CO-independent modification of K^+ channels by tricarbonyldichlororuthenium(II) dimer (CORM-2)

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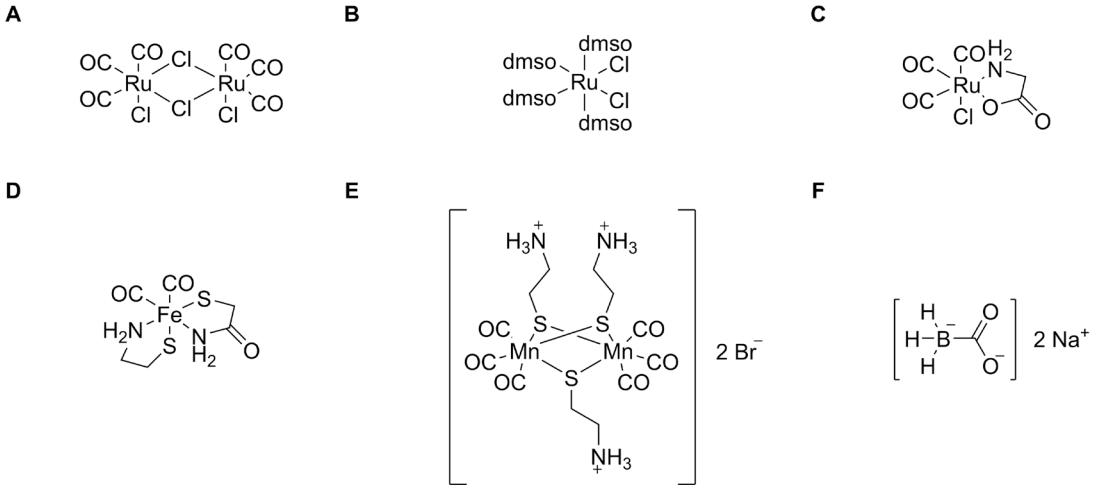
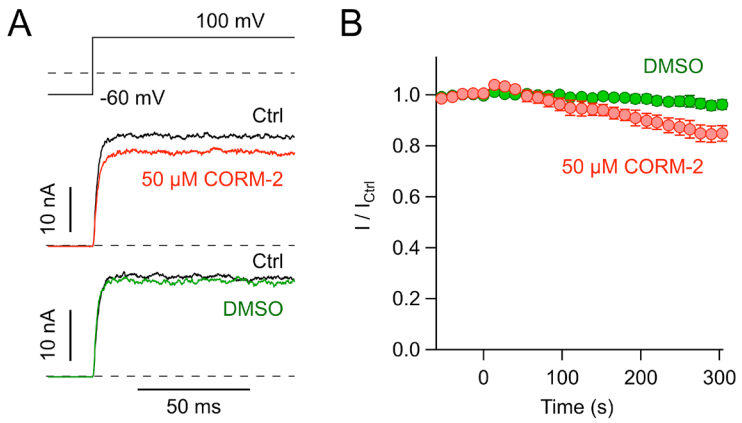
Supplementary Information

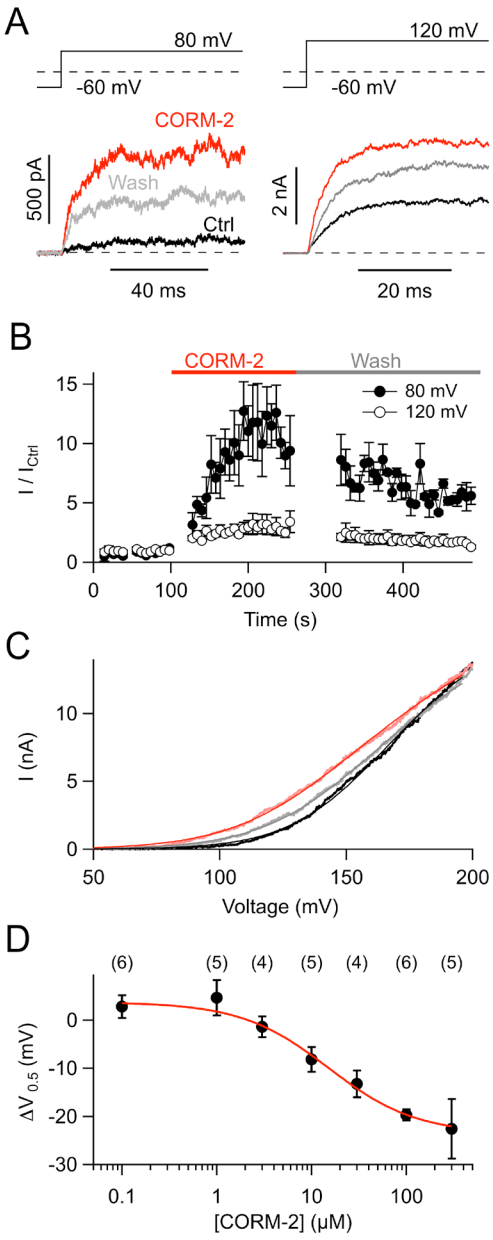
Supplementary Figures



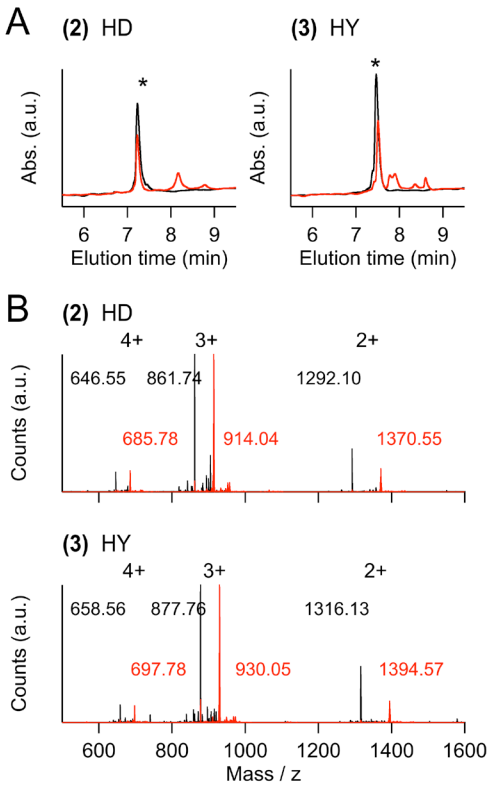
Supplementary Fig. 1. Chemical structures of CORMs and putative degradation products used throughout this study. A) CORM-2 (Tricarbonyldichlororuthenium(II) dimer), B) iCORM-2 (*Trans*-dichlorotetrakis(dimethylsulfoxide) ruthenium(II)), C) CORM-3 (Tricarbonylchloro(glycinato) ruthenium(II)), D) CORM-S1 (Dicarbonylbis(cysteamino) iron(II)), E) CORM-EDE1 ([Bis(tricarbonylmanganese)tris(cysteaminato)]) dihydrobromide, and F) CORM-A1 (Sodium boranocarbonate).



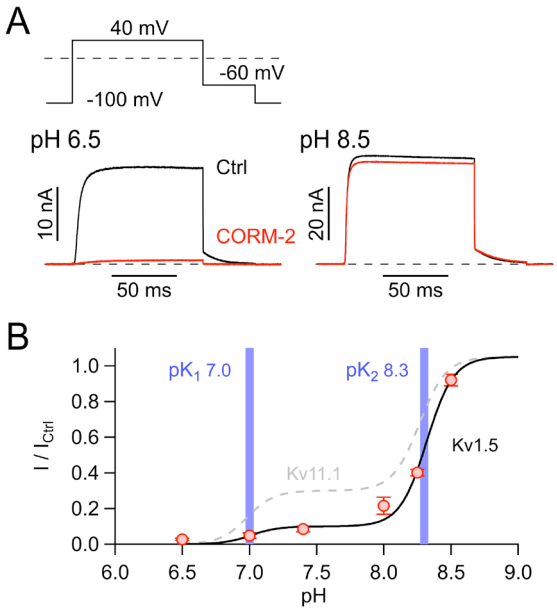
Supplementary Fig. 2. External application of CORM-2 does not activate K_{Ca}1.1 channels. A) Representative K_{Ca}1.1 whole-cell current traces for the indicated protocol before (Ctrl, black) and after application of 50 µM CORM-2 (red) or vehicle (0.1% DMSO, green). The intracellular solution was free of Ca^{2+}. B) Normalized mean current at 100 mV as a function of time with CORM-2 and vehicle application at time = 0; means ± S.E.M. for *n* = 5, each.



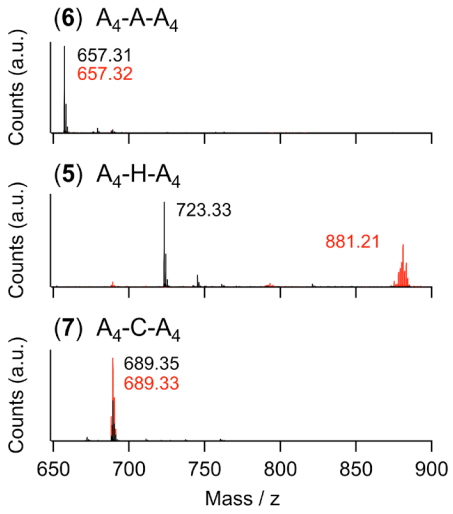
Supplementary Fig. 3. Hallmarks of K_{Ca}1.1 activation by CORM-2. A) Representative K_{Ca}1.1 current traces from inside-out patches before (Ctrl, black), in the presence (red), and after washout (grey) of 50 µM CORM-2. B) Time course of mean current at 80 and 120 mV for CORM-2 application and washout. Means ± S.E.M. for *n* = 3. C) Current responses to voltage ramps with superimposed fit according to: $I(V) = (V-V_{rev}) * (G_{max}/(1+\exp((V_{0.5}-V)/V_e))$, with the maximal conductance G_{max}, the half-activating voltage $V_{0.5}$, a slope factor V_e and the reversal potential V_{rev}. D) Change in $V_{0.5}$ as a function of CORM-2 concentration with superimposed Hill fit yielding a maximal shift of $V_{0.5}$ by -23.6 mV with an EC_{50} of 15.1 µM and a Hill coefficient of 0.94. Data are means ± S.E.M. with *n* indicated in parentheses. The intracellular solution was free of Ca^{2+}.



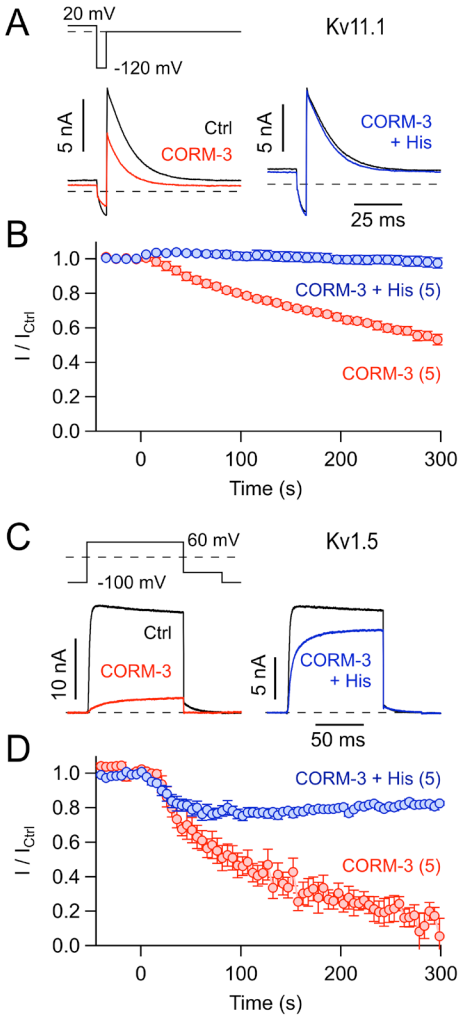
Supplementary Fig. 4. Peptide modification by CORM-2. A) HPLC elution profiles of 22-residue peptides corresponding to the pore loop sequences of mutant Kv11.1 channels (HD (**2**), corresponding to H578D; HY (**3**), corresponding to H587Y). Red traces are from peptides preincubated with CORM-2, black: peptides without preincubation. The elution peak for the non-modified peptides is marked with an asterisk. B) Mass spectra of wild-type and double-mutant peptides (as in (A)) before (black) and after incubation with CORM-2 (red). The labeled peaks correspond to $(M+4H^+)^{4+}$, $(M+3H^+)^{3+}$ and $(M+2H^+)^{2+}$ of the peptides or the peptide:$Ru(CO)_2$ complexes.



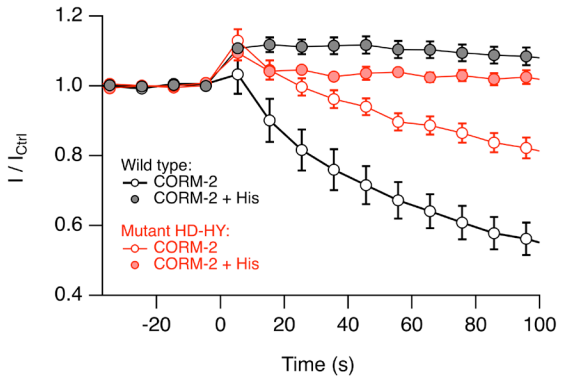
Supplementary Fig. 5. pH dependence of Kv1.5 inhibition by CORM-2. A) Representative traces of whole-cell Kv1.5 currents before (black) and 90 s after (red) application of 50 µM CORM-2. B) Normalized mean remaining currents for various pH values fit with a two-component Hill function (solid line). Resulting pK values are indicated by vertical bars. Data in B are means ± S.E.M. for *n* = 6-8. The dashed fit curve represents the result for Kv11.1 channels (cf. Fig. 6).



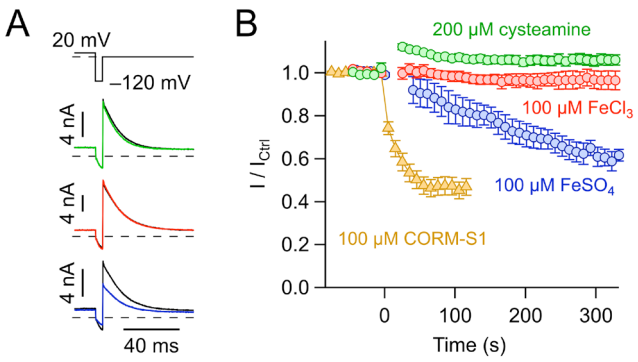
Supplementary Fig. 6. Peptide modification by CORM-2. Superimposed mass spectra of 9mer peptides (Ala (**6**), His (**5**) or Cys (**7**), flanked with 4 Ala on each side) before (black) and after 15-min incubation with 12.5 µM CORM-2 (red). Mass increase equivalent to a $Ru(CO)_2$ adduct is detected in A_4-H-A_4 only.



Supplementary Fig. 7. Histidine-dependent current inhibition of Kv11.1 and Kv1.5 channels by CORM-3. A) Pulse protocol and representative current traces of Kv11.1 channels before (black) and 5 min after application of 50 µM CORM-3 without (red) or with 1 µM histidine in the bath solution (blue). Depolarization to 20 mV lasted 400 ms (not shown). B) Time course of mean normalized currents with application of CORM-3 at time zero. C, D) As in A and B for Kv1.5 channels. Data as B and D are means ± S.E.M. with *n* indicated in parentheses; straight lines connect the data points for clarity.



Supplementary Fig. 8. Effect of CORM-2 on wild-type and mutant Kv11.1 channels. Peak tail current of Kv11.1 channels with application of 50 µM CORM-2 at time=0 for the wild type (black) and mutant H578D:H587Y (red) without (open) and with (filled circles) 1 mM free histidine in the bath. Data are means ± S.E.M. with *n* = 5 or 6.



Supplementary Fig. 9. Effect of CORM-S1 breakdown products on Kv11.1 channels. A) Pulse protocol and representative current traces of Kv11.1 channels before (black) and 5 min after application of 200 µM cysteamin (green), 100 µM $FeCl_3$ (red), or 100 µM $FeSO_4$ (blue). Depolarization to 20 mV lasted 400 ms (not shown). B) Time course of mean normalized currents with application of the indicated substances at time zero. Data are means ± S.E.M. with *n* = 5 or 6.