Supplemental Information

α1ACT is essential for survival and early cerebellar programming in a critical neonatal window

Xiaofei Du[1], Cenfu Wei[1], Daniel Parviz Hejazi Pastor[1], Eshaan R. Rao[1], Yan Li[2], Giorgio Grasselli[3,6], Jack Godfrey[1], Ann C. Palmenberg[4], Jorge Andrade[2,5], Christian Hansel[3], Christopher M. Gomez[1,*]

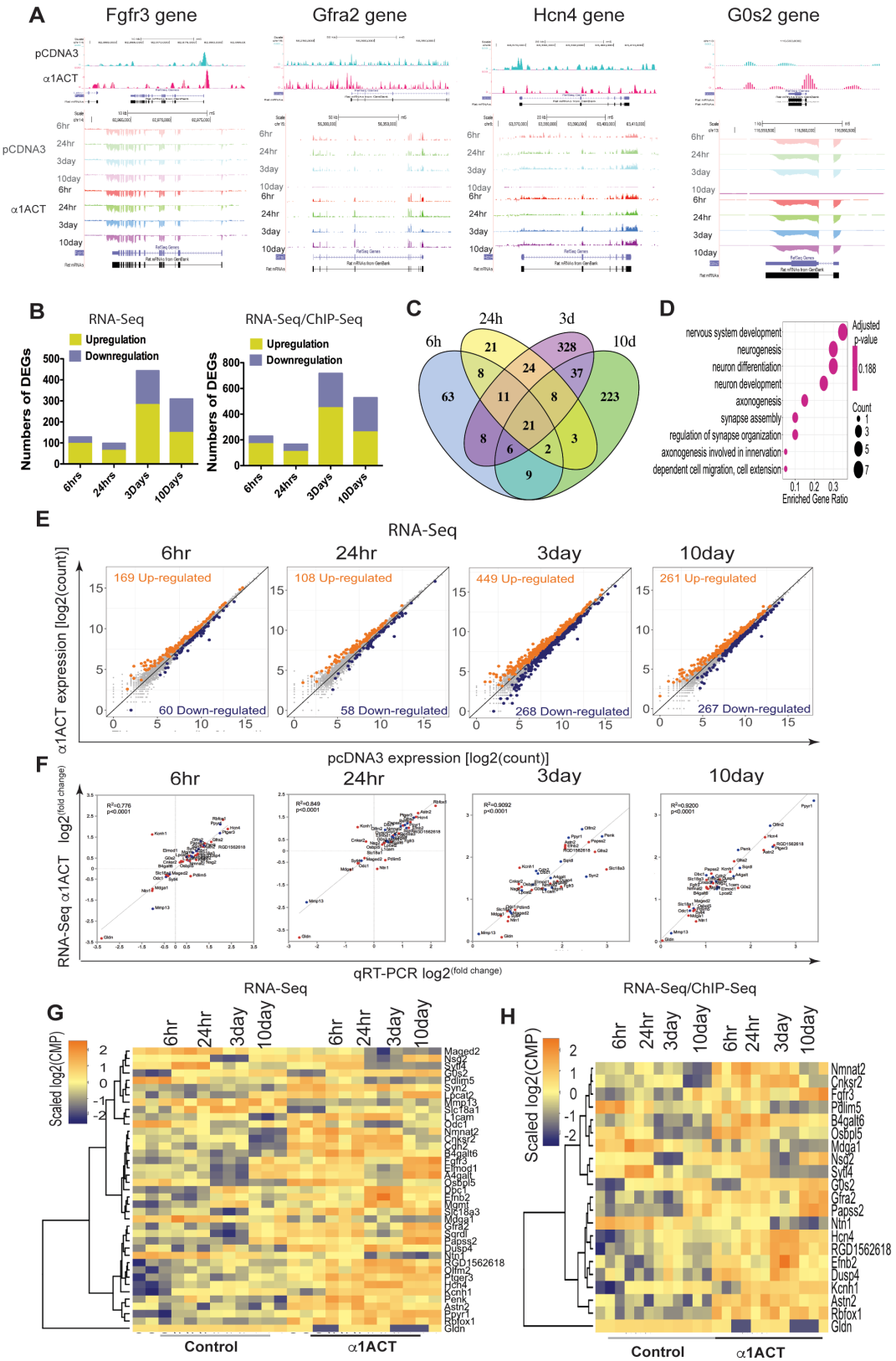

Figure S1. Integrated RNA-seq and ChIP-seq analysis of α1ACT DEGs and verification in pc12 cells, related to Figure 1.

Figure S1. Integrated RNA-seq and ChIP-seq analysis of α1ACT DEGs and verification in pc12 cells, related to Figure 1.

(A) Representative α1ACT binding profiles of 4 selected target genes (*Fgfr3, Gfra2, Hcn4,* and *G0s2*) from whole-genome ChIP-Seq analysis (upper) and corresponding RNA-Seq reads expressions (lower). In ChIP-Seq analysis peaks in blue represent pc12 cell line expressing pcDNA3 while those in red represent pc12 cell lines expressing α1ACT. In RNA-seq analysis pc12- pcDNA3 peaks are displayed within dashed rectangle above and pc12-α1ACT peaks displayed within solid line rectangle below, with all 3 biological replicates overlaid with each other.
(B) Left Differentially-expressed genes (DEGs) from RNA-Seq as days in culture. Right α1ACT-targeted DEGs as days in culture.
(C) RNA-seq/ChIP-seq integration analysis. Venn diagrams of potential putative targeted genes of α1ACT across 6hr, 24hr, 3d, and 10d. There are 21 persistent DEGs across the 4 time points.
(D) Top enriched gene ontologies GOs based on 21 commonly identified potentially α1ACT-regulated DEGs.
(E) Scatter plots of RNA-seq DEGs in log2 normalized count per million (CPM) expression values in pc12 cell lines expressing α1ACT compared to samples expressing pcDNA3 at 4 time points respectively.
(F) Fold changes from RNA-Seq compared to fold changes from qRT-PCR for 39 RNA-Seq identified persistent DEGs, where genes marked in red are those also identified as α1ACT target genes using ChIP-Seq.
(G) The heat map of 1272 RNA-Seq DEGs (rows) across the 4 time points (columns) in the pc12 cell lines expressed with α1ACT and pcDNA3 control samples, where 3 replicates were used in the experiment.
(H) Heat map of log2 normalized CPM expression values of 21 persistent DEGs apparently regulated by α1ACT across 4 time points in biological replicates of pc12 cell line samples expressing α1ACT and EV. Gene dendrogram tree drawn based on hierarchical clustering.

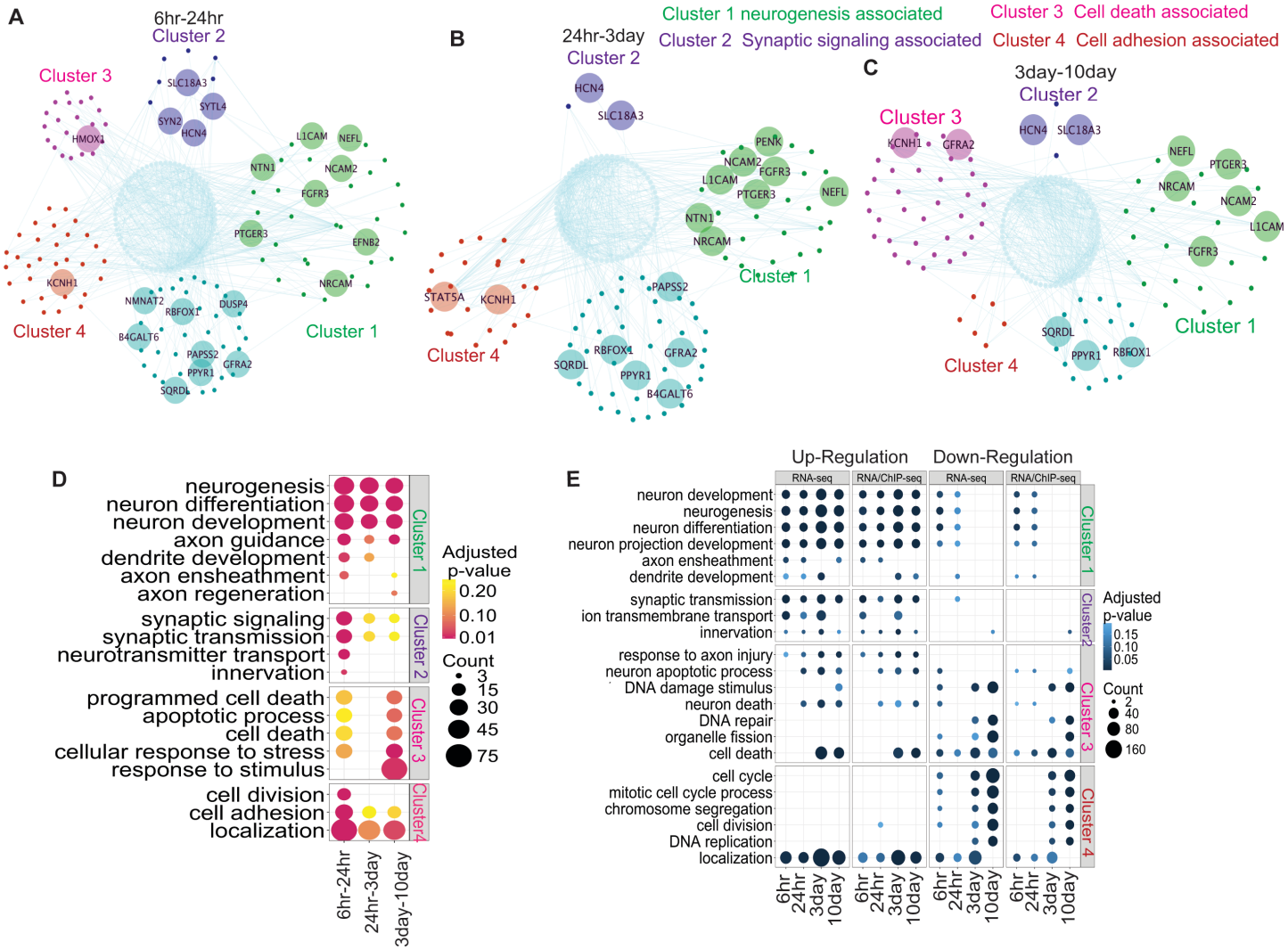

Figure S2. Time-dependent regulation of neurodevelopment and neuronal differentiation clusters by α1ACT, related to Figure 1.

(A, B, and C) Functional interaction network visualization of top modules/clusters of highly correlated DEGs across time point from 6 to 24hr, 24hr to 3d, and 3d to 10d, where genes enriched in top selected GO in D) with respect to different GO clusters are highlighted with corresponding colors. The aqua green category represents uncategorized genes.
(D) Top enriched GOs based on RNA-Seq identified DEGs across time point from 6 to 24hr, 24hr to 3d, and 3d to 10d.
(E) Top enriched GOs based on RNA-Seq identified DEGs and ChIP-Seq/RNA-Seq integrated genes at 4 different time points, where up- and down- indicates dynamic transcriptome regulation by α1ACT.

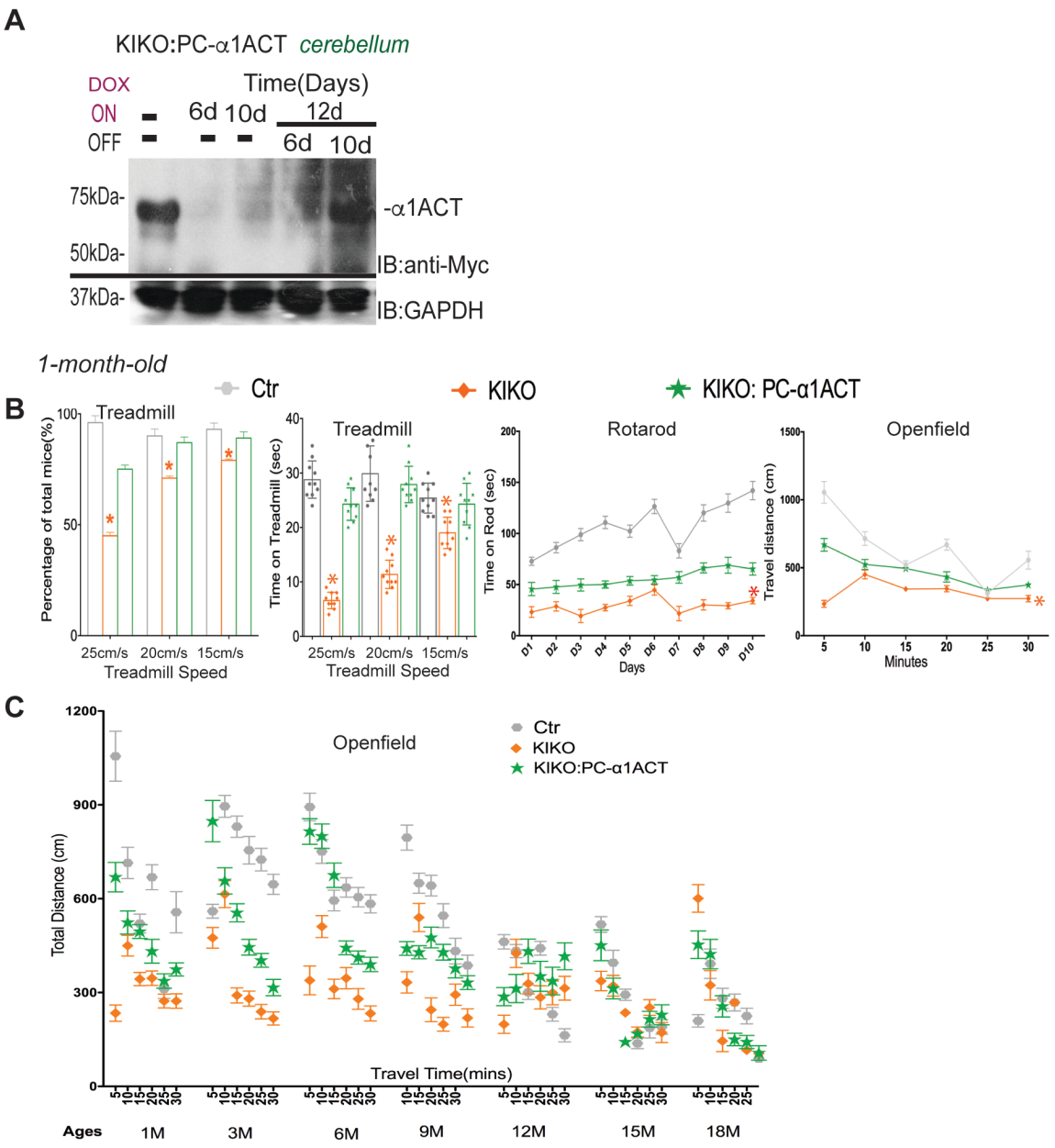

Figure S3. Reintroduction of α1ACT to Purkinje cells restores survival, improves motor function, and rescues normal growth of KIKO mice, related to Figure 2.

Figure S3. Reintroduction of α1ACT in Purkinje cells restores survival, improves motor function, and recovers normal growth of KIKO mice, related to Figure 2.

(A) The expression of α1ACT is inhibited by 6 days of doxycycline (Dox) treatment, but it is recovered after discontinuation of Dox treatment for 10 Days. Cytoplasmic proteins were extracted from cerebellum and were used to assess the expression of α1ACT by western blot.
(B) Left Motor impairment of KIKO mice on treadmill is rescued by α1ACT expression. At 1 month, KIKO:PC-α1ACT mice had significantly improved function in gait speed and time spent on treadmill compared to KIKO mice (n=100,p<0.001). Middle and Right Impaired rotarod and openfield performance of KIKO mice at 1 month is rescued by α1ACT expression. (n=30, p<0.001.

(C) Motor impairment of KIKO mice in open field is corrected by expression of α1ACT. Open field recordings were performed on littermate Ctr, KIKO, KIKO:PC-α1ACT mice at different ages. The differences in motor function were more prominent between KIKO and KIKO:PC-α1ACT mice aged between 1-month and 9-months. There was no significant motor function difference between Ctr and KIKO:PC-α1ACT mice.
Values are represented as mean±SEM. *p<0.05, **p<0.01; ***p<0.001. (n=30, P<0.001).

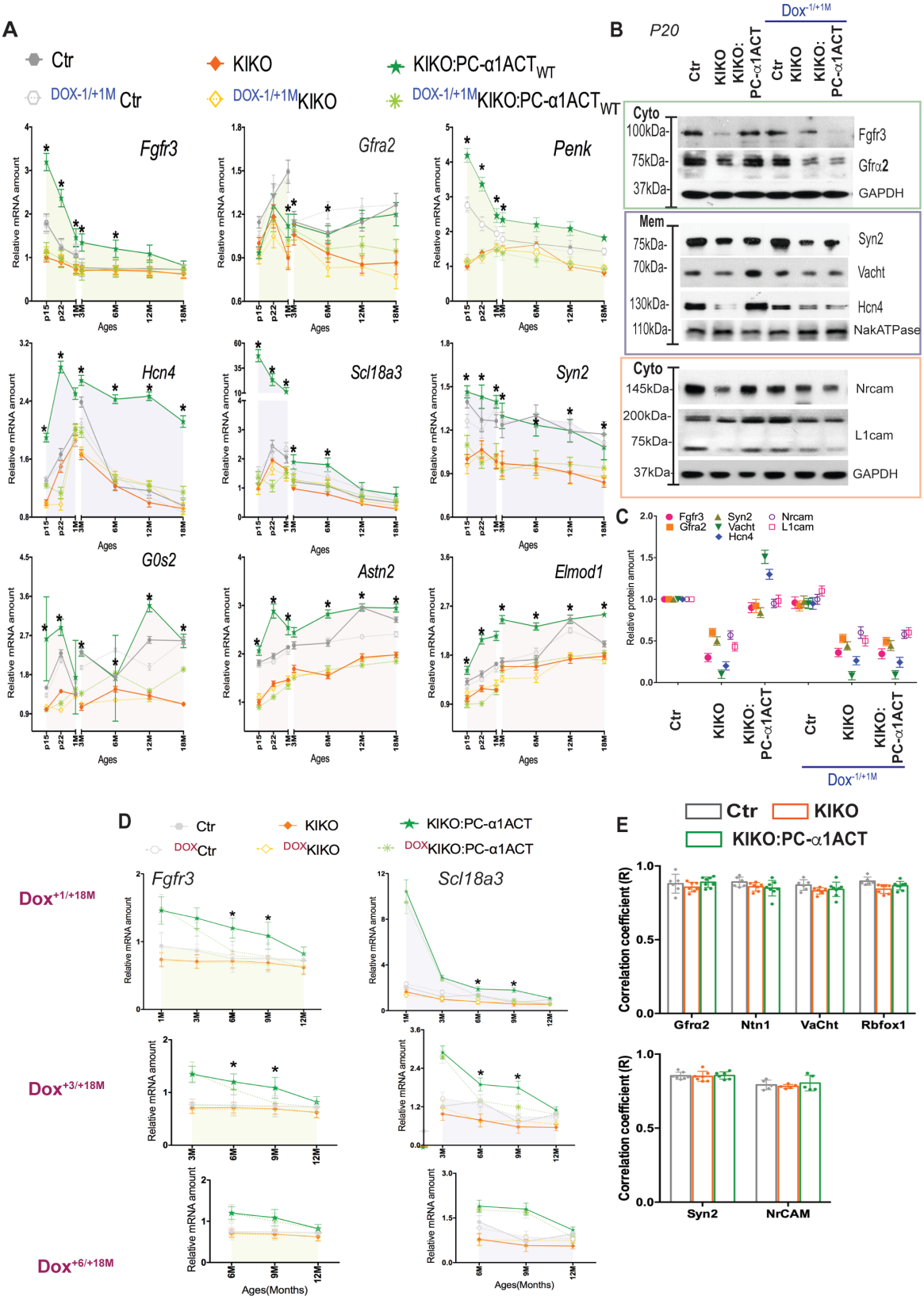

Figure S4. α1ACT-regulated DEG mRNAs and proteins were restored by expressing α1ACT in cerebellum of KIKO mouse during perinatal period, related to Figure 4.

Figure S4. α1ACT-regulated DEG mRNAs and proteins were restored by expressing α1ACT in cerebellum of KIKO mouse during perinatal period, related to Figure 4.
(A) Expression of α1ACT target genes: *Fgfr3*, *Gfra2*, *Penk*, *Hcn4*, *Scl18a3*, *Syn2*, *G0s2*, *Astn2,* and *Elmod1* were determined comparing between Ctr, KIKO and KIKO:PC-α1ACT mice with/without prenatal Dox treatment at indicated ages (n=10) Normalized to the mRNA of KIKO mice at p15.
(B and C) Western blot (B) and Quantitation (C) in cerebellum of Ctr, KIKO and KIKO:PC- α1ACT mice with and without prenatal DOX treatment at P20 day old for Fgfr3, Gfra2, Hcn4, Scl18a3, Syn2, Nrcam, L1cam and G0s2 with normalization to GAPDH (Cyt) and NaKATPase (Mem) (n=10).
(D) α1ACT impact on cerebellar gene expression wanes with age in mouse. Representative target gene Fgfr3 and Scl18a3 expression in the cerebellum of mice at the different ages. Expression was determined by qRT-PCR comparing between Ctr, KIKO and KIKO:PC-α1ACT mice with/without different DOX treatment starting time +1/+18M, +3/+18M, and +6/+18M at indicated ages (n=10).
(E) Correlation coefficient of target gene protein expression with PC marker calbindin (upper) and climbing fiber marker vGlut2 (lower).
Values are represented as mean±SEM. *p<0.05, **p<0.01; ***p<0.001.

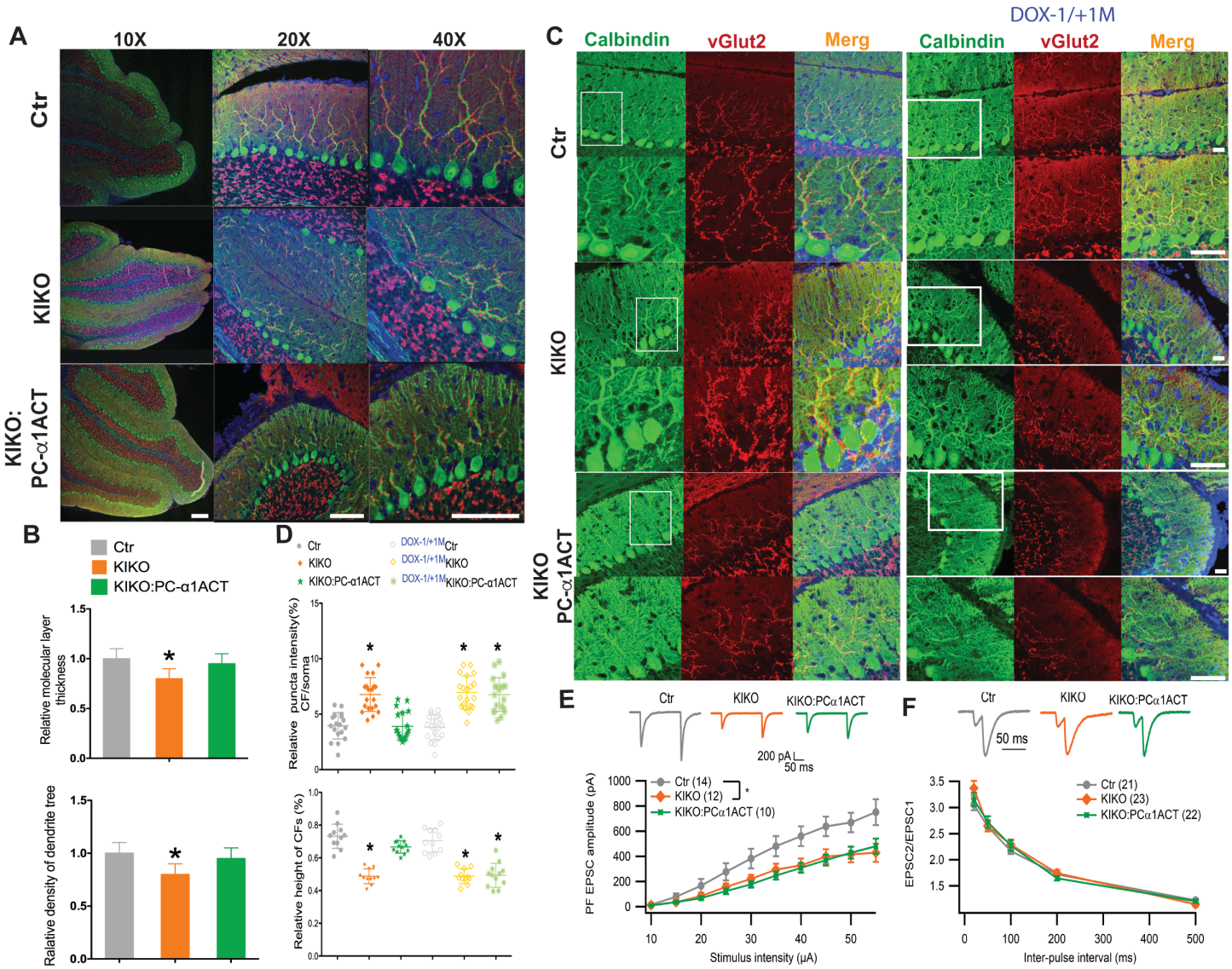

Figure S5. Absence of α1ACT in cerebellum of KIKO mice impairs cerebellar Purkinje cell morphology and electrophysiology, related to Figure 5.

Figure S5. Absence of α1ACT in cerebellum of KIKO mice impairs cerebellar Purkinje cell morphology and electrophysiology, related to Figure 5.

(A) Calbindin and vGlut2 staining of sagittal cerebellar sections from Ctr, KIKO, KIKO:PC-α1ACT littermates at p20. The scale bar represents 100μm.

(B) Quantification of the relative height of dendritic tree (upper), and the density of the PC dendritic tree (lower) were diminished in KIKO, but restored in KIKO:PC-α1ACT mice.

(C) Representative immunostaining of CFs and PC dendrites using anti-vGlut2 (red) and anti-calbindin (green) antibodies in Ctr, KIKO, and KIKO:PC-α1ACT mice with/without DOX-1/+1M at age of 17 days. The scale bar represents 25μm.

(D) Quantification of CF synapses. Immature CF synaptic contacts of KIKO mice are normalized by α1ACT at p17. Bar graphs show the relative intensity of CF puncta in PC soma (left) and the relative height of CFs (right) in KIKO mice compared to those in Ctr and KIKO:PC-α1ACT mice with/without DOX-1/+1M at p17($n=10, p<0.05$).

(E) Mean values and representative traces of PF innervation (PF-EPSC at increasing intensities of stimulation; traces at 55 μA of intensity).

(F) Paired-pulse ratio of PF-EPSC at increasing inter-pulse intervals (traces at 20 ms);

Values are represented as mean±SEM. *$p<0.05$, **$p<0.01$; ***$p<0.001$

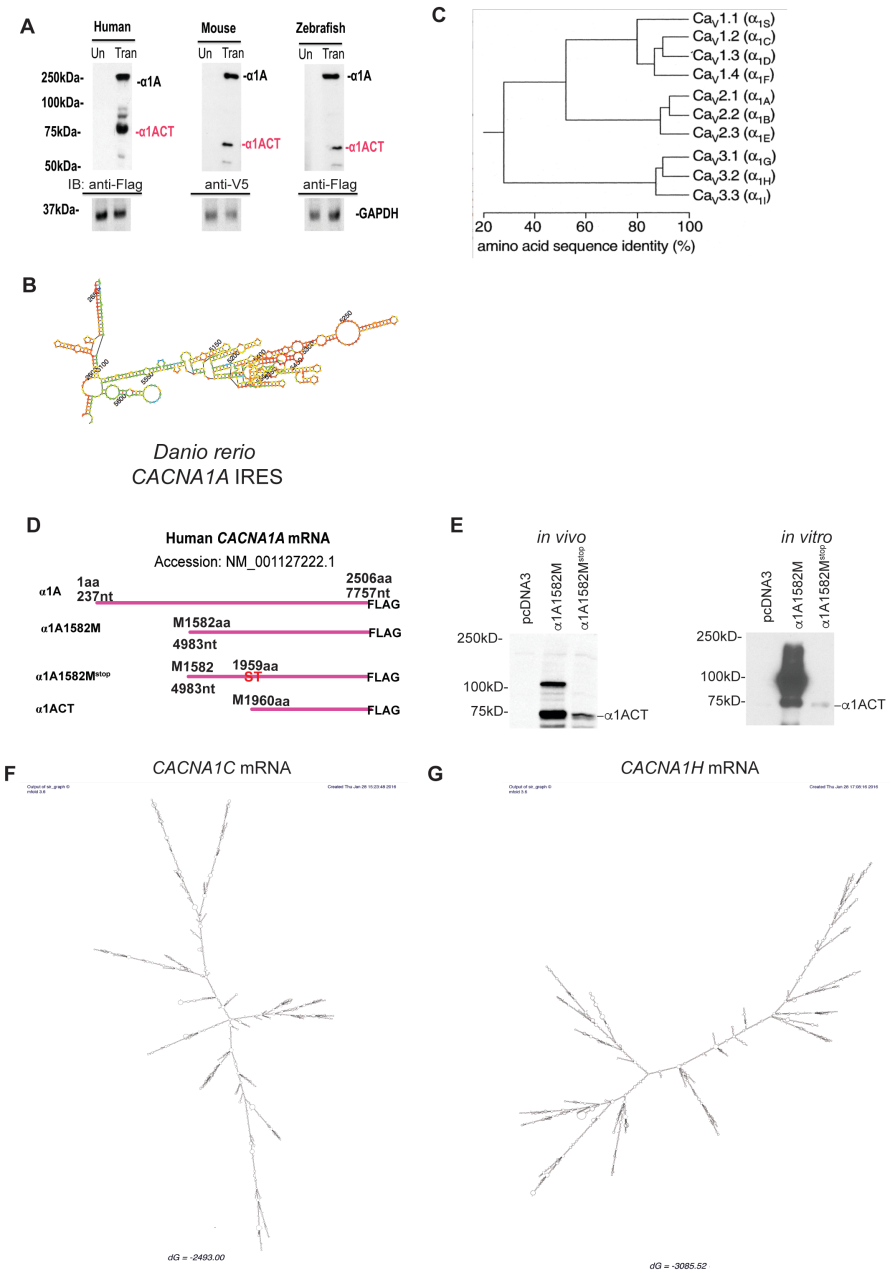

Figure S6. α1ACT is conserved from Zebrafish to Human and secondary proteins in *CACNA1C*, *CACNA1H* genes, related to Figure 6.

(A) Western blot analysis of α1ACT from mouse, zebra fish and human.
(B) M-fold analysis for the secondary structure of zebrafish *CACNA1A* mRNA.
(C) VGCC phylogenetic tree.
(D) Schematic representation of the constructs with *CACNA1A* IRES plus sequence of α1ACT and a stop codon before N-terminus of α1ACT.
(E) Left *in vivo,* Western blot analysis of HEK293 cells transiently transfected with truncated C-terminally Flag tagged α1A. Right *in-vitro* transcription and translation of truncated C-terminally Flag tagged α1A, which generates the C-terminal proteins, α1ACT.
(F and G) Secondary structure of *CACNA1C* and *CACNA1H* mRNA by M-Fold.

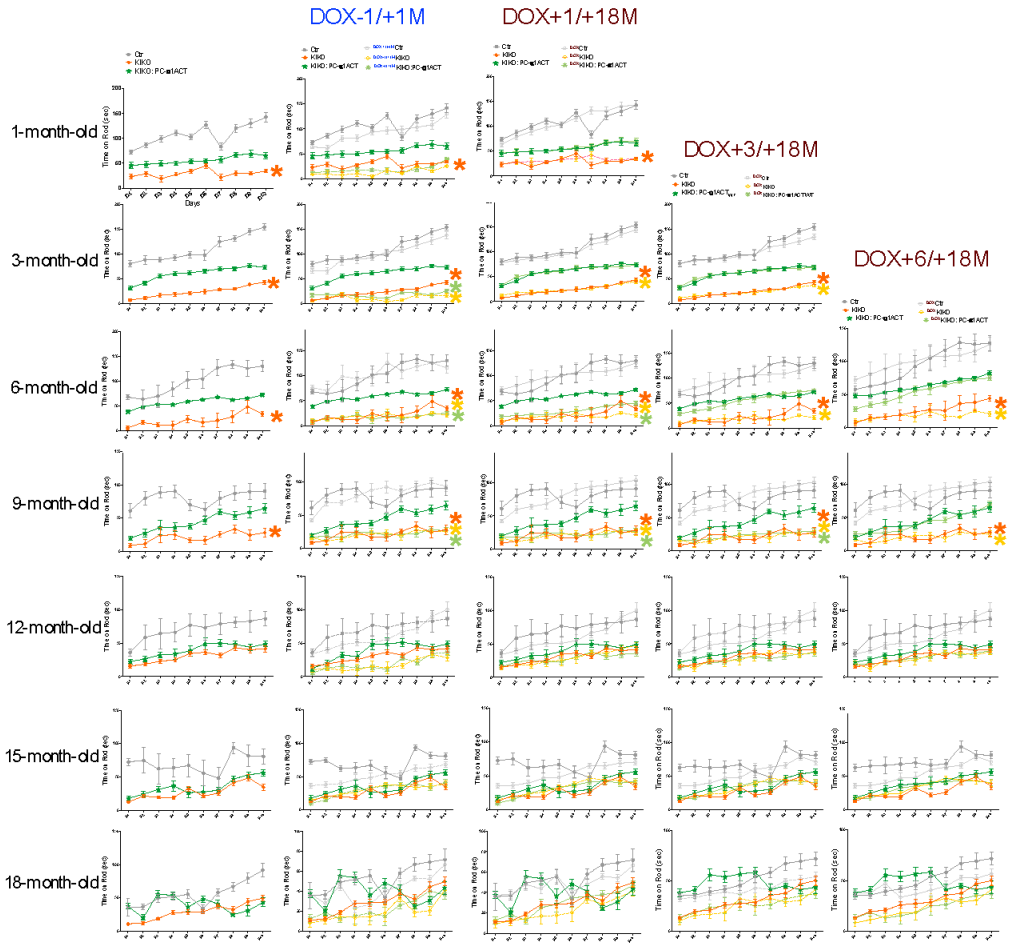

Figure S7. Rotarod data of indicated treatment groups of mice at different ages, related to Figure 3.

Values are represented as mean±SEM. *p<0.05, **p<0.01; ***p<0.001.

Table S1. Fold changes of RNA-Seq and fold changes of qRT-PCR in pc12 cells for 39 RNA-Seq identified persistent DEGs, related to Figure 1.

PC12 Cells	6hr		24hr		3d		10d	
	qRT-PCR	RNA-seq	RT-PCR	RNA-seq	RT-PCR	RNA-seq	RT-PCR	RNA-seq
A4galt	0.75	0.5702595	0.6817	0.5922017	1.7421	1.505421	1.621	1.548662
Astn2	1.237	1.043549	1.5481	1.674815	2.0679	2.289126	2.29	2.169615
B4galt6	0.267	0.3362908	0.4895	0.3919837	1.7345	1.370645	1.050904	1.250904
Cdh2	0.67	0.6137105	0.7463	0.7364938	1.4179	1.674179	1.16	1.527916
Cnksr2	0.3312	0.3391275	-0.2786	0.4165389	1.03141	1.363141	1.25	1.312725
Dbc1	0.93512	0.8359292	0.9398	1.099338	1.4583	1.612583	1.0143	1.573443
Dusp4	0.894	0.6316289	0.6649	0.6786954	1.8914	1.428914	1.3152	1.474305
Efnb2	0.9733	0.6684167	0.7352	0.7439235	2.0643	2.242586	1.14005	1.414005
Elmod1	0.489	0.5582946	0.6374	0.629837	1.346344	1.346344	1.288522	1.288522
Fgfr3	0.932	0.4180547	0.604	0.3967142	1.97665	1.297665	1.0165	1.293055
G0s2	0.26	0.3598383	0.421	0.4499513	1.321143	1.221143	1.72055	1.272055
Gfra2	1.6	1.273488	1.279069	1.279069	2.7308	2.127608	1.63272	1.903272
Gldn	-3.3	-3.320905	-2.6197	-3.8281	0.649	0.09649999	0.036	0.0240986 4
Hcn4	2.34	1.891413	1.4019	1.471019	3.68398	3.268398	2.32	2.473258
Kcnh1	-1.036	1.626352	-0.573	1.051973	1.049209	1.749209	1.54455	1.654455
L1cam	0.579	0.3806072	0.49393	0.390393	1.626	1.23526	1.263	1.296842
Lpcat2	0.51023	0.5610234	0.4309	0.4313009	1.09875	1.209875	1.2482	1.248268
Maged2	-0.2573273	-0.2673273	-0.44086	-0.4308786	0.8637	0.6708637	0.7332	0.7833229
Mdga1	-1.02	-0.9640492	-0.8236	-0.8309336	0.6302	0.6072302	0.6173662	0.6173662
Mgmt	0.6114	0.6131507	0.4583	0.446258	1.77017	1.277017	1.12947	1.412947
Mmp13	-1.012316	-1.912316	-2.3643	-2.268754	0.136	0.1750603	0.2323	0.1989323
Nmnat2	0.6446	0.3666446	0.3517	0.3628397	1.54878	1.254878	1.0234	1.266934
Nsg2	0.5152	0.4142965	0.3652	0.352953	1.0189	1.250189	1.164	1.383754
Ntn1	-1.0369	-1.053992	0.1154	-0.7931574	0.7869	0.4786964	0.785123	0.4785123
Odc1	-0.42	-0.3918093	-0.5665	-0.547988	0.8367	0.7086552	0.6478	0.7339275
Olfm2	1.1345	1.305065	0.392	0.9319932	2.443	2.665744	2.434	2.746516

Osbpl5	0.1962161	0.2962161	0.2924	0.2842042	1.095192	1.295192	1.10368	1.310368
Papss2	1.13	1.105238	1.0243	1.12465	2.59186	2.359186	1.12521	1.612521
Pdlim5	0.678	-0.3583117	0.3976	-0.4074455	0.94885	0.7494885	0.7544489	0.7544489
Penk	1.27	1.098139	1.085	1.148067	2.89	2.495001	1.72165	2.172165
Ppyr1	2.00389	2.109389	1.1738	1.222278	2.143	2.465131	3.40398	3.340398
Ptger3	1.989172	1.689172	1.479	1.503262	3.68632	3.088662	2.49215	2.249215
Rbfox1	2.128435	2.328435	2.1637	1.994617	7.765	7.524765	4.302065	5.302065
RGD1562618	1.306012	1.206012	1.221	1.022007	2.06706	2.206706	2.523	2.285273
Slc18a1	-0.4080302	-0.3080302	-0.3314	-0.3155318	0.7753	0.7200741	0.6294	0.8266777
Slc18a3	1	0.713668	0.8721	0.7695011	2.9893	1.709893	1.0182	1.461182
Sqrdl	1.0296	0.9296004	1.14086	1.014086	1.96785	1.896785	1.7598	1.74598
Syn2	0.78	0.7608895	0.75	0.7172784	2.537345	1.537345	1.23041	1.423041
Sytl4	-0.324368	-0.3477368	-0.5402	-0.5392402	0.803041	0.5803041	0.7268808	0.7268808

Table S2. Primers used in paper, related to STAR Methods.

name	Sequence
C1177M	aatGGTACCATCGCCTTCTTCATGATGAACATCTTCGTG
C1386M	aatGGTACCCGCCTGTTCCGGGTCATGCGTCTGGTGAAGCTG
C1731	aatGGTACCCGACGGGCCATCTCTGGAGATCTCACC
KpnIRpCMV6R	ttaggtaccccggTCACTTGTCGTCGTCGTCCTTGT
H1343M	aatAAGCTTgcggagatgatggtgaaggtggtg
H1495M	aatAAGCTTggccaggccctgatgtcgctg
H1924M	aatAAGCTTcaaggtgtccgtgtccaggatgctctcgct
FlagXbaIR	atacTCTAGATCACTTGTCGTCGTCGTCCTTGT
HumanC5883MSTF	ggaaaggcacgttccgtagtgtgaggatctggag
HumanC5883MSTR	ctccagatcctcacactacggaacgtgcctttcc
3309STHumanCF	ttggcatccagtccagtgcatagaatgtcgtgaagatcttgcg,
3309STHumanCR	cgcaagatcttcacgacattctatgcactggactggatgccaa
5235STHumanCF	gctggaccaggtgtagccccctgcaggt
5235STHumanCR	acctgcaggggggctacacctggtccagc
6105dblSTHumanHF	ctgcaggaggtggagtagtagacctatggggccg
6105dblSTHumanHR	cggccccataggtctactactccacctcctgcag
5898STHumanHF	gatcgagctggagtaggcgcagggcccc
5898STHumanHR	ggggccctgcgcctactccagctcgatc
C1177M	aatGGTACCATCGCCTTCTTCATGATGAACATCTTCGTG
C1386M	aatGGTACCCGCCTGTTCCGGGTCATGCGTCTGGTGAAGCTG
Efnb2F	actgcttagtggccgc
Efnb2R	ctatagatccaggaca
NrcamF	gaccgtgcagaaacggaga
NrcamR	tcactggagagcagcacaa
Fgfr3F	gctggttagtttgata
Fgfr3R	ggtacaaagcctgacagt
Rbfox1F	ccagacatgtagtacacaga
Rbfox1R	gagactggggctccaagca
Gfra2F	ggctgatggtgaacatcctg
Gfra2R	gagtaaccatcacacagagt

Table S3. Probes used in paper, related to STAR Methods.

Taqman probes	catalog number	gene name
	Mm01241874_m1	cacna1a FAM
	Mm0127403_g1	cacna1a FAM
	Mm00432190_m1	cacna1a
	Mm00484537_g1	G0s2
	Mm00502443_m1	Sqrd1
	Mm00615393_m1	Nmnat2
	Mm00491465_s1	Slc18a3
	Mm00500896_m1	Ntn1
	Mm00433586_m1	Gfra2 FAM
	Mm00433294_m1	Fgfr3
	Mm99999915_g1	Gapdh
	Mm00625163_m1	Elmod1
	Mm00625046_m1	Cnksr2
	Mm01176086_m1	Hcn4
	Mm00476554_m1	Nsg2
	Mm00449780_m1	Syn2
	Mm03307804_pri	mmu-mir-485
	Mm00438670_m1	FEfnb2
	Mm01303346_m1	Mdga1 FAM
	Mm01312650_m1	Pdlim5 FAM
	Mm01197820_m1	Papss2 FAM
	Mm01349181_m1	Rab3a
	Mm00463805_m1	Sv2b
	Mm00723761_m1	Dusp4
	Mm00616548_m1	Gldn
	Mm01316769_m1	Kcnh1
	Mm00493049_m1	L1cam
	Hs01588138_m1	CACNA1A FAM
	Hs01588146_m1	CACNA1A FAM
	Hs01579431_m1	CACNA1A
	Hs01579433_g1	CACNA1A FAM
	Hs00179829_m1	FGFR3
	Hs00176393_m1	GFRA2
	Hs00975492_m1	HCN4
	Hs00268179_s1	SLC18A3
	Hs01024740_m1	ASTN2
	Hs00274782_s1	GOS2
	Hs00989928_m1	PAPSS2
	Hs00924151_m1	NTN1
	Hs00380505_m1	ELMOD1

	Hs00322752_m1	Nmnat2
	Hs00738960_m1	GLDN
	Hs01109748_m1	L1CAM
	Hs99999903_m1	ACTB
	Mm01268569_m1	Kcnma1
	Hs01119504_m1	Kcnma1
	Hs99999901_s1	18S

Table S4. Cerebellar tissue blocks, related to Figure 4.

Number	Age	Sex	Cerebellar pathology	Ethnicity
1805	Term/38 weeks gestation	Male	none	unknown
1681	Term/36 weeks gestation	Male	none	unknown
15607	40 weeks gestation	Female	none	unknown
8715	23 years	Female	none	African American
6715	24 years	Male	none	African American
4516	20 years	Male	none	African American
8716	50 years	Female	none	African American
1646	50 years	Male	none	Caucasian
15016	53 years	Male	none	Caucasian
46356	80 years	Male	none	unknown
9763	81 years	Female	none	unknown
13110	83 years	Male	none	unknown

Supplemental Videos, related to Figure 2.

Movie S1.

Motor abnormalities are pervasive at in KIKO mice at p18 and at p30, related to Figure 2.

At p18, KIKO mouse (present at lower left corner at the start) continues to display decreased mobility and a wide ataxic gait when compared to an age-matched wildtype mouse. At p30, KIKO mouse (present in middle right at the start) continues to display decreased mobility and a wide ataxic gait when compared to an age-matched wildtype mouse.

Movie S2

Rescue effects of reintroduction of α1ACT (KIKO:PC-α1ACT) at p18 and at p30, related to Figure 2.

At p18, KIKO rescue mouse (KIKO:PC-α1ACT) introduced in the video as the third mouse still has a similar phenotype to the wildtype mouse and improved gait stance and mobility compared to KIKO. At p30, KIKO rescue mouse (KIKO:PC-α1ACT) introduced in the video as the third mouse still has a similar phenotype to the wildtype mouse and improved gait stance and mobility compared to KIKO.

Movie S3

Perinatal (Dox-1/+1M) Dox exposure in KIKO:PC-α1ACT leads to motor abnormalities at p18 and at p30, related to Figure 2.

At p18, Perinatal Dox induced inhibition of α1ACT in rescue mouse (fourth mouse) reverses the rescue phenotype and reintroduces the motor abnormalities of KIKO mice. At p30, Perinatal Dox induced inhibition of α1ACT in rescue mouse (fourth mouse) reverses the rescue phenotype and reintroduces the motor abnormalities of KIKO mice.