Supplementary Information online

Copy Number Variation modifies expression time-courses

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Supplementary Figures and Tables

Supplementary Figure S1: Gene expression variance between and within strains indicates that most of the variance is due to expression changes between strains

Boxplots comparing the distributions of the standard deviations of expression signals within CNVs (CNV+), outside CNVs (CNV-), and of both groups combined (ALL) for brain and liver at four developmental time-points. The “between strains” standard deviations (white boxes) and the “within strain” standard deviations (gray boxes) were calculated using standard ANOVA (** $P < 0.001$).

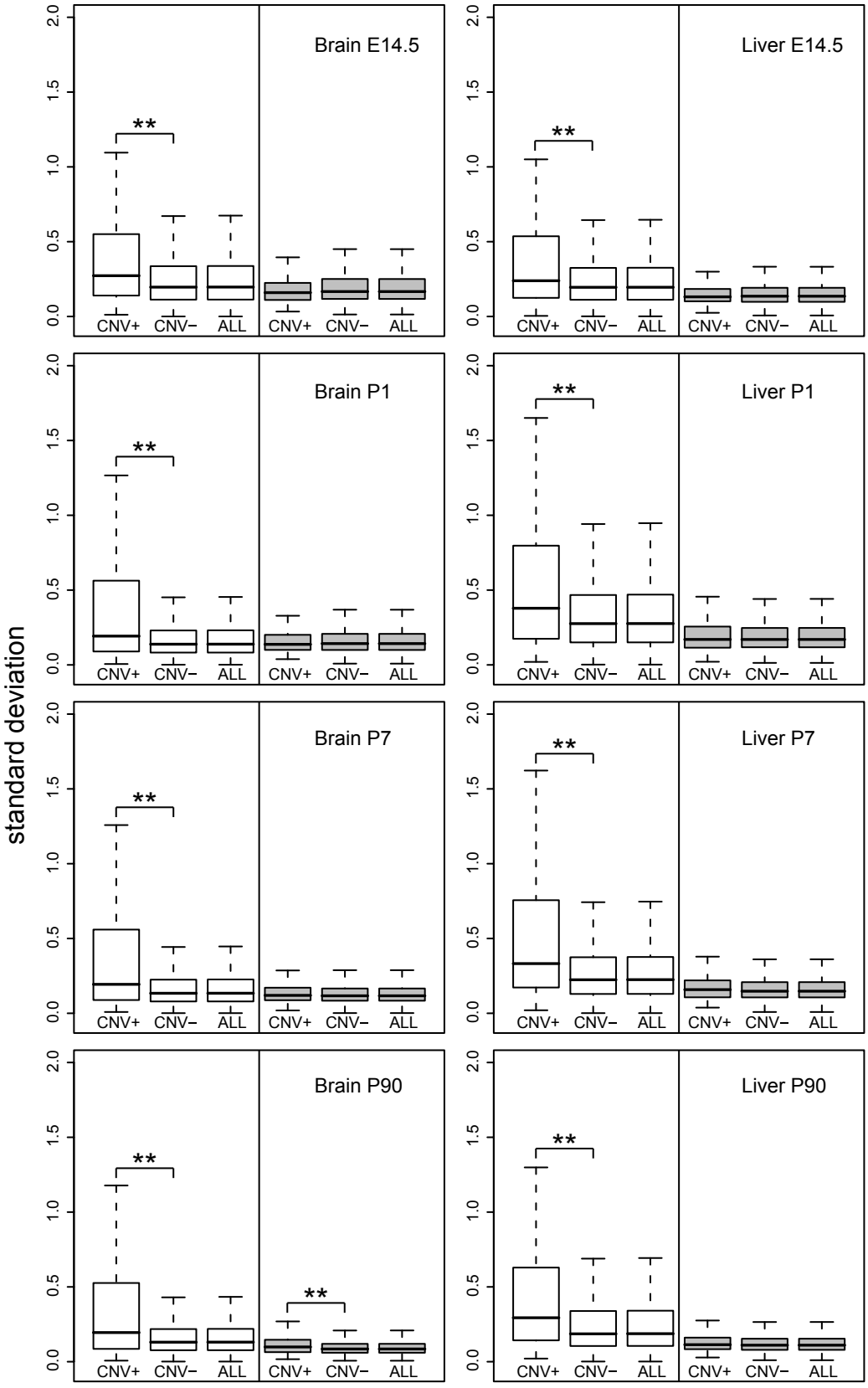
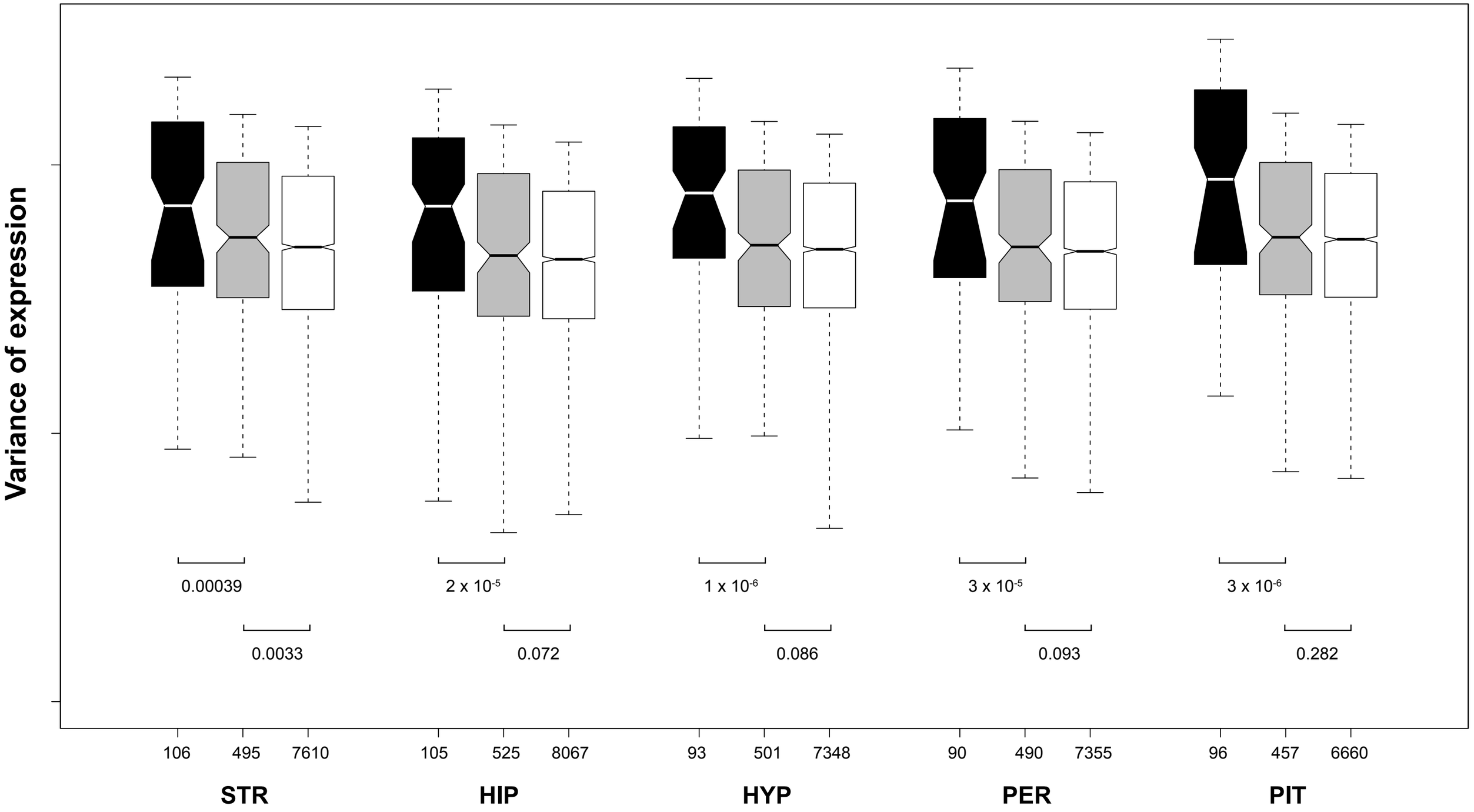

standard deviation
Brain E14.5
Liver E14.5
Brain P1
Liver P1
Brain P7
Liver P7
Brain P90
Liver P90
CNV+
CNV−
ALL
**

Figure S1

Supplementary Figure S2: Variance of expression in different parts of the CNS of genes within CNVs, in neighboring regions, and elsewhere in the genome

Boxplot distribution of signal variances for transcripts expressed in the bed nucleus of the stria terminalis (STR), the hippocampus (HIP), the hypothalamus (HYP), the periaqueductal gray (PER) and the pituitary gland (PIT), and mapping within CNVs (black), 50 - 250 kb of the nearest CNV breakpoint (gray), or further away (white) are shown. The data were computed using the GSE4734 mouse expression dataset (Hovatta et al., *Genome Biol*, 2007). The black (largest two-tailed $P = 4 \times 10^{-4}$, Mann-Whitney *U* test (*U* = 2396773)) and the gray distributions (largest two-tailed *P* = 0.094, Mann-Whitney *U* test; one exception is pituitary gland) are significantly different from the white in all monitored tissues. The numbers of transcripts for which expression could be detected are indicated.

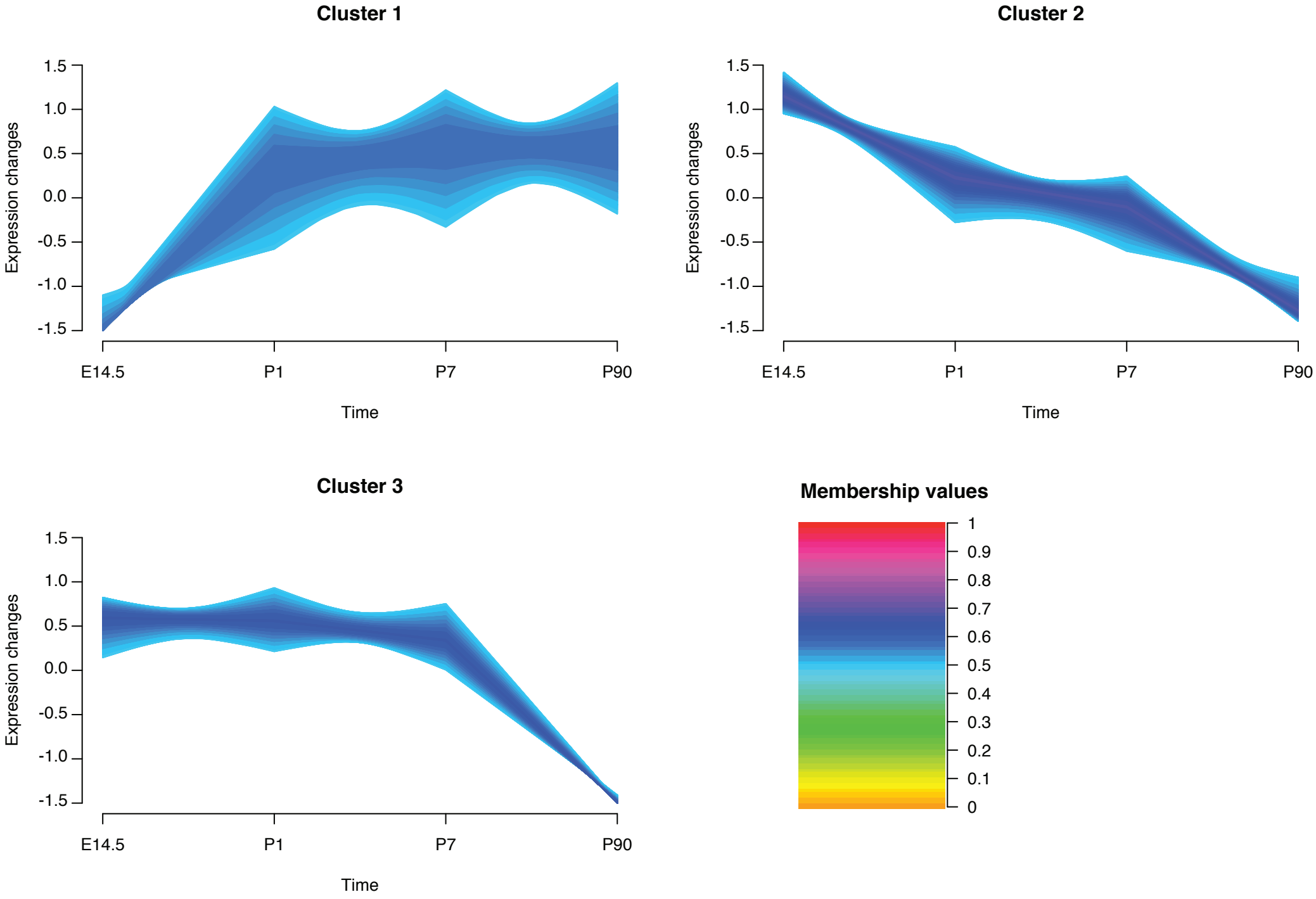
Figure S2


Variance of expression
0.00039
2 x 10-5
1 x 10-6
3 x 10-5
3 x 10-6
0.0033
0.072
0.086
0.093
0.282
106
495
7610
105
525
8067
93
501
7348
90
490
7355
96
457
6660
STR
HIP
HYP
PER
PIT

Supplementary Figure S3: Clustering results produced by Mfuzz

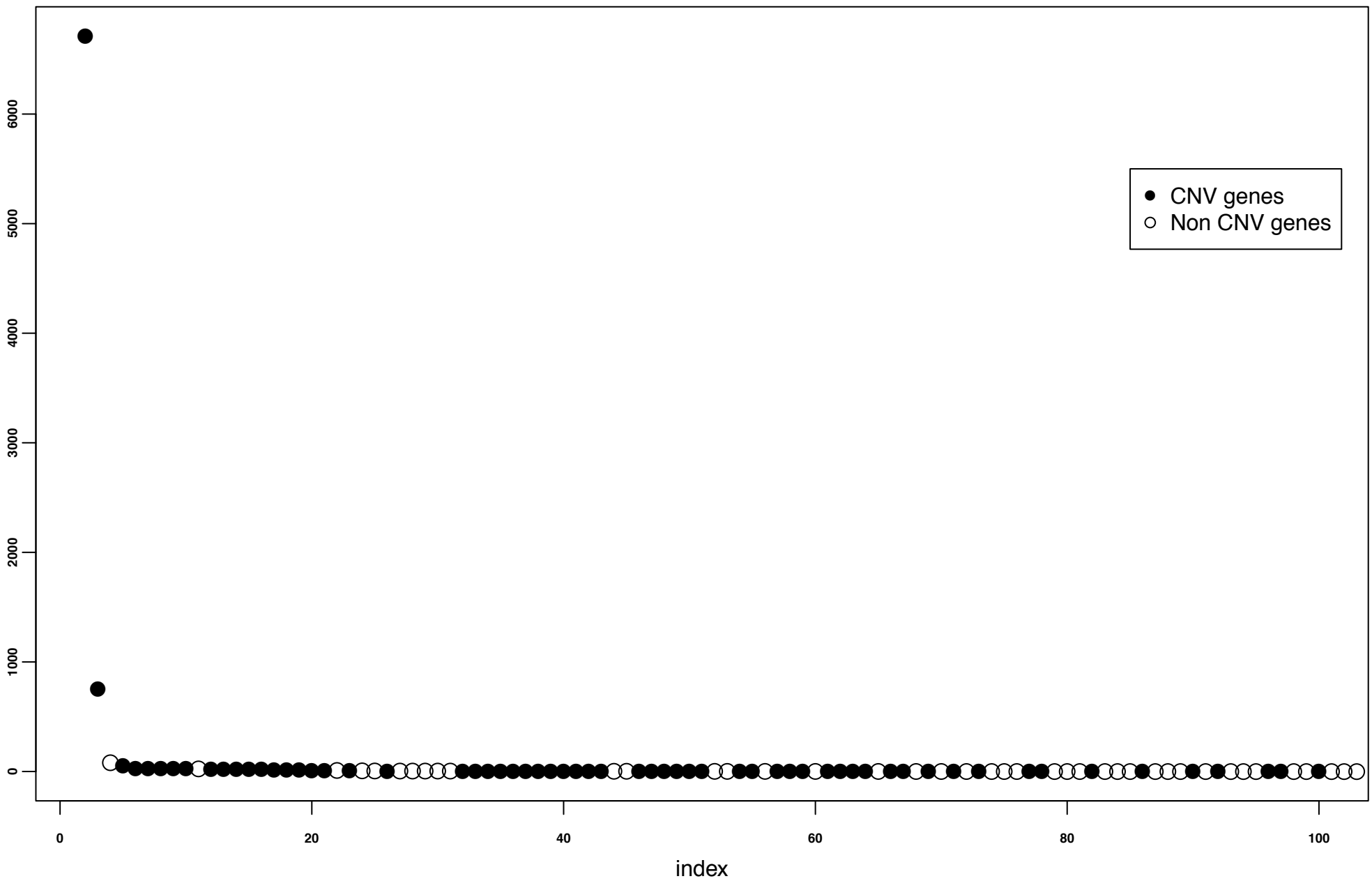
Graphs showing the three clusters of liver time-course expression profiles obtained with Mfuzz (Futschik & Carlisle, *J Bioinform Comput Biol*, 2005). Membership values are color-coded, with red and orange shades denoting high and low membership values, respectively (see inset on the bottom right). Time-courses were included in a given cluster only if their membership values were ≥ 0.5 (see Methods section for details).

Figure S3


Cluster 1
Cluster 2
Cluster 3
Membership values
Expression changes
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
E14.5
P1
P7
P90
Time
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0

Supplementary Figure S4:

Assessed transcripts were ranked decreasingly by the sum of squares deviations from the mean between strains for each developmental time-points. CNV transcripts are represented by a disk and non-CNV transcripts by a circle, respectively. Data from brain are shown, while data from liver are presented in **Figure 4**. We observe in both brain and liver a statistically significant enrichment of CNV-transcripts amongst the transcripts with the highest score, i.e. the transcripts that vary more between strains (see text for details).


Ranking of the sum of squared deviations from the mean
CNV genes
Non CNV genes
6000
5000
4000
3000
2000
1000
0
0
20
40
60
80
100
index

Supplementary Table S1: Proportion of variance of expression due to copy number changes through development

Supplementary Table S2: Proportion of variance of expression due to copy number changes for different brain substructures

Supplementary Table S3: Genes and quantitative PCR assays

Supplementary Table S1: Proportion of variance of expression due to copy number changes through development									
Measure	**Labels/Formulas**	**Brain**				**Liver**			
		E14.5	**P1**	**P7**	**P90**	**E14.5**	**P1**	**P7**	**P90**
sum sum sq between CNV-	B	9862.0	6493.6	6397.5	6601.5	7930.8	11820.1	9447.4	10837.9
sum sum sq within CNV-	W	13596.9	10000.3	8805.1	2351.4	8142.4	9710.1	7505.2	3404.3
sum sum sq between CNV+	b	310.2	278.0	292.1	394.3	394.2	550.1	643.4	624.2
sum sum sq within CNV+	w	189.7	94.0	91.8	119.7	69.2	97.8	89.4	36.7
proportion var strain effect	B/(B+W)=P	0.42	0.39	0.42	0.74	0.49	0.55	0.56	0.76
proportion var strain and CNV effect	b/(b+w)=p	0.62	0.75	0.76	0.77	0.85	0.85	0.88	0.94
expected sum sum sq between CNV+ without CNV effect	w*P/(1-P)=q	137.6	61.1	66.7	336.1	67.4	119.1	112.6	117.0
calculated sum sum sq CNV effect	b-q=r	172.6	216.9	225.4	58.2	326.7	431.1	530.9	507.2
proportion var CNV effect	r/(b+w)	0.35	0.58	0.59	0.11	0.71	0.67	0.72	0.77

Supplementary Table S2: Proportion of variance of expression due to copy number changes for different brain substructures						
Measure	**Labels/Formulas**	**bed nucleus stria terminalis**	**hippocampus**	**hypothalamus**	**periaqueductal gray**	**pituitary gland**
sum sum sq between CNV-	B	1077297852	834903243	799499082	709684856	1158445140
sum sum sq within CNV-	W	516340834	265124929	444897630	204881887	299077978
sum sum sq between CNV+	b	43218033	33791982	30209699	35393980	62856751
sum sum sq within CNV+	w	6966316	4019601	10254441	4211387	7904670
proportion var strain effect	B/(B+W)=P	0.68	0.76	0.64	0.78	0.79
proportion var strain and CNV effect	b/(b+w)=p	0.86	0.89	0.75	0.89	0.89
expected sum sum sq between CNV+ without CNV effect	w*P/(1-P)=q	14534581	12658099	18427646	14587708	30617858
calculated sum sum sq CNV effect	b-q=r	28683452	21133883	11782052	20806272	32238893
proportion var CNV effect	r/(b+w)	0.57	0.56	0.29	0.53	0.46

Supplementary Table S3: genes and quantitative PCR assays

Gene ID	Gene name	Forward primer sequence	Reverse primer sequence	CNV-gene
ENSMUSG00000000326	*Comt1*	GAGATTAACCCTGACTACGC	GATGAGGATGGAAACTTTGTC	No
ENSMUSG00000001100	*Poldip2*	CTCAGACAGAAGCTGTGAC	CTGACATAGTCCAACCCTG	No
ENSMUSG00000001909	*Trmt1*	GTGATGGAGGACTTCTGTG	ACTTGCTGTAGCATGTCTC	Yes
ENSMUSG00000001910	*Nacc1*	TGTCAAGTACTACTGCCAG	GTTCTATCTTGCAAGTGTCG	Yes
ENSMUSG00000001911	*Nfix*	ACTTAAGTTTCCAGGACTGC	CAACTGGAGTCTGTGATACTC	Yes
ENSMUSG00000002395	*Use1*	GACAATCAGACCCTGTCAC	AGTTCACTGACTTCTGTGC	Yes
ENSMUSG00000002617	*Zfp40*	ACTGTAGCAAAGGATCCAG	AATGTCAGTAGATCCTGGG	Yes
ENSMUSG00000003068	*Stk11*	CGTCAAGAAGGAGATCCAG	CATCACCATATACATCTTCTGC	Yes
ENSMUSG00000003072	*Atp5d*	TAAGTACTTTGTGAGCAGC	ATACGGATCTGGATCTCAG	Yes
ENSMUSG00000004677	*Myo9b*	TAGACAGTAGGGTTAGCCC	GTATCGTTGGATCTGGGTG	Yes
ENSMUSG00000004934	*Pias4*	TATGGCAAGAGCTACTCTG	CAATCGTCTTCAACCTCTG	Yes
ENSMUSG00000005204	*Senp3*	AGGAGTTCTCTACACCCTC	TGAAGATGTCCACCATAGC	Yes
ENSMUSG00000007670	*Khsrp*	CAAGAAGCAAGCACAAGTG	GTCTGTCCGTAGTAAGCAG	No
ENSMUSG00000010047	*Hyal2*	CAACTTTGTCAGTTTCCGTG	AGAGATAAGGTCCACCTGG	Yes
ENSMUSG00000013833	*Med16*	ATAACCATGGGAAGCTCAG	TCACCATGCAGTATTCCAG	Yes
ENSMUSG00000013858	*ORF-61*	TACCACTACCGCTTTAACG	GTAGATCATGGAATGCTGGA	Yes
ENSMUSG00000014418	*Hps5*	TGAATCCAAGGATGCTGAC	TCTTTCTCCTCAGTGTCCT	No
ENSMUSG00000015766	*Eps8*	CTTCTCAACTGAATGGTTACG	CCTTTGCACTTGTTCTTGAG	Yes
ENSMUSG00000017548	*Suz12*	TATTCTTGTTTGCAGGCCA	TGTATGTTCTCTGCTGCTC	No
ENSMUSG00000018761	*Mpdu1*	TCTGTTCAGGAAACTGGAG	TCCAGTAGAAGAGGACCTG	Yes
ENSMUSG00000018774	*Cd68*	CAAGCATAGTTCTTTCTCCAG	GGTTGCAAGAGAAACATGG	Yes
ENSMUSG00000019975	*Ikbip*	ATTTCCAGCAAGTACCAGG	CAGCAGCTTTACATGAGCC	No
ENSMUSG00000020672	*Snrpg2*	GGTAAGACAAAGAACGTACTC	CTTTACTCGAGTCTTCCCA	Yes
ENSMUSG00000021018	*Polr2h*	GGACTTAGGTGACAAGTTCC	CGATCCTGTACACTTTCCC	No
ENSMUSG00000021477	*Ctsl*	ATGATCCAGCTACACAACG	TCCTCATTGGTCATGTCAC	Yes
ENSMUSG00000021576	*Pdcd6*	AAATTTGACAGGCAAGGAC	TATGTCTGTCAACCTCTGC	No
ENSMUSG00000021771	*Vdac2*	TTCAGTAAACCTCGCTTGG	GTTGTTGACCTTTGCAGAG	No
ENSMUSG00000022307	*Oxr1*	GAGTGGGAGATTACTACAAGG	TTGGTCGGAAAGATTCAGG	Yes
ENSMUSG00000022856	*Tmem41a*	CTGGTTCTTGAACCTCTCG	AAGGGATCAAACCGATGAG	No
ENSMUSG00000023892	*Zfp51*	ACAGTCATTCTACCTGAAAGAG	TGAATGTCAACAGACCCTG	No
ENSMUSG00000024187	*Ifig3*	TTCATCATCCCATGTCCAG	GAAGTCGTACATGACTGCA	No
ENSMUSG00000024350	*Dnajc18*	GGGTACAAAGAATCAAGAAGTG	TCTTAAGCTCTTCATCGCTG	No
ENSMUSG00000024645	*1700034H14Rik*	GAAGTGGAAGAGAACCCAG	TAACTTTGTTCGGAACGGT	No
ENSMUSG00000025381	*Cnpy2*	GAGCTTATCGAATTCTTCTCC	ATGGTCACATAGATCTGTCC	No
ENSMUSG00000025403	*Shmt2*	GAGAAGGACAGACAGTGTC	CCGTAGTATCTCTTGCCAG	Yes
ENSMUSG00000025757	*Hspa4l*	GTGTTTATCGACATGGGAC	GTAGTAGCCAACACTTTCAG	No
ENSMUSG00000025958	*Creb1*	CAGAAGAGGAGACTTCAGC	GTAATGGCAATGTACTGCC	No
ENSMUSG00000026192	*Atic*	ACTGTGTTCTTCAGATGGAC	TCTGGCTTAAACGAAGACC	No
ENSMUSG00000026535	*Ifi202b*	TCACAGTTTCATCAAGGGAG	TTGTTGCTTTCAATGCCAC	Yes
ENSMUSG00000027472	*Pdrg1*	GGACACCAAGAGGAATCAG	CAAACCATCACATCTTCAGAG	No
ENSMUSG00000027679	*Dnajc19*	TTAGGTGTAAGCCCTACTG	GTTTCAGACCTTCTTCCAC	Yes
ENSMUSG00000027716	*Trpc3*	CCAAGAGATCGAGGATGAC	TTTCCATCATCGAAGTAGGAG	Yes
ENSMUSG00000028312	*Smc2*	TAATTCTACAGCCACAGCTC	TTAGCAGTTACCTCTGTGTC	No
ENSMUSG00000028393	*Alad*	CTTTGTCACGGATGTTCCT	TCTTCTAGCTGGTTTACGC	Yes
ENSMUSG00000028399	*Ptprd*	TGAGGCCATTTATGAATGTG	TAGGAATCTGATCCTCACG	Yes
ENSMUSG00000029014	*Dnajc2*	CATTATGCGGTTCTTGGAC	GACCATTGCTTTATGAGCTG	No
ENSMUSG00000029144	*Mpv17*	AACTACTATCTCTGGCCTG	TTCCAGACAATAGCAACAC	No
ENSMUSG00000030167	*Klrc1*	CTTTCACTGATCTCATGGAC	GAGAATCTCTGCTATCTTTGG	Yes
ENSMUSG00000030401	*Rtn2*	TCTTGTCATCCTAGGAGTG	CACATACTGGTCAATCTGG	Yes
ENSMUSG00000030697	*Ppp4c*	TTCATGGGAGACTTTGTGG	AGGATAGCGAACCTTAAGAG	No
ENSMUSG00000031508	*Ankrd10*	CTAGAACCAGAGTTCCGAG	TATTCAACAGGGAGCTACTG	No
ENSMUSG00000031584	*Gsr*	GAATAAAGTGGGCATTCAGAC	TAGACGCCTTTGACATTGG	Yes
ENSMUSG00000031851	*2310079N02Rik*	TAGGATTCGATGTCGTCAC	CTTCTTGATGGTTTCTTAGGAC	Yes
ENSMUSG00000031939	*Taf1d*	CTTTCTCCTATTGAAGAGCCA	AAGCAACTGTCTTCAACCA	No
ENSMUSG00000033938	*Ndufb7*	ACAAGAGATGATGGATGCC	CGTTTCACGTAATCCAGGT	Yes
ENSMUSG00000034041	*Lyl1*	AGTGATAAACCTGGGACAC	AATGTAGACACTGTTGAGGA	Yes
ENSMUSG00000034101	*Ctnnd1*	CTTTGGAGGAAGAGTTGGA	GCTAAATCTTCTGCATGGG	No
ENSMUSG00000034377	*Tulp4*	GACTTACCAGTTACAGGAGC	TGTGGATTGGTGCAATAGG	Yes
ENSMUSG00000034748	*Sirt6*	AAGTGTAAGACGCAGTACG	CAGTCCAGAATGGTGTCTC	Yes
ENSMUSG00000034771	*Tle2*	CATTATGTGATGGCTGCAC	TACTGCAAAGGAGACAGTC	Yes
ENSMUSG00000034994	*Eef2*	ACAACATATTATTGCTGGCG	GGTCACATTTCTTGATGGG	Yes
ENSMUSG00000035032	*Nek11*	GTCTGACATCTGGTCACTG	ACAGACAAGAAACTGGAGC	Yes
ENSMUSG00000035151	*Elmod2*	TCCAAGAATAAGGTCCTACAG	TTCCTTCATGATGTTCGCT	Yes
ENSMUSG00000035443	*Thyn1*	GGTATAGACATGAAGTTCAGCA	CTGATAGTTGCGAACACCA	No
ENSMUSG00000035640	*Dos*	GCATTTCGTTAGATGCAGG	TTCTTGACCTTGAAGTAGGG	Yes
ENSMUSG00000035754	*Wdr18*	ACATATTCTGTGGAGGCAG	ATGTCACTTGGTTCCTGTG	Yes
ENSMUSG00000035835	*BC005764*	TCGAACCCTTACATCACAC	TTGAAGTACATCGACACGT	Yes
ENSMUSG00000035863	*Palm*	ATGATGAAAGCAGTGGTCC	TGTGAATGAGTTCGTCCAC	Yes
ENSMUSG00000036019	*Tmtc2*	GACATGCTGTATAATCTAGGGT	CAATGGCCAGTTTGTAATAGTG	Yes
ENSMUSG00000036322	*H2-Ea-ps*	CCACTTTCTGACCTTCCTG	TTCTCTTCAAACTCCCAGTG	Yes
ENSMUSG00000037257	*Aagab*	AAATGAAAGACAAGGCTGC	CCTATTGCCATCCAGAAGG	No
ENSMUSG00000038406	*Scaf1*	GGACACTGACAAGTACCTG	TGGTAGTATGGCTTGATGG	No
ENSMUSG00000038781	*Stap2*	GAGAAGGTTCTAGGCTTCG	CTTCAGGACATTAGCAGGG	Yes
ENSMUSG00000038871	*Bpgm*	CATCTGGAAGGTATCTCAGAC	GGTTCTCATCCAACTCCAG	No
ENSMUSG00000039114	*Nrn1*	GATGCAGTCTTTAAGGGCT	AAATCCTCCCAGTATGTGC	No
ENSMUSG00000039154	*Shd*	GAAACCAGAAGATGGAGGG	GGACCATCAAATTGCACTG	Yes
ENSMUSG00000039230	*Tbcd*	GAACCTGTCTACTCAAACTG	ACCACCAAGTAAGACTCTG	No
ENSMUSG00000039620	*6430573F11Rik*	GTCATGGTATGTGACAACC	ATGATGTATGACTCCTATGGAG	Yes
ENSMUSG00000040249	*Lrp1*	GTGTGACAACCACAATGAC	CACTTGAACTGGGTACTGG	Yes
ENSMUSG00000040809	*Chi3l3*	GATCTATGCCTTTGCTGGA	CTCAGTGTTCTTGTCTTTCAG	Yes
ENSMUSG00000041763	*Ipp2*	CAGCCATTCTTTGTTACTTCC	GATCACATCAGCTTTCTTTCC	No
ENSMUSG00000042642	*Flad1*	GTACATTCGTAGCATCTCG	AACACCTGGAGATTATACCTC	Yes
ENSMUSG00000042729	*Wdr74*	TTGGGACCAAGATACACAG	GATCATAGACACGGACCTG	No
ENSMUSG00000044138	*Sirpb1a*	TGTTAGTCCTTGCTAAACCA	TAAAGGTCACTGTCTGCTG	Yes
ENSMUSG00000044308	*Ubr3*	CAGATCAAGAGTATAAGGTTGC	GACATTGTATCTGATTCGTGAC	No
ENSMUSG00000046139	*Patl1*	TCTGAATTCCAGCATCTGG	ACTGTAGGCATTTCCTGAG	No
ENSMUSG00000049313	*Sorl1*	GGGAAATACCACATCATTGTC	TGCTGATAACGAAACTGTG	Yes
ENSMUSG00000052459	*Atp6v1a*	AGTCAATGTGTCTGCTCTC	GTGATATGACTACCAACCCG	No
ENSMUSG00000056144	*Trim34*	CCTACTGGGACAACTTCAC	ATGTCACTTGTCTGTGGTC	Yes
ENSMUSG00000056899	*Immp2l*	TTGTGTCATTGGTGTCTCC	ACTGTGTCGATGATGATCG	No
ENSMUSG00000056940	*Gm3696*	AAGAGAATGGCGATGAAGG	GTATTTCTAGGAGACCTGTGC	Yes
ENSMUSG00000057596	*Trim30d*	TATGTTCAGCATCAGTTGGA	CATGGTCTGAATGCTTGTC	Yes
ENSMUSG00000057729	*Prln3*	CATATGCTTCGGAGACTCG	GAAATCAGGGAACTGGAGG	Yes
ENSMUSG00000060212	*Pcnxl2*	AGCTATAATACAAGGCGCA	AGGTTGTTGTCATCACTCC	Yes
ENSMUSG00000060459	*Kng2*	TAGAGGTAATGCTTTCCTGG	AAATCATCTCCTGGATGAAGG	No
ENSMUSG00000062270	*Morf4l1*	GTGAGGATATTGTAGCCCT	TTCATCCCAACTAGAGGTC	Yes
ENSMUSG00000062604	*Srpk2*	CAGTGACCCAAACAAAGAC	CATGCAGACATGTATCCCA	Yes
ENSMUSG00000066667	*Gm16432*	CCATATGGGTGTATGATCCAG	CCTATGATGACAGTGAAGGT	Yes
ENSMUSG00000068114	*Ccdc134*	AGCTGAAGGATGCTTTCTC	AGTGGTGGACAATCTTTGG	No
ENSMUSG00000069206	*Zfp874*	ATTCCTCCAGGAACAACAG	CTGGCATTGCATACTTAGAG	Yes
ENSMUSG00000070348	*Ccnd1*	GAGACCATTCCCTTGACTG	GAAGCAGTTCCATTTGCAG	No
ENSMUSG00000072735	*Gm10406*	TCCTACAGCATTAAGGAGGA	GACACTTATAGCCAGCCTG	Yes
ENSMUSG00000029580	*Actb*	CTAAGGCCAACCGTGAAAAGAT	CACAGCCTGGATGGCTACGT	Normalization
ENSMUSG00000037742	*Eef1a1*	CCTGGCAAGCCCATGTGT	TCATGTCACGAACAGCAAAGC	Normalization
ENSMUSG00000025630	*Hprt*	GCTCGAGATGTCATGAAGGAGAT	AAAGAACTTATAGCCCCCCTTGA	Normalization
ENSMUSG00000000740	*Rpl13*	CTCATCCTGTTCCCCAGGAA	GGGTGGCCAGCTTAAGTTCTT	Normalization
ENSMUSG00000014767	*Tbp*	TTGACCTAAAGACCATTGCACTTC	TTCTCATGATGACTGCAGCAAA	Normalization