Supporting Online Material for:

HEDGEHOG SIGNALLING REGULATES MYOD EXPRESSION AND ACTIVITY

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Running title: Hh regulates MyoD expression and activity

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This PDF file includes supplementary:
Figure Legends
Figures S1-S6

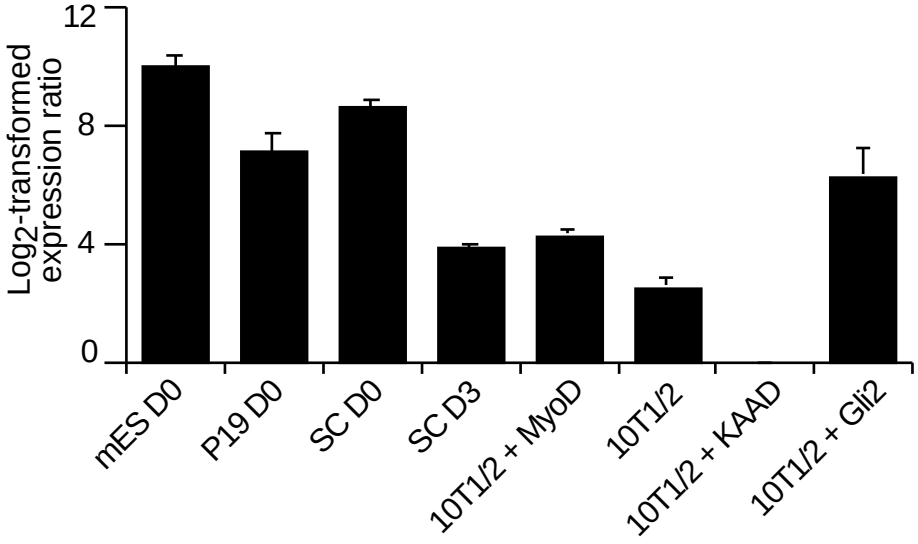

Fig. S1 QPCR analysis of Gli1 mRNA expression in mouse ES (mES), P19, satellite cells (SC) and 10T1/2 fibroblasts transfected with MyoD or Gli2 or treated with or without KAAD-cyclopamine (KAAD). Data is relative to 10T1/2 cells treated with KAAD-cyclopamine. Error bars represent SEM from three biological replicas (n=3). D=day.

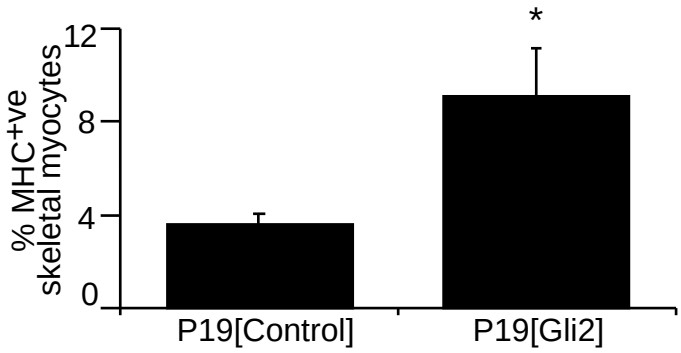

Fig. S2 MHC-positive skeletal myocytes from Fig. 2A were counted in 10 random fields and expressed as % of the total number of nuclei, n=4. In total, 20,000 cells were counted.*p<0.05

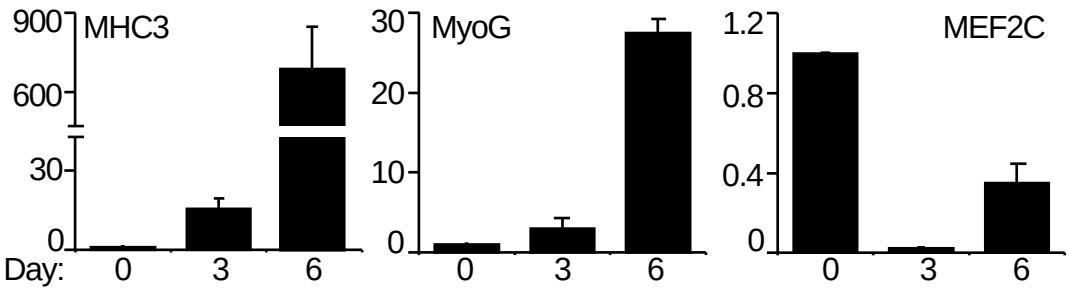

Fig. S3. Freshly isolated SCs were cultured in the absence of growth factors for 6 days. QPCR analysis of the expression of indicated genes in SCs at the time shown. Error bars represent +/- SEM (n=3).

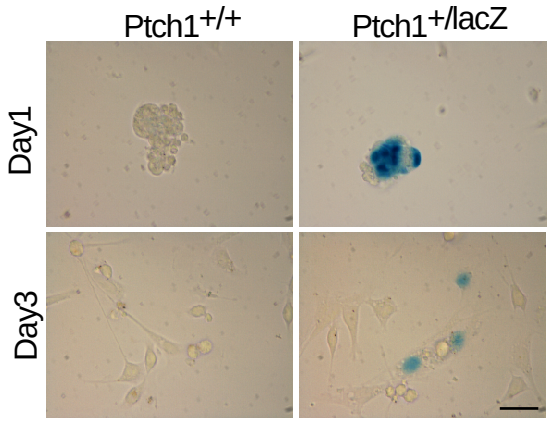

Fig. S4 Freshly isolated SCs from $Ptch1^{+/+}$ or $Ptch1^{+/LacZ}$ mice were cultured in the absence of growth factors and assayed for β-gal activity on day 1 and day 3. Scale bar is 30 μm.

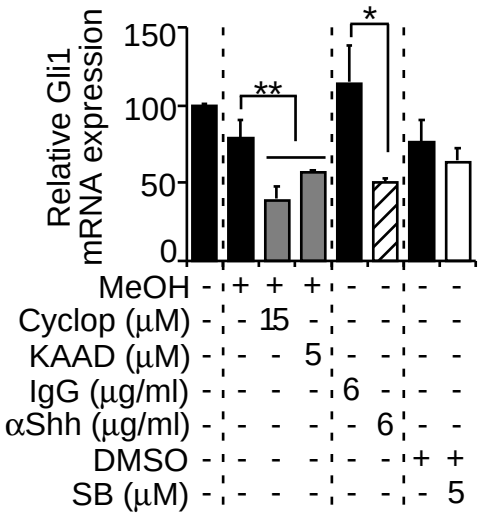

Fig. S5 QPCR analysis of Gli1 mRNA on day 3 of cultured mouse SCs in the presence or absence of cyclopamine (cyclop), KAAD-cyclopamine (KAAD) (grey bars), SB203580 (SB) (white bars) and their respective vehicles (MeOH; methanol) or in the presence of Shh-specific (striped bars) or IgG non-specific antibodies for 3 days. Data from treatment with a specific reagent and its respective vehicle is separated by dashed line and expressed as a percentage of day 3 levels. Error bars represent +/- SEM (n=3). *p<0.05, **p<0.01.

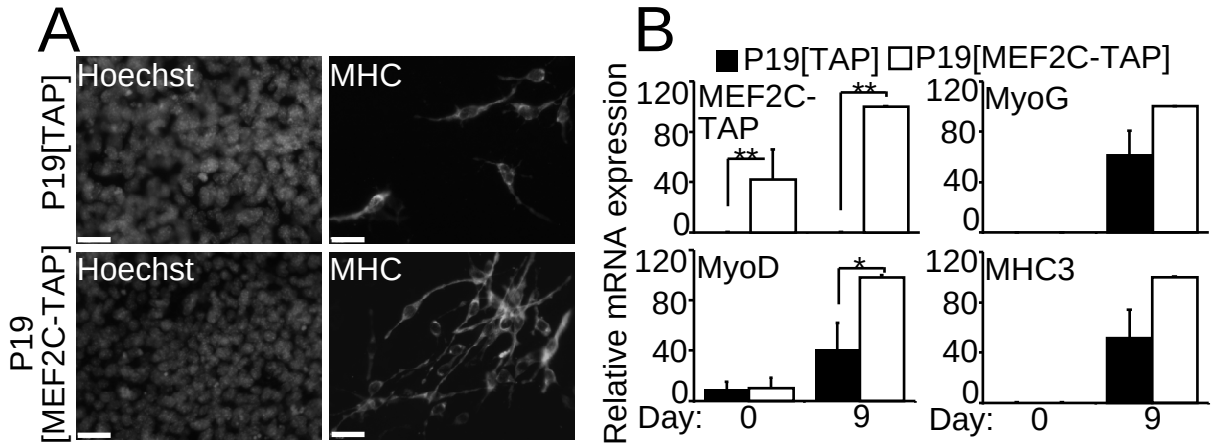

Fig. S6 (A): Day 9 differentiated P19[TAP] and P19[MEF2C-TAP] cells were reacted with MF20 antibodies to detect MHC expression. Nuclei were stained with Hoechst dye. Scale bar is 30 μM. (B): Overexpression of MEF2C upregulates MyoD and MyoG expression while enhancing skeletal myogenesis in P19 EC cells. QPCR analysis of the expression of indicated genes in P19[TAP] (black bars) and P19[MEF2C-TAP] (grey bars) cells at the time shown. Error bars represent +/- SEM from two clonal populations and two biological replicas (n=4), *p<0.05 and **p<0.01.