

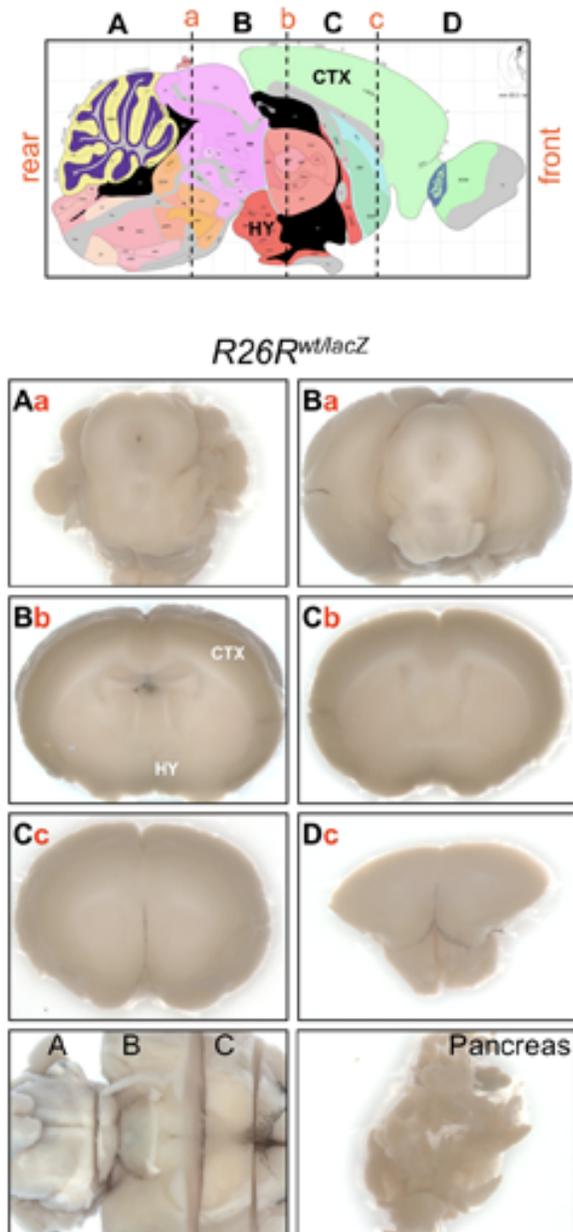
ONLINE APPENDIX

Supplemental Table 1. Source of Mice Used in This Report

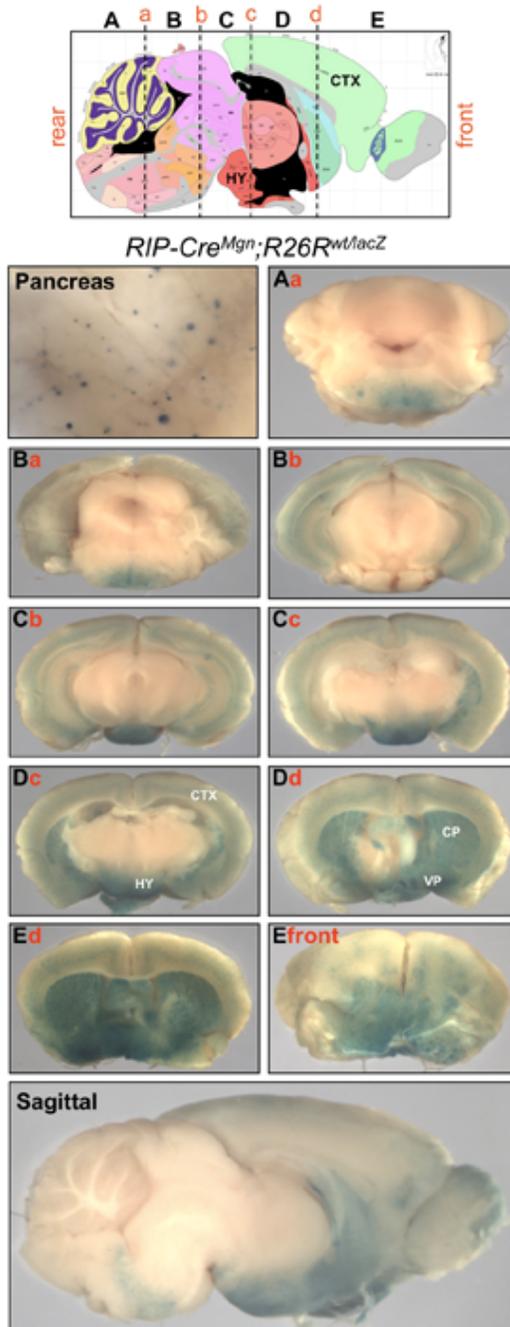
MGI Nomenclature	Synonym Used in This Report	Source of Mice Used in This Report*
<i>Tg(Ins2-cre)^{25Mgn}</i>	<i>RIP-Cre^{Mgn}</i>	The Jackson Laboratory
<i>Tg(Ins2-cre)^{1Herr}</i>	<i>RIP-Cre^{Herr}</i>	Mutant Mouse Regional Resource Center, University of Missouri
<i>Tg(Ins2-creEsr1)^{1Dam}</i>	<i>RIP-Cre/ERT</i>	The Jackson Laboratory
<i>Tg(Ins1-cre/ERT)^{1Lphi}</i>	<i>MIP-Cre/ERT</i>	Dr. Louis Philipson, University of Chicago
<i>Tg(Pdx1-cre)^{89.1Dam}</i>	<i>Pdx1-Cre^{Dam}</i>	Dr. Douglas Melton, Harvard University, Dr. Guiping Guoqiang, Vanderbilt University
<i>Tg(lpf1-cre)^{1Tuv}</i>	<i>Pdx1-Cre^{Tuv}</i>	Drs. David Tuveson, Cambridge Research Institute, UK and Andrew Lowy, UCSD
<i>Tg(Pdx1-cre/ERT)^{1Mga}</i>	<i>Pdx1^{AI-III}-Cre/ERT</i>	Drs. Christopher Wright and Maureen Gannon, Vanderbilt University
<i>Pdx1^{tm1Cvw}</i>	<i>Pdx1^{wt/lacZ}</i>	Drs. Christopher Wright, Vanderbilt University
<i>Gt(ROSA)26Sor^{tm1(EYFP)Cos}</i>	<i>R26R^{YFP}</i>	The Jackson Laboratory
<i>Gt(ROSA)26Sor^{tm1Sor}</i>	<i>R26R^{lacZ}</i>	The Jackson Laboratory

* Refers to source where mice were obtained; see Table 1 for information about creation of mouse strain.

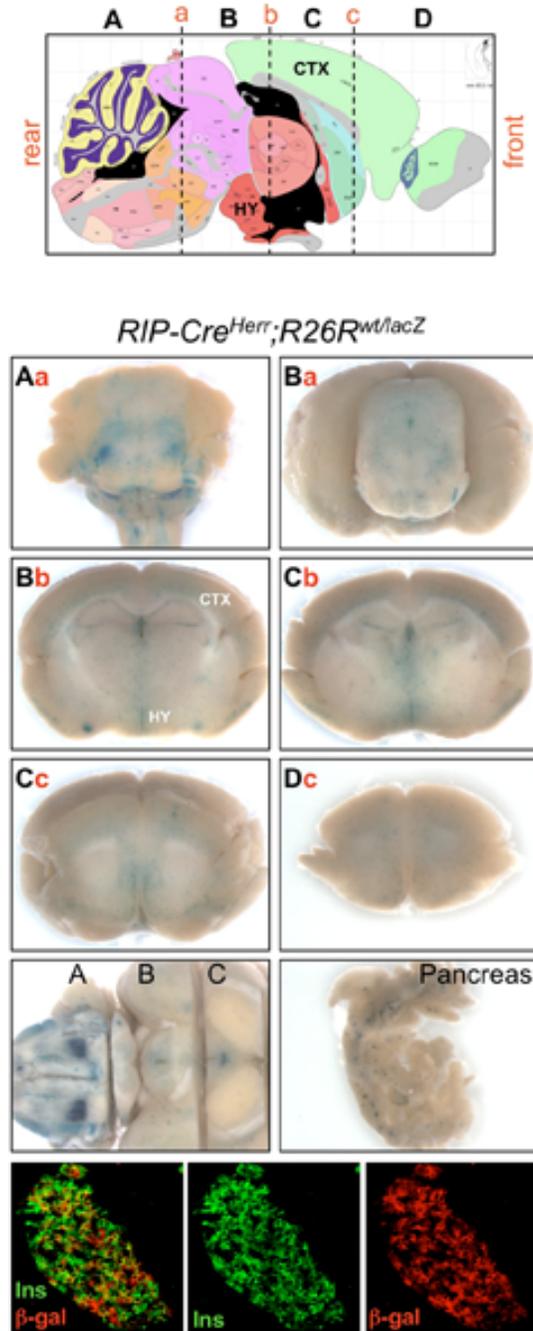
Supplemental Figure 1. Detection of Cre-mediated recombination in $R26R^{wt/lacZ}$ brain. Adult brains were sliced into 4 or 5 coronal sections and subjected to whole mount X-gal staining. (*Top panel*) Sagittal view of mouse brain (from the Allen Mouse Brain Atlas; <http://www.brain-map.org/>)(1). For each Cre transgenic line and controls, brain slices examined are shown in capital letters (A, B, C, etc.). A vertical dashed line marks coronal sectioning plane designated as face in lower case letters (a, b, c, etc.). (*Bottom panel*) Images of individual brain slices from each coronal sectioning plane. In the Bb panel, the cortex (CTX) and hypothalamus (HY) are labeled and correspond to regions marked in the *top panel*. $R26R^{wt/lacZ}$ brains (n=17) and pancreata (n=17) were negative for X-gal staining. The schematics of the mouse brain are from the Allen Mouse Brain Atlas (<http://www.brain-map.org/>).



Supplemental Figure 2. Detection of Cre-mediated recombination in *RIP-Cre^{Mgn}* brain. Adult brains were sliced, labeled and imaged as described in Supplemental Figure 1. (*Top panel*) Sagittal view of mouse brain (from the Allen Mouse Brain Atlas; <http://www.brain-map.org/>)(1) with designated brain slices and coronal sectioning planes. (*Middle panel*) Images of pancreas and individual brain slices from each coronal sectioning plane. The cortex (CTX), hypothalamus (HY), caudate putamen (CP), and ventral pallidum (VP) are labeled in panels Dc or Dd. (*Bottom panel*) Sagittal brain section. *RIP-Cre^{Mgn};R26R^{wt/lacZ}* mice (n=8) showed X-gal staining throughout the brain with high signal intensity in the mid-brain and ventral regions in (*middle and bottom panels*).

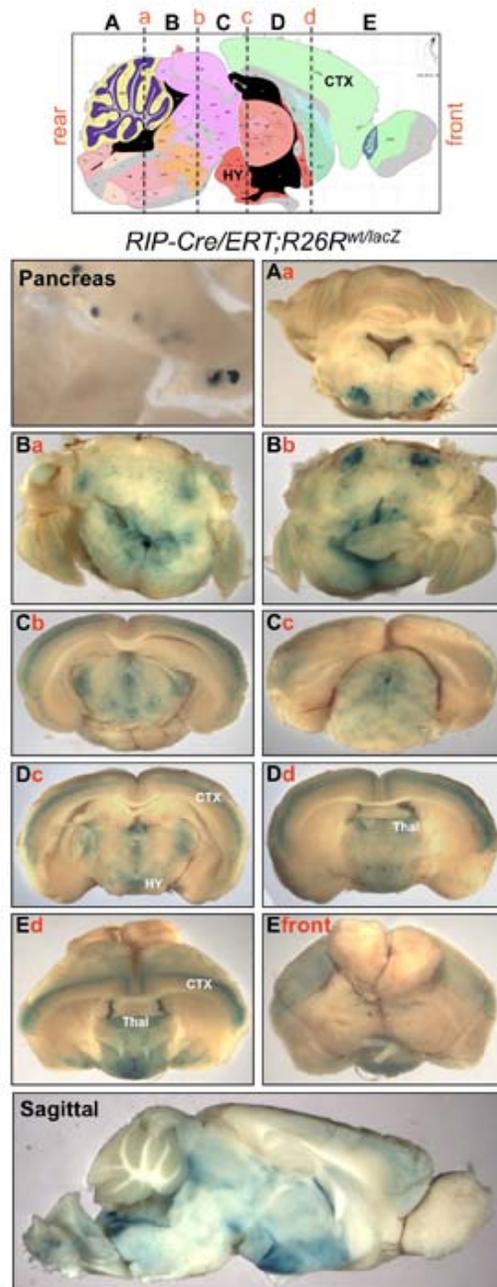


Supplemental Figure 3. Detection of Cre-mediated recombination in *RIP-Cre^{Herr}* brain. Adult brains were sliced, labeled and imaged as described in Supplemental Figure 1. (*Top panel*) Sagittal view of mouse brain (from the Allen Mouse Brain Atlas; <http://www.brain-map.org/>)(1) with designated brain slices and coronal sectioning planes. (*Middle panel*) Images of pancreas and individual brain slices from each coronal sectioning plane. *RIP-Cre^{Herr};R26R^{wt/lacZ}* mice (n=14) showed punctate X-gal staining throughout the brain without obvious regionalization. In the Bb panel, the cortex (CTX) and hypothalamus (HY) are labeled and correspond to regions marked in the *top panel*. (*Bottom panel*) Co-localization of β -gal (red) and insulin (green) in a pancreatic islet; 40x magnification.

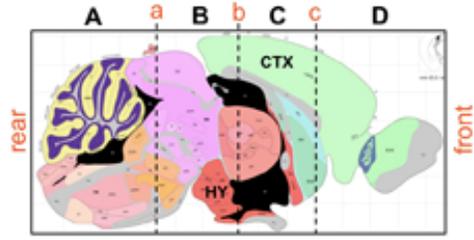


Supplemental Figure 4. Detection of Cre-mediated recombination in *RIP-Cre/ERT* brain.

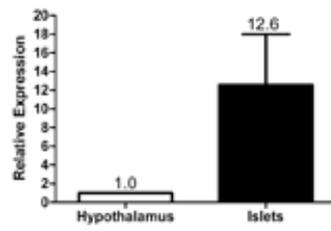
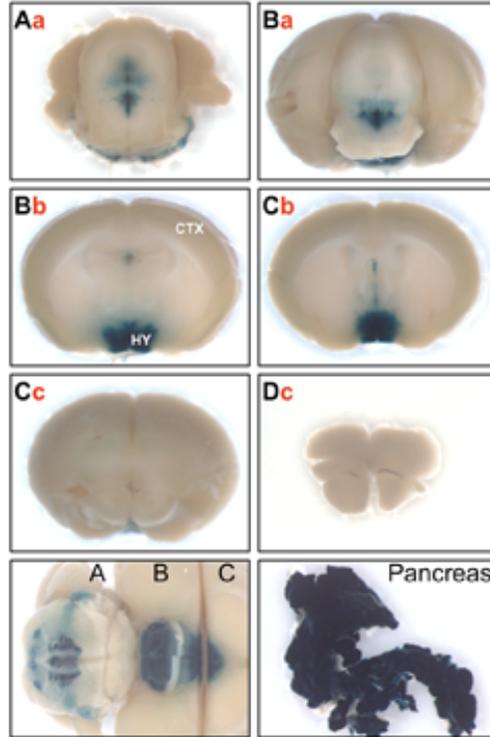
Adult brains were sliced, labeled and imaged as described in Supplemental Figure 1. (*Top panel*) Sagittal view of mouse brain (from the Allen Mouse Brain Atlas; <http://www.brain-map.org/>)(1) with designated brain slices and coronal sectioning planes. (*Middle panel*) Images of pancreas and individual brain slices from each coronal sectioning plane. The cortex (CTX), hypothalamus (HY), and thalamus (Thal) are labeled in panels Dc or Dd. (*Bottom panel*) Sagittal brain section. *RIP-Cre/ERT;R26R^{wt/lacZ}* mice (n=4) were injected i.p. with three 8-mg doses of tamoxifen and analyzed for LacZ expression. Strong, punctate X-gal staining was observed throughout the brain in (*middle and bottom panels*) but the expression pattern was more restricted than that in *RIP-Cre^{Mgn}; R26R^{wt/lacZ}* mice. Brains from littermate controls injected with corn oil vehicle were negative for X-gal staining (data not shown).



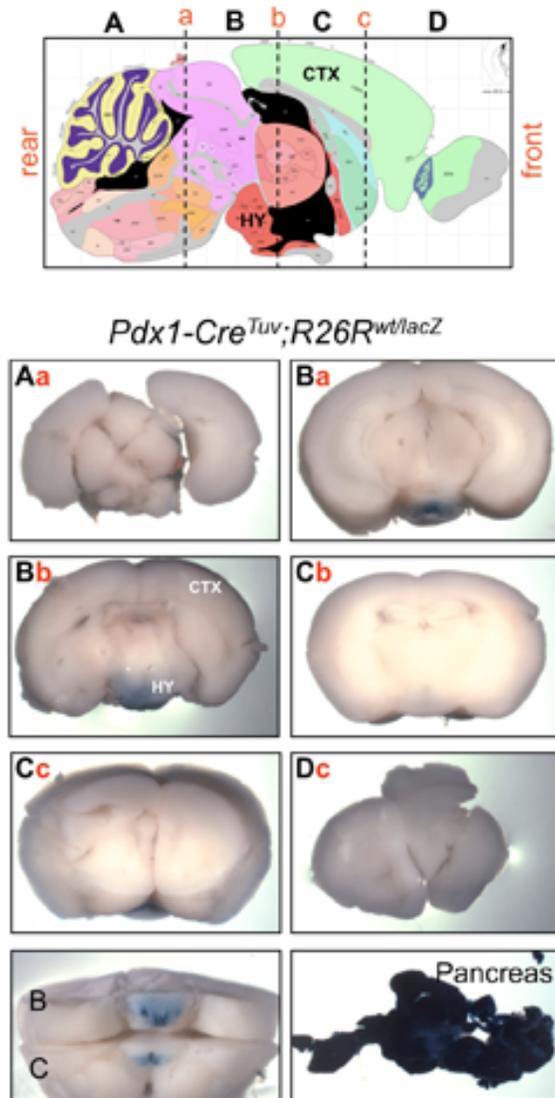
Supplemental Figure 5. Detection of Cre-mediated recombination in *Pdx1-Cre^{Dam}* brain. Adult brains were sliced, labeled and imaged as described in Supplemental Figure 1. (*Top panel*) Sagittal view of mouse brain (from the Allen Mouse Brain Atlas; <http://www.brain-map.org/>)(1) with designated brain slices and coronal sectioning planes. (*Middle panel*) Images of pancreas and individual brain slices from each coronal sectioning plane. X-gal staining in *Pdx1-Cre^{Dam};R26R^{wt/lacZ}* brain (n=7) was localized to the brain stem and hypothalamus. In the Bb panel, the cortex (CTX) and hypothalamus (HY) are labeled and correspond to regions marked in Figure 1A. (*Bottom panel*) Relative *Cre* mRNA expression in *Pdx1-Cre^{Dam}* hypothalamus and islets (n=3). *Cre* mRNA was undetectable in hypothalamus and islets of wild type controls (n=2) (data not shown).



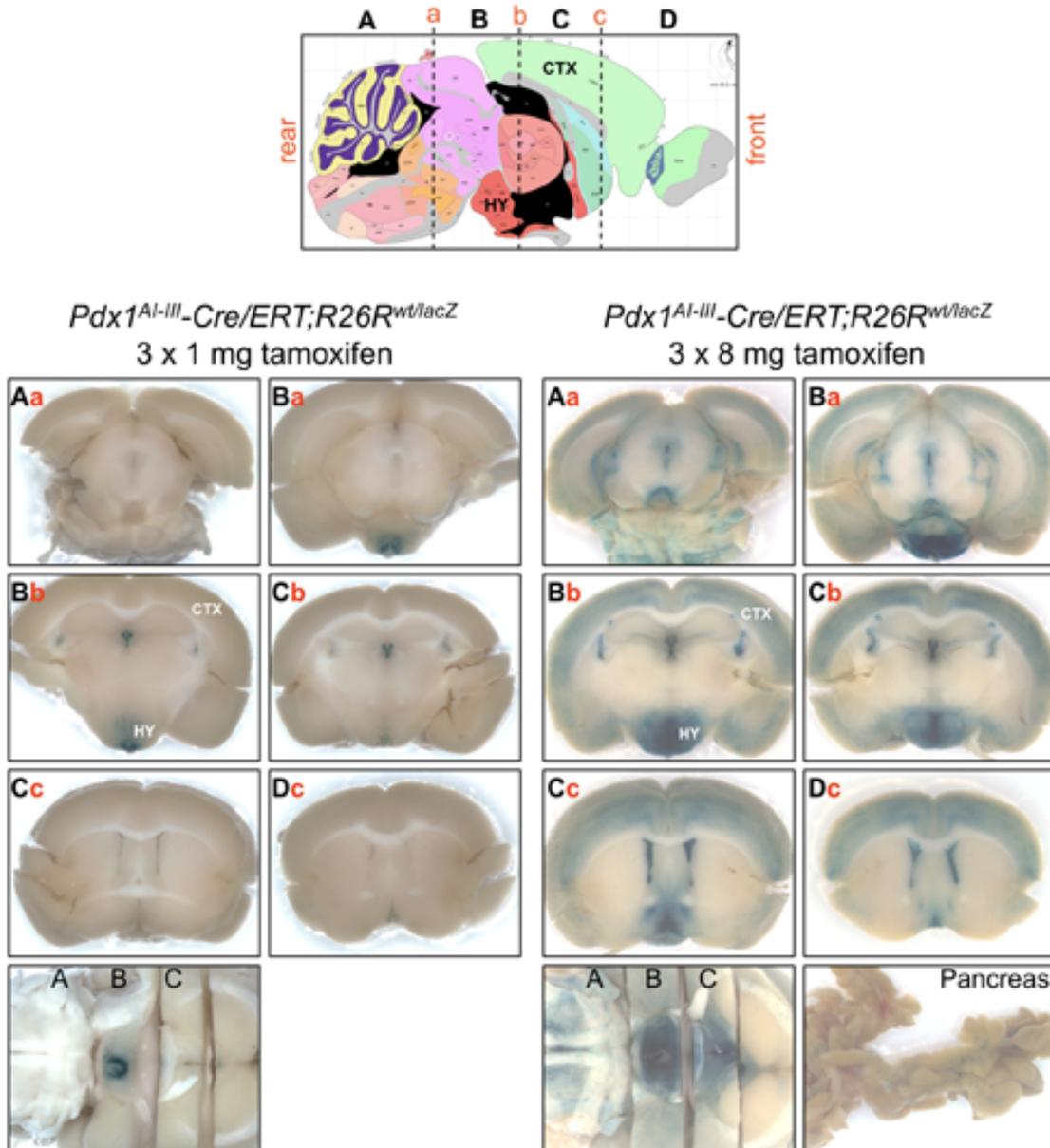
Pdx1-Cre^{Dam};R26R^{wt/lacZ}



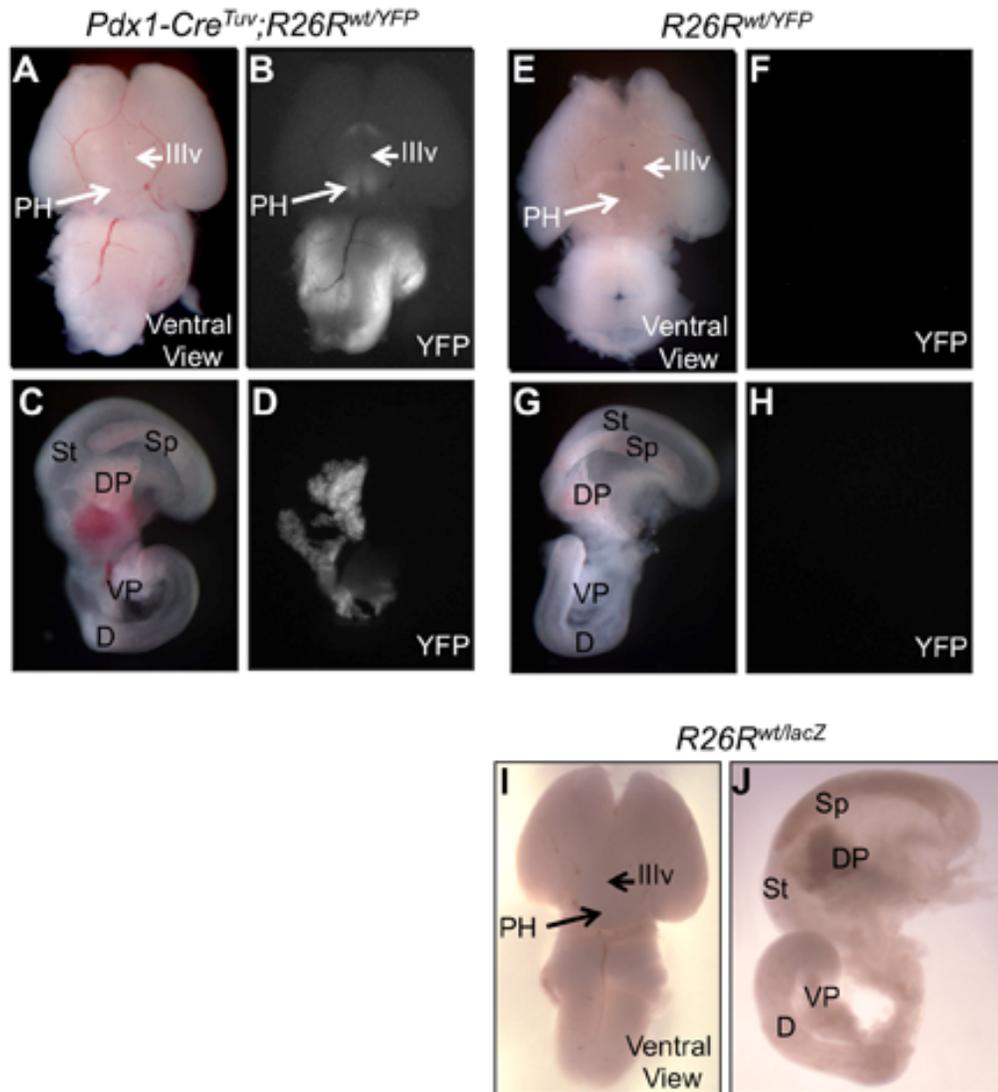
Supplemental Figure 6. Detection of Cre-mediated recombination in *Pdx1-Cre^{Tuv}* brain. Adult brains were sliced, labeled and imaged as described in Supplemental Figure 1. (*Top panel*) Sagittal view of mouse brain (from the Allen Mouse Brain Atlas; <http://www.brain-map.org/>)(1) with designated brain slices and coronal sectioning planes. In the Bb panel, the cortex (CTX) and hypothalamus (HY) are labeled and correspond to regions marked in the *top panel*. (*Bottom panel*) Images of pancreas and individual brain slices from each coronal sectioning plane. X-gal positive cells in *Pdx1-Cre^{Tuv};R26R^{wt/lacZ}* brain (n=4) were localized to hypothalamic region.



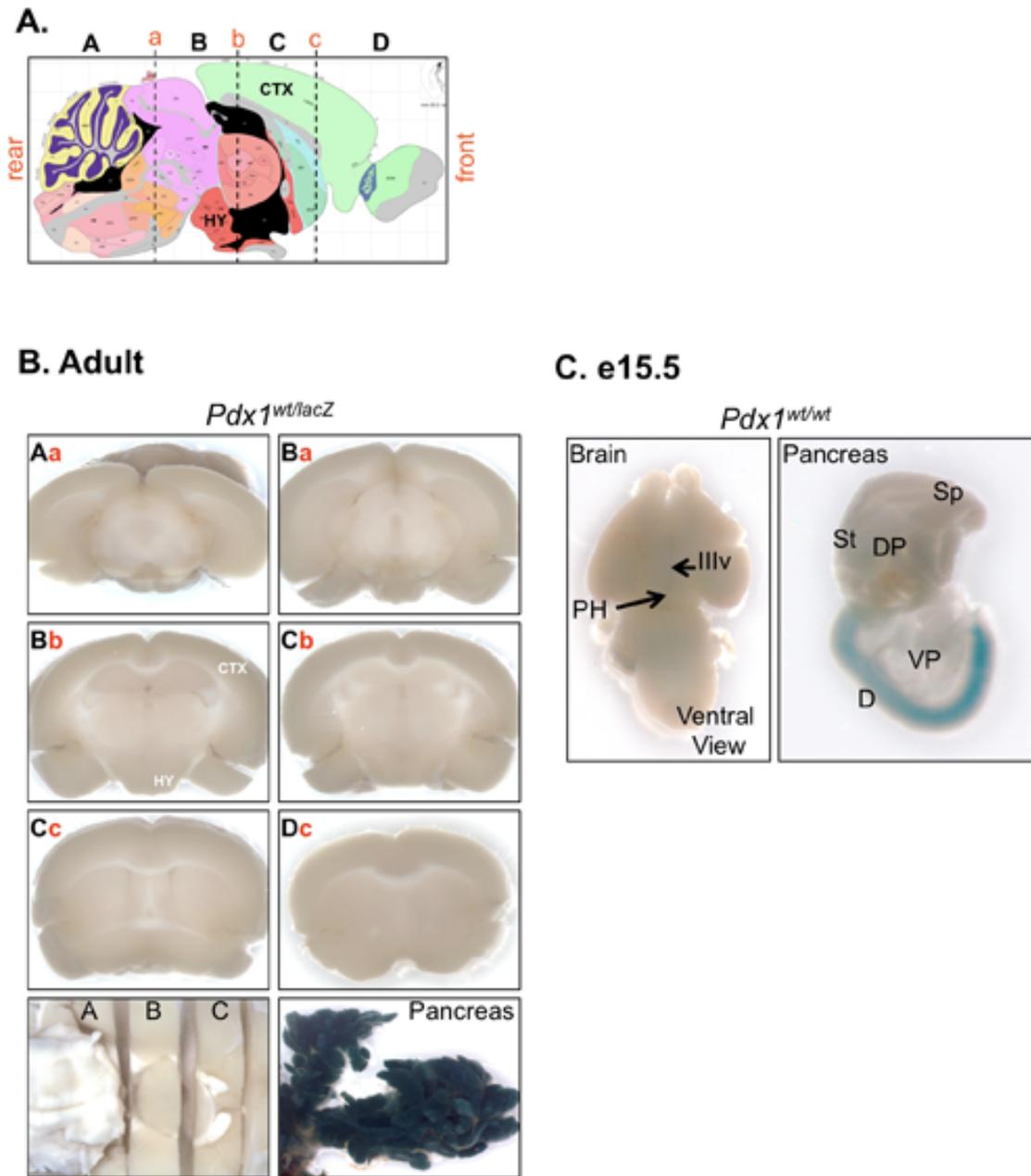
Supplemental Figure 7. Detection of Cre-mediated recombination in *Pdx1^{AI-III}-Cre/ERT* brain. Adult brains were sliced, labeled and imaged as described in Supplemental Figure 1. (*Top panel*) Sagittal view of mouse brain (from the Allen Mouse Brain Atlas; <http://www.brain-map.org/>)(1) with designated brain slices and coronal sectioning planes. Adult *Pdx1^{AI-III}-Cre/ERT;R26R^{wt/lacZ}* mice (n=4) were injected s.c. with 3 doses of either 1-mg in (*left panel*) or 3 doses of 8-mg tamoxifen in (*right panel*) and analyzed for LacZ expression. At the lower tamoxifen dose, X-gal staining was localized primarily to the hypothalamus in (*left panel*) whereas a broader punctate staining pattern was observed using the high tamoxifen dose in (*right panel*). Brains from littermate controls injected with corn oil vehicle (n=2) were negative for X-gal staining (data not shown). In the Bb panels, the cortex (CTX) and hypothalamus (HY) are labeled and correspond to regions marked in the *top panel*.



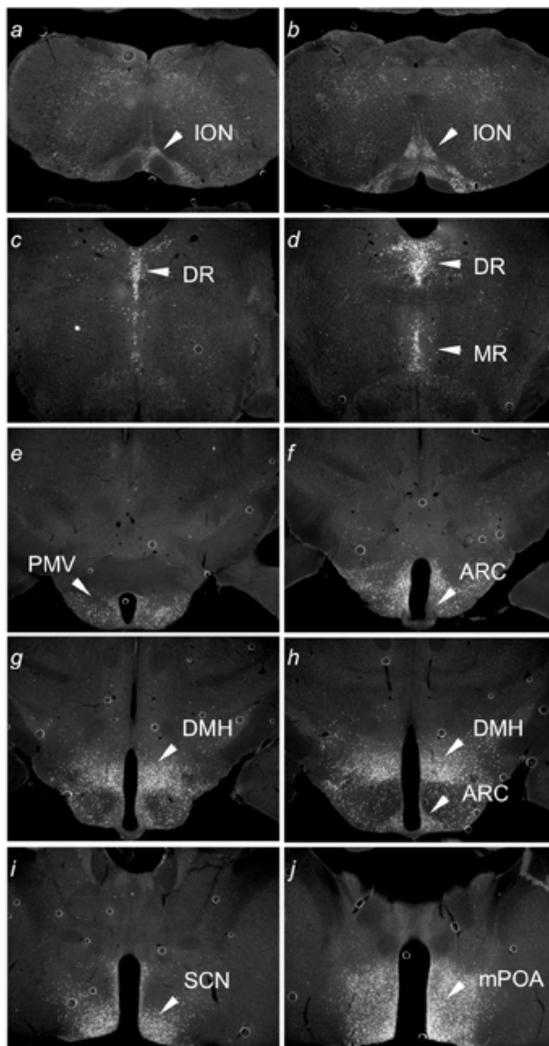
Supplemental Figure 8. Detection of Cre activity in *Pdx1-Cre^{Tuv}* brain at e15.5. Brains and pancreata from *Pdx1-Cre^{Tuv};R26R^{wt/YFP}* embryos (n=8) in (A-D) and their controls (n=6) in (E-H) at e15.5 were analyzed for YFP expression. *R26R^{YFP}* reporter strains indicated expression of *Pdx1-Cre^{Tuv}* transgene in the brain stem and ventral region of the brain that gives rise to the hypothalamus (arrows). (I and J) Brain and pancreas from *R26R^{wt/lacZ}* e15.5 controls (n=5) were negative for X-gal staining. *Abbreviations:* PH-posterior hypothalamic region, St-stomach, Sp-spleen, D-duodenum, DP-dorsal pancreas, VP-ventral pancreas.



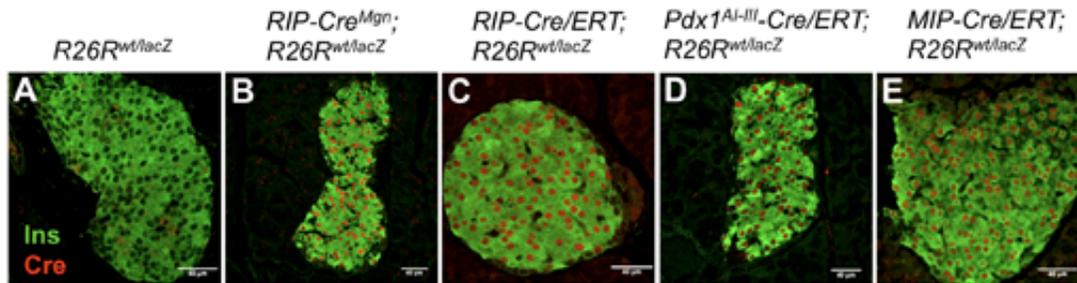
Supplemental Figure 9. LacZ expression is undetectable in brain of adult *Pdx1^{lacZ/wt}* mice. Adult brains were sliced, labeled and imaged as described in Supplemental Figure 1. (A) Sagittal view of mouse brain with designated brain slices and coronal sectioning planes. Sagittal view of mouse brain (from the Allen Mouse Brain Atlas; <http://www.brain-map.org/>)(1) with designated brain slices and coronal sectioning planes. (B) *Pdx1^{lacZ/wt}* brains (n=4) were negative for X-gal staining, while pancreas showed expected X-gal positivity. In the Bb panel, the cortex (CTX) and hypothalamus (HY) are labeled and correspond to regions marked in the *top panel*. (C) In e15.5 *Pdx1^{wt/wt}* embryos (n=10), both brain and pancreas was X-gal negative. *Abbreviations:* PH-posterior hypothalamic region, St-stomach, Sp-spleen, D-duodenum, DP-dorsal pancreas, VP-ventral pancreas.



Supplemental Figure 10. Detection of β -gal signal in brain sections of adult *Pdx1-Cre^{Dam};R26R^{wt/lacZ}* mice. Adult *Pdx1-Cre^{Dam};R26R^{wt/lacZ}* mice were perfusion-fixed, brains isolated and subjected to immunohistochemical labeling for β -gal (β -gal) as a marker for Cre recombination. All coordinates are relative to bregma as based on “The Mouse Brain in Stereotaxic Coordinates” Franklin and Paxinos, 3rd Edition, 2008 (2). All abbreviations are for brain regions are either commonly used or from the mouse brain atlas. (a-b) β -Gal labeling in the brainstem shows signal in the inferior olivary nucleus (ION); bottom center of each image. a = -8.00mm and b = -7.48mm from bregma. (c-d) β -Gal labeling in the hindbrain showing strong signal in the Dorsal Raphe nucleus (DR in (c) and Dorsal and Median Raphe nuclei (DR and MR, respectively) in (d). c = -4.96mm and d = -4.72mm from bregma. (e-f) β -Gal labeling in the caudal hypothalamus showing signal in the ventral premammillary nucleus (PMV) in (e) and arcuate nucleus (ARC) in (f). e = -2.54mm and f = -2.30 mm from bregma. (g-h) β -Gal labeling in the rostral hypothalamus showing signal in the dorsomedial hypothalamus (DMH) in (g) and the ARC/DMH in (h). g = -1.94mm and h = -1.58 mm from bregma. Note: In (e-h), β -gal expression is shown at different levels through the hypothalamus with limited excision within the arcuate nucleus. (i-j) Extensive β -gal labeling in the preoptic area showing strong signal in the suprachiasmatic nucleus (SCN) in (i) and medial preoptic area (mPOA) in (j). i = -0.46mm and j = -0.22mm from bregma. Scale bar is 500 μ m.



Supplemental Figure 11. Cre-recombinase expression in the islets of Langerhans. (A-E) Pancreatic sections prepared from $R26R^{wt/lacZ}$ in (A), $RIP-Cre^{Mgn}$ in (B), $RIP-Cre/ERT$ in (C), $Pdx1^{AI-III}-Cre/ERT$ in (D) and $MIP-Cre/ERT$ in (E) were co-labeled for insulin (green) and Cre recombinase (red). Mice expressing tamoxifen-inducible Cre/ERT in (C-E) were sacrificed the day after receiving the third (last) 2-mg dose of tamoxifen. Data was expressed as the percent of Cre positive β -cells per islet, n=10 islets per each mouse strain (see Results sections). Scale bar in (A-E) represents 40 μ m.



SUPPLEMENTAL REFERENCES

1. Allen Brain Atlas Resources [Internet]. <http://www.brain-map.org>, Allen Institute for Brain Science. Seattle, WA. , 2009
2. Franklin KBJ, Paxinos G: *The Mouse Brain in Stereotaxic Coordinates*. New York, Academic Press, 2007