Supporting Information

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SI Materials and Methods

Culture and Transfection of HEK293 Cells. GFP-Kv2.1loopBAD was constructed as previously described (1, 2). HEK293 cells (American Type Culture Collection, passage 38–45) were transfected with GFP-Kv2.1loopBAD (1 μg plasmid DNA per 10 cm^2 dish cells) by electroporation with a Bio-Rad Genepulser Xcell in a 0.2-cm gap cuvette using a 110-mV, 25-ms pulse. For experiments involving Quantum dot (Qdot) labeling, cells were cotransfected with a plasmid carrying the bacterial biotin ligase BirA (3). Following electroporation, cells were plated on Matrigel-coated 35-mm glass bottom coverslip dishes (MatTek) in DMEM + 10% FBS and were used 24 h after transfection.

Quantum Dot Labeling of GFP-Kv2.1-loopBAD. Live HEK cells transfected with GFP-Kv2.1-loopBAD and BirA were incubated for 15 min with streptavidin-conjugated 655 Qdots at a dilution of 1:1,000 as previously described (1, 2). Imaging was performed with an Olympus FV1000 confocal microscope. GFP was excited using the 488-nm line of an Ar laser set at 0.5–1% transmission, and emission was collected using the variable band pass set at 500–530 nm. The Qdots were excited using a 633-nm HeNe laser set at 100% transmission and emission was collected using a LP650 filter in front of a conventional PMT. The detector voltage was adjusted as needed to use the entire 12-bit dynamic range. For live-cell imaging, cells were imaged at 37 °C in Hepes-buffered saline containing 146 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 0.6 mM $MgSO_4$, 1.6 mM $NaHCO_3$, 0.15 mM NaH_2PO_4, 0.1 mM ascorbic acid, 8 mM glucose, and 20 mM Hepes, pH 7.4.

Whole-Cell Ionic Current Measurements. HEK cells transfected with GFP-Kv2.1-loopBAD were trypsinized 24 h after electroporation and replated on 35-mm glass bottom dishes coated with ECL cell attachment matrix (Millipore). After 1 h, cells were washed extensively with whole-cell external recording solution, containing 130 mM NaCl, 5 mM KCl, 10 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM glucose, and 10 mM Hepes, pH 7.4. Pipettes were pulled from thin-walled borosilicate glass and had a resistance of 1.8–2.5 MΩ when filled with intracellular solution (140 mM KCl, 1 mM $MgCl_2$, 4 mM NaCl, 0.5 mM EGTA, and 10 mM Hepes, pH 7.4).

Whole-cell K^+ currents were recorded using an Axopatch200B amplifier (Axon Instruments). Ionic currents were capacitance and series-resistance compensated, sampled at 10 kHz (Digidata 1440; Axon Instruments), and filtered at 2 kHz. Leak subtraction was performed online using the P/4 method in pClamp10 but non-subtracted records were used to assess data quality. Cells were held at −80 mV and depolarized to +80 mV in 10-mV steps with an interpulse interval of 10 s. Peak ionic currents were converted to conductance using the equation $G = I/(V - E_K)$. E_K was calculated to be −85 mV. The normalized conductance (G/G_{max}) was plotted as a function of voltage and fitted using Eq. **S1**,

$$G(V) = \frac{G_{max}}{1 + e^{(V - V_{1/2})/k}}, \quad \textbf{[S1]}$$

where G_{max} is the maximal conductance, $V_{1/2}$ is the potential for half-maximal activation, and k is a slope factor. Alternatively, voltage-activation curves were obtained by plotting peak tail currents obtained at −40 mV against the previous command potential. A standard Boltzmann fit was used to determine the voltage for half-maximal activation.

Single-Channel Measurements. HEK293 cells expressing GFP-Kv2.1-loopBAD were trypsinized, replated, and allowed to settle for at least 1 h and no more than 3 h before being used for experiments. A magnification factor of ×10–12 on the FV1000 in conjunction with a ×40 0.9-NA dry objective was used to visualize the GFP-Kv2.1-containing clusters during cell-attached voltage clamp.

Patch pipettes were pulled from thick-wall borosilicate glass coated with Sylgard and typically had a resistance of 5–10 MΩ when filled with pipette solution containing 100 mM NaCl, 30 mM $CaCl_2$, 5 mM KCl, 2 mM $MgCl_2$ and 10 mM Hepes, pH 7.4. The external solution was identical to that described for whole-cell current measurements in *SI Text*. Seal resistances under these conditions were typically 50–100 GΩ. Currents were measured using an Axopatch200B amplifier, acquired at 10 kHz, and filtered at 1 kHz. Recordings were leak and capacitance corrected off-line by subtracting an average of at least five null sweeps from each sweep in the record.

Whole-Cell Gating Current Measurements. To prevent saturation of the amplifier, the voltage command was rounded with a time constant of 318 μs (f_c = 500 Hz). Immobilization-resistant charge movement was measured by integration of the outward current generated following the onset of the test pulse (Q_{on}). Maximal Q_{on} at each potential was plotted as a function of voltage and fitted by the equation

$$Q(V) = \frac{Q_{max}}{1 + e^{(V - V_{1/2})/k}}, \quad \textbf{[S2]}$$

where Q_{max} is the maximal charge movement, $V_{1/2}$ is the potential for half-maximal activation, and k is a slope factor.

Estimation of channel number from charge movement was done using the equation

$$Channel\# = \frac{Q_{on} * 6.2 \times 10^{18} q_e}{12.5 q_e}, \quad \textbf{[S3]}$$

where Q_{on} is the charge movement at +60 mV in coulombs. The value of 12.5 q_e is the estimated number of gating charges moved per Kv2.1 channel (4).

Statistics. Data are presented as mean ± SEM. Statistical analysis was performed using an unpaired t test, with $P < 0.01$ considered statistically significant, as indicated in the figure legends.

1. O'Connell KM, Rolig AS, Whitesell JD, Tamkun MM (2006) Kv2.1 potassium channels are retained within dynamic cell surface microdomains that are defined by a perimeter fence. *J Neurosci* 26:9609–9618.
2. Tamkun MM, O'connell KM, Rolig AS (2007) A cytoskeletal-based perimeter fence selectively corrals a sub-population of cell surface Kv2.1 channels. *J Cell Sci* 120:2413–2423.
3. Chen I, Howarth M, Lin W, Ting AY (2005) Site-specific labeling of cell surface proteins with biophysical probes using biotin ligase. *Nat Methods* 2:99–104.
4. Islas LD, Sigworth FJ (1999) Voltage sensitivity and gating charge in Shaker and Shab family potassium channels. *J Gen Physiol* 114:723–742.

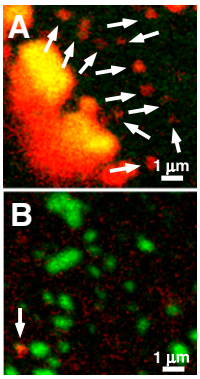

Fig. S1. Kv2.1 is expressed on the plasma membrane outside the cluster domain. Biotinylated GFP-Kv2.1-loopBAD channels expressed on the surface of live HEK cells were saturated with 655 quantum dots (Qdots) and imaged with an Olympus FV1000 confocal microscope. Green represents the GFP fluorescence whereas red shows the bound quantum dots. (*A*) An image where the detector sensitivity was set to image individual Qdots outside the cluster. Note the overexposed cluster Qdot signal superimposed onto the GFP fluorescence. (*B*) A control for nonspecific Qdot labeling, i.e., label obtained after a 1-μM neutravidin block before Qdot binding. The single Qdot seen in the cluster at the lower left is likely due to a single channel here escaping the initial neutravidin block. In both *A* and *B* the arrows point to single quantum dots presumably bound to single Kv2.1 channels.

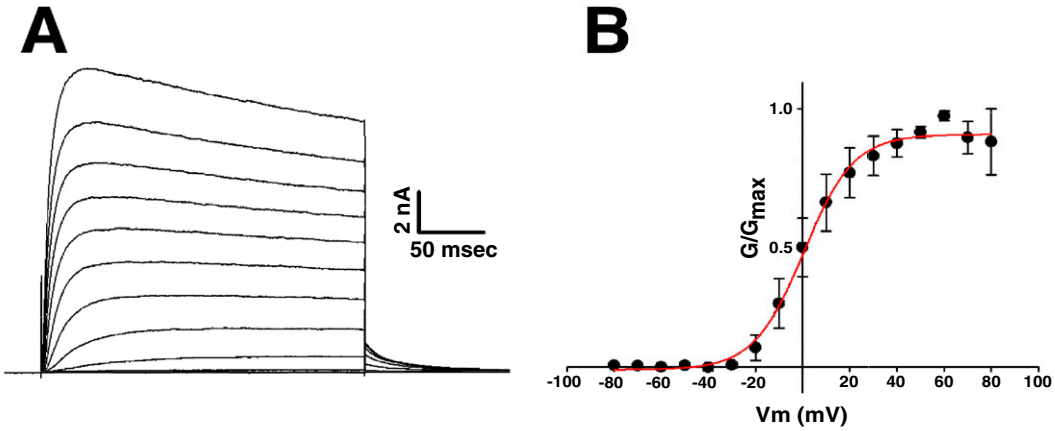

Fig. S2. High-threshold delayed rectifier K^+ currents recorded from HEK cells expressing Kv2.1. (*A*) Representative K^+ currents from an HEK cell transfected with GFP-Kv2.1-loopBAD and BirA and then incubated with SA-QDot655. (*B*) Normalized conductance–voltage curve for GFP-Kv2.1-loopBAD currents. $V_{1/2}$ = +6.7 ± 6.8 mV (n = 6).