

ATCGGGGAATAACGTTGGACCTTTTTGGGTTTCTGATACAAATATCTTTTCGATCCAACAGCCTCTTCGCCCCACCACACATTCTCCAGAAACATGAACAAGTGCATCTCCATCCTCCTCGCCACGGCAACCTCTACCTCTGCCTTCGTGACACATCAAGGTRTCGGTGGCAGATATGCAAGTCACCCCTACACGCCGAGAAGCAAGTGTATAGGCCCTTATTTGCAACCTGGAATAACCCAAAGACTATGTTTATAGAAAAGCTAGGTTGTCGGAGAAGCGGGTGTTGTGTAAAGAGTCTGTTGACTTGTTACAGTAGAGGTAGGAGGAGCGGTCGCCGTTGGAGATGGAGACGGAAGCAGTGTGTAGTTCCAYAGCCACCGTCTATACGTCAAGTGGGATGTGCGGCTCTTCGTTTCA

1 5 10 15 20 25 30 35
M N K C I S I L L A T A T S T S A F V T H Q G X G R Y A V T L H A E K Q V

Exon1

GGCGTCTTCTTCGGTACTTCCACAGGAAGCACAGAGGAGGACGCCGAGCTCATCGTATCGGAATTCTGGTGATGTTGCAGCAGGACCTATTGATATTGATGGTGTAGCCGCGCAGTGTGCCAAAGAGTTTGCAGAAATGATGCTTTAGTGGTAAGATKTGTTTCATCGTCGTACATATCATAATGTGCCCTWGATCCTGTGCATCACATGCCGAGAAGAAGCCATGAAGGTGTCCTTCGTCTCCTCCGTCGGCTCGAGTAGCATAGCCTTAAGCCACTACAACGTCGTCTCGGATAACTATACTACCACATCGGCCGTCACAACGGTTTCTCAAACGCTTCATACTACGAAATCACCATTCTAMACAAGTAGCAGCATGTATAGTATTACACGGGAWCTAGGACAGCGTAGTGTAC

40 45 50 55 60 65 70 75 80 85
G V F F G T S T G S T E E A A E L I V S E F G D V A A G P I D I D G V A G S V A K E F A K Y D A L V

Exon1

FMN bind

Targeted deletion

TGTCTGATGCCGTTCTCCGATATCTTCTCATTAAACAGTGAACACCAACACTGGAACACTGCTGACACTGAACGTAGTGGACGGGCTGGGATGAGATCTACTACTCTGAAATGCAAGATCTCGACATTGCAAGGGAAGAGTGGCCGTTTTCCGATTAGGAGATTCAGTTAGTTATTGTTGAGAAYATGCTGATGCGACTGCTG
 TACACAGACTACGGCAAGAGGCTATAGAAGAGTAATTGTCCATCCTTGTGGTTGTACCTTGTGACCACGACTGTGACTTGCATCACCTTGCCCGACCCCTACTCTAGATGATGAGACTTTACGTTCTAGAGCTGTAACGTCCTTCTCCACCGGCAAAAGCCATCTCTAAGTCAATCAATAACACTCTTTRACGACTACGCTGACC

1 5 10 15 20 25 30 35 40 45 50 55
 V G T P T W N T G A D T E R S G T G W D E I Y Y S E M Q D L D I A G K K V A V F G L G D S V S Y C E N Y A D A T G

AGAGGTACGTGGATTGGTTCTTTTGGTTGGTGTGCTTTATGATCACAACCTAAGCATCTGTGACTTCAACAATAGTGCATGATGTTTTGAAAGCACTCGGGTGAAGATGATGGGTTACACCTCTGTGCGAMGGATATCTCCAGCAAGAACTAAAAGGCTCAGCGTGGAGAAAAGTTCTGTGGAAGTCCATTAGATGCGGTGAATCAAA
 TCTCCATGCACCTAACCAAGAAACAAAACACACWGAAATACTAGTGTGGATTGCGTAGACACATGAAGTTGTTATCGACGCTACTACAAAACTCTGTTGAGCCACATTTACTACCCAAATGTGGAGACAGCTKCCTATAGAGGTGCTTCTTAGTTTCCGAGTGCACCTCTTTTCAAGACACCTGACGGTAATCTACGCCACTTAGTT

E
 Exon2

1 5 10 15 20 25 30 35 40
 L H D V F E A L G C K M M G Y T S V X G Y L H E E S K A Q R G E K F C G L P L D A V N Q

sgRNA2

GAGGAATTGACCGAGGAAAGAGTTCAAAAGTGGGTAGCTGCTCTCATCGCGGAAGGAATCTTGGAGGGTGGCGGTGAAAGTACTTCTACGGCAGCAGTGTCTGTGCCAATTGCTTCTCTGTTGCTGCAAGTTGCAACAACAGCCGTTGAAGTTGAGATCGTTTTCAGTTTTCAGAGCCAGCTTCAGGATATGTTGGGCACTACAACCTTAGA
 CTCCTTAACTGGCTCCTTTCTCAAGTTTTACCCATCGACGAGAGTAGCGCCTTCTTAGAACCTCCACCGCCACTTCGATGAAGATGCCGTCGTACAGACACGGTAAACGAAGAGGACAACGACGTCACAGCTTGTGTCGGCACTTCAACTCTAGCAAAAGTCAAAGTCTCGGTCGAAGTCTATACAACCCGTGATGTTGGGATCT

45 50 55 60 65 70 75 80 85 90 95 100 105 110
 E E L T E E R V Q K W V A A L I A E G I L E G G G E A T S T A A V S V P I A S P V A A V A T T A V E V E I V S V S E P A S G Y V G H Y N P R

Exon3

AGTGACAAAAAATGTGGATCAGTGTGACGGGCTTCATCATATGTTACCAAGTGGAGCTCCTTAGACAAGTCAAAATGATTGTACAGTTCGTTGAAAGAGATAGAGAATGAAAGTTATACATAAACTACATTCATATCAGTCGTGGATTCTCGGCTGGTTCTCCCATCACTACATCTTCTTCTGTTGCACACAGCA
 TCACTGTTTGTGTACACCTAGTCACAACCTGCCCGCAAGTAGTATACAATGGTCACCTCGAGGAATCTGTTCAAGTTTACTAACATGTCAAGCACAACTTTCTATCTTACTTTTCAATATGATTGATGTAAGTATAGTCAGCACCTAAGGAGCCGACCAAGAGGGTAGTGATGTAGAAGGAAGACAACGTGTGCTCGT

115 120 125 130 135 140 145 150 155 160
 S D K T M W I S V D G R S S Y V T S G A P T S Q M I V Q F V L K E I E N E K L Y I N Y I H I S

Exon3

3'
 146
 5'

	WT		Flav KO			-
Colony:	5	16	9	14	1	
750	+	+	+	+	+	
500			+			

The diagram illustrates the human CYP11A1 gene structure and the binding sites for two sgRNAs. The gene structure shows Exon 1 (1-14 nt) and Exon 2 (15-61 nt) separated by a 47-nt intron. sgRNA1 targets the 5' end of Exon 1 (nt 1-14), and sgRNA2 targets the 5' end of Exon 2 (nt 15-61). The diagram shows the sgRNA sequences and their binding sites on the gene structure.

sgRNA1 (top):
 Sequence: 5'-ATTAAACAGGTAGGAACACCAACATGGAACACTGTTGCTGACACTGAAC-3'
 Binding site: 1-14 nt of Exon 1

sgRNA2 (bottom):
 Sequence: 5'-GTAGTGGAACGGGCTGGGATGAGATCTACTACTCTGAAATGCAAGATCTCGACATTGCAAGGGAAGAGGTGGCCGTTTTTCGGATTAGGAGATTCAAGTTAGTTATTGTGAGAAATATGCTGATGCGACTGGAGAGGTACGTGGATTGGTTCTTTTGTTTTG-3'
 Binding site: 15-61 nt of Exon 2

Time (d)	Control (Fv/Fm)	Drought (Fv/Fm)
0	0.65	0.65
1	0.64	0.63
2	0.63	0.62
3	0.64	0.61
4	0.62	0.59
5	0.60	0.57
6	0.58	0.55
7	0.58	0.52

Time (d)	Control (grey)	Drought (orange)	Drought + GA ₃ (yellow)
0	0.60	0.60	0.60
2	0.58	0.60	0.60
4	0.55	0.58	0.58
6	0.45	0.42	0.45
8	0.45	0.40	0.42

H ₂ O ₂ (μM)	Genotype	Median % Dead cells	Q1 % Dead cells	Q3 % Dead cells	Min % Dead cells	Max % Dead cells
0	Δp1	0	0	0	0	0
0	Δp2	0	0	0	0	0
25	Δp1	0	0	0	0	0
25	Δp2	0	0	0	0	0
50	Δp1	0	0	0	0	0
50	Δp2	0	0	0	0	0
75	Δp1	15	5	20	0	25
75	Δp2	2	0	5	0	10
100	Δp1	30	25	50	20	60
100	Δp2	15	10	20	10	25
150	Δp1	95	85	100	70	100
150	Δp2	85	80	95	75	100
200	Δp1	95	90	100	90	100
200	Δp2	95	90	100	90	100
250	Δp1	95	90	100	90	100
250	Δp2	95	90	100	90	100

Box plot showing the percentage of dead cells in response to H_2O_2 treatment for Flav-KO and WT strains across different colonies. The y-axis represents % Dead cells (0 to 100), and the x-axis represents H_2O_2 (μM) (100 to 200). The legend indicates Colony (1, 14, 9, 0, 16, 5) and Strain (Flav-KO, WT). Asterisks (*) indicate significant differences between Flav-KO and WT strains at each H_2O_2 concentration.

H_2O_2 (μM)	Strain	Colony	% Dead cells (approx. median)
100	Flav-KO	1	18
100	Flav-KO	14	20
100	Flav-KO	9	15
100	WT	0	10
100	WT	16	12
100	WT	5	10
100	Flav-KO	1	85
100	Flav-KO	14	88
100	Flav-KO	9	90
100	WT	0	75
100	WT	16	72
100	WT	5	70
200	Flav-KO	1	85
200	Flav-KO	14	88
200	Flav-KO	9	90
200	WT	0	70
200	WT	16	68
200	WT	5	65