Supplementary Fig. S2 (Kasper)

(a) Slc2a1

(b) Pfkfb3

(c) Hig1

(d) Vim

(e) Wildtype, normoxia; p300 CH1/CH1, normoxia; p300 CH1/CH1, hypoxia
Supplementary Fig. S2. The ΔCH1 mutation attenuates hypoxia-dependent recruitment of p300 to HIF-binding sites but not to the control gene Vim. a-d, Quantitative ChIP assays using WT and p300ΔCH1/ΔCH1 MEFs treated four hours with normoxia or hypoxia (mean ± S.E.M. N=3, 3 independent experiments). Control (NRS) and specific (anti-CBP, anti-p300) IP antisera indicated. Hypoxia-dependent ChIP signal was determined by subtracting the normoxia signal from the hypoxia signal after normalizing to input DNA signal for HIF-target genes (a-c). Vim is a non-HIF target gene (d). e, Co-immunoprecipitation of HIF1α with CBP in MEFs treated 2 hrs with 100µM dipyridyl is attenuated by the ΔCH1 mutation. Immunoprecipitations were performed as described in Yang et al. (1998) MCB 18:2218-2229, except that buffers for nuclear extracts and IPs were made without EDTA or EGTA using HIF-1α (HI α67) antibody from Novus and CBP A-22 and C-20 antibodies from Santa Cruz.