Supplementrary materials

Identification of novel epitopes with agonistic activity applicable to developing immunotherapy targeting TRAIL-R1

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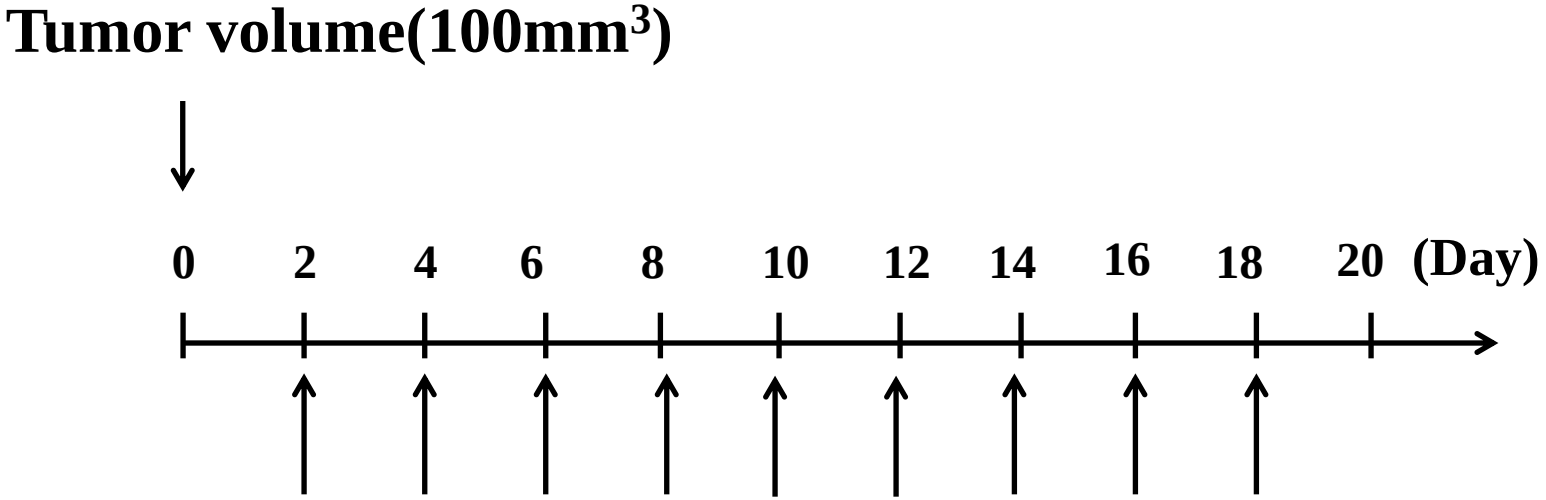
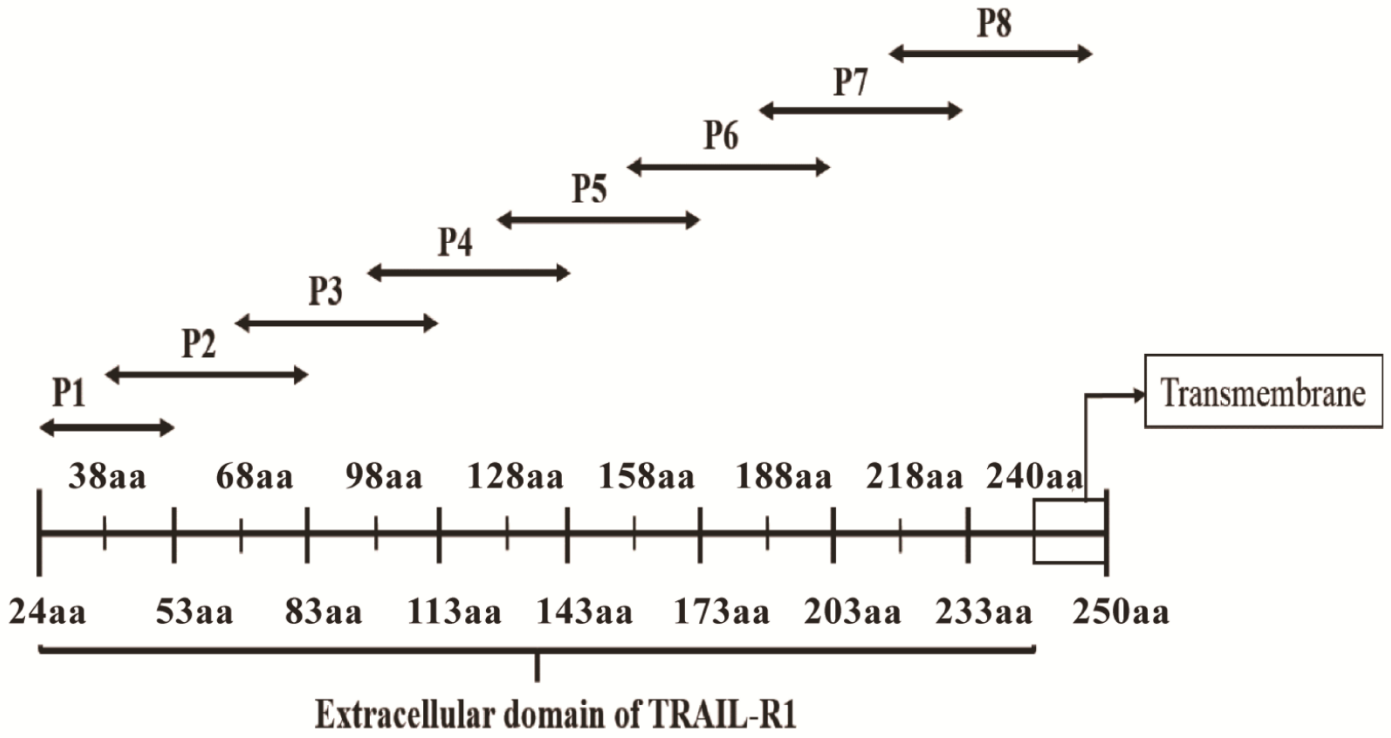
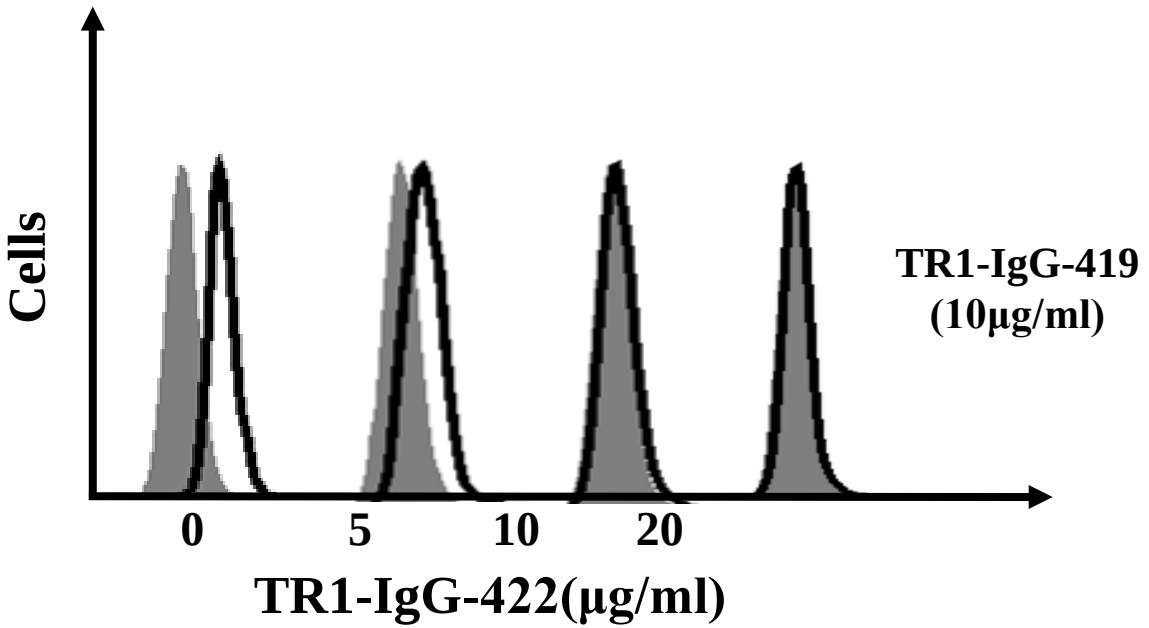


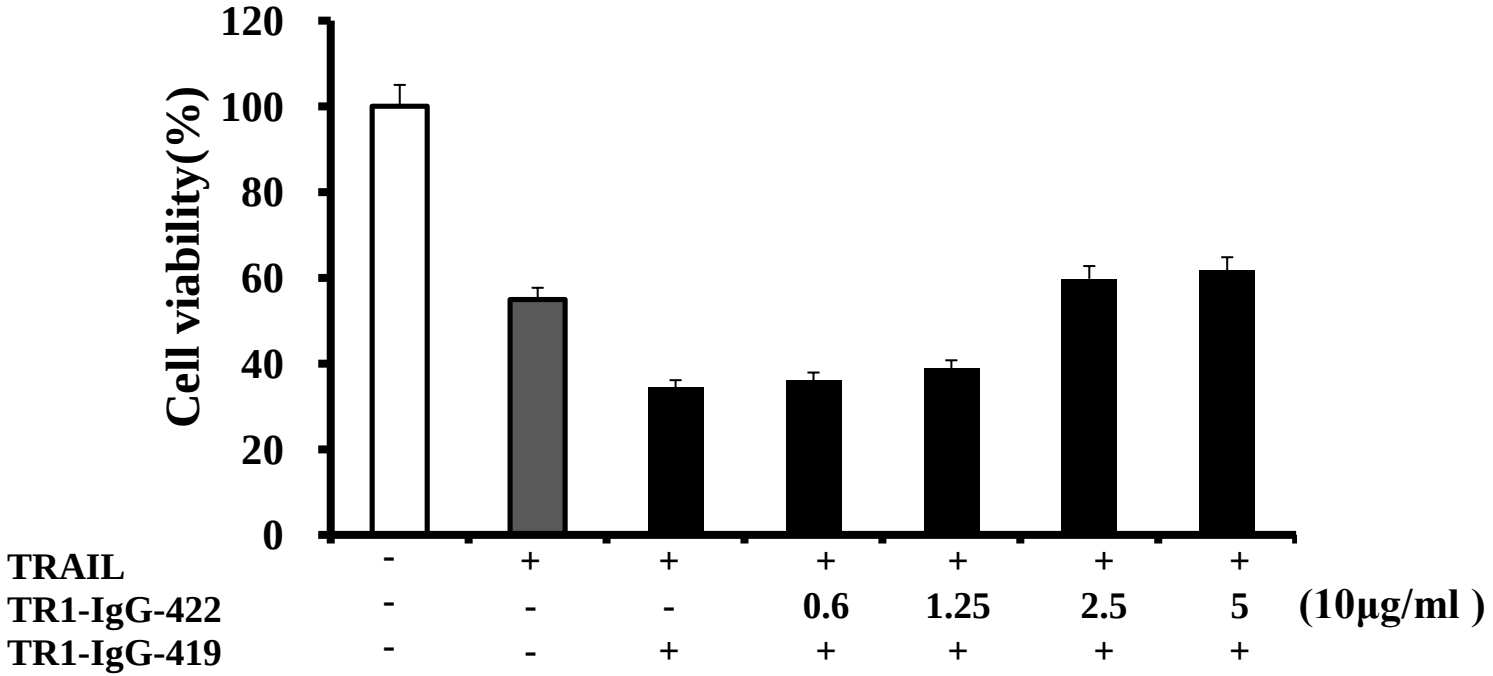
Supplemental Figure 1. The scheme of administration for xenograft tumor-bearing mouse model.



Supplemental Figure 2. Schematic of epitope mapping. The ectodomain of TRAIL-R1 was divided into eight peptide fragments (named Parts 1-8, P1-8) designed to overlap by 15 amino acids. P1 (24-53 aa), P2 (39-83 aa), P3 (69-113 aa), P4 (99-143 aa), P5 (129-173 aa), P6 (159-203 aa), P7 (189-233 aa), P8 (219-250 aa).



Supplemental Figure 3. TR1-IgG-422 blocked the binding of TR1-IgG-419 to TRAIL-R1 on HeLa cells. HeLa cells were pre-incubated with various concentrations of TR1-IgG-422, and then were treated with 10 μg/ml of biotinylated TR1-IgG-419 followed by FITC-conjugated streptavidin. The binding activity of TR1-419 was analysis by flow cytometry. The data presented are representative of three independent experiments.

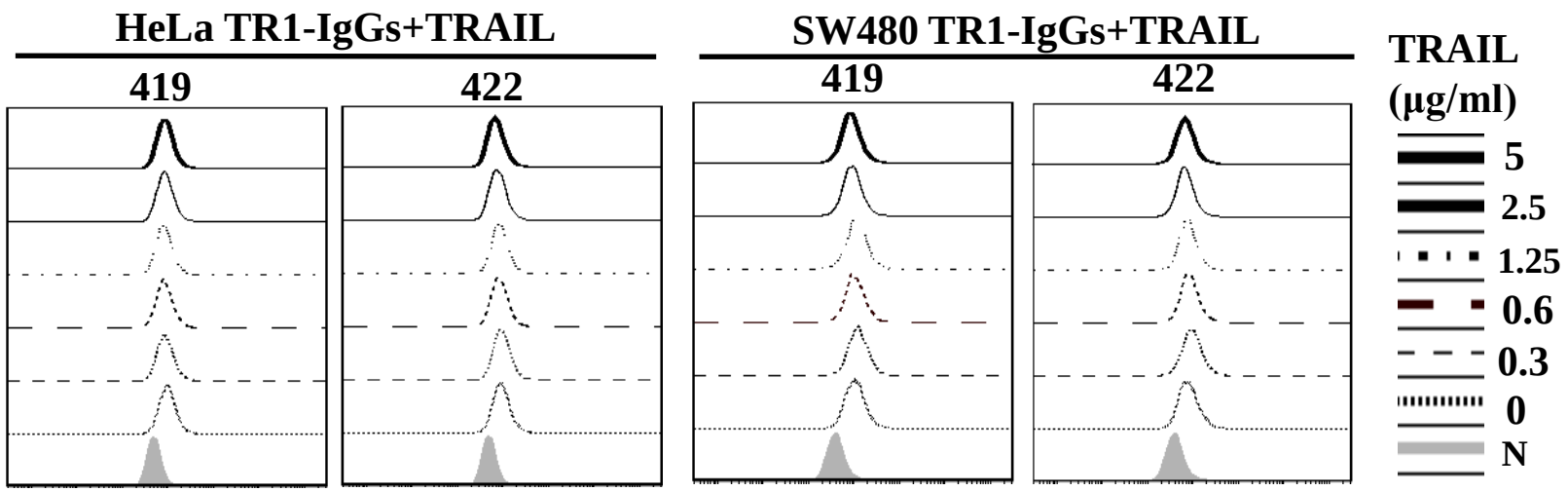


Supplemental Figure 4. TR1-IgG-422 blocked apoptotic effect of TR1-IgG-419 on TRAIL-induced cell death. HeLa cells were pre-cultured with various concentrations of TR1-IgG-422 for 2 h, and then TRAIL and TR1-IgG-419 were added and incubated for 24 h in 96-well plates. Cell viability was detected by MTT assay. The data presented are representative of three independent experiments.

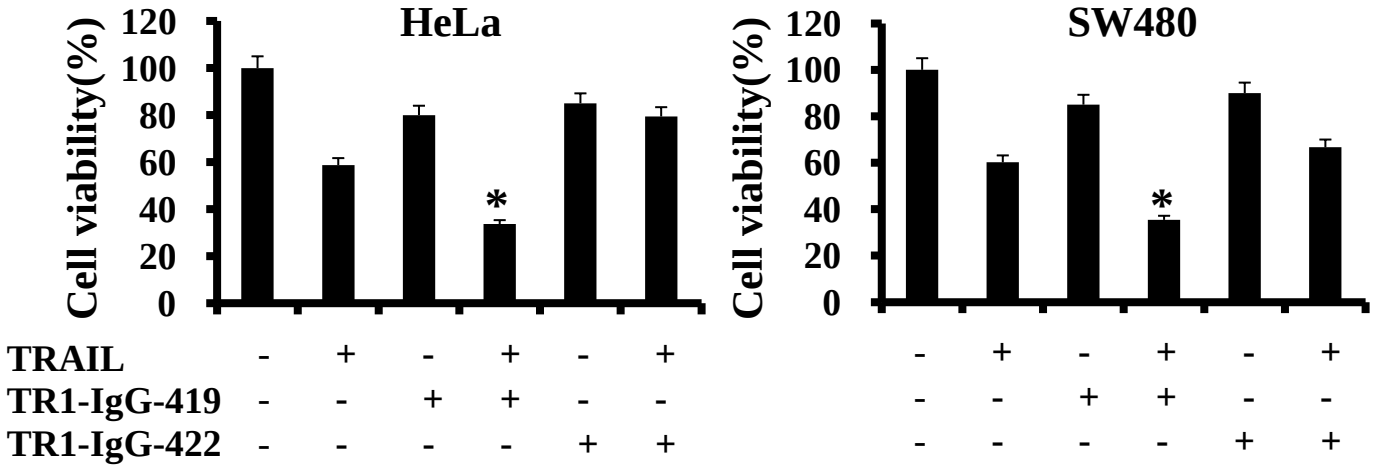
Supplemental Table 1 Binding activity of TR1-mAbs.

TR1-mAbs	HeLa(%)*	SW480(%)*
TR1-IgG-419	49.2	51.1
TR1-IgG-422	52.5	47.9
TR1-IgM-419	74.3	70.4
TR1-IgM-422	97.4	95.9

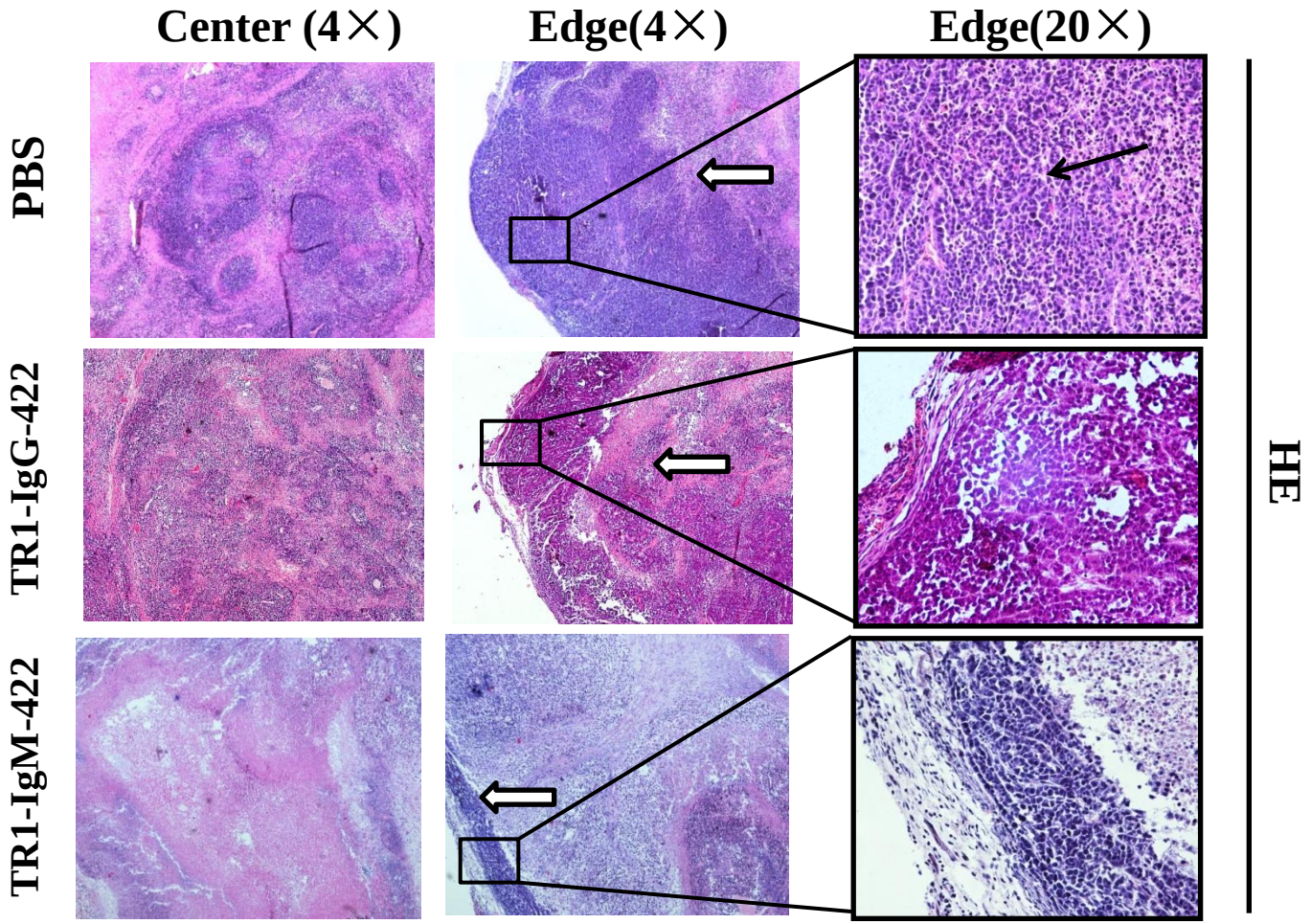
*Log fluorescence intensity(%)



Supplemental Figure 5. Analysis of the competitive inhibition by TRAIL of TR1-IgGs binding to TRAIL-R1 on HeLa and SW480 cells. Cells were pre-cultured in different concentrations of soluble TRAIL, then 1μg/ml of TR1-IgG-419, IgG-422 was added. The results were assessed by flow cytometry. The data presented are representative of three independent experiments.



Supplemental Figure 6. TRAIL interferes with TR1-IgGs inducing tumor cells apoptosis. HeLa and SW480 cells were incubated with alone or a combination of TRAIL (1μg/ml) and TR1-IgGs(1μg/ml) for 24 h. Cell viability was detected by MTT assay. The data presented are representative of three independent experiments.



Supplemental Figure 7. Hematoxylin and eosin(HE) staining of tumor sections after the treatment of TR1-IgG-422 or IgM-422. Tumor tissue paraffin sections from mice treated with PBS (first rows) or TR1-IgG-422 (second rows) and IgM-422 (third rows) were stained with hematoxylin and eosin(HE) . The white arrows indicate the tumor edge, and the black arrow indicates the blood vessels in the tumor tissue. The left column indicates the tumor center position, the middle column indicates the tumor edge, and the right column shows the black boxes magnified.