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**Supporting information for article:**

# Structure of the SPRY domain of human DDX1 helicase, a putative interaction platform within a DEAD-box protein

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**Table S1** Different protein constructs used for structure determination.

Construct length is given with residue numbers referring to DDX1.

| construct | length | solubility/ crystals | space group | unit cell parameters (Å,°) | Resolution (Å) |
|---|---|---|---|---|---|
| SPRY_72-283+Tag | 72-283 | +/+ | $P2_12_12_1$ | $a$ = 43.73, $b$ = 75.64, $c$ = 122.73, α = β = γ = 90 | 4.0 |
| SPRY_72-283 | 72-283 | +/+ | $P2_12_12_1$ | $a$ = 45.06, $b$ = 76.14, $c$ = 122.66, α = β = γ = 90 | 2.0 |
| SPRY_84-283+Tag | 84-283 | +/- | | | |
| SPRY_100-283+Tag | 100-283 | - | | | |
| SPRY_72-261+Tag | 72-261 | +/- | | | |
| SPRY_84-261+Tag | 84-261 | +/+ | $P1$ | $a$ = 35.47, $b$ = 99.28, $c$ = 136.36, α = 102.56, β = 97.62 γ = 98.33 | 2.7 |
| SPRY_84-261+Tag (SeMet) | 84-261 | +/+ | $P2_1$ | $a$ = 37.14, $b$ = 133.80, $c$ = 35.96, α = γ = 90, β = 118.26 | 3.4 |
| SPRY_100-261+Tag | 100-261 | - | | | |

**Table S2** Primers used for cloning of the DDX1 SPRY domain and for site-directed-mutagenesis to truncate the N- and C-terminus (mutations are underlined, introduced nucleotides are italic)

| name | Sequence [5' -> 3'] | application |
|---|---|---|
| **DDX1_NheI_fwd** | GGGAAGGGCTAGCATGGCGGCCTTCTCCG<br>-> region of DDX1 | Amplify the DDX1 ORF, primer contains NheI site for cloning into pET28a |
| **DDX1_NotI_rev** | GGGATCGCGGCCGCTCAGAAGGTTCTGAACAGCTG<br>-> region of DDX1 | Amplify the DDX1 ORF, primer contains NotI site for cloning into pET28a |
| **SPRY_ BamH_fwd** | GTTTATGAAACTCTGAAAG*GATCC*CAGGAAGGCAAAAAAG | Generate BamHI site after Lys69 to clone SPRY-coding region of DDX1 |
| **SPRY_ BamH_rev** | CTTTTTTGCCTTCCTG*GGATC*CTTTCAGAGTTTCATAAAC | see above |
| **SPRY_ XhoI_fwd** | CAAACAAAGTTTCTC*GA*GAATGCTCCGAAAGCTC | Generate XhoI site downstream of the SPRY domain to clone it as BamHI-XhoI fragment |
| **SPRY_ XhoI_rev** | GAGCTTTCGGAGCATT*CAG*GAGAAACTTTGTTTG | see above |
| | | |
| **SPRY_Gly84_BamHI_fwd** | GAAAAACAACAATTAAA*GGATCC*GCTTCAGTGCTG | Generate BamHI site after Gly84 to truncate N-terminus of SPRY domain |
| **SPRY_Gly84_BamHI_rev** | CAGCACTGAAGC*GGATCC*TTTAATTGTTGTTTTTC | see above |
| **SPRY_Ser100_BamHI_fwd** | CATATGACAGA*GGATCC*GCTTTTGCAATTGGG | Generate BamHI site after Ser100 to truncate N-term of SPRY domain |
| **SPRY_Ser100_BamHI-rev** | CCCAATTGCAAAAGC*GGATCC*TCTGTCATATG | see above |
| **SPRY_Ala261_Stop_fwd** | CTCTTTCCAAGGCA*TGATAA*GGTTACATTG | Generate double stop-codon after Ala261 to truncate C-term of SPRY domain |
| **SPRY_Ala261_Stop_rev** | CAATGTAACC*TTATC*ATGCCTTGGAAAGAG | see above |

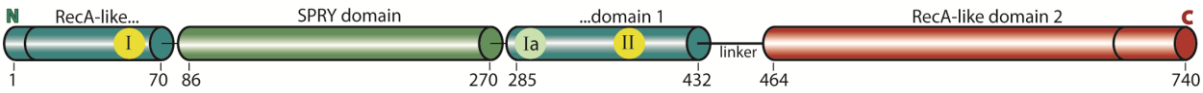



**Figure S1** Domain organization of human DDX1 is shown schematically. Position of conserved helicase motifs I, Ia and II is indicated.

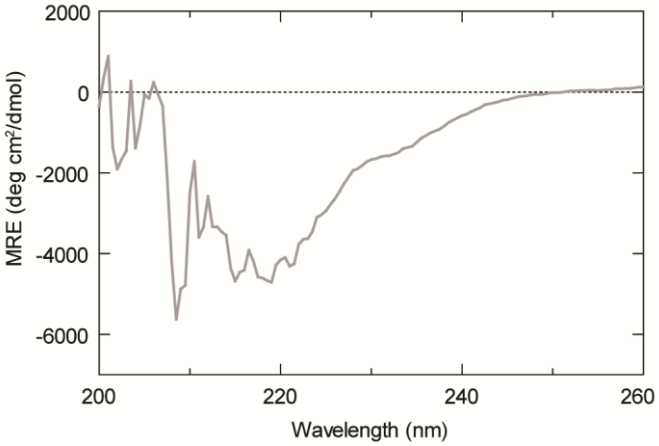



**Figure S2** CD spectrum of construct SPRY_72-283 at 130 µg/ml (5 µM) protein concentration in CD buffer (50 mM $K_2HPO_4/KH_2PO_4$ pH 8.0, 250 mM KF, 3 mM DTE) is shown. Please note that UV signals is noisy in the far-UV (below 215 nm) regime. Data traces represent the average of five spectra and the CD signal was converted to mean-residue-ellipticity (MRE).

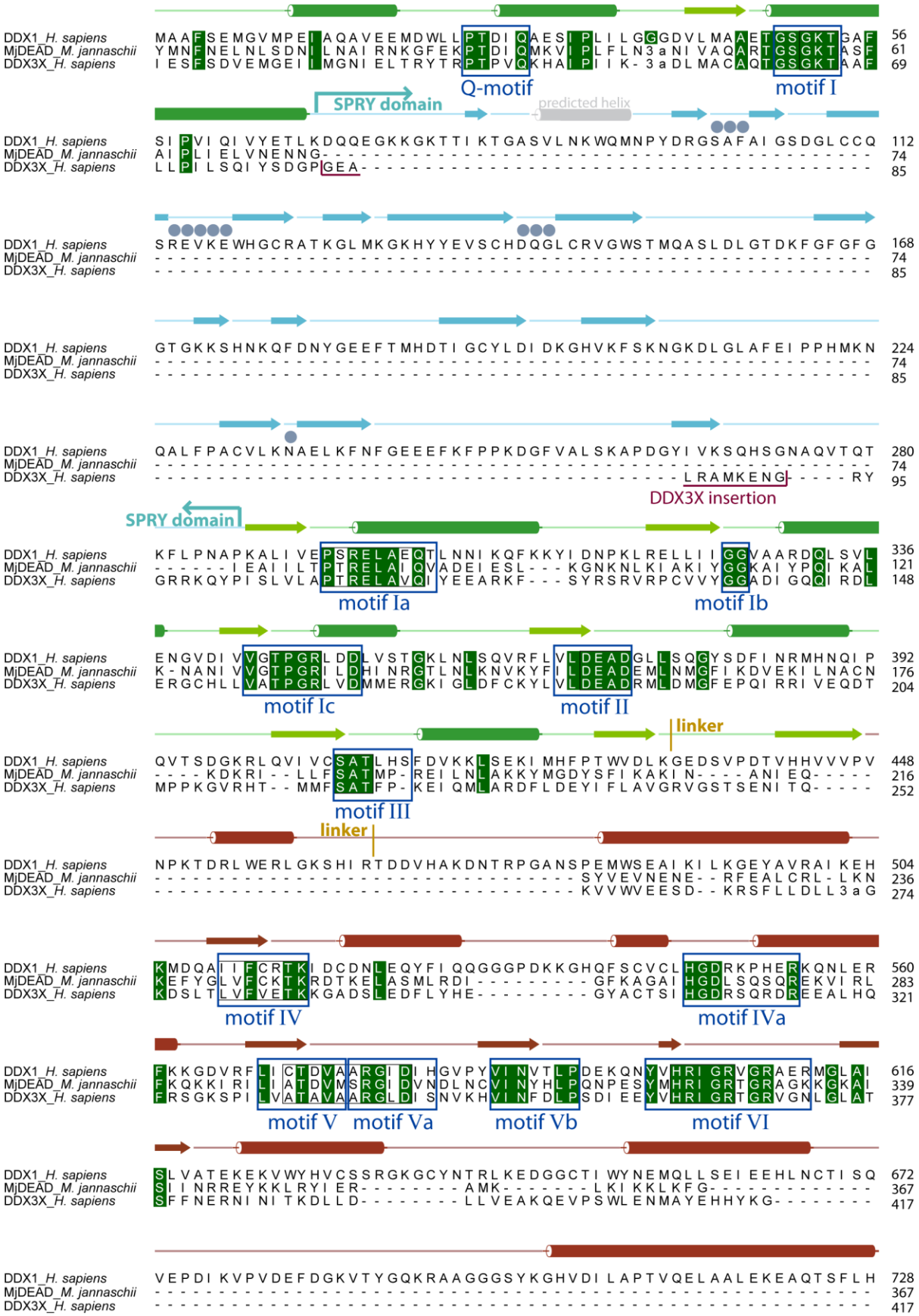
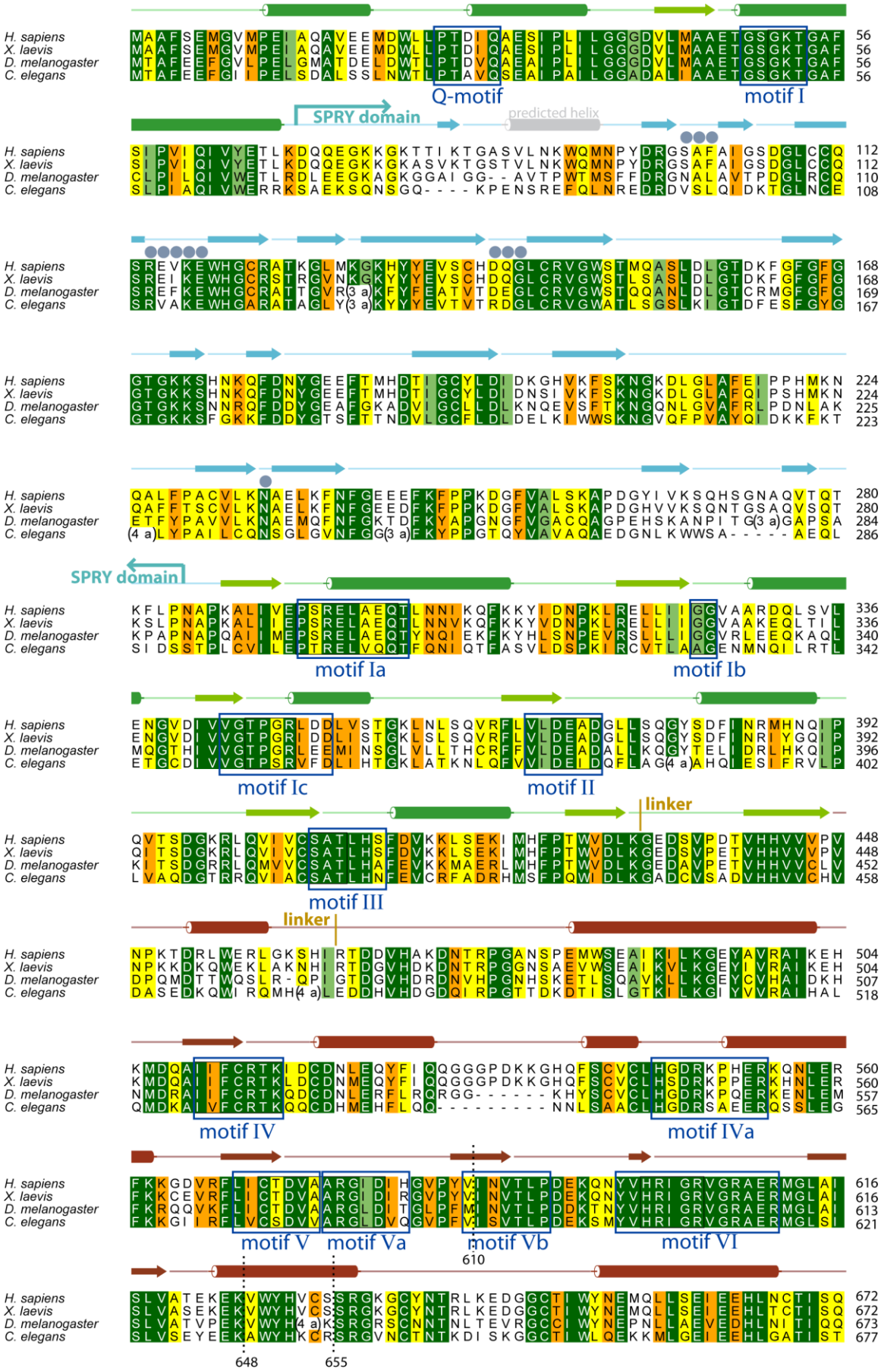



**Figure S3** Amino acid sequence alignment of DDX1 and homologous DEAD-box proteins MjDEAD from *Methanococcus jannaschii* (Story *et al.*, 2001) and DDX3X from *Homo sapiens*

(Hogbom *et al.*, 2007). Identical residues are highlighted in green. Secondary structure elements (arrows indicating β-strands, tons indicating α-helices) as predicted for human DDX1 by PSIPRED (Buchan *et al.*, 2010) are shown above the sequence. Colouring is performed according to domain allocation; RecA-like domain 1 is depicted in green; the SPRY domain is depicted in blue; the RecA-like domain 2 is depicted in red. A DDX3X specific insertion, which forms an elongated positively charged cavity(Hogbom *et al.*, 2007), is indicated in dark red.


H. sapiens
X. laevis
D. melanogaster
C. elegans
Q-motif
motif I
SPRY domain
predicted helix
motif Ia
motif Ib
motif Ic
motif II
linker
motif III
motif IV
motif IVa
motif V
motif Va
motif Vb
motif VI
610
648
655

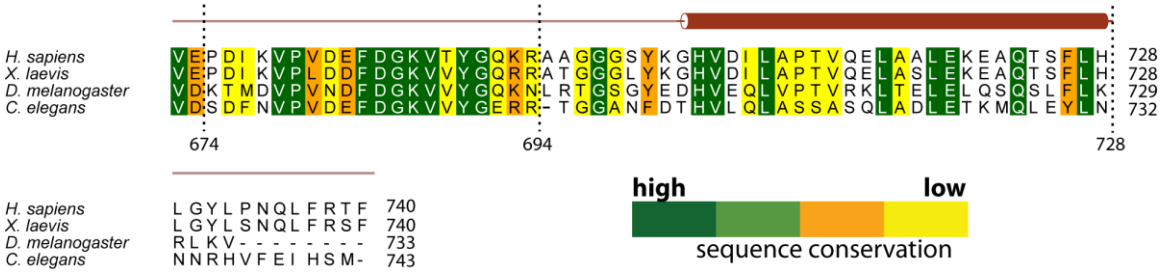



**Figure S4** Amino acid sequence alignment of DDX1 orthologs from representative eukaryotic model organisms. Conservation values were determined using the AMAS server (Livingstone & Barton, 1993) and are indicated by color coding (dark green for identical residues to yellow for less homologous residues). Secondary structure elements (arrows indicating β-strands, tubes indicating α-helices) as predicted for human DDX1 by PSIPRED (Buchan *et al.*, 2010) are shown above the sequence. Colouring is performed according to domain allocation; RecA-like domain 1 is depicted in green; the SPRY domain is depicted in blue; the RecA-like domain 2 is depicted in red.

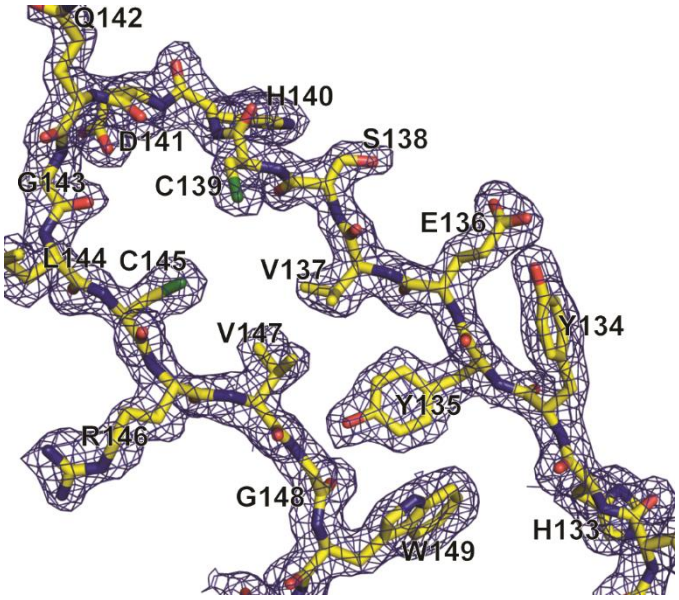



**Figure S5** A fully-refined $2F_o$-$F_c$ electron density map contoured at 1σ sigma covering the strands β6 and β7.

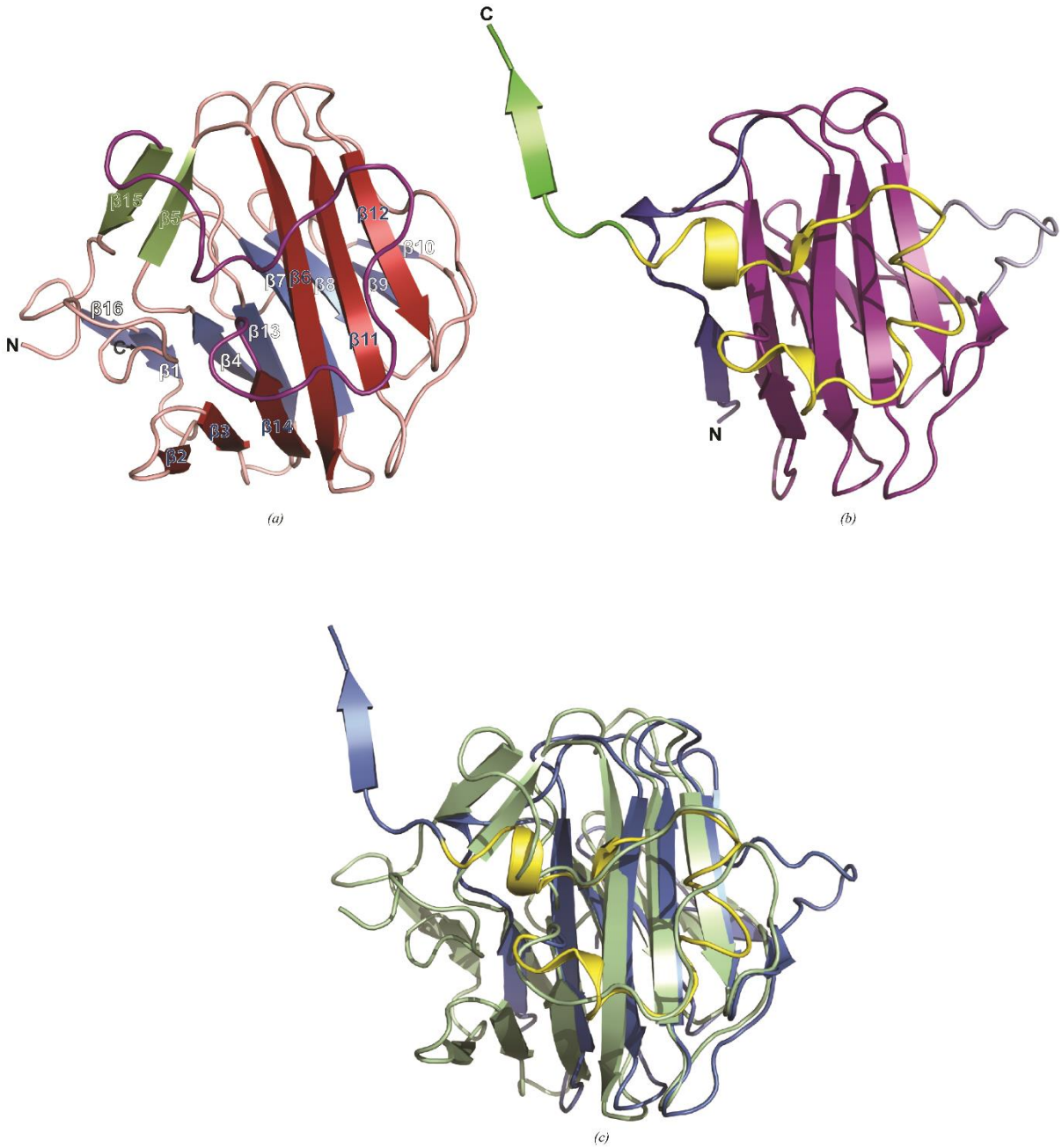



**Figure S6** A potential lid loop similar as described for murine RyR2 SPRY2 (Lau & Van Petegem, 2014) that covers the hydrophobic surface at a convex side of β-sheet 2 of the SPRY domain of DDX1. (*a*) DDX1 colored similar as in **Fig. 2** but rotated horizontally by 180°. The lid loop is highlighted in purple. (*b*) Structure of murine RyR2 SPRY2 (PDB entry 4p9i). The N-terminal extension in depicted in blue, the SPRY domain core in purple, the insertion loop in light blue, the lid in yellow; and the C-terminal tail in green; the domain assignment is adopted from (Lau & Van Petegem, 2014). (*c*) A DALI superposition (Holm & Rosenstrom, 2010) of hDSPRY (green) with the structure of murine RyR2 SPRY2 (blue). The lid loop of murine RyR2 SPRY2 is highlighted in yellow.

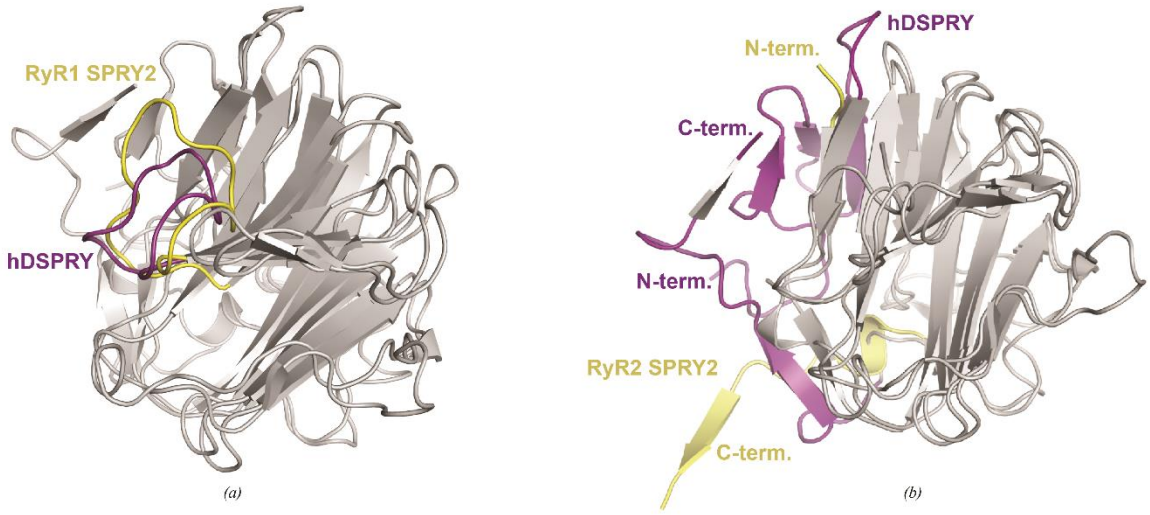



**Figure S7** A DALI superposition (Holm & Rosenstrom, 2010) of hDSPRY with rabbit and mouse RyR SPRY2 domains (Lau & Van Petegem, 2014). (*a*) Superposition of hDSPRY with rabbit RyR1 SPRY2 (PDB entry 4p9j); Loop D is highlighted in purple in case of hDSPRY or RyR1 SPRY2 in yellow, respectively. The common SPRY core of both domains is shown in gray. (*b*) Superposition of hDSPRY with mouse RyR2 SPRY2 (PDB entry 4p9i). Differences in the length and orientation of the N- and C-terminal regions are highlighted purple in case of hDSPRY or RyR2 SPRY2 in yellow, respectively. The common SPRY core of both domains is shown in gray.

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Holm, L. & Rosenstrom, P. (2010). *Nucleic Acids Res* **38**, W545-549.
Lau, K. & Van Petegem, F. (2014). *Nat Commun* **5**, 5397.
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