Supplemental Materials

JPHYSIOL/2010/202432 Eukaryotic initiation factor 2Bε (eIF2Bε) induces cap-dependent translation and skeletal muscle hypertrophy

David L Mayhew, Troy A Hornberger, Hannah C Lincoln, and Marcas M Bamman

As indicated in the Discussion, page 19, we attempted to assess eIF2Bε S539 phosphorylation status in human muscle lysates but the immunoblotting attempts with various primary phospho-antibodies failed to yield satisfactory results. Here we show sample blots using two different primary antibodies on human muscle lysates. In Figure 1A, we blotted human muscle lysates with Ab #4775 (Abcam; Cambridge, MA). The protein of interest, eIF2Bε, migrates to approximately 85 kD. The band found with this antibody was near 150 kD. No band near 85 kD appeared even after overexposing the blot, i.e., extending the exposure time beyond the point of saturation for the 150 kD band. We also blotted human muscle lysates with Ab #3596 (Cell Signaling Technology; Danver, MA) (Figure 1B). In this case, despite manipulating the blocking conditions, a substantial amount of non-specific binding was repeatedly noted. According to the company website, this antibody is no longer available.

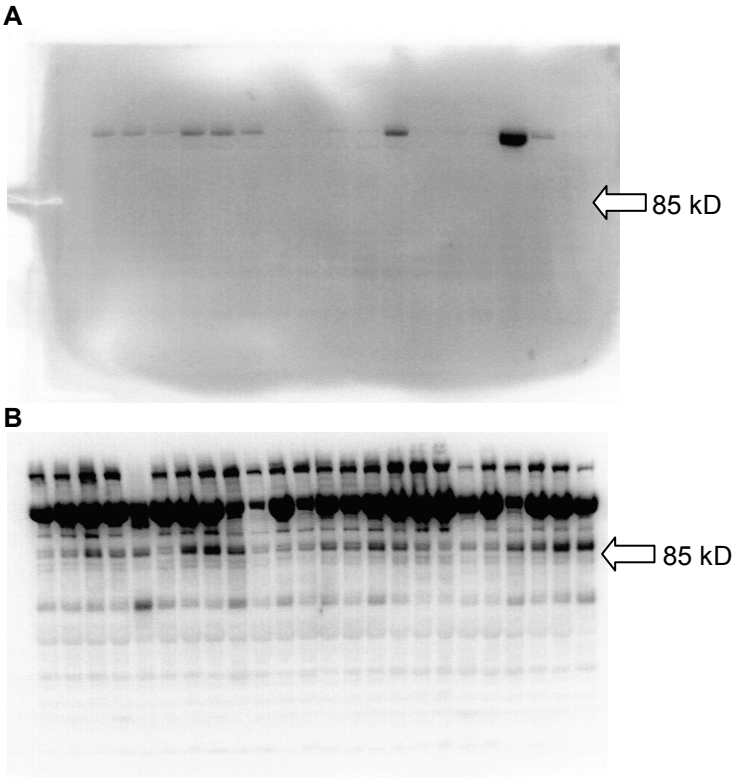

Figure 1. Our attempts to assess S539 phosphorylation status of eIF2Bε in human muscle lysates. Panel A = Ab #4775 (Abcam; Cambridge, MA). Panel B = Ab #3596 (Cell Signaling Technology, Danver, MA). No distinct band was found at the predicted molecular weight (85 kD) using either antibody.