

Table S1. Number of independent founders for each transgene

Transgene	Number of independent transgenic founders	Number of transgenics with staining pattern
RP24-376P4	2	Hindlimbs: 2/2 (weak) Lungs: 2/2 Genital tubercle: 2/2 Umbilical cord: 0/2
RP24-84E15	4	Hindlimbs: 4/4 Lungs: 4/4 Genital tubercle: 4/4 Umbilical cord: 4/4
RP23-136J3	3	Hindlimbs: 3/3 Lungs: 3/3 Genital tubercle: 3/3 Umbilical cord: 3/3
ΔHLEA	4	Hindlimbs: 4/4 (note: posterior only) Lungs: 4/4 Genital tubercle: 4/4 Umbilical cord: 4/4
ΔHLEB	8	Hindlimbs: 8/8 (note: reduced staining in distal half of autopod) Lungs: 8/8 Genital tubercle: 8/8 Umbilical cord: 8/8
ΔHLEA/ΔHLEB	5	Hindlimbs: 1/5 Lungs: 5/5 Genital tubercle: 5/5 Umbilical cord: 5/5
pDBM2	15	Hindlimb: 14/15
pDBM3	10	Lung: 9/10
pDBM5	6	Hindlimb: 6/6 Genital tubercle: 6/6 Umbilical cord: 1/6
pDBM7	6	Hindlimb: 5/6
pDBM36	16	Hindlimb: 15/16
pDBM46	11	Hindlimb: 8/11
pDBM40	3	Hindlimb: 3/3 Genital tubercle: 3/3 Umbilical cord: 3/3 Scattered cells in torso/face: 3/3 (note: ectopic pattern)
pDBM42	9	No clear pattern (genital tubercle: 4/9)
pDBM43	6	No clear pattern (limbs: 2/6)
pDBM44	9	No clear pattern
pDBM45	14	Hindlimb: 10/14 Genital tubercle: 13/14 Umbilical cord: 6/14
pDBM50	10	Hindlimb: 7/10 Genital tubercle: 8/10 Umbilical cord: 2/10
pDBM51	3	Hindlimb: 0/3 Genital tubercle: 0/3 Umbilical cord: 0/3
pDBM20 (stickleback HLEB)	19	Hindlimb: 17/19 Genital tubercle: 18/19

Table S2. Relative hindlimb bone size averages of wild-type, +/ Δ HLEA and Δ HLEA/ Δ HLEA littermates

Measurement	+/+ (n=14)	+/ Δ HLEA (n=15)	Δ HLEA/ Δ HLEA (n=15)	WT-Het <i>P</i> -value	WT- Δ HLEA <i>P</i> -value
Patella length	1.0 \pm 0.012	0.95 \pm 0.0095	0.88 \pm 0.012	0.0018	1.4 \times 10 ⁻⁰⁷
Femur length	1.0 \pm 0.0044	0.98 \pm 0.0044	0.96 \pm 0.0049	0.0040	3.8 \times 10 ⁻⁰⁶
Tibia length	1.0 \pm 0.0032	1.0 \pm 0.0045	0.98 \pm 0.0053	NS	0.013
Tibia width	1.0 \pm 0.012	0.93 \pm 0.013	0.82 \pm 0.0075	9.4 \times 10 ⁻⁴	5.5 \times 10 ⁻¹²
Fibula length	1.0 \pm 0.0031	1.0 \pm 0.0041	0.98 \pm 0.0060	NS	0.030
Pelvis length	1.0 \pm 0.0056	0.99 \pm 0.0064	0.99 \pm 0.0061	NS	NS
Pelvis width (anterior)	1.0 \pm 0.0091	0.97 \pm 0.0097	0.94 \pm 0.012	0.040	2.7 \times 10 ⁻⁴
Pelvis width (center)	1.0 \pm 0.0067	0.98 \pm 0.0067	0.96 \pm 0.0085	0.013	0.0018
Pelvis width (posterior)	1.0 \pm 0.017	1.0 \pm 0.017	1.1 \pm 0.020	NS	5.8 \times 10 ⁻⁴

Hindlimb bone sizes have been normalized relative to forelimb bone lengths and the normalized wild-type (WT) average for each hindlimb bone has been arbitrarily set to 1. The s.e.m. of each normalized average is indicated. The WT-Het and WT- Δ HLEA *P*-value columns refer to comparisons between +/+ and +/ Δ HLEA and between +/+ and Δ HLEA/ Δ HLEA, respectively.

NS, not significant.

Table S3. Average lengths of metatarsal and phalangeal elements of the first, third and fifth digit rays from wild-type and Δ HLEA/ Δ HLEA littermates

		+/+ (n=14)	Δ HLEA/ Δ HLEA (n=15)	WT- Δ HLEA P-value
Digit-ray 1	Metatarsal 1	4.30 \pm 0.033	4.07 \pm 0.063	0.0017
	Phalanx 1	2.14 \pm 0.010	1.93 \pm 0.020	2.5 \times 10 ⁻⁰⁹
	Phalanx 2	1.09 \pm 0.032	0.96 \pm 0.047	2.9 \times 10 ⁻⁰⁹
Digit-ray 3	Metatarsal 3	7.23 \pm 0.034	6.93 \pm 0.053	5.5 \times 10 ⁻⁰⁵
	Phalanx 1	2.98 \pm 0.025	2.74 \pm 0.023	9.1 \times 10 ⁻⁰⁸
	Phalanx 2	1.81 \pm 0.014	1.69 \pm 0.015	2.3 \times 10 ⁻⁰⁶
	Phalanx 3	1.50 \pm 0.017	1.34 \pm 0.014	1.3 \times 10 ⁻⁰⁷
Digit-ray 5	Metatarsal 5	6.42 \pm 0.044	6.32 \pm 0.046	NS
	Phalanx 1	2.55 \pm 0.020	2.47 \pm 0.019	0.0029
	Phalanx 2	1.45 \pm 0.014	1.41 \pm 0.015	0.032
	Phalanx 3	1.14 \pm 0.013	1.04 \pm 0.011	5.5 \times 10 ⁻⁰⁶

Lengths are in millimeters. The s.e.m. of each average is indicated.

NS, not significant.