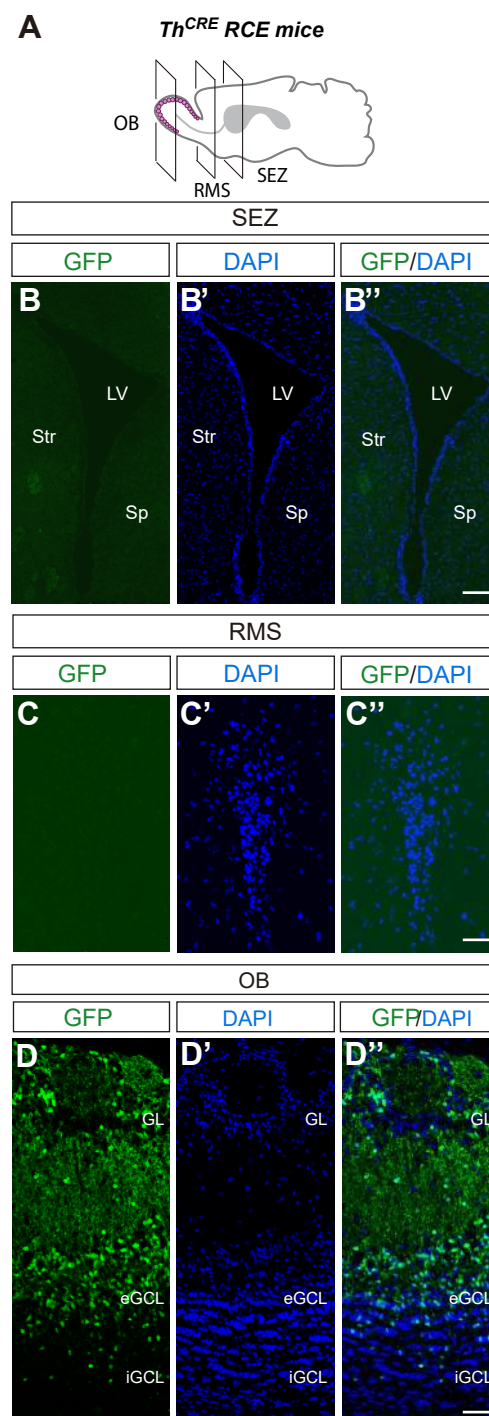


Fig. S1. PBX2 expression analysis in olfactory bulb adult neurogenesis.

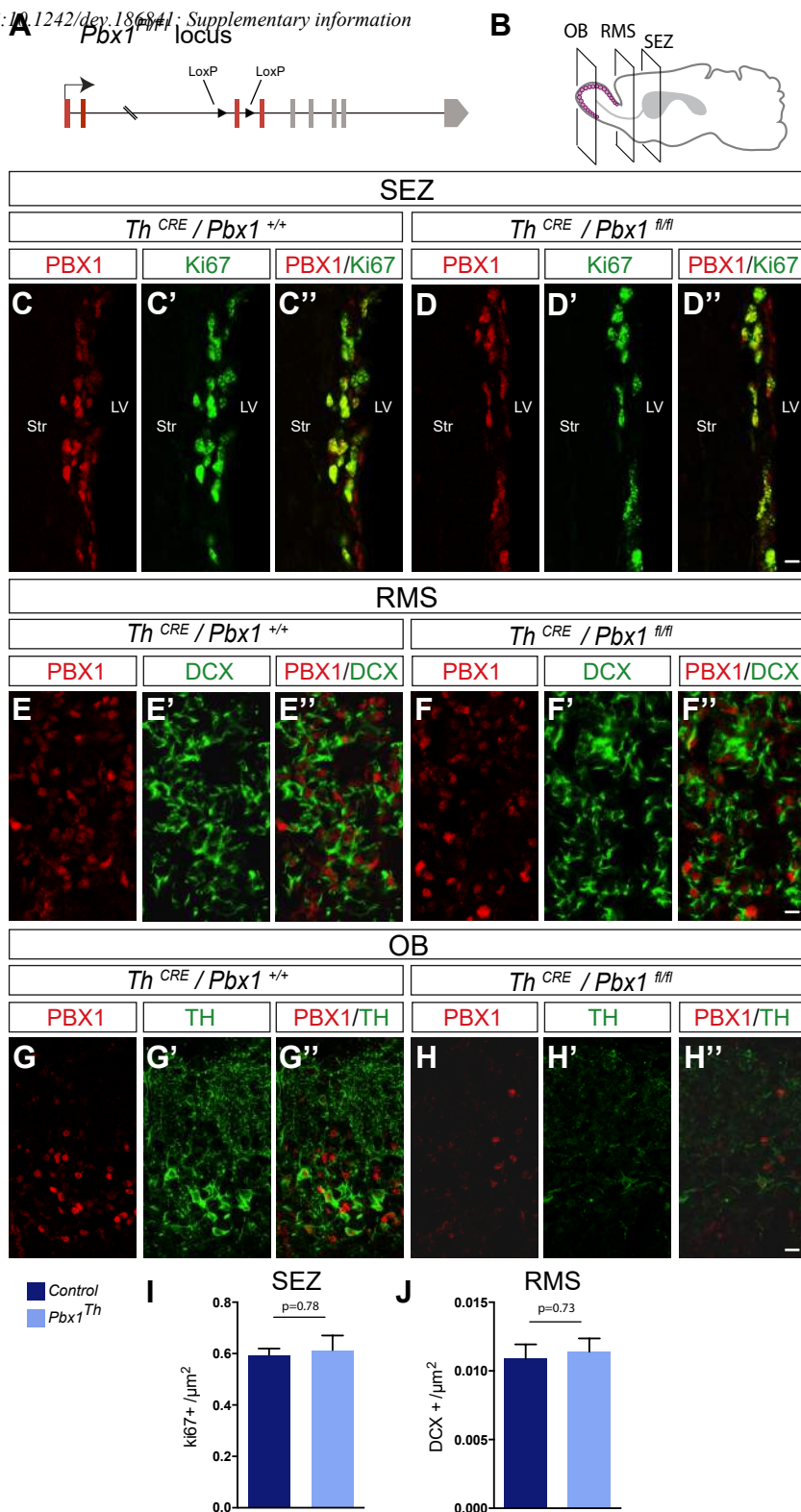
A) Brain representation of the coronal levels analyzed in the micrographs. OB: olfactory bulb, RMS: rostral migratory stream, SEZ: subependymal zone. B) PBX2 expression in the SEZ region. Scale bar: 50 μ m. C) Analysis of PBX2 expression in proliferating progenitors by double PBX2 and Ki67 immunostaining. Scale bar: 10 μ m. D) Quantification of PBX2 expression in proliferating progenitors. E) Analysis of PBX2 expression in RMS shows no-expression in migrating neuroblasts. Scale bar: 20 μ m. F) PBX2 expression in the OB. EPL: external plexiform layer, GCL: granular cell layer, GL: glomerular layer, MCL: mitral cell layer. Scale bar: 75 μ m. G) Double immunostaining of TH and PBX2 in the OB. H) Double immunostaining of TH and PBX3 in the OB. Scale bar: 20 μ m. I) Quantification of PBX2 and PBX3 expression in TH-positive cells.



Supplementary Figure 2

Figure S2. *Th^{CRE}* lineage tracing in olfactory bulb adult neurogenesis.

A) Brain representation of the coronal levels analyzed in the micrographs. OB: olfactory bulb, RMS: rostral migratory stream, SEZ: subependymal zone. **B)** *Th^{CRE}*, RCE animals do not show GFP expression in adult SEZ. Scale bar: 50 μ m. **C)** *Th^{CRE}*, RCE animals do not show GFP expression in adult RMS. Scale bar: 40 μ m. **D)** *Th^{CRE}*, RCE animals show GFP expression in the external granular cell layer and the glomerular layer of the adult olfactory bulb. Scale bar: 75 μ m.



Supplementary Figure 3

Figure S3. *Pbx1*Th mutants specifically remove *Pbx1* from differentiating DA neurons in the OB. **A)** *Pbx1* locus in *Pbx1*^{fl/fl} animals contains LoxP sites flanking the third exon. Red boxes indicate the exons coding for the DNA binding domain. **B)** Brain representation of the coronal levels analyzed in the micrographs. OB: olfactory bulb, RMS: rostral migratory stream, SEZ: subependymal zone. **C)** PBX1 expression in SEZ proliferating progenitors (Ki67 positive) in *Th*^{CRE}, RCE, *Pbx1*^{+/+} animals. **D)** PBX1 expression in SEZ proliferating progenitors (Ki67 positive) in *Th*^{CRE}, RCE, *Pbx1*^{fl/fl} mutants. **E)** PBX1 expression in RMS migrating neuroblasts (DCX positive) of *Th*^{CRE}, RCE, *Pbx1*^{+/+} animals. **F)** PBX1 expression in RMS migrating neuroblasts (DCX positive) of *Th*^{CRE}, RCE, *Pbx1*^{fl/fl} mutants. **G)** PBX1 expression in OB DA lineage labeled by GFP and TH expression in *Th*^{CRE}, RCE, *Pbx1*^{+/+} animals. **H)** PBX1 expression is reduced in OB of *Th*^{CRE}, RCE, *Pbx1*^{fl/fl} animals. **I)** Quantification of proliferating progenitors (Ki67 positive) in controls and *Pbx1*Th mutants. n=3 animals each genotype. Student's t test. **J)** Quantification of RMS migrating neuroblasts (DCX positive) in controls and *Pbx1*Th mutants. n=3 animals. each genotype. Student's t test. Scale bars: 10 μm.

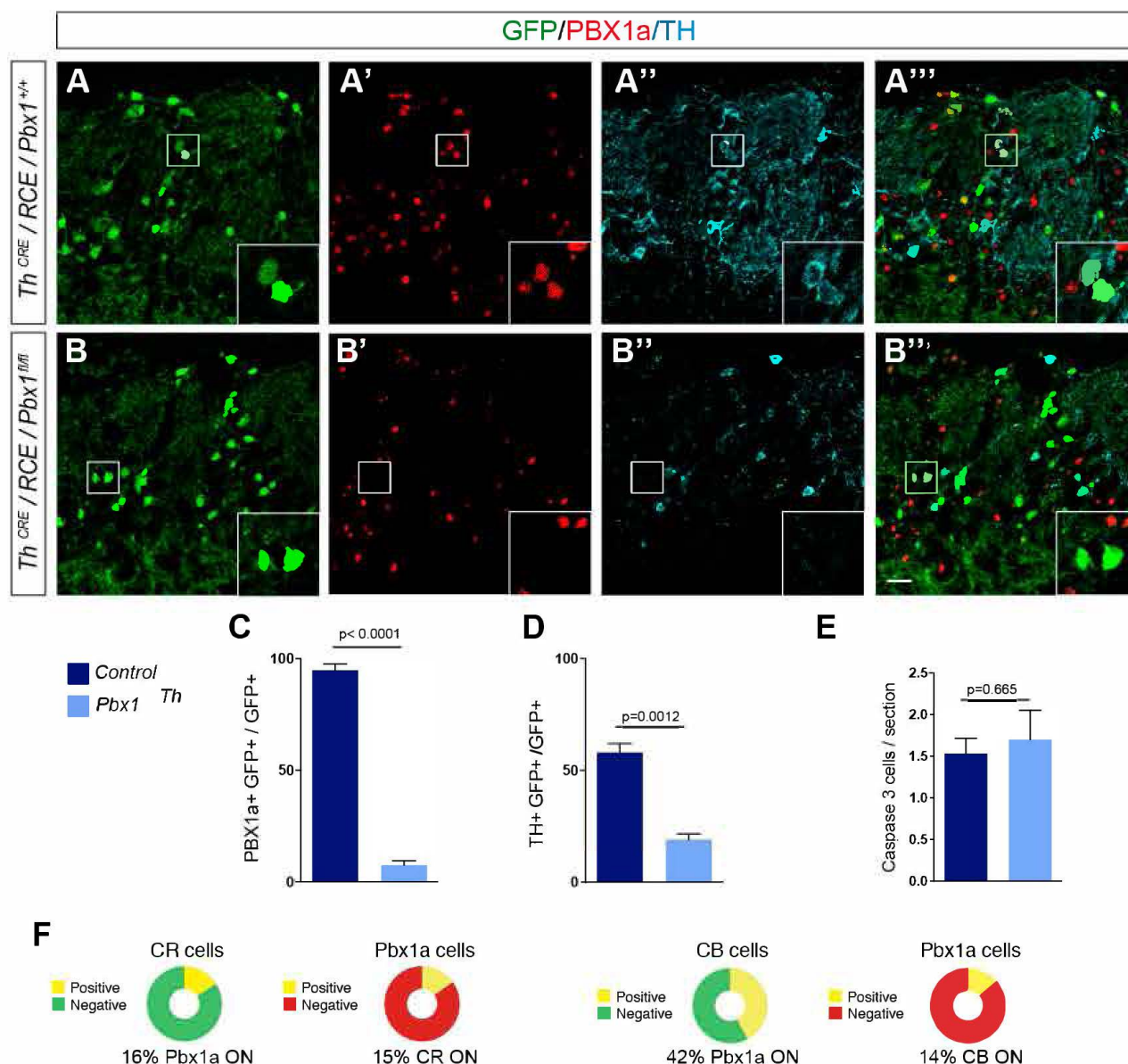
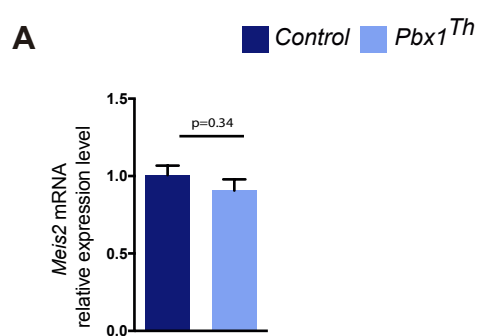


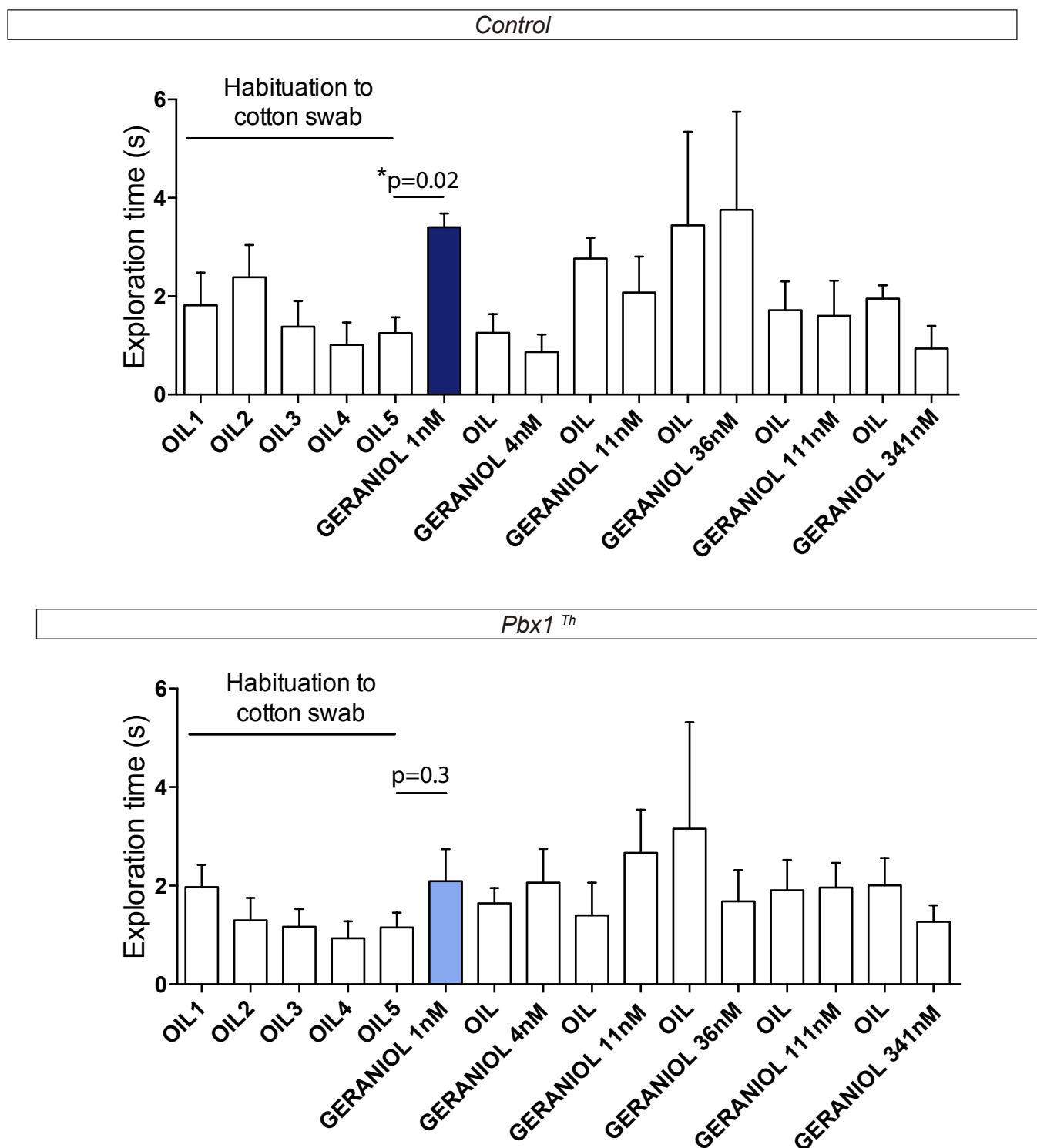
Figure S4. *Pbx1Th* mutants efficiently remove Pbx1 from DA lineage cells in OB.

A) GFP, PBX1a and TH co-expression in *Th^{CRE}, RCE, Pbx1^{+/+}* animals. B) GFP, PBX1a and TH co-expression in *Th^{CRE}, RCE, Pbx1^{fl/fl}* animals show both PBX1a and TH expression defects in the DA lineage. Scale bar: 25 μ m. C) Percentage of GFP positive cells co-expressing PBX1A in controls and *Pbx1Th* mutants. n=3 animals each genotype, Student's t test, unpaired, two tail. D) Percentage of GFP/RCE positive cells co-expressing TH in controls and *Pbx1Th* mutants. n=3 animals each genotype, Student's t test, unpaired, two tail. E) Cell death quantification with Caspase 3 antibody in controls and *Pbx1Th* mutants. n=3 animals each genotype, Student's t test, unpaired, two tail. F) Quantification of double CR/PBX1a and CB/PBX1a positive cells in OB of control animals. n=3 animals each genotype.



Supplementary Figure 5

Figure S5. *Meis2* transcription is unaffected in *Pbx1*Th mutants. A) *Meis2* mRNA expression level in controls and *Pbx1*Th mutants measured by quantitative RT-PCR. Controls n=4 animals, *Pbx1*Th n=3 animals, Student's t test.



Supplementary Figure 6

Figure S6. *Pbx1Th* mutants show odor detection threshold deficits. In the odor threshold paradigm, mice are first habituated to the presence of the cotton swab without odorant (OIL1 to OIL5) and then their reaction to increasing concentrations of geraniol is analyzed. Control mice detect Geraniol at 1 nM while *Pbx1Th* mutants do not significantly react to this concentration. *Pbx1Th* mutants show high variability in their response compared to the control group precluding the establishment of a specific threshold value. n=5 control animals, n=8 *Pbx1Th* mutant animals. Paired Student's t test.

Table S1. Quantification primary data.

| | |
|------------------|---|
| Figure 1E | <i>TH+</i> cells/mm ² : control 418±16, <i>Pbx2</i> ^{-/-} 441±12, <i>Pbx1</i> Th mice 178±6, <i>Pbx1</i> Th and <i>Pbx2</i> ^{-/-} 131±7. n=3 animals each genotype. |
| Figure 1I | <i>GFP+</i> cells/mm ² : control 616±17, <i>Pbx1</i> Th 628±48. n=3 animals each genotype. |
| Figure 1L | <i>TH+</i> cells/mm ² : control 172±8, <i>Pbx1</i> Th 109±7. n=3 animals each genotype. |
| Figure 1N | <i>PGL BrdU+</i> cells/section: control 11±2, <i>Pbx1</i> Th 16±3. n=3 animals each genotype. |
| Figure 1O | <i>TH+/BrdU+</i> cells: control 19±2%, <i>Pbx1</i> Th 8±2%. n=3 animals each genotype. |
| Figure 1P | <i>CR+/BrdU+</i> cells: control 21±3%, <i>Pbx1</i> Th 19±2%. n=3 animals each genotype. |
| Figure 1Q | <i>CB+/BrdU+</i> cells: control 5±1%, <i>Pbx1</i> Th 4±2%. n=3 animals each genotype. |
| Figure 2C | <i>COUPTF1+</i> <i>GFP+/GFP+</i> : control 56±4%, <i>Pbx1</i> Th 53±2%. n=3 animals each genotype. |
| Figure 2F | <i>ETV1+</i> <i>GFP+/GFP+</i> : control 62±6%, <i>Pbx1</i> Th 60±1%. n=3 animals each genotype. |
| Figure 2I | <i>PAX6+</i> <i>GFP+/GFP+</i> : control 83±2%, <i>Pbx1</i> Th 77±1%. n=3 animals each genotype. |
| Figure 2L | <i>DLX+</i> <i>GFP+/GFP+</i> : control 93±0.4%, <i>Pbx1</i> Th 96±0.05%. n=3 animals each genotype. |
| Figure 2O | <i>MEIS2+</i> <i>GFP+/GFP+</i> : control 84±1%, <i>Pbx1</i> Th 39±1%. n=3 animals each genotype. |
| Figure 3D | Relative expression levels: control <i>Ddc</i> 1±0.2, <i>Vmat2</i> 1±0.1, <i>Dat</i> 1±0.1, <i>Gch</i> 1±0.1, n=4 animals; <i>Pbx1</i> Th <i>Ddc</i> 0.5±0.05, <i>Vmat2</i> 0.4±0.1, <i>Dat</i> 0.5±0.1, <i>Gch</i> 1±0.1, n=3 animals. |
| Figure 4D | <i>GFP+</i> pixels/pixels: control 10±1.4%, <i>Pbx1</i> Th 5±0.5%. n=3 animals each genotype. Glomerulus area (μm ²): control 6050±267, <i>Pbx1</i> Th 6514±448. n=3 animals each genotype. |
| Figure 4F | <i>TH+</i> <i>GFP+/GFP+</i> : control 68±3%, <i>Pbx1</i> ^{F/FI} 44±2%. n=5 control animals, 12 mutant animals. |
| Figure 4J | Dendrite length (μm): <i>Pbx</i> ^{+/+} : 684±97, <i>Pbx1</i> ^{F/FI} : 392±91. Dendrite volumen (μm ²): <i>Pbx</i> ^{+/+} : 331±57, <i>Pbx1</i> ^{F/FI} : 81±21. Total branching points: <i>Pbx</i> ^{+/+} : 44±7, <i>Pbx1</i> ^{F/FI} : 27±6. Maximum cell-radius (μm): <i>Pbx</i> ^{+/+} : 64±5, <i>Pbx1</i> ^{F/FI} : 43±5). n=14 cells from three different control animals, and 12 cells from three different mutant animals. |
| Figure 5C | <i>GAD67+</i> <i>GFP+/GFP+</i> : control 52±1%, <i>Pbx1</i> Th 73±1%. n=3 animals each genotype. |
| Figure 5F | <i>CR+</i> <i>GFP+/GFP+</i> : control 4±0.3%, <i>Pbx1</i> Th 13±3%. n=3 animals each genotype. |
| Figure 5I | <i>CB+</i> <i>GFP+/GFP+</i> : control 10±1%, <i>Pbx1</i> Th 10±1%. n=3 animals each genotype. |
| Figure 6A | Time sniffing (s): control 1.3±0.3 (oil) and 3.4±0.3 (geraniol), <i>Pbx1</i> Th 1.2±0.3 (oil) and 2.1±0.7 (geraniol). n=5 control animals and 8 mutant animals. |
| Figure 6B | Time sniffing (s): control 0.7±0.3 (oil) and 1.4±0.5 (carvone), <i>Pbx1</i> Th 0.8±0.3 (oil) and 0.9±0.6 (carvone). n=9 control animals and 8 mutant animals. |
| Figure 6C | Time to descend (s): control: 15.4±1.3, <i>Pbx1</i> Th 14.5±1.2. n=13 control animals and 10 mutant animals. |
| Figure 6D | Total distance (cm): control 380±23, <i>Pbx1</i> Th 315±21. Entries in the center: control 26±7, <i>Pbx1</i> Th 25±3. Speed at the center (cm/s): control 0.68±0.13, <i>Pbx1</i> Th 0.45±0.09. Speed at periphery(cm/s): control 0.28±0.02, <i>Pbx1</i> Th 0.25±0.02. Time in the center: control 2.8%, <i>Pbx1</i> Th 3.9%. n=7 control animals and 6 mutant animals. |
| Figure 6E | Buried marbles: control 2±0.35, <i>Pbx1</i> Th 2±0.27. n=10 control animals and 6 mutant animals. |
| Figure 8G | <i>TH+</i> <i>GFP+/GFP+</i> : Control + CRE 68±3%, <i>Pbx1</i> ^{F/FI} +CRE 44±2%, <i>Pbx1</i> ^{F/FI} +CRE+ <i>Pbx1a</i> 68±2%, <i>Pbx1</i> ^{F/FI} +CRE+ <i>Pbx1b</i> 47±9%. n= 5, 12, 4 and 4 animals respectively. |
| Figure S5 | Relative expression levels: control 1±0.06, <i>Pbx1</i> Th 0.9±0.07. n=4 control animals and 3 mutant animals. |