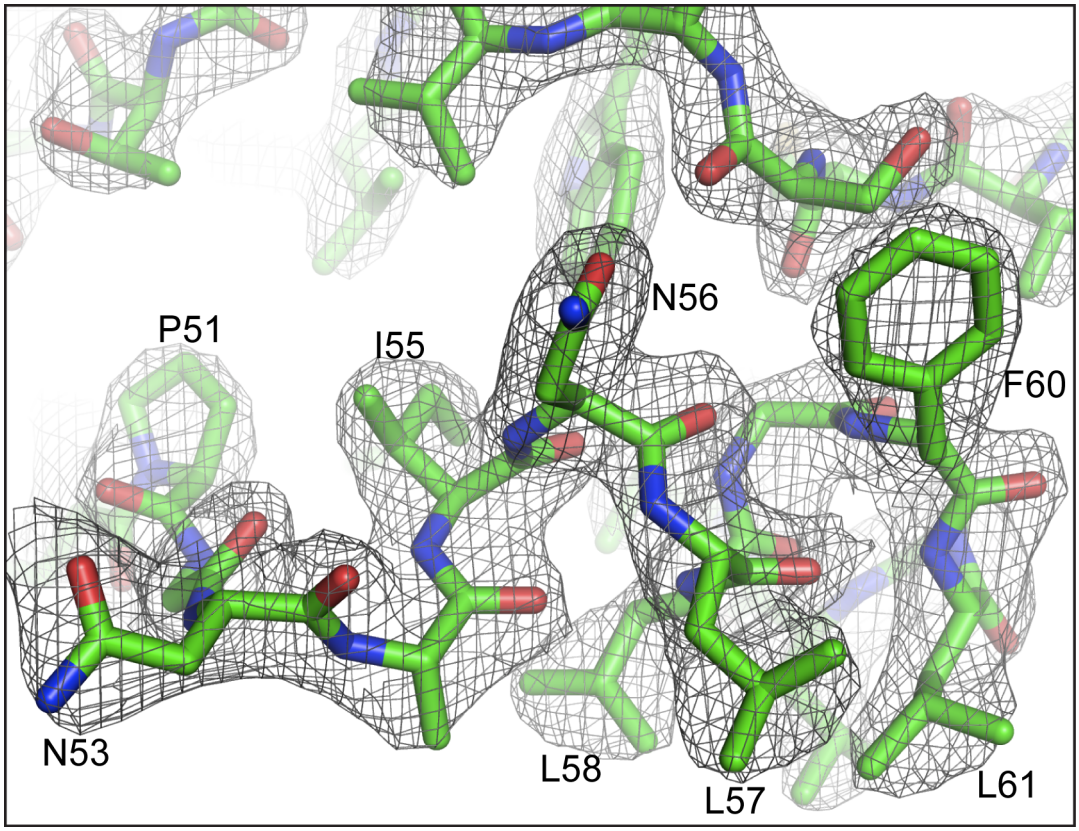
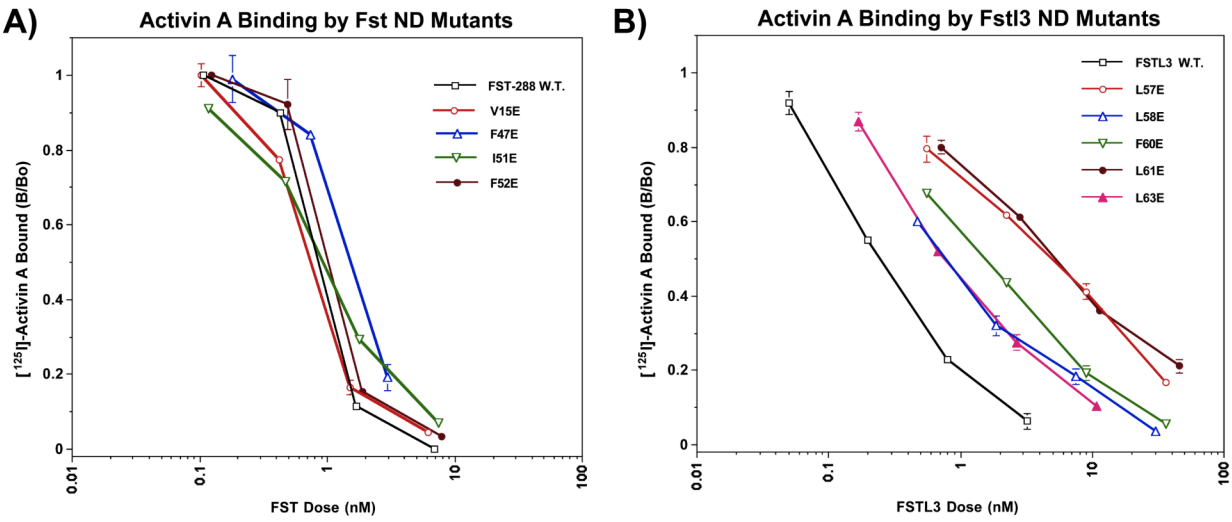
Supplemental Figure 1



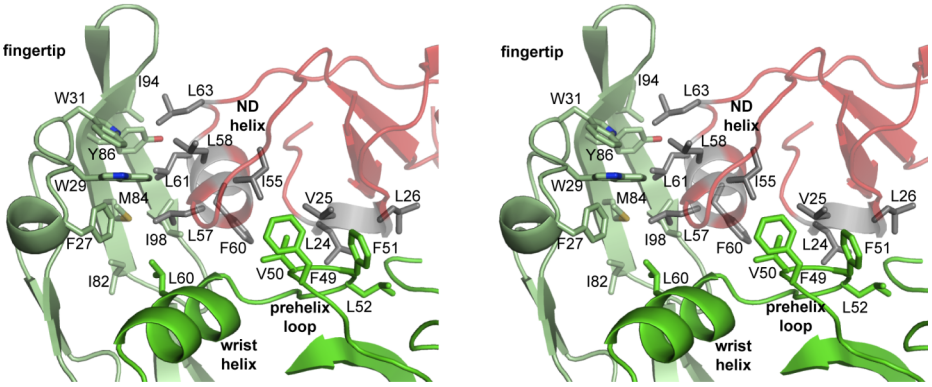
Supplemental Figure 1: Residues in the Fstl3 ND helix are well resolved. 2Fo-Fc electron density map with SIGMAA weighted coefficients contoured at 1.5σ outlining residues in the ND helix region of Fstl3. Fstl3 ND helix residues 49-65 (as well as myostatin residues 26-32) were removed from the initial search model to avoid bias in these areas.

Supplemental Figure 2



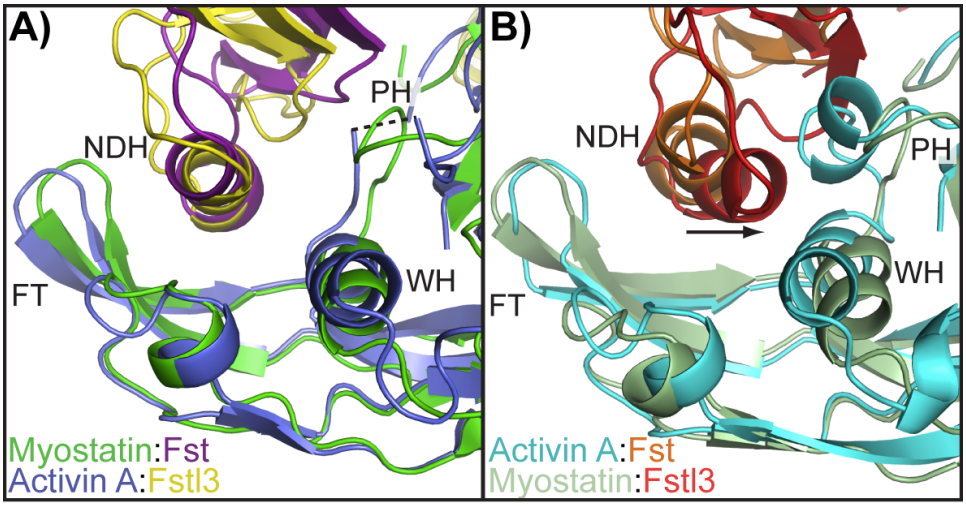
Supplemental Figure 2: Experimental binding data for radiolabeled activin A-binding assays. Binding by Fst-type molecules was determined as described in the Experimental Procedures.

Supplemental Figure 3



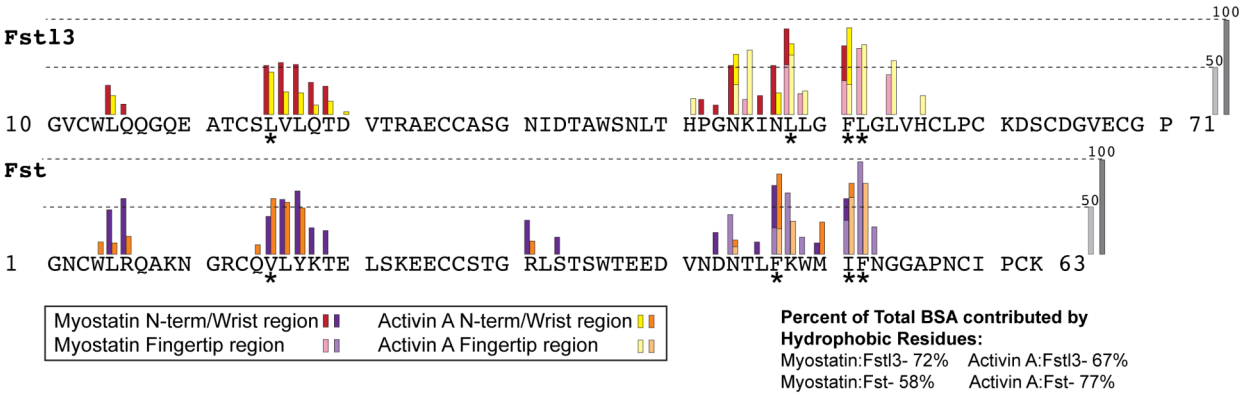
Supplemental Figure 3: Stereoview of the hydrophobic interface between the Fstl3 ND and myostatin. Residues at the hydrophobic interface between myostatin (green) and Fstl3 (red with grey residues) are shown. Fstl3 interacts with the prehelix loop of myostatin, which is disordered in the activin A complex structure.

Supplemental Figure 4



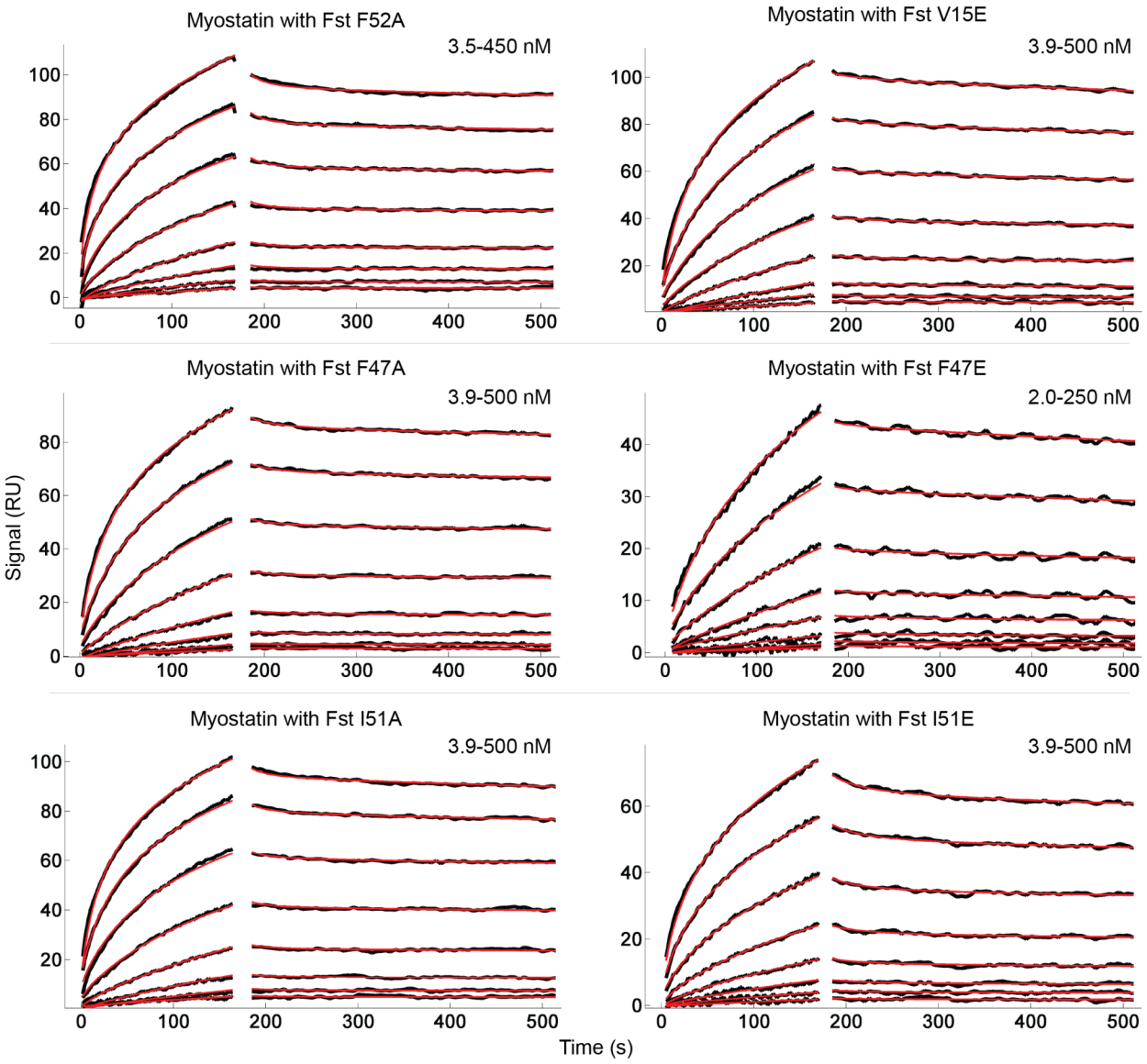
Supplemental Figure 4: Cross-comparisons of the ND helix placement. Ligand monomer opposite the ND helix is aligned in each panel. Structures are shown from the same perspective. The greatest shift in ND helix placement is seen in comparing the activin A:Fst and myostatin:Fstl3 complexes (B) while the most similar placement is seen in the activin A:Fstl3 and myostatin:Fst complexes (A).

Supplemental Figure 5

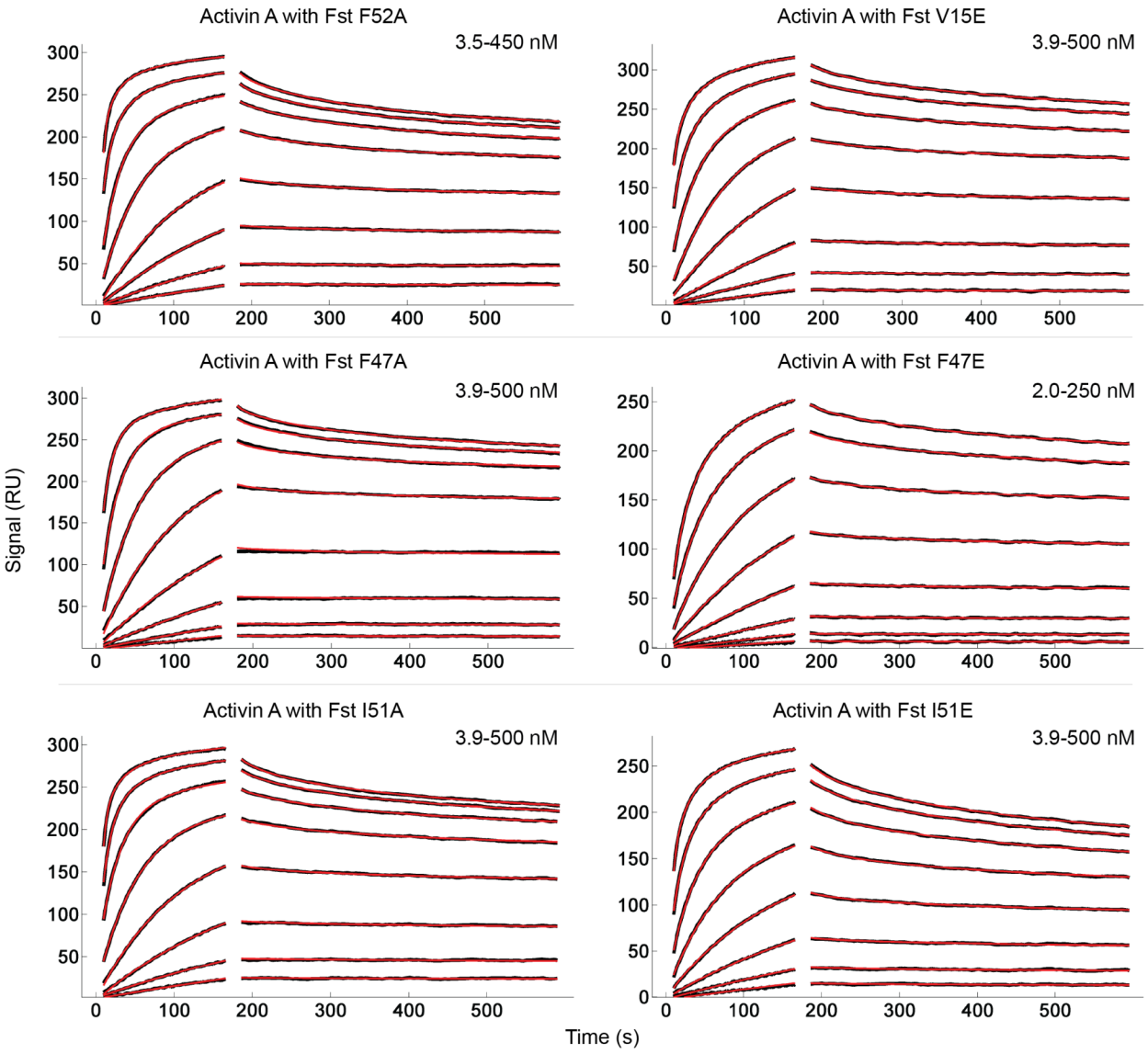


Supplemental Figure 5: Buried surface area (BSA) on the ND of Fst-type complexes on a per residue basis. Sequences for Fstl3 residues 10-71 and Fst residues 1-63 are shown. Surface area buried by ligand on the ND of Fst-type molecules was calculated on a per residue basis (PDB codes 2B0U, 3B4V, and 3HH2). Darker-colored bars represent the amount that a particular residue is buried by the N-terminus and wrist region of a ligand, while lighter-colored bars represent that buried by the fingertip region of a ligand. Gray bars on the right and corresponding dashed lines approximate 50 and 100 $Å^2$ total BSA. The percent of the total surface area buried by ligand on the ND that is contributed by hydrophobic residues is shown in the lower right corner. Residues that were mutated in this study are marked by an asterisk (*).

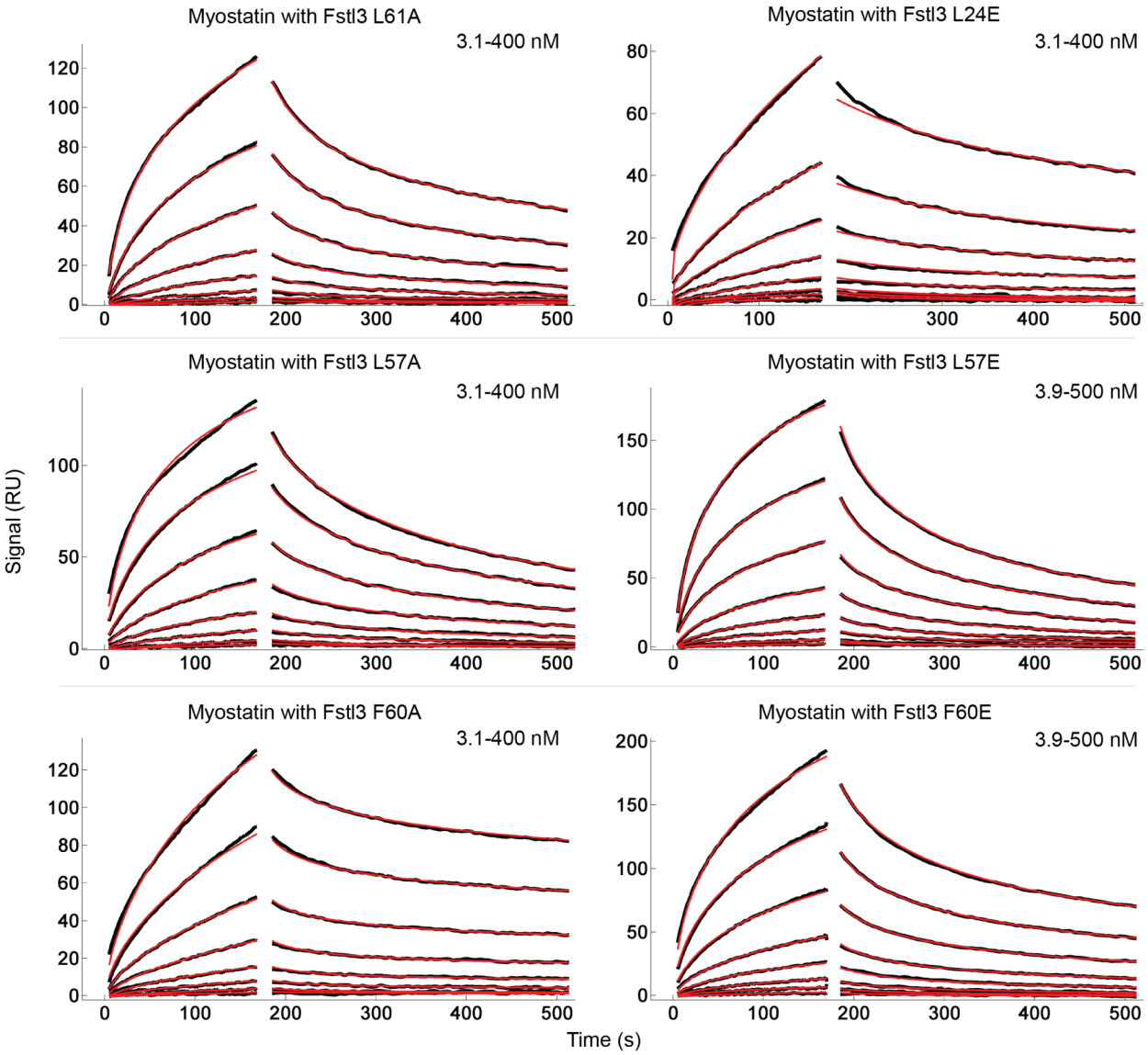
Supplemental Figure 6



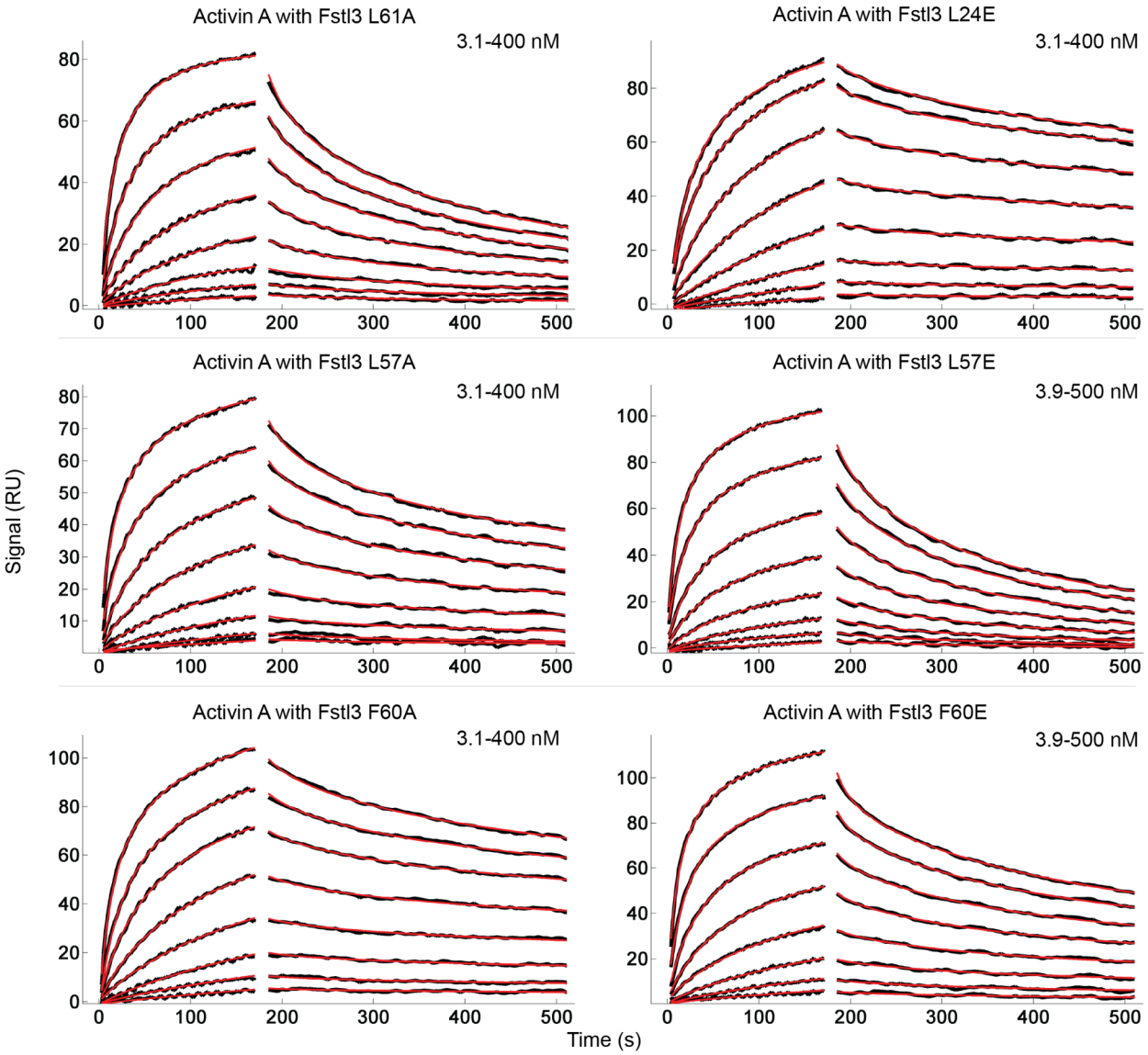
Supplemental Figure 7



Supplemental Figure 8

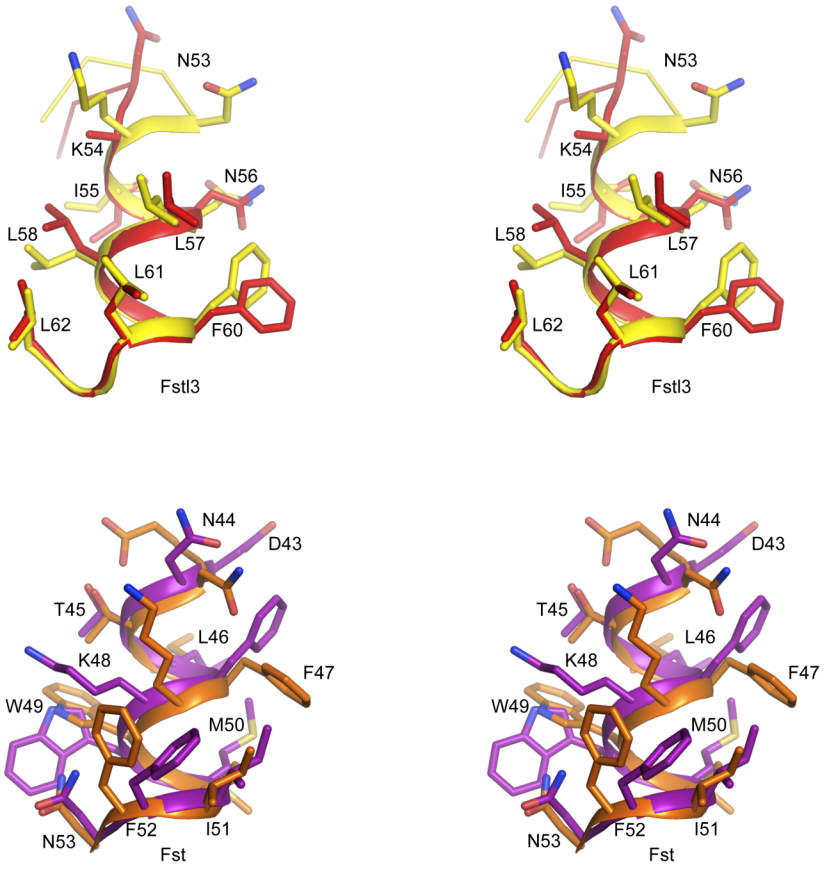


Supplemental Figure 9



Supplemental Figure 6-9: Quantitative SPR measurements of select point mutant Fst-type molecules binding to myostatin. Sensorgrams obtained as WT and altered Fst-type molecules were injected over a CM4 or CM5 sensor chip coupled with myostatin and activin A. Traces shown in black correspond to measurements of a two-fold serial dilution over the concentration range shown in the upper right corner of each sensorgram. Red curves correspond to global fits of each data set to a model as described in Experimental Procedures using the program EVILFIT. Binding constants are shown in Table 2.

Supplemental Figure 10



Supplemental Figure 10: Stereoviews of alignments of the ND helices of each Fst-type molecule from the two different complexes. The ND helix of the myostatin:Fstl3 complex is shown in red, the activin A:Fstl3 complex (3B4V) in yellow, the myostatin:Fst complex (3HH2) in purple, and the activin A:Fst complex (2B0U) in orange. Fstl3 ND helix residue side chains are in similar positions between the two complexes, while those of Fst are in very different positions.

Supplemental Table 1

Data collection and refinement statistics (molecular replacement)

	Native (collected at 100 K)
Data Collection	
Space group	$P6_122$
Cell dimensions (Å,°)	**a**=**b**=82.1, **c**=312.7 $\alpha=\beta=90, \gamma=120$
Resolution (Å)	35.5-2.4 (2.5-2.4)[a]
Observations	672005
Unique reflections	25404
Completeness (%)	98.9(92.6)
Redundancy	14.3(5.2)
R_{merge} (%)	9.0(70.7)
$\langle I/\sigma I \rangle$	19.6(2.8)
Wilson plot B factor (Å^2)	64
Model Refinement	
Reflections (total/free)	25168/1275
R_{work}/R_{free} (%)	24.6/27.0
Atoms (total/protein)	2419/2346
RMSD from ideal	
Bonds (Å)	0.007
Angles (°)	1.012
Ramachandran plot statistics (number/%) [b]	
Favored	298/96
Allowed	12/4
Disallowed	0/0
MolProbity score (number/percentile)	1.34/100th

[a]Values in parentheses are for highest-resolution shell.
[b]Determined using MolProbity.