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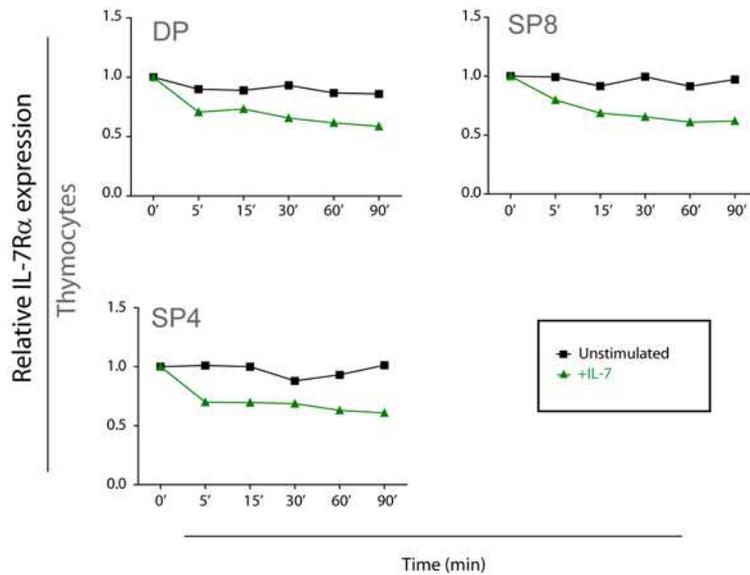
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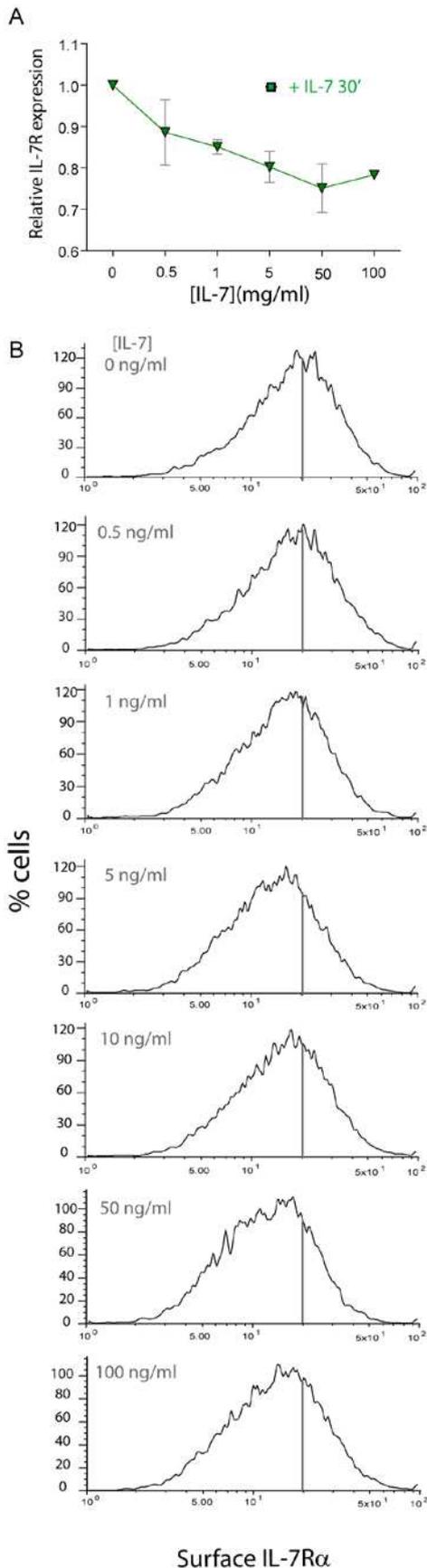


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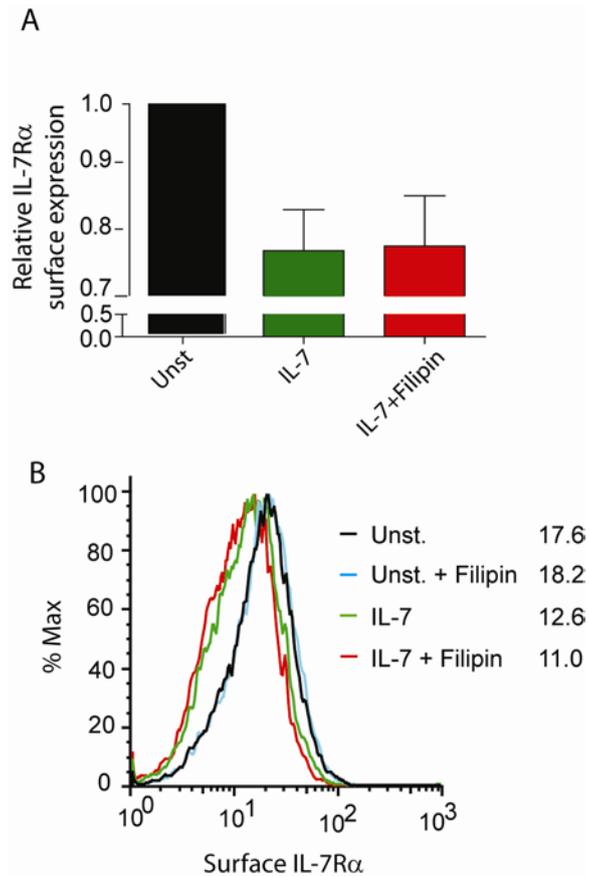
SUPPLEMENTARY FIGURES



Supplementary Figure 1. IL-7 induces rapid IL-7R α down-regulation in primary thymocytes: Primary thymocytes were isolated and cultured in the presence or absence of IL-7 (50ng/ml) in culture medium for the indicated time and subsequently analyzed by flow cytometry for surface IL-7R α expression, as described in 'Materials and Methods'. Thymocytes were incubated with anti-CD4 FITC-conjugated and CD8 APC-conjugated antibodies to determine the double positive (DP), CD4 single positive (SP4) and CD8 single positive (SP8) sub-populations. Relative IL-7R α expression was calculated as the geometric mean intensity of fluorescence normalized to the zero (unstimulated) time point. Results are representative of two independent thymuses.



Supplementary Figure 2. IL-7 induces surface IL-7R α downregulation in a dose-dependent manner. (A) HPB-ALL cells were cultured in the presence of increasing doses of IL-7 (0 to 100ng/ml) in culture medium for 30 minutes and subsequently analyzed by flow cytometry for surface IL-7R α expression, as described in ‘Materials and Methods’. Relative IL-7R α expression was calculated as the geometric mean intensity of fluorescence normalized to the zero (unstimulated) time point. Data are mean \pm sem from two independent experiments. (B) Representative flow cytometry histograms of IL-7R α surface expression in HPB-ALL cells stimulated for 30 minutes with IL-7 at the indicated concentrations. The vertical line in each histogram was arbitrarily set at the same value in all histograms to facilitate their visual comparison.



Supplementary Figure 3. IL-7-induced-IL-7R α internalization is lipid-raft independent. (A) HPB-ALL cells were cultured in the presence or absence of IL-7 (50ng/ml), with or without pre-treatment (1h) with the lipid-raft inhibitor Filipin (5 μ g/ml). Samples were analyzed by flow cytometry for surface IL-7R α expression, as described in ‘Materials and Methods’. Relative IL-7R α expression was calculated as the geometric mean intensity of fluorescence normalized to the zero (unstimulated) time point. Data are mean \pm sem from two independent experiments. (B) Representative flow cytometry histogram overlay of IL-7R α surface expression in HPB-ALL cells stimulated for 30 minutes with IL-7 (50ng/ml), with or without 1h of pre-treatment with Filipin (5 μ g/ml). The indicated values correspond to the geometric mean intensity of fluorescence for each condition.