

A Covalent Molecular Clamp for TOE1 Illuminates Tunable Spliceosome Regulation



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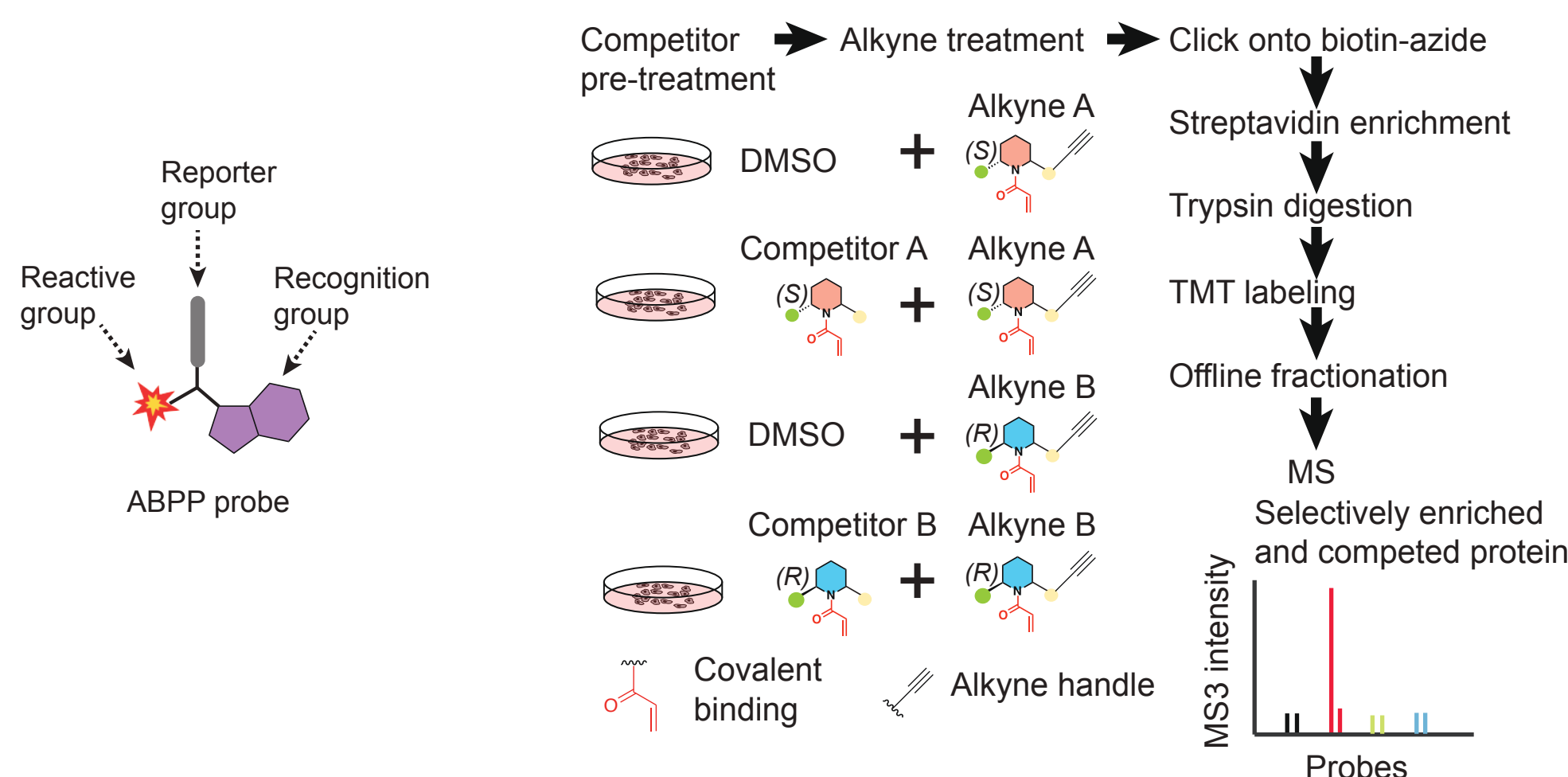
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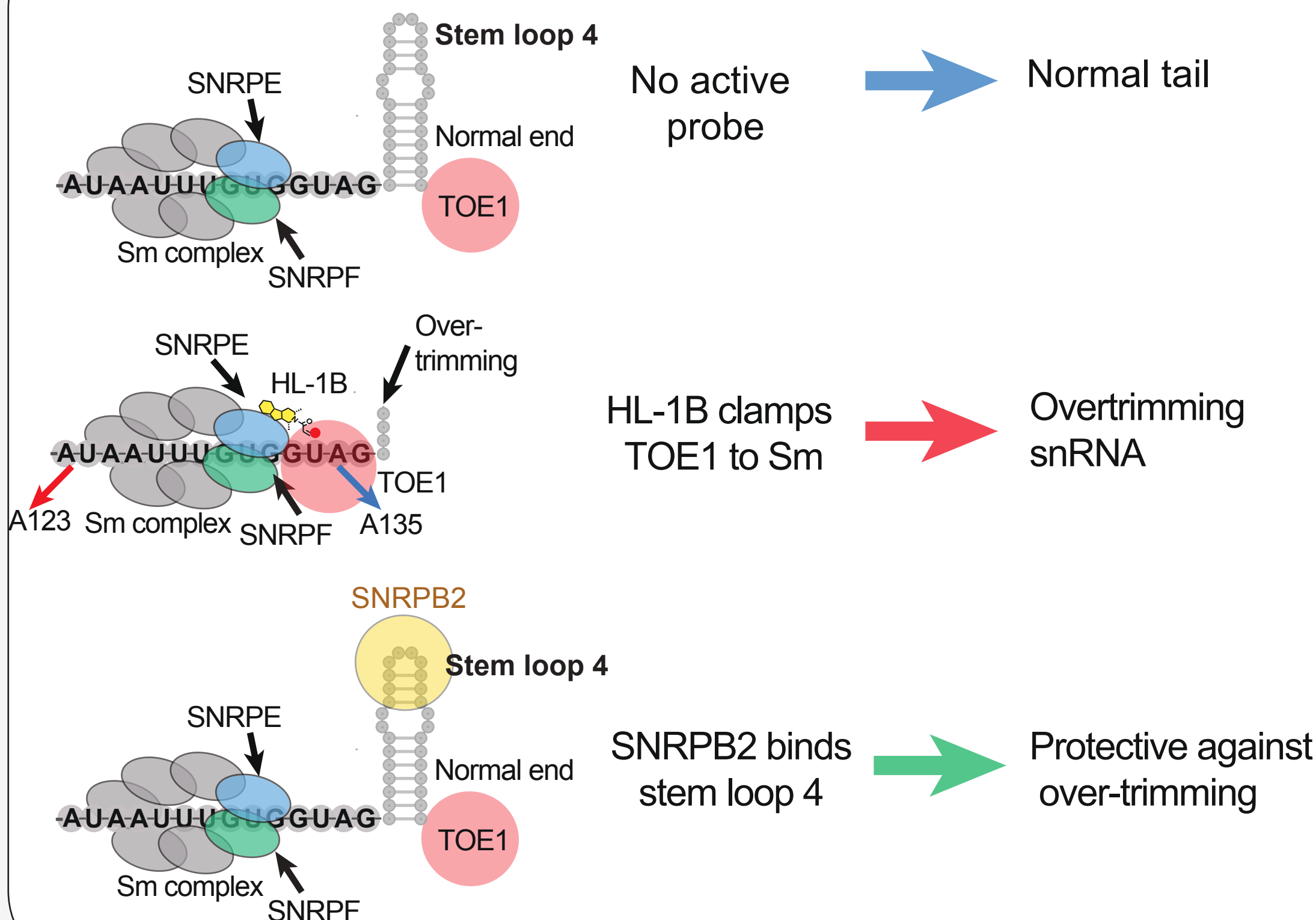
Abstract:

The human spliceosome relies on precisely regulated small nuclear RNAs (snRNAs), yet chemical tools to modulate this machinery have been lacking. I previously developed a base editing method to characterize ligandable cysteines in a proteome-wide manner, which led to the discovery of a functional cysteine (C80) on TOE1, an RNA exonuclease that trims snRNA 3' ends. Here we report the discovery of HL-1B, a potent and selective covalent probe that functions as a "molecular clamp", stabilizing TOE1's association with the Sm complex and thereby reprogramming spliceosome architecture. This induced proximity triggers a striking biochemical outcome: precise over-trimming of snRNA 3' termini in cells. We show that this gain-of-function activity is tunable, with sensitivity dictated by TOE1 abundance and auxiliary splicing regulators such as SNRPB2. Our findings demonstrate the feasibility of chemically controlling spliceosome function with unprecedented precision. This work uncovers an unexpected layer of RNA processing regulation and opens a new frontier for drugging RNA-protein complexes in cancer, neurodegeneration, and other diseases.

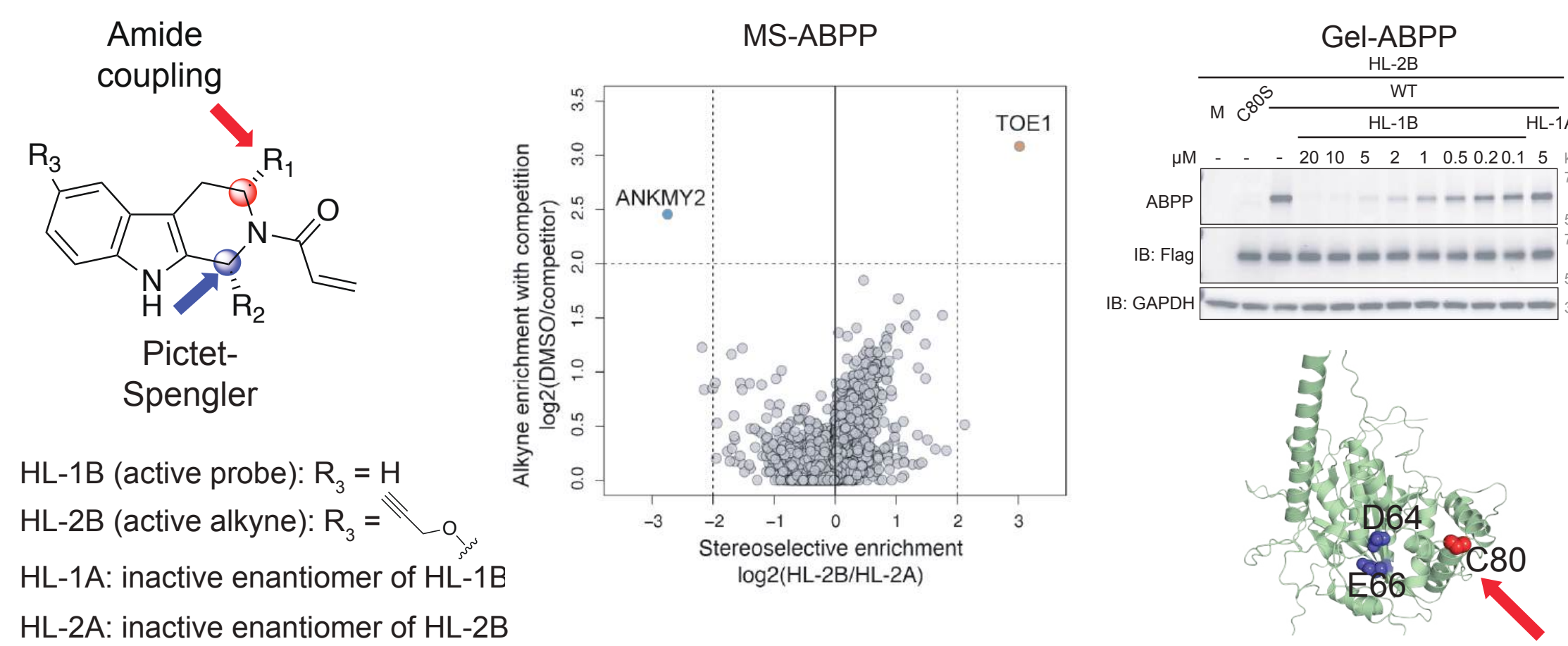
Method (Activity-Based Protein Profiling, ABPP):



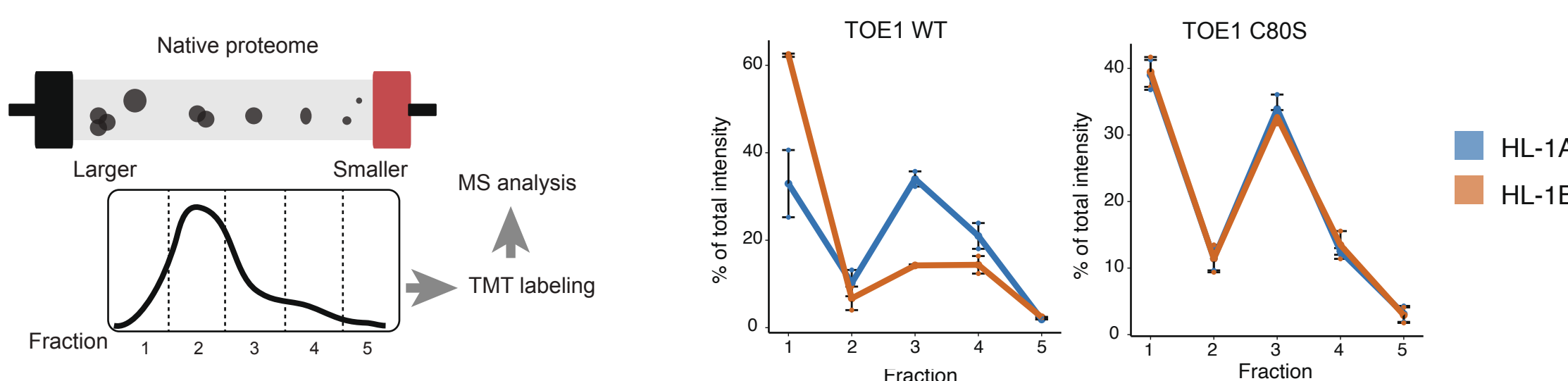
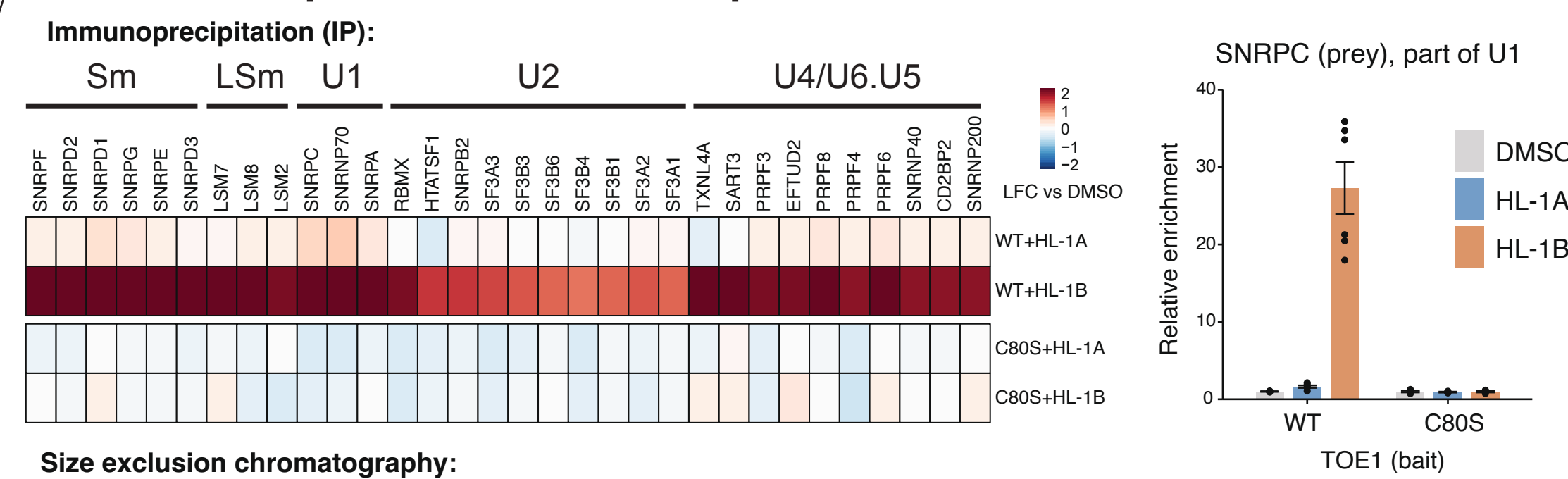
Conclusions:



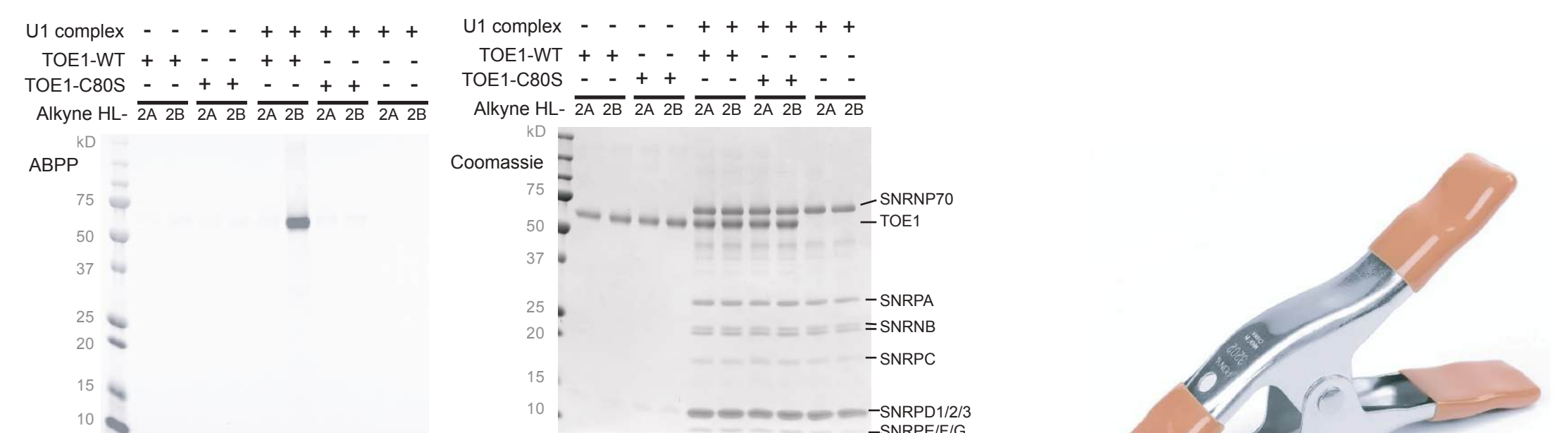
Optimization of tryptoline acrylamide stereoprobes targeting TOE1_C80



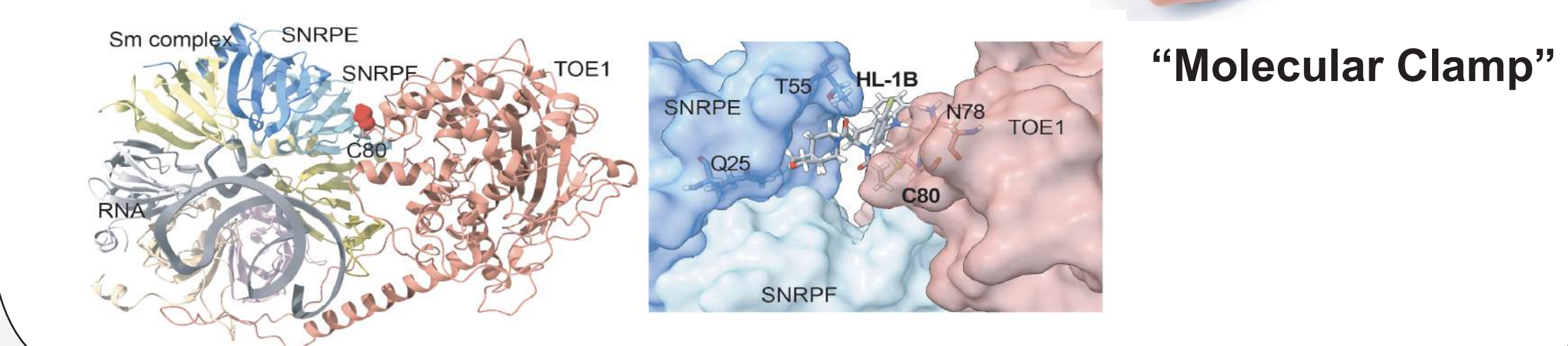
HL-1B clamps TOE1 to Sm complex



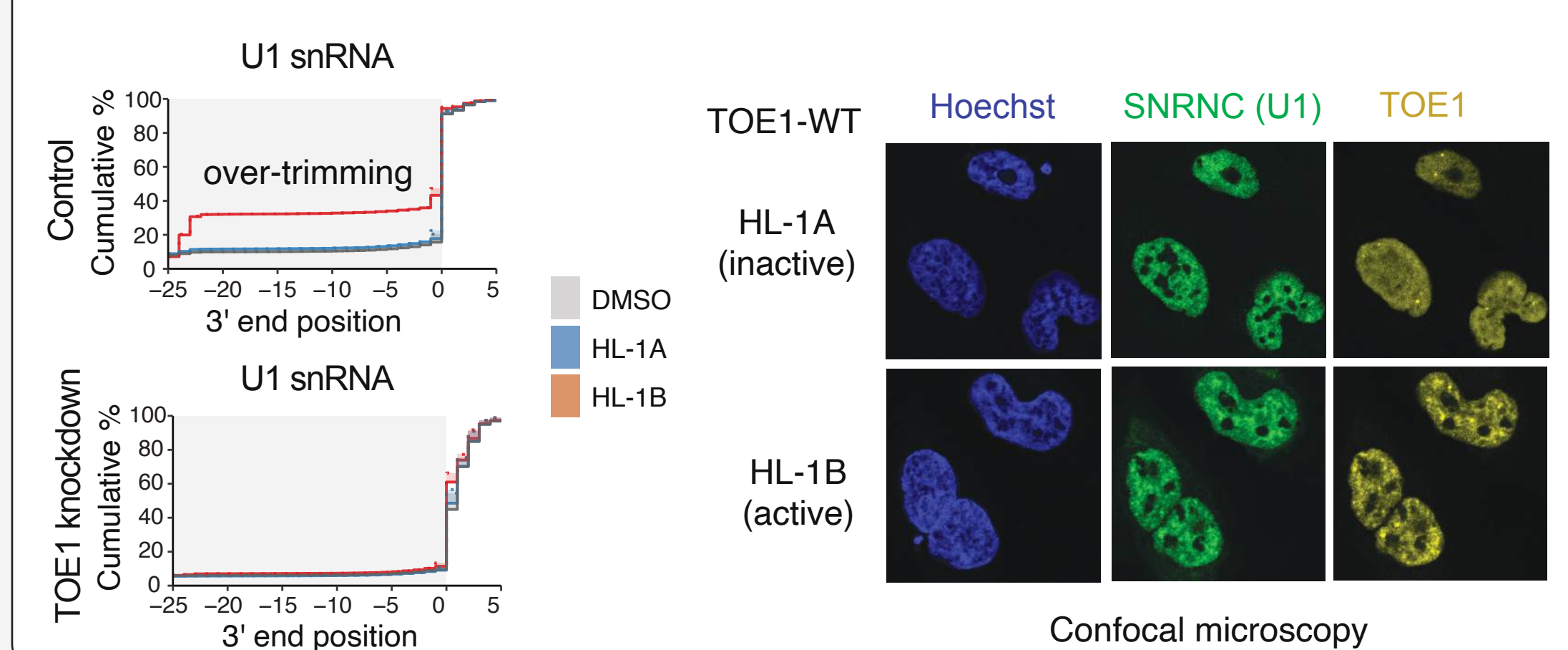
In vitro reconstitution:



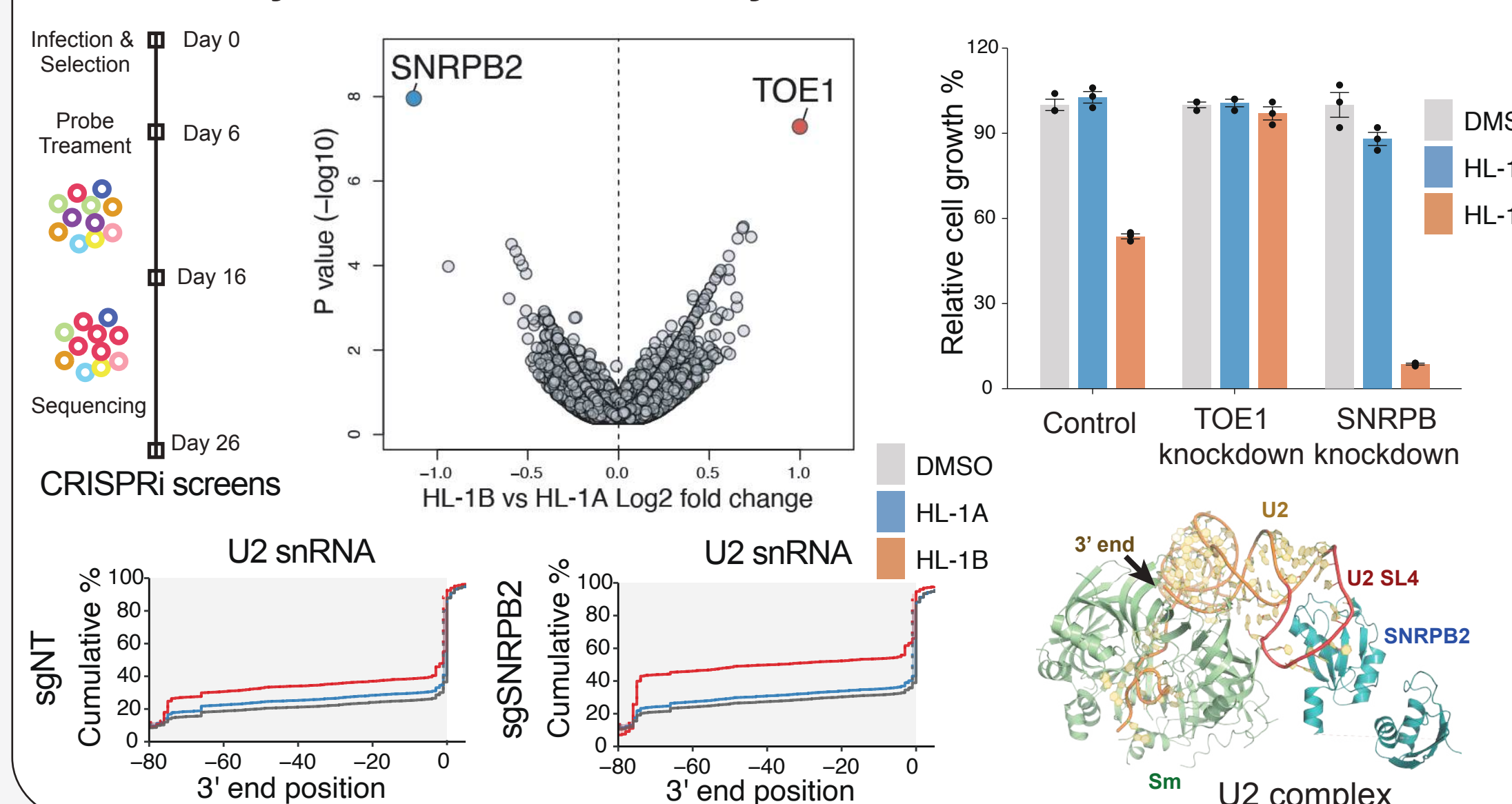
Computational modeling:



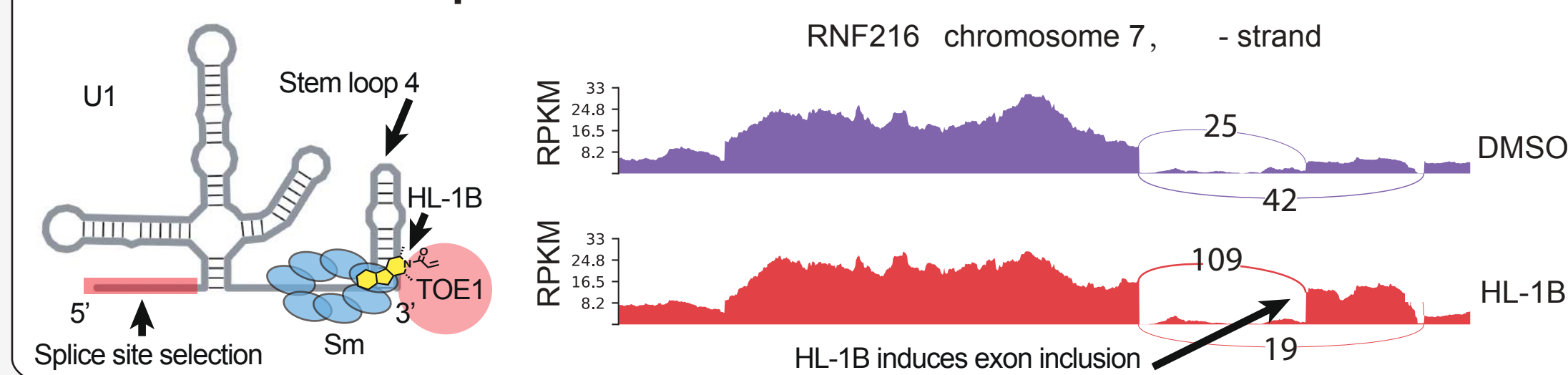
Proximity-inducing HL-1B promotes over-trimming of snRNAs



The activity of HL-1B is tunable by TOE1 and SNRPB2 levels



HL-1B modulates spliceosome functions



Rereferences:

- Li H, Ma T, Remsburg JR, Won SJ, DeMeester KE, Njomen E, Ogasawara D, Zhao KT, Huang TP, Lu B, Simon GM, Melillo B, Schreiber SL, Lykke-Andersen J, Liu DR, Cravatt BF. Assigning functionality to cysteines by base editing of cancer dependency genes. Nat Chem Biol. 2023 Nov;19(11):1320-1330.
- Li H, Tse J, Hayward R, Melillo B, Liu DR, Cravatt BF. Endogenous Site-selective Competition Assays using Prime Editors (ESCAPE). Manuscript in preparation. 2025.
- Li H, Ma T, Tse J, Melillo B, Lykke-Andersen J, Cravatt BF. A covalent molecular glue for TOE1 illuminates tunable spliceosome regulation. Manuscript in preparation. 2025.

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