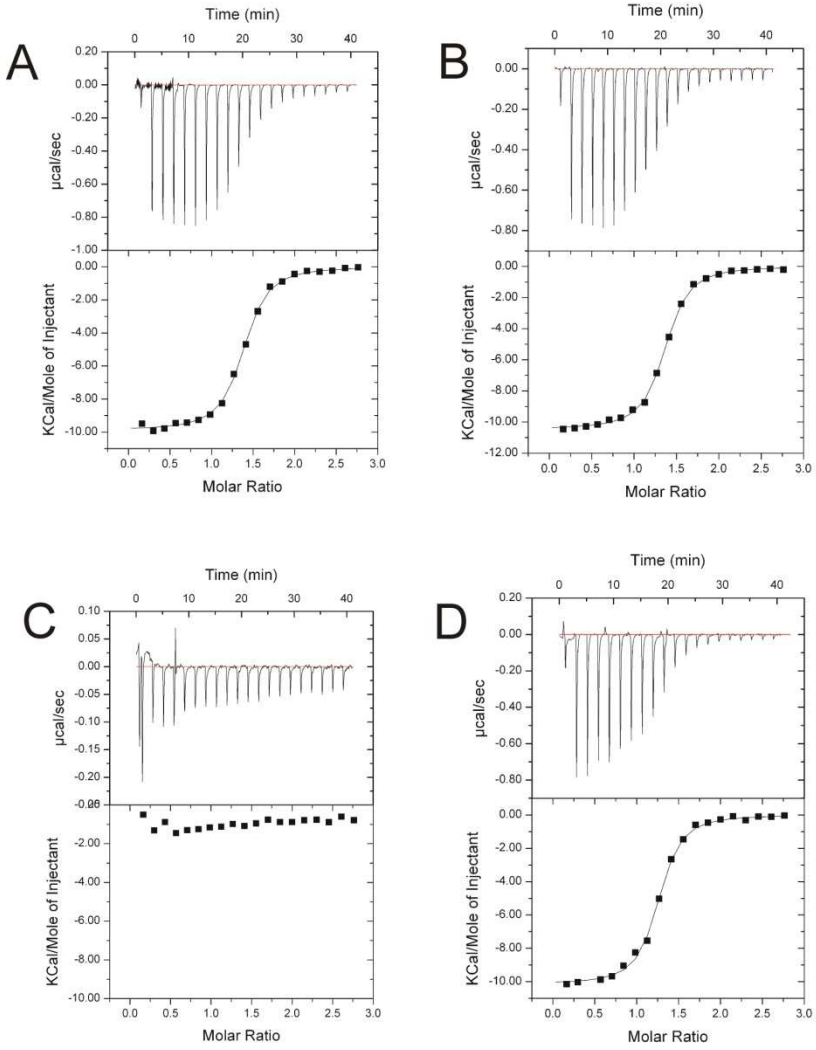
Supplementary Information

Allosteric modulation of chain motions within the transactivation domain of the tumor suppressor protein p53

Jenifer K. Lum, Hannes Neuweiler, and Alan R. Fersht

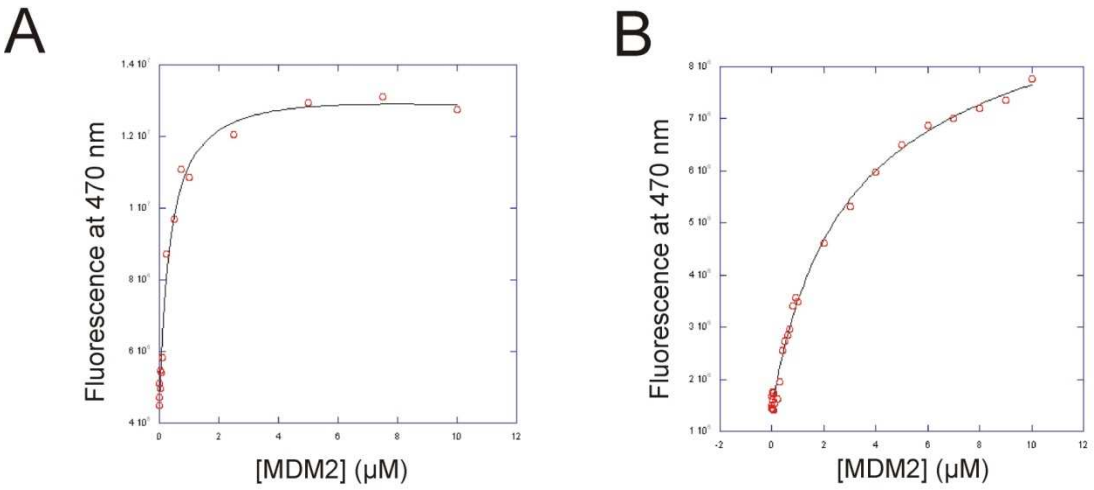
Supplementary Figures

Supplementary Figure 1



ITC binding data for MDM2(2-125) with wild type p53(1-93) and p53(1-93) mutants. A) Raw binding isotherm and fitted binding data for 400μM MDM2(2-125) titrated into 30μM wild type p53(1-93) at 35°C. B) Raw binding isotherm and fitted binding data for 400μM MDM2(2-125) titrated into 30μM phosphorylated p53(1-93) at 35°C. C) Raw binding isotherm and fitted binding data for 400μM MDM2(2-125) titrated into 30μM p53(1-93) W23F at 35°C. D) Raw binding isotherm and fitted binding data for 400μM MDM2 (2-125) titrated into 30μM p53 (1-93) W53F at 35°C.

Supplementary Figure 2



Fluorescence titration of MDM2(2-125) with p53(1-93) V31C (A) and p53(1-93) V31C W23F W91F (B). MDM2(2-125) was manually titrated into 25nM p53(1-93) V31C or 25nM p53(1-93) V31C W23F, W91F, both CPM labeled, in 50mM MES pH 6.8, 100mM NaCl, 1mM DTT, 15°C. Each data point was measured using a time-based acquisition, 1-second increment and 1-second integration time for a total of 20 seconds.

Supplementary Tables

Supplementary Table 1. Summary of ITC binding data for p53(1-93) and MDM2(2-125)

ITC	
p53 construct	K_d (μM)
p53 (1-93)	0.47±0.05
p53 (1-93) 3P	0.43±0.04
p53(1-93) W53F W91F	0.43±0.04
p53(1-93) W23F W91F	ND[1]
Fluorescence	
p53 construct	K_d (μM)
P53 (1-93) V31C	0.36±0.04
P53 (1-93) V31C W23F W91F	2.6±0.3

[1] Not determined (ND): K_d could not be determined from the data
Given errors are standard errors

Supplementary Methods

Isothermal titration calorimetry. ITC experiments were performed on a Microcal ITC200 calorimeter. Experiments were performed in 50mM MES pH 6.8, 100mM NaCl, 2.5mM DTT, 35^{o}C and the resulting data were fit with the one-site binding model within the Origin software.

Fluorescence titration. For fluorescence experiments, 25nM p53(1-93) V31C, both with and without W53F mutations were labeled with CPM and titrated manually with 0-10μM MDM2(2-125) containing 25nM of the relevant p53 construct over 17 (wild type) or 27 steps (W23F, W91F) in 50mM MES pH 6.8, 100mM NaCl, 1mM DTT, 15^{o}C. Fluorescence titrations were run on a Horiba Jobin Yvon fluorimeter. The excitation wavelength was 280nm and the emission wavelength was 470nm with slit widths of 5mm and 15mm respectively. The time based acquisition function was used to monitor emission, with a 1-second increment and 1-second integration over 20 seconds. The data were fit to the quadratic solution to the standard 1:1 binding model, as described previously (Ref. 22 in main text).