

## Supplementary Data

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# RNA-Seq Analysis of Frontal Cortex and Cerebellum from 5XFAD Mice at Early Stage of Disease Pathology

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Supplementary Table 1A  
Top 20 upregulated genes of frontal cortex in 5XFAD mice

Low expression (0.01–1 RPKM)			Moderate expression (1–10 RPKM)			High expression (10–100 RPKM)			Extremely High expression (>100 RPKM)		
Gene	Accession	FC	Gene	Accession	FC	Gene	Accession	FC	Gene	Accession	FC
MIR-434	NR_029953	17.509*	SNORD71	NR_028532	6.808*	MIR-29B-2	NR_029809	2.377	THY1	NM_009382	2.976*
INHBC	NM_010565	5.880*	TTR	NM_013697	5.140*	A330023F24RIK	NR_015566	2.149	MEG3	NR_003633	1.644
1810033B17RIK	NM_026985	5.218*	MIR541	NR_030263	4.561*	ATP5G1	NM_007506	1.967	MEG3	NR_027651	1.641
PLG	NM_008877	5.105*	4930579C15RIK	NM_027089	4.125	MIR-29C	NR_029745	1.959	STMN1	NM_019641	1.433
GM15104	NM_001101501	4.667*	MIR-453	NR_030559	3.806*	A730008H23RIK	NM_172505	1.959	MEG3	NR_027652	1.402
MIR-3070A	NR_037230	4.212*	MIR-491	NR_030478	3.687*	MIR-770	NR_030427	1.854	MIR-1906-1	NR_035440	1.363
MIR-1894	NR_035445	4.173	MIR-138-2	NR_029552	3.607*	ZFP949	NM_001163061	1.716	MIR-1906-2	NR_037313	1.363
FPR2	NM_008039	4.013	MIR-27B	NR_029531	3.189	ROBO3	NM_001164767	1.707	SNHG11	NM_175692	1.304
MIR-126	NR_029541	3.993*	MIR-23B	NR_029530	3.182*	ZFP949	NM_001142943	1.689	MALAT1	NR_002847	1.264
ACSS3	NM_198636	3.820*	MIR-1224	NR_035407	3.039	ATP5G1	NM_001161419	1.636	RNF112	NM_009548	1.200
GM1082	NR_036630	3.803*	MIR-125A	NR_029539	2.964	LENG8	NM_172736	1.582	MTAP1A	NM_001173506	1.199
MIR-1968	NR_035494	3.717*	MIR-124A-1	NR_029813	2.927	CORO6	NM_139129	1.558	MTAP1A	NM_032393	1.199
FSIP1	NM_027759	3.681*	SNORD87	NR_004410	2.491*	CORO6	NM_139130	1.556	GRIN1	NM_008169	1.173
1700018C11RIK	NM_029324	3.484*	MIR-3070B	NR_037231	2.415	CORO6	NM_139128	1.550	ATP1A3	NM_144921	1.172
1700001J03RIK	NM_001008547	3.384*	HBQ1A	NM_175000	2.305*	VWA5B2	NM_001144953	1.509	D4WSU53E	NM_023665	1.170
BCL2A1D	NM_007536	3.363*	MIR-212	NR_029794	2.258	4933439C10RIK	NR_015585	1.484	KALRN	NM_001164268	1.145
OLFR1372-PS1	NR_034155	3.309*	PYROXD2	NM_029011	2.188	PER1	NM_001159367	1.482*	GRIN1	NM_001177656	1.144*
SLC25A41	NM_175333	3.262*	MIR-24-1	NR_029575	2.151	PER1	NM_011065	1.481*	GRIN1	NM_001177657	1.144*
4921513I03RIK	NR_038000	3.238*	TNFRSF25	NM_033042	2.099	TMEM145	NM_183311	1.462	CLSTN1	NM_023051	1.140
EFCAB3	NM_001081046	3.237*	SNORA41	NR_028558	2.061	SRRM2	NM_175229	1.449	STX1A	NM_016801	1.135

The fold change (FC) is calculated by the average RPKM value in 5XFAD mice compared with that in non-transgenic (NT) mice. Differentially expressed genes (DEGs) are grouped according to the average of RPKM values in NT mice. Asterisk indicates significantly changed gene in 5XFAD mice compared with NT mice (Wilcoxon rank-sum test,  $p < 0.05$ ).

Supplementary Table 1B  
Top 20 down-regulated genes of frontal cortex in 5XFAD mice

Low expression (0.01–1 RPKM)			Moderate expression (1–10 RPKM)			High expression (10–100 RPKM)			Extremely High expression (>100 RPKM)		
Gene	Accession	FC	Gene	Accession	FC	Gene	Accession	FC	Gene	Accession	FC
ADAM18	NM_010084	-9.455*	1110057K04Rik	NM_172401	-2.775*	TAC1	NM_009311	-1.974	PLP1	NM_011123	-1.529*
PROK2	NM_001170419	-5.995*	1110057K04Rik	NM_001167767	-2.774*	PENK	NM_001002927	-1.721	SCG5	NM_009162	-1.320
D830046C22RIK	NR_033147	-5.798*	1110057K04Rik	NM_001167768	-2.392	PDE10A	NM_011866	-1.695	PTGDS	NM_008963	-1.293
PROK2	NM_015768	-5.628*	MIR-703	NR_030484	-2.037	GAD2	NM_008078	-1.573*	NAPB	NM_019632	-1.282*
PROK2	NM_001037539	-5.625*	SERPINA9	NM_027997	-2.020	BETA-S	NM_001201391	-1.525	PPP1R1B	NM_144828	-1.267
CTSE	NM_007799	-5.609	DRD2	NM_010077	-1.995	SPOCK3	NM_023689	-1.520*	RGS4	NM_009062	-1.254
MIR-1943	NR_035464	-4.772*	FAU	NM_001190436	-1.958	GPR88	NM_022427	-1.495	SNAP25	NM_011428	-1.248
1700123I01RIK	NM_001165919	-4.668	DRD1A	NM_010076	-1.957*	NNAT	NM_010923	-1.478	PGRMC1	NM_016783	-1.248
CLEC12A	NM_177686	-4.392	CLCNKA	NM_001146307	-1.955	NNAT	NM_180960	-1.474	GPM6B	NM_023122	-1.247*
2810433D01RIK	NR_033474	-4.272*	CD4	NM_013488	-1.954*	PON2	NM_183308	-1.456*	GPM6B	NM_001177962	-1.245*
GPR143	NM_010951	-4.127*	CLCNKA	NM_024412	-1.952	SCOC	NM_001039137	-1.454	VSNL1	NM_012038	-1.245
VMN1R90	NM_001039146	-4.098*	ZFP382	NM_001081007	-1.856	SCOC	NM_019708	-1.451	NPY	NM_023456	-1.245
H2-Q6	NM_207648	-4.053*	MYOC	NM_010865	-1.838	NUDT19	NM_033080	-1.442	GPM6B	NM_001177958	-1.238*
NKX2-1	NM_009385	-4.028*	FAU	NM_007990	-1.834*	GNAL	NM_010307	-1.438*	GPM6B	NM_001177961	-1.238*
GBX1	NM_015739	-4.001*	FAU	NM_001160239	-1.829*	GNAL	NM_177137	-1.433*	GPM6B	NM_001177955	-1.237*
CYP19A1	NM_007810	-3.984*	ADORA2A	NM_009630	-1.828	SYNPR	NM_001163032	-1.426	GPM6B	NM_001177960	-1.236*
A430084P05RIK	NM_001045526	-3.958*	HBB-B1	NM_008220	-1.825	PPP1R2	NM_025800	-1.414*	GPM6B	NM_001177957	-1.235*
GPR77	NM_001146005	-3.872*	HBB-B2	NM_016956	-1.824	ZCCHC12	NM_028325	-1.407	NAP1L5	NM_021432	-1.234
NKX2-1	NM_001146198	-3.802*	ECEL1	NM_021306	-1.815	SYNPR	NM_028052	-1.403	GPM6B	NM_001177959	-1.230*
GPR77	NM_176912	-3.722*	FUT10	NM_001012517	-1.808	DGKB	NM_178681	-1.402*	GPM6B	NM_001177956	-1.229*

The fold change (FC) is calculated by the average RPKM value in 5XFAD mice compared with that in non-transgenic (NT) mice. Differentially expressed genes (DEGs) are grouped according to the average of RPKM values in NT mice. Asterisk indicates significantly changed gene in 5XFAD mice compared with NT mice (Wilcoxon rank-sum test,  $p < 0.05$ ).

Supplementary Table 1C  
Top 20 upregulated genes of cerebellum in 5XFAD mice

Low expression (0.01–1 RPKM)			Moderate expression (1–10 RPKM)			High expression (10–100 RPKM)			Extremely High expression (>100 RPKM)		
Gene	Accession	FC	Gene	Accession	FC	Gene	Accession	FC	Gene	Accession	FC
CRYM	NM_016669	19.589*	MIR-1897	NR_035433	2.892*	MIR-770	NR_030427	2.002*	PLP1	NM_011123	1.426*
CRYBA2	NM_021541	11.875*	6330527O06RIK	NM_029530	2.450	1700020I14RIK	NR_027832	1.984	GM1821	NR_002875	1.363
DDN	NM_001013741	9.635*	MIR-124A-1	NR_029813	2.320*	1700020I14RIK	NR_015473	1.955	MEG3	NR_027651	1.347
FBXO40	NM_001037321	6.132*	LNPEP	NM_172827	2.232*	XIST	NR_001570	1.815	MEG3	NR_003633	1.336
MIR-668	NR_030424	6.027	CD24A	NM_009846	2.194*	E030003E18RIK	NR_015502	1.753	ADCY1	NM_009622	1.257
MIR-128-1	NR_029543	5.997*	TSIX	NR_002844	2.173	DDX17	NM_199079	1.719*	ATP2A2	NM_001110140	1.244
CRYBA4	NM_021351	5.948*	CDKL5	NM_001024624	2.151*	MEGF9	NM_172694	1.706*	DDX3X	NM_010028	1.200
SLC18A3	NM_021712	5.851	PTAR1	NM_028208	2.109*	PEX5L	NM_001163516	1.682*	MALAT1	NR_002847	1.200
CPNE7	NM_170684	5.761	KCNA2	NM_008417	2.089*	PEX5L	NM_021483	1.673*	2900097C17RIK	NR_024329	1.197
GLRA4	NM_010297	5.290*	SOX2OT	NR_015580	2.064*	PEX5L	NM_001163517	1.659*	SEP T7	NM_009859	1.169
CHRM1	NM_001112697	5.130*	KIF1B	NM_008441	2.044*	UGT8A	NM_011674	1.641*	THY1	NM_009382	1.154
CHRM1	NM_007698	5.090*	RASSF4	NM_178045	2.032*	6820431F20RIK	NR_030708	1.623	VAMP1	NM_009496	1.147*
DBH	NM_138942	5.064	SLC17A6	NM_080853	2.029	DNM3	NM_172646	1.615*	MBP	NM_001025258	1.141
NTRK1	NM_001033124	4.824*	UHMK1	NM_010633	2.015*	PEG3	NM_008817	1.568*	MBP	NM_001025254	1.141
CH25H	NM_009890	4.771*	HMBOX1	NM_177338	1.970	SPP1	NM_001204233	1.567	MBP	NM_010777	1.141
FUT4	NM_010242	4.746*	KCNK9	NM_001033876	1.969*	SPIN1	NM_146043	1.561	MBP	NM_001025255	1.140
1700016C15RIK	NM_027077	4.563*	CBL	NM_007619	1.960*	SPP1	NM_001204202	1.558	MBP	NM_001025251	1.140
KCNV1	NM_026200	4.407*	MIR-421	NR_030558	1.956*	SPP1	NM_001204201	1.557	MBP	NM_001025259	1.140
MSR1	NM_031195	4.099*	ERC1	NM_178085	1.947*	GRIA2	NM_001039195	1.557	MBP	NM_001025256	1.139
TK1	NM_009387	4.093*	EIF2C2	NM_153178	1.944*	GLG1	NM_009149	1.557*	GABRA1	NM_010250	1.134

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Supplementary Table 1D  
Top 20 down-regulated genes of cerebellum in 5XFAD mice

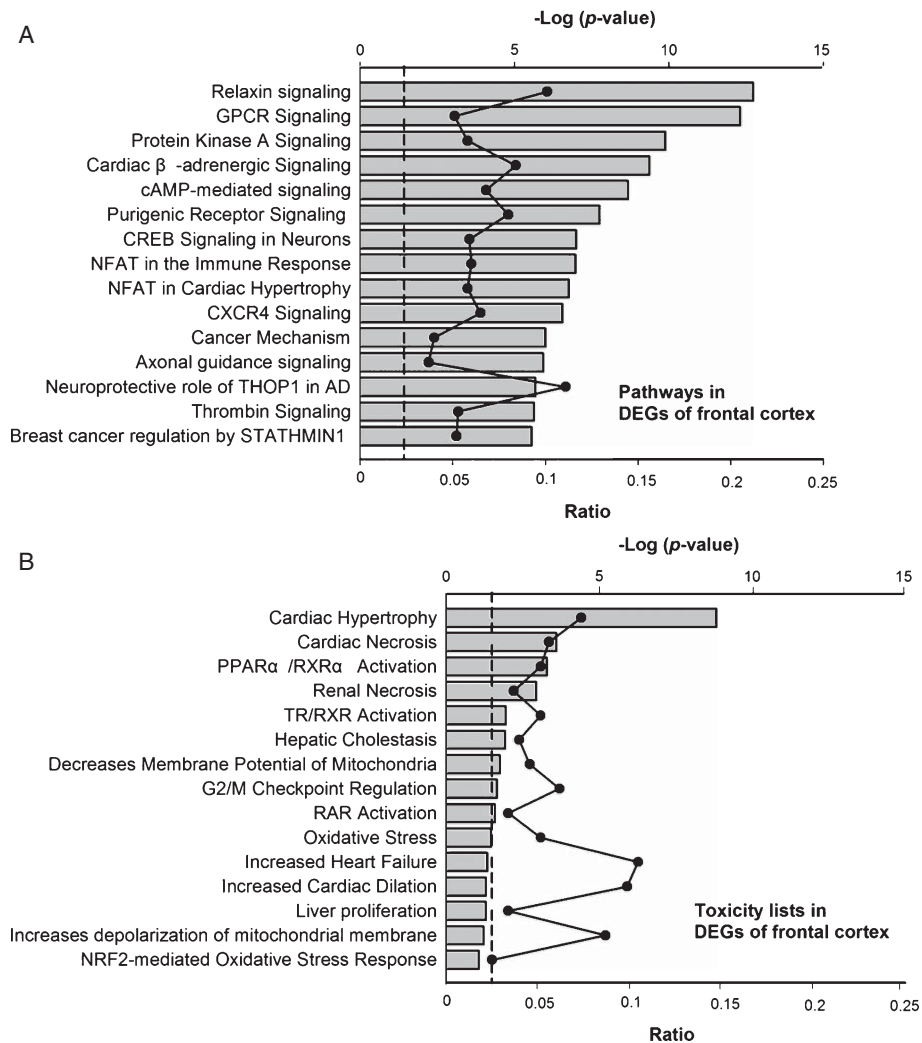
Low expression (0.01–1 RPKM)			Moderate expression (1–10 RPKM)			High expression (10–100 RPKM)			Extremely High expression (>100 RPKM)		
Gene	Accession	FC	Gene	Accession	FC	Gene	Accession	FC	Gene	Accession	FC
D1PAS1	NM_033077	-9.476	4930503E24RIK	NR_028310	-12.906	COX5B	NM_009942	-2.899*	ENPP2	NM_015744	-1.844
WDR86	NM_001081441	-9.369	GM6787	NR_003632	-5.286	RPS17	NM_009092	-1.925*	ENPP2	NM_001136077	-1.825
1700084J12RIK	NR_033608	-8.961*	MIR-207	NR_029594	-5.283	HBA-A2	NM_001083955	-1.855	PCP2	NM_001129804	-1.515*
KRT18	NM_010664	-8.366	GM4987	NM_001085510	-5.053*	HBA-A1	NM_008218	-1.855	PCP2	NM_008790	-1.513*
BC048507	NM_001001185	-8.251*	GSTP2	NM_181796	-4.123	FXYD1	NM_052991	-1.853*	PCP2	NM_001129803	-1.511*
LYZL4	NM_026915	-7.970*	TNNI3	NM_009406	-4.008*	TTC9B	NM_028417	-1.850*	SELM	NM_053267	-1.478*
HCST	NM_011827	-7.721	PRDX6	NR_033713	-3.981*	ENPP2	NM_015744	-1.844	SEPW1	NM_009156	-1.471
TMEM72	NM_178768	-7.138	PRDX6	NM_177256	-3.981*	ENPP2	NM_001136077	-1.825	MT3	NM_013603	-1.311*
CLDN1	NM_016674	-6.062	PRL	NM_001163530	-3.889	FXYD1	NM_194321	-1.808*	CKB	NM_021273	-1.279
OCA2	NM_021879	-5.871	PRL	NM_011164	-3.887	PCSK1N	NM_013892	-1.797	METRNL	NM_133719	-1.206*
4933417G07RIK	NR_033592	-5.241	PIN1-PS1	NM_001033768	-3.436	FXYD1	NM_019503	-1.747*	CALB2	NM_007586	-1.172*
CLDN2	NM_016675	-4.968*	EGR4	NM_020596	-3.327*	FXYD1	NM_052992	-1.706*	ATP5D	NM_025313	-1.158*
SHISA3	NM_001033415	-4.780	LOC100134990	NR_004857	-3.227	C1QTNF4	NM_026161	-1.670	RABAC1	NM_010261	-1.153*
KCNK15	NM_001030292	-4.719*	4930481A15RIK	NR_027940	-3.152*	VIM	NM_011701	-1.571*	PTMS	NM_026988	-1.141*
SLC22A2	NM_013667	-4.673*	4930481A15RIK	NR_027939	-3.143*	2900053A13RIK	NM_001195244	-1.499	SNCB	NM_033610	-1.139*
SEMA3B	NM_009153	-4.666*	HBA-A1	NM_008218	-3.135*	SELM	NM_053267	-1.478	MT1	NM_013602	-1.126
SEMA3B	NM_001042779	-4.598*	HBA-A2	NM_001083955	-3.135*	RPS8	NM_009098	-1.463*	RPL8	NM_012053	-1.118*
1700016K19RIK	NM_198637	-4.570*	RPS16	NM_013647	-3.029*	CCK	NM_031161	-1.419*	ATPIF1	NM_007512	-1.103*
SOCS1	NM_009896	-4.562*	GM6644	NR_028277	-2.988*	A1413582	NM_001002895	-1.414*	ABHD16A	NM_178592	-1.099
CAV3	NM_007617	-4.508*	GM6644	NR_037965	-2.988*	1110065P20RIK	NM_001142727	-1.410	MIR-682	NR_030451	-1.097

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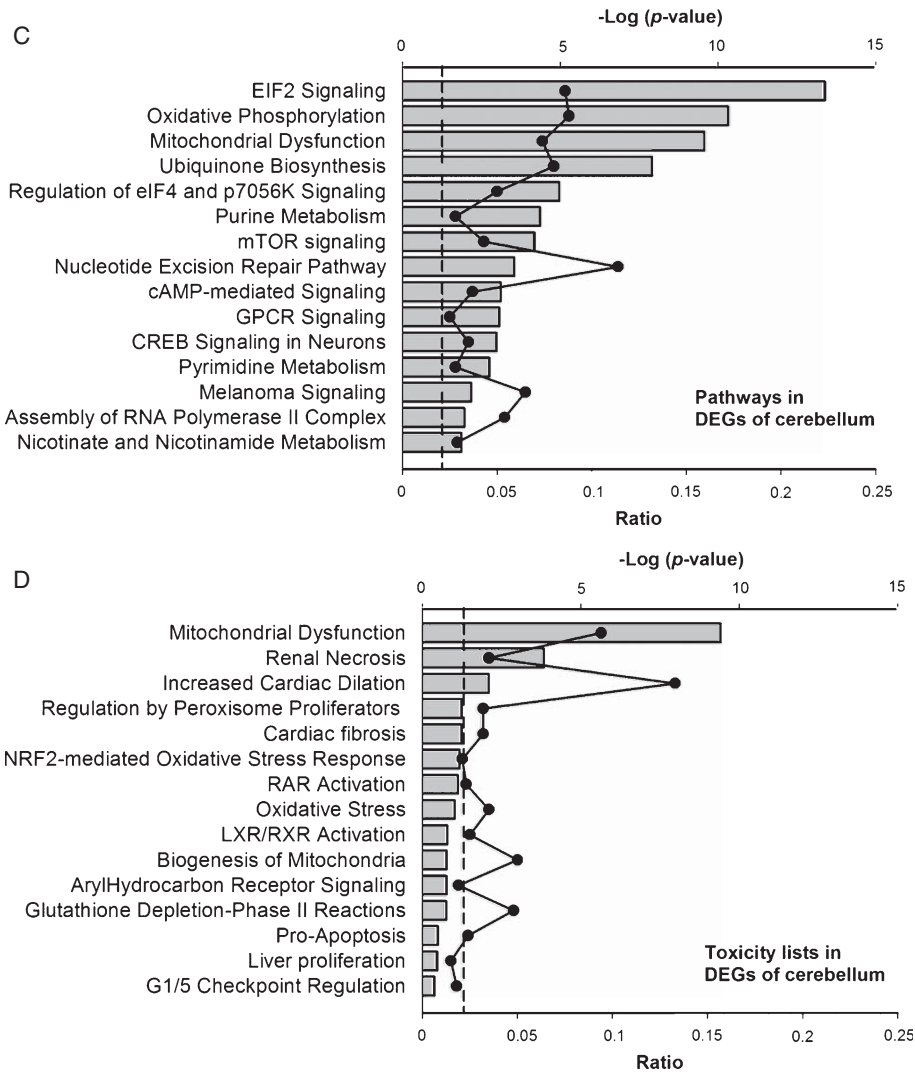
Supplementary Table 2  
Prediction of common transcription factor

Frontal cortex	Transcription factor	<i>p</i> value	E value	Q value	FDR	Genes in the intersection
	CREB1	<10 <sup>-5</sup>	<10 <sup>-5</sup>	<10 <sup>-5</sup>	6.17E-04	ADORA2A, BRCA1, CD4, FOS, GAL, GNRH1, NPPA, NR4A2, PENK, PER1, SST, TAC1
	SP1	<10 <sup>-5</sup>	<10 <sup>-5</sup>	<10 <sup>-5</sup>	1.23E-03	COL6A1, FOS, GAD2, GNRH1, IGFBP2, KRT18, MERTK, MIP, MME, NPY, PER1, PTTG1, TAC1, THY1, TTR
	FOS	3.00E-05	2.43E-03	4.16E-05	1.85E-03	NPPA, PENK, PTGDS, SST, TAC1
	AP2A	5.90E-04	4.78E-02	4.72E-04	2.47E-03	MIP, TAC1
	DLX2	5.90E-04	4.78E-02	4.72E-04	3.09E-03	GNRH1, MSX2
Cerebellum	MYC	<10 <sup>-5</sup>	<10 <sup>-5</sup>	<10 <sup>-5</sup>	1.09E-03	CDK4, DUSP1, IRF3, MT3, NACA, POLR2F, PTOV1, RPL18, RPL3, RPL7, RPL8, RPLP1, RPS15, RPS25, RPS9

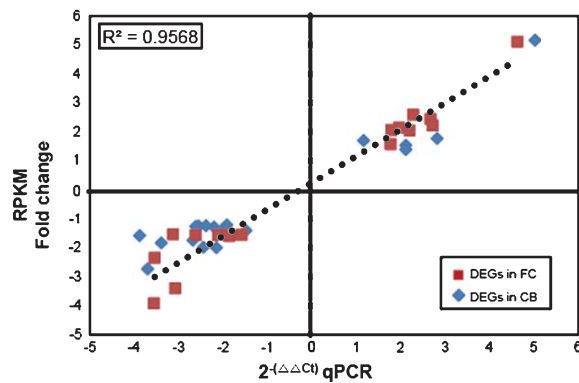
The functionally annotated differentially expressed genes (DEGs) are submitted to TFactS using default settings. Transcription factors significantly regulated with *p*-value, E value, Q value, and FDR (Benjamini-Hochberg) rate (<0.05) are represented.



Supplementary Figure 1. Canonical pathways and toxicity lists of combined DEGs of frontal cortex and cerebellum. The combined DEGs (up- and down-regulated DEGs) of frontal cortex or cerebellum were analyzed with the canonical pathways (A or C) and toxicity lists (B or D) using Ingenuity Pathway Analysis (IPA), respectively. The top 15 lists are represented with *p*-values as a bar graph and ratios as a linear graph. The black line indicates the threshold of  $-\log(p\text{-value} = 0.05)$ .



Supplementary Figure 1. *Continued.*



Supplementary Figure 2. qRT-PCR verification of RNA-Seq analysis. The x-axis represents  $2^{-\Delta\Delta Ct}$  from qRT-PCR, and the y-axis denotes fold changes of RPKM values from RNA-Seq. The squares correspond to the values of differentially expressed genes (DEGs) of frontal cortex (FC) and the rhombuses denote the values of DEGs of cerebellum (CB). The black dotted line represents the linear trend. Note that the expression profiles show a strong and significant correlation ( $R^2 = 0.9568$ ,  $p < 0.05$ ).