



Figure S2. MHC class II contribution to SEB-mediated activation of NF-AT, NF-κB and AP-1 transcription factors. (A) FACS analysis of 5.3-1/B7 cells stained with FITC-conjugated isotype control (Iso), anti-B7.1-FITC Abs or anti-HLA-DR-FITC Abs. (B-D) CD28WT cells were transfected with 5 μg GFP together with 10 μg NF-AT (B) or 2 μg NF-κB (C) or 10 μg AP-1 (D) luciferase constructs and then stimulated for 6 hours with Dap3/B7 or 5.3-1/B7 cells in the absence (Med) or presence of 1 μg ml⁻¹ SEB. Luciferase activity was measured and fold induction (F.I.) over the basal level of unstimulated cells was calculated after normalization of GFP values. Bars show the mean ± SEM and statistical significance was calculated by Student's *t* test. Mean values: NF-AT, Dap3/B7 = 0.9, Dap3/B7 SEB = 12, 5.3-1/B7 = 0.8, 5.3-1/B7 SEB = 12.4; NF-κB, Dap3/B7 = 4.2, Dap3/B7 SEB = 8.6, 5.3-1/B7 = 5.2, 5.3-1/B7 SEB = 7.3; AP-1, Dap3/B7 = 2.6, Dap3/B7 SEB = 7.3, 5.3-1/B7 = 2.6, 5.3-1/B7 SEB = 5. (E, F) CD28WT cells were stimulated for 24 hours with Dap3/B7 or 5.3-1/B7 cells in the absence or presence of SEB. IL-2 (E) and IL-8 (F) levels in culture supernatant were measured by ELISA. Data show the mean ± SEM and statistical significance was calculated by Student's *t* test. Means values (pg ml⁻¹): IL-2, Dap3/B7 = 0, Dap3/B7 SEB = 420.7, 5.3-1/B7 = 4, 5.3-1/B7 SEB = 1000; IL-8, Dap3/B7 = 205.1, Dap3/B7 SEB = 2449, 5.3-1/B7 = 238.8, 5.3-1/B7 SEB = 1793. (*) *p* < 0.05, (**) *p* < 0.01, (***) *p* < 0.001, (****) *p* < 0.0001, NS = not significant.