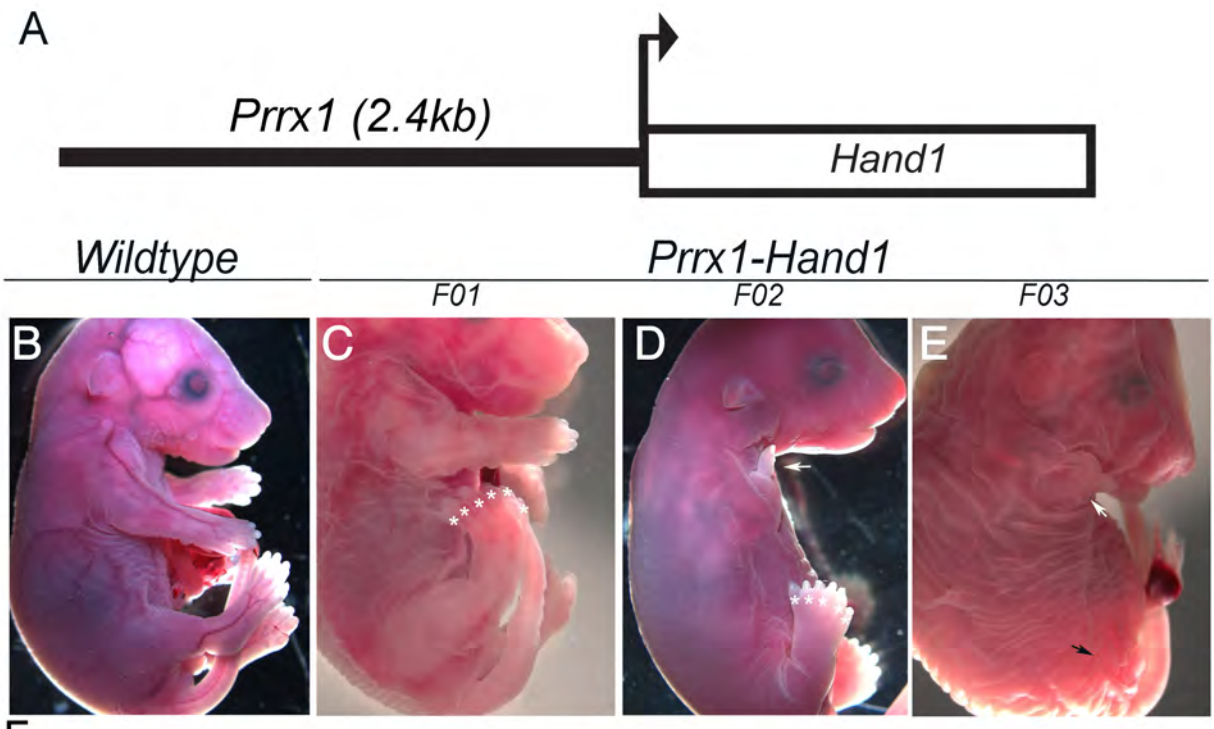
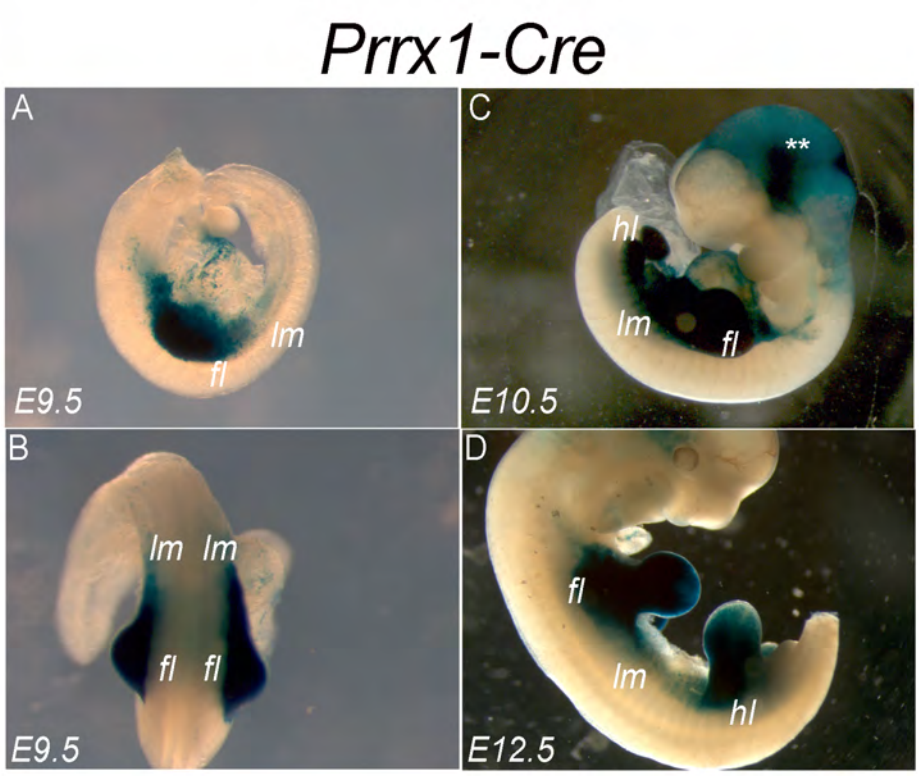


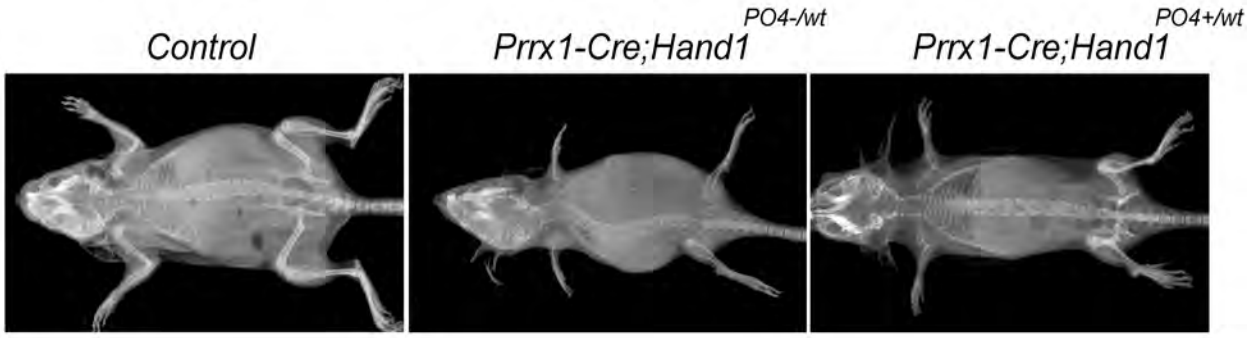
F

Prx1-hand1	Highly regressed forelimbs	Highly regressed hindlimbs	shortened forelimbs	shortened hindlimbs	polydactyl forelimbs	polydactyl hindlimb
n=6	4	4	2	2	1	2

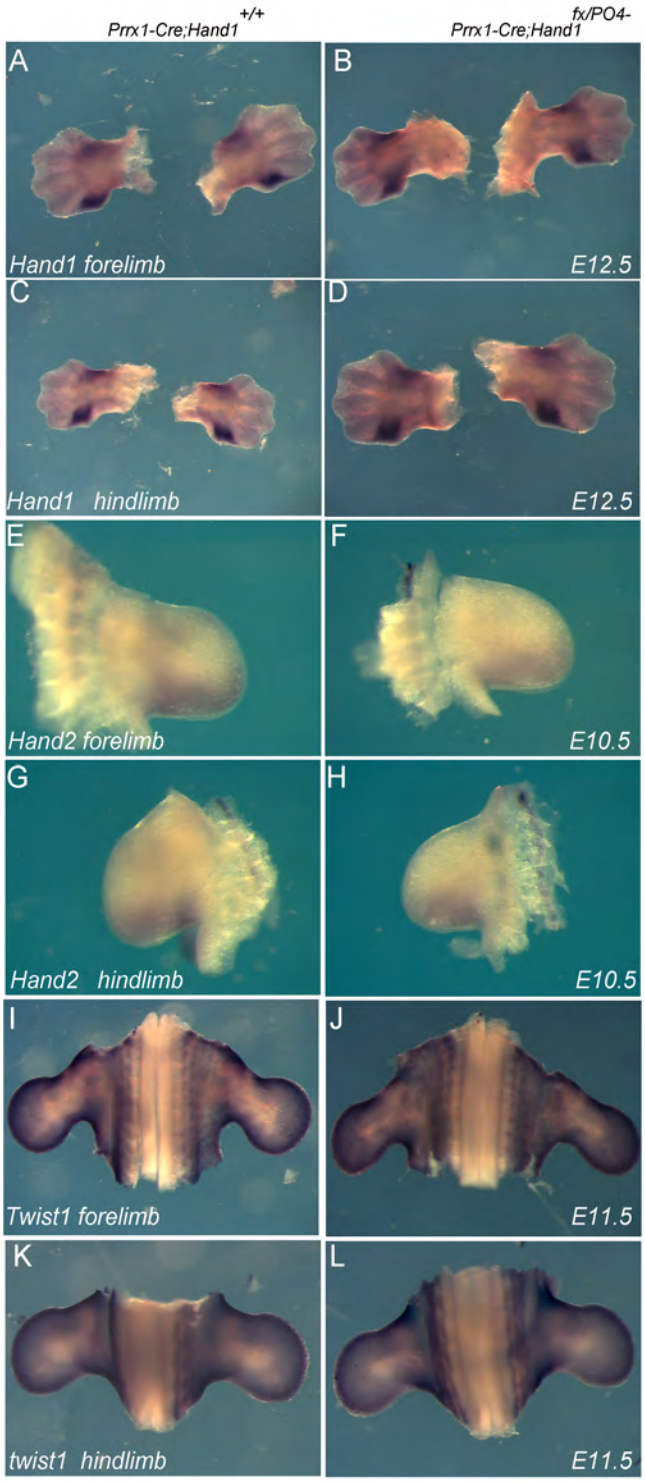
Supplemental Figure 1: Comparison of Hand1 gain-of-function limb phenotypes. A series of transgenic positive and control (transgenic negative) P0 littermates show a range of limb phenotypes with the expression of a *Prrx1-Cre-Hand1* transgene construct (A). B) Control mouse. C) F01 *Prrx1-Cre-Hand1* neonate, which displays hindlimb polydactyly (white asterisk) with normal autopods on forelimbs. D) F01 *Prrx1-Cre-Hand1* neonate that shows hindlimb polydactyly (white asterisk) with severe regression of forelimb outgrowth and patterning (white arrow). E) F03 *Prrx1-Cre-Hand1* neonate that displays a lack of hindlimb outgrowth (black arrow) and remedial development of forelimbs (white arrow). F) Phenotype descriptions of the 6-F0 *Prrx1-Hand1* transgenics generated in this experiment. These data collectively show that transgenic expression of *Hand1* within the developing limbs can present with a wide-spectrum of phenotypes likely based on expression level variation imposed by both integration site and copy number making this approach for functional analysis unreliable.



Supplemental Figure 2: Lineage expression pattern of *Prrx1-Cre*;*R26R* during limb development. A and B) Forelimbs (fl) are robustly stained by Xgal at E9.5 indicating robust β-galactosidase activity with some lateral mesoderm (lm) activity also visible. C) At E10.5 both fore and hindlimbs (hl) as well as lm are easily detectable. D) E12.5 shows uniform robust β-galactosidase activity with the entirety of the limbs.

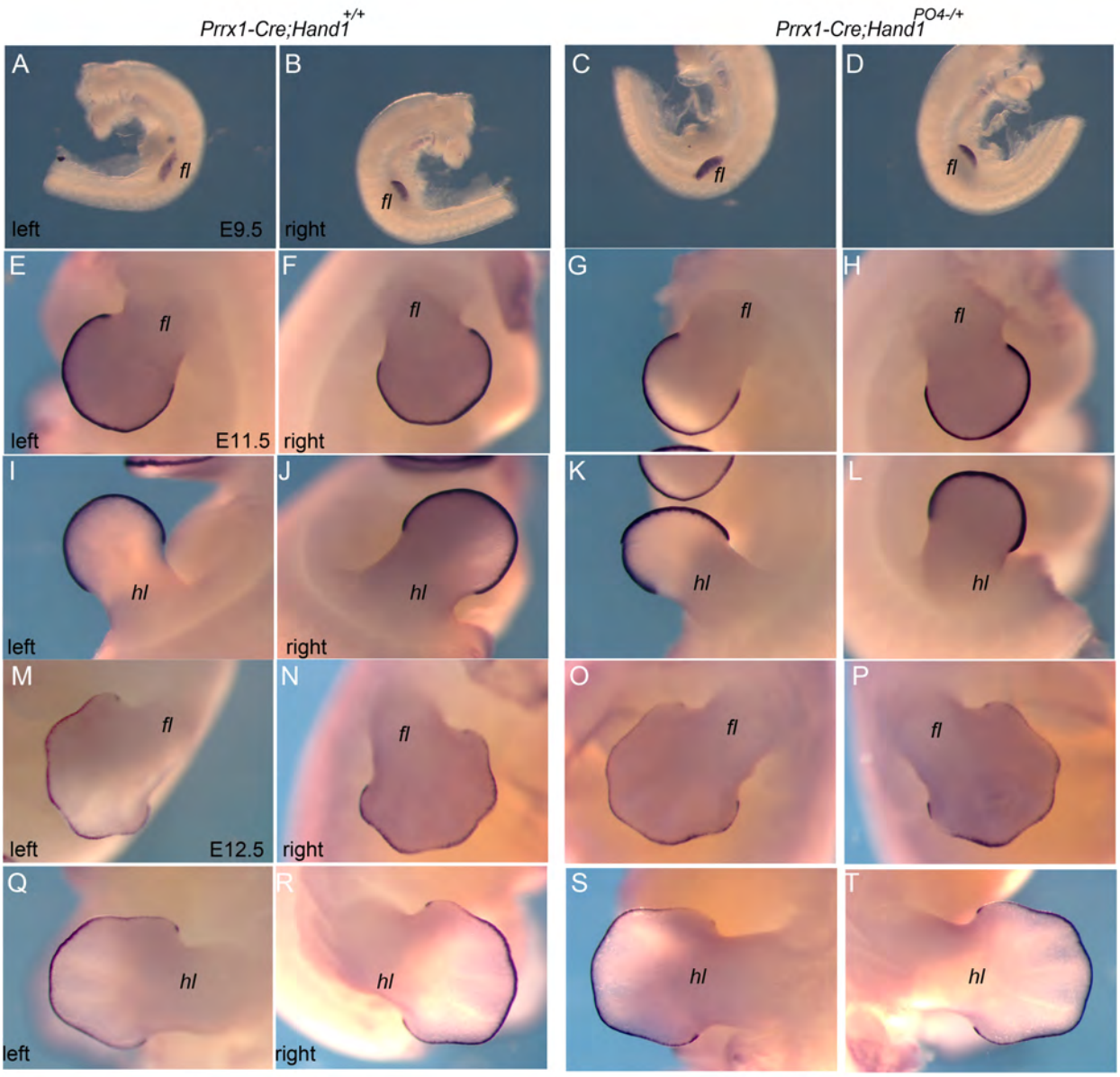


Supplemental Figure 3: X-ray images of control and Hand1 phospho-mutant mice displaying proximal anterior limb defects. Left most image is a ventral view of normal control mouse. Middle image shows the most severe fore- and hindlimb phenotypes in the hypophosphorylation mimic. Right most image shows phosphorylation mimic limb defects.



Supplemental Figure 4: Hand1, Hand2, and Twist1 expression patterns are unaltered in Hand1 phospho-mutant embryos. A-D) E12.5 fore- and hindlimbs comparing *Hand1* expression between control and single copy *Hand1* hypophosphorylation mutants. In these

embryos the only copy of Hand1 present is the mutant allele and its expression shows no differences from wildtype controls (not shown). E-H) Fore- and hindlimb expression of *Hand2* in control *Hand1* hypophosphorylation mutants. No observable changes in *Hand2* expression or expression patterns are observed. I-L) Fore- and hindlimb expression of *Twist1* in control *Hand1* hypophosphorylation mutants. No observable changes in *Twist1* expression or expression patterns are observed.



Supplemental Figure 5: Wholemount *in situ* hybridization of *Fgf8* in forelimbs at E9.5 (A-D), 11.5 (E-H), and E12.5 (M-P) as well as hindlimbs at E11.5 (I-L) and E12.5 (Q-T) in both controls (two left most columns) and *Prrx1-Cre;Hand1*$^{PO4/+}$ mice. Fgf8 marks the AER and no significant difference in expression is observed.