

**Supplementary Table 1.** List of known disordered protein regions and disordered protein binding sites therein used in this study.

| UniProtKB | Disprot | PDB chain    | known disordered regions     | disordered protein binding sites | PubMed identifier  |
|-----------|---------|--------------|------------------------------|----------------------------------|--------------------|
| O43236-6  | NA      | NA           | 248-274                      | 267-274                          | 21949740           |
| P01094    | DP00179 | 1dpjB        | 1-68                         | 2-34                             | 10655612           |
| P01730    | DP00123 | 3b71D        | 421-458                      | 431-441                          | 9622505; 18078954  |
| P02686-5  | DP00236 | 1ymmC        | 1-171                        | 84-108                           | 7500383; 15821740  |
| P02686-6  | DP00237 | NA           | 1-171                        | 146-166                          | 22405011           |
| P04637    | DP00086 | 1dt7X        | 367-388                      | 367-388                          | 10876243           |
| P04638    | DP00087 | 1ycqB        | 1-73                         | 17-27                            | 8875929            |
| P04639    | DP00088 | 2b3gB        | 1-73                         | 33-56                            | 16234232           |
| P05221    | DP00217 | 1ee5B        | 120-200                      | 153-171                          | 10745017           |
| P07766    | DP00506 | 1a81B        | 153-207                      | 186-203                          | 14967045; 9698567  |
| P09883    | DP00342 | 3o0eL; 2ivzE | 1-83                         | 2-16; 32-47                      | 21098297; 16894158 |
| P45976    | DP00625 | 3c66C        | 1-220                        | 81-85; 92-97                     | 18537269           |
| P46108    | DP00748 | 1ju5A        | 1-9; 65-85; 121-133; 192-304 | 65-85                            | 12384576           |
| P46937    | DP00702 | 3kisB        | 61-100                       | 86-100                           | 20123905           |
| P60896    | DP00617 | 1miuB        | 1-70                         | 7-25; 37-63                      | 12228710           |
| P9WHN5    | DP00293 | 3m91B        | 1-64                         | 21-51                            | 19580545; 20953180 |
| Q06253    | DP00288 | 3dd7B        | 1-73                         | 52-73                            | 9915794; 18757857  |
| Q13573    | DP00608 | NA           | 1-172                        | 59-79                            | 20007319           |
| Q6BBK3    | NA      | 3b1jC        | 1-75                         | 51-75                            | 22153507           |
| Q98XH7    | NA      | 3o61C        | 1-72                         | 2-16                             | 16423825; 21035463 |
| Q99LM3    | DP00742 | 1jv9A        | 1-346; 449-459               | 449-459                          | 18477568           |
| Q9Y3M2    | DP00709 | NA           | 1-63                         | 16-22; 21-29                     | 21182262; 19940019 |
| Q9Y6Q9-3  | DP00343 | 1kbhA        | 1023-1093                    | 1045-1091                        | 11823864           |

Rows shaded in light blue correspond to proteins used for comparison with other tools.

**Supplementary Table 2.** Comparison of DISOPRED3 and DISOPRED2 performance divided by IDR length ranges in the CASP9 dataset.

| Subste                     | Method    | Sensitivity | Specificity | Precision | MCC   | AUC   |
|----------------------------|-----------|-------------|-------------|-----------|-------|-------|
| No IDR shorter than 4 aas  | DISOPRED2 | 0.343       | 0.952       | 0.422     | 0.324 | 0.733 |
|                            | DISOPRED3 | 0.347       | 0.992       | 0.823     | 0.508 | 0.854 |
| No IDR shorter than 20 aas | DISOPRED2 | 0.264       | 0.952       | 0.192     | 0.186 | 0.681 |
|                            | DISOPRED3 | 0.432       | 0.992       | 0.711     | 0.540 | 0.871 |
| No IDR shorter than 30 aas | DISOPRED2 | 0.170       | 0.952       | 0.070     | 0.079 | 0.622 |
|                            | DISOPRED3 | 0.436       | 0.992       | 0.548     | 0.479 | 0.831 |
| No IDR shorter than 40 aas | DISOPRED2 | 0.147       | 0.952       | 0.027     | 0.043 | 0.539 |
|                            | DISOPRED3 | 0.265       | 0.992       | 0.237     | 0.244 | 0.736 |

**Supplementary Table 3.** Performance comparison between DISOPRED releases by IDR position along CASP target sequences.

| Subset                   | Method    | Sensitivity | Specificity | Precision | MCC   | AUC   |
|--------------------------|-----------|-------------|-------------|-----------|-------|-------|
| Terminal protein regions | DISOPRED2 | 0.604       | 0.762       | 0.615     | 0.367 | 0.749 |
|                          | DISOPRED3 | 0.646       | 0.914       | 0.825     | 0.594 | 0.868 |
| Internal protein regions | DISOPRED2 | 0.199       | 0.964       | 0.278     | 0.190 | 0.688 |
|                          | DISOPRED3 | 0.181       | 0.997       | 0.820     | 0.369 | 0.807 |

Terminal protein regions consist of the 10 amino acids closest to the target sequence termini; internal positions are all the remaining ones.

**Supplementary Table 4.** DISOPRED3 IDR prediction accuracy on the test protein chains used for comparison with tools predicting disordered regions folding upon protein binding..

| UniProtKB | TP  | FP  | FN  | TN  | Sensitivity | Specificity | Precision | F1    | MCC    |
|-----------|-----|-----|-----|-----|-------------|-------------|-----------|-------|--------|
| O43236-6  | 14  | 84  | 13  | 163 | 0.519       | 0.660       | 0.143     | 0.224 | 0.111  |
| P45976    | 159 | 92  | 61  | 15  | 0.723       | 0.140       | 0.633     | 0.675 | -0.152 |
| P46108    | 13  | 0   | 143 | 148 | 0.083       | 1.000       | 1.000     | 0.154 | 0.206  |
| P46937    | 39  | 150 | 1   | 314 | 0.975       | 0.677       | 0.206     | 0.341 | 0.364  |
| P60896    | 6   | 0   | 64  | 0   | 0.086       | NA          | 1.000     | 0.158 | NA     |
| Q06253    | 1   | 0   | 72  | 0   | 0.014       | NA          | 1.000     | 0.027 | NA     |
| Q98XH7    | 26  | 0   | 46  | 0   | 0.361       | NA          | 1.000     | 0.531 | NA     |
| Q99LM3    | 230 | 0   | 127 | 102 | 0.644       | 1.000       | 1.000     | 0.784 | 0.536  |
| Q9Y3M2    | 21  | 6   | 42  | 57  | 0.333       | 0.905       | 0.778     | 0.467 | 0.290  |

**Supplementary Table 5.** Number and percentage of false positive assignments of disordered protein binding residues that different classifiers make as a function of their sequence separation from the validated sites.

| <b>Method</b> | <b>Maximum sequence separation</b> | <b>Number of false positives</b> | <b>Percentage of false positive</b> |
|---------------|------------------------------------|----------------------------------|-------------------------------------|
| ANCHOR        | 5                                  | 29                               | 3.06                                |
|               | 10                                 | 46                               | 4.85                                |
|               | 20                                 | 87                               | 9.17                                |
|               | 35                                 | 139                              | 14.65                               |
| DISOPRED3     | 5                                  | 14                               | 16.28                               |
|               | 10                                 | 21                               | 24.42                               |
|               | 20                                 | 24                               | 27.91                               |
|               | 35                                 | 25                               | 29.07                               |
| MoRFpred      | 5                                  | 3                                | 1.88                                |
|               | 10                                 | 7                                | 4.38                                |
|               | 20                                 | 16                               | 10.00                               |
|               | 35                                 | 16                               | 10.00                               |
| MSFPSSMpred   | 5                                  | 10                               | 5.62                                |
|               | 10                                 | 10                               | 5.62                                |
|               | 20                                 | 22                               | 12.36                               |
|               | 35                                 | 32                               | 17.98                               |