Correspondence on Chen *et al.* "**Hepatitis B virus X protein promotes the progression and immune escape of hepatocellular carcinoma by activating KLF16-C12orf49-PD-L1 axis**"

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In this recent publication, Chen *et al.* reported that HBV protein X promotes progression and immune escape in HCC through a KLF16-C12ORF49-PD-L1 axis, a potentially targetable pathway [1]. In brief, Chen *et al.* demonstrate that protein X enhances expression of the transcription factor KLF16 in HBV-infected HCC cells. In turn, KLF16 induces expression of SPRING (official name for C12ORF49), and that SPRING, through its interaction with SPOP, attenuates ubiquitylation and subsequent degradation of the immunomodulatory receptor PD-L1. This suggests a model in which SPRING and PD-L1 compete for binding to SPOP, and may explain, at least in part, how HBV-infection promotes immune escape in HCC.

This model is at odds with our current understanding of SPRING's subcellular localization and mechanism of action. We initially identified SPRING as a regulator of the lipogenic SREBP pathway [2], a finding independently confirmed by other groups [3, 4]. Functionally, SPRING promotes Site-1-protease maturation by displacing the inhibitory S1P pro-domain that blocks its active site, thereby licensing S1P-mediated cleavage of SREBPs *in vitro* and *in vivo* [5-7] (Figure 1). Overwhelming evidence indicates SPRING is a glycosylated type I transmembrane protein that resides in the Golgi: 1) SPRING is heavily glycosylated [2, 4-6]; 2) SPRING can be secreted following its proteolysis by S1P [6], an enzyme whose localization in the secretory lumen is beyond dispute; and 3) SPRING forms a direct complex with S1P that we have visualized using cryo-EM [8].

The current study raises several technical and conceptual questions. Technically, using the same antibody reported by the authors of the study (GeneTex; GTX45975), we detected by immunoblotting a comparable band that is consistent with the expected size of SPRING. However, this band was unaffected by SPRING knockout in Hap1 cells or by SPRING knockdown in Huh7 cells, with the latter also used in the study (Figure 2). Moreover, Chen *et al* show SPRING to migrate as a single band both when over-expressed (*e.g.* Fig 6F) and at endogenous level (*e.g.* Fig 5K). This is inconsistent with the detection of a glycosylated protein as shown for over-expressed SPRING (Figure 2) [2, 4-6]. Conceptually, SPOP is a predominantly nuclear protein, and which domains and where in the cell it interacts with the short (16 amino acids) cytosol-facing tail of SPRING is not clear. Additionally, it would be important to dissociate SPRING-dependent SREBP-mediated effects from the effects of any SPRING-SPOP interaction. Indeed, SPRING has been recently implicated in HCC as an anti-ferroptosis factor by enabling the SREBP-dependent expression of the fatty acid desaturase SCD1 [9]. Clearly, investigating the mechanistic role of SPRING in HCC progression warrants additional investigation.

Figure 1

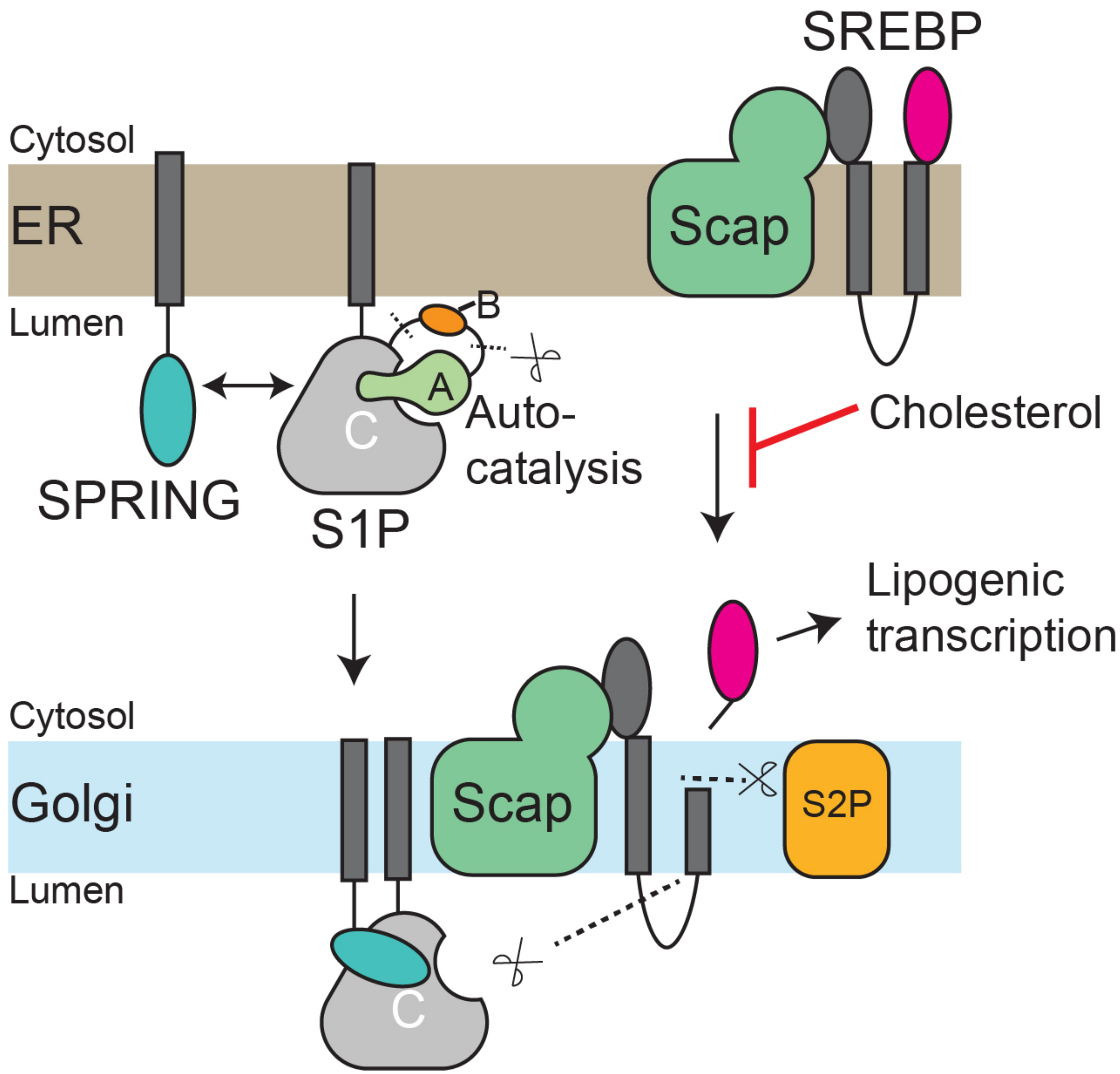

Figure 1. Schematic for the role of SPRING activating S1P by displacing the inhibitory prodomain. The Golgi-localized S1P/SPRING complex carries out proteolytic activation of SREBPs. “A”; inhibitory prodomains, “C”; catalytically active S1P

Figure 2

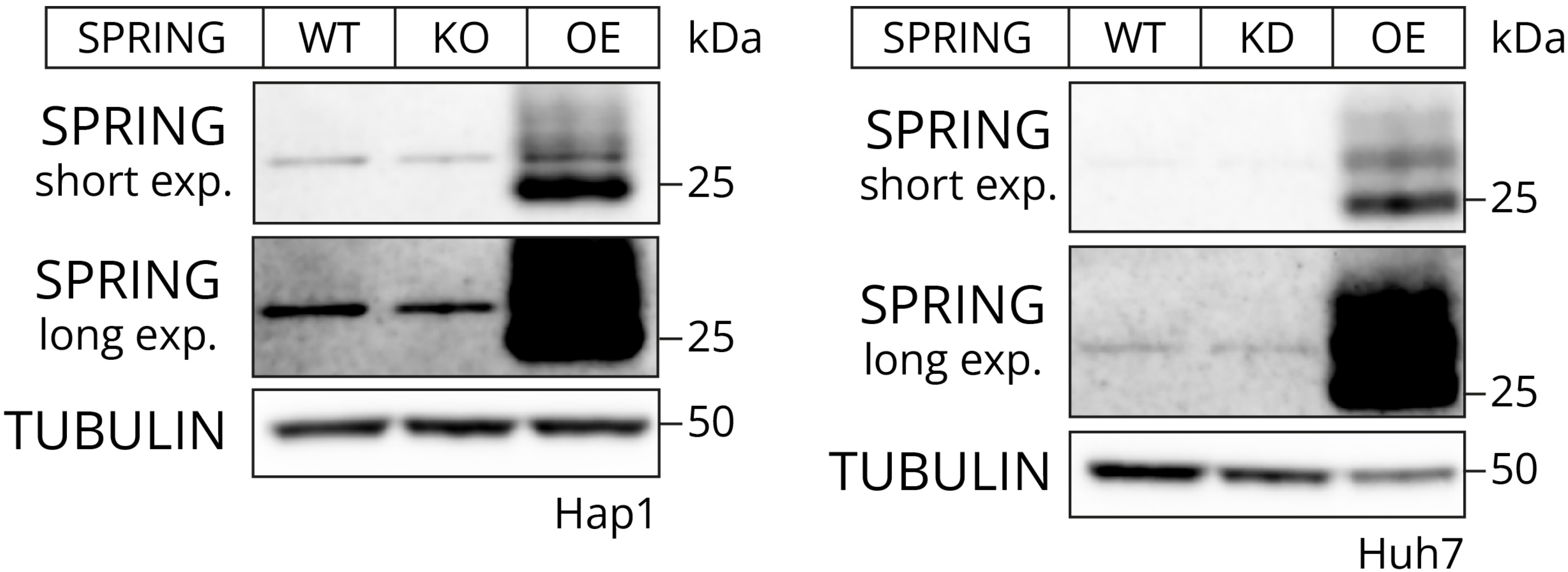

Figure 2. Representative immunoblot of SPRING (C12ORF49) in total cell lysates of Hap1 (left) and Huh7 (right) cells. A polyclonal antibody against SPRING from GeneTex (GTX45975) was used. TUBULIN served as a loading control. WT: Wildtype; KO: Knock-out [3]; OE: Over-expression [7]; KD: shRNA mediated knock-down resulting in ~85% reduction in SPRING mRNA (not shown).

References:

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