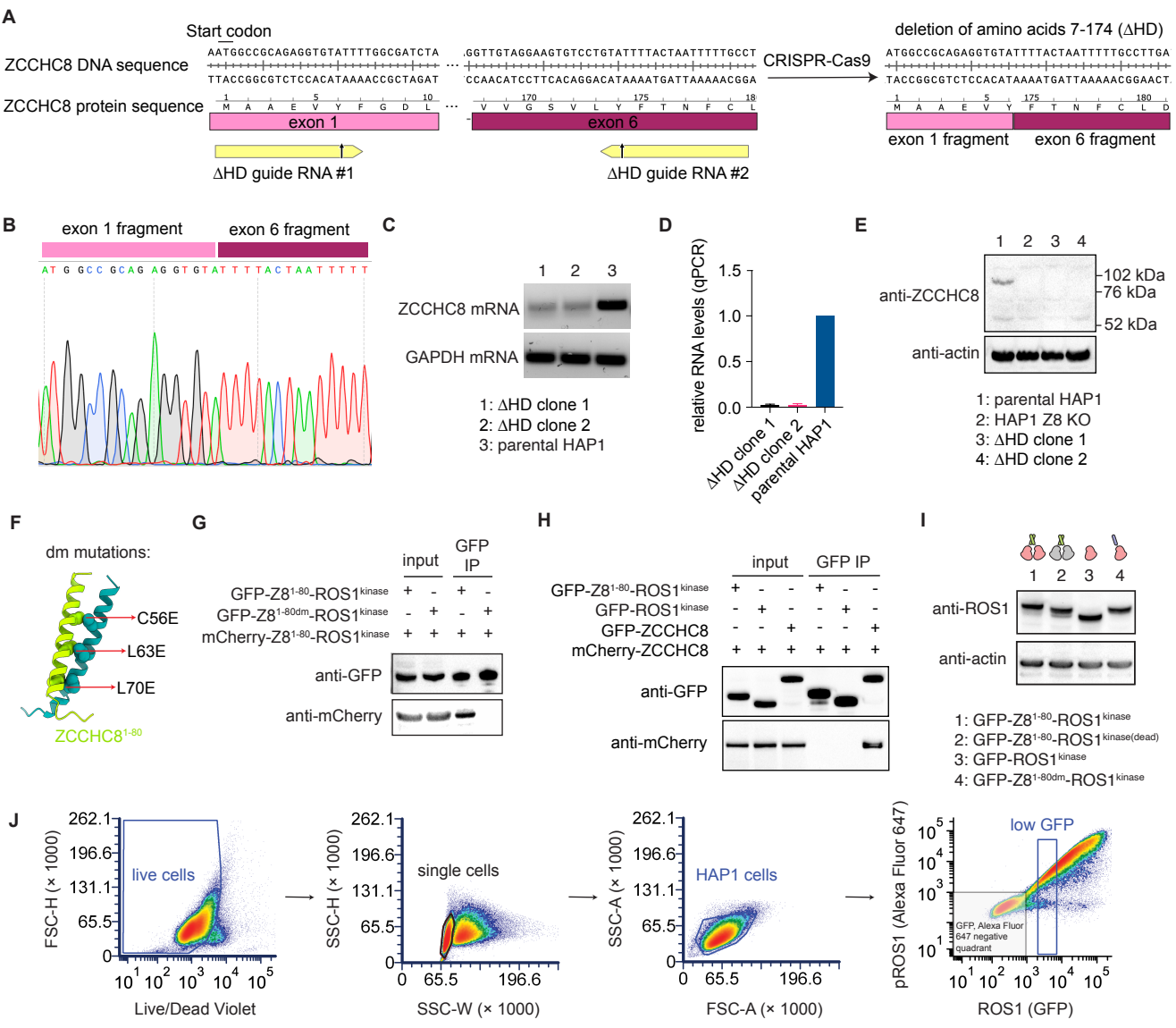
A
Start codon
ZCCHC8 DNA sequence
AATGGCCGCAGAGGTGTATTTTGGCGATCTA
TTACCGGCGTCTCCACATAAAACCGCTAGAT
GGTTGTAGGAAGTGTCCTGTATTTTACTAATTTTTGCCT
CCAACATCCTTCACAGGACATAAAATGATTAAAAACGGA
CRISPR-Cas9
deletion of amino acids 7-174 (ΔHD)
ATGGCCGCAGAGGTGTATTTTACTAATTTTTGCCTTGA
TACCGGCGTCTCCACATAAAATGATTAAAAACGGAACT
ZCCHC8 protein sequence
M A A E V Y F G D L
V V G S V L Y F T N F C L
M A A E V Y F T N F C L D
exon 1
exon 6
exon 1 fragment
exon 6 fragment
ΔHD guide RNA #1
ΔHD guide RNA #2
B
exon 1 fragment
exon 6 fragment
C
1 2 3
ZCCHC8 mRNA
GAPDH mRNA
1: ΔHD clone 1
2: ΔHD clone 2
3: parental HAP1
D
relative RNA levels (qPCR)
1.5
1.0
0.5
0.0
ΔHD clone 1
ΔHD clone 2
parental HAP1
E
1 2 3 4
anti-ZCCHC8
anti-actin
102 kDa
76 kDa
52 kDa
1: parental HAP1
2: HAP1 Z8 KO
3: ΔHD clone 1
4: ΔHD clone 2
F
dm mutations:
C56E
L63E
L70E
ZCCHC8 1-80
G
input
GFP IP
GFP-Z8 1-80-ROS1 kinase
GFP-Z8 1-80dm-ROS1 kinase
mCherry-Z8 1-80-ROS1 kinase
anti-GFP
anti-mCherry
H
input
GFP IP
GFP-Z8 1-80-ROS1 kinase
GFP-ROS1 kinase
GFP-ZCCHC8
mCherry-ZCCHC8
anti-GFP
anti-mCherry
I
1 2 3 4
anti-ROS1
anti-actin
1: GFP-Z8 1-80-ROS1 kinase
2: GFP-Z8 1-80-ROS1 kinase(dead)
3: GFP-ROS1 kinase
4: GFP-Z8 1-80dm-ROS1 kinase
J
live cells
FSC-H (× 1000)
Live/Dead Violet
single cells
SSC-H (× 1000)
SSC-W (× 1000)
HAP1 cells
SSC-A (× 1000)
FSC-A (× 1000)
low GFP
GFP, Alexa Fluor 647 negative quadrant
pROS1 (Alexa Fluor 647)
ROS1 (GFP)