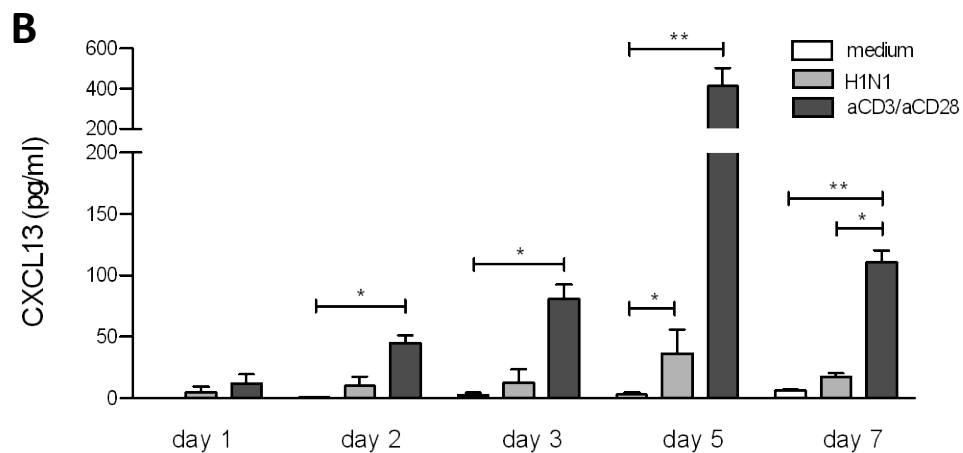
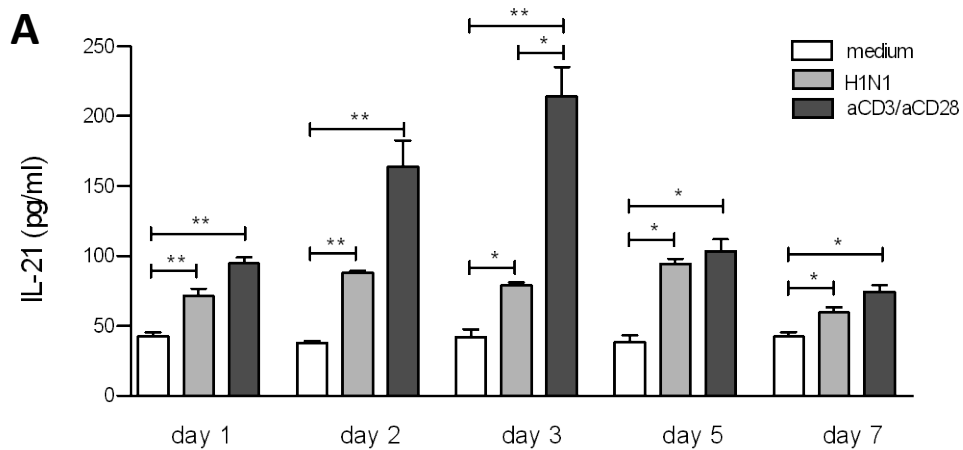
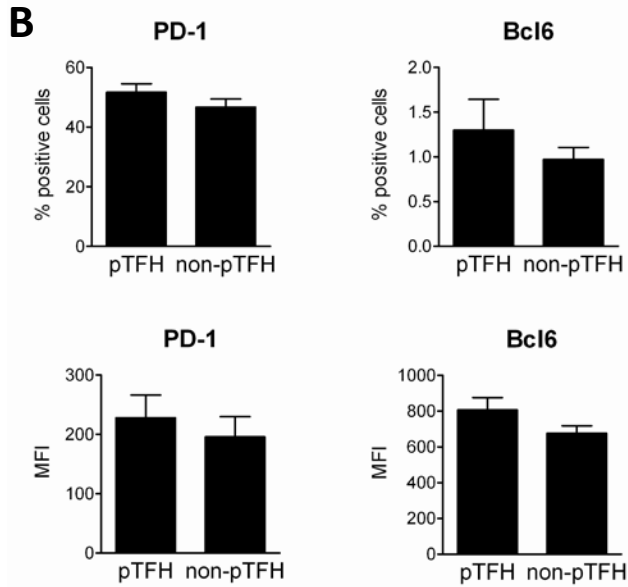
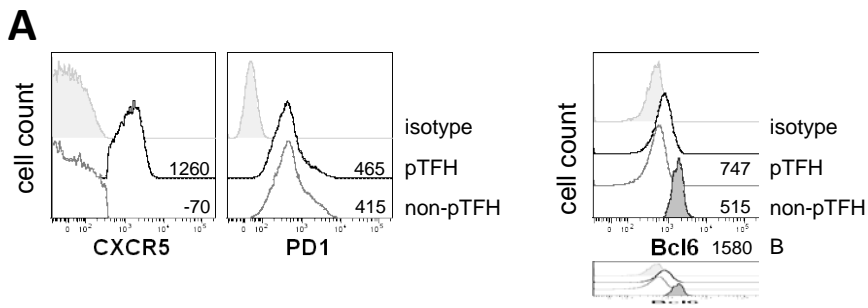


Supplemental Figure 1. Frequencies of live (ViViD^{neg}) CD20⁺ B cells are not significantly different after co-culture with pTFH or non-pTFH cells. Cryopreserved PBMC obtained 4 weeks after H1N1/09 vaccination were thawed and rested overnight. B cells (CD20⁺) were isolated with magnetic beads, while pTFH (CD3⁺CD4⁺CD45RA⁻CXCR5⁺) and non-pTFH (CD3⁺CD4⁺CD45RA⁻CXCR5⁻) cells were purified by cell sorting. B cells were co-cultured with either pTFH or non-pTFH cells at a 1:1 ratio in medium alone or in the presence of 5 µg/ml H1N1 vaccine Ag or 1 µg/ml SEB for 7 days. Cells were harvested on day 7 and stained with live dead cell dye ViViD along with other markers. Frequency of live (ViViD^{neg}) CD20⁺ within the B cells was evaluated by flow cytometry in the pTFH + B (**A**) or non-pTFH + B (**B**) cell co-cultures. Bars represent the mean and error bars indicate the standard deviation of 3 healthy controls (HC), 3 HIV⁺ H1N1/09 vaccine responders (R) and 3 HIV⁺ vaccine non responders (NR).



Supplemental Figure 2: Kinetics of cytokine and chemokine production in response to H1N1/09 antigen stimulation. Cryopreserved PBMC obtained 4 weeks after H1N1/09 vaccination were thawed, rested overnight and cultured for the indicated time in medium alone (open bars) or in the presence of 5 $\mu\text{g/ml}$ H1N1 antigen + 1 $\mu\text{g/ml}$ antiCD28 (light grey bars) or 0.1 $\mu\text{g/ml}$ antiCD3 + 1 $\mu\text{g/ml}$ antiCD28 (dark grey bars). Levels of IL-21 (**A**) and CXCL13 (**B**) in the culture supernatants were measured by ELISA. Bars represent the mean and error bars indicate the standard deviation of 3 healthy controls. * denotes a p value < 0.05, ** p < 0.01.



Supplemental Figure 3: Characterization of THF-associated markers. (A) Freshly isolated PBMC were stained for TFH-associated markers and analyzed by flow cytometry. Histograms representative of one donor show the expression of the indicated markers in peripheral TFH (pTFH, CD4⁺CXCR5⁺ cells, black line) and non pTFH (CD4⁺CXCR5⁻ cells, grey line) cells. Isotype antibodies (filled gray line) were used as negative controls. CD38⁺ B cells (CD20⁺CD38⁺ cells, filled black line) were used as positive control for Bcl6 detection. Mean fluorescence intensity values are indicated for each marker and cell population. **(B)** Graphs show percentage of positive cells (top graphs) and mean fluorescence intensity (MFI, bottom graphs) for the markers analyzed, in both pTFH and non pTFH cells. Graphs summarize the mean standard deviation of 6 healthy controls.