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Supporting Material

Intra- and Intersubunit Dynamic Binding in Kv4.2 Channel Closed-State Inactivation

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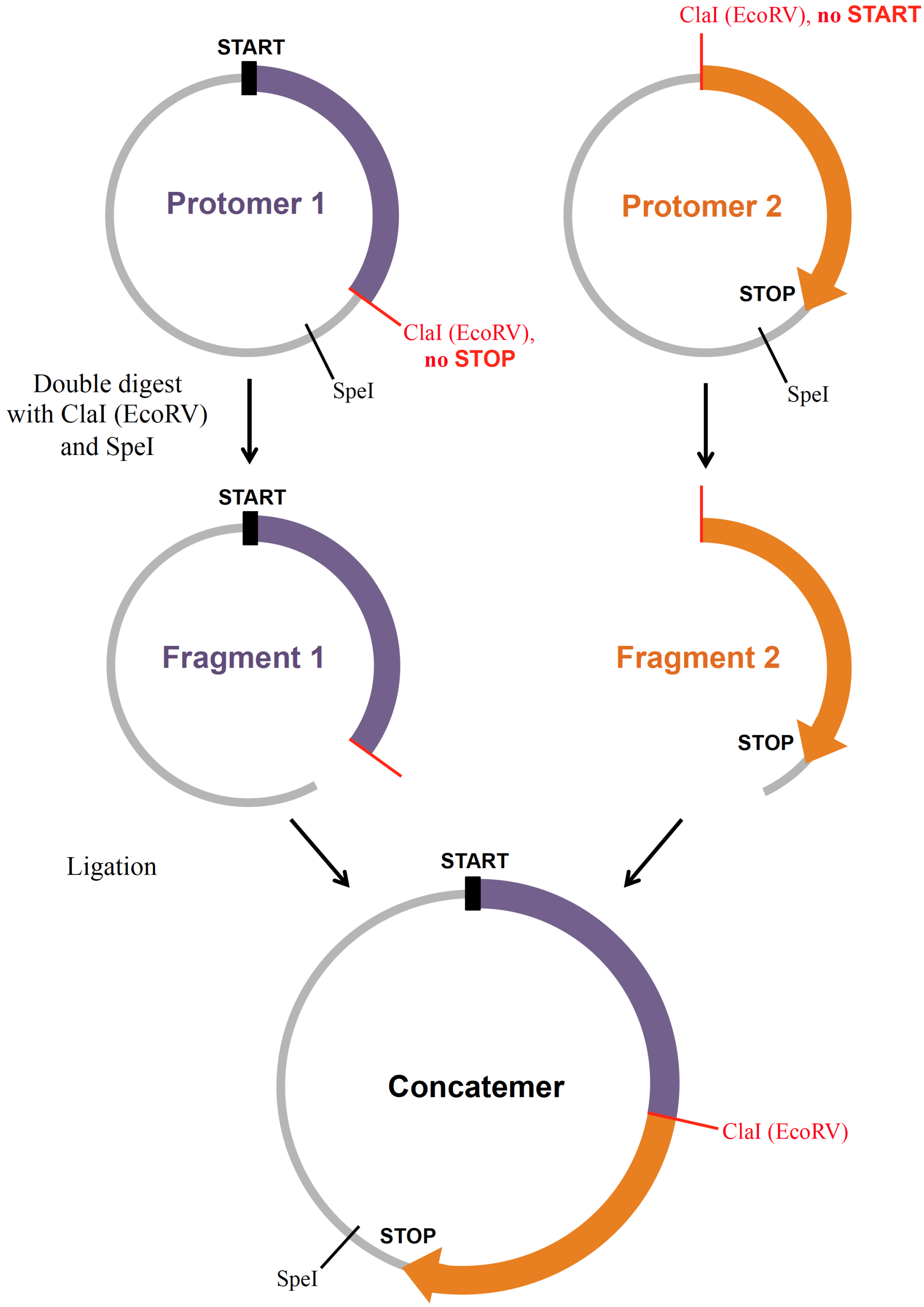

FIGURE S1 Cloning strategy for the generation of concatenated Kv4.2 tandem-dimer cDNA. Protomer 1: Endogenous SpeI site and engineered unique six-bases restriction site for ClaI (or EcoRV) instead of a stop codon (red); Kv4.2 coding sequence 1 in purple. Protomer 2: Endogenous SpeI site and engineered unique six-bases restriction site for ClaI (or EcoRV) instead of a start codon (red); Kv4.2 coding sequence 2 in orange. Double digest with ClaI (or EcoRV) and SpeI yields two different fragments: Fragment 1: START - Kv4.2 - ClaI (EcoRV); Fragment 2: ClaI (EcoRV) - Kv4.2 - STOP. Ligation (Fragment 2 into Fragment 1) results in concatenated cDNA (Concatemer). Point mutations were introduced before concatenation (i.e., in the protomers).

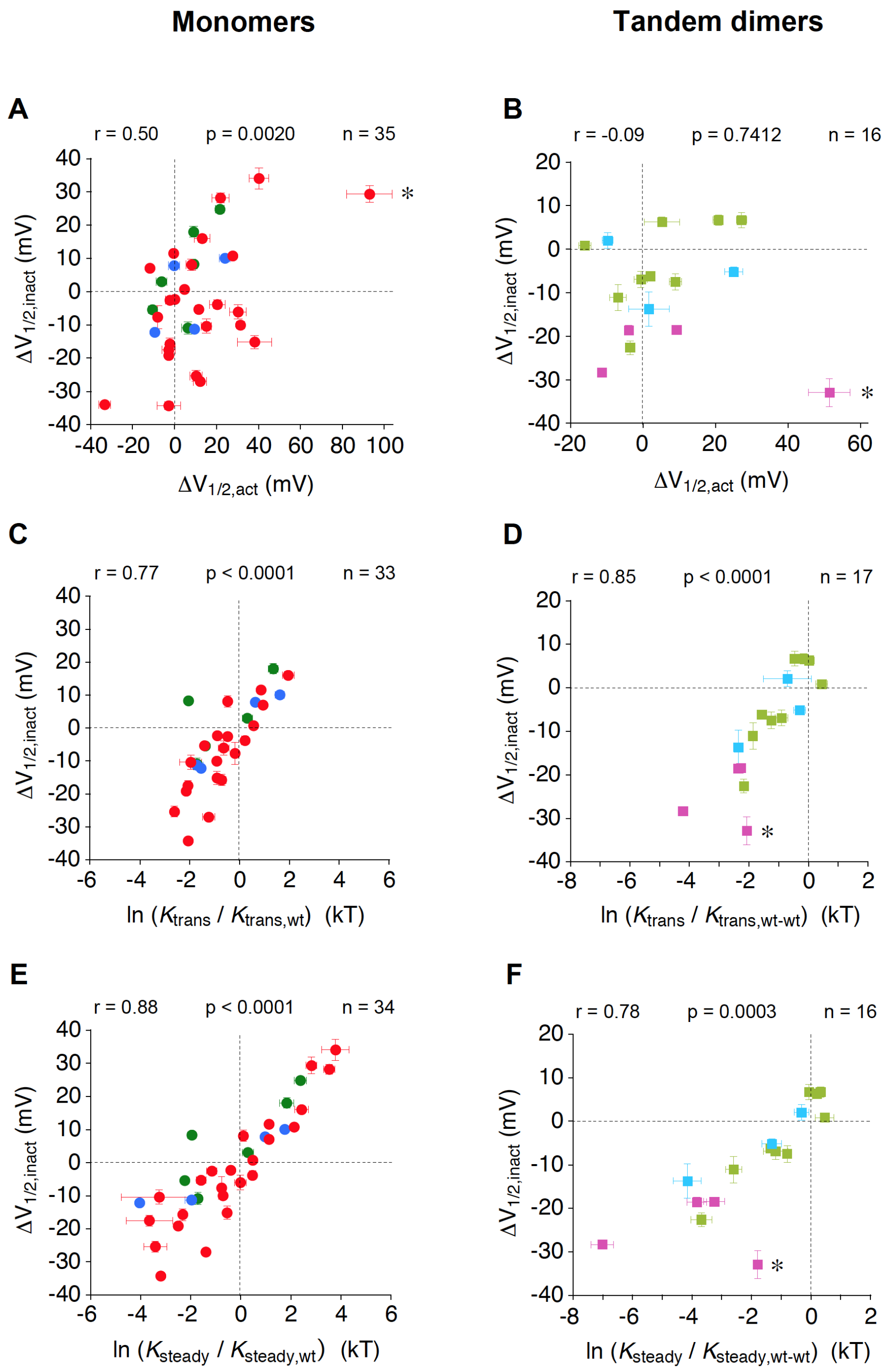

FIGURE S2 Correlation analyses for effects on low-voltage inactivation and effects on the voltage dependences of activation and inactivation for Kv4.2 mutants. Left: monomers (green: S4S5 single mutants, blue: S6 single mutants, red: double mutants); right: tandem dimers (light green: single mutants, light blue: double mutants intra-subunit configuration, magenta: double mutants inter-subunit configuration). For each construct the mutation-induced shift in the voltage dependence of isochronal (30 s prepulse) inactivation ($\Delta V_{1/2,inact}$) is plotted against the corresponding shift in the voltage dependence of peak conductance activation ($\Delta V_{1/2,act}$; *A* and *B*), the corresponding change in affinity for the transitory state expressed as ln ($K_{trans}/K_{trans,wt}$) (*C*) or ln ($K_{trans}/K_{trans,wt\text{-}wt}$) (*D*) and the corresponding change in affinity for steady-state inactivation expressed as ln ($K_{steady}/K_{steady,wt}$) (*E*) or ln ($K_{steady}/K_{steady,wt\text{-}wt}$) (*F*; see Table S1 for the analysis of voltage dependences, and Materials and Methods for the quantification of mutation-induced effects on low-voltage inactivation). Intersection points of dashed lines represent the wild-type monomer (left) or the wild-type dimer (in the absence or presence of auxiliary subunits, right). Two-tailed Pearson correlation analysis (r = Pearson correlation coefficient) was employed to test for an interdependence between the plotted parameters ($p < 0.05$). Significant correlations were found between the mutation-induced shift in the voltage dependence of inactivation and change in affinity for transitory and steady-state inactivation for both monomers and dimers. A significant correlation between the mutation-induced shifts in the voltage dependence of inactivation and activation was only found for the monomers. Asterisk in *A*: [311:408]; asterisks in *B*, *D* and *F*: [404]-[326] (see also Table S1).

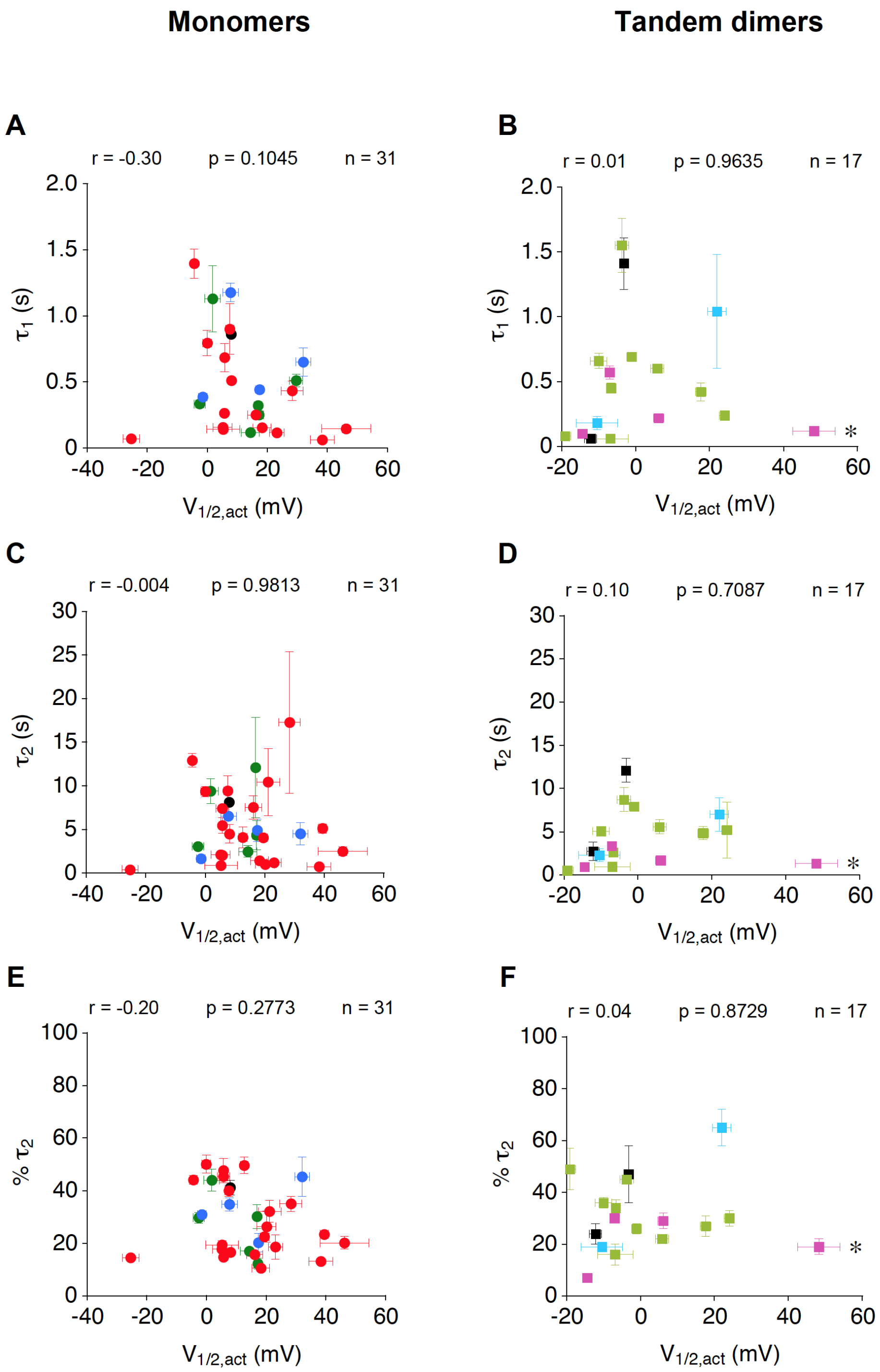

FIGURE S3 Correlation analyses of low-voltage inactivation kinetics and the voltage dependence of activation for wild-type and mutant Kv4.2 channels. Left: monomers (black: wild-type, green: S4S5 single mutants, blue: S6 single mutants, red: double mutants); right: tandem dimers (black: wild-type dimer in the absence and presence of auxiliary subunits, light green: single mutants, light blue: double mutants intra-subunit configuration, magenta: double mutants inter-subunit configuration). For each construct τ_1 (*A* and *B*), τ_2 (*C* and *D*) and the percentage of the total decay (i.e., to reach ss) accounted for by τ_2 (*E* and *F*) is plotted against the corresponding voltage for half-maximal peak conductance activation ($V_{1/2,act}$, see also Tables S1 and S2). Two-tailed Pearson correlation analysis (r = Pearson correlation coefficient) was employed to test for an interdependence of the plotted parameters ($p < 0.05$). No significant correlations were found. Asterisks in *B*, *D* and *F*: [404]-[326].

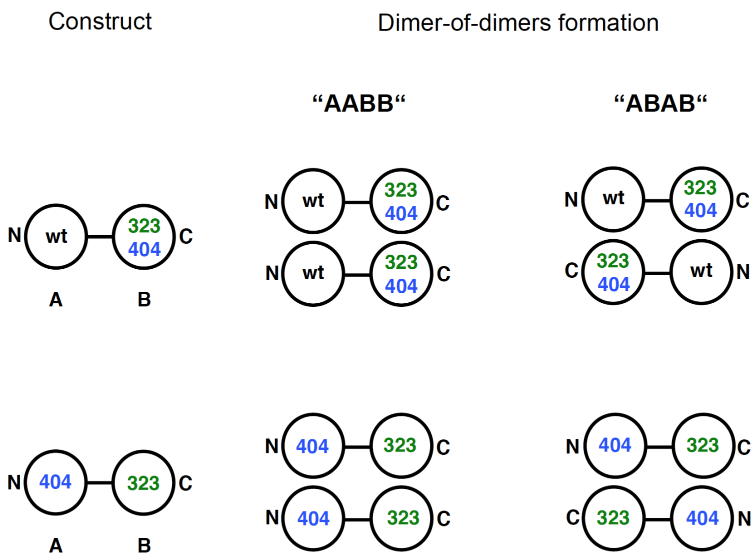

FIGURE S4 Dimer-of-dimers formation with Kv4.2 constructs. Dimer formation is schematically illustrated for the intra dimer [wt]-[323:404] and the corresponding inter dimer [404]-[323] as examples. The dimers are defined by the two entities: A (harboring the N-terminus, N) and B (harboring the C-terminus, C). Two different principal arrangements (AABB or ABAB) can be distinguished. In the "adjacent" AABB configuration the two N-termini point in the same direction. In the ABAB configuration, which reflects the rotational symmetry of voltage-gated sodium and calcium channels, the two N-termini point in opposite directions.

TABLE S1

Voltage dependence of activation and inactivation for monomeric and tandem-dimer Kv4.2 constructs

	$V_{1/2,act}$ (mV)	k_{act} (mV)	n	$V_{1/2,inact}$ (mV)	k_{inact} (mV)	n
[wt]	+8.03 ± 0.56	29.9 ± 1.21	10	-67.3 ± 0.58	5.91 ± 0.36	7
[309]	-2.47 ± 1.90	29.6 ± 0.61	5	-75.0 ± 0.61	4.90 ± 0.13	5
[311]	+17.0 ± 1.08	29.8 ± 0.33	6	-51.6 ± 1.57	7.44 ± 0.39	4
[313]	+14.4 ± 3.01	39.3 ± 3.62	5	-80.4 ± 1.82	9.66 ± 1.93	4
[322]	+1.84 ± 2.62	25.7 ± 0.94	8	-64.3 ± 1.03	6.05 ± 0.82	5
[323]	+17.3 ± 1.34	37.8 ± 1.52	7	-79.1 ± 0.62	6.76 ± 0.24	6
[326]	+29.7 ± 2.16	35.4 ± 2.43	5	-44.8 ± 0.50	6.86 ± 0.09	5
[400]	-	-		-96.3 ± 2.36	8.81 ± 0.52	3
[404]	+17.5 ± 0.68	36.3 ± 0.86	5	-78.6 ± 0.54	6.60 ± 0.33	4
[407]	+7.80 ± 2.62	28.9 ± 1.09	3	-61.8 ± 0.07	5.75 ± 0.18	3
[408]	+32.1 ± 2.49	35.8 ± 1.38	8	-57.2 ± 0.87	6.48 ± 0.67	7
[412]	-1.44 ± 1.45	29.5 ± 1.07	3	-81.7 ± 0.56	4.85 ± 0.35	3
[309:404]	+20.2 ± 3.20	54.3 ± 3.92	6	-96.6 ± 0.84	5.25 ± 0.26	6
[309:407]	+5.80 ± 2.10	31.0 ± 0.68	5	-72.1 ± 0.88	4.29 ± 0.34	5
[309:408]	+21.2 ± 3.86	30.8 ± 1.17	5	-53.5 ± 1.19	4.49 ± 0.32	5
[309:412]	+39.5 ± 1.38	50.8 ± 2.71	3	-79.6 ± 1.10	11.4 ± 0.66	4
[311:400]	+12.7 ± 1.34	38.1 ± 1.20	3	-68.9 ± 0.50	11.8 ± 0.24	3
[311:404]	+19.5 ± 1.46	36.7 ± 0.64	4	-74.9 ± 0.65	5.95 ± 0.35	4
[311:407]	+30.0 ± 4.10	29.9 ± 2.26	4	-41.4 ± 1.47	5.57 ± 0.75	4
[311:408]	+101 ± 10.9	55.6 ± 3.33	3	-40.2 ± 2.51	9.64 ± 1.13	5
[311:412]	+28.4 ± 3.67	42.6 ± 2.31	4	-71.1 ± 1.36	13.2 ± 0.60	3
[313:400]	-25.3 ± 2.72	54.4 ± 9.11	4	-104 ± 1.05	6.40 ± 0.28	4
[313:404]	-	-		-	-	
[313:407]	+16.2 ± 2.74	37.1 ± 3.97	4	-61.5 ± 1.69	8.82 ± 0.22	4
[313:408]	+38.4 ± 4.01	43.5 ± 2.27	5	-75.6 ± 2.18	10.5 ± 1.33	4
[313:412]	+5.25 ± 3.18	32.6 ± 1.15	4	-84.8 ± 1.61	10.2 ± 0.70	5
[322:400]	+5.80 ± 0.50	33.1 ± 1.04	5	-83.1 ± 1.73	6.88 ± 0.24	5
[322:404]	+18.3 ± 2.97	39.6 ± 1.38	5	-92.7 ± 1.66	7.37 ± 0.58	4
[322:407]	+7.56 ± 1.34	28.3 ± 0.40	3	-58.0 ± 0.87	8.99 ± 0.75	3
[322:408]	-	-		-55.9 ± 1.05	8.41 ± 0.47	3
[322:412]	0.002 ± 1.43	29.0 ± 1.04	6	-77.2 ± 3.42	8.72 ± 1.55	5
[323:400]	+5.32 ± 5.48	49.9 ± 6.72	4	-104 ± 1.10	6.52 ± 0.30	5
[323:404]	+23.2 ± 2.38	44.2 ± 1.54	10	-77.7 ± 2.12	6.78 ± 0.93	4
[323:407]	+8.14 ± 1.16	33.4 ± 1.67	3	-69.6 ± 0.11	6.32 ± 0.28	4
[323:408]	-	-		-74.3 ± 0.88	7.17 ± 0.56	3
[323:412]	+5.75 ± 0.68	34.4 ± 0.20	4	-88.8 ± 0.52	9.27 ± 0.26	4
[326:400]	-4.31 ± 0.55	24.2 ± 0.16	5	-62.6 ± 0.34	5.51 ± 0.23	5
[326:404]	+46.3 ± 8.20	53.0 ± 2.66	8	-84.2 ± 2.01	8.75 ± 1.05	6
[326:407]	+48.3 ± 4.63	43.0 ± 3.08	8	-35.5 ± 3.20	13.6 ± 2.07	5
[326:408]	-	-		-	-	
[326:412]	+35.8 ± 1.38	48.3 ± 2.40	5	-56.6 ± 0.98	9.24 ± 0.46	7

[wt]-[wt]	-3.13 ± 1.28	32.3 ± 0.75	3	-62.9 ± 0.60	4.72 ± 0.18	3
[wt]-[309]	-3.69 ± 1.84	23.7 ± 1.85	3	-69.8 ± 1.81	4.63 ± 0.25	3
[412]-[wt]	-6.63 ± 0.76	26.8 ± 1.26	7	-85.5 ± 1.56	7.44 ± 0.81	7
[412]-[309]	-6.98 ± 0.96	27.2 ± 1.14	5	-81.5 ± 1.09	7.10 ± 0.16	5
[wt]-[wt] (+K+D)	-12.1 ± 1.65	23.7 ± 0.51 [a]	4	-64.3 ± 0.24	7.16 ± 0.31	4
[322]-[wt] (+K+D)	-	-		-71.6 ± 0.68	6.11 ± 0.20	3
[404]-[wt] (+K+D)	-6.80 ± 4.81	31.8 ± 1.93 [a]	6	-58.1 ± 1.11	10.9 ± 0.77	5
[322:404]-[wt] (+K+D)	-10.4 ± 5.55	30.4 ± 2.24	7	-78.0 ± 3.95	5.21 ± 0.43	7
[wt]-[322]	-1.04 ± 1.29	27.5 ± 0.21	4	-69.1 ± 0.73	6.45 ± 0.19	4
[404]-[wt]	+24.1 ± 1.42	42.7 ± 1.95	12	-56.2 ± 1.70	12.0 ± 0.96	4
[404]-[322]	+6.20 ± 1.40	33.1 ± 0.85	8	-81.4 ± 0.97	5.12 ± 0.52	6
[wt]-[323]	-9.94 ± 2.29	25.5 ± 0.83	5	-74.0 ± 3.03	5.54 ± 0.37	3
[wt]-[404]	-19.1 ± 1.67	18.3 ± 0.57	3	-62.1 ± 0.54	4.60 ± 0.19	3
[wt]-[323:404]	-12.8 ± 1.45	21.9 ± 0.36	5	-60.9 ± 1.84	4.47 ± 0.28	4
[404]-[323]	-14.4 ± 1.12	28.9 ± 0.39	8	-91.2 ± 0.47	5.13 ± 0.11	8
[326]-[wt]	+17.7 ± 1.40	34.1 ± 0.91	5	-56.2 ± 1.17	7.11 ± 0.37	6
[326:404]-[wt]	+22.0 ± 2.52	31.3 ± 2.27	6	-68.1 ± 1.07	4.64 ± 0.30	6
[wt]-[326]	+5.90 ± 1.74	31.0 ± 1.33	4	-70.4 ± 1.88	6.83 ± 0.54	3
[404]-[326]	+48.3 ± 5.78	50.2 ± 2.92	8	-95.8 ± 3.22	10.0 ± 0.52	5

Voltages of half-maximal activation ($V_{1/2,act}$) and inactivation ($V_{1/2,inact}$) and corresponding slope-factors (k_{act} and k_{inact}) for all Kv4.2 constructs used in the present study. Analysis of voltage dependences: Peak conductance-voltage (GV) relationships were determined based on the equation $G = I_P / (V - V_{rev})$; where I_P is the peak current amplitude at the test voltage V, and V_{rev} is the potassium reversal potential (-90 mV in our experiments). GV relationships were analysed with a fourth-order Boltzmann-function of the form $G / G_{max} = (m / (1 + \exp((V-V')/k_{act})))^4$; where G / G_{max} is the normalized peak conductance at the test voltage V. In some cases the GV curve did not saturate within the range of test voltages studied, and the GV data were re-normalized to m (extrapolated maximum) and re-fitted by the same equation. The voltage dependence is defined by V' (6.5% of the maximal conductance) and the slope factor k_{act}, however, the $V_{1/2,act}$ values given in the table are the voltages where the above function has a value of 0.5. The voltage dependence of isochronal (30 s prepulse) inactivation was analysed with a first-order Boltzmann-function of the form $I / I_{max} = 1 / (1 + \exp((V-V_{1/2,inact})/k_{inact}))$; where I / I_{max} is the relative current amplitude obtained with the prepulse voltage V. $V_{1/2,inact}$ and k_{inact} are the voltage of halfmaximal inactivation and the slope factor, respectively; [a] note that auxiliary subunit co-expression (+K+D) causes a negative shift in the voltage dependence of activation for [wt]-[wt] and [404]-[wt].

TABLE S2

Kinetics of onset of low-voltage inactivation in monomeric and tandem-dimer Kv4.2 constructs

	τ_1 (s)	τ_2 (s)	% τ_2	n
[wt]	0.86 ± 0.02	8.12 ± 0.33	41 ± 3	7
[309]	0.33 ± 0.03	3.03 ± 0.14	30 ± 2	5
[311]	0.32 ± 0.03	12.1 ± 5.76	30 ± 5	4
[313]	0.12 ± 0.01	2.47 ± 0.66	17 ± 1	4
[322]	1.13 ± 0.25	9.37 ± 1.42	44 ± 4	7
[323]	0.25 ± 0.01	4.35 ± 1.69	12 ± 2	6
[326]	-	-	-	4
[400]	0.62 ± 0.14	7.61 ± 1.02	49 ± 6	3
[404]	0.44 ± 0.03	4.90 ± 1.29	20 ± 4	4
[407]	1.18 ± 0.07	6.51 ± 0.45	35 ± 3	5
[408]	0.65 ± 0.11	4.50 ± 1.27	45 ± 8	7
[412]	0.39 ± 0.02	1.66 ± 0.03	31 ± 1	5
[309:404]	0.09 ± 0.01	1.00 ± 0.19	26 ± 6	6
[309:407]	1.03 ± 0.02	7.40 ± 0.38	45 ± 2	6
[309:408]	0.47 ± 0.05	10.4 ± 3.84	32 ± 4	5
[309:412]	0.45 ± 0.02	5.12 ± 0.55	23 ± 1	5
[311:400]	0.38 ± 0.11	4.10 ± 1.18	50 ± 3	4
[311:404]	0.26 ± 0.02	4.04 ± 0.36	23 ± 0.4	5
[311:407]	-	-	-	6
[311:408]	-	-	-	5
[311:412]	0.43 ± 0.08	17.3 ± 8.12	35 ± 3	6
[313:400]	0.07 ± 0.002	0.39 ± 0.03	15 ± 1	4
[313:404]	0.26 ± 0.03	4.98 ± 1.98	27 ± 7	3
[313:407]	0.25 ± 0.01	7.53 ± 1.34	16 ± 1	6
[313:408]	0.06 ± 0.003	0.71 ± 0.06	13 ± 1	5
[313:412]	0.15 ± 0.01	2.09 ± 0.17	18 ± 1	5
[322:400]	0.68 ± 0.11	5.45 ± 0.88	48 ± 5	5
[322:404]	0.15 ± 0.02	1.41 ± 0.28	11 ± 1	4
[322:407]	0.90 ± 0.19	9.41 ± 1.75	40 ± 2	5
[322:408]	0.64 ± 0.19	14.8 ± 3.22	37 ± 12	3
[322:412]	0.79 ± 0.09	9.35 ± 0.60	50 ± 3	6
[323:400]	0.14 ± 0.004	0.85 ± 0.05	19 ± 2	6
[323:404]	0.11 ± 0.02	1.18 ± 0.31	19 ± 5	3
[323:407]	0.51 ± 0.03	4.48 ± 1.07	17 ± 1	6
[323:408]	0.43 ± 0.03	3.12 ± 0.62	16 ± 2	6
[323:412]	0.26 ± 0.003	2.02 ± 0.11	15 ± 0.3	5
[326:400]	1.40 ± 0.11	12.9 ± 0.77	44 ± 2	5
[326:404]	0.15 ± 0.03	2.49 ± 0.53	20 ± 2	6
[326:407]	-	-	-	5
[326:408]	-	-	-	5
[326:412]	-	-	-	5

[wt]-[wt]	1.41 ± 0.20	12.1 ± 1.35	47 ± 11	3
[wt]-[309]	1.55 ± 0.21	8.71 ± 1.40	45 ± 1	4
[412]-[wt]	0.45 ± 0.04	2.63 ± 0.38	34 ± 3	6
[412]-[309]	0.57 ± 0.05	3.33 ± 0.38	30 ± 1	5
[wt]-[wt] (+K+D)	0.06 ± 0.01	2.71 ± 1.05	24 ± 4	6
[322]-[wt] (+K+D)	0.67 ± 0.03	5.86 ± 0.56	24 ± 2	4
[404]-[wt] (+K+D)	0.06 ± 0.003	0.93 ± 0.50	16 ± 4	4
[322:404]-[wt] (+K+D)	0.18 ± 0.05	2.26 ± 0.73	19 ± 1	5
[wt]-[322]	0.69 ± 0.01	7.91 ± 0.36	26 ± 2	4
[404]-[wt]	0.24 ± 0.03	5.18 ± 3.22	30 ± 3	4
[404]-[322]	0.22 ± 0.02	1.66 ± 0.31	29 ± 3	5
[wt]-[323]	0.66 ± 0.06	5.07 ± 0.35	36 ± 2	4
[wt]-[404]	0.08 ± 0.01	0.48 ± 0.18	49 ± 8	4
[wt]-[323:404]	-	-	-	5
[404]-[323]	0.10 ± 0.004	0.92 ± 0.09	7 ± 1	8
[326]-[wt]	0.42 ± 0.07	4.83 ± 0.78	27 ± 4	6
[326:404]-[wt]	1.04 ± 0.44	6.98 ± 1.96	65 ± 7	4
[wt]-[326]	0.60 ± 0.03	5.56 ± 0.80	22 ± 0.3	3
[404]-[326]	0.12 ± 0.01	1.31 ± 0.17	19 ± 3	5

Time constants of low-voltage inactivation (τ_1 and τ_2) obtained by double-exponential fitting of the onset of low-voltage inactivation; % τ_2, relative contribution of τ_2 to the total decay (i.e., to reach ss).

TABLE S3

S4S5/S6 functional coupling analysis for monomeric and tandem-dimer Kv4.2 constructs

	1 - a_1	K_{trans}	Ω_{trans}	**ss**	K_{steady}	Ω_{steady}	**n**
[wt]	0.685 ± 0.008	2.19 ± 0.08		0.457 ± 0.032	0.88 ± 0.11		7
[309]	0.358 ± 0.025	0.57 ± 0.06		0.088 ± 0.009	0.10 ± 0.01		5
[311]	0.891 ± 0.017	9.09 ± 2.08		0.837 ± 0.033	6.33 ± 2.06		4
[313]	0.286 ± 0.023	0.41 ± 0.04		0.142 ± 0.018	0.17 ± 0.02		4
[322]	0.740 ± 0.042	3.31 ± 0.49		0.540 ± 0.054	1.35 ± 0.27		7
[323]	0.224 ± 0.024	0.30 ± 0.04		0.117 ± 0.016	0.13 ± 0.02		6
[326]	-	-		0.899 ± 0.020	10.5 ± 2.79		4
[400]	0.583 ± 0.068	1.56 ± 0.49		0.195 ± 0.039	0.25 ± 0.06		3
[404]	0.292 ± 0.051	0.44 ± 0.11		0.117 ± 0.026	0.14 ± 0.03		4
[407]	0.804 ± 0.010	4.16 ± 0.29		0.700 ± 0.010	2.34 ± 0.12		5
[408]	0.911 ± 0.014	12.5 ± 2.56		0.832 ± 0.016	5.31 ± 0.62		7
[412]	0.320 ± 0.014	0.47 ± 0.03		0.016 ± 0.002	0.02 ± 0.002		5
[309:404]	0.392 ± 0.058	0.77 ± 0.26	6.78 ± 2.92	0.182 ± 0.018	0.23 ± 0.03	15.2 ± 5.03	6
[309:407]	0.576 ± 0.029	1.41 ± 0.14	1.31 ± 0.22	0.228 ± 0.031	0.31 ± 0.05	1.18 ± 0.28	6
[309:408]	0.934 ± 0.013	17.5 ± 4.27	5.41 ± 1.83	0.901 ± 0.020	11.9 ± 3.68	20.2 ± 7.47	5
[309:412]	0.468 ± 0.020	0.89 ± 0.07	7.26 ± 1.08	0.306 ± 0.023	0.45 ± 0.04	250 ± 59.4	5
[311:400]	0.791 ± 0.024	3.97 ± 0.58	1.63 ± 0.26	0.588 ± 0.029	1.47 ± 0.20	1.22 ± 0.37	4
[311:404]	0.350 ± 0.019	0.54 ± 0.05	3.34 ± 0.10	0.161 ± 0.025	0.20 ± 0.04	4.98 ± 0.09	5
[311:407]	-	-	-	0.964 ± 0.009	34.0 ± 6.86	2.01 ± 0.81	6
[311:408]	-	-	-	0.932 ± 0.014	16.2 ± 3.10	2.37 ± 0.17	5
[311:412]	0.731 ± 0.010	2.74 ± 0.15	1.40 ± 0.34	0.584 ± 0.020	1.43 ± 0.11	12.7 ± 4.77	6
[313:400]	0.146 ± 0.010	0.17 ± 0.01	1.69 ± 0.20	0.002 ± 0.002	0.002 ± 0.002	26.8 ± 0.04	4
[313:404]	0.604 ± 0.048	1.61 ± 0.36	20.0 ± 7.00	0.459 ± 0.038	0.86 ± 0.12	33.7 ± 11.6	3
[313:407]	0.575 ± 0.034	1.43 ± 0.20	1.85 ± 0.35	0.495 ± 0.041	1.05 ± 0.17	2.36 ± 0.60	6
[313:408]	0.534 ± 0.051	1.29 ± 0.33	1.80 ± 0.19	0.465 ± 0.056	0.98 ± 0.27	1.03 ± 0.34	5
[313:412]	0.221 ± 0.026	0.29 ± 0.05	3.31 ± 0.70	0.053 ± 0.027	0.06 ± 0.03	19.6 ± 11.6	5
[322:400]	0.518 ± 0.043	1.14 ± 0.19	2.07 ± 0.19	0.080 ± 0.003	0.09 ± 0.003	4.37 ± 0.08	5
[322:404]	0.140 ± 0.018	0.16 ± 0.02	1.76 ± 0.19	0.039 ± 0.020	0.04 ± 0.02	5.37 ± 0.12	4
[322:407]	0.835 ± 0.021	5.48 ± 0.88	1.15 ± 0.20	0.728 ± 0.028	2.83 ± 0.40	1.28 ± 0.22	5
[322:408]	0.866 ± 0.044	8.55 ± 3.37	2.21 ± 0.21	0.794 ± 0.045	4.26 ± 0.94	1.92 ± 0.18	3
[322:412]	0.646 ± 0.026	1.89 ± 0.18	2.64 ± 0.50	0.292 ± 0.016	0.42 ± 0.03	16.7 ± 4.74	6
[323:400]	0.222 ± 0.018	0.29 ± 0.03	1.37 ± 0.50	0.036 ± 0.004	0.04 ± 0.01	1.00 ± 0.34	6
[323:404]	0.255 ± 0.075	0.37 ± 0.13	6.26 ± 2.88	0.090 ± 0.044	0.10 ± 0.05	5.01 ± 2.94	3
[323:407]	0.475 ± 0.015	0.91 ± 0.05	1.62 ± 0.28	0.372 ± 0.013	0.59 ± 0.03	1.66 ± 0.34	6
[323:408]	0.422 ± 0.021	0.74 ± 0.07	2.27 ± 0.12	0.311 ± 0.015	0.46 ± 0.03	1.79 ± 0.13	6
[323:412]	0.205 ± 0.006	0.26 ± 0.01	4.04 ± 0.66 [a]	0.068 ± 0.005	0.07 ± 0.01	29.8 ± 7.48	5
[326:400]	0.850 ± 0.005	5.69 ± 0.25	-	0.730 ± 0.013	2.75 ± 0.19	1.08 ± 0.36	5
[326:404]	0.471 ± 0.029	0.92 ± 0.11	-	0.339 ± 0.019	0.52 ± 0.05	3.12 ± 0.13	6
[326:407]	-	-	-	0.961 ± 0.017	73.0 ± 43.5	2.60 ± 1.73	5
[326:408]	-	-	-	0.829 ± 0.009	4.92 ± 0.31	12.9 ± 0.02	5
[326:412]	-	-	-	0.879 ± 0.015	7.76 ± 0.99	40.2 ± 14.0 [a]	5

[wt]-[wt]	0.830 ± 0.030	5.34 ± 1.34		0.664 ± 0.065	2.18 ± 0.52		3
[wt]-[309]	0.679 ± 0.045	2.30 ± 0.46		0.413 ± 0.090	0.83 ± 0.27		4
[412]-[wt]	0.382 ± 0.032	0.64 ± 0.08		0.064 ± 0.015	0.07 ± 0.02		6
[412]-[309]	0.339 ± 0.023	0.52 ± 0.05	1.90 ± 0.68	0.054 ± 0.016	0.06 ± 0.02	2.20 ± 1.28	5
[wt]-[wt] (+K+D)	0.735 ± 0.031	3.02 ± 0.44		0.646 ± 0.045	2.01 ± 0.29		6
[322]-[wt] (+K+D)	0.406 ± 0.029	0.70 ± 0.08		0.223 ± 0.029	0.29 ± 0.05		4
[404]-[wt] (+K+D)	0.752 ± 0.034	3.24 ± 0.49		0.709 ± 0.030	2.53 ± 0.30		4
[322:404]-[wt] (+K+D)	0.226 ± 0.020	0.30 ± 0.03	2.53 ± 0.11	0.041 ± 0.013	0.04 ± 0.02	8.37 ± 0.05	5
[wt]-[322]	0.527 ± 0.029	1.14 ± 0.15		0.361 ± 0.028	0.57 ± 0.07		4
[404]-[wt]	0.770 ± 0.016	3.42 ± 0.31		0.670 ± 0.029	2.11 ± 0.29		4
[404]-[322]	0.358 ± 0.035	0.58 ± 0.10	1.26 ± 0.27	0.094 ± 0.032	0.11 ± 0.04	5.07 ± 0.10	5
[wt]-[323]	0.453 ± 0.039	0.86 ± 0.13		0.149 ± 0.033	0.18 ± 0.04		4
[wt]-[404]	0.889 ± 0.021	8.84 ± 1.62		0.762 ± 0.058	3.96 ± 1.08		4
[wt]-[323:404]	-	-	-	0.607 ± 0.058	1.80 ± 0.43	5.48 ± 2.68	5
[404]-[323]	0.076 ± 0.009	0.08 ± 0.01	6.62 ± 0.05	0.004 ± 0.003	0.01 ± 0.003	39.4 ± 0.02	8
[326]-[wt]	0.817 ± 0.016	4.66 ± 0.49		0.751 ± 0.013	3.07 ± 0.22		6
[326:404]-[wt]	0.794 ± 0.039	4.23 ± 0.80	1.42 ± 0.49	0.382 ± 0.071	0.68 ± 0.18	4.40 ± 0.09	4
[wt]-[326]	0.606 ± 0.026	1.56 ± 0.17		0.496 ± 0.032	1.00 ± 0.13		3
[404]-[326]	0.406 ± 0.026	0.70 ± 0.07	1.44 ± 0.21 [a]	0.269 ± 0.013	0.37 ± 0.02	2.62 ± 0.12 [a]	5

Coupling coefficients for the transitory state (Ω_{trans}) and steady-state inactivation (Ω_{steady}) were obtained with double-mutant cycle analysis based on K_{trans} and K_{steady} values; $K_{trans} = (1 - a_1) / a_1$; $K_{steady} = ss / (1 - ss)$. Fractions a_1 and ss were obtained with double-exponential fitting of onset of low-voltage inactivation (see Materials and Methods). [a] SEM values based on linear and independent error propagation.