

Supplemental Information

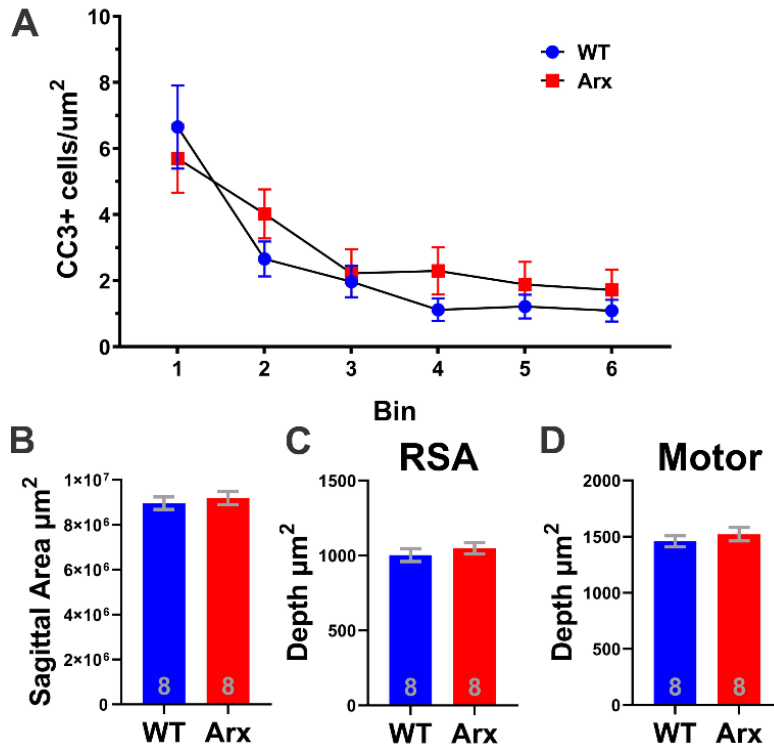


Fig. S1. *Arx*^{(GCG)10+7} mutants do not exhibit differences in CC3 distribution or neocortical size compared to WT littermates at P7. (A) Distribution of CC3+ density in Arx mutants and WT littermates in 6 laminar bins (1 most superficial, 6 most deep bin). There was no difference between genotypes ($F(1,96)=1.681$, $P=0.1979$), a significant effect of bin ($F(5,96)=13.81$, $P<0.0001$), but no interaction between bin and genotype ($F(5,96)=0.7028$, $P=0.6226$) (Two Way ANOVA). $N=10$ WT, 8 Arx. Mean and SEM are displayed. (B) Area (μm^2) of WT and *Arx*^{(GCG)10+7} mutant sagittal slices ($P=0.5812$, Unpaired t test). (C-D) Depth (μm) of two different cortical regions showing no difference between genotypes: (C) RSA cortex ($P=0.4316$, Unpaired t test) and (D) Motor cortex ($P=0.4321$, Unpaired t test) cortex. $N=8$ WT, 8 Arx. Mean and SEM are displayed.

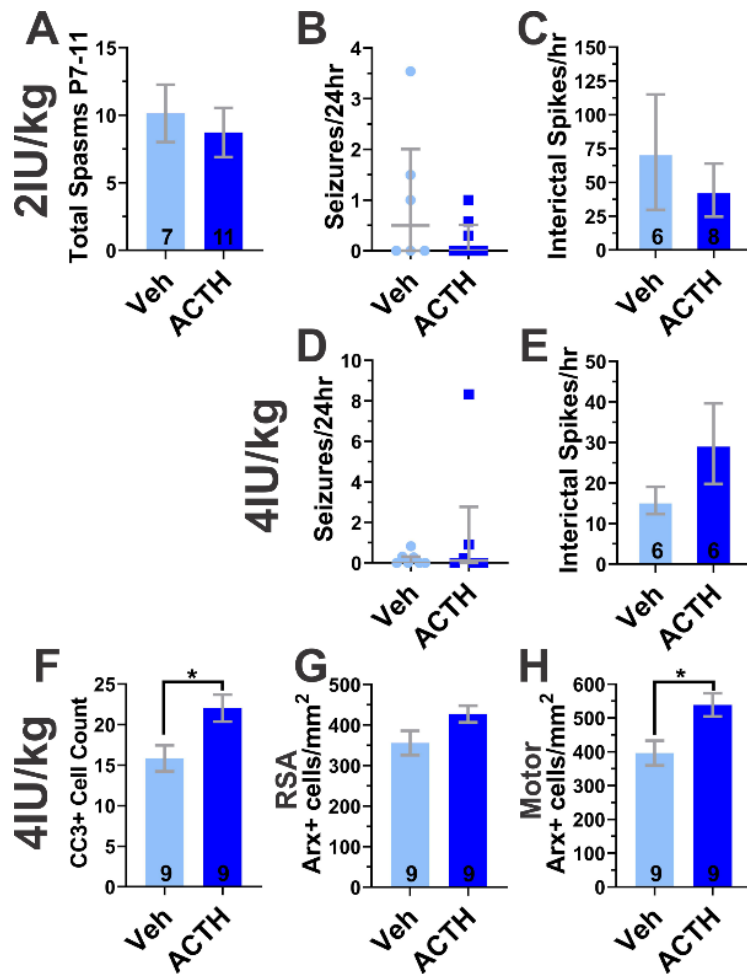


Fig. S2. Neonatal ACTH(1-24) treatment modifies cellular pathology but has no effect on neonatal spasms and electrographic phenotypes in adult *Arx^{(GCG)10+7}*. Effect of daily P3-10 ACTH(1-24) treatment at (A-C) 2IU/kg/day and (D-H) 4IU/kg/day compared to saline vehicle (Veh) control injections. (A) Average cumulative P7-11 spasms ($p=0.6253$, Unpaired T Test). (B&D) Average daily seizure frequency (seizures/24hr) from weekly P45-72 (average of 89 hours of total recording per mouse) video EEG recordings following neonatal 2IU/kg/day or 4IU/kg/day ACTH(1-24) treatment. (B) 2IU/kg/day ACTH(1-24) treatment ($p=0.3287$, Mann Whitney Test) (N=6 Veh, 8 ACTH(1-24)). (D) 4IU/kg/day ACTH(1-24) treatment ($p=0.8701$, Mann Whitney Test) (N=6 Veh, 6 ACTH(1-24)). (C&E) Average interictal spike frequency (interictal spikes/hr) following neonatal 2IU/kg/day or 4IU/kg/day ACTH(1-24) treatment. (C)

2IU/kg/day ACTH(1-24) treatment ($p=0.4908$, Mann Whitney Test). (E) 4IU/kg/day ACTH(1-24) treatment ($p=0.3939$, Mann Whitney Test). **(F-H)** Effect of P3-7 4IU/kg/day ACTH(1-24) treatment compared to vehicle (Veh) treatment (saline) on CC3+ cell counts and Arx+ cell density in $Arx^{(GCG)10+7}$ at P7. **(F)** Mean CC3+ counts in neocortex with ACTH(1-24) treatment ($p=0.0166$, Unpaired T Test) **(G-H)** Mean Arx+ cell density (Arx+ cells/mm²) with ACTH(1-24) treatment in (G) retrosplenial agranular (RSA) region ($p=0.0673$, Unpaired T Test) and (H) motor cortex ($p=0.0117$, Unpaired T Test). Mean and SEM are displayed. With the exception of B&D, all Ns are displayed on graph.

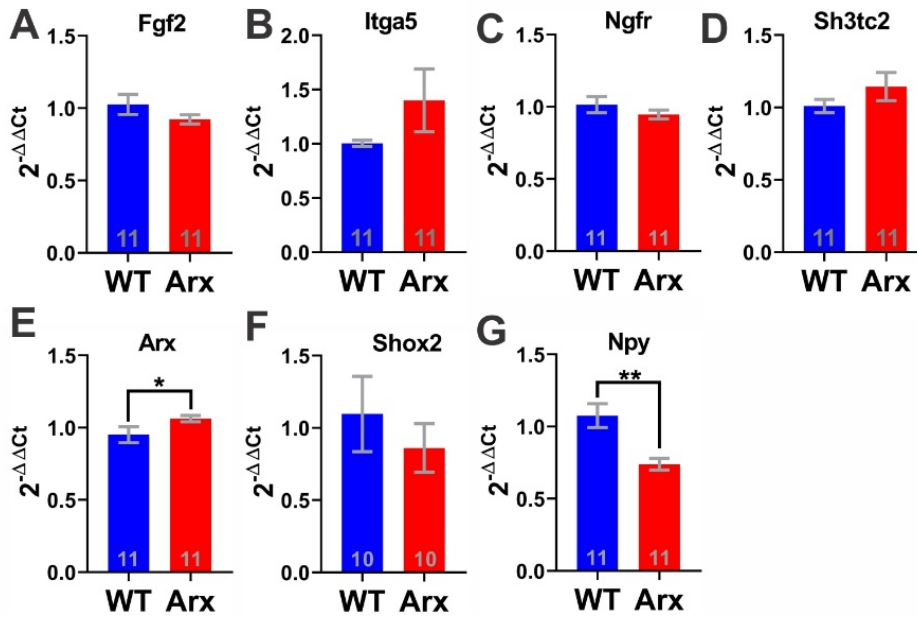


Fig. S3. Validation of Nanostring candidates using qPCR did not reveal mRNA expression changes in *Fgf2*, *Itga5*, *Ngfr*, *Sh3tc2*, and *Shox2* but did reveal changes in *Arx* and *Npy* expression in *Arx*^{(GCG)10+7} P7 cortex compared to WT littermates. (A-G) Average expression ($2^{-\Delta\Delta Ct}$) of each mRNA candidate is displayed. (A) *Fgf2* ($p=0.1330$, Mann Whitney Test) (B) *Itga5* ($p=0.4385$, Mann Whitney Test) (C) *Ngfr* ($p=0.3316$, Mann Whitney Test) (D) *Sh3tc2* ($p=0.3653$, Mann Whitney Test) (E) *Arx* ($p=0.0400$, Mann Whitney Test) (F) *Shox2* ($p=0.6842$, Mann Whitney Test) (G) *Npy* ($p=0.0019$, Mann Whitney Test). Mean and SEMs are displayed. Ns are displayed on graph.

<i>Ccl12</i>	<i>Cx3cr1</i>	<i>Fasl</i>	<i>Il4ra</i>	<i>Prl</i>
<i>Ccl5</i>	<i>Cxcl10</i>	<i>Flt1</i>	<i>Il6</i>	<i>Tgfb1</i>
<i>Ccr2</i>	<i>Cxcl11</i>	<i>Flt4</i>	<i>Il6ra</i>	<i>Tgfb2</i>
<i>Ccr5</i>	<i>Cxcl12</i>	<i>Hgf</i>	<i>Inhbb</i>	<i>Tnf</i>
<i>Cd40</i>	<i>Cxcl16</i>	<i>Il10</i>	<i>Lif</i>	<i>Tnfrsf10b</i>
<i>Cntf</i>	<i>Cxcr4</i>	<i>Il10ra</i>	<i>Ltbr</i>	<i>Tnfrsf11b</i>
<i>Csf1</i>	<i>Egf</i>	<i>Il13ra1</i>	<i>Ngfr</i>	<i>Tnfrsf12a</i>
<i>Csf1r</i>	<i>Egfr</i>	<i>Il15ra</i>	<i>Osmr</i>	<i>Tnfrsf1a</i>
<i>Csf2rb</i>	<i>Epo</i>	<i>Il1b</i>	<i>Pdgfrb</i>	<i>Tnfrsf1b</i>
<i>Cx3cl1</i>	<i>Fas</i>	<i>Il1r1</i>	<i>Plekho2</i>	<i>Vegfa</i>

Table S1.

List of 50 cytokines and cytokine-related mRNA's from NCounter mouse Neuropathology panel used in Nanostring pathway analysis (See Fig. 5A).

mRNA	Correction Set	Uncorrected P-value	Corrected P-Value	Log2 fold change	Linear fold change
Ngfr-mRNA	Overlapping	0.00138	1	-1.110	0.465
Sh3tc2-mRNA	Overlapping	0.00707	1	-0.628	0.647
Fgf2-mRNA	Litter Correction	0.00454	1	-0.325	0.798
Itga5-mRNA	Overlapping	0.00205	1	-0.319	0.801
Hdac7-mRNA	Overlapping	0.00135	1	-0.173	0.887
Npy-mRNA	Batch Correction	0.00462	1	-0.171	0.888
Sirt1-mRNA	Batch Correction	0.00681	1	-0.162	0.894
Vegfa-mRNA	Overlapping	0.00583	1	-0.141	0.907
Atf4-mRNA	Overlapping	0.00878	1	-0.118	0.921
Htr1a-mRNA	Batch Correction	0.00797	1	-0.115	0.923
Acaa1a-mRNA	Batch Correction	0.00493	1	-0.093	0.937
Pnkd-mRNA	Batch Correction	0.00945	1	-0.094	0.937
Ran-mRNA	Overlapping	0.00594	1	-0.092	0.938
Ap4s1-mRNA	Overlapping	0.00354	1	-0.081	0.945
Ikbkb-mRNA	Batch Correction	0.00959	1	0.080	1.06

Table S2. Differentially expressed mRNA candidates between WT and $Arx^{(GCG)10+7}$ using Nanostring. Data was corrected for batch and litter effects and 15 differentially expressed candidates were chosen based on uncorrected P-value less than 0.01. Highlighted candidates were chosen for validation by qRT-PCR.

Gene Name	Accession ID	Probe Target Sequence
Arx	NM_007492.3	TACTGAAAATAACCCGGGAGGGGTTCTCCCAATA TGCTTAGTATTGAAGTTTAAAAATTAAGCCCAAC CTTCTCTTCTCCGGATACCCCACTTAGC
Birc6	NM_007566.3	TCGGCGCTCAGCGCTAAACCAGGTGGACAGGTGA AATGTCAGTATATTTCTGCTGTGGATAAAGTGATAT TTGTGGATGATTACGCCGTGGGCTGTAGGA
Csrnp3	NM_178634.2	AAAGGACTTTGGGTTTGTTCATGCTATTGAAAGCAGT AATGGGGGCTGACTACTCCTTTTGGTTGACCGGTG GGGCTAATCTGGTGGGAATTCTGTGGTAA
Cxcr5	NM_007551.2	ACCGTGCCTTTCTCTTAACCAAGCAGAAAGCTGAAA CCGATCTACTTTAGGTAGCTGTCTGGTTCCAACCTA ACCAGCATTGGGTGAGCCCATGTTACT
Drap1	NM_024176.1	TGCCAAAACCATGACCACGTCCCACCTGAAGCAGT GCATTGAACTCGAGCAGCAGTTTGACTTCTTGA GACTTGGTGGCATCAGTGCCTGACATGCAG
Ebf1	NM_007897.2	CATAGGGGACAATTTCTTTGATGGGTTACAGGTCAT ATTCGGTACCATGCTGGTCTGGAGTGAGTTGATAA CTCCTCATGCCATCCGAGTTCAGACACCT
Endog	NM_007931.1	GGAACAACCTTGAGAGGTACAGCCGCAGCTTGACG CGAATTACAAAATGTCTATGTCTGCACGGGGCC GCTTTTCTGCCAGGACCGAGGCTGATGG
Erbp4	NM_010154.1	TCCTGGTGATCATGGCTTTGACATTTGCTGTCTATG TCAGAAGAAAGAGCATCAAAAAGAAACGTGCTTTG AGGAGATTCTGGAGACAGAGCTGGTAGA
Esr1	NM_007956.4	ACTCGCTACTGTGCCGTGTGCAATGACTATGCCTC TGGCTACCATTATGGGGTCTGGTCTGCGAAGGCT GCAAGGCTTTCTTAAAGAGAAGCATTCAAG
Esr2	NM_010157.3	TGCTTCTTATAACTGTAGCCAGTCCATCCTACCCTT GGAGCATGGTCCCATCTATATCCCTTCTCCTATGT AGAGAGCCGTCACGAATACTCAGCCATG
Ghrh	NM_010285.2	GGATGCAGCGACACGTAGATGCCATCTTACCACC AACTACAGGAACTCCTGAGCCAGCTGTATGCCCG GAAAGTGATCCAGGACATCATGAACAAGCA
Gnmt	NM_010321.1	GGGTCATTGAAGAAGCCAACCTGGTTGACGCTGGAC AAAGATGTGCTTTCAGGAGATGGCTTTGATGCTGT CATCTGCCTTGGGAACAGTTTTGCTCACTT
Hdac4	NM_207225.1	AGACGCTGAGTACTTGGCAGCCTCAGAACGGTGG TTATGCCTATCGCAAATGAGTTTGCCCCAGATGTG GTACTGGTGTTCATCGGGCTTCGATGCTGTG
Hipk2	NM_010433.2	AGGAAGCAAGAAAGTACATTTTCAACTGTTTGGATG ATATGGCCCAGGTGAACATGACAACAGATTTGGAA GGGAGTGACATGTTAGTAGAGAAGGCAGA
Kcnma1	NM_001253361.1	CGCATGTGGTGGGCTTTCTTGGCCTCCTCCATGGT GACTTTCTTCGGGGGCTTTCATCATCTTGCTCTG GCGGACGCTCAAGTACCTGTGGACCGTTT
Kif26b	NM_001161665.1	CATGATCGGAAGGGACGACTCTATGCAGAACCTGG GCATTATTCCATGTGCTATTTCTTGGCTGTTCAAGC TTATCAATGAGCGCAAAGAGAAGACGGGG

Mef2c	NM_001170 537.1	TTCTACTACTAAAGGTATCAATGGAACATGAAGACG AGTATTTAGGCAGAAGCAAACAGGAAACCATCCTT ACAAACATGCTTACCTGCACATCTGTTT
Msx2	NM_013601. 2	CTACCCCTTCCATAGACCTGTGCTCCCCATCCCGC CTGTTGGACTCTATGCCACGCCGGTTGGATATGGC ATGTACCATCTATCCTAAGGAAGACCAGAT
Myt1l	NM_001093 775.1	ATCAGTGACAGAAGTTATGCTGAGGGGATGTCACA GCAGGACAGTAGAAATATGAACTATGTCATGCTAG GGAAGCCCATGAACAATGGACTCATGGAGA
Ndufa1 3	NM_023312. 2	CAGATTCTCCGGGAAAACCTGGAGGAGGAAGCCAT CATCATGAAGGATGTGCCCAACTGGAAGGTGGGC GAGTCTGTGTTCCATACCACACGATGGGTGC
Pcdhg a5	NM_033588. 4	CAGACTTTTCGAGCACCGTCCATATCAGCGTGACC GTTCTTGATGCAAACGATAACGCACCCTTGTTTACC CAAAGCGAATATAGGGTGAGTGTTCCGGA
Pde3a	NM_018779. 1	ATATAGGAAGAAAATGTGGCCGTATTCTGAGCCAG GTATCATACAGACTGTTTGAAGACATGGGGCTCTTT GAAGCCTTTAAATCCCGGTTAGGGAGTT
Perp	NM_022032. 4	CTTCACGATAACCCTGCTGTTAATTACATCTATAAC TGGGCCTATGGCTTCGGATGGGCGGCCACCATCAT CTTGATTGGTTGTTCCCTTCTTCTTCTGCT
Prdx5	NM_012021. 2	AAAGAAGGTGAACTTGGCAGAGCTGTTCAAGGGCA AGAAAGGTGTTTTGTTTGGAGTCCCTGGGGCATT ACACCTGGCTGTTCTAAGACCCACCTGCCT
Samd5	NM_177271. 3	GATGAGATGCGGAAGACTGGAAAGGGGCGTATTTA GGACCTTCTTTCACAAAGGGAAGTGGATGGTGACT CTGCAAACACTTATCAGCTTAACGTTTTCT
Sfn	NM_018754. 2	AATCTGATTTGGTAATCCAAGACGCTCCTGCAATGC AGCCAGCCCTGAACTGCAGGGGGCAGTCTGGAGC CGAAAGGTGCCTTTGCAGGTGGGACCTGCG
Shox2	NM_013665. 1	ATTTTACCCTGGAACAACCTCAACGAGCTGGAGAGG CTTTTCGATGAGACCCACTATCCAGACGCTTTCATG CGCGAGGAATTGAGCCAGCGACTGGGGCT
Twist1	NM_011658. 2	AATGGACAGTCTAGAGACTCTGGAGCTGGATAACT AAAAATAAATCTATATGACAAAGATTTTCATGGAAAT TAGAAGAGCAGAGACCAAATTCACAAGA
Zfp369	NM_178364. 5	GTTACAAGGTTTCATCTGCACAAAATCACCAGATGG GGTCTAGGGCAGGAAGAGCCAGGGACAACAGCAT CTTAACACATGTAAAAATTCACCAGAAAGGC

Table S3

List of 29 additional mRNA probes added to the Nanostring Mouse Neuropathology panel.

Probe	TaqMan™ Assay ID	Use
<i>Lars</i>	Mm00506560_m1	Normalizer/Housekeeping
<i>Tnf</i>	Mm00443258_m1	Target
<i>Il6</i>	Mm00446190_m1	Target
<i>Il1b</i>	Mm00434228_m1	Target
<i>Fgf2</i>	Mm00433287_m1	Target
<i>Itga5</i>	Mm00439797_m1	Target
<i>Ngfr</i>	Mm00446294_m1	Target
<i>Sh3tc2</i>	Mm01261770_m1	Target
<i>Arx</i>	Mm00545903_m1	Target
<i>Shox2</i>	Mm00443183_m1	Target
<i>Npy</i>	Mm00445771_m1	Target

Table S4

TaqMan™ Gene Expression Assay ID's used for qPCR validation. *Lars* mRNA expression was used as a normalization/housekeeping gene as it was computationally identified by Nanostring assay as having adequate expression and low variability between samples.