Supplementary Information

Title:

A Fas ligand (FasL)-fused humanized antibody against tumor-associated glycoprotein 72 (hcc49scFV-FasL) selectively exhibits the cytotoxic effect against oral cancer cells with a low FasL/Fas ratio

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Supplementary Materials and Methods

MMP7 proteolytic analysis

Recombinant hcc49scFV-FasL (20 μg) fusion or pro-form SPP1 (0.2 μg) proteins were resuspended in 50 μl of MMP7 cleavage buffer (200 mM NaCl, 50 mM Tris-HCl, 5 mM $CaCl_2$, pH=7.6) and incubated with matrix metalloproteinase-7 (MMP-7) (40 ng) at 37°C for 20 min. The mixtures were then subjected to Western blot analysis using FasL (Abcam, MA, USA) and SPP1 (GeneTex, Hsinchu, Taipei)-specific antibodies.

Supplementary Figures and Figure Legends

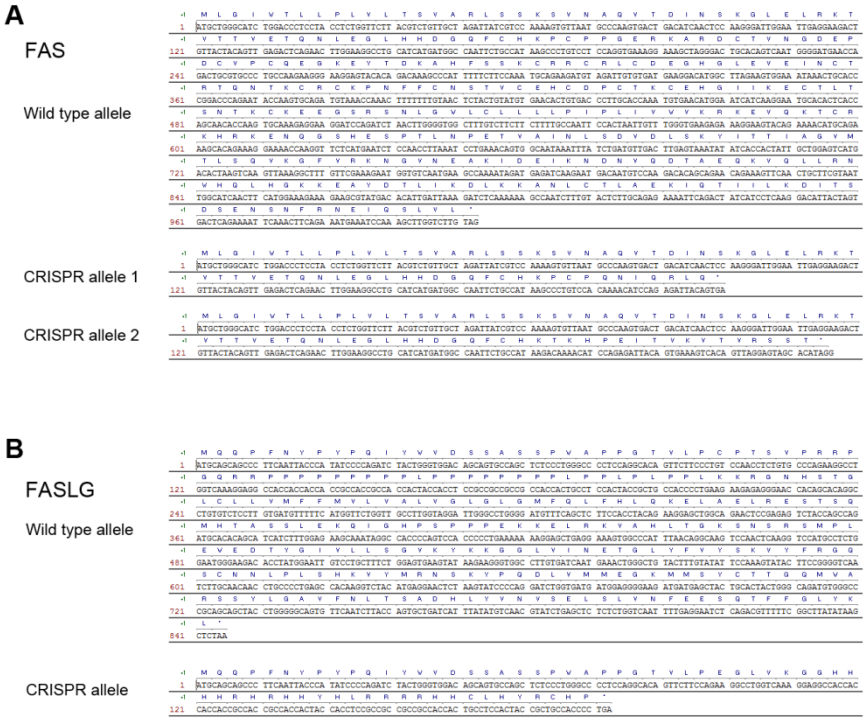

Figure S1. DNA sequence alignment of Fas or FasL Crispr knockout gene with their parent gene. Computer simulation of amino acid translation for Fas (A) and FasL (B) coding genes identified from genomic DNA sequencing of SAS and Cal-27 cells without or with Fas and FasL Crispr knockout, respectively. The symbol "*" denotes translational termination codon.

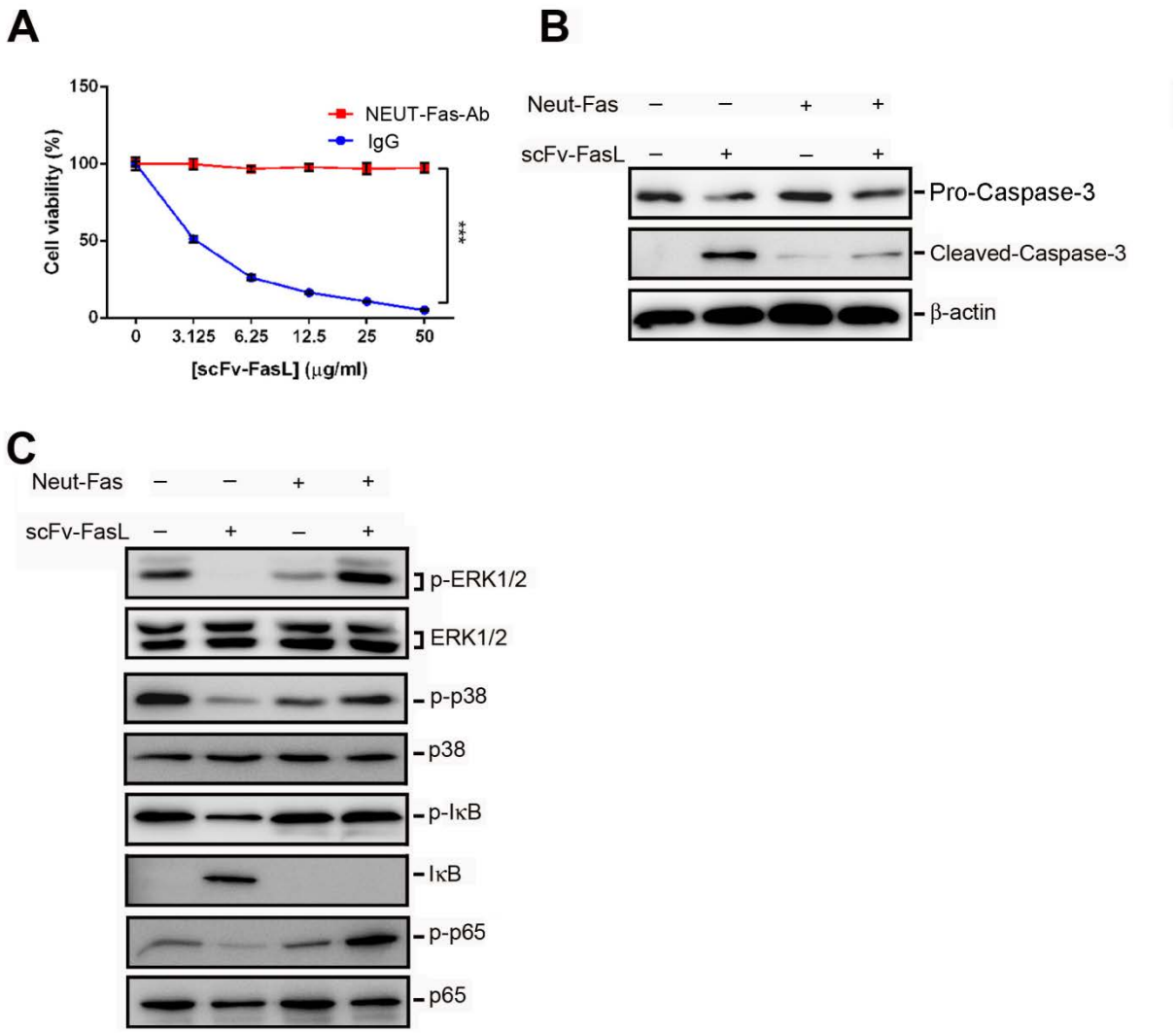

Figure S2. Cell viability, caspase-3 cleavage and the phosphorylation of ERK1/2, p38, I-κB and NF-κB in Cal-27 cells with Fas neutralization. Cell viability (A), caspase-3 cleavage (B) and the phosphorylation of ERK1/2, p38, I-κB and NF-κB (C) in Cal-27 cells treated with hcc49scFV-FasL at designated concentrations or 50 μg/ml for 24 hours in the presence of control IgG or Fas-blocking antibody.

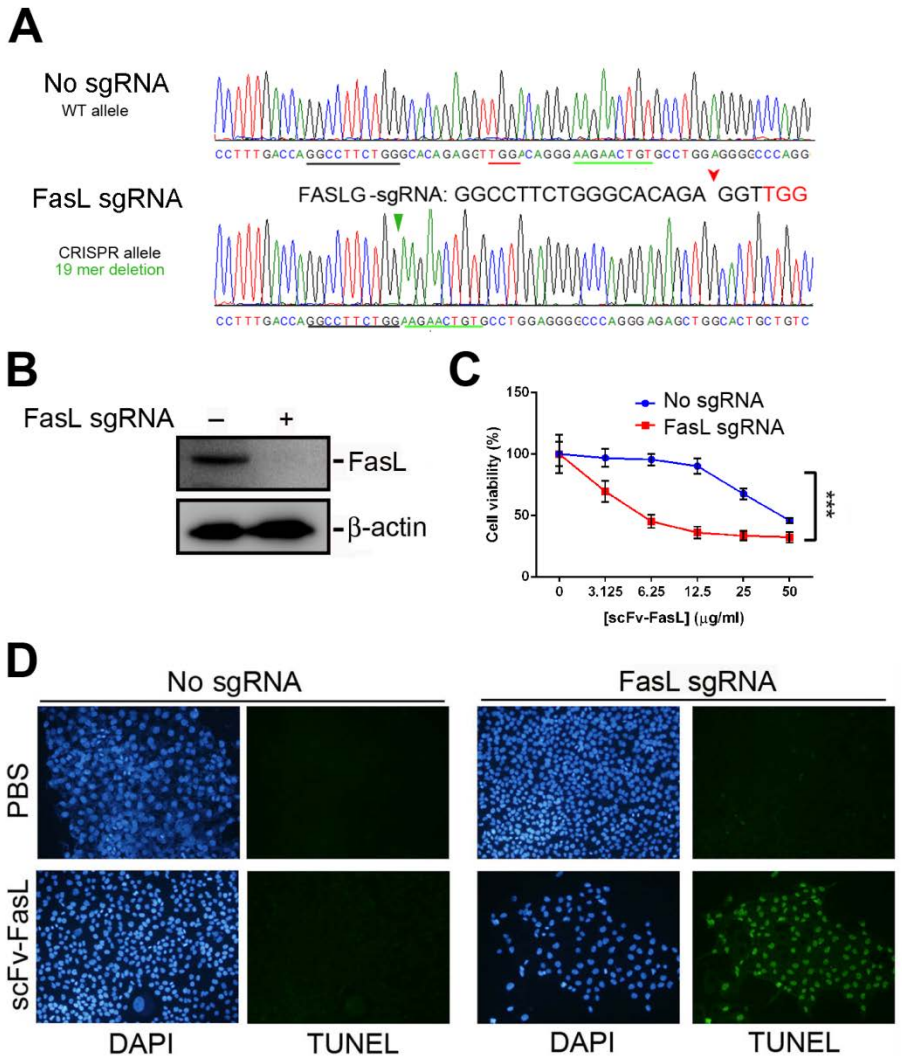

Figure S3. FasL Crispr knockout enhances the cytotoxic effectiveness of hcc49scFv-FasL on Cal-27 cells. (A) Genomic DNA sequencing results of Cal-27 cells without (No sgRNA) or with FasL (FasL sgRNA) Crispr knockout. The protospacer adjacent motif (PAM) sequence was marked in red and the red arrow indicated the cutting site of Crispr nuclease. The oligonucleotides underlined in green and black denote the original DNA sequences of FasL. (B) Western blot analysis for FasL and β-actin protein expression in Fas-sgRNA SAS cells. β-actin was used as an internal control of protein loading. (C) The killing effectiveness of hcc49scFv-FasL at various concentrations on parental (no-sgRNA) and FasL Crispr knockout (Fas-sgRNA) Cal-27 cells. Data from three independent experiments were shown in mean ± SE. Statistical differences were analyzed by one-way ANOVA with Tukey's post hoc tests and the symbol "***" represents $p < 0.001$. (D) TUNEL assay for Cal-27 cells without (no-sgRNA) or with Fas (Fas-sgRNA) Crispr knockout in the absence (PBS) or presence of hcc49scFv-FasL (50 μg/ml) treatment for 24 hours.

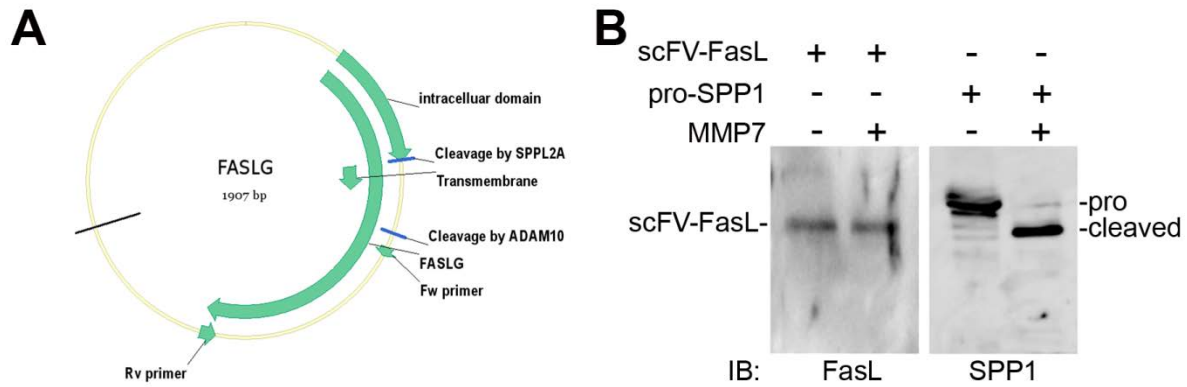

Figure S4. hcc49scFV-FasL fusion protein cannot be cleaved by ADAM10 and MMP-7. (A) The map of *FASLG* cDNA established from Vector NTI. The coding region is shown in the middle green arrow. The fusion FasL was cloned from the DNA sequence encoding the FasL extracellular domain which does not include the cleavage site of ADAM10 protease by using paired forward (Fw) and reversed (Rv) primers. (B) Recombinant proteins hcc49scFV-FasL (scFV-FasL) fusion and pro-form SPP1 (Osteopontin) were subjected to MMP7 proteolytic analysis. The proteolytic products were further analyzed by immunoblotting (IB) using FasL and SPP1-specific antibodies.