**Supplementary figure legends**

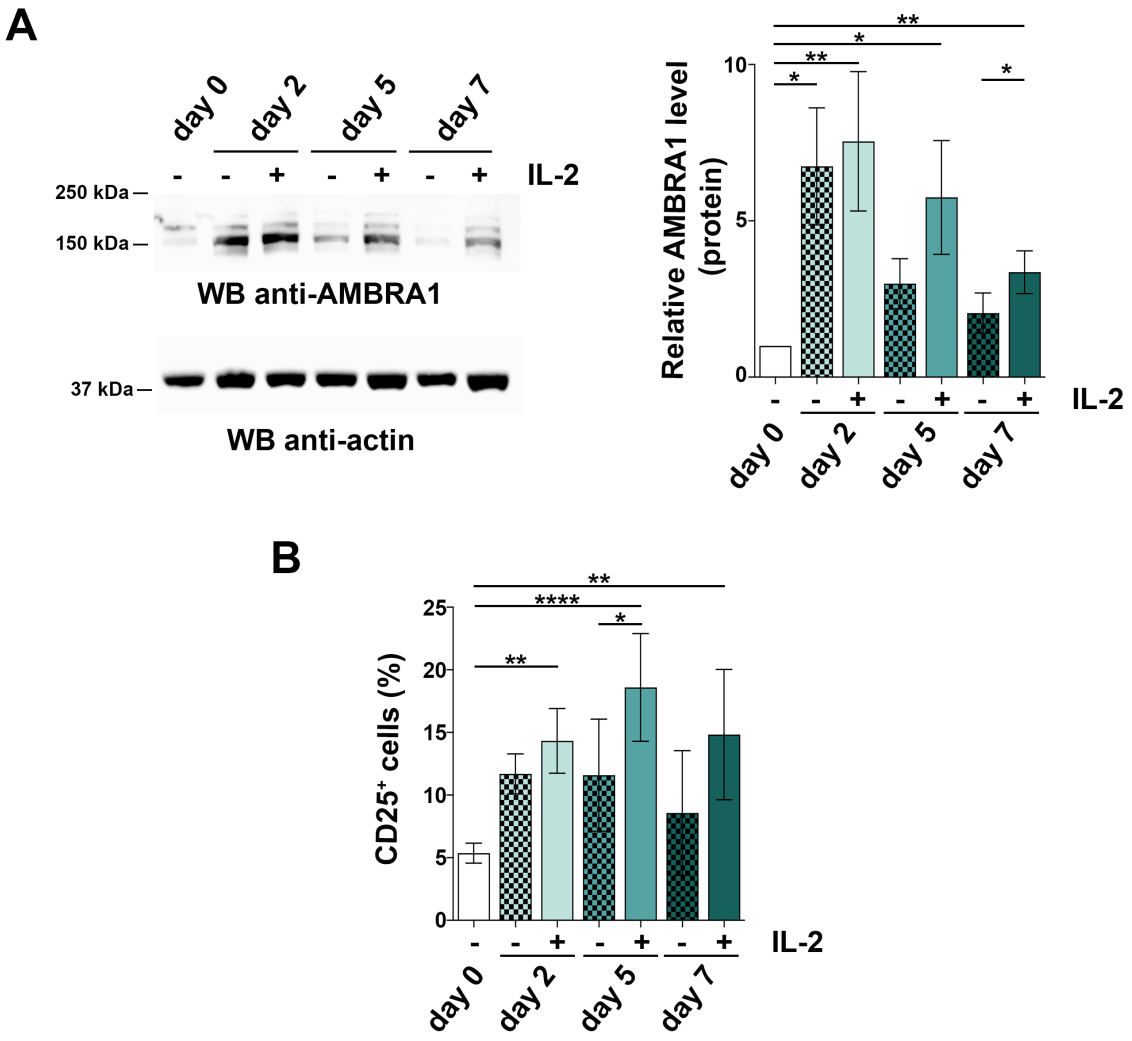
**Figure S1. IL-2 signaling enhances the effects of TCR/CD28 co-stimulation on AMBRA1 expression. A.** Immunoblot analysis of AMBRA1 protein levels in $CD8^+$ T cells collected at days 0, 2, 5 and 7 after stimulation with anti-CD3/anti-CD28 coated beads and cultured in the presence or absence of 50 U/ml IL-2. Actin was used as loading control. The migration of molecular mass markers is indicated. The histograms show the quantification of AMBRA1 expression during CTL differentiation ($n_{donor}$=3, one-way ANOVA test, day 0 value=1). **B.** Flow cytometry analysis of IL-2 receptor (CD25) expression in $CD8^+$ T cells collected at days 0, 2, 5 and 7 after stimulation in the presence or absence of 50 U/ml IL-2. The histograms show the percentage (%) of $CD25^+$ cells ($n_{donor}$=5, one-way ANOVA test). Data are shown as mean fold ± SD. * P≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001; **** P ≤ 0.0001.

**Figure S2. Neither CTL expansion nor IL-2R expression and signaling are affected by AMBRA1 depletion. A.** Flow cytometry analysis of live cell number (propidium $iodide^-$ cells) in control (ctr, scramble RNAi) and AMBRA1 KD CTLs 48h after transfection. The histograms show the quantification of live cells in ctr and AMBRA1 KD CTLs ($n_{donor}$=3, one-sample t test, ctr value=1). **B.** Flow cytometry analysis of surface CD25 expression in control and AMBRA1 KD CTLs. The histograms show the percentage (%) of $CD25^+$ cells ($n_{donor}$=5, Student's t test). **C.** Immunoblot analysis of STAT3 phosphorylation (Tyr705) in response to IL-2 stimulation for 15 minutes. Actin was used as loading control. The migration of molecular mass markers is indicated. The histograms show the quantification of STAT3 phosphorylation in control and AMBRA1 KD CTLs ($n_{donor}$=3, one-way ANOVA test, day 0 value=1). Data are shown as mean fold ± SD. * P≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001; **** P ≤ 0.0001.

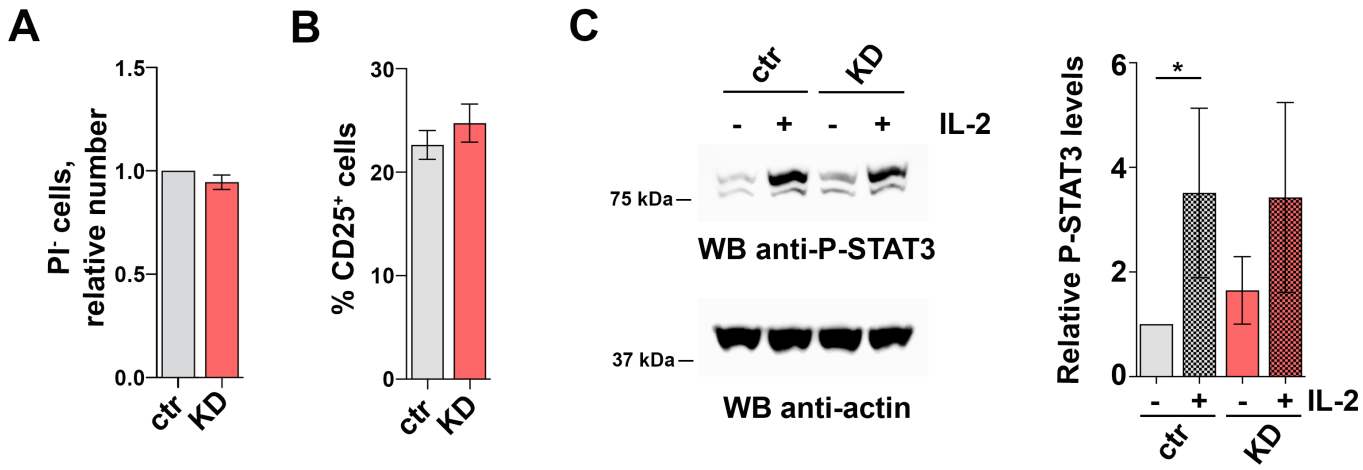
**Figure S3. Control of SBI-0206965 and LB-100 activity and effect on cell viability in CTLs. A.** Immunoblot analysis of the autophagy marker LC3B in lysates of CTLs untreated or treated with SBI-0206965 or LB-100 for 48 h, as described in Fig.3A. Actin was used as loading control. The migration of molecular mass markers is indicated. The histogram shows the quantification of LC3II in treated vs untreated CTLs. Data are shown as mean fold ± SD (n=3, one sample t test, vehicle value in CTLs set as 1). **B**. Immunoblot analysis of AKT (S473) and ERK1/2 phosphorylation in lysates of CTLs untreated or treated with SBI-0206965 or LB-100 for 48 h, as described in Fig.3A. Actin was used as loading control. The migration of molecular mass markers is indicated. The histogram shows the quantification of phospho-proteins in treated vs untreated CTLs. Data are shown as mean fold ± SD (n=3, one sample t test, vehicle value in CTLs set as 1). **C.** Quantification of cell viability of CTLs treated for 48h with LB-100 or SBI-0206965, assessed with trypan blue dye staining. Data are shown as mean fold ± SD (n>3, one-way ANOVA test). **D.** Immunoblot analysis of pFOXO3A S253 and FOXO3A in CTLs untreated or treated with LB-100 or SBI-0206965 for 48 h, as described in Fig.4A. Actin was used as loading control. The migration of molecular mass markers is indicated. The histograms show the quantification of pFOXO3A S253/FOXO3A and FOXO3A/actin in LB-100 and SBI-0206965 treated CTLs related to untreated CTLs. Data are shown as mean fold ± SD (n=3, one sample t test, vehicle value=1). * P≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001; **** P ≤ 0.0001.

**Figure S4. AMBRA1 depletion, ULK1 or PP2A inhibition enhance the mTORC1 pathway. A.** Immunoblot analysis of P-p70S6K T389 in CTLs untreated or treated with LB-100 or SBI-0206965 for 48 h, as described in Fig.4A. Actin was used as loading control. The migration of molecular mass markers is indicated. The histogram shows the quantification of P-p70S6K in treated vs untreated CTLs. Data are shown as mean fold ± SD (n=3, one sample t test, vehicle value=1). **B.** Immunoblot analysis of P-p70S6K T389 in lysates from

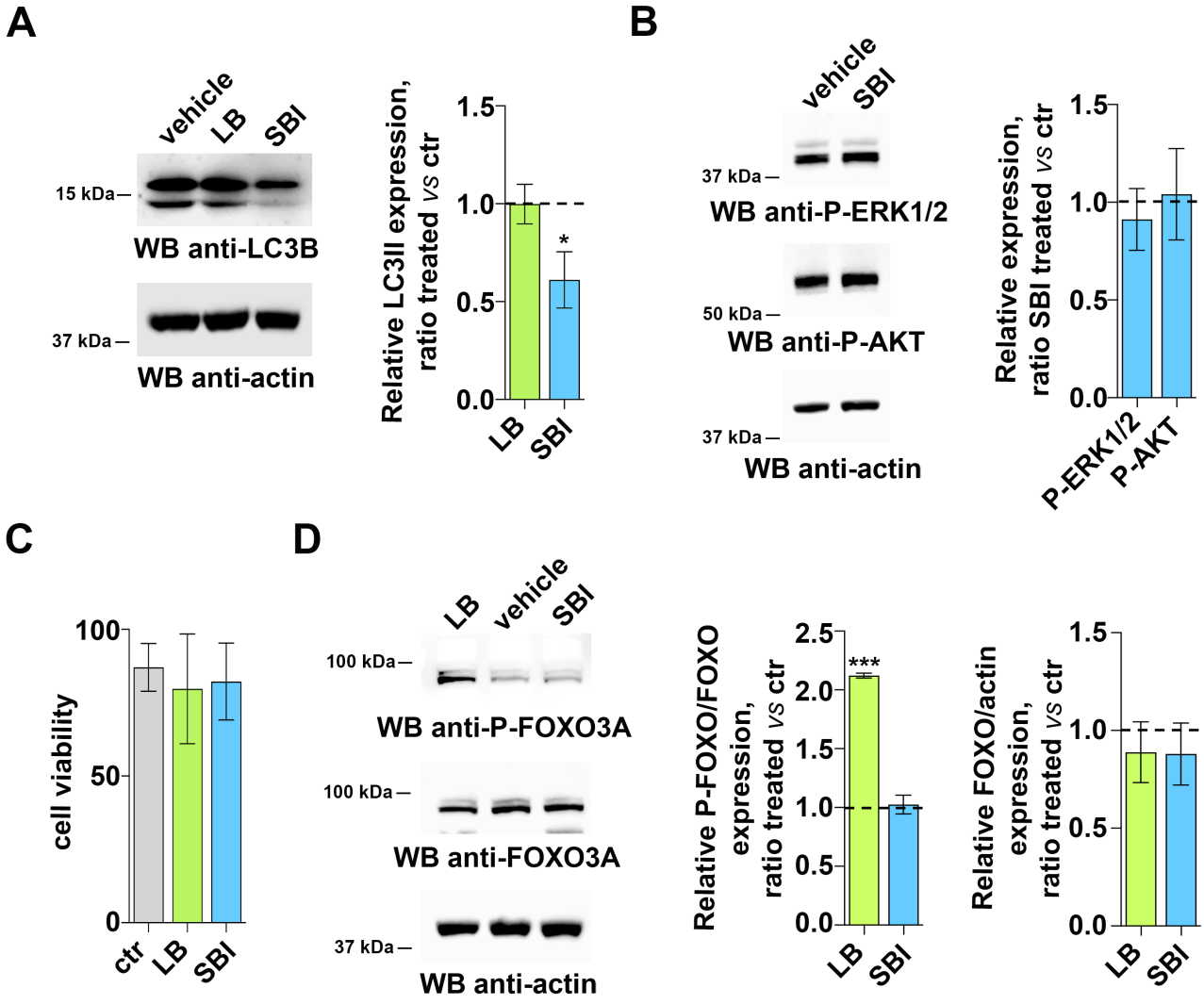
control and AMBRA1 KD CTLs. Actin was used as loading control. The migration of molecular mass markers is indicated. The histogram shows the quantification of P-p70S6K in control and AMBRA1 KD CTLs. Data are shown as mean fold ± SD (n=3, one sample t test, vehicle value=1). * P≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001; **** P ≤ 0.0001.



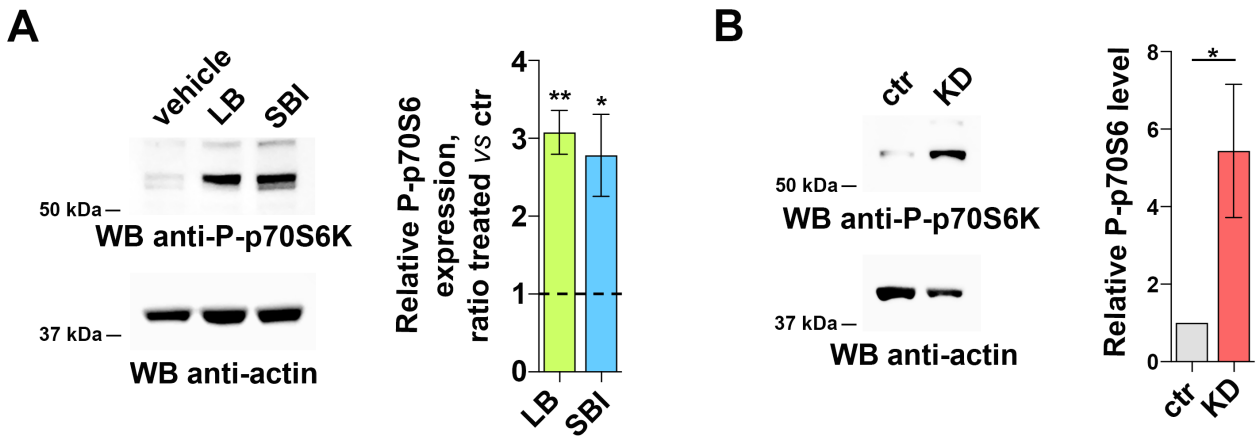

Supplementary figure 1




Supplementary figure 2




Supplementary figure 3




Supplementary figure 4

**Supplementary Tables**

**Supplementary Table 1. List of the primary antibodies used in this work**

| Antibody anti- | Supplier | Identifier | Host | Diluition | Application |
|---|---|---|---|---|---|
| **AMBRA1** | Merck | ABC131 | Rabbit | 1:1000 | WB |
| **β-actin** | Sigma-Aldrich | MAB1501 | Mouse | 1:10000 | WB |
| **Granzyme B** | Cell Signaling | 17215S | Rabbit | 1:2000 | WB |
| **Perforin 1** | Santa Cruz | sc-136994 | Mouse | 1:500 | WB |
| **p-FoxO3a (Ser253)** | Cell Signaling | 9466 | Rabbit | 1:1000 | WB |
| **FoxO3a** | Cell Signaling | 2497 | Rabbit | 1:1000 | WB |
| **LC3B** | Cell Signaling | 3868 | Rabbit | 1:500 | WB |
| **GFP** | Invitrogen | A11120 | Mouse | 1:100 | IF |
| **β-tubulin** | Cell Signaling | 15115 | Rabbit | 1:2000 | WB |
| **TFEB** | Cell Signaling | 37785 | Rabbit | 1:1000 | WB |
| **P-p70S6K (T389)** | Cell Signaling | 9234S | Rabbit | 1:500 | WB |
| **P-AKT (S473)** | Cell Signaling | 9271S | Rabbit | 1:500 | WB |
| **P-ERK1/2** | Cell Signaling | 9101 | Rabbit | 1:1000 | WB |
| **P-STAT3 (Tyr705)** | Cell Signaling | 9145S | Rabbit | 1:1000 | WB |
| **CD25 (PE)** | BioLegend | 302606 | Mouse | 1:40 | FC |

**Supplementary Table 2. List of the primers used in this study**

| Gene | Forward 5'-3' | Reverse 5'-3' |
|---|---|---|
| ***18S*** | CGCCGCTAGAGGTGAAATT | CTTGGCAAATGCTTTCGC |
| ***AMBRA1*** | AACCCTCCACTGCGAGTTGA | TCTACCTGTTCCGTGGTTCTCC |
| ***TFEB*** | GGAGTACCTGTCCGAGACCT | GGGCTATTGGGAGCACTGTT |
| ***TBET*** | TAATAACCCCTTTGCCAAAG | TCCCCCAAGGAATTGACAGT |
| ***RUNX3*** | CCCCTCCGTTCCTAACTGTT | ACAGAGAGTGGATGCGTTGA |
| ***GZMB*** | TCAAAGAACAGGAGCCGACC | TTGGCCTTTCTCTCCAGCTG |
| ***GZMA*** | AACCAGGAACCATGTGCCAA | GGCTTCCAGAATCTCCATTGC |
| ***PRF1*** | CCTGCAGTCACAGCTACACA | GGGGCTCCAGTTAAGGCAAT |
| ***SGLN*** | GACGAGAATCCAGGACTTGAA | GGGCAGATTCCTGTCAAGAG |
| ***GNLY*** | GGATAAGCCCACCCAGAGAAG | ACAGATCTGCTGGGCAGTTT |
| ***EOMES*** | TTGATTCCTTAACCCCCGGC | TCTCTCCTGAGTCCCACTGG |
| ***CTSD (CHIP)*** | GCAGCTTCCAGGTCATAGGG | CTGACCTCAGGTGATCTGCC |
| ***TBET (CHIP)*** | ATCGTCCTATCTCCGAGGCA | GAGAGGGTGTACGTGTGCAG |
| ***RUNX3 (CHIP)*** | CTGGTGAGTCAGAGGCTGAA | GGGAAAGGTGGCGGCTTTTA |