Supplementary material

Supplementary methods

Assessment of catenin mRNA expression using qRT-PCR

Patient RNA was isolated from the blasts of primary AML samples (n=19) or leukemic cell lines as previously described.[1] RNA samples were prepared for, and analysed by, qRT-PCR also as previously described[2] using the following primers (Eurofins MWG Operon, Ebersberg, Germany) for *γ-catenin*: forward; 5'-ACTGAACTCCACCGACCAAC-3' and reverse; 5'-GTCGAAGAGAGAGGTCCCAC-3', and for the housekeeping gene *ABL:* forward; 5'-CCCAACCTTTTCGTTGCACTGT-3' and reverse 5'-AGATGCAGAGGAGGCTCTCGGC-3'. Normalized expression values were determined by crossing-point analysis of *γ-catenin* or *β-catenin* signal compared with the *ABL* housekeeping gene control in duplicate samples. Normalized *γ-catenin* and *β-catenin* qRT-PCR values were compared to the corresponding, microarray values for the patient,[1] and the degree of correlation between the two techniques assessed using Pearson's correlation coefficient (R).

Supplementary material

Supplemental Table S1. AML patient clinical and demographic data. Cohort 1 contains clinical characteristics of the patients used in whole cell Western blotting and cohort 2 the patients (for which there is clinical data available) included in nuclear/cytosolic Western blotting.

Parameter	Patient cohort 1 (n=44)	Patient cohort 2 (n=49)
Age		
<15	0	0
15-29	2	8
30-39	7	5
40-49	12	5
50-59	15	18
60-69	5	7
70+	3	6
Median (range)	53 (23-77)	55 (17-78)
Sex		
Female	20	27
Male	24	22
WHO performance status		
0	20	27
1	17	20
2	3	2
3	4	0
4	0	0
White blood cell count ($x10^9$/L)		
<10	9	6
10-49.9	15	16
50-99.9	9	16
100+	11	11
Unknown	0	0
Median (range)	34.3 (1.2-188.0)	64.1 (3.8-257.0)
Cytogenetics		
Favourable	7	4
Intermediate	23	31
Adverse	3	6
Unknown	11	8
Secondary Disease		
No	38	46
Yes	6	3
FAB Type		
M0	3	6
M1	14	19
M2	6	10
M3	0	0
M4	10	8
M5	3	4
M6	1	0
M7	0	0
ALL	0	0
Bilineage	0	0
RAEB/RAEB-t	0	0
Unknown/Other	7	2

Supplementary material

Supplemental Table S2. AML patient clinical and demographic data. Specific clinical characteristics of the patient samples presented in Western blot Figure 1A.

Lane number (left to right on blot)	Age	Sex	WHO performance status	WBC Count ($x10^9$/L)	Cytogenetic status	Secondary disease (Y/N)	FAB type
1	55	M	3	48.4	Intermediate	N	M2
2	48	F	0	50	Intermediate	N	M2
3	54	F	0	7.6	Intermediate	N	U
4	56	F	0	25	U	Y	M4
5	59	F	0	124.9	Favourable t(8;21)	N	M2
6	42	F	1	58	Favourable	N	M1
7	42	F	1	184	U	N	M1
8	64	M	0	61.4	Intermediate	N	M1
9	35	M	0	10.7	Favourable	N	M2
10	57	M	2	183.7	Intermediate	N	U
11	61	F	1	7.4	Adverse	Y	M0
12	55	M	1	188	U	N	M0

U=Unknown

Supplementary material

Supplemental Table S3. AML patient clinical and demographic data. Specific clinical characteristics of the patient samples presented in Western blot Figure 2A.

Lane number (left to right on blot)	Age	Sex	WHO performance status	WBC Count ($x10^9$/L)	Cytogenetic status	Secondary disease (Y/N)	FAB type
1	32	M	0	257	Intermediate	N	M0
2	59	F	1	29.4	Intermediate	N	M0
3	24	F	0	25.8	Intermediate	N	M2
4	71	M	1	8.8	Adverse	N	M2
5	58	F	0	2	Adverse	N	M1
6	63	M	0	68.2	U	N	M4
7	U	U	U	U	U	U	U

U=Unknown

Supplemental Table S4. AML patient clinical and demographic data. Specific clinical characteristics of the patient samples used for CLSM image Figure 2B.

AML patient number	Age	Sex	WHO performance status	WBC Count ($x10^9$/L)	Cytogenetic status	Secondary disease (Y/N)	FAB type
1	74	F	U	123	Adverse	N	M0
2	73	M	U	120	Adverse	N	M0

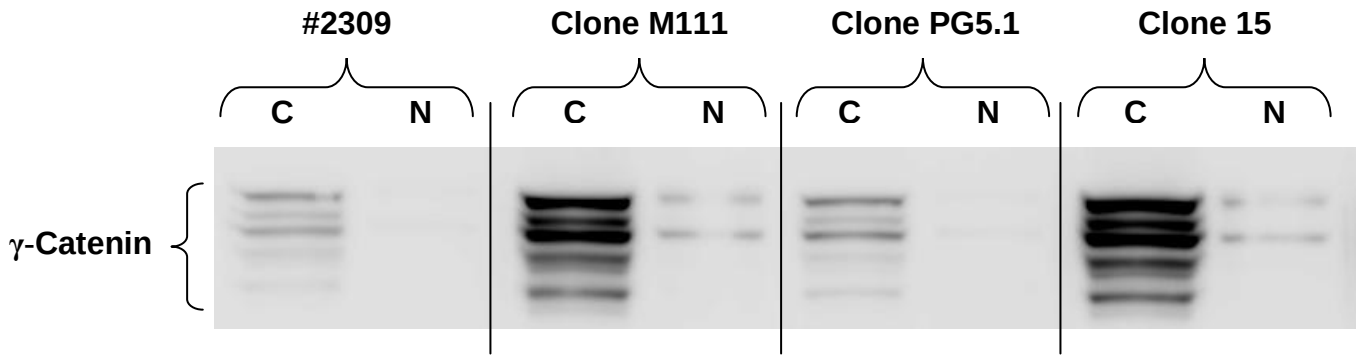
U=Unknown

Supplementary material

Supplemental Table S5. AML patient clinical and demographic data. Specific clinical characteristics of patient samples presented in Western blot Figure 3A.

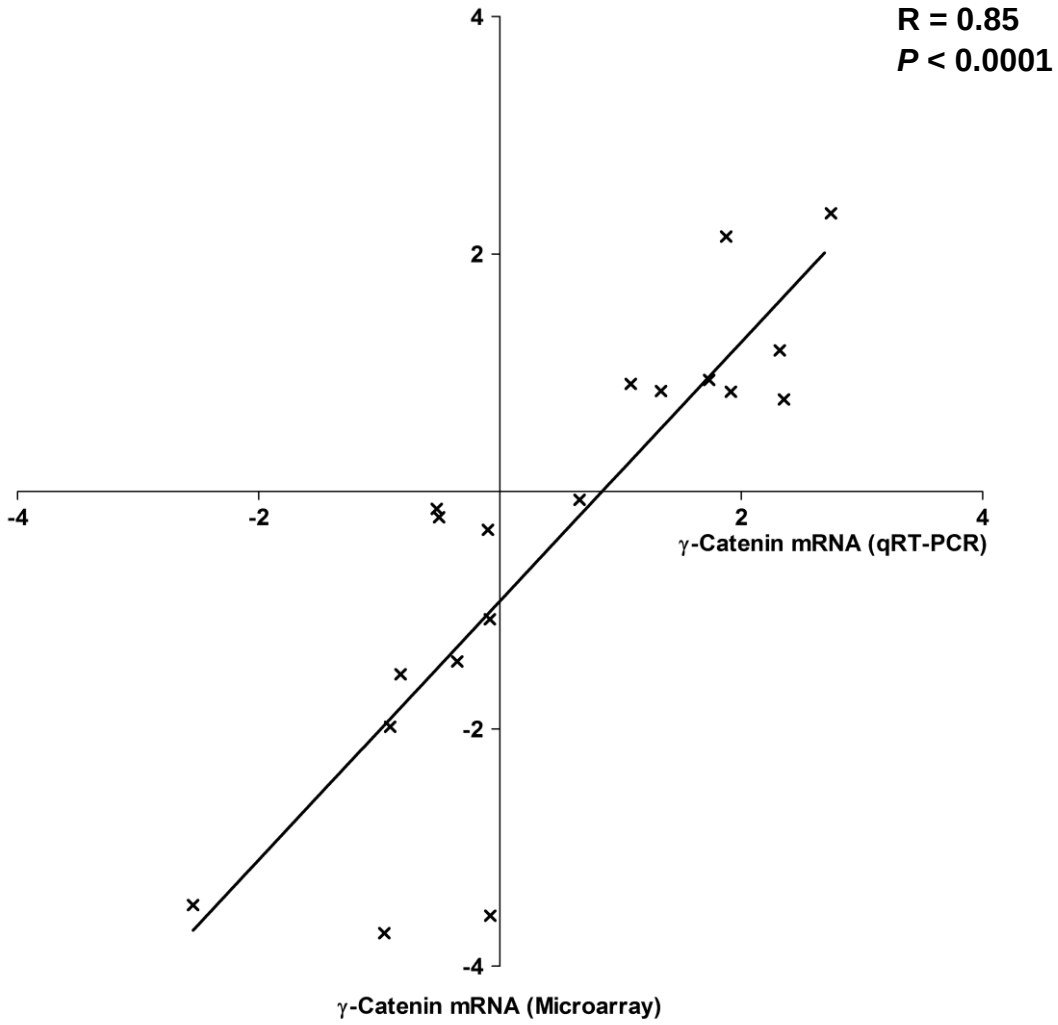
Lane number (left to right on blot)	Age	Sex	WHO performance status	WBC Count (x10^9/L)	Cytogenetic status	Secondary disease (Y/N)	FAB type
1	17	M	0	75	Intermediate	N	M1
2	17	M	0	29	Intermediate	N	M1
3	28	F	0	74	Favourable inv(16)	Y	M1
4	35	M	0	55.5	Adverse	N	M1
5	53	F	0	113.4	Intermediate	N	M1
6	28	F	0	28	Adverse	N	M0
7	68	M	1	193	Intermediate	N	M1
8	U	U	U	U	U	U	M4

U=Unknown

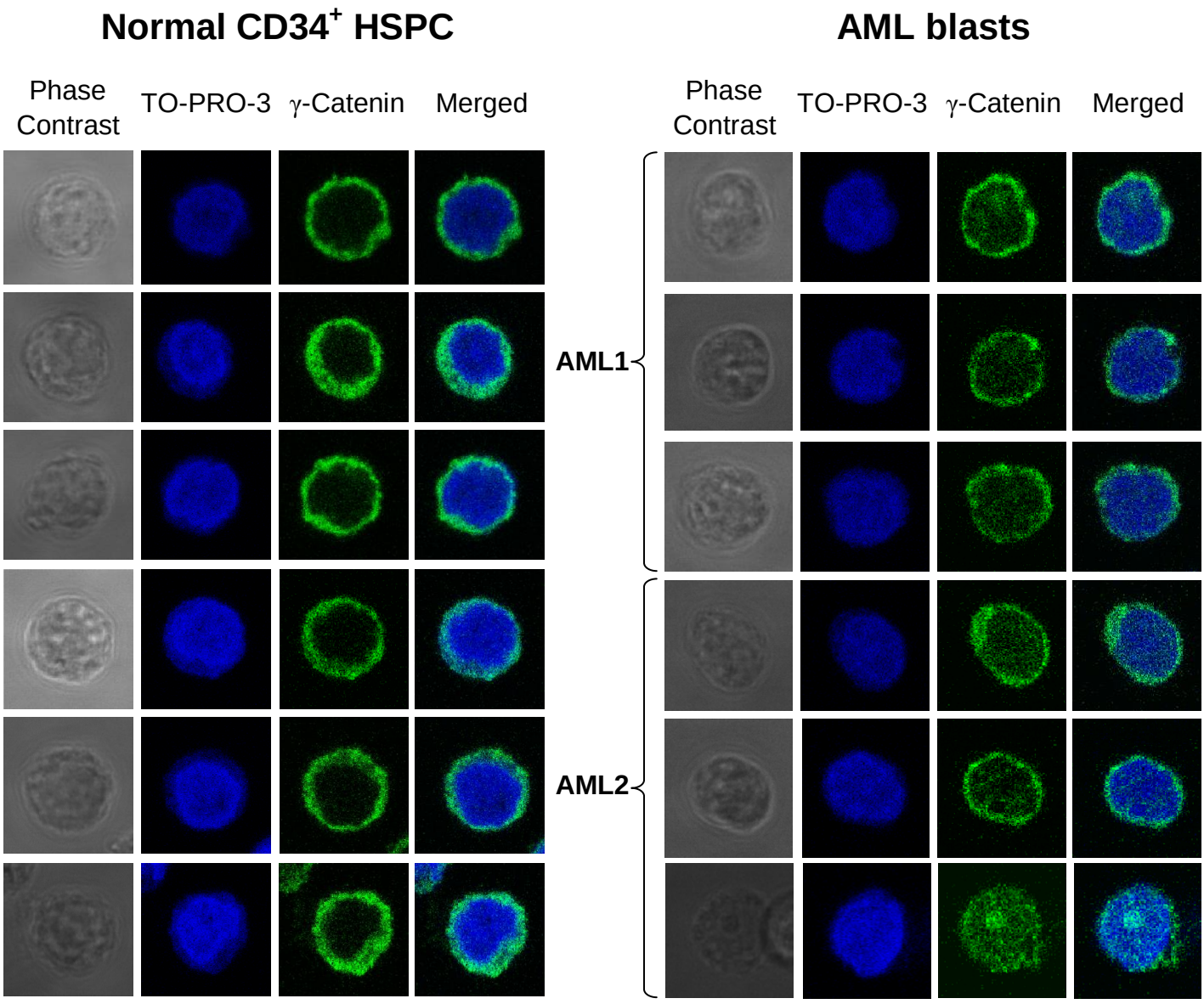


Supplemental Figure S1. The multiple banding observed during γ-catenin detection is not due to non-specific binding by antibodies. Cytosolic and nuclear fractions of K562 cells were separately probed with the following γ-catenin antibodies: #2309 (polyclonal, Cell signalling), clone M111 (monoclonal, Abcam), clone PG 5.1 (monoclonal, AbD Serotec) and Clone 15 (monoclonal, BD). All antibodies produced the same pattern of γ-catenin protein bands (albeit to varying intensities).

Supplementary material

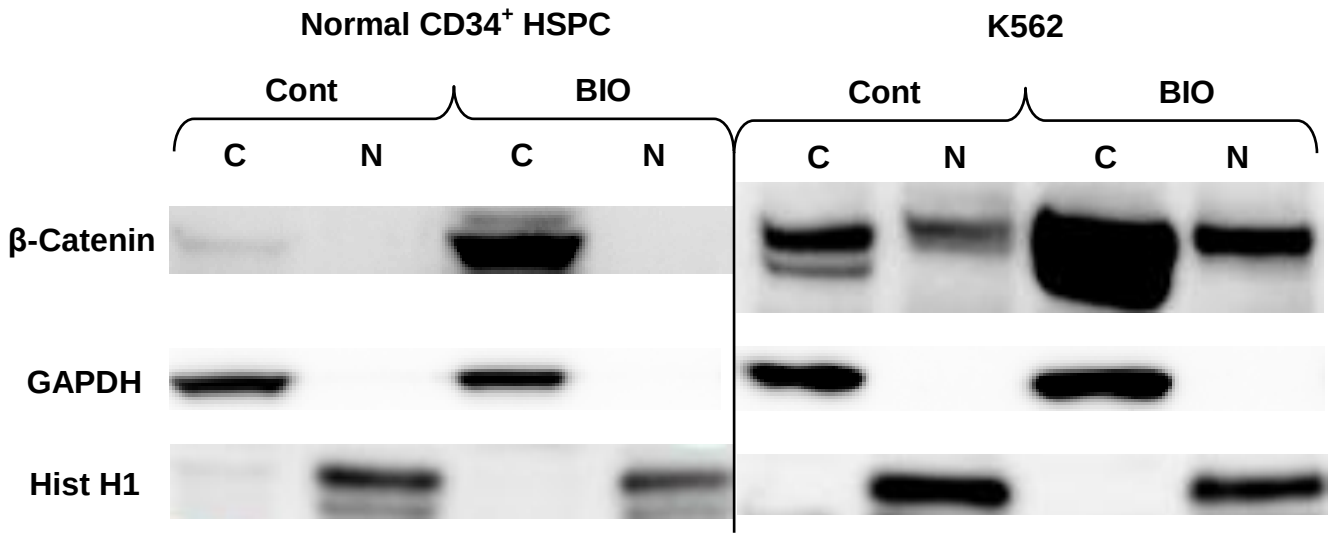


Supplemental Figure S2. Validation of γ-catenin mRNA expression values by qRT-PCR. Patient RNA was isolated from the blasts of primary AML samples (n=19) and the measurement of *γ-catenin* transcript validated by both microarray and qRT-PCR. The degree of correlation between the two techniques is shown on the graph above.



Supplemental Figure S3. γ-Catenin subcellular localisation is dysregulated in AML blasts. AML blasts or normal CD34$^+$ HSPC were fixed, permeabilised, immunolabelled with γ-catenin antibody and analysed by CLSM as described in Methods. Phase contrast, TO-PRO-3 (DNA stain), γ-catenin and merged images are shown. Primary AML blasts (n=5, six Z-sections shown from 2 AML patients, AML1 and AML2) demonstrated significantly (P<0.05) more nuclear γ-catenin staining than their normal CD34$^+$ HSPC counterparts (n=3, six Z-sections shown from one CD34$^+$ HSPC preparation).

Supplementary material



Supplemental Figure S4. Sensitivity of β-catenin protein level to the GSK inhibitor, BIO, in hematopoietic cells. Cytosolic (C) and nuclear (N) expression of β-catenin hematopoietic cells treated with the GSK inhibitor BIO (which inhibits the destruction complex) for 16 hours. GAPDH and Histone indicate the purity/relative loading of each fraction. The extensive stabilization of β-catenin following BIO treatment demonstrates that β-catenin is rapidly degraded in both normal and leukemic cells, however only in leukemic cells does β-catenin stabilization result in its translocation.

References

1. Tonks A, Pearn L, Musson M, et al. Transcriptional dysregulation mediated by RUNX1-RUNX1T1 in normal human progenitor cells and in acute myeloid leukaemia. *Leukemia*. 2007;21(12):2495-2505.

2. Liddiard K, Hills R, Burnett AK, Darley RL, Tonks A. OGG1 is a novel prognostic indicator in acute myeloid leukaemia. *Oncogene*. 2010;29(13):2005-2012.