SUPPLEMENTAL TABLE S1

Table S1. Relative expression of 84 genes associated with the Wnt/ß-catenin pathway in 7 AML samples

	Patient						
Gene	**#5**	**#7**	**#8**	**#13**	**#11**	**#4**	**#10**
AES	2.8898	1.5898	0.9912	0.433	0.4704	4.5859	1.6703
APC	17.8086	0.989	5.267	1.8938	1.3758	11.4686	18.3713
AXIN1	51.1383	3.7442	28.6748	16.7855	13.2442	39.0622	36.5263
BCL9	70.7628	2.0084	5.8987	1.8997	0.2516	30.1529	51.3225
BTRC	5.9205	0.7784	1.394	2.7281	0.7198	8.1209	7.3404
FZD5	4.8975	0.9386	2.9324	2.2406	0.7259	3.1861	3.4484
CCND1	9.1	0.1571	1.6025	0.0521	0.5063	4.5353	3.9255
CCND2	183.8635	7.8028	22.406	42.1507	58.3261	184.5835	17.0555
CCND3	6.7626	6.4422	3.2394	3.29	2.2237	10.8548	8.5251
CSNK1A1	7.4387	3.4525	6.9633	8.9675	5.2633	26.2932	8.793
CSNK1D	3.8233	1.2837	1.9419	1.1814	1.4012	4.2657	2.6599

CSNK1G1	0.0432	0.3583	4.0896	0.7492	1.3523	15.6412	14.8397
CSNK2A1	4.3953	4.4195	4.1966	4.7366	1.4809	15.9658	6.3049
CTBP1	16.7873	0.3264	5.5599	0.0634	0.1431	1.6296	2.2904
CTBP2	2.1124	0.8748	1.0852	0.4401	0.5677	0.8623	1.6976
ß-CATENIN	36.0645	9.2593	7.2676	1.071	2.2232	20.8382	23.3639
ICAT	8.0081	3.409	7.5671	6.298	2.2505	6.3812	8.6936
CXXC4	409.8568	5.762	59.8331	0.2373	0.361	5.2828	37.8154
DAAM1	1.3661	1.4321	3.176	1.0899	1.3508	3.5596	3.703
DIXDC1	18.1481	4.5433	5.8863	7.4109	2.5861	14.3264	10.182
DKK1	101.2115	2.4893	12.8723	0.7176	0.1751	2.2822	13.8032
DVL1	3.0158	0.358	0.7963	0.1293	0.1344	0.3427	1.8405
DVL2	6.64	3.1661	3.3612	2.4508	1.945	11.1586	3.0469
EP300	5.0337	0.0842	12.3928	16.0114	9.2147	15.4061	19.3271
FBXW11	11.2032	6.8249	6.581	5.8894	2.5797	24.793	11.6415
FBXW2	404.1948	5.762	48.3108	5.1444	27.0576	16.8211	137.0258
FGF4	426.4595	5.762	49.3491	0.2373	0.361	5.2828	37.6674
FOSL1	5.3271	10.152	2.9571	5.8096	4.6629	0.6757	5.8938
FOXN1	415.2983	5.762	47.057	0.2373	0.361	15.8028	34.4075
FRAT1	16.2531	6.0711	7.4109	2.7421	2.299	17.5254	26.6046
FRZB	86.6293	1.0482	10.0418	0.0432	0.0657	0.9611	7.1731
FSHB	213.1607	5.762	22.3186	0.2373	0.361	5.2828	20.0674
FZD1	19.3745	2.2068	2.9182	4.7119	0.8093	0.1611	15.5385
FZD2	78.0887	1.5973	80.0045	40.5244	4.803	8.8209	16.1429
FZD3	15.1735	0.2747	1.6384	28.0897	0.0484	0.2519	6.0626
FZD4	0.6949	5.762	38.2819	0.2373	0.361	5.2828	35.1582
FZD6	10.5482	0.1993	17.0294	23.1915	3.6879	50.9174	7.4259
FZD7	10.5236	2.8313	1.9019	0.8054	0.5078	2.5577	6.2455
FZD8	26.5372	0.4726	7.2874	19.84	2.0967	0.4333	22.8
GSK3A	2.5296	2.6627	2.6118	2.1026	1.8674	11.8304	2.9606
GSK3B	1.141	1.0576	0.5835	0.4105	0.4666	3.5684	1.4579
JUN	6.3492	33.4533	17.6869	9.2525	6.8466	73.449	11.0059
KREMEN1	62.4102	0.7607	7.9454	1.0392	0.0477	3.7159	11.8999
LEF1	0.2935	0.0031	0.0286	0.0092	0.0152	0.4753	0.0812
LRP5	107.3384	5.762	14.5368	0.5479	1.1216	5.2828	7.4624
LRP6	70.3742	0.9537	38.2035	0.4102	29.2301	0.8744	11.8444
MYC	49.6587	2.0278	12.3073	8.4742	14.7333	0.5429	41.8601
NKD1	173.9066	3.1704	20.8518	10.1615	1.0017	23.6775	73.4527
NLK	9.9057	0.8448	1.2136	0.5987	0.708	6.091	2.6869
PITX2	320.7459	5.762	37.9018	0.2373	0.361	5.2828	32.5504
PORCN	8.3153	2.3299	4.7181	2.6813	1.5997	7.1498	9.8043
PPP2CA	3.3136	1.7577	2.8578	4.4886	1.3098	17.0635	7.4259
PPP2R1A	3.5603	1.7463	2.754	2.5088	2.1076	11.306	3.2167
PYGO1	510.1066	5.762	77.2113	0.2373	0.361	5.2828	55.8012
RHOU	15.5353	1.3147	5.696	8.7794	1.7416	3.2201	15.3561
SENP2	18.0554	3.9076	13.9005	21.9547	7.0153	42.2874	33.0878
SFRP1	452.3768	5.762	73.0385	0.2373	0.361	5.2828	44.1283
SFRP4	496.5303	5.762	47.1359	2.3457	0.6839	5.2828	88.7748
FBXW4	0.4619	0.0686	0.3545	0.5315	0.2957	2.3434	1.585
SLC9A3R1/EBP50	4.4556	24.9939	11.1519	4.3385	5.73	19.7802	7.439
SOX17	291.2752	5.762	50.225	0.2373	0.5686	12.4288	27.5118
TFT	439.9607	6.1646	75.723	2.181	3.2241	18.6724	116.9778
TCF1	743.3192	13.0184	58.3137	2275.5428	63.6716	1388.7488	202.1132
TCF3	10.805	2.3921	1.7365	0.7087	1.3768	14.7953	11.2327
TLE1	1.0202	0.0091	0.255	0.2016	0.2348	0.2145	0.5173
TLE2	36.0036	0.5432	4.4913	0.0499	0.1824	3.9596	4.7501
WIF1	225.8868	3.6185	28.4411	0.149	0.2942	3.3176	18.7274
WISP1	326.4782	4.2665	34.686	0.1757	0.814	20.4797	21.6543

WNT1	116.7156	1.3854	14.5251	0.108	0.0868	4.9101	24.0684
WNT10A	383.5567	5.762	57.3588	4.7209	2.7489	44.0789	127.0745
WNT11	15.842	0.2617	2.3129	0.8135	0.4044	0.1345	5.8435
WNT16	246.927	5.762	50.224	5.9647	11.5423	93.0332	234.9786
WNT2	581.3411	5.762	88.3335	0.2373	0.361	5.2828	38.0988
WNT2B	370.2441	32.2956	53.0682	8.3726	11.8279	114.068	216.5306
WNT3	486.111	5.762	52.8012	0.2373	0.361	14.1521	32.3064
WNT3A	238.8235	5.762	27.4146	0.2373	0.361	5.2828	22.0148
WNT4	480.0743	5.762	61.8598	0.2373	1.4278	8.2171	40.5844
WNT5A	501.7566	6.5939	50.8366	0.5003	0.361	5.2828	32.804
WNT5B	22.812	0.3227	5.3111	1.3665	0.7932	1.0471	8.0733
WNT6	68.1776	5.762	6.5153	0.2373	0.361	5.2828	10.932
WNT7A	287.3432	5.762	62.4601	0.2373	24.1934	5.2828	47.9782
WNT7B	235.7131	9.403	22.3461	0.2373	0.361	5.2828	82.1887
WNT8A	76.6547	0.9703	9.5917	0.3212	0.1634	12.5338	19.7146
WNT9A	222.1252	4.635	39.9524	0.6858	0.3453	5.5001	40.0025

mRNA levels data are expressed as fold changes *versus* NBM CD34$^+$ levels. GAPDH expression was used for normalization and given the value 1.

SUPPLEMENTAL FIGURES LEGENDS

Figure S1. Absence of correlation between mRNA and protein levels of ß-catenin in AML patients

17 AML patients' samples (black squares, black triangle) were analyzed for their MFI of ß-catenin, indicating protein expression, and for their relative ß-catenin mRNA level normalized to that of CD34$^+$ NBM and to GAPDH. Linear regression R^2 was 0.0026 (black line, *p* value of the slope being non-zero: 0.8457). No correlation was found either after exclusion of the outlier point (black triangle) (grey line, R^2: 0.1388, *p* value of a non-zero slope: 0.1554).

Figure S2. Measurement of nuclear internalized ß-catenin signal in patients #1 and #6

CD34-PE$^+$ gated events from AML patients #1 (A) and #6 (B) were assessed for ß-catenin FITC positivity and gated appropriately (left panel), nuclear internalization repartition (mid panels) and image analysis (right panels) of ß-catenin positive cells within the CD34$^+$ population (see also Supplemental Materials & Methods and Figure 2).

Figure S3. Differential mRNA expression of pro-apoptotic and anti-apoptotic genes after ß-catenin silencing in HL60

HL60 cells were transduced at day 0 with sh47 lentivirus and qPCR were performed after sorting for GFP$^+$ and control GFP$^-$ cells at day 8. ß-actin was used as normalizing control.

Figure S4. Effective knockdown of ß-catenin in AML *ex vivo* patients at week 1

qPCR were done for transduced patients' samples #5, #9 and #1 on sh22 *versus* scramble control GFP$^+$ sorted cells, after 1 week of co-culture with MS-5 stroma. ß-actin was used as normalizing control.

Figure S5. β-catenin knockdown impairs human AML leukemic cells

Cells of patients #5 (left panel), #9 (middle panel), #1 (right panel) were plated in methylcellulose after shRNA transduction and counted at day 14. Data from three individual experiments are shown ± standard deviation. * $p<0.05$. Empty vector was used as control.

Figure S6. Maintenance of the percentage of GFP$^+$ after primary transplant (12 weeks post-transplant)

Individual GFP fold changes were assessed as the ratio of the percentage of GFP$^+$ engrafted cells from patients #5, #9 and #1 (within the bone marrow CD45$^+$CD33$^+$CD19$^-$ population) at week 12 to that of GFP$^+$ cells at day 4. Control and sh ß-catenin data are shown as pooled from several independent experiments (same animals as in Figure 5B). Means are shown ± SEM.

SUPPLEMENTAL MATERIAL & METHODS

Primers pairs used for qPCR

The sequences of the human primers used for qPCR, listed from 5' to 3', were:

Gene	Forward and reverse primers' sequence	
ß-catenin	TCTGATAAAGGCTACTGTTGGATTGA	TCACGCAAAGGTGCATGATT
Cyclin D1	AACTACCTGGACCGCTTCCT	CCACTTGAGCTTGTTCACCA
Cyclin D2	TGGGGAAGTTGAAGTGGAAC	ATCATCGACGGTGGGTACAT
Cyclin D3	ATGCTGGCTTACTGGATGCT	TGCACAGTTTTTCGATGGTC
BCL2	AGTACCTGAACCGGCACCTG	TTCAGAGACAGCCAGGAGAAAT
BAD	CCAGAGTTTGAGCCGAGTGA	CCATCCCTTCGTCGTCCTC
BAK	GTTTTCCGCAGCTACGTTTTT	TCATAGCGTCGGTTGATGTC
BCL XL	TCGCATTGTGGCCTTTTTCT	TGCTGCATTGTTCCCATAGAG
TCF3	GAGGACGAGGAGAACACGTC	AAAGGCCTCGTTGATGTCAC
SFRP4	CTGCCCCATCAAGATGTTCT	TCGGGTTTGTTCTCTTCTGG
DKK1	TTCCGAGGAGAAATTGAGGA	TATCCGGCAAGACAGACCTT
MYC	CCTACCCTCTCAACGACAGC	CTCTGACCTTTTGCCAGGAG
ß-actin	GGACTTCGAGCAAGAGATGG	AGCACTGTGTTGGCGTACAG
GAPDH	GGGAAGGTGAAGGTCGGAGT	GGGTCATTGATGGCAACAATA

Superarray analysis

The expression profile of 84 Wnt/ß-catenin pathway-related genes was determined using a 96-well format human Wnt signaling pathway RT^2 Profiler PCR array (SABiosciences, USA) according to the manufacturer’s instructions. The array also included 6 housekeeping genes and 3 RNA as internal controls. qPCR were run on an ABI 7900HT qPCR instrument equipped with SDS 2.3 software, using RT^2 SYBR Green/ROX qPCR master mix (Applied Biosystems, UK). Data analysis was done by the $2^{-\Delta\Delta Ct}$ method on the manufacturer’s Web portal

http://www.SABiosciences.com/pcrarraydataanalysis.php (Applied Biosystems, UK).

Calculation of ß-catenin internalization equation using ImageStream and IDEAS 4.0 software

To best define the nuclear area within the cell, we eroded the DAPI channel default system mask by 2 pixels widths. This was a non-intensity based modification of the mask. We then instructed the IDEAS analysis software package to calculate the internalization score of the ß-catenin FITC signal within the stringently masked nuclear area. The internalization is defined by the following equation, where I is the constructed nuclear mask and B the area of original segmentation outside of the input mask I.

Internalization = $\log((a / (1-a)) \times (pi / p\beta))$, where $a = mi / (mi + m\beta)$

mi = mean intensity of upper quartile pixels in I, $m\beta$ = mean intensity of upper quartile pixels in B, pi = peak intensity of upper quartile pixels in I, $p\beta$ = peak intensity of upper quartile pixels in B.

Increasingly positive values indicate that proportionally more of the given fluorescent signal is within the input mask, whereas increasingly negative values indicate the signal is without. Values of 0 are indicative of an equal apportionment. In all cases, we have quoted the median internalization score for the population of interest.