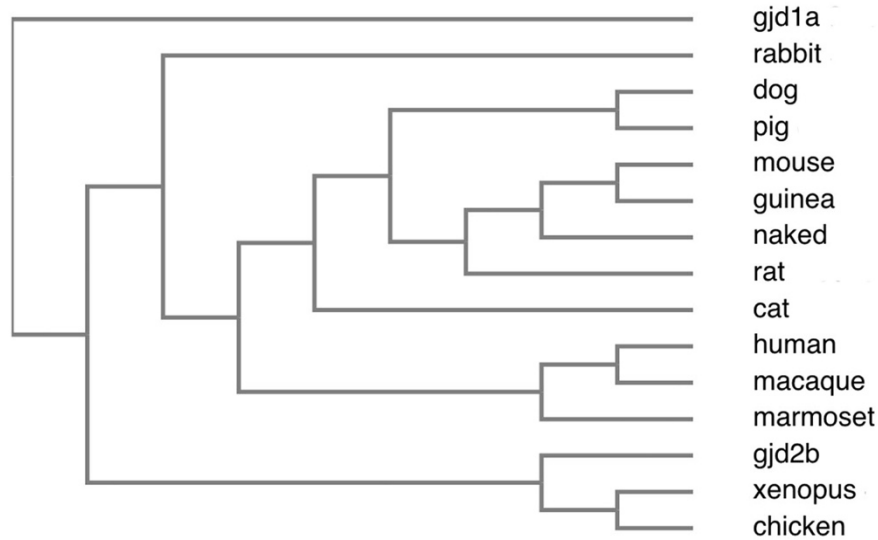


**Supplementary Figure S1: Top)** Cladogram of Cx35/36 protein sequences representing mammalian and lower vertebrates used as animal models in neurobiology research. **Bottom)** Multiple sequence alignment using Clustal Omega with default settings at the European Bioinformatics Institute (EBI). Homology regions for *CaM/CaMKII*-binding and S110/T111 phosphorylation in red.



gjd1a	MGEWTILERLLEAAVQQHSTMIGRILLTVVVFIRILIVGIVGEKVYEDEQIMFICNTLQP	60
rabbit	MGEWTILERLLEAAVQQHSTMIGRILLTVVVFIRILIVAIVGETVYDDEQTMFVCNTLQP	60
dog	-----ERLLEAAVQQHSTMIGRILLTVVVFIRILIVAIVGETVYDDEQTMFVCNTLQP	53
mouse	MGEWTILERLLEAAVQQHSTMIGRILLTVVVFIRILIVAIVGETVYDDEQTMFVCNTLQP	60
rat	MGEWTILERLLEAAVQQHSTMIGRILLTVVVFIRILIVAIVGETVYDDEQTMFVCNTLQP	60
human	MGEWTILERLLEAAVQQHSTMIGRILLTVVVFIRILIVAIVGETVYDDEQTMFVCNTLQP	60
macaque	MGEWTILERLLEAAVQQHSTMIGRILLTVVVFIRILIVAIVGETVYDDEQTMFVCNTLQP	60
marmoset	MGEWTILERLLEAAVQQHSTMIGRILLTVVVFIRILIVAIVGETVYDDEQTMFVCNTLQP	60
cat	MGEWTILERLLEAAVQQHSTMIGRILLTVVVFIRILIVAIVGETVYDDEQTMFVCNTLQP	60
pig	MGEWTILERLLEAAVQQHSTMIGRILLTVVVFIRILIVAIVGETVYDDEQTMFVCNTLQP	60
guinea	MGEWTILERLLEAAVQQHSTMIGRILLTVVVFIRILIVAIVGETVYDDEQTMFVCNTLQP	60
naked	MGEWTILERLLEAAVQQHSTMIGRILLTVVVFIRILIVAIVGETVYDDEQTMFVCNTLQP	60
gjd2b	MGEWTILERLLEAAVQQHSTMIGRILLTVVVFIRILIVAIVGETVYDDEQSMFVCNTLQP	60
xenopus	MGEWTILERLLEAAVQQHSTMIGRILLTVVVFIRILIVAIVGETVYDDEQTMFVCNTLQP	60
chicken	MGEWTILERLLEAAVQQHSTMIGRILLTVVVFIRILIVAIVGETVYDDEQTMFVCNTLQP	60
	*****.***.*** **.*	

#### S110/T111

gjd1a	GCNQACYDKAFPISHIRYWVFQIILVCTPSLCFITYSVHQSAKHKDQRYTLLHGPy----	116
rabbit	GCNQACYDRAFPISHIRYWVFQIIMVCTPSLCFITYSVHQSAQRERRYSTVFLSLDRDP	120
dog	GCNQACYDRAFPISHIRYWVFQIIMVCTPSLCFITYSVHQSAQRERRYSTVFLALDRDP	113
mouse	GCNQACYDRAFPISHIRYWVFQIIMVCTPSLCFITYSVHQSAQRERRYSTVFLALDRDP	120
rat	GCNQACYDRAFPISHIRYWVFQIIMVCTPSLCFITYSVHQSAQRERRYSTVFLALDRDP	120
human	GCNQACYDRAFPISHIRYWVFQIIMVCTPSLCFITYSVHQSAQRERRYSTVFLALDRDP	120
macaque	GCNQACYDRAFPISHIRYWVFQIIMVCTPSLCFITYSVHQSAQRERRYSTVFLALDRDP	120
marmoset	GCNQACYDRAFPISHIRYWVFQIIMVCTPSLCFITYSVHQSAQRERRYSTVFLALDRDP	120
cat	GCNQACYDRAFPISHIRYWVFQIIMVCTPSLCFITYSVHQSAQRERRYSTVFLTLDRDP	120

[illegible]

gjdl	-----IDHGHG-----PSRKL--RNINGILVHPE---SKDDRECLDLKDIPNIPAG	157
rabbit	PDSMGGPGGTGGGGSGGKREDKKLQNAIVNGVLQNPENPSKETEPDCLEVKELTPHPSG	180
dog	PESMGGPGGTGSGASGSSKREDKKLQNAIVNGVLQNTENTSKEVEPCLEVKELTPHPSG	173
mouse	AESIGPGGTGGGGSGGSKREDKKLQNAIVNGVLQNTETTSKETEPDCLEVKELTPHPSG	180
rat	AESIGPGGTGGGGSGGSKREDKKLQNAIVNGVLQNTETTSKETEPDCLEVKELAPHPSG	180
human	PESIGPGGTGGGGSGGKREDKKLQNAIVNGVLQNTENTSKETEPDCLEVKELTPHPSG	180
macaque	PESIGPGGTGGGGSGGKREDKKLQNAIVNGVLQNTENTSKETEPDCLEVKELTPHPSG	180
marmoset	PESIGPGGATGGGGSGGKREDKKLQNAIVNGVLQNTENTSKETEPDCLEVKELTPHPSG	180
cat	PESMGGPGGTGGGGSGGKRDDKKLQNAIVNGVLQNTENTSKETEPDCLEVKELTPHPSG	180
pig	PESMGGPGGTGGGGSGGKREDKKLQNAIVNGVLQNTENTSKETEPDCLEVKELTPHPSG	180
guinea	PESMGGPGGTGGGGSGGKREDKKLQNAIVNGVLQNTENTSKETEPDCLEVKELTPHPSG	180
naked	PESMGGPGGTGGSSGGGKREDKKLQNAIVNGVLQNTENTSKETEPDCLEVKELTPHPSG	180
gj2b	DTMR-----RDSKKIKNTIVNGVLQNTENSTKESEPCLEVKEIPN---S	163
xenopus	DTVK-----REDSKKIKNTLVNGVLQNTENSTKEAEPDCLEIKEIPN---P	163
chicken	DSMK-----REDSKKIKNTIVNGVLQNTENSTKEAEPDCLEVKEIPN---P	163

**T-site homology region**

gjdl	VITYSKSAKIRQQEGISRFYIIQVVFRNVALEIGFLAGQYFLYGFNVPAMFECDRYPCKVEV	217
rabbit	MRTAAKSLLRRQEGISRFYIIQVVFRNALEIGFLVGQYFLYGFSVPGLYEENRYPCIKEV	240
dog	LRTAARSKLRRQEGISRFYIIQVVFRNALEIGFLVGQYFLYGFSVPGLYECDRYPCKIEV	233
mouse	LRTAARSKLRRQEGISRFYIIQVVFRNALEIGFLVGQYFLYGFSVPGLYEENRYPCIKEV	240
rat	LRTAARSKLRRQEGISRFYIIQVVFRNALEIGFLVGQYFLYGFSVPGLYEENRYPCIKEV	240
human	LRTASKSLRRQEGISRFYIIQVVFRNALEIGFLVGQYFLYGFSVPGLYEENRYPCIKEV	240
macaque	LRTASKSLRRQEGISRFYIIQVVFRNALEIGFLVGQYFLYGFSVPGLYEENRYPCIKEV	240
marmoset	LRTASKSLRRQEGISRFYIIQVVFRNALEIGFLVGQYFLYGFSVPGLYEENRYPCIKEV	240
cat	LRTAARSKLRRQEGISRFYIIQVVFRNALEIGFLVGQYFLYGFSVPGLYECDRYPCKIEV	240
pig	LRTAARSKLRRQEGISRFYIIQVVFRNALEIGFLVGQYFLYGFSVPGLYECDRYPCKIEV	240
guinea	LRTAARSKLRRQEGISRFYIIQVVFRNALEIGFLVGQYFLYGFSVPGLYEENRYPCIKEV	240
naked	LRTAARSKLRRQEGISRFYIIQVVFRNALEIGFLVGQYFLYGFSVPGLYEENRYPCIKEV	240
gdj2b	AMRTTTSKMRRQEGISRFYIIQVVFRNALEIGFLVGQYFLYGFNVPAPVECDRYPCKIDV	223
xenopus	SIRTTSKMRRQEGISRFYIIQVVFRNALEIGFLVGQYFLYGFNVPSPMYECDRYPCKIEV	223
chicken	AIRTTTSKMRRQEGISRFYIIQVVFRNALEIGFLVGQYFLYGFNVPSPMYECDRYPCKIEV	223

**S-site homology region**

gjdl	ECYVSRPTEKTVFLVFMFAVSGICVVLNLAELNHLGWRKI KTAIRGVQARRKSICEIRK	277
rabbit	ECYVSRPTEKTVFLVFMFAVSGICVVLNLAELNHLGWRKI KLAVRGAQAQRKRSVYEIRNK	300
dog	ECYVSRPTEKTVFLVFMFAVSGICVVLNLAELNHLGWRKI KLAVRGAQAQRKRSVYEIRNK	293
mouse	ECYVSRPTEKTVFLVFMFAVSGICVVLNLAELNHLGWRKI KLAVRGAQAQRKRSVYEIRNK	300
rat	ECYVSRPTEKTVFLVFMFAVSGICVVLNLAELNHLGWRKI KLAVRGAQAQRKRSVYEIRNK	300
human	ECYVSRPTEKTVFLVFMFAVSGICVVLNLAELNHLGWRKI KLAVRGAQAQRKRSIYEIRNK	300
macaque	ECYVSRPTEKTVFLVFMFAVSGICVVLNLAELNHLGWRKI KLAVRGAQAQRKRSIYEIRNK	300
marmoset	ECYVSRPTEKTVFLVFMFAVSGICVVLNLAELNHLGWRKI KLAVRGAQAQRKRSIYEIRNK	300
cat	ECYVSRPTEKTVFLVFMFAVSGICVVLNLAELNHLGWRKI KLAVRGAQAQRKRSVYEIRNK	300
pig	ECYVSRPTEKTVFLVFMFAVSGICVVLNLAELNHLGWRKI KLAVRGAQAQRKRSVYEIRNK	300
guinea	ECYVSRPTEKTVFLVFMFAVSGICVVLNLAELNHLGWRKI KLAVRGAQAQRKRSVYEIRNK	300
naked	ECYVSRPTEKTVFLVFMFAVSGICVVLNLAELNHLGWRKI KLAVRGAQAQRKRSVYEIRNK	300
gd2b	ECYVSRPTEKTVFLVFMFAVSGICVVLNLAELNHLGWRKI KTAVRGVQARRKSIYEIRNK	283
xenopus	ECYVSRPTEKTVFLVFMFAVSGLCVVLNLAELNHLGWRKI KMAVRGVQAKRKSIYEIRNK	283
chicken	ECYVSRPTEKTVFLVFMFAVSGICVVLNLAELNHLGWRKI KMAVRGVQAKRKSIYEIRNK	283
*****.*****.*****.*****.*****.*****.***.*****.****.*		

gjd1a	DVSHLSSVPNLGRTQSSESAYV	299
rabbit	DLPRV-SVPNFGRTQSSDSAYV	321
dog	DLPRV-SVPNFGRT-----	306
mouse	DLPRV-SVPNFGRTQSSDSAYV	321
rat	DLPRV-SVPNFGRTQSSDSAYV	321
human	DLPRV-SVPNFGRTQSSDSAYV	321
macaque	DLPRV-SVPNFGRTQSSDSAYV	321
marmoset	DLPRV-SVPNFGRTQSSDSAYV	321
cat	DLPRV-SVPNFGRTQSSDSAYV	321
pig	DLPRV-SVPNFGRTQSSDSAYV	321
guinea	DLPRV-SVPNFGRTQSSDSAYV	321
naked	DLPRV-SVPNFGRTQSSDSAYV	321
gjd2b	DLPRM-SMPNFGRTQSSDSAYV	304
xenopus	DLPRM-GVPNFGRTQSSDSAYV	304
chicken	DLPRM-SMPNFGRTQSSDSAYV	304
	*: :: .:***:***	

Protein sequences were retrieved from the NCBI Protein Database. Human (NP\_065711.1), Mouse NP\_034420.2; Rat NP\_062154.1, Macaque XP\_001087723.1, Marmoset JAB32260.1 Xenopus XP\_002932880, Chicken NP\_989913, Dog XP\_038297692.1, Pig XP\_020953959.1, Guinea pig XP\_003475711, Naked mole (rat) XP\_004861563.1, gjd2b/Cx35.1/Cx35b NP\_919401.1, gjd1a/Cx34.1 NP\_001122238.

**Supplementary Table S1:** *Cx36 mRNA expression during early development of the mouse brain*

Structure	Stage	Assay Type	Reference
brain	E14, E16, E18	NB	[1]
future brain	E7.5	RT-PCR	[2]
optic cup	E8.5	ISH	[2]
optic stalk	E8.5	ISH	[2]
forebrain	E10.5	ISH	[2]
midbrain	E10.5	ISH	[2]
thalamus	E12.5	ISH	[2]
telencephalon	E12.5	ISH	[2]
midbrain-hindbrain junction	E12.5	ISH	[2]
spinal cord	E12.5	ISH	[2]
spinal ganglion	E12.5	ISH	[2]
midbrain roof plate	E13.5	ISH	[2]
diencephalon	E13.5	ISH	[2]
main olfactory bulb	E13.5	ISH	[2]
cerebellum	E13.5	ISH	[2]
diencephalon lateral wall mantle layer	E14.5	ISH	[3]
olfactory cortex marginal layer	E14.5	ISH	[3]
telencephalon mantle layer	E14.5	ISH	[3]
metencephalon rest of alar plate mantle layer	E14.5	ISH	[3]
pons mantle layer	E14.5	ISH	[3]
ventral grey horn	E14.5	ISH	[3]
intermediate grey horn	E14.5	ISH	[3]
cervical ganglion	E14.5	ISH	[3]
cervico-thoracic ganglion	E14.5	ISH	[3]
thoracic ganglion	E14.5	ISH	[3]
spinal cord	E17.5	ISH	[2]

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**Supplementary Table S2: Cx36 mRNA and protein expression in the adult rodent nervous system**

<b>Tissue</b>	<b>Subdivision</b>	<b>RNA</b>	<b>Protein</b>	<b>reference</b>
Telencephalon	cortex	high density labelling in layers IV, V and VI	pyramidal neurons, GABAergic interneurons PV positive interneurons	[1] [2] [3-5]
	olfactory system including olfactory epithelium, accessory systems	mitral cell layer, glomerular layer, external plexiform layer, periglomerular cells, tufted cells, granular cell layer, olfactory epithelial layer	olfactory nerve bundles underlying the olfactory epithelium, olfactory nerve layer, glomerular layer of the olfactory bulb, accessory olfactory bulb, vomeronasal nerve, gap junctions between mitral cells	[1] [2, 6-9]
	hippocampus	all regions of the hippocampal formation, including the enthorinal cortex	CA3/4, mossy fiber terminals	[1, 2, 6, 7, 10-12]
	amygdala	medial amygdaloid nucleus, the anterior and posteromedial cortical amygdaloid nucleus, the amygdalo-hippocampal transition area, medial and lateral part of the central nucleus	basomedial nuclei basolateral nuclei	[7, 13]
	basal ganglia	caudate putamen, globus pallidus and nucleus accumbens labeling was localized in a subpopulation of scattered cells with large pale nuclei.		[1, 14]
Diencephalon	thalamus	all major nuclei, reticular thalamic nucleus, including subthalamic nucleus	reticular thalamic nucleus	[13, 15]
	hypothalamus	lateral and anterior arcuate nucleus		[15] [7]

	other midbrain structures	nuclei of the preoptic region, of the anterior region of the paraventricular nucleus, anterior hypothalamic nucleus, lateral hypothalamic area, supraoptic nucleus, of the tuberal region such as arcuate nucleus, ventromedial and dorsomedial nucleus, perifornical nucleus. Mammillary region.		[7, 15]
	habenular nuclei, pineal gland	medial habenular nucleus, scattered in the lateral nucleus. intense signal in the pineal gland	tryptophan hydroxylase-positive and 5-hydroxytryptamine-positive pinealocytes	[1, 6, 16]
	pituitary gland	no data	anterior pituitary gland	[1, 17]
	retina	ganglion cell layer and in the inner border of the inner nuclear layer	All amacrine cells; ganglion cells, bipolar cells, photoreceptor	[1] [2, 6, 18-24] [25-28]
Hindbrain	cerebellum	molecular layer, granular layer, deep nuclei	molecular layer Purkinje neurons	[29] [1, 2, 6]
	inferior olive	all olivary neurons	all olivary neurons	[1] [2, 6, 18, 22]
	pons	raphe, trochlear, reticulocemental motor trigeminal basilar pontine	trigeminal ganglion	[30]
	medulla	most nuclei such as the red, parabrachial and oculomotor nuclei, the periaqueductal grey and the superior and inferior colliculi	lacZ transgenic	[31]

Spinal cord	spinal cord	all the lamina of the gray matter of the spinal cord, with the highest grain density in the motor neurons of the anterior horn	detected in the IML, IC and CA and in laminae VIII and IX ventral horn	[18]
	ganglions	dorsal root ganglia	dorsal root ganglia lumbar dorsal root; trigeminal sensory ganglia	[32, 33]
Other	neural crest-derived neuroendocrine organ	carotid body	carotid body	[34]
	neural crest-derived neuroendocrine organ	myenteric plexus	myenteric plexus	[34]

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