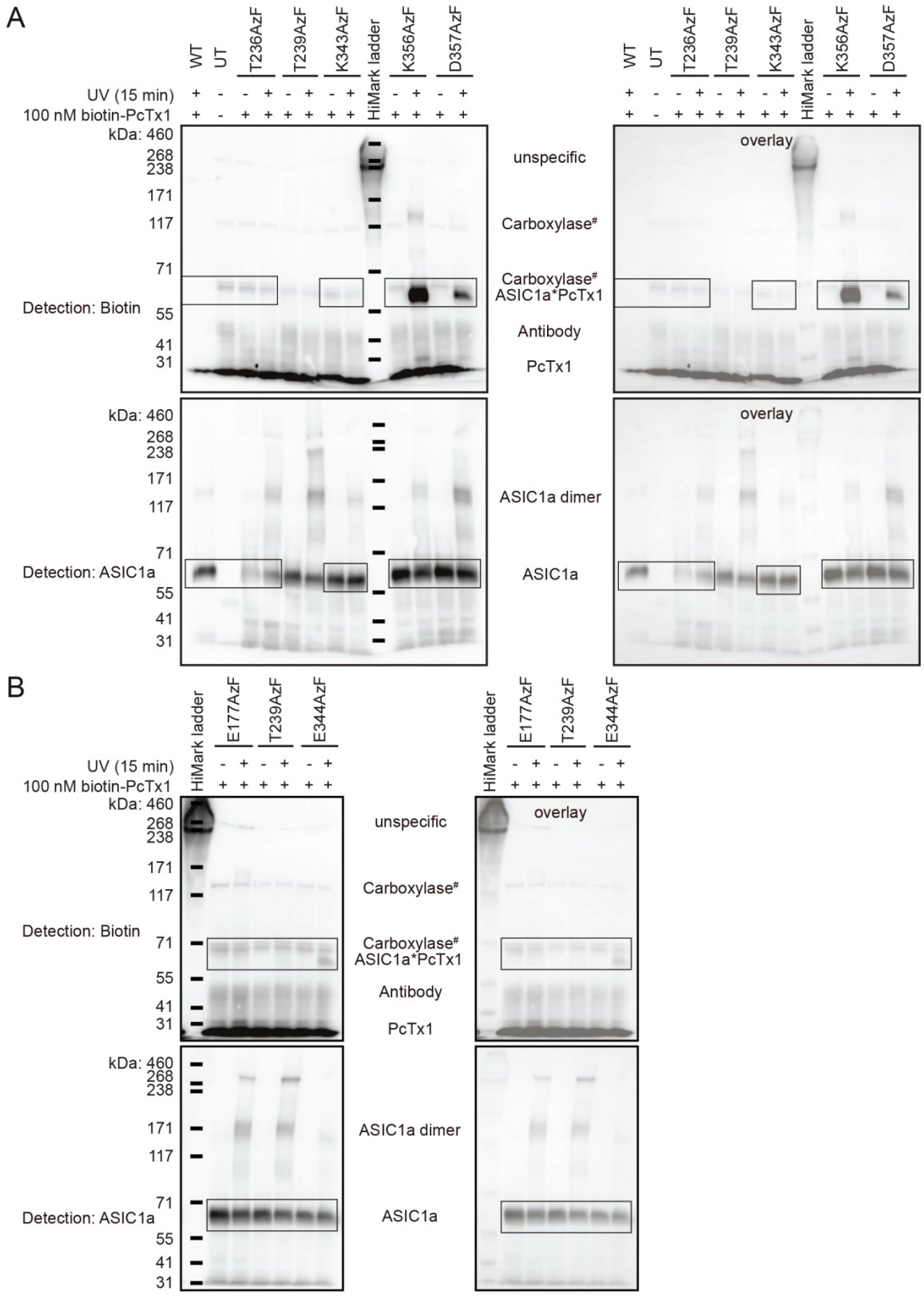
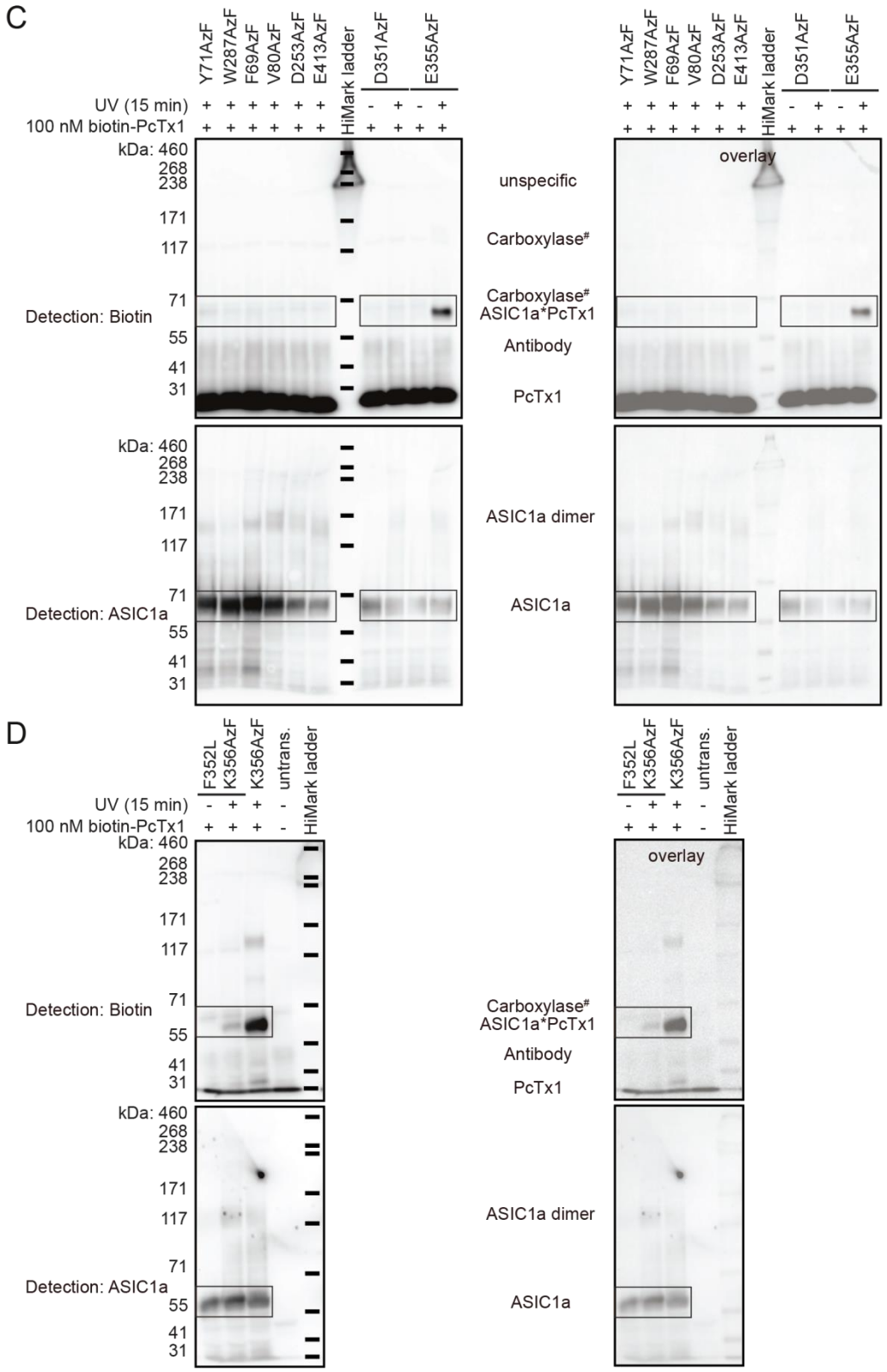
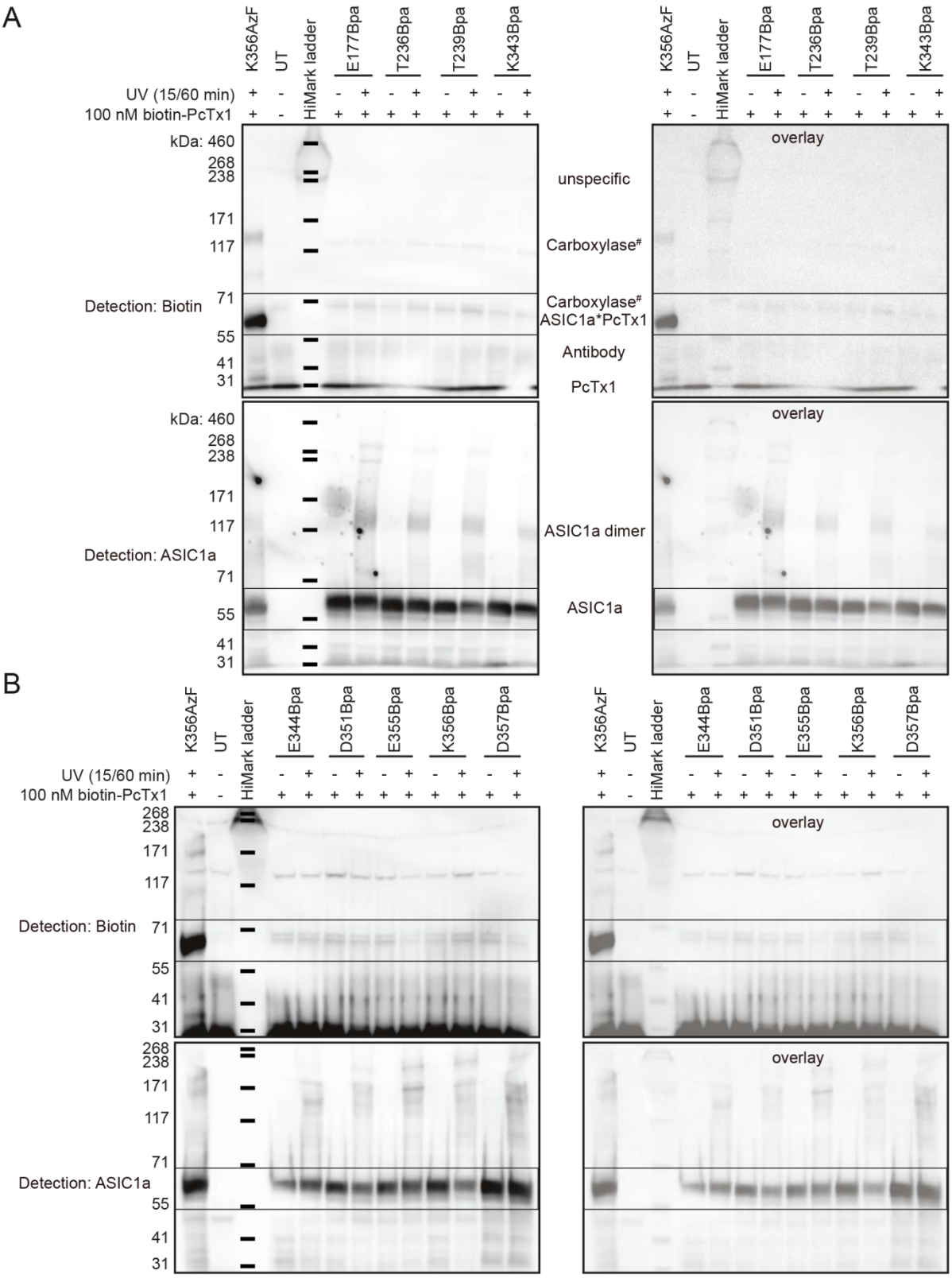
Figure S14 (next two pages): Original Western blots for AzF crosslinking. Black bars indicate protein ladders for clarity (left panel), original markers (coomassie) are overlayed with the blot (chemiluminescence, right panel). Areas cropped for Figure 5 are marked with boxes. (A) Western blot for positions 236, 239, 343, 356 and 357. (B) Western blot for positions 177, 239 and 344. (C) Western blot for positions 71, 287, 69, 80, 253, 413, 351 and 355. (D) Western blot for the F352L K356AzF double mutant. Data is representative of 2-3 individual experiments. Control experiments demonstrating efficient AzF incorporation for all above positions are published in [6].


A
WT
UT
T236AzF
T239AzF
K343AzF
HiMark ladder
K356AzF
D357AzF
UV (15 min)
100 nM biotin-PcTx1
kDa: 460
268
238
171
117
71
55
41
31
Detection: Biotin
Detection: ASIC1a
unspecific
Carboxylase#
Carboxylase#
ASIC1a*PcTx1
Antibody
PcTx1
ASIC1a dimer
ASIC1a
overlay
B
E177AzF
T239AzF
E344AzF


C
Y71AzF
W287AzF
F69AzF
V80AzF
D253AzF
E413AzF
HiMark ladder
D351AzF
E355AzF
UV (15 min)
100 nM biotin-PcTx1
kDa: 460
268
238
171
117
71
55
41
31
Detection: Biotin
Detection: ASIC1a
overlay
unspecific
Carboxylase#
Carboxylase#
ASIC1a*PcTx1
Antibody
PcTx1
ASIC1a dimer
ASIC1a
D
F352L
K356AzF
K356AzF
untrans.
HiMark ladder
UV (15 min)
100 nM biotin-PcTx1
kDa: 460
268
238
171
117
71
55
41
31
Detection: Biotin
Detection: ASIC1a
overlay
Carboxylase#
ASIC1a*PcTx1
Antibody
PcTx1
ASIC1a dimer
ASIC1a


A
K356AzF
UT
HiMark ladder
E177Bpa
T236Bpa
T239Bpa
K343Bpa
UV (15/60 min)
100 nM biotin-PcTx1
kDa: 460
268
238
171
117
71
55
41
31
Detection: Biotin
Detection: ASIC1a
overlay
unspecific
Carboxylase#
Carboxylase#
ASIC1a*PcTx1
Antibody
PcTx1
ASIC1a dimer
ASIC1a
B
K356AzF
UT
HiMark ladder
E344Bpa
D351Bpa
E355Bpa
K356Bpa
D357Bpa
UV (15/60 min)
100 nM biotin-PcTx1
268
238
171
117
71
55
41
31
Detection: Biotin
Detection: ASIC1a
overlay

C

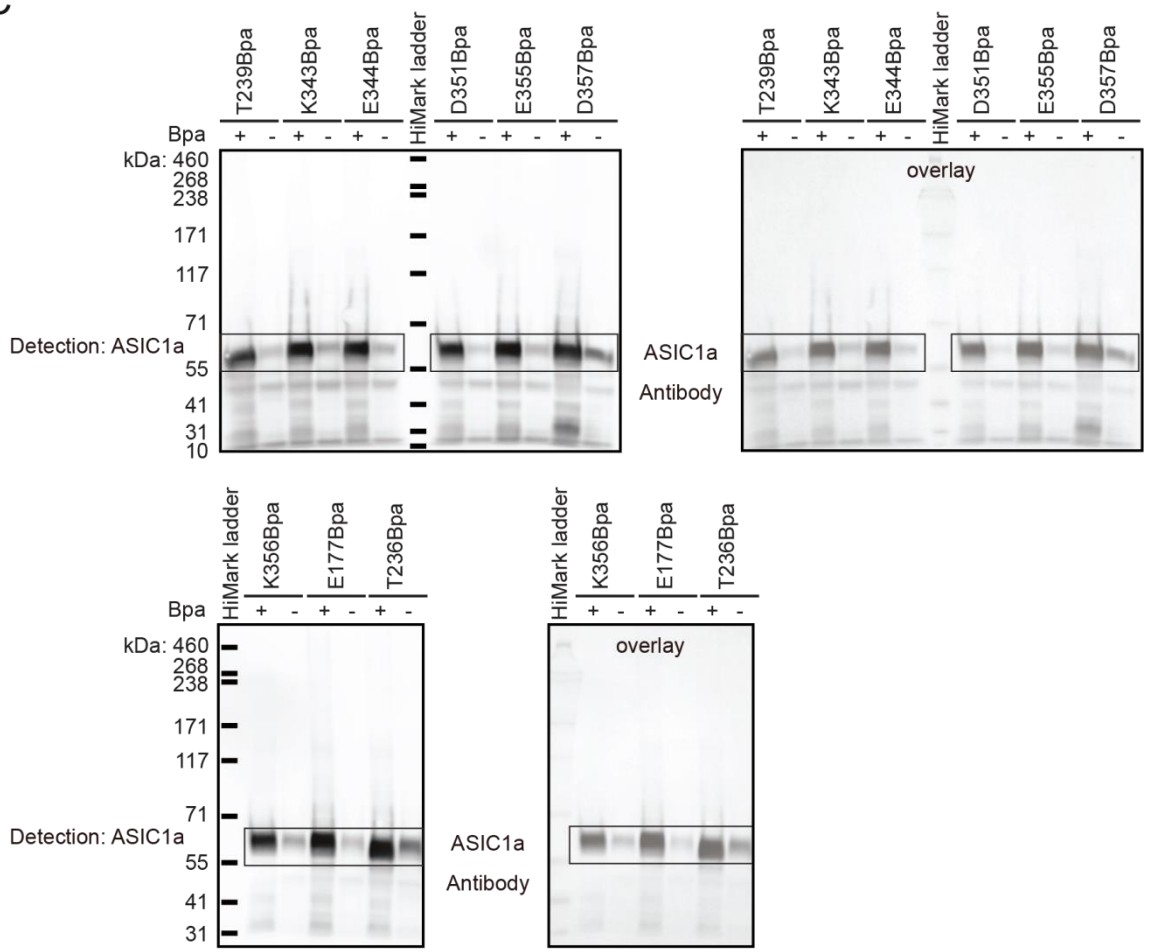


Figure S15: Original Western blots for Bpa crosslinking. Black bars indicate protein ladders for clarity (left panel), original markers (coomassie) are overlayed with the blot (chemiluminescence, right panel). Areas cropped for Figure S12 are marked with boxes. (A) Western blot for positions 177, 236, 239 and 343, including K356AzF as a positive control. (B) Western blot for positions 344, 351, 355, 356 and 357, including K356AzF as a positive control. (C) Western blot demonstrating efficient Bpa incorporation at positions 239, 343, 344, 351, 355 and 357 (upper panel) and at positions 177, 236 and 356 (lower panel). Data is representative of 2-3 individual experiments.