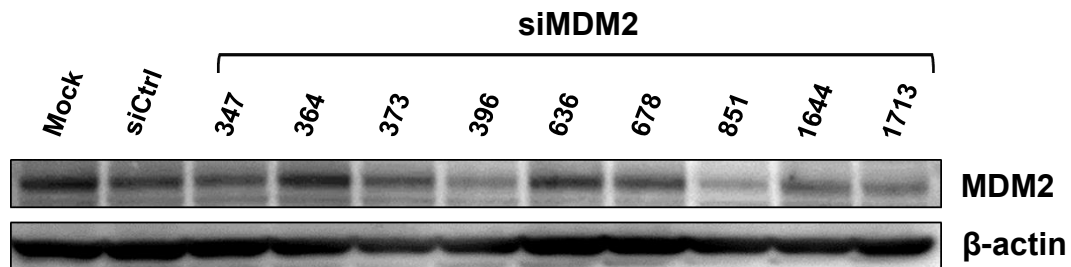
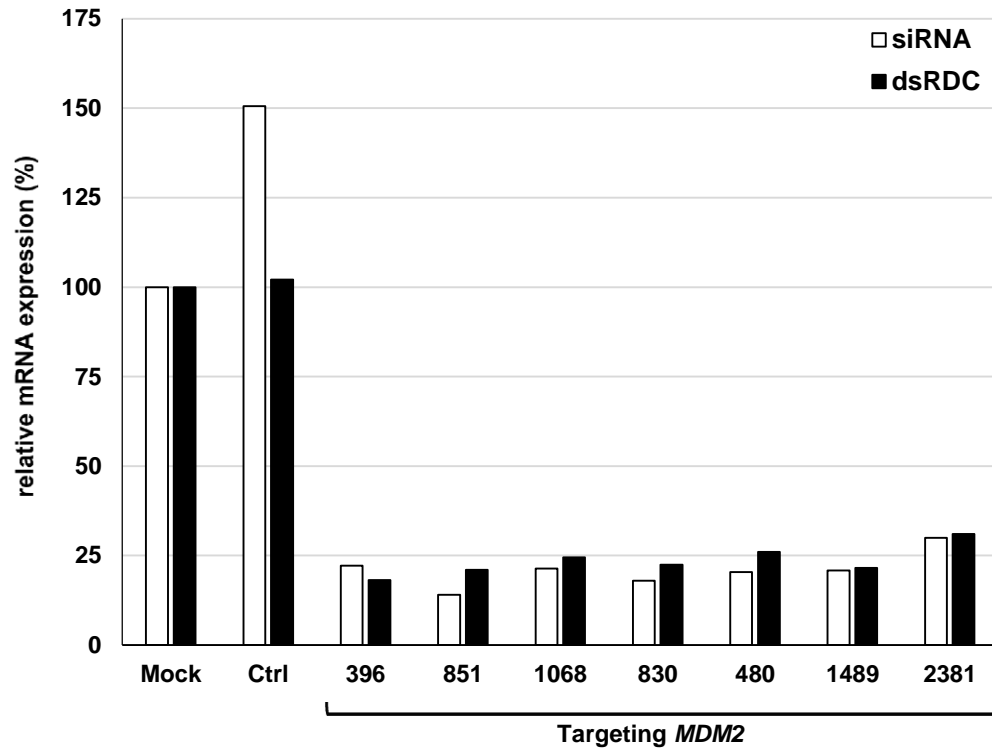


MDM4 expression as an indicator of *TP53* reactivation by combined targeting of *MDM2* and *MDM4* in cancer cells without *TP53* mutation

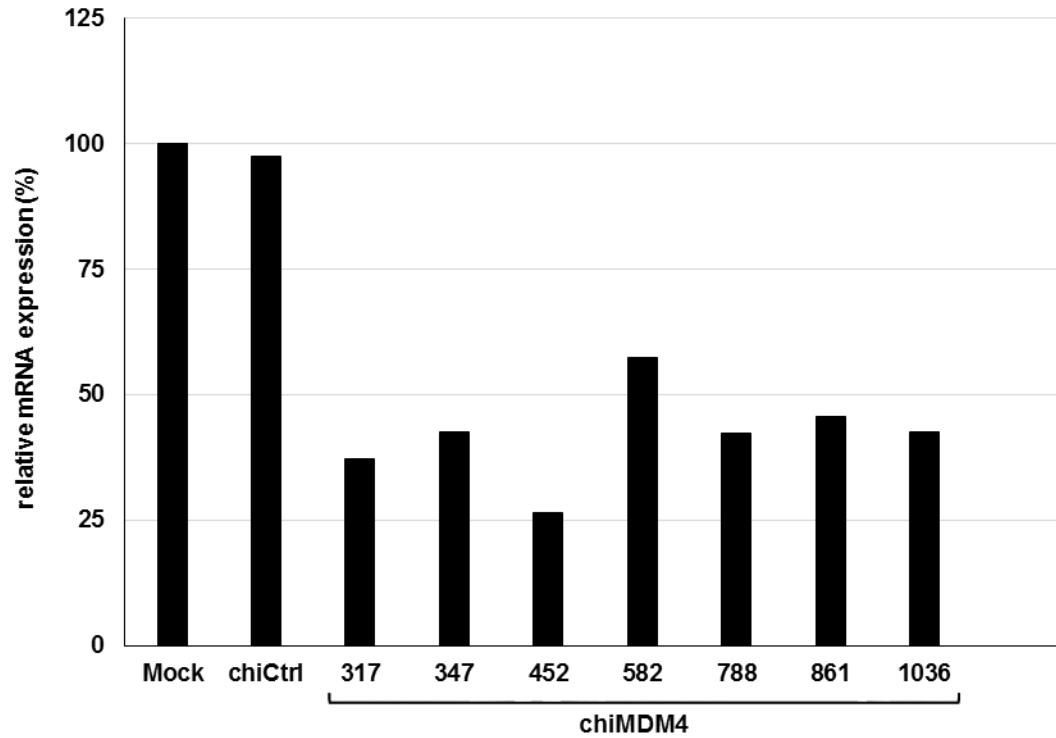
Supplementary Material



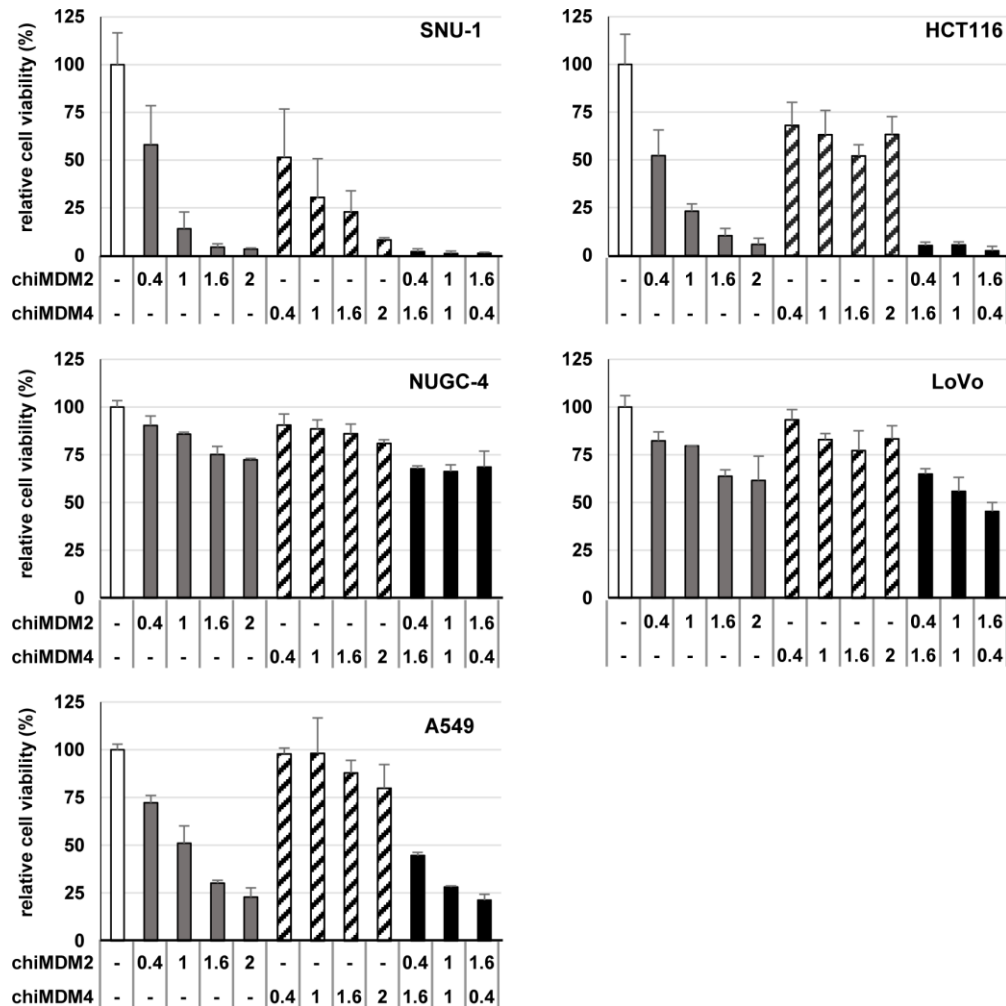
Supplementary Figure 1: Knockdown efficiency of siRNAs targeting *MDM2*. SJS-1 cells were transfected with mock, control siRNA (siCtrl), and nine reported siMDM2s at 1 nM for 48 h and then examined for MDM2 expression by immunoblotting.



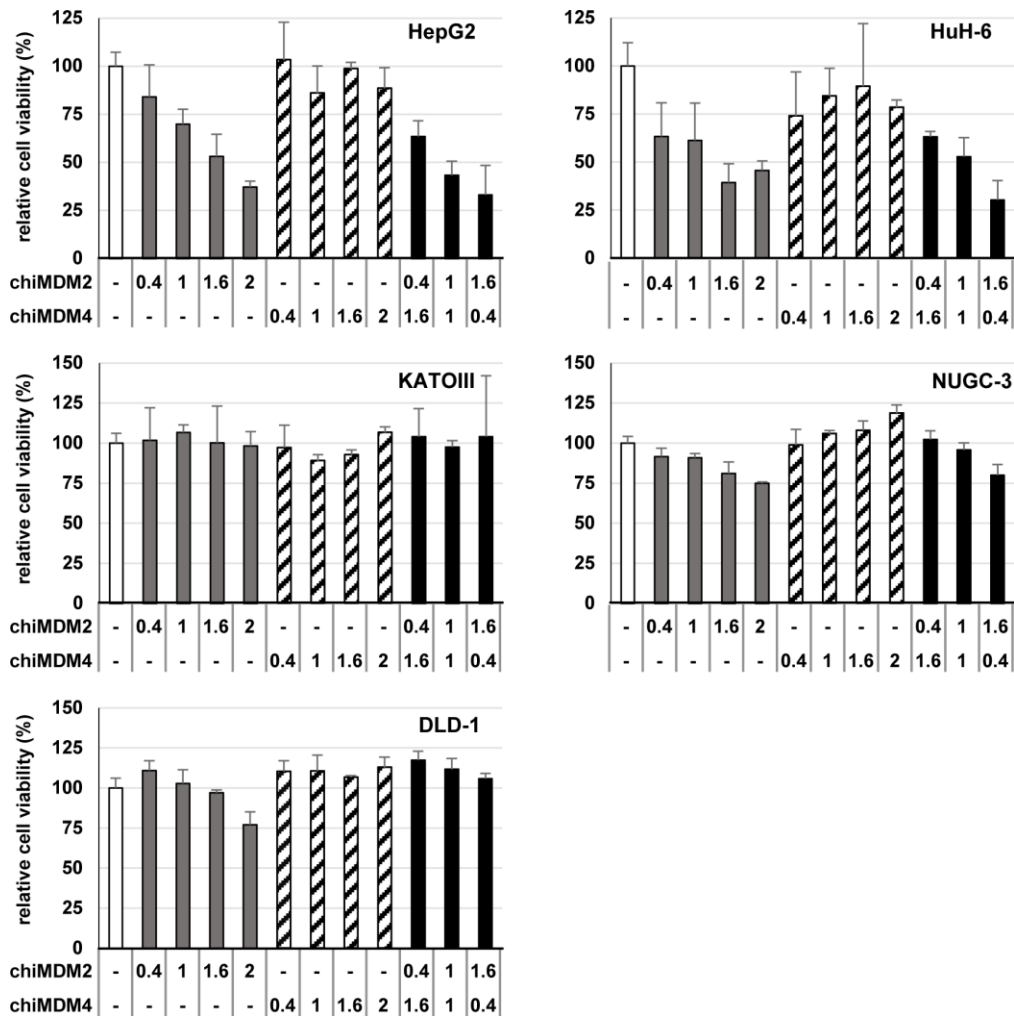
Supplementary Figure 2: Knockdown efficiency of siRNAs targeting *MDM2* and their dsRDC forms. SJSA-1 cells were transfected with mock, control (Ctrl) siRNA, seven siMDM2s, and their dsRDC forms at 1 nM for 48 h and then examined for *MDM2* mRNA expression by qRT-PCR. All experiments were performed in duplicate.



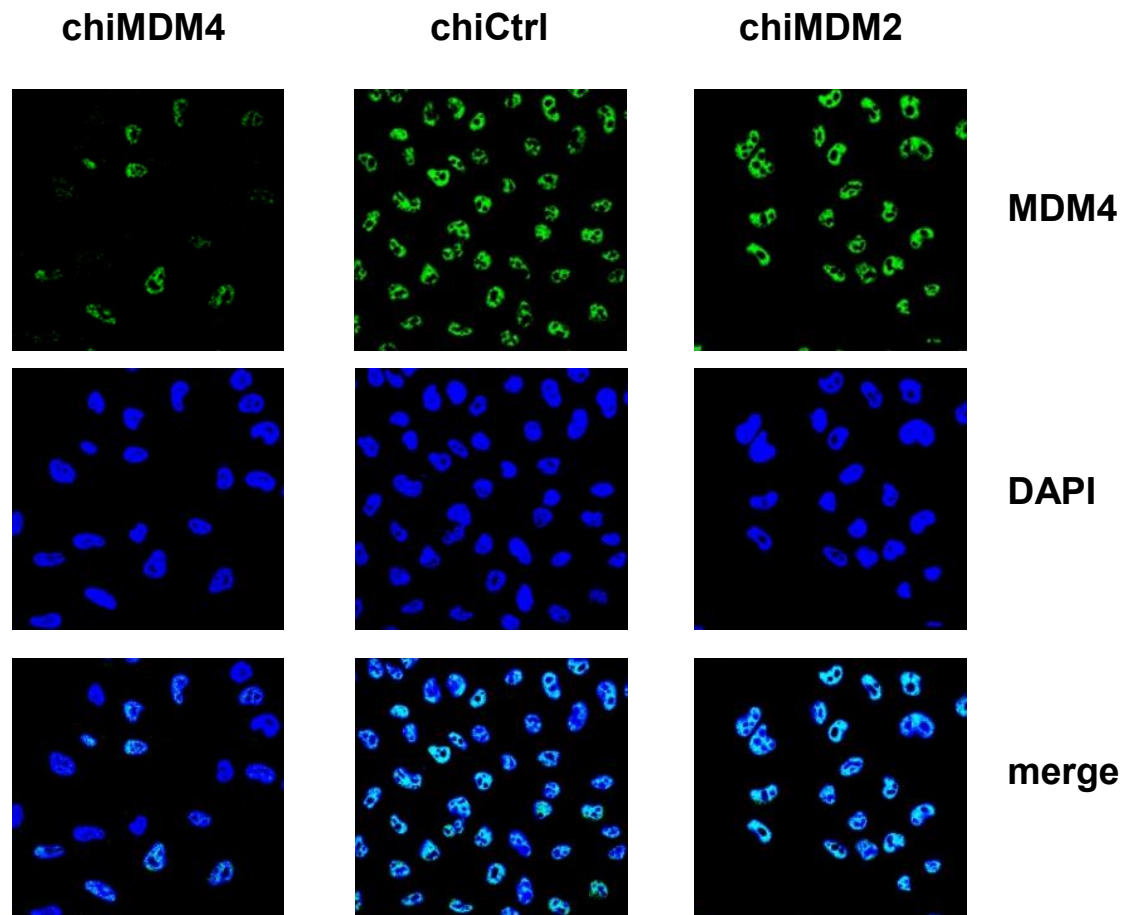
Supplementary Figure 3: Knockdown efficiency of dsRDC-modified siRNAs targeting *MDM4*. MCF-7 cells were transfected with mock, control dsRDC (chiCtrl), and seven dsRDCs targeting *MDM4* (chiMDM4) at 1 nM for 48 h and then examined for *MDM4* mRNA expression by qRT-PCR. All experiments were performed in duplicate.



Supplementary Figure 4: Evaluation of combined knockdown of *MDM2* and *MDM4* on the growth of wtTP53 cell lines with high *MDM4* expression. Effects of individual and simultaneous knockdown of *MDM2* and *MDM4* on growth were examined in five wtTP53 cell lines with high *MDM4* expression. Cells were transfected with *MDM4* dsRDC (chiMDM4-452) alone, *MDM2* dsRDC (chiMDM2-1489) alone, or both. The total amount of dsRDC was adjusted to 2 nM by adding control dsRDC (chiCtrl). Cell viability was determined 5 days after transfection using the WST-8 assay. The number of viable chiCtrl (2 nM) transfected cells was defined as 100% (mean \pm SD; n = 3).



Supplementary Figure 5: Evaluation of combined knockdown of *MDM2* and *MDM4* on the growth of wtTP53 cells with low *MDM4* expression and mt *TP53* cells. Effects of individual and simultaneous knockdown of *MDM2* and *MDM4* on the cell growth were examined in wtTP53 cells with low *MDM4* expression (HepG2 and HuH-6) and mt *TP53* cells (KATOIII, NUGC-3, and DLD-1). Cells were transfected with *MDM4* dsRDC (chiMDM4-452) alone, *MDM2* dsRDC (chiMDM2-1489) alone, or both. The total amount of dsRDCs was adjusted to 2 nM by adding control dsRDC (chiCtrl). Cell viability was determined 5 days after transfection using the WST-8 assay. The number of viable chiCtrl (2 nM) transfected cells was defined as 100% (mean \pm SD; n = 3).



Supplementary Figure 6: Subcellular localization of MDM4 after *MDM2* knockdown. A375 cells were transfected with control dsRDC (chiCtrl), *MDM2* dsRDC (chiMDM2), or *MDM4* dsRDC (chiMDM4) at 1 nM for 24 h. Then, the localization of MDM4 was examined by immunofluorescence. Cellular nuclei were identified by DAPI staining.

Supplementary Table 1: Sequence of siRNAs

siRNAs	Nucleotide position	Passenger strand (5'→3')	Guide strand (5'→3')	References
siMDM2-347	347-369	CCACCUCACAGAUUCCAGCUU	GUCGGAAUCUGUGAGGUGGUU	Linares LK et al.
siMDM2-364	364-386	GCUUCGGAACAAGAGACCCUG	GGGUCUCUUGUUCGAAGCUG	Yin JQ et al.
siMDM2-373	373-395	CAAGAGACCCUGGUUAGACCA	GUCUAACCAGGGUCUCUUGUU	Linares LK et al.
siMDM2-396	396-418	GCCAUUGCUUUUGAAGUUUU	UAACUUCAAAAGCAAUGGCUU	Jin Y et al.
siMDM2-636	636-658	UCAGCAGGAAUCAUCGGACUC	GUCCGAUGAUUCCUGCUGAUU	Carrol VA et al.
siMDM2-678	678-700	CAGGUGUCACCUUGAAGGUGG	ACCUUCAAGGUGACACCUGUU	Toh WH et al.
siMDM2-851	851-873	GCCACAAAUCUGAUAGUUAUU	AUACUAUCAGAUUUGUGGCGU	Warburton HE et al.
siMDM2-1644	1644-1666	UGGUUGCAUUGUCCAUGGCAA	GCCAUGGACAAUGCAACCAUU	Kurki S et al.
siMDM2-1713	1713-1735	AAGGAAUAAGCCCUGCCCAGU	UGGGCAGGGCUUAUUCUUUUU	Uchida C et al.
siMDM2-480	480-502	GUUAUUUAUGACUAAACGAUU	UCGUUUAGUCAUAAUAUACUG	
siMDM2-643	643-665	GAAUCAUCGGACUCAGGUACA	UACCUGAGUCCGAUGAUUCCU	
siMDM2-691	691-713	GAAGGUGGGAGUGAUCAAAAG	UUUGAUCACUCCCACCUUCA	
siMDM2-830	830-852	CUGGUGAACGACAAAGAAAAC	UUUCUUUGUCGUUCACCAGAU	
siMDM2-1068	1068-1090	CUCAGAAGAUUAUAGCCUUG	AAGGCUAUAUUCUUCUGAGUC	
siMDM2-1274	1274-1296	CCCUUCGUGAGAAUUGGCUUC	AGCCAAUUCUCACGAAGGGCC	
siMDM2-1275	1275-1297	CCUUCGUGAGAAUUGGCUUCC	AAGCCAAUUCUCACGAAGGGC	
siMDM2-1489	1489-1511	CAGCCAUCAACUUCUAGUAGC	UACUAGAAGUUGAUGGCUGAG	
siMDM2-1625	1625-1647	GUCAAGGUCGACCUAAAAUG	UUUUUAGGUCGACCUUGACAA	
siMDM2-2370	2370-2392	GGCCUAAAUGUCACUUAGUAC	ACUAAGUGACAUUUAGGCCGG	
siMDM2-2381	2381-3003	CACUUAGUACCUUUGAUUAA	AUAUCAAAAGGUACUAAGUGAC	
siMDM2-3412	3412-3434	CCACCAUUUACCCGUAAGACA	UCUUACGGGUAAAUGGUGGCU	
siMDM2-5187	5187-5209	CUCCAAAGGUAAAAGUACUAA	AGUACUUUUACCUUUGGAGGU	
siMDM2-6068	6068-6090	GGUUCUUUAUAGUACACGUGU	ACGUGUACUAUAAAGAACCUA	
siMDM2-6281	6281-6303	GCAGUUGGGAGCCUCCAUGA	AUUGGAGGCUCCCAACUGCUU	
siMDM2-6393	6393-6415	GUGAUCGUGAAUGGUCUAUAA	AUAGACCAUUCACGAUCACUU	
siMDM2-7414	7414-7436	GUACUCAAUAUUUAACGUUA	ACGUUAAAUAUUUGAGUACAG	
siMDM4-317	317-339	GGUCAUGCACUAUUUAGGUCA	ACCUAAAUAGUGCAUGACCUC	
siMDM4-347	347-369	GGUGAAGCAACUUUAUGAUCA	AUCAUAAAGUUGCUUCACCAU	

siMDM4-452	452-474	CCCUCUCUAUGAUUGCUAAG	UAGCAUAUCAUAGAGAGGGCU
siMDM4-525	525-547	GCUCUCGCACAGGAUCACAGU	UGUGAUCCUGUGCGAGAGCGA
siMDM4-582	582-604	GCAGAGGAAAGUCCACUUC	AAGUGGAACUUCCUCUGCAC
siMDM4-788	788-810	CAACUAUACACCUAGAAGUAA	ACUUCUAGGUGUAUAGUUGCU
siMDM4-861	861-883	GAUACUACAGAUGACUUGUGG	ACAAGUCAUCUGUAGUAUCUG
siMDM4-1036	1036-1058	GUGAUGAUACCGAUGUAGAGG	UCUACAUCGGUAUCAUCACUU
siMDM4-1492	1492-1514	GACCACGAGACGGGAACAUUA	AUGUCCCCGUCUCGUGGUCUU
siMDM4-2263	2263-2285	GAGACUAUAGACUAGCAUAA	UAUGCUAGUCUAUAGUCUCAG
siControl		GUACCGCACGUAUUCGUAUC	UACGAAUGACGUGCGGUACGU
siControl-R		CCGUACUAGCCAUAUGCGUC	CGCAUAAUGGCUAGUACGGGU

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