Left-Right Asymmetry of Maturation Rates in Human Embryonic Neural Development

Supplemental Information

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Supplementary Methods

Tissue collection

Embryos were collected by the MRC-Wellcome Trust Human Developmental Biology Resource http://www.hdbr.org (1). The embryos were obtained anonymously from voluntary medical terminations (a combination of mifepristone and misoprostol) following appropriate informed consent by the donors, and with ethical approval from the Newcastle and North Tyneside NHS Health Authority Joint Ethics Committee. Donors to HDBR are asked to give written consent for the embryonic material to be collected, and are only approached once a decision to terminate their pregnancy has been made. Donors' ages ranged from 16 to 30 years old (Supplementary Table S1), and none of the abortions were due to observed congenital malformations or suspected genetic disorders. All karyotypes were normal. The development of the embryos was assessed and designated to the relevant Carnegie stage (CS) (2), using a practical staging guide devised to enable staging to a particular CS and using the external morphology of a single sample (3).

The spinal cords and hindbrains from eighteen embryos, ranging from CS13 to CS23 at the times of the terminations (Supplementary Table S1), were dissected out and separated from each other, after which they were also separated into left and right halves down the midline. Morphological landmarks were used to determine the spinal cord and hindbrain regions. The flexure of the brain and constriction of the neural tube at the isthmus was used to determine the superior limit of the hindbrain (i.e. midbrain/hindbrain junction). To separate hindbrain from spinal cord, the first dorsal root ganglion was used as landmark. The first dorsal root ganglion was not itself included in the tissue that was dissected, but the neural tube directly superior to this was used as the end point of the hindbrain, so that the neural tissue adjacent to the first dorsal root ganglion was included in the spinal cord tissue. The inferior limit of the spinal cord could not be defined in relation to visible external morphological features, but included the cervical and part of the thoracic region.

RNA-extraction and sequencing

RNA was extracted at HDBR Newcastle. Tissue samples were divided into sub-samples each weighing thirty milligrams. The sub-samples were homogenised using a Precellys 24 bead mill homogeniser (Bertin Corp. Rockville, MD, USA) using ceramic 1.4mm beads for soft tissue homogenising (CK14) with 600µl of RTL plus Buffer with 10µl/ml of β-Mercaptoethanol and 5µl/ml of reagent DX (Qiagen, Venlo, the Netherlands). RNA and DNA was extracted from the tissue with a QIAcube using an AllPrep DNA/RNA Mini Kit (Qiagen) following the manufacturer's recommended protocol. RNA was then pooled from all 30 mg sub-samples belonging to a given embryo's left or right spinal cord, or left or right hindbrain. RNA quality was assessed using an Agilent 2100 Bioanalyzer (Applied Biosystems,

Santa Clara, CA, USA), and then RNA was shipped on dry ice to Beijing Genomics Institute (BGI) Shenzhen/HongKong, China. At BGI, the RNA was treated with DNAse and quality determined again on an Agilent 2100 Bioanalyzer. Two within-embryo left-right pairs of samples (i.e. 1x hindbrain, 1x spinal cord) were excluded because of insufficient quality. All other samples passed the quality filters of: ≥4 μg RNA; concentration≥80 ng/μL; RIN≥7.0; 28 S/18 S≥1.0; smooth baseline and normal 5S peak.

For library preparation, Illumina TruSeq RNA-kits (Illumina, San Diego, CA, USA) were used according to the manufacturer's instructions. Magnetic beads with Oligo(dT) were used to isolate mRNA from total RNA. Mixed with the fragmentation buffer, the mRNA was fragmented. cDNA was synthesized using the mRNA fragments as templates, with random hexamer primers. Short fragments were purified and resolved with elution buffer for end-reparation and single nucleotide A (adenine) addition. After that, the short fragments were connected with adapters. After agarose gel electrophoresis, fragments were selected for PCR amplification as templates. During the quality control (QC) steps, Agilent 2100 Bioanalyzer and ABI StepOnePlus Real-Time PCR Systems (Applied Biosystems, Foster City, CA, USA) were used for quantification and qualification of the sample library. The library was sequenced using Illumina HiSeq^{TM} 2000, yielding paired reads of 90bp.

Bioinformatics & statistics

From each embryo, four libraries were sequenced: left and right spinal cord and left and right hindbrain, except for those failing quality control (Supplementary Table S1), making a total of 68 libraries. Primary sequencing data produced by Illumina HiSeq^{TM} 2000, called as raw reads, were subjected to QC to determine if a resequencing step was needed. After QC, raw reads were filtered into clean reads by removing reads with adapters, and reads with > 10% unknown bases or > 50% bases with quality < 10. The clean reads were aligned to the reference sequence GRCh37 (hg19) with SOAPaligner/SOAP2 v2.21 (4), using default settings. QC of alignment was performed to determine if resequencing was needed (5). Tophat (v2.08) (6) and Cufflink (v2.0.2) (7, 8) were used to refine alignments. SOAPsnp v1.05 was used to call genotypes of single nucleotide polymorphisms (SNPs) (9), and output files were reformatted for PLINK (10).

To inspect data quality, multidimensional scaling (MDS) analysis was used for the transcriptome-wide SNP data with PLINK 1.07 (10). Another MDS analysis was performed based on normalised and filtered (see below) gene expression level data (Bioconductor, plotMDS function). These QC steps uncovered an earlier tube labelling error, which led us to exclude one sample and its contralateral mate from further analysis. This resulted in data from sixteen embryos for spinal cord, and data from seventeen embryos for hindbrain (Supplementary Table S1). MDS analysis of the

expression data was then performed again, based only on the cleaned dataset (Supplementary Figure S1).

Supplementary Results

Gene Ontology (GO) enrichment

In the right spinal cord we observed enrichment for 129 GO-terms, and in the left spinal cord for 18 GO-terms (family-wise error rate (FWER) < 0.05) (Supplementary Table S2). In the hindbrain the numbers were 112 on the left and 17 on the right (Supplementary Table S3). GO-term clustering highlighted 'glutamate receptor signalling pathway' and 'neurotransmitter transport' for the left spinal cord, and processes related to the cell cycle, transcription and translation for the right spinal cord (Supplementary Figure S2). In hindbrain, GO-term clustering highlighted the process 'DNA-dependent DNA replication' on the left side and diverse processes on the right side (Supplementary Figure S2).

Fifty-one significantly upregulated GO-terms were shared between right spinal cord and left hindbrain. Examples are shown Figure 2 in the main text. Two terms were shared between left spinal cord and right hindbrain. None were shared between ipsilateral sides of spinal cord and hindbrain. For the shared GO-terms for left spinal cord and right hindbrain, the clustering highlighted 'synaptic transmission', 'neuron maturation' and 'developmental cell growth'. For right spinal cord and left hindbrain, cell cycle and RNA processing terms were clustered, as well as chromosome segregation terms (Supplementary Figure S3).

Transcription factor targets

The gene-sets in the MSigDB-collection 'transcription factor targets' v5.0 represent genes with particular motifs within 2 kb of their transcription start sites. Not all motifs match known transcription factors (TFs), while some motifs match multiple TFs, and some TFs bind multiple motifs. In the spinal cord, genes with motifs targeted by NRSF (also known as REST) were strongly upregulated in the left side of the tissue (FWER<0.0005) (Supplementary Table S4). Also upregulated in the left spinal cord were targets for *SF1* and *TCF3* (E47) (Supplementary Table S4). *NRSF* encodes a transcriptional repressor that represses neuronal genes in non-neuronal tissues (11). *SF1* encodes a nuclear pre-mRNA splicing factor, and is involved in sex determination (12); the encoded protein of *TCF3* is required for B and T lymphocyte development, but also plays a role in other processes such as cell differentiation (13, 14). In the right spinal cord we observed upregulation of targets for *E2F1* (Fig

2, Supplementary Table S4) and *TFDP1.* The complete list for the spinal cord can be found in Supplementary Table S4.

In the left hindbrain, 27 sets of genes located near particular motifs were upregulated, whereas only one set was upregulated in the right hindbrain (Supplementary Table S5). The motifs on the left matched *E2F1, E2F4, TFDP1, TFDP2, RB1, YY1* (Figure 3, Supplementary Table S5). *E2F1* can mediate both cell proliferation and p53-dependent/independent apoptosis (15, 16); *E2F4* plays an important role in the suppression of proliferation-associated genes. It helps *E2F1* to associate with *RB1* (17); *TFDP1* and *TFDP2* dimerize with E2F transcription factors, resulting in transcriptional activation of cell cycle regulated genes (18); *RB1* is a negative regulator of the cell cycle, which interacts with *E2F* factors. It is also required for cells to resist G1 to S transition (phases of the cell cycle) in response to un-programmed proliferative signals (19); *YY1* acts both as a repressor and an activator at various promoters, and is a negative regulator of p53 (20, 21). Two of the gene sets having motifs for *E2F1/TFDP1* were also significantly enriched in right spinal cord, as mentioned above.

The asynchrony between left and right spinal cord

For all genes within the most left-lateralised GO-term (GO:0007269 (Neurotransmitter_secretion (5), and separately for the most right-lateralised GO-term GO:0006261 (DNA_Dependent_DNA_Replication (7)), we calculated the median expression level per RNA-seq library, and then fitted a line of median expression vs Carnegie stage for the left and the right libraries separately, using either linear regression or allowing a quadratic term for Carnegie stage. In this approach, the distance between the left and the right line is an indicator of the developmental left-right asynchrony. As can be seen in Supplementary Figure S4 A-D, the size of the asynchrony varies by stage because the left and right lines are not parallel. Averaging over all of this analysis, the right-side delay is roughly 0.4 Carnegie stages, but the overall subtlety of lateralization as measured in 18 embryos makes it difficult to put one single and concrete value on the asynchrony.

Comparing spinal cord and hindbrain

Regarding developmental changes of gene expression over Carnegie stages, most genes behaved fairly similarly in hindbrain and spinal cord (Supplementary Figure S5), with a correlation of r=0.95 between the two tissues for their linear slopes of per-gene developmental changes in expression. However, for per-gene lateralization, the similarity between spinal cord and hindbrain was much weaker, as can be seen in Supplementary Figure S6, r=-0.26 for the per-gene left/right t-values in the two tissues. Yet, at the gene-set level, strongly lateralised sets in spinal cord also showed similar, but mirrored, patterns in hindbrain, as shown in Figures 2 and 3 and Supplementary Figures S2 and S3.

Supplementary Tables

Table S1. Information on samples

Embryo #	Carnegie Stage	Karyotype	Age of donor (years)	Batch	Time until freezing (min)	Note
11903	CS13	46, XX	20	1	315	
11993	CS13	46, XY	26	2	150	
12301	CS15	46, XY	19	2	240	
11898	CS16	46, XY	23	3	120	
11962	CS17	46, XX	24	1	225	spinal cord only
11978	CS17	46, XX	20	1	150	
12341	CS17	46, XY	30	3	240	
11905	CS18	46, XY	20	1	300	hindbrain only
12254	CS19	46, XY	19	2	120	
12292	CS20	46, XX	21	2	120	
12347	CS21	46, XX	22	3	600	
11954	CS22	46, XY	20	1	230	hindbrain only
11963	CS22	46, XX	23	1	60	
12307	CS22	46, XY	17	3	120	
12232	CS23	46, XX	28	2	105	
12285	CS23	46, XY	23	2	180	
11985	CS23	46, XY	Unknown	3	120	
12255	CS23	46, XY	16	3	135	

Table S2. Gene Ontology gene sets showing lateralization in spinal cord

GO description	GO number	# genes	NES[a]	FWER[b] p	Result in hindbrain[c]
Up on left side:					
NEUROTRANSMITTER_SECRETION (5)	GO:0007269	66	-2.83	0	
NEUROTRANSMITTER_TRANSPORT (4)	GO:0006836	77	-2.80	0	
REGULATION_OF_NEUROTRANSMITTER_LEVELS (4)	GO:0001505	86	-2.71	0	
GLUTAMATE_RECEPTOR_SIGNALING_PATHWAY (6)	GO:0007215	47	-2.70	0	
SYNAPTIC_TRANSMISSION (3)	GO:0007268	385	-2.65	0	+
REGULATION_OF_SYNAPTIC_TRANSMISSION (3)	GO:0050804	89	-2.56	0	+
REGULATION_OF_ALPHA_AMINO_3_HYDROXY_5_METHYL_4_ISOXAZOLE_PROPIONATE_SELECTIVE_GLUTAMATE_RECEPTOR_ACTIVITY (5)	GO:2000311	17	-2.37	0.004	
MEMBRANE_DEPOLARIZATION (5)	GO:0051899	63	-2.35	0.004	
ADULT_BEHAVIOR (4)	GO:0030534	27	-2.34	0.004	
SIGNAL_RELEASE (5)	GO:0023061	176	-2.33	0.004	
GLUTAMATE_SECRETION (5)	GO:0014047	21	-2.31	0.006	
DENDRITE_MORPHOGENESIS (5)	GO:0048813	56	-2.30	0.007	
REGULATION_OF_GLUTAMATE_RECEPTOR_SIGNALING_PATHWAY (5)	GO:1900449	21	-2.30	0.007	
LEARNING (5)	GO:0007612	29	-2.23	0.018	
DICARBOXYLIC_ACID_TRANSPORT (6)	GO:0006835	38	-2.23	0.019	
NEURON_NEURON_SYNAPTIC_TRANSMISSION (4)	GO:0007270	37	-2.19	0.038	
CENTRAL_NERVOUS_SYSTEM_NEURON_DIFFERENTIATION (6)	GO:0021953	47	-2.17	0.047	
POTASSIUM_ION_TRANSPORT (7)	GO:0006813	90	-2.16	0.049	
Up on right side:					
COTRANSLATIONAL_PROTEIN_TARGETING_TO_MEMBRANE (6)	GO:0006613	107	3.32	0	
SRP_DEPENDENT_COTRANSLATIONAL_PROTEIN_TARGETING_TO_MEMBRANE (6)	GO:0006614	106	3.30	0	
PROTEIN_TARGETING_TO_ER (5)	GO:0045047	109	3.29	0	
ESTABLISHMENT_OF_PROTEIN_LOCALIZATION_TO_ENDOPLASMIC_RETICULUM (6)	GO:0072599	113	3.21	0	
PROTEIN_LOCALIZATION_TO_ENDOPLASMIC_RETICULUM (7)	GO:0070972	124	3.11	0	
TRANSLATIONAL_TERMINATION (7)	GO:0006415	171	3.01	0	

GO description	GO number	# genes	NES[a]	FWER[b] p	Result in hindbrain[c]
NUCLEAR_ TRANSCRIBED_MRNA_CATABOLIC_PROCESS_NONSENSE_MEDIATED_DECAY (9)	GO:0000184	114	3.00	0	
TRANSLATIONAL_ELONGATION (6)	GO:0006414	176	2.95	0	
TRANSLATIONAL_INITIATION (4)	GO:0006413	234	2.95	0	
NUCLEAR_ TRANSCRIBED_MRNA_CATABOLIC_PROCESS (8)	GO:0000956	178	2.78	0	
PROTEIN_TARGETING_TO_MEMBRANE (5)	GO:0006612	151	2.76	0	
MRNA_CATABOLIC_PROCESS (7)	GO:0006402	188	2.71	0	
RNA_CATABOLIC_PROCESS (6)	GO:0006401	206	2.70	0	
TRANSLATION (6)	GO:0006412	412	2.68	0	
VIRAL_TRANSCRIPTION (5)	GO:0019083	177	2.68	0	-
CELLULAR_PROTEIN_COMPLEX_DISASSEMBLY (7)	GO:0043624	215	2.67	0	
MULTI_ORGANISM_METABOLIC_PROCESS (3)	GO:0044033	188	2.65	0	
PROTEIN_COMPLEX_DISASSEMBLY (6)	GO:0043241	233	2.63	0	
ANAPHASE_PROMOTING_COMPLEX_DEPENDENT_PROTEASOMAL_UBIQUITIN_DEPENDENT_PROTEIN_CATABOLIC_PROCESS (8)	GO:0031145	105	2.62	0	-
MACROMOLECULAR_COMPLEX_DISASSEMBLY (5)	GO:0032984	243	2.62	0	
VIRAL_GENE_EXPRESSION (4)	GO:0019080	187	2.61	0	
RRNA_PROCESSING (6)	GO:0006364	51	2.60	0	
MULTI_ORGANISM_CELLULAR_PROCESS (3)	GO:0044764	434	2.60	0	
VIRAL_PROCESS (4)	GO:0016032	425	2.59	0	
MRNA_METABOLIC_PROCESS (6)	GO:0016071	422	2.58	0	-
VIRAL_LIFE_CYCLE (5)	GO:0019058	309	2.58	0	
RRNA_METABOLIC_PROCESS (7)	GO:0016072	54	2.57	0	
INTERSPECIES_INTERACTION_BETWEEN_ORGANISMS (3)&SYMBIOSIS_ENCOMPASSING_MUTUALISM_THROUGH_PARASITISM (4)	GO:0044419	453	2.56	0	
AROMATIC_COMPOUND_CATABOLIC_PROCESS (5)	GO:0019439	337	2.50	0	
REGULATION_OF_UBIQUITIN_PROTEIN_LIGASE_ACTIVITY_INVOLVED_IN_MITOTIC_CELL_CYCLE (5)	GO:0051439	74	2.49	0	
ORGANIC_CYCLIC_COMPOUND_CATABOLIC_PROCESS (5)	GO:1901361	349	2.48	0	

GO description	GO number	# genes	NES[a]	FWER[b] p	Result in hindbrain[c]
NUCLEOBASE_CONTAINING_COMPOUND_CATABOLIC_PROCESS (5)	GO:0034655	318	2.47	0	
HETEROCYCLE_CATABOLIC_PROCESS (5)	GO:0046700	335	2.47	0	
POSITIVE_REGULATION_OF_UBIQUITIN_PROTEIN_LIGASE_ACTIVITY_INVOLVED_IN_REGULATION_OF_MITOTIC_CELL_CYCLE_TRANSITION (7)	GO:0051437	68	2.46	0	
NEGATIVE_REGULATION_OF_LIGASE_ACTIVITY (5)&NEGATIVE_REGULATION_OF_UBIQUITIN_PROTEIN_TRANSFERASE_ACTIVITY (6)	GO:0051352	70	2.45	0	
CELLULAR_NITROGEN_COMPOUND_CATABOLIC_PROCESS (5)	GO:0044270	335	2.45	0	
CHROMOSOME_SEGREGATION (4)	GO:0007059	166	2.44	0	-
ESTABLISHMENT_OF_PROTEIN_LOCALIZATION_TO_MEMBRANE (5)	GO:0090150	262	2.42	0	
DNA_STRAND_ELONGATION (6)	GO:0022616	35	2.41	0	-
DNA_STRAND_ELONGATION_INVOLVED_IN_DNA_REPLICATION (7)	GO:0006271	32	2.41	0	-
MITOCHONDRIAL_TRANSLATIONAL_INITIATION (5)	GO:0070124	84	2.41	0	
NEGATIVE_REGULATION_OF_UBIQUITIN_PROTEIN_LIGASE_ACTIVITY_INVOLVED_IN_MITOTIC_CELL_CYCLE (6)	GO:0051436	65	2.40	0	-
RIBOSOME_BIOGENESIS (5)	GO:0042254	68	2.40	0	
MITOTIC_CELL_CYCLE_CHECKPOINT (6)	GO:0007093	141	2.39	0	-
MITOTIC_SISTER_CHROMATID_SEGREGATION (6)	GO:0000070	101	2.38	0	-
ANTIGEN_PROCESSING_AND_PRESENTATION_OF_PEPTIDE_ANTIGEN_VIA_MHC_CLASS_I (5)	GO:0002474	79	2.38	0	
REGULATION_OF_CHROMOSOME_SEGREGATION (4)	GO:0051983	65	2.38	0	-
REGULATION_OF_PROTEIN_UBIQUITINATION_INVOLVED_IN_UBIQUITIN_DEPENDENT_PROTEIN_CATABOLIC_PROCESS (8)	GO:2000058	80	2.36	0	
NEGATIVE_REGULATION_OF_MITOTIC_CELL_CYCLE_PHASE_TRANSITION (6)	GO:1901991	127	2.36	0	-
ANTIGEN_PROCESSING_AND_PRESENTATION_OF_EXOGENOUS_PEPTIDE_ANTIGEN_VIA_MHC_CLASS_I (6)	GO:0042590	63	2.36	0	
SISTER_CHROMATID_SEGREGATION (5)	GO:0000819	102	2.35	0	-

GO description	GO number	# genes	NES[a]	FWER[b] p	Result in hindbrain[c]
MITOCHONDRIAL_TRANSLATIONAL_ELONGATION (6)	GO:0070125	84	2.35	0	
POSITIVE_REGULATION_OF_PROTEIN_UBIQUITINATION_INVOLVED_IN_UBIQUITIN_DEPENDENT_PROTEIN_CATABOLIC_PROCESS (8)	GO:2000060	75	2.35	0	
MITOCHONDRIAL_TRANSLATIONAL_TERMINATION (6)	GO:0070126	84	2.34	0	
CELL_CYCLE_CHECKPOINT (5)	GO:0000075	199	2.34	0	-
NEGATIVE_REGULATION_OF_CELL_CYCLE_PHASE_TRANSITION (5)	GO:1901988	133	2.34	0	-
NUCLEAR_TRANSCRIBED_MRNA_CATABOLIC_PROCESS_EXONUCLEOLYTIC (9)	GO:0000291	30	2.34	0	
ANTIGEN_PROCESSING_AND_PRESENTATION_OF_EXOGENOUS_PEPTIDE_ANTIGEN_VIA_MHC_CLASS_I_TAP_DEPENDENT (7)	GO:0002479	62	2.33	0	
RIBONUCLEOPROTEIN_COMPLEX_BIOGENESIS (4)	GO:0022613	161	2.32	0	
NEGATIVE_REGULATION_OF_MITOTIC_CELL_CYCLE (5)	GO:0045930	174	2.32	0	-
POSITIVE_REGULATION_OF_UBIQUITIN_PROTEIN_TRANSFERASE_ACTIVITY (6)	GO:0051443	81	2.32	0	
POSITIVE_REGULATION_OF_LIGASE_ACTIVITY (5)	GO:0051351	83	2.31	0	
MITOCHONDRIAL_TRANSLATION (5)	GO:0032543	106	2.30	0	
SIGNAL_TRANSDUCTION_INVOLVED_IN_DNA_INTEGRITY_CHECKPOINT (6)&SIGNAL_TRANSDUCTION_INVOLVED_IN_DNA_DAMAGE_CHECKPOINT (7)	GO:0072401	65	2.30	0	-
MITOTIC_DNA_DAMAGE_CHECKPOINT (7)	GO:0044773	80	2.30	0	-
ESTABLISHMENT_OF_PROTEIN_LOCALIZATION_TO_ORGANELLE (5)	GO:0072594	406	2.30	0	
G1_DNA_DAMAGE_CHECKPOINT (7)	GO:0044783	68	2.30	0	
DNA_DEPENDENT_DNA_REPLICATION (7)	GO:0006261	88	2.29	0	-
EXONUCLEOLYTIC_NUCLEAR_TRANSCRIBED_MRNA_CATABOLIC_PROCESS_INVOLVED_IN_DEADENYLATION_DEPENDENT_DECAY (10)	GO:0043928	29	2.29	0	
DNA_REPLICATION (6)	GO:0006260	188	2.29	0	-
SPINDLE_ORGANIZATION (5)	GO:0007051	77	2.28	0	-
MITOTIC_DNA_INTEGRITY_CHECKPOINT (7)	GO:0044774	84	2.28	0	-

GO description	GO number	# genes	NES[a]	FWER[b] p	Result in hindbrain[c]
MRNA_SPLICING_VIA_SPLICEOSOME (8)&RNA_SPLICING_VIA_TRANSESTERIFICATION_REACTIONS_WITH_BULGED_ADENOSINE_AS_NUCLEOPHILE (9)	GO:0000398	187	2.28	0	-
INTRACELLULAR_SIGNAL_TRANSDUCTION_INVOLVED_IN_G1_DNA_DAMAGE_CHECKPOINT (7)&SIGNAL_TRANSDUCTION_INVOLVED_IN_MITOTIC_G1_DNA_DAMAGE_CHECKPOINT (8)	GO:1902400	61	2.28	0	
MITOTIC_SPINDLE_ORGANIZATION (6)	GO:0007052	46	2.28	0	-
SIGNAL_TRANSDUCTION_INVOLVED_IN_MITOTIC_CELL_CYCLE_CHECKPOINT (6)&SIGNAL_TRANSDUCTION_INVOLVED_IN_MITOTIC_DNA_INTEGRITY_CHECKPOINT (7)&SIGNAL_TRANSDUCTION_INVOLVED_IN_MITOTIC_DNA_DAMAGE_CHECKPOINT (8)	GO:0072413	63	2.27	0	-
MITOTIC_G1_DNA_DAMAGE_CHECKPOINT (8)	GO:0031571	67	2.27	0	
RNA_SPLICING_VIA_TRANSESTERIFICATION_REACTIONS (8)	GO:0000375	191	2.27	0.001	-
SIGNAL_TRANSDUCTION_INVOLVED_IN_CELL_CYCLE_CHECKPOINT (5)	GO:0072395	66	2.27	0.001	-
MUSCLE_FILAMENT_SLIDING (7)&ACTIN_MYOSIN_FILAMENT_SLIDING (7)	GO:0030049	30	2.27	0.001	
MICROTUBULE_CYTOSKELETON_ORGANIZATION_INVOLVED_IN_MITOSIS (5)	GO:1902850	28	2.26	0.001	-
MITOTIC_G1_S_TRANSITION_CHECKPOINT (7)	GO:0044819	68	2.26	0.001	
SIGNAL_TRANSDUCTION_IN_RESPONSE_TO_DNA_DAMAGE (6)	GO:0042770	108	2.25	0.001	-
DNA_DAMAGE_RESPONSE_SIGNAL_TRANSDUCTION_BY_P53_CLASS_MEDIATOR_RESULTING_IN_CELL_CYCLE_ARREST (6)	GO:0006977	60	2.25	0.001	-
REGULATION_OF_LIGASE_ACTIVITY (5)	GO:0051340	98	2.25	0.001	
REGULATION_OF_UBIQUITIN_PROTEIN_TRANSFERASE_ACTIVITY (6)	GO:0051438	95	2.24	0.001	
CELLULAR_RESPIRATION (5)	GO:0045333	128	2.24	0.001	
METAPHASE_PLATE_CONGRESSION (5)	GO:0051310	35	2.24	0.002	-
REGULATION_OF_CELL_CYCLE_PHASE_TRANSITION (6)	GO:1901987	175	2.24	0.002	-

GO description	GO number	# genes	NES[a]	FWER[b] p	Result in hindbrain[c]
ELECTRON_TRANSPORT_CHAIN (4)&RESPIRATORY_ELECTRON_TRANSPORT_CHAIN (5)	GO:0022900	92	2.23	0.002	
REGULATION_OF_MITOTIC_CELL_CYCLE_PHASE_TRANSITION (6)	GO:1901990	164	2.23	0.002	-
G1_S_TRANSITION_OF_MITOTIC_CELL_CYCLE (7)	GO:0000082	186	2.23	0.002	-
DNA_DAMAGE_RESPONSE_SIGNAL_TRANSDUCTION_BY_P53_CLASS_MEDIATOR (7)	GO:0030330	93	2.22	0.003	-
CHROMOSOME_LOCALIZATION (5)	GO:0050000	38	2.22	0.003	-
ESTABLISHMENT_OF_CHROMOSOME_LOCALIZATION (5)	GO:0051303	38	2.21	0.003	-
CELL_CYCLE_G1_S_PHASE_TRANSITION (6)	GO:0044843	191	2.21	0.003	-
DNA_INTEGRITY_CHECKPOINT (6)	GO:0031570	132	2.21	0.004	-
NEGATIVE_REGULATION_OF_CELL_CYCLE_PROCESS (4)	GO:0010948	174	2.20	0.005	-
RNA_SPLICING (7)	GO:0008380	242	2.20	0.007	-
REGULATION_OF_PROTEOLYSIS_INVOLVED_IN_CELLULAR_PROTEIN_CATABOLIC_PROCESS (7)	GO:1903050	179	2.20	0.01	
ATTACHMENT_OF_SPINDLE_MICROTUBULES_TO_KINETOCHORE (5)	GO:0008608	21	2.19	0.01	-
POSITIVE_REGULATION_OF_PROTEIN_UBIQUITINATION (8)	GO:0031398	126	2.19	0.013	
PROTEIN_TARGETING (5)	GO:0006605	412	2.18	0.02	
REGULATION_OF_CELLULAR_PROTEIN_CATABOLIC_PROCESS (6)	GO:1903362	187	2.18	0.02	
MITOTIC_SPINDLE_ASSEMBLY (6)	GO:0090307	24	2.17	0.02	-
DNA_DAMAGE_CHECKPOINT (6)	GO:0000077	125	2.17	0.021	-
PROTEIN_LOCALIZATION_TO_MEMBRANE (4)	GO:0072657	327	2.16	0.022	-
MITOTIC_SISTER_CHROMATID_SEPARATION (6)	GO:0051306	50	2.16	0.022	
POSITIVE_REGULATION_OF_CELL_CYCLE_ARREST (5)	GO:0071158	75	2.16	0.022	
POSITIVE_REGULATION_OF_PROTEOLYSIS (6)	GO:0045862	219	2.16	0.022	
MITOTIC_NUCLEAR_DIVISION (5)	GO:0007067	184	2.16	0.024	-
NEGATIVE_REGULATION_OF_PROTEIN_UBIQUITINATION (8)	GO:0031397	103	2.15	0.025	
MITOTIC_METAPHASE_PLATE_CONGRESSION (4)	GO:0007080	30	2.15	0.025	-

GO description	GO number	# genes	NES[a]	FWER[b] p	Result in hindbrain[c]
POSITIVE_REGULATION_OF_PROTEOLYSIS_INVOLVED_IN_CELLULAR_PROTEIN_CATABOLIC_PROCESS (7)	GO:1903052	117	2.14	0.03	
NEGATIVE_REGULATION_OF_G1_S_TRANSITION_OF_MITOTIC_CELL_CYCLE (7)	GO:2000134	82	2.14	0.03	
PROTEIN_UBIQUITINATION_INVOLVED_IN_UBIQUITIN_DEPENDENT_PROTEIN_CATABOLIC_PROCESS (9)	GO:0042787	112	2.14	0.031	
CENTROMERE_COMPLEX_ASSEMBLY (5)	GO:0034508	35	2.14	0.033	-
POSITIVE_REGULATION_OF_CELLULAR_PROTEIN_CATABOLIC_PROCESS (6)	GO:1903364	120	2.13	0.038	
CENP_A_CONTAINING_CHROMATIN_ORGANIZATION (5)&CENP_A_CONTAINING_NUCLEOSOME_ASSEMBLY (6)	GO:0061641	28	2.13	0.039	-
NEGATIVE_REGULATION_OF_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL (7)	GO:1903321	107	2.13	0.039	
METAPHASE_ANAPHASE_TRANSITION_OF_CELL_CYCLE (6)&REGULATION_OF_MITOTIC_SISTER_CHROMATID_SEPARATION (7)	GO:0044784	49	2.13	0.039	
POSITIVE_REGULATION_OF_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL (7)	GO:1903322	135	2.13	0.044	
REGULATION_OF_METAPHASE_ANAPHASE_TRANSITION_OF_CELL_CYCLE (7)	GO:1902099	48	2.12	0.045	
CHROMATIN_REMODELING_AT_CENTROMERE (7)	GO:0031055	30	2.12	0.046	-
REGULATION_OF_PROTEIN_CATABOLIC_PROCESS (5)	GO:0042176	243	2.12	0.046	
METAPHASE_ANAPHASE_TRANSITION_OF_MITOTIC_CELL_CYCLE (5)	GO:0007091	49	2.12	0.047	
MITOTIC_CELL_CYCLE_PHASE_TRANSITION (6)	GO:0044772	355	2.12	0.049	-
CELL_CYCLE_PHASE_TRANSITION (5)	GO:0044770	368	2.12	0.049	-
OSTEOBLAST_DIFFERENTIATION (5)	GO:0001649	105	2.11	0.05	

[a] NES = normalised enrichment score; positive = upregulated on the right; negative = upregulated on the left.

[b] FWER = familywise error rate. Included in the table are terms with FWER <= 0.05.

[c] + = GO-term is also significantly enriched in right hindbrain; - = GO-term is also significantly enriched in left hindbrain.

Table S3. Gene Ontology gene sets showing lateralization in hindbrain

GO description	GO number	# genes	NES[a]	FWER[b] p
Up on left side:				
DNA_DEPENDENT_DNA_REPLICATION (7)	GO:0006261	88	-2.74	0
DNA_STRAND_ELONGATION_INVOLVED_IN_DNA_REPLICATION (7)	GO:0006271	32	-2.73	0
MRNA_PROCESSING (7)	GO:0006397	276	-2.73	0
RNA_PROCESSING (6)	GO:0006396	378	-2.69	0
DNA_STRAND_ELONGATION (6)	GO:0022616	35	-2.68	0
CELL_CYCLE_DNA_REPLICATION (5)	GO:0044786	39	-2.67	0
RNA_SPLICING (7)	GO:0008380	243	-2.65	0
DNA_RECOMBINATION (6)	GO:0006310	122	-2.65	0
MRNA_SPLICING_VIA_SPLICEOSOME (8)&RNA_SPLICING_VIA_TRANSESTERIFICATION_REACTIONS_WITH_BULGED_ADENOSINE_AS_NUCLEOPHILE (9)	GO:0000398	187	-2.64	0
SPINDLE_ORGANIZATION (5)	GO:0007051	77	-2.64	0
CHROMOSOME_SEGREGATION (4)	GO:0007059	168	-2.63	0
RNA_SPLICING_VIA_TRANSESTERIFICATION_REACTIONS (8)	GO:0000375	191	-2.63	0
MRNA_3_END_PROCESSING (8)	GO:0031124	91	-2.62	0
TELOMERE_MAINTENANCE_VIA_TELOMERE_LENGTHENING (5)	GO:0010833	35	-2.61	0
CHROMOSOME_LOCALIZATION (5)	GO:0050000	39	-2.60	0
MITOTIC_SPINDLE_ORGANIZATION (6)	GO:0007052	46	-2.60	0
TELOMERE_MAINTENANCE (4)	GO:0000723	58	-2.59	0
SISTER_CHROMATID_SEGREGATION (5)	GO:0000819	102	-2.58	0
MITOTIC_SISTER_CHROMATID_SEGREGATION (6)	GO:0000070	101	-2.57	0
ESTABLISHMENT_OF_CHROMOSOME_LOCALIZATION (5)	GO:0051303	39	-2.56	0
SPINDLE_ASSEMBLY (5)	GO:0051225	46	-2.55	0
MRNA_METABOLIC_PROCESS (6)	GO:0016071	423	-2.54	0
RNA_3_END_PROCESSING (7)	GO:0031123	102	-2.54	0
METAPHASE_PLATE_CONGRESSION (5)	GO:0051310	36	-2.54	0
NUCLEAR_DNA_REPLICATION (6)	GO:0033260	31	-2.51	0
TELOMERE_MAINTENANCE_VIA_SEMI_CONSERVATIVE_REPLICATION (5)	GO:0032201	23	-2.51	0
MITOTIC_RECOMBINATION (7)	GO:0006312	34	-2.50	0
TELOMERE_ORGANIZATION (5)	GO:0032200	59	-2.50	0
MITOTIC_METAPHASE_PLATE_CONGRESSION (4)	GO:0007080	30	-2.49	0
MRNA_TRANSPORT (6)	GO:0051028	64	-2.48	0
DNA_REPAIR (4)	GO:0006281	288	-2.48	0
DNA_REPLICATION (6)	GO:0006260	189	-2.46	0

GO description	GO number	# genes	NES[a]	FWER[b] p
REGULATION_OF_CHROMOSOME_SEGREGATION (4)	GO:0051983	65	-2.45	0
MRNA_EXPORT_FROM_NUCLEUS (6)	GO:0006406	63	-2.44	0
CELL_CYCLE_PHASE_TRANSITION (5)	GO:0044770	370	-2.42	0
DNA_CONFORMATION_CHANGE (5)	GO:0071103	123	-2.42	0.001
MITOTIC_CELL_CYCLE_PHASE_TRANSITION (6)	GO:0044772	356	-2.42	0.001
CENTROMERE_COMPLEX_ASSEMBLY (5)	GO:0034508	35	-2.42	0.001
CELL_CYCLE_G1_S_PHASE_TRANSITION (6)	GO:0044843	192	-2.41	0.001
RNA_LOCALIZATION (4)	GO:0006403	80	-2.41	0.001
TERMINATION_OF_RNA_POLYMERASE_II_TRANSCRIPTION (8)	GO:0006369	46	-2.40	0.001
ATTACHMENT_OF_SPINDLE_MICROTUBULES_TO_KINETOCHORE (5)	GO:0008608	21	-2.39	0.001
G1_S_TRANSITION_OF_MITOTIC_CELL_CYCLE (7)	GO:0000082	187	-2.39	0.001
CHROMATIN_ASSEMBLY (5)	GO:0031497	59	-2.38	0.001
ANAPHASE_PROMOTING_COMPLEX_DEPENDENT_PROTEASOMAL_UBIQUITIN_DEPENDENT_PROTEIN_CATABOLIC_PROCESS (8)	GO:0031145	105	-2.38	0.001
TELOMERE_MAINTENANCE_VIA_RECOMBINATION (5)	GO:0000722	27	-2.38	0.001
CELL_CYCLE_CHECKPOINT (5)	GO:0000075	199	-2.37	0.001
PROTEIN_DNA_COMPLEX_SUBUNIT_ORGANIZATION (5)	GO:0071824	87	-2.37	0.002
MICROTUBULE_CYTOSKELETON_ORGANIZATION_INVOLVED_IN_MITOSIS (5)	GO:1902850	28	-2.37	0.002
NEGATIVE_REGULATION_OF_CELL_CYCLE_PROCESS (4)	GO:0010948	174	-2.35	0.002
MITOTIC_NUCLEAR_DIVISION (5)	GO:0007067	185	-2.35	0.003
MITOTIC_CELL_CYCLE_CHECKPOINT (6)	GO:0007093	141	-2.35	0.003
MITOTIC_SPINDLE_ASSEMBLY (6)	GO:0090307	24	-2.34	0.004
PROTEIN_DNA_COMPLEX_ASSEMBLY (6)	GO:0065004	66	-2.34	0.004
REGULATION_OF_SPINDLE_ORGANIZATION (6)	GO:0090224	19	-2.33	0.004
RNA_EXPORT_FROM_NUCLEUS (6)	GO:0006405	70	-2.32	0.004
ESTABLISHMENT_OF_RNA_LOCALIZATION (4)&RNA_TRANSPORT (5)&NUCLEIC_ACID_TRANSPORT (7)	GO:0051236	77	-2.32	0.004
TRANSCRIPTION_COUPLED_NUCLEOTIDE_EXCISION_REPAIR (6)	GO:0006283	47	-2.31	0.005
MISMATCH_REPAIR (5)	GO:0006298	23	-2.31	0.005
NEGATIVE_REGULATION_OF_CELL_CYCLE_PHASE_TRANSITION (5)	GO:1901988	133	-2.30	0.006
DNA_PACKAGING (4)	GO:0006323	81	-2.30	0.007

GO description	GO number	# genes	NES[a]	FWER[b] p
REGULATION_OF_MITOTIC_CELL_CYCLE_PHASE_TRANSITION (6)	GO:1901990	165	-2.29	0.007
CHROMATIN_REMODELING_AT_CENTROMERE (7)	GO:0031055	30	-2.29	0.007
DNA_INTEGRITY_CHECKPOINT (6)	GO:0031570	132	-2.28	0.007
NUCLEAR_DIVISION (5)	GO:0000280	222	-2.28	0.007
MEMBRANE_DISASSEMBLY (4)&MITOTIC_NUCLEAR_ENVELOPE_DISASSEMBLY (5)&NUCLEAR_ENVELOPE_DISASSEMBLY (5)	GO:0030397	42	-2.28	0.007
NEGATIVE_REGULATION_OF_MITOTIC_CELL_CYCLE_PHASE_TRANSITION (6)	GO:1901991	127	-2.27	0.007
REGULATION_OF_CELL_CYCLE_PHASE_TRANSITION (6)	GO:1901987	177	-2.27	0.007
CHROMATIN_ASSEMBLY_OR_DISASSEMBLY (5)	GO:0006333	81	-2.27	0.008
ATP_DEPENDENT_CHROMATIN_REMODELING (7)	GO:0043044	53	-2.27	0.008
NUCLEOSOME_ORGANIZATION (5)	GO:0034728	68	-2.25	0.009
AMINO_ACID_ACTIVATION (5)&TRNA_AMINOACYLATION (6)	GO:0043038	45	-2.23	0.012
TRNA_AMINOACYLATION_FOR_PROTEIN_TRANSLATION (7)	GO:0006418	42	-2.23	0.012
CENP_A_CONTAINING_CHROMATIN_ORGANIZATION (5)&CENP_A_CONTAINING_NUCLEOSOME_ASSEMBLY (6)	GO:0061641	28	-2.23	0.012
HISTONE_EXCHANGE (6)	GO:0043486	33	-2.23	0.012
NUCLEAR_ENVELOPE_ORGANIZATION (4)	GO:0006998	57	-2.23	0.012
MITOTIC_DNA_INTEGRITY_CHECKPOINT (7)	GO:0044774	84	-2.22	0.013
MICROTUBULE_CYTOSKELETON_ORGANIZATION (5)	GO:0000226	233	-2.21	0.013
REGULATION_OF_MICROTUBULE_CYTOSKELETON_ORGANIZATION (5)	GO:0070507	78	-2.21	0.013
REGULATION_OF_RNA_STABILITY (4)	GO:0043487	34	-2.21	0.013
NUCLEOSOME_ASSEMBLY (6)	GO:0006334	47	-2.21	0.013
SPINDLE_CHECKPOINT (6)	GO:0031577	45	-2.21	0.013
DNA_CATABOLIC_PROCESS (6)	GO:0006308	77	-2.20	0.016
DNA_TEMPLATED_TRANSCRIPTION_TERMINATION (7)	GO:0006353	87	-2.20	0.018
SIGNAL_TRANSDUCTION_INVOLVED_IN_CELL_CYCLE_CHECKPOINT (5)	GO:0072395	66	-2.19	0.021
SIGNAL_TRANSDUCTION_INVOLVED_IN_DNA_INTEGRITY_CHECKPOINT (6)&SIGNAL_TRANSDUCTION_INVOLVED_IN_DNA_DAMAGE_CHECKPOINT (7)	GO:0072401	65	-2.19	0.021
DNA_DAMAGE_CHECKPOINT (6)	GO:0000077	125	-2.19	0.023
VIRAL_TRANSCRIPTION (5)	GO:0019083	178	-2.19	0.023
CHROMATIN_REMODELING (6)	GO:0006338	92	-2.19	0.025

GO description	GO number	# genes	NES[a]	FWER[b] p
REGULATION_OF_MRNA_STABILITY (5)	GO:0043488	33	-2.19	0.026
REGULATION_OF_TRANSCRIPTION_INVOLVED_IN_G1_S_TRANSITION_OF_MITOTIC_CELL_CYCLE (5)	GO:0000083	22	-2.18	0.028
NUCLEIC_ACID_PHOSPHODIESTER_BOND_HYDROLYSIS (6)	GO:0090305	118	-2.17	0.029
MITOTIC_SPINDLE_ASSEMBLY_CHECKPOINT (7)	GO:0007094	38	-2.17	0.029
ORGANELLE_FISSION (4)	GO:0048285	248	-2.17	0.031
NUCLEOBASE_CONTAINING_COMPOUND_TRANSPORT (6)	GO:0015931	93	-2.16	0.032
NUCLEOTIDE_EXCISION_REPAIR_DNA_GAP_FILLING (6)	GO:0006297	19	-2.16	0.033
DNA_REPLICATION_INDEPENDENT_NUCLEOSOME_ORGANIZATION (6)&DNA_REPLICATION_INDEPENDENT_NUCLEOSOME_ASSEMBLY (7)	GO:0034724	39	-2.16	0.033
REGULATION_OF_MITOTIC_SPINDLE_ORGANIZATION (6)	GO:0060236	15	-2.15	0.038
DNA_DAMAGE_RESPONSE_SIGNAL_TRANSDUCTION_BY_P53_CLASS_MEDIATOR_RESULTING_IN_CELL_CYCLE_ARREST (6)	GO:0006977	60	-2.15	0.039
NEGATIVE_REGULATION_OF_MITOTIC_CELL_CYCLE (5)	GO:0045930	174	-2.15	0.039
NEGATIVE_REGULATION_OF_UBIQUITIN_PROTEIN_LIGASE_ACTIVITY_INVOLVED_IN_MITOTIC_CELL_CYCLE (6)	GO:0051436	65	-2.15	0.039
MITOTIC_DNA_DAMAGE_CHECKPOINT (7)	GO:0044773	80	-2.14	0.039
SIGNAL_TRANSDUCTION_INVOLVED_IN_MITOTIC_CELL_CYCLE_CHECKPOINT (6)&SIGNAL_TRANSDUCTION_INVOLVED_IN_MITOTIC_DNA_INTEGRITY_CHECKPOINT (7)&SIGNAL_TRANSDUCTION_INVOLVED_IN_MITOTIC_DNA_DAMAGE_CHECKPOINT (8)	GO:0072413	63	-2.14	0.042
NEGATIVE_REGULATION_OF_SISTER_CHROMATID_SEGREGATION (5)&NEGATIVE_REGULATION_OF_MITOTIC_SISTER_CHROMATID_SEPARATION (5)&NEGATIVE_REGULATION_OF_MITOTIC_SISTER_CHROMATID_SEGREGATION (6)&NEGATIVE_REGULATION_OF_MITOTIC_METAPHASE_ANAPHASE_TRANSITION (6)&MITOTIC_SPINDLE_CHECKPOINT (6)	GO:0033046	40	-2.14	0.042
REGULATION_OF_SISTER_CHROMATID_SEGREGATION (5)&REGULATION_OF_MITOTIC_SISTER_CHROMATID_SEGREGATION (6)	GO:0033045	50	-2.14	0.044
SPINDLE_ASSEMBLY_CHECKPOINT (7)	GO:0071173	39	-2.14	0.044

GO description	GO number	# genes	NES[a]	FWER[b] p
NUCLEOTIDE_EXCISION_REPAIR (5)	GO:0006289	69	-2.14	0.045
SIGNAL_TRANSDUCTION_IN_RESPONSE_TO_DNA_DAMAGE (6)	GO:0042770	109	-2.13	0.046
REGULATION_OF_CHROMOSOME_ORGANIZATION (5)	GO:0033044	140	-2.13	0.047
REGULATION_OF_CELL_CYCLE_PROCESS (5)	GO:0010564	339	-2.13	0.048
MULTI_ORGANISM_METABOLIC_PROCESS (3)	GO:0044033	189	-2.12	0.05
DNA_DAMAGE_RESPONSE_SIGNAL_TRANSDUCTION_BY_P53_CLASS_MEDIATOR (7)	GO:0030330	94	-2.12	0.05
Up on right side:				
REGULATION_OF_SYSTEM_PROCESS (4)	GO:0044057	168	2.26	0.004
REGULATION_OF_CELL_JUNCTION_ASSEMBLY (4)	GO:1901888	43	2.25	0.004
MUSCLE_CONTRACTION (6)	GO:0006936	163	2.24	0.007
RENAL_WATER_HOMEOSTASIS (6)	GO:0003091	25	2.20	0.014
MUSCLE_SYSTEM_PROCESS (5)	GO:0003012	187	2.20	0.014
REGULATION_OF_SYNAPTIC_TRANSMISSION (3)	GO:0050804	89	2.19	0.017
BLOOD_CIRCULATION (6)	GO:0008015	178	2.16	0.023
WATER_TRANSPORT (5)	GO:0006833	24	2.16	0.023
MULTICELLULAR_ORGANISMAL_WATER_HOMEOSTASIS (5)&WATER_HOMEOSTASIS (6)	GO:0050891	31	2.16	0.026
CIRCULATORY_SYSTEM_PROCESS (5)	GO:0003013	178	2.16	0.027
FLUID_TRANSPORT (4)	GO:0042044	25	2.16	0.028
SYNAPTIC_TRANSMISSION (3)	GO:0007268	391	2.15	0.034
CELL_JUNCTION_ORGANIZATION (4)	GO:0034330	168	2.14	0.037
HEART_PROCESS (6)	GO:0003015	96	2.14	0.037
CELL_JUNCTION_ASSEMBLY (5)	GO:0034329	154	2.13	0.043
METAL_ION_TRANSPORT (6)	GO:0030001	333	2.12	0.049
MULTICELLULAR_ORGANISMAL_MOVEMENT (4)&MUSCULOSKELETAL_MOVEMENT (5)	GO:0050879	16	2.12	0.05

[a] NES = normalised enrichment score; positive = upregulated on the right; negative = upregulated on the left.

[b] FWER = familywise error rate. Included in the table are terms with FWER <= 0.05.

Table S4. Transcription factor targets significantly lateralised in spinal cord

NAME	Description[a]	# genes	NES[b]	FWER[c] p	Result in hindbrain[d]
Up on left side:					
V$NRSF_01	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif TTCAGCACCACGGACAGMGCC which matches annotation for **REST: RE1-silencing transcription factor**	73	-3.15	0	
CAGNWMCNNNGAC_UNKNOWN	Genes with promoter regions [-2kb,2kb] around transcription start site containing motif CAGNWMCNNNGAC. Motif does not match any known transcription factor	64	-2.66	0	
GTTRYCATRR_UNKNOWN	Genes with promoter regions [-2kb,2kb] around transcription start site containing motif GTTRYCATRR. Motif does not match any known transcription factor	135	-2.38	0	
V$SF1_Q6	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif TGRCCTTG which matches annotation for **SF1: splicing factor 1**	220	-1.89	0.02	
V$E47_01	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif VSNGCAGGTGKNCNN which matches annotation for **TCF3: transcription factor 3** (E2A immunoglobulin enhancer binding factors E12/E47)	209	-1.86	0.024	
YGCANTGCR_UNKNOWN	Genes with promoter regions [-2kb,2kb] around transcription start site containing motif YGCANTGCR. Motif does not match any known transcription factor	116	-1.85	0.033	
Up on right side:					
SGCGSSAAA_V$E2F1DP2_01	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif SGCGSSAAA which matches annotation for **E2F1: E2F transcription factor 1 - TFDP1: transcription factor Dp-1 RB1: retinoblastoma 1** (including osteosarcoma)	151	1.92	0.038	-
V$E2F1_Q6	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif NTTTCGCGCS which matches annotation for **E2F1: E2F transcription factor 1**	212	1.83	0.046	-

[a] Grey text: motifs without known transcription factors.

[b] NES=normalised enrichment score.

[c] FWER=family-wise error rate.

[d] - = also significantly enriched in left- side hindbrain.

Table S5. Transcription factor targets significantly lateralised in hindbrain

NAME	Description[a]	# genes	NES[b]	FWER[c] p-
Up on left side:				
V$E2F1_Q6	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif TTTSGCGS which matches annotation for **E2F1: E2F transcription factor 1**	213	-2.66	0
SGCGSSAAA_V$E2F1DP2_01	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif SGCGSSAAA which matches annotation for **E2F1: E2F transcription factor 1- TFDP1: transcription factor Dp-1 - RB1: retinoblastoma 1** (including osteosarcoma)	152	-2.64	0
V$E2F1DP1_01	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif TTTCspinal cordGC which matches annotation for **E2F1: E2F transcription factor 1 - TFDP1: transcription factor Dp-1**	212	-2.59	0
V$E2F_02	Genes with promoter regions [-2kb,2kb] around transcription start site containing motif TTTSGCGC. Motif does not match any known transcription factor	212	-2.57	0
V$E2F4DP1_01	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif TTTSGCGC which matches annotation for **E2F4: E2F transcription factor 4, p107/p130-binding - TFDP1: transcription factor Dp-1**	218	-2.56	0
V$E2F4DP2_01	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif TTTCspinal cordGC which matches annotation for **E2F4: E2F transcription factor 4, p107/p130-binding - TFDP2: transcription factor Dp-2 (E2F dimerization partner 2)**	212	-2.56	0
V$E2F1DP1RB_01	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif TTTSGCGC which matches annotation for **E2F1: E2F transcription factor 1 - TFDP1: transcription factor Dp-1 - RB1: retinoblastoma 1** (including osteosarcoma)	212	-2.54	0
V$E2F1DP2_01	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif TTTSspinal cordGC which matches annotation for **E2F1: E2F transcription factor 1 0 - TFDP2: transcription factor Dp-2** (E2F dimerization partner 2)	212	-2.54	0
V$E2F_Q6	Genes with promoter regions [-2kb,2kb] around transcription start site containing motif TTTSGCGS. Motif does not match any known transcription factor	213	-2.50	0

NAME	Description[a]	# genes	NES[b]	FWER[c] p-
V$E2F_Q4	Genes with promoter regions [-2kb,2kb] around transcription start site containing motif TTTSGCGS. Motif does not match any known transcription factor	217	-2.47	0
V$E2F_Q4_01	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif NCspinal cordGCSAAAN which matches annotation for **E2F - TFDP1: transcription factor Dp-1**	218	-2.39	0
V$E2F1_Q4_01	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif TTTSGCGSG which matches annotation for **E2F - TFDP1: transcription factor Dp-1**	206	-2.39	0
V$E2F1_Q3	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif NKTSspinal cordGC which matches annotation for **E2F1: E2F transcription factor 1**	224	-2.39	0
GCGspinal cordMNTTT_UNKNOWN	Genes with promoter regions [-2kb,2kb] around transcription start site containing motif GCGspinal cordMNTTT. Motif does not match any known transcription factor	67	-2.35	0
V$E2F_Q3_01	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif TTTSGCGSG which matches annotation for **E2F - TFDP1: transcription factor Dp-1**	212	-2.34	0
V$E2F1_Q6_01	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif NTTTCGCGCS which matches annotation for **E2F1: E2F transcription factor 1**	216	-2.32	0
V$E2F_Q3	Genes with promoter regions [-2kb,2kb] around transcription start site containing motif TTTCGCGC. Motif does not match any known transcription factor	207	-2.24	0
KTGGYRSGAA_UNKNOWN	Genes with promoter regions [-2kb,2kb] around transcription start site containing motif KTGGYRSGAA. Motif does not match any known transcription factor	68	-2.19	0
V$E2F_Q6_01	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif NKCGCGCSAAAN which matches annotation for **E2F - TFDP1: transcription factor Dp-1**	222	-2.19	0
V$E2F_03	Genes with promoter regions [-2kb,2kb] around transcription start site containing motif TTTSGCGCGMNR. Motif does not match any known transcription factor	225	-2.16	0
V$E2F_01	Genes with promoter regions [-2kb,2kb] around transcription start site containing motif TWSGCGCGAAAAYKR. Motif does not match any known transcription factor	60	-2.07	0.002

NAME	Description[a]	# genes	NES[b]	FWER[c] p-
V$NFY_C	Genes with promoter regions [-2kb,2kb] around transcription start site containing motif NCTGATTGGYTASY. Motif does not match any known transcription factor	198	-2.03	0.003
GCCATNTTG_V$YY1_Q6	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif GCCATNTTG which matches annotation for YY1: **YY1 transcription factor**	404	-1.97	0.011
KCCGNSWTTT_UNKNOWN	Genes with promoter regions [-2kb,2kb] around transcription start site containing motif KCCGNSWTTT. Motif does not match any known transcription factor	100	-1.92	0.024
TAANNYSGCG_UNKNOWN	Genes with promoter regions [-2kb,2kb] around transcription start site containing motif TAANNYSGCG. Motif does not match any known transcription factor	76	-1.90	0.035
GGCNKCCATNK_UNKNOWN	Genes with promoter regions [-2kb,2kb] around transcription start site containing motif GGCNKCCATNK. Motif does not match any known transcription factor	112	-1.89	0.036
V$E2F1_Q4	Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif NTTSGCGG which matches annotation for **E2F1: E2F transcription factor 1**	223	-1.89	0.037
Up on right side:				
V$HMEF2_Q6	Genes with promoter regions [-2kb,2kb] around transcription start site containing motif SKYTAAAAATAACYCH. Motif does not match any known transcription factor	113	1.93	0.037

[a] Grey text: motifs without known transcription factors.

[b] NES=normalised enrichment score.

[c] FWER=family-wise error rate.

Supplementary Figures

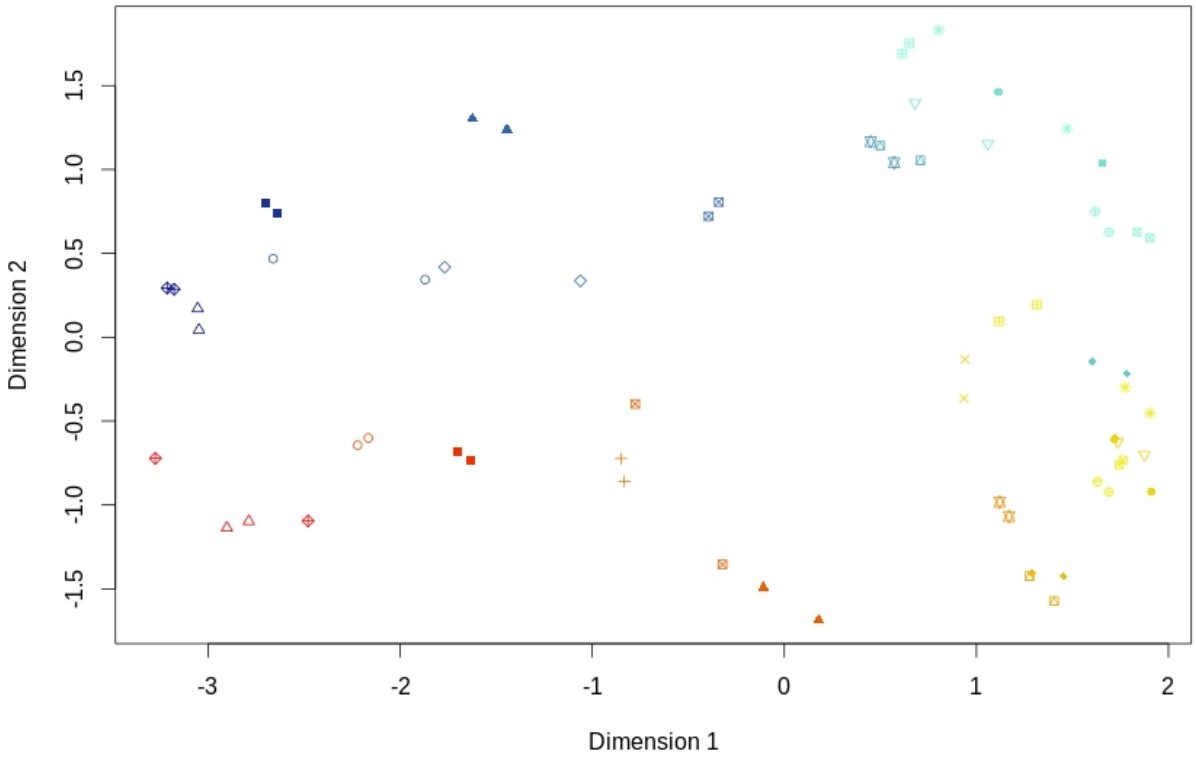


Figure S1. Multidimensional scaling plot based on log2 (cpm) expression values for genes surviving filtering for all RNASeq libraries, and after all quality control stages.
Spinal cord samples are shown in a spectrum of blue shades from dark (earlier Carnegie Stages (CS)) to light (later CS stages). Hindbrain samples are shown in a color spectrum through red-orange-yellow representing earlier (dark red) to later (yellow) CS stages. The same shaped icons represent samples from the same individual embryo (left and right spinal cord, and left and right hindbrain). This analysis of the expression data was purely data-driven, receiving no information regarding sample CS or tissue identity. Nonetheless, dimension 1 clearly ordered samples according to embryonic age, while dimension 2 largely distinguished samples from spinal cord and hindbrain. MDS was also run separately for spinal cord and hindbrain samples to check if major dimensions might emerge reflecting laterality, but none of the top ten dimensions for either structure was associated with the left-right distinction (not shown).

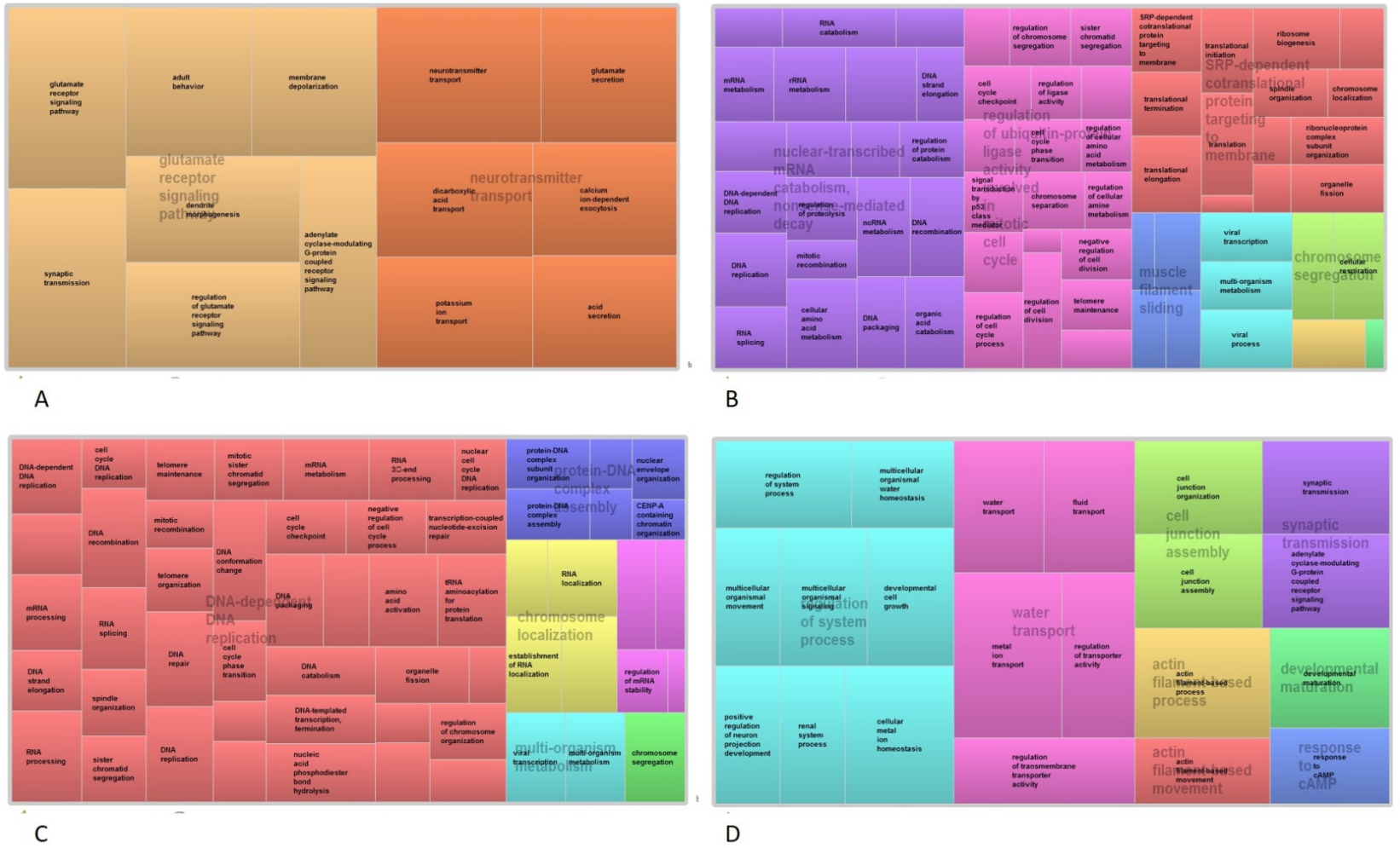
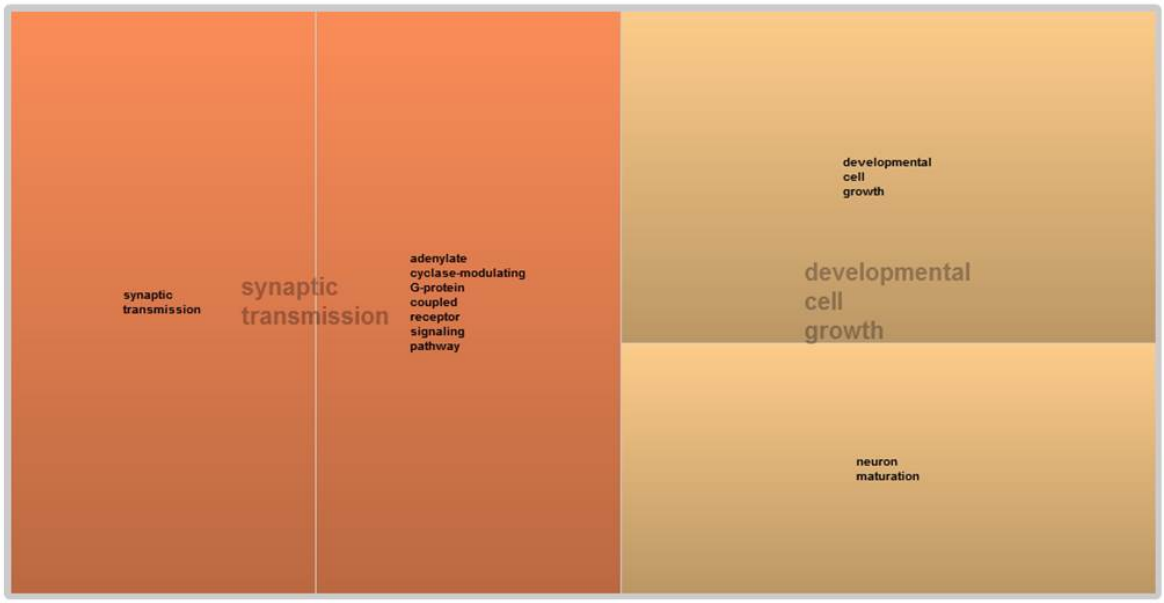
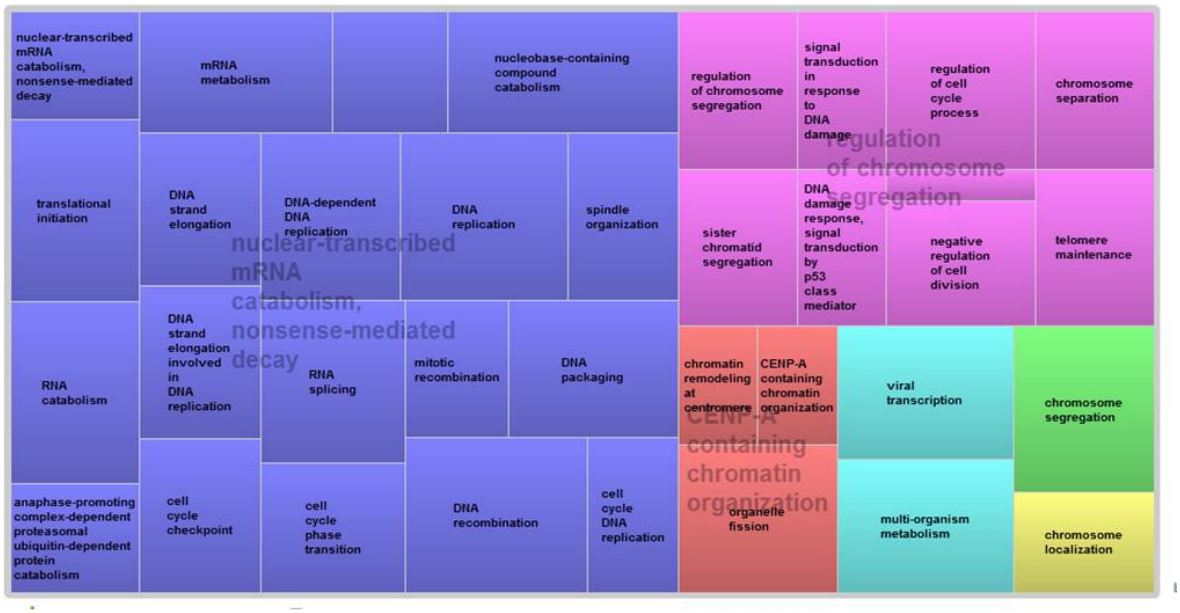


Figure S2. REVIGO (Treemap) clustered map of Gene Ontology terms with more than 2-fold difference between left and right.
(A) Upregulated in left spinal cord. (B) Upregulated in right spinal cord. (C) Upregulated in left hindbrain. (D) Upregulated in right hindbrain.



A



B

Figure S3. REVIGO clustered map of Gene Ontology terms with more than 2-fold difference between left and right in both spinal cord and hindbrain.
(A) Upregulated in left spinal cord and right hindbrain. (B) Upregulated in right spinal cord and left hindbrain.

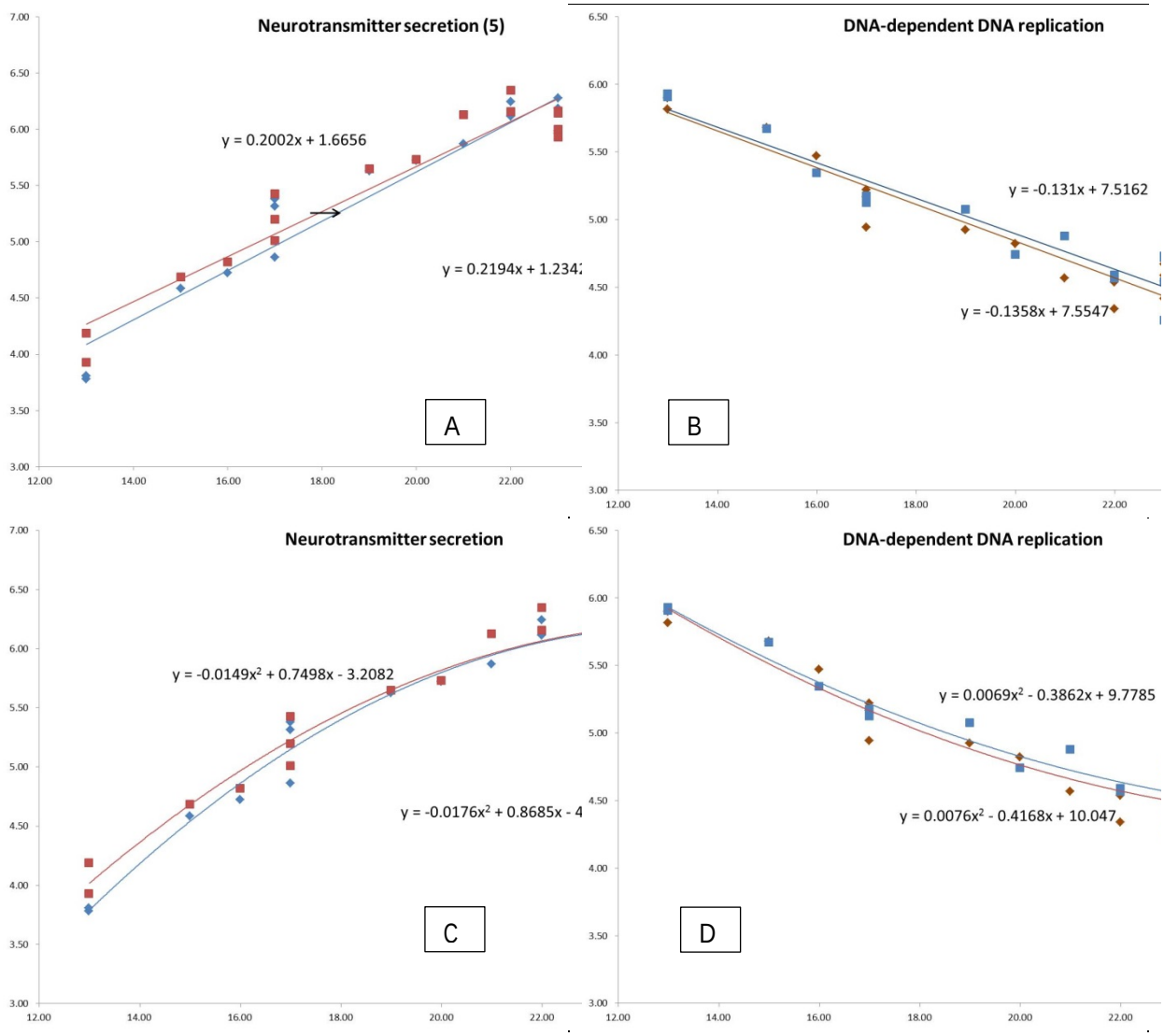


Figure S4. Estimate of asynchrony between left and right spinal cord.
X axis: Carnegie stage of embryo. Y-axis: median expression as $\log_2$ (cpm). A (linear) and C (quadratic) fit for median expression of genes in gene-set 'Neurotransmitter secretion', the most asymmetrically expressed GO-set with higher expression in the left spinal cord, vs Carnegie stage. The black arrow (in A) indicates the asynchrony: i.e. the time the blue line needs to reach the same expression level as the red line. B (linear) and D (quadratic) fit for median expression of genes in the gene-set 'DNA-dependent DNA replication', the most asymmetrically expressed gene-set showing higher expression in the right spinal cord. Cpm = counts per million.

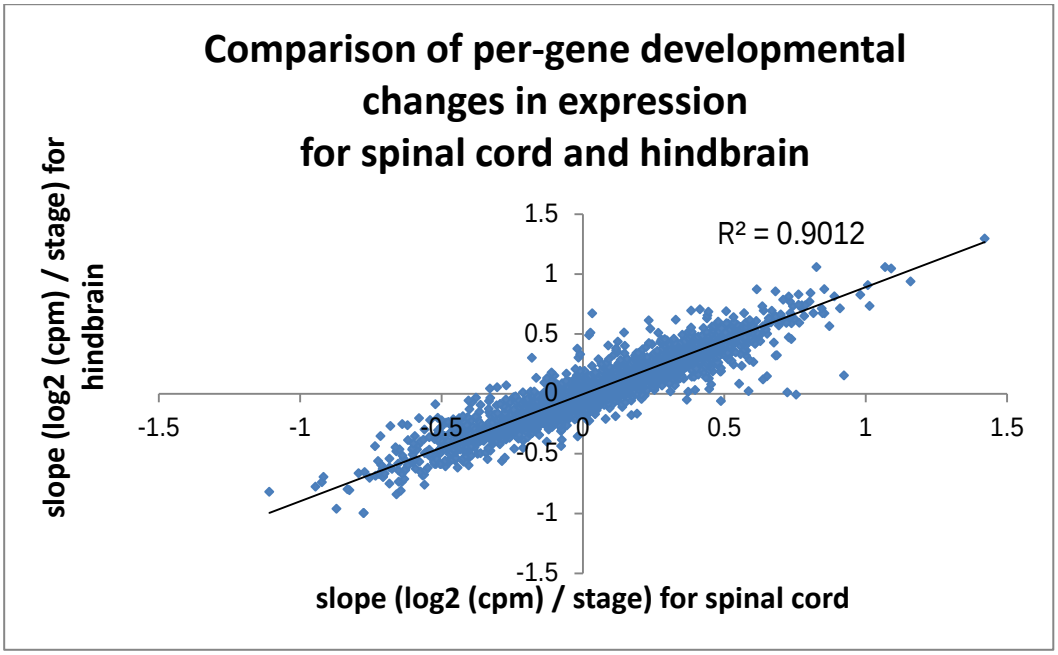


Figure S5. Comparison of per-gene developmental changes of expression in hindbrain vs spinal cord.
The expression trajectory with age is very similar in the two tissues.

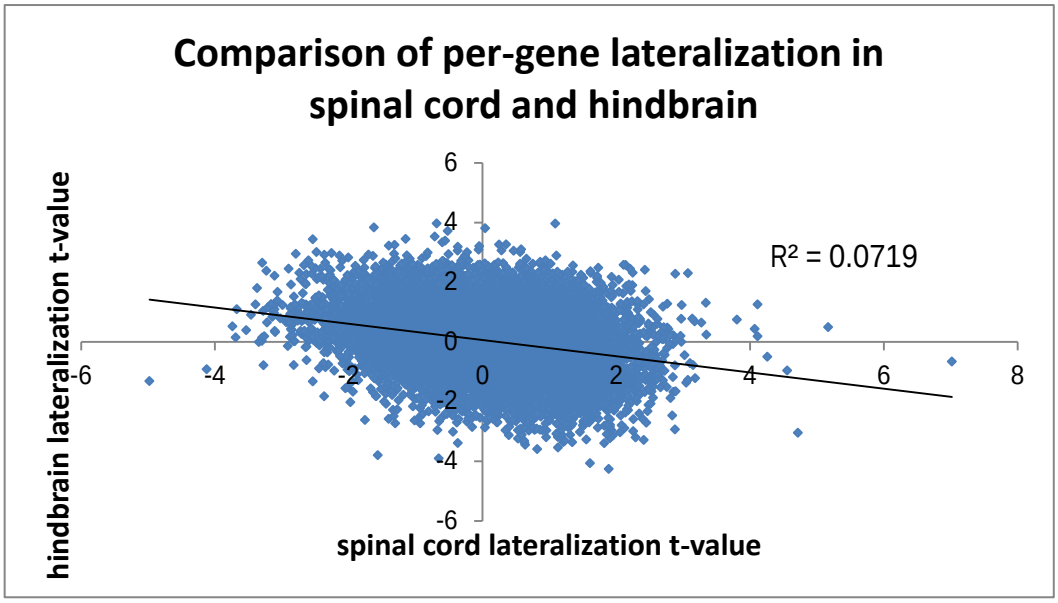


Figure S6. Comparison of per-gene lateralization t-values for hindbrain vs spinal cord.
The left-right differences for most genes were not strongly correlated between the two tissues, and were overall mirrored (weak negative correlation). Yet, at the gene-set level, strongly lateralised sets in spinal cord also showed similar, but mirrored, patterns in hindbrain, as shown in Figures 2 and 3 and Supplemental Figures S2 and S3. (DE=differential expression).

Supplementary References

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