Santos et al., http://www.jgp.org/cgi/content/full/jgp.200810007/DC1

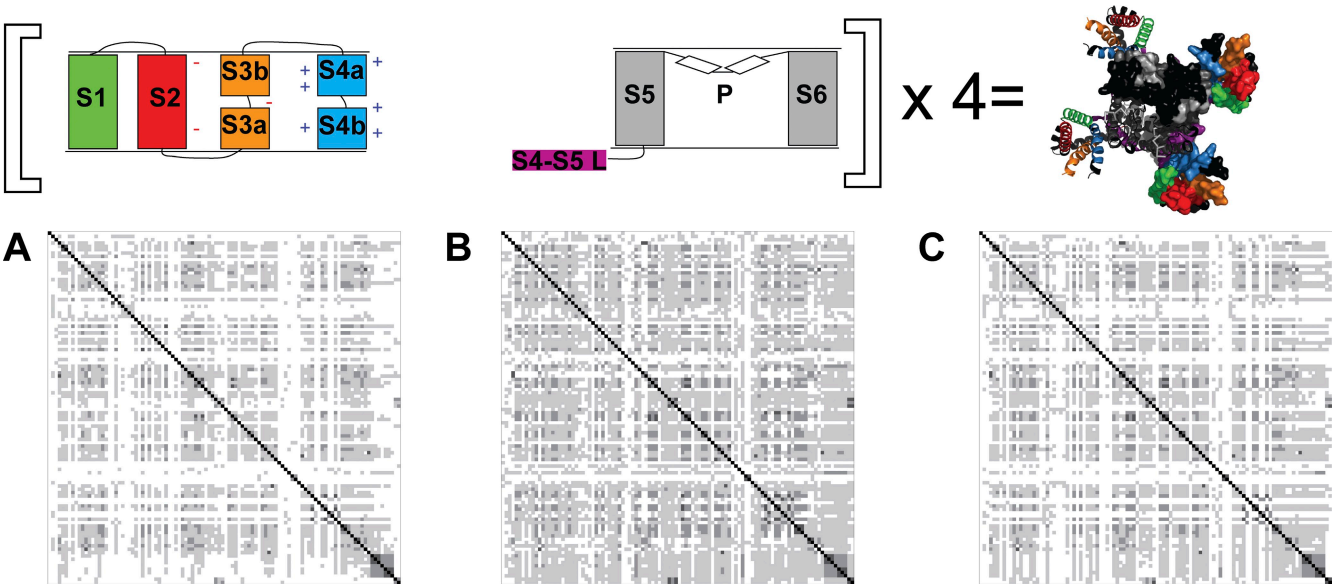

Figure S1. The voltage sensor module sequence contributes strongly to Kv sequence diversity. In Kv channels, four subunits assemble, each contributing one quarter of the pore (S5-P-S6) and a full voltage sensor (S1-S4) module. In the crystal structure as well as in a model in a lipid bilayer, the four sensor modules of the rodent Kv 1.2 (pdb 2A79) sit peripherally to the pore, one never touching the other (A–C, top). The near independent structural arrangement of the two modules could suggest that each module evolved with some degree of independence. To explore the contribution of each Kv module sequence variability to the overall sequence diversity observed in bacterial Kv channels, 85 full-length sequences of candidate bacterial Kv's were aligned with the sequences of KvAP and KvLm using Clustal X (v.1.83) (Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1997. *Nucleic Acids Res.* 25:4876–4882), and the resulting alignment was filtered to contain no two sequences sharing >90% sequence identity. The N and C termini of all sequences in the alignment were eliminated using as guidelines the structurally determined boundaries for the transmembrane segments in KvAP (Jiang, Y., A. Lee, J. Chen, V. Ruta, M. Cadene, B.T. Chait, and R. MacKinnon. 2003. *Nature.* 423:33–41) to generate the full-length alignment of the coupled modules. To generate the separate alignments of voltage sensor and pore module sequences, this alignment was dissected into two: the beginning of the pore module sequence, to include all of the linker between the two modules (S4-S5 linker), was determined from an alignment of the bacterial Kv sequences with the sequence of Kv1.2 channel from rat and from the crystal structure of this eukaryotic Kv channel that shows serine-311 (Fig. 1 B) to be the starting residue of the linker (Long, S.B., E.B. Campbell, and R. Mackinnon. 2005. *Science.* 309:897–903). The percent sequence identity for each of the two sequences in the three alignments was calculated and plotted as a matrix in which each matrix element, corresponding to a sequence identity value between two sequences in the alignment, is color coded: 0–25% identity in white, 25–50% in light gray, 50–75% in dark gray, and 75–100% in black. These pairwise sequence identity matrices for voltage sensor module (A, VSM: S1-S4), pore module including S4-S5 linker (B, PM:S4-S5L-S5-P-S6), and full-length without N and C termini (C) show that sequence identity in the pore module is markedly higher than in the voltage sensor module or full-length sequence, suggesting that the sequence of the voltage sensor contributes more strongly to Kv channel diversity.