# Appendix A. Supplementary data

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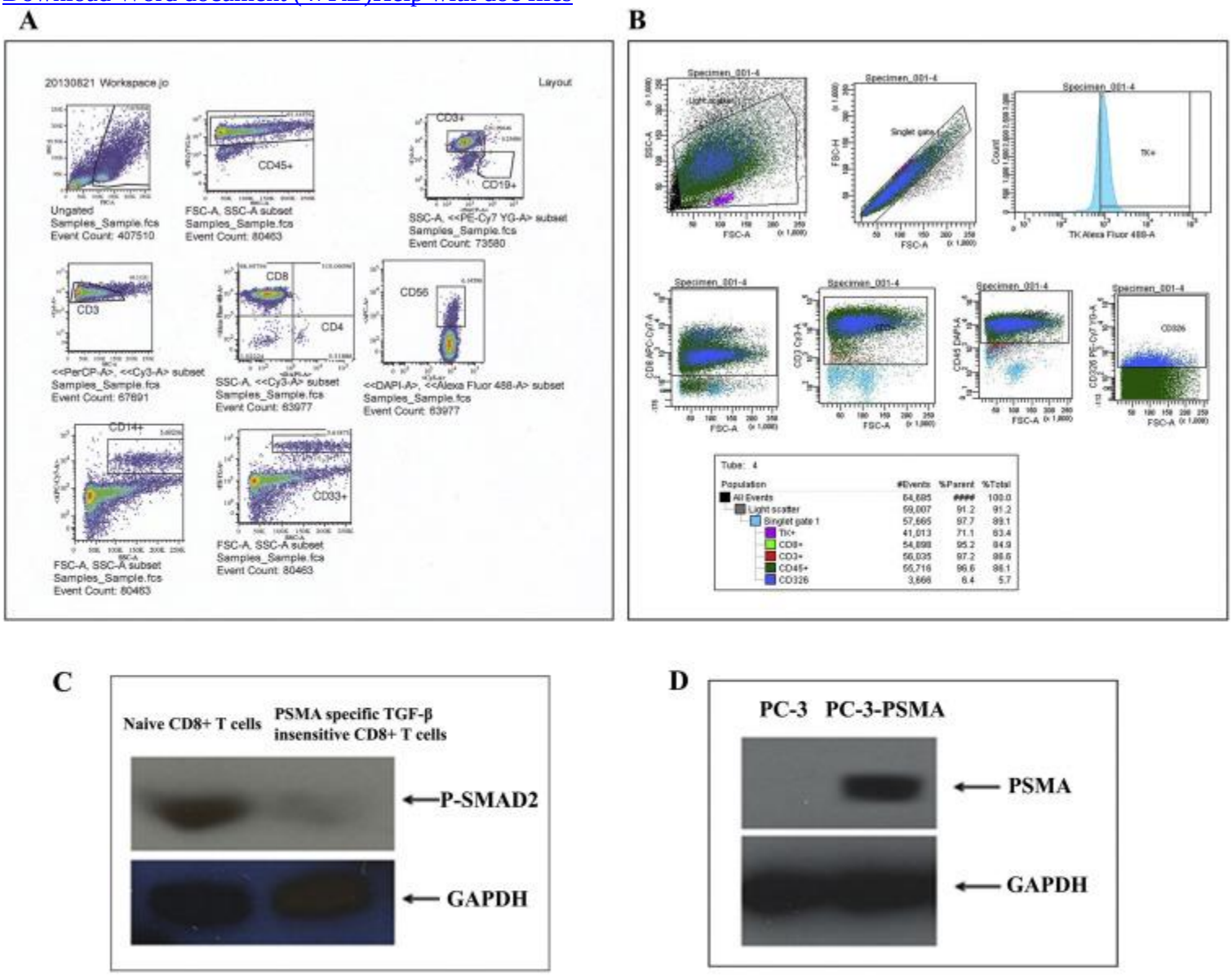


**Supplementary Figure 1**

**A.** Highly purified CD8+ T cells (CD3⁺: 94.51%; CD8⁺ : 98.59%; CD14⁺: 5.69%; CD56⁺: 6.14%; CD45⁺: 91.44%; CD14⁺: 5.69%) were isolated from a patient with mCRPC after leukapheresis. **B.** A total of 71.1% PSMA-specific, TGF-β insensitive CD8⁺ T cells were TK positive 71.1%. **C.** After infection of TßRIIDN-TK-IRES-PZ1, the p-smad2 were significantly blocked in PSMA-specific TGF-β insensitive CD8⁺ T cells in compared to naïve CD8⁺ T cells. **D.** High level expression of PSMA was detected in PC-3-PSMA cells when there is no PSMA expression in PC-3 cells.

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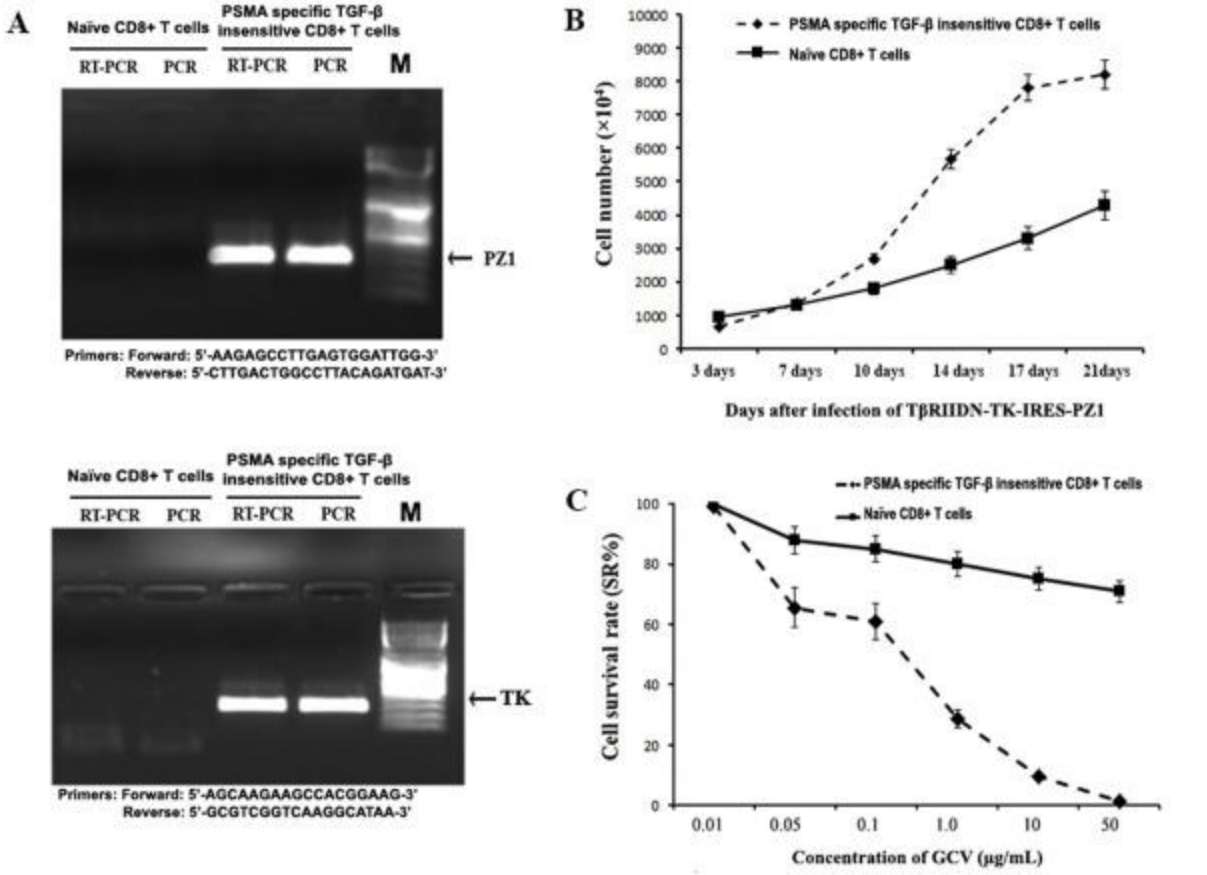


## Supplementary Figure 2

**A.** After infection of TβRIIDN-TK-IRES-PZ1, high level expression of PZ1 and RT-PCR and PCR in RNA and DNA level was identified in PSMA specific TGF-β insensitive $CD8^+$ T cells (Right two lanes). There was no significant expression of PZ1 or TK detected in naïve $CD8^+$ T cells (Left two lanes). **B.** After infection of TβRIIDN-TK-IRES-PZ1, the amount of cells were counted twice/weekly. After Day 7, PSMA-specific TGF-β insensitive $CD8^+$ T cells from mCRPC were expanded ex vivo in CPWS with a significantly greater rate of cell division; 23.4 fold in Day 21 (from $3.5 \times 10^6$ to $8.2 \times 10^7$). This was significantly greater than naïve $CD8^+$ T cells which could only be expanded 12.2 fold (from $3.5 \times 10^6$ to $4.3 \times 10^7$). **C.** The survival rate of PSMA specific TGF-β insensitive $CD8^+$ T cells decreased sharply to 1.3% under the treatment of GCV at 50μg/ml, while the growth of naïve $CD8^+$ T cells was not affected significantly and kept the survival rate above 80%.

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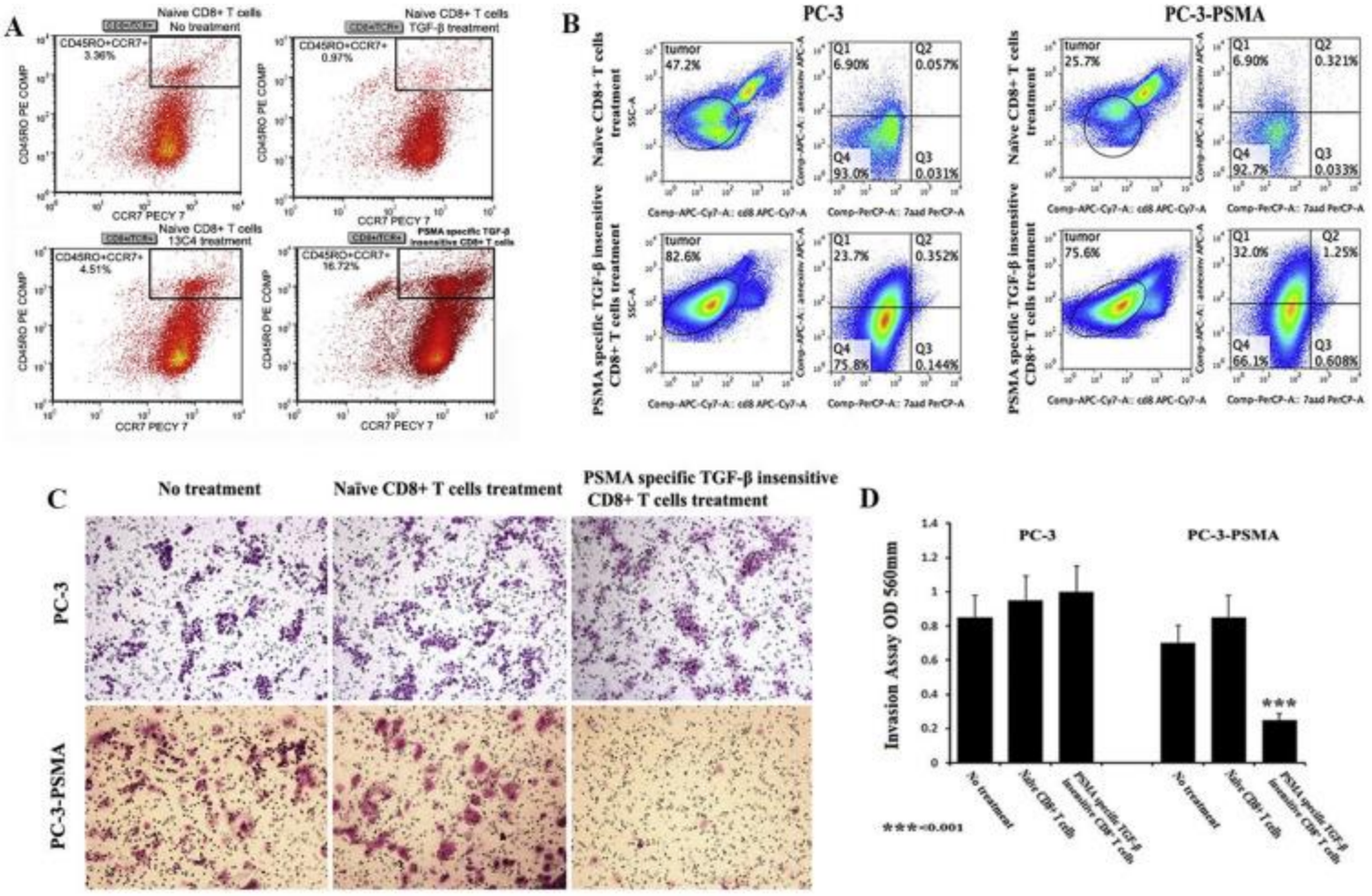


## Supplementary Figure 3

**A.** Expression of CD8, TCR, CD45RO and CCR7 in PSMA specific TGF-β insensitive CD8⁺ T cells, naïve CD8⁺ T cells with treatment of TGF-β or control 13C4 antibody. Analysis was performed by immunofluorescent FACS (CD45RO-PE, CCR7-PECY7; see Supplementary Materials and Methods). **B.** Markers for early apoptosis (Annexin V) and late apoptosis (7-aad) were evaluated by double staining immunofluorescent FACS. Incubation with PSMA-specific, TGF-ß-insensitive CD8⁺ T cells induced 23.7% and 32.0% expression of Annexin V in PC-3 and PC-3-PSMA respectively in compared to 6.9% and 6.9% respectively when incubation with naïve CD8⁺ T cells. There is no significant difference on expression of 7-aad between above treatment groups. **C.** PC-3 and PC-3-PSMA possessed equal invasive capabilities. There were no significant changes in cell motility through a Matrigel-coated polycarbonate membrane when co-cultured with naïve CD8⁺ T cells. The invasion of PC-3-PSMA cells, but not PC-3 cells, could be inhibited by co-culture with PSMA specific TGF-β insensitive CD8⁺ T cells. **D.** Absorbance values. This result indicates PSMA specific TGF-β insensitive CD8⁺ T cells suppressed invasion of PSAM positive PCa specifically.

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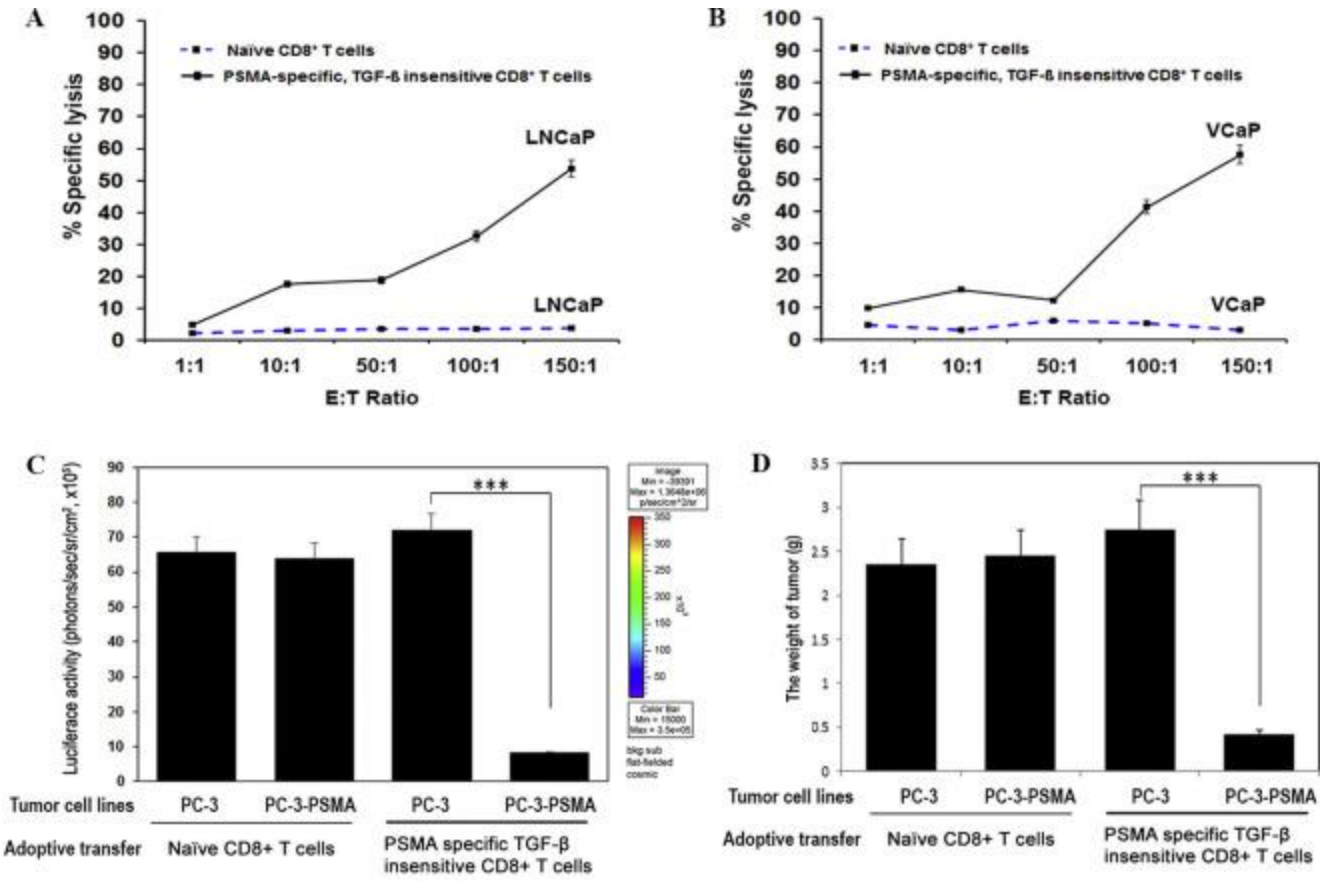


**Supplementary Figure 4**

**A.** CTL for LNCaP and VCaP cell lines was done by using the conventional $^{51}Cr$ release assay (see Supplementary Materials and Methods). Naïve CD8$^+$ T cells and PSMA specific, TGF-β insensitive CD8$^+$ T cells were co-cultured with $^{51}Cr$-labeled targets at the specific E/T ratios. LNCaP cells or VcaP cells were used as the targets respectively; PSMA specific, TGF-β insensitive CD8$^+$ T cells generated 53.7% specific lysis against LNCaP cells and generated 57.6% specific lysis against VcaP cells (**B**) respectively when there was lower than 10% lysis against both PCa cell lines was found by treatment with naïve CD8$^+$ T cells. **C.** Right before the mice were sacrificed at the end of the 35-day treatment period, IVIS 100 imaging system was used to measure the luciferase activity of each tumor. The average luciferase density was $71.8\times10^5$(photons/sec/sr/cm$^2$) for PC-3 tumor and $8.1\times10^5$ for PC-3-PSMA tumor under the treatment of PSMA-specific TGF-ß-insensitive CD8$^+$ T cells. The average luciferase density was $65.5\times10^3$(photons/sec/sr/cm$^2$) for PC-3-PSMA tumor and $63.8\times10^5$ for PC-3 tumor under the treatment of naïve CD8$^+$ T cells ($P<0.05$). **D.** Mice were sacrificed and tumors were isolated. The average tumor weight of PC-3-PSMA tumor was 0.413g. In comparison, PC-3 tumors were found in all mice treated with PSMA-specific TGF-ß-insensitive CD8$^+$ T cells, and the average weight of PC-3 tumors was 2.75g correspondingly ($P<0.05$). The corresponding values in the mice that received adoptive transfer of naïve CD8$^+$ T cells were 2.45g and 2.36g, respectively.

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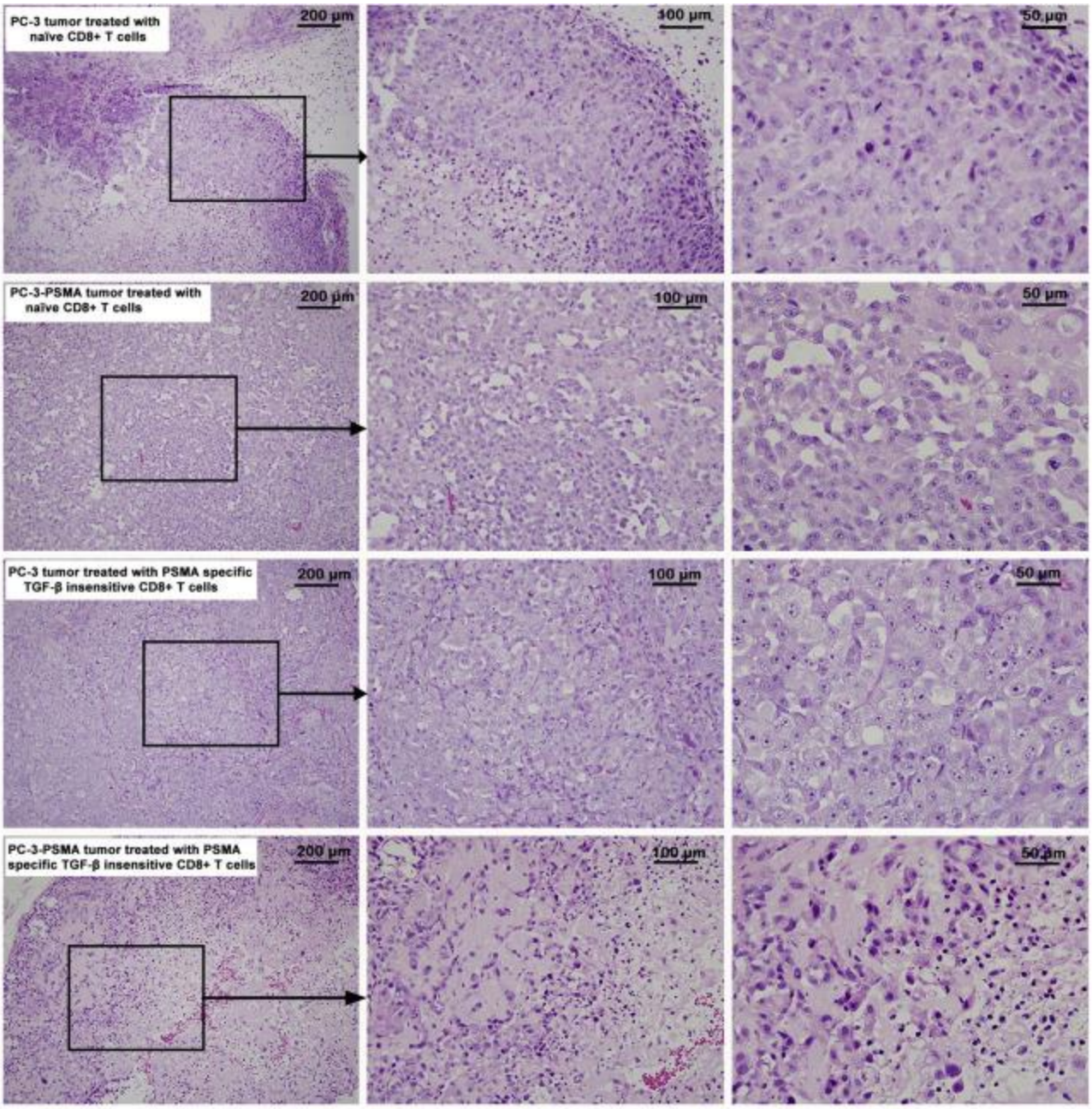


# Supplementary Figure 5

Representative histologic features (H&E staining) of tumor nodules from animals that received adoptive transfer of naive CD8⁺ T cells or adoptive transfer with PSMA-specific TGF-ß-insensitive CD8⁺ T cells (time point 35 days following the treatment). These animals received injection of tumor cells 7 days before the adoptive transfer. Large amount of nuclear fusion, fragmentation and necrosis were found in PC-3-PSMA tumors but not PC-3 tumors that received the adoptive transfer of PSMA-specific TGF-ß-insensitive CD8⁺ T cells. No degenerative changes or necrosis was noted in either PC-3 or PC-3-PSMA tumors of which received adoptive transfer of naive CD8⁺ T cells.

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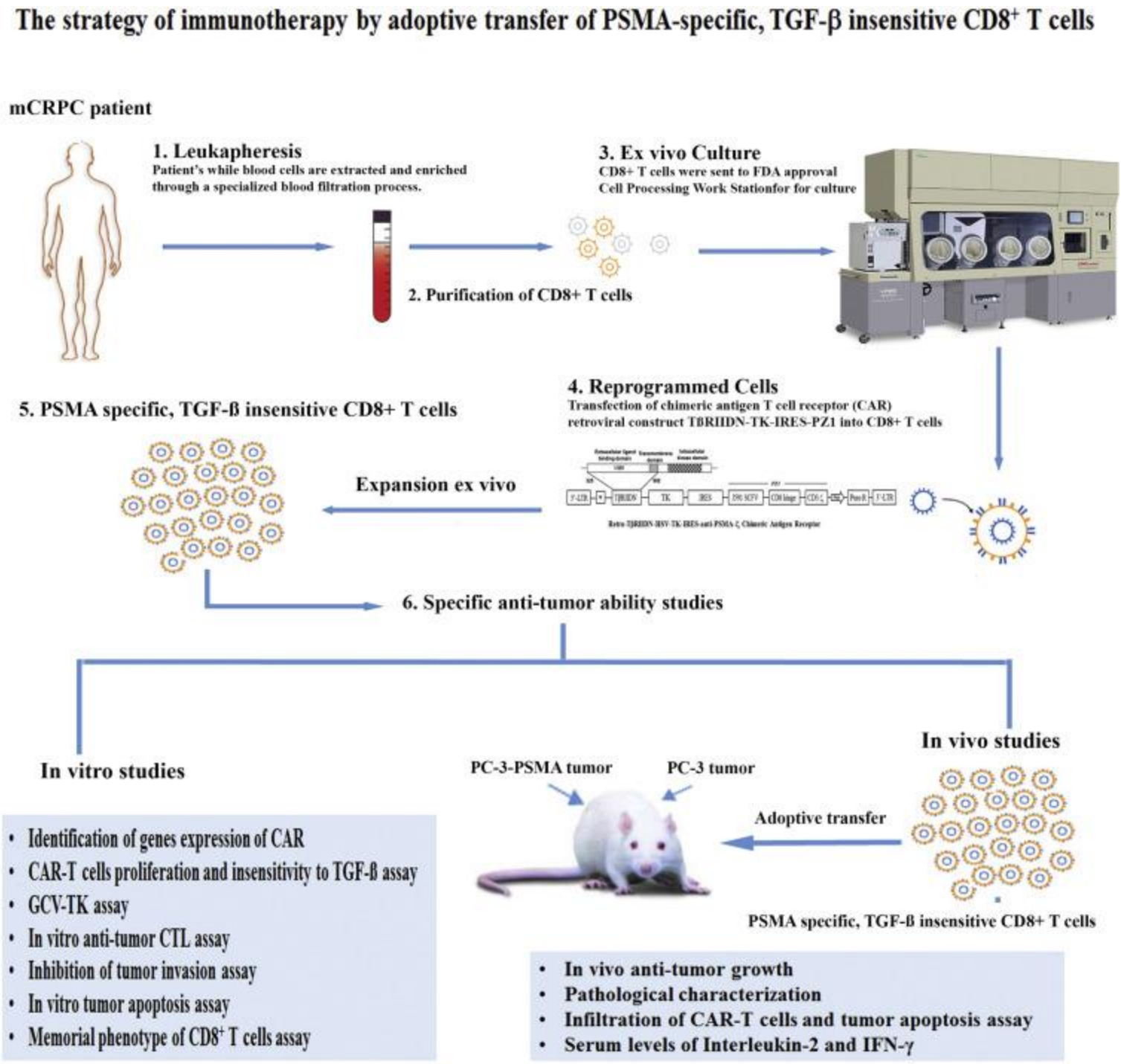


## Supplementary Figure 6

Schematic Summary Diagram for strategy of immunotherapy by PSMA-specific TGF-ß-insensitive CD8+ T cells. 1. Collection of white blood cells from mCRPC patient by leukapheresis; 2. Purification of CD8+ T cells; 3. Ex vivo culture of CD8+ T cells in FDA approval Cell Processing Work Station (CPWS); 4. Reprogrammed cells by transfection of TßRIIDN-TK-IRES-PZ1 CAR retroviral construct into the CD8+ T cells; 5. Generation and ex vivo expansion of PSMA specific, TGF-ß insensitive CD8+ T cells; 6. Specific anti-tumor ability studies in vitro and in vivo.

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