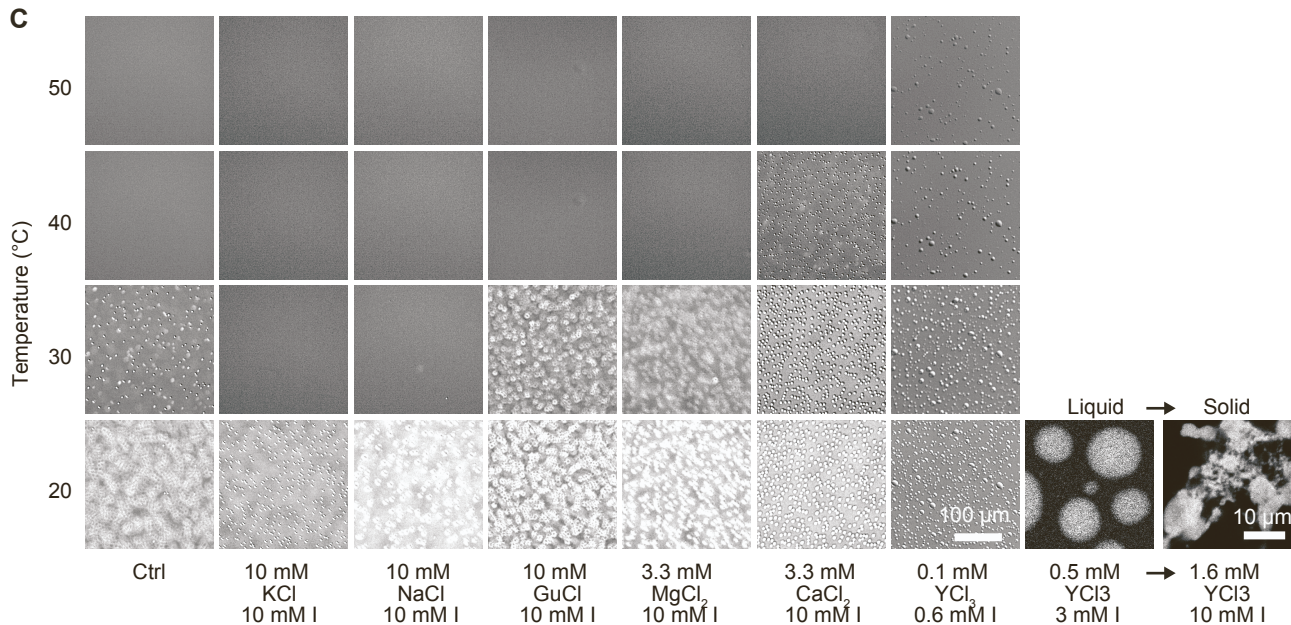
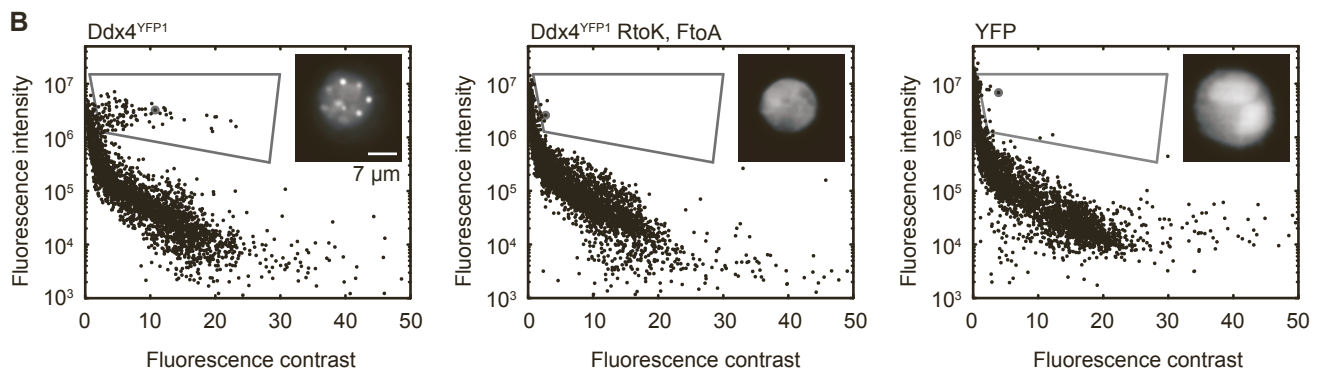
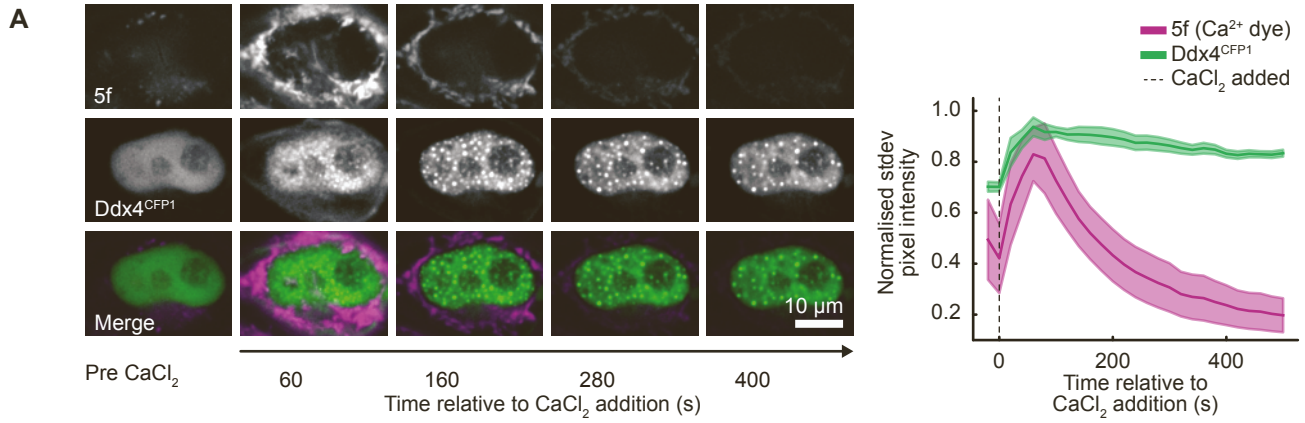


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Supplemental information

**Ion binding with charge inversion
combined with screening modulates
DEAD box helicase phase transitions**

Michael D. Crabtree, Jack Holland, Arvind S. Pillai, Purnima S. Kompella, Leon Babl, Noah N. Turner, James T. Eaton, Georg K.A. Hochberg, Dirk G.A.L. Aarts, Christina Redfield, Andrew J. Baldwin, and Timothy J. Nott

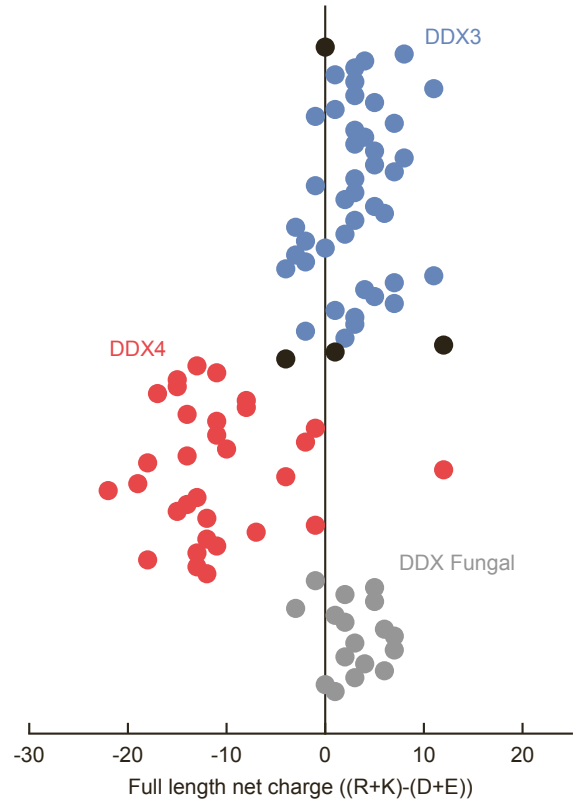
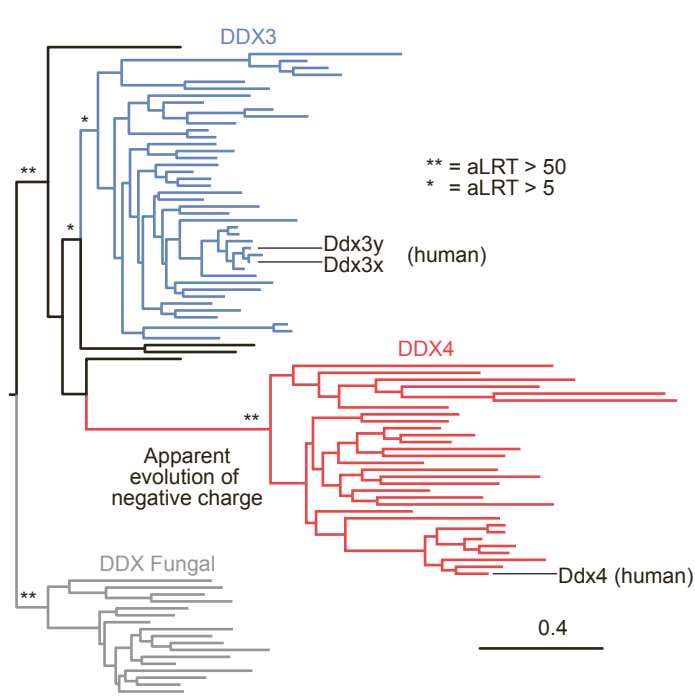
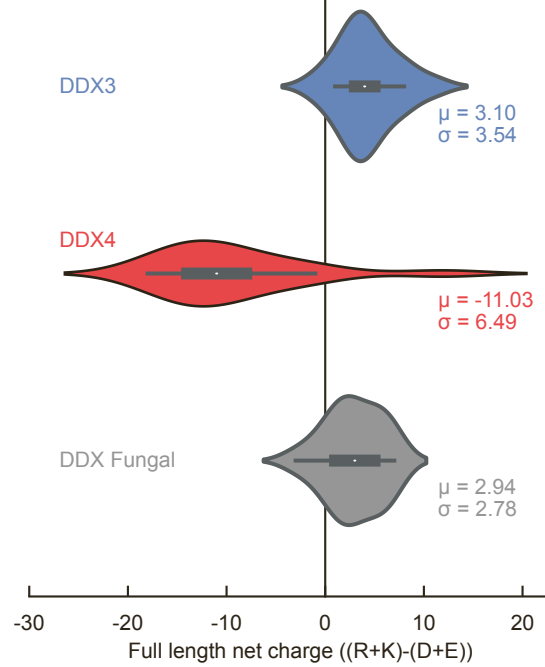
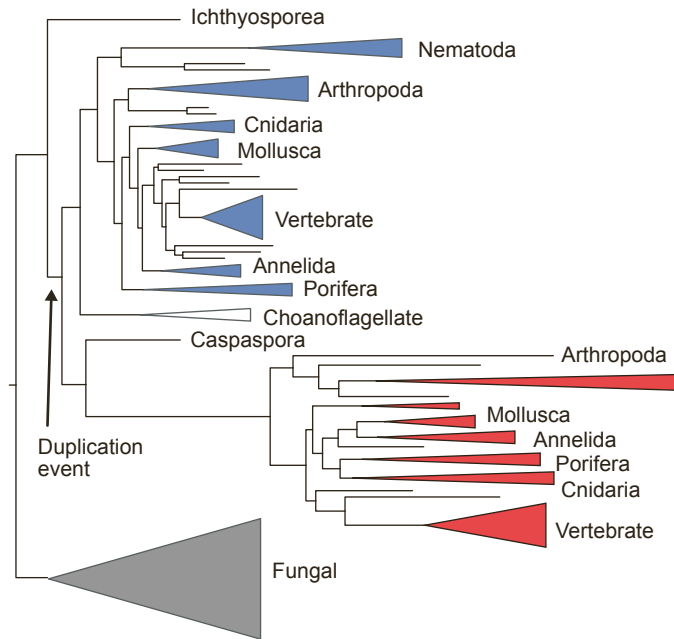


Supp Fig 1. Cation valency modulates Ddx4^{N1} phase separation propensity.

(A) Snapshots of a live cell imaging experiment in which CaCl₂ was added to the growth media surrounding live HeLa cells expressing Ddx4^{CFP1} and continuously monitored (left). Quantitation of the fluorescence emission of a Ca²⁺ ion sensitive dye (Fluo 5F) and Ddx4^{CFP1} showing the coincidence of condensate formation and intracellular influx of Ca²⁺ ions. Scale bar 10 μm. Errors indicate SEM.

(B) Flow cytometry data for HeLa cells expressing Ddx4^{YFP1} indicated a subset of cells with high fluorescence intensity and contrast that were not present in cells expressing either an RtoK + FtoA mutant known to reduce the phase separation propensity of Ddx4^{1,2}, or YFP alone. Images taken during flow cytometry indicated that this subset was reporting on cells with condensate-like features, allowing the number of cells with condensates to be determined in a high throughput manner. Scale bar 7 μm. A grey box indicates the region where cells with condensates were identified and markers with grey outlines indicate the data point that corresponds to each cell image. Data represents a run of 100,000 cells for each construct, with approximately 1% of Ddx4^{YFP1} cells falling within the region outlined with the grey box.

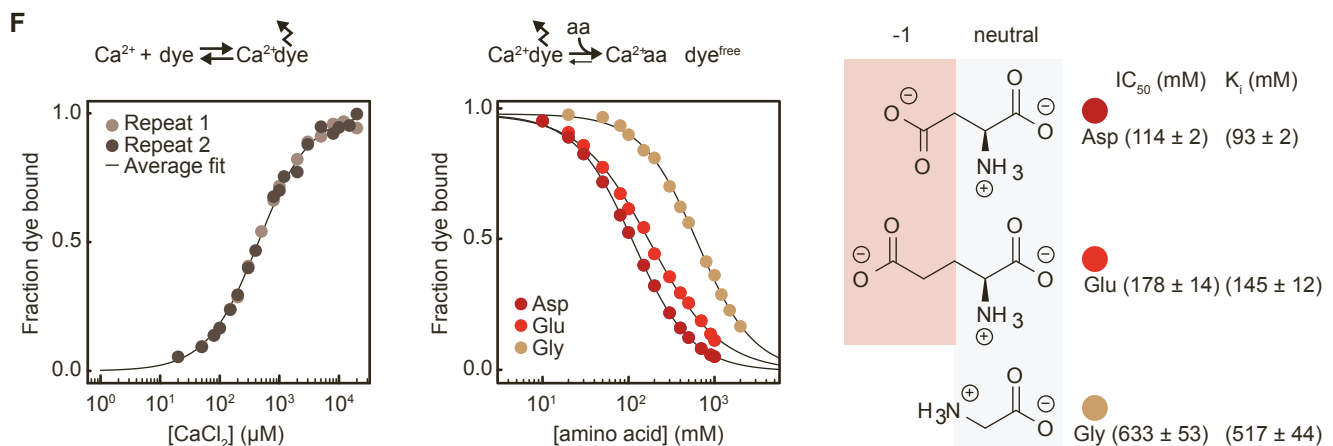
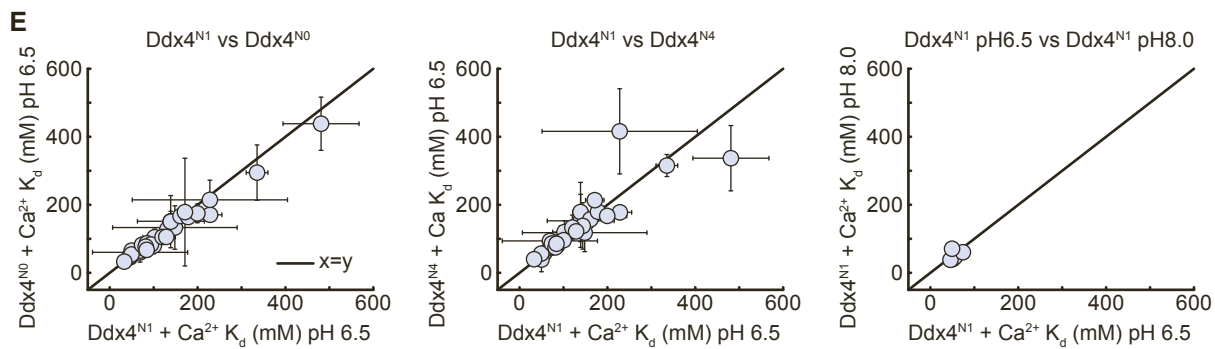
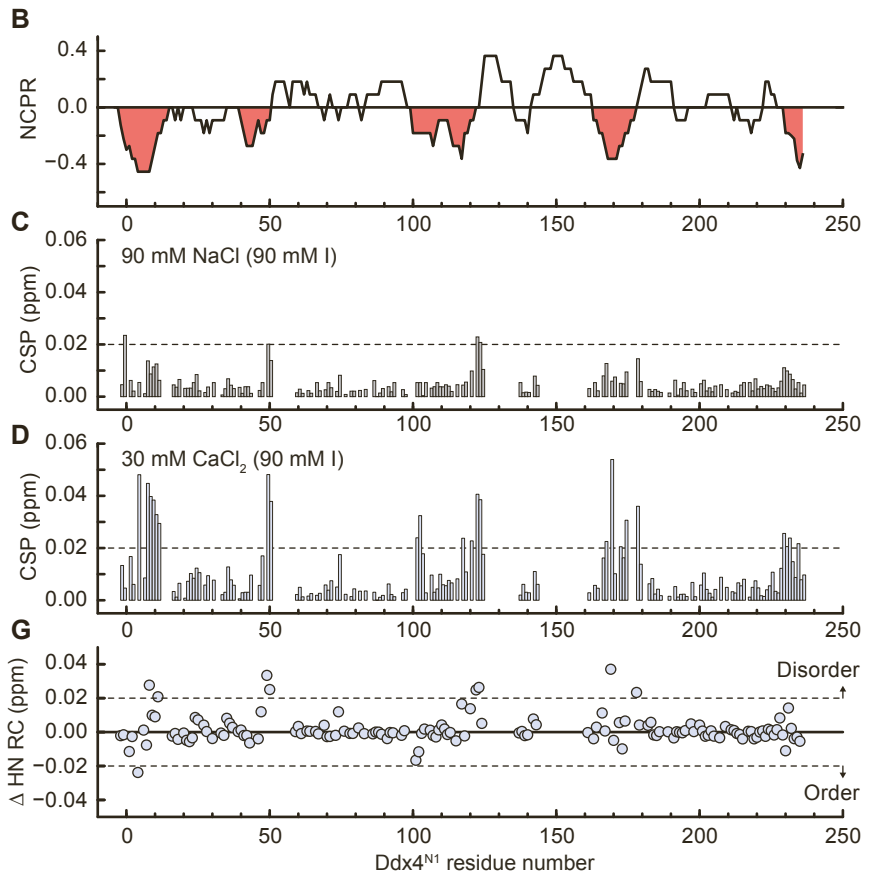
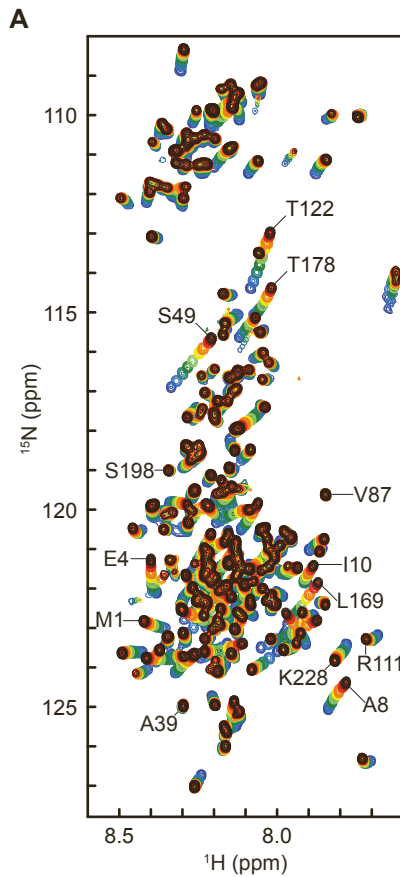
(C) DIC images indicate the effect of ions on the stability of Ddx4^{N1} condensates. Salt concentrations and ionic strengths (*I*) are indicated. Scale bar 100 μm. Note the transition from liquid-like to solid-like morphology observed upon increasing YCl₃ concentration from 0.5 to 1.6 mM (fluorescence micrographs where the fluorescence signal comes from free Alexa 488 dye, scale bar 10 μm).

A**B**

Supp Fig 2. Evolution of Ddx4 and Ddx3 proteins

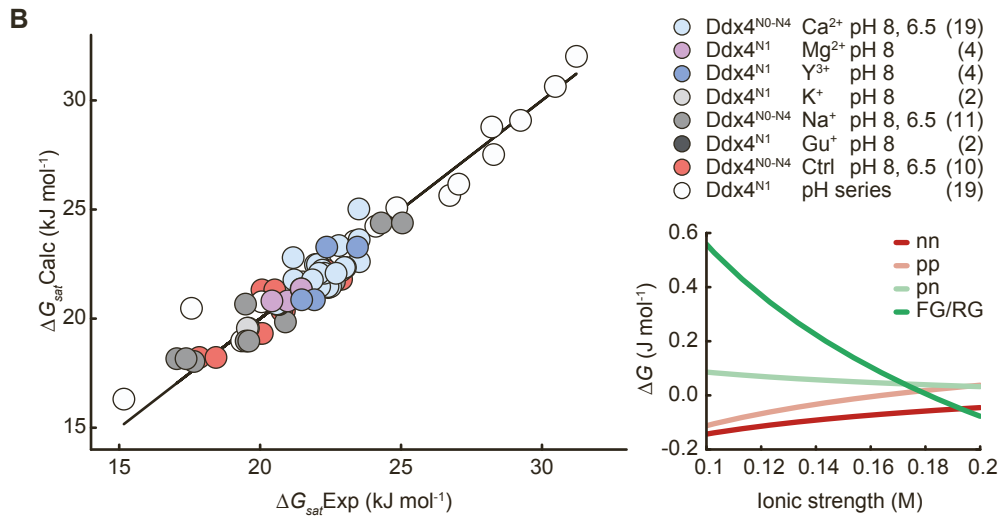
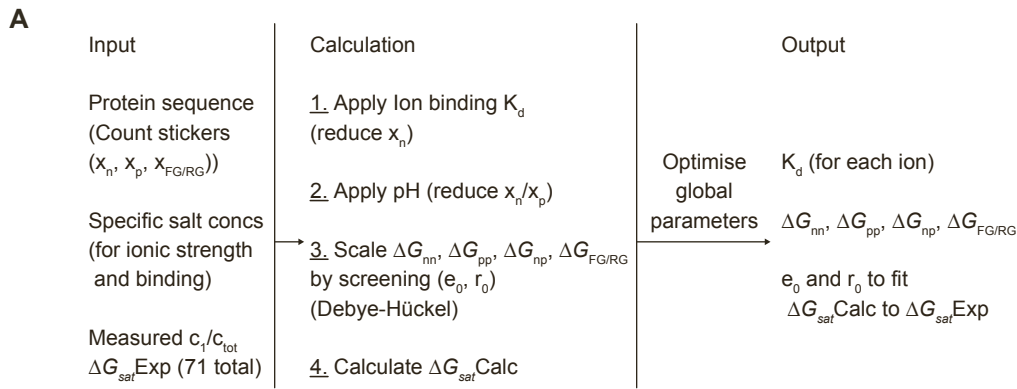
(A) Reconstruction of the evolutionary history of DEAD box helicases Ddx4 and Ddx3. Left: Maximum likelihood (ML) tree was constructed based on sequence alignments of the folded domains from 94 animal and fungal species. The animal Ddx4, Ddx3 and fungal clades are shown in red, blue and grey respectively. Eukaryotic clades that are neither animals nor fungi are shown in black. Approximate likelihood-ratio test (aLRT) statistics for selected nodes are indicated. Right: Scatterplot of simple net charge expressed as $(R (arg) + K (lys)) - (D (asp) + E (glu))$ for full length Ddx proteins; the values are aligned with branch-tips to which they correspond.

(B) Left: ML phylogeny simplified to show major clades of animals that appear to have one copy of each Ddx paralog. The node that corresponds to the Ddx3/Ddx4 duplication event is indicated. Right: Violin plots of net charge of Ddx4 proteins compared to Ddx3x and fungal Ddx proteins, with mean (μ) and s.d. (σ) indicated.



Supp Fig 3. The divalent ion Ca^{2+} binds to individual negatively charged sidechains.

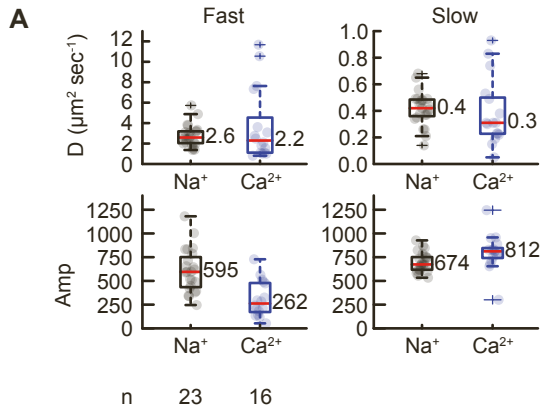
- (A) ^1H - ^{15}N BEST-TROSY spectra of ^{15}N Ddx4^{N1} at pH 6.5 in the presence of 0 (black), 3.3 (dark red), 10 (red), 30 (orange), 50 (yellow), 100 (light green), 150 mM (green), 250 mM (aqua), 400 mM (light blue), and 600 (dark blue) CaCl_2 . Residues that show relatively large chemical shift perturbations (CSP) are labelled, as are residues that show minimal CSPs (A39, V87, S198).
- (B) Net charge per residue (NCPR, 10 amino acid window, 1 amino acid step) for Ddx4^{N1}, calculated using IDDomainSpotter³.
- (C) Per residue CSP for ^{15}N Ddx4^{N1} at pH 6.5 at 90 mM additional NaCl compared to 0 mM additional salt. Dashed line indicates a CSP of 0.02 ppm.
- (D) Per residue CSP for ^{15}N Ddx4^{N1} at pH 6.5 at 30 mM additional CaCl_2 compared to 0 mM additional salt. Dashed line indicates a CSP of 0.02 ppm.
- (E) Correlations of residue-specific K_d for different samples or conditions compared to the Ddx4^{N1} reference sample. Error bars indicate the estimated standard error for the best-fit value.
- (F) Binding isotherm for Ca^{2+} ions and the calcium sensitive dye, Fluo 5N (left). Data from two repeats are shown. Solid line represents the fit values using the mean K_d . A competition-based experiment was used to monitor the binding of amino acids to calcium (right). IC_{50} values were extracted using equation 10 and converted to K_i using equation 11. Carboxylic acid sidechains of D (asp) and E (glu) carry a charge of -1 (pink box). The +1 and -1 charges of the amino and carboxy groups of the amino acid backbone result in a net charge for this region of the molecule of 0 (grey box). Errors represent the error of the fit.
- (G) Change in combined ^1H and ^{15}N chemical shift relative to the Neighbor Corrected intrinsically disordered protein (IDP) Library ($\Delta\text{HN RC}$)⁴ at pH 6.5 at 30 mM additional CaCl_2 compared to 0 mM additional salt. Dashed line indicates a CSP of 0.02 ppm. Positive and negative values indicate moving towards or away from the reference value for a random coil, respectively.



Supp Fig 4. Modeling condensate stability.

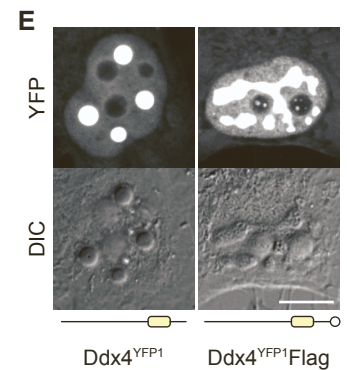
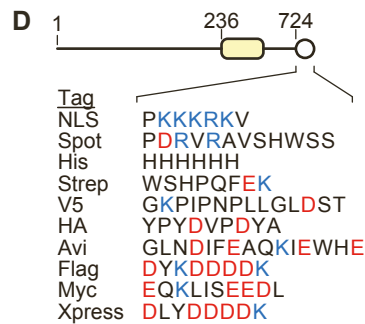
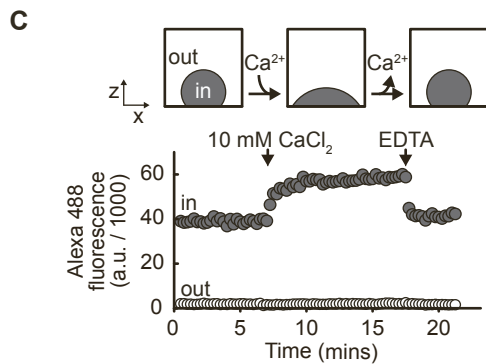
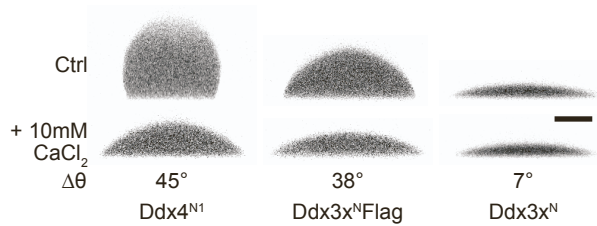
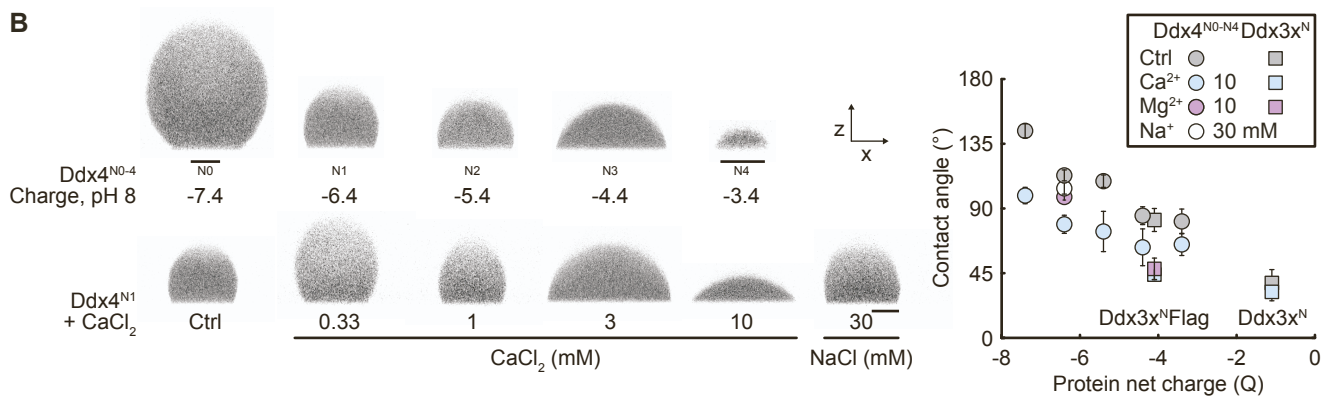
(A) Schematic representation of the stickers and spacers model used to explain condensate stability.

(B) Correlation of experimental and calculated ΔG_{sat} , fitted values, and the impact of ionic strength on attractive and repulsive interactions in the model.



Chi squared values for Na⁺ versus Ca²⁺ FRAP datasets:

	Na ⁺ mono	Na ⁺ bi	Na ⁺ ratio: mono/bi	Ca ²⁺ mono	Ca ²⁺ bi	Ca ²⁺ ratio: mono/bi
Mean	124041	14575	8.1	40392	9317	4.4
SD	86039	5262	2.6	27526	4709	1.6



Supp Fig 5. Emergent properties of Ddx4^{N0-N4} biomolecular condensates.

(A) Box plots showing fast and slow diffusion coefficients ($\mu\text{m}^2 \text{sec}^{-1}$) and amplitudes from biexponential fits of individual FRAP curves. Red bar indicates median values (indicated for each dataset), box limits indicate interquartile range, whiskers indicate max/min values, crosses indicate outliers. n indicates the number of condensates measured. Comparison of chi squared values from mono and bi exponential fits are tabulated.

(B) Confocal fluorescence microscopy xz images of Ddx4^{N0-N4} charge series, Ddx3x^N and Ddx3x^NFlag condensates sitting on siliconized glass coverslips indicate the correlation between divalent ion addition, net charge and condensate contact angles. Image contrast, generated using Alexa 488, was adjusted to highlight the profile of the condensates. Scale bars apply to the respective rows of images and indicate 5 μm . Ddx4^{N0} has a unique scale bar in the top row. Plot showing droplet contact angles following addition of 30 mM NaCl, 10 mM MgCl₂ or 10 mM CaCl₂. Error bars indicate standard deviation.

(C) Summary of the data from Movie S1. Addition of 10 mM CaCl₂ to a sample containing Ddx4^{N1} condensates increased the maximum Alexa 488 fluorescence intensity originating inside Ddx4^{N1} condensates (grey) whereas no change in Alexa 488 fluorescence intensity was observed in the dilute phase surrounding Ddx4^{N1} condensates (white). Addition of EDTA (20 mM) returned the condensates to their initial appearance and Alexa 488 fluorescence intensity (partitioning).

(D) Ddx4^{YFP1} was tagged at its C-terminus with a variety of common peptide affinity purification tags. Position and sequences of the tags are indicated. Full protein sequences are given in Table S1.

(E). Morphologies of Ddx4^{YFP1} and Ddx4^{YFP1}Flag condensates inside HeLa cells. The spherical appearance of Ddx4^{YFP1} condensates is consistent with a higher surface tension than the less stable Ddx4^{YFP1}Flag condensates.

Protein name	Sequence
Ddx4 ^{N1}	GAMGSMGDEDWEAEINPHMSSYVPIFEKDRYSENGDNFNRTPASSEMDDGPSRRDHFMSKGFASGRNFGNRDAGECNKR DNTSTMGGFVGVKSGFNRFNSRFEDGDSSGFWRRESSNDCEDNPNTRNRGFSKRGGYRDGNNSEASGPYRRGGGRGSRGCRG GFGLGSPNNDLDPDECMQRTGGLFGSRRPVLSTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEGGES
Ddx3x ^N	GAMGSMSHVAVENALGLDQQFAGLDLNSSDNQSGGSTASKGRYIPPHLRNREATKGFYDKDSSGWSSSKDKDAYSSFGSRSDS RGKSSFFSDRGSGRGRFDDRGRSDYDGIISRGRDSGFGKFERGGNSRWCDKSDDEDDWS
Ddx3x ^{N_FLAG}	GAMGSMSHVAVENALGLDQQFAGLDLNSSDNQSGGSTASKGRYIPPHLRNREATKGFYDKDSSGWSSSKDKDAYSSFGSRSDS RGKSSFFSDRGSGRGRFDDRGRSDYDGIISRGRDSGFGKFERGGNSRWCDKSDDEDDWSYKDDDDK
Ddx3y ^N	GAMGSMSHVVVKNDELDQQLANLDLNEKQSGGASTASKGRYIPPHLRNREASKGFHDKDSSGWSSCKDKDAYSSFGSRDSR GKPGYFSESGSRGRFDDRGRSDYDGIIGNRERPGFRFERSGHSRWCDKSVEDDWS
Vasa ^N	GAMGSMDDWDEPIVDTRGARGGDWSDDEDATAKSFSGEAGDVGSGGEGGYQGGNRDVFGRIGGGRRGGAGGYRG GNRDGGGFHGGRRERGERDFRGGEGGFRGGQGGSRGGQGGSRGGQGGFRGGEGGFRGLYENEDGDERRRGLDREERGGER RGLDREERGGGERGERDGGFARRRNEDDINNINNIV
Bel ^N	GAMGSMNAINQNGTGLEQQVAGLDLNGGSADYSGPITSTNSVTGGVYVPPHLRGGGNNNAADAESQGGQGGQGG FDSRSGNPRQETRDPPQSRGGGGEYRRGGGGGRRGNRQSGDYGYGSGGGRRGGGRFEDNYNGGFEFSRRGGDWNRS GGGGGGRRGFRGSPYRGGGGGSSNLNEQTAEDGQAQQQQPRNDRWQEPERPAFGDSEGGQAGGNRSYNNRGER GGGGYNSRWKEGGGSNVDT
drDdx4 ^N	GAMGSMDDWEEDQSPVVCSSGFLGNSDGGKFSYTTGGAGNDKSNSEGTEGSSWKMTGDSFRGRGGRRGGSRGGGGF SGFKSEIDENGSDGGWNGGESRGRGRGGFRGGFRSGSRDENENRNDGDKGGERGRGRGGGGFRGGFRDGGNEDTG RRGFRENENENDEGEGRGRGRGGFRGGFRDGGGDESGRKFRGGFRGRNEEVFSKVTADKLDQEG
Ddx4 ^{N0}	GAMGSMGDEDWEAEINPHMSSYVPIFEKDRYSENGDNFNRTPASSEMDDGPSRRDHFMSKGFASGRNFGNRDAGECNKR DNTSTMGGFVGVKSGFNRFNSRFEDGDSSGFWRRESSNDCEDNPNTRNRGFSKRGGYRDGNNSEASGPYRRGGGRGSRGCRG GFGLGSPNNDLDPDECMQRTGGLFGSRRPVLSTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEGGESD
Ddx4 ^{N1}	GAMGSMGDEDWEAEINPHMSSYVPIFEKDRYSENGDNFNRTPASSEMDDGPSRRDHFMSKGFASGRNFGNRDAGECNKR DNTSTMGGFVGVKSGFNRFNSRFEDGDSSGFWRRESSNDCEDNPNTRNRGFSKRGGYRDGNNSEASGPYRRGGGRGSRGCRG GFGLGSPNNDLDPDECMQRTGGLFGSRRPVLSTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEGGES
Ddx4 ^{N2}	GAMGSMGDEDWEAEINPHMSSYVPIFEKDRYSENGDNFNRTPASSEMDDGPSRRDHFMSKGFASGRNFGNRDAGECNKR DNTSTMGGFVGVKSGFNRFNSRFEDGDSSGFWRRESSNDCEDNPNTRNRGFSKRGGYRDGNNSEASGPYRRGGGRGSRGCRG GFGLGSPNNDLDPDECMQRTGGLFGSRRPVLSTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEGG
Ddx4 ^{N3}	GAMGSMGDEDWEAEINPHMSSYVPIFEKDRYSENGDNFNRTPASSEMDDGPSRRDHFMSKGFASGRNFGNRDAGECNKR DNTSTMGGFVGVKSGFNRFNSRFEDGDSSGFWRRESSNDCEDNPNTRNRGFSKRGGYRDGNNSEASGPYRRGGGRGSRGCRG GFGLGSPNNDLDPDECMQRTGGLFGSRRPVLSTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEA
Ddx4 ^{N4}	GAMGSMGDEDWEAEINPHMSSYVPIFEKDRYSENGDNFNRTPASSEMDDGPSRRDHFMSKGFASGRNFGNRDAGECNKR DNTSTMGGFVGVKSGFNRFNSRFEDGDSSGFWRRESSNDCEDNPNTRNRGFSKRGGYRDGNNSEASGPYRRGGGRGSRGCRG GFGLGSPNNDLDPDECMQRTGGLFGSRRPVLSTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKS
YFP (mCitrine; recombinantly expressed and purified)	GAMGSMVSKGEELFTGVVILVELDGDVNGHKFSVSGEGEDATYKGLTKFICTTGKLPVPWPTLVTTFGYGLMCFARYPDHM KQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDGLVNRIELKIDFKEDGNILGHKLEYNYNHNVYIMADKQKNGIKV NFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDMVLLFVTAAGIT
Ddx4 ^{YFP0}	MGDEDWEAEINPHMSSYVPIFEKDRYSENGDNFNRTPASSEMDDGPSRRDHFMSKGFASGRNFGNRDAGECNKRNTST MGGFVGVKSGFNRFNSRFEDGDSSGFWRRESSNDCEDNPNTRNRGFSKRGGYRDGNNSEASGPYRRGGGRGSRGCRG SPNNDLDPDECMQRTGGLFGSRRPVLSTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEGGESDMSVSKGEELF TGVPVILVELDGDVNGHKFSVSGEGEDATYKGLTKFICTTGKLPVPWPTLVTTFGYGLMCFARYPDHMVILVELDGDVNGHKFSV VQERTIFFKDDGNYKTRAEVKFEGDGLVNRIELKIDFKEDGNILGHKLEYNYNHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLA DHYQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDMVLLFVTAAGITFTYIPGFSGSTRGVNFASVDTRKKGKSTLNTAGFSS SQAPNPVDDSWD
Ddx4 ^{YFP1}	MGDEDWEAEINPHMSSYVPIFEKDRYSENGDNFNRTPASSEMDDGPSRRDHFMSKGFASGRNFGNRDAGECNKRNTST MGGFVGVKSGFNRFNSRFEDGDSSGFWRRESSNDCEDNPNTRNRGFSKRGGYRDGNNSEASGPYRRGGGRGSRGCRG SPNNDLDPDECMQRTGGLFGSRRPVLSTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEGGESMSVSKGEELFTG VVPILVELDGDVNGHKFSVSGEGEDATYKGLTKFICTTGKLPVPWPTLVTTFGYGLMCFARYPDHMVILVELDGDVNGHKFSV ERTIFFKDDGNYKTRAEVKFEGDGLVNRIELKIDFKEDGNILGHKLEYNYNHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADH YQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDMVLLFVTAAGITFTYIPGFSGSTRGVNFASVDTRKKGKSTLNTAGFSSQ APNPVDDSWD
Ddx4 ^{YFP2}	MGDEDWEAEINPHMSSYVPIFEKDRYSENGDNFNRTPASSEMDDGPSRRDHFMSKGFASGRNFGNRDAGECNKRNTST MGGFVGVKSGFNRFNSRFEDGDSSGFWRRESSNDCEDNPNTRNRGFSKRGGYRDGNNSEASGPYRRGGGRGSRGCRG SPNNDLDPDECMQRTGGLFGSRRPVLSTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEGGMSVSKGEELFTG VVPILVELDGDVNGHKFSVSGEGEDATYKGLTKFICTTGKLPVPWPTLVTTFGYGLMCFARYPDHMVILVELDGDVNGHKFSV RTIFFKDDGNYKTRAEVKFEGDGLVNRIELKIDFKEDGNILGHKLEYNYNHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADH YQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDMVLLFVTAAGITFTYIPGFSGSTRGVNFASVDTRKKGKSTLNTAGFSSQ

	QQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDHMLLEFVTAAGITFSTYIPGFSGSTRGNVFASVDTRKKGSTLNTAGFSSSQAPNPVDESWD
Ddx4 ^{YFP3}	MGDEDWEAEINPHMSSYVPIFEKDRYSGENGNFNRTPASSEMDDGSPRRDHFMSKGFASGRNFGNRDAGECNKRDNTSTMGGFGVGKSFNGRFGNSRFEDGSSGFWRSSNDCEPNTRNRGFSKRGGYRDGNNSSEASGPYRRGGRSFRGCRGGFGLGSPNNLDLPDECMQRTGGLFGSRRPVLSTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGLTLKFICTTGKLPVWPWPTLVTTFGYGLMCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGVSQVLADHYQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDHMLLEFVTAAGITFSTYIPGFSGSTRGNVFASVDTRKKGSTLNTAGFSSSQAPNPVDESWD
Ddx4 ^{YFP4}	MGDEDWEAEINPHMSSYVPIFEKDRYSGENGNFNRTPASSEMDDGSPRRDHFMSKGFASGRNFGNRDAGECNKRDNTSTMGGFGVGKSFNGRFGNSRFEDGSSGFWRSSNDCEPNTRNRGFSKRGGYRDGNNSSEASGPYRRGGRSFRGCRGGFGLGSPNNLDLPDECMQRTGGLFGSRRPVLSTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGLTLKFICTTGKLPVWPWPTLVTTFGYGLMCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGVSQVLADHYQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDHMLLEFVTAAGITFSTYIPGFSGSTRGNVFASVDTRKKGSTLNTAGFSSSQAPNPVDESWD
YFP (mCitrine; expressed in HeLa cells)	MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGLTLKFICTTGKLPVWPWPTLVTTFGYGLMCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGVSQVLADHYQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDHMLLEFVTAAGITLERPLESRGPV
Ddx4 ^{YFP1} RtoK, FtoA	MGDEDWEAEINPHMSSYVPIFEKDRYSGENGNFNRTPASSEMDDGSPRRDHFMSKGAASGNAGNRDAGECNKRDNTSTMGGAGVGKSAAGNSKGFEDGSSGFWRSSNDCEPNTRNRGASKKGGYRDGNNSSEASGPYRRGGRSFRGCRGGGAGLSPNNLDLPDECMQRTGGLAGSRRPVLSTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEAGGESDQGPVKVTLMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGLTLKFICTTGKLPVWPWPTLVTTFGYGLMCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGVSQVLADHYQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDHMLLEFVTAAGITLEASTYIPGASGSGTKGNVFASVDTRKKGSTLNTAGFSSSQAPNPVDESWD
Ddx4 ^{CFP1}	MGDEDWEAEINPHMSSYVPIFEKDRYSGENGNFNRTPASSEMDDGSPRRDHFMSKGFASGRNFGNRDAGECNKRDNTSTMGGFGVGKSFNGRFGNSRFEDGSSGFWRSSNDCEPNTRNRGFSKRGGYRDGNNSSEASGPYRRGGRSFRGCRGGFGLGSPNNLDLPDECMQRTGGLFGSRRPVLSTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEAGGESMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGLTLKFICTTGKLPVWPWPTLVTTLVGWVQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNILGHKLEYNAINSDNVYITADKQKNGIKANFKIRHNIEDGVSQVLADHYQQNTPIGDGPVLLPDNHLYSTQSKLSKDPNEKRDHMLLEFVTAAGITFSTYIPGFSGSTRGNVFASVDTRKKGSTLNTAGFSSSQAPNPVDESWD
Ddx4 ^{YFP1} His	MGDEDWEAEINPHMSSYVPIFEKDRYSGENGNFNRTPASSEMDDGSPRRDHFMSKGFASGRNFGNRDAGECNKRDNTSTMGGFGVGKSFNGRFGNSRFEDGSSGFWRSSNDCEPNTRNRGFSKRGGYRDGNNSSEASGPYRRGGRSFRGCRGGFGLGSPNNLDLPDECMQRTGGLFGSRRPVLSTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEAGGESMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGLTLKFICTTGKLPVWPWPTLVTTFGYGLMCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGVSQVLADHYQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDHMLLEFVTAAGITFSTYIPGFSGSTRGNVFASVDTRKKGSTLNTAGFSSSQAPNPVDESWDGSGSGHHHHHH
Ddx4 ^{YFP1} Myc	MGDEDWEAEINPHMSSYVPIFEKDRYSGENGNFNRTPASSEMDDGSPRRDHFMSKGFASGRNFGNRDAGECNKRDNTSTMGGFGVGKSFNGRFGNSRFEDGSSGFWRSSNDCEPNTRNRGFSKRGGYRDGNNSSEASGPYRRGGRSFRGCRGGFGLGSPNNLDLPDECMQRTGGLFGSRRPVLSTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEAGGESMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGLTLKFICTTGKLPVWPWPTLVTTFGYGLMCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGVSQVLADHYQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDHMLLEFVTAAGITFSTYIPGFSGSTRGNVFASVDTRKKGSTLNTAGFSSSQAPNPVDESWDGSGSGEQKLISEEDL
Ddx4 ^{YFP1} Flag	MGDEDWEAEINPHMSSYVPIFEKDRYSGENGNFNRTPASSEMDDGSPRRDHFMSKGFASGRNFGNRDAGECNKRDNTSTMGGFGVGKSFNGRFGNSRFEDGSSGFWRSSNDCEPNTRNRGFSKRGGYRDGNNSSEASGPYRRGGRSFRGCRGGFGLGSPNNLDLPDECMQRTGGLFGSRRPVLSTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEAGGESMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGLTLKFICTTGKLPVWPWPTLVTTFGYGLMCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGVSQVLADHYQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDHMLLEFVTAAGITFSTYIPGFSGSTRGNVFASVDTRKKGSTLNTAGFSSSQAPNPVDESWDGSGSGDYKDDDDK
Ddx4 ^{YFP1} HA	MGDEDWEAEINPHMSSYVPIFEKDRYSGENGNFNRTPASSEMDDGSPRRDHFMSKGFASGRNFGNRDAGECNKRDNTSTMGGFGVGKSFNGRFGNSRFEDGSSGFWRSSNDCEPNTRNRGFSKRGGYRDGNNSSEASGPYRRGGRSFRGCRGGFGLGSPNNLDLPDECMQRTGGLFGSRRPVLSTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEAGGESMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGLTLKFICTTGKLPVWPWPTLVTTFGYGLMCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGVSQVLADHYQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDHMLLEFVTAAGITFSTYIPGFSGSTRGNVFASVDTRKKGSTLNTAGFSSSQAPNPVDESWDGSGSGYPYDVPDYA

Ddx4 ^{YFP1} Strep	<u>MG</u> DEDWEAEINPHMSSYVPIFEKDRYSGENGDNFRNTPASSEMDDGPSRRDHF <u>FMKSGFASGRNFGNRDAGECNKRDNTST</u> <u>MGGFGVGKSGFN</u> RGFSNSRFEDGDSSGFWRESSNDCEDNPNRNRGFSKRGGYRDGNNSEASGPYRRGGGRGSFRGCRGGFGLG SPNNLDLPDECMQRTGGLFGSRRPVLSGTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEGGESMVSKGEELFTG VVPILVELDGDVNGHKFSVSGEGEGDATYGKLT LF ICTTGKLPVPWPTLVTTFFGYGLMCFARYPDHMKQHDFFKSAMPEGYVQ ERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGVSQ LADH YQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDHMLLEFVTAAGITFSTYIPGFSGSTRGNVFAVSDTRKKGKSTLNTAGFSSSQ APNPVDESWDGSGSGWHPQFEK
Ddx4 ^{YFP1} Xpress	<u>MG</u> DEDWEAEINPHMSSYVPIFEKDRYSGENGDNFRNTPASSEMDDGPSRRDHF <u>FMKSGFASGRNFGNRDAGECNKRDNTST</u> <u>MGGFGVGKSGFN</u> RGFSNSRFEDGDSSGFWRESSNDCEDNPNRNRGFSKRGGYRDGNNSEASGPYRRGGGRGSFRGCRGGFGLG SPNNLDLPDECMQRTGGLFGSRRPVLSGTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEGGESMVSKGEELFTG VVPILVELDGDVNGHKFSVSGEGEGDATYGKLT LF ICTTGKLPVPWPTLVTTFFGYGLMCFARYPDHMKQHDFFKSAMPEGYVQ ERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGVSQ LADH YQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDHMLLEFVTAAGITFSTYIPGFSGSTRGNVFAVSDTRKKGKSTLNTAGFSSSQ APNPVDESWDGSGSGDLVDDDDK
Ddx4 ^{YFP1} Avi	<u>MG</u> DEDWEAEINPHMSSYVPIFEKDRYSGENGDNFRNTPASSEMDDGPSRRDHF <u>FMKSGFASGRNFGNRDAGECNKRDNTST</u> <u>MGGFGVGKSGFN</u> RGFSNSRFEDGDSSGFWRESSNDCEDNPNRNRGFSKRGGYRDGNNSEASGPYRRGGGRGSFRGCRGGFGLG SPNNLDLPDECMQRTGGLFGSRRPVLSGTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEGGESMVSKGEELFTG VVPILVELDGDVNGHKFSVSGEGEGDATYGKLT LF ICTTGKLPVPWPTLVTTFFGYGLMCFARYPDHMKQHDFFKSAMPEGYVQ ERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGVSQ LADH YQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDHMLLEFVTAAGITFSTYIPGFSGSTRGNVFAVSDTRKKGKSTLNTAGFSSSQ APNPVDESWDGSGSGGLNDFEAQKIEWHE
Ddx4 ^{YFP1} V5	<u>MG</u> DEDWEAEINPHMSSYVPIFEKDRYSGENGDNFRNTPASSEMDDGPSRRDHF <u>FMKSGFASGRNFGNRDAGECNKRDNTST</u> <u>MGGFGVGKSGFN</u> RGFSNSRFEDGDSSGFWRESSNDCEDNPNRNRGFSKRGGYRDGNNSEASGPYRRGGGRGSFRGCRGGFGLG SPNNLDLPDECMQRTGGLFGSRRPVLSGTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEGGESMVSKGEELFTG VVPILVELDGDVNGHKFSVSGEGEGDATYGKLT LF ICTTGKLPVPWPTLVTTFFGYGLMCFARYPDHMKQHDFFKSAMPEGYVQ ERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGVSQ LADH YQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDHMLLEFVTAAGITFSTYIPGFSGSTRGNVFAVSDTRKKGKSTLNTAGFSSSQ APNPVDESWDGSGSGGKPIPNLLGLDST
Ddx4 ^{YFP1} NLS	<u>MG</u> DEDWEAEINPHMSSYVPIFEKDRYSGENGDNFRNTPASSEMDDGPSRRDHF <u>FMKSGFASGRNFGNRDAGECNKRDNTST</u> <u>MGGFGVGKSGFN</u> RGFSNSRFEDGDSSGFWRESSNDCEDNPNRNRGFSKRGGYRDGNNSEASGPYRRGGGRGSFRGCRGGFGLG SPNNLDLPDECMQRTGGLFGSRRPVLSGTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEGGESMVSKGEELFTG VVPILVELDGDVNGHKFSVSGEGEGDATYGKLT LF ICTTGKLPVPWPTLVTTFFGYGLMCFARYPDHMKQHDFFKSAMPEGYVQ ERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGVSQ LADH YQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDHMLLEFVTAAGITFSTYIPGFSGSTRGNVFAVSDTRKKGKSTLNTAGFSSSQ APNPVDESWDGSGSGPKKPKKRV
Ddx4 ^{YFP1} Spot	<u>MG</u> DEDWEAEINPHMSSYVPIFEKDRYSGENGDNFRNTPASSEMDDGPSRRDHF <u>FMKSGFASGRNFGNRDAGECNKRDNTST</u> <u>MGGFGVGKSGFN</u> RGFSNSRFEDGDSSGFWRESSNDCEDNPNRNRGFSKRGGYRDGNNSEASGPYRRGGGRGSFRGCRGGFGLG SPNNLDLPDECMQRTGGLFGSRRPVLSGTGNGDTSQSRSGSGSERGGYKGLNEEVITGSGKNSWKSEAEGGESMVSKGEELFTG VVPILVELDGDVNGHKFSVSGEGEGDATYGKLT LF ICTTGKLPVPWPTLVTTFFGYGLMCFARYPDHMKQHDFFKSAMPEGYVQ ERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGVSQ LADH YQQNTPIGDGPVLLPDNHLYSYQSKLSKDPNEKRDHMLLEFVTAAGITFSTYIPGFSGSTRGNVFAVSDTRKKGKSTLNTAGFSSSQ APNPVDESWDGSGSGPDRVRAVSHWSS

Table S1. Amino acid sequences of proteins used in the paper. Residual cloning tags at the N-termini of recombinantly expressed and purified protein sequences (GAMGS) are underlined. Residue numbering indicated in the main text starts from the first non-underlined amino acid.

Protein name / amino acid numbers in construct	Ddx4 ^{N1} /6-241 vs other sequences used in the study	Alignment length	% identity	% similarity
>Ddx4 ^{N1} /1-241	Ddx4 ^{N1} /6-241	236	100.0	100.0
>Ddx3x ^N /1-142	Ddx3x ^N /6-142	237	12.2	23.6
>Ddx3x ^N _FLAG/1-150	Ddx3x ^N _FLAG/6-150	237	12.2	24.5
>Ddx3y ^N /1-140	Ddx3y ^N /6-140	237	10.6	22.8
>Vasa ^N /1-197	Vasa ^N /6-197	247	17.0	30.8
>Bel ^N /1-257	Bel ^N /6-257	288	15.6	32.3
>drDdx4 ^N /1-230	drDdx4 ^N /6-230	255	20.8	38.4
>Ddx4 ^{N0} _238/1-243	Ddx4 ^{N0} _238/6-243	238	99.2	99.2
>Ddx4 ^{N1} _236/1-241	Ddx4 ^{N1} _236/6-241	236	100.0	100.0
>Ddx4 ^{N2} _234/1-239	Ddx4 ^{N2} _234/6-239	236	99.2	99.2
>Ddx4 ^{N3} _231/1-236	Ddx4 ^{N3} _231/6-236	236	97.9	97.9
>Ddx4 ^{N4} _229/1-234	Ddx4 ^{N4} _229/6-234	236	97.0	97.0

Table S2. Amino acid sequence similarity and identity scores for Ddx4 and Ddx3 homologues, related to Figure 2. Amino acid groupings were: AVLIM, FYW, GSTCHNQ, P, KR, DE. https://www.bioinformatics.org/sms2/ident_sim.html, ref ⁵.

Parameter	Fitted value	\pm	$x0$	Mean	Stdev
ΔG_{nn}	-21.91	1.12	-22.45	-22.43	1.07
ΔG_{pp}	-27.02	3.64	-26.13	-25.79	3.23
ΔG_{pn}	16.22	0.93	17.25	16.98	0.90
$\Delta G_{FG/RG}$	55.82	7.40	53.62	51.66	7.24
Ca^{2+}	73.22	14.08	73.18	74.37	16.51
Mg^{2+}	105.21	18.56	108.71	112.59	29.69
γ^{3+}	5.47	1.99	5.83	4.83	2.36
K^+	487.27	103.39	503.24	2739.25	15083.85
Na^+	3763.19	1700.23	4207.47	231054.69	871979.16
Gu^+	173.06	21.10	175.53	180.93	34.66
$r0_{nn}$	67.52	3.67	63.63	63.71	3.78
$r0_{pp}$	54.44	9.32	56.04	56.04	8.44
$r0_{pn}$	91.46	5.95	84.23	84.48	6.10
$r0_{FG/RG}$	25.97	4.53	27.31	27.72	4.71
erval	46.53	2.10	48.36	48.30	1.81

Table S3. Stickers and spacers model parameters.

Supplementary references

1. Nott, T. J. *et al.* Phase Transition of a Disordered Nuage Protein Generates Environmentally Responsive Membraneless Organelles. *Mol. Cell* **57**, 936–947 (2015).
2. Brady, J. P. *et al.* Structural and hydrodynamic properties of an intrinsically disordered region of a germ cell-specific protein on phase separation. *Proc. Natl. Acad. Sci. U. S. A.* **114**, E8194–E8203 (2017).
3. Millard, P. S. *et al.* IDDomainSpotter: Compositional bias reveals domains in long disordered protein regions—Insights from transcription factors. *Protein Sci.* **29**, 169–183 (2020).
4. Tamiola, K., Acar, B. & Mulder, F. A. A. A. Sequence-specific random coil chemical shifts of intrinsically disordered proteins. *J. Am. Chem. Soc.* **132**, 18000–18003 (2010).
5. Stothard, P. The sequence manipulation suite: JavaScript programs for analyzing and formatting protein and DNA sequences. *Biotechniques* **28**, (2000).