

**Supplemental Figure 1:** Gene targeting and genotype analysis of cell lines and mice. (A) Schematic representations of the *Hoxc10* genomic region (top), the targeting vector (middle), and the fragment sizes resulting from enzymatic digestion (bottom). An in-frame fusion of *LacZ* and *neo<sup>R</sup>* was inserted into the coding sequence (black shading) of *Hoxc10* exon 2. The position of 5' and 3' flanking probes pro5 (lavender) and pro4 (yellow) used for Southern analysis are indicated in association with the genomic region schematic. The engineered sequence was flanked by thymidine kinase genes (TK) in the assembled targeting vector. Restriction sites for BamH I (B), Mlu I (M), Sal I (S), Sca I, Spe I, and Sph I are indicated. (B) Southern blot analysis of wild-type and heterozygous mice using the 5' flanking probe, pro5. ScaI digestion reveals an upshift from 11.5 to 18.9 kb following vector integration. A 5' fragment at 14 kb, which is unchanged following vector integration, is also revealed with this probe. Mlu I/Spe I digestion reveals a downshift from 25 kb, from the first exon of *Hoxc11* to 3' of *Hoxc10*, to 17 kb, from first exon of *Hoxc11* to a Mlu I site in *LacZ*. See fragment maps in A for positions of restriction sites. (C) Genotyping with 3' flanking probe pro 4. BamHI digestion produces an upshift from 6.5 kb to 8.9 kb, and Xho I/Sph I reveals a downshift from 18.5 kb to 13.0 kb. CC1.2 is the ES cell line used for targeting. 266-1d-2 is the correctly targeted cell line that was used to produce chimera 1800, whose agouti pup 1800N1 is the founder of the colony. See fragment maps in A for positions of restriction sites. (D) Genotyping by PCR using the primers and conditions described in Methods and Materials produces a wild-type band of 327 bp and a mutant band of 228 bp. Genotypes are indicated above the lanes.

