

SUPPLEMENTARY DATA (Garg *et al.*)

Supplementary Figures

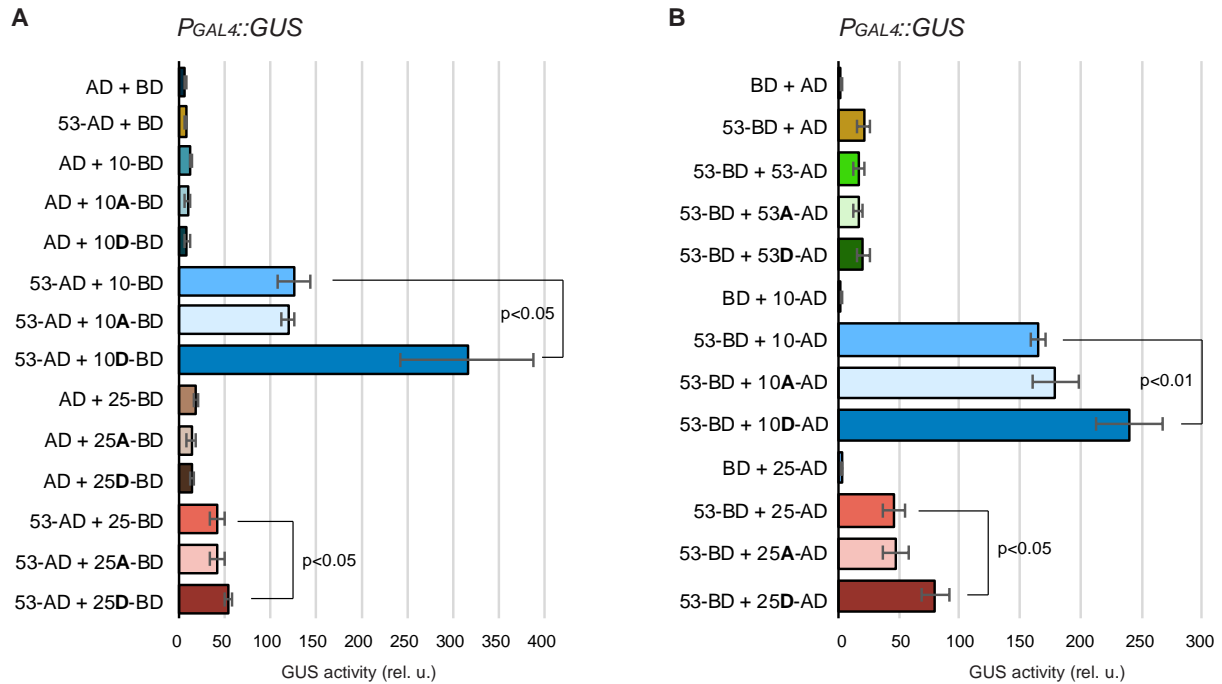


Fig. S1. Analysis of the heterodimerization of bZIP53 with bZIP10 and 25 by *protoplast two-hybrid* assay. $P_{GAL4}:GUS$ is used as reporter. (A) bZIP53 is fused to GAL4 activation domain (AD); bZIP10, 25 and their mutated forms are fused to GAL4 DNA-binding domain (BD). (B) Reciprocal assays applying bZIP53 as BD-fusion, and bZIP10, 25, their mutated forms and bZIP53 mutated forms as AD-fusions. **A**: substitution of Ser15 and Ser19 to Ala, **D**: substitution of Ser15 and Ser19 to Asp.

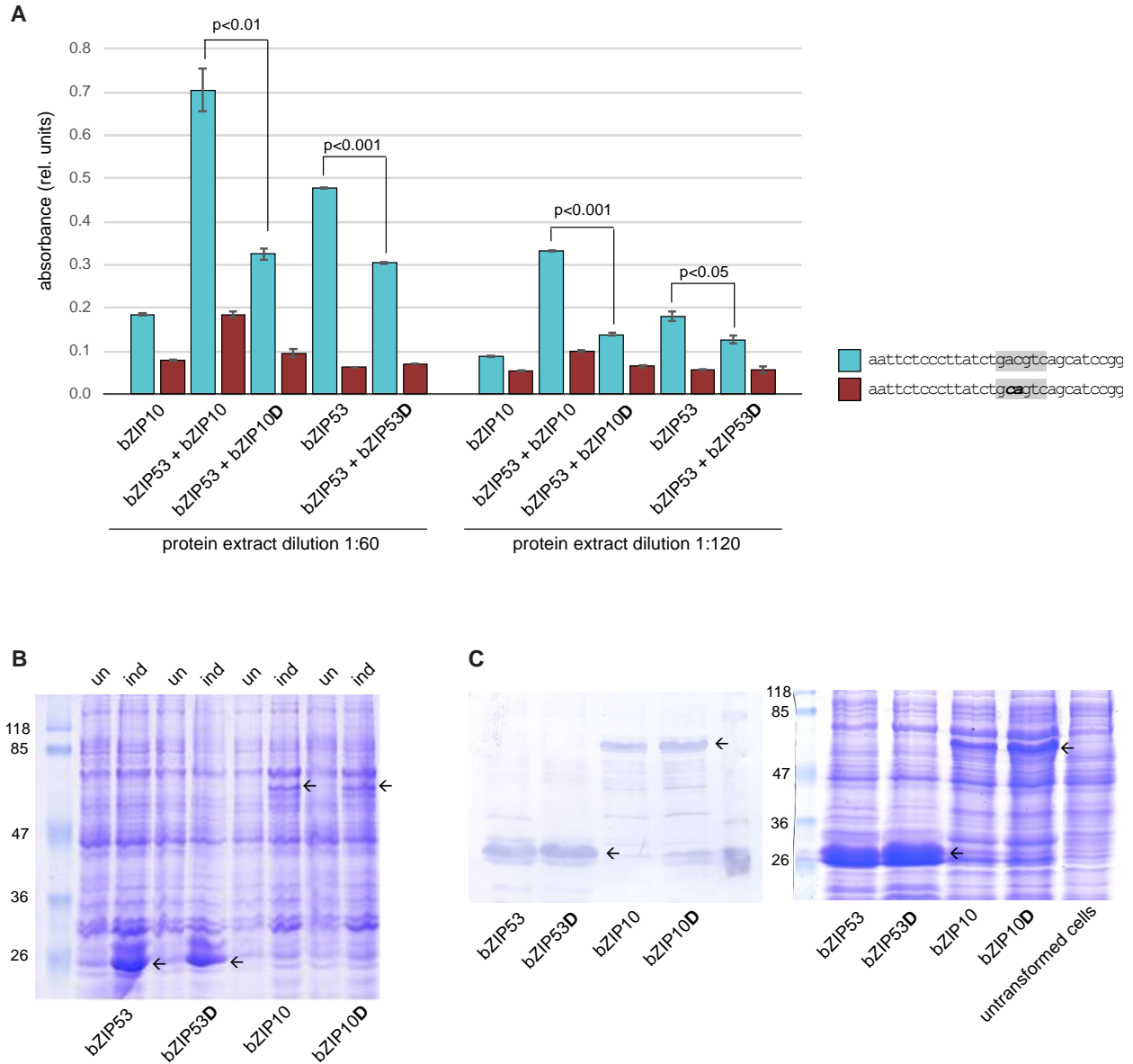


Fig. S2. Binding of bZIP heterodimers to the cognate DNA sequence. (A) DPI-ELISA of the interaction of the bZIP53-bZIP10 heterodimers and corresponding mutated forms to the C-box (blue bars) and mutated C-box (dark-red bars). The recombinant C-terminally His-tagged proteins were extracted from *E. coli* under denatured conditions in the DNA-binding buffer containing 6 M urea, mixed as indicated on the X-axis and heated for 3-4 min at 50°C, following by stepwise dilutions in the DNA-binding buffer. The final protein dilutions were 1:60 and 1:120. Data are presented as mean values \pm standard deviation (StD); $n=2$. (B) The expression of the recombinant proteins was verified by the Coomassie staining of total protein extracts separated by SDS-polyacrylamide gels. (C) The detection of corresponding bZIP proteins (indicated by arrows) in the denatured extracts by the Coomassie staining (right panel) and western blotting using monoclonal mouse anti-His antibody (Roche) and anti-mouse-AP conjugate (BioRad). **D**: substitution of Ser15,Ser19 to Asp; un: uninduced; ind: induced.

Supplementary Tables

Table S1: Primer sequences used for Gateway cloning and mutagenesis.

Construct	Primer Sequences
pENTR/bZIP53	5'- CACCATGGGGTCGTTGCAAATG -3' 5'- GCAATCAAACATATCTGCAGAAGCTCTGATT -3'
Construct and mutation	
bZIP10 ^{S15,19A}	5'-AATCGTGAGGCAGCTAGGCGAGCTAGAAGGAGAA-3' 5'-TTCTCCTTCTAGCTCGCCTAGCTGCCTCACGATT-3'
bZIP10 ^{S15,19D}	5'-AATCGTGAGGATGCTAGGCGAGATAGAAGGAGAA-3' 5'-TTCTCCTTCTATCTCGCCTAGCATCCTCACGATT-3'
bZIP25 ^{S15,19A}	5'-GAAGCCGCTAGGCGCGCTAGGAGAAGAAAGCAAG-3' 5'-CTTGCTTTCTTCTCCTAGCGCGCCTAGCGGCTTC-3'
bZIP25 ^{S15,19D}	5'-GAAGACGCTAGGCGCGATAGGAGAAGAAAGCAAG-3' 5'-CTTGCTTTCTTCTCCTATCGCGCCTAGCGTCTTC-3'
bZIP53 ^{S15,19A}	5'-AGAGAAGCTGCTCGGAGGGCAAGGATGAGGAAAC-3' 5'-GTTTCCTCATCCTTGCCCTCCGAGCAGCTTCTCT-3'
bZIP53 ^{S15,19D}	5'-AGAGAAGATGCTCGGAGGGATAGGATGAGGAAAC-3' 5'-GTTTCCTCATCCTATCCCTCCGAGCATCTTCTCT-3'

Table S2: Primer sequences used for qPCR.

Gene	Primer Sequence	Product size (nt)
<i>ProDH1</i>	GCTGCCAAATCTTTACCAACA TGAAGTTCGGACTTTTGTATTCC	127
<i>ProDH2</i>	AGCTACGCATAACACAGACTCG TTATTGATCCCTAGCTCACTTGC	66
<i>BCAT2</i>	TCACAAATTATGCGCCAGTT CGAGATAAAGAACGTCTGAAAACC	75
<i>bZIP53</i>	CAATGTCTTGAGGGCACAGG TGCATTGGACAAGGCATCTG	156
<i>UBI10</i>	TCACCGGAAAGACCATCACT CGGTGGGATACCCTCTTTG	92
<i>EF-1-alpha</i>	GTAACAAGATGGATGCCACCACCCC TCCCTCGAATCCAGAGATTGGCACA	140

Table S3: Oligonucleotides used for the protein-DNA binding assay.

Oligo-label	Sequence
C-box	5' -biotAATTCTCCCTTATCTGACGTCAGCATCCGG-3' 3' -TTAAGAGGGAATAGACTGCAGTCGTAGGCC-5'
C-mut	5' -biotAATTCTCCCTTATCTGACGTCAGCATCCGG-3' 3' -TTAAGAGGGAATAGACGTCAGTCGTAGGCC-5'

Table S4: Accession numbers of gene sequences used in this study.

Gene name	Accession number*
<i>bZIP10</i>	At4G02640
<i>bZIP25</i>	At3G54620
<i>bZIP53</i>	AT3G62420
<i>BCAT2</i>	AT1G10070
<i>ProDH1</i>	AT3G30775
<i>ProDH2</i>	AT5G38710
<i>EF-1-alpha</i>	AT5G60390
<i>UBQ10</i>	AT4G05320

*Sequence data can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases

Table S5. Summary of amino acid analysis in seven weeks old *Arabidopsis* leaf rosettes harvested at the dawn ($\mu\text{M}/\text{mg}$ of dry weight).

<i>p</i>	StDev	mean	bZIP53ox						StDev	mean	Col-0	a. a.
0,164	1,64	19,03	16,54	18,62	17,88	23,13	18,97	3,35	23,05	18,26	22,82	Ala
0,023	0,16	5,94	6,02	6,13	6,05	5,58	5,90	1,18	7,93	8,73	8,91	Arg
0,261	1,68	21,84	20,48	21,51	19,83	21,34	26,02	1,94	19,49	16,70	22,40	Asn
0,022	5,34	52,09	55,58	47,90	46,87	61,96	48,15	5,70	67,84	59,30	68,99	Asp
0,003	7,15	75,53	76,27	58,20	83,77	84,41	74,99	6,49	41,42	31,69	46,74	Gln
0,003	9,65	151,89	155,66	144,62	135,03	159,12	165,01	0,93	186,75	185,74	186,37	Glu
0,021	0,93	3,28	5,25	2,71	2,84	3,64	1,98	0,23	5,62	5,28	5,79	Gly
0,005	0,10	2,09	2,20	2,00	2,06	1,96	2,22	0,75	3,90	4,36	4,56	His
0,000	0,12	1,21	1,26	1,35	1,31	1,21	0,90	0,46	3,31	3,74	3,56	Ile
0,000	0,35	1,52	1,95	1,79	0,64	1,56	1,67	0,54	4,45	5,03	4,67	Leu
0,000	0,21	1,79	2,12	1,72	1,98	1,48	1,66	0,14	3,39	3,46	3,53	Lys
0,840	0,24	0,74	1,13	0,45	0,95	0,73	0,46	0,22	0,79	1,08	0,46	Met/ Cys
0,001	0,27	1,55	1,78	1,79	0,88	1,66	1,62	0,15	3,03	3,26	2,86	Phe
0,000	0,48	4,77	4,12	5,02	4,22	5,10	5,38	0,50	8,59	8,96	8,97	Pro
0,007	2,90	29,69	33,08	31,63	31,59	24,86	27,27	0,36	38,05	37,98	38,59	Ser
0,035	4,69	32,33	37,63	38,77	31,87	28,15	25,23	2,06	22,30	20,10	21,42	Thr
0,011	0,21	0,71	0,74	0,76	1,13	0,59	0,31	0,22	1,52	1,82	1,55	Tyr
0,004	1,14	0,82	0,10	0,10	0,10	0,10	3,68	1,72	6,95	9,52	6,06	Val
0,026	15,80	406,79	421,90	385,06	389,00	426,58	411,39	15,58	448,39	425,02	458,26	total

Table S5. (continue)

<i>p</i>	StDev	mean	bZIP53^{S15,19D}Ox			<i>p</i>	StDev	mean	bZIP53^{S15,19A}Ox			a. a.				
0,578	1,64	21,62	21,87	20,11	22,59	19,03	24,52	0,021	1,16	15,91	16,15	16,46	17,07	16,87	13,02	Ala
0,427	1,52	9,00	11,24	10,55	7,98	7,09	8,14	0,789	0,39	7,73	8,03	7,47	7,10	7,66	8,41	Arg
0,022	6,11	33,09	28,83	25,65	29,53	41,51	39,95	0,081	3,05	25,61	33,23	25,27	23,74	24,21	21,61	Asn
0,066	6,61	81,60	77,19	85,11	78,56	72,51	94,63	0,356	3,33	63,61	61,49	66,85	57,96	68,69	63,04	Asp
0,055	43,32	112,05	78,98	76,31	72,56	169,39	162,99	0,005	3,93	62,49	60,69	69,04	62,78	65,45	54,46	Gln
0,880	11,24	185,22	176,43	185,18	174,11	177,07	213,33	0,037	7,78	204,44	207,50	214,10	185,89	203,54	211,16	Glu
0,457	1,45	4,77	7,42	3,93	5,76	3,48	3,28	0,000	0,51	2,28	2,13	3,55	1,79	2,22	1,72	Gly
0,268	0,53	4,59	5,08	4,72	4,10	3,75	5,30	0,061	0,26	2,84	3,31	2,55	2,85	2,48	3,00	His
0,176	1,08	4,75	7,31	4,06	4,89	3,50	3,99	0,018	0,31	2,23	2,75	1,97	1,96	1,97	2,48	Ile
0,166	1,16	6,09	8,99	5,26	6,00	4,98	5,21	0,002	0,34	2,41	2,76	2,10	2,17	2,12	2,93	Leu
0,110	0,97	4,97	7,34	3,72	5,02	4,18	4,61	0,050	0,41	2,65	3,01	2,17	2,31	2,43	3,32	Lys
0,300	0,10	0,62	0,47	0,55	0,57	0,67	0,82	0,710	0,56	0,98	0,70	0,67	2,38	0,35	0,80	Met/ Cys
0,097	0,54	3,97	5,33	3,29	3,71	3,62	3,92	0,415	0,31	2,78	3,55	2,73	2,54	2,30	2,76	Phe
0,047	0,99	10,97	12,99	8,71	11,37	10,77	11,01	0,000	0,75	2,88	1,96	1,94	3,65	2,92	3,95	Pro
0,066	6,33	48,33	57,29	39,17	46,02	55,21	43,97	0,087	3,45	32,95	32,10	29,85	28,28	37,08	37,45	Ser
0,019	2,34	28,94	29,55	27,11	30,91	24,92	32,20	0,001	4,15	41,73	47,20	45,58	35,67	42,77	37,40	Thr
0,250	0,52	2,13	3,43	1,48	2,14	2,01	1,59	0,027	0,17	1,00	1,14	1,14	0,74	0,82	1,14	Tyr
0,255	1,36	8,70	10,95	6,85	9,86	8,46	7,39	0,029	2,17	1,91	4,07	0,10	0,11	0,10	5,17	Val
0,023	54,45	571,43	550,72	511,77	515,67	612,15	666,83	0,127	16,02	477,08	491,78	493,57	438,99	483,97	473,82	total

