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 Prx1-Cre;Hand1PO4/+ mice. Fgf8 marks the AER and no significant difference in expression is observed.