



Supplemental Figure 1 – PECAM-1 cross-linking does not appear to significantly affect the phosphorylation or total cellular levels of I κ B α at late timepoints. HUVEC were stimulated with IL-1 β (1 ng/ml) + PAF (10⁻¹⁰M). After 4 hours, mAb Hec7 or mAb PECAM 1.3 was added and 30 minutes later were cross-linked with goat anti-mouse IgG. Cells were lysated at 5 hours, lysates were made, and western blots were obtained as described in Materials and Methods. Images at top of figure show representative blots from two independent experiments of phospho-I κ B α (top), total I κ B α antigen (middle), and α -tubulin (bottom). Bottom two graphs depict the levels of phospho-I κ B α and total I κ B α antigen, respectively, normalized to α -tubulin from two independent experiments. It does appear that PECAM-1 cross-linking with mAb Hec7 might slightly decrease levels of total I κ B α antigen at 5 hours, though these results were not statistically significant by one-way ANOVA analysis. If anything, these findings would indicate a more “active” state of NF κ B, thus lending further support that PECAM-1 engagement likely does not inhibit NF κ B activity in endothelial cells.