

## **Supporting Information**

### **A Single-Domain Antibody-Based Anti-PSMA Recombinant Immunotoxin**

#### **Exhibits Specificity and Efficacy for Prostate Cancer Therapy**

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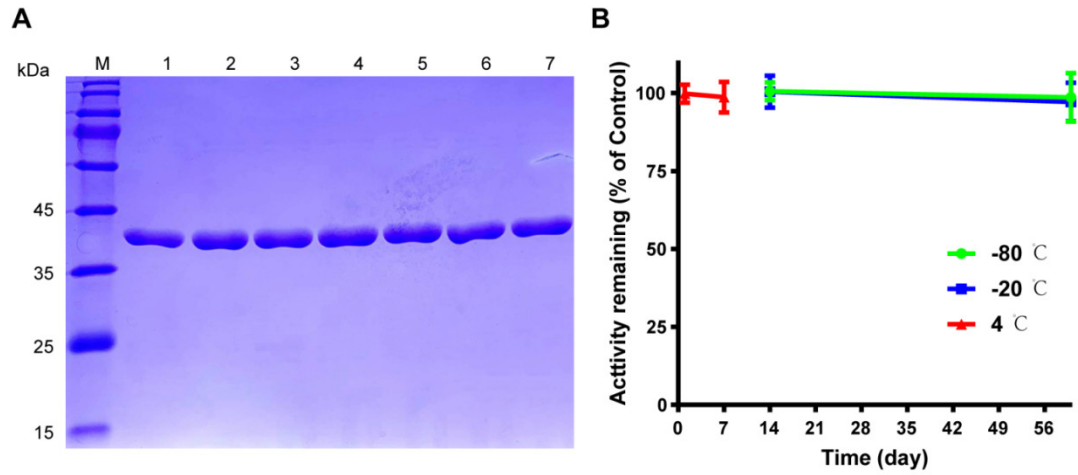
**Amino acid sequence of JVM-PE24X7 and JVM-PE38** (Sequence of JVM is labeled as red and sequence of PE24X7 and PE38 is labeled as blue).

**JVM-PE24X7:**

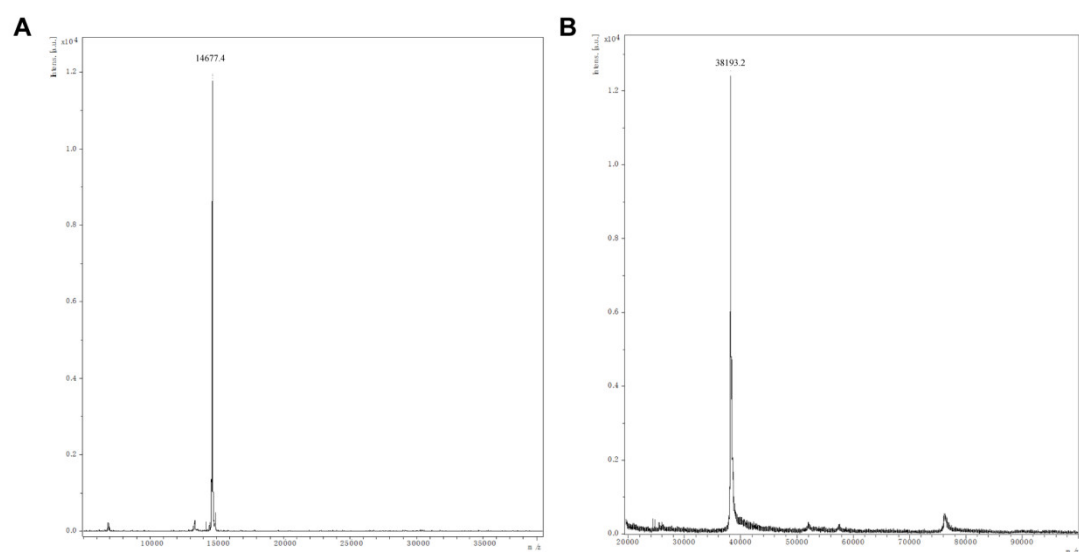
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PKDEL

**JVM-PE38:**

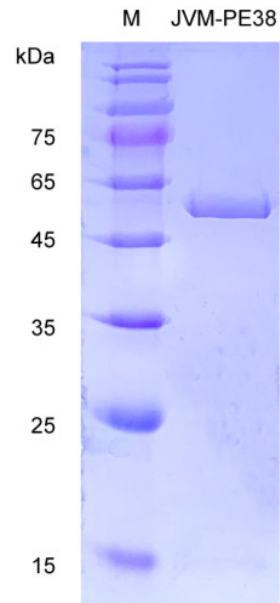
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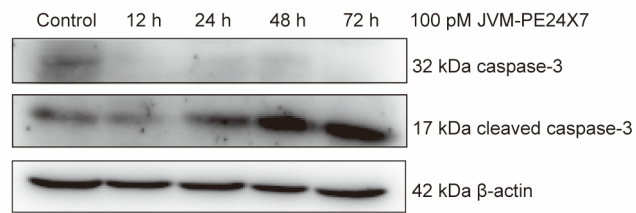
**Figure S1.** Storage stability analysis of JVM-PE24X7 by SDS-PAGE and cytotoxicity assay. **(A)** Freshly purified JVM-PE24X7 (lane 1) was stored at 4 °C for 24 h (lane 2) and 1 week (lane 3), at -20 °C for 2 weeks (lane 4) and 2 months (lane 5), and at -80 °C for 2 weeks (lane 6) and 2 months (lane 7). The proteins were loaded onto a 12.5% polyacrylamide gel with protein weight standards and detected by Coomassie brilliant blue staining **(B)** Evaluation of residual activity of JVM-PE24X7 after storage at different temperatures for different durations. Data is presented as a percentage of the remaining activity compared to the freshly purified JVM-PE24X7.



**Figure S2.** MALDI-TOF MS of purified JVM-PE24X7 and JVM. (A) Theoretical molecular mass of JVM-PE24X7 is 38172.4 Da and detected molecular mass is 38193.2 Da. (B) Theoretical molecular mass of JVM is 14621.4 Da and detected molecular mass is 14677.4 Da.



**Figure S3.** SDS-PAGE of purified JVM-PE38. The protein was loaded onto a 12.5% polyacrylamide gel with protein weight standards, and detected through Coomassie brilliant blue staining.



**Figure S4.** Western blot analyses of apoptosis-related proteins in C4-2B cells. Western blot showed the dynamic changes of caspase-3 and cleaved caspase-3 in C4-2B cells treated with 100 pM JVM-PE24X7 for 12 h, 24 h, 48 h or 72 h.