Supplemental figure 1. Mitochondrial membrane potential stained with $DiIC_1(5)$. ***A,*** Measurement of mitochondrial membrane potential $\Delta\Psi_m$ in isolated mitochondria by a double staining method using $\Delta\Psi_m$-sensitive dye, 100 nM $DiIC_1(5)$ and $\Delta\Psi_m$-non sensitive mitochondrial marker, 100 nM NAO. Each particle was identified as a mitochondrion based on the mitochondria-selective stain with NAO in a buffer containing 20 mM glutamate plus 2 mM malate. The emission signals of NAO were plotted on the x-axis and those of DiIC1(5) were plotted on the y-axis. Each point indicates the fluorescent intensity derived from each mitochondrion under steady-state (*control*) and treatment with 1 μM FCCP (*+FCCP*) or 6.25 μM Ca^{2+} (*+*Ca^{2+}). Most of the NAO-positive particles were determined as energized mitochondria under steady-state condition. ***B,*** Frequency distributions of $DiIC_1(5)$ fluorescence. Fluorescent intensities of $DiIC_1(5)$ under steady-state condition (*control*) and treatment with 6.25 μM Ca^{2+} (*+*Ca^{2+}) are displayed on the x-axis. The y-axis represents the number of particles at the respective intensities. Treatment with Ca^{2+} increased the population of depolarized mitochondria from wild-type (*WT*) and mutPOLG Tg (*Tg*) mice. ***C***, Quantification of $\Delta\Psi_m$. The mean intensities of $DiIC_1(5)$ fluorescence were compared between wild-type (*WT*) and mutPOLG Tg (*Tg*) under steady-state condition (*control*) and treatment with Ca^{2+}(*+*Ca^{2+}) or FCCP (*+FCCP*) (n = 4 for each genotype). No significant differences were detected in the mean intensities between genotypes under any of the conditions. Data are expressed as mean ± SEM.

Supplemental figure 2. Action potential-induced $[Ca^{2+}]_i$ transient in hippocampal pyramidal neurons. ***A***, Fluorescence image of a pyramidal neuron filled with bis-fura-2 in a hippocampal slice from a wild-type (*WT*) mouse. Using simultaneous whole-cell patch clamp recording with optical imaging, $[Ca^{2+}]_i$ transients (*top* and *middle*) and voltage responses (*bottom*) were induced by ten action potentials (APs) at 5–100 Hz. Regions of

interest are indicated by white rectangles at the soma (*s*) and the apical dendrite (*d*) shown in the DIC image, respectively. ***B***, Fluorescence image of a pyramidal neuron in a hippocampal slice from a mutPOLG Tg (*Tg*) mouse (littermate pair analyzed in *A*). $[Ca^{2+}]_i$ transients and voltage responses were observed as well as those in *A* at the soma (*s*) and apical dendrite (*d*) shown in the DIC image. The arrangement of images is the same as that in *A*. ***C***, Kinetics of $[Ca^{2+}]_i$ transients in a rising phase by the APs ranging from 5–100 Hz. The peak amplitude and the rise time mesured at the soma (*left*) and the dendrite (*right*) were compared with the neurons of wild-type (*WT*) and mutPOLG Tg (*Tg*) mice (n = 8). Data expressed as mean ± SEM were plotted against firing frequency of APs.