Supporting Information

Antibody-Linked Fluorogen-Activating Proteins for Antigen Detection and Cell Ablation

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A

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B

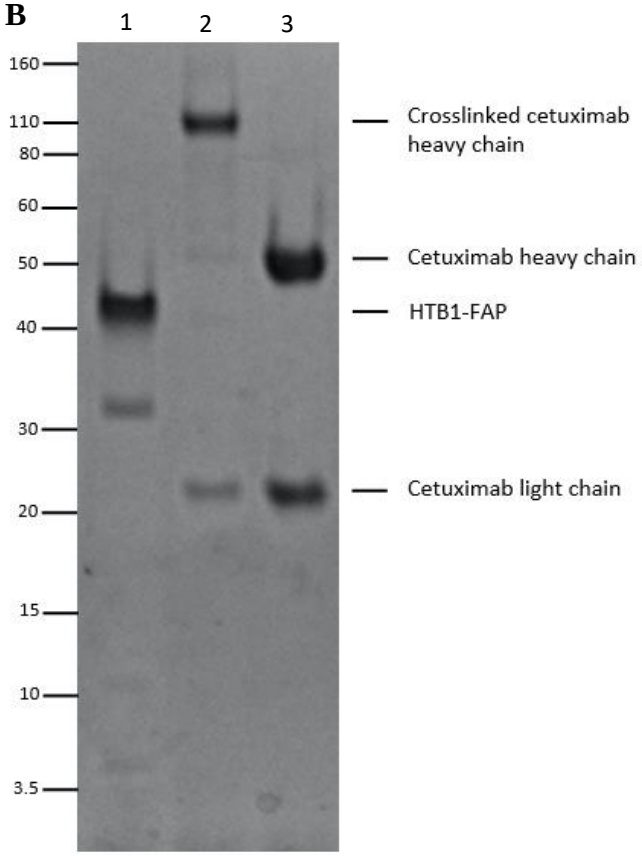

Figure S1. HTB1-FAP(dL5**) reagent conjugated to cetuximab. (A) Amino acid sequence of HTB1-FAP polypeptide (316 amino acids). The location of the p-benzoyl-L-phenylalanine (BPA) residue is indicated by an asterisk. (B) SDS PAGE showing crosslinked polypeptides. Lane 1: HTB1-FAP polypeptide. Lane 2: Crosslinked cetuximab. Lane 3: Cetuximab alone.

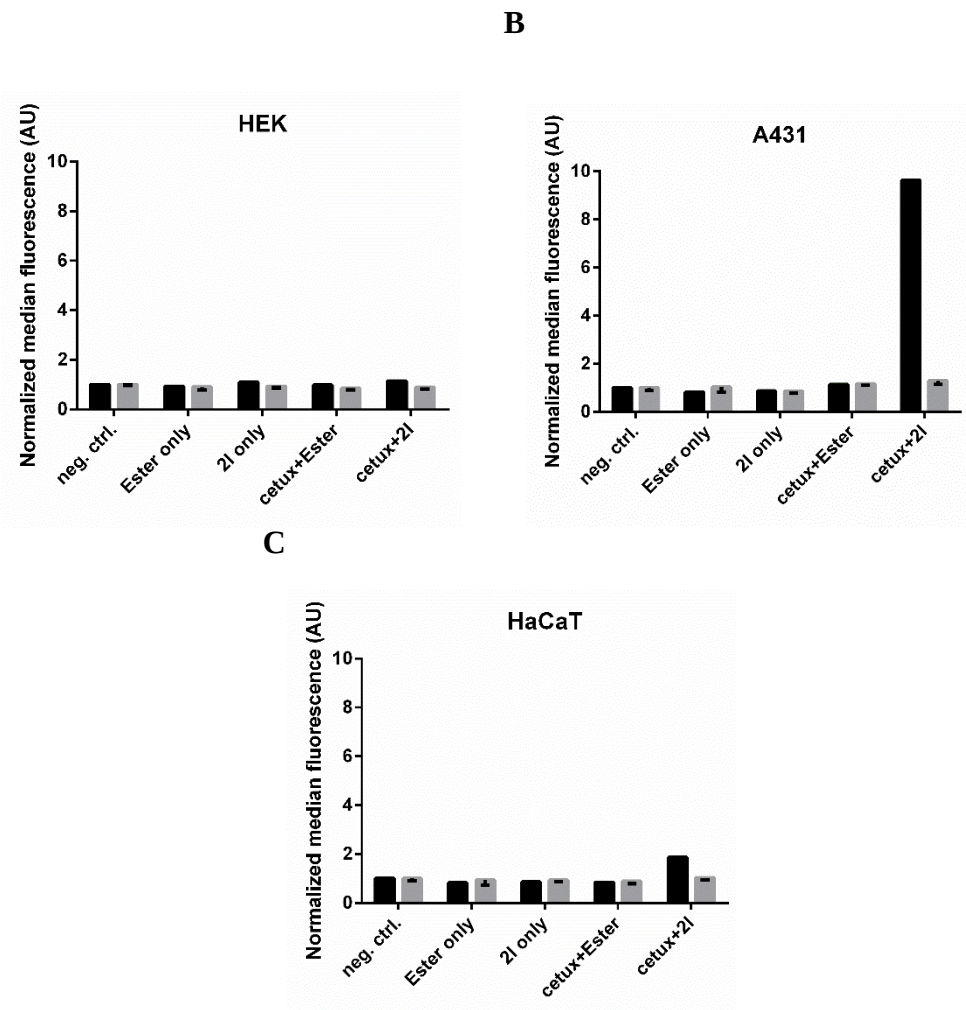

Figure S2. Flow cytometric analysis of cytotoxic response via Eth-D staining in HEK, HaCaT, and A431 cells after dL5**-cetuximab and MG-2I treatment and photoirradiation. Gray bars are from irradiated cells, and black bars are from non-irradiated cells. Neg. ctrl. = no antibody or dye. Ester/2I only = only fluorogen, no FAP/antibody. Cetux+Ester/2I = antibody and indicated fluorogen. Data from (A) HEK-293 cells, (B) HaCaT cells, and (C) A431 cells are shown. Numbers shown are the normalized average median FL2 signal (EthD-1 fluorescence) in arbitrary units normalized to the negative controls. Each bar is therefore the fold increase in EthD-1 signal over the negative control. Each bar is the average of three replicates $\pm$ SD.

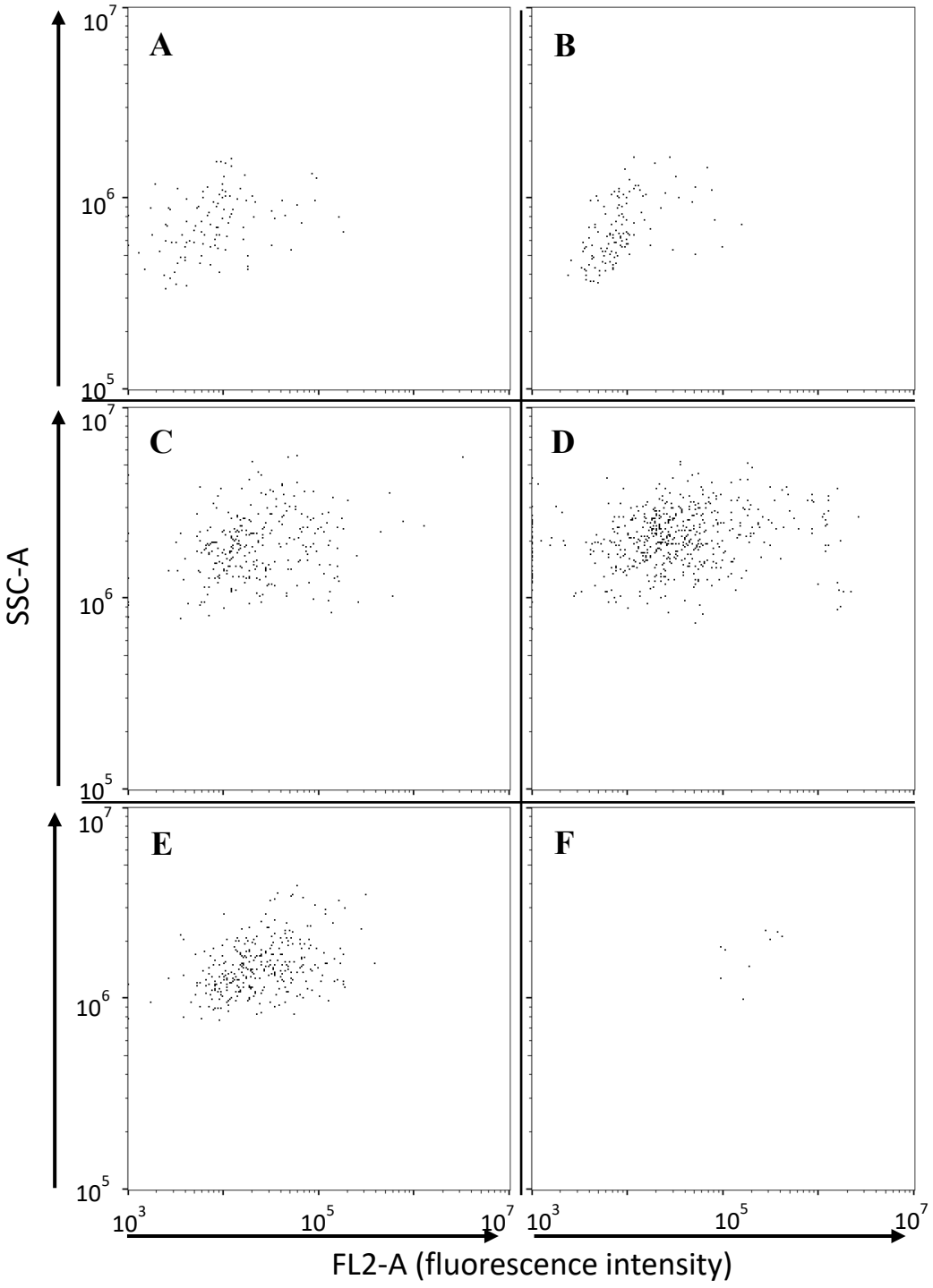

Figure S3. Representative dot plots from flow cytometry data shown in Figure S2. (A) and (B) HEK-293 cells. (C) and (D) HaCaT cells. (E) and (F) A431 cells. All cells shown were exposed to dL5**-cetuximab and the photosensitizing fluorogen MG-2I. Cells in the right column were irradiated with 640nm light, while cells in the left column were not irradiated. Axes are side scatter vs. fluorescence intensity in the FL-2 channel (both in arbitrary units). The FL-2 channel shows signal from the dead cell stain EthD-1.

Table S1. Total number of singlet cells analyzed per condition to generate the data shown in Figure S2. Irr. = cells irradiated at 640nm. Non-irr. = cells not irradiated. Neg. ctrl. = no antibody or dye. Ester/2I only = only fluorogen, no FAP/antibody. Cetux+Ester/2I = antibody and indicated fluorogen.

HEK-293 cells		
	Irr.	Non-irr.
Neg. ctrl.	551	314
Ester only	433	248
2I only	601	387
Cetux+Ester	534	331
Cetux+2I	455	392
HaCaT cells		
	Irr.	Non-irr.
Neg. ctrl.	1350	901
Ester only	1546	1317
2I only	1385	1451
Cetux+Ester	1644	1047
Cetux+2I	1779	857
A431 cells		
	Irr.	Non-irr.
Neg. ctrl.	1105	907
Ester only	946	1020
2I only	709	1089
Cetux+Ester	497	802
Cetux+2I	12	725