

Fig. S5.

a

```
WT BAK          TCCTGCTCCTACAGCACCATGGGGCAGGTGGGACGGCAGCTCGCCATC-----  
"2+5" KO BAK a  TCCTGCTCCTACAGCACCATGGGGCAGGTGGGACGGCAGCTCGCCATCAGGTGGTGCAGATGAACCTCAGGGTCAGCTTGCCGTAGGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGA  
"2+5" KO BAK b  TCCTGCTCCTACAGCACCATGGGGCAGGTGGGACGGCAGCTCGCCATC-----  
  
WT BAK          -----  
"2+5" KO BAK a  ACTTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACCATGCTAGAGTCGACTTCCTTTGCCCTCGGACGAGTGCTGG  
"2+5" KO BAK b  -----  
  
WT BAK          -----  
"2+5" KO BAK a  GCGCTCGGTTTCCACTATCGGGCGACCTTTGGTTCGCCCGCCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGGGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATT  
"2+5" KO BAK b  -----  
  
WT BAK          -----  
"2+5" KO BAK a  TCACACCGCATACGTCAAAGCAACCATAGTACGCGCCCTGTAGCGGTTTACCAGGGTGTTCGCCCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTGCCGTCGTCCTTGAAGAAGATGGT  
"2+5" KO BAK b  -----  
  
WT BAK          -----ATCGGGGACGACATCAACCGACGCTATGACTCAGAGTTCAGACCATGTTGCAGCACCTGCAGCCACGGCAGAGAATGCCTATGAG  
"2+5" KO BAK a  GCGCTCCTGGACGCAGCCTTCGGGCATGGCGGAATCGGGGACGACATCAACCGACGCTATGACTCAGAGTTCAGACCATGTTGCAGCACCTGCAGCCACGGCAGAGAATGCCTATGAG  
"2+5" KO BAK b  -----AATCGGGGACGACATCAACCGACGCTATGACTCAGAGTTCAGACCATGTTGCAGCACCTGCAGCCACGGCAGAGAATGCCTATGAG
```

b

```
WT BAX          TTTCCACCATCAGCCTGATGCCTGCTCCCCGGCACTGGTTCTCCTCTCTCCTGCAGGATGATTGCCG--CCGTGGACACAGACTCCCCCGAGAGGTCTTTTTCCGAGTGGCAGCTGA  
"2+5" KO BAX a  TTTCCACCATCAGCCTGATGCCTGCTCCCCGGCACTGGTTCTCCTCTCTCCTGCAGGATGATTGCCG-GCCGTGGACACAGACTCCCCCGAGAGGTCTTTTTCCGAGTGGCAGCTGA  
"2+5" KO BAX b  TTTCCACCATCAGCCTGATGCCTGCTCCCCGGCACTGGTTCTCCTCTCTCCTGCAGGATGATTGCCGCGCCGTGGACACAGACTCCCCCGAGAGGTCTTTTTCCGAGTGGCAGCTGA
```

Fig. S5.

c

```
WT BCL-xL          GAAGAGTTCATTCACTACCTGTTCAAAGCTCTGATATGCTGTCCCTGGGGTGATGTGGAGCTGGGATGTCAGGTCCTGAATGCCCGCCGGTACC-----  
"2+5" KO BCL-xL  GAAGAGTTCATTCACTACCAGTTCAAAGCTCTGATATGCCGTCCCTGGGGTGATGTGGAGCTGGGATGTCAGGTCCTGAATGCCCGCCGGTACCAGCTCTGCTTATATAGACCTCCAC  
  
WT BCL-xL          -----  
"2+5" KO BCL-xL  CGTACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTGGTGCCAAAACAACTCCCATTGACGTCAATGGGGTGGAGACTTGG  
  
WT BCL-xL          -----GCAGTTCAAACCTCGTCGCCTGCCTCCC  
"2+5" KO BCL-xL  AATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCACCATGGTAATAGCGATGACTAATACGTAGATGTCCGCAGTTCAAACCTCGTCGCCTGCCTCCC
```

d

```
WT BCL-2          TTTCCCTCTGGGAAGGATGGCGCACGCTGGGAGAACAGGGTACGATAA--CCGGGAGATAGTGATGAAGTACATCCATTATAAGCTGTCGCAGAGGGGCTACGAGTGGGATGCGGGAG  
"2+5" KO BCL-2 a  TTTCCCTCTGGGAAGGATGGCGCACGCTGGGAGAACAGGGTACGATAAAACCGGGAGATAGTGATGAAGTACATCCATTATAAGCTGTCGCAGAGGGGCTACGAGTGGGATGCGGGAG  
"2+5" KO BCL-2 b  TTTCCCTCTGGGAAGGATGGCGCACGCTGGGAGAACAGGGTACGATA---CCGGGAGATAGTGATGAAGTACATCCATTATAAGCTGTCGCAGAGGGGCTACGAGTGGGATGCGGGAG
```

e

```
WT BCL-w          CTCGGCCCCAGACACACGGGCTCTGGTGGCAGACTTTGTAGGTTATAAGCTGAGGCAGAAAGGGTTATGTCTGTGGAGCTGGCCCCGGGGAGGGCCAGCAGCTGACCCGCTGCACCAAGC  
"2+5" KO BCL-w  CTCGGCCCCAGACACACGGGCTCTGGTGGCAGACTTTGTAGGTTATA-----AGAAGGGTTATGTCTGTGGAGCTGGCCCCGGGGAGGGCCAGCAGCTGACCCGCTGCACCAAGC
```

- Cont.

Fig. S5.

f

```
WT MCL-1          AGGAGCTGGACGGGTACGAGCCGGAGCCTCTCGGGAAGCGGCCGGCTGTCTGCCGCTGCTGGAGTTGGTCGGGGAATCTGGTAATAACACCAGTAC-----  
"2+5" KO MCL-1 a  AGGAGCTGGACGGGTACGAGCCGGAGCCTCTCGGGAAGCGGCCGGCTGTCTGCCGCTGCTGGAGTTGGTCGGGGAATCTGGTAATAACACCAGTAC-----  
"2+5" KO MCL-1 b  AGGAGCTGGACGGGTACGAGCCGGAGCCTCTCGGGAAGCGGCCGGCTGTCTGCCGCTGCTGGAGTTGGTCGGGGAATCTGGTAATAACACCAGTACTCGCTCCAGTCAATGACCGCTGT
```

```
WT MCL-1          -----  
"2+5" KO MCL-1 a  -----  
"2+5" KO MCL-1 b  TATGCGGCCATTGTCCGTCAAGACATTGTTGGGGCCG.....742bp insertion .....ACAGACGTCGCGGTGAGTTCAGGCTTTTCATGCTAGCAGGACCGGGTTACTTTCAAC
```

```
WT MCL-1          -----GGACGGGTCACTACCCTCGACGCCGCCAGCAGAGGAGGAGGAGGACGAGTTCAACGCG  
"2+5" KO MCL-1 a  -----CCTCGACGCCGCCAGCAGAGGAGGAGGAGGACGAGTTCAACGCG  
"2+5" KO MCL-1 b  ATCGCCAGCGAGTTTCAACAAAGCGTAGTTAGTACATTGAGGATCTGTGACCCGTCAACGGACGGGTCACTACCCTCGACGCCGCCAGCAGAGGAGGAGGAGGACGAGTTCAACGCG
```

g

```
WT A1            CTGTGAATTTGGATATATTTACAGGCTGGCTCAGGACTATCTGCAGTGCGTCCTACAGATACCACA-ACCTGGATCAGGTCCAAGCAAACGTCCAGAGTGCTACAAAATGTTGCGTCT  
"2+5" KO A1      CTGTGAATTTGGATATATTTACAGGCTGGCTCAGGACTATCTGCAGTGCGTCCTACAGATACCACACACCTGGATCAGGTCCAAGCAAACGTCCAGAGTGCTACAAAATGTTGCGTCT
```

- Cont.

Fig. S5 Mutations in the targeted loci of “2+5” KO cells.

(a-g) Genomic sequences of *BAX*, *BAK*, *BCL-xL*, *BCL-2*, *BCL-w*, *MCL-1* and *AI* around the CRISPR target sites aligned with the corresponding wild-type sequences. Each CRISPR target sequence is underlined. Insertions are labeled in green and the stop codons are labeled in red.