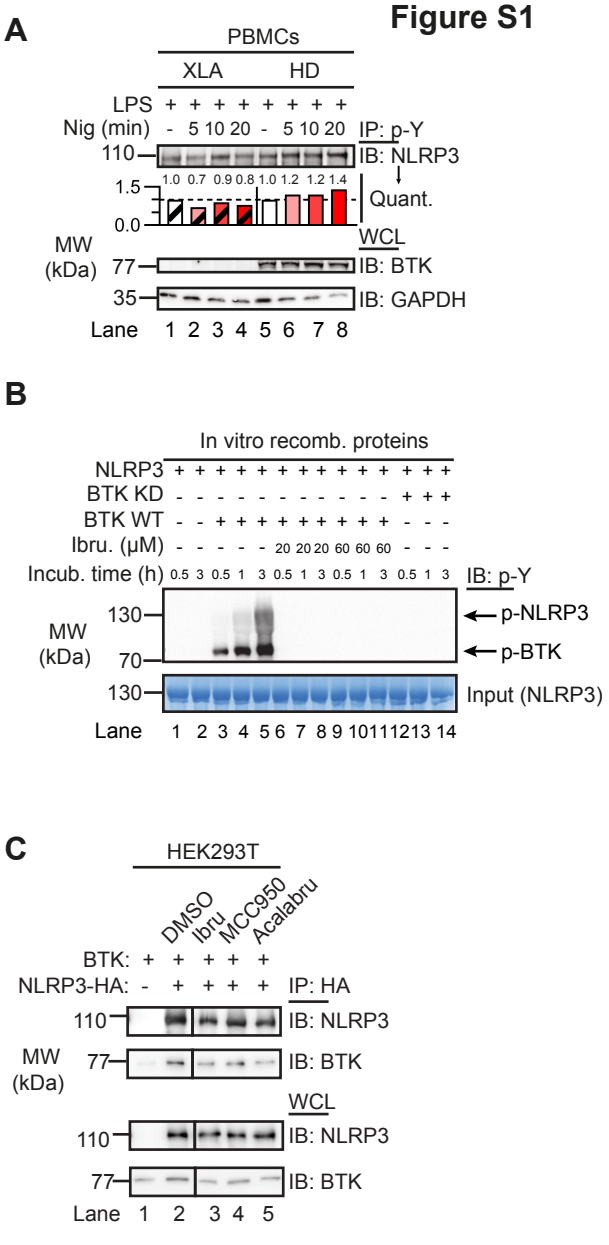
Figure S1


A
PBMCs
XLA
HD
LPS + + + + + + + +
Nig (min) - 5 10 20 - 5 10 20
IP: p-Y
110
IB: NLRP3
1.5
0.0
1.0 0.7 0.9 0.8 1.0 1.2 1.2 1.4
Quant.
WCL
MW (kDa)
77
IB: BTK
35
IB: GAPDH
Lane 1 2 3 4 5 6 7 8
B
In vitro recomb. proteins
NLRP3 + + + + + + + + + + + + + +
BTK KD - - - - - - - - - - - + + +
BTK WT - - + + + + + + + + + - - -
Ibru. (μM) - - - - - - 20 20 20 60 60 60 - - -
Incub. time (h) 0.5 3 0.5 1 3 0.5 1 3 0.5 1 3 0.5 1 3
IB: p-Y
MW (kDa)
130
70
p-NLRP3
p-BTK
130
Input (NLRP3)
Lane 1 2 3 4 5 6 7 8 9 10 11 12 13 14
C
HEK293T
DMSO
Ibru
MCC950
Acalabru
BTK: + + + + +
NLRP3-HA: - + + + +
IP: HA
110
IB: NLRP3
MW (kDa)
77
IB: BTK
WCL
110
IB: NLRP3
77
IB: BTK
Lane 1 2 3 4 5

Human NLRP3 (Uniprot ID Q96P20)

Figure S2

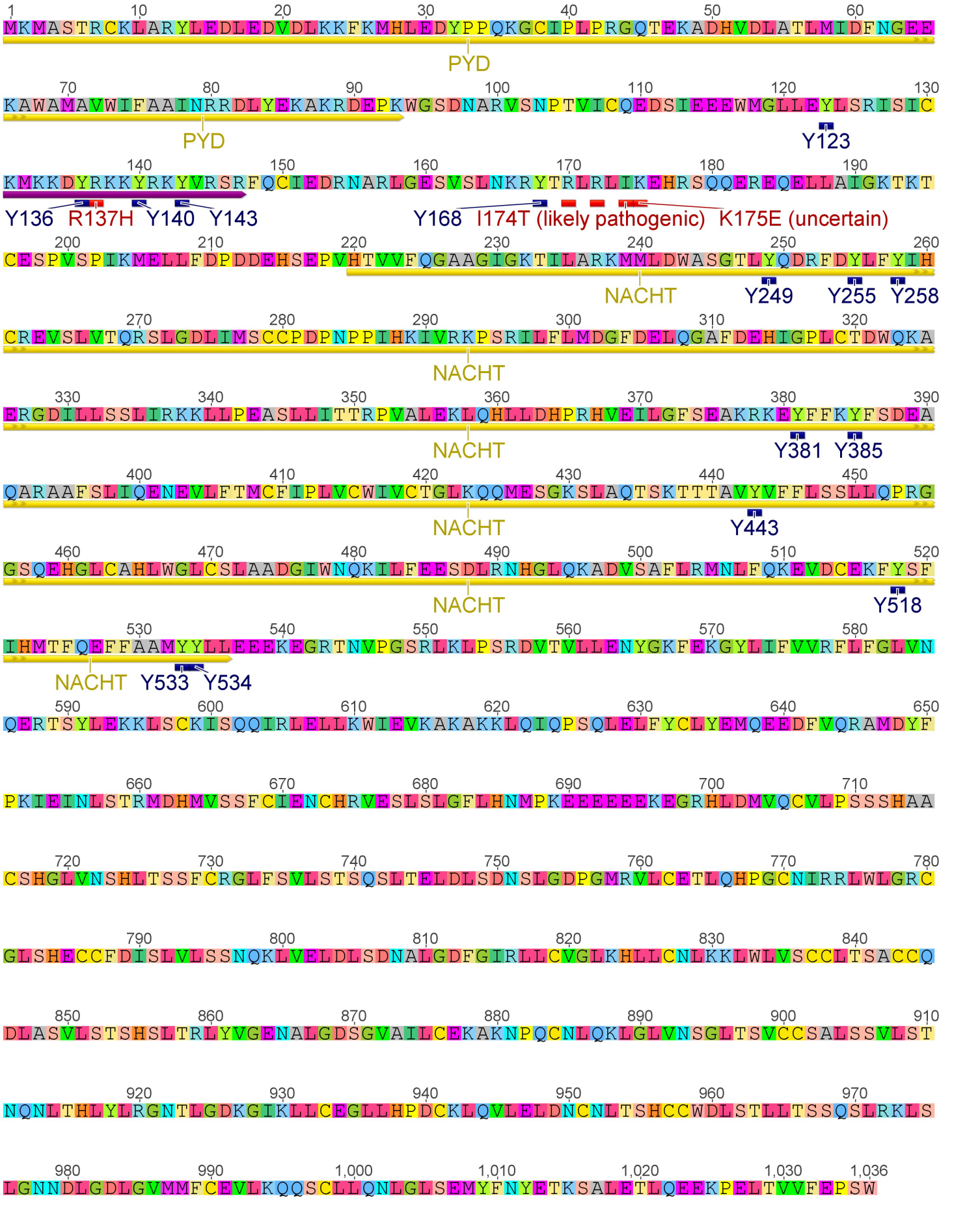


Figure S3

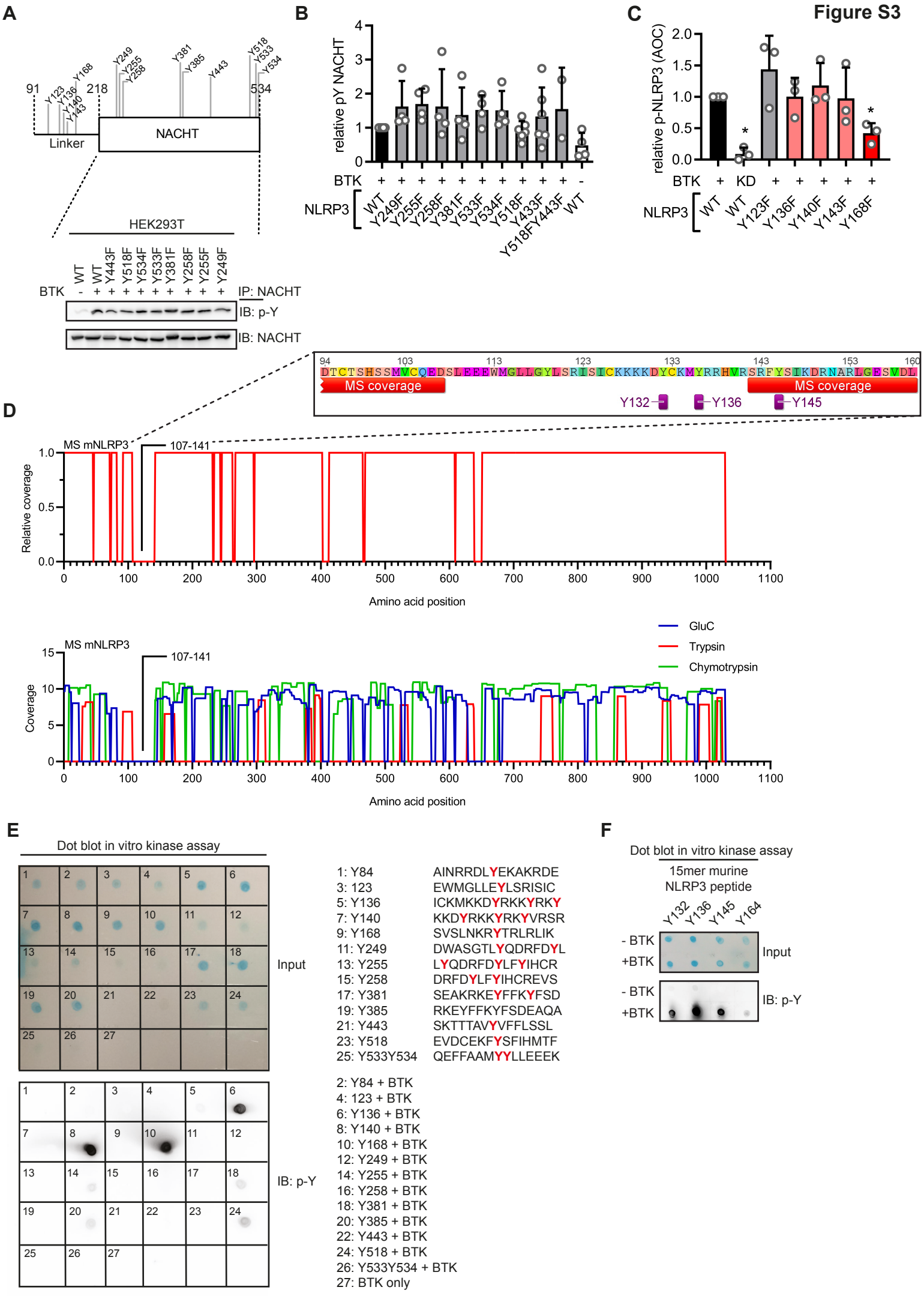
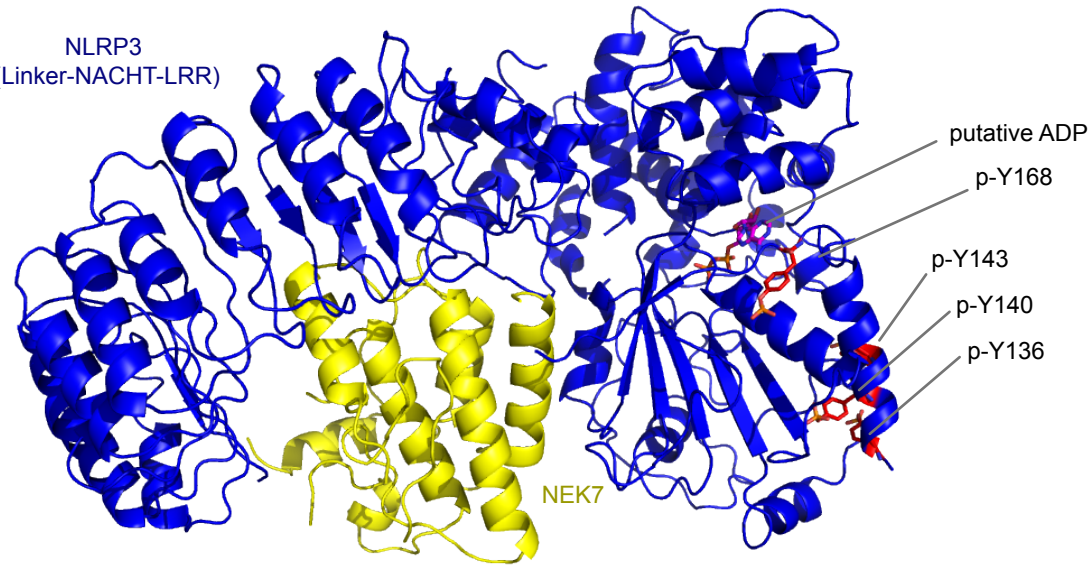

A
91
218
534
Y123
Y136
Y140
Y143
Y168
Y249
Y255
Y258
Y381
Y385
Y443
Y518
Y533
Y534
Linker
NACHT
HEK293T
BTK
WT
WT
Y443F
Y518F
Y534F
Y533F
Y381F
Y258F
Y255F
Y249F
IP: NACHT
IB: p-Y
IB: NACHT
B
relative pY NACHT
BTK
NLRP3
WT
Y249F
Y255F
Y258F
Y381F
Y533F
Y534F
Y518F
Y443F
Y518FY443F
WT
C
relative p-NLRP3 (AOC)
BTK
KD
NLRP3
WT
WT
Y123F
Y136F
Y140F
Y143F
Y168F
D
MS coverage
MS coverage
Y132
Y136
Y145
MS mNLRP3
107-141
Relative coverage
Amino acid position
GluC
Trypsin
Chymotrypsin
Coverage
E
Dot blot in vitro kinase assay
Input
IB: p-Y
1: Y84 AINRRDLYEKAKRDE
3: 123 EWMGLLEYLSRISIC
5: Y136 ICKMKKDYRKKYRKY
7: Y140 KKDYRKKYRKYVRSR
9: Y168 SVSLNKRYTRLRLIK
11: Y249 DWASGTLYQDRFDYL
13: Y255 LYQDRFDYLFYIHCR
15: Y258 DRFDYLFYIHCREVS
17: Y381 SEAKRKEYFFKYFSD
19: Y385 RKEYFFKYFSDEAQA
21: Y443 SKTTTAVYVFFLSSL
23: Y518 EVDCEKFYSFIHMTF
25: Y533Y534 QEFFAAMYYLLEEEK
2: Y84 + BTK
4: 123 + BTK
6: Y136 + BTK
8: Y140 + BTK
10: Y168 + BTK
12: Y249 + BTK
14: Y255 + BTK
16: Y258 + BTK
18: Y381 + BTK
20: Y385 + BTK
22: Y443 + BTK
24: Y518 + BTK
26: Y533Y534 + BTK
27: BTK only
F
Dot blot in vitro kinase assay
15mer murine
NLRP3 peptide
Y132
Y136
Y145
Y164
- BTK
+BTK
Input
- BTK
+BTK
IB: p-Y

Figure S4

A


NLRP3
(Linker-NACHT-LRR)
putative ADP
p-Y168
p-Y143
p-Y140
p-Y136
NEK7

B

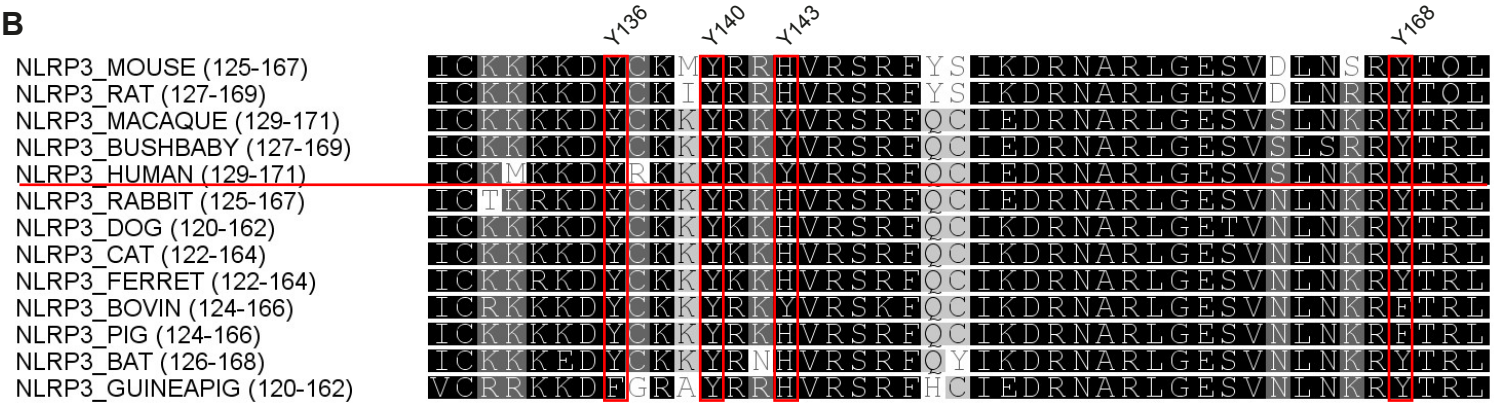
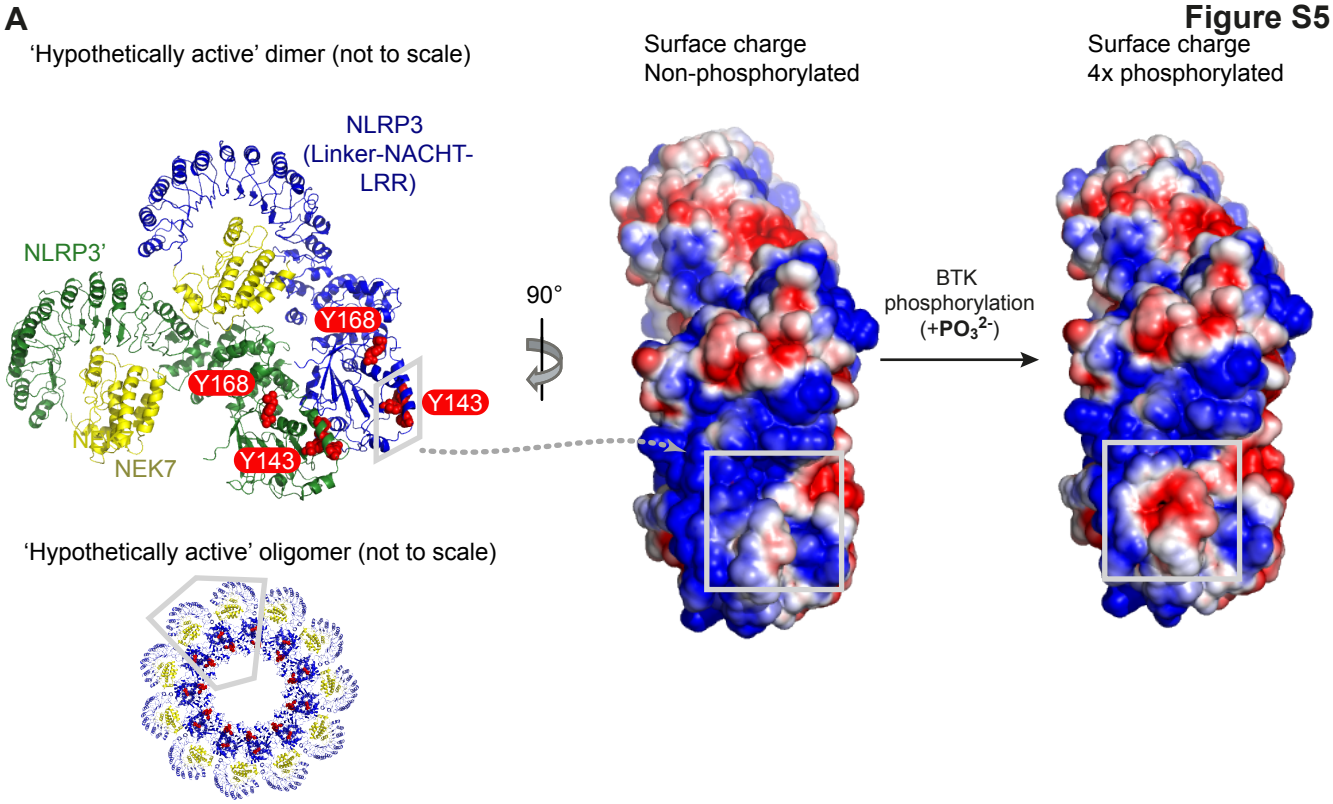
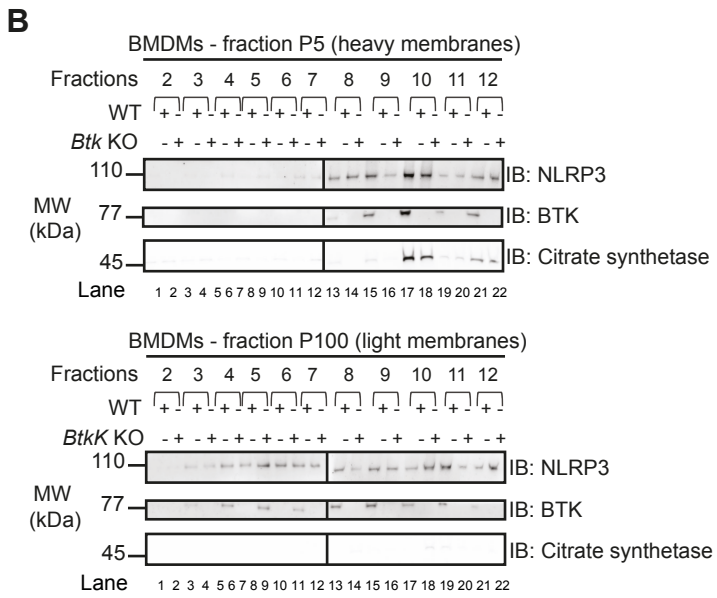

Y136
Y140
Y143
Y168
NLRP3_MOUSE (125-167)
NLRP3_RAT (127-169)
NLRP3_MACAQUE (129-171)
NLRP3_BUSHBABY (127-169)
NLRP3_HUMAN (129-171)
NLRP3_RABBIT (125-167)
NLRP3_DOG (120-162)
NLRP3_CAT (122-164)
NLRP3_FERRET (122-164)
NLRP3_BOVIN (124-166)
NLRP3_PIG (124-166)
NLRP3_BAT (126-168)
NLRP3_GUINEAPIG (120-162)

Figure S5


A
'Hypothetically active' dimer (not to scale)
NLRP3
(Linker-NACHT-
LRR)
NLRP3'
Y168
Y168
Y143
Y143
NEK7
90°
Surface charge
Non-phosphorylated
BTK
phosphorylation
(+PO3 2-)
Surface charge
4x phosphorylated
'Hypothetically active' oligomer (not to scale)


B
BMDMs - fraction P5 (heavy membranes)
Fractions 2 3 4 5 6 7 8 9 10 11 12
WT
Btk KO
110
77
45
MW (kDa)
IB: NLRP3
IB: BTK
IB: Citrate synthetase
Lane 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
BMDMs - fraction P100 (light membranes)
Fractions 2 3 4 5 6 7 8 9 10 11 12
WT
BtkK KO
110
77
45
MW (kDa)
IB: NLRP3
IB: BTK
IB: Citrate synthetase
Lane 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

Graphical abstract

Figure S6