Supporting Information

An improved strategy for fluorescent tagging of membrane proteins for overexpression and purification in mammalian cells

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Table S1

Protein	Coding Sequence
mVenus	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTG GACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGC CACCTaCGGCAAGCTGACCCTGAAGCTGATCTGCACCACCGGCAAGCTGCCCGTGCC CTGGCCCACCCTCGTGACCACCCTGGGCTACGGCCTGCAGTGCTTCGCCCGCTACCCC GACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAG GAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAA GTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGG AGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTC TATATCACCGCCGACAAGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCA CAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCA TCGGCGACGGCCCCGTGCTGCTGCCCGACAaCCACTACCTGAGCTACCAGTCCAAGCT GAGCAAAGAcCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC CGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG
mCerulean	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTG GACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGC CACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCC CTGGCCCACCCTCGTGACCACCCTGACCTGGGGCGTGCAGTGCTTCGCCCGCTACCCC GACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAG GAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAA GTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGG AGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACGCCATCAGCGACAACGTC TATATCACCGCCGACAAGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCA CAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCA TCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCAAGC TGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCG CCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG
mEGFP	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTG GACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGC CACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCC CTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCC GACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAG GAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAA GTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGG AGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTC TATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCA CAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCA TCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCAAGC TGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCG CCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG
mCherry	ATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTT CAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGG GCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGT GGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGG CCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGG GCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCC AGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCA ACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCT CCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTG AAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAA GAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTC CCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACT CCACCGGCGGCATGGACGAGCTGTACAAG

Table S2.

Oligo ID	Oligo sequence	Use
OMR503	GCAGCATATGGTGAGCAAGGGCGAGG	Insert Fluorescent protein cDNA into pET 28 vector, NdeI site
OMR504	GGTGCTCGAGTTACTTGTACAGCTCGTC	Insert Fluorescent protein cDNA into pET 28 vector, XhoI site
OMR349	CATCATCACCACCATACTGCTGCCGCCGCTGTGAGCAAGGGCGAGGAG	Replace N-terminal mEGFP cDNA using overlap extension PCR in pPICZ vector
OMR347	CGAGATGGACCTTGAAACAAAACTTCCAACTTGTACAGCTCGTCCAT	Replace N-terminal mEGFP cDNA using overlap extension PCR in pPICZ vector
OMR352	AGGTCCAACTGCTGCCGCCGCTGTGAGCAAGGGCGAG	Replace C-terminal mEGFP cDNA using overlap extension PCR in pPICZ vector
OMR353	ATGGTGATGGTGACCACCTCCGGACTTGTACAGCTCGTCCAT	Replace C-terminal mEGFP cDNA using overlap extension PCR in pPICZ vector
OMR236	CAGGTGTCCACTCCCAGTTCAATTACAAACAACTAATTATTCGAAACCATGCATCAC	Insert mEGFP-DHHC20 expression cassette into pEG-Bacmam vector using overlap extension PCR
OMR237	GCTTGTCGAGACTGCAGGCTCTATGTTCTAGAAAGCTGGCGGCCTAAGAATTCGA	Insert mEGFP-DHHC20 expression cassette into pEG-Bacmam vector using overlap extension PCR
OMR238	CAGGTGTCCACTCCCAGTTCAATTACGAAGCTAGCCTCGAGCCACCATG	Insert DHHC20-mEGFP expression cassette into pEG-Bacmam vector using overlap extension PCR
OMR239	GCTTGTCGAGACTGCAGGCTCTATTTGTTCTAGACTATTAGTGATGGTG	Insert DHHC20-mEGFP expression cassette into pEG-Bacmam vector using overlap extension PCR
OMR635a	CAGGTGTCGTGAGACCGGTGCCACCATGCATCACCACCACCATCATCATC	Gibson assembly into modified pSP vector. Insert amplification.
OMR635b	GTCGAGGCTGATCAGCGAGCTCTAGCTAAGAATTCGACCTCTGCAGTTTAG	Gibson assembly into modified pSP vector. Insert amplification.
OMR635c	CTAAACTGCAGAGGTCGAATTCTTAGCTAGAGCTCGCTGATCAGCCTCGAC	Gibson assembly into modified pSP vector. Vector amplification.
OMR635d	GATGATGATGGTGGTGGTGATGCATGGTGGCACCGGTCTCACGACACCTG	Gibson assembly into modified pSP vector. Vector amplification.

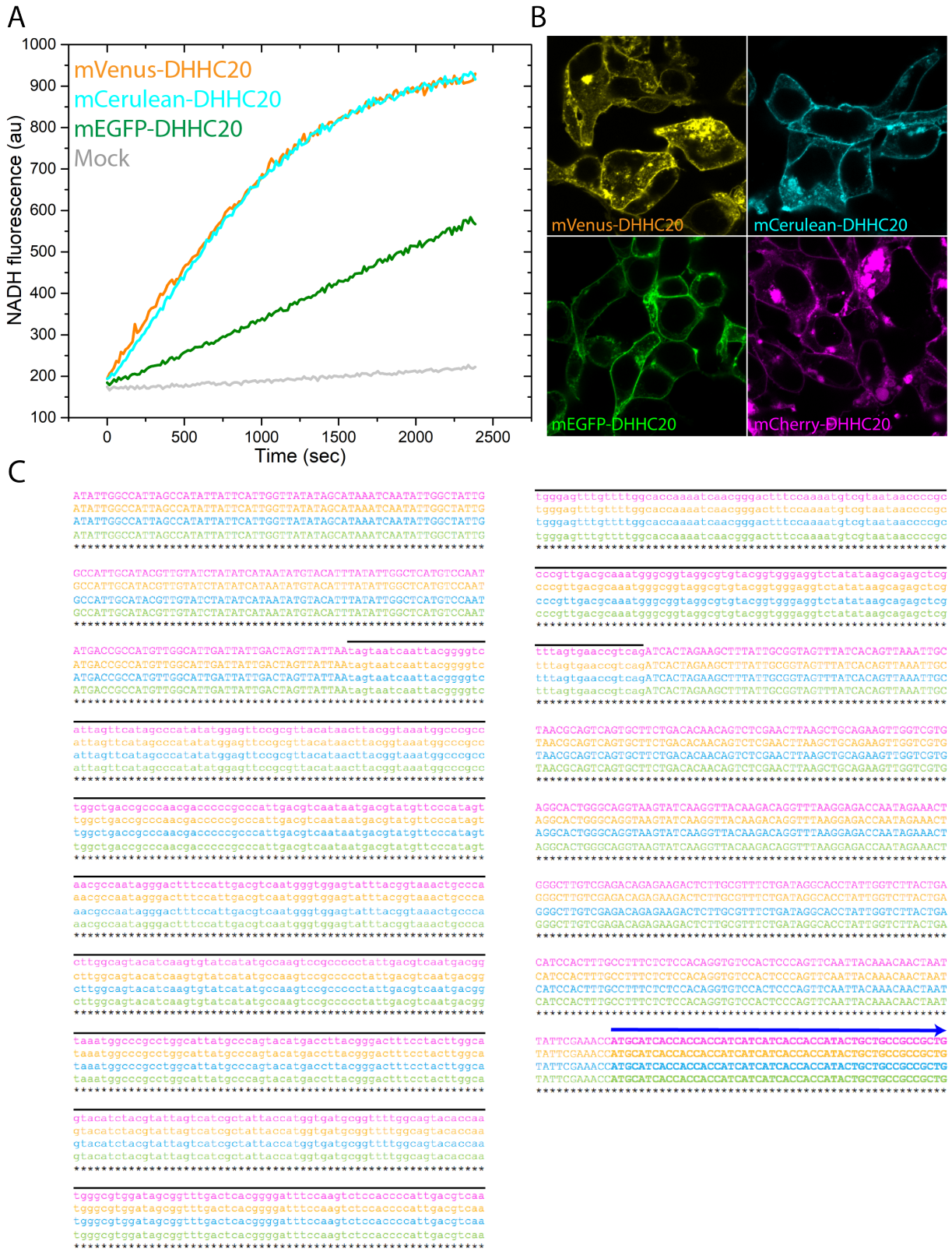

Figure S1. Activity and expression of human DHHC20 protein tagged with fluorescent proteins. (**A**) Auto-palmitoylation activity of cell lysates from HEK293T cells transiently expressing

DHHC20 palmitoyltransferase enzyme tagged with the different fluorescent proteins. (**B**) Confocal microscopy of HEK293T cells over-expressing DHHC20 tagged with the different fluorescent proteins showing that they localize similarly. (**C**) Sequence of the DNA region upstream of the start codon (blue arrow). The CMV promoter (black line) is intact for all the constructs. Orange, mVenus-DHHC20; Cyan, mCerulean-DHHC20; Green, mEGFP-DHHC20; Magenta, mCherry-DHHC20.

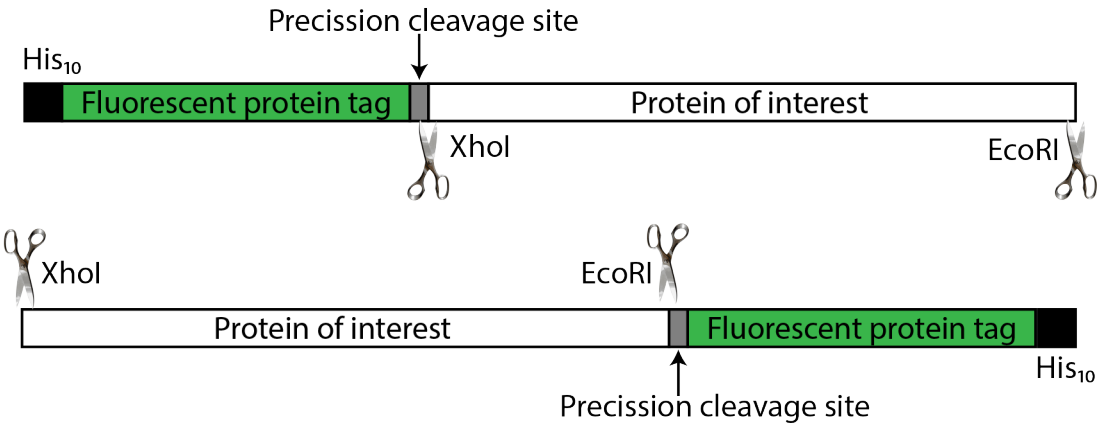

Figure S2. Design of the fluorescent protein tag expression cassette. Once the fluorescent protein tags have been inserted by overlap extension PCR, any protein of interest can be ligated in using the XhoI and EcoRI restriction sites.

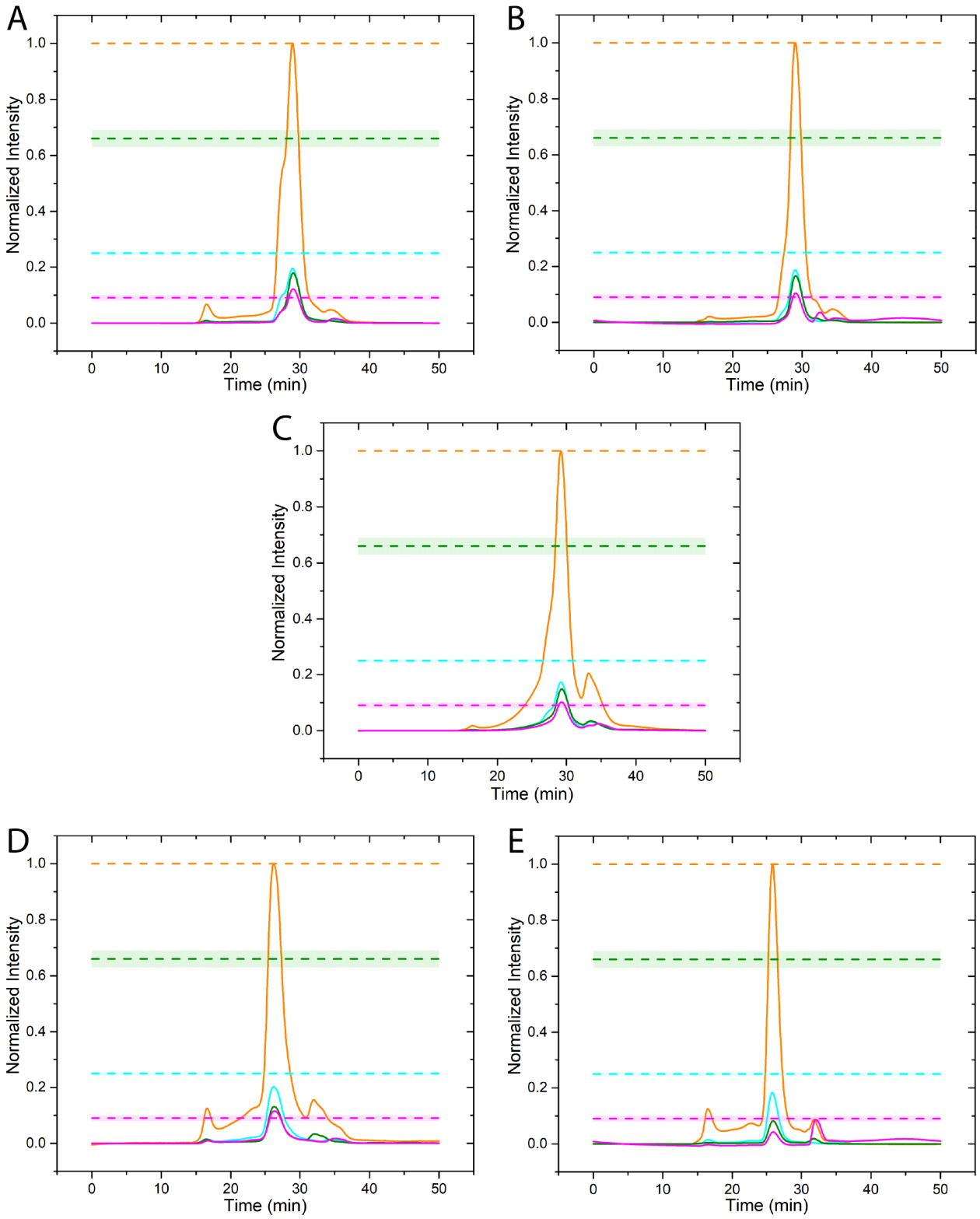

Figure S3. Decreased expression of mEGFP tagged proteins is independent of the detergent used for extraction. FSEC chromatogram of human DHHC20 tagged at the N-terminus with mVenus

(orange), mCerulean (cyan), mEGFP (green), and mCherry (magenta) extracted using (**A**) Cymal 7, (**B**) Triton X-100, (**C**) Anzergent 3-14. FSEC chromatogram of *Cyanidioschyzon merolae* CLC (cmCLC) tagged with mVenus (orange), mCerulean (cyan), mE
GFP (green), and mCherry (magenta) extracted using (D) Cymal 7, (E) Triton X-100. The dashed lines represent the normalized peak height expected if all the differently tagged proteins expressed equally well. The mVenus:mCerulean:mEGFP:mCherry relationship is ~1.0:0.25(±0.01):0.65(±0.03):0.1±(0.01).

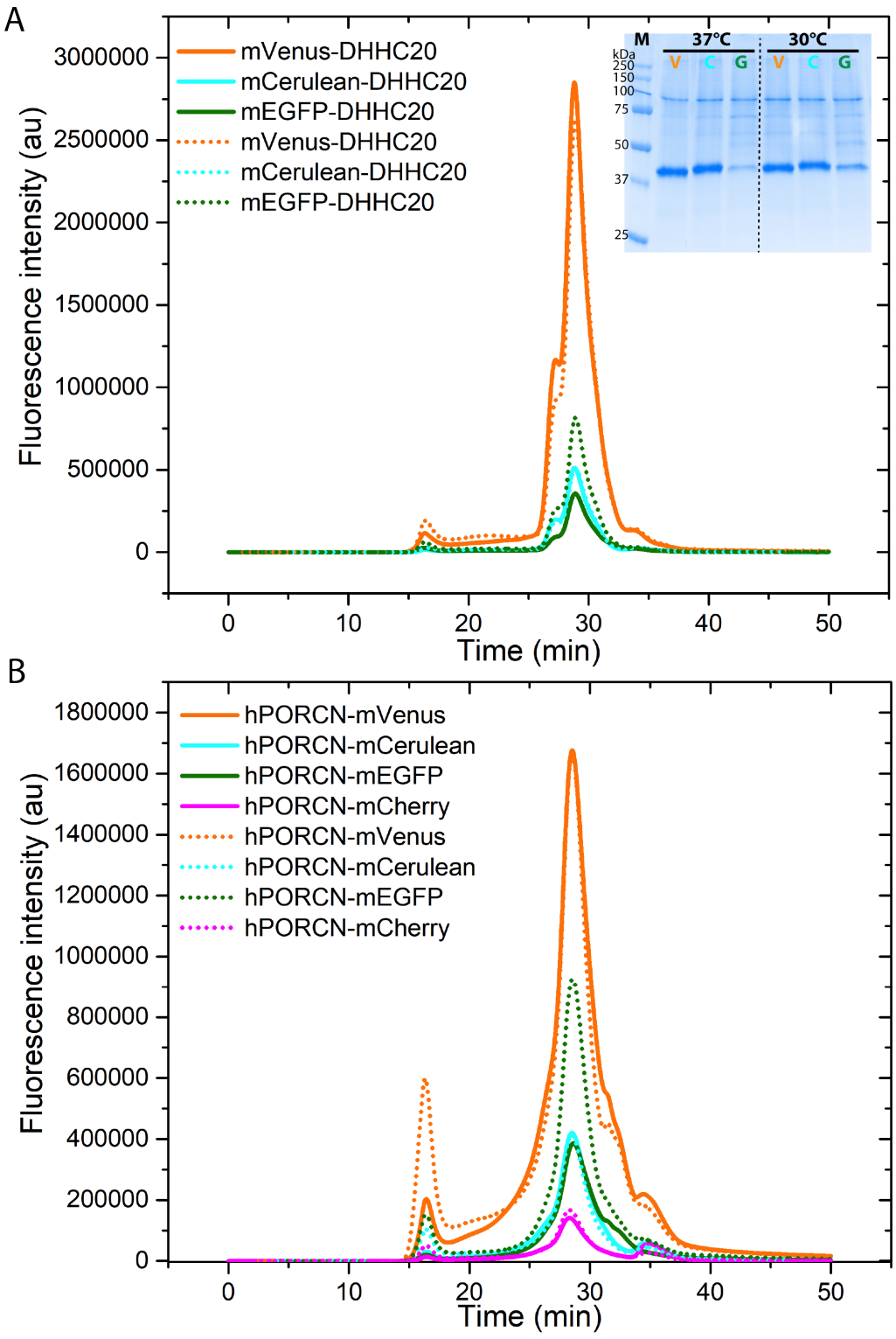

Figure S4. FSEC traces showing temperature independence of mVenus and mCerulean tagged proteins. (**A**) Temperature dependence of FP-DHHC20 expressed at 37 (solid) and 30°C (dotted) in HEK 293S GnTi- cells by baculovirus transduction assessed by FSEC and protein purification

of metal affinity chromatography and SDS-PAGE analysis. Only the mEGFP-DHHC20 trace (green, dotted) increases upon incubation at 30°C. (**B**) Temperature dependence of hPORCN-FP expressed at 37 (solid) and 30°C (dotted) in HEK 293T adherent cells using PEI mediated transient transfection. Only the hPORCN-mEGFP trace (green, dotted) increases upon incubation at 30°C. The chromatograms show the fluorescence values without normalization.