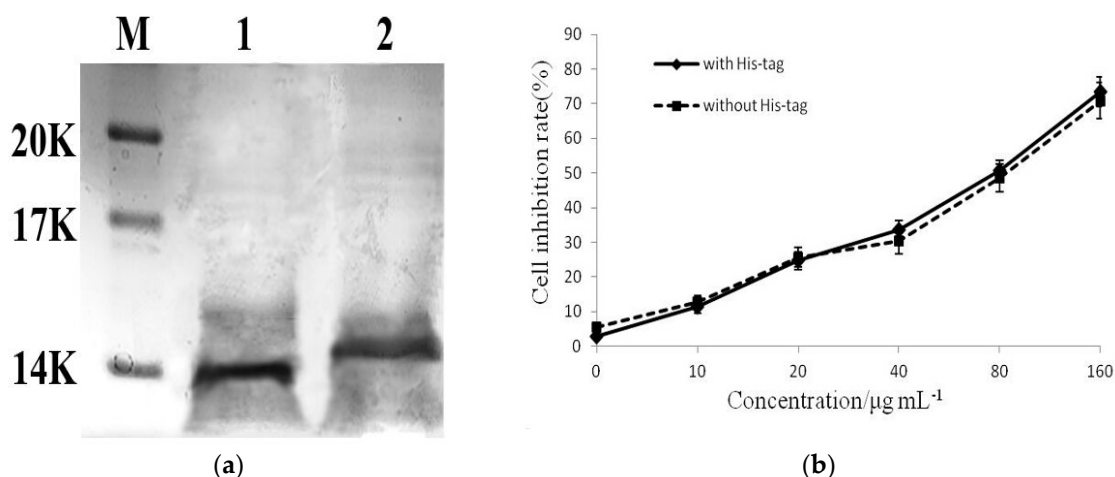


# Supplementary Materials: Preparation and Antitumor Activity of CS5931, a Novel Polypeptide from Sea Squirt *Ciona Savignyi*

Xiaoshuang Chen, Huanli Xu, Bo Li, Feng Wang, Xiaoliang Chen, Dexin Kong and Xiukun Lin



**Figure S1.** Purification and cytotoxicity of fusion protein His-tag-DDDK-CS5931. The expression plasmid, pET28a/His-tag-DDDK-CS5931 was constructed, and transformed into *E. Coli* BL21 cells. The strain was cultured using optimized conditions. After purification using  $\text{Ni}^{2+}$  column, the polypeptide was cut using enterokinase, and renatured using the optimized renaturation conditions. (a) SDS-PAGE analysis. Lane 1 represented inhibitory effect of CS5931 without his-tag on HCT116 cancer cells while lane 2 indicated the effect of his-tag CS5931; (b) Comparison of the cytotoxicity of his-tag CS5931 with CS5931 without his-tag on human colon carcinoma HCT116 cells. MTT assay was performed to determine the cytotoxicity of the polypeptides.