

Supporting Information for

Live Cell Off-target Identification of Lapatinib Using Ligand-Directed Tosyl Chemistry

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Experimental

Materials and Methods: All chemical and biochemical reagents were purchased from commercial sources (Wako pure chemical, TCI chemical, Sigma-Aldrich) and were used without further purification. All cell lines were obtained from ATCC. Thin layer chromatography (TLC) was performed on silica gel 60 F254 precoated aluminum sheets (Merck). Chromatographic purification was performed using flash column chromatography on silica gel 60 N (neutral, 40–50 μm , Kanto Chemical).

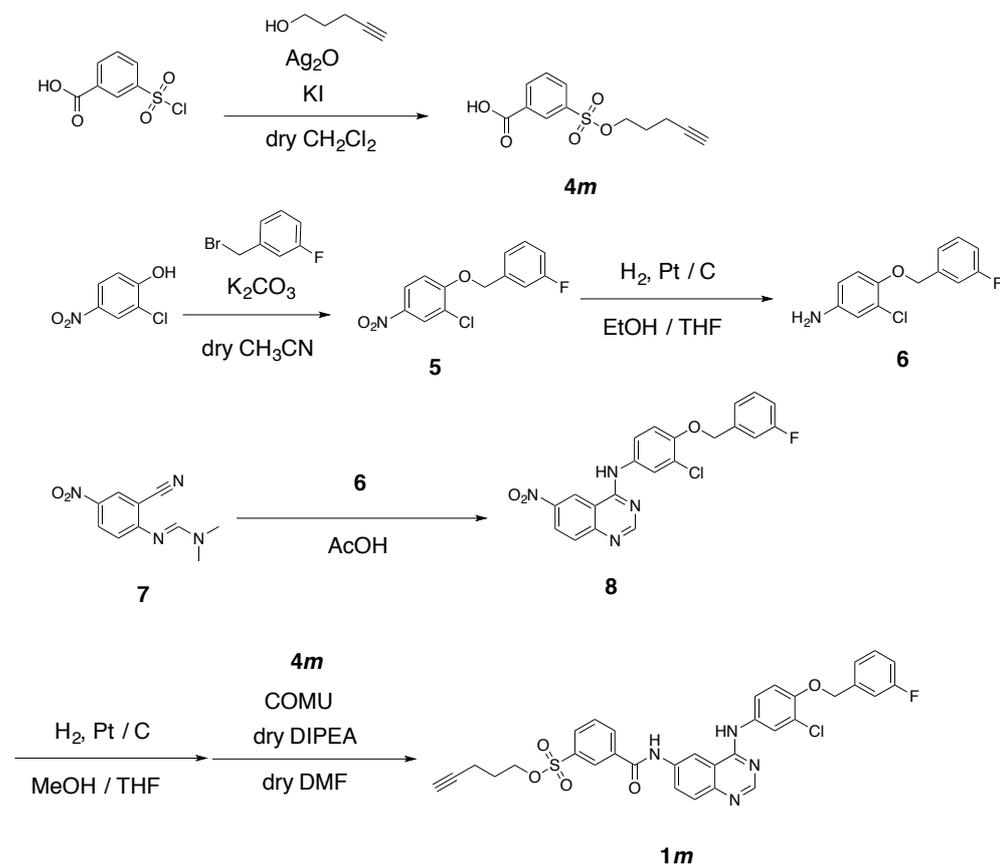
Physical Measurements: ^1H -NMR spectra were recorded on 400 MHz Varian Mercury spectrometer. Chemical shifts were referenced to residual solvent peaks or tetramethylsilane ($\delta = 0$ ppm). UV-vis absorption spectra were acquired on a Shimadzu UV-2550 spectrophotometer. High resolution mass spectra were measured on an Exactive (Thermo Scientific) equipped with

electron spray ionization (ESI). Reversed-phase HPLC (RP-HPLC) was carried out on a Hitachi Chromaster system equipped with a diode array, and an YMC-Pack Triat C18 or ODS-A column. All runs used linear gradients of acetonitrile containing 0.1% TFA (solvent A) and 0.1% aqueous TFA (solvent B).

Biochemical experiments: SDS-PAGE and western blotting were performed using a Bio-Rad Mini-Protean III electrophoresis apparatus. Fluorescence and chemical luminescent signals were detected with Imagequant LAS 4000 (GE Healthcare).

Synthesis and Characterization:

Synthesis of **1m**.



3-((pent-4-yn-1-yloxy)sulfonyl)benzoic acid (**4m**)

To a stirred solution of 3-(chlorosulfonyl)benzoic acid (1.00 g, 4.53 mmol) in CH_2Cl_2 (45 ml) were added 4-Pentyn-1-ol (847 μl , 9.06 mmol), Potassium Iodide (KI) (150 mg, 0.90 mmol), Ag_2O (1.26 g, 5.44 mmol). The reaction mixture was allowed to stir for overnight at room temperature. The solution was filtered by Celite and purified by silica gel column

chromatography (CHCl₃ : MeOH : AcOH = 20:2:1) and evaporated. To remove residual 3-(chlorosulfonyl)benzoic acid, the residue was dissolved in saturated aqueous NaHCO₃. The aqueous layer was washed with CHCl₃, acidified with 1N aqueous HCl, and extracted with CHCl₃. The organic layer was dried over Na₂SO₄, filter, evaporated to yield compound **4m** (62 mg, 0.23 mmol, 5 %) as a white powder. ¹H-NMR (400 MHz, CDCl₃): δ 8.64 (s, 1H), 8.38 (d, 1H, *J* = 8.0 Hz), 8.16 (d, 1H, *J* = 8.0 Hz), 7.71 (dd, 1H, *J* = 8.0, 8.0 Hz), 4.26 (t, 2H, *J* = 6.0 Hz), 2.28 (m, 2H), 1.87-1.93 (m, 3H).

2-chloro-1-((3-fluorobenzyl)oxy)-4-nitrobenzene (5)

To a stirred solution of 2-chloro-4-nitrophenol (9.37 g, 54.0 mmol), K₂CO₃ (18.66 g, 135 mmol) in dry CH₃CN (40 ml) were added *m*-fluorobenzyl bromide (8.50 g, 45.0 mmol) in dry CH₃CN (10 ml). The reaction mixture was allowed to stir for 18 h at 40 °C. The solution was evaporated and dissolved in AcOEt. The solution was washed with H₂O, saturated aqueous NaHCO₃, brine. The organic layer was dried over MgSO₄, filtered, evaporated to yield the compound **5** (12.6 g, 44.5 mmol, 99 %) as a pale yellow powder. ¹H-NMR (400 MHz, CDCl₃): δ 8.33 (d, 1H, *J* = 2.8 Hz), 8.14 (dd, 1H, *J* = 2.8, 9.2 Hz), 7.36-7.42 (m, 1H), 7.21-7.24 (m, 1H), 7.08-7.20 (m, 1H), 7.04-7.08 (m, 1H), 7.01 (d, 1H, *J* = 9.2 Hz), 5.26 (s, 2H).

3-chloro-4-((3-fluorobenzyl)oxy)aniline (6)

To a stirred solution of the compound **5** (6.00 g, 21.3 mmol) in THF : EtOH = 1 : 1 (35 ml) was added 5 % platinum-carbon (600 mg) at room temperature. The reaction mixture was allowed to stir for 3.5 h under hydrogen atmosphere. The solution was filtered by Celite and evaporated to yield the compound **6** (5.37 g, 21.3 mmol, quant.) as a brown solid. ¹H-NMR (400 MHz, CDCl₃): δ 7.30-7.36 (m, 1H), 7.17-7.22 (m, 2H), 6.97-7.02 (m, 1H), 6.79 (s, 1H), 6.76 (d, 1H, *J* = 2.8 Hz), 6.50 (dd, 1H, *J* = 2.8, 8.8 Hz), 5.03 (s, 2H).

***N*-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-nitroquinazolin-4-amine (8)**

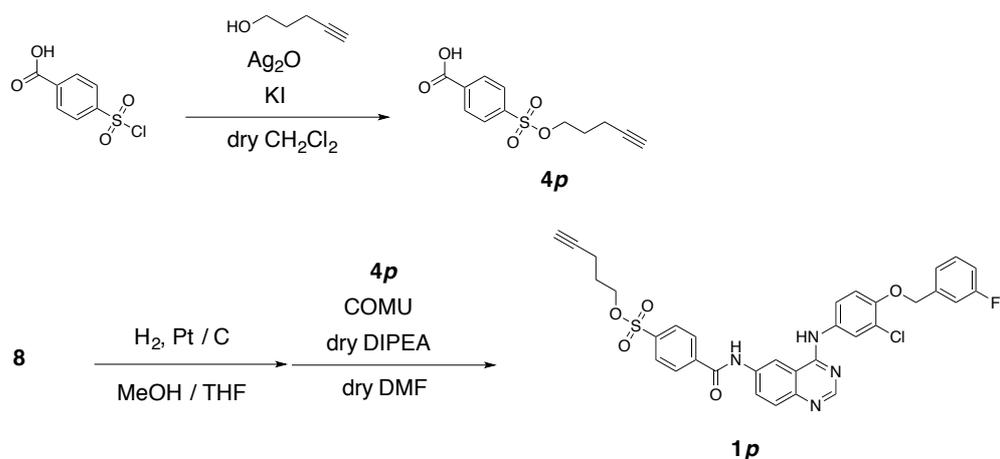
To a stirred solution of compound **7**^[1] (236 mg, 1.08 mmol) in AcOH (2 ml) was added compound **6** (300 mg, 1.2 mmol) at room temperature. The reaction mixture was allowed to stir for 1h under reflux. The solid was filter and washed with Et₂O to yield compound **8** (227 mg, 0.53 mmol, 49 %) as yellow powder. ¹H-NMR (400 MHz, DMSO): δ 9.61 (s, 1H), 8.71 (s, 1H), 8.55 (dd, 1H, *J* = 2.8, 8.8 Hz), 8.00 (s, 1H), 7.92 (d, 1H, *J* = 8.8 Hz), 7.73 (dd, 1H, *J* = 2.8, 8.8 Hz), 7.46 (m, 1H), 7.30-7.34 (m, 3H), 7.18 (m, 1H), 5.26 (s, 2H).

pent-4-yn-1-yl

3-((4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)carbamoyl)benzenesulfonate (1m)

To a stirred solution of compound **8** (12 mg, 0.03 mmol) in THF : MeOH = 1 : 1 (1 ml) was added 5% platinum-carbon (1.5 mg) at room temperature. The reaction mixture was allowed to stir for 1h under hydrogen atmosphere. The solution was filtered by Celite and evaporated. The residue was dissolved in dry DMF (0.3 ml). then, compound **4m** (8.5 mg, 0.03 mmol), (1-Cyano-2-ethoxy-2-oxoethylideneaminoxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) (17 mg, 0.05 mmol), dry N,N-diisopropylethylamine (DIPEA) (16 μ l, 0.09 mmol) were added to the solution. The reaction mixture was allowed to stir for 1 h at room temperature. The reaction mixture was diluted with CHCl₃ and washed with saturated aqueous NaHCO₃ and 5% citric acid. The organic layer was dried over Na₂SO₄, filtered, evaporated. The residue was purified by silica gel column chromatography (CHCl₃ : MeOH = 40:1 \rightarrow CHCl₃ : MeOH = 10:1) to yield Compound **1m** (2.5 mg, 3.9 μ mol, 13 %) as a brown solid. ¹H-NMR (400 MHz, CD₃OD : CDCl₃ = 1 : 1): δ 9.05 (s, 1H), 8.65 (s, 1H), 8.55 (s, 1H), 8.37 (m, 1H), 8.29 (m, 1H), 8.12 (m, 1H), 7.77-7.86 (m, 3H), 7.11-7.37 (m, 5H), 7.01 (m, 1H), 5.21 (s, 2H) 4.25 (t, 2H, *J* = 5.6 Hz), 2.24 (m, 2H), 2.00 (s, 1H), 1.87 (m, 2H). HR-ESI MS: calcd for C₃₃H₂₆ClFN₄O₅S [M+H]⁺ = 645.1369: obsd 645.1360.

Synthesis of 1p



4-((pent-4-yn-1-yloxy)sulfonyl)benzoic acid (4p)

To a stirred solution of 4-(chlorosulfonyl)benzoic acid (1.18 g, 5.34 mmol) in CH₂Cl₂ (54 ml) were added 4-Pentyn-1-ol (500 μ l, 5.34 mmol), KI (177 mg, 1.07 mmol), Ag₂O (1.5 g, 6.41

mmol). The reaction mixture was allowed to stir overnight at room temperature. The solution was filtered by Celite and purified by silica gel column chromatography (CHCl₃ : MeOH = 10:1) and evaporated. To remove residual 4-(chlorosulfonyl)benzoic acid, the residue was dissolved in saturated aqueous NaHCO₃. The aqueous layer was washed with CHCl₃, acidified with 1N aqueous HCl, and extracted with CHCl₃. The organic layer was dried over Na₂SO₄, filter, evaporated to yield compound **4p** (70 mg, 0.27 mmol, 5 %) as a white powder. ¹H-NMR (400 MHz, CDCl₃): δ 8.38 (d, 2H, *J* = 8.0 Hz), 8.16 (d, 2H, *J* = 8.0 Hz), 4.25 (t, 2H, *J* = 6.0 Hz), 2.28 (m, 2H), 1.87-1.93 (m, 3H).

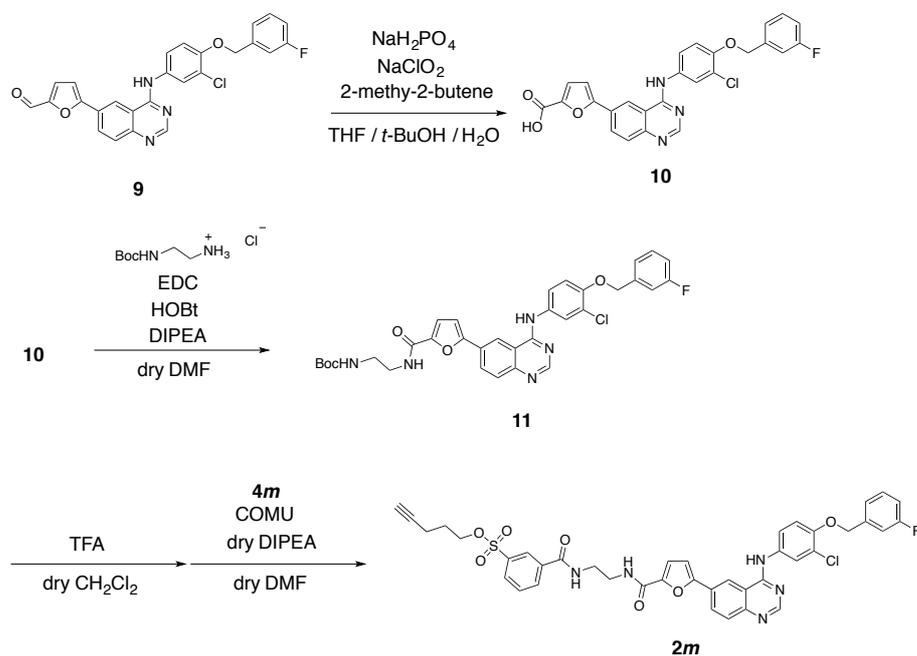
pent-4-yn-1-yl

4-((4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)carbamoyl)benzenesulfonate (1p)

To a stirred solution of compound **8** (12 mg, 0.03 mmol) in THF : MeOH = 1 : 1 (1 ml) was added 5% platinum-carbon (1.5 mg) at room temperature. The reaction mixture was allowed to stir for 1h under hydrogen atmosphere. The solution was filtered by Celite and evaporated. The residue was dissolved in dry DMF (0.3 ml). Then, compound **4p** (8.5 mg, 0.03 mmol), COMU (17 mg, 0.05 mmol), dry DIPEA (16 μl, 0.09 mmol) were added to the solution. The reaction mixture was allowed to stir for 1 h at room temperature. The reaction mixture was diluted with CHCl₃ and washed with saturated aqueous NaHCO₃ and 5% citric acid. The organic layer was dried over Na₂SO₄, filtered, evaporated. The residue was purified by silica gel column chromatography (CHCl₃ : MeOH = 40:1 → CHCl₃ : MeOH = 10:1) to yield Compound **1p** (1.0 mg, 1.7 μmol, 5 %) as a brown solid. ¹H-NMR (400 MHz, CD₃OD : CDCl₃ = 1 : 1): δ 9.09 (s, 1H), 8.67 (s, 1H), 8.22 (d, 2H, *J* = 8.4 Hz), 8.07 (d, 2H, *J* = 8.4 Hz), 7.82-7.87 (m, 2H), 8.12 (m, 1H), 7.77-7.86 (m, 3H), 7.55 (m, 1H), 7.11-7.37 (m, 5H), 7.02 (m, 1H), 5.23 (s, 2H), 4.24 (t, 2H, *J* = 5.6 Hz), 2.26 (m, 2H), 2.04 (s, 1H), 1.87 (m, 2H).

HR-ESI MS: calcd for C₃₃H₂₆ClFN₄O₅S [M+H]⁺ = 645.1369: obsd 645.1361.

Synthesis of **2m**



5-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)furan-2-carboxylic acid (**10**)

To a stirred solution of compound **9**¹ (250 mg, 0.528 mmol) in THF : *t*-BuOH : H₂O = 5 : 3 : 2 (10 ml) were added monosodium phosphate (320 mg, 2.67 mmol), 2-methyl-2-butene (370 mg, 5.28 mmol), sodium chlorite (181 mg, 1.60 mmol) at room temperature. The reaction mixture was allowed to stir for 1 h at room temperature. The reaction mixture was washed with brine and filtered. The residue was dissolved with THF and precipitated by hexane to yield compound **10** (255 mg, 0.521 mmol, 98 %) as an orange powder. ¹H-NMR (400 MHz, CD₃OD): δ 8.51 (s, 1H), 8.27 (m, 1H), 7.89 (m, 1H), 7.84 (d, 1H, *J* = 4.0 Hz), 7.82 (d, 1H, *J* = 4.0 Hz), 7.61 (m, 1H), 7.38-7.44 (m, 1H), 7.25-7.28 (m, 2H), 7.19 (m, 1H), 7.03-7.08 (m, 2H), 7.19 (m, 1H), 5.24 (s, 2H).

tert-butyl

(2-(5-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)furan-2-carboxamido)ethyl)carbamate (**11**)

To a stirred solution of compound **10** (100 mg, 0.2 mmol) in dry DMF (3 ml) were added *tert*-butyl (2-aminoethyl)carbamate hydrochloride (59 mg, 0.3 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (57 mg, 0.3 mmol), 1-hydroxybenzotriazole (HOBt) (46 mg, 0.3 mmol), DIPEA (50 μl, 0.3 mmol). The reaction

mixture was allowed to stir for 10 h at room temperature. The solution was purified by silica gel column chromatography (CHCl₃: MeOH = 50:1) to yield compound **11** (89 mg, 0.14 mmol, 70 %) as a yellow solid. ¹H-NMR (400 MHz, CD₃OD): δ 8.77 (s, 1H), 8.67 (m, 1H), 8.28 (m, 1H), 7.93 (m, 1H), 7.73 (m, 1H), 7.60 (m, 1H), 7.36 (m, 1H), 7.21 (m, 2H), 7.18 (d, 1H, *J* = 4.0 Hz), 6.98-7.03 (m, 2H), 6.83 (d, 1H, *J* = 4.0 Hz), 5.17 (s, 2H), 3.49 (m, 2H), 3.33 (m, 2H), 1.23 (s, 9H).

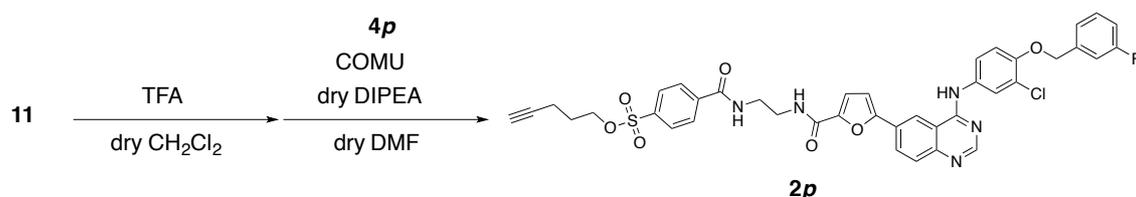
pent-4-yn-1-yl

3-((2-(5-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)furan-2-carboxamido)ethyl)carbamoyl)benzenesulfonate (**2m**)

To a stirred solution of compound **11** (32 mg, 0.05 mmol) in dry CH₂Cl₂ (1 ml) was added TFA (0.5 ml) at room temperature. The reaction mixture was allowed to stir for 1 h at room temperature. After azeotropic removal of TFA with toluene, the residue was dissolved in dry DMF (1.0 ml). Then, compound **4m** (13 mg, 0.05 mmol), COMU (28 mg, 0.08 mmol), dry DIPEA (8.7 μl, 0.05 mmol) were added to the solution. The reaction mixture was allowed to stir for 1 h at room temperature. The solution was diluted with CHCl₃ and washed with saturated aqueous NaHCO₃ and 5% citric acid. The organic layer was dried over Na₂SO₄, filtered, evaporated. The residue was purified by silica gel column chromatography (Hexane : AcOEt = 2:1 → CHCl₃ : MeOH = 10:1) and HPLC to yield compound **2m** (4 mg, 5.00 μmol, 10 %) as a yellow solid. ¹H-NMR (400 MHz, CD₃OD : CDCl₃ = 1 : 1): δ 9.18 (s, 1H), 8.68 (s, 1H), 8.43 (m, 1H), 8.28 (s, 1H), 8.03 (m, 1H), 7.95 (m, 1H), 7.89 (m, 1H), 7.60-7.69 (m, 2H), 7.45 (m, 1H), 7.36 (m, 1H), 7.00-7.26 (m, 6H), 5.19 (s, 2H), 4.05 (t, 2H, *J* = 6.0 Hz), 3.78 (m, 2H), 3.65 (m, 2H), 2.12 (m, 2H), 1.95 (m, 1H), 1.74 (m, 2H).

HR-ESI MS: calcd for C₄₀H₃₃ClFN₅O₇S [M+H]⁺ = 782.1846: obsd 782.1840.

Synthesis of **2p**



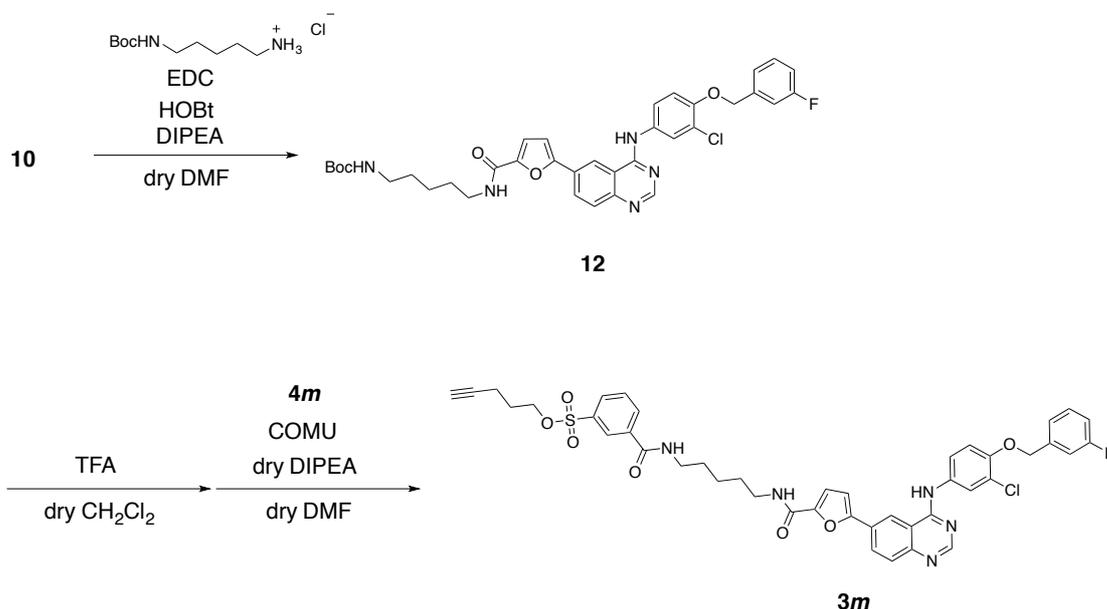
pent-4-yn-1-yl

4-((2-(5-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)furan-2-carboxamido)ethyl)carbamoyl)benzenesulfonate (**2p**)

To a stirred solution of compound **11** (32 mg, 0.05 mmol) in dry CH₂Cl₂ (3 ml) was added TFA (1.5 ml) at room temperature. The reaction mixture was allowed to stir for 1 h at room temperature. After azeotropic removal of TFA with toluene, the residue was dissolved in dry DMF : dry CH₂Cl₂ = 1:1 (1.0 ml). Then, compound **4p** (13 mg, 0.05 mmol), COMU (28 mg, 0.08 mmol), dry DIPEA (8.7 μl, 0.05 mmol) were added to the solution. The reaction mixture was allowed to stir for 1 h at room temperature. The solution was diluted with CHCl₃ and washed with saturated aqueous NaHCO₃ and 5% citric acid. The organic layer was dried over Na₂SO₄, filtered, evaporated. The residue was purified by silica gel column chromatography (Hexane : AcOEt = 2:1 → CHCl₃ : MeOH = 10:1) and HPLC to yield compound **2p** (1.5 mg, 2.00 μmol, 4 %) as a yellow solid. ¹H-NMR (400 MHz, CDCl₃): δ 9.18 (s, 1H), 8.51 (s, 1H), 8.33 (m, 2H), 7.82-7.97 (m, 5H), 7.46 (m, 1H), 7.34 (m, 1H), 7.00-7.24 (m, 5H), 6.85 (m, 1H), 5.11 (s, 2H), 4.15 (t, 2H, *J* = 6.0 Hz), 3.87 (m, 2H), 3.76 (m, 2H), 2.17-2.22 (m, 3H), 1.74 (m, 2H).

HR-ESI MS: calcd for C₄₀H₃₃ClFN₅O₇S [M+H]⁺ = 782.1846: obsd 782.1826.

Synthesis of **3m**



tert-butyl

(5-(5-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)furan-2-carboxamido)pentyl)carbamate (**12**)

To a stirred solution of compound **10** (80 mg, 0.16 mmol) in dry DMF (2.3 ml) were added *tert*-butyl (5-aminopentyl)carbamate hydrochloride (69 mg, 0.25 mmol), EDC (48 mg, 0.25

mmol), HOBT (38 mg, 0.25 mmol), DIPEA (43 μ l, 0.25 mmol). The reaction mixture was allowed to stir for 10 h at room temperature. The solution was purified by silica gel column chromatography (CHCl_3 : MeOH = 50:1) to yield compound **12** (55 mg, 0.08 mmol, 51 %) as a yellow solid. $^1\text{H-NMR}$ (400 MHz, CD_3OD): δ 8.55 (s, 1H), 8.40(s, 1H), 8.11 (m, 1H), 7.84 (m, 1H), 7.64 (m, 1H), 7.52 (m, 1H), 7.35 (m, 1H), 7.23 (m, 2H), 7.12 (d, 1H, J = 4.0 Hz), 6.98-7.04 (m, 2H), 6.94 (d, 1H, J = 4.0 Hz), 5.07 (s, 2H), 3.36 (m, 2H), 3.03 (m, 2H), 1.47-1.70 (m, 6H), 1.23 (s, 9H).

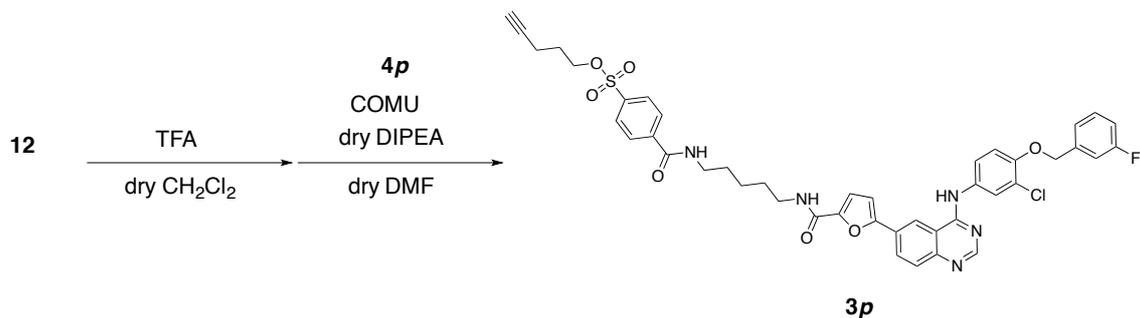
pent-4-yn-1-yl

3-((5-(5-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)furan-2-carboxamido)pentyl)carbamoyl)benzenesulfonate (3m)

To a stirred solution of compound **12** (13 mg, 0.02 mmol) in dry CH_2Cl_2 (1 ml) was added TFA (0.5 ml) at room temperature. The reaction mixture was allowed to stir for 1 h at room temperature. After removal of TFA with toluene, the residue was dissolved in dry DMF (0.4 ml). Then, compound **4m** (5.3 mg, 0.02 mmol), COMU (11 mg, 0.03 mmol), dry DIPEA (10 μ l, 0.06 mmol) were added to the solution. The reaction mixture was allowed to stir for 1.5 h at room temperature. The solution was diluted with CHCl_3 and washed with saturated aqueous NaHCO_3 and 5% citric acid. The organic layer was dried over Na_2SO_4 , filtered, evaporated. The residue was purified by silica gel column chromatography (CHCl_3 : MeOH = 40:1 \rightarrow CHCl_3 : MeOH = 10:1) to yield compound **3m** (8.4 mg, 11.00 μ mol, 54 %) as a yellow solid. $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 8.80 (s, 1H), 8.62 (s, 1H), 8.07 (s, 1H), 7.97 (m, 1H), 7.83-7.85 (m, 3H), 7.60-7.64 (m, 2H), 7.33-7.40 (m, 2H), 6.97-7.25 (m, 6H), 5.11 (s, 2H), 4.03 (t, 2H, J = 6.0 Hz), 3.51 (m, 2H), 3.41 (m, 2H), 2.19 (s, 2H), 1.84 (m, 1H), 1.77 (m, 2H), 1.59-1.64 (m, 4H), 1.34-1.40 (m, 2H).

HR-ESI MS: calcd for $\text{C}_{43}\text{H}_{39}\text{ClFN}_5\text{O}_7\text{S}$ $[\text{M}+\text{H}]^+$ = 824.2316: obsd 824.2299.

Synthesis of **3p**



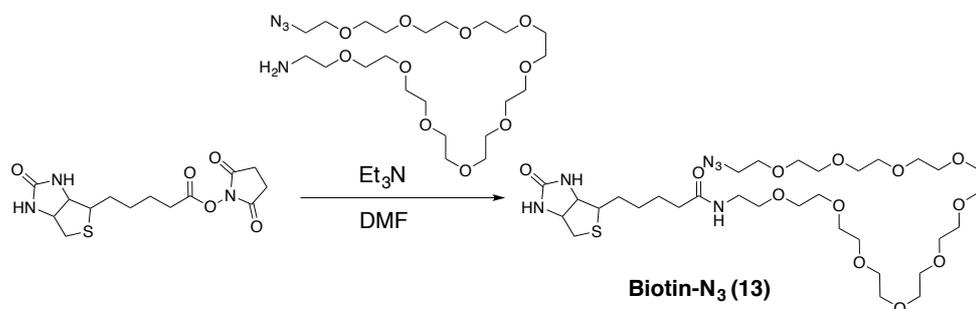
pent-4-yn-1-yl

4-((5-(5-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)furan-2-carboxamido)pentyl)carbamoyl)benzenesulfonate (**3p**)

To a stirred solution of compound **12** (34 mg, 0.05 mmol) in dry CH_2Cl_2 (1 ml) was added TFA (0.5 ml) at room temperature. The reaction mixture was allowed to stir for 2 h at room temperature. After azeotropic removal of TFA with toluene, the residue was dissolved in dry DMF (1.0 ml). Then, compound **4p** (13.0 mg, 0.05 mmol), COMU (28 mg, 0.08 mmol), dry DIPEA (8.7 μl , 0.05 mmol) were added to the solution. The reaction mixture was allowed to stir for 1.5 h at room temperature. The solution was diluted with CHCl_3 and washed with saturated aqueous NaHCO_3 and 5% citric acid. The organic layer was dried over Na_2SO_4 , filtered, evaporated. The residue was purified by silica gel column chromatography (Hexane : AcOEt = 2:1 \rightarrow CHCl_3 : MeOH = 10:1) and HPLC to yield compound **3p** (1.0 mg, 1.20 μmol , 3 %) as a yellow solid. $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 8.92 (s, 1H), 8.57 (s, 1H), 8.41 (m, 1H), 8.29 (m, 1H), 7.84 (m, 2H), 7.72-7.76 (m, 3H), 7.30-7.43 (m, 2H), 6.93-7.19 (m, 6H), 5.15 (s, 2H), 4.07 (t, 2H, $J = 6.0$ Hz), 3.30-3.42 (m, 4H), 2.09-2.17 (m, 3H), 1.91 (m, 2H), 1.48-1.78 (m, 6H).

HR-ESI MS: calcd for $\text{C}_{43}\text{H}_{39}\text{ClFN}_5\text{O}_7\text{S}$ $[\text{M}+\text{H}]^+ = 824.2316$: obsd 824.2298.

Synthesis of Biotin- N_3 (**13**)

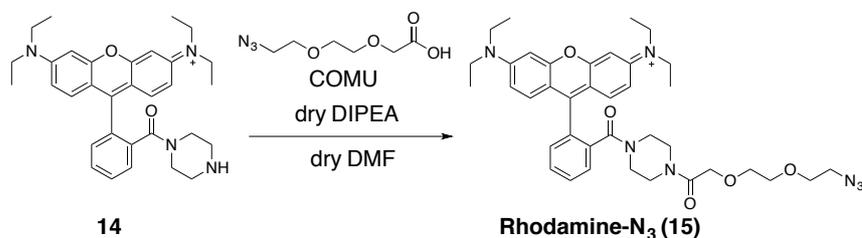


Biotin- N_3 (**13**)

To a stirred solution of 32-Azido-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontan-1-amine

(102.3 mg, 0.194 mmol) in dry DMF (1 ml) were added 2,5-dioxopyrrolidin-1-yl 5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate (65.3 mg, 0.191 mmol), Et3N (30 μ L, 0.216 mmol). The reaction mixture was allowed to stir for 2.5 h at room temperature. The reaction mixture was purified directly by HPLC to yield compound Biotin-N₃ (119.1 mg, 0.160 mmol, 83%) as a white gum. ¹H-NMR (400 MHz, CDCl₃): δ 6.94 (1H, t, *J* = 5.0 Hz), 6.32 (1H, s), 5.43 (1H, s), 4.47-4.53 (1H, m), 4.28-4.34 (1H, m), 3.61-3.69 (36H, m), 3.56 (2H, t, *J* = 5.0 Hz), 3.41-3.48 (2H, m), 3.39 (2H, t, *J* = 5.0 Hz), 3.11-3.18 (1H, m), 2.91 (1H, dd, *J* = 12.8, 5.0 Hz), 2.74 (1H, d, *J* = 12.8 Hz), 2.12-2.41 (4H, m), 1.62-1.81 (4H, m), 1.41-1.48 (2H, m).

Synthesis of Rhodamine-N₃ (15)



Rhodamine-N₃ (15)

To a stirred solution of compound **14**^[2] (20 mg, 0.04 mmol) in dry DMF (0.8 ml) were added COMU (23 mg, 0.06 mmol), dry DIPEA (20 μ l, 0.12 mmol) and 2-(2-(2-azidoethoxy)ethoxy)acetic acid^[3] (7.5 mg, 0.04 mmol, 1.0 eq). The reaction mixture was allowed to stir for 1 h at room temperature. The solution was diluted with CHCl₃ and washed with saturated aqueous NaHCO₃ and 5% citric acid. The organic layer was dried over Na₂SO₄, filtered, evaporated. The residue was purified by silica gel column chromatography (1% NH₃ aq. CHCl₃: MeOH = 40:1 \rightarrow 1% NH₃ aq. CHCl₃: MeOH = 10:1) to yield Rhodamine-N₃ (27 mg, 0.04 mmol, 98 %) as a purple solid. ¹H-NMR (400 MHz, CDCl₃): δ 7.68-7.78 (m, 3H), 7.51 (m, 1H), 7.27 (d, 2H, *J* = 9.2 Hz), 7.07 (s, 2H, *J* = 9.2 Hz), 6.95 (s, 2H), 4.2 (s, 2H), 3.62-3.71 (m, 16H), 3.39 (m, 8H), 3.30-3.33 (m, 6H), 1.30 (t, 12H, *J* = 6.8 Hz).

Chemical labeling of recombinant HER2 kinase domain in test tube:

Biotin tethered labeling reagents (**1m-Bt**, **1p-Bt**, **2m-Bt**, **2p-Bt**, **3m-Bt**, **3p-Bt**) were prepared from alkyne labeling reagents (**1m**, **1p**, **2m**, **2p**, **3m**, **3p**) by click chemistry in test tube (Scheme S1). Click chemistry condition were as follows: to a solution of alkyne labeling reagents (1 μ l, 5 mM in DMSO) was added Cu(MeCN)₄BF₄ (1 μ l, 75 mM in DMSO), bathophenanthroline disulfonic acid (BPAA) (1 μ l, 7.5 mM in DMSO : H₂O = 1:1), Tris(2-carboxyethyl)phosphine Hydrochloride (TCEP) (1 μ l, 37.5 mM in H₂O), Biotin-N₃ (1 μ l, 15 mM in DMSO) for 1 h at room temperature. The solution was diluted with 100 mM HEPES (pH 7.5) containing 5 mM MgCl₂, and used for HER2 kinase domain labeling without any purification. Recombinant human HER2 (SignalChem, E27-11G) was diluted with 100 mM HEPES buffer containing 5 mM MgCl₂ and used for HER2 kinase domain labeling. Recombinant human HER2 (100 nM) was incubated with labeling reagents (1 μ M) and in the presence or absence of 20 μ M lapatinib for 10 h at 25 °C. Each sample was mixed with 5x SDS-PAGE loading buffer (325 mM Tris-HCl, 15% SDS, 20% sucrose, 0.5 M DTT, and 0.02% BPB, pH 6.8) and incubated for 1 h at room temperature. The samples were applied to SDS-PAGE and electrotransferred onto immune-blot PVDF membranes (Bio-rad), followed by blocking with 5% non-fat dry milk in TBS containing 0.05% Tween (Sigma-Aldrich, St. Louis, MO). The membranes were stained with SA_v-HRP (GE Healthcare) or with rabbit HER2/ErbB2 antibody (cell signaling technology, 2242) followed by a HRP-conjugated goat anti-rabbit IgG (santa cruz, SC-2004). The membranes were developed with ECL prime western blotting detection system (GE Healthcare).

Chemical labeling of HER2 endogenously expressed in live NCI-N87 cells:

NCI-N87 cells (3.2×10^5 cells) were incubated on 35-mm dishes (BD Falcon) at 37 °C. After 72 hours, the medium was replaced by serum-free DMEM containing labeling reagents with or without lapatinib at 37 °C. After 8 hours, the media was removed, and the cells were washed twice with PBS. Then, the cells were lysed with RIPA buffer (25 mM Tris/HCl, 150 mM NaCl, 1% NP-40, 0.25% DOC, 0.1% SDS, pH7.4) containing protease inhibitor (Calbiochem, 39134), and rotated at 4 °C for 30 min. After centrifugation (10,000 g, 10 min), the supernatant was incubated with Neutra-Avidin agarose resins 50 μ L (Thermo scientific, 29202, 50 % slurry) at 4 °C for 1 h to remove endogenously expressing biotinylated protein. To the supernatant were added Cu(MeCN)₄BF₄ (500 μ M), BPAA (50 μ M), TCEP (250 μ M), Biotin-N₃ (100 μ M) for 1h at room temperature. Each sample was mixed with 5x SDS-PAGE loading buffer (325 mM

Tris-HCl, 15% SDS, 20% sucrose, 0.5 M DTT, and 0.02% BPB, pH 6.8) and incubated for 1 h at room temperature. The samples were applied to SDS-PAGE and western blotting as described above.

Confirmation of chemical labeling of HER2 by immunoprecipitation analysis:

NCI-N87 cells (3.2×10^5 cells) were incubated on 35-mm dishes (BD Falcon) at 37 °C. After 72 hours, the medium was replaced by serum-free DMEM containing labeling reagents with or without lapatinib at 37 °C. After 8 hours, the media was removed, and the cells were washed twice with PBS. Then, the cells were lysed with RIPA buffer (25 mM Tris/HCl, 150 mM NaCl, 1% NP-40, 0.25% DOC, 0.1% SDS, pH7.4) containing protease inhibitor (Calbiochem, 39134), and rotated at 4 °C for 30 min. After centrifugation (10,000 g, 10 min), the supernatant was incubated with Neutra-Avidin agarose resins 50 μ L (Thermo scientific, 29202, 50 % slurry) at 4 °C for 1 h to remove endogenously expressing biotinylated protein. To the supernatant were added $\text{Cu}(\text{MeCN})_4\text{BF}_4$ (500 μ M), BPAA (50 μ M), TCEP (250 μ M), Biotin- N_3 (100 μ M) for 1h at room temperature. The sample was incubated with Herceptin at 4 °C for 12 hour, followed by addition of nProtein A sepharose 4 Fast flow and further incubation at 4 °C for 1 hours. The sepharose was washed 5 times with RIPA. Protein was eluted by addition of 2x SDS-PAGE sample buffer containing 250 mM DTT and boiling in heatblock for 5 min. The samples were applied to SDS-PAGE and western blotting as described above.

HER2 labeling in live SK-BR-3 cells:

SK-BR-3 cells (4.0×10^5 cells) were incubated on 35-mm dishes (BD Falcon) at 37 °C. After 60 hours, the medium was replaced by serum-free McCoy's 5A Medium containing labeling reagents with or without lapatinib at 37 °C. After 8 hours, the media was removed, and the cells were washed twice with PBS. Then, the cells were lysed with RIPA buffer (25 mM Tris/HCl, 150 mM NaCl, 1% NP-40, 0.25% DOC, 0.1% SDS, pH7.4) containing protease inhibitor (Calbiochem, 39134), and rotated at 4 °C for 30 min. After centrifugation (10,000 g, 10 min), the supernatant was incubated with Neutra-Avidin agarose resins 50 μ L (Thermo scientific, 29202, 50 % slurry) at 4 °C for 1 h to remove endogenously expressing biotinylated protein. To the supernatant were added $\text{Cu}(\text{MeCN})_4\text{BF}_4$ (500 μ M), BPAA (50 μ M), TCEP (250 μ M), Biotin- N_3 (100 μ M) for 1h at room temperature. Each sample was mixed with 5x SDS-PAGE loading buffer (325 mM Tris-HCl, 15% SDS, 20% sucrose, 0.5 M DTT, and 0.02% BPB, pH 6.8) and incubated for 1 h at room temperature. The samples were applied to SDS-PAGE and

western blotting as described above.

Labeling in Live NCI-N87 cells and partial purification of off-target proteins by Fast Protein Liquid Chromatography (FPLC):

Prior to labeling experiments, NCI-N87 cells (2.0×10^6 cells) were incubated on three 10-cm dishes (BD Falcon) at 37 °C. After 72 hours, the medium was replaced by serum-free DMEM containing **2m** (5 μM) at 37 °C. After 8 hours, the media was removed, then lysis buffer cells were washed twice with PBS. Then, the cells were lysed with 50 mM Tris/HCl (pH7.5), 150 mM NaCl, 0.1% Triton containing protease inhibitor (Calbiochem, 39134), and rotated at 4 °C for 30 min. The lysate was centrifuged (10,000 G, 10 min). To the supernatant were added $\text{Cu}(\text{MeCN})_4\text{BF}_4$ (500 μM), BPAA (50 μM), TCEP (250 μM), Biotin- N_3 (100 μM) for 1h at room temperature. The solution was dialyzed against 50 mM Tris/HCl (pH8.0) with a Spectra/Por dialysis membrane (MWCO: 8,000). The resulting solution was purified by FPLC. The chromatographic material was used AKTA purifier (GE Healthcare). Chromatographic separation with the Mono Q 4.6/100 PE column was achieved with a step gradient of B in A at a flow rate of 1.5 ml/min (A. 50 mM HEPES buffer (pH 8.0), B. Buffer A containing 1M NaCl). Mono Q fraction was detected by western blotting as described above. Fraction containing labeled off-target protein was concentrated using a Amicon Ultra-4 (10K, Millipore).

2D-PAGE analysis of off-target proteins:

Partially purified fraction was precipitated using ReadyPrep 2-D cleanup kit (Bio-rad, 163-2130). The resulting pellet was solubilized ReadyPrep protein extraction kit (Bio-rad, 163-2086). To the solution was added 0.2 % (w/v) Bio-Lyte 3/10 ampholyte and applied to a 7-cm immobilized pH gradient (IPG) strip, pH 3-10. Rehydration was carried out for 12 hours using a PEOTEAN i12 IEF system, followed by isoelectric focusing (IEF). All IEF equipments and reagents were purchased from Bio-rad. Immediately after IEF, IPG strips were equilibrated with in buffer solution I and II containing 375 mM Tris-HCl (pH 8.8), 6 M urea, 20% glycerol, 2% SDS, and 130 mM DTT for buffer solution I or 137 mM iodoacetamide for buffer solution II. The equilibrated IPG strips were placed on the top of acrylamide SDS-PAGE minigels, sealed with low melt agarose. After second-dimension separation, proteins were detected by silver staining (Wako) and western blotting.

Identification of off-target proteins by peptide fingerprint analyses:

To identify the labeled proteins, The band pattern of silver stained 2D gel was carefully compared with the corresponding western blot. The labeled protein spots thus identified were excised from the gel, destained, and subjected to in-gel tryptic digestion using in-gel digestion kit (Thermo Scientific). Samples were analyzed by nano-flow reverse phase liquid chromatography followed by tandem MS, using a Triple TOF 5600+ (AB SCIEX, Concord, Canada).

A capillary reverse phase HPLC-MS/MS system composed of an Eksigent Ekspert nano-LC 400 HPLC system (AB SCIEX) which was directly connected to an AB SCIEX quadrupole time-of-flight (QqTOF) TripleTOF 5600+ mass spectrometer (AB SCIEX) in Trap and Elute mode. In the Trap and Elute mode, samples were automatically injected using Ekspert 400 system into a peptide trap column (ChromeXP, C18-CL, 200 μm I.D. x 0.5 mm, 3 μm particle size, 120 \AA pore size, AB SCIEX) attached to a cHiPLC system (AB SCIEX) for desalinating and concentrating peptides. After washing the trap with MS-grade water containing 0.1% trifluoroacetic acid and 2% acetonitrile (solvent C), the peptides were loaded into a separation capillary reverse phase column (ChromeXP, C18-CL, 75 μm I.D. x 150 mm, 3 μm particle size, 120 \AA pore size, AB SCIEX) by switching the valve. The eluents used were: A, 100% water containing 0.1% formic acid, and B, 100% acetonitrile containing 0.1% formic acid. The column was developed at a flow rate of 0.3 $\mu\text{L}/\text{min}$ with the concentration gradient of acetonitrile: from 2% B to 32% B in 20 min, 32% B to 80% B in 1 min, sustaining 80% B for 10 min, from 80% B to 2% B in 1 min, and finally re-equilibrating with 2% B for 15 min. Mass spectra and tandem mass spectra were recorded in positive-ion and “high-sensitivity” mode with a resolution of $\sim 35,000$ full-width half-maximum. The nanospray needle voltage was typically 2,300 V in HPLC-MS mode. After acquisition of ~ 5 samples, TOF MS spectra and TOF MS/MS spectra were automatically calibrated during dynamic LC-MS & MS/MS autocalibration acquisitions injecting 50 fmol BSA. Analyst TF1.6 system (AB SCIEX) was used to record peptide spectra over the mass range of m/z 400–1250, and MS/MS spectra in information-dependent data acquisition over the mass range of m/z 100–1600. For CID-MS/MS, the mass window for precursor ion selection of the quadrupole mass analyzer was set to 0.7 ± 0.1 Da. The precursor ions were fragmented in a collision cell using nitrogen as the collision gas. Advanced information dependent acquisition (IDA) was used for MS/MS collection on the TripleTOF 5600+ to obtain MS/MS spectra for the 10 most abundant parent ions following each survey MS1 scan (250 msec acquisition time per MS1 scan, and typically

100 msec acquisition time per each MS/MS). Dynamic exclusion features were based on value m/z and were set to an exclusion mass width 50 mDa and an exclusion duration of 12 sec. Searches were performed by using the Mascot server version 2.4.0 (Matrix Science, MA, USA) against latest Swissprot database for protein identification. Searching parameters were set as follows: enzyme selected as used with three maximum missing cleavage sites, species limited to human, a mass tolerance of 40 ppm for peptide tolerance, 0.1 Da for MS/MS tolerance, variable modification of carbamidomethyl (C), oxidation (M) and chemical modification (C, E, H, K, S, T, Y). The maximum expectation value for accepting individual peptide ion scores $[-10 \cdot \text{Log}(p)]$ was set to ≤ 0.05 , where p is the probability that the observed match is a random event. Protein identification and modification information returned from Mascot were manually inspected and filtered to obtain confirmed protein identification and modification lists of CID MS/MS.

Immunoprecipitation analysis of off-target protein:

NCI-N87 cells (3.2×10^5 cells) were incubated on 35-mm dishes (BD Falcon) at 37 °C. After 72 hours, the medium was replaced by serum-free DMEM containing labeling reagents with or without lapatinib at 37 °C. After 8 hours, the media was removed, and the cells were washed twice with PBS. Then, the cells were lysed with RIPA buffer (25 mM Tris/HCl, 150 mM NaCl, 1% NP-40, 0.25% DOC, 0.1% SDS, pH7.4) containing protease inhibitor (Calbiochem, 39134), and rotated at 4 °C for 30 min. After centrifugation (10,000 g, 10 min), the supernatant was incubated with Neutra-Avidin agarose resins 50 μL (Thermo scientific, 29202, 50 % slurry) at 4 °C for 1 h to remove endogenously expressing biotinylated protein. To the supernatant were added $\text{Cu}(\text{MeCN})_4\text{BF}_4$ (500 μM), BPAA (50 μM), TCEP (250 μM), Biotin- N_3 (100 μM) for 1h at room temperature. The sample was incubated with rabbit PDI antibody (cell signaling technology, 2446) at 4 °C for 18 hour, followed by addition of nProtein A sepharose 4 Fast flow and further incubation at 4 °C for 1 hours. The sepharose was washed 5 times with RIPA. Protein was eluted by addition of 2x SDS-PAGE sample buffer containing 250 mM DTT and boiling in heatblock for 5 min. The samples were applied to SDS-PAGE and western blotting as described above.

Reciprocal immunoblot of 2D-PAGE western blots:

To further confirm the labeled protein by **2m**, the 2D-PAGE westernblots with SA ν -HRP were reprobed by a standard procedure. In brief, the membrane was washed with stripping buffer (25 mM glycine-HCl, pH 2.0 containing 1.0% SDS) with shaking. After 30 min, the stripping buffer

was replaced with PBS and the membrane was washed twice with PBS for 10 min. The membranes were stained with rabbit PDI antibody (cell signaling technology, 2446) followed by a HRP-conjugated goat anti-rabbit IgG (santa cruz, SC-2004).

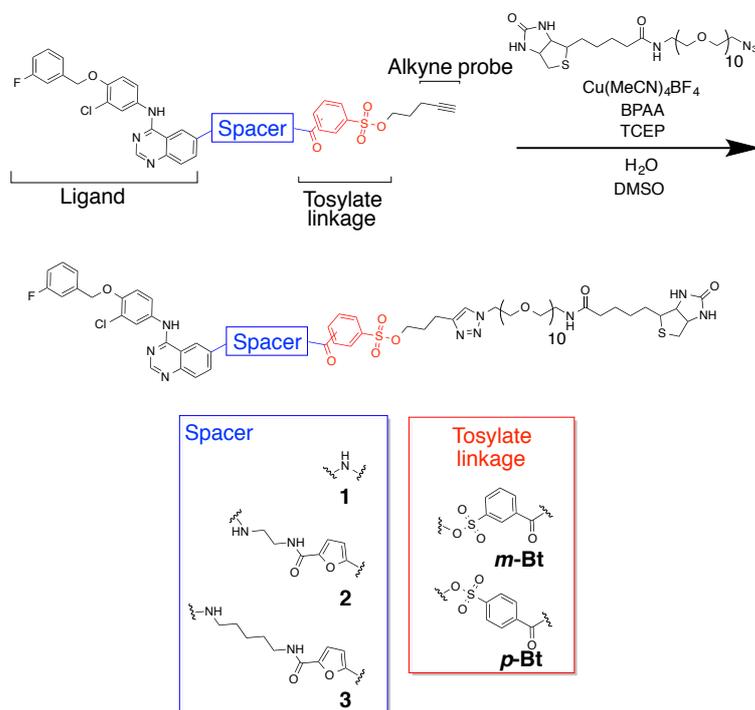
Chemical labeling of recombinant PDI in test tube:

Rhodamine tethered labeling reagent **2m-Rh** was prepared from alkyne labeling reagents **2m** by click chemistry in test tube (Scheme S2). Click chemistry condition were as follows: to a stirred solution of alkyne labeling reagents (1 μ l, 5 mM) was added $\text{Cu}(\text{MeCN})_4\text{BF}_4$ (1 μ l, 75 mM in DMSO), bathophenanthroline disulfonic acid (BPAA) (1 μ l, 7.5 mM in DMSO : H₂O = 1:1), Tris(2-carboxyethyl)phosphine Hydrochloride (TCEP) (1 μ l, 37.5 mM in H₂O), Rhodamine-N₃ (5 μ l, 3.3 mM in DMSO) for 1h at room temperature. The solution was diluted with 50 mM HEPES (pH 7.4) containing 3 mM DTT, and used for PDI labeling without any purification. Recombinant human protein disulfide isomerase (44 μ M, R&D systems, 4236-DI) was diluted with 50 mM HEPES (pH 7.4) containing 3 mM DTT, and used for PDI labeling. Recombinant human protein disulfide isomerase (220 nM) was incubated with labeling reagents in the presence or absence of 20 μ M lapatinib or 20 μ M estradiol for 18 h at 25 °C. Each sample was mixed with 5x SDS-PAGE loading buffer (325 mM Tris-HCl, 15% SDS, 20% sucrose, 0.5 M DTT, and 0.02% BPB, pH 6.8) and incubated for 1 h at room temperature. The samples were subjected to SDS-PAGE and detected by in-gel fluorescence image. After fluorescence imaging, the gels were stained by silver stain.

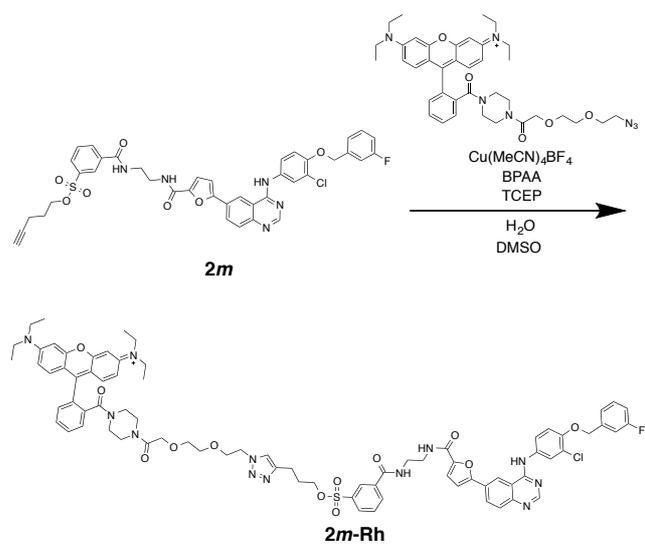
Insulin turbidity assay:

PDI activity was assessed by measuring the PDI-catalyzed reduction of insulin in the presence of DTT, Reduction-induced aggregation of insulin chain was monitored in a 96-well plate at 650 nm, using microplate reader (Tecan, infinite 200). The incubation mixture contained 100 mM NaH₂PO₄ (pH 7.0), 1 mM DTT, 2 mM EDTA, 1.0 mg/ml bovine insulin (Sigma), and 830 ng/ml Recombinant human PDI (R&D systems, 4236-DI).

Supporting Schemes and Figures



Scheme S1 The biotin tethered labeling reagents (**1*m*-Bt**, **1*p*-Bt**, **2*m*-Bt**, **2*p*-Bt**, **3*m*-Bt**, **3*p*-Bt**) prepared from alkyne-type of LDT reagents (**1*m***, **1*p***, **2*m***, **2*p***, **3*m***, **3*p***) by click chemistry.



Scheme S2 The rhodamine tethered labeling reagent (**2m-Rh**) prepared from alkyne-type of LDT reagent (**2m**) by click chemistry.

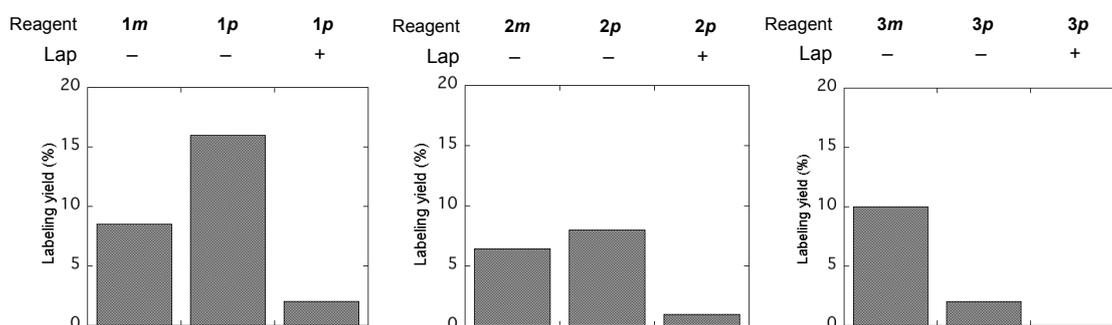


Figure S1 Reaction yield of HER2 kinase domain labeling by biotin tethered labeling reagents. The reaction was carried out with 100 nM HER2 kinase domain and 1 μ M labeling reagents in the presence or absence of 20 μ M Lap in HEPES buffer (100 mM HEPES, 5 mM $MgCl_2$, pH 7.5) at 25 $^{\circ}C$ for 10 h. The labeling yields were determined by using biotin-labeled bovine albumin (Sigma) as a standard marker.

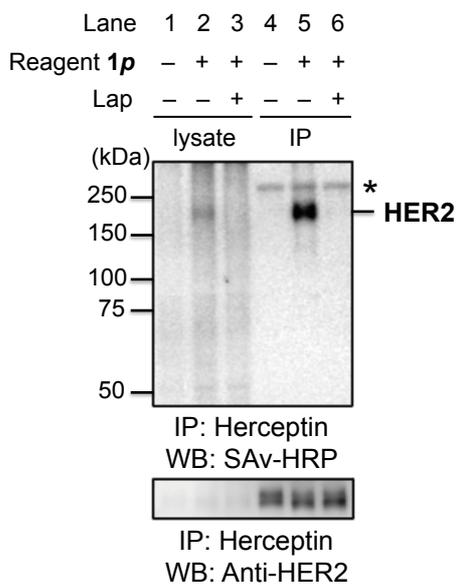


Figure S2 Confirmation of chemical labeling of HER2 by immunoprecipitation (IP) by HER2 antibody. After labeling reaction using 1 μ M **1p** in the presence or absence of 20 μ M Lap followed by biotin modification, the cell lysate was immunoprecipitated with human anti-HER2 antibody, Herceptin. The immunoprecipitate was analyzed by biotin blotting with SAV-HRP (upper) or western blotting with rabbit anti-HER2 antibody (lower). * indicates non-specific band derived from anti-HER2 antibody.

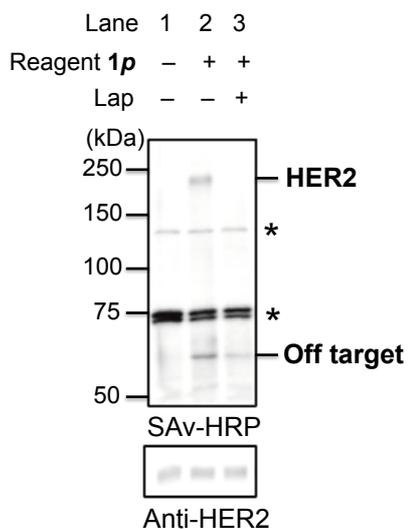


Figure S3 Chemical labeling of HER2 in live SK-BR-3 cells by **1p**. SK-BR-3 cells were treated with 300 nM **1p** in serum free DMEM at 37 °C for 8 h with or without 30 μM Lap. * indicates biotinylated proteins endogenously expressed in SK-BR-3 cells. Upper, biotin-blotting analysis. Lower, western blotting analysis using anti-HER2 antibody.

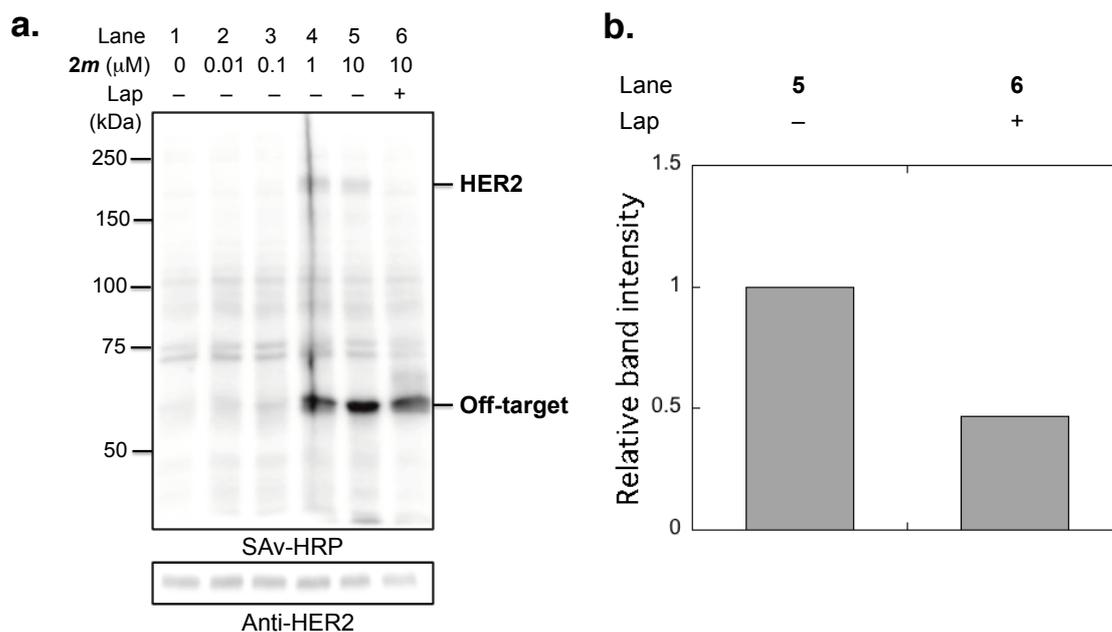


Figure S4 Concentration-dependent chemical labeling of HER2 and an off-target protein by **2m**. (a) NCI-N87 cells were treated with each concentration of **2m** in DMEM at 37 °C for 8 h with or without 30 μM Lap. Upper, biotin-blotting analysis. Lower, western blotting analysis using anti-HER2 antibody. (b) The relative band intensities of off-target labeling were determined from band intensity in Fig. S4a. Band intensity of off-target in lane 5 was used as the standard.

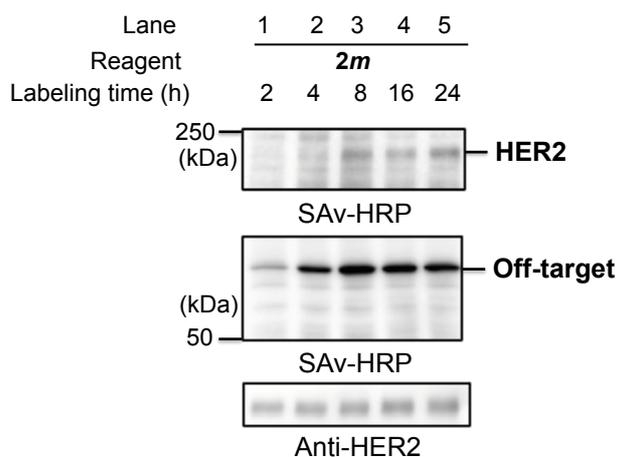


Figure S5 Time-dependent chemical labeling of HER2 and an off-target protein by **2m**. NCI-N87 cells were treated with 1 μ M **2m** in serum free DMEM at 37 °C for each time. Upper, biotin-blotting analysis for HER2. Middle, biotin-blotting analysis for off-target. Lower, western blotting analysis using anti-HER2 antibody.

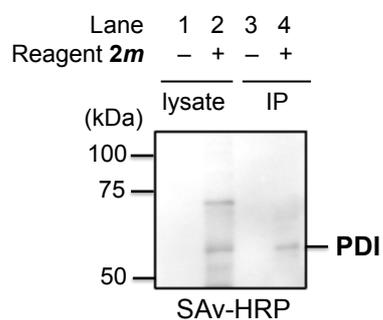


Figure S6 Confirmation of chemical labeling of PDI in live NCI-N87 cells by immunoprecipitation by PDI antibody. After labeling reaction using 5 μ M **2m** followed by biotin modification, the cell lysate was immunoprecipitated with human anti-PDI antibody. The immunoprecipitate was analyzed by biotin blotting with SAv-HRP.

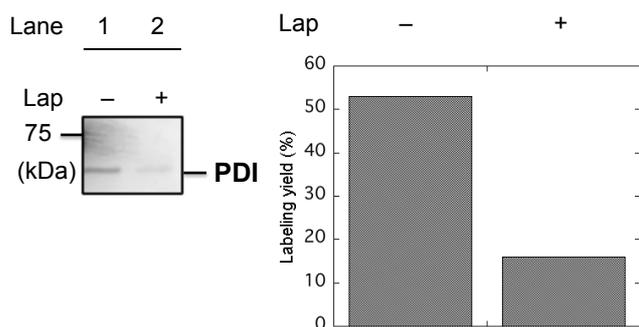


Figure S7 Inhibition of PDI labeling by Lap. The reaction was carried out with 220 nM PDI and 2.2 μM **2m-Rh** in HEPES buffer (50 mM HEPES, 3 mM DTT, pH 7.4) in the presence or absence of 20 μM Lap at 25 °C for 18 h. The labeling yields were determined by using rhodamine labeled bovine albumin as a standard marker.

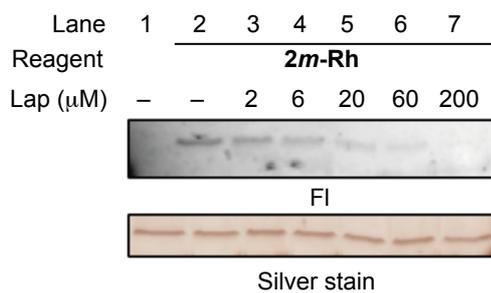


Figure S8 Concentration-dependent inhibition of PDI labeling by Lap. SDS-PAGE analysis of PDI labeling by in gel fluorescence imaging (FI, upper) and silver stain (lower). The reaction was carried out with 220 nM PDI and 2.2 μ M **2*m*-Rh** in HEPES buffer (50 mM HEPES, 3 mM DTT, pH 7.4) in the presence or absence of each concentration of Lap at 25 °C for 18 h.

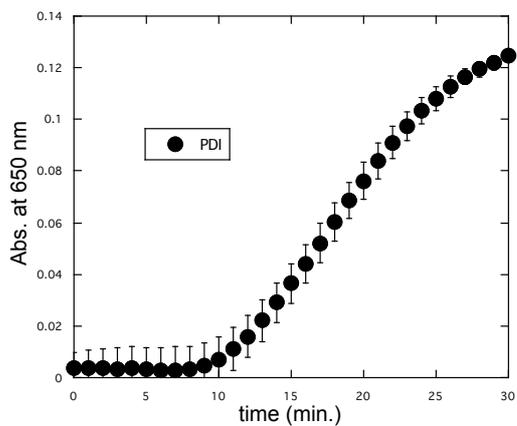


Figure S9 Time course of PDI reductase activity. Reductase activity was assessed by measuring the PDI-catalyzed reduction of insulin. In this assay, the aggregation of reduced insulin chains was measured using microplate reader at 650 nm. The experiments were performed in phosphate buffer (100 mM NaH_2PO_4 , 1 mM DTT, 2 mM EDTA, pH 7.0) containing 830 ng/ml PDI and 1.0 mg/ml bovine insulin.

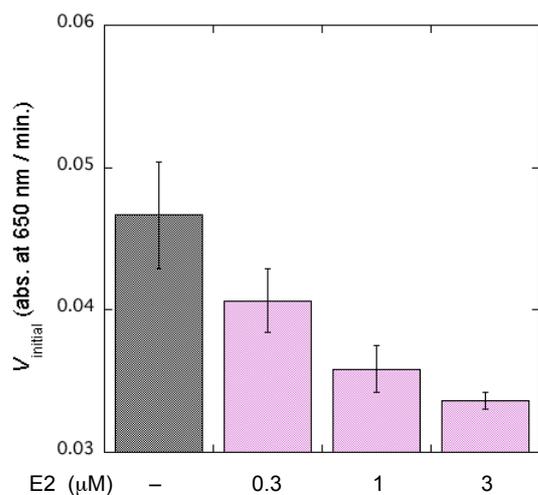


Figure S10 Effect of E2 on the initial velocity of PDI reductase activity. Reductase activity was assessed by measuring the PDI-catalyzed reduction of insulin (See Figure S9). The initial velocity was calculated from the slope of the linear part of the curve. The experiments were performed in phosphate buffer (100 mM NaH_2PO_4 , 1 mM DTT, 2 mM EDTA, pH 7.0) containing 830 ng/ml PDI and 1.0 mg/ml bovine insulin in the presence or absence of E2 at 25 °C.

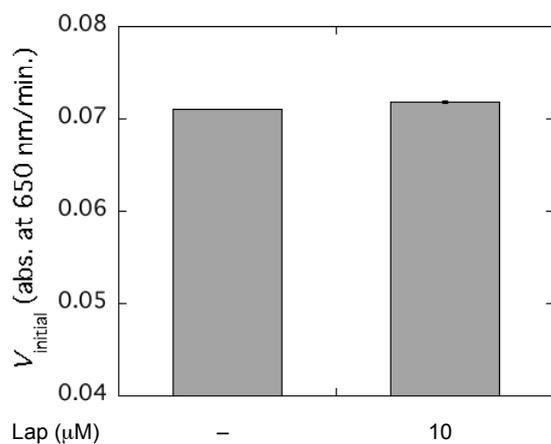


Figure S11 Effect of Lap on the initial velocity of PDI reductase activity. Reductase activity was assessed by measuring the PDI-catalyzed reduction of insulin (See Figure S9). The initial velocity was calculated from the slope of the linear part of the curve. The experiments were performed in phosphate buffer (100 mM NaH_2PO_4 , 1 mM DTT, 2 mM EDTA, pH 7.0) containing 830 ng/ml PDI and 1.0 mg/ml bovine insulin in the presence or absence of Lap at 25 °C.

Table S1 MSMS analysis of off-target for Lap.

Uniprot Accession	Score	coverage	Protein Mass	Num. of matches	Num. of significant matches	emPAI
PDIA1 HUMAN	7311	75%	57081	271	271	31.4
K1C10 HUMAN	525	*	58792	14	14	1.36
TBB5 HUMAN	466	34%	49639	20	20	1.97
TBB4B HUMAN	416	30%	49799	19	19	1.75
K2C1 HUMAN	461	*	65999	19	19	1.4
K22E HUMAN	322	*	65393	11	11	0.84
K2C5 HUMAN	116	*	62340	4	4	0.26
K1C9 HUMAN	335	*	62027	11	11	0.9
KPYM HUMAN	318	37%	57900	15	15	1.55
TBA1B HUMAN	280	32%	50120	10	10	1.05
TBA4A HUMAN	173	25%	49892	8	8	0.78
TMX3 HUMAN	254	26%	51839	11	11	1
CAPR1 HUMAN	158	10%	78318	7	7	0.38
SNX7 HUMAN	149	12%	45274	5	5	0.49
1433Z HUMAN	132	23%	27728	5	5	0.9
1433B HUMAN	79	*	28065	4	4	0.66
T2EA HUMAN	108	*	49421	6	6	0.55
NP1L1 HUMAN	93	*	45346	5	5	0.49
NP1L4 HUMAN	63	*	42797	3	3	0.29
RD23B HUMAN	93	*	43145	3	3	0.28
BASI HUMAN	92	*	42174	5	5	0.53
PSMD2 HUMAN	87	*	100136	2	2	0.08
ZPR1 HUMAN	87	*	50893	3	3	0.24
SQSTM HUMAN	83	*	47657	4	4	0.35
IMA7 HUMAN	74	*	59991	5	5	0.35
PDCD4 HUMAN	66	*	51703	3	3	0.23
TCPD HUMAN	66	*	57888	1	1	0.06
HEX11 HUMAN	66	*	40598	2	2	0.19
DBNL HUMAN	64	*	48178	3	3	0.25
GDIA HUMAN	62	*	50550	4	4	0.33
CALR HUMAN	58	*	48112	2	2	0.16
MCP HUMAN	54	*	43719	1	1	0.09
IMA4 HUMAN	51	*	57775	2	2	0.13
IMA3 HUMAN	44	*	57851	4	4	0.28
SCRN1 HUMAN	49	*	46353	1	1	0.08
RBBP4 HUMAN	49	*	47626	2	2	0.16
DCD HUMAN	48	*	11277	2	2	0.83
PRS4 HUMAN	48	*	49154	1	1	0.08
CH60 HUMAN	44	*	61016	2	2	0.13
HS90A HUMAN	37	*	84607	3	3	0.09
BICR2 HUMAN	37	*	56800	1	1	0.07
CASP8 HUMAN	37	*	55356	2	2	0.14
CE022 HUMAN	37	*	49935	1	1	0.07
MFAP1 HUMAN	35	*	51927	1	1	0.07
SFSWA HUMAN	33	*	104758	1	1	0.04
SRC8 HUMAN	33	*	61549	1	1	0.06
S4A4 HUMAN	32	*	121383	1	1	0.03
TCPH HUMAN	31	*	59329	1	1	0.06
FKBP4 HUMAN	29	*	51772	1	1	0.07
LRBA HUMAN	28	*	318906	1	1	0.01

* not determined

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