Supplemental Materials

Molecular Biology of the Cell

Ly et al.

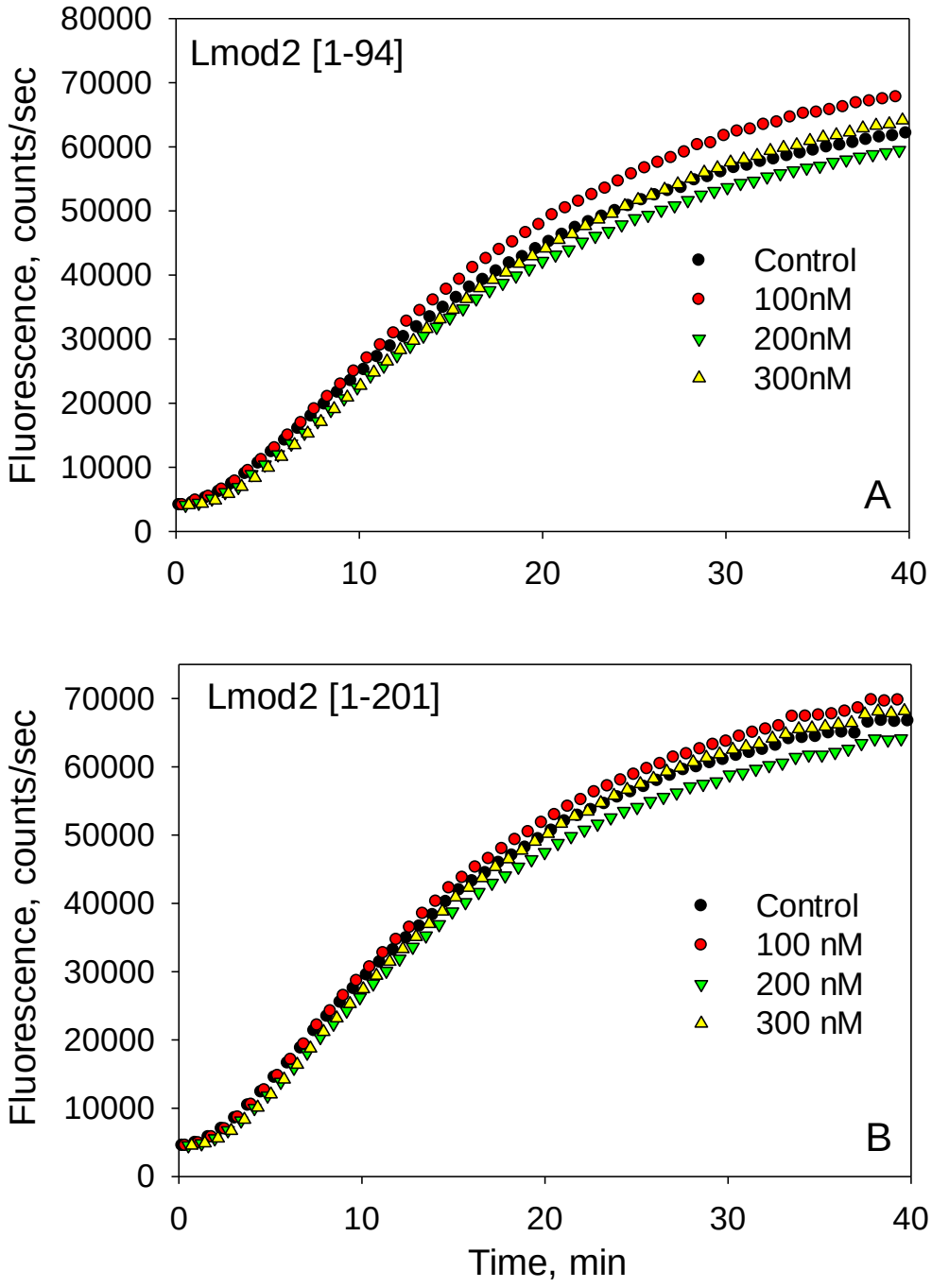

Figure S1. Polymerization of 1.5 µM G-actin/0.15 µM pyrene-G-actin by adding the 20X polymerization buffer (final concentration: 100 mM KCl, 1 mM MgCl2, 1 mM EGTA, 25 mM imidazole, pH 7.0) in the presence of different concentrations of Lmod2 [1-94] (A) or Lmod2 [1-201] (B). Control: actin alone. Both fragments did not have any concentration-dependent nucleating activity.

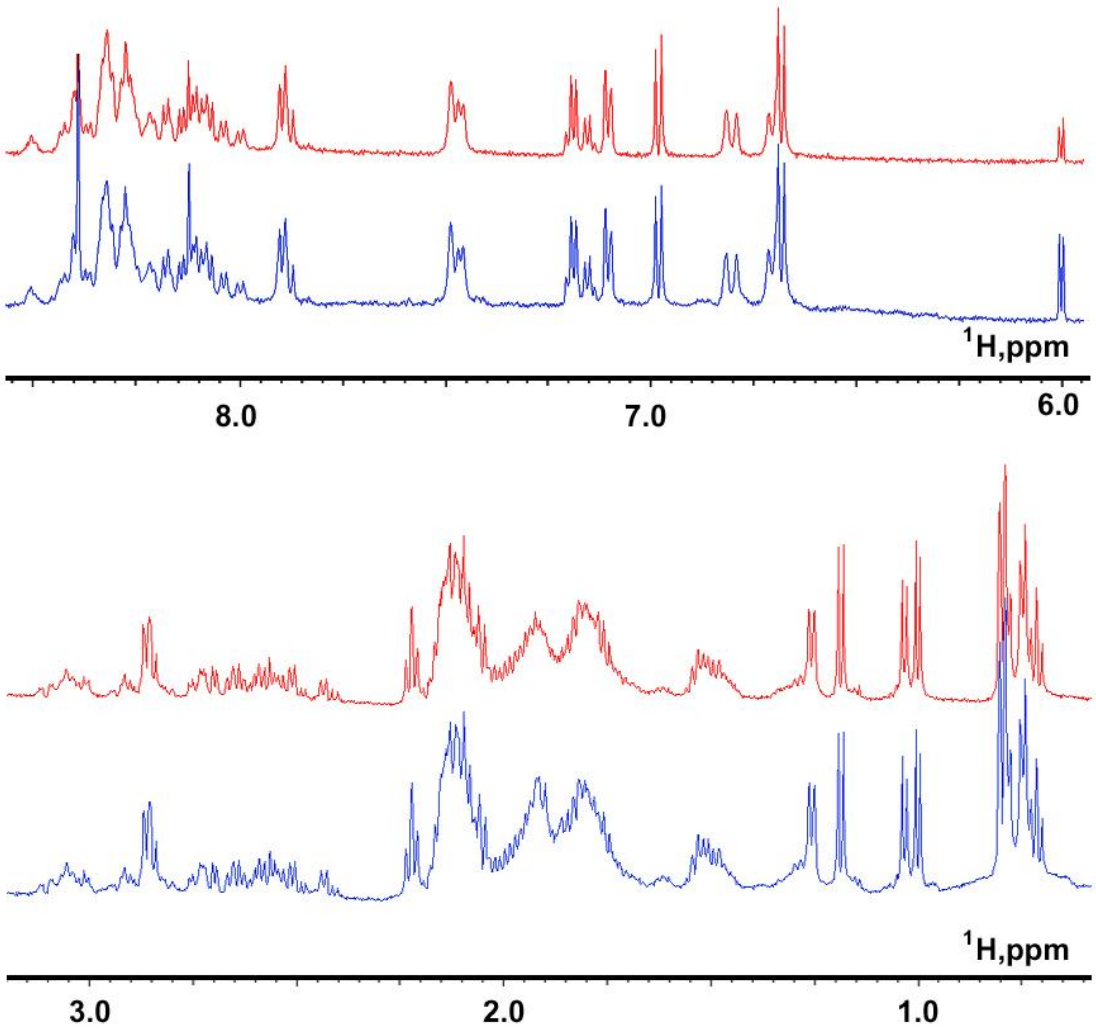

Figure S2. The fragment Lmod2 [85-123] and G-actin do not interact within the range of affinities detectable by NMR. NMR proton spectra of 0.2 mM Lmod2 [85-123] in the absence (red) and presence (blue) of 10 μM G-actin were compared in the amide (top panel) and side-chain (bottom panel) chemical shift regions in 2 mM potassium phosphate, 0.1 mM ATP, 0.1 mM $CaCl_2$, 0.01% NaN_3, pH 6.8. The width/position of peaks did not alter, suggesting that there is no detectable binding between actin and the Lmod2 [85-123]. The NMR spectra were recorded at 25^{o}C.

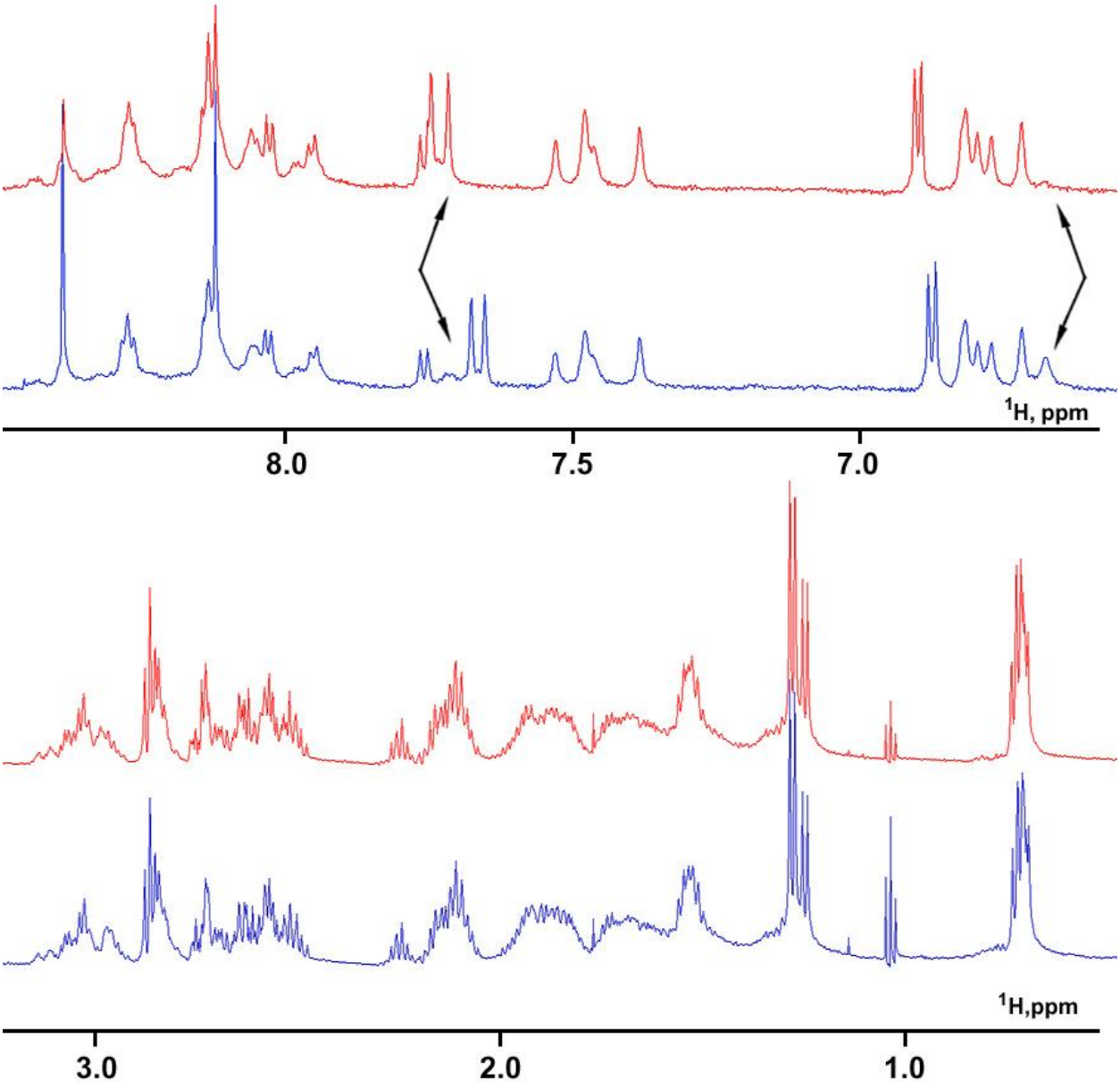

Figure S3. NMR proton spectra of 0.2 mM Lmod2 [140-180] in the absence (red) and presence (blue) of 10 μM G-actin were compared in the amide (top panel) and side-chain (bottom panel) chemical shift regions in 2 mM potassium phosphate, 0.1 mM ATP, 0.1 mM $CaCl_2$, 0.01% NaN_3, pH 6.8. In the presence of actin some resonance peaks (indicated with arrows) manifested differential broadening/shifts. The NMR spectra were recorded at 25^{o}C.

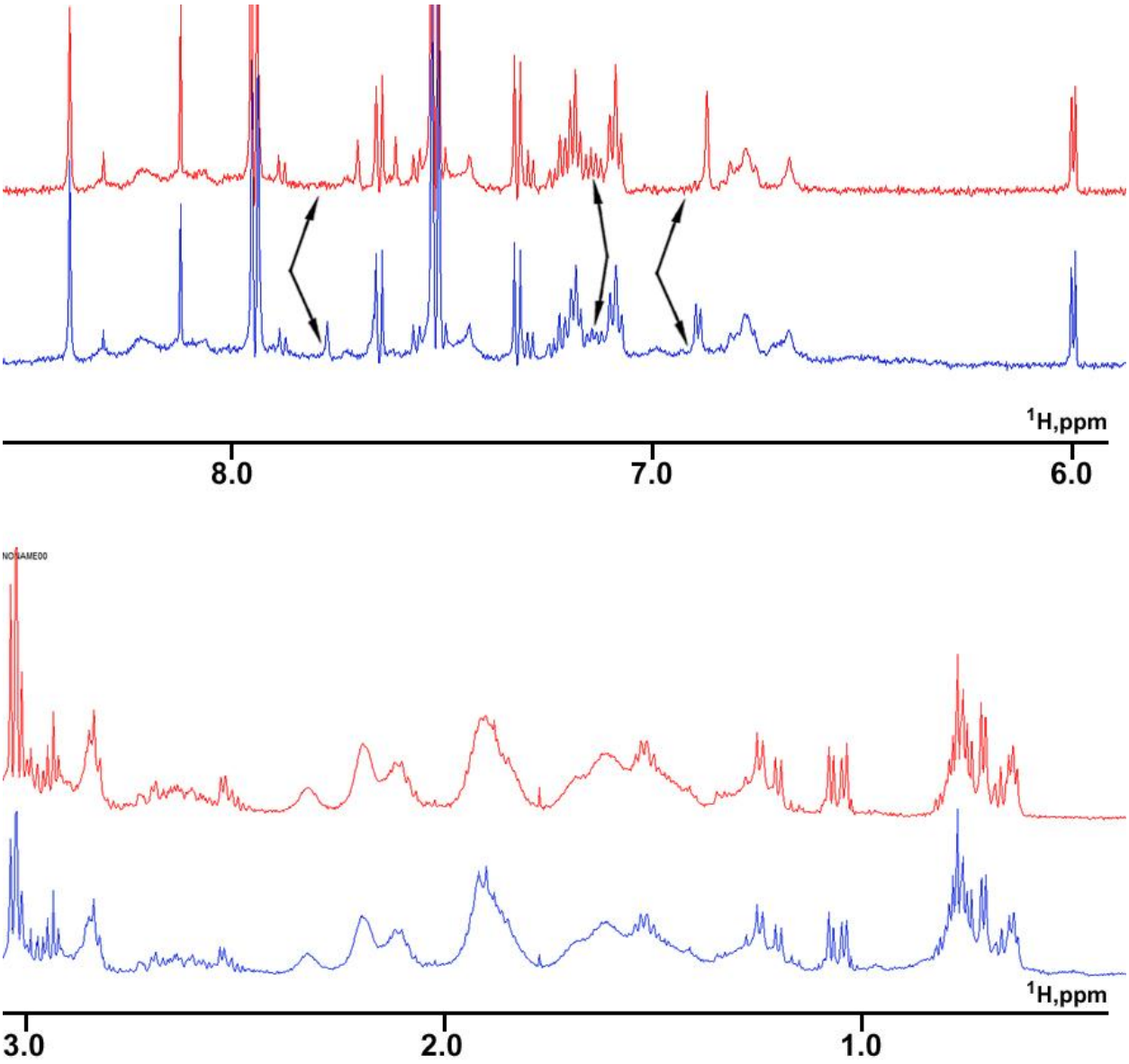

Figure S4. NMR proton spectra of 0.2 mM Lmod2 [172-210] in the absence (red) and presence (blue) of 10 μM G-actin were compared in the amide (top panel) and side-chain (bottom panel) chemical shift regions in 2 mM potassium phosphate, 0.1 mM ATP, 0.1 mM $CaCl_2$, 0.01% NaN_3, pH 6.8. In the presence of actin some resonance peaks (indicated with arrows) manifested differential broadening/shifts. The NMR spectra were recorded at 25^{o}C.

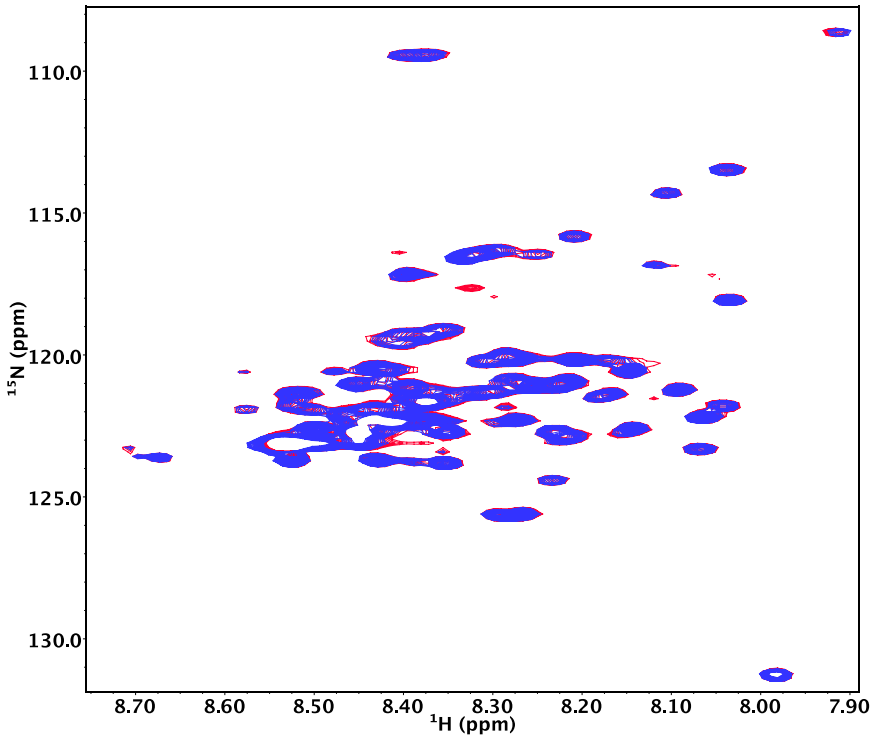

Figure S5. An overlay of 2D ^{15}N-HSQC spectra of 0.1 mM ^{15}N-labeled Lmod2 [103-201] in the absence (red) and presence (blue) of 10 μM G-actin in 2 mM potassium phosphate, 0.1 mM ATP, 0.1 mM $CaCl_2$, 0.01% NaN_3, pH 6.8. The NMR spectra were recorded at 25^{o}C.

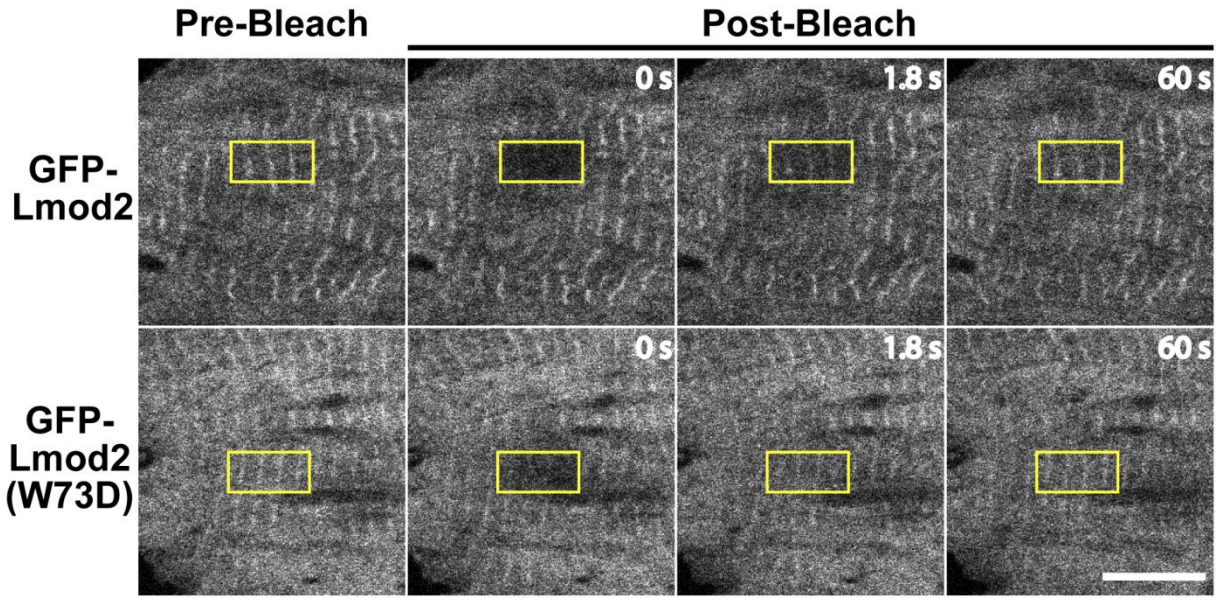

Figure S6. Representative images of GFP-Lmod2 and GFP-Lmod2(W73D) before and after photobleaching. The yellow box indicates area of photobleach. Scale bar, 10 μm.