Supporting Information Figure 1

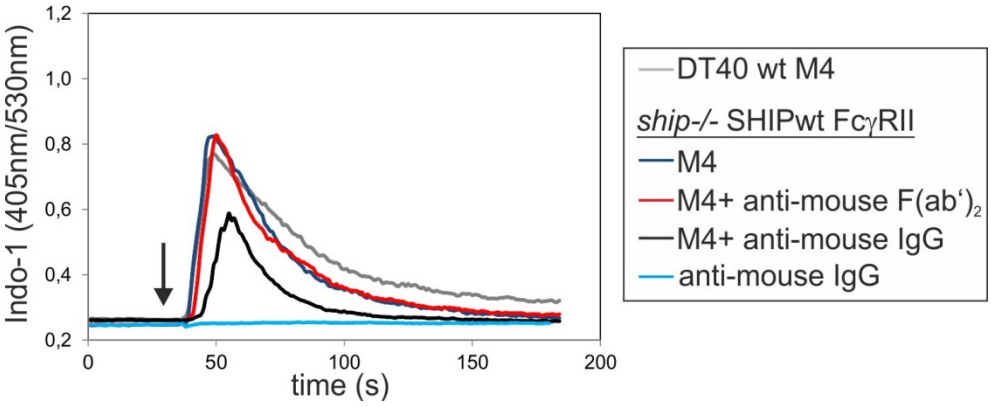

Figure 1. BCR/FcγRIIb coligation attenuates BCR-induced Ca^{2+} mobilization. Ratiometric Ca^{2+} flux profiles of SHIP-deficient DT40 B cells expressing FcγRIIb together with EGFP-tagged SHIP. For stimulation cells were treated at the indicated time point with either mouse anti-chicken IgM (M4; blue line) or M4 together with $F(ab')_2$ fragments of anti-mouse (red line) to trigger the BCR alone. Treatment with M4 together with anti-mouse IgG was used to coengage the BCR and the FcγRIIb (black line). Treatment with anti-mouse IgG and M4-stimulated DT40 wild-type B cells served as controls (light blue and grey lines, respectively).

Supporting Information Figure 2

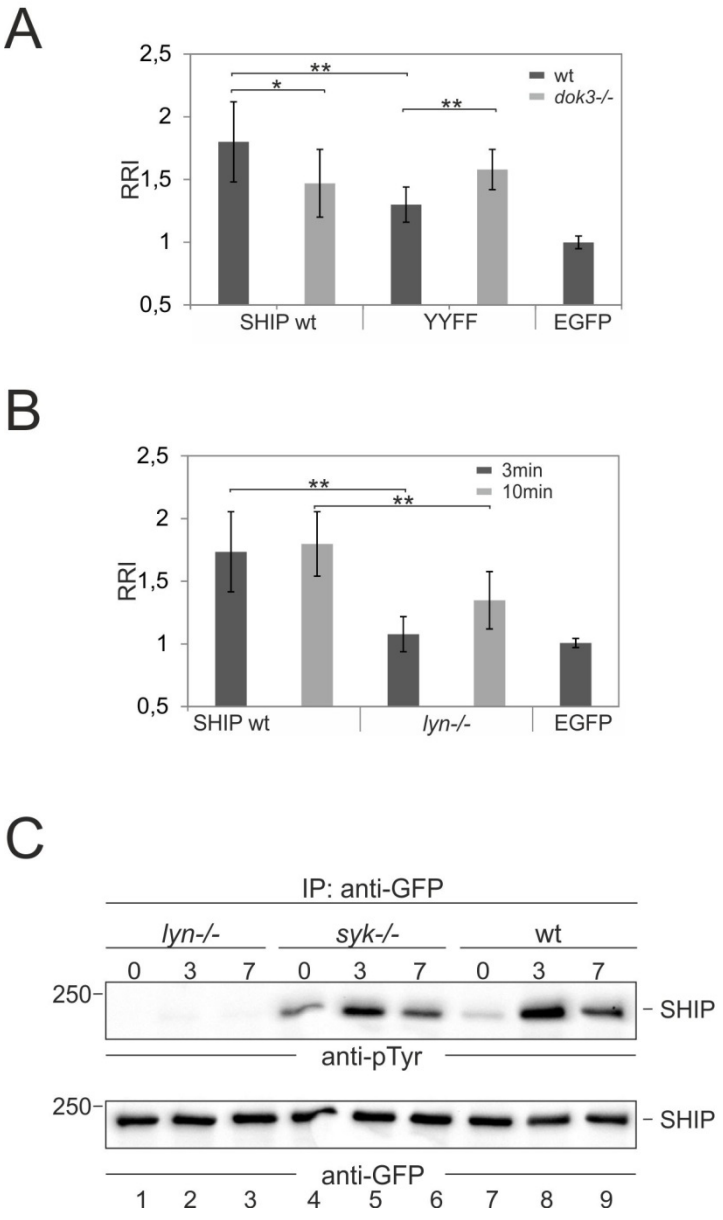

Figure 2. Lyn-dependent phosphorylation of SHIP promotes its BCR-induced plasma membrane recruitment. (A) Wild-type (dark grey bars) or Dok-3-deficient DT40 B cells (light grey bars) were transfected with constructs encoding EGFP-tagged versions of wild-type SHIP or the SHIP variant Y917,1020F (YYFF), respectively, and analyzed by confocal laser scanning microscopy (LSM). EGFP-expressing wild-type DT40 B cells served as control. Images from 20 cells were taken before and after BCR stimulation and the ratios of the mean fluorescence signal intensities at the plasma membrane and in the cytosol were determined by the ImageJ software. The Relative Recruitment Index (RRI), defined by the quotient of the

ratio values after and before stimulation, was plotted as bar diagram (± SD). The statistical significance was evaluated by student's t test (*p<0,01; **p<0,001). (B) Wild-type or Lyn-deficient DT40 B cells expressing EGFP-tagged SHIP or EGFP alone were analyzed by confocal laser scanning microscopy as described in (A). Dark and light grey bars indicate the RRIs after 3 and 10 minutes of stimulation, respectively (** p<0,001). (C) Lyn-deficient (lanes 1-3), Syk-deficient (lanes 4 to 6), and DT40 wild-type B cells (lanes 7 to 9) expressing EGFP-tagged SHIP were left untreated (0; lanes 1, 4, 7) or stimulated via their BCR for 3 or 7 minutes, lysed and subjected to anti-GFP immunopurification. Obtained material was analyzed by anti-phosphotyrosine (pTyr) or anti-GFP immunoblotting (upper and lower panel, respectively). Relative molecular mass of marker protein is indicated on the left in kDa.

Supporting Information Figure 3

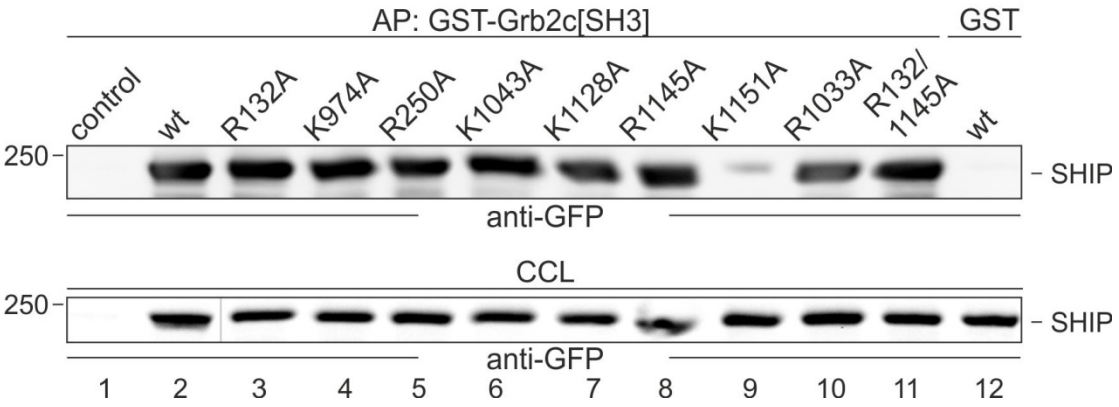

Figure 3. The C-terminal SH3 domain of Grb2 interacts with a specific proline-rich motif in SHIP (^{1146}PPLPVK^{1151}). SHIP-deficient DT40 B cells were retrovirally transduced with constructs encoding EGFP alone, a EGFP-tagged version of wild-type SHIP (lanes 2 and 12), or variants with disrupted SH3 domain-binding motifs due to K/R-to-A amino acid exchanges in the indicated positions (lanes 3 to 11). Cleared cellular lysates (CCL) were subjected to affinity purification (AP) using a GST fusion protein encompassing the C-terminal SH3 domain of Grb2 (lanes 1 to 11), which has previously been reported to mediate interaction with SHIP. GST alone served as control (lane 12). Obtained materials or cleared cellular lysates or cleared lysates (upper and lower panel, respectively) were analyzed by anti-GFP immunoblotting. Relative molecular mass of marker proteins is indicated on the left in kDa.

Suppporting Information Figure 4

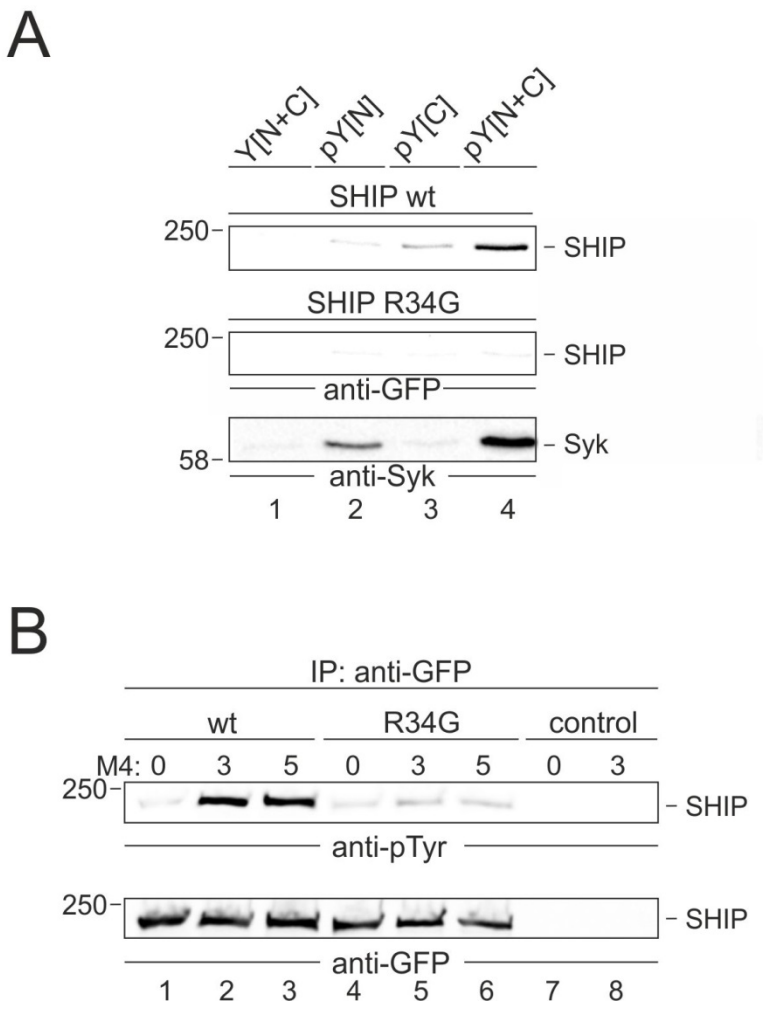

Figure 4. SHIP interacts with phosphorylated ITAMs and the SH2 domain of SHIP is required for its BCR-induced tyrosine phosphorylation. (A) SHIP-deficient DT40 B cells expressing EGFP-tagged versions of wild-type SHIP (upper panel) or a SHIP variant with a dysfunctional SH2 domain (R34G; middle panel) were lysed and subjected to affinity purifications using biotinylated peptides resembling the intracellular part of Igα in non-phosphorylated (Y[N+C]; lane 1) or phosphorylated configurations (N- or C-terminal ITAM phosphotyrosine, pY[N] and pY[C], lanes 2 and 3, respectively; dual ITAM phosphorylation, pY[N+C], lane 4). Immunoblot analysis of obtained proteins was performed using antibodies to GFP (upper panels) or Syk (lower panel). (B) DT40 B cells described in (A) (lanes 1-6) and transfectants with empty vector as control cells (lanes 7-8) were left untreated (0; lanes 1, 4, and 7) or stimulated via their BCR for the indicated time points. After lysis SHIP proteins

were immunopurified using anti-GFP antibodies and subjected to anti-phosphotyrosine (pTyr) or anti-GFP immunoblotting (upper and lower panel, respectively). Relative molecular mass of marker proteins is indicated on the left in kDa.