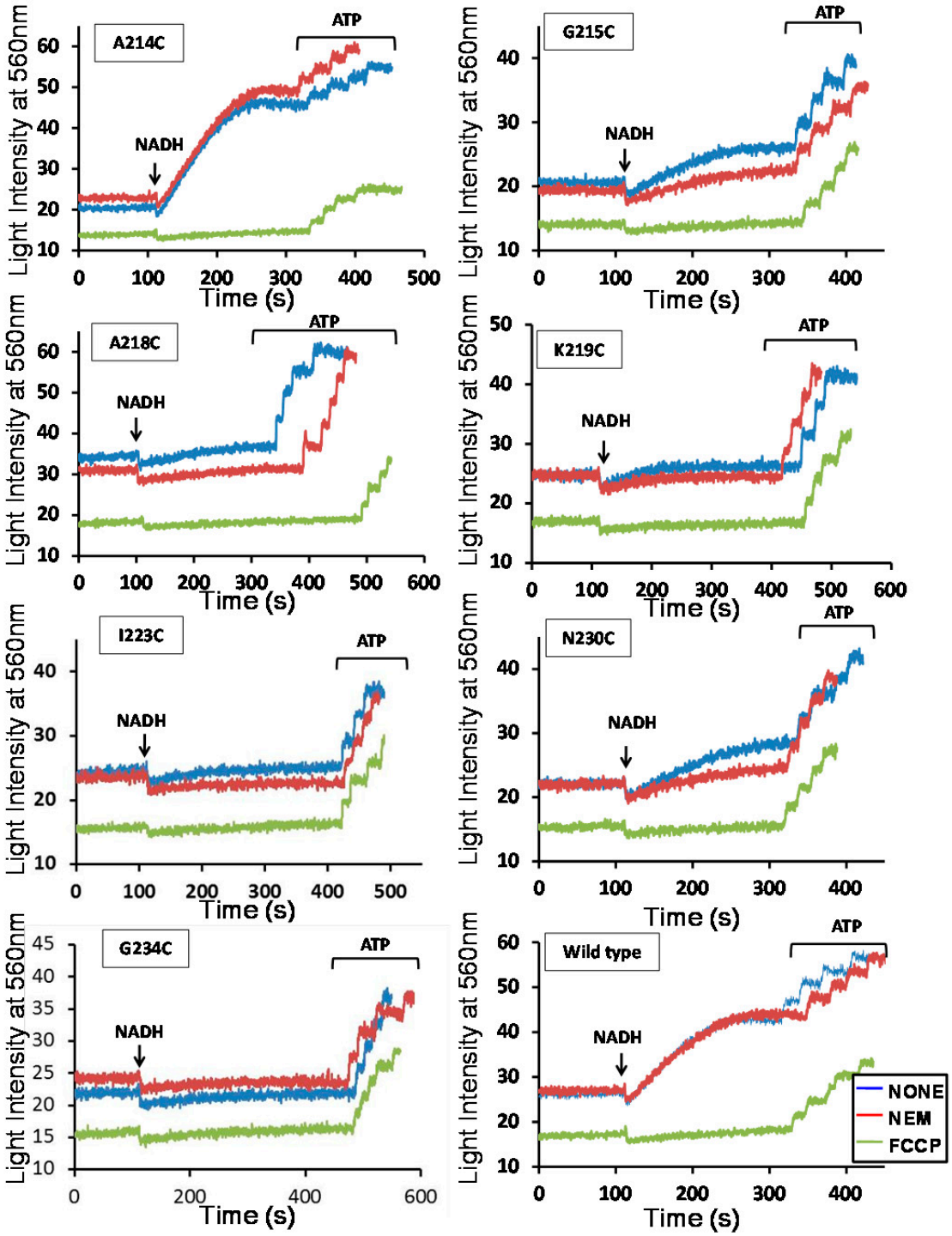
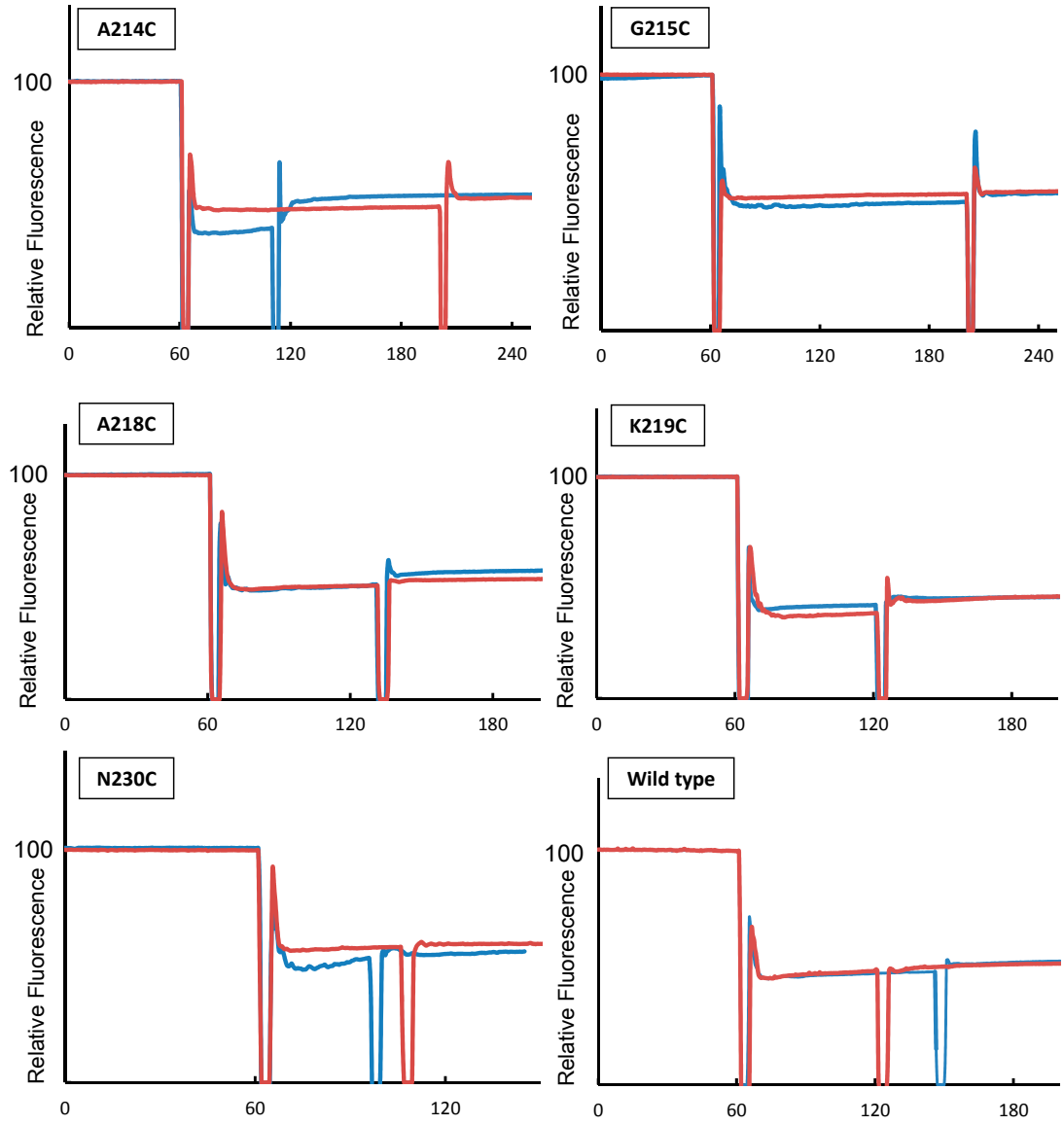


Supplementary Figure S1. ATP synthesis assay data for the wild-type protein and mutant proteins. Without NEM treatment, blue; with NEM treatment, red; PA3-NO_3 buffer containing 12.5 μL of 200 μg/mL FCCP, green. After starting the measurement, 1.5 μL of 0.5 M NADH was added. When the 560 nm value reached stability, 5 μL of 96.2 μM ATP was added for calibration, and this calibration was conducted four times.



Supplementary Figure S2. Proton pump-mediated ACMA quenching data for the wild-type protein and mutant proteins. Without NEM treatment, blue; with NEM treatment, red. The reaction was initiated by adding 1 mM K^+-ATP and terminated with FCCP. %ACMA shows the fluorescent intensity of ACMA, in which the initial intensity before the addition of ATP is calibrated as 100%.