

Mechanistic Insights into Pregnenolone-Mediated Conidiation

Inhibition in *Fusarium oxysporum*

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Data S1: Sequencing of *Fusarium oxysporum* through ITS

>K6525-FO-1_TSS20241210-027-02838-BSND

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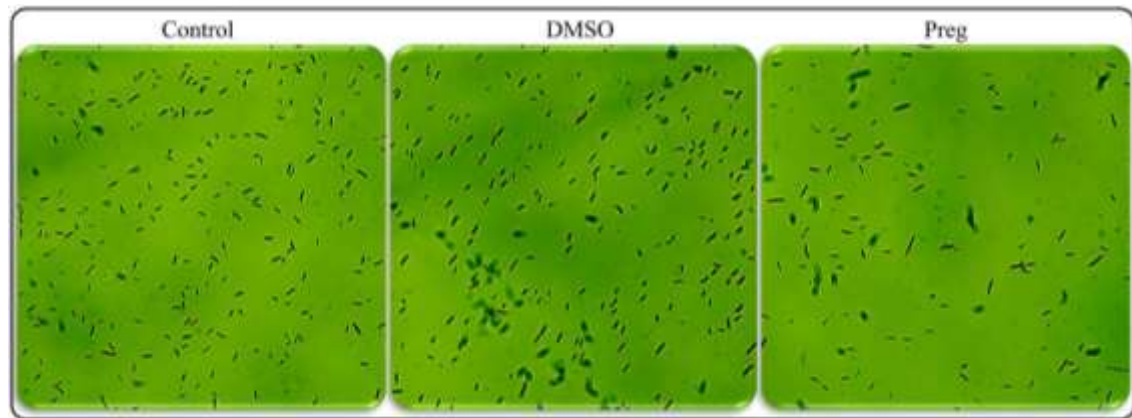


Figure S2: Effect of pregnenolone on conidiation of *Fusarium oxysporum*

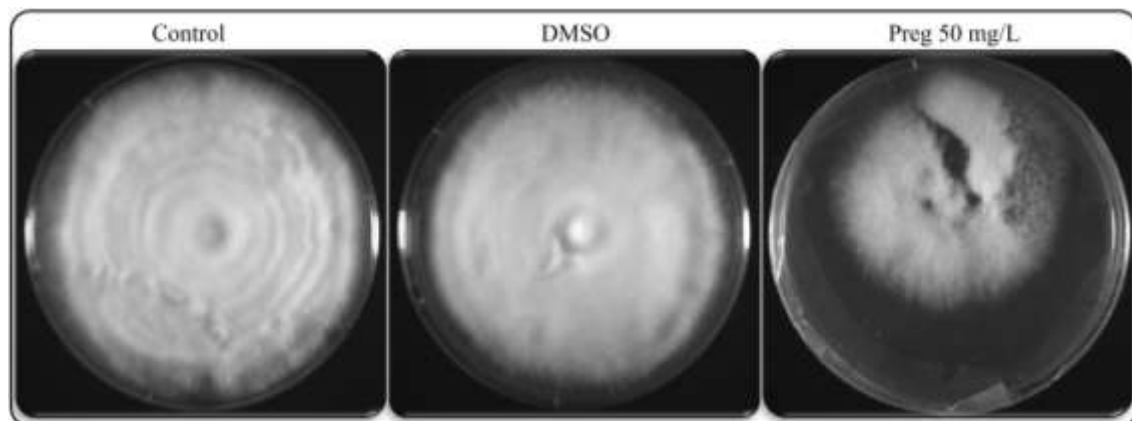


Figure S3: Effect of pregnenolone on colony growth of *Fusarium oxysporum*

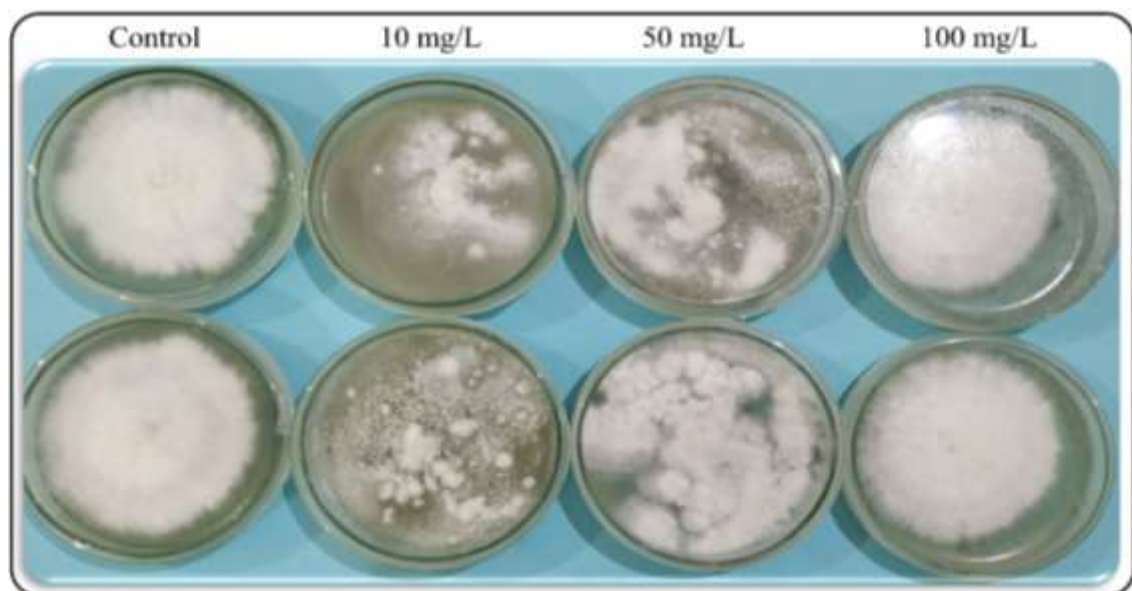


Figure S4: Effect of Pregnenolone spray on colony growth of *Fusarium oxysporum*

Table S1: Genes and Transcripts Statistics

Sample	Total	Known	Novel	Total	Known	Novel
	gene No	gene No	gene No	transcript No	transcript No	transcript No
Control1	14,903	14,700	203	15,473	14,435	1038
Control2	15,239	15,042	197	15,841	14,787	1054
Control3	15,510	15,306	204	16,121	15,047	1074

Control4	15,584	15,384	200	16,199	15,118	1081
Preg1	15,782	15,585	197	16,390	15,329	1061
Preg2	15,498	15,297	201	16,090	15,049	1041
Preg3	15,180	14,982	198	15,766	14,730	1036
Preg4	15,687	15,483	204	16,300	15,229	1071

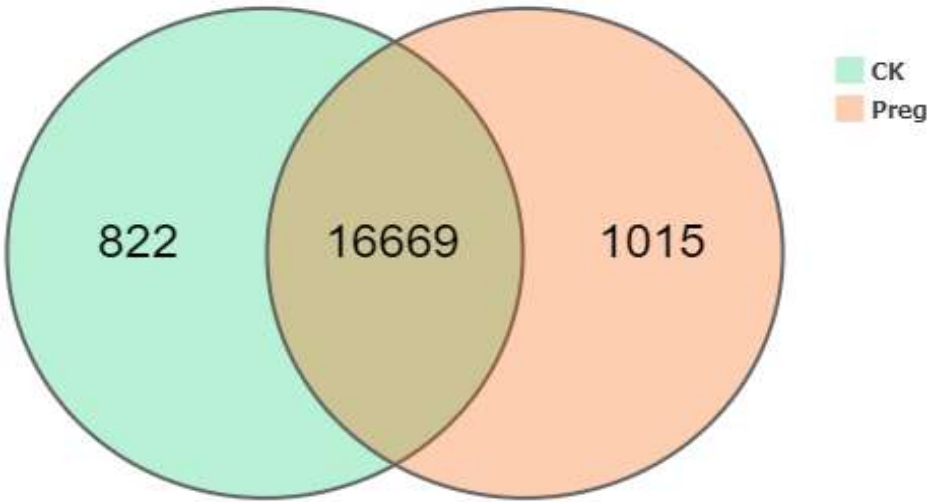


Figure S5: Venn diagram of sample expression

Table S2: Most Up-regulated and down-regulated genes

	Gene ID	F value	P value	Gene annotation	Pathway
Up-regulated genes	Gene_9990	4.42	1.50E-09	Delta24(24(1))-sterol reductase	Steroid biosynthesis
	Gene_9823	2.83	4.05E-05	white-opaque regulator 2	Steroid biosynthesis
	Gene_467	2.98	1.56E-09	cholestenol Delta-isomerase	Steroid biosynthesis
	Gene_13440	2.61	0.00122	ATP-binding cassette, subfamily C	ABC transporters
	Gene__2073	2.27	1.54E-07	ATP-binding cassette G	ABC transporters
	gene_21264	1.36	1.96E-05	ATP-binding cassette, subfamily B	ABC transporters
	Gene_12526	2.45	2.95E-05	succinate-semialdehyde dehydrogenase	Lysin degradation
	Gene_13315	1.52	1.097E-05	lysine4 N-trimethyltransferase SETD1	Lysin degradation
	Gene_16542	2.075	4.04E-05	aldehyde dehydrogenase	Lysin degradation
Down-regulated genes	Gene_26245	-1.30	0.0001526156	Delta7-sterol 5-desaturase	Steroid biosynthesis
	Gene_8233	-1.32	3.47E-05	Enolase	Glycolysis / Gluconeogenesis
	Gene_502	-1.2	9.37E-05	glyceraldehyde 3-phosphate dehydrogenase	Glycolysis / Gluconeogenesis
	Gene_23487	-2.23	3.21E-08	tRNA wybutosine-synthesizing protein 1	Taurine and hypotaurine metabolism
	Gene_16721	-1.74	3.21E-06	acetate kinase	Taurine and hypotaurine metabolism
	Gene_15148	-1.03	0.009789	retinol dehydrogenase 12	Biosynthesis of cofactors

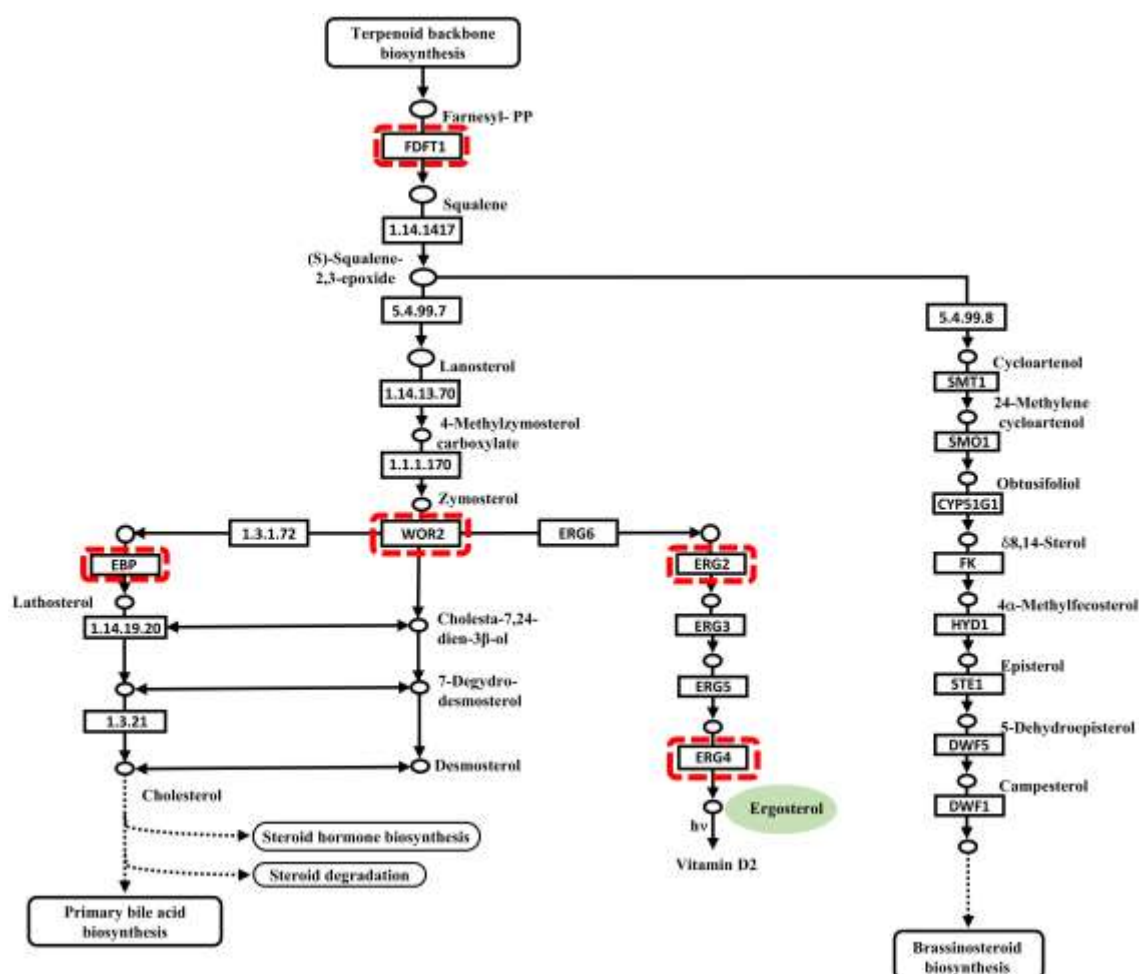


Figure S6: Steroid biosynthesis pathway in Fungi

Table S3. Primers used for the construction of knockout cassettes and PCR detection

Name	Primers
HYG-F	ATGAAAAAGCCTGAACTCAC
HYG-R	CTATTCCTTTGCCCTCGG
FDFT1-UP-F	CTCGCGGTGTAAAGAGACC
FDFT1-UP-R	gtgagttcaggcttttcatTCGTCTGGTAGGGTGGG
FDFT1-DW-F	ccgagggcaaaggaatagAGATGATGGCAGAACTTTGG
FDFT1-DW-R	CGTGCTGATTTCTCCAACCTTTG
ERG4-UP-F	AGGTGGGAGAACAAAATCAAGG
ERG4-UP-R	gtgagttcaggcttttcatCGATGGGGAATCCGAATAAG
ERG4-DW-F	ccgagggcaaaggaatagGCATTTGCATACTTGTTATGAGGC
ERG4-DW-R	CCGTCATTATGGCCGGTATC
WOR2-UP-F	CTTCGATTTGGCGGTGATTAC

WOR2-UP-R	gtgagttcaggcttttcatGCGCAGTACAGCGCTAG
WOR2-DW-F	ccgagggcaaaggaatagCTGGTAGTCACTGCTCGAG
WOR2-DW-R	GCTACGAGATGTTTCCCGC
ERG2-UP-F	TACGGAGATAAGACTCCAGGG
ERG2-UP-R	gtgagttcaggcttttcatACCGTTCCGCATCGC
ERG2-DW-F	ccgagggcaaaggaatagTATTGCTGTTTGGGTGTACCAAC
ERG2-DW-R	CACAACAGCGGTTGGTTC
EBP-UP-F	AGGATGCAGCAGCTACAG
EBP-UP-R	gtgagttcaggcttttcatACAGAGCCCCTCGGC
EBP-DW-F	ccgagggcaaaggaatagGATCCTGAGCCGGGG
EBP-DW-R	AAGGAATCAGAGGCCGG

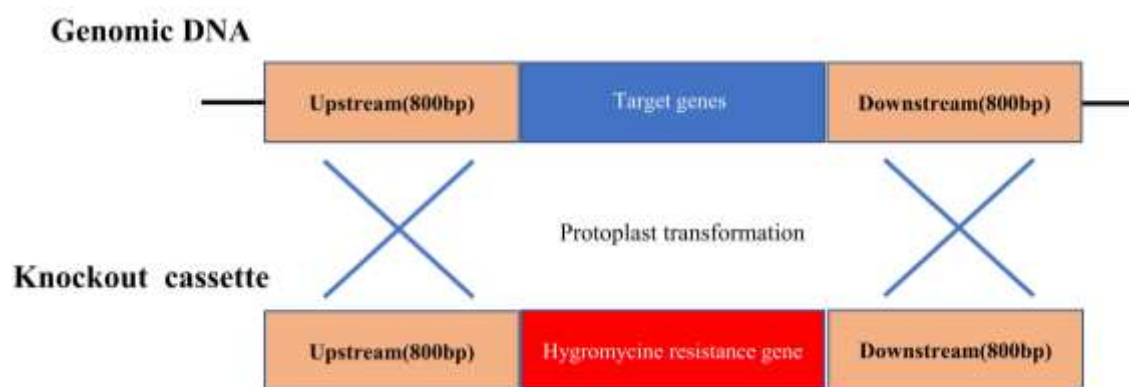


Figure S7: Schematic diagram of the principle of gene knockout

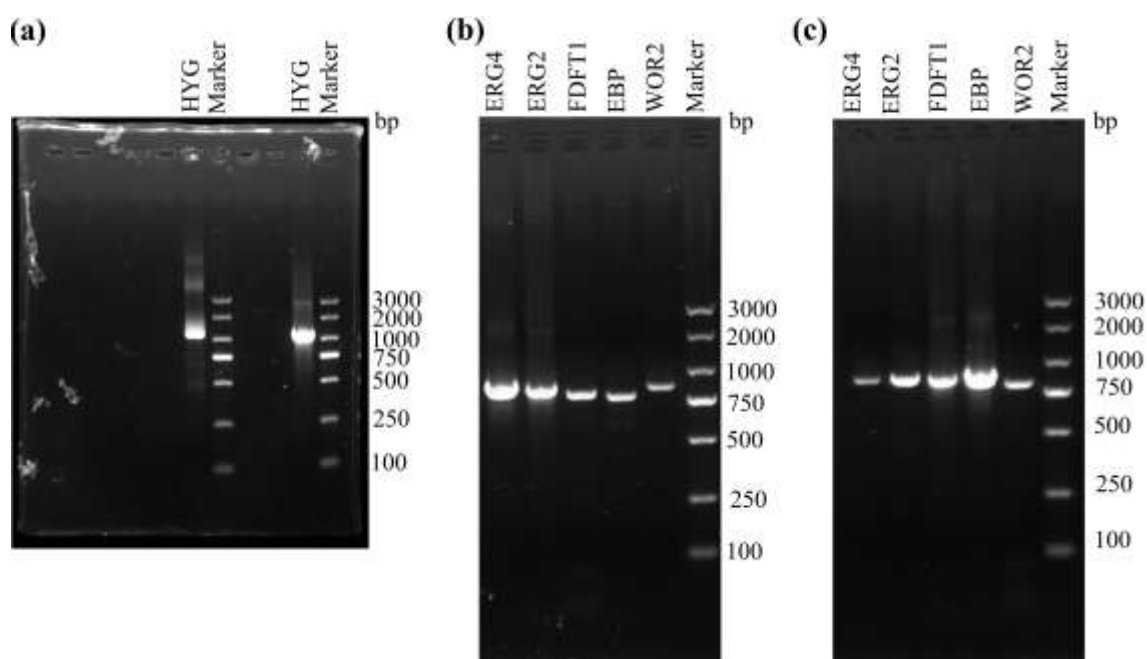


Figure S8: PCR amplification of DNA fragments

(a) Hygromycin resistance gene (1026 bp), (b) Upstream homologous recombination sequences (800 bp) of the five target genes, (c) Downstream homologous recombination sequences (800 bp) of the six target genes

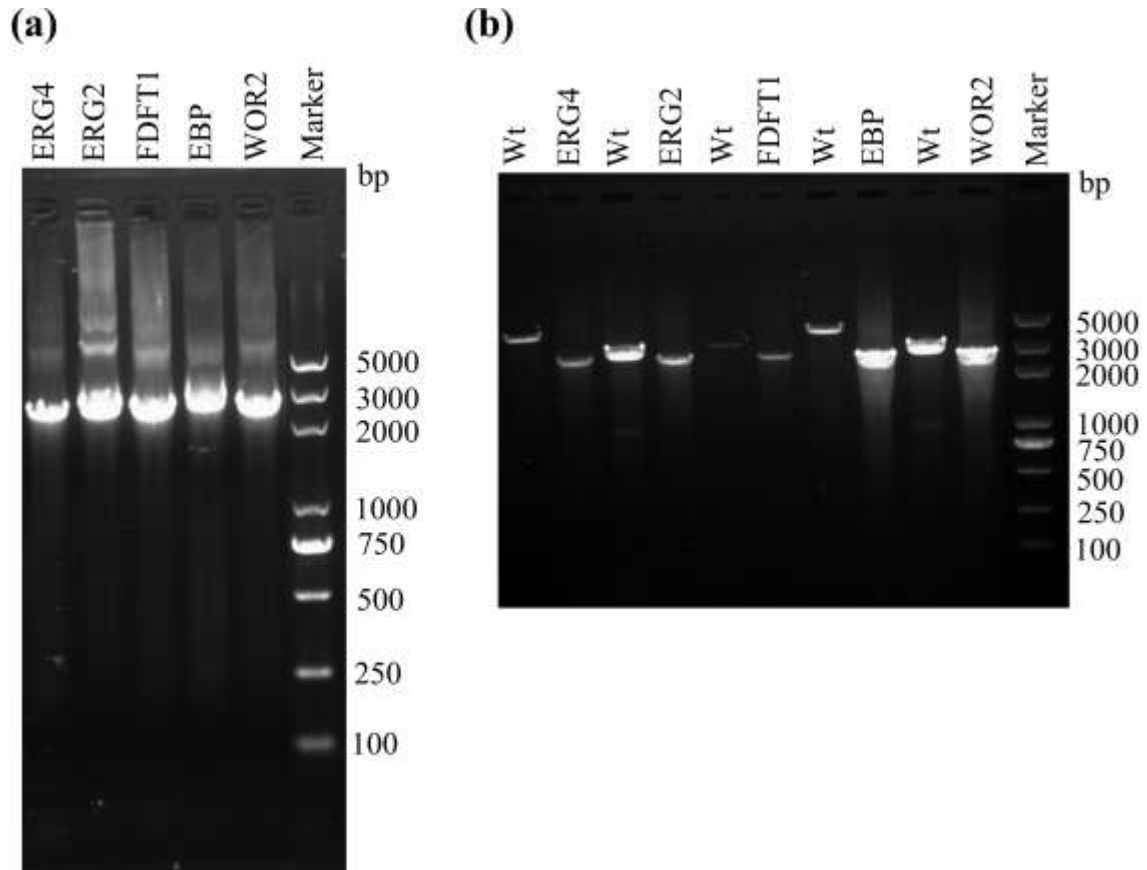


Figure S9: Fusion PCR and confirmation of gene knockouts

(a) Knockout box, (b) PCR validation of mutants compared with wild type.

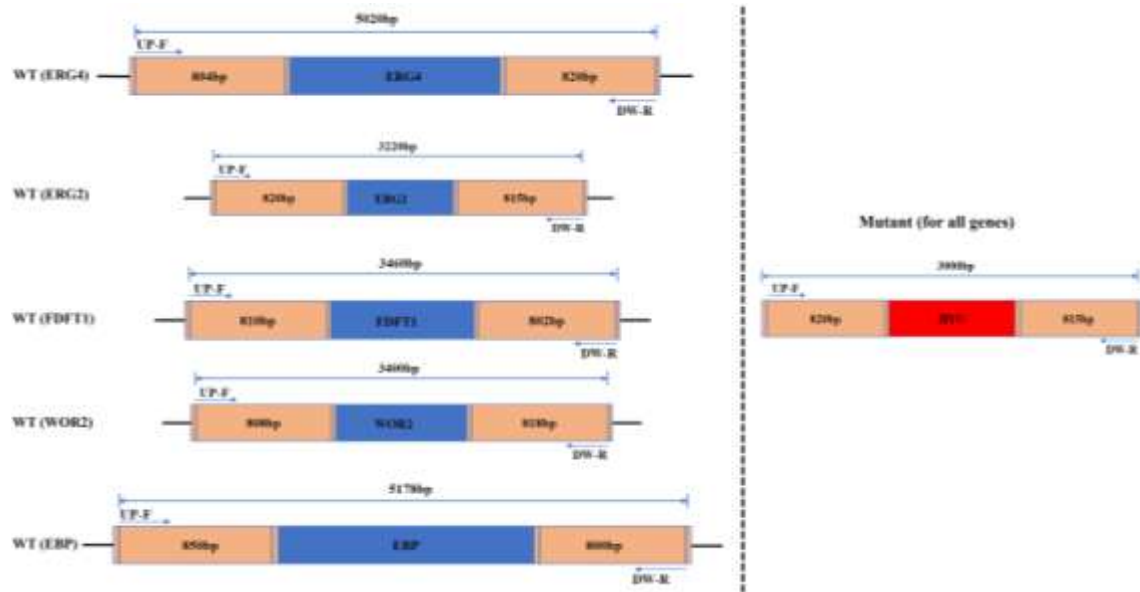


Figure S10: Schematic diagram for PCR verification of knockout strains

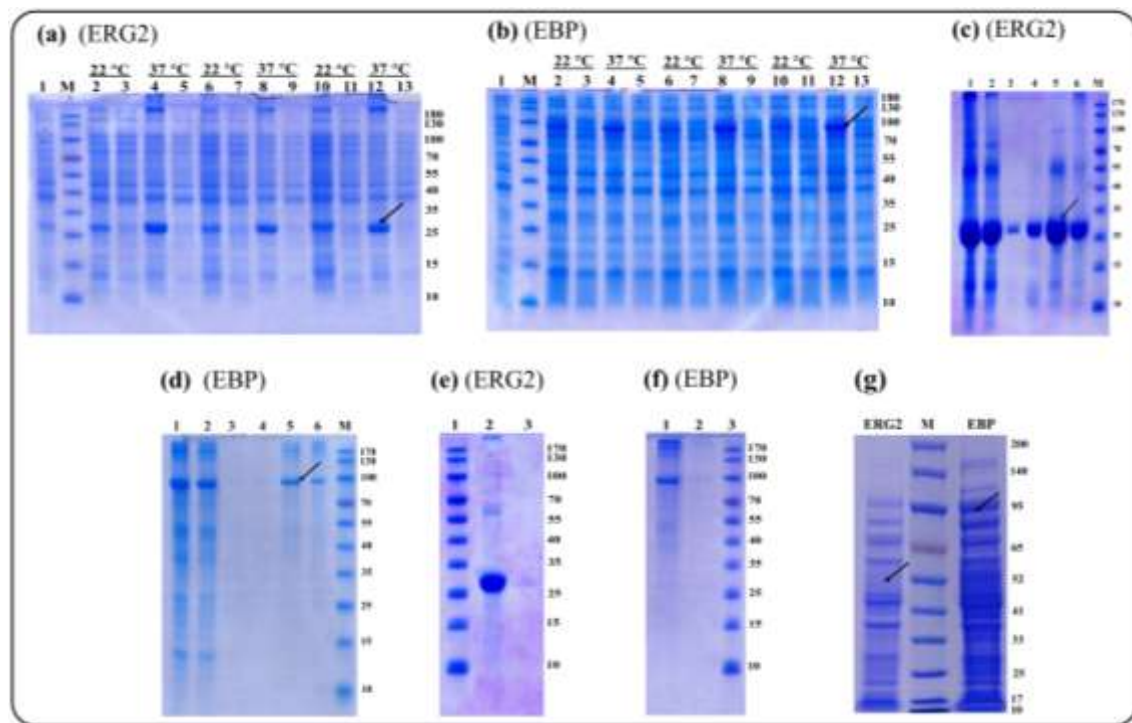


Figure S11: Expression and purification of ERG2 and EBP

Note: (a, b); Lane 1 (Without induction), Lane M (Marker); Lane 2 (whole cell lysate 22°C), Lane 3 (supernatant 22°C); Lane 4 (whole cell lysate 37°C), Lane 5 (supernatant 37°C); Lane 6 (whole cell lysate 22°C), Lane 7 (supernatant 22°C); Lane 8 (whole cell lysate 37°C), Lane 9 (supernatant 37°C); Lane 10 (whole cell lysate 22°C), Lane 11 (supernatant 22°C); Lane 12 (whole cell lysate 37°C), Lane 13 (supernatant 37°C).

Purification of denature protein from inclusion body of ERG2 **(c)** and EBP **(d)**; Lane 1 Supernatant, Lane 2 Flow through, Lane 3 Elution by 2 mM imidazole, Lane 4 Elution by 20mM imidazole, Lane 5 Elution by 250mM imidazole, Lane 6 Beads, Lane 7 marker. Refolding of denatured protein via dialysis of ERG2 **(e)** and EBP **(f)**: Lane 1 marker, Lane 2 before dialysis, Lane 3 after dialysis, **(g)**: Expression of ERG2-GFP and EBP-GFP in E. coli (BL21)

Table S4: Analysis of binding ability

Fluor. Molecule	Ligand	K _D [μM]	K _D Confidence [μM]	Signal/Noise
ERG2-GFP	Pregnenolone	16.3	± 12.85	13.320
EBP-GFP	Pregnenolone	0.15	± 0.051	9.734
GFP-tag	Pregnenolone	No binding	/	/