

Supplementary Information for:

BMP-mediated induction of GATA4/5/6 blocks somitic responsiveness to Shh

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Supplemental Figure Legends:

Supplemental Figure 1. Diagram of GATA4-full length, GATA4-Zinc Finger (ZF) Domain, and GATA4-Zinc Finger (ZF) Domain-VP16 fusion proteins.

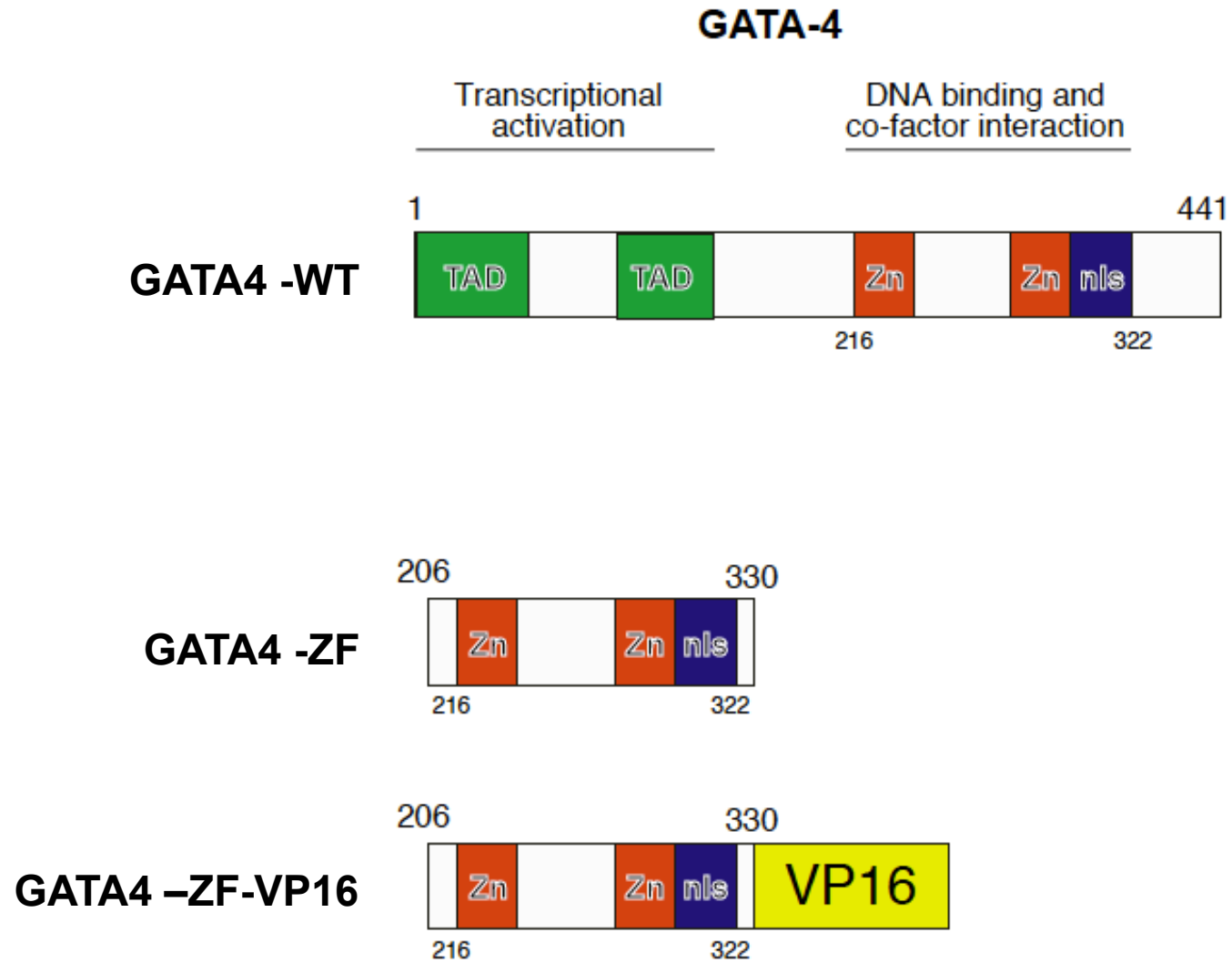
Supplemental Figure 2. GATA6-WT and GATA6-DBD-mutant are expressed at approximately equivalent levels in transfected NIH 3T3 cells. NIH 3T3 cells were transfected with expression vehicles encoding either GATA6-WT-Flag or GATA6-DBD-mutant-Flag. 48 hours post-transfection, the cells were lysed and total cell lysate was separated on SDS-PAGE. Relative levels of GATA6-Flag or β -actin were evaluated by Western Blot.

Supplemental Figure 3. shRNA targeting FOG1 successfully knocks-down expression of FOG1 protein in *Sufu*^{-/-} MEFs programmed to express either GATA4 or GATA5. Either parental *Sufu*^{-/-} MEFs (lanes 1 and 2) or *Sufu*^{-/-} MEFs programmed to express either GATA4 or GATA5 (lanes 3, 4 and 5, 6, respectively) were infected with lentiviruses encoding both hygromycin resistance and shRNAs targeting either GFP or FOG1, as indicated. After selection of polyclones expressing the shRNA, gene expression was assayed by Western Blot. Note that expression of the 160 kD FOG1 protein is induced in *Sufu*^{-/-} MEFs programmed to express either

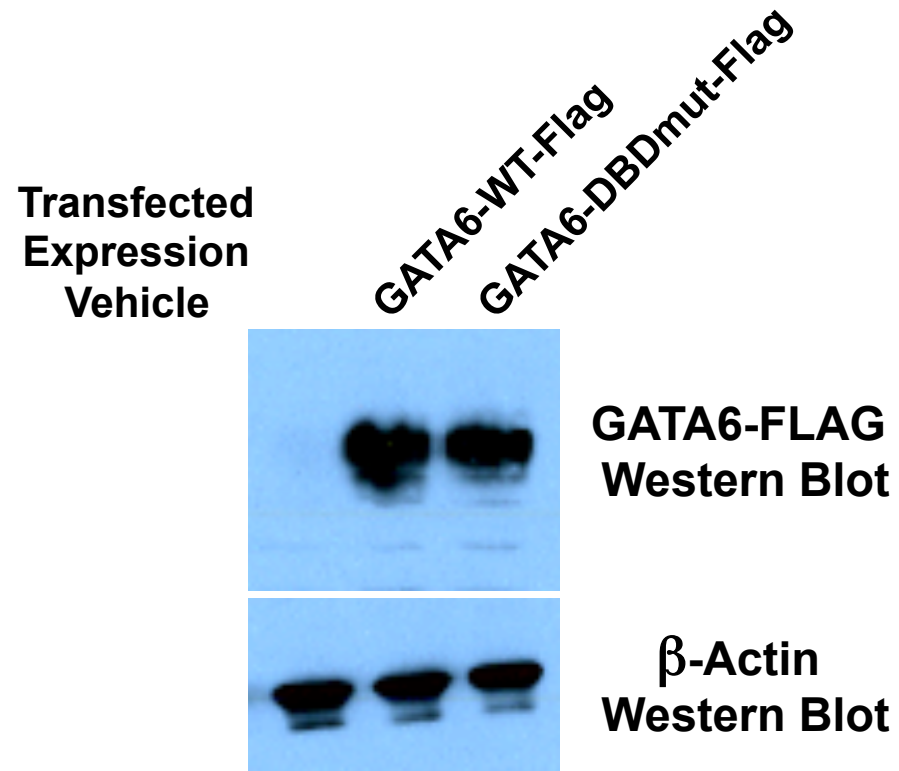
GATA4 or GATA5, and that an shRNA targeting FOG1 RNA significantly decreased expression of this protein.

Supplemental Figure 4. Exogenous GATA4/5 expression in NIH3T3 cells increases the expression of both FOG1 and FOG2. Expression of FOG1/2 in either parental NIH 3T3 cells (CTL) or in polyclones of such cells programmed to express either pOZ-GATA4 or pOZ-GATA5. Gene expression was evaluated by RT-qPCR (relative to TATA Binding Protein; TBP).

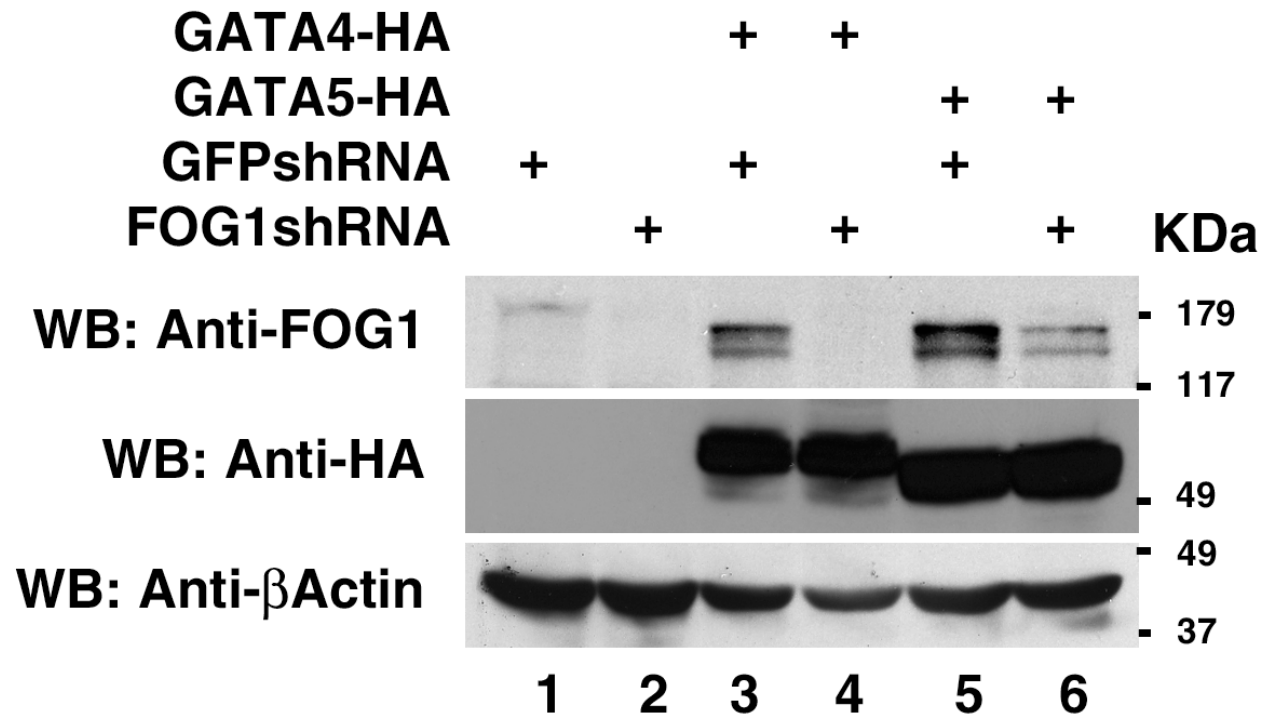
Supplemental Figure 5. (A) GATA factors can repress induction of promyogenic genes by Wnt and Shh in PSM explants. Chicken presomitic mesoderm (PSM) explants were infected with either RCAS-GFP or RCAS-GATA4 and cultured for 5 days in the presence of either Wnt-1 expressing fibroblasts and Shh. Gene expression was evaluated by RT-PCR. **(B) Pax3 blocks induction of GATA4/5/6 by BMP signals.** Chicken presomitic mesoderm (PSM) explants were infected with either RCAS-GFP or RCAS-Pax3, as indicated. BMP4 was added at the beginning of the culture period as indicated and the explants were cultured a total of 5 days. Gene expression was evaluated by RT-PCR. (The results of six independent explants are displayed.)



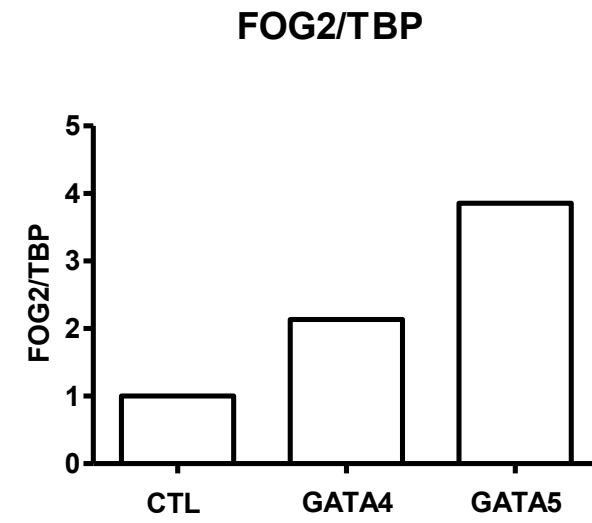
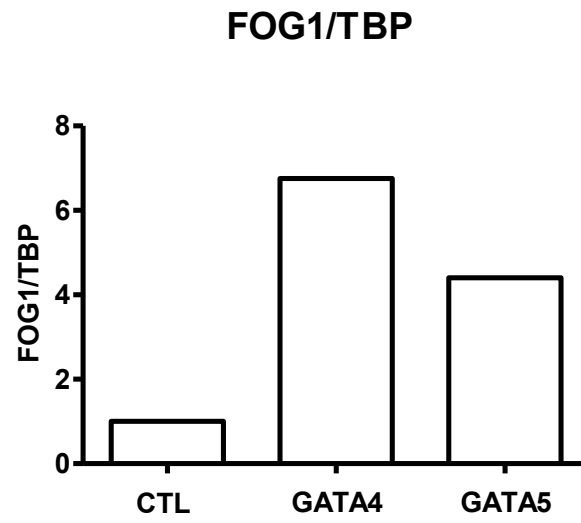
Daoud_Supplemental Fig 2



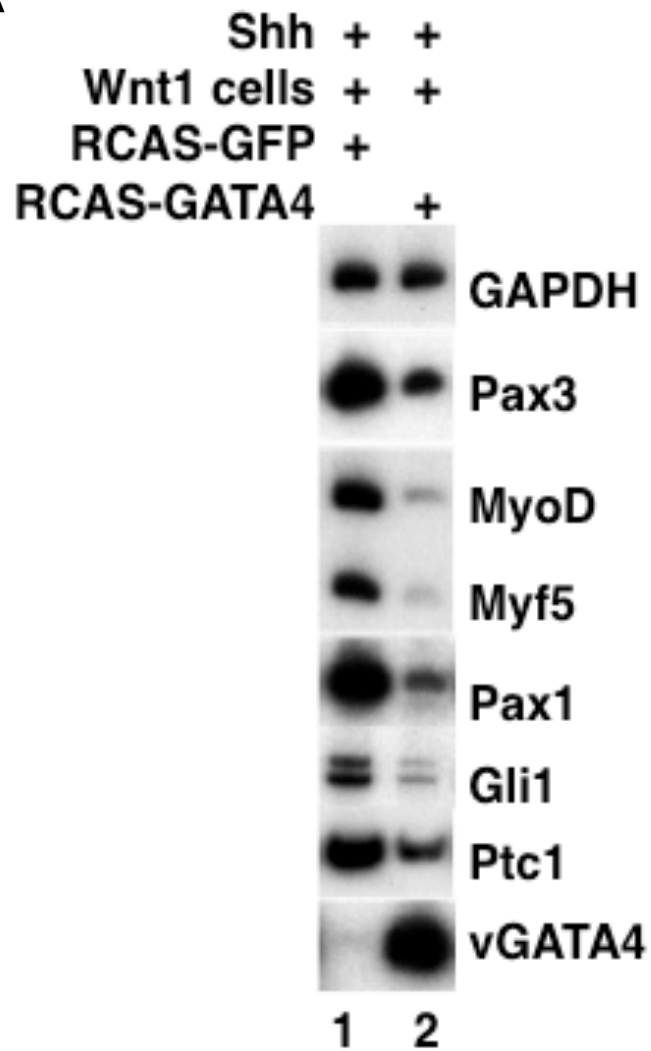
Daoud_Supplemental Fig 3



Daoud_Supplemental Fig 4



A



B

