# A link between LEAFY and B-gene homologs in *Welwitschia mirabilis* sheds light on ancestral mechanisms prefiguring floral development

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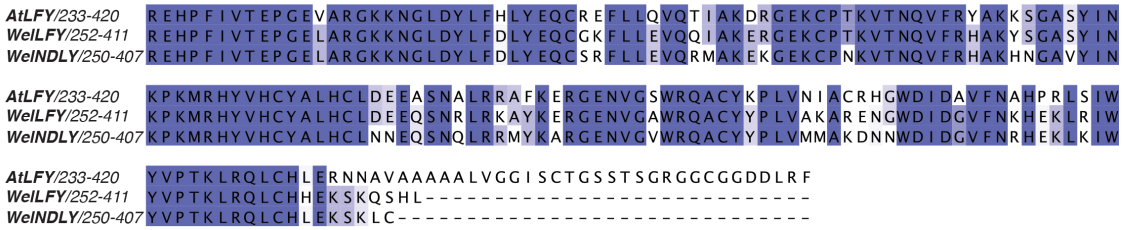
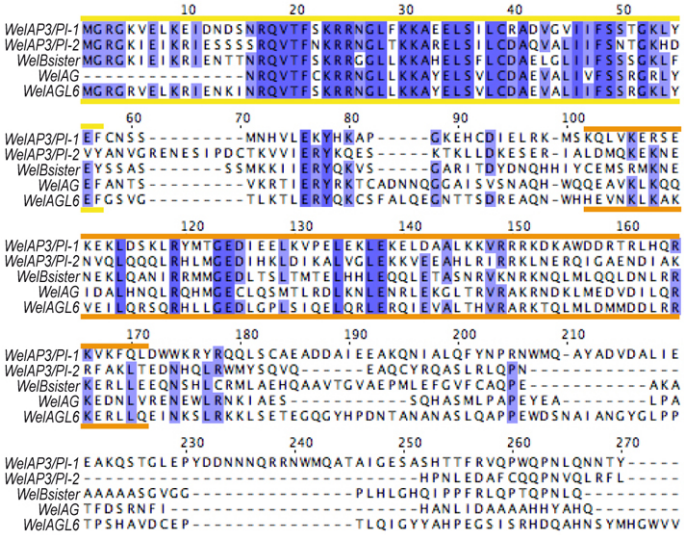
## Supporting Information

This file contains:

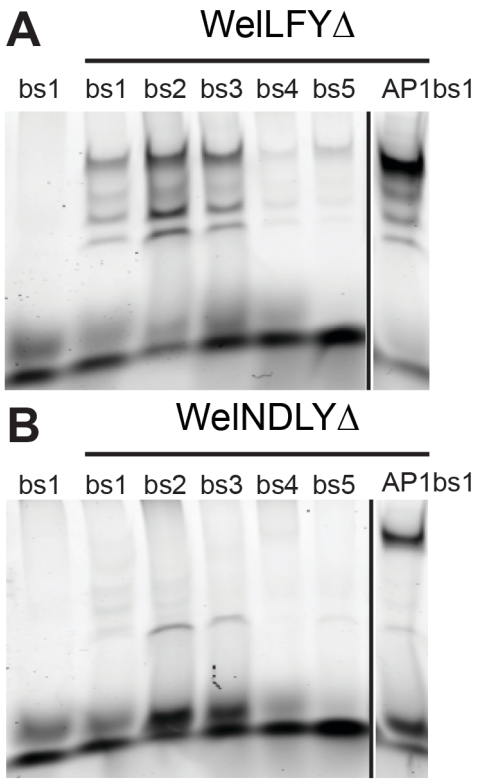
**Supplementary Figure 1 – Alignment of the DNA Binding Domain (DBD) of AtLFY, WelLFY and WelNDLY**.



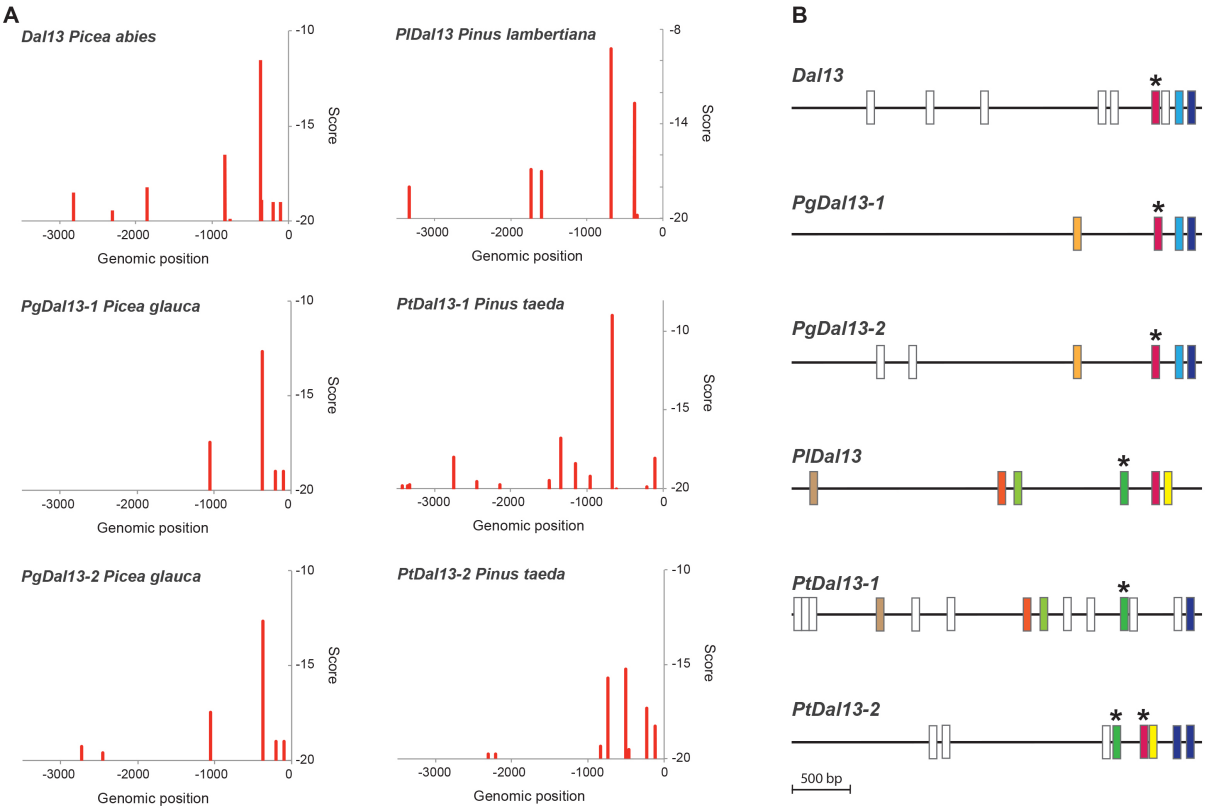

**Supplementary Figure 2** - **Alignment of the predicted amino acid sequences of the five newly identified *Welwitschia mirabilis* genes reveals the conserved domain structure of MIKC-type MADS-box proteins**. The yellow box indicates the MADS-domain and the orange box indicates the K-domain according to (Becker *et al*., 2000). The *WelAG* sequence is incomplete at its N- and C-termini and the C-terminus of the *WelBsister* sequence is missing.



**Supplementary Figure 3 – Comparison of WelLFY and WelNDLY binding to individual sites from *WelAP3/PI* promoters**. EMSA with 10 nM fluorescent DNA corresponding to the five sites indicated in Fig. 5C or AP1bs1 (used as a positive control) and 1 μM of WelLFYΔ (**A**) or WelNDLYΔ (**B**) in lanes 2-7. The relative intensities of the binding by WelLFY are slightly different as compared to the main text figure. This is due to different fluorescent labelling efficiency of the different probes.



**Supplementary Figure 4 – Conservation of potential LEAFY binding sites in the promoters of conifer B genes**. (**A**) Identification of potential LEAFY binding sites, using the WelLFYΔ matrix, in the 3.5kb upstream of the coding sequences of *DAL13* from *Picea abies* and its homologs in *Picea glauca* (*PgDAL13-1*, *PgDAL13-2*), *Pinus lambertiana* (*PlDAL13*) and *Pinus taeda* (*PtDAL13-1*, *PtDAL13-2*). (**B**) Schematic representation of sites identified in (A). Sites that are species-specific are indicated with white boxes. Sites that are conserved between two or more species are depicted with the same coloured box. For each homolog, the site with the best score is indicated with a star.



**Supplementary Table 1** – List of oligonucleotides used in this study.

| Oligonucleotides used to isolate *Welwitschia* genes | | | |
|---|---|---|---|
| Name | Sequence | Name | Sequence |
| WelAG deg1F | ATGGGMCGHGGVAARATYGA | WelBsis deg1F | ATGGGHMGAGGMAARATHGA |
| WelAG deg2F | AACMGACARGTHACWTTYTG | WelBsis deg2F | AAYAGRCARGTBACHTTYTC |
| WelAG deg1R | TCYTGWTGDGCRTARTGRTG | WelBsis deg1R | TCYTGVAGRTTWGGYTGWGT |
| WelB1 aF | CCGAGAAATTGGATGCAAGC | WelB2 aF | GTCATAGAGAGGTACAAGCAA |
| WelB1 bF | ACTGGATTAGAACCCTATGACGAC | WelB2 bF | GATATGCAAAAAGAGAAAAATGAGAAT |
| WelActinGeneF2 | ATGGCCGATGCTGAGGACATTCAA | WelAGL6ATG-F | ATGGGTCGAGGCAGAGTTGAACT |
| WelActinGeneR2 | CTAAAAGCACTTTCTGTGGACAATAG | WelAGL6StopR | TCAGACTACCCATCCATGCATGT |
| WelLFY-F | GCGGGATCCTCAGGGATGGCTCCTGAAAGTT | WelNdly-F | TAAAGCTTTCTGCATCCATGGTCG |
| WelLFY-R | GGGAGGATCCTGTTCTTGACAAATCAAAGATGGGACTGCT | WelNdly-R | CCCTCGAGTCAACATAATTTGCTTTT |

| Oligonucleotides used for Semi-Quantitative RT-PCR | | | | | |
|---|---|---|---|---|---|
| Gene | Oligonucleotide forward | | Oligonucleotide reverse | | Product size |
| | Name | Sequence | Name | Sequence | |
| *WelLFY* | WelLFYprobe2-F | CGCAAGATCGATGAAAATG | WelLFYprobe2-R | GGAAACCCGCAAATCCGCT | 204 bp |
| *WelNDLY* | WelNdlyprobe2-F | GGACTTGCAGAGGCTTGAAC | WelNdlyprobe2-R | ATCGCAGTTTCTTTGCAGGT | 179 bp |
| *WelAG* | ProbWelAG-F | CGCTCTGCACAATCAACT | WelAGprofil-R | CCTGATGAGCATAGTGATGGGCA | 340 bp |
| *WelAP3/PI-1* | WelB1aF | CCGAGAAATTGGATGCAAGC | WelB1profil-R | GTAAGTATTATTTTGAAGGTTG | 197 bp |
| *WelAP3/PI-2* | WelB2bF | GATATGCAAAAAGAGAAAAATGAGAAT | ProbWelB2-R | CTGAACGTTGGGTTGCT | 339 bp |
| *WelBsister* | ProbWelBsister-F | GGAGCAGCAGCTGGAAACCGCAT | ProbeWelBsister-R | GGCTGAGTGGGTTGCAG | 299 bp |
| *WelAGL6* | ProbeWelAGL6-F | GGAAATCAACAAATCTCTACGCAAA | oEDW-WelAGL6-Stop | TCAGACTACCCATCCATGCATG | 271 bp |
| *WelActin* | WelActin-F | TTGCAATTCAGGCAGTTTTG | WelActin-R | GGCCACATATGCCAACTTCT | 260 bp |

| Oligonucleotides used for in situ probe synthesis | | | |
|---|---|---|---|
| Gene | Probe size | PCR oligonucleotides | |
| | | Name | Sequence (Sequence of T7 promoter in bold) |
| *WelLFY* | 291 bp | WLFinsR | CAAGTCTTTCCATGTAAGTCCT |
| | | WLFinsLT7 | **TAATACGACTCACTATAGGG**AGCCGAAAAGAAAAGGTTG |
| *WelNDLY* | 263 bp | WNDinsR2 | GTTTGTCCTCTGTTCATCACTGT |
| | | WelfxT7 | **TAATACGACTCACTATAGGG**ACTTGCAGAGGCTTG |
| *WelAP3/PI-1* | 225 bp | WelB1 aF | CCGAGAAATTGGATGCAAGC |
| | | ProbWelB1 RT7 | **TAATACGACTCACTATAGGG**CCACTCTCTGAAAGAGT |
| *WelAP3/PI-2* | 339 bp | WelB2 bF | GATATGCAAAAAGAGAAAAATGAGAAT |
| | | ProbWelB2 RT7 | **TAATACGACTCACTATAGGG**CTGAACGTTGGGTTGCT |
| *WelAG* | 341 bp | ProbWelAG F | CGCTCTGCACAATCAACT |
| | | ProbWelAG RT7 | **TAATACGACTCACTATAGGG**CCTGATGAGCATAGTGAT |

| Oligonucleotides used to construct expression plasmids | | | |
|---|---|---|---|
| Name | Sequence | Name | Sequence |
| oFL1001 | CCGCCATGGGGGAAGACAGGCAGAGGGAA | oFL1002 | GGCTCGAGTCAAAGATGGGACTGCTTGCT |
| oFL1003 | GGGCCATGGGAGAGGAGAGACCCAGAGAA | oFL1004 | CCCTCGAGTCAACATAATTTGCTTTTTTCCAAGTGAC |
| oETH1001 | CCACTACTGAGAATCTTTATTTTCAGGGCCAGTTCAG | oETH1002 | CCCAAACCACTACCTCCGTTGCCGTTATCCTGTTTGTATAGTTCATCCAT |
| oEDW-WelLFYmin40-F | ACACATATGAAGGAAATGGTTTGCCTAGAGGAGC | WelLFYnostop-Xho1-R | GGCTCGAGAAGATGGGACTGCTTGCT |
| oEDW-WelNDLYmin40-F | TTACATATGAAGGATCTGAAATCGCTTGAAGAT | WelNDLYnostop-Xho1-R | CCCTCGAGACATAATTTGCTTTTTTCCA |

| Oligonucleotides used in EMSA assays | | |
|---|---|---|
| Name | 5'-labeled TAMRA | Non-fluorescent |
| *AP1bs1* | TTGGGGAAGGACCAGTGGTCCGTACAATGT | ACATTGTACGGACCACTGGTCCTTCCCCAA |
| *AGbs2* | TGGATTTATACCCAATGTGTTAATGGGTTGT | ACAACCCATTAACACATTGGGTATAAATCCA |
| *AP3bs1* | CCTTCTTAAACCCTAGGGGTAATATTCTAT | ATAGAATATTACCCCTAGGGTTTAAGAAGG |

| Oligonucleotides used to clone *WelAP3/PI* upstream sequences and *WelTubulin* gene sequence | |
|---|---|
| Name | Sequence |
| Lambda786 | CGGAGTGGCTCACAGTCGGTGGTCCGGCAGTACAA |
| WelB1screenR2 | CGGAGTTTGGAGTCCAGCTTTTCCTTTTCG |
| WelB2screenR3 | TAAGAACCGTAACTGAACGTTGGGTTG |
| oEDW-WelTubulin-gene-F1 | GCCTTTAAACGACTTCTGTAAATA |
| oEDW-WelTubulin-gene-R1 | GGCTAACACAACAACAGAAGCAGAT |

| Oligonucleotides used to synthesize the DNA sequences for SPR analysis | | |
|---|---|---|
| Gene | 5'-Biotin (Forward) | Non-labelled (Reverse) |
| *WelAP3/PI-1* | CGGATCTGGGTCGACTCTAGGCCT | CCAGTCCACGCAAATAATCAGA |
| *WelAP3/PI-2* | GTAAAACGACGGCCAG | GGAGTTCTTGCTTAGAAGATCAC |
| *WelTubulin* | GGAAACAGCTATGACCATG | AGTGAACAAATGCCCGCTTGG |

**Supplementary Table 2** – B gene homologs identified in conifer species with sequenced genomes. The number of sites with scores > -20 in the 3.5kb upstream of the start codon is indicated. Best scores in upstream region of each gene are given to the right. Best scores across all B gene homologs within a species are indicated in white. na = not assessed as sequence identified was incomplete at 3' end.

| Species | Gene name | Accession or Scaffold number | Nb of sites with score > -20 | Best score |
|---|---|---|---|---|
| *Picea abies* | DAL11 | AF158539 | 4 | -16.60 |
| | DAL12 | AF15854 | 6 | -16.69 |
| | DAL13 | AF15843 | 9 | **-11.54** |
| *Picea glauca* | PgDAL11-1 | gb\|ALWZ025600346.1 | 4 | -18.24 |
| | PgDAL11-2 | gb\|ALWZ024195817.1 | 3 | -16.60 |
| | PgDAL12-1 | gb\|ALWZ026117334.1 | 3 | -16.69 |
| | PgDAL12-2 | gb\|ALWZ025965213.1 | 3 | -16.69 |
| | PgDAL13-1 | gb\|ALWZ022140061.1 | 4 | **-12.67** |
| | PgDAL13-2 | gb\|ALWZ026682569.1 | 6 | **-12.67** |
| *Pinus taeda* | PtDAL11 | tscaffold8598 | 6 | -15.95 |
| | PtDAL12 | C32411524 | na | na |
| | PtDAL13-1 | tscaffold786067 | 13 | **-8.97** |
| | PtDAL13-2 | tscaffold241828.2 | 8 | -15.26 |
| *Pinus lambertiana* | PlDAL11 | scaffold1561304 13 | 7 | -13.07 |
| | PlDAL12-1 | scaffold489614 12 | 5 | -18.30 |
| | PlDAL12-2 | scaffold1694510 12 | 0 | <-20 |
| | PlDAL13 | scaffold1190273 11 | 6 | **-9.22** |

**Supplementary Table 3** – Size-exclusion chromatography assays with recombinant $WelLFY_{DBD}$ and $WelNDLY_{DBD}$.

| | Elution Vol. (ml) | | Equivalent MW (kDa) | | Predicted nb. of molecules | |
|---|---|---|---|---|---|---|
| | Assay 1 | Assay 2 | Assay 1 | Assay 2 | Assay 1 | Assay 2 |
| $WelLFY_{DBD}$ | 88.5 | 91.5 | 24.0 | 18.6 | 1.05 | 0.81 |
| $WelNDLY_{DBD}$ | 91.5 | 90 | 18.6 | 21.1 | 0.82 | 0.93 |

**Supplementary Table 4** – Position Weight Matrix (PWM) obtained for WelLFYΔ and AtLFYΔ.

| | WelLFYΔ | | | |
|---|---|---|---|---|
| | **A** | **T** | **C** | **G** |
| **1** | -2.43 | 0 | -0.25 | -0.48 |
| **2** | 0 | -1.27 | -3.61 | -2.80 |
| **3** | -2.59 | 0 | -2.33 | -3.21 |
| **4** | Lateral triplet 4-5-6 | | | |
| **5** | | | | |
| **6** | | | | |
| **7** | -3.48 | -4.46 | 0 | -4.46 |
| **8** | -4.68 | -4.55 | 0 | -4.55 |
| **9** | Central triplet 9-10-11 | | | |
| **10** | | | | |
| **11** | | | | |
| **12** | -3.71 | -4.68 | -4.55 | 0 |
| **13** | -4.39 | -3.48 | -4.46 | 0 |
| **14** | Lateral triplet 14-15-16 | | | |
| **15** | | | | |
| **16** | | | | |
| **17** | 0 | -2.59 | -3.21 | -2.33 |
| **18** | -1.27 | 0 | -2.80 | -3.61 |
| **19** | 0 | -2.43 | -0.48 | -0.25 |

| | AtLFYΔ | | | |
|---|---|---|---|---|
| | **A** | **T** | **C** | **G** |
| **1** | -1.40 | 0 | -0.92 | -0.01 |
| **2** | 0 | -2.45 | -2.25 | -1.44 |
| **3** | -0.76 | -1.63 | -1.97 | 0 |
| **4** | Lateral triplet 4-5-6 | | | |
| **5** | | | | |
| **6** | | | | |
| **7** | -2.31 | 0 | -4.80 | -3.85 |
| **8** | -5.03 | 0 | -5.14 | -2.13 |
| **9** | Central triplet 9-10-11 | | | |
| **10** | | | | |
| **11** | | | | |
| **12** | -2.13 | -5.14 | 0 | -5.03 |
| **13** | -3.85 | -4.80 | 0 | -2.31 |
| **14** | Lateral triplet 14-15-16 | | | |
| **15** | | | | |
| **16** | | | | |
| **17** | 0 | -1.97 | -1.63 | -0.76 |
| **18** | -1.44 | -2.25 | -2.45 | 0 |
| **19** | -0.01 | -0.92 | 0 | -1.40 |

Lateral triplet 4-5-6

| WelLFYΔ | | | | | | |
|---|---|---|---|---|---|---|
| | | 2nd position | | | | |
| | | **A** | **C** | **G** | **T** | |
| 1st position | **A** | -5.68 | -6.09 | -3.12 | -6.09 | **A** |
| | | -4.99 | -6.09 | -5.68 | -6.09 | **C** |
| | | -6.09 | -6.09 | -6.09 | -6.09 | **G** |
| | | -6.09 | -6.09 | -5.17 | -6.09 | **T** |
| | **C** | -6.09 | -6.09 | -4.58 | -6.09 | **A** |
| | | -4.48 | -6.09 | -5.68 | -6.09 | **C** |
| | | -6.09 | -6.09 | -5.68 | -6.09 | **G** |
| | | -6.09 | -6.09 | -5.40 | -6.09 | **T** |
| | **G** | -5.17 | -5.17 | -0.71 | -3.89 | **A** |
| | | -3.79 | -5.40 | -3.56 | -6.09 | **C** |
| | | -6.09 | -6.09 | -3.29 | -5.68 | **G** |
| | | -5.40 | -5.68 | -3.29 | -5.68 | **T** |
| | **T** | -2.32 | -5.40 | -2.36 | -5.68 | **A** |

3rd position

| AtLFYΔ | | | | | | |
|---|---|---|---|---|---|---|
| | | 2nd position | | | | |
| | | **A** | **C** | **G** | **T** | |
| 1st position | **A** | -3.29. | -6.15 | -1.45 | -4.36 | **A** |
| | | -3.55 | -5.23 | -4.01 | -5.75 | **C** |
| | | -4.65 | -6.15 | -6.15 | -5.75 | **G** |
| | | -3.62 | -6.15 | -4.07 | -6.15 | **T** |
| | **C** | -4.54 | -6.15 | -3.41 | -3.90 | **A** |
| | | -3.62 | -6.15 | -4.07 | -6.15 | **C** |
| | | -5.75 | -5.75 | -6.15 | -5.75 | **G** |
| | | -4.28 | -6.15 | -3.95 | -5.05 | **T** |
| | **G** | -3.23 | -5.75 | -0.84 | -2.95 | **A** |
| | | -3.06 | -6.15 | -3.62 | -6.15 | **C** |
| | | -5.23 | -6.15 | -3.71 | -6.15 | **G** |
| | | -3.32 | -6.15 | -2.84 | -6.15 | **T** |
| | **T** | -1.19 | -5.46 | -1.62 | -4.28 | **A** |

3rd position

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | -3.14 | -2.29 | -3.65 | **C** | |
| | | -3.84 | -6.09 | -3.95 | -6.09 | **G** | |
| | | -1.22 | -4.70 | -1.14 | -4.70 | **T** | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | -3.51 | -1.94 | -2.44 | **C** | |
| | | -3.38 | -5.75 | -4.76 | -5.75 | **G** | |
| | | -1.50 | -6.15 | -1.53 | -4.90 | **T** | |

Central Triplet 9-10-11

| | | WelLFYΔ 2nd position | | | | | |
|---|---|---|---|---|---|---|---|
| | | **A** | **C** | **G** | **T** | | |
| 1st position | **A** | -5.73 | -5.73 | -3.94 | -5.73 | **A** | 3rd position |
| | | -1.03 | -2.16 | 0 | -2.42 | **C** | |
| | | -5.73 | -4.48 | -3.78 | -5.73 | **G** | |
| | | -3.78 | -1.91 | -1.91 | -3.78 | **T** | |
| | **C** | -5.73 | -5.73 | -5.73 | -5.73 | **A** | |
| | | -5.73 | -4.23 | -3.59 | -5.04 | **C** | |
| | | -5.73 | -5.73 | -5.73 | -5.73 | **G** | |
| | | -5.73 | -3.78 | -4.48 | -5.73 | **T** | |
| | **G** | -5.04 | -3.20 | -3.33 | -5.04 | **A** | |
| | | -3.86 | -2.84 | -2.84 | -3.86 | **C** | |
| | | -5.04 | -3.59 | -4.23 | -5.73 | **G** | |
| | | -2.42 | 0 | -2.16 | -1.03 | **T** | |
| | **T** | -5.73 | -5.73 | -5.73 | -5.73 | **A** | |
| | | -5.04 | -3.33 | -3.20 | -5.04 | **C** | |
| | | -5.73 | -5.73 | -5.73 | -5.73 | **G** | |
| | | -5.73 | -3.94 | -5.73 | -5.73 | **T** | |

| | | AtLFYΔ 2nd position | | | | | |
|---|---|---|---|---|---|---|---|
| | | **A** | **C** | **G** | **T** | | |
| 1st position | **A** | -3.74 | -3.84 | -3.47 | -4.43 | **A** | 3rd position |
| | | -0.15 | -1.12 | 0 | -1.46 | **C** | |
| | | -3.55 | -3.27 | -2.90 | -4.65 | **G** | |
| | | -2.13 | -1.19 | -1.19 | -2.13 | **T** | |
| | **C** | -4.94 | -5.34 | -5.34 | -5.34 | **A** | |
| | | -3.96 | -2.30 | -2.71 | -3.74 | **C** | |
| | | -4.65 | -4.65 | -4.65 | -4.65 | **G** | |
| | | -4.65 | -2.90 | -3.27 | -3.55 | **T** | |
| | **G** | -3.09 | -2.90 | -2.30 | -3.96 | **A** | |
| | | -1.88 | -1.40 | -1.40 | -1.88 | **C** | |
| | | -3.74 | -2.71 | -2.30 | -3.96 | **G** | |
| | | -1.46 | 0 | -1.12 | -0.15 | **T** | |
| | **T** | -4.94 | -5.34 | -5.34 | -4.94 | **A** | |
| | | -3.96 | -2.30 | -2.90 | -3.09 | **C** | |
| | | -5.34 | -5.34 | -5.34 | -4.94 | **G** | |
| | | -4.43 | -3.47 | -3.84 | -3.74 | **T** | |

Lateral Triplet 14-15-16

| | | WelLFYΔ 2nd position | | | | | |
|---|---|---|---|---|---|---|---|
| | | **A** | **C** | **G** | **T** | | |
| 1st position | **A** | -4.70 | -1.14 | -4.70 | -1.22 | **A** | 3rd position |
| | | -5.68 | -3.29 | -5.68 | -5.40 | **C** | |
| | | -6.09 | -5.40 | -6.09 | -6.09 | **G** | |
| | | -6.09 | -5.17 | -6.09 | -6.09 | **T** | |
| | **C** | -6.09 | -3.95 | -6.09 | -3.84 | **A** | |
| | | -5.68 | -3.29 | -6.09 | -6.09 | **C** | |
| | | -6.09 | -5.68 | -6.09 | -6.09 | **G** | |
| | | -6.09 | -6.09 | -6.09 | -6.09 | **T** | |
| | **G** | -3.65 | -2.29 | -3.14 | 0 | **A** | |
| | | -6.09 | -3.56 | -5.40 | -3.79 | **C** | |
| | | -6.09 | -5.68 | -6.09 | -4.48 | **G** | |
| | | -6.09 | -5.68 | -6.09 | -4.99 | **T** | |
| | **T** | -5.68 | -2.36 | -5.40 | -2.32 | **A** | |
| | | -3.89 | -0.71 | -5.17 | -5.17 | **C** | |
| | | -6.09 | -4.58 | -6.09 | -6.09 | **G** | |
| | | -6.09 | -3.12 | -6.09 | -5.68 | **T** | |

| | | AtLFYΔ 2nd position | | | | | |
|---|---|---|---|---|---|---|---|
| | | **A** | **C** | **G** | **T** | | |
| 1st position | **A** | -4.90 | -1.53 | -6.15 | -1.50 | **A** | 3rd position |
| | | -6.15 | -2.84 | -6.15 | -3.32 | **C** | |
| | | -5.05 | -3.95 | -6.15 | -4.28 | **G** | |
| | | -6.15 | -4.07 | -6.15 | -3.62 | **T** | |
| | **C** | -5.75 | -4.76 | -5.75 | -3.38 | **A** | |
| | | -6.15 | -3.71 | -6.15 | -5.23 | **C** | |
| | | -5.75 | -6.15 | -5.75 | -5.75 | **G** | |
| | | -5.75 | -6.15 | -6.15 | -4.65 | **T** | |
| | **G** | -3.44 | -1.94 | -3.51 | 0 | **A** | |
| | | -6.15 | -3.62 | -6.15 | -3.06 | **C** | |
| | | -6.15 | -4.07 | -6.15 | -3.62 | **G** | |
| | | -5.75 | -4.01 | -5.23 | -3.55 | **T** | |
| | **T** | -4.28 | -1.62 | -5.46 | -1.19 | **A** | |
| | | -2.95 | -0.84 | -5.75 | -3.23 | **C** | |
| | | -3.90 | -3.41 | -6.15 | -4.54 | **G** | |
| | | -4.36 | -1.45 | -6.15 | -3.29 | **T** | |

**Supplementary Table 5** – Examples of sequences (chosen from the 100 most frequent sequences) isolated in the SELEX-seq assays performed with WelNDLYΔ. Eighteen sequences clearly resembling those bound by $AtLFY_{DBD}$ are highlighted in blue, while the majority of sequences (in yellow) are very different (score < -20).

| **Sequence ID** | **Sequence** | **Score calculated with $AtLFY_{DBD}$ Matrix** |
|---|---|---|

| | | |
|---|---|---|
| WelNDLY-NC 19 | TATTACCCAGTGGATAATAGTTCTCTAGAT | -2,31 |
| WelNDLY-NC 1 | CCAGCGCAGGATTACCCAGCGGTCCATGCT | -2,58 |
| WelNDLY-NC 97 | AGGTCGCATGGACCGATGGGTAATAGCGTC | -3,25 |
| WelNDLY-NC 13 | GTTTATCCGTTGGGTAATAGCTCTCTAGAT | -3,94 |
| WelNDLY-NC 2 | TTTTACCCAGCGGACAATCAATGGGTGTAT | -4,39 |
| WelNDLY-NC 73 | AACTACGGACCGCTGGGTAAAGGCGTGGTC | -4,41 |
| WelNDLY-NC 7 | TCTTGTTGGGCATTGTCCGCTGGGTAAAGA | -4,51 |
| WelNDLY-NC 74 | TCTTGTTGGGCATTGTCCGCTGGGTAAAGA | -4,51 |
| WelNDLY-NC 80 | CGTTATCCAGCGGGTAATAGGGGGGGAGAC | -4,59 |
| WelNDLY-NC 23 | TTTGAGTATTACCCGTTGGTTAACAGCCAA | -4,89 |
| WelNDLY-NC 65 | AATGGTGCTAGACGGACCGCTGGGTAATCG | -4,96 |
| WelNDLY-NC 67 | GATGGACCGATGGATAATATCCGTGTCGCT | -4,98 |
| WelNDLY-NC 18 | TAGTGCATAGACCGTTGGGTAATGACCGCA | -5,04 |
| WelNDLY-NC 43 | AGTGTGCAATTGTCCAGCGGTTAATAAGGG | -5,68 |
| WelNDLY-NC 77 | CCAGCGCAGGATTACCCAGCGGGCCATGCT | -6,61 |
| WelNDLY-NC 78 | CCAACGGTTATTGCCCGGTGGTCCTTACGC | -7,05 |
| WelNDLY-NC 45 | CAAACGAGGGGACGGACCGCTGGATAACGA | -8,35 |
| WelNDLY-NC 55 | ATATGCTTCGGACCGACGGTTAATAGCGGA | -9,21 |
| WelNDLY-NC 85 | ACGTTCAGGTACACAGTAGTTACAACATC | -20,79 |
| WelNDLY-NC 53 | TGTTCGCCGGGGCTATGTCCGAGGTTGTAAG | -21,17 |
| WelNDLY-NC 87 | AATAACGGAAGGCTGGAACGTTTCAGCCAA | -21,84 |
| WelNDLY-NC 63 | TTTGTGCCGCTGAATCGATTCCGTAGCTGT | -22,22 |
| WelNDLY-NC 91 | TGATGCTACGCCGAGCAGAACCTTGGGACA | -22,40 |
| WelNDLY-NC 21 | TGATATACCAAGTCTAATGCATAGGTCTCT | -22,58 |
| WelNDLY-NC 22 | TGATATACCAAGTCTAATGCATAGGTCTCT | -22,58 |
| WelNDLY-NC 54 | GTGATATACCAAGTCTAATGCATAGGTCTC | -22,58 |
| WelNDLY-NC 86 | GTGCCATCTAAGAGTCCGATGTTGTAACTA | -22,88 |
| WelNDLY-NC 39 | ACCTTTAGTCCGCAGGCAGCGGTCCGGAT | -23,13 |
| WelNDLY-NC 16 | GGATTCGTACAACGGCACGCGCGGAGCATC | -23,21 |
| WelNDLY-NC 47 | TCGGATTCGTACAACGGCACGCGCGGAGCAT C | -23,21 |
| WelNDLY-NC 41 | GTTATAACGTTCGTTAGGGCCTCCCCTACTC | -23,59 |
| WelNDLY-NC 44 | GTTATAACGTTCGTTAGGGCCTCCTTCAGAT | -23,59 |
| WelNDLY-NC 68 | GTTATAACGTTCGTTAGGGCCTCCTCTGGAT | -23,59 |
| WelNDLY-NC 72 | TTCGTCGTTGGAACAACTCGTTAGAATTGC | -24,37 |
| WelNDLY-NC 95 | TGAATCGATTTAGTCGGACACAGGGTTGTG | -24,90 |
| WelNDLY-NC 90 | AAGATTGTAGCAAGCGTTAAATTGCCGTCT | -25,45 |
| WelNDLY-NC 5 | TGACCGCTGTGTAATAAGATGGTCTAGAG | -25,51 |
| WelNDLY-NC 42 | TGACCGCTGTGTAATAAGATGGTCTAGAT | -25,51 |
| WelNDLY-NC 69 | TATCCGAGAGGCTCAGCCCGTGGCACGACA | -25,51 |
| WelNDLY-NC 31 | TGGACCATGTCGCCAAGTTCGTTAATGTCAA | -25,53 |
| WelNDLY-NC 30 | TAACAAGACACCTGCCAGTTTTCGTAACAT | -25,65 |
| WelNDLY-NC 38 | TAACAAGACACCTGCCAGCTTTCGTACCAT | -25,74 |
| WelNDLY-NC 94 | ACACCCCTACCAAGGTTAGTTACAACATCG | -25,82 |
| WelNDLY-NC 70 | TTATCGCGGCCTGATGTATGTTGTAACTAT | -26,07 |
| WelNDLY-NC 28 | CGCGTTATAACGTTCGTTAGGGCCTCCTTC | -26,08 |
| WelNDLY-NC 36 | CGCGTTATAACGTTCGTTAGGGCCTCCCCT | -26,08 |
| WelNDLY-NC 58 | CGCGTTATAACGTTCGTTAGGGCCTCCTCT | -26,08 |
| WelNDLY-NC 61 | CGCGTTATAACGTTCGTTAGGGCCTCCCCT | -26,08 |
| WelNDLY-NC 12 | TTTTGCTCATCGCTGCTATGGGCATGAACT | -26,09 |
| W12 | CGCAAGATGCTGTACCGATTCGGCCGCAAC | -26,23 |

| | | |
|---|---|---|
| WelNDLY-NC 71 | ATGTAAGCGTCGTGTTGAATCGTTTCAGTT | -26,33 |
| WelNDLY-NC 66 | AATTAGCAGATGGGTGCAAAATCGATTCAG | -26,36 |
| WelNDLY-NC 75 | AGTTACCCTAACGAGCTCTTAGCGACGGGC | -26,39 |
| WelNDLY-NC 82 | TGACCGCTGGGTAAAGACGGAGGAGGCATGT | -26,39 |
| WelNDLY-NC 46 | TTGCTGTAGCACGGTGGGAACGTTCCCGTTT | -26,51 |
| WelNDLY-NC 3 | TAACAAGACACCTGCCAGTTTTCGTACCAT | -26,85 |
| WelNDLY-NC 4 | TAACAAGACACCTGCCAGTTTTCGTACCAT | -26,85 |
| WelNDLY-NC 6 | GGATAACAAGACACCTGCCAGTTTTCGTAC | -26,85 |
| WelNDLY-NC 10 | GATAACAAGACACCTGCCAGTTTTCGTACC | -26,85 |
| WelNDLY-NC 14 | TAACAAGACACCTGCCAGTTTTCGTACCAT | -26,85 |
| WelNDLY-NC 24 | GATAACAAGACACCTGCCAGTTTTCGTACC | -26,85 |
| WelNDLY-NC 83 | ATAACAAGACACCTTCCAGTTTTCGTACCA | -26,85 |
| WelNDLY-NC 49 | TGACCGCTGGTCAATATCCACTTCGTGAGTC | -26,93 |
| WelNDLY-NC 64 | GTGCTGACGGAGGACACGTGTTAGGTAAGAA | -26,94 |
| WelNDLY-NC 9 | CTCATCGCTGCTATGGGCATGAACTCTAGAT | -27,40 |
| WelNDLY-NC 17 | GCTCATCGCTGCTATGGGCATGAACTCTAG | -27,40 |
| WelNDLY-NC 20 | TTGCTCATCGCTGCTATGGGCATGAACTCTA | -27,40 |
| WelNDLY-NC 33 | GCTCATCGCTGCTATGGGCATGAACTCTAG | -27,40 |
| WelNDLY-NC 35 | CTCATCGCTGCTATGGGCATGAACTCTAGAC | -27,40 |
| WelNDLY-NC 62 | TCATCGCTGCTATGGGCATGAACTCTAGAC | -27,40 |
| WelNDLY-NC 59 | ACGGACGATGGCAGATGGATGTTGTAACTA | -27,42 |
| WelNDLY-NC 60 | GTCGACCTGCTTATCTGTACTCACTCCTCT | -27,54 |
| WelNDLY-NC 52 | ATGCTCGTTAGCGTTAACGCGTCGCATATC | -27,92 |
| WelNDLY-NC 32 | GCTCATCGCTGCTATGGGCATGGACTCTAG | -28,06 |
| WelNDLY-NC 56 | CTCATCGCTGCTATGGGCATGGACTCTAGA | -28,06 |
| WelNDLY-NC 48 | GACCACGAACGGCTTTGTATGTTGTAACTA | -28,12 |
| WelNDLY-NC 96 | TAACAAGACACCTGCCAGTTTTCGCACCAT | -28,17 |
| WelNDLY-NC 15 | AGTCGACCTGCTTATCTGTGCTCACTCCTCT | -28,20 |
| WelNDLY-NC 25 | GAGTCGACCTGCTTATCTGTGCTCACTCCTCT | -28,20 |
| WelNDLY-NC 26 | GAGTCGACCTGCTTATCTGTGCTCACTC | -28,20 |
| WelNDLY-NC 50 | AGTCGACCTGCTTATCTGTGCTCACTCCTCT | -28,20 |
| WelNDLY-NC 81 | GAGTCGACCTGCTTATCTGTGCTCACTCCTC | -28,20 |
| WelNDLY-NC 84 | GTCGACCTGCTTATCTGTGCTCACTCCTCT | -28,20 |
| WelNDLY-NC 40 | TGAATCGTTACAGCAGCACGGGCGGTCAAA | -28,27 |
| WelNDLY-NC 27 | CGACCTGCTTATCTATGCTCACTCCTCTGG | -29,13 |
| WelNDLY-NC 76 | ATGGGCCACATGCAAAATCGTTTCAGCACT | -29,13 |
| WelNDLY-NC 92 | ACTAACGCATTGGGATCGTTCCCCCTGTGT | -29,43 |
| WelNDLY-NC 89 | GACAGGGGATAGCCGAATCGTTTCAGCGTC | -29,98 |
| W10 | CGCAAGATGCTGAACCGCTCCAGCCGCAAC | -30,41 |
| WelNDLY-NC 29 | TGTCCGCTGGGTAATAGTGCACCTCCAACTC | -30,85 |
| WelNDLY-NC 93 | TAACAAGACACCTGCCAGGTTTCGTACCAT | -30,94 |
| WelNDLY-NC 88 | CATACGAAGACACGTGACGAATCGATTCAG | -31,51 |
| WelNDLY-NC 51 | TTTGCTGAATCGTTTCTGCGCCCTCCGAAC | -32,20 |

## Supplementary Methods

### Isolation of *Welwitschia* sequences and phylogenetic analyses

Total RNAs were extracted with TRIzol reagent (Invitrogen) from leaves or dissected cones. For each sample, 2 µg of total RNA was treated with RNAse-free DNAse (Ambion) and quantified using a NanoDrop-ND100 (NanoDrop Technologies). cDNA was synthesized from 1 µg of total RNA with the RevertAid M-MuLV RT (Fermentas), using oligo-dT primers. *WelAG* (KF145186), *WelAP3/PI-1* (KF145184), *WelAP3/PI-2* (KF145185) and *WelBsister* (KF145187) were isolated using degenerate primers (see SI Table 5) and 3' and 5' RACE kits (Invitrogen). The sequences of *P. abies* actin (ACP1972) and *G. gnemon AGL6* (*GGM13*, CAB44459) were used as query for a BLAST search against a *Welwitschia* EST database (Albert *et al*., 2005). EST contig assembly was performed with DNA BASER Sequence Assembler 2.6 and the complete coding sequences of *WelActin* and *WelAGL6* (KF145188) were subsequently obtained by PCR using cDNA from mixed cones as template, Phusion® DNA polymerase (Ozyme) and specific primers (see SI Table 5). Phylogenetic placement was evaluated using three different methods. First, our *Welwitschia* and GenBank *Gnetum gnemon* and *G. parvifolium* MADS-box amino acid sequences (CAB44448, CAB44449, CAB44455, CAB44457, CAB44459, CAC13991, CAC13992, CAC13993, BAA85629, BAA85630, BAA85631) were aligned with MUSCLE 3.6 using default settings (Edgar, 2004) and the nucleotide sequences were then force-aligned based on the amino acid alignment using PAL2NAL (Suyama *et al*., 2006). Poorly aligned regions were removed, third codon positions were down weighted, and 1000 parsimony bootstraps were performed using PAUP* 4.0, and using amino acid characters with a Blosum62 derived stepmatrix (Hill *et al*., 2006). A second phylogenetic analysis was performed from the amino acid alignment of MADS genes from (Winter *et al*., 2002). The *Welwitschia* genes were added by hand, and 1000 neighbour-joining bootstraps were performed using PAUP* 4.0, with a character weight matrix based on the Blosum62 matrix. In our third phylogenetic analysis, the alignment from Becker and Theissen (Becker & Theißen, 2003), with added *Welwitschia* and *Gnetum* genes (except GGM17) was analyzed within SeaView 4.3.0 (Gouy *et al*., 2010). First it was degapped and realigned by MUSCLE, then a tree was calculated by PhyML with branch support evaluated by the approximate likelihood ratio test (aLRT). The model used was blosum62; otherwise defaults were used.

### Semi-quantitative RT-PCR

PCR reactions were carried out in 50 µl using cDNA derived from 50 ng of DNAse-treated total RNA as template. 5 µl were taken after 25, 30 and 35 cycles and visualized on 2% agarose gels stained with SYBR®Safe (Invitrogen). A fragment of *Welwitschia* actin cDNA was amplified as a control under the same conditions but samples were taken after 20, 25 and 30 cycles. Primer combinations used are given in SI Table 5.

### *In situ* hybridization

Following a fixation step in 4% paraformaldehyde, tissues were dehydrated and progressively embedded in Paraplast X-Tra™ (Tyco/Healthcare). Embedded cones were cut at 7 µm thick and sections mounted on polylysine coated glass slides (ProbeOn Plus, Fisher Biotech). Wax removal, proteinase K treatment (Invitrogen), paraformaldehyde fixation and probe hybridization were performed according to the J. Long protocol (www.its.caltech.edu/~plantlab/protocols/insitu.pdf). Probes were designed against the most specific region of each gene of interest: for *WelLFY* and *WelNDLY*, a probe corresponding to the variable sequence spanning exons 1 and 2 of each gene was amplified. For *Welwitschia* MADS-box genes, a probe matching the characteristic C-terminal end of each gene was used. Probes were synthesized according to (Drea *et al*., 2005): cDNA corresponding to

*WelAP3/PI-1*, *WelAP3/PI-2* and *WelAG* cloned in pCR2.1 vectors (Invitrogen), pEDW84 or pEDW87 were used as templates in PCR reactions with GoTaq DNA polymerase (Promega) and the primers given in SI Table 5. The PCR products obtained were used as templates for the *in vitro* transcription step with DIG RNA Labelling Kit (Roche) according to the manufacturer's instructions. Probe quality and concentration were checked with a BioAnalyzer 2100 (Agilent). The Frohlich and Moyroud illumination method (Frohlich & Moyroud, 2008) was used to minimize the effects of light scattering that can make dense tissues appear dark. Multiple *in situ* hybridization attempts with female cones were unsuccessful.

**Expression plasmid construction**

*$WelLFY_{DBD}$ and $WelNDLY_{DBD}$ expression plasmids*.

Residues 247-411 from *WelLFY* cDNA (AF109130) and residues 245-407 from *WelNDLY* cDNA (AF108227) were amplified with Phusion® DNA polymerase (Ozyme) and primers given in SI Table 5, subcloned into pCR-Blunt (Invitrogen) and shuttled to pETM-11 (Dümmler *et al*., 2005) as *Nco*I/*Xho*I fragments to yield the pFLO3 and pFLO4 expression vectors.

*GFP-$WelNDLY_{DBD}$ expression plasmid*.

A GFP fragment was amplified from pBS-GLFY plasmid obtained from X. Wu (Wu *et al*., 2003) using primers oETH1001 and oETH1002. This fragment was subsequently used as a megaprimer to amplify plasmid pFLO4 and yield pETH25.

*AtLFYΔ, WelLFYΔ and WelNDLYΔ expression plasmids*.

Residues 40-424 from *AtLFY* cDNA (AAM27940), residues 58-411 from *WelLFY* cDNA and residues 56-407 from *WelNDLY* cDNA were amplified with Phusion® DNA polymerase (Ozyme) and primers given in SI Table 5, subcloned into pCR-Blunt (Invitrogen) and shuttled to pETM-30a+ as a *Nde*I/*Xho*I fragment to yield the pETH79, pEDW29 and pEDW49 expression vectors.

**Protein expression and purification**

pFLO3 and pFLO4 vectors were used to produce recombinant $WelLFY_{DBD}$ and $WelNDLY_{DBD}$ proteins according to the protocol previously described for $LFY_{DBD}$ from *A. thaliana* (Hamès *et al*., 2008). AtLFYΔ, WelLFYΔ and WelNDLYΔ were expressed using *Escherichia coli* strain Rosetta™ 2(DE3)pLys (Novagen) transformed with pETH79, pEDW29 or pEDW49 respectively. After induction by 0.5 mM IPTG, cells were grown overnight at 17°C. The pellet from a 1 l culture was sonicated in 50 ml lysis buffer (50 mM Tris-HCl pH8, 5 mM Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) with one protease inhibitor cocktail table Complete EDTA-free (Roche) and centrifuged for 45 minutes at 16500 rpm. The supernatant was loaded on a column with 1 ml Ni-NTA resin (Qiagen), then washed with 35 ml of wash buffer (50 mM Tris-HCl pH8, 20 mM imidazole, 5 mM TCEP) and eluted with the same buffer containing 350 mM imidazole. The fractions containing the protein were pooled and dialysed overnight in 50 mM Tris-HCl pH8, 5 mM TCEP buffer at 4°C. Next, the proteins were exposed to a salt shock with 0.8 M NaCl to remove the bacterial DNA bound to the proteins and the Ni-NTA purification step was repeated as previously except that 0.8 M NaCl was added to the wash and elution buffers. The fractions containing the proteins were pooled and applied to a Hi-load Superdex-200 16/60 prep grade column (GE Healthcare) equilibrated with 20 mM Tris-HCl pH8, 0.8 M NaCl and 5 mM TCEP to eliminate aggregated protein by size exclusion chromatography.

**EMSA assay**

The double stranded DNA probes used (Fig. 4A-C) were generated by annealing single-stranded oligonucleotides, 5'-labeled with TAMRA (Sigma), to non-fluorescent complementary oligonucleotides in annealing buffer (10 nM Tris pH7.5, 150 mM NaCl and 1 mM EDTA). The corresponding sequences are listed in SI Table 5. For each reaction, 10 nM fluorescent dsDNA was incubated with the protein in 20 $\mu$l binding buffer (20 mM Tris–HCl pH 7.5, 150 mM NaCl, 1% glycerol, 0.25 mM EDTA, 2 mM MgCl2, 28 ng/ml fish sperm DNA (Roche) and 3 mM DTT). For Fig. 4D and Fig. 5D, DNA probes were generated by annealing single-stranded oligonucleotides (with a protruding G on one end) in annealing buffer (10 nM Tris pH7.5, 150 mM NaCl and 1 mM EDTA). Labelling of 4 pmol dsDNA was then performed by end-filling using 8 pmol Cy3-dCTP and 1 U of Klenow fragment for 1 h at 37 °C, followed by enzyme inactivation at 65 °C for 10 min. For each reaction, 10 nM fluorescent dsDNA was incubated with the protein in 20 $\mu$l binding buffer (10 mM Hepes pH 7.5, 1 mM spermidine, 1% glycerol, 14 mM EDTA pH 8, 0.3 mg/ml BSA, 0.25 % CHAPS, 28 ng/ml fish sperm DNA (Roche) and 3 mM TCEP). After 15 min incubation on ice, binding reactions were loaded onto native 6% polyacrylamide gels 0.5X TBE and electrophoresed at 90 V for 90 min at 4°C. Gels were scanned on a Typhoon 9400 scanner (Molecular Dynamics, Sunnyvale, CA).

**Cloning of *WelAP3/PI-1* and *WelAP3/PI-2* upstream sequences and *WelTubulin* genomic locus**

A $^{32}$P labelled DNA probe corresponding to the *WelAP3/PI-1* and *WelAP3/PI-2* PCR products (see *in situ* hybridization) was used to probe four lambda genomic sublibraries as in (Frohlich & Meyerowitz, 1997). The upstream sequences of the two corresponding genes were retrieved by PCR with Phusion® DNA polymerase (Ozyme), using the selected lambda phage plaques as templates and primer Lambda786 matching within the lambda vector and the gene-specific primer WelB1screenR2 or WelB2screenR3 (see SI Table 5). Both PCR fragments were cloned in pCR-Blunt to yield the new vectors pEDW131 and pEDW130, containing the *WelAP3/PI-1* or *WelAP3/PI-2* upstream sequence respectively (KF145189, KF145190). The *WelTubulin* sequence was identified using the *Picea wilsonii* tubulin sequence (ABX57816) to perform a BLAST search against a *Welwitschia* EST database (Albert *et al*., 2005). The *WelTubulin* genomic locus was amplified with Phusion® DNA polymerase (Ozyme) using genomic DNA as a template and the primers oEDW-WelTubulin-gene-F1 and oEDW-WelTubulin-gene-R1 (see SI Table 5). The fragment obtained was cloned in pCR-Blunt to yield the vector pEDW64.

**SPR analysis of DNA-protein interaction**

Double-stranded DNA molecules for SPR analysis were synthesized by PCR amplification from pEDW131, pEDW130 and pEDW64 plasmids using the primers listed in SI Table 5. PCR products were purified using a NucleoSpin® Extract II kit (Macherey-Nagel) and quantified with a NanoDrop-ND100 (NanoDrop Technologies). Chip immobilisation and sensogram recording were performed according to (Moyroud *et al*., 2009). Real-time SPR interaction curves were analysed using the BiacoreT100 analysis software. For each analysis, the response of the reference channel was subtracted from the interaction curves obtained from the 3 experimental channels. These normalized curves were fitted globally to a heterogeneous ligand interaction model, permitting the determination of two apparent dissociation constants ($K_D^{App}$), corresponding to the presence of two types of binding sites. The validity of the interaction model was verified by data fitting ($\chi^2$<10). $K_D^{App}$ values in the nM range were considered as indicative of high affinity binding events, while $K_D^{App}$ values in the uM range were regarded as low affinity interactions (Moyroud *et al*., 2009).

**Identification of WelLFY binding sites in *WelAP3/PI-1*, *WelAP3/PI-2* and in conifer homologs of *DAL11*, *12* and *13* upstream sequences**

The predicted affinity of every possible 19-mer within *WelAP3/PI-1*, *WelAP3/PI-2* and conifer homologs of *DAL11*, *DAL12* and *DAL13* upstream sequences was calculated as previously described (Moyroud *et al*., 2011) using WelLFYΔ PWM as a model and previously established scripts in Python (http://biodev.cea.fr/morpheus/) for automatic data processing.

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