Supporting Information

Table S1. Parameters from Boltzmann Equation fits to GV data.

Boltzmann Coefficients		Offset			G/G_{max}			$V_{1/2}$ (mV)			Slope (mV)			
Construct		mean	±	sem	mean	±	sem	mean	±	sem	mean	±	sem	n =
SpIHwt	No cNMP*	-0.013	±	0.016	0.033	±	0.021	-74	±	13	-38.3	±	34.8	11
	cAMP	0.008	±	0.003	1	±	0	-67	±	2	-6.1	±	0.2	11
	cGMP	-0.001	±	0.003	0.372	±	0.020	-71	±	1	-6.6	±	0.2	11
SpIHΔCterm	No cNMP	-0.015	±	0.006	1	±	0	-97	±	3	-6.6	±	0.7	4
359:360	No cNMP*	0.023	±	0.012	0.069	±	0.015	-33	±	4	-5.9	±	2.1	6
1:1	cAMP	0.186	±	0.024	1	±	0	-38	±	3	-8.3	±	0.6	6
	cGMP	0.069	±	0.020	0.414	±	0.075	-46	±	2	-10.1	±	0.8	6
359:360	No cNMP*	0.027	±	0.005	0.052	±	0.007	-19	±	12	-3.0	±	1.5	3
4:1	cAMP	0.187	±	0.003	1	±	0	-40	±	1	-9.0	±	0.1	3
	cGMP	0.082	±	0.009	0.565	±	0.021	-50	±	1	-10.9	±	0.3	3
359:363	No cNMP*	0.009	±	0.015	0.035	±	0.007	-71	±	17	-9.0	±	5.2	4
	cAMP	0.022	±	0.017	1	±	0	-66	±	1	-7.0	±	0.5	4
	cGMP	0.015	±	0.015	0.362	±	0.045	-80	±	1	-8.0	±	0.6	4
355:360	No cNMP				0.030	±	0.001							3
	cAMP				0.959	±	0.013							3
	cGMP				0.211	±	0.049							3
350:360	No cNMP				0.056	±	0.011							3
	cAMP				0.942	±	0.037							3
	cGMP				0.283	±	0.016							3
350:363	No cNMP				0.210	±	0.046							5
	cAMP				0.958	±	0.011							5
	cGMP				0.490	±	0.063							5
350:367	No cNMP				0.685	±	0.043							3
	cAMP				0.979	±	0.007							3
	cGMP				0.893	±	0.007							3
359:367	No cNMP	0.265	±	0.034	0.515	±	0.051	-79	±	4	-8.8	±	0.4	7
1:1	cAMP	0.780	±	0.021	1	±	0	-54	±	2	-11.8	±	0.7	7
	cGMP	0.488	±	0.039	0.874	±	0.030	-69	±	3	-9.2	±	0.6	7
359:367	No cNMP	0.196	±	0.024	0.366	±	0.019	-85	±	4	-7.7	±	0.9	3
4:1	cAMP	0.740	±	0.008	1	±	0	-59	±	4	-13.9	±	1.4	3
	cGMP	0.423	±	0.014	0.852	±	0.006	-72	±	4	-10.9	±	0.7	3
A4	No cNMP‡	0.034	±	0.016				-48	±	6	6.8	±	0.4	3
	No cNMP‡	0.014	±	0.007				-73	±	8	-15.4	±	5.0	3
	cAMP	0.090	±	0.019	1	±	0	-40	±	4	-6.9	±	0.5	3
	cGMP ‡	0.274	±	0.003				-59	±	4	6.8	±	1.1	3
	cGMP ‡	0.047	±	0.011				-61	±	3	-9.5	±	2.9	3
A6	No cNMP‡	0.232	±	0.049				-62	±	8	11.3	±	0.7	3
	No cNMP‡	0.336	±	0.020				-34	±	3	-17.3	±	2.4	3
	cAMP	0.469	±	0.027	1	±	0	-3	±	7	-16.0	±	2.4	3
	cGMP	0.333	±	0.004	0.906	±	0.019	-23	±	5	-12.9	±	1.6	3
R350A	No cNMP‡	0.012	±	0.017				11	±	5	11.5	±	1.0	3
	No cNMP‡	0.006	±	0.001				-11	±	9	-11.4	±	1.2	3
	cAMP	0.079	±	0.013	1	±	0	-6	±	7	-10.2	±	-13.7	3
	cGMP	0.079	±	0.008	0.317	±	0.048	-19	±	7	0.5	±	2.6	3
F351A	No cNMP*	0.023	±	0.002				93	±	75	47.5	±	17.6	3
	No cNMP‡*	0.013	±	0.010				-71	±	3	-3.7	±	0.3	3
	cAMP	0.011	±	0.001	1	±	0	-81	±	1	-6.7	±	-6.0	3
	cGMP	0.005	±	0.003	0.005	±	0.029	-80	±	1	0.2	±	0.3	3
Q354A	No cNMP*	0.002	±	0.001	0.035	±	0.005	-62	±	3	-7.2	±	1.6	3
	cAMP	0.012	±	0.005	1	±	0	-65	±	1	-6.5	±	0.7	3
	cGMP	0.004	±	0.003	0.262	±	0.043	-70	±	1	-7.8	±	1.5	3
W355A	No cNMP*	0.008	±	0.012	0.011	±	0.001	-190	±	171	-135.3	±	134.7	3
	cAMP	0.026	±	0.004	1	±	0	-40	±	2	-5.2	±	0.5	3
	cGMP*	0.006	±	0.021	0.061	±	0.012	-25	±	4	-7.0	±	6.1	3
E356A	No cNMP‡	0.081	±	0.015		±		-52	±	4	8.9	±	3.6	3
	No cNMP‡	0.028	±	0.003		±		-67	±	3	-7.0	±	2.6	3
	cAMP	0.104	±	0.021	1	±	0	-47	±	1	-8.2	±	0.3	3
	cGMP	0.047	±	0.008	0.555	±	0.058	-64	±	1	-6.9	±	0.3	3
R367A	No cNMP	0.051	±	0.008	0.880	±	0.018	-70	±	3	-7.7	±	1.5	3
	cAMP	0.058	±	0.009	1	±	0	-63	±	3	-8.1	±	0.4	3
	cGMP	0.051	±	0.012	1.024	±	0.009	-69	±	2	-6.8	±	0.7	3
D471A	No cNMP	0.011	±	0.006	0.451	±	0.051	-64	±	2	-6.9	±	0.4	3
	cAMP	0.016	±	0.004	1	±	0	-59	±	1	-6.5	±	0.6	3
	cGMP	0.013	±	0.003	0.991	±	0.043	-68	±	1	-6.8	±	0.6	3
ΔQWE	No cNMP				0.572	±	0.039							5
	cAMP				0.970	±	0.008							5
	cGMP				0.872	±	0.029							3
ΔQAF	No cNMP	0.319	±	0.029	0.650	±	0.038	-37	±	2	7.7	±	0.5	5
	cAMP				1	±	0							5

Data shown as mean ± s.e.m. * not well determined. ‡ product of two Boltzmann eqn.

Supplemental figures:

Fig. S1. Sequence alignment of the S4-S5 regions of several HCN and related channels.

HCN1, human NM_021072.3; HCN2, human NM_001194.3; HCN2, human NM_020897.2; HCN4, human NM_005477.2; HCN1, mouse NM_010408.3; HCH2, mouse NM_008226.2; SpHCN, invertebrate NM_214564.1; CNGA1, bovine 1.NM_174278.2; Tax4, C. elegans NM_066632.4; EAG1, mouse NM_001038607.2; EAG, rat 1.NM_031742.1; ERG, human NM_172057.2; Kv1.2, rat NM_023954.1

Fig. S2. $SpHCN_{WT}$ current families recorded in the absence and presence of ligand along with block by ZD7288, an HCN channel specific blocker.

(*A*-*D*) $SpHCN_{WT}$ currents measured (*A*), in the absence of ligand, (*B*) presence of 1 mM cGMP, (*C*) 1 mM cAMP, and (*D*) 1 mM cAMP + 100 µM ZD7288 in response to test voltage steps from -20 mV and -120 mV in increments of -10 mV followed by a voltage step to +40 mV. (*E*) $SpHCN_{WT}$ currents measured during voltages steps to -100 and +100 mV as ZD7288 was applied to inside of patch. (*F*) Normalized current amplitudes measured at the end of the -100 mV pulse first in 1 mM cAMP (red bar) then in 1 mM cAMP + 100 µM ZD7288 (black bar). The data were fit by a single exponential with tau = 5.7 s.

Fig. S3. Functional split channels require co-expression of both VSD and PD, and reduced VSD-PD coupling is not a property of the VSD:PD ratio.

Oocytes were injected with different ratios of VSD:PD cRNAs: 4:1, 1;1, 0:1. (*A*) ZD-subtracted currents from split $spHCN_{(359:360)}$ channels with a break in the S4-S5 linker, (*B*) ZD-subtracted currents from split $spHCN_{(359:367)}$ channels with the S4-S5 linker + $S5_{N\text{-}term}$ region deleted. (*C*) GV curves from split $spHCN_{(359:360)}$ channels with cRNA ratios 4:1 (open symbols) and 1:1 (filled symbols). (*D*) GV curves from split $spHCN_{(359:367)}$ channels.

Fig. S4. Simple 4 state model for closed state inactivation.

(A) Model showing closed (C_0, C_1), open (O), and inactivated (I) states with equilibrium constants *H(V)*, *L*, and *J*.
(B) Po vs voltage plot shows activation without inactivation (red, *J* = 0), activation with voltage-independent inactivation (black, dashed, *J* = 200), and activation with voltage-dependent inactivation (black, solid, $J(V) = 0.1 * e^{(-V/8)}$). For all traces, $H(V) = 10^{-6}e^{(-V/8)}$ and *L* = 100).

Fig. S5. A simple state diagram which corresponds to the HA model shown in Fig. 2A.

State diagram shows how the equilibrium constants, *H*, *K*, and *L*, with coupling factors *F*, *C*, and *E* explicitly affect specific transitions.

Fig. S1

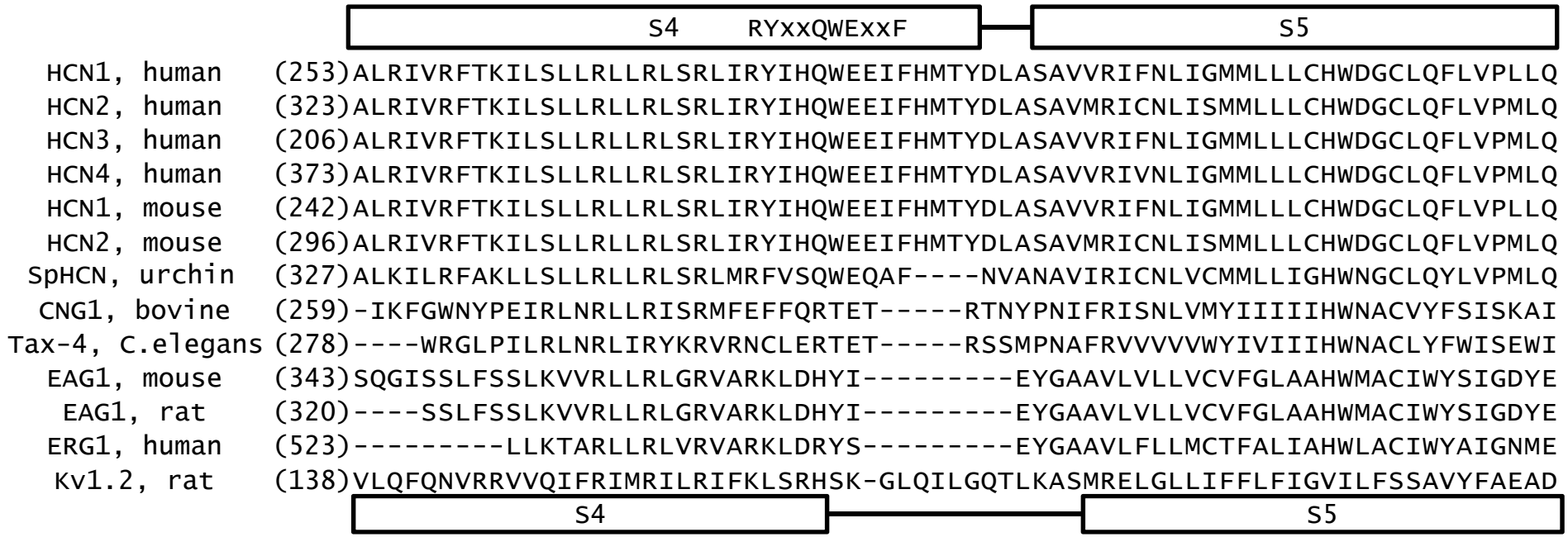

S4 RYxxQWExxF
S5
HCN1, human (253)ALRIVRFTKILSLLRLLRLSRLIRYIHQWEEIFHMTYDLASAVVRIFNLIGMMLLLCHWDGCLQFLVPLLQ
HCN2, human (323)ALRIVRFTKILSLLRLLRLSRLIRYIHQWEEIFHMTYDLASAVMRICNLISMMLLLCHWDGCLQFLVPMLQ
HCN3, human (206)ALRIVRFTKILSLLRLLRLSRLIRYIHQWEEIFHMTYDLASAVVRIFNLIGMMLLLCHWDGCLQFLVPMLQ
HCN4, human (373)ALRIVRFTKILSLLRLLRLSRLIRYIHQWEEIFHMTYDLASAVVRIVNLIGMMLLLCHWDGCLQFLVPMLQ
HCN1, mouse (242)ALRIVRFTKILSLLRLLRLSRLIRYIHQWEEIFHMTYDLASAVVRIFNLIGMMLLLCHWDGCLQFLVPLLQ
HCN2, mouse (296)ALRIVRFTKILSLLRLLRLSRLIRYIHQWEEIFHMTYDLASAVMRICNLISMMLLLCHWDGCLQFLVPMLQ
SpHCN, urchin (327)ALKILRFAKLLSLLRLLRLSRLMRFVSQWEQAF----NVANAVIRICNLVCMMLLIGHWNGCLQYLVPMLQ
CNG1, bovine (259)-IKFGWNYPEIRLNRLLRISRMFEFFQRTET-----RTNYPNIFRISNLVMYIIIIIHWNACVYFSISKAI
Tax-4, C.elegans (278)----WRGLPILRLNRLIRYKRVRNCLERTET-----RSSMPNAFRVVVVVWYIVIIIHWNACLYFWISEWI
EAG1, mouse (343)SQGISSLFSSLKVVRLLRLGRVARKLDHYI---------EYGAAVLVLLVCVFGLAAHWMACIWYSIGDYE
EAG1, rat (320)----SSLFSSLKVVRLLRLGRVARKLDHYI---------EYGAAVLVLLVCVFGLAAHWMACIWYSIGDYE
ERG1, human (523)---------LLKTARLLRLVRVARKLDRYS---------EYGAAVLFLLMCTFALIAHWLACIWYAIGNME
Kv1.2, rat (138)VLQFQNVRRVVQIFRIMRILRIFKLSRHSK-GLQILGQTLKASMRELGLLIFFLFIGVILFSSAVYFAEAD
S4
S5

Fig. S2

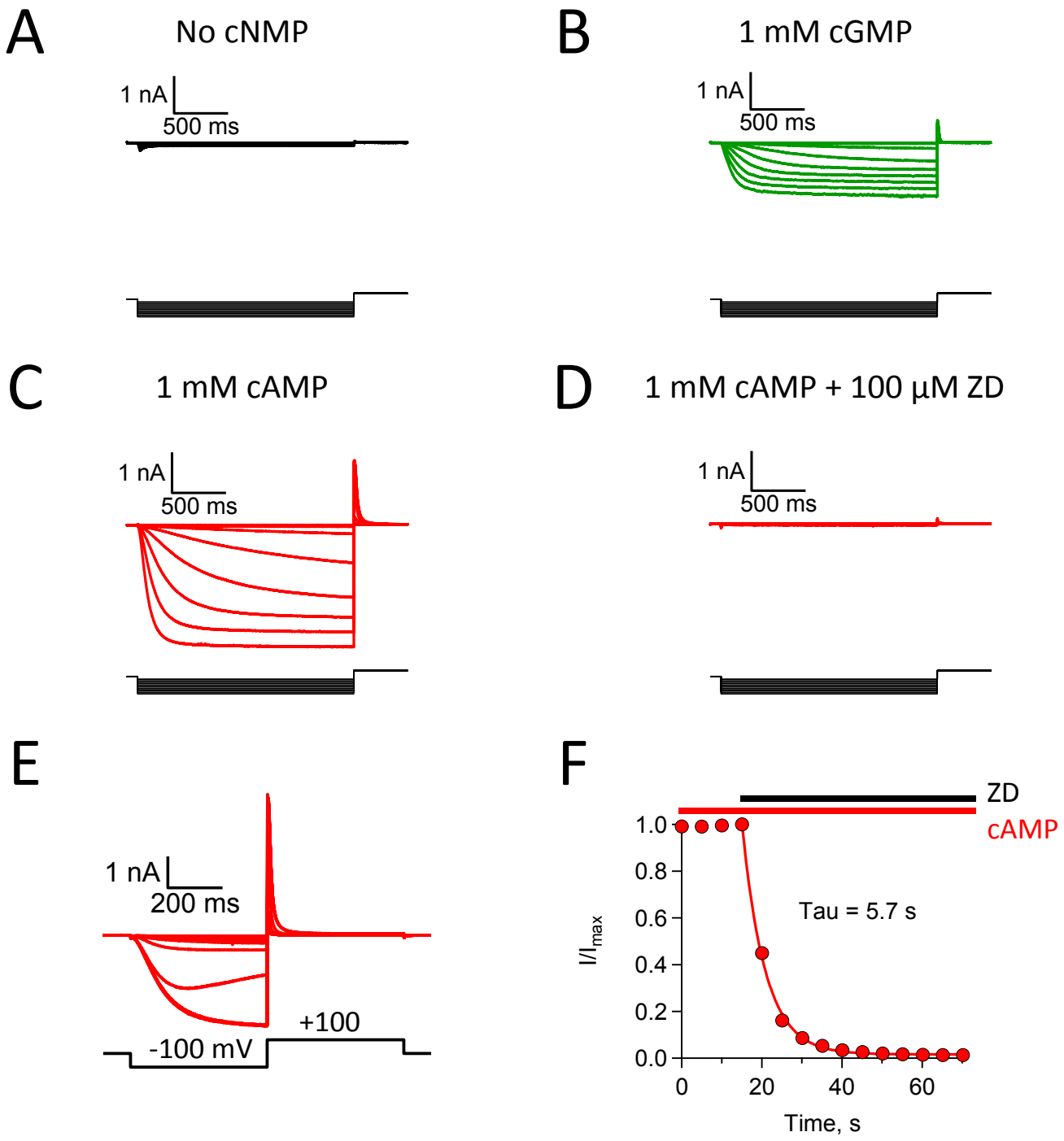

A
No cNMP
1 nA
500 ms
B
1 mM cGMP
1 nA
500 ms
C
1 mM cAMP
1 nA
500 ms
D
1 mM cAMP + 100 µM ZD
1 nA
500 ms
E
1 nA
200 ms
+100
-100 mV
F
ZD
cAMP
1.0
0.8
0.6
0.4
0.2
0.0
I/I_max
Tau = 5.7 s
0
20
40
60
Time, s

Fig. S3

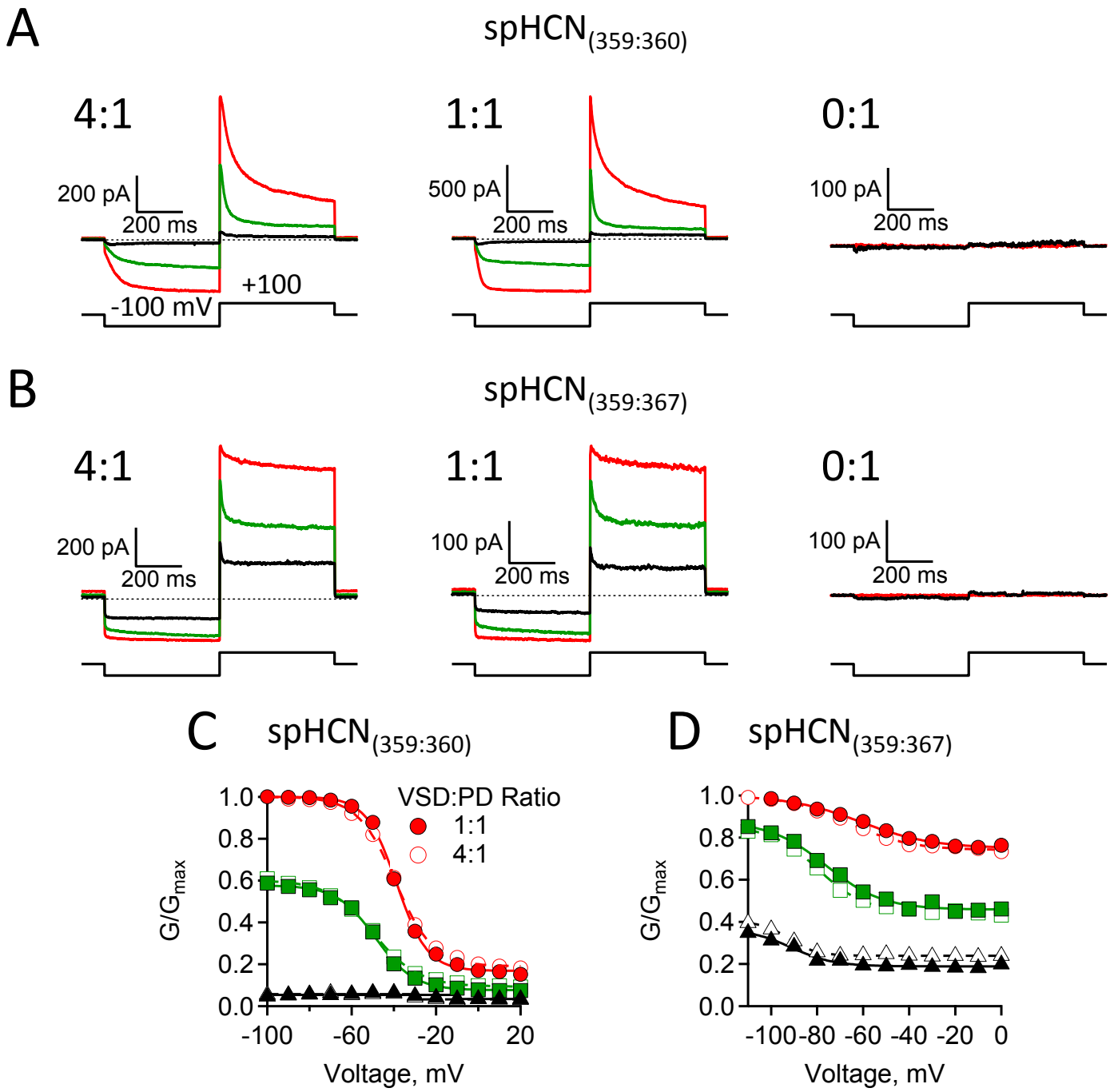

A
spHCN(359:360)
4:1
200 pA
200 ms
+100
-100 mV
1:1
500 pA
200 ms
0:1
100 pA
200 ms
B
spHCN(359:367)
4:1
200 pA
200 ms
1:1
100 pA
200 ms
0:1
100 pA
200 ms
C
spHCN(359:360)
VSD:PD Ratio
1:1
4:1
G/Gmax
1.0
0.8
0.6
0.4
0.2
0.0
-100
-60
-20
20
Voltage, mV
D
spHCN(359:367)
G/Gmax
1.0
0.8
0.6
0.4
0.2
0.0
-100 -80 -60 -40 -20 0
Voltage, mV

Fig. S4

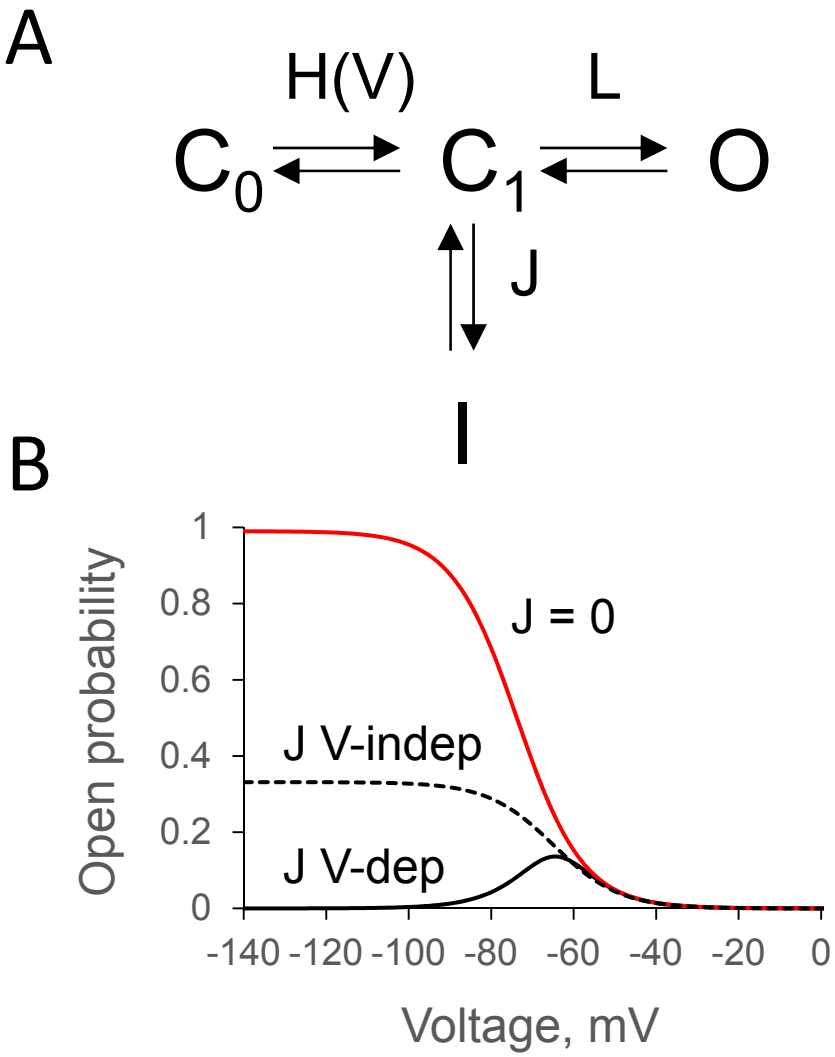
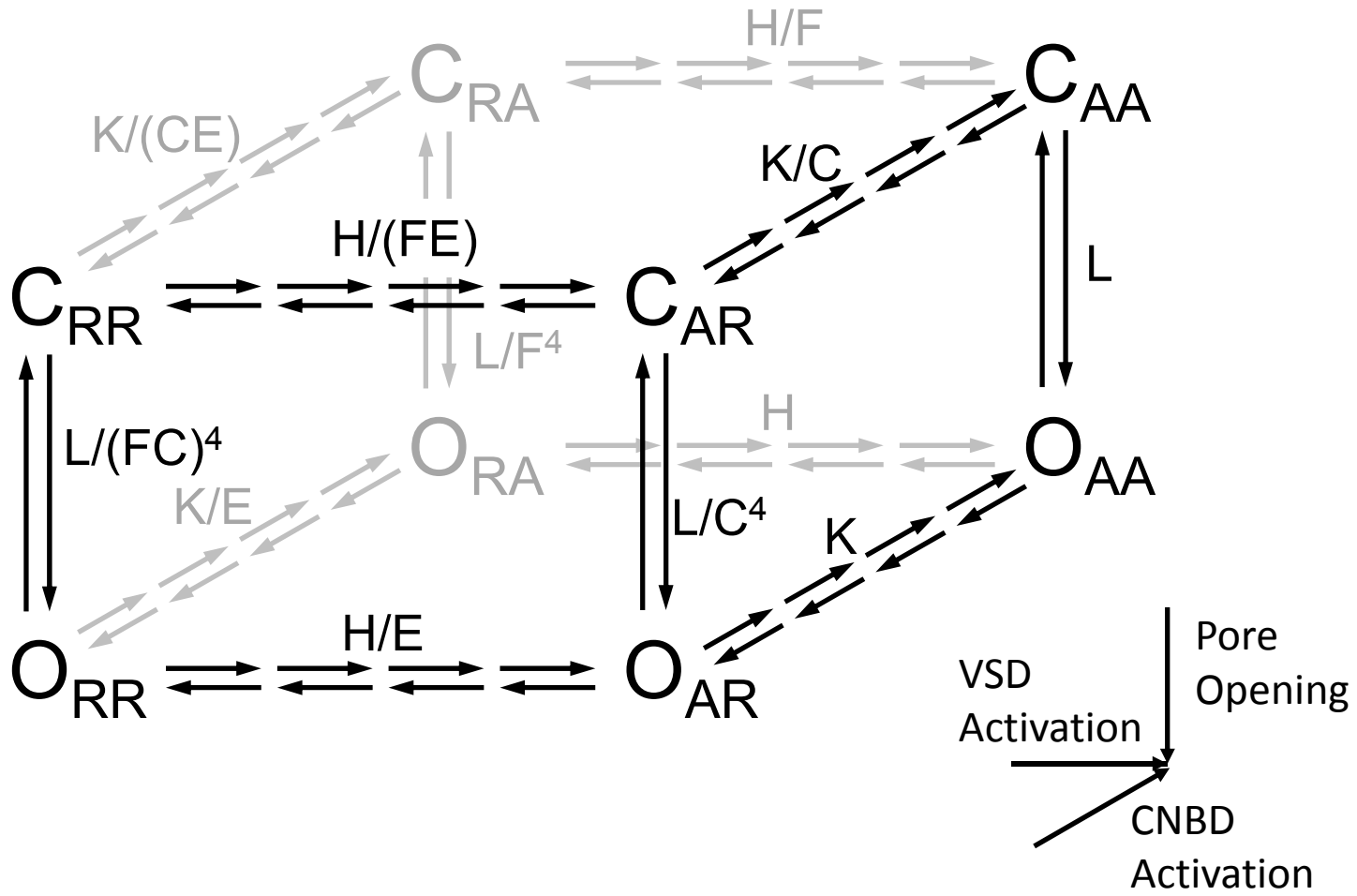

A
H(V)
L
C0
C1
O
J
I
B
Open probability
1
0.8
0.6
0.4
0.2
0
J = 0
J V-indep
J V-dep
-140 -120 -100 -80 -60 -40 -20 0
Voltage, mV

Fig. S5


H/F
C_RA
C_AA
K/(CE)
K/C
H/(FE)
C_RR
C_AR
L
L/F^4
H
L/(FC)^4
O_RA
O_AA
K/E
L/C^4
K
H/E
O_RR
O_AR
Pore
Opening
VSD
Activation
CNBD
Activation