Supplementary Materials and Methods

Cloning of CLL donor BCR as scFv. Briefly, for the first round of PCR, the VH region was amplified using a 5' primer containing a XmaI site, CLL655_VH_FR1_XmaI_Fwd (GTCCTCGCAACTGCCCCATCCCGGGGCCCAACCAGCGATGGCCCAGGTGCAGCTGGTGCAG TCTGG) and 3' primer containing a $(Gly_4\text{-}Ser)_3$ linker sequence, CLL655_VH_scFv_Rev (CCGCCGGATCCACCTCCGCCTGAACCGCCTCCACCTGAGGAGACGGTGAC) with the following cycle profile: 94°C for 5 min, (94°C, 1 min; 55°C, 1 min; and 72°C, 3 min) × 25, 72°C for 7 min. For VL region amplification, a 5' primer containing a $(Gly_4\text{-}Ser)_3$ linker sequence, CLL655_VL_scFv_Fwd (GGAGGCGGTTCAGGCGGAGGTGGATCCGGCGGTGGCGGATCGGACATCCAGATGACCCAG TCTCC) and 3' primer containing a SalI site and FLAG tag, CLL655_VL_FR4_SalI_Rev (GAGTCATTCTCGACTGCTATGTCGACTTTATCATCATCATCTTTATAATCACGTTTGATATCC ACTTTGGT) were used with the following cycle profile: 94°C for 5 min, (94°C, 1 min; 55°C, 1 min; and 72°C, 3 min) × 25, 72°C for 7 min. For the second round of PCR, the scFv gene was assembled by overlap-extension based on the $(Gly_4\text{-}Ser)_3$ linker sequence homology between the VH and VL PCR products using the following cycle profile: 94°C for 5 min, (94°C, 1 min; 60°C, 1 min; and 72°C, 3 min) × 6, 72°C for 7 min. In the third round of PCR, the assembled scFv gene was PCR amplified using the outside primers, CLL655_VH_FR1_XmaI_Fwd and CLL655_VL_FR4_SalI_Rev using the following cycle profile: 94°C for 5 min, (94°C, 1 min; 55°C, 1 min; and 72°C, 3 min) × 25, 72°C for 7 min. PCR products were purified by QIAquick Gel Extraction Kit (Qiagen), digested with XmaI and SalI (New England Biolabs), and cloned into pDISPLAY vector (Invitrogen) using T4 DNA ligase (New England Biolabs). CLL donor scFv plasmid was purified from transformed NovaBlue competent cells (Novagen) with the QIAprep Spin Miniprep Kit (Qiagen).

Construction of scFv mutants. The two scFv mutants were constructed by amplifying the VH and VL regions from the wildtype CLL donor scFv plasmid utilizing overlap-extension PCR as described above, except using different primer sets for the first round of PCR. For the 1324 HCDR3 Swap scFV mutant, the VH region was amplified using a 5' primer containing a XmaI site, CLL655_VH_FR1_XmaI_Fwd (GTCCTCGCAACTGCCCCATCCCGGGGCCCAACCAGCGATGGCCCAGGTGCAGCTGGTGCAG TCTGG) and 3' primer containing the CLL1324 VH CDR3 sequence as overhang, CLL655_FR3-1324_CDR3_Rev (TTGTTGATAATAACTCCCTGAACCATAGGACGCCTCTCTCGCACAGTAATA). The VL region was amplified using a 5' primer containing the CLL1324 VH CDR3 sequence as overhang, CLL1324_CDR3-655_FR4_Fwd (GAGGCGTCCTATGGTTCAGGGAGTTATTATCAACAATACTTTGACTACTGG) and 3' primer containing a SalI site and FLAG tag, CLL655_VL_FR4_SalI_Rev (GAGTCATTCTCGACTGCTATGTCGACTTTATCATCATCATCTTTATAATCACGTTTGATATCC ACTTTGGT). The 1324 HCDR3 Swap scFv gene was assembled by overlap-extension based on the CLL1324 VH CDR3 sequence homology between the VH and VL PCR products. For the Donor HCDR3 Swap scFv mutant, the VH region was amplified using a 5' primer containing a XmaI site, CLL655_VH_FR1_XmaI_Fwd (GTCCTCGCAACTGCCCCATCCCGGGGCCCAACCAGCGATGGCCCAGGTGCAGCTGGTGCAG TCTGG) and 3' primer containing the CLL1324 VH FR4 sequence as overhang, CLL655_CDR3-1324_FR4_Rev (TGAGGAGACGGTGACCGTGGTCCCTTTGCCCCAGACGTCCATGTAGTAGTAGTAGTAGTAG TACCCCCAAAAATC). The VL region was amplified using a 5' primer containing the CLL1324 VH FR4 sequence as overhang and 5' end of a $(Gly_4\text{-}Ser)_3$ linker sequence, CLL1324_FR4-GlySer_Fwd (TACTACTACTACTACTACATGGACGTCTGGGGCAAAGGGACCACGGTCACCGTCTCCTCAG GTGGAGGCGGTTCA) and 3' primer containing a SalI site and FLAG tag, CLL655_VL_FR4_SalI_Rev

(GAGTCATTCTCGACTGCTATGTCGACTTTATCATCATCATCTTTATAATCACGTTTGATATCC ACTTTGGT). The Donor HCDR3 Swap scFv gene was assembled by overlap-extension based on the $(Gly_4\text{-}Ser)_3$ linker sequence homology between the VH and VL PCR products.

Quantitative real-time PCR. RNA from CLL donor MNCs were converted into cDNA as described in Materials and Methods. Quantitative real-time PCR was done using the Customized TaqMan® Gene Expression Assay (Invitrogen) on a LightCycler® 480 Instrument II (Roche). The sequences for the upstream IGHV1 allele-specific primer, downstream IGHJ4 allele-specific primer, and the TaqMan probe complementary to the hypervariable V–N–D region of the CLL donor HCDR3 are as follows: upstream primer (GCTGAGCAGCCTGAGATCTG), downstream primer (GGCCCCAGTAGTCAAAGTAGTAC), and probe (CTGTGCGAGAGTTACAGTCAAG). Two independent quantitative real-time PCR reactions with duplicate samples were performed, and the value of the target gene was normalized to the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene. The fold change in the CLL donor HCDR3 transcript relative to the GAPDH endogenous control was determined by the following formulas:

$$\text{Fold Change} = 2^{-\Delta(\Delta CT)}$$

$$\Delta C_T = C_{T,\ \text{CLL donor HCDR3}} - C_{T,\ \text{GAPDH}}$$

$$\Delta(\Delta C_T) = \Delta C_{T,\ \text{stimulated}} - \Delta C_{T,\ \text{control}}$$

Supplementary Figure Legends

Figure S1: Flow cytometric analysis of monoclonal VLR39 reactivity with different cell types and cell lines. VLR39 binding (red line) was compared with that of control VLR4 having *Bacillus anthracis* BclA-specificity (gray shading). Histogram of VLR39 binding to (A) five different healthy donor lymphocytes gated on B cells ($CD3^-/CD19^+$), T cells ($CD3^+/CD19^-$), and non-B/T cells ($CD3^-/CD19^-$), (B) four EBV transformed B cells, and (C) four different human B cell leukemia/lymphoma lines, including pre-B cell leukemia (697), Burkitt's Lymphoma (Daudi, Ramos), and diffuse large B cell lymphoma (SU-DHL-6).

Table S1: CLL VH gene families and HCDR3 sequences. The *IGHV*, *IGHD*, and *IGHJ* genes and the HCDR3 amino acid sequences expressed in the leukemic cells of 26 CLL patients tested for VLR39 reactivity are shown. Shared VH gene family usage between CLL donor and other CLL patients are highlighted in yellow (*IGHV*), blue (*IGHD*), and green (*IGHJ*).

Figure S2: Flow cytometric analysis of monoclonal VLR39 reactivity with CLL cells with different *VH* gene sets. VLR39 binding (red line) was compared with that of control VLR4 having *Bacillus anthracis* BclA-specificity (gray shading). Histogram of VLR39 binding to 26 CLL patients with known *VH* gene sequences (gated on $CD5^+/CD19^+$ lymphocytes).

Figure S3: CLL *VH* gene sequence analysis. Sequence alignment between the IGVH-D-J gene rearrangements of CLL donor and those of the 26 CLL patients tested for VLR39 reactivity by flow cytometry are shown. Shared *VH* gene family usage between CLL donor and other CLL patients are highlighted in yellow (*IGVH*), blue (*IGHD*), and green (*IGHJ*). Identical CDR1, CDR2, and IGHJ amino acid sequences are shown in red.

Figure S4: IGHV-D-J sequence alignment of VLR39 sorted cells. Sequence alignment between the IGHV-D-J gene rearrangement of CLL donor and those of the $VLR39^+/CD5^{hi}$ cells sorted from the CLL

donor B cells at 58 months after treatment. No significant alignment was possible for the VLR39^{-}/CD5^{lo} cells. Nucleotide identity to the original CLL donor IGHV-D-J sequencing results is highlighted in gray.

Table S2: Detection of CLL recurrence by quantitative real-time PCR. Cryopreserved MNCs from the CLL donor after treatment were analyzed by qPCR for the presence of CLL donor HCDR3 transcript. Each time point is calculated as months after end of CFAR treatment regimen. The C_T , ΔC_T, and $\Delta(\Delta C_T)$ of the qPCR reaction using the CLL donor HCDR3-specific probe and GAPDH probes were used to calculate the fold change in the CLL donor HCDR3 transcript relative to the GAPDH endogenous control.

Figure S1

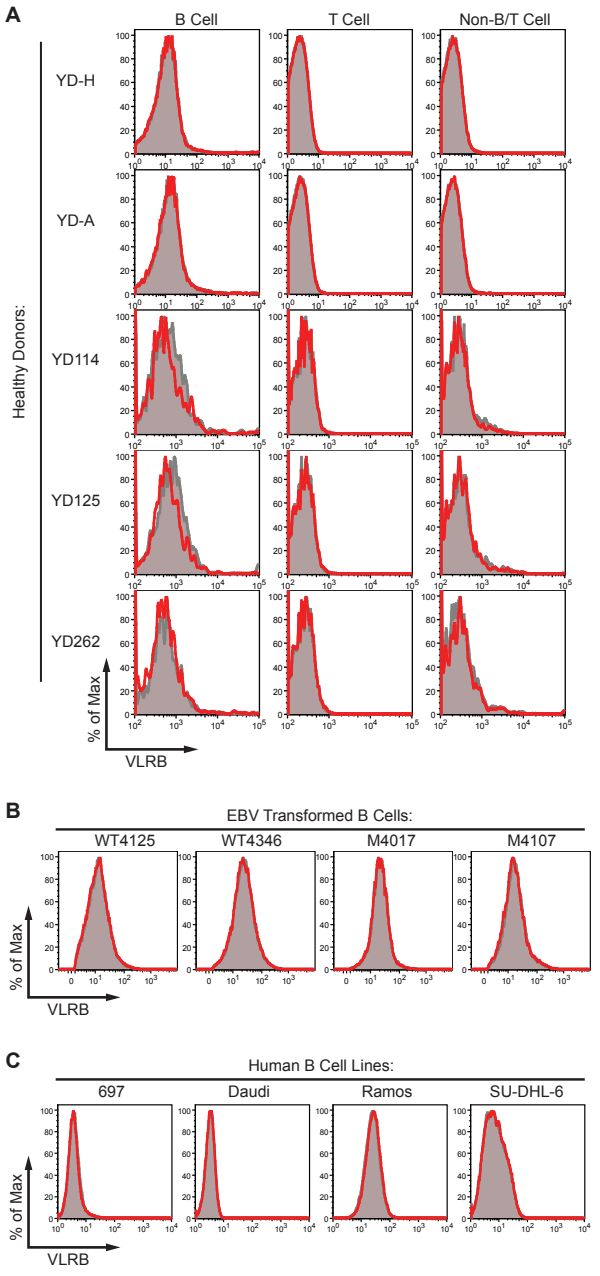
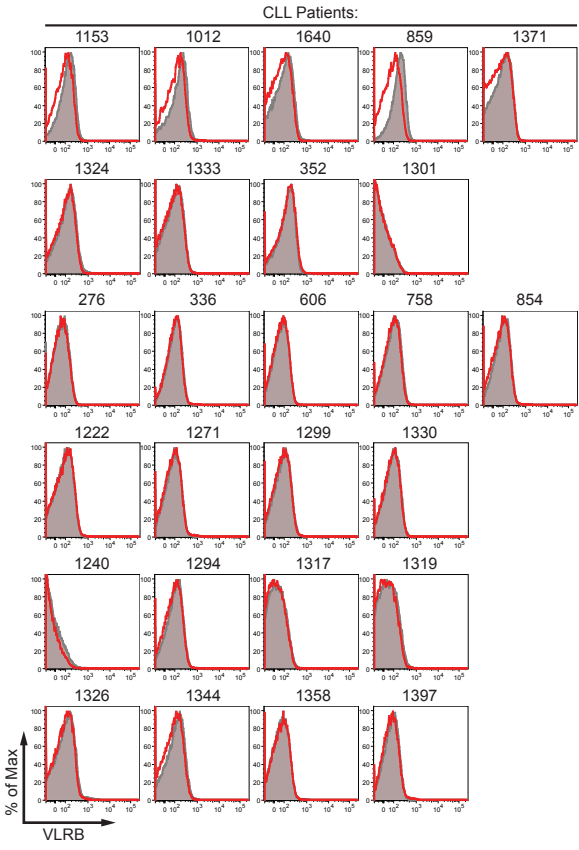

A
B Cell
T Cell
Non-B/T Cell
Healthy Donors:
YD-H
YD-A
YD114
YD125
YD262
% of Max
VLRB
B
EBV Transformed B Cells:
WT4125
WT4346
M4017
M4107
% of Max
VLRB
C
Human B Cell Lines:
697
Daudi
Ramos
SU-DHL-6
% of Max
VLRB

Table S1. CLL *VH* gene families and HCDR3 sequences

CLL ID	IGHV Gene	IGHD Gene	IGHJ Gene	HCDR3 amino acid sequence
Donor	IGHV1-69*01	IGHD3-3*01	IGHJ4*02	ARVTVKYYDFWGYYFDY
1153	IGHV1-69*01	IGHD3-3*01	IGHJ4*02	DDSYYDFWSGWYY
1012	IGHV1-69*01	IGHD3-3*01	IGHJ6*03	ARVEIFGVVGLSYYYYYMDV
1640	IGHV1-69*01	IGHD3-3*01	IGHJ6*03	ARGAIFGVVIIPVTPFYMDV
859	IGHV3-09*01	IGHD3-3*01	IGHJ4*02	AKDASSNYDFWSGYYDY
1371	IGHV4-b*02	IGHD3-3*01	IGHJ4*02	ARVMEKYYDFWSGYYYFD
1324	IGHV1-69*01	IGHD3-10*01	IGHJ6*03	AREASYGSGSYYQQYYYYYYMDV
1333	IGHV1-69*01	IGHD3-10*01	IGHJ6*02	AVGVLWFGELLFSYYYYYGMD
352	IGHV1-69*05	IGHD3-3*01	IGHJ6*02	AGRLIFGVVITAGGDYGMDV
1301	IGHV4-31*03	IGHD3-3*01	IGHJ3*02	ARAPIGSTIFGVVIIRFAFDI
276	IGHV1-2*02	IGHD2-21*02	IGHJ4*02	ARTQIGDCGGDCYPFDY
336	IGHV1-3*01	IGHD6-19*01	IGHJ4*02	AREQWLVLSYFDY
606	IGHV1-2*04	IGHD3-10*01	IGHJ4*02	ARDLRYSYGSGSTPFLDS
758	IGHV1-18*01	IGHD3-16*01	IGHJ4*02	ARKSWVGAYYFDY
854	IGHV1-3*01	IGHD2-2*01	IGHJ4*02	VSHYCTSSTCDQMY
1222	IGHV1-2*02	IGHD6-19*01	IGHJ4*02	AREQWLASPNLDY
1271	IGHV4-34*02	IGHD6-6*01	IGHJ4*02	ARGRWSPKFVL
1299	IGHV3-23*01/IGHV3-23*02	IGHD2-2*01	IGHJ4*03	AKGLVIGLPDV
1330	IGHV3-7*03	IGHD2-8*01	IGHJ4*02	ARSSRDGTNDYDGEYRYFDY
1240	IGHV3-33*01/IGHV3-33*06	IGHD3-9*01	IGHJ2*01	ATRPQLNYDILTGYYIGGGYFDL
1294	IGHV3-21*01	IGHD7-27*01	IGHJ6*03	ARDPYRGLYGMFYFYYMDV
1317	IGHV4-59*01	IGHD2-21*02	IGHJ3*02	ARNPYCGGDCYSDAFDI
1319	IGHV4-31*03	IGHD2-2*02	IGHJ6*02	ARDLYGWTYCSSTSCYRYYGMDV
1326	IGHV3-53*04	IGHD5-12*01	IGHJ6*02	ARDRVDIVATTTYYYYYYGMDV
1344	IGHV1-69*02	IGHD2-21*02	IGHJ5*02	ARSTNLDYFFAAVTGNWFDP
1358	IGHV4-34*02	IGHD2-8*01	IGHJ6*02	TRAMDYYYGMDV
1397	IGHV1-69*06	IGHD3-22*01	IGHJ6*02	ATPPRGTYDSSGYYYGGLDNYYGMDV

Figure S2


CLL Patients:
1153
1012
1640
859
1371
1324
1333
352
1301
276
336
606
758
854
1222
1271
1299
1330
1240
1294
1317
1319
1326
1344
1358
1397
% of Max
VLRB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1324 QVQLVQSGAEVKKPGSSVKVSCKASGGTFSS..YAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAREASYGSG...SYYQQYYYYYYMDVWGKGT.....
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352 QVQLVQSGAEVKKPGSSVKVSCKASGGTFSS..YAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAGRLIFGV.....VITAGGDYGMDVWGQGTTVTVSS
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336 QVQLVQSGAEVKNPGASVKVSCKASGYTFIT..YAMHWVRQAPGQRLEWMGWINAGNGNTKYSQKFQGRVTITRDTSASTAYMELSSLRSEDTAVYYCAREQWLVLS.....YFDYWGQGTLVTVSS
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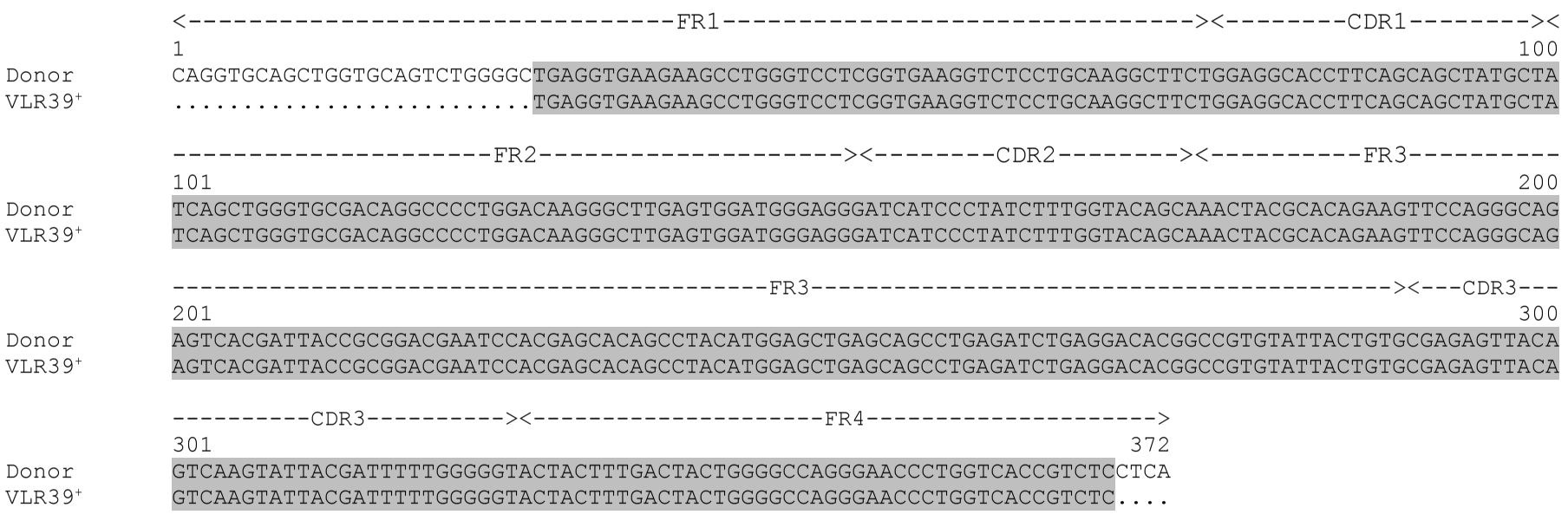
Figure S3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VLR39+ .....TGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTA
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101 200
Donor TCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCTTTGGTACAGCAAACTACGCACAGAAGTTCCAGGGCAG
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301 372
Donor GTCAAGTATTACGATTTTTGGGGGTACTACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
VLR39+ GTCAAGTATTACGATTTTTGGGGGTACTACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTC....

Figure S4

Table S2. Detection of CLL recurrence by quantitative real-time PCR

Months After End of CFAR Treatment	Donor CLL HCDR3 (C_T)	GAPDH (C_T)	ΔC_T	$\Delta(\Delta C_T)$	Fold Change	CLL HCDR3 Detection
38	not detectable	18.7 ± 0.1	0.00	0	0	negative
51	37.48	25.4 ± 0.4	12.10	2.02	0.25	weak positive
56	26.4 ± 0.1	18.4 ± 0.2	8.02	-2.02	4.05	positive