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Clustered gamma-protocadherins regulate cortical interneuron programmed cell death

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Abstract Cortical function critically depends on inhibitory/excitatory balance. Cortical inhibitory interneurons (cINs) are born in the ventral forebrain and migrate into cortex, where their numbers are adjusted by programmed cell death. Here, we show that loss of clustered gamma protocadherins (Pcdhg), but not of genes in the alpha or beta clusters, increased dramatically cIN BAX-dependent cell death in mice. Surprisingly, electrophysiological and morphological properties of Pcdhg-deficient and wild-type cINs during the period of cIN cell death were indistinguishable. Co-transplantation of wild-type with Pcdhg-deficient interneuron precursors further reduced mutant cIN survival, but the proportion of mutant and wild-type cells undergoing cell death was not affected by their density. Transplantation also allowed us to test for the contribution of Pcdhg isoforms to the regulation of cIN cell death. We conclude that Pcdhg, specifically Pcdhg3, Pcdhg4, and Pcdhg5, play a critical role in regulating cIN survival during the endogenous period of programmed cIN death.

Introduction GABAergic cortical inhibitory interneurons (cINs) regulate neuronal circuits in the neocortex. The ratio of inhibitory interneurons to excitatory neurons is crucial for establishing and maintaining proper brain function (Rossignol, 2011; Chao et al., 2010; Marín, 2012; Hattori et al., 2017; Huang et al., 2007; Rubenstein and Merzenich, 2003). Alterations in the number of cINs have been linked to epilepsy (Dudek and Shao, 2003), schizophrenia (Beasley and Reynolds, 1997; Hashimoto et al., 2003; Enwright et al., 2016) and autism (Gao and Penzes, 2015; Cellot and Cherubini, 2014; Fatemi et al., 2009). During mouse embryonic development, the brain produces an excess number of cINs and ~40% of those are subsequently eliminated by apoptosis during early postnatal life, between postnatal day (P) 1 and 15 (Southwell et al., 2012; Denaxa et al., 2018; Wong et al., 2018). What makes the death of these cells intriguing is its timing and location. In normal development, cINs are generated in the medial and caudal ganglionic eminences (MGE; CGE) of the ventral forebrain, far from their final target destination in the cortex. cINs migrate tangentially from their site of origin to reach the neocortex, where they
become synthetically integrated and complete their maturation (Anderson et al., 1997; Wichterle et al., 2001; Butt et al., 2005; Nery et al., 2003). The ganglionic eminences are also an important source of interneurons in the developing human brain, where migration and differentiation extend into postnatal life (Hansen et al., 2013; Paredes et al., 2016; Ma et al., 2013). How is the final number of clNs regulated once these cells arrive in the cortex?

Since clNs play a pivotal role in regulating the level of cortical inhibition, the adjustment of their number by programmed cell death is a key feature of their development and essential for proper brain physiology. While recent work suggests that activity-dependent mechanisms regulate clN survival through their connectivity to excitatory neurons (Wong et al., 2018; Denaxa et al., 2018; Duan et al., 2020; Priya et al., 2018) studies indicate that clN survival is mediated by a population-autonomous (or cell-autonomous) mechanism (Southwell et al., 2012). Heterochronically transplanted MGE clN precursors undergo a wave of apoptosis coinciding with their age, which is asynchronous from endogenous clNs. Whereas it is well established that neuronal survival in the peripheral nervous system (PNS) is regulated through limited access to neurotrophic factors secreted by target cells (Huang and Reichardt, 2001; Aloe and Chaldakov, 2013; Oppenheim and Carlonne, 2013), clN survival is independent of TrkB, the main neurotrophin receptor expressed by neurons of the CNS (Southwell et al., 2012; Rauskolb et al., 2010). Moreover, the proportion of clNs undergoing apoptosis remains constant across graft sizes that vary 200-fold (Southwell et al., 2012). Taken together, this work suggests that clN developmental death is intrinsically determined and that cell-autonomous mechanisms within the maturing clN population contribute to the regulation of their survival.

The clustered protocadherins (Pcdh) (Wu and Maniatis, 1999) are a set of cell surface homophilic-binding proteins implicated in neuronal survival and self-avoidance in the spinal cord, retina, cerebellum, hippocampus, and olfactory bulb glomeruli (Ing-Esteves et al., 2018; Wang et al., 2002b; Lefebvre et al., 2012, Lefebvre et al., 2008; Katori et al., 2017; Mountoufaris et al., 2017; Chen et al., 2017). In the mouse, the Pcdh locus encodes a total of 58 isoforms that are arranged in three gene clusters, alpha, beta and gamma: Pcdha, Pcdhb, and Pcdhg (Wu et al., 2001). The Pcdha and Pcdhg isoforms are each composed of a set of variable exons, which are spliced to three common constant cluster-specific exons (Tasic et al., 2002; Wang et al., 2002a). Each variable exon codes for the extracellular, transmembrane and most-proximal intracellular domain of a protocadherin protein. The Pcdhb isoforms are encoded by single exon genes encoding both extracellular, transmembrane and cytoplasmic domains (Wu and Maniatis, 1999). Of the 58 Pcdh genes, it has been suggested that a combinatorial, yet stochastic, set of isoforms is expressed in each neuron (Esumi et al., 2005; Kaneko et al., 2006; Mountoufaris et al., 2017), suggesting a source for neuronal diversity in the CNS (Canzio et al., 2019). Interestingly, Pcdh genes, and specifically isoforms Pcdhgc3, Pcdhgc4, and Pcdhgc5, are required for the postnatal survival of mice (Wang et al., 2002b; Hasegawa et al., 2016; Chen et al., 2012). Whether Pcdh genes are required for the regulation of clN elimination remains unknown.

In the present study, we used a series of genetic deletions of the Pcdh gene locus to probe the role of clustered Pcdhs in the regulation of clN cell death in mice. We show that Pcdhg, but not Pcdha or Pcdhb, are required for the survival of approximately 50% of clNs through a BAX-dependent mechanism. Using co-transplantation of Pcdhg-deficient and wild-type (WT) clNs of the same age, we show that they compete for survival in a mechanism that involves Pcdhg. Taking advantage of the transplantation assay, we show that removal of the three Pcdhg isoforms, Pcdhgc3, Pcdhgc4, and Pcdhgc5, is sufficient to increase cell death of MGE-derived clNs. Three-dimensional reconstructions and patch-clamp recordings indicate that the Pcdhg mutant cells have similar morphology, excitability and receive similar numbers of inhibitory and excitatory synaptic inputs compared to wild type clNs. We conclude that clN cell death is regulated by all or some of the C-isofoms in the Pcdhg cluster and that this process is independent of the structural complexity or intrinsic physiological properties of the cell or the strength of its excitatory and inhibitory synaptic inputs.

Results

Pcdhg expression in developing clNs

Expression of clustered protocadherins (Pcdh) in the brain starts in the embryo and continues postnatally (Hirano et al., 2012; Frank et al., 2005; Wang et al., 2002b; Kohmura et al., 1998). RT-
PCR analysis revealed the expression of each of the 58 isoforms in the Pcdh gene locus in the adult cortex (P30) (Figure 1A). Of the 58 Pcdh genes, those in the Pcdhg cluster are essential for postnatal survival (Hasegawa et al., 2016; Chen et al., 2012), and are implicated in cell death in the retina and spinal cord (Lefebvre et al., 2008; Prasad et al., 2008). We, therefore, determined whether Pcdh genes are expressed in cINs during the period of cIN cell death. Using Gad1-GFP mice to label GABAergic cINs (Tamamaki et al., 2003), we FACS-sorted GFP-positive (GFP+) and GFP-

Figure 1. Expression of clustered Pcdhs in the mouse cortex and purified cortical GABAergic cells. (A) PCR analysis of clustered Pcdh and Gapdh gene expression in P30 whole cortex extracts. (B) PCR analysis of Pcdhg and Gapdh gene expression in purified P7 cortical GABAergic cells. (C) Quantification of target gene mRNA levels at various postnatal stages (P2, P5, P8, P12, P15) in purified cortical GABAergic cells. P2 mRNA levels used as a reference for each gene (Kruskal-Wallis test, P value = 0.0007 [Pcdhgfc4], P value < 0.0001 [Pcdhgfc5], P value = 0.014 [Pcdhga1], P value = 0.024[Pcdhga2], P value = 0.003[Pcdhga3], P value = 0.038[Pcdhgb6]; n = 3 technical replicas). Significant p values are marked with*. See Figure 1—source data 1 for followup of comparisons.

The online version of this article includes the following source data and figure supplement(s) for figure 1:

Source data 1. Pcdhg expression in cortex.

Figure supplement 1. GABAergic markers are enriched in GFP positive FACS-sorted cells from Gad1-GFP mice.
negative (GFP-) cells from P7 mice at the peak of cIN cell death (Figure 1-figure supplement 1A). We confirmed that GABAergic cell markers (Gad1, Gad2) were enriched in the GFP+ population, while markers of excitatory neurons (Tbr1, Satb2, Otx2), astrocytes (Gfap, Aldh1f1), and oligodendrocytes (Olig2, Mbp) were enriched in the GFP- population (Figure 1-figure supplement 1B). With the exception of Pcdhga9 isoform, we detected the expression of all other 21 Pcdh in cINs (Figure 1B). To determine the expression pattern of Pcdh at different stages during the period of cell death, we measured the expression level of 8 Pcdh mRNAs (Pcdhgc3, Pcdhgc4, Pcdhgc5, Pcdhga1, Pcdhga2, Pcdhga3, Pcdhgb6, and Pcdhgb7) at P2, P5, P8, P12 and P15 using qPCR (Figure 1C). All eight isoforms were expressed in cINs at each of the five ages studied. Interestingly, the expression of Pcdhgc5 increased dramatically between P8 and P15. An increase in expression of Pcdh isoforms Pcdhga1, Pcdhga2 and Pcdhgc4 was also observed at P12, compared to other ages, but this increase was less pronounced than that observed for Pcdhgc5. The above results show that all Pcdh isoforms are expressed in cINs and that the expression of Pcdh isoforms Pcdhga1, Pcdhga2 and Pcdhgc4 and Pcdhgc5 increases during the period of postnatal cell death.

Reduced number of cINs in the cortex of Pcdh mutants

Most cINs are produced between embryonic days (E) 10.5 and 16.5 by progenitors located in the medial and caudal ganglionic eminences (MGE and CGE) (Anderson et al., 1997; Wichterle et al., 2001; Nery et al., 2002; Miyoshi et al., 2010). To address the potential role of Pcdh in cIN development, we used the Pcdh conditional allele (Pcdhgc^con3^) to block production of all 22 Pcdh isoforms (Lefebvre et al., 2008). In the Pcdhgc^con3^ allele, the third common exon shared by all Pcdh isoforms contains the sequence coding for GFP and is flanked by loxP sites (Lefebvre et al., 2008; Figure 2A). In unrecombined Pcdhgc^con3^ mice, all Pcdh isoforms are thus fused to GFP. However, when these animals are crossed to a Cre driver line, expression of the entire Pcdh cluster is abolished in Cre-expressing cells (Prasad et al., 2008). Robust GFP expression was detected throughout the brain in E13.5 embryos, including cells in the MGE and CGE (Figure 3B), indicating expression of Pcdh isoforms in cIN progenitors. We crossed Pcdhgc^con3^ mice to Gad2^Cre^ mice (Taniguchi et al., 2011) to conditionally ablate all Pcdh in GABAergic cells throughout the CNS at an early embryonic stage (E10.5) (Katarova et al., 2000). Recombined cells were visualized thanks to the conditional Ai14 (tdTomato) reporter expression (Figure 2A). Heterozygous Gad2^Cre^;Ai14; Pcdhgc^con3^/+ mice were viable and fertile. However, homozygous Gad2^Cre^;Ai14;Pcdhgc^con3^/+ mice displayed growth retardation after birth, a hind limb paw-clasping phenotype when held by the tail and were infertile (Figure 2B). Brain size as well as cerebral cortex thickness of homozygous Gad2^Cre^;Ai14;Pcdhgc^con3^/+ mice was similar to those of control mice (Figure 2B). However, the density of tdTomato positive cells in somatosensory and visual cortex was roughly halved in homozygous Gad2^Cre^;Ai14;Pcdhgc^con3^/+ animals, compared to wild type and heterozygous littermates (Figure 2C and C'). The density of cINs stained positive for parvalbumin (PV) and somatostatin (SST) (MGE-derived), vasoactive intestinal peptide (VIP) (CGE-derived) or reelin (RLN) (derived from both the MGE and CGE) was significantly reduced in the visual cortex of homozygous Gad2^Cre^;Ai14; Pcdhgc^con3^/+ mice (Figure 2D and Figure 2-figure supplement 1). Taken together, these experiments indicate that the embryonic loss of Pcdh function in GABAergic progenitor cells leads to a drastically reduced number of cINs in the neocortex, affecting all cIN subtypes similarly.

The developmental defects observed in Gad2^Cre^;Ai14;Pcdhgc^con3^/+ mutant mice may indirectly affect the survival of cINs in a non-cell autonomous manner. We thus decided to restrict the Pcdh loss of function to MGE/POA (preoptic area) progenitors by means of the Nkx2.1^Cre^ mouse (Xu et al., 2008). MGE/POA progenitors give rise to the majority of mouse cINs, including PV and SST interneurons. Nkx2.1 expression is detected in the ventral telencephalon from E9.5 (Sandberg et al., 2016; Shimamura et al., 1995) and is downregulated in most cINs as they migrate into the developing neocortex (Nóbrega-Pereira et al., 2008). Pcdhgc^con3^ mice were crossed to Nkx2.1^Cre^ mice. As described above, tdTomato expression was again used to visualize the recombinated cells (Figure 3A). Homozygous Nkx2.1^Cre^;Ai14;Pcdhgc^con3^/+ embryos lost GFP expression specifically in the MGE and the preoptic regions (Figure 3B), consistent with full recombination and loss of Pcdh function in cells derived from the Nkx2.1 lineage.

At P30, the number of MGE-derived tdTomato+ cells in Nkx2.1^Cre^;Ai14;Pcdhgc^con3^/+ mice was dramatically reduced (~50%) in both the visual and somatosensory cortex (Figure 3C and C'). MGE-derived PV and SST interneuron number was similarly reduced in these animals. However CGE-
Figure 2. Reduced number of GABAergic cINs in Pcdhg-deficient mice. (A) Mutant mice with loss of Pcdhg in GABAergic neurons were generated by crossing conditional Pcdhg<sup>flon3</sup> mice to Pan-GABAergic Cre driver (Gad2) mice. The conditional Ai14 reporter was used to fluorescently label Gad2-expressing cells. (B) Photographs of P21 Gad2<sup>Cre</sup>;Ai14 mice that are wild type (WT) or mutant (Pcdhg<sup>flon3/flon3</sup>) for Pcdhg. (B') Body weight and cortical thickness measurements in P30 Gad2<sup>Cre</sup>;Ai14;Pcdhg<sup>flon3/flon3</sup> (Pcdhg WT), Gad2<sup>Cre</sup>;Ai14;Pcdhg<sup>flon3/+-</sup> (Pcdhg HET), and Gad2<sup>Cre</sup>;Ai14;Pcdhg<sup>flon3/flon3</sup> (Pcdhg mutant) mice (Kruskal-Wallis test, P value=0.0027, adjusted p values **p=0.0017, n = 12 mice [Pcdhg WT], n = 7 mice [Pcdhg HET] and n = 5 mice [Pcdhg mutant]). (C) Photographs of coronal sections in primary visual cortex (V1) of P30 Gad2<sup>Cre</sup>;Ai14; Pcdhg<sup>flon3/flon3</sup> (Pcdhg mutant) mice (Kruskal-Wallis test, P value = 0.0006 for V1 and 0.0099 for S1BF), adjusted p values **p<0.0180, *p=0.0365, n = 3–5 mice of each genotype). (C') Quantifications of tdTomato+ cell density in V1 and somatosensory (S1BF) cortex of P30 Gad2<sup>Cre</sup>;Ai14 Pcdhg WT (black), Pcdhg HET (grey), and Pcdhg mutant (magenta) mice (Kruskal-Wallis test; for V1 (P value = 0.006), for S1BF (P value = 0.009), adjusted p values **p=0.0180, *p=0.036, n = 3–5 mice of each genotype). (D) Quantifications of cIN subtype density in V1 cortex at P30. All four non-overlapping cIN subtypes (PV, SST, RLN, and VIP) were similarly reduced in numbers in Gad2<sup>Cre</sup>;Ai14;Pcdhg<sup>flon3/flon3</sup> mice (Pcdhg mutant, magenta) compared to WT controls (Kruskal-Wallis test; for PV (P value = 0.0002), for SST (P value = 0.0001), for RLN (P value = 0.002), and for VIP (P value = 0.0093); adjusted p values **p=0.004 (PV), **p=0.0093 (SST), **p=0.0093 (RLN), *p=0.0365 (VIP), n = 3–5 mice of each genotype).

The online version of this article includes the following source data and figure supplement(s) for figure 2:

**Source data 1.** Quantification of GABAergic cINs, body weight and cortical thickness measurements in controls and Pcdhg deficient mice.

**Figure supplement 1.** Reduced number of GABAergic cIN subtypes in Pcdhg-deficient mice.

**Figure supplement 2.** Reduced number of GABAergic cINs across cortical layers in Pcdhg-deficient mice.

**Figure supplement 2—source data 1.** Quantification of all GABAergic cINs by cortical layer in the visual cortex of controls and Pcdhg-deficient mice.
Figure 3. Loss of Pcdhg genes targeted to Nkx2.1 expressing cells results in selective loss of cIN derived from the MGE. (A) Mutant mice with loss of Pcdhg in MGE-derived cIN were generated by crossing Pcdhg<sup>fcon3</sup> mice to Nkx2.1<sup>Cre</sup> mice. The conditional Ai14 line was used to fluorescently label MGE-derived cells. (B) Pcdhg<sup>fcon3/fcon3</sup> mice (at E13.5; top panels) carrying the Pcdhg mutant allele, but not Cre, show robust expression of GFP in the MGE. In contrast, in Nkx2.1<sup>Cre;Ai14;Pcdhg<sup>fcon3/fcon3</sup> mice (at E13.5; bottom panels), carrying the Pcdhg mutant allele and expressing Cre, GFP expression was eliminated from the MGE. Note NKX2.1 staining (magenta) in the panels on the right delineates MGE/POA (preoptic area). The few cells left expressing GFP in the MGE are blood vessels and are tdTomato negative. (C) Photographs of coronal sections of the primary visual cortex (V1) in Nkx2.1<sup>Cre;Ai14;Pcdhg<sup>fcon3</sup> mice (Pcdhg WT), Nkx2.1<sup>Cre;Ai14;Pcdhg<sup>fcon3/fcon3</sup> (Pcdhg HET) and Nkx2.1<sup>Cre;Ai14;Pcdhg<sup>fcon3/fcon3</sup> (Pcdhg mutant). Scale bar, 100 μm. (C') Quantification of the density of tdTomato+ cells in V1 and S1BF cortex of P30 Nkx2.1<sup>Cre;Ai14;Pcdhg<sup>WT</sup>, Pcdhg HET (grey) and Pcdhg mutant (magenta) mice. The number of Nkx2.1-derived cells was significantly reduced in Nkx2.1<sup>Cre;Ai14;Pcdhg</sup> mutant mice compared to WT controls (Kruskal-Wallis test; for V1 (P value=0.002), for S1BF (P value=0.0065), adjusted p values *p=0.0232, **p=0.0168 (S1), n = 4–6 mice of each genotype). (D) Body weight and cortical thickness measurements in Nkx2.1<sup>Cre;Ai14;Pcdhg</sup> WT (black) and Pcdhg mutant (magenta) mice at P30. Body weight and cortical thickness were not significantly affected by loss of Pcdhg (Mann-Whitney test, body weight (p=0.0547, n = 10 mice of each genotype), cortical thickness (p=0.2857, n = 4–5 mice of each genotype). (E) Quantification of tdTomato+ cIN subtypes in V1 mouse cortex at P30. Nkx2.1<sup>Cre;Ai14;Pcdhg</sup> Figure 3 continued on next page.
mutant mice (magenta) had significantly reduced numbers of MGE-derived parvalbumin (PV)+, somatostatin (SST)+, and Reelin (RLN)+ cells compared to WT controls. In contrast VIP+ cells, which are derived from the CGE, were not significantly affected (Kruskal-Wallis test; for PV (P value = 0.0113), for SST (P value=0.0009), for RLN (P value = 0.0014), and for VIP (P value=0.636); adjusted p values *p=0.0113 (PV), **p=0.0055 (SST), **p=0.0055 (RLN), n = 4–5 mice of each genotype).

The online version of this article includes the following source data and figure supplement(s) for figure 3:

**Source data 1.** Quantification of Nkx2.1-derived cINs, body weight and cortical thickness measurements in controls and Pcdhg-deficient mice.

**Figure supplement 1.** Reduced number of MGE cIN subtypes after loss of Pcdhg in Nkx2.1-derived cells.

**Figure supplement 2.** Numbers of Nkx2.1-derived RLN positive cINs are reduced in layers II–VI in Pcdhg-deficient mice.

**Figure supplement 2—source data 1.** Quantification of Reelin positive cINs by cortical layer in the visual cortex of controls and Pcdhg loss of function mice.

**Figure supplement 3.** Cortical layer distribution of Nkx2.1-derived cINs in Pcdhg WT and mutant mice.

**Figure supplement 3—source data 1.** Quantification of Nkx2.1-derived cINs by cortical layer in the visual cortex of controls and Pcdhg loss of function mice.

**Figure supplement 4.** Increased survival of non-Nkx2.1-derived SST and PV cINs in Pcdhg-deficient mice.

**Figure supplement 4—source data 1.** Quantification of non-Nkx21-derived PV and SST positive cINs in the visual cortex of controls and Pcdhg loss of function mice.

**Pcdhg function is not required for the proliferation and migration of cIN precursors**

The reduction in the number of cINs in Nkx2.1<sup>Cre</sup>;Ai14;Pcdhg<sup>fcon3/fcon3</sup> mice was not a result of abnormal cortical thickness or abnormal layer distribution, as these measures were similar across genotypes in P30 mice (Figure 3C). Next, we asked whether migration or proliferation defects in the cIN progenitor population could lead to a reduced cIN density in Pcdhg mutant mice. Quantification of the number of dividing cells in the ventricular or subventricular zones at E13.5 and E15.5, using the mitotic marker Phosphohistone H3 (PH3), showed no difference in the number of mitotic cells in the MGE between Nkx2.1<sup>Cre</sup>;Ai14; Pcdhg<sup>fcon3/fcon3</sup> mice and controls (Figure 4A and B). Migration of young cINs into cortex was also not affected in Nkx2.1<sup>Cre</sup>;Ai14;Pcdhg<sup>fcon3/fcon3</sup> mice. The tdTomato+ cells in the cortex displayed a similar migratory morphology in Nkx2.1<sup>Cre</sup>;Ai14; Pcdhg<sup>fcon3/fcon3</sup> embryos and controls. Consistent with the absence of an effect of Pcdhg on cIN migration, the number of migrating cells in cortex in the marginal zone (MZ), the subplate (SP), and the intermediate and subventricular zone (IZ/SVZ) was equivalent between Pcdhg mutant embryos and controls at E15.5 (Figure 4C and D). These findings indicate that loss of Pcdhg did not affect the proliferation of MGE progenitors or the migration of young MGE-derived cINs into the developing neocortex.
Figure 4. Proliferation and migration are not affected by the loss of Pcdhg in NKX2.1 expressing cells. (A) Photographs of coronal sections through the embryonic forebrains of E13.5 Nkx2.1^{Cre};Ai14;Pcdhg^{+/+} (Pcdhg WT, top panels) and Nkx2.1^{Cre};Ai14;Pcdhg^{fcon3/fcon3} (Pcdhg mutant, bottom panels). Close-up photographs of the MGE from Nkx2.1^{Cre};Ai14;Pcdhg WT (right panels) and Pcdhg mutant (bottom, right panels) embryos. Robust reporter activity (tdTomato) was observed in the MGE. Dividing cells were labeled using the mitotic marker PH3. Note that the size and number of PH3+ cells in the MGE was similar in the mutant and control brains. Scale bars, 50 μm. (B) Quantification of PH3+ cells from MGE ventricular (VZ) and subventricular zone (SVZ) in E13.5 (top) and E15.5 (bottom) Nkx2.1^{Cre};Ai14;Pcdhg WT (black bars) and Pcdhg mutant (magenta bars) embryos (Mann-Whitney test, p = 0.4000 (E13.5 VZ), p = 0.8571 (E13.5 SVZ), p = 0.8571 (E15.5 VZ), p > 0.999 (E15.5 SVZ), n = 3–4 embryos of each genotype). (C) Photographs of coronal sections of dorsal cortex at E15.5 showing the migrating MGE-derived cIN in Nkx2.1^{Cre};Ai14;Pcdhg WT (left) and Pcdhg mutant (right) embryos. Note the robust migratory streams of young neurons in the SVZ and in the marginal zone (MZ). From these regions, cells disperse into the intermediate zone (IZ) and cortical plate (CP). Similar numbers of migrating cINs were observed in mutants and controls. Scale bar, 100 μm. (D) Quantifications of number of migrating MGE-derived cINs in the CP and in the IZ/SVZ of Nkx2.1^{Cre}, Ai14;Pcdhg WT (black) and Pcdhg mutant (magenta) mice. No significant differences were detected in the number of tdTomato+ migrating cells in Pcdhg mutant and WT controls (Mann-Whitney test, p > 0.990 (E15.5 CP), p = 0.7000 (E15.5 IZ-SVA), n = 3 embryos of each genotype).

The online version of this article includes the following source data for figure 4:

Source data 1. Quantification of PH3 positive cells in the embryonic MGE and number of Nkx2.1-derived cINs in the embryonic dorsal cortex of controls and Pcdhg loss of function mice.
Accentuated cIN cell death in Pcdh mutants

A wave of programmed cell death eliminates ~40% of the young cINs shortly after their arrival in the cortex (Southwell et al., 2012; Wong et al., 2018). This wave starts at ~P0, peaks at P7, and ends at ~P15. Next, we asked if the reduced cIN density observed in Pcdhg mutant mice could stem from a heightened number of mutant cINs undergoing apoptosis at the normal time. Such cells were immunolabeled using an antibody directed against cleaved-Caspase 3 (CC3). Since CC3 positive and 15. Similarly to their wild type littermates, Nkx2.1;Pcdha-/- mice had significantly higher numbers of tdTomato+/CC3+ cells compared to controls. We also examined the proportion of CC3+ cells that were tdTomato negative (un-recombined cells that would notably include pyramidal cells, CGE-derived cINs, and glial cells). With the exception of a small, but significant increase observed at P0, we found no significant difference in the number of CC3+/tdTomato- cells between genotypes (Figure 6B, bottom graph). This suggests that the survival of neighboring Pcdhg-expressing cells is not impacted by the loss of Pcdhg-deficient MGE/POA-derived cINs. Importantly, the homozygous deletion of the pro-apoptotic Bcl-2-associated X protein (BAX) rescued cIN density in the Pcdhg mutant mice to levels similar to those observed in control Bax-/-;Pcdha+/+ mice or in mice carrying only the Bax mutation (Bax+/−) (Southwell et al., 2012; Figure 5C). The above results indicate that loss of Pcdhg in MGE/POA-derived cIN enhances their demise through programmed cell death during the developmental period when these cells are normally eliminated.

Loss of Pcdhg does not affect survival of cINs after the period of programmed cell death

We then asked whether Pcdhg-expression is also required for the survival of cINs past the period of programmed cell death. To address this question we took advantage of the PVCre transgene (Hippenmeyer et al., 2005) that becomes activated specifically in PV interneurons starting at around ~P16 (Figure 6 and Figure 6—figure supplement 1). Quantifications of tdTomato+ cell density in PVcre/Ai14;Pcdha+/+ and PVcre/Ai14 mice at P60-P100 revealed no significant differences between homozygous and control mice (V1 and S1BF) (Figure 6D and E). In contrast, the SstCre line, like Nkx2.1Cre;Pcdhg+/+ mice, was viable, fertile, and displayed normal weight (Figure 7B, top graph). cIN density in the visual cortex of Nkx2.1Cre;Ai14;Pcdha+/+ mice at P30 was similar to that of Nkx2.1Cre;Ai14 mice (Figure 7B). To determine whether Pcdhg genes affected MGE/POA-derived cIN survival, constitutive Pcdhb gene cluster knockout (Pcdhbdel/del) mice were crossed to Nkx2.1Cre;Ai14 mice (Figure 7A). Mice carrying a deletion of the entire Pcdhb cluster were viable, fertile and of normal weight (Figure 7C, top graph) (Chen et al., 2017). The density of cINs was similar between mice lacking Pcdhb and controls (Figure 7C). The above results indicate that unlike the Pcdhg cluster, which is essential for the regulation of cIN elimination, the function of Pcdha or Pcdhb is dispensable for the survival of MGE/POA-derived cINs.
Increased programmed cell death in Pcdhg mutants is rescued in Pcdhg-bax null animals. (A) Photographs of coronal sections through a Nkx2.1<sup>Cre<sup>;Ai14</sup>;Pcdhg<sup>f<sub>con3</sub>/f<sub>con3</sub></sup> (Pcdhg mutant) P7 mouse cortex (top), showing tdTomato+ cINs and cleaved caspase 3 positive cells (CC3+). Close-up photographs (bottom) of tdTomato+, CC3+ (white Arrowheads) and tdTomato-, CC3+ (blue Arrowheads) cells. Scale bar 25 μm. (B) Quantification of the density of tdTomato+, CC3+ (MGE-derived, top graph) cells from Nkx2.1<sup>Cre<sup>;Ai14</sup>;Pcdhg WT (black line) and Pcdhg mutant (magenta line) mice. Quantification of the density of tdTomato-, CC3+ (non-MGE-derived, bottom graph) cells from Nkx2.1<sup>Cre<sup>;Ai14</sup>;Pcdhg WT (black line) and Pcdhg mutant (magenta line) mice. Note that the number of CC3+ cells was significantly increased in the MGE-derived population in Pcdhg mutant mice, and coincides with the normal period of programmed cell death for cINs in WT mice (Each age was analyzed with a nested 1-way ANOVA (mouse ID nested within genotype), P value<0.0001. Significant comparisons are marked with *, **p=0.0004 ***p=0.0014, ****p=0.0009, n = 3–5 mice of each genotype). (C) Coronal sections through the primary visual cortex (V1) of Nkx2.1<sup>Cre<sup>;Ai14</sup>;Pcdhg<sup>f<sub>con3</sub>/f<sub>con3</sub></sup> (Pcdhg mutant, left) and Nkx2.1<sup>Cre<sup>;Ai14</sup>;Pcdhg<sup>f<sub>con3</sub>/f<sub>con3</sub></sup>;Bax<sup>-/-</sup> (Pcdhg mutant, Bax null, right) mice at P30. Quantifications of the density of cINs in V1 (top) and S1BF (bottom) cortex. Note that genetic removal of Bax in both Pcdhg<sup>f<sub>con3</sub>/f<sub>con3</sub></sup> (Pcdhg HET) and Pcdhg<sup>f<sub>con3</sub>/f<sub>con3</sub></sup> (Pcdhg mutant) mice rescues cell death to similar levels (Kruskal-Wallis test, P value<0.001 (for V1 an S1BF), adjusted p values for V1 (**p=0.0109, *p=0.0286) and for S1BF (**p=0.0109, *p=0.0286); n = 4–5 mice of each genotype).

The online version of this article includes the following source data for figure 5:

**Source data 1.** Analysis of cIN programmed cell death in controls, Pcdhg mutant and Bax null mice.
Figure 6. Pcdhg function is not required for the survival of PV cINs after the period of programmed cell death. (A) Mutant mice with loss of Pcdhg in SST or PV cells were generated by crossing conditional Pcdhg\(^{fcon3}\) mice to mice carrying Cre under Sst (Sst\(^{Cre}\)) or Pvalb (Parvalbumin, PV\(^{Cre}\)). The conditional Ai14 line was used to fluorescently label SST or PV cells. (B) Photographs of coronal sections of the primary visual cortex (V1) of P30 Sst\(^{Cre}\); Ai14; Pcdhg\(^{+/+}\) (Pcdhg WT, top left) and Sst\(^{Cre}\); Ai14; Pcdhg\(^{fcon3/fcon3}\) (Pcdhg mutant, bottom left) mice. Scale bars, 50 \(\mu\)m. (C) Quantifications of the density of tdTomato+ cINs in V1 cortex of Pcdhg WT (black) and Pcdhg mutant (magenta) Sst\(^{Cre}\); Ai14 mice at P30 (Mann-Whitney test, **p=0.0286, n = 4 mice of each genotype). (D) Photographs of coronal sections of V1 in PV\(^{Cre}\); Ai14; Pcdhg\(^{+/+}\) (Pcdhg WT, top right) and PV\(^{Cre}\); Ai14; Pcdhg\(^{fcon3/fcon3}\) (Pcdhg mutant, bottom right) mice at P60. Scale bars, 50 \(\mu\)m. (E) Quantifications of the density of tdTomato+ cIN in V1 cortex of Pcdhg WT and Pcdhg mutant PV\(^{Cre}\); Ai14 mice at P60-100 (Mann-Whitney test, p=0.4206, n = 5 mice of each genotype).

The online version of this article includes the following source data and figure supplement(s) for figure 6:

**Source data 1.** Analysis of PV and SST-derived cINs at P30 in controls and Pcdhg mice.

**Figure supplement 1.** Late postnatal expression of Parvalbumin in cINs.
Loss of Pcdhg does not affect cIN dispersion after transplantation but affects their survival

In order to compare the timing and extent of migration, survival, and maturation of cINs of different genotypes within the same environment, we co-transplanted into the cortex of host animals, MGE-derived cIN precursor cells expressing red and green fluorescent proteins. MGE cIN precursors were derived from E13.5 Gad1-GFP embryos (Pcdhg WT controls) or from Nkx2.1CAG;Ai14 embryos that were either Pcdhg WT or Pcdhg mutant (Figure 8A). We first confirmed that MGE cells WT for Pcdhg, but carrying the two different fluorescent reporters, displayed no differences in their survival.
**Figure 8.** Pcdhg are required for cIN survival after transplantation. (A) Schematic of co-transplantation of MGE-derived cIN precursors. MGE cells were derived from Nkx2.1Cre;Ai14;Pcdhg\textsuperscript{fox3/flox3} (Pcdhg WT) or Nkx2.1Cre;Ai14;Pcdhg\textsuperscript{fox3/flox3} (Pcdhg mutant) embryos. These cells were mixed in equal proportions with MGE cells from Gad1-GFP embryos (Pcdhg WT, green) and transplanted into WT black (Blk) six host recipient mice. Cell survival was analyzed before (3 DAT) and throughout the period of cell death (6–21 DAT). (B,B') Survival fraction of co-transplanted MGE-derived cIN precursors. (B) MGE cells were derived from Gad1-GFP (green) and Nkx2.1\textsuperscript{Cre};Ai14;Pcdhg\textsuperscript{fox3/flox3} (magenta) embryos; both GFP+ and tdTomato+ cells carry WT Pcdhg. In this control experiment the survival fraction was similar for both genotypes carrying the different fluorescent reporters (2-way ANOVA, F\textsubscript{genotype} = 2.54, P value > 0.999; n = 4–6 mice per time point from two transplant cohorts). (B') MGE cells were derived from Gad1-GFP WT (green) and Nkx2.1\textsuperscript{Cre};Ai14;Pcdhg\textsuperscript{flox3/flox3} (Pcdhg mutant, magenta) embryos. GFP+ and tdTomato+ cells showed dramatic differences in their survival; the majority of cells carrying the Pcdhg mutant allele (magenta) were eliminated between 6 and 21 DAT (2-way ANOVA, F\textsubscript{genotype} = 2738.02, P value < 0.0001; adjusted p values ***p<0.0001; n = 4–5 mice per time point from two transplant cohorts. Quantifications in (B and B') were done at 3, 6, 13 and 21 DAT and are represented as fractions of GFP+ or tdTomato+ cells from total cells (GFP + tdTomato+) per brain section. The increase in the proportion of WT cells during this period is not a reflection of increased cell numbers (WT cIN also undergo elimination by programmed cell death (See Figure 8—figure supplement 1), but rather that WT cells account for a larger fraction of all transplant-derived cells (WT + Pcdhg mutant). (C) Representative photographs of cortical sections from transplanted host mice at 6 (left) and 21 (right) DAT. Transplanted MGE cells were derived from Gad1-GFP (Pcdhg WT, green) and Nkx2.1\textsuperscript{Cre};Ai14;Pcdhg\textsuperscript{flox3/flox3} (Pcdhg mutant, red) embryos. Scale bars, 50 \textmu m.

The online version of this article includes the following source data and figure supplement(s) for figure 8:

**Source data 1.** Survival of transplanted MGE-derived cIN precursor cells carrying WT or mutant Pcdhg.

**Figure supplement 1.** Number of cIN drops for both the Pcdhg WT and Pcdhg mutant transplanted population.

**Figure supplement 1—source data 1.** Quantification of transplanted MGE-derived cIN precursor cells carrying WT or mutant Pcdhg.
Equal proportions of Gad1-GFP cells (Pcdhg WT, GFP+) and Nkx2.1\textsuperscript{Cre};Ai14 cells (Pcdhg WT, tdTomato+) were co-transplanted into the neocortex of neonatal recipients. While equivalent numbers of red and green cells were mixed before being transplanted, the absolute number of cells transplanted varied from transplant to transplant. In order to compare the survival, we use the fraction of green or red cells, among all co-transplanted cells (red + green). The fraction of surviving GFP+ and tdTomato+ cells at 3, 6, 13, and 21 days after transplantation (DAT) was measured (Figure 8A and B, top graph). The contribution of each cell population to the overall pool of surviving cells was found to be ~50% at 3 DAT, and remained constant at 6, 13, and 21 DAT (Figure 8B, top graph). This experiment indicates that the fluorescent reporters (GFP or tdTomato) or breeding background does not affect the survival of MGE cINs in this assay. Next, we co-transplanted equal numbers of Gad1-GFP cells (Pcdhg WT) and Nkx2.1\textsuperscript{Cre};Ai14;Pcdhg\textsuperscript{fcon3/fcon3} cells (Pcdhg mutant) into the cortex of WT neonatal recipients. As above, we measured the proportion of surviving GFP+ and tdTomato+ cells at 3, 6, 13, and 21 DAT (Figure 8A). Similar numbers of GFP+ and tdTomato+ cells were observed at 3 and 6 DAT. However, the fraction of Pcdhg mutant cINs (tdTomato+) surviving was dramatically lower when the transplanted cells reached a cellular age equivalent to that of endogenous cINs after the normal wave of programmed cell death (6DAT is roughly equivalent to P0; 21DAT is roughly equivalent to P15) (Southwell et al., 2012). Note that in this experiment the proportion of WT cells increases during this same period. This change in proportion is not a reflection of increased survival, as these cells also undergo elimination by programmed cell death (see below) (Figure 8—figure supplement 1), but that, with the increased loss of mutant cells, the WT cells account for a larger fraction of the total.

We next determined whether the survival of Pcdhg WT (GFP+) or Pcdhg mutant (tdTomato+) cINs was affected by their density (Figure 9). At 6 DAT, WT and Pcdhg mutant MGE-derived cells had migrated away from the injection site establishing a bell-shaped distribution of density as a function of tangential distance from the injection site (Figure 9B and B'). The dispersion of developing cINs lacking Pcdhg was indistinguishable from that of control WT cells at this time (Figure B', top graph), consistent with our observation that Pcdhg expression is not required for the migration of MGE-derived cINs. Strikingly, the survival fraction at 6 DAT of control Pcdhg WT (GFP+) and Pcdhg mutant (Nkx2.1\textsuperscript{Cre};Ai14;Pcdhg\textsuperscript{fcon3/fcon3}) cINs at the injection site or at multiple locations anterior or posterior to the site of injection were also similar (Figure 9B', bottom graph). By 21 DAT the survival of Pcdhg mutant (Nkx2.1\textsuperscript{Cre};Ai14;Pcdhg\textsuperscript{fcon3/fcon3}) cells was dramatically reduced, and to a similar extent at all distances from the injection site (Figure 9B and B'). Since the density of cIN varies five-fold over regions measured, we conclude that the survival of control Pcdhg WT and Pcdhg mutant cIN does not depend on their density over this range.

In order to determine the absolute number of cINs eliminated in our co-transplantation experiments, we co-transplanted 50 K cells of each genotype (Pcdhg WT and Pcdhg mutant) into WT host mice (Figure 10A). Our baseline for survival was established at 6 DAT, before the period of cIN programmed cell death. In control experiments where cIN precursors WT for the Pcdhg allele, derived from Nkx2.1\textsuperscript{Cre};Ai14 or Gad1-GFP embryos, were transplanted, 39% of the transplanted cIN population was eliminated between 6 and 21 DAT (Figure 10A–C and Figure 10—figure supplement 1). Therefore, transplanted MGE cINs not only undergo programmed cell death during a period defined by their intrinsic cellular age but are also eliminated in a proportion that is strikingly similar to that observed during normal development (Wong et al., 2018; Southwell et al., 2012). Given these observations, we next asked how the presence of Pcdhg mutant cIN affected the survival of WT cIN in the transplantation setting. We co-transplanted 50K Gad1-GFP Pcdhg WT (GFP+) with 50K Nkx2.1\textsuperscript{Cre};Ai14 Pcdhg mutant (tdTomato+) MGE cIN precursors and compared the survival of each population at 6 and 21 DAT. At 6 DAT the total number of tdTomato+ cells in the cortex of recipient mice was similar to that of GFP+ cells (Figure 10A,D and E). However, between 6 and 21DAT, the total number of GFP+ cells had decreased by an average of ~63% (Figure 10E, compared to Figure 10C). Compared to the ~40% of endogenous or transplanted WT cINs that are normally eliminated (present study and previous work Southwell et al., 2012; Wong et al., 2018), this experiment suggests that WT cells die at a higher rate (63%) when co-transplanted with Pcdhg mutant MGE cells. However, this observation would require additional animals for statistical confirmation. Regardless, the number of Pcdhg mutant (tdTomato+ cells) cINs decreased dramatically, by ~96% (Figure 10E). This experiment confirms that MGE cells lacking Pcdhg function are eliminated in far greater numbers than control MGE cells and show that the presence of Pcdhg WT cINs within a
mixed population also affects the survival of mutant cINs (compare Figure 8 and Figure 10). These observations are consistent with the hypothesis that cINs interact with others of the same age discussed below.
Loss of Pcdhg isoforms Pcdhgc3, Pcdhgc4, and Pcdhgc5 is sufficient to increase cell death

The results above indicate that the loss of function of all 22 Pcdh isoforms encoded from the Pcdhg gene cluster significantly increased cell death among cINs. Whether all 22 Pcdhg are equally involved in the regulation of cIN survival remains unclear. Our qPCR expression analysis suggests that the expression of Pcdhga1, Pcdhga2, Pcdhgc4 and Pcdhgc5 in cINs increases during, or soon after, the period of cell death (Figure 1C). To test if Pcdhga1, Pcdhga2, and Pcdhga3 were required...
for the normal survival of MGE-derived cINs, we crossed the Pcdhgtako/tako mouse line (Pcdhga1, Pcdhga2, and Pcdhga3 isofom KO) to Nkx2.1Cre; Ai14 mice (Figure 7A). At P30, the density of cINs in visual cortex of Nkx2.1Cre; Ai14;Pcdhgtako/tako (Pcdhga1, Pcdhga2, and Pcdhga3 mutant) mice was not significantly different from that of control Nkx2.1Cre; Ai14 mice that are WT for Pcdhga (Figure 7D). Consistent with this finding, co-transplanted E13.5 Nkx2.1Cre; Ai14;Pcdhgtako/tako MGE cells and Pcdhga WT (GFP+) displayed surviving fractions of similar sizes (Figure 11A,B & B'). Therefore, the removal of the first three isoforms (Pcdga1, Pcdhga2 and Pcdhga3) of the Pcdhga cluster does not significantly affect cIN survival.

We next tested if removal of the last three isoforms of the Pcdhga cluster (Pcdhgc3, Pcdhgc4, and Pcdhgc5) affected cIN survival. Pcdhgtako/+ mice, heterozygous for excision of Pcdhgc3, Pcdhgc4, and Pcdhgc5 were crossed to the Nkx2.1Cre; Ai14 mouse line to label MGE/POA-derived cINs (Figure 11A). Even though homozygous Nkx2.1Cre; Ai14;Pcdhgtako/tako mice develop normally (normal weight and no evidence of brain abnormalities) and are born in normal Mendelian ratios, these mice die shortly after birth (Chen et al., 2012). To bypass neonatal lethality, and study the role of Pcdhgc3, Pcdhgc4, and Pcdhgc5 isoforms during the normal period of cIN programmed cell death, we co-transplanted Pcdhgtako/tako (tdTomato+, mutant) and Pcdhga WT (GFP+) E13.5 MGE cells into the cortex of WT neonatal recipients (Figure 11A). At 6 DAT, the dispersion and density of tdTomato+ and GFP+ cells were indistinguishable. However, the number of tdTomato+ MGE-derived Pcdhgtako/tako mutant cINs dropped dramatically between 6 and 21 DAT, compared to the Pcdhga WT (GFP+) population (Figure 11C). The survival of Pcdhgtako/tako mutant cIN at 21 DAT was strikingly similar to that observed after transplantation of MGE cells lacking the entire Pcdhga cluster (Nkx2.1Cre; Ai14;Pcdhgc3/tako); compare Figure 8 and Figure 11. These results indicate that unlike Pcdga1, Pcdhga2 and Pcdhga3 isoforms, Pcdhgc3, Pcdhgc4, and Pcdhgc5 are essential for cIN survival.

Morphological and physiological maturation of cINs lacking Pcdhga

The above results indicate that cINs lacking Pcdhga genes have increased cell death, specifically when the transplanted cells reach an age equivalent to that of endogenous cINs undergoing their normal period of programmed cell death. We therefore asked whether the loss of Pcdhga in cINs affected their morphological maturation during this period. We first determined the survival fraction for co-transplanted control Gad1-GFP (Pcdhga WT) and Nkx2.1Cre; Ai14;Pcdhgc3fcon3/fcon3 (Pcdhga mutant) MGE-derived cIN precursors at two-day intervals during the intrinsic period of cIN cell death in the transplanted population (6, 8, 10 and 12 DAT). When equal proportions of Pcdhga WT and Pcdhga mutant cells were co-transplanted, their survival fraction remained similar up to 6 DAT, but the proportion of the Pcdhga mutant cells dropped steadily throughout the period of cell death (Figure 12B). Morphological reconstructions of the transplanted cells during this period of cIN programmed cell death (Figure 12A) revealed no obvious differences between the Pcdhga mutant and control Pcdhga WT cells in neuritic complexity, including neurite length (Figure 12C), the number of neurites (Figure 12D), number of nodes (Figure 12E) and number of neurite ends (Figure 12F). These results suggest that Pcdhga genes do not play a major role in the morphological maturation of cINs during the period of cIN death.

Next, we utilized co-transplantation of cINs that were either Pcdhga-deficient (Nkx2.1Cre; Ai14; Pcdhgc3fcon3/fcon3) or WT (Gad1-GFP) to investigate whether the loss of Pcdhga affected the integration or intrinsic physiological properties of these cells at time points around the peak of Pcdhga-mediated cell death. To test how integration was affected, we made acute cortical slices of mouse visual cortex at 8, 9, 10, 11, and 12 DAT and measured the frequency of spontaneous excitatory (glutamatergic) and inhibitory (GABAergic) synaptic events, comparing mutant and WT cINs within the same slice. There was no effect of Pcdhga loss of function on the frequency of spontaneous excitatory (glutamatergic) synaptic events or on the frequency of spontaneous inhibitory (GABAergic) synaptic events (Figure 13B and Tables 1 and 2). We next investigated whether the loss of Pcdhga function altered intrinsic physiological properties in co-transplanted cINs. There was no effect of the loss of Pcdhga on the maximum firing rate (Figure 13C and Tables 1 and 2), membrane time constant (Tau) (Figure 13D and Table 1), or input resistance (Figure 13F and Table 1). A difference in capacitance was observed between WT and Nkx2.1Cre; Ai14;Pcdhgc3fcon3/fcon3 cINs at 8DAT, but this difference was not statistically significant following multiple comparisons correction and was not seen at later
Figure 11. Loss of Pcdhc3, Pcdhc4, and Pcdhc5 is sufficient to increase cIN cell death. (A) Diagram of the mutant alleles Pcdhg\textsuperscript{tako} (Pcdhga1, Pcdhga2, and Pcdhga3 KO) and Pcdhg\textsuperscript{tcko} (Pcdhc3, Pcdhc4, and Pcdhc5 KO). Below - schematic of transplantation of MGE cIN precursors from Nkx2.1\textsuperscript{Cre};Ai14;Pcdhg\textsuperscript{tako/tako} (Pcdhga1, Pcdhga2, and Pcdhga3 deleted) and Nkx2.1\textsuperscript{Cre};Ai14;Pcdhc3, Pcdhc4, and Pcdhc5 deleted embryos. These cells were mixed in equal proportions with MGE cells from Gad1-GFP embryos (Pcdhg WT, green) and transplanted into WT Blk6 host recipients. Survival of the GFP and tdTomato-labeled cells was analyzed at 6 and 21 DAT. (B, B') Representative photographs of cortical sections from transplanted host animals at 6 (left) and 21 (right) DAT. Note the similar proportions of Pcdhg WT (GFP+) and Pcdhga1, Pcdhga2, and Pcdhga3 deleted cells (tdTomato+) at 6 and 21DAT. Scale bars, 100 μm. (B') Quantifications of the survival fraction of the GFP (green) and tdTomato (magenta)-labeled MGE-derived cells at 6 and 21 DAT. Note, survival fraction remains similar and constant for both genotypes (Pcdhg WT and Pcdhga1, Pcdhga2, and Pcdhga3 deleted cells) between 6 and 21 DAT (Mann-Whitney test, p=6571, n = 4 mice per time point from one transplant cohort). (C, C') Representative photographs from coronal brain sections of transplanted host animals at 6 (left) and 21 (right) DAT. Scale bars, 100 μm. Survival of MGE-derived cINs from Pcdhc3, Pcdhc4, and Pcdhc5 deleted embryos (tdTomato+) is markedly different from MGE-derived cINs from Pcdhg WT embryos (GFP+). (C') Survival fraction at 6 and 21 DAT of the Pcdhc WT (green) and Pcdhc3, Pcdhc4, and Pcdhc5 deleted cells (magenta) (Mann-Whitney test, ****p=0.0286, n = 4 mice per time point from one transplant cohort). The online version of this article includes the following source data for figure 11:

Source data 1. Survival analysis of transplanted MGE-derived cIN precursor cells deficient in Pcdha1, Pcdha2 and Pcdha3 or deficient in Pcdhc3, Pcdhc4 and Pcdhc5.
Figure 12. Loss of Pcdhg does not affect the morphological maturation of cIN during the period of programmed cell death. (A) Photographs of representative images and morphological reconstructions of co-transplanted Gad1-GFP cells (Pcdhg WT, left columns) with Nkx2.1<sup>Cre</sup>;<sup>Ai14</sup>;Pcdhg<sup>fcon3/fcon3</sup> cells (Pcdhg mutant, right columns) at 6, 8, 10 and 12DAT. Scale bars, 50 μm. (B) Quantifications of Pcdhg WT (green) and Pcdhg mutant cells (magenta) from co-transplanted animals, represented as survival fraction from total number of cells per section at 6, 8, 10 and 21 DAT. Pcdhg mutant cells begin to increase their elimination between 6 and 8 DAT and this increased death occurs through 21 DAT. (C–F) Measurements of neurite complexity during the period of programmed cell death, including neurite length (C), neurite number (D), node number (E) and neurite ends (F) in Pcdhg WT (green) and Pcdhg mutant (magenta) neurons at 6, 8, 10 and 12 DAT. Two-tailed unpaired Student’s t-test, n = 32 (WT), n = 35 (Pcdhg mutant) cells at 6 DAT, n = 27 (WT and Pcdhg mutant) cells at 8 DAT, n = 26 (WT), n = 27 (Pcdhg mutant) cells at 10 DAT, and n = 27 (WT), n = 31 (Pcdhg mutant) cells at 12 DAT; cells analyzed from two transplant cohorts. All statistical comparisons were not significant following Benjamini-Hochberg multiple comparisons correction at alpha of 0.05.

The online version of this article includes the following source data for figure 12:

Source data 1. Morphological reconstruction of transplanted MGE-derived cIN precursor cells carrying WT or mutant Pcdhg.
time points (Figure 13E and Table 1). We conclude that the synaptic integration and morphological - functional maturation of cINs lacking Pcdhg is similar to that of WT controls.

**Discussion**

The findings above indicate that Pcdhg genes play a critical role in regulating cIN survival during the endogenous period of cIN programmed cell death. Specifically, Pcdhgc3, Pcdhgc4, and Pcdhgc5 isoforms within the Pcdhg cluster are essential for the selection of those cINs that survive past the period of programmed cell death and become part of the adult cortical circuit. Pcdhg genes do not
affect the production or migration of cINs and appear to be dispensable for the survival of cINs after the period of cell death. Together with previous work in the spinal cord and retina, these results suggest that PcdhgC3, PcdhgC4, and PcdhgC5 isoforms are key to the regulation of programmed cell death in the CNS. In contrast, deletions of the alpha and beta Pcdh gene clusters did not alter cell death during this period.

Our initial approach involved the removal of Pcdhg function from all GAD2 expressing cells using the Gad2;Cre;Ai14;Pcdhg<sup>focon3/fcon3</sup> mice. These mice displayed a dramatic reduction of cortical interneurons of all subtypes, including a significant decrease in the number of VIP+ cells, which are derived from the CGE. A similar observation has been recently reported by Carriere et al. (2020). In these mice, Pcdhg function was also removed from most other GABAergic neurons throughout the nervous system, as well as from a small fraction of astrocytes (Taniguchi et al., 2011). Since the removal of Pcdhg function in all Gad2-CRE expressing cells could affect the survival of cINs indirectly, we used Nkx2.1<sup>Cre</sup>;Ai14 ; mice to more specifically remove Pcdhg function from MGE derived cINs. As in the Gad2<sup>Cre</sup>;Ai14;Pcdhg<sup>focon3/fcon3</sup> mice, a sharp decrease in cINs was observed, but now only MGE-derived PV, SST and a subpopulation of RLN cIN were affected. The number of VIP cells was not affected in these mice, suggesting that the reduction in the number of MGE-derived cINs does not affect the survival of those derived from the CGE. Consistent with recent observations (Carriere et al., 2020), the number of un-recombined PV and SST (PV+/tdTomato- and SST+/tdTomato- ) cINs in Nkx2.1<sup>Cre</sup>;Ai14;Pcdhg<sup>focon3/fcon3</sup> mice, increased compared to WT mice (Figure 3—figure supplement 4). These cells, which are likely derived from the dorsal Nkx6.2+/Nkx2.1- MGE domain (Hu et al., 2017a; Hu et al., 2017b; Fogarty et al., 2007; Sousa et al., 2009) may increase their survival in compensation for the loss of Nkx2.1-derived cINs lacking Pcdhg function. However, we cannot exclude that the increased number of un-recombined PV and SST cells in Nkx2.1<sup>Cre</sup>;Ai14;Pcdhg<sup>focon3/fcon3</sup> mice resulted from increased production or migration of cINs derived from regions of low, or no, expression of Nkx2.1. Further experiments will be required to understand the origin of these un-recombined PV+/Ai14- and SST+/Ai14- cINs and whether the observed increase in their numbers is due to compensatory survival mechanisms.

Pcdhg genes are not required for the normal production of young Nkx2.1-derived cINs in the MGE, or for their migration into the mouse cerebral cortex. The extent of proliferation in the MGE was essentially the same in Pcdhg WT and mutant mice, and the number of migrating MGE-derived cINs into the cortex was also indistinguishable between control and mutant mice. We did not directly address whether interference with the Pcdha and Pcdhb gene clusters affected the birth and migration of cINs, but we infer these two clusters also have no, or minimal effects on cIN production and migration because the final numbers of MGE-derived cINs were not significantly affected after the loss of either Pcdha or Pcdhb. However, the aggregate loss of Pcdh genes in multiple clusters might in principle be required for phenotypes to be manifested (Ing-Esteves et al., 2018). Our findings, therefore, cannot exclude the possibility that simultaneous elimination of the Pcdha and Pcdhb isoforms might have an effect on cIN production, migration, or apoptosis. However, the elimination of Pcdhg alone, and specifically of the PcdhgC3, PcdhgC4, and PcdhgC5 isoforms increases cell death among cINs, establishing that removal of a limited number of isoforms in the Pcdhg cluster is sufficient to reveal the cell-death phenotype.

Importantly, the increase in programmed cell death observed following the loss of Pcdhg function was fully rescued when the pre-apoptotic gene Bax was also eliminated. Not only was the increased Pcdh-dependent cell death eliminated in Bax mutant animals, but these animals had ~40% increase in the numbers of surviving cINs compared to WT controls, identical to the effect of the Bax mutation in wild type animals. This observation is consistent with previous observation showing that ~ 40% of cINs are eliminated during the period of programmed cell death (Southwell et al., 2012; Wong et al., 2018). Moreover, the increased death of cINs after removal of Pcdhg function occurs precisely during the normal period of programmed cell death. These observations indicate that Pcdh isoforms are required specifically to regulate cIN numbers during the critical window of programmed cell death. This is consistent with previous studies in the retina and spinal cord that have pointed to Pcdhg, and specifically the C-isoforms (PcdhgC3, PcdhgC4 and PcdhgC5), as key mediators of programmed cell death (Lefebvre et al., 2008; Chen et al., 2012). Interestingly, in all three neural structures, the cortex, the spinal cord, and the retina, Pcdhg C-isoforms appear to be the key regulators of survival of local circuit interneurons.
A recent study suggests that the Pcdhgc4 isoform is the key mediator in the regulation of neuronal cell death in the spinal cord (Garrett et al., 2019). How the specific Pcdhgc4 isoform in the Pcdh gene cluster mediates cell death remains a fundamental question for future research. Interestingly, the Pcdhgc4 isoform appears to be unique in that it is the only Pcdh isoform that does not bind in a homophilic manner (Garrett et al., 2019) and it is not translocated to the membrane unless it is associated with other Pcdha or Pcdhg (Aye et al., 2014). The mechanism by which the Pcdhgc4 isoform regulates cell-cell interactions among young cINs, leading to the adjustment of local circuit neuron numbers, remains unclear.

Heterochronic transplantation of cIN from the MGE into the cortex of WT mice allowed us to test for the survival of Pcdhg WT and Pcdhg mutant cIN simultaneously and in the same environment. As previously reported (Southwell et al., 2012), cINs die following their own time-course of maturation. Consistent with this, the transplanted cells (extracted from the MGE at E13.5) died with a delay of 6–12 days compared to the endogenous host cINs when transplanted into P0-P6 WT mice. This is consistent with the notion that the cellular age of cIN determines the timing of programmed cell death (Southwell et al., 2012). Interestingly, the role of Pcdhg function was clearly evident in these co-transplants in that the survival of the mutant cells was extremely low compared to that of WT cells. The dramatic decrease in the survival of Pcdhg mutant cIN occurs precisely during the period of programmed cell death for the transplanted population. The survival of transplanted WT cINs as well as that of Pcdhg mutant cINs was constant over a wide range of densities, as evidenced by the fact that while the density of transplanted cIN decreases as a function of the distance from the transplantation site, the proportion of dying cells of both phenotypes was similar at different distances from the site of transplantation.

Interestingly, the survival of WT cIN may also be reduced when co-transplanted with Pcdhg deficient MGE-cells although this difference did not reach statistical significance with the numbers of cases studied. If true, these findings would be consistent with the notion that cell-cell interactions among young cIN after their migration is an essential step in determining their final numbers. However, we cannot exclude that the increase in the elimination of WT cells may result from a non-specific (e.g., toxic) effect of the increased cell death among Pcdhg mutant cells. If the latter occurs, the process is specific to the population of Nkx2.1+ MGE-derived cINs because there was no effect on cell death of WT CGE-derived VIP cINs (Figure 3E, Figure 3—figure supplement 1) or on non-Nkx2.1-derived SST or PV cells (Figure 3—figure supplement 4). Interactions mediated by Pcdhg, and specifically among the C-isoforms, may directly or indirectly regulate survival of cINs of the same age and origin.

Unlike the spinal cord where cell death takes place prenatally (Wang et al., 2002b; Prasad et al., 2008), cIN programmed cell death occurs mostly postnatally. Since mice lacking Pcdhgc3, Pcdhgc4, and Pcdhgc5 isoforms die soon after birth, we could not study normal cIN cell death directly in these mutant animals. We, therefore, took advantage of transplantation and co-transplantation to compare the survival of cells lacking these three isoforms. The loss of cINs lacking Pcdhgc3, Pcdhgc4, and Pcdhgc5 isoforms was identical to that when the function of the entire Pcdh cluster is lost. This further suggests that these Pcdhgc C-isoforms (Pcdhgc3, Pcdhgc4, and Pcdhgc5) are the key to the regulation of cIN death. The co-transplantation assay, implemented in the present study, provides strong evidence that Pcdhg in cINs are key to their selection by programmed cell death. Pcdhg could be mediating initial cell-cell interactions that are important for the survival of cINs. Two non-exclusive possibilities exist: (1) Pcdhg mediate cell-cell interaction among young cINs to adjust their population size, and levels of inhibition, according to the numbers that reached the cortex; (2) Pcdhg mediate interactions with locally produced excitatory pyramidal neurons to adjust final numbers according to local levels of excitation. For the latter, MGE-derived cIN could interact with pyramidal neurons via Pcdhg C-isoforms. However, alternative # 2 is unlikely to explain how Pcdhg adjust cIN numbers since using conditional removal of Pcdhg in pyramidal cells shows no effect on the survival of cINs (Carriere et al., 2020). However, we cannot exclude that initial connectivity with excitatory pyramidal neurons may indeed require the proper expression of Pcdhg among cINs through non-homophilic interactions.

A recent study has shown that coordinated activity of synaptically connected assemblies of cINs is essential for their survival (Duan et al., 2020). Pyramidal cells receive information from these assemblies via GABA,γ2-signaling and through the de-synchronization of their activity regulate cIN programmed cell death. Pcdhg could be important in bringing together cINs of a common origin and at
similar stages of maturation for the formation of initial cIN functional assemblies. The formation of these assemblies of synchronously firing cINs and the subsequent selection by pyramidal driven desynchronization could explain both cell/population autonomous (Southwell et al., 2012), and non-cell autonomous (Wong et al., 2018) mechanisms of cIN programmed cell death. Interestingly, PCDHGCS binds to the GABA\textsubscript{A}\textgamma subunit of the GABA receptor (Li et al., 2012), but the role of PCDHGC5-GABA\textsubscript{A}\textgamma interaction on neuronal survival remains unknown. The transplantation assay provides a powerful tool to further study how Pcdhg, cell-cell interactions, and cellular age contribute to cIN selection. It will be interesting, for example, to determine if heterochronically transplanted cINs form functional assemblies and whether these assemblies are affected by the removal of different Pcdhg isoforms.

During the evolution of multiple mammalian species including that of humans, the cerebral cortex has greatly expanded in size and in the number of excitatory and inhibitory neurons it contains. Interestingly, the proportion of cINs to excitatory pyramidal neurons has remained relatively constant. Appropriate numbers of inhibitory cINs are considered essential in the modulation of cortical function. The embryonic origin of cINs, far from the cerebral cortex, raises basic questions about how their numbers are ultimately controlled in development and during evolution. Coordinated increased production of inhibitory interneurons in the MGE and CGE is an essential step to satisfy the demand of an expanded cortex (Hansen et al., 2013). In addition, MGE and CGE derived interneurons in larger brains require longer and more protracted migratory periods (Paredes et al., 2016). Interneurons arrive in excess of their final number. This is ultimately adjusted by a period of programmed cell death once the young cINs have arrived in the cortex. Here we have identified the C-isoforms (Pcdhgc3, Pcdhgc4 and Pcdhgc5) in the Pcdhg cluster as an essential molecular component that regulates programmed cell death among cINs. The fact that a cell surface adhesion protein plays a key role in this regulation suggests that interactions with other cells, possibly other cINs of the same age (Southwell et al., 2012), or possibly excitatory pyramidal cells (Wong et al., 2018), is part of the logic to adjust the final number of these essential GABAergic cells for proper brain function. An understanding of the cell-cell interactions that use Pcdhg C-isoform to regulate cIN cell death should give fundamental insights into how the cerebral cortex forms and evolves.

Materials and methods

### Key resources table

| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Genetic reagent (mouse)           | Gad2\textsuperscript{Cre} | PMID:21943598       |             | Also referred to as Gad2-IRES-Cre knock-in |
| Genetic reagent (mouse)           | Nkx2.1\textsuperscript{Cre} | PMID:17990269       |             | Also referred to as C57BL/6J-Tg(Nkx2-1-cre)2Sand/J |
| Genetic reagent (mouse)           | PV\textsuperscript{Cre}   | PMID:15836427       |             | Also referred to as B6;129P2-Pvalbtm1(cre)Arbr/J |
| Genetic reagent (mouse)           | Sst\textsuperscript{Cre}  | PMID:21943598       |             | Also referred to as Ssttm2.1(cre)Zjh/J |
| Genetic reagent (mouse)           | Ai14         | The Jackson Laboratory |             | Also referred to as Ai14, Ai14 D or Ai14 (RCL-tdT)-D |
| Genetic reagent (mouse)           | Gad1-GFP     | The Jackson Laboratory |             | Also referred to as G42 line. |
| Genetic reagent (mouse)           | Bax\textsuperscript{−/−} | The Jackson Laboratory |             | Also referred to as B6;129-Baxtm2Sjk Bak1tm1Thsn/J |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|-------------------|-------------|------------------------|
| Genetic reagent (mouse)           | Pcdha<sub>acon/acon</sub> | PMID: 28450636    |             | Referred to as Pcdha<sup>a</sup> in original publication. |
| Genetic reagent (mouse)           | Pcdh<sup>b</sup><sub>del/del</sub> | PMID: 28450637    |             | Referred to as Pcdh<sup>b</sup> in original publication. |
| Genetic reagent (mouse)           | Pcdh<sup>g</sup><sub>tako/tako; Pcdhga1, Pcdhga2, and Pcdhga3 mutant; Pcdhga1, Pcdhga2, and Pcdhga3 KO</sub> | PMID: 22884324  |             | Referred to as Pcdh<sup>g</sup> in original publication. |
| Genetic reagent (mouse)           | Pcdh<sup>g</sup><sub>tako/tako; Pcdhgc3, Pcdhgc4, and Pcdhgc5 mutant; Pcdhgc3, Pcdhgc4, and Pcdhgc5 KO</sub> | PMID: 22884324  |             | Referred to as Pcdh<sup>g</sup> in original publication. |
| Genetic reagent (mouse)           | Pcdh<sup>g</sup><sub>fcon3/fcon3</sub> | PMID: 19029044    |             | Referred to as Pcdh<sup>g</sup> in original publication. |
| Antibody                          | anti-GFP (chicken polyclonal) | Aves Lab          | Cat#: GFP-1020, RRID: AB_10000240 | IF(1:2500) |
| Antibody                          | Anti-Reelin (mouse monoclonal) | MBL International | Cat#: MBL, D223-3, RRID: AB_843523 | IF(1:500) |
| Antibody                          | Anti-PV (rabbit antiserum)    | Swant             | Cat#: PV27, RRID: AB_2631173 | IF(1:1000) |
| Antibody                          | Anti-PV (mouse monoclonal)    | Sigma-Aldrich     | Cat#: P3088, RRID: AB_477329 | IF(1:500) |
| Antibody                          | Anti-SST (rat, polyclonal)    | Santa Cruz Biotechnology | Cat#: sc-7819, RRID: AB_2302603 | IF(1:500) |
| Antibody                          | Anti-cleaved caspase 3 (rabbit polyclonal) | Cell Signaling Technology | Cat#: 9661L, RRID: AB_2341188 | IF(1:400) |
| Antibody                          | Anti-phosphohiston-H3 (rabbit polyclonal) | EDM Millipore | Cat#: 06–570, RRID: AB_310177 | IF(1:500) |
| Antibody                          | Anti-NKX2-1 (rabbit polyclonal) | Life Technologies | Cat#: sc-13040, ARRID: AB_230472 | IF(1:250) |
| Chemical compound, drug           | DNAse I | Sigma Millipore | Cat#: 260913-10MU | 180 ug/mL |
| Commercial assay or kit           | QuantiTect Rev. Transcription Kit | Qiagen         | Cat#: 205311 |             |
| Software, algorithm               | Stereo Investigator | MBF bioscience |             |             |
| Software, algorithm               | NeuroLucida | MBF bioscience |             |             |

Continued on next page


Animals

R26-Ai14, Gad1-GFP, Gad2-ires-Cre (Gad2<sup>Cre</sup>), BAC-Nkx2.1-Cre (Nkx2.1<sup>Cre</sup>), Sst-ires-Cre (Sst<sup>Cre</sup>), PV-RES-Cre-pA (PV<sup>Cre</sup>), Bax<sup>/−</sup>, Bax<sup>fl/fl</sup> and WT C57BL/6J breeders were purchased from the Jackson Laboratory. Whenever possible, the number of males and females was matched for each experimental condition. All protocols and procedures followed the University of California, San Francisco (UCSF) guidelines and were approved by the UCSF Institutional Animal Care Committee.

Pcdh<sup>g</sup> loss of function mice were obtained by crossing Pcdhg<sup>fon3/fon3</sup> mice with Gad2<sup>Cre</sup>;Ai14; Pcdhg<sup>fon3/+</sup> mice, Nkx2.1<sup>Cre</sup>;Ai14;Pcdhg<sup>fon3/+</sup>, PV<sup>Cre</sup>;Ai14;Pcdhg<sup>fon3/+</sup> or Sst<sup>Cre</sup>;Ai14;Pcdhg<sup>fon3/+</sup>. Pcdhya<sub>1</sub>, Pcdhya<sub>2</sub> and Pcdhya<sub>3</sub> isoform knockout mice were obtained from crosses of Pcdhg<sup>taiko/tako</sup> to Nkx2.1<sup>Cre</sup>;Ai14;Pcdhg<sup>taiko/+</sup> mice.

Pcdha loss function mice were obtained by crossing Pcdha<sup>acon/acon</sup> mice with Nkx2.1<sup>Cre</sup>;Ai14; Pcdha<sup>acon/+</sup> mice. Pcdhb loss function mice were obtained by crossing Pcdhb<sup>del/del</sup> mice with Nkx2.1<sup>Cre</sup>;Ai14;Pcdhb<sup>del/+</sup> mice. Embryonic donor tissue was produced by crossing WT C57BL/6J to heterozygous mice expressing green fluorescent protein-expressing (GFP) driven by Gad1 promoter. Pcdhg<sup>fon3/fon3</sup> (tdTomato-expressing) tissue was obtained from embryos produced by crossing Nkx2.1<sup>Cre</sup>;Ai14;Pcdhg<sup>fon3/+</sup> mice with Pcdhg<sup>fon3/fon3</sup> mice. Pcdhg<sup>taiko/taiko</sup> (tdTomato-expressing) tissue was obtained from embryos produced by crossing Nkx2.1<sup>Cre</sup>;Ai14;Pcdhg<sup>taiko/taiko</sup> mice. Homozygous Pcdhg<sup>taiko/taiko</sup> mice die around birth. GAD1-GFP and Nkx2.1<sup>Cre</sup>;Ai14 offspring were genotyped under an epifluorescence microscope (Leica), and PCR genotyping was used to screen for Pcdhg<sup>fon3/fon3</sup>, Pcdhg<sup>taiko/taiko</sup> and Pcdhg<sup>taiko/taiko</sup> embryos in GFP or Ai14 positive animals. All cell transplantation experiments were performed using wild type C57Bl/6J recipient mice. All mice were housed under identical conditions.

Immunostaining

Mice were fixed by transcardiac perfusion with 10 mL of ice-cold PBS followed by 10 mL of 4% formaldehyde/PBS solution. Brains were incubated overnight (12–24 hr) for postfixation at 4°C, then rinsed with PBS and cryoprotected in 30% sucrose/PBS solution for 48 hr at 4°C. Unless otherwise stated, immunohistochemistry was performed on 50 μm floating sections in Tris Buffered Saline (TBS) solution containing 10% normal donkey serum, 0.5% Triton X-100 for all procedures on postnatal mice. Immunohistochemistry from embryonic tissue was performed on 20 μm cryostat sections. All washing steps were done in 0.1% Triton X-100 TBS for all procedures. Sections were incubated overnight at 4°C with selected antibodies, followed by incubation at 4°C overnight in donkey secondary antibodies (Jackson ImmunoResearch Laboratories). For cell counting and post hoc examination of marker expression, sections were stained using chicken anti-GFP (1:2500, Aves Labs, GFP-1020, RRID:AB_10009240), mouse anti-Reelin (1:500 MBL, D223–3, RRID:AB_843523), rabbit anti-PV (1:1000, Swant PV27, RRID:AB_2631173), mouse anti-parvalbumin (anti-PV, 1:500, Sigma-Aldrich, P3088, RRID:AB_477329), rat anti-somatostatin (SST, 1:500, Santa Cruz Biotechnology, sc-7819, RRID:AB_2302603), anti-cleaved caspase 3 (1:400, Cell Signaling Technology, 9661L, RRID:AB_2341188), rabbit anti-phosphohistone-H3 (1:500, EDM Millipore, RRID:AB_310177), rabbit anti-NKX2-1 (1:250, Life Technologies, RRID:AB_793532).
Cell counting

For cell density counts in the visual and barrel cortex (Figure 2C & C', Figure 3C & C', Figure 5C, Figure 6 and Figure 7), cells were directly counted using a Zeiss Axiover-200 inverted microscope (Zeiss) and an AxioCam MRM camera (Zeiss), using Stereo Investigator (MBF). tdTomato+ cells were counted in every six sections (i.e., 300 µm apart) along the rostral-caudal axis of visual and barrel cortex. Total cell counts were extrapolated by Stereo Investigator. Cell densities were determined by dividing the total number of tdTomato+ cells by the volume of the region of interest in the visual or barrel cortex, identified by landmarks, for each animal. To measure PV, SST, RLN and VIP-positive cell densities in the visual cortex (Figure 2D and Figure 3E), cells were counted from confocal-acquired images. Cleaved caspase-3-positive (CC3+) cells were counted from images acquired on a Zeiss Axiover-200 inverted microscope (Zeiss) and an AxioCam MRM camera (Zeiss) using Neuroulucida (MBF). Cleaved caspase-3-positive cells were counted in the cortex of every six sections along the rostral-caudal axis for each animal (Figure A and B). Cells in the olfactory bulb, hippocampus, and piriform cortex were not counted. For layer distribution analysis of tdTomato+ cells and for analysis of tdTomato negative (non-recombined) PV and SST positive cells (Figure 2—figure supplement 2, Figure 3—figure supplements 3 and 4), cells were counted from confocal-acquired images and using Fiji software cell counter function.

Cell dissection and transplantation

Unless otherwise mentioned, MGEs were dissected from E13.5 embryos as previously described (Southwell et al., 2012). The day when the sperm plug was observed was considered E0.5. Dissections were performed in ice-cold Leibovitz L-15 medium. MGEs were kept in L-15 medium at 4°C. MGEs were mechanically dissociated into a single cell suspension by repeated pipetting in L-15 medium containing DNase I (180 µg/ml). The dissociated cells were then centrifuged (4 min, 800xg). For all co-transplantations, the number of cells in each suspension (GFP+ or tdTomato+) was determined using a hemocytometer. Concentrated cell suspensions were loaded into beveled glass micropipettes (~70–90 µm diameter, Wiretrol 5 µl, Drummond Scientific Company) prefilled with mineral oil and mounted on a microinjector. Recipient mice (C57Bl/6) were anesthetized by hypothermia (~4 min) and positioned in a clay head mold that stabilizes the skull (Merkle et al., 2007). Micropipettes were positioned at an angle of 0 degrees from vertical in a stereotactic injection apparatus. Unless otherwise stated, injections were performed in the left hemisphere 1 mm lateral and 1.5 mm anterior from Lambda, and at a depth of 0.8 mm from the surface of the skin. After the injections were completed, transplant recipients were placed on a warm surface to recover from hypothermia. The mice were then returned to their mothers until they were perfused or weaned (P21). Transplantation of Nkx2.1Cre;Ai14;Pcdhgtako/tako was performed using frozen cells (Figure 11B). For these experiments, dissected MGEs from each embryo were collected in 500 µL L15 and kept on ice until cryopreserved. MGEs were resuspended in 10% DMSO in L15 and cryopreserved as previously described (Rodriguez-Martinez et al., 2017). Vials were cooled to −80°C at a rate of −1°C/minute in a Nalgene Mr. Frosty Freezing Container and then transferred to liquid nitrogen for long term storage. Prior to transplantation, vials were removed from −80°C and thawed at 37°C for 5 min, after which the freezing medium was removed from the vial and replaced with 37°C L-15. Dissociation was performed as above.

For cell counts from transplanted animals, Gad1-GFP positive cells and tdTomato-positive cells were counted in all layers of the neocortex. Cells that did not display neuronal morphology were excluded from all quantifications. The vast majority of cells transplanted from the E13.5 MGE exhibited neuronal morphologies in the recipient brain. GFP and tdTomato-positive cells were counted from tiles acquired on a Zeiss Axiover-200 inverted microscope (Zeiss) with an AxioCam MRM camera (Zeiss); using Neuroulucida (MBF). For quantification of the absolute numbers of transplanted cells in the neocortex of host recipients, cells from every second coronal section were counted (Figure 9). The raw cell counts were then multiplied by the inverse of the section sampling frequency (2) to obtain an estimate of total cell number (Figure 10). In some experiments the absolute number of grafted cells varies between transplants; hence we report findings as the fraction of cells (GFP or tdTomato) that survive from the total transplant-derived cell number in that animal (GFP + tdTomato-positive) (Figure 8). We determined for these experiments the number of transplant-derived cells of the different genotypes before and, in different animals, after the period of cell death. For
Table 1. Group data for the frequency of spontaneous excitatory and inhibitory synaptic currents (Hz), max firing rate (Hz), tau (ms), capacitance (pF), and input resistance (mOhm) from Figure 13. The mean, standard deviation, sample size, and statistical tests are reported. Comparisons were not statistically significant following Benjamini-Hochberg multiple comparisons correction at an alpha of 0.05.

| Frequency of spontaneous excitatory synaptic currents (Hz) | Frequency of spontaneous inhibitory synaptic currents (Hz) |
|----------------------------------------------------------|----------------------------------------------------------|
| **Pcdhg<sup>fcon3</sup>/fcon3** | **WT** | Mann-Whitney | **Pcdhg<sup>fcon3</sup>/fcon3** | **WT** | Mann-Whitney |
| Mean ± SD | Mean ± SD | p value | Mean ± SD | Mean ± SD | p value |
| 8DAT | 1.0 ± 0.7 (n = 18) | 1.0 ± 0.7 (n = 9) | 0.890 | 0.4 ± 0.2 (n = 18) | 0.4 ± 0.2 (n = 6) | 0.463 |
| 9DAT | 1.4 ± 1.4 (n = 19) | 1.6 ± 1.4 (n = 21) | 0.432 | 0.4 ± 0.2 (n = 15) | 0.4 ± 0.2 (n = 18) | 0.347 |
| 10DAT | 2.2 ± 1.0 (n = 9) | 3.4 ± 2.8 (n = 9) | 0.652 | 0.6 ± 0.2 (n = 9) | 0.6 ± 0.3 (n = 7) | 0.859 |
| 11DAT | 3.2 ± 2.3 (n = 14) | 2.5 ± 1.8 (n = 13) | 0.298 | 0.6 ± 0.5 (n = 11) | 0.7 ± 0.4 (n = 11) | 0.711 |
| 12DAT | 2.6 ± 2.1 (n = 11) | 3.3 ± 2.8 (n = 14) | 0.597 | 0.7 ± 0.1 (n = 10) | 0.7 ± 0.2 (n = 13) | 0.436 |
| **Max Firing Rate (Hz)** | **Tau (ms)** |
| **Pcdhg<sup>fcon3</sup>/fcon3** | **WT** | Mann-Whitney | **Pcdhg<sup>fcon3</sup>/fcon3** | **WT** | Mann-Whitney |
| Mean ± SD | Mean ± SD | p value | Mean ± SD | Mean ± SD | p value |
| 8DAT | 21.8 ± 23 (n = 16) | 21.5 ± 22.9 (n = 12) | 0.789 | 3.6 ± 4.3 (n = 8) | 3.8 ± 2.6 (n = 4) | 0.683 |
| 9DAT | 29.1 ± 25.1 (n = 17) | 35.0 ± 22.3 (n = 19) | 0.416 | 6.8 ± 5.3 (n = 5) | 8.7 ± 6.7 (n = 10) | 0.717 |
| 10DAT | 35.7 ± 22.8 (n = 6) | 37.7 ± 28.6 (n = 7) | 1.000 | 11.0 ± 8 (n = 6) | 7.8 ± 6.7 (n = 6) | 0.387 |
| 11DAT | 24.5 ± 24.4 (n = 11) | 36.3 ± 34.9 (n = 12) | 0.534 | 5.7 ± 4.3 (n = 10) | 7.3 ± 5.7 (n = 9) | 0.707 |
| 12DAT | 35.0 ± 32.3 (n = 10) | 29.8 ± 29.3 (n = 12) | 0.757 | 5.8 ± 4.1 (n = 8) | 5.5 ± 3.9 (n = 9) | 0.880 |
| **Capacitance (pF)** | **Input Resistance (mOhm)** |
| **Pcdhg<sup>fcon3</sup>/fcon3** | **WT** | Mann-Whitney | **Pcdhg<sup>fcon3</sup>/fcon3** | **WT** | Mann-Whitney |
| Mean ± SD | Mean ± SD | p value | Mean ± SD | Mean ± SD | p value |
| 8DAT | 18.3 ± 7.3 (n = 16) | 11.6 ± 2.7 (n = 10) | 0.019 | 257.3 ± 87.1 (n = 23) | 313.6 ± 86.5 (n = 16) | 0.027 |
| 9DAT | 16.4 ± 6.0 (n = 15) | 17.2 ± 5.1 (n = 19) | 0.465 | 327 ± 106.5 (n = 23) | 278.9 ± 83.4 (n = 23) | 0.095 |
| 10DAT | 20.5 ± 2.8 (n = 9) | 20.2 ± 3.9 (n = 7) | 0.837 | 297.5 ± 94.3 (n = 11) | 264.7 ± 52.9 (n = 9) | 0.304 |
| 11DAT | 22.4 ± 5.1 (n = 15) | 20.4 ± 6.5 (n = 13) | 0.503 | 269.1 ± 79.6 (n = 15) | 294.2 ± 59.9 (n = 14) | 0.185 |
| 12DAT | 23.2 ± 5.8 (n = 13) | 21.5 ± 3.4 (n = 15) | 0.338 | 266.5 ± 96.4 (n = 13) | 262.3 ± 83.6 (n = 15) | 0.882 |

Some experiments, we also quantified the number of transplant-derived cells during the period of cell death (Figure 12B). In data presented as the fraction of transplant-derived cells (Figure 8 and Figure 11), GFP positive and tdTomato-positive cells were counted from coronal sections along the rostral-caudal axis in at least 10 sections per animal. The number of GFP or tdTomato-positive cells was divided by the total cell number (GFP + tdTomato) in that section. This fraction does not reflect the absolute number of cells, but their relative contribution to the overall population of transplant-derived cells at different DAT. For one experiment (Figure 10), we calculated the absolute number of transplant-derived WT (GFP) and Pcdhg<sup>fcon3</sup>/fcon3 (tdTomato) cells. For this experiment, 50 K cells of each genotype were counted before transplantation.

**Neuron Morphology analysis**

Recipient brains were co-transplanted with Gad1-GFP (WT Pcdhg) and Nkx2.1<sup>Cre</sup>;Ai14; Pcdhg<sup>fcon3</sup>/fcon3 (mutant Pcdhg) MGE cIN precursors. Transplanted cells were identified in sections (50 µm) stained for GFP and tdTomato and analyzed at 6, 8, 10 and 12 days after transplantation (DAT). Neuron morphology was reconstructed from confocal image (20X objective-4X zoom) stacks taken at 1 µm intervals, using NeuronLucida software (MBF). Sections with a relatively low density of GFP+ (WT) and TdTomato+ (Mutant) transplant-derived neurons (in order to clearly image individual cells) were selected and (145.31 um<sup>2</sup>) fields tiled to cover all the visible processes. All GFP+ (WT) and TdTomato+ (Mutant) positive neurons in these tiles were reconstructed. Neuron morphometric analysis was done using NeuronLucida Explorer.
RT-PCR
Total RNA was prepared from dissected cortex of using Trizol (Invitrogen) and reverse-transcribed by Quantiscript Reverse Transcriptase (Qiagen), using a mix of oligo-dT and random primers, according to the manufacturer’s protocol. Primer sequences for gene expression analysis in Figure 1 are provided in Appendix 1—table 1.

Slice electrophysiology
As in Larimer et al., 2016; Larimer et al., 2017; Priya et al., 2019, host animals were anesthetized and decapitated at 8, 9, 10, 11, or 12 days after transplant. The brain was removed into ice-cold dissection buffer containing (in mM): 234 sucrose, 2.5 KCl, 10 MgSO$_4$, 1.25 NaH$_2$PO$_4$, 24 NaHCO$_3$, 11 dextrose, 0.5 CaCl$_2$, bubbled with 95% O$_2$/5% CO$_2$ to a pH of 7.4. Coronal slices of visual cortex (200 µm thick) were cut via vibratome (Precisionary Instruments) and transferred to artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 3 KCl, 2 MgSO$_4$, 1.23 NaH$_2$PO$_4$, 26 NaHCO$_3$, 10 dextrose, 2 CaCl$_2$ (bubbled with 95% O$_2$/5% CO$_2$), incubated at 33˚C for 30 min, then stored at room temperature. Fluorescently identified transplant-derived MGE-lineage interneurons (tdTomato +; Pcdhg$^{fcon3/fcon3}$ or GFP; WT) were viewed using IR-DIC video microscopy. Whole-cell current-clamp recordings were made with a Multiclamp 700B (Molecular Devices) using an internal solution that contained (in mM): 140 K-gluconate, 2 MgCl$_2$, 10 HEPES, 0.2 EGTA, 4 MgATP, 0.3 NaGTP, 10 phosphocreatine (pH 7.3, 290 mosm). Data were low-pass filtered at 2.6 kHz and digitized at 10 kHz by a 16 bit analog-to-digital converter (National Instruments). Data acquisition and analysis were done with custom software written in Matlab (https://www.mathworks.com/matlabcentral/fileexchange/21903-mphys). A Mann-Whitney (nonparametric) test, followed by multiple comparisons correction using the Benjamini-Hochberg stepdown method for control of false discovery rate (0.05 familywise) was used for the determination of statistical significance for all comparisons of physiological properties.

Table 2.
Ratios are reported for the frequency of spontaneous excitatory and inhibitory synaptic currents where wild type and Pcdhg$^{fcon3/fcon3}$ co-transplants are compared within the same slice. The natural log of each ratio was averaged for 8, 9, 10, 11, and 12 DAT and show that slice variation was not significant.

| Frequency of spontaneous excitatory synaptic currents | Frequency of spontaneous inhibitory synaptic currents |
|-----------------------------------------------------|---------------------------------------------------|
| LN(Pcdhg$^{fcon3/fcon3}$/WT)                        | LN(Pcdhg$^{fcon3/fcon3}$/WT)                      |
| Mean ± SD                                           | Mean ± SD                                         |
| 8DAT -0.305 ± 0.425                                 | 0.162 ± 0.793                                    |
| 9DAT 0.177 ± 0.468                                  | 0.018 ± 0.550                                    |
| 10DAT 0.321 ± 0.706                                 | 0.301 ± 0.436                                    |
| 11DAT 0.120 ± 0.927                                 | -0.075 ± 0.262                                   |
| 12DAT -0.523 ± 0.943                                | -0.072 ± 0.128                                   |

Statistical Analysis
The person carrying quantifications was blinded to the genotype, except for data shown in Figure 2—figure supplement 2, and Figure 3—figure supplements 3 and 4. With the exception of slice electrophysiology data, all results were plotted and tested for statistical significance using Prism 8. All samples were tested for normality using the Shapiro-Wilk normality test. Unpaired comparisons were analyzed using the two-tailed unpaired Student’s t test for normally distributed, and Mann-Whitney test for not normally distributed samples. For multiple comparisons analysis of one variable, either a one-way ANOVA with post hoc Tukey’s test was used to compare the mean of each column with the mean of every other column, or a Dunnett test was used to compare the mean of each column to the mean of the control group for normally distributed samples. For samples with non-Gaussian distributions, a nonparametric Kruskal-Wallis test was performed followed by a post-hoc Dunn’s test. Two-way ANOVA with post hoc Sidak’s test was used for multiple comparisons with more than one variable. Outliers were identified using ROUT method with alpha set to 0.05.
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Additional information

Competing interests
Arturo Alvarez-Buylla: is cofounder, serves on the scientific advisory board, and owns shares in Neuron Therapeutics. The other authors declare that no competing interests exist.

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### Appendix 1—table 1. Clustered protocadherin primer sequences for RT-PCR gene expression analysis in Figure 1.

| Gene name | Oligo name | Sequence forward/reverse |
|-----------|------------|-------------------------|
| Pcdhga1   | Pcdhga1    | CACGAGAGCTGTGAGAAACAGG F |
| Pcdhga2   | Pcdhga2    | CTGATTCCCTCTCAGCACCTCAG F |
| Pcdhga3   | Pcdhga3    | GAAACGAAAGAGCCCCCACGC F |
| Pcdhga4   | Pcdhga4    | CTCCTGTATCTCAGACCTTG C |
| Pcdhga5   | Pcdhga5    | CACACAAAGAGCGCCCCGAGA F |
| Pcdhga6   | Pcdhga6    | GCAAAGAGGAGAGCTCTTGAG F |
| Pcdhga7   | Pcdhga7    | TCAAGAAGTGAGGGAAGAGCC F |
| Pcdhga8   | Pcdhga8    | CATCCATAGATTCTCGAGAAATAA F |
| Pcdhga9   | Pcdhga9    | TCAGTTGAGCCCAAGTTTTCC F |
| Pcdhga10  | Pcdhga10   | CCAAGTCTCTGTAAGAGAGGC F |
| Pcdhga11  | Pcdhga11   | GCAGGCTCTCTGATAACTG F |
| Pcdhga12  | Pcdhga12   | TCTTTACATCGGGTGATTCGG F |
| Pcdhgb1   | Pcdhgb1    | CAGGATCTCCTCTGATAACTG F |
| Pcdhgb2   | Pcdhgb2    | GACTCTGGGTACACAGGACTC F |
| Pcdhgb4   | Pcdhgb4    | TGGACAGTTGAGATACTGAGAAGAAGAGCGGCCACGC F |
| Pcdhgb5   | Pcdhgb5    | CCAAGTTTGCCCTGATACTC F |
| Pcdhgb6   | Pcdhgb6    | CTAAATTCCGCTACCTTGG F |
| Pcdhgb7   | Pcdhgb7    | AGAGATAGCTCTCGCCAGCTGTG F |
| Pcdhgb8   | Pcdhgb8    | CAGAGACTTTTGTAGGGAGGC F |
| Pcdhgc3   | Pcdhgc3    | GCAGCAGGGTGATCTCC F |
| Pcdhgc4   | Pcdhgc4    | CAGGCTGCTCACCTCGATCTT F |
| Pcdhgc5   | Pcdhgc5    | GCTTGGCTCCCGTCCTGATTA F |
| PcdhgcCon | PcdhgcCon  | GTAAAACGCTCCGTCCTGAG F |
| PcdhA1    | PcdhA1_1   | TTTTTGACGACACCCCGTC F |
| PcdhA2    | PcdhA2_2   | TTTCCTCTAGCACTGACCTC F |
| PcdhA3    | PcdhA3_3   | GTGGGAAAGAGCGAGCTTTA F |
| PcdhA4    | PcdhA4_4   | AGCTGTGGGAAGAGTGATCC F |
| PcdhA5    | PcdhA5_5   | AGAGCTGTGAGGAGAGTGAGC F |
| PcdhA6    | PcdhA6_6   | CACGAGTGAGGAGCTCTTG F |
| PcdhA7    | PcdhA7_7   | ATACGACCAAGATGCTGAG F |
| PcdhA8    | PcdhA8_8   | CATCCATAGATTCCCTGAG F |
| PcdhA9    | PcdhA9_9   | TGTGGGAAAGAGCTGCTCTC F |
| PcdhA11   | PcdhA11_11 | GAAACGACGACTCTCTCG F |
| PcdhB1    | PcdhB1_1   | TGTGGGAGGTGTTCTCG F |
| PcdhB2    | PcdhB2_2   | GACTCCGGAAGTTGCTCCTC F |
| PcdhB4    | PcdhB4_4   | CAGGACAAAGATGCTACATTTG F |
| PcdhB5    | PcdhB5_5   | TCTGGACAAAGGCTCTTG F |
| PcdhB6    | PcdhB6_6   | GATCGTTTCGCGTAGCTCTCC F |
| Gene name     | Oligo name | Sequence                                      | forward/reverse |
|--------------|-----------|----------------------------------------------|-----------------|
| Pcdhgb7      | 2-PcdhgbB7 | TCCAGCCGCGAAGATATTC                         | F               |
| Pcdhgb8      | 2-PcdhgbB8 | CGAGACCTTGTACGGAAGC                        | F               |
| Pcdhgc3      | PcdhgcC_3 | TGCGAAGTGTGAGCTCCGTG                       | F               |
| Pcdhg4       | PcdhgC_4  | CAAGCTTGTCACCCTCTGATG                      | F               |
| PcdhgCon     | Pcdhg_COM-R| GAGAGAAACGCCAGTCAAGT                        | R               |
| Pcdha1       | 2-PcdhAlpha_1 | AAAAGATGCACCAGCAGAG                         | F               |
| Pcdha2       | PcdhA2-F   | GGAATCACTACGAGGAGAGAGACAC                 | F               |
| Pcdha3       | PcdhAlpha_3 | ACACCATGCCAGTGTAACTAG                   | F               |
| Pcdha4       | PcdhAlpha_4 | TCTGATTCAGGAGCAGAGACAG                   | F               |
| Pcdha5       | Pcdh_a5    | TTCACTCCAGCAGCTCACGTA                      | F               |
| Pcdha6       | Pcdh_a6    | TGAGCCTAGCAGGAGACGTA                      | F               |
| Pcdha7       | Pcdh_a7    | GTGTTCCAGCAGCTCAGATAAC                     | F               |
| Pcdha8       | Pcdh_a8    | TTCTTGGACTCTCCAGGAG                       | F               |
| Pcdha9       | PcdhAlpha_9 | CGGAAGTGGGATGGAAGG                         | F               |
| Pcdha10      | PcdhAlpha_10 | CAGTGTTCTCTCTGTGTGG                      | F               |
| Pcdha11      | Pcdh_a11   | TCCCAACCTGTGAGTCAAG                       | F               |
| Pcdha12      | PcdhAlpha_12 | TGCAGAGGACACGATGCAAG                   | F               |
| Pcdhac1      | PcdhAlpha_C1 | TGCCCAGTATCTGTGTTCAGAG                     | F               |
| Pcdhac2      | Pcdh_a2    | AAATCCAGCGGCCAAGAGAAG                        | F               |
| PcdhaCon     | Pcdh_a-CON | TGCTTTAGCAGGACGAGG                         | R               |
| Pcdhb1       | pcdh1_F    | TAGGTTCCGAGGAGGTAACAG                     | F               |
| Pcdhb1       | Pcdh_B1_R  | GGATCAGATTTCGCAAGAAG                       | R               |
| Pcdhb2       | 2Pcdh_B2_F | AATGGTTTGCTCCATCCAGAG                     | F               |
| Pcdhb2       | 2Pcdh_B2_R | GATCCAGGGCTGTTGTTGTC                      | R               |
| Pcdhb3       | PcdhB3-F   | TGCTCTCCACAGGACATGCA                      | F               |
| Pcdhb3       | PcdhB3-R   | CAGAGGTTGCTCCAGATGG                       | R               |
| Pcdhb4       | pcdhb4_F   | TCTGGAGGTAGGCCAGATTTCA                    | F               |
| Pcdhb4       | pcdhb4_R   | GATGTCCCTCATAGCGAGGAG                     | R               |
| Pcdhb5       | 3Pcdh_B5_F | TGTTGCTAGGCTTACTCCCTG                     | F               |
| Pcdhb5       | 3Pcdh_B5_R | CTCAGTCCACAGGACATCGC                      | R               |
| Pcdhb6       | PcdhB6_F   | TGCCAGGACAAATCGAAGGAGAAGGCACTT            | F               |
| Pcdhb6       | PcdhB6-R   | CTCAGTCCAGGATGGAGGAG                      | R               |
| Pcdhb7       | pcdhb7_F   | GACCTCATGGAAGACTGAGA                      | F               |
| Pcdhb7       | 2-pcdhb7_R | GAGTTGCTGGCTGACTCCGAA                      | R               |
| Pcdhb8       | 2-pcdhb8_F | ATTCAGATGCCAGAAAGAAGC                     | F               |
| Pcdhb8       | 2-pcdhb8_R | TAGTGTCAGTCCCCACCC                      | R               |
| Pcdhb9       | 2-pcdhb9_F | ACAAAGGAGAAGCGAAGAGAAGC                    | F               |
| Pcdhb9       | 2-pcdhb9_R | TCAGTGTTTGCTGACTCAATC                      | R               |
| Pcdhb10      | 2PcdhB10-F | GTATTTAGGGCATAGCAGG                      | R               |
| Pcdhb10      | 2PcdhB10-R | AGAGGGCGCTTCCTTCTCTAGT                     | R               |
| Pcdhb11      | 3Pcdh_beta11_F | CGACCACTTCCAGAGGTTCC              | F               |
| Pcdhb11      | 3Pcdh_beta11_R | GCTGCCTTCCAGAGGAAACAC              | R               |
| Gene name | Oligo name    | Sequence                     | forward/reverse |
|-----------|--------------|------------------------------|-----------------|
| Pcdhb12   | pcdhb12_F    | CTGGGATATATGGCAATGTCG        | F               |
| Pcdhb12   | pcdhb12_R    | GTCAGACGGATTTCCTGTTG         | R               |
| Pcdhb13   | 2PcdhB13-F   | GATAACGCTCCAGAAGTGATCC       | F               |
| Pcdhb13   | 2PcdhB13-R   | CTCCTGTTATTTCCAGATCC        | R               |
| Pcdhb14   | 2-pcdhb14_F  | CCCAGCACACATAACAGTG          | F               |
| Pcdhb14   | 2-pcdhb14_R  | GATGGTGCCCTATGCAATG         | R               |
| Pcdhb15   | PcdhB15-F    | CTCAGTCCCGTACTGGAGATAT      | F               |
| Pcdhb15   | PcdhB15-R    | AATGGTCTTCCAACAGCAACT       | R               |
| Pcdhb16   | pcdhb16-F    | TCATCGTGAGAACAACAGC         | F               |
| Pcdhb16   | pcdhb16-R    | GCAGCAGCGAGTAAGTGATG        | R               |
| Pcdhb17   | PcdhB17-F    | AAGAGAGCCTTGACAGGGGAAG      | F               |
| Pcdhb17   | PcdhB17-R    | AGACCTGCACTTTATGGTG         | R               |
| Pcdhb18   | PcdhB18-F    | TGCAATGGACTTTCGTGGGAC       | F               |
| Pcdhb18   | pcdhb18-R    | TAGCCATGTGTTAGAAAGCAAGCA    | R               |
| Pcdhb19   | 3Pcdh_beta19_F | TGCCTCTTCCCTCCTCTG           | F               |
| Pcdhb19   | 3Pcdh_beta19_R | AGTCCCAGCAGTTAATGC          | R               |
| Pcdhb20   | PcdhB20-F    | GAAGTGATCATGTGCCTGTAAT      | F               |
| Pcdhb20   | PcdhB20-R    | CTTCCGGTTGTCCCCAGAGTCTT     | R               |
| Pcdhb21   | 2Pcdh_beta21_F | ACAGCAGCGGGCTTTCTAC         | F               |
| Pcdhb21   | 2Pcdh_beta21_R | GCCAGCTCAGAGGTTGTTG         | R               |
| Pcdhb22   | 2Pcdh_beta22_F | GCTCTGCTAGCCGTCAACTG        | F               |
| Pcdhb22   | 2Pcdh_beta22_R | ATCACCTCTGGTGACTGG          | R               |