Cdx2 Regulates Gene Expression through Recruitment of Brg1-associated Switch-Sucrose Non-fermentable (SWI-SNF) Chromatin Remodeling Activity*

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The packaging of genomic DNA into nucleosomes creates a barrier to transcription that can be relieved through ATP-dependent chromatin remodeling via complexes such as the switch-sucrose non-fermentable (SWI-SNF) chromatin remodeling complex. The SWI-SNF complex remodels chromatin via conformational or positional changes of nucleosomes, thereby altering the access of transcriptional machinery to target genes. The SWI-SNF complex has limited ability to bind to sequence-specific elements, and, therefore, its recruitment to target loci is believed to require interaction with DNA-associated transcription factors. The Cdx family of homeodomain transcription factors (Cdx1, Cdx2, and Cdx4) are essential for a number of developmental programs in the mouse. Cdx1 and Cdx2 also regulate intestinal homeostasis throughout life. Although a number of Cdx target genes have been identified, the basis by which Cdx members impact their transcription is poorly understood. We have found that Cdx members interact with the SWI-SNF complex and make direct contact with Brg1, a catalytic member of SWI-SNF. Both Cdx2 and Brg1 co-occupy a number of Cdx target genes, and both factors are necessary for transcriptional regulation of such targets. Finally, Cdx2 and Brg1 occupancy occurs coincident with chromatin remodeling at some of these loci. Taken together, our findings suggest that Cdx transcription factors regulate target gene expression, in part, through recruitment of Brg1-associated SWI-SNF chromatin remodeling activity.

The Cdx genes are vertebrate orthologs of Drosophila caudal and encode homeodomain transcriptional factors. In the mouse, the three members of this family (Cdx1, Cdx2, and Cdx4) are co-expressed in the caudal embryo in all three germ layers commencing at mid-gastrulation and play overlapping roles in a number of developmental programs, including axial elongation, endoderm specification, and anterior-posterior vertebral patterning (1–6). Both Cdx1 and Cdx2 are also expressed in the intestinal epithelium throughout life, where they play critical roles in intestinal homeostasis (7–11) and can function as tumor suppressors (12–15). Although Cdx members play critical roles in governing gene expression during development and in the adult intestine, little is known about the mechanisms by which Cdx members regulate target gene transcription.

The packaging of DNA into nucleosomes creates a barrier to transcription by obstructing access of the basal transcription machinery as well as modulating access of other transcriptional regulators to their DNA binding motifs (16–19). Alteration of the chromatin structure by ATP-dependent remodeling complexes can alleviate these constraints, and such complexes play important roles in the transcriptional regulation of many eukaryotic genes. Such complexes include the switch-sucrose non-fermentable (SWI-SNF)2 chromatin-remodeling complex (16, 20–22), which remodels the chromatin structure via conformational or positional changes of nucleosomes (23, 24). Because the SWI-SNF complex possesses limited sequence-specific DNA binding activity, its recruitment to relevant target loci is accomplished via interaction with DNA-bound transcription factors (17, 23), including Myc, Tbx5, β-catenin, and p53 (25–28).

Although little is known regarding the means by which Cdx members regulate transcription, it is believed that they serve to recruit co-regulators to target genes, and it is the biochemical activity of such co-regulators that impacts the transcription of such targets (12, 29, 30). Using a quantitative affinity purification/MS approach based on stable isotope labeling by amino acids in culture (SILAC) (31), we found that Cdx2 associates with multiple members of the Brg1-containing SWI-SNF complex in HEK293 cells. We further found that Cdx2 and Brg1 associated in vitro, suggesting direct interaction, and that both factors co-occupied a number of Cdx target genes. Recruitment of Brg1 to such targets required Cdx2 and occurred coincident with Brg1- or Cdx2-dependent local chromatin remodeling. Taken together, these findings suggest that Cdx members modulate target gene transcription, at least in part, through recruitment of SWI-SNF-mediated chromatin remodeling.

* The authors declare that they have no conflicts of interest with the contents of this article.

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2 The abbreviations used are: SWI-SNF, switch-sucrose non-fermentable; SILAC, stable isotope labeling by amino acids in culture; IP, immunoprecipitation; CDRE, Cdx response element; RIP, radioimmune precipitation assay; qPCR, quantitative PCR; CRISPR-Cas9, clustered regularly interspaced short palindromic repeats-CRISPR-associated system.
Results

Cdx2 Interacts with Members of the SWI-SNF Complex—To identify potential Cdx2 co-regulators, we carried out a quantitative affinity purification/MS experiment, directly comparing immunoprecipitation (IP) of endogenous Cdx2 from HEK293 cell extracts encoded with a “heavy” SILAC label to a control IgG IP from HEK293 cell extracts encoded with a “light” SILAC label. We identified 544 proteins, of which a subset were enriched specifically with Cdx2, as indicated by heavy (Cdx2 IP):light (control IP) ratios greater than 1. This analysis detected significant enrichment of six members of the SWI-SNF chromatin remodeling complex with CDX2, including BAF-1 (BAF190), BAF53, BAF57, BAF155, BAF170, and BAF250 (Fig. 1A). Given the relatively high level of enrichment of these subunits, the fact that multiple subunits were recovered, and the known role of SWI-SNF complexes in transcriptional regulation, these findings suggested that Cdx2 may recruit SWI-SNF to regulate target gene expression.

| Gene Name | Unique Peptides | Sequence Coverage [%] | Ratio H/L Normalized | Log Ratio |
|-----------|-----------------|------------------------|----------------------|-----------|
| CDX2      | 6               | 17.6                   | 14.40                | 3.85      |
| BAF250    | 16              | 9.9                    | 7.51                 | 2.91      |
| BAF190 (BRG1) | 21             | 16.5                   | 5.27                 | 2.40      |
| BAF53     | 2               | 4.7                    | 4.19                 | 2.07      |
| BAF57     | 2               | 6.1                    | 2.78                 | 1.48      |
| BAF155    | 4               | 6.2                    | 2.70                 | 1.43      |
| BAF170    | 2               | 3.5                    | 2.47                 | 1.30      |

FIGURE 1. CDX2 interacts with the SWI-SNF complex. A, SILAC-MS revealed interaction between Cdx2 and multiple members of the SWI-SNF chromatin remodelling complex. The ratio of heavy to light isotope peptides (H/L) indicates the relative enrichment of CDX2 interactions. B, co-immunoprecipitation of CDX2 and BRG1 from HEK293 cells. IgG was used as a negative control. C, FLAG-BRG1-B2, expressed in HEK293 cells, was immunoprecipitated with α-CDX2 antibody, and interaction was assessed by anti-FLAG. IgG was used as a negative control. D, whole cell lysates from embryonic day 9.5 mouse embryos were immunoprecipitated with antibodies against α-Cdx1, α-Cdx2, or IgG as a negative control, and interaction with BRG1 was assessed by Western blotting. E, [35S]methionine-labeled BRG1 was incubated with full-length Cdx2, Cdx1, and Cdx4-GST fusion proteins, and binding was measured using autoradiography film.
Brg1, the ATPase subunit of SWI-SNF, has been shown to interact with a number of transcription factors (25–28), suggesting that it may serve to bridge the SWI-SNF complex with Cdx2. Co-precipitation of Brg1 with CDX2 was validated by Western blotting analysis using Brg1-specific antibodies (Fig. 1B). Moreover, both Cdx1 and Cdx2 associated with Brg1 in embryonic day 9.5 mouse embryos (Fig. 1D).

A region of Brg1 between amino acids 325 and 611, termed Brg1-B2 hereafter, has been demonstrated to interact with a number of transcription factors (32, 33), suggesting that it may serve as an interface with Cdx2. Consistent with this, we found robust interaction between FLAG epitope-tagged Brg1-B2 and endogenous Cdx2 in immunoprecipitation assays from HEK293 cells (Fig. 1C).

Binding assays using GST-Cdx fusion proteins and in vitro translated Brg1 indicated that full-length GST-Cdx1, -Cdx2, or -Cdx4 were interacting with Brg1 (Fig. 1E). This is consistent with a direct physical interaction between Cdx proteins and Brg1. Moreover, the finding that all Cdx members interacted comparably suggests a mechanistic basis for their well documented functional overlap (4–6).

To further examine the association between Brg1 and Cdx2, immunofluorescence analysis was carried out in C2BBe1 cells or mouse small intestine. Endogenous Brg1 and Cdx2 proteins were found within similar nuclear foci in C2BBe1 cells, with a Pearson coefficient of correlation of 0.89 ± 0.04, indicative of an overlap in their distribution (34) (Fig. 2A). Both Cdx2 and Brg1 were also co-expressed in murine enterocytes (Fig. 2B). Taken together, these results are consistent with Cdx2 and Brg1 forming a complex in vivo.

**Figures 2A and 2B**

Co-localization of CDX2 and BRG1. A, immunohistochemistry was carried out to detect immunofluorescence of CDX2 (green) and BRG1 (red) in C2bbe1 cells. Also shown is a merged image. B, immunofluorescence of Cdx2 (green) and BRG1 (red) in mouse small intestine. Also shown is a merged image. DAPI staining was used to label nuclei.

**Brg1 and Cdx2 Co-occupy Cdx Target Genes**—The above observations suggest that Cdx members physically interact with Brg1 to recruit SWI-SNF activity to their target loci. To further explore this, we aligned a Brg1 ChIP sequencing dataset acquired from an embryonic stem cell differentiation model (35) with known or putative Cdx target genes. In cases where overlap was suggested, we assessed chromatin association for either Cdx2 or Brg1 in HEK293 cells by ChIP. These analyses revealed that the Dll1, Sc1, Cyp26a1, Axin2, and Wnt3a loci, but not the negative control Wnt3a exon 4, were co-occupied by both Cdx2 and Brg1 in HEK293 cells and that this binding correlated with known or putative (by Transfac analysis) Cdx response elements (CDREs) (Fig. 2C and 2D).

**Figures 2C and 2D**

Co-localization of CDX2 and BRG1. A, immunohistochemistry was carried out to detect immunofluorescence of CDX2 (green) and BRG1 (red) in C2bbe1 cells. Also shown is a merged image. B, immunofluorescence of Cdx2 (green) and BRG1 (red) in mouse small intestine. Also shown is a merged image. DAPI staining was used to label nuclei.

**Brg1 Impacts the Expression of Cdx Target Genes**—As Brg1 and Cdx2 physically interact and co-occupy several Cdx target loci, we next examined whether Brg1 was necessary for the expression of any of these genes. To this end, we generated Brg1- and Cdx2-null cell lines using CRISPR-Cas9 gene editing with guide RNAs directed against the first exon of either gene (39). Multiple clones were identified that no longer expressed expression of any of these genes. To this end, we generated Brg1- and Cdx2-null cell lines using CRISPR-Cas9 gene editing with guide RNAs directed against the first exon of either gene (39).

**Figures 2E and 2F**

Co-localization of CDX2 and BRG1. A, immunohistochemistry was carried out to detect immunofluorescence of CDX2 (green) and BRG1 (red) in C2bbe1 cells. Also shown is a merged image. B, immunofluorescence of Cdx2 (green) and BRG1 (red) in mouse small intestine. Also shown is a merged image. DAPI staining was used to label nuclei.

**Meis1, and Tcf4** was attenuated in Brg1-null cells (Fig. 4B). Thus, expression of a subset of Cdx target genes is dependent on both Brg1 and Cdx2, whereas others are independent of either Brg1 (Wnt3a) or Cdx2 (Lef1 and Tcf4), at least in HEK293 cells.

Transfection of a Cdx2 expression vector into Cdx2-null cells (Fig. 4C) resulted in a restoration of expression of genes attenuated by Cdx2 loss (Fig. 4D), confirming the specificity of the CRISPR-generated mutation. This rescue of gene expression could not be accounted for by a nonspecific Cdx2 function, as TCF4, acting as a negative control, was unaffected (Fig. 4D).

A similar rescue was also seen upon transfection of a Brg1 expression vector into the cognate null cell line (Fig. 4, C and E). However, comparable restoration of expression was not seen using a Brg1 cDNA encoding a catalytically inert ATPase domain mutant (K798R) (40) (Fig. 4, C and E). As the ATPase activity of Brg1 is essential for the chromatin remodeling activity of the SWI-SNF complex (20, 22, 41, 42), these observations suggest that the expression of a subset of Cdx target genes depends on Brg1-mediated SWI-SNF remodeling.
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Cdx2 and Brg1 Impact the Chromatin Architecture at Cdx Target Genes—The above observations suggest that Cdx2 recruits Brg1, which may impact the expression at Cdx target loci through local alterations in the chromatin architecture. Consistent with this model, ChIP analysis revealed loss of Brg1 occupancy on a number of target genes in Cdx2-null HEK293 cells, whereas binding of Brg1 to the Cdx2-independent Brg1 target CYP3A4 was unaffected (Fig. 5).

The SWI-SNF complex remodels chromatin via conformational or positional changes of nucleosomes. As a consequence, DNA accessibility to enzymatic activity can be altered. We monitored this by restriction enzyme accessibility assay (43), in agreement with convergent regulation of Brg1-Cdx interactions (30) and suggest that this is a more general mechanism of transcriptional regulation by Cdx family members.

Cdx Members Interact with Brg1—The SWI-SNF complexes are large multimeric protein complexes comprised of over a dozen subunits, the composition of which can vary according to developmental stage and/or cell type (46). Although the Brg1 SWI-SNF subunit contains DNA binding motifs (17, 47), these do not appear to be capable of directing the complex to specific genomic targets. Rather, recruitment of SWI-SNF to specific loci is believed to rely on association with DNA-bound transcription factors (18, 24). Our finding that a number of SWI-SNF members, including Brg1, associated with Cdx2 in HEK293 cells and embryos suggests that Cdx2 may serve to recruit SWI-SNF to specific target genes.

Of particular note was the finding that Brg1 was significantly enriched and relatively abundant in Cdx2 immunoprecipitates from cultured cells. Furthermore, in vitro GST pulldown assays revealed that Brg1 interacted with all three Cdx members. Taken together, these findings suggest that Cdx transcription factors interface with the SWI-SNF complex through direct association with Brg1, likely through the Brg1-B2 domain, although interaction with other SWI-SNF components or regions of Brg1 or additional scaffold proteins cannot be ruled out at present. The promiscuous interaction between Cdx members and Brg1 is also consistent with the functional overlap between family members, as evidenced by the interaction between mutant Cdx alleles (5, 6, 48, 49) and the ability of Cdx2

Discussion

Cdx members play critical roles in many developmental processes and impact intestinal homeostasis and tumorigenesis in the adult (3, 5, 7–10, 37, 45). Little is known, however, regarding the mechanisms by which Cdx members regulate target gene expression. In an effort to better understand this, we utilized a quantitative mass spectrometry approach to identify proteins associated with Cdx2 from HEK293 cells. This cell line expresses abundant levels of Cdx2 and is capable of supporting Cdx-dependent transcription. This exercise recovered multiple members of the SWI-SNF chromatin remodeling complex, including the catalytic subunit Brg1. Additional protein-protein association assays, colocalization analysis, and chromatin occupancy and DNA accessibility assays suggest that Cdx2 (and likely other Cdx members) regulate target gene expression, at least in part, through recruitment of SWI-SNF-mediated chromatin remodeling. These findings extend prior work indicative of Brg1-Cdx interactions (30) and suggest that this is a more general mechanism of transcriptional regulation by Cdx family members.
to completely complement Cdx1 function in vertebral patterning (4). Moreover, all three Cdx members appear equivalent in their ability to occupy target genes (4, 36, 37), further supporting a common mechanistic basis for their impact on transcription.

**Brg1-dependent Regulation of Cdx Target Genes**—Cdx members occupy a number of target genes in the developing embryo (36, 38) and the intestine (9, 10, 50, 51), and this occupation is often predictive of Cdx-dependent expression. ChIP sequencing analyses from embryonic stem cells undergoing mesodermal differentiation (35) suggests that Brg1 is also resident on a number of these genes in a manner that overlaps known (or potential) CDREs. Co-occupation of a number of these loci by Brg1 and Cdx2 was confirmed by ChIP analysis in HEK293 cells. Moreover, and consistent with our model, occupancy of Brg1 at these loci was lost in Cdx2-null cells. The specificity of this relationship was further supported by recovery of Brg1 chromatin occupancy upon re-expression of wild-type Cdx2 into the knockout cell lines.

A comparison of Cdx target gene mRNA levels between wild-type and Cdx2-null cell lines suggests that Brg1 is essential for the normal expression of a number of such targets. The finding that loss of Cdx2 resulted in a comparable reduction in the expression of many of these target genes, together with Cdx-dependent recruitment of Brg1 to these loci, is consistent with a functional requirement for Brg1 in Cdx-dependent gene expression. Finally, a limited number of target genes, although exhibiting co-occupation by Cdx2 and Brg1, were not impacted by loss of one or the other factor. This may be indicative of fortuitous, but non-functional, binding or context-dependent regulation by Cdx that is not faithfully recapitulated in HEK293 cells.

**Cdx2-dependent Chromatin Remodeling**—The SWI-SNF complex remodels the chromatin structure via conformational or positional changes of nucleosomes (46). Enzyme accessibility assays have been used as a surrogate measure of such activity and have revealed, for example, Brg1-dependent changes in chromatin structure (42, 52). Using such an assay, we assessed the consequence of Brg1 or Cdx2 loss of function on the chromatin structure at the *Dll1* locus and found that chromatin accessibility was comparably affected by loss of either factor. Similarly altered chromatin accessibility was also seen in Brg1- and Cdx-deficient murine intestinal epithelial cells. Moreover, wild-type Brg1, but not a catalytically inert mutant, restored both target gene expression and chromatin accessibility in Brg1-null cells, consistent with a requirement for Brg1-dependent chromatin remodeling in Cdx-mediated transcription. Finally, the finding of non-complementation of Cdx and Brg1
mutant alleles regarding goblet cell hypertrophy further supports the relevance of this interaction in vivo. In this regard, mice null for Muc2 exhibit changes in goblet cell morphology (53), and MUC2 is a CDX2 target (54). It is therefore tempting to speculate that Muc2 may be co-regulated by Cdx and SWI-SNF.

Gene expression is tightly regulated at multiple levels to ensure appropriate transcriptomes; the chromatin state is a major determinant of transcription (55). Our findings are consistent with a model wherein Cdx members impact the chromatin state and target gene expression through recruitment of Brg1 and associated SWI-SNF-dependent chromatin remodeling (Fig. 8). In this regard, prior work has shown Cdx2 occupancy of a number of genes in intestinal progenitor cells. Many of these targets, however, are not perturbed in this progenitor population by loss of Cdx2 but were impacted in the differentiated progeny thereof (50). Taken together, these observations suggest that one mechanism of action of Cdx2 may be to recruit SWI-SNF to establish the appropriate chromatin landscape in progenitor cells permissive for subsequent transcription in descendant lineages.

Materials and Methods

CRISPR-Cas9 Gene Editing—HEK293 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% FCS and 1% penicillin/streptomycin at 37 °C with 5% CO₂ in air. Guide oligonucleotides against the first exon of Cdx2 or Brg1 were cloned into PX459 (Addgene). Targeting plasmids (1 μg) were subsequently transfected into HEK293 cells using the calcium phosphate method (56), and clones were selected by culture in complete medium with puromycin (3 μg/ml). Mutation was assessed by Western blotting analysis, and candidates were confirmed by sequencing PCR amplicons generated from the targeted interval. In some experiments, expression vectors
encoding Cdx2, Brg1, or a kinase-dead Brg1 mutant (40) were transfected in wild-type or null cell lines by calcium phosphate-mediated transfection.

**Immunoprecipitation and Mass Spectroscopy**—SILAC-based interactome mapping was conducted as described previously (57, 58). Briefly, HEK293 cells were metabolically labeled by culturing in either heavy medium containing $^{13}$C-arginine and D4-lysine or light medium containing $^{12}$C-arginine and $^{12}$C-lysine for seven passages. The two populations of cells were then collected, and protein was extracted using RIPA buffer (50 mM Tris (pH 7.5), 150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, and protease inhibitors). Experimental (heavy) extracts were incubated with Cdx2 antibody (4), and control (light) extracts were incubated with an equivalent amount of rabbit IgG (Santa Cruz Biotechnology). The samples were then incubated with 50 μl of protein A/G-Sepharose beads for 1 h at 4°C, and the beads were pelleted and washed with RIPA buffer. The two sets of beads were then combined, and proteins were eluted in a 1% SDS solution. Following reduction and alkylation steps with DTT and iodoacetamide, respectively, sample buffer was added, and the proteins were resolved by electrophoresis on a NuPAGE 10% BisTris gel (Thermo Fisher). The gel was stained using SimplyBlue Safestain (Thermo Fisher), and the entire lane was cut into five slices. Each slice was cut into 2 × 2 mm fragments, destained, and digested overnight with Trypsin Gold (Thermo Fisher). 15 μl of each tryptic digest was analyzed by liquid chromatography nanospray ionization tandem MS on an LTQ Orbitrap XL hybrid MS system (Thermo Scientific) with the nanospray coupled to a Dionex Ultimate 3000 RSLC nano HPLC (58). Database searching (against the human Uniprot database) and quantitation were performed using MaxQuant software v1.2.7.4 (59).

**Immunostaining**—Intestines were prepared as described previously (7). Paraffin-embedded material was sectioned at 5 μm, and frozen material was sectioned at 8 μm. C2bbe1 cells were trypsinized and replated onto glass coverslips. Sections or cells were washed with PBS, fixed in 4% PFA/PBS for 10 min, permeabilized with 0.3% Triton-X-100 in PBS, and blocked for 1 h with 1% goat serum (Millipore) in PBS. Samples were then incubated with anti-Brg1 (1:1000, Santa Cruz Biotechnology) overnight at 4 °C in blocking solution, followed by 1 h at room temperature in Alexa Fluor 594-conjugated goat anti-rabbit IgG (1:1000, Invitrogen). Samples were then washed with PBS and incubated with 1:1000 anti-Cdx2 primary antibody overnight at 4 °C in blocking solution, washed with PBS, and incubated with 1:1000 goat anti-rabbit Alexa Fluor® 488. Nuclei were counterstained with DAPI. The nuclei of individual cells (n = 15) were

![Figure 6. BRG1 and CDX2 impact target gene chromatin accessibility.](image-url)

**FIGURE 6.** BRG1 and CDX2 impact target gene chromatin accessibility. **A,** schematic of CDREs in the Dll1 proximal promoter. **B** and **C,** relative accessibility of chromatin at the Dll1 locus is reduced in the absence of BRG1 or CDX2 in HEK293 cells (B) or in Cdx1-Cdx2 compound null mutant intestinal epithelial cells (C), as assessed by restriction enzyme accessibility assay. **D,** chromatin accessibility in the Dll1 promoter is rescued by reintroduction of wild-type CDX2. **E,** wild-type BRG1 but not a BRG1 ATPase-deficient mutant (BRG1 mut) rescues chromatin accessibility in BRG1 null cells. *, p < 0.05 by Student’s t test.
analyzed separately using the ZEN colocalization module on a confocal microscope. The Pearson colocalization coefficient, a measure of the colocalization of the two fluorophores, was recorded (34).

Western Blotting Analysis—HEK293 cells were grown to confluence, rinsed twice with PBS, resuspended in 200 μl of RIPA buffer, and incubated on ice for 20 min. Protein concentration was assessed by Bradford assay and normalized, and proteins were resolved on 10% or 15% SDS-PAGE gels and transferred to Immobilon-P (Millipore) membranes by standard means. Membranes were activated with 100% methanol (Fisher Scientific), blocked with 5% milk powder in PBST (0.1% Tween 20 (Fisher Scientific) in PBS), and incubated overnight at 4 °C with 1:1000 primary antibody (Cdx2 (4), Cyclophilin (Abcam), Brg1 (Santa Cruz Biotechnology), or FLAG (Sigma-Aldrich)). Membranes were then washed with PBST, incubated with 1:20,000 HRP-conjugated secondary antibodies (goat anti-rabbit or goat anti-mouse HRP, Santa Cruz Biotechnology), or FLAG (Sigma-Aldrich). Following a final wash in PBS, HRP reactivity was revealed using Luminata Forte ECL.
substrate (Millipore), and reactivity was revealed using the Odyssey® Fc imaging system.

Quantitative RT-PCR—RNA was isolated from cells using TRIzol according to the recommendations of the manufacturer, and cDNA was generated using standard procedures. cDNA was subsequently amplified by semiquantitative RT-PCR with GoTaq (Promega) or qPCR with SYBR Green (Promega) and quantified using Actin as an input control. qPCR was performed using the MX3005P (Agilent Technologies), and results were analyzed using the 2^−ΔΔCt method. For specificity, the dissociation curve was considered for each amplicon.

Cdx-Brg1 Interaction Assays—Sequences encoding Brg1-B2 (amino acids 325–611) were amplified by PCR, cloned into pCEP4-FLAG, and transfected into HEK293 cells (10 µg of DNA/10-cm plate). Lysates were immunoprecipitated with 5 µg of either IgG (Santa Cruz Biotechnology) or Cdx2 antibodies bound to protein A/G-Sepharose and assessed by Western blotting analysis. For in vitro interaction assays, [35S]methionine-labeled full-length Brg1 was synthesized using the TnT T7 coupled reticulocyte lysate system (Promega) as specified by the manufacturer, and in vitro binding was assessed essentially as described previously (26). Briefly, 0.2 µl of Brg1 was added to GST or GST-Cdx fusion proteins bound to glutathione-agarose beads in 500 µl of TNEN buffer (50 mM Tris, 100 mM NaCl, 5 mM EDTA, 0.1% IGEPAL (Sigma), and protease inhibitors), incubated at 4 °C for 2 h, and beads were washed thoroughly with TNEN buffer. Bound proteins were eluted by boiling in 2× Laemmli buffer and resolved on 10% SDS-PAGE gels. The gels were dried and exposed to autoradiography film overnight at −80 °C.

ChIP—Formaldehyde was added to 10-cm tissue culture plates to a final concentration of 1%, incubated for 10 min, and quenched by addition of 0.125 mM glycine. Cells were then rinsed with PBS, collected by centrifugation, and lysed in 3 ml of RIPA buffer on ice for 20 min. Sheared chromatin was prepared as described previously (60) and preincubated with protein A/G beads for 1 h. Samples were then incubated with Cdx2 or Brg1 antibodies with rocking at 4 °C, and 50 µl of A/G beads were added to each sample and incubated overnight at 4 °C. Samples were then processed for qPCR, with amplification across regions encompassing potential CDREs. Oligonucleotide sequences are available upon request.

Restriction Enzyme Accessibility Assay—Cells were suspended in 1 ml of lysis buffer (10 mM PIPES (pH 8), 85 mM KCl, 1 mM CaCl2, 5% sucrose, 0.5% Nonidet P-40, plus protease inhibitors), disrupted by 10 strokes of a Dounce pestle, and centrifuged to collect nuclei. Nuclei were incubated with XhoI or restriction buffer only, and DNA cleavage was assessed by ligation-mediated qPCR as described previously (43).

Genetic Complementation Analysis—All animals used in this study were maintained according to guidelines established by the Canadian Council on Animal Care. Cdx1^+/−;Cdx2^+/F; Brg1^+/−, acting as a control, and mutant Cdx1^−/−;Cdx2^+/F; Brg1^+/−;Villin-Cre ER^T (abbreviated to Cdx1^−/−;Cdx2^+/−; Brg1^+/−) or Cdx1^−/−;Cdx2^+/−;Brg1^+/−;Villin-Cre ER^T (abbreviated to Cdx1^−/−;Cdx2^+/−;Brg1^+/−) mice were described previously (38, 61–63). As described previously, mice were treated with 2 mg of tamoxifen by oral gavage at 2 months of age (7). Intestines were dissected from the animals 48 h after treatment and processed for staining with Alcian blue, Churukian Silver, or Phloxine-tartrazine as described previously (64).

Statistical Analysis—Statistical analysis was conducted using Student’s t test (paired, two-tailed).

Author Contributions—D. L., T. T. N., L. T. M., and T. B. B. conceived and coordinated the study. T. T. N., T. B. B., R. R., and D. L. wrote the paper. L. T. M., R. R., and T. B. B. designed, performed, and analyzed the experiment shown in Fig. 1. J. G. A. S. designed, performed, and analyzed the experiment shown in Fig. 2. T. B. B. and T. M. designed, performed, and analyzed the experiment shown in Fig. 3. T. M. and T. M. M. designed, performed, and analyzed the experiment shown in Fig. 4. T. T. N. designed, performed, and analyzed the experiments shown in Figs. 5 and 6. T. E. F. designed, performed, and analyzed the experiment shown in Fig. 7. B. L. H. and K. J. M. derived performed initial characterization of CRISPR-Cas9 mutant cell lines. All authors reviewed and approved the final version of the manuscript.

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