Supporting Material

Mapping the processivity determinants of the kinesin-3 motor domain

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Materials and methods

Molecular dynamics simulations

Simulation models were based on the crystallographic structures of human kinesin-1 in complex with the microtubule in the ATP and Apo states (PDB codes 4HNA and 4LNU) (1) (2) and on the CryoEM structures for human kinesin-3 in ATP, Apo and ADP states (PDB codes 4UXP, 4UXO, 4XUS) (3). The kinesin-1 ADP state complex was built from initially merging a kinesin-1 ADP state structure (PDB code 1BG2) with tubulin from a kinesin-3 ADP complex structure (PDB code 4XUS) via superposition based on the α4 helix (residues 255-270 in kinesin-1 and 278-293 in kinesin-3). A loop refinement optimization was performed on the kinesin-1 ADP state complex with Modeller v9.10 (4) for L8c segment (residues 158-162), L11 (residues 237-253), L12 (residues 271-276) and the neck-linker (residues 326-337) and evaluated using the discrete optimized protein energy score (5). All kinesin-tubulin model structures were built with the AMBER 12 package (6) and corresponding all-atom potential function ff99SB (7). Models were inserted in a cubic water box with margins located 12Å from the closest protein atom. The energetic parameters for the nucleotide molecules were obtained from the AMBER parameter database (8). TIP3P water molecules and sodium counter ions were added to each system. Four runs of energy minimization of 4000 cycles each were employed alternating every 100 cycle the steepest descent and the conjugate gradient algorithms. Positional restrains with a force of 500 kcal / (mol $Å^2$) were applied in the first run on all protein and ligand atoms. In the second run the restrains were applied on the protein backbone only and in the third run on the water and counter ions only. In the fourth run no atoms were restrained. A fifth energy minimization run of 4000 cycles was then performed using the conjugate gradient algorithm only and no atomic restrains. Two subsequent molecular dynamics runs of 10ps and 200ps were employed to increase each system temperature from 100K to 300K and to equilibrate each system configuration at 300K. The production runs started from these equilibrated conformations. Periodic boundary conditions and full particle-mesh Ewald electrostatics were used. A 12Å cutoff value was included for truncation of non-bonded interactions. The simulations were run at constant temperature (300 K) and constant pressure (1 atm) with a 2fs time step. The SHAKE algorithm was used to constrain all covalent bonds involving hydrogen atoms. Each simulation was 40ns long, and was reproduced four times with random initial velocity assignments, for a total of 160ns of production phase dynamics for each system.

MM/GBSA calculations

Molecular mechanics with generalized Born and surface area solvation (MM/GBSA) binding energy calculations were performed with the GB^{OBC} model (9) in AMBER 12 (6). For each simulated system, pairwise energy values (resulting from setting idecomp=4) were scaled by the average number of kinesin-microtubule interaction pairs in both in kinesin-1 and kinesin-3 (39 average contacts) and averaged over four replicate simulations. The total binding free energies reported in the main text were scaled by a factor of 0.3. This was obtained from linear fitting to experimental microtubule dissociation constants determined by Hirokawa and coworkers for kinesin-3 (10). Results obtained from the combined analysis of structural interactions across models of all nucleotide states of each kinesin were used to inform experiential site-directed mutagenesis studies. Residue level comparisons of kinesin-1 and kinesin-3 motor domains utilized a Bio3D structure based sequence alignment that mapped all equivalent (aligned) and non-equivalent (un-aligned) regions (11).

Site-directed mutagenesis

Mutations were introduced into plasmids encoding constitutively motile, dimeric versions of kinesin-3 KIF1A(1-393)LZ-3xmCitrine (12). All mutations were introduced using QuikChange site-directed mutagenesis (Agilent Technologies) and verified by DNA sequencing of both strands.

Cell Culture, Transfection and Cell Lysates

COS-7 (monkey kidney fibroblast, ATCC) cells were grown in DMEM + 10% (vol/vol) FBS and 2 mM L-glutamine at 37 °C with 5% (vol/vol) CO_2. Cells were transfected with 1.0 μg of plasmid DNA using Expressfect (Danville Scientific). After 16 hrs of transfection, the cell lysates were prepared as described previously (12). Briefly, COS-7 cells expressing fluorescent protein-tagged motors were trypsinized and pelleted by low-speed centrifugation at 4 °C. The pellet was washed once with cold DMEM culture medium and lysed in ice-cold lysis buffer (25 mM HEPES/KOH, 115 mM potassium acetate, 5 mM sodium acetate, 5 mM $MgCl_2$, 0.5 mM EGTA, and 1% Triton X-100, pH 7.4) freshly supplemented with 1 mM PMSF and protease inhibitors (10 μg/mL leupeptin, 5 μg/mL chymostatin, 3 μg/mL elastatinal, and 1 mg/mL pepstatin). The lysate was clarified by centrifugation at 16,000 × g at 4 °C and either used fresh for assays or aliquots were frozen in liquid nitrogen and stored at −80 °C until further use.

Microtubule polymerization

Microtubules were polymerized from purified tubulin (TL238, Cytoskeleton) in BRB80 buffer (80 mM Pipes/KOH pH 6.8, 1 mM $MgCl_2$, 1 mM EGTA) supplemented with 1mM GTP at 37 ^oC for 20 min, stabilized by addition of 5 volumes of pre-warmed BRB80 containing 20 μM taxol, incubated for 5 min, and then stored at room temperature.

Total Internal Reflection Fluorescence Single-Molecule Motility Assays

All single-molecule assays were performed at room temperature using a Nikon Ti-E objective-type total internal reflection fluorescence (TIRF) microscope with Perfect Focus System, a 100× 1.49 N.A. CFI APO TIRF objective, an Agilent 3-Line (488, 561, and 640 nm) Monolithic Standard Power Laser Launch with AOTF, an EMCCD camera (iXon+ DU897; Andor), and controlled by Nikon Elements image acquisition software. A narrow motility chamber (~10 μl volume) was assembled by attaching a clean #1.5 coverslip to a microscope slide with double-sided tape. Polymerized microtubules were diluted in P12 buffer (12 mM Pipes/KOH pH 6.8, 1 mM $MgCl_2$, 1 mM EGTA) containing 10 μM taxol and then introduced into the motility chamber and incubated for 5 min at room temperature to adsorb onto the coverslip. Subsequently, 50 μl of blocking buffer (10 mg/ml BSA in P12 buffer with 10 μM taxol) was introduced and incubated for 20 min to prevent non-specific binding of kinesin motors onto the coverslip surface. Finally, kinesin motors in a 50 μl Motility Mix (0.1 – 2.0 μl of COS-7 cell lysate with 30 μl of blocking buffer, 15 μl of P12 buffer, 2 mM ATP, 0.5 μl of 100 mM DTT, 0.5 μl of 100 mM $MgCl_2$, and 0.5 μl each of 20 mg/ml glucose oxidase, 8 mg/ml catalase and 1M glucose) was added to the flow chamber and the ends were sealed with molten paraffin wax and imaged at 10 frames/sec without binning and at low laser power to avoid photobleaching during processive motor runs.

Data Analysis

The position of fluorescent motor spots was manually tracked frame-by-frame using a custom-written plugin in ImageJ (nih.gov). The velocities and run lengths of individual motors were binned and histograms were generated for the population by plotting the number of events in each bin. The average velocity and run length were then obtained by fitting either a single Gaussian peak (velocity) or an exponential (run length) to the population histogram. The measurements for each construct come

from at least two independent protein preparations and include motile events lasting at least five frames (500 ms). All data are presented as mean ± standard error of mean (SEM).

Supporting References

1. Gigant, B., W. Wang, B. Dreier, Q. Jiang, L. Pecqueur, A. Pluckthun, C. Wang, and M. Knossow. 2013. Structure of a kinesin-tubulin complex and implications for kinesin motility. Nature Stru Mol Bio 20:1001-1007.
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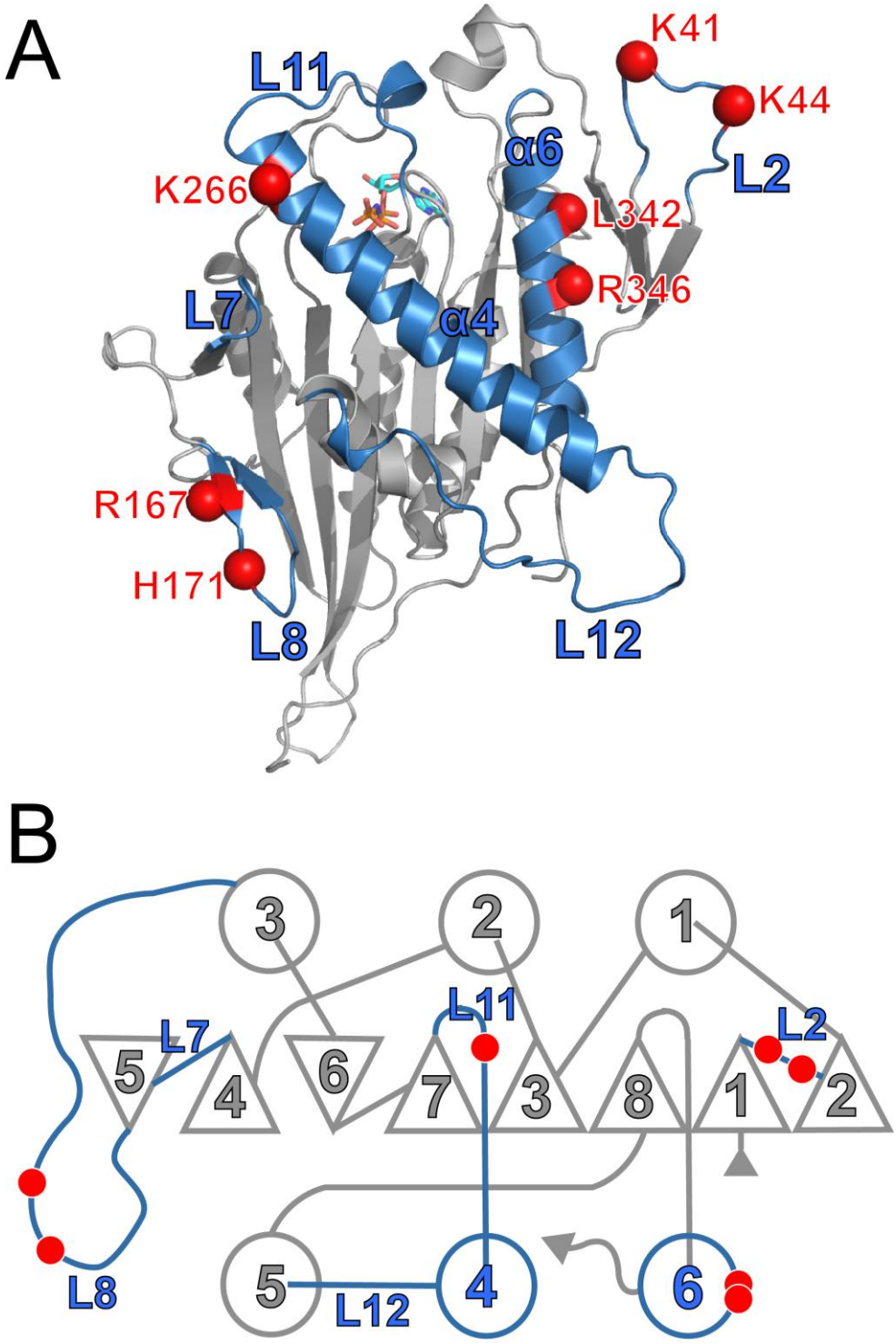

Figure S1. Kinesin-3 motor domain structure. (**A**) Kinesin regions displaying major energetic interactions with microtubule residues are rendered in blue. Red spheres indicate the location of mutation sites investigated in the current study. (**B**) Topological diagram of the kinesin-3 motor domain. Major β-strands are depicted as triangles and α-helices as circles. Regions are colored as in panel **A.**

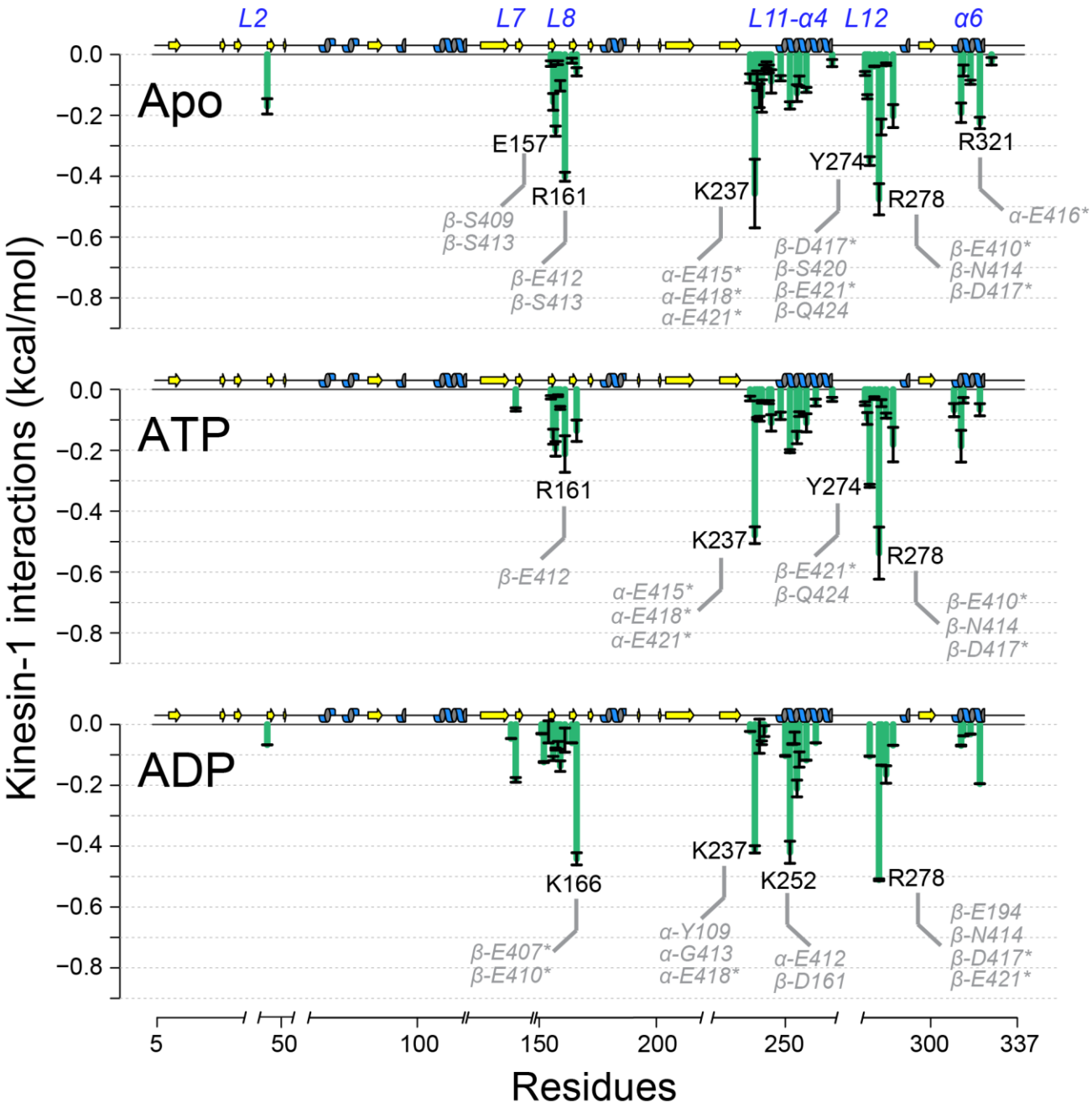

Figure S2. Kinesin-1 residue contributions to tubulin binding energy. Kinesin residues with prominent contributions are labeled in black and their major tubulin interacting partners are labeled in gray.

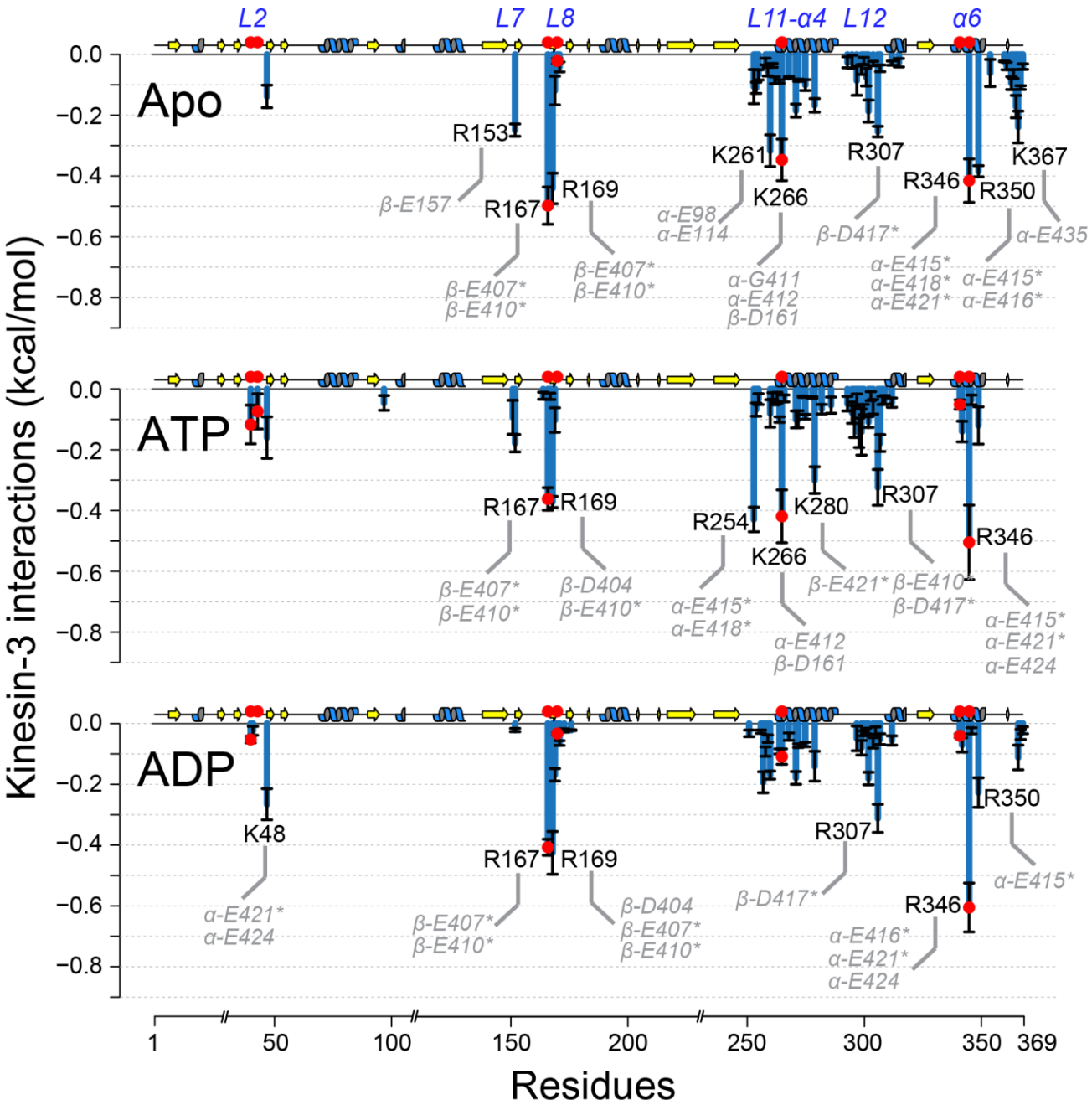

Figure S3. Kinesin-3 residue contributions to tubulin binding energy. Kinesin residues with prominent contributions are labeled in black and their major tubulin interacting partners are labeled in gray. The location of residues subjected to mutagenesis simulations and single molecule experiments are indicated with red points.

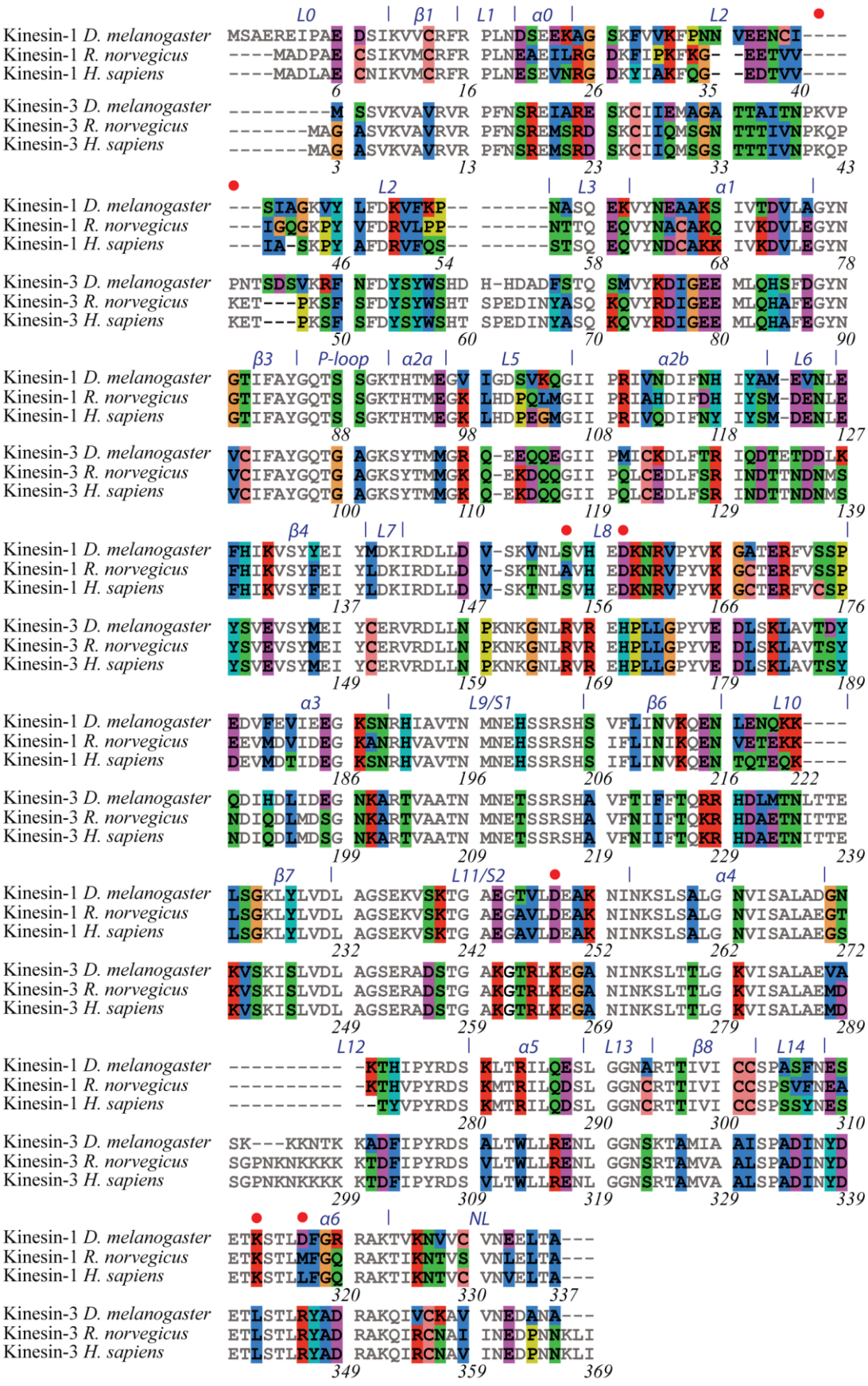
L0 β1 L1 α0 L2
Kinesin-1 D. melanogaster MSAEREIPAE DSIKVVCRFR PLNDSEEKAG SKFVVKFPNN VEENCI----
Kinesin-1 R. norvegicus ----MADPAE CSIKVMCRFR PLNEAEILRG DKFIPKFKG- -EETVV----
Kinesin-1 H. sapiens ----MADLAE CNIKVMCRFR PLNESEVNRG DKYIAKFQG- -EDTVV----
6 16 26 35 40
Kinesin-3 D. melanogaster ---------M SSVKVAVRVR PFNSREIARE SKCIIEMAGA TTAITNPKVP
Kinesin-3 R. norvegicus -------MAG ASVKVAVRVR PFNSREMSRD SKCIIQMSGN TTTIVNPKQP
Kinesin-3 H. sapiens -------MAG ASVKVAVRVR PFNSREMSRD SKCIIQMSGS TTTIVNPKQP
3 13 23 33 43
L2 L3 α1
Kinesin-1 D. melanogaster ---SIAGKVY LFDKVFKP-- ------NASQ EKVYNEAAKS IVTDVLAGYN
Kinesin-1 R. norvegicus ---IGQGKPY VFDRVLPP-- ------NTTQ EQVYNACAKQ IVKDVLEGYN
Kinesin-1 H. sapiens ---IA-SKPY AFDRVFQS-- ------STSQ EQVYNDCAKK IVKDVLEGYN
46 54 58 68 78
Kinesin-3 D. melanogaster PNTSDSVKRF NFDYSYWSHD H-HDADFSTQ SMVYKDIGEE MLQHSFDGYN
Kinesin-3 R. norvegicus KET---PKSF SFDYSYWSHT SPEDINYASQ KQVYRDIGEE MLQHAFEGYN
Kinesin-3 H. sapiens KET---PKSF SFDYSYWSHT SPEDINYASQ KQVYRDIGEE MLQHAFEGYN
50 60 70 80 90
β3 P-loop α2a L5 α2b L6
Kinesin-1 D. melanogaster GTIFAYGQTS SGKTHTMEGV IGDSVKQGII PRIVNDIFNH IYAM-EVNLE
Kinesin-1 R. norvegicus GTIFAYGQTS SGKTHTMEGK LHDPQLMGII PRIAHDIFDH IYSM-DENLE
Kinesin-1 H. sapiens GTIFAYGQTS SGKTHTMEGK LHDPEGMGII PRIVQDIFNY IYSM-DENLE
88 98 108 118 127
Kinesin-3 D. melanogaster VCIFAYGQTG AGKSYTMMGR Q-EEQQEGII PMICKDLFTR IQDTETDDLK
Kinesin-3 R. norvegicus VCIFAYGQTG AGKSYTMMGK Q-EKDQQGII PQLCEDLFSR INDTTNDNMS
Kinesin-3 H. sapiens VCIFAYGQTG AGKSYTMMGK Q-EKDQQGII PQLCEDLFSR INDTTNDNMS
100 110 119 129 139
β4 L7 L8
Kinesin-1 D. melanogaster FHIKVSYYEI YMDKIRDLLD V-SKVNLSVH EDKNRVPYVK GATERFVSSP
Kinesin-1 R. norvegicus FHIKVSYFEI YLDKIRDLLD V-SKTNLAVH EDKNRVPYVK GCTERFVSSP
Kinesin-1 H. sapiens FHIKVSYFEI YLDKIRDLLD V-SKTNLSVH EDKNRVPYVK GCTERFVCSP
137 147 156 166 176
Kinesin-3 D. melanogaster YSVEVSYMEI YCERVRDLLN PKNKGNLRVR EHPLLGPYVE DLSKLAVTDY
Kinesin-3 R. norvegicus YSVEVSYMEI YCERVRDLLN PKNKGNLRVR EHPLLGPYVE DLSKLAVTSY
Kinesin-3 H. sapiens YSVEVSYMEI YCERVRDLLN PKNKGNLRVR EHPLLGPYVE DLSKLAVTSY
149 159 169 179 189
α3 L9/S1 β6 L10
Kinesin-1 D. melanogaster EDVFEVIEEG KSNRHIAVTN MNEHSSRSHS VFLINVKQEN LENQKK----
Kinesin-1 R. norvegicus EEVMDVIDEG KANRHVAVTN MNEHSSRSHS IFLINIKQEN VETEKK----
Kinesin-1 H. sapiens DEVMDTIDEG KSNRHVAVTN MNEHSSRSHS IFLINVKQEN TQTEQK----
186 196 206 216 222
Kinesin-3 D. melanogaster QDIHDLIDEG NKARTVAATN MNETSSRSHA VFTIFFTQRR HDLMTNLTTE
Kinesin-3 R. norvegicus NDIQDLMDSG NKARTVAATN MNETSSRSHA VFNIIFTQKR HDAETNITTE
Kinesin-3 H. sapiens NDIQDLMDSG NKARTVAATN MNETSSRSHA VFNIIFTQKR HDAETNITTE
199 209 219 229 239
β7 L11/S2 α4
Kinesin-1 D. melanogaster LSGKLYLVDL AGSEKVSKTG AEGTVLDEAK NINKSLSALG NVISALADGN
Kinesin-1 R. norvegicus LSGKLYLVDL AGSEKVSKTG AEGAVLDEAK NINKSLSALG NVISALAEGT
Kinesin-1 H. sapiens LSGKLYLVDL AGSEKVSKTG AEGAVLDEAK NINKSLSALG NVISALAEGS
232 242 252 262 272
Kinesin-3 D. melanogaster KVSKISLVDL AGSERADSTG AKGTRLKEGA NINKSLTTLG KVISALAEVA
Kinesin-3 R. norvegicus KVSKISLVDL AGSERADSTG AKGTRLKEGA NINKSLTTLG KVISALAEMD
Kinesin-3 H. sapiens KVSKISLVDL AGSERADSTG AKGTRLKEGA NINKSLTTLG KVISALAEMD
249 259 269 279 289
L12 α5 L13 β8 L14
Kinesin-1 D. melanogaster ---------- -KTHIPYRDS KLTRILQESL GGNARTTIVI CCSPASFNES
Kinesin-1 R. norvegicus ---------- -KTHVPYRDS KMTRILQDSL GGNCRTTIVI CCSPSVFNEA
Kinesin-1 H. sapiens ---------- --TYVPYRDS KMTRILQDSL GGNCRTTIVI CCSPSSYNES
280 290 300 310
Kinesin-3 D. melanogaster SK---KKNTK KADFIPYRDS ALTWLLRENL GGNSKTAMIA AISPADINYD
Kinesin-3 R. norvegicus SGPNKNKKKK KTDFIPYRDS VLTWLLRENL GGNSRTAMVA ALSPADINYD
Kinesin-3 H. sapiens SGPNKNKKKK KTDFIPYRDS VLTWLLRENL GGNSRTAMVA ALSPADINYD
299 309 319 329 339
α6 NL
Kinesin-1 D. melanogaster ETKSTLDFGR RAKTVKNVVC VNEELTA---
Kinesin-1 R. norvegicus ETKSTLMFGQ RAKTIKNTVS VNLELTA---
Kinesin-1 H. sapiens ETKSTLLFGQ RAKTIKNTVC VNVELTA---
320 330 337
Kinesin-3 D. melanogaster ETLSTLRYAD RAKQIVCKAV VNEDANA---
Kinesin-3 R. norvegicus ETLSTLRYAD RAKQIRCNAI INEDPNNKLI
Kinesin-3 H. sapiens ETLSTLRYAD RAKQIRCNAV INEDPNNKLI
349 359 369

Figure S4. (Previous page). **Kinesin-1 and kinesin-3 structure based sequence alignment**. Residue numbers (black text) refer to Kinesin-1 and Kinesin-3 *H. sapiens*. Positions with divergent residue types are colored by their physiochemical properties (red positively charged; purple negatively charged; blue apolar; green polar; cyan Tyrosine and Histidine; Glycine orange; Cysteine pink and Proline yellow). Red dots indicate the positions of experimental mutations noted in the main text. Blue labels reference major secondary structure elements.

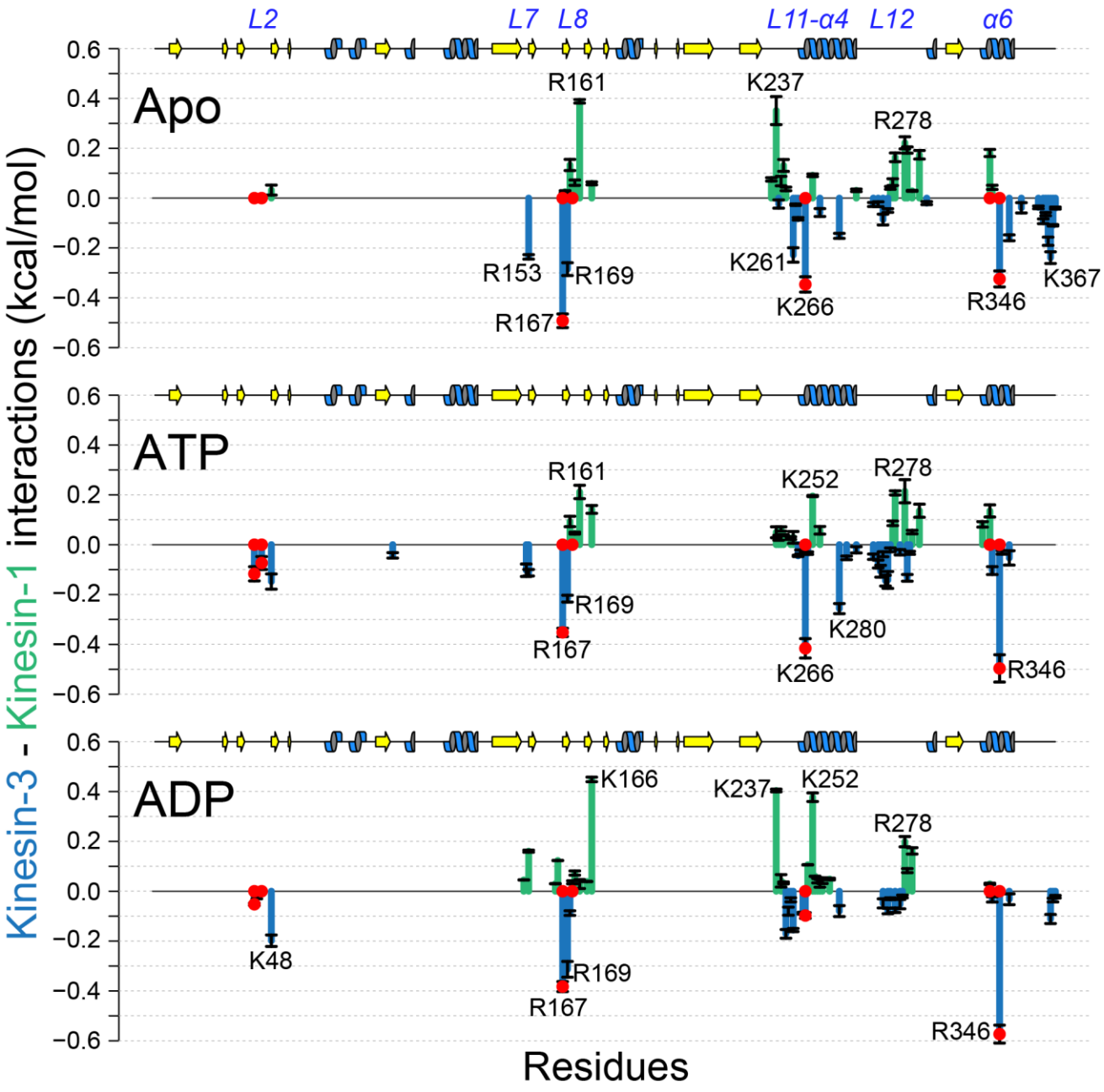

Figure S5. Differences in residue contribution to binding energy for kinesin-1 (green) and kinesin-3 (blue) in the Apo, ATP and ADP states. These values were determined from 4 replicate 40ns molecular dynamics simulations and subsequent energetic calculations. The location of residues subjected to mutagenesis simulations and single molecule experiments are indicated with red points.

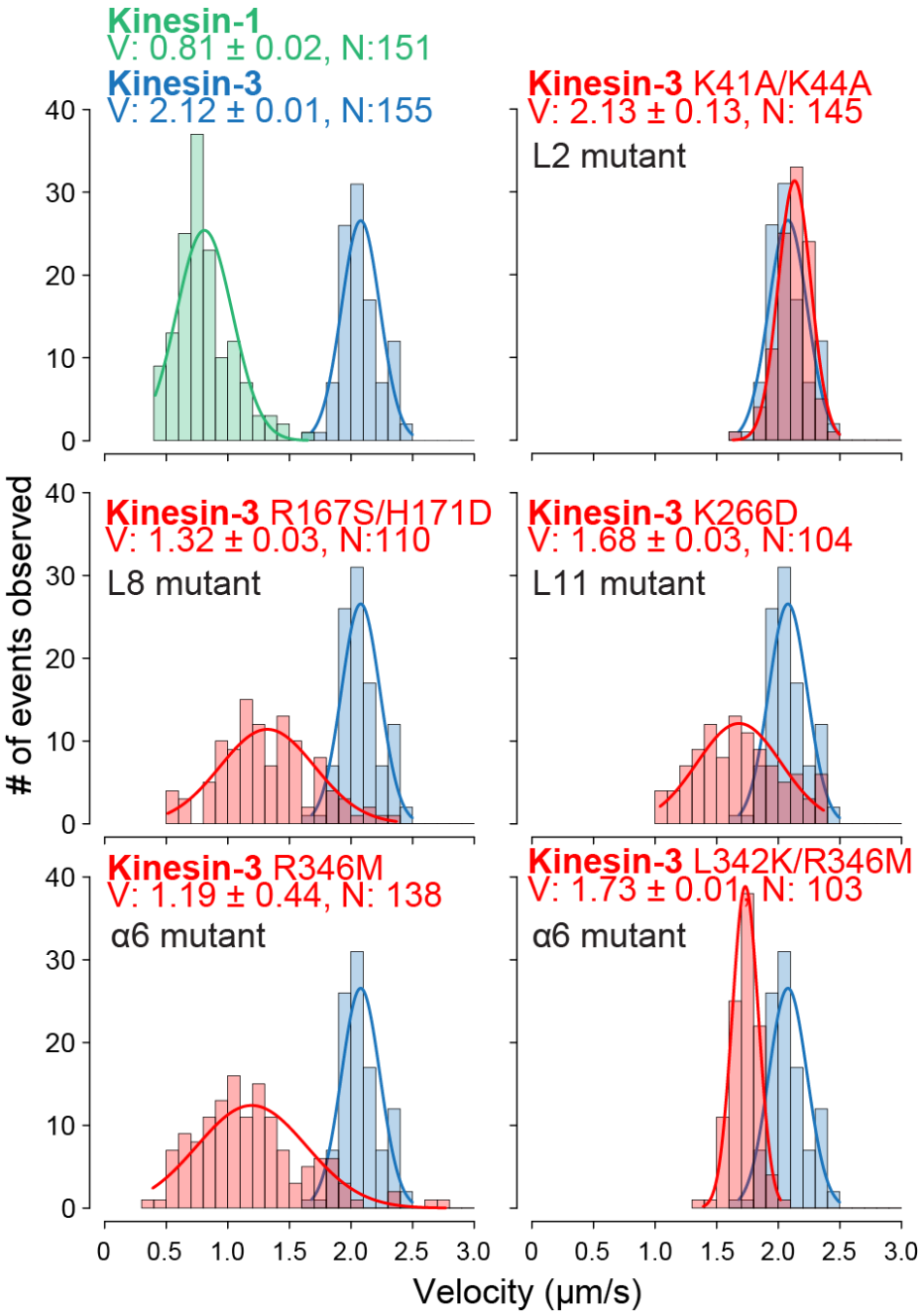

Figure S6. Experimental velocity measurements for kinesin-1 wild type, kinesin-3 wild type and kinesin-3 mutants.

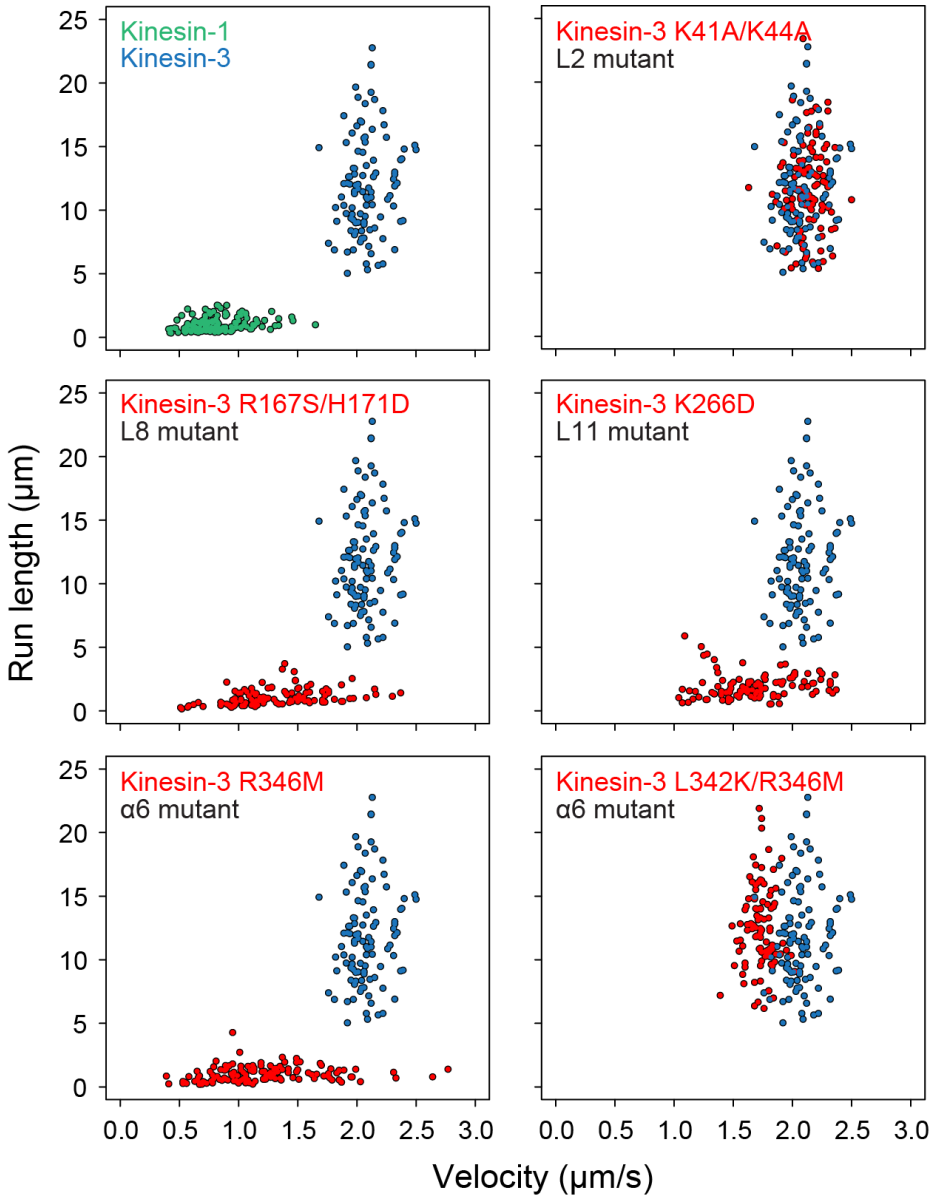

Figure S7. Experimental velocity versus Run length measurements for kinesin-1 wild type (green), kinesin-3 wild type (blue) and kinesin-3 mutants (red).

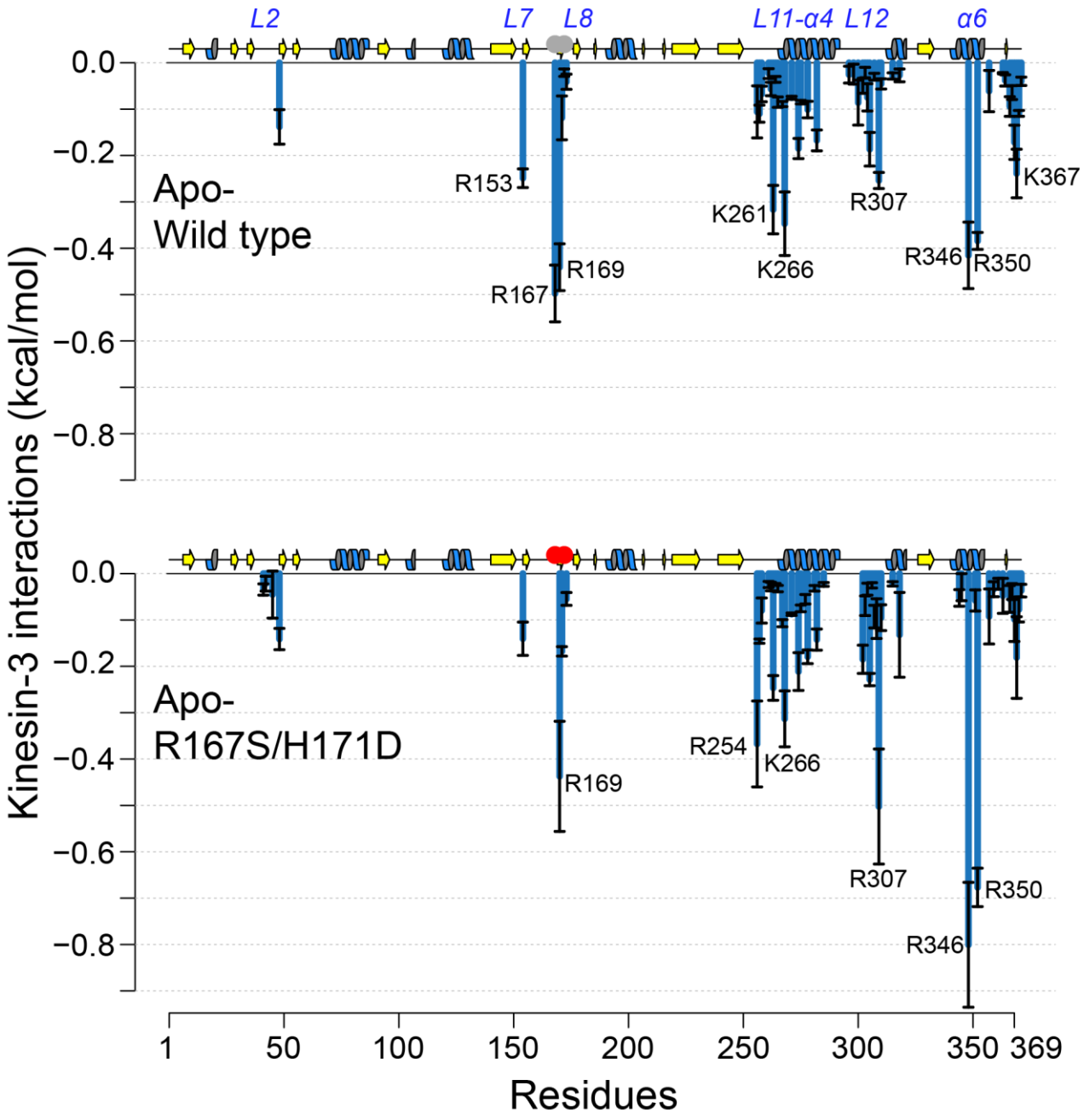

Figure S8. Results for the kinesin-3 L8 double mutant R167S/H171D in the Apo state. Kinesin residues with prominent contributions are labeled in black. The mutation sites are indicated with gray points in the WT plot and with red points in the L8 mutant plot. Note the absence of contributions from position 167 in the mutant but enhanced contributions from R307, R346 and R350.