Supplementary figure legends

Figure S1. Flow cytometry analysis of individual surface marker expression of the iDC and mDC. For detection of the expression of individual surface markers, including CD11c, CD40, CD80, and CD86, the iDC and mDC were either stained with antibodies of each marker or isotype-matched control antibodies or remained unstained, followed by flow cytometry analysis. The representative data of each analysis were represented by both dot and solid curve plots with the boxes and horizontal lines indicating the position of the gating based on the unstained cells (the level of background fluorescence), respectively. The number of cells for a gated region was expressed as the percentage of all analyzed cells. The percentage of cells positive for the indicated markers was calculated by subtracting that of the gated cells stained with isotype antibodies (the level of non-specific binding of antibodies of each marker) from that of the gated cells stained with marker antibodies, and was shown in the upper right corner of the bottom plots merging the data of unstained cells (black in color) and cells stained with either isotype (cyan in color) or marker antibodies (orange and red in color for iDC and mDC, respectively). Abbreviations: iDC, immature DC; mDC, mature DC.

Figure S2. Flow cytometry analysis of concurrent surface marker expression of the mDC. For detection of the concurrent expression of surface markers, including CD11c, CD40, CD80, and CD86, the mDC were either stained with antibodies of CD11c together with CD40, CD80, and CD86, respectively, or isotype-matched control antibodies or remained unstained, followed by flow cytometry analysis. Shown were representative data of each analysis with vertical and horizontal lines indicating the position of the gating based on the unstained cells (the level of background fluorescence). The number of cells for each gated region (Q1 to Q4) was expressed as the percentage of all analyzed cells. The percentage of cells positive for both indicated markers (double positive) was calculated by subtracting that of the upper right gated cells stained with isotype antibodies (the level of non-specific binding of antibodies of both markers) from that of the upper right gated cells stained with both marker antibodies, and was shown in the upper right corner of the bottom plots merging the data of unstained cells (black in color) and cells stained with either isotype (cyan in color) or marker antibodies (red in color). Abbreviations: mDC, mature DC.

Figure S3. Flow cytometry analysis of antigen uptake capacity of the iDC and mDC. To assess the antigen uptake capacity, the iDC and mDC were either remained untreated or incubated with FITC-dextran at 37℃ to allow for phagocytosis or on ice to stop phagocytosis, followed by flow cytometry analysis. The data of each analysis were represented by both dot and solid curve plots with the boxes and horizontal lines indicating the position of the gating based on the untreated cells (the level of background fluorescence), respectively. The number of cells for a gated region was expressed as the percentage of all analyzed cells. The percentage of cells that had successfully phagocytosed dextran was calculated by subtracting that of the gated cells incubated with FITC-dextran on ice (the level of non-phagocytic binding of dextran) from that of the gated cells incubated with FITC-dextran at 37℃, and was shown in the upper right corner of the bottom plots merging the data of untreated cells (black in color) and cells incubated with FITC-dextran on ice (cyan in color) or at 37℃ (red in color). Shown was the representative result of three independent experiments. Abbreviations: iDC, immature DC; mDC, mature DC.

Figure S4. Examination of tumor histopathology of each treatment group of mice. Tumor histopathology of the orthotopic HCC mice following treatment with the mDC (1×10^6 cells/dose) and/or anti-PD-L1 (100 or 200 μg/dose) was examined by H&E staining. Shown were representative results of six mice (denoted as #1 to #6) in each treatment group. Original magnification, ×20. Scale bar, 50 μm.

Figure S5. Evaluation of DC infiltration in tumors of each treatment group of mice. The infiltration of DC in tumor tissues of the orthotopic HCC mice following treatment with the mDC (1×10^6 cells/dose) and/or anti-PD-L1 (100 or 200 μg/dose) was detected by fluorescent IHC staining of CD11c. DC that were positive for CD11c (green in color) were indicated by white arrows. Nuclei were stained with DAPI (blue in color). Shown were representative results of six mice (denoted as #1 to #6) in each treatment group. Original magnification, ×40. Scale bar, 50 μm.

Figure S6. Quantitative analysis of DC in tumors of each treatment group of mice. Graph showing the number of DC (CD11c-positive cells) per microscopic field (original magnification, ×40) in tumor tissues of the orthotopic HCC mice following

treatment with the mDC (1×10^6 cells/dose) and/or anti-PD-L1 (100 or 200 μg/dose). The horizontal lines represented the mean values. The number of DC per field in each group of mice (n=6) was shown as mean±SEM and median (range). The significance of the difference of DC number per field between different treatment groups of mice was analyzed and compared with the control group of mice. A *P* value < 0.05 was considered significant. Abbreviations: mDC, mature DC; vs, versus.

Figure S7. Evaluation of cytotoxic T cell infiltration in tumors of each treatment group of mice. The infiltration of cytotoxic T cells in tumor tissues of the orthotopic HCC mice following treatment with the mDC (1×10^6 cells/dose) and/or anti-PD-L1 (100 or 200 μg/dose) was detected by fluorescent IHC staining of CD3 together with CD8. Cytotoxic T cells that were double positive for CD3 (green in color) and CD8 (red in color) appeared yellow and were indicated by white arrows. Nuclei were stained with DAPI (blue in color). Shown were representative results of six mice (denoted as #1 to #6) in each treatment group. Original magnification, ×40. Scale bar, 50 μm.

Figure S8. Quantitative analysis of cytotoxic T cells in tumors of each treatment group of mice. Graph showing the number of cytotoxic T cells (CD3/CD8 double-positive cells) per microscopic field (original magnification, ×40) in tumor tissues of the orthotopic HCC mice following treatment with the mDC (1×10^6 cells/dose) and/or anti-PD-L1 (100 or 200 μg/dose). The horizontal lines represented the mean values. The number of cytotoxic T cells per field in each group of mice (n=6) was shown as mean±SEM and median (range). The significance of the difference of cytotoxic T cell number per field between different treatment groups of mice was analyzed and compared with the control group of mice. A P value < 0.05 was considered significant. Abbreviations: mDC, mature DC; vs, versus.

Figure S9. Detection of granzyme B-positive cells in tumors of each treatment group of mice. The granzyme B-positive cells (green in color) in tumors of the orthotopic HCC mice following treatment with the mDC (1×10^6 cells/dose) and/or anti-PD-L1 (100 or 200 μg/dose) was detected by fluorescent IHC staining as indicated by white arrows. Nuclei were stained with DAPI (blue in color). Shown were representative results of six mice (denoted as #1 to #6) in each treatment group.

Original magnification, ×40. Scale bar, 50 μm.

Figure S10. Quantitative analysis of granzyme B-positive cells in tumors of each treatment group of mice. Graph showing the number of granzyme B-positive cells per microscopic field (original magnification, ×40) in tumor tissues of the orthotopic HCC mice following treatment with the mDC (1×10^6 cells/dose) and/or anti-PD-L1 (100 or 200 μg/dose). The horizontal lines represented the mean values. The number of granzyme B-positive cells per field in each group of mice (n=6) was shown as mean±SEM and median (range). The significance of the difference of granzyme B-positive cell number per field between different treatment groups of mice was analyzed and compared with the control group of mice. A P value < 0.05 was considered significant. Abbreviations: mDC, mature DC; vs, versus.