**Supplementary Materials and Methods**

**Processing of human blood samples and isolation of T cells**

Blood of CLL patients was drawn using ethylene diamine tetraacetic acid (EDTA)-coated tubes (Sarstedt, Nürnbrecht, Germany). Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient separation (Bio&Sell, Feucht, Germany; # L 6115) as previously described,1, 2 and if needed, EasySep™ Human Tcell Isolation Kit (Stemcell Technologies, Vancouver, Canada; #17951) was used to isolate T cells according to manufacturer’s instructions typically yielding a purity of 97.5%. PBMCs of CLL patients were viably frozen and when needed, frozen PBMCs were thawed and rested for 30 min until further processing.

**Collection of murine tissue samples and preparation of cell suspensions**

Mice were euthanized by increasing concentrations of carbon dioxide (CO2). Single-cell suspensions were generated by mechanical disruption of spleens using gentleMACS (Miltenyi Biotech, Bergisch Gladbach, Germany; #130-093-237). Red blood cells were lysed using RBC Lysis Buffer (Biolegend, London, United Kingdom; #420301), filtered with a 70 µm cell strainer (Corning, USA; #352350) and counted using a Vi-Cell XR 2.03 cell counter (Beckman Coulter Inc.,Brea, USA). If necessary, T cells or CD8+ T cells were isolated from whole splenocytes using EasySep™ Mouse T cell Isolation Kit or Mouse CD8+ T cell Isolation Kit (Stemcell Technologies, Vancouver, Canada; #19851 and #19853) according to manufacturer’s instructions typically yielding a purity of 96%. Splenocytes of Eµ-TCL1 mice were viably frozen and when needed, frozen PBMCs were thawed and rested for 30 min until further processing.

**Activation of T cells**

To assess the activation of T-cells, cells were stimulated with anti-CD3 (1 µg/ml; murine: clone 145-2C11, ThermoFisher Scientific, Dreieich, Germany; #16-0031-86; human: clone: UCHT1, BioLegend, London, United Kingdom; #300438) or a combination of anti-CD3 (1 µg/ml) plus anti-CD28 (2 µg/ml; murine: clone 27.52, ThermoFisher Scientific, Dreieich, Germany; #16-0281-85; human: clone: 28.2, BioLegend, London, United Kingdom; #302934) antibodies in the presence of the different inhibitors or DMSO as vehicle control at 37°C, 5% CO2. Isolated T cells were stimulated using Dynabeads T-Activator CD3/CD28 (ThermoFisher Scientific, Dreieich, Germany; #11456D and #11161D) at a cell:bead ratio of 1:1. For cytokine analysis, cells were stimulated for 6hrs in the presence of eBioscience™ Protein Transport Inhibitor Cocktail (ThermoFisher Scientific, Dreieich, Germany; # 00-4980-93) as previously described.2

**Analyzing T-cell proliferation**

Both, murine and human cells were labeled with Cell Proliferation Dye eFluorTM 670 (ThermoFisher Scientific, Dreieich, Germany) per manufacturer’s instructions to track cell divisions. Cells were stimulated with anti-CD3 (1 µg/ml) or a combination of anti-CD3 (1 µg/ml) plus anti-CD28 (2 µg/ml) antibodies in the presence of the different inhibitors or DMSO as vehicle control. Isolated T cells were stimulated using Dynabeads T-Activator CD3/CD28 (ThermoFisher Scientific, Dreieich, Germany) at a cell:bead ratio of 1:1.

**Cytotoxic T-cell assay**

CD8+ T cells recognizing an LCMV peptide were isolated from P14xCD4-Cre LCMV TCR-transgenic mice with a C57BL/6 background as detailed above. Purified T cells were treated with PI3K inhibitors or DMSO at 6 × 106 cells/mL for 1h at 37°C, 5% CO2. TCL1-355 tumor cells3 were labeled with eBioscience™ Cell Proliferation Dye eFluor 670 according to the manufacturer’s instructions using final concentrations of 5 × 106 cells/mL and 1 µM cell dye for staining. After washing the T cells to remove inhibitors, labeled tumor cells were co-cultured with T cells at an effector:target (E:T) ratio of 1:1 in presence of 2 ng/mL gp33-41 peptide (RP20257, GenScript Biotech Corp, New Jersey, USA). For the analysis of effector cytokines and T cell degranulation, cells were incubated at 37°C, 5% CO2 in the presence of eBioscience™ Protein Transport Inhibitor Cocktail and anti-CD107a antibody for 6hrs. To investigate tumor cell death, co-cultures were analyzed after 24hrs using the PE Annexin V Apoptosis Detection Kit with 7-AAD (Biolegend, BioLegend, London, United Kingdom; #640934) according to the manufacturer’s instructions.

**Flow Cytometry**

After incubation, cells were stained with fluorescently labelled antibodies (Supplementary Table 2) as previously described.1, 2 In brief, single cell suspensions were stained with fluorescently labelled antibodies in phosphate-buffered saline (PBS, Sigma-Aldrich, Taufkirchen, Germany; # D8537) with addition of eBioscienceTM Fixable Viability Dye eFluor® (ThermoFisher Scientific, Dreieich, Germany) at a concentration of 1:1000 for 30 min at 4°C. All Antibodies used for FACS stainings are listed in Supplementary Table 2. If possible, cells were fixed using eBioscienceTM Intracellular Fixation Buffer (ThermoFisher Scientific, Dreieich, Germany; #0-8222-49) for 30min at room temperature, washed and stored at 4°C in the dark until data acquisition. Cells of Nr4a1-GFP mice were stained, washed, and acquired immediately to get accurate readings of intracellular GFP levels.

For PhosphoFlow Cytometry, after incubation of surface markers, cells were fixed with 1% paraformaldehyde for 10min and permeabilized with ice-cold methanol. Staining with phospho-specific antibodies and total-protein controls was performed for 30min at room temperature and samples were acquired immediately at the flow cytometer.

**References**

1. Roessner PM, Llao Cid L, Lupar E, et al. EOMES and IL-10 regulate antitumor activity of T regulatory type 1 CD4(+) T cells in chronic lymphocytic leukemia. *Leukemia*. Aug 2021;35(8):2311-2324. doi:10.1038/s41375-021-01136-1

2. Roessner PM, Hanna BS, Ozturk S, et al. TBET-expressing Th1 CD4(+) T cells accumulate in chronic lymphocytic leukaemia without affecting disease progression in Emicro-TCL1 mice. *British journal of haematology*. Apr 2020;189(1):133-145. doi:10.1111/bjh.16316

3. Chakraborty S, Martines C, Porro F, et al. B-cell receptor signaling and genetic lesions in TP53 and CDKN2A/CDKN2B cooperate in Richter transformation. *Blood*. 2021;138(12):1053-1066. doi:10.1182/blood.2020008276

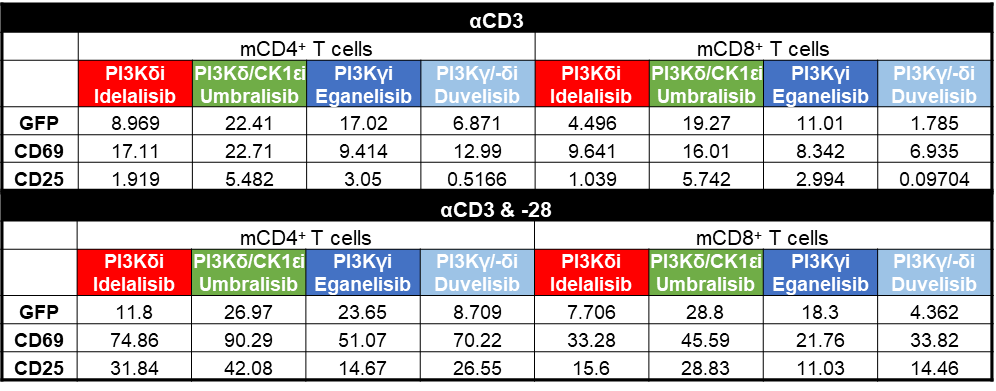
**Clinical information CLL patients**

|  |  |
| --- | --- |
| **Number of samples** | 14 |
| **Sex** | 28.6 % female (4/14) |
| **Age (years)** | mean: 63.3  median: 59.0 |
| **Binet stage at sampling** | 64.3 % A (9/14)  7.1 % B (1/14)  28.6 % C (4/14) |
| **Mutational state of *IGHV*** | 92.9 % mutated (13/14) |
| **Chromosomal aberration** | 85.7 % del13q (12/14)  14.3 % normal karyotype (2/14)  7.14 % del14q (1/14) |
| **TP53 mutation** | 0.0 % (0/14) |
| **Prior treatment** | 0.0 % (0/14) |

**List of flow cytometry products**

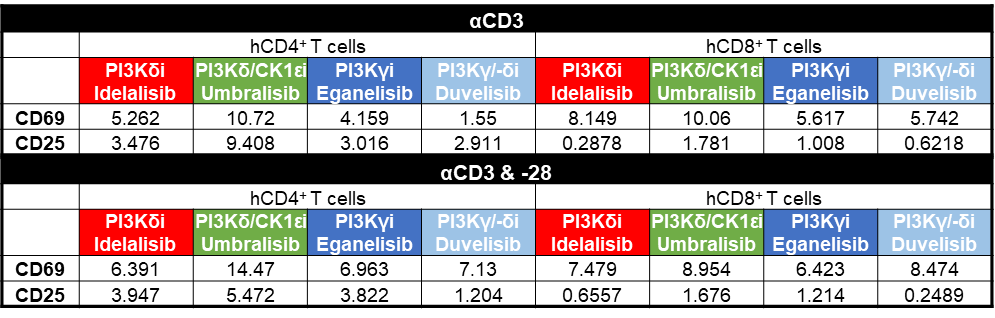
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **product** | **#** | **Supplier** | | |
| AKT Pan (PKB) Antibody, anti-human/mouse/rat, PE, REAfinity™ | 130-126-096 | Miltenyi Biotech, Bergisch Gladbach | | |
| AKT pS473 Antibody, anti-human/mouse, APC, REAfinity™ | 130-119-570 | Miltenyi Biotech, Bergisch Gladbach | | |
| Annexin V Apoptosis Detection Kit with 7-AAD | 640937 | BioLegend, London, United Kingdom |
| anti-human CD19 FITC | 302206 | BioLegend, London, United Kingdom | |
| anti-human CD197 (CCR7) Brilliant Violet 421™ | 353208 | BioLegend, London, United Kingdom | |
| anti-human CD25 PE | 302606 | BioLegend, London, United Kingdom | |
| anti-human CD3 APC | 317318 | BioLegend, London, United Kingdom | |
| anti-human CD4 APC/Cy7 | 317418 | BioLegend, London, United Kingdom | |
| anti-human CD4 Brilliant Violet 650™ | 317436 | BioLegend, London, United Kingdom | |
| anti-human CD45RO APC/Cy7 | 304228 | BioLegend, London, United Kingdom | |
| anti-human CD5 APC | 300612 | BioLegend, London, United Kingdom | |
| anti-human CD5 Brilliant Violet 510™ | 364018 | BioLegend, London, United Kingdom | |
| anti-human CD69 [PE/Dazzle™ 594](https://www.biolegend.com/en-us/products/pe-dazzle-594-anti-human-cd279-pd-1-antibody-9834) | 310942 | BioLegend, London, United Kingdom | |
| anti-human CD8a Brilliant Violet 605™ | 301040 | BioLegend, London, United Kingdom | |
| anti-mouse CD107a PE | 12-1071-82 | ThermoFisher Scientific, Dreieich, Germany | |
| anti-mouse CD127 (IL-7Ra) Brilliant Violet 605™ | 135041 | BioLegend, London, United Kingdom | |
| anti-mouse CD19 FITC | 11-0193-86 | ThermoFisher Scientific, Dreieich, Germany | |
| anti-mouse CD25 eFluor® 450 | 48-0251-82 | ThermoFisher Scientific, Dreieich, Germany | |
| anti-mouse CD3ε Brilliant Violet 605™ | 100351 | BioLegend, London, United Kingdom | |
| anti-mouse CD4 APC/Cy7 | 100526 | BioLegend, London, United Kingdom | |
| anti-mouse CD44 Alexa Fluor® 700 | 103026 | BioLegend, London, United Kingdom | |
| anti-mouse CD5 BD Horizon™ BV605 | 563194 | BD Biosciences, Heidelberg, Germany | |
| anti-mouse CD5 PE | 100608 | BioLegend, London, United Kingdom | |
| anti-mouse CD69 PE/Dazzle™ 594 | 104536 | BioLegend, London, United Kingdom | |
| anti-mouse CD8a Brilliant Violet 605™ | 100744 | BioLegend, London, United Kingdom | |
| anti-mouse CD8a PE | 100708 | BioLegend, London, United Kingdom | |
| anti-mouse TCRβ PE/Cy7 | 25-5961-82 | BioLegend, London, United Kingdom | |
| anti-mouse TNFa eFluor® 450 | 48-7321-82 | ThermoFisher Scientific, Dreieich, Germany | |
| anti-mouse TNFa FITC | 11-7321-82 | ThermoFisher Scientific, Dreieich, Germany | |
| BD Phosflow™ Alexa Fluor® 488 Mouse anti-NF-κB p65 (pS529) | 558421 | BD Biosciences, Heidelberg, Germany | | |
| BD Phosflow™ Alexa Fluor® 647 Mouse Anti-mTOR (pS2448) | 564242 | BD Biosciences, Heidelberg, Germany | | |
| eBioscience™ Cell Proliferation Dye eFluor® 670 | 65-0840-85 | ThermoFisher Scientific, Dreieich, Germany | | |
| eBioscience™ Fixable Viability Dye eFluor® 450 | 65-0863-18 | ThermoFisher Scientific, Dreieich, Germany | | |
| eBioscience™ Fixable Viability Dye eFluor® 506 | 65-0866-18 | ThermoFisher Scientific, Dreieich, Germany | | |
| mTOR (7C10) Rabbit mAb (PE Conjugate) | 15006S | Cell Signaling Technology, Leiden, Netherlands | | |
| NF-κB p65 (D14E12) XP® Rabbit mAb (Alexa Fluor® 647 Conjugate) | 8801S | Cell Signaling Technology, Leiden, Netherlands | | |

**IC50 concentrations of murine T cells**

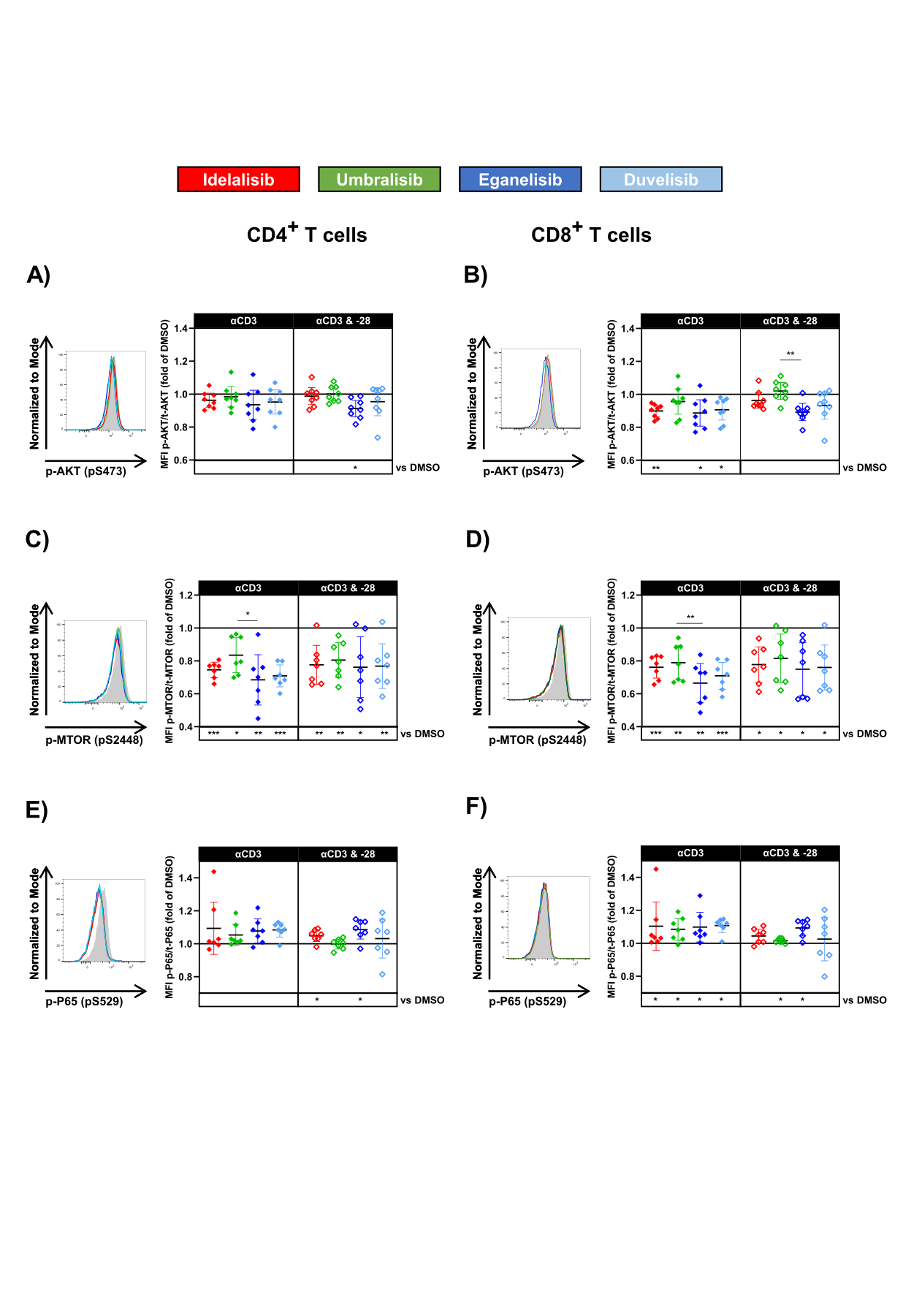


Splenocytes of Nr4a1-GFP mice were stimulated with αCD3 (1 µg/ml) or a combination of αCD3 (1 µg/ml) and αCD28 (2 µg/ml) in the presence of DMSO control or the indicated PI3K inhibitors (n = 3). MFI of Nr4a1-GFP and frequency of CD69+ or CD25+ cells were analyzed after 6hrs. IC50 values are given as µM.

**IC50 concentrations of human T cells**

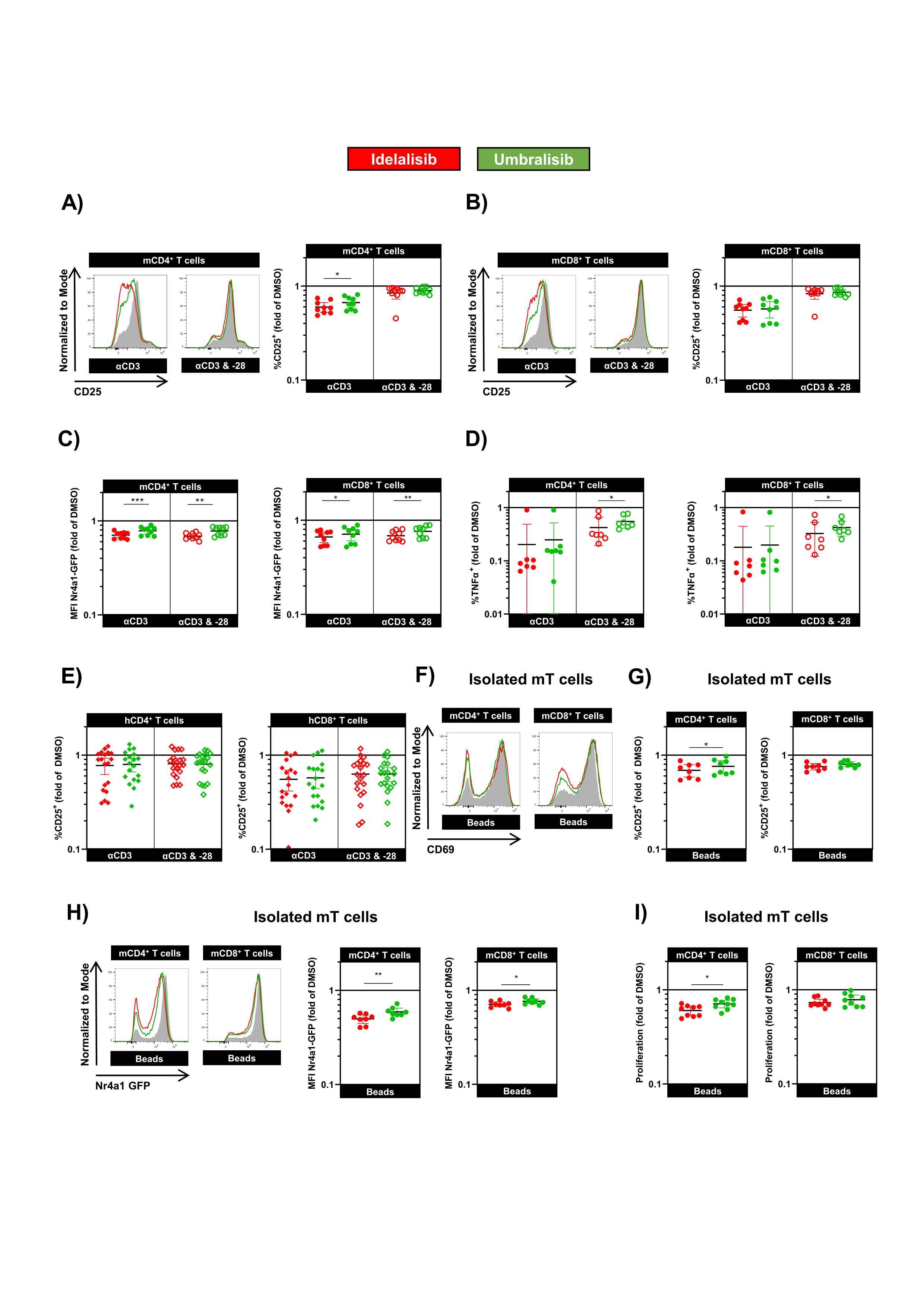


Human PBMCs were stimulated with αCD3 (1 µg/ml) or a combination of αCD3 (1 µg/ml) and αCD28 (2 µg/ml) in the presence of DMSO control or the indicated PI3K inhibitors (n = 4). Frequency of CD69+ or CD25+ cells were analyzed after 6hrs or 72hrs, respectively. IC50 values are given as µM.



**Suppl. Figure 1: PI3K inhibition reduces AKT/MTOR signaling after T-cell stimulation**

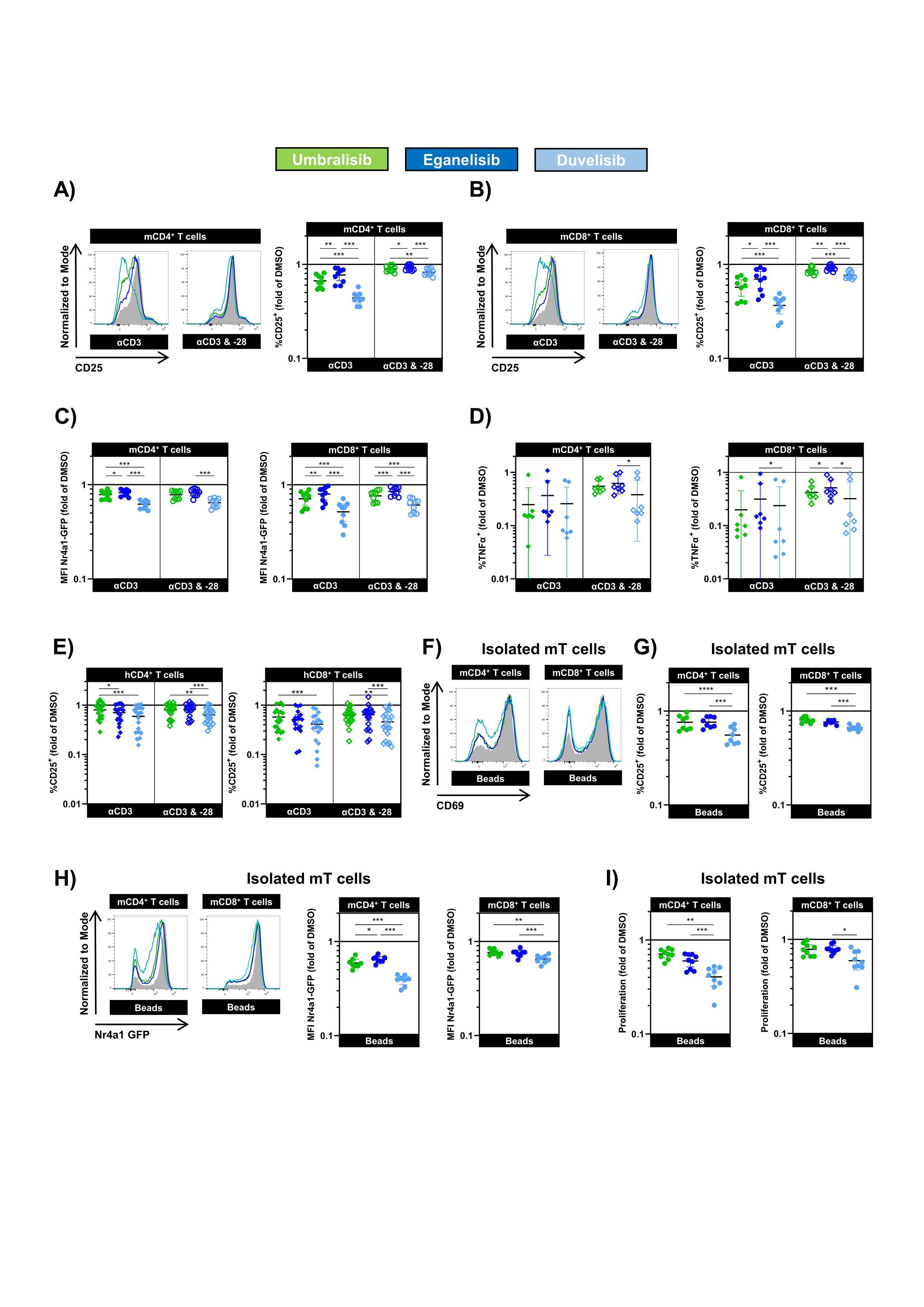
**A-F)** Human PBMCs were incubated with DMSO control, idelalisib, umbralisib, eganelisib or duvelisib (all 1 µM) for 1h and subsequently, stimulated with αCD3 (1 µg/ml) or a combination of αCD3 (1 µg/ml) and αCD28 (2 µg/ml) for 1hr. Representative expression of phospho-proteins analyzed by phosphoFlow as well as Mean fluorescence intensity (MFI) of phospho- versus total-proteins are depicted. Expression of p-AKT of **A)** CD4+ and **B)** CD8+ T cells, p-MTOR of **C)** CD4+ and **D)** CD8+ T cells, as well as p-NF-kB P65 of **E)** CD4+ and **F)** CD8+ T cells were analyzed. Quantification was normalized to DMSO control (n = 7-8). All graphs show mean ± CI. \* p <0.05; \*\* p < 0.01; \*\*\* p < 0.001.



**Suppl. Figure 2: Dual PI3Kδ and CK1ε inhibitor umbralisib reducing T-cell activity similarly to idelalisib**

**A-D)** Murine splenocytes (m; circles) or **E)** human PBMCs (h; rhombi) were stimulated with αCD3 (1 µg/ml) or a combination of αCD3 (1 µg/ml) and αCD28 (2 µg/ml) in the presence of DMSO control, idelalisib or umbralisib (both 1 µM). **A-B)** Representative histogram and frequency of CD25+ cells (n = 9) of **A)** CD4+ and **B)** CD8+ T cells after 6hrs of stimulation relative to DMSO control. **C)** MFI of Nr4a1 GFP of CD4+ (left; n = 9) and CD8+ T cells (right; n = 9) relative to DMSO control. **D)** Frequency of TNFα-producing CD4+ (left; n = 7) and CD8+ T cells (right; n = 7) after 6hrs of stimulation normalized to DMSO control. **E)** Frequency of CD25+ cells out of human CD4+ (left) and CD8+ T cells (right) after 48 hrs of stimulation with αCD3 (n = 19) or a combination of αCD3 and αCD28 (n = 20) normalized to DMSO control. **F-I)** Murine T cells of were isolated using magnetic bead-based negative enrichment and stimulated with αCD3/αCD28 coated beads in the presence of the indicated inhibitors (all 1 µM) **F)** Representative histogram of CD69-expressing CD4+ (left) and CD8+ T cells (right). **G)** CD25 expression normalized to DMSO control (n = 8). **H)** MFI of Nr4a1-GFP depicted as representative histograms as well as quantification normalized to DMSO control (n = 8). I**)** Proliferation of T cells was analyzed by flow cytometry and normalized to DMSO control (n = 9).

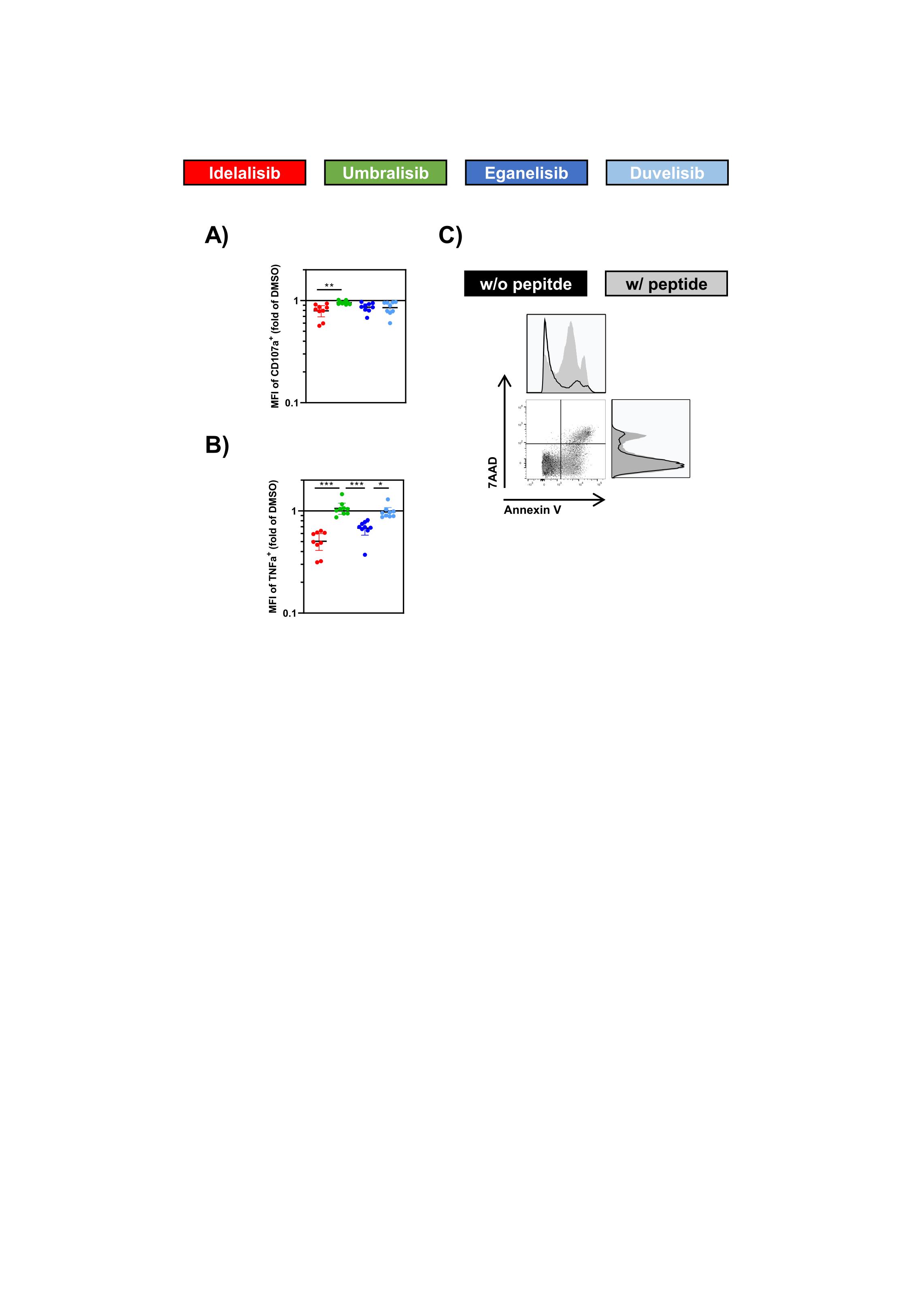
All graphs show mean ± CI. \* p <0.05; \*\* p < 0.01; \*\*\* p < 0.001.



**Suppl. Figure 3: Duvelisib, a dual PI3Kγ and -δ inhibitor, strongly reduces T-cell activity**

**A-D)** Murine splenocytes (m; circles) or **E)** human PBMCs (h; rhombi) were stimulated with αCD3 (1 µg/ml) or a combination of αCD3 (1 µg/ml) and αCD28 (2 µg/ml) in the presence of DMSO control, umbralisib, eganelisib or duvelisib (all 1 µM). **A-B)** Representative histogram and frequency of CD25+ cells (n = 9) of **A)** CD4+ and **B)** CD8+ T cells after 6hrs of stimulation relative to DMSO control. **C)** MFI of Nr4a1 GFP of CD4+ (left; n = 9) and CD8+ T cells (right; n = 9) relative to DMSO control. **D)** Frequency of TNFα-producing CD4+ (left; n = 7) and CD8+ T cells (right; n = 7) after 6hrs of stimulation normalized to DMSO control. **E)** Frequency of CD25+ cells out of human CD4+ (left) and CD8+ T cells (right) after 48 hrs of stimulation with αCD3 (n = 19) or a combination of αCD3 and αCD28 (n = 20) normalized to DMSO control. **F-I)** Murine T cells of were isolated using magnetic bead-based negative enrichment and stimulated with αCD3/αCD28 coated beads in the presence of the indicated inhibitors (all 1µM) **F)** Representative histogram of CD69-expressing CD4+ (left) and CD8+ T cells (right). **G)** Relative CD25 expression normalized to DMSO control (n = 8). **H)** MFI of Nr4a1-GFP depicted as representative histograms as well as quantification normalized to DMSO control (n = 8). **I)** Proliferation of T cells was analyzed by flow cytometry and normalized to DMSO control (n = 9).

All graphs show mean ± CI. \* p <0.05; \*\* p < 0.01; \*\*\* p < 0.001.



**Suppl. Figure 4: Duvelisib reduces the activity of antigen-specific T cells**

Splenic P14 CD8+ T cells were incubated for 1hr with respective PI3K inhibitors and co-cultured with cognate antigen-pulsed TCL1 leukemic cells (n = 9). **A-B)** Degranulation and cytokine production and T cells after 6 hrs and **C-D)** leukemia cell death after 24 hrs were analyzed. Mean fluorescence intensity (MFI) of **A)** CD107a and **B)** TNFα per marker-expressing cell. **C)** Frequency of early apoptotic (Annexin V+ 7AAD-), and all dead cells (Annexin V+/- 7AAD+) of without (black) or with peptide pulsing of leukemic cells (grey).

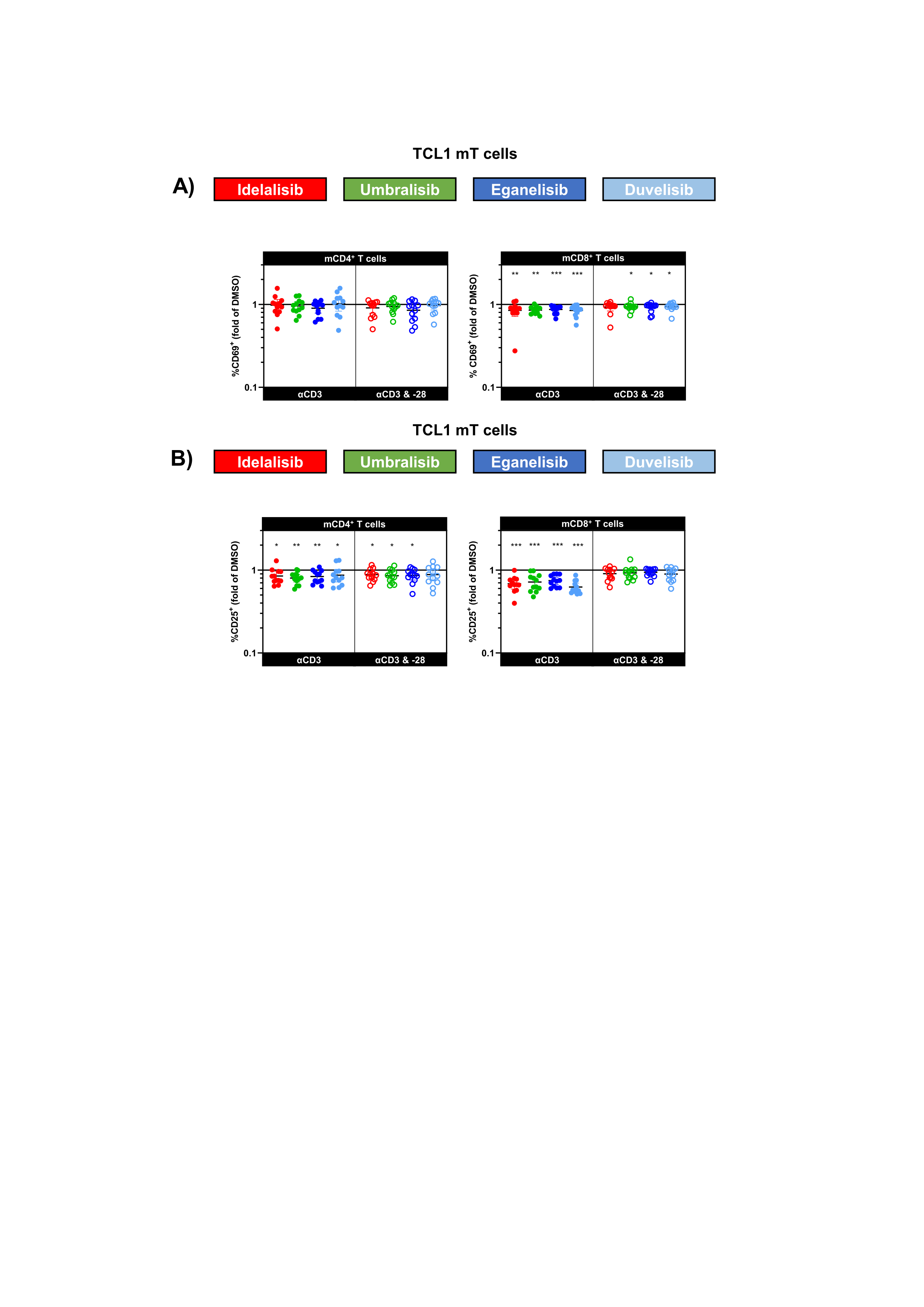
All graphs show mean ± CI. \*p <0.05; \*\* p < 0.01; \*\*\* p < 0.001.



**Suppl. Figure 5: PI3Kγ and -δ additively control T-cell function**

**A)** Human PBMCs (h; rhombi) were stimulated with αCD3 (1 µg/ml; n = 6) or a combination of αCD3 (1 µg/ml) and αCD28 (2 µg/ml; n = 7) in the presence of DMSO control, eganelisib, idelalisib, umbralisib or a combination of those (all 1 µM). Frequency of CD25+ cells out of human CD4+ (left) and CD8+ T cells (right) normalized to DMSO control.

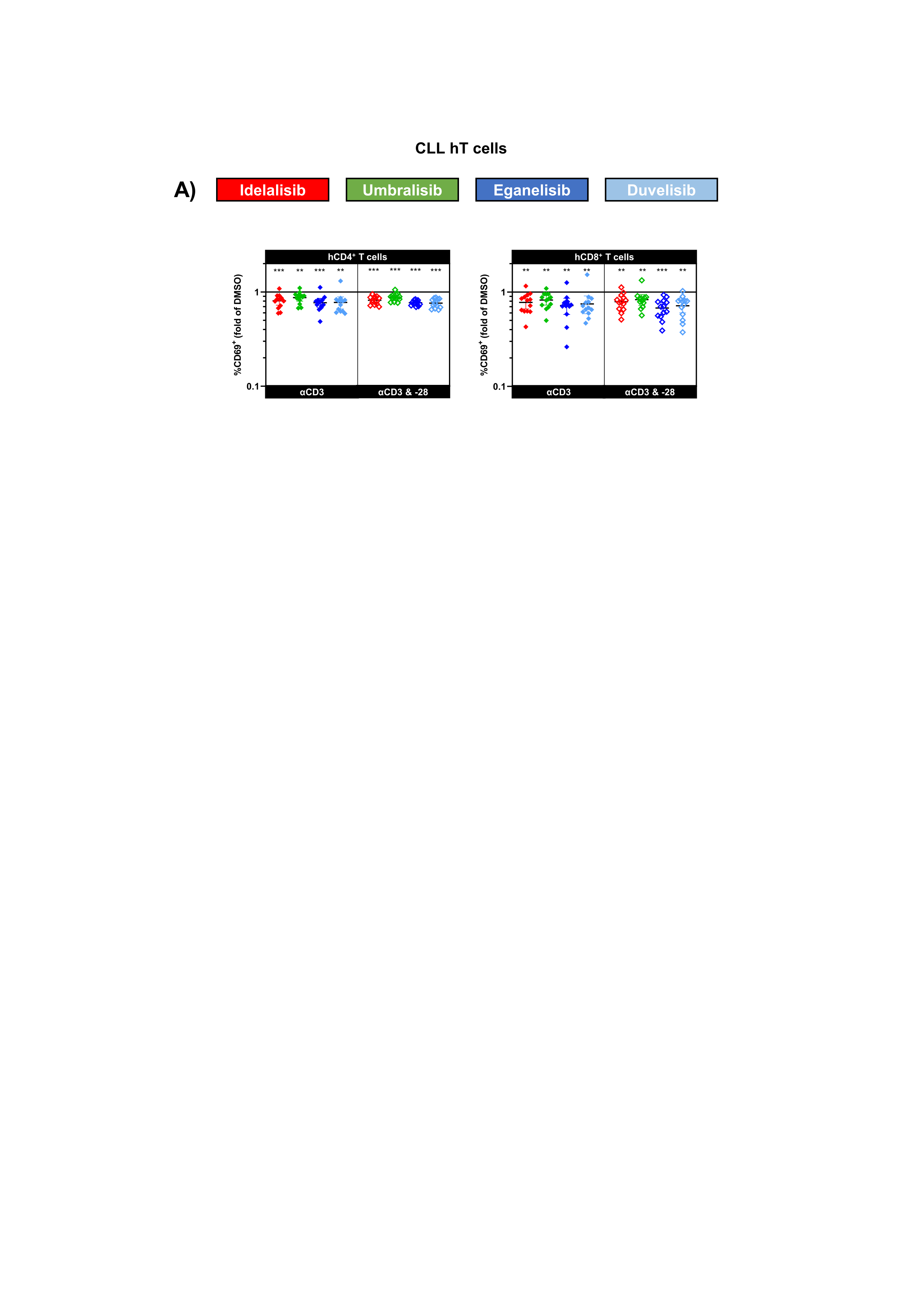
All graphs show mean ± SEM. \* p <0.05; \*\* p < 0.01; \*\*\* p < 0.001.



**Suppl. Figure 6: T cells of Eµ-TCL1 mice show minor sensitivity to PI3K inhibition**

**A-F)** Splenocytes of leukemic Eµ-TCL1 (m; circles; n = 12) were stimulated with αCD3 (1 µg/ml) or a combination of αCD3 (1 µg/ml) and αCD28 (2 µg/ml) in the presence of DMSO control, or the indicated PI3K inhibitors (all 1 µM). **A)** Frequency of CD69+ and **B)** CD25+ cells out of CD4+ (left) and CD8+ T cells (right) after 6 hrs of stimulation.

All graphs show mean ± CI. \* p <0.05; \*\* p < 0.01; \*\*\* p < 0.001.



**Suppl. Figure 7: Class I PI3K inhibition strongly reduces the function of T cells of CLL patients**

PBMCs of CLL patients (n = 13) were stimulated with αCD3 (1 µg/ml) or a combination of αCD3 (1 µg/ml) and αCD28 (2 µg/ml) in the presence of DMSO control, or the indicated PI3K inhibitors (all 1 µM). **A)** Frequency of CD69+ cells out of CD4+ (left) and CD8+ T cells (right) was analyzed after 6 hrs of stimulation and normalized to DMSO control.

All graphs show mean ± CI. \* p <0.05; \*\* p < 0.01; \*\*\* p < 0.001.