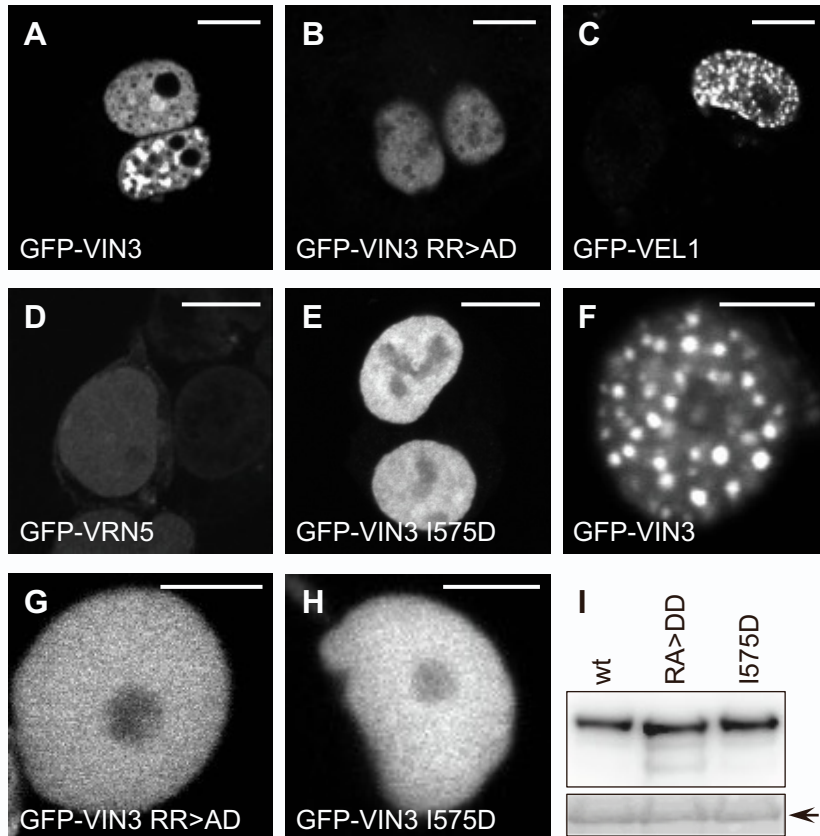


Cell Reports, Volume 41

Supplemental information

**Head-to-tail polymerization by VEL proteins
underpins cold-induced Polycomb
silencing in flowering control**

Marc Fiedler, Elsa Franco-Echevarría, Anna Schulten, Mathias Nielsen, Trevor J. Rutherford, Anna Yeates, Bilal Ahsan, Caroline Dean, and Mariann Bienz



Supplementary Fig. 1 (related to Fig. 1)

VEL-dependent formation of nuclear condensates

(A-E) Representative confocal images of COS-7 (A-C) or HeLa (D-E) cells transfected with wt or mutant GFP-VIN3, GFP-VEL1 or GFP-VRN5, as indicated in panels; scale bars 10 μm . (F-H) Representative confocal images of *N. benthamiana* leaves infiltrated with wt or mutant GFP-VIN3, as indicated in panels; scale bars 5 μm . (I) Western blot showing comparable levels of wt and mutant GFP-VIN3 proteins; arrow, internal control (large subunit of Rubisco, visualized by Ponceau staining).

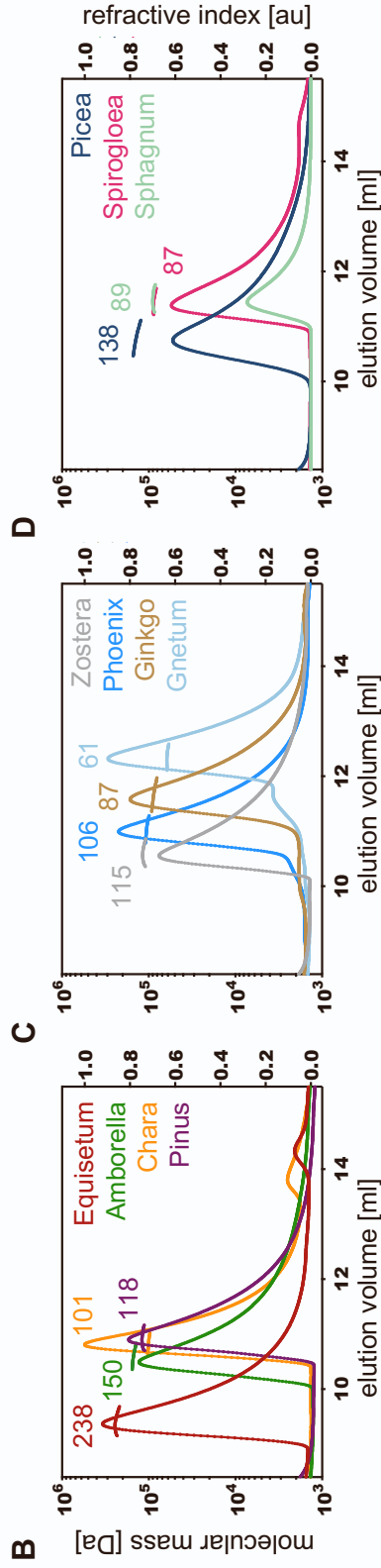
A

| | | | | | |
|-----|-------------|-------|-------|-------|-------|
| | | R554A | R556D | L563D | I575D |
| MYA | At VIN3 | | | | |
| | At VEL1 | | | | |
| | At VEL2 | | | | |
| 43 | At VRN5 | | | | |
| | Phoenix | | | | |
| | Zostera | | | | |
| | Amborella | | | | |
| | Pinus | | | | |
| | Picea | | | | |
| 320 | Gnetum | | | | |
| | Ginkgo | | | | |
| 370 | Cycas | | | | |
| 430 | Selaginella | | | | |
| 530 | Treubia | | | | |
| | Sphagnum | | | | |
| 580 | Spirogløea | | | | |
| | Chara | | | | |

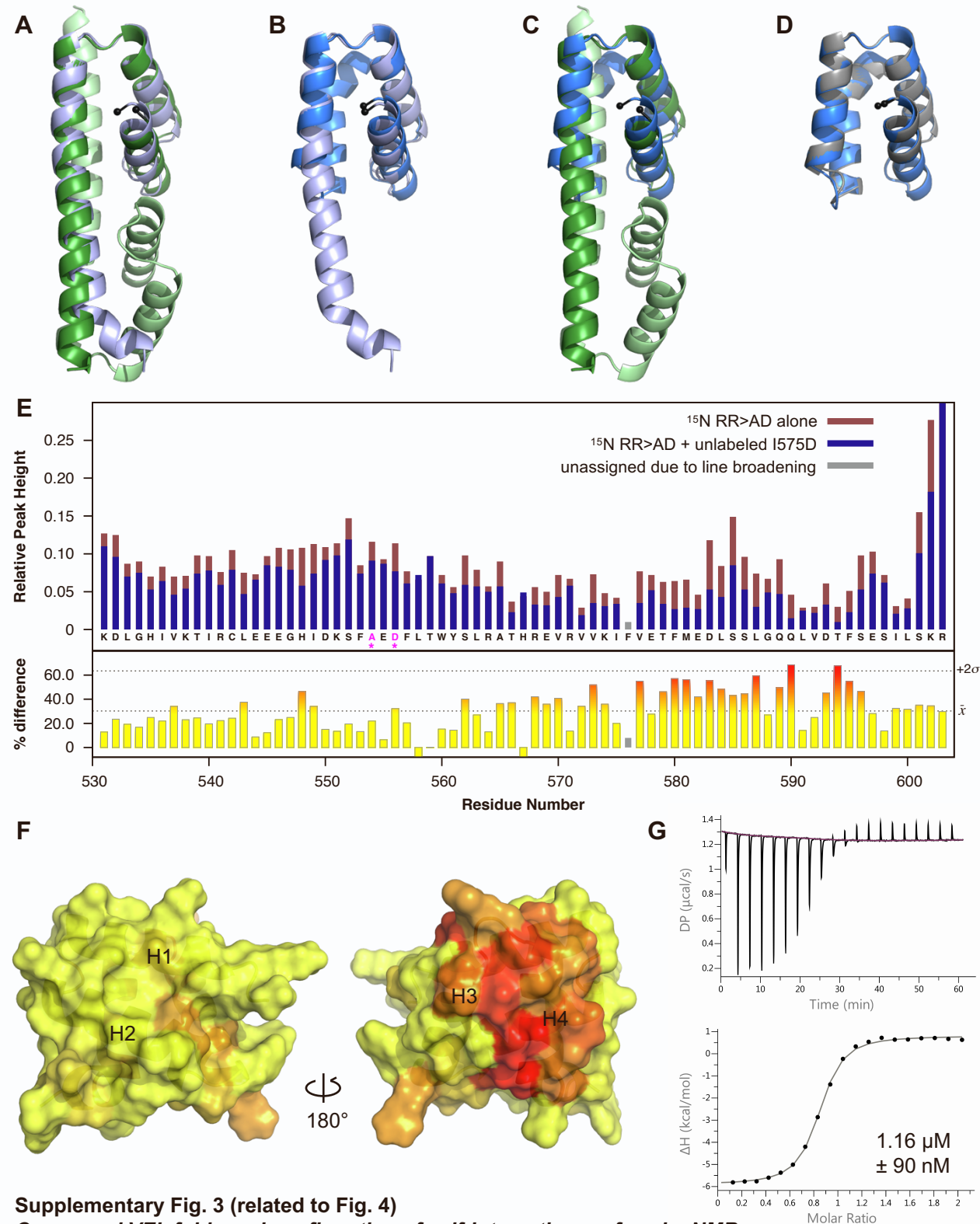
```

GVGDKDLGHI VKTIRCLLEE EGHIDKSFRE RFLTWYSLRATHREVRVVKIFVETFMEDLSLGGQQLVDTFSEIILSKRSSTNG-----VVPAGICLKLWH
GGTESGLEHCWKIIRQLCECSGHI DKNFRQKFLTWYSLRAVTSQEIIRVVKIFIDTFDDPMLAEQLIDTFDDRVSIKRSVGGSGASAVPSPGFCMKLWH
GSGSFGFECCNLIRQLCESGOVKSDFRKKFLTWYGLKAKADKEKHVEIIFDVFDTFKDKEALAKQLIDTFSDDCIRKHPHPIGG-----GSESAGVSLIILQD
SSIDDTLEKCVKVIIRWLEREGHIKTTFRRLFTWFSMSSTAQEQSVVSTFQTLDEDDPGSLAGQLVDAFTDVVSTKRPNN-----GV-M-TSH
GSLEGA YCYC VVIRWLEREGHIETNERIKELTWFSLRATPQERRIVSVVDTLDDPASLAGQLVDTFSEVCSKRPVP-----VPTGFCMKLWH
GELDGHYCYC VRIIRWLEREGHI EKDFRMKFLTWFSLRATPQERRIVSVFDTLDDPGSLAGQLVDSLEIAMYKKP-----RNGFCSKLWH
MGLDESLEYCYC VKIIRWLEREGHI ERDFRMKFLTWFSLRATPQERRIVSVFDTLDDPGSLAGQLMDSFLD IVSSKRP-----RNGFCSKLWH
GSLENNYCYC VVIIRWLEREGHI OKDFRQKFLTWFSLRATPQERRIVSVFDTMDDPASLAGQLDDTFSEERICSKRAPV-----LRNEFCSKLWH
DSELEKNYCYC VKIIRWLERERHI QKEFRQKFLTWFSLRATPQERRIVSVFDTMDDPASLAGQLVDTFSEGICSKRAPV-----LRNGFCSKLWH
VCLEKNPEHS IKVIIRWLEREGHI TEFERKFLTWFSLRASSEERRIVYAFIDALVGD PDLAEQLIDSEFSEIMSNKKPVP-----LPKGFCTRLWH
GSLEKKNYCYC VKIIRWLEREGHI QKEFRVKEFLTWFSLRATPQERRIVSVFDTLDDPASLAGQLVDTFSEGICSKKAQV-----LRNGFCSKLWH
GSLEKKNYCYC VMIIRWLEREGHI QKEFRVKEFLTWFSLRATPQERRIVSVFDTLDDPASLAGQLVDTFSEVCSKKAQV-----LRNGFCSKLWH
LCIAKSPFEFCVKAIRWLELEGLYKKEFRILKFLSWFSLRATENEKRAVSVFIDTLQDSPTNLAEQQLVDTFADTFVAKROOV-----RSNGGFHNQLWH
DYLDKSLEFSIKVIIRWLERQGHLPDFRIKLLTWLGFRAVVEEKKMIIFVYVVRTLLTDPHGLAHQLVDTFGEIVATQRLRA-----EEASS
SIQEKLLLEYCYC VVIRWLEREGHI KSEFRVREVTWFALRA TEDEKRVSIFIKNMGDNPERUAGQLKDTFEEIISVTR
NGSERVEQCVRTIIRWLEREGH LKAFRRSFTWLSLRATEAERKVVHIVMENMLDDPVALAAQLDTFEEIISAPKRLKV-----RH
KESQREVKVLR LVCQLEREGYVSKSFRIDFLMWF TLRASQDEKRAITLFEALQEDLNSLAEQQLQAGYEAAAQVKKLRADG-LTGTPEHDCQKKRR
NEGDR EIVNCLRHVCLLEKSGHLSKDFRIKFLTWFSRRASDAEKKVVAMIICALGDEPATLAEQLVDTFSELIGTLPGGK-TELMEAMHMEGFVNGLRE

```



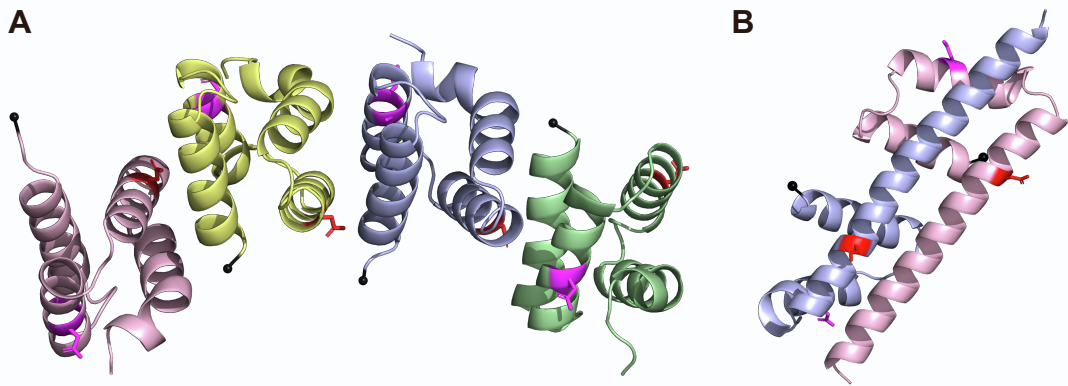
Supplementary Fig. 2 (related to Fig. 2)
Deep conservation of VEL domains in plants
 (A) Sequence alignment of VEL domains from diverse plant species, as indicated (At, *Arabidopsis thaliana*; MYA, million years ago); point mutations disabling self-association of VIN3 are indicated above. (B-D) SEC-MALS of purified Lip-tagged VEL domains from various species as indicated in inset; curves, elution profiles; line traces, molar masses as derived from MALS.



Supplementary Fig. 3 (related to Fig. 4)

Conserved VEL folds and confirmation of self-interacting surface by NMR

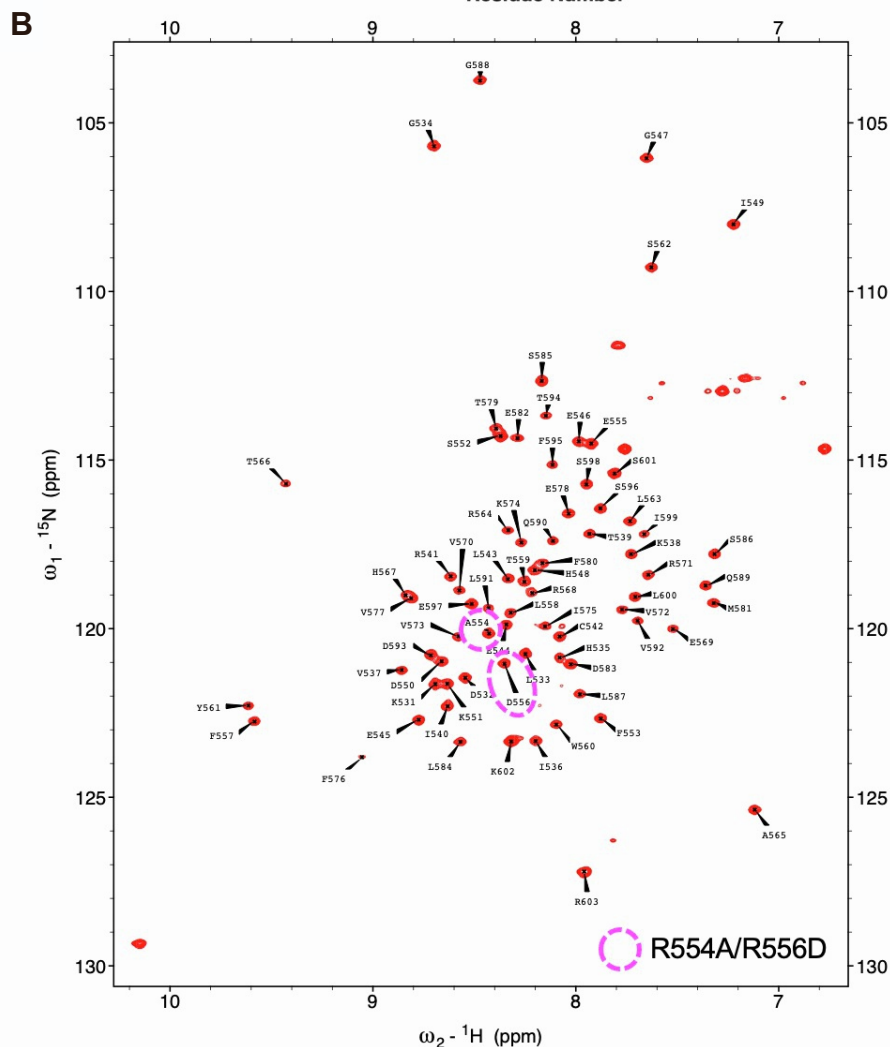
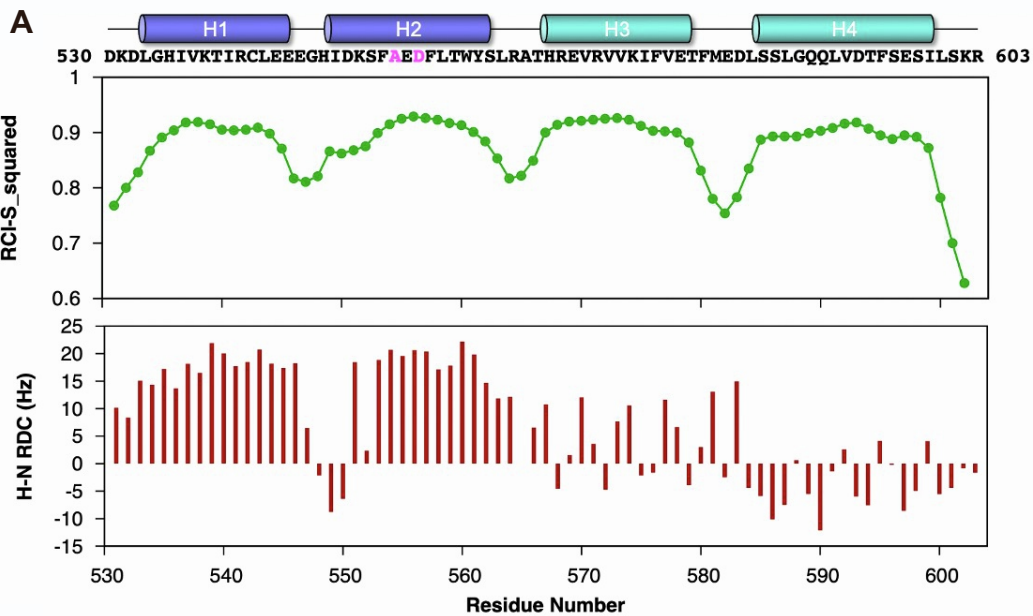
Overlays of ribbon diagrams of different VEL domains; *black balls*, N-termini; **(A)** VIN3_{VEL} RR>AD (7O6U, *light blue*) and VIN3_{VEL} R556D I575D (7O6T, *green*), RMSD 1.69 Å; **(B)** VIN3_{VEL} RR>AD (7O6U, *light blue*) and VEL1_{VEL} I664D (7O6W, *blue*), RMSD 1.63 Å; **(C)** VIN3_{VEL} R556D I575D (7O6T, *green*) and VEL1_{VEL} I664D (7O6W, *blue*), RMSD 1.77 Å; **(D)** VEL1_{VEL} I664D (7O6W, *blue*) and VEL1_{VEL} RK>AD I664D (7O6V, *gray*), RMSD 0.41 Å. **(E)** ¹⁵N-labeled RR>AD probed with unlabeled I575D; *top panel*, relative peak heights from HSQC of unbound (*maroon*) versus HSQC of bound (*blue*); *bottom panel*, differences in percent. **(F)** Percent differences in **(E)** plotted onto the surface of the RR>AD NMR structural model, confirming that the tail surface as defined by crystallography also serves as the polymerisation interface in solution. **(G)** ITC profiles (*top panel*) of VIN3_{VEL} RR>AD binding to VIN3_{VEL} I575D. Fitted data (*bottom panel*) show a *K_d* value of 1.16 μM for the dimeric interface.



Supplementary Fig. 4 (related to Fig. 5)

Abnormal filaments of double-mutant VEL domains

Abnormal head-to-tail interactions in **(A)** VEL1_{VEL} (R643A K645D I664D; 7O6V) or **(B)** VIN3_{VEL} (R556D I575D; 7O6T) crystals bearing point mutations in both head and tail surfaces; *magenta*, R643A K645D **(A)**, or R556D **(B)**; *red*, I664D **(A)**, or I575D **(B)**.



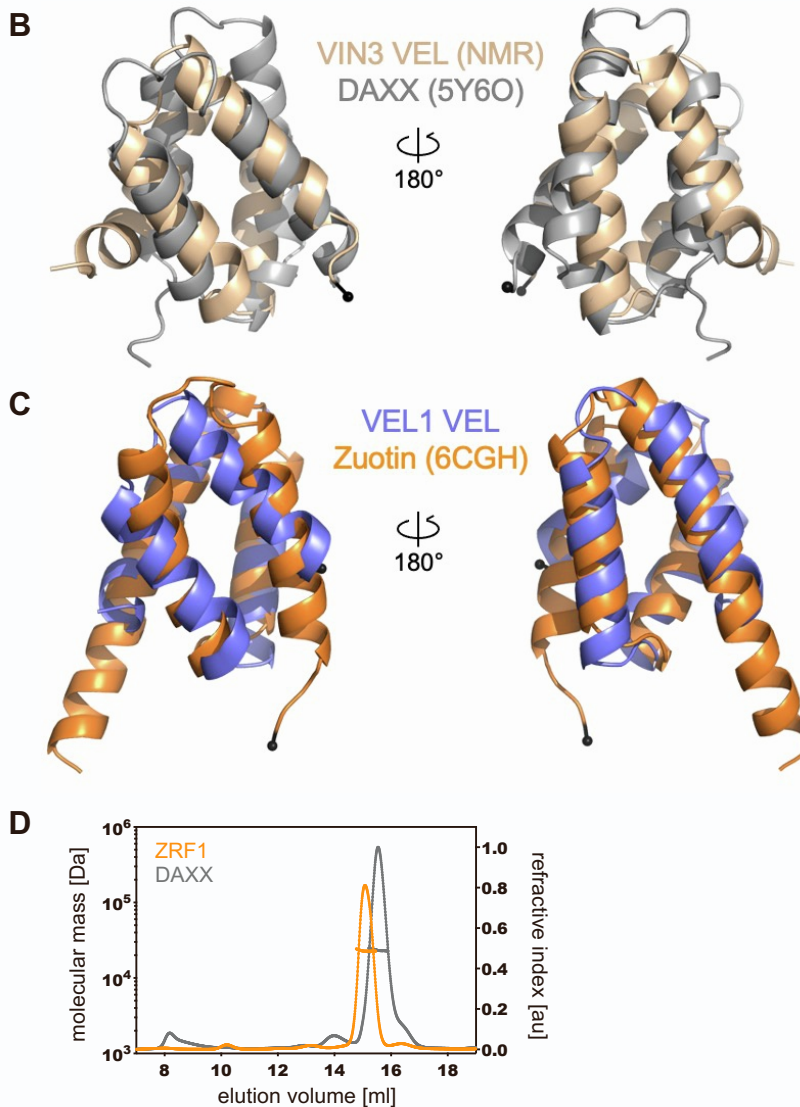
Supplementary Fig. 5 (related to Fig. 6)

NMR spectroscopy of VIN3_{VEL} RR>AD

(A) VIN3_{VEL} RR>AD sequence, with four α -helices above (*magenta*, R554A and R556D); RCI-S² showing the propensity of each residue (green dots) to be within a turn (<0.85) or an α -helix (>0.85); *bottom*, RDC plot further confirming 'H4 tucked under' conformation (see main text). (B) Full assignments of VIN3_{VEL} 529-603, with RR>AD residues marked (*magenta*); spectrum acquired at 300 μ M.

A

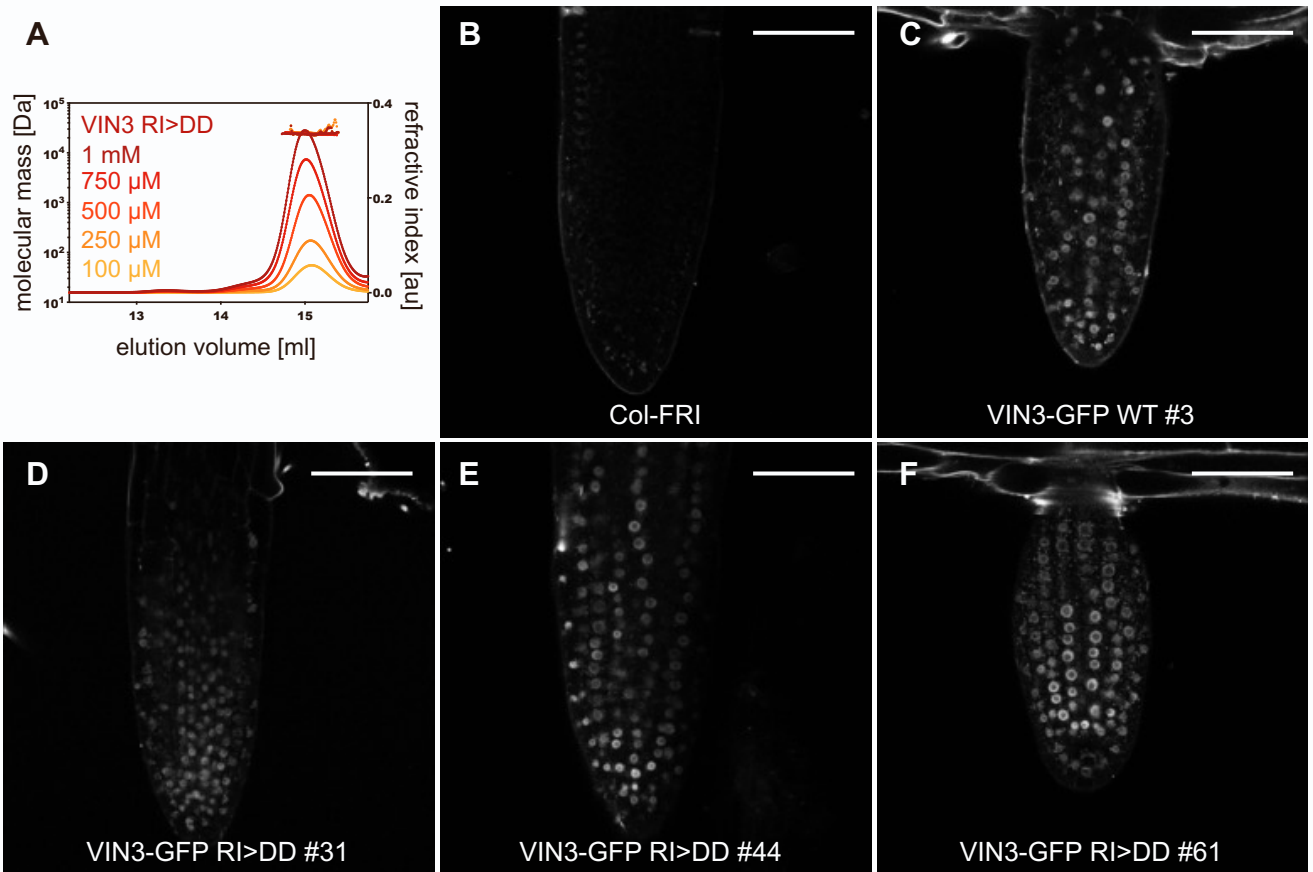
| | VIN3_NMR | Z | VEL1_crystal | Z |
|----|---|-----|---|-----|
| 1 | 4GMQ - RIBOSOME-BINDING DOMAIN OF ZUO1 | 6.1 | 6HPN - ANTIGEN, P35 | 6.4 |
| 2 | 6HPN - ANTIGEN, P35 | 6.0 | 6CGH - SOLUTION STRUCTURE OF THE FOUR-HELIX BUNDLE REGION OF HUMAN J-PROTEIN ZUOTIN | 6.3 |
| 3 | 5Y6O - CRYSTAL STRUCTURE OF DAXX N-TERMINAL FOUR-HELIX BUNDLE DOMAIN (4HB) IN COMPLEX WITH ATRX | 5.9 | 4GMQ - RIBOSOME-BINDING DOMAIN OF ZUO1 | 5.0 |
| 4 | 3TDW - GENTAMICIN RESISTANCE PROTEIN | 5.2 | 5Y6O - CRYSTAL STRUCTURE OF DAXX N-TERMINAL FOUR-HELIX BUNDLE DOMAIN (4HB) IN COMPLEX WITH ATRX | 5.0 |
| 5 | 6CGH - SOLUTION STRUCTURE OF THE FOUR-HELIX BUNDLE REGION OF HUMAN J-PROTEIN ZUOTIN | 5.0 | 1OKS - RNA POLYMERASE ALPHA SUBUNIT | 4.8 |
| 6 | 2IPC - PREPROTEIN TRANSLOCASE SECA SUBUNIT | 4.9 | 3F7W - PUTATIVE FRUCTOSAMINE-3-KINASE | 4.7 |
| 7 | 1OKS - RNA POLYMERASE ALPHA SUBUNIT | 4.7 | 1K30 - GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE | 4.7 |
| 8 | 3F7W - PUTATIVE FRUCTOSAMINE-3-KINASE | 4.7 | 6N9Y - NON-STRUCTURAL PROTEIN 1 OF BLUETONGUE VIRUS | 4.6 |
| 9 | 2LWX - SOLUTION STRUCTURE OF THE C-TERMINAL PDR1-ACTIVATING DOMAIN OF THE J-PROTEIN ZUO1 | 4.6 | 3TDW - GENTAMICIN RESISTANCE PROTEIN | 4.6 |
| 10 | 5LXJ - PHOSPHOPROTEIN | 4.5 | 2IPC - PREPROTEIN TRANSLOCASE SECA SUBUNIT | 4.6 |



Supplementary Fig. 6 (related to Fig. 6)

VEL-related 4HB domains in DNAJ co-chaperones

(A) Table of top hits from DALI searches for VIN3_{VEL} (left) and VEL1_{VEL} (right), with 4HB from Zuotin (orange) and DAXX (gray) highlighted. (B, C) Overlays of (B) VIN3_{VEL} RR>AD (wheat) with 4HB domain of DAXX (5Y6O; gray), RMSD 3.24 Å, (C) VEL1_{VEL} (blue) with 4HB domain of Zuotin (6CGH; orange), RMSD 2.69 Å; black balls, N-termini. (D) SEC-MALS of human DAXX (gray trace) and human ZRF1 (orange trace), both corresponding to monomers; void volume of column at 8 ml.



Supplementary Fig. 7 (related to Fig. 7)

Nuclear localization of wt and RI>DD mutant VIN3-GFP

(A) SEC-MALS of purified Lip-VIN3_{VEL} RI>DD, at increasing concentrations as indicated in panels; *line traces*, molar masses as derived from MALS. Highest concentration is still monomeric (calculated 23.4 kDa, observed 24 kDa); void volume of column at 8 ml. (B-F) Confocal images of Arabidopsis root tips expressing wt VIN3-GFP (homozygous) or polymerization-deficient RI>DD mutant (independent first generation transgenic lines) at 6WT0. Scale bar 50 μ m. Transgene copy numbers in individual RI>DD lines are 3 (#31), 11 (#44) and 3 (#61). Note that all images were taken with the same microscopy settings and on the same day from the same individual plants as those shown in main Fig. 7 whose flowering is delayed compared to wt VIN3-GFP, revealing comparable levels of fluorescence in the nuclei of these plants.

| | VEL1_VEL | | VIN3_VEL | | |
|---|-------------------------------|--|--|-------------------------------|--|
| PDB accession codes | I664D | R643A K645D I664D | I575D | R554A R556D | R556D I575D |
| | 7O6W | 7O6V | 7OQV | 7O6U | 7O6T |
| Crystal data | | | | | |
| Wavelength (Å) | 0.97950 | 0.97950 | 0.97950 | 0.97950 | 0.97950 |
| Resolution (Å) | 27.47 – 2.64 (2.77 – 2.64) | 29.03 – 2.5 (2.6 – 2.5) | 29.07 – 2.4 (2.49 – 2.4) | 28.82 – 1.84 (1.88 – 1.84) | 29.47 – 2.02 (2.07 – 2.02) |
| Space group | P 6 ₃ | P 2 ₁ 2 ₁ 2 ₁ | P 2 ₁ 2 ₁ 2 ₁ | I 4 ₁ | P 2 ₁ 2 ₁ 2 ₁ |
| Unit cell dimensions | | | | | |
| a, b, c (Å) | 79.26, 79.26, 59.96 | 52.07, 58.05, 119.99 | 31.5, 91.27, 98.41 | 40.75, 40.75, 83.96 | 31.42, 51.81, 85.01 |
| α, β, γ (°) | 90, 90, 120 | 90, 90, 90 | 90, 90, 90 | 90, 90, 90 | 90, 90, 90 |
| Total reflections | 239198 (22200) | 338001 (38598) | 101791 (10799) | 82212 (4592) | 349471 (22221) |
| Unique reflections | 6335 (769) | 13183 (1481) | 11740 (1191) | 5941 (354) | 9677 (677) |
| Multiplicity | 37.8 (29.2) | 25.6 (26.1) | 8.7 (9.1) | 13.8 (13) | 36.1 (32.8) |
| Mean I/σ (I) | 37.0 (3.9) | 9.6 (2.7) | 13.3 (2.8) | 39.2 (3.9) | 36.2 (4.6) |
| R merge (%) | 6.8 (104.5) | 24.4 (141.3) | 9.8 (76.2) | 4.5 (66.5) | 7.0 (87.7) |
| CC 1/2 | 1.00 (0.84) | 0.99 (0.84) | 1.00 (0.91) | 1.00 (0.91) | 1.00 (0.93) |
| Completeness (%) | 98.8 (92) | 100 (100) | 100 (100) | 99.7 (95.1) | 99.7 (96.5) |
| Complexes in A.U. | 2 | 4 | 4 | 1 | 2 |
| Refinement | | | | | |
| Resolution | 27.47 – 2.64 | 29.03 – 2.5 | 29.07 – 2.4 | 28.82 – 1.84 | 29.47 – 2.02 |
| Number of reflections | 6281 | 13134 | 11698 | 5913 | 9609 |
| R _{work} / R _{free} (%) | 21.16 – 24.92 | 21.36 – 24.07 | 22.34 – 26.95 | 22.13 – 24.44 | 20.24 – 23.66 |
| N° of atoms | 1151 | 2308 | 2357 | 604 | 1223 |
| Protein | 1140 | 2299 | 2319 | 588 | 1173 |
| Ligand | 5 | - | - | - | 1 |
| Water | 6 | 9 | 38 | 16 | 49 |
| Average B Factors (Å²) | | | | | |
| Wilson/overall | 80.42 | 53.72 | 45.12 | 38.71 | 41.25 |
| Protein | 66.86 | 45.62 | 60.33 | 33.78 | 34.11 |
| Ligand | 161.50 | - | - | - | 52.82 |
| Water molecules | 79.40 | 53.22 | 52.33 | 48.46 | 50.39 |
| All atoms | 67.33 | 45.65 | 60.20 | 34.17 | 34.78 |
| RMSDs deviations | | | | | |
| Bonds lengths (Å) | 0.006 | 0.008 | 0.007 | 0.010 | 0.009 |
| Bond angles (°) | 1.01 | 1.16 | 1.53 | 1.50 | 1.22 |
| Ramachandran plot Statistics (%) | | | | | |
| Favored regions | 97.76 | 100 | 98.89 | 100 | 100 |
| Allowed regions | 2.24 | 0 | 1.11 | 0 | 0 |
| Disallowed regions | 0 | 0 | 0 | 0 | 0 |

Table S1

Crystal data collection and refinement statistics