Supporting Information

Ren et al. 10.1073/pnas.0909570107

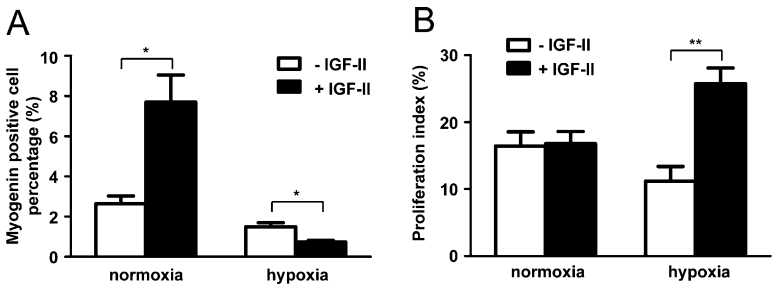

Fig. S1. Hypoxia converts myogenic action of IGF-II into mitogenic action. (*A*) C2C12 myoblasts were induced to differentiate by switching to differentiation medium supplemented with or without IGF-II (300 ng/mL). After culturing under normoxic or hypoxic conditions for 36 h, cells were fixed and stained. Percentage of myogenin-positive cells was quantified. Data are mean ± SE, $n = 3$. *$P < 0.05$. (*B*) C2C12 cells were grown under normoxic or hypoxic conditions for 24 h and pulse labeled with 10 μM BrdU. Proliferation index, defined as percentage of BrdU-positive cells, was determined. Data are mean ± SE, $n = 4$. *$P < 0.05$, **$P < 0.01$.

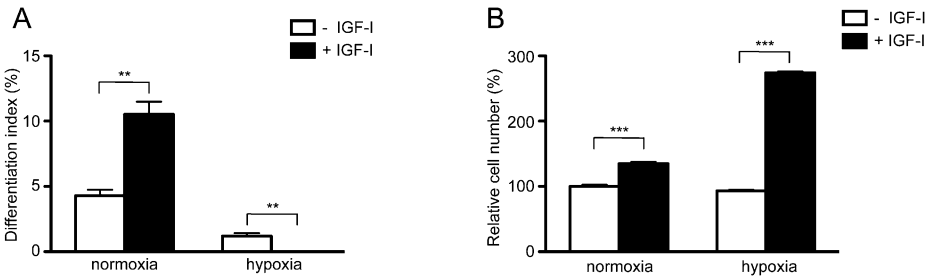

Fig. S2. Hypoxia converts myogenic action of IGF-I into mitogenic action. C2C12 myoblasts were induced to differentiate by adding differentiation medium supplemented with or without IGF-I (300 ng/mL). After culturing under normoxic or hypoxic conditions for 36 h, cells were fixed and stained for MHC and DAPI. Differentiation index was quantified and shown in *A*. Total cell number was quantified and shown in *B*. Values are expressed as relative to that of normoxia control group. Data are means ± SE, $n = 3$. **$P < 0.01$, ***$P < 0.001$.

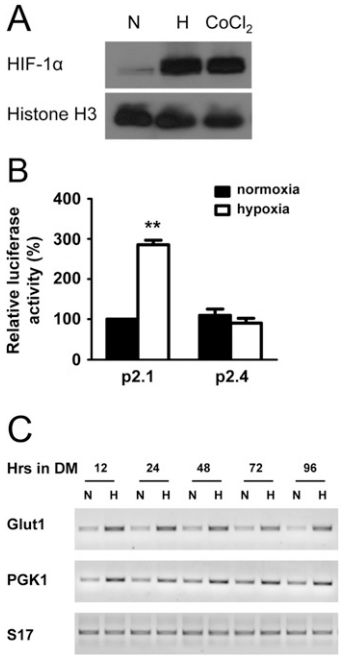

Fig. S3. Hypoxia activates HIF-1–regulated gene expression in differentiating myoblasts. (*A*) Hypoxia increases nuclear HIF-1α accumulation. C2C12 myoblasts were subjected to normoxia, hypoxia, or $CoCl_2$ (100 μM) treatment for 8 h. Nuclear fraction was prepared and analyzed by Western immunoblot using antibodies indicated. (*B*) Hypoxia induces HIF-1-dependent transcription. C2C12 myoblasts were transfected with HIF-1 reporter gene, p2.1, and control p2.4 which has a mutated HRE. Cells were induced to differentiate under normoxic or hypoxic conditions for 2 days. Luciferase activities were measured. Transfection efficiency was normalized by *Renilla* luciferase activity. Data are mean ± SE, $n = 4$. $^{**}P < 0.01$. (*C*) Hypoxia increases expression of HIF-1 target genes. C2C12 cells were induced to differentiate under normoxic or hypoxic conditions. RT-PCR analysis of PGK1 and Glut1 was performed at indicated time points.

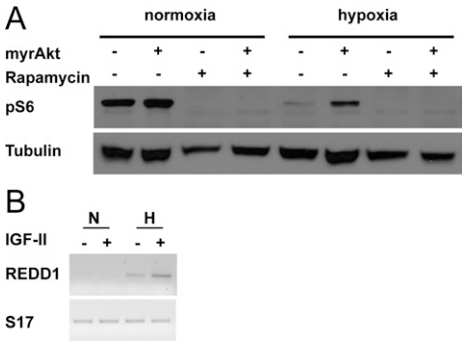

Fig. S4. Inhibition of mTOR activity by hypoxia can be restored by myrAkt expression. (*A*) Effect of overexpression of constitutively active Akt (myrAkt) on mTOR signaling. Cells were transfected with control or myrAkt plasmid and induced to differentiate in presence or absence of rapamycin (200 nM) under normoxic or hypoxic conditions. Cells were lysed and analyzed by Western immunoblot using the indicated antibodies. (*B*) Hypoxia induces REDD1 expression in C2C12 myoblasts. At 36 h after cells were induced to differentiate under conditions indicated, RNA samples were collected and subjected to RT-PCR analysis.

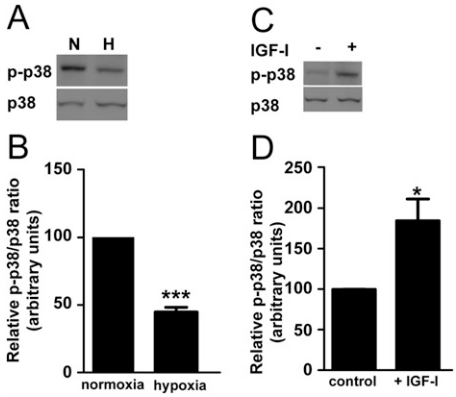

Fig. S5. Regulation of p38 MAPK activity by hypoxia and IGFs. (*A* and *B*) Hypoxia represses p38 MAPK activity. C2C12 cells were induced to differentiate under normoxic or hypoxic conditions for 4 days. Levels of phospho- and total p38 MAPK were measured by Western blot. Representative blot is shown in *A,* and quantified results are shown in *B*. Values are expressed as relative to that of normoxia group. Data are mean ± SE, $n = 6$. $P < 0.0001$. (*C* and *D*) IGF-I activates p38 MAPK in myoblasts. C2C12 cells were serum starved overnight and exposed to IGF-I (50 ng/mL). After 10 min, cells were lysed and ratio of phospho- and total p38 was determined. Data are mean ± SE, $n = 5$. *$P < 0.05$, ***$P < 0.001$.