Fe^{2+}-Mediated Activation of BK_{Ca} Channels by Rapid Photolysis of CORM-S1 Releasing CO and Fe^{2+}

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Supporting Information

Supplementary Methods

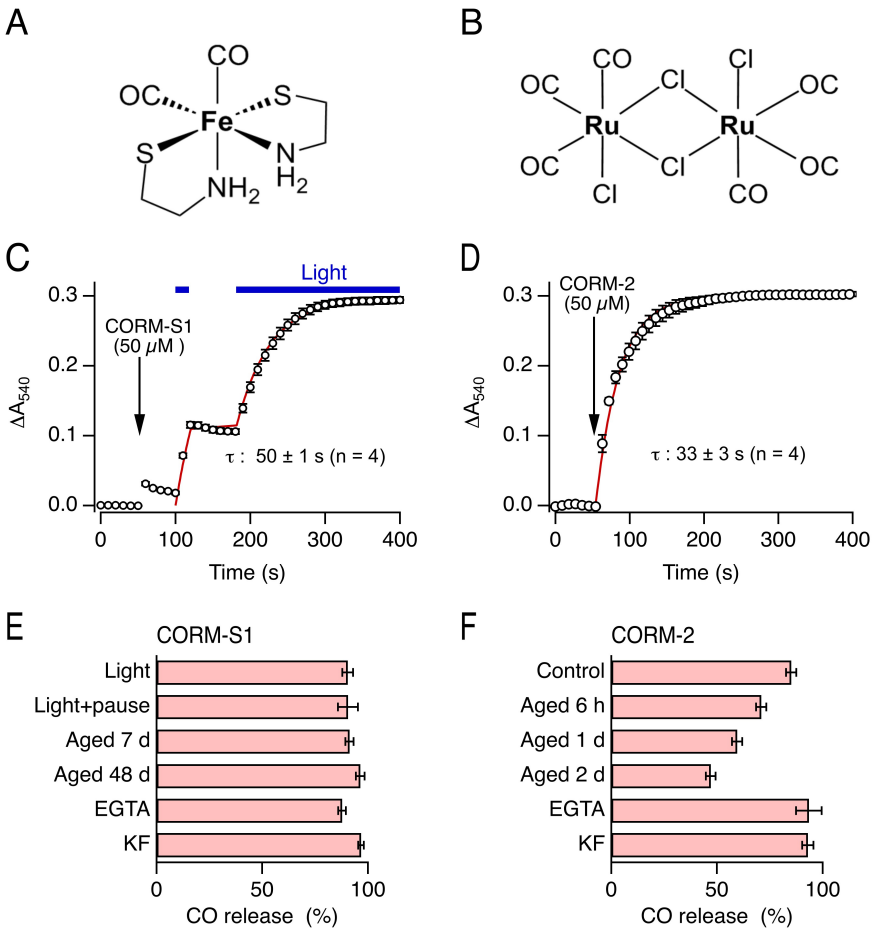
Spectrophotometric Measurements. For spectrophotometric Fe^{2+} stability assays, 2 mM 1,10-phenanthroline was dissolved in KF buffer (pH 6.8 or pH 7.4). 100 µM $FeSO_4$ was dissolved in KF buffer and incubated at ambient temperature. Aliquots were taken every 5 min and immediately mixed 1:1 with 2 mM 1,10-phenanthroline (in KF). Optical density (OD) was determined immediately after mixing.

For CORM-S1 stability experiments, 100 µM CORM-S1 was dissolved in KF buffer (pH 7.4) and incubated at ambient temperature. Aliquots were taken every 5 min and immediately mixed 1:1 with 2 mM 1,10-phenanthroline. $OD_{510\,nm}$ before and after 4.5 min of illumination with a cold-light source was determined. The residual CORM-S1 concentration in solution was calculated by subtracting $OD_{510\,nm}$ before from $OD_{510\,nm}$ after illumination.

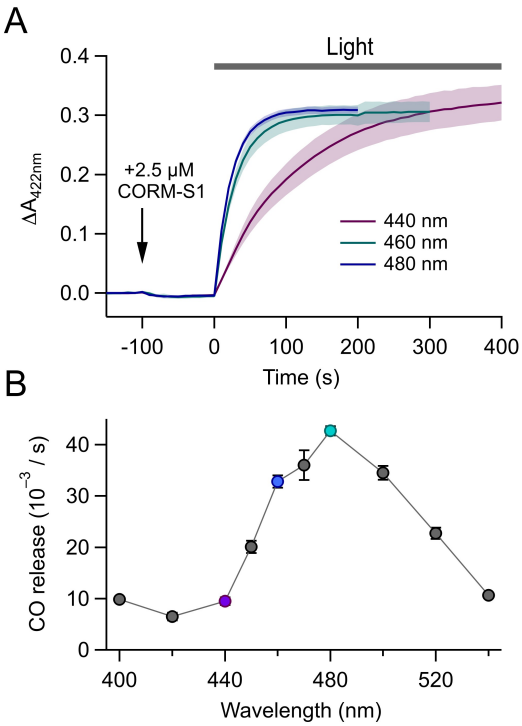
Coexpression of Human Slo β1 Subunits. For coexpression of α and β subunits, the coding sequence of human Slo β1 (*KCNMB*1, NP004128) replaced the kanamycin-resistance gene under control of the SV40 promoter in the hSlo1-α pCI-neo vector. Coexpression was confirmed in each patch by quantifying the time course of channel deactivation at –60 mV (pH 7.4) or –100 mV (pH 6.8), which was about 2.5-fold slower for human Slo β1 coexpression.

Fe^{2+} Release in Patch-Clamp Experiments. Inside-out membrane patches with BK_{Ca} channels were excised to a bath solution composed of KF buffer and CORM-S1 at 50 or 100 µM. The pipette tip with the inside-out patch was placed in the focus of the objective (40x dry or 100x oil). An epifluorescence light source (100 W mercury lamp) and an excitation bandpass filter (450-490 nm) was applied. Light was turned on/off with a shutter within about 2 ms.

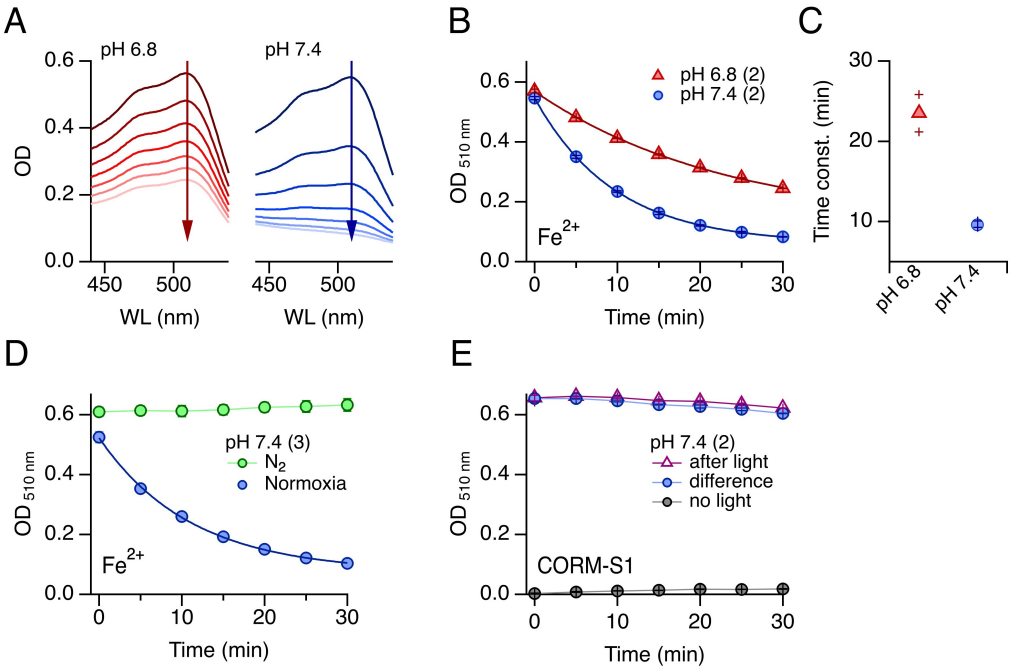
Supplementary Figures



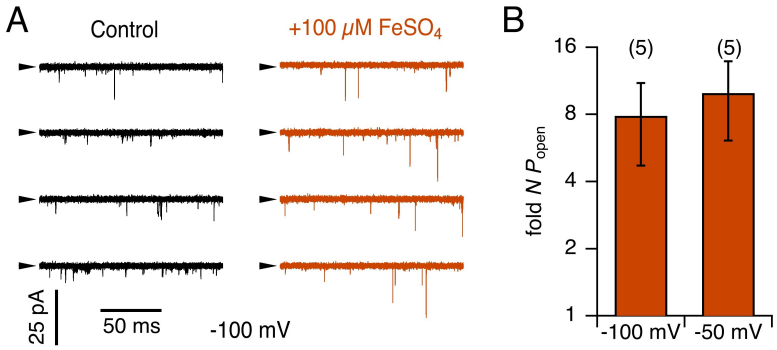
Supplementary Figure 1. CO release and stability of CORM-S1 and CORM-2. (A, B) Structures of CORM-S1 (A) and CORM-2 (B). Decomposition of CORM-S1 yields Fe^{2+}, 2x CO, and 2x cysteamine ($HSCH_2CH_2NH_2$). (C, D) Spectrophotometric assay using the absorbance of CO-bound myoglobin at 540 nm in PBS buffer. (C) CO release from 50 µM CORM-S1 (n = 4); the blue bars indicate episodes of light exposure. The curve indicates a single-exponential fit, interrupted in the dark period. (D) CO release on solvation of 50 µM CORM-2 with underlying single-exponential fit (n = 4). In C and D, arrows denote time of CORM application. (E) Percent release of CO for experiments shown in C using fresh CORM-S1 and samples that were stored at –20 °C in DMSO for 7 and 48 days, as well as fresh CORM-S1 in EGTA and KF solutions (n = 4). (F) Percent release of CO as shown in D for fresh and aged (6 h, 1 d, 2 d at –20 °C) CORM-2, as well as fresh CORM-2 in EGTA and KF solutions (n = 4). Data are means ± standard error (SEM).



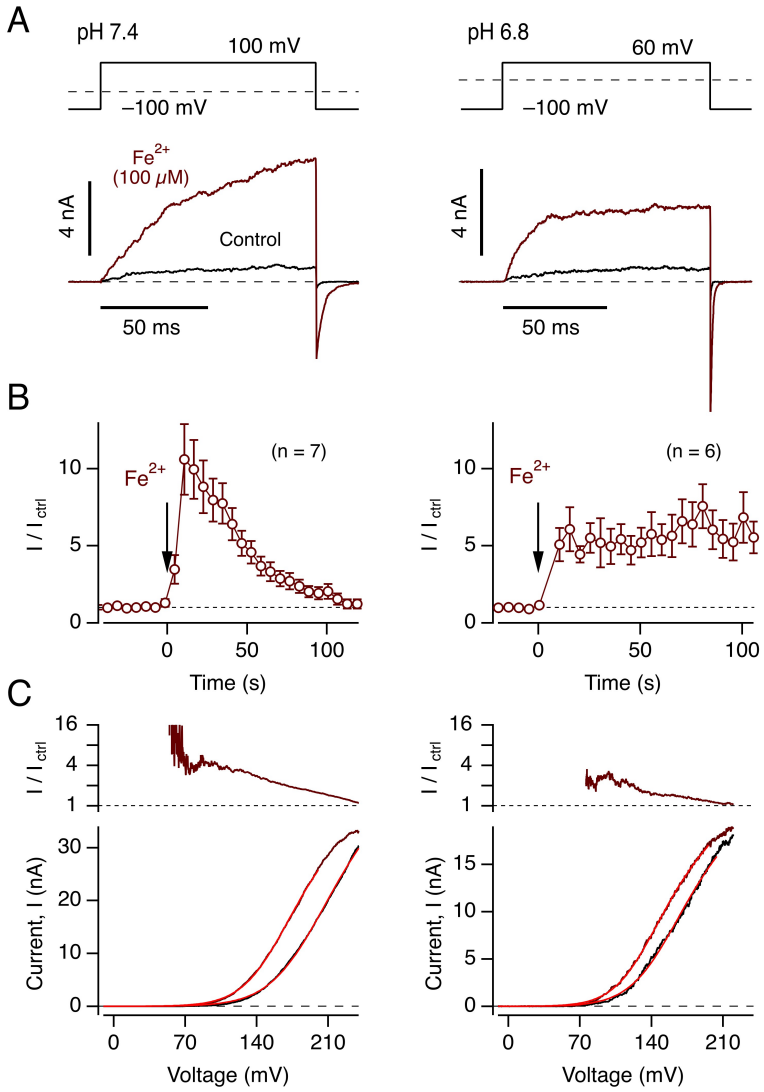
Supplementary Figure 2. Wavelength dependence of CO release from CORM-S1. (A) CO release was measured spectrophotometrically with a myoglobin assay. The sample in a cuvette was illuminated with a light guide connected to a monochromator (Polychrome V, T.I.L.L. Photonics). Superposition of time courses of absorbance changes at 422 nm in cuvettes before and after addition of 2.5 μM CORM-S1 (arrow). Illumination of the sample with light of the indicated wavelengths is denoted by the horizontal bar. Data are means ± standard error (SEM) in shading (n = 4). (B) Rate constants of CO released as a function of illumination wavelength. The release kinetics was corrected for the wavelength-dependent variation in light intensity. Data are means ± standard error (SEM) (n = 4). Straight lines connect data points for clarity.



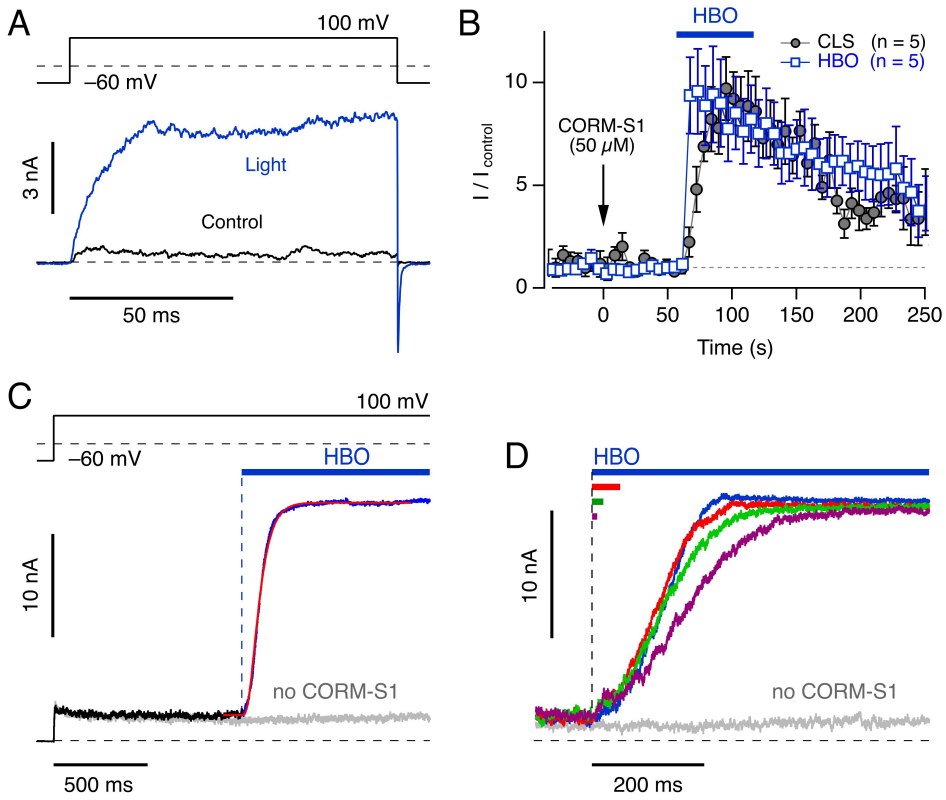
Supplementary Figure 3. Fe^{2+} and CORM-S1 stability in KF buffer. (A) Superposition of ferroin absorption spectra at 0 min (dark line) and up to 30 min (pale lines) at ambient temperature after dissolving Fe^{2+} in KF buffer adjusted to pH 6.8 (red) or 7.4 (blue). For ferroin measurement, 100 µM Fe^{2+} aliquots were mixed 1:1 with 2 mM 1,10-phenanthroline in KF buffer immediately before data acquisition. (B) Absorbance from A at 510 nm as a function of time with superimposed single-exponential fits. (C) Time constants of the single-exponential fits from B. (D) As in B at pH 7.4 for normoxic and hypoxic (N_2-saturated solutions) conditions. (E) Ferroin absorption at 510 nm of Fe^{2+} released from CORM-S1 in KF buffer over time. $OD_{510\,nm}$ before illumination with a cold-light source (gray), after illumination (purple), and the difference of both (blue), the latter representing intact CORM-S1. Straight lines connect data points for clarity. Filled symbols are means and crosses denote individual experiments.



Supplementary Figure 4. Fe^{2+} activates hSlo1 channels with resting voltage sensors. (A) Representative current recordings at –100 mV in the inside-out patch-clamp configuration from an hSlo1 expressing HEK293T cell before (control, black) and after (brown) addition of 100 μM Fe^{2+} solution to the intracellular side. Arrows indicate closed-state current levels, downward deflections channel openings. Every trace is a superposition of 4 recordings because openings at these voltages are rare. Relative open probabilities were determined from at least 30-s recordings before and after addition of Fe^{2+}. (B) Mean changes in relative open probability ($N\,P_{open}$) upon addition of 100 μM Fe^{2+} solution at –100 and –50 mV; means ± standard error (SEM), number of experiments in parentheses.



Supplementary Figure 5. Activation of hSlo1 + Slo β1 channel complexes by Fe^{2+}. (A) Current recordings in the inside-out patch-clamp configuration of HEK293T cells expressing hSlo1 α subunits and human Slo β1 subunits for the indicated pulse protocol and pH value before (Control) and after application of 100 μM Fe^{2+} solution to the intracellular side. (B) Time course of the current at 100 mV and 60 mV, respectively, relative to the control value (I / I_{ctrl}) with application of 100 μM Fe^{2+} solutions at time zero. (C) *bottom:* Representative current recordings in response to stimulation with a voltage ramp (–60 to 240 mV in 150 ms at pH 7.4; –100 to 220 mV in 160 ms at pH 6.8) before (black) and after application of 100 μM Fe^{2+} to the intracellular side (brown) with superimposed fits (red; Eq. 1), used to estimate the shift in half-maximal activation voltage, $\Delta V_{0.5}$. *top:* Fractional current change with Fe^{2+} application. $\Delta V_{0.5}$ were –34 ± 3 mV (n = 7, pH 7.4) and –21 ± 2 mV (n = 6, pH 6.8).



Supplementary Figure 6. Light-triggered rapid release of Fe^{2+} for channel activation. (A) Current recordings of hSlo1 BK_{Ca} channels from an inside-out patch before (Control) and 12 s after start of illumination with blue light (450-490 nm bandpass, 100-W HBO, 40x objective) of 50 µM CORM-S1 in KF solutions. (B) Time course of relative current at 100 mV in the presence of 50 µM CORM-S1 for the indicated light stimulation (bar) using GFP epifluorescence illumination (HBO, blue) compared to illumination with a cold-light source (CLS, data from Figure 1B). Straight lines connect data points for clarity. The dashed gray line marks 1.0, the control level. (C) Current recordings of BK_{Ca} channels in KF solution with 100 µM CORM-S1. Channels were preactivated by depolarization to 100 mV. Start of illumination with blue light (100x objective, 100-W HBO lamp, GFP filter set) is indicated by the bar. Control recordings with illumination but in the absence of CORM-S1 are shown in gray. The superimposed red curve is the result of an exponential fit to the 4^{th} power with a time constant of 48 ms. (D) Time-magnified data as in C for various durations of blue-light application (10 ms, magenta; 20 ms, green; 50 ms, red; continuous, blue). Data traces are from the same patch; solution in the bath was stirred between the individual recordings in order to remove remaining Fe^{2+} and Fe^{3+}, and to replenish CORM-S1 in the area of the objective focus.