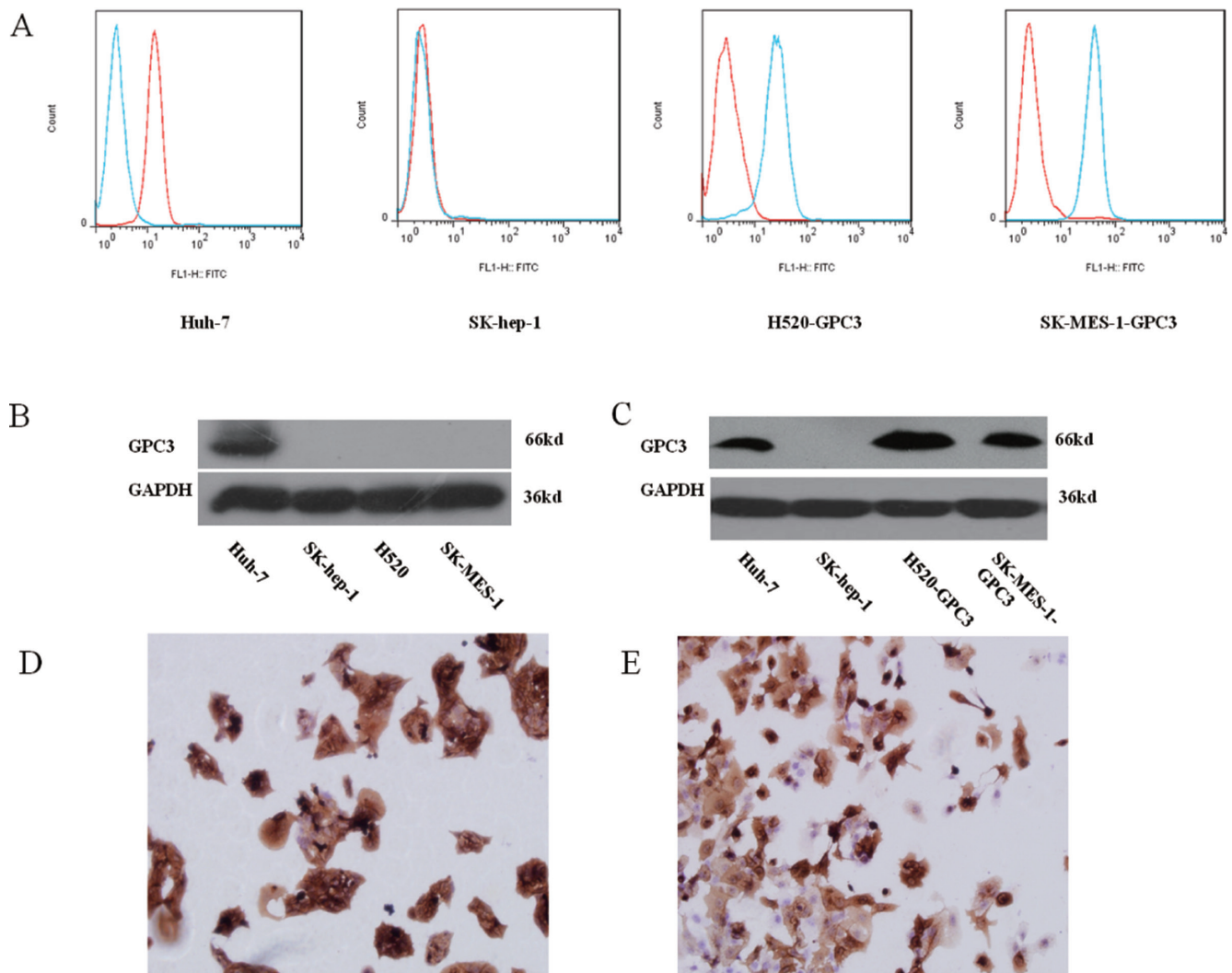


Adoptive immunotherapy using T lymphocytes redirected to glypican-3 for the treatment of lung squamous cell carcinoma

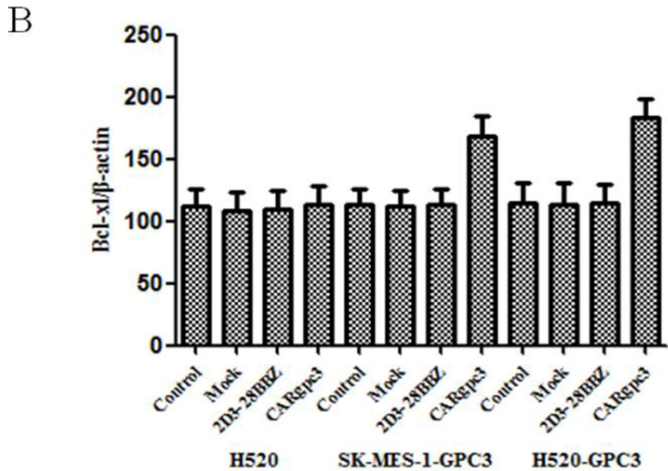
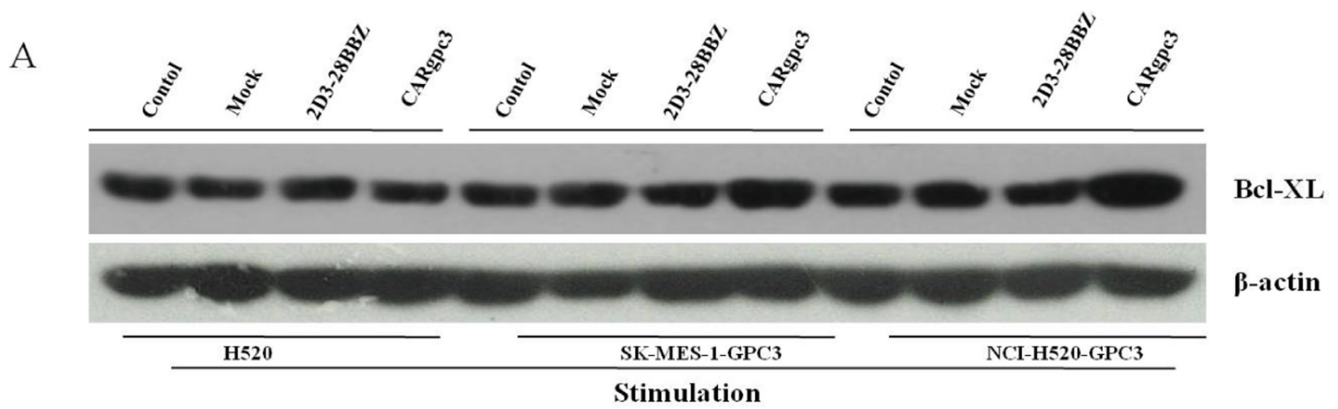
Supplementary Materials

Supplementary Table S1: Clinicopathologic features of 60 patients with lung cancer

Clinicopathologic features	Number of patients
Age (y)	
> 60	41
≤ 60	19
Gender	
M	42
F	18
Pathological type	
Adeno	30
Squamous	30
Tumor histology	
Moderately to poorly	20
Moderately	20
Poorly	16
Poorly to moderately	2
Unidentified	2
Clinical stages	
I	28
II	20
III-IV	12
Lymphatic metastasis	
YES	27
NO	33



Supplementary Figure S1: GPC3 expression was analyzed in lung squamous cell carcinoma cell lines. (A), (C) The expression of GPC3 (red line) on the surface of lung squamous cells as detected by flow cytometry. **(B), (D)** The expression of GPC3 protein in human lung squamous cell lines was detected by Western blot. The human HCC cell lines Huh-7 and SK-hep-1 served as the positive and negative controls, respectively. **(E), (F)** The expression of GPC3 in LSCC cell lines as evaluated by IHC.



Supplementary Figure S2: Bcl-xL protein in CAR_{gpc3} T cells was up-regulated in GPC3-positive tumor cells. CAR_{gpc3} T cells and the control T cells were each co-cultured with tumor cells (at an effector-to-target ratio of 1:1) for 24 h. The T cells were separated from the tumor cells and subjected to Western blot analysis for the measurement of Bcl-xL protein levels.

Supplementary Video: The cytotoxicity of CAR_{gpc3} T cells against NCI-H520-GPC3 cells in the effector-to-target ratio of 1:1 was also demonstrated by live-cell time-lapse imaging. The incubation time was 24 hours. Snapshots over time were obtained from live-cell time-lapse images of target cells that underwent apoptotic cell death after they were engaged by the CAR_{gpc3} T cells.