Supplementary Figure Legends

Supplementary Figure 1 TP53 loss/mutation allows AML cells to escape sub-lethal BCL2 inhibition

A Western blot confirming CRISPR/Cas9-induced loss of TP53 expression in independent clones of MV-4-11 human AML derived cell lines, expressing guide RNAs to target TP53. In all cases, cells were co-treated with an MDM2 inhibitor for 16h to induce expression and activation of TP53, as well as the broad spectrum caspase inhibitor Q-VD-OpH to prevent late stages of apoptosis that are associated with protein degradation.

B Wild-type (WT) or two independently derived TP53 KO clones of MOLM-13 (left) or MV-4-11 cells (right) were treated with 0-10 µM of the MDM2 inhibitor (MDM2-i; RG7388) and their viability determined 72h later by CellTiter-Glo assay. Data shown are means ± SD of ≥3 independent experiments.

C WT or TP53-deficient (KO) clones of MV-4-11 were treated with 0-10 µM venetoclax and their viability determined 24h later; the IC_{50} values are indicated in parentheses.

D Cell competition assays over 28d were performed with TP53 R248W mutant (blue) and WT TP53 (black) MV-4-11 AML cells treated continuously with a sub-lethal (IC_{20}) dose of venetoclax (or vehicle control-treated) and monitored by flow cytometry. Error bars indicate SD of 3 replicate samples.
Supplementary Figure 2  *TP53 KO* conferred complete resistance to MDM2 inhibitor-induced cell death.

**A**  WT or *TP53 KO* clones of the RS4;11 human ALL cell line were treated with 0-10 µM doses of the MDM2i (RG7388) and their viability was determined 72hr later by CellTiter-Glo assay. Data shown are means ± SD of ≥3 independent experiments.

**B**  WT or *TP53 KO* clones of the OCI-LY19 human DLBCL cell line were treated with 0-10 µM doses of the MDM2i (RG7388) and their viability was determined 72h later by CellTiter-Glo assay. Data shown are means ± SD of ≥3 independent experiments.
Supplementary Figure 3 TP53 loss does not impact changes in mitochondrial metabolism that are caused by treatment with BH3-mimetic drugs

A  WT or TP53 KO clones of MV-4-11 cells were treated with IC$_{20}$ doses of venetoclax or DMSO (vehicle control) for 3d. Mitochondrial metabolism was examined by using the Seahorse Mito Stress Test Kit. Error bars indicate SD of 8 replicate samples.

B  Permeabilized WT or TP53 KO clones of RS4;11 or MOLM-13 cells were treated with 0-10 µM BH3$^B$IM peptide or venetoclax, and after 90 min mitochondrial depolarization was determined by staining with the dye JC-1. Data shown are means ± SD of a representative experiment.

C  WT or TP53 KO clones of RS4;11 or MOLM-13 cells were treated with 0-10 µM venetoclax, and after 6h caspase-3/7 activation was measured by using the Caspase Glo 3/7 assay. Data shown are means ± SD of ≥3 independent experiments.

D  WT or TP53-deficient (KO) clones of RS4;11 or MOLM-13 were treated with 0-10 µM venetoclax and their viability determined 6h and 16h later. Error bars indicate SD of 3 replicate samples.
Supplemental Figure 4 Growth advantage of TP53-deficient cells after exposure to sub-optimal doses of an MCL1 inhibitor

A Enrichment of sgRNAs targeting the indicated genes in 3 independently derived WT Trp53 Eµ-Myc lymphoma cell lines treated with an MCL1 inhibitor (MCL1-i, S63845). Eµ-Myc lymphoma cell lines 1-3: AH15A, AF47A, 560, respectively.

B Western blot analysis confirming CRISPR/Cas9-induced loss of TRP53 expression in Eµ-Myc lymphoma cell lines 1-3 expressing sgRNAs targeting Trp53. Cells were treated with (+) or without (-) an MDM2i (Nutlin-3a, 10 µM) for 16h to induce TP53 protein levels and the broad-spectrum caspase inhibitor Q-VD-OPh (see above). Probing for HSP70 served as a protein loading control. Blots shown are representative of ≥3 independent experiments.

C WT or Trp53 KO Eµ-Myc lymphoma cell lines 1-3 were treated with 5 µM of the MDM2i Nutlin-3a and their viability was determined 72h later by Annexin V plus DAPI staining followed by flow cytometric analysis. Data shown are means ± SD of ≥3 independent experiments.

D Trp53 WT or Trp53 KO Eµ-Myc 1 (left), Eµ-Myc 2 (middle) or Eµ-Myc 3 (right) lymphoma cell lines were treated with increasing doses (0-10 µM) of the MCL1-i and their viability determined after 24h by staining with Annexin V and propidium iodide, followed by flow cytometric analyses. Means ± SD of ≥3 independent experiments.

E WT or Eµ-Myc Bax KO lymphoma cell line 3 was treated with 0-10 µM doses of the MCL1-i S63845 and cell viability was determined 24h later by staining with Annexin V plus propidium iodide, followed by flow cytometric analysis. Data shown are means ± SD of ≥3 independent experiments.

F Impact of TP53 loss on MCL1-i sensitivity in human cancer derived cell lines. Similar experiments to those with mouse Eµ-Myc lymphoma cell lines (D) were undertaken with the human cancer cell lines MOLM-13 (left) or BL2 (right) after treatment with 0-10 µM MCL1-i for 24h.

G Similar experiments to those after venetoclax treatment (Supplementary Figure 1D) were undertaken with MCL1-i S63845 in the TP53 R248W mutant MV-4-11 cells. Data shown represent means ± SD of ≥3 independent experiments.

H TP53 WT or TP53 KO RS4;11 cells were treated with increasing doses (0-10 µM) of the MCL1-i S63845 and their viability was determined after 24h by CellTiter-Glo assay (left panel). The outgrowth of TP53 KO RS4;11 cells, seeded in culture at a 50 TP53 KO:50 TP53 WT ratio, and treated continuously with an IC_{20} dose of S63845 (or control vehicle treated) was monitored
by flow cytometric analysis (right panel). Data shown represent means ± SD of ≥3 independent experiments.
Supplementary Figure 5 Defects in TP53 function impair the induction of apoptosis in cancer cells by MCL1-i

A  *Eµ-Myc* lymphoma cells deficient in the apoptosis effectors BAX and BAK were treated with increasing doses (0-10 µM) of the DNA damaging drugs cisplatin or etoposide, or the MCL1-i and after 24h γH2AX staining (a marker of DNA damage) was determined by flow cytometric analysis. Data are representative of ≥3 independent experiments.

B  Activation of BAX (detected by monoclonal antibody 1E5) or BAK (detected by monoclonal antibody G317-2), both early events in apoptosis signaling (x-axes), and loss of Cytochrome c (a later event; y-axes) in WT or *TP53* KO RS4;11 cells were determined by flow cytometry 6h after treatment with IC_{50} doses of MCL1-i. Data are representative of ≥3 independent experiments.

C  BAX or BAK expression in WT or *TP53* KO MOLM-13 and RS4;11 cells was determined by Western blot analysis 6h after treatment with IC_{50} doses of venetoclax or the MCL1-i S63845. Probing for HSP70 served as a protein loading control. Blots shown are representative of ≥3 independent experiments.
Supplementary Figure 6 Growth advantages in *Noxa/Puma/Bim* deficient lymphoma cells

**A** WT, *Trp53* KO or *Noxa/Puma/Bim* KO *Eµ-Myc* 2 lymphoma cell lines were treated with increasing doses (0-10 µM) of the MCL1-i S63845 and their viability determined after 24h by staining with Annexin V and propidium iodide, followed by flow cytometric analyses. Data shown are means ± SD of ≥3 independent experiments.

**B** The growth of WT (black) or *Noxa/Puma/Bim* KO (purple) *Eµ-Myc* 2 lymphoma cell lines treated continuously with a sub-optimal (IC₃₀) dose of MCL1-i (or control treated) was monitored over 14d by flow cytometric analysis.

**C** The growth of *Trp53* KO (red) or *Noxa/Puma/Bim* KO (purple) *Eµ-Myc* 2 lymphoma cell lines treated continuously with a sub-optimal (IC₃₀) dose of MCL1-i (or control treated) was monitored over 14d by flow cytometric analysis.
Supplementary Figure 7 Dual BCL2 and MCL1 inhibition leads to enhanced BAK activation in TP53 deficient cells

Activation of BAK (detected by antibody G317-2; x-axes) or loss of Cytochrome c (y-axes) in TP53 WT or TP53 KO RS4;11 cells were determined by flow cytometry 6 h after treatment with IC_{50} doses of venetoclax, or MCL1-i individually, or with venetoclax combined with MCL1-i at IC_{20} or IC_{50} doses. Data are representative of ≥3 independent experiments.
Supplementary Table 1 Percentage of marrow myeloblasts, *TP53* and *IDH1* VAF and Monosomy 17 after treatment

<table>
<thead>
<tr>
<th>Timepoint</th>
<th>Bone marrow myeloblasts by morphology</th>
<th>TP53 VAF</th>
<th>IDH1 VAF</th>
<th>Monosomy 17 by cytogenetics</th>
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<tbody>
<tr>
<td>Diagnosis</td>
<td>74%</td>
<td>78.95%</td>
<td>27.69%</td>
<td>79%</td>
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<tr>
<td>Post &quot;7+3&quot; induction</td>
<td>79%</td>
<td>77.78%</td>
<td>44.23%</td>
<td>100%</td>
</tr>
<tr>
<td>Post &quot;HiDAC+3&quot; induction*</td>
<td>72%</td>
<td>29.45%</td>
<td>33.33%</td>
<td>Not done</td>
</tr>
<tr>
<td>Post 1&lt;sup&gt;st&lt;/sup&gt; cycle BCL2-i</td>
<td>10%</td>
<td>2.27%</td>
<td>7.27%</td>
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<tr>
<td>Post 2&lt;sup&gt;nd&lt;/sup&gt; cycle BCL2-i</td>
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<td>0%</td>
<td>0%</td>
<td>0%</td>
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<td>Post 3&lt;sup&gt;rd&lt;/sup&gt; cycle BCL2-i</td>
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<td>Not done</td>
<td>0%</td>
</tr>
<tr>
<td>AML ID</td>
<td>Gender</td>
<td>Age</td>
<td>VEN dose (mg)</td>
<td>Blast change at day 8</td>
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<td>--------</td>
<td>--------</td>
<td>-----</td>
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<td>----------------------</td>
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<tr>
<td>CAL-008</td>
<td>F</td>
<td>75</td>
<td>50</td>
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<td>73</td>
<td>50</td>
<td>-13%</td>
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<td>CAL-012</td>
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<td>68</td>
<td>50</td>
<td>+21%</td>
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<td>CAL-011</td>
<td>M</td>
<td>65</td>
<td>100</td>
<td>-73%</td>
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<td>-50%</td>
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<td>CAL-030</td>
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<td>200</td>
<td>-11%</td>
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<tr>
<td>CAU-005</td>
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<td>CVC-001</td>
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<td>69</td>
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### Supplementary Table 3 Sub-clonal change during venetoclax therapy using single cell DNA genomics

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<tr>
<th>AML ID</th>
<th>Clone</th>
<th>Proportion (%) Day 0</th>
<th>Proportion (%) Day 8</th>
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<td>CAL-030</td>
<td>TP53 WT</td>
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<td>42</td>
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<tr>
<td></td>
<td>TP53mut</td>
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<td>42</td>
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<tr>
<td></td>
<td>TP53mut + NRAS</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>TP53mut + PTPN11</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>TP53mut + KRAS</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>CAL-012</td>
<td>TP53 WT</td>
<td>50</td>
<td>20</td>
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<td>59</td>
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<td></td>
<td>TP53mut + KRAS</td>
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<td>13</td>
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<td></td>
<td>TP53mut + PTPN11</td>
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<td>8</td>
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<td>CAU-001</td>
<td>WT</td>
<td>34</td>
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<td></td>
<td>TP53mut</td>
<td>60</td>
<td>76</td>
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<td></td>
<td>TP53mut + CALR del</td>
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<td>7</td>
</tr>
<tr>
<td>CVC-001</td>
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<td>78</td>
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<td></td>
<td>TP53 WT + DNMT3A</td>
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<td>TP53 WT + KMT2A</td>
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<td>TP53mut</td>
<td>16</td>
<td>21</td>
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Green and red filled boxes represent clones which reduced, or increased by ≥5% during venetoclax therapy, respectively.
**Supplementary Table 4 Details of dosing of BH3-mimetic drugs**

<table>
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<tr>
<th>Cell line</th>
<th>VEN IC\textsubscript{20}</th>
<th>VEN IC\textsubscript{50}</th>
<th>MCL1-i IC\textsubscript{20}</th>
<th>MCL1-i IC\textsubscript{30}</th>
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<tbody>
<tr>
<td>MOLM-13</td>
<td>10nM</td>
<td>500nM</td>
<td>10nM</td>
<td>ND</td>
</tr>
<tr>
<td>RS4;11</td>
<td>10nM</td>
<td>1000nM</td>
<td>200nM</td>
<td>ND</td>
</tr>
<tr>
<td>E\textsubscript{M}-Myc 1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>50nM</td>
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<tr>
<td>E\textsubscript{M}-Myc 2</td>
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<td>100nM</td>
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<tr>
<td>E\textsubscript{M}-Myc 3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>50nM</td>
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<tr>
<td>BL2</td>
<td>ND</td>
<td>ND</td>
<td>10nM</td>
<td>ND</td>
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ND = Not done
## Supplementary Table 5 Venetoclax IC<sub>50</sub> value

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<tr>
<th>Cell line</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
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<tr>
<td>MOLM-13 TP53 WT</td>
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<td>255nM</td>
<td>331nM</td>
<td>89nM</td>
<td>192nM</td>
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<tr>
<td>RS4;11 TP53 WT</td>
<td>93nM</td>
<td>97nM</td>
<td>176nM</td>
<td>173nM</td>
<td>156nM</td>
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<tr>
<td>RS4;11 TP53 KO</td>
<td>90nM</td>
<td>88nM</td>
<td>102nM</td>
<td>82nM</td>
<td>92nM</td>
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### Supplementary Table 6 sgRNA sequence (5’-3’)

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<th>Species</th>
<th>Gene</th>
<th>sgRNA sequence</th>
<th>Notes</th>
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<tr>
<td>Human</td>
<td>TP53</td>
<td>CCCCCGACGATATTGAACAA</td>
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<tr>
<td>†Human</td>
<td>TP53</td>
<td>GAGCGCTGCTCACATAGCGA</td>
<td>A vector expressing this guide was used to target TP53 in human BL2 cells.</td>
</tr>
<tr>
<td>Human</td>
<td>TP53</td>
<td>TGGCCATCTACAAGGCACTCA</td>
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<tr>
<td>Human</td>
<td>TP53</td>
<td>ACTCGGATAAGATGCTGAGG</td>
<td></td>
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<tr>
<td>Human</td>
<td>TP53</td>
<td>GATCCACCTCACGTTTCCAT</td>
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<tr>
<td>Human</td>
<td>TP53</td>
<td>GGTGCCCTATGAGCCGCTG</td>
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<tr>
<td>Human</td>
<td>BAX</td>
<td>GTCTGACGGCAACTTCAACT</td>
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<tr>
<td>Human</td>
<td>BAK</td>
<td>GGGCCATGCTGCTAGACGTA</td>
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<tr>
<td>Mouse</td>
<td>Trp53</td>
<td>GAGCGCTGCTCGATGGTGA</td>
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</tr>
<tr>
<td>*Human</td>
<td>BIM</td>
<td>GCCTCCCAAGCTCAGACCTG</td>
<td>A vector expressing this guide was used as the control in experiments with the mouse Eμ-Myc lymphoma-derived cell lines.</td>
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<tr>
<td>Mouse</td>
<td>Bak</td>
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<tr>
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<tr>
<td>Mouse</td>
<td>Puma</td>
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<td>Noxa</td>
<td>GTTGAGCTGCGAATCCAGGT</td>
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</tbody>
</table>

*A vector expressing this guide was used as the control in experiments with the mouse Eμ-Myc lymphoma-derived cell lines.*

†*A vector expressing this guide was used to target TP53 in human BL2 cells.*

#*A vector expressing this guide was used as the control in experiments with human BL2 cells.*
### Supplementary Table 7 Primer sequences

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<tr>
<th>Primer</th>
<th>Sequence</th>
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<tr>
<td>PCR1_F</td>
<td>TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG - locus specific sequence</td>
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<tr>
<td>PCR1_R</td>
<td>GTCTCGTGAGGCTCGGAGATGTGTATAAGAGACAG - locus specific sequence</td>
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<tr>
<td>PCR2_F</td>
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<td>PCR2_R</td>
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<td>TP53_1F</td>
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<td>TP53_1R</td>
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<td>TP53_2R</td>
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<td>TP53_6F</td>
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Supplementary Figure 1

A

<table>
<thead>
<tr>
<th>MV-4-11</th>
<th>WT #1</th>
<th>TP53 KO #1</th>
<th>TP53 KO #2</th>
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<tr>
<td>HSP70</td>
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<td>TP53</td>
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</table>

B

Graph showing viability of MOLM-13 and MV-4-11 cells with varying concentrations of MDM2.

C

Graph showing viability of MV-4-11 cells with varying concentrations of Venetoclax.

D

Graph showing the percentage of cells over days for WT + DMSO, WT + VEN IC_{50}, TP53 mut + DMSO, and TP53 mut + VEN IC_{50}.
Supplementary Figure 2

A

RS4;11

Viability (% untreated)

MDM2 [μM]

WT #1

TP53 KO #1

TP53 KO #2

B

OCI-LY19

Viability (% untreated)

MDM2 [μM]

WT #1

TP53 KO #1
Supplementary Figure 3

A. Graph showing the OCR (pontolin/min) versus time (minutes) for MV-4-11 cells with different treatments.

B. Graphs showing the percentage of polarized mitochondria against BIM concentration [nM] for RS4:11 WT and KO, MOLM-13 WT and KO.

C. Graphs showing the percentage of maximal caspase activation against Venetoclax concentration [nM] for RS4:11 and MOLM-13 cells with WT and TP53 KO.

D. Graphs showing viability (% untreated) against Venetoclax concentration [nM] for RS4:11 and MOLM-13 cells with WT and TP53 KO at 6h and 16h.
Supplementary Figure 4

A. Top Hits:
- E2-Myc 1
- E2-Myc 2
- Ddx8
- Trp53
- Bax
- Mifid62
- Slept4
- Ep-Myc 3

B. Nuclin-3a (10 μM)
- TRP53
- HSP70

C. Nuclin-3a (5 μM)

D. Ep-Myc 1
- WT + [IC50, 10 μM]
- Trp53 KO + [IC50, 10 μM]

E. Ep-Myc 2
- WT + [IC50, 10 μM]
- Trp53 KO + [IC50, 10 μM]

F. Ep-Myc 3
- WT + [IC50, 10 μM]
- Trp53 KO + [IC50, 10 μM]

G. MOLM-13
- WT + MOL1 + [IC50, 10 μM]
- MCL1 + [IC50, 10 μM]

H. MV-4-11
- TPS3 KO + DMISO
- WT + DMISO
- WT + MCL1 + [IC50, 10 μM]

Supplementary data
Supplementary Figure 5

A  

Ep-Myc 3 BAK/BAX KO

Cisplatin  
Etoposide  
MCL1-i

γH2AX-FITC

0 µM  
0.002 µM  
0.02 µM  
0.156 µM  
1.25 µM  
10 µM

B

BAK activation  
BAX activation

DMSO

MCL1-i IC₅₀

Cytochrome C

WT  
TP53 KO

Cytochrome C

WT  
TP53 KO

1.63%  
0.64%  
1.42%  
0.58%  
31.8%  
16.8%  
13.9%  
6.53%

C

MOLM-13  
RS4;11

WT  
TP53KO  
WT  
TP53KO

HSP70  
70 kDa

BAK  
25 kDa

BAX  
20 kDa
Supplementary Figure 6

A

$E\mu$-Myc 2

- WT
- Trp53 KO
- Noxa/Puma/Bim KO

Viability (% untreated)

MCL1-i (µM)

B

$E\mu$-Myc 2

- WT + DMSO
- WT + MCL1-i IC$_{50}$
- Noxa/Puma/Bim KO + DMSO
- Noxa/Puma/Bim KO + MCL1-i IC$_{50}$

% cells

Days

C

$E\mu$-Myc 2

- Trp53 KO + DMSO
- Trp53 KO + MCL1-i IC$_{50}$
- Noxa/Puma/Bim KO + DMSO
- Noxa/Puma/Bim KO + MCL1-i IC$_{50}$

% cells

Days
Supplementary Figure 7

Supplementary data
Supplementary Methods

**Primary AML specimen treated with the BCL2 inhibitor S55746**

Bone marrow samples were collected from the patient with AML treated in the Phase 1 dose-escalation study of orally administered selective BCL2 selective inhibitor S55746 as monotherapy for the treatment of patients with AML and high or very high risk MDS (Protocol CL1-055746-002) after informed consent. The study was conducted in accordance with the approved protocols through the Human Research Ethics Committee (Melbourne Health HREC project 2012.274; Walter and Eliza Hall Institute HREC project 13/01) and the Declaration of Helsinki. Bone marrow morphology assessment and cytogenetics were performed at the Royal Melbourne Hospital and Victorian Cancer Cytogenetics Service respectively. DNA was extracted using the MagAttract DNA Blood M48 Kit or QIAasympohy DSP DNA Mini Kit (Qiagen #951336, #937236) according to the manufacturer’s instructions. Whole genome amplification was performed on DNA from post “HiDAC+3” timepoint using REPLI-g Mini Kit (Qiagen #150023). Library preparation and exome hybridisation was performed using TruSeq Nano kit (Illumina #20015964), SureSelectXT2 Target Enrichment System and Human All Exon v5 Capture Library (Agilent Technologies #G9621B, #5190-6216). 150bp paired-end sequencing was performed on captured libraries using an Illumina HiSeq 2500 at the Australian Genome Research Facility (AGRF). Whole exome sequencing (WES) data were aligned with BWA v0.7.15-r1140\(^1\) and analyzed with SuperFreq\(^2\) for somatic variant calling, copy number calling and clonal tracking.

**Primary AML specimens treated with venetoclax**

Samples were collected from patients with AML treated at the Alfred Hospital in the Chemotherapy And Venetoclax in Elderly Acute myeloid leukemia Trial (CAVEAT) after informed consent\(^3\). Studies were conducted in accordance with the approved protocols through the Human Research Ethics Committee of the Alfred Hospital and the Declaration of Helsinki for all subjects.

**TP53 gene mutational analysis using next generation sequencing**

TP53 mutation analyses were performed on bone marrow aspirates collected from patient-derived xenograft models during and after treatment. Genomic DNA was prepared using the Qiagen DNeasy Blood and Tissue kit (Qiagen #69504) according to the manufacturer’s instructions. Sequencing was performed using a 2-step PCR approach for targeted sequencing. Unique primer sequences for TP53 that contain a common overhang sequence at the 5’ end were used to amplify TP53 exons. Cycling conditions were initial denaturation at 98°C for 30s, 22 cycles of denaturation
at 98°C for 10sec, annealing at 60°C for 30sec, and elongation at 72°C for 20sec, followed by a final elongation step at 72°C for 5min. PCR amplicons were purified using 1.0x Ampure Beads (Beckman Coulter) prior to the second PCR to introduce adaptor sequences and 8 bp dual-index barcodes for multiplexing using the cycling conditions, as indicated above. The barcoded PCR product size was assessed by agarose gel electrophoresis, purified using 0.8x Ampure Beads and quantified by Qubit fluorometry (ThermoFisher Scientific). PCR products from up to 96 samples were pooled equimolar and sequenced on an Illumina MiniSeq 300-cycle Mid-Output kit (Illumina) according to the manufacturer’s recommendations. Read pairs were demultiplexed according to the index sequences using bcl2fastq, and subsequently processed by Trim_galore (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) to remove adaptors and low-quality reads. Then, the clean reads were aligned using the Burrows-Wheeler Aligner to UCSC human assembly hg19 and variant calling was performed using Varscan2. All variant calls were manually inspected in integrated genome viewer (IGV) ver2.6.3. Primer sequences are detailed in Supplementary Table 7.

**Single cell DNA sequencing**

Single cell DNA sequencing was performed using the Tapestri platform (Mission Bio) as previously described. Briefly, cryopreserved bone marrow or peripheral blood mononuclear cells were thawed, counted and 2,000-4,000 cells/mL used for cellular lysis, barcoding and library preparation. The Tapestri 20-gene AML panel (CAL-012 and CAL-030) or the 45-gene Myeloid panel (CVC-001 and CAU-001) were used. Sequencing of equimolar library pools was performed on an Illumina NovaSeq 6000 S1 kit (300 cycles). Fastq files generated by the NovaSeq instrument were processed using the Tapestri pipeline for adaptor trimming, sequence alignment, barcode correction, cell finding and variant calling. Results were visualized using the Tapestri Insights platform.

**Data sharing**

Processed sequencing data will be made available subject to a data transfer agreement and will be restricted to ethically approved research into blood cell malignancies and cannot be used to assess germline variants (exome sequencing case stored at EGA: EGAS00001004841).

**Whole genome CRISPR/Cas9 screens**
Cas9-expressing Eµ-Myc lymphoma cells were split into 6 replicates of 3x10^5 cells, transduced with the mouse YUSA whole genome pooled CRISPR library^6 as described below and then expanded for 4-5d. For each replicate infection, 7x10^6 cells were plated into two T75 flasks and one was treated with DMSO (vehicle) and the other with MCL1-i (S63845, Active Biochem #6044) at a cell line-dependent concentration which achieved ~99% cell death after 24h (Eµ-Myc 1 (WT): 800nM, Eµ-Myc 1 (Trp53 KO): 1µM, Eµ-Myc 2 (WT): 400nM, Eµ-Myc 3 (WT): 400nM). Surviving cells were flow-sorted (FACSAria, BD) for mCherry (Cas9 vector) and BFP (sgRNA vector) double-positive cells. Sorted cells were expanded for 3-4d until at least 1x10^5 live cells were obtained for each replicate. Cells were collected along with DMSO treated control cells, and DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen #69506). Samples underwent an indexing PCR, were purified using Ampure XP beads (Beckman Coulter #A63880) and sequenced using the NextSeq 500 platform (Illumina). Enriched sgRNAs were identified using MAGeCK v.0.5.9^1 and edgeR v.3.30.0^7. sgRNAs were deemed to be significantly enriched when the corresponding false discovery rate (FDR) was less than 0.1. These methods produced concordant results.

**Cell lines**

Human blood cancer-derived cell lines, MV-4-11 (ATCC #CRL-9591), MOLM-13 (DSMZ #ACC 544) and RS4;11 (ATCC #CRL-1873) were cultured in RPMI-1640 medium (WEHI) containing 10% Fetal Calf Serum (FCS; Sigma #F9423); OCI-LY19 (DSMZ #ACC 528) cultured in alpha-MEM medium (Gibco #32561-037) containing 10% FCS; BL2 (kind gift from Prof Alan Rickinson, The University of Birmingham, UK) cultured in RPMI-1640 medium containing 10% FCS, 50µM α-thioglycerol (Sigma-Aldrich #M-6145), 1mM sodium pyruvate (Gibco #11360070), 2mM L-glutamine (Gibco #25030081). All cell lines were kept at 37°C in 5% CO₂. Aliquots of early passages (P3–P4) of the human cancer-derived cell lines were cryopreserved and thawed for experiments to ensure analysis of cells <P15. Mouse lymphoma-derived cell lines, Eµ-Myc 1 (AH15A), Eµ-Myc 2 (AF47A) and Eµ-Myc 3 (560) were cultured in high-glucose Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (FBS; Sigma #F9423), 50µM β-mercapto-ethanol (Sigma-Aldrich #M3148) and 100mM asparagine (Sigma-Aldrich #A4284) at 37°C in 10% CO₂. All cell lines were regularly verified as Mycoplasma negative (MycoAlert mycoplasma detection kit; Lonza #LT07-118).

**Generation of gene knock-out or mutant cell lines using CRISPR-Cas9**
Six sgRNAs targeting human TP53 or guides targeting BAX or BAK were synthesized (Integrated DNA Technology; Supplementary Table 6) and cloned into pKLV-U6gRNA(BbsI)-PGKpuro2ABFP (Addgene Plasmid #50946; permits constitutive expression of the sgRNA of interest, with BFP as a reporter)\(^6\). The following were cloned into FgH1UTG (Addgene Plasmid #70183) which permits doxycycline-inducible expression of the sgRNA and constitutive expression of a GFP reporter \(^6\): for E\(\mu\)-Myc cells, sgRNAs targeting mouse Trp53 exon 5 and a non-targeting (NT) control against human BIM exon 3, and for human BL2 cells, sgRNAs targeting human TP53 exon 5 and a NT control against mouse Bim exon 2. All lentiviruses were produced in 293T cells (ATCC #CRL-3216) and cell lines were transduced using established protocols\(^9\). Stable Cas9-expressing human and mouse target cells were generated firstly through transduction of target cells with FuCas9Cherry (Addgene Plasmid #70182), followed by cell sorting for mCherry expression using a FACS Aria (Becton Dickinson: BD). Human cell lines stably expressing Cas9 were subsequently transduced with lentiviral supernatants containing all 6 sgRNAs targeting TP53, or empty vector-labelled with GFP (pKLV2-U6gRNA5(gBFP)-PGKGF2ABFP-W, Addgene Plasmid #67984). Mouse lymphoma cells stably expressing Cas9 were transduced with lentiviral supernatants containing inducible sgRNAs targeting Trp53 exon 5 or a NT sgRNA as a control\(^8\). Cells were flow-sorted using a FACS Aria (Becton Dickinson) based on fluorochrome expression. Expression of mouse Trp53 and NT sgRNAs were induced by addition of 1\(\mu\)g/mL doxycycline hyclate (Sigma-Aldrich #D9891) to culture medium for 10d. The loss of TP53 or TRP53 protein expression were confirmed by Western blotting following culture of cells for 24r in the presence of the MDM2i Nutlin-3a (10 \(\mu\)M; Sigma-Aldrich #SML0580) and the broad spectrum caspase inhibitor QVD-OPh (25\(\mu\)M; Abcam #ab141421). Puma, Noxa and Bim triple KO mouse cells were generated by lentiviral infection as above, using an inducible sgRNA targeting mouse Bim exon 2 as described above, in addition to constitutively-expressed sgRNAs targeting mouse Puma and Noxa from the Sanger Whole Genome CRISPR Arrayed Library (Sigma-Aldrich).

**In vitro cell growth competition assays**

Cas9-expressing human cancer derived lines were infected with a vector expressing GFP (control) or a vector co-expressing TP53 sgRNAs and BFP (test). Control WT TP53 GFP\(^+\) cells were seeded at indicated ratios with test KO TP53 BFP\(^+\) cells into 24-well plates and treated with BH3-mimetics (or DMSO as controls). The medium and drugs were refreshed every 3-4d when the relative proportion of viable GFP\(^+\) or BFP\(^+\) cells was evaluated by flow cytometry (CytoFLEX or LSR II; BD). Similar experiments were undertaken with WT (GFP\(^0\)) or Trp53 KO (GFP\(^\text{hi}\))
generated by additional infection with a GFP-expressing retrovirus) *Eµ-Myc* mouse lymphoma cells.

**Western blotting**  
Total protein from cells was isolated using lysis buffer (20mM Tris-HCl pH 7.4, 135mM NaCl, 1.5mM MgCl₂, 1mM EDTA, 10% glycerol) or RIPA buffer, supplemented with complete protease inhibitors (Roche #4693132001) and protein concentration was determined with Bradford assay (Bio-Rad #4693132001). Alternatively, cells were lysed in sample buffer (0.15M Tris pH 6.8, 30% glycerol, 1.2% SDS, 0.018mg/mL bromophenol blue, 5% (v/v) 2-mercaptoethanol) at a concentration of 10⁷ cells/mL. Protein samples were size-fractionated by SDS-PAGE and then blotted onto nitrocellulose (Invitrogen #IB23001) or polyvinylidene fluoride (PVDF, GE Healthcare #10600021) membranes. Membranes were blocked with 5% non-fat dry milk (Devondale) in PBS or TBS with 0.1% Tween 20 (Sigma #P9416) and then probed with antibodies against TP53 (clone D07, Novocastra #p53-D07-L-CE-H), TRP53 (clone CM5, Novocastra #NCL-p53-CM5p), BAK (Sigma #B5897), BAX (clone 49F9, WEHI Antibody Services) and HSP70 (protein loading control; clone N6, gift from Drs R Anderson and W Welch). Detection was performed with IR800-conjugated goat anti-mouse IgG antibodies (Rockland #610-132-121) and detected by Odyssey Imaging System (Li-Cor), or HRP-conjugated goat antibodies against mouse IgG (Southern Biotech #1010-05), rabbit IgG (Southern Biotech #4010-05) or rat IgG (Southern Biotech #3010-05) and detected by ChemiDoc Imaging System (Bio-Rad).

**Cell viability assays**  
The response of cells to BH3-mimetic drugs was assessed at 24h and the response to the MDM2 inhibitor at 72h, post-treatment by either CellTiter-Glo assay or by staining cells with Annexin V and 4′,6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich #D9542) or propidium iodide (PI, Merck, #P4864), followed by flow cytometric analysis. For CellTiter-Glo assays, cells were seeded in white 96-well plates at 5,000 cells/well. The cells were treated with 6-point 1:8 serial dilutions of venetoclax (Chemgood #C-1008), MCL1-i (S63845; Active Biochem #A-6044) or MDM2-i (RG7388; Chemgood #C-1287) starting from 10μM. The relative number of viable cells was determined by CellTiter-Glo assay, according to the manufacturer's instructions (Promega, #G9241). Cells were analyzed on a luminometer (LumiStar Optima, BMG Labtech) and values were normalized to DMSO-treated control cells. To measure cell viability using Annexin V and DAPI/PI, cells were seeded in flat-bottomed 96-well plates at 2×10⁴ cells per well and treated with 6-point 1:8 serial dilutions of BH3-mimetics or MDM2 inhibitor (Nutlin-3a; Sigma #SML0580),
starting from doses of 10 µM. Cells were stained with Annexin V-Alexa Fluor 647 (WEHI) and DAPI/PI and cell viability was assessed by DAPI or PI and Annexin V exclusion using a CytoFLEX or LSR II Flow Cytometer (BD). FACS data were analyzed using Flowjo software.

To measure caspase activation, cells were treated with the indicated doses of BH3-mimetics for 6h and stained with the Caspase Glo 3/7 reagent according to the manufacturer’s instructions (Promega #G8090) and analyzed on a luminometer.

**Mouse AML Xenograft model**

All mouse studies were conducted with approval from the Alfred Medical Research and Education Precinct Animal Ethics Committee. For *in vivo* studies, venetoclax was dissolved in 60% Phosal 50PG (Lipoid, Thermo Fisher Scientific # FSHNC0130871), 30% PEG-400 (Sigma #202398) and 10% ethanol. MCL1-i S63845, was dissolved in 50% (2-hydroxypropyl)-β-cyclodextrin (Sigma #332607) and 50% 50 mM HCl. NOD-SCID IL2Rγ-null mice (NSG, 8-10 weeks old) were obtained from the Animal Resources Centre. MOLM-13 WT cells expressing GFP and *TP53* KO cells expressing BFP were competitively transplanted (50:50 ratio, 4x10⁵ cells total) by intravenous injection into non-irradiated NSG mice. Three days after transplantation, mice were treated with vehicle, or with BH3-mimetic drugs venetoclax (75mg/kg by oral gavage QD weekdays) or MCL1-i S63845 (25mg/kg intravenously QW) for 2 weeks. Three days following the last drug treatment, the relative contributions of GFP-positive WT and BFP-positive *TP53* KO MOLM-13 cells to the leukemia burden were assessed by flow cytometric analysis of the human CD45⁺ve cells in the bone marrow and peripheral blood. MV-4-11 cells *TP53* WT or *TP53* KO were intravenously injected at 1 x 10⁵ cells (1:1) into 6-week old female irradiated (100 Rad) non-obese diabetic/severe combined immunodeficient (NOD/SCID/IL2rγnull). Mice were gavaged with venetoxlax (200 µL 75 mg/kg) dissolved in PEG400 (Sigma), Phosal50 PG, and distilled H₂O at a ratio of 40:10:50 or S63845 (200 µL 25 mg/kg) twice weekly or weekly IV dissolved in 50% 2-hydroxypropyl)-β-cyclodextrin (Sigma) and 50% 50mM HCL or the drug combination, or vehicle.

**Patient-derived xenograft models of AML**

To establish mouse models of primary patient AML, 1 x 10⁶ leukemic blasts were injected into 6-week old female NOD-IL2Rcγnull (NRG-SG3) mice (The Jackson Laboratory, Bar Harbor, ME, USA) via tail-vein injection and animals monitored for leukemia progression using flow cytometric analysis of peripheral blood for hCD45⁺ cells. hCD45⁺ cell counts in the bone marrow from the femurs of euthanized animals were used to determine the extent of leukemia.
infiltration. Bone marrow cells were extracted by flushing femurs in PBS supplemented with 2% fetal bovine serum. To determine the efficacy of S55746 and S63845, mice were gavaged daily with S55746 (100 mg/kg) for 5 days or received S63845 25mg/kg twice weekly IV. Drug efficacy was determined by flow cytometric analysis in bone marrow isolated from flushed femurs. Single-cell suspensions of cells were stained with fluorochrome-conjugated antibodies to the following surface markers: mouse CD45 (BD, clone 30-F11) and human CD45 (BD, HI30). Sample data were acquired on an FACSsymphony™ flow cytometer (BD Biosciences) and analyzed using FlowJo software (TreeStar).

**γ-H2AX staining**

Cells deficient for both BAX and BAK were treated with 6-point 1:8 serial dilutions of indicated drugs for 24h before being fixed with 1.6% paraformaldehyde (Electron Microscope Science #15170) for 10min at room temperature for human cells or fixation buffer (Invitrogen/eBiosciences #00-5523) for 30 min on ice for mouse cells. Cells were permeabilized with 1xPerm Buffer (Invitrogen/eBiosciences #00-5523) supplemented with an A647- or A488-conjugated antibodies to detect γ-H2AX (Cell Signalling #9720 or #9719) or similarly labelled Ig isotype-matched control antibodies (Cell Signalling #2985), and stained by incubation for 30min on ice. Cells were analyzed using a CytoFLEX or LSR II Flow Cytometer (BD).

**JC-1 BH3 profiling assay**

Cells were washed and resuspended in pH7.5 TEB buffer (135mM trehalose dehydrate (Sigma #T0167)), 10mM HEPES-KOH (Sigma #H3375), 50mM KCl (Sigma #P9333), 20μM EDTA (Ajax-Finechem #AJA180), 20μM EGTA (Sigma #E3889), 0.1% (w/v) BSA (Sigma #A9418), 5mM succinate (Sigma #S2378). Cells were seeded in round-bottomed 96-well plates at 1×10^5 cells per well. 6-point 1:8 serial dilutions of venetoclax or the BIM-BH3 peptide (DMRPEIWEAQERREGDEENAYYARR; Genscript) starting from 10μM in TEB buffer supplemented with 0.002% digitonin (Sigma #D141) and 20μg/mL oligomycin (Sigma #75351) were used. The cells were incubated in the dark for 90min at room temperature. Cells were stained with 900nM JC-1 dye (Sigma #T4069) for 40min in the dark and analyzed using a FACSFortessa Flow Cytometer (BD).

**Seahorse assays**

MOLM-13 and MV-4-11 cells were treated with DMSO or 20nM of venetoclax for 3d. Drugs were washed out and 2.5x10^5 cells/well were plated for immediate analysis using the Mito Stress Test
Kit (Agilent, #103015-100), according to the manufacturer’s protocols. The following drug concentrations were used: 1μM Oligomycin, 2.2μM FCCP, 0.5μM Rotenone/Antimycin A. Eμ-Myc lymphoma cells were treated with DMSO or 200nM S63845 for 3h. Drug was washed out and 1.6x10^5 cells/well were plated per well for immediate analysis using the Mito Stress Test Kit (Agilent, #103015-100). The following drug concentrations were used: 1.5μM Oligomycin, 2.0μM FCCP, 0.5μM Rotenone/Antimycin A. Mitochondrial function was analyzed using the Seahorse XFe96 (Agilent). Graphs were prepared in GraphPad Prism v.8. Error bars indicate SD of six replicate samples.

**Measurement of BAX and BAK activation by flow cytometry (to supplement the description in the main Methods)**

BH3-mimetic (or DMSO control) treated human cancer cells were permeabilized by resuspension in Fractionation buffer (20 mM HEPES/KOH pH 7.5, 100 mM sucrose, 100 mM KCl, 2.5 mM MgCl₂) supplemented with 0.025% w/v digitonin (Calbiochem #300410), 1x Complete protease inhibitor (Roche #11836145001), 4 mg/mL pepstatin A (Sigma #P5318) followed by incubation for 15 min at 4°C and two washes in Fractionation buffer. Permeabilized cells were then incubated in Fixation buffer (Invitrogen/eBiosciences #00-5523) for 30 min at 4°C prior to two washes with Permeabilization buffer (Invitrogen/eBiosciences #00-5523). Fixed cells were first stained for 30 min at 4°C with antibodies to activated BAX (biotinylated rat monoclonal clone 1E5, WEHI) and activated BAK (mouse monoclonal clone G317-2, BD Biosciences #556382) diluted in Permeabilization buffer (Invitrogen/eBiosciences #00-5523). Cells were washed and incubated for 30 min at 4°C with streptavidin-Brilliant Violet 785 (Biolegend #405249), goat anti-mouse IgG-Pacific Blue (Thermo Fisher Scientific #P31582) together with anti-Cytochrome c-APC (clone REA702, Miltenyi Biotec #130-111-368) diluted in Permeabilization buffer. Stained cells were washed in Permeabilization buffer and analyzed on a FACS Fortessa (BD) before data analysis (FlowJo software). For mouse cancer cells, cells were treated as above, but the fixation step was omitted. Permeabilised cells were stained for activated BAX (mouse monoclonal clone 6A7, BD Biosciences #556467, or rat monoclonal clone 49F9, WEHI) and activated BAK (mouse monoclonal clone G317-2 (BD Biosciences #556382). Stained cells were washed and incubated with PE Goat Anti-Mouse (Southern Biotech #1034-09) or Anti-Rat (BD Biosciences #550767) IgG for analysis.
References


