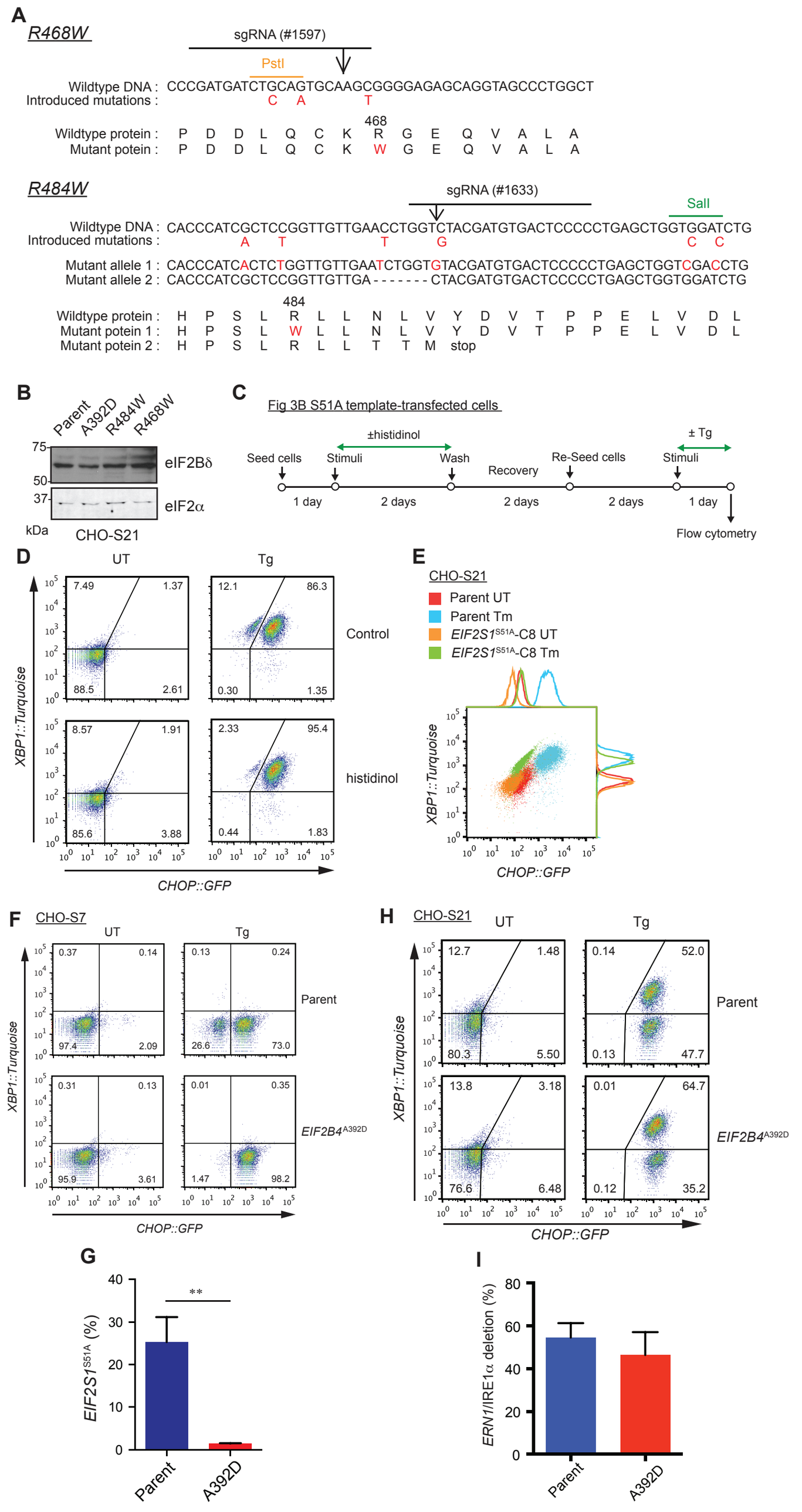
A
R468W
sgRNA (#1597)
PstI
Wildtype DNA : CCCGATGATCTGCAGTGCAAGCGGGGAGAGCAGGTAGCCCTGGCT
Introduced mutations : C A T
468
Wildtype protein : P D D L Q C K R G E Q V A L A
Mutant potein : P D D L Q C K W G E Q V A L A
R484W
sgRNA (#1633)
SalI
Wildtype DNA : CACCCATCGCTCCGGTTGTTGAACCTGGTCTACGATGTGACTCCCCCTGAGCTGGTGGATCTG
Introduced mutations : A T T G C C
Mutant allele 1 : CACCCATCACTCTGGTTGTTGAATCTGGTGTACGATGTGACTCCCCCTGAGCTGGTCGACCTG
Mutant allele 2 : CACCCATCGCTCCGGTTGTTGA - - - - - - - CTACGATGTGACTCCCCCTGAGCTGGTGGATCTG
484
Wildtype protein : H P S L R L L N L V Y D V T P P E L V D L
Mutant potein 1 : H P S L W L L N L V Y D V T P P E L V D L
Mutant potein 2 : H P S L R L L T T M stop
B
Parent
A392D
R484W
R468W
75
50
37
kDa
eIF2Bδ
eIF2α
CHO-S21
C
Fig 3B S51A template-transfected cells
±histidinol
± Tg
Seed cells
Stimuli
Wash
Recovery
Re-Seed cells
Stimuli
1 day
2 days
2 days
2 days
1 day
Flow cytometry
D
UT
Tg
Control
histidinol
XBP1::Turquoise
CHOP::GFP
7.49
1.37
88.5
2.61
12.1
86.3
0.30
1.35
8.57
1.91
85.6
3.88
2.33
95.4
0.44
1.83
E
CHO-S21
Parent UT
Parent Tm
EIF2S1S51A-C8 UT
EIF2S1S51A-C8 Tm
XBP1::Turquoise
CHOP::GFP
F
CHO-S7
UT
Tg
Parent
EIF2B4A392D
XBP1::Turquoise
CHOP::GFP
0.37
0.14
97.4
2.09
0.13
0.24
26.6
73.0
0.31
0.13
95.9
3.61
0.01
0.35
1.47
98.2
H
CHO-S21
UT
Tg
Parent
EIF2B4A392D
XBP1::Turquoise
CHOP::GFP
12.7
1.48
80.3
5.50
0.14
52.0
0.13
47.7
13.8
3.18
76.6
6.48
0.01
64.7
0.12
35.2
G
EIF2S1S51A (%)
**
Parent
A392D
I
ERN1/IRE1α deletion (%)
Parent
A392D