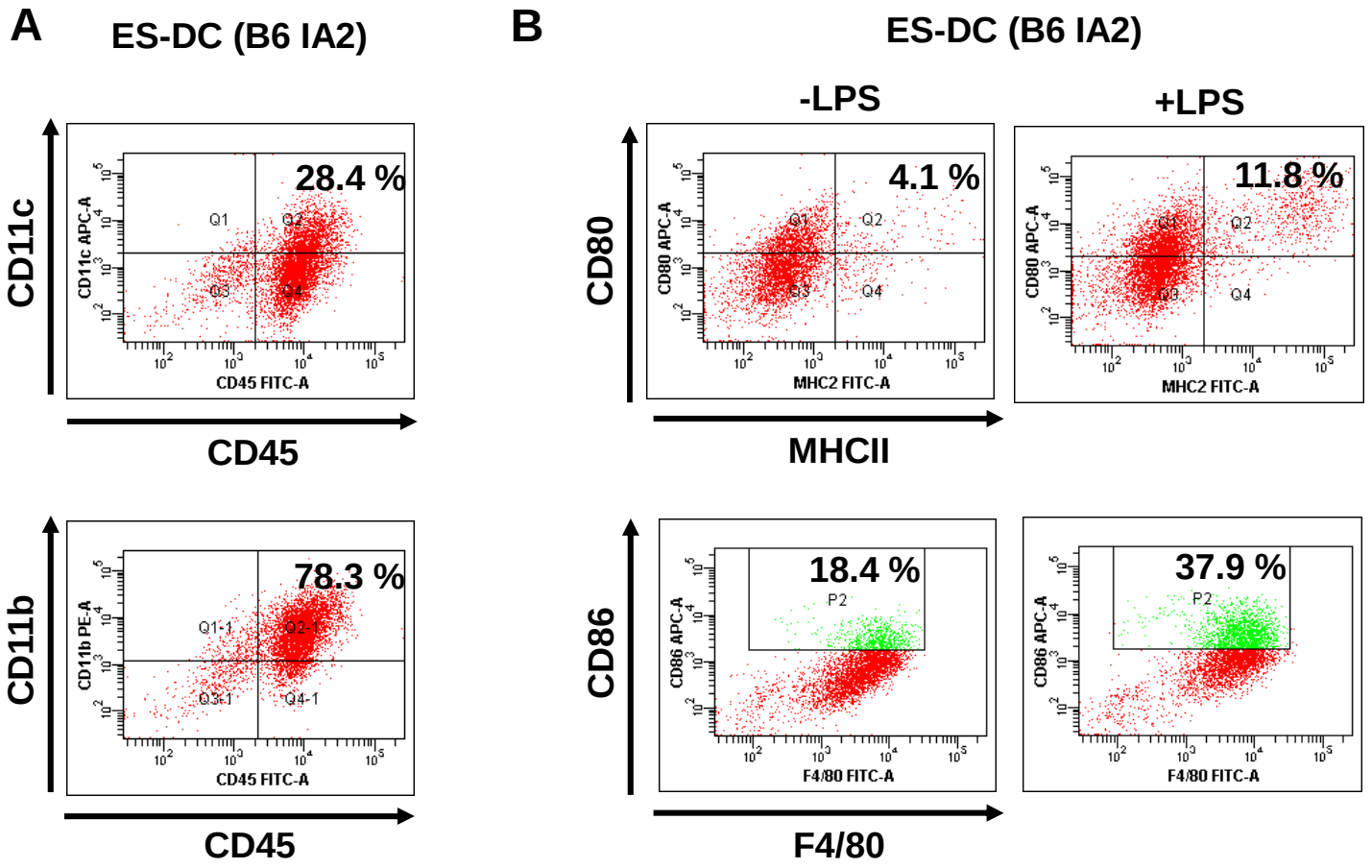
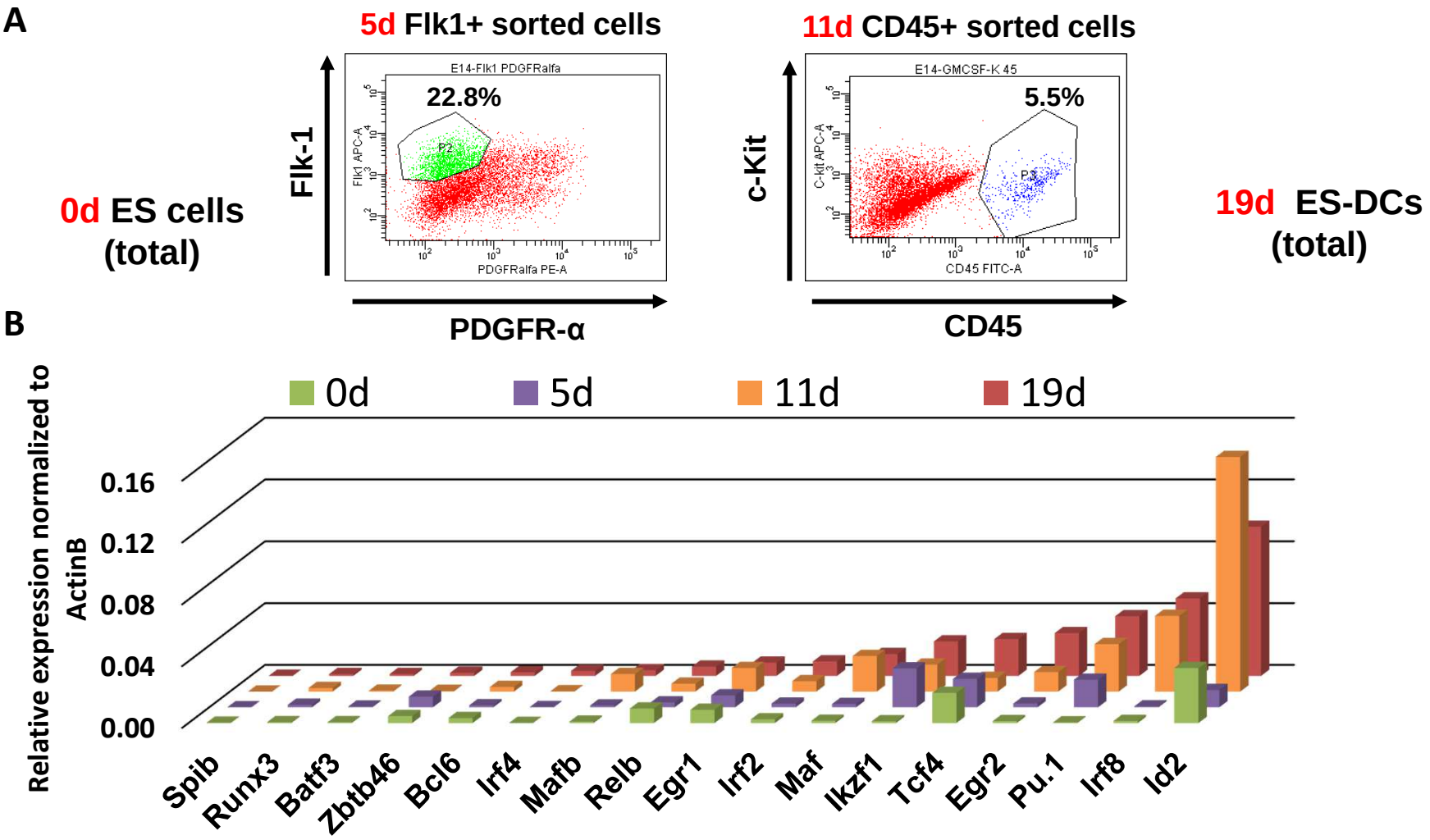
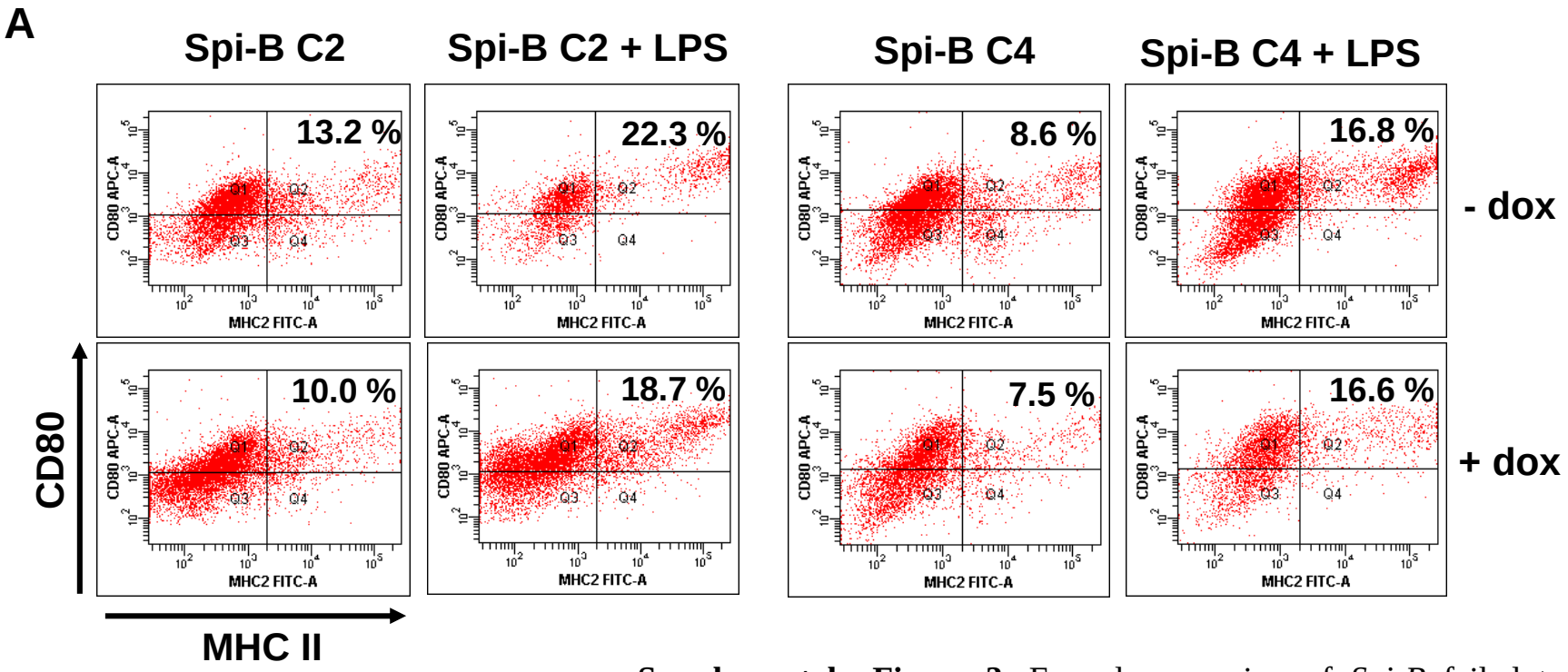


Supplemental Figure 1. Representative cell surface phenotype of ES-DCs derived from B6 IA2 (genetic background: C57BL/6) ES cells. Flow cytometric profiles representative of at least three independent experiments are shown. (**A**) 19 day-differentiated, immature ES-DCs were monitored by flow cytometry (CD45, CD11b and CD11c). (**B**) 19 day-differentiated, ES-DCs were analyzed by flow cytometry (MHCII, CD80. F4/80 and CD86). The indicated cells were treated with 100 ng/ml LPS for 24 hours before harvesting.



Supplemental Figure 2. Gene expression profiles of the selected DC affiliated transcription factors upon ES-DC development. (**A**) 5 day-cultured Flk1 single positive mesodermal and 11-day cultured CD45 positive myeloid cells were separated by FACS for transcript analysis. Representative FACS profiles are presented. In addition, RNAs were obtained from unsorted (total) ES cells and 19-day differentiated ES-DCs. (**B**) Relative expression of 17 DC specific genes was determined by quantitative real-time RT-PCR. Expression data were obtained from 0 (ES cells), 5 (Flk1+ mesodermal cells), 11 (CD45+ myeloid cells) and 19 day-differentiated cells (ES-DCs). Expression was normalized to beta-actin. The average gene expression level was determined from five independent experiments.



Supplemental Figure 3. Forced expression of *Spi-B* failed to modify the maturation capacity of ES-DCs. *Spi-B* transgenic ES cells were differentiated for 19 days. (**A**) *Spi-B* inducible cells were treated with 1 µg/ml doxycycline (+dox). In addition, the indicated cells were treated with LPS (100 ng/µl) at day 18. Cell surface expression of CD80 and MHCII was assessed by flow cytometry at day 19. Representative flow cytometric data were obtained from two *Spi-B* transgenic cell clones (C2 and C4). (**B**) *Spi-B* inducible cells were differentiated and the expression of CD80 and MHCII was assessed as figure part A. The average percent of the CD80/MHCII double positive cells and the SD values were calculated from 6 independent experiments. Significant differences between mean values were evaluated using two-tailed, unpaired Student's t test (NS: $p > 0.05$).

Gene symbol	Assay ID
Batf3	Mm 01318274_m1
Bcl6	Mm00477633_m1
Beta-actin	Mm01205647_g1
Egr1	Mm00656724_m1
Egr2	Mm00456650_m1
Id2	Mm00711781_m1
Ikzf1	Mm01187877_m1
Irf2	Mm00515204_m1
Irf4	Mm00516431_m1
Irf8	Mm00492570_m1
Maf	Mm02581355_s1
Mafb	Mm00627481_s1
Relb	Mm 01305800_m1
Runx3	Mm00490666_m1
Sfpi1 (Pu.1)	Mm00488142_m1
Spi-B	Mm03048233_m1
Tcf4	Mm00443210_m1
Zbtb46	Mm00511327_m1

Supplemental Table 1. Taqman gene expression assays for mRNA transcript detection.