Transcriptional mapping of the primary somatosensory cortex upon sensory deprivation

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Background (162)

Experience-dependent plasticity (EDP) is essential for anatomical and functional maturation of sensory circuits during development. Although the principal synaptic and circuit mechanisms of EDP are experimentally and computationally increasingly well studied, its molecular mechanisms remain largely elusive. EDP can be readily studied in the rodent barrel cortex. Each 'barrel column' topographically is linked to the principal whisker that forms its main source of input. Depriving select whiskers while sparing their neighbours introduces competition between barrel columns, ultimately leading to weakening of intracortical, translaminar (i.e. Cortical Layer (L)4-to-L2/3) feed-forward excitatory projections in the deprived columns. The same synapses are potentiated in the neighbouring spared columns. These experience-dependent, and cortical column- and layer-specific, alterations of synaptic strength are thought to underlie somatosensory map plasticity. We used RNA sequencing in this model system to uncover cortical-column and -layer specific changes on the transcriptome level that are induced by altered sensory experience. This resource will help to systematically address the molecular processes that underlie cortical plasticity.

Findings (47)

From column- and layer-specific barrel cortical tissue, high quality RNA was purified and sequenced. The current dataset entails an average of 50 million paired-end reads per sample, 75 base pairs in length. On average, 90.15% of reads could be uniquely mapped to the mm10 reference mouse genome.

Conclusions (32) – Word total for the abstract: 241 out of 250

The current data reveal the transcriptional changes in gene expression in the barrel cortex upon altered sensory experience in juvenile animals and will help to molecularly map the mechanisms of cortical plasticity.

Data Description

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

In the whisker system experience dependent plasticity is most commonly studied in the barrel cortex subfield of the primary somatosensory cortex where neural representations of whiskers change in response to altered patterns of incoming sensory information. As originally shown in the barrel cortex (Hand 1982), sensory deprivation induced by transient whisker trimming is sufficient to perturb neural receptive fields both during development and in adulthood. Previous work has also shown that the cellular basis of deprivation-induced decreases in whisker evoked representations are primarily due to a reduction of synaptic strength in monosynaptically connected feed-forward neuronal networks in behaving animals (Allen et al. 2003; Celikel et al. 2004). Conversely whisker sparing induced enhancement in whisker representation is mediated at least in part by the long-term synaptic facilitation expressed along the L4 projections *in vivo* (Clem et al. 2008). Identification of the molecular events that mediate these bidirectional changes in synaptic connectivity will benefit from systematic analysis of the gene transcription. Therefore, we performed RNA sequencing in the barrel cortex with or without sensory deprivation across cortical layers 2-4. This database will assist molecular and cellular neurobiologists in addressing the molecular mechanisms associated with experience dependent plasticity, and will enable statistical approaches to determine the dynamics of the coupled changes across molecular pathways as cortical circuits undergo plastic changes in their organization.

Animals

All experiments were performed in accordance with the Animal Ethics Committee of the Radboud University in Nijmegen, the Netherlands. Pregnant wild type mice (Charles River) were kept at a 12-hour light/dark cycle with access to food *ad libitum*. Cages were checked for birth daily. To

induce experience-dependent plasticity, pups underwent bilateral plucking of their C-row whiskers under isoflurane anaesthesia at P12 (**Figure 1**). Control animals were not plucked but anaesthetized and handled similarly. After recovery pups were returned to their home cage. Every other day pups were checked for whisker regrowth, which were plucked if present. At P23-P24, pups were randomly selected from their litter for slice preparation and tissue collection. For each experimental condition (i.e. whisker deprived or control), 4 female pups were used.

Figure 1 is about here

Slice preparation and sample collection

Pups were anaesthetized using isoflurane and then perfused with ice cold carbogenated slicing medium (108 mM ChCl, 3 mM KCl, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 25 mM glucose, 1 mM $CalCl_2$, 6 mM $MgSO_4$ and 3 mM Na-pyruvate). Next, pups were decapitated, after which the brain was quickly dissected out and 400 µm thalamocortical slices from each hemisphere were prepared as described before (Allen et al. 2003; Celikel et al. 2004). Slices were transferred to 37 degrees Celcius carbogenated ACSF (120 mM NaCl, 3.5 mM KCl, 10 mM glucose, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, 25 mM $NaHCO_3$ and 1.25 mM NaH_2PO_4) where they were kept for 30 minutes and recovered at room temperature for another 30 minutes until tissue collection.

After incubation, slices were placed under a Nikon Eclipse FN1 microscope. The holding chamber was continuously perfused with room temperature carbogenated ACSF. Due to the 55 degree cut, slices were obtained in which S1 barrels from specific rows (A-E) could be identified. A thin, long glass pipette was pulled using a Sutter instruments P-2000 pipette puller and was used to make intercolumnar incisions from L1 to the bottom of L4 after which the slice was placed under a binocular dissection microscope where the location of specific barrel columns could now be readily identified by eye. A sterile 32G needle was then used to cut out L2/3 and L4 separately

from each column. Tissue from columns A/E and B/D were pooled as they both constitute second and first order spared whiskers, respectively. Immediately after dissection, tissue samples were snap frozen in liquid nitrogen and stored at -80 degrees Celcius until further use. All tools that came into direct contact with brain tissue were treated using RNAseZap in order to minimize RNAse contamination.

RNA isolation and quality control

Tissue samples originating from the same rows and layers were pooled within each animal. Tissue was quickly dissolved in Qiazol (Qiagen #79306), after which RNA was isolated using the miRNeasy Mini kit (Qiagen #217004), DNAse treated (Thermo Scientific, #EN0521) and cleaned up using RNeasy MinElute kit (Qiagen #74204), all following the manufacturer's instructions. Samples were then stored at -80 degrees Celcius until further processing.

RNA sample integrity was determined using Agilent Tapestation (High Sensitivity RNA Screentape). Sample RINs ranged from 7.1 to 8.8. To further assess RNA purity and integrity, RNA samples were used in RT-PCR to confirm that cDNA could be produced and that a large (~1000 bp) amplicon could be obtained. To produce cDNA, SuperScript® II Reverse Transcriptase (Thermo Scientific #18064014) and random hexamer primers (Roche #11034731001) were used. The resulting cDNA was then added to a PCR reaction mix which further consisted of Jumpstart Ready Mix (Sigma P2893) and exon-exon junction spanning CamKII primers (FW TCCAACATTGTACGCCTCCAT; RV TGTTGGTGCTGTCGGAAGAT). From all cDNA samples a fragment of the expected size could be amplified, suggesting that the RNA samples contained pure RNA of sufficient integrity. All RNA samples thus passed our quality control criteria and were subjected to RNA sequencing.

RNA sequencing

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

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

Figure 2 is about here

Reads were then mapped to the mm10 reference genome using STAR (Dobin et al. 2013), which uniquely mapped between 39,000,000 and 59,000,000 reads, constituting an average 90.15% unique map rate across samples (**Figure 2D**). Since the library preparation protocol entails a PCR enrichment step which can lead to technical duplication, hence an overestimation of observed transcripts, we used Seqmonk (Babraham Bioinformatics) to plot the read density against the duplication levels (i.e. the percentage of duplicate reads) for each transcript. The obtained duplication plots showed a clear positive relation between read density and duplication levels (**Figure 3** and **Supplemental Figure 1**), suggesting that the origin of read duplication is biological, rather than technical.

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

Figure 3 is about here

Analysis of gene expression

Using a 2 read cut-off, we identified 16,900 to 17,600 transcripts per sample (**Figure 4A**). Raw gene counts can be found online (see Supporting Data – DOI to appear). Differential gene expression analyses across groups were performed using EdgeR v3.12.1 (Robinson et al. 2010; McCarthy et al. 2012) using only genes with a count per million (CPM) >1 in all of the samples within each group (**Supplementary Table 1** for details on the commands used). Since laminar identity is an important feature of our experimental setup, we assessed the relative expression of known molecular markers for L2/3 (*Cacna1h*, *Id2*, *Igfbp4*, *Igfn1*, *Mdga1*, *Plcxd1*, *Rasgrf2*, *Rgs8*, *Tle3*) and L4 (*Cartpt*, *Cyp39a1*, *Kcnh5*, *Kcnip2*, *Lmo3*, *Rorb*, *Scnn1a*) (Rowell et al. 2010; Xue et al. 2014; Molyneaux et al. 2015), which showed selective enrichment of the laminar markers in isolated layers (**Figure 4B**).

Figure 4 is about here

To assess the variance in transcript counts between experimental groups, we calculated the coefficient of variation (CV) of each group with a cut-off of 50 as the minimal read count (**Figure 4C**). This analysis showed that 85.93% of transcripts have a CV below 15%, suggesting the overall variance in our dataset is low.

These quality control routines suggest that we have obtained RNA-sequencing data of high read quality, with individual bases being called confidently throughout the length of reads, which uniquely map to the mm10 reference genome at high rates (>90% average). The laminar origin of our samples could be identified through known molecular markers, confirming our samples are of high anatomical specificity. This RNA-seq dataset should prove useful for researchers interested in the molecular underpinnings of cortical experience-dependent plasticity.

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Availability of the supporting data

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

Raw sequence reads were deposited in NCBI GEO.

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

List of abbreviations

EDP Experience dependent plasticity

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

L4 Cortical Layer 4, i.e. granular layer

Competing interests

The authors declare no competing interests.

Author contributions

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

Figure Legends

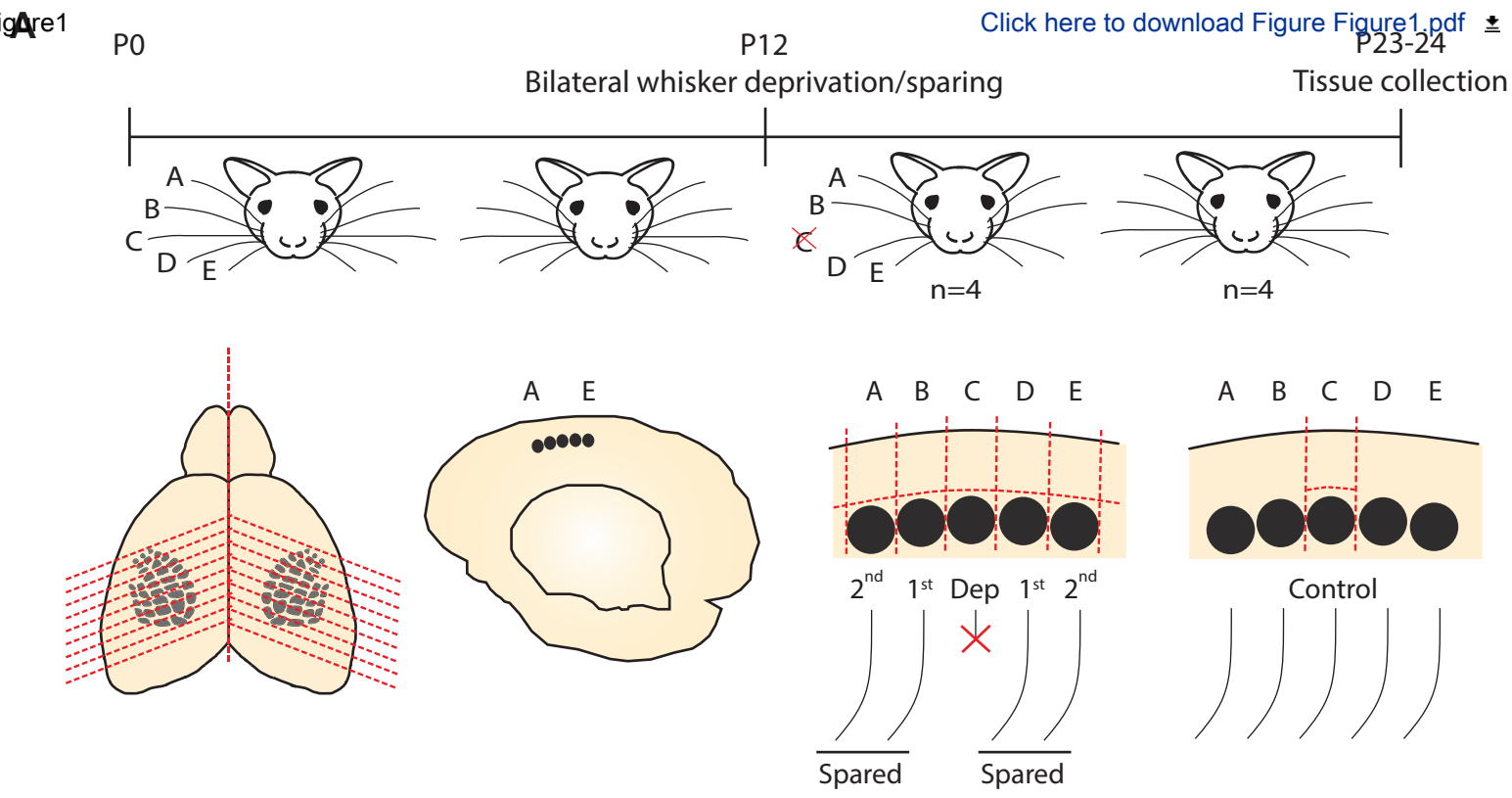
Figure 1. Overview of the experimental design, sample collection and data organization. **(A)** Pups were bilaterally spared or deprived of off their C-row whiskers between P12 and P23-P24, when acute slices are made and column- and layer-specific tissues were excised. **(B)** RNA was isolated, checked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

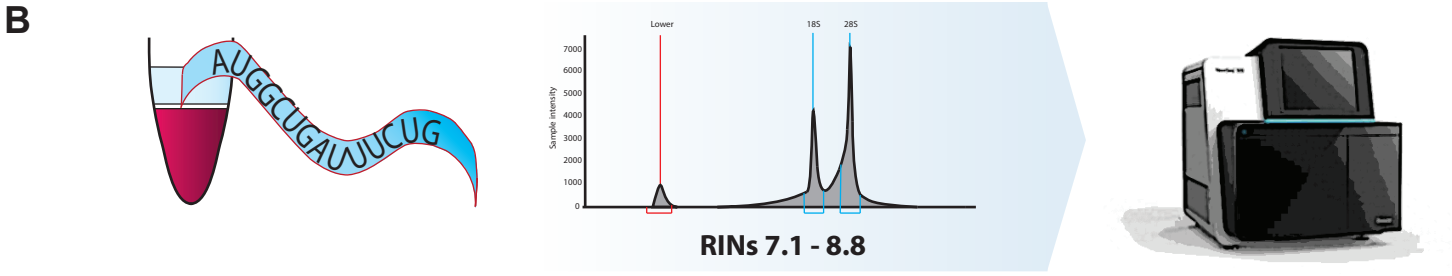
Figure 2. FastQC and STAR output graphs for all samples. **(A-B)** *Phred* scores per base and per sequence. **(C)** Per sequence GC content. **(D)** STAR output of alignment scores.

Figure 3. Overlays of duplication plot contours, showing a positive correlation between read density and duplication levels. Depicted contours enclose 90% of the data points.

Figure 4. Gene expression analyses. **(A)** Histogram of read counts per transcript per sample. With a cut-off of 2 reads, between 16,900 and 17,600 transcripts could be identified across samples. **(B)** Relative expression of known molecular markers for cortical laminae. Layer 4 markers are enriched in samples originating from this layer; the same is true for Layer 2/3 marker expression in Layer 2/3 samples. **(C)** Cumulative plot of the coefficient of variance (CV) averaged across groups. CVs of <15% are found in ~85% of transcripts.

Supplemental Figure 1. Duplication plots for all samples, produced using SeqMonk (Babraham Bioinformatics).





RNA isolation, quality control and sequencing

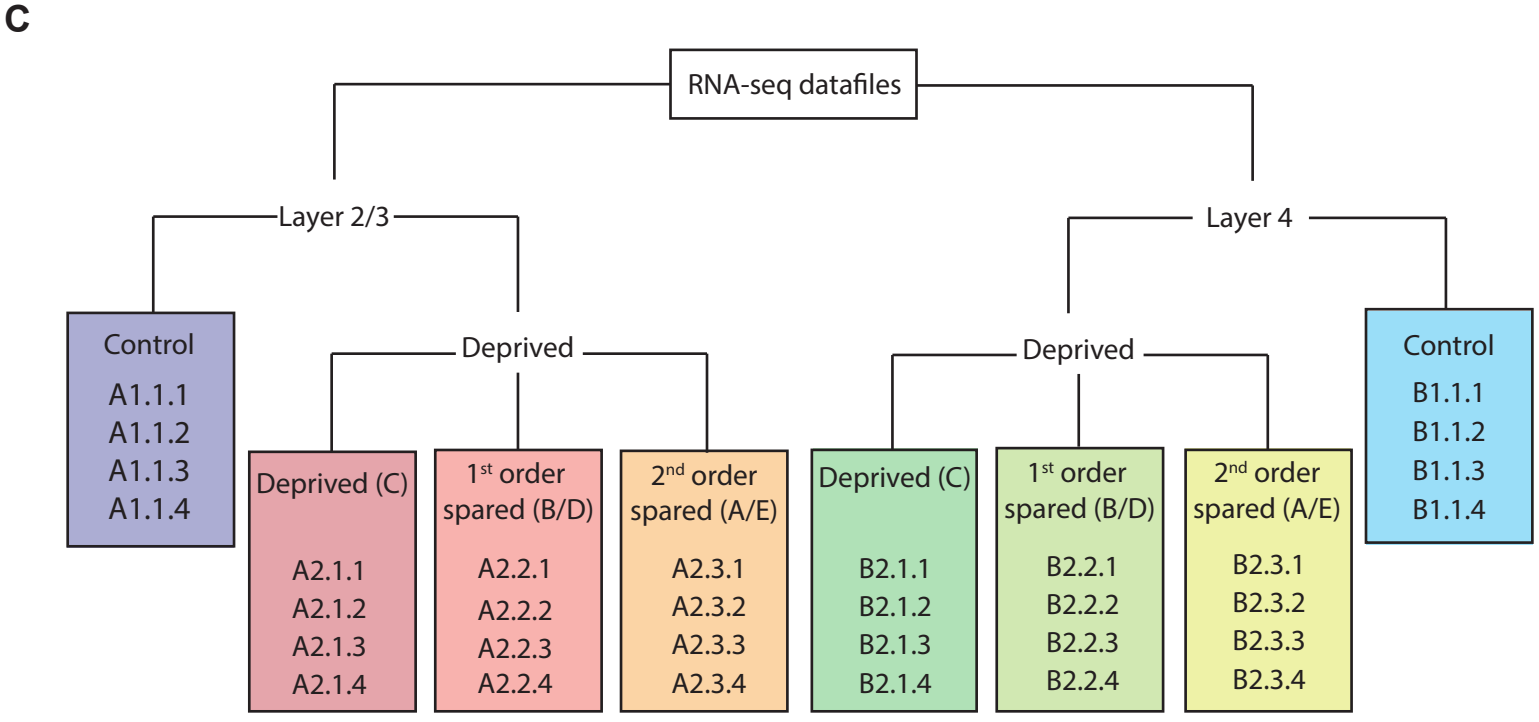


Figure2

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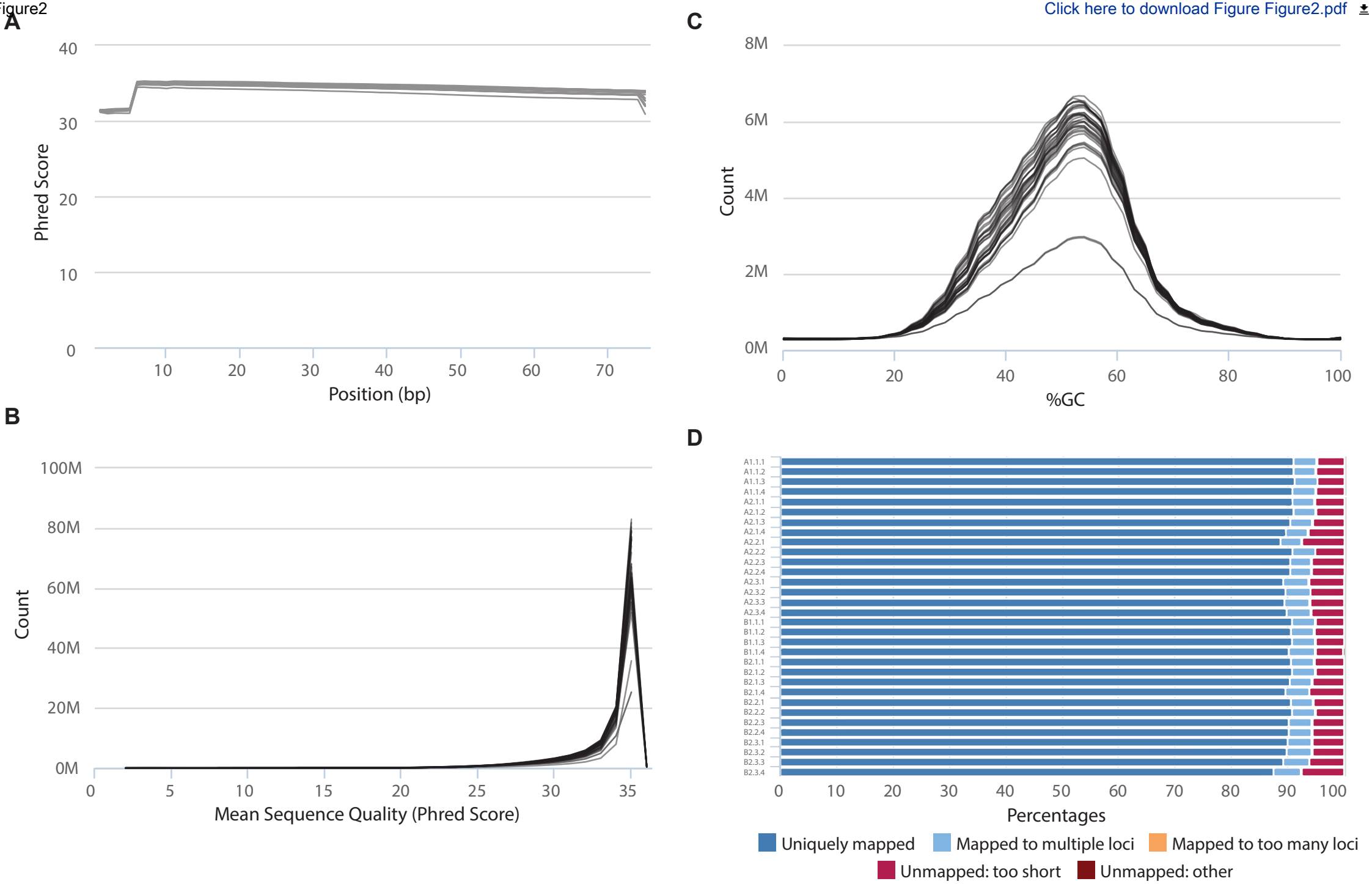

Figure3

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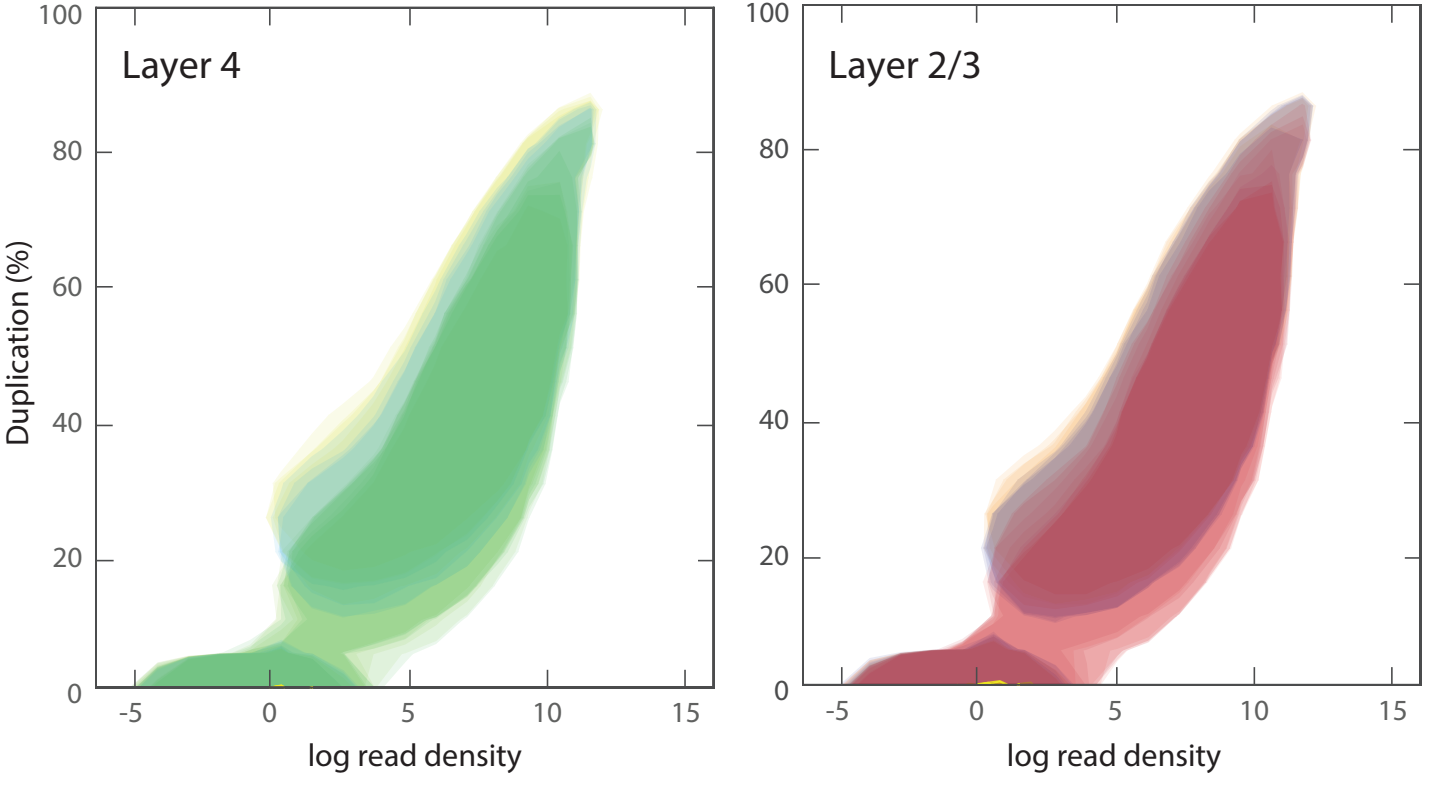

Figure4

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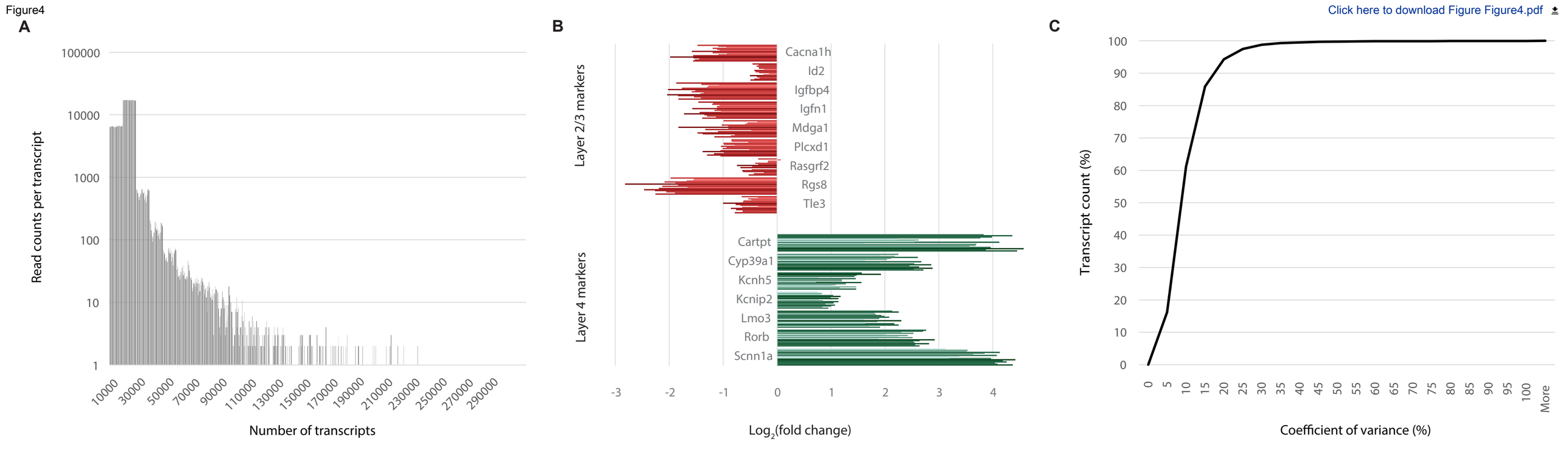

Supplementary Figure 1

Supplementary Table 1