Supplementary Figure 1



B Pro-monocytes (CD115⁺ Ly6C⁺) sorted Cultured for 4 days in GM-CSF



DC isolation, sorting and culturing. (A) Bone marrow monocyte-derived DC (BMDC) were initially enriched using EasySep negative selection kits followed by sorting FSC^{low}/ SSC^{Iow}/Ly6C⁺/CD115⁺ pro-monocytes. Blood monocyte-derived DC (MoDC) were initially enriched from PBMC using Miltenyi CD11b positive selection kit, or EasySep negative selection kits followed by sorting FSC^{low}/SSC^{low}/Ly6C⁺/CD115⁺ cells. Cells were then cultured for 4-5 days with 50ng/mL GM-CSF. Spleen DC were enriched using Miltenyi's Pan-Dendritic Cell negative selection kit followed by FACS sorting of FSClow/SSClow/CD11c+/MHC Ilhigh cells and were used immediately in subsequent assays. Tumor-associated DC (TADC) were enriched by Miltenyi CD11b positive selection kit followed by FACS sorting FSClow/SS-Clow/Ly6Cneg/CD11c+/MHC IIhigh cells. Cells were used fresh or were cultured for 4-5 days with GM-CSF prior to their activation. (B) Standard BMDC preparation resulting from cell sorting of pro-monocytes followed by culture for 4-5 days in GM-CSF. (C) Standard yield of DC populations from the spleen using mechanical disruption through a cell strainer. Arrows denote sequential gating.



BMDC activated with allolgG-IC induce only minimal CD8⁺ T cell proliferation. Related to Figure 2D. Bone marrow DC (BMDC), patrolling blood monocyte-derived DC (Patrolling DC), inflammatory blood monocyte-derived DC (Inflammatory DC), or tumor-associated DC (TADC) subsets were activated with 129/Sv allogeneic IgG-B16 tumor cell immune complexes (alloIgG-IC) overnight, washed, and incubated with splenic CD8⁺ T cells, which were assessed for proliferation rates. Statistical significance was determined by two-way ANOVA with Tukey's multiple comparisons test. * denotes p<0.05, ** denotes p<0.01, **** denotes p<0.001.



DC derived from sorted cells above

Similar to BMDC, bone marrow monocytes are directly activated by allolgG-IC, while blood monocytes are not. Related to Figures 3A&B. (A) Flow cytometry analysis of MHC II and CD86 expression on DC subsets, including bone marrow monocyte-derived DC (BMDC), following overnight incubation with 129/Sv allogeneic IgG-B16 tumor cell immune complexes (allolgG-IC). Graphs show the percentages of cells expressing both MHC II and CD86 and the levels of IL-12 in the supernatants. (B) Percentages of DC, including mobilized hematopoeitic stem cell (HSC)-derived DC, expressing both MHC II and CD86 following overnight incubation with allolgG-IC. Statistical significance was determined by two-way ANOVA with Tukey's multiple comparisons test. * denotes p<0.05, ** denotes p<0.01, *** denotes p<0.001.

Supplementary Figure 4



Similar to BMDC, spleen DC exhibit activation of MAPK and PI3K/Akt pathways following stimulation with allolgG-IC. Related to Figures 4A&B. (A) Histograms showing phosphorylated pERK1/2, pJNK and (p)-p38 levels in spleen DC incubated with C57Bl/6 allogeneic IgG-LMP tumor cell immune complexes (allol-gG-IC). Graph shows arcsinh ratios of phospho-species in splenic DC incubated for 15 min with LMP tumor cells or allolgG-IC over baseline levels from unstimulated DC. (B) pAkt levels in splenic DC incubated with tumor cells or allolgG-IC, as measured by PathScan® Intracellular signaling array. Graph shows the pixel density of pAkt in activated DC. Statistical significance was determined by two-way ANOVA with Sidak's multiple comparisons test. * denotes p<0.05, ** denotes p<0.01, *** denotes p<0.001, **** denotes p<0.0001.

Supplementary Figure 5



С



Dual inhibition of SHP-1 and SHIP-1 enables MoDC activation in response to allolgG-IC. Related to Figures 6F,G&I. (A) Phosphorylated Akt levels in bone marrow monocyte-derived DC (BMDC) and blood monocyte-derived DC (MoDC) incubated with tumor cells or 129/Sv allogeneic IgG-B16 tumor cell immune complexes (alloIgG-IC) in the presence of SHIP-1 and SHP-1/2 inhibitors. Dot plots show arcsinh ratios of pAkt in DC incubated for 10 min with allolgG-IC over levels in tumor cell-stimulated DC. (B) Confocal images of CFSE-labeled tumor cell uptake by MoDC after overnight incubation with allolgG-IC with and without SHIP-1 and SHP-1/2 inhibitors. (C) Confocal images of CFSE-labeled tumor cell uptake by BMDC isolated from WT or FcyRIIb KO mice after overnight incubation with IgG-IC. Images were acquired with a 40X objective.