PNAS

Supporting Information for
The Clustered Gamma Protocadherin PcdhγC4 Isoform Regulates Cortical Interneuron Programmed Cell Death in the Mouse Cortex.

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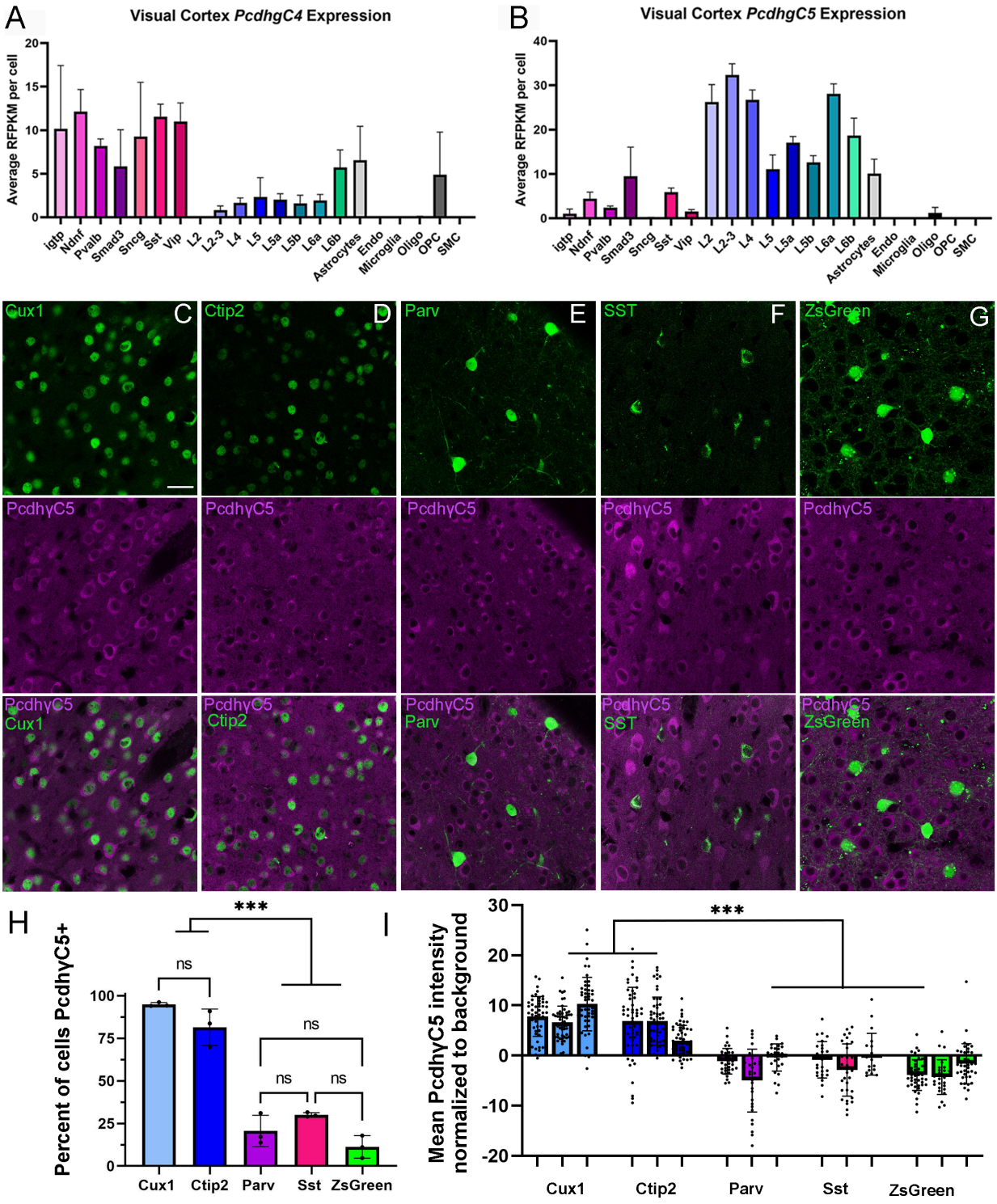
Figures S1 to S6


A
Visual Cortex PcdhgC4 Expression
Average RFPKM per cell
Igtp
Ndnf
Pvalb
Smad3
Sncg
Sst
Vip
L2
L2-3
L4
L5
L5a
L5b
L6a
L6b
Astrocytes
Endo
Microglia
Oligo
OPC
SMC
B
Visual Cortex PcdhgC5 Expression
Average RFPKM per cell
C
Cux1
D
Ctip2
E
Parv
F
SST
G
ZsGreen
PcdhγC5
PcdhγC5 Cux1
PcdhγC5 Ctip2
PcdhγC5 Parv
PcdhγC5 SST
PcdhγC5 ZsGreen
H
Percent of cells PcdhγC5+
***
ns
I
Mean PcdhγC5 intensity normalized to background
Cux1
Ctip2
Parv
Sst
ZsGreen

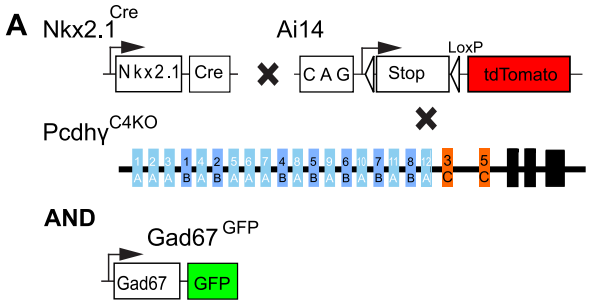
Supplementary Figure 1 - PcdhγC5 expression in excitatory cortical neurons.

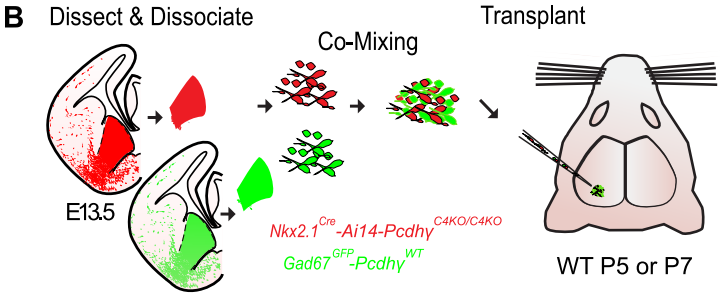
A-B. Inhibitory neuron subtypes show enriched PcdhγC4 expression (A), contrasting glutamatergic neuron subtypes which have enriched PcdhγC5 expression (B). Error bars represent the SEM. Interferon gamma-induced GTPase (Igtp), Neuron-derived neurotrophic factor (Ndnf), Parvalbumin (Pvalb), Somatostatin (SST), and Vasoactive intestinal peptide-expressing (Vip). Error bars represent the SEM.

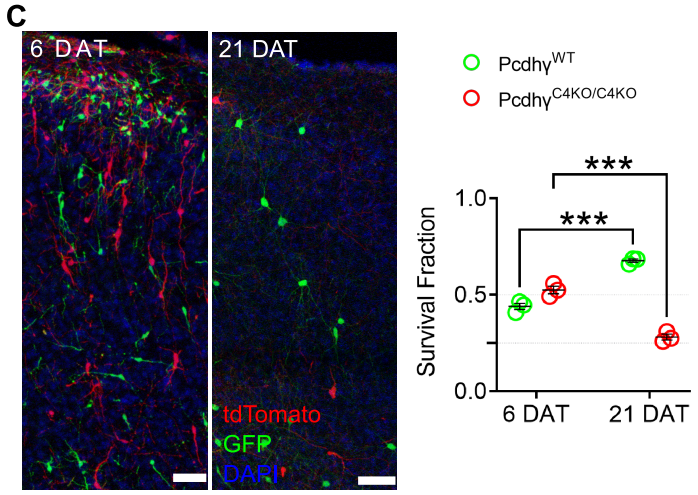
C-G. Immunostainings in P14 mouse visual cortex show PcdhγC5 expression in excitatory neurons (Cux1 and Ctip2)(C-D) and GABAergic neurons (Parv), SST, or MGE derived cINs from the Nkx2.1; ZsGreen reporter mouse) (E-G). Scale bar = 25 um.

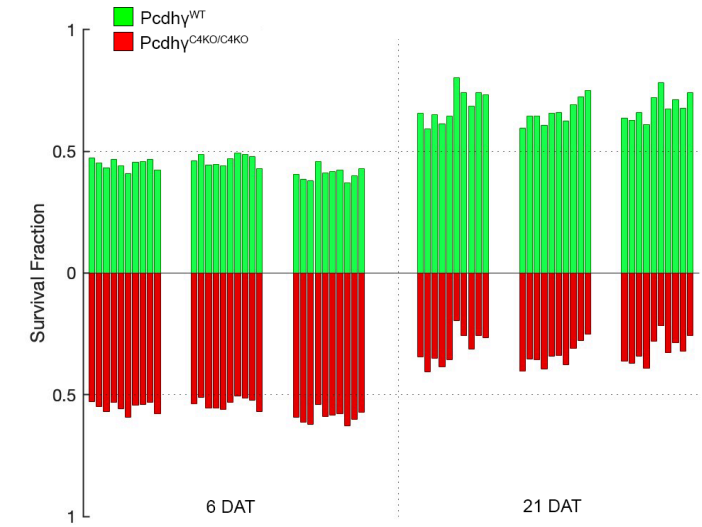
H. Quantification of PcdhγC5 positive neuronal subtypes in the visual cortex of P14 mice. Most upper layer (94.8% of $Cux1^+$) or lower layer (81.5% of $Ctip2^+$) excitatory neurons co-express the PcdhγC5 protein. In contrast, the majority of MGE-derived GABAergic cINs are negative for PcdhγC5 expression. PcdhγC5 is expressed by 11.2% of MGE-derived $ZsGreen^+$ cINs, 20.6% PV^+ cINs, and 30.0% of SST^+ cINs. n = 3 animals per genotype, at least 77 cells quantified per condition. T-test; ***p = 0.003 ; ns = not significant. Error bars represent SD.

I. Quantifications of the mean signal intensity of PcdhγC5 staining in cortical neuron subtypes in P14 mouse visual cortex. Upper (Cux1) and lower (Ctip2) layer excitatory neurons showed higher levels of PcdhγC5 signal than cINs (PV, SST, or MGE-derived $ZsGreen^+$). n = 3 animals per genotype, at least 77 cells quantified per condition. Nested t test; ***p = 0.0006. Error bars represent SD.


A
Nkx2.1 Cre
Nkx2.1
Cre
Ai14
C A G
LoxP
Stop
tdTomato
Pcdhγ C4KO
AND
Gad67 GFP
Gad67
GFP


B
Dissect & Dissociate
Co-Mixing
Transplant
E13.5
Nkx2.1 Cre -Ai14-Pcdhγ C4KO/C4KO
Gad67 GFP -Pcdhγ WT
WT P5 or P7


C
6 DAT
21 DAT
tdTomato
GFP
DAPI
Pcdhγ WT
Pcdhγ C4KO/C4KO
***
***
1.0
0.5
0.0
Survival Fraction
6 DAT
21 DAT


D
Pcdhγ WT
Pcdhγ C4KO/C4KO
1
0.5
0
0.5
1
Survival Fraction
6 DAT
21 DAT

Supplementary Figure 2 - Genetic deletion of PcdhγC4 increased cell death in MGE-derived cINs.

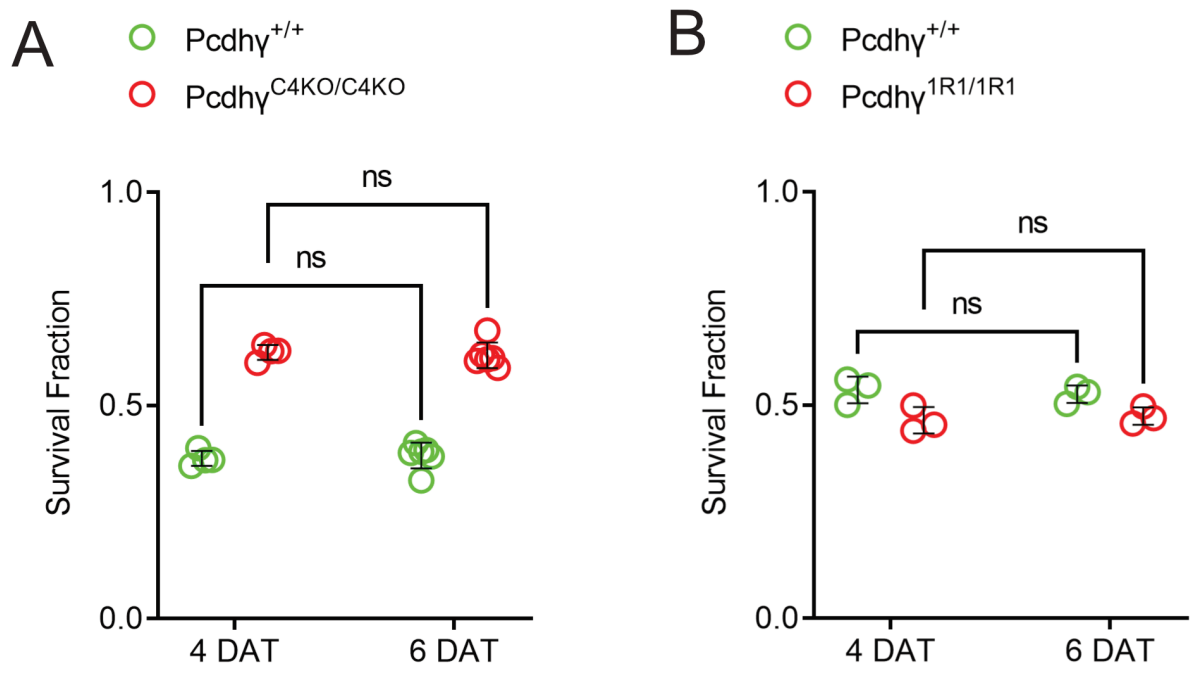
A. Diagram of genetic crosses. Pcdhγ^{C4KO} homozygous MGE cells were labeled via the MGE/POA-specific genetic reporter Nkx2.1^{Cre} mice that also carry the conditional Ai14 allele. Control Pcdhγ^{WT} MGE cells were labeled with GFP via the Gad67-GFP reporter mouse line.

B. Schematics of transplantation protocol. The MGEs of Pcdhγ^{C4KO} homozygous mutant or control E13.5 embryos were dissected, dissociated, and mixed in similar proportions. The mixture of GFP^{+} (Pcdhγ^{WT}) and tdTomato^{+} (Pcdhγ$^{C4KO/C4KO}$) cells were grafted into the cortex of WT neonate mice.

C. Left - Confocal images from the cortex of 6 and 21 DAT mice. The transplanted cells were labeled with GFP (Pcdhγ^{WT}) or tdTomato (Pcdhγ$^{C4KO/C4KO}$). Right - Quantifications (shown as survival fraction) of surviving GFP or tdTomato labeled MGE-derived cINs at 6 and 21 DAT. Both the GFP and tdTomato labeled cells undergo PCD between 6 and 21 DAT, but Pcdhγ$^{C4KO/C4KO}$ cells are eliminated at higher rates.

D. Survival fraction quantification from (C) shown by the brain section (each bar) and separated by animals at 6 and 21 DAT.

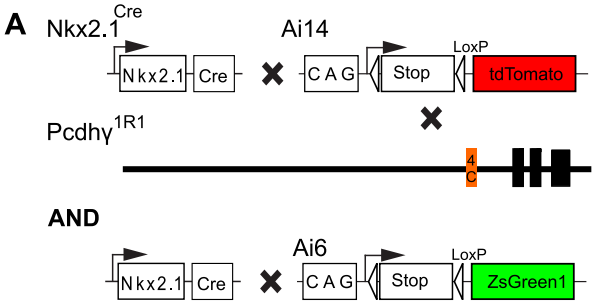
Scale bar = 50 um, Nested-ANOVA, ***p = 0.0002, n = 3 mice per time point, and 10 brain sections quantified per mouse. DAT 6 cell counted = 11,281, DAT 21 cells counted = 2,683

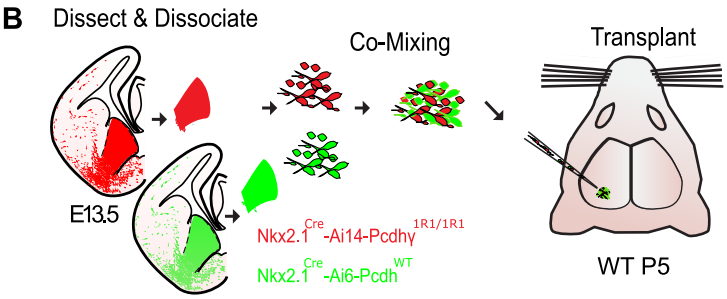


Supplementary Figure 3 - Survival fractions of transplanted MGE-derived cINs remains consistent prior to PCD.

A. Quantification of survival fractions from co-transplanted Pcdhγ$^{C4KO/C4KO}$ (tdTomato) and Pcdhγ^{WT} (GFP) MGE-derived cINs at 4 and 6 DAT. There was no apparent change in the proportion of tdTomato to GFP cells between 4 and 6 DAT, before the period of naturally occurring cell death for transplanted cells. Nested-ANOVA, n $\geq$ 4 mice per time point, and 10 brain sections quantified per mouse.

A. Quantification of survival fractions from co-transplanted Pcdhγ$^{1R1/1R1}$ (tdTomato) and Pcdhγ^{WT} (GFP) MGE-derived cINs at 4 and 6 DAT. There was no apparent change in the proportion of tdTomato to GFP cells between 4 and 6 DAT, before the period of naturally occurring cell death for transplanted cells. Nested-ANOVA, n = 3 mice per time point, and 10 brain sections quantified per mouse.


A
Nkx2.1 Cre
Ai14
Nkx2.1
Cre
C A G
Stop
LoxP
tdTomato
Pcdhγ 1R1
AND
Ai6
ZsGreen1


B
Dissect & Dissociate
Co-Mixing
Transplant
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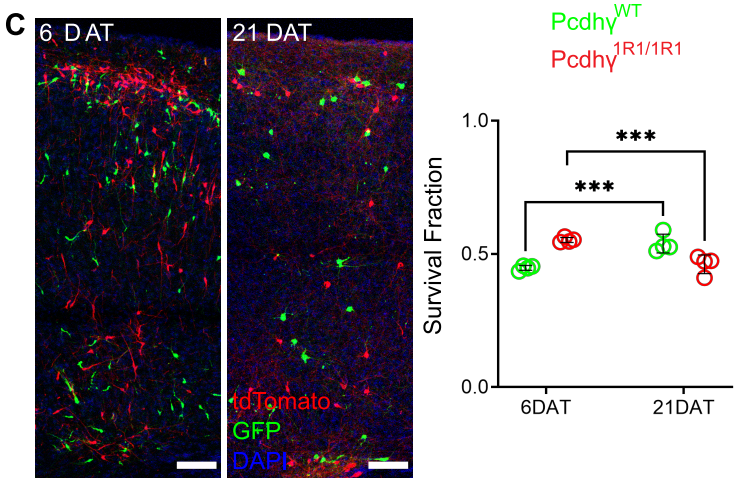
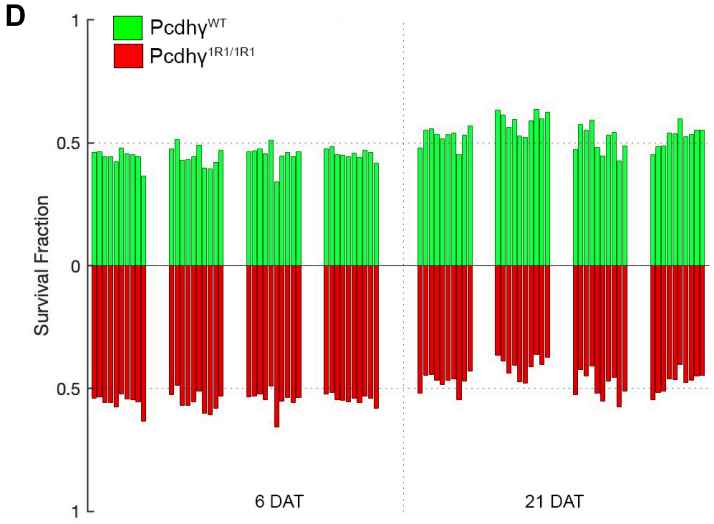
Nkx2.1 Cre -Ai14-Pcdhγ 1R1/1R1
Nkx2.1 Cre -Ai6-Pcdh WT
WT P5


C
6 DAT
21 DAT
tdTomato
GFP
DAPI
Pcdhγ WT
Pcdhγ 1R1/1R1
Survival Fraction
1.0
0.5
0.0
***
***
6DAT
21DAT


D
Pcdhγ WT
Pcdhγ 1R1/1R1
Survival Fraction
1
0.5
0
0.5
1
6 DAT
21 DAT

Supplementary Figure 4 - Expression of PcdhγC4 is sufficient for the survival of most MGE-derived cINs.

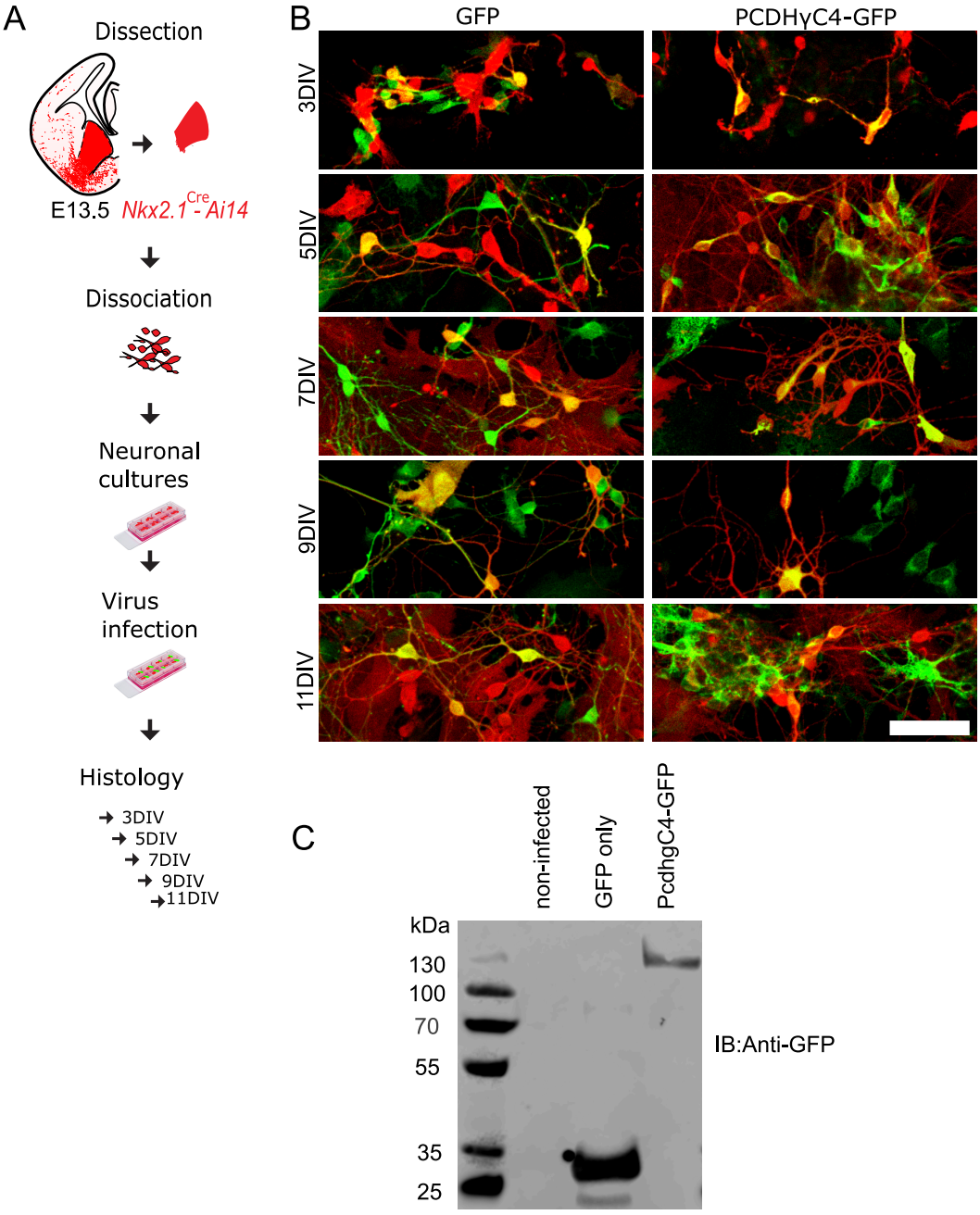
A. Diagram of genetic crosses between MGE/POA-specific reporter Nkx2.1Cre;Ai14 and Pcdhγ^{1R1} mice. Control cells were obtained from Gad67-GFP embryos.

B. Schematics of transplantation protocol. The MGEs from E13.5 Pcdhγ^{1R1} homozygous or control embryos were dissected, dissociated, and mixed in similar proportions. The mixture of GFP^{+} (Pcdhγ^{WT}) and tdTomato^{+} (Pcdhγ$^{1R1/1R1}$) cells was grafted into the cortex of WT neonate mice.

C. Left - Confocal images from the cortex of 6 and 21 DAT mice. The transplanted cells are labeled with GFP (Pcdhγ^{WT}) or tdTomato (Pcdhγ$^{1R1/1R1}$). Right - Quantifications (shown as survival fraction) of surviving MGE-derived cINs at 6 and 21 DAT. Both the transplanted GFP and tdTomato-labeled cells undergo PCD between 6 and 21 DAT, but the Pcdhγ$^{1R1/1R1}$ cells are eliminated at slightly higher rates.

D. Survival fraction quantification from (C) shown by individual brain sections (each bar) and separated by animals at 6 and 21 DAT.

Scale bar = 50 um, Nested-ANOVA , ***p = 0.009 , n = 3 mice per time point and 10 brain sections per mouse. DAT 6 cell counted = 6,030, DAT 21 cells counted = 1,609

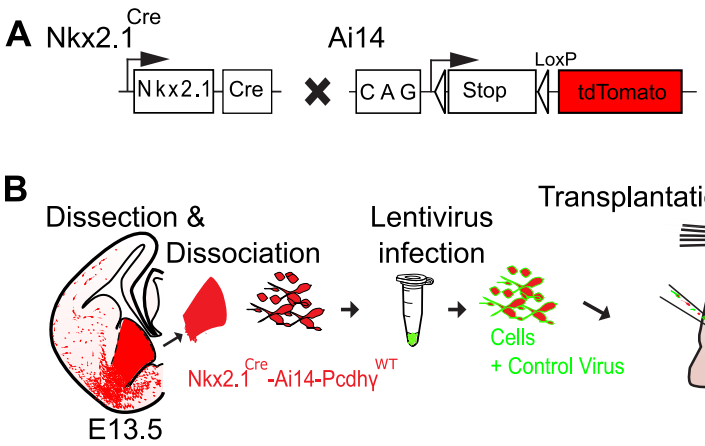

A
Dissection
E13.5 Nkx2.1Cre-Ai14
Dissociation
Neuronal cultures
Virus infection
Histology
3DIV
5DIV
7DIV
9DIV
11DIV
B
GFP
PCDHγC4-GFP
3DIV
5DIV
7DIV
9DIV
11DIV
C
non-infected
GFP only
PcdhgC4-GFP
kDa
130
100
70
55
35
25
IB:Anti-GFP

Supplementary Figure 5 - Infection with lentivirus and expression of GFP in cell cultures of cINs

A. Diagram of the vitro culture assay, infection, and imaging of cINs expressing lentiviral driven GFP, or PcdhγC4-GFP protein. MGE-derived cIN precursor cells at E13.5 were cultured on a layer of cortical feeder cells of an equivalent age, and infected with lentivirus. Cells were imaged at 3, 5, 7, 9, or 11 days in vitro (DIV).

B. Representative images of the transduced cells at 3, 5, 7, 9, or 11 DIV. MGE-derived cells are labeled with tdTomato expression. All transduced cells were identified via the expression of GFP. The transduced MGE-derived cells express both GFP and tdTomato proteins. Note that the lentiviral-driven PcdhγC4-GFP protein appears to be preferentially localized to the cell surface, but it is also found in the cell soma, and perisomatic regions, as well as in the axons and dendrites. In contrast, the GFP signal appears to fill the entire area of cells expressing the control lentiviral-driven GFP protein. Scale bar = 25nm.

C. Immunoblot against GFP from non-infected control neuronal cultures and lentiviral-infected neuronal cultures expressing GFP or PcdhγC4-GFP. No GFP band was detected in the non-infected sample (left lane). A GFP band of ~26k was detected in samples infected with a virus expressing GFP (middle lane). A band size of ~130 kD, corresponds to the expected size for the PcdhγC4 protein fused to GFP, was detected in samples infected with virus expressing PcdhγC4-GFP (right lane). Note that due to the high infection rate of the GFP only lentivirus, the protein sample was diluted 1:2 as compared to the control and PcdhγC4-GFP conditions.


A
Nkx2.1
Cre
Ai14
LoxP
Nkx2.1
Cre
C A G
Stop
tdTomato
B
Dissection &
Dissociation
Lentivirus
infection
Transplantation
Cells
+ Control Virus
Nkx2.1Cre-Ai14-PcdhγWT
E13.5
WT P4
C
6 DAT
21 DAT
tdTomato
GFP
DAPI
Pcdhγ (tdTomato+)
Pcdhγ (tdTomato+GFP+)
Survival Fraction
1.0
0.5
0.0
ns
ns
6 DAT
21 DAT
D
Pcdhγ (tdTomato+)
Pcdhγ(tdTomato+GFP+)
Survival Fraction
1
0.5
0
0.5
1
6 DAT
21 DAT

Supplementary Figure 6 - Infection with lentivirus and expression of GFP does not affect the survival of cINs

A. Diagram of mouse crosses to obtain Pcdhγ^{WT} MGE cells labeled with MGE/POA-specific genetic reporter.

B. Schematic of lentiviral infection and transplantation of MGE cIN precursors. The MGEs of Nkx2.1^{Cre};Ai14 embryos that carry Pcdhγ^{WT} were dissected, dissociated, and infected in suspension with lentivirus-expressing GFP. The infected cells were grafted into the cortex of WT recipient mice P0-P8.

C. Confocal acquired images of the transplanted cINs in the cortex at 6 and 21 DAT. Notice that all transplanted cells are labeled with tdTomato (red), while cells expressing virally-driven GFP are labeled in green. Quantifications of the tdTomato^{+} only (teal arrows) or tdTomato^{+}GFP^{+} (yellow cells, white arrows) cells are shown as the fraction of cells from the total tdTomato^{+} cells at 6 and 21 DAT.

D. Survival fraction quantifications from (C) shown as individual brain sections (each bar) and separated by animals at 6 and 21 DAT.

Scale bar = 50 um, Nested-ANOVA, ns = not significant, n = 4 mice per time point, and 10 brain sections per mouse from one transplant cohort. DAT6 cell counted = 17,288, DAT21 cells counted = 2,779