

Edited HSPCs rescue frataxin deficiency and inflammation-driven neurologic, cardiac and pancreatic pathology in Friedreich's ataxia

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Guide to Supplementary Materials

Supplementary Figure 1 provides supporting characterization of mPPL-001 to assess excision specificity of the sgRNAs (UP4 and DN4) and preservation of HSPC reconstitution potential, *in vitro*.

Supplementary Figure 2 demonstrates relationship between engraftment of mPPL-001-derived cells and neurological functional outcomes.

Supplementary Figure 3 identifies GAA excision threshold in mPPL-001 necessary for consistent therapeutic benefits.

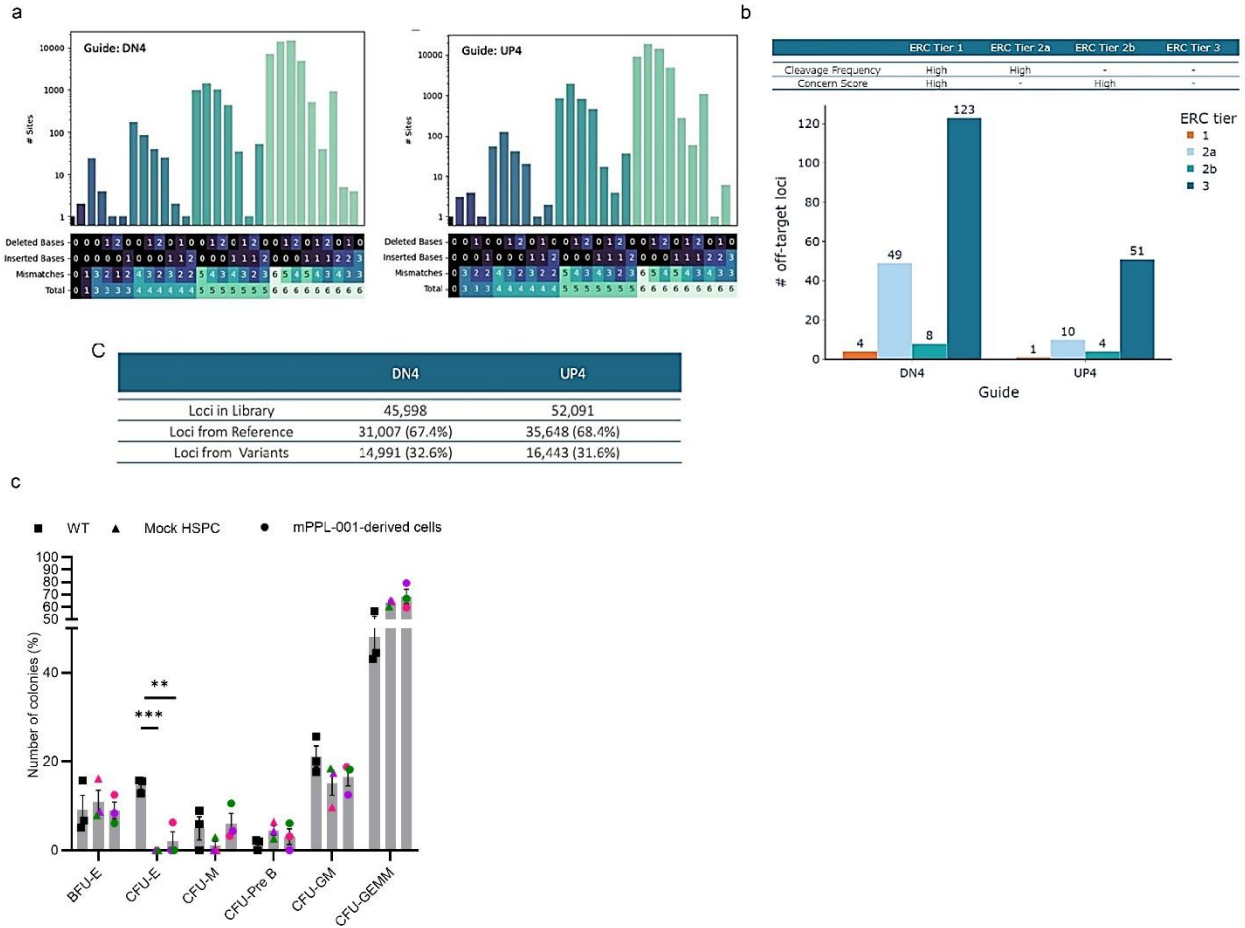
Supplementary Figure 4 provides supporting transcriptomic analysis that demonstrate rescue of disease-associated transcriptional programs in mPPL-001 recipients

Supplementary Figure 5 presents baseline cardiac measurements that support interpretation of the treatment response in mPPL-001 recipients

Supplementary Figure 6 contextualizes effects of mPPL-001 on the pancreatic phenotype in FRDA

Supplementary Tables 1 and 2 provide the gene-level results supporting the transcriptional rescue in mPPL-001 recipients.

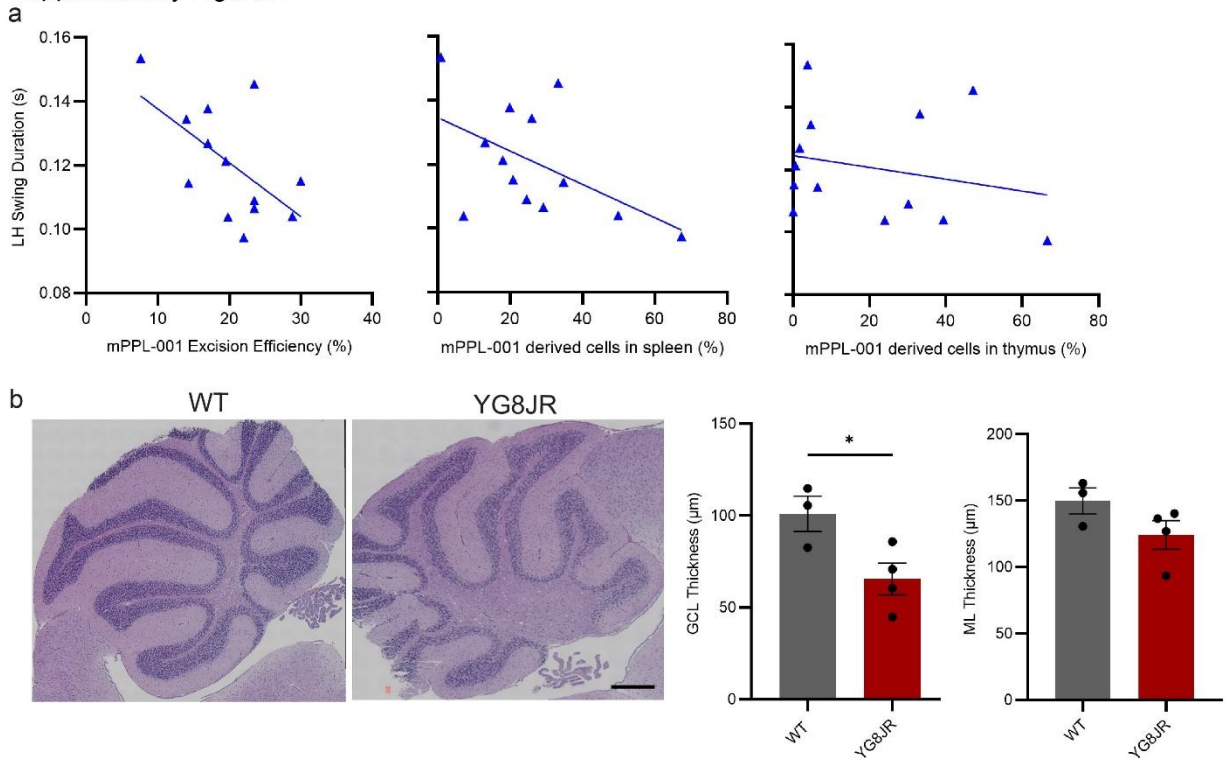
Supplementary Figure 1



Supplementary Figure 1. Characterization of mPPL-001 editing and hematopoietic reconstitution.

(a) ONE-seq nomination profiles for DN4 and UP4 sgRNAs. **(b)** *In vitro* cleavage assay nomination of putative off-target sites for UP4 and DN4. **(c)** Quantification of *in vitro* differentiation potential of HSPCs using Colony forming units (CFU) assay (BFU-E: Burst Forming Unit-Erythroid; CFU-E: Colony Forming Unit-Erythroid; CFU-M: Colony Forming Unit-Macrophage, CFU-Pre B: Colony Forming Unit-Pre-B cell, CFU-GM: Colony Forming Unit- Granulocyte, Macrophage; CFU-GEMM: Colony Forming Unit-Granulocyte, Erythroid, Macrophage, Megakaryocyte).

Supplementary Figure 2



Supplementary Fig 2. Correlation analysis of mPPL-001 transplantation with gait improvements and cerebellar pathology in YG8JR mice. (a) Regression analysis of Left Hind (LH) swing duration vs. mPPL-001 excision efficiency in the input product ($p=0.03$; $F=5.94$; $r^2=0.35$) and mPPL-001-derived cells in spleen ($p=0.06$; $F=4.097$; $r^2=0.27$) and thymus ($p=0.4$; $F=0.6$; $r^2=0.05$). **(b)** Representative H&E-stained cerebellar sections and quantification of granular cell layer (GCL) and molecular layer (ML) thickness in 2.0-2.5 months old WT and YG8JR mice (age of transplant). Scale bar = $100\mu\text{m}$. Data are presented as Mean $\pm$ SEM and analyzed by unpaired t -test. Significance is set at $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$ and $****p \leq 0.0001$.

Supplementary Figure 3

a

Groups	hFXN GAA Excision Efficiency in mPPL-001
Group 1	19.80
Group 2	14.00
Group 3	7.60
Group 4	14.30
Group 5	28.80
Group 6	17.00
Group 7	22.00
Group 8	30.50
Group 9	23.50
Group 10	28.30

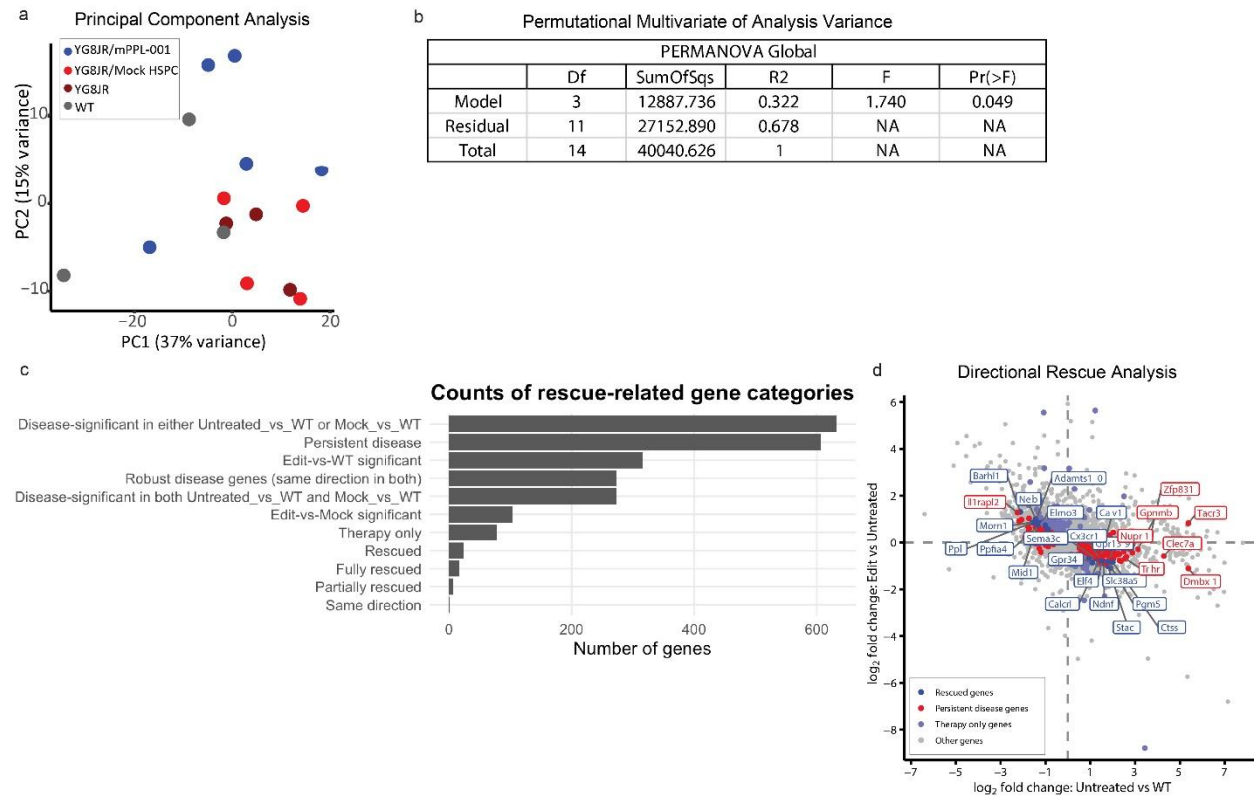
b

mPPL-001 Excision Efficiency (%)	mPPL-001 recipients	Bone marrow chimerism (Mean)	Cerebellar FXN (Change over Mock HSPC)	Left hind swing duration (Shift Towards WT)	Coupling LH-RH (Shift Towards WT levels)
7.60	n=1	0.28			
14.00	n=1	0.90			
14.30	n=1	7.30			
17.00	n=2	3.29			
19.80	n=1	1.90			
22.00	n=1	9.00			
23.00	n=3	13.43			
28.80	n=1	36.10			
28.30	n=1	2.57			
30.50	n=1	3.49			

Color code
Similar to Mock
Partial shift towards WT, improved relative to Mock
Near WT levels

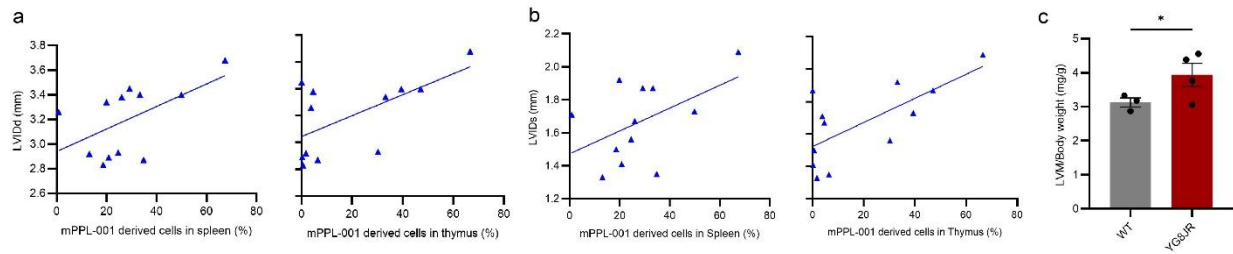
Supplementary Figure 3. Cohort-level relationship between mPPL-001 GAA excision efficiencies and therapeutic response. (a) hFXN GAA excision efficiencies in input mPPL-001 across treated cohorts. (b) Summary of cohort-level therapeutic responses in relation to mPPL-001 excision efficiency.

Supplementary Figure 4



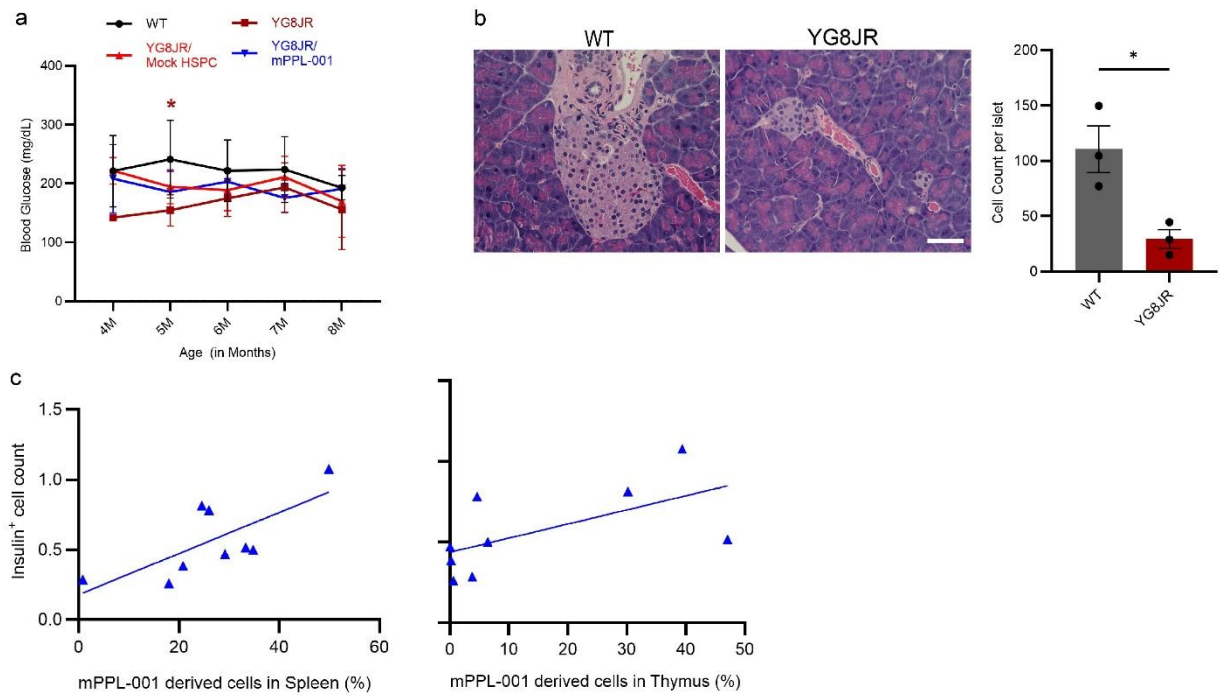
Supplementary Figure 4. mPPL-001 induces partial rescue of disease-associated cerebellar transcriptional changes in YG8JR mice. (a) Principal component analysis of cerebellar transcriptomic profile. **(b)** Global PERMANOVA of cerebellar transcriptomic profiles. **(c)** Counts of rescue-related gene categories. **(d)** Directional rescue analysis comparing disease-associated transcriptional changes (untreated vs. WT) with mPPL-001 associated changes (mPPL-001 vs. untreated).

Supplementary Figure 5



Supplementary Figure 5. Echocardiographic parameters correlate with hematopoietic engraftment of mPPL-001-derived cells. (a) Correlation of LVIDd vs. mPPL-001-derived cells engrafted in spleen ($p=0.06$; $F=4.258$; $r^2=0.29$) and thymus ($p=0.03$; $F=0.616$; $r^2=0.38$). (b) Correlation of LVIDs vs. mPPL-001-derived cells engrafted in spleen ($p=0.1$; $F=3.2$; $r^2=0.24$) and thymus ($p=0.01$; $F=9.511$; $r^2=0.48$). (c) M-mode echocardiography measurement of left ventricular dimensions in 2.0-2.5 month old YG8JR (age of transplant) and age-matched WT mice. Data are presented as Mean $\pm$ SEM and analyzed by unpaired t -test. Significance is set at * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$.

Supplementary Figure 6



Supplementary Figure 6. Abundance of Insulin positive β -cells in pancreas correlate with hematopoietic engraftment of mPPL-001-derived cells. (a) Periodic fasting body glucose measurement of mice of all cohorts up to 8 months of age. * Represents significance at 5 months between WT and untreated YG8JR mice. (b) Representative images of H&E-stained pancreatic sections and quantification of cell count within islets in 2.0-2.5-month-old YG8JR mice compared to age-matched WT controls (WT=3, YG8JR=3). 3-5 islets per mouse were quantified. Scale bars=50 μ m. (c) Regression analysis of Insulin⁺ β -cell numbers vs. engraftment of mPPL-001-derived cells in spleen ($p=0.02$; $F=7.66$; $r^2=0.52$) and thymus ($p=0.08$; $F=4.023$; $r^2=0.36$).