Figure S1
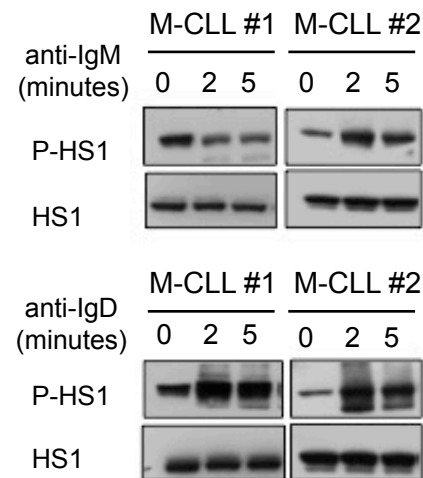

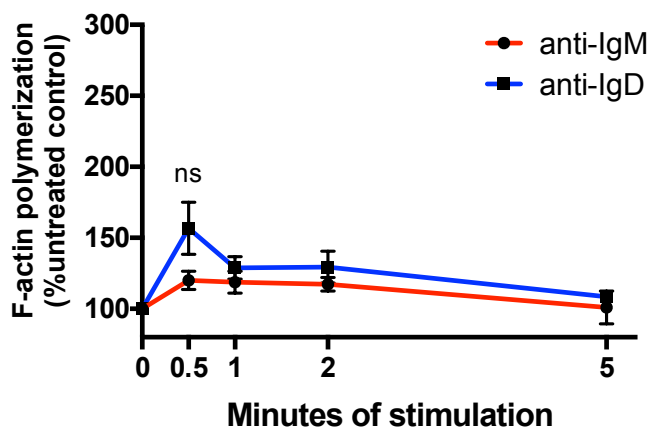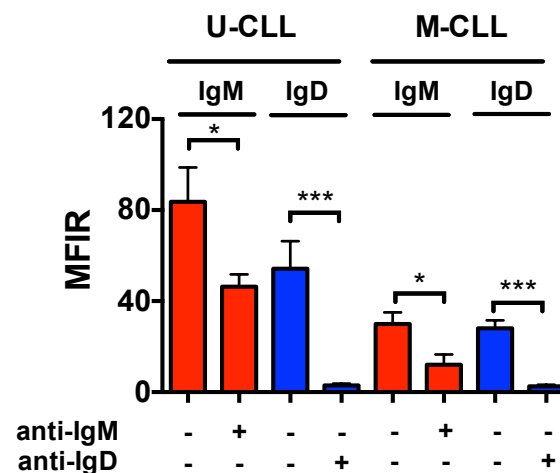**Figure S1. HS1 activation and F-actin polymerization studies in M-CLL cases, and receptor internalization assays.** (A) Mean ± SEM of HS1 protein phosphorylation (P-HS1), after 2 and 5 minutes of anti-IgM or anti-IgD stimulation, as analyzed by flow cytometry on 5 M-CLL cases, and expressed as percentage (%) of the untreated control. ns: not significant; Mann-Whitney test. (B) Western Blot analysis of P-HS1 at the activatory tyrosine residue Y397 and HS1 total levels after 2 and 5 minutes of anti-IgM or anti-IgD stimulation. Two representative M-CLL cases (M-CLL#1, M-CLL#2) out of 5 tested are shown. (C) Mean ± SEM of F-actin polymerization after a period of 5 minutes of anti-IgM or anti-IgD stimulation, as analyzed on 4 M-CLL cases, and expressed as % of the untreated control. ns: not significant; Mann-Whitney test. (D) Mean ± SEM of IgM and IgD receptor expression, expressed as Mean Fluorescent Intensity Ratio (MFIR) before and after 5 minutes of anti-IgM and anti-IgD stimulation, as analyzed by flow cytometry on 9 U-CLL and 6 M-CLL cases. *P <0.05, ***P<0.001; paired t test.
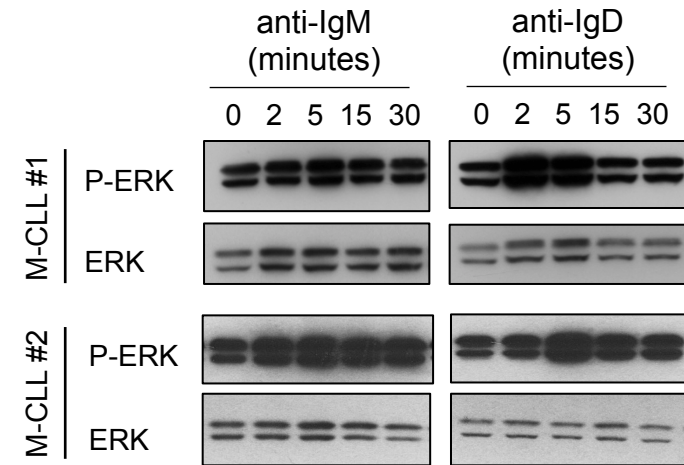
Figure S2

A
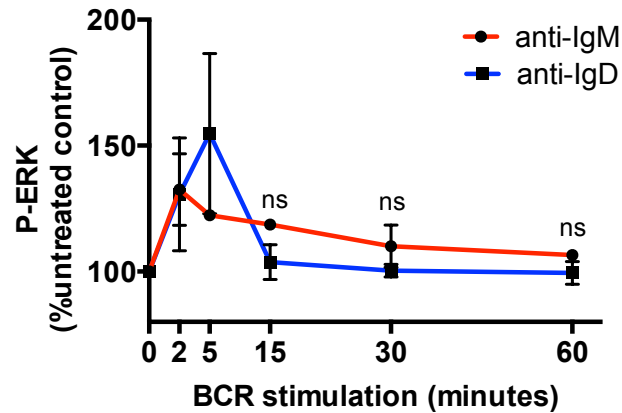
B

Figure S2. ERK kinase activation kinetics in M-CLL cases. **(A)** Mean ± SEM of ERK kinase phosphorylation (P-ERK), at serial time points following IgM or IgD stimulation, as analyzed by flow cytometry on 5 M-CLL samples, and expressed as percentage (%) of the untreated control. ns; not significant; Mann Whitney test. **(B)** Western Blot analysis of P-ERK and ERK total levels at serial time points following anti-IgM or anti-IgD stimulation. Two representative M-CLL cases (M-CLL#1, M-CLL#2) out of 5 tested are shown.
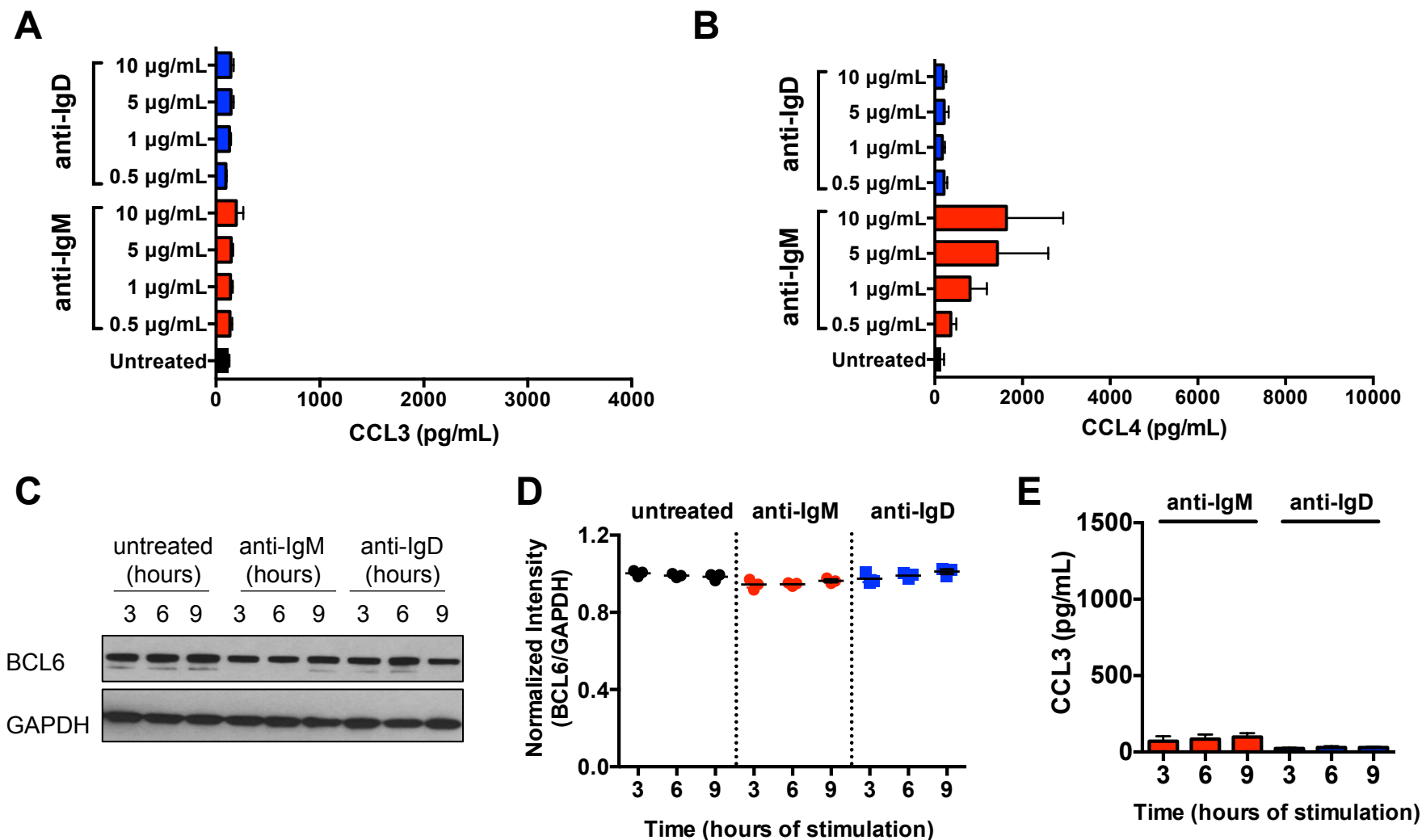
Figure S3

Figure S3. CCL3 and CCL4 chemokine secretion and BCL6 protein expression in M-CLL cells. (A) CCL3 and (B) CCL4 measurement by ELISA in the supernatants of 5 M-CLL samples after 24-hour stimulation with increasing concentrations of anti-IgM and anti-IgD. Displayed are the means ± SEM of chemokine concentrations in pg/mL. (C) Western Blot analysis of BCL6 protein expression at serial time points following anti-IgM or anti-IgD stimulation in one representative M-CLL case. GAPDH protein levels were analyzed, as loading control. (D) Quantification of BCL6 protein expression in 3 M-CLL cases after anti-IgM or anti-IgD, expressed as normalized intensity between BCL6 and GAPDH levels. (E) CCL3 chemokine measurement in the supernatants of 3 M-CLL cases at serial time points following anti-IgM or anti-IgD stimulation. Displayed are the means ± SEM of chemokine concentrations in pg/mL.

Functional assay	U-CLL cases (#)	IgM and IgD MFIR (mean±SEM) of U-CLL	M-CLL cases (#)	IgM and IgD MFIR (mean±SEM) of M-CLL
HS1 phosphorylation (*only flow cytometry; [†]ibrutinib treatment;[‡]combination studies)	5[†‡],14[†‡],17*[†‡],24[†‡],25*[‡], 54, 60	IgM: 91.29±10.66 IgD: 64.50±14.59	3,7,13,16,22	IgM: 37.08±7.17 IgD: 36.87±12.5
F-actin polymerization	37,81,82,83	IgM: 84.26±13.97 IgD: 58.75±16.90	8,42,78,79	IgM: 34.25±6.60 IgD: 38.03±10.78
Receptor internalization (*anti-Igκ stimulation)	2,17,24,27,40*,59, 70*,76*,77*	IgM: 83.63±15.13 IgD: 54.28±12.07	4,7,15,19,22,52	IgM: 29.18±5.41 IgD: 27.72±3.34
ERK phosphorylation ([†]ibrutinib treatment;[‡]combination studies)	5[†‡], 14[†‡],17[†‡],24[†‡],60	IgM: 80.53±7.78 IgD: 63.40±15.25	3,7,13,16,22	IgM: 37.08±7.17 IgD: 36.87±12.5
CCL3 and CCL4 secretion ([‡]combination studies)	17,20[‡],24[‡],25,30,32[‡],40[‡]	IgM: 123.3±19.43 IgD: 74.98±21.40	3,13,21,29,47	IgM: 40.12±11.34 IgD: 40.88±19.58
BCL6 protein expression	27,67,68	IgM: 76.01±20.40 IgD: 55.66±21.40	18,42,50	IgM: 48.19±18.90 IgD: 48.89±15.45
Viability ([†]ibrutinib treatment; [‡]combination studies)	2[†‡],5[†‡],6,10[†],14[†‡],17[†],24[†‡] ,25[†‡],26, 54, 59,70,73	IgM: 84.56±12.27 IgD: 47.11±10.46	7,8,11,12[†],13[†],15[†],16[†],18[†] ,21,22[†],23[†],64,65	IgM: 42.10±7.59 IgD: 34.37±8.54
CLL-NLC co-cultures	27,28,54,59,67,69,70, 72,74,77	IgM: 73.61±9.96 IgD: 55.70±10.32	8,18,42,52,62,63,64,65	IgM: 43.58±10.15 IgD: 43.61±10.34

Table SI. U-CLL and M-CLL cases analyzed in functional assays. Functional assays and number (#) of U-CLL and M-CLL analyzed cases. Arabic numerals refer to the sample list as in Table I. Means ±SEM of IgM and IgD mean fluorescent intensity ratios (MFIR) of the analyzed subgroups are also indicated.