APPENDIX

Table 1: Strains used in present study

Bacterial/yeast/ plant	Strain	Genotype Description	References
Escherichia coli	DH5α	F⁻, *end*A1, *hsd*R17 (r_K^-, m_K^+), *sup*E44, *thi*-1, λ⁻, *rec*A1, *gyr*A96, relA1 Δ(*lacZYA-arg*F) U169 *deo*R (ϕ80d *lac*Δ (lacz) M15	Sambrook et al., 1989
	TG1	K12, *Δ(lac-proAB) Δ(mcrB-hsdSM)5 (rK- mK-) thi-1 supE [FÅL traD36 proAB lacIqZΔM15]*	Rudd M 2000 Nature biotech
	HB2151	K12*, ara, Δ lac-pro), thi/F'/ proA⁺B⁺, lacIᵠZ ΔMI5*	Geneservice Ltd,Cambridge, UK
Pichia pastoris	X33	Wild Type	Invitrogen, San Diego, CA, USA
	GS115	*his4*	Invitrogen, San Diego, CA, USA
	GC7	Strain GS115 transformed with pPIC2 vector	Present work
	CX4	Strain X33 transformed with pPIC2 vector	Present work
	DC2	Strain X33 transformed with dpPIC2 vector	Present work
S. cerevisiae	EBY100	*MATa (GAL1-AGA1::URA3 ura3-52 trp1 leu2Δ1 his3Δ200 pep4::HIS2 prb1Δ1.6R can1*	ATCC® MYA-4941™

	GAL), Trp⁻ Leu⁻	
EBCON2	Strain EBY100 transformed with pCTCON2 vector	Present work
EBC2	Strain EBY100 transformed with pCTCONC2 vector	Present work
EB57	Strain EBY100 transformed with mutated pCTCONC2 vector	Present work

Table 2: Plasmid used in present study

Plasmid	Description	Source / Reference
pBluescript II-KS⁺	Phagemid with M13 origin of replication	Sambrook*et al.*,1989
pUC19	Cloning vector used for alpha-complementation	Sambrook*et al.*,1989
pPICZαC	Expression vector with AOX1 promoter and alpha secretory signal.	Invitrogen,CA,USA
pCAMBIA 2301	Binary vector	
pRTDS 5.0	Plant expression vector	
pPIC2	pPICZαC derivative having single copy of anti TNF scFv gene	Present work
dpPIC2	pPICZαC derivative having two copy of anti TNF scFv gene	Present work
pRTC2	pRTDS 5.0 derivative having single copy of anti TNF scFv gene	Present work
pCAMC2	pCAMBIA 2301 derivative having single copy anti TNF scFv gene	Present work

Table 3. Primers used in present study

Name	Oligonucleotide Sequences (5'-3')
V_H For	CAGGAAACAGCTATGAC
V_H Rev	CGACCCGCCACCGCCGCTG
V_K For	CATCTGTAGGAGACAGAGTC
V_K Rev	CTATGCGGCCCCATTCA
Con2seqF	GTTCCAGACTACGCTCTGCAGG
Con2 seqR	GATTTTGTTACATCTACACTGTTG
YD F'	GGACTGGCTAGCATGGCCGAGGTGCAGCTG
YD R'	CAGATCTCGAGCTATTACAAGTCCTCTTCAGAAATAAGCTTTTGTTCGGATCCCCGTTTGATTTCCACCTTG
T3	GCAATTAACCCTCACTAAAGG
T7	TAATACGACTCACTATAGGG
Con F	CGACGATTGAAGGTAGATACCCATACGACGTTCCAGACTACGCTCTGCAG
ConR	CAGATCTCGAGCTATTACAAGTCCTCTTCAGAAATAAGCTTTTGTTC
C2RT R	CTTCACGGAGTCTGCGTAAG
ATNF1 F	GAGCATCGATGGCCGAGGTGCAGCTG
ATNF1 R	CTGTCTAGATCACCGTTTGATTTCCACCTTGG
c-myc R	TCGACGGCGCTATTCAGATCC

α Factor R	TACTATTGCCAGCATTGCTGC
ATNF2 F	CAGGAATTCATGGCCGAGGTGCAGCTG
ATNF2 R	CTAGGATCCTCACCGTTTGATTTCCACCTTGG

4. REAGENTS AND MEDIA

1. 2XTY medium

Tryptone	16 g
Yeast extract	10 g
NaCl	5 g
H_2O	to 1l

2. TYE medium

Bacto agar	15 g
NaCl	8 g
Tryptone	10 g
Yeast extract	5 g
H_2O	to 1l

3. SDCAA medium

Dextrose	20 g
Yeast nitrogen base	6.7 g
Casamino acids	5 g

Ammonium sulfate	5.3 g
Na_2HPO_4. $7H_2O$	10.19 g
$NaH_2PO_4.H_2$	8.56 g
H_2O	to 1l

4. SGCAA medium

Same as selective media except substitute following for dextrose

Galactose	20 g
H_2O	to 1l

5. **YPD Medium**

Yeast Extract	10 g
Peptone	20 g
Dextrose	20 g

6. PBS

NaCl	5.84 g
Na_2HPO_4	4.72 g
NaH_2PO_4 .$2H_2O$	2.64 g
pH	7.2
H_2O	to 1l

7. 2% PBSM

PBS containing 2% skimmed milk

8. STET

Sucrose	8 %
TritonX-100	5 %
Tris pH 8.0	50 mM
EDTA pH 8.0	50 mM

9. Lysozyme 10 mg/ml

10. TE

Tris pH 8.0	10 mM
EDTA pH 8.0	1 mM

11. Yeast extraction buffer

Tris-HCl pH 8.0	10 mM
NaCl	100 mM
Triton X-100	2 %
SDS	1 %

12. BMGY medium

Yeast extract	1 %
Peptone	2 %
Potassium phosphate pH 6.0	100 mM
YNB	1.34 %

Biotin	4 x 10^{-5} %
Glycerol	1 %

13. BMMY medium

Yeast extract	1 %
Peptone	2 %
Potassium phosphate pH 6.0	100 mM
YNB	1.34 %
Biotin	4 x 10^{-5} %
Methanol	1 %

14. SP Medium

KNO_3	300 mg
$MgSO_4 \cdot 7H_2O$	74 mg
$Ca(NO_3)_2 \cdot 4H_2O$	72 mg
KH_2PO_4	40 mg
H_3BO_3	1.0 mg
$MnSO_4$	0.1 mg
$ZnSO_4 \cdot 7H_2O$	1.0 mg
$Na_2MoO_4 \cdot 2H_2O$	0.1mg
$CuSO_4 \cdot 5H_2O$	0.03 mg
Na EDTA	0.003mg

Ferric citrate	1.0 mg
H_2O	to 1l

15. Solid media (1L)

McCown Woody Plant medium	2.46 g
Activated Charcoal	0.05 g
Sucrose	5 g
Gelrite	3 g

16. YEP agar

Yeast extract	0.2 %
Peptone	0.5 %
Agar	1.5 %

17. X-gluc staining solution

X-Gluc(5-Bromo-4-chloro-1*H*-indol-3-yl β-D-glucopyranosiduronic acid	1mM
Methanol	20 %l
Phosphate buffer pH 7.0	50 mM

18. Callus induction medium (CIM)

Ca Medium

McCown Woody Plant medium	2.46 g

Dicamba	50 mg
(3,6-Dichloro-2-methoxybenzoic acid)	
BA (N6-Benzyladenine)	2 mg
Galactose	15 g
Agar	4 g
Gelrite	3 g
pH	5.6
H_2O	to 1l

19. Cb Medium

McCown Woody Plant medium	2.46 g
Sorbitol	20 g
Maltose	10 g
PCA (p-Chlorophenoxy acetic acid)	5 mg
2iP (N6-(2-Isopentenyl) adenine)	2 mg
Agar	4 g
Gelrite	3 g
pH	5.6
H_2O	to 1l

20. CIM-B Medium

MS (Murashige and Skoog)	4.4g

2, 4 D (2, 4-Dichlorophenoxyacetic acid)	30 µM
BA (N6-Benzyladenine)	3 µM
Sucrose	30 g
Agar	4 g
Gelrite	3 g
pH	5.6
H_2O	to 1l

21. Co-cultivation medium

MS (Murashige and Skoog)	4.4 g
Acetosyringone	100 µM
Dextrose	20 g
Agar	4 g
Gelrite	3 g
H_2O	to 1l

22. Frond Regeneration Media

SH media	4.4 g
Sucrose	5 g
Agar	4 g
Gelrite	3 g

H_2O	to 1l

23. AB minimal medium for *Agrobacterium tumefaciens*

AB liquid	900 ml
20 x AB buffer	50 ml (1 x)
20 x AB salts	50 ml (1 x)

24. AB buffer (20 x)

K_2HPO_4 (anh.)	60 g
NaH_2PO_4 (anh.)	20 g
H_2O	to 1 L

Each salt was dissolved separately in 500ml H_2O and then mixed to obtain 1 L solution with pH 7.0.

25. AB salts (20 x)

NH_4Cl	20 g
$MgSO_4.7H_2O$	6 g
KCl	3 g
$CaCl_2$ (anhydrous)	3 g
$FeSO_4$	0.05 g
H_2O	to 1 L

25. AB liquid media

Glucose	5 g

H_2O	to 1 L

26. Induction medium for *Agrobacterium tumefaciens*

20 x AB salts	50 ml (1 x)
Glucose	1.8 g (10mM)
Glycerol	5 %
H_2O	to 1 L
pH	5.4