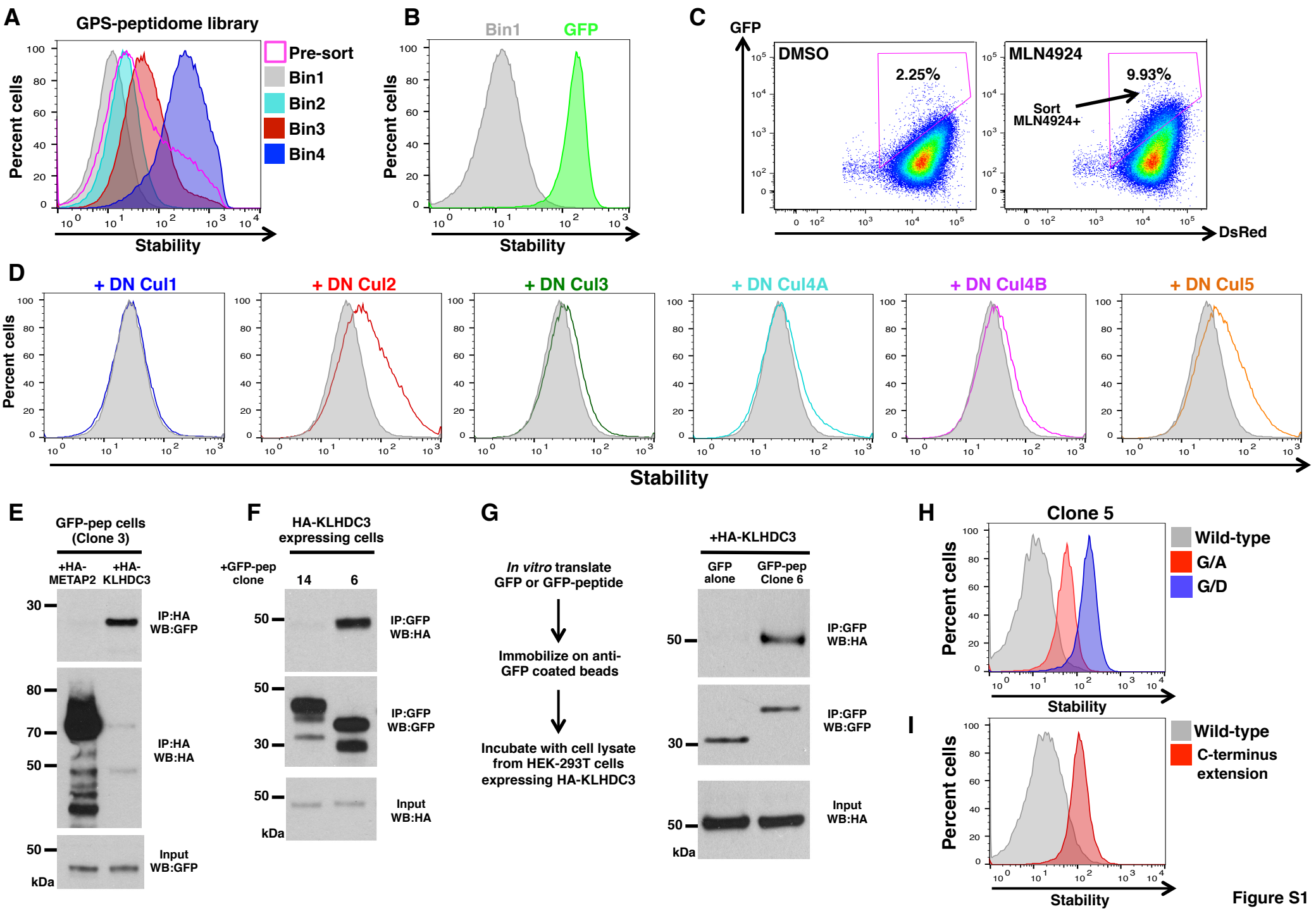
A
GPS-peptidome library
Pre-sort
Bin1
Bin2
Bin3
Bin4
Percent cells
Stability
B
Bin1
GFP
Percent cells
Stability
C
GFP
DMSO
2.25%
MLN4924
9.93%
Sort
MLN4924+
DsRed
D
+ DN Cul1
+ DN Cul2
+ DN Cul3
+ DN Cul4A
+ DN Cul4B
+ DN Cul5
Percent cells
Stability
E
GFP-pep cells
(Clone 3)
+HA-
METAP2
+HA-
KLHDC3
IP:HA
WB:GFP
IP:HA
WB:HA
Input
WB:GFP
kDa
F
HA-KLHDC3
expressing cells
+GFP-pep
clone
14
6
IP:GFP
WB:HA
IP:GFP
WB:GFP
Input
WB:HA
kDa
G
In vitro translate
GFP or GFP-peptide
Immobilize on anti-
GFP coated beads
Incubate with cell lysate
from HEK-293T cells
expressing HA-KLHDC3
+HA-KLHDC3
GFP
alone
GFP-pep
Clone 6
IP:GFP
WB:HA
IP:GFP
WB:GFP
Input
WB:HA
kDa
H
Clone 5
Wild-type
G/A
G/D
Percent cells
Stability
I
Wild-type
C-terminus
extension
Percent cells
Stability

Figure S1