Supporting Information

Site-specific Bioconjugation and Convergent Click Chemistry Enhances Antibody-Chromophore Conjugate Binding Efficiency

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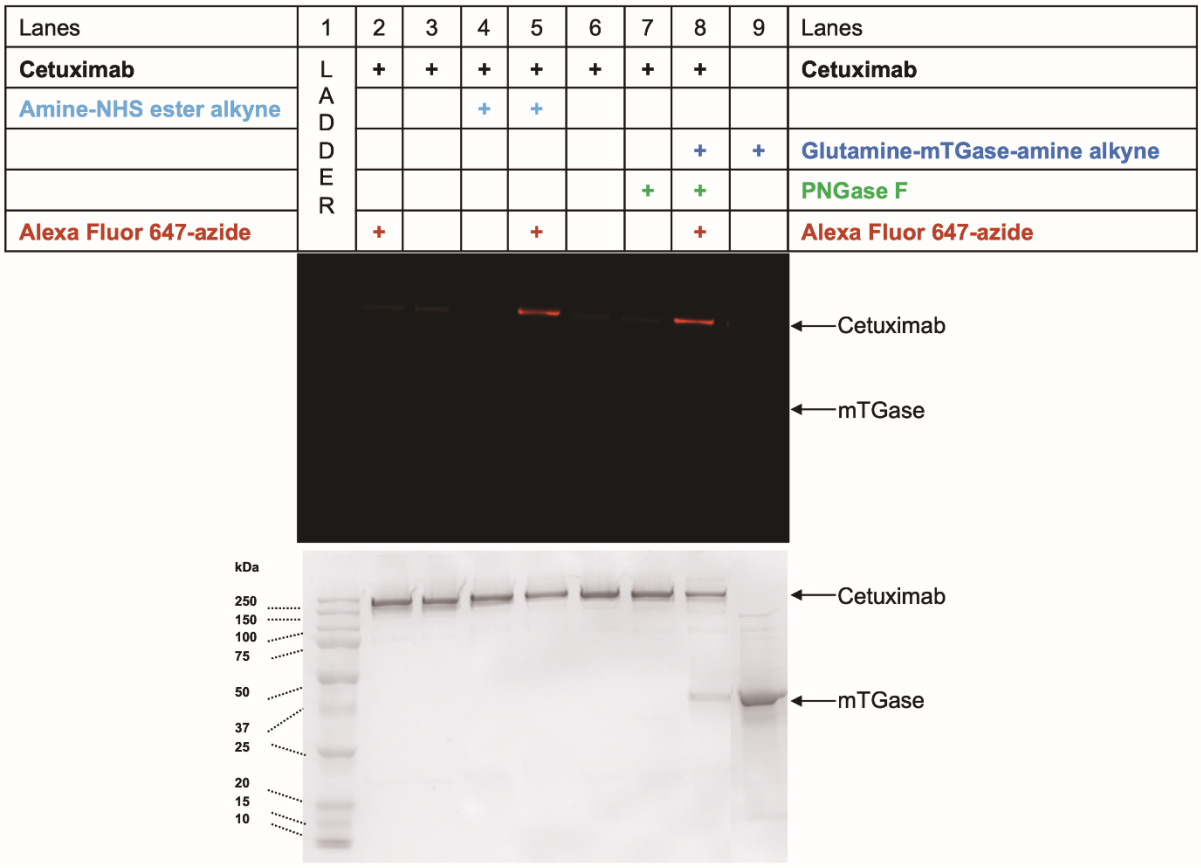

Figure S1. Characterization of antibody–chromophore conjugates by non-reducing SDS-PAGE. Lanes: (1) molecular weight standards; (2) cetuximab incubated with Alexa Fluor 647 azide at 37°C; (3) cetuximab; (4) cetuximab incubated with dibenzylcyclooctyne-PEG4-NHS ester (compound 2); (5) NHS-ester modified cetuximab incubated with Alexa Fluor 647 azide; (6) cetuximab; (7) cetuximab treated with PNGase F; (8) deglycosylated cetuximab incubated with transglutaminase and dibenzylcyclooctyne-PEG4-amine, and subsequently, Alexa Fluor 647 azide; and, (9) microbial transglutaminase (mTGase). The gel was assessed via fluorescence imaging (excitation 610–635 nm and emission 675–720 nm, top) and Coomassie staining (bottom).

Lanes	1	2	3	4	5	6	7	8	9	Lanes
Cetuximab	L	+	+	+	+	+	+	+		**Cetuximab**
Amine-NHS ester alkyne	A			+	+					
	D							+	+	**Glutamine-mTGase-amine alkyne**
	D						+	+		**PNGase F**
Alexa Fluor 647-azide	E R	+			+			+		**Alexa Fluor 647-azide**

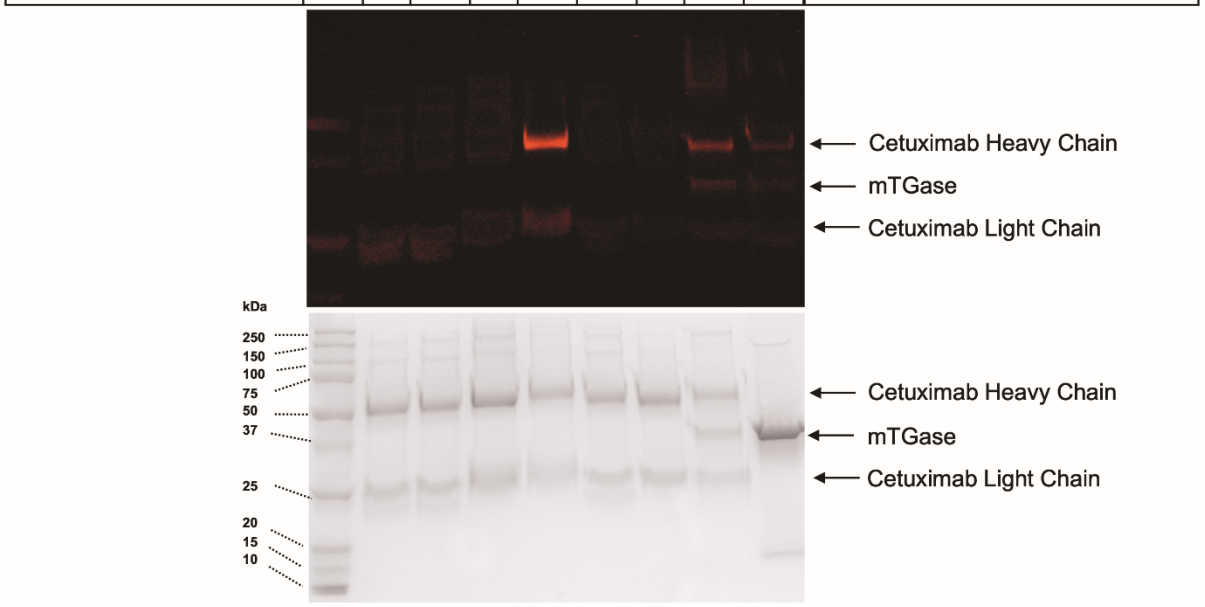

Figure S2. Characterization of antibody–chromophore conjugates by reducing SDS-PAGE. Lanes: (1) Molecular weight standards; (2) cetuximab incubated with Alexa Fluor 647 azide at 37°C; (3) cetuximab; (4) cetuximab incubated with dibenzylcyclooctyne-PEG4-NHS ester (compound 2); (5) NHS-ester modified cetuximab incubated with Alexa Fluor 647 azide; (6) cetuximab; (7) cetuximab treated with PNGase F; (8) deglycosylated cetuximab incubated with transglutaminase and dibenzylcyclooctyne-PEG4-amine, and subsequently, Alexa Fluor 647 azide; and, (9) microbial transglutaminase (mTGase). The gel was assesed via fluorescence imaging (excitation 610–635 nm and emission 675–720 nm, top) and Coomassie staining (bottom).

Lanes	1	2	3	4	5	6	Lanes
Cetuximab	LADDER	+	+	+	+		**Cetuximab**
Amine- NHS ester DBCO				+	+		
			+			+	**Glutamine- mTGase DBCO**
		+	+				**PNGase F**
Alexa Fluor 647-azide			+		+		**Alexa Fluor 647-azide**

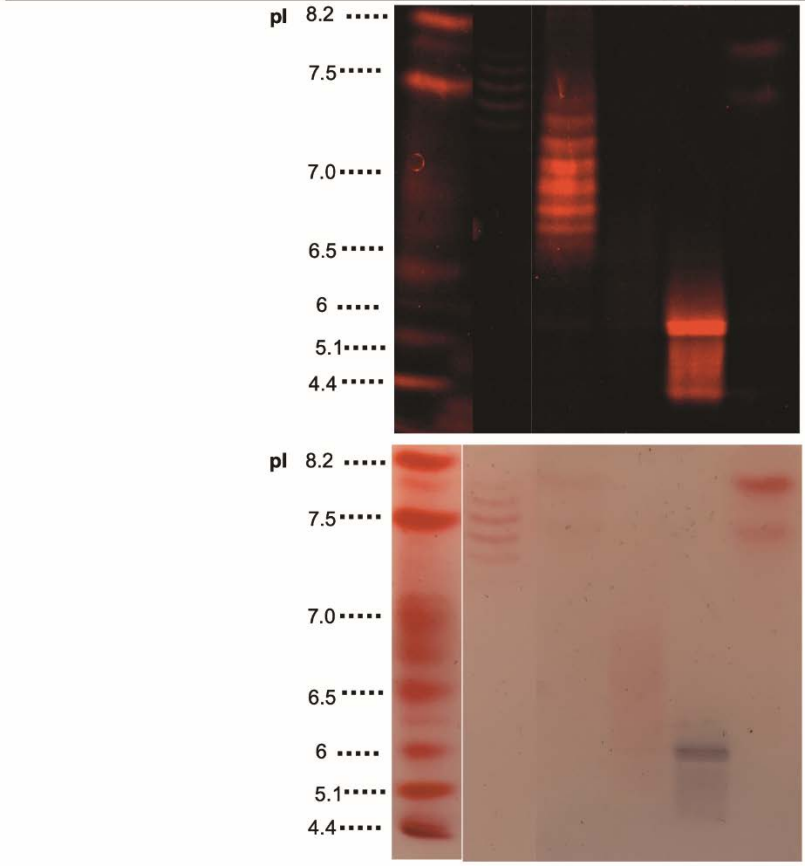

Figure S3. Characterization of antibody–chromophore conjugates by isoelectric focusing (IEF). Lanes: (1) Molecular weight standards; (2) cetuximab treated with PNGase F; (3) deglycosylated cetuximab incubated with transglutaminase and dibenzylcyclooctyne-PEG4-amine, and subsequently, Alexa Fluor 647 azide; (4) cetuximab incubated with dibenzylcyclooctyne-PEG4-NHS ester (compound 2); (5) NHS-ester modified cetuximab incubated with Alexa Fluor 647 azide; and, (6) microbial transglutaminase (mTGase). The gel was assessed with fluorescence imaging (top) and Coomassie and Crocien Scarlet staining (bottom).

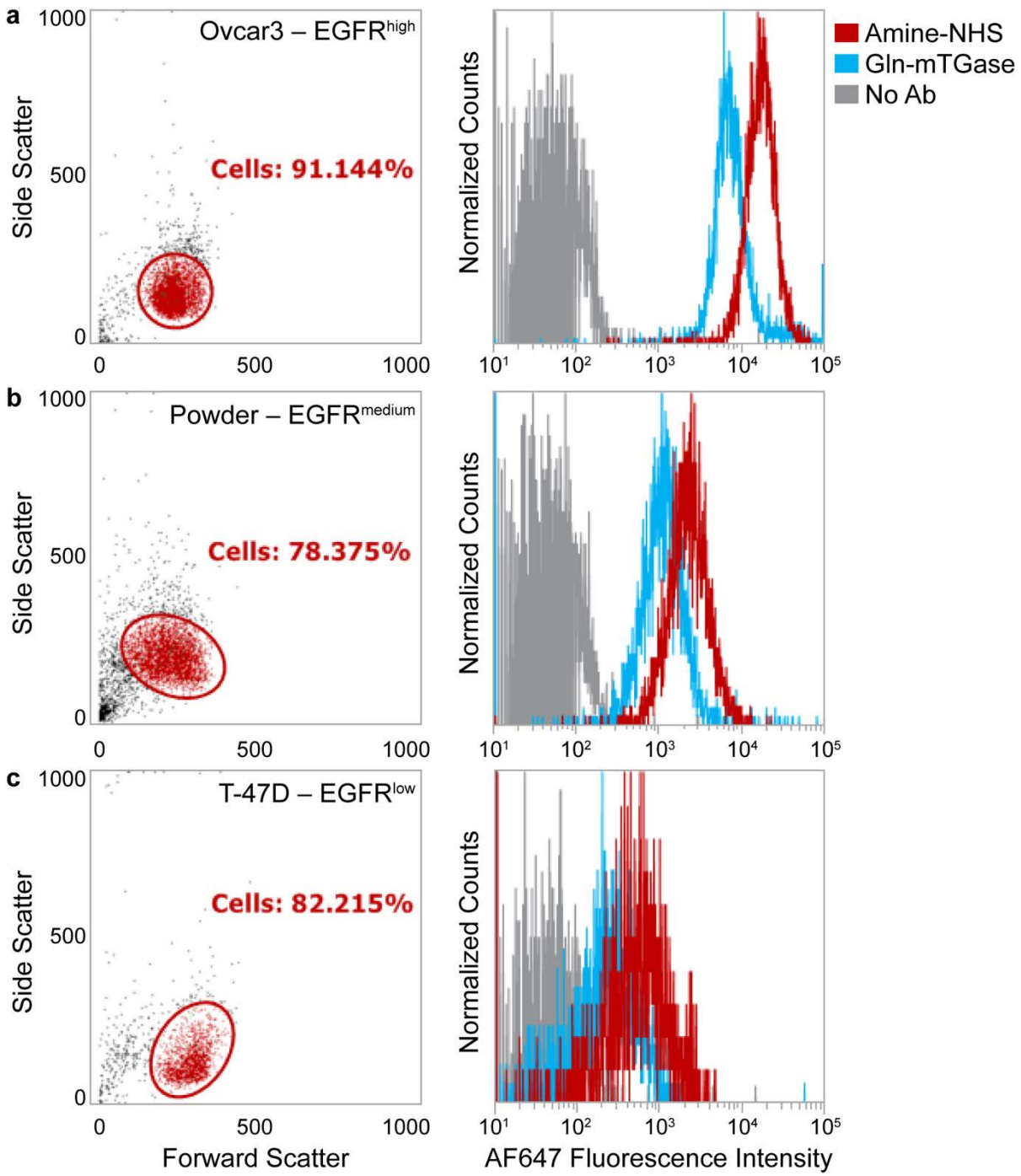

Figure S4. Flow cytometry analysis of (**a**) Ovcar3, (**b**) Powder, and (**c**) T-47D cancer cell lines stained with conjugates prepared with non-specific amine (amine-NHS) or site-specific enzymatic (Gln-mTGase) chemistries. An exemplary forward *vs*. side scatter plot (left) was used to isolate cell-like objects; a gate "Cells" was placed around this population. The percentage of events within the gate is inset. The AF647 fluorescence is then plotted as a histogram for only the events in the "Cells" gate (right). The overlay shows the distribution of fluorescence intensities of cells stained with each conjugate plus the no-stain control.

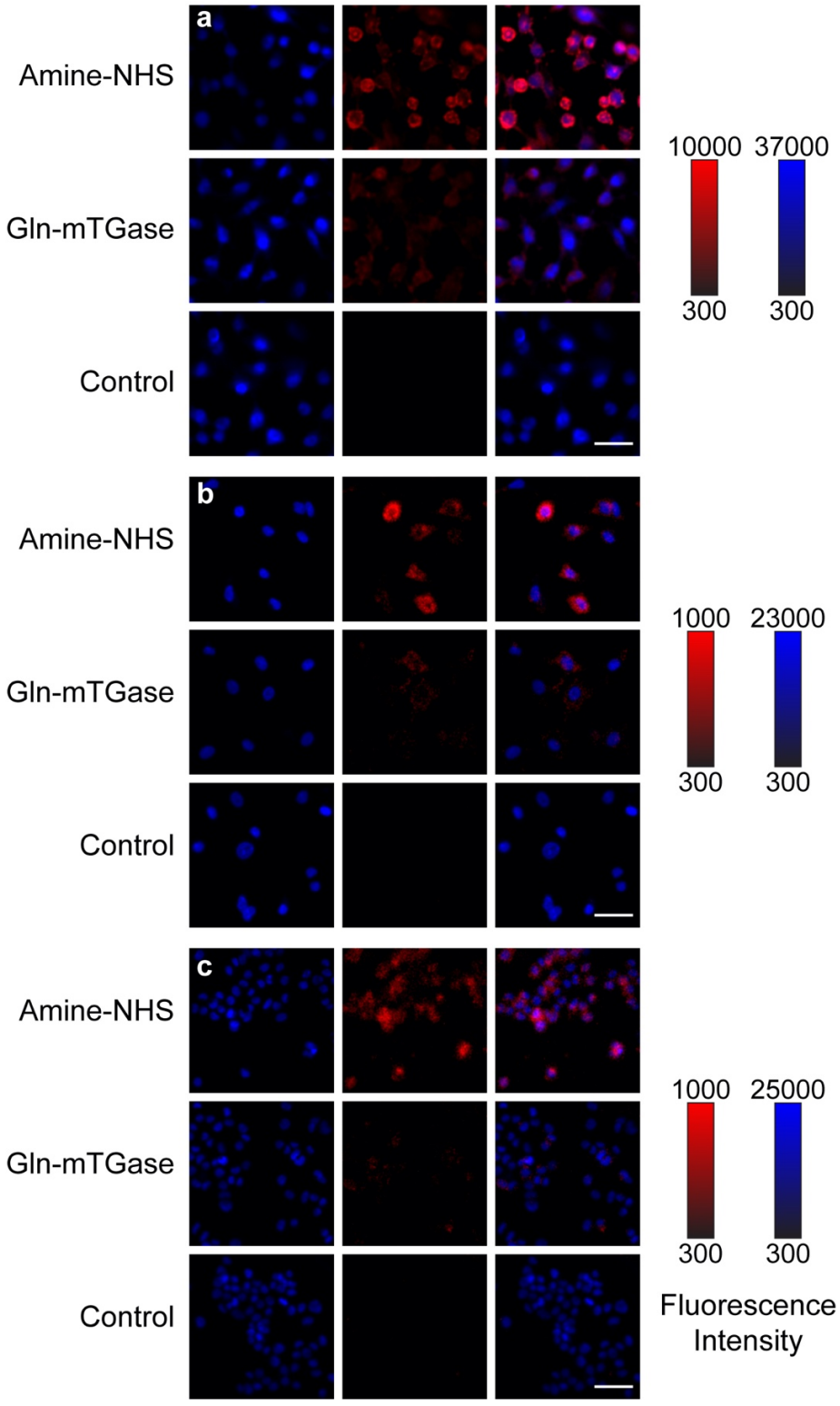

Figure S5. Confocal fluorescence microscopic images of (**a**) Ovcar3, (**b**) Powder, and (**c**) T-47D cell lines stained with Hoechst 33342 nuclear stain (left) and cet–AF647 conjugates (middle). Raw images are merged to reveal Ab uptake into the cytoplasm (right). For each cell line, AF647 fluorescence was collected with identical microscope settings (laser power, detector gain, *etc*.) and the images are displayed on the same scale. 16-bit fluorescence intensity scales of AF647 (red) and Hoechst 33342 (blue) are shown for each cell line. Scale bars, 50 μm.