SUPPLEMENTARY INFORMATION

Figure S1. Plasmids used in this work.

Name	Backbone	Insert sequence
p_mCherry_EGFPn_inteinN	pEF1α-mCherry-C1 (Clontech, Catalog No. 631972)	ATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCT TCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGA GGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAA GGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTC CAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCC CCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGAC CGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGC GCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTG GGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATC AAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACC ACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCA AGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACG CGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTCCGGAGCG GGCAGCGCGGCGGGCAGCGGCGAATTTCCTGGCAGCATGAGCAAAGGAGAAGAA CTGTTTACCGGAGTGGTGCCAATTCTCGTGGAACTGGATGGCGATGTTAATGGGCA CAAATTTTCTGTCAGAGGAGAAGGTGAAGGTGATGCCACAATTGGAAAGCTCACT CTGAAATTCATCTGCACCACTGGAAAACTCCCTGTGCCATGGCCAACACTGGTCAC TACCTTAACCTACGGCGTGCAGTGCTTTTCAAGATATCCAGATCATATGAAAAGGC ATGACTTTTTCAAATCAGCAATGCCCGAGGGGTATGTGCAGGAGCGAACGATTTCT TTCAAAGATGATGGGAAATACAAGACCCGCGCTGTTGTTAAGTTTGAAGGAGACG ATAAAGGCATTGAATGCAGCGTGTTTAGCATTGATAGCAACGGCATTGTGTATACC CAGCCGATTGCGCAGTGGCATCATCGCGGCAAACAGGAAGTGTTTGAATATTGCCT GGAAGATGGCAGCATTATTAAAGCGACCAAAGATCATAAATTTATGACCCAGGAT GGCAAAATGCTGCCGATTGATGAAATTTTTGAACAGGAACTGGATCTGCTGCAGGT GAAAGGCCTGCCGGAATAAG
p_inteinC_EGFPC_SBFP	pCMV-GFP (Addgene #11153)	ATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCT TCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGA GGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAA GGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTC CAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCC CCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGAC CGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGC GCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTG GGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATC AAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACC ACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCA AGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACG CGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTCCGGAGCG GGCAGCGCGGCGGGCAGCGGCGAATTTCCTGGCAGCATGAGCAAAGGAGAAGAA CTGTTTACCGGAGTGGTGCCAATTCTCGTGGAACTGGATGGCGATGTTAATGGGCA CAAATTTTCTGTCAGAGGAGAAGGTGAAGGTGATGCCACAATTGGAAAGCTCACT CTGAAATTCATCTGCACCACTGGAAAACTCCCTGTGCCATGGCCAACACTGGTCAC TACCTTAACCTACGGCGTGCAGTGCTTTTCAAGATATCCAGATCATATGAAAAGGC ATGACTTTTTCAAATCAGCAATGCCCGAGGGGTATGTGCAGGAGCGAACGATTTCT TTCAAAGATGATGGGAAATACAAGACCCGCGCTGTTGTTAAGTTTGAAGGAGACG

		ATAAAGGCATTGAATGCAGCGTGTTTAGCATTGATAGCAACGGCATTGTGTATACC CAGCCGATTGCGCAGTGGCATCATCGCGGCAAACAGGAAGTGTTTGAATATTGCCT GGAAGATGGCAGCATTATTAAAGCGACCAAAGATCATAAATTTATGACCCAGGAT GGCAAAATGCTGCCGATTGATGAAATTTTTGAACAGGAACTGGATCTGCTGCAGGT GAAAGGCCTGCCGGAATAAG
p_mCherry_EGFP1-10_inteinN	pEF1α-mCherry-C1 (Clontech, Catalog No. 631972)	ATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCT TCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGA GGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAA GGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTC CAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCC CCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGAC CGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGC GCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTG GGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATC AAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACC ACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCA AGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACG CGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTCCGGAGCG GGCAGCGCGGCGGGCAGCGGCGAATTTCCTGGCAGCATGAGCAAAGGAGAAGAA CTGTTTACCGGAGTGGTGCCAATTCTCGTGGAACTGGATGGCGATGTTAATGGGCA CAAATTTTCTGTCAGAGGAGAAGGTGAAGGTGATGCCACAATTGGAAAGCTCACT CTGAAATTCATCTGCACCACTGGAAAACTCCCTGTGCCATGGCCAACACTGGTCAC TACCTTAACCTACGGCGTGCAGTGCTTTTCAAGATATCCAGATCATATGAAAAGGC ATGACTTTTTCAAATCAGCAATGCCCGAGGGGTATGTGCAGGAGCGAACGATTTCT TTCAAAGATGATGGGAAATACAAGACCCGCGCTGTTGTTAAGTTTGAAGGAGACA CACTGGTGAATCGTATCGAGTTGAAGGGGACAGACTTTAAGGAAGACGGAAATAT TCTCGGGCACAAGCTGGAATACAACTTTAATAGTCACAATGTGTATATCACAGCGG ACAAGCAAAAGAATGGTATCAAAGCAAACTTCACAGTCAGACACAACGTCGAAG ATGGAAGCGTTCAGCTGGCCGACCATTATCAACAGAACACTCCAATCGGGGACGG CCCTGTGCTCCTCCCAGACAACCATTACCTGTCAACCCAGACTGTCCTAAGTAAAG ATCCAAATGAAAAAGATAAAGGCATTGAATGCAGCGTGTTTAGCATTGATAGCAA CGGCATTGTGTATACCCAGCCGATTGCGCAGTGGCATCATCGCGGCAAACAGGAA GTGTTTGAATATTGCCTGGAAGATGGCAGCATTATTAAAGCGACCAAAGATCATAA ATTTATGACCCAGGATGGCAAAATGCTGCCGATTGATGAAATTTTTGAACAGGAAC TGGATCTGCTGCAGGTGAAAGGCCTGCCGGAATAAG
p_inteinC_EGFP11_SBFP	pCMV-GFP (Addgene #11153)	ATGATTAAAATTGCGACCCGCAAATATCTGGGCAAACAGAACGTGTATGATATTGG CGTGGAACGCGATCATAACTTTGCGCTGAAAAACGGCTTTATTGCGAGCAACTGCT TTAACAAACGGGACCACATGGTGCTGCACGAGTACGTGAACGCCGCCGGCATCAC AGGCAGCGCGGGCAGCGCGGCGGGCAGCGGCGAATTTATGGTGAGCAAAGGCGA AGAACTGTTTACCGGCGTGGTGCCGATTCTGGTGGAACTGGATGGCGATGTGAACG GCCATAAATTTAGCGTGAGCGGCGAAGGCGAAGGCGATGCGACCTATGGCAAACT GACCCTGAAATTTATTTGCACCACCGGCAAACTGCCGGTGCCGTGGCCGACCCTGG TGACCACCCTGAGCCATGGCGTGCAGTGCTTTGCGCGCTATCCGGATCATATGAAA CAGCATGATTTTTTTTAAAAGCGCGATGCCGGAAGGCTATGTGCAGGAACGCACCAT TTTTTTTAAAGATGATGGCAACTATAAAACCCGCGCGGAAGTGAAATTTGAAGGCG ATACCCTGGTGAACCGCATTGAACTGAAAGGCATTGATTTTAAAGAAGATGGCAA CATTCTGGGCCATAAACTGGAATATAACTTTAACAGCCATAACGTGTATATTACCG CGGATAAACAGAAAAACGGCATTAAAGCGAACTTTAAAATTCGCCATAACATTGA AGATGGCGGCGTGCAGCTGGCGGATCATTATCAGCAGAACACCCCGATTGGCGAT GGCCCGGTGCTGCTGCCGGATAACCATTATCTGAGCACCCAGAGCAAACTGAGCA AAGATCCGAACGAAAAACGCGATCATATGGTGCTGCTGGAATTTGTGACCGCGGC GGGCATTACCCTGGGCATGGATGAACTGTATAAAGAATTCCATCATCATCATCATC ATATGTAAT

p_EGFP_inteinN	pCMV-GFP	(Addgene #11153)	ATGCATCATCATCATCATCACGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGT GCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCC GGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCA CCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGC GTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTC CGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGC AACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCA TCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCT GGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAAC GGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGC TCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCC CGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAG CGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCAT GGACGAGCTGTACAAGAGATCTCGAGCTCAAGCTTCGAATTCTGCAGTCGACGGTA CCGCGGGCGATAAAGGCATTGAATGCAGCGTGTTTAGCATTGATAGCAACGGCATT GTGTATACCCAGCCGATTGCGCAGTGGCATCATCGCGGCAAACAGGAAGTGTTTGA ATATTGCCTGGAAGATGGCAGCATTATTAAAGCGACCAAAGATCATAAATTTATGA CCCAGGATGGCAAAATGCTGCCGATTGATGAAATTTTTGAACAGGAACTGGATCTG CTGCAGGTGAAAGGCCTGCCGGAATAA

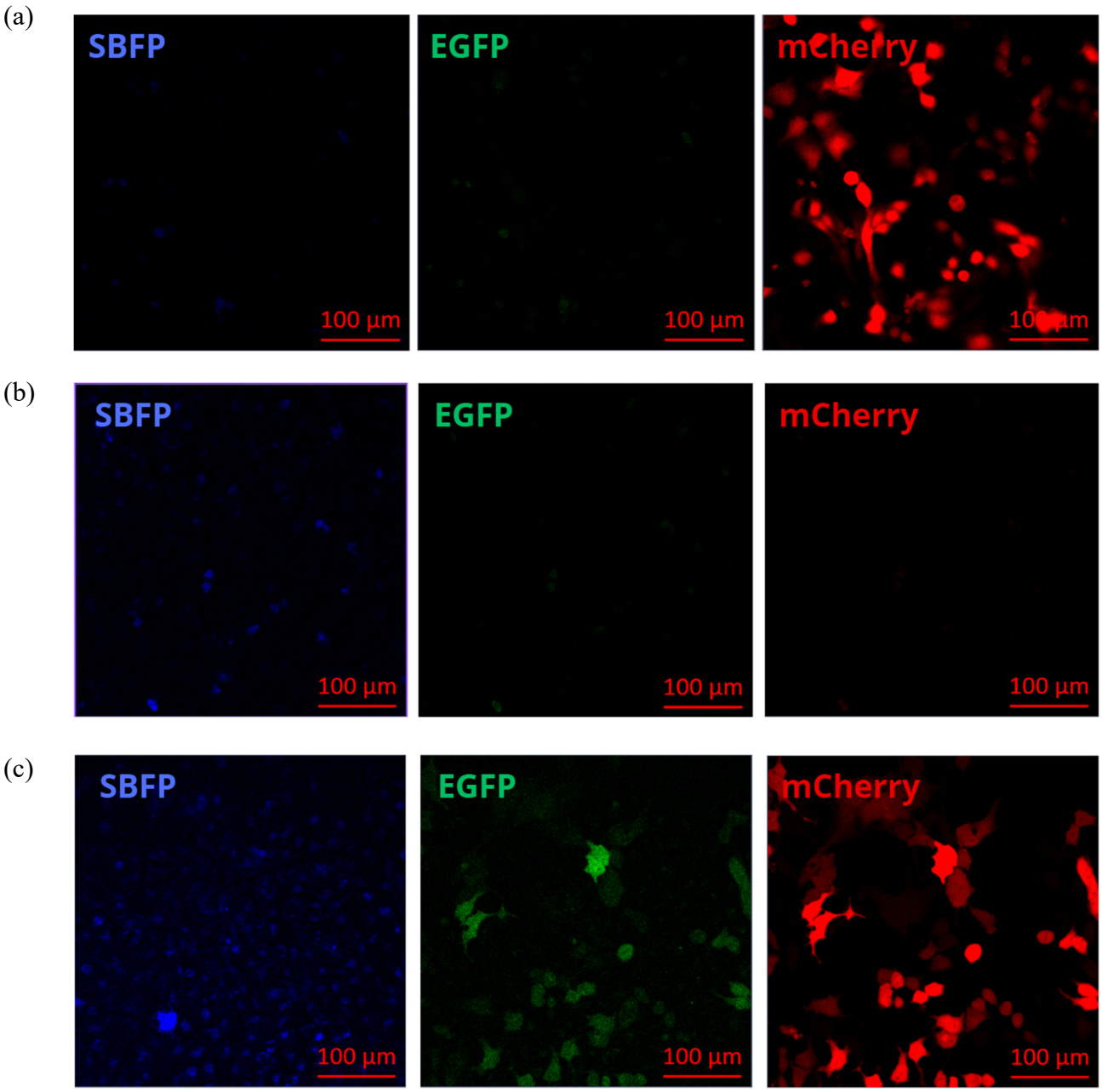

Figure S2. Representative confocal images of HeLa cells treated either with (a) p_egfp1-10_inteinN, (b) p_inteinC_egfp11, or (c) both plasmids simultaneously.

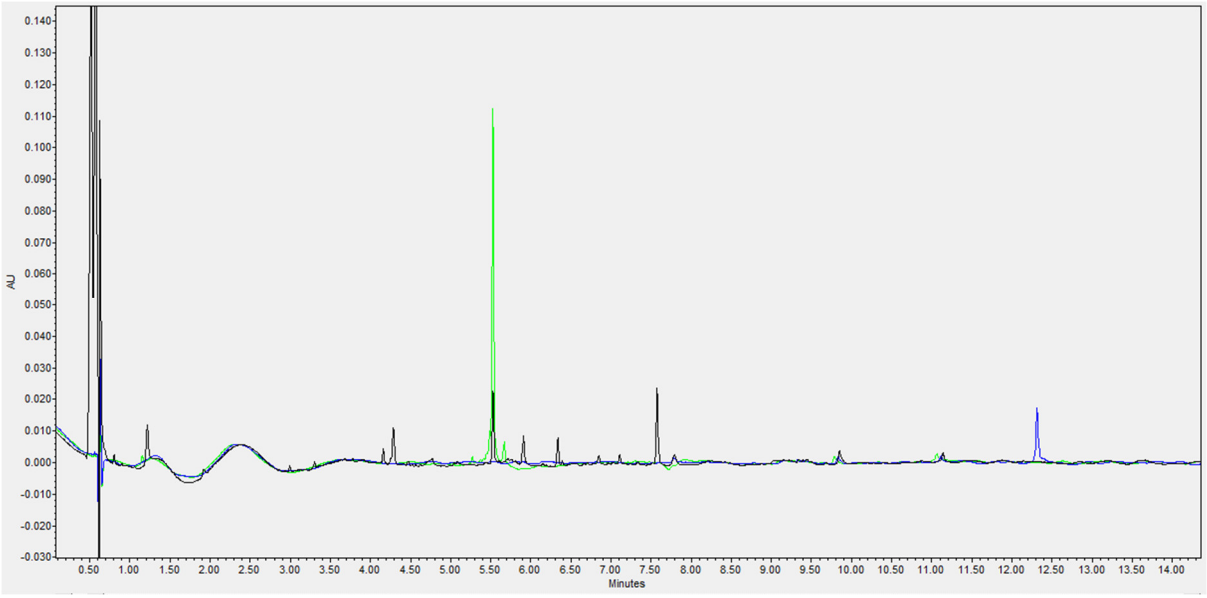

Figure S3. UPLC image. The green line is the intein^{C} peptide, the blue line is the NF55-XK(Rhod)Xpra peptide, and the black line is the purified click reaction mixture. Note the reduction in the amount of intein^{C}, which was initially in excess compared to NF55-XK(Rhod)XPra, and the disappearance of NF55-XK(Rhod)XPra from the reaction mixture. Furthermore, the emergence of a new compound (NF55-XK(Rhod)X–intein^{C} conjugate) can be observed.

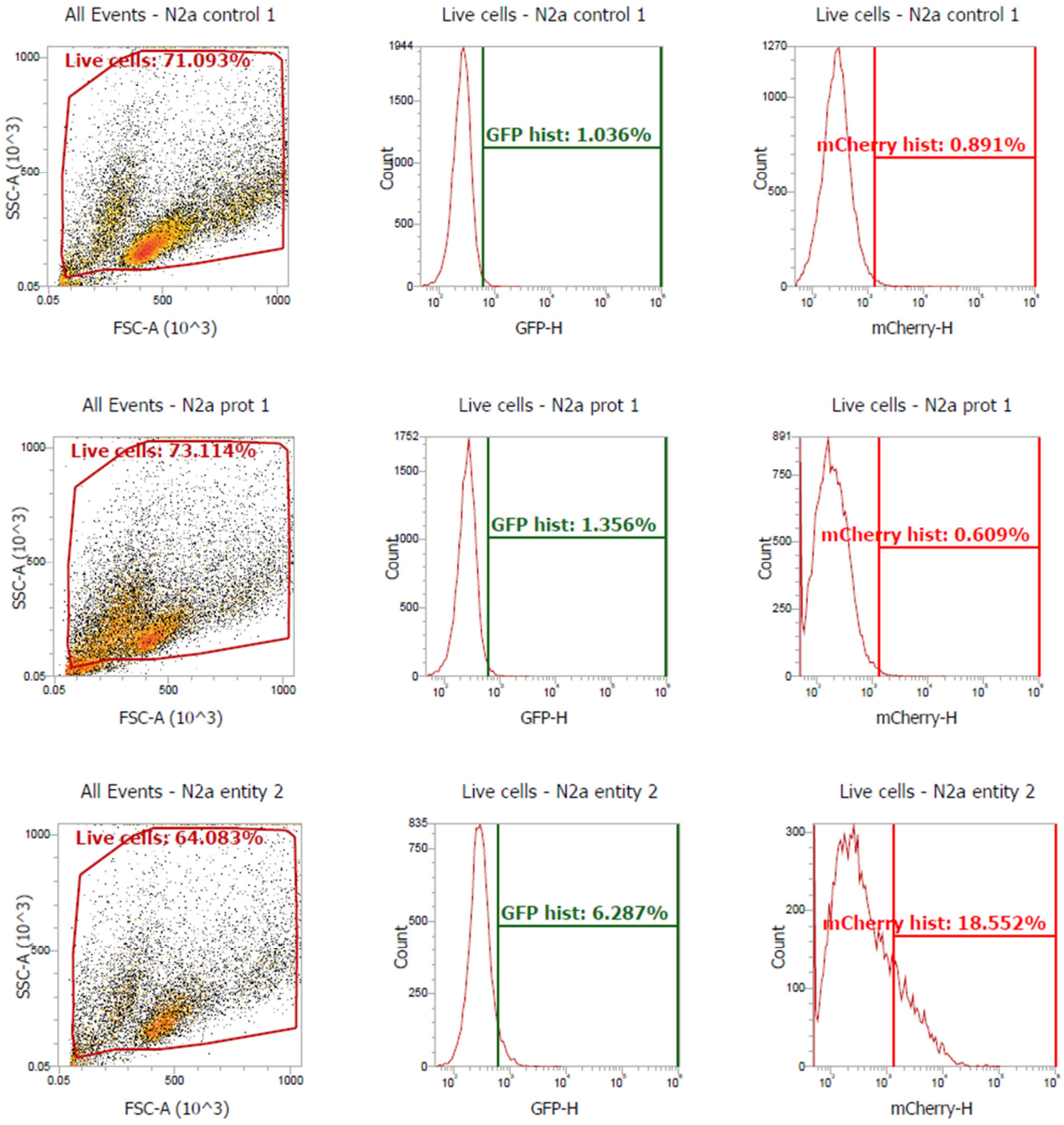

Figure S4. Representative flow cytometry plots of Neuro 2a cells transduction efficacy assay. Flow cytometry was performed using an Attune™ NxT Flow Cytometer equipped with a 488 nm argon laser. The viable cell population was determined from a plot of forward-scattered (FSC) vs. side-scattered (SSC) light. Attune™ NxT Software 3.2.1 software was used to analyze a minimum of 10,000 events per sample. The area scaling factor was 1.28, with a threshold of SSC 0.3, FSC 220, SSC 360, BL1/GFP 460, and RL1/APC/Cy5 520. GFP+ and mCherry show gated events with a signal intensity over the threshold of ~1% of untreated cells.

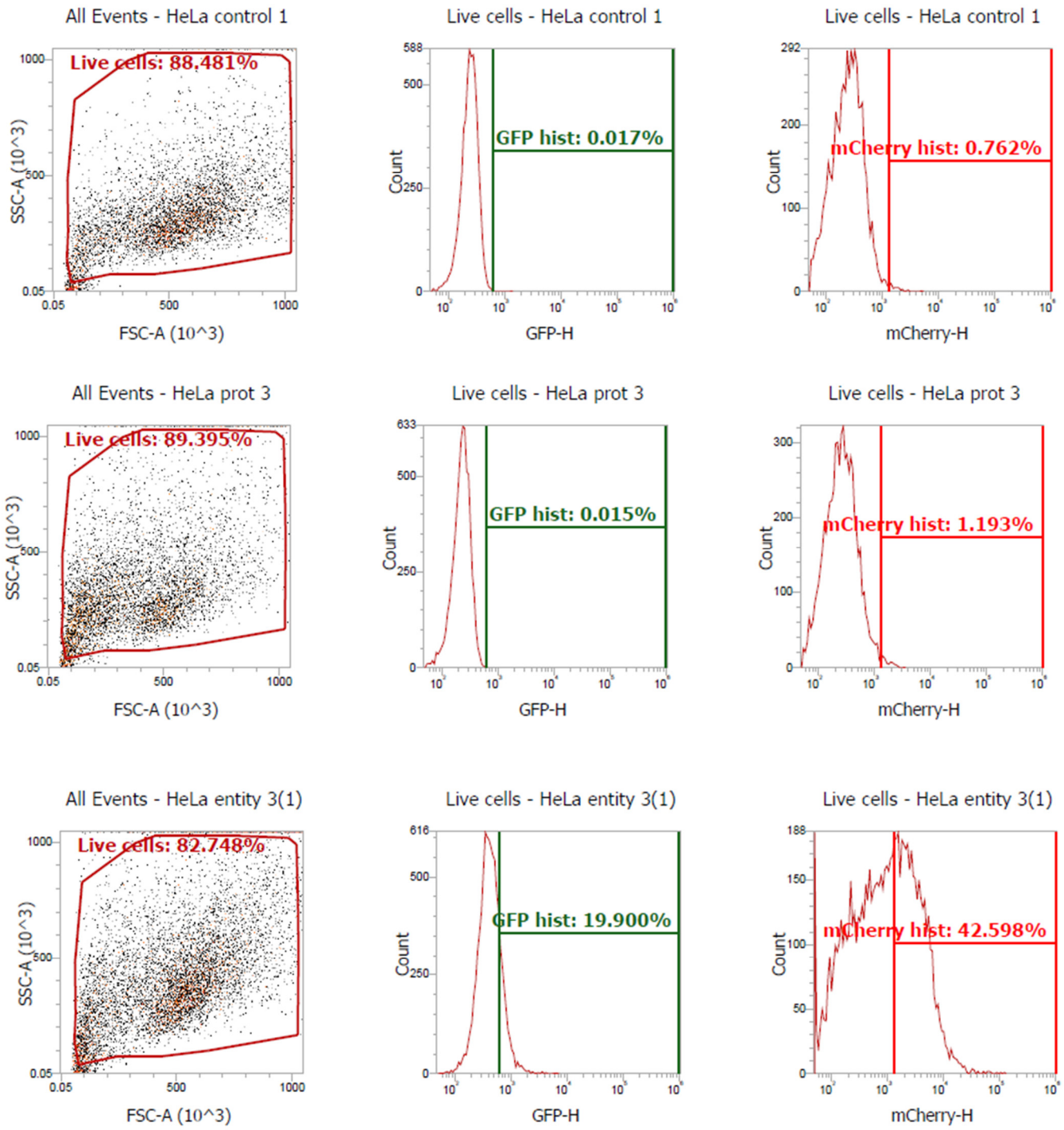

Figure S5. Representative flow cytometry plots of HeLa cells transduction efficacy assay. Flow cytometry was performed using an Attune™ NxT Flow Cytometer equipped with a 488 nm argon laser. The viable cell population was determined from a plot of forward-scattered (FSC) vs. side-scattered (SSC) light. Attune™ NxT Software 3.2.1 software was used to analyze a minimum of 10,000 events per sample. The area scaling factor was 1.28, with a threshold of SSC 0.3, FSC 220, SSC 360, BL1/GFP 460, and RL1/APC/Cy5 520. GFP+ and mCherry show gated events with signal a intensity over the threshold of ~1% of untreated cells.

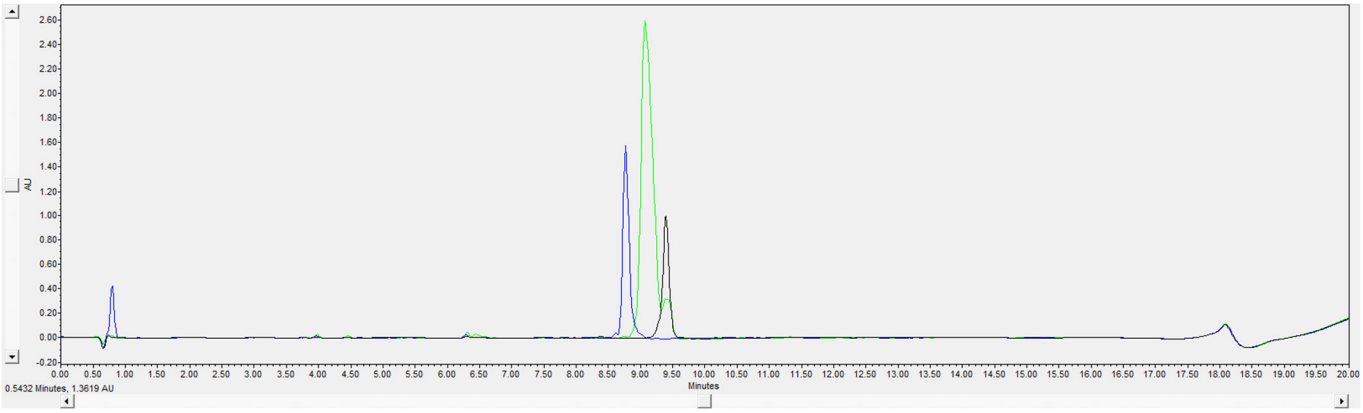

Figure S6. UPLC image. The blue line is the EGFP–intein^{N} protein, the green line is the reaction mixture of EGFP–intein^{N} protein and intein^{C}-activated NF55 peptide, and the black line is the intein^{C}-activated NF55 peptide. In the reaction mixture, no protein or peptide peaks are registered.

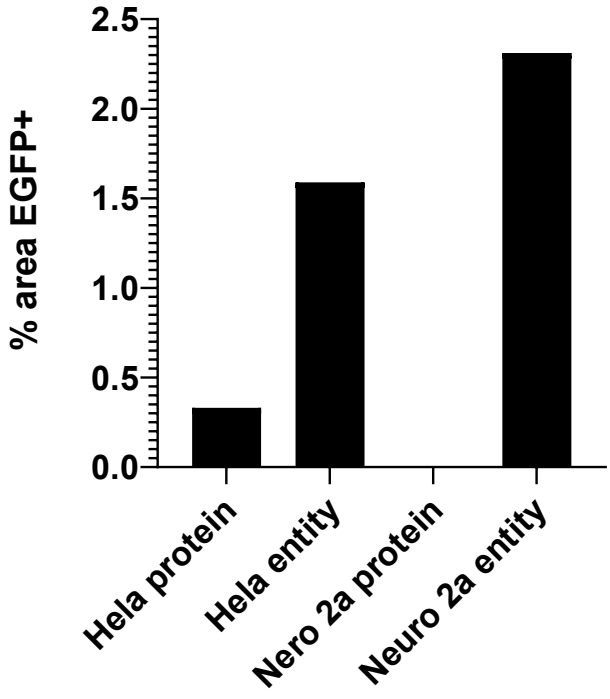

Figure S7. Confocal image analysis showing average percentage of EGFP-positive area. Analysis is performed with ImageJ (Version 1.53a), National Institute of Health, Bethesda, Maryland, USA.