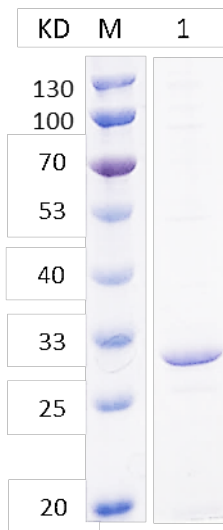


Supporting Information for

Escherichia coli adhesion portion FimH functions as an adjuvant for cancer
immunotherapy

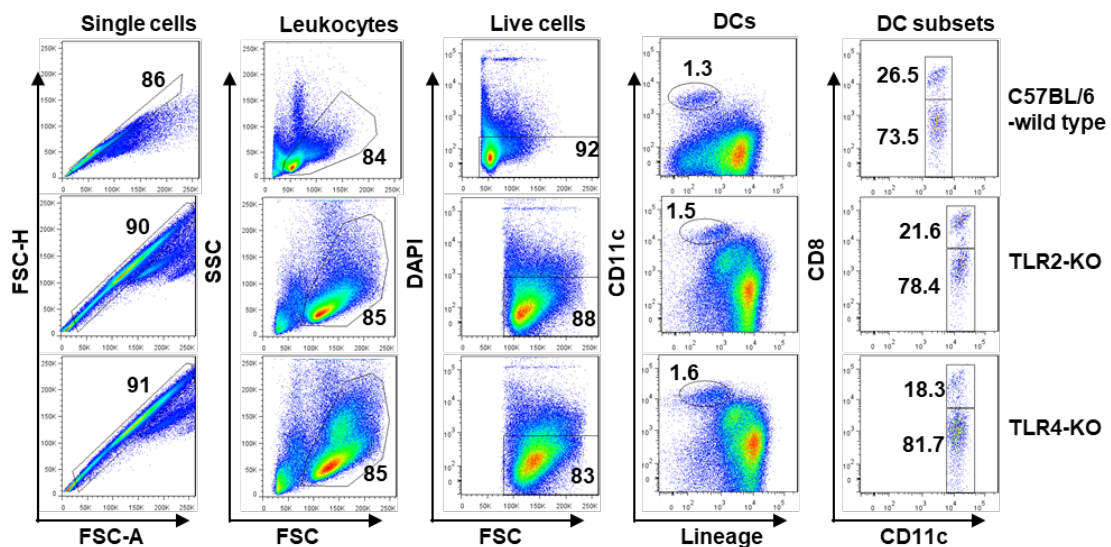
Wei et al.,

Fig. S1



Supplementary figure 1. Purification of recombinant FimH from *E. coli*. pET28a-FimH plasmids were transfected into competent cells and the FimH protein was purified on a Nickel column. Lane 1 shows the purified FimH separated on SDS-PAGE; M = molecular weight marker.

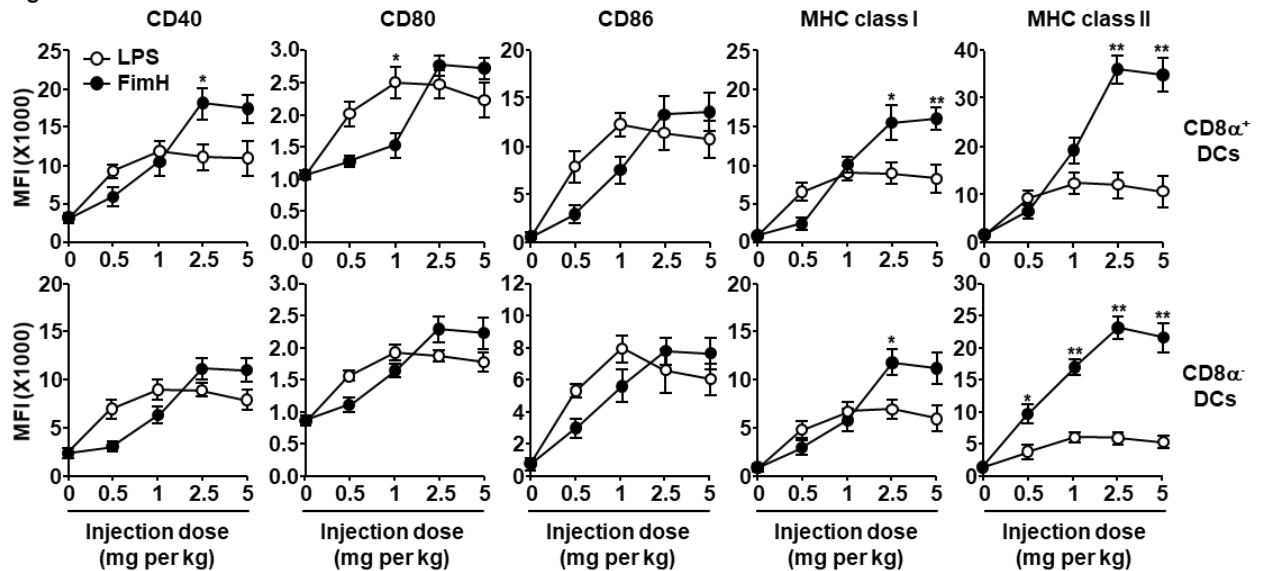
Fig. S2



Supplementary figure 2. Definition of dendritic cells (DCs) in inguinal lymph node (iLN). iLN was harvested from C57BL/6, TLR2-KO and TLR4-KO mice and the cells were

stained with a lineage marker and CD11c. The lineage markers included were anti-B220, anti-CD3, anti-CD49b, anti-Gr1, anti-Thy1.1, and anti-TER-119. Lineage⁻CD11c⁺ live leukocytes were defined as DCs. The DCs were further divided into CD8 α ⁺ and CD8 α ⁻ DCs.

Fig. S3

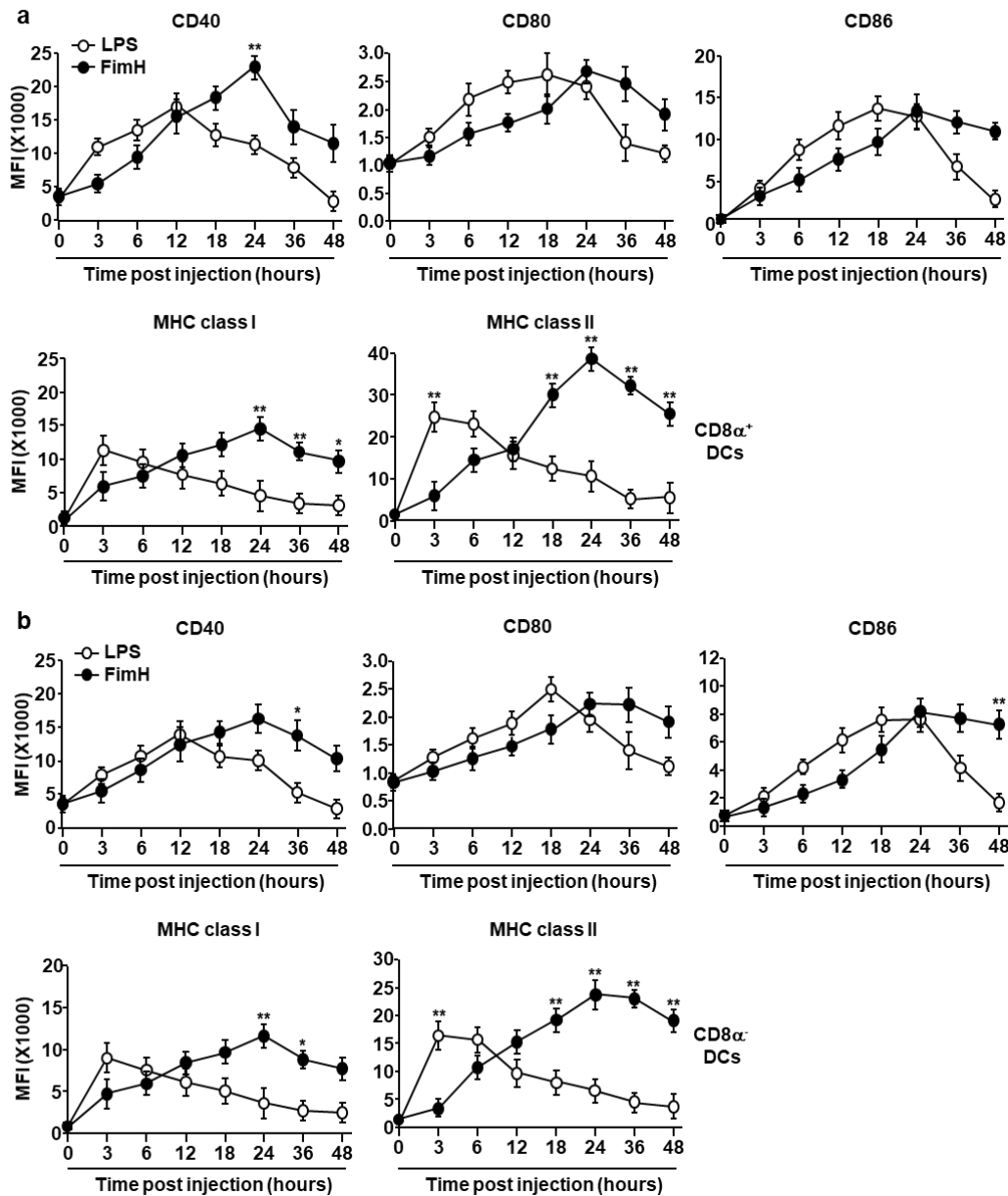


Supplementary figure 3. Dose-dependent effect of FimH in mouse DC activation.

C57BL/6mice were injected subcutaneously (*s.c.*) with the indicated dose of FimH or LPS for 24 h and the iLN was harvested. The expression level of costimulatory molecules and MHC classes I and II in CD8 α ⁺ DCs (upper panel) and CD8 α ⁻ DCs (lower panel) are shown. n = 4.

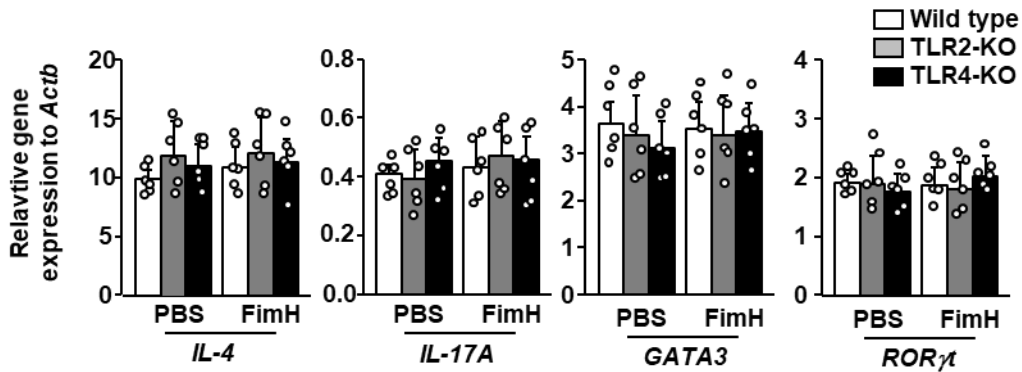
All data are presented as mean \pm SEM. **p* < 0.05, ***p* < 0.01.

Fig. S4



Supplementary figure 4. Time-dependent effect of FimH on the activation of mouse iLN DCs. C57BL/6 mice were injected *s.c.* with 2.5 mg per kg of FimH and 1 mg per kg of LPS and iLN was harvested as indicated by the time points post-injection. The expression level of costimulatory molecules and MHC classes I and II in (a) CD8 α^+ DCs and (b) CD8 α^- DCs are shown. $n = 4$. All data are presented as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$.

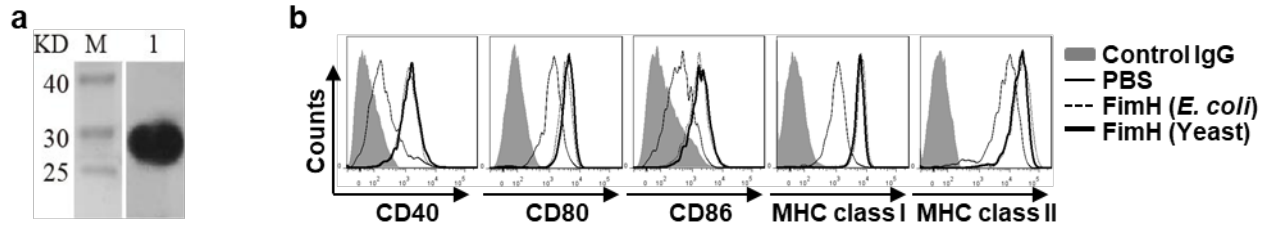
Fig. S5



Supplementary figure 5. FimH does not promote Th2 and Th17 immune responses.

C57BL/6, TLR2-KO, and TLR4-KO mice were injected with 2.5 mg per kg FimH. Twenty-four hours after treatment, the iLN was harvested and analyzed to estimate mRNA levels. n = 6. All data are presented as mean \pm SEM.

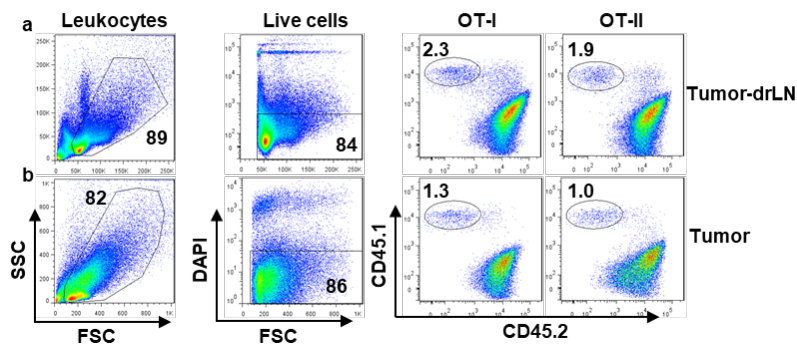
Fig. S6



Supplementary figure 6. FimH from yeast had induced activation of DCs. FimH was

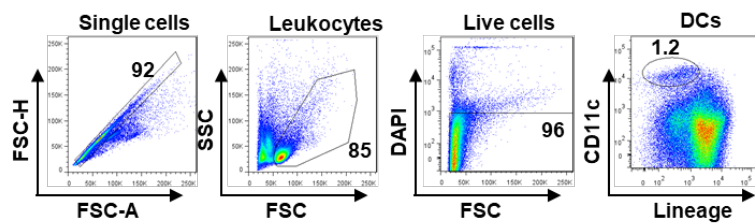
purified from yeast, as indicated in the “Materials and Methods” section. **a** Lane 1 demonstrates the recombinant FimH extracted from yeast, M = molecular weight marker. **b** The FimH from *E. coli* or yeast were injected to C57BL/6 mice and DC activation was analyzed in iLN. The expression of costimulatory molecules and MHC class I and II are shown. n = 6. All data are presented as mean \pm SEM.

Fig. S7



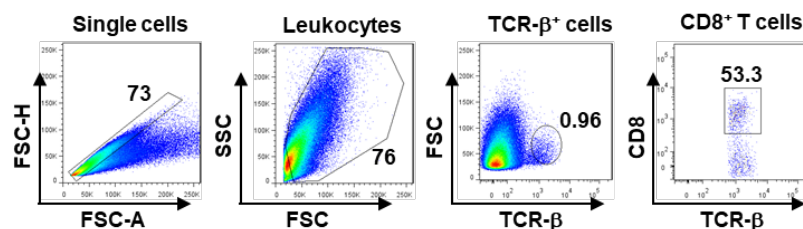
Supplementary figure 7. Flow cytometry analysis of OT-I and OT-II cells in tumor-draining lymph node and tumor. Analysis strategy of flow cytometry for detection of CD45.1-expressing OT-I and OT-II cells in CD45.2 congenic mice (a) tumor-drLN and (b) tumor was shown.

Fig. S8



Supplementary figure 8. Definition of DCs in mediastinal lymph node (mLN). mLN was harvested from C57BL/6 and the cells were stained as shown in supplemental figure 2. Lineage⁻CD11c⁺ live leukocytes were defined as DCs in the mLN.

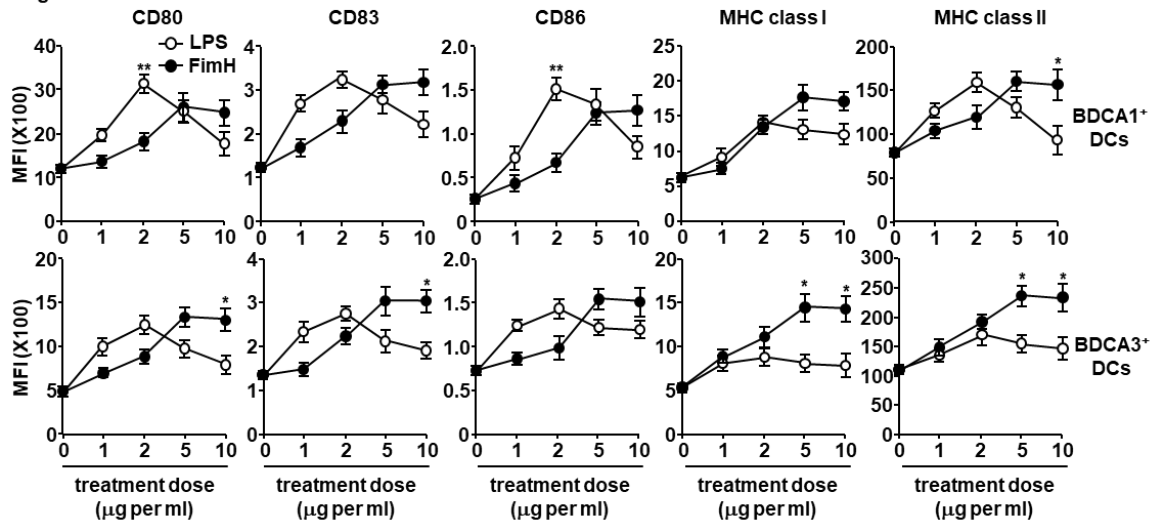
Fig. S9



Supplementary figure 9. Gating strategy for tumor-infiltrated CTLs by combination treatment of FimH and anti-PD-L1 antibodies. CTLs in CT26 tumors were analyzed by

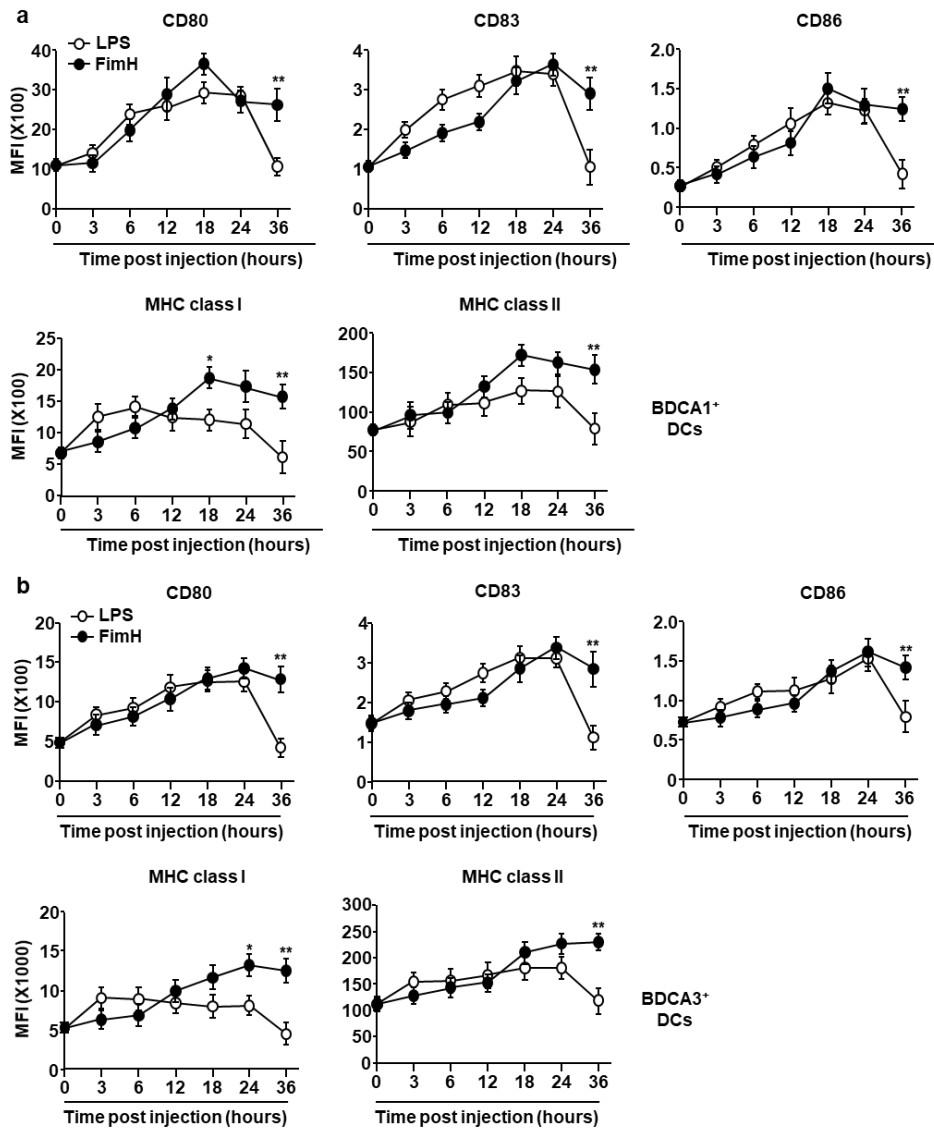
flow cytometry. Strategy of flow cytometry analysis for CTLs in CT26 tumor was shown.

Fig. S10



Supplementary figure 10. Dose-dependent effect of FimH in the activation of human peripheral blood DCs (PBDCs) in a dose-dependent manner. Peripheral blood mononuclear cells (PBMCs) were treated with the indicated dose of FimH or LPS for 24 h. The expression levels of costimulatory molecules and MHC classes I and II in BDCA1⁺ (upper panel) and BDCA3⁺ DCs (lower panel) are measured by flow cytometry. n = 4. All data are presented as mean ± SEM. **p* < 0.05, ***p* < 0.01.

Fig. S11



Supplemental figure 11. Time-dependent effect of FimH in the activation of human PBDC. PBMCs were treated with 5 μ g per ml of FimH and 2 μ g per ml of LPS, and were harvested as indicated by the time point. The expression levels of costimulatory molecules and MHC classes I and II in (a) BDCA1⁺ DCs n = 4 and (b) BDCA3⁺ DCs n = 4 are shown. All data are presented as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$.