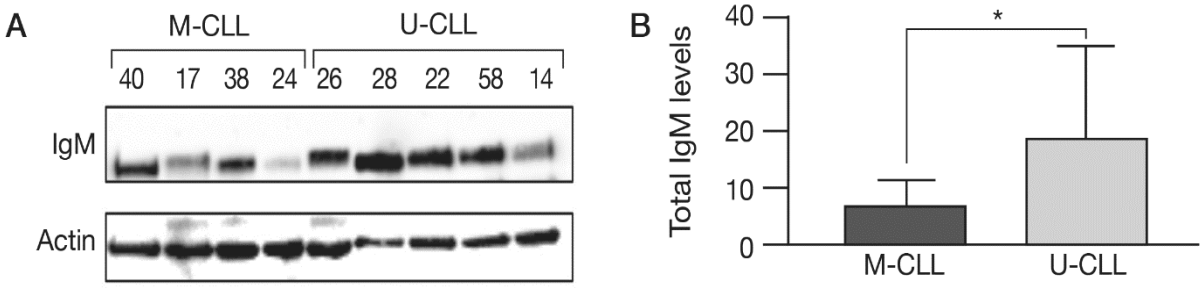
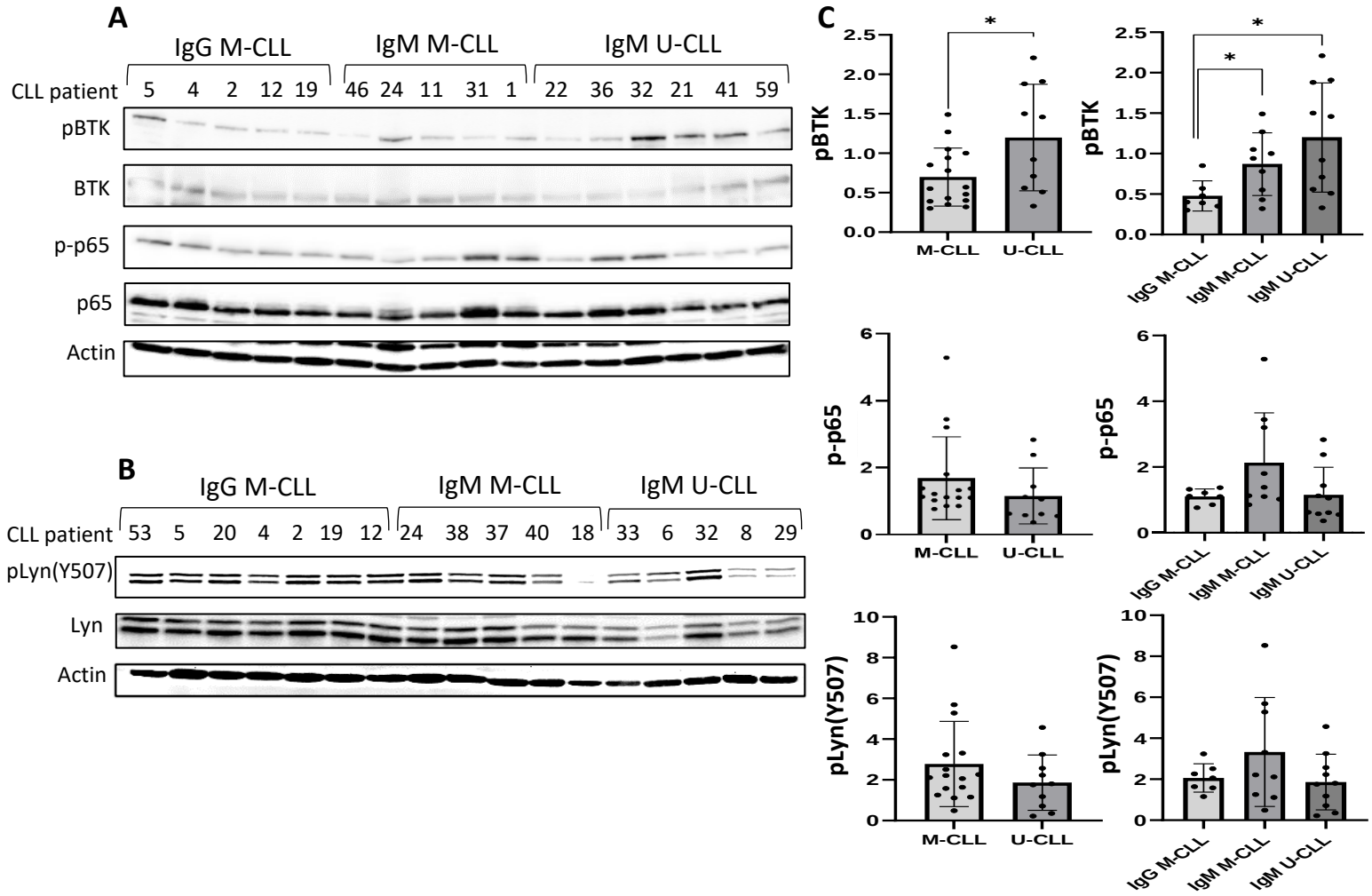
Supplementary Figures

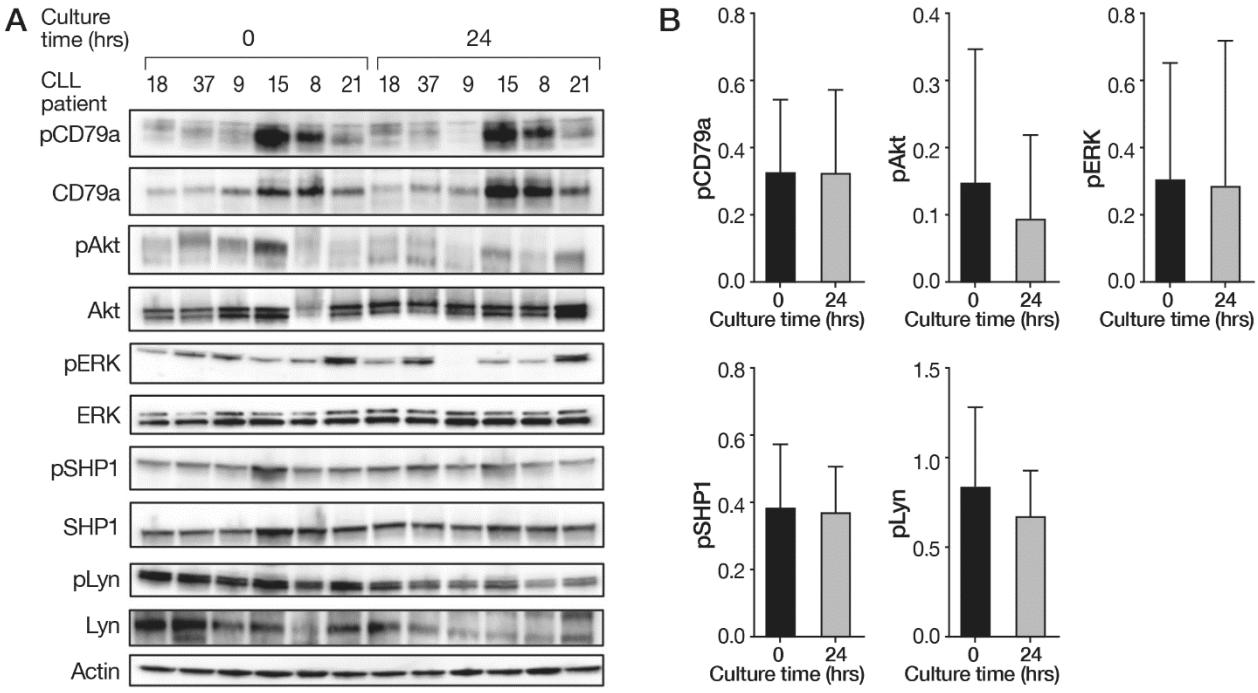


Supplementary Figure S1: IgM levels in M-CLL and U-CLL cells. A. A representative Western blot analysis of primary CD19+ purified cells from M-CLL and U-CLL cases showing IgM levels. Actin was used to verify equal loading. B. Quantification of IgM levels in M-CLL and U-CLL cells in (A) by normalization to actin using myImageAnalysis™ Software (n=23). *p<0.05.

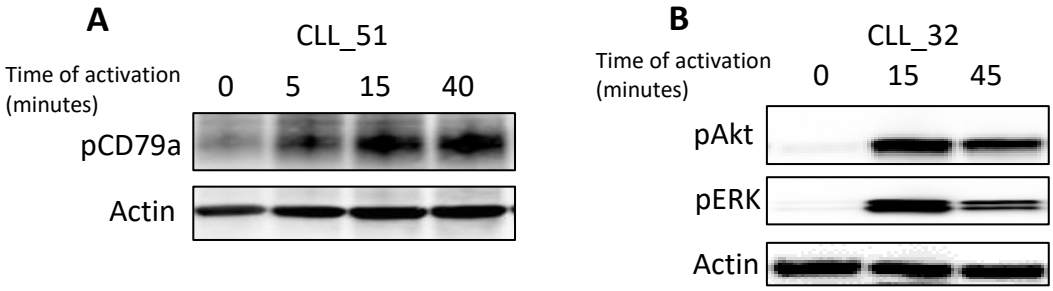


Supplementary Figure S2: BTK , p65 and Lyn phosphorylation in CLL subgroups. Peripheral blood CLL cells were isolated from IgG M-CLL, IgM M-CLL and IgM U-CLL patients. Protein was extracted and analyzed by Western blot.A. A representative Western blot analysis showing BTK(Y223) and p65(S536) phosphorylation. B. A representative Western blot analysis showing Lyn(Y507)

phosphorylation. Actin was used to verify equal loading. C. Quantification of pBTK, p-p65 and pLyn levels in (A) and (B) by normalization to actin using myImageAnalysis™ Software (n=26). *p<0.05.

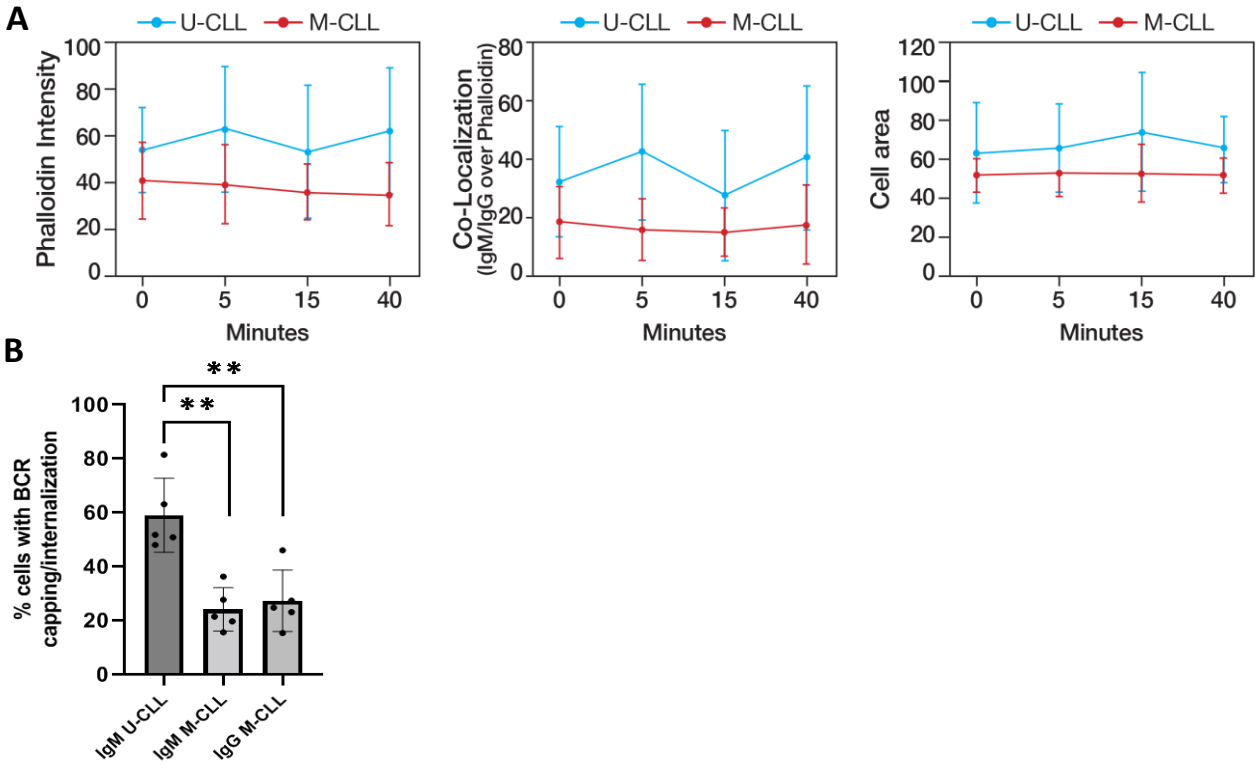


Supplementary Figure S3: Phosphorylation levels of BcR signaling elements in different time points. Peripheral blood CLL cells were isolated from M-CLL and U–CLL patients.Protein was extracted immediately after thawing and following 24 hours of incubation, and analyzed by Western blot. A. A representative Western blot analysis showing CD79a(Y182), Akt (S473), ERK(T202/Y204), SHP1 (Y564) and Lyn (Y396) phosphorylation, as well as total amount of these proteins. Actin was used to verify equal loading. B. Quantification of pCD79a, pAkt, pERK, pSHP1 and pLyn levels in (A) by normalization to actin using myImageAnalysis™ Software (n=14).

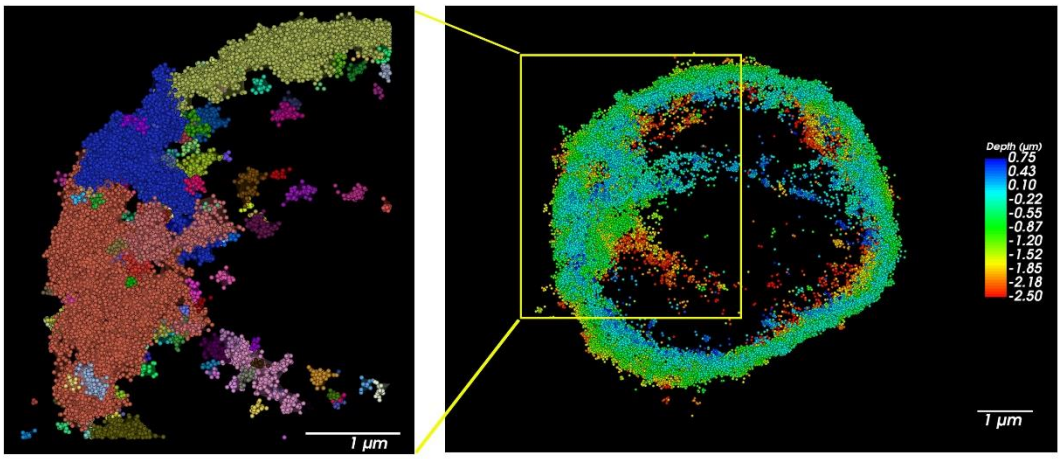


Supplementary Figure S4 : A time-dependent BcR activation pattern in CLL cells. CLL cells were incubated with goat F(ab')2 anti-human IgM (10 µg/mL), for the indicated time points or left untreated. Protein was extracted and analyzed by Western blot.A. A representative Western blot analysis showing CD79a(Y182) phosphorylation after 5, 15 and 40 minutes. Actin was used to verify equal loading (n=3). B. A

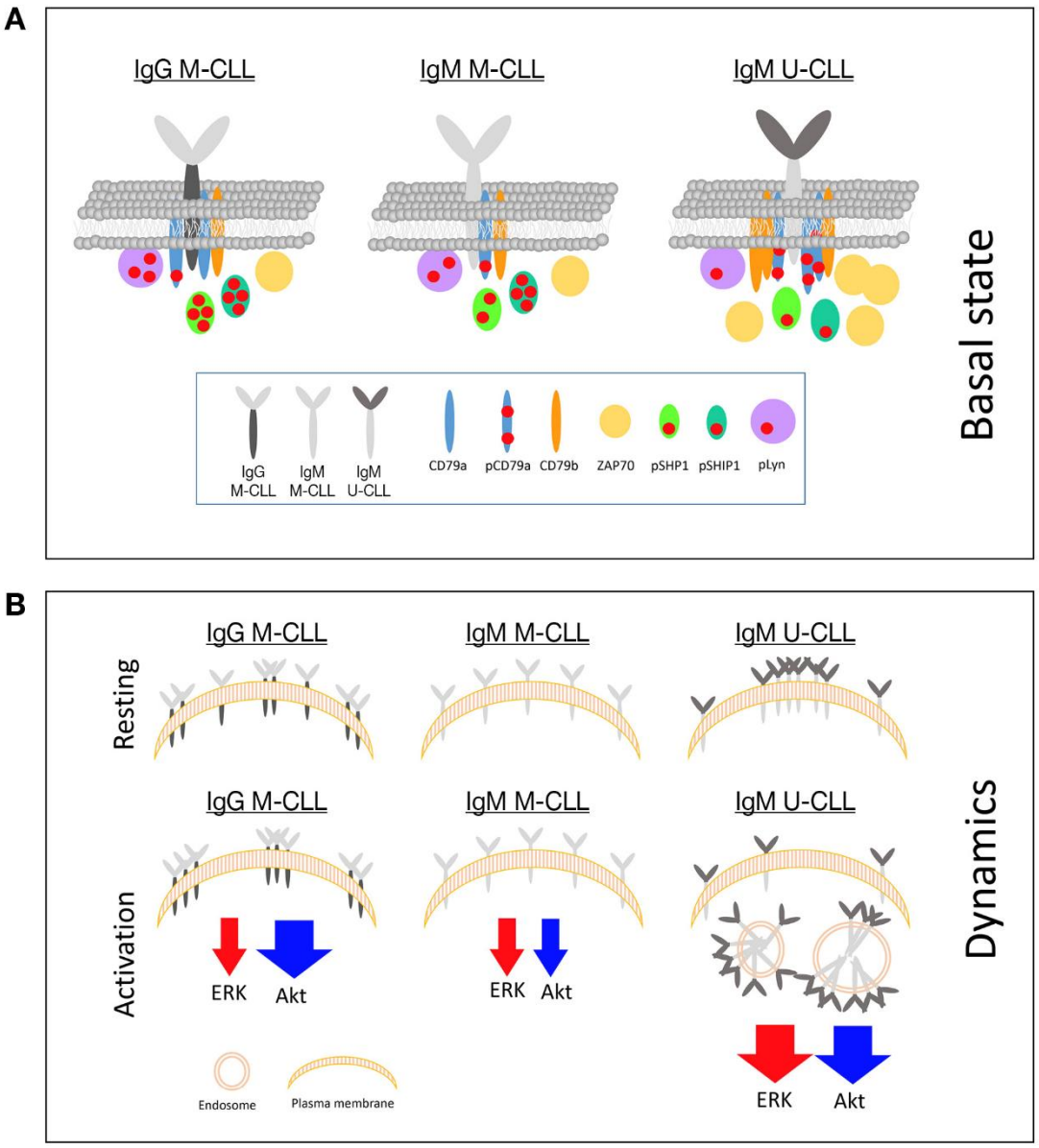
representative Western blot analysis showing Akt (S473) and ERK (T202/Y204) phosphorylation after 15 and 45 minutes. Actin was used to verify equal loading (n=8).



Supplementary Figure S5: BcRs and F-actin dynamic in M-CLL and U-CLL cells before and after activation. A.CLL cells were unstimulated ("0") or activated with F(ab')2 Fragment Goat Anti-Human IgM or IgG at 10µg/mL for 5, 15 and 40 minutes. The cells were stained with human anti-IgM or anti-IgG antibody conjugated to FITC, followed by staining with DyLight 594 Phalloidin. Quantification of Phalloidin intensity,Co-Localization of IgM/IgG over Phalloidin and cell area was done using Imaris software (n=25). B. Analysis of the percentage of cells in the basal state showing capping/ internalization of the BcR's in the three groups of CLL (n=15, mean of two fields per patient) **p<0.01.



Supplementary Figure S6: Visualization of cluster analysis of d-STORM data. Depth representation of a d-STORM image of IgM U-CLL cell (right panel) and its corresponding clusters (left panel). This cell contains both large and small clusters, each individual cluster is marked in a different color.



Supplementary Figure S7:The differences in basal BcR signalosomes and BcR spatial dynamics between immunogenetic CLL subtypes. A. Basal signalosomes compositions of IgG M-CLL, IgM M-CLL and IgM U-CLL are illustrated according to the molecules depicted in the insert. Small red circles indicate phosphorylation. B. The dynamics of IgG M-CLL (left), IgM M-CLL (middle) and U-CLL (right) is depicted in the resting (top) versus the activation (bottom) states of the BcR. Strength of the ERK (red) and Akt (blue) responses are indicated by the arrows width.

Supplementary Table 1: Patient characteristics.

Patient	Gender/Age (y)	ALC(x10^9/L)	WBC	IGHV gene SHM status
CLL_01	M/75	163.6	169.4	M-CLL
CLL_02	M/51	63.7	67.7	IgG M-CLL
CLL_03	F/73	81.8	94.3	M-CLL
CLL_04	F/86	42.1	48.8	IgG M-CLL
CLL_05	F/67	104.8	113	IgG M-CLL
CLL_06	M/86	81.8	88.3	U-CLL
CLL_07	M/60	215.7	219.5	U-CLL
CLL_08	F/72	233.7	253.9	U-CLL
CLL_09	M/71	41.7	47	M-CLL
CLL_10	F/68	124.6	135	M-CLL
CLL_11	M/63	103.3	124.9	M-CLL
CLL_12	F/70	130	138.1	IgG M-CLL
CLL_13	M/64	146.7	157.4	U-CLL
CLL_14	M/71	47.9	56.3	U-CLL
CLL_15	F/69	83.1	103.3	U-CLL
CLL_16	F/59	176.5	188.7	U-CLL
CLL_17	F/72	45.9	52.5	M-CLL
CLL_18	F/72	126.1	134.1	M-CLL
CLL_19	F/82	30.4	38.2	IgG M-CLL
CLL_20	F/72	97.9	106.7	IgG M-CLL
CLL_21	F/62	153.6	178	U-CLL
CLL_22	M/64	235.3	249	U-CLL
CLL_23	M/54	47.1	56.2	U-CLL
CLL_24	F/70	67.1	75.2	M-CLL
CLL_25	F/75	117.8	150.3	M-CLL
CLL_26	F/69	28.6	40.9	U-CLL
CLL_27	M/68	133	143.8	U-CLL
CLL_28	F/41	41.8	48.4	U-CLL
CLL_29	M/58	215.8	229	U-CLL
CLL_30	F/49	165.7	176.8	IgG M-CLL
CLL_31	M/82	21.6	25.9	M-CLL
CLL_32	M/71	94.1	97.9	U-CLL
CLL_33	M/52	137.1	147.1	U-CLL
CLL_34	M/52	126.8	134.8	M-CLL
CLL_35	M/81	84	89.4	U-CLL
CLL_36	F/52	130	141.1	U-CLL
CLL_37	M/72	30.9	38.5	M-CLL
CLL_38	M/51	31.9	37.5	M-CLL
CLL_39	F/86	99.3	124	U-CLL
CLL_40	F/74	42.6	48.6	M-CLL
CLL_41	M/50	80.3	91.9	U-CLL
CLL_42	M/73	103.5	110.1	U-CLL
CLL_43	M/70	100.6	106.1	U-CLL
CLL_44	F/66	171.9	186.2	M-CLL

CLL_45	M/84	98.7	135.7	M-CLL
CLL_46	M/52	50.9	54.6	M-CLL
CLL_47	M/76	81	89	M-CLL
CLL_48	M/75	81.3	86.4	M-CLL
CLL_49	M/80	68	84	U-CLL
CLL_50	M/57	71.4	76.6	U-CLL
CLL_51	M/70	12.7	18.5	U-CLL
CLL_52	F/55	99.5	109	IgG M-CLL
CLL_53	F/81	229.5	259	IgG M-CLL
CLL_54	M/68	43	46	IgG M-CLL
CLL_55	M/73	32.1	42	IgG M-CLL
CLL_56	M/66	12	26	IgG M-CLL
CLL_57	F/54	27.3	34.6	IgG M-CLL
CLL_58	F/76	200.9	210.2	U-CLL
CLL_59	M/67	75.6	81.9	U-CLL
Normal _01	F/27			
Normal _02	M/64			

M-male, F-female, y- years, ALC- absolute lymphocyte count, M-CLL: CLL with mutated IGHV genes; U-CLL: CLL with unmutated IGHV genes.

Supplementary Tables 2-4: Gene Set Enrichment Analysis for immunoglobulin subtypes in CLL .Gene Set Enrichment Analysis (GSEA) was used to identify overrepresentation of gene sets from the online database available at the GSEA Web site (http://www.broadinstitute.org/gsea/). In addition, select gene sets from the gene expression database of the Staudt laboratory (http://lymphochip.nih.gov/signaturedb/index.html) were uploaded to GSEA for inclusion in the analysis. Enriched or overrepresented gene sets between IgM U-CLL, IgM M-CLL and IgG M-CLL were identified using 1000 permutations of the phenotype labels.

Table 2. IgM U-CLL vs. IgM M-CLL		
	NES	FDR q-val
NF-κB signaling pathway	2.83	<0.0001
B-cell receptor signaling	2.39	<0.0001
TGF-β signaling pathways	2.18	<0.0001
Inflammatory response	2.17	<0.0001
Apoptosis regulation	2.1	<0.0001
TLR signaling pathway	1.91	0.003
IFN-γ response	1.81	0.002
RAS signaling pathway	1.77	0.003
IFN-α response	1.76	0.002
P53 signaling Pathway	1.75	0.002

Table 3. IgM U-CLL vs. IgG M-CLL		
	NES	FDR q-val
NF-κB signaling pathway	2.8	<0.0001
B-cell receptor signaling	2.17	<0.0001
TGF-β signaling pathways	2.11	<0.0001
Apoptosis regulation	1.92	<0.0001
Inflammatory response	1.8	0.006
IL4 response	1.73	0.008
P53 signaling Pathway	1.62	0.016
JAK/STAT3 signaling pathway	1.64	0.017
Ras signaling pathway	1.6	0.015
MYD88 signaling pathway	1.59	0.015

Table 4. IgG M-CLL vs. IgM M-CLL		
	NES	FDR q-val
P53 signaling Pathway	1.79	0.02
IFN-γ response	1.73	0.014