Supporting Information

Conditional Glycosylation in Eukaryotic Cells Using a Biocompatible Chemical Inducer of Dimerization

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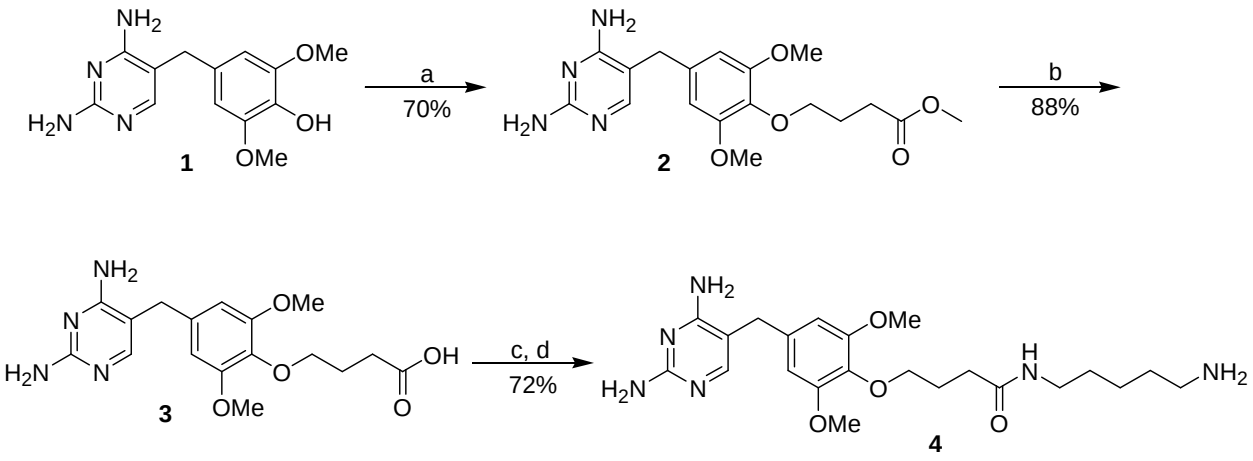
Experimental Procedures

General Synthesis Procedures. All chemical reagents were of analytical grade, obtained from commercial suppliers, and used without further purification unless otherwise noted. Flash chromatography was performed using Merck 60 Å 230-400 mesh silica gel. Analytical thin layer chromatography (TLC) was performed on glass-backed Analtech Uniplate silica gel plates, and compounds were visualized by staining with ceric ammonium molybdate, 10% H_2SO_4 in ethanol, and/or the absorbance of UV light. All reaction solvents were distilled under a nitrogen atmosphere prior to use. Pyridine, dichloromethane, and toluene were dried over CaH_2. Organic extracts were dried over a desiccant, and the drying agent was removed by vacuum filtration. Unless otherwise specified, all solvents were removed under reduced pressure using a rotary evaporator. ^{1}H-NMR and ^{13}C-NMR spectra were obtained at 400 and 100 MHz, respectively, using a Bruker AVQ-400 spectrometer. Chemical shifts are reported in δ ppm relative to tetramethylsilane, and coupling constants (*J*) are reported in hertz (Hz). Low and high-resolution fast atom bombardment (FAB+) mass spectra were obtained at the UC Berkeley Mass Spectrometry Laboratory.

Synthesis of Tmp-SLF. The synthesis of Tmp-SLF takes advantage of previously published protocols[1-4] and is summarized in Schemes 1 and 2.

Scheme S1. Synthesis of 4.[a]



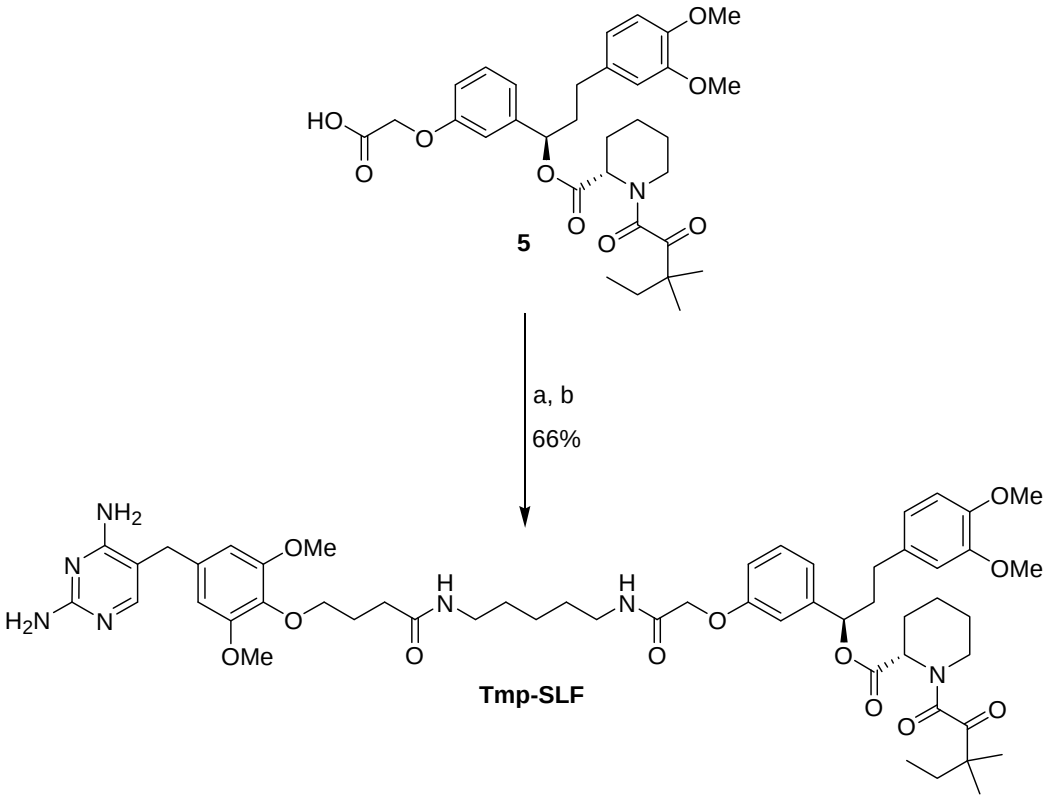
[a]Key: (a) methyl 4-bromobutyrate, KOtBu, DMSO; (b) LiOH, MeOH; (c) pentafluorophenyl acetic acid, pyridine, DMF; (d) 1,5-diaminopentane, DMF.

Synthesis of 2. A solution of trimethoprim[1] (**1**) (100 mg, 0.36 mmol) and KO*t*Bu (47 mg, 0.4 mmol) in anhydrous DMSO (1.3 mL) was stirred for 30 min. To this solution was added methyl 4-bromobutyrate[5] (71.6 mg, 0.40 mmol) and the resulting solution was stirred for an additional 2 h. The mixture was concentrated *in vacuo*, dissolved in acetone, and again concentrated *in vacuo*. The resulting brown solid was purified using silica gel chromatography (dichloromethane:methanol, 20:1) to afford **2** (94.2 mg, 70%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.99 (m, 2 H), 2.59 (t, 2 H, J = 7.4), 3.06 (s, 2 H), 3.65 (s, 3 H), 3.74 (s, 6 H), 3.94 (t, 2 H, J = 6.1), 4.82 (br s, 2 H), 5.11 (br s, 2 H), 6.34 (s, 2 H) 7.72 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.29, 30.40, 34.63, 51.46, 56.01, 72.08, 104.87, 106.22, 133.85, 135.62, 153.68, 156.52, 162.24, 162.75, 174.09; HRMS (FAB): Calcd. for $C_{18}H_{25}N_4O_5$ $[M+H]^+$ 377.1819, found 377.1822.

Synthesis of 3. To a solution of **2** (500 mg, 1.33 mmol) in methanol (5 mL) was added 1 N LiOH (10 mL) dropwise. The resulting mixture was stirred for 2 h and then acidified with 3 N HCl to pH 1.5. The solution was concentrated *in vacuo*, causing the product to precipitate. The resulting white solid was washed with 1 N HCl and dried affording **3** (425 mg, 88%). ^{1}H NMR (400 MHz, DMSO): δ 1.76 (m, 2 H), 2.38 (t, 2 H, *J* = 7.2), 3.51 (s, 2 H), 3.68 (s, 6 H), 3.77 (t, 2 H, *J* = 6.1), 6.43 (br s, 2 H), 6.54 (s, 2 H), 6.79 (br s, 2 H), 7.45 (s, 1 H); ^{13}C NMR (100 MHz, DMSO): δ 25.50, 30.53, 31.11, 33.06, 56.23, 71.92, 106.31, 107.31, 135.13, 150.09, 153.27, 159.70, 163.20, 174.92; HRMS (FAB): Calcd. for $C_{17}H_{23}N_4O_5$ $[M+H]^+$ 363.1663, found 363.1667.

Synthesis of 4. To a stirring solution of **3** (50 mg, 0.14 mmol) in DMF (1 mL) was added pentafluorophenyl trifluoroacetate (26 μL, 0.15 mmol) and pyridine (12 μL, 0.15 mmol). After 7 h, the reaction was quenched with ethyl acetate:methanol (1:1, 5 mL) and concentrated *in vacuo*. The resulting ester (30 mg, 59 μmol) was immediately added to 1,5-diaminopentane (34 μL, 0.293 mmol) in DMF (1 mL). The resulting mixture was stirred for 13 h and concentrated *in vacuo*. The resulting brown oil was purified by C18 reverse-phase HPLC to afford **4** (20 mg, 72%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.39 (m, 2 H), 1.54 (m, 2 H), 1.65 (m, 2 H), 1.95 (m, 2 H), 2.42 (t, 2 H, *J* = 7.2), 2.89 (t, 2 H, *J* = 7.6), 3.16 (t, 2 H, *J* = 7.2), 3.64 (s, 2 H), 3.79 (s, 6 H), 3.91 (t, 2 H, *J* = 6), 6.55 (s, 2 H), 7.89 (s, 1 H); ^{13}C NMR (100 MHz, CD_3OD): δ 23.19, 25.96, 26.67, 28.50, 32.25, 32.49, 38.43, 39.13, 55.15, 72.05, 78.01, 105.75, 109.35, 116.69, 132.54, 135.55, 139.90, 153.60, 161.77, 164.71, 174.49; HRMS (FAB): Calcd. for $C_{22}H_{35}N_6O_4$ $[M+H]^+$ 447.2714, found 447.2720.

Scheme S2. Synthesis of Tmp-SLF.[b]



[b]Key: (a) pentafluorophenyl acetic acid, pyridine, CH_2Cl_2; (b) **4**, pyridine, DMF.

Synthesis of TMP-SLF. To a stirring solution of SLF[2-4] (**5**, 50 µg, 86 µmol) in CH_2Cl_2 (2 mL) was added pentafluorophenyl trifluoroacetate (16 µL, 95 µmol) and pyridine (7.7 µL, 95 µmol). The solution was allowed to react for 15 h, diluted in CH_2Cl_2 (10 mL), and extracted with 1 N HCl. The ester was quickly purified on a silica gel plug (hexane:ethyl acetate, 3:1) and was added (30 mg, 40 µmol) to a solution of **4** (19.6 mg, 44 µmol) and pyridine (3.6 µL, 44 µmol) in DMF (0.5 mL). The resulting reaction was stirred for 14 h and concentrated *in vacuo*. The resulting yellow oil was purified by silica gel chromatography (ethyl acetate→20% methanol) to yield **TMP-SLF** (34 mg, 66%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.85 (t, 3 H, J = 7.5), 1.11 (d, 1 H, J = 3.0), 1.19 (m, 5 H), 1.28 (m, 3 H), 1.48 (m, 5 H), 1.69 (m, 5 H), 1.92 (m, 2 H), 2.05 (m, 6 H), 2.11 (m, 1 H), 2.25 (m, 1 H), 2.36 (t, 2 H, J = 5.4), 2.64 (m, 2 H), 3.15 (t, 3 H,

J = 6.0), 3.24 (m, 3 H), 3.42 (br d, 1 H, *J* = 12.9), 3.72 (s, 2 H), 3.76 (m, 12 H), 3.88 (t, 2 H, *J* = 6.1), 4.50 (s, 2 H), 5.25 (d, 1 H, *J* = 5.2), 5.77 (m, 1 H), 6.63 (s, 2 H), 6.72 (d, 1 H, *J* = 6.3), 6.83 (m, 2 H), 6.92 (d, 1 H, *J* = 7.5), 7.02 (m, 2 H), 7.08 (m, 1 H), 7.31 (t, 1 H, *J* = 7.9); ^{13}C NMR (100 MHz, DMSO): δ 8.09, 21.01, 22.45, 22.76, 23.70, 24.68, 26.19, 30.99, 32.24, 32.36, 33.17, 38.15, 38.32, 38.62, 40.17, 44.01, 46.27, 51.12, 55.07, 55.18, 55.51, 67.18, 71.91, 76.19, 105.99, 112.02, 112.40, 112.95, 113.01, 114.13, 119.40, 120.16, 129.68, 133.61, 135.97, 142.97, 142.22, 147.79, 149.39, 153.64, 157.96, 167.17, 169.49, 207.42; HRMS (FAB): Calcd. for $C_{54}H_{74}N_7O_{12}$ $[M+H]^+$ 1012.5390, found 1012.5403.

Biological Procedures.

Reagents. DNA primers were purchased from Elim Biopharmaceuticals. Restriction enzymes and T4 DNA ligase were from New England BioLabs. Calf intestinal alkaline phosphatase (CIAP) was from Amersham. Pfu polymerase from Stratagene was used for all PCRs except where indicated. DNA sequencing was performed by Elim Biopharmaceuticals, Inc. (Hayward, CA) and the University of California, Berkeley DNA Sequencing Facility. The vectors pcDNA3.1 and pcDNA3.1-Zeo were purchased from Invitrogen. Tmp-SLF was dissolved in DMSO for use in cell culture experiments and the yeast three-hybrid screens. Biotinylated mAb HECA-452 was purchased from Pharmingen, and streptavidin Tricolor was from Caltag. Trimethoprim (Tmp) was dissolved in DMSO for cell culture experiments.

Yeast Three-Hybrid Assays. The plasmid and yeast strain construction, FKBP mutants, and three-hybrid assays have been described previously.[6] Representative X-gal plate assays

comparing the wild type B-42-FKBP and B42-FKBP mutants are shown in Figure S1. The FKBP mutants used here have SLF dissociation constants in the following order from strongest to weakest binder: H87L > wild type > F36Y ≈ R42Q > W59L = D37V > Y26F.[6] The Tmp-SLF transcriptional induction with B42-FKBP(H87L)-containing yeast seems to be consistently stronger than Mtx-SLF at 1 μM. In addition, Tmp-SLF induced transcription in yeast containing the B42-FKBP(F36Y) fusion even though the SLF affinity for the FKBP(F36Y) mutant is 3-fold lower than wild-type FKBP.[6] We are currently making FucT7 constructs with these FKBP mutants for evaluation in the conditional glycosylation system.

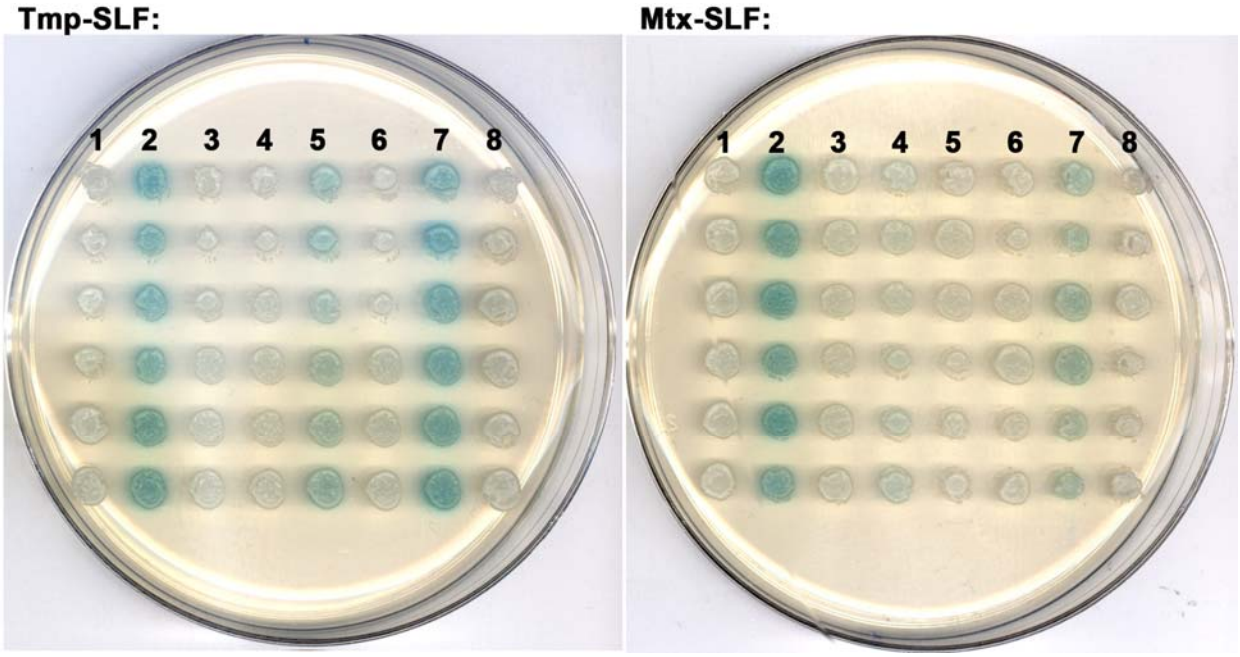


Figure S1. X-gal plate assays comparing Tmp-SLF and Mtx-SLF induced *lacZ* transcription from yeast strains expressing either wild-type FKBP or a mutant of FKBP. Yeast transformants were grown in the presence of either 1 μM Tmp-SLF or Mtx-SLF for 3 days at 30 °C. All strains are derived from the same parent strain, but columns 1-8 differ in the protein that is fused to B42: 1, B42; 2, B42-FKBP; 3, B42-FKBP(D37V); 4, B42-FKBP(W59L); 5, B42-FKBP(F36Y); 6, B42-FKBP(R42Q); 7, B42-FKBP(H87L); 8, B42-FKBP(Y26F).

Tmp-SLF Conditional Glycosylation.

Localization Domain Plasmids. The localization (Loc) domain of human FucT7 as well as the generation of Loc-FKBP and TOPO-FKBP-myc have been described previously.[7] Plasmids encoding Loc-DHFR or the corresponding constructs with multiple copies of DHFR or FKBP were prepared in analogous fashion to the earlier reported FucT7 system for rapamycin.[7] Briefly, a PCR product encoding DHFR-myc was produced using E. coli DH5α genomic DNA (kindly provided by Zeljka Cabrilo) and DHFR_5′ (5′-ACTCTAGAATCAGTCTGATTGCGGCGTT-3′) and DHFRmycstop_3′ (5′-AGACTAGTCTACAGATCCTCTTCTGAGATGAGTTTTTGTTCCCGCCGCTCCAGAATCTCA-3′) as primers. The PCR product was cloned into the plasmid pCR4Blunt-TOPO using the Zero Blunt TOPO PCR Cloning Kit (Invitrogen) to generate TOPO-DHFR-myc, whose identity was confirmed by sequencing. The insert, containing DHFR followed by the myc epitope tag and a stop codon, was excised with *Xba*I and *Spe*I. DHFR-myc was ligated into plasmid pcDNA3.1-Loc[7] that had been digested with *Xba*I, yielding the plasmid Loc-DHFR. The orientation of the insert was confirmed by restriction digest and sequencing.

Catalytic Domain Plasmids. The generation of the FKBP-Cat and TOPO-HA-FKBP plasmids have been described previously.[7] Each catalytic domain contains the secretion signal V_H, derived from the murine Ig κ-chain as described previously.[7] This tag was necessary for the chimeric proteins to enter the endoplasmic reticulum, and was cleaved from the catalytic domain once it entered the secretory pathway. The insert encoding HA-DHFR was produced in a fashion analogous to the production of DHFR-myc (see above). In this case, the primers used were 5′_HA-DHFR (5′-TCTAGATATCCGTACGACGTACCAGACTACGCAATCAGTCTGATTGCGGCGTTA-3′) and 3′_DHFRKpn (5′-GGTACCTCTAGTCCGCCGCTCCAGAAT

CTCAAAGCAATAGCTGTGA-3′), and Phusion polymerase (New England Biolabs) was utilized for amplification. The insert HA-DHFR-Kpn was cloned into the plasmid pCR4Blunt-TOPO, sequenced, and excised using the restriction enzymes *Xba*I and *Kpn*I. The plasmid FKBP-Cat was digested with *Xba*I and *Kpn*I to give a linearized plasmid containing only the Cat domain. The insert HA-DHFR-Kpn was ligated into the digested plasmid to generate the plasmid DHFR-Cat. The insert orientation was confirmed by restriction digest and sequencing.

Production of Constructs with Multiple Repeats of DHFR or FKBP. A series of plasmids were produced containing multiple repeats of DHFR or FKBP. The preparation of 2xFKBP-Cat and 3x-FKBP-Cat plasmids was described earlier.[7] The plasmid construction for Loc-3xDHFR and 3xDHFR-Cat are described here; other plasmids were produced in an analogous manner. The PCR product, DHFR, from the primers DHFR_5′ and 3′_DHFR (5′-TCACTAGTCCGCCGCTCCAGAATCTCAA-3′) was cloned into the plasmid pCR4Blunt-TOPO, sequenced, and excised using the restriction enzymes *Xba*I and *Spe*I. The DHFR insert was ligated into Loc-DHFR that had been digested with *Xba*I. The resulting plasmid, Loc-2xDHFR, was digested with *Xba*I and ligated with the DHFR insert to produce Loc-3xDHFR.

For the 3xDHFR-CAT plasmid, the PCR products from Phusion polymerase amplification of HA-DHFR from primers 5′_HA-DHFR and 3′_DHFR as well as DHFR-Kpn from primers DHFR_5′ and 3′_DHFRKpn were separately cloned into the plasmid pCR4Blunt-TOPO and sequenced. HA-DHFR was excised using the restriction enzymes *Xba*I and *Spe*I; DHFR-Kpn was excised using the restriction enzymes *Xba*I and *Kpn*I. The DHFR-Kpn insert was ligated into FKBP-Cat that had been digested with *Xba*I and *Kpn*I. The resulting plasmid, Vh-DHFR-Cat, was digested with *Xba*I and ligated with the DHFR insert. The resulting plasmid, Vh-2xDHFR-Cat, was digested again with *Xba*I and ligated with the HA-DHFR insert to

produce 3xDHFR-Cat. The orientations of all inserts were confirmed by restriction digests and sequencing. Figure S2 provides the general construction of the plasmids.

Generation "Universal" Loc and Cat Plasmids. To ease the conditional glycosylation system into more general usage, a more universal set of plasmids containing one, two, or three copies of FKBP or DHFR with a multiple cloning site available for insertion of the new Loc and Cat domains were created in an analogous fashion. For the Loc-ready universal plasmid with one copy of FKBP, the FKBP followed by the myc tag and stop codon were excised from the plasmid encoding Loc-FKBP using *Xba*I and then cloned into pcDNA3.1-Zeo. For the Cat-ready

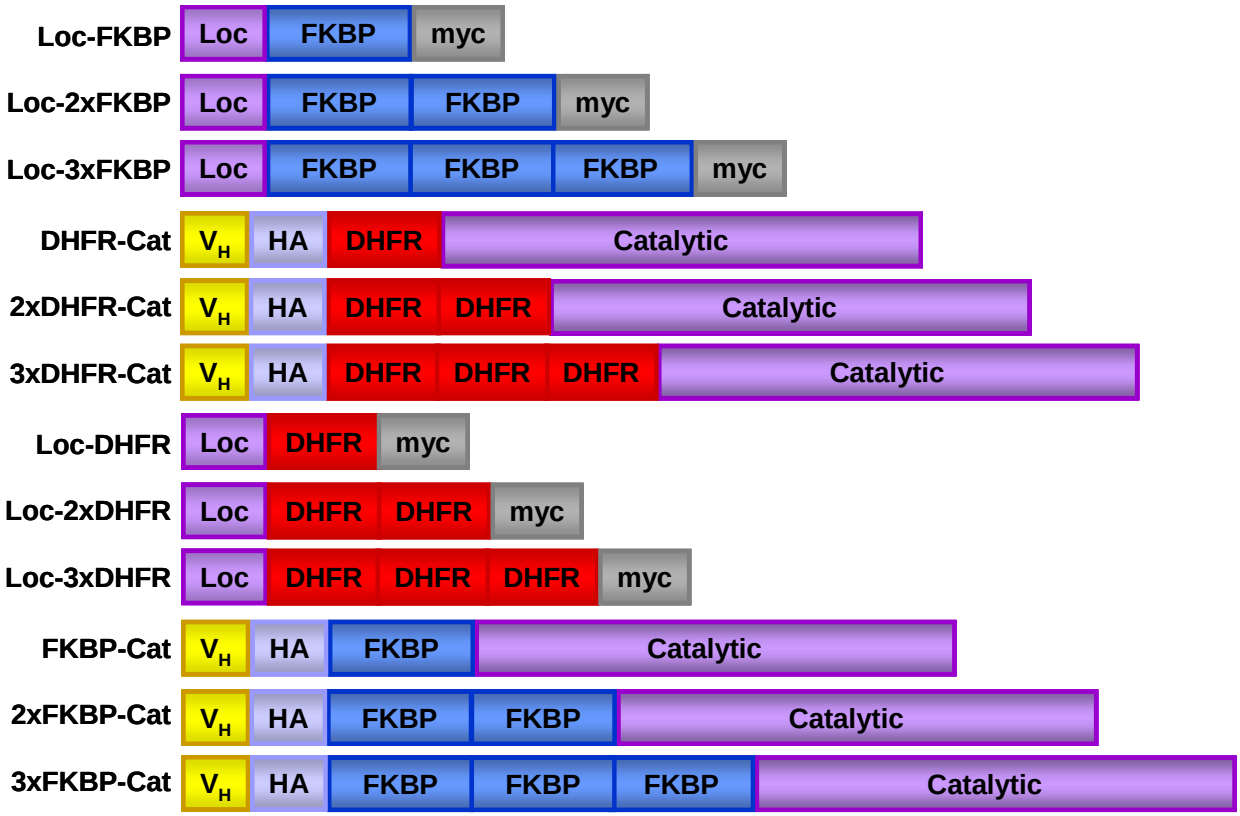


Figure S2. FucT7 plasmid constructs for inducible glycosylation. Fusion proteins are composed of either FKBP or *E. coli* DHFR linked to either the Loc or Cat domain of FucT7. V_H is the secretion signal derived from the murine Ig κ-chain; HA, hemagglutinin peptide epitope tag; myc, *myc* peptide epitope tag.

universal plasmid with one copy of DHFR, the murine V_H chain signal peptide followed by a HA tag and one repeat of DHFR were excised from the plasmid encoding DHFR-Cat using *Nde*I and *Kpn*I and then cloned into pcDNA3.1. Figure S3 gives an example plasmid map for each "Universal" plasmid construct. These plasmids were deposited with Addgene, Inc. (Cambridge, MA).

Cell Culture Procedures. Chinese hamster ovary (CHO) cells were cultured in Ham's F12 nutrient mixture with L-glutamine (GIBCO) containing 10% fetal bovine serum (Hyclone), 100 units/ml penicillin, and 0.1 mg/ml streptomycin. Cell cultures were maintained in a water-saturated, 5% CO_2 atmosphere at 37 °C.

General Procedure for Testing Inducible Expression. One day prior to transfection, CHO cells were seeded at a density of 2.0×10^6 cells per 10-cm plate. For transfection, LipofectAMINE PLUS Reagent (Invitrogen) was used according to the manufacturer's directions, using unsupplemented Opti-MEM I (GIBCO) as media during the transfection. The cells were allowed to recover 18 h after transfection. The cells were then lifted from the plates by trypsinization, counted, and reseeded into 12-well dishes at a density of 100,000 cells per well. Cells were incubated for 24 h in media containing the indicated concentrations of Tmp-SLF. The cells treated with vehicle were incubated with the same concentration of DMSO in media as in the Tmp-SLF-treated cells. For Tmp-SLF dose-response experiments, the DMSO concentration for all samples was held constant.

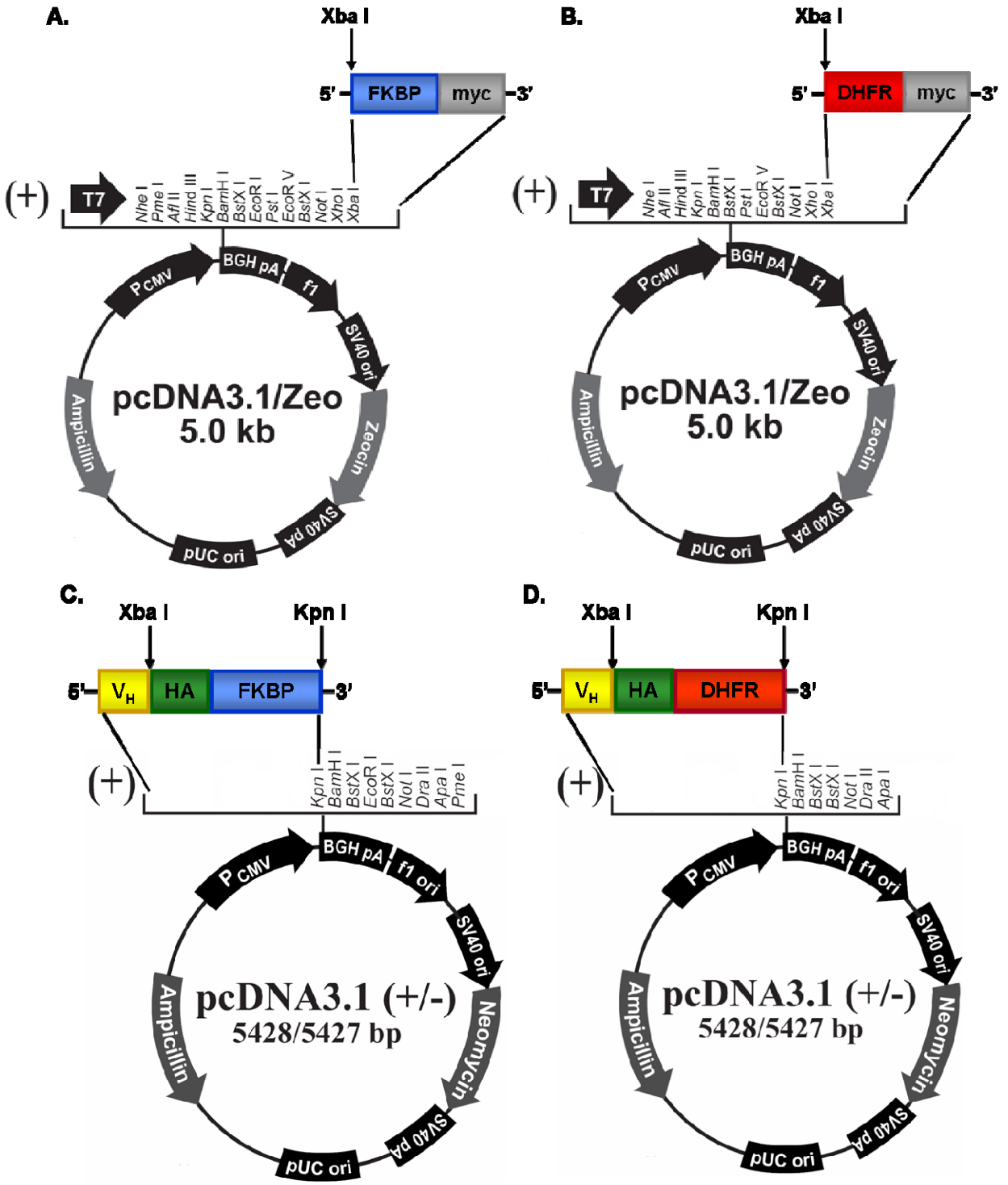


Figure S3. Example plasmid maps for the Universal plasmids created for easier creation of the Loc and Cat constructs with other enzymes: (A) Universal FKBP-myc plasmid for Loc domains, (B) Universal DHFR-myc plasmid for Loc domains, (C) Universal HA-FKBP plasmid for Cat domains, and (D) Universal HA-DHFR plasmid for Cat domains. The Universal Cat domain plasmids contain a murine V_H chain signal peptide as well.

Flow Cytometry. To remove the cells from the plate for flow cytometry analysis, they were washed twice with Dulbecco's Phosphate-Buffered Saline (1× PBS, GIBCO), then incubated with 1× PBS containing 1 mM EDTA (0.2 ml/well) for 15 min at 37 °C. Cells were maintained at 4 °C for all subsequent steps. The cells were washed twice with FACS buffer (1× PBS, 1% BSA). Then, each sample was incubated for 1 hr in 50 µl of FACS buffer containing biotinylated HECA-452 in a dilution of 20 µl per 10^6 cells. The cells were washed twice with FACS buffer, then stained with the secondary reagent, 1 µl of tricolor-streptavidin conjugate in 50 µl of FACS buffer, for 0.5 hr. Following two more washes with FACS buffer, the cells were resuspended in 300 µl of FACS buffer, and the fluorescence of at least 20,000 live cells per sample was measured on a FACSCalibur flow cytometer (BD Biosciences). The mean fluorescence intensity was quantified for all live cells, and experiments were performed in triplicate, with error bars representing the standard deviation of the mean.

Comparison of Various FucT7-derived Constructs. While all 18 possible construct pairings were compared for their ability to induce cell surface sLe^x expression (see Figure S4), only six pairs showed appreciable induced sLe^x expression. The mean fluorescence of these six construct pairs in the presence and absence of Tmp-SLF is shown below in Figure S5. While the pair Loc-DHFR/2xFKBP-Cat had a similar mean fluorescence as Loc-DHFR/3xFKBP-Cat in the presence of Tmp-SLF, the Loc-DHFR/3xFKBP-Cat pair displayed no signal from sLe^x expression above background in the absence of Tmp-SLF. Interestingly, initial Tmp-SLF experiments using similar constructs with another rapamycin-inducible enzyme, GlcNAc6-sulfotransferase 2,[8] indicates that the ideal construct pair may not be the same for each enzyme. differ from that of FucT7 (data not shown). We are currently following up on these initial results.

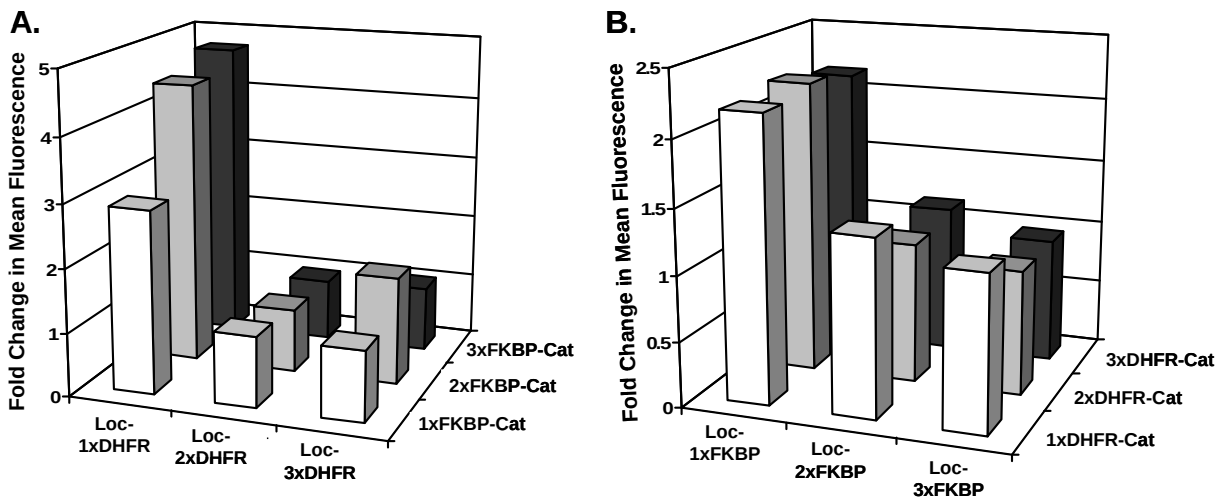


Figure S4. Fold change in mean fluorescence intensity for FucT7 construct pairs in the presence of 1 μM Tmp-SLF over background (absence of Tmp-SLF) for each of the 18 construct pairs assayed. The mean fluorescence of all live cells is plotted and each bar represents triplicate data points of the construct pair in the presence and absence of Tmp-SLF for 24 h. (A) The fold change in mean fluorescence intensity for the construct pairs with DHFR fused to the Loc domain and FKBP to the Cat domain. (B) The fold change in mean fluorescence intensity for the construct pairs with FKBP fused to the Loc domain and DHFR to the Cat domain.

Specificity of Tmp-SLF FucT7 Response. CHO cells were transfected with the Loc-DHFR/3xFKBP-Cat construct pair were incubated with 90 nM Tmp-SLF and varying concentrations of Tmp. A representative data set is shown in Figure S4. The IC_{50} was determined from the combined results of three independent experiments, each with triplicate data points.

Fluorescence Microscopy. Cells were transfected as described for flow cytometry experiments. One day after transfection, cells were seeded on slides mounted with tissue culture wells (Lab-Tek) and allowed to adhere for two days in the presence of 1 μM Tmp-SLF. Cells were washed three times with PBS, then fixed in 3% paraformaldehyde in PBS. After three

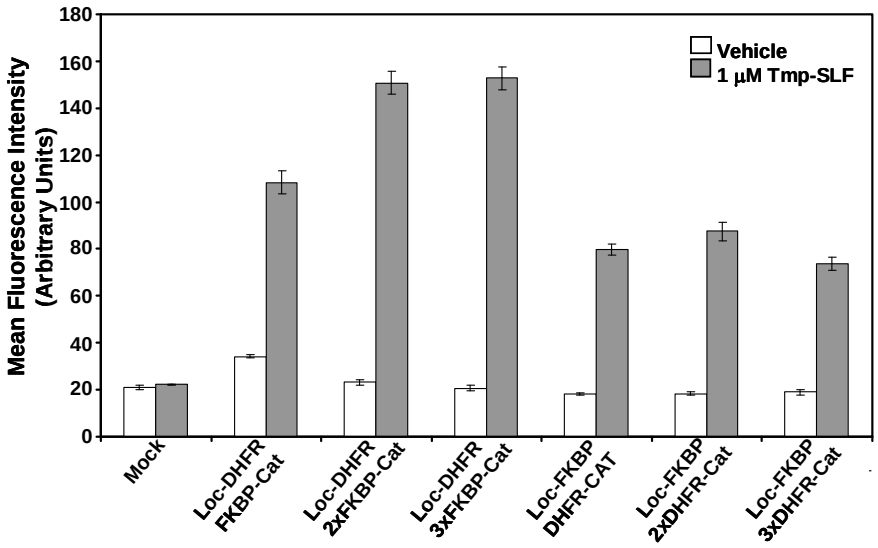


Figure S5. Mean fluorescence intensity of CHO cells transfected with the indicated FucT7-derived plasmids showing the direct comparison of the best six of 18 construct combinations assayed. Open bars represent vehicle treated cells and filled bars those cells treated with 1 μM Tmp-SLF for 24 h, then probed for sLe^x expression. The mean fluorescence of all live cells is plotted. Error bars represent the standard deviations of triplicate data points.

PBS washes, the cells were permeabilized with PBS containing 1% BSA and 0.1% Triton X-100 (Sigma) for 5 min at RT. The cells were blocked in PBS with 1% BSA for 20 min, followed by the addition of the first antibodies diluted in blocking buffer (mouse anti-HA, 1:500 dilution, Covance Research and rabbit anti-Giantin, 1:500 dilution, Covance Research). The cells were incubated at room temperature for 2 h with the primary antibodies. The cells were washed three times with PBS, blocked for 10 min with blocking buffer, then incubated at RT for 1 h with secondary antibodies diluted in blocking buffer (Alexa 546-conjugated goat anti-mouse antibody, 1:500 dilution, Molecular Probes and Alexa 647-conjugated goat anti-rabbit antibody,

1:500 dilution, Molecular Probes). After three PBS washes, cells were mounted using Vectashield with DAPI (Vector Labs).

A Zeiss Axiovert 200M inverted microscope equipped with a 63 x 1.4 numerical aperature Plan-Apochromat oil immersion lens was employed for imaging. A 175-W Xenon lamp housed in a Sutter DG4 illuminator linked to the microscope by an optical fiber assured shuttering and illumination. Image stacks containing 30-40 sections spaced 0.3 μm were acquired using a CoolSNAP HQ CCD charged coupled device camera (Roper Scientific). SLIDEBOOK software (Intelligent Imaging Innovations, Denver) was used to control the microscope and camera. The image stacks were digitally deconvolved using the nearest neighbor algorithm of SLIDEBOOK.

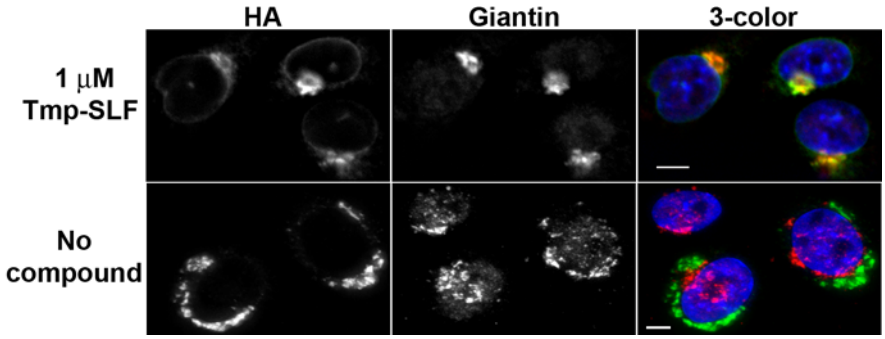


Figure S6. Effects of Tmp-SLF on Cat domain localization. CHO cells were transfected with FucT7 Loc-DHFR/3xFKBP-Cat constructs in the presence and absence of 1 μM Tmp-SLF. The cells were fixed, permeabilized, and stained with anti-HA and anti-Giantin sera, followed by Alexa546-conjugated (against anti-HA) and Alexa647-conjugated secondary antibodies. The top panel shows a single section of a deconvolved data set with the signal from the HA and Giantin shown in monochrome in the first and second columns. The third column shows three color overlays with the HA tag in green, Giantin in red, and the nuclear stain DAPI in blue. The bottom panel is a 3D projection containing the maximum pixel intensities of a deconvolved dataset. (Scale bar = 5 μm) The color scheme is identical to that in the top row. Colocalization studies with the known Golgi-resident protein Giantin confirmed that the Cat domain is Golgi-resident in the presence of Tmp-SLF. In the absence of Tmp-SLF, the Cat domains showed no colocalization with Giantin and were instead localized within a small, discrete portion of the cell that was not the Golgi apparatus.

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