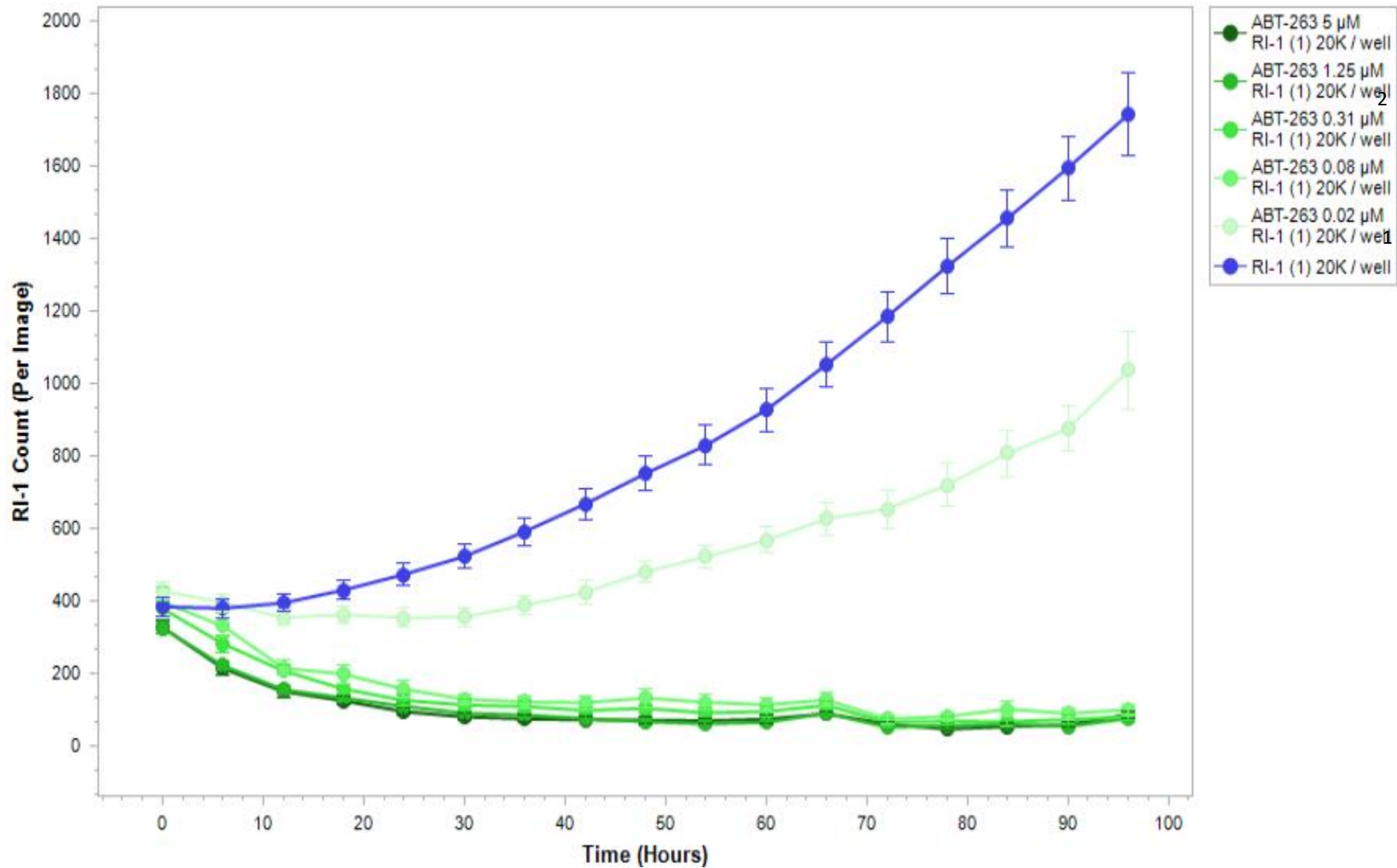


Suppl. Figure 1a

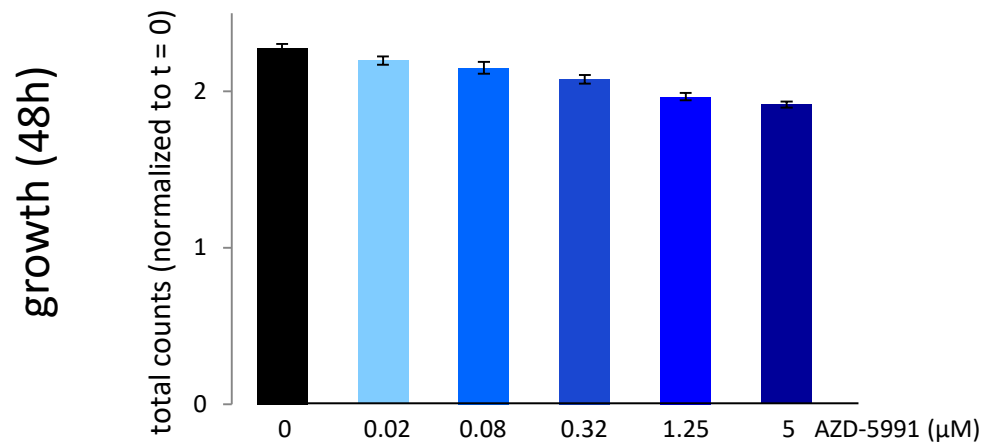
RI-1 DLBCL (BCL2<sup>pos</sup>)



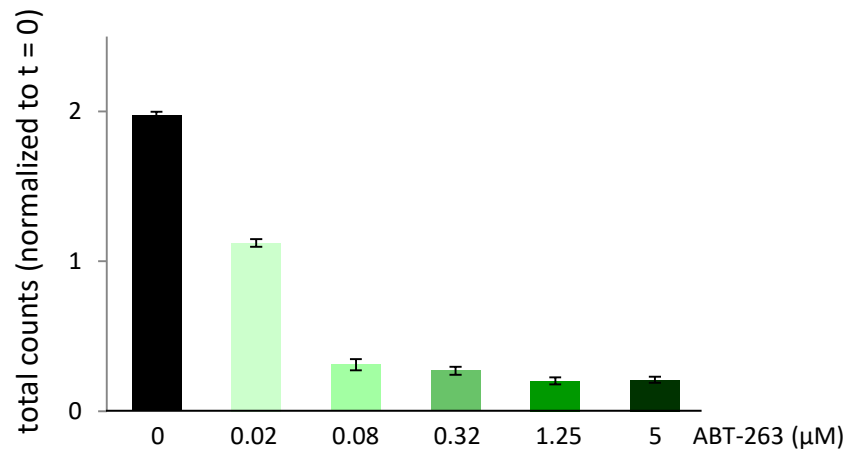
Suppl. Figure 1b

RI-1 DLBCL (BCL2<sup>pos</sup>)

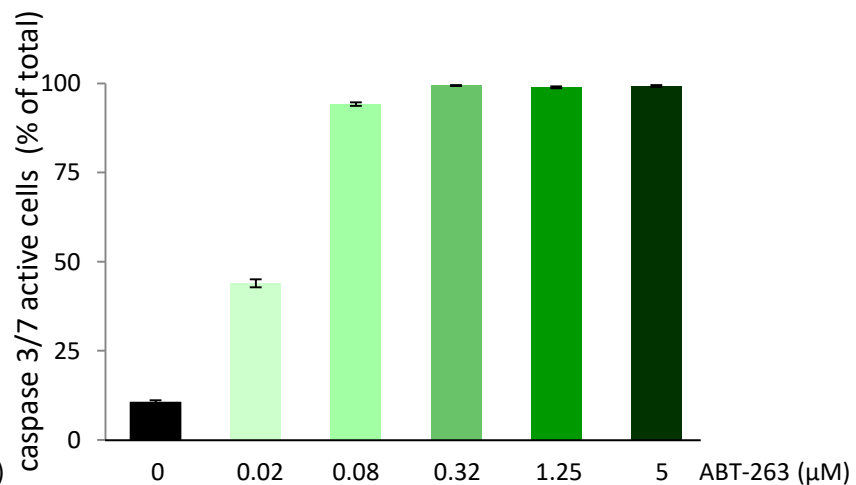
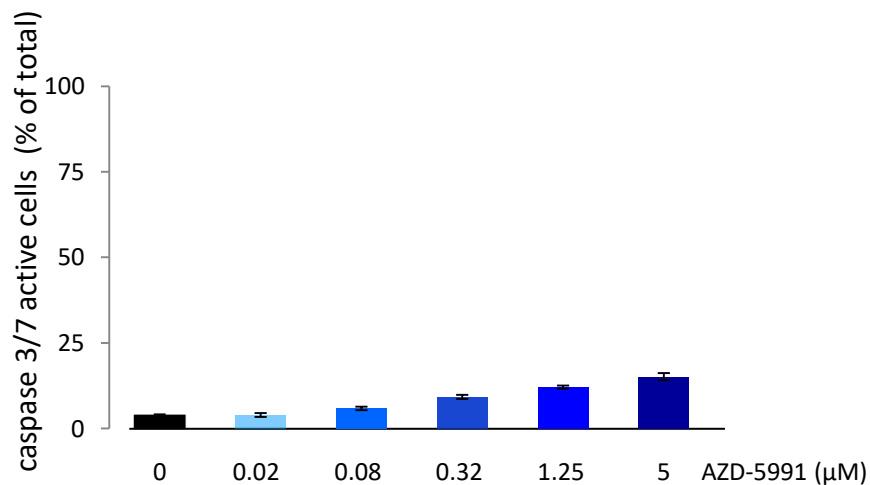
AZD-5991



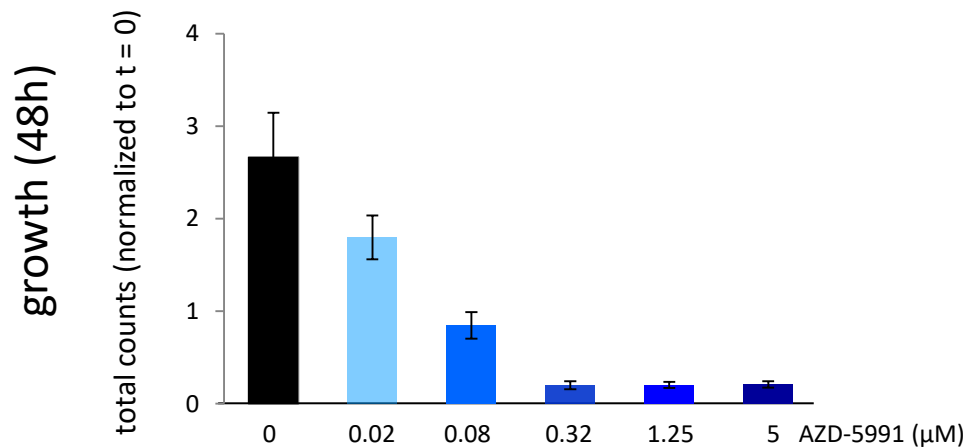
ABT-263



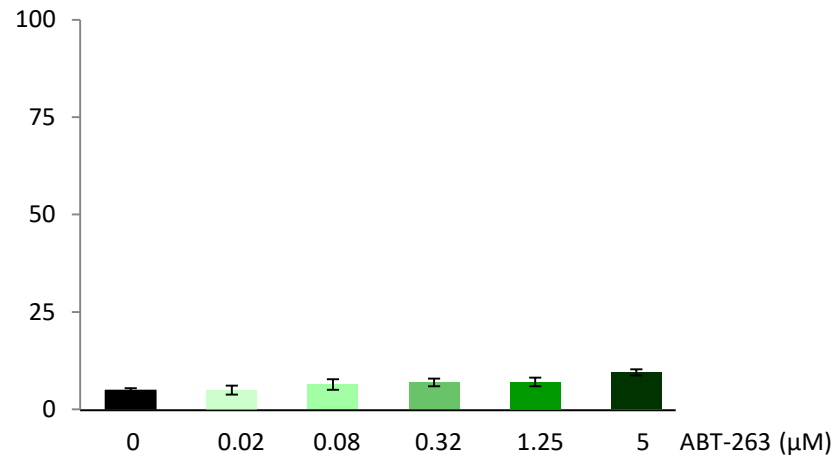
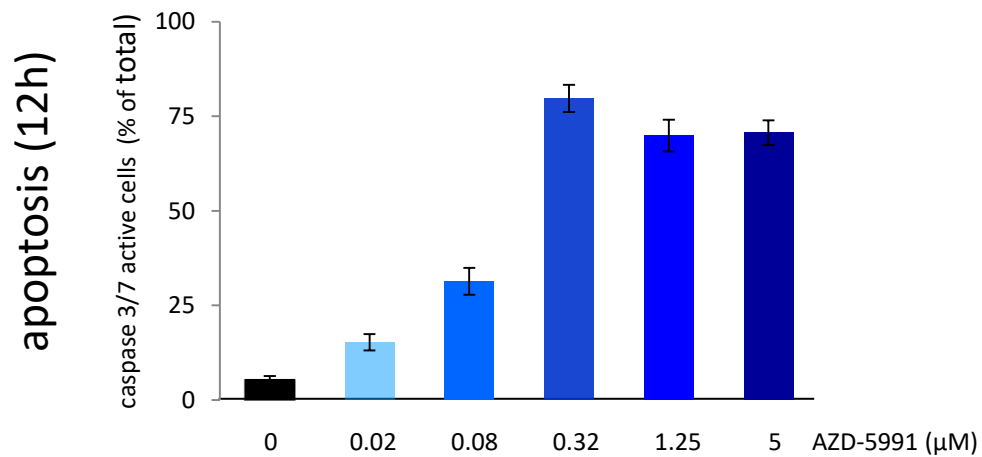
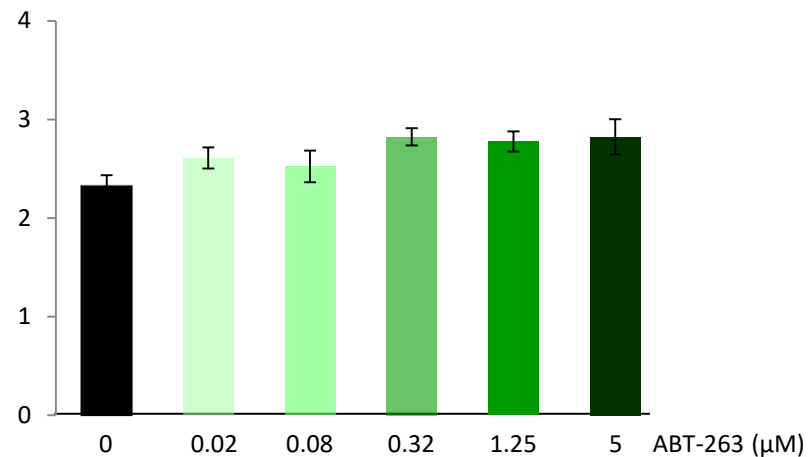
apoptosis (12h)



AZD-5991



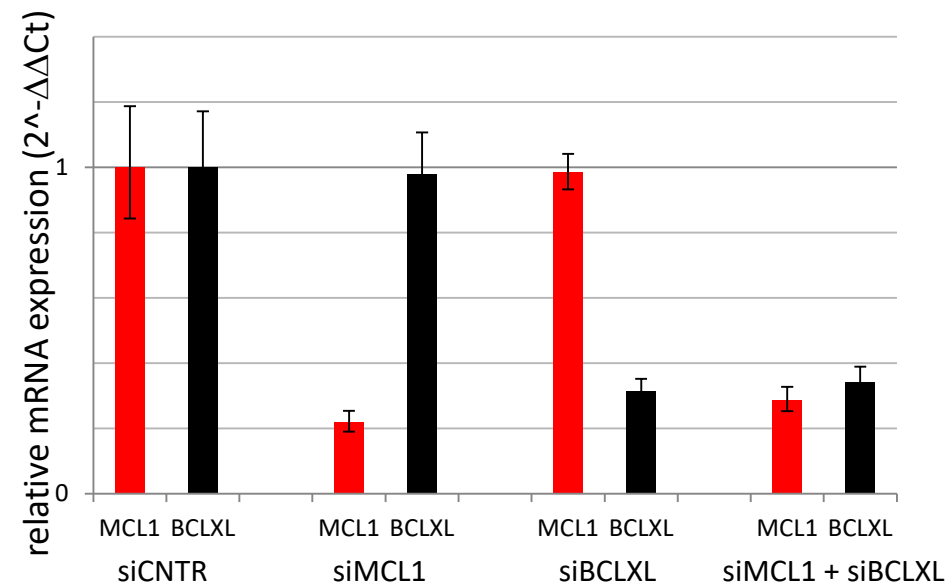
ABT-263



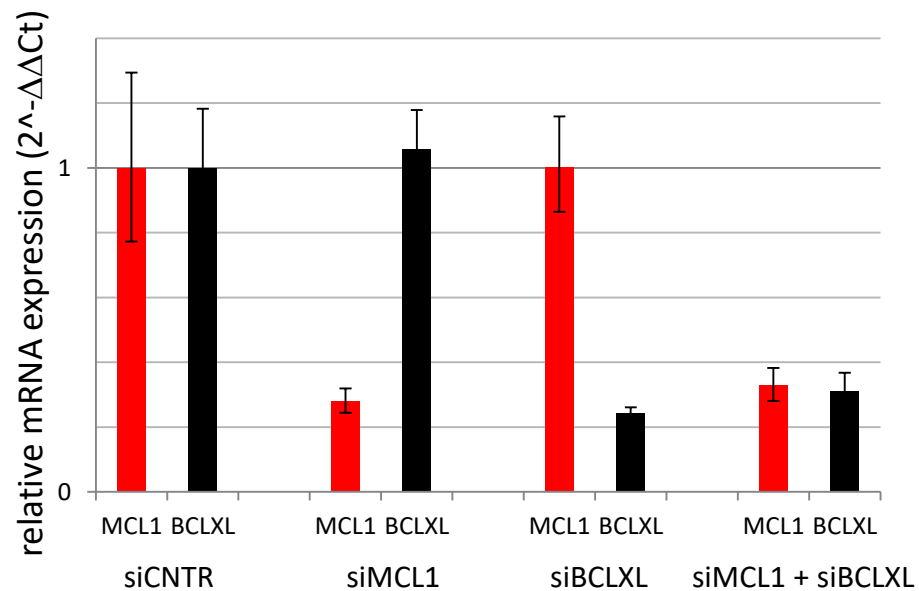


# Suppl. Figure 3

## L-82

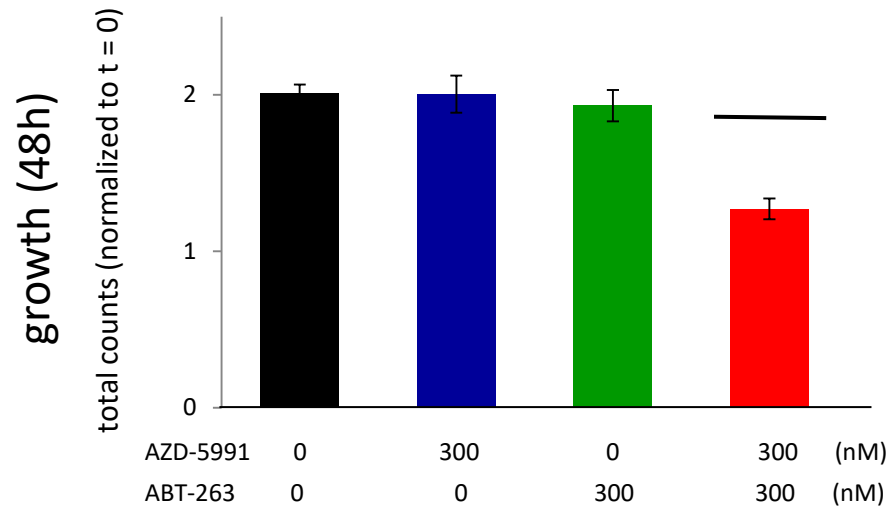


## KARPAS-299

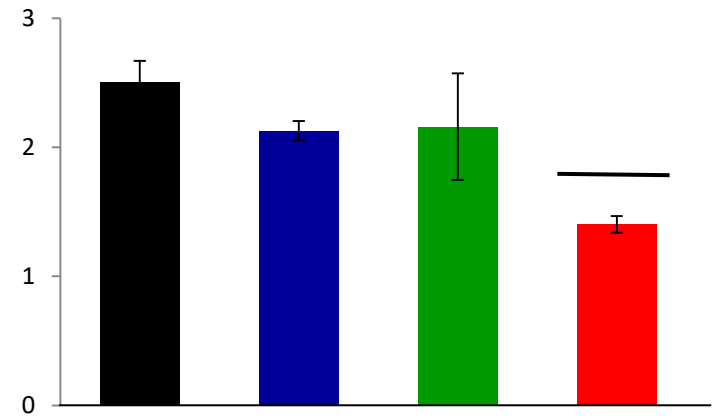


# Suppl. Figure 4a

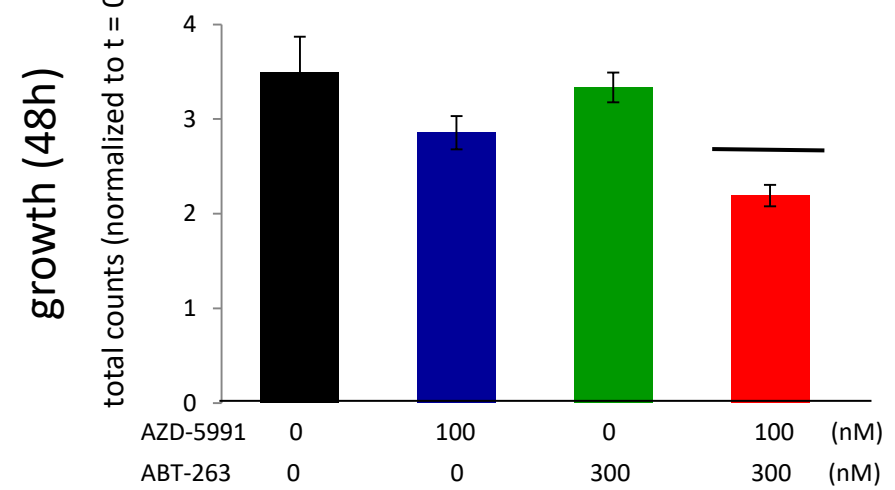
## KI-JK: MCL1<sup>pos</sup>/BCLXL<sup>pos</sup>



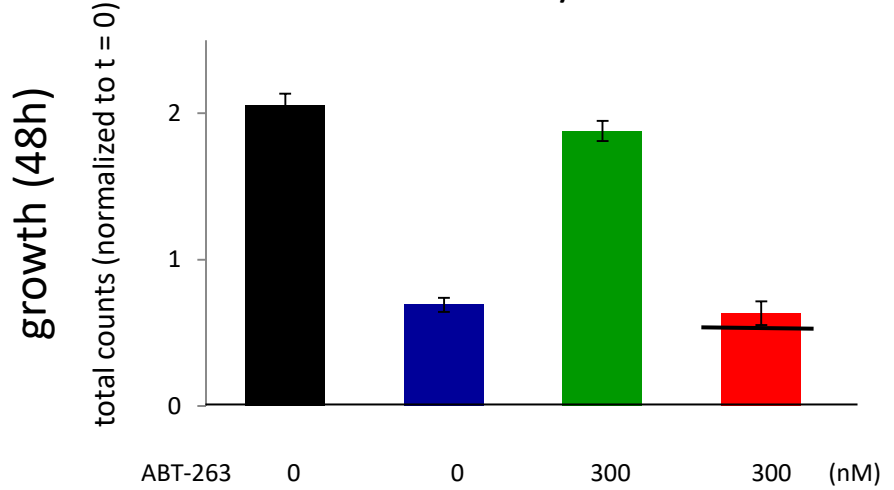
## SUP-M2: MCL1<sup>pos</sup>/BCLXL<sup>pos</sup>



## DEL: MCL1<sup>pos</sup>/BCLXL<sup>pos</sup>

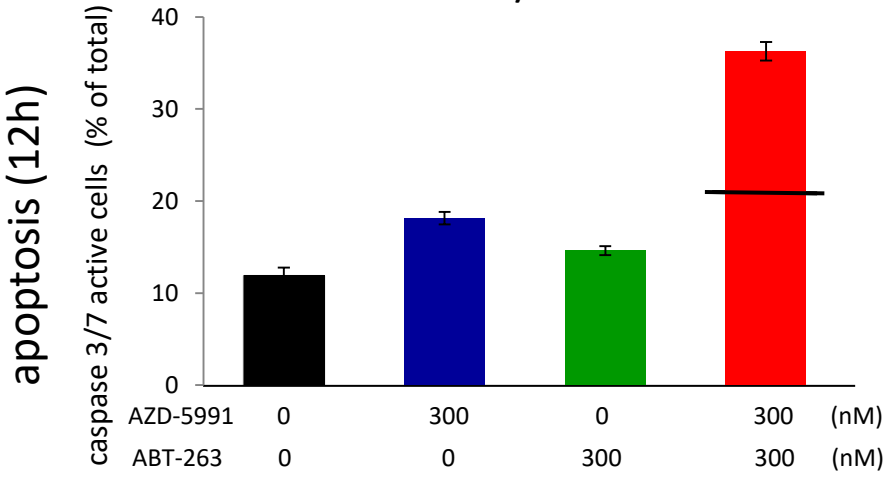


## BC-3: MCL1<sup>pos</sup>/BCLXL<sup>low</sup>

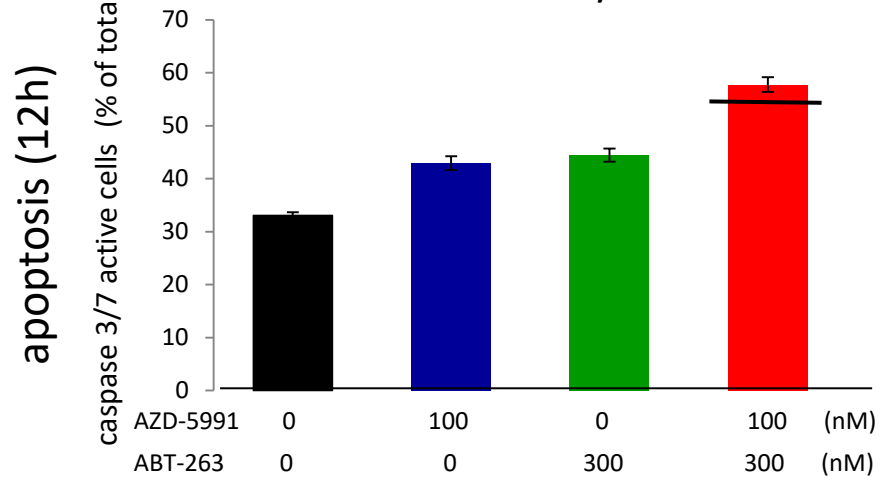


# Suppl. Figure 4b

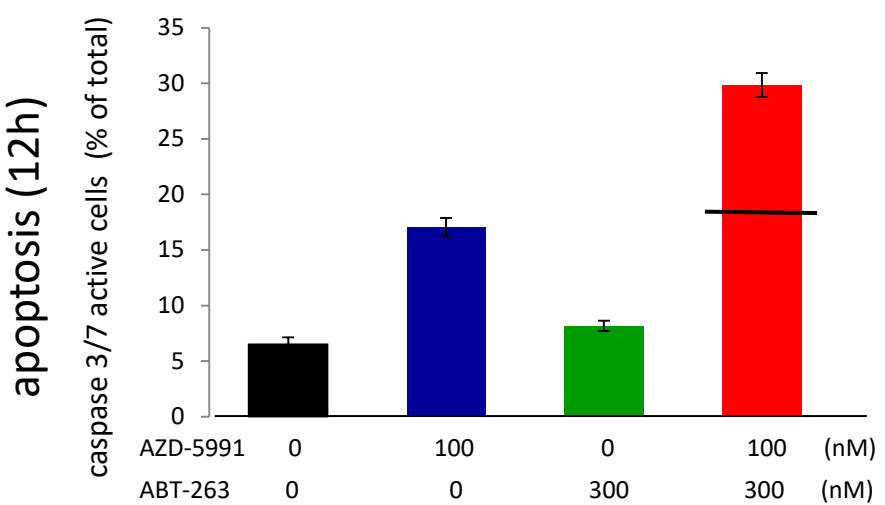
## KI-JK: MCL1<sup>pos</sup>/BCLXL<sup>pos</sup>



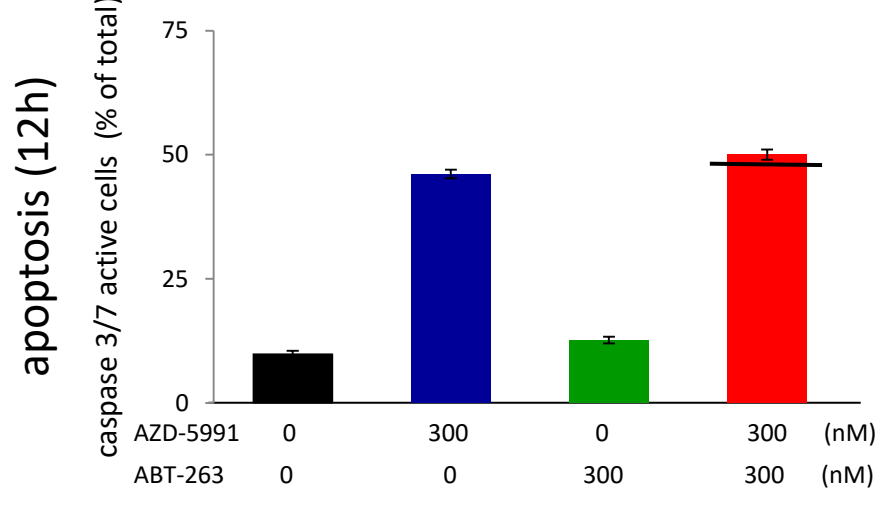
## SUP-M2: MCL1<sup>pos</sup>/BCLXL<sup>pos</sup>



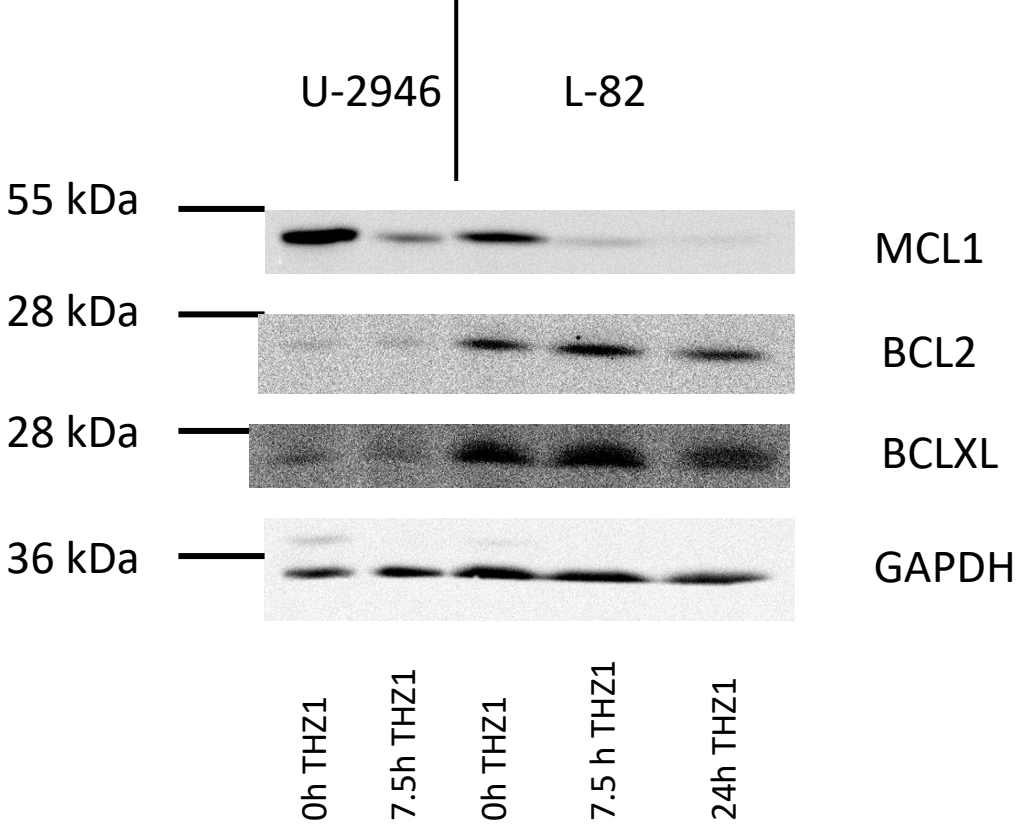
## DEL: MCL1<sup>pos</sup>/BCLXL<sup>pos</sup>



## BC-3: MCL1<sup>pos</sup>/BCLXL<sup>low</sup>

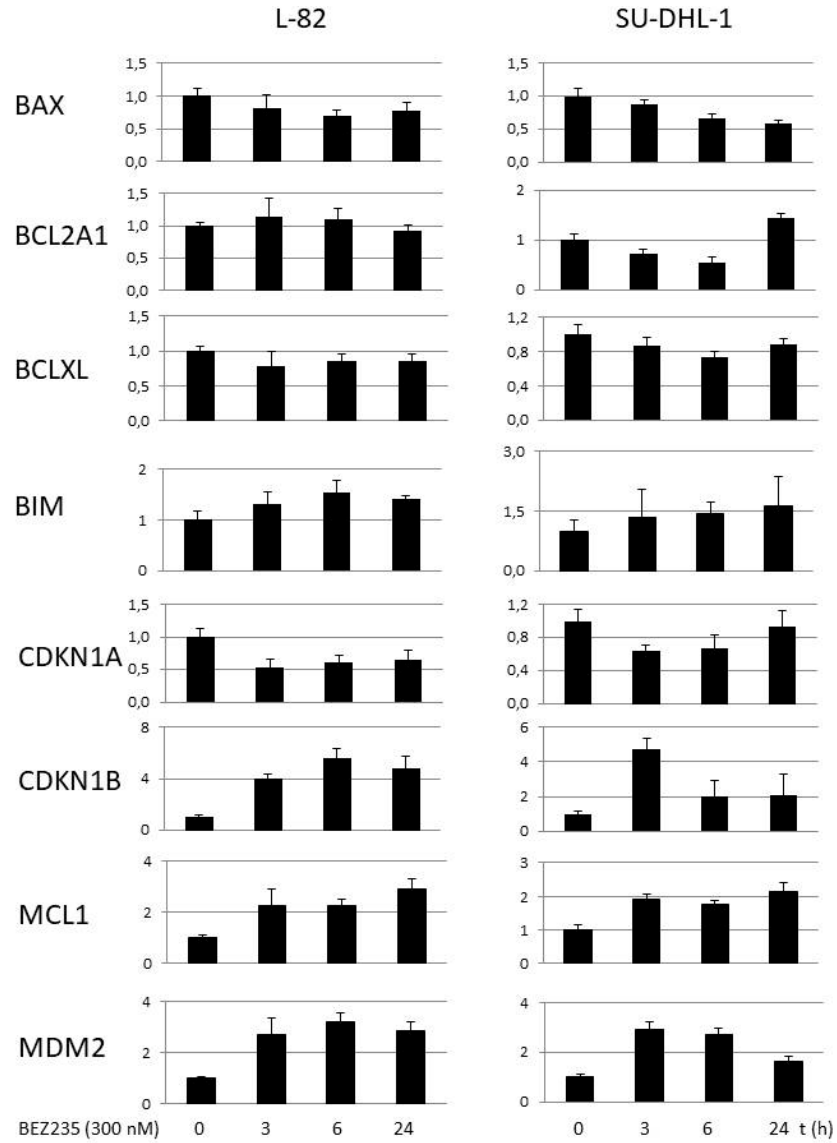


Suppl. Figure 5

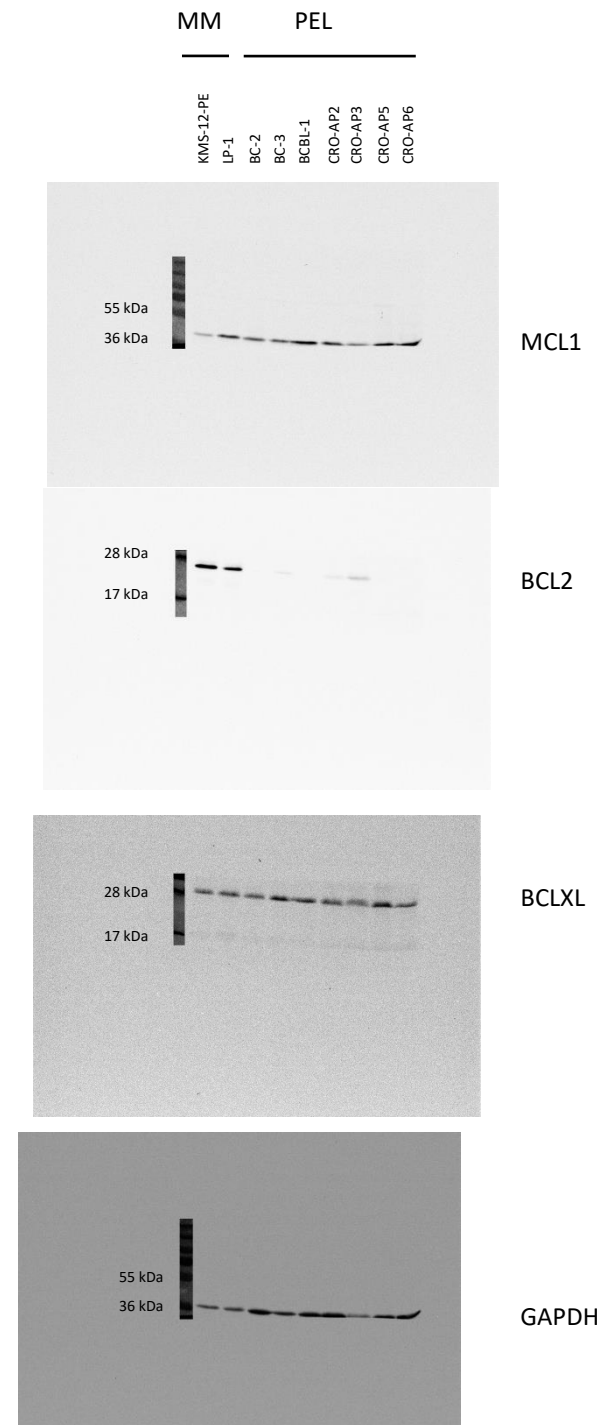
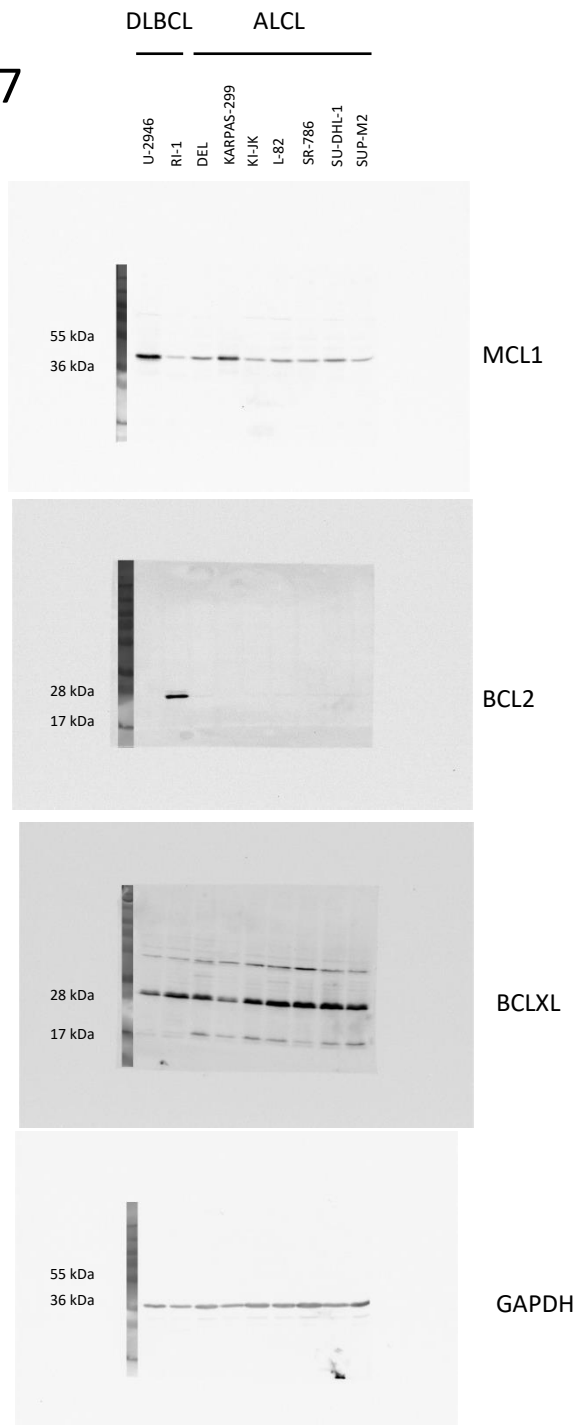




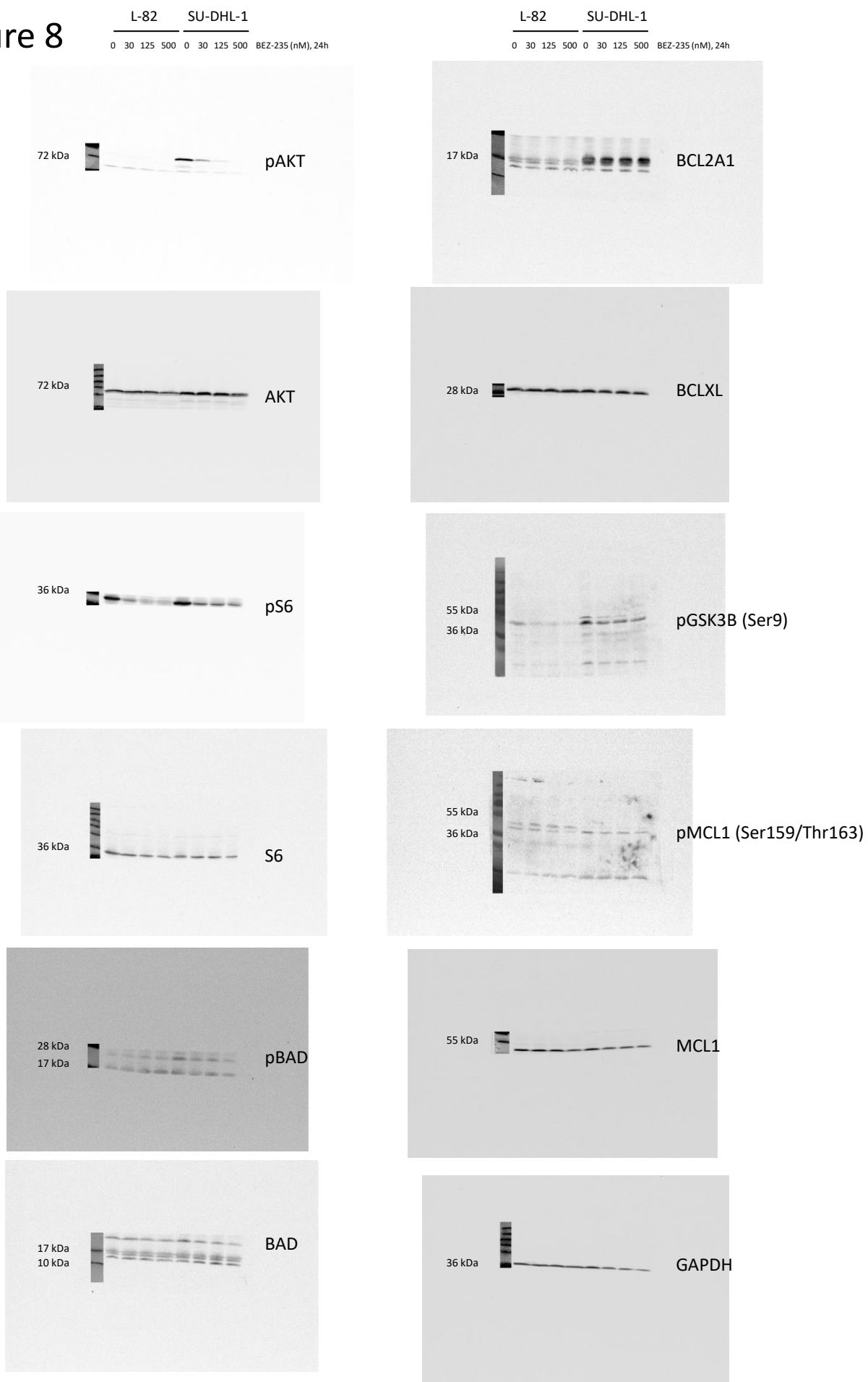
# Suppl. Figure 6



# Suppl. Figure 7



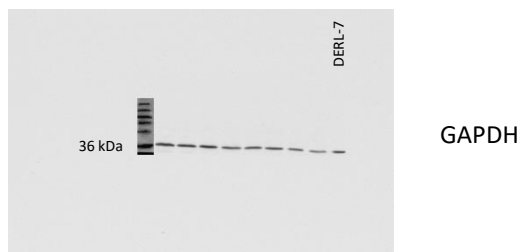
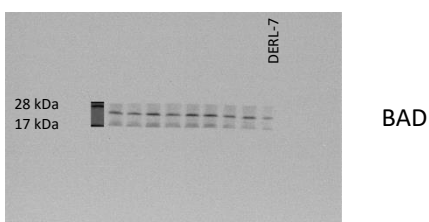
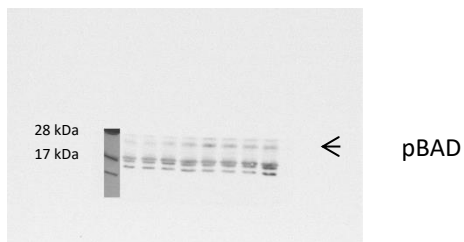
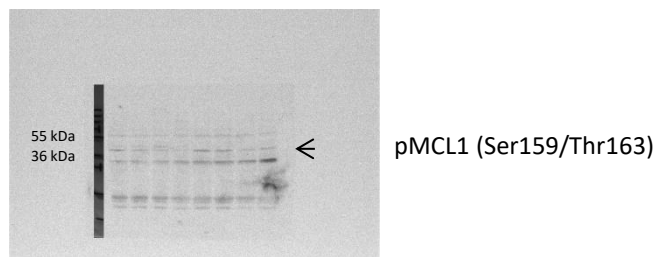
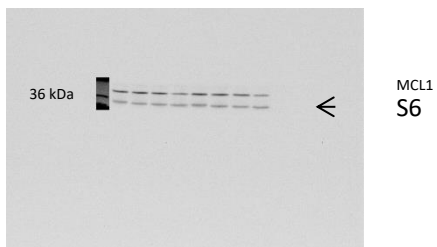
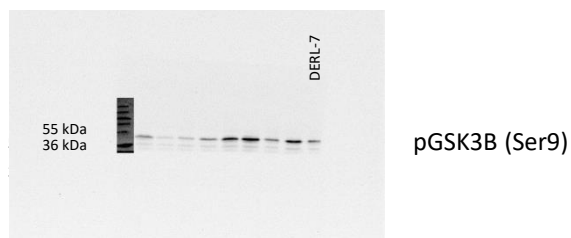
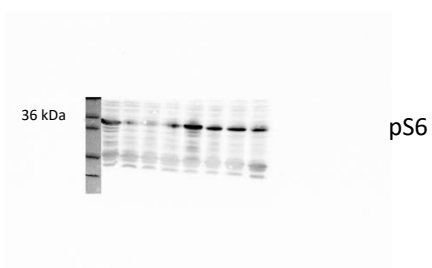
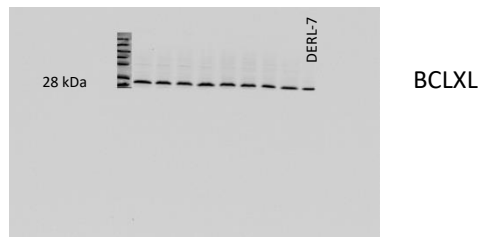
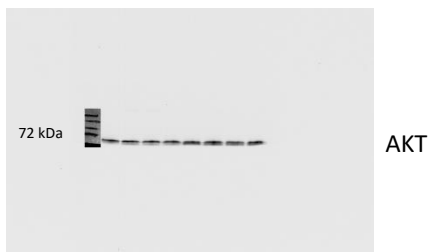
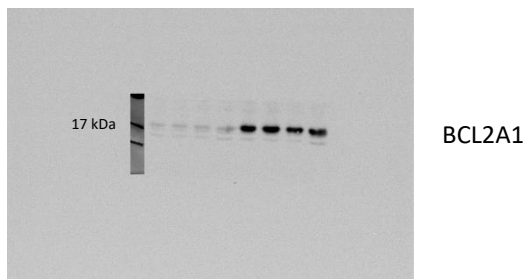
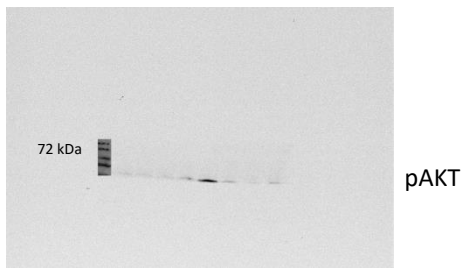
# Suppl. Figure 8



# Suppl. Figure 9

L-82 SU-DHL-1  
0 3 6 24 0 3 6 24 time (h); 300 nM BEZ-235

L-82 SU-DHL-1  
0 3 6 24 0 3 6 24 time (h); 300 nM BEZ-235

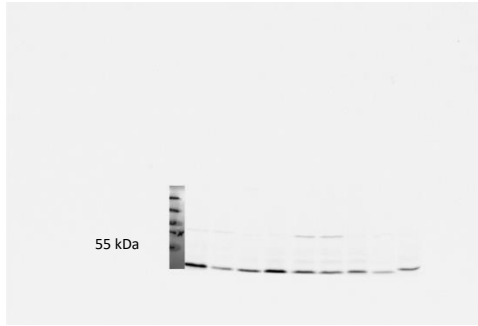


# Suppl. Figure 10

DLBCL                      ALCL

---

U-2946                      KARPAS-299  
RI-1                      KI-1K  
DEL                      L-82  
                                 SR-786  
                                 SU-DHL-1  
                                 SUP-M2

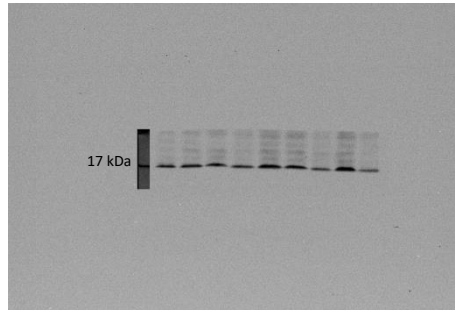


MCL1

28 kDa

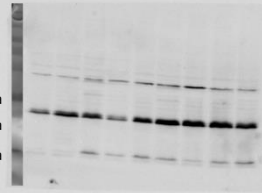


BCL2



BCL2A1

36 kDa  
28 kDa  
17 kDa



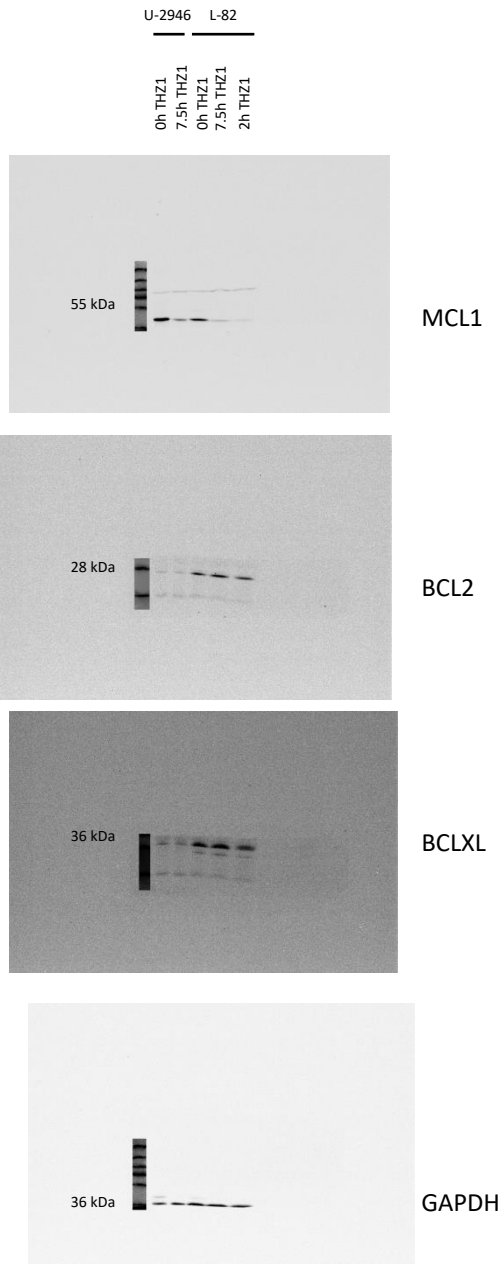
BCLXL  
BCL-W

36 kDa



GAPDH

# Suppl. Figure 11



## Supplementary Files

**Supplementary Figure 1. Effect of AZD-5991 and ABT-263 on growth and apoptosis of DLBCL cell lines.** a) Life cell imaging data showing kinetics of the dose-dependent effects of ABT-263 on cell numbers of the BCL2<sup>pos</sup> DLBCL cell line RI-1. Antiproliferative and proapoptotic effects of the MCL-1 inhibitor AZD-5991 and of the BCL2 inhibitor ABT-263 on the BCL2<sup>pos</sup> cell line RI-1 (b) and on the MCL1<sup>pos</sup> cell line U-2946 (c). Apoptosis was assessed by caspase 3/7 activity.

**Supplementary Figure 2. Expression of BCL2 family members in ALCL.** Western blot analysis showed the expression pattern of BCL2 family members in ALCL cell lines.

**Supplementary Figure 3. Efficiency of MCL1 and BCLXL knockdown.** qRT-PCR confirmed the efficiency of MCL1 and BCLXL knockdown (24h) with siRNA oligonucleotides.

**Supplementary Figure 4. Effect of AZD-5991 and ABT-263 on growth and apoptosis of ALCL cell lines.** Life cell imaging data showing the effect of the MCL1 inhibitor AZD-5991 (300 nM) and the BCL2/BCLXL inhibitor ABT-263 (300 nM) on a) growth (48h) and b) apoptosis (12h) of ALCL cell lines. Apoptosis was assessed by caspase 3/7 activity. The horizontal line shows the expected additive effects of the combination of both inhibitors. Note that the only cell line that does not respond to ABT-263 is the BCLX<sup>low</sup> PEL cell line BC-3.

**Supplementary Figure 5. Effect of transcriptional inhibition on MCL1 expression.** Western blot analysis showed that stimulation with the transcriptional

inhibitor THZ1 (200 nM) induced a rapid decrease of MCL1 levels in the DLBCL cell line U-2946 and in the ALCL cell line L-82.

**Supplementary Figure 6. Effect of BEZ-235 on mRNA expression levels of BCL2 family members and of TP53 targets.** qRT-PCR analysis showing time-dependent effects of BEZ-235 (300 nM) on mRNA expression of BCL2 family members and on potential down-stream targets of AKT. Note that the FOXO target BIM is not upregulated after stimulation with BEZ-235. Also TP53 targets CDKN1A and NOXA (NOXA with ct > 40) are not induced by BEZ-235.

**Supplementary Figure 7. Original blots cropped and presented in Figure 1C.** Note that in some cases blots were cut to allow the simultaneous use of two antibodies.

**Supplementary Figure 8. Original blots cropped and presented in Figure 5A.** Blots were cut to allow the simultaneous use of several antibodies.

**Supplementary Figure 9. Original blots cropped and presented in Figure 5B.** Blots were cut to allow the simultaneous use of several antibodies. On several blots, lysate of cell line DERL-7 was included as positive control.

**Supplementary Figure 10. Original blots cropped and presented in Supplementary Figure 2.** Note that blots were cut to allow the simultaneous use of several antibodies.



**Supplementary Figure 11. Original blots cropped and presented in Supplementary Figure 5.** Note that blots were cut to allow the simultaneous use of several antibodies.

**Supplementary Table 1.** Amplification status of *MCL1* (red), *BCL2* (black) and *BCLXL* (blue)

| Disease / cell line | karyotype            | CGH array        | q gen PCR<br>NC-NC_LINE1 |
|---------------------|----------------------|------------------|--------------------------|
| <b>ALCL</b>         |                      |                  |                          |
| DEL                 | (2n)                 | 2n / 1n / 2n     | 2n / 1n / 2n             |
| KARPAS-299          | (2n)                 | n.d. / n.d.      | 9n / 2n / 2n             |
| KI-JK               | (3n) +1,+1, +20      | n.d. / n.d.      | 6n / 2n / 4n             |
| L-82                | (3n), +1             | 2.5n / 1.5n / 4n | 3n / 1.5n / 3.5n         |
| SR-786              | (3n), +1,-18         | 2.5n / 1n / 4n   | 3n / 1n / 4n             |
| SU-DHL-1            | (3n) +1,-18, -20     | 2.5n / 1n / 4n   | 3n / 1.5n / 2n           |
| SUP-M2              | (2n), +1             | 2.5n / 2n / 2n   | 2.5n / 2n / 2n           |
| <b>DLBCL</b>        |                      |                  |                          |
| RI-1                | (2n)                 | n.d. / n.d.      | 2n / 18n / 2n            |
| U-2946              | (2n)                 | 6n / 2n / 2n     | 14n / 2n / 2n            |
| <b>MM/PCL</b>       |                      |                  |                          |
| ARH-77              | (2n)                 | n.d.             | 3n / 2n / 2n             |
| KMS-12-PE           | (2n)                 | n.d.             | 3n / 9n / 2n             |
| L-363               | (2n)                 | n.d.             | 3n / 2n / 2n             |
| LP-1                | (3n) -1, +18, +20    | n.d.             | 8n / 3n / 4n             |
| MOLP-8              | (2n)                 | n.d.             | 3n / 2n / 2n             |
| OPM-2               | (3n) +1, +1, +1, +18 | n.d.             | 5n / 2n / 2n             |
| U-266               | (2n)                 | n.d.             | 3n / 2n / 2n             |
| <b>PEL</b>          |                      |                  |                          |
| BC-1                | (2n)                 | n.d.             | 3n / 1n / 2n             |
| BC-2                | (4n) -18             | n.d.             | 2n / 1n / 2n             |
| BC-3                | (2n)                 | n.d.             | 2n / 1.5n / 2n           |
| BCBL-1              | (4n)                 | n.d.             | 3n / 1n / 2n             |
| CRO-AP2             | (2n)                 | n.d.             | 3n / 1.5n / 2n           |
| CRO-AP3             | (4n) -1              | 2.5n / 2n / 2n   | 3n / 1.5n / 2n           |
| CRO-AP5             | (2n)                 | n.d.             | 3n / 1.5n / 2n           |
| CRO-AP6             | (2n)                 | n.d.             | 3n / 1.5n / 2n           |

*MCL1* (chr1q21.3), *BCL2* (chr18q21.33), *BCLXL* (chr20q11.21); for detailed karyotypes see [www.dsmz.de](http://www.dsmz.de);

q gen PCR: endogenous control: LINE1; diploid reference cell line: NC-NC; n.d. not done.





|                   |       |            |  |                 |       |       |       |       |       |
|-------------------|-------|------------|--|-----------------|-------|-------|-------|-------|-------|
| TC1300008225.hg.1 | chr13 | PSPC1      | paraspeckle component 1                                    | NM_001042414    | 8.73  | 9.55  | 9.88  | -0.82 | -1.16 |
| TC0800012362.hg.1 | chr8  | KHDRBS3    | KH domain containing, RNA binding, signal trans            | NM_006558       | 6.81  | 6.92  | 7.96  | -0.11 | -1.15 |
| TC1100013133.hg.1 | chr11 | HBG2       | hemoglobin, gamma G  | NM_000184       | 5.67  | 6.05  | 6.82  | -0.38 | -1.15 |
| TC0100007645.hg.1 | chr1  | TINAGL1    | tubulointerstitial nephritis antigen-like 1                | NM_001204414    | 7.00  | 7.18  | 8.15  | -0.18 | -1.15 |
| TC1100006760.hg.1 | chr11 | PPFIBP2    | PTPRF interacting protein, binding protein 2 (lipri)       | NM_001256568    | 5.05  | 5.20  | 6.20  | -0.15 | -1.15 |
| TC2000009023.hg.1 | chr20 | SAMHD1     | SAM domain and HD domain 1                                 | NM_015474       | 6.04  | 6.62  | 7.19  | -0.58 | -1.15 |
| TC0300013255.hg.1 | chr3  | ZMAT3      | zinc finger, matrin-type 3                                 | NM_022470       | 7.64  | 8.42  | 8.78  | -0.79 | -1.15 |
| TC0800008715.hg.1 | chr8  | ZHX2       | zinc fingers and homeoboxes 2                              | NM_014943       | 6.50  | 7.32  | 7.64  | -0.82 | -1.14 |
| TC0X00009000.hg.1 | chrX  | VCX3A      | variable charge, X-linked 3A                               | NM_016379       | 7.08  | 7.03  | 8.22  | 0.05  | -1.14 |
| TC1200012424.hg.1 | chr12 | TMEM132D   | transmembrane protein 132D                                 | NM_133448       | 5.45  | 4.96  | 6.59  | 0.49  | -1.14 |
| TC0100007638.hg.1 | chr1  | SERINC2    | serine incorporator 2                                      | NM_001199037    | 10.30 | 10.95 | 11.43 | -0.65 | -1.13 |
| TC0400009543.hg.1 | chr4  | TLR3       | toll-like receptor 3                                       | NM_003265       | 5.32  | 6.55  | 6.44  | -1.24 | -1.12 |
| TC0900007618.hg.1 | chr9  | PCSK5      | proprotein convertase subtilisin/kexin type 5              | NM_001190482    | 5.65  | 6.05  | 6.78  | -0.40 | -1.12 |
| TC0200012997.hg.1 | chr2  | TGFA       | transforming growth factor alpha                           | NM_001099691    | 9.21  | 9.35  | 10.33 | -0.14 | -1.12 |
| TC1200008081.hg.1 | chr12 | DYRK2      | dual specificity tyrosine-(Y)-phosphorylation regul        | NM_003583       | 5.85  | 6.23  | 6.96  | -0.38 | -1.11 |
| TC0200013298.hg.1 | chr2  | ST3GAL5    | ST3 beta-galactoside alpha-2,3-sialyltransferase           | NM_001042437    | 5.69  | 5.17  | 6.79  | 0.52  | -1.10 |
| TC2000009058.hg.1 | chr20 | TGM2       | transglutaminase 2   | NM_004613       | 10.49 | 11.00 | 11.58 | -0.51 | -1.09 |
| TC0300007617.hg.1 | chr3  | KCTD6      | potassium channel tetramerization domain contai            | NM_001128214    | 4.87  | 5.37  | 5.96  | -0.50 | -1.09 |
| TC0Y00006495.hg.1 | chrY  | TGIF2LY    | TGFB-induced factor homeobox 2-like, Y-linked              | NM_139214       | 4.42  | 4.63  | 5.51  | -0.22 | -1.09 |
| TC0100015991.hg.1 | chr1  | SMG5       | SMG5 nonsense mediated mRNA decay factor                   | NM_015327       | 8.52  | 9.04  | 9.61  | -0.51 | -1.09 |
| TC1900006864.hg.1 | chr19 | MAP2K7     | mitogen-activated protein kinase kinase 7                  | NM_001297555    | 6.75  | 7.23  | 7.83  | -0.48 | -1.09 |
| TC1100007200.hg.1 | chr11 | DEPDC7     | DEP domain containing 7                                    | NM_001077242    | 5.38  | 5.39  | 6.46  | -0.01 | -1.08 |
| TC0900011623.hg.1 | chr9  | PTGES2     | prostaglandin E synthase 2                                 | NM_001256335    | 6.96  | 7.77  | 8.04  | -0.80 | -1.08 |
| TC1500006925.hg.1 | chr15 | THBS1      | thrombospondin 1   | NM_003246       | 11.87 | 12.06 | 12.95 | -0.19 | -1.08 |
| TC1900007382.hg.1 | chr19 | PGPEP1     | pyroglutamyl-peptidase I                                   | NM_001300927    | 8.14  | 8.66  | 9.22  | -0.52 | -1.07 |
| TC2000007343.hg.1 | chr20 | DHX35      | DEAH (Asp-Glu-Ala-His) box polypeptide 35                  | NM_001190809    | 6.34  | 6.74  | 7.41  | -0.40 | -1.07 |
| TC0600014318.hg.1 | chr6  | FAM46A     | family with sequence similarity 46, member A               | NM_017633       | 7.49  | 7.82  | 8.55  | -0.34 | -1.06 |
| TC1100012574.hg.1 | chr11 | SORL1      | Memczak2013 ANTISENSE, CDS, coding, INTEF hsa_circ_0000366 |                 | 6.70  | 7.20  | 7.76  | -0.50 | -1.06 |
| TC1100013163.hg.1 | chr11 | APIP       | APAF1 interacting protein                                  | NM_015957       | 7.54  | 8.58  | 8.60  | -1.04 | -1.06 |
| TC1900009558.hg.1 | chr19 | ZNF558     | zinc finger protein 558                                    | NM_001304350    | 6.06  | 6.41  | 7.12  | -0.35 | -1.06 |
| TC2000008104.hg.1 | chr20 | PRPF6      | pre-mRNA processing factor 6                               | NM_012469       | 7.90  | 8.32  | 8.95  | -0.42 | -1.05 |
| TC0800012363.hg.1 | chr8  | KHDRBS3    | KH domain containing, RNA binding, signal trans            | ENST00000522578 | 5.78  | 5.99  | 6.83  | -0.21 | -1.05 |
| TC1200011812.hg.1 | chr12 | CRY1       | cryptochrome circadian clock 1                             | NM_004075       | 6.14  | 6.56  | 7.19  | -0.42 | -1.05 |
| TC1100009929.hg.1 | chr11 | OR5111     | olfactory receptor, family 51, subfamily I, membe          | NM_001005288    | 5.84  | 5.94  | 6.89  | -0.10 | -1.05 |
| TC1100009926.hg.1 | chr11 | OR51B2     | olfactory receptor, family 51, subfamily B, membe          | NM_003180       | 5.09  | 5.42  | 6.14  | -0.33 | -1.04 |
| TC0400009765.hg.1 | chr4  | MXD4       | MAX dimerization protein 4                                 | NM_006454       | 7.73  | 8.45  | 8.78  | -0.72 | -1.04 |
| TC0200008680.hg.1 | chr2  | SLC9A2     | solute carrier family 9, subfamily A (NHE2), cation        | NM_003048       | 4.39  | 4.09  | 5.43  | 0.30  | -1.04 |
| TC0900009785.hg.1 | chr9  | LINGO2     | leucine rich repeat and Ig domain containing 2             | NM_001258282    | 4.40  | 4.58  | 5.44  | -0.19 | -1.04 |
| TC0100018443.hg.1 | chr1  | PLPP3      | phospholipid phosphatase 3                                 | NM_003713       | 8.56  | 7.29  | 9.59  | 1.27  | -1.04 |
| TC0300008853.hg.1 | chr3  | TMEM108    | transmembrane protein 108                                  | NM_001136469    | 6.39  | 6.25  | 7.42  | 0.13  | -1.03 |
| TC1700008209.hg.1 | chr17 | NGFR       | nerve growth factor receptor                               | NM_002507       | 6.71  | 6.68  | 7.74  | 0.02  | -1.03 |
| TC1100012478.hg.1 | chr11 | CD3D       | CD3d molecule, delta (CD3-TCR complex)                     | NM_000732       | 5.23  | 5.91  | 6.26  | -0.68 | -1.03 |
| TC1400010774.hg.1 | chr14 | FOXP3      | forkhead box N3  | NM_001085471    | 6.12  | 6.58  | 7.14  | -0.46 | -1.03 |
| TC0400010242.hg.1 | chr4  | PPARGC1A   | peroxisome proliferator-activated receptor gamma           | NM_013261       | 7.69  | 7.64  | 8.72  | 0.05  | -1.02 |
| TC2000009646.hg.1 | chr20 | CTSZ       | cathepsin Z  | NM_001336       | 9.79  | 10.31 | 10.81 | -0.51 | -1.02 |
| TC1200012161.hg.1 | chr12 | TRIP1      | TP53 regulated inhibitor of apoptosis 1                    | NM_016399       | 8.40  | 9.23  | 9.43  | -0.83 | -1.02 |
| TC0500007452.hg.1 | chr5  | MAP3K1     | mitogen-activated protein kinase kinase kinase 1,          | NM_005921       | 5.37  | 5.46  | 6.39  | -0.09 | -1.02 |
| TC0X00007053.hg.1 | chrX  | MAOA       | monoamine oxidase A  | NM_000240       | 6.80  | 7.16  | 7.82  | -0.36 | -1.02 |
| TC0800010002.hg.1 | chr8  | DUSP4      | dual specificity phosphatase 4                             | NM_001394       | 10.66 | 10.90 | 11.68 | -0.24 | -1.02 |
| TC0600007067.hg.1 | chr6  | GMPR       | guanosine monophosphate reductase                          | NM_006877       | 6.93  | 7.42  | 7.95  | -0.49 | -1.02 |
| TC0500009610.hg.1 | chr5  | NSD1       | nuclear receptor binding SET domain protein 1              | NM_022455       | 7.60  | 7.82  | 8.61  | -0.23 | -1.02 |
| TC0200012339.hg.1 | chr2  | SLC8A1     | solute carrier family 8 (sodium/calcium exchange)          | NM_001112800    | 4.60  | 4.60  | 5.62  | 0.01  | -1.02 |
| TC0600012434.hg.1 | chr6  | ME1        | malic enzyme 1, NADP(+)-dependent, cytosolic               | NM_002395       | 8.55  | 9.62  | 9.56  | -1.07 | -1.01 |
| TC1900006932.hg.1 | chr19 | ZNF559-ZNF | ZNF559-ZNF177 readthrough                                  | NM_001172650    | 5.47  | 5.72  | 6.48  | -0.26 | -1.01 |
| TC1200012741.hg.1 | chr12 | ANO2       | anoctamin 2, calcium activated chloride channel            | NM_001278596    | 4.84  | 4.89  | 5.86  | -0.05 | -1.01 |
| TC1900009518.hg.1 | chr19 | FBN3       | Zhang2013 ALT_ACCEPTOR, ALT_DONOR, coc hsa_circ_0092332    |                 | 5.13  | 5.36  | 6.14  | -0.23 | -1.01 |
| TC0300008561.hg.1 | chr3  | PARP14     | poly(ADP-ribose) polymerase family member 14               | NM_017554       | 8.95  | 9.34  | 9.96  | -0.38 | -1.01 |
| TC0200015093.hg.1 | chr2  | ZNF385B    | zinc finger protein 385B                                   | NM_001113397    | 4.41  | 4.94  | 5.42  | -0.53 | -1.01 |
| TC0900011192.hg.1 | chr9  | LPAR1      | lysophosphatidic acid receptor 1                           | NM_001401       | 7.19  | 7.56  | 8.20  | -0.37 | -1.01 |
| TC0300011859.hg.1 | chr3  | ZBTB11     | zinc finger and BTB domain containing 11                   | NM_014415       | 5.66  | 6.29  | 6.66  | -0.63 | -1.01 |
| TC0900009479.hg.1 | chr9  | RANBP6     | RAN binding protein 6                                      | NM_001243202    | 5.17  | 5.63  | 6.17  | -0.46 | -1.00 |
| TC0200010421.hg.1 | chr2  | AOX1       | aldehyde oxidase 1   | NM_001159       | 7.96  | 9.02  | 8.96  | -1.06 | -1.00 |
| TC0200009865.hg.1 | chr2  | SCN2A      | sodium channel, voltage gated, type II alpha subu          | NM_001040142    | 7.06  | 7.54  | 8.07  | -0.47 | -1.00 |

**Supplementary Table 3.** Sequence of primers for quantitative genomic PCR.

| gene                   |         |             | primer                               |
|------------------------|---------|-------------|--------------------------------------|
| <i>BCL2</i> intron 2/3 | forward | NM_000633   | 5'- CCA GGT TGG GTC TTG ACA G - 3'   |
| <i>BCL2</i> intron 2/3 | reverse | NM_000633   | 5'- ATG ATG CCC TTG GTC TTC TGT - 3' |
| <i>BCLXL</i> exon 2    | forward | NM_138578.3 | 5'- CCG GGA GCT GGT GGT TG - 3'      |
| <i>BCLXL</i> exon 2    | reverse | NM_138578.3 | 5'- CCT GTT CTC TTC CAC ATC ACT - 3' |
| <i>MCL1</i> exon 1     | forward | NM_021960   | 5'- TGG CGT GCA GCG CAA CC - 3'      |
| <i>MCL1</i> intron 1/2 | reverse | NM_021960   | 5'- GGA GTG AGG CCT TGG CG - 3'      |
| <i>LINE1</i>           | forward |             | 5'- TGC TTT GAA TGC GTC CCA GAG -3'  |
| <i>LINE1</i>           | reverse |             | 5'- AAA GCC GCT CAA CTA CAT GG - 3'  |

PCR conditions: 59°C, 35 cycles.