

Expanded View Figures

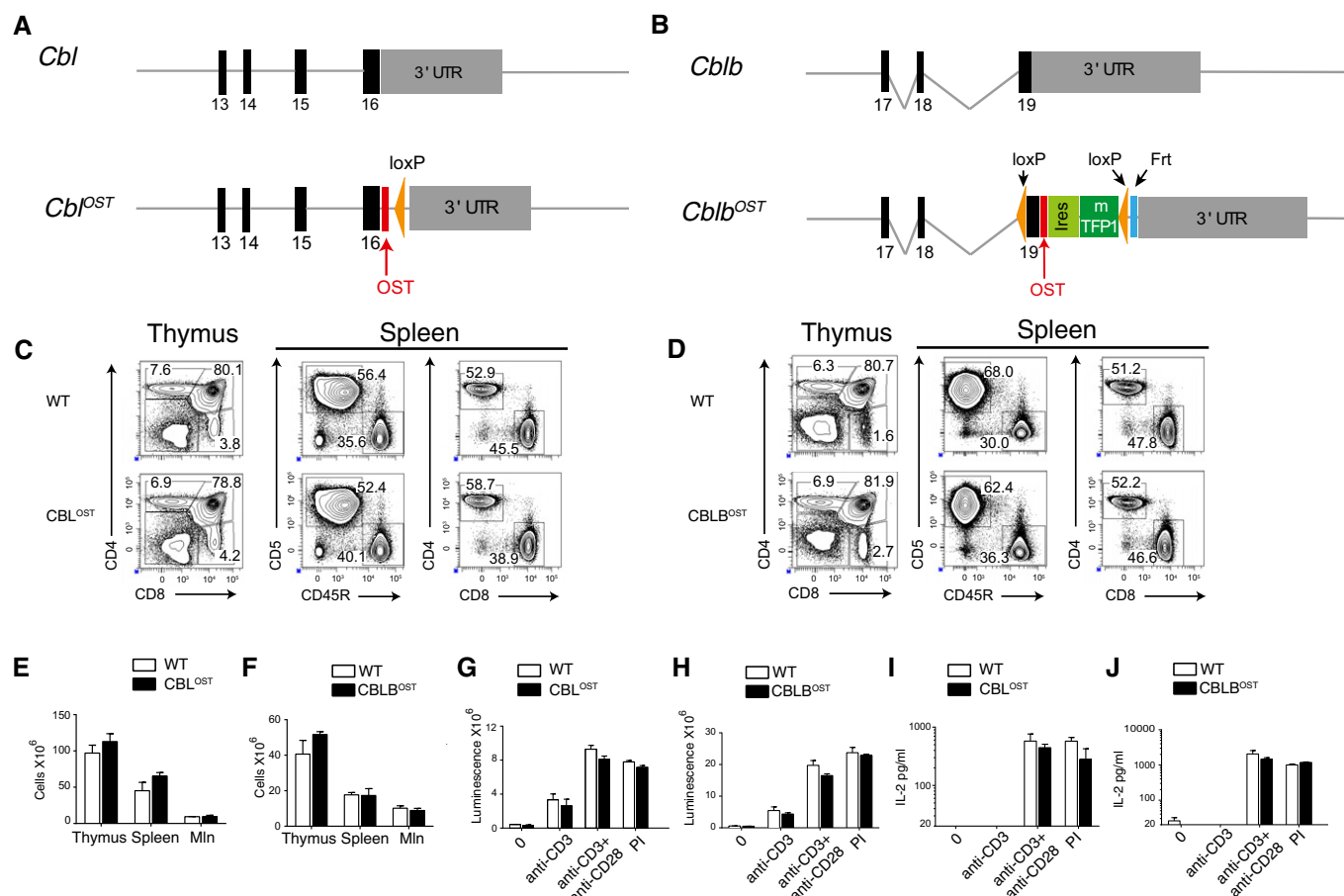
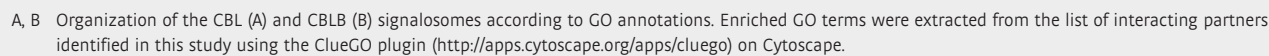


Figure EV1. T cells from *CBL*^{OST} and *CBLB*^{OST} mice develop and function normally.

- A Structure of the 3' end of the wild-type *Cbl* allele and of the targeted *Cbl*^{OST} allele following homologous recombination and CRE-mediated excision of the loxP-neo^r-loxP cassette. Exons are shown as filled black boxes and numbered. In the *Cbl*^{OST} allele, the One-STrEP-tag (OST) is shown in red and the remaining loxP site in orange.
- B Structure of the 3' end of the wild-type *Cblb* allele and of the targeted *Cblb*^{OST} allele following homologous recombination and FLP-mediated excision of the frt-neo^r-frt cassette. Exons are shown as filled black boxes and numbered. In the *Cblb*^{OST} allele, the One-STrEP-tag (OST) is shown in red, the IRES-mTFP1 cassette in green, the two loxP sites in orange, and the remaining frt site in blue.
- C Flow cytometry analysis of thymus and spleen from wild-type (WT) and *CBL*^{OST} mice for expression of CD4 versus CD8 and CD5 versus CD45R. Numbers adjacent to outlined areas indicate percentage of cells.
- D Flow cytometry analysis of thymus and spleen from wild-type (WT) and *CBLB*^{OST} mice for expression of CD4 versus CD8 and CD5 versus CD45R. Numbers adjacent to outlined areas indicate percentage of cells.
- E Cellularity of thymus, spleen, and pooled mesenteric lymph nodes (Mln) from wild-type (WT) and *CBL*^{OST} mice. Data are expressed as mean value ± SEM.
- F Cellularity of thymus, spleen, and pooled mesenteric lymph nodes (Mln) from wild-type (WT) and *CBLB*^{OST} mice. Data are expressed as mean value ± SEM.
- G ATP content of CD4⁺ T cells purified from WT and *CBL*^{OST} mice and activated for 48 h with PMA and ionomycin (PI) or with plate-bound anti-CD3 (0.3 µg/ml) in the presence or absence of soluble anti-CD28 (1 µg/ml). ATP content is directly proportional to the numbers of proliferating cells in the well and assessed by luminescence. Data are expressed as mean value ± SEM.
- H ATP content of CD4⁺ T cells purified from WT and *CBLB*^{OST} mice and activated for 48 h with PMA and ionomycin (PI) or with plate-bound anti-CD3 (0.3 µg/ml) in the presence or absence of soluble anti-CD28 (1 µg/ml), assessed by luminescence. Data are expressed as mean value ± SEM.
- I IL-2 in supernatants of WT and *CBL*^{OST} CD4⁺ T cells activated as in (G). Data are expressed as mean value ± SEM.
- J IL-2 in supernatants of WT and *CBLB*^{OST} CD4⁺ T cells activated as in (H). Data are expressed as mean value ± SEM.

Data information: Data in (C-J) are representative of at least three experiments with at least two mice per genotype.





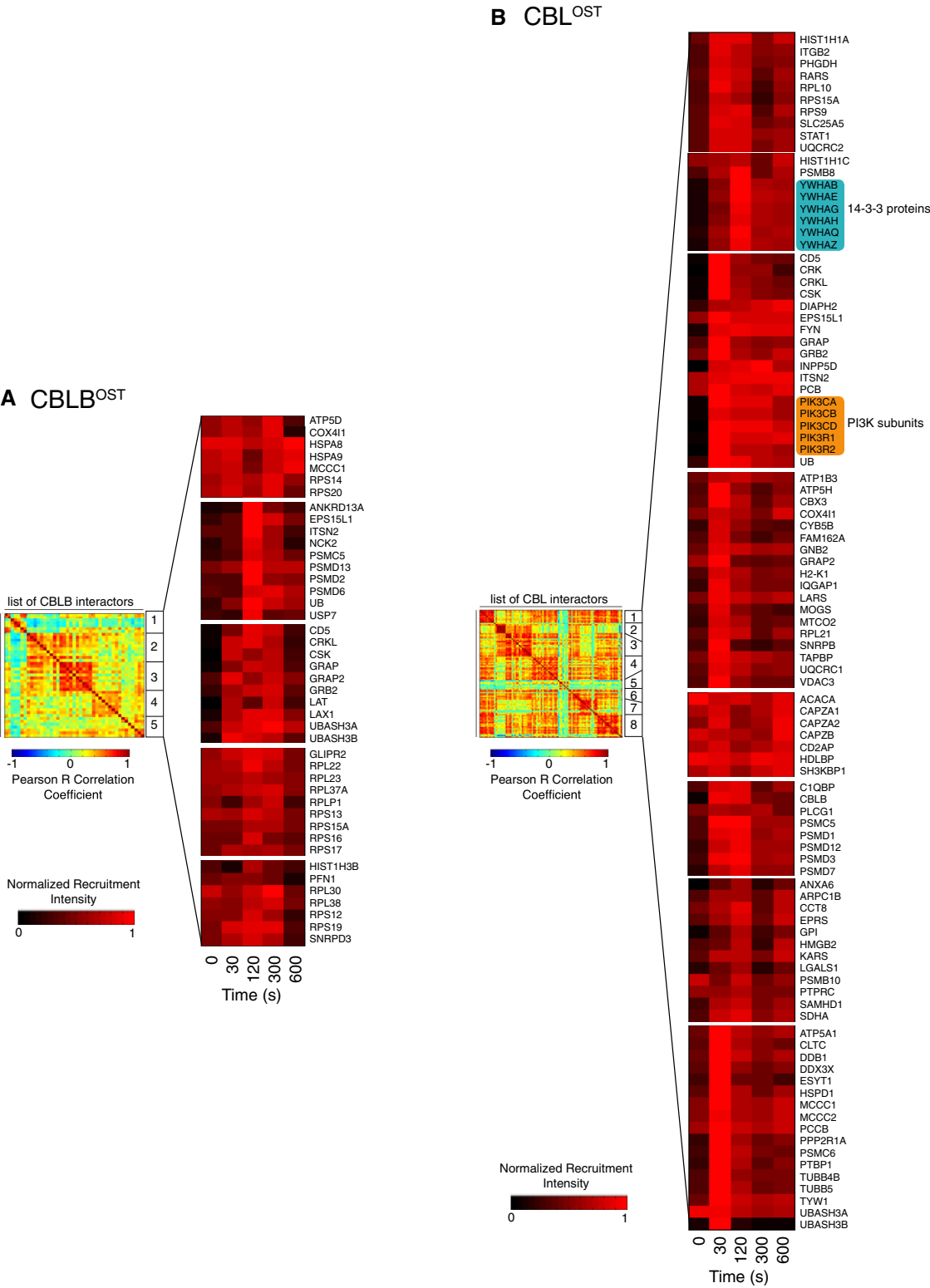


Figure EV4. K-means clustering analysis of the CBL and CBLB signalosomes.

A, B Representation of the CBLB (A) and CBL (B) correlation matrix (R_{ij}) partitioned into different clusters using a K-means clustering algorithm. The normalized recruitment intensity to the bait as a function of time is represented for the different interactors grouped into corresponding clusters. Within each cluster, interactors are listed in alphabetical order. Proteins from the 14-3-3 family and PI3K subunits are highlighted within the CBL signalosome.

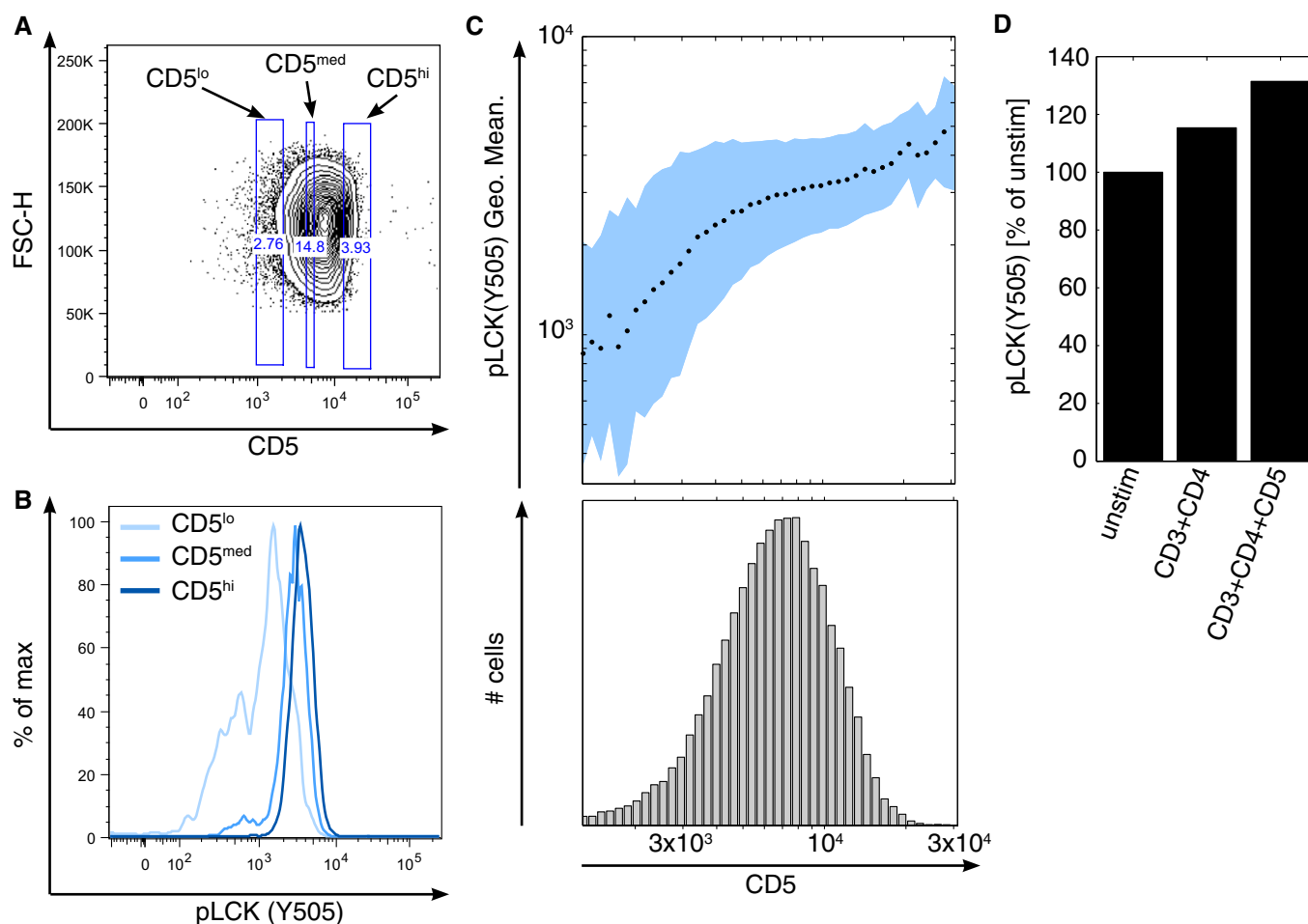


Figure EV5. CD5 contributes to control the phosphorylation of the negative-regulatory tyrosine found at position 505 of LCK via CSK.

- A Expression of CD5 on short-term expanded CD4⁺ T cells stimulated by cross-linking biotinylated anti-CD3 and anti-CD4 antibodies with streptavidin for 1 min at 37°C. The depicted gates define three populations of T cells with different expression of CD5 at their surface (CD5^{lo}, CD5^{med} and CD5^{hi}). Numbers adjacent to outlined areas indicate percentage of cells.
- B In addition to having been stained with anti-CD5, the cells described in (A) were permeabilized and stained with an anti-pLCK(Y505). The histogram represents the levels of phospho-LCK(Y505) found in the three populations defined in (A) on the basis of CD5 levels.
- C The upper panel represents the mean ($\pm$ SD) of the log fluorescence intensity of phospho-LCK(Y505) as a function of the geometric mean fluorescence intensity of CD5. Mean and SD were computed from populations of cells defined using a regular binning of the CD5 expression histogram (lower panel).
- D Effect of CD5 cross-linking on the phosphorylation of Y505 of LCK. Short-term expanded CD4⁺ T cells were stimulated with 2 μ g biotinylated anti-CD3 plus 2 μ g biotinylated anti-CD4 (as in A) in the presence or absence of 2 μ g biotinylated anti-CD5 (clone 53-7.3). The intensity of phospho-LCK(Y505) is represented as percent of phospho-LCK(Y505) intensity in the unstimulated condition.