

Juli E. Jones¹, Xie Xie², Courtney Heard¹, Sofie Ekström², Barbara Küppers-Munther², S. Jordan Kerns¹, Laleh Arabanian², Carol Geukens², Anja V. Gruszczuk², Benjamin Munro³, Camilla Koolmeister³, Roberta Filograna³, Jelena Masic³, Sirelin Sillamaa⁴, Nils-Göran Larsson³, Claes Gustafsson⁴, Maria Falkenberg⁴, Simon Giroux¹, Andrew M. Griffin¹, Thomas A. Keating¹, & Gabriel Martinez Botella¹

¹Pretzel Therapeutics, Waltham, MA, USA, ²Pretzel Therapeutics, Mölndal, Sweden, ³Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden, ⁴Department of Medical Biochemistry and Cell Biology, University of Gothenburg, Gothenburg, Sweden

ABSTRACT

Mitochondrial DNA depletion syndromes (MDDS) are severe primary mitochondrial disorders caused by reduced mitochondrial DNA (mtDNA) copy number, leading to impaired oxidative phosphorylation and multi organ failure. Mutations in DNA polymerase γ (POLG), the sole polymerase responsible for mtDNA replication, are the most common cause, with over 300 pathogenic variants identified. These disorders are associated with profound morbidity, early mortality, and multi-system symptoms, notably with predominant neurological involvement including seizures, neuropathy, cognitive impairment and ataxia, often accompanied by hepatic and muscular disease. The genetic diversity of MDDS highlights the urgent need for mutation agnostic therapies. PX578 is a first-in-class, orally bioavailable, brain penetrant small molecule activator of Poly designed to restore mtDNA replication and mitochondrial function independent of patient genotype. PX578 binds an allosteric site at the interface of the POLGA catalytic subunit and the proximal POLGB subunit, enhancing enzyme activity and processivity. In POLG patient derived fibroblasts, hepatocytes, and POLG mutant neural stem cells, PX578 increases mtDNA copy number and improves mitochondrial function. In PolgA449T/G826S compound heterozygous mice, PX578 robustly increased mtDNA across multiple tissues. Taken together, PX578 elicits robust efficacy in disease backgrounds with wild-type or POLG mutations, thereby supporting its potential as a broad disease-modifying therapy. PX578 has completed Phase 1 in healthy volunteers and is advancing to studies in individuals living with POLG disease. By directly addressing the underlying defect in mtDNA depletion, PX578 represents a novel, mechanism based, disease modifying therapeutic strategy for a broad spectrum of MDDS patients.

METHODS

mtDNA Depletion and Recovery Experiments: Fibroblasts were treated with 50 ng/mL ethidium bromide (EtBr) for 7 days (T7). After EtBr removal, cells were cultured in low serum to induce quiescence for 3 days (T7 +3), then PX578 or 0.01% DMSO vehicle was added for 14 days followed by mtDNA quantification via RT-qPCR.

Neural Stem Cells: Oxygen consumption rate (OCR) was measured using the Agilent Seahorse assay - XF Cell Mito stress test kit. Neural stem cells (NSCs) homozygous for the G848S POLG mutation were treated with PX578 or 0.01% DMSO vehicle for 10 days.

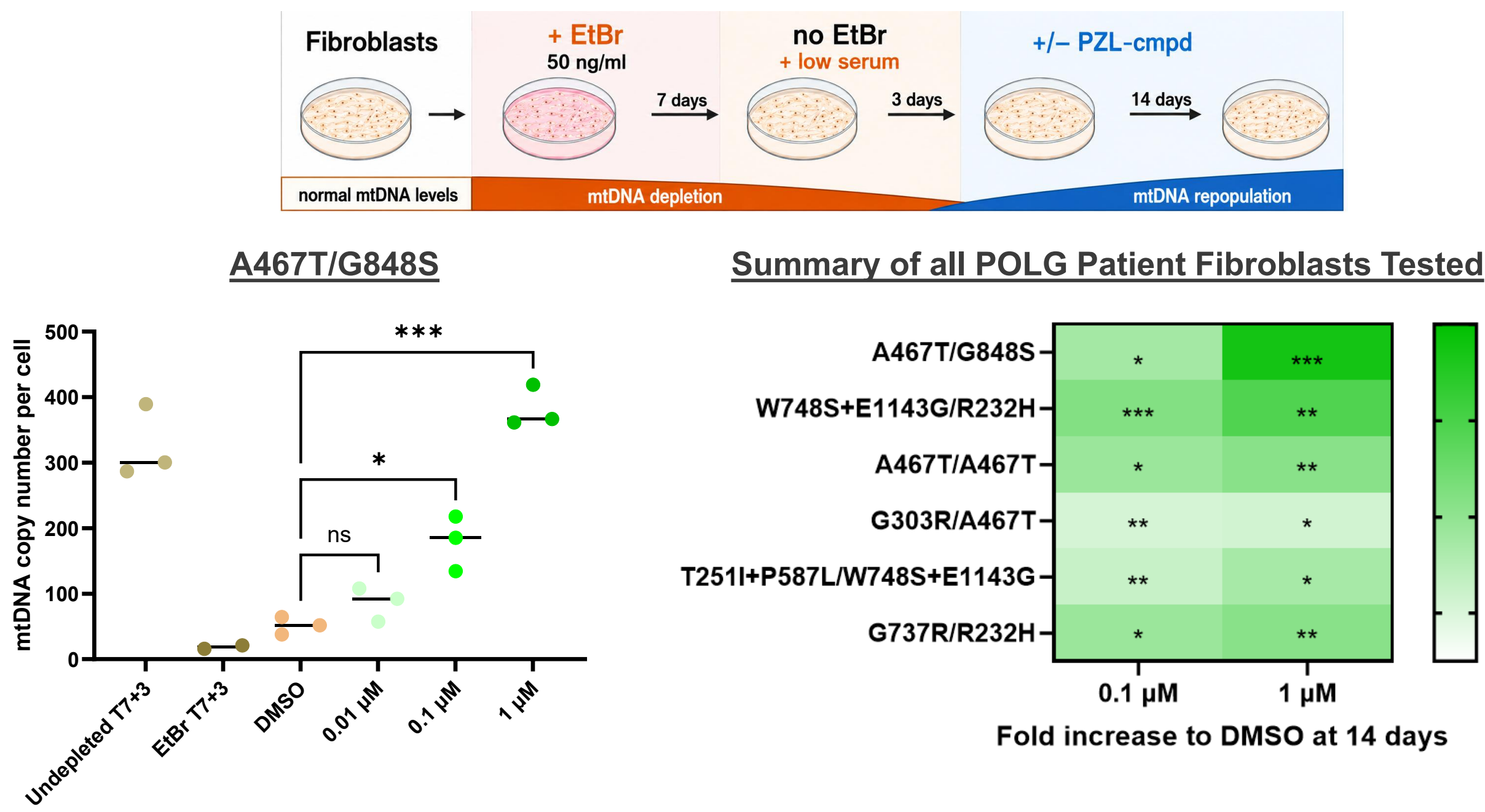
Hepatocytes: OCR was measured using the Resipher cell culture oxygen monitoring system and Albumin/AST secretion was measured using ELISA. iPSC derived hepatocyte homozygous for W748S+E1143G POLG mutation were treated with PX578 or 0.01% DMSO vehicle throughout the total 28 days of differentiation.

POLG Mouse: Twelve-week-old mice were treated with vehicle or PX578 (100 mg/kg) by daily oral gavage for 29 days. Samples were collected on Day 29, followed by mtDNA quantification via RT-qPCR.

mtDNA: Copy number was determined by a reference gene for nuclear DNA (*B2M*) and a target gene for mtDNA (*CYTB*). The relative measurement of the qPCR was transformed into absolute copy numbers by running a standard curve with plasmid DNA with known concentrations of the reference genes (*CYTB* and *B2M*). The final mtDNA copy number per cell was calculated using the following equation: (Copies of *CYTB*) *2 / (Copies of *B2M*)

RESULTS

PX578 Accelerates mtDNA Recovery in POLG Patient Fibroblasts

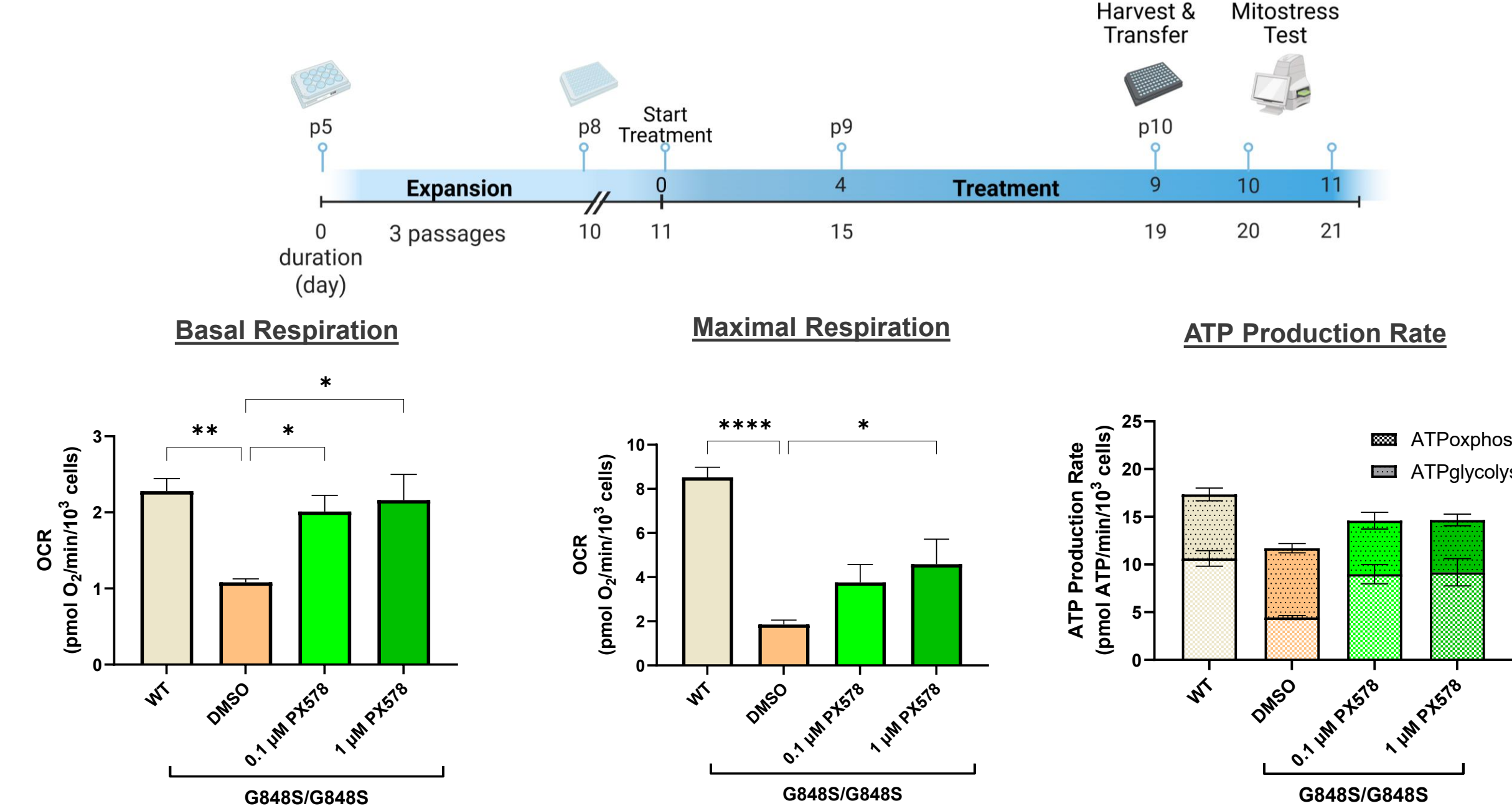


EtBr-depleted quiescent patient fibroblasts carrying POLG mutations were treated with either DMSO or 0.01, 0.1 and 1 μ M PX578 for 14 days

T7+3: 7-day depletion in proliferation phase and 3-day induction of quiescence phase without EtBr.

Data were analyzed using Brown-Forsythe and Welch one-way ANOVA where compound-treated groups were compared to the DMSO control using * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$).

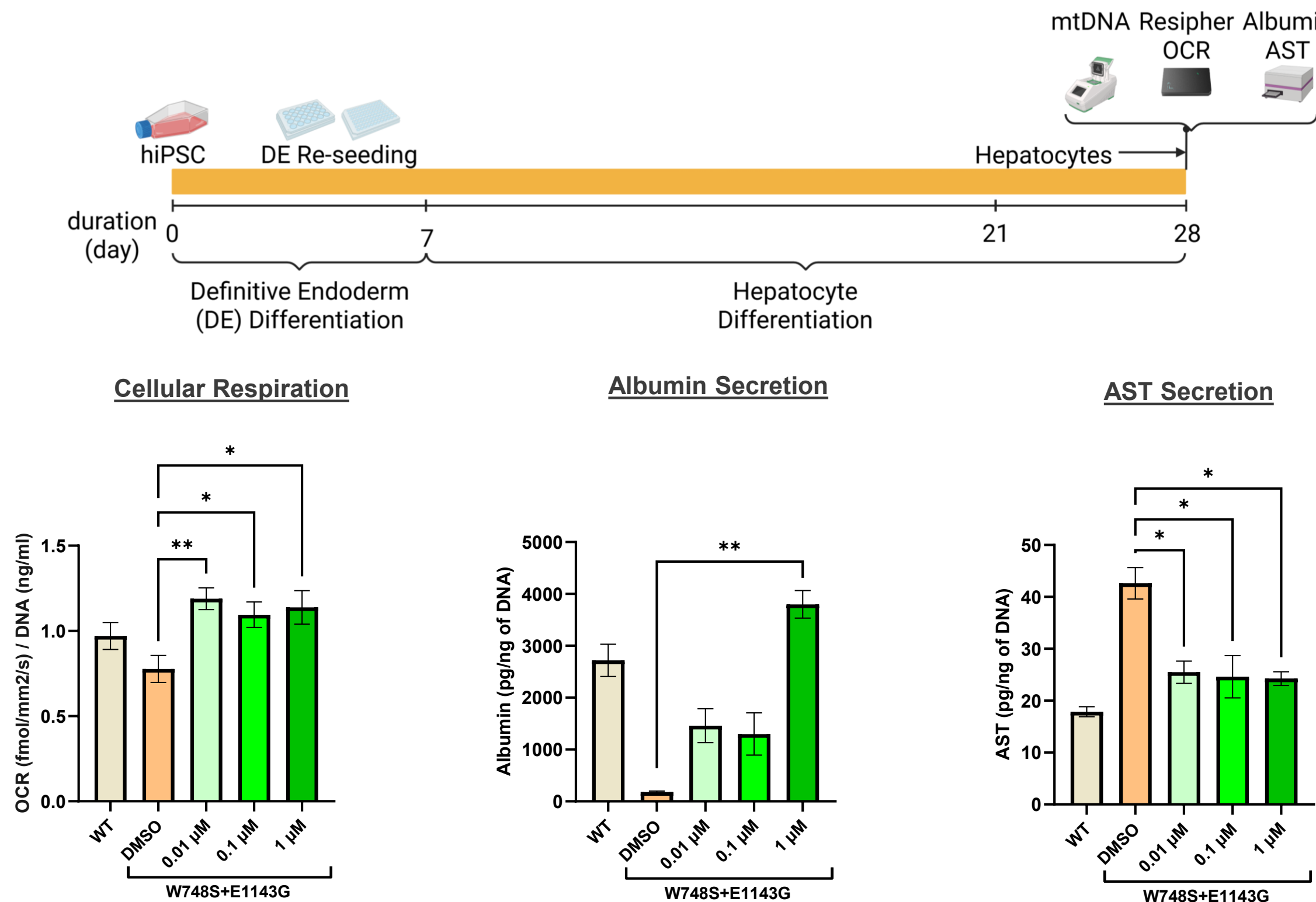
PX578 Enhances Cellular Respiration in Neural Stem Cells



Human neural stem cells homozygous for the G848S POLG mutation were cultured with 0, 0.1 and 1.0 μ M of PX578 for 10 days prior to Seahorse assay.

Data were analyzed using ordinary one-way ANOVA with multiple comparisons and Fisher's LSD test. All data graphed as mean + SEM; * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$), and **** ($P \leq 0.0001$).

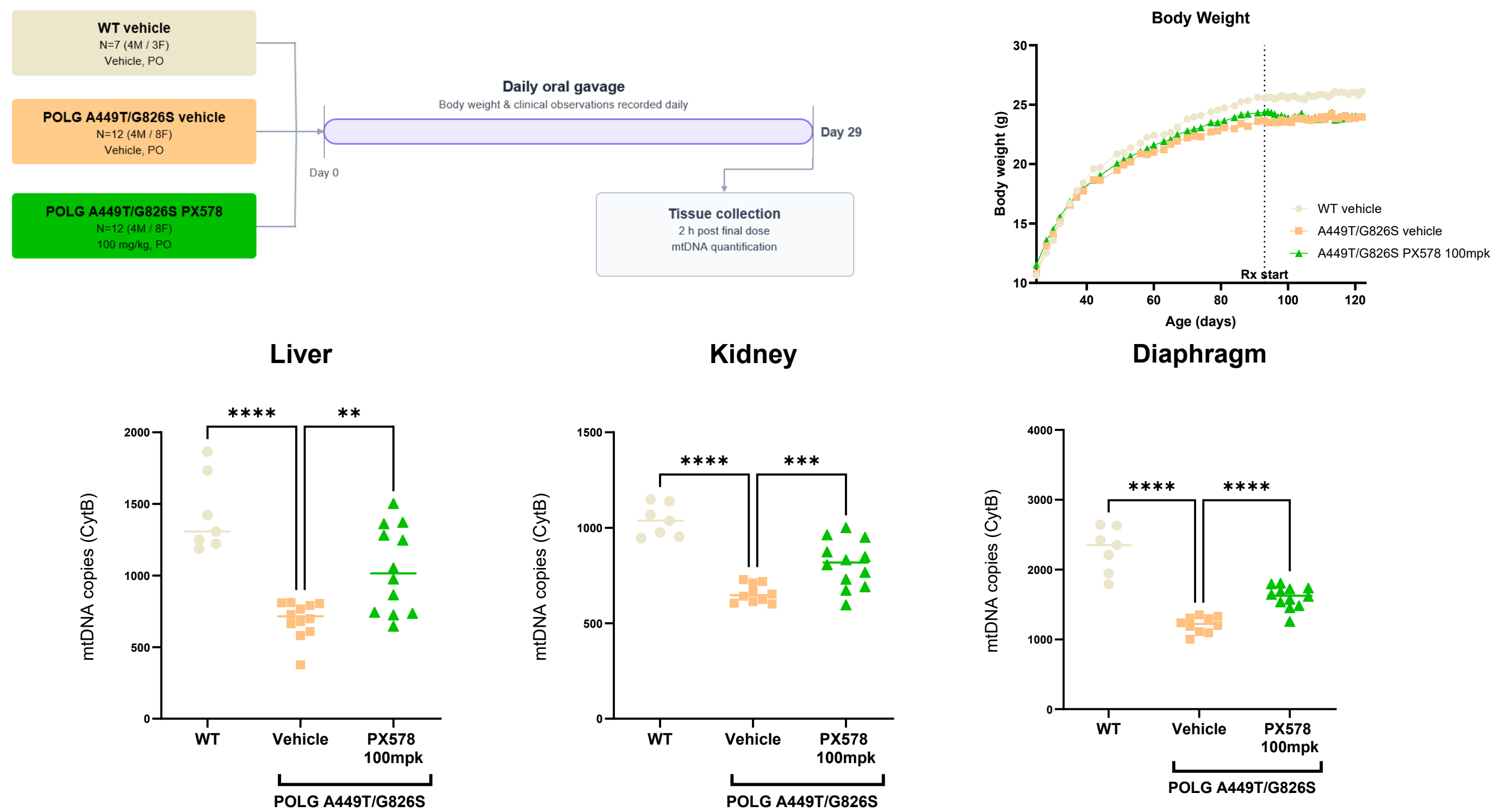
PX578 Rescues Cellular Health in iHepatocytes



Human iPSC-derived hepatocytes homozygous for the W748S +E1143G POLG mutation were cultured with 0, 0.01, 0.1 and 1.0 μ M of PX578 for 28 days prior to measuring OCR by Resipher system, albumin and AST secretion. There was no detectable change in mtDNA (data not shown).

Data were analyzed by a Brown–Forsythe test and one-way Welch’s ANOVA comparing each group to DMSO-treated cells. All data graphed as mean + SEM; * ($P \leq 0.05$), ** ($P \leq 0.01$).

PX578 Increases mtDNA in A449T/G826S POLG Mice



12-week-old mice given daily oral dose of PX578 (100 mg/kg) or vehicle for 29 days. Tissue mtDNA assessed on day 29.

mtDNA copy numbers were analyzed by a Brown–Forsythe test, followed by a one-way Welch’s ANOVA comparing each group to vehicle-treated A449T/G826S PoLG mice in each tissue type; ** ($P \leq 0.01$), *** ($P \leq 0.001$), and **** ($P \leq 0.0001$).

CONCLUSIONS

PX578 is a first-in-class, orally-administered, brain-penetrant small molecule activator of mtDNA POLy - sole DNA polymerase responsible for mtDNA replication

PX578 binds to POL γ leading to increased mtDNA synthesis in POLG patient-derived fibroblasts

PX578 improves cellular energetics in iHepatocytes and human neural stems cells with POLG mutations, which is at the core of metabolic dysfunction in PMD

In A449T/G826S POLG mice, PX578 increases mtDNA in liver, kidney and diaphragm, which are tissues where mtDNA is below wildtype levels

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