Chlamydomonas Cellular Phenotypes Enable *in vivo* Validation of Computationally Designed Therapeutic ADA1 Variants

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Background

The Problem

- Adenosine Deaminase (ADA) deficiency leads to SCID due to the accumulation of toxic purine metabolites that destroy T-cells.
- Current Enzyme Replacement Therapy (ERT) relies on bovine (cow) ADA. This is frequently immunogenic, leading to antibody neutralization and treatment failure.
- Machine Learning (ML) can design stabilized human ADA variants to evade immune rejection.
- We lack a rapid, high-throughput *in vivo* system to screen these variants.

The Approach

- We leveraged our Zoogle dataset (zoogle.arcadiascience.com) to establish *C. reinhardtii* as an ideal model based on high ADA1 evolutionary conservation.
- We obtained and characterized an ADA1-deficient mutant (CLIP2 library) to establish baseline disease phenotypes.
- We engineered a multi-modal phenotyping pipeline to quantify motility and metabolic health in high-throughput.
- We transformed the mutant strain with ML-designed human ADA1 variants to assess their ability to restore wild-type function *in vivo* .

C. reinhardtii ranks better than super model organisms in similarity to human ADA1

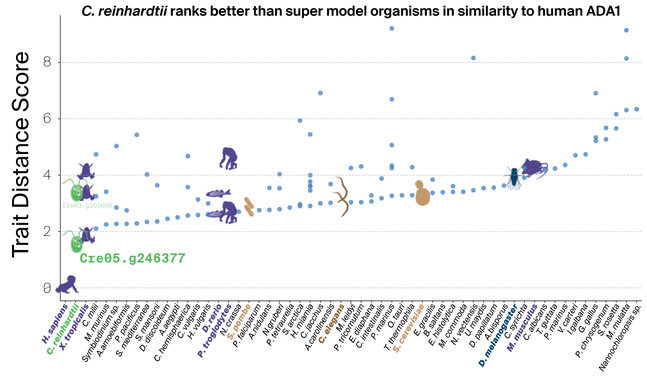


Figure 1) ADA1 orthologs were detected in 56 of the 62 organisms analyzed in the Zoogle dataset. Trait distance scores quantify similarity to human ADA1; lower scores indicate greater similarity.
The *Chlamydomonas reinhardtii* gene with the highest similarity, Cre05.g246377, has been previously annotated as ADA2; here we refer to it as ADA1 for simplicity.

ADA1 Mutant Phenotypes

ADA1 mutant cells show distinct Raman spectral signatures relative to WT.

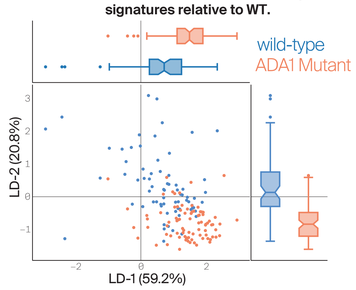


Figure 2) Linear Discriminant Analysis (LDA) of filtered Raman spectra. Spectra were collected using a Wasatch 785 nm system.

ADA1 mutants exhibit disrupted starch sheaths.

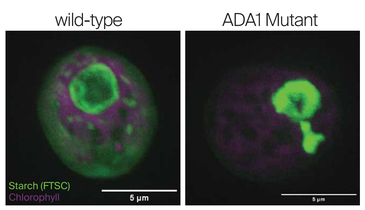


Figure 3) Representative images of fluorescent staining of starch (FTSC, green) and intrinsic chlorophyll fluorescence (magenta). Maximum Intensity Projections of Z-stack images.

ADA1 mutants show increased starch accumulation.

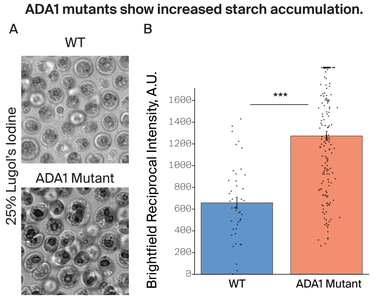


Figure 4) A) Nitrogen-starved cells stained with 25% Lugol's iodine. B) Quantification of whole cell brightfield reciprocal intensity. ***p<0.001.

Increased starch in ADA1 mutants is detectable by Raman spectroscopy.

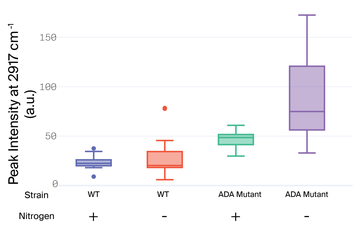


Figure 5) Peak intensity at 2917 cm^{-1}—associated with starch-rich biochemical features—is elevated in ADA1 mutants relative to wild-type cells, both under nitrogen-replete and nitrogen-starved conditions.

ADA1 mutants show prolonged swimming endurance compared to WT

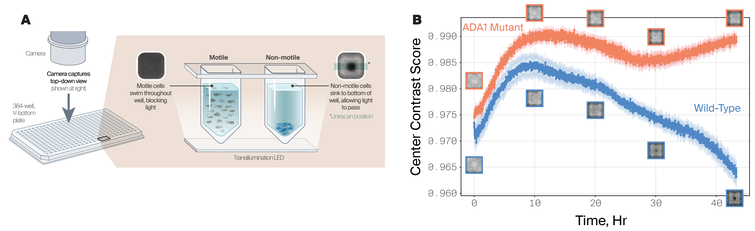
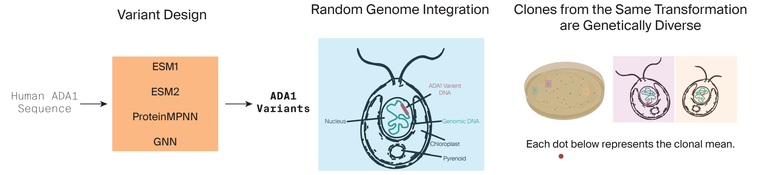


Figure 6) A) Schematic of the imaging assay from [3] depicts the experimental setup. B) Mean Center Contrast Score (Center Intensity Divided by Edge Intensity) over time of WT (n=132, blue) and ADA1 mutant (n=96, orange) wells. Representative wells are included at the appropriate time point.

Rescuing ADA1 with Designer Variants



ADA1 mutants consistently accumulate excess starch, and several ADA1 variants show trends toward phenotypic rescue despite clonal variability.

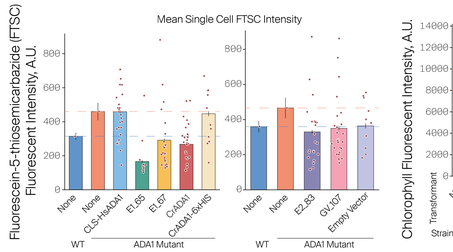


Figure 7) Bars represent the mean FTSC fluorescence across all recovered clones for each strain or construct. Each dot corresponds to the mean single-cell fluorescence measured from an individual transformation clone, reflecting clone-to-clone variability. Error bars indicate the standard deviation.

ADA1 mutants have increased chlorophyll autofluoresnce, with several ADA1 variant transformants showing trends of phenotypic rescue.

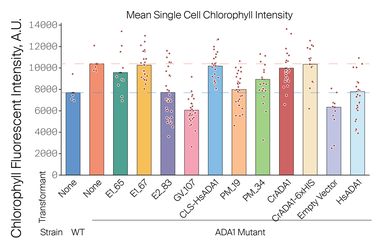


Figure 8) Bars represent the average chlorophyll fluorescence intensity across all recovered clones for each strain or construct. Each dot corresponds to the mean single-cell chlorophyll signal measured from an individual transformation clone, reflecting clone-to-clone variation. Error bars indicate the standard deviation.

ADA1 mutation alters swimming behavior, with ADA1 variant transformants showing variable trends toward resuce

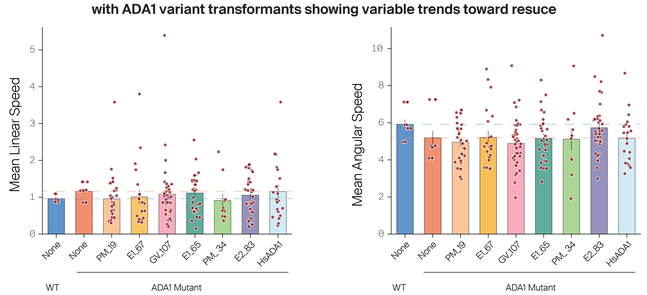


Figure 9) Bars represent the mean linear (left) and angular (right) swimming speeds across all recovered clones for each strain or construct. Each dot corresponds to the clone-level mean speed, reflecting variability among independent transformation clones. Error bars indicate the standard deviation.

Conclusions/Summary

The Results

- ADA1 mutants exhibit significantly altered motility and metabolic attributes compared to wild-type controls.
- Treatment with ML-generated variants results in phenotypic recovery, shifting the mutant behavior back toward WT levels in some clones.

The Impact

- Establishes *Chlamydomonas* as a robust, non-mammalian host for modeling human metabolic errors.
- Validates a rapid, cost-effective pipeline to screen large libraries of ML-designed variants
- Bridges the gap between computational design and *in vivo* validation

Next Steps



Mutating ADA1 in *Chlamydomonas* induces distinct cellular phenotypes, characterized by increased starch accumulation, enhanced swimming longevity, and elevated linear velocity.

We successfully rescued these phenotypes by co-expressing ML-designed ADA1 variants, with several candidates surprisingly surpassing wild-type function. However, because standard *Chlamydomonas* transformation results in random genomic integration, we observe significant variability between clones expressing the same variant. Consequently, it remains unclear which clonal rescues are intrinsic to the designed enzyme kinetics or artifacts of high-copy expression and off-target genomic disruption.

To validate these findings, we are currently mapping insertion sites to confirm that phenotypic recovery is driven strictly by the ADA1 variant rather than positional effects. Additionally, we are performing quantitative molecular assays to confirm protein expression levels directly.

Simultaneously, we are developing open-source methods for targeted gene integration in *C. reinhardtii* to standardize expression across clones

Additionally, we are performing *in vitro* experiments on purified ADA1 variants to characterize properties like stability and *in vitro* activity.

We are also using this pipeline to test potential enzyme replacement variants for additional lysosomal disorders caused by other enzymes, including UBE2A, PPT1, and DNASE2, in *S. pombe*, *S. cerevisiae*, *C. reinhardtii*, and *C. elegans*.

Download the Poster:

bit.ly/chlamy-ADA

All published work: research.arcadiascience.com

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Contributors (A–Z)

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