**Supplementary data for “*XCL1/glypican-3* fusion gene immunization generates potent antitumor cellular immunity and enhances anti-PD1 efficacy ”** byKun Chen1, Zhiyuan Wu1, Hong Zhao2, Yanmei Wang1, Yutong Ge3, Dongmei Wang1, Zhengjiang Li4, Changming An4, Yuying Liu5, Feifei Wang1, Xinyu Bi2, Hongying Wang6, Jianqiang Cai2, Chunhong Ma3, and Chunfeng Qu1, \*

**Supplementary Tables**

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| **Supplementary Table S1. List of quantitative RT-PCR primers** | | | | | |
| Genes | Gene ID | Primer sequence (5' to 3') | Location | F/R | Product Size |
| *mouse Xcl1* | 16963 | TTTGTCACCAAACGAGGACTAAA | 178-200 | F | 164 bp |
| CCAGTCAGGGTTATCGCTGTG | 341-321 | R |
| *mouse Gpc3* | 14734 | CAGCCCGGACTCAAATGGG | 133-151 | F | 126 bp |
| CAGCCGTGCTGTTAGTTGGTA | 258-238 | R |
| *mouse Gapdh* | 14433 | AGGTCGGTGTGAACGGATTTG | 8-28 | F | 123 bp |
| TGTAGACCATGTAGTTGAGGTCA | 130-108 | R |
| *human GPC3* | 2719 | CCCGTGCCAGGATCAGATTT | 311-330 | F | 204 bp |
| CTTGGCATGGCGAACAACAA | 514-495 | R |
| *human GAPDH* | 2597 | GGAGCGAGATCCCTCCAAAAT | 108-128 | F | 197 bp |
| GGCTGTTGTCATACTTCTCATGG | 304-282 | R |

**Supplementary Table S2. Antibodies and ELISA kits**

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| --- | --- | --- | --- | --- |
| **Reagents** | **Clone** | **RRID** | **Source** | **Application** |
| Anti-mouse CD45 | 30-F11 | AB\_10371770 | Thermo Fisher Scientific | FC |
| Anti-mouse CD45.1 | A20 | AB\_2534250 | Thermo Fisher Scientific | FC |
| Anti-mouse CD45.2 | 104 | AB\_2534252 | Thermo Fisher Scientific | FC |
| Anti-mouse CD3 | 145-2C11 | AB\_465496 | Thermo Fisher Scientific | FC |
| Anti-mouse CD4 | GK1.5 | AB\_464892 | Thermo Fisher Scientific | FC |
| Anti-mouse CD8α | 53-6.7 | AB\_1107004 | Thermo Fisher Scientific | FC, IF |
| Anti-mouse IFN-γ | XMG1.2 | AB\_1257211 | Thermo Fisher Scientific | FC |
| Anti-mouse granzyme B | NGZB | AB\_10870787 | Thermo Fisher Scientific | FC |
| Anti-mouse CD11c | N418 | AB\_469346 | Thermo Fisher Scientific | FC, IF |
| Anti-mouse NK1.1 | PK136 | AB\_466050 | Thermo Fisher Scientific | FC |
| Anti-mouse I-A/I-E | m5/114.15.2 | AB\_465928 | Thermo Fisher Scientific | FC |
| Anti-mouse PD1 | J43 | AB\_10853805 | Thermo Fisher Scientific | FC |
| Anti-mouse CD16/32 | 93 | AB\_467135 | Thermo Fisher Scientific | FC |
| Anti-mouse PD1 | RMP1-14 | AB\_10949053 | BioXcell | Neu |
| Rat IgG2a | 2A3 | AB\_1107769 | BioXcell | Neu |
| Anti-human HLA-A2 | BB7.2 | AB\_1877228 | BioLegend | FC |
| Anti-human CD141 | M80 | AB\_10900238 | BioLegend | FC |
| Anti-human CD45 | HI30 | AB\_2715892 | BioLegend | FC |
| Anti-human HLA-DR | LN3 | AB\_314684 | BioLegend | FC |
| Anti-human CD11c | 3.9 | AB\_2129792 | BioLegend | FC |
| Anti-His tag | J099B12 | AB\_2716151 | Easybio | FC |
| Anti-human/mouse/rat GPC3 | 307801 | AB\_11217192 | R&D Systems | IB, IHC, ICC |
| Anti-β actin | AC-40 | AB\_262137 | Sigma-Aldrich | IB |
| Mouse IFN gamma | Cat. Num | 88-7314-22 | Thermo Fisher Scientific | ELISA |
| Mouse Granzyme B | Cat. Num | BMS6029 | Thermo Fisher Scientific | ELISA |
| Mouse IL-12p70 | Cat. Num | 88-7121 | Thermo Fisher Scientific | ELISA |
| Mouse IL-18 | Cat. Num | BMS618/3 | Thermo Fisher Scientific | ELISA |
| Mouse CCL5 | Cat. Num | KMC1031 | Thermo Fisher Scientific | ELISA |
| Mouse CXCL9 | Cat. Num | EMCXCL9 | Thermo Fisher Scientific | ELISA |
| Human IL-12 | Cat. Num | 88-7126-22 | Thermo Fisher Scientific | ELISA |
|  |  |  |  |  |
| FC: Flow Cytometry; IF: Immunofluorescence; IB: Immunoblot; Neu: Neutralization; IHC: Immunohistochemistry; ICC: Immunocytochemistry; ELISA: Enzyme Linked Immunosorbent Assay | | | | |

**Supplementary Figures**

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**Supplementary Figure S1. The constructions of XCL1/E2crimson fusion molecules.**

**A.** Schematic representation of the constructions. The fusion molecules are linked with glycine (5)–serine-glycine (5). **B.** The predicted 3D structures of constructed fusion proteins analyzed with the RaptorX structure prediction server (<http://raptorx.uchicago.edu/>). **C.** Predicted interactions between mouse XCL1 in the fusion proteins (gray ribbon) and mouse XCR1 (black ribbon) analyzed using the ZDOCK program (<http://zdock.umassmed.edu/>).

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**Supplementary Figure S2. The mXCL1/E2crimson fusion protein expression in transfected HEK293T cells at different time points.**

HEK293T cells were transfected with *mXcl1-E2crimson* or *E2crimson-mXcl1* plasmids, the expression of fusion proteins at different time points post-transfection were monitored with by immunofluorescent microscopy.

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**Supplementary Figure S3. Expression of the mXCL1-E2crimson fusion protein in the immunized sites and draining lymph nodes at different time points in vivo.**

**A.** The expressed mXCL1-E2crimson or E2crimson in the plasmid DNA injected muscles at different time points detected by immunofluorescent microscopy. **B.** The expressed mXCL1-E2crimson in their draining lymph nodes at different time points detected by immunofluorescent microscopy.

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**Supplementary Figure S4.** The percentage of the CD11c+CD8α+DCs and CD11c+CD8α-DCs that taken the protein in the draining lymph nodes 6 days post-injection was shown. Each dot represents one mouse.

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**Supplementary Figure S5. The variation of serum ALT, body weight in different time points, and tumor numbers in different size measured on week 22.**

Two days before each immunization, the serum ALT level was measured. **A.** The serum ALT level in mice immunized starting from week 6. **B.** The serum ALT level in mice immunized starting from week 14. C. Body weights were measured before they received the first immunization at week 6 and when they were sacrificed at week 22.D. Body weights were measured before they received the first immunization at week 14 and when they were sacrificed at week 22. E. Tumor numbers in different size at wk22, each dot represents one mouse. \*\**P*<0.01, *t*-test was conducted.

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**Supplementary Figure S6. A**)The percentage and the total numbers of GPC3-specific CD8+IFNγ-producing and **B**) CD8+GrzB-producing T cells in the livers were shown. Each dot represents one mouse. \**P*<0.05 and \*\**P*<0.01 conducted using *t-*test.

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**Supplementary Figure S7. Experimental scheme of GPC3-specific cytotoxicity induced by hXCL1-GPC3 proteins *in vitro*.**

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**Supplementary Figure S8.** **The tumor volume and incidence in 3 independent HCC-PDX models in NSG immunodeficient mice.**

**A.** The mRNA expression level of GPC3 in HCC cancerous (Ca) and paracancerous (Pa) tissues from 3 donors and HepG2 cell line determined by qRT-PCR assay. **B.** The tumor volume and incidence in 3 independent PDX models are shown. Each dot represents the tumor value in one mouse. The GPC3-expressing HCC tissues were inoculated into NSG mice to establish the HCC-PDX. Their lymph nodes were obtained in the same time. Autologous T cells were co-cultured with the DCs, which were chemoattracted by *hXCL1-GPC3*- or by *GPC3*-transfected cell lysates, at a ratio of 1 DC to 20 T cells for 5 days. The mice (n=3) received 106 stimulated T cells intravenously on day 6 when the xenografts were measurable. The tumor volume and incidence are shown.