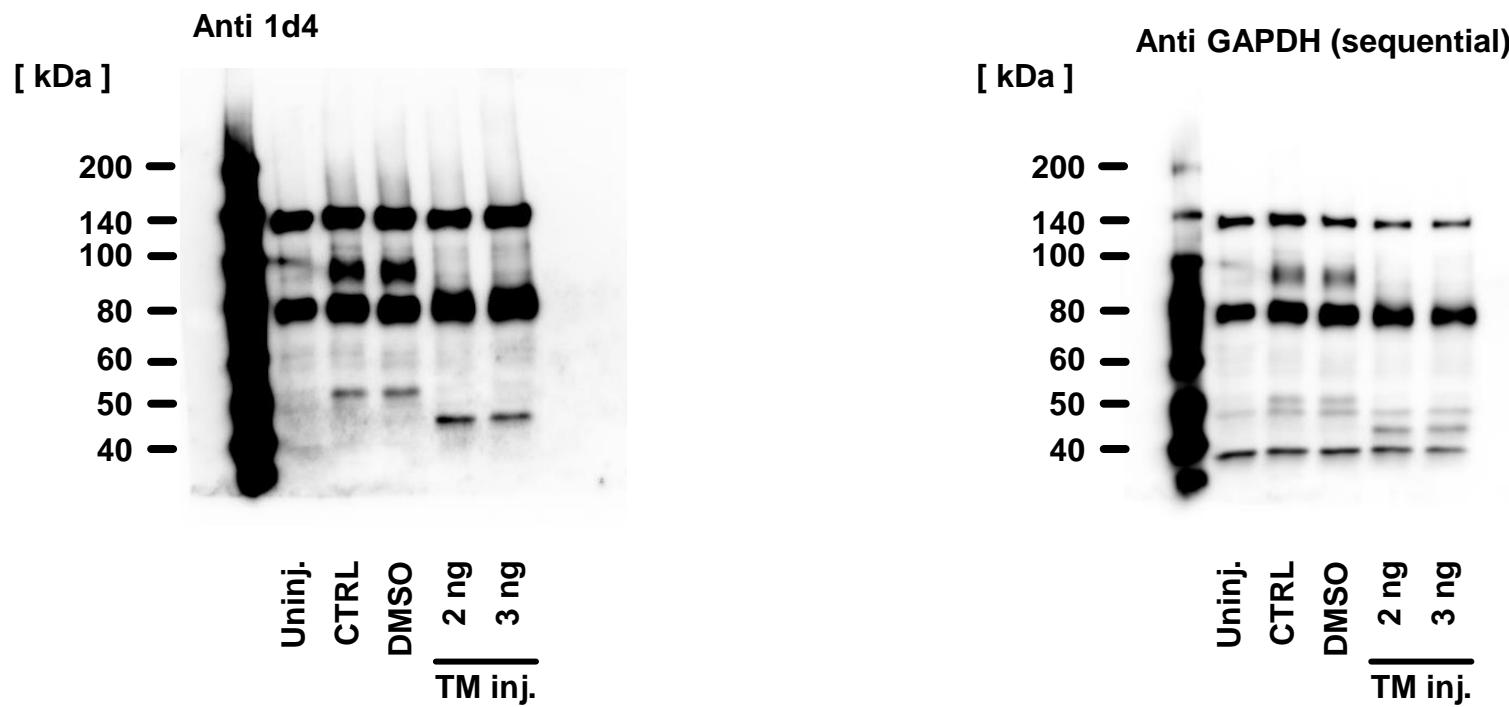


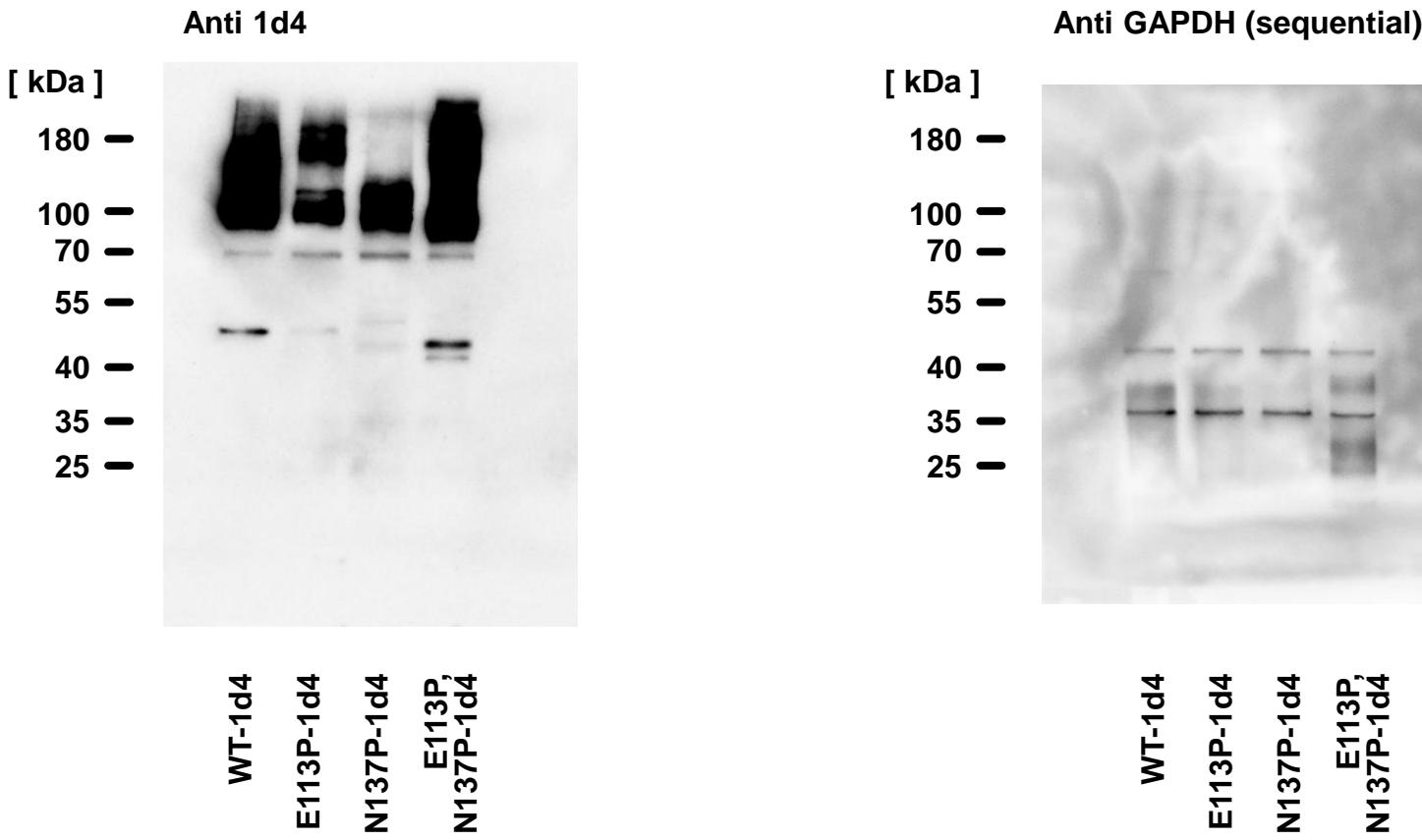
Supplementary figure 1

Western blot: Tunicamycin treatment of TREK-1, expressed in *Xenopus* oocytes:
(from figure 2a)



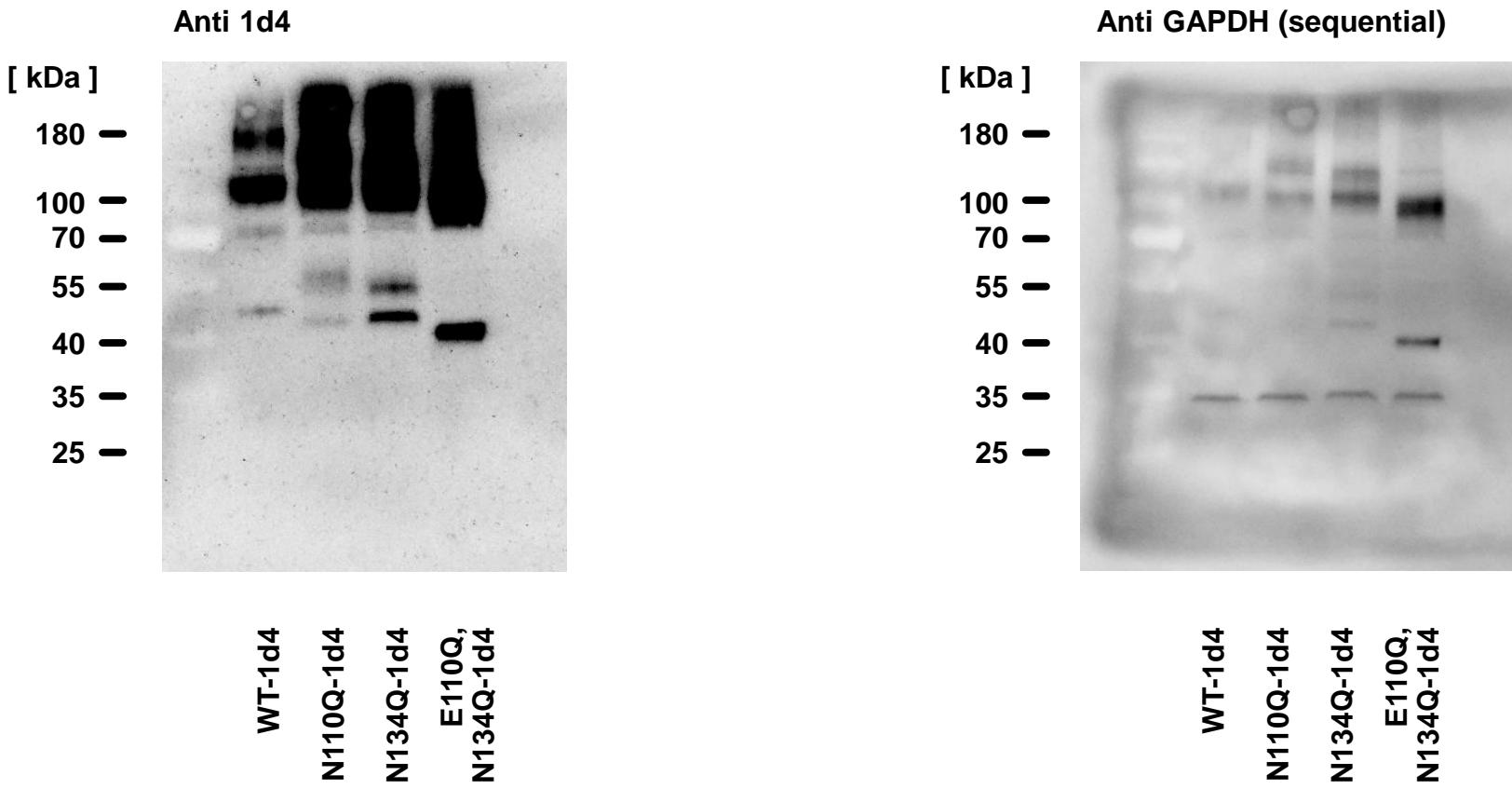
Supplementary figure 2

Western blot: Glycosylation deficient TREK-1 mutants, expressed in *Xenopus* oocytes:
(from figure 3a)



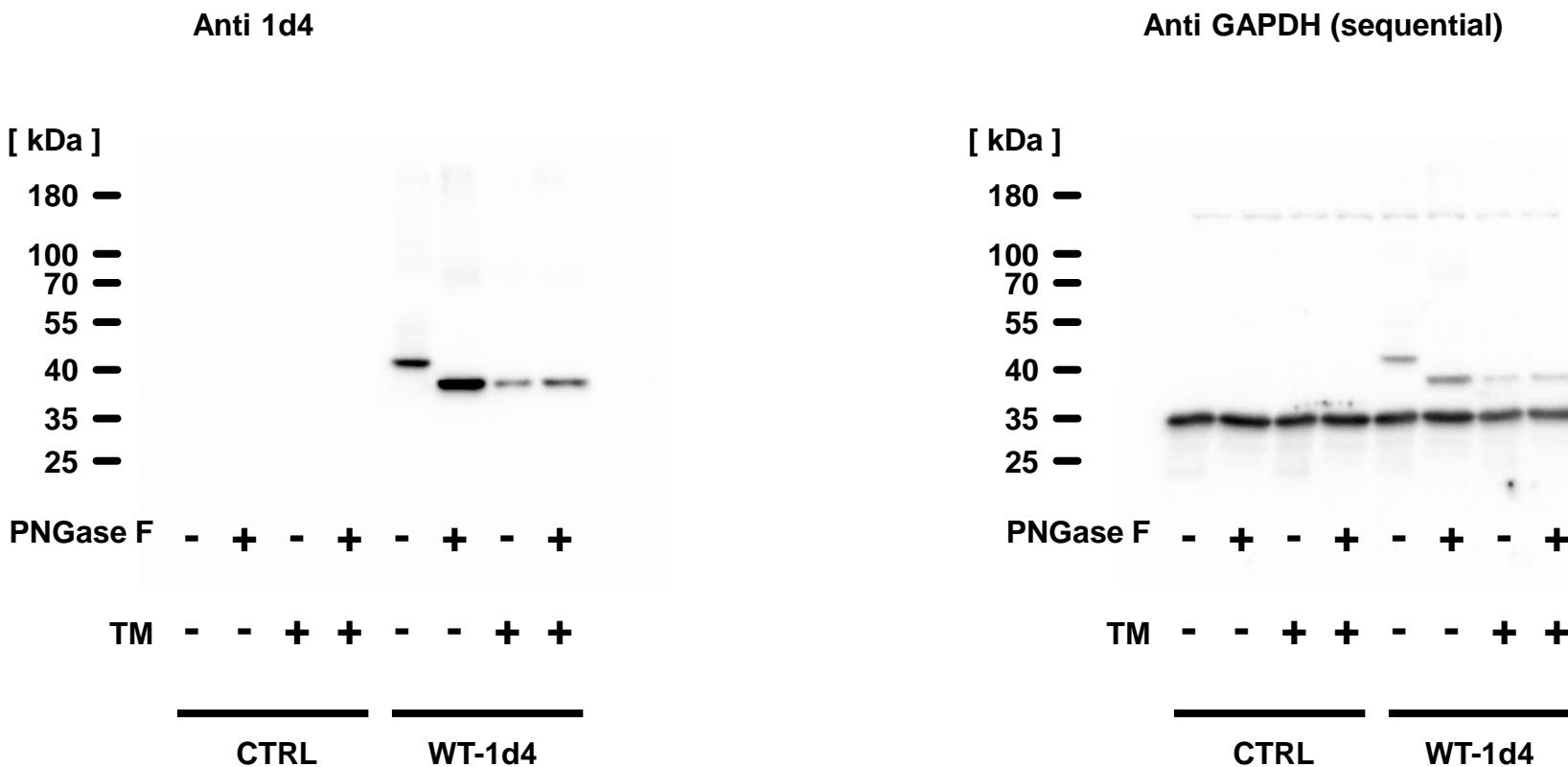
Supplementary figure 3

Western blot: Glycosylation deficient TREK-1 mutants, expressed in *Xenopus* oocytes:
(from figure 3b)



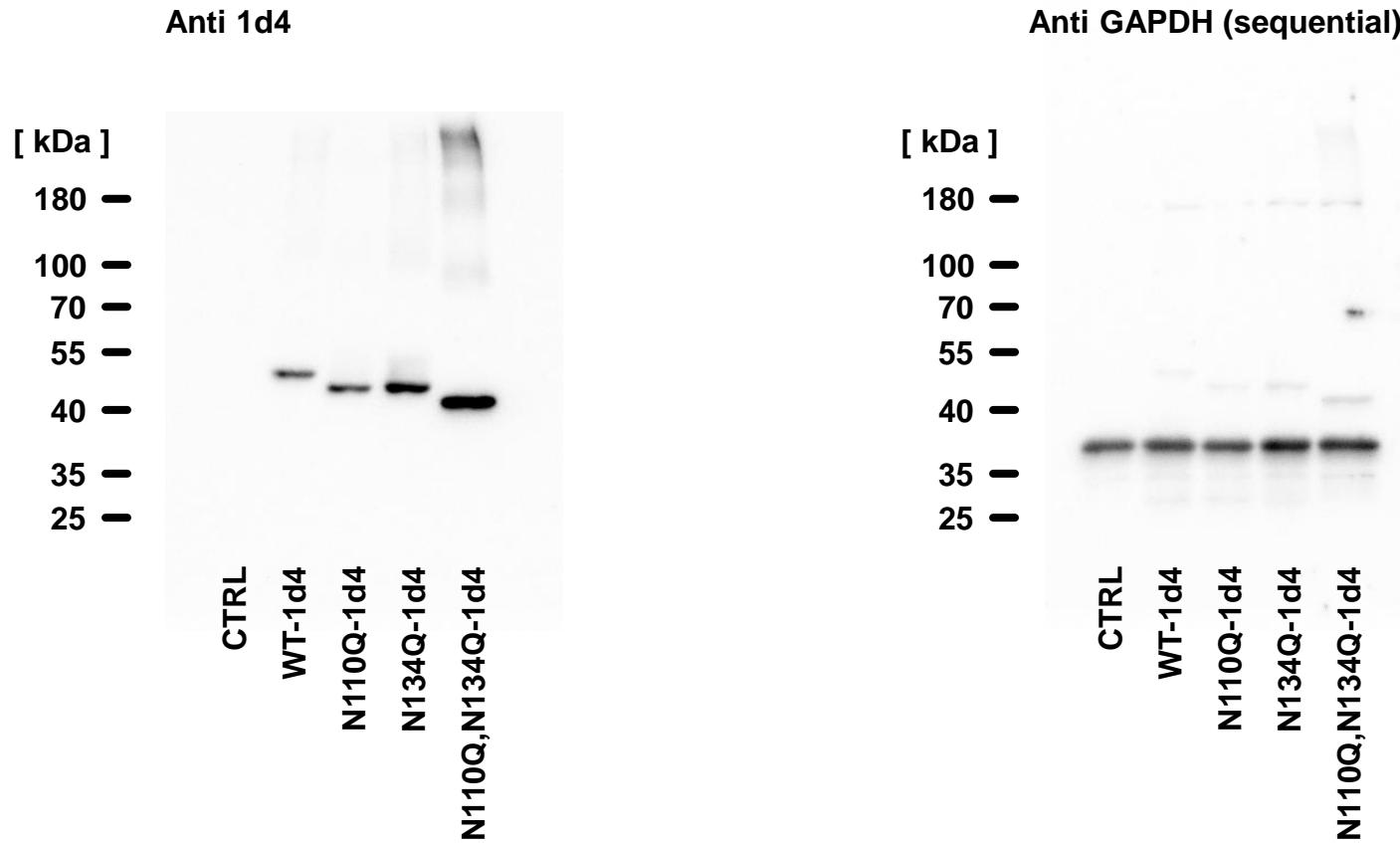
Supplementary figure 4

Western blot: PNGase F and tunicamycin treatment of TREK-1, expressed in HEK 293T cells:
(from figure 4a)



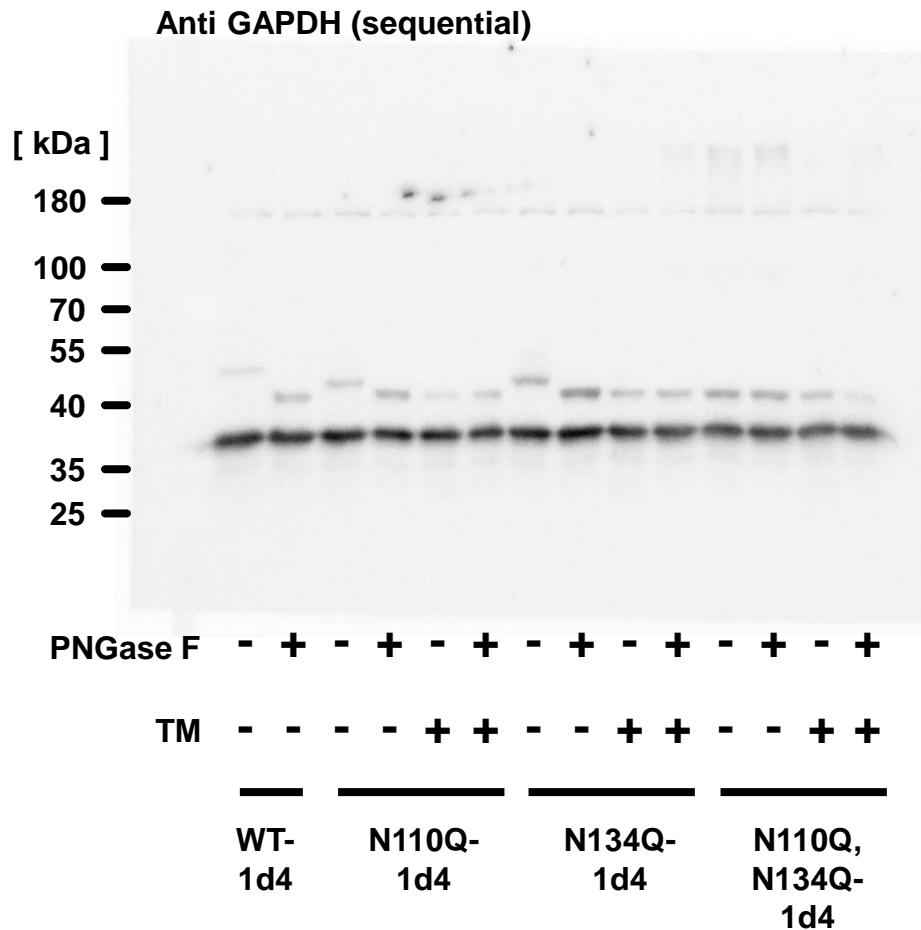
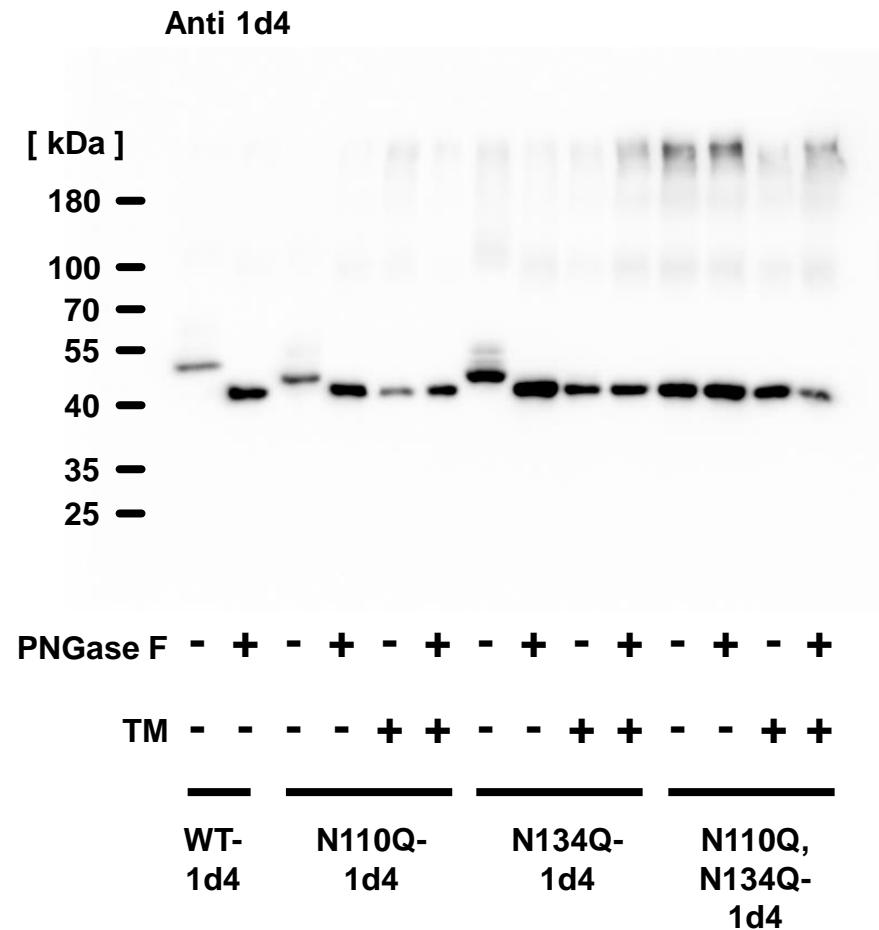
Supplementary figure 5

Western blot: Glycosylation deficient TREK-1 mutants, expressed in HEK 293T cells:
(from figure 4b)



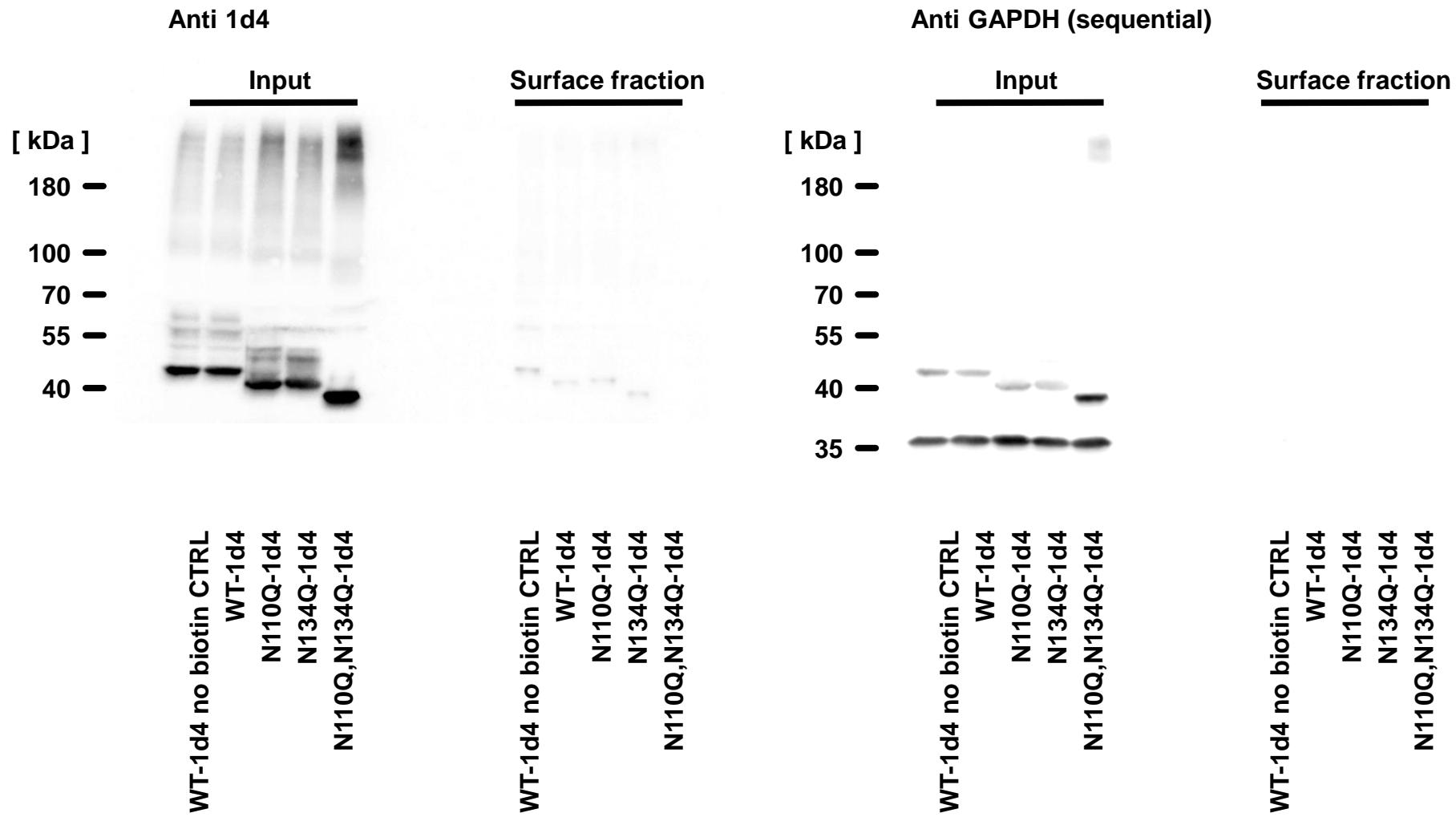
Supplementary figure 6

**Western blot: PNGase F and tunicamycin treatment of glycosylation deficient TREK-1 mutants:
(from figure 4c)**



Supplementary figure 7

Western blot membrane 1: Surface expression (from figure 5b):



Supplementary figure 8

Western blot membrane 2: Surface expression (from figure 5b):

Anti 1d4



Input

Surface fraction

Anti GAPDH (sequential)

[kDa]

180

100

70

55

40

35

Input

Surface fraction

WT-1d4 no biotin CTRL
WT-1d4
N110Q-1d4
N134Q-1d4
N110Q,N134Q-1d4

Supplementary figure 9

Western blot membrane 3: Surface expression (from figure 5b):

Anti 1d4



Input

Surface fraction

Anti GAPDH (sequential)

[kDa]

180 —

100 —

70 —

55 —

40 —

35 —

Input

Surface fraction

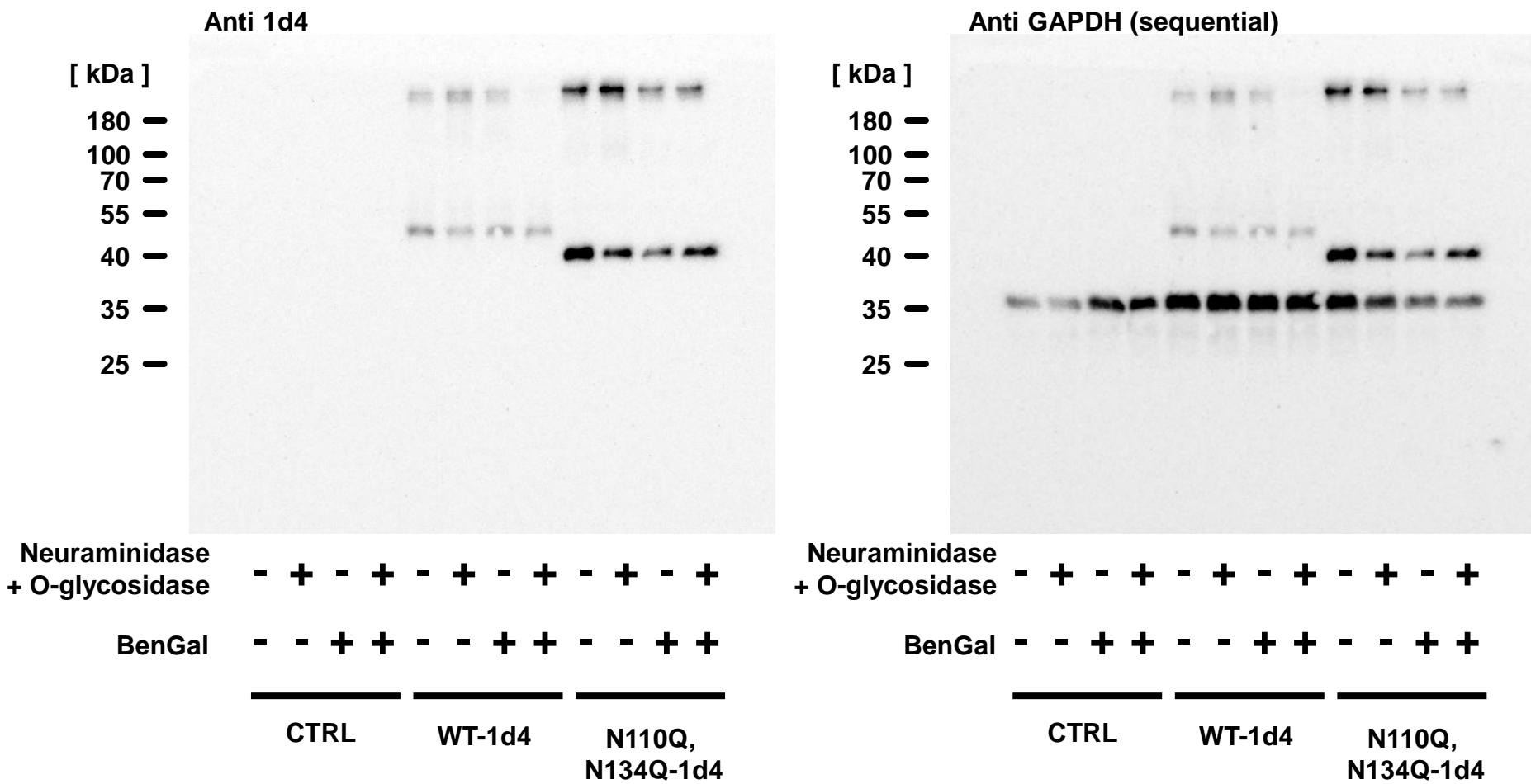
WT-1d4 no biotin CTRL
WT-1d4
N110Q-1d4
N134Q-1d4
N110Q,N134Q-1d4

WT-1d4 no biotin CTRL
WT-1d4
N110Q-1d4
N134Q-1d4
N110Q,N134Q-1d4

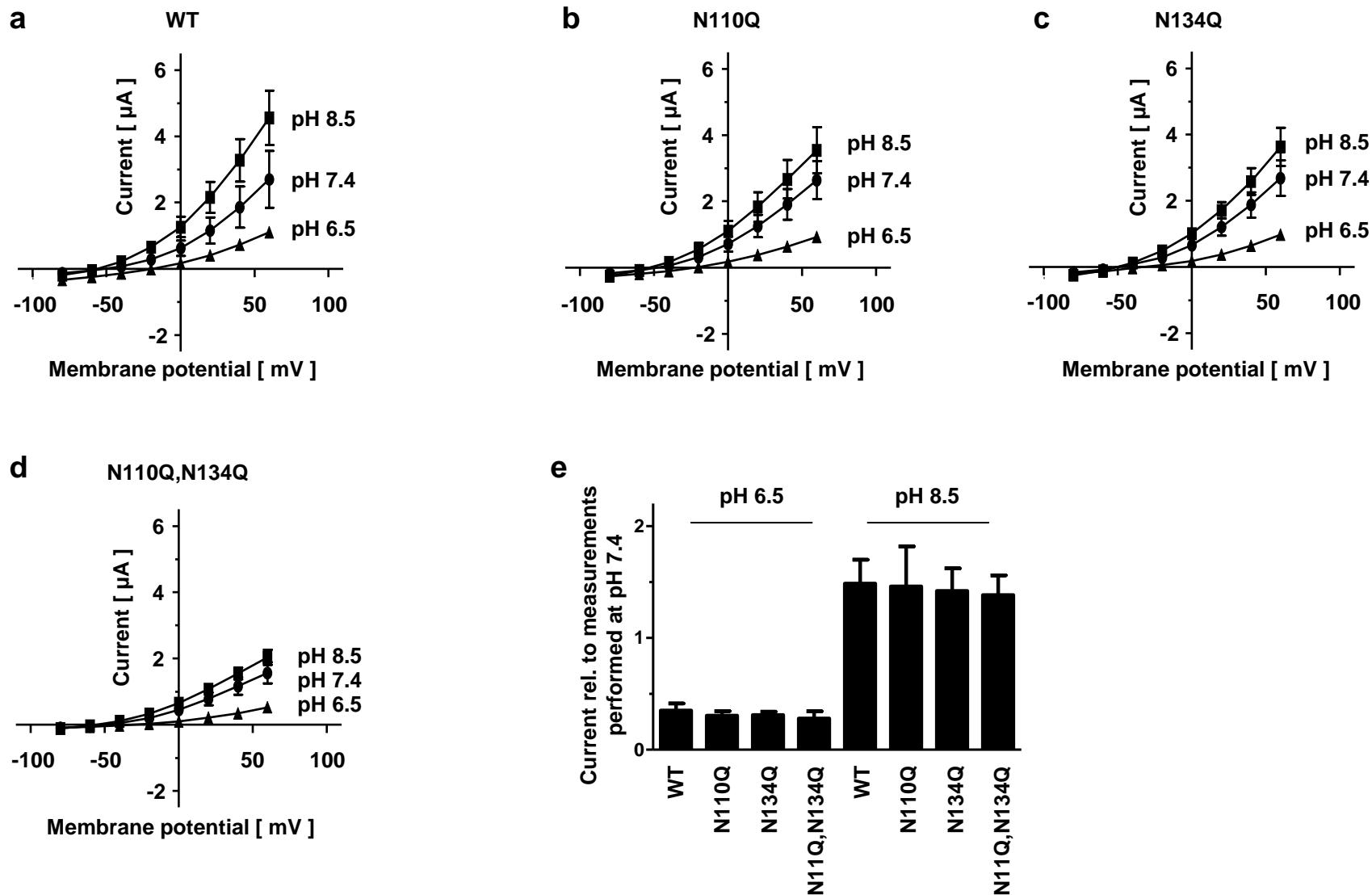
WT-1d4 no biotin CTRL
WT-1d4
N110Q-1d4
N134Q-1d4
N110Q,N134Q-1d4

Supplementary figure 10

Western blot membrane: O-glycosylation of TREK-1 (from figure 6)

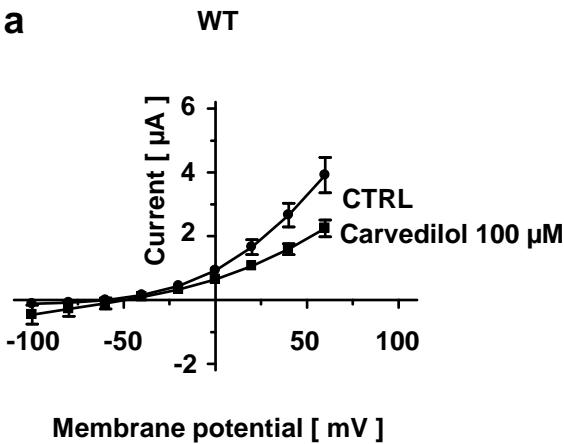


Supplementary figure 11

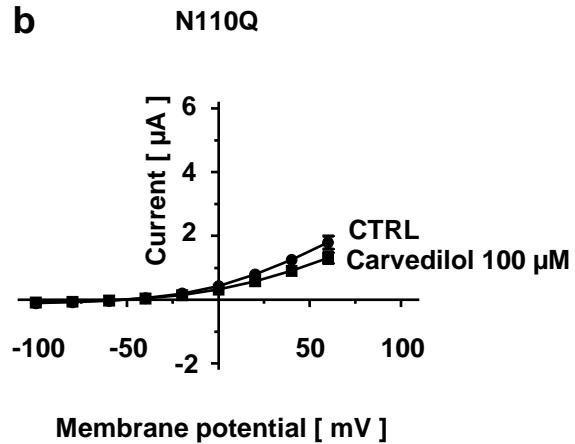


Supplementary figure 12

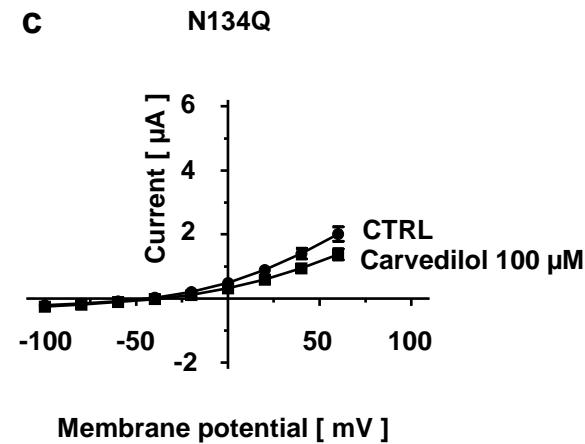
a



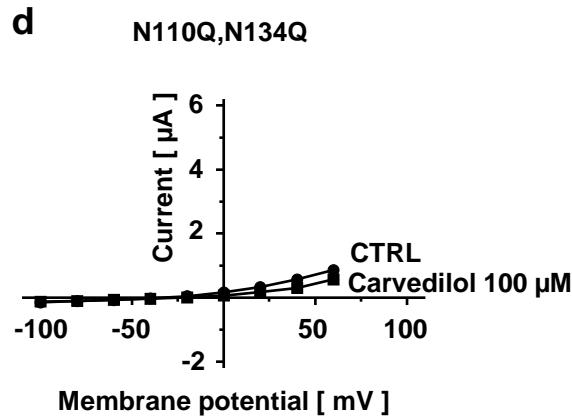
b



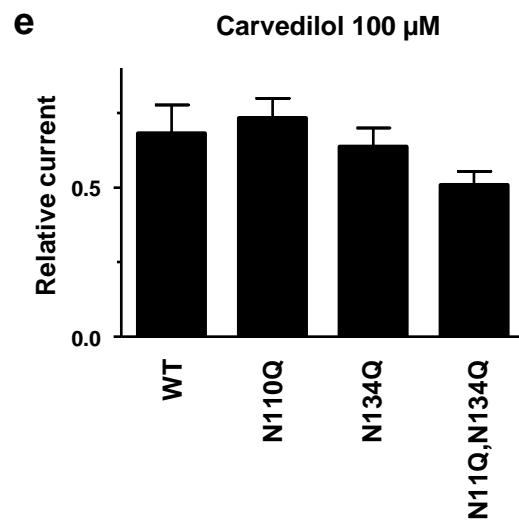
c



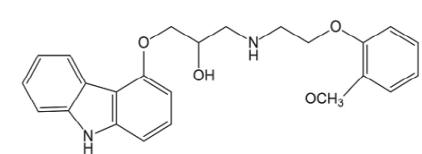
d



e

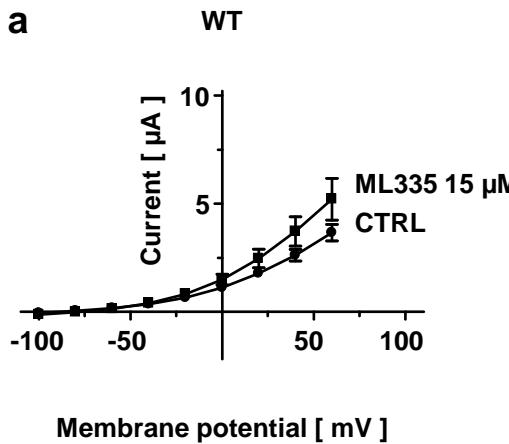


f

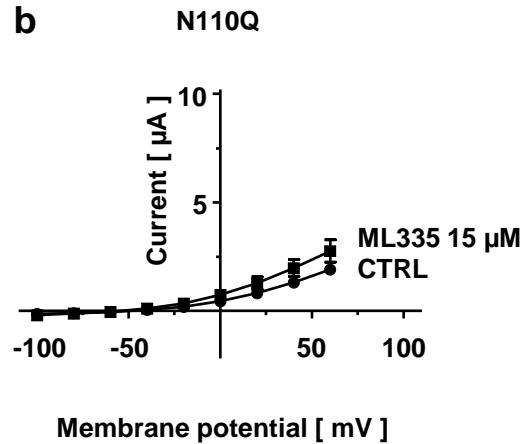


Supplementary figure 13

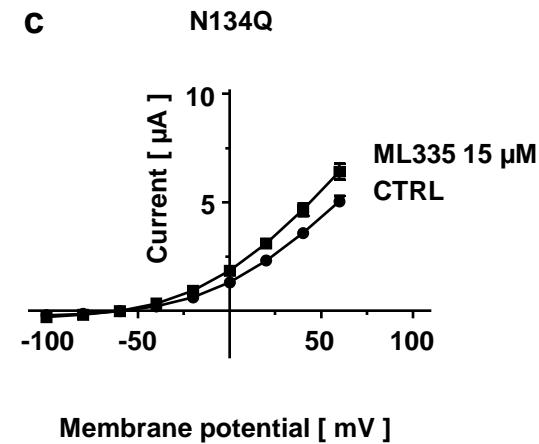
a



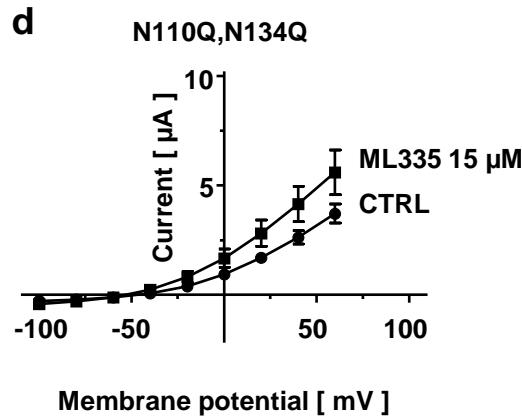
b



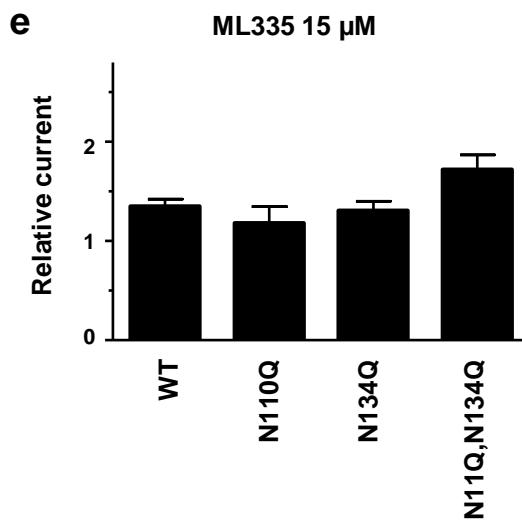
c



d



e



f

