

Supplemental information

**Improved safety of induced pluripotent
stem cell-derived antigen-presenting
cell-based cancer immunotherapy**

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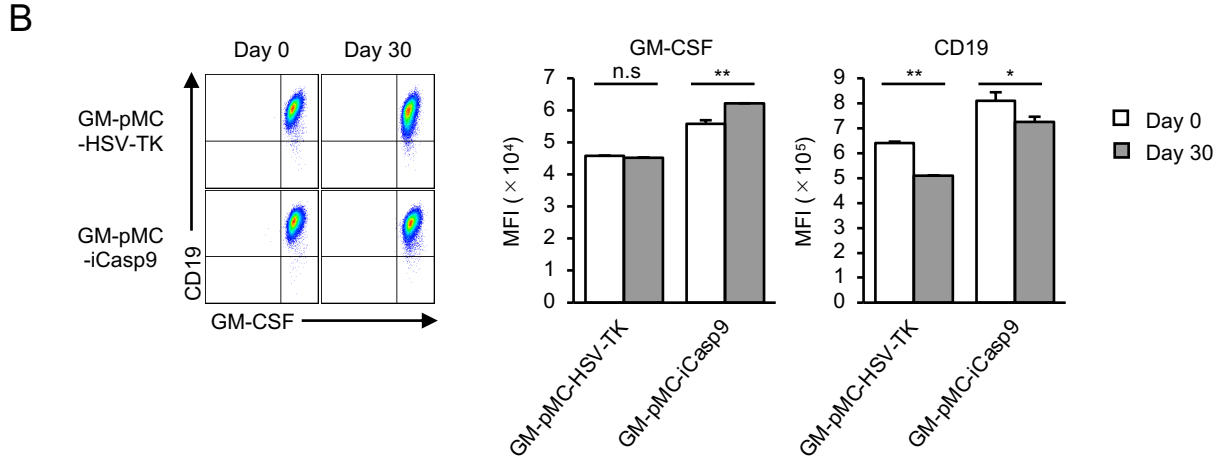
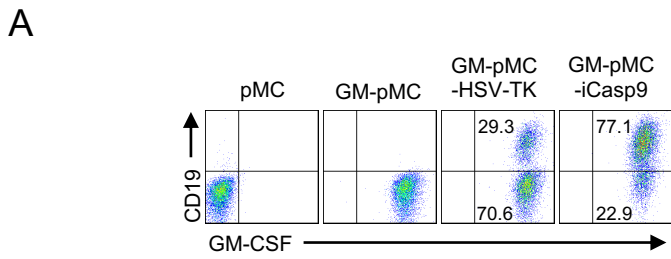
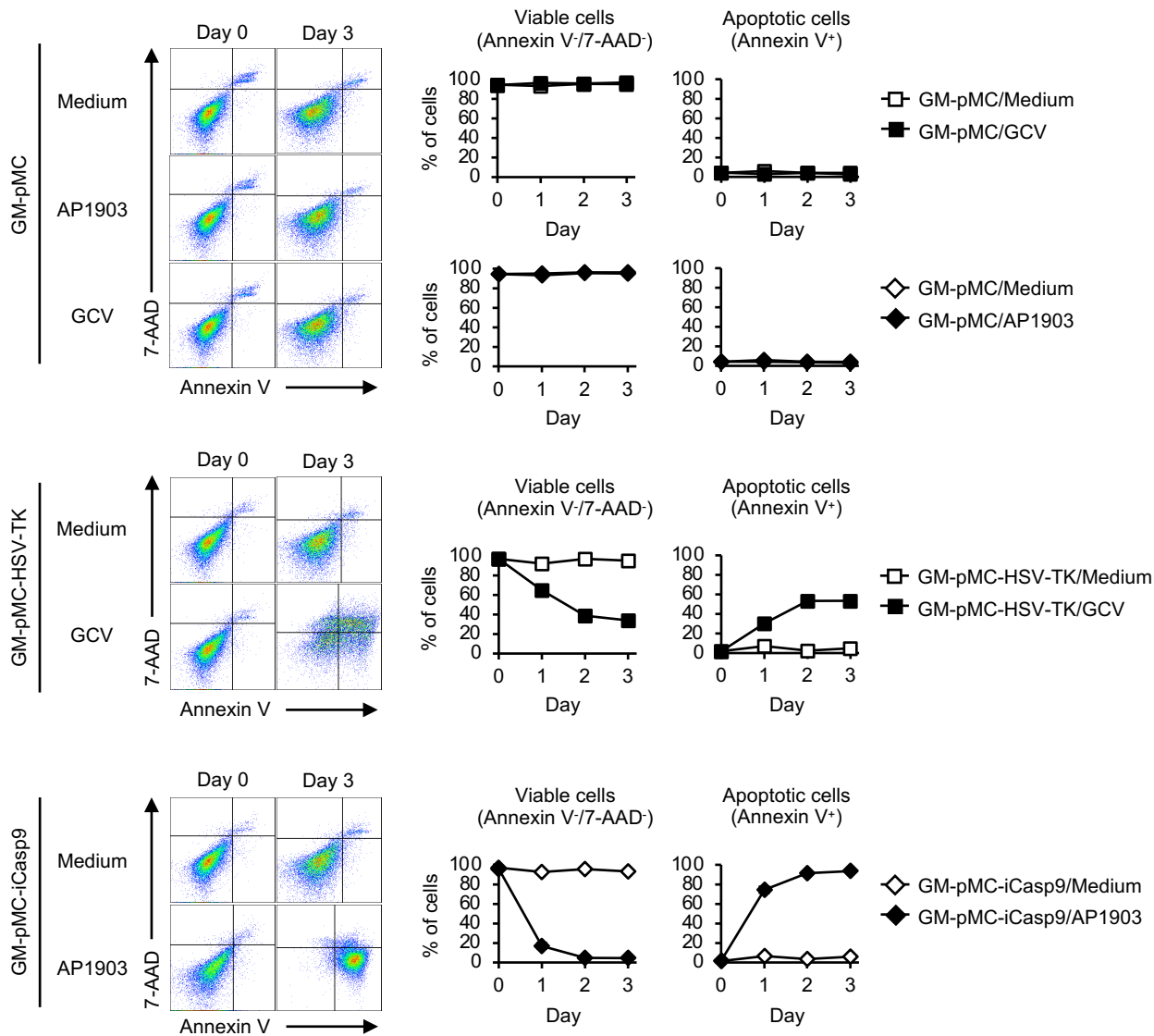


Figure S1, related to Figure 1. Efficiency and stability of EF1 α promotor-mediated suicide gene expression in GM-pMCs

(A) Initial transduction rates for GM-pMC-HSV-TK and GM-pMC-iCasp9 were evaluated using flow cytometry.

(B) Cells were cultured for 30 days in the presence of GM-CSF and M-CSF. The expression levels of GM-CSF and each suicide gene were evaluated using flow cytometry at the indicated time points. Left-hand panels, representative flow cytometry profiles. Right-hand panels, mean fluorescence intensity (MFI). Data shown are the means \pm S.D. of triplicate cultures. * $p < .05$, ** $p < .01$.

A



B

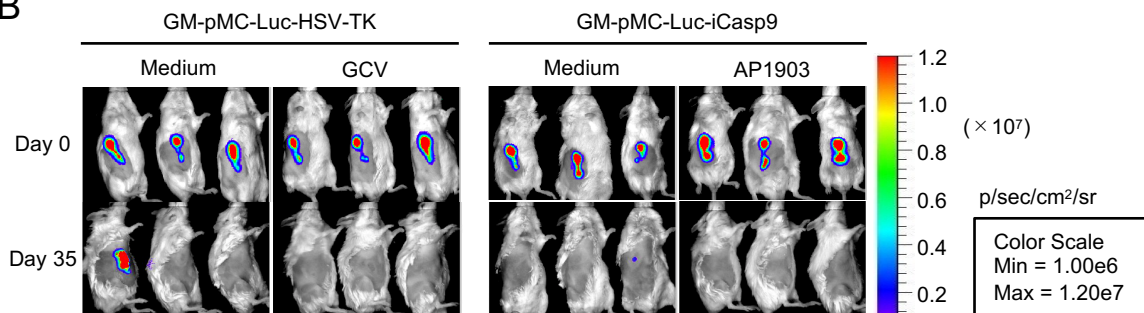
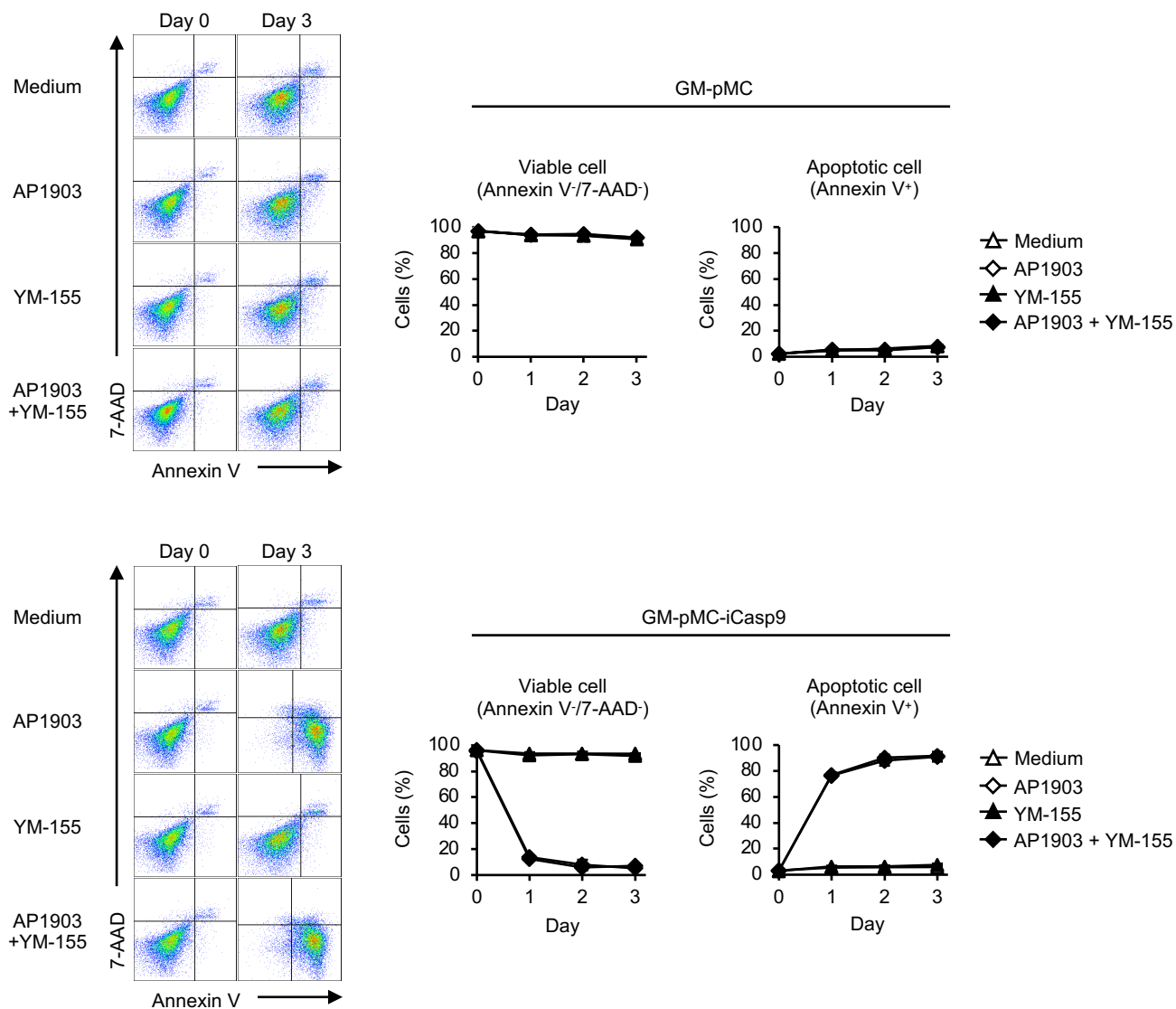


Figure S2, related to Figure 3 and Figure 4. Both HSV-TK/GCV and iCasp9/AP1903 induce apoptosis specifically in suicide gene-expressing cells.

(A) Cells (2.0×10^5 /well) were seeded in 24-well culture plates in the presence of 10 μ M GCV or 10 nM AP1903. The viability of the cells was evaluated using Annexin/7-AAD staining at the indicated time points. The medium served as a control. Left panels, representative flow cytometry profiles. Right panels show the percentage of viable cells (Annexin V⁻/7-AAD⁻) and apoptotic cells (Annexin V⁺) at the indicated time points. Data shown are the means \pm S.D. of triplicate cultures and are representative of two independent experiments.

(B) BALB/c Rag2^{-/-}Jak3^{-/-} mice were inoculated s.c. with the luciferase-expressing GM-pMC-HSV-TK or GM-pMC-iCasp9, and treated with GCV or AP1903 as in **Figure 4**. Bioluminescence images of representative mice from one experiment are shown (n = 7 to 8 mice).

A



B

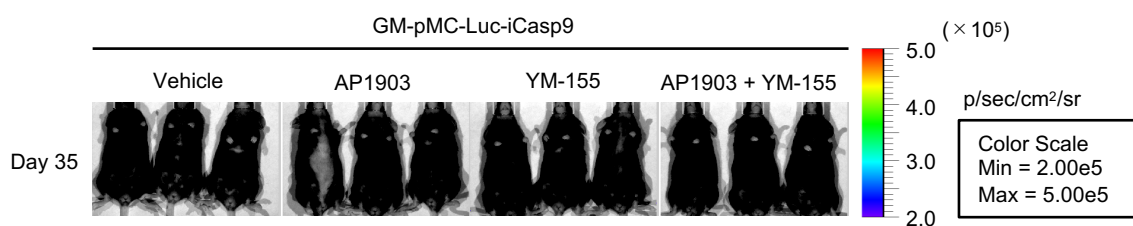


Figure S3, related to Figure 5. Survivin inhibitor does not affect the viability of GM-pMC.

(A) Cells (2.0×10^5 /well) were seeded in 24-well culture plates in the presence of 10 nM AP1903 and/or 10 nM YM-155. Medium served as a control. The viability of the cells was evaluated using Annexin/7-AAD staining at the indicated time points as in Figure S1. Left panels, representative flow cytometry profiles. Right panels, the graphs show the percentage of viable cells (Annexin V⁻/7-AAD⁻) and apoptotic cells (Annexin V⁺) at the indicated time points. Data shown are the means \pm S.D. of triplicate cultures and are representative of two independent experiments.

(B) C57BL/6 mice were s.c. inoculated with the luciferase-expressing GM-pMC-iCasp9 (1.0×10^6 cells) and i.p. treated with 2.5 mg/kg AP1903 and/or 5 mg/kg YM-155 for 5 consecutive days as in Figure 5C. Bioluminescence images of representative mice from one experiment are shown (n = 8 to 9 mice).

Table S1. Antibodies for flow cytometry

Antibody	Company	Product #
APC anti-mouse/human CD11b antibody	BioLegend	101211
APC Rat IgG2b, kappa Isotype Ctrl antibody	BioLegend	400611
PE anti-mouse CD11c antibody	BioLegend	117307
Armenian Hamster IgG, Isotype Control, PE Conjugated, Clone HTK888 antibody	BioLegend	400907
PE anti-mouse/human CD45R/B220 antibody	BioLegend	103207
PE Rat IgG2a, κ Isotype Ctrl Antibody	BioLegend	400508
PE anti-mouse F4/80 antibody	BioLegend	123109
PE anti-mouse Ly-6G/Ly-6C (Gr-1) antibody	BioLegend	108407
PE anti-mouse CD205 (DEC-205) antibody	BioLegend	138213
CD33 Monoclonal Antibody (9A11-CD33), PE, eBioscience™	ThermoFisher Scientific	12-0331-82
Rat IgG1 kappa Isotype Control (eBRG1), PE, eBioscience™ antibody	ThermoFisher Scientific	12-4301-82
CD40 Monoclonal Antibody (1C10), PE, eBioscience™	ThermoFisher Scientific	12-0401-82
Rat IgG2a kappa Isotype Control (eBR2a), PE, eBioscience™ antibody	ThermoFisher Scientific	12-4321-80
CD80 (B7-1) Monoclonal Antibody (16-10A1), PE, eBioscience™	ThermoFisher Scientific	12-0801-82
Armenian Hamster IgG Isotype Control (eBio299Arm), PE, eBioscience™ antibody	ThermoFisher Scientific	12-4888-81
CD86 (B7-2) Monoclonal Antibody (GL1), PE, eBioscience™	ThermoFisher Scientific	12-0862-81
PE anti-mouse H-2K ^b /H-2D ^b antibody	BioLegend	114607
PE Mouse IgG2a, κ Isotype Ctrl Antibody	BioLegend	400212
PE anti-mouse I-A/I-E antibody	BioLegend	107607
PE Rat IgG2b, kappa Isotype Ctrl antibody	BioLegend	400607
APC anti-human CD19 Antibody	BioLegend	302211
APC Mouse IgG1, κ Isotype Ctrl Antibody	BioLegend	400119
PC Annexin V Apoptosis Detection Kit with 7-AAD	BioLegend	640930