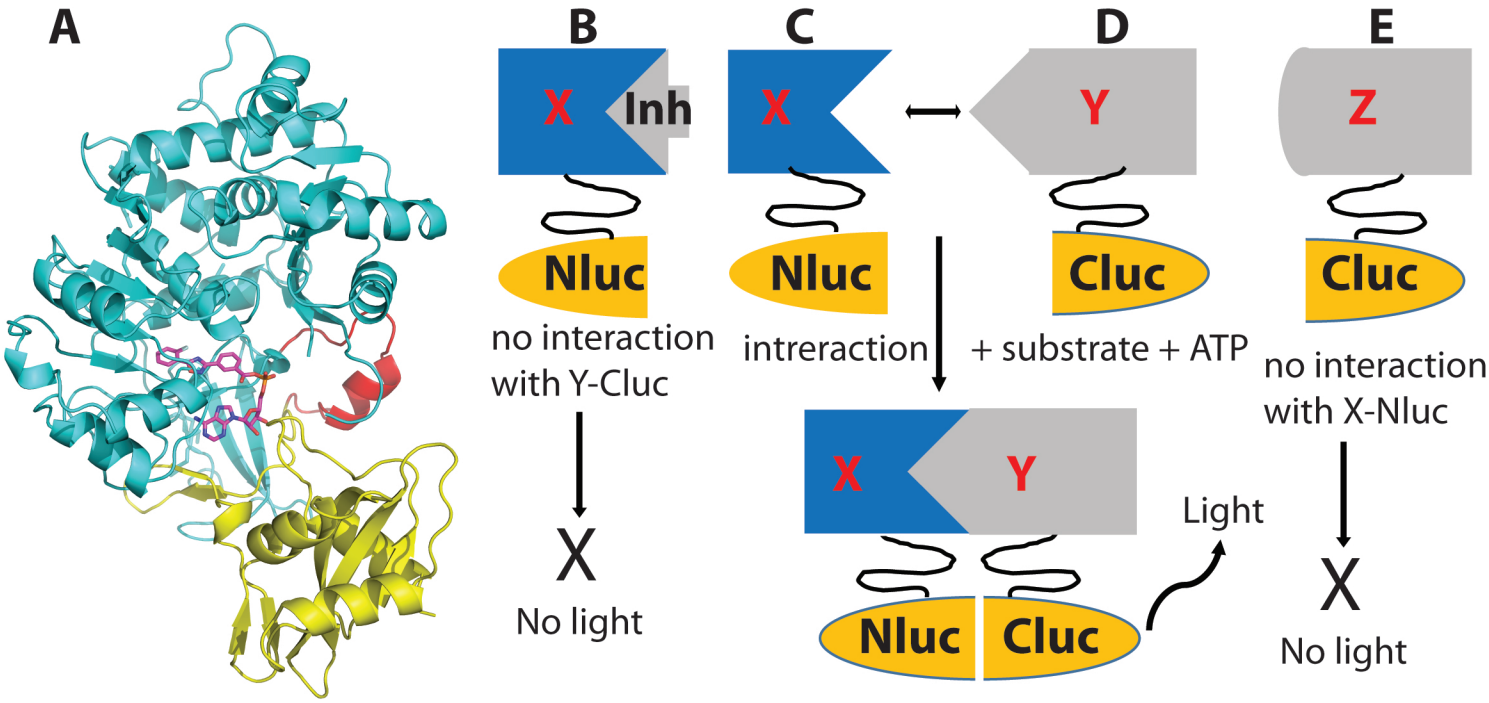
Figure S1



Supplemental Fig. S1. Schematic diagram showing the firefly SLC strategy to monitor PPIs and principle to screen inhibitors. (**A**) Cartoon representation of firefly luciferase (FLuc) with Nluc (aa. 1-416) in cyan and Cluc (aa. 398-550 in yellow, and overlapping peptide 398-416 showing in red. Luciferase inhibitor was shown in stick (magenta). (**B**) Small molecule Inh occupies the Y molecule binding pocket on X molecule. (**C&D**), X-Y interaction results in active luciferase. Luminescent light will be generated in the presence of substrate luciferin and ATP. (**E**) Non-interacting molecule Z-CLuc cannot complement with A-NLuc.

To explore whether orthosteric inhibitors abolishing the NS2B-NS3 interactions can inhibit the protease function, we developed a SLC-based NS2B-NS3 interaction assay. The principle of SLC and its application in identification of protein-protein interaction inhibitors is illustrated above. Firefly luciferase (FLuc) is composed of 550 aa (**Fig. S1A**), which can be split into two fragments: the N-terminal fragment (NLuc) consisting of aa 1-416 and the C-terminal fragment (CLuc) composed of aa 398-550 for SLC assay of protein-protein interactions (Luker et al., 2004). Assuming X and Y molecules are binding pairs, X-Y interactions will bring the NLuc fused to X molecule and the CLuc fused to Y molecule together to reconstitute a fully active FLuc. When luciferase substrate is added, the reconstituted FLuc will catalyze the reaction, generating luminescence signal (**Fig. S1C,D**). However, when non-interacting protein Z fused to CLuc is mixed with X-NLuc, interaction will not occur; and SLC will not occur, leading to no light (**Fig. S1E**).

In principle, SLC can be developed as HTS to screen inhibitors. As shown in Fig. S1b, when the Y-binding site on X molecule is occupied by a small molecule (e.g. Inh) (or Y competitor), interaction will not occur between molecules X and Y, resulting in inactive Luc fragments and no light. Indeed, it has been reported previously that SLC could be used to monitor inhibitor-induced dissociation of binding partners (Chan et al., 2012; Porter et al., 2008).