Supplemental material

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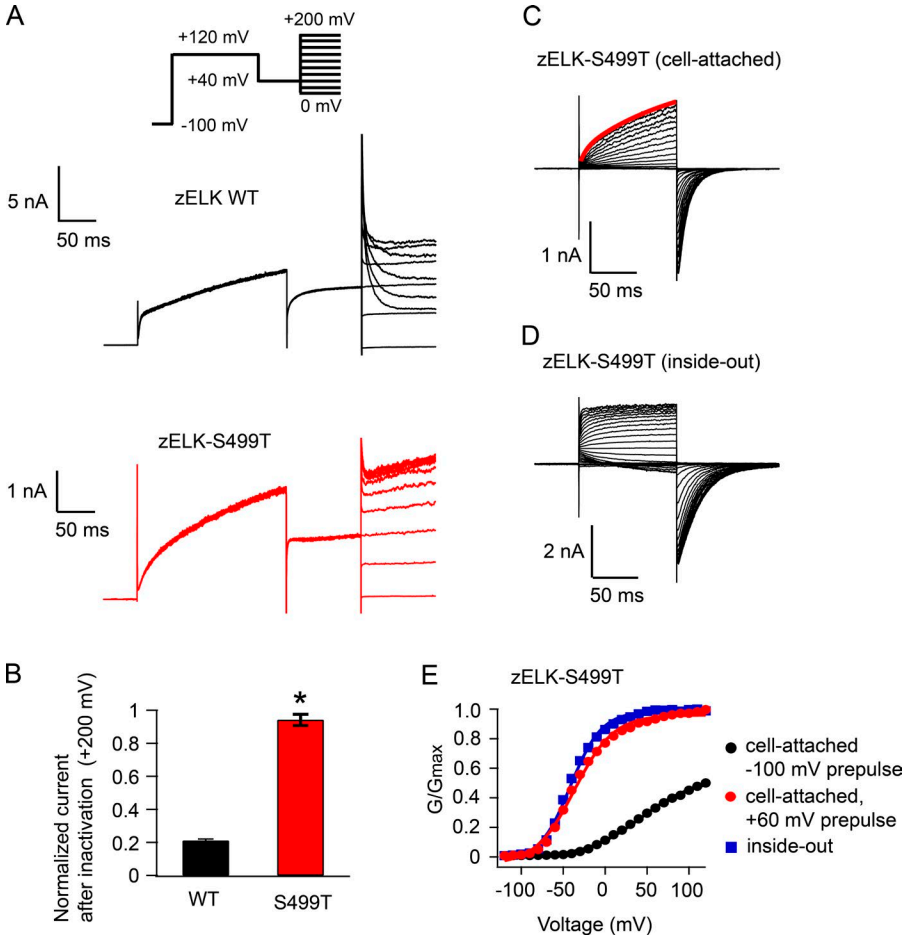

Figure S1. **S499T mutation abolished the inactivation of zELK channels. (A)** Representative current traces elicited by a three-component voltage protocol, consisting of a +120-mV depolarizing pulse from the resting −100 mV, a +40-mV pulse for the recovery of the inactivation, and a family of depolarizing voltage steps from 0 to +200 mV to visualize the inactivation, which is unmasked by the recovery pulse. S499T removed the inactivation almost completely. **(B)** Summary of the current amplitude at the end of the +200-mV pulse in the family of voltage steps in A, normalized to the peak of the current amplitude in the beginning of the +200-mV voltage pulse, for zELK wild-type and zELK–S499T channels (n = 4). *, P < 0.01. **(C)** Representative I-V traces for the activation of zELK–S499T channels in the cell-attached configuration using the voltage protocol in Fig. 1 B. The red curve is the fit of the current activation at +120 mV by a double-exponential function with τ1 = 6.4 ms and τ2 = 103 ms. **(D)** Representative I-V traces for the activation of zELK–S499T channels after run-up in the inside-out configuration using the same voltage protocol in C. **(E)** G-V curve of the zELK-S499T channel activation in the control cell–attached configuration ($V_{1/2}$ = 38 mV), after a +60-mV prepulse ($V_{1/2}$ = −37 mV), and after run-up ($V_{1/2}$ = −41 mV).

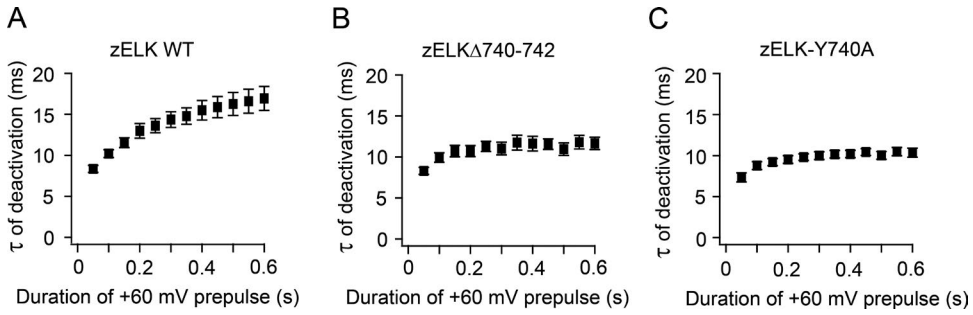

Figure S2. **Summary of the time constants of the tail currents.** Summary of the time constants of the tail current using the protocol in Fig. 2 C for zELK wild-type (n = 12; A), zELKΔ740–742 (n = 5; B), and zELK–Y740A channels (n = 5; C). Data are shown as mean ± SEM.

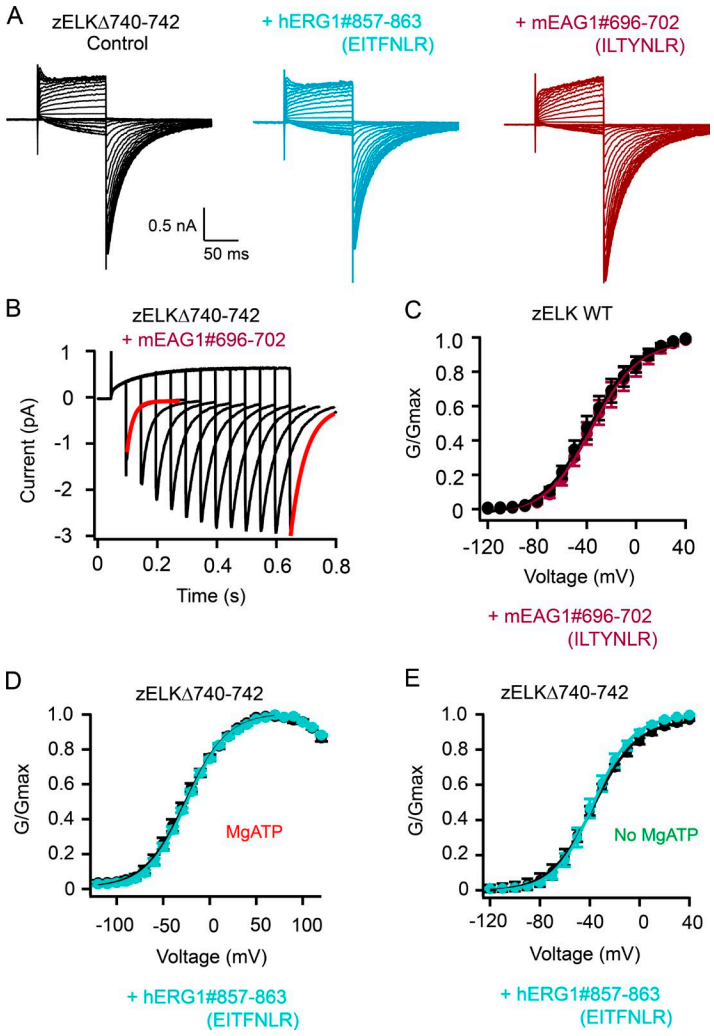

Figure S3. **Effects of the intrinsic-ligand peptide. (A)** Representative I-V traces for the activation of zELKΔ740–742 channels before and after adding hERG1 and mEAG1 peptides, all in the presence of MgATP. **(B)** Current traces of zELKΔ740–742 channels after applying the mEAG1 peptide, elicited by a voltage protocol with increasing durations of +60-mV pulse in the presence of MgATP. **(C)** The intrinsic-ligand peptide from hEAG1 did not significantly affect the voltage-dependent gating of zELK wild-type channels in the absence of MgATP. **(D)** The intrinsic-ligand peptide from hERG1 did not significantly affect the voltage-dependent gating of zELKΔ740–742 channels in the presence of MgATP. **(E)** The intrinsic-ligand peptide from hERG1 did not significantly affect the voltage-dependent gating of zELKΔ740–742 channels in the absence of MgATP. Data are shown as mean ± SEM, n = 3–5.

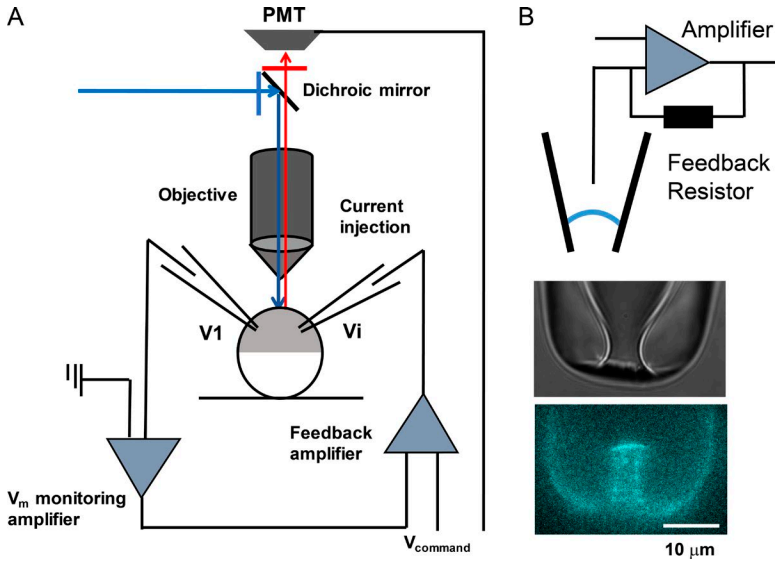

Figure S4. **Comparison of PCF and VCF. (A)** Diagram showing the design of VCF. The oocyte is voltage clamped with a two-electrode voltage-clamp amplifier. Vi is the microelectrode for current injection, and V1 is the microelectrode for monitoring membrane voltages. PMT, photomultiplier tube. **(B)** Diagram illustrating PCF. The upper image is a giant patch electrode in brightfield, whereas the lower image shows the L-Anap fluorescence from the same patch.

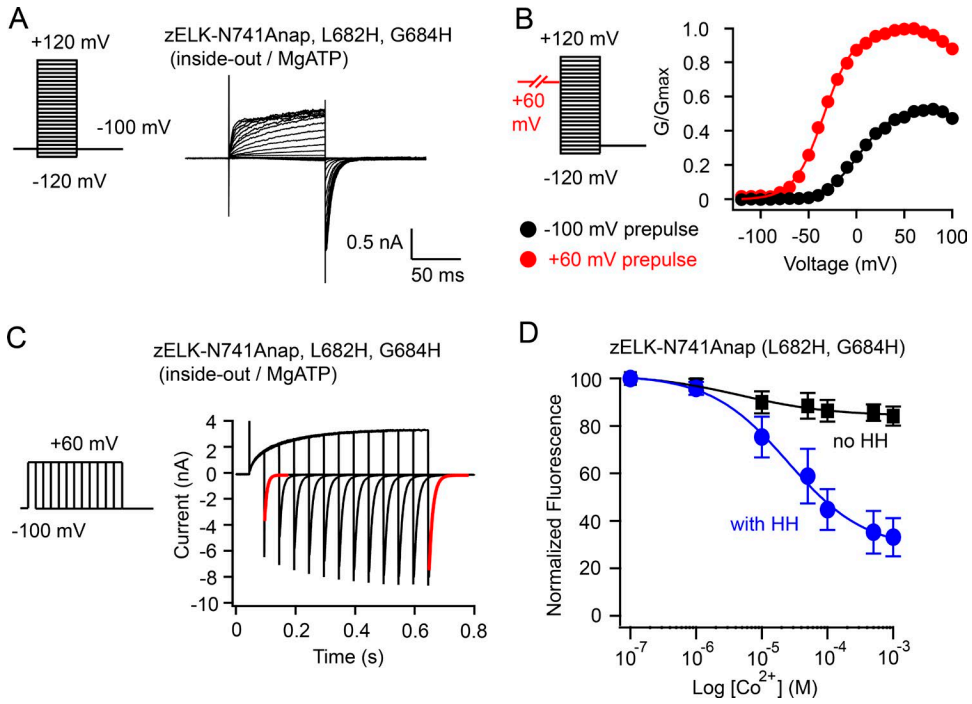

Figure S5. **Properties of zELK-N741Anap channels. (A)** Representative I-V recordings for zELK-N741Anap,L682H,G684H channel activation in the inside-out configuration using the voltage protocol with steps of depolarizing voltages. MgATP is present in the bath solution. **(B)** G-V curve of zELK-N741Anap,L682H,G684H activation with ($V_{1/2}$ = −34 mV) and without ($V_{1/2}$ = 2 mV) the 500-ms, +60-mV prepulse. **(C)** Currents of zELK-N741Anap,L682H,G684H channels elicited by a voltage protocol with increasing durations of a +60-mV pulse. τ of the tail current recovery (red traces) increased from 7 to 13 ms. **(D)** Quenching of Anap fluorescence measured using PCF by different concentrations of Co^{2+} with or without dihistidines and in the presence of 2 mM MgATP at the resting holding voltage of −100 mV. Data are shown as mean ± SEM, *n* = 4.

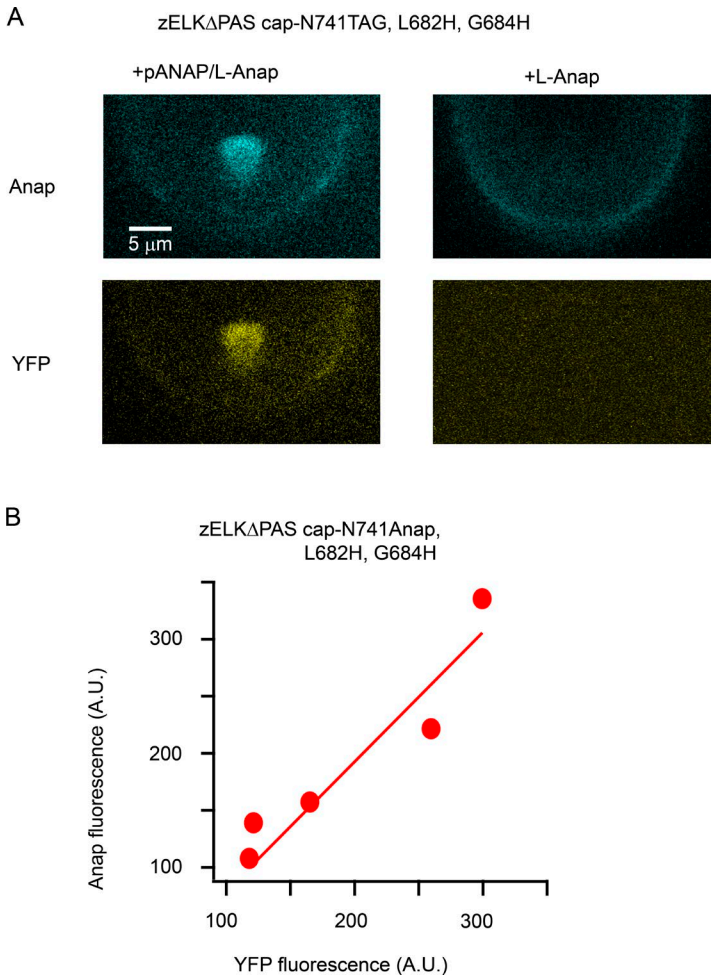

Figure S6. **Successful incorporation of Anap for zELKΔPAS cap-N741TAG,L682H,G684H channels. (A)** Representative PCF images with oocytes injected with mRNA of zELKΔPAS cap-N741TAG,L682H,G684H, L-Anap, and with (left) or without (right) pANAP vector. **(B)** Relationship of Anap fluorescence versus YFP fluorescence measured by PCF for zELKΔPAS cap-N741Anap,L682H,G684H channels. A.U., arbitrary units.