## Table S1. Tabulation of skeletal analysis for BAC15 mice

| | | | | Craniofacial defects | | | | | Sternum | Limbs |
|---|---|---|---|---|---|---|---|---|---|---|
| Genotype | Code | Age | Sex | Sutures | Bony Isl. | Zygo. Fus. | Face | O.g. Teeth | Fusion | L/R (Type) |
| Wild type | Tg83-3/3-b.3 | P4 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg83-3/3-c.1 | P4 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg83-3/3-c.2 | P4 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg83-3/6B-a.4 | P6 | n.d. | _ | Y | _ | _ | _ | _ | _ |
| | Tg83-3/6B-a.9 | P6 | n.d. | _ | Y | _ | _ | _ | _ | _ |
| | Tg83-3/3-a.1 | P6 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg83-3/6B-a.9 | P6 | n.d. | _ | Y | _ | _ | _ | _ | _ |
| Total = 7 | | | | | | | | | | |
| 1C-BAC15 | Tg83-3/3-b.1 | P4 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg83-3/3-b.2 | P4 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg83-3/3-b.5 | P4 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg83-3/3-b.7 | P4 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg83-3/3-c.4 | P4 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg83-3/6B-a.1 | P6 | n.d. | _ | Y | _ | _ | _ | _ | _ |
| | Tg83-3/6B-a.2 | P6 | n.d. | _ | Y | _ | _ | _ | _ | _ |
| | Tg83-3/6B-a.3 | P6 | n.d. | _ | Y | _ | _ | _ | _ | _ |
| | Tg83-3/6B-a.5 | P6 | n.d. | _ | Y (v. small) | _ | _ | _ | _ | _ |
| | Tg83-3/6B-a.7 | P6 | n.d. | _ | Y | _ | _ | _ | "Y, rib misaligned" | _ |
| Total = 10 | | | | | | | | | | |
| 2C-BAC15 | Tg83-3/3-a.2 | P6 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg83-3/3-a.3 | P6 | n.d. | _ | _ | _ | _ | _ | 4+5 | _ |
| | Tg83-3/3-a.4 | P6 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg83-3/3-b.4 | P4 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg83-3/3-b.5 | P4 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg83-3/3-c.3 | P4 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg83-3/6B-a.6 | P6 | n.d. | _ | Y | _ | _ | _ | "4+5, ribs abnormal" | _ |
| Total = 7 | | | | | | | | | | |
| 3C-BAC15 | Tg83-3/6B-a.8 | P6 | n.d. | _ | Y | _ | _ | _ | _ | _ |
| Total = 1 | | | | | | | | | | |

Wild Type are litter mates of 2C- and 4C- transgenic mice.
n.d., not determined; –, no phenotype; Y, yes; Bony Isl., bony islands; Zygo. Fus., zygomatic fusion; O.g., over grown teeth; R, right; L, left.
For sternal defects: numbers indicate which sternabrae were fused.
Limb L/R, Hind-limb polydactyly with sides of occurrence indicated as L, left and/or, R, right.
For BAC15 data, 3C-BAC15 is derived from breeding 1C and 2C founders.

## Table S2. Tabulation of skeletal analysis for BAC16 mice

| | | | | Craniofacial defects | | | | | Sternum | Limbs |
|---|---|---|---|---|---|---|---|---|---|---|
| Genotype | Code | Age | Sex | Sutures | Bony Isl. | Zygo. Fus. | Face | O.g. Teeth | Fusion | L/R (Type) |
| Wild type | Tg90-3/2A-a.2 | P1 | n.d. | _ | Y | _ | _ | _ | 4+5 | _ |
| | Tg90-3/8G-b.8 | P24 | n.d. | Bridged | Y | _ | _ | _ | _ | _ |
| | Tg90-3/8G-b.10 | P24 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg90-3/8A-a.6 | 2 months | F | _ | _ | n.d. | _ | _ | n.d. | _ |
| Total = 4 | | | | | | | | | | |
| 1C-BAC16 | Tg90-3/11H.5 | E18.5 | n.d. | _ | Y | _ | _ | n.a. | _ | _ |
| | Tg90-3/8G-b.3 | P8 | M | Fused | Y | Unilateral | _ | _ | _ | _ |
| | Tg90-3/8G-b.4 | P8 | F | Bridged | Y | Ant. only | Curv. | _ | 4+5 | _ |
| Total = 3 | | | | | | | | | | |
| 2C-BAC16 | Tg90-3/11H.2 | E18.5 | n.d. | _ | Y | _ | _ | n.a. | 4+5 | _ |
| | Tg90-3/11H.3 | E18.5 | n.d. | _ | Y | _ | _ | n.a. | 4+5 | _ |
| | Tg90-3/2A-a.1 | P1 | n.d. | _ | Y | _ | _ | _ | _ | _ |
| | Tg90-3/2A-a.3 | P1 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg90-3/2A-a.5 | P1 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg90-3/2A-a.10 | P1 | n.d. | _ | Y | _ | _ | _ | 4+5 | _ |
| | Tg90-3/2A-a.11 | P1 | n.d. | _ | Y | _ | _ | _ | _ | _ |
| | Tg90-3/2B-b.1 | P7 | n.d. | Fused | Y | Ant. only | _ | _ | 4+5 | _ |
| | Tg90-3/2B-b.5 | P7 | n.d. | Fused | Y | Ant. only | _ | _ | 4+5 | _ |
| | Tg90-3/8G-a.6 | P24 | n.d. | _ | _ | _ | _ | _ | _ | _ |
| | Tg90-3/8G-a.7 | P24 | n.d. | Bridged | Y | _ | _ | _ | 4+5 | _ |
| | Tg90-3/8G-a.9 | P24 | n.d. | _ | _ | _ | _ | _ | 4+5 | _ |
| | Tg90-3/8B-a.5 | 1.5 months | F | Bridged | _ | n.d. | _ | Upp. Incisors | n.d. | L+R (I) |
| | Tg90-3/8A-a.2 | 2 months | M | Bridged | _ | n.d. | _ | _ | n.d. | R |
| | Tg90-3/8C-a.2 | 2 months | M | Bridged | Y | Ant. only | _ | _ | 4+5 | Curved |
| | Tg90-3/3I-a.8 | 5 months | F | n.d. | _ | _ | _ | _ | 4+5 | R (III) |
| Total = 15 | | | | | | | | | | 3 |
| 4C-BAC16 | Tg90-3/11H.1 | E18.5 | n.d. | Closer appos. | Y | _ | _ | n.a. | Extra 5+4+5 | R (II) |
| | Tg90-3/11H.4 | E18.5 | n.d. | Closer appos. | Y | Ant. only | _ | n.a. | 4+5 | R (I) |
| | Tg90-3/2B-b.2 | P7 | n.d. | Fused | Y | Ant. only | Slight Curv; S. Max | _ | 4+5 | R (slight I) |
| | Tg90-3/2B-b.3 | P7 | n.d. | Fused | Y | Ant. only | _ | _ | 4+5 | L+R (I+III) |
| | Tg90-3/2B-b.4 | P7 | n.d. | Bridged | _ | Ant.+ post. | _ | _ | 4+5 | R (II) |
| | Tg90-3/2B-b.6 | P7 | n.d. | Bridged | _ | Ant. only | Curv.; S. Max | _ | 1+2; 4+5 | R (IV) |
| | Tg90-3/8G-a.1 | P8 | M | Fused | Y | R. ant only/L.both | _ | _ | 4+5 | L+R (I+III) |
| | Tg90-3/8G-a.2 | P8 | F | Fused | Y | Ant.+ post. | _ | _ | 1+2; 4+5 | L+R (I+III) |
| | Tg90-3/8G-a.5 | P24 | n.d. | Fused | Y | Ant. only | Curv. | Upp. Incisors | 4+5 | _ |
| | Tg90-3/8B-a.4 | 1.5 months | F | Bridged | _ | n.d. | Slight Curv. | Upp. Incisors | n.d. | L+R (I+III) |
| | Tg90-3/4-a.1 | 2 months | M | Bridged | Y | n.d. | Slight Curv. | Upp. Incisors | 4+5 | _ |
| | Tg90-3/7A-a.5 | 2 months | F | Bridged | _ | Ant.+ post. | Short Max. | Upp. Incisors | 1+2; 3+4+5 | L (I) |
| | Tg90-3/7B-a.1 | 2 months | M | n.d. | Y | Ant.+ post. | _ | Upp. Incisors | 1+2; 4+5 | L+R (I+III) |
| | Tg90-3/8A-a.1 | 2 months | M | Fused | Y | n.d. | Curv. Left | Upp. Incisors | n.d. | L+R |
| | Tg90-3/8C-a.1 | 2 months | M | _ | Y | Ant.+ post. | Short Max. | Upp. Incisors | 4+5 | R (I) |
| | Tg90-3/3D-a.1 | 2.5 months | M | _ | _ | Ant. only | _ | Upp. Incisors | 4+5 | L+R (I+II) |
| | Tg90-3/3D-a.2 | 2.5 months | M | Fused | _ | Ant.+ post. | _ | Upp. Incisors | Rib mis 4+5 | R (II) |
| | Tg90-3/2B-c.2 | 3.5 months | M | n.d. | _ | Ant. only | Curv. Left | Upp. Incisors | 4+5 | L+R (I+III) |
| | Tg90-3/3D-a.7 | 4 months | F | Bridged | _ | n.d. | Curv. Right | Upp. Incisors | n.d. | n.d. |
| | Tg90-3/3A-a.4 | 10 months | F | n.d. | _ | Ant. only | Mild Left Curv. | Upp. Incisors | 4+5 | R (III) |
| | Tg90-3/7C-a.1 | 13 months | M | n.d. | Y | Ant. only | Curv.; S. Max | Upp. Incisors | 4+5 | _ |
| | Tg90-3/7C-a.5 | 13 months | F | Fused | _ | Ant. only | _ | _ | 1+2; 4+5 | L (II) |
| Total = 21 | | | | | | | | | | 17 |

Wild type are litter mates of 2C- and 4C-transgenic mice.

n.a., not applicable; n.d., not determined; –, no phenotype; Y, yes; Bony Isl., bony islands; Zygo. Fus., zygomatic fusion; Ant., anterior; post., posterior; R, right; L, left; O.g., over grown teeth; Upp., upper.

For facial defects: Curv., curved (with side indicated); S. Max., short maxilla.

For sternal defects: numbers indicate which sternabrae were fused; Rib mis., rib misalignment (as in Fig. 4C).

Limb L/R, Hind-limb polydactyly with sides of occurrence indicated as L, left and/or, R, right.

The type/combinations of polydactyly observed are indicated by symbols I to IV. Each type, I, II, III and IV, is depicted in Fig. 5, in parts F, D, H, and J, respectively.

# Table S3. Candidate genes governing limb development and polydactyly

| Gene | E10-E10.5 | E11-E11.5 | E12-E12.5 |
|---|---|---|---|
| *Alx4* | + | | |
| *Bmp2* | | + | |
| *Bmp4* | + | + | |
| *BmpRI-B* | | + | |
| *Cdx1* | – | – | |
| *CdX2* | – | – | |
| *Dkk1* | | + | + |
| *Fgf4* | + | + | + |
| *Fgf8* | + | | |
| *Fgfr1* | + | | |
| *Gli3* | + | + | |
| *Gremlin* | | + | |
| *dHAND* | + | + | |
| *Hoxd12* | | + | |
| *Hoxd13* | | + | + |
| *Lmbr1* | – | – | |
| *Lef1* | | + | |
| *Msx1* | | + | |
| *Msx2* | | + | |
| *Sall1* | + | | |
| *Shh* | + | + | |
| *Twist* | + | + | |
| *Wnt5a* | | + | |

List of genes whose expression was examined in wild-type, 2C- and 4C-BAC16 embryonic mice in this study. Studies of engineered or spontaneous mutations (Biesecker, 2002) have implicated a subset of such genes in polydactyly.

+, embryonic stages examined where expression was detected.

–, stages examined where no expression was detected.

Blanks represent stages that were not studied.

A minimum of two embryos were examined at each stage indicated.