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Supporting information

Therapeutic effect of IL-4 receptor targeting pro-apoptotic peptide (AP1-ELP-KLAK) in glioblastoma tumor model

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Materials and methods:

Cell viability assay *in vitro*

D54/effluc and U87MG/effluc (1×10^4/well) cells were seeded in 96-well plates. After 24 hours, the cells were treated with ELP-KLAK and AP1-ELP-KLAK (2.5, 5, 10 μM) at 37 ºC for 4 h. Then, bioluminescence signals were obtained after adding 3 μL D-luciferin/well (30 mg/mL) using IVIS Lumina III.

Confocal microscopy

D54 and U87MG (4×10^4) cells were plated in 4 well-chambered slides. Next day, when the cells reached 70% confluency, 10 μM Alexa 488 labeled ELP-KLAK and AP1-ELP-KLAK were treated for 1 h at 4 °C and 37 °C. Cells were fixed with 4% paraformaldehyde, followed by cell membrane and nuclear staining with Wheat germ agglutinin Alexa Fluor 594 conjugate (Molecular Probes, Inc.) and Hoechst 33342 respectively. Confocal images were obtained and analyzed using a Zeiss LSM-510 Meta confocal microscope.

Apoptosis assay

D54 and U87MG (5×10^5/well) cells were seeded in 6-well plates. Next day, when the cell reached about 80 percent confluence, different concentrations of ELP-KLAK and AP1-ELP-KLAK (5, 10, 20 μM) were treated for 4 h. All the cells were harvested in PBS, washed thoroughly, and diluted with Annexin V binding buffer. Necrotic and apoptotic cells were stained by adding 5 μL of FITC Annexin V reagent, followed by Propidium iodide (PI). Annexin

V and PI-positive cells were measured using flow cytometer and analyzed using FlowJo software.

TUNEL staining

For ex vivo analysis of apoptosis in D54/effluc tumors, excised tumor tissue was fixed with 4% paraformaldehyde and rapidly frozen. Cryo-microtome derived tumor sections (5-μm thick) were subjected to TUNEL staining. Apoptotic cells were seen as strong green signal by deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) assay conjugated with FITC.

Cell Proliferation Assay

Cell proliferation assay were performed using a Cell Counting Kit (CCK)-8 (Dojindo Laboratories, Tokyo, Japan). D54, D54/effluc, U87MG and U87MG/effluc were seeded at 1 $x10^4$ cells per well in 96-well plates. 10 μL of CCK-8 solution was added to each well at 24 and 48 h then plates were incubated at 37 °C for 2 h. Absorbance was measured at 450 nm using a microplate reader (BMG Labtech, Offenburg, Germany).

MALDI TOF/TOF MS analysis

Molecular weights of ELP-KLAK and AP1-ELP-KLAK were examined using an UltrafleXtreme (Bruker). Protein samples were dissolved with 0.1% trifluoroacetic acid and mixed with an equal

volume of matrix solution (1:1). Mixture (1 µl) was then applied to a standard steel target for drying at room temperature. The MS spectra were obtained after calibration with standards.

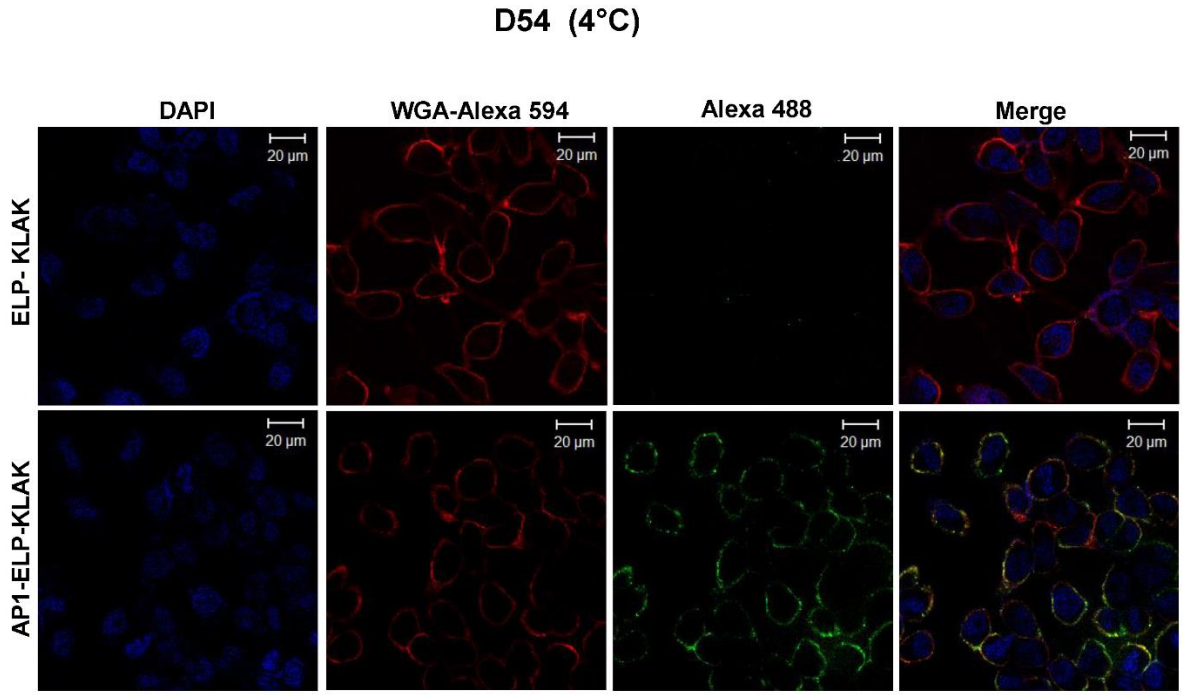

Figure S1. Cell binding activity of AP1-ELP-KLAK. D54 glioblastoma cells ($8x10^4$) was incubated with 10 µM of Alexa Fluor 488 labeled ELP-KLAK and AP1-ELP-KLAK (Green) for 1 h at 4 °C. The cells were then fixed with 4% paraformaldehyde and cell nuclei were stained with Hoechst 33342 (Blue), and cell membranes (Red) were stained with Wheat Germ Agglutinin Alexa Fluor 594. Cell binding activities of respective polypeptides was analyzed confocal microscopy. Scale bar, 20 µm.

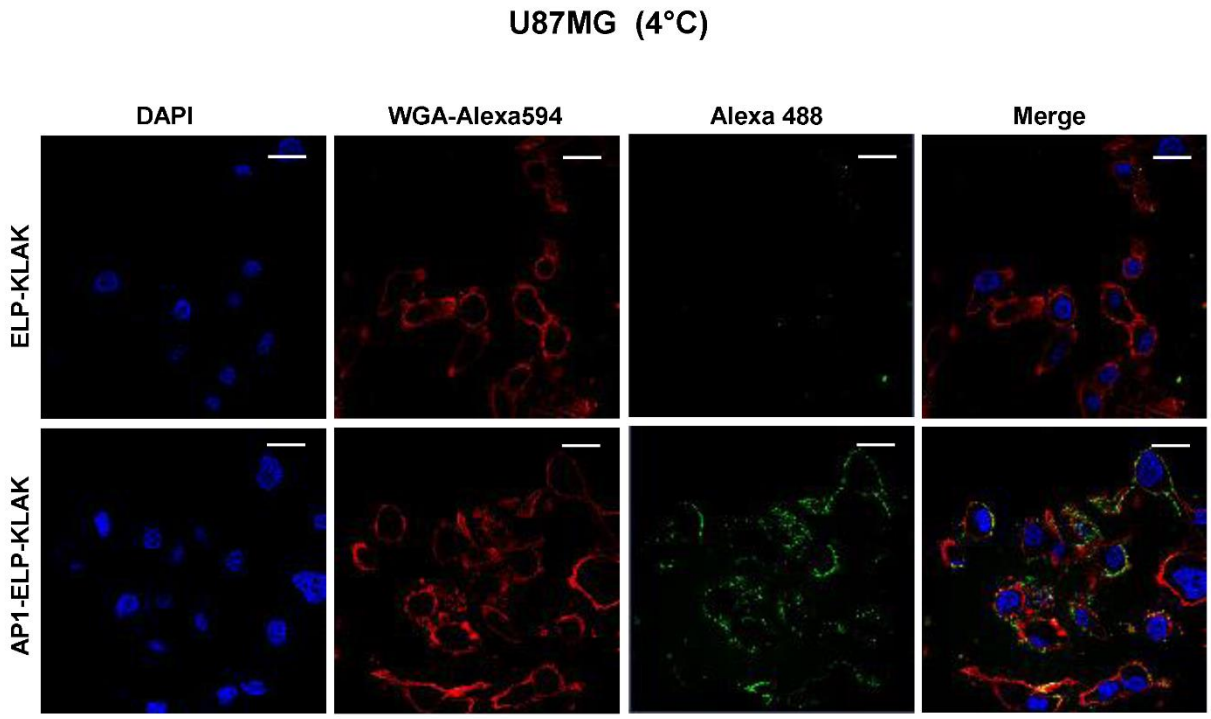

Figure S2. Cell binding activity of AP1-ELP-KLAK. U87MG glioblastoma cells ($5x10^4$) was incubated with 10 μM of Alexa Fluor 488 labeled ELP-KLAK and AP1-ELP-KLAK (Green) for 1 h at 4 °C. The cells were then fixed with 4% paraformaldehyde and cell nuclei were stained with Hoechst 33342 (Blue), and cell membranes (Red) were stained with Wheat Germ Agglutinin Alexa Fluor 594. Cell binding activities of respective polypeptides was analyzed confocal microscopy. Scale bar, 20 μm.

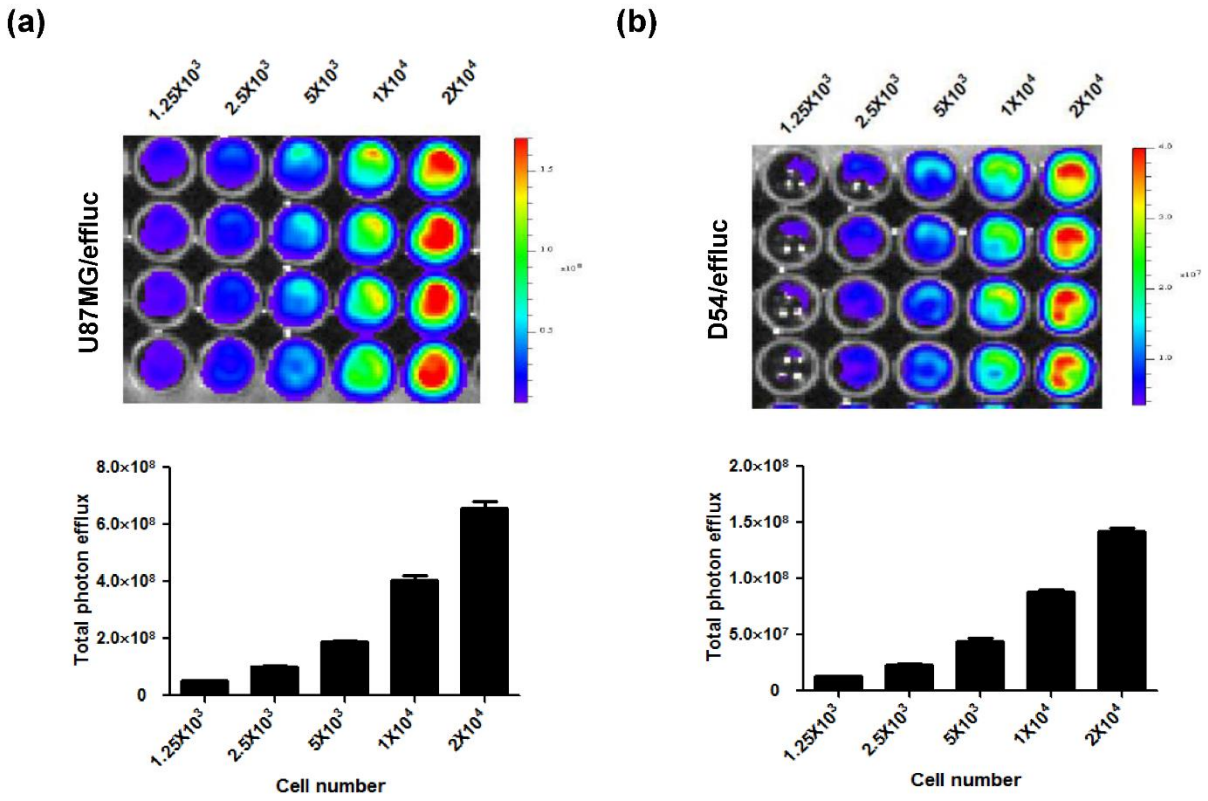

Figure S3. Luciferase assay. To confirm the stable expression of enhanced firefly luciferase (effluc) reporter gene, a specified number of (a) U87MG/effluc and (b) D54/effluc cells were seeded in 96-well black plates. After 24 h incubation, each well was supplemented with 3 µL D-luciferin and then the bioluminescence signals were measured using an IVIS Lumina III in vivo imaging system.

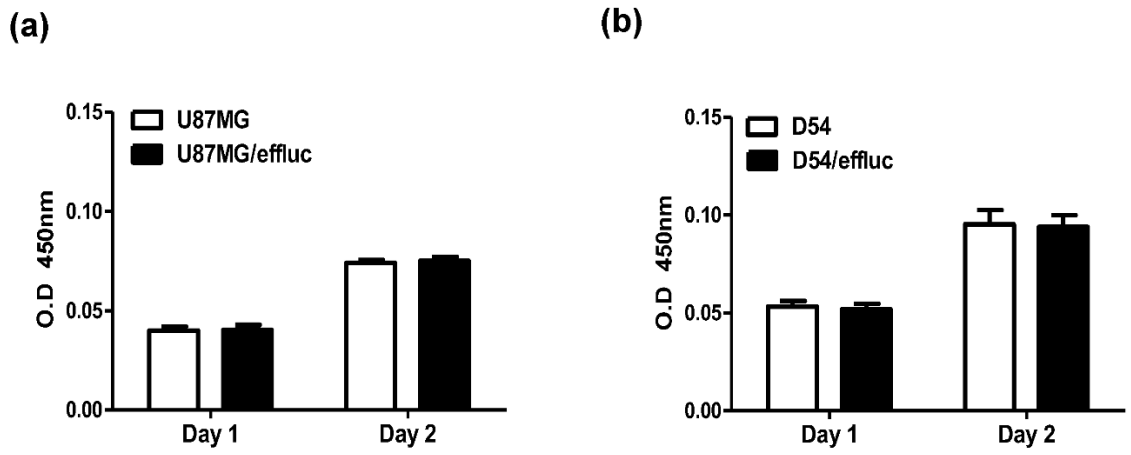

Figure S4. Cell proliferation assay. (a-b) The stable D54/effluc and U87MG/effluc cells and respective parental D54 and U87MG cells of equal numbers ($3X10^3$) were seeded in 96 well plates. Cellular viabilities were assessed by measuring WST-8 absorbance at 450 nm (n=5).

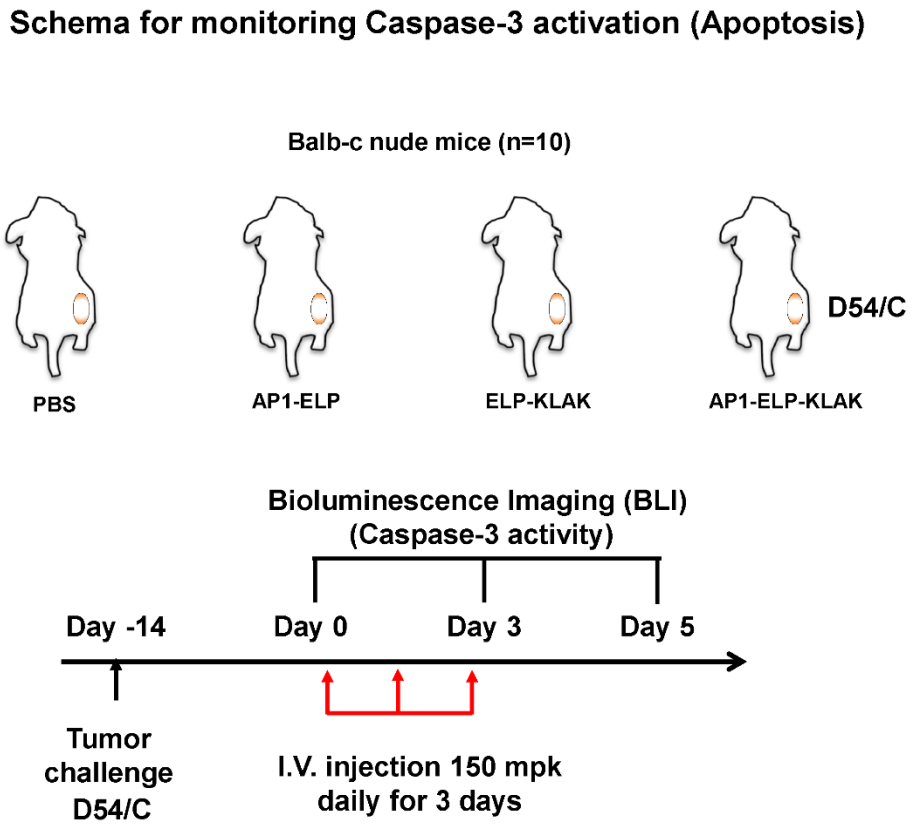

Figure S5. Schematic representation of monitoring caspase 3-activation. D54-C cells ($5x10^6$) were subcutaneously implanted into the right hind flanks of nude mice (n=30). When tumor formation was detected, the mice were divided into groups in each group: a control group for ELP treatment and 2 therapy groups (ELP-KLAK and AP1-ELP-KLAK respectively) treatment. Treatments were initiated by daily IV injection of respective proteins (150 mg kg−1) for 3 days.

After injection, BLI was acquired to monitor caspase-3 activation at the indicated times (day 0 and day 3, day 5) using an IVIS Lumina III.

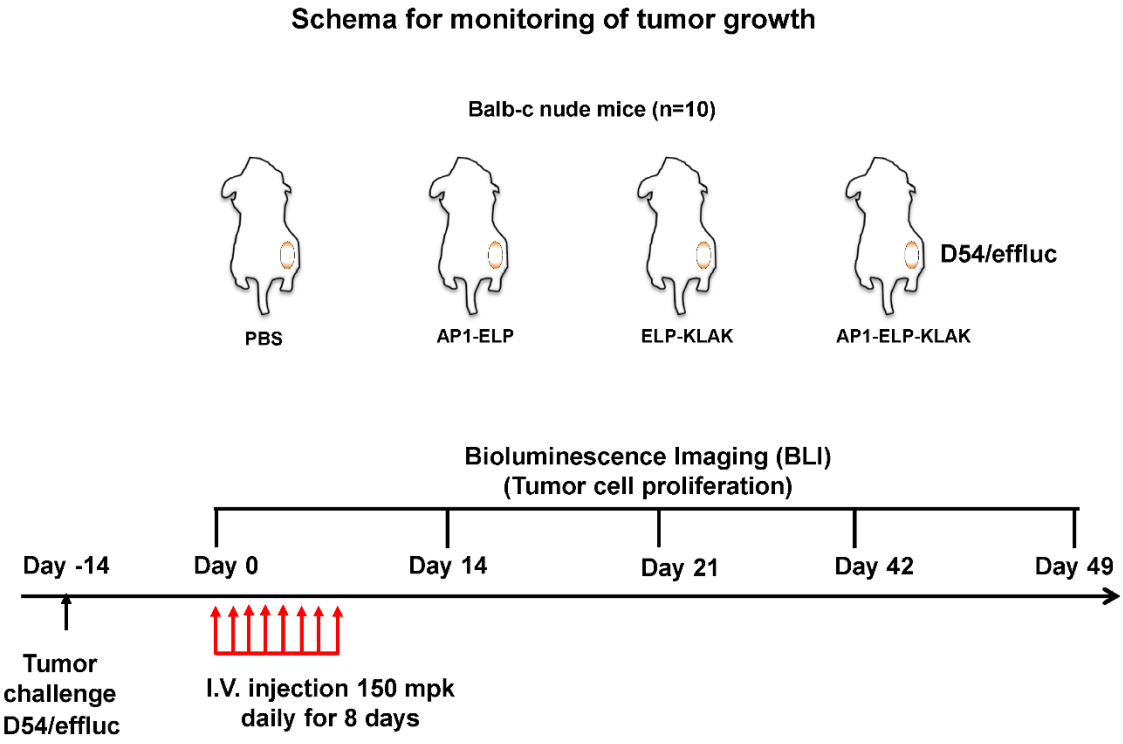

Figure S6. In vivo therapy. Female BALB/c nude mice were implanted with D54/effluc cells, when tumor size reached a volume of ~50 - 100 mm3, mice were divided into four groups, 2 control groups: PBS and ELP only and 2 therapy groups: ELP-KLAK and AP1-ELP-KLAK respectively (n = 15 per group). Treatments were initiated by daily IV injection of respective proteins (150 mg kg−1) or PBS as a control for 8 days. Before and during therapy, BLI was acquired to monitor cellular viability for therapeutic response at the indicated times.

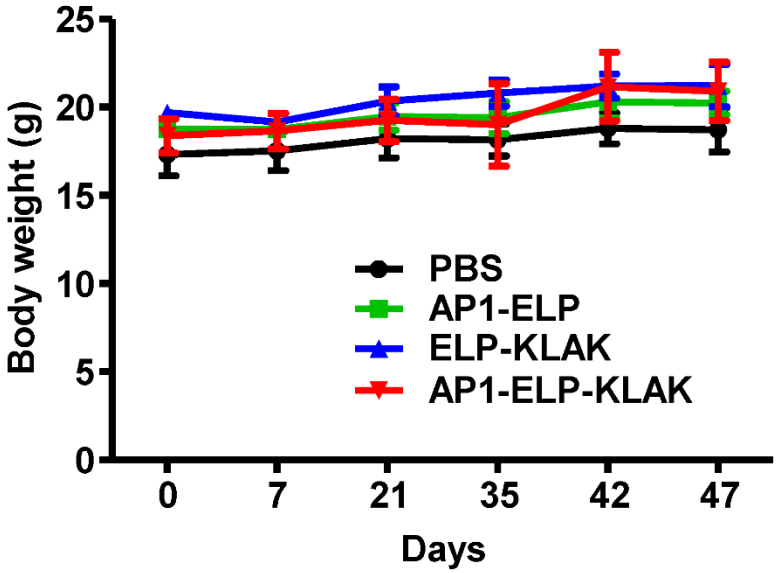

Figure S7. Female BALB/c nude mice were implanted with D54/effluc cells and treatments were initiated by daily IV injection of respective proteins (150 mg kg−1) or PBS as a control for 8 days. Before and during therapy, the body weight of the mice was measured at the indicated times to monitor toxicity effect.