

Supplementary Figure 2. Effect of SHP-2(C/S) expression on phosphorylation and association of signaling molecules with Gads and SLP-76. Jurkat T cells were transiently transfected with an empty vector (Vector) or an expression vector encoding SHP-2(C/S) or SHP-2 WT. Cells were stimulated for 5 min by anti-CD3 crosslinking. Postnuclear cell lysates were pre-cleared on protein G, and then immunoprecipitated with either (a) anti-Gads or (b) anti-SLP-76 antibody. Immunoprecipitates were separated by SDS/PAGE and immunoblotted for phosphotyrosine. (c) Jurkat cells (wild type) or SLP-76-deficient cells (J.14) were stimulated by anti-CD3 crosslinking for 5 min. Cell lysates were immunoprecipitated with antibody to Gads. Immune complexes were resolved on SDS-PAGE

and blotted with anti-phosphotyrosine, and the membranes were stripped and reprobed with antibodies to the indicated proteins.