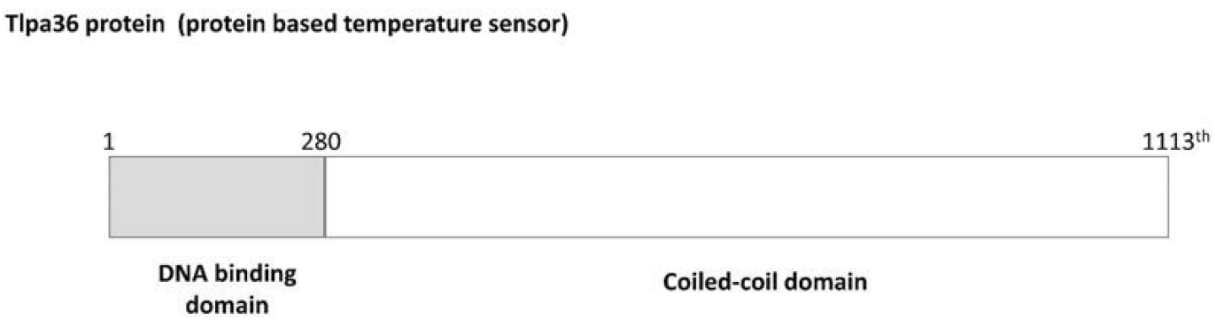
FIGURE 1

A


Tlpa36 protein (protein based temperature sensor)
1
280
1113th
DNA binding domain
Coiled-coil domain

B

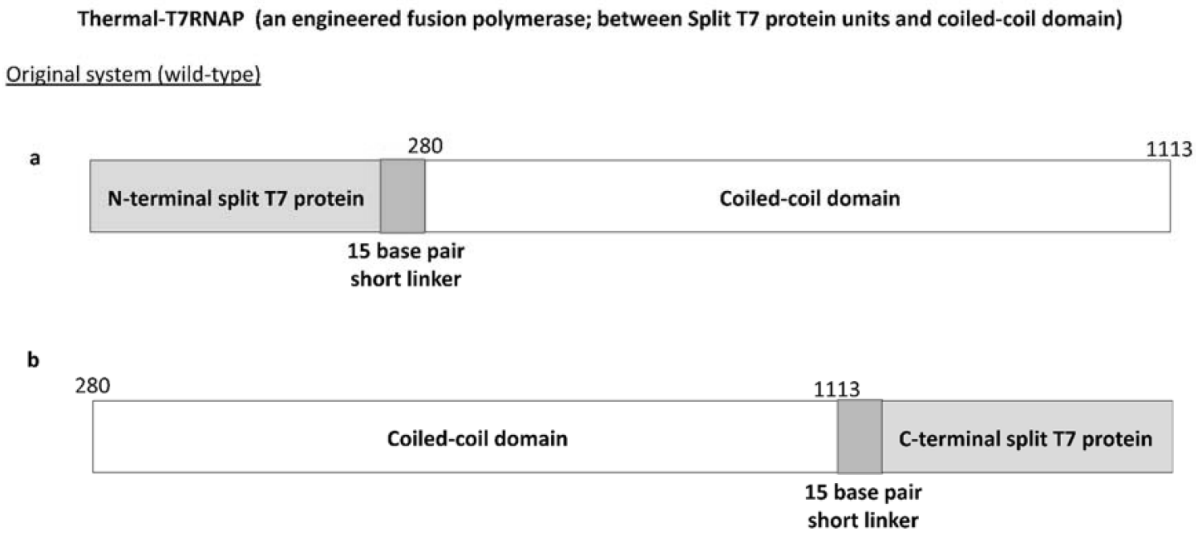

Thermal-T7RNAP (an engineered fusion polymerase; between Split T7 protein units and coiled-coil domain)
Original system (wild-type)
a
280
1113
N-terminal split T7 protein
Coiled-coil domain
15 base pair short linker
b
280
1113
Coiled-coil domain
C-terminal split T7 protein
15 base pair short linker

FIGURE 2

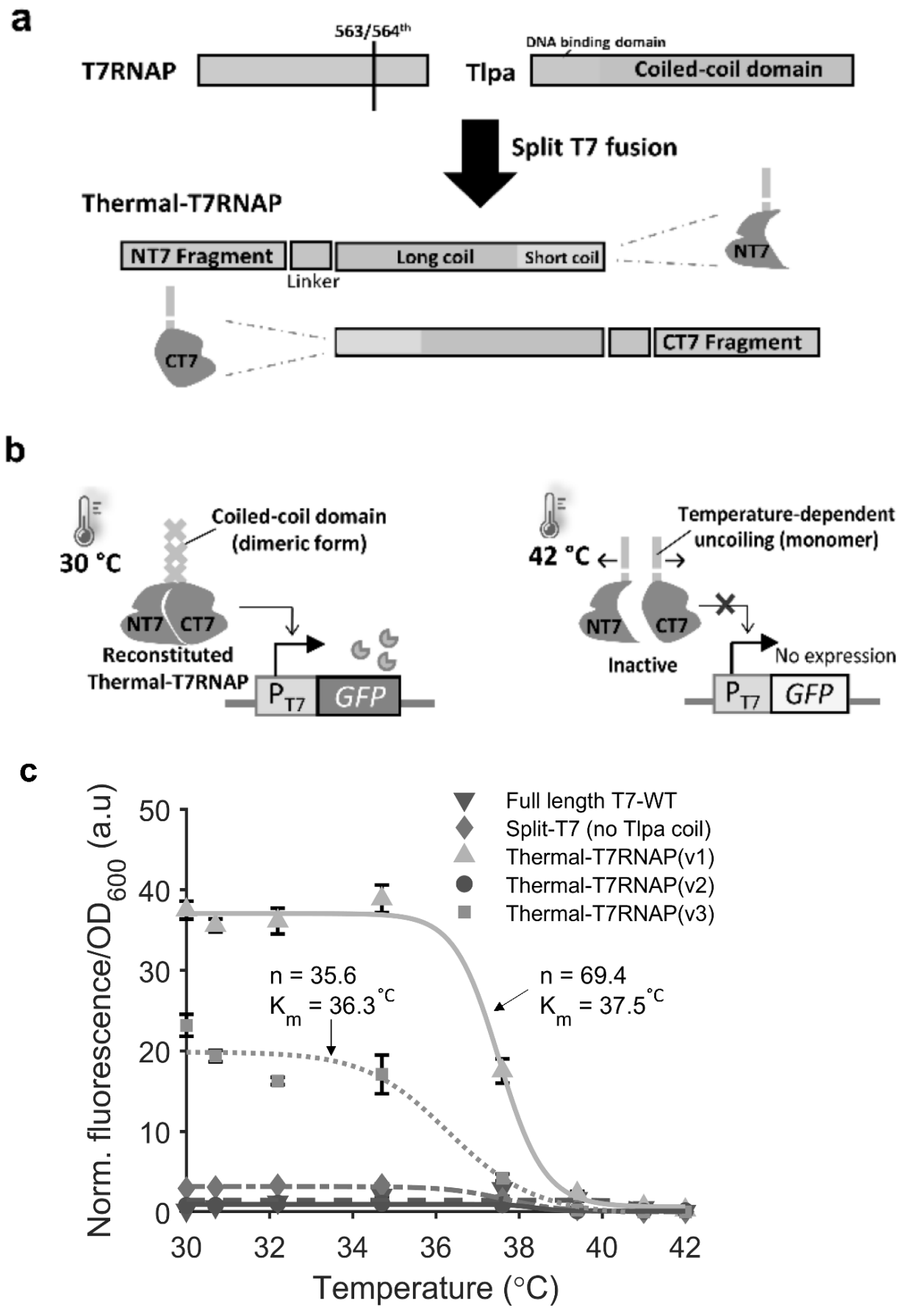

a
563/564th
DNA binding domain
T7RNAP
Tlpa
Coiled-coil domain
Split T7 fusion
Thermal-T7RNAP
NT7 Fragment
Linker
Long coil
Short coil
NT7
CT7
CT7 Fragment
b
30 °C
Coiled-coil domain
(dimeric form)
NT7
CT7
Reconstituted
Thermal-T7RNAP
P T7
GFP
42 °C
Temperature-dependent
uncoiling (monomer)
NT7
CT7
Inactive
No expression
P T7
GFP
c
Norm. fluorescence/OD600 (a.u)
Temperature (°C)
Full length T7-WT
Split-T7 (no Tlpa coil)
Thermal-T7RNAP(v1)
Thermal-T7RNAP(v2)
Thermal-T7RNAP(v3)
n = 35.6
Km = 36.3 °C
n = 69.4
Km = 37.5 °C

FIGURE 2 (continued)

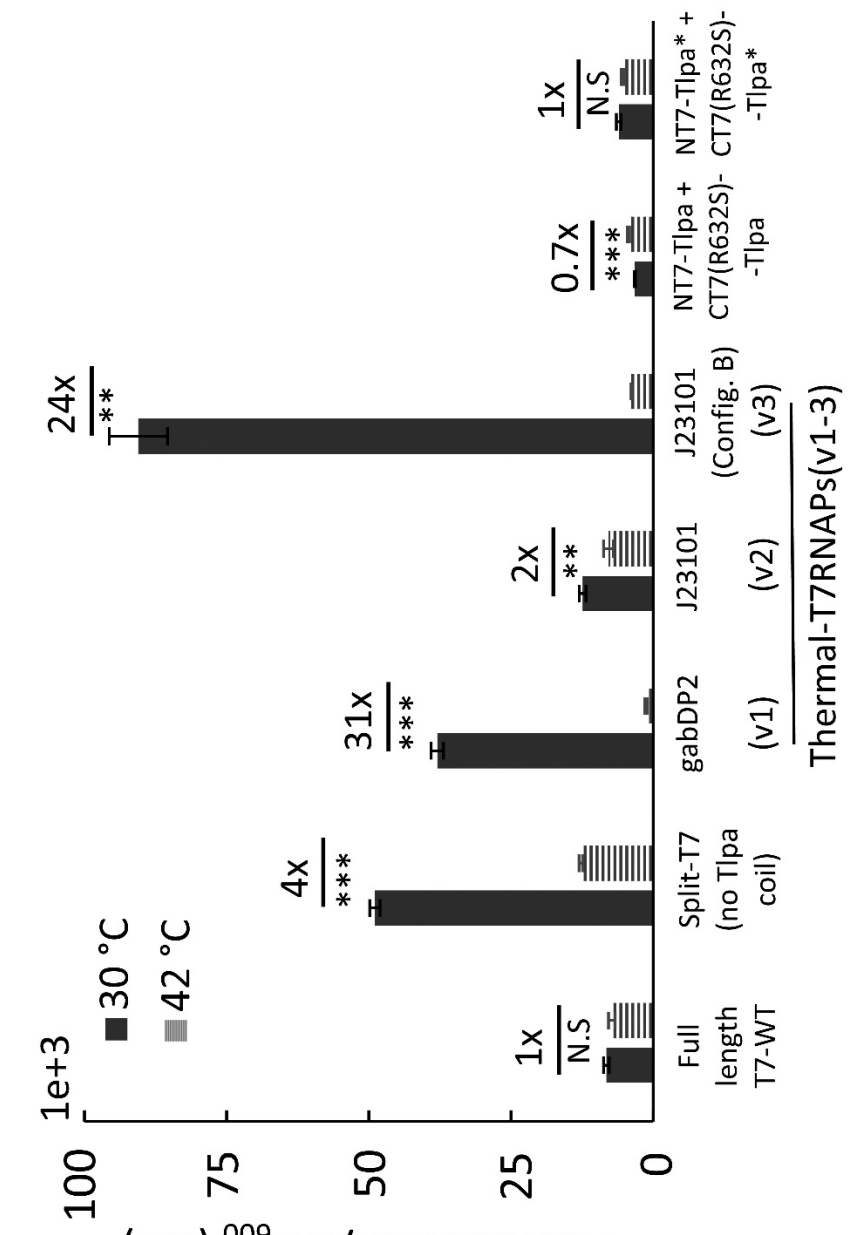
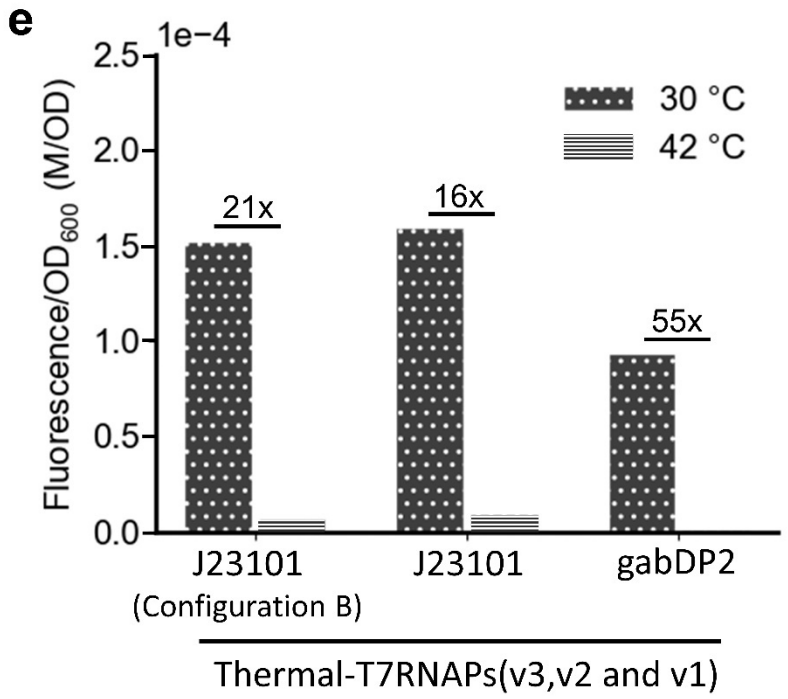


FIGURE 2 (continued)



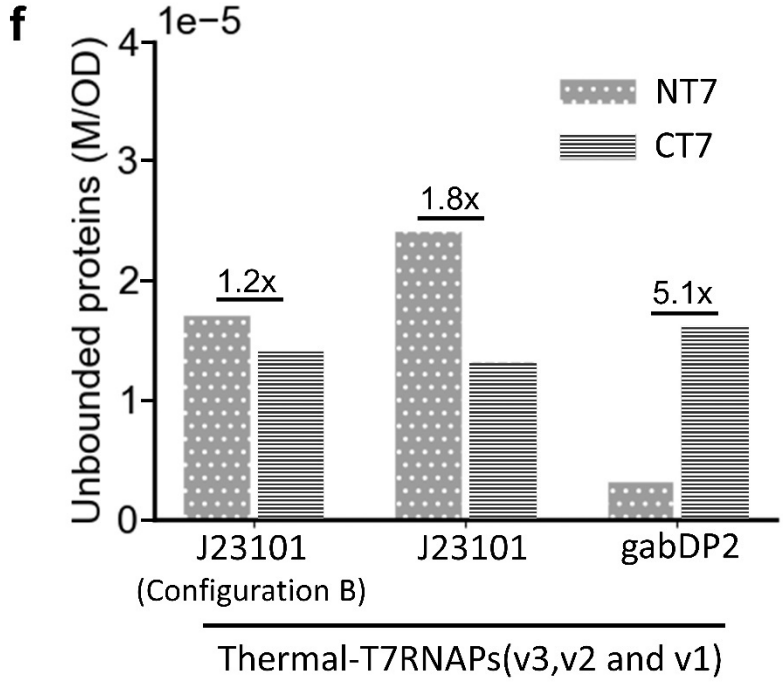


FIGURE 2 (continued)

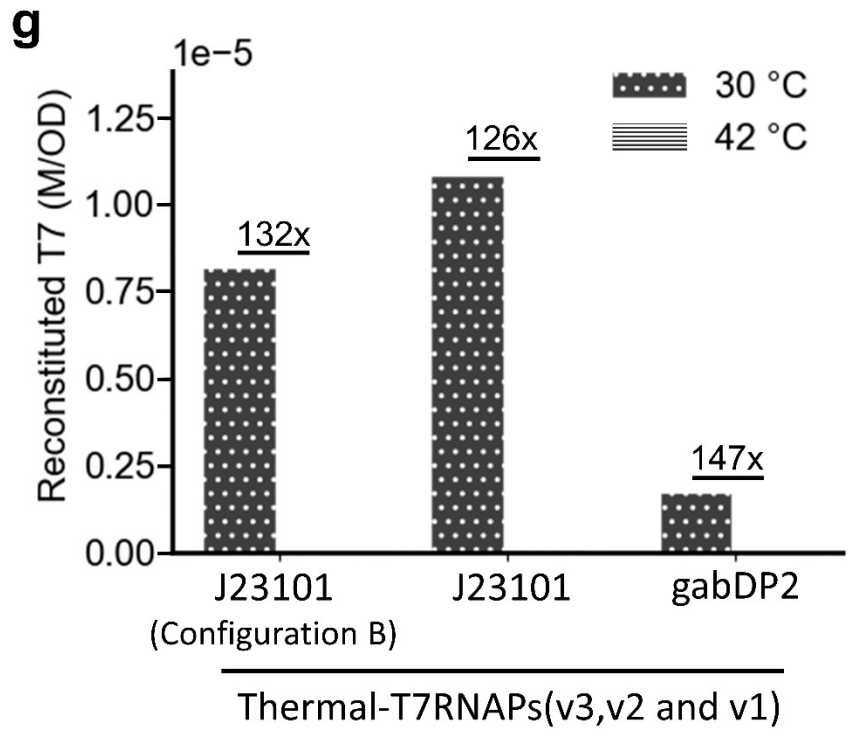


FIGURE 3

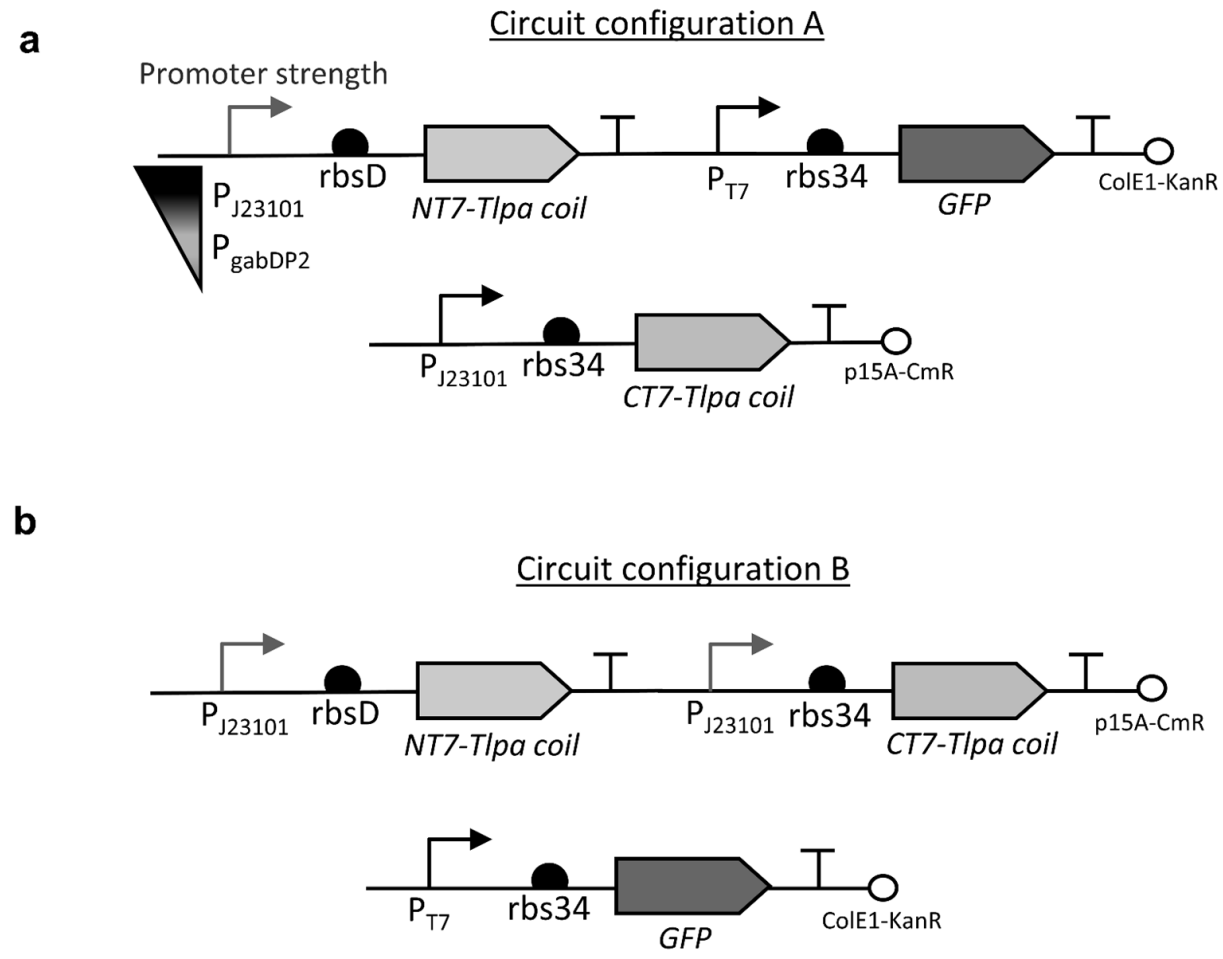

a
Circuit configuration A
Promoter strength
P J23101
P gabDP2
rbsD
NT7-Tlpa coil
P T7
rbs34
GFP
ColE1-KanR
P J23101
rbs34
CT7-Tlpa coil
p15A-CmR
b
Circuit configuration B
P J23101
rbsD
NT7-Tlpa coil
P J23101
rbs34
CT7-Tlpa coil
p15A-CmR
P T7
rbs34
GFP
ColE1-KanR

FIGURE 4

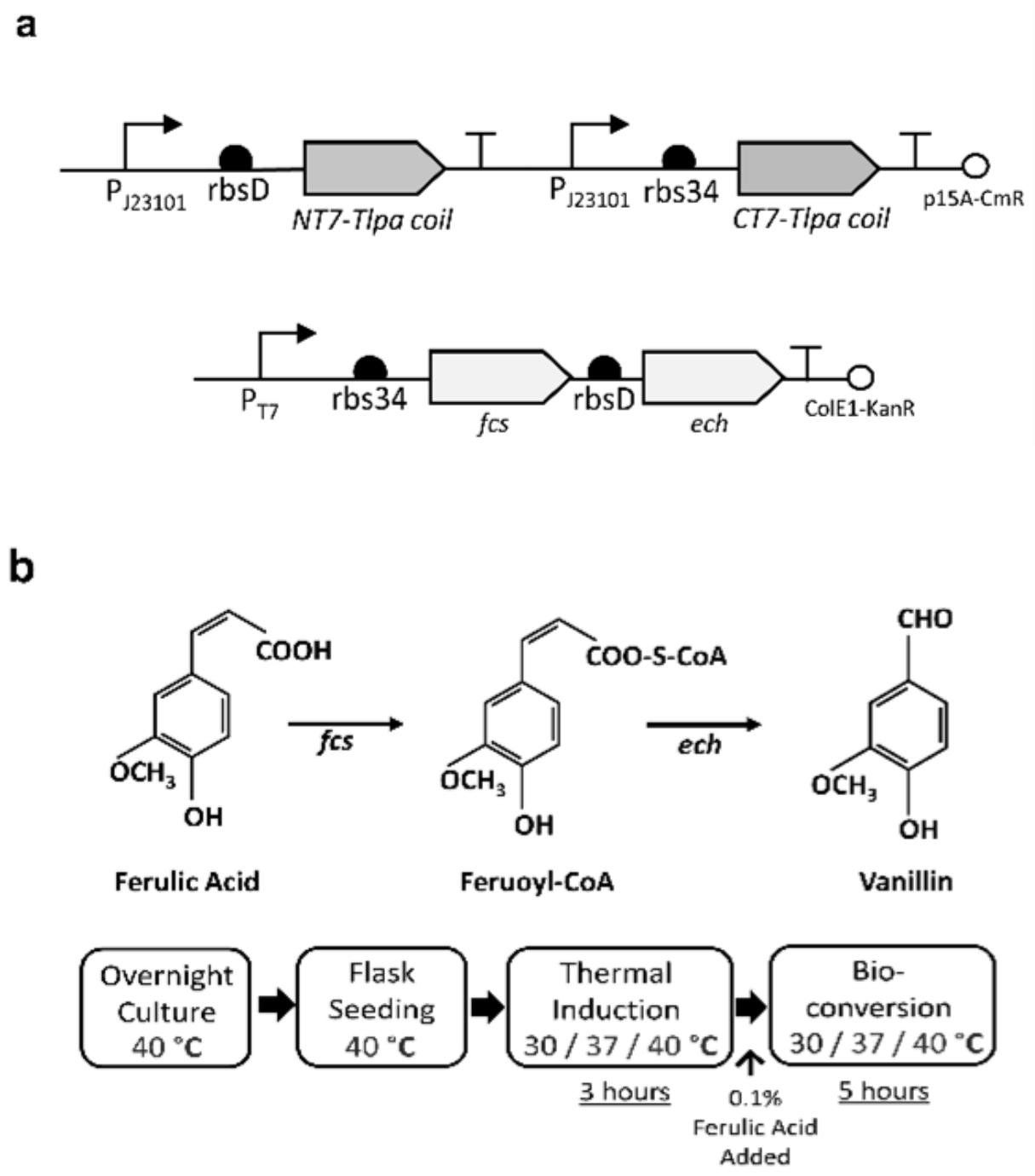

a
P J23101
rbsD
NT7-Tlpa coil
P J23101
rbs34
CT7-Tlpa coil
p15A-CmR
P T7
rbs34
fcs
rbsD
ech
ColE1-KanR
b
COOH
OCH3
OH
fcs
COO-S-CoA
OCH3
OH
ech
CHO
OCH3
OH
Ferulic Acid
Feruoyl-CoA
Vanillin
Overnight Culture 40 °C
Flask Seeding 40 °C
Thermal Induction 30 / 37 / 40 °C
Bio-conversion 30 / 37 / 40 °C
3 hours
0.1% Ferulic Acid Added
5 hours

FIGURE 4 (continued)

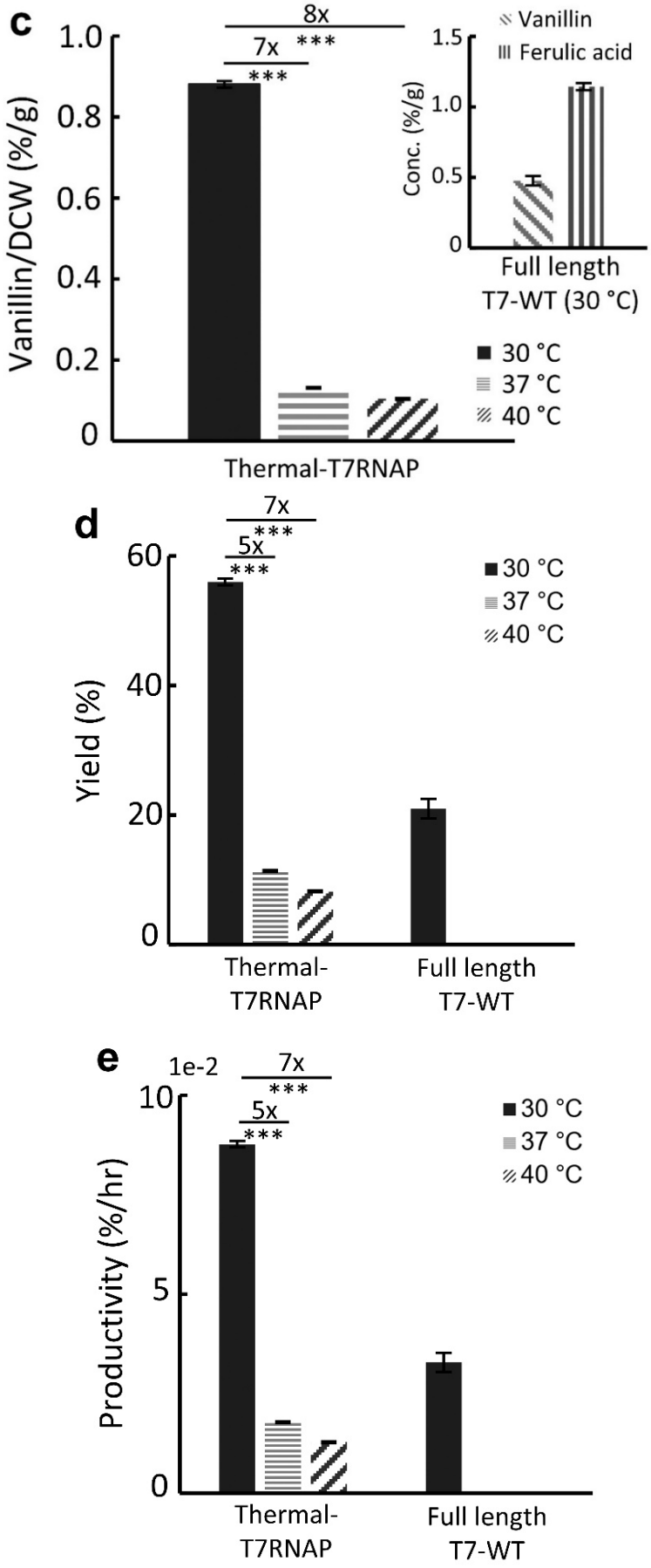


FIGURE 5

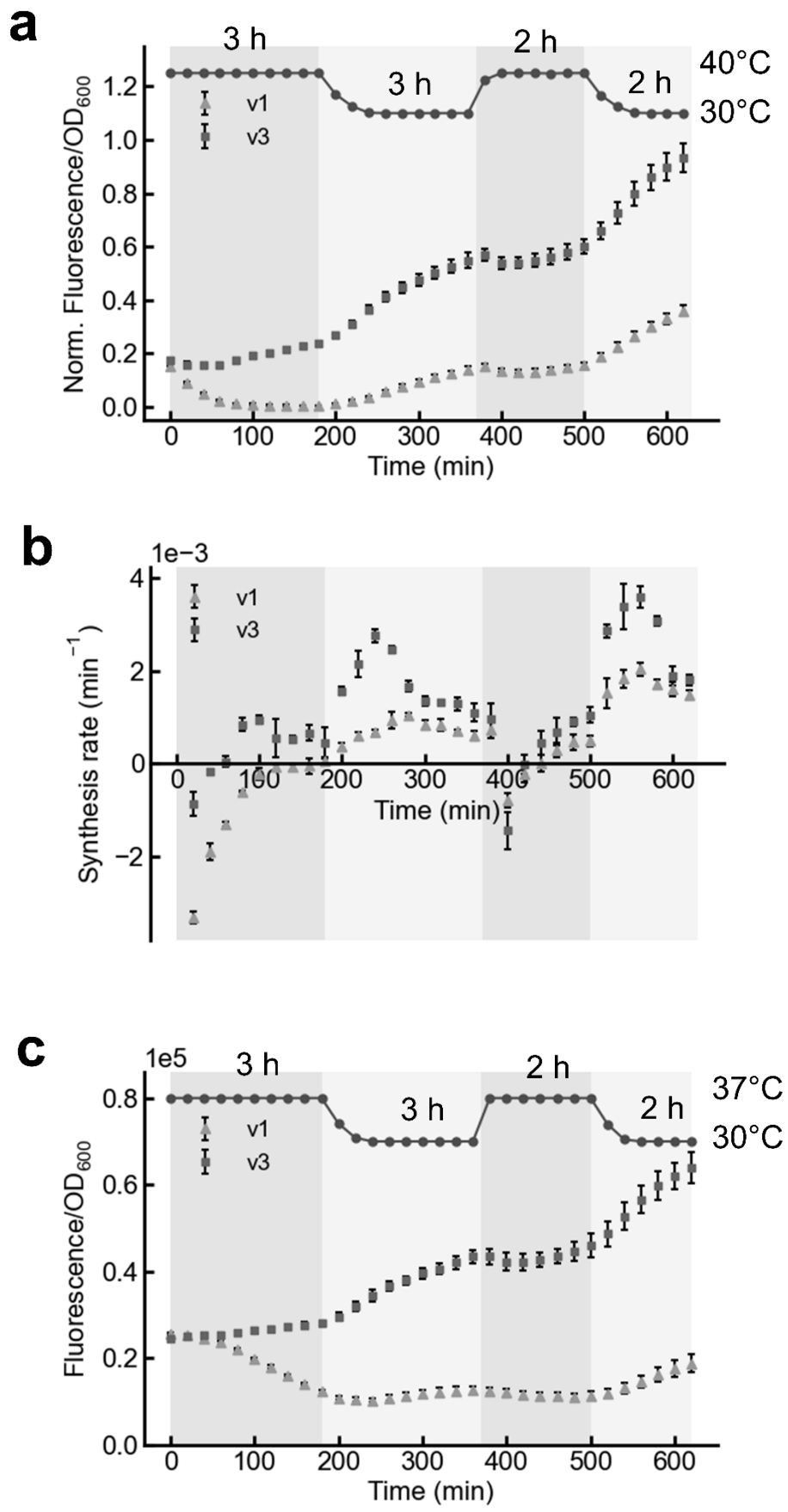

a
3 h
2 h
3 h
2 h
40°C
30°C
v1
v3
Norm. Fluorescence/OD600
Time (min)
b
1e−3
v1
v3
Synthesis rate (min−1)
Time (min)
c
1e5
3 h
2 h
3 h
2 h
37°C
30°C
v1
v3
Fluorescence/OD600
Time (min)

FIGURE 5 (continued)

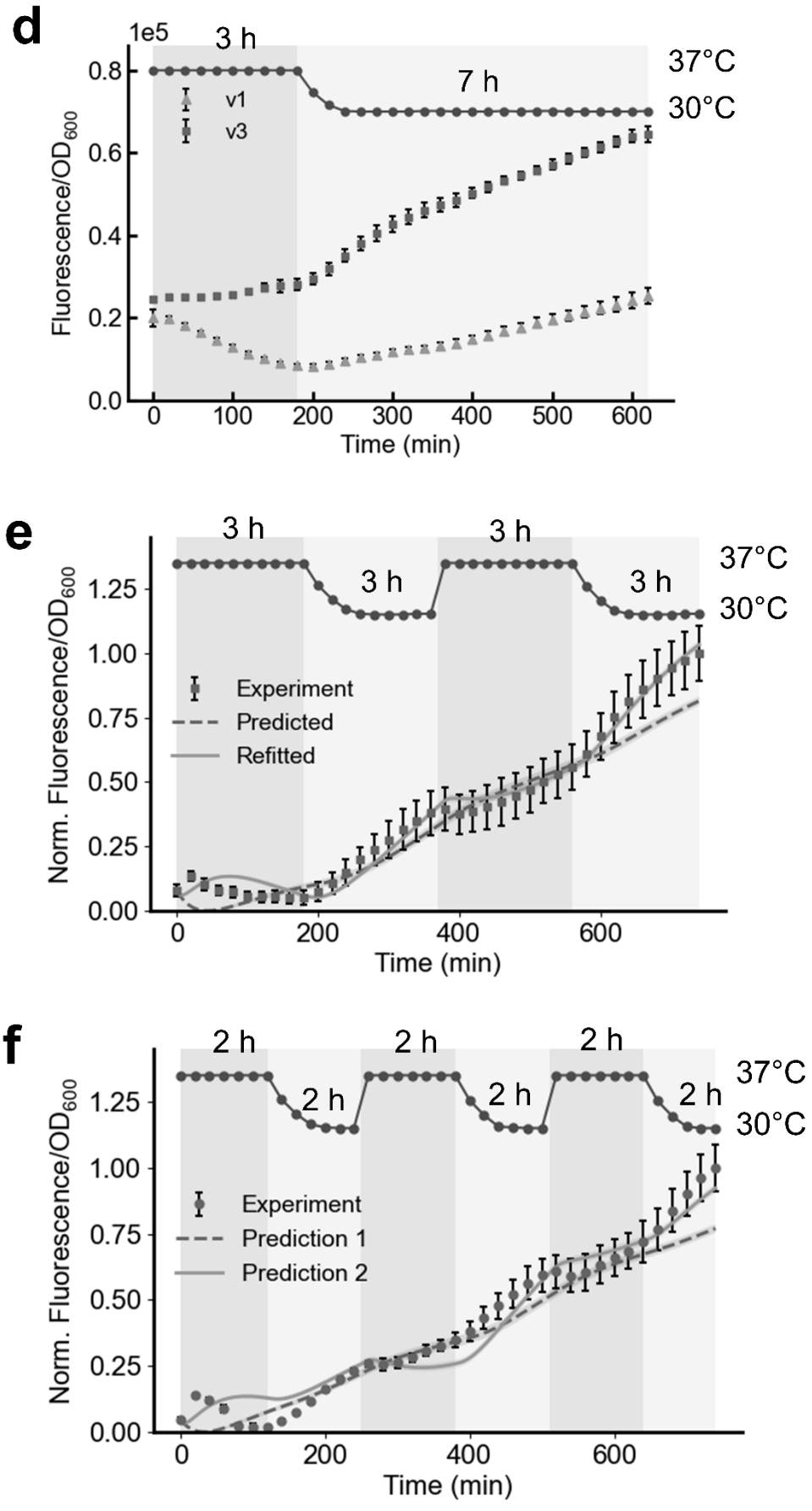


FIGURE 5 (continued)

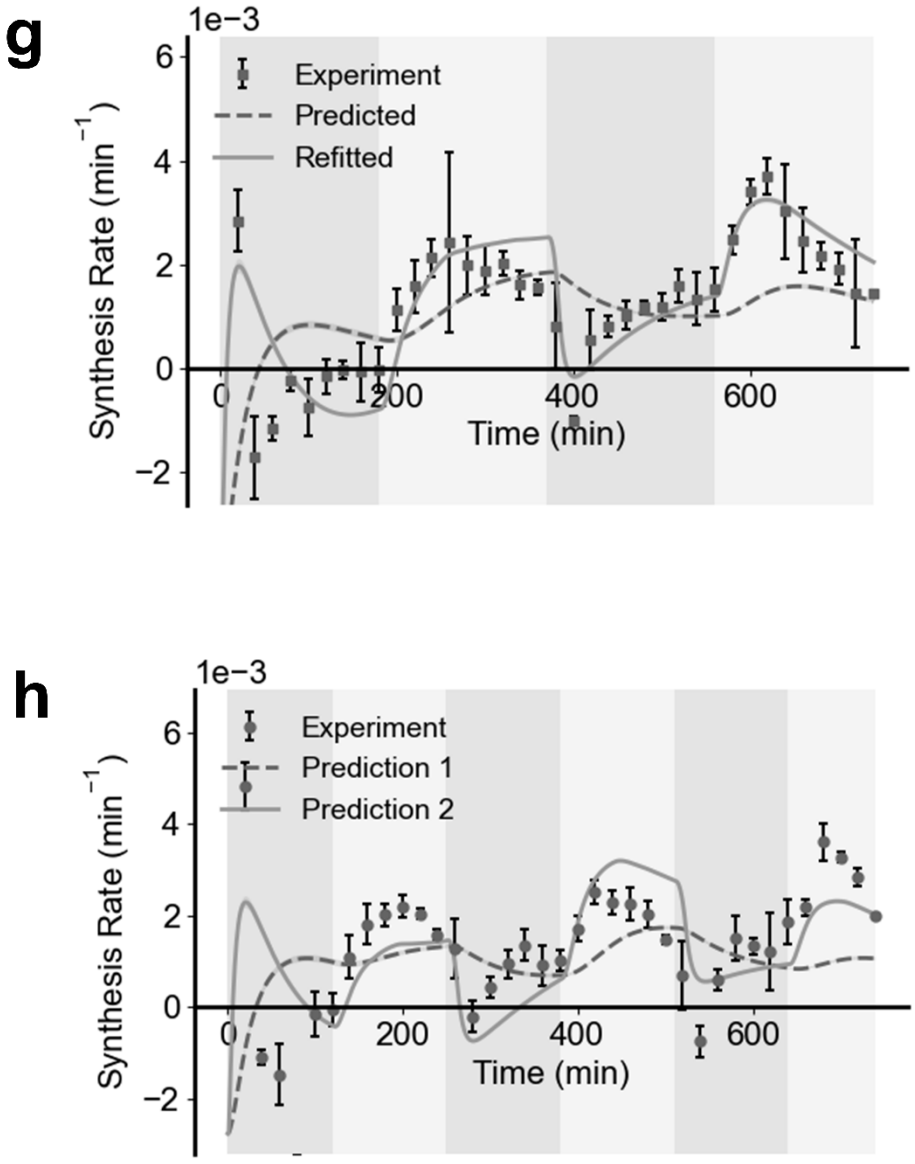
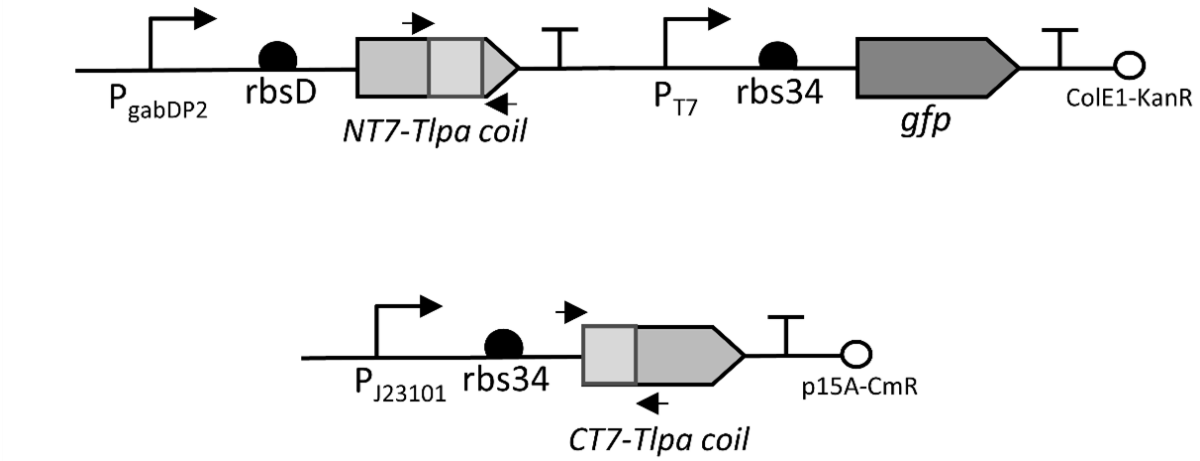


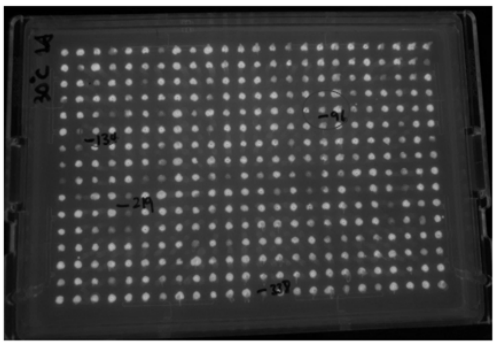
FIGURE 6

a

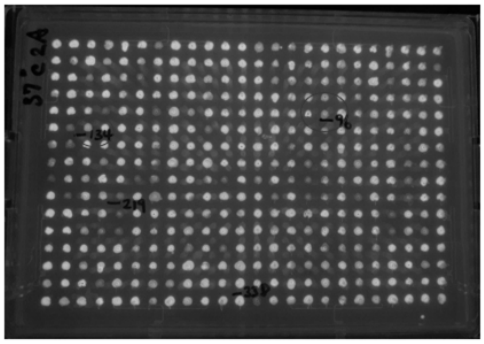


b

30 °C



37 °C



40 °C

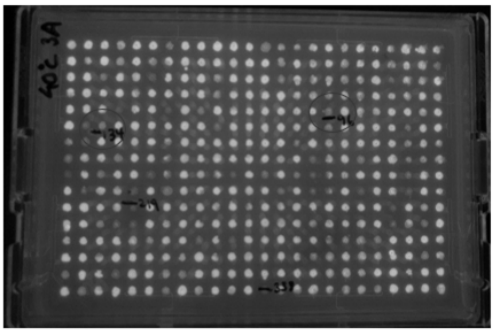


FIGURE 7

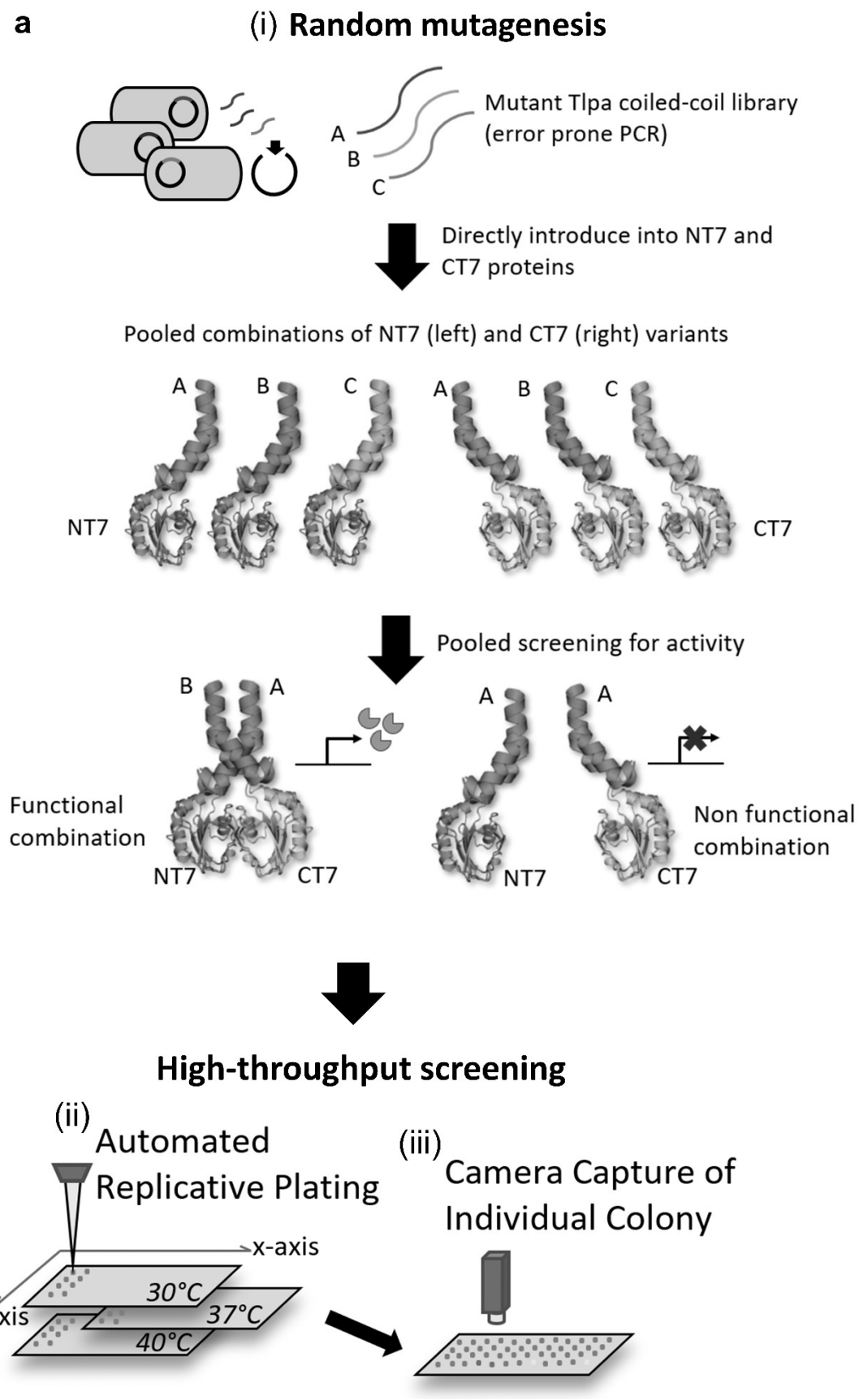

a
(i) Random mutagenesis
A
B
C
Mutant Tlpa coiled-coil library
(error prone PCR)
Directly introduce into NT7 and
CT7 proteins
Pooled combinations of NT7 (left) and CT7 (right) variants
NT7
CT7
Pooled screening for activity
Functional
combination
Non functional
combination
High-throughput screening
(ii)
Automated
Replicative Plating
x-axis
y-axis
30°C
37°C
40°C
(iii)
Camera Capture of
Individual Colony

FIGURE 7 (continued)

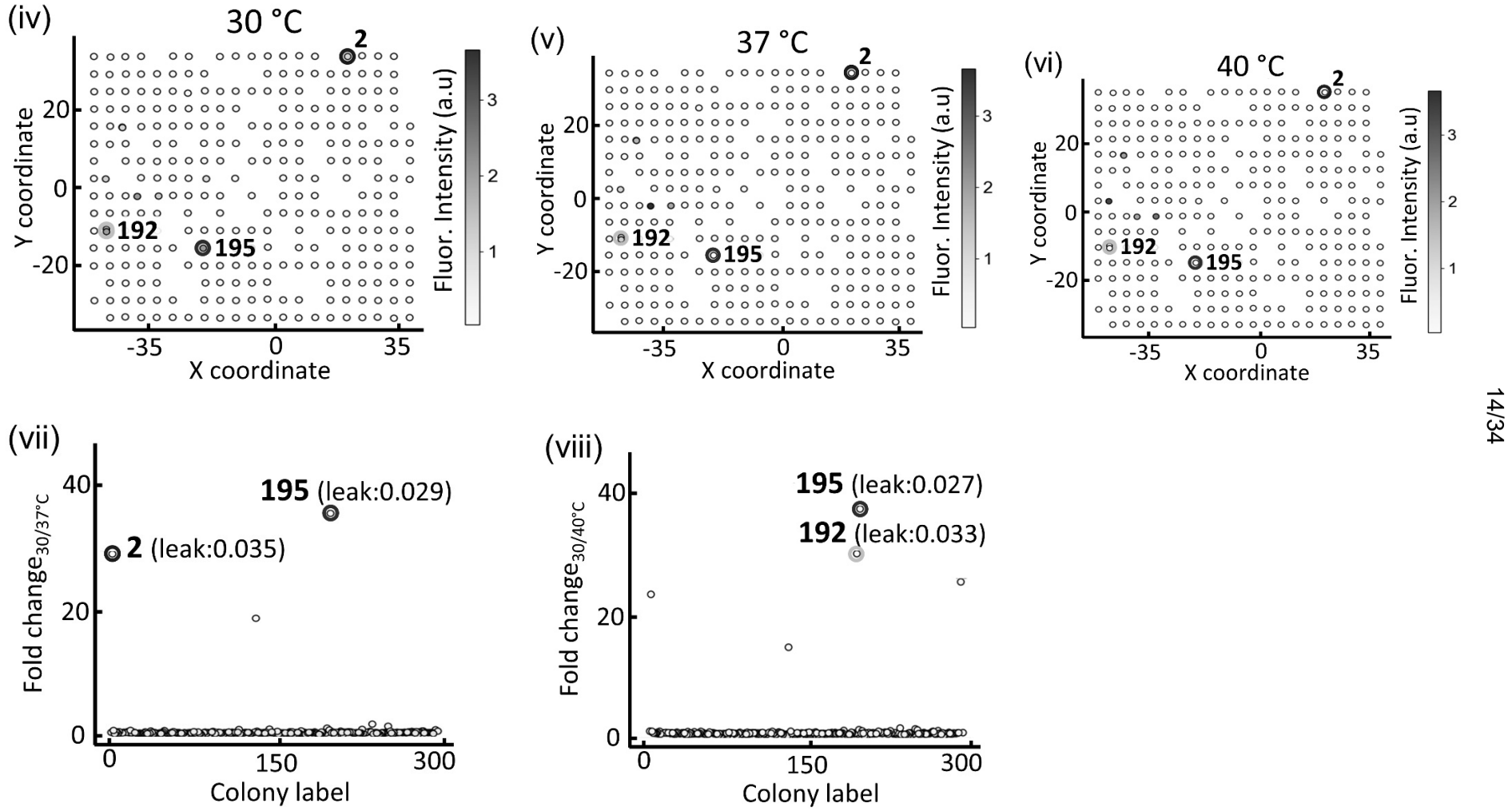


FIGURE 7 (continued)

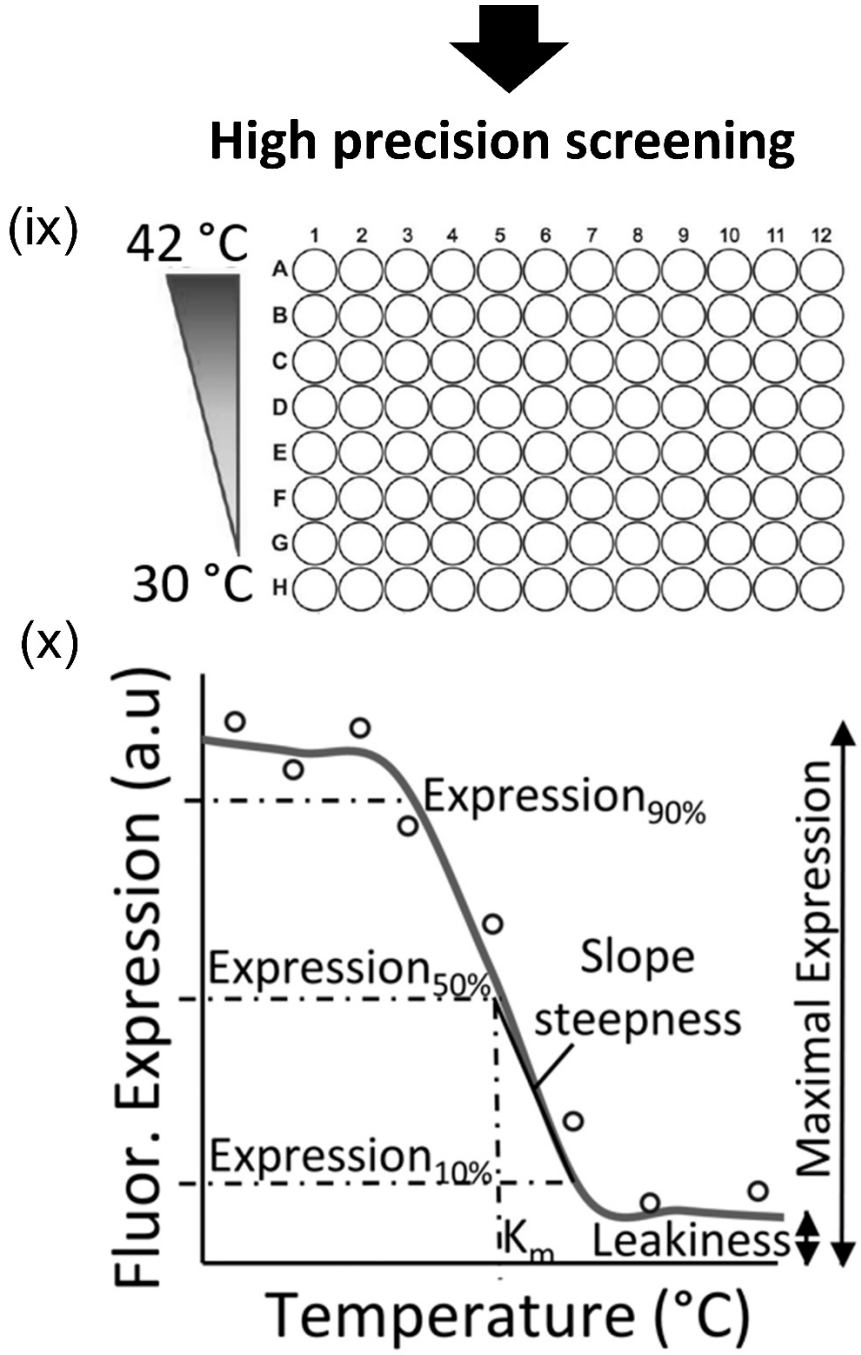
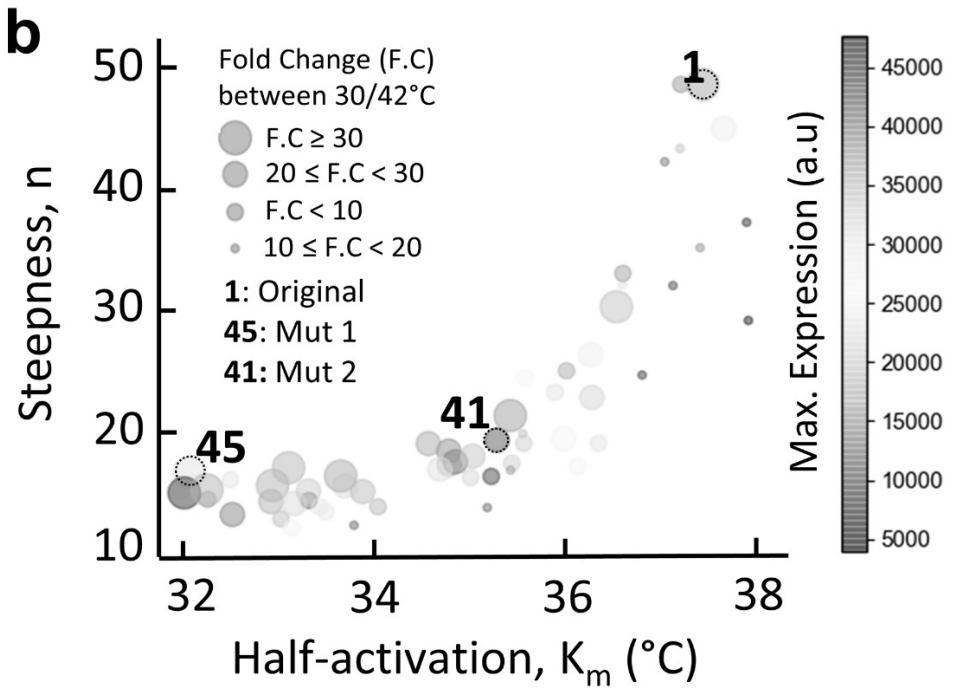


FIGURE 7 (continued)



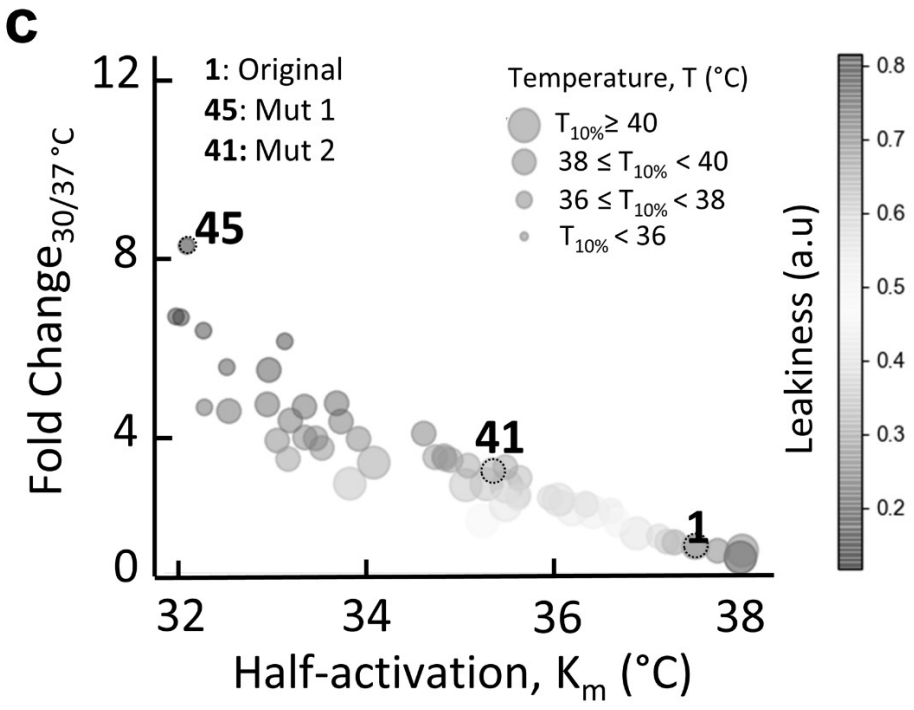


FIGURE 7 (continued)

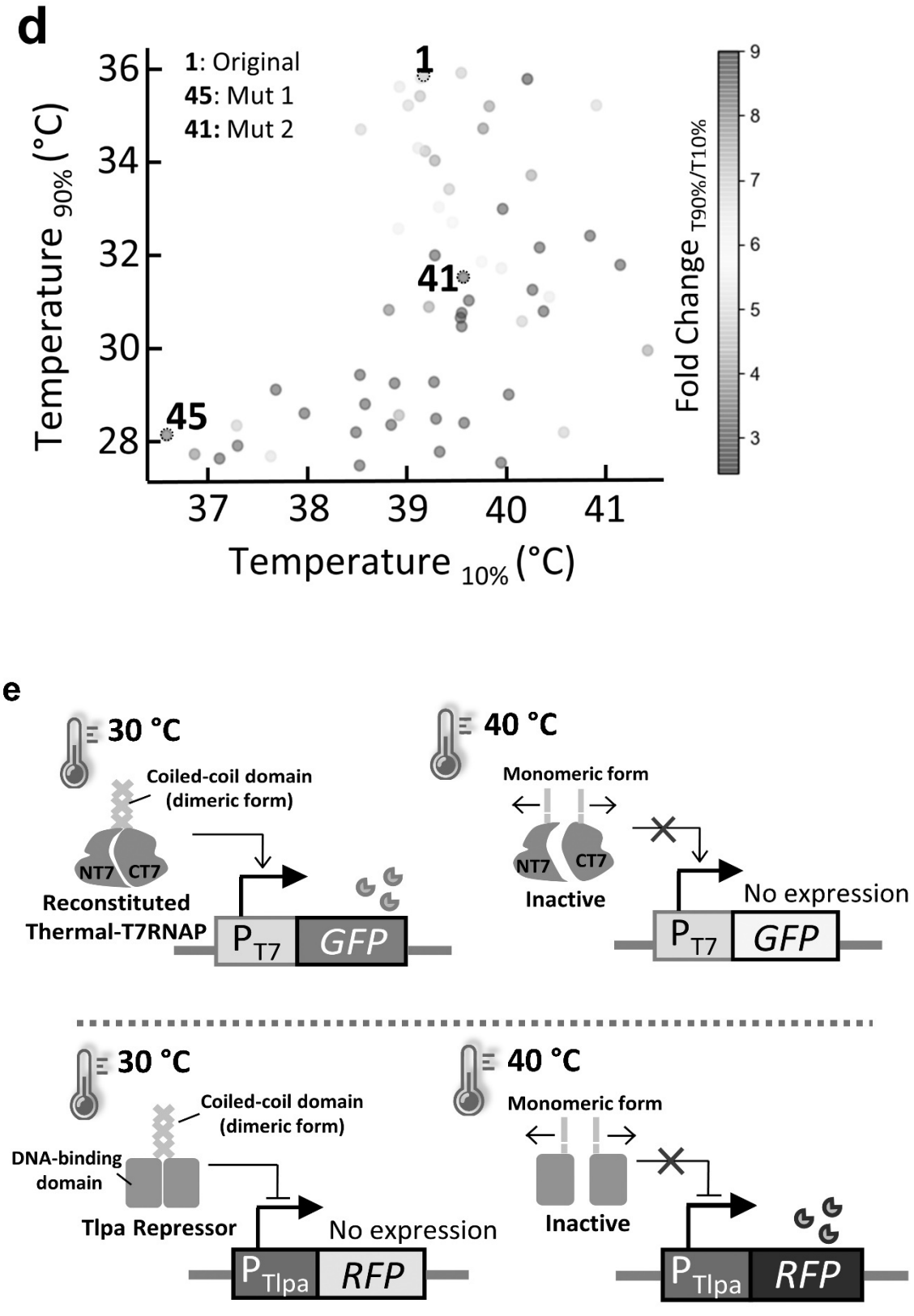
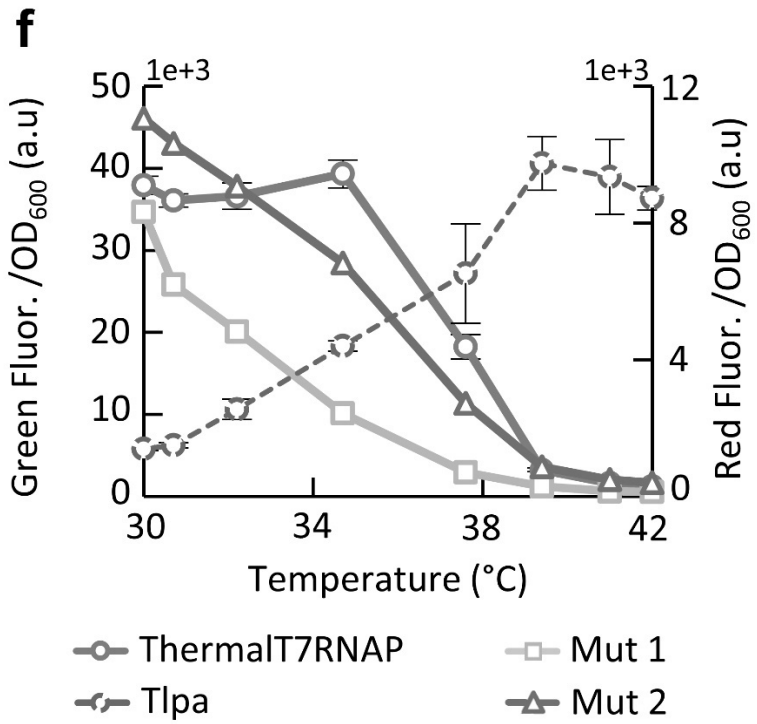


FIGURE 7 (continued)



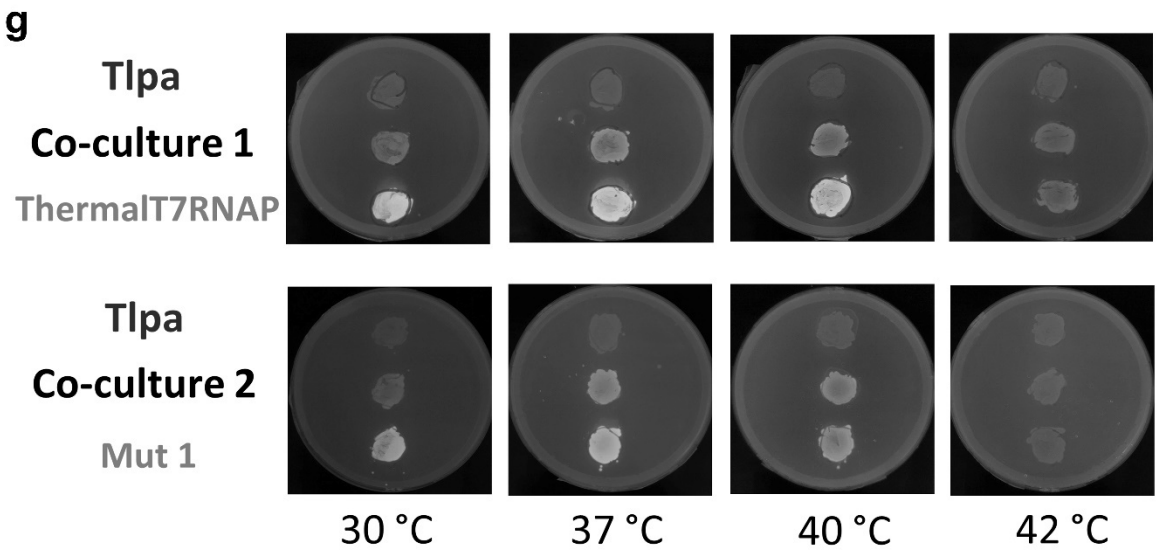


FIGURE 8

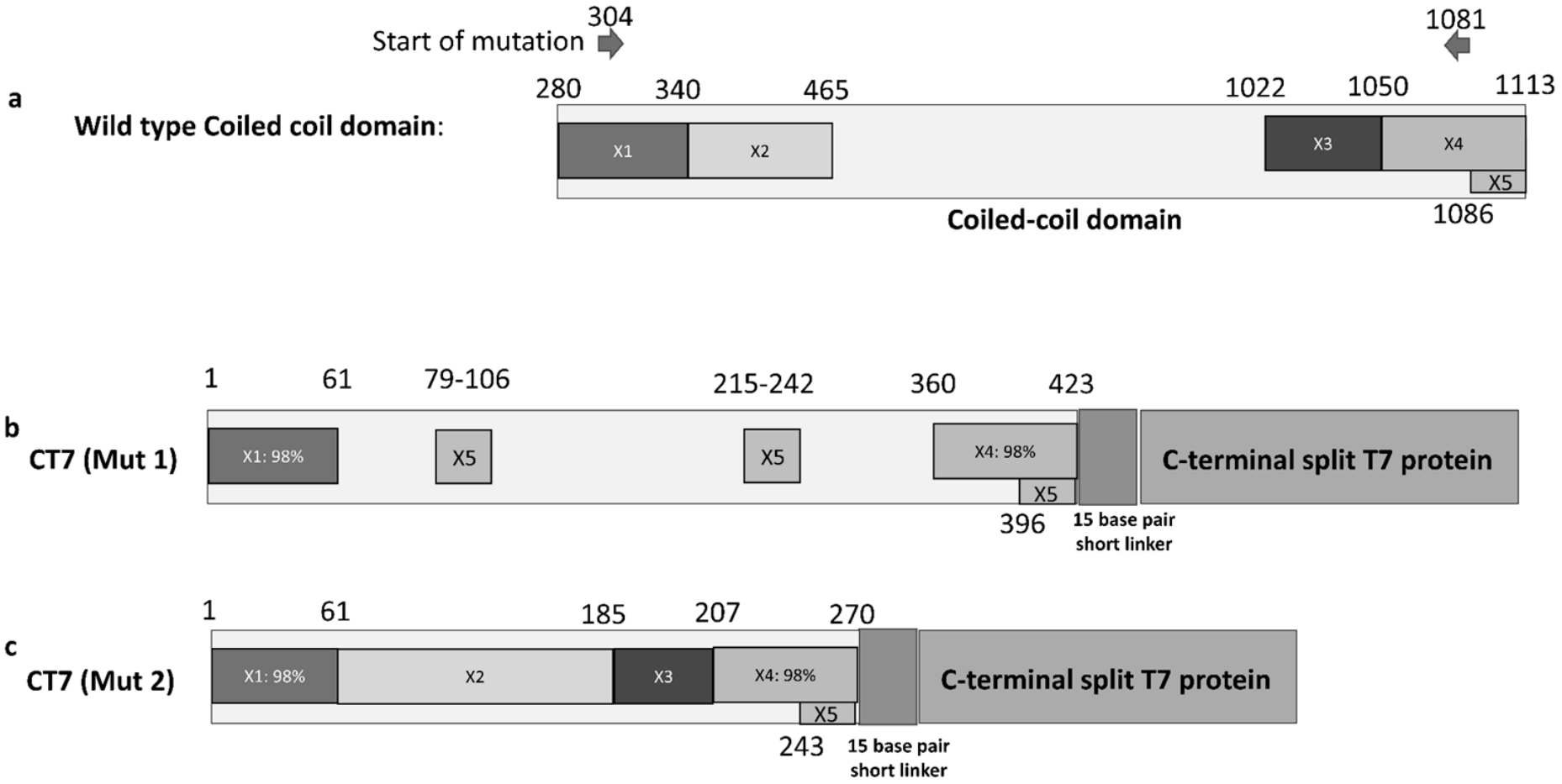

a
Wild type Coiled coil domain:
Start of mutation
304
1081
280
340
465
1022
1050
1113
X1
X2
X3
X4
X5
Coiled-coil domain
1086
b
CT7 (Mut 1)
1
61
79-106
215-242
360
423
X1: 98%
X5
X5
X4: 98%
X5
C-terminal split T7 protein
396
15 base pair
short linker
c
CT7 (Mut 2)
1
61
185
207
270
X1: 98%
X2
X3
X4: 98%
X5
C-terminal split T7 protein
243
15 base pair
short linker

FIGURE 9

A

Region	Protein	Nucleotide Position	Sequences producing significant alignments	Sequence Homology (%)	SEQ ID NO:
1	Tlpa	1050-1113	GCGTCAGAACCAGTCACTGATGGC GGCGCTTTCAGGCAATAAACAGAC CGGTGGCCAGAATGCG	98	19
	Mut1	360- 423	GCGACAGAACCAGTCACTGATGG CGGCGCTTTCAGGCAATAAACAGA CCGGTGGCCAGAATGCG		20
2	Tlpa	280-340	GCGGTCCGGGCTGCAGAACGCCG GGTTGCGGAAGTCACGCGTGCTG CCGGTGAACAGACCG	98	21
	Mut1	1-61	GCGGTCCGGGCTGCAGAACGCCG GGTTGCGGAAGTCACGCGTGCTG CCGGTAAACAGACCG		22
3	Tlpa	1086-1113	CAATAAACAGACCGGTGGCCAGAA TGCG	100	23
	Mut1	79-106	Conserved		-
4	Tlpa	1086-1113	CAATAAACAGACCGGTGGCCAGAA TGCG	100	23
	Mut1	215-242	Conserved		-

B

Region	Protein	Nucleotide Position	Sequences producing significant alignments	Sequence Homology (%)	SEQ ID NO:
1	Tlpa	280-465	GCGGTCCGGGCTGCAGAACGCCG GGTTGCGGAAGTCACGCGTGCTG CCGGTGAACAGACC GCACAGGCAGAGCGGGAGCTGGC CGACGCCGCGCAGACAGTCGACG ACCTGGAAGAAAAA CTGGTTGAACTGCAGGACAGATAT GACAGTTTGACGCTGGCGCTGGA GTCAGAACGTTCA CTGCGT	100	24
	Mut2	1-186	Conserved		-
2	Tlpa	1022-1113	CACTGCGTGGTGAGGCTGAAGCC CTGAAGCGTCAGAACCAGTCACTG ATGGCGGCGCTTT CAGGCAATAAACAGACCGGTGGC CAGAATGCG	100	25
	Mut2	179-270	Conserved		-

FIGURE 9 (continued)

C

Region	Protein	Nucleotide Position	Sequences producing significant alignments	Sequence Homology (%)	SEQ ID NO:
1	Mut1	341-465	CACAGGCAGAGCGGGAGCTGGC CGACGCCGCGCAGACAGTCGAC GACCTGGAAGAAAAAC TGGTTGAACTGCAGGACAGATAT GACAGTTTGACGCTGGCGCTGG AGTCAGAACGTTCAC TGCGT	100	26
	Mut2	1-125	Conserved		-
2	Mut1	1022-1049	CACTGCGTGGTGAGGCTGAAGC CCTGAA	100	27
	Mut2	118-145	Conserved		-

FIGURE 10

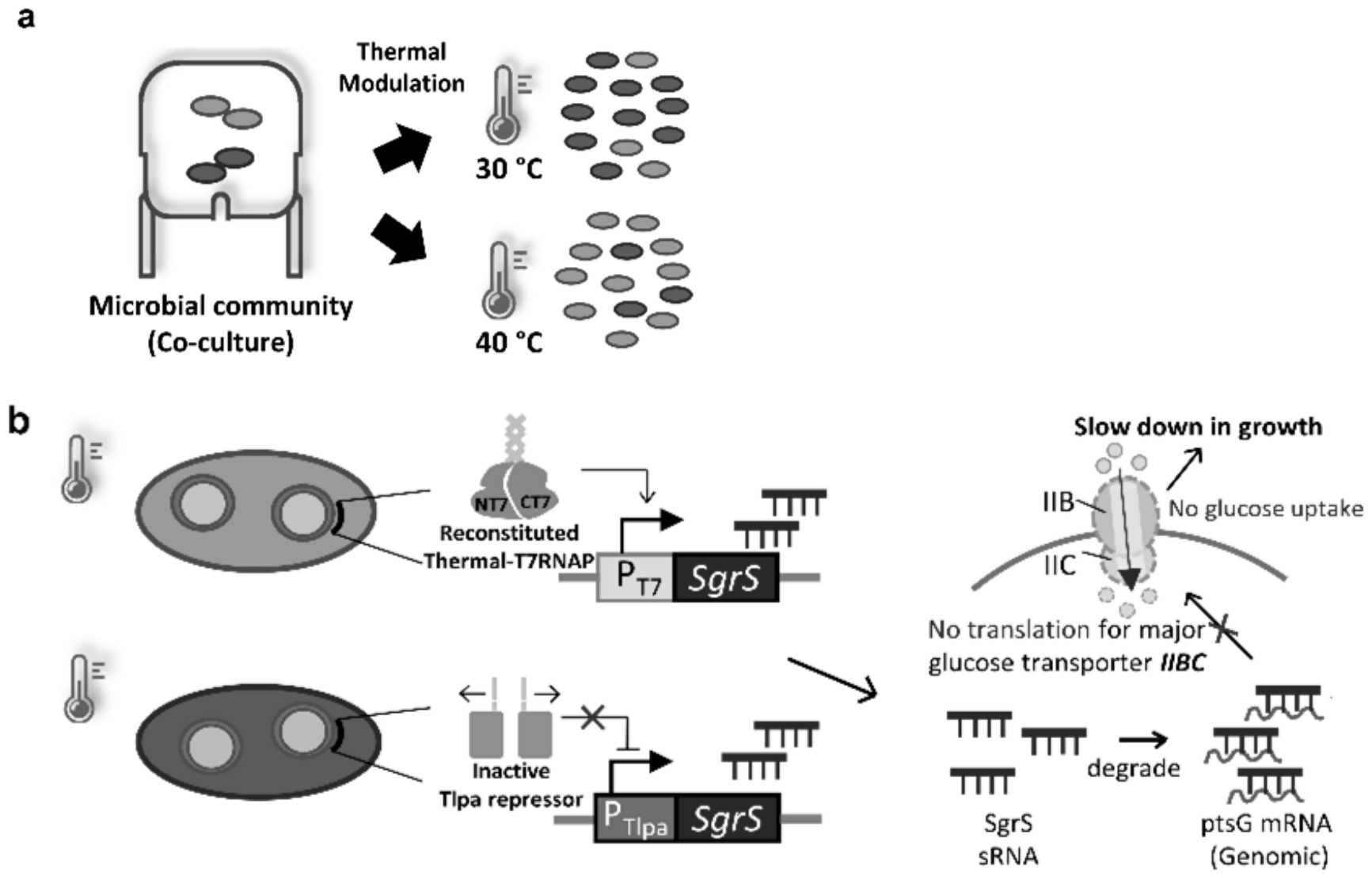

a
Thermal
Modulation
30 °C
40 °C
Microbial community
(Co-culture)
b
NT7
CT7
Reconstituted
Thermal-T7RNAP
P T7
SgrS
Inactive
Tlpa repressor
P Tlpa
SgrS
Slow down in growth
IIB
IIC
No glucose uptake
No translation for major
glucose transporter IIBC
degrade
SgrS
sRNA
ptsG mRNA
(Genomic)

FIGURE 10 (continued)

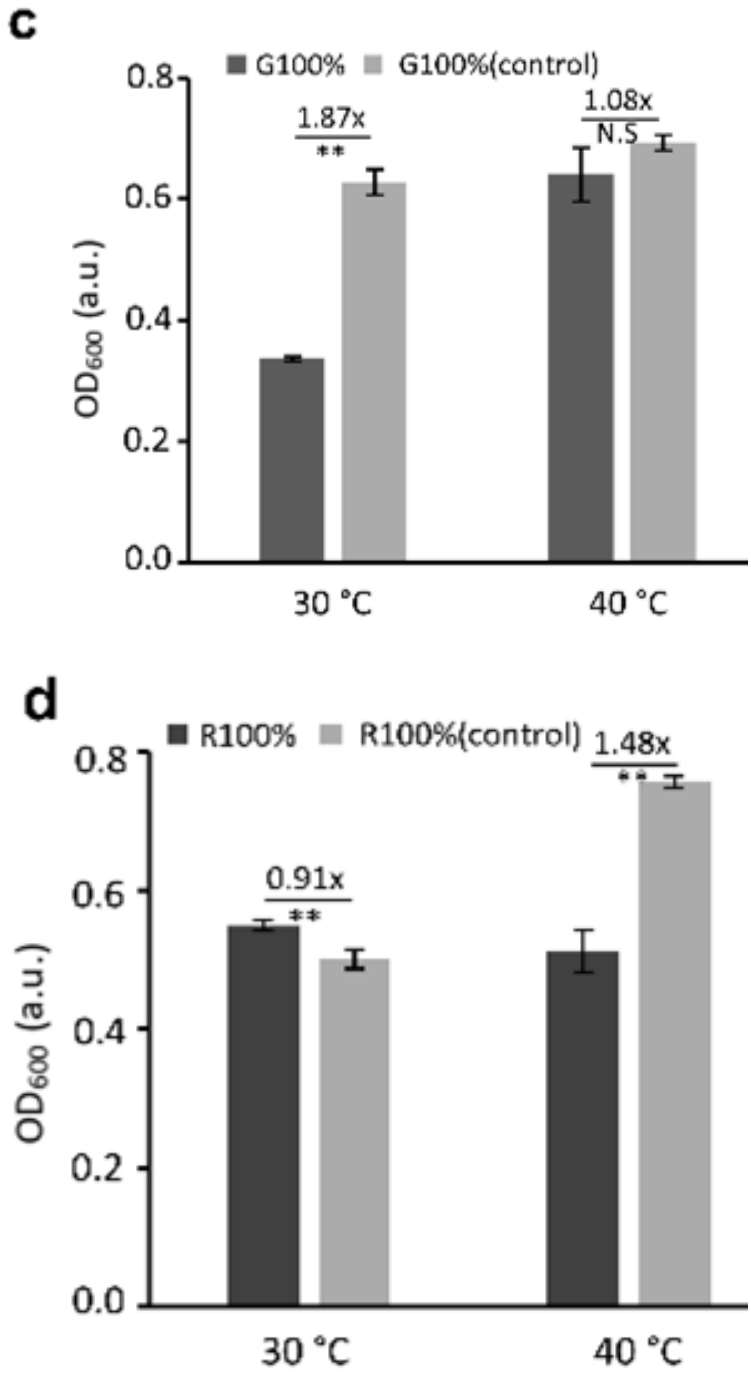
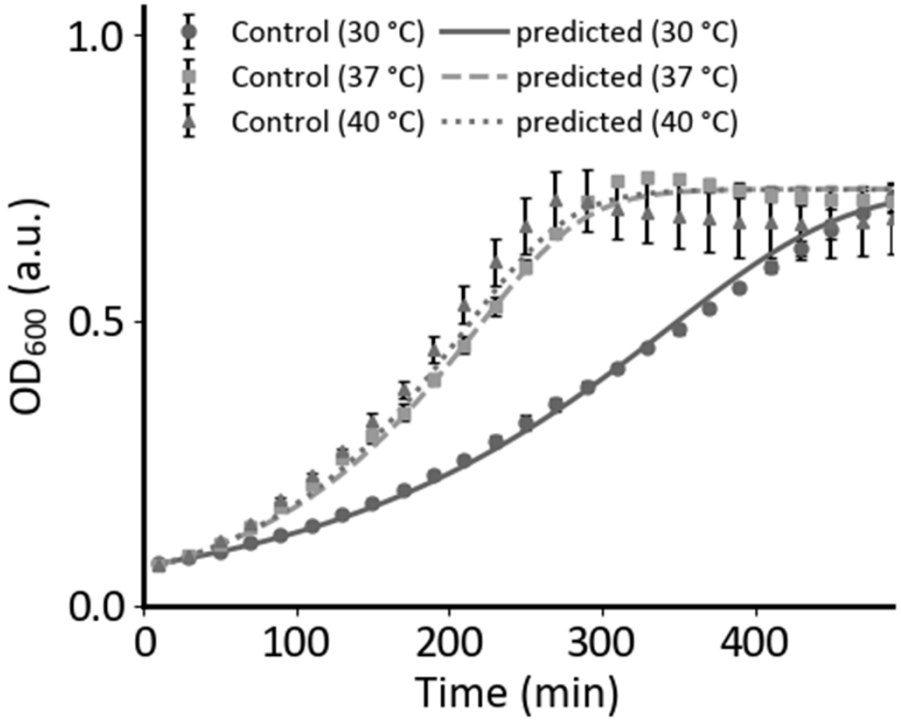


FIGURE 10 (continued)

e



f

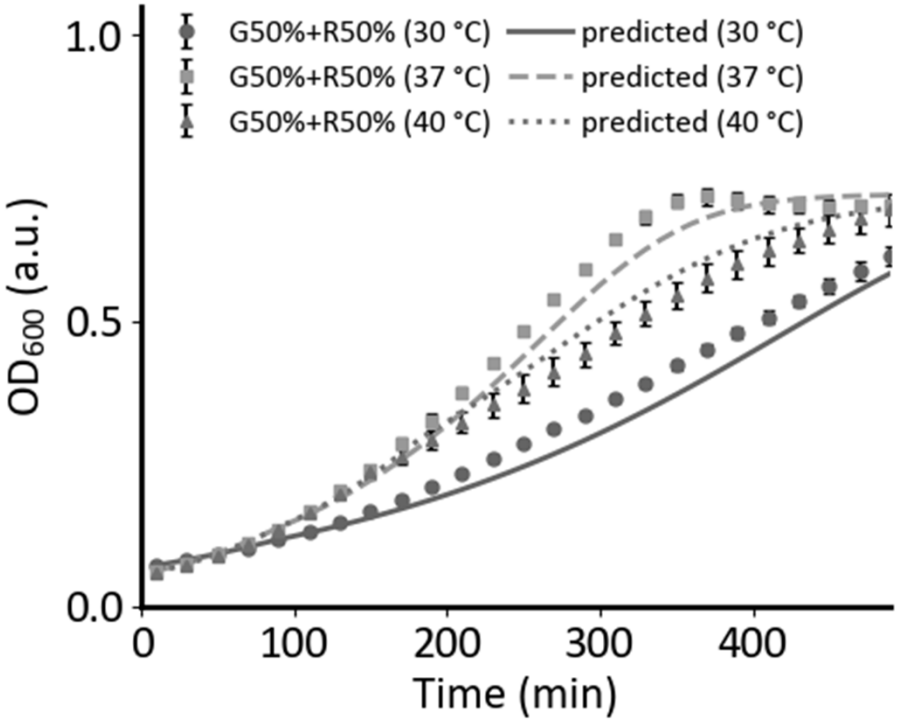
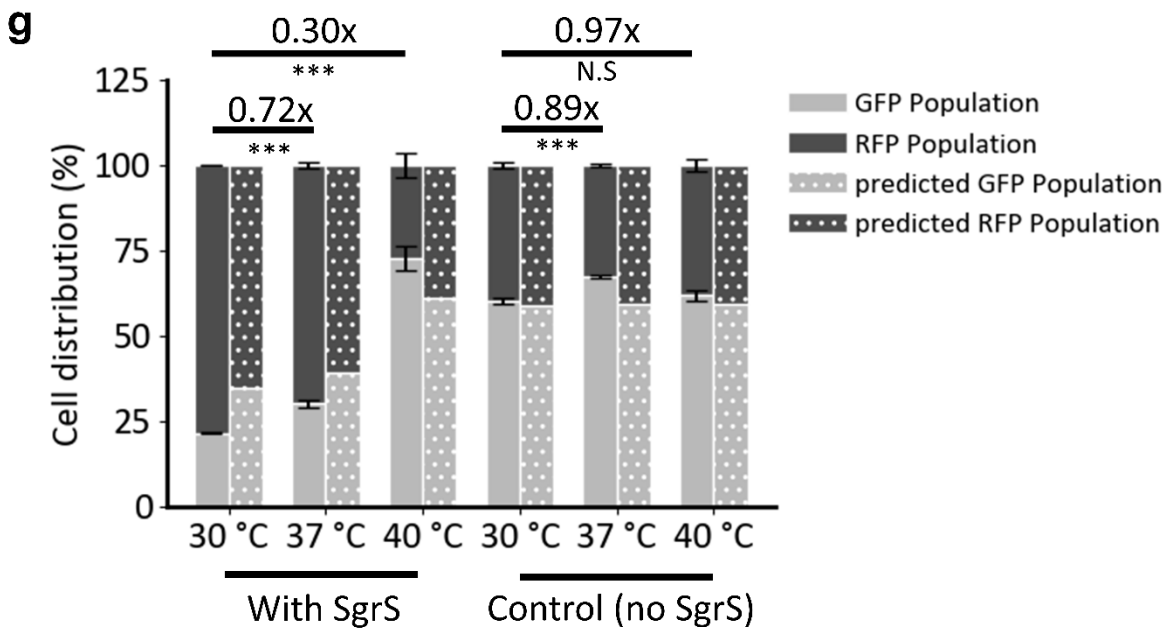


FIGURE 10 (continued)



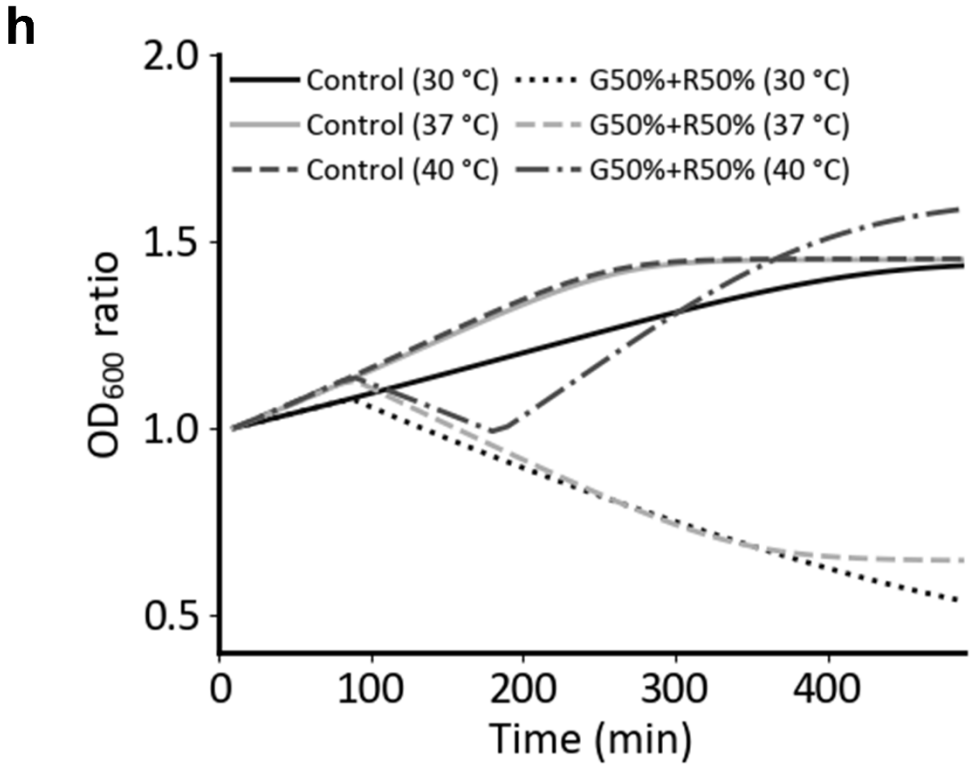


FIGURE 10 (continued)

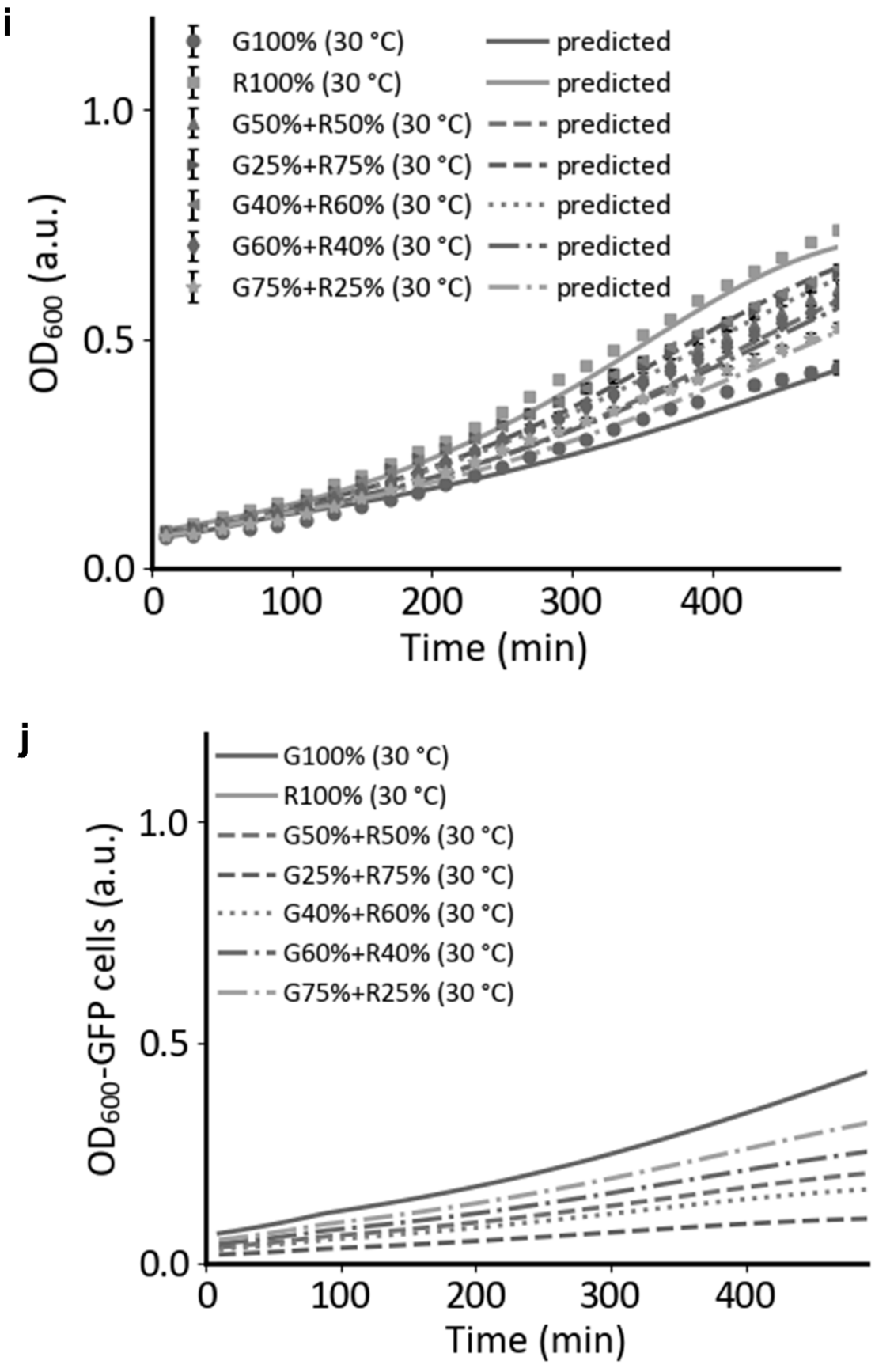
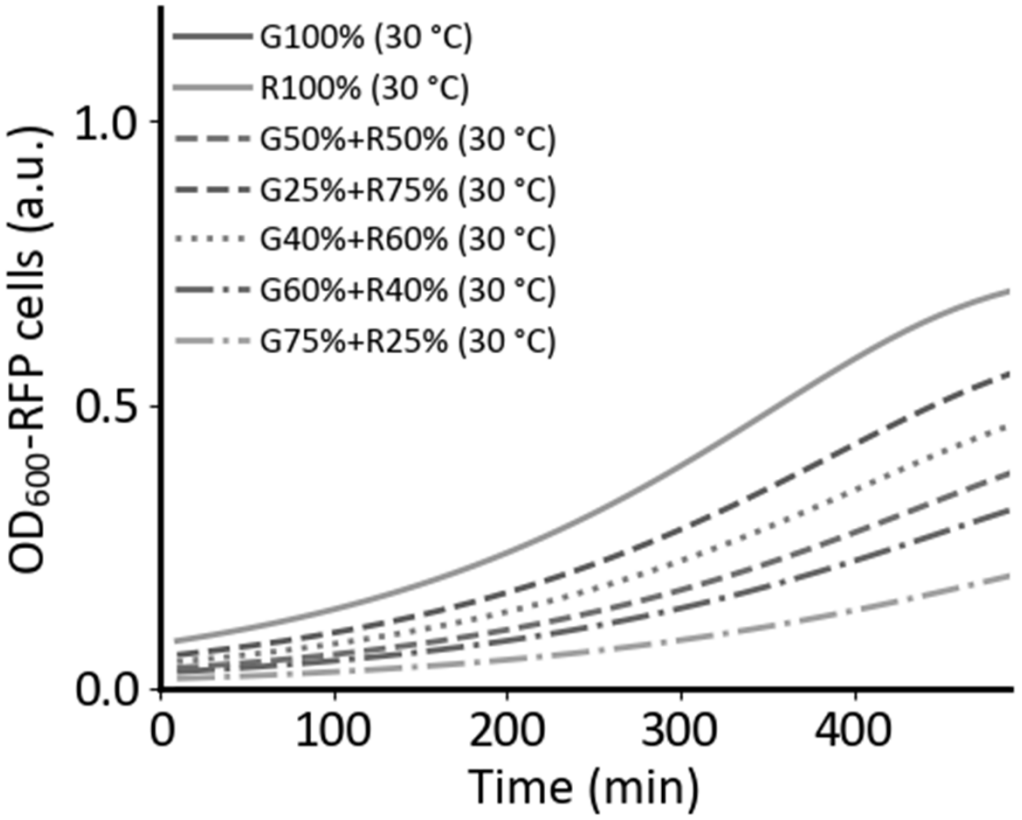


FIGURE 10 (continued)

k



m

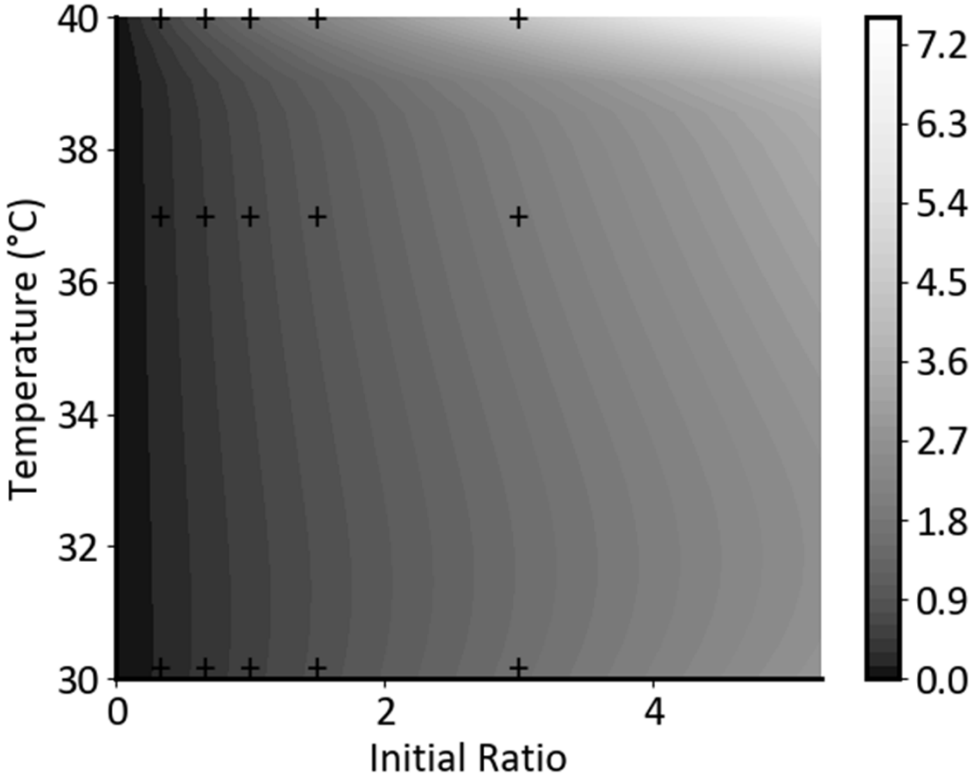


FIGURE 10 (continued)

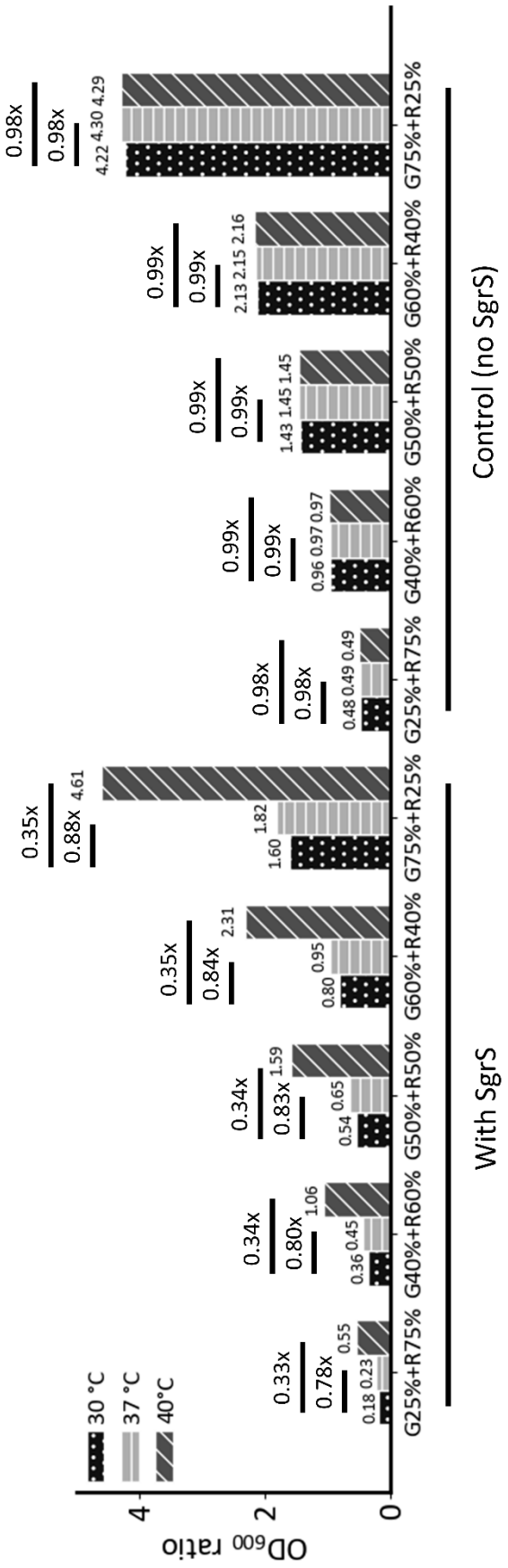


FIGURE 11

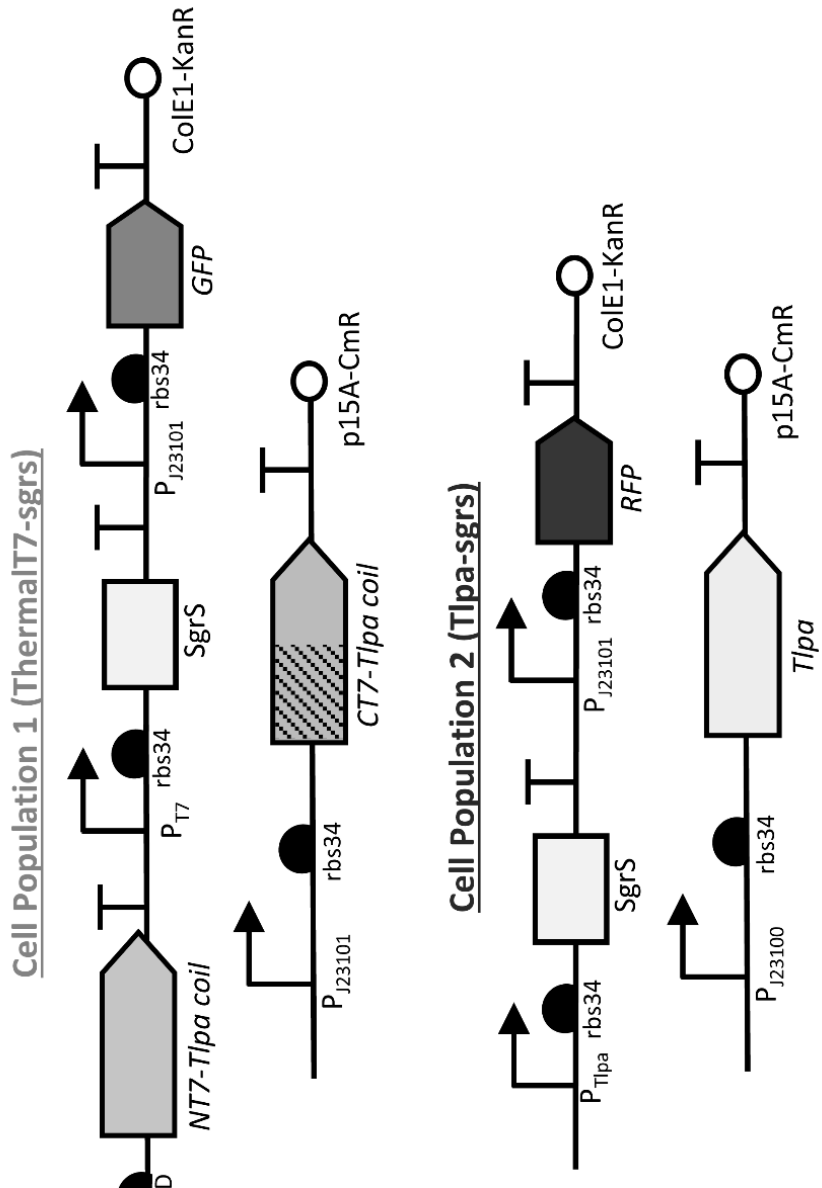

Cell Population 1 (ThermalT7-sgrs)
P gabDP2
rbsD
NT7-Tlpa coil
P T7
rbs34
SgrS
P J23101
rbs34
GFP
ColE1-KanR
P J23101
rbs34
CT7-Tlpa coil
p15A-CmR
Cell Population 2 (Tlpa-sgrs)
P Tlpa
rbs34
SgrS
P J23101
rbs34
RFP
ColE1-KanR
P J23100
rbs34
Tlpa
p15A-CmR

FIGURE 12

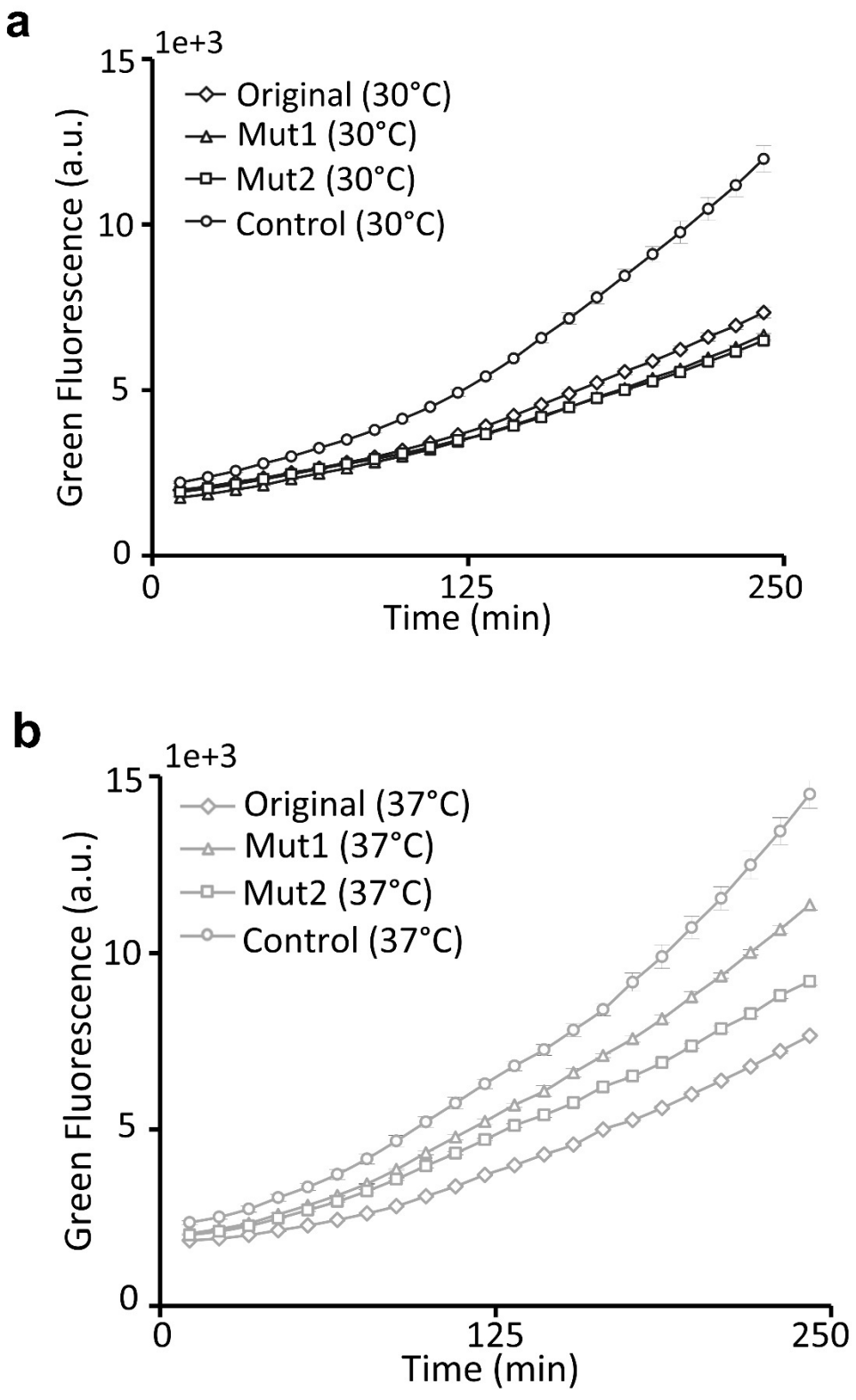
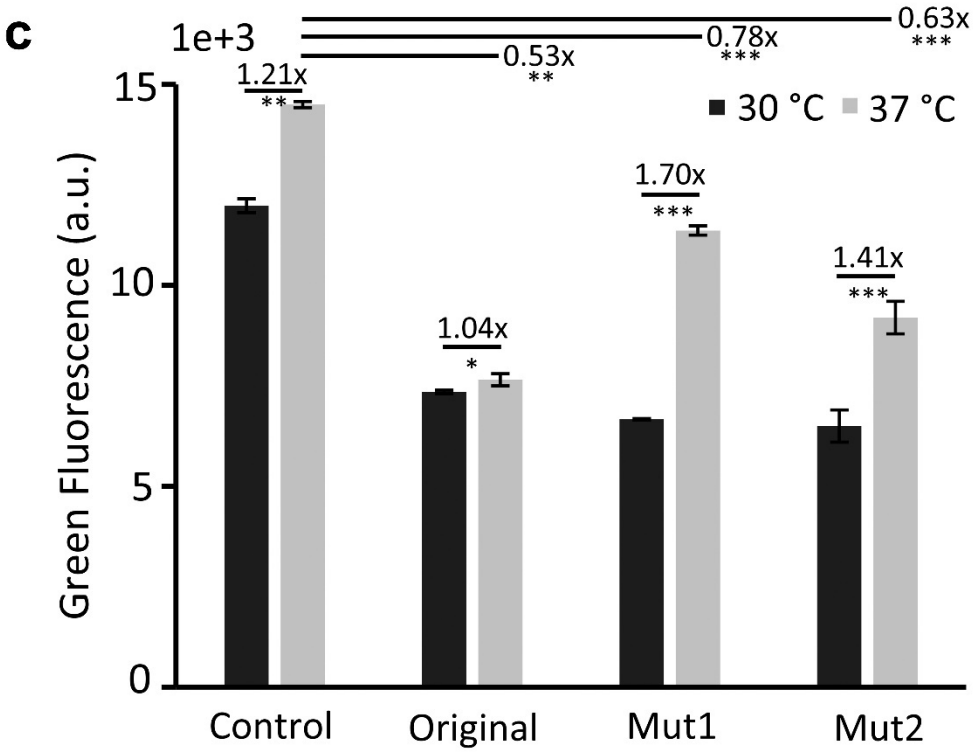

a
1e+3
15
10
5
0
Green Fluorescence (a.u.)
Original (30°C)
Mut1 (30°C)
Mut2 (30°C)
Control (30°C)
0
125
250
Time (min)
b
1e+3
15
10
5
0
Green Fluorescence (a.u.)
Original (37°C)
Mut1 (37°C)
Mut2 (37°C)
Control (37°C)
0
125
250
Time (min)

FIGURE 12 (continued)



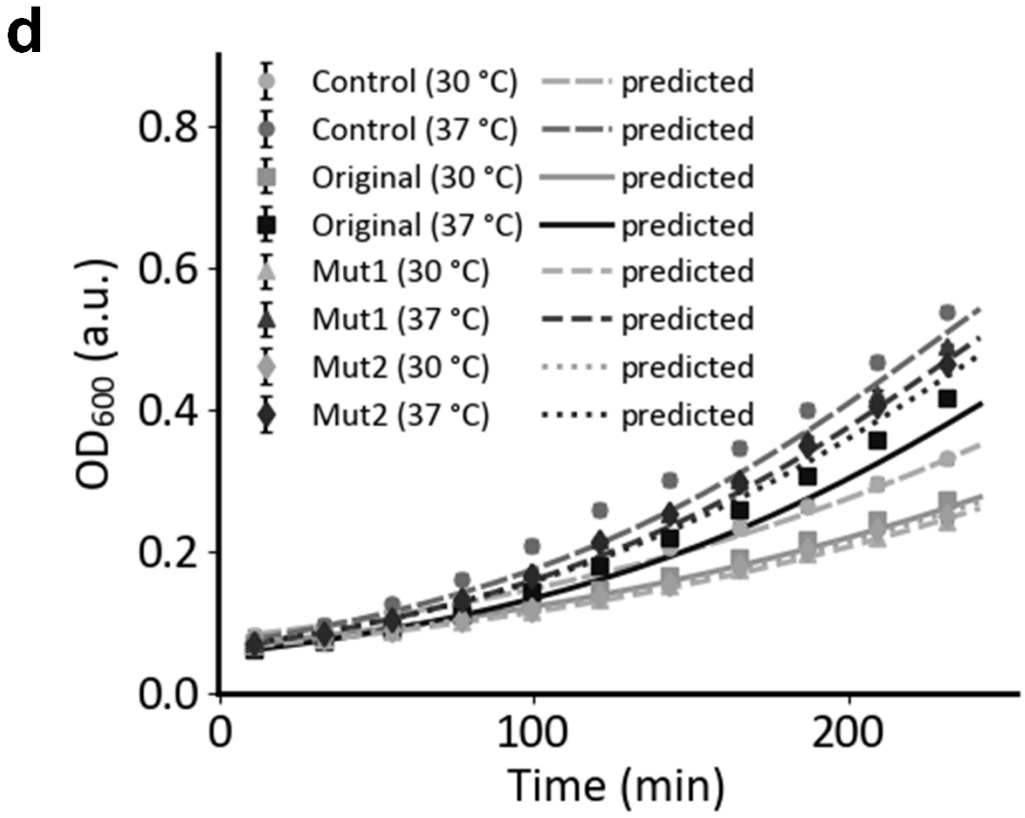


FIGURE 12 (continued)

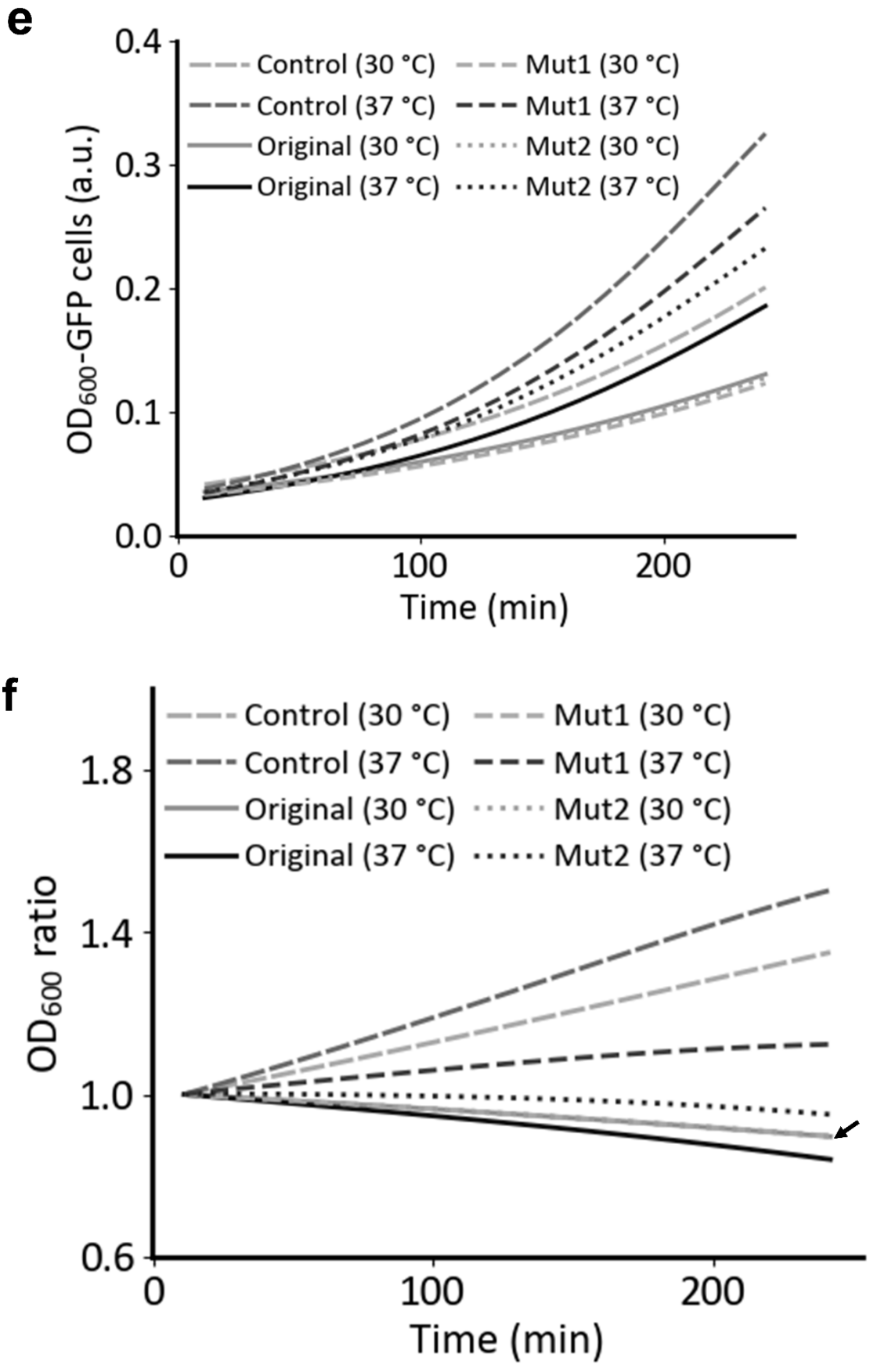
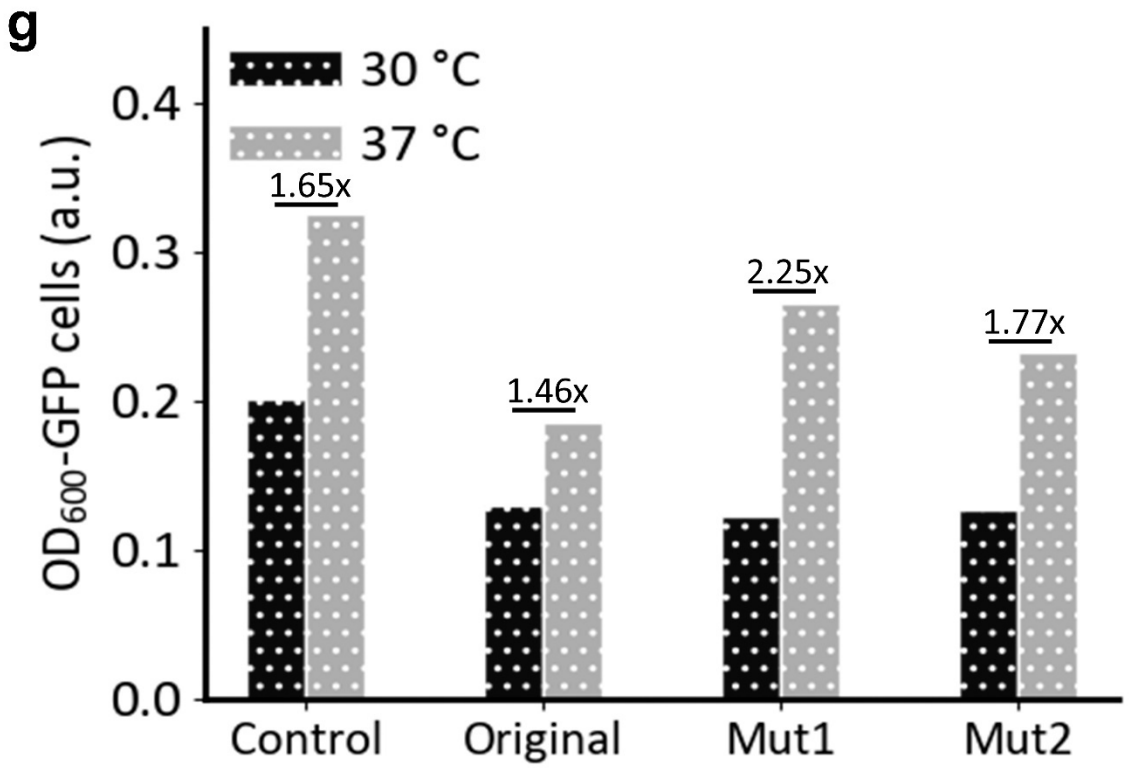


FIGURE 12 (continued)



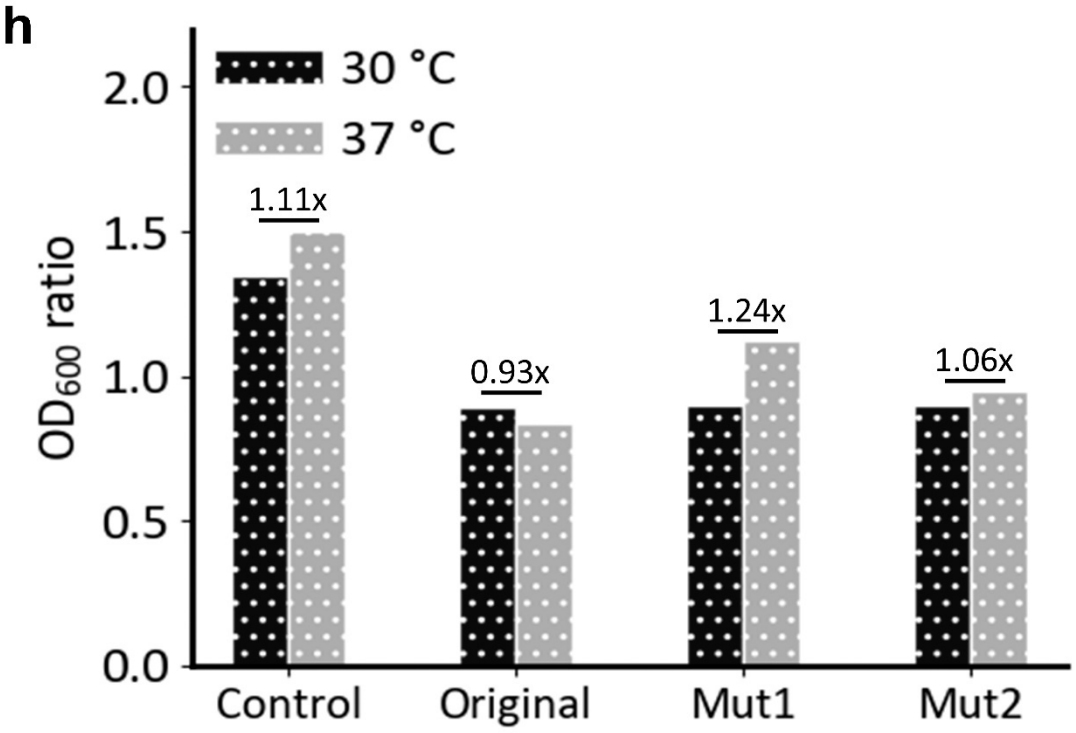


FIGURE 12 (continued)

