Supplementary Figure and Table Legends

Supplementary Figure 1: Cloning, expression and purification of recombinant B7-H4

A. cDNA expression of B7-H4 in macrophages (**1**) after 2 or 5 hrs of stimulation with IL10/IL4 or (**2**) after 72 hrs in transwell co-culture with OVCAR3 cell line. Simultaneous β-actin amplification was used as control. **B.** Detection of recombinant B7-H4 (0.5 µg) after electrophoresis by western blot (**1**) in 293-F cell supernatant with anti-HIS mouse Ab followed by HRP anti-mouse Ig Ab or (**2**) after HIS purification with goat anti-human B7-H4 polyclonal Ab followed by HRP rabbit anti-goat polyclonal Ab; or (**3**) by coumassie staining.

Supplementary Figure 2: Analysis of B7-H4 expression in human ovarian cancer cells

Upper panel: Cell lines (as indicated) were labeled with PE-conjugated anti-B7-H4 mAb (**open histogram**s) or PE-conjugated isotype control (**grey filled histogram**s) and analyzed by flow cytometry. EBV-transformed B cells (18), *in vitro* differentiated macrophages stimulated with IL-4 and IL-10 (M2 MΦ (8)), and B7-H4-transduced C3O ovarian cancer cells, were used as positive controls. **Lower panel**: Cell lysates from four ovarian cancer cell lines (OVCAR5, SKOV3, A1857, OVCAR3) and M2 MΦ were probed with polyclonal goat anti-human B7-H4 antibody (1 µg/ml) followed by HRP-conjugated rabbit anti-goat polyclonal antibody (1 µg/ml). β-actin detection was used as endogenous protein loading control.

Supplementary Figure 3: Strategies for isolation of anti-B7-H4 scFvs from a new yeast-display scFv library derived from ovarian cancer patients.

A. Schematic representation of protein-based and cell-based isolation strategies: **I.** Magnetic sortings of the yeast-display scFv library were performed using 293, 146 and 66 pM of biotinylated rB7-H4 protein for the first, second, and third magnetic sorting, respectively. Flow cytometry sorting were performed using 13.33 pM of biotinylated rB7-H4; **IIa.** Depletion of non-specific binders by panning on B7-H4^{neg} C30 cells; **IIb.** Enrichment in B7-H4 specific binders by panning on B7-H4-transduced C30 cells.

Supplementary Figure 4: ELISA validation of isolated anti-B7-H4 scFvs.

A-B. Protein-based (**A**) and cell-based (**B**) isolated anti-B7-H4 scFvs were plastic-immobilized and incubated with serial dilutions of biotinylated recombinant B7-H4 (**black diamonds**) or irrelevant control antigen (BSA, **grey triangles**). Protein binding to scFv was detected with SA-HRP. Colorimetric signal was developed with TMB substrate solution, quenched with sulfuric acid and read at 450 nm on a Biotek ELISA reader.

Supplementary Figure 5: *In vitro* tumor-polarized TAMs downregulate antigen-specific T cells proliferation and co-stimulation.

MART-1 TCR specific T cells were stimulated with T2 APCs pulsed with serial dilutions (0.0025-1 µM) of MART-1 peptide or 1 µM of HER-2 peptide as control, in the absence or in the presence of tumor-polarized B7-H4^{+} TAMs (as indicated). **A**. T cell proliferation

was analyzed by CFSE dilution; **B**. T cell co-stimulation was analyzed by detection of CD137 expression.

Supplementary Table 1: B7-H4 expression on tumor cells derived from human ovarian cancer ascites and solid tumors.

Supplementary Table 2: Germline immunoglobulin gene usages of the predicted amino-acid sequence of the anti-B7-H4 scFvs.

Kabat analysis of the homology of heavy (H) and light (L) chain variable regions to germline immunoglobulin genes are displayed for each anti-B7-H4 scFv clone.