Supplementary Figure 1. *C. albicans*-primed naïve T cells acquire a mixed phenotype. (a) Naïve CD4⁺ T cells from healthy donors were labeled with CFSE and cultured with autologous monocytes pulsed with heat-inactivated *C. albicans*. CFSElo proliferating T cells were expanded with IL-2. (b) On day 17, CFSElo cells were restimulated for 5h with PMA and ionomycin and analyzed for IL-17, IL-4 and IFN-γ production by intracellular staining. Viable cytokine-producing cells were then isolated using the MACS Cytokine Secretion Assay.
Supplementary Figure 2. Phosphoprotein antibody profiling identifies sustained ERK phosphorylation in activated Th17 cells. (a-b) Three Th17 (a) and Th2 (b) clones were either left resting or were stimulated for 5 days with plate-bound anti-CD3 (5μg/mL) and anti-CD28 (1μg/mL) antibodies prior protein extraction and analysis of protein phosphorylation. Protein lysate and analysis of phosphorylated proteins was performed using a proteome profiler human phospho-kinase antibody array (ARY003B, R&D systems) following exactly manufacturer’s instructions. Shown is the ratio in phospho-protein signal between activated (day 5) and resting (day 0) cells (mean ± s.e.m.). (c) Same data as in (a-b), except that the phospho-protein signal raw values are shown for those proteins that showed differences in the two states (resting and activated). Results for ERK1/2 are highlighted by a red box.
Supplementary Figure 3. MiR-181a expression modulates T cell responses towards tetanus toxoid. Memory T cells were sorted from peripheral blood, loaded with CFSE and stimulated with autologous monocytes in the presence of tetanus toxoid. Five days after stimulation, antigen-specific cells (CFSE-low) were recovered by sorting, divided into three aliquots and transfected with either a scrambled control, a miR-181a mimic or a miR-181a antagonir. Forty-eight hours after transfection cells were restimulated with autologous monocytes and increasing concentrations of tetanus toxoid, and proliferation was assessed four days later by $^3$H-thymidine incorporation assay.
Supplementary Figure 4. Culture of Th17 cells with repolarizing cytokines induces loss of the Th17 phenotype and modest IFN\(\gamma\) and IL-4 production. Individual C. albicans-specific central memory Th17 cells were cloned and expanded. Each clone was then divided into aliquots that were cultured under non-polarizing conditions (control) or with addition of IL-12 or IL-4. Percentage of cytokine-producing cells (IL-17, INF\(\gamma\) or IL-4) in individual clones was assessed upon stimulation with PMA and ionomycin by intracellular staining. Each dot represents one clone and subclones are connected by a line. A total of 45 clones were analyzed.
Supplementary Figure 5. T cell clones with identical Vβ CDR3 show intrinsic proliferative capacities that are dependent on the phenotype. \(C.\) \textit{albicans}–specific \(\text{T}_{\text{H}2}\) and \(\text{T}_{\text{H}17}\) cells were cloned and the TCR Vβ CDR3 region was determined by Sanger sequencing of Vβ-specific PCR products. Two clones were identified with identical CDR3 sequence but displaying a \(\text{T}_{\text{H}2}\) or \(\text{T}_{\text{H}17}\) phenotype, based on their ability to produce IL-4 or IL-17/IL-22 and to express \(GATA3\) or \(RORC\) mRNA, respectively (not shown) (Vβ CDR3 #1: CASSYVSQDKNEAFF; Vβ CDR3 #2: CATSRQTVGETQYF). (a) The identified clones were restimulated with increasing concentrations of \(C.\) \textit{albicans} lysate in the presence of autologous monocytes. Three days after stimulation, T cell proliferation was assessed by \(^{3}\text{H}\)-thymidine incorporation assay. (b) Total RNA was extracted from the same clones as in (a) and miR-181a expression was evaluated by TaqMan qRT-PCR (relative to RNU48 as endogenous control).