Supporting Material and Methods

BAT-gal construct. BAT-gal was constructed by fusing 7 TCF/LEF-binding sites upstream of a 0.13 kb (0.13-sia) fragment containing the minimal promoter-TATA box of *siamois* (1). We used this minimal promoter for two reasons: first, this fragment has already been shown to be inactive *in vivo* during the development of *Xenopus* embryos (1); second, *siamois* is not present in mammals, making BAT-gal a completely heterologous reporter system that may be less subject to regulatory mechanisms in mammalian cells. BAT-gal drives the expression of β-gal in the nucleus to enhance detection of the reporter. Multimerized LEF/TCF-binding sites were generated by ligating synthetic double-stranded oligonucleotides (5'-CAGAATCATCAAAGGACCT-3'). The mutant LEF/TCF reporter was generated similarly to BAT-gal but using oligonucleotides mutated in the LEF consensus (2).

Analysis of BAT-gal in mutant mice. BAT-gal mice carrying the APC(Min/+) mutation were generated by crossing male mice bearing a point mutation in the APC gene with BAT-gal transgenic females and pups were genotyped by using PCR (3). Adult animals were dissected and intestines opened and cleaned as previously described (4). The BAT-gal transgene on the LRP6 mutant background was obtained by crossing BAT-gal with LRP6+/- mice (5). The LRP6 mutation was identified by PCR on yolk-sac DNA.

***LacZ* detection**. Dissected embryos, or vibratome sections, were washed in PBS, fixed for 30 min in 4% paraformaldehyde, and incubated in the 5-bromo-4-chloro-3-indolyl-β-d-galactoside (X-Gal) staining solution for 4-16 h (6). Individual organs were dissected after fixation from embryos older than embryonic day (E)11.5 to enhance penetration of the staining solution.

Histological analysis and immunohistochemistry. *LacZ*-positive embryos and newborn and adult tissues were extensively washed in PBS after X-Gal staining and postfixed overnight in 4% paraformaldehyde in PBS at 4°C. The samples were then

dehydrated, embedded in paraffin and sectioned. Five-micrometer sections were stained sequentially with hematoxylin/eosin and mounted in Permount. For immunohistochemistry, embryos were fixed for 2 h in 4% paraformaldehyde in PBS at 4°C. Embryos were cryoprotected in 30% sucrose in PBS (overnight), included in OCT (Bioptica, Cambridge, UK) and stored at -80°C before sectioning in a cryotome (5-15 μm). Sections were incubated with the following primary antibodies: anti-platelet endothelial cell adhesion molecule (PECAM) (generous gift from A. Vecchi, Inst. Mario Negri, Milan) and anti-β-gal (Molecular Probes) and analyzed by confocal microscopy (Bio-Rad).

***In situ* hybridization.** *In situ* hybridization was performed as described (7) by using sense and antisense cRNA transcribed from *lacZ, FGF8, or Tbx5* cDNA.

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