Supplemental Notes

Clinical report for case subject with L245P variant of IMPDH2

The proband was a 3-year-old female, the firstborn and only child of non-consanguineous parents of Jewish Ashkenazi origin. Pregnancy was significant for intrauterine growth retardation (IUGR), dilation of the lateral ventricles, and mild pulmonic stenosis on fetal ultrasound. Delivery was at 33+6 weeks, at a birthweight of 1520 grams (7th %ile) and with a head circumference of 28.5 cm (9th %ile). Complications of prematurity included bronchopulmonary dysplasia (BPD) with oxygen dependency for approximately one month. She was born with congenital dysplasia of the hip (CDH) which required surgical intervention, and with left sided torticollis. Echocardiogram revealed pulmonic stenosis, for which she underwent interventional catheterization (balloon angioplasty). There was also an atrial septal defect which closed spontaneously. Head ultrasound showed mild dilation of the left ventricle. Renal ultrasound was normal.

The proband had significant hypotonia and global developmental delay. She walked at around 2-years-7-months of age, and at 3 years of age, said less than 10 words, although she used nonverbal communication and seemed to have a higher receptive ability. Development quotient (DQ) was 42 at 14 months, and 60 at repeat evaluation. Hearing evaluation showed bilateral type B tympanograms and normal free field audiometry. She had abnormal posturing of the head, which she held tilted back and had a downward gaze. Ophthalmology exam including ultrasound of the orbits was normal. There were no convulsive episodes, and EEG was normal.

Family history was positive for CDH in the proband's father and the paternal uncle. The mother's half-brother was born with omphalocele and died at 2 years of age. His cognition was reported to be normal, and no DNA was available for testing. The maternal grandfather died in his 20's of cancer affecting his leg; no further information was available.

On physical examination at 3-years-2-months, the child had failure to thrive, with a preserved head circumference of 47.5 cm (~10th %ile). She was responsive, had good eye contact yet minimal facial mimicry. She held her head tilted back with abnormal posturing. She had a double hair whorl, sparse thin hair, high forehead, hypertelorism, bilateral epicanthal folds, a broad nasal bridge, open mouth, small low-set and posteriorly rotate ears, a systolic murmur, and axial hypotonia.

Clinical report for case subject with K238R variant of IMPDH2

The proband was the product of a naturally conceived single gestation to a then 27-year-old G2P3 mother. Pregnancy and neonatal history were uncomplicated. Family history was non-contributory. He was born at 40+1 weeks gestation weighing 2920g (25th percentile) and was 51.4cm in length (75th-90th percentile). HC at birth was 33cm (25th percentile). APGAR scores were 8 at 1 minute and 9 at 5 minutes. He was discharged home at day of life 2.

Concerns for his development were first raised at 6.5 months of age due to gross motor delays. He sat at 8-9 months, crawled at 12 months, and walked at 18 months. Significant speech delay was also noted. Medical history was notable for tachypnea and stridor with feeds as a neonate, milk protein allergy and gastroesophageal reflux, meatal stenosis and penile adhesion s/p correction, easy bruising, torticollis and plagiocephaly, eczema, and hypotonia.

At 9 months of age, he weighed 8.396kg (24th percentile), was 74.8cm in length (80th percentile), and head circumference was 44.1cm (18 percentile). Physical examination was notable for slight upslant to palpebral fissures, prominent cheeks, supernumerary nipple, and mild hypotonia.

Follow-up at 23 months was notable for persistent developmental delay with most skills clustering around the 16-20 month age range per Developmental Pediatrician and concern for possible autism spectrum disorder. He was referred to Neurology for evaluation of possible movement disorders and was again noted to have hypotonia. He was started on Sinemet (6mg BID) due to the possibility of L-DOPA responsive symptoms in IMPDH2 related disorders with reported improvements in energy, interaction, speech skills, and movement.

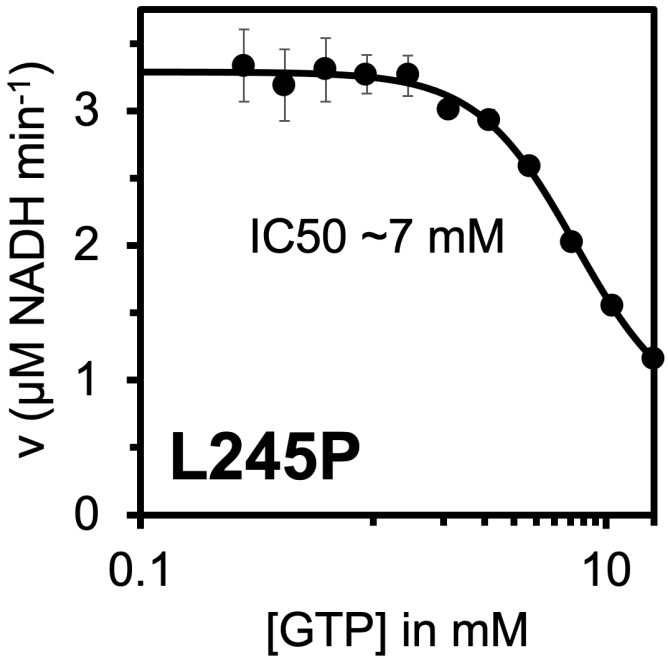
Supplemental Figures

Supplemental Fig. 1: Photos of the patient with the K238R variant at different ages.

[Images were removed for submission to bioRxiv]

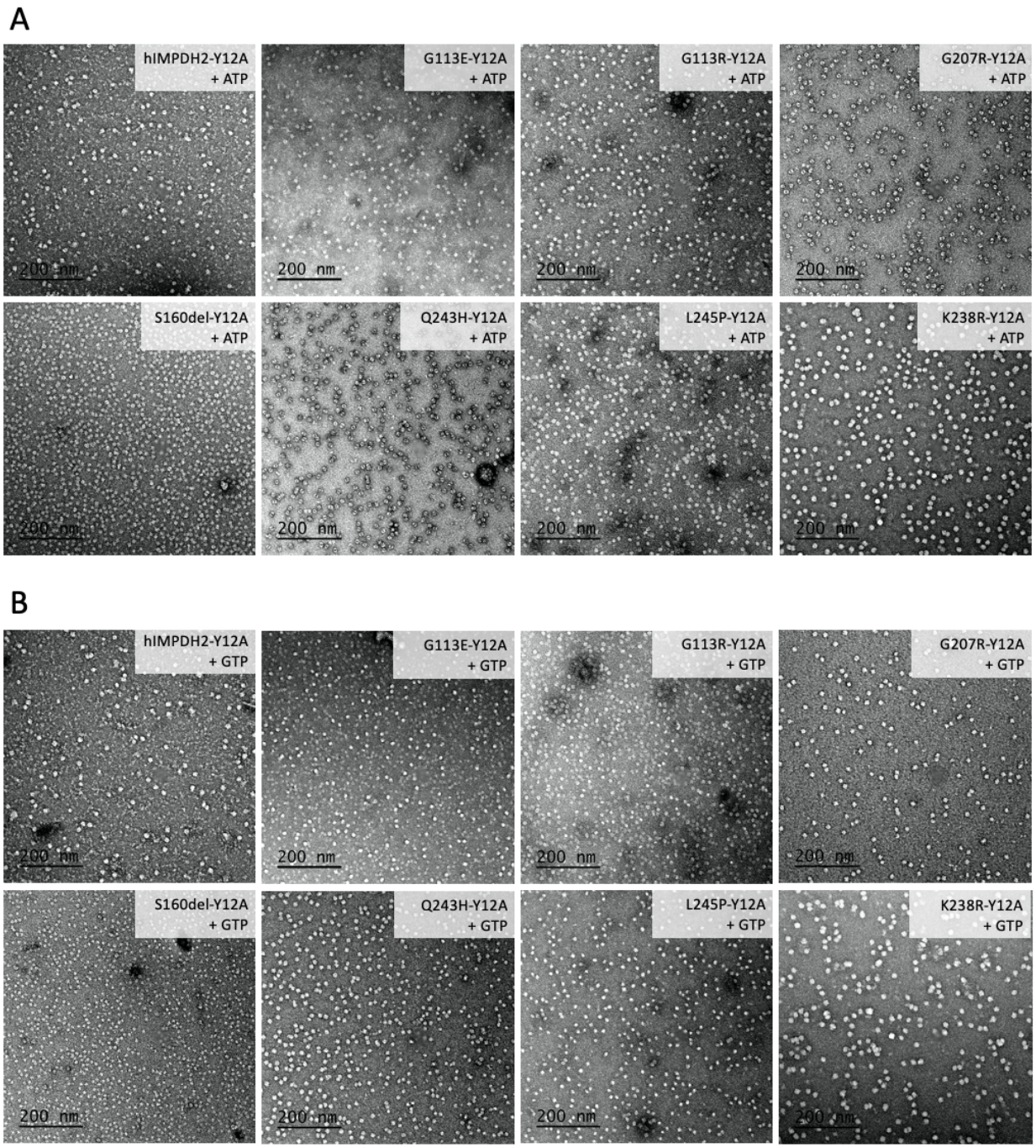
Photos of the patient at 9 months (A), 16 months (B), and 26 months of age (C).

Supplemental Fig. 2: The L245P mutant is sensitive to high concentrations of GTP.



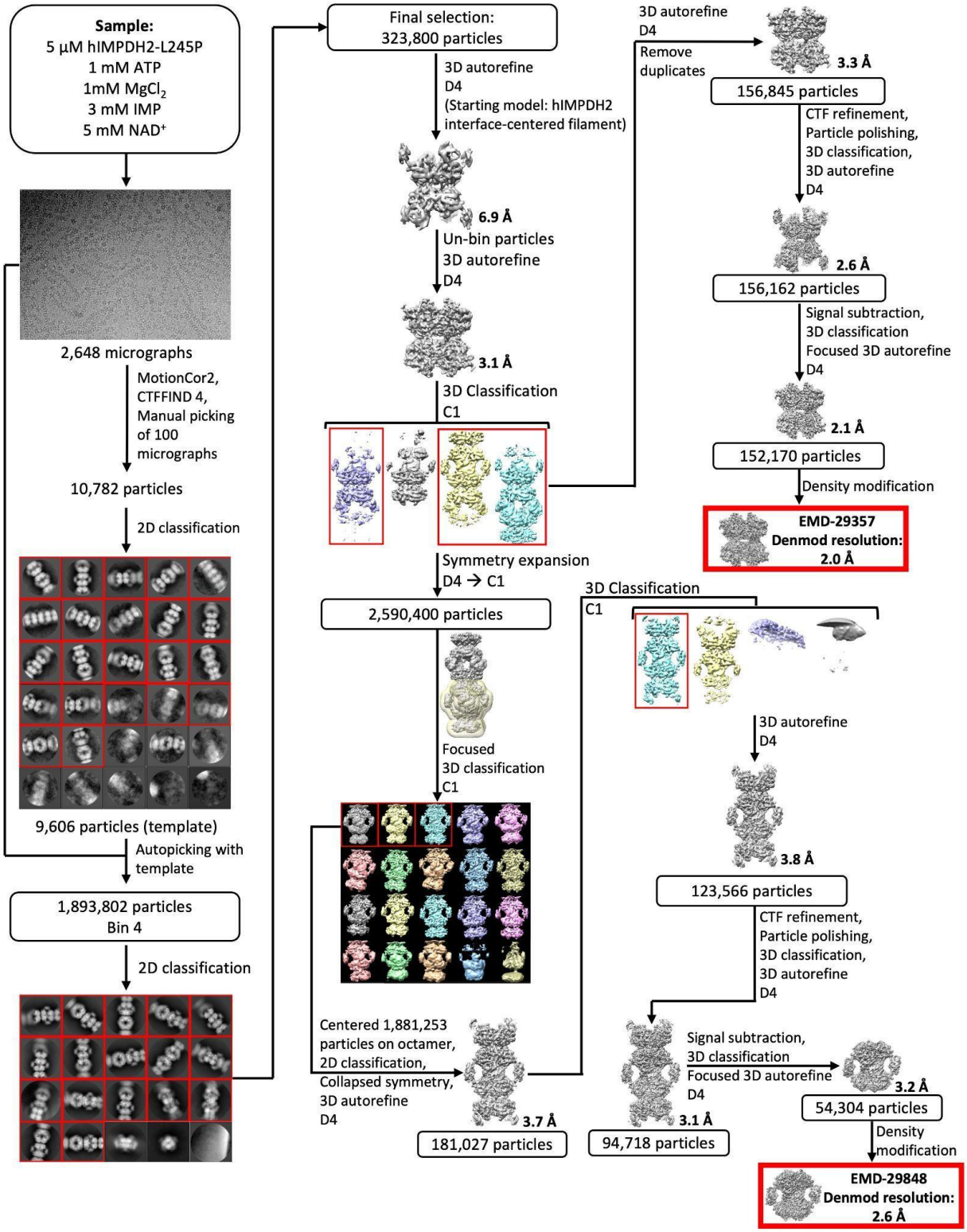
GTP inhibition curve of the L245P variant up to 16 mM GTP. Each data point represents the average initial rate of three reactions. Error bars represent standard deviation for n=3 technical replicates. Velocities were calculated from the change in absorbance at 340 nm. Reactions were initiated with 300 µM NAD+ and contained 1 µM enzyme, 1 mM ATP, 1 mM IMP, 1 mM MgCl2 and varying concentrations of GTP.

Supplemental Fig. 3: Non-assembly disease mutants imaged using negative stain EM.

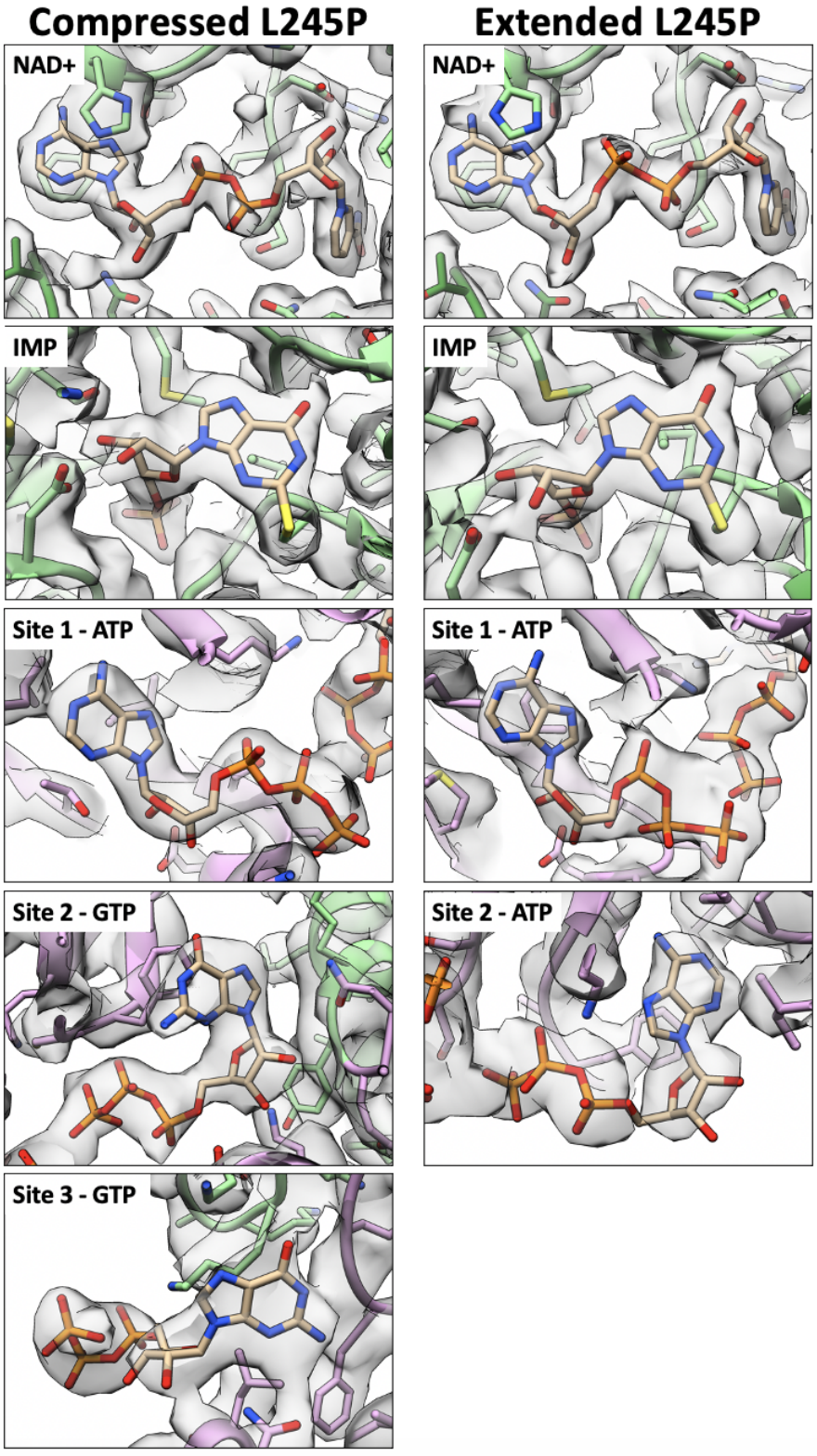


Representative negative stain images of 0.5 µM enzyme with either 1 mM ATP and 1 mM $MgCl_2$ (A) or 5 mM GTP (B). Filaments were not observed in either condition.

Supplemental Fig. 4: Extended L245P filament cryo-EM data processing.

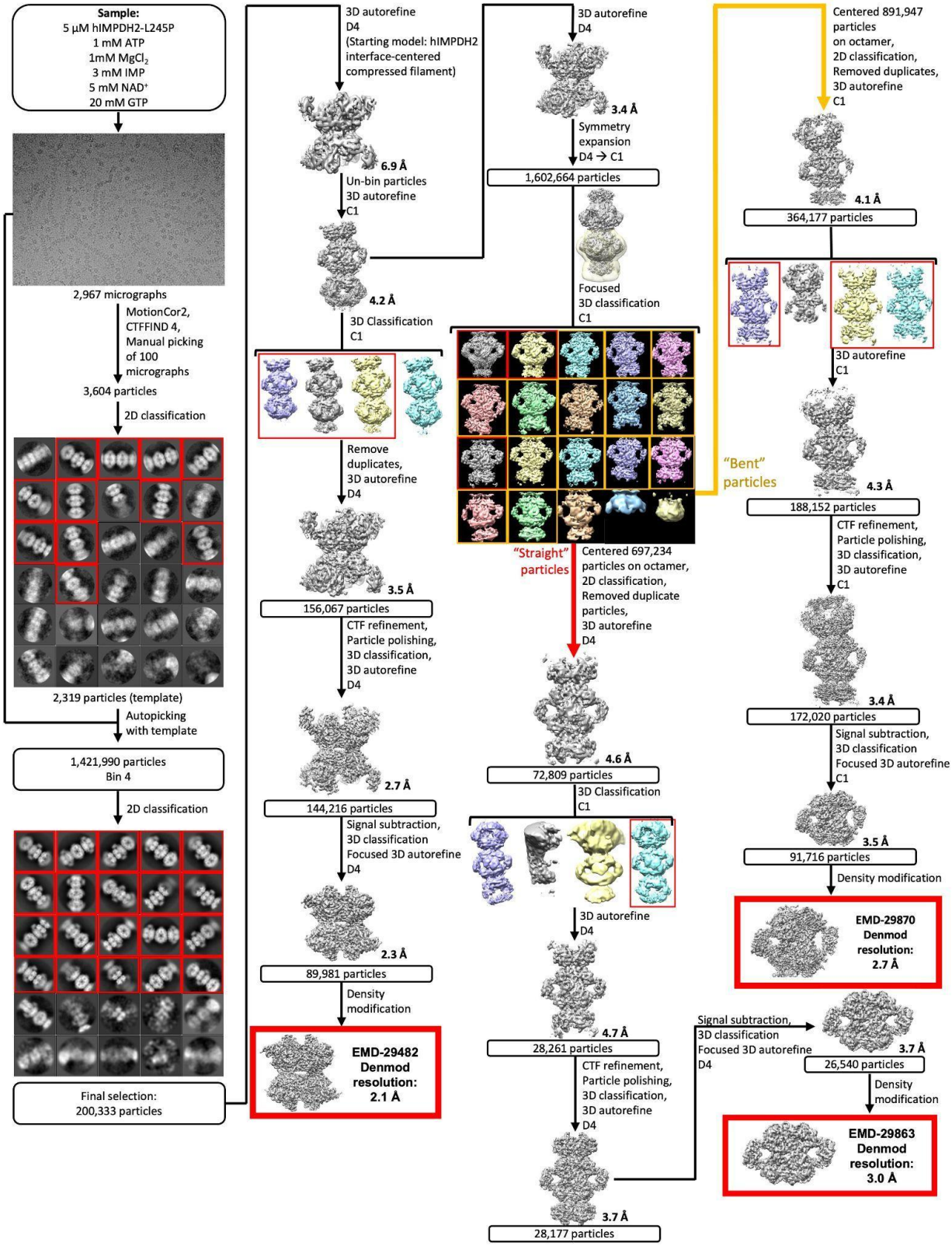


Supplemental Fig. 5: Volume around ligands in extended and compressed L245P structures.

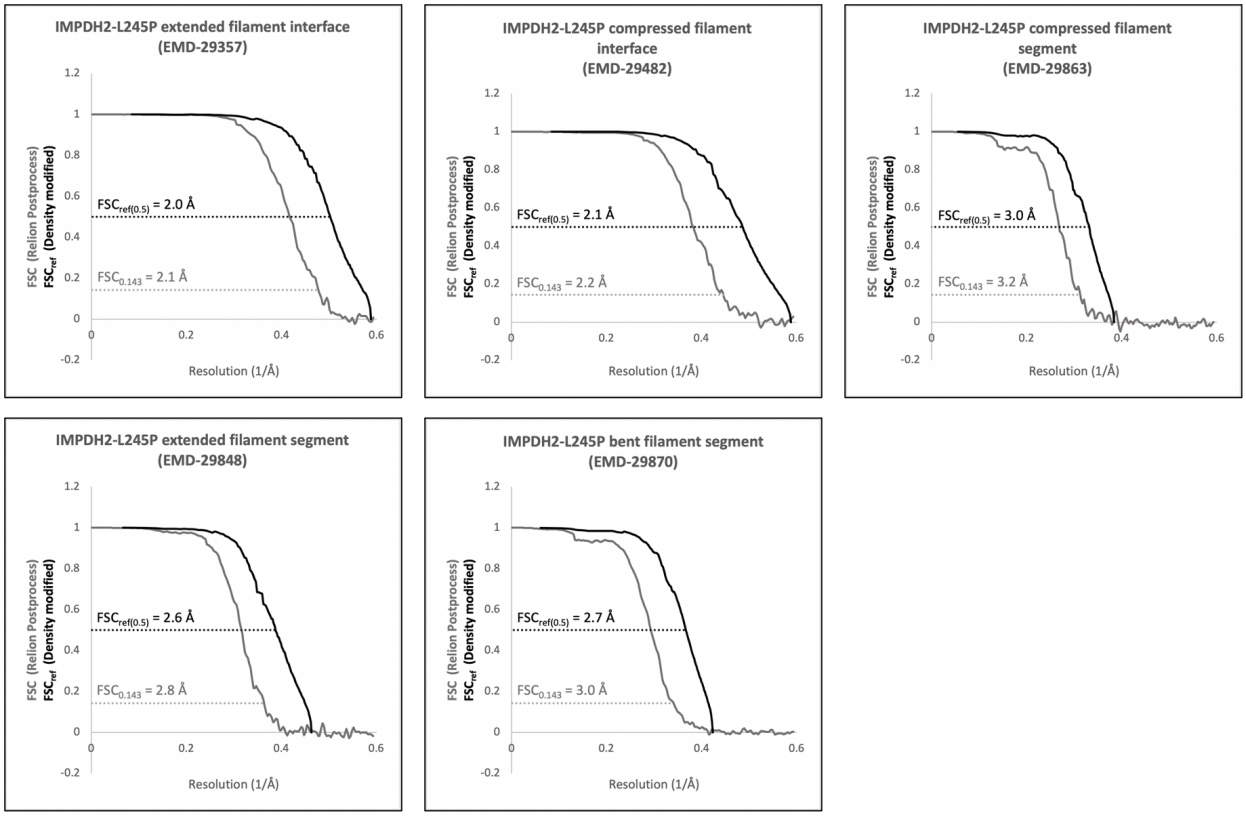


All ligands are resolved in the L245P extended and compressed structures. The catalytic domain is colored in green, and the regulatory domain is colored in pink.

Supplemental Fig. 6: L245P+GTP/ATP/IMP/NAD+ data processing.



Supplemental Fig. 7: FSC curves of cryo-EM reconstructions.



Supplemental Fig. 8: Filament assembly interface reconstruction of the L245P filament in the presence of 20 mM GTP, 1 mM ATP, 3 mM IMP, 5 mM NAD, and 1 mM MgCl2.