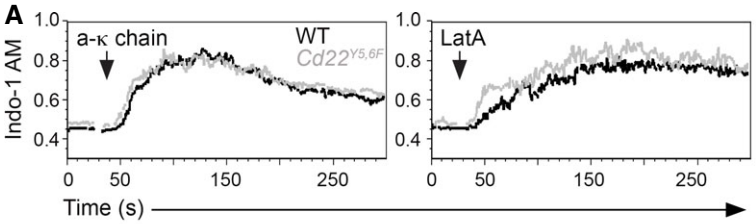
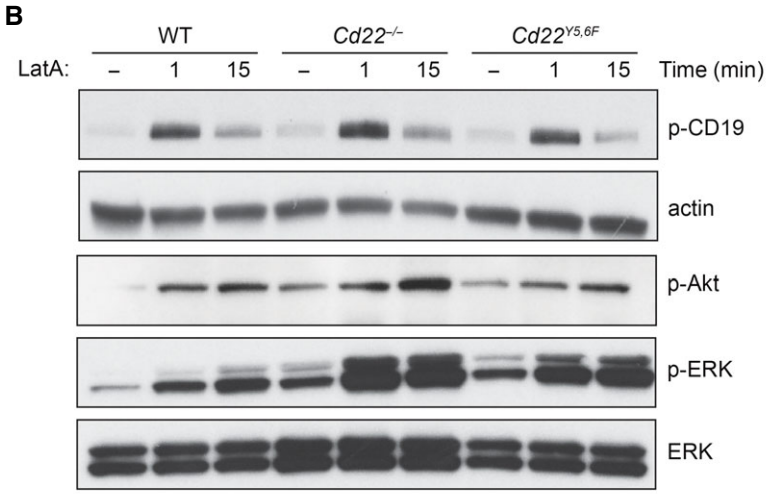
Expanded View Figures





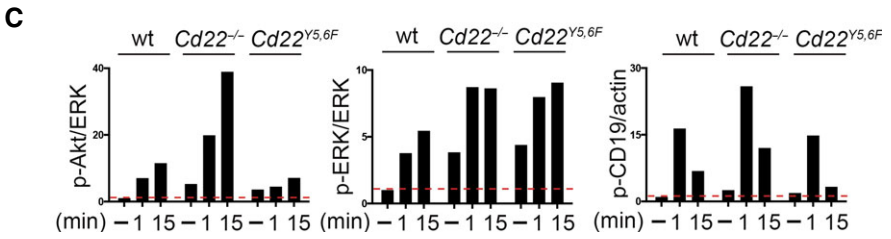


Figure EV1. BCR signalling in *Cd22^{Y5,6F}* B cells upon cytoskeleton disruption and ligand-dependent stimulation.

A Wild-type and *Cd22^{Y5,6F}* primary B cells were treated with 5 μg/ml anti-kappa or 1 μM LatA and intracellular calcium was measured by flow cytometry.

B, C Wild-type, *Cd22$^{-/-}$* and *Cd22^{Y5,6F}* primary B cells were treated with vehicle control (–) or 1 μM LatA for the indicated times. Cells were lysed and analysed by SDS–PAGE followed by immunoblotting with phospho-CD19 (p-CD19), phospho-Akt (p-Akt), phospho-ERK (p-ERK) and actin or total ERK as loading controls. The intensity of phosphorylated proteins, normalized to actin or ERK, was referred to the unstimulated sample of the wild-type cells, set as 1.

Source data are available online for this figure.

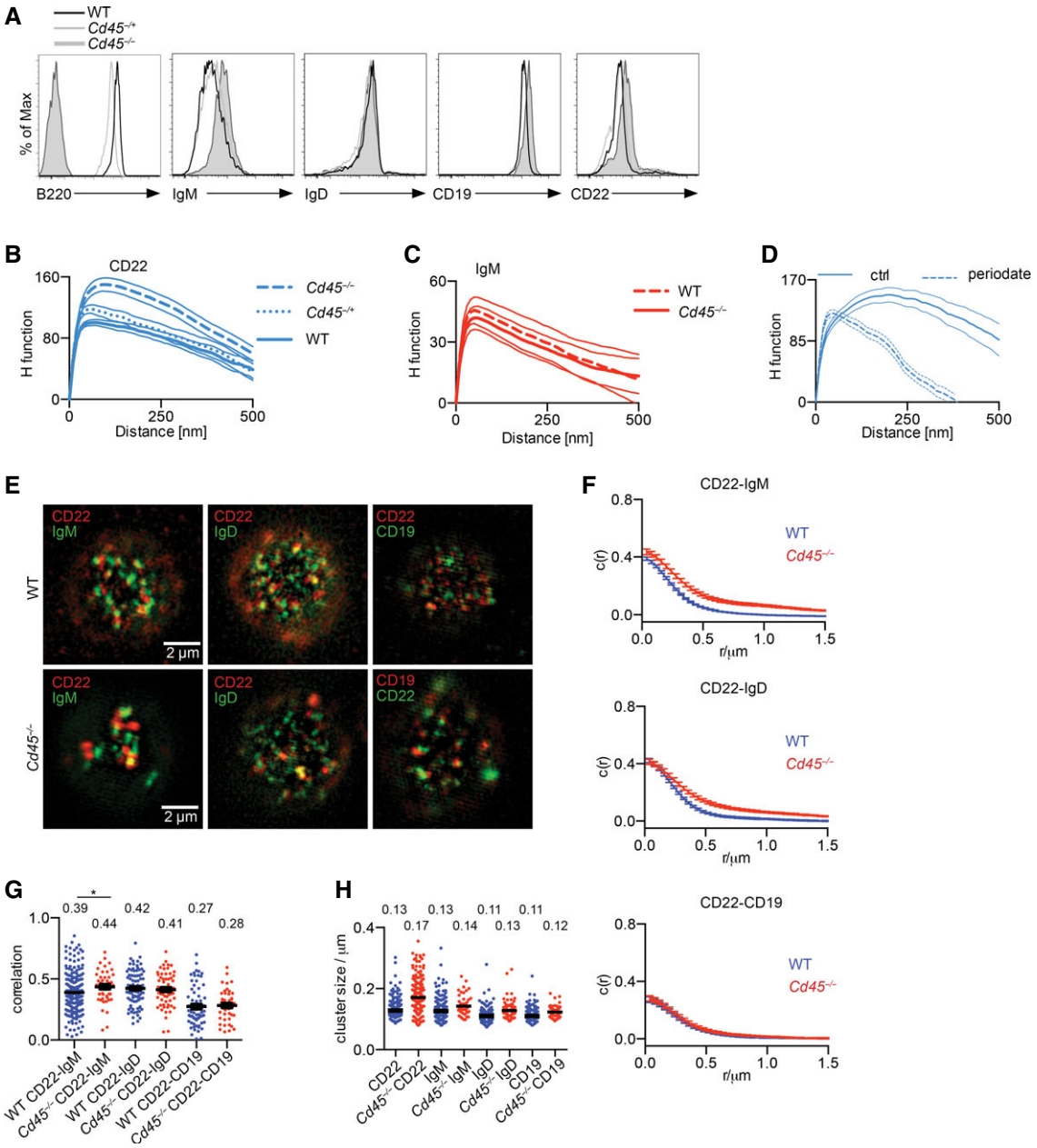


Figure EV2. CD45 is required for organizing CD22.

A Expression levels of cell surface proteins were measured by flow cytometry. Wild-type, $Cd45^{-/+}$ and $Cd45^{-/-}$ primary B cells were labelled with antibodies against B220, IgM, IgD, CD19 and CD22.

B Wild-type, $Cd45^{-/+}$ and $Cd45^{-/-}$ primary B cells were stained with Alexa 647-conjugated antibody against CD22 and settled onto non-stimulatory coverslips. Cells were then fixed, imaged with dSTORM and analysed. Quantification of the distribution of CD22 with H function. Thin lines denote mean ± SEM.

C Wild-type and $Cd45^{-/-}$ primary B cells were stained with Cy5-conjugated Fab fragment against IgM and settled onto non-stimulatory coverslips. Cells were then fixed, imaged with dSTORM and analysed. Quantification of the distribution of IgM with H function. Thin lines denote mean ± SEM.

D CD45-deficient primary B cells were treated with vehicle control or periodate, stained with Alexa 647-conjugated antibody against CD22 and settled onto non-stimulatory coverslips. Cells were then fixed, imaged with dSTORM and analysed. Quantification of the distribution of IgM with H function. Thin lines denote mean ± SEM.

E–H Dual-colour SIM analysis of CD22, IgM, IgD and CD19 in CD45-deficient primary B cells. Cells were fixed, stained with Atto633- or -488-conjugated antibody against CD22, IgM or IgD and Alexa 647-conjugated antibody against CD19 and settled onto non-stimulatory coverslips. Cells were then embedded in agarose, imaged with SIM and analysed. (E) SIM images of CD22, IgM, IgD and CD19. (F) Cross-correlation function. (G) Pearson correlation coefficient. (H) Cluster size calculated from autocorrelation analysis. Bars and numbers indicate the median. Data are from two independent experiments. *$P < 0.05$ (Student's t-test).

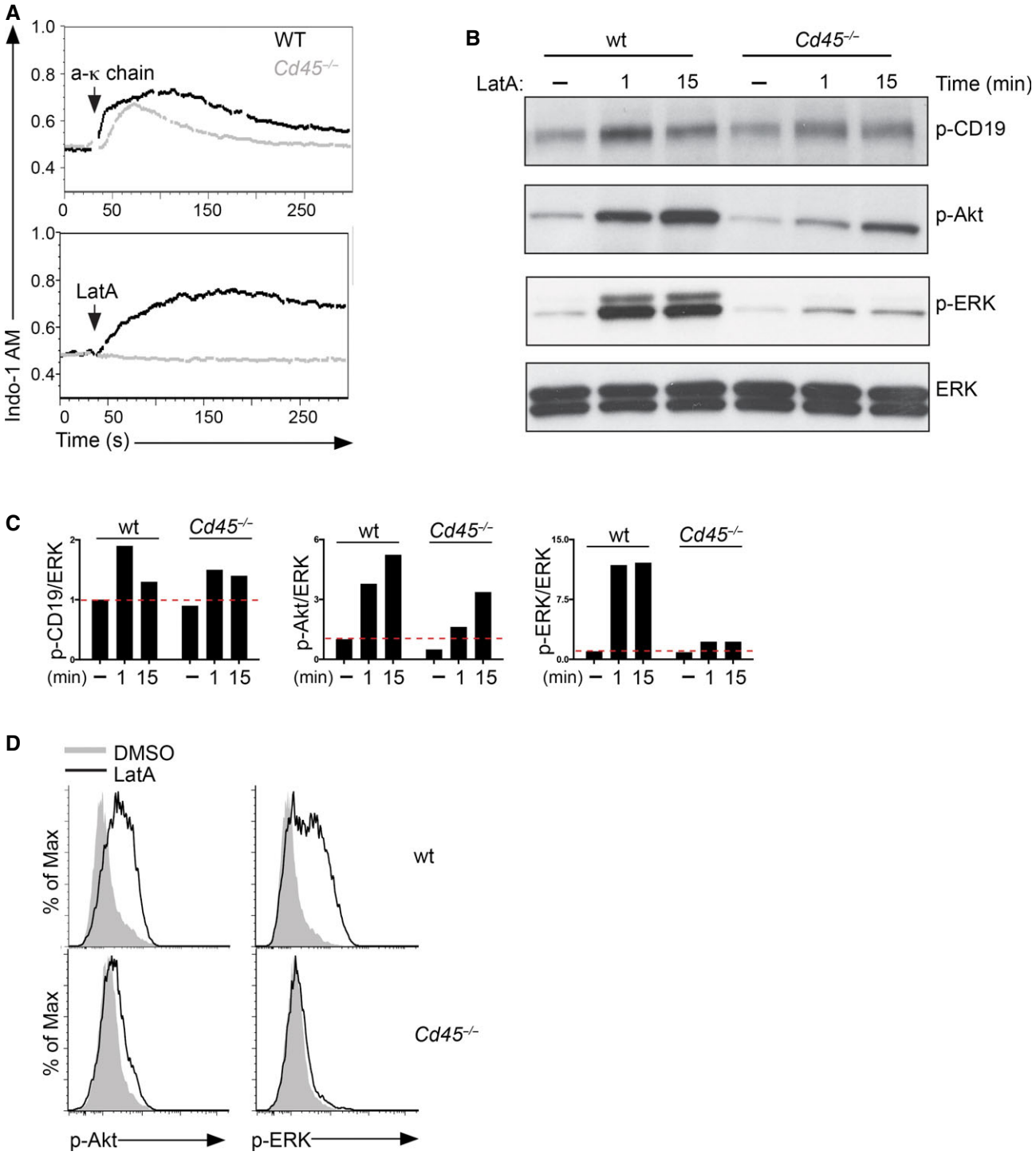


Figure EV3. CD45 is dispensable for BCR signalling after ligand stimulation but essential for receptor signalling after cytoskeleton disruption.

A Wild-type and *Cd45*$^{-/-}$ primary B cells were treated with 5 μg/ml anti-kappa or 1 μM LatA. Intracellular calcium flux was measured by flow cytometry.

B, C Wild-type and CD45-deficient primary B cells were treated with vehicle control (–) or 1 μM LatA for the indicated times. Cells were lysed and analysed by SDS–PAGE followed by immunoblotting with phospho-CD19 (p-CD19), phospho-Akt (p-Akt), phospho-ERK (p-ERK) and total ERK as a loading control. The intensity of phosphorylated proteins, normalized to ERK, was referred to the unstimulated sample of the wild-type cells, set as 1.

D Wild-type and CD45-deficient primary B cells were treated with vehicle control (–) or 1 μM LatA for 5 min, fixed, permeabilized, stained for phospho-Akt (p-Akt) and phospho-ERK (p-ERK) and analysed by flow cytometry. Data are representative of three independent experiments.

Source data are available online for this figure.

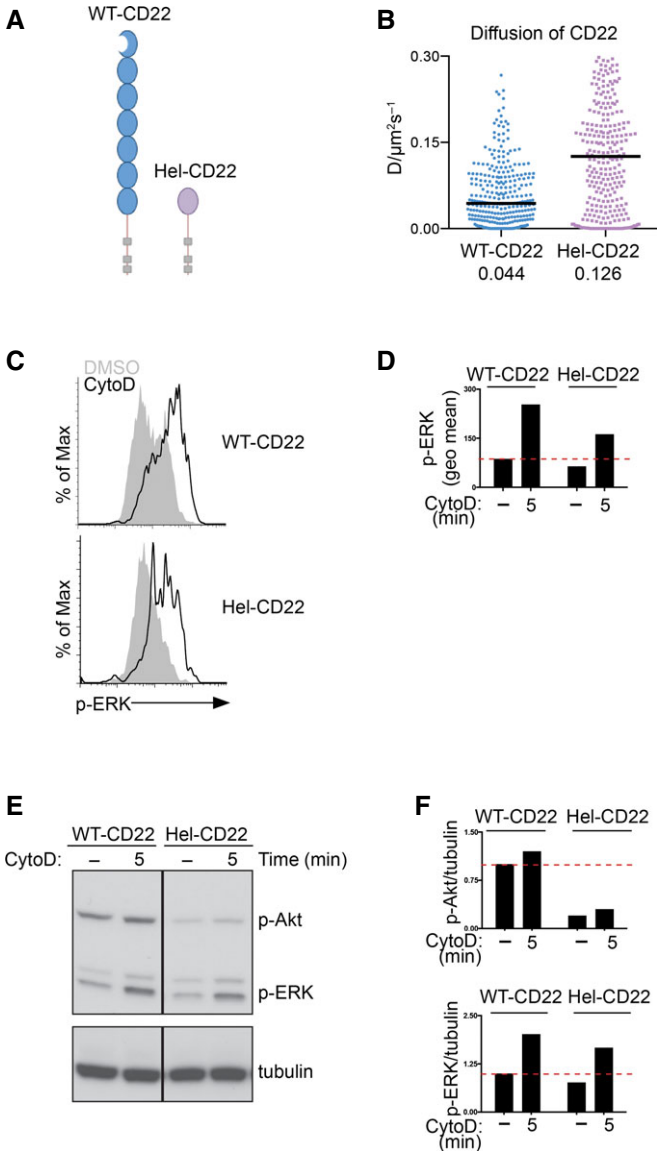


Figure EV4. Extracellular domain of CD22 regulates its diffusion.

A Schematic structure of wild-type CD22 and chimeric Hel-CD22 construct.

B Single-particle tracking of wild-type CD22 and Hel-CD22 in A20 B cells. A20 B cells co-expressing endogenous wild-type CD22 and Hel-CD22 were simultaneously labelled with Atto 488-conjugated Fab fragments against CD22 or Atto 633-conjugated Fab fragment against Hel, settled onto non-stimulatory coverslips and imaged. Single-particle trajectories were then analysed. Bars and numbers indicate the median. Data are pooled from two experiments.

C, D A20 B cells co-expressing wild-type CD22 and Hel-CD22 were treated with vehicle control (–) or 10 μM cytochalasin D (CytoD) for 5 min, fixed, permeabilized, stained for phospho-ERK (p-ERK) and analysed by flow cytometry.

E, F A20 B cells co-expressing wild-type CD22 and Hel-CD22 B cells were treated with vehicle control (–) or 10 μM CytoD for 5 min. Cells were lysed and analysed by SDS–PAGE followed by immunoblotting with phospho-Akt (p-Akt), phospho-ERK (p-ERK) and tubulin as a loading control. The intensity of phosphorylated proteins, normalized to tubulin, was referred to the unstimulated sample of the wild-type cells, set as 1. Data are representative of two independent experiments.

Source data are available online for this figure.

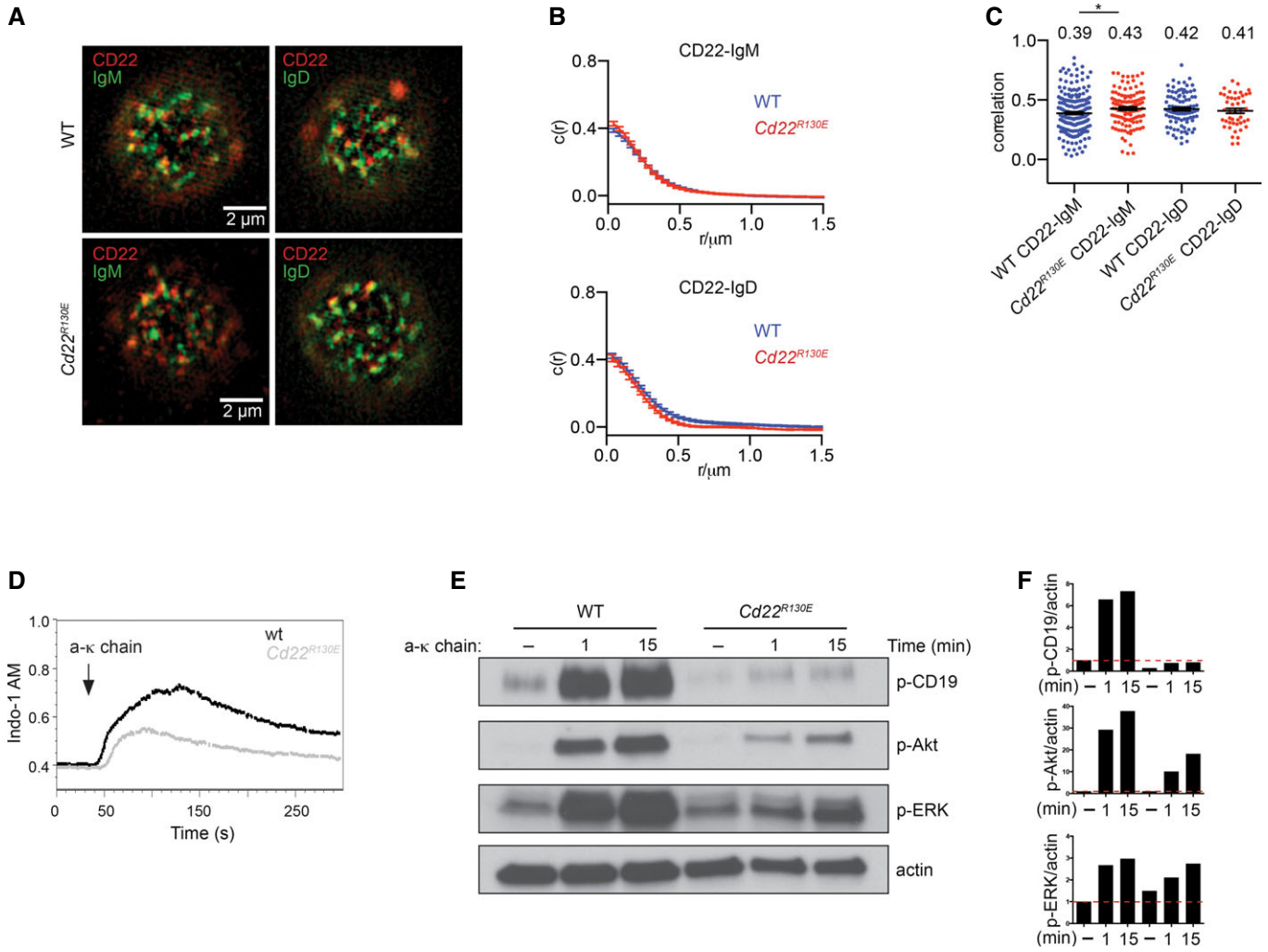


Figure EV5. CD22 colocalization with BCR and ligand-dependent BCR signalling in primary *Cd22*^{R130E} B cells.

A–C Dual-colour SIM analysis of CD22, IgM and IgD. Cells were fixed, stained with Atto633- or -488-conjugated antibody against CD22, IgM or IgD and settled onto non-stimulatory coverslips. Cells were then embedded in agarose, imaged with SIM and analysed. (A) SIM images of CD22, IgM and IgD. (B) Cross-correlation function. (C) Pearson correlation coefficient. Bars and numbers indicate the mean. Data are from three independent experiments. *$P < 0.05$ (Student's *t*-test).

D Wild-type and *Cd22*^{R130E} primary B cells were treated with 5 μg/ml anti-kappa, and intracellular calcium flux was measured by flow cytometry.

E, F Wild-type and *Cd22*^{R130E} primary B cells were stimulated with 5 μg/ml anti-kappa for the indicated times. Cells were lysed and analysed by SDS–PAGE followed by immunoblotting with phospho-CD19 (p-CD19), phospho-Akt (p-Akt), phospho-ERK (p-ERK) and actin as a loading control. The intensity of phosphorylated proteins, normalized to actin, was referred to the unstimulated sample of the wild-type cells, set as 1. Data are representative of two independent experiments.

Source data are available online for this figure.