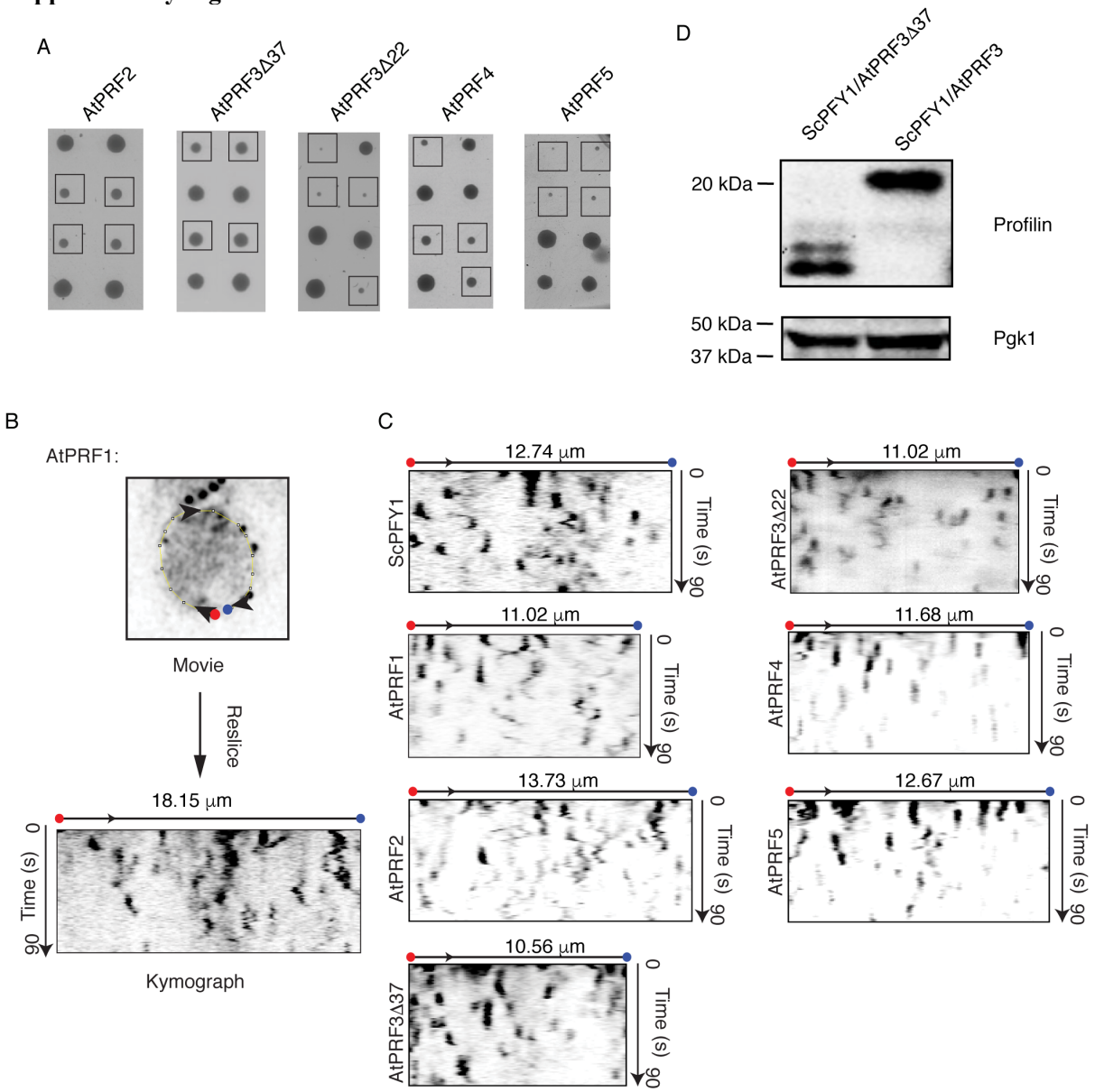
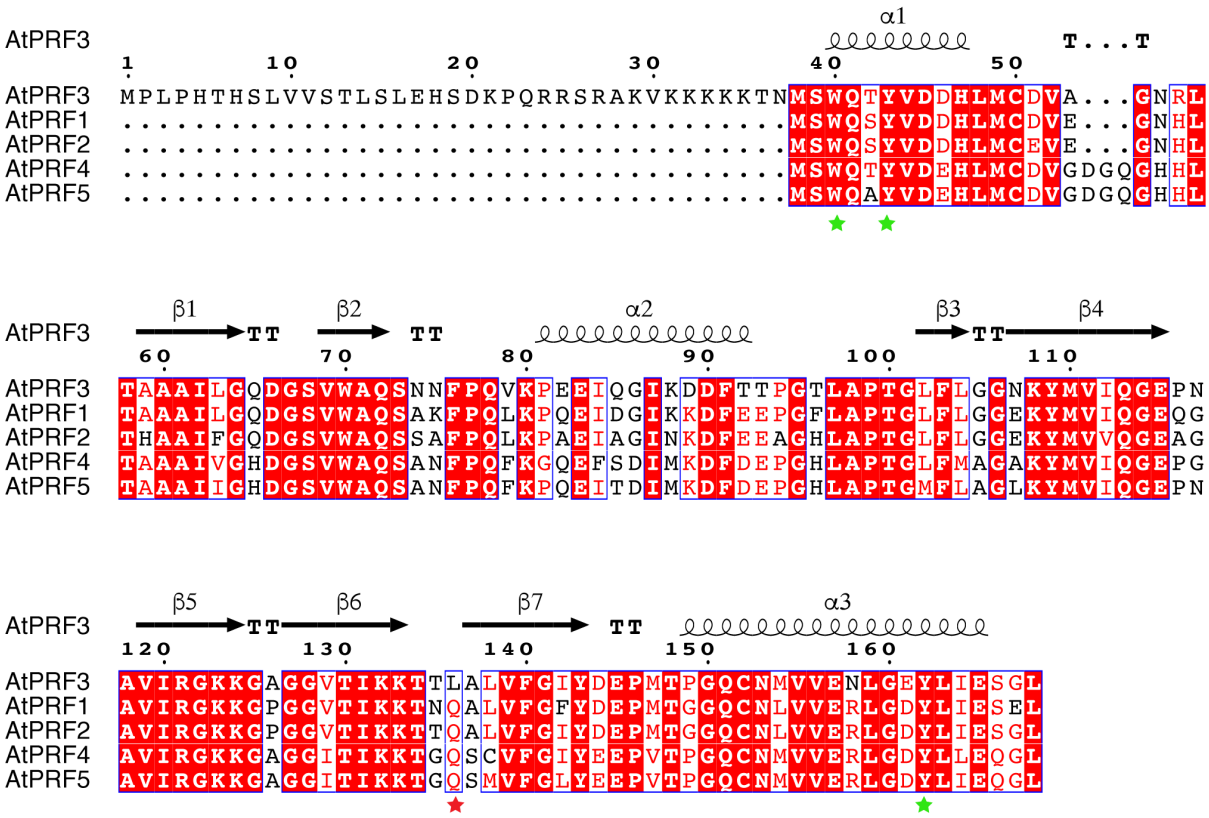
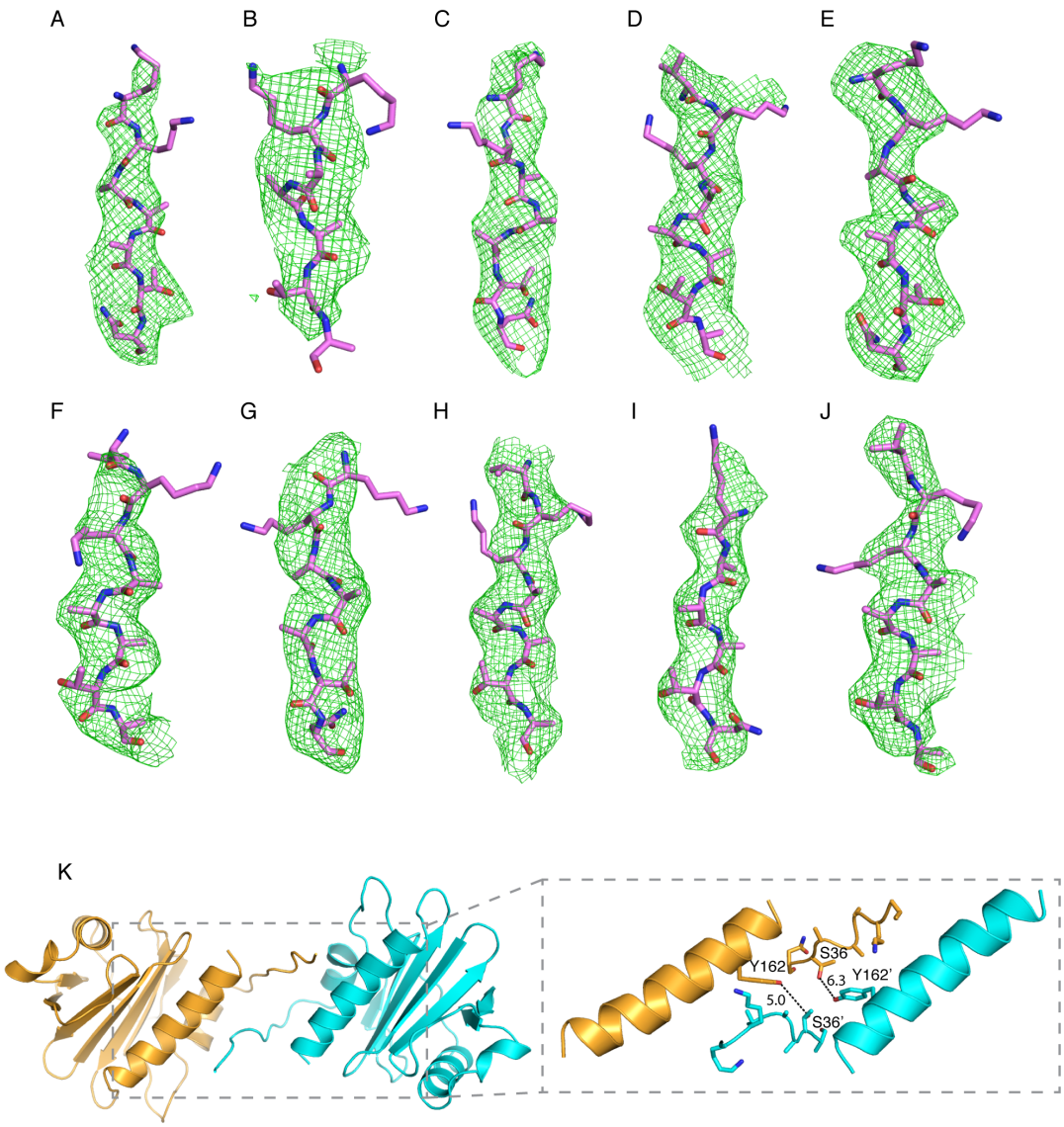
Supplementary Figures



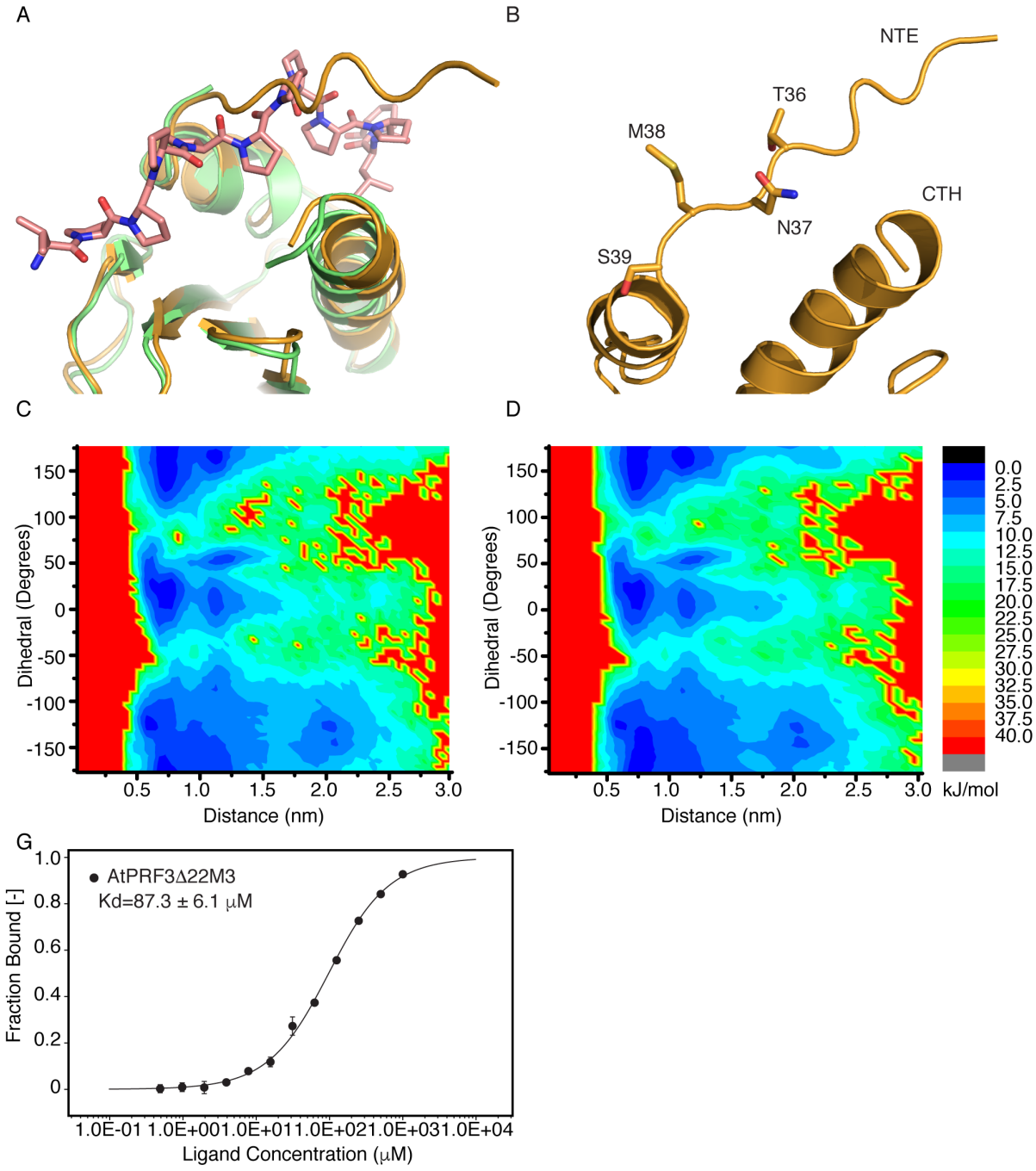
Supplementary Figure 1. *In Vivo* Functional Analysis of AtPRF3 in Budding Yeast. (A) Tetrad analysis of heterozygous diploid strains expressing AtPRFs. Squares highlight the colonies expressing the AtPRFs. (B, C) Dynamic internalization of actin patches at cell cortex was streamed during image acquisition with 1s of exposure and 90 frames. Kymographs of representative Abp1-mRFP were shown for each test strain. (D) Western blot detection of AtPRF isoforms in the indicated diploid strains using antibodies against Myc and loading control Pgk1. The protein markers were indicated.



Supplementary Figure 2. Multiple Sequence Alignment of AtPRFs with secondary structural features generated based on AtPRF3Δ37. The conserved Poly-P binding sites were labeled by the green star. The L136 of AtPRF3 was labeled by the red star



Supplementary Figure 3. Unbiased Fo-Fc Map and Crystallographic Dimer of AtPRF3Δ22M2. (A) to (J) The unbiased omit Fo-Fc electron density map countered at 2.2σ of all ten chains with N terminus from K31 to N37 in one asymmetrical unit of AtPRF3Δ22M2. (K) The crystallographic dimer of AtPRF3Δ22M2 on the left with the dimer interface shown on the right.



Supplementary Figure 4. Molecular Dynamic Simulation of AtPRF3Δ22. (A) Structural alignment of Poly-P (salmon) bound AtPRF2 (lime) to AtPRF3Δ22M2 (light orange). (B) Reaction coordinates for free energy surface projection. Dihedral angle formed by the α carbons of residues 36 to 39. And center of mass distance between N-Terminal extension (NTE) and C-Terminal Helix (CTH). (C) and (D) Convergence of hamiltonian replica exchange molecular dynamics simulation which was evaluated by plotting the free energy surface over various time blocks, 100-550 ns (C) and 100-650 ns (D). (E) Microscale thermophoresis binding curves of Poly-P to AtPRF3Δ22M3 with the dissociation constant indicated.

Supplementary Table 1. Minima Counts for Each Minimum of Free Energy Surface

Minimum	Count	Percentage of Total
minimum1	15895	4.54
minimum2	36473	10.42
minimum3	18440	5.27
minimum4	3973	1.14
minimum5	6980	1.99
minimum6	7148	2.04
Minimum7	1993	0.57