**Online supplemental figure 1: Gating strategy and exemplary FACS plots of *in vitro* differentiated T helper cell subsets.**

Following 48h or 72h of *in vitro* polarization of naïve CD4$^+$ T cells isolated from spleens from WT or *Cblb*-deficient mice, cells were restimulated with PMA (500ng/ml) and Ionomycin (500ng/ml) in the presence of Brefeldin A and Monensin for 4 hours and stained with fluorescently labelled antibodies against CD45, CD25, IFNγ, IL-13, IL-9, IL-17, FOXP3 and with the Zombie Aqua™ Fixable Viability Kit. (A) Lymphocytes were gated according to their SSC-A/FSC-A profile, single cells are identified (FSC-H/FSC-A) and gated for viable CD45$^{hi}$ cells. After (B) 48h or (C) 72h T helper cell subsets were identified according to their signature cytokine IFNγ (Th1), IL-13 (Th2), IL-9 (Th9), IL-17 (Th17) or the expression of CD25 and Foxp3 (Treg). Plots are taken from at least 5 independent experiments with at least n=10 biological replicates.

**Online supplemental figure 2: Analysis of T helper cell differentiation after 48h and 72h**

Naïve (CD4$^+$CD62L$^+$CD44$^-$) T cells from WT and Cbl-b$^{-/-}$ mice are differentiated for 48h or 72h in the presence of a defined cocktail of cytokines and functional antibodies. The frequency (of CD45$^+$LD$^-$) of IFNγ$^+$ (Th1), IL-13$^+$ (Th2), IL-9$^+$ (Th9), IL-17$^+$ (Th17) and CD25$^+$Foxp3$^+$ (Treg) cells was determined by flow cytometry and plotted as (A) heatmap or (B) individual values. (C) *Ifng*, *Il13*, *Il9*, *FOXP3* and *Il17* expression of *in vitro* differentiated T helper cells was determined after 48h and 72h by qPCR and x-fold induction in relation to Th0 cells was determined by the ddtc method using the housekeeping gene GAPDH. Data for Th9 cells and Treg was already shown in Figure 1 and is plotted here again for the sake of completeness. Data is representative of at least 2 independent experiments with n=2-11 biological replicates. Results are shown as mean +/- SEM and significance was calculated by

two-way ANOVA and Multiple comparison test, corrected by Sidak post hoc analysis, as recommended by GraphPad Prism software (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

**Online supplemental figure 3: Measurement of cytokines released during T helper cell differentiation**

Supernatant of T helper cell cultures is taken after (A) 48h or (B) 72h and stored at -80°C until further analysis. The concentration of different cytokines (IFNγ, IL-13, IL-9, IL-2 and TNFα) released into the supernatant over this time period is determined using different BD™ Cytometric Bead Array (CBA) Flex Sets according to the manufacturer's instructions. (C) For the sake of clarity, the concentration values for TNFα from figures (A) and (B) are shown here again in a slightly different presentation. Data is representative of at least 3 independent experiments with n=3-5 biological replicates. Results are shown as mean +/- SEM and significance was calculated by two-way ANOVA and Multiple comparison test, corrected by Sidak post hoc analysis, as recommended by GraphPad Prism software (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

**Online supplemental figure 4: Cbl-b deficiency does not enhance expression or nuclear entry of NFAT**

Naïve ($CD4^+CD62L^+CD44^-$) T cells from WT and *Cblb*$^{-/-}$ mice are differentiated for 48h in the presence of a defined cocktail of cytokines and functional antibodies. (A) Expression of *NFATc1, NFATc1* alphaP1, *NFATc1* betaP2 and *NFATc2* is determined by qPCR and determined by the ddCt method using the house keeping gene GAPDH. Fold induction is shown in relation to WT Th0 cells. Results are pooled from two independent experiments (n=4 biological replicates). (B) Detection of NFATc1 in the nuclear fraction of Th0 and Th9 cells. (C) Expression of NFATc1 is

quantified with ImageJ and normalized to Lamin B1 (abcam16048). (D) Detection of NFATc1 in the cytosolic fraction of Th0 and Th9 cells. (E) Expression of NFATc1 is quantified with ImageJ and normalized to actin. Results from B-E are representative for 2 independent experiments (n=4 biological replicates). (A-E) Results are shown as mean +/- SEM and significance was calculated by two-way ANOVA and Multiple comparison test, corrected by Sidak post hoc analysis, as recommended by GraphPad Prism software (*p<0.05).

**Online supplemental figure 5: scRNA sequencing of *in vitro* differentiated Th9 cells**

Single-cell RNA sequencing of *in vitro* differentiated Th9 cells was performed on 7500 cells per sample (six biological replicates, 3*WT and 3*$Cblb^{-/-}$ mice) and a depth of approximately 80.000 reads/cell. (A) Graph based clustering led to formation of six major and one minor clusters, which are shown in two-dimensional t-distributed stochastic neighbour embedding (tSNE) space and Uniform Manifold Approximation and Projection (UMAP) space. The graphs show all cells or just WT or KO cells. The seven colour-code shows the 7 identified clusters, while the two colour-code differentiates WT and KO cell. (B) Volcano plot depicting differentially expressed genes in Cbl-b deficient Th9 cells compared to WT Th9 cells. Grey dots represent genes with non-significant difference in expression between $Cbl\text{-}b^{-/-}$ and WT cells ($P_{adj} \geq 0.05$). Red dots represent genes with $P_{adj}<0.05$ and $|\log_2 FC| \geq 0.25$ while blue dots represent genes with $P_{adj}<0.05$ and $|\log_2 FC| < 0.25$. Gene names are shown for differentially expressed genes with $P_{adj}<0.05$ and $|\log_2 FC| \geq 0.75$. Data points with p-value below the technical representation limit are displayed as triangles. Y-axis denotes “-log10 $P_{adj}$“ values while X-axis shows “$\log_2$ FC” values.

**Online supplemental figure 6: *Cblb* expression in WT IL-9 positive cells is significantly lower compared to all remaining cells**

Single cell RNA sequencing was performed on three times 7500 WT Th9 cells (n=3 biological replicates) at a depth of approximately 80.000 reads/cell. (A) Graph based clustering based on all WT and KO cells led to formation of six major and one minor clusters. Here, the two-dimensional t-distributed stochastic neighbour embedding (tSNE) space was filtered to show only WT cells. Expression levels of (B) *Cblb* and (C) *Il9* are depicted on a per cell level. (D) Expression level of *Cblb* is depicted for each of the identified seven clusters in a violin plot representation. (E) *Il9* and *Cblb* expression are plotted in a scatter plot with cluster 2 (IL-$9^{+}$) shown in red and all remaining cells in green. R denotes the Pearson correlation. (F) Expression level of *Cblb* is depicted for cluster 2 (IL-$9^{+}$) and all remaining cells in a violin plot representation. Significance of differential expression between both groups was calculated via a Wilcoxon Rank Sum test and adjusted for multiple testing via Bonferroni correction based on the total number of genes in the dataset.

**Online supplemental figure 7: Neither WT nor *Cblb*$^{-/-}$ Th9 cells kill B16-OVA tumor cells *in vitro***

OT-II and OT-II**Cblb*$^{-/-}$ naïve CD4$^{+}$ T cells are polarised *in vitro* into Th9 cells for 48h. B16-OVA and B16-WT tumor cells are labelled with CFSE, mixed at a 1:1 ratio and seeded into 96-well plates (50.000 cells/well; 25.000 CFSE low B16-OVA and 25.000 CFSE high B16-WT). 100.000 Th9 cells are added and incubated for 16h. The degree of dead/killed B16-OVA and B16-WT cells is determined by flow cytometry. (A) Percentage of killed B16-WT and B16-OVA cells in coculture with OT-II and OT-II**Cblb*$^{-/-}$ Th9 cells. (B) Exemplary plots of B16 tumors cells after 16h co-culture with Th9 cells. Data is taken from one experiment (n=2 or 3 biological replicates).

Significance is determined by one-way ANOVA as suggested by GraphPad prism or not determined (n.d.) due to low number of replicates.