

Supplementary information for Monitoring Protein Secretion in Living Cells With a HiBiT-Integrated RUSH System

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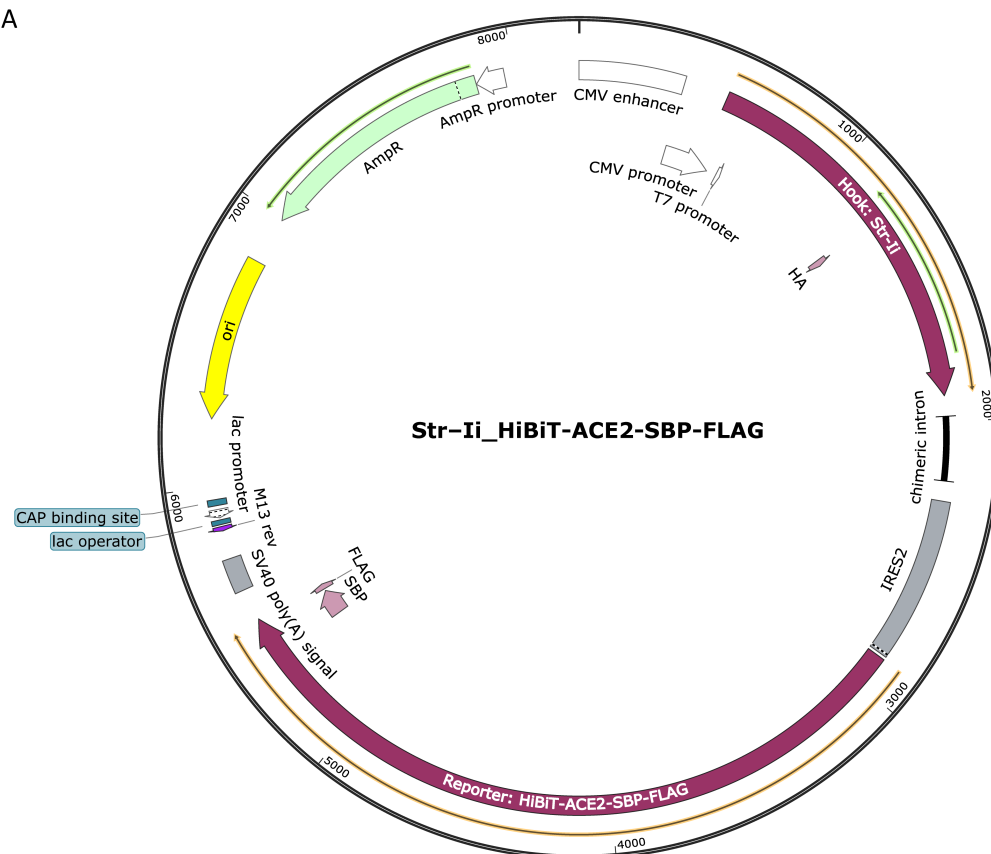
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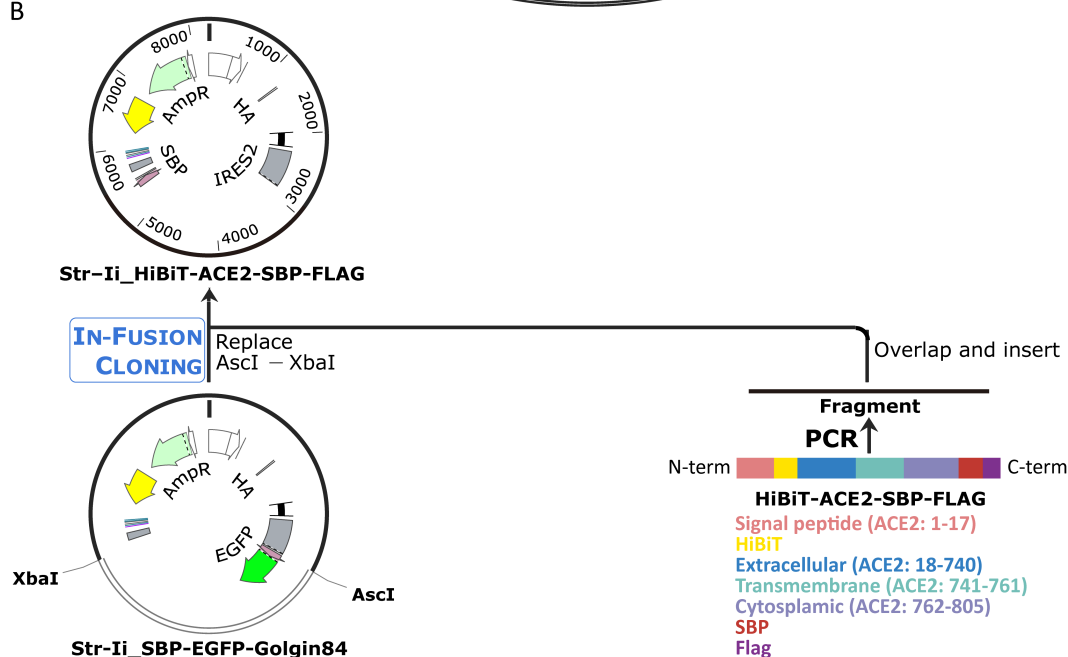
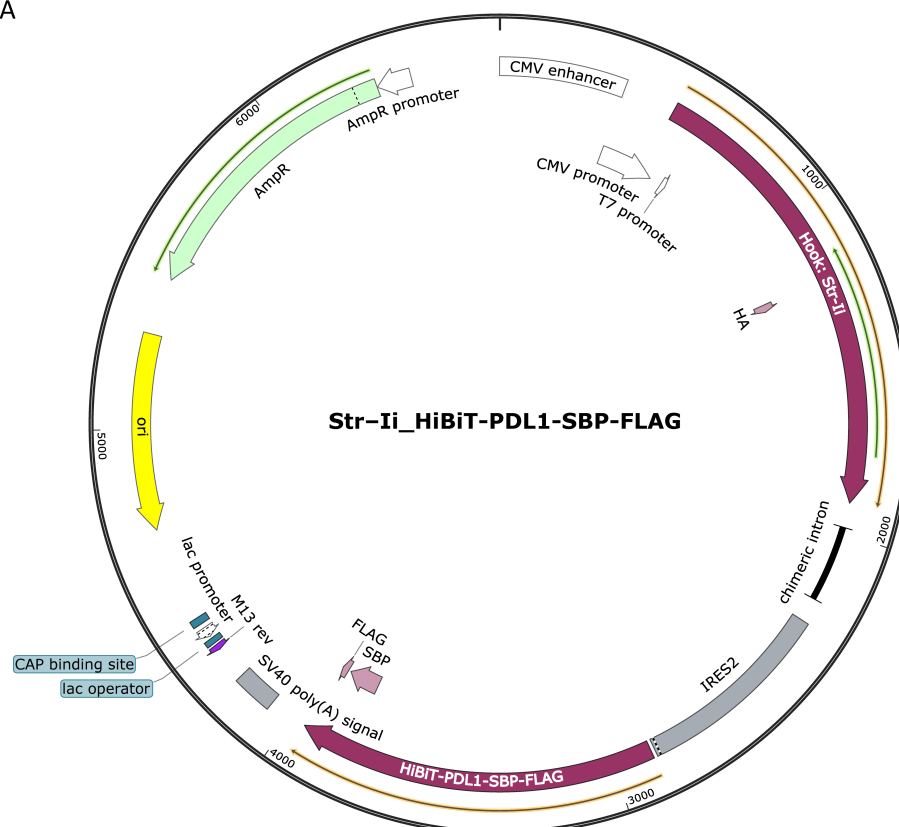


Figure S1. Plasmid map and cloning strategy of Str-Ii_HiBiT-ACE2-SBP-FLAG. (A) Plasmid map of Str-Ii_HiBiT-ACE2-SBP-FLAG generated using SnapGene software. (B) Schematic diagram of the In-Fusion cloning strategy. The parental vector Str-Ii_SBP-EGFP-Golgin84 was digested with AscI and XbaI to excise the SBP-EGFP-Golgin84 cassette. The HiBiT-ACE2-SBP-FLAG fragment (amplified by PCR with overlapping ends) was inserted into the linearized vector via In-Fusion cloning to generate Str-Ii_HiBiT-ACE2-SBP-FLAG. The domain organization of the inserted fragment is color-coded from the N-terminus to the C-terminus: signal peptide (ACE2: 1-17), HiBiT, extracellular domain (ACE2: 18-740), transmembrane domain (ACE2: 741-761), cytoplasmic domain (ACE2: 762-805), SBP tag, and FLAG tag. The complete nucleotide sequence of the HiBiT-ACE2-SBP-FLAG construct is provided below, with functional elements and linkers annotated. The sequence has been verified by Sanger sequencing.

ATGTCAAGCTCTTCCTGGCTCCTTCTCAGCCTTGTTGCTGTAAGTCTGCTGCT (ACE2: 1-17, Signal peptide)
 GTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGC (HiBiT) GGGAGCTCCGGT (GSSG linker) CAGTCCAC
 CATTGAGGAACAGGCCAAGACATTTTTGGACAAGTTTAACCACGAAGCCGAAGACCTGTTCTATCAAA
 GTTCACCTTGCTTCTTGGAATTATAACACCAATATTACTGAAGAGAATGTCCAAAACATGAATAATGCT
 GGGGACAAATGGTCTGCCTTTTTAAAGGAACAGTCCACACTTGCCCAAATGTATCCACTACAAGAAAT
 TCAGAATCTCACAGTCAAGCTTCAGCTGCAGGCTCTTCAGCAAAATGGGTCTTCAGTGCTCTCAGAAG
 ACAAGAGCAAACGGTTGAACACAATTCTAAATACAATGAGCACCATCTACAGTACTGGAAAAGTTTGT
 AACCCAGATAATCCACAAGAATGCTTATTACTTGAACCAGGTTTGAATGAAATAATGGCAAACAGTTT
 AGACTACAATGAGAGGCTCTGGGCTTGGGAAAGCTGGAGATCTGAGGTCCGCAAGCAGCTGAGGCCA
 TTATATGAAGAGTATGTGGTCTTGA AAAATGAGATGGCAAGAGCAAATCATTATGAGGACTATGGGGA
 TTATTGGAGAGGAGACTATGAAGTAAATGGGGTAGATGGCTATGACTACAGCCGCGGCCAGTTGATT
 GAAGATGTGGAACATACCTTTGAAGAGATTAAACCATTATATGAACATCTTCATGCCTATGTGAGGGC
 AAAGTTGATGAATGCCTATCCTTCCTATATCAGTCCAATTGGATGCCTCCCTGCTCATTGCTTGGTGA
 TATGTGGGGTAGATTTTGGACAAATCTGTACTCTTTGACAGTTCCCTTTGGACAGAAACCAAACATAG
 ATGTTACTGATGCAATGGTGGACCAGGCCTGGGATGCACAGAGAATATTCAAGGAGGCCGAGAAGTT
 CTTTGTATCTGTTGGTCTTCCTAATATGACTCAAGGATTCTGGGAAAATTCCATGCTAACGGACCCAG
 GAAATGTTTCAGAAAGCAGTCTGCCATCCCACAGCTTGGGACCTGGGGAAGGGCGACTTCAGGATCCT
 TATGTGCACAAAGGTGACAATGGACGACTTCCTGACAGCTCATCATGAGATGGGGCATATCCAGTATG
 ATATGGCATATGCTGCACAACCTTTTCTGCTAAGAAATGGAGCTAATGAAGGATTCCATGAAGCTGTT
 GGGGAAATCATGTCACTTTTCTGCAGCCACACCTAAGCATTTAAAATCCATTGGTCTTCTGTCACCCGA
 TTTTCAAGAAGACAATGAAACAGAAATAAACTTCCTGCTCAAAACAAGCACTCACGATTGTTGGGACTC
 TGCCATTTACTTACATGTTAGAGAAGTGGAGGTGGATGGTCTTTAAAGGGGAAATTCCTCAAAGACCAG
 TGGATGAAAAAGTGGTGGGAGATGAAGCGAGAGATAGTTGGGGTGGTGGAACCTGTGCCCCATGATG
 AAACATACTGTGACCCCGCATCTCTGTTCCATGTTTCTAATGATTACTCATTTCATTCGATATTACACAA
 GGACCCTTTACCAATTCCAGTTTCAAGAAGCACTTTGTCAAGCAGCTAAACATGAAGGCCCTCTGCAC
 AAATGTGACATCTCAAACTCTACAGAAGCTGGACAGAAACTGTTCAATATGCTGAGGCTTGGA AAATC
 AGAACCTGGACCCTAGCATTGGAAAATGTTGTAGGAGCAAAGAACATGAATGTAAGGCCACTGCTC
 AACTACTTTGAGCCCTTATTTACCTGGCTGAAAGACCAGAACAAAGAATTCTTTTGTGGGATGGAGTAC
 CGACTGGAGTCCATATGCAGACCAAAGCATCAAAGTGAGGATAAGCCTAAAATCAGCTCTTGGAGAT
 AAAGCATATGAATGGAACGACAATGAAATGTACCTGTTCCGATCATCTGTTGCATATGCTATGAGGCA
 GTACTTTTTTAAAGTAAAAAATCAGATGATTCTTTTTGGGGAGGAGGATGTGCGAGTGGCTAATTTGA
 AACCAAGAATCTCCTTTAATTTCTTTGTCACTGCACCTAAAAATGTGTCTGATATCATTCTAGAACTG
 AAGTTGAAAAGGCCATCAGGATGTCCCGGAGCCGTATCAATGATGCTTTCCGTCTGAATGACAACAGC
 CTAGAGTTTCTGGGGATACAGCCAACACTTGGACCTCCTAACCAGCCCCCTGTTTCC (ACE2: 18-740, Ex
 tracellular) ATATGGCTGATTGTTTTTGGAGTTGTGATGGGAGTGATAGTGGTTGGCATTGTCATCCTGA
 TC (ACE2: 741-761, Transmembrane) TTCACTGGGATCAGAGATCGGAAGAAGAAAAATAAAGCAAGAAG
 TGGAGAAAATCCTTATGCCTCCATCGATATTAGCAAAGGAGAAAAATAATCCAGGATTCAAAAACACTG
 ATGATGTTTCAGACCTCCTTT (ACE2: 762-805, Cytoplasmic) ctgaattcc (LNS linker) atggacgagaagaccactgggtggc
 gaggtggacaggtgtgaaggactggctggggaactgaacaacttcgtgcagactggagcatcaccacaaggtcaacgtgaacca (SBP) cctgcaggt (P
 AG linker) GATTACAAGGACGACGATGACAAG (FLAG) TAG

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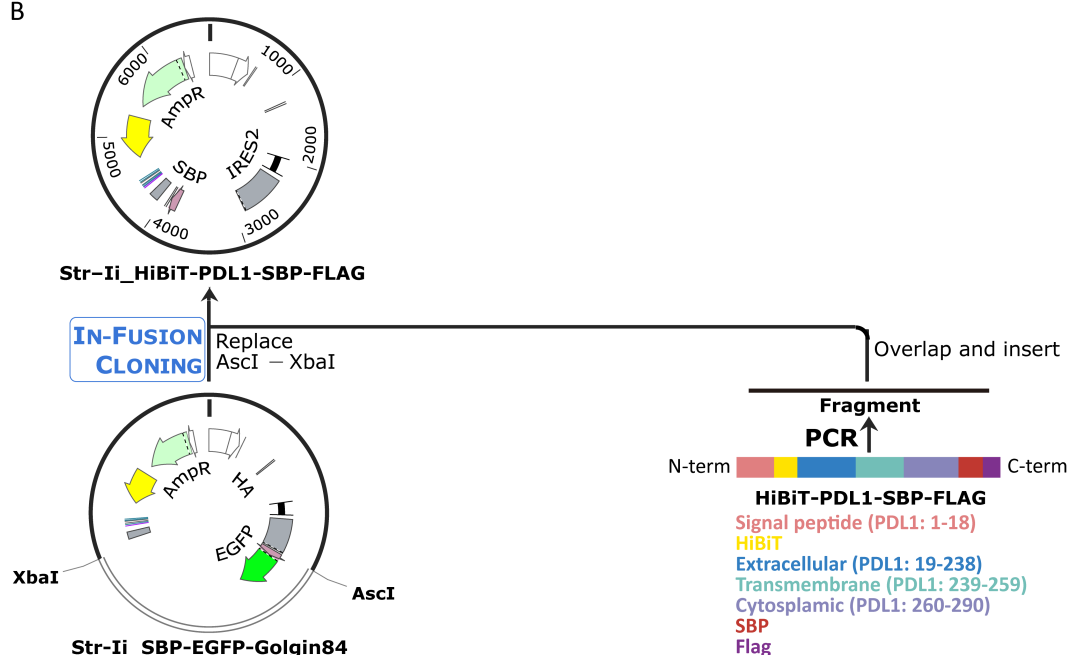
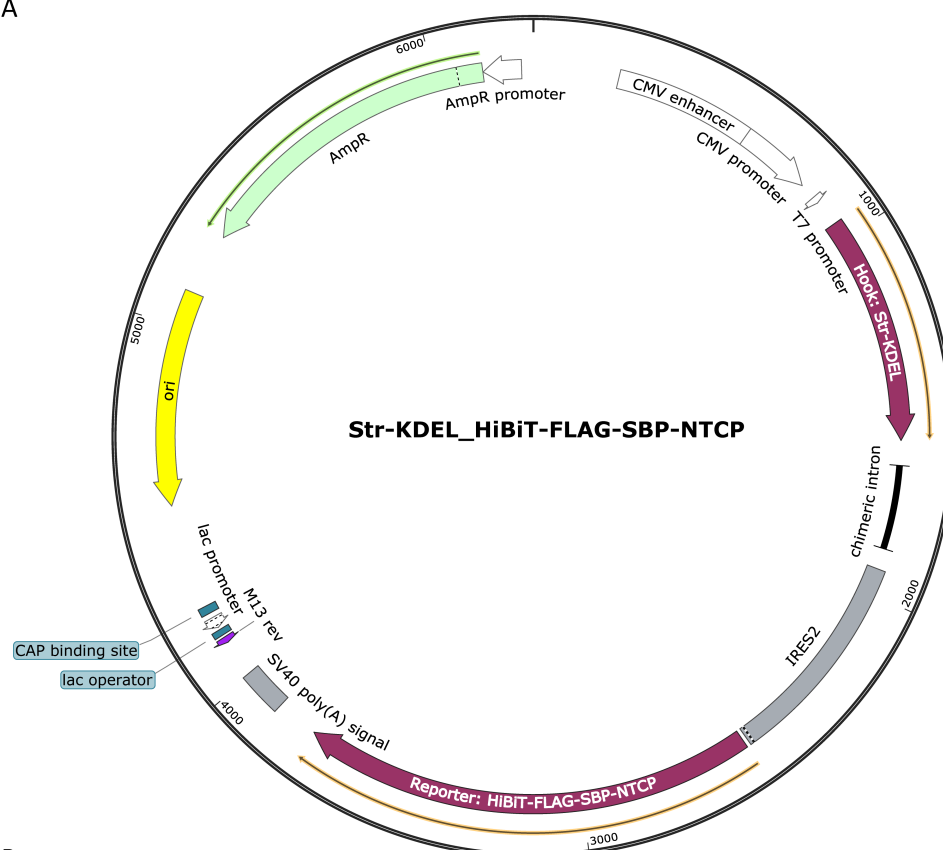


Figure S2. Plasmid map and cloning strategy of Str-Ii_HiBiT-PDL1-SBP-FLAG. (A) Plasmid map of Str-Ii_HiBiT-PDL1-SBP-FLAG generated using SnapGene software. (B) Schematic diagram of the In-Fusion cloning strategy. The parental vector Str-Ii_SBP-EGFP-Golgin84 was digested with AscI and XbaI to excise the SBP-EGFP-Golgin84 cassette. The HiBiT-PDL1-SBP-FLAG fragment (amplified by PCR with overlapping ends) was inserted into the linearized vector via In-Fusion cloning to generate Str-Ii_HiBiT-PDL1-SBP-FLAG. The domain organization of the inserted fragment is color-coded from the N-terminus to the C-terminus: signal peptide (PDL1: 1-18), HiBiT, extracellular domain (PDL1: 19-238), transmembrane domain (PDL1: 239-259), cytoplasmic domain (PDL1: 260-290), SBP tag, and FLAG tag. The complete nucleotide sequence of the HiBiT-PDL1-SBP-FLAG construct is provided below, with functional elements and linkers annotated. The sequence has been verified by Sanger sequencing.

ATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTGCTGAACGCA (PDL1: 1-18, Signal peptide) GTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGC (HiBiT) GGGAGCTCCGGT (GSSG linker) TTTAC TGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTAGCAATATGACAATTGAATGCAAATTCC CAGTAGAAAAACAATTAGACCTGGCTGCACTAATTGTCTATTGGGAAATGGAGGATAAGAACATTATT CAATTTGTGCATGGAGAGGAAGACCTGAAGGTTTCAGCATAGTAGCTACAGACAGAGGGCCCGGCTGT TGAAGGACCAGCTCTCCCTGGGAAATGCTGCACTTCAGATCACAGATGTGAAATTGCAGGATGCAGG GGTGTACCGCTGCATGATCAGCTATGGTGGTGCCGACTACAAGCGAATTACTGTGAAAGTCAATGCC CCATACAACAAAATCAACCAAAGAATTTTGGTTGTGGATCCAGTCACCTCTGAACATGAACTGACATG TCAGGCTGAGGGCTACCCCAAGGCCGAAGTCATCTGGACAAGCAGTGACCATCAAGTCCTGAGTGGT AAGACCACCACCACCAATTCCAAGAGAGAGGAGAAGCTTTTCAATGTGACCAGCACACTGAGAATCA ACACAACAATAATGAGATTTTCTACTGCACTTTTAGGAGATTAGATCCTGAGGAAAACCATACAGCT GAATTGGTCATCCCAGAACTACCTCTGGCACATCCTCCAAATGAAAGG (PDL1: 19-238, Extracellular) AC TCACTTGGTAATTCTGGGAGCCATCTTATTATGCCTTGGTGTAGCACTGACATTCATCTTC (PDL1: 239-259, Transmembrane) CGTTTAAGAAAAGGGAGAATGATGGATGTGAAAAAATGTGGCATCCAAGATACA AACTCAAAGAAGCAAAGTGATACACATTTGGAGGAGACG (PDL1: 260-290, Cytoplasmic) ctgaattcc (LNS linker) atggacgagaagaccactgggtggcgaggtggacacgtgtgaaggactggctggggaactgaacaactcgtgcacgactggagcatcaccacaaggt caacgtgaacca (SBP) cctgcaggt (PAG linker) GATTACAAGGACGACGATGACAAG (FLAG) TAG

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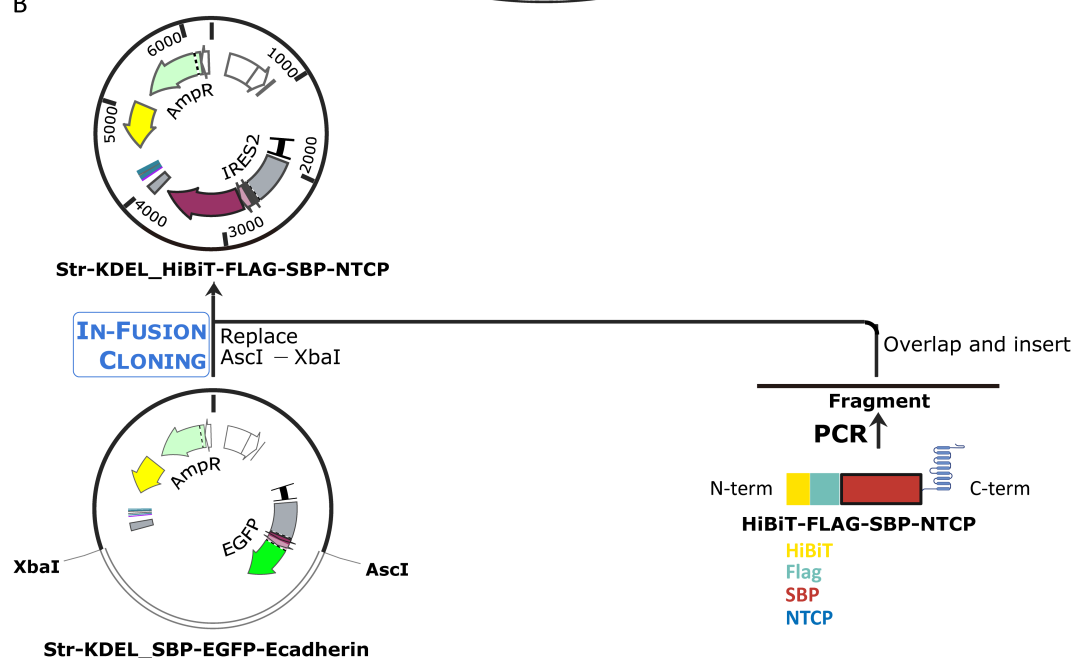
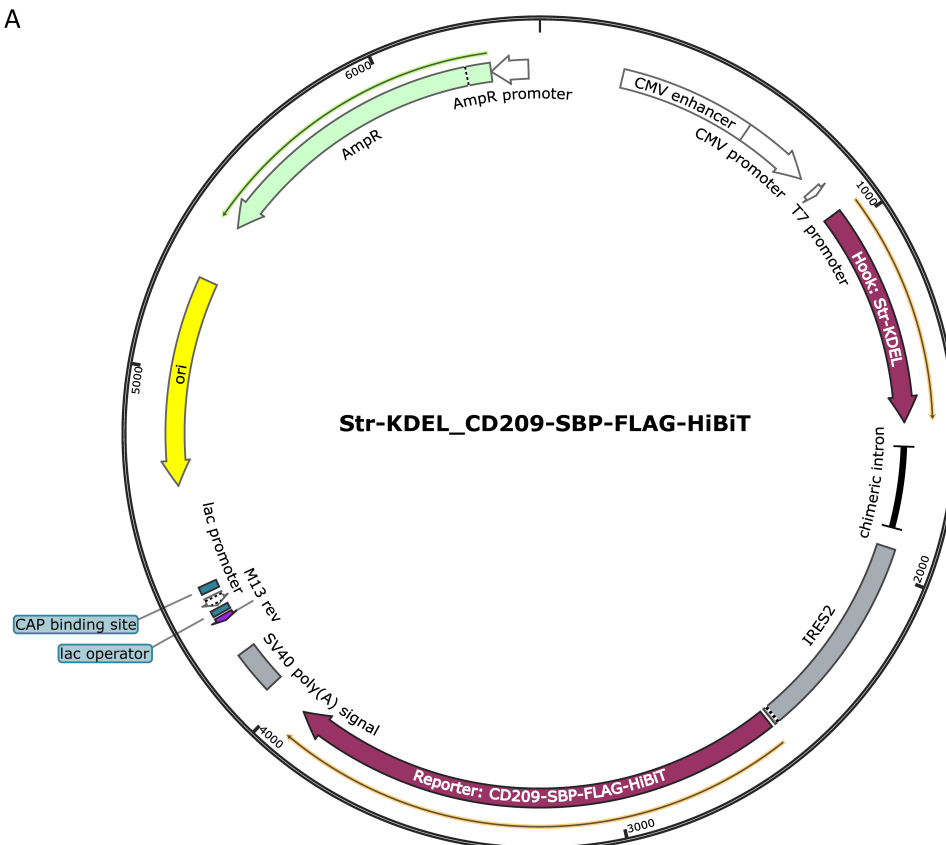


Figure S3. Plasmid map and cloning strategy of Str-KDEL_HiBiT-FLAG-SBP-NTCP. (A) Plasmid map of Str-KDEL_HiBiT-FLAG-SBP-NTCP generated using SnapGene software. (B) Schematic diagram of the In-Fusion cloning strategy. The parental vector Str-KDEL_SBP-EGFP-Ecadherin was digested with AscI and XbaI to excise the SBP-EGFP-Ecadherin cassette. The HiBiT- FLAG-SBP-NTCP fragment (amplified by PCR with overlapping ends) was inserted into the linearized vector via In-Fusion cloning to generate Str-KDEL_HiBiT-FLAG-SBP-NTCP. The domain organization of the inserted fragment is color-coded from the N-terminus to the C-terminus: HiBiT, FLAG tag, SBP tag, and NTCP (full length). The complete nucleotide sequence of the HiBiT-FLAG-SBP-NTCP construct is provided below, with functional elements and linkers annotated. The sequence has been verified by Sanger sequencing.

ATG**GTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGC (HiBiT)** GGATCC (GS linker) **GATTACAAGGAC GACGATGACAAG (FLAG)** GGT**ag**t (GS linker) **gacgagaagaccactgggtggcgaggtggacacgtgttgaaggactggctggggaactt gaacaacttcgtgcacgactggagcatcaccacaaggtcaacgtgaacca (SBP)** gga**agc** (GS linker) **ATGGAGGCCCCACAACGCGT CTGCCCCATTCAACTTCACCCTGCCACCCAACCTTGGCAAGCGCCCCACAGACCTGGCACTGAGCGTC ATCCTGGTGTTCATGTTGTTCTTCATCATGCTCTCGCTGGGCTGCACCATGGAGTTCAGCAAGATCAA GGCTCACTTATGGAAGCCTAAAGGGCTGGCCATCGCCCTGGTGGCACAGTATGGCATCATGCCCTC ACGGCCTTTGTGCTGGGCAAGGTCTTCCGGCTGAAGAACATTGAGGCACTGGCCATCTTGGTCTGTG GCTGCTCACCTGGAGGGAACCTGTCCAATGTCTTCAGTCTGGCCATGAAGGGGGACATGAACCTCAG CATTGTGATGACCACCTGCTCCACCTTCTGTGCCCTTGGCATGATGCCTCTCCTCCTGTACATCTACTC CAGGGGGATCTATGATGGGGACCTGAAGGACAAGGTGCCCTATAAAGGCATCGTGATATCACTGGTC CTGGTTCTCATTCCTTGACCATAGGGATCGTCCTCAAATCCAAACGGCCACAATACATGCGCTATGT CATCAAGGGAGGGATGATCATCATTCTTGTGCAGTGTGGCCGTCACAGTTCTCTCTGCCATCAATG TGGGGAAGAGCATCATGTTTGCCATGACACCACTCTTGATTGCCACCTCCTCCCTGATGCCTTTTATT GGCTTTCTGCTGGGTTATGTTCTCTCTGCTCTCTTCTGCCTCAATGGACGGTGCAGACGCACTGTCAG CATGGAGACTGGATGCCAAAATGTCCAACCTCTGTTCCACCATCCTCAATGTGGCCTTTCCACCTGAAG TCATTGGACCACTTTTCTTCTTCCCTCCTCTACATGATTTTCCAGCTTGGAGAAGGGCTTCTCCTCA TTGCCATATTTGGTGCTATGAGAAATTCAAGACTCCCAAGGATAAAACAAAATGATCTACACAGCT GCCACAACCTGAAGAAACAATTCCAGGAGCTCTGGGAAATGGCACCTACAAAGGGGAGGACTGCTCCC CTTGCACAGCCTAG (NTCP: full length)**

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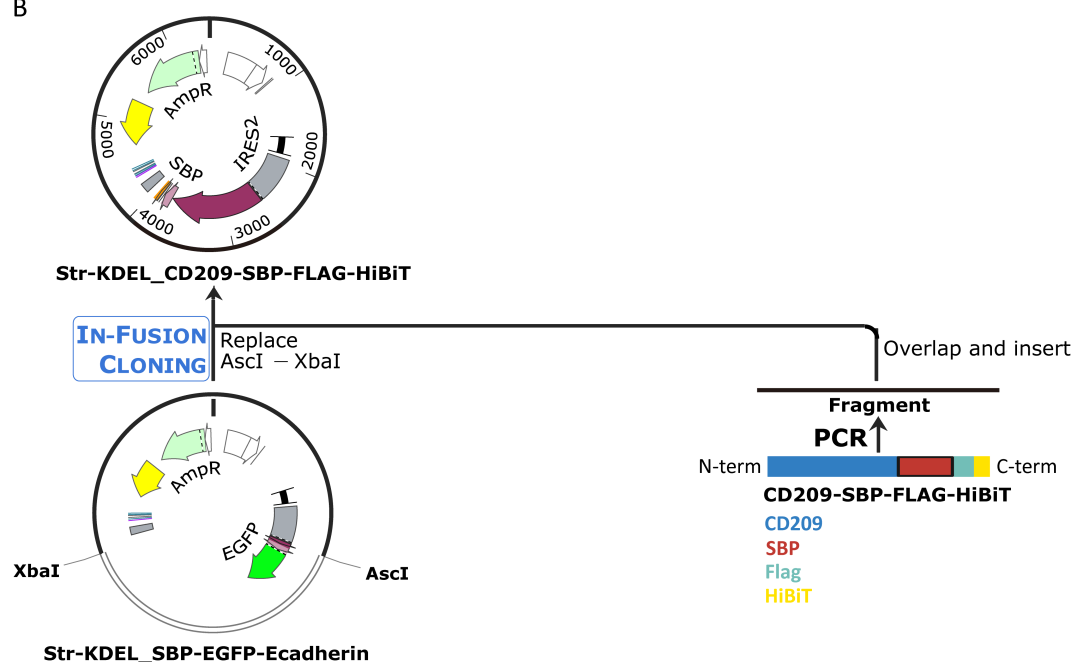


Figure S4. Plasmid map and cloning strategy of Str-KDEL_CD209-SBP-FLAG-HiBiT. (A) Plasmid map of Str-KDEL_CD209-SBP-FLAG-HiBiT generated using SnapGene software. (B) Schematic diagram of the In-Fusion cloning strategy. The parental vector Str-KDEL_SBP-EGFP-Ecadherin was digested with AscI and XbaI to excise the SBP-EGFP-Ecadherin cassette. The CD209-SBP-FLAG-HiBiT fragment (amplified by PCR with overlapping ends) was inserted into the linearized vector via In-Fusion cloning to generate Str-KDEL_CD209-SBP-FLAG-HiBiT. The domain organization of the inserted fragment is color-coded from the N-terminus to the C-terminus: CD209 (full length), SBP tag, FLAG tag, and HiBiT. The complete nucleotide sequence of the CD209-SBP-FLAG-HiBiT construct is provided below, with functional elements and linkers annotated. The sequence has been verified by Sanger sequencing.

ATGAGTGACTCCAAGGAACCAAGACTGCAGCAGCTGGGCCTCCTGGAGGAGGAACAGCTGAGAGGCC
TTGGATTCCGACAGACTCGAGGATACAAGAGCTTAGCAGGGTGTCTTGGCCATGGTCCCCTGGTGCT
GCAACTCCTCTCCTTCACGCTCTTGGCTGGGCTCCTTGTCCAAGTGTCCAAGGTCCCCAGCTCCATAA
GTCAGGAACAATCCAGGCAAGACGCGATCTACCAGAACCTGACCCAGCTTAAAGCTGCAGTGGGTGA
GCTCTCAGAGAAATCCAAGCTGCAGGAGATCTACCAGGAGCTGACCCAGCTGAAGGCTGCAGTGGGT
GAGCTTCCAGAGAAATCTAAGCTGCAGGAGATCTACCAGGAGCTGACCCGGCTGAAGGCTGCAGTGG
GTGAGCTTCCAGAGAAATCTAAGCTGCAGGAGATCTACCAGGAGCTGACCTGGCTGAAGGCTGCAGT
GGGTGAGCTTCCAGAGAAATCTAAGATGCAGGAGATCTACCAGGAGCTGACTCGGCTGAAGGCTGCA
GTGGGTGAGCTTCCAGAGAAATCTAAGCAGCAGGAGATCTACCAGGAGCTGACCCGGCTGAAGGCTG
CAGTGGGTGAGCTTCCAGAGAAATCTAAGCAGCAGGAGATCTACCAGGAGCTGACCCGGCTGAAGGC
TGCAGTGGGTGAGCTTCCAGAGAAATCTAAGCAGCAGGAGATCTACCAGGAGCTGACCCAGCTGAAG
GCTGCAGTGGAAACGCCTGTGCCACCCCTGTCCCTGGGAATGGACATTCTTCCAAGGAAACTGTTACTT
CATGTCTAACTCCCAGCGGAACTGGCAGCACTCCATCACCGCCTGCAAAGAAGTGGGGGCCAGCTC
GTCGTAATCAAAAGTGCTGAGGAGCAGAACTTCTACAGCTGCAGTCTTCCAGAAGTAACCGCTTAC
CTGGATGGGACTTTCAGATCTAAATCAGGAAGGCACGTGGCAATGGGTGGACGGCTCACCTCTGTTG
CCCAGCTTCAAGCAGTATTGGAACAGAGGAGAGCCCAACAACGTTGGGGAGGAAGACTGCGCGGAAT
TLAGTGGCAATGGCTGGAACGACGACAAATGTAATCTTGCCAAATTCTGGATCTGCAAAAAGTCCGCA
GCCTCCTGCTCCAGGGATGAAGAACAGTTTCTTTCTCCAGCCCCTGCCACCCCAAAACCCCCCTCCTGC
G (CD209: full length) CTGAATAGC (LNS linker) gacgagaagaccactggtggcgaggtggacacgttggtgaaggactggtgggaac
ttgaacaacttcgtgcacgactggagcatcaccacaaggtaacgtgaacca (SBP) CCCGCCGGC (PAG linker) GATTACAAGGAC
GACGATGACAAG (FLAG) GGATCC (GS linker) GTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGC (HiBi
T) TAG

Table S1. Primers used for molecular cloning

Plasmid	Forward	Reverse
Str-Ii_HiBiT-ACE2-SBP-FLAG	5'-cttgccacaacccggATGTCAAGCTC TTCCTGG-3'	5'-tgatcagttatctagCTACTTGTCAT CGTCGTCC-3'
Str-Ii_HiBiT-PDL1-SBP-FLAG	5'-cttgccacaacccggATGAGGATATT GCTGTCTTTAT-3'	5'-tgatcagttatctagCTACTTGTCAT CGTCGTCC-3'
Str-KDEL_HiBiT-FLAG-SBP-NTCP	5'-aacccgggagggcgcgATGGTGAGCG GCTGGC-3'	5'-tgatcagttatctagCTAGGCTGTGC AAGGGGAG-3'
Str-KDEL_CD209-SBP-FLAG-HiBiT	5'-aacccgggagggcgcgATGAGTGACT CCAAGGAACC-3'	5'-tgatcagttatctagCTAGCTAATCT TCTTGAACAG-3'