# Supporting Information

## Zhang and Chook 10.1073/pnas.1207247109

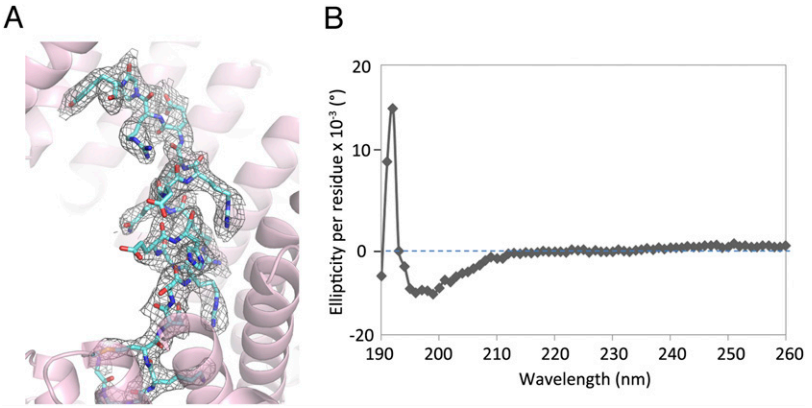


**Fig. S1.** Structure of the atypical PY-NLS of FUS. (*A*) $F_o - F_c$ electron density map for the FUS PY-NLS displayed with a 2.5σ cutoff. Kapβ2 is drawn as a pink cartoon and the PY-NLS is drawn as cyan sticks. (*B*) The circular dichroism spectrum of the FUS PY-NLS. FUS PY-NLS (20 μM) in buffer containing 20 mM Tris, pH 7.5, 100 mM NaCl, 10% (vol/vol) glycerol, and 2 mM β-mercaptoethanol was scanned at 25 °C from 190 nm to 260 nm with five repeats. The buffer background was subtracted to generate the final spectrum.

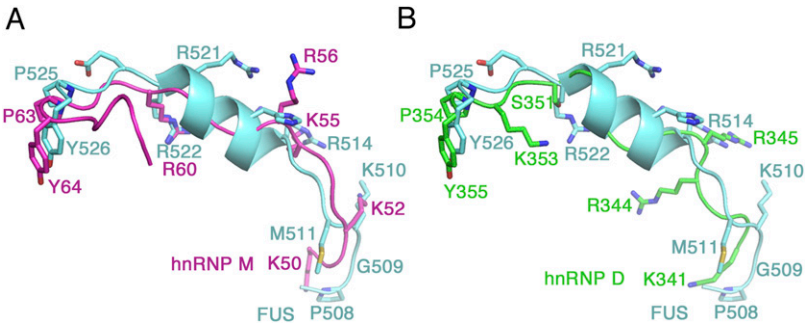


**Fig. S2.** Structure of the atypical PY-NLS of FUS. Kapβ2 residues 297–823 for Kapβ2-PY NLS complexes are superimposed. (*A*) Details of the superposition shown to compare PY-NLSs of FUS (cyan; PDB ID 4FDD) and hnRNP M (magenta; PDB ID 2OT8). (*B*) Details of the superposition to compare PY-NLSs of FUS and hnRNP D NLS (green; PDB ID 2Z5N).

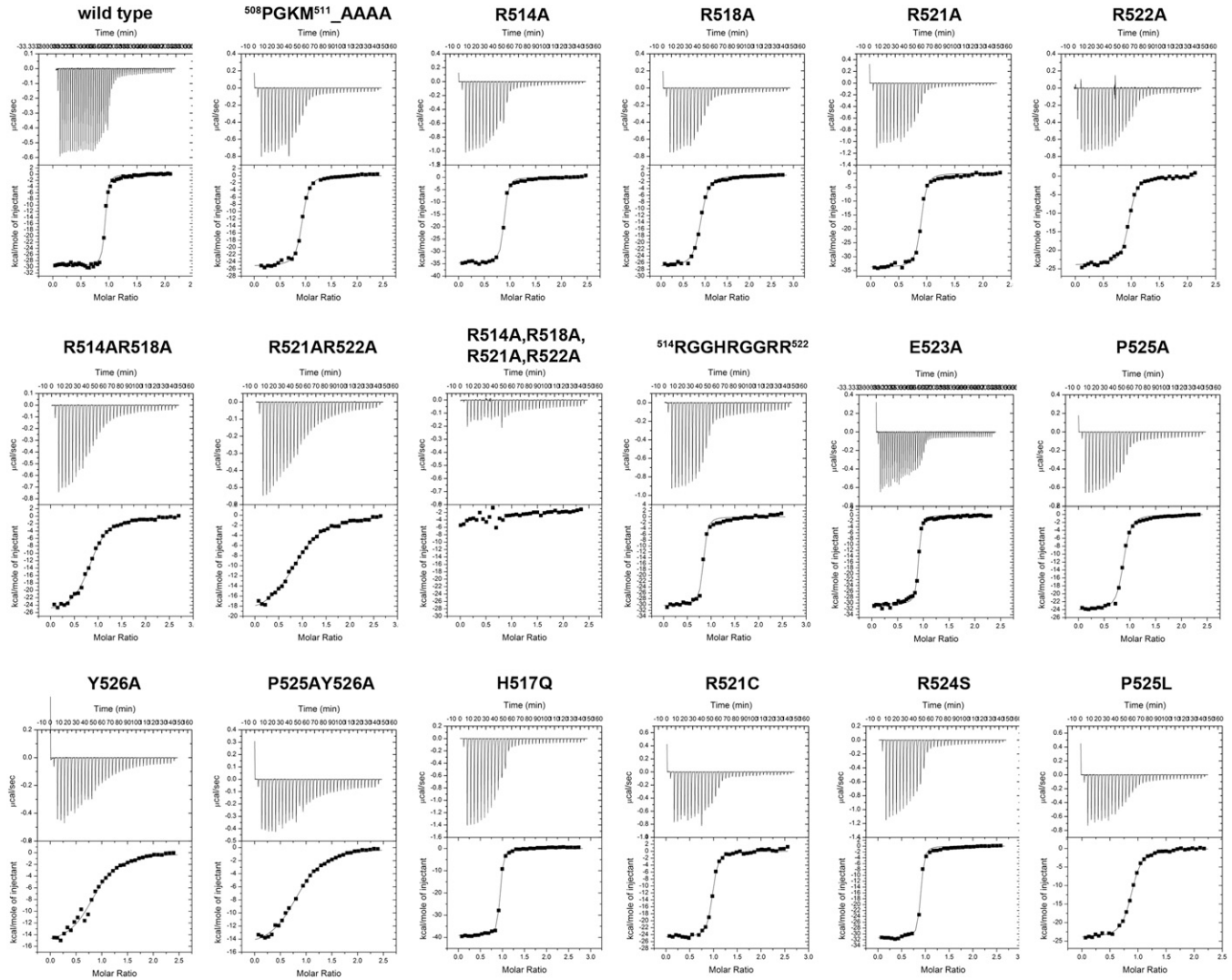


**Fig. S3.** Isothermal titration calorimetry measurements of wild-type and mutant FUS PY-NLSs. About 100 μM of wild-type and mutant MBP-FUS PY-NLS proteins was titrated into the sample cell of a MicroCal Omega VP-ITC calorimeter containing 10 μM full-length Kapβ2 at 20 °C. Data were plotted and analyzed using the single binding-site model of the MicroCal Origin software version 7.0. Experiments for wild-type and mutant FUS PY-NLSs were performed in triplicate except for experiments with the $^{514}$RGGHRGGRR$^{522}$ mutant, which were performed twice. Representative traces for the wild-type and mutant FUS PY-NLSs are shown.

**Table S1. Structure and crystallographic statistics of the Kapβ2-FUS PY-NLS complex**

| | Kapβ2-FUS-NLS |
|---|---|
| Space group | $P2_12_12$ |
| Cell dimensions | |
| *a*, *b*, *c*, Å | 128.720, 157.255, 67.543 |
| α, β, γ, ° | 90.00, 90.00, 90.00 |
| Data collection | |
| Resolution range, Å | 50–2.3 |
| Completeness, % | 97.6 (78.9)* |
| Redundancy | 5.8 (3.0) |
| $R_{sym}$ † | 0.074 (0.453) |
| $I/\sigma I$ | 22.6 (1.95) |
| Refinement | |
| Resolution range, Å | 50–2.3 |
| No. of reflections | 57,369 |
| $R_{work}$ ‡ | 0.20783 |
| $R_{free}$ § | 0.24465 |
| rms deviation | |
| Bond lengths, Å | 0.0075 |
| Bond angles, ° | 1.2227 |
| Average B-factors, Å$^2$ | 57.78 |
| Solvent content, % | 64.92 |
| Ramachandran plot | |
| Most favored regions, % | 93.1 |
| Allowed regions, % | 6.4 |
| General allowed regions, % | 0.5 |
| Disallowed regions, % | 0.0 |

*Values in parentheses are for highest-resolution shell.

†$R_{sym} = \Sigma|I_i - \langle I\rangle|/\Sigma|I_i|$, where $I_i$ is the intensity of the *i*th measurement, and $\langle I\rangle$ is the mean intensity for that reflection.

‡$R_{work} = \Sigma|F_o - F_c|/|F_o|$, where $F_c$ and $F_o$ are the calculated and observed structure factor amplitudes, respectively.

§$R_{free}$ calculated as for $R_{work}$ but for 5.0% of the total reflections chosen at random and omitted from refinement for all datasets.