**Figure EV1. Synthetic monovalent antigens activate B1-8 splenic B cells.**

A, B Schematic illustration of the synthetic monovalent antigens. C–E Calcium flux measured by FACScan for splenic B cells isolated from B1-8 transgenic mice after stimulation with (C) 1NIP-pep (80 nM) with or without 1% FCS in the medium; (D) 1NIP-pep (80 nM) (with or without the staining of anti-B220); (E) 1NIP-DNA (80 nM) and control DNA (same sequence, without NIP, 80 nM). The addition of the stimuli to the cells is indicated by arrows. Data are representative of three independent experiments.

**Figure EV2. Monovalent antigens induce weak phosphorylation of AKT, ERK and Igα.**

Western blot analysis of AKT, ERK and Igα phosphorylation for B1-8 splenic B cells upon 1- and 5-min treatment with different stimuli. Western blot analysis of Syk and β-actin expression functions as a loading control. Data are representative of three independent experiments.

Source data are available online for this figure.

**Figure EV3. Monovalent antigen-induced BCR signalling is Lyn dependent.**

A, B Calcium flux measured by FACScan for splenic B cells isolated from B1-8 and Lyn-deficient B1-8 mice upon stimulation with (A) NIP15-BSA (30 pM) or (B) 1NIP-pep (80 nM). Stimuli were added at time points indicated by the arrows. Data are representative of three independent experiments.
Figure EV4. The kinase activity of Lyn is crucial for monovalent antigen-induced calcium response and BCR opening.

A, B Calcium flux measured by FACScan for splenic B cells isolated from B1-8 transgenic mice after the stimulation with NIP15-BSA (30 pM), 1NIP-pep (80 nM), Ac146Fab (25 nM) or Ac38Fab (25 nM) after 45-min incubation with 1 mM PP2. Arrows indicate the addition of the stimuli to the cells.

C Representative microscopic images showing Fab-PLA results monitoring the BCR proximity for IgM-BCR and IgD-BCR on untreated or treated cells. PLA signals are shown as red dots and nuclei were visualized by DAPI staining. Scale bar: 5 μm.

D Quantified PLA results presented as box plots. The median values are highlighted as thick lines and the whiskers represent the minimum and maximum value. PLA signals (dots/cell) were counted from at least 100 cells for each sample. *P*-values were calculated by Kruskal–Wallis one-way analysis of variance (ANOVA).

Data information: Data are representative of three independent experiments.
Figure EV5. Monovalent antigen induced calcium signalling only in IgM-BCR-expressing cells.

A. FACScan analysis of 3046S cells transduced with IgM-BCR components. The GFP-positive gate represents the transduced cells (3046SM).

B, C. Calcium flux measured by FACScan for the transduced 3046SM cells upon stimulation with (B) 1NIP-Pep (80 nM) or (C) Ac146 Fab (25 nM). The non-transduced cells in the same tube function as internal control.

D–F. Calcium flux measured by FACScan for 3046SM and 3046SD cells upon stimulation with (D) anti-HC (2 μl/ml), or (E) 200 nM or (F) 40 nM of 1NIP-pep.

Data information: Arrows indicate the addition of the stimuli to the cells. Data are representative of a minimum of three independent experiments.