Notch1-Induced Transformation of RKE-1 Cells Requires Upregulation of Cyclin D1.

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Phenotype of Embryos with an Inactivating Mutation in the *Notch1* Gene.

The targeting mutation in the *Notch1* locus deleted 192 amino acids of the coding region including EGF-like repeats 11 and 12 in the Notch ligand binding domain will be described elsewhere. Homozygous mutant embryos died at mid-gestation and exhibited a classic *Notch1*$^{-/-}$ phenotype (31, 32) (Fig. S1). At E9.5 *Notch 1*$^{-/-}$ embryos had a reduced number of irregularly demarcated somites [12-13 (n=3) versus 21-23 (n=3) in controls]. Mutant embryos were severely growth retarded and anemic (Fig. S1A), and their yolk sac vascularization was disorganized (Fig. S1B and S1C). They also had an underdeveloped, looped heart with a distended pericardial sac (Fig. S1A), and kinked neuroepithelium (Fig 3D).

Controls for *In Situ* Hybridization and Immunohistochemistry.

Fig. S2 shows embryos probed by *in situ* hybridiztion with a sense probe to cyclin D1 in embryos lacking Notch1 (S2. A) or Pofut1 (S2. B). These embryos were treated in the same experiments for the same time as embryos hybridized to the anti-sense cyclin D1 probe (Fig. 3). Fig. S2. C shows immunohistochemistry using only the secondary antibody that was used to detect anti-cyclin D1 antibodies in Fig. 3.

Figure Legends

Fig. S1. Phenotype of *Notch1*$^{-/-}$ embryos (A) Lateral view of control (left) and *Notch1*$^{-/-}$ embryo (right) at E9.5. (B) Wild type yolk sac at E9.5. (C) *Notch1*$^{-/-}$ yolk sac at E9.5 with impaired vasculogenesis.

Fig. S2. Embryos probed with cyclin D1 sense oligonucleotides or secondary antibody alone. (A) Wild type embryo from Notch1 mating at E8.5 subjected to *in situ* hybridization with a cyclin D1 sense probe and incubated with anti-digoxigenin antibody for the same time as *Notch1* embryos incubated with antisense probe. (B) Wild type

embryo from Pofut1 mating at E9.5 subjected to *in situ* hybridization with a cyclin D1 sense probe and incubated with anti-digoxigenin antibody for the same time as *Pofut1* embryos incubated with antisense probe (Fig. 3). (C) Wild type embryo from Pofut1 mating at E9.5 subjected to immunohistochemical analysis incubated with horseradish peroxidase-conjugated anti-mouse IgG secondary antibody (Zymed) only and developed with DAB.

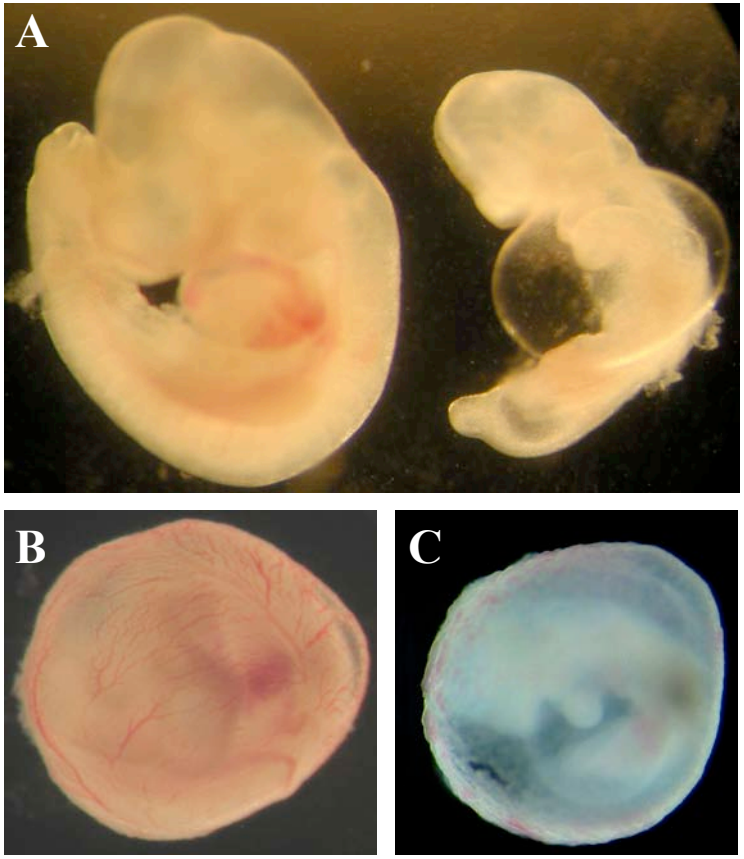

FIG. S1

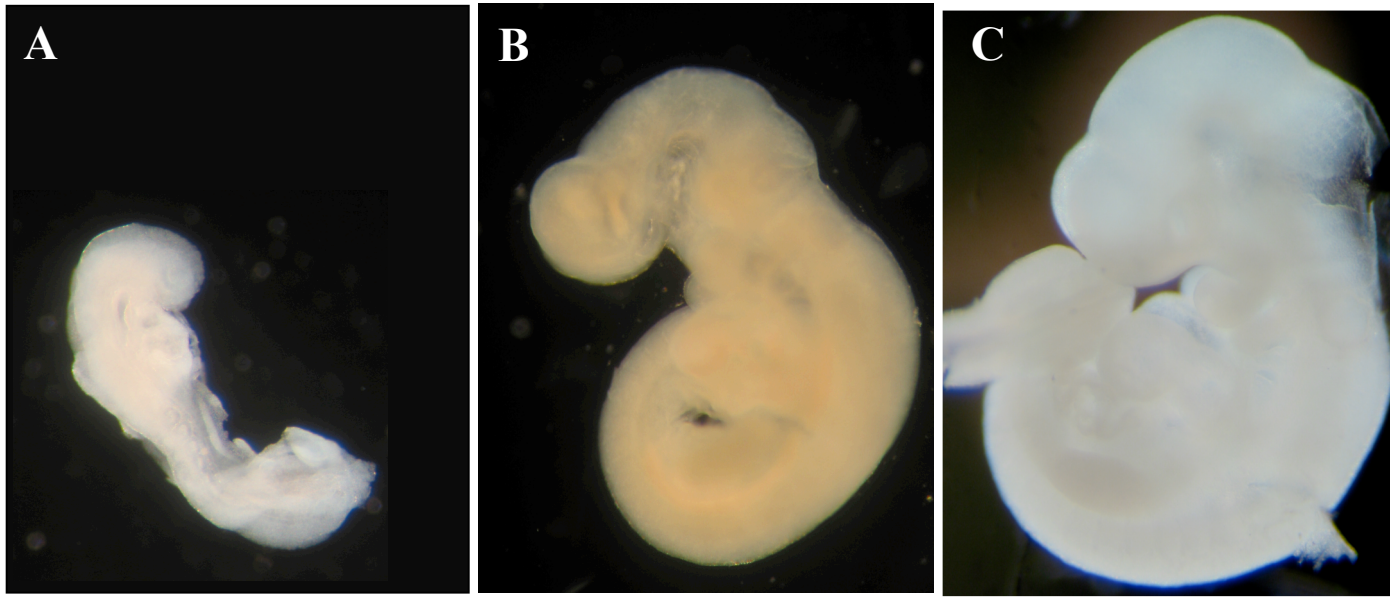

FIG. S2