**Ska3 Ensures Timely Mitotic Progression by Interacting Directly With Microtubules and Ska1 Microtubule Binding Domain**

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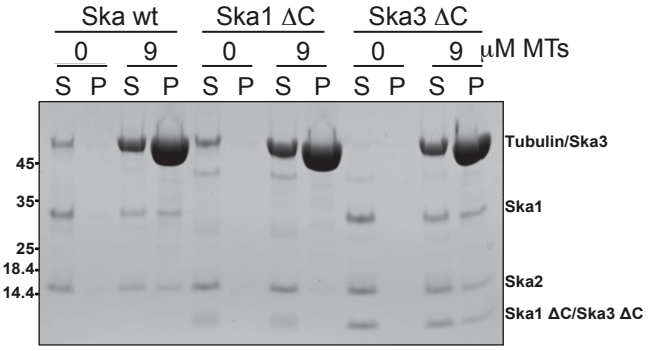
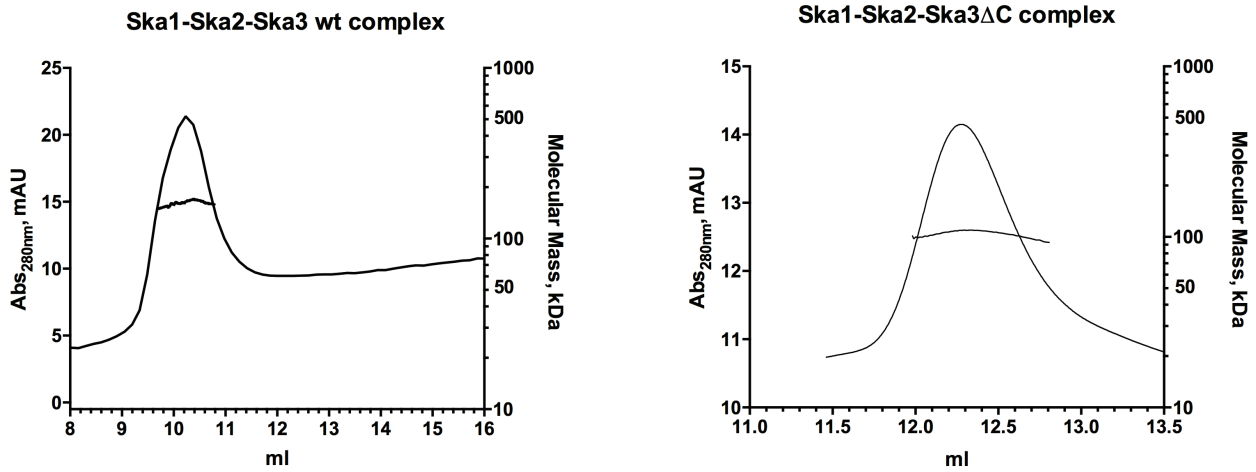
Running title: Ska3-microtubule interactions

**a**



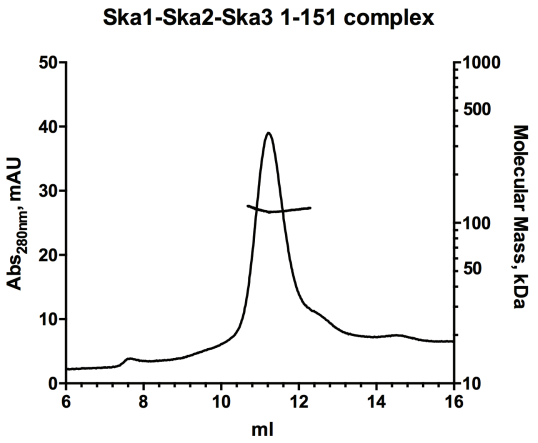

**b**

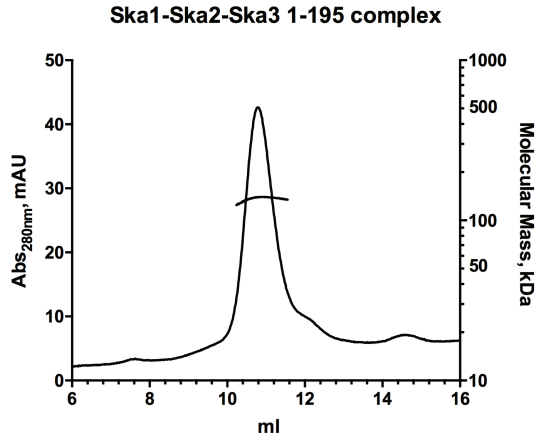



| | Measured MW in kDa (Calculated MW) | Quaternary Structure |
|---|---|---|
| Ska1-Ska2-Ska3 | 169kDa (180KDa) | Homodimer |
| Ska1-Ska2-SKa3ΔC | 121kDa (112kDa) | Homodimer |

**Figure S1**. **a**) Representative SDS-PAGE gels of cosedimentation assays comparing the microtubule binding ability of the wt Ska complex, Ska1ΔC and Ska3ΔC as shown in **Fig. 1d**. **b**) SEC-MALS profile of the wt Ska complex (left) and Ska3ΔC (right). The analysis shows that the deletion of the C-terminal domain of Ska3 does not alter the overall oligomeric state of the complex.

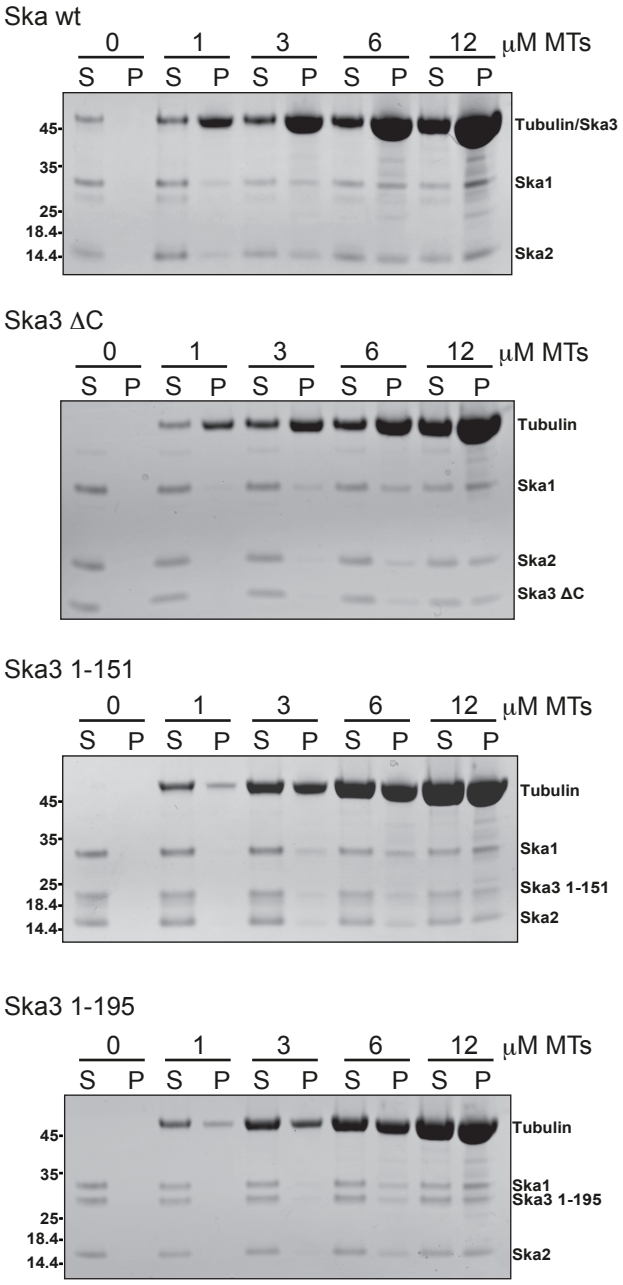
**a**




| | Measured MW in kDa (Calculated MW) | Quaternary Structure |
|---|---|---|
| Ska1-Ska2-Ska3 1-151 | 121.8kDa (122KDa) | Homodimer |
| Ska1-Ska2-SKa3 1-195 | 132.9kDa (132kDa) | Homodimer |

**b**




**c**

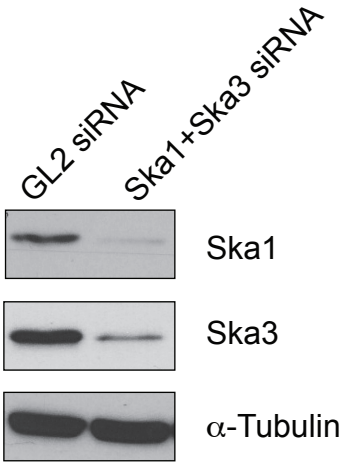



**Figure S2**. **a)** SEC-MALS profiles of the Ska1-Ska2-Ska3 1-151 (top) and Ska1-Ska2-Ska3 1-195 (bottom) complexes. The analysis shows that the truncations do not affect the oligomeric structure of the complex. **b)** Representative SDS-PAGE gels of cosedimentation assays comparing wt Ska complex and Ska3ΔC with Ska1-Ska2-Ska3 1-151 and Ska1-Ska2-Ska3 1-195 as shown in **Fig. 2a**. **c)** Representative western blot showing the level of depletion obtained in HEK293T with Ska1 and Ska3 double siRNA after 72 hours of treatment.

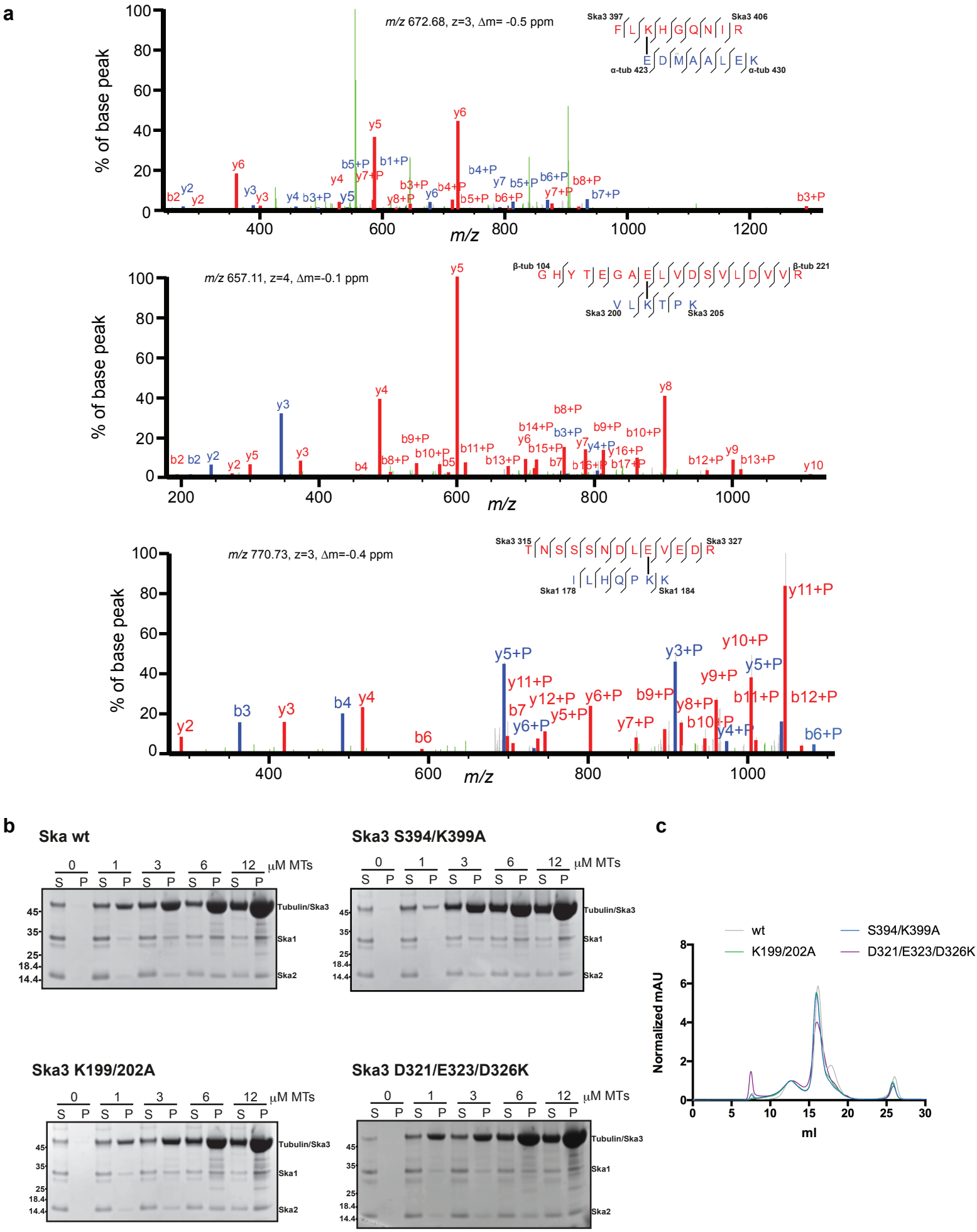



**Figure S3**. **a**) High resolution fragmentation spectra of a few representative cross-linked peptides. **b**) Representative SDS-PAGE gels of MT-cosedimentation assays comparing wt Ska complex and Ska3 point mutants (Ska3 K199/202A, S394/K399A and D321/E323/D326K) as shown in **Fig. 3c** and **d**. **c**) Comparison of the size exclusion chromatograms of all the mutants tested in MT-cosedimentation assays shown in **Fig. 3c** and **d**. Analysis shows that all mutants behave identically to the wt Ska complex.

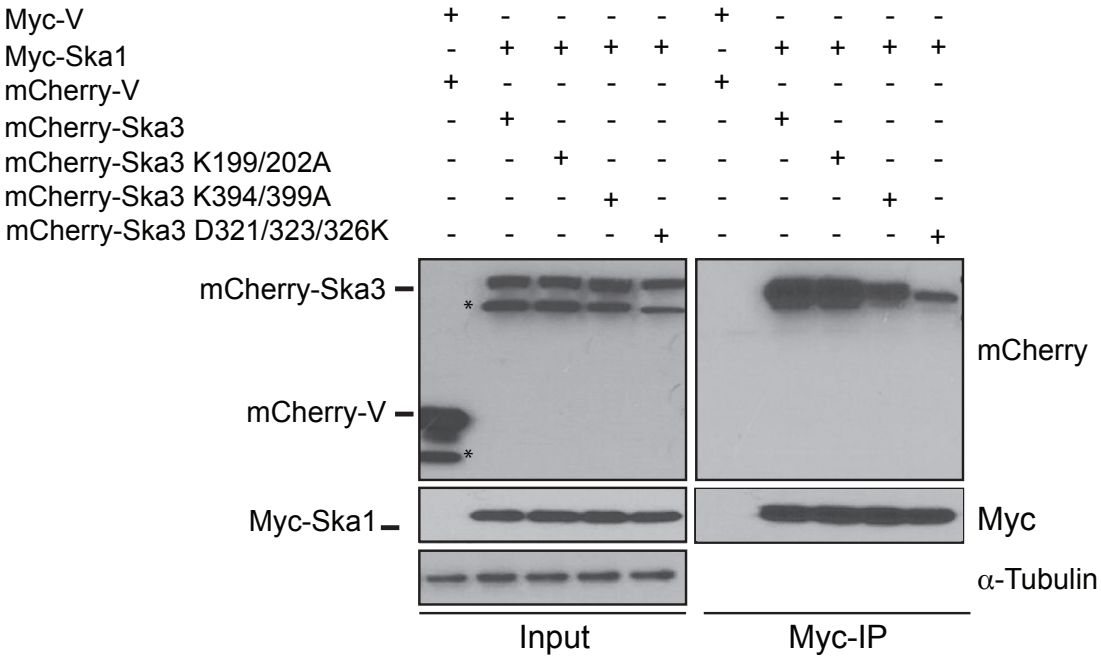



**Figure S4**. Combinations of Myc-Ska1 and mCherry-Ska3 versions as depicted in the figure were expressed in HEK-293T cells and cells were harvested after 36 hours. Myc-empty vector or Myc-Ska1 was then immunoprecipitated using anti-Myc antibodies, resolved by SDS-PAGE and co-immunoprecipitation of mCherry-Ska3 versions was resolved by Western blotting.