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Supporting information for article:

A tetramerization domain in prokaryotic and eukaryotic transcription regulators homologous to p53

Nerea Bernardo, Isidro Crespo, Anna Cuppari, Wilfried J. J. Meijer and D. Roeland Boer



**Figure S1** Analytical size exclusion chromatography (SEC) of RCO at different stages of degradation. The chromatogram for fresh Rco is shown in blue, and the chromatogram of a 30 days old Rco is shown in red. The shaded gray area covers the range of molecular weights from 24 kDa (2.168 ml) to 10 kDa (3.0 ml, corresponding to the total column volume according to the manufacturer). The monomeric crystallized fragment has a molecular weight of 4.3 kDa, the tetramer therefore corresponds to a molecular weight of 12.2 kDa.



**Figure S2** Cartoon and stick representation of the core strand-loop-helix motif of the  $\text{RcoTet}_{pLS20}$ -like structures. The angle between the helix and strand is shown. CEP-1 and Dmp53 are proteins that do not have a glycine in the loop region, and are highlighted by a square box.



**Figure S3** AlphaFold2 model of the full-length Rco dimer. A) Cartoon representation of the tetrameric model of the full length Rco<sub>pLS20</sub> as calculated by AlphaFold2, colored by chain. B) Closeup of the TetDloop of the AlphaFold2 model (green), superposed on the X-ray structure presented herein (PDB code 8BNY, blue).

**Table S1**Summary of the results obtained by SEC for  $Rco_{pLS20}$  at different pHs. The table showsthe  $V_{el}$  of  $Rco_{pLS20}$  at pH5, pH8 and pH10, together with an estimation of MW and predictedoligomerization state. The theoretical MW of  $Rco_{pLS20}$  is 20.32 kDa.

| рН | V <sub>el</sub> (ml) | Estimated M <sub>W</sub> based on<br>V <sub>el</sub> (kDa) | Estimated oligomerization state | Inferred oligomerization state in solution |  |
|----|----------------------|--|---------------------------------|--|--|
| 5  | 1.97                 | 56.99  | 2.8                             | Dimer                                      |  |
| 8  | 1.80                 | 85.43  | 4.2                             | Tetramer                                   |  |
| 10 | 1.66                 | 151.57   | 7.5                             | Octamer                                    |  |

**Table S2**Hits retrieved by the eFold search using the RcoTet $p_{LS20}$  structure as subject andapplying the standard cutoff of 70 % sse.

| ## | RMSD      | Nalign | Ν   | %seq  |      | Query |       | Protein | Organism   |
|----|-----------|--------|-----|-------|------|-------|-------|---------|------------|
|    |           |        |     |       | %sse | PDB   | Nnes  |         |            |
| 1  | 1.09-2.88 | 22-29  | 1   | 8-23  | 100  | 2wqj  | 26-32 | P73Tet  | H. sapiens |
| 2  | 1.32-2.67 | 23-28  | 1   | 4-15  | 100  | 3zy0  | 26-29 | P63Tet  | H. sapiens |
| 3  | 1.38-2.92 | 22-26  | 1   | 12-14 | 100  | 4cz7  | 30-31 | P53Tet  | D. rerio   |
| 4  | 1.54-1.70 | 29-30  | 1-2 | 13-14 | 100  | 1saf  | 42    | P53Tet  | H. sapiens |
| 5  | 1.61-2.22 | 22-24  | 1   | 14-17 | 100  | 4cz6  | 26-33 | P53Tet  | D. rerio   |
| 6  | 1.63-1.90 | 29-30  | 1   | 17    | 100  | 2j10  | 31    | P53Tet  | H. sapiens |
| 7  | 1.64-1.90 | 29-30  | 1   | 13-14 | 100  | 2j0z  | 31    | P53Tet  | H. sapiens |
| 8  | 1.69      | 29     | 1   | 14    | 100  | 1sal  | 42    | P53Tet  | H. sapiens |
| 9  | 1.69-1.85 | 29     | 1   | 14    | 100  | 2j11  | 31    | P53Tet  | H. sapiens |
| 10 | 1.69      | 29     | 1   | 14    | 100  | 1 sak | 42    | P53Tet  | H. sapiens |
| 11 | 1.70-1.71 | 29     | 1   | 14    | 100  | 1sae  | 42    | P53Tet  | H. sapiens |
| 12 | 1.72      | 29     | 1   | 14    | 100  | 3sak  | 42    | P53Tet  | H. sapiens |
| 13 | 1.73-1.8  | 29-30  | 1   | 13-14 | 100  | 1 olh | 42    | P53Tet  | H. sapiens |
| 14 | 1.82-2.70 | 19-29  | 1   | 14-21 | 100  | 10lg  | 42    | P53Tet  | H. sapiens |
| 15 | 1.9       | 23     | 1   | 17    | 100  | 2wtt  | 29    | P73Tet  | H. sapiens |
| 16 | 2.18-2.95 | 24-26  | 1   | 12-13 | 100  | 4cz5  | 28-32 | P53Tet  | D. rerio   |
| 17 | 2.25      | 29     | 1   | 14    | 100  | 1hs5  | 34    | P53Tet  | H. sapiens |
| 18 | 2.85      | 27     | 1   | 4     | 100  | lalu  | 29    | P53Dim  | H. sapiens |