Epac2a-knockout mice are resistant to dexamethasone-induced skeletal muscle atrophy and short-term cold stress

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Supplementary document 1

- The sequences of the primers used for real-time PCR:

  uncoupling protein-1 (UCP-1; forward, 5'-AGG ATG GTG AAC CCG ACA AC-3' and reverse, 5'-TTG GAT CTG AAG GCG GAC TT-3'), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α; forward, 5'-TAT GGA GTG ACA TAG AGT GTG CT-3' and reverse, 5'-CCA TTG CAA TCC ACC CAG AAG G-3'), CCAAT/enhancer-binding protein beta (C/EBPβ; forward, 5'-GGT TTC GGG ACT TGA TGC A GTG CT-3' and reverse, 5'-CCA CAA CCC CGC AGG AAC-3'), carnitine palmitoyltransferase I b (CPT1b; forward, 5'-CGA GGA TTC TCT GGA ACT GC-3' and reverse, 5'-GGT CGC TTC TTC AAG GTC TG-3'), carnitine palmitoyltransferase II (CPT2; forward, 5'-CAA CTC GTA TAC CCA AAC CCA GCA GAG AAC-3') and reverse, 5'-GTG CCC ATC TTG ATC GAG GAC ATC-3'), Peroxisome proliferator-activated receptor alpha (PPARα; forward, 5'-GCT GTG GAG ATC GGC CTG-3' and reverse, 5'-GCA ACT TCT TCT AAT GTA GCC TAT GTT T-3'), cluster of differentiation 36 (Cd36; forward, 5'-TCC TCT GAC ATT TGC AGG TCT ATC-3' and reverse, 5'-AAA GGC ATT GGC TGG AAG AA-3'), cytochrome c oxidase subunit VIIIb (Cox8b; forward, 5'-GAA CCA TGA AGC CAA GCA CT-3' and reverse, 5'-GCG AAG TTC ACA GTG GTT C-3'), cytochrome complex (CytoC; forward, 5'-TGG CCC CTC CCA
TCT ACA C-3’ and reverse, 5’-ATC CTT GGC TAT CTG GGA CAT G-3’), myogenin (forward, 5’-GGA CTG GAC GCC CTC ATT C-3’ and reverse, 5’-CGC TCT GGT CCC CTG CTT-3’), GAPDH (forward, 5’-TGC ACC ACC AAC TGC TTA GC-3’ and reverse, 5’-GGC ATG GAC TGT GGT CAT GAG-3’).

• Primary antibodies for western blot:
  anti-UCP-1 (Abcam, Cambridge, MA, USA), anti-PGC-1α (Abcam), anti-C/EBPβ (Cell Signaling Technology, Danvers, MA, USA), anti-CPT1b (NOVUS, Littleton, CO, USA), anti-CPT2 (Abcam), anti-PPARα (Abcam), anti-Epac2A (Cell Signaling Technology), anti-Akt (Cell Signaling Technology), anti-phosphorylated Akt (Cell Signaling Technology), anti-mTOR (Cell Signaling Technology), anti-p-mTOR (Cell Signaling Technology), anti-eukaryotic initiation factor 4E (eIF4E; Cell Signaling Technology), anti-p-eIF4E (Cell Signaling Technology), anti-Glut4 (Cell Signaling Technology), anti-p-Stat3 (Cell Signaling Technology), anti-Stat3 (Cell Signaling Technology), anti-atrogin 1 (Abcam), anti-MuRF (Abcam), anti-myogenin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-GAPDH (Cell Signaling Technology).
Fig. S1. (A) pPKA protein expression in iBAT before and after cold exposure. (B) pPKA protein expression in gastrocnemius muscle before and after 7-day dexamethasone treatment.
*p<0.05, **p<0.01 compared to WT; #p<0.05 compared to WT treated with cold or dexamethasone stress. For details, see the main text.
Fig. 2S. Triglyceride content (TG) of iBAT before and after cold stress. **p<0.01 compared to KO at ambient temperature. For details, see the main text.
Fig. S3. Expression of Epac2a protein in iBAT and gastrocnemius muscle. WT and Epac2a-KO mice were exposed to cold or dexamethasone stress. For details, see the main text.