**Supporting Information**

# Cytotoxic conjugates of fibroblast growth factor 2 with monomethyl auristatin E for effective killing of cells expressing FGF receptors

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*DPR analysis by reverse-phase high-performance liquid chromatography*

Drug to protein ratio was analyzed by RP-HPLC, which allowed us to separate conjugate species containing a different number of the hydrophobic auristatin E moiety. Conjugates were eluted from the C18 column (Aeris PEPTIDE 3.6u XB-C18 250 x 4.6 mm; Phenomenex Inc., Torrance, CA, USA) with 35%–50% gradient of water-acetonitrile supplemented with 0.1% TFA. Absorption measurements at 280 nm was used for conjugate detection. Table S1 summarizes the yields of conjugation reaction calculated based on integration of peaks area.

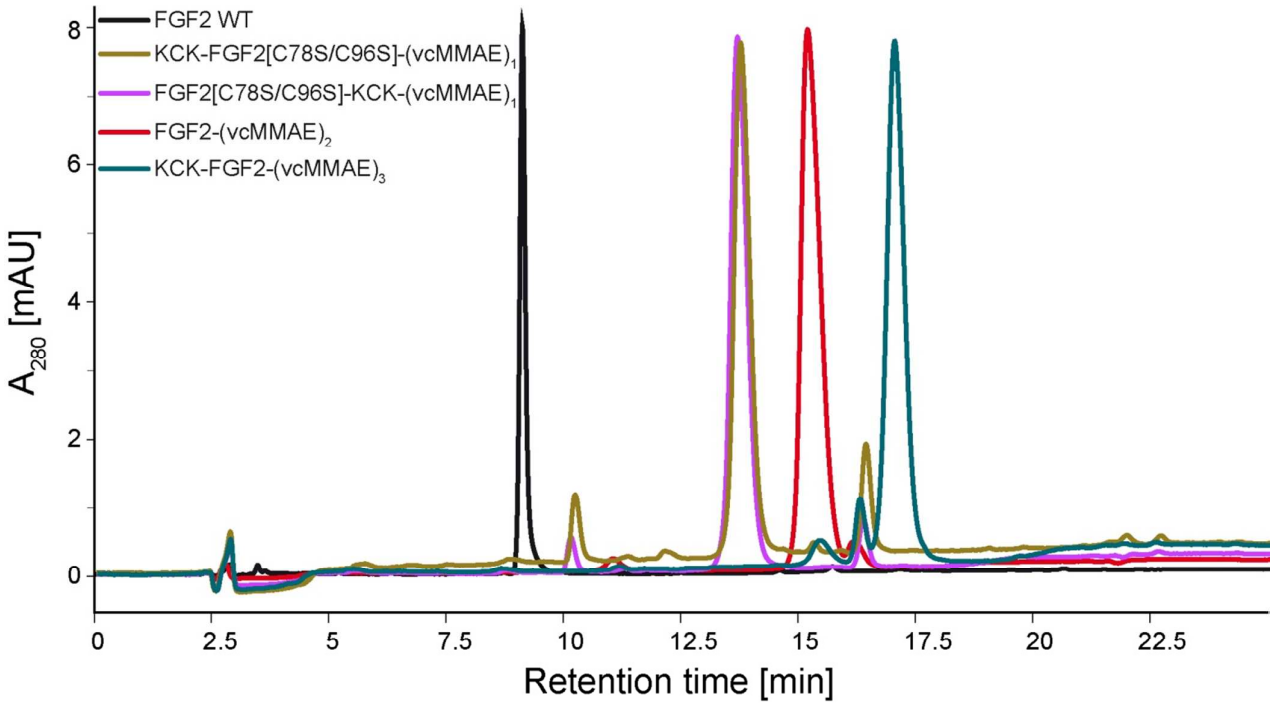


**Figure S1.** HPLC analysis of DPR in FGF2 conjugates.

**Table S1.** Conjugation yield calculated form HPLC analysis.

| **Conjugate** | Conjugation yield [%] |
|---|---|
| KCK-FGF2-$(vcMMAE)_3$ | 93 |
| FGF2 -$(vcMMAE)_2$ | 95 |
| KCK-FGF2[C78S/C96S]-$(vcMMAE)_1$ | 90 |
| FGF2[C78S/C96S]-KCK-$(vcMMAE)_1$ | 92 |

*Co-localization of FGF2 conjugates with lysosomes by structured illumination microscopy (SIM)*

U2OS-R1 cells grown on coverslips were incubated with 500 ng/mL of KCK-FGF2[C78S/C96S]-$(vcMMAE)_1$ or KCK-FGF2-$(vcMMAE)_3$ in DMEM medium supplemented with 10% FBS and 50 U/mL heparin at 37°C for 90 min and then fixed with 4% formaldehyde in PBS. The fixed cells were treated with 0.05% saponin for permeabilization and then incubated with primary antibodies: goat anti-FGF2 and anti-LAMP-1 (mouse monoclonal H4A3, Developmental Studies Hybridoma Bank), and subsequently incubated with secondary antibodies: donkey anti-goat coupled with Alexa Fluor 488 and donkey anti-mouse coupled with Alexa Fluor 568. The coverslips were mounted with ProLong Gold Antifade Mountant. Images were captured on a Deltavision OMX V4 microscope (GE Healthcare, UK), equipped with an Olympus 60x 1.42 NA Plan-Apochromat objective, and cooled sCMOS cameras, using 488 nm and 568 nm laserlines. Z-stacks were recorded with a z-spacing of 125 nm. For each focal plane, 15 raw images (five phases for three different angular orientations of the illumination pattern) were captured. SIM images were reconstructed and aligned using softWoRx software (GE Healthcare, UK), and further processed for illustrations in Fiji software.[67] Images shown are Maximum Intensity Projections of 9 (for KCK-FGF2[C78S/C96S]-$(vcMMAE)_1$) and 17 (for KCK-FGF2-$(vcMMAE)_3$) z-sections, respectively. Bar corresponds to 2 μm.

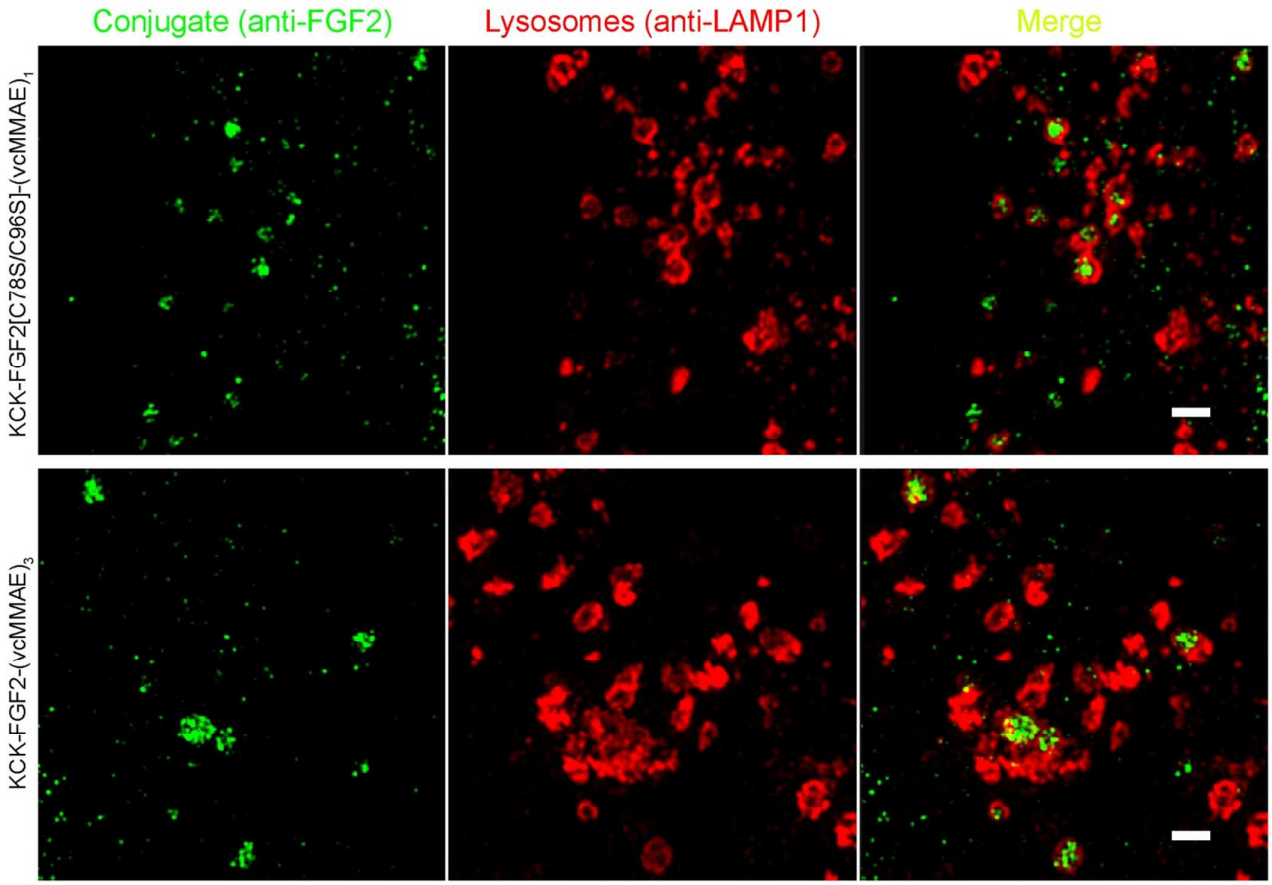


**Figure S2.** Co-localization of KCK-FGF2[C78S/C96S]-$(vcMMAE)_1$ and KCK-FGF2-$(vcMMAE)_3$ with lysosomes in U2OS-R1 cells visualized by structured illumination microscopy (SIM). The cells were stained with anti-FGF2 (green) and anti-LAMP-1 (red) antibodies. Bar corresponds to 2 μm.

*Quantification of FGFR1 level in U2OS-R1, BJ and cell lines*

An equal number of BJ, U2OS or U2OS-R1 cells were lysed with Laemmli sample buffer and sonicated. Total cell lysate was separated by SDS-PAGE and analyzed by Western blotting using the following antibodiesanti-FGFR1. 1:1000 dilution of primary antibodies was used. Specific protein bands were visualized with HRP-conjugated secondary antibodies and an enhanced chemoluminescence substrate using ChemiDoc station (BioRad, Hercules, CA, USA). Amount of FGFR1 protein was analyzed by densitometry. Intensity of bands corresponding to FGFR1 was quantified using Fiji software and normalized to receptor level in BJ cells.

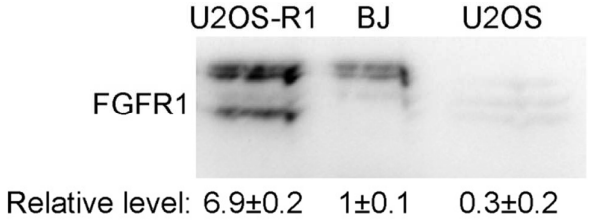


**Figure S3.** The FGFR1 expression level in BJ, U2OS and U2OS-R1 cell lines. An equal number of cells were separated by SDS-PAGE, analyzed by Western blotting and the bands corresponding to FGFR1 were quantified by densitometry. Data were normalized to the receptor level in BJ cells and are expressed as the mean ± SD of triplicate independent experiments.

*Flow Cytometry Analysis of binding of FGF2 and FGF2-conjugate to U2OS-R1, BJ and U2OS cells*

Flow cytometry measurements were performed for all three cell lines used in this study to assess a level of FGFR1 on cell surface accessible for FGF2 WT and FGF2 conjugate loaded with three vcMMAE molecules. FGF2 WT and KCK-FGF2-$(vcMMAE)_3$ were labeled with fluorescein. 1 μL of NHS-fluorescein at a concentration of 1 mg/mL in DMAc (*N,N*-dimethylacetamide) was added to 100 μL of FGF2 or conjugate at a concentration of 1 mg/mL in 50 mM monosodium phosphate, 10 mM $Na_2SO_4$, pH=7.8, and incubated for 1 h at room temperature in the dark. U2OS-R1, BJ and U2OS cells were incubated with 1 μg/mL of labeled ligand in DMEM medium supplemented with 10% FBS and 10 U/mL heparin for 15 min at 37 °C. Then, the cells were washed three times with DMEM medium supplemented with 10% FBS and 10 U/mL heparin and analyzed by flow cytometry using NovoCyte 2060R Flow Cytometer (ACEA Biosciences, San Diego, CA, USA). Data were evaluated with NovoExpress software (ACEA Biosciences, San Diego, CA, USA). U2OS-R1 cells exhibited much higher level of FGFR1 accessible to FGF2 or FGF2 conjugate as compared to BJ or U2OS cells. There is no difference in the binding profile between FGF2 and KCK-FGF2-$(vcMMAE)_3$ conjugate in tested cell lines.

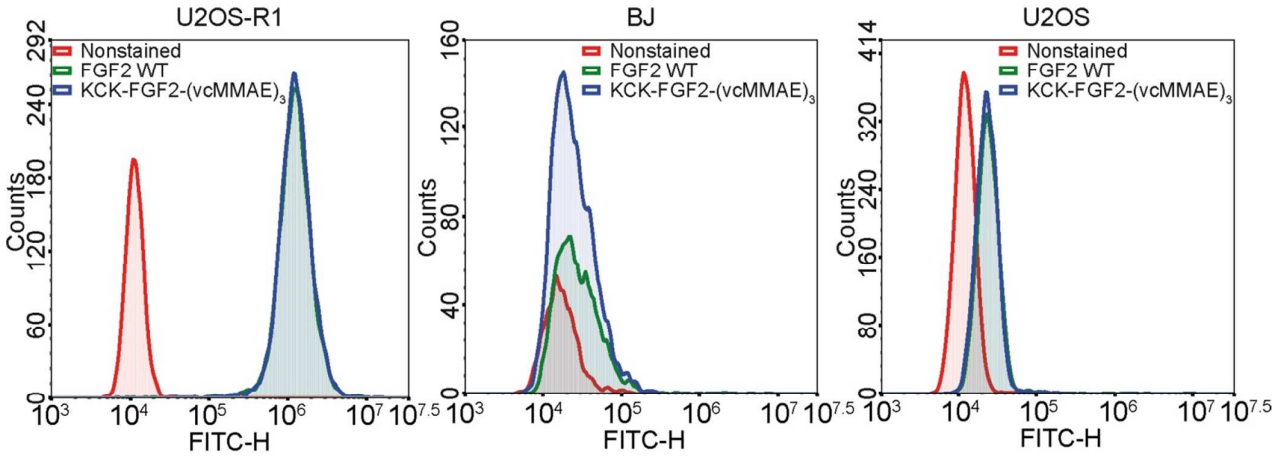


**Figure S4.** Binding of fluorescently labeled FGF2 WT or KCK-FGF2-(vcMMAE)$_3$ to U2OS-R1, BJ and U2OS cells analyzed by flow cytometry.