Supplemental Materials

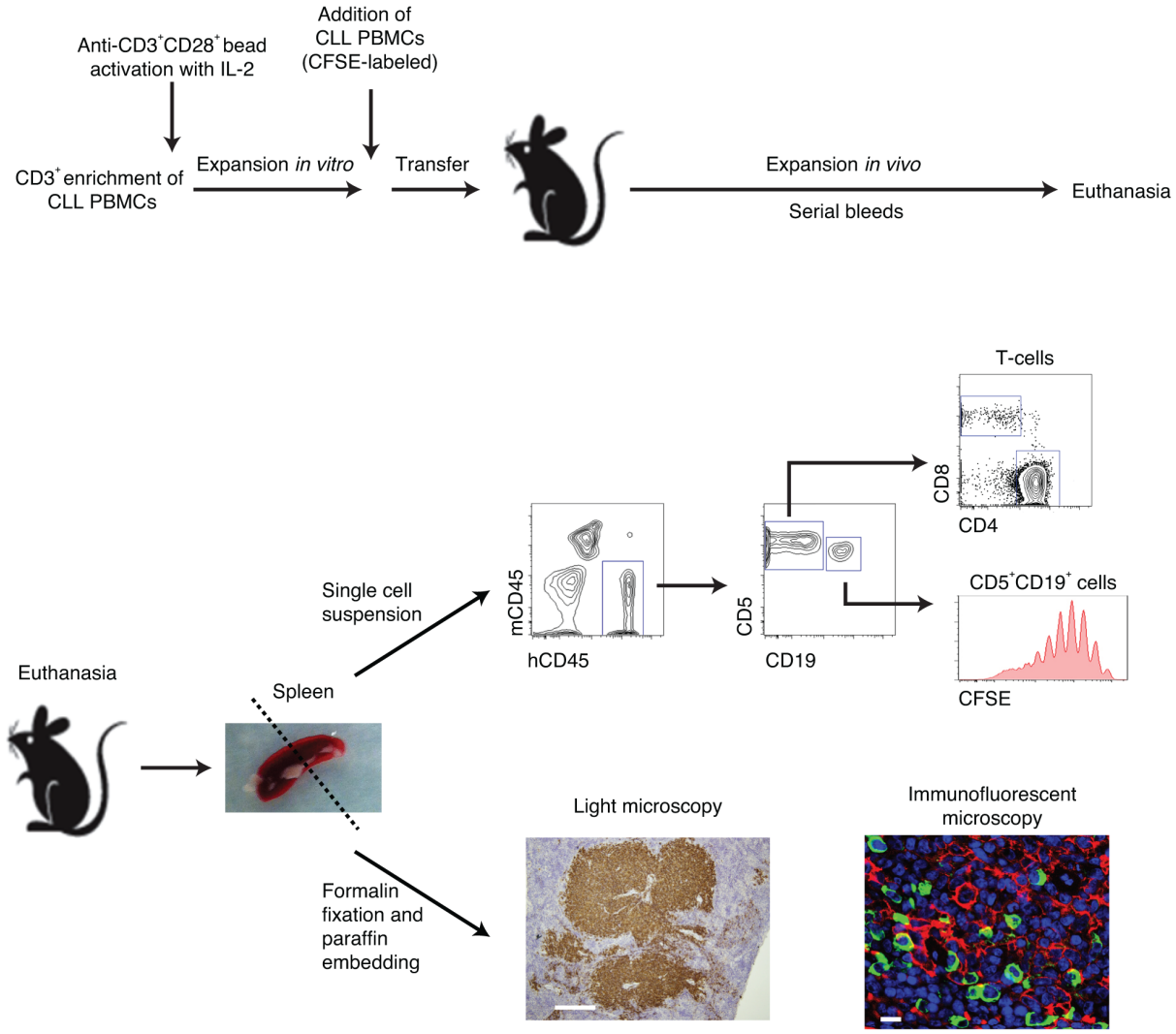

Figure S1. The methodology of xenogeneic transplantation is shown in the upper panel. CLL T cells were first obtained using anti-CD3 microbeads and then activated *in vitro* using anti-CD3/CD28 beads and IL-2. Following *in vitro* stimulation, activated $CD3^+$ cells were mixed with freshly thawed and CFSE-labelled CLL PBMCs and then transferred into NSG mice. Following a period of *in vivo* expansion of both B and T cells (see Table 1 and Table S2), tracked by peripheral blood sampling, mice were euthanized. The lower panel shows the methodology for analysis of splenic tissue by both FC, with the gating strategy indicated for identification of human B and T cells, and by IHC. Note that the gating strategy for identification of human PCs is shown in Figure 1A.

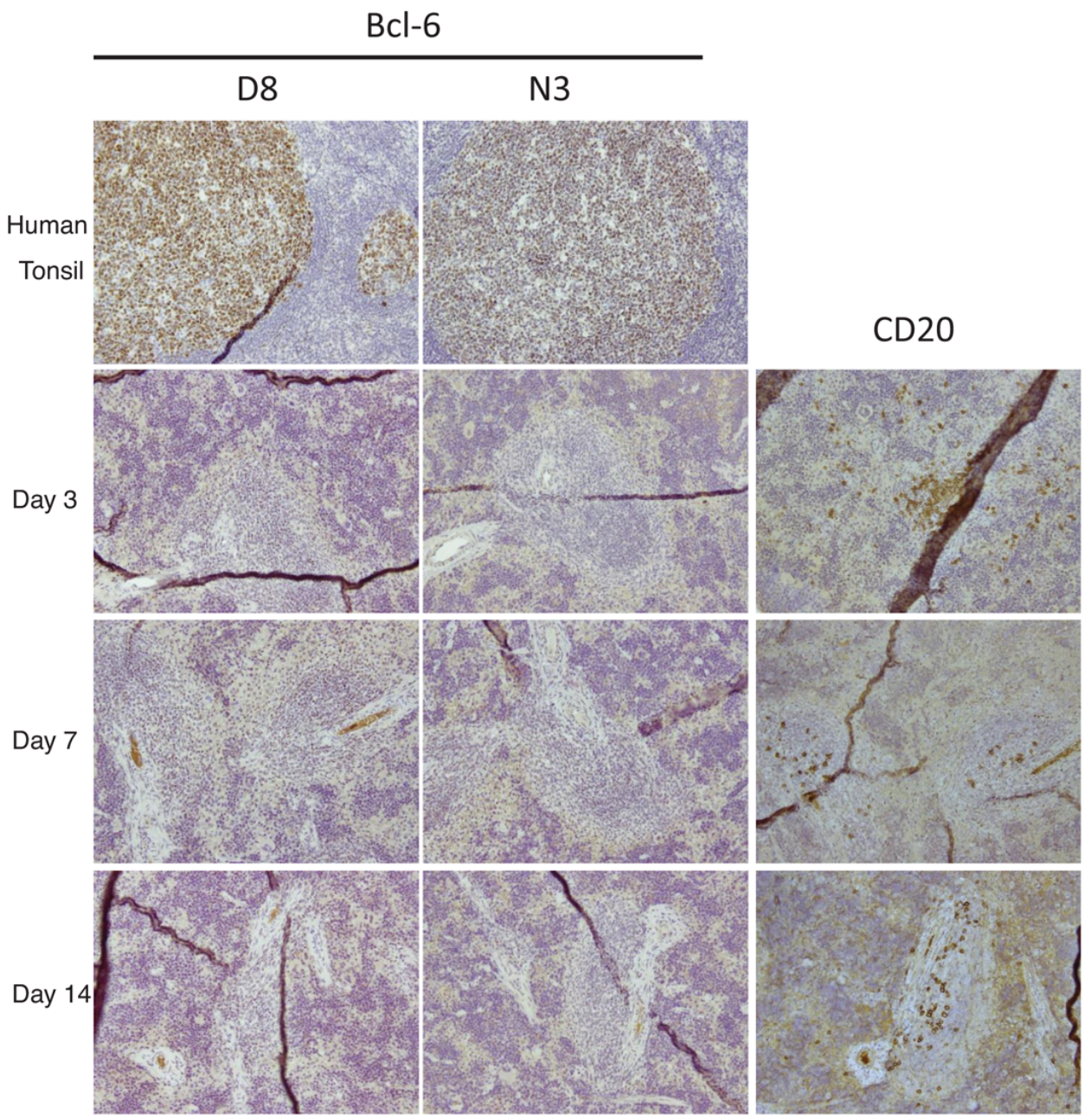

Figure S2. Bcl-6^{+} cells are not found in CD20^{+}PAX5^{+}PVAs using additional anti-human BCL-6 mAbs. Human tonsil tissue shows Bcl-6^{+} cells within normal GCs with anti-Bcl-6 (clone D-8) and anti-Bcl-6 (clone N-3) mAbs (Santa Cruz). These antibodies do not identify Bcl-6^{+} cells in PVAs at days 3, 7 and 14 after xenografting CLL cells.

Table S1. Ultra Deep Sequencing Data

Samples	U-CLL 0515		M-CLL 0854		U-CLL1083	
Isotype	IgM	IgM	IgM	IgM	IgM	IgM
Pre or Post (mouse number) Transfer	Pre	Post (1)	Pre	Post (1)	Pre	Post (1)
Total sequence reads	118,433	146,233	138,108	138,272	88,365	86,107
Minimum block reads	87,633	97,643	75,170	90,155	37,782	32,119
Block read length (bp)	396/396	396/396	413/413	413/413	193/220	193/220
Dominant clone reads	82,935	89,556	68,625	82,092	36,774	31,306
% Dominant clone / block reads	94.6%	91.7%	91.3%	91.1%	97.3%	97.5%
Total individual subclones	538	810	584	560	171	160
New unique subclones	-	408	-	120	-	82
Number of new unique subclones	-	3177	-	404	-	301
Total new unique subclones / block reads	-	3.25E-02	-	4.48E-03	-	9.37E-03
IGHV-D-J block read length (bp)	316/316	316/316	333/333	333/333	193/117	193/117
IGHV-D-J mutations in new unique subclones	-	165	-	125	-	74
IGHV-D-J mutation frequency minus background		6.06E-04		1.07E-04		1.90E-03
AID hotspot sites (HS)		36		41		34
AID HS mutations		25		13		15
Fold AID HS vs random mutation		1.3300		0.8447		1.8482
AID coldspot sites (CS)		57		53		64
AID CS mutations		31		21		15
Fold AID CS vs random mutation		1.0416		1.0555		0.9818

Total primer sequence reads are sequences with primer sequence at the 5' end
Minimum block reads = the number of reads with the minimum block read length, total of both forward and reverse.
Block read length = the sequence length of X/Y with X being the forward primer reads and Y being the reverse primer reads. Read lengths do not include primer sequence.
Dominant clone reads = the number of reads identical to original CLL clone.
% Dominant clone / block reads = frequency of dominant clone in the total block reads.
Total individual subclones = number of individual block reads that occur at least **2** times.
New unique subclones = the number of individual subclones Post-transfer that are not found in Pre-transfer sample.
Number of new unique subclones = the total number of new unique subclones sequence reads.
Total new unique subclones / block reads = a measure of the fraction of the total sequence reads represented by new unique subclones.
IGHV-D-J block read length = X/Y with X being the forward primer read and Y being the reverse primer read. All are full length except for the U-CLL1083 sample.
IGHV-D-J mutations in new unique subclones = the number of unique mutations found in *the* IGHV-D-J sequence of new unique subclones.
IGHV-D-J mutation frequency minus background = IGHV-D-J mutations in new unique subclones / (number of new unique subclones x IGHV-D-J bp length) - background.
Background = IGHM mutations in new unique subclones / (number of new unique subclones x IGHM bp length)
AID hotspot sites (HS) = number of WRC or GYW motifs in IGHV-D-J dominant clone sequence.
AID HS mutations = number of unique mutations found at WRC or GYW motifs in IGHV-D-J new unique subclone sequences.
Fold AID HS vs random mutation = (AID HS mutations / IGHV-D-J mutations in new unique subclones) / (AID hotspot sites / IGHV-D-J bp length)
AID coldspot sites (CS) = number of SYC or GRS motifs in IGHV-D-J dominant clone sequence.
AID CS mutations = number of unique mutations found at SYC or GRS motifs in IGHV-D-J new unique subclone sequences.

Table S1. Ultra Deep Sequencing Data (Continued)

Sample	U-CLL 1122							
Isotype	IgM	IgM	IgG	IgM	IgG	IgM	IgM	IgM
Pre or Post (mouse number) Transfer	Pre	Post (1)	Post (1)	Post (2)	Post (2)	Post (3)	Post (4)	Post (5)
Total sequence reads	153,719	191,628	94,972	115,303	93,903	126,314	135,997	148,673
Minimum block reads	117,347	146,912	67,252	89,991	65,422	98,089	107,176	116,556
Block read length (bp)	387/387	387/387	387/387	387/387	387/387	387/387	387/387	387/387
Dominant clone reads	110,715	135,488	43,891	81,615	50,303	89,714	95,541	100,696
% Dominant clone / block reads	94.3%	92.2%	65.3%	90.7%	76.9%	91.5%	89.1%	86.4%
Total individual subclones	563	666	910	630	644	600	657	813
New unique subclones	-	209	208	209	109	163	229	391
Number of new unique subclones	-	1432	1409	1191	792	974	2660	5787
Total new unique subclones / block reads	-	9.75E-03	2.10E-02	1.32E-02	1.21E-02	9.93E-03	2.48E-02	4.96E-02
IGHV-D-J block read length (bp)	307/307	307/307	307/307	307/307	307/307	307/307	307/307	307/307
IGHV-D-J mutations in new unique subclones	-	185	176	140	127	141	198	163
IGHV-D-J mutation frequency minus background		6.11E-04	4.83E-04	8.66E-04	2.48E-03	6.70E-04	1.18E-03	5.91E-04
AID hotspot sites (HS)		31	31	31	31	31	31	31
AID HS mutations		30	50	21	38	33	40	30
Fold AID HS vs random mutation		1.6059	2.8134	1.4855	2.9632	2.3178	2.0007	1.8227
AID coldspot sites (CS)		50	50	50	50	50	50	50
AID CS mutations		23	22	15	16	16	30	20
Fold AID CS vs random mutation		0.7634	0.7675	0.6579	0.7735	0.6967	0.9303	0.7534

Table S1. Ultra Deep Sequencing Data (Continued)

Sample	M-CLL 1164					
Isotype	IgM	IgM	IgG	IgM	IgG	IgM
Pre or Post (mouse number) Transfer	Pre	Post (1)	Post (1)	Post (2)	Post (2)	Post (3)
Total sequence reads	164,953	110,727	100,863	119,144	94,146	157,831
Minimum block reads	124,273	78,936	69,385	20,463	59,461	112,774
Block read length (bp)	393/393	393/393	393/393	393/393	393/393	393/393
Dominant clone reads	119,665	74,590	39,341	17,515	34,320	98,877
% Dominant clone / block reads	96.3%	94.5%	56.7%	85.6%	57.7%	87.7%
Total individual subclones	537	491	737	332	779	740
New unique subclones	-	112	106	137	170	342
Number of new unique subclones	-	356	557	992	698	1373
Total new unique subclones / block reads	-	4.51E-03	8.03E-03	4.85E-02	1.17E-02	1.22E-02
IGHV-D-J block read length (bp)	313/313	313/313	313/313	313/313	313/313	313/313
IGHV-D-J mutations in new unique subclones	-	115	166	102	183	249
IGHV-D-J mutation frequency minus background		1.38E-03	3.11E-03	2.20E-03	3.26E-03	7.91E-04
AID hotspot sites (HS)		29	29	29	29	29
AID HS mutations		17	28	20	30	40
Fold AID HS vs random mutation		1.5955	1.8205	2.1163	1.7694	1.7338
AID coldspot sites (CS)		50	50	50	50	50
AID CS mutations		9	18	3	20	27
Fold AID CS vs random mutation		0.4899	0.6788	0.1841	0.6842	0.6788

Table S1. Ultra Deep Sequencing Data (Continued)

Samples	U-CLL 1279				M-CLL 1623	
Isotype	IgM	IgM	IgG	IgA	IgM	IgM
Pre or Post (mouse number) Transfer	Pre	Post (1)	Post (1)	Post (1)	Pre	Post (1)
Total sequence reads	87,867	190,772	70,197	193,676	97,678	96,292
Minimum block reads	57,669	134,724	40,689	142,563	53,439	42,451
Block read length (bp)	410/410	410/410	410/410	347/347	409/409	409/409
Dominant clone reads	54,486	125,733	29,997	127,567	50,386	40,265
% Dominant clone / block reads	94.5%	93.3%	73.7%	89.5%	94.3%	94.9%
Total individual subclones	477	714	517	646	410	362
New unique subclones	-	284	78	256	-	99
Number of new unique subclones	-	1330	647	5409	-	584
Total new unique subclones / block reads	-	9.87E-03	1.59E-02	3.79E-02	-	1.38E-02
IGHV-D-J block read length (bp)	330/330	330/330	330/330	330/330	329/329	329/329
IGHV-D-J mutations in new unique subclones	-	254	85	253	-	107
IGHV-D-J mutation frequency minus background		2.89E-04	8.81E-04	5.74E-04		2.40E-03
AID hotspot sites (HS)		38	38	38		28
AID HS mutations		29	15	37		11
Fold AID HS vs random mutation		0.9915	1.5325	1.2700		1.2079
AID coldspot sites (CS)		57	57	57		55
AID CS mutations		41	8	40		11
Fold AID CS vs random mutation		0.9345	0.5449	0.9153		0.6150

Table S2

Characteristics of CLL samples transferred into mice euthanized at or before 28 days after xenografting

Patient sample (Gender)	Ig isotype	*IGHV*	*IGHV* mutation status[2]	Cytogenetic abnormality defined by FISH	CD38%	Number of NSG recipients	Days at assessment (number of recipients in brackets)
0827(M)	Unknown	3-23	M-CLL	none detected	3.2	15	3 (5), 7(5), 14(5)
1024(F)	IgM	3-30	M-CLL	none detetced	7.3	15	3 (5), 7(5), 14(5)
1083[1](M)	IgM	4-38-2	U-CLL	del13q14.3; del11q23.3	62	3	14(1), 21(1), 28(1)
1122[1](M)	IgM	3-09	U-CLL	tri12	77	20	3(5), 7(5), 14(5), 21(5)
1164[1](M)	IgM	4-34	M-CLL	none detected	99	20	3(5), 7(5), 14(5), 21(5
1301[1](M)	IgM	4-31	U-CLL	tri12	88	3	14(1), 21(1), 28(1)
1523[1](M)	IgM	3-48	U-CLL	del13q14.3	20	2	14(1), 21(1)

1: Mice also used for studies with euthanasia after day 28 (see Table 1)
2: M-CLL if *IGHV* sequence is >2% different from most similar germline gene; U-CLL if *IGHV* sequence is ≤2% different from germline gene

Table S3

Antibodies used for surface labelling and intracellular labelling of cells in FC

Surface labelling

Target Antigen	Species	Isotype	Clone	Company	Fluorochrome
CD4	Mouse	IgG1	RPA-T4	BD Biosciences	PE
CD4	Mouse	IgG1	RPA-T4	BD Biosciences	APC-H7
CD5	Mouse	IgG1	UCHT2	Biolegend	PE-Cy7
CD8	Mouse	IgG1	RPA-T8	BD Biosciences	V450
CD8	Mouse	IgG1	SK1	BD Biosciences	APC-H7
CD8	Mouse	IgG1	SK1	BD Biosciences	APC
CD19	Mouse	IgG1	HIB19	BD Biosciences	APC
CD19	Mouse	IgG1	J3-119	Beckman Coulter	ECD
CD38	Mouse	IgG1	HIT2	BD Biosciences	A700
CD45 (human)	Mouse	IgG1	H130	Invitrogen	PO
CD45 (mouse)	Rat	IgG2b (Rat)	30-F11	BD Biosciences	PerCP
CD45 (mouse)	Rat	IgG2b (Rat)	30-F11	BD Biosciences	PE-Cy7
CD45 RA	Mouse	IgG2b	H1100	BD Biosciences	PE-Cy7
CD57	Mouse	IgM	NK-1	BD Biosciences	APC
CD138	Mouse	IgG1	MI15	BD Biosciences	CD138
CCR7	Mouse	IgG2A	150503	R&D	PE
CXCR5	Mouse	IgG2b	51505	R&D	FITC
ICOS	Mouse	IgG1	ISA-3	Ebioscience	PE-Cy7
PD-1 (CD279)	Mouse	IgG1	MIH4	Ebioscience	PE

Intracellular labelling

Target Antigen	Species	Isotype	Clone	Company	
Bcl-6	Mouse	IgG1	BCL-UP	eBioscience	APC
T-Bet	Mouse	IgG1	4B10	eBioscience	PE
AID	Rat	IgG2b (Rat)	EK2 5G9	Cell signaling	Unconjugated
IFN-γ	Mouse	IgG1	B27	BD Biosciences	PE
Isotype controls					
PE-Isotype	Mouse	IgG1	MOPC-21	BD Biosciences	PE
APC-Isotype	Mouse	IgG1	X40	BD Biosciences	APC
PE-Isotype	Mouse	IgG1	P3.6.2.8.1	eBioscience	PE
Isotype	Rat	IgG2b (Rat)	clone KLH/G2b-1-2	Southern Biotechnology	

Following AID labelling, R-PE-conjugated goat $F(ab')_2$ anti-rat IgG (H+L) from Southern Biotechnology was used

Table S4

Antibodies used for microscopy

Target Antigen	Species	Isotype	Clone	Company
AID	Mouse	IgG1	ZA-001	Invitrogen
Bcl-6	Mouse	IgG1	PG-B6p	Dako
Bcl-6	Mouse	IgG3	D-8	Santa Cruz
Bcl-6	Rabbit	Polyclonal	N-3	Santa Cruz
Bimp-1	Mouse	IgG1	3H2-E8	Abcam
CD3	Rat	IgG1	CD3-12	AbD Serotec
CD4	Mouse	IgG1	4B12	Leica
CD8	Mouse	IgG1	1A5	Leica
CD20	Mouse	IgG2a	L26	Dako
CD38	Mouse	IgG1	SPC32	Leica
CD57	Mouse	IgM	TB01	Dako
CD138	Mouse	IgG1	MI15	Dako
Human Ig λ	Rabbit	Polyclonal		Dako
Human Ig κ	Rabbit	Polyclonal		Dako
Human Ig γ	Rabbit	Polyclonal		Dako
Human Ig μ	Rabbit	Polyclonal		Dako
ICOS	Rabbit	IgG	SP98	Abcam
IRF4	Mouse	IgG1	MUM1p	Abcam
Ki67	Mouse	IgG1	MIB-1	Dako
Ki67	Rabbit	IgG	SP6	Abcam
PAX5	Mouse	IgG1	DAK-Pax5	Abcam
PD-1	Mouse	IgG1	NAT105	Abcam
Plasma Cell	Mouse	IgG1	VS38c	Dako
T-Bet	Rabbit	IgG	EPR9302	Abcam

For light microscopy, antibodies were visualized using the Vectorstain ABC system with ImmPACT DAB substrate and counterstaining with hematoxylin (All Vector Laboratories).

For immunofluorescent microscopy, antibodies were visualized with appropriate donkey whole IgG affinity purified antibodies multiply absorbed against alternate species conjugated to DyLightTM 488, 594 and 649 (All Jackson Immunoresearch).