SUPPLEMENTARY DATA

Research Design and Methods for Supplementary Figure 1

Promoter cloning, plasmid transfection and gene reporter assay

A 4.5-kb fragment of the mouse promoter of Pdx1 upstream of the transcription start site was generated by PCR from mouse genomic DNA, subcloned in pCR2.1 TOPO (Invitrogen, CA, USA) and transferred into a luciferase reporter vector pGL3Basic (Promega, WI, USA). The plasmid containing the mouse GR cDNA under the control of CMV was obtained from Dr C Jewell and Dr JA Cidlowski (National Institute of Environmental Health Sciences, Bethesda, USA). Plasmids containing deletions in the Pdx1 promoter: pPdx1-delAII-2, pPdx1-delAII-3, pPdx1-delAII-3', pPdx1-delAII-4, pPdx1-delAIII-1 and pPdx1-delAIII-2 were generated using the QuickChange Site-Directed mutagenesis kit (Stratagene, Agilent, CA, USA). pTK constructs containing deletions in the Pdx1 promoter : Pst-Bst :pTK (PstBstTKCAT) ; areaIPdx1 :TK (mAIpTKCAT) ; areaIIPdx1 :TK (mAIIpTKCAT) ; areaIIIPdx1 :TK (mAIIIpTKCAT) were obtained from Pr R. Stein (Vanderbilt University Medical Center, Nashville, USA).

Cell transfection and promoter activity measurements

Min6 or INS-1 cells were transfected with the different plasmids using Lipofectamine (Life Technologies, CA, USA). Extracts were prepared 48h after transfection. The CAT activity from the reporter constructs was normalized to the β-galactosidase activity of the co-transfected internal control plasmid. Cell transfection, luciferase, CAT and β-galactosidase enzymatic assays were performed according to the manufacturer's procedures.

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Supplementary Figure 1. GCs inhibit Pdx1 promoter activity through GR binding in area II.
(A) Pdx1 promoter activity in untreated (white bars) or Dex-treated (black bars) Min6 (left panel) or INS-1 (right panel) cells. (B) Pdx1 promoter activity in the presence of increasing amounts of a mouse GR-expressing plasmid (mGR). (C) Block deletions in area II or area III and inhibition of the corresponding promoter activity in untreated (white bars) or Dex-treated (black bars) cells. (D) Consequences of deletions of areas I, II or III on the inhibitory effect of Dex on Pdx1 promoter. Left panel shows a summary of the plasmids used for these experiments. Right graph shows the relative CAT activity with each plasmid in the presence (black bars) or the absence (white bars) of Dex. Dex treatment (10^{-7}M) was performed 24h after transfection and extracts were collected 48h post transfection. Results are expressed as means ± SD for n=4 independent experiments. * $p<0.05$ and ** $p<0.01$ using a Mann-Whitney non parametric test for Dex *vs* control experiments.