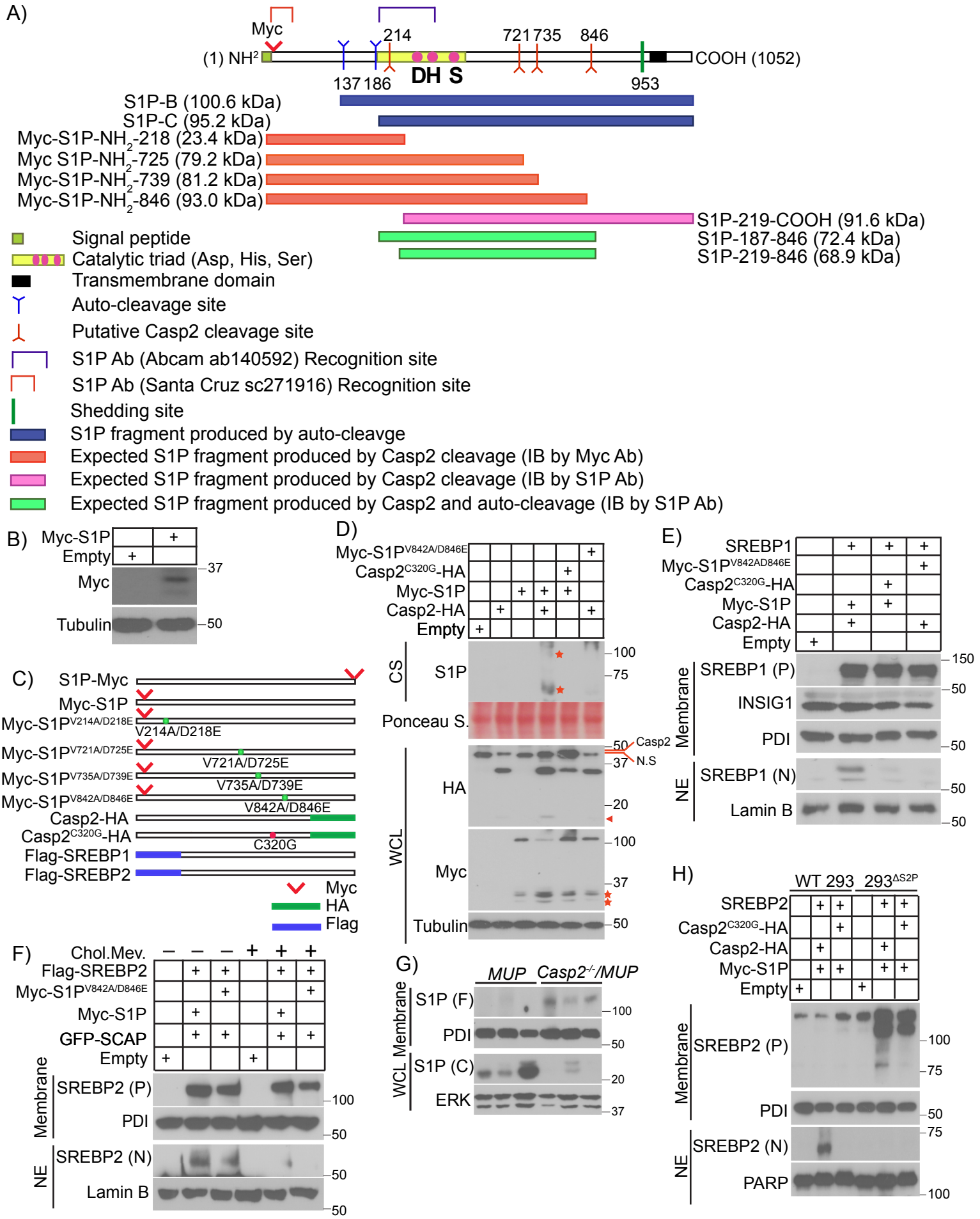
A)
Myc
214
721 735
846
(1) NH2
COOH (1052)
137 186
DH S
953
S1P-B (100.6 kDa)
S1P-C (95.2 kDa)
Myc-S1P-NH2-218 (23.4 kDa)
Myc S1P-NH2-725 (79.2 kDa)
Myc-S1P-NH2-739 (81.2 kDa)
Myc-S1P-NH2-846 (93.0 kDa)
S1P-219-COOH (91.6 kDa)
S1P-187-846 (72.4 kDa)
S1P-219-846 (68.9 kDa)
Signal peptide
Catalytic triad (Asp, His, Ser)
Transmembrane domain
Auto-cleavage site
Putative Casp2 cleavage site
S1P Ab (Abcam ab140592) Recognition site
S1P Ab (Santa Cruz sc271916) Recognition site
Shedding site
S1P fragment produced by auto-cleavge
Expected S1P fragment produced by Casp2 cleavage (IB by Myc Ab)
Expected S1P fragment produced by Casp2 cleavage (IB by S1P Ab)
Expected S1P fragment produced by Casp2 and auto-cleavage (IB by S1P Ab)
B)
Myc-S1P
Empty
Myc
Tubulin
37
50
C)
S1P-Myc
Myc-S1P
Myc-S1PV214A/D218E
V214A/D218E
Myc-S1PV721A/D725E
V721A/D725E
Myc-S1PV735A/D739E
V735A/D739E
Myc-S1PV842A/D846E
V842A/D846E
Casp2-HA
Casp2C320G-HA
C320G
Flag-SREBP1
Flag-SREBP2
Myc
HA
Flag
D)
Myc-S1PV842A/D846E
Casp2C320G-HA
Myc-S1P
Casp2-HA
Empty
CS
S1P
Ponceau S.
WCL
HA
Myc
Tubulin
Casp2
N.S
100
75
50
37
20
E)
SREBP1
Myc-S1PV842AD846E
Casp2C320G-HA
Myc-S1P
Casp2-HA
Empty
Membrane
SREBP1 (P)
INSIG1
PDI
NE
SREBP1 (N)
Lamin B
150
50
F)
Chol.Mev.
Flag-SREBP2
Myc-S1PV842A/D846E
Myc-S1P
GFP-SCAP
Empty
Membrane
SREBP2 (P)
PDI
NE
SREBP2 (N)
Lamin B
100
50
G)
MUP
Casp2-/-/MUP
Membrane
S1P (F)
PDI
WCL
S1P (C)
ERK
100
50
20
37
H)
WT 293
293ΔS2P
SREBP2
Casp2C320G-HA
Casp2-HA
Myc-S1P
Empty
Membrane
SREBP2 (P)
PDI
NE
SREBP2 (N)
PARP
100
75
50