

Figure 3 - figure supplement 2

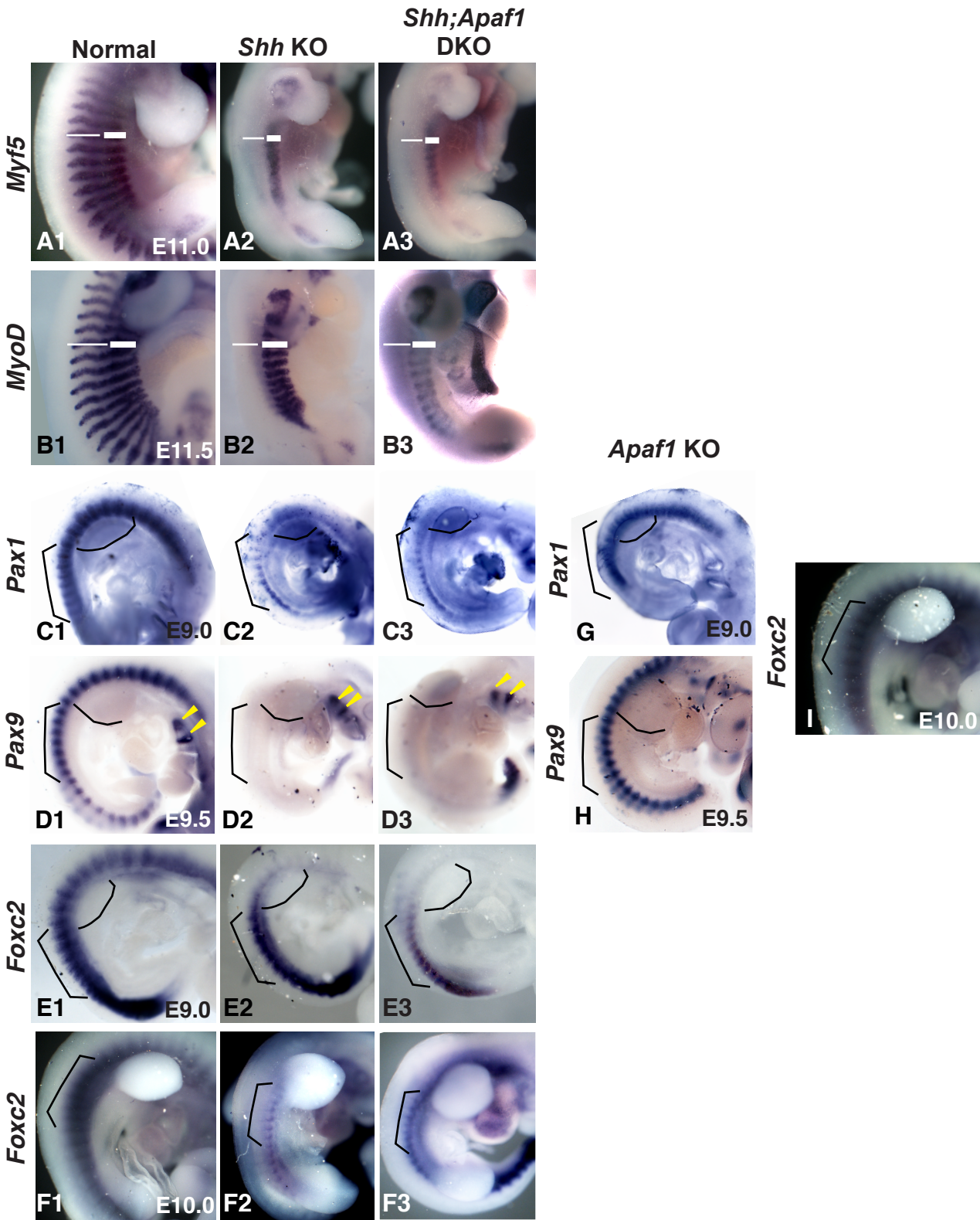


Figure 3 -supplement 2

Somite patterning in *Apaf1;Shh* KO embryos is similar to *Shh* KO embryos

A1-B3. E11.0 and E11.5 embryos showing *Myf5* (**A1-A3**) and *MyoD* (**B1-B3**) expression in the developing epaxial and hypaxial muscles. **A1 and B1.** Normal embryos. Loss of *Shh* leads to a lack of *Myf5* (**A2**) and *MyoD* (**B2**) in the epaxial region (thin line) but not the hypaxial region (thick line). *Shh;Apaf1* DKO embryos also display a loss of *Myf5* (**A3**) and *MyoD* (**B3**) in epaxial but not hypaxial progenitors.

C1-C3. Analysis of *Pax1* expression at E9.0. **C1.** *Pax1* expression in normal embryos. **C2.** *Pax1* expression in the sclerotome of *Shh* KO embryos. **C3.** *Shh;Apaf1* DKO embryos also express *Pax1* in a similar pattern.

D1-D3. Analysis of *Pax9* expression at E9.5. **D1.** *Pax9* is expressed in the sclerotome of normal embryos. **D2.** *Shh* KO embryos do not have *Pax9* expression in the sclerotome. Note *Pax9* expression in the brachial arches (arrowheads). **D3.** *Shh;Apaf1* DKO embryos exhibit a similar pattern.

E1-F3. Analysis of *Foxc2* expression at E9.0 and E10.0. *Foxc2* is expressed throughout the developing somite at E9.0 (**E1**) and is restricted to the sclerotome by E10.5 (**F1**) in normal embryos. *Foxc2* expression is detected in similar patterns in both the *Shh* KO (**E2, F2**) and the *Shh;Apaf1* DKO (**E3, F3**) embryos at both E9.0 and after de-epithelialization of the thoracic somites (E10.0).

G. *Pax1* expression at E9.0 in *Apaf1* KO embryos is similar to controls. Compare to C1. **H.** *Pax9* expression at E9.5 in *Apaf1* KO embryos is similar to controls. Compare to D1. Tail truncated due to collection for genotyping. **I.** *Foxc2* expression at E10.0 in *Apaf1* KO embryos is similar to controls. Compare to F1.

Brackets outline comparable somite regions, curved line indicates forelimb bud location. Phenotypes were consistently observed in at least 3/3

When null for skeletal muscle genes, embryos can also display skeletal abnormalities, including rib fusions, truncations and sternal defects (*Pax3*: (Henderson et al., 1999; Vivian et al., 2000)). In *Shh* KO embryos epaxial progenitors are reportedly absent, while the hypaxial progenitors are reduced (Borycki et al., 1999; Gustafsson et al., 2002) and our results looking at *Myf5* and *MyoD* expression confirmed this observation (**A2, B2**). Perhaps absence of the epaxial compartment explains the failure of the proximal rib to form in both situations while additional defects in the hypaxial compartment are responsible for lost distal segments in the *Shh;Apaf1* DKO embryos. However, we found that *Shh;Apaf1* DKO embryos displayed the same pattern as *Shh* KO embryos (**A3, B3**) suggesting that a failure to specify the hypaxial muscle compartment is not the cause of the *Shh;Apaf1* DKO phenotype.

Pax9 and *Pax1* are expressed in similar patterns in the sclerotome and are required for rib and vertebrae development (Furumoto et al., 1999; Peters et al., 1999; Rodrigo et al., 2003). Previous studies have shown that *Pax1* is initially expressed in the *Shh* KO embryo but then lost (Chiang et al., 1996; Borycki et al., 1998; Zhang et al., 2001). As previously reported, we found *Pax1* expressed in a smaller domain in *Shh* KO embryos compared to normal or *Apaf1* KO embryos at E9.0 (**C2**). However, in *Shh;Apaf1* DKO embryos, *Pax1* expression was similar suggesting that some sclerotome specification still occurs (**C3**). As development continues, *Pax1* expression is eventually lost in *Shh* KO embryos as well as in the *Shh;Apaf1* DKO embryos. Neither *Shh* KO nor *Shh;Apaf1* DKO embryos exhibited *Pax9* expression in somites at E9.5 (**B2-B3**).

We then determined if the expression of *Foxc2*, a winged helix/forkhead transcription factor required for axial skeletal development (Kume et al., 2001; Wilm et al., 2004) is altered. *Foxc2*, which is expressed in the early unsegmented paraxial mesoderm and later becomes restricted to the sclerotome, may be a more comprehensive sclerotome marker than *Pax1/9* (**C1, D1**), (Furumoto et al., 1999). Like *Pax1*, *Foxc2* was expressed in a reduced domain in both *Shh* KO and the *Shh;Apaf1* DKO embryos compared to normal embryos prior to E9.0, (**E2, E3**). However, in contrast to *Pax1*, the smaller domain of *Foxc2* expression continued to be present after E10.0 in both *Shh* KO embryos and somite-matched *Shh;Apaf1* DKO embryos (**F2, F3**). In summary, the loss of *Shh* correlates with smaller *Pax1* and *Foxc2* expression domains. In addition, the *Pax1* domain disappears while the smaller *Foxc2* expression domain persists, suggesting that at least a portion of the sclerotome is maintained in both contexts.

Compared to control embryos, *Apaf1* KO embryos did not display disruptions in sclerotome patterning as assessed by RNA *in situ* marker expression analysis (**G, H, I**).