Supporting information for:

A hereditary spastic paraplegia–associated atlastin variant exhibits defective allosteric coupling in the catalytic core

John P. O'Donnell[1], Laura J. Byrnes[1, 2], Richard B. Cooley[1, 3], and Holger Sondermann[1, 4, §]

[1]From the Department of Molecular Medicine, Cornell University, Ithaca, NY 14853, USA
[2]Present address: Worldwide Medicinal Chemistry, Pfizer, Eastern Point Rd, Groton, CT 06340, USA
[3]Present address: Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, USA
[4]Lead contact
[§]Correspondence: hs293@cornell.edu

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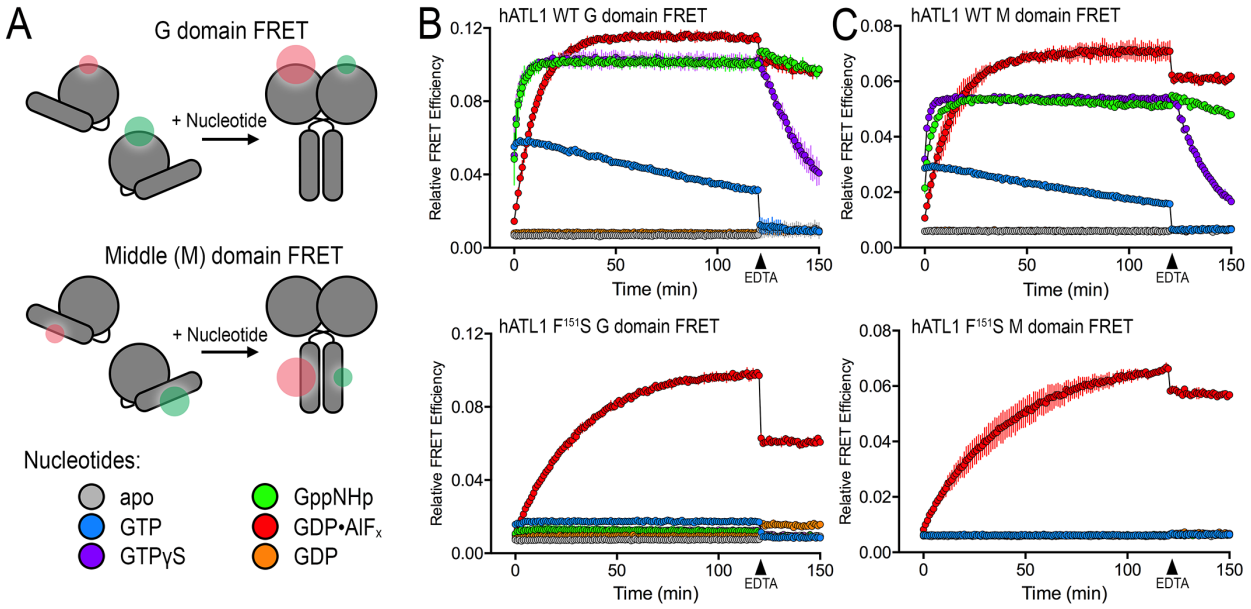

Figure S1 (associated with Figure 3). Determination of nucleotide-dependent equilibrium and steady-state dimerization of wild-type and F^{151}S variants of ATL. (A) Donor- and acceptor-labeled proteins on either G or middle domain (1 μM) were incubated with indicated nucleotides (500 μM). After 120 minutes, EDTA (10 mM) was added, indicated by the arrow on the x-axis. **(B)** G domain FRET. **(C)** Middle domain FRET. Graphs showing means and standard deviation (SD) are plotted from two biological replicates with three technical repeats each.

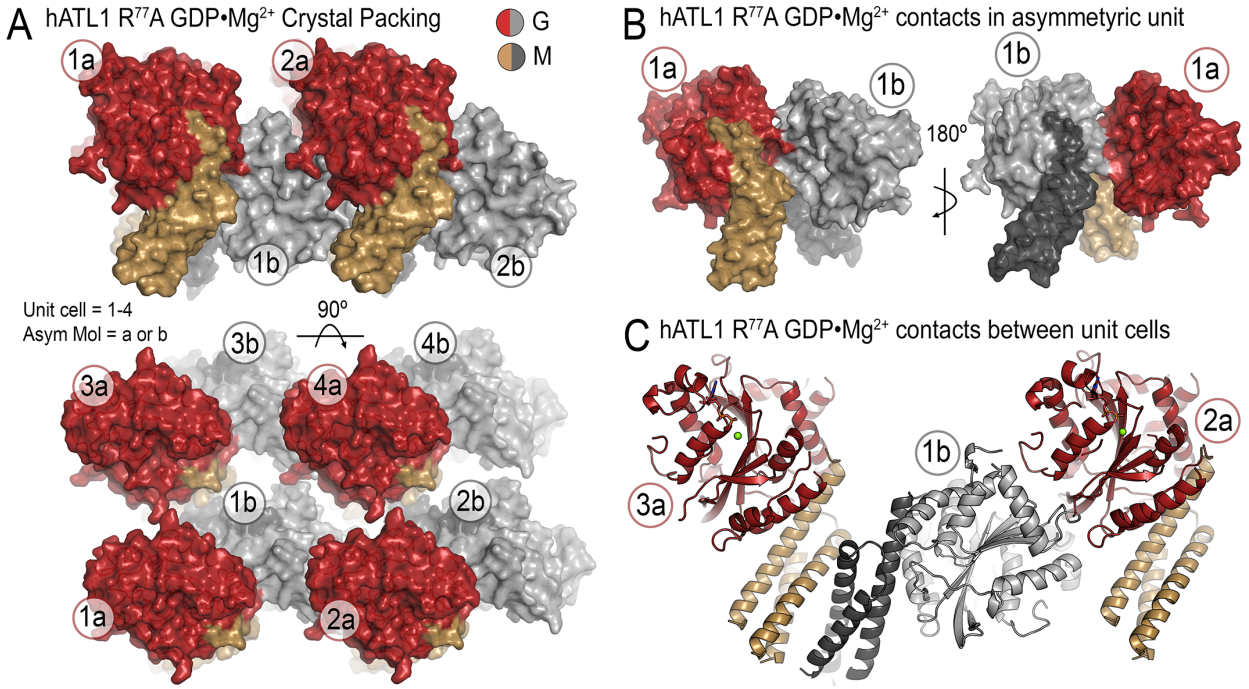

Figure S2 (associated with Figure 5). Novel ATL crystal packing and asymmetric contents of crystals containing ATL1-R^{77}A bound to GDP•Mg^{2+}. (A) Schematic of crystal packing and contacts made in in the ATL1-R^{77}A structure. Two protomers form a unit cell and their G domains are colored either red or light gray, and their middle domains are colored tan or dark gray. Each protomer is labeled with a number and letter corresponding to its position within the crystal lattice. **(B)** Asymmetric unit contents illustrate a novel contact between protomers. **(C)** Asymmetric units pack via middle domain-mediated (molecules 3a and 1b) and G domain-mediated (molecules 1b and 2a) contacts.

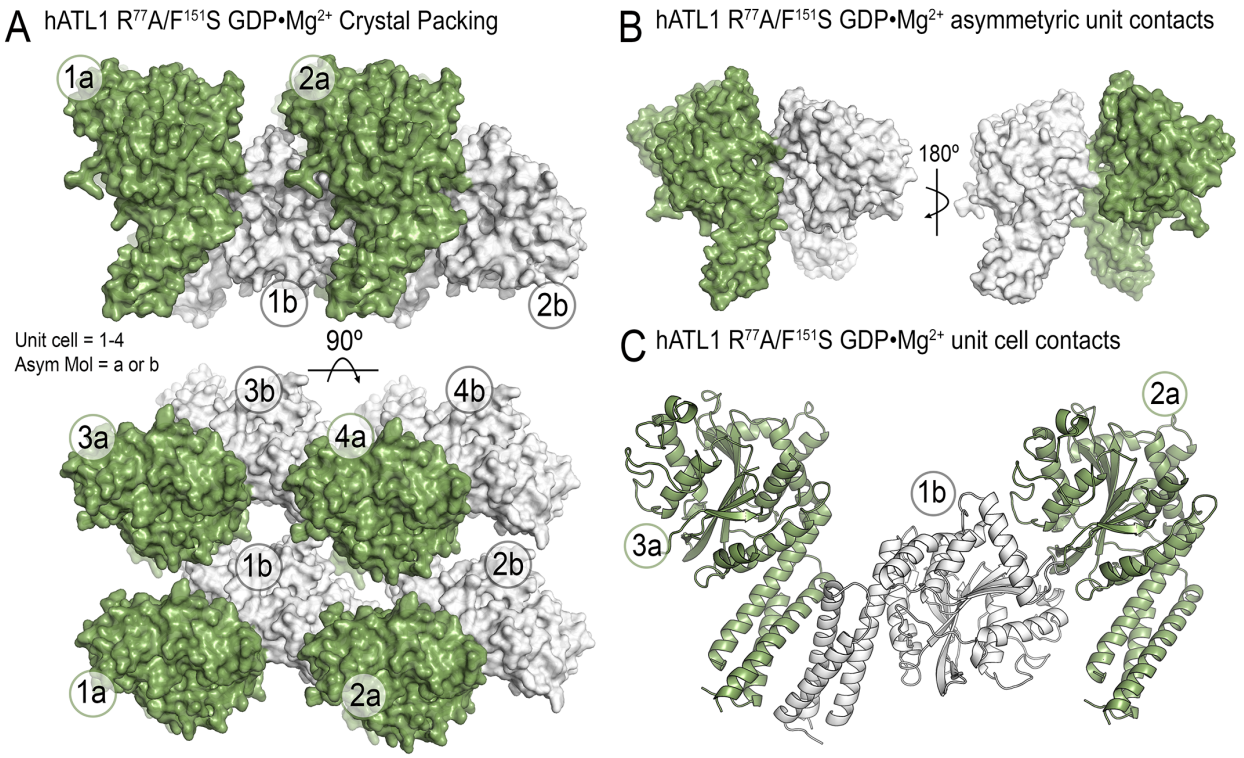

Figure S3 (associated with Figure 5). Crystals containing ATL1-R^{77}A/F^{151}S bound to GDP•Mg^{2+} exhibit the same crystal packing and asymmetric contents as seen for the ATL1-R^{77}A structure. (A) Crystal packing is the same as for the ATL1-R^{77}A structure. Each protomer is labeled with a number and letter corresponding to its position within the crystal lattice, and colored either green or white. **(B)** Asymmetric unit contents mirrors the ATL1-R^{77}A structure. **(C)** Crystal contacts made between asymmetric units are also consistent with ATL1-R^{77}A structure.

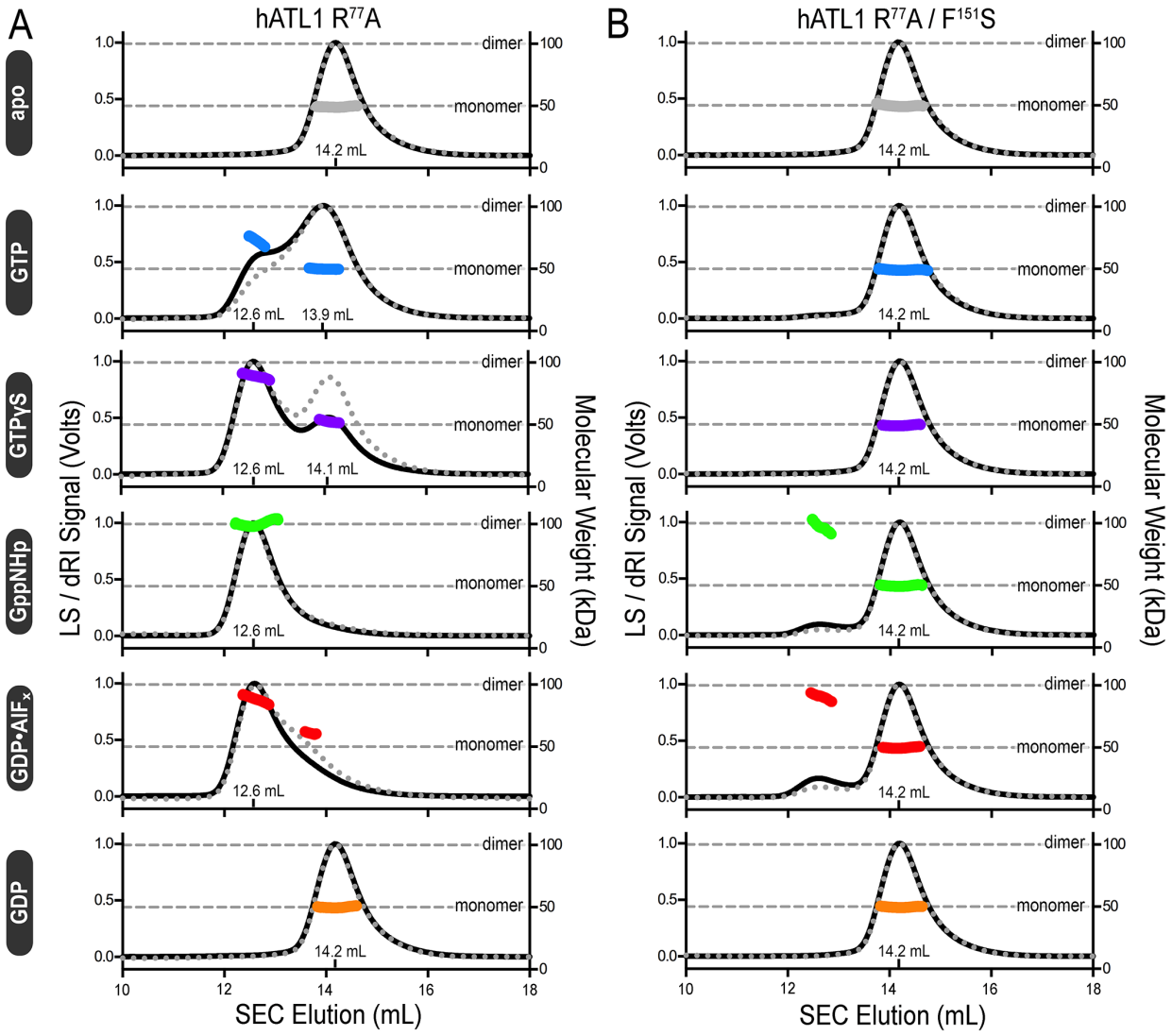

Figure S4 (associated with Figure 5). Molecular weight determination indicates R77 involvement in nucleotide-dependent conformational changes of ATL1-F^{151}S. (A) Absolute molecular weights (colored data points across elution peaks are plotted on the right axis; theoretical monomer and dimer molecular weights, horizontal gray lines) of ATL-R^{77}A catalytic core fragment (injection: 40 µM ± 1 mM nucleotide) were determined using SEC-MALS (90º-light scattering, black solid, and refractive index signal, grey dotted, are plotted on the left axis). **(B)** SEC-MALS data for the corresponding ATL1-R^{77}A/F^{151}S construct using the same experimental conditions as in (A).

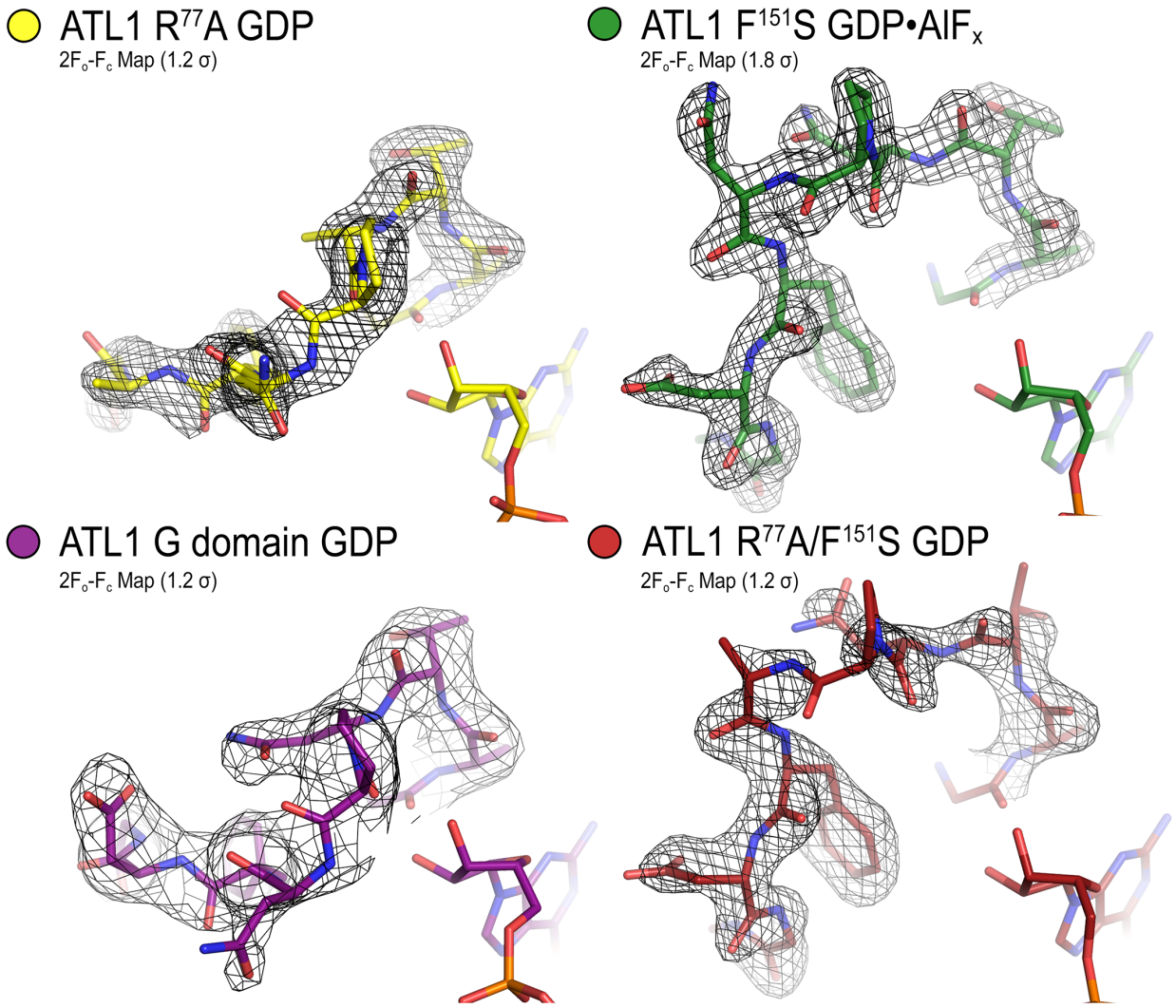

Figure S5 (associated with Figure 5E). Guanine cap electron densities. Electron densities ($2F_o$-F_c) for the guanine cap (residues 277-284) are depicted for ATL1-$R^{77}A$ bound to GDP (yellow, 1.2σ), ATL1-$F^{151}S$ bound to GDP•AlF_4^- (green, 1.8σ), ATL1 G domain (1-339) bound to GDP (purple, 1.2σ), and ATL1-$R^{77}A/F^{151}S$ bound to GDP (red, 1.2σ).