

Figure S1. Electron density for the MDM2_E69AK70A complexes. A) All atom cylinder representation of the MDM2_E69AK70A/p53₁₅₋₂₉ complex, with carbon atoms of the MDM2 domain coloured ice blue and carbon atoms of the p53-derived peptide coloured yellow. Final $2mF_o-DF_c$ electron density is contoured at 0.25 electrons per cubic angstrom. B) All atom cylinder representation of the MDM2_E69AK70A/Nutlin-3a complex with carbon atoms of the mdm2 domain coloured ice blue and carbon atoms of Nutlin-3a peptide coloured yellow. Final $2mF_o-DF_c$ electron density is contoured at 0.47 electrons per cubic angstrom.

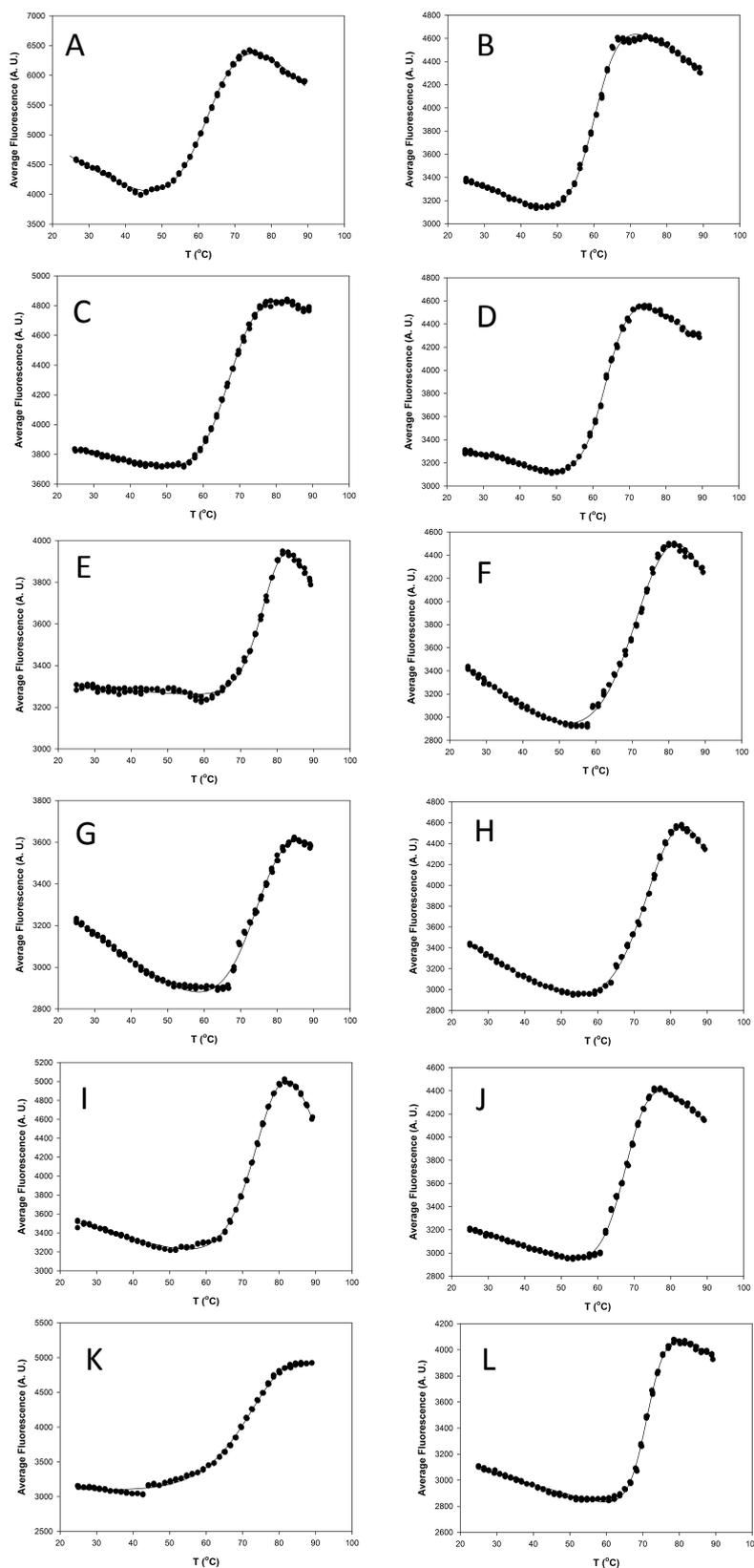


Figure S2. Differential scanning fluorimetry traces for MDM2₁₇₋₁₀₉ and MDM2_E69AK70A. Temperature-dependent fluorescence of SyproOrange (10 μ M) in the presence of MDM2₁₇₋₁₀₉ (panels a, c, e, g, i, k) or MDM2_E69AK70A (panels b, d, f, h, j, l) and either no ligand (a, b), p53₁₅₋₂₉ (c, d), Nutlin-3a (e, f), MI-63 (g, h), Compound 1 (i, j) or Compound 2 (k, l).

Table S1. Melting temperatures for MDM2₁₇₋₁₀₉ and MDM2_E69AK70A in the presence of various ligands.

Ligand	T _m MDM2 ₁₇₋₁₀₉ (°C)	T _m MDM2_E69K70A (°C)
apo	64.5 ± 2.0	60.6 ± 1.4
p53	68.1 ± 1.2	63.5 ± 1.0
DMSO	66.0 ± 1.4	63.1 ± 1.0
Nutlin3a	77.9 ± 1.6	70.4 ± 1.1
MI63	80.0 ± 0.3	72.0 ± 1.1
Compound 1	74.3 ± 1.9	67.1 ± 2.7
Compound 2	77.0 ± 2.1	70.7 ± 0.6

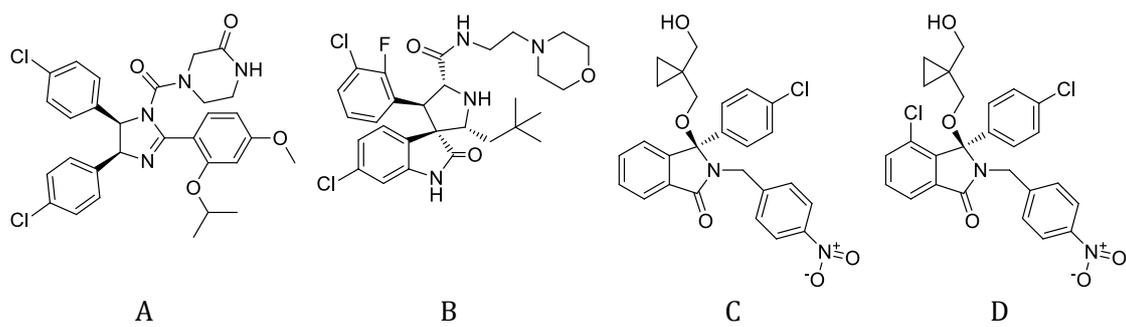


Figure S3. Structures of small molecular inhibitors used in this study. a) Nutlin-3a, b) MI-63, c) Compound 1, d) Compound 2.

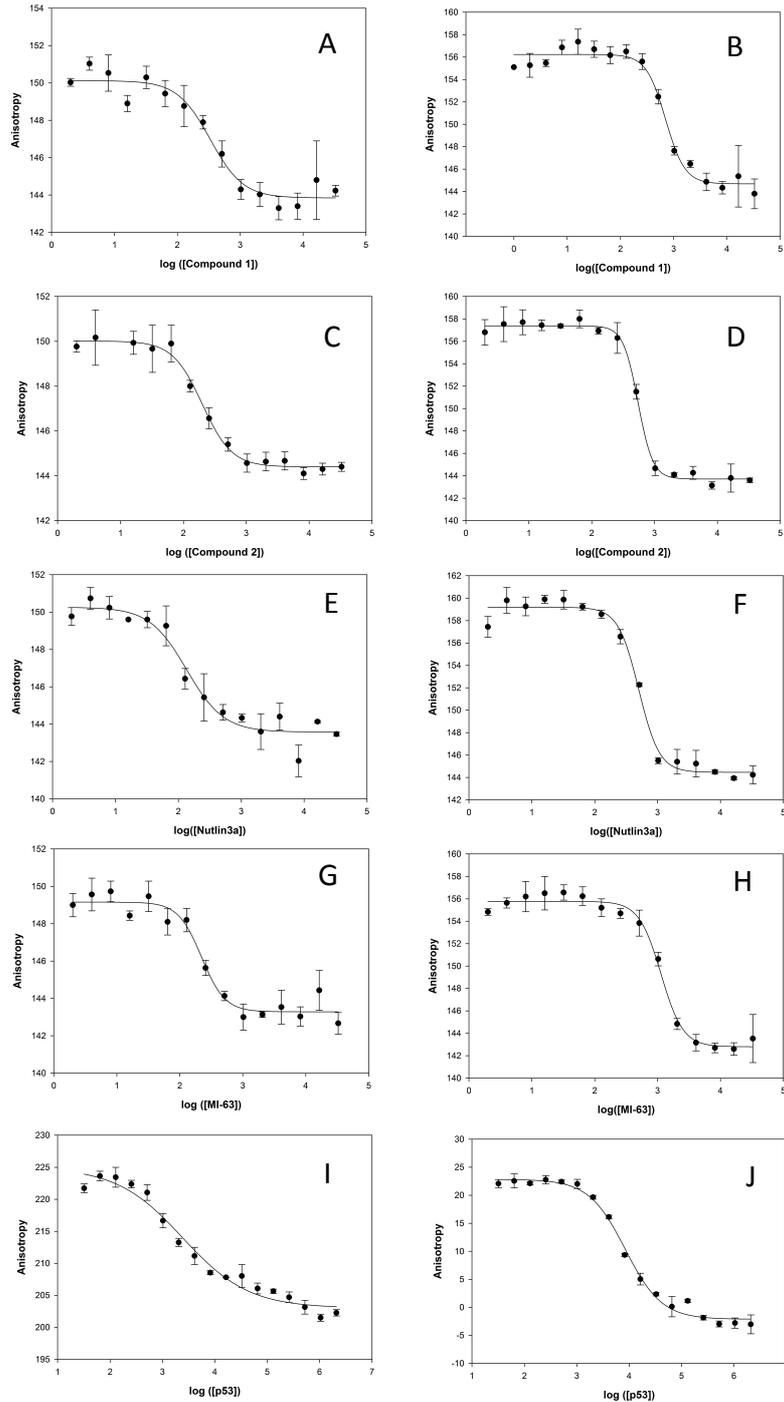


Figure S4. Competitive binding of ligands to MDM2₁₇₋₁₀₉ and MDM2_E69AK70A followed by fluorescence polarization anisotropy.(c-l) Fluorescence anisotropy of fluorescein-labelled p53₁₅₋₂₉ in the presence of a fixed concentration of MDM2₁₇₋₁₀₉ (a, c, e, g, i) or MDM2_E69AK70A (b, d, f, h, j) and in differing concentrations of Compound 1 (a, b), Compound 2 (c, d), Nutlin-3a (e, f), MI-63 (g, h), and unlabeled p53₁₅₋₂₉ (i, j).

Table S2. Inhibition constants for interference with the interaction between fluorescein-labeled p53₁₅₋₂₉ and MDM2₁₇₋₁₀₉ or MDM2_E69AK70A

Ligand	Ki MDM2₁₇₋₁₀₉ (nM)	Ki MDM2_E69AK70A (nM)
p53 ₁₅₋₂₉	1280 ± 250	4440 ± 290
Nutlin-3a	71 ± 11	264 ± 14
MI-63	86 ± 8	555 ± 33
Compound 1	175 ± 28	380 ± 46
Compound 2	103 ± 11	287 ± 11

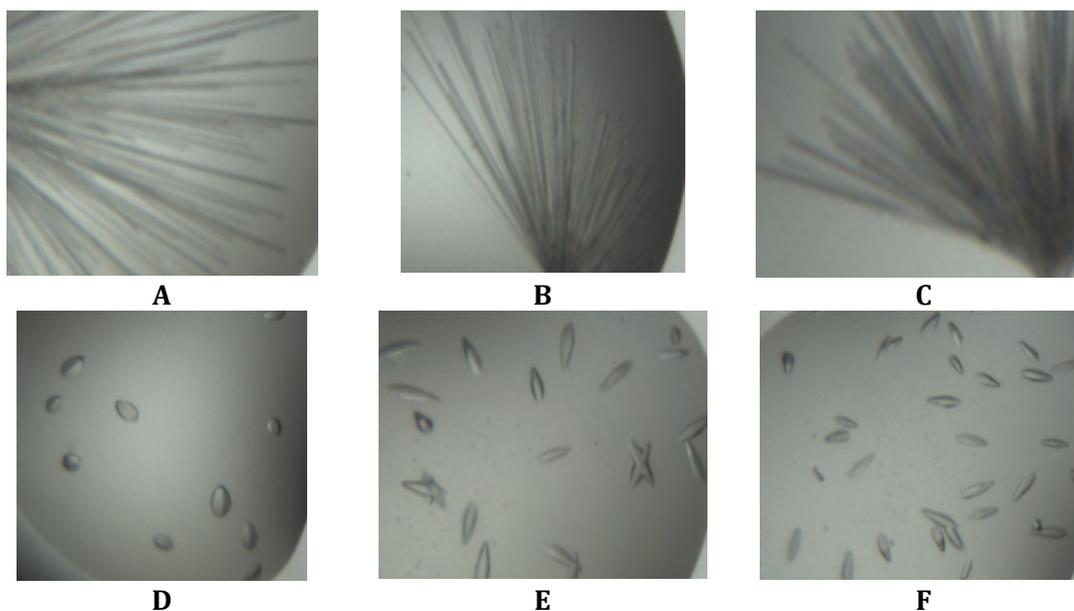
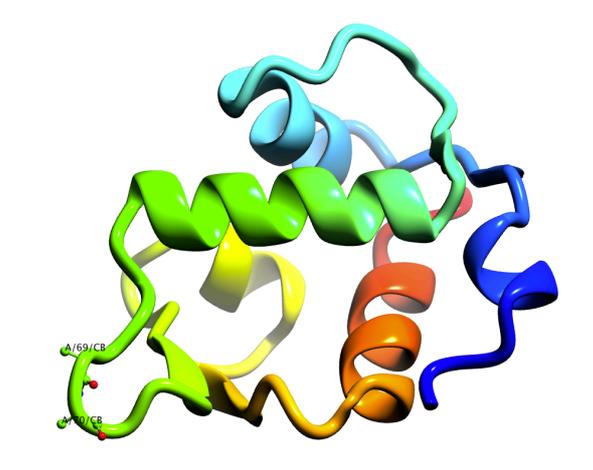


Figure S5. Crystallization screening hits for MDM2_E69AK70A complexed with p53₁₅₋₂₉ or Nutlin-3a. Hits identified by sparse matrix screening of MDM2_E69AK70A complexed with p53₁₅₋₂₉ (a-c) or Nutlin-3a (d-f). The corresponding conditions are a) 0.2 M ammonium sulfate, 30 % w/v PEG8000, b) 30 % w/v PEG4000, 0.2 M imidazole maleate pH 6, c) 36 % w/v polyethylene glycol monomethyl ether 5000, 0.1 M sodium acetate pH 5.5, d) 0.5 M ammonium sulfate, 0.1 M sodium citrate pH 5.6, 1.0 M lithium sulphate, e) 2.0 M ammonium sulfate, 100 mM cacodylate pH 6.5, 200 mM NaCl, final pH: 6.3, 2:1 protein:well-solution in crystallization droplet, and f) 2.0 M ammonium sulfate, 100 mM cacodylate pH 6.5, 200 mM NaCl, final pH: 6.3, 1:1 protein:well-solution in crystallization droplet.

MCNTNMSVPT	DGAVTTSQIP	ASEQETLVRP
KPLLLKLLKS	VGAQKDTYTM	KEVLFYLGQY
IMTKRLYDEK	QQHIVYCSND	LLGDLFGVPS
FSVKEHRKIY	TMIYRNLVVV	NQESSDSGT
SVSENRCHE	GGSDQKDLVQ	ELQEEKPSSS
HLVSRPSTSS	RRRAISETEE	NSDELSEGERQ
RKRHKSDSIS	LSFDESLALC	VIREICCERS
SSSESTGTPS	NPDLDAVSE	HSGDWLDQDS
VSDQFSVEFE	VESLSESDYS	LSEEGQELSD
EDDEVYQVTV	YQAGESDTS	FEEDPEISLA
DYWKCTSCNE	MNPPLPCHN	RCWALRENWL
PEDKGDKGE	ISEKAKLENS	TQAEEGFDVP
DCKKTIVNS	RESCVEEND	KITQASQSQE
SEDYSQPSTS	SSIIYSSQED	VKEFEREETQ
DKEESVSSL	PLNAIEPCVI	CQGRPKNGCI
VHGKTGHLMA	CFTCAKLLK	RNKPCPVCRQ
PIQMIVLTYFP		



A

B

Figure S5. Crystallizable fragment of MDM2, indicating location of SER mutations.

A) The sequence of human MDM2 (Uniprot reference Q00987) is presented, with the fragment 17-109 highlighted in red, and residues E69 and K70 highlighted in yellow. B) The locations of the mutated residues E69 and K70 are presented in the context of a ribbon rendering of the MDM2_E69AK70A/Nutlin-3a structure, ramped in colour from blue to red from residue 17 to residue 108.