



**Figure S1: Characterization of the Cdx dominant negative fusion protein.** (A) The Cdx dominant negative fusion protein consists of the repressor domain of Engrailed (EnR) and the homeodomain of Cdx1. (B) Anti-Flag western blot showing detection of EnRCdx1 and EnR proteins in Cos7 cells transfected with respective expression vectors. (C) Electrophoretic mobility shift assay using a consensus Cdx binding site as probe and nuclear extracts (NE) from Cos7 cells transfected or not with an EnRCdx1 expressing vector: 1-free probe; 2-mock NE; 3-5-increasing amount of EnRCdx1 NE; 6-7-maximum amount of EnRCdx1 NE and competition with wt (6) or mutated (7) cold probes. EnRCdx1 binding is indicated by an arrow. (D) Co-transfection assay in P19 cells using a *Hoxb8* reporter construct bearing multiple Cdx binding sites. Cdx2 expression results in strong transactivation (lane2). Co-transfection of Cdx2 and increasing amounts of EnRCdx1 expression construct results in dose-dependent repression (lanes 3-5) whereas expression of the EnR domain without the Cdx1 homeodomain does not affect Cdx2-mediated transactivation (lanes 6-8). (E) Semi-quantitative RT-PCR analysis showing repression of endogenous *Cdx1* expression in N2a cells transfected with EnRCdx1 expression vector. Note that *Cdx1* is known to autoregulate via binding of Cdx1-Lef1 complexes to Lef/Tcf binding sites in the *Cdx1* promoter (46). *Cdx1* expression levels are normalized against *Gapdh* expression. Number above each lane represents the number of PCR cycles. (F) Co-transfection assays in N2a cells using luciferase reporter plasmids driven by Galectine1 or Syncytin2 promoter (provided by B. Barbeau, UQAM) to confirm the specificity of EnRCdx1. Increasing amounts of EnRCdx1 do not affect the expression of these non-Cdx target reporters.