

1 **Supplementary Figure 1 | Structure and lentiviral co-transduction of E-HDF**
 2 **monolayers. (a,b)** Representative images of Vimentin⁺ E-HDFs in isotropic (a) or anisotropic
 3 (b) monolayers. (c,d) Strong, ubiquitous co-expression of eGFP (left) and mCherry (right) in
 4 isotropic (c) and anisotropic (d) E-HDF monolayers co-transduced with pRRL-CMV-NavSheP
 5 D60N-P2A-Kir2.1-T2A-eGFP and pRRL-CMV-Cx43-P2A-mCherry lentiviruses. (e) Mixed
 6 culture of HDFs transduced with pRRL-CMV-NavSheP D60N-P2A-Kir2.1-T2A-eGFP and
 7 HDFs transduced with pRRL-CMV-Cx43-P2A-mCherry virus shown as a control to demonstrate
 8 no cross-talk between read and green channels. Scale bars, 100 μm.
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NaChBac -----M--KMEARQKQNSFTSKMVKIVNHRAFFTFVIALILFNALVIGIETYPRIYADHKWLFYRIDLVLLWIFITIEIAMRFLASNPKSAFFRSSMNFDFLIVAAGHIFAGA-QFVT 110
NavSheP -----MSTSLLNAPTGLQARVINLVEQNIWFGHFLTLLINAVQLGMEETSASLMAQYGALLMSLDKVLVSVFVVELLLRIYAYRG--KFFKDPNMFVDFTVIVIALIPAS--GPLA 107
NavRosD -----MGVREQLDIWLDRNIVNVIIVAVIIIFNAILLGMEETSQIMAEAGDLIIMLDKACLTFVVEIFLKLVARGR--RFFKSGMNFDFVLVIGAAALVPGS--QTMS 98
NavBacL -----MNTHQNRYSQSKVSALINCRLFQSFITTVIVLNAISIGLETYA--FFKPYQKVFVIVDCLFLTFVVEIELSLKLFV--QKQHFKNMNFDFVIVAGSLIYNT-NFVS 105
NavBp -----ME--NMPAEQQVPLVALAQRIVFHKAFTPTIITLIIINAIVIGLEIETPYTYQGYNDIFVYAADLALLWIFITIEITLRFIAARPTKSFKSSMNFDFLVLVAGHVFAGA-HFVT 111
NavSi1P -----MQRMQAFIDRPGGFRGITAVIMLNAVVLGMEETSPYLMERMGGLLAADRICLGFIVEIALKLTARQ--RFFLNGMNLDFDVIGIALIPAA--GGLS 95
NavSu1P -----MVGTVNMRERLGRWVEGRAVSNFIIGVIVFNAALLGMEETAPALMEKYGTLLIFLDRVCLFIFIAEITLKIARGV--GFFRNGMNFDFTVVAIALVPAV--GGLS 102
NavPz -----MSLRARLDALVHGRRAQGVITGVILFNVALLGLSETSGRVMVAVGPIILLDAACLAVFAEIAAKLIARGP--RFFRDMNFDFVSVVAIALMPAG--QGLS 98
NavAb1 -----MQATPSSV-PAANAWRQLSAHLNSSLFANSITAILLNAIILGVETSSVAQERVQWLLTVNQVILFAFTLEIALKLVAFGP--RFFRSGMNFDFLIVAISLTPAS--GPLA 108
NavAb -----MYLRITNIVESSFFTKFIIVLVLNGITMGLSETSKTFMQSGVGYTLLNQVITVITFIEIILRIYVHRI--SFFKDPWSLDFDFVVAISLTPAS--SGFE 96
NavMs -----MSRKIRDLESKRFQNVITAIIVLNGAVLGLTDTLTSASSQNLLEVDQLCLTFIVEISLKIYAYGVR--GFFRSGMNLDFDVIVAIALMPAQ--GGLS 97
NavCt -----MSHI--HERHSQKHPIEAMCDLWVHPRFTSAIIVLIVINAVVGMETYPGIYQYQDWFYLDIIRMLWVFAEIIKLVATRPRYHFFKDSMNFDFLIVASGLFVGA-QFVT 113
NavAe MSEQPDLVGHKQVHPEDETLRGLAWFIDRPGTQYFVGLILVNAITLGHUITSPEVTAYLQPHLGMWNTFIIAAFFVEISLRIADGP--RFRVSGMNLDFDVVAISLVPDS--GAFS 116
NavRh -----HTPFSSLKDNRIQFTVWSIILNVALIGATITVEL-DPLFLETIHLLDYGITIFFVVEIILIRFIEGKQKADFFKSGMNFDFTVIVAISLTPINNSSFL 99

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NaChBac VLRLRLVRLVRLRAISVVPRLRLVLDALVMTIPALGNILILMSIIFYFVAVIGTMLFQVHSPEYFGNLQLSLLTLFQVVTLESMSGVMRPIFAEVPWSWLYFVSVFLIGTFIIFNLFIGV 230
NavSheP VLRLRLVRLVRLVRLVIVPSSMKRVVVALGSLPLGASIAATVLLIIFYFVAVIATKIFGDAFPEWFGTIDASFYTLFQIMTLESMSGIRPVMVEVYPAWVFFVFPFLVATFVNLNLFIAI 227
NavRosD VLRLRLVRLVRLVRSVAPSLRRVVEGFVALPGMGSVFLMAIIFYFGVISTKLFASFQWFGSLPQSGYTLFQIMTLESMSGIVRPMVEVYPAWVFFVFPFLVATFVNLNLFIAI 218
NavBacL VLRLRLVRLVRLVRLVRSVAPSLRRVVEGFVALPGMGSVFLMAIIFYFGVISTKLFASFQWFGSLPQSGYTLFQIMTLESMSGIVRPMVEVYPAWVFFVFPFLVATFVNLNLFIAI 225
NavBp VLRLRLVRLVRLVRAISVVPRLRLVLDALVMTIPALGNILILMSIIFYFVAVIGTMLFQVHSPEYFGNLQLSLLTLFQVVTLESMSGVMRPIFAEVPWSWLYFVSVFLIGTFIIFNLFIGV 231
NavSi1P VLRLRLVRLVRLVRSVAPSLRRVVEGFVALPGMGSVFLMAIIFYFGVISTKLFASFQWFGSLPQSGYTLFQIMTLESMSGIVRPMVEVYPAWVFFVFPFLVATFVNLNLFIAI 215
NavSu1P VLRLRLVRLVRLVRSVAPSLRRVVEGFVALPGMGSVFLMAIIFYFGVISTKLFASFQWFGSLPQSGYTLFQIMTLESMSGIVRPMVEVYPAWVFFVFPFLVATFVNLNLFIAI 222
NavPz VLRLRLVRLVRLVRSVAPSLRRVVEGFVALPGMGSVFLMAIIFYFGVISTKLFASFQWFGSLPQSGYTLFQIMTLESMSGIVRPMVEVYPAWVFFVFPFLVATFVNLNLFIAI 218
NavAb1 ILRLRLVRLVRLVRLVRSVAPSLRRVVEGFVALPGMGSVFLMAIIFYFGVISTKLFASFQWFGSLPQSGYTLFQIMTLESMSGIVRPMVEVYPAWVFFVFPFLVATFVNLNLFIAI 228
NavAb ILRLRLVRLVRLVRLVRSVAPSLRRVVEGFVALPGMGSVFLMAIIFYFGVISTKLFASFQWFGSLPQSGYTLFQIMTLESMSGIVRPMVEVYPAWVFFVFPFLVATFVNLNLFIAI 216
NavMs VLRLRLVRLVRLVRSVAPSLRRVVEGFVALPGMGSVFLMAIIFYFGVISTKLFASFQWFGSLPQSGYTLFQIMTLESMSGIVRPMVEVYPAWVFFVFPFLVATFVNLNLFIAI 217
NavCt VLRLRLVRLVRLVRSVAPSLRRVVEGFVALPGMGSVFLMAIIFYFGVISTKLFASFQWFGSLPQSGYTLFQIMTLESMSGIVRPMVEVYPAWVFFVFPFLVATFVNLNLFIAI 233
NavAe VLRLRLVRLVRLVRSVAPSLRRVVEGFVALPGMGSVFLMAIIFYFGVISTKLFASFQWFGSLPQSGYTLFQIMTLESMSGIVRPMVEVYPAWVFFVFPFLVATFVNLNLFIAI 236
NavRh VLRLRLVRLVRLVRSVAPSLRRVVEGFVALPGMGSVFLMAIIFYFGVISTKLFASFQWFGSLPQSGYTLFQIMTLESMSGIVRPMVEVYPAWVFFVFPFLVATFVNLNLFIAI 218

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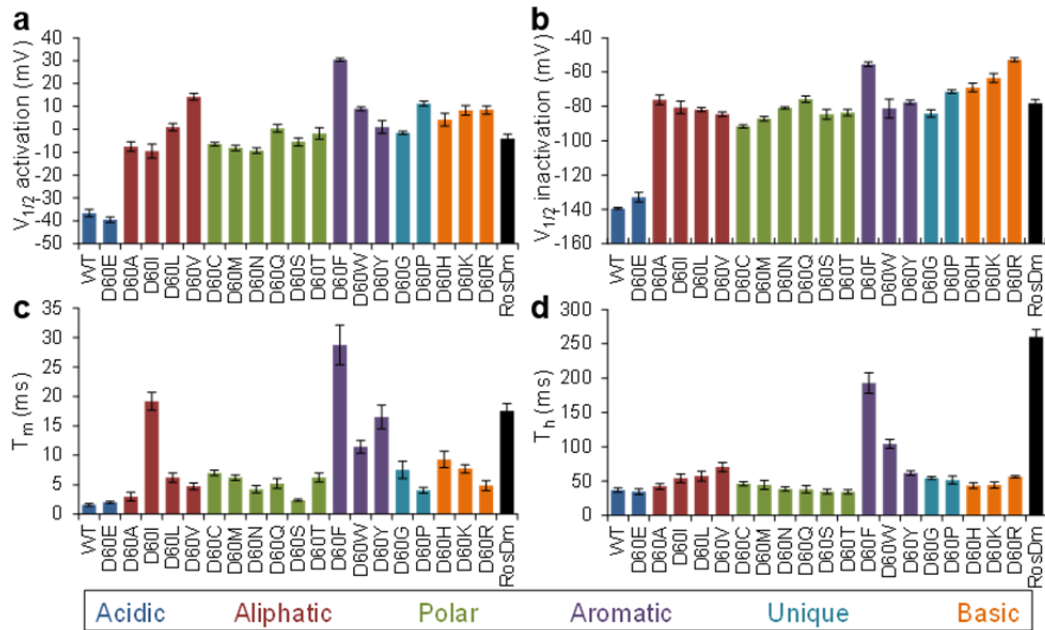
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NaChBac IVNVMKEAELTDN--EEDGEA-----DGLKQEISALRKDVAEL-----KSLLKQSK----- 274
NavSheP IVNMTQTFSDDEHALEREQD-----KQILEQEQRWHEELKAIKLELQQLTLRNAAGDSSNVSTKGNIGSD 295
NavRosD IVNSMQDAHQQEAGEQTDAY-----RDEVL SRLIAIEEKL----- 253
NavBacL IVNNAQKISQEIETDGTGL-----KEDTKEIKELRAEVKEL-----KALLQTYITQNEKDD----- 276
NavBp IVNVMKEANEELKSELDDKE-----ADTKEELASLRNEVAEM-----KDLIKM'KHQQT'KGG----- 284
NavSi1P IVNSMQDAHHAEDGERTDAY-----RDEVLARLEQIDQRLNAL-----GETTK----- 258
NavSu1P IVNSMQDAHNEEETVRTDAY-----RLEVMEKLEAIEKK----- 256
NavPz IVNSMQDAHQAESAATDAY-----RDEVLMLRAIEKQLDES-----GGRGRV----- 262
NavAb1 IVSATQEVHSEQRAREANNI---LIAHDERQEMLDL'RAM'HA'KIVALEAAQQGKA-----GQ----- 283
NavAb IVDAMAILNQKEEQHIIDEVQ---SHEDININNEI---IKLREEIIVEL-----KELIKTSLKNI----- 267
NavMs IVDAMAITKEQEEAATGHH-----QEPISQTLHLGLDRDLRIEKQLAQHNELLQRQQPK-----K----- 274
NavCt IVSNVRAETEDAEEQEGREERQGLFEGDSSVSAEEIAKLRQEIKEL-----RQLL'KEL'KD'HSQSST----- 298
NavAe IIESMQSAHNEAEDAKRIEQE---QRAHDERLEMLQLIRDLSKVDRLERRSGKR----- 288
NavRh LVDVWIQKLL----- 228

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14 **Supplementary Figure 2 | Homology of different BacNa_v orthologs.** Residues E43 and D60
15 are indicated by green and red arrows respectively. The main regions labeled are: S1-S6
16 transmembrane helices, S4/S5 linker, P1 and P2 pore helices, and selectivity filter (SF). The
17 BacNa_v orthologs listed have been previously characterized either structurally or functionally:
18 NaChBac (*Bacillus halodurans*), NavSheP (*Shewanella putrefaciens*), NavRosD (*Roseobacter*
19 *denitrificans*), NavBacL (*Bacillus licheniformis*), NavBp (*Bacillus pseudofirmus*), NavSi1P
20 (*Silicibacter pomeroyi*), NavSu1P (*Sulfitobacter pontiacus*), NavPz (*Paracoccus*
21 *zeaxanthinifaciens*), NavAb1 (*Alcanivorax borkumensis*), NavAb (*Arcobacter butzleri*), NavMs
22 (*Magnetococcus marinus*), NavCt (*Caldalkalibacillus thermarum*), NavAe (*Alkalilimnicola*
23 *ehrllichii*), and NavRh (*Rickettsiales sp.*)

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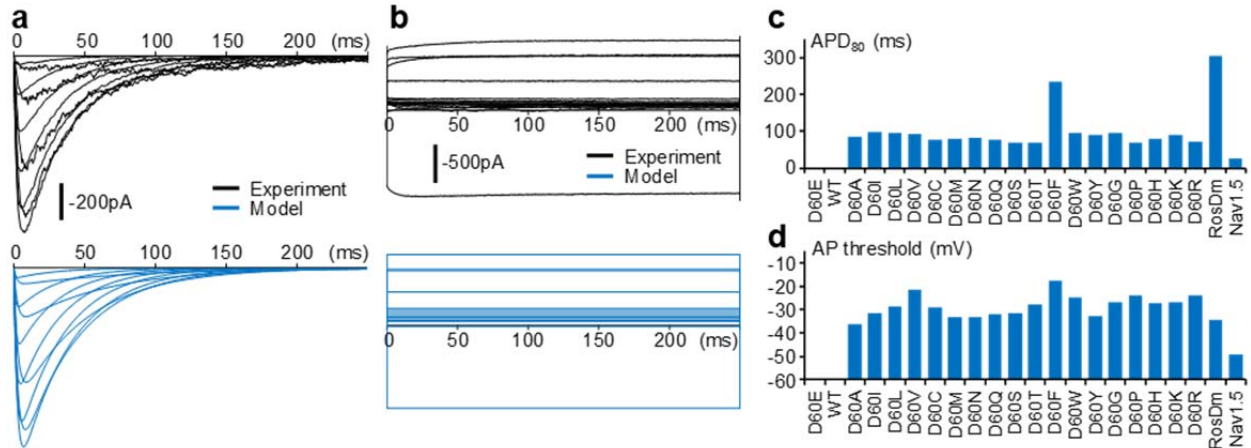


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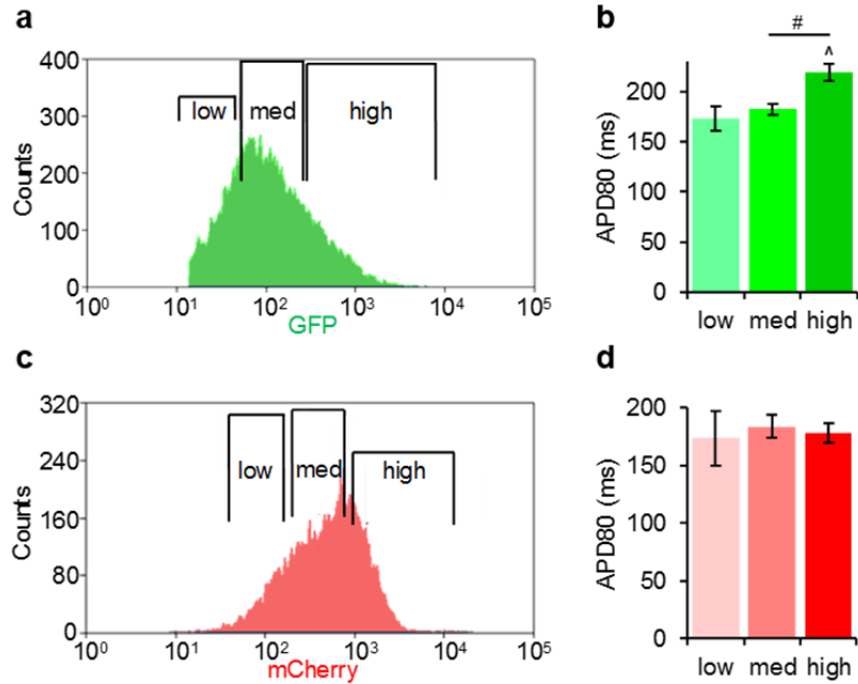
27 **Supplementary Figure 3 | Electrophysiological properties of Na_vSheP mutants.** (a,b) Mid-
 28 points ($V_{1/2}$) of activation (a) and inactivation (b) curves of Na_vSheP mutants exhibit different
 29 depolarization shifts relative to the $V_{1/2}$ of the wild-type (WT) channel. (c,d) Time constants of
 30 activation (τ_m , c) and inactivation (τ_h , d) measured at +20mV. Color-coding into groups is based
 31 on the characteristics of the respective amino acid side chains at residue 60. Recordings were
 32 performed at 25°C (n = 4-10). Supplementary Table 1 shows a complete list of all activation and
 33 inactivation parameters. Error bars indicate s.e.m.

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 37 **Supplementary Figure 4 | Computational modeling of E-HDF electrophysiology.** (a)
 38 Representative whole-cell voltage clamp recording of $\text{Na}_v\text{SheP D60A}$ current in E-HDFs and the
 39 corresponding computational traces. (b) Representative whole-cell voltage clamp recording of I_K
 40 current in E-HDFs and the corresponding computational traces. (c,d) AP characteristics of E-
 41 HDFs expressing different BacNa_v mutants or $\text{Na}_v1.5$, obtained by computational modeling:
 42 APD_{80} (c) and AP threshold (d). For modeling BacNa_v currents, parameters derived from
 43 voltage-clamp measurements at 25°C were scaled for the temperature of 37°C . Highly
 44 hyperpolarized inactivation of WT and D60E channels prevented AP initiation.
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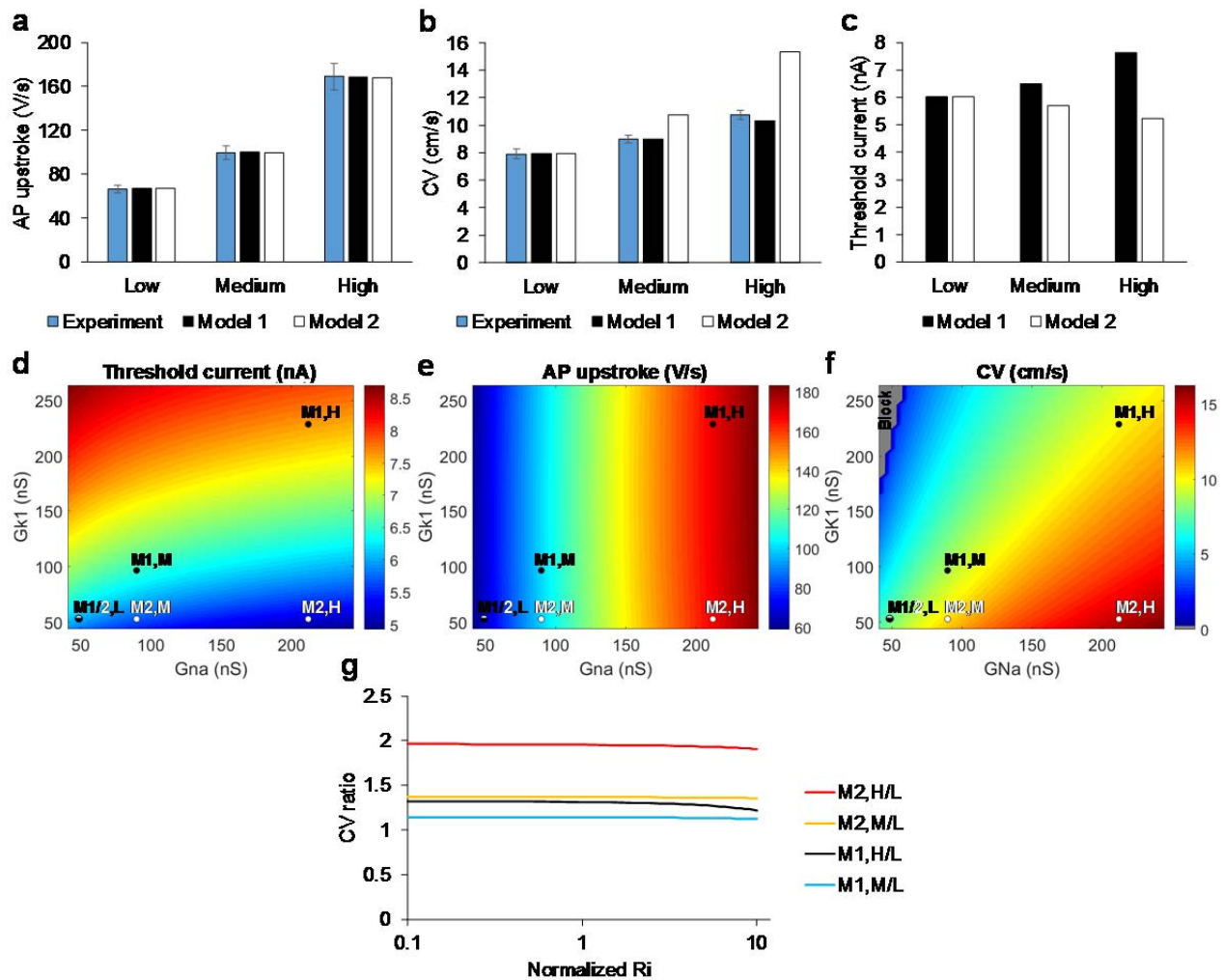


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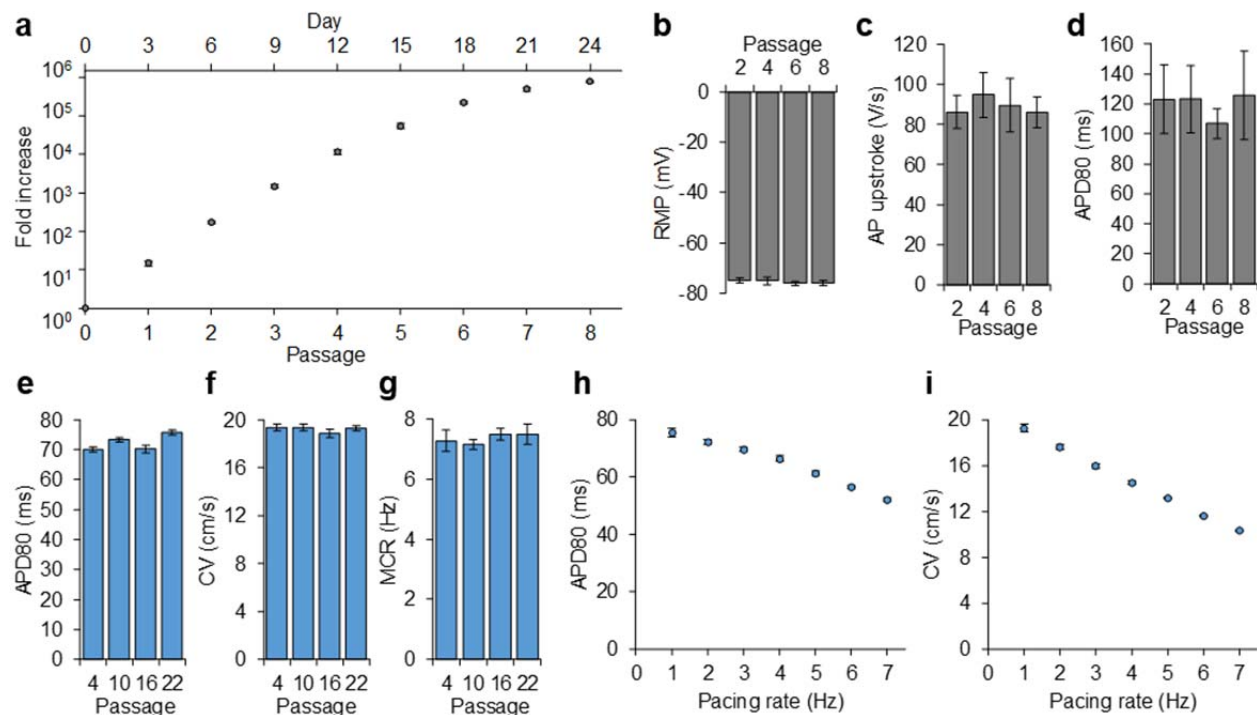
47 **Supplementary Figure 5 | Use of FACS to tune electrophysiological properties of E-Fibs.** (a)
 48 E-HDFs generated by co-transduction with Na_vSheP D60N-P2A-eGFP-Kir2.1 and Cx43-P2A-
 49 mCherry were sorted into three groups based on the level of eGFP expression (low, medium,
 50 high). (b) Optical mapping of eGFP-sorted E-HDFs showed longer APD₈₀ associated with higher
 51 eGFP intensity (n = 6-10). (c-d) When E-HDFs were sorted into three groups based on mCherry
 52 expression level (low, medium, high, c), APD₈₀ was comparable among the groups (d, n = 5).
 53 #P<0.01; ^P<0.05 vs low eGFP group in b. Error bars indicate s.e.m; statistical significance was
 54 determined by one-way ANOVA, followed by Tukey's *post hoc* test to calculate P values.

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59 **Supplementary Figure 6 | Computational studies of BacNav and Kir2.1 effects on E-HDF**
60 **electrical properties.** (a-c) Maximum AP upstroke velocity (a), conduction velocity (b), and
61 threshold current required to elicit AP (c) in E-HDFs expressing varying levels (low, medium,
62 and high) of both NavSheP D60N and Kir2.1 (model 1) or the low level of Kir2.1 and varying
63 levels of only NavSheP D60N (model 2). Experimental data for AP upstroke and CV shown in
64 Fig. 3e,h are included for comparison. (d-f) Simulated effects of varying BacNav and Kir2.1
65 conductance values (G_{Na} and G_{K1}) on threshold current (d), AP upstroke (e), and CV (f). Solid
66 circles denote low (L), medium (M), or high (H) groups using either model 1 (M1, black circles)
67 or model 2 (M2, white circles) shown in panels a-c. (g) Effects of varying intercellular
68 resistivity, R_i (shown relative to nominal R_i value used in panels b and f), on CV ratios between
69 the groups. Error bars indicate s.e.m.



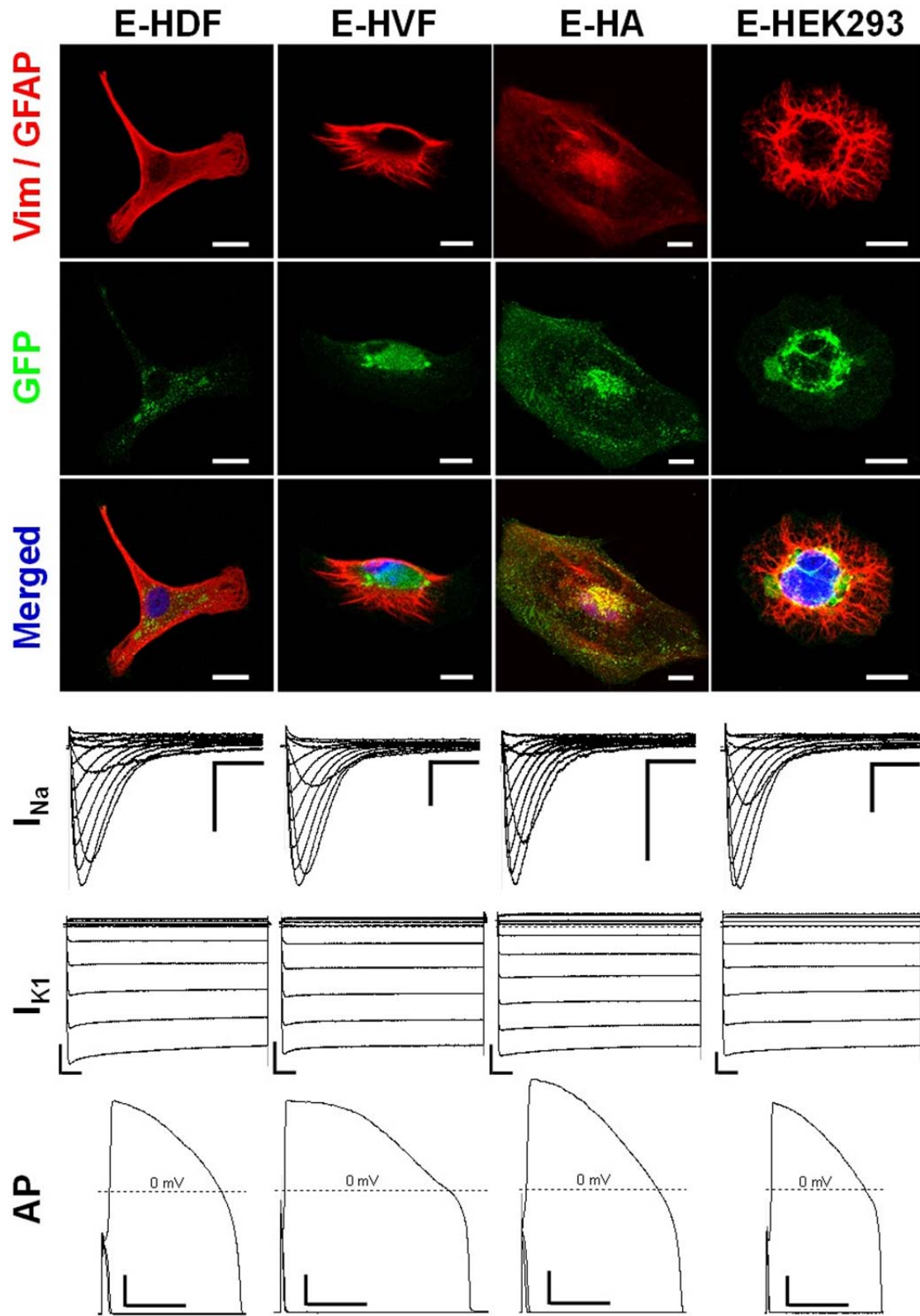
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73 **Supplementary Figure 7 | Stability of engineered cell phenotypes during long-term**
 74 **expansion.** (a) Growth curve of E-HDFs transduced with Na_vSheP D60N-Kir2.1 lentivirus
 75 following initial expansion and FACS. Cells senesced at passage eight. (b-d) Resting membrane
 76 potential (b), maximum AP upstroke velocity (c), and APD₈₀ (d) of E-HDFs at various passages
 77 throughout expansion assessed by patch clamp recordings (n = 8-10). (e-g) Optical mapping
 78 studies in monolayers of monoclonal E-HEK293 cells expressing Na_vSheP D60A, Kir2.1, and
 79 Cx43, showing stable APD₈₀ (e), CV (f), and maximum capture rate (MCR, g) during 22
 80 passages (n = 6-8). Properties beyond passage 22 were not examined and were presumed to
 81 remain stable. (h,i) Dependence of APD₈₀ (h) and CV (i) on pacing rate in monolayers made
 82 from passage 22 E-HEK293 cells (n = 6). AP conduction at each pacing rate was mapped after
 83 30s of pacing. All data were obtained at 37°C. Error bars indicate s.e.m; statistical significance
 84 was determined by one-way ANOVA.

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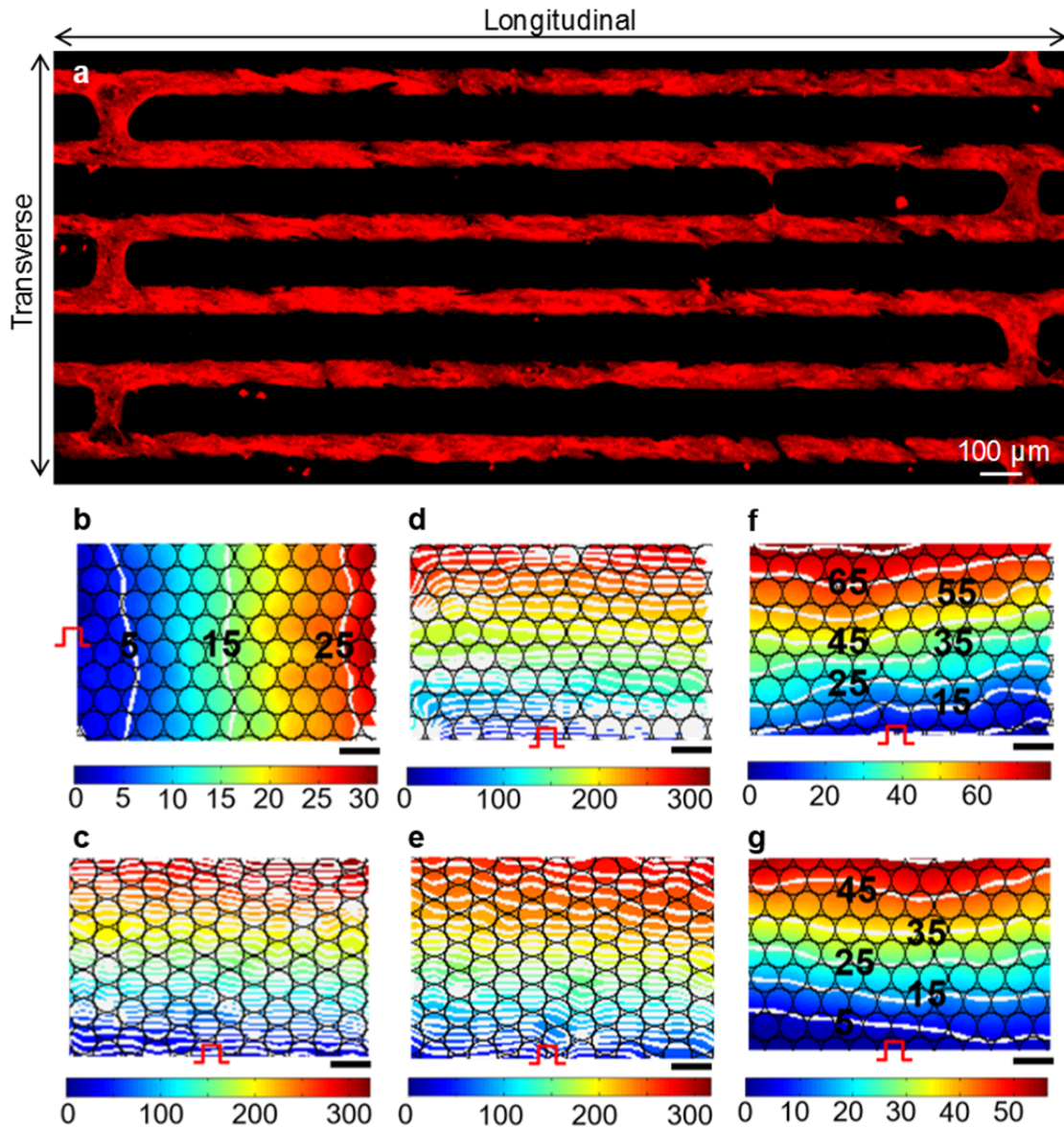
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 89 **Supplementary Figure 8 | Induction of excitability in various types of unexcitable human**
 90 **cells.** Each human cell type (shown in different columns) was transduced with Na_v SheP D60N-
 91 P2A-eGFP- $K_{ir}2.1$ lentivirus, expanded, and analyzed by voltage and current clamp recordings.

92 Engineered human astrocytes (E-HAs) were labeled for Glial fibrillary Acidic Protein (GFAP),
93 while engineered human dermal fibroblasts (E-HDFs), human ventricular fibroblast (E-HVFs),
94 and human embryonic kidney 293 cells (E-HEK293s) were labeled for vimentin. Scale bars:
95 20 μ m (immunostaining images), 100ms and 2 nA (I_{Na} and I_K recordings), 25ms and 20mV (AP
96 recordings).
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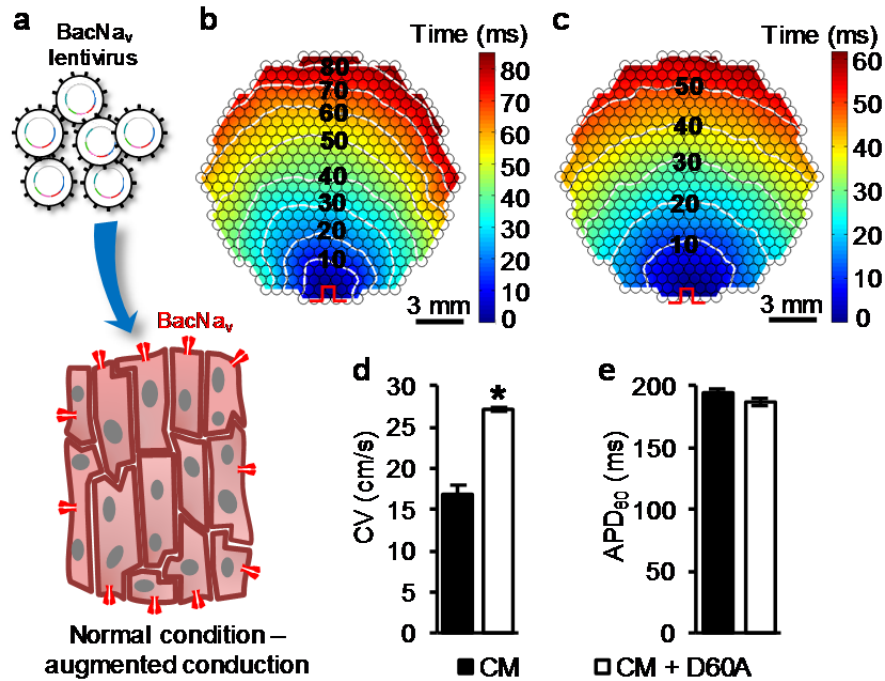


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99 **Supplementary Figure 9 | Use of E-HDFs to improve impaired cardiomyocyte conduction.**

100 (a) A zig-zag pattern of neonatal rat cardiomyocytes (CMs) used to simulate fast longitudinal
 101 and slow transverse AP conduction characteristic of cardiac tissue with interstitial fibrosis. Cells
 102 were stained with α -sarcomeric actin (red). (b,c) Representative fast longitudinal conduction (b)
 103 and slow transverse conduction (c) in CM monoculture. (d,e) Slow unaltered transverse
 104 conduction in CM + wt HDF co-culture (d) and CM + Na_vShePD60A+Kir2.1 HDF co-culture
 105 (e). (f,g) Moderately and significantly improved transverse conduction in CM + Cx43 HDF (f)
 106 and CM + E-HDF (g) co-cultures, respectively. In b-g: Scale bars, 1 mm. Pulse signs indicate
 107 positions of line stimulation. Circles denote recording sites.

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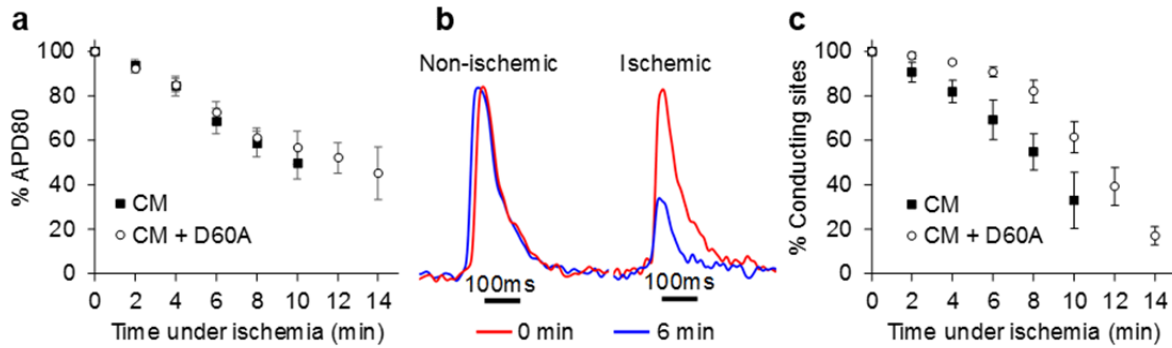
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110 **Supplementary Figure 10 | Enhancement of cardiac AP conduction by BacNav expression.**

111 (a) Schematic depicting exogenous expression of BacNav_v in cardiomyocytes (CMs) to augment
 112 conduction. (b,c) Representative isochrone maps of AP conduction in electrically stimulated
 113 isotropic monolayers of control neonatal rat CMs (b) and CMs transduced with Na_vSheP D60A
 114 lentivirus (c). (d,e) CM monolayers transduced with Na_vSheP D60A lentivirus (CM + D60A)
 115 show increased CV (d) and unaltered APD₈₀ (e) compared to control CM monolayers (n = 5).
 116 *P<0.001 vs control CM. Pulse signs indicate location of stimulating electrode. Circles denote
 117 504 recording sites. Error bars indicate s.e.m; statistical significance was determined by an
 118 unpaired Student's t-test to calculate P value.

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122 **Supplementary Figure 11 | Consequences of ischemia on AP duration and firing in CM**
 123 **monolayers with regional ischemia.** (a) With time of ischemia, AP duration gradually
 124 decreases in the ischemic region of monolayers made of either control cardiomyocytes (CM) or
 125 cardiomyocytes transduced with NavSheP D60A (CM + D60A) (n = 6). (b) Representative
 126 optical AP traces in the non-ischemic (peripheral) and ischemic (central) regions of the
 127 monolayer before (0 min) and during ischemia (6 min). (c) With time of ischemia, number of
 128 actively conducting recording sites within ischemic region is progressively reduced (n = 6). Error
 129 bars indicate s.e.m.

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Channel	Activation			Inactivation		
	$V_{1/2}$ (mV)	Slope factor (mV)	Time constant (ms)	$V_{1/2}$ (mV)	Slope factor (mV)	Time constant (ms)
SheP WT	-36.7	8.1	1.6	-139.5	8.9	31.7
D60E	-39.6	7.4	2.0	-132.9	9.5	27.8
D60A	-7.6	7.1	3	-76.1	9	42.4
D60I	-9.5	6.8	19.2	-80.6	7.8	54
D60L	0.9	7.3	6.2	-81.9	10.1	57.3
D60V	14.2	8.2	4	-84.6	10.4	70.5
D60C	-6.5	7.3	7	-91.7	9.2	46.3
D60M	-8.1	7.6	6.2	-87.1	8.6	44.5
D60N	-9.3	6.2	4.2	-80.8	9.8	38.6
D60Q	0.5	7.7	5.2	-75.8	8.5	38.2
D60S	-5.5	7	2.4	-84.6	8.6	34.9
D60T	-1.8	6.8	6.2	-83.6	8.2	34.3
D60F	30.4	8.9	28.8	-55.4	8.6	193
D60W	8.9	11	11.4	-81.2	9.7	104.5
D60Y	1	8.7	16.5	-77.5	8.9	61.9
D60G	-1.5	6.6	7.5	-84.2	9.4	54.6
D60P	11.3	7.5	4	-71.4	10.5	51.6
D60H	4.3	6.9	9.3	-69	8	43.6
D60K	8.3	7.5	7.7	-63.4	7.5	44.3
D60R	8.4	6.4	4.8	-52.8	7.2	56.4
E43A	0.3	8.2	3.5	-57.7	13.2	53.1
E43L	2.9	8.2	2.4	-87.3	7.5	92
E43V	10	8.3	4	-106.4	10.5	40.2
E43N	0	8.2	2.6	-61.3	15.8	58.2
E43Q	25.1	9.2	2.5	-44.1	15.5	82.9
E43S	5.5	10	4.5	-58.7	14	23.6
E43T	-2.5	7.6	2.7	-68.8	4.5	51.8
E43H	16.7	11.3	4.4	-69.9	14.6	53.9
E43K	12.8	10.5	3	-50.3	11.2	24.1
RosD G217A	-16.6	10.5	17.5	-78.2	9.8	260.2

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Supplementary Table 1 | Biophysical properties of BacNa_v mutants. Activation and inactivation time constants were measured at +20mV clamp potential. Recordings were performed at 25⁰C. Data shown are mean values (n = 4-10).

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E43X	Primers	D60X	Primers
E43A	5'-ggctggctgaggtgccatacctaactgc-3' 5'-gcagttaggtatggcgacctcagccagcc-3'	D60A	5'-aaatacactcagtagcaacttagcaagactatcaacaagacc-3' 5'-ggtactttgtgatgagtcctgctaagttgactcagtgattt-3'
E43C	5'-catcaggctggctgaggtgcacatacctaactgcaccgc-3' 5'-gcggctcagttaggtatgtgcacctcagccagcctgatg-3'	D60C	5'-cacaatacactcagtagcaacttaaaagactcatcaacaagaccgtat-3' 5'-atacggctactttgtgatgagtccttgaagttgactcagtgattttg-3'
E43D	5'-ctgctgaggtatccatacctaactgcaccgc-3' 5'-cgctggctcagttaggtatggatacctcagccagc-3'	D60E	5'-gtactttgtgatgagtcctgagaagttgactcagtgattttg-3' 5'-caatacactcagtagcaactctcaagactcatcaacaagacc-3'
E43F	5'-catcaggctggctgaggtgaacatacctaactgcaccgc-3' 5'-gcggctcagttaggtatgtgcacctcagccagcctgatg-3'	D60F	5'-cacaatacactcagtagcaacttaaaagactcatcaacaagaccgtat-3' 5'-atacggctactttgtgatgagtccttgaagttgactcagtgattttg-3'
E43G	5'-ggctggctgaggtccccatacctaactgc-3' 5'-gcagttaggtatggggacctcagccagcc-3'	D60G	5'-aaatacactcagtagcaacttaaaagactcatcaacaagacc-3' 5'-ggtactttgtgatgagtcctgctaagttgactcagtgattt-3'
E43H	5'-tcaggctggctgaggtatgcatacctaactgcacc-3' 5'-ggtcagttaggtatgcatacctcagccagcctga-3'	D60H	5'-atacactcagtagcaactatgaagactcatcaacaagacc-3' 5'-ggtactttgtgatgagtcctataagttgactcagtgattt-3'
E43I	5'-catcaggctggctgaggtatcatacctaactgcaccgc-3' 5'-gcggctcagttaggtatgatacctcagccagcctgatg-3'	D60I	5'-cacaatacactcagtagcaacttaaaagactcatcaacaagaccgtat-3' 5'-atacggctactttgtgatgagtccttataagttgactcagtgattttg-3'
E43K	5'-ggctggctgaggtcttcatacctaactgcac-3' 5'-gtcagttaggtatgaagacctcagccagcc-3'	D60K	5'-cactcagtagcaactcttaagactcatcaacaagaccgtattg-3' 5'-gccaatacggctactttgtgatgagtccttaagagttgactcagtg-3'
E43L	5'-catcaggctggctgaggttaacatacctaactgcaccgc-3' 5'-gcggctcagttaggtatgtaaacctcagccagcctgatg-3'	D60L	5'-cactcagtagcaacttaaaagactcatcaacaagaccgtattg-3' 5'-gccaatacggctactttgtgatgagtcctttaaagttgactcagtg-3'
E43M	5'-caggctggctgaggtcatacctaactgcacc-3' 5'-ggtcagttaggtatgatcactcagccagcctg-3'	D60M	5'-cactcagtagcaactcataagactcatcaacaagaccgtattg-3' 5'-gccaatacggctactttgtgatgagtccttaagagttgactcagtg-3'
E43N	5'-atcaggctggctgaggtattcatacctaactgcaccgc-3' 5'-cgctcagttaggtatgatacctcagccagcctgat-3'	D60N	5'-aatacactcagtagcaacttaaaagactcatcaacaagacc-3' 5'-cggtactttgtgatgagtcctataagttgactcagtgattt-3'
E43P	5'-caggctggctgaggtggcatacctaactgcacc-3' 5'-ggtcagttaggtatgccacctcagccagcctg-3'	D60P	5'-caatacactcagtagcaacttagaagactcatcaacaagaccgt-3' 5'-acggctactttgtgatgagtcctcaagttgactcagtgattttg-3'
E43Q	5'-ggctggctgaggtctgcatacctaactgcac-3' 5'-gtcagttaggtatgcagacctcagccagcc-3'	D60Q	5'-actcagtagcaactctgaagactcatcaacaagaccgtattg-3' 5'-gcaatacggctactttgtgatgagtcctgaagttgactcagtg-3'
E43R	5'-caggctggctgaggtcctcatacctaactgcacc-3' 5'-ggtcagttaggtatgagacctcagccagcctg-3'	D60R	5'-caatacactcagtagcaacttagaagactcatcaacaagaccgt-3' 5'-acggctactttgtgatgagtcctcaagttgactcagtgattttg-3'
E43S	5'-caggctggctgaggtgcacatacctaactgcacc-3' 5'-ggtcagttaggtatgtgcacctcagccagcctg-3'	D60S	5'-cacaatacactcagtagcaacttaaaagactcatcaacaagaccgtat-3' 5'-atacggctactttgtgatgagtccttagaagttgactcagtgattttg-3'
E43T	5'-caggctggctgaggtgcatacctaactgcacc-3' 5'-ggtcagttaggtatgacacctcagccagcctg-3'	D60T	5'-cacaatacactcagtagcaacttagaagactcatcaacaagaccgtat-3' 5'-atacggctactttgtgatgagtcctcaagttgactcagtgattttg-3'
E43V	5'-ggctggctgaggtcaccatacctaactgc-3' 5'-gcagttaggtatggtacctcagccagcc-3'	D60V	5'-ggtactttgtgatgagtcctgtaagttgactcagtgattt-3' 5'-aaatacactcagtagcaacttaaaagactcatcaacaagacc-3'
E43W	5'-caggctggctgaggtccacatacctaactgcacc-3' 5'-ggtcagttaggtatgagacctcagccagcctg-3'	D60W	5'-cactcagtagcaactcacaagactcatcaacaagaccgtattg-3' 5'-gccaatacggctactttgtgatgagtccttgaagttgactcagtg-3'
E43Y	5'-atcaggctggctgaggtatacatacctaactgcaccgc-3' 5'-cggtcagttaggtatgatacctcagccagcctgat-3'	D60Y	5'-aatacactcagtagcaacttaaaagactcatcaacaagacc-3' 5'-cggtactttgtgatgagtccttataagttgactcagtgattt-3'

Supplementary Table 2 | List of mutagenesis primers used to create Na_vSheP E43X and D60X libraries (designed by QuikChange® Primer Design Program).

Ionic concentrations

Intracellular Na ⁺ concentration	10.7 mM
Extracellular Na ⁺ concentration	135.3 mM
Intracellular K ⁺ concentration	120 mM
Extracellular K ⁺ concentration	5.4 mM

Membrane properties

Total membrane surface area (A)	2134 μm^2
Total membrane capacitance (C_m)	22 pF
BacNa _v maximum conductance (\bar{G}_{Na})	81.51 nS
K _{ir} 2.1 maximum total conductance (\bar{G}_{K1})	87.93 nS
Resting membrane potential (V_{rest})	-80.1 mV
Sodium Nernst potential (E_{Na}) at 37 ⁰ C	67.8 mV
Potassium Nernst potential (E_{K}) at 37 ⁰ C	-82.8 mV

Core-conductor model parameters

Time step (Δt)	0.0025 ms
Space step (Δx)	24 μm
Cable radius	10.0 μm
Cable length	0.72 cm
Specific membrane capacitance (c_m)	1.031 $\mu\text{F}/\text{cm}^2$
Intracellular resistivity (R_i)	1.48 k Ω .cm

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147 **Supplementary Table 3 | Computational modeling parameters.** Cell membrane properties (A,
 148 C_m , \bar{G}_{Na} , \bar{G}_{K1} , V_{rest} , E_{Na} , and E_{K}) used in the model were mean experimental values derived from
 149 whole-cell voltage-clamp recordings in a monoclonal HEK293 line expressing K_{ir}2.1, Cx43, and
 150 Na_vSheP D60A. Extracellular Na⁺ and K⁺ concentrations used in the model were the same as
 151 used in the bath solution during patch clamp and optical mapping experiments. Intracellular Na⁺
 152 and K⁺ concentrations in the model were derived using Nernst equation, extracellular Na⁺ and K⁺
 153 concentrations and E_{Na} and E_{K} , respectively. Specific membrane capacitance (c_m) used in the
 154 model was obtained by dividing mean cell membrane capacitance (C_m) with mean estimated cell
 155 membrane surface area (A). Intracellular resistivity (R_i) was adjusted to exactly simulate the
 156 mean conduction velocity (21.04 cm/s) recorded by optical mapping in confluent isotropic
 157 monolayers of the above-mentioned monoclonal HEK293 line expressing K_{ir}2.1, Cx43, and
 158 Na_vSheP D60A.

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