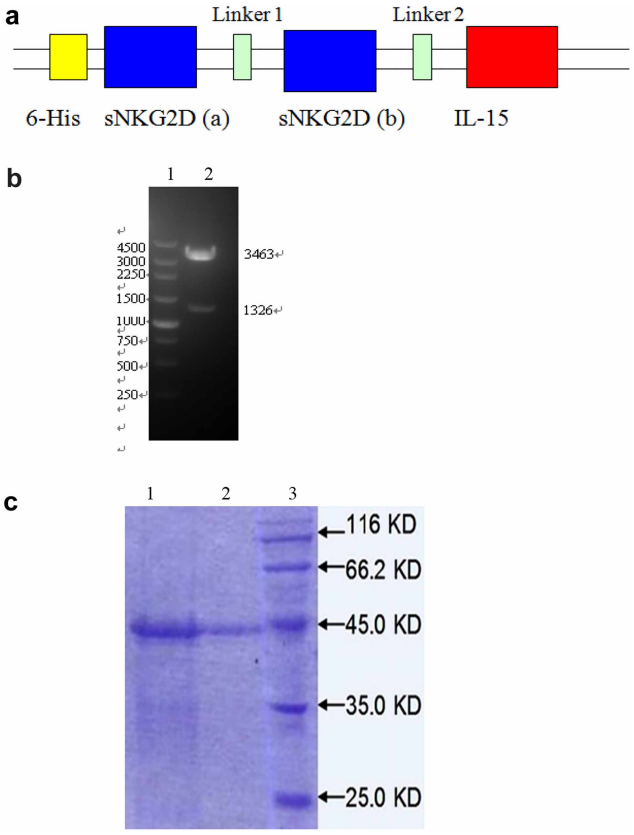
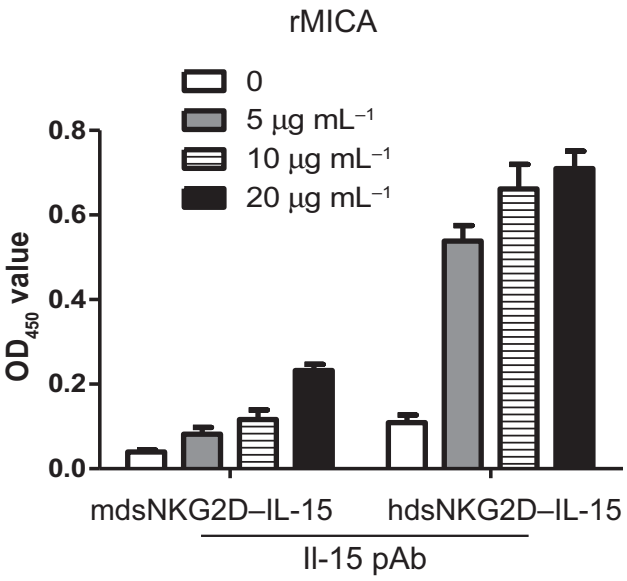
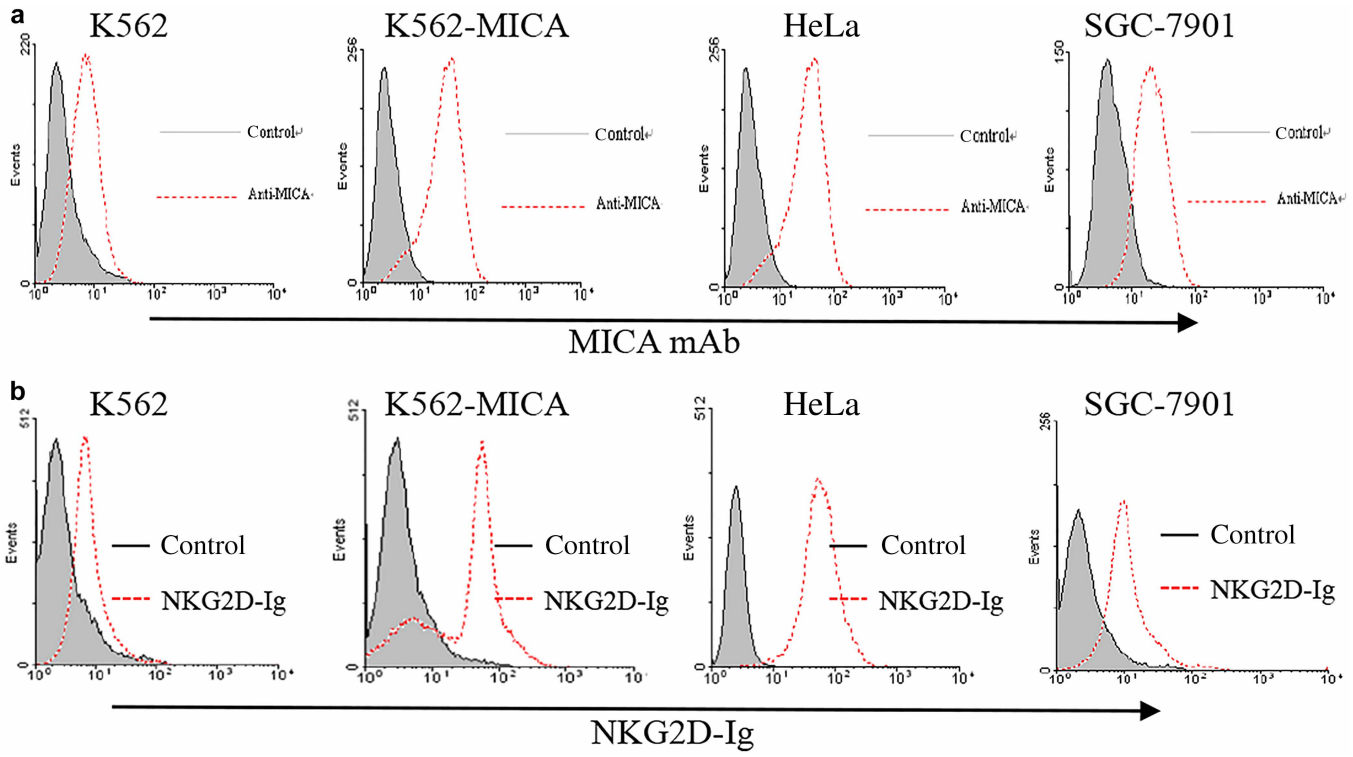


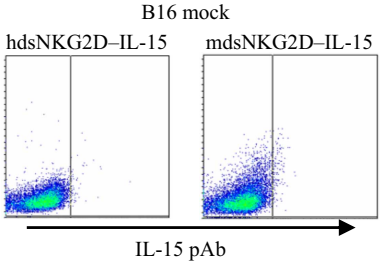
Supplementary Figure 1 Generation of the human dsNKG2D–IL-15 fusion protein. (**a**) Schematic representation of the cloning strategy and domain assembly for scFv-GM-CSF fusion proteins. (**b**) The recombinant human dsNKG2D–IL-15 was digested by *Bamh* I and *Hind* III restrictive enzymes, and the results of electrophoresis are shown. (**c**) SDS-PAGE of the purified recombinant human dsNKG2D–IL-15 protein. Lane 1, denatured dsNKG2D–IL-15; Lane 2, renatured dsNKG2D–IL-15; Lane 3, Standard protein marker.



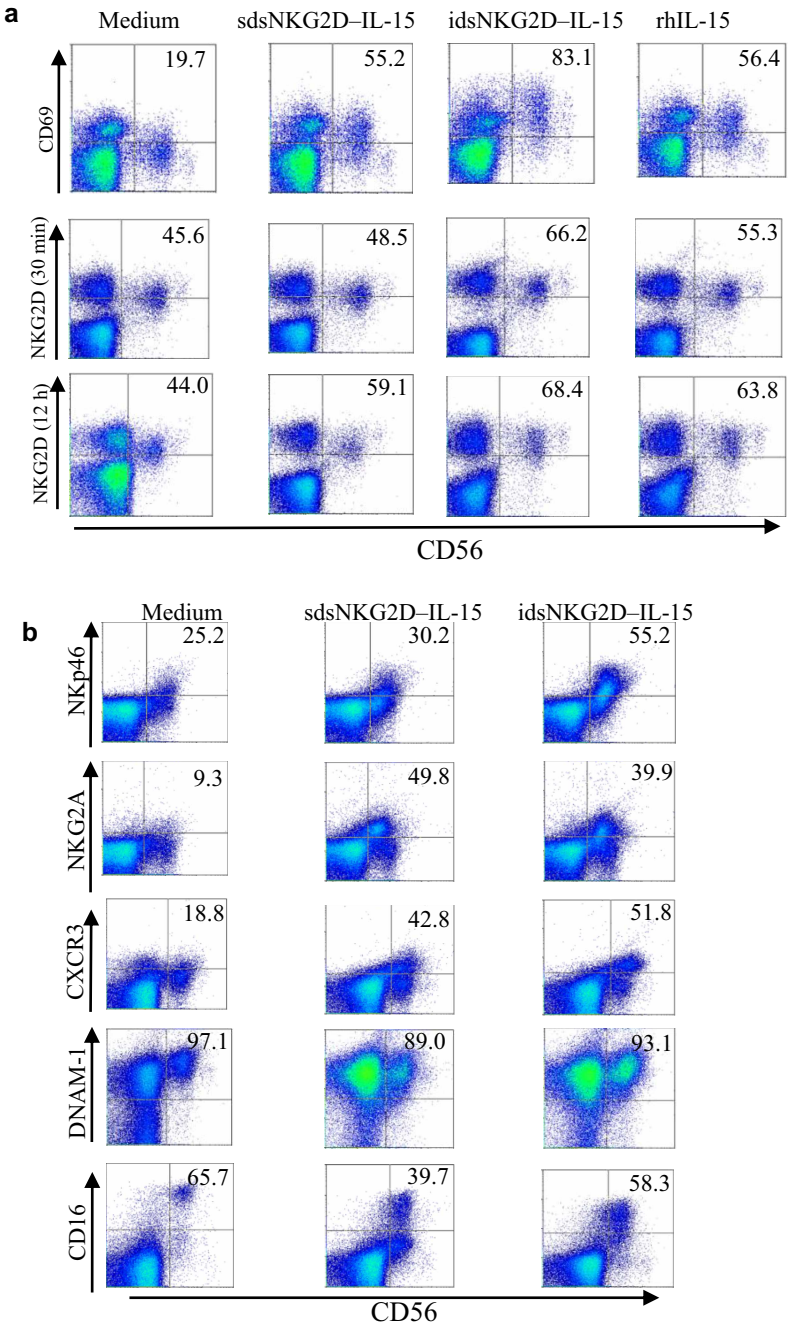
Supplementary Figure 2 Soluble MICA protein (1 μg) was coated on wells. Human or mouse dsNKG2D–IL-15 was added and incubated at 37°C for 1 h. Polyclonal antibody against both human and mouse IL-15, and HRP-secondary antibodies were added sequentially into the wells. OD450 value of each well was read after adding the substrate and stop solution.



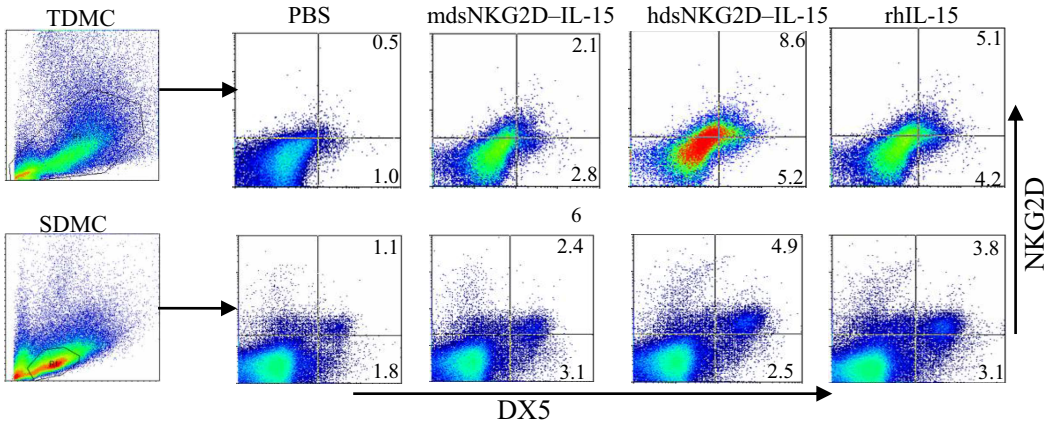
Supplementary Figure 3 Expression of MICA and NKG2D ligands on tumor cells. (**a**) MICA on K562, K562-MICA, Hela, SGC-7901 cells is stained by MICA mAb. Gray area: Isotype antibody staining; red line: MICA mAb staining. (**b**) Total NKG2D ligands on K562, K562-MICA, Hela, SGC-7901 cells are confirmed by staining with commercial recombinant NKG2D–Ig fusion protein and detected by goat anti-human IgG antibody. Gray area: Isotype antibody staining; red line: NKG2D–Ig staining.



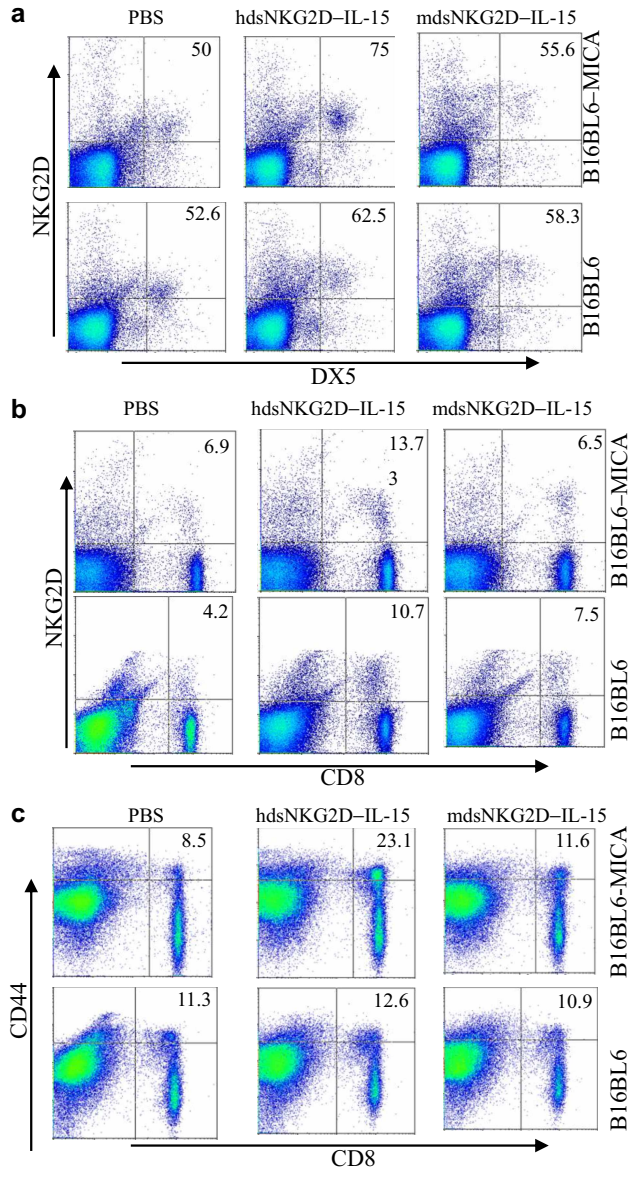
Supplementary Figure 4 Binding of human or mouse dsNKG2D–IL-15 with mock-transfected B16 cells. Cells were incubated with human or mouse dsNKG2D–IL-15 for 30 min, then stained by rabbit anti-human and mouse IL-15 polyclonal antibody and fluorescent secondary goat anti-rabbit antibody.



Supplementary Figure 5 Representative results of receptor expression on $CD56^{+}$ cells after stimulations with soluble or immobilized dsNKG2D–IL-15. (**a**) CD69 or NKG2D expression. (**b**) Nkp46, NKG2A, CXCR3, DNAM-1, CD16 expression.



Supplementary Figure 6 Representative results of NK cell frequencies in tumor tissue and spleen of SGC-7901 cell-transplanted tumor mice treated by human dsNKG2D–IL-15, mouse dsNKG2D–IL-15, or recombinant human IL-15. Upper panel: tumor-derived mononuclear cells (TDMC); lower panel: splenic mononuclear cells (SDMC). NK cell frequency is calculated by total frequencies of upper right quadrant and lower right quadrant. Frequency of $NKG2D^{+}DX5^{+}$ cells is from frequency of upper right quadrant divided by total NK cell frequency.



Supplementary Figure 7 Representative results of NK and $CD8^+$ T-cell frequencies in spleen of mice transplanted with B16-MICA or B16 cells treated by human or mouse dsNKG2D–IL-15. (**a**) Variations of $NKG2D^+DX5^+$ cells. (**b**) Variations of $NKG2D^+CD8^+$ cells. (**c**) Variations of $CD44^+CD8^+$ cells. Numbers indicate upper right quadrant divided by sum of upper right quadrant and lower right quadrant.