# Supporting Information

# Determinants of specificity of MDM2 for the activation domains of p53 and p65: Proline27 disrupts the MDM2-binding motif of p53

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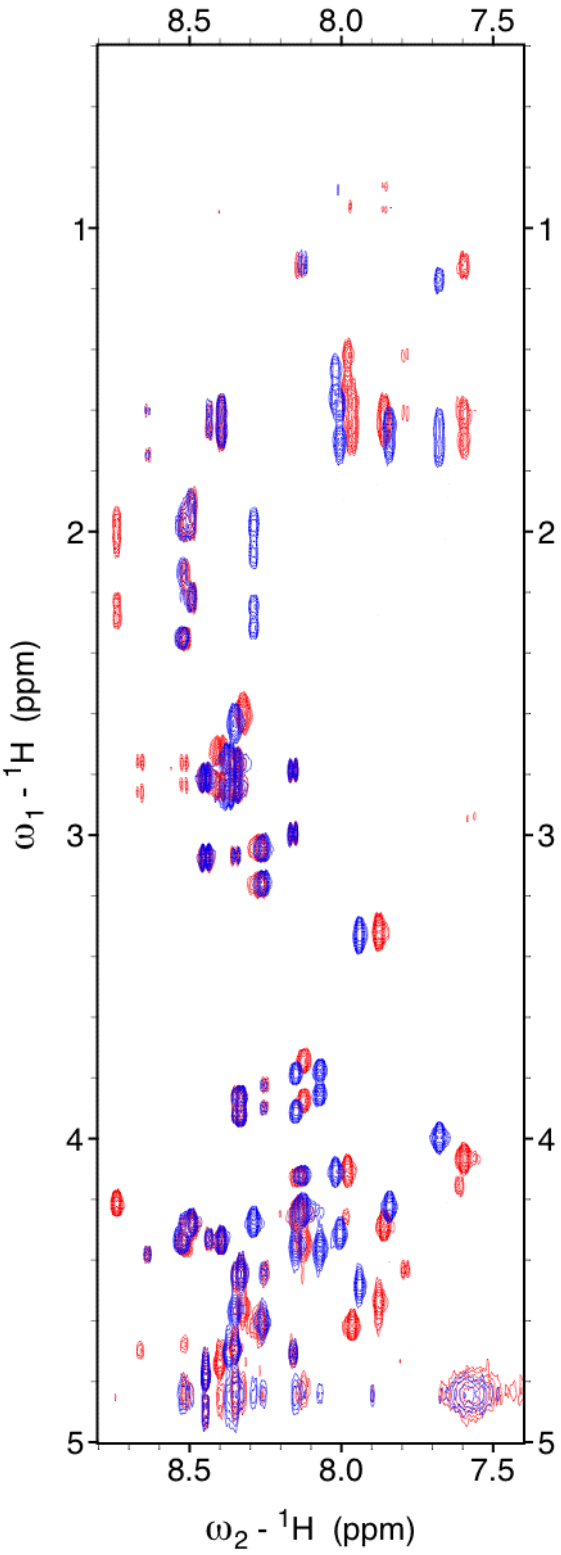


***Figure S1***. Comparative TOCSY spectra of the peptides 333 ($p53_{12\text{-}30}$) (red) and p53-P27S (blue). Experiments were conducted at 23 ˚C in water containing 5 mM phosphate (pH 6.0) and 25 mM NaCl. Peptides were analyzed as the cysteine-alkylated acetamides.

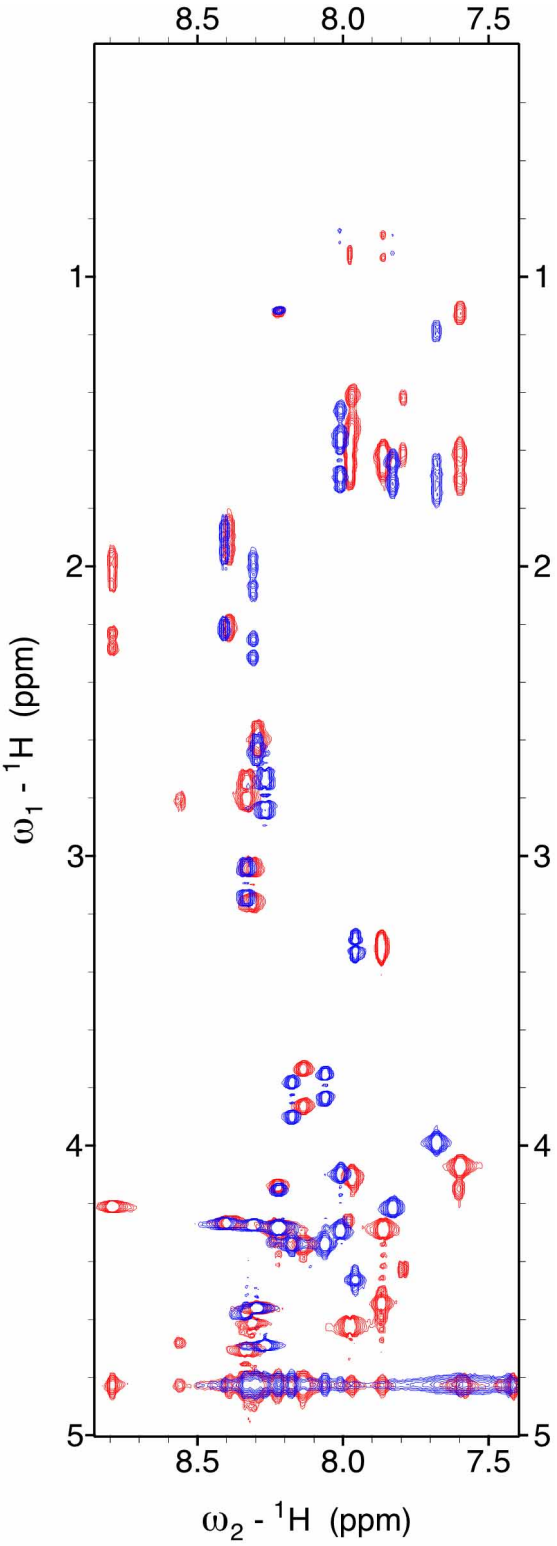


***Figure S2***. Comparative TOCSY spectra of the peptides $p53_{17-29}$ (red) and $p53_{17-29}$-P27S (blue). Experiments were conducted at 23 ˚C in water containing 20 mM phosphate (pH 6.0) and 10 mM NaCl.

| | δ, $H^N$ ppm | $^3J_{\alpha N}$ Hz | δ, $H_\alpha$ ppm | δ, $C_\alpha$ ppm | $H_\alpha$ CSI ppm | $C_\alpha$ CSI ppm |
|---|---|---|---|---|---|---|
| E17 | 8.39 | 6.4 | 4.26 | 56.9 | -0.16 | 0.7 |
| T18 | 8.22 | 7.9 | 4.28 | 61.8 | -0.15 | -0.2 |
| F19 | 8.31 | n.d. | 4.61 | 58.2 | -0.04 | 0.1 |
| S20 | 8.14 | 5.7 | 4.34 | 58.7 | -0.17 | 0.0 |
| D21 | 8.30 | 6.8 | 4.56 | 55.0 | -0.26 | 2.0 |
| L22 | 7.97 | n.d. | 4.11 | 56.6 | -0.27 | 1.1 |
| W23 | 7.87 | n.d. | 4.54 | 57.8 | -0.16 | 0.2 |
| K24 | 7.60 | n.d. | 4.07 | 56.9 | -0.29 | 0.2 |
| L25 | 7.86 | n.d. | 4.29 | 55.1 | -0.09 | -0.4 |
| L26 | 7.98 | n.d. | 4.63 | 53.1 | +0.25 | -2.4 |
| P27 | n.a. | n.a. | 4.38 | 63.7 | -0.07 | 0.0 |
| E28 | 8.79 | 5.5 | 4.21 | 57.3 | -0.21 | 1.1 |
| N29 | 8.33 | n.d. | 4.71 | 53.2 | -0.08 | -0.1 |

***Table S1***. NMR-derived data for $p53_{17\text{-}29}$. Data were collected at 296 K. n.a. = not applicable. n.d. = not determined due to spectral overlap. $H_\alpha$ and $C_\alpha$ CSI (chemical shift index) indicate the deviations observed from random coil chemical shifts for the indicated resonances.

| | δ, $H^N$ ppm | $^3J_{\alpha N}$ Hz | δ, $H_\alpha$ ppm | δ, $C_\alpha$ ppm | $H_\alpha$ CSI ppm | $C_\alpha$ CSI ppm |
|---|---|---|---|---|---|---|
| E17 | 8.41 | 6.4 | 4.27 | 56.9 | -0.15 | 0.7 |
| T18 | 8.22 | 7.9 | 4.28 | 61.8 | -0.15 | -0.2 |
| F19 | 8.33 | 6.4 | 4.58 | 58.5 | -0.07 | 0.4 |
| S20 | 8.17 | 5.7 | 4.34 | 59.1 | -0.17 | 0.4 |
| D21 | 8.30 | n.d. | 4.56 | 55.3 | -0.26 | 2.3 |
| L22 | 8.00 | 5.7 | 4.10 | 57.1 | -0.28 | 1.6 |
| W23 | 7.95 | 5.0 | 4.46 | 58.6 | -0.24 | 1.0 |
| K24 | 7.67 | 5.7 | 3.99 | 57.9 | -0.37 | 1.2 |
| L25 | 7.82 | 6.1 | 4.22 | 56.2 | -0.16 | 0.7 |
| L26 | 8.01 | 5.7 | 4.29 | 55.9 | -0.09 | 0.4 |
| S27 | 8.06 | 6.1 | 4.34 | 59.1 | -0.17 | 0.4 |
| E28 | 8.31 | n.d. | 4.28 | 57.2 | -0.14 | 1.0 |
| N29 | 8.27 | 7.5 | 4.69 | 53.4 | -0.10 | 0.1 |

***Table S2***. NMR-derived data for $p53_{17\text{-}29}$-P27S. Data were collected at 296 K. n.d. = not determined due to spectral overlap in the 1-D spectrum. $H_\alpha$ and $C_\alpha$ CSI (chemical shift index) indicate the deviations observed from random coil chemical shifts for the indicated resonances.

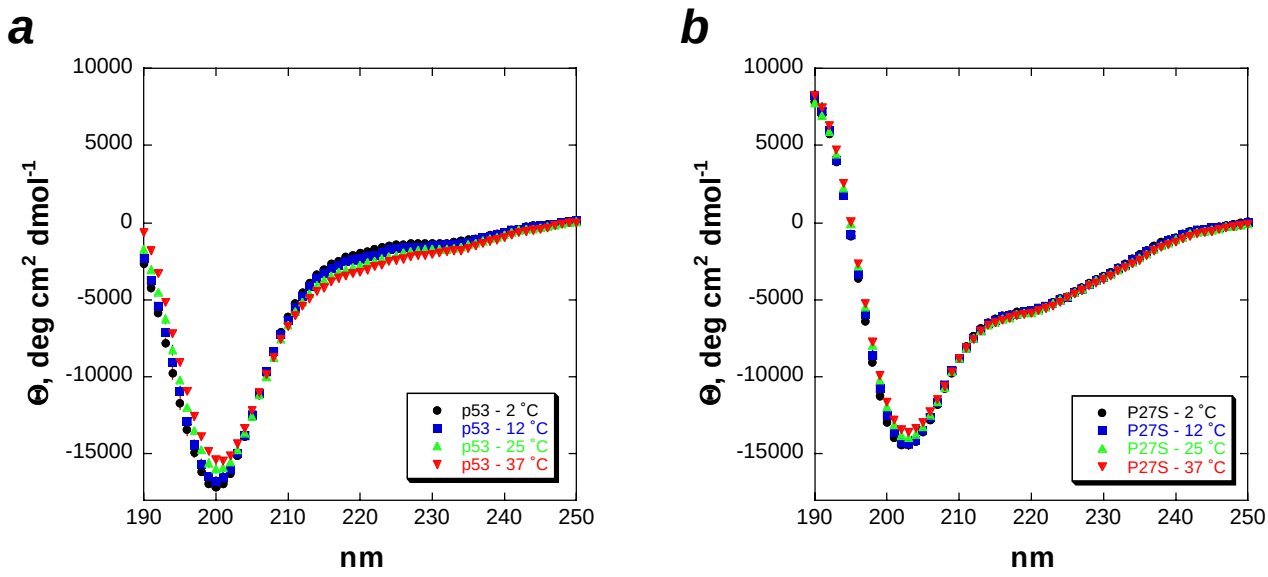


***Figure S3***. Temperature-dependent CD spectra of the peptides (a) $p53_{17-29}$ and (b) $p53_{17-29}$-P27S. Experiments were conducted in water containing 5 mM phosphate (pH 7.0) and 25 mM KF. Data were background-corrected but were not smoothed. Data are the average of three independent trials.

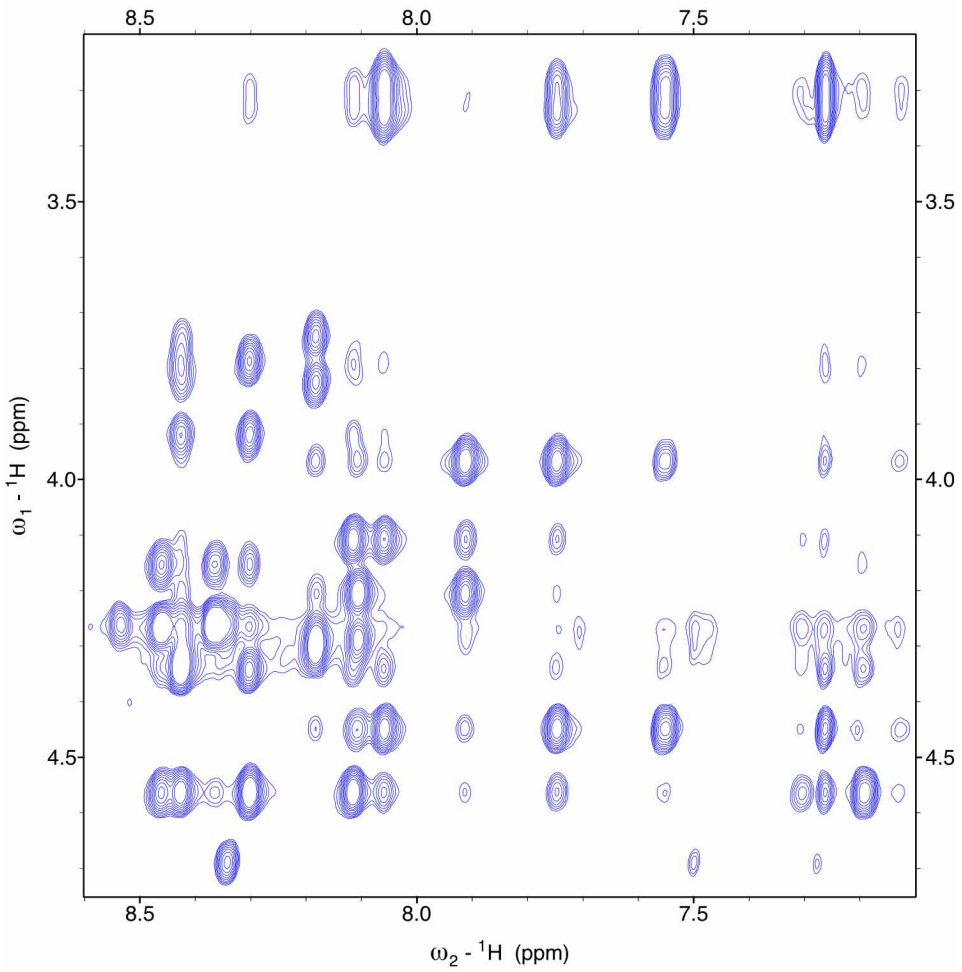


***Figure S4***. NOESY spectrum of $p53_{17\text{-}29}$-P27S. The experiment was conducted at 4 ˚C in water containing 20 mM phosphate (pH 6.0) and 10 mM NaCl.

| | δ, $H^N$ ppm | δ, $H_\alpha$ ppm | δ, others ppm |
|---|---|---|---|
| E17 | 8.53 | 4.26 | |
| T18 | 8.37 | 4.27 | 4.16 ($H_\beta$) |
| F19 | 8.46 | 4.57 | 3.15, 3.05 ($H_\beta$) |
| S20 | 8.30 | 4.35 | 3.92, 3.79 ($H_\beta$) |
| D21 | 8.43 | 4.56 | |
| L22 | 8.12 | 4.11 | |
| W23 | 8.06 | 4.45 | 3.34, 3.29 ($H_\beta$) |
| K24 | 7.74 | 3.97 | 2.96 ($H_\varepsilon$) |
| L25 | 7.91 | 4.21 | |
| L26 | 8.11 | 4.29 | |
| S27 | 8.18 | 4.31 | 3.83, 3.74 ($H_\beta$) |
| E28 | 8.43 | 4.26 | |
| N29 | 8.34 | 4.69 | |

***Table S3***. $H^N$, $H_\alpha$ and select $H_\beta$ assignments for $p53_{17\text{-}29}$-P27S at 4 ˚C.

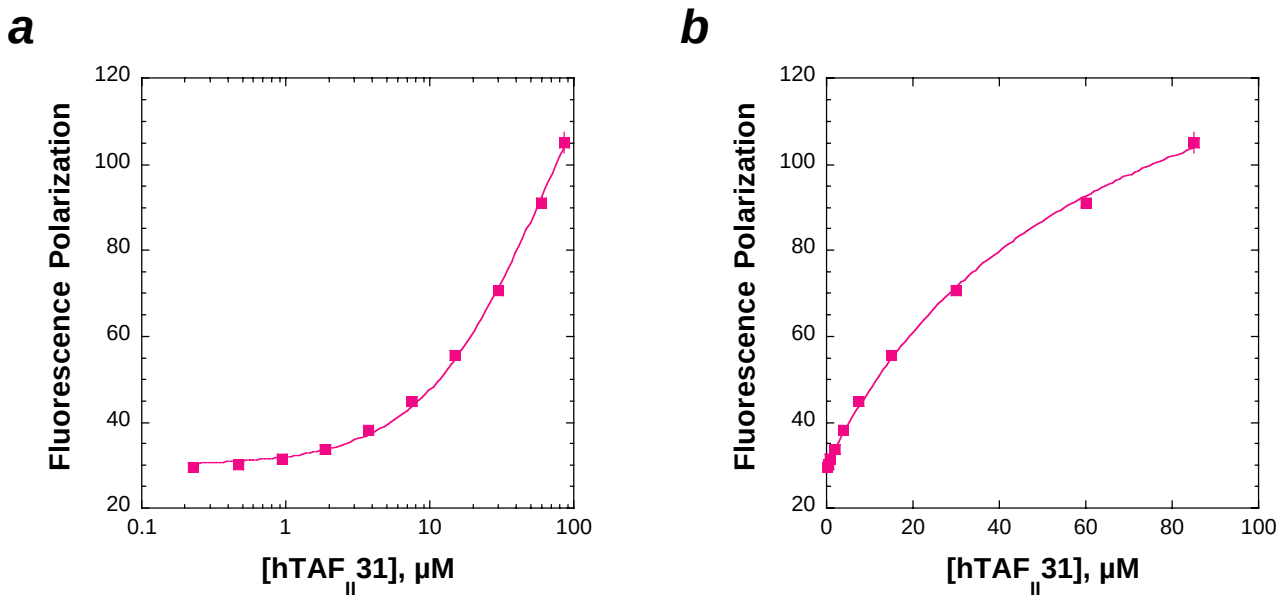


***Figure S5***. Fluorescence polarization data for $hTAF_{II}31_{1-140}$ binding to $p53_{17-29}$-P27S, shown on a (a) logarithmic and (b) linear scale. Polarization data are in millipolarization units. Data represent the average of 4 independent trials. Error bars are shown and indicate standard error.

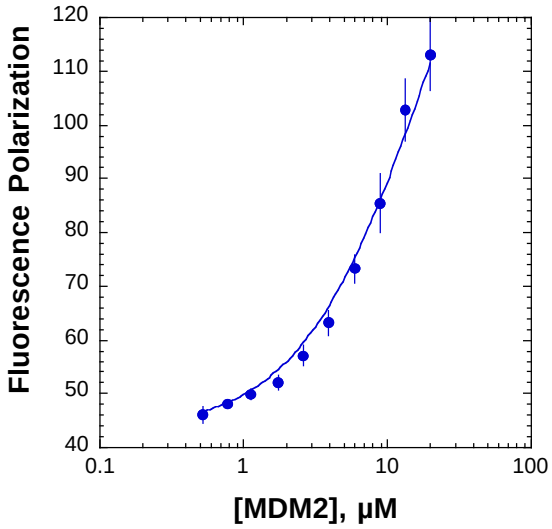


***Figure S6***. Competitive binding of $p65_{532-551}$ to MDM2 in the presence of 20 μM $hTAF_{II}31$. The experiment was conducted under standard solution conditions (Experimental section of the manuscript) with 100 nM fluorescein-labeled $p65_{532-551}$. Polarization data are in millipolarization units. Data represent the average of 3 independent trials. Error bars indicate standard error. Binding to MDM2 is indicated by an increase in the fluorescence polarization over the polarization observed (43 mP in this experiment) in the presence of 20 μM $hTAF_{II}31$ and in the absence of MDM2. The increase in polarization indicates binding to MDM2 and is observable due to a larger observed polarization of $p65_{532-551}$ when fully bound to MDM2 ($polarization_{max}$ = 173 mP; Figure 2d in manuscript) than when $p65_{532-551}$ is fully bound to $hTAF_{II}31$ ($polarization_{max}$ = 76 mP; Figure 7c in manuscript). Therefore, the observation of polarization values greater than 76 mP is only consistent with the presence of $p65_{532-551}$ bound to MDM2 in the solution. Binding analysis indicates a dissociation constant ($K_d$) of 18 μM for the $p65_{532-551}$•MDM2 complex in the presence of 20 μM $hTAF_{II}31$.