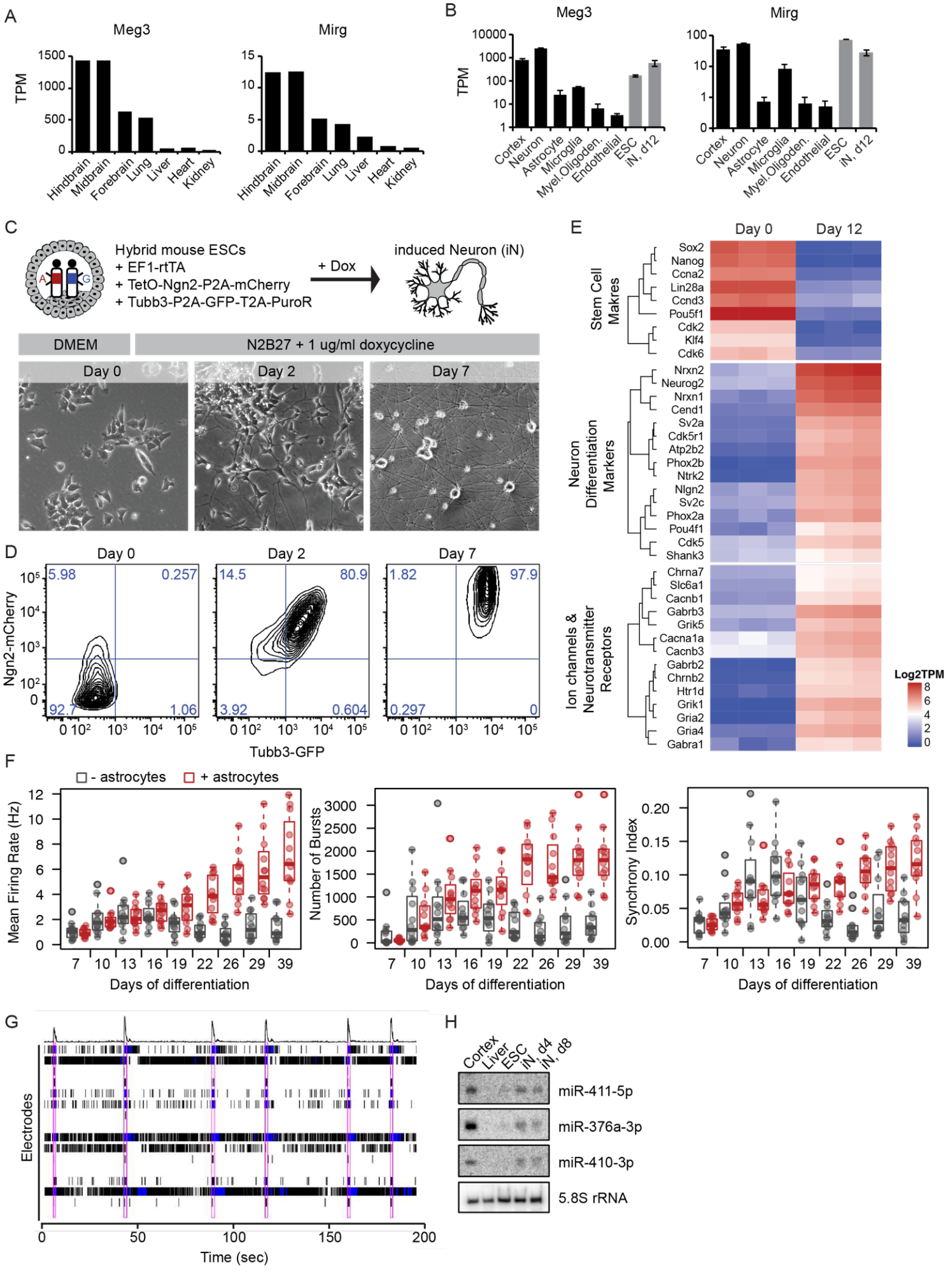
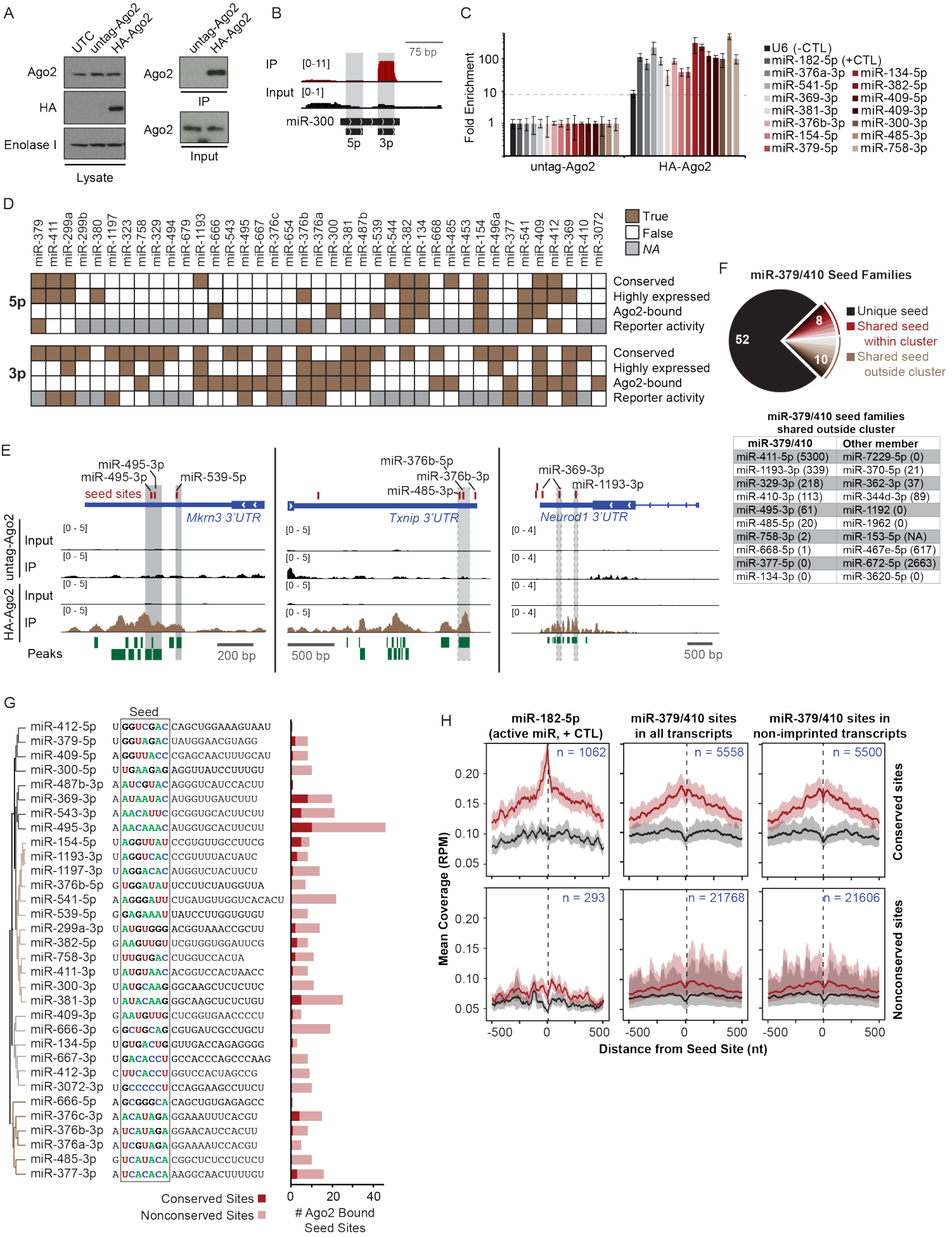

A
Meg3
Mirg
TPM
Hindbrain
Midbrain
Forebrain
Lung
Liver
Heart
Kidney
B
Meg3
Mirg
TPM
Cortex
Neuron
Astrocyte
Microglia
Myel.Oligoden.
Endothelial
ESC
iN, d12
C
Hybrid mouse ESCs
+ EF1-rtTA
+ TetO-Ngn2-P2A-mCherry
+ Tubb3-P2A-GFP-T2A-PuroR
+ Dox
induced Neuron (iN)
DMEM
N2B27 + 1 ug/ml doxycycline
Day 0
Day 2
Day 7
D
Day 0
Day 2
Day 7
Ngn2-mCherry
Tubb3-GFP
5.98
0.257
92.7
1.06
14.5
80.9
3.92
0.604
1.82
97.9
0.297
0
E
Day 0
Day 12
Stem Cell Makres
Sox2
Nanog
Ccna2
Lin28a
Ccnd3
Pou5f1
Cdk2
Klf4
Cdk6
Neuron Differentiation Markers
Nrxn2
Neurog2
Nrxn1
Cend1
Sv2a
Cdk5r1
Atp2b2
Phox2b
Ntrk2
Nlgn2
Sv2c
Phox2a
Pou4f1
Cdk5
Shank3
Ion channels & Neurotransmitter Receptors
Chrna7
Slc6a1
Cacnb1
Gabrb3
Grik5
Cacna1a
Cacnb3
Gabrb2
Chrnb2
Htr1d
Grik1
Gria2
Gria4
Gabra1
Log2TPM
F
- astrocytes
+ astrocytes
Mean Firing Rate (Hz)
Number of Bursts
Synchrony Index
Days of differentiation
G
Electrodes
Time (sec)
H
Cortex
Liver
ESC
iN, d4
iN, d8
miR-411-5p
miR-376a-3p
miR-410-3p
5.8S rRNA

Figure S1. An *in vitro* model system for studying imprinted gene function in neurons, Related to Figure 1

A. Quantification of *Meg3* and *Mirg* expression as transcripts per million (TPM) from postnatal day 0 mouse tissues. **B.** TPM quantification of *Meg3* and *Mirg* expression from cell types isolated from the mouse cortex, in vitro ESCs, and iNs (day 12). Mean ± abs error (n = 2) for neuron, astrocyte, microglia, myelinating oligodendrocyte, and endothelial samples; Mean ± std deviation (n = 3) for whole cortex, ESCs, and iNs. **C.** Schematic overview of Ngn2-induced differentiation and brightfield images of ESCs and iNs (day 2 and 7). **D.** Fluorescence activated cells sorting (FACS) of ESCs and iNs (day 2 and 7) based on Ngn2-mCherry and endogenously tagged Tubb3-GFP. Percentage of cells in each gated quadrant is indicated. **E.** Heatmap of transcripts per million (TPM) from rRNA-depleted RNA-seq of ESCs (day 0, n = 3) and iNs (day 12, n = 3) for stem cell markers, neuron differentiation markers, and ion channels or neurotransmitter receptors. **F.** Analysis of multielectrode array (MEA) recordings for mean firing rate (Hz), number of electrode bursts, and synchrony index across a time course of differentiation. Black, iNs alone; Red, iNs co-cultured with astrocytes. Individual data points are the mean of 16 electrodes in an individual well, and boxplot summarize the data of 12 wells per condition. **G.** An example raster plot of iNs (day 19). The first row is the population histogram and all subsequent rows are tracks of the 16 individual electrodes in the well. Electrode bursts are shown in blue and network bursts are outlined in pink. **H.** Northern blot for three mature miRNAs from miR-379/410 in adult mouse cortex and liver, *in vitro* ESCs, and iNs (day 4 and 8) relative to 5.8S rRNA.



miR-379/410	Other member
miR-411-5p (5300)	miR-7229-5p (0)
miR-1193-3p (339)	miR-370-5p (21)
miR-329-3p (218)	miR-362-3p (37)
miR-410-3p (113)	miR-344d-3p (89)
miR-495-3p (61)	miR-1192 (0)
miR-485-5p (20)	miR-1962 (0)
miR-758-3p (2)	miR-153-5p (NA)
miR-668-5p (1)	miR-467e-5p (617)
miR-377-5p (0)	miR-672-5p (2663)
miR-134-3p (0)	miR-3620-5p (0)

Figure S2. miR-379/410 targeting by Ago2 seCLIP in iNs, Related to Figure 2

A. Western blot from cells with overexpression of untagged-Ago2 or HA-Ago2 from a stably integrated transgene. Left: whole cell lysates probed with antibodies for Ago2, HA, or Enolase I (loading control). UTC, untransfected control with endogenous Ago2 expression. Right: Confirmation of Ago2 immunoprecipitation in seCLIP-seq. Cross-linked cell lysates were immunoprecipitated with an HA antibody. The input and IP samples were then probed with Ago2 antibody. **B.** Genomic alignment of seCLIP-seq reads to miR-300. Scale is reads per million. **C.** RNA immunoprecipitation using an HA antibody for iNs expressing untagged-Ago2 or HA-Ago2 followed by miRNA qRT-PCR. Dotted line indicates enrichment threshold established by U6 snRNA negative control. miR-182-5p is a positive control from outside the miR-379/410 cluster. **D.** Summary of data for each miRNA from miR-379/410 organized according to their 5'-to-3' position within the cluster. Conserved, true if miRNA is conserved among eutherian mammals (from TargetScanMouse, release 7.2); Highly expressed, from Figure 1B; Ago2-bound, from Figure 2A; Reporter activity, from Figure 1G. **E.** Genomic alignment of Ago2 seCLIP-seq reads to the 3' UTR of *Mkrn3, Txnip*, and *Neurod1*. Reads are scaled to reads per million (RPM). Predicted binding sites for active miR-379/410 miRNAs are indicated in red. Significant Ago2 peaks are shown as green boxes. Significant Ago2 peaks overlapping a seed site for miR-379/410 are highlighted in gray. **F.** Top: Pie chart of seed families in miR-379/410 cluster. Unique seed families contain one miR-379/410 miRNA (n = 52, black), seed families with two miR-379/410 family members (n = 8, red), seed families with one miR-379/410 family member and one member outside the cluster (n = 10, gold). Bottom: Table of miR-379/410 seed families that share a seed sequence with a miRNA outside the cluster. Normalized expression counts in iNs for each miRNA are indicated in parenthesis. **G.** Active miR-379/410 miRNAs were clustered according to the similarity of their seed sequence. The mature miRNA sequence is shown and the seed sequence is highlighted. The bar graph indicates the number of significant Ago2 peaks that overlap a conserved or nonconserved seed match for each active miRNA. **H.** Metagene plot of Ago2 seCLIP-seq coverage (reads per million, RPM) at seed sites for the indicated miRNAs, including coverage 500 nt upstream and downstream of the seed site. The number of sites included in each plot is indicated. Top, conserved sites; Bottom, nonconserved sites with TargetScan score ≥ 90; Black, untagged-Ago2 IP; Red, HA-Ago2 IP.

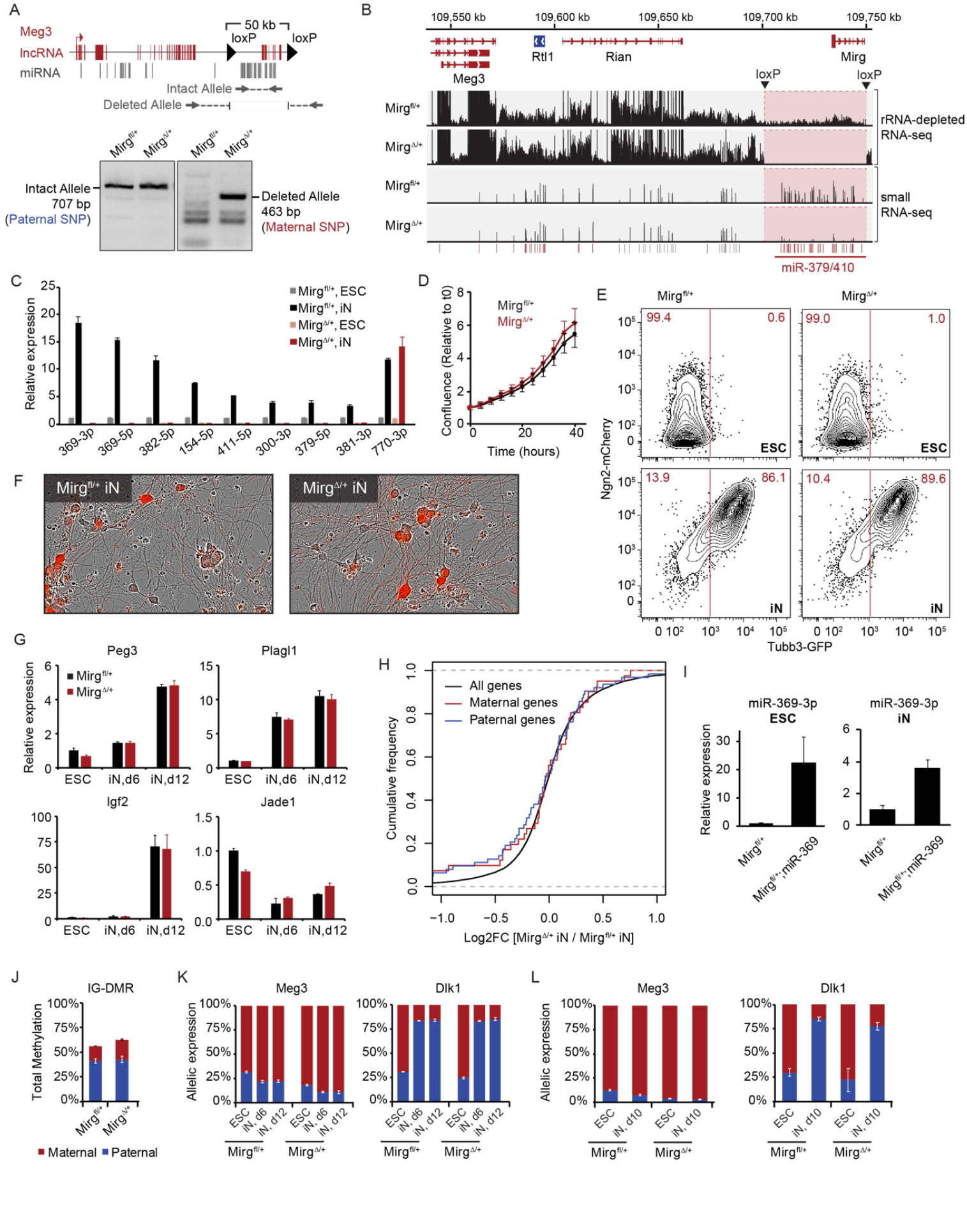

A
Meg3
lncRNA
miRNA
50 kb
loxP
loxP
Intact Allele
Deleted Allele
Mirg^fl/+
Mirg^Δ/+
Intact Allele
707 bp
(Paternal SNP)
Deleted Allele
463 bp
(Maternal SNP)
B
109,550 kb
109,600 kb
109,650 kb
109,700 kb
109,750 kb
Rtl1
Rian
Mirg
Meg3
loxP
loxP
rRNA-depleted RNA-seq
small RNA-seq
miR-379/410
C
Relative expression
Mirg^fl/+, ESC
Mirg^fl/+, iN
Mirg^Δ/+, ESC
Mirg^Δ/+, iN
369-3p
369-5p
382-5p
154-5p
411-5p
300-3p
379-5p
381-3p
770-3p
D
Confluence (Relative to t0)
Time (hours)
E
Mirg^fl/+
Mirg^Δ/+
99.4
0.6
99.0
1.0
13.9
86.1
10.4
89.6
ESC
iN
Ngn2-mCherry
Tubb3-GFP
F
Mirg^fl/+ iN
Mirg^Δ/+ iN
G
Peg3
Plagl1
Igf2
Jade1
Relative expression
ESC
iN,d6
iN,d12
H
All genes
Maternal genes
Paternal genes
Cumulative frequency
Log2FC [Mirg^Δ/+ iN / Mirg^fl/+ iN]
I
miR-369-3p
ESC
miR-369-3p
iN
Relative expression
Mirg^fl/+
Mirg^fl/+;miR-369
J
IG-DMR
Total Methylation
Maternal
Paternal
K
Meg3
Dlk1
Allelic expression
ESC
iN, d6
iN, d12
L
Meg3
Dlk1
Allelic expression
ESC
iN, d10

Figure S3. Characterization of maternal miR-379/410 deletion in ESCs, Related to Figure 3 **A.** Top: Schematic of *Meg3* genomic locus showing location of maternal loxP insertions and primer locations to confirm presence of intact and deleted allele following Cre-mediated recombination. Bottom: Agarose gel of PCR products for intact and deleted allele in $Mirg^{fl/+}$ and $Mirg^{\Delta/+}$ ESCs. Allelic identity was obtained by Sanger sequencing across allelic SNPs from gel isolated bands. **B.** Genomic alignment of rRNA-depleted RNA-seq and small RNA-seq reads to the Chr12 *Meg3* locus in $Mirg^{fl/+}$ and $Mirg^{\Delta/+}$ iNs. **C.** qRT-PCR for mature miRNAs from miR-379/410 in $Mirg^{fl/+}$ and $Mirg^{\Delta/+}$ ESCs and iNs. miR-770-3p is a miRNA transcribed outside the miR-379/410 cluster as an assay control. Mean ± SEM (n = 3). **D.** Proliferation rate was compared between $Mirg^{fl/+}$ and $Mirg^{\Delta/+}$ ESCs by quantifying the confluency of the cell culture dish over time on the Incucyte Imaging System. Mean ± SEM (n = 3). **E.** FACS of ESCs and iNs based on Ngn2-mCherry and endogenously tagged Tubb3-GFP. Percentage of Tubb3-GFP negative or positive cells is indicated. **F.** Overlay of brightfield and Ngn2-mCherry fluorescence images for $Mirg^{fl/+}$ and $Mirg^{\Delta/+}$ iNs. **G.** qRT-PCR for paternally-expressed transcripts in ESCs and iNs (d6, day 6; d12, day 12). **H.** CDF plot of gene expression upon miR-379/410 deletion in iNs for all genes (black, n = 14,325), maternally-expressed genes (red, n = 41, p = 0.9), and paternally-expressed genes (blue, n = 63, p = 0.3). P-values were calculated using two-sided Kolmogorov-Smirnov test. **I.** qRT-PCR for miR-369-3p in $Mirg^{fl/+}$ cells with overexpression of miR-369. **J.** Quantification of allele-specific DNA methylation at the intergenic differentially methylated region (IG-DMR) for the *Dlk1*/*Meg3* locus in $Mirg^{fl/+}$ and $Mirg^{\Delta/+}$ ESCs using pyrosequencing. Mean ± SEM (n = 3). **K.** Allele-specific qRT-PCR for *Meg3* and *Dlk1* expression in $Mirg^{fl/+}$ and $Mirg^{\Delta/+}$ ESCs and iNs. Mean ± SEM (n = 3). **L.** Allele-specific expression of *Meg3* and *Dlk1* by quantification of SNP-containing RNA-sequencing reads in $Mirg^{fl/+}$ and $Mirg^{\Delta/+}$ ESCs and iNs. Mean ± SEM (n = 3).

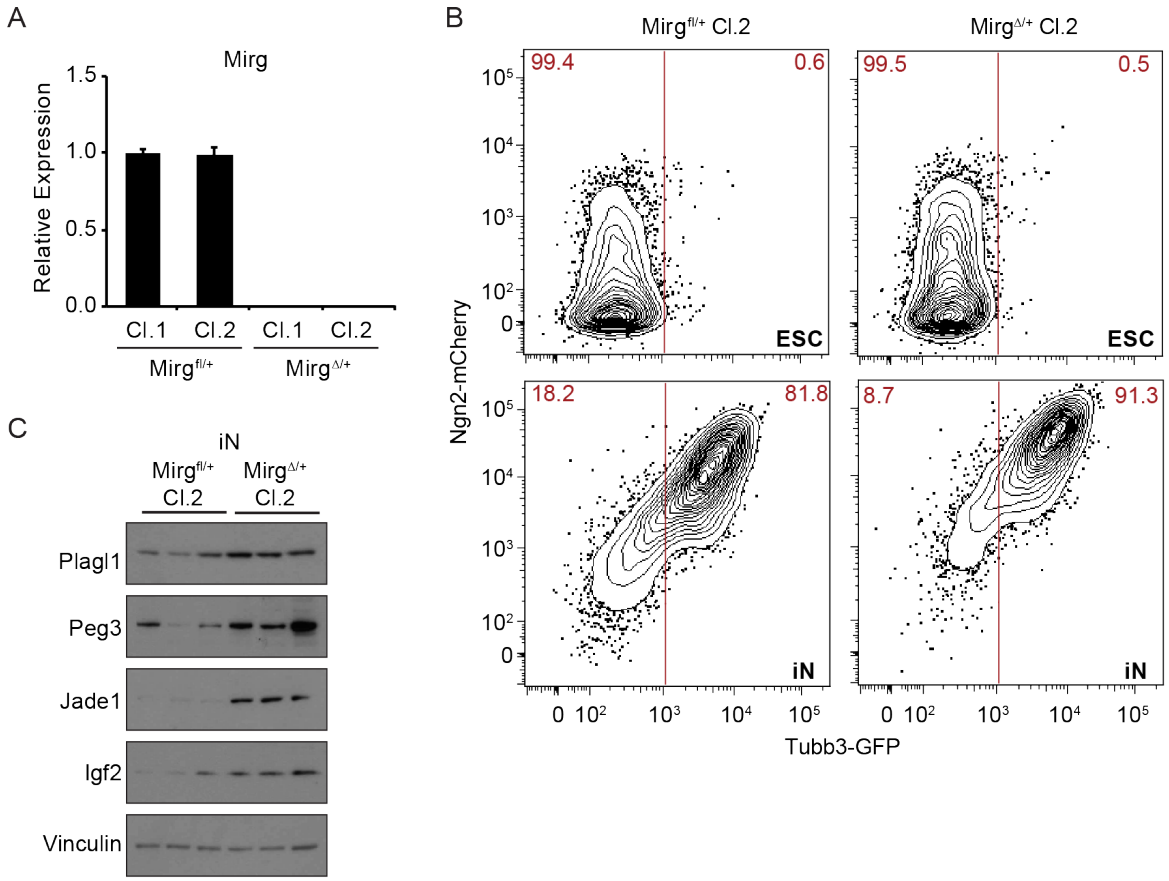


Figure S4. Confirmation of observed changes in a second subclonal Mirg$^{fl/+}$ and Mirg$^{\Delta/+}$ cell line, Related to Figure 3

A. qRT-PCR for *Mirg* in two subclonal cell lines with maternal allele floxed or deleted *Mirg* locus. **B.** FACS of ESCs and iNs based on Ngn2-mCherry and endogenously tagged Tubb3-GFP. Percentage of Tubb3-GFP negative or positive cells is indicated. **C.** Western blot of protein lysates from Mirg$^{fl/+}$ (Clone 2) and Mirg$^{\Delta/+}$ (Clone 2) iNs probed with the indicated antibodies. Vinculin was used as a loading control. Biological triplicates are shown for each condition.

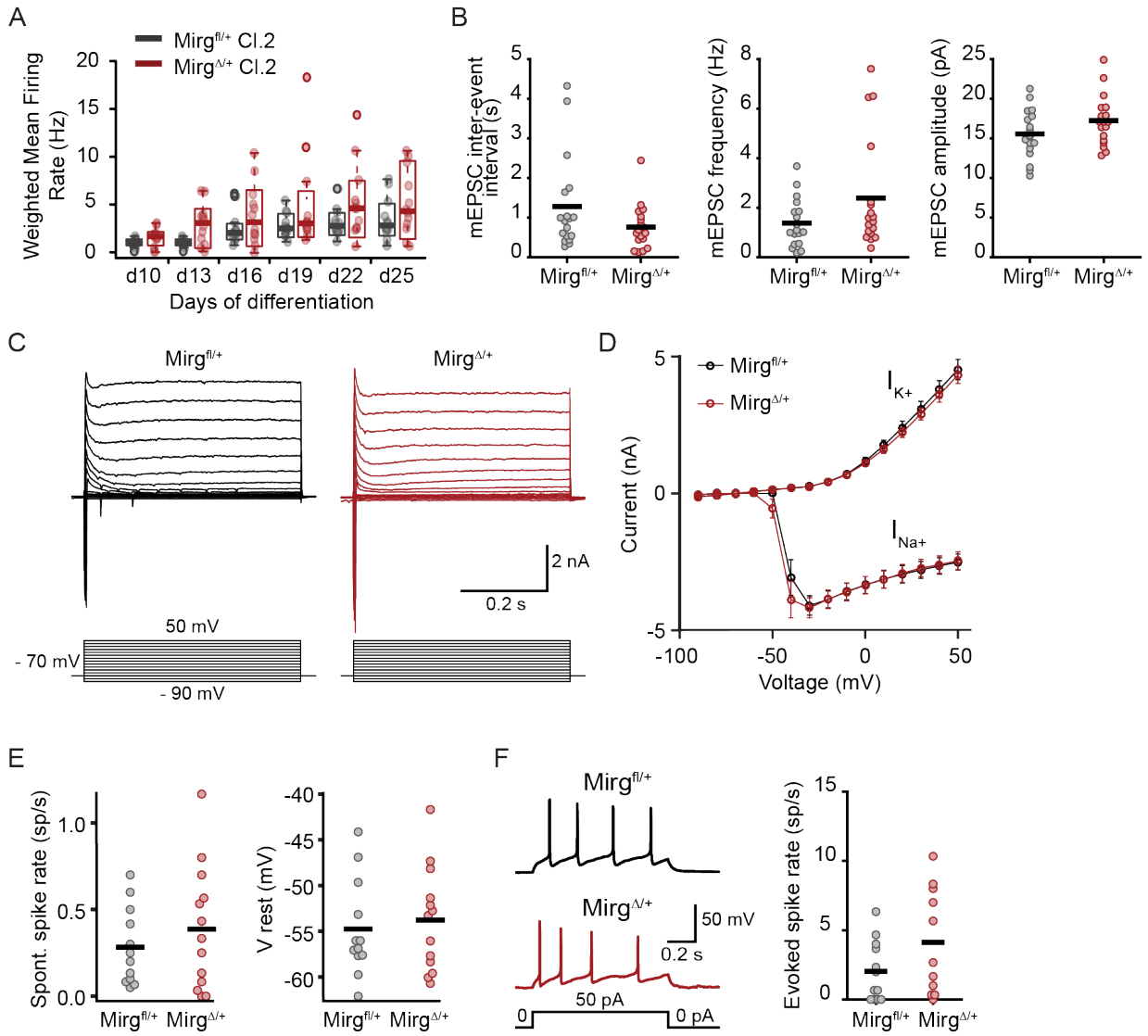


Figure S5. miR-379/410 deletion increases synaptic events but preserves spiking and membrane properties, Related to Figure 5

A. Boxplot of weighted mean firing rate from MEA recordings at day 10 to 25 of differentiation. Individual data points are the mean of 16 electrodes in a single well. Black, $Mirg^{fl/+}$ Clone 2 iNs (n = 12 wells); Red, $Mirg^{\Delta/+}$ Clone 2 iNs (n = 12 wells). **B**. Quantification of inter-event interval (p = 0.10, t_{34} = 1.6773), mEPSC frequency (p = 0.09, t_{34} = -1.7588), and amplitude (p = 0.11, t_{34} = 1.6237) from mEPSC voltage-clamp recordings. Individual data points are the mean values for each cell, n = 18 cells for each condition. Solid black bar represents the mean value for each condition. P-values were calculated using unpaired two-tailed Student's t-test. **C**. Example voltage-clamp trace in response to voltage steps for $Mirg^{fl/+}$ and $Mirg^{\Delta/+}$ iNs. **D**. Amplitude of sodium (I_{Na+}) and potassium (I_{K+}) currents at different voltage steps. Black, $Mirg^{fl/+}$ iNs (n = 12);

Red, $Mirg^{\Delta/+}$ iNs (n = 13); Mean ± SEM. **E**. Spontaneous spike rate ($p = 0.40$, $t_{23} = -0.86$) and resting membrane potential ($p = 0.67$, $t_{23} = -0.44$) measured during one-minute-long current-clamp recordings without current injection. Black, $Mirg^{fl/+}$ iNs (n = 12); Red, $Mirg^{\Delta/+}$ iNs (n = 13). **F**. Left: Representative current-clamp traces of the spiking activity in response to +50 pA current steps for each condition. Right: Average evoked spike rate at 50 pA current injection ($p = 0.12$, $t_{21} = -1.6335$). Black, $Mirg^{fl/+}$ iNs (n = 12); Red, $Mirg^{\Delta/+}$ iNs (n = 11). P-values in (**E**) and (**F**) were calculated using unpaired two-tailed Student's t-test. Solid black bars in (**E**) and (**F**) represent the average value for each condition.