

Methods

Mouse embryology. Mouse embryos were transfected with separate expression constructs for each factor and EGFP, and cultured as described³⁰ with modifications mainly in the timing and location of the transfection. At embryonic day (E) 6.5-7.5, embryos were injected posteriorly under the visceral endoderm. Embryos were cultured for 36-48 hours. It should be noted that there is variation between embryos as they are cultured, primarily reflecting slight differences in the stages of initial isolation and injection and in their individual development under culture conditions. Co-transfection of constructs was observed in at least 90% of transfected cells (Figure S2); the degree of EGFP signal does not always correlate with the level of induction of target genes, probably due to different transfection efficiencies for each vector in each cell. Expression constructs were all under the control of a CMV promoter.

Immunohistochemistry, antibodies, histochemistry, and *in situ* hybridization.

Embryos were collected, fixed with 4% paraformaldehyde, and processed for paraffin sections or cryosections. Immunohistochemistry was performed on whole embryos or sections after antigen retrieval by microwave heating (for paraffin sections). Antisera used were anti- α TM and anti-cTnT (both from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA 52242). Whole-mount *in situ* hybridization was performed with digoxigenin-labeled probes generated by *in vitro* transcription and standard procedures.

Chromatin immunoprecipitation. Proteins were cross-linked to DNA from cells FACS-sorted from 25–30 embryos by adding formaldehyde/PBS (final concentration 1%) to the culture medium. To stop cross-linking, glycine was added to a final concentration of 0.125 M. Cells were washed with PBS with protease inhibitors, and centrifuged. Cells were resuspended in 1% SDS, 10 mM EDTA, and 50 mM Tris-HCl,

pH8.0, with protease inhibitor and sonicated. Samples were diluted (1/10 in 0.01% SDS, 1.1% Triton X, 2 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl, pH8.0, and protease inhibitor), precleared with protein G beads and salmon sperm DNA, and centrifuged. Supernatants were incubated with primary antibody against the protein of interest for 4 h to O/N at 4C. Antisera were anti-Brg1 (Upstate), anti-Gata4 (Santa Cruz C-20), or normal rabbit IgG (Santa Cruz #2027). Following multiple washes samples were eluted, and crosslinks reversed with 5 μ l 5M NaCl. Samples were purified prior to PCR amplification. Primer sequences are as follows: 1. *Nppa* primers: 340F TGT TGC CAG GGA GAA AGA ATC CT; 36R CAG CTC TTT GAG AAG GCA. 2. *Tnnt2* primers: 50F ACC AAC CTC CAT CCG AAA GGT; 595R AGA TGC CTT TGT TAT TCC TGA AA.