

Title: Structural basis of human separase regulation by securin and Cdk1-cyclin B1

Authors:

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Supplementary Information Guide

1. Supplementary Figure 1

This file includes uncropped gels and blots for [Fig. 2f](#), and [Extended Data Figs. 1a, 2b, 9a-c, 10a,b](#) and [11c,d](#).

2. Supplementary Video 1 Overall structure of inhibitory separase complexes.

This video shows the overall architecture of the human separase-securin and the separase-Cdk1-cyclin B1-Cks1 complexes as electron density and ribbon representation while rotating around the X-axis.

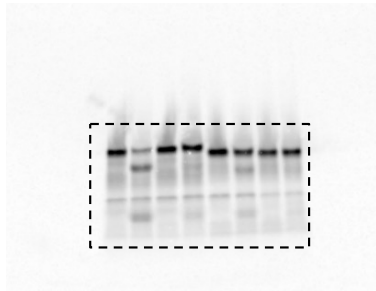
3. Supplementary Video 2 Autoinhibitory loops mimic securin binding.

This video shows a superposition of the autoinhibitory loop 1 and 3 with securin and highlights common binding motifs that recognise structural elements in separase adjacent to the catalytic site.

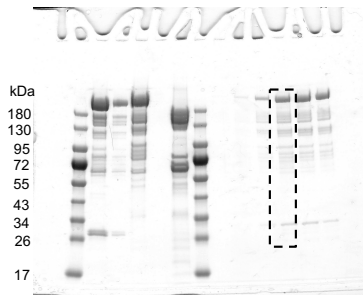
4. Supplementary Video 3 Separase and Cdk1-cyclin B1-Cks1 complex assembly.

This video describes the structural assembly of the separase-Cdk1-cyclin B1-Cks1 complex. The phosphate-binding pocket in cyclin B1 and the Cdc6-like domain are highlighted. A superposition between a Cdk1 structure bound to a substrate peptide and the separase Cdc6-like domain bound to the catalytic site of Cdk1 illustrates the structural similarities between these two binding partners.

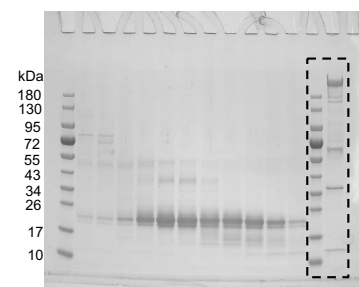
Figure 2f autoradiogram



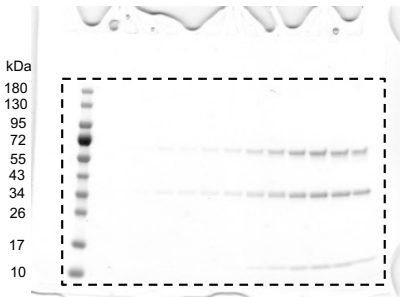
Extended Data Fig. 1a left SDS-PAGE



Extended Data Fig. 1a right SDS-PAGE

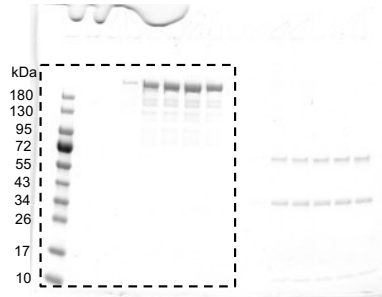


Extended Data Fig. 2b SDS-PAGE



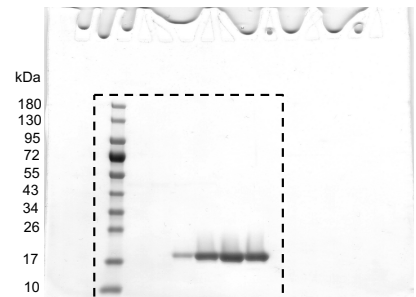
Hs Cdk1-cyclin B1-Cks1

Extended Data Fig. 2b SDS-PAGE



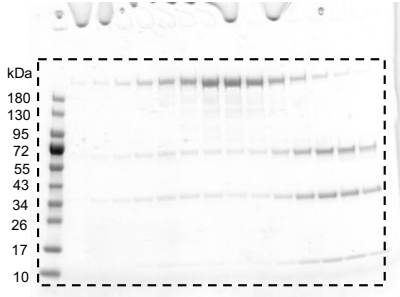
Hs Securin^{Δ160}-Separase^{C2029S}

Extended Data Fig. 2b SDS-PAGE



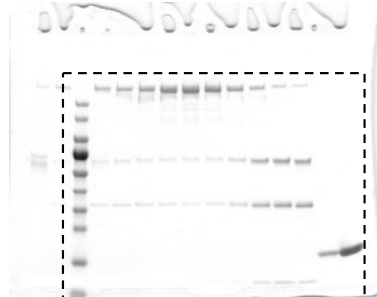
Hs Pin1

Extended Data Fig. 2b SDS-PAGE



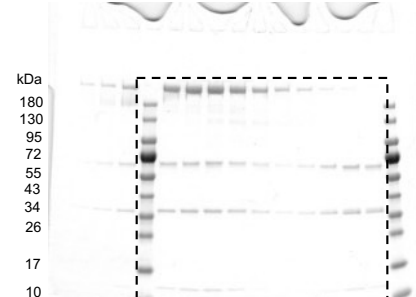
Hs Securin^{Δ160}-Separase^{C2029S} + Cdk1-cyclin B1-Cks1

Extended Data Fig. 2b SDS-PAGE



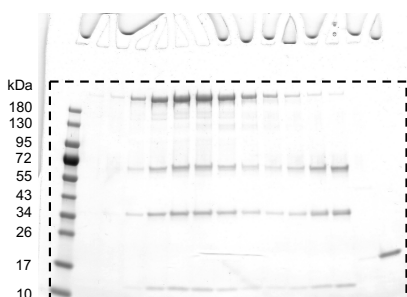
Hs Securin^{Δ160}-Separase^{C2029S} + Cdk1-cyclin B1-Cks1+Pin1

Extended Data Fig. 2b SDS-PAGE



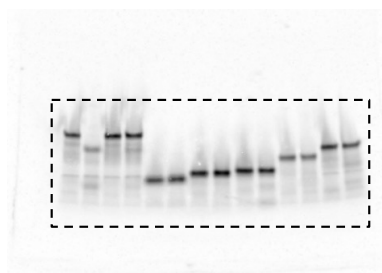
Hs Securin^{Δ160}-Separase^{C2029S} + Cdk1-cyclin B1-Cks1+ATP

Extended Data Fig. 2b SDS-PAGE

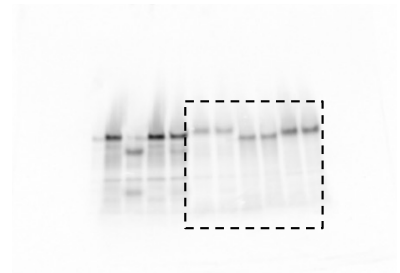


Hs Securin^{Δ160}-Separase^{C2029S} + Cdk1-cyclin B1-Cks1+Pin1+ATP

Extended Data Fig. 9a left autoradiogram

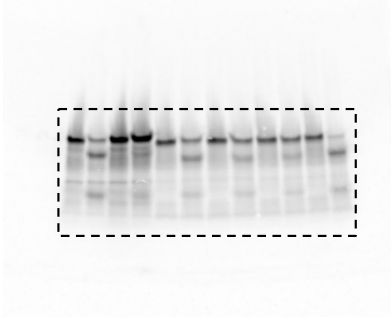


Extended Data Fig. 9a right autoradiogram

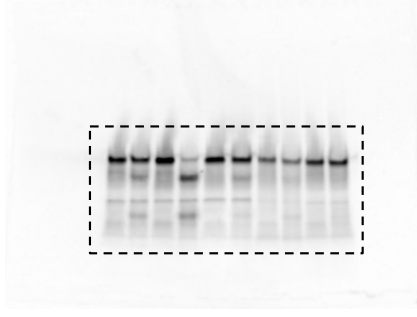


(continued)

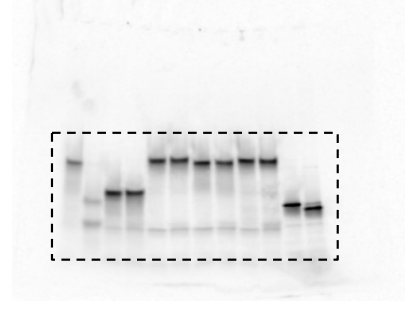
Extended Data Fig. 9b
left autoradiogram



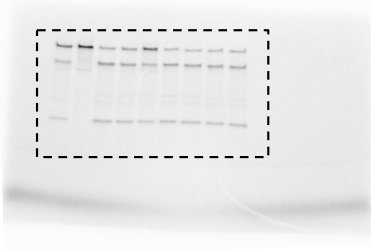
Extended Data Fig. 9b
right autoradiogram



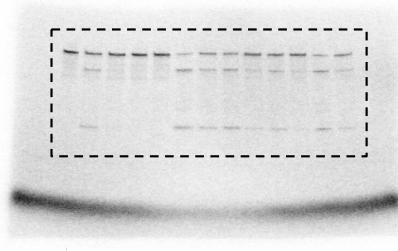
Extended Data Fig. 9c
autoradiogram



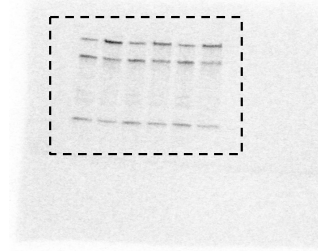
Extended Data Fig. 10a
autoradiogram



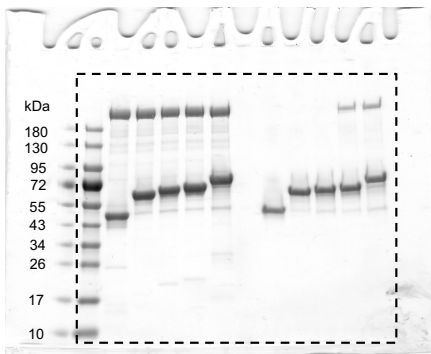
Extended Data Fig. 10b left
autoradiogram



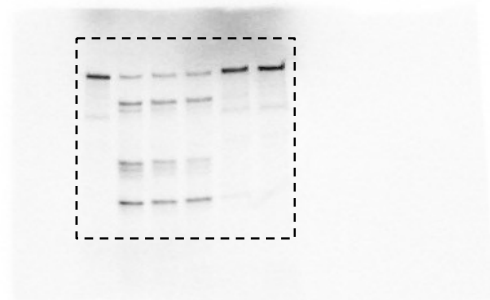
Extended Data Fig. 10b right
autoradiogram



Extended Data Fig. 11c
SDS-PAGE



Extended Data Fig. 11d
autoradiogram



Supplementary Figure 1 Uncropped gel and blot images.

This file includes uncropped gels and blots for Fig. 2f, and Extended Data Figs. 1a, 2b, 9a-c, 10a-b, and 11c-d.