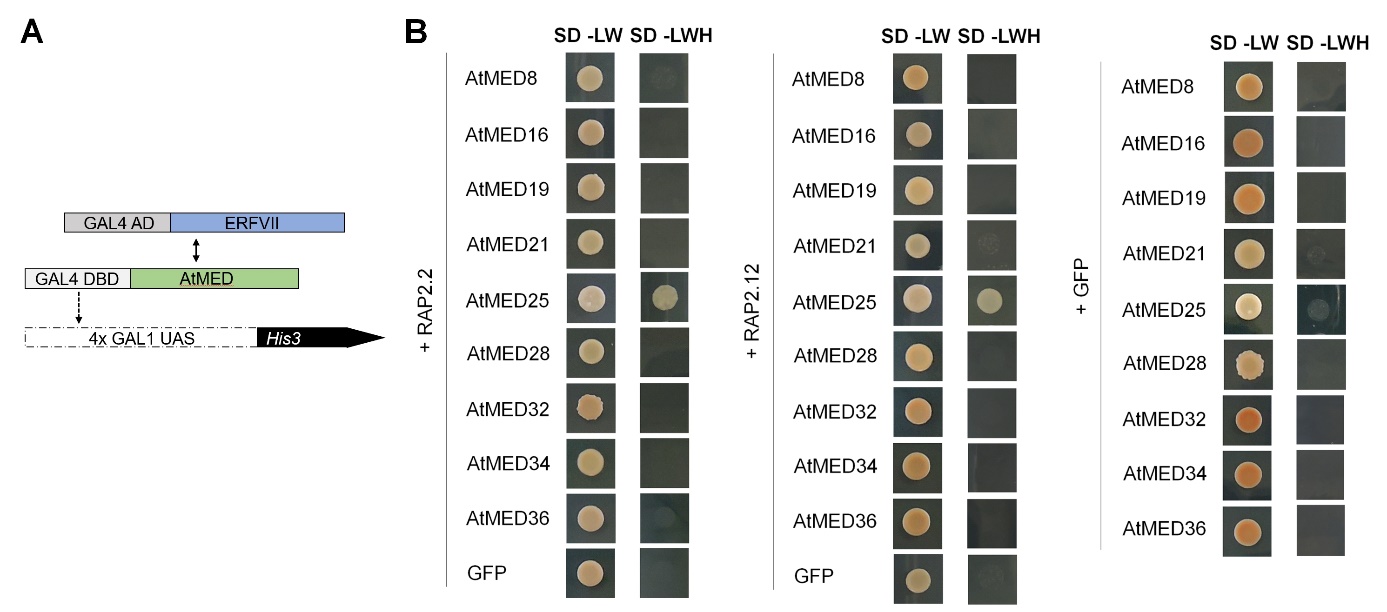
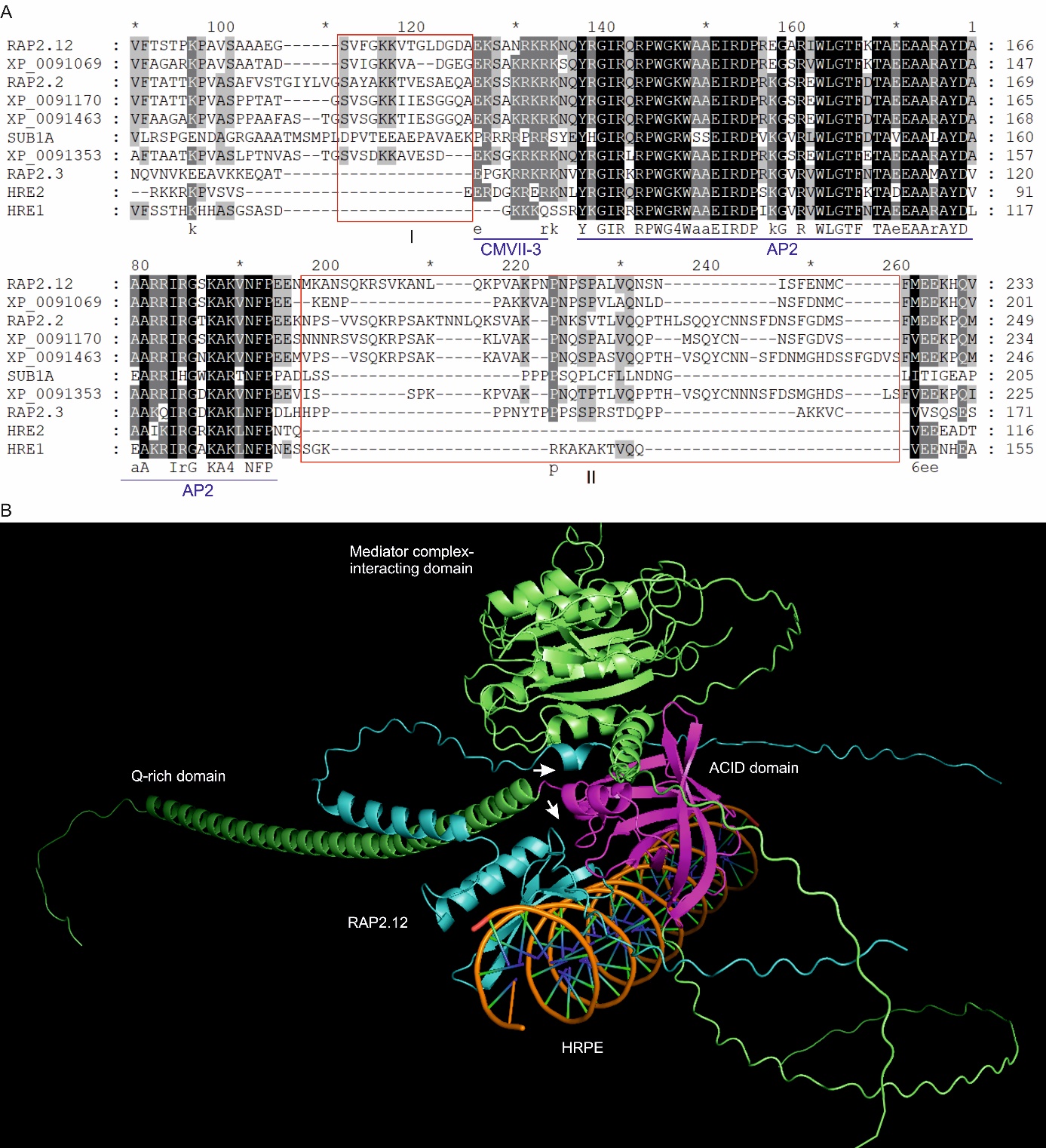
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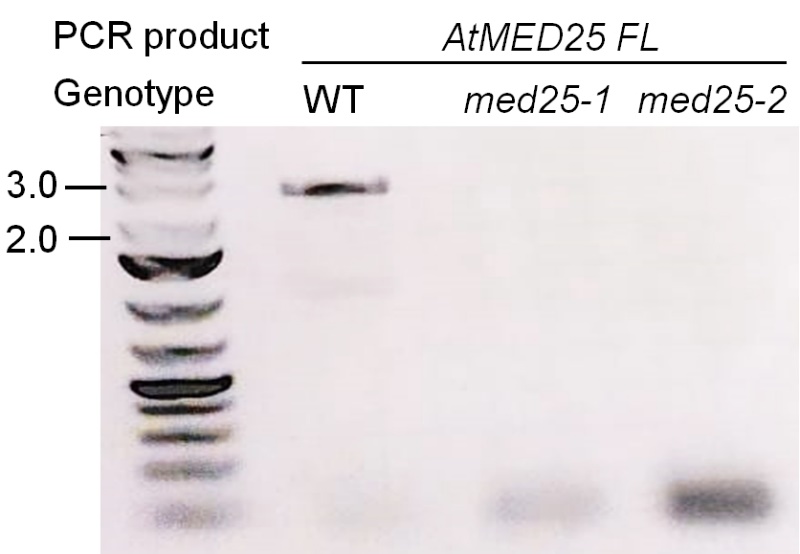
**Supporting Figure S1.** Yeast-two-hybrid assays for testing interaction between Mediator complex subunits with the ERFVII factors RAP2.2 and RAP2.12. (Supports Figure 1)

(**A**) Schematic representation of yeast-two-hybrid assay performed with ERFVIIs and *At*MED subunits. (**B**) Growth of yeast colonies on selection medium (-LWH) lacking leucine (L), tryptophan (W), and histidine (H) with 65 mM 3-aminotriazole (3-AT) indicates interaction.

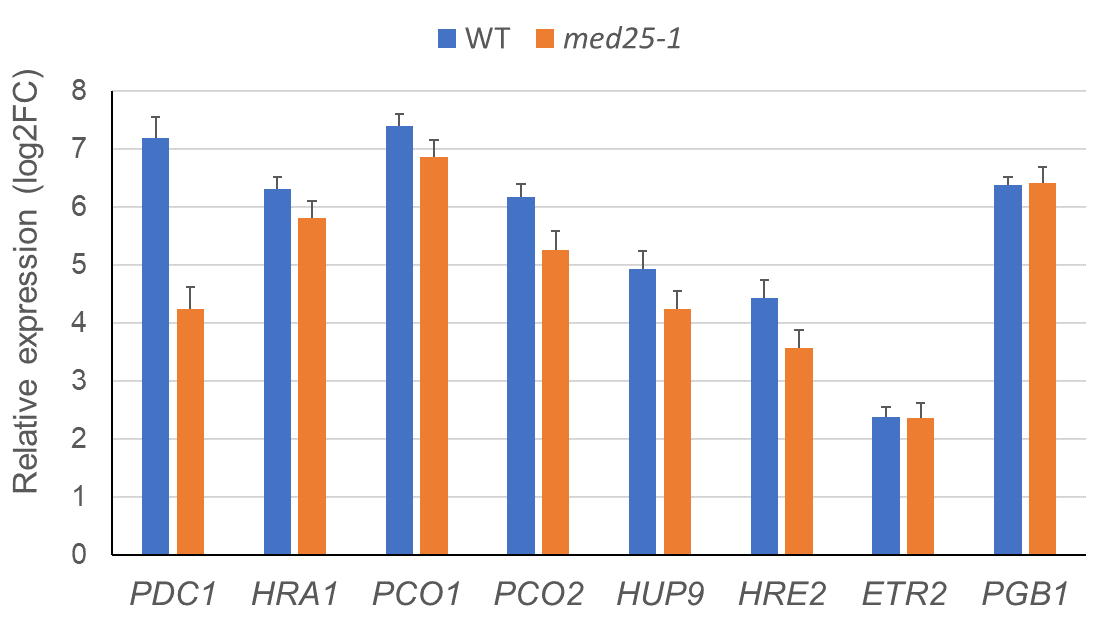


**Supporting Figure S2.** Multiple sequence alignment of the ERFVII-DNA binding domain and AlphaFold3 joint structure prediction for RAP2.12, *At*MED25 and target DNA. (Supports Figure 1)

(**A**) Multiple sequence alignment of the ERFVII DNA binding domain and flanking sequences of the five Arabidopsis ERFVII family members, rice SUB1A and orthologous for RAP2.2 and RAP2.12 from *Brassica* *rapa* (XP\_009106954; XP\_009117071; XP\_009135370; XP\_009146390). The underlined blue domains, CMVII-3 and AP2 domain, are highly conserved and present in all selected ERFVII members, whereas the flanking sequences highlighted by a red box (I and II), represent sequence variation between the ERFVII members. Especially the sequence in box I is of interest, as it is only present in RAP2.2 and RAP2.12 from *Arabidopsis* and *Brassica rapa*, and SUB1A in rice, but not in Arabidopsis RAP2.3, HRE1 and HRE2 which do not interact with *At*MED25. (**B**) AlphaFold3 modelling of the joint structure of the RAP2.12-*At*MED25 complex on the HRPE motif. Modelling was done using the AlphaFold3 server (Abramson et al., 2024) using as input RAP2.12 (93-253), full-length *At*MED25, and a DNA sequence containing the HRPE motif of the *LBD41* promoter. RAP2.12 is depicted in teal (dark shade cyan), while *At*MED25 is shown in lime (light green) except for the ACID domain, which is shown in light magenta. The target DNA containing the HRPE motif is presented in orange colour. For *At*MED25, the Q-rich domain, the Mediator complex-interacting domain and the ACID domain are labelled in the figure. In addition, the contact sites between the ACID domain of *At*MED25 and RAP2.12 are indicated by white arrowheads. Modelling the RAP2.2-*At*MED25 complex on the HRPE motif of *LBD41* revealed a result similar to RAP2.12-specific complexes. Protein models were visualized using PyMol (Schrödinger, 2015).

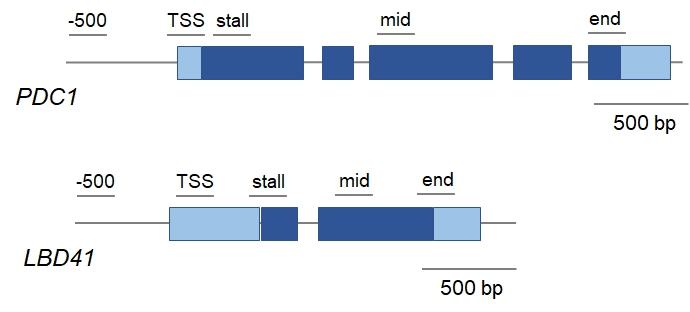


**Supporting Figure S3.** Semi-quantitative RT-PCR analysis of *AtMED25* transcript levels in the T-DNA insertion lines *med25-1* and *med25-2*. Used primer sequences can be found in Table S1. In both T-DNA lines, no full-length *AtMED25* transcript was detected. Band sizes of the ladder (left) are given in kb.

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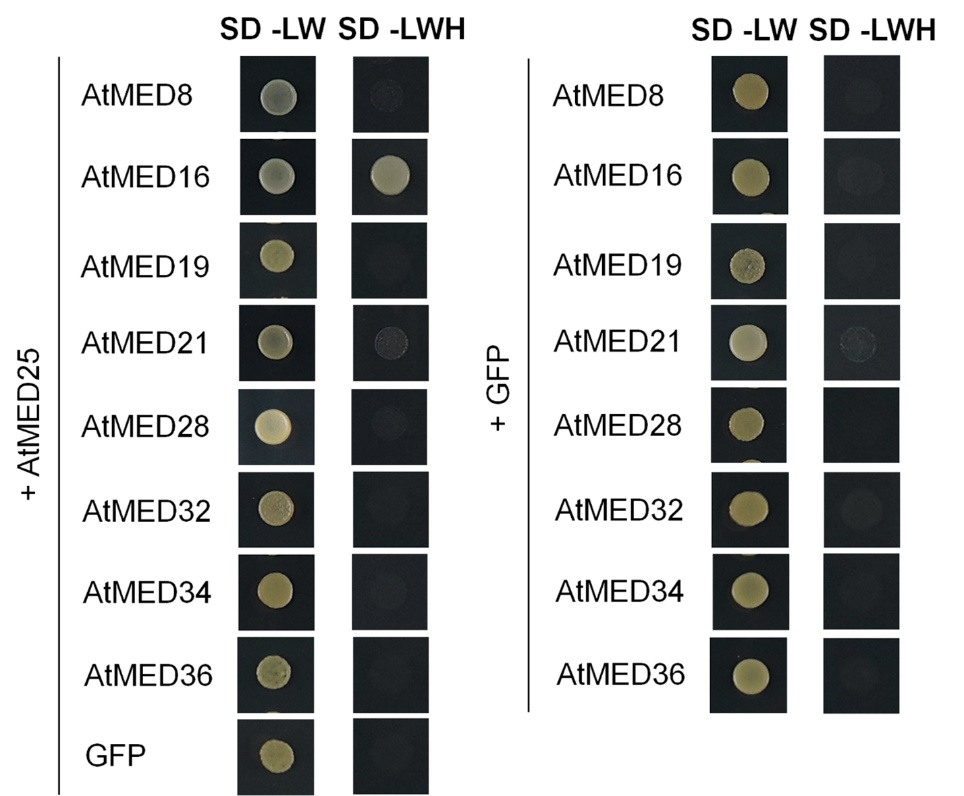
**Supporting Figure S4.** Plot ofRNA-Seq data for selected hypoxia core genes in hypoxia-treated *med25-1* and wildtype. (Supports Figure 2).

Plotted are the log2FC (FC, fold change) values for selected hypoxia core genes as reported in Supplemental Dataset 1. The error bars indicate the standard error (SE). n = 3.



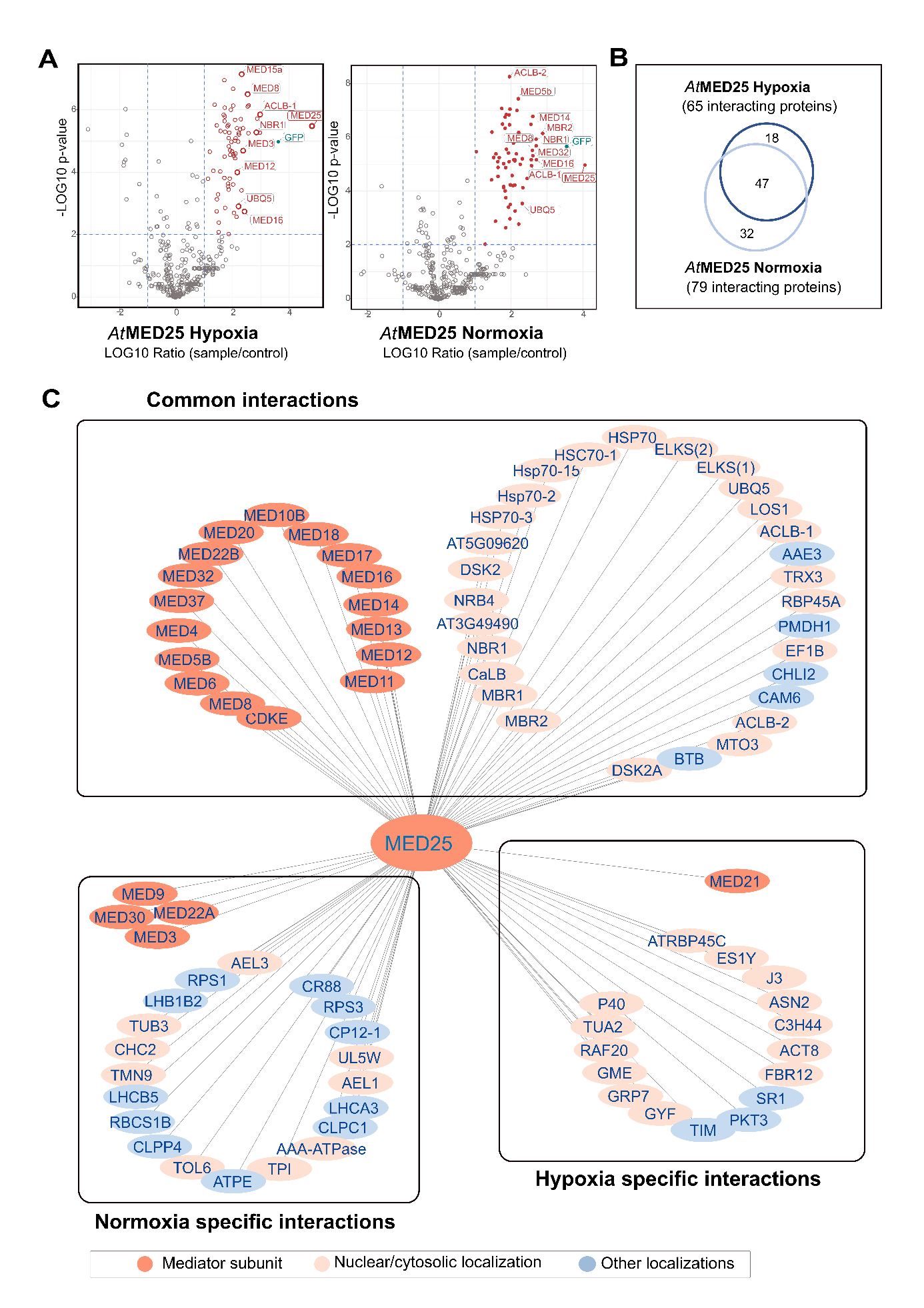
**Supporting Figure S5.** Position of primer pairs used in RNA Pol II ChIP assay. (Supports Figure 3).

Overview of primer pair locations along two hypoxia-responsive genes. Dark blue boxes indicate the exons of the genes, while light blue boxes represent the UTRs. Thin black lines above show the regions amplified by the primers in each ChIP reaction.

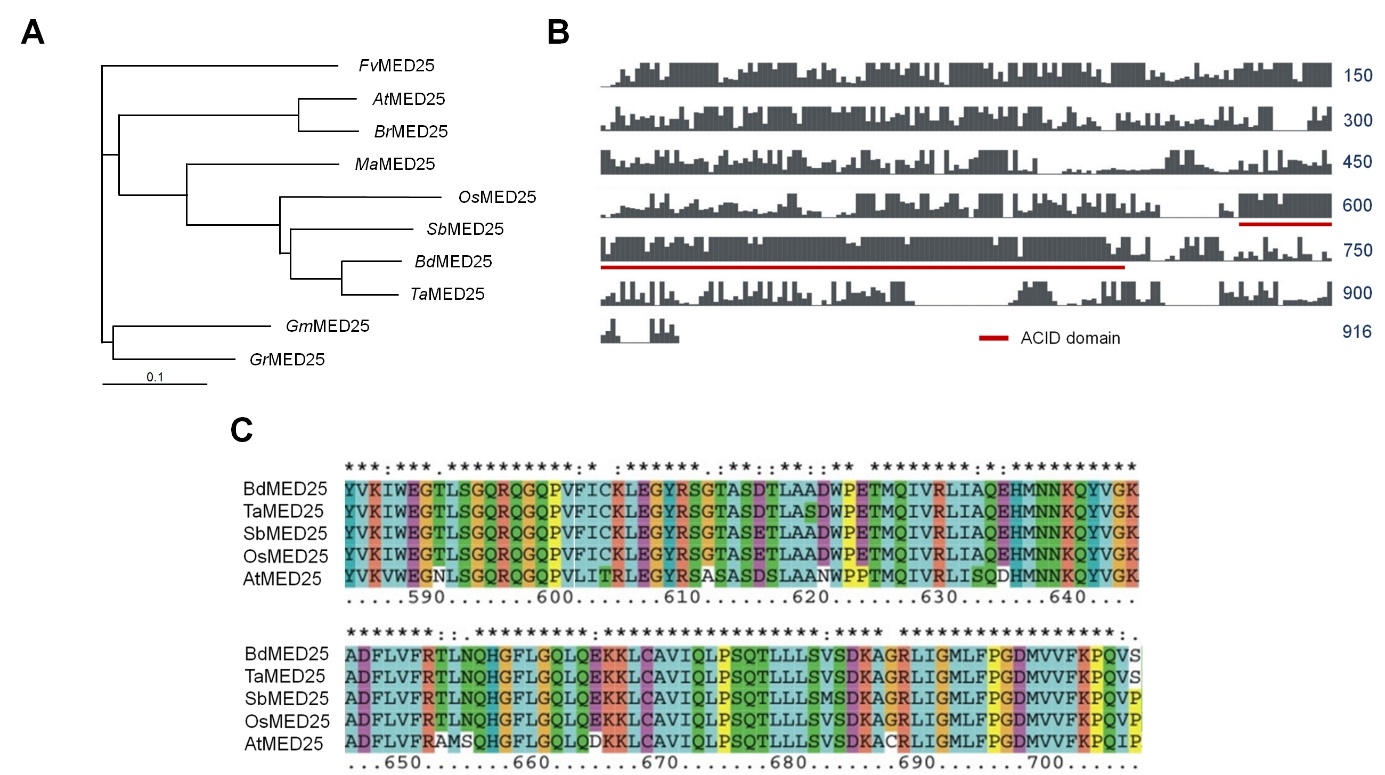


**Supporting Figure S6.** Yeast-two-hybrid assays for testing interaction between Mediator complex *At*MED25 with other Mediator subunits from Arabidopsis. (Supports Figure 5)

Growth of yeast colonies on selection medium (-LWH) lacking leucine (L), tryptophan (W), and histidine (H) with 65 mM 3-aminotriazole (3-AT) indicates interaction.



**Supporting Figure S7. Identified potential interaction partners of *At*MED25 under normoxic and hypoxic conditions.** Immunopurification of *At*MED25-GFP followed by mass-spectrometry (IP-MS) revealed an enrichment of common and specific proteins under normoxic and hypoxic conditions as presented in Supporting DataSet S3. All data is based on three biological replicates and statistically significant enriched proteins are presented. The *At*MED25 interaction network was constructed with the Cytoscape software (Shannon et. al, 2003). Dark pink indicates Mediator subunits, light pink indicates proteins present in the nucleus/cytosol, blue-coloured proteins have other cellular localisations.



**Supporting Figure S8.** Multiple sequence alignment of MED25 proteins from different plant species. (Supports Figure 6)

(**A**) Phylogenetic tree of *At*MED25 and its homologous proteins from selected species. Dendrogram obtained using neighbour-joining analysis. (**B**) Schematic representation of the sequence alignment of *At*MED25 and homologous proteins. Black boxes indicate conserved residues and the red underlined domain represents the activator-interacting domain (ACID). (**C**) Multiple sequence alignment indicates a strong conservation of the ACID of MED25 from *Brachypodium distachyon* (*Bd*), *Triticum aestivum* (*Ta*), *Sorghum bicolor* (*Sb*), *Oryza sativa* (*Os*) and *Arabidopsis thaliana* (*At*).

**Supplementary Table 1. Oligonucleotide sequences used for cloning constructs in pENTR-D/TOPO and other vectors.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Constructs** | **Length (aa)** | **Primer name** | **5’→3’ sequence** | **Usage** |
| RAP2.2 1-379 (stop codon) | 1-379 | RAP2.2\_FL\_F | caccATGTGTGGAGGAGCTATAATCT | Y2H, transactivation assay |
| RAP2.2\_FL\_stop\_R | TCAAAAGTCTCCTTCCAGCATGA |
| RAP2.2 1-379 (no stop codon) | 1-379 | RAP2.2\_Fl\_F | caccATGTGTGGAGGAGCTATAATCT | BiFC |
| RAP2.2\_FL\_nostop\_R | AAAGTCTCCTTCCAGCATGAAA |
| RAP2.2\_1-356 (stop codon) | 1-356 | RAP2.2\_FL\_F | caccATGTGTGGAGGAGCTATAATCT | Y2H, transactivation assay |
| RAP2.2\_1-356\_stop\_R | TTATTCCTCTTCCTGAGTCACAGC |
| RAP2.2\_1-331 (stop codon) | 1-331 | RAP2.2\_FL\_F | caccATGTGTGGAGGAGCTATAATCT | Y2H, transactivation assay |
| RAP2.2\_1-331\_stop\_R | TTAGTCAAGGTATGCCATCAGATCGTC |
| RAP2.2\_267-379 (stop codon) | 267-379 | RAP2.2\_267-379\_F | caccATGGGATACCAGTATTTCAGTTCCGA | Y2H, transactivation assay |
| RAP2.2\_267-379\_stop\_R | TCAAAAGTCTCCTTCCAGCATGAAA |
| RAP2.2\_44-303 (stop codon) | 44-303 | RAP2.2\_44-303\_F | caccATGGATTTCTTCGATCTTGACGATGATT | Y2H |
| RAP2.2\_44-303\_stop\_R | TCAATTGACAAGCATTGAAGAGATCTC |
| RAP2.2\_44-267 (stop codon) | 44-267 | RAP2.2\_44-303\_F | caccATGGATTTCTTCGATCTTGACGATGATT | Y2H |
| RAP2.2\_44-267\_stop\_R | TCATCCATTGTTACCTCCAGCATCGAA |
| RAP2.2\_90-303 (stop codon) | 90-303 | RAP2.2\_90-303\_F | caccATGGCTTCCGCTTTCGTCTCCACT | Y2H |
| RAP2.2\_44-303\_stop\_R | TCAATTGACAAGCATTGAAGAGATCTC |
| RAP2.2\_90-267 (stop codon) | 90-267 | RAP2.2\_90-267\_F | caccATGGCTTCCGCTTTCGTCTCCACT | Y2H |
| RAP2.2\_44-267\_stop\_R | TCATCCATTGTTACCTCCAGCATCGAA |
| RAP2.2\_336-379 (stop codon) | 336-379 | RAP2.2\_336-379\_F | caccATGGCCTTGTGGGACACCCCACT | Y2H, transactivation assay |
| RAP2.2\_FL\_stop\_R | TCAAAAGTCTCCTTCCAGCATGA |
| RAP2.2\_355-379 (stop codon) | 355-379 | RAP2.2\_355-379\_F | caccATGGTGACTCAGGAAGAGGAAAAC | Y2H, transactivation assay |
| RAP2.2\_FL\_stop\_R | TCAAAAGTCTCCTTCCAGCATGA |
| RAP2.12 1-358 (stop codon) | 1-358 | RAP2.12\_FL\_F | caccATGTGTGGAGGAGCTATAATATC | Y2H, transactivation assay |
| RAP2.12\_FL\_stop\_R | TCAGAAGACTCCTCCAATCATG |
| RAP2.12 1-358 (no stop codon) | 1-358 | RAP2.12\_FL\_F | caccATGTGTGGAGGAGCTATAATATC | BiFC |
| RAP2.12\_FL\_nostop\_R | GAAGACTCCTCCAATCATG |
| RAP2.12\_1-339 (stop codon) | 1-339 | RAP2.12\_FL\_F | caccATGTGTGGAGGAGCTATAATATC | Y2H, transactivation assay |
| RAP2.12\_1-339\_stop\_R | TTAGTTTGCACCATTGTCCTGAGTC |
| RAP2.12\_1-307 (stop codon) | 1-307 | RAP2.12\_FL\_F | caccATGTGTGGAGGAGCTATAATATC | Y2H, transactivation assay |
| RAP2.12\_1-307\_stop\_R | TTACTTGAGCTTCTTAGCTGGATTGGC |
| RAP2.12\_44-289 (stop codon) | 44-289 | RAP2.12\_44-289\_F | caccATGAATTTCTTCGATTTTGACGCTGAG | Y2H |
| RAP2.12\_44-289\_stop\_R | TCAGTTGATAACCGCAGAAGAGATGT |
| RAP2.12\_44-253 (stop codon) | 44-253 | RAP2.12\_44-253\_F | caccATGAATTTCTTCGATTTTGACGCTGAG | Y2H |
| RAP2.12\_44-253\_stop\_R | TCACCCATTACATCCAGCATCAACGGA |
| RAP2.12\_93-289 (stop codon) | 93-289 | RAP2.12\_93-289\_F | caccATGGTCTCCGCCGCTGCGGAAGGTT | Y2H |
| RAP2.12\_44-289\_stop\_R | TCAGTTGATAACCGCAGAAGAGATGT |
| RAP2.12\_93-253 (stop codon) | 93-253 | RAP2.12\_93-253\_F | caccATGGTCTCCGCCGCTGCGGAAGGTT | Y2H |
| RAP2.12\_44-253\_stop\_R | TCACCCATTACATCCAGCATCAACGGA |
| RAP2.12\_1-179 (stop codon) | 1-179 | RAP2.12\_1-179\_F | caccTGTGTGGAGGAGCTATAATATC | Y2H, transactivation assay |
| RAP2.12\_1-179\_stop\_R | TTAATTCACCTTAGCTTTAGATCCA |
| RAP2.12\_180-358 (stop codon) | 180-358 | RAP2.12\_180-358\_F | caccATGTTCCCTGAAGAAAACATGAAGGCT | Y2H, transactivation assay |
| RAP2.12\_FL\_stop\_R | TCAGAAGACTCCTCCAATCATG |
| RAP2.12\_225-358 (stop codon) | 225-358 | RAP2.12\_225-358\_F | caccATGTGTTTCATGGAGGAGAAACACCA | Y2H, transactivation assay |
| RAP2.12\_FL\_stop\_R | TCAGAAGACTCCTCCAATCATG |
| RAP2.12\_255-358 (stop codon) | 255-358 | RAP2.12\_255-358\_F | caccATGCAGTATTTCAGCTCTGACCAGG | Y2H, transactivation assay |
| RAP2.12\_FL\_stop\_R | TCAGAAGACTCCTCCAATCATG |
| RAP2.12\_309-358 (stop codon) | 309-358 | RAP2.12\_309-358\_F | caccATGATGGATTTCGAGACACCTTACAA | Y2H, transactivation assay |
| RAP2.12\_FL\_stop\_R | TCAGAAGACTCCTCCAATCATG |
| RAP2.12\_335-358 (stop codon) | 335-358 | RAP2.12\_335-358\_F | caccATGCAGGACAATGGTGCAAACCCT | Y2H, transactivation assay |
| RAP2.12\_FL\_stop\_R | TCAGAAGACTCCTCCAATCATG |
| RAP2.12\_1-23 (stop codon) | 1-23 | RAP2.12\_FL\_F | caccATGTGTGGAGGAGCTATAATATC | Y2H |
| RAP2.12\_1-23\_stop\_R | CTAAAACTCGCTAGTAAC |
| RAP2.12\_1-79 (stop codon) | 1-79 | RAP2.12\_FL\_F | caccATGTGTGGAGGAGCTATAATATC | Y2H |
| RAP2.12\_1-79\_stop\_R | CTAATCGGCGAAAACATC |
| RAP2.12\_1-103 (stop codon) | 1-103 | RAP2.12\_FL\_F | caccATGTGTGGAGGAGCTATAATATC | Y2H |
| RAP2.12\_1-103\_stop\_R | CTAACCAAAAACTGAACC |
| RAP2.12\_1-123 (stop codon) | 1-123 | RAP2.12\_FL\_F | caccATGTGTGGAGGAGCTATAATATC | Y2H |
| RAP2.12\_1-123\_stop\_R | CTAATTCTTCCTCTTCCTATT |
| AtMED25 full length (stop codon) |  | AtMED25\_FL\_F | caccATGTCGTCGGAGGTGAAACAG | Y2H, transactivation assay, semi-quantitative RT-PCR |
| AtMED25\_FL\_stop\_R | TTATCCCATGAAGCCAGCTCC |
| AtMED25 full length (no stop codon) |  | AtMED25\_FL\_F | caccATGTCGTCGGAGGTGAAACAG | GFP fusion, in planta localization, BiFC, ChIP |
| AtMED25\_FL\_nostop\_R | TCCCATGAAGCCAGCTCCAG |
| AtMED25 promoter |  | pAtMED25\_F | caccTTGTTTAGATTTATTCGGATTTTA | GUS analysis |
| pAtMED25\_R | AGAAAATGGGATATACAAAGGA |
| ADH1 promoter |  | pADH1\_F | caccTGGGCCTATGATTCAACACAACA | Transactivation assay |
| pADH1\_R | TATCAACAGTGAAGAACTTGCTT |
| HB1 promoter |  | pHB1\_F | caccGAAATTAAACACCTTCTTCTGTAA | Transactivation assay |
| pHB1\_R | AATATTTCACAACCTCTAAATGAT |
| OsMED25 full length (stop codon) |  | OsMED25\_FL\_F | caccATGGCGGCGGCGGCGGCCGAGA | Y2H, transactivation assay, BiFC |
| OsMED25\_FL\_stop\_R | TCAAGATAGGTAGCCACCCCCA |
| RAP2.2-GFP |  | RAP2.2-CFP\_F | TTTGCGATCGCATGTGTGGAGGAGCTATAATCT | Co-IP |
| RAP2.2-CFP\_R | AAAGTTTAAACTTACTTGTACAGCTCGTCCATGC |
| flag-AtMED25Δq |  | flag-AtMED25Δq\_F | AAAGCGATCGCATGGATTACAAGGATGACGATGACAA  GGCAGCCGGTTCGTCGGAGGTGAAACAGCTGA | Co-IP |
| flag-AtMED25Δq\_F | TGTGGTTTAAATTACACAACCATATCCCCTGGGAAAAG  CATTCCAA |

**Supplementary Table 2. Sequences of oligonucleotides used for RT-qPCR expression analysis of ethylene-induced genes.**

|  |  |  |
| --- | --- | --- |
| **Gene** | **Forward primer 5'→3'** | **Reverse primer 5'→3'** |
| *ETR2* | tgttagattctccggcggctatg | ttcccatgaatcaactgcaccac |
| *PGB1* | ggctcttgtagtgaagtcttgga | cttcgttgttggtgcaatctca |
| *HRE1* | tccgatgagccatttgtcttctcc | ccatcttccccaaggccttc |
| *RAP2.2* | cctagcgtcgtatcccagaa | agggtttgcaccattgtcctgag |
| *RAP2.3* | aactcacggctgaggaactctg | acgttaacttggttggtgggatgg |

**Supplementary Table S3. Sequences and positions of motifs and oligonucleotides used for ChIP-qPCR analysis.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Promoter** | **Protein** | **Motif** | **Motif sequence 5'→3'** | **Motif position 5'→3'** | **Forward primer 5'→3'** | **Reverse primer 5'→3'** |
| *PDC1* | *At*MED25-GFP | R1 | GCAGATGGTTTT | -685 till - 674 | aagaagcaaaccccaaaacc | gccatttgcatttggtcag |
| *PDC1* | *At*MED25-GFP | R2 | GGGAGAGGTTTT | -125 till - 114 | gaatgtttgggtcaaatctcaa | aacaaaatgagaatgggagagg |
| *LBD41* | *At*MED25-GFP | R1 | GCCGACGCTTTC | -898 till - 887 | aaagcgtcggctaacagaga | tccggtttttgatcttttcttc |
| *LBD41* | *At*MED25-GFP | R2 | GCTCCTGGTTTT | -748 till - 736 | tttccagaaaaccaggagcta | tgggatttgagtcatttgagg |
| *LBD41* | *At*MED25-GFP | R3 | GCCGCTGTTTTT | -349 till - 338 | cattggatagagtggggacaa | ccctgattcaaggttttgct |
| *SAD6* | *At*MED25-GFP | R1 | none | none | tggcattctcgtgtacaaagtc | tgtgatcttgctgttcaattgtt |
| *SAD6* | *At*MED25-GFP | R2 | CCCCACGGTTTG | -153 till - 142 | cccacagaatctcaaaccag | tggttggctgttgttcgtta |
| *UBI10* | *At*MED25-GFP | R1 | none | none | aaccactttgacgccgttta | acggctggatcttatgacga |
| *LBD16\** | *At*MED25-GFP | R1 | TGTCTC | -989 till - 984 | cccaataaattagaagtctcatgttgc | caaatttatcgagtgagccaaagg |
| *PDC1* | RNAPII | up500 | none | none | accccaaaaccatctgcatct | caagagacgtgatgccatttg |
| *PDC1* | RNAPII | TSS | none | none | tcacacacatacacaaacttgaca | gtcggcttgcaatcatcgatc |
| *PDC1* | RNAPII | stall | none | none | gatgattgcaagccgacgaac | gatggtgatagcggaggaagg |
| *PDC1* | RNAPII | mid | none | none | tcaatactctgtttctgcgaga | ccgcagcttctaaacccatct |
| *PDC1* | RNAPII | end | none | none | agaggagttagtggaggcgat | gccccactcaagcaactct |
| *LBD41* | RNAPII | up500 | none | none | gtgctacaaaatgggtctcaca | tccagacaaccaaaactcagct |
| *LBD41* | RNAPII | TSS | none | none | atgacacgcgcattggataga | gcttcttgtgtttcttcccca |
| *LBD41* | RNAPII | stall | none | none | tcggaaagggtgtagtgagga | ttcaggcgatttgatccaagc |
| *LBD41* | RNAPII | mid | none | none | gtggaggctgtgatgaaagga | cgtagatcttaagaggcggacc |
| *LBD41* | RNAPII | end | none | none | agagcacgtgtaagactgagc | agttaccggctcgacttgaag |

\* Ito et al., 2016