Supplementary Fig. 1: Detection of the interaction of ZAP-70-YFP and CD3ε or ζ (lysate: 2×10^5 ZAP-70-YFP-expressing 3a9 cells/20μl). Lysates were prepared from resting (−PV) or pervanadate-treated (+PV) cells. (a) Detection by FCCS. Antibodies used for indirect immunolabeling were: hamster anti-CD3ε (ε) and Cy5-labeled anti-hamster, or mouse anti-CD3ζ and Cy5-labeled anti-mouse. Average curves of 10 repeated measurements in one representative experiment are shown. (b) Reproducibility of cross-correlation amplitudes for the detection of the interaction between ZAP-70-YFP and CD3ε or CD3ζ. Averages of data from 3 independent experiments are shown. Anti-CD3ε or -ζ primary and Cy5-conjugated
secondary antibodies were used for labeling (black columns). In controls, primary antibodies were omitted (white columns). Optionally, ITAM- (I) or pITAM-peptide (pI) was added to a final concentration of 1 µM. Within each of the 3 data sets, values were normalized to the cross-correlation amplitude of the lysate of pervanadate-treated cells in the presence of anti-CD3ε and in the absence of peptide. Error bars correspond to the error propagation of the standard deviations of the single experiments.

(c) Interaction of ZAP-70-YFP with CD3ε detected by mass-tag FCS. pITAM (pI) was used at a concentration of 2 µM. Lysates were incubated with anti-CD3ε antibody (ε) or a non specific isotype (iso) and protein G beads. Normalized average autocorrelation curves from ten repeated measurements of one representative experiment. Pervanadate treatment by itself led to a shift of the ZAP-70-YFP autocorrelation due to incorporation of the protein into complexes. For the lysate of pervanadate-treated cells, a pronounced additional shift was observed in the presence of anti-CD3ε antibody and protein G beads. 130 µl of the same samples were subjected to immunoprecipitation of CD3ε and Western Blotting for ZAP-70.

(d) Influence of the addition of primary antibodies with and without mass-tag on the relative diffusion time of ZAP-70-YFP-containing particles. Means and standard deviations of ten repeated measurements of one representative experiment are shown.