To confirm the specificity of the anti-PGC-1α antibody (Abcam, Cambridge, UK) used in our study a competitive assay was performed in presence of 0 or 1.5 μg/mL of the synthetic human PGC-1α peptide (diluted 1 μg/mL in 4% BSA in tris buffered saline (TBS) with 0.1 % of Tween 20 (BSA blocking buffer)) (Abcam, Cambridge, UK) pre-incubated with the anti-PGC-1α antibody (diluted 1:500 in BSA-blocking buffer) overnight at 4ºC. PGC-1α protein expression in human skeletal muscle (deltoid and vastus lateralis), mouse skeletal muscle (gastrocnemious) and in C2C12 cells protein extracts, was analyzed by western blot with the pre-incubation solution (Supplementary Figure 1C). Anti-alpha-tubulin antibody was used as loading control.

Our results showed that the PGC-1α antibody was able to detect a single band with an estimated molecular weight of 110 KDa in the four protein extracts used (left side of the Figure 1). The predicted band size for PGC-1α is 91 KDa and we have obtained an observed band size for PGC-1α of approximately 110 KDa. It is important to take into consideration that western blotting is a technique that separates proteins based on size - in general, the smaller the protein the faster it migrates through the gel. However, migration is also affected by other factors and in fact, the actual band size observed may differ from that predicted. Common factors include: post-translational modification (e.g. phosphorylation, glycosylation etc, which increase the size of the protein), post-translation cleavage (e.g. many proteins are synthesized as pro-proteins, and then cleaved to give the active form, e.g. pro-caspases), splice variants (alternative splicing may create different sized proteins from the same gene), relative charge (the composition of amino acids (charged vs. non-charged)), multimers (dimerization of a protein).

The competitive assay with synthetic human PGC-1α peptide as a competitive blocker of the antigen-antibody interaction showed that the antibody was able to bind specifically to the ~110 KDa band (right side of the Figure 1). This implies that the PGC-1α band detected in all of the protein extracts used share a common epitope with the synthetic human PGC-1α blocking peptide, which contains an amino acid sequence within residues 750 of the C-terminus of human PGC-1α.
Legend to figure

The anti-PGC-1α antibody recognized specifically the PGC-1α band detected in muscle protein extracts (human and mouse) and in whole cell C2C12 protein extract. This figure shows a representative Western blot analysis with different preincubation solutions (0 μg/mL or 1.0 μg/mL of SH PGC-1α) and with a monoclonal mouse anti-alpha-tubulin antibody as a loading control in human deltid muscle (HMD) (40 μg), human vastus lateralis muscle (HMV) (40 μg), mouse gastrocnemious muscle (MMG) (40 μg) and C2C12 cells whole protein extracts (40 μg).