Camarata et al., http://www.jcb.org/cgi/content/full/jcb.200511109/DC1

Supplemental results

Polyclonal antibodies specifically recognize LMP4

In order to better understand the function of LMP4 binding to Tbx5, we generated LMP4-specific antibodies. To avoid cross-reactivity, we compared protein sequences of the closely related members of the PDZ-LIM protein family and identified a 17-amino-acid peptide from the proline-rich (P-rich) region, between the PDZ and LIM domains, to be highly conserved among the LMP protein homologues from different species but not to the related ENH proteins (Fig. S1 A). The respective chicken LMP4 peptide was used for the generation of specific antisera in rabbits, followed by affinity purification using the peptide, and then tested for specificity against LMP4 proteins expressed in both *Escherichia coli* and COS-7 cells (Fig. S1, B and C). On Western blots, the antibodies recognized a recombinant form of LMP4 containing the PDZ and P-rich domains but did not interact with the LIM domains or the GST tag (Fig. S1 B). Furthermore, the LMP4 PDZ/P-rich protein band was absent after preincubation of the serum with the peptide used for immunization, demonstrating exclusive specificity for this antigen (Fig. S1 B, lane 6). The LMP4 antibodies also recognized full-length LMP4 proteins expressed from chicken and zebrafish cDNAs transfected into COS-7 cells (Fig. S1 C, lanes 1 and 2). Based on our Western blot data with recombinant LMP4 proteins expressed in prokaryotic and eukaryotic cells, the generated antiserum appeared to be specific for its LMP4 target. LMP4 is a member of a larger family of PDZ-LIM proteins, and we wished to ensure that the serum would not interact with a related family member. For this reason, the evolutionarily closest PDZ-LIM protein from mouse, ENH1 (Nakagawa et al., 2000), recombinant protein was produced in *E. coli*. Similar to LMP4, ENH1 contains conserved N-terminal PDZ and three C-terminal LIM domains. However, the proline-rich regions of mouse ENH1 and chicken LMP4 vary significantly in sequence (Fig. S1 A). In Western blot analyses, the LMP4 antiserum did not cross-react with any portion of mouse ENH1 (Fig. S1 B). Additionally, preimmune serum did not react with the *E. coli* or COS-7 cell lysates (unpublished data). Equal protein loading was determined by either probing for GST or by BCA assay (unpublished data). Thus, the polyclonal antibody specifically recognizes LMP4 and does not cross-react with the highly related PDZ-LIM protein family member ENH1.