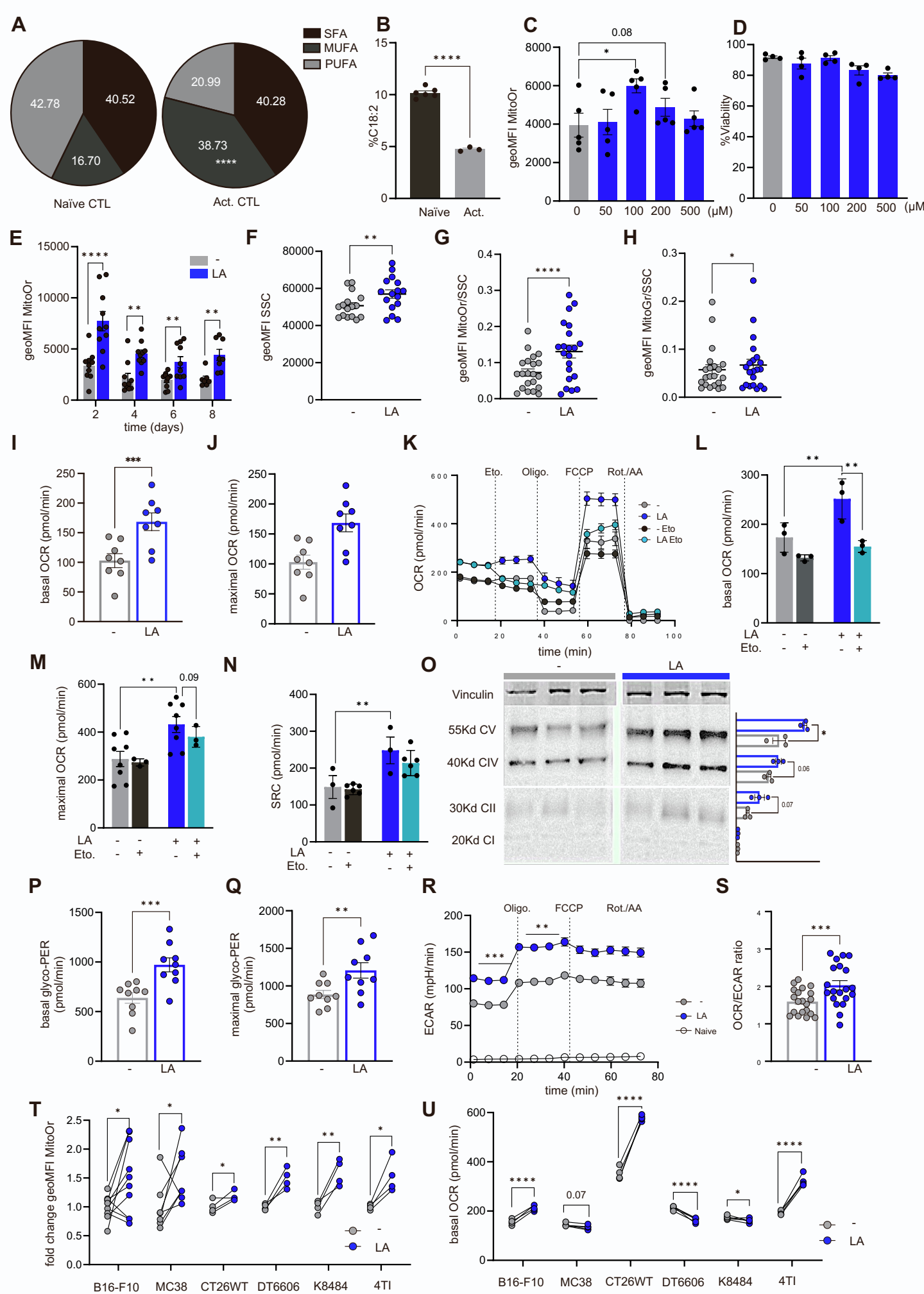


**Supplemental information**

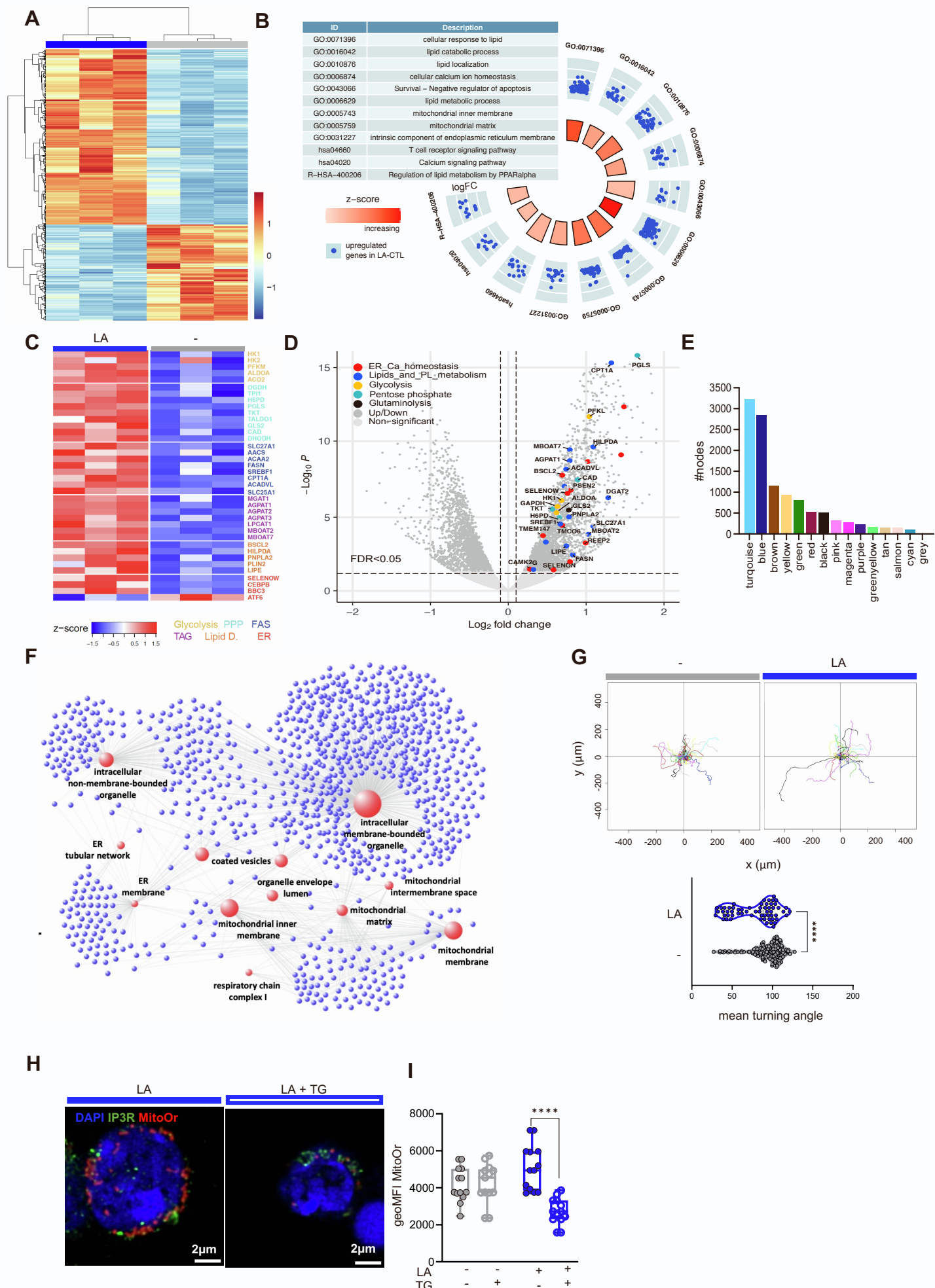
**Linoleic acid potentiates CD8<sup>+</sup> T cell  
metabolic fitness and antitumor immunity**

**Carina B. Nava Lauson, Silvia Tiberti, Paola A. Corsetto, Federica Conte, Punit Tyagi, Markus Machwirth, Stefan Ebert, Alessia Loffreda, Lukas Scheller, Dalia Sheta, Zeinab Mokhtari, Timo Peters, Ayush T. Raman, Francesco Greco, Angela M. Rizzo, Andreas Beilhack, Giovanni Signore, Nicola Tumino, Paola Vacca, Liam A. McDonnell, Andrea Raimondi, Philip D. Greenberg, Johannes B. Huppa, Simone Cardaci, Ignazio Caruana, Simona Rodighiero, Luigi Nezi, and Teresa Manzo**



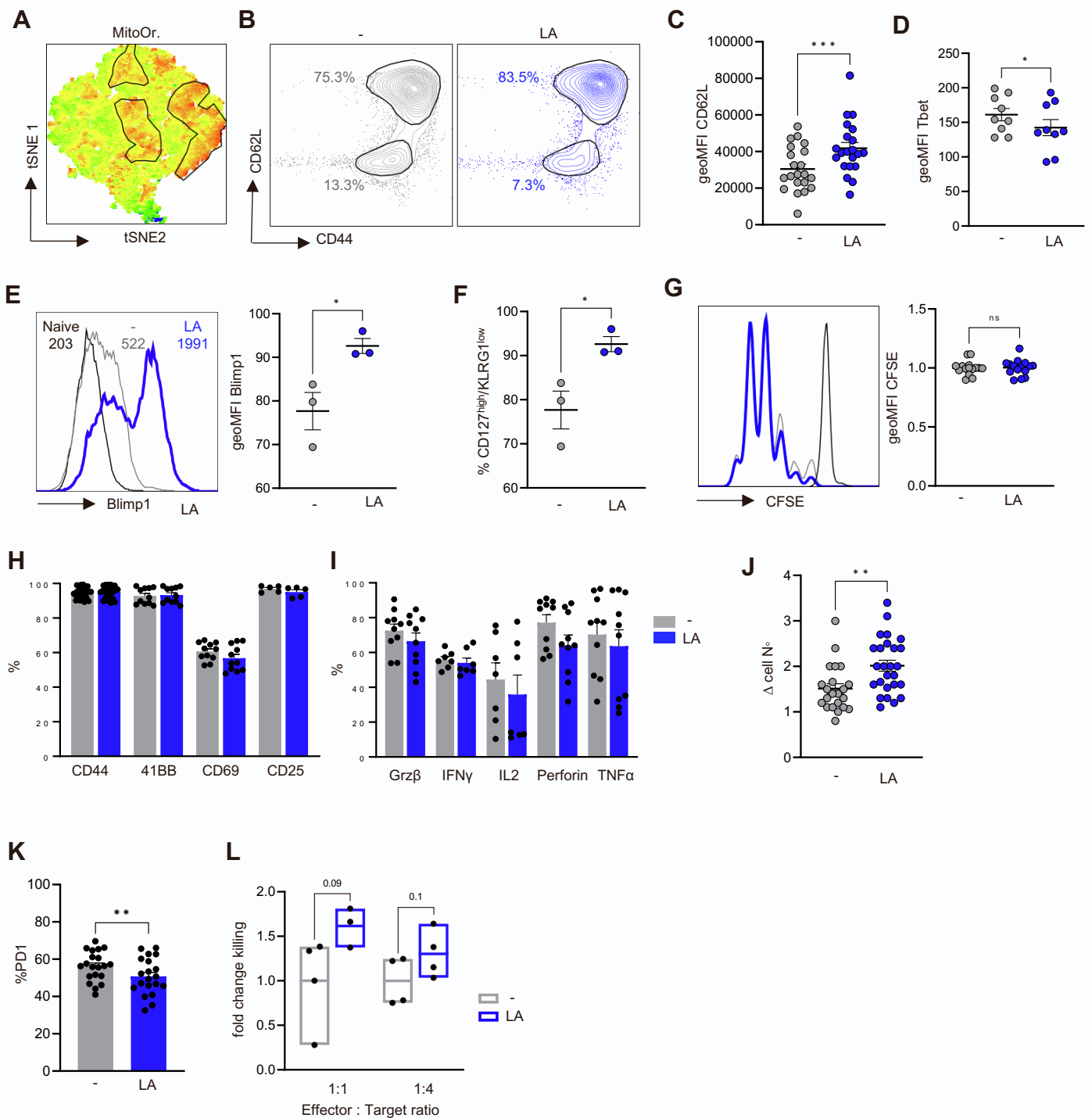
## **Supplementary figure 1. LA promotes mitochondrial rewiring in CTL. Related to Figure 1.**

(A-B) LS-MS quantification of SFA, MUFA and PUFA (A) and C18:2 (B) in naïve (n=5) and activated (n=3) CTL. MitoOr. staining (C) and viability percentage (D) upon LA titration (n=4).  
(E) MitoOr. staining in long term culture (n=6/10; 3 independent experiments).  
SSC values (F) were used to normalize MitoOr (G) and MitoGr (H) (n=16/20; 5 independent experiments).  
(I-J) CTL metabolic profile: basal (I) and maximal (J) OCR (n=8/12; 3 independent experiments).  
(K-N) FAO dependency test: OCR (K-M) and SRC (N). n=4.  
(O) OXPHOS representative western blot (n=3; 2 independent experiments).  
(P-R) Glycolytic activity: basal (P) and maximal (Q) PER (n=9; 3 independent experiments), as well as ECAR (R, n=4, representative of 5 independent experiments).  
(S) OCR/ECAR ratio (n=20; 5 independent experiments).  
(T-U) LA effect on tumor cell lines. MitoOr. (T) staining and basal OCR (U).  
Data are represented as mean  $\pm$  SEM. Paired, two-tailed Student's t test \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.



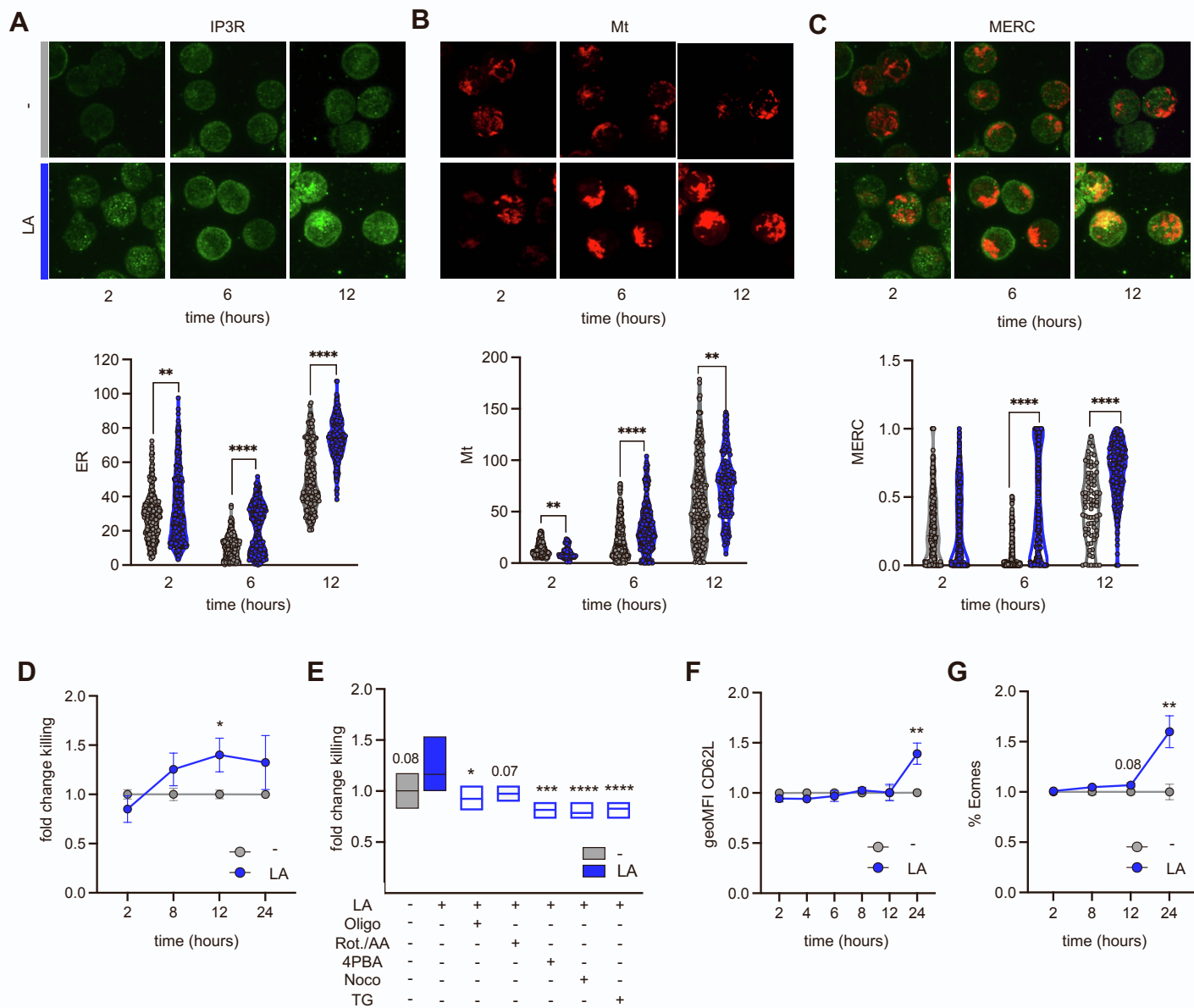
## **Supplementary figure 2. LA favors ER-Mitochondria network in CTL. Related to Figure 2.**

- (A) Topmost variable genes scoring from unsupervised clustering analysis (n= 3).
  - (B) Top biological processes upregulated within total DEGs.
  - (C) Heatmaps representing genes related to glycolysis, PPP, FAS, TAG, lipid droplets and ER.
  - (D) Volcano plot highlighting most representative genes for ER-Ca<sup>2+</sup> homeostasis, lipids and PL metabolism, glycolysis, PPP and glutaminolysis pathways.
  - (E) Modules identified from WGCNA and respective DEG number.
  - (F) GO cellular components network from blue module.
  - (G) Rose plots and mean turning angle based on live cell tracking assays.
  - (H-I) Effect of TG on MitoOr measured by confocal microscopy (H) and flow cytometry (I).
- Data are represented as mean ± SEM. Paired (H) or unpaired (F), two-tailed Student's t test \*\*\*\*p < 0.0001.



### Supplementary figure 3. LA pushes memory differentiation in CTL. Related to Figure 2 and 4.

(A) tSNE representation showing MitoOr. MFI (n=5).  
 (B-F) CTL memory phenotype assessed as: representative FACS plot showing CD44<sup>high</sup>/CD62L<sup>high</sup> (B) and CD62L (C, n=21; 5 independent experiments), Tbet (D, n=9; 3 independent experiments), Blimp1 (E, n=4; 4 independent experiments) and CD127<sup>high</sup>/KLRG1<sup>low</sup> (F) expression assessed by flow cytometry.  
 (G) CFSE profile (n=14; 4 independent experiments).  
 (H-I) Activation status measures as: canonic activation markers (H) and effector cytokines (I).  
 (J) Fold change in cell numbers after 48 hours of activation (n=26; 5 independent experiments).  
 (K) Percentage of PD1 positive cells (n=20; 5 independent experiments).  
 (L) Effector function assessed as killing assays 48 hours post-activation (n=4).  
 Data are represented as mean ± SEM. Paired, two-tailed Student's t test \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.



**Supplementary figure 4. Dynamic and fate of LA-redirected CTL.**  
Related to Figure 4.

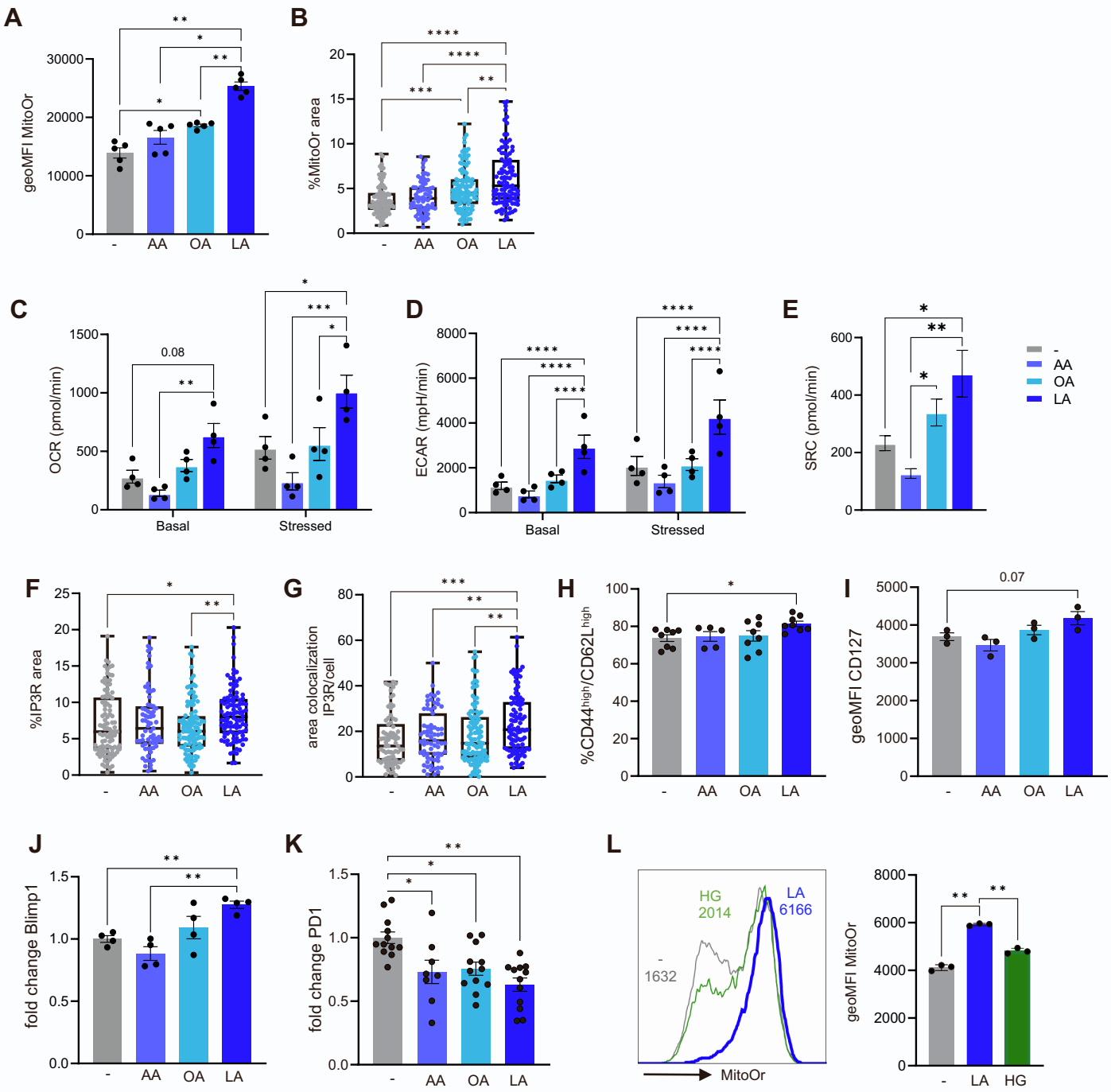
(A-C) Representative confocal images (top panels) and respective quantification (bottom graphs) of kinetics experiments for IP3R (A), MitoOr (B) and MAM formation (C).

(D) Effector functions assessed by killing assay (n=7).

(E) Effect of mitochondrial activity blockage (Oligo and Rot./AA) and MAM disruption (4PBA, Noco and TG) on CTL effector function (n=3/5).

(F-G) Kinetics on memory phenotype assessed by CD62L (F) and Eomes (G) expression.

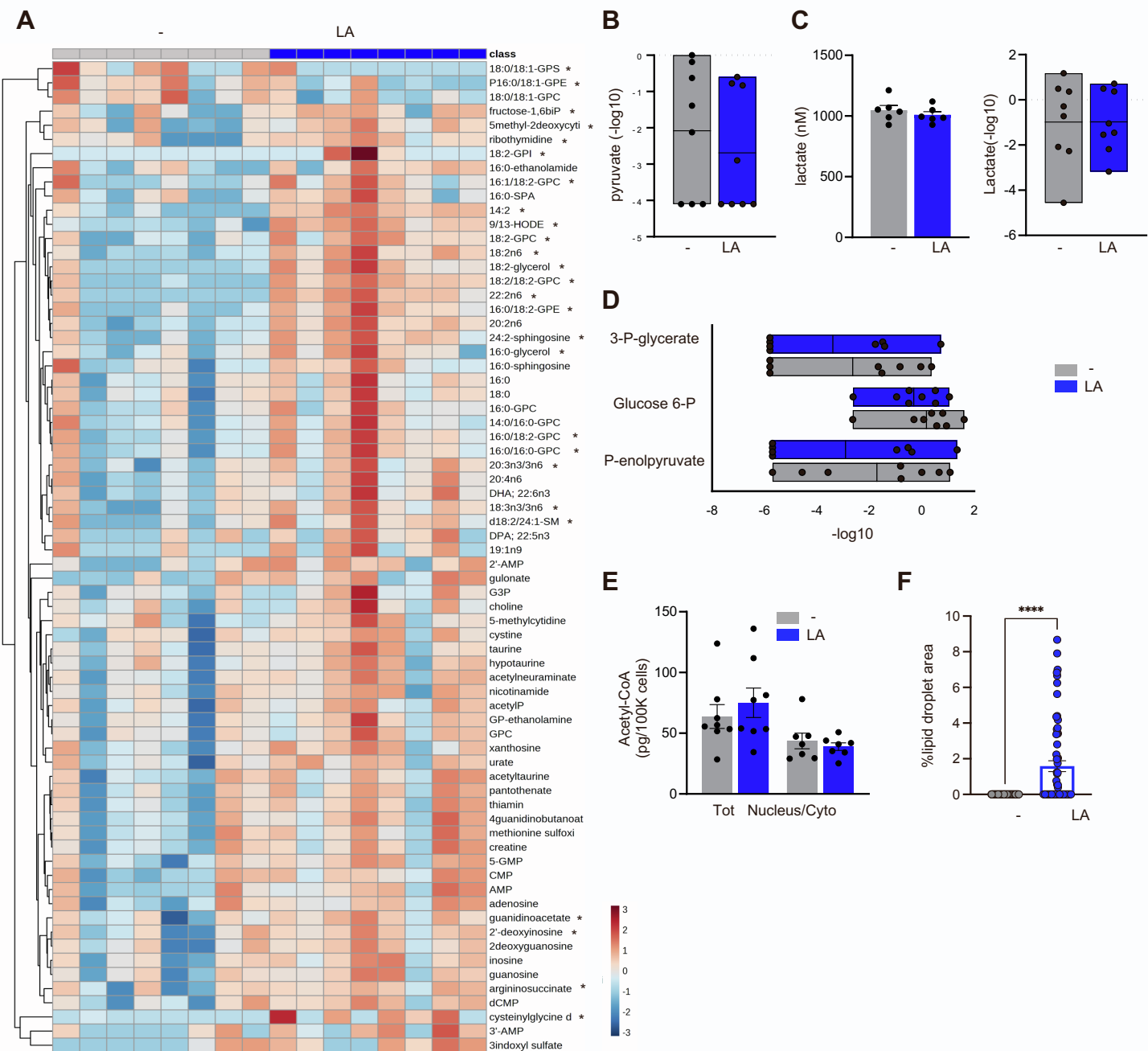
Data are represented as mean  $\pm$  SEM. Paired, two-tailed Student's t test (A,B,C and D) or one-way ANOVA using LA as control group (E) \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001

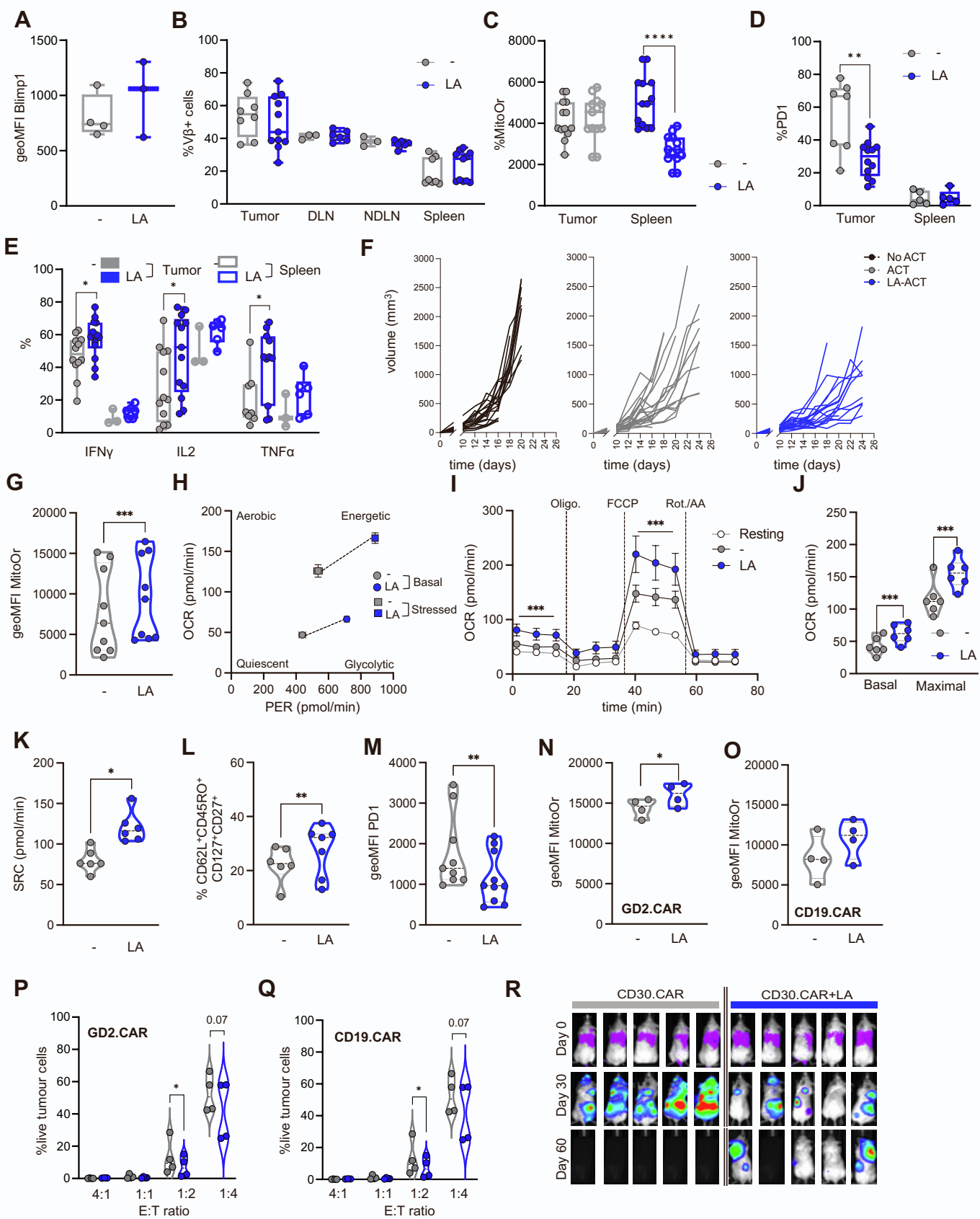


**Supplementary figure 5. LA has a unique role in regulating CTL responses. Related to Figure 1 and 2.**

(A-B) MitoOr. staining MFI -calculated by FACS - (A, n=5) and area -calculated by confocal microscopy (B, n=5).  
 (C-E) Metabolic profile assessed as: OCR (C), ECAR (D) and SRC (E). n= 4.  
 (F-G) IP3R area (F) and MAM quantification (G) by confocal microscopy (n=5; 2 independent experiments).  
 (H-K) Memory phenotype assessed as CD44<sup>high</sup>/CD62L<sup>high</sup> (H), CD127 (I) and Blimp1 (J),  
 (K) PD1 expression on different LC-FAs.  
 (L) MitoOr. staining on cells cultured in high-glucose conditions.  
 Data are represented as mean ± SEM. Paired, two-tailed Student's t test \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.







## Supplementary figure 7. LA-CTL improves ACT efficacy. Related to Figure 6 and 7.

(A) Blimp1 expression.

(B) Percentage of adoptively transferred CTL.

(C-E) Phenotypic characterization: MitoOr. (C), PD1 (D) and effector cytokines (E).

(F) Spider plots representing tumor growth curves.

(G-K) Metabolic characterization of human CD8 T cells (n=6/10; 3 independent experiments): MitoOr. (G), energetic map (H), OCR (I-J) and SRC (K).

(L-M) Phenotypic characterization of human CD8 T cells: percentage of TCM (L), expression levels of PD1(M).

(N-O) MitoOr of CAR-T cells: GD2 (N) and CD19 (O).

(P-Q) Long term killing assays in GD2 (P) and CD19 (Q) CAR T cells.

(R) Bioluminescence images assessing tumor burden.

Data are represented as mean  $\pm$  SEM. B, C and D show percentages within adoptively transferred CTL 14 days post-ACT. Statistics are based on paired (g-) or unpaired (A-F and R), two-tailed Student's t test \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.