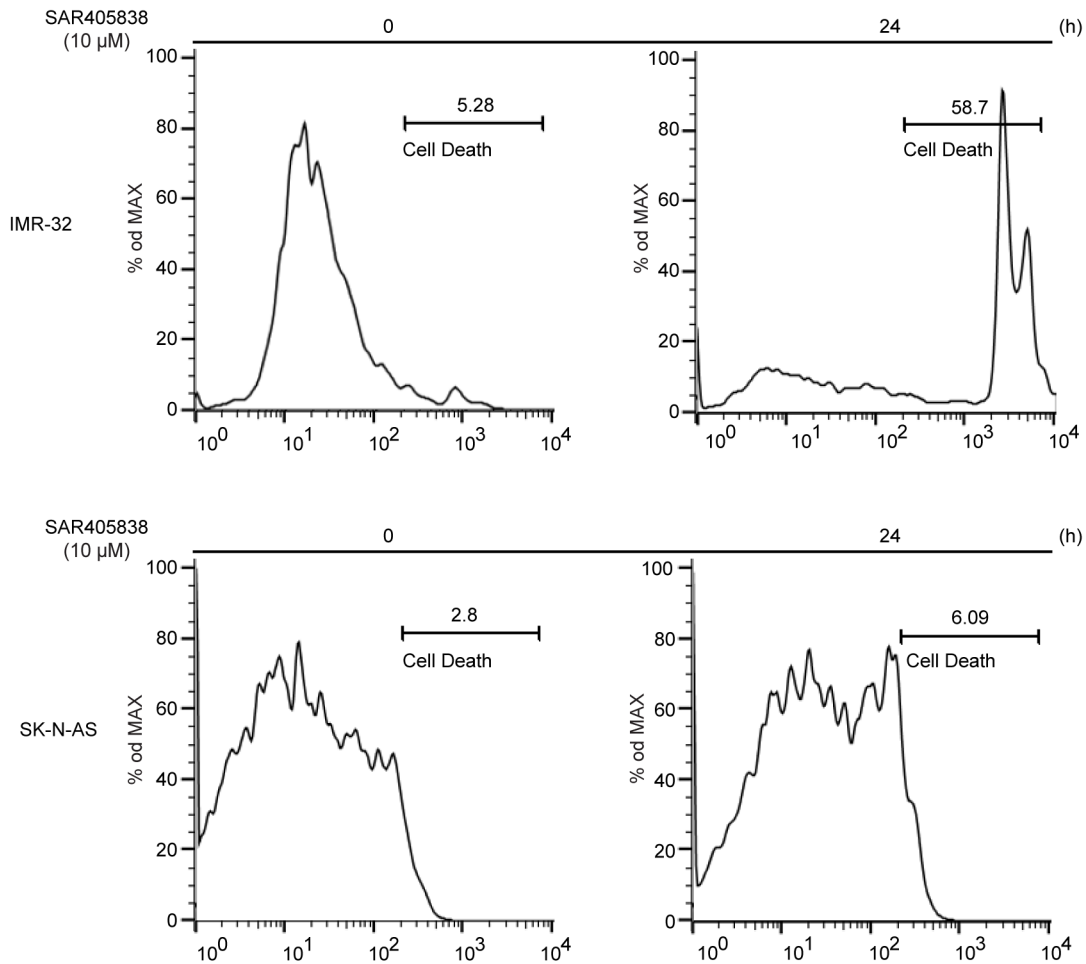


Novel MDM2 inhibitor SAR405838 (MI-773) induces p53-mediated apoptosis in neuroblastoma

SUPPLEMENTARY FIGURES



Supplementary Figure S1: SAR405838 induces cell death of p53 wild-type NB cell line IMR-32. The p53 wild-type IMR-32 and p53 mutant SK-N-AS cell lines were treated with 10 μ M SAR405838 for 0 or 24 hours. The percentage of cell death in the treated cells was detected by flow cytometry analysis.

1. p53 knockout compared with p53 wild-type
(knockout encodes a fusion p53 with 242 aa p53 + 95 aa non-p53 aa due to a frameshift of a 19 nt deletion)

CDS: Putative 1 214 H S V V V P Y E P P E V G S D C T T I H
Query 661 ACATAGTGTGGTGGCCCTATGAGCCGCTGAGTGGCTCTGACTGTACACCATCA 720
Sbjct 841 ACATAGTGTGGTGGCCCTATGAGCCGCTGAGTGGCTCTGACTGTACACCATCA 900
CDS:cellular tumor a 214 H S V V V P Y E P P E V G S D C T T I H

CDS: Putative 1 234 Y N Y M C N S S C G P S S
Query 721 CTACACTACATGTGTAAAGTCTCTG-----GGCCCATCTCTAC 761
Sbjct 901 CTACACTACATGTGTAAAGTCTCTG-----GGCCCATCTCTAC 960
CDS:cellular tumor a 234 Y N Y M C N S S C M G G H N R R P I L T

CDS: Putative 1 247 P S S H N K T P V V I Y W D G T A L R C
Query 762 CATCATCACCTGGAGACTCCAGTGTAACTACTGGACCGAAGACTTTGAGGTGG 821
Sbjct 961 CATCATCACCTGGAGACTCCAGTGTAACTACTGGACCGAAGACTTTGAGGTGG 1020
CDS:cellular tumor a 254 I T T L E D S S G N L L G R H S P F E V R

CDS: Putative 1 267 V F V P V L G E T G A Q R K R I S A R K
Query 822 TGTFTTGTGCTCTCTGGAGAGCCGGCCACAGAGAGATCCCGAAGAAAG 881
Sbjct 1021 TGTFTTGTGCTCTCTGGAGAGCCGGCCACAGAGAGATCCCGAAGAAAG 1080
CDS:cellular tumor a 274 V C A C P G R D R R T E E N L R K K G

CDS: Putative 1 287 G S L T T S C P Q G A L S E H C P T T P
Query 882 GGAGCTCACACAGACTGCCCGAGGACTAAGCCAGACTGCCAAGACACAG 941
Sbjct 1081 GGAGCTCACACAGACTGCCCGAGGACTAAGCCAGACTGCCAAGACACAG 1140
CDS:cellular tumor a 294 E P H H E L P P G S T K R A L P N N T S

CDS: Putative 1 307 A P L P S Q R R N H W M E N I S P F R S
Query 942 CTCTCTCCACAGCCAGGACTCCAGGACTGATGAGGACTGATGAGGACTG 1001
Sbjct 1141 CTCTCTCCACAGCCAGGACTCCAGGACTGATGAGGACTGATGAGGACTG 1200
CDS:cellular tumor a 314 S S P Q P K K K P L D G E Y F T L Q I R

CDS: Putative 1 327 V G V S A S R C S E S *
Query 1002 TGGGCTGAGGCTTCCAGATGTTCCAGAGCTGAATGAGGCTTGAAGTCAAGGATG 1061
Sbjct 1201 TGGGCTGAGGCTTCCAGATGTTCCAGAGCTGAATGAGGCTTGAAGTCAAGGATG 1260
CDS:cellular tumor a 334 G R E R F E M P F R E L N E A L E L K D A

CDS: Putative 1 346 CCAGGCTGGAGAGGAGCCAGGGGGAGCGGCTCACTCCAGCCACTGAAGTCAAAA 1121
Query 1062 CCAGGCTGGAGAGGAGCCAGGGGGAGCGGCTCACTCCAGCCACTGAAGTCAAAA 1320
Sbjct 1261 CCAGGCTGGAGAGGAGCCAGGGGGAGCGGCTCACTCCAGCCACTGAAGTCAAAA 1380
CDS:cellular tumor a 354 Q A G K E P F G G S R A N S S H L K S K K

CDS: Putative 1 365 GGCTCAGTCTACTCCCGCATAAAAACATGTTCAAGCAGAGAGGCTGACTCAGA 1181
Query 1122 GGCTCAGTCTACTCCCGCATAAAAACATGTTCAAGCAGAGAGGCTGACTCAGA 1380
Sbjct 1321 GGCTCAGTCTACTCCCGCATAAAAACATGTTCAAGCAGAGAGGCTGACTCAGA 1440
CDS:cellular tumor a 374 G Q S T S R H K K L M P K F E G P D S D

CDS: Putative 1 389 CTGACATTTCCACTTCTGTGTTCCCACTGACAGGCTCCACCCCATCTCTCCCTCCC 1241
Query 1182 CTGACATTTCCACTTCTGTGTTCCCACTGACAGGCTCCACCCCATCTCTCCCTCCC 1380
Sbjct 1381 CTGACATTTCCACTTCTGTGTTCCCACTGACAGGCTCCACCCCATCTCTCCCTCCC 1440

2. p53 knockout compared with p53 wild-type
(knockout encodes a fusion p53 with 243 aa p53 + 95 aa non-p53 aa due to a frameshift of a 16 nt deletion)

CDS: Putative 1 214 H S V V V P Y E P P E V G S D C T T I H 780
Query 661 ACATAGTGTGGTGGCCCTATGAGCCGCTGAGTGGCTCTGACTGTACACCATCA 780
Sbjct 841 ACATAGTGTGGTGGCCCTATGAGCCGCTGAGTGGCTCTGACTGTACACCATCA 900
CDS:cellular tumor a 214 H S V V V P Y E P P E V G S D C T T I H

CDS: Putative 1 234 Y N Y M C N S S C M G P S S 824
Query 781 CTACACTACATGTGTAAAGTCTCTGATG-----GGCCCATCTCTAC 824
Sbjct 901 CTACACTACATGTGTAAAGTCTCTGATG-----GGCCCATCTCTAC 960
CDS:cellular tumor a 234 Y N Y M C N S S C M G G H N R R P I L T

CDS: Putative 1 248 P S S H N K T P V V I Y W D G T A L R C 884
Query 825 CATCATCACCTGGAGACTCCAGTGTAACTACTGGACCGAAGACTTTGAGGTGG 884
Sbjct 961 CATCATCACCTGGAGACTCCAGTGTAACTACTGGACCGAAGACTTTGAGGTGG 1020
CDS:cellular tumor a 254 I T T L E D S S G N L L G R N S F E V R

CDS: Putative 1 268 V F V P V L G E T G A Q R K R I S A R K 944
Query 885 TGTFTTGTGCTCTCTGGAGAGCCGGCCACAGAGAGATCCCGAAGAAAG 944
Sbjct 1021 TGTFTTGTGCTCTCTGGAGAGCCGGCCACAGAGAGATCCCGAAGAAAG 1080
CDS:cellular tumor a 274 V C A C P G R D R R T E E N L R K K G

CDS: Putative 1 288 G S L T T S C P Q G A L S E H C P T T P 1004
Query 945 GGAGCTCACACAGACTGCCCGAGGACTAAGCCAGACTGCCAAGACACAG 1004
Sbjct 1081 GGAGCTCACACAGACTGCCCGAGGACTAAGCCAGACTGCCAAGACACAG 1140
CDS:cellular tumor a 294 E P H H E L P P G S T K R A L P N N T S

CDS: Putative 1 308 A P L P S Q R R N H W M E N I S P F R S 1064
Query 1005 CTCTCTCCACAGCCAGGACTCCAGGACTGATGAGGACTGATGAGGACTG 1064
Sbjct 1141 CTCTCTCCACAGCCAGGACTCCAGGACTGATGAGGACTGATGAGGACTG 1200
CDS:cellular tumor a 314 S S P Q P K K K P L D G E Y F T L Q I R

CDS: Putative 1 328 V G V S A S R C S E S * 1124
Query 1065 TGGGCTGAGGCTTCCAGATGTTCCAGAGCTGAATGAGGCTTGAAGTCAAGGATG 1124
Sbjct 1201 TGGGCTGAGGCTTCCAGATGTTCCAGAGCTGAATGAGGCTTGAAGTCAAGGATG 1260
CDS:cellular tumor a 334 G R E R F E M P F R E L N E A L E L K D A

CDS: Putative 1 347 CCAGGCTGGAGAGGAGCCAGGGGGAGCGGCTCACTCCAGCCACTGAAGTCAAAA 1184
Query 1125 CCAGGCTGGAGAGGAGCCAGGGGGAGCGGCTCACTCCAGCCACTGAAGTCAAAA 1320
Sbjct 1261 CCAGGCTGGAGAGGAGCCAGGGGGAGCGGCTCACTCCAGCCACTGAAGTCAAAA 1380
CDS:cellular tumor a 354 Q A G K E P F G G S R A N S S H L K S K K

CDS: Putative 1 366 GGCTCAGTCTACTCCCGCATAAAAACATGTTCAAGCAGAGAGGCTGACTCAGA 1244
Query 1185 GGCTCAGTCTACTCCCGCATAAAAACATGTTCAAGCAGAGAGGCTGACTCAGA 1380
Sbjct 1321 GGCTCAGTCTACTCCCGCATAAAAACATGTTCAAGCAGAGAGGCTGACTCAGA 1440
CDS:cellular tumor a 374 G Q S T S R H K K L M P K F E G P D S D

CDS: Putative 1 386 CTGACATTTCCACTTCTGTGTTCCCACTGACAGGCTCCACCCCATCTCTCCCTCCC 1304
Query 1245 CTGACATTTCCACTTCTGTGTTCCCACTGACAGGCTCCACCCCATCTCTCCCTCCC 1380
Sbjct 1381 CTGACATTTCCACTTCTGTGTTCCCACTGACAGGCTCCACCCCATCTCTCCCTCCC 1440

Supplementary Figure S2: The p53-knockout information in SH-SY5Y cells via the CRISPR-Cas9-mediated genomic editing technology. The lenti-viral vectors carrying the cassettes for a p53 gRNA and Cas9-2A-puromycin were transfected with packaging vectors into 293T cells for generating virus. The wild-type SH-SY5Y cells were transduced with lenti-virus for p53 knockout and then selected with puromycin. The single cell clone with p53 knockout was verified by PCR flanking the target site followed by cloning the PCR products and sequencing analysis.