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Supplemental information

The molecular basis of pH sensing by the human fungal pathogen *Candida albicans* TOK potassium channel

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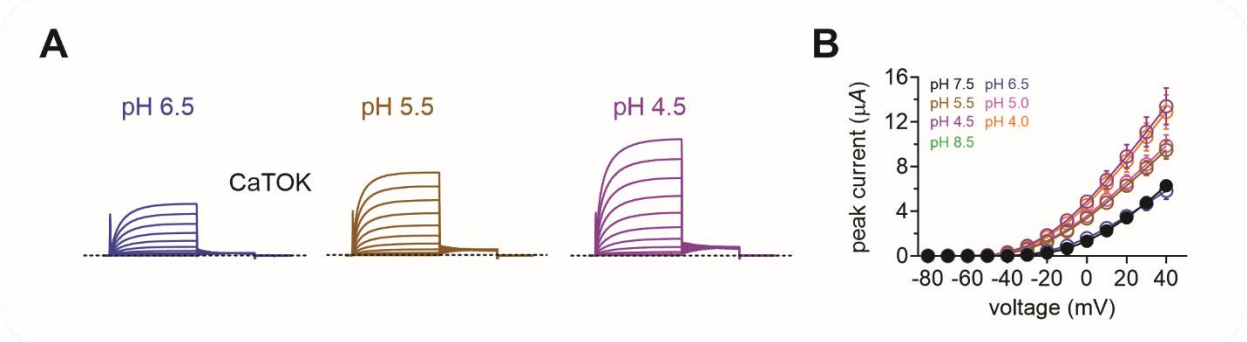
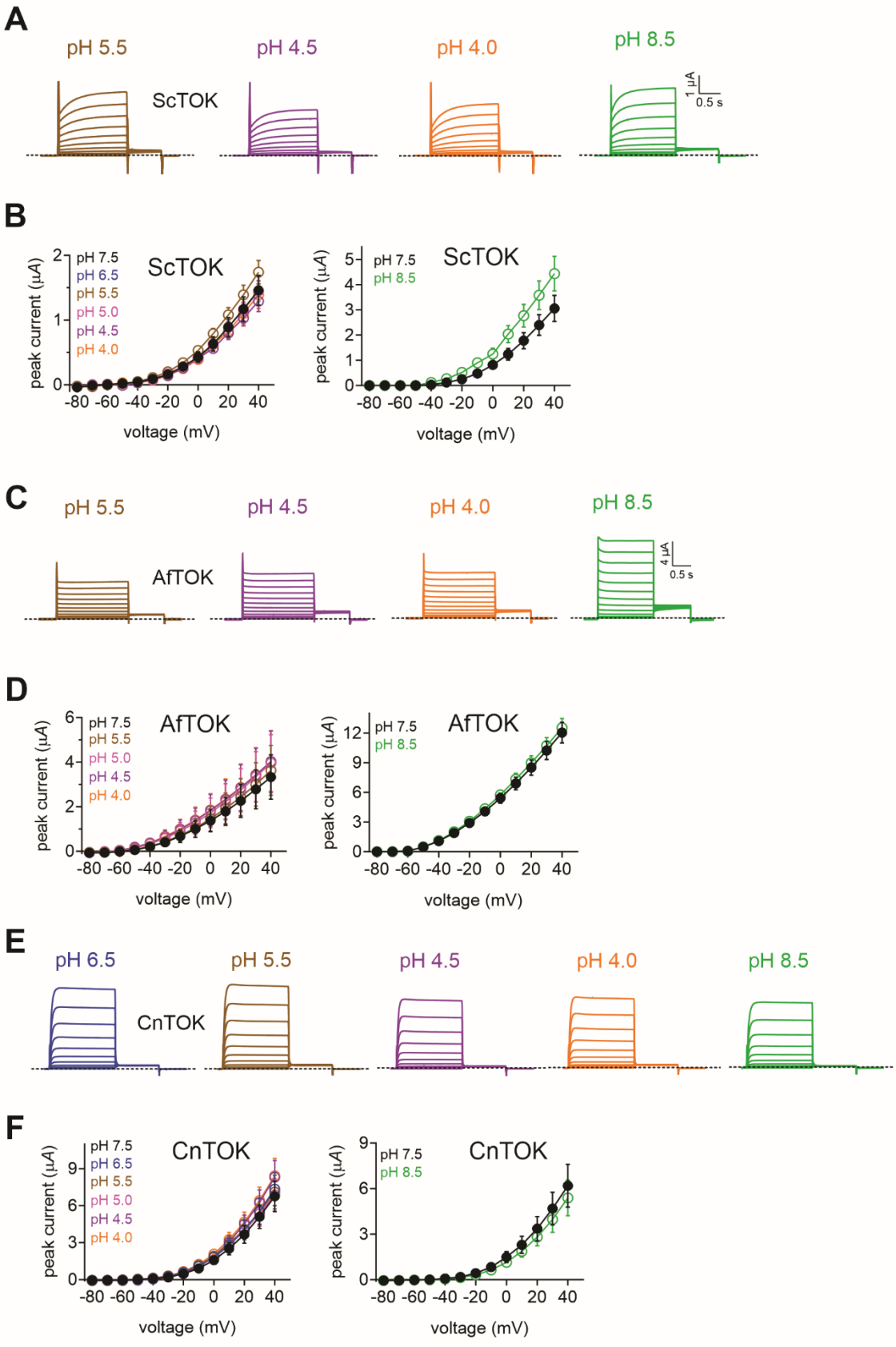


Supplementary Figure 1. CaTOK channels are activated by extracellular acidification.

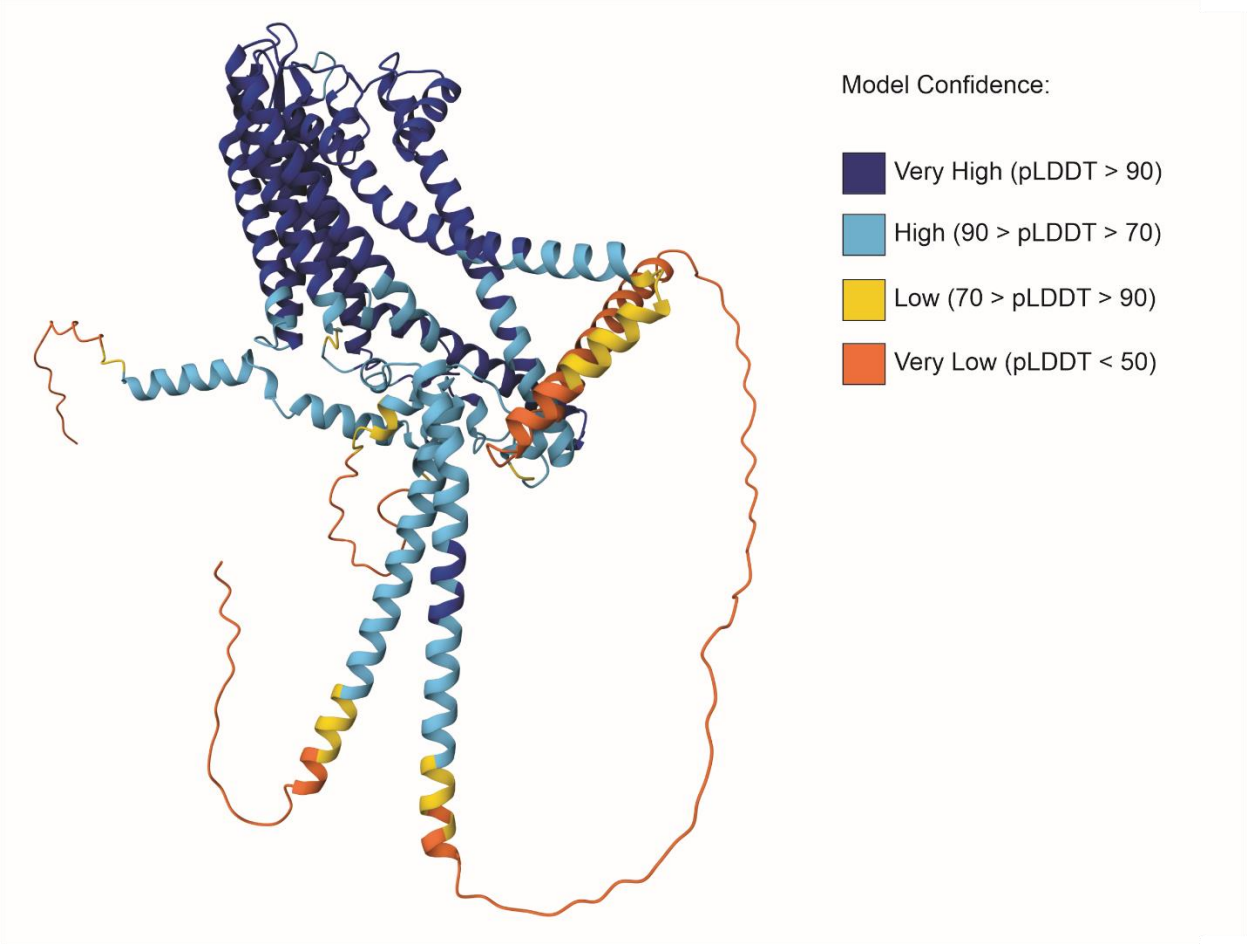
A. Averaged traces for CaTOK channels as indicated, expressed in oocytes in the presence of pH 8.5 – 4.0. Scale bar lower left; *n* = 8-26 per group.

B. Mean peak current (measured during prepulse) from traces as in A; *n* = 8-26 per group.

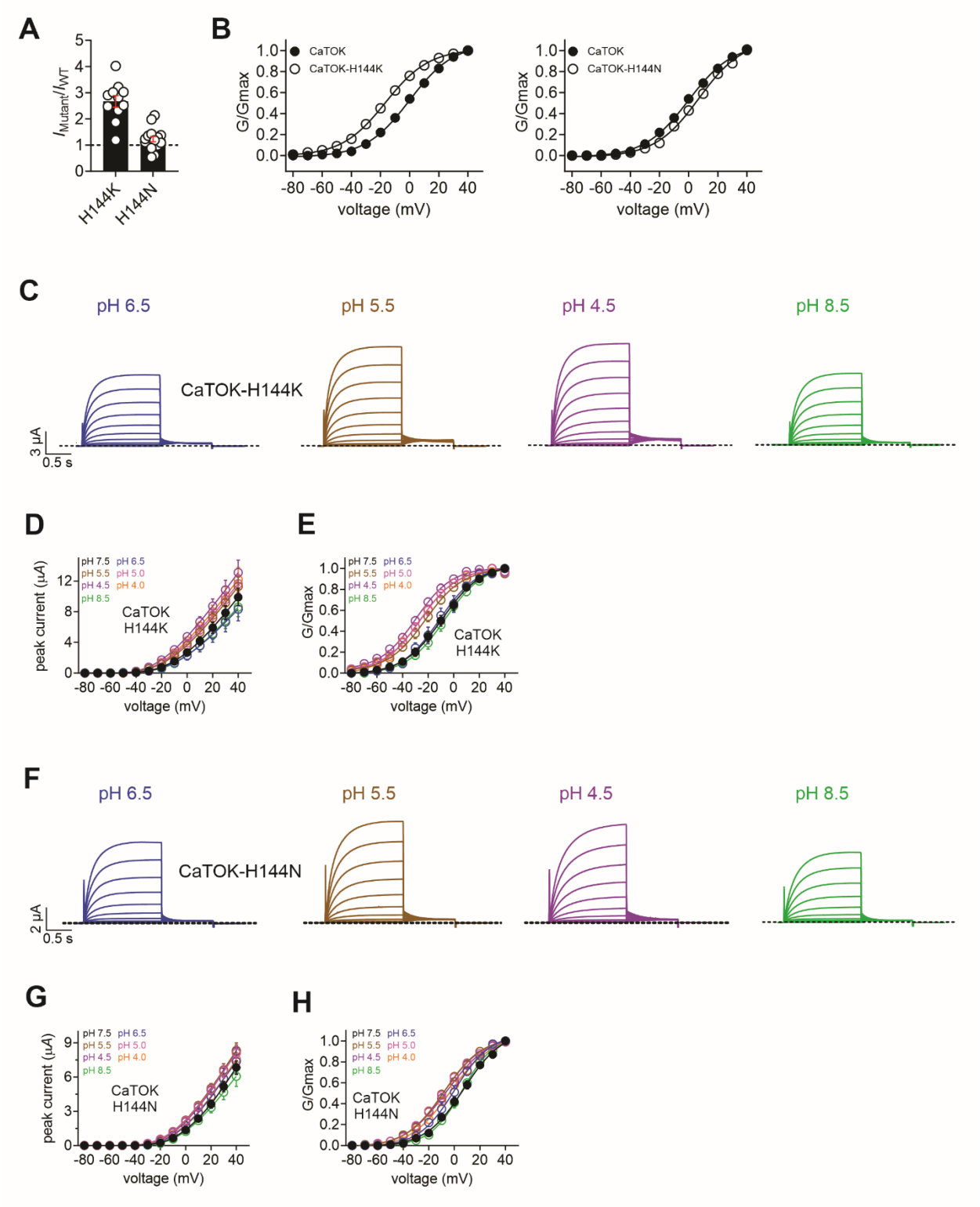

A
pH 5.5
pH 4.5
pH 4.0
pH 8.5
ScTOK
1 µA
0.5 s
B
ScTOK
pH 7.5
pH 6.5
pH 5.5
pH 5.0
pH 4.5
pH 4.0
peak current (µA)
voltage (mV)
ScTOK
pH 7.5
pH 8.5
peak current (µA)
voltage (mV)
C
pH 5.5
pH 4.5
pH 4.0
pH 8.5
AfTOK
4 µA
0.5 s
D
AfTOK
pH 7.5
pH 5.5
pH 5.0
pH 4.5
pH 4.0
peak current (µA)
voltage (mV)
AfTOK
pH 7.5
pH 8.5
peak current (µA)
voltage (mV)
E
pH 6.5
pH 5.5
pH 4.5
pH 4.0
pH 8.5
CnTOK
F
CnTOK
pH 7.5
pH 6.5
pH 5.5
pH 5.0
pH 4.5
pH 4.0
peak current (µA)
voltage (mV)
CnTOK
pH 7.5
pH 8.5
peak current (µA)
voltage (mV)

Supplementary Figure 2. ScTOK, AfTOK, and CnTOK are all insensitive to extracellular pH.

A. Averaged traces for ScTOK channels as indicated, expressed in oocytes in the presence of pH 8.5 – 4.0. Scale bar upper right; *n* = 5-8 per group.
B. Mean peak current (measured during prepulse) from traces as in A; *n* = 5-8 per group.
C. Averaged traces for AfTOK channels as indicated, expressed in oocytes in the presence of pH 8.5 – 4.0. Scale bar upper right; *n* = 6-7 per group.
D. Mean peak current (measured during prepulse) from traces as in B; *n* = 6-7 per group.
E. Averaged traces for CnTOK channels as indicated, expressed in oocytes in the presence of pH 8.5 – 4.0. Scale bar upper right; *n* = 5-11 per group.
F. Mean peak current (measured during prepulse) from traces as in E; *n* = 5-11 per group.



Supplementary Figure 3. Predicted structure of CaTOK channel protein using AlphaFold. Key in the top left shows the per-residue model confidence score (pLDDT) between 0 and 100.



Supplementary Figure 4. Protonation and deprotonation of H144 in the S1-S2 linker perturbs but does not abolish CaTOK pH sensitivity.

A. Current density at +40 mV for H144K and H144N normalized to wild type. Black dashed line indicates no change; *n* = 11-16 per group.

B. Mean normalized tail current (G/Gmax) for H144K (open circles; left) and H144N (open circles; right) versus wild type (filled circles); *n* = 11-16 per group.

C. Averaged traces for CaTOK-H144K channels as indicated, expressed in oocytes in the presence of pH 8.5 – 4.0. Scale bar lower left; *n* = 6-11 per group.

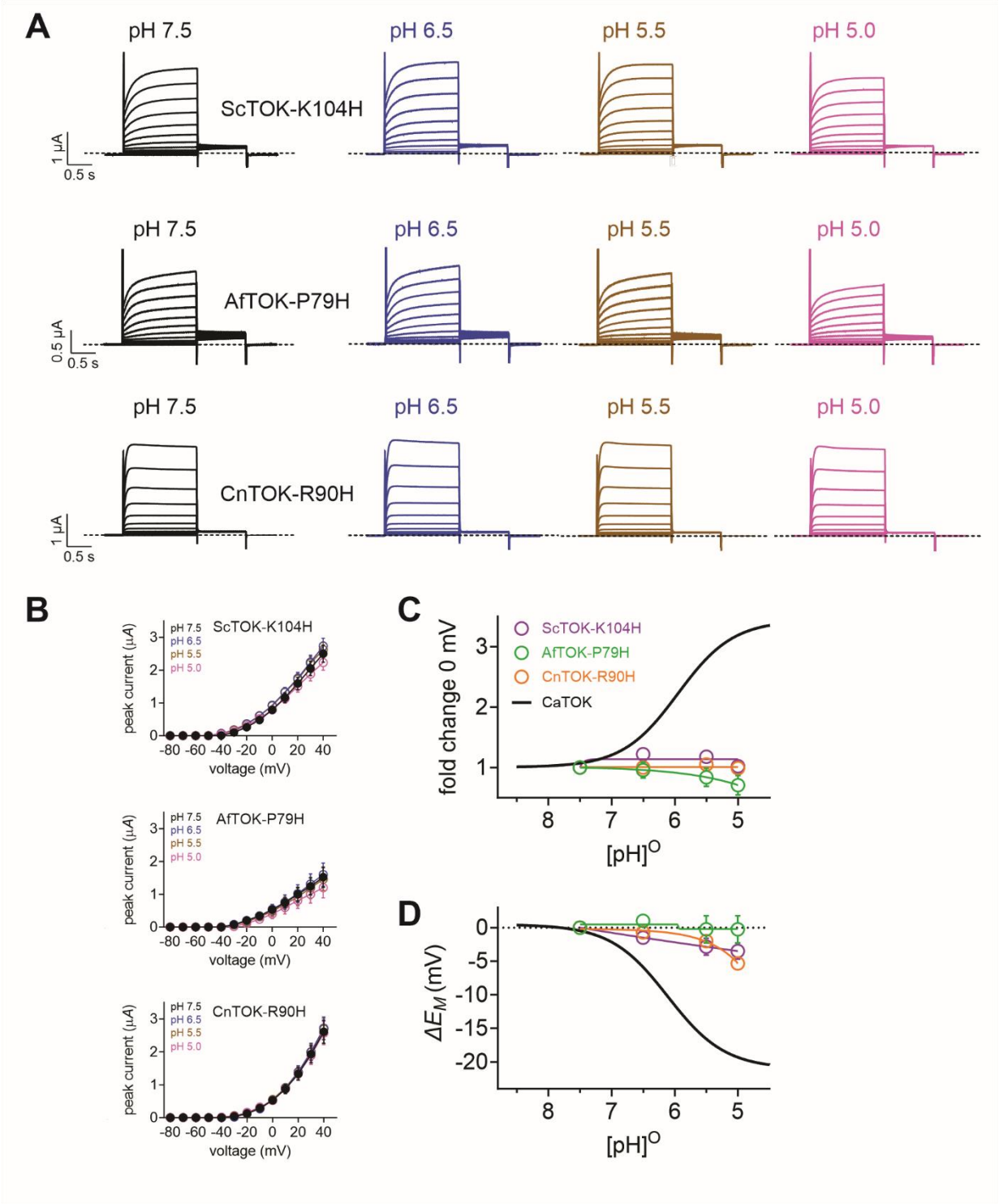
D. Mean peak current (measured during prepulse) from traces as in C; *n* = 6-11 per group.

E. Mean normalized tail current (G/Gmax) for traces as in C; *n* = 6-11 per group.

F. Averaged traces for CaTOK-H144N channels as indicated, expressed in oocytes in the presence of pH 8.5 – 4.0. Scale bar lower left; *n* = 8-16 per group.

G. Mean peak current (measured during prepulse) from traces as in F; *n* = 8-16 per group.

H. Mean normalized tail current (G/Gmax) for traces as in F; *n* = 8-16 per group.



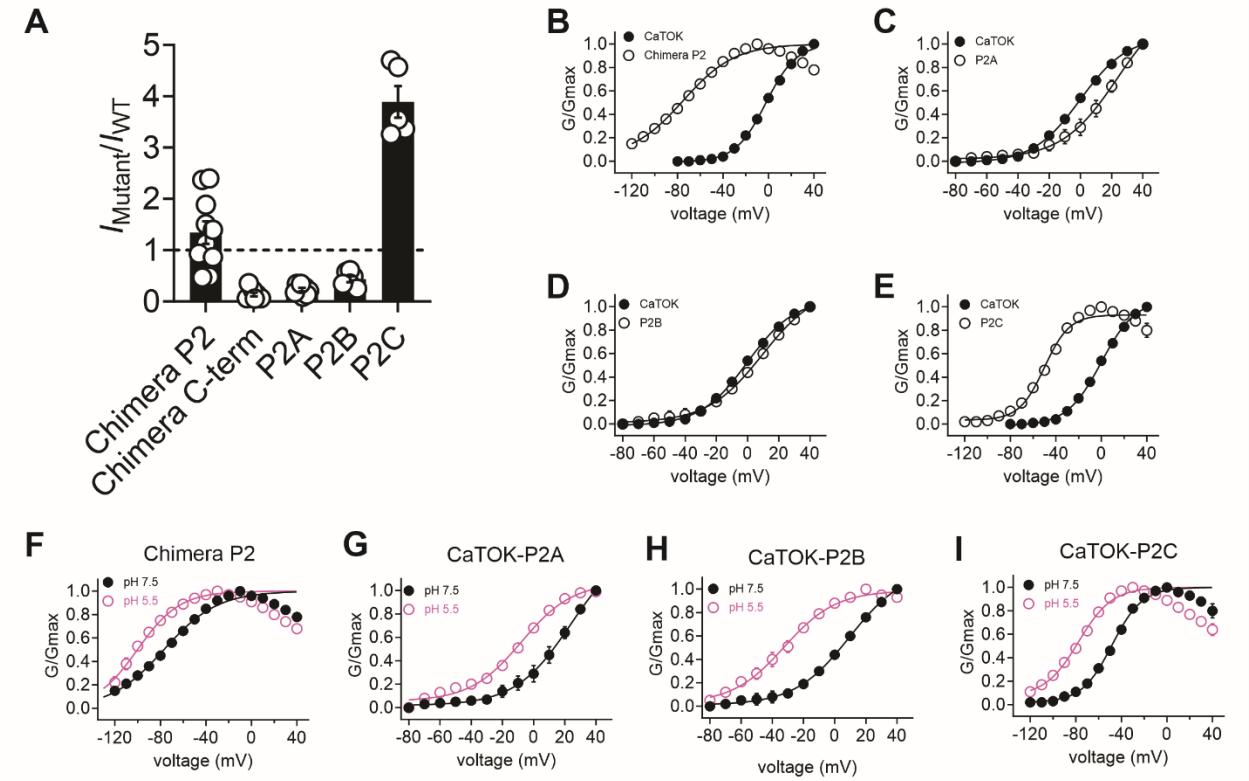
Supplementary Figure 5. Introduction of a histidine alone cannot confer pH sensitivity to pH insensitive ScTOK, AfTOK, and CnTOK.

A. Averaged traces for ScTOK-K104H (Top), AfTOK-P79H (Middle), and CnTOK-R90H (Bottom) channels as indicated, expressed in oocytes in the presence of pH 8.5 – 4.0. Scale bar lower left; *n* = 4-7 per group.

B. Mean peak current (measured during prepulse) from traces as in A; *n* = 4-7 per group.

C. Mean current increase versus $[pH]^{O}$ at 0 mV from traces as in A, with CaTOK data from Figure 1; *n* = 4-7 per group.

D. Mean E_M versus $[pH]^{O}$ at 0 mV from traces as in A, CaTOK data from Figure 1; *n* = 4-7 per group.



Supplementary Figure 6. Current augmentation in response to extracellular acidification is conferred by the distal end of the second pore.

A. Current density at +40 mV for mutant chimeras and second pore domain cluster mutants normalized to wild type. Black dashed line indicates no change; *n* = 5-10 per group.

B. Mean normalized tail current (G/Gmax) for Chimera P2 (open circles) versus wild type (filled circles); *n* = 10 per group.

C. Mean normalized tail current (G/Gmax) for Chimera P2A (open circles) versus wild type (filled circles); *n* = 8 per group.

D. Mean normalized tail current (G/Gmax) for Chimera P2B (open circles) versus wild type (filled circles); *n* = 6 per group.

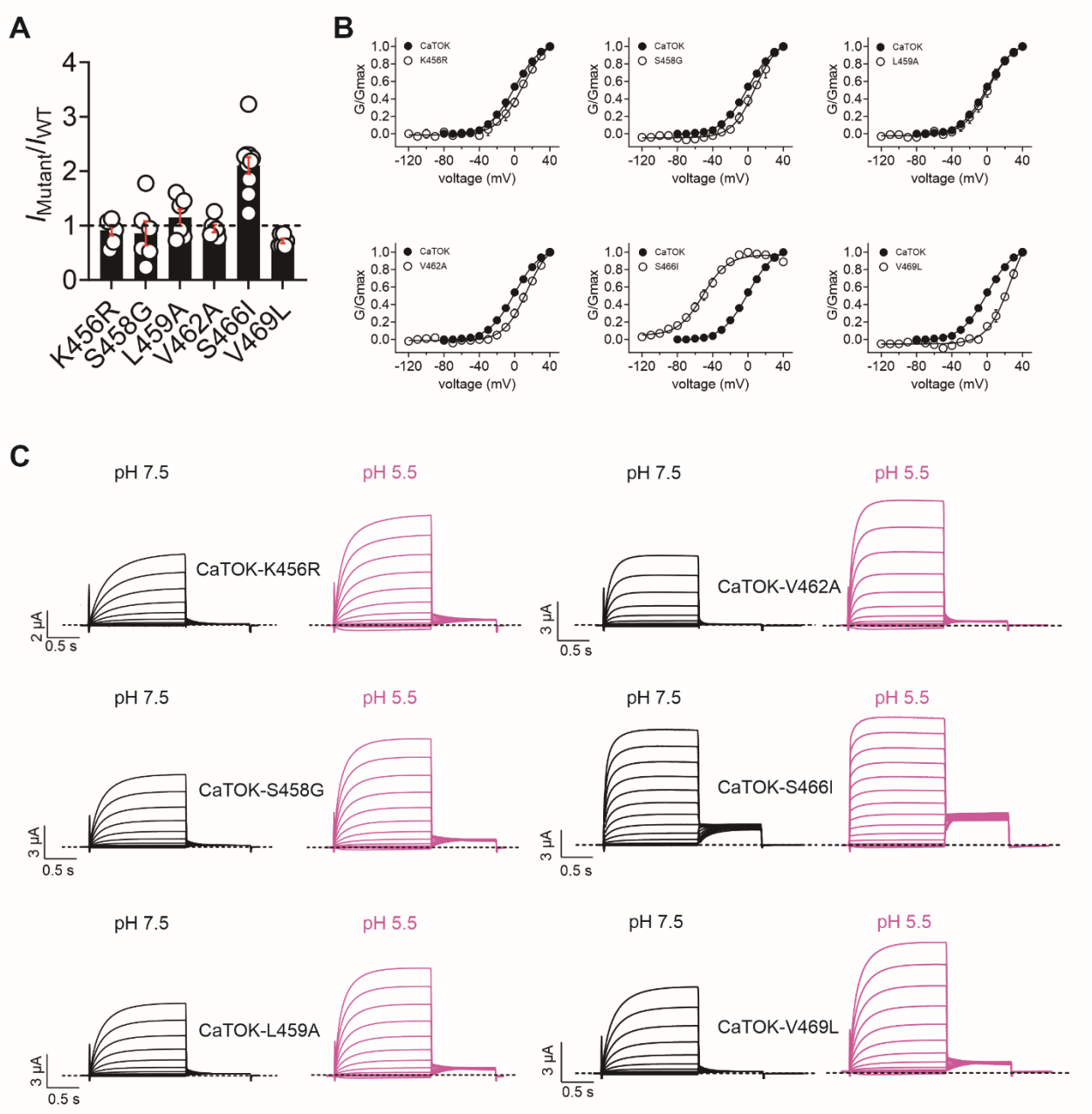
E. Mean normalized tail current (G/Gmax) for Chimera P2C (open circles) versus wild type (filled circles); *n* = 5 per group.

F. Mean normalized tail current (G/Gmax) for chimera P2 mutant in the presence and absence of pH 5.5; *n* = 10 per group.

G. Mean normalized tail current (G/Gmax) for P2A cluster mutant in the presence and absence of pH 5.5; *n* = 8 per group.

H. Mean normalized tail current (G/Gmax) for P2B cluster mutant in the presence and absence of pH 5.5; *n* = 6 per group.

I. Mean normalized tail current (G/Gmax) for P2C cluster mutant in the presence and absence of pH 5.5; *n* = 5 per group.

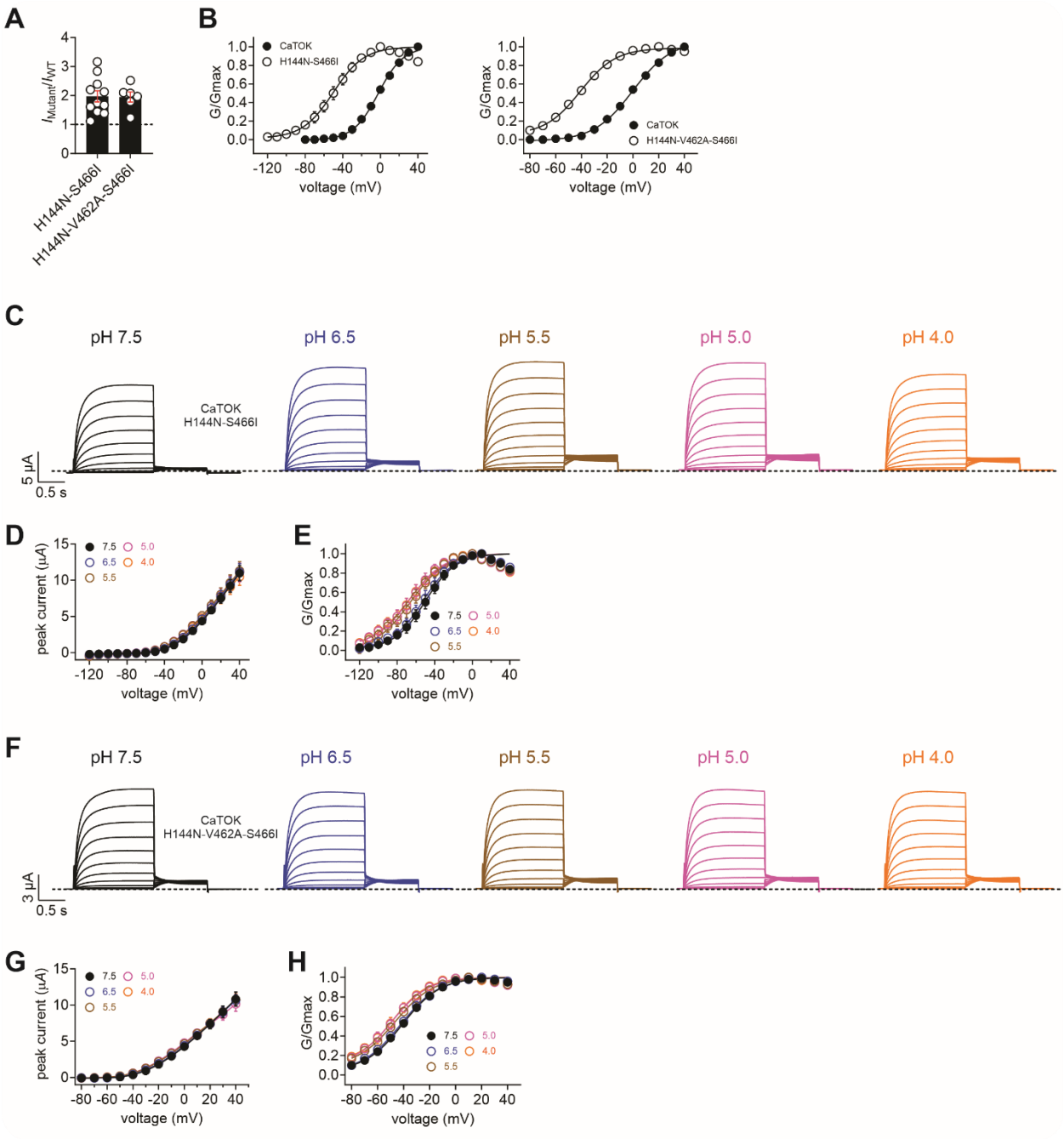


Supplementary Figure 7. V462 and S466 in the proximal cap of S8 form part of the pH sensor for CaTOK.

A. Current density at +40 mV for P2C single point mutants normalized to wild type. Black dashed line indicates no change; *n* = 6-11 per group.

B. Mean normalized tail current (G/Gmax) P2C single point mutants (open circles) versus wild type (filled circles); *n* = 6-11 per group.

C. Averaged traces for K456R, S458G, L459A, V462A, S466I, and V469L channels as indicated, expressed in oocytes in the absence and presence of pH 5.5. Scale bar lower left; *n* = 6-11 per group.



Supplementary Figure 8. CaTOK-H144N-V462-S466I mutant abolishes pH sensitivity.

A. Current density at +40 mV for H144N-S466I and H144N-V462A-S466I mutants normalized to wild type. Black dashed line indicates no change; *n* = 5-11 per group.

B. Mean normalized tail current (G/Gmax) for H144N-S466I (open circles; left) and H144N-V462A-S466I (open circles; right) versus wild type (filled circles); *n* = 5-11 per group.

C. Exemplar traces for H144N-S466I channels as indicated, expressed in oocytes in the presence of pH 7.6 – 4.0. Scale bar lower left; *n* = 11 per group.

D. Mean peak current (measured during prepulse) from traces as in C; *n* = 11 per group.

E. Mean normalized tail current (G/Gmax) for traces as in C; *n* = 11 per group.

F. Exemplar traces for H144N-V462A-S466I channels as indicated, expressed in oocytes in the presence of pH 7.6 – 4.0. Scale bar lower left; *n* = 5-6 per group.

G. Mean peak current (measured during prepulse) from traces as in F; *n* = 5-6 per group.

H. Mean normalized tail current (G/Gmax) for traces as in F; *n* = 5-6 per group.