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# Reversal of cell, circuit and seizure phenotypes in a mouse model of DNM1 epileptic encephalopathy

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1                    Reversal of cell, circuit and seizure  
2                    phenotypes in a mouse model of *DNM1*  
3                    epileptic encephalopathy

4  
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25 **ABSTRACT**

26 Dynamin-1 is a large GTPase with an obligatory role in synaptic vesicle endocytosis at mammalian  
27 nerve terminals. Heterozygous missense mutations in the dynamin-1 gene (*DNM1*) cause a novel  
28 form of epileptic encephalopathy, with pathogenic mutations clustering within regions required for  
29 its essential GTPase activity. We reveal the most prevalent pathogenic *DNM1* mutation, R237W,  
30 disrupts dynamin-1 enzyme activity and endocytosis when overexpressed in central neurons. To  
31 determine how this mutation impacted cell, circuit and behavioural function, we generated a mouse  
32 carrying the R237W mutation. Neurons from heterozygous mice display dysfunctional endocytosis,  
33 in addition to altered excitatory neurotransmission and seizure-like phenotypes. Importantly, these  
34 phenotypes are corrected at the cell, circuit and *in vivo* level by the drug, BMS-204352, which  
35 accelerates endocytosis. Here, we demonstrate a credible link between dysfunctional endocytosis  
36 and epileptic encephalopathy, and importantly reveal that synaptic vesicle recycling may be a viable  
37 therapeutic target for monogenic intractable epilepsies.

## 38 INTRODUCTION

39 Heterozygous pathogenic missense mutations in the *DNM1* gene result in a specific form of  
40 developmental epileptic encephalopathy, characterised by severe to profound intellectual disability,  
41 hypotonia and epilepsy<sup>1-3</sup>. Epilepsy in these individuals typically starts with infantile spasms  
42 progressing to Lennox-Gastaut syndrome. The *DNM1* gene encodes the large GTPase dynamin-1, a  
43 mechanochemical enzyme that undergoes a conformational change on GTP hydrolysis, providing  
44 force for the final stages of vesicle fission<sup>4,5</sup>. It has a modular structure with an N-terminal GTPase  
45 domain, followed by domains essential for self-assembly (middle and GTPase effector domains,  
46 GED), membrane lipid binding (pleckstrin homology, PH) and protein interactions (C-terminal  
47 proline-rich domain). All domains perform key roles in mediating dynamin-1 function<sup>6-9</sup>, however its  
48 GTPase activity is essential for SV fission reaction during endocytosis<sup>4,5</sup>. To date, all identified  
49 pathogenic mutations in the *DNM1* gene are localised to either the GTPase or middle domain, with  
50 one each in the PH and GED domains<sup>10,11</sup>. Importantly, almost all individuals with these mutations in  
51 *DNM1* have intractable epilepsy<sup>1</sup>, making the identification of novel therapeutic interventions an  
52 urgent unmet challenge.

53

54 Considering the essential role for dynamin-1 in SV endocytosis and the clustering of mutations  
55 within the GTPase domain in individuals with epileptic encephalopathy, a logical prediction is that  
56 defects in SV endocytosis underpin this disorder. However, to date, no determination of the role of  
57 these predicted dominant negative GTPase domain mutations has been performed at the neuron,  
58 circuit or behavioural level. To investigate this, we generated a mouse carrying the most prevalent  
59 mutation in the *DNM1* gene (R237W).

60

61 In this work, heterozygous *Dnm1*<sup>+/*R237W*</sup> mice display defective SV endocytosis, altered  
62 neurotransmission and seizure-like activity. Importantly, these phenotypes are all reversed by the  
63 small molecule BMS-204352, which we reveal accelerates SV endocytosis. We therefore reveal a  
64 preclinical model and potential therapy that will provide impetus to future small molecule screening  
65 studies and clinical trials to generate interventions for *DNM1* epileptic encephalopathy.

66

## 67 RESULTS

### 68 R237W dynamin-1 displays reduced basal GTPase activity

69 Heterozygous mutations in the *DNM1* gene give rise to a specific form of epileptic encephalopathy<sup>1</sup>,  
70 however little is known regarding how these mutations translate into this neurodevelopmental  
71 disorder. To determine this, we investigated the most prominent pathogenic *DNM1* mutation in

72 human disease - R237W<sup>1,2</sup>. The R237 residue is critical for GTPase function, directly participating in  
73 GTP binding and stabilising the transition state of this region during GTP hydrolysis<sup>12</sup>. Because of this  
74 key role, we reasoned that substitution of a larger residue such as R237W would disrupt GTPase  
75 activity. To test this, we assayed the GTPase activity of full-length dynamin-1 in a heterologous  
76 expression system. We chose to use the splice variant dynamin-1aa, since this version is the  
77 predominant isoform in mammalian brain<sup>3</sup>. This version of dynamin-1 was fused to the fluorescent  
78 protein mCerulean (Dyn1-mCer) and then immunoprecipitated using nanobodies against the mCer  
79 moiety. As a positive control, we assessed the ability of the K44A dynamin-1 mutant (which is  
80 deficient in both GTP binding and hydrolysis<sup>13</sup>) to hydrolyse GTP. When these experiments were  
81 performed, a level of baseline GTPase activity was discovered in immunoprecipitates of the mCer  
82 protein. However, this was increased two-fold in Dyn1<sub>WT</sub>-mCer immunoprecipitates (**Figure 1a,b**). In  
83 contrast, the Dyn1<sub>K44A</sub>-mCer mutant displayed a significantly reduced ability to hydrolyse GTP (**Figure**  
84 **1a**). Importantly, Dyn1<sub>R237W</sub>-mCer displayed reduced GTPase activity at a similar level to Dyn1<sub>K44A</sub>-  
85 mCer (**Figure 1b**). Therefore, the R237W mutation disrupts the GTPase activity of dynamin-1.

86

#### 87 **R237W dynamin-1 is dominant negative for SV endocytosis**

88 Dynamin-1 GTPase activity is essential for SV endocytosis<sup>5</sup>. To determine whether Dyn1<sub>R237W</sub> exerts a  
89 dominant-negative effect on this process, and in an attempt to mimic heterozygous individuals with  
90 *DNM1* mutations, Dyn1-mCer mutants were overexpressed in primary cultures of hippocampal  
91 neurons, in approximately 2-3 fold in excess of endogenous dynamin-1 (**Supplementary Figure 1a,b**).  
92 The genetically-encoded reporter synaptophysin-pHluorin (sypHy) was used to monitor activity-  
93 dependent SV recycling. SypHy is the SV protein synaptophysin that has an exquisitely pH-sensitive  
94 GFP inserted into a luminal domain<sup>14</sup>. The acidic interior of SVs results in the quenching of sypHy  
95 fluorescence in resting nerve terminals. During SV exocytosis, the reporter is exposed to the  
96 extracellular space and the subsequent unquenching provides a readout of SV fusion. SypHy remains  
97 fluorescent during endocytosis and is quenched on acidification of the newly formed SV. The speed  
98 of SV endocytosis is rate limiting when compared to SV acidification<sup>14,15</sup>, meaning that the loss of  
99 sypHy fluorescence is indicative of the rate of SV endocytosis.

100

101 Neurons were challenged with a train of 300 action potentials (10 Hz) with the total SV recycling  
102 pool revealed by subsequent application of an alkaline solution (NH<sub>4</sub>Cl). Neurons expressing the  
103 mCer empty vector displayed a typical sypHy response, with a rapid increase in fluorescence  
104 (reflecting SV exocytosis) followed by a slow decrease (SV endocytosis, **Figure 1c**). When neurons  
105 overexpressing Dyn1<sub>WT</sub>-mCer were monitored, there was no difference in either SV endocytosis

106 (measured as the amount of sypHy left to retrieve (**Figure 1c,d**), or SV exocytosis (measured as the  
107 extent of the evoked sypHy peak as a proportion of the total SV pool, **Figure 1e**). In neurons  
108 expressing Dyn1<sub>K44A</sub>-mCer, SV endocytosis was significantly retarded, whereas SV exocytosis was  
109 unaffected (**Figure 1c-e**). When neurons overexpressing Dyn1<sub>R237W</sub>-mCer were assessed, SV  
110 endocytosis was greatly reduced compared to Dyn1<sub>WT</sub>-mCer control, with no significant effect on SV  
111 exocytosis (**Figure 1f-h**). In contrast, overexpression of a middle domain dynamin-1 mutant (A408T),  
112 from the *Fitful* mouse<sup>16</sup> which has normal GTPase activity (**Supplementary Figure 1c**), had no  
113 dominant negative impact on either SV endocytosis or exocytosis (**Supplementary Figure 1d-f**).  
114 Therefore, the pathogenic *DNM1* GTPase mutant R237W, has a selective, dominant-negative effect  
115 on SV endocytosis.

116

### 117 ***Dnm1*<sup>+/<sub>R237W</sub></sup> mice display defective SV endocytosis**

118 The overexpression of Dyn1<sub>R237W</sub>-mCer in *Dnm1*<sup>+/<sub>+</sub></sup> neurons does not accurately recapitulate the *in*  
119 *vivo* situation, where this mutation would be expressed via its endogenous locus. Furthermore, it  
120 does not allow a direct investigation of how reduced SV endocytosis could culminate in epileptic  
121 encephalopathy. To address this, we generated a mouse line that expressed a *Dnm1*<sup>R237W</sup> allele,  
122 using CRISPR-Cas9 technology. Heterozygous *Dnm1*<sup>+/<sub>R237W</sub></sup> mice were fertile and were born in  
123 Mendelian proportions. No gross alterations in brain architecture were observed using Nissl staining  
124 (**Figure 2a**). Furthermore, the *Dnm1*<sup>+/<sub>R237W</sub></sup> mouse is not a hypomorph, since quantitative Western  
125 blotting and mass spectrometry analysis revealed no change in dynamin-1 expression in either  
126 primary hippocampal neurons from *Dnm1*<sup>+/<sub>R237W</sub></sup> mice, brain lysates from either 3 week- or 6 week-  
127 old *Dnm1*<sup>+/<sub>R237W</sub></sup> mice (**Supplementary Figure 2a,b**) or *Dnm1*<sup>+/<sub>R237W</sub></sup> nerve terminals (**Supplementary**  
128 **Data 1**).

129

130 Western blotting for common SV recycling proteins and dynamin-1 interaction partners in primary  
131 hippocampal neurons from *Dnm1*<sup>+/<sub>R237W</sub></sup> mice revealed no differences in their protein levels (**Figure**  
132 **2b,c**). This was also the case in brain lysates from either 3 week-old or 6 week-old *Dnm1*<sup>+/<sub>R237W</sub></sup> mice  
133 (**Supplementary Figure 2c-g**). To determine more global changes in presynaptic protein expression,  
134 quantitative mass spectrometry was performed on nerve terminals isolated from either *Dnm1*<sup>+/<sub>+</sub></sup> or  
135 *Dnm1*<sup>+/<sub>R237W</sub></sup> littermates (**Figure 2d**). We established a list of 4237 quantified proteins associated to  
136 synapses, mitochondria and vesicular structures and transport (**Supplementary Figure 3**). This  
137 revealed 151 proteins that were significantly increased in *Dnm1*<sup>+/<sub>R237W</sub></sup> nerve terminals, with 39  
138 significantly decreased (**Figure 2e, Supplementary Data 1**). To identify cellular functions that may be  
139 either upregulated or perturbed, network analysis using the STRING web tool (v.11.5) was

140 performed on the proteins that were significantly altered. Upregulated proteins in *Dnm1*<sup>+/<sup>R237W</sup></sup> nerve  
141 terminals clustered around cell functions such as the proteasome core complex and the ErbB  
142 signalling pathway, whereas downregulated proteins were associated with G-protein coupled  
143 receptor signalling pathway and the dynactin complex (**Figure 2f**). Therefore, while there are no  
144 gross changes in architecture or protein expression in *Dnm1*<sup>+/<sup>R237W</sup></sup> mice, subtle variations are  
145 present at their nerve terminals that may reflect either disrupted cell signalling or potential  
146 compensatory mechanisms.

147

148 When hippocampal brain sections of *Dnm1*<sup>+/<sup>R237W</sup></sup> mice were examined at the ultrastructural level,  
149 their nerve terminals repeatedly displayed misshapen SVs and endosomal-like compartments, in  
150 contrast to *Dnm1*<sup>+/<sup>+</sup></sup> controls (**Figure 3a**). When quantified, there was a 250% increase in the number  
151 of presynaptic endosomes in *Dnm1*<sup>+/<sup>R237W</sup></sup> nerve terminals when compared to littermate controls  
152 (**Figure 3b**). There was also a small (17%) but significant increase in the number of SVs (**Figure 3c**).  
153 SVs in *Dnm1*<sup>+/<sup>R237W</sup></sup> nerve terminals were larger than *Dnm1*<sup>+/<sup>+</sup></sup> controls (**Figure 3d**), with no significant  
154 change in endosome area (**Supplementary Figure 4a**). This morphological phenotype suggested that  
155 there was a deficit in SV endocytosis, therefore primary hippocampal cultures from *Dnm1*<sup>+/<sup>R237W</sup></sup> mice  
156 and *Dnm1*<sup>+/<sup>+</sup></sup> littermates were prepared. As before, SV exocytosis and endocytosis were monitored  
157 using the sypHy reporter (**Figure 3e**). Neurons from *Dnm1*<sup>+/<sup>R237W</sup></sup> mice displayed a significant slowing  
158 in SV endocytosis during challenge with two different stimulus trains (300 action potentials at 10 Hz  
159 or 400 action potentials at 40 Hz, **Figure 3f,g,i,j**). Furthermore, there was no significant effect on SV  
160 exocytosis during either stimulus train (**Figure 3h,k**). This was also the case when SV exocytosis was  
161 isolated in the presence of bafilomycin A1, which prevents acidification of SVs after endocytosis,  
162 removing contamination from retrieving SVs<sup>17</sup> (**Supplementary Figure 4b-e**). Therefore *Dnm1*<sup>+/<sup>R237W</sup></sup>  
163 neurons display a specific defect in SV endocytosis across a range of stimulus frequencies.

164

165 To confirm the endocytosis defect via a complementary approach, morphological analysis was  
166 performed using activity-dependent uptake of the fluid phase marker horse radish peroxidase (HRP).  
167 After its accumulation, HRP can be converted to an electron dense product, to reveal the number of  
168 endocytic intermediates generated during stimulation<sup>18</sup>. Recent studies have revealed that the  
169 majority of intermediates formed directly from the presynaptic plasma membrane are endosomes,  
170 which then shed SVs to refill the recycling pool<sup>19,20</sup>. *Dnm1*<sup>+/<sup>R237W</sup></sup> neurons displayed a significant  
171 reduction in HRP-labelled endosomes compared to *Dnm1*<sup>+/<sup>+</sup></sup> controls (**Figure 3l-n**), with no change in  
172 HRP endosome size (**Supplementary Figure 4f**), indicating activity-dependent generation of

173 endosomes was impacted. Therefore *Dnm1*<sup>+/<sup>R237W</sup> neurons have an intrinsic and specific deficit in SV  
174 endocytosis.</sup>

175

### 176 ***Dnm1*<sup>+/<sup>R237W</sup> mice have altered excitatory neurotransmission</sup>**

177 SV endocytosis is essential to sustain neurotransmitter release<sup>21,22</sup>, therefore we predicted that the  
178 endocytosis defects observed in *Dnm1*<sup>+/<sup>R237W</sup> neurons would translate into dysfunctional  
179 neurotransmission. To determine this, we examined neurotransmission at the excitatory Schaffer  
180 Collateral CA3-CA1 synapse, using whole-cell patch clamp recordings. We first determined the  
181 intrinsic properties of *Dnm1*<sup>+/<sup>R237W</sup> neurons (**Supplementary Table 1**). Most parameters were  
182 unaffected, however differences in both Tau (membrane decay time) and capacitance (cell size)  
183 were detected. Alterations in capacitance may reflect dysfunctional endocytosis, whereas the  
184 elevated Tau value suggests the plasma membrane is slower to charge, reflecting a decrease in cell  
185 excitability. This increase in Tau is consistent with the increased half-width of action potentials in  
186 *Dnm1*<sup>+/<sup>R237W</sup> neurons, in addition to the decay rate and rise time (**Supplementary Table 1**).  
187 Therefore, *Dnm1*<sup>+/<sup>R237W</sup> neurons display alterations in their intrinsic properties, which may be an  
188 adaptation to defects at the cell or circuit level. However, there was no significant difference in the  
189 amount of current required to trigger action potential firing between *Dnm1*<sup>+/<sup>R237W</sup> and *Dnm1*<sup>+/<sup>+  
190 synapses when either spike frequency or rheobase was quantified (**Figure 4a-c, Supplementary**  
191 **Table 1**). Therefore, the R237W *Dnm1* allele does not result in intrinsic hyperexcitability of  
192 *Dnm1*<sup>+/<sup>R237W</sup> neurons.</sup></sup></sup></sup></sup></sup></sup></sup>

193

194 Next we investigated both miniature excitatory or inhibitory postsynaptic currents (mEPSCs, mIPSCs)  
195 at CA1 neurons, since these can reflect deficiencies in SV recycling. The frequency, but not  
196 amplitude, of mEPSCs was significantly reduced at *Dnm1*<sup>+/<sup>R237W</sup> synapses (**Figure 4d-f**), suggesting a  
197 presynaptic SV recycling defect with no obvious postsynaptic phenotype. In contrast, we observed  
198 no significant difference in the frequency of mIPSC events and an increase in mIPSC amplitude at  
199 *Dnm1*<sup>+/<sup>R237W</sup> synapses (**Figure 4g-i**). This suggests that there was a selective perturbation of  
200 excitatory neurotransmission in *Dnm1*<sup>+/<sup>R237W</sup> mice. To determine this, we assessed whether evoked  
201 excitatory or inhibitory neurotransmission was impacted in *Dnm1*<sup>+/<sup>R237W</sup> mice. Somewhat  
202 surprisingly, *Dnm1*<sup>+/<sup>R237W</sup> synapses displayed an increase in evoked EPSC amplitudes across a range  
203 of stimulus intensities (**Figure 4j,k**). In contrast, *Dnm1*<sup>+/<sup>R237W</sup> synapses displayed no alteration in  
204 evoked IPSC amplitude across an identical stimulus range (**Figure 4l,m**). Finally, we determined the  
205 paired-pulse ratio (PPR) for excitatory and inhibitory neurotransmission by applying pairs of pulses at  
206 a range of inter-stimulus intervals. This analysis revealed a significant decrease in the EPSC PPR and a</sup></sup></sup></sup></sup></sup>



207 significant increase the IPSC PPR in *Dnm1*<sup>+/<sup>R237W</sup> synapses when compared to *Dnm1*<sup>+/ <sup>(Figure 5a-d).  
208 This suggests that the presence of the R237W *Dnm1* allele results in an increased release probability  
209 (Pr) for excitatory neurotransmission and decreased Pr for inhibitory neurotransmission, providing a  
210 potential mechanism for imbalanced excitability at these synapses.</sup></sup></sup>

211

212 Since *Dnm1*<sup>+/<sup>R237W</sup> synapses appear to have increased excitatory neurotransmission across a range of  
213 stimuli, we next investigated whether neurotransmission could be sustained during a prolonged  
214 train of high frequency action potentials (600 APs at 40 Hz). *Dnm1*<sup>+/<sup>R237W</sup> synapses displayed an  
215 inability to support neurotransmission during the stimulus train, when compared to *Dnm1*<sup>+/ <sup>controls  
216 (Figure 5e-g). This finding, when considered with the reduced PPR, could be due to either increased  
217 Pr, or an inability to replenish SV pools. To determine this, the amplitude of the first evoked EPSC  
218 was divided by the effective readily releasable pool (RRP) size, estimated from the 40 Hz action  
219 potential train<sup>23</sup> (Figure 5g). Pr was unaffected in *Dnm1*<sup>+/<sup>R237W</sup> circuits (Supplementary Figure 5a),  
220 whereas both the size and replenishment rate of the RRP was significantly reduced (Figure 5h,i).  
221 Therefore, excitatory neurotransmission in *Dnm1*<sup>+/<sup>R237W</sup> circuits is initially augmented (most likely via  
222 increased Pr), however this enhancement cannot be sustained during an action potential train (most  
223 likely via reduced SV endocytosis), resulting in its depression.</sup></sup></sup></sup></sup></sup>

224

### 225 ***Dnm1*<sup>+/<sup>R237W</sup> mice display myoclonic jumping</sup>**

226 Heterozygous mutations in the *DNM1* gene cause epileptic encephalopathies<sup>1,2</sup>. However many  
227 preclinical rodent models of monogenic epilepsies and neurodevelopmental disorders do not  
228 recapitulate the seizure activity observed in individuals with these disorders<sup>24</sup>. When examined,  
229 *Dnm1*<sup>+/<sup>R237W</sup> mice do not display overt spontaneous tonic-clonic seizures, however they do display  
230 “myoclonic jumping”, which involves bursts of highly active jumping (Figure 6a, Supplementary  
231 Movie 1). Importantly, *in vivo* electrophysiological recordings from *Dnm1*<sup>+/<sup>R237W</sup> mice during these  
232 myoclonic jumps revealed increased generalised spiking activity during these events (Figure 6b).  
233 *Dnm1*<sup>+/<sup>R237W</sup> mice had increased power during “myoclonic jumping” events in the low-frequency  
234 delta and theta bands compared to the rare jumping events observed in *Dnm1*<sup>+/ <sup>mice (Figure 6c-d).  
235 Low frequency bands are not associated with muscular activity, suggesting the increased power  
236 observed during myoclonic jumping is not a consequence of jumping *per se*. In addition, the  
237 electrophysiological activity associated with myoclonic jumping in *Dnm1*<sup>+/<sup>R237W</sup> mice was also longer  
238 than in *Dnm1*<sup>+/ <sup>mice (Figure 6e). Taken together, this suggests that this behaviour is a consequence  
239 of hyperexcitability and potential seizure activity. Therefore, the *Dnm1*<sup>+/<sup>R237W</sup> mouse has both  
240 construct and face validity as a preclinical model of *DNM1* epileptic encephalopathy.</sup></sup></sup></sup></sup></sup></sup></sup></sup>

241

242 **BMS-204352 corrects phenotypes in *Dnm1*<sup>+/<sup>R237W</sup> mice</sup>**

243 The presynaptic and circuit phenotypes observed in *Dnm1*<sup>+/<sup>R237W</sup> neurons are strongly supportive of  
244 dysfunctional SV endocytosis being the key driver of the myoclonic jumping observed in *Dnm1*<sup>+/<sup>R237W</sup>  
245 mice. Therefore, we next determined whether correction of SV endocytosis could restore normal  
246 neurotransmission and ablate the observed behavioural phenotypes. The small molecule BMS-  
247 204352 ((3S)-(+)-(5-chloro-2-methoxyphenyl)-1,3-dihydro-3-fluoro-6-(trifluoromethyl)-2H-indol-2-  
248 one) was chosen for this task since it is a therapeutic safe for use in humans <sup>25</sup> and can correct  
249 behavioural defects in a preclinical model of fragile X syndrome <sup>26</sup>. This latter effect prompted us to  
250 investigate its action, since a number of fragile X syndrome model systems display circuit  
251 hyperexcitability <sup>27</sup>. We first examined the effect of BMS-204352 in primary cultures of *Dnm1*<sup>+/<sup>+</sup>  
252 hippocampal neurons overexpressing both Dyn1<sub>WT</sub>-mCer and sypHy. Intriguingly, a dose-dependent  
253 acceleration of SV endocytosis was observed at time points after stimulation, with a reduction in SV  
254 exocytosis also observed at the highest dose (**Supplementary Figure 5b-d**). Therefore BMS-204352  
255 may have the potential to correct presynaptic defects in *Dnm1*<sup>+/<sup>R237W</sup> neurons.</sup></sup></sup></sup>

256

257 BMS-204352 displays positive modulatory effects on both neuronal K<sub>v</sub>7 channels and BK channels,  
258 whereas it is a negative modulator of both K<sub>v</sub>7.1 channels and GABA<sub>A</sub> receptors <sup>28, 29</sup>. This spectrum  
259 of activity across multiple potassium channel subtypes suggest that it may accelerate SV endocytosis  
260 via a series of different mechanisms. To determine this, we examined SV endocytosis and exocytosis  
261 in *Dnm1*<sup>+/<sup>+</sup> hippocampal neurons expressing sypHy in the presence of a series of potassium channel  
262 modulators. These were: two structurally-unrelated BK channel agonists (NS11021 and BMS-  
263 191011), a BK channel antagonist (Paxilline), a K<sub>v</sub>7 channel activator (Retigabine), and a K<sub>v</sub>7 channel  
264 inhibitor (XE-991). No modulator was able to accelerate SV endocytosis in the manner observed  
265 with BMS-204352 (**Supplementary Figure 6**). Therefore, the action of BMS-204352 on SV  
266 endocytosis is not due to modulation of a specific class of ion channels, suggesting its presynaptic  
267 effects are an amalgamation of the modulation of some or all of these channels, or an as yet  
268 unidentified off-target effect.</sup>

269

270 Regardless of the BMS-204352 mechanism of action, we next examined whether it was able to  
271 correct defective SV endocytosis due to expression of the R237W dynamin-1 mutant. We first  
272 determined its effect on *Dnm1*<sup>+/<sup>+</sup> cells overexpressing Dyn1<sub>R237W</sub>-mCer. In these neurons, BMS-  
273 204352 fully restored SV endocytosis kinetics (**Figure 7a,b**), suggesting it may be a viable  
274 intervention to correct dysfunction in *Dnm1*<sup>+/<sup>R237W</sup> neurons. When the effect of BMS-204352 on SV  
275 endocytosis was examined in primary cultures of *Dnm1*<sup>+/<sup>R237W</sup> neurons, a full correction of SV</sup></sup></sup>

276 endocytosis kinetics was again observed when compared to *Dnm1*<sup>+/+</sup> neurons (**Figure 7d,e**). BMS-  
277 204352 had no significant effect on SV exocytosis in either *Dnm1*<sup>+/+</sup> neurons with overexpressed  
278 Dyn1<sub>R237W</sub>-mCer, or *Dnm1*<sup>+/R237W</sup> neurons (**Figure 7c,f**). Therefore BMS-204352 restores SV  
279 endocytosis that was previously rendered dysfunctional via the mutant R237W *Dnm1* allele.

280

281 We next determined whether BMS-204352 could correct the observed dysfunction of excitatory  
282 neurotransmission in *Dnm1*<sup>+/R237W</sup> mice, since we predicted that defects in circuit activity were a  
283 result of impaired SV endocytosis. When applied to *Dnm1*<sup>+/+</sup> hippocampal slices, BMS-204352 had no  
284 effect on evoked EPSC amplitudes across a range of stimuli (**Figure 8a,b**). However, BMS-204352  
285 fully restored normal evoked EPSC amplitudes in *Dnm1*<sup>+/R237W</sup> slices to *Dnm1*<sup>+/+</sup> levels, across the  
286 same range of stimulus intensities (**Figure 8c,d**). Therefore BMS-204352 can restore normal evoked  
287 excitatory neurotransmission in *Dnm1*<sup>+/R237W</sup> circuits.

288

289 We next examined whether BMS-204352 could reverse short-term plastic changes in excitatory  
290 neurotransmission by monitoring synaptic facilitation evoked via a 10 Hz AP train (15 sec). In  
291 *Dnm1*<sup>+/+</sup> slices, a pronounced facilitation was observed (**Figure 8e,f**), in agreement with previous  
292 studies<sup>30</sup>. In contrast, no facilitation of excitatory neurotransmission was observed in *Dnm1*<sup>+/R237W</sup>  
293 slices (**Figure 8e,f**). Application of BMS-204352 to *Dnm1*<sup>+/R237W</sup> hippocampal slices fully restored  
294 facilitation to *Dnm1*<sup>+/+</sup> levels and had no effect on the *Dnm1*<sup>+/+</sup> response (**Figure 8e,f**). Therefore  
295 BMS-204352 corrects fundamental defects in evoked excitatory neurotransmission and short-term  
296 plasticity in *Dnm1*<sup>+/R237W</sup> circuits.

297

298 Finally, we determined whether BMS-204352 was able to correct the myoclonic jumping phenotype  
299 in *Dnm1*<sup>+/R237W</sup> mice. *Dnm1*<sup>+/R237W</sup> mice and *Dnm1*<sup>+/+</sup> littermate controls were habituated in an open  
300 field arena for 30 minutes on day 1. This protocol was repeated for 5 days. On days 2 and 4, mice  
301 were dosed with either BMS-204352 or a vehicle control in a counterbalanced manner (**Figure 9a**).  
302 Mice were also monitored on days 3 and 5 to examine baseline behaviour (Washout, **Figure 9a**).  
303 When baseline behaviour of *Dnm1*<sup>+/+</sup> and *Dnm1*<sup>+/R237W</sup> mice were examined, robust differences in  
304 both the number of myoclonic jumps and bursts of jumps (defined as a train of at least 2 myoclonic  
305 jumps with less than 2s between consecutive jumps) were observed (**Supplementary Figure 7a,b**).  
306 This phenotype was not due to increased general activity, since there is no significant change in the  
307 distance travelled by *Dnm1*<sup>+/R237W</sup> when compared to *Dnm1*<sup>+/+</sup> controls (**Supplementary Figure 7c**).

308

309 During the test phase, the phenotypes that were observed during washout were retained in vehicle-  
310 treated *Dnm1*<sup>+/+</sup> and *Dnm1*<sup>+/R237W</sup> mice. Specifically, vehicle-treated *Dnm1*<sup>+/R237W</sup> mice displayed a  
311 significant increase in the total number of myoclonic jumps (**Figure 9b**) and the number of jumping  
312 bursts (**Figure 9c**) when compared to *Dnm1*<sup>+/+</sup> controls. Delivery of BMS-204352 to *Dnm1*<sup>+/+</sup> mice had  
313 no significant effect on these parameters (**Figure 9b,c**). In contrast, BMS-204352 fully corrected both  
314 jumping phenotypes in *Dnm1*<sup>+/R237W</sup> mice to the levels observed in *Dnm1*<sup>+/+</sup> mice (**Figure 9b,c**).  
315 Importantly, this correction was not due to depression of locomotive activity, since the distance  
316 travelled was not significantly different when *Dnm1*<sup>+/R237W</sup> mice treated with or without BMS-204352  
317 were compared (**Supplementary Figure 7c**). Furthermore, BMS-204352 had no effect on the time  
318 spent in the middle of the open area, indicating that the correction of seizure phenotypes was not  
319 due to previously documented anxiolytic effects of the molecule<sup>29</sup> (**Supplementary Figure 7d**). In  
320 summary, these results suggest that BMS-204352 has high potential for therapy in *DNM1* epileptic  
321 encephalopathy, since it corrects dysfunction at the cellular, circuit and behavioural level in a  
322 preclinical model of this disorder.

323

## 324 **DISCUSSION**

325 Heterozygous *DNM1* mutations are responsible for a novel form of epileptic encephalopathy<sup>1,2</sup>.  
326 Here, we confirmed that the most common pathogenic *DNM1* mutation, R237W, disrupts dynamin-1  
327 enzyme activity and SV endocytosis. Furthermore, using the *Dnm1*<sup>+/R237W</sup> mouse, we revealed that  
328 dysfunctional SV endocytosis translates into altered excitatory neurotransmission and ultimately  
329 seizure-like phenotypes. Importantly, these phenotypes were corrected at the cell, circuit and *in vivo*  
330 level via the acceleration of SV endocytosis using BMS-204352. This study therefore provides a  
331 compelling link between dysfunctional SV endocytosis and epileptic encephalopathy, but moreover  
332 reveals that SV endocytosis may be a viable therapeutic route for monogenic intractable epilepsies.

333

334 The R237W mutation was chosen for our mouse model, since it is the most prevalent missense  
335 mutation in the *DNM1* gene (8 from 33 cases<sup>1,2</sup>). The *Dnm1*<sup>+/R237W</sup> mouse appears to have both face  
336 and construct validity and therefore is predicted to be of high value for future therapeutic studies.  
337 These mice displayed a selective defect in SV endocytosis, excitatory neurotransmission and a  
338 characteristic jumping phenotype. This behavioural phenotype occurred co-incident with increased  
339 generalised spiking activity, providing evidence that it may be precipitated via seizure-like events.  
340 We named this jumping phenotype “myoclonic jumping” since it appears similar to phenotypes in  
341 several preclinical epilepsy models observed either in isolation or in progression towards full tonic-

342 clonic seizures<sup>31-33</sup>. Furthermore, it is also observed in autism / neurodegeneration models as a  
343 measure of repetitive and stereotypic behaviour<sup>34, 35</sup>.

344

345 It is informative to contrast the *Dnm1*<sup>+/<sup>R237W</sup></sup> mouse with a previously characterised mouse model of  
346 *DNM1* epileptic encephalopathy, the *Fitful* mouse<sup>16</sup>. This mouse does not model a human mutation,  
347 but instead arose from a spontaneous mutation (A408T) in the middle domain of the ax isoform of  
348 *Dnm1*, with mice homozygous for this mutation displaying spontaneous convulsive seizures resulting  
349 in lethality after 2-3 weeks<sup>16</sup>. Heterozygous *Fitful* mice also display spontaneous seizures that are  
350 detectable via EEG, or convulsive episodes on routine handling after 2-3 months. The A408T  
351 mutation appears to be responsible for this phenotype, since homozygous *Fitful* mice display less  
352 SVs in inhibitory nerve terminals and overexpression of the A408T mutant inhibited receptor-  
353 mediated endocytosis in COS7 cells<sup>36</sup>. The absence of a dominant-negative effect of Dyn1<sub>A408T</sub>-mCer  
354 on SV endocytosis in our study was therefore surprising. However, overexpression of dynamin-1  
355 mutants in heterologous expression systems (where dynamin-2 is the dominant isoform) may result  
356 in more severe phenotypes when compared to the expression of these mutants in their natural  
357 context. This is supported by the relatively mild effect of both Dyn1<sub>R237W</sub>-mCer and Dyn1<sub>K44A</sub>-mCer on  
358 SV endocytosis in our study, which contrasts with the ablation of receptor-mediated endocytosis  
359 observed with the K44A mutant in non-neuronal heterologous expression systems<sup>13</sup>.

360

361 One intriguing finding was the alteration in both spontaneous and evoked excitatory  
362 neurotransmission in *Dnm1*<sup>+/<sup>R237W</sup></sup> mice with no parallel effect on inhibitory neurotransmission. The  
363 absence of effect on spontaneous or evoked inhibitory neurotransmission agrees with previous  
364 studies in the homozygous *Fitful* mouse (but see<sup>37</sup>), although increased rundown occurs during  
365 prolonged AP trains with a concomitant delay in the recovery of IPSC amplitude<sup>16</sup>. This relatively  
366 mild effect contrasts with studies in *Dnm1*<sup>-/-</sup> neurons, where there was a large reduction in evoked  
367 IPSCs when compared to EPSCs, and faster and more extensive depression of inhibitory  
368 neurotransmission during action potential trains<sup>38</sup>. Furthermore, in *Dnm1/3*<sup>-/-</sup> synapses, a strong  
369 facilitation of excitatory neurotransmission was observed during both low and high frequency  
370 stimulation, with mEPSC frequency, evoked EPSC amplitude, RRP size and Pr all decreased<sup>39</sup>.  
371 *Dnm1*<sup>+/<sup>R237W</sup></sup> synapses also display reduced mEPSC frequency and decreased RRP, however in  
372 contrast, we observe an increase in both Pr and evoked EPSCs in addition to an absence of STP  
373 during action potential trains. Furthermore, we observe a decreased Pr at inhibitory *Dnm1*<sup>+/<sup>R237W</sup></sup>  
374 synapses. Therefore, even when SV endocytosis is disrupted to a similar extent between *Dnm1*<sup>+/<sup>R237W</sup></sup>  
375 and *Dnm1*<sup>-/-</sup> neurons, the dominant-negative R237W mutation exerts discrete effects on circuit

376 activity not observed in models of loss of dynamin-1 function. Our observation of increased Pr at  
377 excitatory synapses with a concomitant Pr decrease at inhibitory synapses, therefore provides a  
378 potential microenvironment for epileptogenesis, making it critical for future studies to determine  
379 how *Dnm1*<sup>+/<sup>R237W</sup> neurons modify brain circuit properties and higher order functions.</sup>

380

381 We observed a full correction of cellular, circuit and *in vivo* phenotypes via the delivery of BMS-  
382 204352. BMS-204352 was developed as a BK channel agonist for the treatment of stroke <sup>40</sup>, however  
383 in Phase III trials it failed to display efficacy superior to placebo <sup>25</sup>. Nevertheless, the drug exhibited  
384 an excellent safety profile, identifying it as a promising candidate for repurposing studies. Because  
385 BMS-204352 displayed both positive and negative modulatory effects against a number of  
386 potassium channel subtypes <sup>28, 29</sup>, we employed a series of channel openers and blockers to  
387 elucidate its mechanism of action. Intriguingly, no drug from this palette of modulators recapitulated  
388 its observed modifying activity on SV endocytosis. Therefore BMS-204352 may have additional off-  
389 target effects responsible for its reversal of phenotypes in the *Dnm1*<sup>+/<sup>R237W</sup> mouse. This question is  
390 under active investigation.</sup>

391

392 The fact that BMS-204352 accelerates SV endocytosis and corrects cell, circuit and behavioural  
393 phenotypes in the *Dnm1*<sup>+/<sup>R237W</sup> mouse, provides strong evidence of a direct causal link between  
394 dysfunctional SV endocytosis and these outcomes. However, other interpretations are possible,  
395 since the R237W allele should perturb any dynamin-dependent endocytosis mode at the synapse.  
396 For example, altered trafficking of voltage-gated ion channels, and either pre- or post-synaptic  
397 receptors may also contribute to the observed phenotypes. *Dnm1*<sup>+/<sup>R237W</sup> excitatory synapses display  
398 no significant alterations in excitability in terms of action potential threshold, suggesting an absence  
399 of intrinsic hyperexcitability observed in other NDDs models<sup>26</sup>. Action potential broadening and a  
400 decrease in action potential decay rate were observed however, which may contribute towards the  
401 enhancement in evoked EPSCs. Intriguingly, BK channels perform a key role in sculpting action  
402 potential shape<sup>41</sup>. However, BMS-204352 is not acting via this mechanism to correct function, since  
403 the observed broadening reflects reduced, rather than enhanced BK channel function. An increase in  
404 mIPSC amplitude is also observed, similar to the *Dnm1*<sup>-/-</sup> mouse<sup>38</sup>. This may reflect a compensatory  
405 recruitment of postsynaptic GABA<sub>A</sub> receptors to offset increased excitability. Conversely, it may be a  
406 consequence of increased quantal release, due to larger SVs being formed via dysfunctional  
407 endocytosis<sup>16</sup>. The unaltered mEPSC amplitude in *Dnm1*<sup>+/<sup>R237W</sup> CA1 neurons suggests that  
408 postsynaptic stranding of AMPA receptors does not contribute to the increased evoked EPSC  
409 response however. One potential explanation for the increase in evoked EPSCs is the dysregulated</sup></sup></sup>

410 retrieval of modulatory presynaptic receptors. A number of these have direct effects on Pr, via  
411 regulation of either ion channels or signalling cascades<sup>42, 43</sup>. In support, we observed an upregulation  
412 of ErbB signalling molecules (which have direct effects on synaptic function<sup>44</sup>) in *Dnm1*<sup>+/<sup>R237W</sup></sup>  
413 synaptosomes and a concomitant decrease in metabotropic receptor signalling molecules. It will be  
414 important to determine which of these signalling cascades directly contribute to increased excitatory  
415 Pr and decreased inhibitory Pr, and which are compensatory changes that adjust for circuit  
416 hyperexcitability.

417

418 BMS-204352 is therefore a promising lead compound for future trials in *DNM1* epileptic  
419 encephalopathy. There is also potential for its use to be wider than this specific condition, since a  
420 cohort of monogenic neurodevelopmental disorders are predicted to have SV endocytosis defects at  
421 their core. For example, a series of frameshift, nonsense and missense mutations in essential SV  
422 endocytosis and SV cargo clustering genes have been identified in individuals with intellectual  
423 disability, autism and epilepsy<sup>24</sup>, including the coat protein clathrin<sup>45</sup>, adaptor protein complexes<sup>46</sup>,  
424 SV cargo retrieval<sup>47-49</sup> and regulators of endocytosis such as TBC1D24<sup>50, 51</sup>. Furthermore, neurons  
425 derived from preclinical models for prevalent monogenic conditions such as fragile X syndrome and  
426 CDKL5 deficiency disorder have recently been discovered to display defects in SV retrieval<sup>18, 52</sup>. With  
427 dysfunctional SV endocytosis emerging as a key convergence point in these monogenic conditions,  
428 upregulation of SV endocytosis via BMS-204352 may therefore provide a potential treatment to  
429 restore essential recycling mechanisms and normal function.

430

431 In conclusion, we have key cell, circuit and behavioural defects in a mouse model of *DNM1* epileptic  
432 encephalopathy, which provide important information on the molecular locus of seizure activity.  
433 Furthermore, an agent that accelerates SV endocytosis corrects all of these defects, suggesting  
434 intervention via this trafficking pathway is a promising therapeutic route.

435

## 436 **METHODS**

### 437 **Materials**

438 Unless otherwise specified, all cell culture reagents were obtained from Invitrogen (Paisley, UK).  
439 Foetal bovine serum was from Biosera (Nuaille, France). Papain was obtained from Worthington  
440 Biochemical (Lakewood, NJ, USA). Caesium-gluconate, tetrodotoxin (TTX) and picrotoxin were from  
441 Hello Bio (Bristol, UK). Na<sub>2</sub>GTP was from Scientific Laboratory Supplies (Newhouse, UK), whereas  
442 Na<sub>2</sub>-creatine was from (Merck, London, UK). BMS-204352 was from Bio-Techne Ltd (Abingdon, UK).  
443 All other reagents were obtained from Sigma-Aldrich (Poole, UK) unless specified. Synaptophysin-

444 pHluorin (sypHy) was provided by Prof. L. Lagnado (University of Sussex, UK). Rat dynamin-1aa fused  
445 to mCerulean at its C-terminus<sup>53</sup> was subjected to site-directed mutagenesis to generate both  
446 R237W (forward primer ATTGGCGTGGTGAAGCTGGAGCCAGAAGGACATA, reverse primer  
447 TATGTCCTTCTGGCTCCAGTTCACCACGCCAAT) and K44A mutations (forward primer  
448 GGCCAGAGCGCCGGCGGAGCTCGGTGCTGGAC, reverse primer  
449 CTCCAGCACCGAGCTCGCGCCGGCGCTCTGGCC). Base changes were confirmed by Source Bioscience  
450 Sanger Sequencing (Glasgow, UK).

451

#### 452 **Generation of *Dnm1*<sup>+/<sup>R237W</sup></sup> mice**

453 The *Dnm1*<sup>+/<sup>R237W</sup></sup> mouse was generated by Horizon Discovery (St. Louis, USA). Briefly, the codon  
454 encoding R237 within the *Dnm1* gene was targeted using CRISPR-Cas9 technologies on a C57Bl/6J  
455 genetic background using the guide sequence cgtggtgaaccggagccagaagg. This resulted in the  
456 modification of the *Dnm1* gene sequence from CGGAGC (equivalent amino acids 237/238 - RS) to  
457 TGGTCT (amino acids 237/238 - WS). In total, 44 animals were screened for the point mutation, with  
458 4 found to be positive. Two founders were backcrossed to *Dnm1*<sup>+/<sup>+</sup></sup> mice to generate F1  
459 heterozygous progeny. The F1 progeny of one of the founder lines was taken forward to establish  
460 the colony. Mice were maintained as heterozygotes by crossing *Dnm1*<sup>+/<sup>R237W</sup></sup> mice with C57Bl/6J  
461 *Dnm1*<sup>+/<sup>+</sup></sup> mice, with 3 backcrosses every 5 generations. Genotyping was performed by Transnetyx  
462 (Cordova, TN, USA). A separate in-house colony of C57Bl/6J *Dnm1*<sup>+/<sup>+</sup></sup> mice were used as a source of  
463 tissue for hippocampal cultures in experiments where dynamin-1 variants were overexpressed.

464

465 Animal work was performed in accordance with the UK Animal (Scientific Procedures) Act 1986,  
466 under Project and Personal Licence authority and was approved by the Animal Welfare and Ethical  
467 Review Body at the University of Edinburgh (Home Office project licences – 7008878 and PP5745138  
468 to Prof. Cousin and PP1538548 to Dr. Gonzalez-Sulser). Specifically, all animals were killed by  
469 Schedule 1 procedures in accordance with UK Home Office Guidelines; adults were killed by cervical  
470 dislocation or exposure to CO<sub>2</sub> followed by decapitation, whereas embryos were killed by  
471 decapitation followed by destruction of the brain. The in-house colony of C57Bl/6J *Dnm1*<sup>+/<sup>+</sup></sup> mice and  
472 the *Dnm1*<sup>+/<sup>R237W</sup></sup> mouse colony were housed in standard open top caging on a 14/10 h light/dark  
473 cycle (light 7 A.M. to 9 P.M.). Breeders were fed RM1 chow, whereas stock mice were maintained on  
474 RM3 chow. The ambient temperature ranged between 19 – 23°C with humidity 55 ± 10%.

475

#### 476 **Cell culture and transfections**



477 Heterozygous *Dnm1*<sup>+/<sup>R237W</sup> mice were mated with *Dnm1*<sup>+/ <sup>mice to produce either *Dnm1*<sup>+/ <sup>or  
478 *Dnm1*<sup>+/<sup>R237W</sup> offspring. Hippocampi from each embryo were processed separately to avoid  
479 contamination across genotypes. Dissociated primary hippocampal cultures were prepared from  
480 embryos as previously described<sup>18</sup>. Briefly, isolated hippocampi were digested in a 10 U/mL papain  
481 solution (Worthington Biochemical, LK003178) at 37°C for 20 min. The papain was then neutralised  
482 using DMEM F12 (ThermoFisher Scientific, 21331-020) supplemented with 10 % Foetal bovine serum  
483 (BioSera, S1810-500) and 1 % penicillin/streptomycin (ThermoFisher Scientific, 15140-122). Cells  
484 were triturated to form a single cell suspension and plated at 5 x 10<sup>4</sup> cells per coverslip on laminin  
485 (10 µg/ mL; Sigma Aldrich, L2020) and poly-D-lysine (Sigma Aldrich, P7886) coated 25 mm glass  
486 coverslips (VWR International Ltd, Lutterworth, UK). Cultures were maintained in Neurobasal media  
487 (ThermoFisher Scientific, 21103-049) supplemented with 2 % B-27 (ThermoFisher Scientific, 17504-  
488 044), 0.5 mM L-glutamine (ThermoFisher Scientific, 25030-024) and 1% penicillin/streptomycin.  
489 After 2-3 days *in vitro* (DIV), 1 µM of cytosine arabinofuranoside (Sigma Aldrich, C1768) was added  
490 to each well to inhibit glial proliferation. Hippocampal neurons were transfected with  
491 synaptophysin-pHluorin (sypHy) and/or Dyn1-mCer using Lipofectamine 2000 (ThermoFisher  
492 Scientific, 11668027) as per manufacturer's instructions and imaged at DIV 13-15.</sup></sup></sup></sup></sup></sup>

493

#### 494 **Imaging of SV recycling using sypHy**

495 Imaging of SV recycling was monitored using sypHy as previously described<sup>18</sup>. SypHy-transfected  
496 hippocampal cultures were mounted in a Warner Instruments (Hamden, CT, USA) imaging chamber  
497 with embedded parallel platinum wires (RC-21BRFS) and were mounted on a Zeiss Axio Observer D1  
498 inverted epifluorescence microscope (Cambridge, UK). Neurons were challenged with field  
499 stimulation using a Digitimer LTD MultiStim system-D330 stimulator (current output 100 mA, current  
500 width 1 ms) either at 10 Hz for 30 s or 40 Hz for 10 s. Neurons were visualised at 500 nm band pass  
501 excitation with a 515 nm dichroic filter and a long-pass >520 nm emission filter, with images  
502 captured using an AxioCam 506 mono camera (Zeiss) with a Zeiss EC Plan Neofluar 40x/1.30 oil  
503 immersion objective. Image acquisition was controlled using Zen Pro software (Zeiss). Imaging time  
504 courses were acquired at 4 s intervals while undergoing constant perfusion with imaging buffer (119  
505 mM NaCl, 2.5 mM KCl, 2 mM CaCl<sub>2</sub>, 2 mM MgCl<sub>2</sub>, 25 mM HEPES, 30 mM glucose at pH 7.4,  
506 supplemented with 10 µM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Abcam, Cambridge, UK,  
507 ab120271) and 50 µM DL-2-Amino-5-phosphonopentanoic acid (AP5, Abcam, Cambridge, UK,  
508 ab120044). Alkaline buffer (50 mM NH<sub>4</sub>Cl substituted for 50 mM NaCl) was used to reveal the  
509 maximal pHluorin response. SV fusion during stimulation was measured by stimulating sypHy-  
510 transfected neurons (10 Hz, 90 s) in the presence of 1 µM bafilomycin A1 (Cayman Chemical

511 Company, Ann Arbor Michigan, USA, 11038). BMS-204352 and other potassium channel modulators  
512 in imaging buffer were perfused over neurons 2 min prior to and during imaging until addition of  
513 alkaline buffer.

514

515 Time traces were analysed using the FIJI distribution of Image J (National Institutes of Health).  
516 Images were aligned using the Rigid body model of the StackReg plugin  
517 (<https://imagej.net/StackReg>). Nerve terminal fluorescence was measured using the Time Series  
518 Analyser plugin (<https://imagej.nih.gov/ij/plugins/time-series.html>). Regions of interest (ROIs) 5  
519 pixels in diameter were placed over nerve terminals that responded to the electrical stimulus. A  
520 response trace was calculated for each cell by averaging the individual traces from each selected  
521 ROI. Inhibition of SV endocytosis was calculated as remaining fluorescence 140 s after termination of  
522 stimulation. The time constant of SV endocytosis could not be calculated, since individual sypHy  
523 traces within each set of experiments did not conform to first order kinetics.

524

#### 525 **HRP uptake in hippocampal cultures**

526 Hippocampal cultures were mounted in the RC-21BRFS stimulation chamber and challenged with  
527 400 action potentials (40 Hz) in the presence of 10 mg/ml HRP (Sigma Aldrich, P8250) supplemented  
528 imaging buffer. Immediately following the end of stimulation, cultures were washed in imaging  
529 buffer to remove non-internalised HRP and fixed with a solution of 2 % glutaraldehyde (Electron  
530 Microscopy Sciences, Hatfield, USA, 16019) and 2% PFA in 0.1 M in phosphate buffer (PB). After  
531 washing in 0.1 M PB, HRP was developed with 0.1 % 3,3'-diaminobenzidine (Fluka Chemica,  
532 Gillingham, UK, 22204001) and 0.2 % v/v hydrogen peroxide (Honeywell, Muskegon, USA, 216763) in  
533 PB. After further washing in PB, cultures were stained with 1 % osmium tetroxide (TAAB laboratory  
534 and microscopy, Aldermaston, UK, O015/1) for 30 min. Samples were then dehydrated using an  
535 ethanol series and polypropylene oxide (Electron Microscopy Sciences, Hatfield, USA, 20411) and  
536 embedded using Durcupan resin (Sigma Aldrich, 44610). Samples were sectioned, mounted on grids,  
537 and viewed using an FEI Tecnai 12 transmission electron microscope (Oregon, USA). Intracellular  
538 structures that were <61 nm in diameter were arbitrarily designated to be SVs, whereas larger  
539 structures were considered endosomes. The area of individual endosomes was obtained by tracing  
540 the circumference using the freehand selections tool in ImageJ and measuring the resulting area.  
541 Typically, 20 fields of view were acquired for one coverslip of cells. In nerve terminals that contained  
542 HRP, the average number of HRP-labelled endosomes and SVs per nerve terminal was calculated for  
543 each coverslip and represents the experimental n.

544

545 **Quantification of endocytic profiles**

546 Two-month-old *Dnm1*<sup>+/+</sup> or *Dnm1*<sup>+/R237W</sup> mice were terminally anaesthetised by intraperitoneal  
547 overdose of sodium pentobarbital. Mice were then perfused through the left ventricle with ice-cold  
548 0.1 M phosphate buffer, followed by a fixative solution consisting of 2% paraformaldehyde and 2%  
549 glutaraldehyde in 0.1 M phosphate buffer. Brains were dissected and post-fixed in the same fixative  
550 solution overnight at 2-4 °C, at which point the fixative solution was replaced with 0.1 M phosphate  
551 buffer. Brains were then processed for electron microscopy as described above. Individual  
552 endosomes and SVs were counted and normalized to the nerve terminal area. The area of individual  
553 endosomes and SVs was obtained by fitting a region of interest over each individual structure using  
554 the round area selection tool in ImageJ and measuring the resulting area. Intracellular structures  
555 that were <2922 nm<sup>2</sup> in area were arbitrarily designated to be SVs, whereas larger structures were  
556 considered endosomes.

557

558 **Immunocytochemistry**

559 Immunofluorescence staining and analysis was performed as previously described<sup>18</sup>. Briefly,  
560 hippocampal neurons were fixed with 4% paraformaldehyde (PFA) in PBS for 15 min at room  
561 temperature. PFA was then removed and cells were quenched 2x 5 min with 50 mM NH<sub>4</sub>Cl in PBS.  
562 Cells were then washed 4x 5 min with PBS. Before staining, cells were permeabilised in 1% bovine  
563 serum albumin (BSA) in PBS-Triton 1% for 5 min. Cells were then washed in PBS before blocking in  
564 1% BSA in PBS at room temperature for 1 h. After blocking, cells were left to incubate in primary  
565 antibody diluted in blocking solution for 30-45 min (chicken anti-GFP (Abcam ab13970) 1:500; rabbit  
566 anti-SV2A (Abcam ab32942) 1:200; goat anti-dynamin-1 (Santa Cruz sc-6402) 1:200). Following 4 x 5  
567 min washes, cells were left to incubate in secondary antibody (goat anti-chicken Alexa-Fluor-488  
568 (Invitrogen A11039) 1:1000; goat anti-rabbit Alexa-Fluor-568 (Invitrogen A21069) 1:1000; donkey  
569 anti-goat Alexa-Fluor-647 (Invitrogen A21447) 1:1000) diluted in blocking buffer for 30-45 min at  
570 room temperature in the dark. After washing, coverslips were mounted to slides using FluorSave  
571 Reagent (Millipore). Alexa Fluor 488 and 568 images were acquired using a dual camera imaging  
572 system (Zeiss). The signal was filtered by a double band pass excitation filter (470/27 + 556/25) with  
573 beam splitter (490 + 575) and emission filters 512/30 and 630/98 (Zeiss) respectively. Alexa Fluor  
574 647 was visualised with a 640 nm excitation and a 690/50 band pass emission filter. For each image  
575 analysed, ROIs were placed over the transfected neuron, a non-transfected neuron and the  
576 background. This allowed measurement of levels of overexpression of mCer-Dyn1 within neurons on  
577 the same coverslip by comparing the overexpression to normal expression levels. Background

578 fluorescence was subtracted from all signals. For each coverslip, 4-6 fields with transfected neurons  
579 were acquired. The n is the number of transfected cells imaged.

580

### 581 **Immunohistochemistry**

582 Two-month-old *Dnm1*<sup>+/+</sup> or *Dnm1*<sup>+/R237W</sup> littermate male mice were administered a lethal dose of  
583 sodium pentobarbital and transcardially perfused with cold PBS followed by cold PFA (PFA, 4% in  
584 0.1M PB). Brains were extracted and fixed for 24 hours in PFA at 4°C, washed with PBS, and  
585 transferred to a 30 % sucrose / PBS solution for 48 hours at 4°C. Brains were embedded in tissue  
586 freezing compound and 50 µm coronal sections were generated using a freezing microtome. Free  
587 floating thin sections were permeabilised for 4-5 hours in block solution (PBS, 10 % horse serum, 0.5  
588 % BSA, 0.5 % Triton X-100, 0.2 M glycine) then incubated with NeuN primary antibody diluted in  
589 block solution (1:1000; Merck; Cat # MAB377) overnight at 4 °C. Slices were washed 4-5 times in PBS  
590 for 2 hours then incubated for 3-4 hours with secondary antibody (anti-rabbit Alexa Fluor 568;  
591 1:1000; Invitrogen; Cat #A10042) and NeuroTrace Green Fluorescent Nissl Stain (1:2000; Invitrogen;  
592 Cat #N21480) at room temperature. Slices were then washed 4-5 times in PBS for 2 hours and  
593 mounted onto glass slides using ProLong Gold Antifade Mountant (Invitrogen; Cat #P36930).  
594 Sections were imaged on a Leica SP8 upright confocal laser scanning microscope using a X10/NA  
595 0.45 objective. The tile function within the Leica software was used to acquire overlapping images  
596 over the whole section followed by the merge image processing function to stitch the tiles together.

597

### 598 **Protein biochemistry**

599 Cultured hippocampal neurons from *Dnm1*<sup>+/+</sup> or *Dnm1*<sup>+/R237W</sup> sex-matched littermates at DIV 14 were  
600 lysed directly into SDS (sodium dodecylsulfate) sample buffer (67 mM Tris, pH 7.4, 2 mM EGTA, 9.3%  
601 glycerol, 12% β-mercaptoethanol, bromophenol blue, 67 mM SDS) and boiled at 95°C for 10 minutes  
602 prior to Western blotting. Whole brain lysates were prepared from the brains of 3 week old and 6  
603 week old age-matched *Dnm1*<sup>+/+</sup> or *Dnm1*<sup>+/R237W</sup> mice. Brain homogenates were prepared in RIPA  
604 buffer (10 mM Tris-HCl, pH 8.0, 1mM EDTA, 0.5 mM EGTA, 15 Triton X-100, 0.1% sodium  
605 deoxycholate, 0.1% SDS, 140 mM NaCl and 1 mM PMSF) and centrifuged in a Beckman-Coulter  
606 Optima-Max ultracentrifuge at 116,444 g for 40 min at 4°C. Protein concentration was determined  
607 using a Bradford (Applichem, Germany; A6932) assay following manufacturer's instructions. SDS  
608 sample buffer was added to the lysates and samples were boiled for 10 min before loading on SDS-  
609 PAGE and blotting onto nitrocellulose membranes. Membranes were incubated with primary  
610 antibodies overnight at 4°C (Goat anti-amphyphysin-1 (Santa Cruz sc-8536) 1:500; rabbit anti-Eps15  
611 (Santa Cruz sc-534) 1:1000; Goat anti-dynamin-1 (Santa Cruz sc-6402) 1:1000; ; mouse anti-

612 synaptotagmin-1 (Abcam ab13259) 1:500; rabbit anti-syndapin-1 (Abcam ab137390) 1:4000; goat  
613 anti-endophilin-A1 (Santa Cruz sc-10874) 1:1000; rabbit anti-C-src (Santa Cruz sc-19) 1:100; mouse  
614 anti-actin (Sigma Aldrich A4325) 1:50000). Secondary antibodies were incubated for 1h at room  
615 temperature (all Li-Cor, 1:10000; donkey anti-goat (IRDye® 680RD, 926-68074); donkey anti-goat  
616 (IRDye® 800CW, 926-32214); donkey anti-rabbit (IRDye® 800CW, 926-32213); donkey anti-mouse  
617 (IRDye® 800CW, 926-32212); goat anti-mouse (IRDye® 680RD, 926-68070). Membranes were imaged  
618 on an Odyssey 9120 Infrared Imaging System (LI-COR Biosciences) using *LI-COR* Image Studio Lite  
619 software (version 5.2) and analysed using ImageJ. The integrated density of signals was measured in  
620 rectangular ROIs of an identical size set around the protein expression bands.

621

### 622 **Mass Spectrometry**

623 Synaptosomes were prepared from two-month-old *Dnm1<sup>+/+</sup>* or *Dnm1<sup>+R237W</sup>* littermate male mice as  
624 described<sup>30</sup>. Briefly, animals were culled by cervical dislocation with death confirmed by destruction  
625 of the brain via homogenization in ice-cold 0.32 M sucrose, 5 mM EDTA (pH 7.4) after removal of the  
626 cerebellum. The homogenate was centrifuged at 950 x *g* for 10 min at 4 °C at which point the  
627 supernatant was saved and the pellet was resuspended in the same sucrose buffer. The resuspended  
628 pellet solution was centrifuged at 950 x *g* for 10 min at 4 °C and the resulting supernatant was  
629 combined with the first. The combined supernatant was then centrifuged at 20,400 x *g* for 30 min at  
630 4 °C and the pellet (crude synaptosomal fraction) was retained. Synaptosome pellets were dissolved  
631 in Urea lysis buffer (8M Urea in 50mM Tris-Cl and 1% sodium deoxycholate) and were quantified  
632 using the BCA method. 20 µg of total protein was used for proteomic sample preparation by  
633 suspension trapping (S-Trap)<sup>54</sup>, as recommended by the supplier (ProtiFi, Huntington NY, USA).  
634 Samples were reduced with 5 mM Tris (2-carboxyethyl)phosphine (Pierce) for 30 min at 37°C, and  
635 subsequently alkylated with 5 mM IAM (Iodoacetamide) for 30 min at 37°C in the dark. After  
636 acidification with phosphoric acid, sample was cleaned and digested using Trypsin (1:20) as  
637 mentioned by in the manufacturer's protocol using S-trap filter for 2 hours at 47°C and the digested  
638 peptides are eluted using 0.2% Formic acid and 50% Acetonitrile:0.2% formic acid. The eluted  
639 digested peptides were dried in speed vac and stored at -80°C.

640

641 The peptides were reconstituted in 30 µL of 0.1% formic acid and vortexed and 5 µL of each sample  
642 was injected on the mass spectrometer. Peptides were analysed by nanoflow-LC-MS/MS using a  
643 Orbitrap Q-Exactive-HF™ Mass Spectrometer (Thermo Scientific™) coupled to a Dionex™ Ultimate™  
644 3000. Samples were injected on a 100 µm ID × 5mm trap (Thermo Trap Cartridge 5mm) and  
645 separated on a 75 µm × 50 cm nano LC column (EASY-Spray™ LC Columns #ES803). All solvents used

646 were HPLC or LC-MS Grade (Millipore™). Peptides were loaded for 5 minutes at 10 µL/min using  
647 0.1% FA, 2% Acetonitrile in Water. The column was conditioned using 100% Buffer A (0.1% FA, 3%  
648 DMSO in Water) and the separation was performed on a linear gradient from 0 to 35% Buffer B  
649 (0.1% FA, 3% DMSO, 20% Water in Acetonitrile), over 140 minutes at 250 nL/min. The column was  
650 then washed with 90% Buffer B for 5 minutes and equilibrated 10 minutes with 100% Buffer A in  
651 preparation for the next analysis. Full MS scans were acquired from 350 to 1500 m/z at resolution  
652 60,000 at m/z 200, with a target AGC of  $3 \times 10^6$  and a maximum injection time of 50 ms. MS/MS scans  
653 were acquired in HCD mode with a normalized collision energy of 25 and resolution 15000 using a  
654 Top 20 method, with a target AGC of  $2 \times 10^5$  and a maximum injection time of 50 ms. The MS/MS  
655 triggering threshold was set at 5E3 and the dynamic exclusion of previously acquired precursor was  
656 enabled for 45 s for DDA (Data-Dependent Acquisition) mode. For DIA (Data Independent  
657 Acquisition) mode the scan range was 385 to 1015 m/z, where MS/MS data was acquired in 24 m/z  
658 isolation windows at a resolution of 30,000.

659

660 Pooled peptides from all samples were fractionated on a Basic Reverse Phase column (Gemini C18,  
661 3µm particle size, 110Å pore, 3 mm internal diameter, 250 mm length, Phenomenex #00G-4439-Y0)  
662 on a Dionex Ultimate 3000 Off-line LC system. All solvent used were HPLC grade (Fluka). Peptides  
663 were loaded on column for 1 minute at 250 µL/min using 99% Buffer A (20mM Ammonium Formate,  
664 pH=8) and eluted for 48 minutes on a linear gradient from 2 to 50% Buffer B (100% ACN). The  
665 column is then washed with 90% Buffer B for 5 minutes and equilibrated for 5 minutes for the next  
666 injection. Peptide elution was monitored by UV detection using at 214 nm. Fractions were collected  
667 every 45 s from 2 min to 60 min for a total of 12 fractions. Non-consecutive concatenation of every  
668 13th fraction was used to obtain 12 pooled fractions (Pooled Fraction 1: Fraction 1 + 13 + 25 + 37,  
669 Pooled Fraction 2 : Fraction 2 + 14 + 26 + 38 ...).

670

## 671 **Data Analysis**

672 Label-free quantitative analysis was performed using the data set acquired in DIA mode. Peptide  
673 identification was carried out using a library generated using both DDA and DIA datasets using  
674 Spectronaut™ version 15.0. The library was generated using the Pulsar algorithm integrated in  
675 Spectronaut using Mus musculus FASTA using 1% FDR. The maximum of missed cleavage was set to  
676 2 using Trypsin/P enzyme. Carbamidomethylation (C) was set as fixed modification and acetylation  
677 (Protein N term), oxidation (M), deamination (NQ), were set as variable modifications. The library  
678 consisted spectra information of 5906 proteins in total. DIA data set for both WT and HET was  
679 searched using this library quantified 4237 proteins in total. Statistical analysis was done using R

680 script and limma package was used for making contrasts. Raw proteomic data was deposited on  
681 PRIDE (<https://www.ebi.ac.uk/pride/>) as outlined below.

682

### 683 **Analysis of mass spectrometry data**

684 Gene Ontology (GO) terms enrichment analysis on the upregulated and downregulated proteins was  
685 performed against *Mus musculus* background using Database for Annotation, Visualization  
686 and Integrated Discovery (DAVID). Detailed enrichment analysis are available in **Supplementary Data**  
687 **2**. Network analysis of the upregulated and downregulated proteins were performed using the  
688 STRING web tool (v.11.5).

689

690 Enrichment analysis of the full protein list were performed using ShinyGO v0.76.2 for the Cellular  
691 Component and Biological Pathways, selected by FDR and sorted by FoldEnrichment and using the  
692 synapse specific database SynGO<sup>55</sup> against the “brain expressed” background, setting medium  
693 stringency and second level terms as labels for Cellular Component representation and top levels  
694 terms as labels for Biological Pathways representation (**Supplementary Figure 3**).

695

### 696 **GTPase assays**

697 A colorimetric assay was used to quantify GTPase activity of the different mCer-Dyn1 mutants<sup>56</sup>.  
698 HEK293T cells transfected with mCer-Dyn1 plasmids were harvested 48 h after transfection with a  
699 1:1 ratio of Lipofectamine2000 to plasmid. The cells were resuspended in 1 ml of sucrose lysis buffer  
700 (250 mM sucrose, 3 mM imidazole pH 7.4 supplemented with 2 µl/ml protease inhibitors and 1 mM  
701 phenylmethane sulfonyl fluoride) and mechanically broken using a primed ball-bearing cell cracker  
702 (EMBL, Heidelberg, Germany). Anti-GFP VHH coupled to agarose beads for immunoprecipitation of  
703 GFP-fusion proteins (GFP-Trap; ChromoTek GmbH, Germany; gta-20) was used for  
704 immunoprecipitation of mCer, mCer-Dyn1WT or mutant mCer-Dyn1 according to manufacturer’s  
705 instructions. A Bradford (Applichem, Germany; A6932) assay was performed according to  
706 manufacturer’s instructions to determine protein concentration of GFP-Trap-bound mCer or mCer-  
707 Dyn1. GFP-Trap-bound mCer-Dyn1 mutants were diluted to a concentration of 1 µM in GTPase assay  
708 buffer (20 mM HEPES pH 7.5, 50 mM KCl - this low salt concentration allows for the oligomerisation  
709 of dynamin<sup>57</sup>, 2 mM MgCl<sub>2</sub>). For each reaction, 20 µl of 2 mM GTP diluted in GTPase assay buffer  
710 and 20 µl of 1 µM stock of mCer-Dyn1 was incubated for 30 min at 37°C after which 0.5 M EDTA pH  
711 8.0 was added to terminate the reaction. 300 µl of filtered Malachite green solution (34 mg  
712 Malachite green carbinol base dissolved in 40 ml of 1 N HCl added to 1 g of ammonium molybdate  
713 tetrahydrate diluted in 14 mL of 4 N HCl up to 100 mL with ddH<sub>2</sub>O) was added to each reaction. The

714 change in colour of malachite green was quantified using a plate reader to measure the absorbance  
715 at 650 nm. The amount of inorganic phosphate released was calculated using the standard curve.

716

### 717 **Acute slice preparation**

718 Horizontal hippocampal slices (350  $\mu\text{m}$ ) were prepared from *Dnm1*<sup>+/<sup>R237W</sup> and *Dnm1*<sup>+/<sup>+</sup> littermate  
719 control mice (P19-25 of either sex). Animals were culled by cervical dislocation with death confirmed  
720 by removal of the brain. Excised brains were rapidly transferred to chilled (2 – 5°C) carbogenated  
721 sucrose-modified artificial cerebrospinal fluid (saCSF in mM: NaCl 86, NaH<sub>2</sub>PO<sub>4</sub> 1.2, KCl 2.5, NaHCO<sub>3</sub>  
722 25, glucose 25, sucrose 50, CaCl<sub>2</sub> 0.5, and MgCl<sub>2</sub> 7) for 2 minutes and subsequently sliced in the same  
723 solution using a vibrating microtome (Leica VT1200S). Slices were allowed to recover for 1 hour at  
724 33°C in carbogenated standard aCSF which contained (mM): NaCl 126, KCl 3, NaH<sub>2</sub>PO<sub>4</sub> 1.2, NaHCO<sub>3</sub>  
725 25, glucose 15, CaCl<sub>2</sub> 2, and MgCl<sub>2</sub> 2.</sup></sup>

726

### 727 **Electrophysiology**

728 For recording, slices were transferred to an immersion chamber continuously perfused with  
729 standard aCSF (MgCl<sub>2</sub> 1 mM) maintained at 32°C using an in-line Peltier heater (Scientifica, Uckfield,  
730 UK). A cut was made between CA2 and CA1 (identified as the medial termination of stratum  
731 lucidum) to ablate recurrent activity. Whole-cell patch-clamp recordings were made from visually  
732 identified pyramidal neurons in the CA1 region using pulled borosilicate electrodes (4-7 M $\Omega$ ). The  
733 intracellular solution for evoked and intrinsic properties experiments consisted of (mM): K-gluconate  
734 142, KCl 4, EGTA 0.5, HEPES 10, MgCl<sub>2</sub> 2, Na<sub>2</sub>ATP 2, Na<sub>2</sub>GTP 0.3, and Na<sub>2</sub>-creatine 10. For miniature  
735 excitatory post-synaptic current (mEPSC) recordings, a caesium-based intracellular solution was used  
736 (mM): Cs-gluconate 140, CsCl 3, EGTA 0.2, HEPES 10, QX-314 chloride 5, MgATP 2, NaATP 2, Na<sub>2</sub>GTP  
737 0.3, and phosphocreatine 10. Excitatory currents were recorded in the presence of picrotoxin (50  
738  $\mu\text{M}$ ) with cells voltage-clamped at -70 mV, inhibitory currents were recorded in the presence of  
739 CNQX (10  $\mu\text{M}$ ) and D-AP5 (50  $\mu\text{M}$ ) with cells voltage-clamped at -10 mV. A further addition of TTX  
740 (300 nM) for mPSC recording. For experiments with BMS-204352, the drug was dissolved in DMSO  
741 and TWEEN® 80 before adding to standard aCSF. Final drug concentration was 30  $\mu\text{M}$ , with the  
742 vehicles both at 0.03 % v/v.

743

744 Recording protocols: Intrinsic properties were recorded in current-clamp mode. All other recordings  
745 were made under voltage-clamp. Currents were low pass filtered at 3–10 kHz and sampled at 10-20  
746 kHz, using Clampex 10 software (pClamp 10, Molecular Devices, San Jose, USA). For evoked  
747 recordings, Schaffer collaterals were stimulated with a patch electrode (~1–2 M $\Omega$ ) filled with aCSF



748 and positioned in stratum radiatum, connected to an isolated constant current stimulator (Digitimer,  
749 Welwyn Garden City, UK). In all cases, the stimulus intensity was set to evoke a current of ~200 pA  
750 following a 50  $\mu$ s pulse. Stimulus was delivered at either: paired pulses (interval 10 – 500 ms, pairs  
751 30 s apart), or long trains (either 10 or 40 Hz for 15 s, four repeats delivered 4 minutes apart). Data  
752 were analysed offline using either the open source Stimfit software package (intrinsic properties) or  
753 Clampfit from the pClamp 10 software suite (all EPSCs). Cells were excluded from analysis if series  
754 resistance varied by more than 20% during recording.

755

756 RRP size and its replenishment were determined using approaches described in <sup>23</sup>. Briefly RRP was  
757 calculated by plotting the cumulative EPSC amplitude from 40 Hz 15 s trains, and performing a linear  
758 regression on the last 1 s of that plot. The y-intercept of this regression line denotes RRP size (**Figure**  
759 **5g**). Replenishment rate is represented by the slope of the regression line. Pr was calculated as  
760 amplitude of the first evoked EPSC divided by the effective RRP size <sup>58</sup>.

761 For quantification of IPSC PPR, the amplitude of the second response was measured from the lowest  
762 point immediately following the second stimulation artefact. This was because the typical decay  
763 kinetics of inhibitory responses meant that the response had not returned to baseline prior to the  
764 onset of the second stimulus.

765

### 766 **Surgery for *in vivo* electrophysiology**

767 *Dnm1*<sup>+/<sup>R237W</sup></sup> and *Dnm1*<sup>+/<sup>+</sup></sup> littermate control mice of either sex aged 8 weeks were anaesthetized with  
768 isoflurane and mounted on a stereotaxic frame (David Kopf Instruments, USA). Pairs of local LFP  
769 electrodes ( $\emptyset$  = 50.8  $\mu$ m, Teflon insulated stainless steel, A-M Systems, USA) were implanted  
770 targeting dorsal hippocampus bilaterally (1.85 mm caudal, 1.25 mm lateral from bregma and 1.40  
771 mm ventral from brain surface), ventral hippocampus bilaterally (3.3 mm caudal, 3.3 mm lateral  
772 from bregma and 2.9 mm ventral from the brain surface), left motor cortex (1.55 mm caudal, 1.88  
773 mm left from bregma and on the brain surface), right somatosensory cortex (1.3 mm caudal, 2.0 mm  
774 lateral from bregma and on the brain surface) and the midline cerebellum (5.7 mm caudal, 0 mm  
775 lateral from bregma and on the brain surface). Two miniature ground screws (Yahata Neji, M1 Pan  
776 Head Stainless Steel Cross, RS Components, Northants, UK) were attached over the cerebellum (5.0  
777 mm caudal, 2 mm lateral) to serve as ground as well as three additional screws for structural  
778 support. The electrodes were attached to an electronic interface board (EIB-16, Neuralynx, USA).  
779 The electrode assemblies were fixed to the skull using a combination of UV activated cement (3M  
780 Relyx Unicem 2 Automix, Henry Schein, Gillingham, UK) and dental cement (Simplex Rapid, Kemdent,  
781 Swindon, UK).

782

### 783 ***In vivo* LFP recordings**

784 Mice were placed in 50 x 50 cm square arenas and connected for recordings to an RHD 16-channel  
785 recording headstage (Intantech, USA) through an electrical commutator (Adafruit, USA) and an  
786 acquisition board (Open Ephys, USA). LFP signals were sampled at 1 kHz and referenced to ground  
787 using OpenEphys GUI (Open Ephys, USA). Mice were video-recorded during stimulation sessions at  
788 9.98 frames/s (C270 HD webcam, Logitech, USA). A 1 s light pulse from a blue LED (blue = 465 nm,  
789 Plexon, USA) mounted on each commutator was triggered by a Master-8 (AMPI) every five min to  
790 synchronize jump timestamps in video and LFP recordings.

791

### 792 **Analysis of *in vivo* LFP recordings**

793 Jump timestamps were identified by visual analysis of concurrent videos in 1 hr recordings. Between  
794 2-8 jumps per animal were analysed and values were averaged per mouse. The power spectrum,  
795 with a 1 s non-overlapping Hann window, was calculated from the dorsal hippocampus for 1 s after  
796 the start of a jump, using the SciPy Python function Periodogram. The duration of  
797 electrophysiological activity was manually measured by plotting the data with the plot function from  
798 the MNE Python package.

799

### 800 **Behavioural experiments**

801 For the open field assay, 6- to 8-week-old *Dnm1*<sup>+/<sup>R237W</sup></sup> and *Dnm1*<sup>+/<sup>+</sup></sup> littermate controls of either sex  
802 were placed in an open field arena 50 cm x 50 cm for 30 mins for 5 consecutive days. The first day in  
803 the arena served as habituation. On days 2 (test 1) and 4 (test 2) mice received 2 mg/kg BMS-204352  
804 or vehicle (DMSO 1/80; Tween 80 1/80; 0.9% NaCl) administered by intraperitoneal injection (as  
805 described in <sup>26</sup>) in a counterbalanced manner as described in **Figure 9a**. No injections were  
806 administered on days 3 and 5 (washout). Injections were administered 20 min prior to start of  
807 experiment to ensure maximal brain BMS-204352 concentration for duration of time in open field  
808 arena. Activity was recorded at 9.89 fps from both the top view and side view of the arena using  
809 Logitech cameras (C270 HD webcam, Logitech) with up to 4 animals being recorded simultaneously  
810 in individual arenas. Jumping behaviour was scored using Behavioral Observation Research  
811 Interactive Software (BORIS v.7.9.24, University of Torino <sup>59</sup>) which allowed for each jump to be  
812 logged in time.

813

### 814 **Analysis of mouse movement and location in behavioural tasks**

815 DeepLabCut (DLC v.2.1.10.4) was used to compare the movement and position of mice<sup>60</sup>. The tail  
816 base was used for analysis, since it provided the most accurate approximation of movement and  
817 position in two-dimensions. DLC tracked the movement of the animal for the duration of each video  
818 and provided an output for its X- and Y-coordinates at every frame. A loop was used to iterate the  
819 predicted tail base coordinates in each video and calculate the distance an animal travelled between  
820 each frame. These distances were summed across frames to determine total distance covered in the  
821 30 minute experiment. Videos were then grouped based on their camera angle. For each angle, DLC  
822 was used to assign coordinates to the corners of the animal's arena, which allowed conversion of  
823 DLC units into centimetres. To determine the time an animal spent in the centre and along the walls  
824 of the arena, different camera angles were used. For each angle, the dimensions of the animal's  
825 arena were approximated to create an "outer" and an "inner" box. Each box contained half the total  
826 area of the arena. The number of tail base coordinates found within both boxes were totalled (time  
827 spent in centre), as well as those only found within the large box (time spent at edges).

828

### 829 **Statistical analysis**

830 Experimenters were blinded to the genotype of both animals and cells for all experiments and data  
831 analysis. Statistical analysis was performed using GraphPad Prism 8.4.3. Statistical analysis for paired  
832 behaviour data was analysed using IBM SPSS Statistics v29. No statistical methods were used to  
833 predetermine sample sizes and no randomization procedures were applied. Statistical tests were  
834 applied based on the distribution of the datasets measured using D'Agostino-Pearson normality test.  
835 Significance was set at ns  $P > 0.05$ , \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\*  $P < 0.0001$ . Mann-Whitney  
836 (two-tailed), Wilcoxon matched-pairs signed rank (two-tailed), and Kruskal-Wallis with Dunns *post-*  
837 *hoc* tests were used to compare non-Gaussian data sets. Student's t test (two-tailed) and analyses of  
838 variance (ANOVA) followed by Dunnett's *post-hoc* test were used to compare normally distributed  
839 data sets. General linear model (repeated measures) was used to determine genotype effects,  
840 treatment effects and interactions. Bonferroni multiple comparisons test was used for multi-group  
841 comparisons where appropriate. Information about sample sizes, statistical tests used to calculate P  
842 values and the numeric values of the results are specified in figure legends and **Supplementary Table**  
843 **2**.

844

### 845 **DATA AVAILABILITY**

846 All relevant data are included in the article and/or its supplementary information files. Source data  
847 are provided within this paper. The one exception is the raw proteomic data, which is deposited on  
848 PRIDE (Project accession: PXD039667; Project title: Reversal of cell, circuit and seizure phenotypes in

849 a mouse model of DNM1 epileptic encephalopathy; Project webpage:

850 <http://www.ebi.ac.uk/pride/archive/projects/PXD039667>).

851

## 852 **CODE AVAILABILITY**

853 No custom code or software were used.

854

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1000 **AUTHOR CONTRIBUTIONS**

1001 Conceptualization, KB, MAC; Methodology, KB, KLD, MP, MS, EB, AG, ECD, MT, AGS; Formal Analysis,  
1002 KB, KLD, AG, ECD, MP, MS, AGS; Investigation, KB, KLD, AGS, MAC; Resources, MAC, MT, AGS;  
1003 Writing – Original Draft, KB, MAC; Writing – Review & Editing, all authors; Funding Acquisition, MAC,  
1004 MT, AGS.

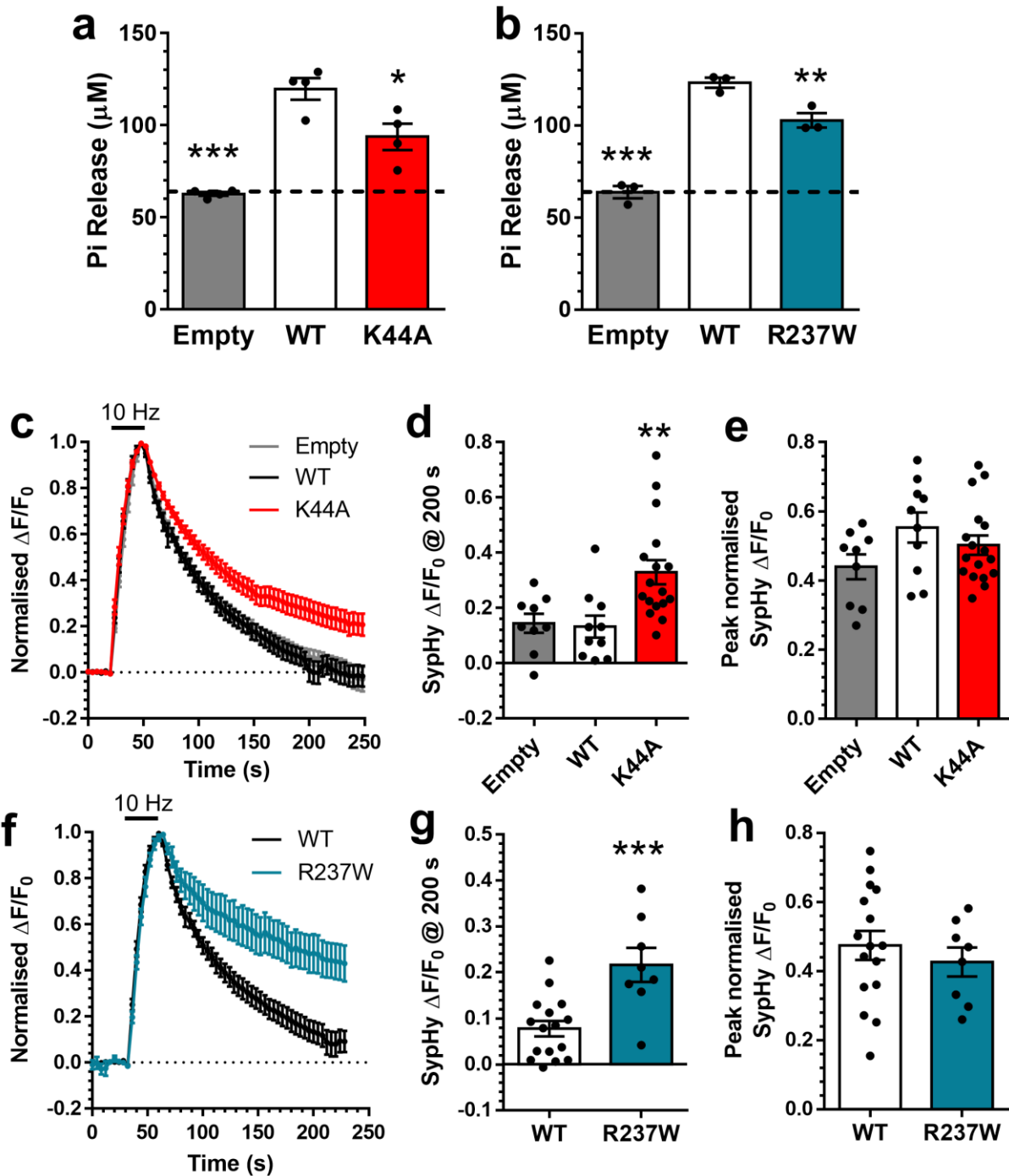
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1006 **COMPETING INTERESTS**

1007 The authors declare no competing interests.

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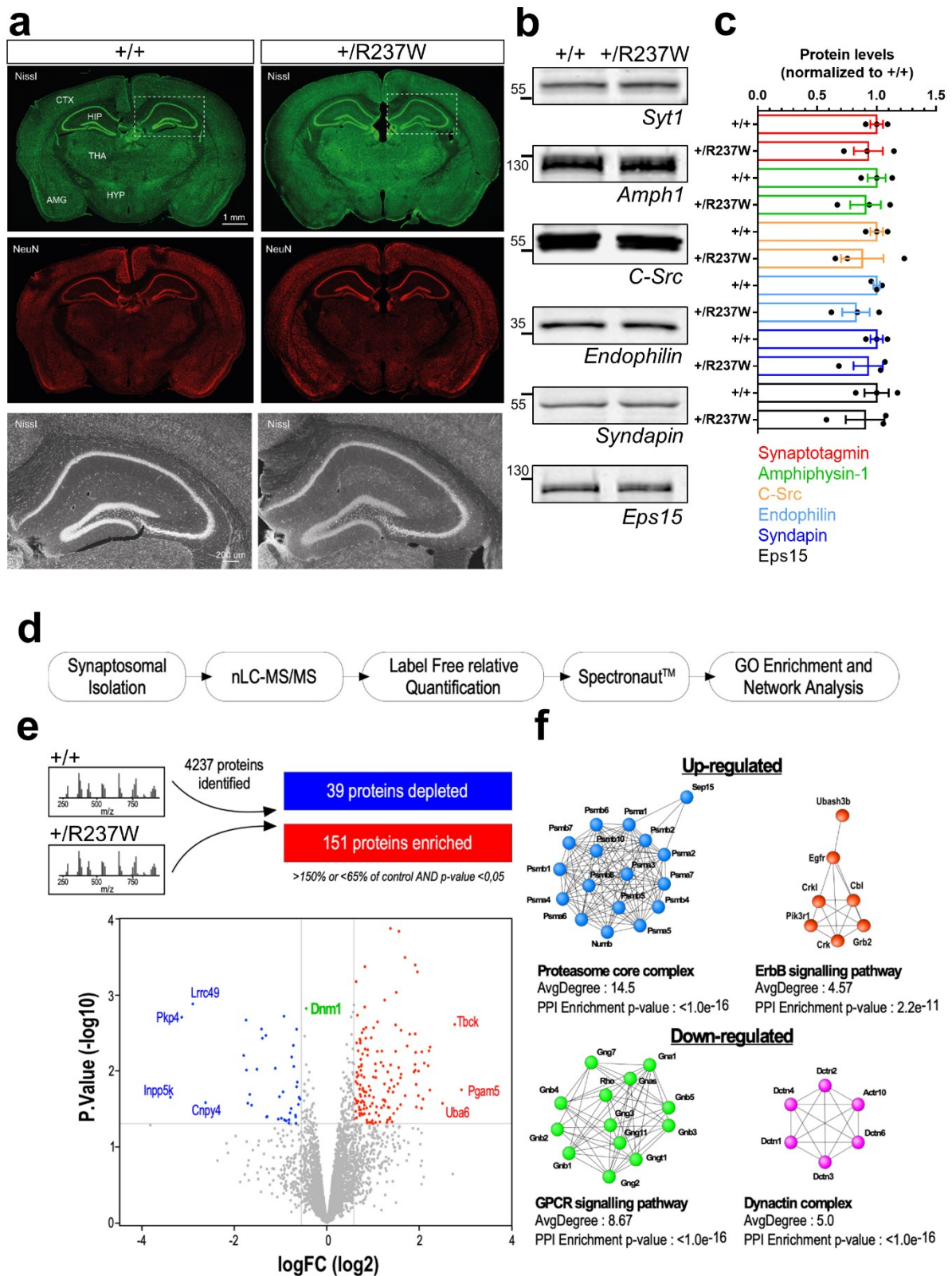
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1012 **Figure 1** – The R237W DNM1 GTPase domain mutation impairs SV endocytosis in a dominant  
 1013 negative manner. (a,b) HEK293T cells were transfected with either mCer (Empty), Dyn1<sub>WT</sub>-mCer,  
 1014 Dyn1<sub>K44A</sub>-mCer or Dyn1<sub>R237W</sub>-mCer. After 48 h the cells were lysed and mCer was  
 1015 immunoprecipitated. The GTPase activity of the immunoprecipitate is displayed as released Pi  $\pm$  SEM  
 1016 (one-way ANOVA, a all n=4 separate experiments, \*\*\*p<0.0001 WT to Empty, \*p=0.0138 WT to  
 1017 K44A; b all n=3 separate experiments, \*\*\*p<0.0001 WT to Empty, \*\*p=0.0093 WT to R237W). (c-h)  
 1018 Primary cultures of hippocampal neurons were transfected with synaptophysin-pHluorin (sypHy) and



1019 either mCer (Empty), Dyn1<sub>WT</sub>-mCer, Dyn1<sub>K44A</sub>-mCer or Dyn1<sub>R237W</sub>-mCer between 11-13 DIV. At 13-15  
1020 DIV, cultures were stimulated with a train of 300 action potentials (10 Hz). Cultures were pulsed with  
1021 NH<sub>4</sub>Cl imaging buffer 180 s after stimulation. **(c,f)** Average sypHy response ( $\Delta F/F_0 \pm SEM$ ) normalised  
1022 to the stimulation peak. Bar indicates stimulation (**c**, n=9 Empty, n=10 Dyn1<sub>WT</sub>-mCer, n=17 Dyn1<sub>K44A</sub>-  
1023 mCer; **f**, n=16 Dyn1<sub>WT</sub>-mCer, n=8 Dyn1<sub>R237W</sub>-mCer). **(d,g)** The average level of sypHy fluorescence  
1024 ( $\Delta F/F_0 \pm SEM$ ) at 200 s (**d** one-way ANOVA, n=9 Empty, n=10 Dyn1<sub>WT</sub>-mCer, n=17 Dyn1<sub>K44A</sub>-mCer,  
1025 \*\*p=0.0046 WT to K44A; **g** Unpaired two-sided t test, n=16 Dyn1<sub>WT</sub>-mCer, n=8 Dyn1<sub>R237W</sub>-mCer,  
1026 \*\*\*p=0.0007). **(e,h)** The peak level of sypHy fluorescence ( $\Delta F/F_0 \pm SEM$ ) normalised to the NH<sub>4</sub>Cl  
1027 challenge (**e** one-way ANOVA, n=9 Empty, n=10 Dyn1<sub>WT</sub>-mCer, n=17 Dyn1<sub>K44A</sub>-mCer, all ns; **h**  
1028 Unpaired two-sided t test, n=16 Dyn1<sub>WT</sub>-mCer, n=8 Dyn1<sub>R237W</sub>-mCer, p=0.48).  
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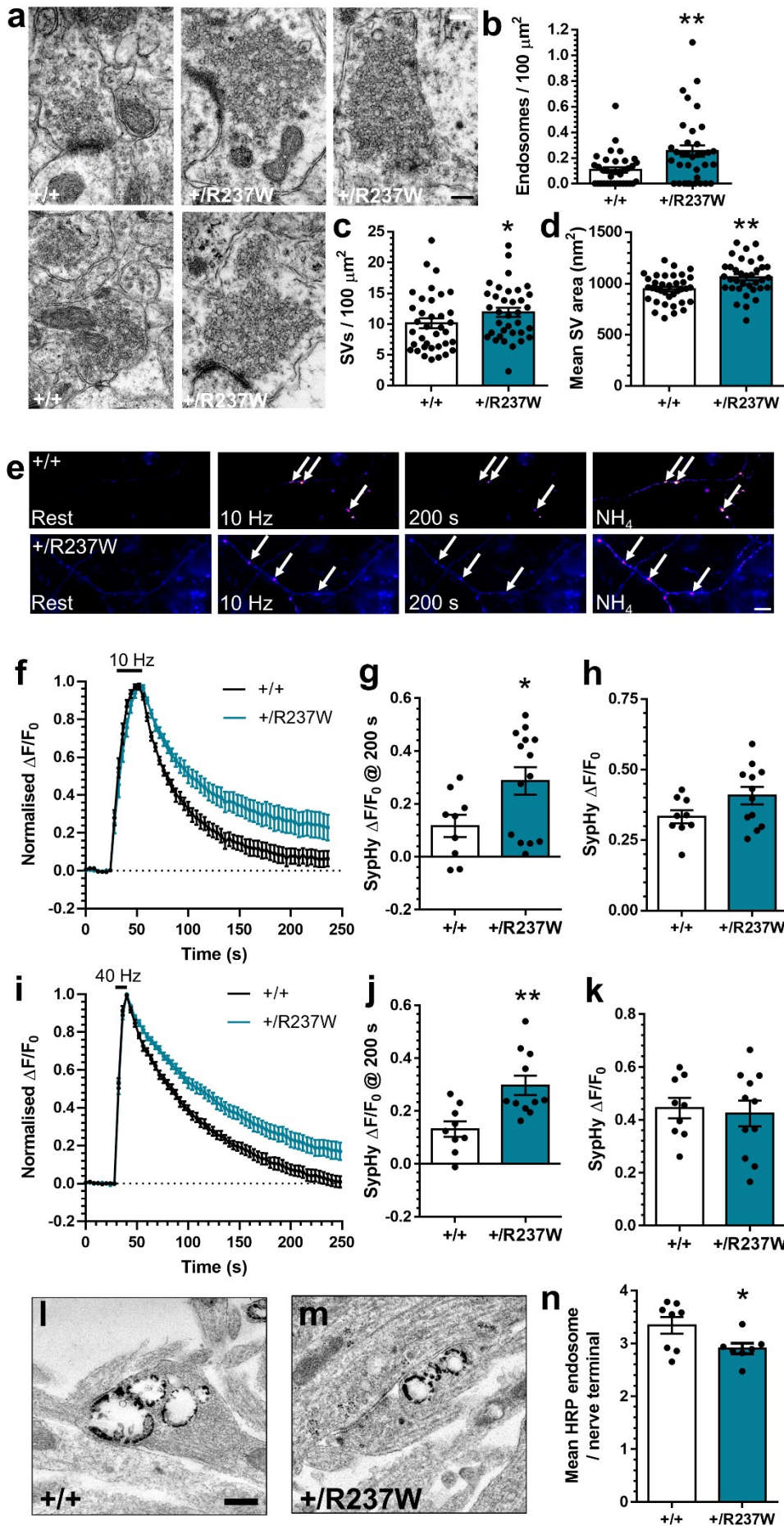
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**Figure 2 – *Dnm1*<sup>+/R237W</sup> mice display no gross abnormalities but altered protein expression.** (a) Brains from 2 month-old *Dnm1*<sup>+/+</sup> and *Dnm1*<sup>+/R237W</sup> mice were perfusion-fixed and 50  $\mu$ m brain sections were stained with Nissl and a NeuN antibody to label gross neuronal architecture. Cortex (CTX), thalamus (THA), hypothalamus (HYP), amygdala (AMG) and hippocampus (HPC) are labelled, scale

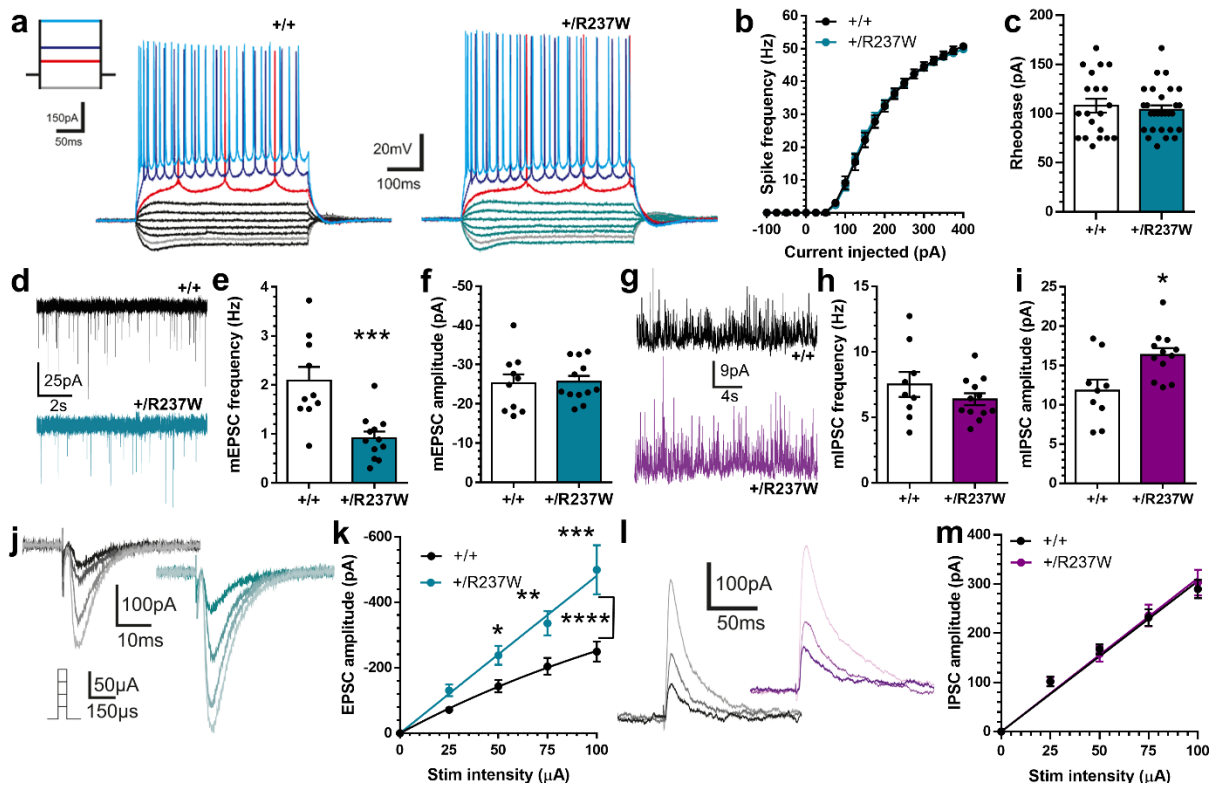
1035 bar 1 mm. Lower panels represent zoom images of the hippocampus, scale bar 200  $\mu\text{m}$ . **(b,c)** Lysates  
1036 from primary cultures of hippocampal neurons prepared from either *Dnm1*<sup>+/+</sup> and *Dnm1*<sup>+/R237W</sup>  
1037 embryos were prepared and blotted for common presynaptic proteins and dynamin-1 interaction  
1038 partners. **(b)** Representative blots displays levels of Synaptotagmin-1 (Syt1), Amphiphysin-1  
1039 (Amph1), C-src, Endophilin, Syndapin and Eps15. **(c)** Quantification of protein levels normalised to  
1040 *Dnm1*<sup>+/+</sup>  $\pm$  SEM (n=3 independent cultures for all, all ns, two-sided Mann-Whitney test). **(d,e)**  
1041 Workflow of quantitative proteomic analysis. Total protein content was cleaned onto SDS-PAGE gel  
1042 before tryptic digestion. Proteins were analysed by high-resolution tandem MS, with significant  
1043 differences revealed using a two-sided unpaired t test corrected for multiple comparisons. **(e)**  
1044 Volcano plot displays 4237 quantified proteins, 39 which were depleted in *Dnm1*<sup>+/R237W</sup> (blue), while  
1045 151 were enriched (red). *Dnm1* level is displayed in green. **(f)** STRING network analysis of up and  
1046 down regulated proteins in *Dnm1*<sup>+/R237W</sup> synaptosomes. The resulting sub-complexes were subjected  
1047 to MCL clustering at granularity 4, which results in 18 clusters including 2 clusters with an average  
1048 superior to 4 for the up-regulated proteins (proteasome core complex, ErbB signaling pathways) and  
1049 6 clusters including 2 clusters with an average superior to 4 for the down-regulated proteins (GPCR  
1050 signaling pathway, dynactin complex). Source data are provided as a Source Data file.

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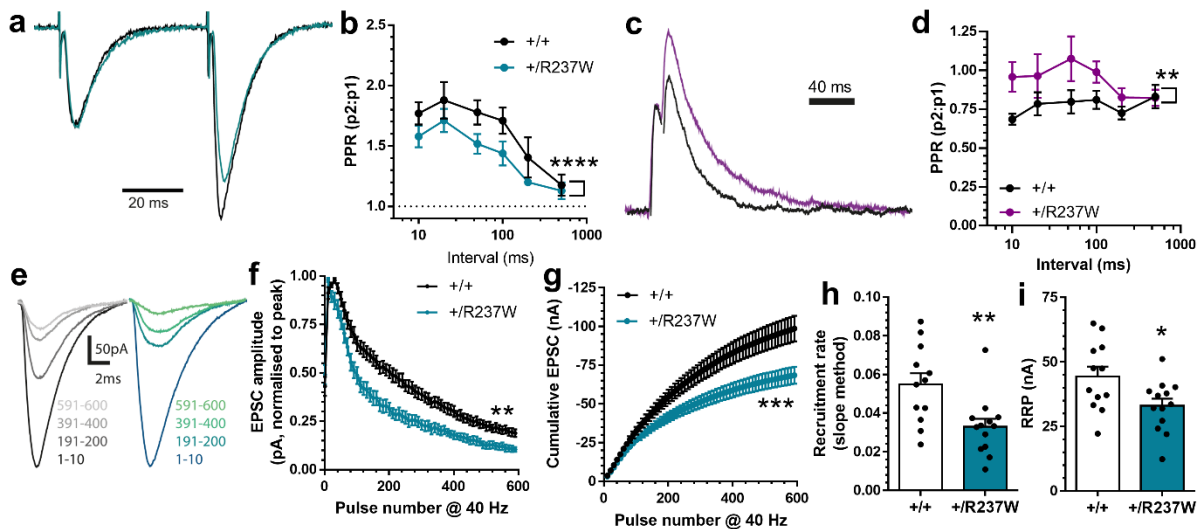
1054 **Figure 3** – *Dnm1*<sup>+/R237W</sup> neurons display dysfunctional SV endocytosis. **(a)** Brains from 2 month-old  
1055 *Dnm1*<sup>+/+</sup> and *Dnm1*<sup>+/R237W</sup> mice were perfusion fixed and processed for electron microscopy.  
1056 Representative images reveal enlarged endosomes in *Dnm1*<sup>+/R237W</sup> excitatory hippocampal nerve  
1057 terminals, scale bar 250 nm. The number of presynaptic SVs **(b)** and endosomes **(c)** and size **(d)** of  
1058 SVs were quantified ± SEM (n=36 profiles *Dnm1*<sup>+/+</sup>, n=35 *Dnm1*<sup>+/R237W</sup>, **b** p=0.005, **c** p=0.044 Mann-  
1059 Whitney test, **d** p=0.0024 Unpaired two-sided t test). **(c-i)** Primary cultures of hippocampal neurons  
1060 prepared from either *Dnm1*<sup>+/+</sup> and *Dnm1*<sup>+/R237W</sup> embryos were transfected with synaptophysin-  
1061 pHluorin (sypHy) between 7-9 DIV. At 13-15 DIV, cultures were stimulated with a train of either **(f-h)**  
1062 300 action potentials (10 Hz) or **(i-k)** 400 action potentials (40 Hz). Cultures were pulsed with NH<sub>4</sub>Cl  
1063 imaging buffer 180 s after stimulation. **(e)** Representative images of the sypHy response in *Dnm1*<sup>+/+</sup>  
1064 and *Dnm1*<sup>+/R237W</sup> neurons are displayed at Rest, during 10 Hz stimulation, at 200 s and during NH<sub>4</sub>Cl.  
1065 Arrows indicate responsive nerve terminals. Scale bar 10 μm. **(f,i)** Average sypHy response ( $\Delta F/F_0 \pm$   
1066 SEM) normalised to the stimulation peak **(f, n=9 *Dnm1*<sup>+/+</sup>, n=12 *Dnm1*<sup>+/R237W</sup>; i, n=9 *Dnm1*<sup>+/+</sup>, n=11**  
1067 ***Dnm1*<sup>+/R237W</sup>**). **(g,j)** Average level of sypHy fluorescence ( $\Delta F/F_0 \pm$  SEM) at 200 s **(g Two-sided Mann-**  
1068 **Whitney test, n=9 *Dnm1*<sup>+/+</sup>, n=12 *Dnm1*<sup>+/R237W</sup> \*p=0.045; j Unpaired two-sided t test, n=9 *Dnm1*<sup>+/+</sup>,**  
1069 **n=11 *Dnm1*<sup>+/R237W</sup> \*\*p=0.003). **(h,k)** Peak level of sypHy fluorescence ( $\Delta F/F_0 \pm$  SEM) normalised to  
1070 the NH<sub>4</sub>Cl challenge **(h Unpaired two-sided t test, n=9 *Dnm1*<sup>+/+</sup>, n=12 *Dnm1*<sup>+/R237W</sup> p=0.237; k**  
1071 **Unpaired two-sided t test, n=9 *Dnm1*<sup>+/+</sup>, n=11 *Dnm1*<sup>+/R237W</sup> p=0.751). **(l-n)** *Dnm1*<sup>+/+</sup> and *Dnm1*<sup>+/R237W</sup>  
1072 neurons were stimulated with a train of 400 action potentials (40 Hz) in the presence of 10 mg/ml  
1073 HRP. Representative images display HRP-labelled endosomes in *Dnm1*<sup>+/+</sup> **(l)** and *Dnm1*<sup>+/R237W</sup> **(m)**  
1074 nerve terminals, scale bar 250 nm. **(n)** Average number of HRP-labelled endosomes per nerve  
1075 terminal ± SEM (Unpaired two-sided t test, n=8 *Dnm1*<sup>+/+</sup>, n=7 *Dnm1*<sup>+/R237W</sup> \* p=0.039). Source data  
1076 are provided as a Source Data file.  
1077****



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1079 **Figure 4 – *Dnm1*<sup>+/*R237W*</sup> mice display dysfunctional excitatory neurotransmission.** Neurotransmission  
 1080 at CA3/CA1 synapses was monitored using whole-cell patch clamp recording in acute hippocampal  
 1081 slices from *Dnm1*<sup>+/*+*</sup> and *Dnm1*<sup>+/*R237W*</sup> mice. (a) Representative voltage responses of CA1 pyramidal  
 1082 neurons in response to 500 ms hyper/depolarizing current injections, with average spike frequency  
 1083 (b) and rheobase (c) ± SEM (n=20 *Dnm1*<sup>+/*+*</sup>, n=28 *Dnm1*<sup>+/*R237W*</sup>, b Two-way ANOVA, p=0.997, c  
 1084 Unpaired two-sided t test, p=0.613). (d) Example mEPSC events. Average frequency (e) and  
 1085 amplitude (f) of mEPSC events ± SEM (Two-sided Mann-Whitney test, n=10 *Dnm1*<sup>+/*+*</sup>, n=12  
 1086 *Dnm1*<sup>+/*R237W*</sup>, b p=0.0008, c p=0.665). (g) Example mIPSC events. Average frequency (h) and  
 1087 amplitude (i) of mIPSC events ± SEM (Two-sided Mann-Whitney test, n=9 *Dnm1*<sup>+/*+*</sup>, n=12  
 1088 *Dnm1*<sup>+/*R237W*</sup>, h p=0.379, i p=0.023). (j-m) Acute hippocampal slices were stimulated at a range of  
 1089 intensities (25, 50, 75, and 100 μA, 3 repeats at each intensity, frequency 0.05 Hz) in a pseudo  
 1090 random order. Representative traces (j) and evoked EPSC amplitude ± SEM (k) is displayed (Two-way  
 1091 ANOVA with Fishers LSD, n=34 *Dnm1*<sup>+/*+*</sup>, n=39 *Dnm1*<sup>+/*R237W*</sup>, \*\*\*\*p=0.018, \*p=0.043 50 μA, \*\*p=0.005  
 1092 75 μA, \*\*\*p=0.0004 100 μA). Representative traces (l) and evoked IPSC amplitude ± SEM (m) are  
 1093 displayed (Two-way ANOVA with Fishers LSD, n=36 *Dnm1*<sup>+/*+*</sup>, n=40 *Dnm1*<sup>+/*R237W*</sup>, overall and all  
 1094 pairwise comparisons p>0.92).

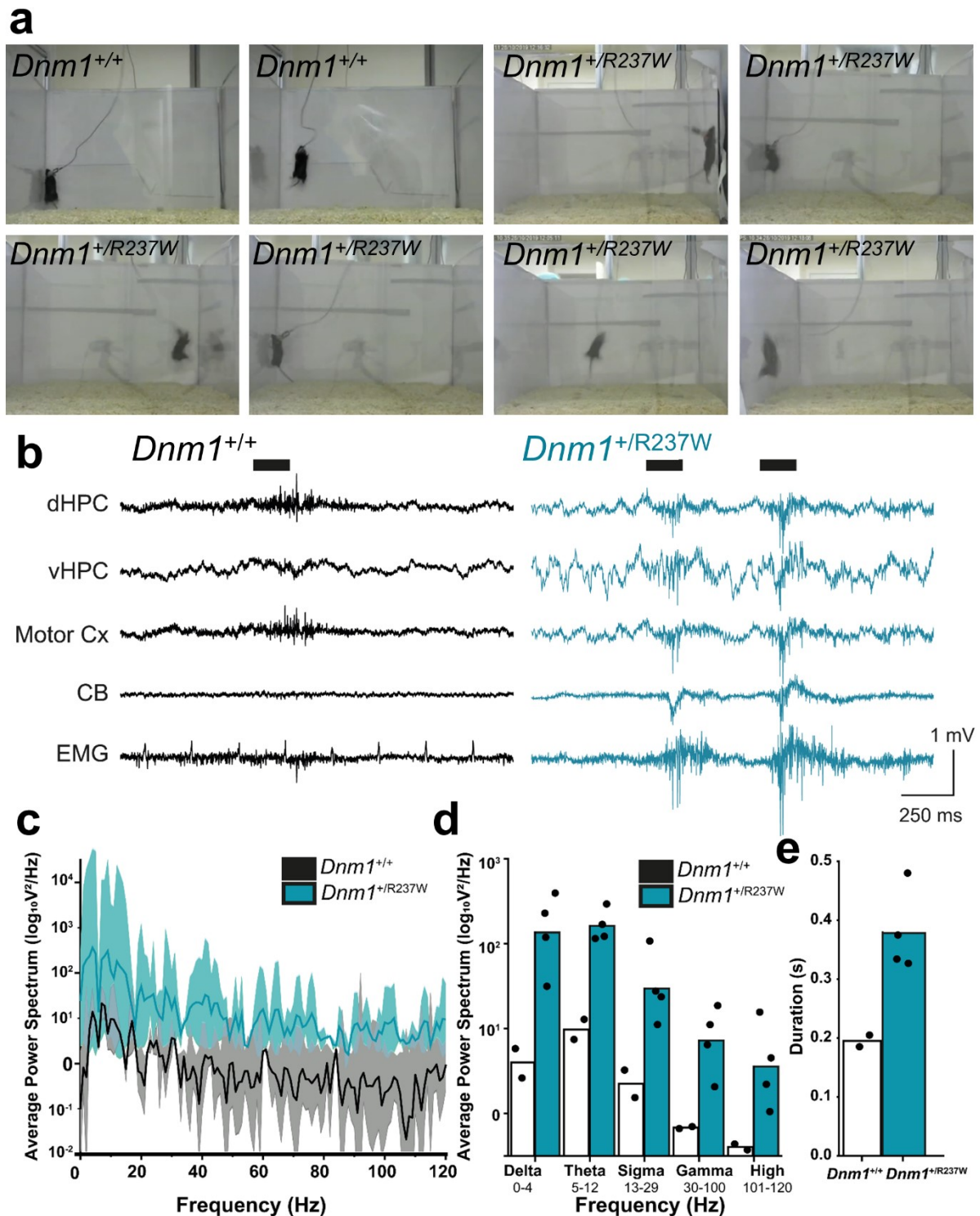
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1097 **Figure 5 – *Dnm1*<sup>+/R237W</sup> mice display altered short-term plasticity.** Neurotransmission at CA3/CA1  
 1098 synapses was monitored using whole-cell patch clamp recording in acute hippocampal slices from  
 1099 *Dnm1*<sup>+/+</sup> and *Dnm1*<sup>+/R237W</sup> mice. (a,b) Paired pulse ratio (PPR) of evoked EPSCs as a function of the  
 1100 inter-stimulus interval (10-500 ms) ± SEM (Two-way ANOVA with Fishers LSD, n=11 *Dnm1*<sup>+/+</sup>, n=10  
 1101 *Dnm1*<sup>+/R237W</sup>, \*\*\*\*p<0.0001). (c,d) PPR of evoked IPSCs as a function of the inter-stimulus interval  
 1102 (10-500 ms) ± SEM (Two-way ANOVA with Fishers LSD, n=9 *Dnm1*<sup>+/+</sup>, n=13 *Dnm1*<sup>+/R237W</sup>, \*\*p=0.0018).  
 1103 (e-g) Slices were stimulated with 600 APs (40 Hz). Representative traces (e) are shown as averages of  
 1104 10 consecutive responses from the pulse ranges stated. Average evoked EPSC amplitude (f)  
 1105 normalized to peak response is displayed ± SEM (Two-way ANOVA, n=12 *Dnm1*<sup>+/+</sup>, n=13 *Dnm1*<sup>+/R237W</sup>,  
 1106 \*\*p<0.0043). (g) Linear regression on the last 1 s of the cumulative EPSC plot in f ± SEM (Two-way  
 1107 ANOVA, \*\*\*\*p<0.0001). (h,i) The average rate of readily releasable pool (RRP) replenishment (h) and  
 1108 mean RRP size (i) ± SEM were estimated from the linear regression plot in g (h Two-sided Mann-  
 1109 Whitney test, \*\*p=0.0045; i Unpaired two-sided t test, \*p=0.0229). Source data are provided as a  
 1110 Source Data file.

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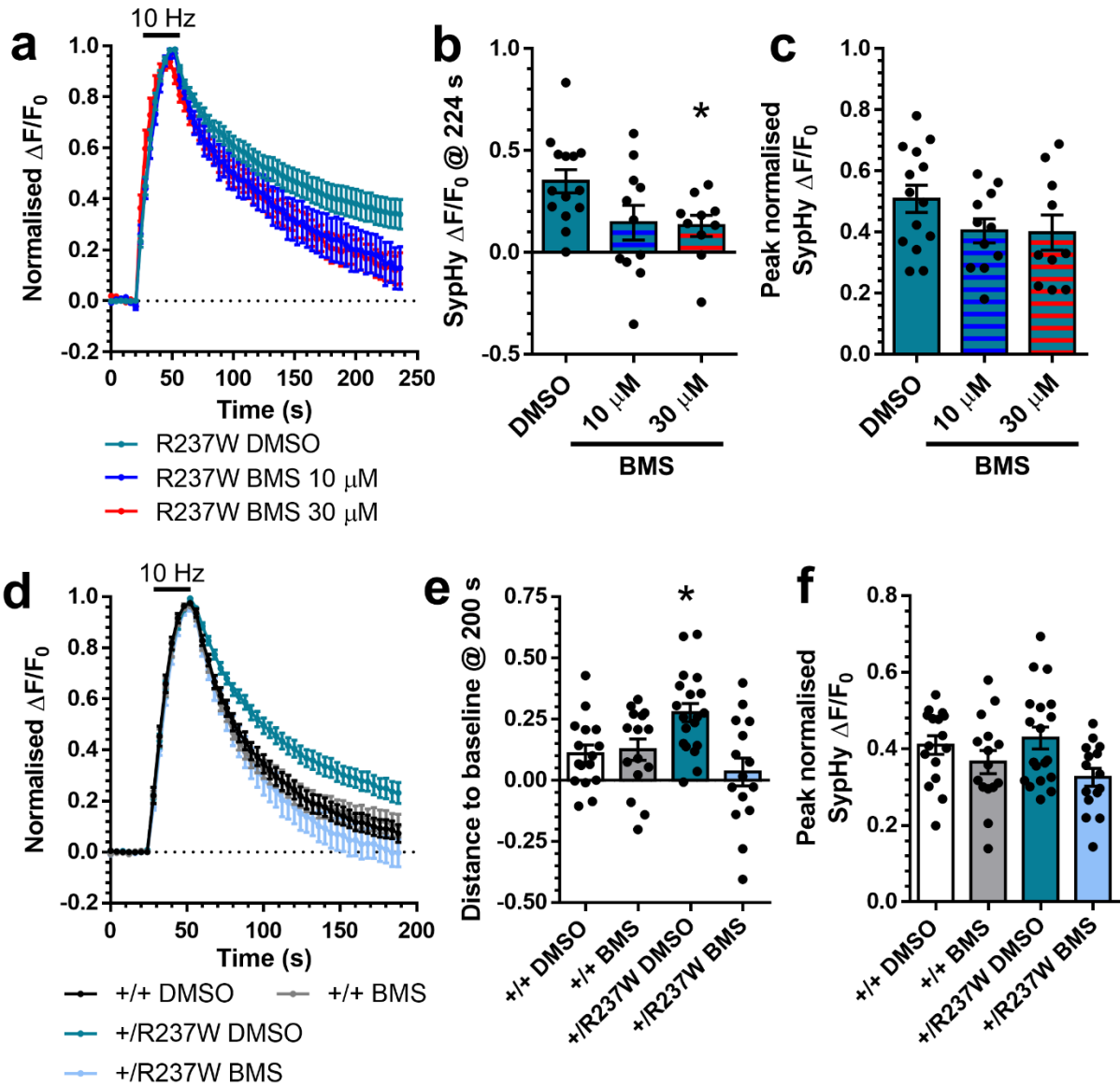
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**Figure 6** – *Dnm1*<sup>+/R237W</sup> mice display myoclonic jumping seizure-like activity. (a) Representative still images displaying the typical jumping behaviour of both *Dnm1*<sup>+/+</sup> and *Dnm1*<sup>+/R237W</sup> mice. *Dnm1*<sup>+/+</sup> mice occasionally jumped, however *Dnm1*<sup>+/R237W</sup> mice displayed stereotypical and burst-like events. (b) Example traces of *in vivo* LFP and electromyogram (EMG) recordings from dorsal hippocampus (dHPC, ventral hippocampus (vHPC), motor cortex (Motor Cx) and cerebellum (CB) during jumping activity in *Dnm1*<sup>+/+</sup> and *Dnm1*<sup>+/R237W</sup> mice. (c) Power spectrum estimate across all jump epochs. Lines



1119 indicate mean values  $\pm$  SEM. **(d)** Plot of average power in commonly used frequency bands during  
1120 jumps. Bars indicate mean from  $n=2$  *Dnm1*<sup>+/+</sup> and  $n=4$  *Dnm1*<sup>+/R237W</sup>. **(e)** Plot of average duration of  
1121 electrographical activity associated with jumps. Bars indicate mean from  $n=2$  *Dnm1*<sup>+/+</sup> and  $n=4$   
1122 *Dnm1*<sup>+/R237W</sup>. Source data are provided as a Source Data file.  
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1125 **Figure 7** – BMS-204352 corrects dominant negative effect of R237W mutation on SV endocytosis. (a-

1126 c) Primary cultures of hippocampal neurons prepared from *Dnm1*<sup>+/+</sup> embryos were transfected with

1127 synaptophysin-pHluorin (sypHy) and Dyn1<sub>R237W</sub>-mCer between 11-13 DIV. At 13-15 DIV, cultures

1128 were stimulated with a train of 300 action potentials (10 Hz) in the presence of either 10  $\mu$ M or 30

1129  $\mu$ M BMS-204352 or a vehicle control (DMSO). Cultures were pulsed with NH<sub>4</sub>Cl imaging buffer 180 s

1130 after stimulation. (a) Average sypHy response ( $\Delta F/F_0 \pm$  SEM) normalised to the stimulation peak

1131 (stimulation indicated by bar, n=14 DMSO, n=11 10  $\mu$ M, n=10 30  $\mu$ M). (b) Average level of sypHy

1132 fluorescence ( $\Delta F/F_0 \pm$  SEM) at 224 s (One-way ANOVA, n=14 DMSO, n=11 10  $\mu$ M, n=10 30  $\mu$ M,

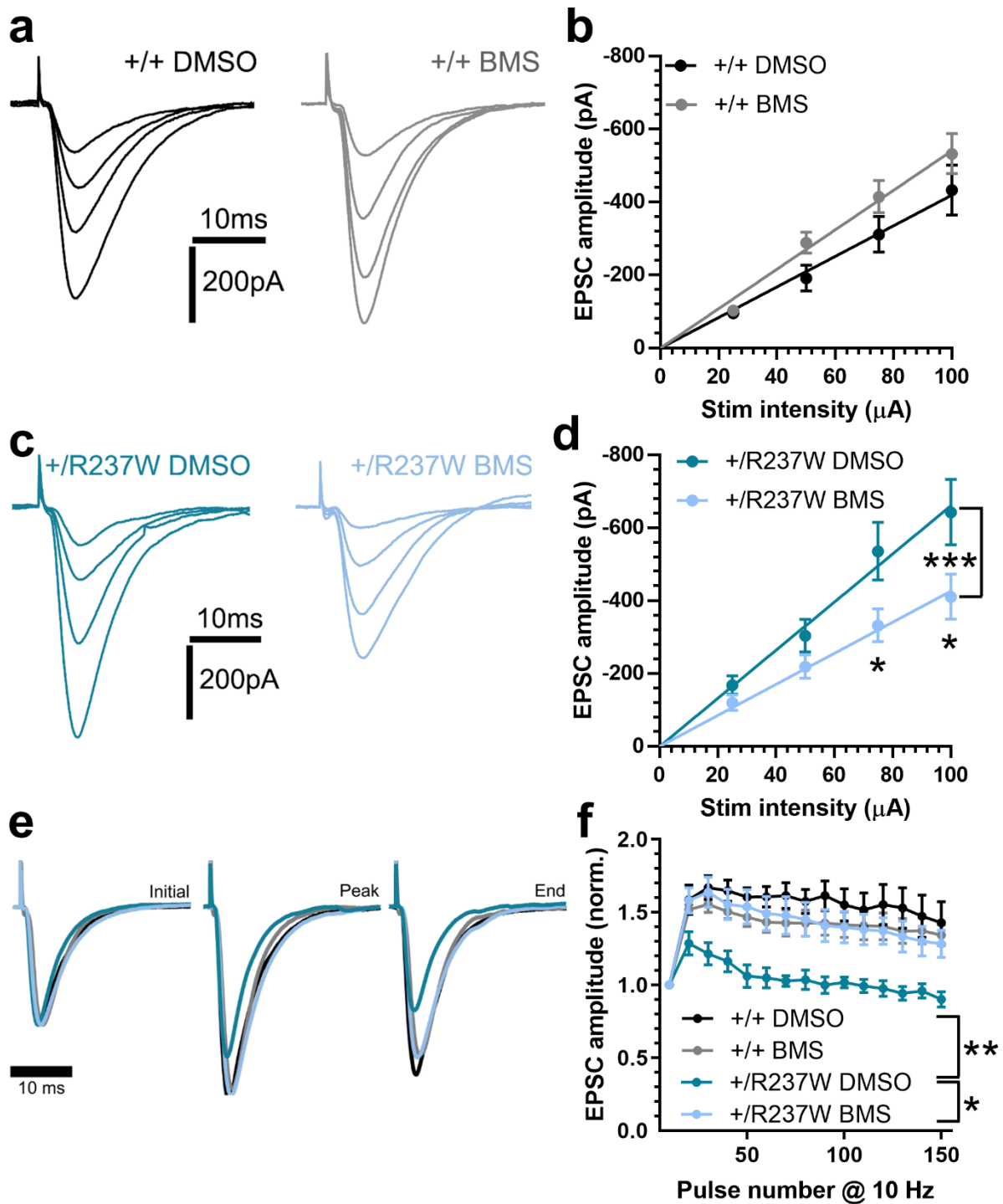
1133 \*p=0.0467 DMSO vs 30  $\mu$ M). (c) Peak level of sypHy fluorescence ( $\Delta F/F_0 \pm$  SEM) normalised to the

1134 NH<sub>4</sub>Cl challenge (One-way ANOVA, n=10 DMSO, n=8 10  $\mu$ M, n=6 30  $\mu$ M, all ns). (e-f) Primary cultures

1135 of hippocampal neurons prepared from either *Dnm1*<sup>+/+</sup> or *Dnm1*<sup>+/R237W</sup> embryos were transfected

1136 with sypHy between 7-9 DIV. At 13-15 DIV, cultures were stimulated with a train of 300 action

1137 potentials (10 Hz) in the presence of either 30  $\mu$ M BMS-204352 or a vehicle control (DMSO). Cultures  
1138 were pulsed with NH<sub>4</sub>Cl imaging buffer 180 s after stimulation (stimulation indicated by bar). **(d)**  
1139 Average sypHy response ( $\Delta F/F_0 \pm$  SEM) normalised to the stimulation peak (n=19 DMSO *Dnm1*<sup>+/+</sup>,  
1140 n=15 BMS *Dnm1*<sup>+/+</sup>, n=16 DMSO *Dnm1*<sup>+/R237W</sup>, n=15 BMS *Dnm1*<sup>+/R237W</sup>). **(e)** Average level of sypHy  
1141 fluorescence ( $\Delta F/F_0 \pm$  SEM) at 200 s (One-way ANOVA, n=19 DMSO *Dnm1*<sup>+/+</sup>, n=15 BMS *Dnm1*<sup>+/+</sup>,  
1142 n=16 DMSO *Dnm1*<sup>+/R237W</sup>, n=15 BMS *Dnm1*<sup>+/R237W</sup>, \*p=0.0183 DMSO *Dnm1*<sup>+/+</sup> vs DMSO *Dnm1*<sup>+/R237W</sup>).  
1143 **(f)** Peak level of sypHy fluorescence ( $\Delta F/F_0 \pm$  SEM) normalised to the NH<sub>4</sub>Cl challenge (One-way  
1144 ANOVA n=19 DMSO *Dnm1*<sup>+/+</sup>, n=15 BMS *Dnm1*<sup>+/+</sup>, n=16 DMSO *Dnm1*<sup>+/R237W</sup>, n=15 BMS *Dnm1*<sup>+/R237W</sup>,  
1145 all ns). Source data are provided as a Source Data file.  
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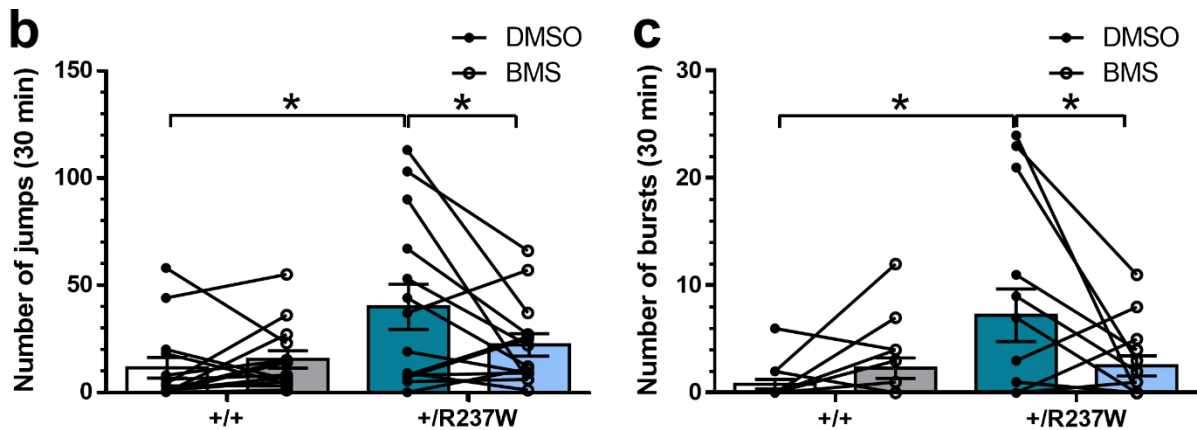
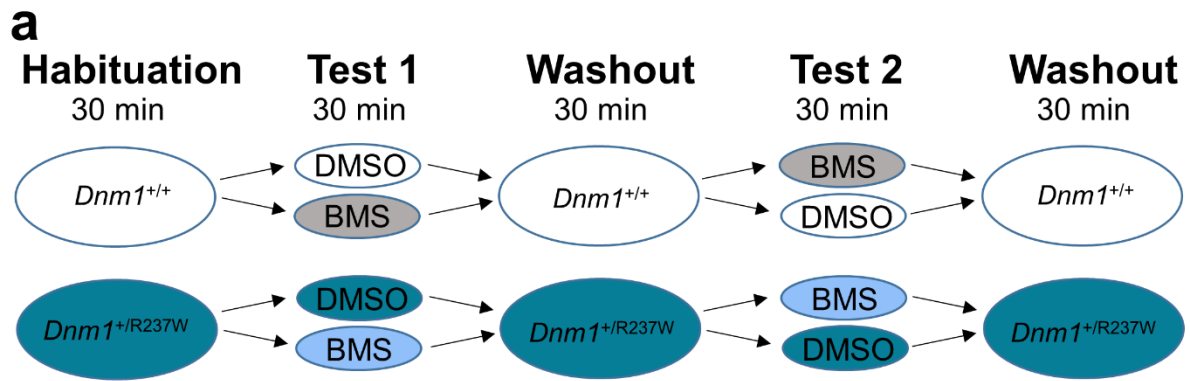


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1148 **Figure 8 – BMS-204352 corrects neurotransmission defects in  $Dnm1^{+/R237W}$  mice. (a-d)**

1149 Neurotransmission at CA3/CA1 synapses was monitored in acute hippocampal slices from either  
 1150  $Dnm1^{+/+}$  (a,b) or  $Dnm1^{+/R237W}$  (c,d) mice. Slices were stimulated at a range of intensities (25, 50, 75,  
 1151 and 100  $\mu$ A, 3 repeats at each intensity, frequency 0.05 Hz) in a pseudo random order in the  
 1152 presence of 30  $\mu$ M BMS-204352 or a vehicle control (DMSO). Representative traces are displayed for  
 1153 either  $Dnm1^{+/+}$  (a) or  $Dnm1^{+/R237W}$  (c) slices. (b,d) Evoked EPSC amplitude  $\pm$  SEM is displayed (Two-  
 1154 way ANOVA with Sidak's multiple comparison test, b n=13 DMSO, n=11 BMS, all ns; d n=13 DMSO,

1155 n=13 BMS, \*\*\*p=0.0004, \*p=0.041 75  $\mu$ A, \*p=0.0145 100  $\mu$ A). (e,f) *Dnm1*<sup>+/+</sup> or *Dnm1*<sup>+/R237W</sup>  
1156 hippocampal slices stimulated with a 10 AP train (10 Hz), in the presence of either 30  $\mu$ M BMS-  
1157 204352 or a vehicle control (DMSO). (e) Representative traces, (f) evoked EPSC amplitude for  
1158 normalized to first pulse  $\pm$  SEM. Two-way ANOVA with Dunnett's multiple comparison test, n=5  
1159 DMSO *Dnm1*<sup>+/+</sup>, n=6 BMS *Dnm1*<sup>+/+</sup>, n=5 DMSO *Dnm1*<sup>+/R237W</sup>, n=7 BMS *Dnm1*<sup>+/R237W</sup>, \*\*p=0.002 DMSO  
1160 *Dnm1*<sup>+/+</sup> vs DMSO *Dnm1*<sup>+/R237W</sup>, \*p=0.013 DMSO *Dnm1*<sup>+/R237W</sup> vs BMS *Dnm1*<sup>+/R237W</sup>. Source data are  
1161 provided as a Source Data file.  
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**Figure 9** – BMS-204352 corrects seizure-like events in *Dnm1*<sup>+/R237W</sup> mice. *Dnm1*<sup>+/+</sup> and *Dnm1*<sup>+/R237W</sup> mice were placed in an open field chamber for a 30 min period for 5 days. After habituation on day 1, mice were dosed with either BMS-204352 or a vehicle control (DMSO) on days 2 and 4, with drug washout on days 3 and 5. Delivery of drug treatment was interleaved between days 2 and 4. (a) Schematic of the experimental protocol. (b,c) Average number of myoclonic jumps ± SEM (b) or bursts ± SEM (c). (b) General linear model (repeated measures) with Bonferroni multiple comparisons n=14 for all, \* p=0.019 DMSO *Dnm1*<sup>+/R237W</sup> vs BMS *Dnm1*<sup>+/R237W</sup>, \* p=0.021 DMSO *Dnm1*<sup>+/+</sup> vs DMSO *Dnm1*<sup>+/R237W</sup>, all other ns (c) General linear model (repeated measures) with Bonferroni multiple comparisons n=14 for all, \* p=0.016 DMSO *Dnm1*<sup>+/+</sup> vs DMSO *Dnm1*<sup>+/R237W</sup> \* p=0.011 DMSO *Dnm1*<sup>+/R237W</sup> vs BMS *Dnm1*<sup>+/R237W</sup> all other ns. Source data are provided as a Source Data file.