Expanded View Figures

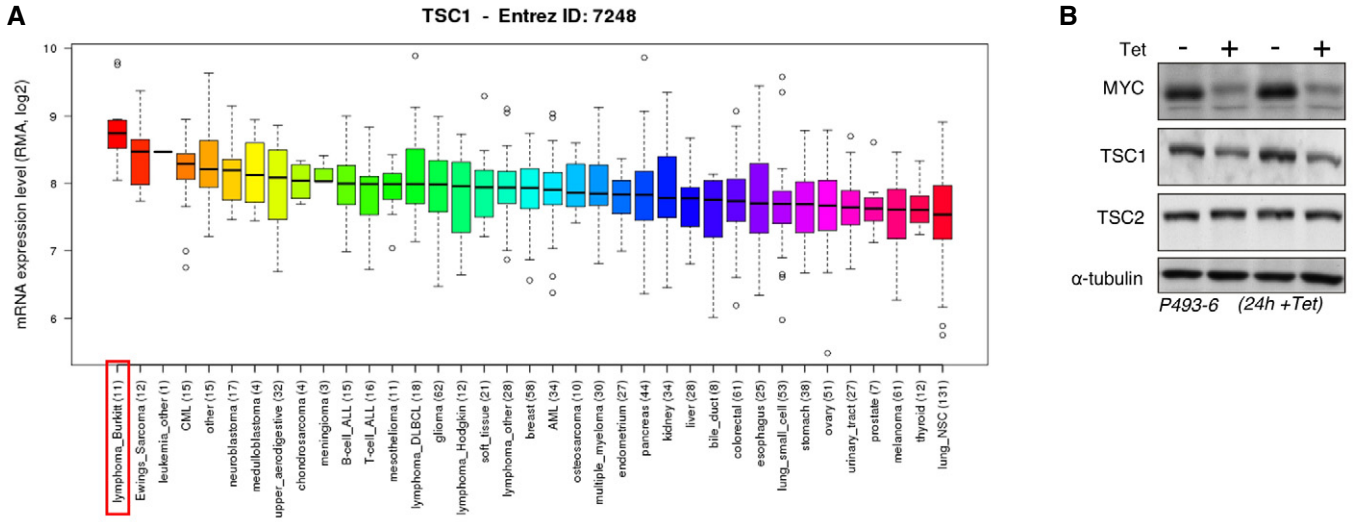

Figure EV1. TSC1 is highly expressed in Burkitt's lymphoma.

A Box plots showing the relative *TSC1* mRNA expression levels across different cancer cell line types with the horizontal line showing the median, whiskers showing upper and lower non-outlier limits, the box representing the first to the third quartiles, and open circles representing outliers. Data extracted from CCLE_Expression_Entrez_2012-10-18.res, with gene-centric robust multi-array analysis (RMA)-normalized mRNA expression data (the number of different cell lines is indicated in parentheses).

B TSC1 protein reduction precedes TSC2 reduction following repression of MYC (+Tet, 24 h) in P493-6 cells. Immunoblots showing expression levels of MYC, TSC1, TSC2, or α-tubulin in low (+Tet) versus high MYC (−Tet) P493-6 cells (in comparison with 72 h MYC repression shown in Fig 1B).

Figure EV2. Reduced activity of the mTORC1 pathway in BL patient samples.

A H&E-stained pictures of the samples in Figs 2A and EV2D. Two representative sections of the indicated sample are presented in 100× (left) and 200× (right) magnification, scale bar = 100 μm.

B Immunoblot of Fig 2B. Frozen tissue slices of individual Burkitt's lymphoma (BL), reactive lymph nodes (LN), or tonsils were lysed and subjected to immunoblotting with the indicated antibodies. Quantification shown in Fig 2B was done with ImageJ.

C Immunoblot of the same samples as in (B) with the indicated antibodies. Graph at the right shows the quantification of the P-S685K/S6K ratio performed with ImageJ (mean ± SD, $n = 3$ for reactive LN, $n = 10$ for lymphomas). ***$P < 0.001$, statistical relevance was determined by unpaired t-test (two-tailed).

D Example of immune staining of P-S6, the B-cell marker CD20, and DAPI nuclear DNA-staining in control lymph nodes (upper row) and Burkitt's lymphoma (lower rows). Of eight investigated lymphoma samples, we found seven with virtual absence of P-S6 staining and one with positive P-S6 staining. The samples belong to the same cohort used in Figs 2A and EV2A, scale bar = 100 μm.

E Wild-type (wt) MEFs and TSC1-deficient MEFs immunostained with anti-TSC1 antibody to confirm its specificity. Scale bar, 50 μm.

F wt MEFs untreated and wt MEFs treated with 20 nM rapamycin for 12 h were immunostained with anti-phosphorylated-S6 antibody to confirm its specificity. Scale bar, 50 μm.

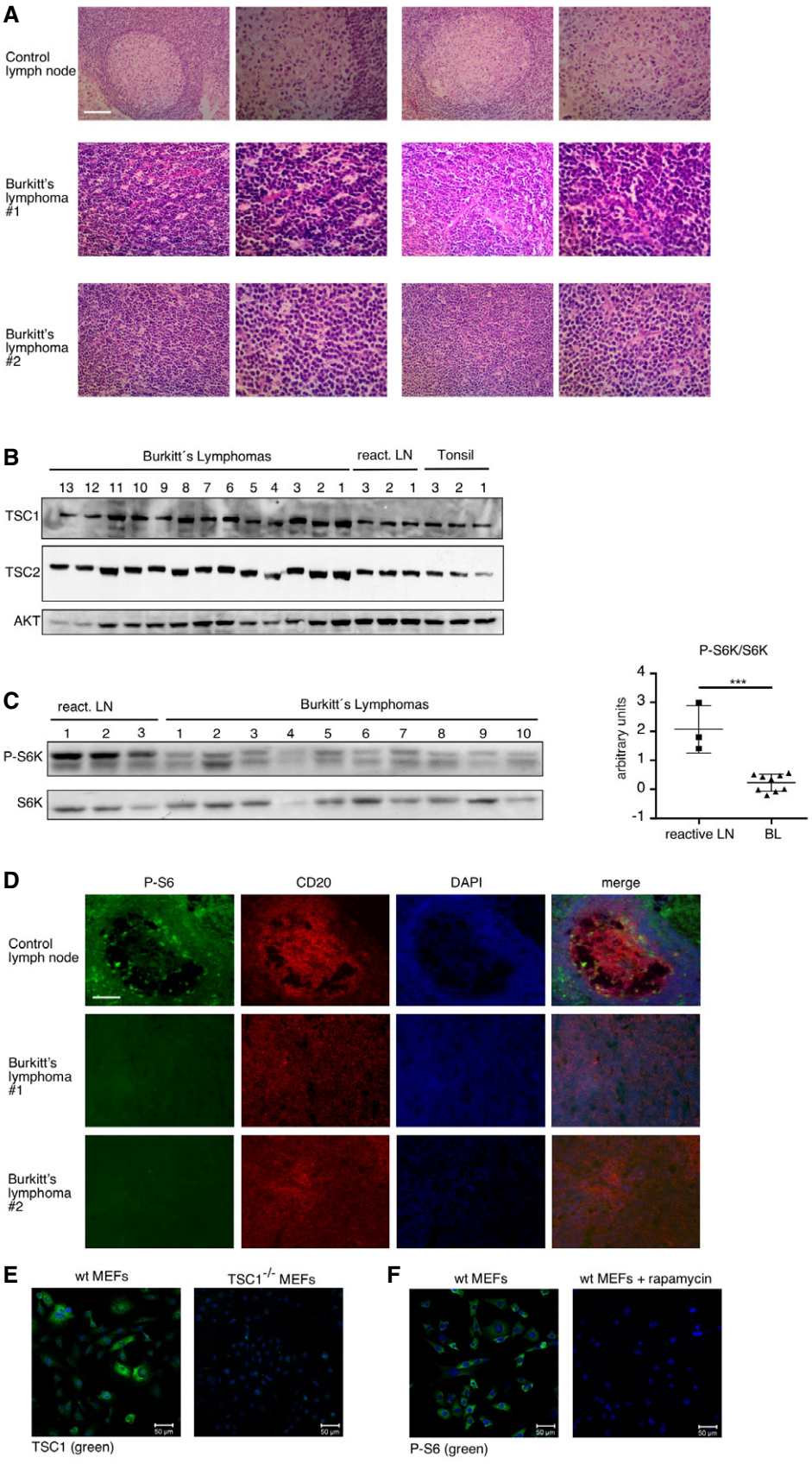

Figure EV2.

Figure EV3. TSC1 is required for survival of BL cells.

A Immunoblots of the experiment in Fig 3C. U2OS-MYC-ER cells expressing scrambled control shRNA or TSC1-specific shRNA, treated with hydroxytamoxifen (4-OHT) or rapamycin where indicated. Immunoblots showing expression of TSC1, S6K/P-S6K, or α-tubulin.

B–E Immunoblots of the experiments in Fig 3D to confirm *TSC1* knockdown. BL cells expressing scrambled control shRNA or TSC1-specific shRNA and subjected to immunoblotting with the indicated antibodies. The corresponding immunoblots for Raji and DG75 are shown in Fig EV4D and E, respectively.

F At the top, immunoblots of indicated proteins in Hodgkin lymphoma (HL) cell lines KMH2 and L540 expressing either a TSC1-specific shRNA or scrambled control shRNA. The bar graphs below show that TSC1 knockdown does not affect cell viability in KMH2 or L540 cells (mean ± SD, $n = 3$ biological replicates). See Fig 1A for MYC and TSC1 expression levels.

G Immunoblots show the reduction of TSC2 levels upon expression of a TSC2-specific shRNA compared to scrambled control shRNA in the indicated BL cell lines and in addition the expression levels of S6K/PS6K and β-actin as loading control. The bar graphs at the right show the relative cell viability of the same BL cell lines expressing either TSC2-specific shRNA or scrambled control shRNA 2 days after seeding equal amounts of viable cells as determined by a cell viability assay (mean ± SD, biological replicates $n = 3$ for Ramos, $n = 5$ for BL2 and $n = 6$ for CA46).

H Control- or TSC1-shRNA expressing Raji BL cells treated with different concentrations of rapamycin to either completely inhibit mTORC1 activity (10 nM) or titrate the activity to control levels (30 pM), and survival rate of these cells over 7 days (mean ± SD, $n = 3$ biological replicates).

Data information: In all graphs $^{**}P < 0.01$; $^{***}P < 0.001$, statistical relevance was determined by unpaired *t*-test (two-tailed).

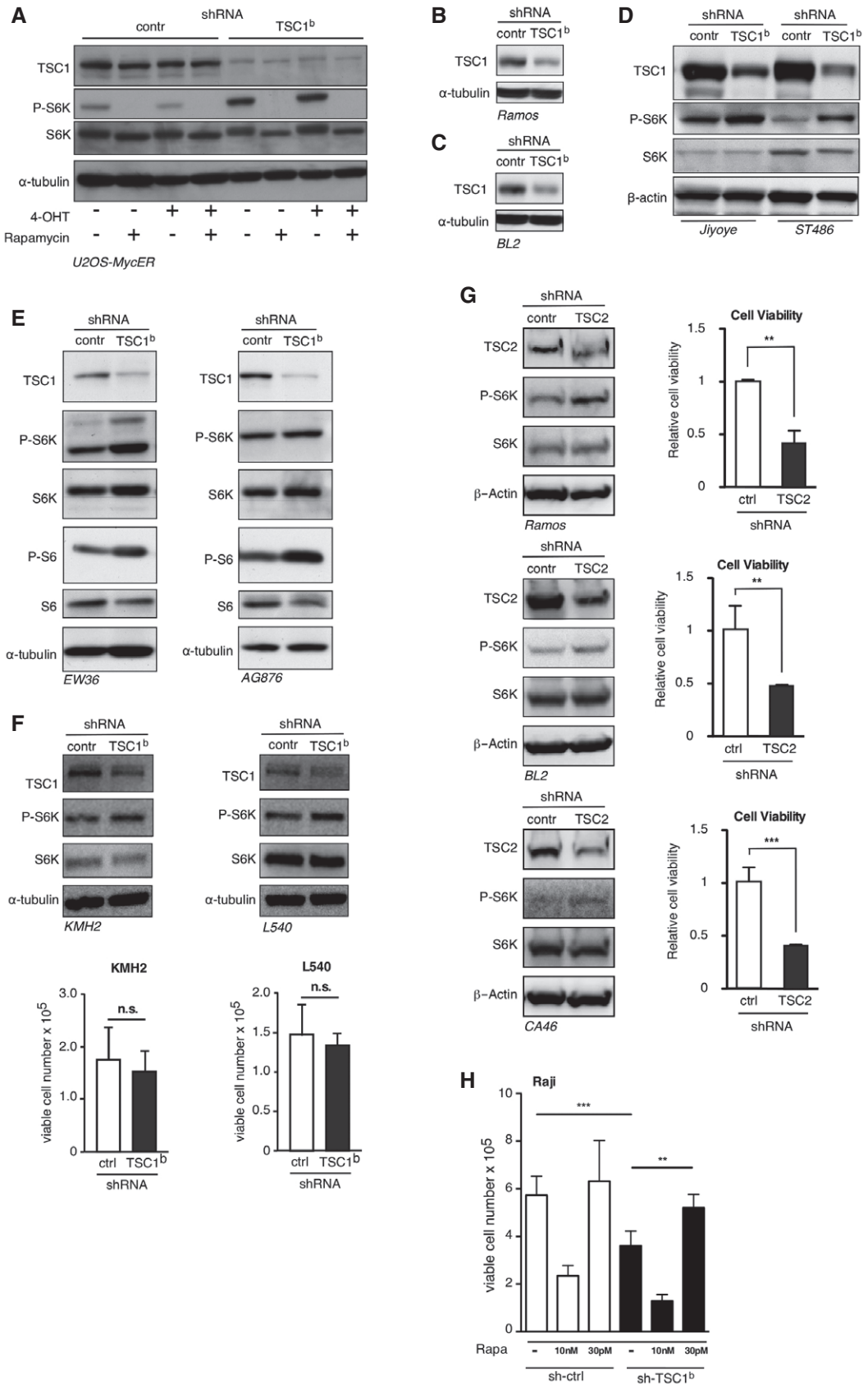

Figure EV3.

Figure EV4. TSC1 knockdown increases mitochondrial function.

A Immunoblots of control- or TSC1-shRNA expressing BL cells as indicated showing the effect of the TSC1 knockdown on phosphorylation of AKT (P-Ser473). Antibodies specific for AKT and β-actin served as controls. The incubation of the same immunoblots with an antibody specific for P-Thr308 AKT did not produce any detectable signal.

B *TSC1* knockdown increases ratio of oxygen consumption to lactate production (as measured by acidification) in a rapamycin-dependent manner. Ratio of oxygen consumption to lactate production rates determined in the experiment of Figure 4A in high MYC P493-6 (−Tet) cells expressing scrambled control shRNA or TSC1-specific shRNA, treated with 20 nM rapamycin for 12 h where indicated (mean ± SD, $n = 6$ biological replicates).

C Relative mRNA expression determined by qRT–PCR of *ATP5G1* (left graph) or cytochrome C (*CYCS*) (right graph) in Raji cells expressing scrambled control shRNA or TSC1-specific shRNA (mean ± SD, $n = 3$).

D From left to right in Raji cells expressing scrambled control shRNA or TSC1-specific shRNA: immunoblots showing expression of TSC1, S6K/P-S6K, or α-tubulin; rate of oxygen consumption, basal and in response to 10 μM DNP or 10 μM oligomycin where indicated; ratio of oxygen consumption to lactate production rates (mean ± SD, $n = 8$).

E From left to right in DG75 cells expressing scrambled control shRNA or TSC1-specific shRNA: immunoblots showing expression of TSC1, S6K/P-S6K, S6/P-S6, P-4E-BP1, or α-tubulin; rate of oxygen consumption, basal and in response to 10 μM DNP or 10 μM oligomycin where indicated; ratio of oxygen consumption to lactate production rates (mean ± SD, $n = 8$).

F Left graphs show increased ROS levels in BL cell lines as indicated expressing a TSC2-specific shRNA compared to scrambled control shRNA expressing cells as determined by FACS analysis of CellRox-stained cells (mean ± SD, $n = 6$ biological replicates). Right graphs show increased rate of basal and maximal (in response to 600 nM of the chemical uncoupler FCCP) oxygen consumption in TSC2 knockdown cells compared to scrambled control shRNA expressing cells. Oxygen consumption in response to the ATPase inhibitor oligomycin (10 μM) was comparable between TSC2 knockdown and control cells (mean ± SD, $n = 8$). Immunoblots at the bottom left show expression levels of TSC2, PS6K/S6K, and β-actin as loading control for the EW36 cell line. Corresponding immunoblots for Ramos and CA46 cell lines are shown in Fig EV3G.

Data information: In all graphs $*P < 0.05$; $**P < 0.01$; $***P < 0.001$, statistical relevance was determined by unpaired *t*-test (two-tailed).

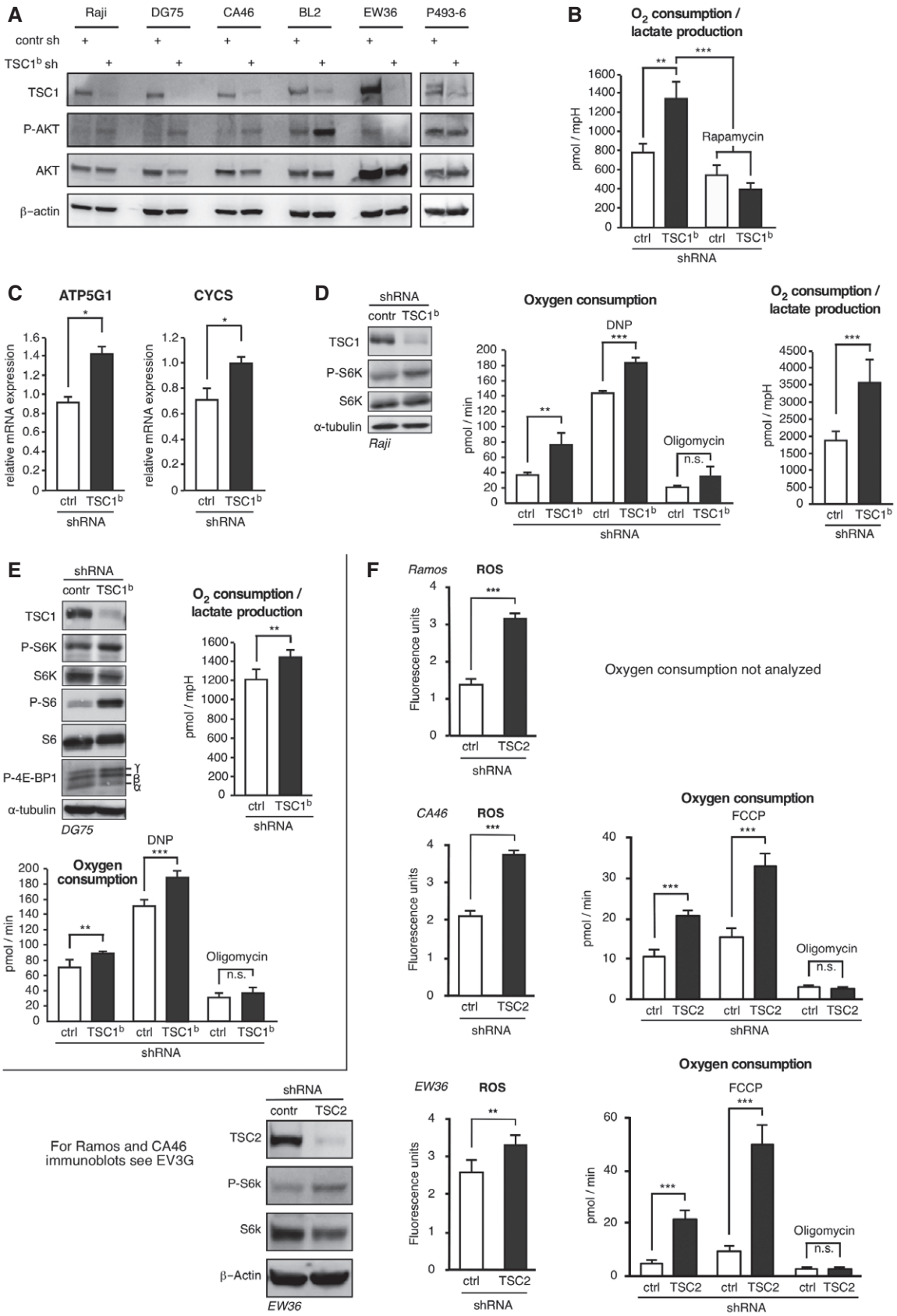

Figure EV4.

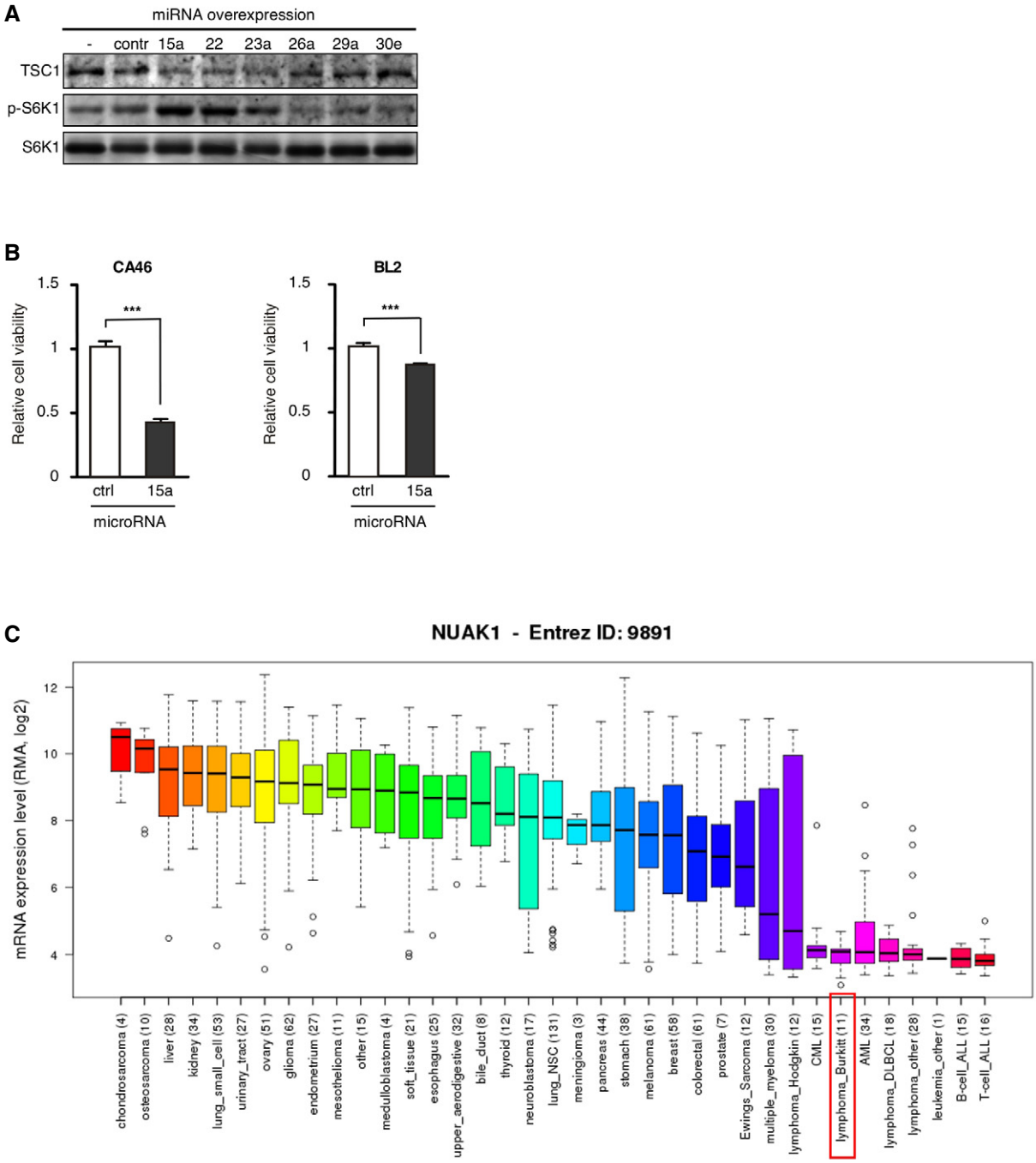

Figure EV5. Effect of MYC-suppressed microRNAs on mTORC1 activity and BL cell survival and NUAK mRNA expression levels in different types of cancer cell lines.

A MYC-suppressed microRNAs regulate the TSC1-mTORC1 axis. Immunoblot with the indicated antibodies of HEK293T cells transfected with the indicated microRNAs or a control miRNA.

B Reduced relative cell viability of the BL cell lines CA46 (left) and BL2 (right) expressing miR-15a compared to control miR expressing cells (mean ± SD, n = 5 biological replicates). ***$P < 0.001$, statistical relevance was determined by unpaired t-test (two-tailed).

C Box plots showing the relative NUAK1/ARK5 mRNA expression levels across different cancer cell line types with the horizontal line showing the median, whiskers showing upper and lower non-outlier limits, the box representing the first to the third quartiles, and open circles representing outliers. Data extracted from CCLE_Expression_Entrez_2012-10-18.res, with gene-centric robust multi-array analysis (RMA)-normalized mRNA expression data. The number of cell lines used for each tumor type is indicated in parentheses.