SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1

Construction of standard curves for quantitation of BCL2 family survival proteins.

Recombinant protein standards (BCL2, BCL2L1, BCL2L2, BCL2A1) were obtained from Drs. Peter Czabotar and David Huang (WEHI, Melbourne, Australia) and recombinant MCL1 was purchased from Bioclone Inc, San Diego, CA, USA. Serial dilutions were made and run on westerns with CLL cell lysate. Densitometry was used to construct standard curves.

Supplemental Figure 2

miRNA significantly altered by culture conditions

(a) miRNA with potential binding sites in the 3' UTR of BCL2 were obtained fron TargetScanv5.0. Two statistical tests were applied: firstly ANOVA across the three groups (peripheral blood, NT and CD154 culture) and secondly Kruskal Wallis to compare NT and CD154 cultures. The results were integrated in the Venn diagram to show (in the intersection) those miRNA significantly altered across all groups as well as between NT and CD154 culture. (b) Amount of miR-17, miR20a and miR-15b in fractions from sucrose density gradients produced from nuclear poor lysates of CLL cells cultured under different conditions. Squares are from freshly isolated cells, triangles from stromal cell culture and circles from stromal cells with CD154. (c) Luciferase assays demonstrating the lack of effect of miR-17 and miR-20a at the BCL2 3'UTR (left-hand panels). MiR-15b reduced luciferase expression by about 30% (right-hand panel).

Supplemental Figure 3

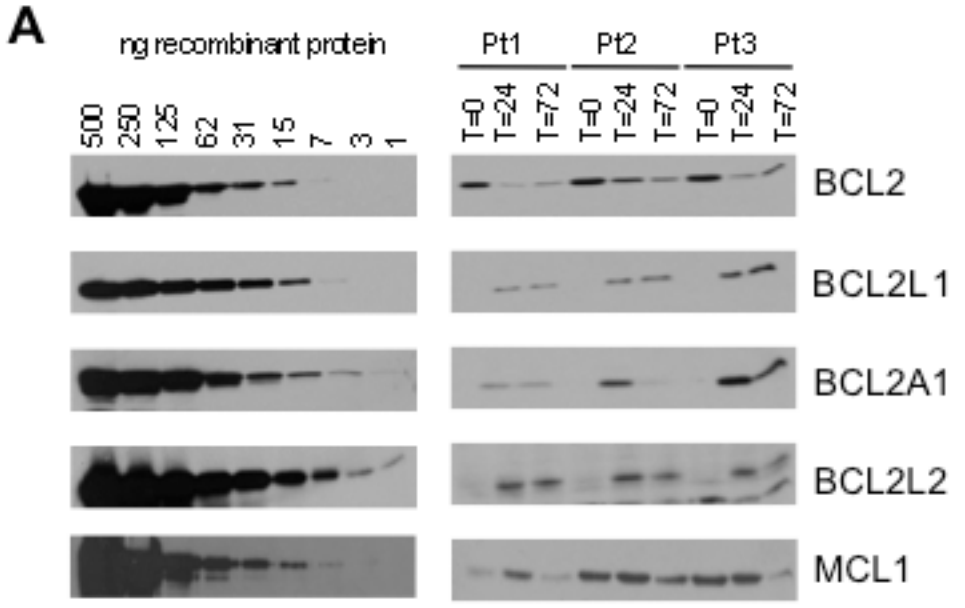
miRNA standard curves.

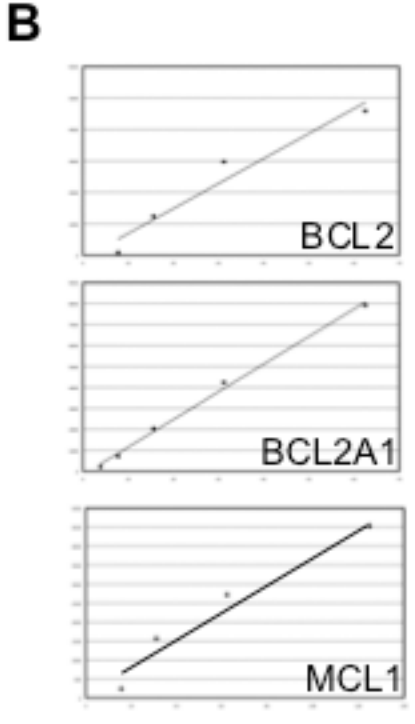
MiRNAs were isolated from freshly isolated and stimulated CLL cells using a miRvana miRNA isolation kit and reverse transcribed using a Taqman MicroRNA Reverse Transcription Kit (Applied Biosystems). Commercial miRNA primer/probe sets were used for analysis by real-time PCR (Applied Biosystems: miR 155 #002623, miR 15b #000390, miR 125b #000449). Data were normalized to the control RNU44 (Applied Biosystems) and are expressed as fold change in expression relative to freshly isolated CLL cells. To quantitate miRNA expression, standard curves were created using serial dilutions of the Applied Biosystems mirVana miRNA reference panel (v9.1). Known quantities of miR were plotted against Ct values and the slope values generated were used to calculate the absolute amount of miR-15b, miR-17, miR-20a, miR-125b and miR-155 in freshly isolated and CD stimulated CLL cells. Data were expressed as amol miRNA/ng total RNA.

Supplemental Table 1

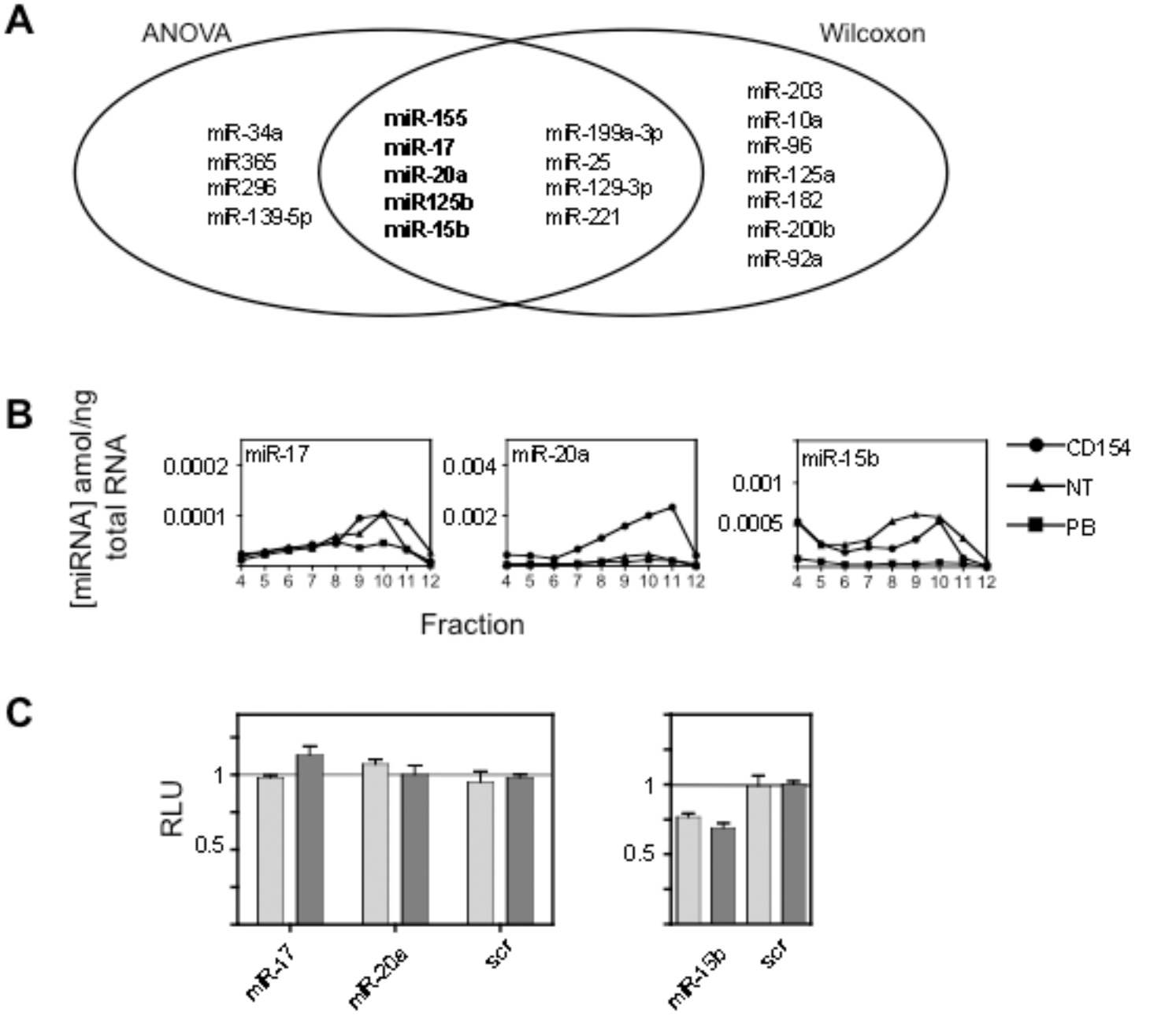
Clinical characteristics of patients used in the study. Immunoglobulin hene mutational ststus is either M, mutated or U, unmutated. The results of fluorescence in situ hybridisation (FISH) for the commonest chromosomal abnormalities in CLL (11q deletion, 13q deletion, 17p deletion and trisomy 12) are presented.

Patient Number	Gender	Age at diagnosis	Stage diagnosis	CD38 (%)	Immunoglobulin gene mutational status	11q del	13q del	17p del	trisomy 12
1	M	64	A	<1	M	-	+	-	-
2	M	47	A	12	U	-	+	-	-
3	F	49	A	1	M	-	+	-	-
4	M	64	A	6	U	+	-	-	-
5	F	34	A	36	M	-	+	-	-
6	M	81	B	99	U	+	+	-	-
7	M	73	A	100	M	-	+	-	-
8	M	59	B	<1	U	-	+	-	-
9	M	52	B	16	U	-	+	-	-
10	M	33	A	16	M	-	-	-	-
11	M	56	A	4	M	-	-	-	+
12	F	56	A	<1	M	-	+	-	-
13	M	65	A	ND	M	-	+	-	-
14	M	78	A	20	U	-	-	-	+
15	F	50	A	ND	M	-	-	+	-
16	M	78	A	ND	M	-	+	-	-
17	F	74	C	95	U	+	-	-	-
18	F	57	A	ND	M	-	-	-	-
19	M	68	A	ND	M	ND	ND	ND	ND
20	M	65	A	1	U	+	+	-	-
21	M	81	A	39	U	+	_	-	-
22	F	61	A	1	M	-	+	-	-
23	M	48	A	42	U	+	-	-	-
24	F	61	A	2	U	+	+	-	-
25	F	71	A	8	M	-	+	-	-
26	M	77	A	1	U	+	-	-	-

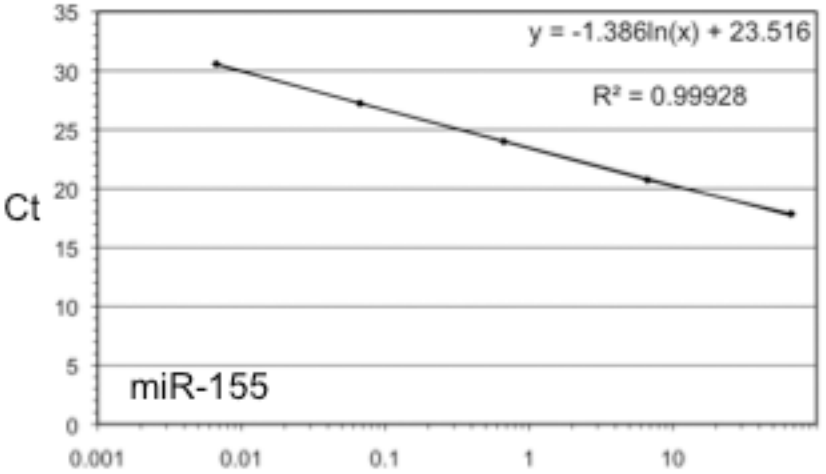


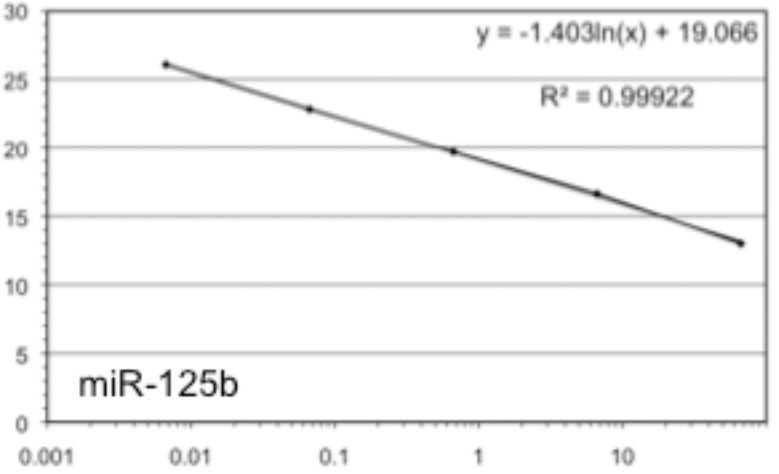


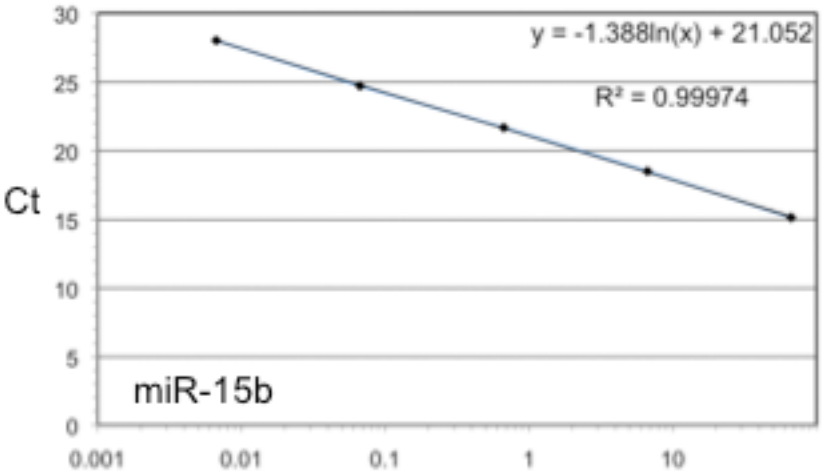
Supplemental Figure 1
Willimott & Wagner



Supplemental Figure 2
Willimott & Wagner







miR (amole)

miR (amole)

Supplemental Figure 3
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