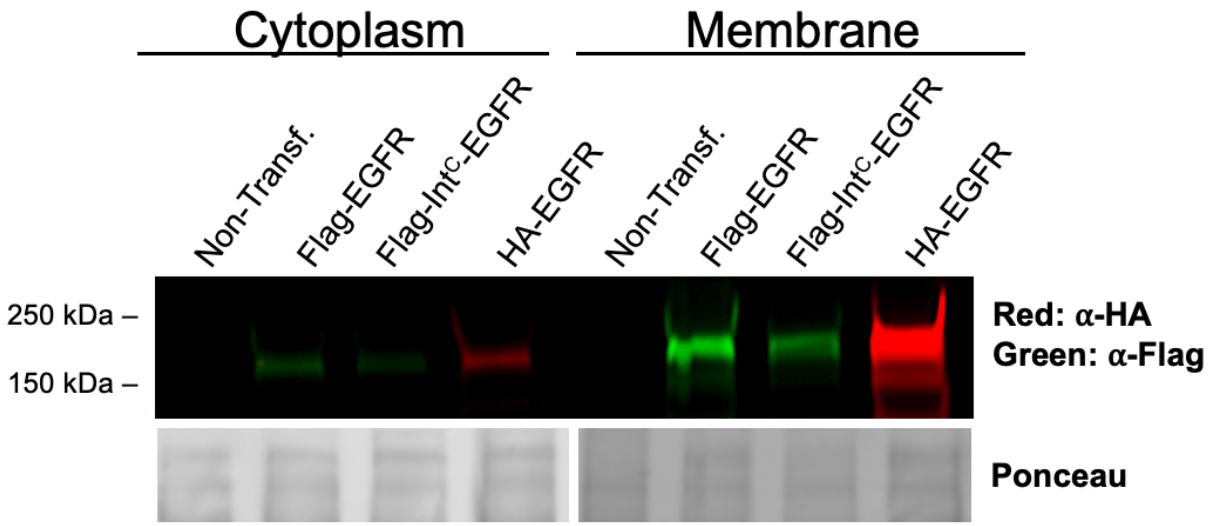
Supplemental Figures

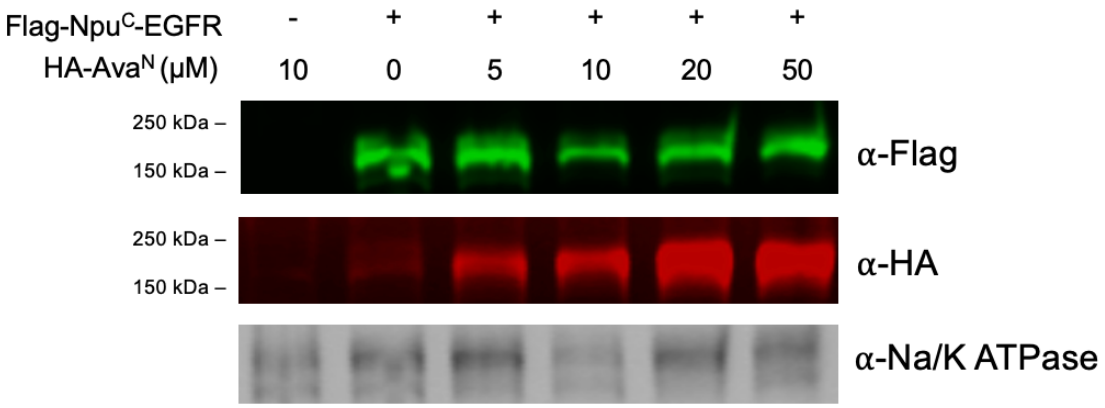
Supplemental Figure 1

Western blot comparison of membrane expression levels of Flag-EFGR vs Flag-Npu^{C}-EGFR



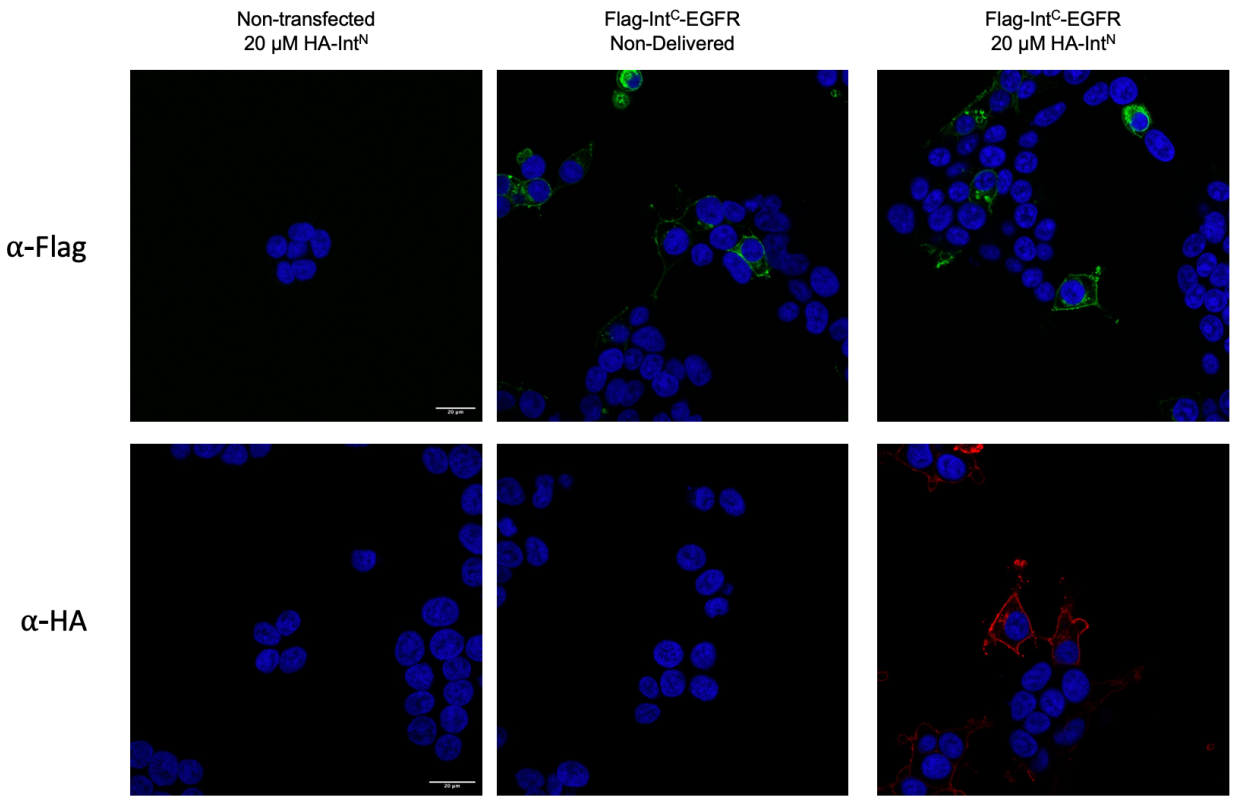
HEK293T cells were transfected with the different EGFR constructs (Flag-EGFR, Flag-Int^{C}-EGFR, and HA-EGFR). The cytoplasm and membrane fractions were isolated and analyzed by western analysis (anti-Flag – green, anti-HA – red, Ponceau – loading control).

Supplemental Figure 2
Optimization of HA-Ava^{N} concentration in intein splicing



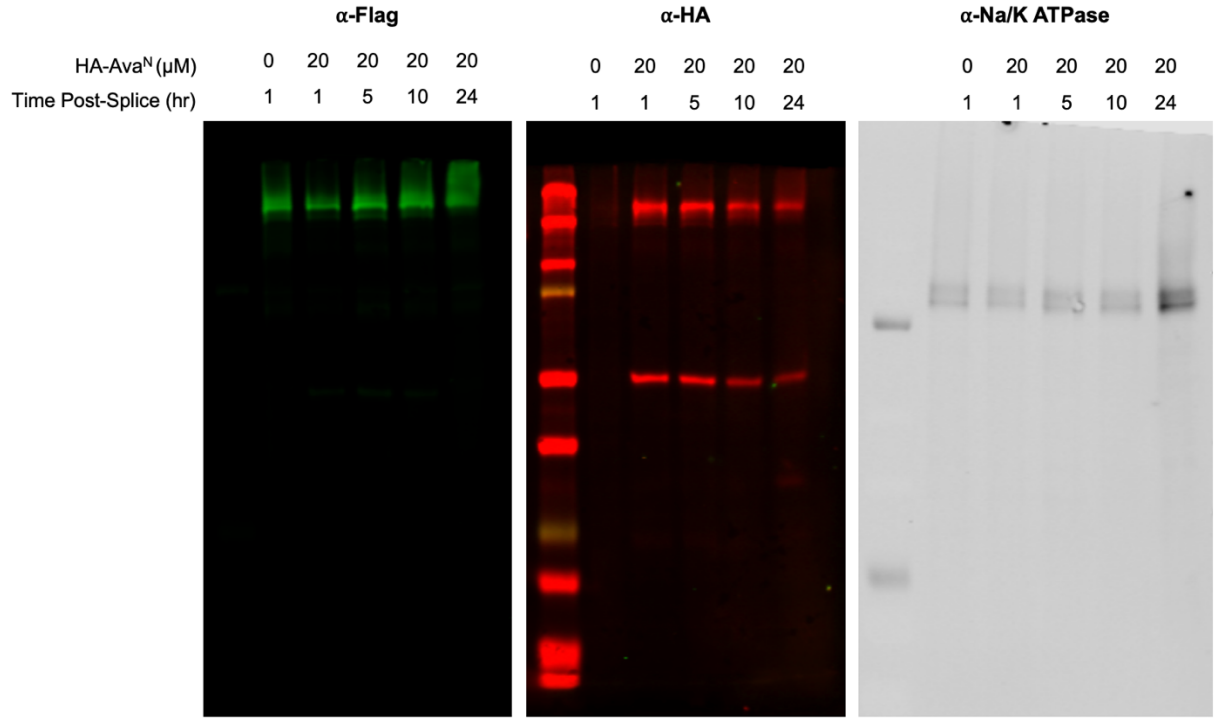
HEK293T cells were transfected with Flag-Npu^{C}-EGFR and treated with increasing concentrations of HA-Ava^{N} (0-50 mM) for 1 hour. The membrane fractions were isolated and analyzed by western blot (anti-Flag – green, anti-HA – red, Na/K ATPase – membrane fraction loading control).

Supplemental Figure 3
Full view immunofluorescence of HA-Ava^{N} splicing.



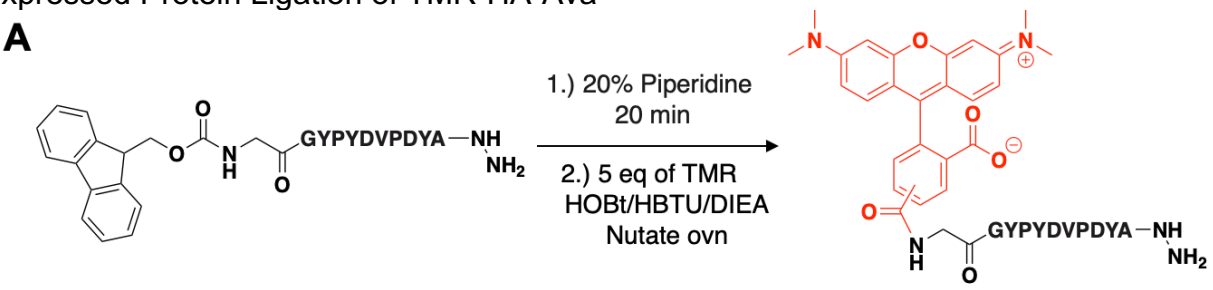
Immunofluorescence HEK293T cells transfected with Flag-Npu^{C}-EGFR and incubated with HA-Ava^{N}. Cells were fixed with paraformaldehyde and then stained with either anti-Flag (green) or anti-HA (red) and DAPI (blue) followed by confocal imaging.

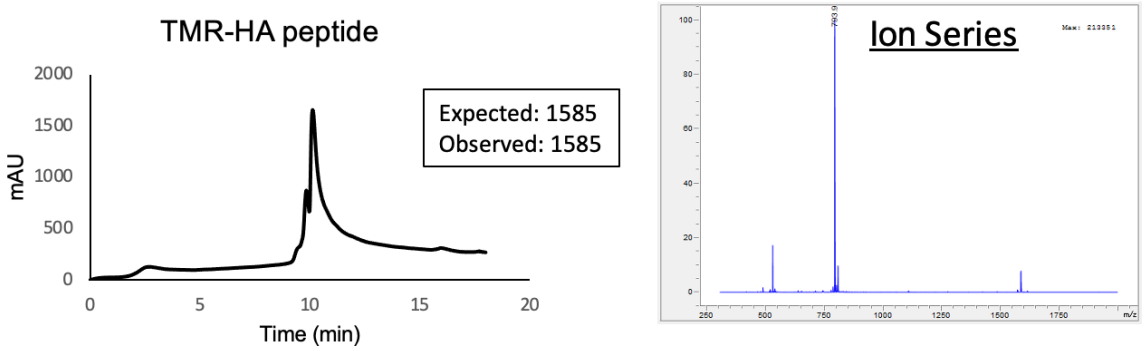
Supplemental Figure 4
Uncropped, unedited Figure 2B

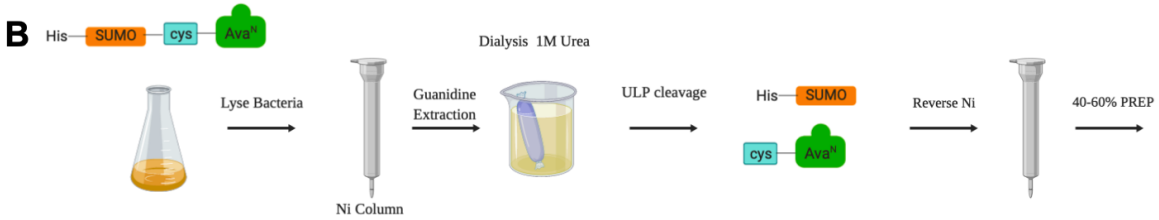


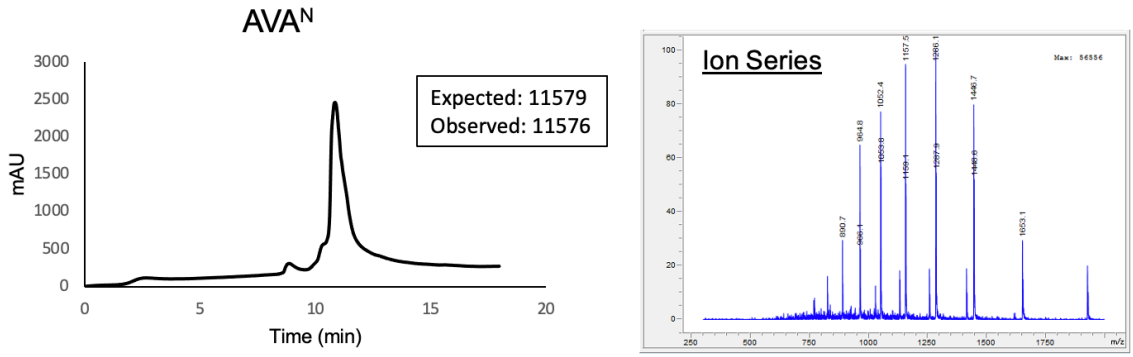
HEK293T cells were transfected with Flag-Npu^C-EGFR and then treated with 20 μM of HA-Ava^N for 1 hr. Cells were then harvested at the indicated time points post splicing and the membrane fraction was extracted and analyzed by western blot (anti-Flag – green, anti-HA – red, anti-Na/K ATPase – gray scale)

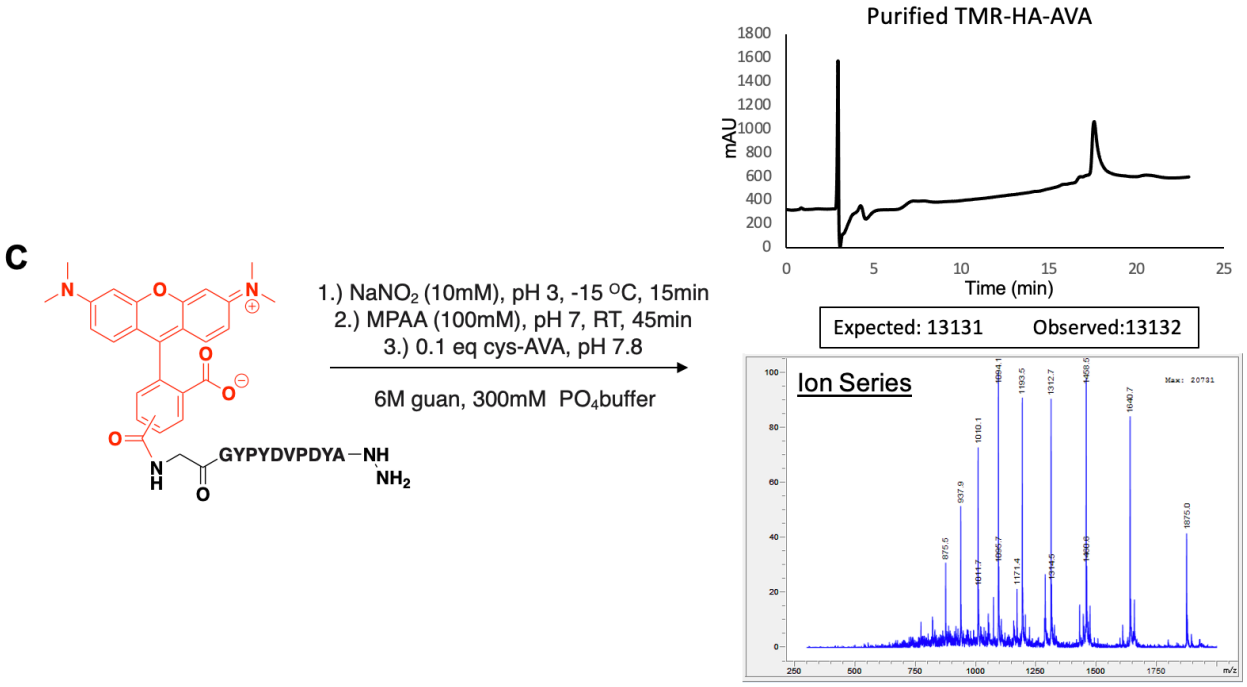
Supplemental Figure 5
Expressed Protein Ligation of TMR-HA-Ava^N





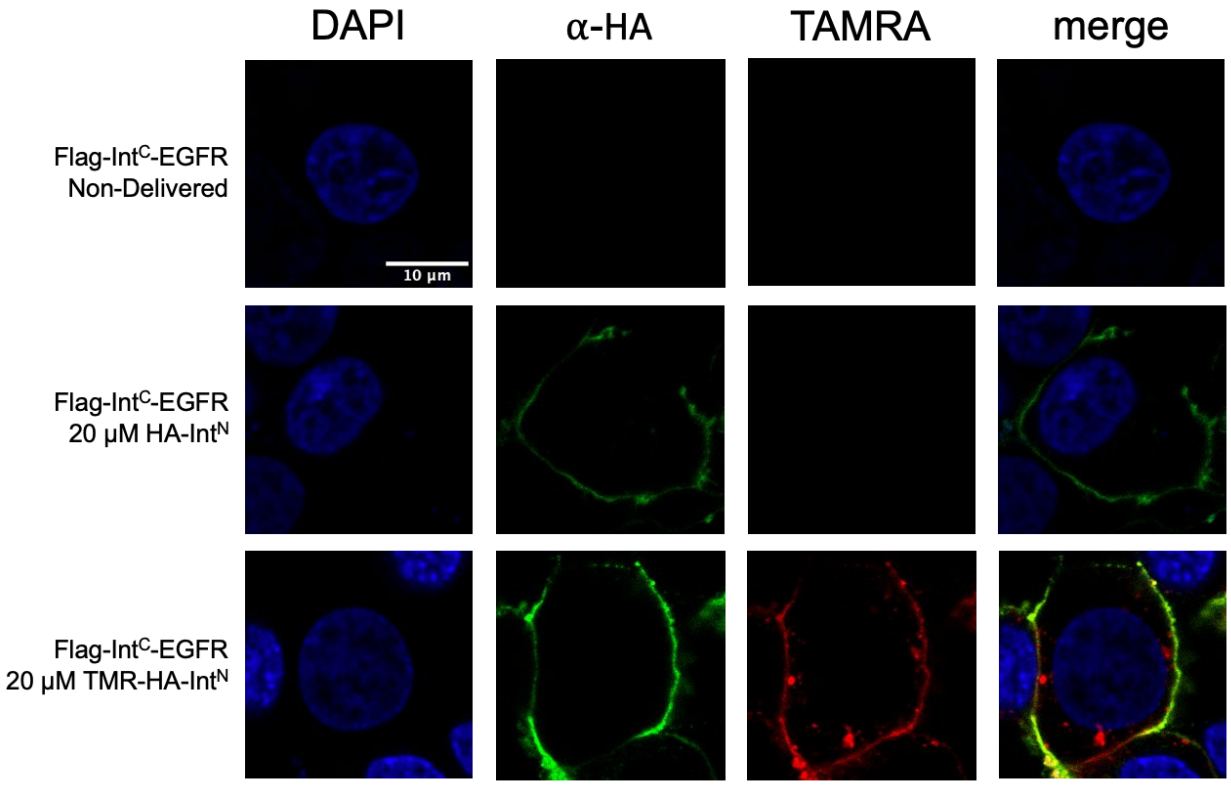






A) Synthesis of TMR-HA peptide. The HA peptide was synthesized via F-moc based solid phase peptide synthesis, followed by a coupling of the TMR fluorophore to the N-terminal glycine. Representative Liquid-Chromatography/Mass-Spectrometry chromatogram (measured at 214nm) and Ion Series of purified peptide are presented. **B)** Purification of Ava^N with N-terminal cysteine. Representative LCMS (214nm) and Ion Series of purified protein. **C)** Expressed protein ligation of TMR-HA peptide and cys-Ava^N. Representative LCMS (214nm) and Ion Series of purified product.

Supplemental Fig 6
TMR spliced EGFR mimics recombinant HA-Ava^{N} spliced EGFR



HEK293T cells expressing Flag-Int^{C}-EGFR were treated with 20 μM of HA-Ava^{N} or TMR-HA-Ava^{N} for 60 minutes. Cells were fixed with paraformaldehyde, stained with anti-HA (green), DAPI (blue) and imaged (red-TMR).