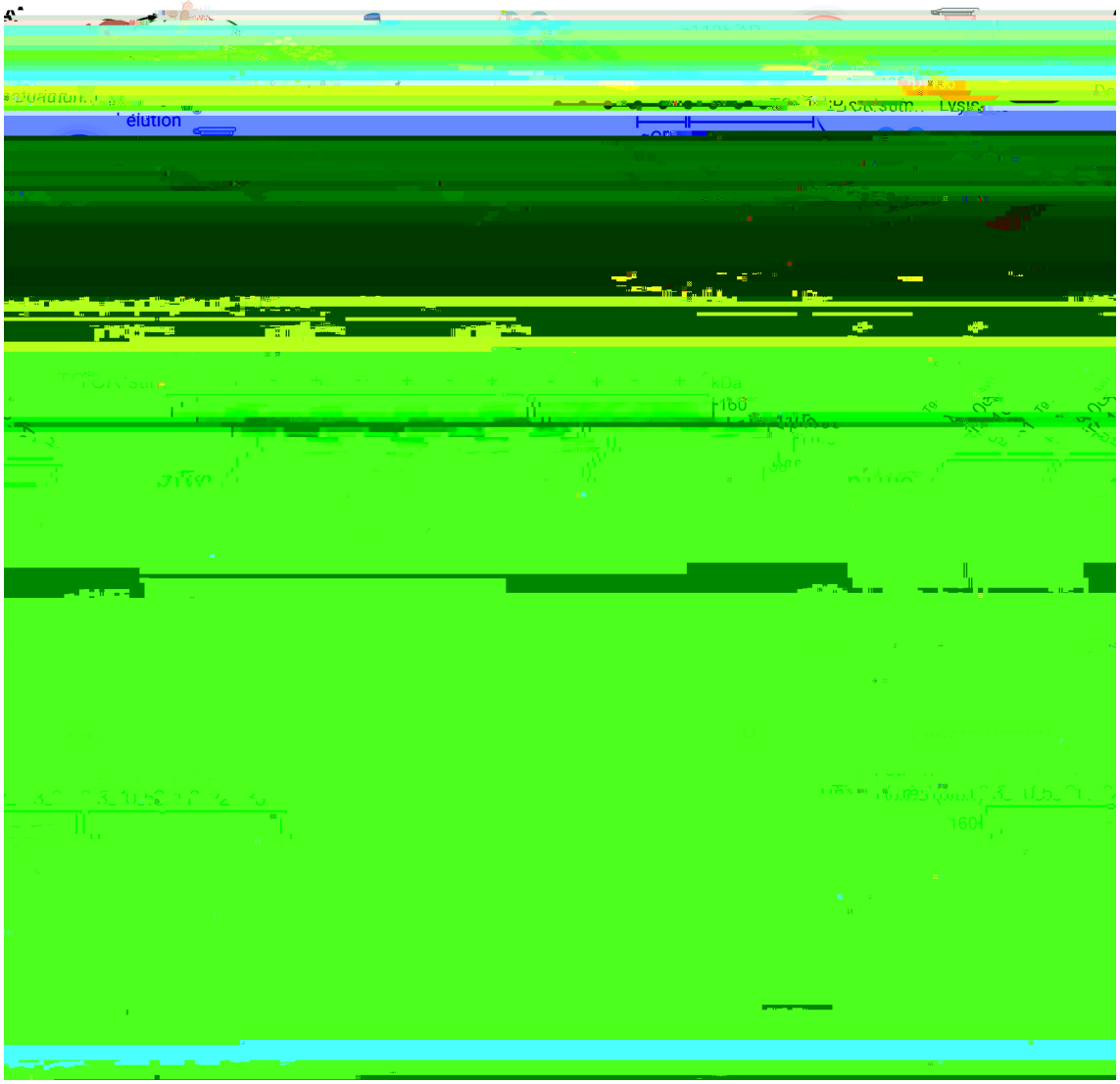
élution
Lysis
kDa
160
160

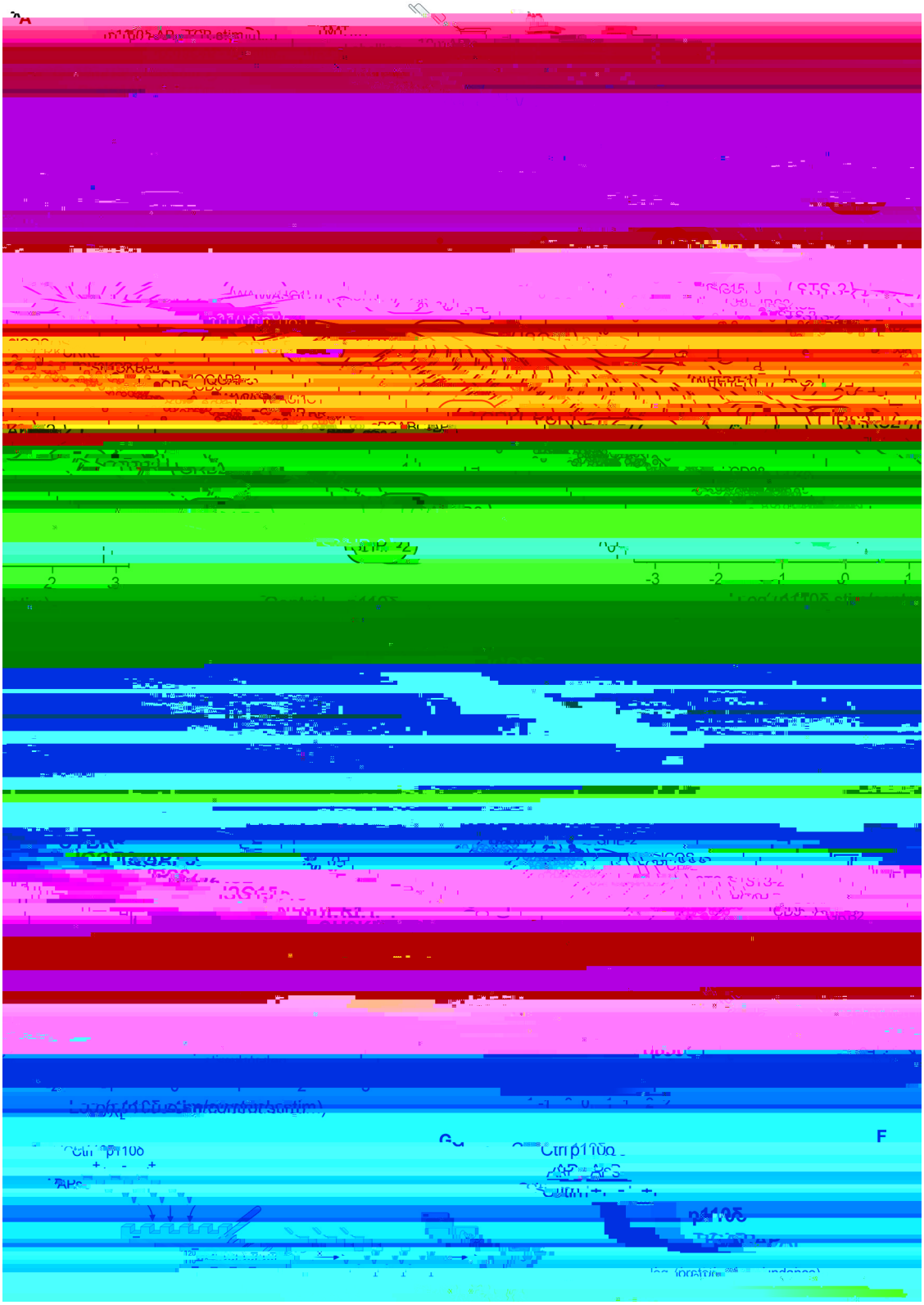

FIGURE 2 | The p110δ interactome in CD4$^+$ T cell blasts. (A) Schematic of samples analysed by quantitative MS. p110δ and control APs were produced from unstimulated and TCR-stimulated CD4$^+$ T cell blasts in three independent biological-repeat experiments (detailed in **Supplementary Figures 3A,B**). Peptides were

(Continued)

FIGURE 2 |

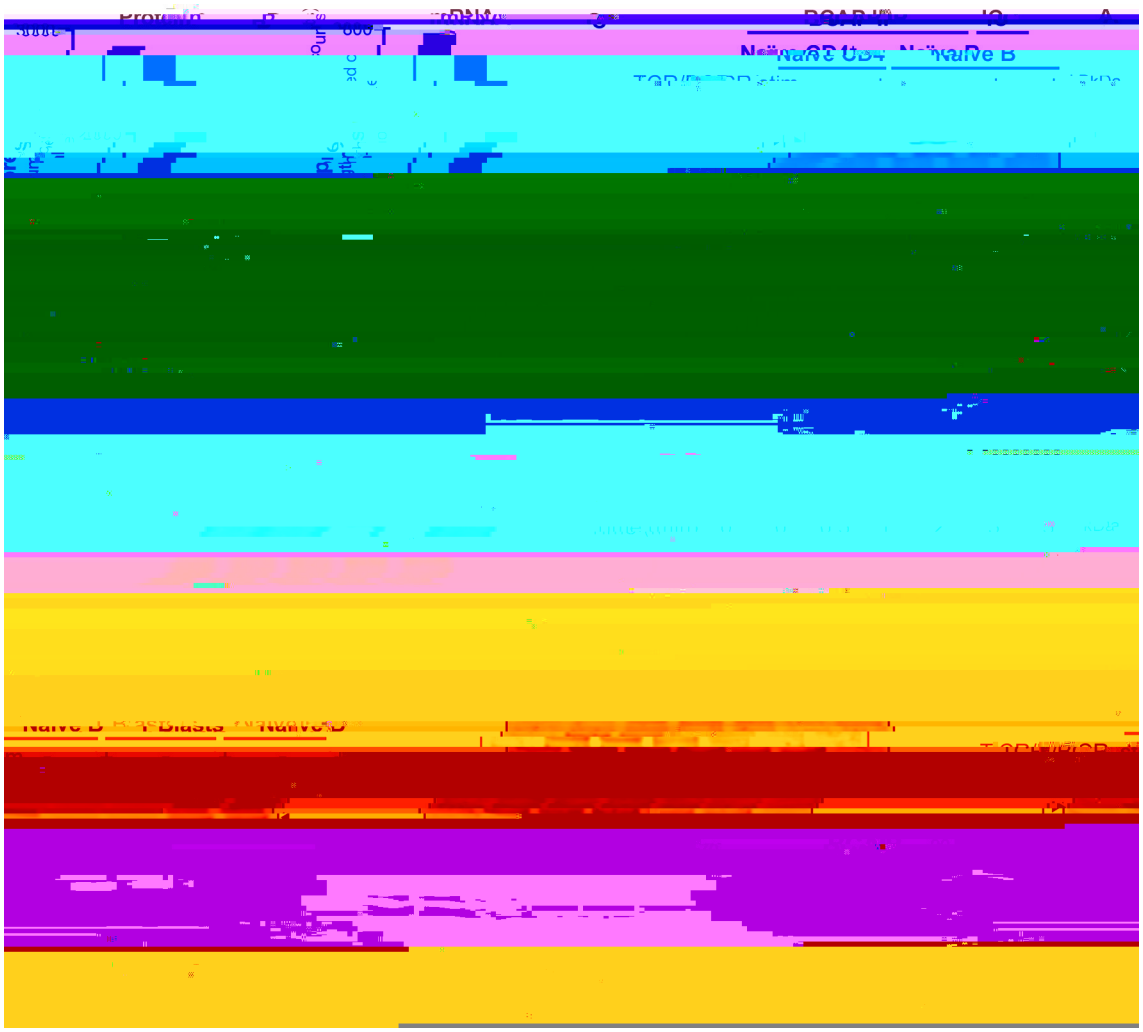

FIGURE 3 | BCAP is upregulated in activated CD4$^+$ T cells and tyrosine-phosphorylated upon TCR stimulation. (A) BCAP protein copy number in mouse naïve CD4$^+$ T cells, 24 h-antigen-activated OT-II CD4$^+$ T cells, and differentiated Th1, Th2, and Th17 CD4$^+$ T cells. Data are from ImmPRes [http://immpres.co.uk; (4)]. (B) Expression of Pik3ap1 mRNA in murine naïve splenic CD4$^+$ T cells, in vitro-activated and polarised Th1, Th2, and Th17 CD4$^+$ T cells, and splenic Tregs. Data are from Th-Express [https://th-express.org; (33)]. Expression levels in each subtype are calculated as read counts normalised by size factor and transcript length. (C) Immunoblot of immunoprecipitates (IPs) from naïve CD4$^+$ T cells and naïve splenic B cells using anti-BCAP (BCAP IP) or IgG isotype-control antibody (IC). Naïve CD4$^+$

downstream of the TCR (Figure 4C). The ability of Pik3ap1-knockout cells to nevertheless induce pAKT implies that the role of BCAP is redundant, but could also reflect an incomplete loss of BCAP protein.

These results support the hypothesis that BCAP positively regulates p110δ in $CD4^+$ T cell blasts following TCR engagement. Furthermore, the observations are consistent with the p110δ interactome, which revealed that multiple adaptor proteins are involved in the recruitment and activation of PI3Kδ during TCR signalling and therefore the elimination of just one of these adaptors would be expected to only partially reduce PI3Kδ signalling output. Finally, these experiments demonstrate that novel p110

FIGURE 5 | The p110δ interactome in naïve CD4$^+$ T cells. (A) Volcano plot of proteins identified by mass spectrometry in APs from naïve CD4$^+$ T cells. The plot shows the log_2-difference in abundance of each protein in p110δ APs compared to control APs from TCR-stimulated cells from three independent repeat experiments [Log_2(p110δ stim/control stim)] vs. the $–Log_{10}$-p value, determined by a two-tailed Student's t-test. Cells had been stimulated for 1 min by CD3-CD4-crosslinking. The thresholds used to determine specific p110δ-interactors (upper-right quadrant) are drawn at 1.5-fold enrichment and $p = 0.05$. Proteins of interest are represented by red points. Proteins identified as specific p110δ-interactors in CD4$^+$ T blasts that fall outside of the thresholds in naïve cells (CRKL, NHERF1) are filled dark grey. The fr6.934(N)-0.637146(H)-0.6(a)0.206547(d)-275.048(d)-279.0204797(r)0.108525(a)0.204797(c)0.20304.224051(r)0.15(r)0.10852504crol0.367584(t)0.465607(i)0.451604(m)02dactified

bind to a proline-rich region in the CD3ε chain that is exposed during TCR engagement (35), so it is possible that NCK could bridge BCAP and the TCR-CD3 complex to recruit p110δ to the TCR signalosome. Previous studies have shown that BCAP-deficient DT40 B cells, which do not express the PI3K-adaptor CD19, exhibit reduced PIP_3 production and AKT activation following BCR stimulation (29), although activation of PI3K and AKT appears to be unaffected in $BCAP^{-/-}$

may be explained as an indirect interaction via CBL, given that STS-2 binds constitutively to CBL in $CD4^+$ T cells via a direct SH3:proline-rich domain interaction (57, 59).

It was very interesting to find that p110δ associated constitutively with the multidomain adaptor proteins CRKL and SH3KBP1

Naïve B Cell Isolation and BCR Stimulation

Mouse spleens were homogenised through 40 μm cell strainers and red blood cells were lysed by incubation with Red Blood Cell Lysing Buffer (R7757, Sigma-Aldrich) for 5 min at room temperature. Naïve B cells were isolated by negative selection using a mouse B Cell Isolation Kit (130-090-862, Miltenyi Biotec) and LS magnetic columns (Miltenyi Biotec) according to the manufacturer's protocol.

Before BCR stimulation, purified naïve B cells were resuspended at 4×10^6 cells/ml in 0.5% FBS RPMI media and rested for 75 min at 37°C. Cells were collected by centrifugation then incubated at 2×10^7 cells/ml in 0.5% FBS RPMI containing 50 μg/mL goat anti-mouse IgM [$F(ab')_2$ fragment; 115-036-075, Jackson ImmunoResearch] for 30 min on ice. B cells were then stimulated by incubation at 37°C for 2.5 min. Cells were immediately centrifuged at 800 × g for 2 min at 4°C and then resuspended in ice-cold lysis buffer (100 μl per 1×10^7 cells), incubated on ice for 10 min and cleared by centrifugation at 20,000 × g for 10 min at 4°C.

Affinity Purification

Fresh, cleared lysates from 1×10^8 T cell blasts or 0.19×10^8 naïve T cells were immediately incubated with 60 or 19 μl, respectively, of washed streptavidin-conjugated Dynabeads® (M-280 Streptavidin, 11205D, Invitrogen) for 30 min on a rotating wheel at 4°C. Beads were separated using a magnetic tube rack and "post" lysates were retrieved. Beads were washed three times with 1 ml ice-cold lysis buffer then resuspended in 1× NuPAGE LDS Sample Buffer (NP0007, Invitrogen) and boiled at 95°C for 5 min. Beads were allowed to return to room temperature then separated on the magnetic rack and eluates were retrieved to low-protein binding microcentrifuge tubes and

specific signals in Th cells and regulates their responses. Int Immunol.