

Supplementary Information

AUTS2 confers gene activation to Polycomb group proteins in the CNS

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Running Title: PRC1-AUTS2 activates transcription and regulates neurodevelopment

Table S1 MS analysis of RING1B phosphorylation sites by CK2

<i>In vitro</i>									
start site	end site	sequence	Site	phospho?	m/z	Charge	RT	Peak area	
27	43	TPQEAITDGLIIVVSPR	41	N	912.9904	2+	36.5	873345680	
27	43	TPQEAITDGLIIVVSPR	41	Y	952.973	2+	36.3	23113931	
27	43	TPQEAITDGLIIVVSPR	41	N	608.9961	3+	36.5	1064733343	
27	43	TPQEAITDGLIIVVSPR	41	Y	635.6514	3+	36.3	50966276	
162	195	QQIENGSGAEDNGDSSHC SNASTHSNQEAGPSNK	168	Y	899.3583	4+	20.09	2321354	
162	195	QQIENGSGAEDNGDSSHC SNASTHSNQEAGPSNK	168	N	879.364	4+	20.06	1334494	
162	196	QQIENGSGAEDNGDSSHC SNASTHSNQEAGPSNKR	168	N	914.1344	4+	20.84	1215029	pryo-Q
162	196	QQIENGSGAEDNGDSSHC SNASTHSNQEAGPSNKR	168	Y	934.1255	4+	21.11	2829748	pryo-Q
<i>In vivo</i>									
start site	end site	sequence	Site	phospho?	m/z	Charge	RT	Peak area	
162	196	QQIENGSGAEDNGDSSHC SNASTHSNQEAGPSNKR	168	N	914.1344	4+	23.13	1497222	pryo-Q
162	196	QQIENGSGAEDNGDSSHC SNASTHSNQEAGPSNKR	168	Y	934.1255	4+	23.5	213321	pryo-Q
162	195	QQIENGSGAEDNGDSSHC SNASTHSNQEAGPSNK	168	N	899.3583	4+	21.29	207541	
162	195	QQIENGSGAEDNGDSSHC SNASTHSNQEAGPSNK	168	Y	879.364	4+	21.81	33822	

Table-S2-AUTS2 target genes of human phenotypes

Abnormality of the forebrain-117/372 genes	Abnormality of the cerebrum-116/370 genes	Muscular hypotonia-99/346 genes	Aplasia/Hypoplasia involving the central nervous system-92/290 genes
<p>Acad9 Acvrl1 Adnp Aldh18a1 Ap3b1 Ass1 Atpaf2 B3galt1 Bckdhb Blm Casr Ccbe1 Chmp2b Cln8 Cttd D2hgdh Dguok Dhcr24 Dhcr7 Dlat Dld Dolk Dpm1 Dync2h1 Efnb1 Ehmt1 Eif2ak3 Eif2b3 Eif2b5 Ercc2 Ercc6 Esco2 Etfb Etfdh Fam20c Fgfr3 Fh1 Fktn Frem2 Ftl1 Gli2 Gpr56 Hibch Hspd1 Hyls1 Igf1r Kdm5c Kras Large Map2k2 Mapk10 Mapt Mcoln1 Mcph1 Mks1 Mmadhc Moc2 Mrps16 Mycn Nduf3 Nduf4 Nduf6 Ndufv1 Nsd1 Pafah1b1 Pank2 Pccb Pcnt Pex1 Pex10 Pex13 Pex14 Pex19 Pla2g6 Pomt1 Ppp2r2b Prps1l3 Psap Psat1 Pten Pts Rab23 Rab3gap2 Recql4 Rpia Sall1 Sdha Sdhaf1 Shh Six3 Slc17a5 Slc35c1 Slc9a6 Smad4 Snap29 Spg11 Spg7 Spr Stxbp1 Sucla2 Tbce Tcf4 Thrb Tinf2 Tsc2 Tsen54 Tuba1b Tubb2a Tubb2b Tubb3 Tymp Ube3a Upb1 Vangl1 Vps13a Wfs1 Zbtb16</p>	<p>Acad9 Acvrl1 Adnp Aldh18a1 Ap3b1 Ass1 Atpaf2 B3galt1 Bckdhb Blm Casr Ccbe1 Chmp2b Cln8 Cttd D2hgdh Dguok Dhcr24 Dhcr7 Dlat Dld Dolk Dpm1 Dync2h1 Efnb1 Ehmt1 Eif2ak3 Eif2b3 Eif2b5 Ercc2 Ercc6 Esco2 Etfb Etfdh Fam20c Fgfr3 Fh1 Fktn Frem2 Ftl1 Gli2 Gpr56 Hibch Hspd1 Hyls1 Igf1r Kdm5c Kras Large Map2k2 Mapk10 Mapt Mcoln1 Mcph1 Mks1 Mmadhc Moc2 Mrps16 Mycn Nduf3 Nduf4 Nduf6 Ndufv1 Nsd1 Pafah1b1 Pank2 Pccb Pcnt Pex1 Pex10 Pex13 Pex14 Pex19 Pla2g6 Pomt1 Ppp2r2b Prps1l3 Psap Psat1 Pten Pts Rab23 Rab3gap2 Recql4 Rpia Sall1 Sdha Sdhaf1 Shh Six3 Slc17a5 Slc35c1 Slc9a6 Smad4 Snap29 Spg11 Spg7 Spr Stxbp1 Sucla2 Tbce Tcf4 Tinf2 Tsc2 Tsen54 Tuba1b Tubb2a Tubb2b Tubb3 Tymp Ube3a Upb1 Vangl1 Vps13a Wfs1 Zbtb16</p>	<p>2310046K01Rik 6330578E17Rik Abcc8 Abcd3 Adnp Ahi1 Aldh18a1 Alg6 Atpaf2 Bckdhb Bin1 Bsnd Cant1 Casr Cpt1a D2hgdh Dguok Dhcr7 Dlat Dld Dolk Dpm1 Efnb1 Ehmt1 Eif2b3 Eif2b5 Ercc6 Etfb Etfdh Fgfr3 Fh1 Fktn Gaa Gale Gamt Gnptab Hlcs Hspd1 Ikbkap Inpp5e Kcnc3 Kcnj11 Kras Large Lifr Map2k2 Mccc1 Mccc2 Mcoln1 Mmaa Mmab Mmadhc Mrps16 Mtm1 Mtrr Myo5a Ndn Nduf3 Nduf4 Nduf6 Nduf8 Ndufv1 Nphp1 Nsd1 Ogdh Pafah1b1 Pccb Pex1 Pex10 Pex13 Pex14 Pex19 Pla2g6 Polg Pomt1 Prodh Prps1l3 Psap Pten Pts Ret Sdha Sdhaf1 Sepn1 Six3 Slc17a5 Slc35c1 Smpd1 Snap29 Snurf Sox9 Spr Sucla2 Suox Tcf4 Tor1a Tymp Ube3a Upb1</p>	<p>Adnp Aldh18a1 Ap3b1 Atpaf2 B3galt1 Blm Chmp2b Cln8 Cttd Dguok Dhcr24 Dhcr7 Dlat Dolk Dpm1 Efnb1 Ehmt1 Eif2ak3 Eif2b3 Eif2b5 Ercc2 Ercc6 Esco2 Fam20c Fgfr3 Fh1 Fktn Frem2 Gli2 Gpr56 Hibch Hspd1 Hyls1 Igf1r Inpp5e Kdm5c Kras Large Map2k2 Mapk10 Mcoln1 Mcph1Mks1 Mmadhc Mocs2 Mrps16 Mycn Nsd1 Nsdhl Pafah1b1 Pank2 Pccb Pcnt Pex1 Pex10 Pex13 Pex14 Pex19 Pla2g6 Pomt1 Ppp2r2b Psap Psat1 Pts Rab23 Rab3gap2 Recql4 Sall1 Shh Six3 Slc17a5 Slc35c1 Slc9a6 Snap29 Spg11 Spg7 Spr Stxbp1 Sucla2 Tbce Tcf4 Tinf2 Tsen54 Tuba1b Tubb2a Tubb2b Tubb3 Ube3a Upb1 Vangl1 Wfs1 Zbtb16</p>

Table S3 Antibodies used in the study

Antibody	Suppliers	Cat. No.	Lot No.	Applications
CBX2	Abcam	ab80044		WB
CK2B	Bethyl Laboratories	A301-984A		WB, ChIP
FLAG	Sigma	F3165		WB
FLAG beads	Sigma	A2220		IP
GAL4	Millipore	06-262		ChIP
GAL4	Santa Cruz	Sc-510		WB
GFAP	Abcam	ab4674		IF
H2AK119ub1	Cell Signaling	8240	1	WB, ChIP, ChIP-seq
H3	Abcam	ab1791		ChIP
H3K4me3	Abcam	ab8580	GR86940-1	ChIP, ChIP-seq
H3K27ac	Abcam	ab4729	GR63945-1	ChIP-seq
H3K27me3	Millipore	07-449	JBC1873477	ChIP, ChIP-seq
H3K36me3	abcam	ab9050	GR75636-1	ChIP-seq
H4K16ac	Millipore	07-329		ChIP
HA	Covance	MMS-101P		WB
HA	Abcam	ab9110	GR98618-3	ChIP-seq
HA beads	Sigma	A2095		IP
NeuN	Millipore	MAB377		IF
P300	Santa Cruz	sc584		WB
P300	Santa Cruz	sc585		IP, ChIP, ChIP-seq
PCGF4/BMI1	Bethyl Laboratories	A301-694A		WB
PoII	Santa Cruz	sc899	G2414	ChIP, ChIP-seq
RING1B	MBL International	D139-3		WB, ChIP
RYBP	Sigma	PRS2227		WB
β -Tubulin	Abcam	ab6046		WB
AUTS2 [#]	Reinberg Lab			WB,IP,ChIP,ChIP-seq,IHC,IF
PCGF5 ^{\$}	Reinberg Lab			WB
PCGF1	Bardwell Lab			WB
RING1B	Di Croce Lab			ChIP-seq
<p>[#], The AUTS2 antibody was raised in rabbits using a bacteria-expressed and His-fused fragment encoding the last 100 amino acids of human AUTS2. A GST-fused fragment was used for affinity purification of the anti-serum prior various application.</p> <p>^{\$}, The PCGF5 antibody was raised in rabbits using a synthetic peptide encoding a C-terminal sequence (GPLYQSYPMVLQYRP) of human PCGF5. The antibody was affinity purified using the same peptide.</p>				

Table S4 List of primers

For RT-qPCR

<i>hRYBP</i>	5'-CAACGTCACCGTCATTATCACAG-3'/5'-CATCATTCACTGCTGACATGTGCG-3'
<i>hRING1B</i>	5'-AGGCTGTGCAGACAAACGGAAC-3'/5'-GTCATGGTGTTCCTCAACATATCC-3'
<i>hAUTS2</i>	5'-TCAGGTGCAGAGGCCACCCAG-3'/5'-TTAAACTGTTGAGGCTTAAGCTACTG-3'
<i>hHOXA6</i>	5'-GGGCCTCGTGTCTTCTATTCT-3'/5'-TGCTGTCGGGTTTGTACTG-3'
<i>hTFAP2A</i>	5'-GGACCACCTGGTATTCTGTATTT-3'/5'-CTGGGCAACAAAGGACTATGA-3'
<i>hTLE3</i>	5'-CCGGAAACTCTGAGTGTGTATT-3'/5'-CCAGGTGAGAGCATCTGTTTAT-3'
<i>hXIST</i>	5'-CTCGGACAGCTGTAAAGAAGAG-3'/5'-GAATGTCCAAGAGGAGCCTAAG-3'
<i>hEEF2</i>	5'-GTGCCATCACTCAACCATAAC-3'/5'-GGCCATTAAGTCCCTACTAAG-3'
<i>hHOXD11</i>	5'-TGGAACCTCACGATTCTTCC-3'/5'-GTAACGGGACGTTTGCATTTAC-3'
<i>hNR2F2</i>	5'-TTCCAAGAGCAAGTGGAGAAG-3'/5'-CATCTGAGGTGAACAGGACTATG-3'
<i>hGATA6</i>	5'-CACACCACAACCTACCACCTTAT-3'/5'-TCCTGGTTTGAATTCCCTCTTT-3'
<i>hSMAD7</i>	5'-CTCCATCAAGGCTTTCGACTAC-3'/5'-AGCTGATCTGCACGGTAAAG-3'
<i>hGAPDH</i>	5'-GATGACATCAAGAAGGTGGTAA-3'/5'-GTCTTACTCCTTGAGGCCATGT-3'
<i>mDynll1</i>	5'-CTAGCCTCGAACTCAGAAATCC -3'/5'-GCAGGGACAAAGGAACATTTAAG -3'
<i>mNat8l</i>	5'-TGGTAGATGGCTGGCTTTG -3'/5'-TCCTCTGCCCTATCCCTTATC -3'
<i>mMex3b</i>	5'-GTAGGGAGGCAAGGTTGTAAG-3'/5'-TGCCCGTCACAACAAAGA -3'
<i>mPpp1r8</i>	5'-ACCACAAACACCTGAAGAGAG-3'/5'-CGCCAAATGAGACCGTAGAA-3'
<i>mGrif1</i>	5'-GAGGAGAGCTTTGTGCCTATAC -3'/5'-CCATCTTTCTTCCAGTGTTCTT -3'
<i>mAlkbh5</i>	5'-CTGATGCTGTGGTGAGAGAAA -3'/5'-GAGGACTGGTGGGCAATAAA -3'
<i>mHspa5</i>	5'-GAGACTGCTGAGGCGTATTT -3'/5'-TGACATTCAGTCCAGCAATAGT-3'
<i>mDnahc8</i>	5'-GGAGCATTGGAAGCGTATGT -3'/5'-GCGCACGTTATCCTTTGATT -3'
<i>mGh</i>	5'-ATGCCCTTGCCAGTCTGTT-3'/5'-GTTCTCTGCTGGGCCTCCT-3'

For qPCR following ChIP

<i>GAL4</i>	5'-CACCGAGCGACCCTGGATAAGC-3'/5'-GCTTCTGCCAACCGAACGGAC-3'
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