Fig. S1. Pax3<sup>Cre/+</sup> deletion of Hif1α or Arnt fails to induce embryonic muscle phenotype. (A) Representative images of sections from wholemount X-gal treated E18.5 fetuses. (B) Table lists numbers of surviving mice at different developmental ages by genotype. Representative images of hematoxylin and Von Kossa stains (C) and Slow Myosin IHC staining (D) of E18.5 sections. ** denotes rib bone hypocalcification. Table lists frequency of bone defects across genotypes. (E) Genotyping PCR of Cre and Hif1α alleles performed on genomic DNA from whole embryo and forelimb muscle harvested from E18.5 fetus. Arnt PCR is used as a control. Representative images and quantification of MHC staining (F,G) and PAX7 staining (H) of E18.5 muscle sections. Error bars represent s.e.m. *Not significantly different by Student’s t-test (P>0.05). Scale bars: 20 μm.
Fig. S2. Role of HIF1α in hindlimb ischemia model. (A) Limb perfusion assessed by Diffuse Correlation Spectroscopy before FAL (Pre-Surgery), immediately following FAL (Post-Surgery), 2 days (Day 2) and 7 days (Day 7) following FAL. Perfusion ratio refers to flow in ligated limb to that in non-ligated collateral limb. Error bars represent s.e.m. (B) Western Blot for HIF1α and Actin expression in tissue lysates from EDL muscle before FAL (U) and 2, 4, 7 and 14 days after FAL (Day 2, Day 4, Day 7, Day 14). Lysates from 3-5 mice were assessed at each time point. (C) Relative Myogenin expression was detected in mouse EDL muscle before FAL (Uninjured) and 2, 4, 7 and 14 days after FAL. Group averages were graphed (n=3-12). Error bars represent s.e.m. (D) Genotyping PCR of Cre and Hif1a alleles performed on genomic DNA from primary myoblasts and skin harvested from indicated mice. Arnt PCR demonstrates equivalent DNA template amount across samples. (E) Low-magnification representative images of Dystrophin IF on injured EDL muscle 7 days following FAL. Scale bars: 100 μm. Representative images and quantification of PAX7/Laminin IF (F) and Van Gieson stain (G) on injured EDL muscle 7 days following FAL. Arrows point to PAX7/Laminin/DAPI triple-positive cells. Error bars represent s.d. Scale bars represent 10 μm (PAX7/Laminin IF) or 20 μm (Van Gieson stain). (H) Genotyping PCR of Cre, Hif1a and Arnt alleles performed on genomic DNA from EDL muscle isolated from indicated mice. (I) Western Blot for Hif1α expression in tissue lysates from EDL muscle isolated from indicated mice before FAL (NL) and 2 days after FAL (L). Non-specific bands observed with Ponceau S staining (Ponceau) were used as loading control. *P<0.05, #P>0.05 (Student’s t-test).
**Fig. S3. HIF1α modulates Wnt activity in adult myogenesis.** (A) Relative expression of Hif1a in C2C12 myoblasts transduced with scrambled shRNA (scr) or Hif1a targeting shRNA (shHIF). (B) Relative expression of Axin2 in C2C12 myoblasts that were differentiated and treated with Wnt inhibitors sFRP3 or DKK1 for 48 hours. (C) Representative images and quantification of MHC IF on C2C12 myoblasts transduced with scrambled shRNA (scr) or Hif1a targeting shRNA (shHIF), which were then differentiated for 48 hours in 21% or 0.5% O₂. (D) Representative images and quantification of MHC IF on C2C12 myoblasts transduced with scrambled shRNA (scr) or Hif1a targeting shRNA (shHIF), which were then differentiated and treated with Wnt inhibitors sFRP3 or DKK1 for 48 hours in 21% or 0.5% O₂. Fusion index was calculated to quantify differentiation. Error bars represent s.d. Scale bars: 50 μm. *P<0.05 (Student's t-test).