## **Supplementary Note 1**

Assessing the purified SPSB3-ELOBC complex by Coomassie blue staining, we noted that a protein of ~ 35 kDa co-eluted with SPSB3, which was competed off by cGAS (See asterisk below and figure panels in Fig. 2d, Extended Data Fig. 4a, b, Extended Data Fig. 5c, and Extended Data Fig. 6a). Mass spectrometry identified the protein as the kanamycin-resistance protein (KanR (see below)). When using an alternative antibiotic resistance gene to express SPSB3 in bacteria, our attempts to purify the SPSB3-ELOBC complex failed. Probably, through engaging with SPSB3, KanR stabilises SPSB3 and prevents protein aggregation. Critically, since the binding of KanR to SPSB3 can be potently outcompeted by cGAS, the presence of KanR does not confound the usage of the SPSB3-ELOB/C complex in further downstream experiments.

b



