle with access to food *ad libitum*. Cages were checked for birth daily. To  
63 induce experience-dependent plasticity, pups underwent bilateral plucking of their C-row whiskers  
64 under isoflurane anaesthesia at P12 (**Figure 1**). Control animals were not plucked but  
65 anaesthetized and handled similarly. After recovery pups were returned to their home cage. Every  
66 other day pups were checked for whisker regrowth, which were plucked if present. At P23-P24,  
67 pups were randomly selected from their litter for slice preparation and tissue collection. For each  
68 experimental condition (i.e. whisker deprived or control), 4 female pups were used, [thus each](#)  
69 [group consisted of 4 independent biological samples \(also known as biological replicates\).](#)  
70 [Samples from cortical layer \(L\) 4 and L2/3 were treated independently with their own](#)  
71 [corresponding groups of control, deprived, 1<sup>st</sup> order spared, 2<sup>nd</sup> order spared columns as detailed](#)  
72 [in Figure 1.](#)

73  
74 *Figure 1 is about here*

### 76 *Slice preparation and sample collection*

77 Pups were anaesthetized using isoflurane and then perfused with ice-cold carbogenated slicing  
78 medium (108 mM ChCl, 3 mM KCl, 26 mM NaHCO<sub>3</sub>, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 25 mM glucose, 1 mM  
79 CaCl<sub>2</sub>, 6 mM MgSO<sub>4</sub> and 3 mM Na-pyruvate). Next, pups were decapitated [before](#) the brain was  
80 quickly dissected out and 400 µm thalamocortical slices from each hemisphere were prepared as  
81 described before [\[2, 3\]. Slices were transferred to 37 degrees Celsius](#) carbogenated ACSF (120  
82 mM NaCl, 3.5 mM KCl, 10 mM glucose, 2.5 mM CaCl<sub>2</sub>, 1.3 mM MgSO<sub>4</sub>, 25 mM NaHCO<sub>3</sub> and 1.25

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4 83 mM NaH<sub>2</sub>PO<sub>4</sub>) where they were kept for 30 minutes and recovered at room temperature for  
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6 84 another 30 minutes until tissue collection.

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10 86 After incubation, slices were placed under a Nikon Eclipse FN1 microscope. The holding chamber  
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12 87 was continuously perfused with room temperature carbogenated ACSF. Due to the 55 degree  
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14 88 cut, slices were obtained in which S1 barrels from specific rows (A-E) could be identified [2]. A  
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16 89 thin, long glass pipette was pulled using a Sutter instruments P-2000 pipette puller and was used  
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18 90 to make intercolumnar incisions from L1 to the bottom of L4 after which the slice was placed under  
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20 91 a binocular dissection microscope where the location of specific barrel columns could now be  
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22 92 readily identified by eye. A sterile 32G needle was then used to cut out L2/3 and L4 separately  
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24 93 from each column. Tissue from columns A/E and B/D were pooled as they both constitute second  
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26 94 and first order spared whiskers, respectively. Immediately after dissection, tissue samples were  
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28 95 snap frozen in liquid nitrogen and stored at -80 degrees [Celsius](#) until further use. All tools that  
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30 96 came into direct contact with brain tissue were treated using RNaseZap in order to minimize  
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32 97 RNase contamination.

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40 99 *RNA isolation and quality control*

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42 100 Tissue samples originating from the same rows and layers were pooled within each animal. [From](#)  
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44 101 [control animals, only the C column tissues were used \(also see Re-use potential\)](#). Tissue was  
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46 102 quickly dissolved in Qiazol (Qiagen #79306), after which RNA was isolated using the miRNeasy  
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48 103 Mini kit (Qiagen #217004), DNase treated (Thermo Scientific, #EN0521) and cleaned up using  
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50 104 RNeasy MinElute kit (Qiagen #74204), all following the manufacturer's instructions. Samples were  
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52 105 then stored at -80 degrees [Celsius](#) until further processing.

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58 107 RNA sample integrity was determined using Agilent TapeStation (High Sensitivity RNA  
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60 108 Screentape). Sample RINs ranged from 7.1 to 8.8. To further assess RNA purity and integrity,

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109 RNA samples were used in RT-PCR to confirm that cDNA could be produced and that a large  
110 (~1000 bp) amplicon could be obtained. To produce cDNA, SuperScript® II Reverse  
111 Transcriptase (Thermo Scientific #18064014) and random hexamer primers (Roche  
112 #11034731001) were used. The resulting cDNA was then added to a PCR reaction mix which  
113 further consisted of Jumpstart Ready Mix (Sigma P2893) and exon-exon junction spanning  
114 CamKII primers (FW TCCAACATTGTACGCCTCCAT; RV TGTTGGTGCTGTCGGAAGAT).  
115 From all cDNA samples a fragment of the expected size could be amplified, suggesting that the  
116 RNA samples contained pure RNA of sufficient integrity. All RNA samples thus passed our quality  
117 control criteria and were subjected to RNA sequencing.

118

119 *RNA sequencing*

120 RNA sequencing was conducted at the Genomics Core Facility of the EMBL, Heidelberg,  
121 Germany. The cDNA library was generated using the non-stranded NEBNext Ultra RNA Library  
122 Preparation Kit for Illumina (New England Biolabs, catalogue #E7530), which includes oligo-dT  
123 bead selection of mRNA. For library enrichment, 13-14 PCR cycles were performed. Pooled  
124 libraries were sequenced on the Illumina NextSeq 500 instrument in a 75bp paired-end mode  
125 using High output flow cells.

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127 *Data validation and quality control*

128 Sequencing read quality was assessed using FastQC (Babraham Bioinformatics), the results of  
129 which were merged using MultiQC (<http://multiqc.info>). Results are displayed in **Figure 2**. Per  
130 base quality *phred* scores range from 34.80 to 35.15, indicating base call accuracies of >99.9%  
131 (**Figure 2A**). Overall 91.48-94.03% of reads had a mean *phred* score of 30 or above (**Figure 2B**).  
132 In line with these scores, per base N content (i.e. percentage of bases that could not confidently  
133 called) was very low, with a maximum value 0.053%.

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4 135 *Figure 2 is about here*

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9 137 Reads were then mapped to the mm10 reference genome using STAR [5], which uniquely  
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11 138 mapped between 39,000,000 and 59,000,000 reads, constituting an average 90.15% unique map  
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13 139 rate across samples (**Figure 2D**). Since the library preparation protocol entails a PCR enrichment  
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15 140 step, which can lead to technical duplication, hence an overestimation of observed transcripts,  
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17 141 we used Seqmonk (Babraham Bioinformatics) to plot the read density against the duplication  
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19 142 levels (i.e. the percentage of duplicate reads) for each transcript. The obtained duplication plots  
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21 143 showed a clear positive relation between read density and duplication levels (**Figure 3** and  
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23 144 **Supplemental Figure 1**), suggesting that the origin of read duplication is biological, rather than  
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25 145 technical.  
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29 146 Based on the above quality control measures we determined that our RNA-sequencing data was  
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31 147 of sufficient quality to be used in downstream analyses, therefore we continued with gene  
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33 148 expression analysis.  
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38 150 *Figure 3 is about here*

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44 153 *Analysis of gene expression*

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46 154 Using a 2 read cut-off we identified 16,900 to 17,600 transcripts per sample (**Figure 4A**). Raw  
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48 155 gene counts can be found online (see Supporting Data – DOI to appear). Differential gene  
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50 156 expression analyses across groups were performed using EdgeR v3.12.1 [6, 7] using only genes  
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52 157 with a count per million (CPM) >1 in [at least 4 samples](#) (**Supplementary Table 1** for details on  
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54 158 the commands used). Since laminar identity is an important feature of our experimental setup, we  
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56 159 assessed the relative expression of known molecular markers for L2/3 (*Cacna1h*, *Id2*, *Igfbp4*,  
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58 160 *Igfn1*, *Mdga1*, *Plcx1*, *Rasgrf2*, *Rgs8*, *Tle3*) and L4 (*Cartpt*, *Cyp39a1*, *Kcnh5*, *Kcnp2*, *Lmo3*,  
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4 161 *Rorb*, *Scnn1a*) [8–10], which showed selective enrichment of the laminar markers in isolated  
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6 162 layers (**Figure 4B**).

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11 164 *Figure 4 is about here*  
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15 166 To assess the variance in transcript counts, we calculated the coefficient of variation (CV) [for](#)  
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17 167 each [transcript](#) with a cut-off of 50 as the minimal read count [separately for each group](#) (**Figure**  
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19 168 **4C**). This analysis showed that, [on average](#), 85.93% of transcripts have a CV below 15%,  
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21 169 suggesting [low](#) variance [across transcript counts for individual genes](#). [Principal component](#)  
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23 170 [analysis \(PCA\) showed that samples cluster based on layer, and the first two components](#)  
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25 171 [explained ~88% variance the data \(Figure 4C, Supplemental Figure 2B\)](#).  
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31 173 These quality control routines suggest that we have obtained RNA-sequencing data of high read  
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33 174 quality, with individual bases being called confidently throughout the length of reads, which  
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35 175 uniquely map to the mm10 reference genome at high rates (>90% average). The laminar origin  
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37 176 of our samples could be identified through known molecular markers, confirming our samples are  
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40 177 of high anatomical specificity.  
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#### 42 178 43 44 179 [Re-use potential](#)

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46 180 [The current RNA-seq dataset might help address the molecular underpinnings of cortical](#)  
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48 181 [experience-dependent plasticity. For example, it could be used \(1\) to identify genes whose](#)  
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50 182 [transcription is modulated in an experience-dependent manner, \(2\) to statistically map the](#)  
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52 183 [transcriptional networks at laminar resolution, \(3\) creating synergy with the single neuron RNA-](#)  
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54 184 [seq datasets \[11, 12\], to address the molecular diversity of the cortical networks, \(4\) combined](#)  
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56 185 [with the proteomic analysis performed under comparable experimental conditions in the](#)  
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58 186 [accompanying manuscript \(Kole et al, submitted\), to systematically study the transcriptional and](#)  
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187 translational regulation of the genome upon altered sensory experience, and finally (5) to identify  
188 and quantify splice isoforms, given the sequencing depth of the current dataset. Since splicing  
189 and other posttranscriptional mechanisms govern which proteins are ultimately produced,  
190 combining the current transcriptomic dataset with a proteomics approach would also be of high  
191 importance.

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193 The current dataset focuses on isolated cortical columns and layers, which are necessarily  
194 diverse samples containing neuronal and non-neuronal cell classes. In terms of experience  
195 dependent plasticity, although most previous studies focus on excitatory projections, inhibitory  
196 cells and even non-neuronal cells have been implicated in plasticity [13–15]. This heterogeneity  
197 might be particularly important for L2/3, as also shown by the principal component analysis  
198 (Figure 4D), given the relative diversity of cellular populations in supragranular layers and their  
199 heterogeneous connectivity patterns [16].

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201 Researchers reusing our dataset should be aware that comparisons between control column C  
202 and spared columns (A/E, B/D) may have to be approached with caution, as this would involve  
203 two different columnar identities (whose transcriptomic dissimilarities are currently unknown),  
204 each coming from cortices that have had different sensory experience. However direct  
205 comparisons between the C columns across experimental conditions (i.e control versus deprived)  
206 as well as within-animal across-column comparisons in deprived animals control for these  
207 confounding variables.

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209 Taken together we hope that this data will prove useful in discovering novel molecular targets  
210 responsible for cortical plasticity and will lead to targeted control of plasticity in health and disease.

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**273 Availability of the supporting data**

274 Supporting data are available online (<https://goo.gl/tBof51>) and will be distributed via GigaScience  
275 DB.

276 Raw sequence reads were deposited in NCBI GEO.

277 Link: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE90929>

**279 List of abbreviations**

280 EDP Experience dependent plasticity

281 L2/3 Cortical Layer 2/3, also known as supragranular layers

282 L4 Cortical Layer 4, i.e. granular layer

**284 Competing interests**

285 The authors declare no competing interests.

**287 Author contributions**

288 KK performed all experimental manipulations, sample acquisition, biological and bioinformatic  
289 quality controls, and prepared the tables and figures. YK and JaP performed bioinformatic  
290 analysis. JeP performed library prep. VB supervised RNA-seq. PT contributed bioinformatic  
291 analysis and co-supervised the project. TC designed and supervised the project. KK and TC wrote  
292 the manuscript. All authors edited otherwise approved the final version of the manuscript.

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4 **293 Figure Legends**

5 **294**  
6  
7 **295 Figure 1.** Overview of the experimental design, sample collection and data organization. **(A)** Pups  
8 **296** were bilaterally spared or deprived of off their C-row whiskers between P12 and P23-P24, when  
9 **297** acute slices are made and column- and layer-specific tissues were excised. **(B)** RNA was isolated,  
10 **298** checked for integrity and purity, and subsequently sequenced. **(C)** Organization of the database.  
11 **299** Colour codes denote experimental groups. Same denominations are used in the read counts  
12 **300** matrix file (see **Supplemental Data**).  
13 **301**

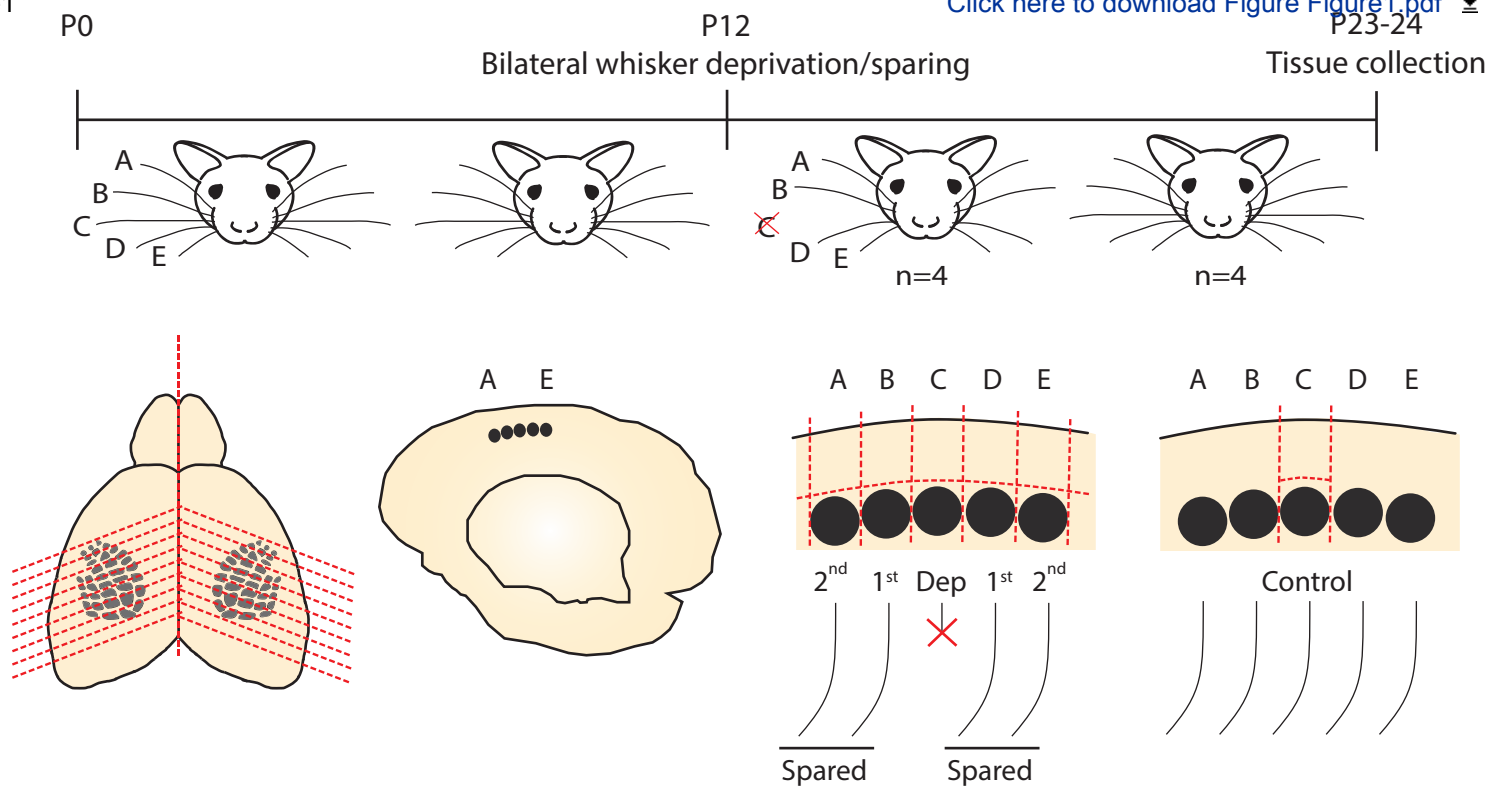
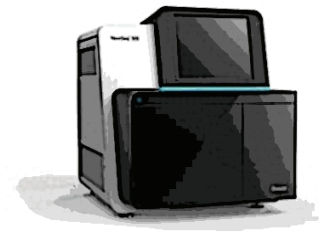
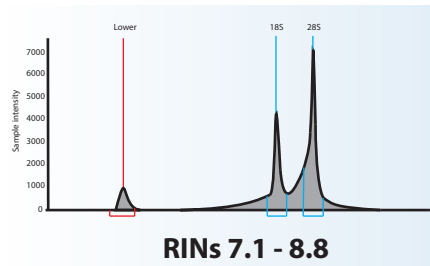
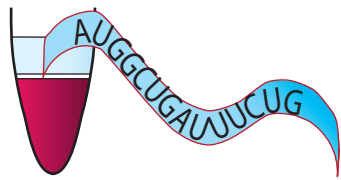
14 **302 Figure 2.** FastQC and STAR output graphs for all samples. **(A-B)** *Phred* scores per base and  
15 **303** per sequence. **(C)** Per sequence GC content. **(D)** STAR output of alignment scores.  
16 **304**

17 **305 Figure 3.** Overlays of duplication plot contours, showing a positive correlation between read  
18 **306** density and duplication levels. Depicted contours enclose 90% of the data points.  
19 **307**

20 **308 Figure 4.** Gene expression analyses. **(A)** Histogram of read counts per transcript per sample.  
21 **309** With a cut-off of 2 reads, between 16,900 and 17,600 transcripts could be identified across  
22 **310** samples. **(B)** Relative expression of known molecular markers for cortical laminae. Layer 4  
23 **311** markers are enriched in samples originating from this layer; the same is true for Layer 2/3 marker  
24 **312** expression in Layer 2/3 samples. **(C)** Cumulative [plots](#) of the coefficient of variance (CV) [of](#)  
25 **313** [individual experimental](#) groups. [Including only transcripts identified by 50 reads or more, average](#)  
26 **314** [CVs of <15% are found in ~85% of transcripts.](#) **(D)** [Principal component analysis \(PCA\) showing](#)  
27 **315** [sample clustering by layer, including only transcripts identified by at least 50 reads. Principal](#)  
28 **316** [component \(PC\) 1 and 2 account for 88% of overall variance.](#)  
29 **317**

30 **318 Supplemental Figure 1.** Duplication plots for all samples, produced using SeqMonk (Babraham  
31 **319** Bioinformatics).  
32 **320**

33 **321 [Supplemental Figure 2. \(A\) Cumulative plots of the coefficient of variance \(CV\) of experimental](#)**  
34 **322 [each group, including transcripts identified by at least one read. Average CVs of <25% are](#)**  
35 **323 [found in ~85% of transcripts. \(B\) Principal component analysis \(PCA\) including transcripts](#)**  
36 **324 [identified by at least one read. The majority \(88%\) of overall variance is explained by Principal](#)**  
37 **325 [components \(PC\) 1 and 2.](#)**  
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**B**

RNA isolation, quality control and sequencing

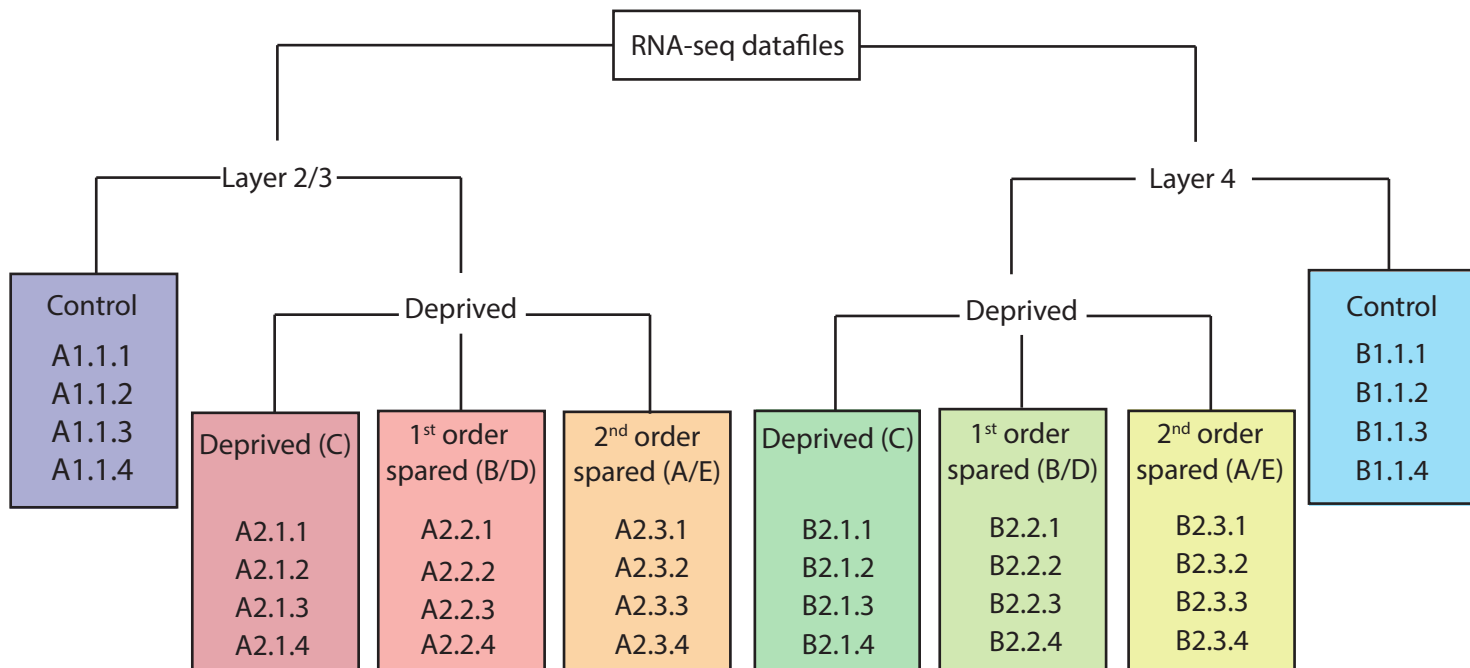
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Figure2

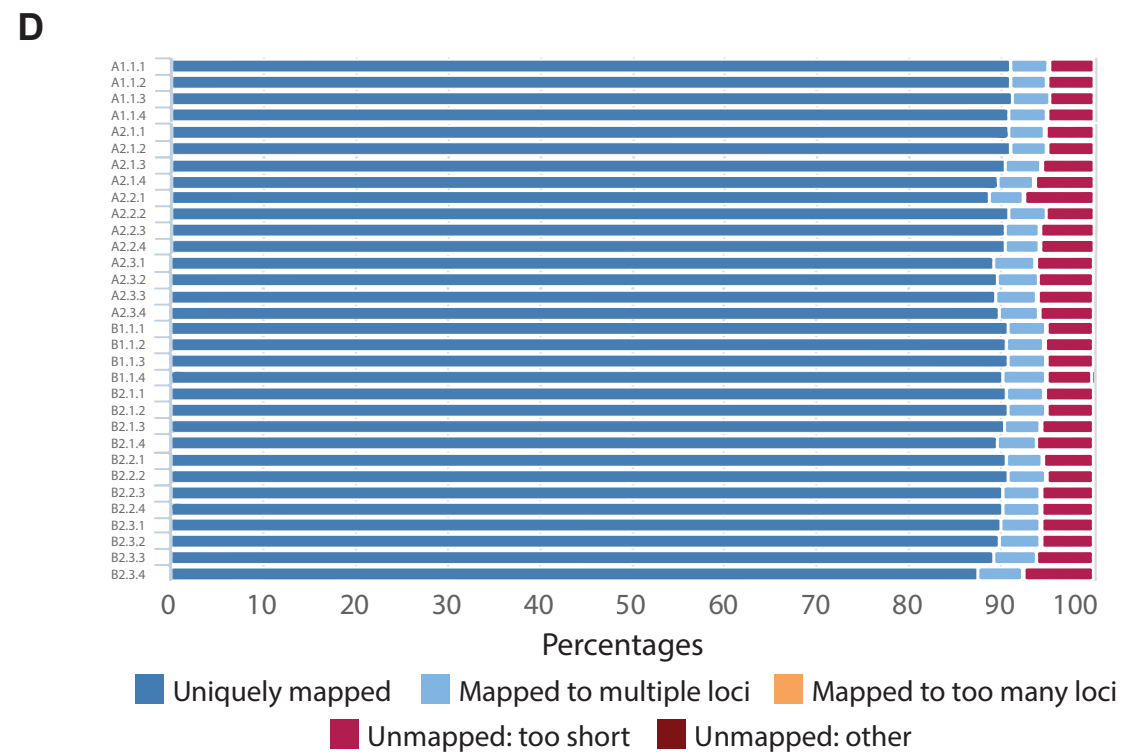
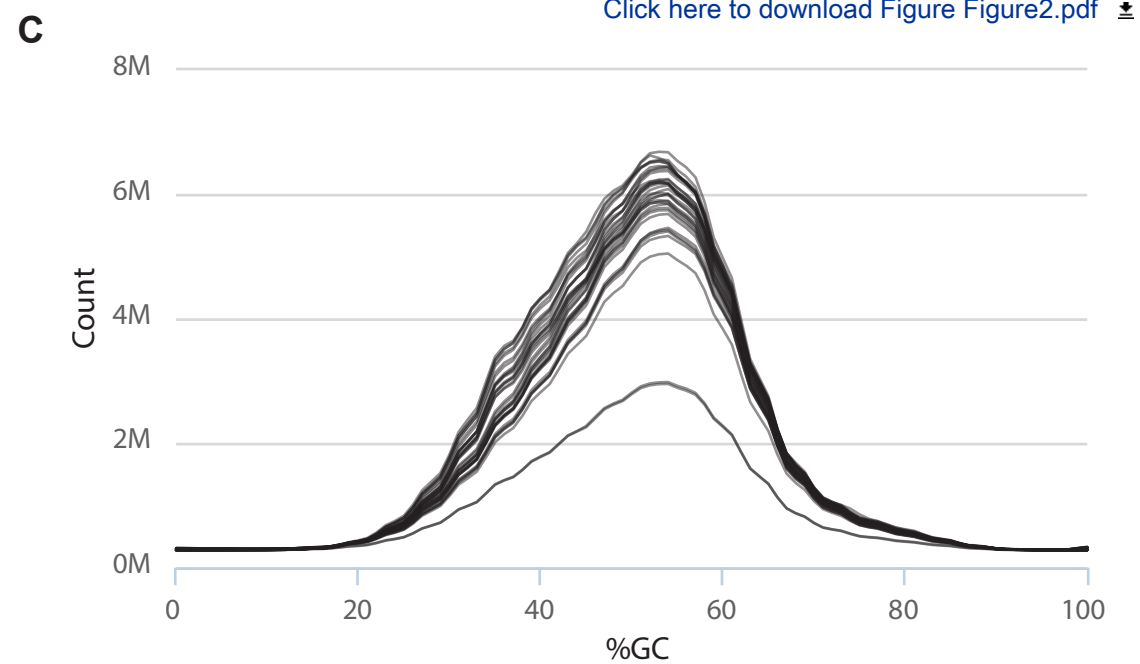
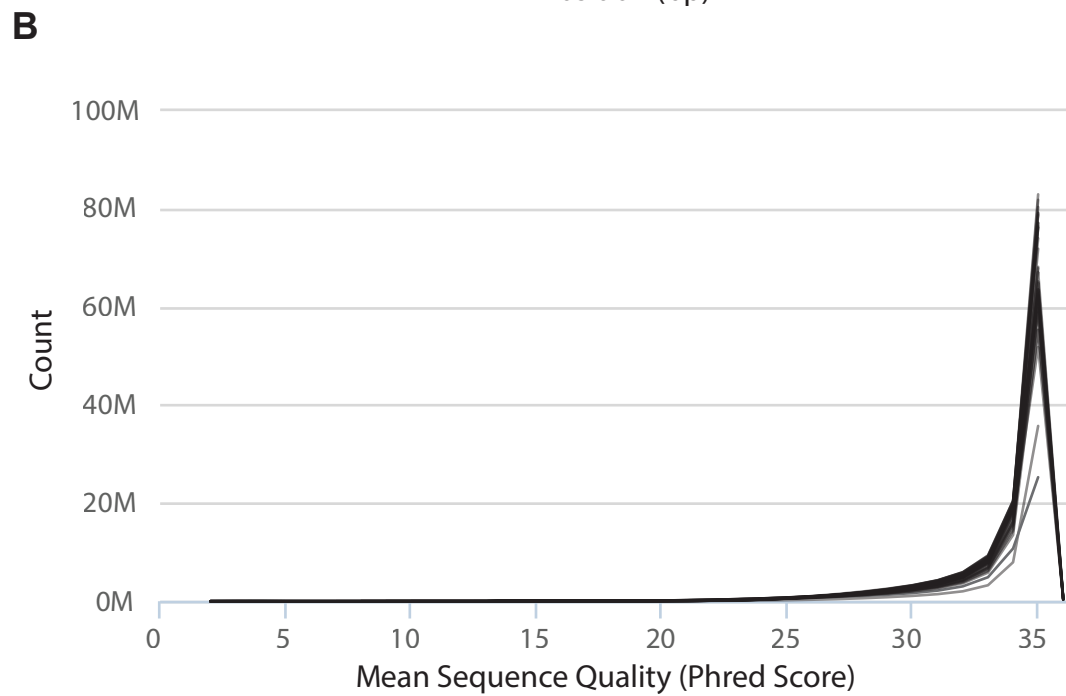
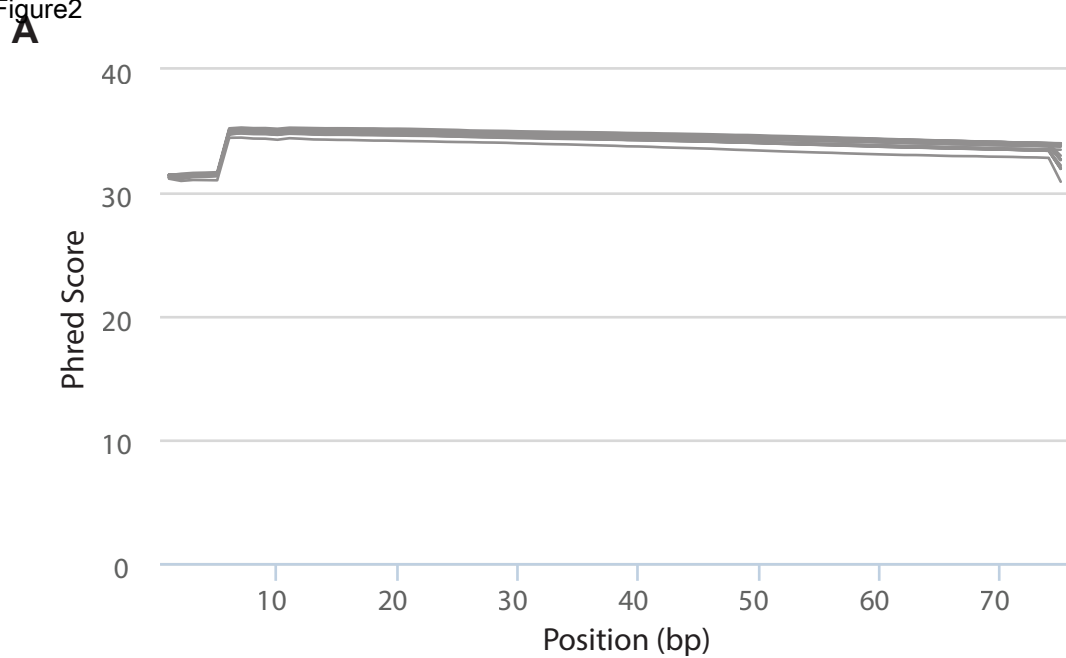


Figure3

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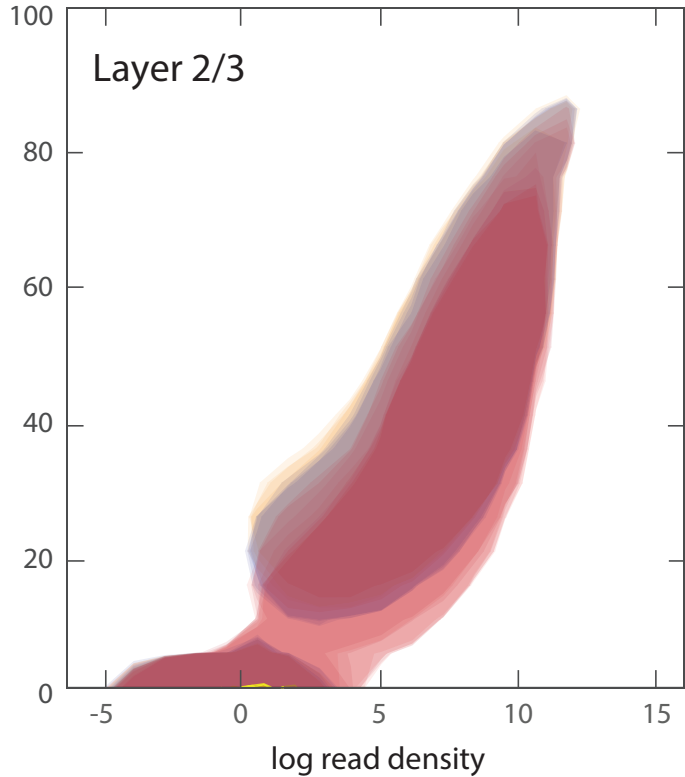
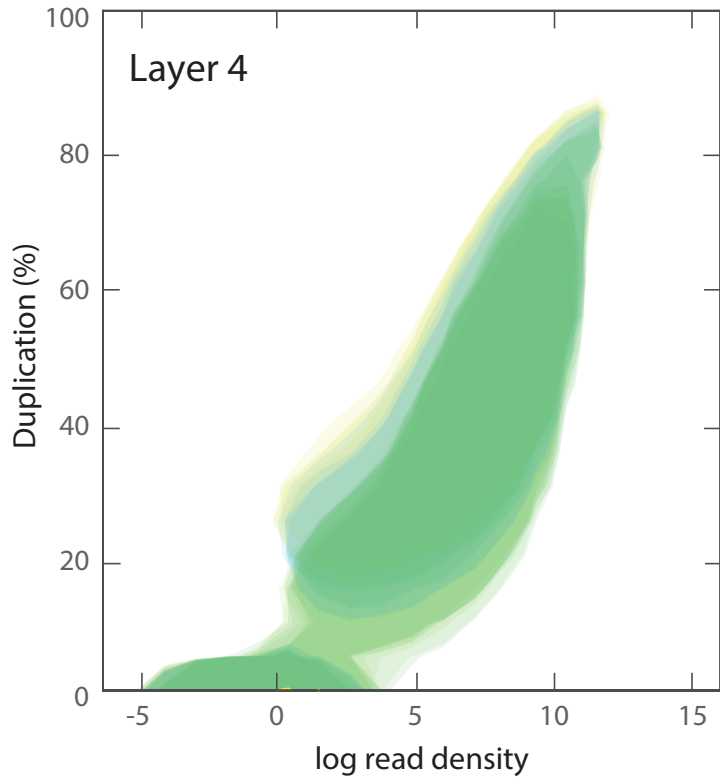
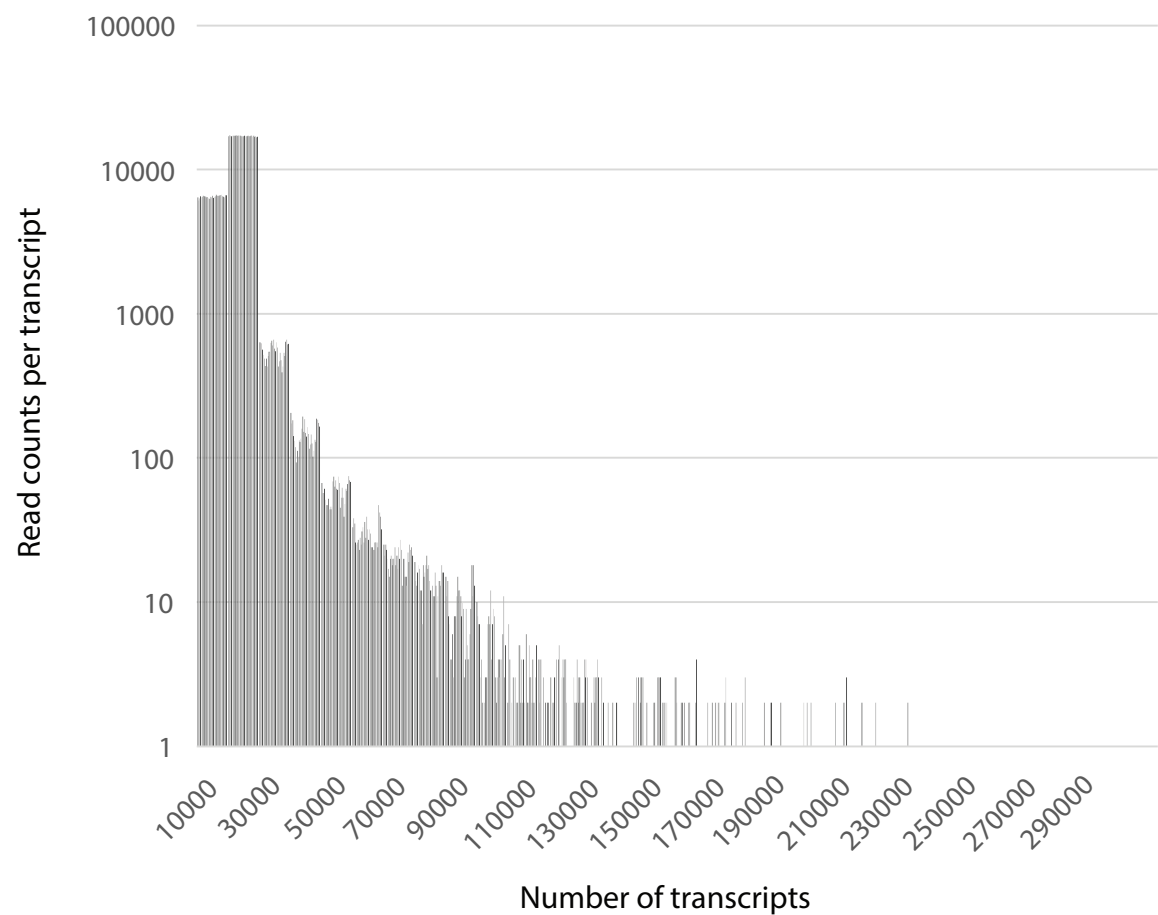
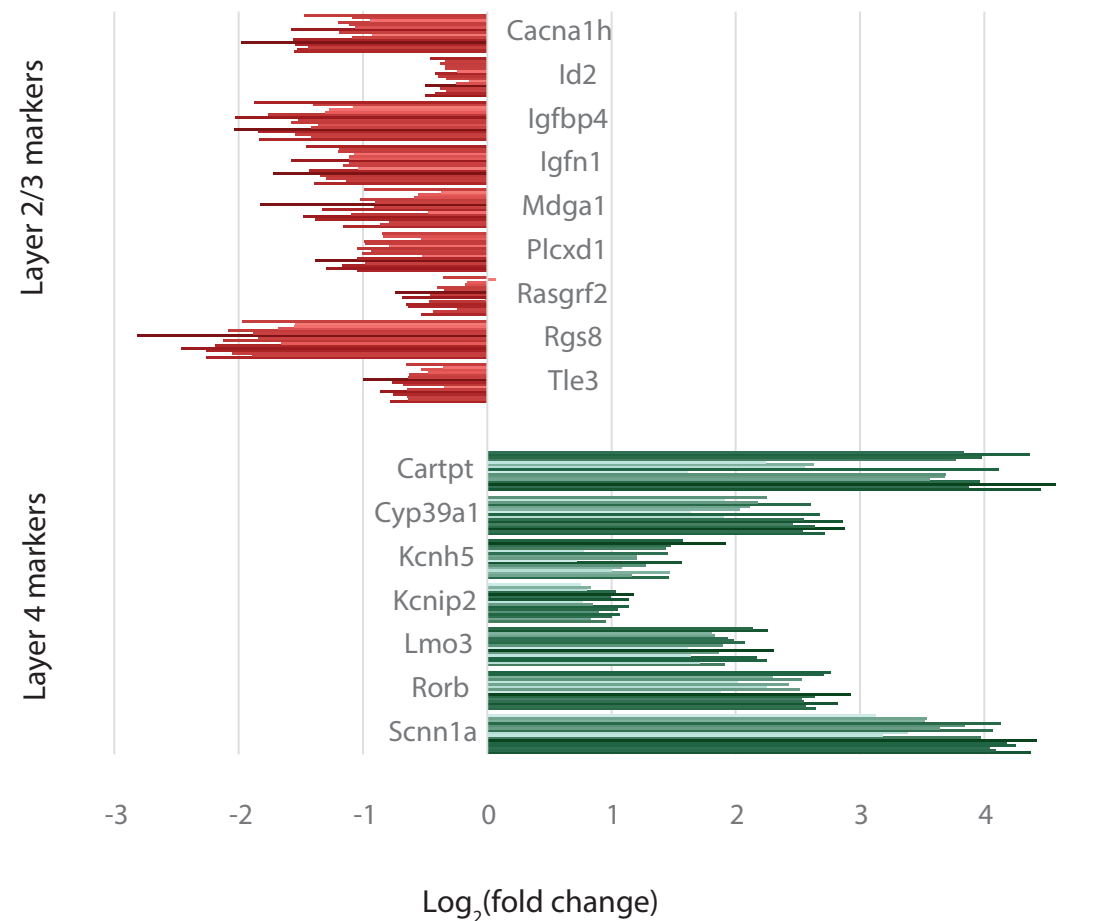
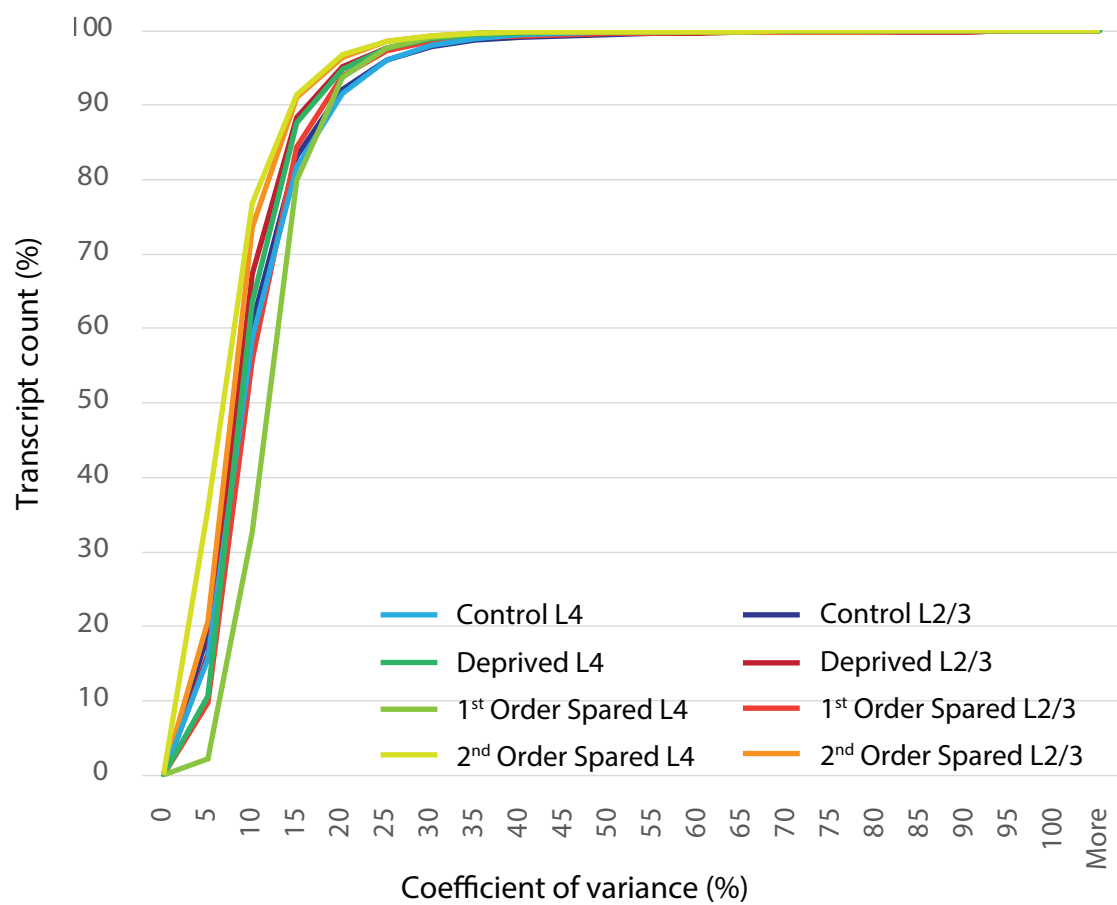
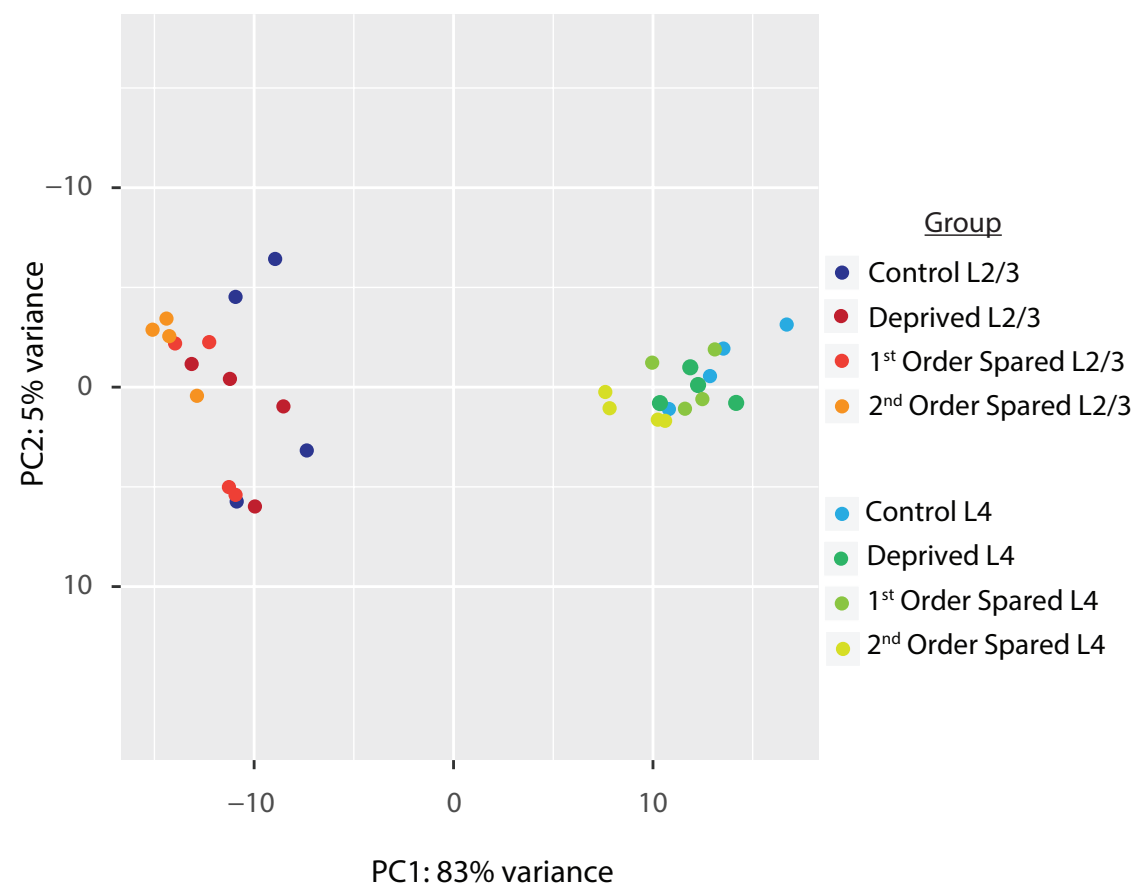


Figure 4

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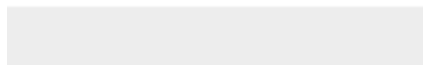




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