Supplementary Information
for
"Somatic mutation as a mechanism of Wnt/β-catenin pathway activation in chronic lymphocytic leukemia"

Contents

METHODS

Whole-genome and -exome DNA sequencing

In brief, whole genome shotgun (WG) and whole exome (WE) capture libraries were constructed. Whole genome libraries were sequenced on an average of 39 lanes of an Illumina GA-II sequencer, using 101 bp paired-end reads, with the aim of reaching 30X genomic coverage of distinct molecules per sample.[1,2] Exome sequencing libraries were sequenced on three lanes of the same instrument, using 76 bp paired-end reads. Sequencing data subsequently was processed using the "Picard" pipeline, developed at the Broad Institute's Sequencing Platform (Fennell T, unpublished; Cambridge, MA), which includes base-quality recalibration,[3] alignment to the NCBI Human Reference Genome Build hg18 using MAQ,[4] and aggregation of lane- and library-level data.

Plasmid constructs

DNA plasmids encoding *DKK2*, *BCL9,* (Human Orfeome Collection, DFCI Center for Cancer Systems Biology), *RYK, CSNK1E* (ORIGENE, Rockville, MD), *WNT1*, *WNT10A* (gift from Xi He, Children's Hospital Boston) and *FZD5* were subjected to site-directed mutagenesis (QuickChange Site-Directed Mutagenesis Kit, Stratagene, La Jolla, CA) to generate constructs bearing the patient mutations. Wildtype and mutant *DKK2* cDNA sequences were subcloned into the expression vector N-terminal p3×FLAG-CMV (Sigma, St. Louis, MO) or C-terminal FLAG (pCB2). Wildtype and mutant *BCL9* cDNA sequences were subcloned into pcDNA3.1 (Invitrogen, Carlsbad, CA). *RYK* and *CSNK1E* cDNA sequences were subcloned in a vector with a C-terminal DDK tag (pCMV6-

ENTRY). *FZD5* was amplified with cDNA from normal donor and subcloned into a pcDNA3.1 directional TOPO expression vector with a C-terminal V5 tag. *WNT1* and *WNT10A* sequences are in pcDNA3.1 vector with a C-terminal V5 tag.

Microarray data analysis

All expression profiles were processed using RMA (Robust Multi-array Average)[5], implemented by the ExpressionFileCreator module in GenePattern[5,6] and Affymetrix probes were collapsed to unique genes (Gene Symbol) by selecting the probe with the maximal average expression for each gene. Batch effects were removed using the ComBat module[7] in GenePattern.[6] Differential expression between normal and CLL B-cell samples was analyzed using the ComparativeMarkerSelection and ExtractComparativeMarkerResults modules in GenePattern.[6,8] We identified Wnt pathway genes that were differentially expressed between mutated and unmutated CLL-B cells with a permutation *t* test using the ComparativeMarkerSelection module in GenePattern.[6,8] We devised a 'Wnt score' as a statistical measure of differential expression between a set of 37 known Wnt activators versus the 23 known repressors. We generated a *t* test score for each CLL sample by comparing the differential expression of activators that are upregulated to repressors that are downregulated in each sample according to FDR-corrected permutation test *P*-values assessing significance of *t*-test scores, with FDR≤0.05. We performed unsupervised and supervised hierarchical clustering by extracting Wnt pathway genes from a total of 70 samples (12 mutated, 58 unmutated CLL RNAs).

Detection of Wnt activation

Activation of the Wnt pathway was detected by the following assays:

- ***Wnt reporter assay***

Wnt reporter assay was measured by using the reporter plasmids SuperTOPflash and pRL-TK. For measuring Wnt pathway activation in HEK293T cells, 1×10^5 cells were plated/well of a 24-well plate. 24 hours later, 200ng SuperTOPflash, 20ng pRL-TK, together with expression plasmids encoding Wnt1 and Wnt pathway members (NT and/or MUT), were introduced into cells (Lipofectamine 2000; Invitrogen, Carlsbad, CA). For each experiment, a matrix of plasmid amounts between Wnt1 (from 0 to 10ng) and the pathway gene (from 0 to 100ng) was run. For some experiments, recombinant Wnt3a (R&D Systems, Minneapolis, MN) was added 24 hours after the transfection. For characterization of *FZD5* function, 10ng of *LRP6* expression plasmid (gift from Xi He, Children's Hospital Boston) was also included. After 48 hours, cells were lysed and their luciferase activity assayed (Dual–Luciferase Reporter Assay System; Promega, Madison, WI) using a microplate luminometer (WALLAC 1420 Multilabel Counter, Perkin Elmer Life Science, Turku, Finland). Firefly luciferase levels were normalized against Renilla luciferase activity before normalization with an empty vector control.

For measuring Wnt activity in primary B cells, 5 million freshly isolated $CD19^+$ normal B or CLL-B cells were nucleofected with SuperTOPflash (20μg) and pRL-TK (2 μg) plasmids, with or without 1μg Wnt1 expression plasmid (Amaxa; Lonza, Walkersville, MD; U-017, B buffer). Immediately following nucleofection, cells were cultured for 24

hours in B cell media.[9,10] Live cells were isolated by Ficoll/Hypaque centrifugation and lysed prior to measurement of luciferase activity. In other experiments, luciferase-reporter transfected normal B cells were cultured in media containing either wildtype or mutant DKK2 protein supernatant for 24 hours before measuring luciferase activity. Supernatants were obtained from HEK293T cells transfected with wildtype or mutant *DKK2* expression plasmid and were concentrated (4ºC, 3000 rpm) in centricon tubes (Millipore, Billerica, MA), with 10kD size exclusion.

- ***Quantitative RT-PCR of downstream Wnt targets***

Quantitative RT-PCR was used to evaluate siRNA silencing efficiency and Wnt pathway downstream targets expression. RNA and cDNA were prepared as previously reported.[11].Taqman RT-PCR assays for *DKK2, AXIN2, JUN, DVL1, CTNNB1* and *LEF1* were purchased from Life Technologies (Applied Biosystems, Foster City, CA) and assays were performed using 7500 Fast Real-time PCR cycler (Applied Biosystems, Foster City, CA).

- ***Immunoblotting of downstream Wnt targets***

Immunoblotting was performed as previously described.[12] Total cell lysates were prepared by lysing cells using RIPA buffer (50mM Tris-HCl, pH7.4, 150mM NaCl, 1%NP-40, 0.5% sodium deoxycholate, 0.1% SDS and 1mM EDTA) supplemented with protease inhibitors and phosphatase inhibitors, followed by centrifugation at 20,000g for 10 minutes. Equal amount of proteins were resolved by SDS-PAGE, transferred to nitrocellulose membranes, and incubated with primary antibodies overnight at 4°C.

Secondary antibodies conjugated with horseradish peroxidase were used for signal visualization by ECL film. The sources of primary antibodies were: anti-DVL2 (Dallas, TX) anti-V5 (Life technologies, Grand Island, NY), anti-DDK (ORIGENE, Rockville, MD).

Immunofluorescence staining and imaging

Normal CD19+ B cells or CLL-B cells were treated with either non-targeting siRNA or gene-specific siRNA via NWs for 48 hours before staining. Cells on top of NWs were washed with PBS briefly and then fixed with 4% formaldehyde in PBS for 10 minutes followed by washing with PBS three times. Next, the fixed cells on NWs were blocked in a PBS buffer contained 10% goat serum (Life Technologies, Grand Island, NY), 0.25% Triton X-100 for 1 hour at room temperature and then probed with primary antibody at 1:100 dilution in a PBS buffer contained 3% BSA, 0.2% Triton X-100 (Ab Buffer) for overnight at 4°C. The next day, the cells was washed for three times with PBS and then probed with Alexa-Fluor 546 labeled goat anti-rabbit antibody at 1:250 in Ab Buffer for one hour (Life Technologies, Grand Island, NY). After extensive rinsing with PBS, the cells were stained with DAPI for 5 minutes prior to imaging. Antibodies against LEF1 (ab53293), DVL1 (ab170694), BCL9 (ab37305), RYK (ab5513), FZD5 (ab75234), CSNK1E (ab70110) were purchased from Abcam (Cambridge, MA). Anti-CTNNB1 antibody (AV14001) was purchased from Sigma (St. Louis, MO). Anti-DVL2 antibody (3216) was purchased from Cell Signaling Technology (Danvers, MA).

Statistical considerations

Regarding the experiments to compare cell viability following siRNA-mediated silencing

for *DVL1, CTNNB1,* or *LEF1* in both normal and CLL-B cells, we used Welch t-test (two-sided) since the variability of one group differed substantially as indicated by the error bars (**Figure 4F**). The use of the Welch test allows the variability to differ between groups, unlike a Student t-test; if the variances are similar the Welch t-test converges to the Student t-test.

In a separate set of experiments, cell viability was examined after gene silencing of *BCL9* (**Figure 6A**) or *DKK2* (**Figure 6B**), a single patient sample with either *DKK2* or *BCL9* mutation compared to a group of CLL samples. These experiments were performed twice in triplicate. The variability between groups was similar which supported the use of a Student t-test. Furthermore, since we hypothesized a decrease in cell viability relative to control for those patients with a mutation in *BCL9* or *DKK2* compared to WT, we further performed a one-sided test.

For experiments examining gene silencing of *RYK*, *CSNK1E*, *FZD5*, *WNT1* and *WNT10A* in CLL samples, measurements were performed once in triplicate for a single patient due to limited availability of samples. In this setting, we used a 95% CI in lieu of formal testing; a p-value<0.05 denotes the exclusion for the average of the triplicates samples for the patient (n=1) with a mutation from the 95% CI of the CLL without mutation group.

Table S1. Wnt pathway gene list used to perform mutation significance (MutSig) analysis. Highlighted genes were found to be mutated by sequencing. Genes were selected based on database demonstrating robust experimental evidence of involvement with core Wnt pathway members. (http://www.stanford.edu/group/nusselab/cgi-bin/wnt/; http://www.sabiosciences.com/rt_pcr_product/HTML/PAHS-043A.html; http://www.string-db.org).

Gene Symbol	Gene Name	HGNC ID
APC	adenomatous polyposis coli	HGNC:583
APC2	adenomatosis polyposis coli 2	HGNC:24036
AXIN1	axin 1	HGNC:903
AXIN2	axin 2	HGNC:904
BCL9	B-cell CLL/lymphoma 9	HGNC:1008
BRD7	bromodomain containing 7	HGNC:14310
BTRC	beta-transducin repeat containing	HGNC:1144
CBY1	chibby homolog 1 (Drosophila)	HGNC:1307
CHD8	chromodomain helicase DNA binding protein 8	HGNC:20153
CREBBP	CREB binding protein	HGNC:2348
CSNK1A1	casein kinase 1, alpha 1	HGNC:2451
CSNK1E	casein kinase 1, epsilon	HGNC:2453
CTNNB1	catenin (cadherin-associated protein), beta 1, 88kDa	HGNC:2514
CTNNBIP1	catenin, beta interacting protein 1	HGNC:16913
CXXC4	CXXC finger protein 4	HGNC:24593
DAAM1	dishevelled associated activator of morphogenesis 1	HGNC:18142
DIXDC1	DIX domain containing 1	HGNC:23695
DKK1	dickkopf homolog 1 (Xenopus laevis)	HGNC:2891
DKK2	dickkopf homolog 2 (Xenopus laevis)	HGNC:2892
DVL1	dishevelled, dsh homolog 1 (Drosophila)	HGNC:3084
DVL2	dishevelled, dsh homolog 2 (Drosophila)	HGNC:3086
DVL3	dishevelled, dsh homolog 3 (Drosophila)	HGNC:3087
FRAT1	frequently rearranged in advanced T-cell lymphomas	HGNC:3944
FRAT2	frequently rearranged in advanced T-cell lymphomas 2	HGNC:16048
FZD1	frizzled homolog 1 (Drosophila)	HGNC:4038
FZD4	frizzled homolog 4 (Drosophila)	HGNC:4042
FZD5	frizzled homolog 5 (Drosophila)	HGNC:4043
FZD8	frizzled homolog 8 (Drosophila)	HGNC:4046
FZD9	frizzled homolog 9 (Drosophila)	HGNC:4047
GSK3A	glycogen synthase kinase 3 alpha	HGNC:4616
GSK3B	glycogen synthase kinase 3 beta	HGNC:4617
INVS	inversin	HGNC:17870
KREMEN1	kringle containing transmembrane protein 1	HGNC:17550
KREMEN2	kringle containing transmembrane protein 2	HGNC:18797
LEF1	lymphoid enhancer-binding factor 1	HGNC:6551
LRP5	low density lipoprotein receptor-related protein 5	HGNC:6697
LRP6	low density lipoprotein receptor-related protein 6	HGNC:6698
LRRFIP2	leucine rich repeat (in FLII) interacting protein 2	HGNC:6703
NDP	Norrie disease (pseudoglioma)	HGNC:7678
NKD1	naked cuticle homolog 1 (Drosophila)	HGNC:17045
NKD2	naked cuticle homolog 2 (Drosophila)	HGNC:17046
NLK	nemo-like kinase	HGNC:29858
PITX2	paired-like homeodomain 2	HGNC:9005
PORCN	porcupine homolog (Drosophila)	HGNC:17652

Gene Symbol	Gene Name	HGNC ID
PRICKLE1	prickle homolog 1 (Drosophila)	HGNC:17019
PSEN1	presenilin 1	HGNC:9508
PYGO1	pygopus homolog 1 (Drosophila)	HGNC:30256
RSPO4	R-spondin family, member 4	HGNC:16175
RYK	RYK receptor-like tyrosine kinase	HGNC:10481
SFRP1	secreted frizzled-related protein 1	HGNC:10776
SFRP2	secreted frizzled-related protein 2	HGNC:10777
SOX17	SRY (sex determining region Y)-box 17	HGNC:18122
TCF4	transcription factor 4	HGNC:11634
TCF7	transcription factor 7 (T-cell specific, HMG-box)	HGNC:11639
TCF7L1	transcription factor 7-like 1 (T-cell specific, HMG-box)	HGNC:11640
TCF7L2	transcription factor 7-like 2 (T-cell specific, HMG-box)	HGNC:11641
TLE1	transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	HGNC:11837
TMEM132A	transmembrane protein 132A	HGNC:31092
WNT1	wingless-type MMTV integration site family, member 1	HGNC:12774
WNT2	wingless-type MMTV integration site family member 2	HGNC:12780
WNT3	wingless-type MMTV integration site family, member 3	HGNC:12782
WNT4	wingless-type MMTV integration site family, member 4	HGNC:12783
WNT5A	wingless-type MMTV integration site family, member 5A	HGNC:12784
WNT7A	wingless-type MMTV integration site family, member 7A	HGNC:12786
WNT10A	wingless-type MMTV integration site family, member 10A	HGNC:13829
ZBTB33	zinc finger and BTB domain containing 33	HGNC:16682

Table S2. Primers and probes used for pyrosequencing

Target gene	Sequence (5'→3')	
FZD5	Forward	TTCCTGTCAGCCTGCTACCTGT
	Reverse	**Bio**-AACCAGGTGAGCGACAGGATGA
	Probe	CTTCCTGGTGCGTC
BCL9	Forward	ACATGCCACATTCTCTGCC
	Reverse	**Bio**-GGCCCTTCCATTTCAGAGTTT
	Probe	ACATTCTCTGCCCCC
RYK	Forward	**Bio**-AGAAGGGAAGTCATCCACAAAGA
	Reverse	AACTGGCCTGTTTTCATTGTTCC
	Probe	GAACAACAAGTCTCTGGA
BRD7	Forward	GCGGCTTGTGAACAGTCAG
	Reverse	**Bio**-CAGTAGCCTGGCTCTCCTACA
	Probe	GAACAGTCAGTGCGAAT
DKK2	Forward	**Bio**-CACATATAAAGGGCATGAAGG
	Reverse	TTCTTGCGTTGTTTGGTA
	Probe	GGGTTTGCTGTGC

Table S3. Clinical characteristics of the samples with or without Wnt pathway mutations among 91 CLL samples that underwent whole tumor sequencing.

Clinical characteristics of patients undergo whole exome sequencing			
	Wnt pathway mutations absent N (%)	**Wnt pathway mutation present N (%)**	**p-value**
N	78 (86)	13 (14)	
Age at Diagnosis ≥55 yrs. (%)	32 (41)	8 (62)	0.23
Sex			
Female	30 (38)	5 (38)	0.99
Male	48 (62)	8 (62)	
Rai Stage at Sample			
0-2	67 (86)	13 (100)	0.35
3-4	11 (14)	0 (0)	
Time from Dx to 1st Therapy (months), median (range)	24 (0.4, 154)	57 (1.6, 110)	0.21
# Patients initiating first therapy	48 (62)	10 (77)	0.36
IGHV			
Mutated	32 (41)	6 (46)	0.99
Unmutated	34 (44)	6 (46)	
Unknown	12 (15)	1 (8)	
ZAP-70			
Positive	30 (38)	8 (62)	0.21
Negative	40 (51)	4 (31)	
Unknown	8 (10)	1 (8)	
FISH Cytogenetics			
del (13q-) het	48 (58)	8 (62)	0.99
del (13q-)homo	11 (14)	1 (8)	0.99
trisomy 12	12 (15)	1 (8)	0.68
del (11q)	18 (23)	4 (31)	0.51
del(17p)	12 (15)	5 (38)	0.062
mutated *TP53* alone or mutated *TP53*+del(17p)	15 (19)	5 (38)	0.15

Table S4. Results from studying differential gene expression of 132 Wnt pathway members in normal B cells and CLL-B cells. Shaded genes are significantly differentially expressed between normal and CLL-B cells (FDR<0.05).

Rank	Feature	Score	Feature P	FDR (BH)	Fold Change	Normal Mean	Normal Std	CLL Mean	CLL Std	Activator Repressor
176	SMAD3	13.61	0.002	0.008	4.63	1533.05	425.34	331.17	217.08	Activator
1681	HBP1	6.47	0.002	0.008	1.36	1769.04	333.17	1301.58	325.78	repressor
2746	SOX4	5.12	0.002	0.008	7.41	601.25	495.09	81.18	145.74	Repressor
2771	SMARCA4	5.09	0.002	0.008	1.15	659.34	75.36	573.33	93.49	Activator
2785	NKD1	5.08	0.002	0.008	1.15	101.15	11.94	87.61	14.39	Repressor
2831	KREMEN2	5.05	0.002	0.008	1.17	305.69	41.75	261.11	31.25	Repressor
3202	AES	4.70	0.002	0.008	1.25	3410.62	671.48	2732.26	608.13	Repressor
3343	NKD2	4.58	0.002	0.008	1.18	221.03	35.65	186.56	25.43	Repressor
4372	SENP2	3.89	0.002	0.008	1.15	339.65	52.64	294.69	56.72	Repressor
4638	FGF4	3.77	0.002	0.008	1.12	237.23	31.93	210.93	33.51	Neutral or Unknown
4731	SOSTDC1	3.72	0.002	0.008	1.13	61.32	8.70	54.34	8.08	Repressor
4811	DVL1	3.69	0.006	0.018	1.14	229.21	34.14	200.25	48.36	Activator
5054	TCF7L1	3.57	0.002	0.008	1.14	75.60	12.31	66.11	11.55	Activator
5222	HNF1A	3.49	0.002	0.008	1.10	189.26	21.93	172.71	21.20	Activator
5317	BTRC	3.45	0.002	0.008	1.12	291.21	42.22	260.28	33.24	Repressor
5958	FRAT1	3.19	0.002	0.008	1.26	285.58	87.41	226.81	60.56	Activator
6050	DKK4	3.16	0.002	0.008	1.10	85.50	10.86	77.84	13.13	Repressor
6423	LRP5	3.03	0.004	0.013	1.10	148.85	21.19	134.88	21.49	Activator
6595	TBP	2.97	0.010	0.028	1.11	405.16	64.46	363.51	64.83	Neutral or unknown
7376	LRP6	2.71	0.006	0.018	1.13	189.67	38.11	167.48	33.76	Activator
7610	FZD8	2.64	0.008	0.023	1.11	199.64	36.30	179.26	29.31	Activator
7773	WNT8A	2.59	0.008	0.023	1.10	74.06	11.84	67.59	8.62	Activator
7814	CHD8	2.58	0.010	0.028	1.07	825.53	92.02	773.46	99.45	Repressor
7885	CPZ	2.56	0.006	0.018	1.10	198.10	32.65	180.21	28.50	Activator
7937	NLK	2.54	0.018	0.045	1.16	239.16	60.55	206.50	47.14	Repressor
7972	PPP2CA	2.53	0.008	0.023	1.14	1916.79	442.44	1674.20	425.66	Repressor
8569	WNT11	2.37	0.012	0.032	1.08	111.55	14.99	103.74	16.20	Activator
9155	TGFB1I1	2.23	0.010	0.028	1.17	48.87	14.36	41.87	15.11	Activator
9157	FZD5	2.23	0.006	0.018	1.14	52.69	13.73	46.31	8.01	Activator
9446	TLE2	2.15	0.018	0.045	1.07	342.46	50.50	318.84	50.22	Repressor
9461	ROR2	2.15	0.024	0.056	1.07	23.10	3.32	21.59	2.37	Activator
9546	TLE1	2.13	0.020	0.049	1.32	1350.82	694.41	1025.16	776.38	Repressor
9609	RARA	2.11	0.004	0.013	1.49	334.03	233.15	224.73	271.98	Repressor
9670	CREBBP	2.10	0.014	0.037	1.08	1420.87	213.06	1318.57	296.71	Activator
9822	DKK2	2.06	0.038	0.082	1.05	16.19	1.56	15.47	1.85	Repressor
10760	CTNNB1	1.84	0.070	0.135	1.10	824.60	182.55	751.86	179.61	activator
11248	WISP1	1.73	0.072	0.138	1.07	105.23	18.00	98.62	13.71	Activator
11272	DAAM1	1.73	0.070	0.135	1.07	338.68	57.67	317.00	57.90	Activator
11308	WNT6	1.72	0.068	0.132	1.07	597.68	103.21	559.64	88.67	Activator
11337	PPP2R1A	1.72	0.090	0.165	1.06	889.27	147.16	835.23	125.92	Repressor
11839	NDP	1.62	0.090	0.165	1.05	17.60	2.52	16.73	2.07	Activator
12041	INVS	1.58	0.128	0.218	1.08	216.78	46.45	200.75	48.40	Activator
12552	TCF3	1.47	0.140	0.234	1.06	3263.49	571.43	3073.20	749.48	Activator
12912	TMEM132A	1.40	0.126	0.215	1.05	214.65	37.98	203.47	25.91	Activator
12994	PYGO1	1.38	0.164	0.265	1.04	45.87	4.97	44.27	7.43	Activator
13084	WNT7B	1.37	0.144	0.239	1.04	313.31	35.63	302.57	39.58	Activator
13112	FRAT2	1.36	0.130	0.221	1.14	291.17	124.75	254.85	106.41	Activator
13430	CSNK1A1	1.30	0.208	0.319	1.08	1497.00	385.63	1389.86	334.45	Repressor
14209	RSPO4	1.15	0.238	0.354	1.03	130.04	16.56	125.89	17.04	Activator
14239	SFRP1	1.14	0.220	0.334	1.04	95.78	12.79	92.47	16.63	Repressor
14325	FZD9	1.13	0.222	0.336	1.04	87.54	12.70	84.43	12.50	Activator
15020	CTBP1	1.00	0.325	0.451	1.03	3756.51	519.75	3637.88	722.96	Repressor
15287	FZD4	0.95	0.295	0.419	1.03	137.21	18.43	133.40	19.34	Activator
15635	BRD7	0.88	0.355	0.482	1.03	902.37	145.38	874.51	142.44	Repressor
16052	DKK3	0.80	0.301	0.426	1.04	55.25	11.64	53.08	16.98	Repressor

Rank	Feature	Score	Feature P	FDR (BH)	Fold Change	Normal Mean	Normal Std	CLL Mean	CLL Std	Activator Repressor
16076	WNT7A	0.80	0.441	0.569	1.03	149.81	28.98	144.88	23.42	Activator
16215	TLE3	0.77	0.425	0.553	1.06	747.29	269.87	702.50	239.59	Repressor
16305	APC2	0.76	0.457	0.583	1.03	131.49	25.84	127.33	21.22	Repressor
16784	FZD6	0.67	0.413	0.541	1.08	49.61	22.77	46.06	33.48	Repressor
16920	WNT2B	0.65	0.479	0.602	1.02	125.33	16.15	122.97	20.12	Activator
17401	DIXDC1	0.57	0.549	0.664	1.01	219.59	25.21	216.47	25.67	Activator
17798	WNT1	0.50	0.567	0.681	1.02	32.89	5.76	32.27	5.66	Activator
17883	CXXC4	0.48	0.607	0.714	1.02	116.14	16.87	114.37	16.75	Repressor
18053	DKK1	0.45	0.619	0.724	1.02	37.14	5.58	36.59	5.52	Repressor
18820	CSNK1G1	0.33	0.758	0.833	1.01	445.38	70.20	440.28	80.76	Repressor
20567	RUVBL1	0.03	0.990	0.993	1.00	261.44	68.51	260.96	61.36	Activator
20651	SFRP5	0.02	0.964	0.976	1.00	155.66	18.78	155.58	23.13	Repressor
20705	KREMEN1	0.01	0.982	0.988	1.00	155.59	22.72	155.53	25.54	Repressor
20748	SFRP4	0.00	0.788	0.855	1.00	87.12	12.83	87.13	25.08	Repressor
19764	FBXW11	-0.17	0.890	0.929	1.01	876.27	192.45	883.40	221.68	Repressor
19063	SOX17	-0.29	0.902	0.935	1.01	28.29	4.02	28.56	6.70	Repressor
18953	CTNNBIP1	-0.31	0.754	0.831	1.01	470.36	68.71	474.82	50.94	Repressor
18518	SFRP2	-0.38	0.972	0.982	1.02	83.93	15.24	85.43	32.57	Repressor
18469	FBXW4	-0.39	0.689	0.781	1.02	796.08	175.29	810.75	159.38	Repressor
17754	FZD2	-0.50	0.667	0.764	1.03	55.87	16.73	57.81	24.07	Activator
17682	ZBTB33	-0.52	0.659	0.757	1.03	689.41	153.95	707.05	176.98	Repressor
17390	CER1	-0.57	0.593	0.703	1.02	94.50	13.69	96.20	14.18	Repressor
17162	PORCN	-0.61	0.525	0.643	1.02	279.63	53.03	286.47	37.33	Activator
17024	APC	-0.63	0.583	0.694	1.01	331.02	34.45	335.92	43.56	Repressor
16825	WNT2	-0.67	0.575	0.688	1.02	110.11	16.21	112.48	17.75	Activator
16520	GSK3A	-0.72	0.491	0.614	1.02	458.87	65.82	469.06	61.09	Repressor
16242	NR2C2	-0.77	0.449	0.576	1.05	580.70	174.04	609.79	173.94	Neutral or unknown
15846	CSNK1D	-0.84	0.417	0.545	1.04	1406.76	287.82	1460.54	333.94	Repressor
15689	FRZB	-0.87	0.385	0.513	1.03	65.01	9.29	66.76	8.92	Repressor
15432	AKT1	-0.92	0.371	0.499	1.04	496.18	113.84	518.49	94.25	Activator
15298	GPR177	-0.94	0.499	0.621	1.08	54.85	21.50	59.16	16.64	Repressor
15202	SLC9A3R1	-0.96	0.429	0.557	1.07	481.97	172.40	517.18	138.35	Neutral or unknown
14800	TCF7L2	-1.04	0.523	0.642	1.36	150.36	242.25	204.09	208.27	Activator
14212	CSNK2A1	-1.15	0.259	0.379	1.05	460.54	96.64	484.88	104.65	Repressor
13763	FZD1	-1.24	0.439	0.567	1.18	50.31	34.83	59.55	30.74	Activator
13686	PSEN1	-1.25	0.238	0.354	1.06	452.85	105.38	480.74	78.44	Repressor
13363	GSK3B	-1.31	0.206	0.317	1.07	570.61	131.68	608.76	149.96	Repressor
11819	PITX2	-1.62	0.132	0.223	1.03	13.64	1.27	14.09	1.43	Activator
11093	RHOU	-1.76	0.251	0.371	1.41	53.29	58.18	74.91	40.56	Activator
10976	AXIN2	-1.79	0.176	0.280	1.30	218.62	161.05	284.77	227.32	Repressor
10910	WNT16	-1.80	0.170	0.273	1.11	135.03	36.08	150.39	57.47	Activator
10068	SNAI2	-2.00	0.108	0.190	1.07	16.48	2.41	17.69	4.71	Repressor
9914	FZD7	-2.04	0.164	0.265	1.19	27.72	6.72	33.08	30.05	Activator
9829	WNT4	-2.05	0.052	0.106	1.07	112.08	17.35	119.80	16.93	Activator
9800	CTBP2	-2.06	0.106	0.188	1.31	284.65	201.56	374.07	183.09	Repressor
9367	DVL2	-2.17	0.034	0.075	1.08	583.43	103.04	632.53	111.00	Activator
9083	AXIN1	-2.24	0.036	0.079	1.05	615.27	67.41	649.09	82.58	Repressor
6884	PIN1	-2.86	0.010	0.028	1.11	291.76	48.60	322.49	54.62	Activator
6126	MAP3K7IP1	-3.13	0.018	0.045	1.08	181.54	21.06	196.32	26.22	Repressor
6089	BCL9	-3.14	0.014	0.037	1.09	94.10	11.84	102.38	14.05	Activator
5732	RYK	-3.29	0.010	0.028	1.16	394.72	86.44	459.68	119.10	Activator
5686	RUVBL2	-3.30	0.006	0.018	1.12	518.93	83.28	580.12	99.10	Activator
5403	WNT5A	-3.42	0.002	0.008	1.79	15.20	2.26	27.27	46.85	Activator
4948	EP300	-3.62	0.004	0.013	1.16	470.41	85.99	543.40	133.36	Activator
4935	WIF1	-3.62	0.010	0.028	1.12	24.89	3.55	27.95	5.76	Repressor
4479	PYGO2	-3.84	0.002	0.008	1.16	457.79	88.62	532.10	92.56	Activator
3773	TCF7	-4.28	0.010	0.028	1.33	1471.25	475.67	1952.80	760.12	Activator
3442	SMAD4	-4.51	0.004	0.013	1.17	459.71	76.04	537.81	102.62	Activator

Rank	Feature	Score	Feature P	FDR (BH)	Fold Change	Normal Mean	Normal Std	CLL Mean	CLL Std	Activator Repressor
3403	HDAC1	-4.54	0.002	0.008	1.18	1757.45	305.34	2075.86	430.44	Repressor
3142	LRRFIP2	-4.76	0.002	0.008	1.23	212.19	48.09	261.43	43.89	Activator
2980	CBY1	-4.90	0.002	0.008	1.18	188.17	30.48	222.77	44.66	Repressor
2935	PRKCB	-4.95	0.002	0.008	1.17	5747.79	828.29	6734.40	1416.06	Repressor
2728	DVL3	-5.13	0.002	0.008	1.24	473.70	101.98	588.30	108.71	Activator
2512	CSNK1E	-5.34	0.002	0.008	1.38	465.51	124.52	642.22	283.36	Repressor
2011	FBXW2	-6.02	0.002	0.008	1.43	384.26	125.01	548.87	132.12	Repressor
1376	PRKCA	-7.05	0.002	0.008	2.55	62.61	30.29	159.91	165.12	Repressor
1015	WNT9A	-7.98	0.002	0.008	1.51	53.91	11.18	81.53	34.84	Activator
888	CALCOCO1	-8.36	0.002	0.008	1.24	1002.20	121.68	1245.54	203.10	Activator
864	WNT5B	-8.42	0.002	0.008	1.59	43.22	7.70	68.82	34.82	Activator
631	SMAD2	-9.42	0.002	0.008	1.38	697.66	125.64	964.36	160.94	Activator
569	WNT10A	-9.79	0.002	0.008	1.87	331.54	110.82	621.54	255.74	Activator
338	FZD3	-11.33	0.002	0.008	1.88	24.61	6.04	46.17	19.37	Activator
215	WNT3	-12.96	0.002	0.008	9.44	70.46	36.35	664.90	605.69	Activator
50	TCF4	-18.78	0.002	0.008	2.29	3436.81	885.37	7872.96	2034.24	Activator
11	ROR1	-24.98	0.002	0.008	9.18	138.39	52.28	1270.74	589.51	Activator
1	LEF1	-30.93	0.002	0.008	18.52	193.43	118.29	3581.77	1429.73	Activator

Table S5. Gene expression of 132 Wnt pathway members in 70 CLL patients. This gene list was curated from literature and from websites (http://www.stanford.edu/group/nusselab/cgi-bin/wnt/;http://www.sabiosciences.com/rt_pcr_product/HTML/PAHS-043A.html).

Rank	Feature	Score	Feature P	FDR (BH)	Fold Change	Mutated		Unmutated	
						Mean	Std	Mean	Std
2	FBXW2	2.12	0.08	0.98	1.14	636.56	112.34	559.51	123.67
4	CTNNBIP1	2.02	0.05	0.98	1.06	496.21	44.75	467.38	46.41
8	WNT4	1.85	0.07	0.98	1.08	126.19	14.70	117.33	16.74
11	NLK	1.64	0.17	0.98	1.09	231.28	32.99	213.08	43.09
13	CBY1	1.59	0.15	0.98	1.10	247.56	43.38	225.30	47.95
17	WNT9A	1.45	0.13	0.98	1.20	97.47	34.55	81.45	36.49
22	DKK1	1.34	0.20	0.98	1.06	38.41	5.23	36.20	5.03
23	GSK3B	1.31	0.24	0.98	1.09	677.51	133.17	621.22	148.45
25	CSNK1A1	1.29	0.26	0.98	1.08	1526.45	291.04	1407.46	296.04
26	LRRFIP2	1.26	0.27	0.98	1.05	275.78	34.84	261.71	37.18
28	CTBP1	1.19	0.29	0.98	1.06	3856.40	512.89	3651.88	663.89
30	RUVBL2	1.16	0.28	0.98	1.06	607.31	99.97	571.16	88.81
31	PSEN1	1.13	0.31	0.98	1.06	522.12	88.86	490.71	80.39
33	TLE3	1.11	0.26	0.98	1.09	753.10	171.90	691.76	184.59
35	TBP	1.09	0.35	0.98	1.05	386.28	45.80	369.39	60.63
36	PIN1	1.09	0.23	0.98	1.06	336.85	59.87	316.68	49.08
40	WNT16	0.98	0.32	0.98	1.13	167.44	63.64	148.05	55.67
46	FZD5	0.93	0.35	0.98	1.06	46.91	8.89	44.41	6.32
47	TGFB1I1	0.92	0.27	0.98	1.16	47.74	23.57	41.10	17.46
48	GSK3A	0.92	0.31	0.98	1.03	472.51	48.31	457.94	57.26
50	RARA	0.88	0.25	0.98	1.31	242.24	218.62	184.51	143.72
51	CPZ	0.87	0.38	0.98	1.03	180.38	18.80	174.58	29.14
52	RSPO4	0.85	0.39	0.98	1.03	127.29	13.76	123.40	17.21
53	FRAT2	0.83	0.37	0.98	1.16	297.18	161.77	257.05	100.00
54	FZD1	0.82	0.28	0.98	1.17	73.38	41.31	62.45	44.45
55	PYGO2	0.82	0.46	0.99	1.05	562.43	102.20	536.38	87.85
56	WNT5A	0.81	0.32	0.98	1.90	44.01	89.16	23.17	19.11
57	RUVBL1	0.79	0.45	0.99	1.06	280.48	61.05	265.07	64.01
59	PRKCB	0.77	0.48	0.99	1.05	6954.57	1175.47	6652.66	1467.74
61	KREMEN2	0.74	0.42	0.98	1.03	267.41	32.31	259.95	28.56
62	GPR177	0.73	0.42	0.98	1.11	66.15	29.85	59.69	16.26
63	WNT5B	0.72	0.37	0.98	1.23	89.20	77.81	72.60	40.83
69	WNT2	0.61	0.53	0.99	1.03	114.25	19.23	110.67	13.84
78	CALCOCO1	0.51	0.59	0.99	1.03	1259.29	213.45	1224.67	205.21
81	RHOU	0.46	0.63	0.99	1.11	84.76	61.46	76.27	36.68
85	TCF7L2	0.44	0.60	0.99	1.17	233.13	256.09	199.75	156.01
87	DAAM1	0.41	0.69	0.99	1.02	323.01	49.84	316.19	63.06
90	SOX4	0.39	0.62	0.99	1.17	84.21	108.29	71.69	63.43
91	PPP2R1A	0.36	0.74	0.99	1.02	839.41	113.99	826.26	116.81
96	WNT11	0.32	0.73	0.99	1.01	102.52	14.11	101.11	14.27
97	SLC9A3R1	0.29	0.75	0.99	1.03	531.50	194.07	514.41	143.04
99	CTNNB1	0.28	0.78	0.99	1.01	762.86	102.40	752.41	178.00
102	PPP2CA	0.25	0.76	0.99	1.02	1703.12	429.47	1669.50	402.52
103	NR2C2	0.25	0.84	0.99	1.02	648.87	136.43	637.75	163.10
104	ZBTB33	0.24	0.81	0.99	1.01	737.97	79.31	730.31	173.68
105	WNT8A	0.22	0.81	0.99	1.01	67.43	10.11	66.75	8.42
106	BCL9	0.21	0.80	0.99	1.01	104.39	14.33	103.39	16.01
107	TCF7L1	0.21	0.80	0.99	1.01	66.15	13.42	65.29	11.76
109	CTBP2	0.20	0.77	0.99	1.05	399.40	304.21	381.07	177.43
112	FRAT1	0.15	0.86	0.99	1.02	226.56	75.59	223.02	54.75
113	FBXW11	0.15	0.89	0.99	1.01	929.12	220.31	918.24	252.75
114	FZD8	0.15	0.83	0.99	1.01	179.38	22.79	178.20	32.44

Rank	Feature	Score	Feature P	FDR (BH)	Fold Change	Mutated Mean	Mutated Std	Unmutated Mean	Unmutated Std
117	WIF1	0.11	0.87	0.99	1.01	28.56	9.18	28.26	5.81
119	AKT1	0.09	0.89	0.99	1.00	512.78	65.86	510.71	96.59
120	FZD2	0.09	0.88	0.99	1.02	63.49	34.39	62.53	28.74
121	FRZB	0.09	0.92	0.99	1.00	66.72	7.23	66.51	8.97
123	DKK2	0.08	0.91	0.99	1.00	15.57	1.81	15.53	1.79
126	PITX2	0.06	0.95	0.99	1.00	14.23	0.95	14.21	1.50
127	HBP1	0.05	0.95	0.99	1.00	1314.26	347.79	1308.53	310.82
132	TCF4	0.00	0.89	0.99	1.00	7943.40	2604.66	7945.63	2041.83
131	DVL3	-0.01	0.97	1.00	1.00	601.31	84.35	601.47	126.70
130	CSNK2A1	-0.01	0.98	1.00	1.00	490.74	65.60	491.09	120.00
129	CER1	-0.02	1.00	1.00	1.00	94.72	12.80	94.81	12.56
128	INVS	-0.04	0.98	1.00	1.00	195.48	55.40	196.15	45.38
125	BTRC	-0.07	0.93	0.99	1.00	257.41	47.60	258.44	31.85
124	SNAI2	-0.07	0.71	0.99	1.01	18.32	3.04	18.41	6.91
122	EP300	-0.09	0.90	0.99	1.01	535.29	126.59	538.75	115.25
118	FZD7	-0.10	0.99	1.00	1.01	32.99	13.68	33.46	16.28
116	FZD4	-0.12	0.90	0.99	1.01	130.84	19.89	131.59	19.20
115	CSNK1G1	-0.14	0.88	0.99	1.01	447.04	77.61	450.66	85.21
111	LRP6	-0.15	0.90	0.99	1.01	160.14	21.90	161.33	33.46
110	NKD1	-0.16	0.92	0.99	1.01	83.64	13.79	84.32	14.10
108	SMAD2	-0.20	0.83	0.99	1.01	962.37	163.86	972.75	142.12
101	PRKCA	-0.25	0.94	0.99	1.07	174.75	156.10	187.68	185.97
100	FGF4	-0.27	0.75	0.99	1.01	201.91	25.23	204.20	33.45
98	KREMEN1	-0.29	0.83	0.99	1.01	150.69	23.22	152.76	20.77
95	SMAD4	-0.32	0.75	0.99	1.02	539.16	89.69	548.57	111.82
94	FZD9	-0.33	0.78	0.99	1.02	81.68	11.79	82.93	12.33
93	MAP3K7IP1	-0.34	0.76	0.99	1.01	193.73	25.91	196.51	26.64
92	CXXC4	-0.36	0.75	0.99	1.02	111.16	21.79	113.56	16.30
89	RYK	-0.39	0.75	0.99	1.04	444.90	138.97	461.43	109.03
88	BRD7	-0.41	0.67	0.99	1.02	858.29	119.76	874.45	148.53
86	SMARCA4	-0.42	0.71	0.99	1.02	581.17	88.70	593.35	98.24
84	WNT2B	-0.44	0.71	0.99	1.02	122.76	16.26	125.34	26.50
83	AXIN1	-0.46	0.68	0.99	1.02	630.85	77.72	642.54	94.83
82	LRP5	-0.46	0.64	0.99	1.02	130.71	18.72	133.43	18.37
80	DVL2	-0.46	0.61	0.99	1.03	607.90	106.79	623.66	110.67
79	TCF3	-0.47	0.67	0.99	1.05	2973.56	965.46	3114.56	806.52
77	LEF1	-0.52	0.55	0.99	1.08	3487.50	1649.35	3751.96	1398.43
76	NDP	-0.54	0.61	0.99	1.02	16.43	1.65	16.73	1.97
75	CSNK1E	-0.55	0.66	0.99	1.07	574.81	238.18	615.55	220.50
74	CSNK1D	-0.55	0.57	0.99	1.03	1435.58	240.30	1479.38	288.70
73	WNT6	-0.56	0.61	0.99	1.03	539.80	81.27	553.94	72.27
72	WNT10A	-0.57	0.57	0.99	1.09	603.14	315.48	658.63	268.69
71	PYGO1	-0.57	0.58	0.99	1.02	42.75	5.10	43.72	6.65
70	SENP2	-0.58	0.74	0.99	1.02	291.13	32.16	297.93	54.54
68	FZD3	-0.61	0.61	0.99	1.09	43.31	20.38	47.35	22.42
67	ROR1	-0.63	0.53	0.99	1.10	1209.50	615.09	1330.68	570.59
66	HDAC1	-0.65	0.54	0.99	1.04	2058.67	380.51	2138.69	432.30
65	WISP1	-0.67	0.53	0.99	1.03	94.23	11.40	96.69	12.46
64	SMAD3	-0.72	0.54	0.99	1.14	315.20	187.94	358.77	210.96
60	AES	-0.77	0.45	0.99	1.06	2568.26	559.04	2709.76	676.50
58	PRICKLE1	-0.78	0.53	0.99	1.11	533.87	214.20	594.10	349.31
49	ROR2	-0.89	0.42	0.98	1.02	20.85	1.65	21.35	2.09
45	WNT1	-0.93	0.40	0.98	1.05	30.16	4.98	31.64	5.26
44	SOSTDC1	-0.93	0.39	0.98	1.04	52.54	7.29	54.71	7.77
43	DKK4	-0.94	0.38	0.98	1.04	73.74	9.89	76.90	13.38
42	APC2	-0.95	0.43	0.98	1.04	120.76	16.73	125.97	19.86
41	AXIN2	-0.97	0.40	0.98	1.27	229.96	200.33	292.08	210.87
39	DIXDC1	-1.03	0.34	0.98	1.04	211.08	22.25	218.72	28.52
38	SFRP2	-1.03	0.64	0.99	1.10	79.50	10.71	87.31	52.56

Rank	Feature	Score	Feature P	FDR (BH)	Fold Change	Mutated		Unmutated	
						Mean	Std	Mean	Std
37	FBXW4	-1.04	0.31	0.98	1.06	745.20	143.69	792.08	134.73
34	APC	-1.10	0.33	0.98	1.04	326.37	29.55	337.97	46.71
32	CREBBP	-1.12	0.35	0.98	1.07	1217.80	238.18	1304.97	278.87
29	PORCN	-1.18	0.26	0.98	1.05	269.51	40.12	284.03	31.87
27	HNF1A	-1.22	0.24	0.98	1.04	166.02	15.13	172.34	21.05
24	SOX17	-1.29	0.39	0.98	1.07	27.86	3.05	29.83	9.51
21	DVL1	-1.36	0.21	0.98	1.07	180.44	26.33	193.70	46.72
20	SFRP1	-1.39	0.32	0.98	1.05	88.71	7.80	93.18	17.57
19	NKD2	-1.40	0.24	0.98	1.05	176.77	16.45	184.94	25.93
18	WNT7B	-1.44	0.23	0.98	1.05	289.15	27.91	302.66	36.87
16	TLE1	-1.45	0.19	0.98	1.38	831.13	673.71	1149.83	781.88
15	CHD8	-1.50	0.18	0.98	1.04	740.48	64.25	772.61	81.81
14	SFRP4	-1.55	0.20	0.98	1.10	80.46	7.81	88.60	36.27
12	SFRP5	-1.59	0.22	0.98	1.07	146.63	19.06	156.85	25.36
10	TMEM132A	-1.68	0.16	0.98	1.06	192.15	20.60	203.87	27.51
9	WNT3	-1.80	0.17	0.98	1.49	489.04	370.59	729.03	607.60
7	DKK3	-1.89	0.13	0.98	1.14	48.85	4.60	55.84	26.33
6	TLE2	-1.90	0.14	0.98	1.07	301.73	34.35	323.55	44.39
5	WNT7A	-1.93	0.06	0.98	1.08	133.45	16.42	144.11	21.54
3	FZD6	-2.05	0.13	0.98	1.24	38.42	9.98	47.73	26.66
1	TCF7	-3.07	0.02	0.98	1.29	1587.22	402.34	2055.13	752.87

Table S6. Putative function of mutated Wnt pathway genes and their possible interaction with other pathways. This information was compiled based on mining of the STRING , UNIPROT and NCBI-GENE databases (http://string-db.org/, http://www.uniprot.org/, http://www.ncbi.nlm.nih.gov/gene). Shaded region - genes selected for functional studies.

Gene	Putative function in Wnt pathway	Interaction with other pathways
WNT1	Wnt ligand	Unknown
FZD5	Wnt receptor	Unknown
DKK2	Repressor of Wnt signaling by interaction with Wnt co-receptor *LRP6*	Unknown
WNT10A	Wnt ligand	Unknown
RYK	Protein tyrosine kinase whose extracellular domain has a Wnt binding region and is involved in stimulating Wnt pathway signaling	Unknown
BCL9	Nuclear co-factor that promotes the transcriptional activity of β-catenin	Unknown
CSNK1E	Casein kinase that phosphorylates a large number of proteins, including *DVL1*.	Signal transduction, DNA repair, cell cycle, cellular protein localization.
PRICKLE1	Nuclear receptor that is a negative regulator of the Wnt pathway through binding DVL protein.	Regulation of transcription protein ubiquitination
BRD7	Activator of the Wnt signaling in a *DVL1*-dependent manner by negatively regulating the GSK3B phosphotransferase activity	Cell cycle, transcription regulation.
RSPO4	In the canonical Wnt pathway: acts via a direct interaction with WNT; In a Wnt independent b-catenin pathway: acts through receptor signaling that may not use Frizzled/LRP receptors	Sensory transduction
CHD8	DNA helicase that acts as a chromatin remodeling factor and regulates transcription. Negatively regulates *CTNNB1* -targeted gene expression	Transcriptional regulation

CREBBP	Mediator for interaction between the Wnt and TGF-beta signaling pathways; Acetylates histones and non-histone proteins.	Host-virus interaction, Transcription regulation

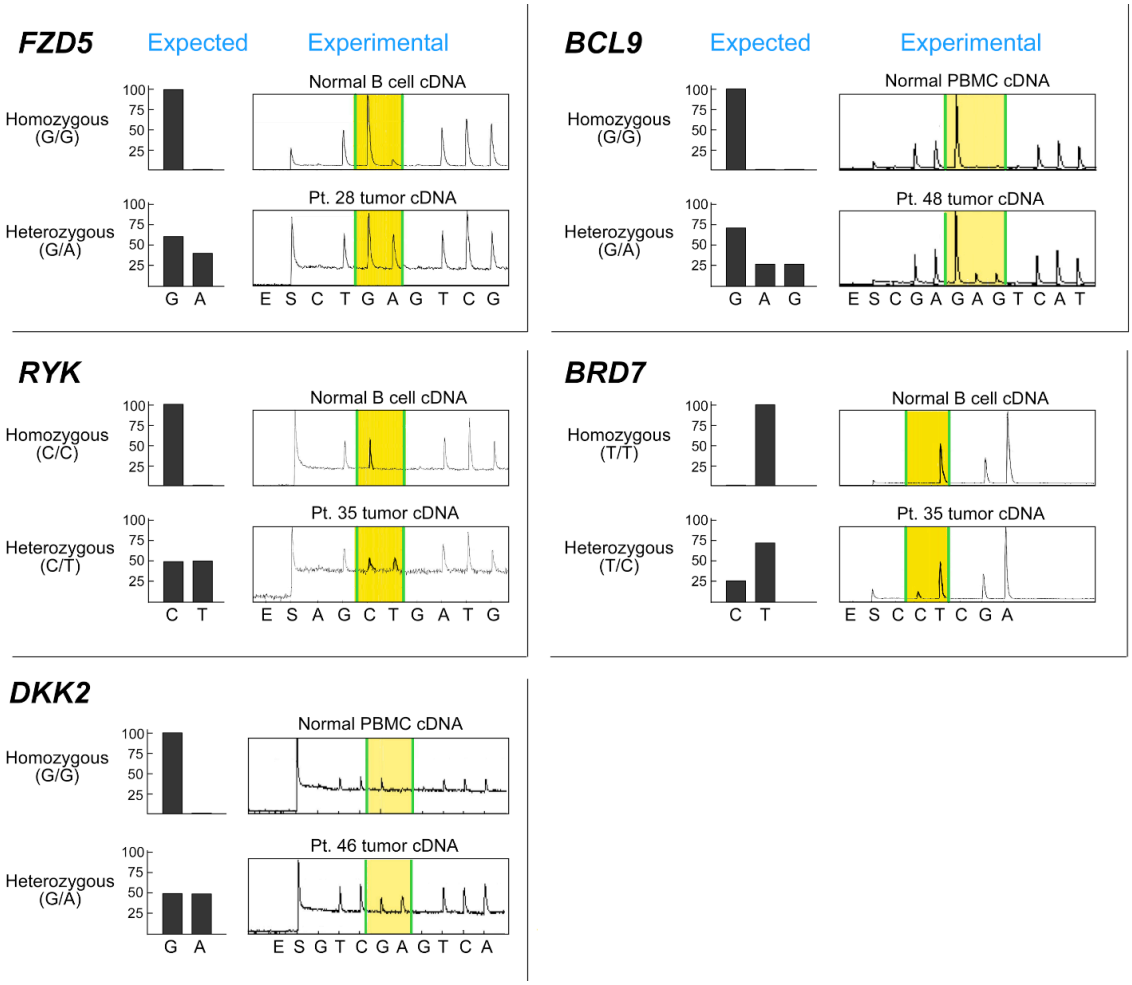

Figure S1. Wnt pathway mutations are detectable in transcripts from patient leukemia cells by quantitative targeted sequencing. Quantitative targeted sequencing to detect a region of genetic change within cDNA was performed, as previously described [13]. In brief, biotinylated amplicons generated from PCR of the regions of transcript surrounding the mutation of interest were generated. Immobilized biotinylated single-stranded DNA fragments were isolated per manufacturer's protocol and sequencing undertaken using an automated pyrosequencing instrument (PSQ96; Qiagen, Valencia CA), followed by quantitative analysis using Pyrosequencing software (Qiagen). Primers and probes used for Wnt pathway member amplifications are provided in **Table S2**. The presence of mutations in CLL-B cell transcripts was confirmed by pyrosequencing of *FZD5* (from P28), *BCL9* (from P48), *RYK* (from P35), *BRD7* (from P35) and *DKK2*

(from P46). Pyrosequencing results were compared between cDNA from tumor and normal PBMC or CD19-B cells. Left panels - Expected pyrograms for homozygous (i.e. wildtype) and heterozygous (i.e. mutated) sequences; right panels - pyrograms of reverse transcribed RNA from normal B cells, PBMC or CLL samples.

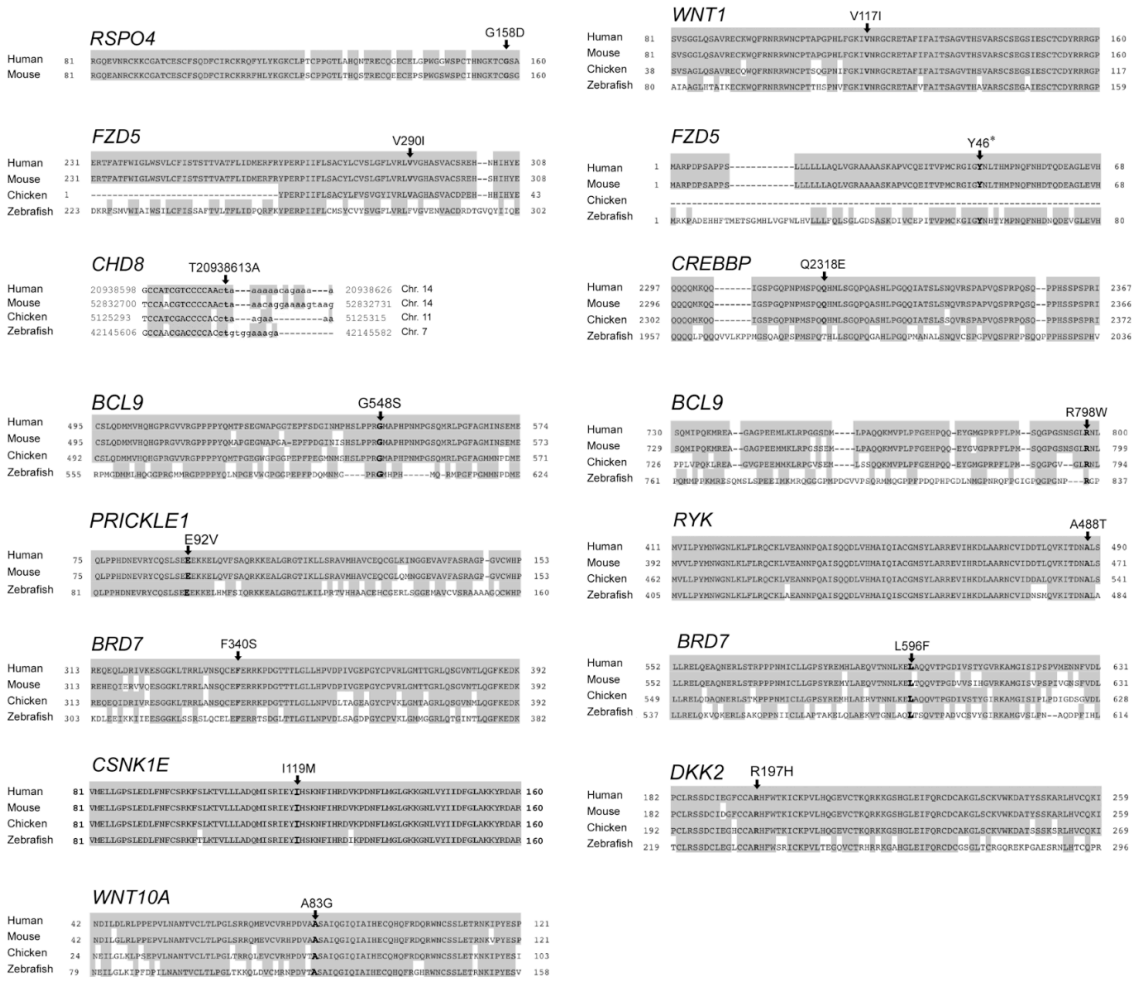

Figure S2. Wnt mutations in CLL occur in evolutionarily conserved regions. All 15 discovered Wnt pathway mutations localized to conserved regions of genes. When available, alignments of gene sequences around each mutation are shown for human, mouse, chicken and zebrafish genes (UCSC Genomic Bioinformatics: http://genome.ucsc.edu).

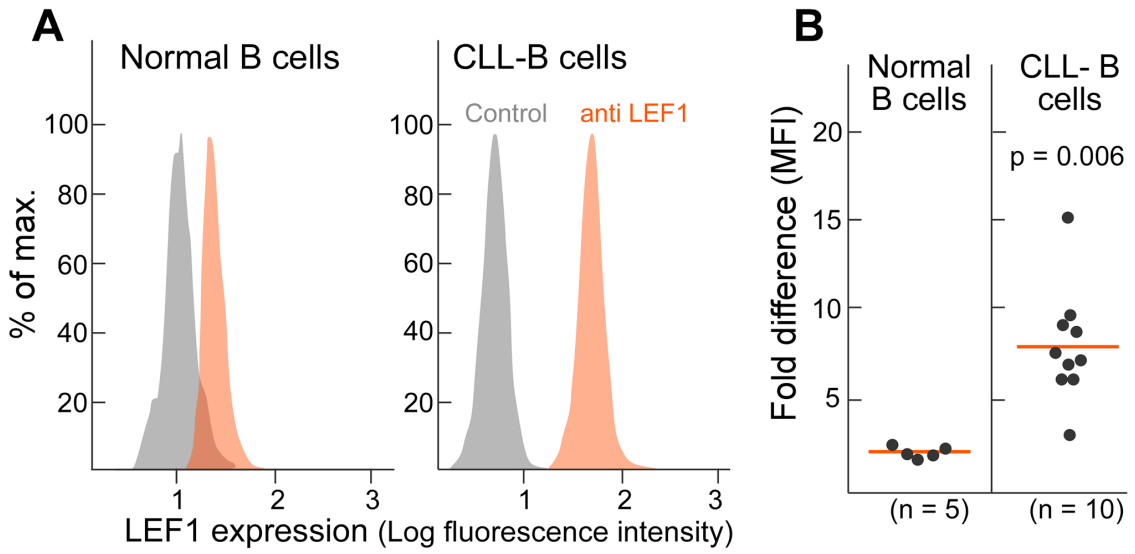

Figure S3. **LEF1 is highly expressed in chronic lymphocytic leukemia cells. A**. Representative FACS plots of intranuclear staining of *LEF1* coupled with surface CD19 staining, performed using PBMCs from normal donors and CLL patients. **B**. Greater fold difference between *LEF1* and control antibody staining within CD19 positive cells in CLL samples compared to normal CD19+ B cells (p=0.006, two-tailed Student t-test). Median values (red line) are shown.

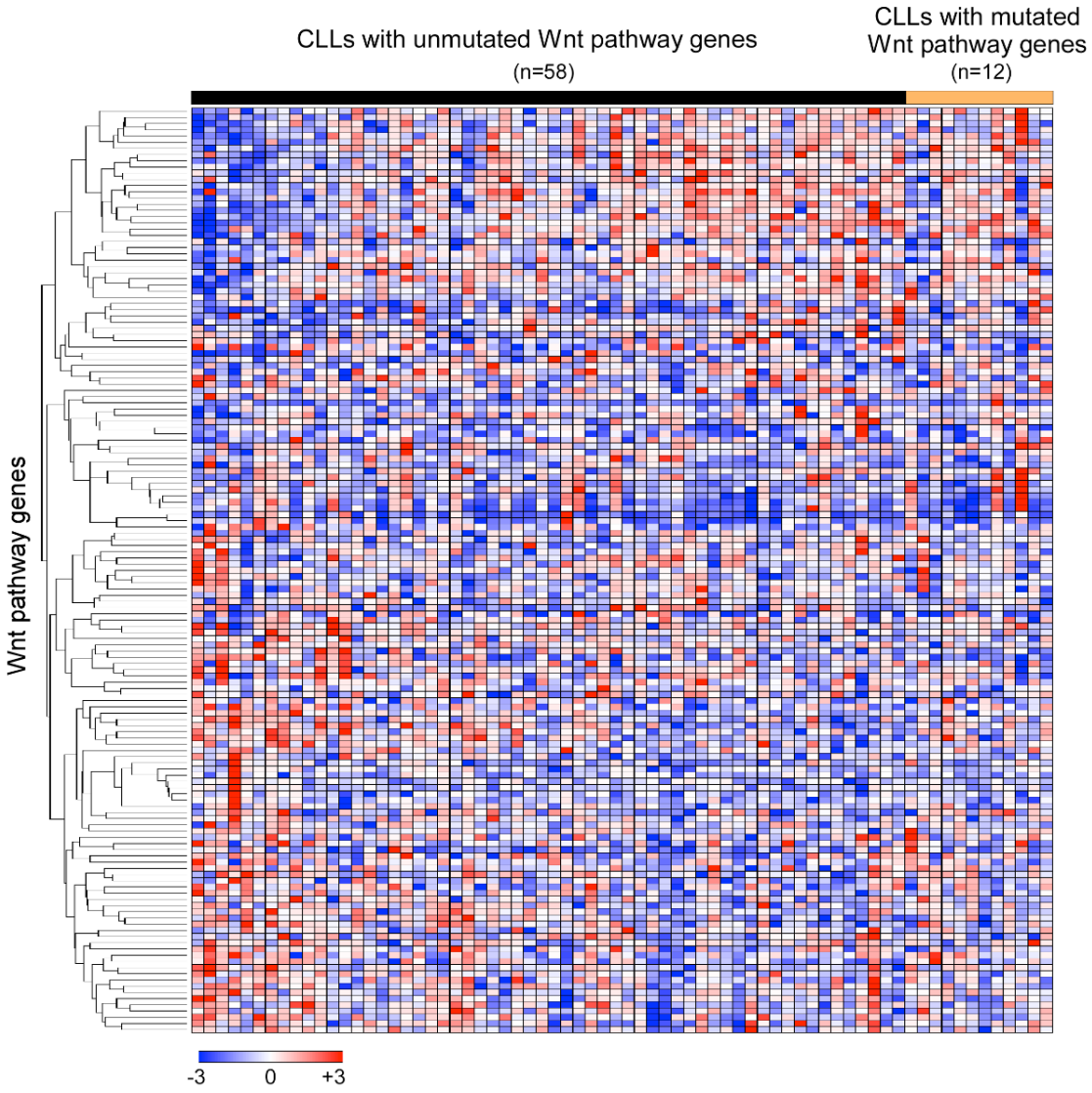

Figure S4. Wnt pathway mutations do not contribute to pathway gene expression. Supervised hierarchical clustering of Wnt pathway gene expression profiles from 70 CLL-B cells (12 with mutations and 58 without mutations), performed using Pearson linear correlation with average linkage. Expression levels are log2 transformed and mean-centered for each gene prior to clustering and for visualization.

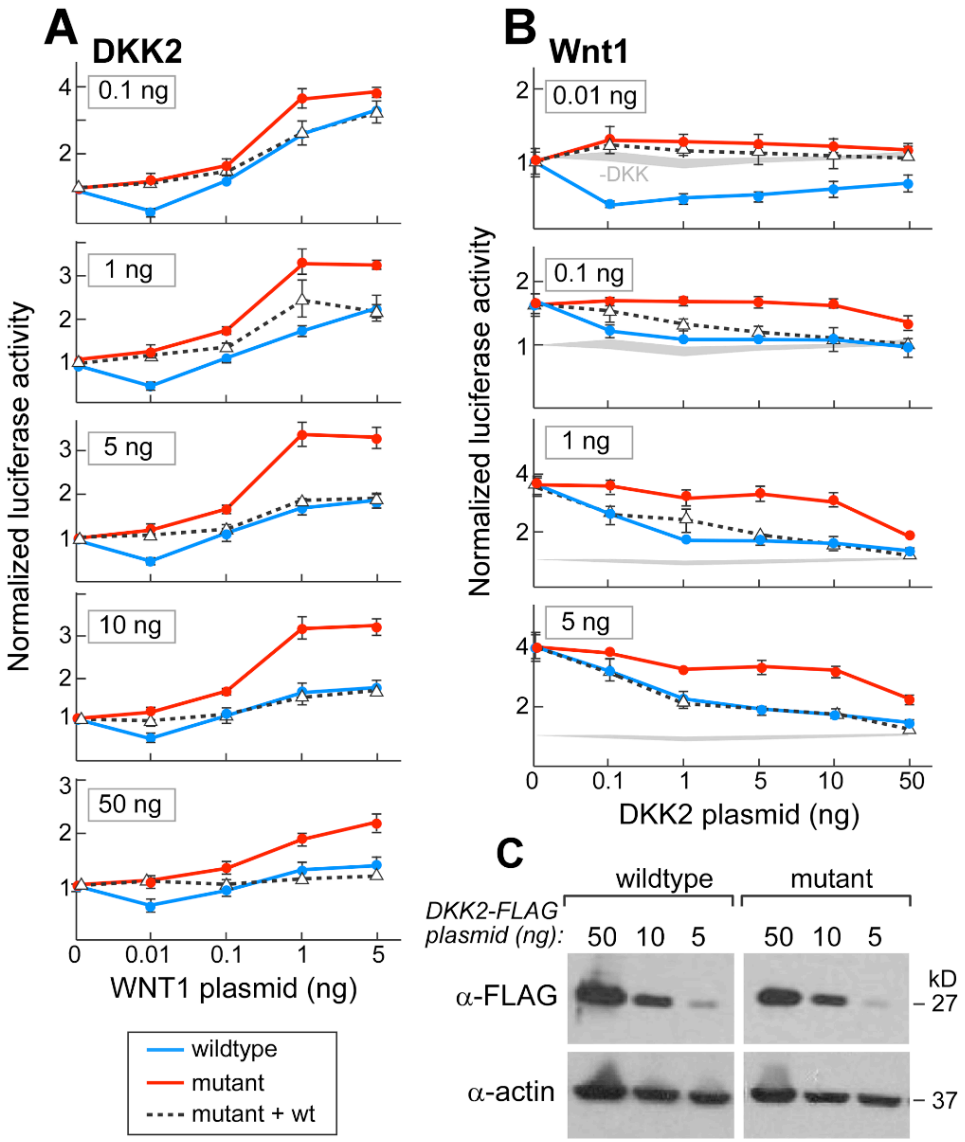

Figure S5. **Mutant *DKK2* abolishes Wnt pathway repression. (A, B)** HEK293T cells were transfected with different doses (0.1-50ng) of wildtype (dotted line), mutant (red line) or both wildtype and mutant (blue line) *DKK2* expression plasmid along with different dosage of *WNT1* expression plasmid (0.01-5ng) and reporter plasmids. Luciferase activity was measured 48 hours after transfection and was normalized to empty vector transfected HEK293T cells. Shown are median results from three

independent experiments ($p<0.01$, Wald test). **(C)** Equivalent wildtype and mutant *DKK2* protein expression detected in the transfected HEK293T cells. DKK2 protein expression was determined by immunoblotting using α-FLAG and α-actin antibodies against lysates generated from HEK293T cells that had been transfected with different doses of either wildtype or mutant DKK2 expression plasmid.

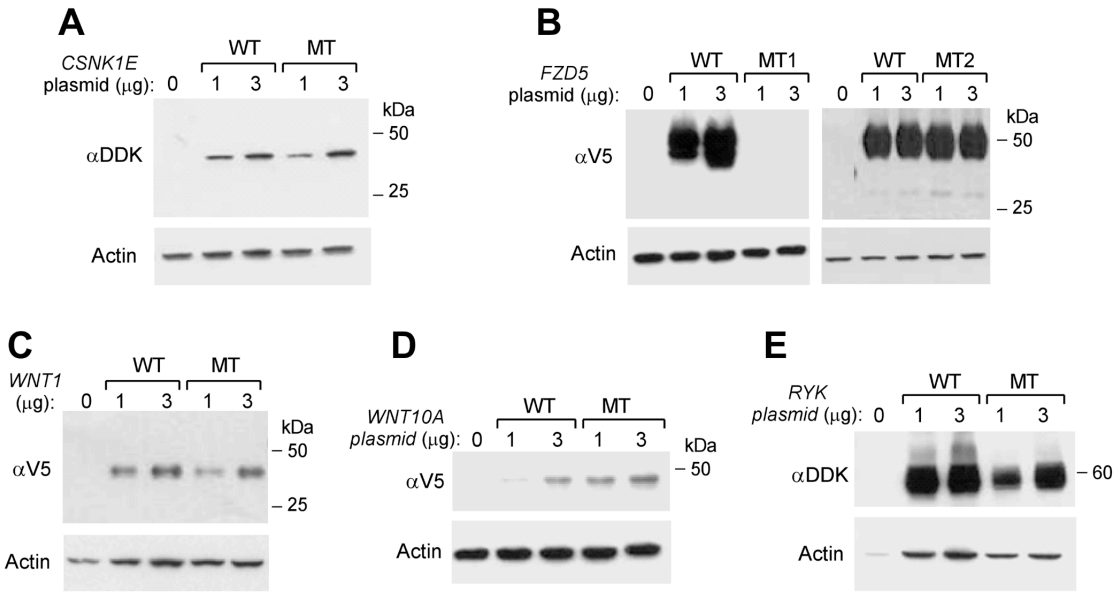

Figure S6. Wildtype and mutated constructs are equivalently expressed in transfected HEK293T cells. HEK293T cells were transfected with different doses of wildtype or mutant constructs for 48 hours and cell lysates were generated. *CSNK1E* and *RYK* protein expression were analyzed by immunoblotting using α-DDK antibody while *FZD5*, *WNT1* and *WNT10A* protein expression were analyzed using α-V5 antibody.

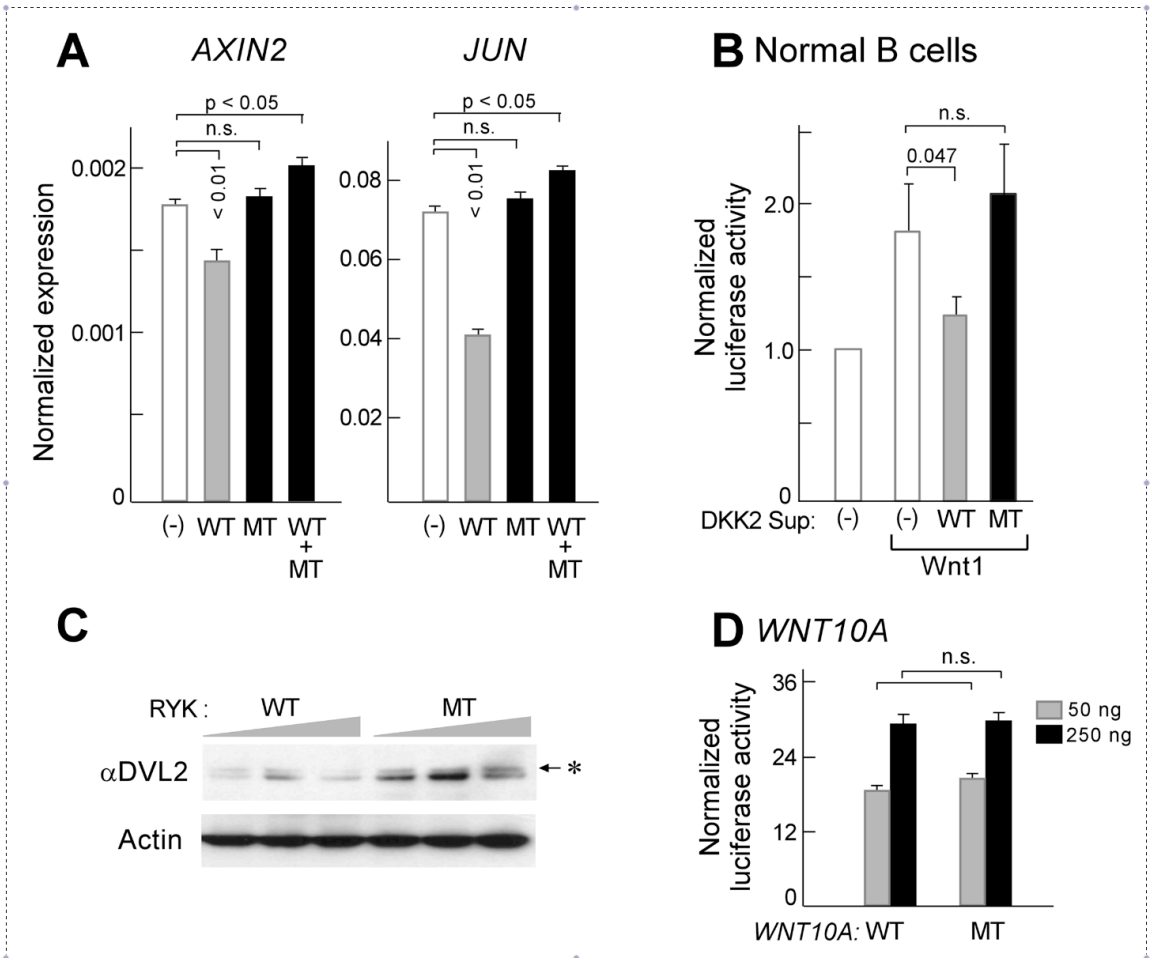

Figure S7. **Wnt pathway downstream targets are activated in wildtype and mutated constructs transfected cells**. **A.** Downstream Wnt pathway target gene expression is increased with expression of mutated *DKK2*. Expression of *AXIN2* and *JUN* transcripts was examined in samples from HEK293T cells transfected with Wnt1 0.1ng in the absence or presence of wildtype, mutant *DKK2* or both. Representative results from three independent experiments are shown. **B.** Secreted mutant *DKK2* abolishes repression of Wnt pathway activation in normal $CD19^+$ B cells. B cells were nucleofected with Wnt1 expression (1μg, Amaxa) and reporter plasmids and cultured for 24 hours in supernatant containing wildtype or mutant *DKK2*, followed by detection of luciferase activity. Shown are the average results of 8 independent experiments. **C.** Mutant *RYK* increases

the level of *DVL2* protein expression in HEK293T cells. The upper band of *DVL2* (indicated by an asterisk) is the phosphorylated form, and the lower band is the non-phosphorylated form. **D.** Wildtype and mutated *WNT10A* have similar potential to activate Wnt pathway. HEK293T cells were cotransfected with 50 or 250ng of WT or MT *WNT10A* along with reporter plasmids and luciferase activity was measured 48 hours after transfection.

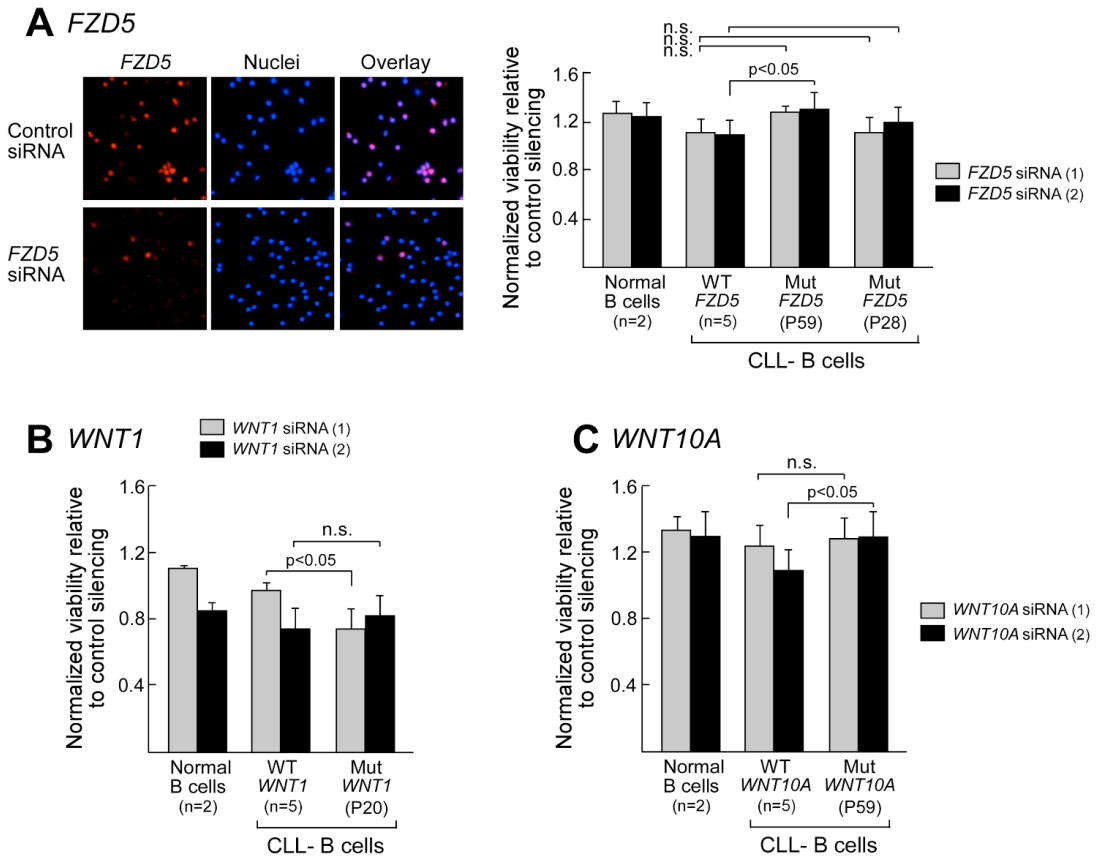

Figure S8. Silencing of mutated allele expression in samples with or without Wnt pathway mutation. A. Left panel: siRNA silencing of *FZD5* was confirmed by immunofluorescence staining. Two different siRNAs targeting *FZD5* gene ('*FZD5* siRNA') or non-targeting control siRNA were delivered into normal CD19+ B cells via NWs. Representative results of one siRNA is shown. **A-Right panel, B-C.** Cell viability was measured via CellTiterGlo assay after NW-mediated delivery of gene-specific or non-targeting control siRNA in normal CD19+ B cells and CLL-B cells with and without Wnt pathway mutations. Percentage of cell survival was normalized to non-targeting siRNA control.

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