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## Supplementary information

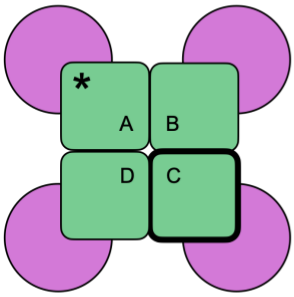
# IMPDH1 retinal variants control filament architecture to tune allosteric regulation

In the format provided by the authors and unedited

| | | IMPDH1 | IMPDH2 |
|---|---|---|---|
| IMP | **Vmax** (μM NADH/min/μM IMPDH) | 3.5 | 2.8 |
| | **K0.5** (μM) | 60 | 38 |
| | **Hill** | 3.5 | 3.0 |
| | | | |
| NAD | | **IMPDH1** | **IMPDH2** |
| | **Vmax** (μM NADH/min/μM IMPDH) | 3.4 | 5.3 |
| | **K0.5** (μM) | 16.3 | 27 |
| | **Hill** | 2.6 | 1.4 |

**Supplemental Table 1. IMPDH1 and IMPDH2 have similar kinetics**

(A-E) Table comparing $V_{max}$, K0.5, and Hill coefficients for IMP and NAD+ for IMPDH1 and IMPDH2. Reactions performed with 1 μM protein, 1 mM ATP, 1 mM or varying IMP, and 300 μM or varying NAD+. All data points reported are an average of 3 measurements from the same protein preparation. Fits for activity assays were calculated using the Hill-Langmuir equation $V = Vmax*[S]n/((K0.5)n+[S]n)$ and $IC_{50}$ was calculated using a modified Hill equation $V=Vmin*(Vmax-Vmin)/(1+(I/IC50)hill$[63].




| | | IMPDH2 | | IMPDH1(514) | | IMPDH1(546) | | IMPDH1(595) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Active large interface | Inhibited large interface | Active large interface | Inhibited small interface | Active large interface | Inhibited small interface | Active large interface | Inhibited large interface |
| IMPDH2 | Active large interface | — | 0.061 | 0.878 | 3.314 | 2.145 | 3.246 | 2.712 | 0.693 |
| | Inhibited large interface | — | — | 1.266 | 1.721 | 2.371 | 2.371 | 2.595 | 0.816 |
| IMPDH1(514) | Active large interface | — | — | — | 3.787 | 1.705 | 3.7 | 2.249 | 1.065 |
| | Inhibited small interface | — | — | — | — | 5.046 | 1.368 | 5.506 | 2.208 |
| IMPDH1(546) | Active large interface | — | — | — | — | — | 4.723 | 0.554 | 1.698 |
| | Inhibited small interface | — | — | — | — | — | — | 5.174 | 1.924 |
| IMPDH1(595) | Active large interface | — | — | — | — | — | — | — | 0.560 |
| | Inhibited large interface | — | — | — | — | — | — | — | — |

**Supplemental Table 2. RMSD between catalytic domains of models**
Top-down view of tetramer with catalytic domains (green) and regulatory domains (pink). Models are aligned on catalytic domain with asterisk and alpha carbon RMSD is calculated by catalytic domain outlined in bold. IMPDH2 active is 6u8n, IMPDH2 inactive is 6u8s RMSD to determine how tetramer flexing (bent/flat) compares.

| | WT | Y12A |
|---|---|---|
| **IMPDH2** | 805 | 219 |
| **IMPDH1(514)** | 188 | 196 |
| **IMPDH1(546)** | 903 | 585 |
| **IMPDH1(563)** | 632 | 151 |
| **IMPDH1(595)** | 1104 | 550 |

**Supplemental Table 3. IMPDH1 and IMPDH2 $IC_{50}$ for GTP in μM**

a

| | | WT | R105W | T116M | N198K | R224P | D226N | R231P | K238E | V268I | H372P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 514 | Vmax | 3.4 | 3.7 | 3.4 | 3.4 | 3.2 | 3.4 | 4.2 | 5.3 | 4.6 | 4.4 |
| | K0.5 | 16.3 | 15.5 | 9.3 | 10.8 | 9.9 | 8.8 | 12.9 | 20.7 | 16.9 | 16.2 |
| | Hill | 2.6 | 3.1 | 1.7 | 3.2 | 1.9 | 1.7 | 2.1 | 2.7 | 2.4 | 3.2 |
| | | | | | | | | | | | |
| | | WT | R105W | T116M | N198K | R224P | D226N | R231P | K238E | V268I | H372P |
| 546 | Vmax | 5.5 | 5.3 | 4.2 | 5.1 | 6.4 | 5.1 | 5.8 | 5.4 | 6.0 | 8.8 |
| | K0.5 | 32.9 | 22.8 | 19.5 | 31.0 | 31.5 | 26.2 | 31.9 | 22.5 | 24.9 | 46.9 |
| | Hill | 1.9 | 1.1 | 1.3 | 1.0 | 1.0 | 1.1 | 0.9 | 1.0 | 0.9 | 1.1 |
| | | | | | | | | | | | |
| | | WT | R105W | T116M | N198K | R224P | D226N | R231P | K238E | V268I | H372P |
| 595 | Vmax | 5.2 | 5.3 | 4.0 | 4.8 | 1.5 | 5.5 | 6.8 | 26.9 | 5.1 | 7.4 |
| | Km | 30.4 | 32.6 | 28.8 | 36.7 | 28.1 | 23.1 | 39.6 | 1.6 | 36.7 | 66.2 |
| | Hill | 1.2 | 1.0 | 1.1 | 0.6 | 0.7 | 0.9 | 1.1 | 0.9 | 1.3 | 1.3 |

b

| | | WT | R105W | T116M | N198K | R224P | D226N | R231P | K238E | V268I | H372P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 514 | Vmax | 3.5 | 4.2 | 2.5 | 3.0 | 3.1 | 2.6 | 3.5 | 5.0 | 3.3 | 3.5 |
| | K0.5 | 59.5 | 13.6 | 28.0 | 19.9 | 12.3 | 10.0 | 20.2 | 17.9 | 25.4 | 25.0 |
| | Hill | 3.5 | 2.7 | 2.7 | 2.1 | 2.0 | 1.9 | 2.2 | 2.0 | 2.5 | 3.0 |
| | | | | | | | | | | | |
| | | WT | R105W | T116M | N198K | R224P | D226N | R231P | K238E | V268I | H372P |
| 546 | Vmax | 4.8 | 5.3 | 4.7 | 4.7 | 4.5 | 4.6 | 4.5 | 4.5 | 4.8 | 6.1 |
| | K0.5 | 33.0 | 23.3 | 34.4 | 34.4 | 27.6 | 22.6 | 17.9 | 26.8 | 39.2 | 45.1 |
| | Hill | 1.3 | 1.0 | 0.8 | 0.8 | 1.3 | 1.1 | 1.2 | 1.5 | 1.4 | 1.3 |
| | | | | | | | | | | | |
| | | WT | R105W | T116M | N198K | R224P | D226N | R231P | K238E | V268I | H372P |
| 595 | Vmax | 5.2 | 4.9 | 3.6 | 3.7 | 5.0 | 4.6 | 6.0 | 6.2 | 5.0 | 7.1 |
| | K0.5 | 27.9 | 20.4 | 23.4 | 18.9 | 22.3 | 22.6 | 26.0 | 35.8 | 20.3 | 28.6 |
| | Hill | 1.4 | 1.5 | 1.6 | 1.2 | 1.1 | 1.1 | 1.3 | 1.5 | 1.3 | 1.5 |

c

| | | WT | R105W | T116M | N198K | R224P | D226N | R231P | K238E | V268I | H372P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 514 | Vmax | 3.6 | 4.1 | 2.7 | 3.0 | 2.8 | 2.2 | 3.2 | 4.5 | 3.1 | 3.3 |
| | K0.5 | 187.8 | 15.9 | 132.4 | N/A | N/A | 134.0 | N/A | N/A | 150.8 | 151.2 |
| | Hill | 4.7 | 25.2 | 3.0 | N/A | N/A | 32.1 | N/A | N/A | 5.7 | 2.6 |
| | | | | | | | | | | | |
| | | WT | R105W | T116M | N198K | R224P | D226N | R231P | K238E | V268I | H372P |
| 546 | Vmax | 4.4 | 4.8 | 4.4 | 3.9 | 5.2 | 4.5 | 3.9 | 4.5 | 5.3 | 7.3 |
| | K0.5 | 903.4 | 759.6 | 888.3 | N/A | N/A | N/A | N/A | N/A | 870.9 | 630.8 |
| | Hill | 5.3 | 2.7 | 3.8 | N/A | N/A | N/A | N/A | N/A | 3.2 | 5.4 |
| | | | | | | | | | | | |
| | | WT | R105W | T116M | N198K | R224P | D226N | R231P | K238E | V268I | H372P |
| 595 | Vmax | 4.5 | 4.9 | 4.1 | 3.6 | 4.9 | 4.5 | 5.0 | 6.0 | 5.2 | 7.4 |
| | K0.5 | 1104.0 | 1274.4 | 1760.5 | N/A | N/A | N/A | N/A | N/A | 1313.9 | 1843.0 |
| | Hill | 3.9 | 2.4 | 3.5 | N/A | N/A | N/A | N/A | N/A | 2.4 | 1.8 |

**Supplemental Table 4. IMPDH1 RP mutations do not change NAD+ or IMP kinetics in any variants**
**a-c**, $V_{max}$ (μM NADH/min/μM IMPDH), K0.5 (μM), and Hill coefficient for NAD+ for WT and RP mutants in all variants. **a**, For NAD+. Reactions performed with 1 μM protein, 1 mM ATP, 1 mM IMP, and varying NAD+. **b**, For IMP. Reactions performed with 1 μM protein, 1 mM ATP, 300 μM NAD+, and varying IMP. **c**, For GTP. Reactions performed with 1 μM protein, 1 mM ATP, 1 mM IMP, 300 μM NAD+, and varying GTP. All data points reported are an average of 3 measurements from the same protein preparation. Fits for activity assays were calculated using the Hill-Langmuir equation V = Vmax·[S]n/((K0.5)n+[S]n) and $IC_{50}$ was calculated using a modified Hill equation V=Vmin*(Vmax-Vmin)/(1+(I/IC50)hill[63].