Figure 1S. B cell proliferation in response to BCR stimulation. B cells isolated from healthy donors and patients with and without cGVHD, stained with CFSE and stimulated with (A) α-IgM (10 µg/mL) or (B) α-IgM + α-IgG (10 µg/mL) for 6 days. (A-B) Left: representative histograms of B cell proliferation from patients without cGVHD (gray area, thin line) and with cGVHD (white area, bold line). Right: frequency of B cell proliferation quantified in healthy donors, (HD, filled triangles) patients without cGVHD (-cGVHD, open circles) and with cGVHD (+cGVHD, filled squares).
Figure 2S. BCR-driven phosphorylation of BLNK and Syk in CD27+ and CD27- B cells from healthy donors. Phosphorylation of BLNK (tyrosine 84) and Syk (tyrosine 348) in CD27+ and CD27- B cells (CD20+) stimulated with α-IgM (5 µg/mL) or PBS as a control as indicated for 5 min. (A) Top: representative dot plots of pBLNK in CD27+ B cells from healthy donors. Numbers indicate frequency of CD20+ CD27+ B cells that are pBLNK+. Bottom: frequency of BLNK phosphorylation in CD27+ B cells quantified in healthy donors (n = 4). (B) Top: representative dot plots of pBLNK in CD27- B cells from healthy donors. Numbers indicate frequency of CD20+ CD27- B cells that are pBLNK+. Bottom:
Figure 3S. Surface expression of IgD and IgG is similar in CD27- and CD27+ B cells from patients with and without cGVHD. Flow cytometric analysis of PBMCs for surface (A) IgD and (B) IgG expression as indicated. Isotypes are depicted as thin gray lines. Left: representative histogram of CD27- B cells (CD19+ CD20+) from a patient without cGVHD (gray area, thin line) and with cGVHD (white area, bold line). Center: representative histogram of CD27+ B cells (CD19+ CD20+) from a patient without cGVHD (gray area, thin line) and with cGVHD (white area, bold line). Right: frequency of (A) IgD or (B) IgG surface expression on CD27- and CD27+ B cells (CD19+ CD20+) quantified in patients without cGVHD (-cGVHD, n = 5, open circles) and with cGVHD.
(+cGVHD, n = 7, filled squares). IgD: ANOVA, p < 0.0001; * p < 0.05. IgG ANOVA, p < 0.0001; * p < 0.05. Data are median +/- range pooled from 2 independent experiments.
Figure 4S. R406 inhibits phosphorylation of Syk and BLNK in B cells from a healthy donor ex vivo. B cells purified from a healthy donor, treated with R406 (0, 0.01, 0.1, 1 and 10 µM) for 30 min and stimulated with α-IgM (50 µg/mL) for 5 min as indicated. (A) Frequency of Syk phosphorylation (tyrosine 348). (B) Frequency of BLNK phosphorylation (tyrosine 84). (C) Proliferation of B cells purified from a healthy donor, stained with CFSE, treated with R406 (0, 0.01, 0.1, 1 and 10 µM) for 30 min and stimulated with α-IgM (50 µg/mL) for 6 days as indicated.