Supporting Information

Fibroblast Growth Factor 2 Conjugated with Monomethyl Auristatin E Inhibits Tumor Growth in a Mouse Model

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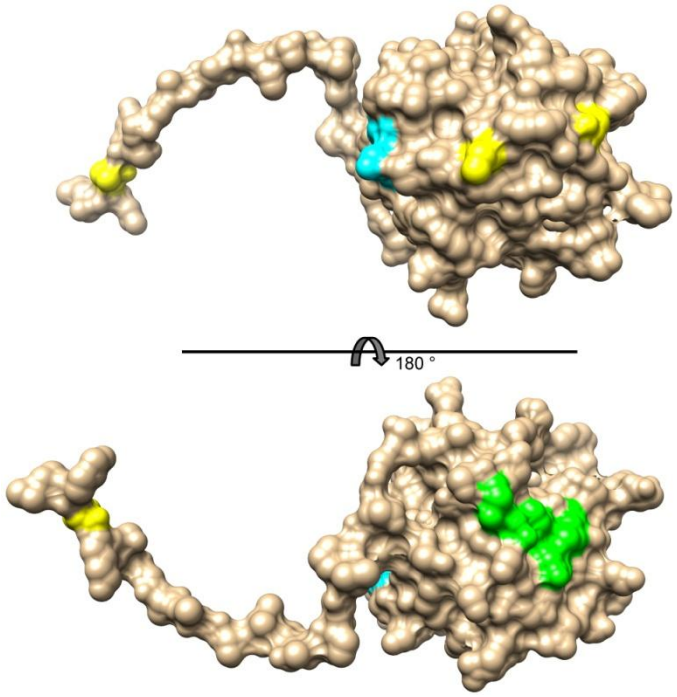

Figure S1. Spatial distribution of conjugation sites on the surface of FGF2. The structure of FGF2 with N-terminal extension of KCKSGG was predicted using the IntFOLD5 server and visualized by UCSF Chimera 1.15 software. Conjugation sites (surface-exposed Cys residues) are shown in yellow, primary FGFR1 binding sites in green, and secondary FGFR1 binding sites in cyan.

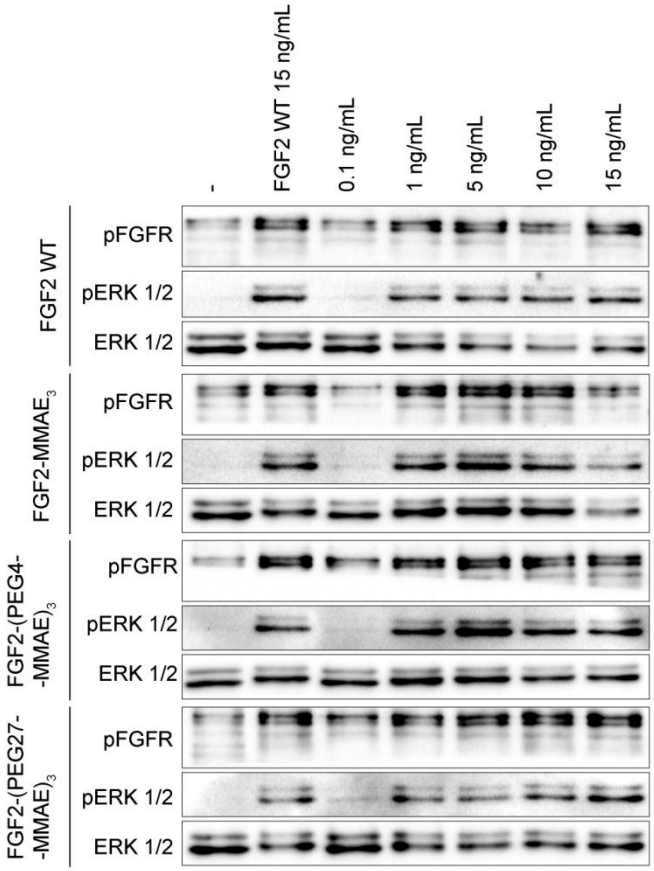

Figure S2. Analysis of concentration-dependent activation of downstream signaling in NIH 3T3 cells after 15 min stimulation with 0.1, 1, 5, 10, 15 ng/mL FGF2 WT or FGF2-based conjugates in the presence of 10 U/mL heparin detected by western blotting using anti-phospho-FGFR (pFGFR) and anti-phospho-ERK1/2 (p-ERK1/2) antibodies. Total ERK 1/2 served as a loading control.

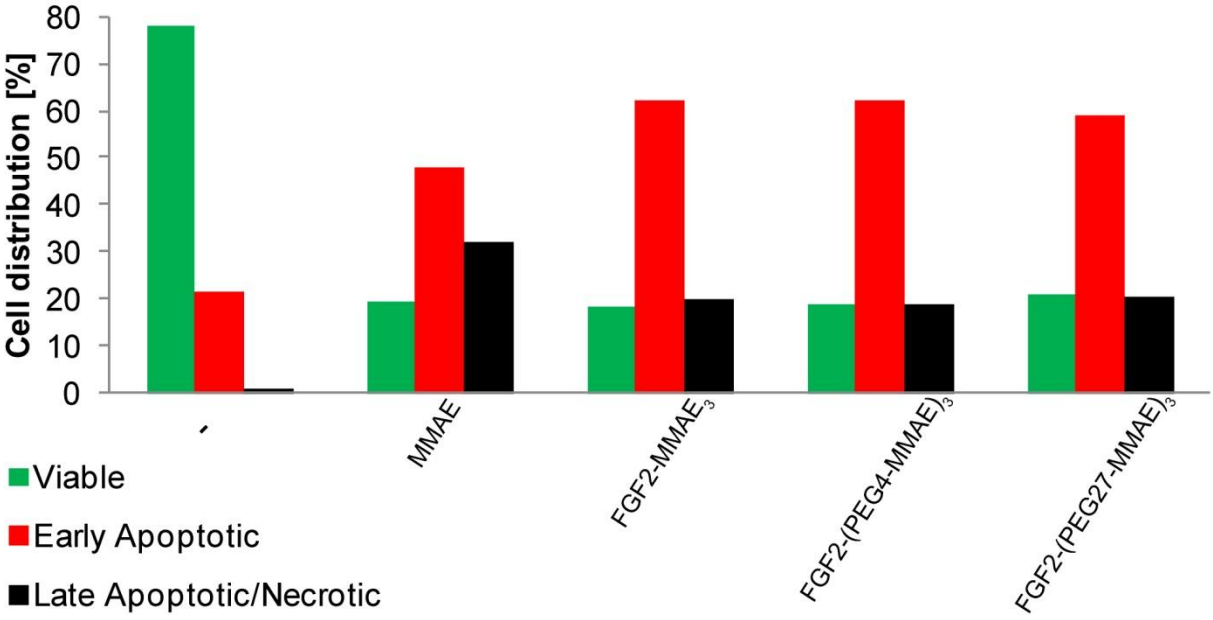

Figure S3. The mechanism of killing by MMAE and FGF2-based conjugates was verified by Annexin V and propidium iodide assay. MCF7-R1 cells were treated with 10 nM of MMAE, FGF2-$MMAE_3$, FGF2-$(PEG4\text{-}MMAE)_3$, or FGF2-$(PEG27\text{-}MMAE)_3$ for 72 h, then cells were stained with Annexin V-FITC and propidium iodide and then analyzed by flow cytometry.

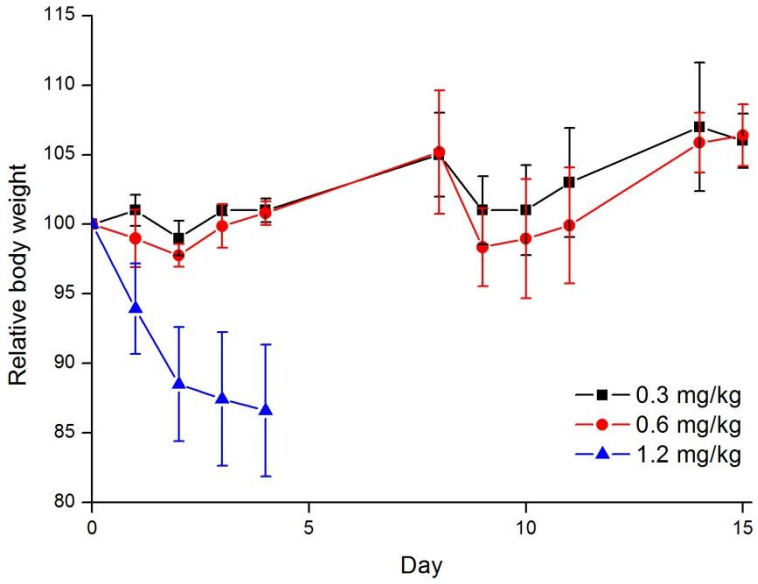

Figure S4. Determination of the maximum tolerated dose of free MMAE in NGC mice. Free MMAE was administered (i.v.) as a single dose (day 0) to three mice per group at a concentration of 0.3, 0.6 or 1.2 mg/kg body weight. Then body mass was monitored. A decrease in body weight greater than 15% resulted in termination of the experiment.