



Supplementary Figure 4 Specificity of anti-phospho-threonine1939-MyH9. a. To ensure the specificity of the antiserum raised against the mouse MyH9 RIVRKGTGDCSDE phosphopeptide, lysates from untreated, carbachol- or PMA- treated rat basophilic leukemia cells (RBL-2H3m1) were immunoblotted with the anti-pThr1939-MyH9 and then stripped and reblotted with anti-MyH9. The phosphopeptide used as an immunogen is identical between mouse and rat MyH9 sequences. The same lysates were also probed with anti-pSer-MyH9. Carbachol stimulation induces threonine and serine phosphorylation of MyH9, while PMA stimulation only induces serine phosphorylation of MyH9, which is recognized specifically by the pSer-MyH9 antibody.
b. To further verify the specificity of the antibody, MyH9 was immunoprecipitated from lysates of untreated or CD3-stimulated DO.11.10 T cell blasts. The MyH9 immunoprecipitates were then immunoblotted with anti-pThr1939-MyH9, stripped and reblotted with anti-MyH9 and finally stripped and reblotted with a generic anti-phospho-threonine antibody.