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Nkx2.2:Cre Knock-In Mouse Line: A Novel Tool for Pancreas- and CNS-Specific Gene Deletion

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Summary

Nkx2.2 is a homeodomain-containing transcriptional regulator necessary for the appropriate differentiation of ventral neuronal populations in the spinal cord and hindbrain, and endocrine cell populations in the pancreas and intestine. In each tissue, Nkx2.2 inactivation leads to reciprocal cell fate alterations. To confirm the cell fate changes are due to respecification of Nkx2.2-expressing progenitors and to provide a novel tool for lineage tracing in the pancreas and CNS, we generated an Nkx2.2:Cre mouse line by knocking in a Cre-EGFP cassette into the Nkx2.2 genomic locus and inactivating endogenous Nkx2.2. The R26R-CAG-LSL-tdTomato reporter was used to monitor the specificity and efficiency of Nkx2.2:Cre activity; the tomato reporter faithfully recapitulated endogenous Nkx2.2 expression and could be detected as early as embryonic day (e) 9.25 in the developing CNS and was initiated shortly thereafter at e9.5 in the pancreas. Lineage analyses in the CNS confirmed the cell populations thought to be derived from Nkx2.2-expressing progenitor domains. Furthermore, lineage studies verified Nkx2.2 expression in the earliest pancreatic progenitors that give rise to all cell types of the pancreas; however they also revealed more robust Cre activity in the dorsal versus ventral pancreas. Thus, the Nkx2.2:Cre line provides a novel tool for gene manipulations in the CNS and pancreas. genesis 00:00-00.

Keywords

Nkx2.2; Cre-lox; CNS; pancreas; lineage

INTRODUCTION

Nkx2.2 is a tissue specific homeodomain transcription factor expressed in the central nervous system (CNS), pancreas, and intestine. In each of these tissues, *Nkx2.2* is dynamically expressed and plays essential roles in cell fate decisions. In the developing neural tube, *Nkx2.2* is expressed in a ventral stripe along the anterior-posterior axis at the ~13 somite stage where it specifies the fates of neural progenitors in response to sonic hedgehog (Shh) signaling (Briscoe *et al*., 1999; Ericson *et al*., 1997a; Pattyn *et al*., 2003a; Shimamura *et al*., 1995). Nkx2.2 function has been most extensively characterized in the hindbrain and spinal cord regions, where disruption of *Nkx2.2* causes progenitor populations

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to undergo fate switches to produce ectopic motor neurons (Briscoe *et al*., 1999; Pattyn *et al*., 2003a,b).

In the developing pancreas, *Nkx2.2* expression can first be detected in the dorsal pancreatic rudiment around embryonic day (e) 8.75 and subsequently in the ventral pancreatic rudiment at e9.5 (Jorgensen *et al*., 2007). At the earliest stages, *Nkx2.2* is expressed in the *Pancreatic duodenal homeobox 1* (*Pdx1*)—expressing pancreatic progenitor cells, but is subsequently restricted to the *Neurogenin3* (*Neurog3*) —expressing endocrine progenitor cells. Ultimately, Nkx2.2 is localized to specific endocrine populations within the islet, including insulin-producing beta cells, glucagon-producing alpha cells, PP-producing PP cells, and ghrelin-producing epsilon cells (Arnes *et al*., 2012; Jorgensen *et al*., 2007; Sussel *et al*., 1998). In mice lacking *Nkx2.2*, islet cell specification is disrupted; beta cells are not specified, alpha and PP cells form in reduced numbers, and the majority of endocrine cells express ghrelin (Prado *et al*., 2004). *Nkx2.2* is also expressed in several enteroendocrine cell populations within the small intestine. Disruption of *Nkx2.2* function also leads to the loss of many hormone expressing cell types in the duodenum at the expense of increased ghrelin cell numbers (Desai *et al*. 2008).

The well-defined expression of Nkx2.2 in several essential progenitor domains makes it a good candidate to study progenitor cell fates in normal and perturbed developmental conditions. To provide a tool for manipulating gene expression in the *Nkx2.2*+ progenitor cells of the CNS and pancreas, we expressed Cre recombinase from the endogenous *Nkx2.2* regulatory elements. Although caveats exist, the Cre/LoxP site-specific recombinase system remains a powerful tool to facilitate cell-specific gene inactivation and lineage analyses in the mouse (Harno *et al*., 2013; Magnuson and Osipovich, 2013; Smedley *et al*., 2011). Previously, a *Nkx2.2-Cre* transgenic mouse line was generated to express Cre recombinase under the transcriptional control of a *Nkx2.2* CNS regulatory element; however, this element does not drive expression in the pancreas (Lei *et al*., 2006; Wang *et al*., 2011). In this study, we knocked *Cre* into the first coding exon of *Nkx2.2* to recapitulate Cre expression in the entire Nkx2.2 expression domain and allow for the study of lineage decisions in the presence and absence of Nkx2.2 function. This novel mouse line provides a valuable tool for studying coordinated gene functions and lineage decisions in the CNS, pancreas and intestine.

RESULTS AND DISCUSSION

The *Nkx2.2:Cre-EGFP* knockin line was generated using recombination mediated cassette exchange (RMCE) technology in *Nkx2.2* acceptor allele (*Nkx2.2*^{LCA}) ES cells (Fig. 1a) (Arnes *et al*., 2012; Chen *et al*., 2011). To generate the *Nkx2.2:Cre-EGFP* allele, we engineered a *Cre-EGFP* fusion protein into the first coding exon of *Nkx2.2* such that *Cre* would be expressed from the endogenous *Nkx2.2* start codon and the majority of coding exon 1 would be deleted (Fig. 1a). This strategy allowed for inactivation of *Nkx2.2*, while retaining all potential non-coding regulatory elements. We confirmed the Cre-mediated exchange event in *Nkx2.2*^{LCA} ES cells by PCR analysis (Fig. 1c) and ES cell clone 4H9/IE4 was used to generate mice carrying the *Nkx2.2*^{CreEGFP} allele. Following germline transmission of the *Nkx2.2*^{CreEGFP} allele, the *hygromycin* selection cassette was excised from the genome by mating to FLPe mice (Dymecki, 1996), as described previously (Arnes *et al*., 2012). Subsequent PCR genotyping was performed using primers P5 and P6 (326 bp PCR product) to determine the presence of the *Nkx2.2*^{CreEGFP} allele (Fig. 1c).

Similar to the conventional *Nkx2.2*$^{+/-}$ mice (Sussel *et al*., 1998), heterozygous *Nkx2.2*$^{CreEGFP/+}$ mice were viable and did not display overt developmental or postnatal defects (data not shown). To determine the activity and integrity of the novel *Nkx2.2*$^{CreEGFP/+}$ allele, we crossed the *Nkx2.2*$^{CreEGFP/+}$ mice to *R26R-tdTomato* Cre-

inducible reporter mice (Madisen *et al*., 2010). *Nkx2.2*$^{Cre/+}$; *R26R:Tomato* embryos and mice showed robust Cre activity; we could detect reporter expression in all previously characterized Nkx2.2-expressing tissues, whereas reporter expression was undetectable in littermates lacking the *Nkx2.2-Cre* allele (Fig. 2a,b, data not shown). Although Cre appeared to be highly active, we could only detect extremely low levels of EGFP by direct fluorescence and FAC sorting (Supporting Information Fig. 1a-c). Furthermore, any potential EGFP nuclear signal was quenched during tissue processing and therefore did not interfere with subsequent immunofluorescence analyses (Supporting Information Fig. 1d-i). Analogous to mice deleted for both copies of *Nkx2.2* (*Nkx2.2*$^{-/-}$; (Prado *et al*., 2004; Sussel *et al*., 1998), homozygous *Nkx2.2*$^{CreEGFP/CreEGFP}$ mice died shortly after birth and displayed pancreatic phenotypes indistinguishable from the *Nkx2.2*$^{-/-}$ null mice, including the absence of insulin-producing beta cells, decreased glucagon-producing alpha cells and a concomitant increase in ghrelin-producing epsilon cells (Fig. 2c-h).

To determine the onset of Nkx2.2-Cre activity in *Nkx2.2*$^{Cre/+}$; *R26R:Tomato* embryos, we visualized the conditional tomato reporter using direct fluorescence of whole mount embryos. We could first detect Cre activity along the entire A/P axis of the CNS by e9.25 (19 somites; Fig. 3a, b). By e9.5, shortly after detection of reporter expression in the CNS, we could visualize tomato expression in the pancreatic rudiments (Fig. 3c). Robust reporter expression in Nkx2.2-expressing domains and in Nkx2.2-derived lineages was maintained at subsequent embryonic stages, confirming the contribution of Nkx2.2-expressing progenitor cells in the ventral hindbrain to vagal motor neuron populations (Ericson *et al*., 1997b; Fig. 3d-e).

To monitor the efficiency and specificity of the *Nkx2.2-Cre* allele in each of the organs expressing *Nkx2.2*, we assessed tomato expression in tissue sections. At e9.5, tomato expression was visible in the dorsal but not ventral pancreatic rudiment, consistent with the previously reported delay of *Nkx2.2* expression in the ventral bud (Jorgensen *et al*., 2007; Fig. 4a). In the dorsal bud at this stage, tomato was co-expressed with all glucagon-producing cells and a subset of Pdx1-expressing cells (Fig. 4a', a"'). Interestingly, the two populations do not appear to overlap, suggesting that the "early" alpha cells are derived from Nkx2.2-expressing populations that are negative for Pdx1. By e12.5, Nkx2.2-Cre was active in the majority, but not all Pdx1-expressing cells of the dorsal pancreatic bud. Surprisingly, however, even at this later stage of pancreas development, the tomato reporter was only expressed in a small subset of cells in the ventral pancreatic bud, perhaps indicating Cre is not expressed or there is lower Nkx2.2-Cre activity in these cells (Fig. 4b). By 14.5, we can detect tomato activity throughout the dorsal and ventral pancreas in the central and tip domains. Tomato expression appeared most robust in the central epithelium where it was coexpressed with Neurog3 in the endocrine progenitors and insulin, glucagon, and ghrelin in the differentiated endocrine populations (Fig. 4c-e). Tomato expression could also be detected in the amylase-positive acinar cells (Fig. 4d). Endogenous *Nkx2.2* expression is normally extinguished from the differentiating acinar cells, however tomato staining of the exocrine lineage is consistent with expression of *Nkx2.2* in the pancreatic precursors that give rise to all pancreatic lineages, including the acini (Gu *et al*., 2003).

In the adult pancreas, tomato-expressing cells are present throughout the exocrine and endocrine compartments. In the islet, tomato is coexpressed with glucagon, insulin, and PP in the alpha, beta, and PP cell populations, as previously reported for endogenous Nkx2.2 (Sussel *et al*., 1998; Fig. 5a and data not shown). Nkx2.2 is also expressed in the enteroendocrine cells of the small intestine (Desai *et al*. 2008). Consistently, tomato is expressed in scattered enteroendocrine cell populations within the villi of the duodenum at birth (Fig. 5b).

As evident in whole mount embryos (Fig. 2), Nkx2.2-Cre is active along the A/P axis of the CNS. In transverse sections at the level of the thoracic spinal cord, we could detect tomato reporter expression adjacent to the floor plate in the midline Sox9+ progenitor domain and extended laterally into the V3 interneurons that are derived from the Nkx2.2-expressing P3 population (Fig. 6a, e). Nkx2.2-Cre remains active in the Olig2+/Sox10+ oligodendrocyte precursor cells (OPCs), which develop from the P3 domain at later stages (data not shown). Furthermore, consistent with endogenous Nkx2.2 expression in ventral hindbrain progenitors that give rise to the visceral motor neurons (vMN), tomato expression could be detected in the axons of the vMNs that project along the vagus (Xth cranial) nerve and co-express the neuronal marker Tuj1 (Ericson *et al*., 1997b; Fig. 6b-d). Tomato expression was also maintained in efferent neurons extending into the stomach, face and ear (Fig. 6f-g, Supporting Information Fig. 2).

In summary, we have generated and characterized a novel *Nkx2.2:CreEGFP* knockin mouse line. We have demonstrated that Cre activity is highly efficient and restricted to the known Nkx2.2 expression domains in the CNS, pancreas and intestine. Although Cre and EGFP are expressed as a fusion protein, EGFP signal is almost undetectable and cannot be used to label Nkx2.2-expressing cells *in vivo*. This may indicate *Cre-EGFP* expression from the Nkx2.2 regulatory domain is generally low, but produces sufficient Cre recombinase to catalyze recombination of the *R26R:tomato* reporter. Furthermore, our data showing a paucity of tomato expression in the e12.5 ventral pancreatic bud may also suggest that *Nkx2.2* is expressed at even lower levels within the ventral pancreas resulting in sub-threshold Cre activity. This unexpected feature of the *Nkx2.2:CreEGFP* allele may prove to be useful in studies wishing to delete pancreatic genes primarily from the early dorsal pancreas primordia. We also demonstrate the usefulness of the *Nkx2.2:CreEGFP* allele to perform lineage analyses of the neuronal populations derived from the *Nkx2.2*-expressing progenitor domains in the ventral spinal cord and hindbrain.

MATERIALS AND METHODS

Generation of Nkx2.2:Cre-EGFP Mice

Nkx2.2:Cre-EGFP mice were generated using the RMCE protocol as described previously (Arnes *et al*., 2012; Chen *et al*., 2011). Schematic representation of the strategy is shown in Figure 1. The *Nkx2.2:Cre-eGFP* allele was created using recombineering to insert the *CreEGFP* fusion gene into the Nkx2.2 basal exchange vector pNkx2.2.Ex1.H (Arnes *et al*., 2012). The *CreEGFP* gene was fused in frame with the *Nkx2.2* start ATG and replaced all but 25 base pairs of the *Nkx2.2* first coding exon (Fig. 1 “targeting vector”). The modified *Nkx2.2:CreEGFP* targeting vector was sequence verified. For RMCE, the targeting vector was co-injected with a Cre-expressing vector (Sauer, 1998) into ES cells carrying the *Nkx2.2*^{LCA} allele (4H9). Cells were screened for loss of the selection cassette in the *Nkx2.2*^{LCA} allele and for hygromycin resistance, as described previously (Arnes *et al*., 2012). Surviving clones were PCR verified for the correct insertion. The 5′ primers (P1 and P2) verified integration of the *hygromycin* cassette within the correct genomic context (606 bp PCR product). The 3′ primers (P3 and P4) verified incorporation of the 3′ Lox2272 site (479 bp wild-type PCR product versus 556 bp Nkx2.2^{CreEGFP} allele), as described previously (Arnes *et al*., 2012; Fig. 1A,B). Correctly exchanged ES clone 4H9/1E4 was injected into C57/B16/J1 blastocysts and four resulting >95% chimeric male mice were mated to C57/B16/J1 mice (Jackson Labs). Germline passage of the allele from two chimeric males was confirmed by PCR using primers P5 (oDB1) (5′-CAGTCACAGACTACATTTCT-3′) and P6 (ODB128) 5′-TCATCACTCGTTGCATCGA-3′). Removal of the *FRT*-flanked *Hygromycin* cassette was

accomplished by breeding with *ACTB:FLPe* mice (Jackson Labs). The *Nkx2.2:Cre-EGFP* mice will be available to the research community upon request.

Animals

All mice were maintained on a C57/B16/J1 background and housed and treated according to the Columbia University Institutional Animal Care and Use Committee approval protocol AAAC0259 and AAAG3206. Nkx2.2-Cre activity was assessed using the *R26R:Tomato* reporter strain: *R26*-TOM (B6.Cg-*Gt(RO-SA)26Sor*$^{tm14(CAG-tdTomato)Hze}$/J) obtained from the Jackson Laboratory.

Immunofluorescence

Tissue processing and immunofluorescence was performed as previously described (Mastracci *et al*., 2011). The primary antibodies used were: rabbit α-amylase (1:500; Sigma-Aldrich), goat α-ghrelin (1:500; Santa Cruz Biotechnologies), guinea pig α-glucagon (1:1000; Linco) rabbit α-glucagon (1:1000; DAKO), guinea pig α-insulin (1:1000; Linco), rabbit α-insulin (1:1000; Cell Signaling Technology), rabbit α-pancreatic polypeptide (1:200; Zymed), rabbit α-somatostatin (1:200; Phoenix Pharmaceuticals), rabbit α-Pdx1 (1:1000; Millipore), rabbit α-Sox9 (1:500; Santa Cruz), rabbit α-Neurog3 (1:500; BCBC Antibody Core), rabbit α-Tuj1(1:200; Covance). Sections were incubated with appropriate secondary antibodies conjugated to DyLight-488, DyLight-549 and DyLight-649, Alexa-488, Alexa-647 (Jackson Immunoresearch). Fluorescein-Dolichos Biflorus Agglutinin (DBA, Vector Labs, Burlingame, CA) was added with the primary antibodies. DAPI (1:1000; Invitrogen) was applied for 30 min following secondary antibody incubation. Tomato signal was detected by direct fluorescence of the protein. Images were acquired with either an epifluorescence (Leica DM5500) or a confocal (Zeiss LSM710) microscope.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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LITERATURE CITED

Arnes L, Leclerc K, Friel JM, Hipkens SB, Magnuson MA, Sussel L. Generation of Nkx2.2:lacZ mice using recombination-mediated cassette exchange technology. Genesis. 2012 DOI:10.1002/dvg.22037.

Briscoe J, Sussel L, Serup P, Hartigan-O'Connor D, Jessell TM, Rubenstein JL, Ericson J. Homeo-box gene Nkx2.2 and specification of neuronal identity by graded Sonic hedgehog signalling. Nature. 1999; 398:622–627. [PubMed: 10217145]

Chen SX, Osipovich AB, Ustione A, Potter LA, Hipkens S, Gangula R, Yuan W, Piston DW, Magnuson MA. Quantification of factors influencing fluorescent protein expression using RMCE to generate an allelic series in the ROSA26 locus in mice. Dis Model Mech. 2011 DOI:dmm.006569 [pii] 10.1242/dmm.006569.

Desai S, Loomis Z, Pugh-Bernard A, Schrunk J, Doyle MJ, Minic A, McCoy E, Sussel L. Nkx2.2 regulates cell fate choice in the enteroendocrine cell lineages of the intestine. Dev Biol. 2008; 313:58–66. [PubMed: 18022152]

Dymecki SM. Flp recombinase promotes site-specific DNA recombination in embryonic stem cells and transgenic mice. Proc Natl Acad Sci USA. 1996; 93:6191–6196. [PubMed: 8650242]

Ericson J, Briscoe J, Rashbass P, van Heyningen V, Jessell TM. Graded sonic hedgehog signaling and the specification of cell fate in the ventral neural tube. Cold Spring Harbor Symp Quant Biol. 1997a; 62:451–466. [PubMed: 9598380]

Ericson J, Rashbass P, Schedl A, Brenner-Morton S, Kawakami A, van Heyningen V, Jessell TM, Briscoe J. Pax6 controls progenitor cell identity and neuronal fate in response to graded Shh signaling. Cell. 1997b; 90:169–180. [PubMed: 9230312]

Gu G, Brown JR, Melton DA. Direct lineage tracing reveals the ontogeny of pancreatic cell fates during mouse embryogenesis. Mech Dev. 2003; 120:35–43. [PubMed: 12490294]

Harno E, Cottrell EC, White A. Metabolic pitfalls of CNS cre-based technology. Cell Metab. 2013; 18:21–28. [PubMed: 23823475]

Jorgensen MC, Ahnfelt-Ronne J, Hald J, Madsen OD, Serup P, Hecksher-Sorensen J. An illustrated review of early pancreas development in the mouse. Endocr Rev. 2007; 28:685–705. [PubMed: 17881611]

Lei Q, Jeong Y, Misra K, Li S, Zelman AK, Epstein DJ, Matise MP. Wnt signaling inhibitors regulate the transcriptional response to morphogenetic Shh-Gli signaling in the neural tube. Dev Cell. 2006; 11:325–337. [PubMed: 16950124]

Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, Ng LL, Palmiter RD, Hawrylycz MJ, Jones AR, Lein ES, Zeng H. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. Nat Neurosci. 2010; 13:133–140. [PubMed: 20023653]

Magnuson MA, Osipovich AB. Pancreas-specific cre driver lines and considerations for their prudent use. Cell metabolism. 2013; 18:9–20. [PubMed: 23823474]

Mastracci TL, Wilcox CL, Arnes L, Panea C, Golden JA, May CL, Sussel L. Nkx2.2 and Arx genetically interact to regulate pancreatic endocrine cell development and endocrine hormone expression. Dev Biol. 2011; 359:1–11. [PubMed: 21856296]

Pattyn A, Vallstedt A, Dias JM, Samad OA, Krumlauf R, Rijli FM, Brunet JF, Ericson J. Coordinated temporal and spatial control of motor neuron and serotonergic neuron generation from a common pool of CNS progenitors. Genes Dev. 2003a; 17:729–737. [PubMed: 12651891]

Pattyn A, Vallstedt A, Dias JM, Sander M, Ericson J. Complementary roles for Nkx6 and Nkx2 class proteins in the establishment of motoneuron identity in the hindbrain. Development. 2003b; 130:4149–4159. [PubMed: 12874134]

Prado CL, Pugh-Bernard AE, Elghazi L, Sosa-Pineda B, Sussel L. Ghrelin cells replace insulin-producing beta cells in two mouse models of pancreas development. Proc Natl Acad Sci USA. 2004; 101:2924–2929. [PubMed: 14970313]

Sauer B. Inducible gene targeting in mice using the Cre/lox system. Methods. 1998; 14:381–392. [PubMed: 9608509]

Shimamura K, Hartigan DJ, Martinez S, Puelles L, Rubenstein JL. Longitudinal organization of the anterior neural plate and neural tube. Development. 1995; 121:3923–3933. [PubMed: 8575293]

Smedley D, Salimova E, Rosenthal N. Cre recombinase resources for conditional mouse mutagenesis. Methods. 2011; 53:411–416. [PubMed: 21195764]

Sussel L, Kalamaras J, Hartigan-O'Connor DJ, Meneses JJ, Pedersen RA, Rubenstein JL, German MS. Mice lacking the homeodomain transcription factor Nkx2.2 have diabetes due to arrested differentiation of pancreatic beta cells. Development. 1998; 125:2213–2221. [PubMed: 9584121]

Wang H, Lei Q, Oosterveen T, Ericson J, Matise MP. Tcf/Lef repressors differentially regulate Shh-Gli target gene activation thresholds to generate progenitor patterning in the developing CNS. Development. 2011; 138:3711–3721. [PubMed: 21775418]

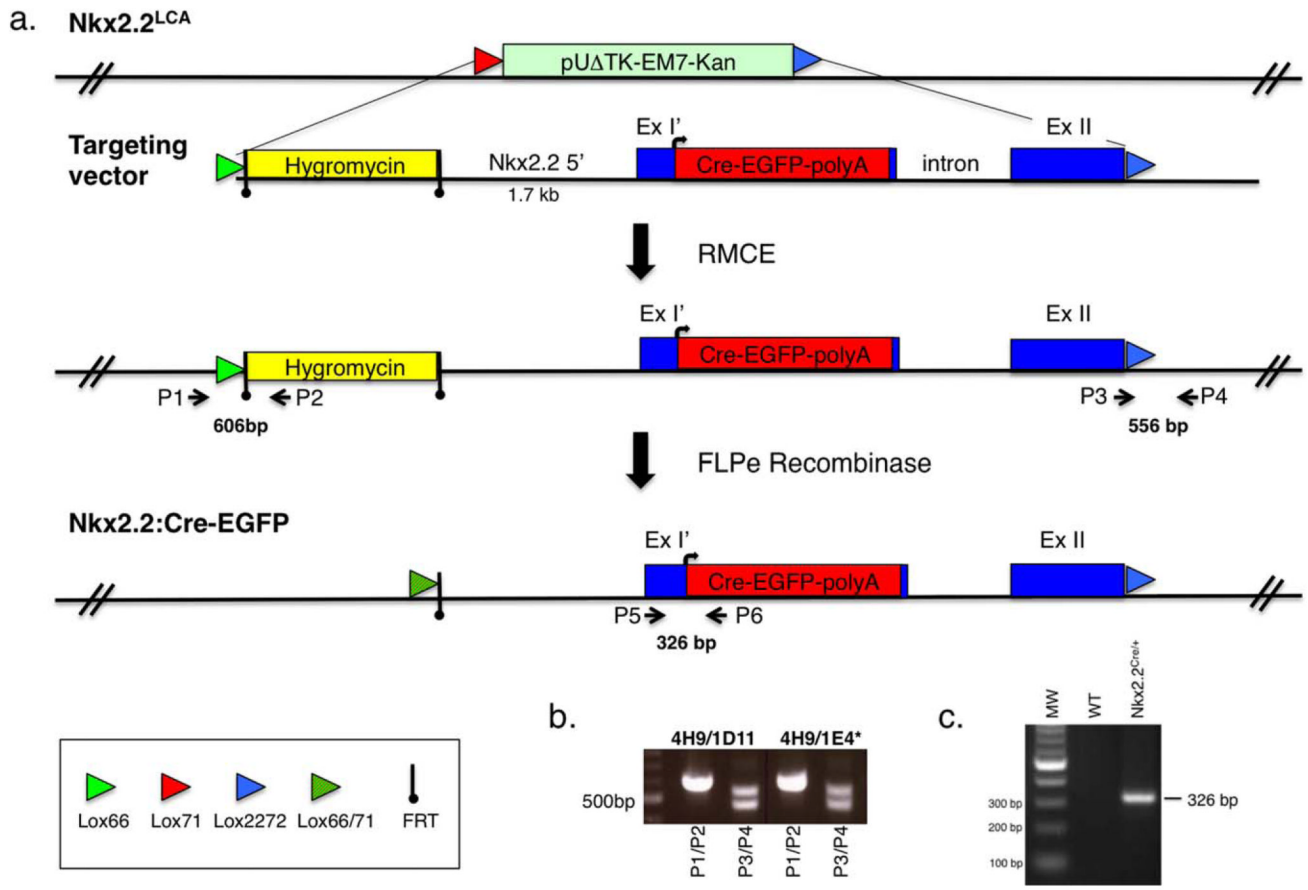

FIG. 1.

Generation of the *Nkx2.2:CreEGFP* knockin allele. (a) Schematic of the strategy used to create the *Nkx2.2:CreEGFP* knockin allele. *Nkx2.2*^{LCA} refers to the RMCE *Nkx2.2* acceptor allele integrated into the genomic locus of the 4H9 ES cell line (Arnes et al., 2012). The RMCE lox sites are indicated by colored arrows. The targeting vector includes a FRT flanked *hygromycin* cassette for positive selection of the targeting event. The *CreEGFP* gene was recombinered in frame at the *Nkx2.2* ATG codon and deleted most of coding exon 1. ExI' signifies the truncated region of *Nkx2.2* coding exon 1 and ExII refers to the *Nkx2.2* coding exon 2. The *CreEGFP* cassette replaces the *Nkx2.2*^{LCA} allele using RMCE. P1 and P2 indicate the 5′ PCR primers that verify integration of the *hygromycin* cassette into the correct genomic locus. P3 and P4 indicate the 3′ primers that distinguish the wild-type Nkx2.2 allele and the newly integrated *Nkx2.2:CreEGFP* allele containing the 2722 lox site. The Flpe-recombinase is used to remove the FRT-flanked *hygromycin* cassette. P5 and P6 refer to the genotyping primer set used to determine for the presence of the *Nkx2.2:CreEGFP* allele. (b) Representative PCR amplification results of *Nkx2.2*^{LCA} ES cells containing RMCE-mediated recombination of the *Nkx2.2:CreEGFP* allele. For each RMCE ES cell clone, two sets of primers (P1/P2 and P3/P4) were used. Primers 1 and 2 detect a 606 bp product. Primers 3 and 4 detect a 556 bp for the *Nkx2.2:CreEGFP* allele and 479 bp for the *Nkx2.2* wild-type allele. (c) Representative PCR amplification results of *Nkx2.2:CreEGFP* mice and wild-type littermates. Primer P5 and P6 amplify a 326 bp band from only the mice carrying the *Nkx2.2:CreEGFP* allele.

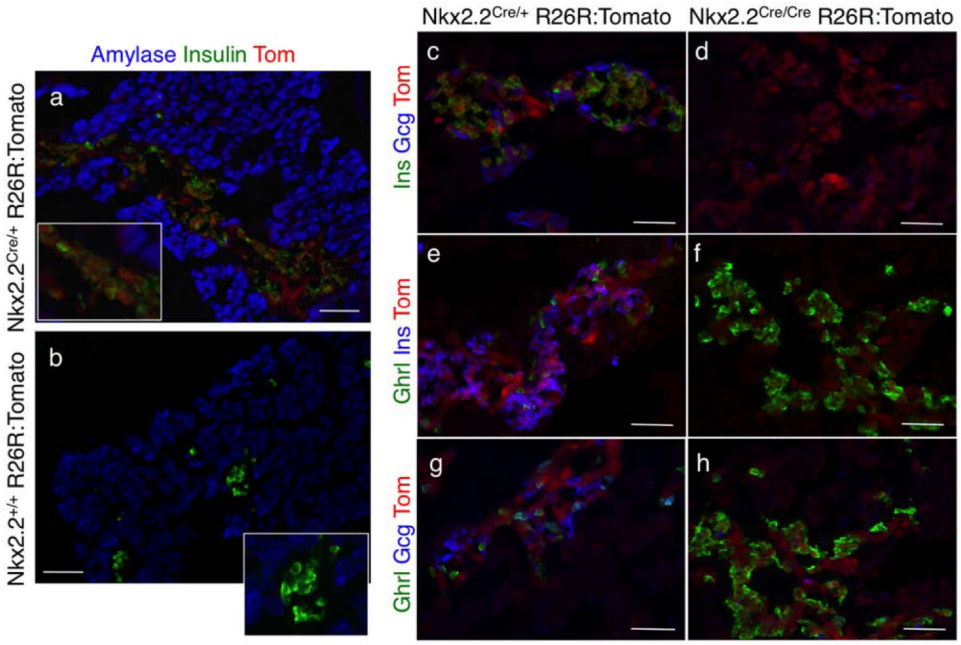

FIG. 2.

Characterization of the *Nkx2.2*^{CreEGFP}; *R26R:Tomato* mice. (a,b) Pancreata derived from *Nkx2.2*$^{CreEGFP/+}$; *R26R:Tomato* e18.5 embryos and *Nkx2.2*$^{+/+}$; *R26R:Tomato* littermate controls were sectioned and immunolabeled with antibodies against amylase (blue) and insulin (green). Tomato reporter expression (red; direct fluorescence) could only be visualized in the embryos containing the *Nkx2.2:CreEGFP* allele and not the *Nkx2.2*$^{+/+}$ littermate controls, verifying the Tomato reporter is only activated in the presence of Cre recombinase. Magnification: 20×; Scale bar = 100 μm. Insets are 40×. (c-h) Pancreata derived from *Nkx2.2*$^{CreEGFP/+}$; *R26R:Tomato* e18.5 embryos and *Nkx2.2*$^{CreEGFP/EGFP}$; *R26R:Tomato* littermate controls were sectioned and immunolabeled with antibodies against endocrine hormones as indicated. Similar to the phenotype of the *Nkx2.2*$^{-/-}$ mice, insulin+ cells are absent, glucagon+ cells are reduced and ghrelin+ cells are elevated. Magnification: 40×; Scale bar = 500 μm.

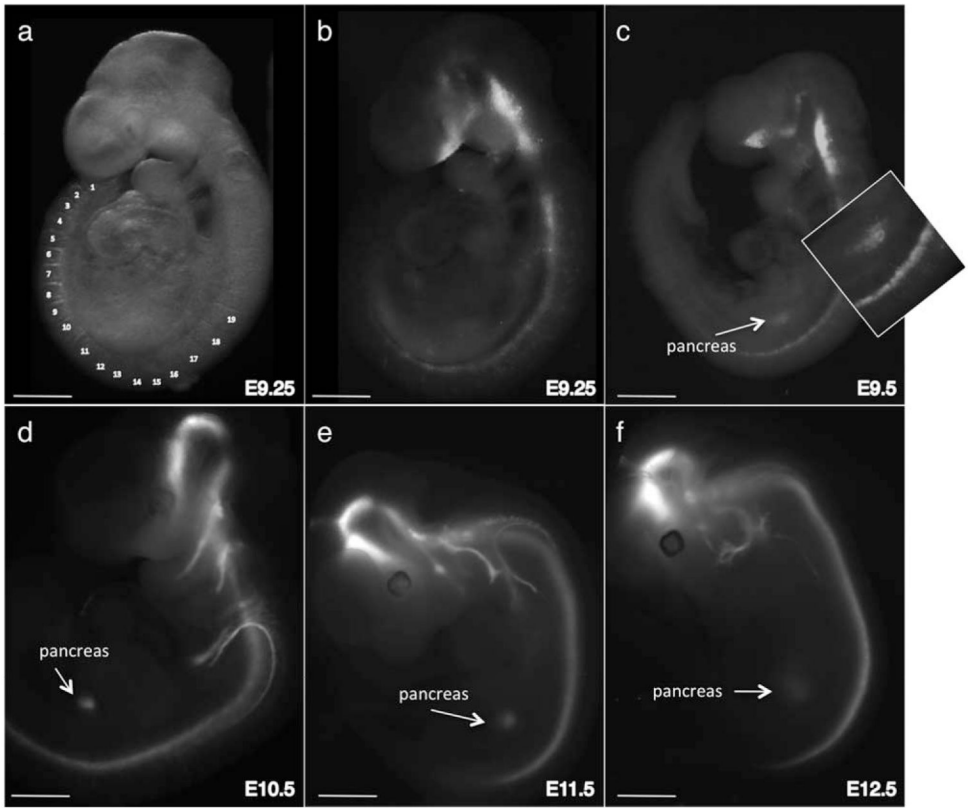

FIG. 3.

Characterization of the onset of Nkx2.2-Cre activity. (a) Whole mount bright field image of an ~9.25 (19 somites) *Nkx2.2*$^{CreEGFP/+}$; *R26R:Tomato* embryo with the somite numbers indicated. (b) Detection of the tomato reporter in the same embryo shown in (a) using direct fluorescence imaging. The reporter is visible along the A/P axis of the CNS. (c) Fluorescence imaging of an e9.5 *Nkx2.2*$^{CreEGFP/+}$; *R26R:Tomato* embryo. Tomato reporter expression is now visible in the pancreas. The inset shows higher magnification of the pancreas and spinal cord. (d,e) Fluorescence imaging of tomato expression in whole mount *Nkx2.2*$^{CreEGFP/+}$; *R26R:Tomato* embryos at successively later embryonic stages. The tomato reporter is also visible in the cells and axons derived from Nkx2.2-expressing progenitor domains in the CNS. Scale bars = 250 μm (E9.25); 450 μm (E9.5); 600 μm (E10.5); 1 mm (E11.5, E12.5).

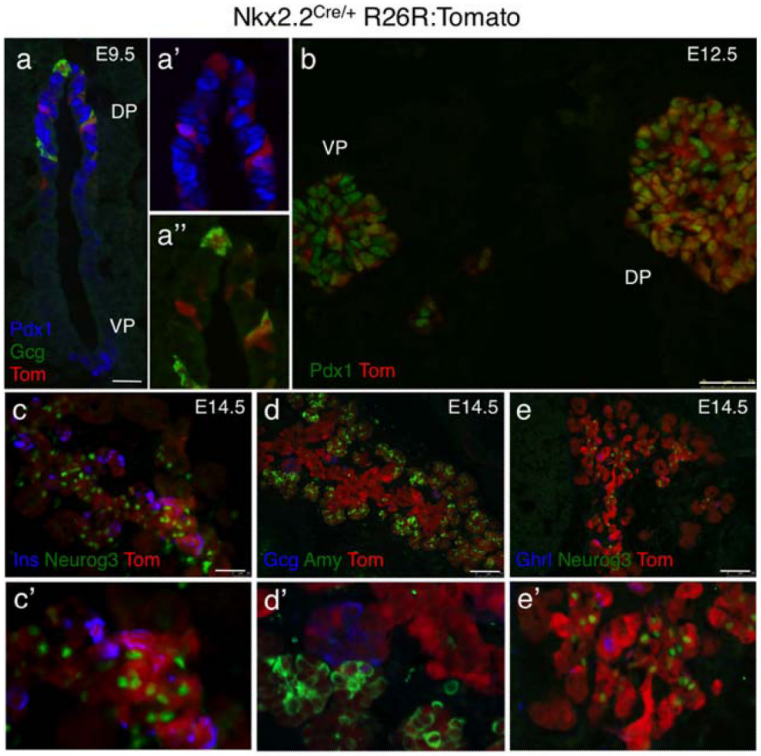

FIG. 4.

Assessment of Nkx2.2-Cre activity in the developing pancreas. Combined direct fluorescence (tomato reporter; red) and immunofluorescence of different pancreatic markers in *Nkx2.2*$^{CreEGFP/+}$; *R26R:Tomato* embryos to illustrate cell type specific Cre activity. (a) In a transverse section through an e9.5 embryo, tomato expression can be visualized in the dorsal pancreatic epithelium (DP) and not in the ventral pancreas epithelium (VP). In the dorsal pancreas, tomato is co-expressed with Pdx1 (blue) and glucagon (green). Scale bar = 25 μm. (a') Higher magnification two channel image showing that tomato is co-expressed with a subset of Pdx1+ cells. (a") Higher magnification two channel image showing tomato is co-expressed with glucagon+, Pdx1− cells. (b) Tomato is extensively co-expressed with Pdx1 (green) in the dorsal (DP) pancreatic bud at e12.5; only a small subset of Pdx1 cells does not express the tomato reporter. Scale bar = 75 μm. (c-e) In sections through E14.5 pancreas, tomato is co-expressed with Neurog3, insulin, glucagon, ghrelin, and amylase. Magnification 20×; Scale bars = 75 μm. (c'-e') Higher power images showing co-expressing cells within the pancreas (40×).

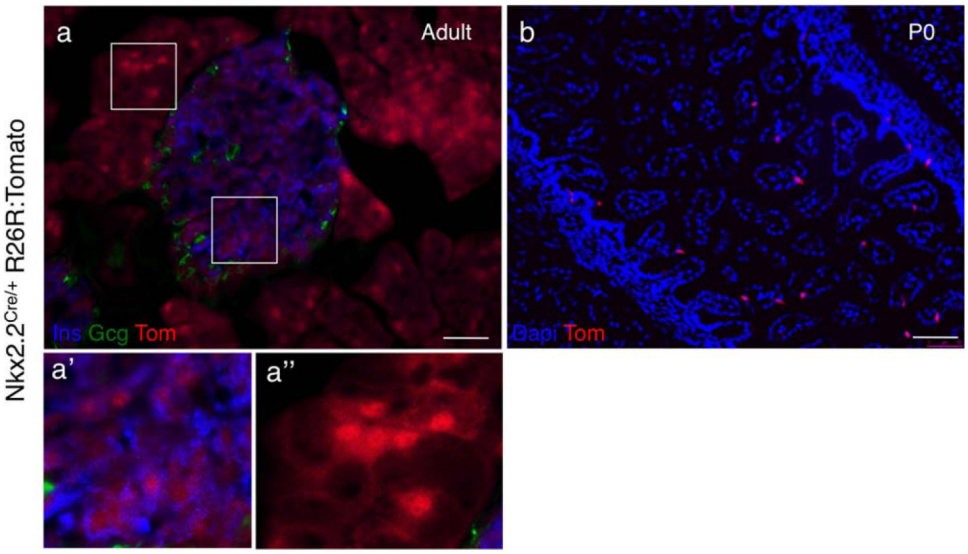

FIG. 5.

Nkx2.2-Cre activity in the postnatal pancreas and intestine. Combined direct fluorescence (tomato reporter; red) and immunofluorescence of in *Nkx2.2*$^{CreEGFP/+}$; *R26R:Tomato* mice. (a) The tomato reporter is expressed in the exocrine and endocrine compartments of the adult pancreas. Within the islet, tomato is co-expressed with insulin and glucagon. White squares indicate magnified fields in a' (islet cells) and a" (acinar cells). (b) Tomato expression can be detected in enteroendocrine cells within the villi of the small intestine. Dapi staining (blue) labels all cell nuclei to help visualize the intestinal morphology. Magnification 20×; Scale bars = 100 μm.

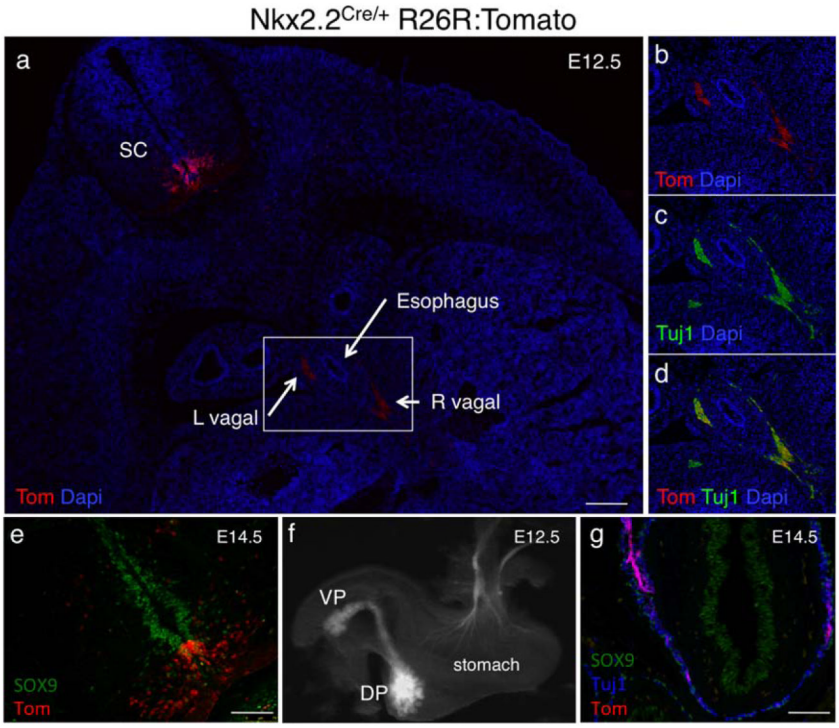

FIG. 6.

Nkx2.2-Cre activity in the central nervous system. Analysis of tomato expression in the CNS of *Nkx2.2*$^{CreEGFP/+}$; *R26R:Tomato* embryos. (a) Transverse section through the thoracic region of an e14.5 embryo. The tomato reporter is expressed in the ventral spinal cord in the P3 progenitor domain adjacent to the floor plate and in the more lateral V3 interneuron domain. Tomato expression can also be detected adjacent to the esophagus in the left (L) and right (R) vagal nerves. Dapi (blue) labels cell nuclei. Magnification 5×; Scale bar = 500 μm. The rectangle represents the region shown at higher magnification in b-d. (b-d) The tomato reporter is co-expressed with Tuj1 in motor neuron axons extending from Nkx2.2-derived visceral motor neurons in the hindbrain. Magnification 20×; Scale bar = 100 μm. (e) Transverse section of an e14.5 thoracic spinal cord showing co-expression of Tomato and Sox9 (red) in the ventricular zone containing neural precursors. Tomato expressing cells are also present in the more lateral domain containing differentiated interneuron populations. Magnification 20×; Scale bar = 100 μm. (f) Whole mount of dissected e12.5 visceral tissue from a *Nkx2.2*$^{CreEGFP/+}$; *R26R:Tomato* embryo. Tomato is expressed in the dorsal (DP) and ventral (VP) and in a subset of axons extending into the stomach. (g) Section through the distal stomach of an e14.5 *Nkx2.2*$^{CreEGFP/+}$; *R26R:Tomato* embryo. Tomato+ axons extend into the Tuj1 expressing neurons innervating the stomach. Magnification 20×; Scale bar = 100 μm.