Supplemental Figure Legends

Supplemental Figure S1. Analysis of primary myoblasts treated with Wnt3a using the Essen Incuyte FLR to measure confluence over time. Cultures were incubated in growth media (GM) or differentiation media (DM) supplemented with 1:5 diluted Wnt3a-conditioned or control L-cell-conditioned media for 18hrs. Data is the average of two experiments performed in duplicate.

Supplemental Figure S2. Expression of Wnt target genes in whole TA muscle from Barx2 wildtype and null mice (P21). The number of mice examined is indicated for each gene (average n~20); approximately half are wildtype and half null. To more easily compare between litters, the expression level in null mice is normalized to a wildtype sibling control in each litter which is set to one (horizontal line).

Supplemental Figure S3. Barx1 and Msx2 but not Msx1 can induce TOPflash promoter activity in C2C12 cells. All data were normalized to a Renilla luciferase internal control and then to pcDNA3 transfection. Data were collected in triplicate and 3 assays were performed with similar results. * indicates significant at $P<0.05$ when compared to the control.

Supplemental Figure S4. A. The FOPflash control reporter gene that contains mutated TCF/LEF binding sites is not activated by co-transfection of Barx2 constructs either alone or in combination with MyoD in C2C12 cells. All data were normalized to a Renilla luciferase internal control and then to pcDNA3 transfection. Data were collected in triplicate and two assays were performed with similar results. **B.** Effect of differentiation on TOPflash, Axin2 and cyclinD1 promoter activities. C2C12 cells were transfected with each of the three promoter constructs and incubated in growth (GM) or differentiation (DM) media for 48 hours before assay. All data were normalized to a Renilla luciferase internal control. Data were collected in triplicate and is the average of 2 assays. Promoter activities in GM and DM are not significantly different (T-test). **C.** Barx2 can induce TOPflash promoter activity in COS7 cells. All data were normalized to a Renilla luciferase internal control and then to pcDNA3 transfection. Data were collected in triplicate and 3 assays were performed with similar results. * indicates significant at $P<0.05$ when compared to the control.

Supplemental Figure S5. A. Barx2 and β-catenin can be co-immunopreciptated in extracts from transfected COS7 cells that have been sonicated and also treated with ethidium bromide to disrupt protein-DNA interactions. **B.** Endogenous Barx2 and β-catenin can be co-immunoprecipitated from embryonic limb bud mesenchymal cells

Supplemental Figure S6. ChIP was performed with antibodies to MyoD in C2C12 cells transfected with MyoD expression plasmid alone, or with the combination of Barx2 and MyoD plasmids. Enrichment of a known MyoD target promoter (desmin) was compared to enrichment of the integrated TOPflash promoter. Data are PCR amplification values for the relevant target promoter normalized to amplification values for a control non-target locus, and subsequently normalized to mock ChIP with preimmune IgG. Data are derived from 2 experiments; * indicates enrichment significant at $P<0.05$ when compared to the mock ChIP.