Supplementary Material

Harrington AE et al.: Structural basis for the inhibition of activin signalling by follistatin

Figure 1. **Biochemical and biophysical analysis of R192A mutant of follistatin Fs12 fragment**

A) Analytical size exclusion chromatography on Superdex 75 10/30 column in 100 mM ammonium acetete, 5 mM EDTA, pH 7.0. 200 µl of indicated proteins (activin A dimer concentration 50 µM, Fs12 fragments 110 µM) or protein mixtures were loaded onto the column and eluted at flow rate of 0.8 ml/min.
B) SDS-PAGE analysis of the size exclusion chromatography with Fs12(R192A) mutant and activin A. Activin A (lane 1) and mutant Fs12 protein (lane 2) were mixed together (lane 3), incubated for a few minutes and centrifuged at 15,000 rpm for three minutes to separate soluble protein (lane 4) from precipitated protein (lane 5, only activin A). The soluble fraction (lane 4) was loaded onto Superdex 75 10/30 column. 0.8 ml fractions (one fraction per minute) from 13 minutes onwards are shown in lanes 6-11. Lanes 7 and 8 show only Fs12(R192A) protein (elution peak at 14.8 min), whereas free activin A elutes as a much broader peak at 17.6 minutes, shown in lanes 9-11.
C) Isothermal titration calorimetric analysis of Fs12(R192A) interaction with activin A. 70 µM Fs12(R192A) was titrated into 3.7 µM activin A in 100 mM Tris-HCl, 5 mM EDTA, 3.2 µM BSA, pH 8.0 at 25°C. Raw ITC data on the left panel shows only dilution heats, similar to Fs12 titration into the same buffer in the absence of activin A. Integrated heats after substraction of dilution heats from control experiment are shown on the right panel. It is clear there is no binding of R192A mutant to activin A under these conditions.

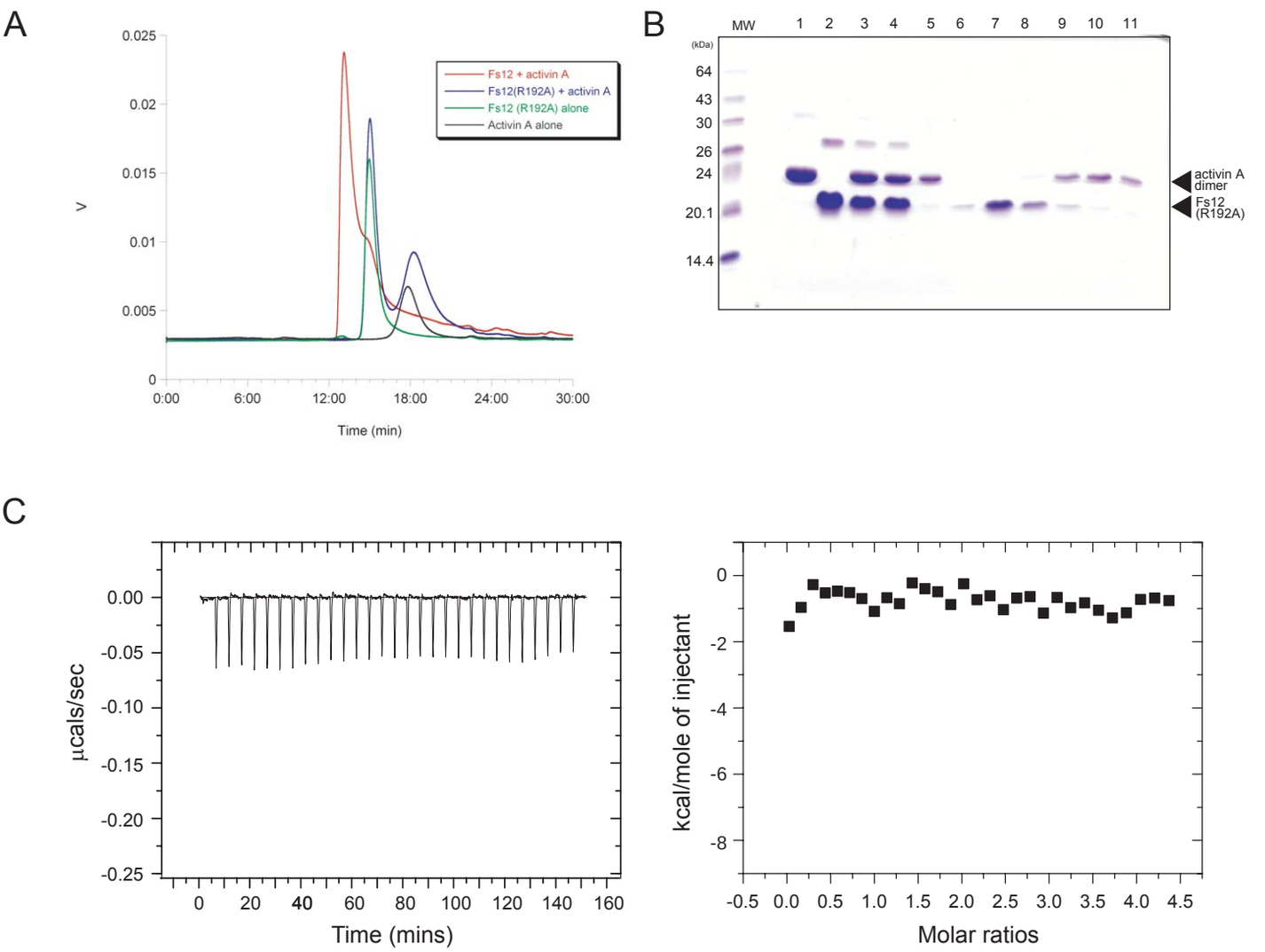

Figure 2. **Phenotypic and RT-PCR analysis of R192A mutant of follistatin Fs12 fragment in *Xenopus* animal cap assay**

All experiments performed as described in the Materials and Methods section of the main article.
A) Phenotypic analysis of the effect of R192A mutation on the ability of Fs12 fragment to inhibit activin A induced animal cap elongation. While 100 nM wild type Fs12 inhibits the elongation of animals caps completely (panel c), similar concentration of R192A mutant shows only minimal effect (panel f).
B) Quantitation of the phenotypic data shown in (A) using larger number of animal caps (n=35)
C) RT-PCR analysis of activin A induced gene expression in the presence of either wild-type or R192A mutated Fs12 protein. Although Fs12(R192A) is unable to inhibit activin A induced animal cap elongation, it does inhibit expression of *Goosecoid* (*Gsc*) and *Chordin* (*Chd*) expression to certain extent. The inhibition is, however, much less pronounced than with the wild type protein, indicating lower affinity for activin A. WOC, water only control; WE, whole embryo; ODC, ornithine decarboxylase used for normalisation of RNA level; ODC-RT, ornithine decarboxylase PCR without reverse-transcriptase reaction.