

This is a repository copy of *Inhibitory effect of eslicarbazepine acetate and S-licarbazepine on Nav1.5 channels*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/165462/>

Version: Accepted Version

Article:

Leslie, Theresa, Bruckner, Lottie, Chawla, Sangeeta orcid.org/0000-0003-4077-736X et al. (1 more author) (Accepted: 2020) Inhibitory effect of eslicarbazepine acetate and S-licarbazepine on Nav1.5 channels. *Frontiers in Pharmacology*. ISSN 1663-9812 (In Press)

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

1 **Inhibitory effect of eslicarbazepine acetate and S-licarbazepine on** 2 **Na_v1.5 channels**

3 **Theresa K. Leslie¹, Lotte Brückner¹, Sangeeta Chawla^{1,2}, William J. Brackenbury^{1,2*}**

4 ¹Department of Biology, University of York, Heslington, York, YO10 5DD, UK

5 ²York Biomedical Research Institute, University of York, Heslington, York, YO10 5DD, UK

6 * **Correspondence:** Dr William J. Brackenbury, Department of Biology and York Biomedical
7 Research Institute, University of York, Wentworth Way, Heslington, York YO10 5DD, UK. Email:
8 william.brackenbury@york.ac.uk. Tel: +44 1904 328284.

9 **Keywords:** Anticonvulsant, cancer, epilepsy, eslicarbazepine acetate, Na_v1.5, S-licarbazepine,
10 voltage-gated Na⁺ channel.

11 **Abstract**

12 Eslicarbazepine acetate (ESL) is a dibenzazepine anticonvulsant approved as adjunctive treatment for
13 partial-onset epileptic seizures. Following first pass hydrolysis of ESL, S-licarbazepine (S-Lic)
14 represents around 95 % of circulating active metabolites. S-Lic is the main enantiomer responsible
15 for anticonvulsant activity and this is proposed to be through the blockade of voltage-gated Na⁺
16 channels (VGSCs). ESL and S-Lic both have a voltage-dependent inhibitory effect on the Na⁺ current
17 in N1E-115 neuroblastoma cells expressing neuronal VGSC subtypes including Na_v1.1, Na_v1.2,
18 Na_v1.3, Na_v1.6 and Na_v1.7. ESL has not been associated with cardiotoxicity in healthy volunteers,
19 although a prolongation of the electrocardiographic PR interval has been observed, suggesting that
20 ESL may also inhibit cardiac Na_v1.5 isoform. However, this has not previously been studied. Here,
21 we investigated the electrophysiological effects of ESL and S-Lic on Na_v1.5 using whole-cell patch
22 clamp recording. We interrogated two model systems: (1) MDA-MB-231 metastatic breast
23 carcinoma cells, which endogenously express the ‘neonatal’ Na_v1.5 splice variant, and (2) HEK-293
24 cells stably over-expressing the ‘adult’ Na_v1.5 splice variant. We show that both ESL and S-Lic
25 inhibit transient and persistent Na⁺ current, hyperpolarise the voltage-dependence of fast inactivation,
26 and slow the recovery from channel inactivation. These findings highlight, for the first time, the
27 potent inhibitory effects of ESL and S-Lic on the Na_v1.5 isoform, suggesting a possible explanation
28 for the prolonged PR interval observed in patients on ESL treatment. Given that numerous cancer
29 cells have also been shown to express Na_v1.5, and that VGSCs potentiate invasion and metastasis,
30 this study also paves the way for future investigations into ESL and S-Lic as potential invasion
31 inhibitors.

32 **1 Introduction**

33 Eslicarbazepine acetate (ESL) is a member of the dibenzazepine anticonvulsant family of compounds
34 which also includes oxcarbazepine and carbamazepine (1). ESL has been approved by the European
35 Medicines Agency and the United States Federal Drug Administration as an adjunctive treatment for
36 partial-onset epileptic seizures (2). ESL is administered orally and rapidly undergoes first pass
37 hydrolysis to two stereoisomeric metabolites, R-licarbazepine and S-licarbazepine (S-Lic; also
38 known as eslicarbazepine; Figure 1A, B) (3-5). S-Lic represents around 95 % of circulating active
39 metabolites following first pass hydrolysis of ESL and is the enantiomer responsible for

40 anticonvulsant activity (6, 7). S-Lic also has improved blood brain barrier penetration compared to R-
 41 licarbazepine (8). Although S-Lic has been shown to inhibit T type Ca²⁺ channels (9), its main
 42 activity is likely through blockade of voltage-gated Na⁺ channels (VGSCs) (10). ESL offers several
 43 clinical advantages over other older VGSC-inhibiting antiepileptic drugs, e.g. carbamazepine,
 44 phenytoin; it has a favourable safety profile (10, 11), reduced induction of hepatic cytochrome P450
 45 enzymes (12), low potential for drug-drug interactions (13, 14), and takes less time to reach a steady
 46 state plasma concentration (15).

47 VGSCs are composed of a pore-forming α subunit in association with one or more auxiliary β
 48 subunits, the latter modulating channel gating and kinetics in addition to functioning as cell adhesion
 49 molecules (16). There are nine α subunits (Nav1.1-Nav1.9), and four β subunits (β 1-4) (17, 18). In
 50 postnatal and adult CNS neurons, the predominant α subunits are the tetrodotoxin-sensitive Nav1.1,
 51 Nav1.2 and Nav1.6 isoforms (19) and it is therefore on these that the VGSC-inhibiting activity of ESL
 52 and S-Lic has been described. In the murine neuroblastoma N1E-115 cell line, which expresses
 53 Nav1.1, Nav1.2, Nav1.3, Nav1.6 and Nav1.7, ESL and S-Lic both have a voltage-dependent inhibitory
 54 effect on the Na⁺ current (10, 20). In this cell model, S-Lic has no effect on the voltage-dependence
 55 of fast inactivation, but significantly hyperpolarises the voltage-dependence of slow inactivation (10).
 56 S-Lic also has a lower affinity for VGSCs in the resting state than carbamazepine or oxcarbazepine,
 57 thus potentially improving its therapeutic window over first- and second-generation dibenzazepine
 58 compounds (10). In acutely isolated murine hippocampal CA1 neurons, which express Nav1.1,
 59 Nav1.2 and Nav1.6 (21-23), S-Lic significantly reduces the persistent Na⁺ current, a very slow-
 60 inactivating component ~1 % the size of the peak transient Na⁺ current (24, 25). Moreover, in
 61 contrast to carbamazepine, this effect is maintained in the absence of β 1 (24, 26).

62 In healthy volunteers, ESL has not been associated with cardiotoxicity and the QT interval remains
 63 unchanged on treatment (27). However, a prolongation of the PR interval has been observed (27),
 64 suggesting that caution should be exercised in patients with cardiac conduction abnormalities (13).
 65 Prolongation of the PR interval suggests that ESL may also inhibit the cardiac Nav1.5 isoform,
 66 although this has not previously been studied. Nav1.5 is not only responsible for the initial
 67 depolarisation of the cardiac action potential (28), but is also expressed in breast and colon carcinoma
 68 cells, where the persistent Na⁺ current promotes invasion and metastasis (29-32). Inhibition of Nav1.5
 69 with phenytoin or ranolazine decreases tumour growth, invasion and metastasis (33-35). Thus, it is of
 70 interest to specifically understand the effect of ESL on the Nav1.5 isoform.

71 In the present study we investigated the electrophysiological effects of ESL and S-Lic on Nav1.5 [1]
 72 endogenously expressed in the MDA-MB-231 metastatic breast carcinoma cell line, and [2] stably
 73 over-expressed in HEK-293 cells. We show that both ESL and S-Lic inhibit transient and persistent
 74 Na⁺ current, hyperpolarise the voltage-dependence of fast inactivation, and slow the recovery from
 75 channel inactivation. These findings highlight, for the first time, the potent inhibitory effects of ESL
 76 and S-Lic on the Nav1.5 isoform.

77 **2 Materials and methods**

78 **2.1 Pharmacology**

79 ESL (Tokyo Chemical Industry UK Ltd) was dissolved in DMSO to make a stock concentration of
 80 67 mM. S-Lic (Tocris) was dissolved in DMSO to make a stock concentration of 300 mM. Both
 81 drugs were diluted to working concentrations of 100-300 μ M in extracellular recording solution. The
 82 concentration of DMSO in the recording solution was 0.45 % for ESL and 0.1 % for S-Lic. Equal

83 concentrations of DMSO were used in the control solutions. DMSO (0.45 %) had no effect on the
 84 Na⁺ current (Supplementary Figure 1).

85 2.2 Cell culture

86 MDA-MB-231 cells and HEK-293 cells stably expressing Nav1.5 (a gift from L. Isom, University of
 87 Michigan) were grown in Dulbecco's modified eagle medium supplemented with 5 % FBS and 4
 88 mM L-glutamine (36). Molecular identity of the MDA-MB-231 cells was confirmed by short tandem
 89 repeat analysis (37). Cells were confirmed as mycoplasma-free using the DAPI method (38). Cells
 90 were seeded onto glass coverslips 48 h before electrophysiological recording.

91 2.3 Electrophysiology

92 Plasma membrane Na⁺ currents were recorded using the whole-cell patch clamp technique, using
 93 methods described previously (32, 35). Patch pipettes made of borosilicate glass were pulled using a
 94 P-97 pipette puller (Sutter Instrument) and fire-polished to a resistance of 3-5 MΩ when filled with
 95 intracellular recording solution. The extracellular recording solution for MDA-MB-231 cells
 96 contained (in mM): 144 NaCl, 5.4 KCl, 1 MgCl₂, 2.5 CaCl₂, 5.6 D-glucose and 5 HEPES (adjusted to
 97 pH 7.2 with NaOH). For the extracellular recording solution for HEK-293 cells expressing Nav1.5,
 98 the extracellular [Na⁺] was reduced to account for the much larger Na⁺ currents and contained (in
 99 mM): 60 NaCl, 84 Choline Cl, 5.4 KCl, 1 MgCl₂, 2.5 CaCl₂, 5.6 D-glucose and 5 HEPES (adjusted
 100 to pH 7.2 with NaOH). The intracellular recording solution contained (in mM): 5 NaCl, 145 CsCl, 2
 101 MgCl₂, 1 CaCl₂, 10 HEPES, 11 EGTA, (adjusted to pH 7.4 with CsOH) (39). Voltage clamp
 102 recordings were made at room temperature using a Multiclamp 700B or Axopatch 200B amplifier
 103 (Molecular Devices) compensating for series resistance by 40–60%. Currents were digitized using a
 104 Digidata interface (Molecular Devices), low pass filtered at 10 kHz, sampled at 50 kHz and analysed
 105 using pCLAMP 10.7 software (Molecular Devices). Leak current was subtracted using a P/6 protocol
 106 (40). Extracellular recording solution ± drugs was applied to the recording bath at a rate of ~1.5
 107 ml/min using a ValveLink 4-channel gravity perfusion controller (AutoMate Scientific). Each new
 108 solution was allowed to equilibrate in the bath for ~4 min following switching prior to recording at
 109 steady state.

110 2.4 Voltage clamp protocols

111 Cells were clamped at a holding potential of -120 mV or -80 mV for ≥ 250 ms, dependent on
 112 experiment (detailed in the Figure legends). Five main voltage clamp protocols were used, as
 113 follows:

- 114 1. To assess the effect of drug perfusion and wash-out on peak current in real time, a simple one-
 115 step protocol was used where cells were held at -120 mV or -80 mV for 250 ms and then
 116 depolarised to -10 mV for 50 ms.
- 117 2. To assess the voltage-dependence of activation, cells were held at -120 mV for 250 ms and then
 118 depolarised to test potentials in 10 mV steps between -120 mV and +30 mV for 50 ms. The
 119 voltage of activation was taken as the most negative voltage which induced a visible transient
 120 inward current.
- 121 3. To assess the voltage-dependence of steady-state inactivation, cells were held at -120 mV for 250
 122 ms followed by prepulses for 250 ms in 10 mV steps between -120 mV and +30 mV and a test
 123 pulse to -10 mV for 50 ms.

124 4. To assess recovery from fast inactivation, cells were held at -120 mV for 250 ms, and then
 125 depolarised twice to 0 mV for 25 ms, returning to -120 mV for the following intervals between
 126 depolarisations (in ms): 1, 2, 3, 5, 7, 10, 15, 20, 30, 40, 50, 70, 100, 150, 200, 250, 350, 500. In
 127 each case, the second current was normalised to the initial current and plotted against the interval
 128 time.

129 2.5 Curve fitting and data analysis

130 To study the voltage-dependence of activation, current-voltage (I-V) relationships were converted to
 131 conductance using the following equation:

132 $G = I / (V_m - V_{rev})$, where G is conductance, I is current, V_m is the membrane voltage and V_{rev}
 133 is the reversal potential for Na⁺ derived from the Nernst equation. Given the different recording
 134 solutions used, V_{rev} for Na⁺ was +85 mV for MDA-MB-231 cells and +63 mV for HEK-Na_v1.5 cells.

135 The voltage-dependence of conductance and availability were normalised and fitted to a Boltzmann
 136 equation:

137 $G = G_{max} / (1 + \exp((V_{1/2} - V_m) / k))$, where G_{max} is the maximum conductance, $V_{1/2}$ is the
 138 voltage at which the channels are half activated/inactivated, V_m is the membrane voltage and k is the
 139 slope factor.

140 Recovery from inactivation data ($I_t / I_{t=0}$) were normalised, plotted against recovery time (Δt) and
 141 fitted to a single exponential function:

142 $\tau = A_1 + A_2 \exp(-t / t_0)$, where A_1 and A_2 are the coefficients of decay of the time constant
 143 (τ), t is time and t_0 is a time constant describing the time dependence of τ .

144 The time course of inactivation was fitted to a double exponential function:

145 $I = A_f \exp(-t / \tau_f) + A_s \exp(-t / \tau_s) + C$, where A_f and A_s are maximal amplitudes of the slow
 146 and fast components of the current, τ_f and τ_s are the fast and slow decay time constants and C is the
 147 asymptote.

148 2.6 Statistical analysis

149 Data are presented as mean and SEM unless stated otherwise. Statistical analysis was performed on
 150 the raw (non-normalised) data using GraphPad Prism 8.4.0. Pairwise statistical significance was
 151 determined with Student's paired t -tests. Multiple comparisons were made using ANOVA and Tukey
 152 post-hoc tests, unless stated otherwise. Results were considered significant at $P < 0.05$.

153 3 Results

154 3.1 Effect of eslicarbazepine acetate and S-licarbazepine on transient and persistent Na⁺ 155 current

156 Several studies have clearly established the inhibition of neuronal VGSCs (Na_v1.1, Na_v1.2, Na_v1.3,
 157 Na_v1.6, Na_v1.7 and Na_v1.8) by ESL and its active metabolite S-Lic (10, 20, 24, 41). Given that ESL
 158 prolongs the PR interval (27), potentially via inhibiting the cardiac Na_v1.5 isoform, together with the
 159 interest in inhibiting Na_v1.5 in carcinoma cells to reduce invasion and metastasis (33, 34, 42-44), it is
 160 also relevant to evaluate the electrophysiological effects of ESL and S-Lic on this isoform. We

161 therefore evaluated the effect of both compounds on $\text{Na}_v1.5$ current properties using whole-cell patch
 162 clamp recording, employing a two-pronged approach: (1) recording $\text{Na}_v1.5$ currents endogenously
 163 expressed in the MDA-MB-231 breast cancer cell line (29, 30, 45), and (2) recording from $\text{Na}_v1.5$
 164 stably over-expressed in HEK-293 cells (HEK- $\text{Na}_v1.5$) (46).

165 Initially, we evaluated the effect of both compounds on the size of the peak Na^+ current in MDA-
 166 MB-231 cells. Na^+ currents were elicited by depolarising the membrane potential (V_m) to -10 mV
 167 from a holding potential (V_h) of -120 mV or -80 mV. Application of the prodrug ESL (300 μM)
 168 reversibly inhibited the transient Na^+ current by 49.6 ± 3.2 % when the V_h was -120 mV ($P < 0.001$;
 169 $n = 13$; ANOVA + Tukey test; Figure 2A, D). When V_h was set to -80 mV, ESL (300 μM) reversibly
 170 inhibited the transient Na^+ current by 79.5 ± 4.5 % ($P < 0.001$; $n = 12$; ANOVA + Tukey test; Figure
 171 2C, E). We next assessed the effect of ESL in HEK- $\text{Na}_v1.5$ cells. Application of ESL (300 μM)
 172 inhibited $\text{Na}_v1.5$ current by 74.7 ± 4.3 % when V_h was -120 mV ($P < 0.001$; $n = 12$; Figure 2F, I) and
 173 by 90.5 ± 2.8 % when V_h was -80 mV ($P < 0.001$; $n = 14$; Figure 2H, J). However, the inhibition was
 174 only partially reversible ($P < 0.001$; $n = 14$; Figure 2F, H-J). Application of ESL at a lower
 175 concentration (100 μM) elicited a similar result (Supplementary Figure 2A-J & Supplementary Table
 176 1). Together, these data suggest that ESL preferentially inhibited $\text{Na}_v1.5$ in the open or inactivated
 177 state, since the current inhibition was greater at more depolarised V_h .

178 We next tested the effect of the active metabolite S-Lic. S-Lic (300 μM) inhibited the transient Na^+
 179 current in MDA-MB-231 cells by 44.4 ± 6.1 % when the V_h was -120 mV ($P < 0.001$; $n = 9$;
 180 ANOVA + Tukey test; Figure 3A, D). When V_h was set to -80 mV, S-Lic (300 μM) inhibited the
 181 transient Na^+ current by 73.6 ± 4.1 % ($P < 0.001$; $n = 10$; ANOVA + Tukey test; Figure 3C, E).
 182 However, the inhibition caused by S-Lic (300 μM) was only partially reversible ($P < 0.05$; $n = 10$;
 183 ANOVA + Tukey test; Figure 3A, C-E). In HEK- $\text{Na}_v1.5$ cells, S-Lic (300 μM) inhibited $\text{Na}_v1.5$
 184 current by 46.4 ± 3.9 % when V_h was -120 mV ($P < 0.001$; $n = 13$; ANOVA + Tukey test; Figure 3F,
 185 I) and by 74.0 ± 4.2 % when V_h was -80 mV ($P < 0.001$; $n = 12$; ANOVA + Tukey test; Figure 3H,
 186 J). Furthermore, the inhibition in HEK- $\text{Na}_v1.5$ cells was not reversible over the duration of the
 187 experiment. Application of S-Lic at a lower concentration (100 μM) elicited a broadly similar result
 188 (Supplementary Figure 3A-J & Supplementary Table 1). Together, these data show that channel
 189 inhibition by S-Lic was also more effective at more depolarised V_h . However, unlike ESL, channel
 190 blockade by S-Lic persisted after washout, suggesting higher target binding affinity for the active
 191 metabolite and/or greater trapping of the active metabolite in the cytoplasm.

192 We also assessed the effect of both compounds on the persistent Na^+ current measured 20-25 ms after
 193 depolarisation to -10 mV from -120 mV. In MDA-MB-231 cells, ESL (300 μM) inhibited the
 194 persistent Na^+ current by 77 ± 34 % although the reduction was not statistically significant ($P = 0.13$;
 195 $n = 12$; paired t test; Figure 2B, Table 1). In HEK- $\text{Na}_v1.5$ cells, ESL (300 μM) inhibited persistent
 196 current by 76 ± 10 % ($P < 0.01$; $n = 12$; paired t test; Figure 2G, Table 1). S-Lic (300 μM) inhibited
 197 the persistent Na^+ current in MDA-MB-231 cells by 66 ± 16 % ($P < 0.05$; $n = 9$; paired t test; Figure
 198 3B, Table 2). In HEK- $\text{Na}_v1.5$ cells, S-Lic (300 μM) inhibited persistent current by 35 ± 16 % ($P <$
 199 0.05 ; $n = 11$; Figure 3G, Table 2). Application of both compounds at a lower concentration (100 μM)
 200 elicited a similar result (Supplementary Table 1). In summary, both ESL and S-Lic also inhibited the
 201 persistent Na^+ current.

202 3.2 Effect of eslicarbazepine acetate and S-licarbazepine on voltage dependence of activation 203 and inactivation

204 We next investigated the effect of ESL (300 μM) and S-Lic (300 μM) on the I-V relationship in
 205 MDA-MB-231 and HEK-Nav1.5 cells. A V_h of -120 mV was used for subsequent analyses to ensure
 206 that the elicited currents were sufficiently large for analysis of kinetics and voltage dependence,
 207 particularly for MDA-MB-231 cells, which display smaller peak Na⁺ currents (Tables 1, 2). Neither
 208 ESL nor S-Lic had any effect on the threshold voltage for activation (Figure 4A-D; Tables 1, 2). ESL
 209 also had no effect on the voltage at current peak in either cell line (Figure 4A-D; Tables 1, 2).
 210 Although S-Lic had no effect on voltage at current peak in MDA-MB-231 cells, it was significantly
 211 hyperpolarised in HEK-Nav1.5 cells from -18.0 ± 4.2 mV to -30.0 ± 5.6 mV (P < 0.001; n = 9; paired
 212 t test; Figure 4A-D; Tables 1, 2).

213 ESL had no significant effect on the half-activation voltage (V_{1/2}) or slope factor (k) for activation in
 214 MDA-MB-231 cells (Figure 5A; Table 1). The activation k in HEK-Nav1.5 cells was also unchanged
 215 but the activation V_{1/2} was significantly hyperpolarised by ESL from -39.4 ± 1.3 to -44.2 ± 1.8 mV (P
 216 < 0.05; n = 10; paired t test; Figure 5B; Table 1). S-Lic also had no significant effect on the activation
 217 V_{1/2} or k in MDA-MB-231 cells (Figure 5C; Table 2). However, the V_{1/2} of activation in HEK-Nav1.5
 218 cells was significantly hyperpolarised from -32.8 ± 3.1 mV to -40.5 ± 3.4 mV (P < 0.01; n = 9; paired
 219 t test; Figure 5D; Table 2) and k changed from 5.9 ± 0.9 mV to 4.5 ± 1.1 mV (P < 0.05; n = 9; paired
 220 t test; Figure 5D; Table 2).

221 As regards steady-state inactivation, in MDA-MB-231 cells, ESL significantly hyperpolarised the
 222 inactivation V_{1/2} from -80.6 ± 0.7 mV to -86.7 ± 1.2 mV (P < 0.001; n = 13; paired t test) without
 223 affecting inactivation k (Figure 5A; Table 1). ESL also hyperpolarised the inactivation V_{1/2} in HEK-
 224 Nav1.5 cells from -78.2 ± 2.5 mV to -88.3 ± 2.7 mV (P < 0.001; n = 10; paired t test), and changed
 225 the inactivation k from -6.9 ± 0.4 mV to -9.8 ± 0.7 mV (P < 0.001; n = 10; paired t test; Figure 5B;
 226 Table 1). S-Lic also significantly hyperpolarised the inactivation V_{1/2} in MDA-MB-231 cells from -
 227 71.8 ± 2.5 mV to -76.8 ± 2.2 mV (P < 0.05; n = 7; paired t test) without affecting inactivation k
 228 (Figure 5C; Table 2). However, the inactivation V_{1/2} in HEK-Nav1.5 cells was not significantly
 229 altered by S-Lic, although the inactivation k significantly changed from -6.5 ± 0.4 mV to -8.1 ± 0.5
 230 mV (P < 0.05; n = 9; paired t test; Figure 5D; Table 2). In summary, both ESL and S-Lic affected
 231 various aspects of the voltage dependence characteristics of Na_v1.5 in MDA-MB-231 and HEK-
 232 Nav1.5 cells, predominantly hyperpolarising the voltage dependence of inactivation.

233 3.3 Effect of eslicarbazepine acetate and S-licarbazepine on activation and inactivation 234 kinetics

235 We next studied the effect of both compounds on kinetics of activation and inactivation. In MDA-
 236 MB-231 cells, ESL (300 μM) significantly accelerated the time to peak current (T_p), upon
 237 depolarisation from -120 mV to -10 mV, from 2.1 ± 0.2 ms to 1.9 ± 0.2 ms (P < 0.01; n = 13; paired t
 238 test; Table 1). However, in HEK-Nav1.5 cells, ESL significantly slowed T_p from 1.4 ± 0.2 ms to 1.5 ±
 239 0.2 ms (P < 0.001; n = 14; paired t test; Table 1). S-Lic (300 μM) had no significant effect on T_p in
 240 MDA-MB-231 cells but significantly slowed T_p in HEK-Nav1.5 cells from 1.8 ± 0.5 ms to 2.3 ± 0.6
 241 ms (P < 0.01; n = 13; paired t test; Table 2).

242 To study effects on inactivation kinetics, the current decay following depolarisation from -120 mV to
 243 -10 mV was fitted to a double exponential function to derive fast and slow time constants of
 244 inactivation (τ_f and τ_s). Neither ESL nor S-Lic had any significant effect on τ_f or τ_s in MDA-MB-231
 245 cells (Tables 1, 2). However, in HEK-Nav1.5 cells, ESL significantly slowed τ_f from 0.9 ± 0.1 ms to
 246 1.2 ± 0.1 ms (P < 0.001; n = 12; paired t test; Table 1) and slowed τ_s from 6.6 ± 0.8 ms to 20.8 ± 8.5
 247 ms, although this was not statistically significant. S-Lic significantly slowed τ_f from 1.0 ± 0.04 ms to

248 1.3 ± 0.06 ms (P < 0.001; n = 11; paired t test; Table 2) and τ_s from 6.3 ± 0.5 ms to 7.3 ± 0.5 ms (P <
 249 0.05; n = 11; paired t test; Table 2). In summary, both ESL and S-Lic elicited various effects on
 250 kinetics in MDA-MB-231 and HEK-Na_v1.5 cells, predominantly slowing activation and inactivation.

251 3.4 Effect of eslicarbazepine acetate and S-licarbazepine on recovery from fast inactivation

252 To investigate the effect of ESL and S-Lic on channel recovery from fast inactivation, we subjected
 253 cells to two depolarisations from V_h of -120 mV to 0 mV, changing the interval between these in
 254 which the channels were held at -120 mV to facilitate recovery. Significance was determined by
 255 fitting a single exponential curve to the normalised current/time relationship and calculating the time
 256 constant (τ_r). In MDA-MB-231 cells, ESL (300 μM) significantly slowed τ_r from 6.0 ± 0.5 ms to 8.7
 257 ± 0.7 ms (P < 0.05; n = 10; paired t test; Figure 6A, Table 1). Similarly, in HEK-Na_v1.5 cells, ESL
 258 significantly slowed τ_r from 4.5 ± 0.4 ms to 7.1 ± 0.6 ms (P < 0.001; n = 10; paired t test; Figure 6B,
 259 Table 1). S-Lic (300 μM) also significantly slowed τ_r in MDA-MB-231 cells from 6.8 ± 0.4 ms to
 260 13.5 ± 1.0 ms (P < 0.01; n = 7; paired t test; Figure 6C, Table 2). Finally, S-Lic also significantly
 261 slowed τ_r in HEK-Na_v1.5 cells from 5.7 ± 0.7 ms to 8.0 ± 1.2 ms (P < 0.01; n = 10; paired t test;
 262 Figure 6D, Table 2). In summary, both ESL and S-Lic slowed recovery from fast inactivation of
 263 Na_v1.5.

264 4 Discussion

265 In this study, we have shown that ESL and its active metabolite S-Lic inhibit the transient and
 266 persistent components of Na⁺ current carried by Na_v1.5. We show broadly similar effects in MDA-
 267 MB-231 cells, which express endogenous Na_v1.5 (29, 30, 45), and in HEK-293 cells over-expressing
 268 Na_v1.5. Notably, both compounds were more effective when V_h was set to -80 mV than at -120 mV,
 269 suggestive of depolarised state-dependent binding. In addition, the inhibitory effect of ESL was
 270 reversible whereas inhibition by S-Lic was less so. As regards voltage-dependence, both ESL and S-
 271 Lic shifted activation and steady-state inactivation curves, to varying extents in the two cell lines, in
 272 the direction of more negative voltages. ESL and S-Lic had various effects on activation and
 273 inactivation kinetics, generally slowing the rate of inactivation. Finally, recovery from fast
 274 inactivation of Na_v1.5 was significantly slowed by both ESL and S-Lic.

275 To our knowledge, this is the first time that the effects of ESL and S-Lic have specifically been tested
 276 on the Na_v1.5 isoform. A strength of this study is that both the prodrug (ESL) and the active
 277 metabolite (S-Lic) were tested using two independent cell lines, one endogenously expressing
 278 Na_v1.5, the other stably over-expressing Na_v1.5. MDA-MB-231 cells also express Na_v1.7, although
 279 this isoform is estimated to be responsible for only ~9 % of the total VGSC current (30, 45). MDA-
 280 MB-231 cells also express endogenous β1, β2 and β4 subunits (47-49). MDA-MB-231 cells
 281 predominantly express the developmentally regulated ‘neonatal’ Na_v1.5 splice variant, which differs
 282 from the ‘adult’ variant over-expressed in the HEK-Na_v1.5 cells by seven amino acids located in the
 283 extracellular linker between transmembrane segments 3 and 4 of domain 1 (30, 42, 45). Notably,
 284 however, there were no consistent differences in effect of either ESL or S-Lic between the MDA-
 285 MB-231 and HEK-Na_v1.5 cells, suggesting that the neonatal vs. adult splicing event, and/or
 286 expression of endogenous β subunits, does not impact on sensitivity of Na_v1.5 to these compounds.
 287 This finding contrasts another report showing different sensitivity of the neonatal and adult Na_v1.5
 288 splice variants to the amide local anaesthetics lidocaine and levobupivacaine (44). Our findings
 289 suggest that the inhibitory effect of S-Lic on Na_v1.5 is less reversible than that of ESL. This may be
 290 explained by the differing chemical structures of the two molecules possibly enabling S-Lic to bind
 291 the target with higher affinity than ESL. Most VGSC-targeting anticonvulsants, including phenytoin,

292 lamotrigine and carbamazepine, block the pore by binding via aromatic-aromatic interaction to a
 293 tyrosine and phenylalanine located in the S6 helix of domain 4 (50). However, S-Lic has been
 294 proposed to bind to a different site given that it was found to block the pore predominantly during
 295 slow inactivation (10). Alternatively, the hydroxyl group present on S-Lic (but not ESL) may become
 296 deprotonated, potentially trapping it in the cytoplasm.

297 The findings presented here broadly agree with *in vitro* concentrations used elsewhere to study
 298 effects of ESL and S-Lic on Na⁺ currents. For example, using a V_h of -80 mV, 300 μM ESL was
 299 shown to inhibit peak Na⁺ current by 50 % in N1E-115 neuroblastoma cells expressing Nav1.1,
 300 Nav1.2, Nav1.3, Nav1.6 and Nav1.7 (20). S-Lic (250 μM) also blocks peak Na⁺ current by ~50 % in
 301 the same cell line (10). In addition, S-Lic (300 μM) reduces persistent Na⁺ current by ~25 % in
 302 acutely isolated murine hippocampal CA1 neurons expressing Nav1.1, Nav1.2 and Nav1.6 (21-24).
 303 Similar to the present study, ESL was shown to hyperpolarise the voltage-dependence of steady-state
 304 inactivation in N1E-115 cells (20). On the other hand, similar to our finding in HEK-Nav1.5 cells, S-
 305 Lic has no effect on steady-state inactivation in N1E-115 cells (10). Again, in agreement with our
 306 own findings for Nav1.5, S-Lic slows recovery from inactivation in N1E-115 cells (10). These
 307 observations suggest that the sensitivity of Nav1.5 to ESL and S-Lic is broadly similar to that
 308 reported for neuronal VGSCs. In support of this, Nav1.5 shares the same conserved residues proposed
 309 for Nav1.2 to interact with ESL (Figure 7) (51).

310 Notably, the concentrations used in this study are at or above those achieved in clinical use (e.g. ESL
 311 1200 mg once daily gives a peak plasma concentration of ~100 μM) (10). However, it has been
 312 argued that the relatively high concentrations which have been previously tested *in vitro* are clinically
 313 relevant given that S-Lic has a high (50:1) lipid:water partition co-efficient and thus would be
 314 expected to reside predominantly in the tissue membrane fraction *in vivo* (15). Our study suggests
 315 that a clinically relevant plasma concentration (100 μM) would inhibit peak and persistent Nav1.5
 316 currents. Future work investigating the dose-dependent effects of ESL and S-Lic would be useful to
 317 aid clinical judgements.

318 The data presented here raise several implications for clinicians. The observed inhibition of Nav1.5 is
 319 worthy of note when considering cardiac function in patients receiving ESL (13). Although the QT
 320 interval remains unchanged for individuals on ESL treatment, prolongation of the PR interval has
 321 been observed (27). Further work is required to establish whether the basis for this PR prolongation
 322 is indeed via Nav1.5 inhibition. In addition, it would be of interest to investigate the efficacy of ESL
 323 and S-Lic in the context of heritable arrhythmogenic mutations in *SCN5A*, as well as the possible
 324 involvement of the β subunits (24, 26, 52, 53). The findings presented here are also relevant in the
 325 context of Nav1.5 expression in carcinoma cells (54). Given that cancer cells have a relatively
 326 depolarised V_m, it is likely that Nav1.5 is mainly in the inactivated state with the persistent Na⁺
 327 current being functionally predominant (55, 56). Increasing evidence suggests that persistent Na⁺
 328 current carried by Nav1.5 in cancer cells contributes to invasion and several studies have shown that
 329 other VGSC inhibitors reduce metastasis in preclinical models (29-35, 57). Thus, use-dependent
 330 inhibition by ESL would ensure that channels in malignant cells are particularly targeted, raising the
 331 possibility that it could be used as an anti-metastatic agent (43). This study therefore paves the way
 332 for future investigations into ESL and S-Lic as potential invasion inhibitors.

333 5 Author Contributions

334 TL, SC and WB contributed to the conception and design of the work. TL, LB and WB contributed
 335 to acquisition, analysis, and interpretation of data for the work. TL, SC and WB contributed to

336 drafting the work and revising it critically for important intellectual content. All authors approved the
337 final version of the manuscript.

338 **6 Abbreviations**

339 ESL, eslicarbazepine acetate; HEK-Nav1.5, HEK-293 cells stably expressing Nav1.5; I-V, current-
340 voltage; k, slope factor; PSS, physiological saline solution; S-Lic, S-licarbazepine, T_p: time to peak
341 current; τ_f: fast time constant of inactivation; τ_s: slow time constant of inactivation; τ_r: time constant
342 of recovery from inactivation; VGSC, voltage-gated Na⁺ channel; V_m, membrane potential; V_h,
343 holding potential; V_{peak}: voltage at which current was maximal; V_{rev}, reversal potential; V_{thres}:
344 threshold voltage for activation; V_{1/2}, half-activation voltage.

345 **7 Acknowledgements**

346 This work was supported by Cancer Research UK (A25922) and Breast Cancer Now
347 (2015NovPhD572).

348 **8 Conflict of interest statement**

349 The authors declare that the research was conducted in the absence of any commercial or financial
350 relationships that could be construed as a potential conflict of interest.

351 **9 Data availability statement**

352 The datasets used and/or analysed during the current study are available from the corresponding
353 author on reasonable request.

354 **10 References**

- 355 1. Almeida L, Soares-da-Silva P. Eslicarbazepine acetate (BIA 2-093). *Neurotherapeutics*.
356 2007;4(1):88-96.
- 357 2. Sperling MR, Abou-Khalil B, Harvey J, Rogin JB, Biraben A, Galimberti CA, et al.
358 Eslicarbazepine acetate as adjunctive therapy in patients with uncontrolled partial-onset seizures:
359 Results of a phase III, double-blind, randomized, placebo-controlled trial. *Epilepsia*. 2015;56(2):244-
360 53.
- 361 3. Almeida L, Falcao A, Maia J, Mazur D, Gellert M, Soares-da-Silva P. Single-dose and
362 steady-state pharmacokinetics of eslicarbazepine acetate (BIA 2-093) in healthy elderly and young
363 subjects. *J Clin Pharmacol*. 2005;45(9):1062-6.
- 364 4. Almeida L, Minciu I, Nunes T, Butoianu N, Falcão A, Magureanu S-A, et al.
365 Pharmacokinetics, Efficacy, and Tolerability of Eslicarbazepine Acetate in Children and Adolescents
366 With Epilepsy. *The Journal of Clinical Pharmacology*. 2008;48(8):966-77.
- 367 5. Perucca E, Elger C, Halász P, Falcão A, Almeida L, Soares-da-Silva P. Pharmacokinetics of
368 eslicarbazepine acetate at steady-state in adults with partial-onset seizures. *Epilepsy Res*.
369 2011;96(1):132-9.
- 370 6. Potschka H, Soerensen J, Pekcec A, Loureiro A, Soares-da-Silva P. Effect of eslicarbazepine
371 acetate in the corneal kindling progression and the amygdala kindling model of temporal lobe
372 epilepsy. *Epilepsy Res*. 2014;108(2):212-22.

- 373 7. Sierra-Paredes G, Loureiro AI, Wright LC, Sierra-Marcuño G, Soares-da-Silva P. Effects of
374 eslicarbazepine acetate on acute and chronic latrunculin A-induced seizures and extracellular amino
375 acid levels in the mouse hippocampus. *BMC Neurosci.* 2014;15(1):134.
- 376 8. Alves G, Figueiredo I, Falcao A, Castel-Branco M, Caramona M, Soares-Da-Silva P.
377 Stereoselective disposition of S- and R-licarbazepine in mice. *Chirality.* 2008;20(6):796-804.
- 378 9. Brady K, Hebeisen S, Konrad D, Soares-da-Silva P. The effects of eslicarbazepine, R-
379 licarbazepine, oxcarbazepine and carbamazepine on ion transmission Cav3.2 channels. *Epilepsia.*
380 2011;52:260.
- 381 10. Hebeisen S, Pires N, Loureiro AI, Bonifacio MJ, Palma N, Whyment A, et al. Eslicarbazepine
382 and the enhancement of slow inactivation of voltage-gated sodium channels: a comparison with
383 carbamazepine, oxcarbazepine and lacosamide. *Neuropharmacology.* 2015;89:122-35.
- 384 11. Brown ME, El-Mallakh RS. Role of eslicarbazepine in the treatment of epilepsy in adult
385 patients with partial-onset seizures. *Ther Clin Risk Manag.* 2010;6:103-9.
- 386 12. Galiana GL, Gauthier AC, Mattson RH. Eslicarbazepine Acetate: A New Improvement on a
387 Classic Drug Family for the Treatment of Partial-Onset Seizures. *Drugs R D.* 2017;17(3):329-39.
- 388 13. Zaccara G, Giovannelli F, Cincotta M, Carelli A, Verrotti A. Clinical utility of
389 eslicarbazepine: current evidence. *Drug Des Devel Ther.* 2015;9:781-9.
- 390 14. Falcao A, Fuseau E, Nunes T, Almeida L, Soares-da-Silva P. Pharmacokinetics, drug
391 interactions and exposure-response relationship of eslicarbazepine acetate in adult patients with
392 partial-onset seizures: population pharmacokinetic and pharmacokinetic/pharmacodynamic analyses.
393 *CNS Drugs.* 2012;26(1):79-91.
- 394 15. Bialer M, Soares-da-Silva P. Pharmacokinetics and drug interactions of eslicarbazepine
395 acetate. *Epilepsia.* 2012;53(6):935-46.
- 396 16. Catterall WA. Structure and function of voltage-gated sodium channels at atomic resolution.
397 *Exp Physiol.* 2014;99(1):35-51.
- 398 17. Goldin AL, Barchi RL, Caldwell JH, Hofmann F, Howe JR, Hunter JC, et al. Nomenclature
399 of voltage-gated sodium channels. *Neuron.* 2000;28:365-8.
- 400 18. Brackenbury WJ, Isom LL. Na Channel beta Subunits: Overachievers of the Ion Channel
401 Family. *Front Pharmacol.* 2011;2:53.
- 402 19. Van Wart A, Matthews G. Impaired firing and cell-specific compensation in neurons lacking
403 nav1.6 sodium channels. *J Neurosci.* 2006;26(27):7172-80.
- 404 20. Bonifacio MJ, Sheridan RD, Parada A, Cunha RA, Patmore L, Soares-da-Silva P. Interaction
405 of the novel anticonvulsant, BIA 2-093, with voltage-gated sodium channels: comparison with
406 carbamazepine. *Epilepsia.* 2001;42(5):600-8.
- 407 21. Royeck M, Horstmann MT, Remy S, Reitze M, Yaari Y, Beck H. Role of axonal Nav1.6
408 sodium channels in action potential initiation of CA1 pyramidal neurons. *J Neurophysiol.*
409 2008;100(4):2361-80.
- 410 22. Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, et al. Reduced
411 sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in
412 infancy. *Nat Neurosci.* 2006;9(9):1142-9.
- 413 23. Westenbroek RE, Merrick DK, Catterall WA. Differential subcellular localization of the RI
414 and RII Na⁺ channel subtypes in central neurons. *Neuron.* 1989;3(6):695-704.

- 415 24. Doeser A, Soares-da-Silva P, Beck H, Uebachs M. The effects of eslicarbazepine on
 416 persistent Na(+) current and the role of the Na(+) channel beta subunits. *Epilepsy Res.*
 417 2014;108(2):202-11.
- 418 25. Saint DA. The cardiac persistent sodium current: an appealing therapeutic target? *Br J*
 419 *Pharmacol.* 2008;153(6):1133-42.
- 420 26. Uebachs M, Opitz T, Royeck M, Dickhof G, Horstmann MT, Isom LL, et al. Efficacy loss of
 421 the anticonvulsant carbamazepine in mice lacking sodium channel beta subunits via paradoxical
 422 effects on persistent sodium currents. *J Neurosci.* 2010;30(25):8489-501.
- 423 27. Vaz-Da-Silva M, Nunes T, Almeida L, Gutierrez MJ, Litwin JS, Soares-Da-Silva P.
 424 Evaluation of Eslicarbazepine acetate on cardiac repolarization in a thorough QT/QTc study. *J Clin*
 425 *Pharmacol.* 2012;52(2):222-33.
- 426 28. George AL, Jr. Inherited disorders of voltage-gated sodium channels. *J Clin Invest.*
 427 2005;115(8):1990-9.
- 428 29. Roger S, Besson P, Le Guennec JY. Involvement of a novel fast inward sodium current in the
 429 invasion capacity of a breast cancer cell line. *Biochim Biophys Acta.* 2003;1616(2):107-11.
- 430 30. Fraser SP, Diss JK, Chioni AM, Mycielska ME, Pan H, Yamaci RF, et al. Voltage-gated
 431 sodium channel expression and potentiation of human breast cancer metastasis. *Clin Cancer Res.*
 432 2005;11(15):5381-9.
- 433 31. House CD, Vaske CJ, Schwartz AM, Obias V, Frank B, Luu T, et al. Voltage-gated Na⁺
 434 channel SCN5A is a key regulator of a gene transcriptional network that controls colon cancer
 435 invasion. *Cancer Res.* 2010;70(17):6957-67.
- 436 32. Nelson M, Yang M, Millican-Slater R, Brackenbury WJ. Nav1.5 regulates breast tumor
 437 growth and metastatic dissemination in vivo. *Oncotarget.* 2015;6(32):32914-29.
- 438 33. Nelson M, Yang M, Dowle AA, Thomas JR, Brackenbury WJ. The sodium channel-blocking
 439 antiepileptic drug phenytoin inhibits breast tumour growth and metastasis. *Mol Cancer.*
 440 2015;14(1):13.
- 441 34. Driffort V, Gillet L, Bon E, Marionneau-Lambot S, Oullier T, Joulin V, et al. Ranolazine
 442 inhibits Nav1.5-mediated breast cancer cell invasiveness and lung colonization. *Mol Cancer.*
 443 2014;13(1):264.
- 444 35. Yang M, Kozminski DJ, Wold LA, Modak R, Calhoun JD, Isom LL, et al. Therapeutic
 445 potential for phenytoin: targeting Na(v)1.5 sodium channels to reduce migration and invasion in
 446 metastatic breast cancer. *Breast Cancer Res Treat.* 2012;134(2):603-15.
- 447 36. Simon A, Yang M, Marrison JL, James AD, Hunt MJ, O'Toole PJ, et al. Metastatic breast
 448 cancer cells induce altered microglial morphology and electrical excitability in vivo. *J*
 449 *Neuroinflammation.* 2020;17(1):87.
- 450 37. Masters JR, Thomson JA, Daly-Burns B, Reid YA, Dirks WG, Packer P, et al. Short tandem
 451 repeat profiling provides an international reference standard for human cell lines. *Proc Natl Acad Sci*
 452 *U S A.* 2001;98(14):8012-7.
- 453 38. Uphoff CC, Gignac SM, Drexler HG. Mycoplasma contamination in human leukemia cell
 454 lines. I. Comparison of various detection methods. *J Immunol Methods.* 1992;149(1):43-53.
- 455 39. Brackenbury WJ, Djamgoz MB. Activity-dependent regulation of voltage-gated Na⁺ channel
 456 expression in Mat-LyLu rat prostate cancer cell line. *J Physiol.* 2006;573(Pt 2):343-56.

- 457 40. Armstrong CM, Bezanilla F. Inactivation of the sodium channel. II. Gating current
458 experiments. *J Gen Physiol.* 1977;70(5):567-90.
- 459 41. Soares-da-Silva P, Pires N, Bonifácio MJ, Loureiro AI, Palma N, Wright LC. Eslicarbazepine
460 acetate for the treatment of focal epilepsy: an update on its proposed mechanisms of action.
461 *Pharmacol Res Perspect.* 2015;3(2).
- 462 42. Djamgoz MBA, Fraser SP, Brackenbury WJ. In Vivo Evidence for Voltage-Gated Sodium
463 Channel Expression in Carcinomas and Potentiation of Metastasis. *Cancers (Basel).* 2019;11(11).
- 464 43. Martin F, Ufodiana C, Watt I, Bland M, Brackenbury WJ. Therapeutic value of voltage-gated
465 sodium channel inhibitors in breast, colorectal and prostate cancer: a systematic review. *Front*
466 *Pharmacol.* 2015;6:273.
- 467 44. Elajnaf T, Baptista-Hon DT, Hales TG. Potent Inactivation-Dependent Inhibition of Adult
468 and Neonatal Nav1.5 Channels by Lidocaine and Levobupivacaine. *Anesth Analg.* 2018;127(3):650-
469 60.
- 470 45. Brackenbury WJ, Chioni AM, Diss JK, Djamgoz MB. The neonatal splice variant of Nav1.5
471 potentiates in vitro metastatic behaviour of MDA-MB-231 human breast cancer cells. *Breast Cancer*
472 *Res Treat.* 2007;101(2):149-60.
- 473 46. Patino GA, Brackenbury WJ, Bao YY, Lopez-Santiago LF, O'Malley HA, Chen CL, et al.
474 Voltage-Gated Na⁺ Channel beta 1B: A Secreted Cell Adhesion Molecule Involved in Human
475 Epilepsy. *J Neurosci.* 2011;31(41):14577-91.
- 476 47. Nelson M, Millican-Slater R, Forrest LC, Brackenbury WJ. The sodium channel beta1
477 subunit mediates outgrowth of neurite-like processes on breast cancer cells and promotes tumour
478 growth and metastasis. *Int J Cancer.* 2014;135(10):2338-51.
- 479 48. Chioni AM, Brackenbury WJ, Calhoun JD, Isom LL, Djamgoz MB. A novel adhesion
480 molecule in human breast cancer cells: voltage-gated Na⁺ channel beta1 subunit. *Int J Biochem Cell*
481 *Biol.* 2009;41(5):1216-27.
- 482 49. Bon E, Driffort V, Gradek F, Martinez-Caceres C, Anachelin M, Pelegrin P, et al. SCN4B acts
483 as a metastasis-suppressor gene preventing hyperactivation of cell migration in breast cancer. *Nature*
484 *communications.* 2016;7:13648.
- 485 50. Lipkind GM, Fozzard HA. Molecular model of anticonvulsant drug binding to the voltage-
486 gated sodium channel inner pore. *Mol Pharmacol.* 2010;78(4):631-8.
- 487 51. Shaikh S, Rizvi SM, Hameed N, Biswas D, Khan M, Shakil S, et al. Aptiom (eslicarbazepine
488 acetate) as a dual inhibitor of beta-secretase and voltage-gated sodium channel: advancement in
489 Alzheimer's disease-epilepsy linkage via an enzoinformatics study. *CNS Neurol Disord Drug*
490 *Targets.* 2014;13(7):1258-62.
- 491 52. Brackenbury WJ, Isom LL. Voltage-gated Na⁺ channels: potential for beta subunits as
492 therapeutic targets. *Expert Opin Ther Targets.* 2008;12(9):1191-203.
- 493 53. Rivaud MR, Delmar M, Remme CA. Heritable arrhythmia syndromes associated with
494 abnormal cardiac sodium channel function: ionic and non-ionic mechanisms. *Cardiovasc Res.* 2020.
- 495 54. Fraser SP, Ozerlat-Gunduz I, Brackenbury WJ, Fitzgerald EM, Campbell TM, Coombes RC,
496 et al. Regulation of voltage-gated sodium channel expression in cancer: hormones, growth factors
497 and auto-regulation. *Philos Trans R Soc Lond B Biol Sci.* 2014;369(1638):20130105.

- 498 55. Yang M, Brackenbury WJ. Membrane potential and cancer progression. *Front Physiol.*
 499 2013;4:185.
- 500 56. Yang M, James AD, Suman R, Kasprowicz R, Nelson M, O'Toole PJ, et al. Voltage-
 501 dependent activation of Rac1 by Nav 1.5 channels promotes cell migration. *J Cell Physiol.*
 502 2020;235(4):3950-72.
- 503 57. Besson P, Driffort V, Bon E, Gradek F, Chevalier S, Roger S. How do voltage-gated sodium
 504 channels enhance migration and invasiveness in cancer cells? *Biochim Biophys Acta.* 2015;1848(10
 505 Pt B):2493-501.

506

507 10.1 Figure legends

508 **Figure 1.** Chemical structures of eslicarbazepine acetate and S-licarbazepine. (A) eslicarbazepine
 509 acetate; (9S)-2-carbamoyl-2-azatricyclo[9.4.0.0^{3,8}]pentadeca-1(15),3,5,7,11,13-hexaen-9-yl acetate.
 510 (B) S-licarbazepine; (10R)-10-hydroxy-2-azatricyclo[9.4.0.0^{3,8}]pentadeca-1(11),3,5,7,12,14-hexaene-
 511 2-carboxamide. Structures were drawn using Chemspider software.

512 **Figure 2.** Effect of eslicarbazepine acetate on Nav1.5 currents. (A) Representative Na⁺ currents in an
 513 MDA-MB-231 cell elicited by a depolarisation from -120 mV to -10 mV in physiological saline
 514 solution (PSS; black), eslicarbazepine acetate (ESL; 300 μM; red) and after washout (grey). Dotted
 515 vertical lines define the time period magnified in (B). (B) Representative persistent Na⁺ currents in an
 516 MDA-MB-231 cell elicited by a depolarisation from -120 mV to -10 mV. (C) Representative Na⁺
 517 currents in an MDA-MB-231 cell elicited by a depolarisation from -80 mV to -10 mV. (D)
 518 Normalised Na⁺ currents in MDA-MB-231 cells elicited by a depolarisation from -120 mV to -10
 519 mV. (E) Normalised Na⁺ currents in MDA-MB-231 cells elicited by a depolarisation from -80 mV to
 520 -10 mV. (F) Representative Na⁺ currents in a HEK-Nav1.5 cell elicited by a depolarisation from -120
 521 mV to -10 mV in PSS (black), ESL (300 μM; red) and after washout (grey). Dotted vertical lines
 522 define the time period magnified in (G). (G) Representative persistent Na⁺ currents in a HEK-Nav1.5
 523 cell elicited by a depolarisation from -120 mV to -10 mV. (H) Representative Na⁺ currents in a HEK-
 524 Nav1.5 cell elicited by a depolarisation from -80 mV to -10 mV. (I) Normalised Na⁺ currents in HEK-
 525 Nav1.5 cells elicited by a depolarisation from -120 mV to -10 mV. (J) Normalised Na⁺ currents in
 526 HEK-Nav1.5 cells elicited by a depolarisation from -80 mV to -10 mV. Results are mean + SEM. *P
 527 ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; one-way ANOVA with Tukey tests (n = 12-14). NS, not
 528 significant.

529 **Figure 3.** Effect of S-licarbazepine on Nav1.5 currents. (A) Representative Na⁺ currents in an MDA-
 530 MB-231 cell elicited by a depolarisation from -120 mV to -10 mV in physiological saline solution
 531 (PSS; black), S-licarbazepine (S-Lic; 300 μM; red) and after washout (grey). Dotted vertical lines
 532 define the time period magnified in (B). (B) Representative persistent Na⁺ currents in an MDA-MB-
 533 231 cell elicited by a depolarisation from -120 mV to -10 mV. (C) Representative Na⁺ currents in an
 534 MDA-MB-231 cell elicited by a depolarisation from -80 mV to -10 mV. (D) Normalised Na⁺
 535 currents in MDA-MB-231 cells elicited by a depolarisation from -120 mV to -10 mV. (E)
 536 Normalised Na⁺ currents in MDA-MB-231 cells elicited by a depolarisation from -80 mV to -10 mV.
 537 (F) Representative Na⁺ currents in a HEK-Nav1.5 cell elicited by a depolarisation from -120 mV to -
 538 10 mV in PSS (black), S-Lic (300 μM; red) and after washout (grey). Dotted vertical lines define the
 539 time period magnified in (G). (G) Representative persistent Na⁺ currents in a HEK-Nav1.5 cell
 540 elicited by a depolarisation from -120 mV to -10 mV. (H) Representative Na⁺ currents in a HEK-
 541 Nav1.5 cell elicited by a depolarisation from -80 mV to -10 mV. (I) Normalised Na⁺ currents in HEK-

542 Na_v1.5 cells elicited by a depolarisation from -120 mV to -10 mV. (J) Normalised Na⁺ currents in
 543 HEK-Na_v1.5 cells elicited by a depolarisation from -80 mV to -10 mV. Results are mean + SEM. *P
 544 ≤ 0.05; ***P ≤ 0.001; one-way ANOVA with Tukey tests (n = 9-13). NS, not significant.

545 **Figure 4.** Effect of eslicarbazepine acetate and S-licarbazepine on the current-voltage relationship.
 546 (A) Current-voltage (I-V) plots of Na⁺ currents in MDA-MB-231 cells in physiological saline
 547 solution (PSS; black circles) and in eslicarbazepine acetate (ESL; 300 μM; red squares). (B) (I-V)
 548 plots of Na⁺ currents in HEK-Na_v1.5 cells in PSS (black circles) and ESL (300 μM; red squares). (C)
 549 I-V plots of Na⁺ currents in MDA-MB-231 cells in PSS (black circles) and S-licarbazepine (S-Lic;
 550 300 μM; red squares). (D) I-V plots of Na⁺ currents in HEK-Na_v1.5 cells in PSS (black circles) and
 551 S-Lic (300 μM; red squares). Currents were elicited using 10 mV depolarising steps from -80 to +30
 552 mV for 30 ms, from a holding potential of -120 mV. Results are mean ± SEM (n = 7-13).

553 **Figure 5.** Effect of eslicarbazepine acetate and S-licarbazepine on activation and steady-state
 554 inactivation. (A) Activation and steady-state inactivation in MDA-MB-231 cells in physiological
 555 saline solution (PSS; black circles) and in eslicarbazepine acetate (ESL; 300 μM; red squares). (B)
 556 Activation and steady-state inactivation in HEK-Na_v1.5 cells in PSS (black circles) and ESL (300
 557 μM; red squares). (C) Activation and steady-state inactivation in MDA-MB-231 cells in PSS (black
 558 circles) and S-licarbazepine (S-Lic; 300 μM; red squares). (D) Activation and steady-state
 559 inactivation in HEK-Na_v1.5 cells in PSS (black circles) and S-Lic (300 μM; red squares). For
 560 activation, normalised conductance (G/G_{max}) was calculated from the current data and plotted as a
 561 function of voltage. For steady-state inactivation, normalised current (I/I_{max}), elicited by 50 ms test
 562 pulses at -10 mV following 250 ms conditioning voltage pulses between -120 mV and +30 mV,
 563 applied from a holding potential of -120 mV, was plotted as a function of the prepulse voltage.
 564 Results are mean ± SEM (n = 7-13). Activation and inactivation curves are fitted with Boltzmann
 565 functions.

566 **Figure 6.** Effect of eslicarbazepine acetate and S-licarbazepine on recovery from inactivation. (A)
 567 Recovery from inactivation in MDA-MB-231 cells in physiological saline solution (PSS; black
 568 circles) and in eslicarbazepine acetate (ESL; 300 μM; red squares). (B) Recovery from inactivation in
 569 HEK-Na_v1.5 cells in PSS (black circles) and ESL (300 μM; red squares). (C) Recovery from
 570 inactivation in MDA-MB-231 cells in PSS (black circles) and S-licarbazepine (S-Lic; 300 μM; red
 571 squares). (D) Recovery from inactivation in HEK-Na_v1.5 cells in PSS (black circles) and S-Lic (300
 572 μM; red squares). The fraction recovered (I_t/I_c) was determined by a 25 ms pulse to 0 mV (I_c),
 573 followed by a recovery pulse to -120 mV for 1-500 ms, and a subsequent 25 ms test pulse to 0 mV
 574 (I_t), applied from a holding potential of -120 mV, and plotted as a function of the recovery interval.
 575 Data are fitted with single exponential functions which are statistically different between control and
 576 drug treatments in all cases. Results are mean ± SEM (n = 7-10).

577 **Figure 7.** Clustal alignment of amino acid sequences of Na_v1.1-Na_v1.9 (*SCN1A-SCN11A*). ESL was
 578 proposed previously (51) to interact with the highlighted amino acids in Na_v1.2. An alignment of
 579 Na_v1.2 (UniProtKB - Q99250 (SCN2A_HUMAN)) with Na_v1.1 (UniProtKB - P35498
 580 (SCN1A_HUMAN)), Na_v1.3 (UniProtKB - Q9NY46 (SCN3A_HUMAN)), Na_v1.4 (UniProtKB -
 581 P35499 (SCN4A_HUMAN)), Na_v1.5 (UniProtKB - Q14524 (SCN5A_HUMAN)) Na_v1.6
 582 (UniProtKB - Q9UQD0 (SCN8A_HUMAN)), Na_v1.7 (UniProtKB - Q15858 (SCN9A_HUMAN)),
 583 Na_v1.8 (UniProtKB - Q9Y5Y9 (SCN10A_HUMAN)), and Na_v1.9 (UniProtKB - Q9UI33
 584 (SCN11A_HUMAN)) shows that the interacting amino acids highlighted in yellow are conserved
 585 between Na_v1.2 and Na_v1.5, along with most other isoforms. Asterisks indicate conserved residues.
 586 Colon indicates conservation between groups of strongly similar properties - scoring > 0.5 in the

587 Gonnet PAM 250 matrix. Period indicates conservation between groups of weakly similar properties
588 - scoring ≤ 0.5 in the Gonnet PAM 250 matrix.

589

590 **Table 1.** Effect of eslicarbazepine acetate (300 μM) on Na⁺ current characteristics in MDA-MB-231
 591 and HEK-Na_v1.5 cells.¹

A. MDA-MB-231 cells				
<i>Parameter</i>	<i>Control</i>	<i>ESL</i>	<i>P value</i>	<i>N</i>
V _{thres} (mV)	-45.7 ± 1.7	-45.0 ± 1.4	0.58	13
V _{peak} (mV)	3.1 ± 2.1	-3.9 ± 2.7	0.056	13
Activation V ^{1/2} (mV)	-19.3 ± 1.4	-22.0 ± 1.5	0.095	12
Activation k (mV)	10.6 ± 0.7	9.3 ± 0.8	0.076	12
Inactivation V ^{1/2} (mV)	-80.6 ± 0.7	-86.7 ± 1.2	<0.001	13
Inactivation k (mV)	-4.8 ± 0.4	-7.4 ± 1.7	0.139	13
Peak current density at -10 mV (pA/pF)	-14.8 ± 3.9	-8.0 ± 2.5	<0.001	13
Persistent current density at -10 mV (pA/pF)	-0.15 ± 0.05	-0.02 ± 0.07	0.13	12
T _p at -10 mV (ms)	2.1 ± 0.2	1.9 ± 0.2	<0.01	13
τ _f at -10 mV (ms)	1.3 ± 0.1	1.3 ± 0.2	0.954	13
τ _s at -10 mV (ms)	10.0 ± 2.3	6.9 ± 2.0	0.289	13
τ _r (ms)	6.0 ± 0.5	8.7 ± 0.7	<0.05	10
B. HEK-Na_v1.5 cells				
<i>Parameter</i>	<i>Control</i>	<i>ESL</i>	<i>P value</i>	<i>N</i>
V _{thres} (mV)	-55.0 ± 1.7	-54.0 ± 2.2	0.758	10
V _{peak} (mV)	-26.0 ± 2.2	-24.0 ± 4.3	0.591	10
Activation V ^{1/2} (mV)	-39.4 ± 1.3	-44.2 ± 1.8	<0.05	10
Activation k (mV)	5.3 ± 1.3	3.8 ± 0.7	0.361	10
Inactivation V ^{1/2} (mV)	-78.2 ± 2.5	-88.3 ± 2.7	<0.001	10
Inactivation k (mV)	-6.9 ± 0.4	-9.8 ± 0.7	<0.001	10
Peak current density at -10 mV (pA/pF)	-154.4 ± 24.0	-33.1 ± 4.7	<0.001	12
Persistent current density at -10 mV (pA/pF)	-0.61 ± 0.15	-0.12 ± 0.05	<0.01	12
T _p at -10 mV (ms)	1.4 ± 0.2	1.9 ± 0.2	<0.001	14
τ _f at -10 mV (ms)	0.9 ± 0.1	1.2 ± 0.1	<0.001	12
τ _s at -10 mV (ms)	6.6 ± 0.8	20.8 ± 8.5	0.128	12
τ _r (ms)	4.5 ± 0.4	7.1 ± 0.6	<0.001	10

592 ¹ESL: eslicarbazepine acetate (300 μM); V_{thres}: threshold voltage for activation; V_{peak}: voltage at
 593 which current was maximal; V^{1/2}: half (in)activation voltage; k: slope factor for (in)activation; T_p:
 594 time to peak current; τ_f: fast time constant of inactivation; τ_s: slow time constant of inactivation; τ_r:
 595 time constant of recovery from inactivation. The holding potential was -120 mV. Results are mean ±
 596 SEM. Statistical comparisons were made with paired t-tests.

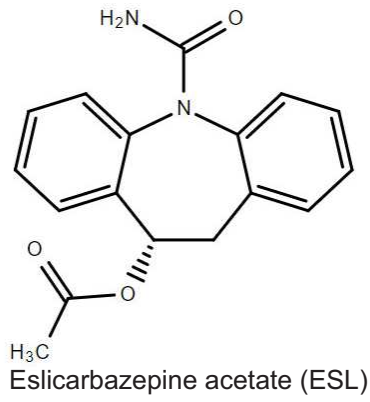
597 **Table 2.** Effect of S-licarbazepine (300 μM) on Na⁺ current characteristics in MDA-MB-231 and
 598 HEK-Nav1.5 cells.¹

A. MDA-MB-231 cells				
<i>Parameter</i>	<i>Control</i>	<i>S-Lic</i>	<i>P value</i>	<i>N</i>
V _{thres} (mV)	-34.4 ± 2.0	-35.7 ± 2.0	0.603	7
V _{peak} (mV)	11.43 ± 4.4	10.0 ± 4.9	0.818	7
Activation V ¹ / ₂ (mV)	-12.9 ± 1.3	-13.7 ± 1.4	0.371	7
Activation k (mV)	11.0 ± 0.5	11.9 ± 0.8	0.520	7
Inactivation V ¹ / ₂ (mV)	-71.8 ± 2.5	-76.8 ± 2.2	<0.05	7
Inactivation k (mV)	-6.8 ± 0.9	-6.0 ± 1.2	0.302	7
Peak current density at -10 mV (pA/pF)	-12.0 ± 3.1	-6.9 ± 2.5	<0.001	9
Persistent current density at -10 mV (pA/pF)	-1.3 ± 0.4	-0.6 ± 0.2	<0.05	9
T _p at -10 mV (ms)	4.5 ± 0.4	5.1 ± 0.7	0.103	9
τ _f at -10 mV (ms)	3.8 ± 1.1	3.2 ± 0.4	0.553	7
τ _s at -10 mV (ms)	25.7 ± 7.0	27.1 ± 12.0	0.920	7
τ _r (ms)	6.8 ± 0.4	13.5 ± 1.0	<0.01	7
B. HEK-Nav1.5 cells				
<i>Parameter</i>	<i>Control</i>	<i>S-Lic</i>	<i>P value</i>	<i>N</i>
V _{thres} (mV)	-50.0 ± 1.9	-51.3 ± 3.5	0.598	9
V _{peak} (mV)	-18.0 ± 4.2	-30.0 ± 5.6	<0.001	9
Activation V ¹ / ₂ (mV)	-32.8 ± 3.1	-40.5 ± 3.4	<0.01	9
Activation k (mV)	5.9 ± 0.9	4.5 ± 1.1	<0.05	9
Inactivation V ¹ / ₂ (mV)	-75.9 ± 2.6	-79.3 ± 4.1	0.116	9
Inactivation k (mV)	-6.5 ± 0.4	-8.1 ± 0.5	<0.05	9
Peak current density at -10 mV (pA/pF)	-140.9 ± 26.8	-77.2 ± 17.0	<0.001	13
Persistent current density at -10 mV (pA/pF)	-0.9 ± 0.2	-0.5 ± 0.2	<0.05	11
T _p at -10 mV (ms)	1.8 ± 0.5	2.3 ± 0.6	<0.01	13
τ _f at -10 mV (ms)	1.0 ± 0.04	1.3 ± 0.06	<0.001	11
τ _s at -10 mV (ms)	6.3 ± 0.5	7.3 ± 0.5	<0.05	11
τ _r (ms)	5.7 ± 0.7	8.0 ± 1.2	<0.01	10

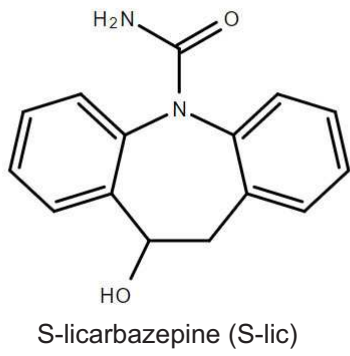
599 ¹S-Lic: S-licarbazepine (300 μM); V_{thres}: threshold voltage for activation; V_{peak}: voltage at which
 600 current was maximal; V¹/₂: half (in)activation voltage; k: slope factor for (in)activation; T_p: time to
 601 peak current; τ_f: fast time constant of inactivation; τ_s: slow time constant of inactivation; τ_r: time
 602 constant of recovery from inactivation. The holding potential was -120 mV. Results are mean ± SEM.
 603 Statistical comparisons were made with paired t-tests.

604 Figure 1

A



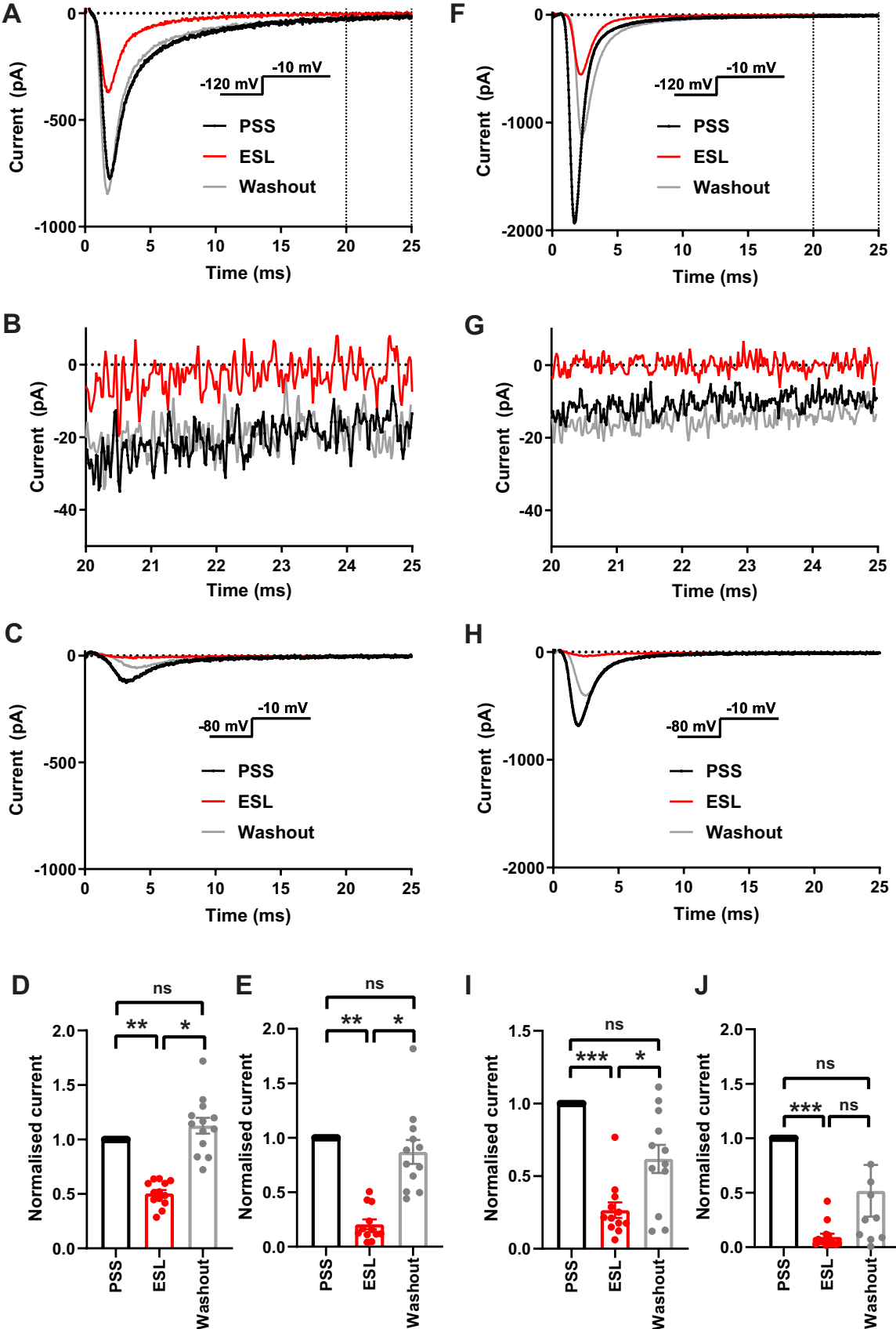
B



605

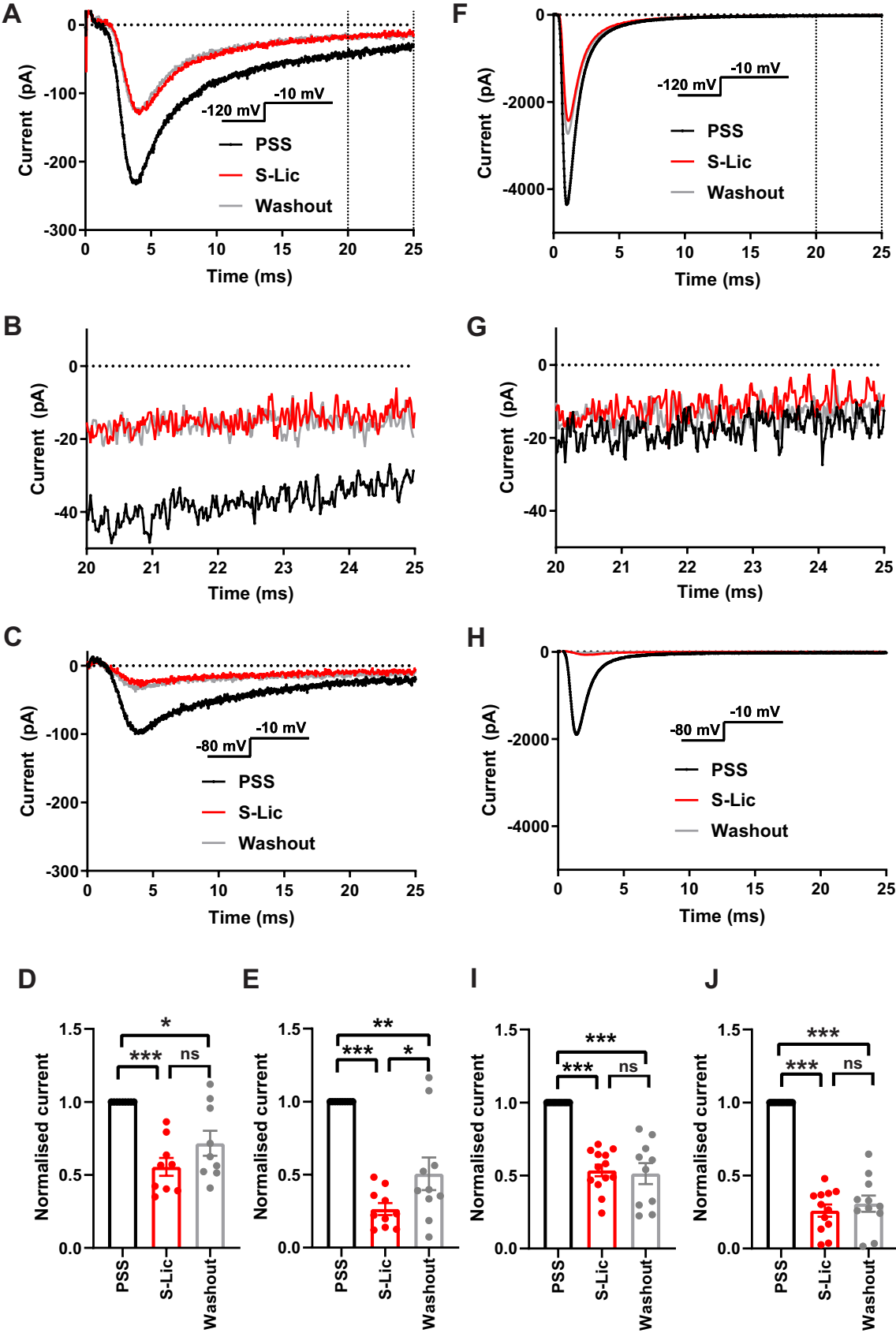
606

607 Figure 2



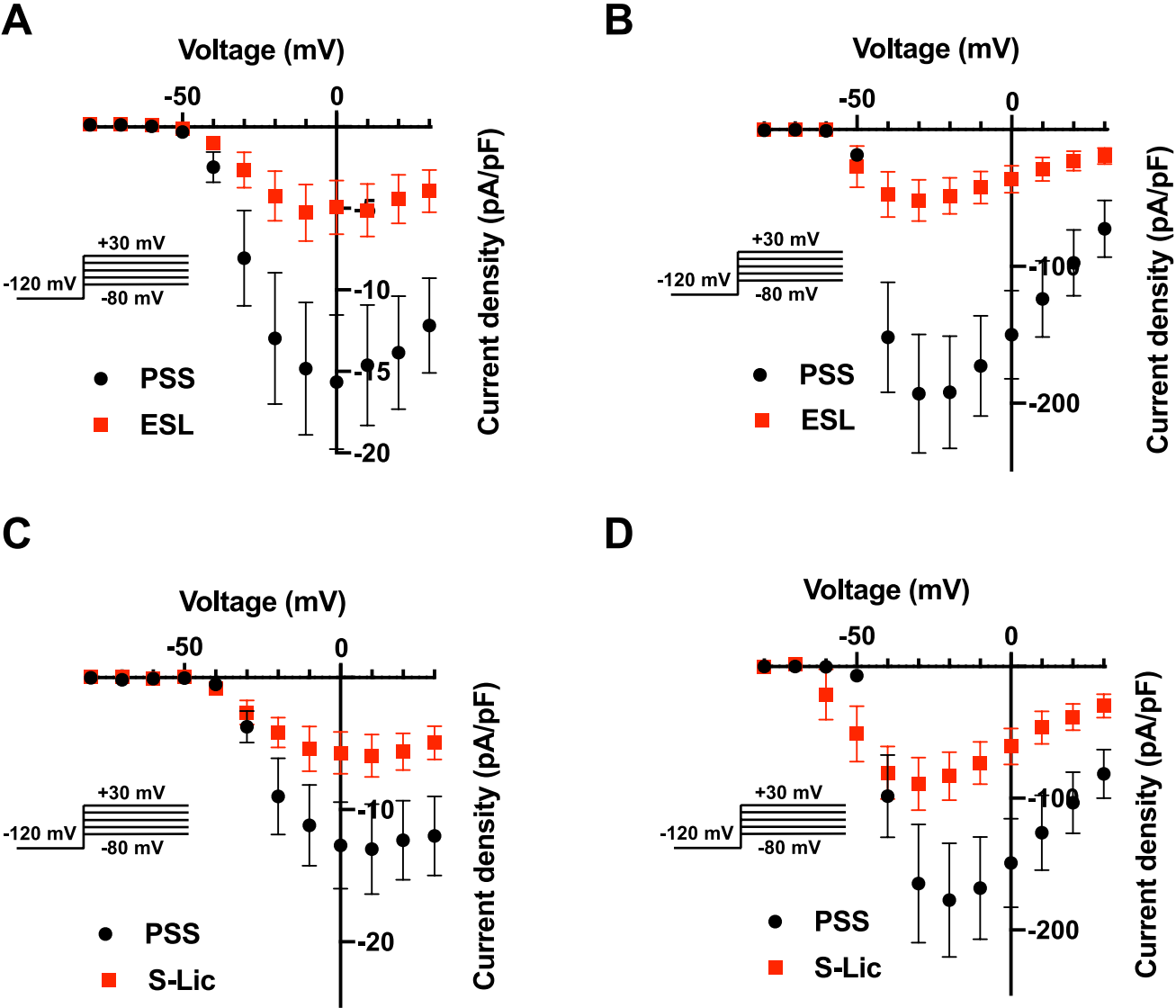
608

609 Figure 3



610

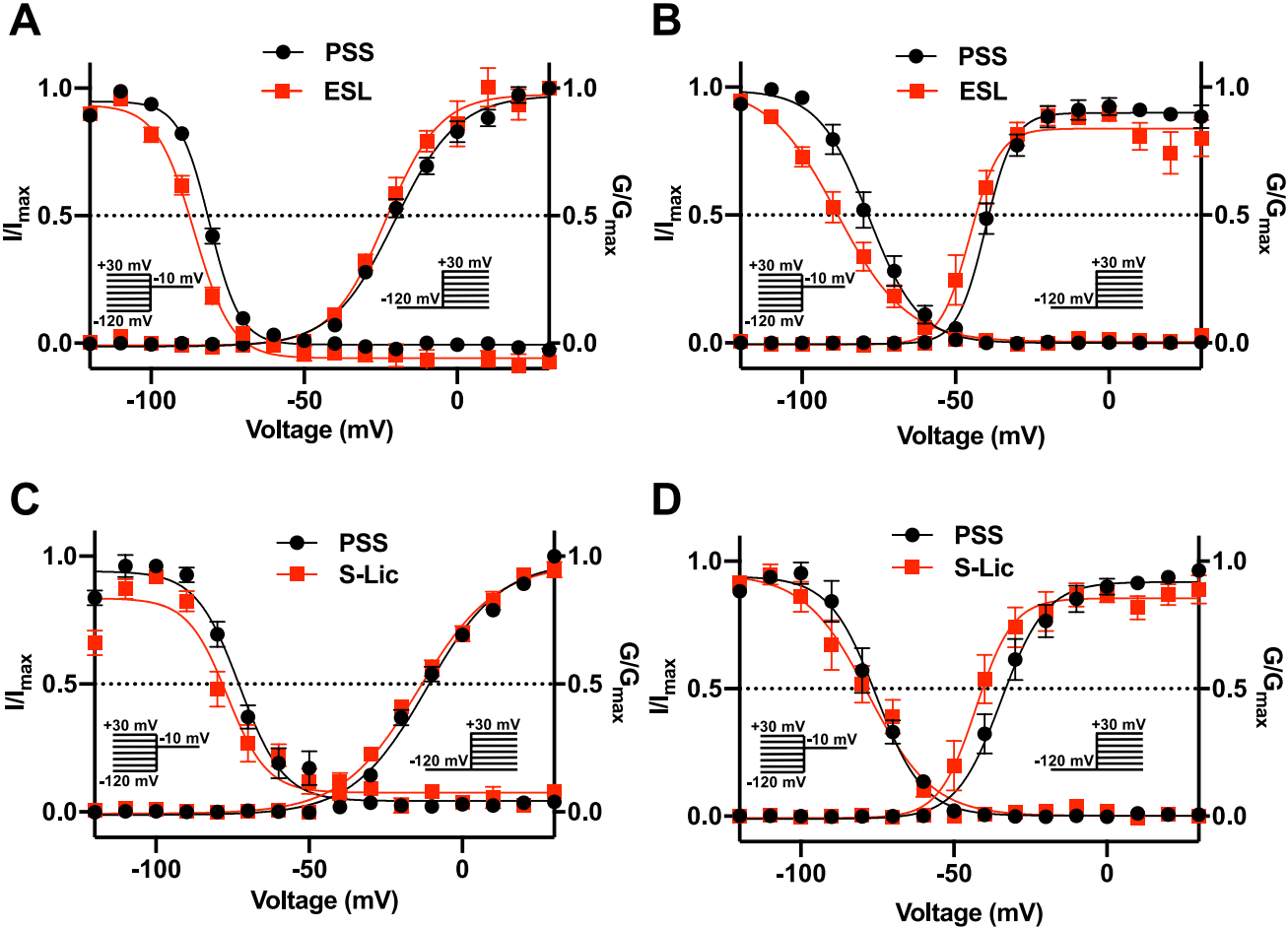
611 Figure 4



612

613

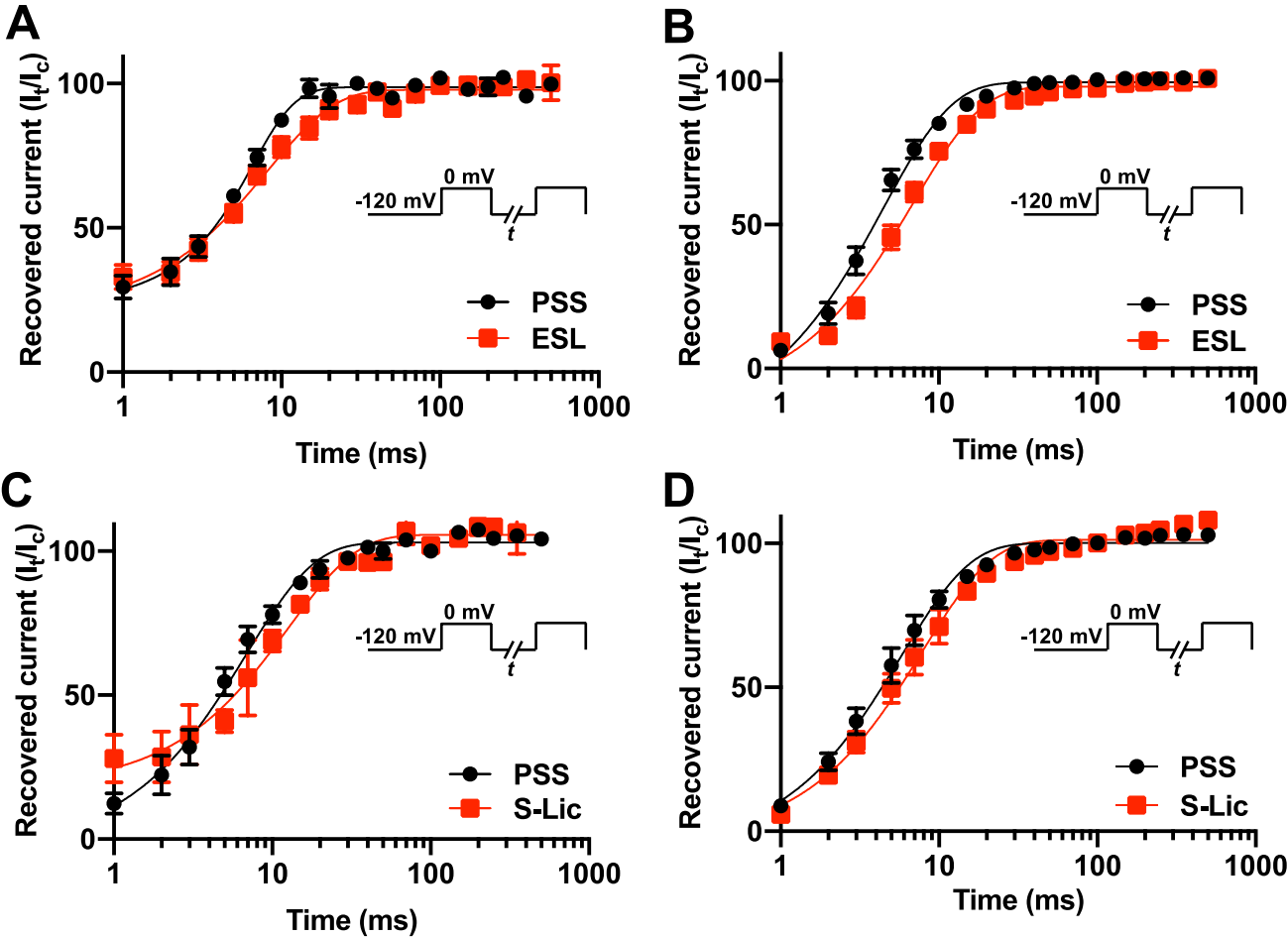
614 Figure 5



615

616

617 Figure 6



618

619

620 Figure 7

SCN1A	I L E N F S V A T E E S A E P L S E D D F E M F Y E V W E K F D P D A T Q F M E F E K L S Q F A A A L E P P L N L P Q P	1844
SCN2A	I L E N F S V A T E E S A E P L S E D D F E M F Y E V W E K F D P D A T Q F I E F A K L S D F A D A L D P P L L I A K P	1834
SCN3A	I L E N F S V A T E E S A E P L S E D D F E M F Y E V W E K F D P D A T Q F I E F S K L S D F A A A L D P P L L I A K P	1829
SCN4A	I L E N F N V A T E E S S E P L G E D D F E M F Y E T W E K F D P D A T Q F I A Y S R L S D F V D T L Q E P L R I A K P	1656
SCN5A	I L E N F S V A T E E S T E P L S E D D F D M F Y E I W E K F D P E A T Q F I E Y S V L S D F A D A L S E P L R I A K P	1830
SCN8A	I L E N F S V A T E E S A D P L S E D D F E T F Y E I W E K F D P D A T Q F I E Y C K L A D F A D A L E H P L R V P K P	1824
SCN9A	I L E N F S V A T E E S T E P L S E D D F E M F Y E V W E K F D P D A T Q F I E F S K L S D F A A A L D P P L L I A K P	1818
SCN10A	I L E N F N V A T E E S T E P L S E D D F D M F Y E T W E K F D P E A T Q F I T F S A L S D F A D T L S G P L R I P K P	1780
SCN11A	I L E N F N T A T E E S E D P L G E D D F D I F Y E V W E K F D P E A T Q F I K Y S A L S D F A D A L P E P L R V A K P	1662

***** . . ***** : ** . ***** : *** ***** . ***** : : * : * . : * ** : : *

SCN1A	N K L Q L I A M D L P M V S G D R I H C L D I L F A F T K R V L G E S G E M D A L R I Q M E E R F M A S N P S K V S Y Q	1904
SCN2A	N K V Q L I A M D L P M V S G D R I H C L D I L F A F T K R V L G E S G E M D A L R I Q M E E R F M A S N P S K V S Y E	1894
SCN3A	N K V Q L I A M D L P M V S G D R I H C L D I L F A F T K R V L G E S G E M D A L R I Q M E D R F M A S N P S K V S Y E	1889
SCN4A	N K I K L I T L D L P M V P G D K I H C L D I L F A L T K E V L G D S G E M D A L K Q T M E E K F M A A N P S K V S Y E	1716
SCN5A	N Q I S L I N M D L P M V S G D R I H C M D I L F A F T K R V L G E S G E M D A L K I Q M E E K F M A A N P S K I S Y E	1890
SCN8A	N T I E L I A M D L P M V S G D R I H C L D I L F A F T K R V L G D S G E L D I L R Q Q M E E R F V A S N P S K V S Y E	1884
SCN9A	N K V Q L I A M D L P M V S G D R I H C L D I L F A F T K R V L G E S G E M D S L R S Q M E E R F M S A N P S K V S Y E	1878
SCN10A	N R N I L I Q M D L P L V P G D K I H C L D I L F A F T K N V L G E S G E L D S L K A N M E E K F M A T N L S K S S Y E	1840
SCN11A	N K Y Q F L V M D L P M V S E D R L H C M D I L F A F T A R V L G G S D G L D S M K A M M E E K F M E A N P L K K L Y E	1722

* : : * : * : * * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *

621

622

623 **Supplementary Table 1A.** Effect of eslicarbazepine acetate (100 μM) on peak and persistent Na⁺
 624 current in MDA-MB-231 and HEK-Nav1.5 cells.
 625

A. MDA-MB-231 cells				
<i>Parameter</i>	<i>Control</i>	<i>ESL</i>	<i>P value</i>	<i>N</i>
Peak current density at -10 mV, V _h -120 mV (pA/pF)	-22.1 ± 13.5	-11.6 ± 7.9	<0.05	7
Peak current density at -10 mV, V _h -80 mV (pA/pF)	-7.1 ± 4.1	-2.1 ± 2.0	< 0.05	7
Persistent current density at -10 mV, V _h -120 mV (pA/pF)	-0.5 ± 0.3	-0.4 ± 0.2	0.277	7
B. HEK-Nav1.5 cells				
<i>Parameter</i>	<i>Control</i>	<i>ESL</i>	<i>P value</i>	<i>N</i>
Peak current density at -10 mV, V _h -120 mV (pA/pF)	-158.4 ± 85.7	-77.7 ± 51.3	<0.01	8
Peak current density at -10 mV, V _h -80 mV (pA/pF)	-59.0 ± 50.7	-12.2 ± 11.9	<0.05	8
Persistent current density at -10 mV, V _h -120 mV (pA/pF)	-1.0 ± 0.3	-0.4 ± 0.1	<0.001	8

626 ¹ESL: eslicarbazepine acetate (100 μM). Results are mean ± SEM. Statistical comparisons were made
 627 with paired t-tests.
 628

629 **Supplementary Table 1B.** Effect of S-licarbazepine (100 μM) on peak and persistent Na⁺ current in
 630 MDA-MB-231 and HEK-Nav1.5 cells.
 631

A. MDA-MB-231 cells				
<i>Parameter</i>	<i>Control</i>	<i>S-Lic</i>	<i>P value</i>	<i>N</i>
Peak current density at -10 mV, V _h -120 mV (pA/pF)	-17.2 ± 8.7	-12.3 ± 7.4	0.084	8
Peak current density at -10 mV, V _h -80 mV (pA/pF)	-7.8 ± 4.7	-3.5 ± 2.6	<0.05	8
Persistent current density at -10 mV, V _h -120 mV (pA/pF)	-0.6 ± 0.3	-0.4 ± 0.2	<0.01	8
B. HEK-Nav1.5 cells				
<i>Parameter</i>	<i>Control</i>	<i>S-Lic</i>	<i>P value</i>	<i>N</i>
Peak current density at -10 mV, V _h -120 mV (pA/pF)	-108.5 ± 20.3	- 75.6 ± 30.9	<0.05	8
Peak current density at -10 mV, V _h -80 mV (pA/pF)	-30.2 ± 0.9	-11.8 ± 1.3	<0.001	8
Persistent current density at -10 mV, V _h -120 mV (pA/pF)	-0.5 ± 0.1	-0.3 ± 0.1	<0.05	7

632 ¹S-Lic: S-licarbazepine (100 μM). Results are mean ± SEM. Statistical comparisons were made with
 633 paired t-tests.
 634

635 **Supplementary Figure Legends**

636 **Supplementary Figure 1.** Effect of 0.45% DMSO on VGSC current-voltage relationship and gating
 637 in MDA-MB-231 cells. (A) Current-voltage (I-V) plots of Na⁺ currents in MDA-MB-231 cells in
 638 physiological saline solution (PSS; black circles) and in PSS with 0.45% DMSO (0.45% DMSO;
 639 green squares). Currents were elicited using 10 mV depolarising steps from -80 to +30 mV for 30 ms,
 640 from a holding potential of -120 mV. Results are mean ± SEM (n = 13-17). (B) Activation and
 641 steady-state inactivation in physiological saline solution (PSS; black circles) and in PSS with 0.45%
 642 DMSO (0.45% DMSO; green squares). For activation, normalised conductance (G/G_{max}) was
 643 calculated from the current data and plotted as a function of voltage. For steady-state inactivation,
 644 normalised current (I/I_{max}), elicited by 50 ms test pulses at -10 mV following 250 ms conditioning
 645 voltage pulses between -120 mV and +30 mV, applied from a holding potential of -120 mV, was
 646 plotted as a function of the prepulse voltage. Results are mean ± SEM (n = 10-13). Activation and
 647 inactivation curves are fitted with Boltzmann functions.

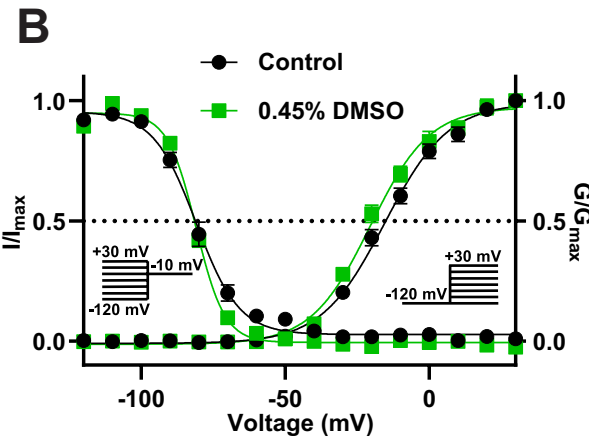
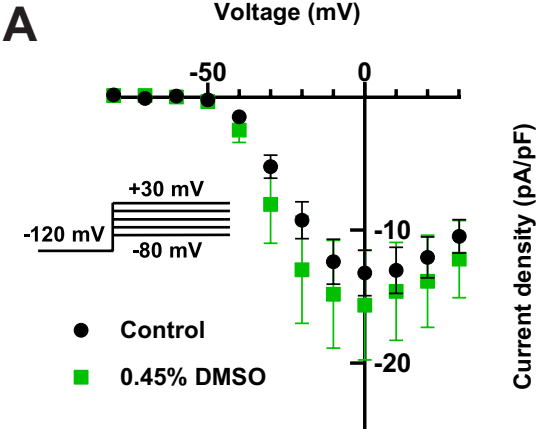
648 **Supplementary Figure 2.** Effect of 100 μM eslicarbazepine acetate on Na_v1.5 currents. (A)
 649 Representative Na⁺ currents in an MDA-MB-231 cell elicited by a depolarisation from -120 mV to -
 650 10 mV in physiological saline solution (PSS; black), eslicarbazepine acetate (ESL; 100 μM; red) and
 651 after washout (grey). Dotted vertical lines define the time period magnified in (B). (B) Representative
 652 persistent Na⁺ currents in an MDA-MB-231 cell elicited by a depolarisation from -120 mV to -10
 653 mV. (C) Representative Na⁺ currents in an MDA-MB-231 cell elicited by a depolarisation from -80
 654 mV to -10 mV. (D) Normalised Na⁺ currents in MDA-MB-231 cells elicited by a depolarisation from
 655 -120 mV to -10 mV. (E) Normalised Na⁺ currents in MDA-MB-231 cells elicited by a depolarisation
 656 from -80 mV to -10 mV. (F) Representative Na⁺ currents in a HEK-Na_v1.5 cell elicited by a
 657 depolarisation from -120 mV to -10 mV in PSS (black), ESL (100 μM; red) and after washout (grey).
 658 Dotted vertical lines define the time period magnified in (G). (G) Representative persistent Na⁺
 659 currents in a HEK-Na_v1.5 cell elicited by a depolarisation from -120 mV to -10 mV. (H)
 660 Representative Na⁺ currents in a HEK-Na_v1.5 cell elicited by a depolarisation from -80 mV to -10
 661 mV. (I) Normalised Na⁺ currents in HEK-Na_v1.5 cells elicited by a depolarisation from -120 mV to -
 662 10 mV. (J) Normalised Na⁺ currents in HEK-Na_v1.5 cells elicited by a depolarisation from -80 mV to
 663 -10 mV. Results are mean + SEM. *P ≤ 0.05; **P ≤ 0.01; one-way ANOVA with Tukey tests (n = 7-
 664 8). NS, not significant.

665 **Supplementary Figure 3.** Effect of 100 μM S-licarbazepine on Na_v1.5 currents. (A) Representative
 666 Na⁺ currents in an MDA-MB-231 cell elicited by a depolarisation from -120 mV to -10 mV in
 667 physiological saline solution (PSS; black), S-licarbazepine (S-Lic; 100 μM; red) and after washout
 668 (grey). Dotted vertical lines define the time period magnified in (B). (B) Representative persistent
 669 Na⁺ currents in an MDA-MB-231 cell elicited by a depolarisation from -120 mV to -10 mV. (C)
 670 Representative Na⁺ currents in an MDA-MB-231 cell elicited by a depolarisation from -80 mV to -10
 671 mV. (D) Normalised Na⁺ currents in MDA-MB-231 cells elicited by a depolarisation from -120 mV
 672 to -10 mV. (E) Normalised Na⁺ currents in MDA-MB-231 cells elicited by a depolarisation from -80
 673 mV to -10 mV. (F) Representative Na⁺ currents in a HEK-Na_v1.5 cell elicited by a depolarisation
 674 from -120 mV to -10 mV in PSS (black), S-Lic (100 μM; red) and after washout (grey). Dotted
 675 vertical lines define the time period magnified in (G). (G) Representative persistent Na⁺ currents in a
 676 HEK-Na_v1.5 cell elicited by a depolarisation from -120 mV to -10 mV. (H) Representative Na⁺
 677 currents in a HEK-Na_v1.5 cell elicited by a depolarisation from -80 mV to -10 mV. (I) Normalised
 678 Na⁺ currents in HEK-Na_v1.5 cells elicited by a depolarisation from -120 mV to -10 mV. (J)
 679 Normalised Na⁺ currents in HEK-Na_v1.5 cells elicited by a depolarisation from -80 mV to -10 mV.

680 Results are mean + SEM. * $P \leq 0.05$; *** $P \leq 0.001$; one-way ANOVA with Tukey tests (n = 7-8). NS,
681 not significant.

682

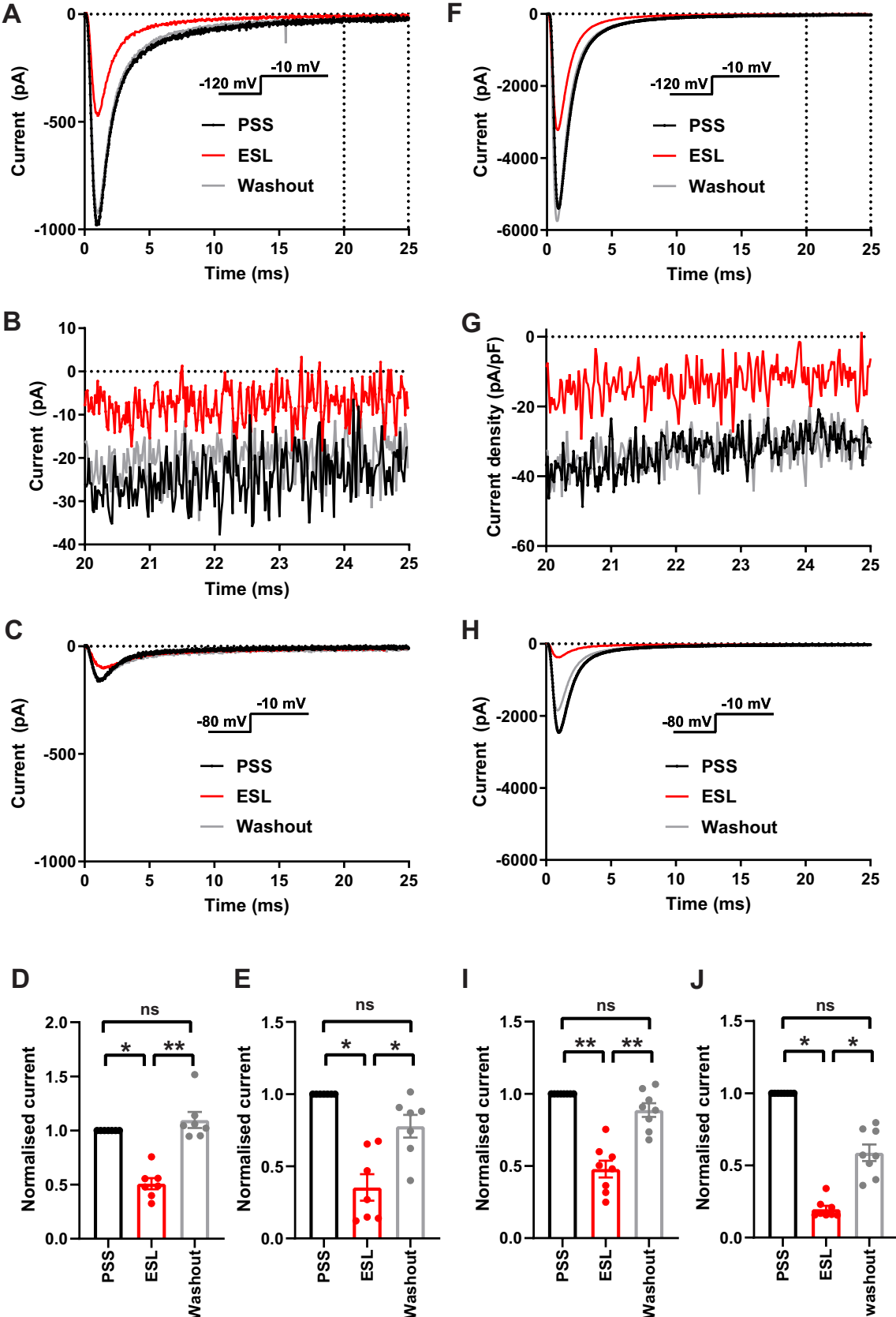
683 Supplementary Figure 1



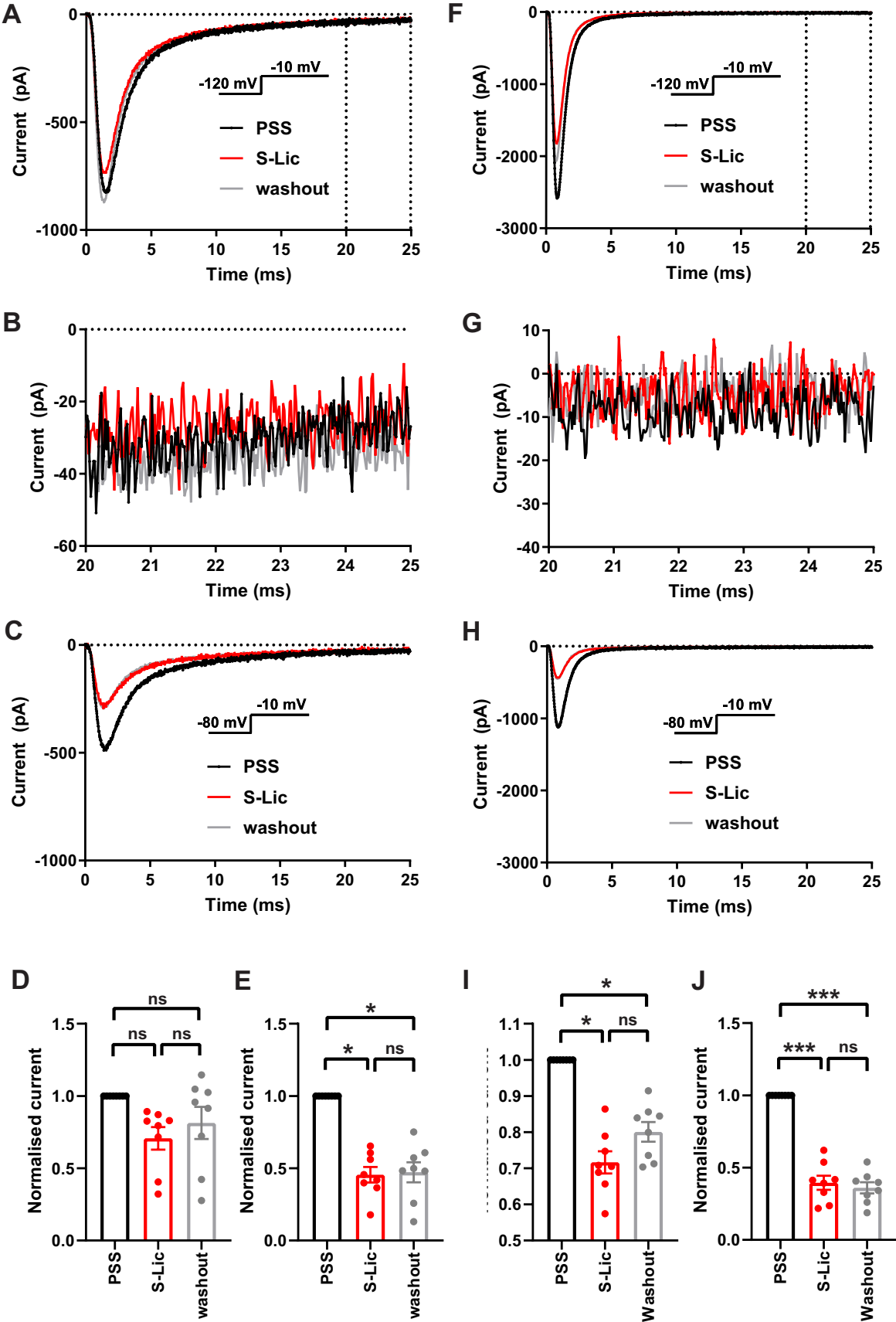
684

685

686 Supplementary Figure 2



688 Supplementary Figure 3



689