SUPPLEMENTAL TABLE S1

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

| Gene   | #5   | #7   | #8   | #13  | #11  | #4   | #10  |
|--------|------|------|------|------|------|------|------|
| AES    | 2.8998 | 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 |
| Gene     | Log2 Fold Change | p-value | Adjusted p-value |
|----------|-----------------|---------|------------------|
| CSNK1G1  | 0.0432          | 0.3583  | 4.0896           |
| CSNK2A1  | 4.3953          | 4.4195  | 4.1966           |
| CTPB1    | 16.7873         | 0.3264  | 5.5599           |
| CTPB2    | 2.1124          | 0.8748  | 1.0852           |
| β-CATENIN| 36.0645         | 9.2593  | 7.2676           |
| ICAT     | 8.0081          | 3.409   | 7.5671           |
| CXXC4    | 409.8568        | 5.762   | 59.8331          |
| DAAM1    | 1.3661          | 1.4321  | 3.176            |
| DIXDC1   | 18.1481         | 4.5433  | 5.8863           |
| DKK1     | 101.2115        | 2.4893  | 12.8723          |
| DVL1     | 3.0158          | 0.358   | 0.7963           |
| DVL2     | 6.64            | 3.1661  | 3.3612           |
| EP300    | 5.0337          | 0.0842  | 12.3928          |
| FBXW11   | 11.2032         | 6.8249  | 6.581            |
| FBXW2    | 404.1948        | 5.762   | 48.3108          |
| FGF4     | 426.4595        | 5.762   | 49.3491          |
| FOSL1    | 5.3271          | 10.152  | 2.9571           |
| FOXN1    | 415.2983        | 5.762   | 47.057           |
| FRAT1    | 16.2531         | 6.0711  | 7.4109           |
| FRZB     | 86.6293         | 1.0482  | 10.0418          |
| FSHB     | 213.1607        | 5.762   | 22.3186          |
| FZD1     | 19.3745         | 2.2068  | 2.9182           |
| FZD2     | 78.0897         | 1.5973  | 80.0045          |
| FZD3     | 15.1735         | 0.2747  | 1.6384           |
| FZD4     | 0.6949          | 5.762   | 38.2819          |
| FZD6     | 10.5482         | 0.1993  | 17.0294          |
| FZD7     | 10.5236         | 2.8313  | 1.9019           |
| FZD8     | 26.5372         | 0.4726  | 7.2874           |
| GSK3A    | 2.5296          | 2.6627  | 2.6118           |
| GSK3B    | 1.141           | 1.0576  | 0.5835           |
| JUN      | 6.3492          | 33.4533 | 17.6869          |
| KREMEN1  | 62.4102         | 0.7607  | 7.9454           |
| LRP5     | 107.3384        | 5.762   | 14.5368          |
| LRP6     | 70.3742         | 0.9537  | 38.2035          |
| MYC      | 49.6587         | 2.0278  | 12.3073          |
| NKD1     | 173.9066        | 3.1704  | 20.8518          |
| NLK      | 9.9057          | 0.8448  | 1.2136           |
| PITX2    | 320.7459        | 5.762   | 37.9018          |
| PORCN    | 8.3153          | 2.3299  | 4.7181           |
| PPP2CA   | 3.3136          | 1.7577  | 2.8578           |
| PPP2R1A  | 3.5603          | 1.7463  | 2.754             |
| PYG1     | 510.1066        | 5.762   | 77.2113          |
| RHOU     | 15.5353         | 1.3147  | 5.696            |
| SENP2    | 18.0554         | 3.9076  | 13.9005          |
| SFRP1    | 452.3768        | 5.762   | 73.0385          |
| SFRP4    | 496.5303        | 5.762   | 47.1369          |
| FBXW4    | 0.4619          | 0.0686  | 0.3545           |
| SLC9A3R1/EGBP5 | 4.4566 | 24.9939 | 11.1519 |
| SOX17    | 291.2752        | 5.762   | 50.225           |
| TFF      | 439.9607        | 6.1646  | 75.723           |
| TCF1     | 743.3192        | 13.0184 | 58.3137          |
| TCF3     | 10.805          | 2.3921  | 1.7365           |
| TLE1     | 1.0202          | 0.0091  | 0.255            |
| TLE2     | 36.0036         | 0.5432  | 4.4913           |
| WIF1     | 225.8868        | 3.6185  | 28.4411          |
| WISP1    | 326.4782        | 4.2665  | 34.868           |

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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 β-catenin in AML patients

17 AML patients’ samples (black squares, black triangle) were analyzed for their MFI of β-catenin, indicating protein expression, and for their relative β-catenin mRNA level normalized to that of CD34+ NBM and to GAPDH. Linear regression R² 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, R²: 0.1388, p value of a non-zero slope: 0.1554).

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

CD34-PE+ gated events from AML patients #1 (A) and #6 (B) were assessed for β-catenin FITC positivity and gated appropriately (left panel), nuclear internalization repartition (mid panels) and image analysis (right panels) of β-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 β-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. β-actin was used as normalizing control.
**Figure S4. Effective knockdown of β-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. β-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 β-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 |
|------------|--------------------------------------|
| β-catenin  | TCTGATAAAGGCTACTGTTGGATTGA TCA CGCA AAGG GTGCATGATT |
| Cyclin D1  | AACTACCTGGACCGCTTCTT CC ACTT GAGGTTGGTTCACCA |
| Cyclin D2  | TGGGGAAATTTGAAAGTTGAAC AT C AT C GACG TGGGTACAT |
| Cyclin D3  | ATGCTTGCTTTACTGGATGCT TGCACAGTTTTCGATGCT |
| BCL2       | AGTACCTGAACC GGCACCTG TTCAGAGACAGCCAGGAAAT |
| BAD        | CCAGAGT TTTGAGCGAGCACTGTA CCATCCCTTTGC GTGCCTC |
| BAK        | G TTTTCCGCAGCAGCTACGTTT TTCAGAGCAGCCAGGAAAT |
| BCL XL     | TCG CATTTG TGGCCTTTTT TGCAT GCAGGTTGGTTCGCTC |
| TCF3       | GAGGACGAGGAAGACACGT CAA GGCCAGCTGAGTCAC |
| SFRP4      | CTGCCCC ATCAAGAT TT CT CGGTTGTTCTCTTCAGAG |
| DKK1       | TCTCGAGG GAGAAATTGAGGA TA TCCGGAAGACAGACCTT |
| MYC        | CCTACCCCTCTCAACGAGAGC C TCTGACCATTTGGCCAGGAG |
| β-actin    | GGA CTGAGCAAGAGATG G AGC A C TGTGTTGGCGTGACAG |
| GAPDH      | GGGAG GTGAGG CAGTGAGT GGG TCATTTAGGCAACAATA |
**Superarray analysis**

The expression profile of 84 Wnt/ß-catenin pathway-related genes was determined using a 96-well format human Wnt signaling pathway RT² 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 RT² 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](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.

$$\text{Internalization} = \log \left( \frac{a}{1-a} \right) \times \frac{\pi}{p_b}, \text{ where } a = \frac{m_i}{m_i + m_B}$$

$m_i$ = mean intensity of upper quartile pixels in I, $m_B$ = mean intensity of upper quartile pixels in B, $pi$ = peak intensity of upper quartile pixels in I, $p_B$ = 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.