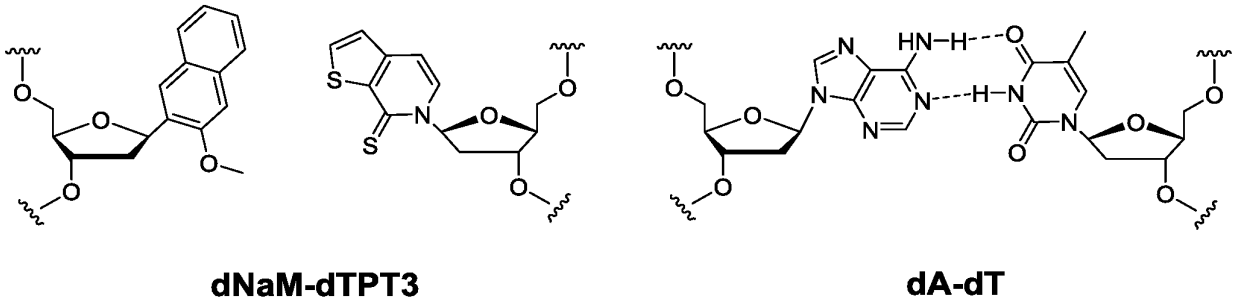

dNaM-dTPT3
dA-dT

FIG. 1A

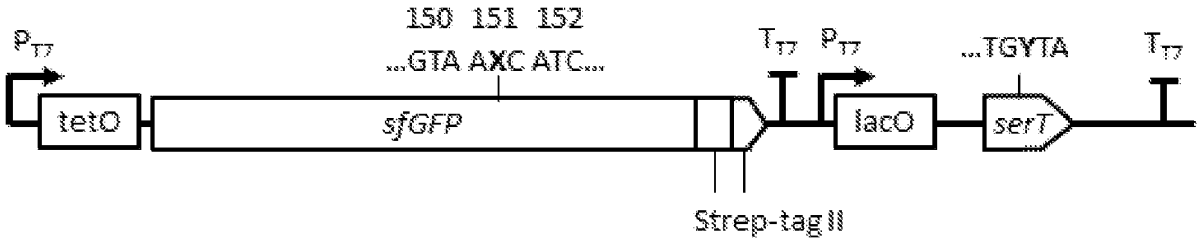

150 151 152
...GTA AXC ATC...
...TGYTA
tetO
sfGFP
lacO
serT
Strep-tag II

FIG. 1B

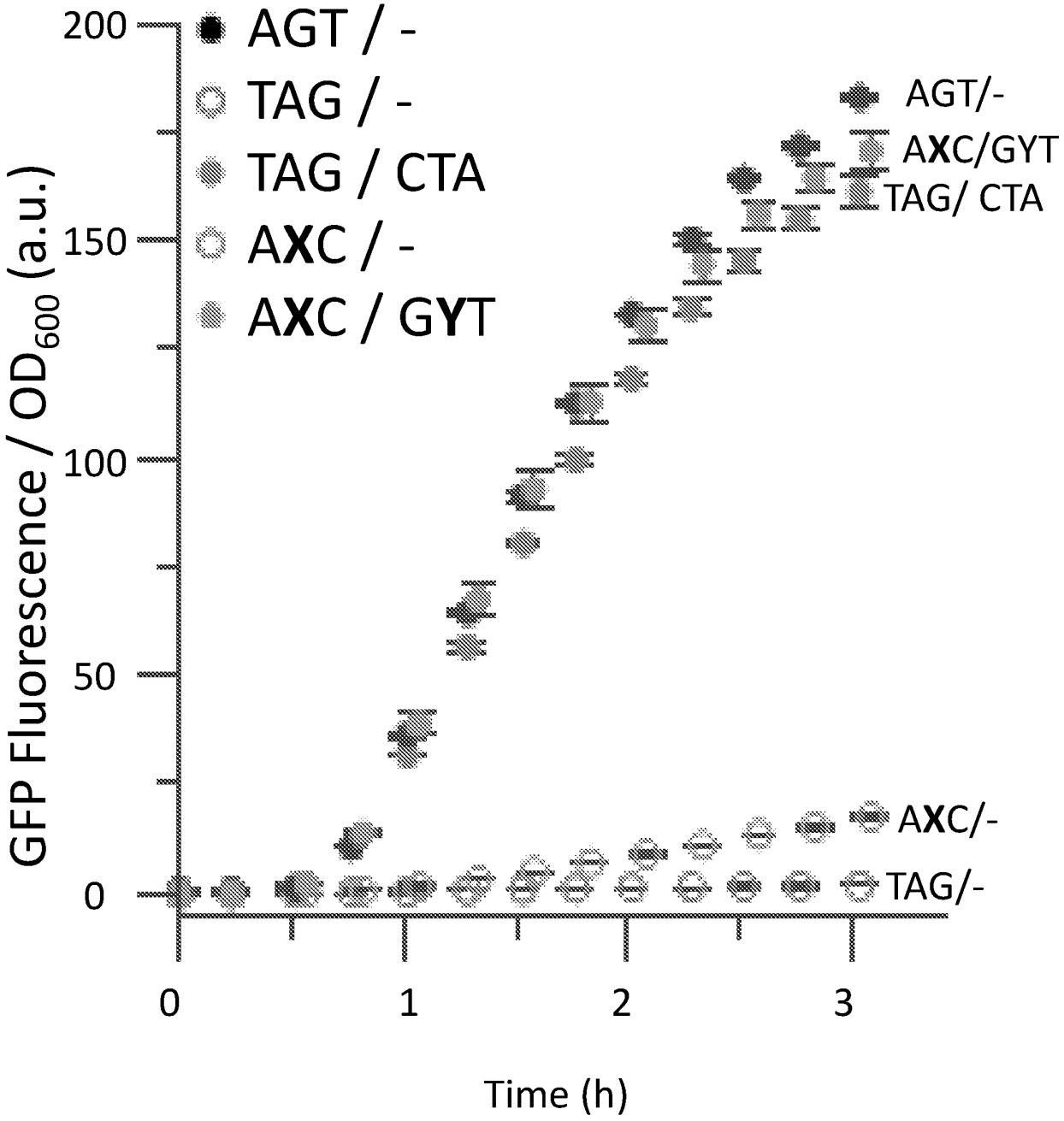

AGT / -
TAG / -
TAG / CTA
AXC / -
AXC / GYT
AGT/-
AXC/GYT
TAG/ CTA
AXC/-
TAG/-
GFP Fluorescence / OD600 (a.u.)
200
150
100
50
0
0
1
2
3
Time (h)

FIG. 1C

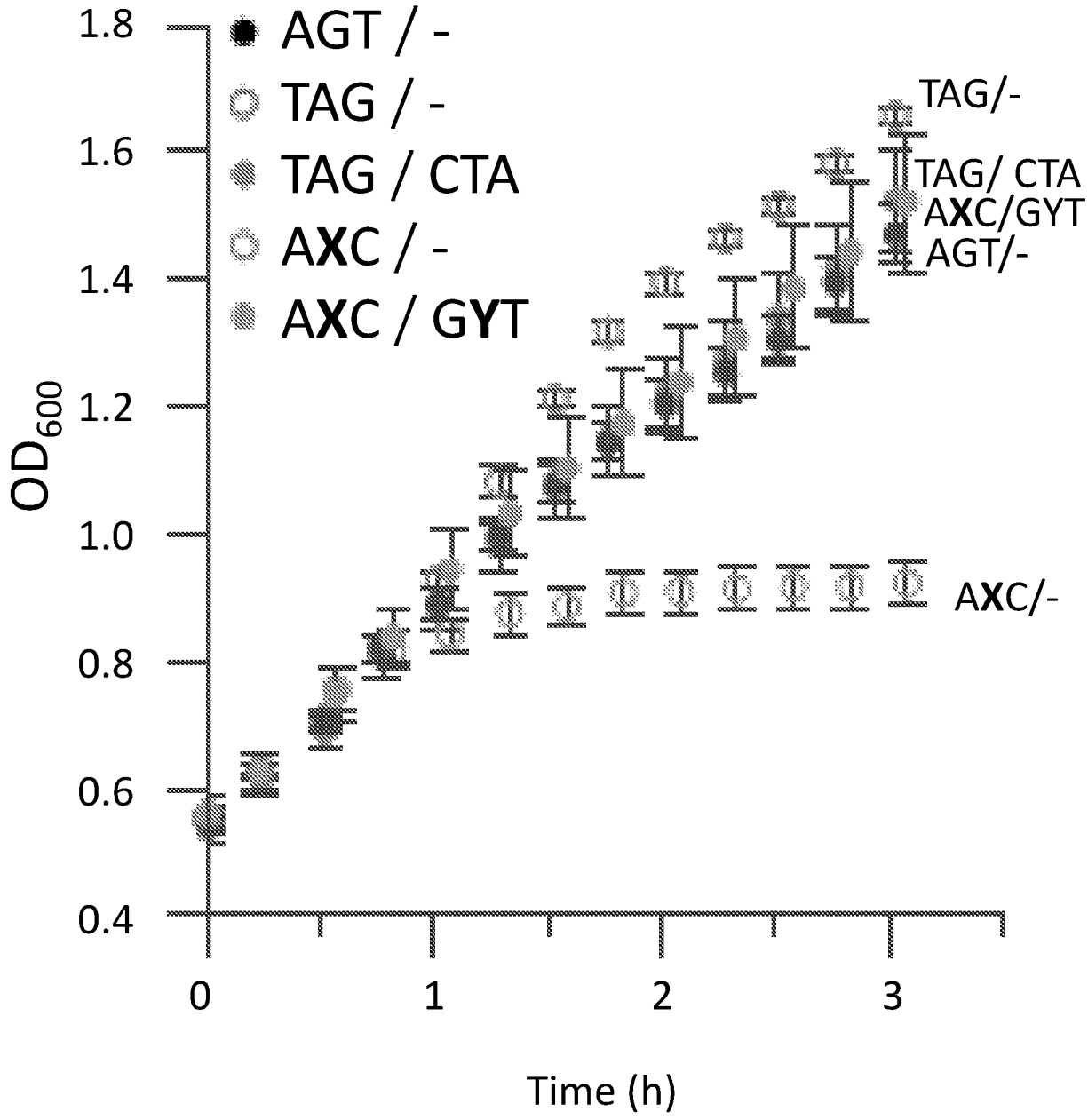

AGT / -
TAG / -
TAG / CTA
AXC / -
AXC / GYT
TAG/-
TAG/ CTA
AXC/GYT
AGT/-
AXC/-
OD600
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0
1
2
3
Time (h)

FIG. 1D

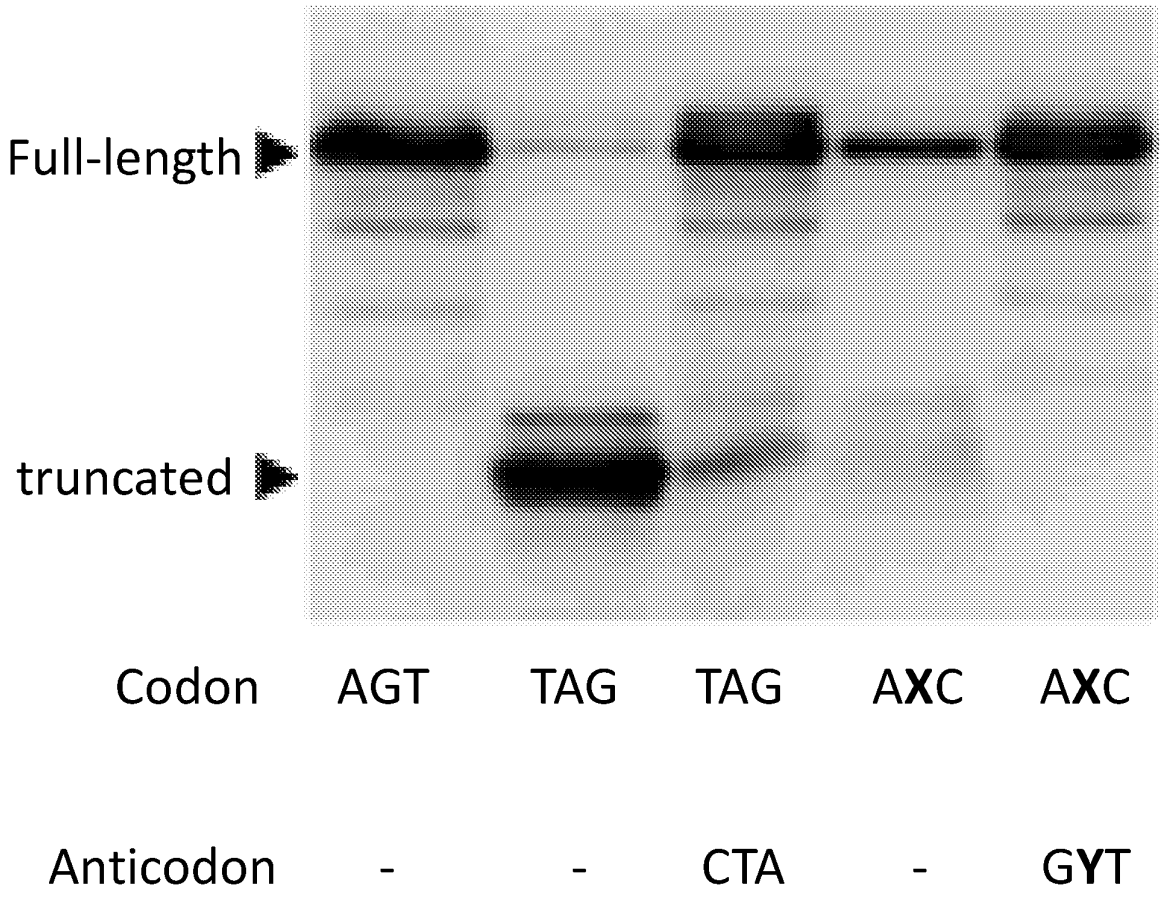

Full-length
truncated
Codon AGT TAG TAG AXC AXC
Anticodon - - CTA - GYT

FIG. 1E

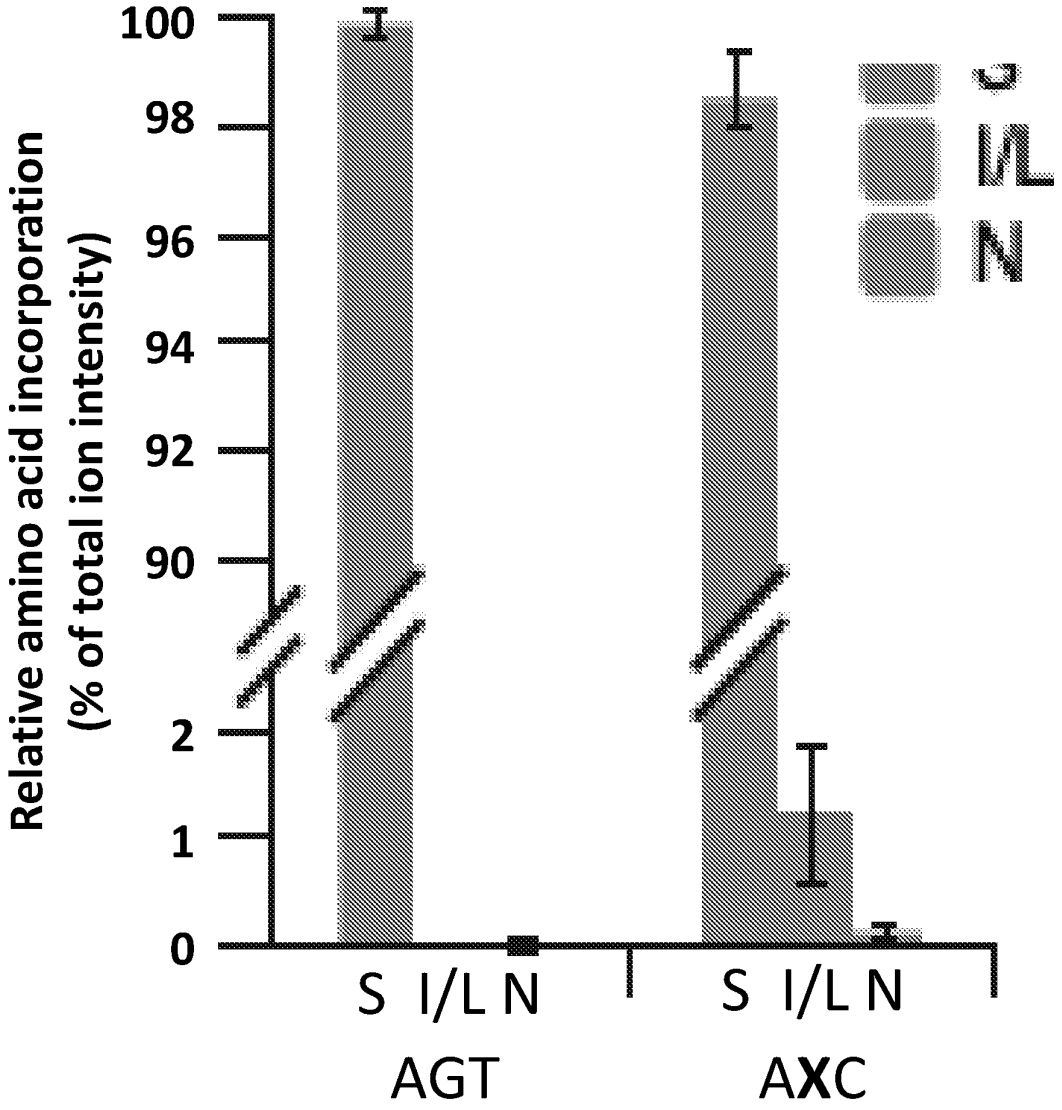

Relative amino acid incorporation
(% of total ion intensity)
100
98
96
94
92
90
2
1
0
S
I/L
N
S I/L N
AGT
AXC

FIG. 1F

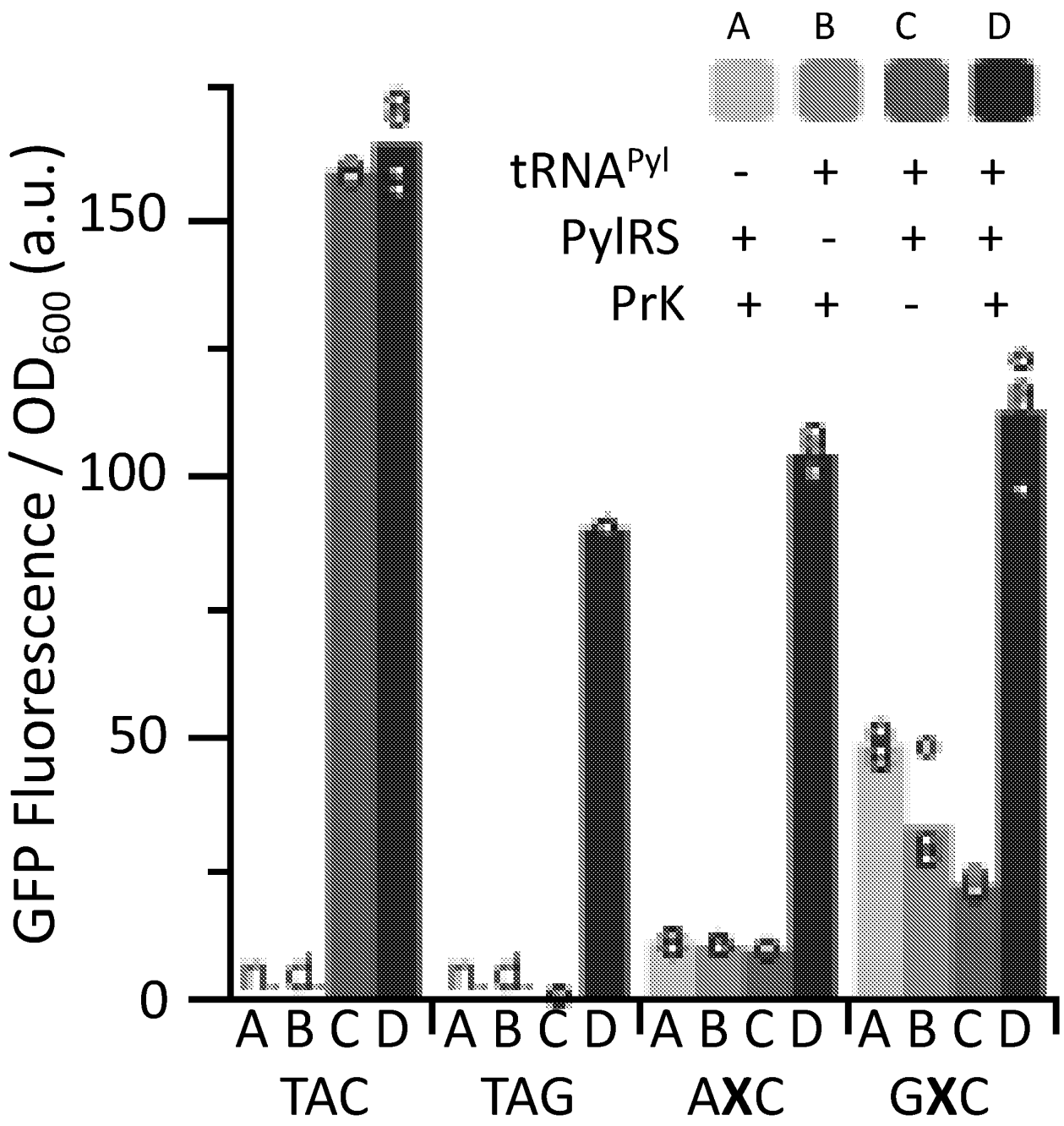

A B C D
tRNA^{Pyl} - + + +
PylRS + - + +
PrK + + - +
GFP Fluorescence / OD_{600} (a.u.)
150
100
50
0
n.d.
n.d.
A B C D A B C D A B C D A B C D
TAC TAG AXC GXC

FIG. 2A

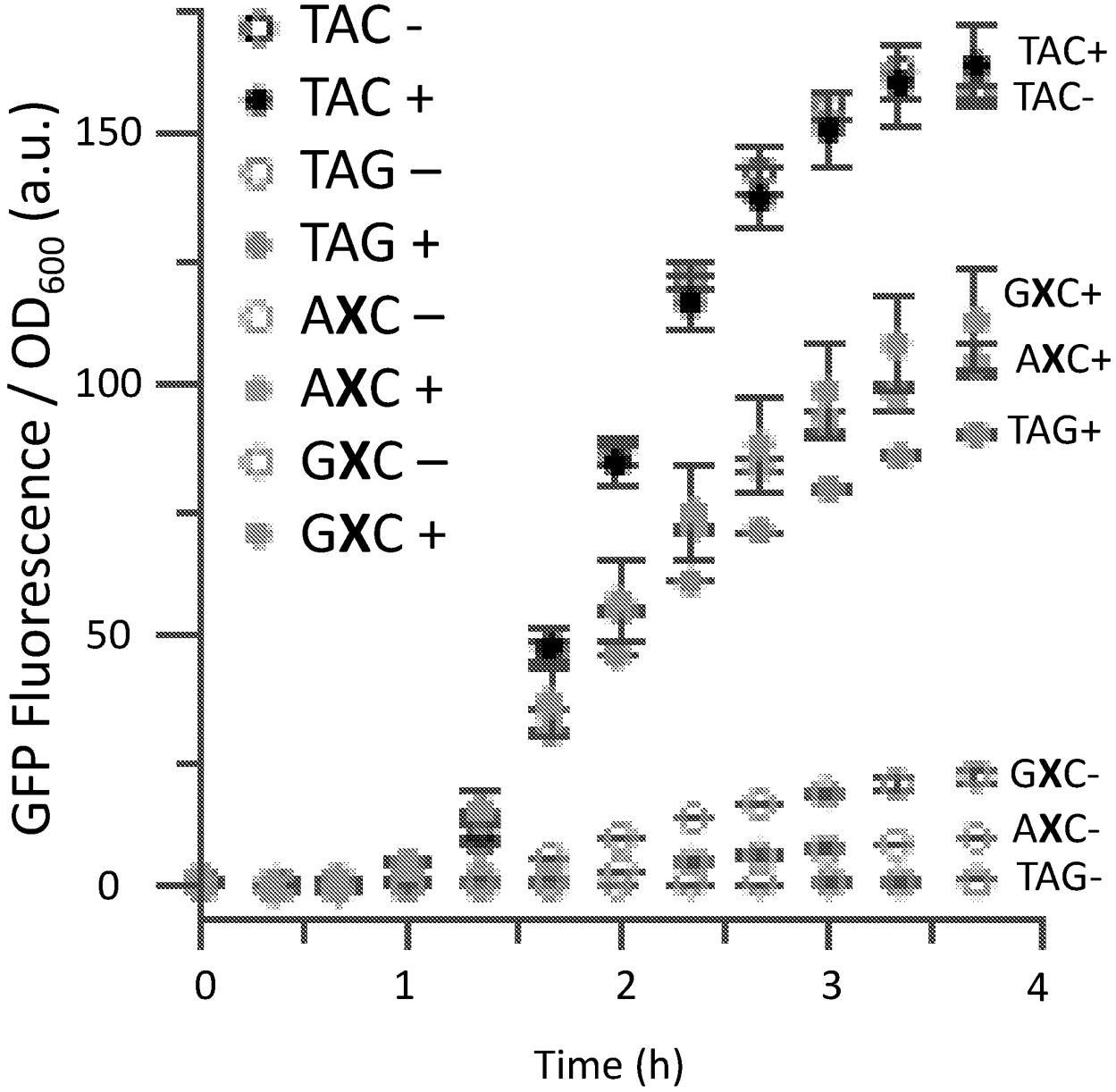
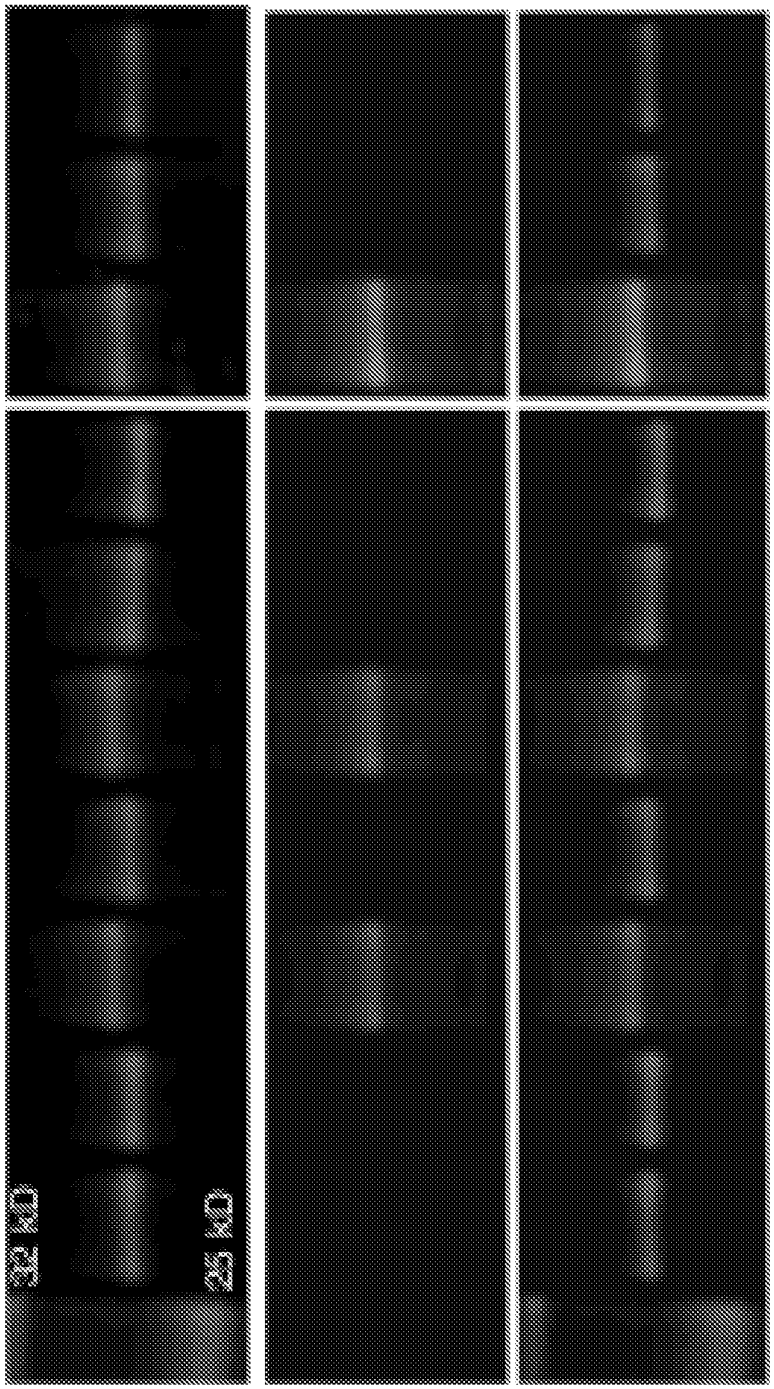

TAC -
TAC +
TAG –
TAG +
AXC –
AXC +
GXC –
GXC +
TAC+
TAC-
GXC+
AXC+
TAG+
GXC-
AXC-
TAG-
GFP Fluorescence / OD600 (a.u.)
0
50
100
150
0
1
2
3
4
Time (h)

FIG. 2B



codon	Anti-codon	PrK	TAMRA -N_3
TAC	GYC	+	+
GXC	GYC	+	-
GXC	GYC	+	+
TAC	GYT	+	+
AXC	GYT	+	-
AXC	GYT	+	+
TAG	CTA	+	-
TAG	CTA	+	+
TAC	-	+	+
TAC	-	-	+

FIG. 2C

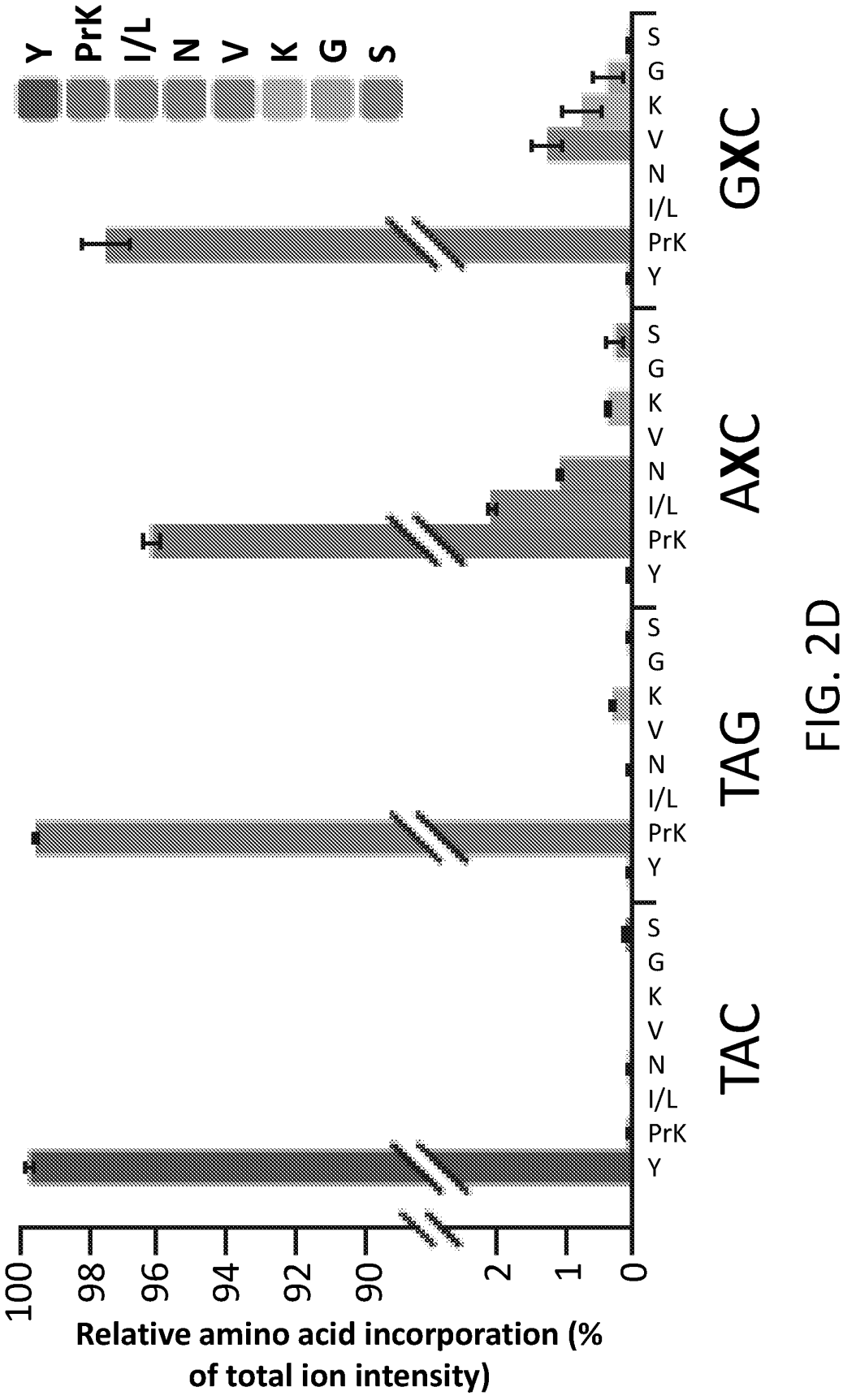

Y
PrK
I/L
N
V
K
G
S
TAC
TAG
AXC
GXC
Y
PrK
I/L
N
V
K
G
S
100
98
96
94
92
90
2
1
0
Relative amino acid incorporation (% of total ion intensity)

FIG. 2D

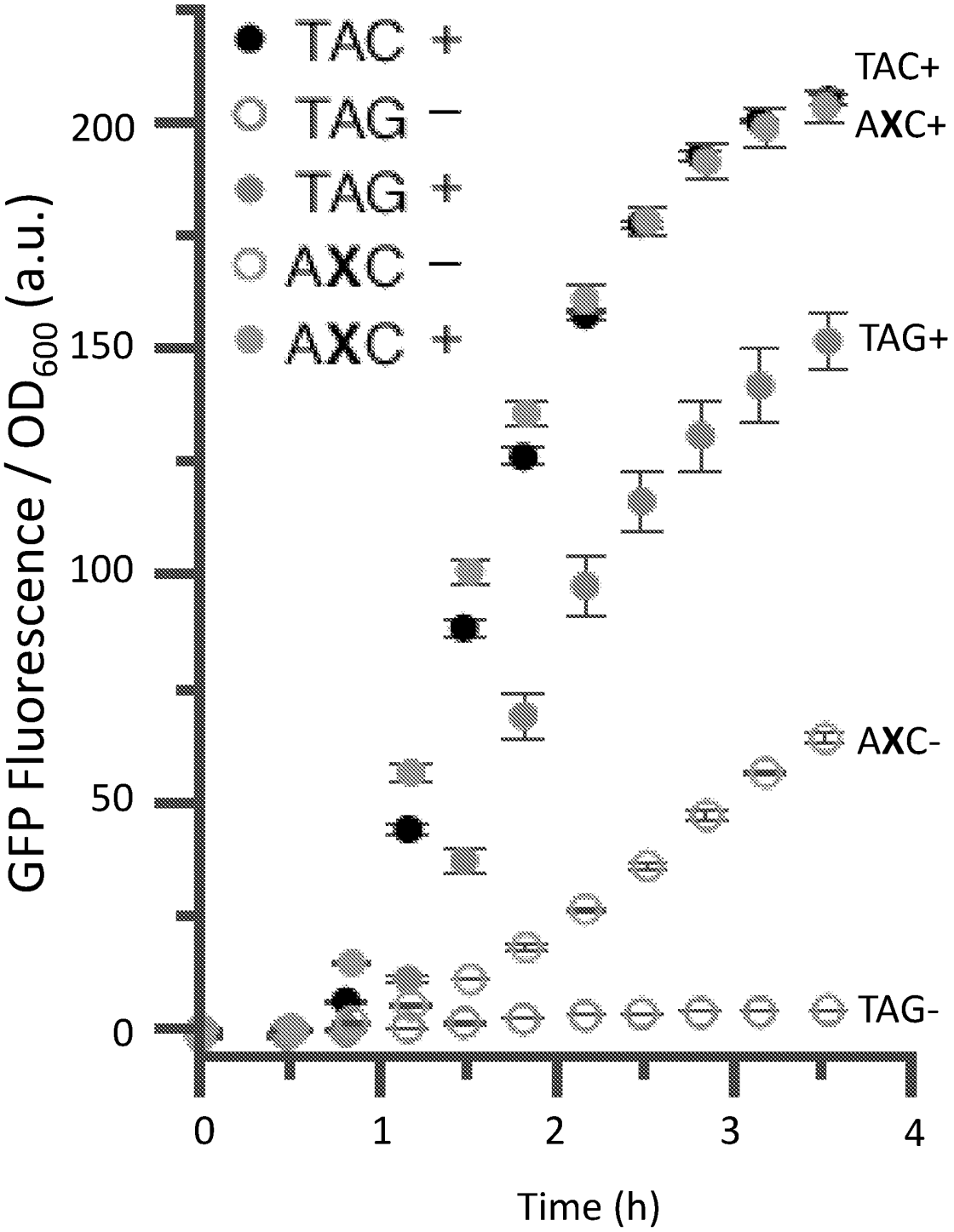
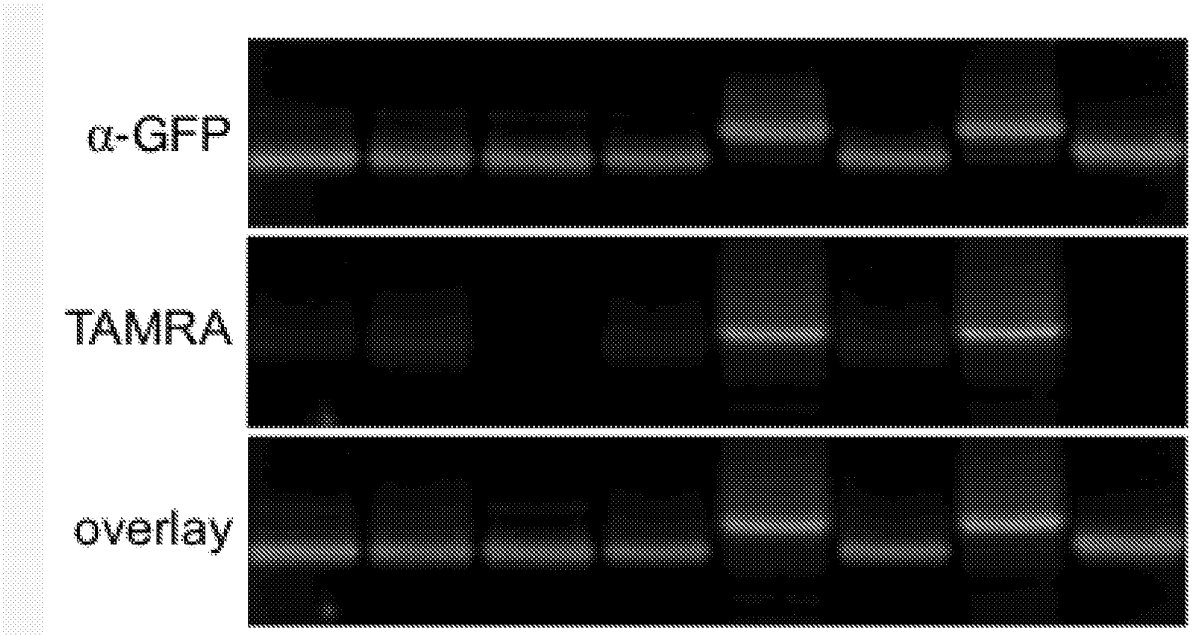

TAC +
TAG −
TAG +
AXC −
AXC +
TAC+
AXC+
TAG+
AXC-
TAG-
GFP Fluorescence / OD600 (a.u.)
0
50
100
150
200
0
1
2
3
4
Time (h)

FIG. 3A



codon	TAC	TAC	TAC	TAC	TAG	TAC	AXC	AXC
anticodon	–	–	–	CTA	CTA	GYT	GYT	GYT
*p*AzF	–	+	+	+	+	+	+	+
TAMRA-DBCO	+	+	–	+	+	+	+	–

FIG. 3B

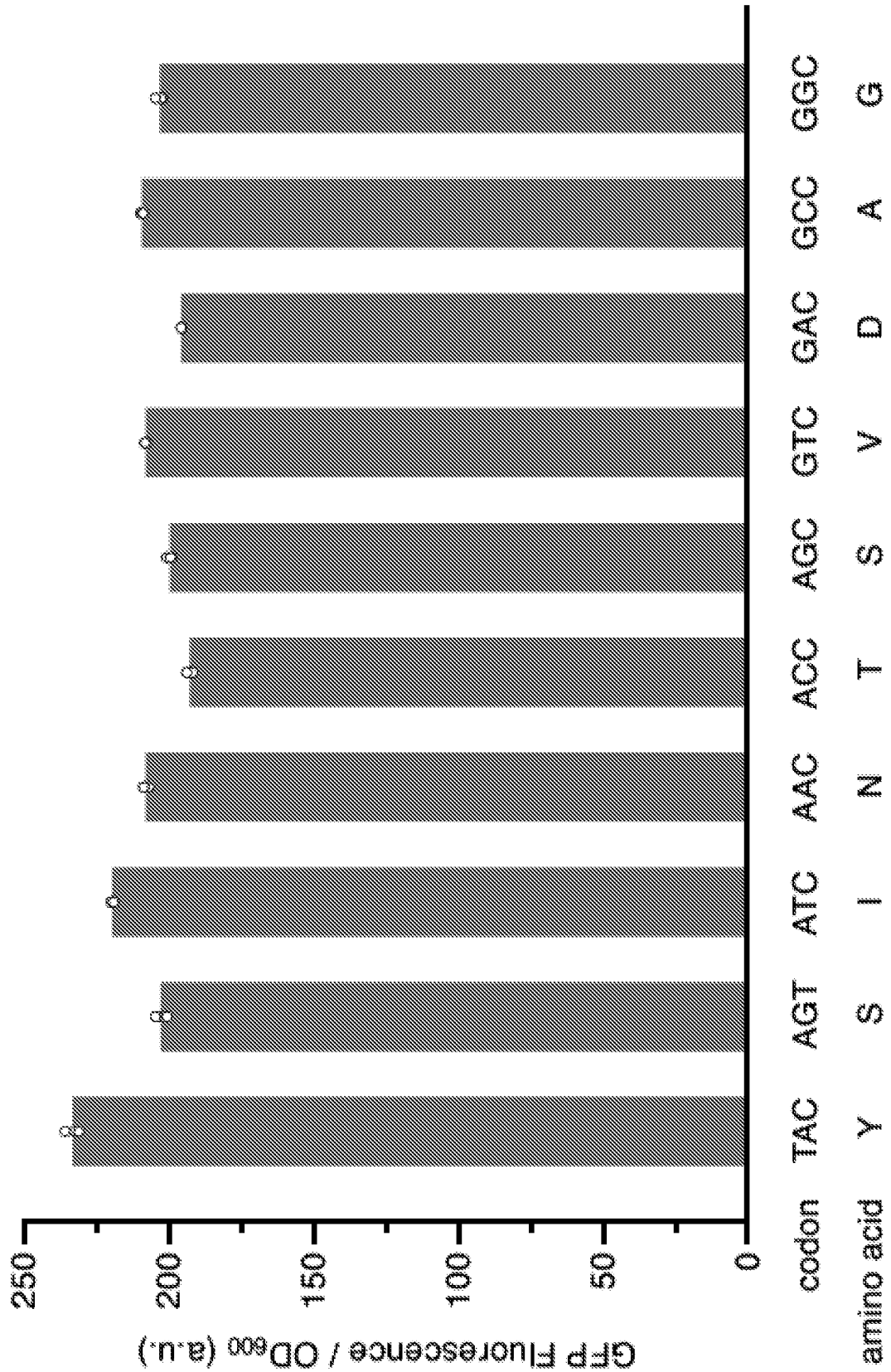

GFP Fluorescence / OD600 (a.u.)
250
200
150
100
50
0
codon
amino acid
TAC
Y
AGT
S
ATC
I
AAC
N
ACC
T
AGC
S
GTC
V
GAC
D
GCC
A
GGC
G

FIG. 4

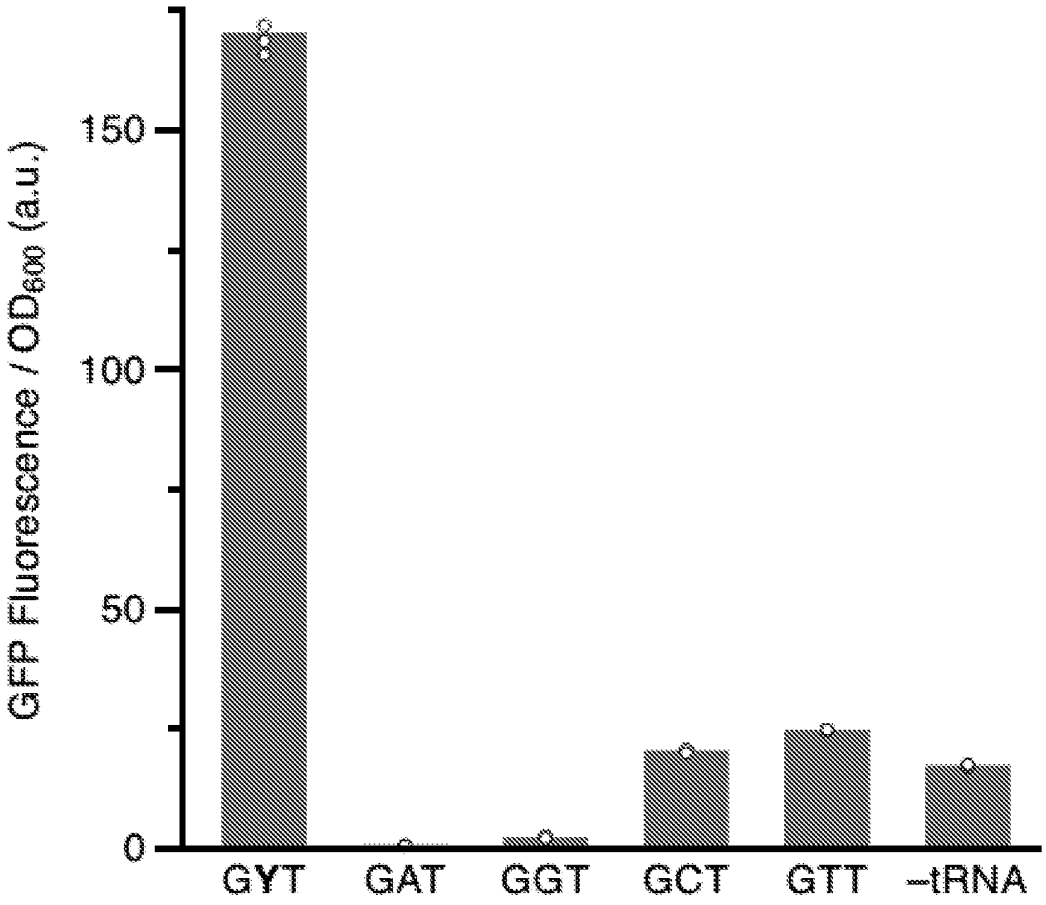

GFP Fluorescence / OD_{600} (a.u.)
150
100
50
0
GYT
GAT
GGT
GCT
GTT
–tRNA

FIG. 5A

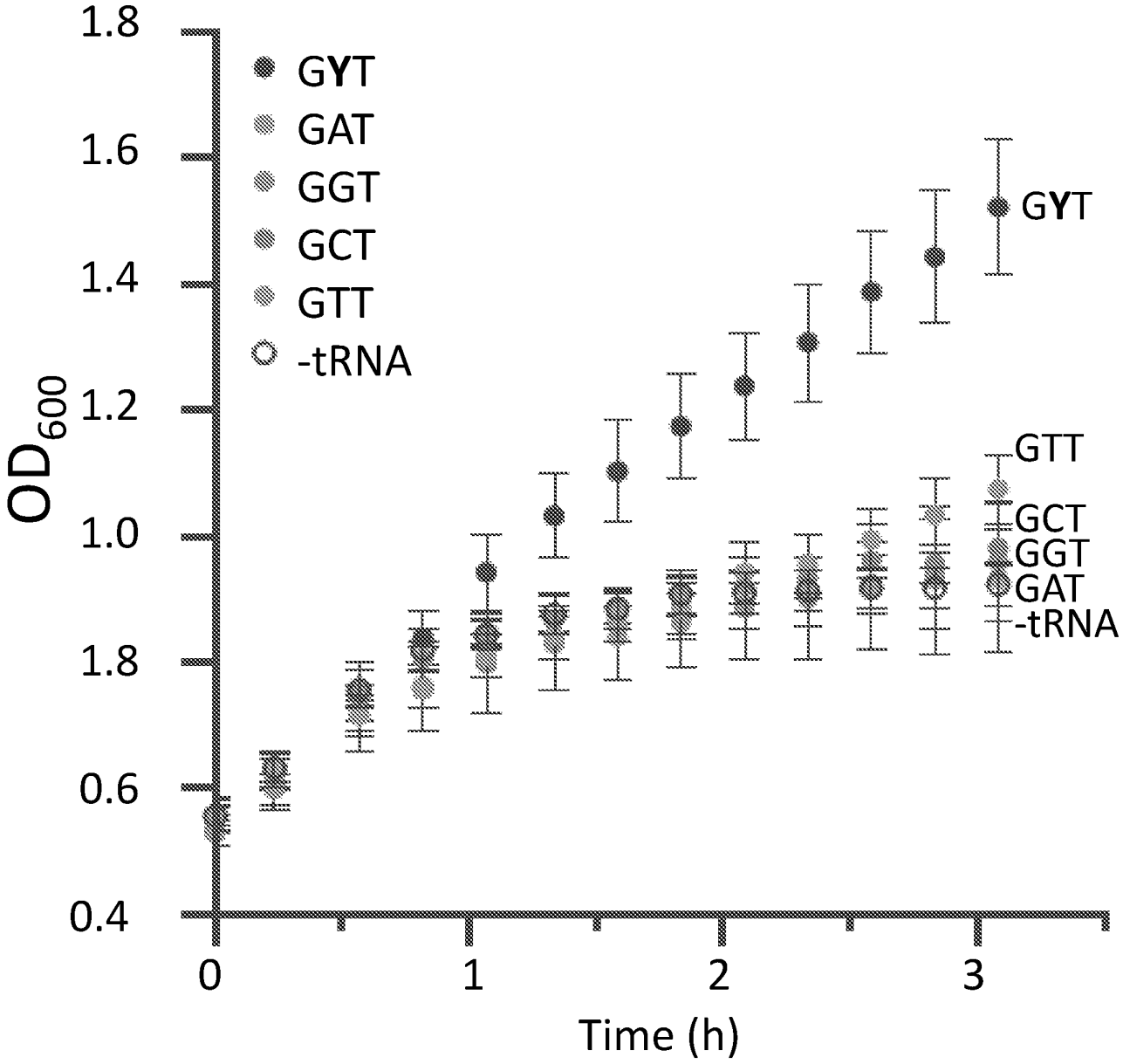

1.8
1.6
1.4
1.2
1.0
1.8
0.6
0.4
OD600
GYT
GAT
GGT
GCT
GTT
-tRNA
0
1
2
3
Time (h)
GYT
GTT
GCT
GGT
GAT
-tRNA

FIG. 5B

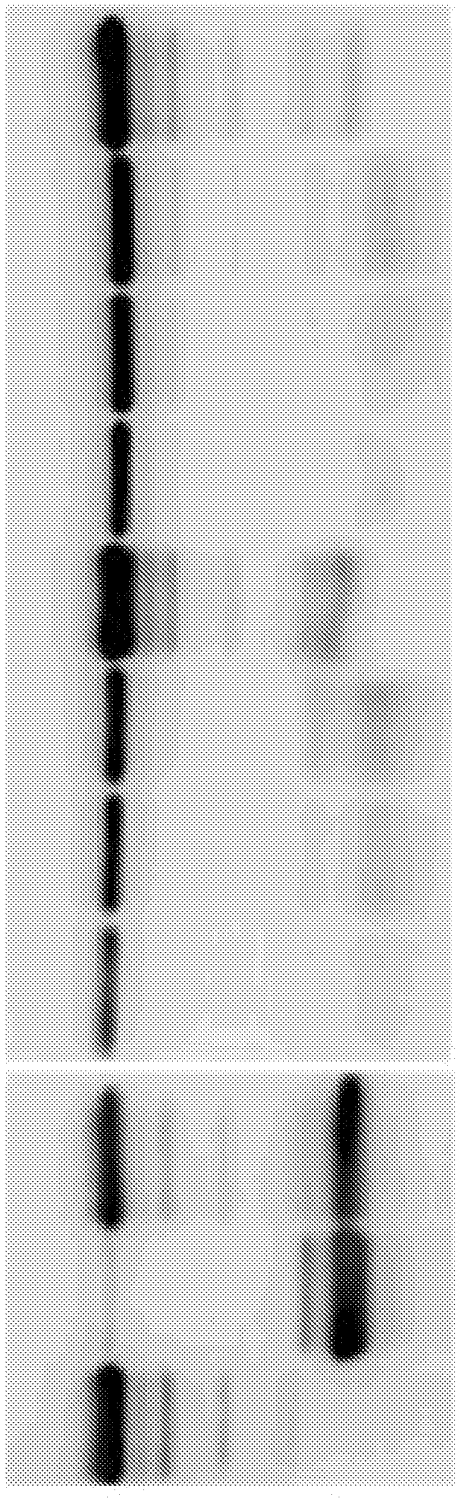

Full-length
truncated
Codon
Anticodon
tRNA^{Pyl}
PylRS
PrK
TAC
TAG
CTA
AXC
GYT
GXC
GYC

FIG. 6A

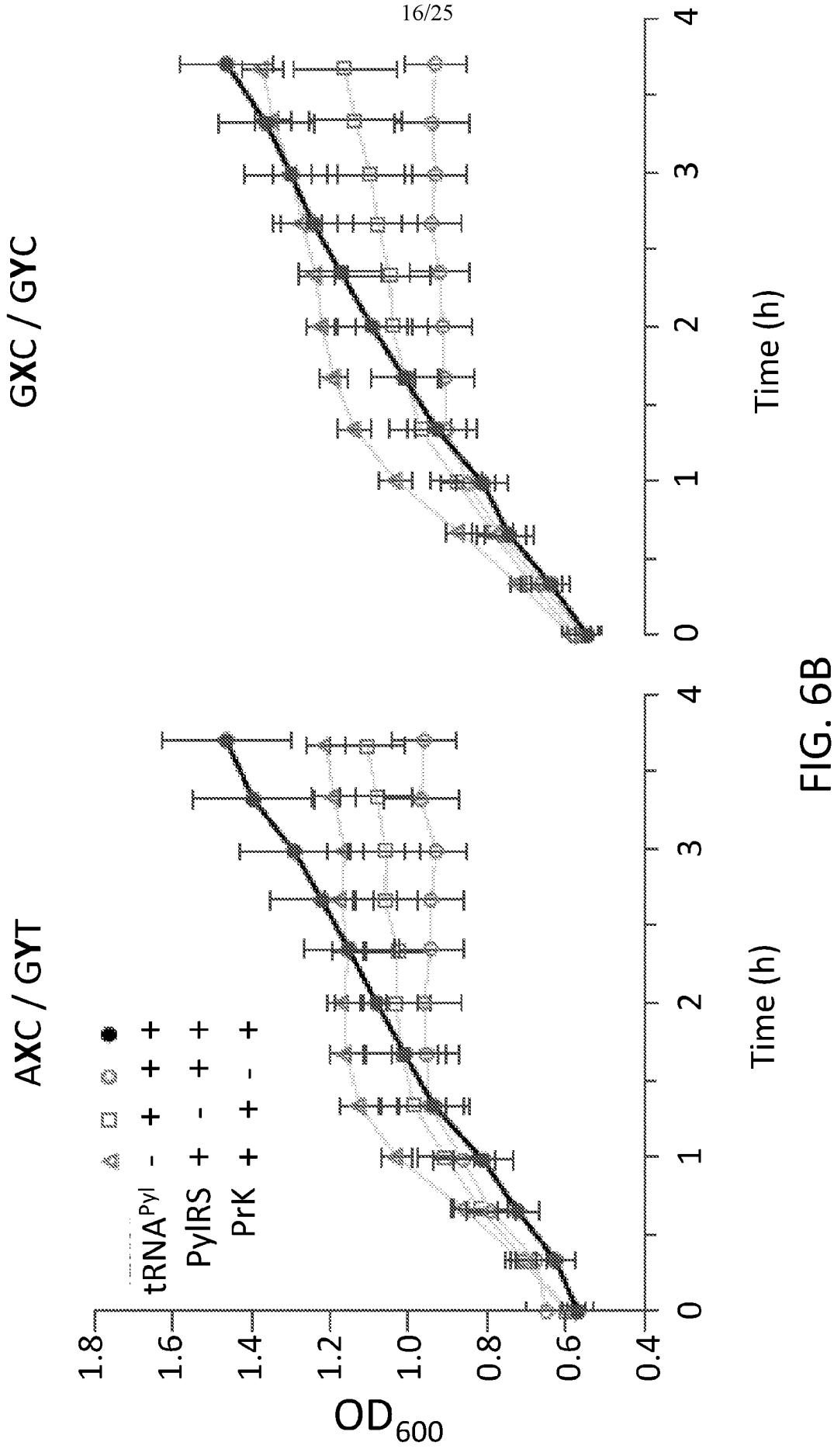

AXC / GYT
GXC / GYC
OD600
Time (h)
Time (h)
tRNAPyl - + + +
PylRS + - + +
PrK + + - +
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0
1
2
3
4

FIG. 6B

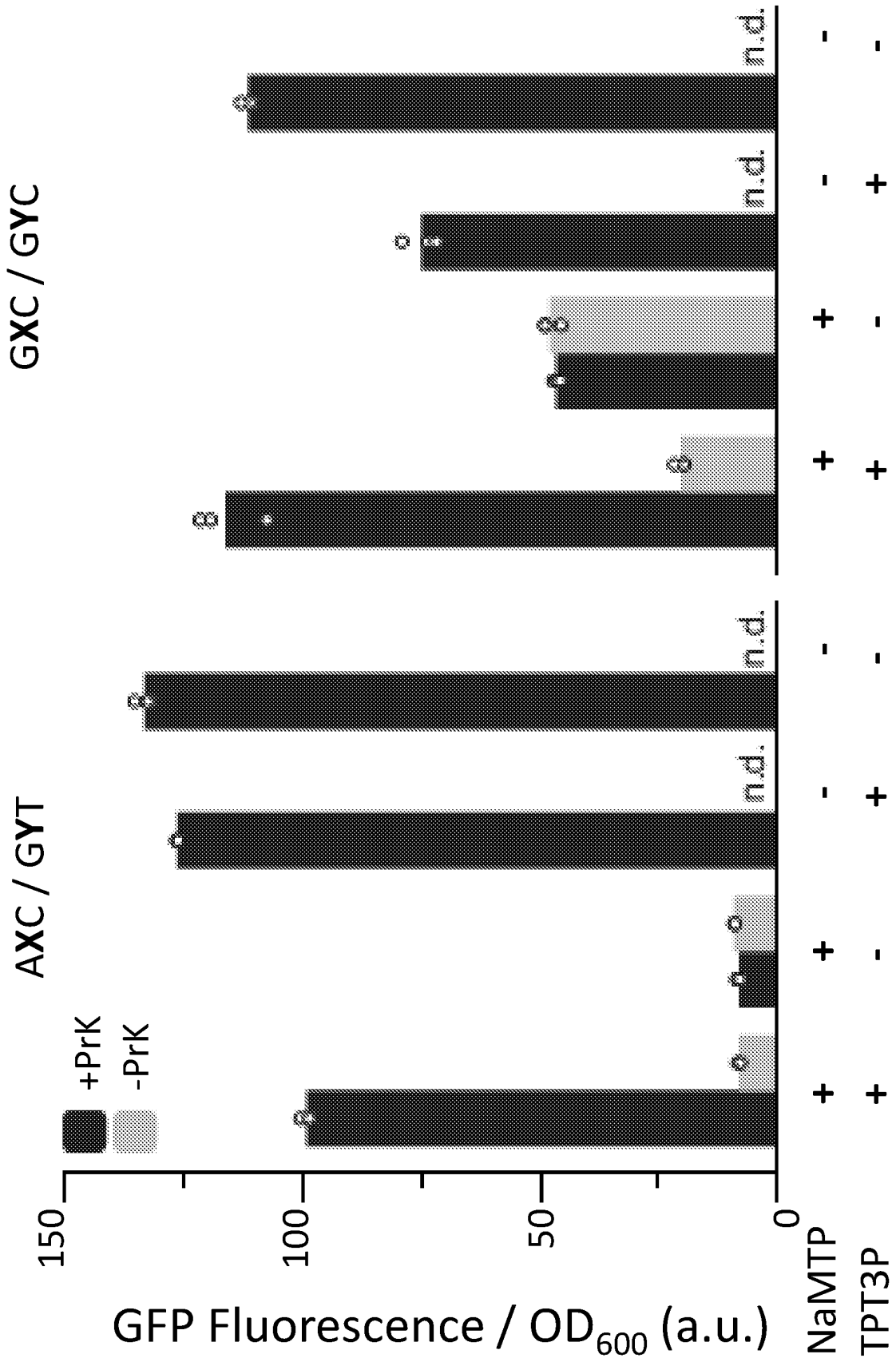

AXC / GYT
GXC / GYC
+PrK
-PrK
GFP Fluorescence / OD600 (a.u.)
150
100
50
0
NaMTP
TPT3P
+
-
m.d.

FIG. 7A

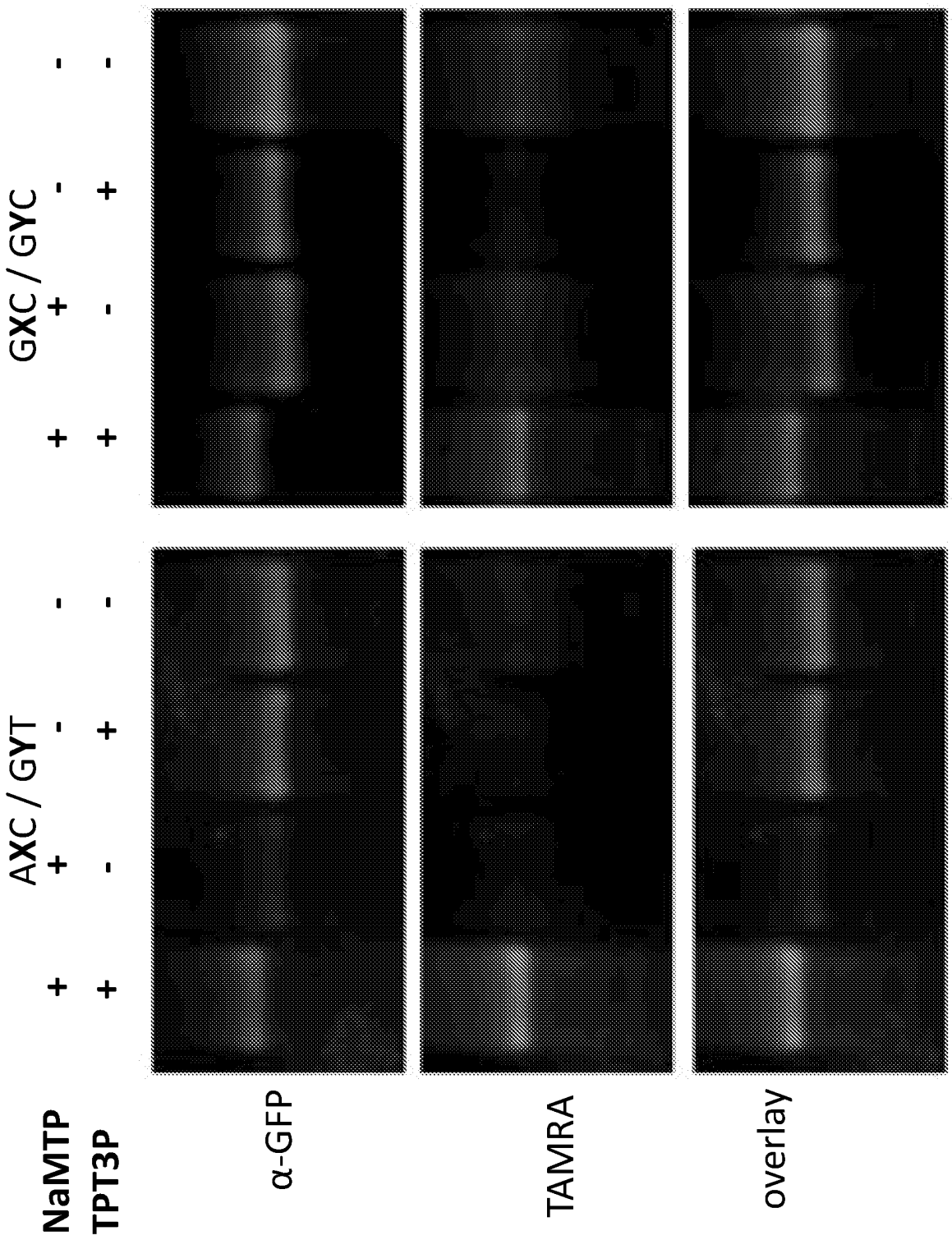
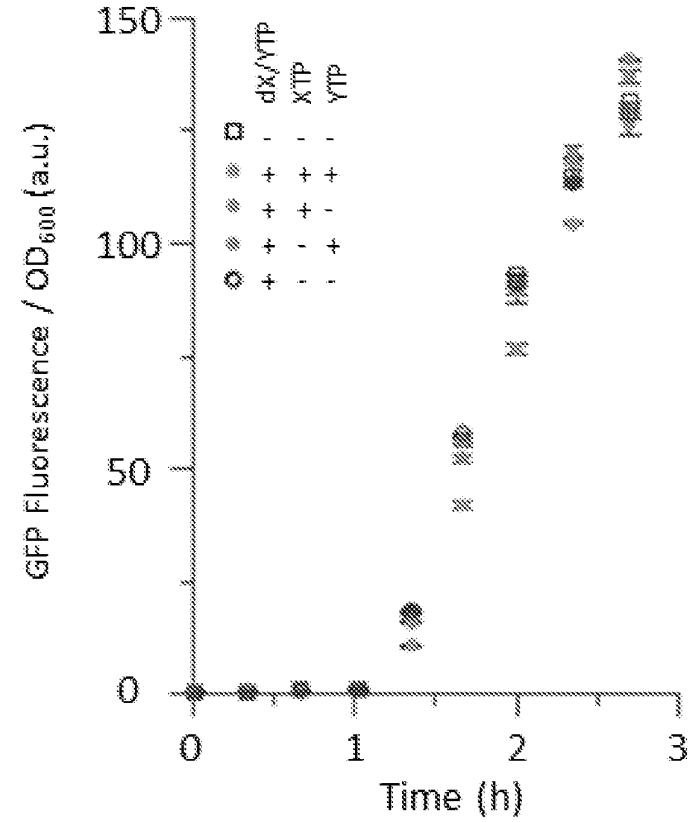


FIG. 7B


150
100
50
0
GFP Fluorescence / OD_{600} (a.u.)
dX/YTP
XTP
YTP
0
1
2
3
Time (h)

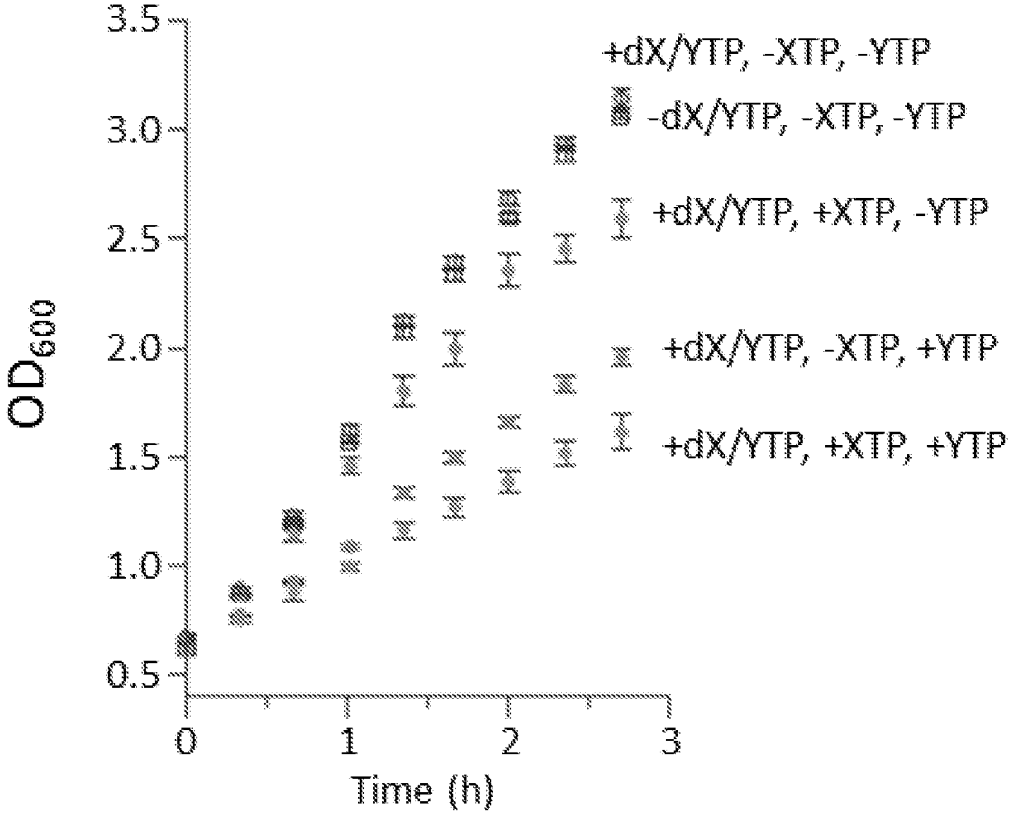

3.5
3.0
2.5
2.0
1.5
1.0
0.5
OD_{600}
+dX/YTP, -XTP, -YTP
-dX/YTP, -XTP, -YTP
+dX/YTP, +XTP, -YTP
+dX/YTP, -XTP, +YTP
+dX/YTP, +XTP, +YTP
0
1
2
3
Time (h)

FIG. 7C

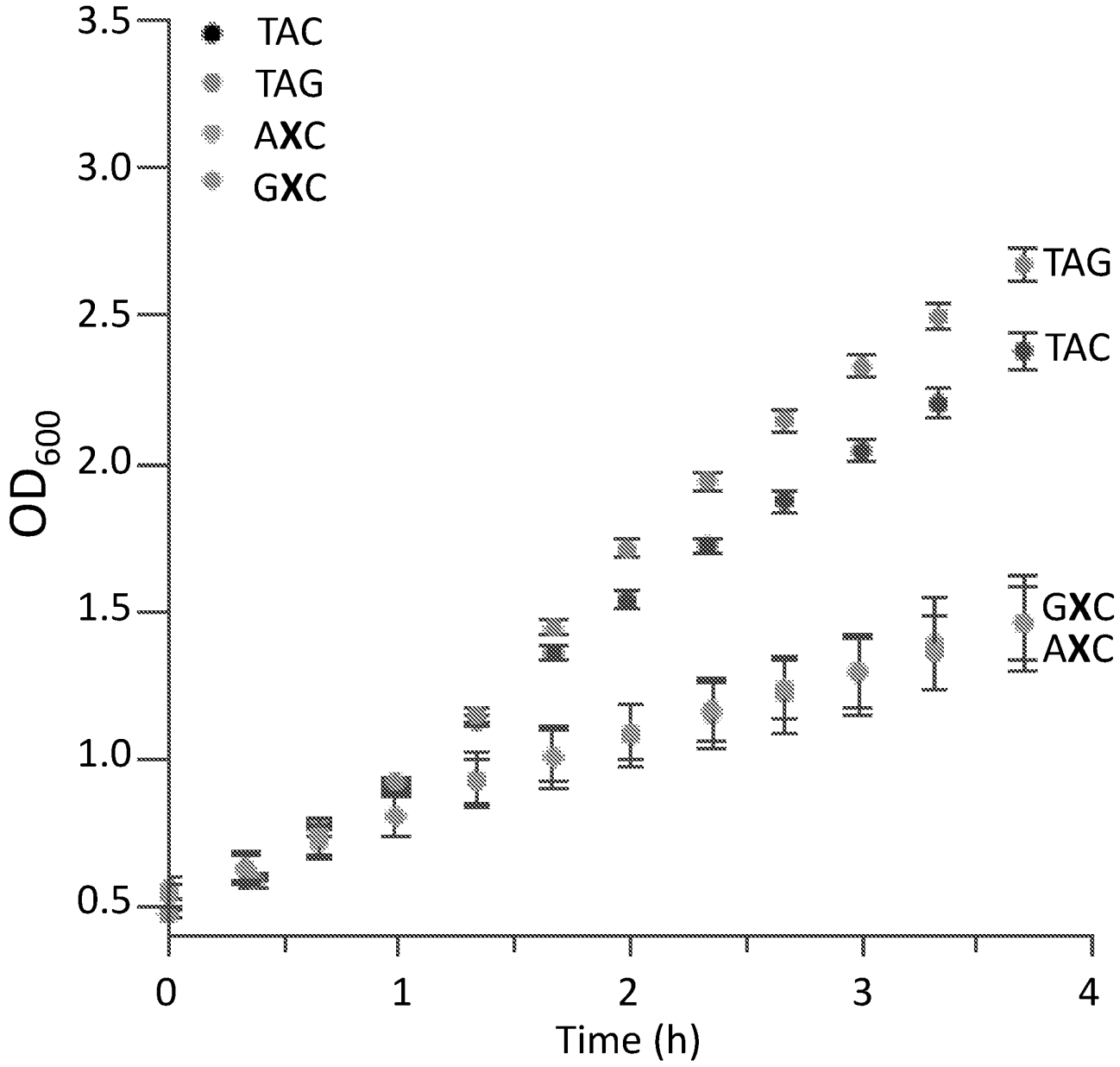

TAC
TAG
AXC
GXC
3.5
3.0
2.5
2.0
1.5
1.0
0.5
OD600
0
1
2
3
4
Time (h)
TAG
TAC
GXC
AXC

FIG. 7D

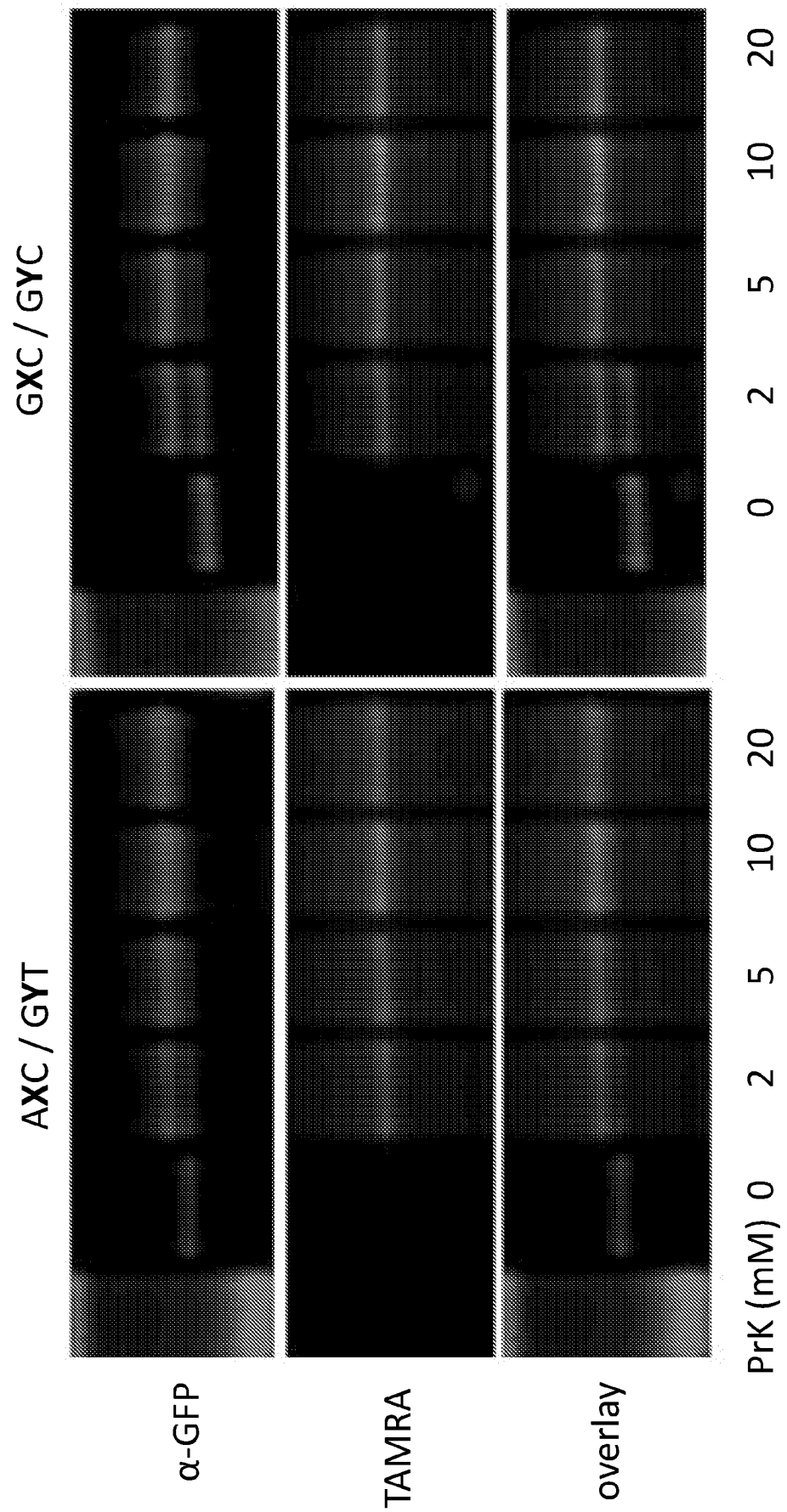


FIG. 8A

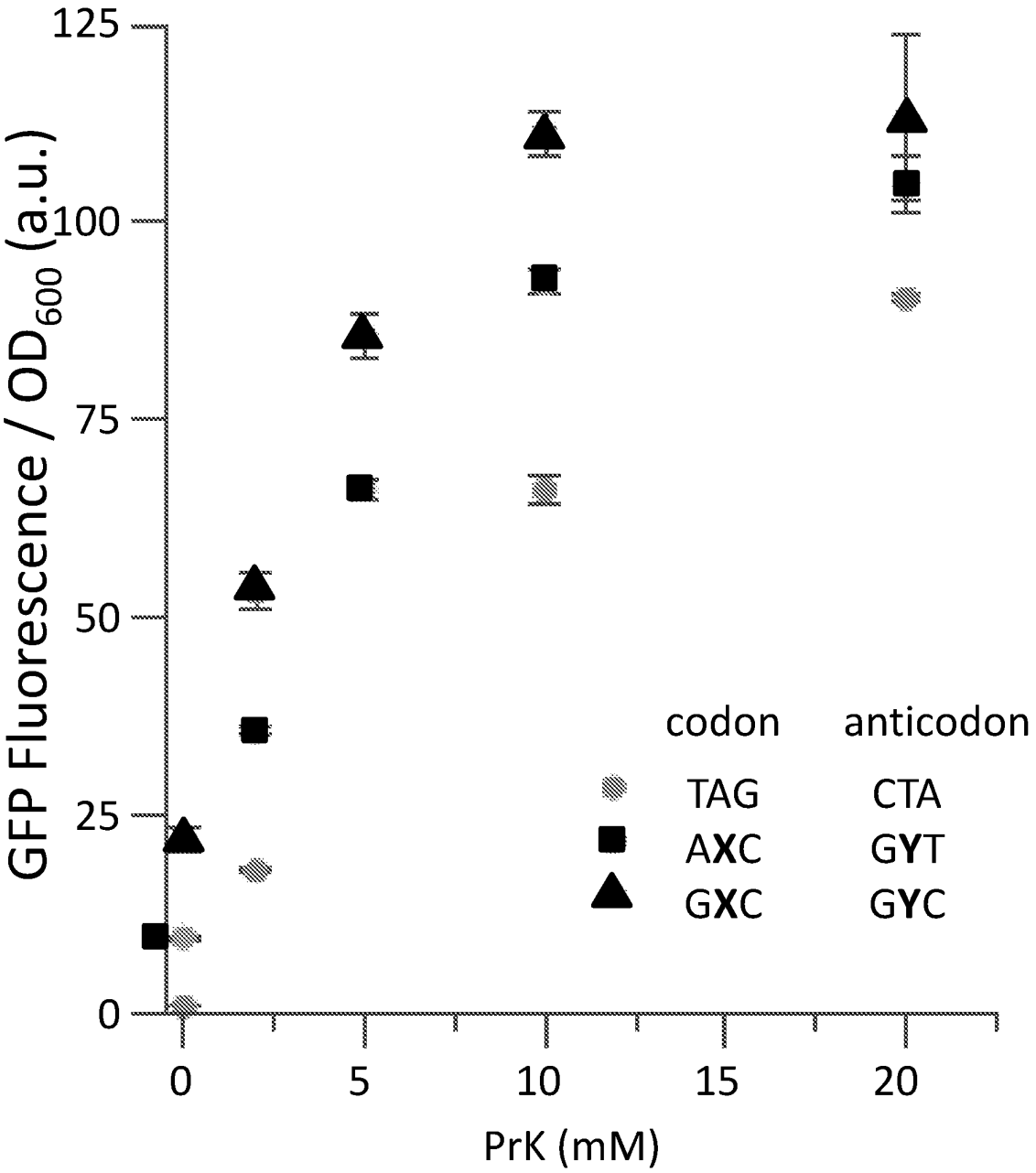

125
100
75
50
25
0
GFP Fluorescence / OD600 (a.u.)
0
5
10
15
20
PrK (mM)
codon
anticodon
TAG
CTA
AXC
GYT
GXC
GYC

FIG. 8B

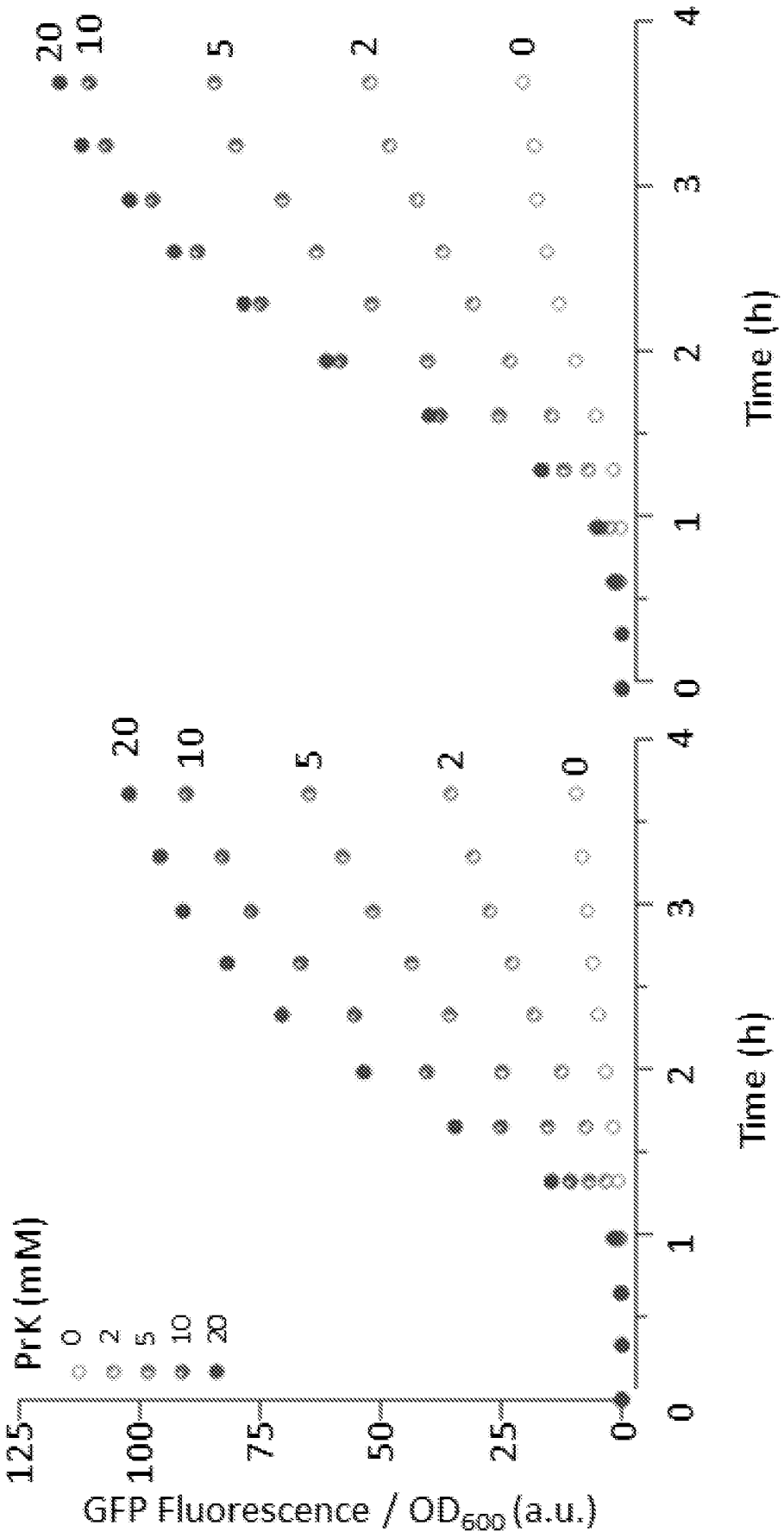

AXC / GYT
GXC / GYC
PrK (mM)
0
2
5
10
20
GFP Fluorescence / OD600 (a.u.)
125
100
75
50
25
0
Time (h)
0
1
2
3
4

FIG. 8C

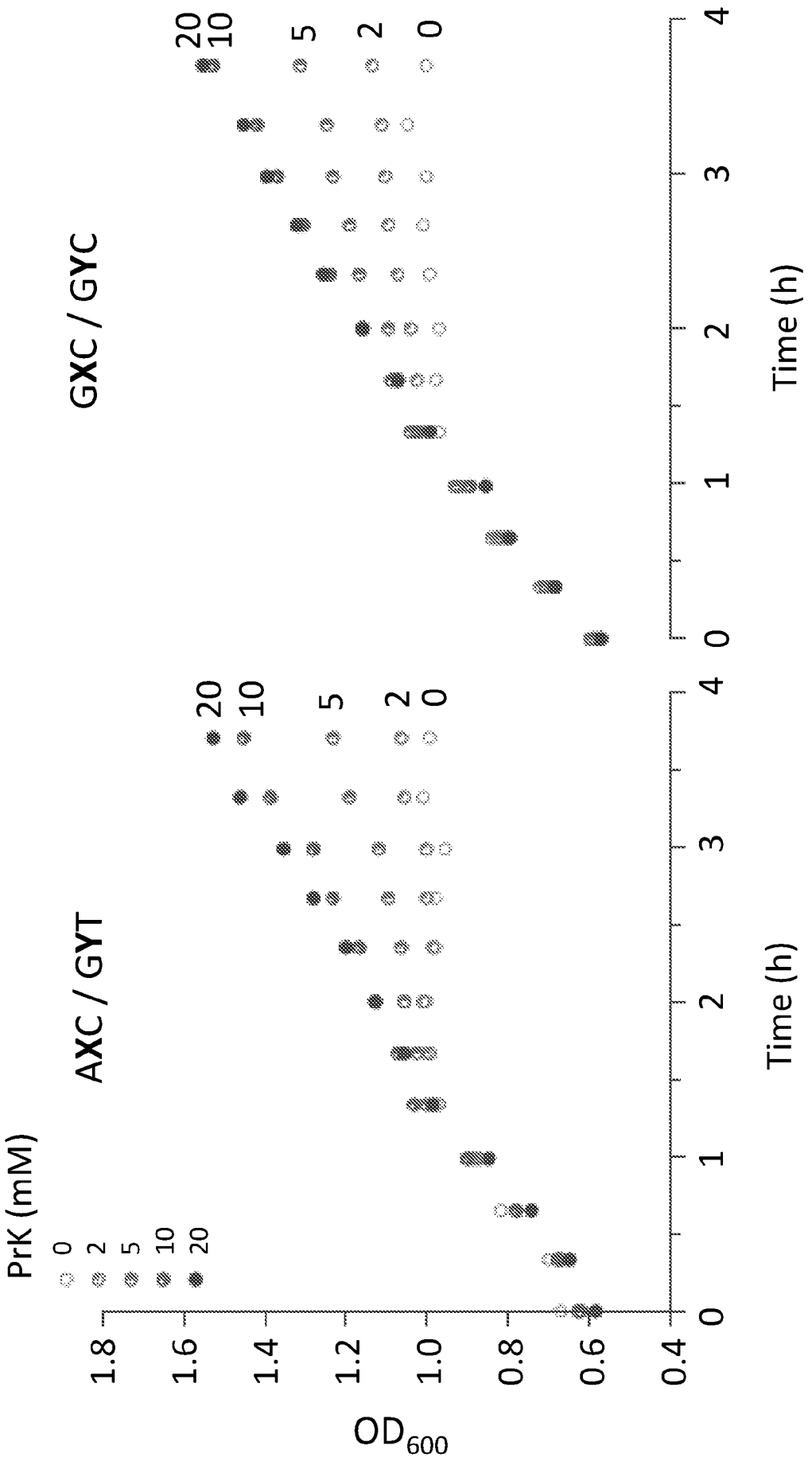

AXC / GYT
GXC / GYC
PrK (mM)
0
2
5
10
20
OD600
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0
1
2
3
4
Time (h)

FIG. 8D

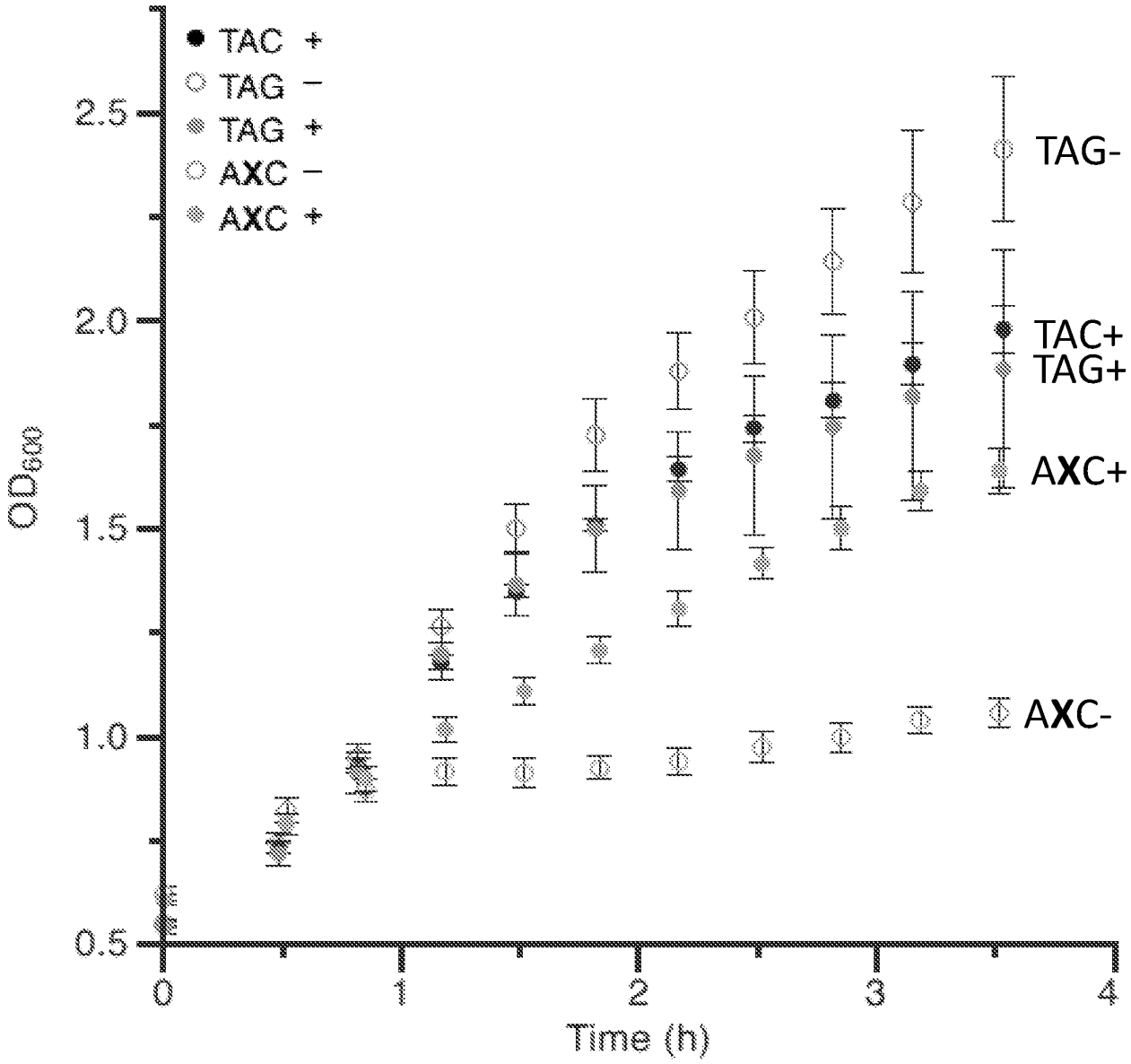

TAC +
TAG −
TAG +
AXC −
AXC +
2.5
2.0
1.5
1.0
0.5
OD_{600}
0
1
2
3
4
Time (h)
TAG-
TAC+
TAG+
AXC+
AXC-

FIG. 9