Asymmetric cell division, the partitioning of cellular components in response to polarizing cues during mitosis, has roles in differentiation and development. It is important for the self-renewal of fertilized zygotes in Caenorhabditis elegans and neuroblasts in Drosophila, and in the development of mammalian nervous and digestive systems. T lymphocytes, upon activation by antigen-presenting cells (APCs), can undergo asymmetric cell division, wherein the daughter cell proximal to the APC is more likely to differentiate into an effector-like T cell and the distal daughter is more likely to differentiate into a memory-like T cell. Upon activation and before cell division, expression of the transcription factor c-Myc drives metabolic reprogramming, necessary for the subsequent proliferative burst. Here we find that during the first division of an activated T cell in mice, c-Myc can sort asymmetrically. Asymmetric distribution of amino acid transporters, amino acid content, and activity of mammalian target of rapamycin complex 1 (mTORC1) is correlated with c-Myc expression, and both amino acids and mTORC1 activity sustain the differences in c-Myc expression in one daughter cell compared to the other. Asymmetric c-Myc levels in daughter T cells affect proliferation, metabolism, and differentiation, and these effects are altered by experimental manipulation of mTORC1 activity or c-Myc expression. Therefore, metabolic signalling pathways cooperate with transcription programs to maintain differential cell fates following asymmetric T-cell division.

To visualize c-Myc levels in activated T cells, we isolated T cells from c-Myc–GFP fusion knock-in (c-Myc–GFP) mice and activated them in vitro with anti-CD3 antibodies, anti-CD28 antibodies, and ICAM. As T cells completed the first division (indicated by dilution of CellTrace Violet), the c-Myc–GFP signal was brightest in cells that expressed higher levels of CD8, a marker of asymmetric cell division. This difference between CD8high and CD8low cells dissipated in subsequent divisions, as did the difference in c-Myc expression. We observed the GFP signal was diffuse from prophase through anaphase, and only upon cytokinesis and re-formation of the nuclear envelope were c-Myc levels distinguishable in the daughter cells. Real-time analysis of GFP expression during mitosis revealed that the signal was diffuse throughout the cell until after division. The signal then increased in one daughter cell, establishing an asymmetric distribution (Fig. 2a and Supplementary Video 7). In fixed T cells, we observed the GFP signal was diffuse from prophase through anaphase, and only upon cytokinesis and re-formation of the nuclear envelope were c-Myc levels distinguishable in the daughter cells (Extended Data Fig. 3). It is therefore likely that c-Myc is differentially regulated in the two daughters by asymmetrically inherited upstream signalling proteins, rather than being polarized itself.

To determine if differences in the levels of c-Myc following the first division are relevant to c-Myc function, we examined undivided and first-division T cells sorted into c-Mychigh and c-Myclow populations for expression of several metabolic genes that were previously found to be controlled by c-Myc. We found similar differences in expression of most of these genes influenced by c-Myc–GFP in both undivided and first-division cells (Extended Data Fig. 4a–c). The difference between c-Myclow and c-Mychigh upon asymmetric cell division is relevant for expression of c-Myc target genes.

We assessed several activation markers on c-Mychigh and c-Myclow T cells before and after the first division. CD44 expression was comparable among all populations, and both c-Mychigh and c-Myclow populations exhibited increased expression of CD69 compared with undivided cells. Although all activated cells also displayed increased CD25 and CD98, c-Mychigh T cells displayed elevated levels of both (Fig. 2c), as previously described for CD25 (ref. 2). Interleukin (IL)-2 can drive the expression of c-Myc, but neither transient inhibition of IL-2 receptor signalling with Janus kinase (JAK) inhibitors (Extended Data Fig. 5a) nor increased IL-2 (Extended Data Fig. 5b) influenced the asymmetric assortment of c-Myc in T cells following the first division.

We then further examined the CD98 asymmetry. Microscopy confirmed that surface expression of CD98 was elevated on the proximal daughter T cell and correlated with c-Myc–GFP expression (Fig. 2d, e, Extended Data Fig. 5c, d). CD98 is a heterodimer composed of CellTrace Violet and anti-CD3 antibodies.
of SLC3A2 and SLC7A5. Analyses of these components in undivided cells revealed that SLC3A2 protein is polarized to the region of the mitochondria. Of the metabolites revealed that levels of several, but not all, amino acids were at least 1.5-fold higher in first-division c-Myc\textsubscript{low} versus c-Myc\textsubscript{high} T cells (Fig. 2f). We therefore examined the effects of amino acid availability on the asymmetric expression of c-Myc. Although high leucine or glutamine had no effect on c-Myc expression in first-division T cells, transient depletion of all amino acids greatly diminished c-Myc asymmetry (Fig. 2g). Asymmetric distribution of c-Myc–GFP was sensitive to transient inhibition of glutaminolysis by the glutamine analogue 6-diazo-5-oxo-l-norleucine (DON) (Fig. 2g). Expression levels of CD98 were unchanged in these conditions (Fig. 2h). Thus, amino acid availability as well as glutaminolysis appears to sustain high c-Myc levels following asymmetric cell division.
We then acutely inhibited mTORC1 activity following the first division. Inhibition of mTORC1 activity with either rapamycin or Torin2 reduced c-Myc expression in CD8\(^{\text{high}}\) T cells compared to that of CD8\(^{\text{low}}\) cells (Fig. 3f), but did not influence CD98 asymmetry (Fig. 3g). To determine if elevation of mTORC1 activity affects c-Myc asymmetry, CD4-Cre, Tsc\(^{1/2}\)floX/floX mice, which exhibit hyperactive mTORC1 signalling\(^{12}\), were crossed with c-Myc–GFP mice. Unlike those from wild-type littermates, Tsc\(^{1/2}\) CD8\(^{\text{high}}\) and CD8\(^{\text{low}}\) T cells in the first division had similarly high expression of c-Myc–GFP (Fig. 3h). Therefore, asymmetric mTORC1 activity sustains the asymmetric assortment of c-Myc following T-cell division.

Various signals determine when and how much c-Myc is expressed\(^{13}\). Although phosphorylation of c-Myc by glycogen synthase kinase 3 (GSK3) promotes its proteasomal degradation\(^{14,15}\), GSK3 inhibition had no effect on c-Myc asymmetry (Fig. 3f). Further, although acute inhibition of the proteasome with MG132 elevated c-Myc expression in both CD8\(^{\text{high}}\) and CD8\(^{\text{low}}\) daughter cells, asymmetry was unaffected. Strikingly, acute inhibition of translation by cycloheximide attenuated c-Myc expression and asymmetry, whereas acute inhibition of transcription by actinomycin D had no effect (Fig. 3f). None of the short-term treatments tested had any effect on the expression or asymmetric distribution of CD98 (Fig. 3f). Therefore, it is likely that mTORC1 activity regulates c-Myc at the level of translation, although a role for transcription over a longer timeframe could not be assessed. Consistently, we observed that mRNA for c-Myc and both components of CD98 (SLC3A2 and SLC7A5), and several other metabolic genes (which are c-Myc targets\(^{15}\)) were enriched in first-division c-Myc\(^{\text{high}}\) T cells compared to c-Myc\(^{\text{low}}\) T cells (Extended Data Fig. 4a, c).

T-cell proliferation depends on c-Myc\(^{6}\). We therefore examined if the relative differences in c-Myc levels in the first division influence subsequent proliferation. At 35 h after activation, CellTrace-Violet-labelled activated T cells were pulsed with BrdU for 1 h. The cell cycle status of c-Myc\(^{\text{high}}\) and c-Myc\(^{\text{low}}\) T cells that had divided once was examined, and nearly ten times the frequencies of c-Myc\(^{\text{high}}\) T cells were cycling (in S or G2/M phases) compared with c-Myc\(^{\text{low}}\) T cells (Fig. 4a). Additionally, sorted c-Myc\(^{\text{high}}\) T cells proliferated much more rapidly than c-Myc\(^{\text{low}}\) T cells (Fig. 4b, c) when placed back in culture for 48 h. In contrast, sorted CD8\(^{\text{high}}\) and CD8\(^{\text{low}}\) first-division TSC1-null T cells, which display similar c-Myc levels (Fig. 3h), showed equivalent proliferation (Fig. 4c).

As c-Myc regulates metabolic reprogramming during T-cell activation\(^{6,16,17}\), we tested whether metabolic differences exist in first-division T cells expressing high or low levels of c-Myc. We found that c-Myc\(^{\text{high}}\) cells possessed diminished levels of glucose and glycolytic intermediates, but elevated levels of downstream metabolites (3-phosphoglycerate and phosphoenolpyruvate) and end products pyruvate and lactate, which may reflect enhanced glycolysis in c-Myc\(^{\text{high}}\) cells. Elevated levels of tricarboxylic acid cycle intermediates such as \(α\)-ketoglutarate, fumarate, and malate in c-Myc\(^{\text{high}}\) T cells may reflect enhanced gluconeogenesis (Extended Data Fig. 9). Additionally, pentose phosphate pathway metabolites including 6-phosphogluconate and sedoheptulose 7-phosphate accumulated in c-Myc\(^{\text{high}}\) cells. We then examined the extracellular acidification rate in c-Myc\(^{\text{high}}\) and c-Myc\(^{\text{low}}\) T cells under conditions of glycolytic stress\(^{18,19}\) and found that c-Myc\(^{\text{high}}\) T cells tended to be more glycolytic (Fig. 4e, Extended Data Fig. 10a). Even during basal respiration with glucose, c-Myc\(^{\text{high}}\) T cells exhibited higher extracellular acidification rate with no changes in oxygen consumption rate (Extended Data Fig. 10b). Similarly, metabolic flux assays tracking radiolabelled substrates revealed increased glycolysis and glutamine uptake in c-Myc\(^{\text{high}}\) T cells (Fig. 4d). Although it has been suggested that mitochondria sort asymmetrically\(^{20}\), we did not detect any differences in mitochondrial mass or DNA in c-Myc\(^{\text{low}}\) compared with c-Myc\(^{\text{high}}\) first-division T cells (Extended Data Fig. 10c, d), but this does not exclude the possibility that ‘older’ mitochondria may sort preferentially into one daughter\(^{20}\). However, we suggest that the differences in

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**Figure 3** | mTORC1 activity is required for the maintenance of c-Myc asymmetry in activated CD8 T cells. a–c, Representative image and quantification of asymmetry based on fluorescence intensity (difference/total) of pS6 (red); 88.9% both bright in proximal daughter (\(x^2 = 19.89, \text{DF} = 3, P = 0.0002, \chi^2\)-square goodness of fit; \(R^2 = 0.1388, P = 0.1388, \text{linear regression}\) (a, b) and p70S6K (red); 85.7% both bright in proximal daughter (\(x^2 = 14.14, \text{DF} = 3, P = 0.0027, \chi^2\)-square goodness of fit; \(R^2 = 0.3526, P = 0.1598, \text{linear regression}\)) (c) in T cells co-cultured with BMDCs. d, e, Representative image and quantification of asymmetry based on fluorescence intensity (difference/total) of pFOXO1 (pS256) (red) in CD8 T cells (antibody-coated plates); 75.8% concordance of markers (\(P = 0.0081, \text{two-tailed binomial test}\); \(R^2 = 0.5153, P < 0.0001, \text{linear regression}\)). Original magnification, \(<1000\). f, g, MFI of first division c-Myc–GFP (f) or CD98 (g) in CD8\(^{\text{high}}\) and CD8\(^{\text{low}}\) after 35 h activation on antibody-coated plates and the indicated agent for 45 min before flow cytometric analysis. *\(P < 0.05\), unpaired Student’s t-test. h, CellTrace-Violet-labelled T cells from lymphoid tissues of CD4-Cre\(^{+}\) (WT) or CD4-Cre\(^{+}\) Tsc\(^{1/2}\)floX/floX animals 36 h after activation. The ratio of MFI of c-Myc–GFP in first-division CD8\(^{\text{high}}\)-CD8\(^{\text{low}}\) T cells is plotted (left), and representative flow plots are depicted (right).

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One consequence of amino acid availability is activation of the mTORC1 complex, which is also activated by glutaminolysis\(^{21}\). Both phospho (p)-mTOR distribution (Extended Data Fig. 7a, b) and mTORC1 activity\(^{8}\), assessed by pS6 (Fig. 3a, b) or p70S6K (Fig. 3c), associated with c-Myc\(^{\text{high}}\) daughter T cells (Extended Data Fig. 8a, b). Reciprocally, inhibition of c-Myc expression by treatment with the inhibitor IQ1 resulted in reduced mTORC1 signalling (Extended Data Fig. 7c), indicating a positive feedback mechanism. Another indirect target, phosphorylated when mTORC1 is active, is the transcription factor FOXO1\(^{9}\). FOXO1 is important for maintaining quiescence in T cells\(^{10}\) and negatively regulates c-Myc target genes in its non-phosphorylated form\(^{11}\). Phosphorylated FOXO1 (pFOXO1) was also correlated with c-Myc–GFP expression (Fig. 3d, e, Extended Data Fig. 8e).
lymphoid organs of mice that had received c-Myc$^{\text{high}}$ T cells (Fig. 4f).

After an additional two weeks, recipient animals were challenged with a heterosubtypic virus (restricting responses to T cells) expressing SIINFEKL. Nine days later, an inverse pattern to pre-challenge results was observed, with increased frequencies of cells derived from the c-Myc$^{\text{low}}$ T cells (Fig. 4g). Thus, the asymmetric distribution of c-Myc in the first division was directly correlated with the eventual fate of these cells, with high levels of c-Myc conferring increased proliferation in the primary response and low levels of c-Myc conferring decreased proliferation in the primary response (but increased persistence and secondary response). Importantly, when T cells were wild type or heterozygous for c-Myc (exhibiting reduced c-Myc expression), sorted CD8$^{\text{high}}$ and CD8$^{\text{low}}$ T cells from c-Myc$^{1/2^-}$ T cells contributed equally to the recall response, whereas wild-type CD8$^{\text{high}}$ T cells did not (Fig. 4h). *In vivo* inhibition of mTORC1 in recipient animals with rapamycin also restored the ability of CD8$^{\text{high}}$ T cells to contribute to a secondary response (Fig. 4i), underscoring the relationship between mTORC1 signalling and c-Myc expression in regulating cell fate. These findings corroborate studies that show distinct fates of T cell daughters that have undergone asymmetric cell division, with the proximal and distal daughters displaying effector-like and memory-like gene expression and function$^{21}$, but suggest that these fates remain amenable to regulation (for example, of mTORC1 or c-Myc). Although the fates of asymmetrically divided T cells are distinct, we do not suggest this is necessarily the only source of memory T-cell differentiation. Other studies have shown that memory cells have expressed proteins such as granzyme B and interferon (IFN)$^{22-24}$, and are thus probably products of effector cells$^{25,26}$. Our data support a model in which T cells proceed through an activation phase, but asymmetric division results in a segregation of activating signals and amino acid transporters that result in divergent mTORC1 activity and c-Myc expression that ultimately make a daughter cell more or less likely to become terminally differentiated.

Our findings suggest that the interplay of amino acid transport and glutaminolysis, mTORC1 activity, and c-Myc expression sustains the fates of T cells that have undergone asymmetric cell division. c-Myc drives the expression of the amino acid transporters CD98 and SLC1A5$^{27,28}$, and sustains mTORC1 activity during early activation$^{28}$. Conversely, amino acids and glutaminolysis sustain mTORC1 activity, which sustains c-Myc translation$^{29}$, with possible effects on c-Myc through inhibition of FOXO1$^{11}$. During activation, some amino acid transporters sort asymmetrically, and a positive feedback arises, causing asymmetry in mTORC1 activity and c-Myc expression, which is then sustained by c-Myc function (or lack thereof) in the daughter cells. Notably, c-Myc was among the top candidates likely to drive divergence in gene expression between the daughters of first division in activated T cells$^{21}$. Consistently, T cells lacking CD98 or treated with inhibitors of mTORC1$^{30}$ display poor primary responses but generate memory T cells, whereas T cells lacking TSC1 (and elevated mTORC1 activity)$^{12}$ or lacking FOXO1, display robust primary responses but poor memory responses$^{31}$. As c-Myc is also clearly consequential for oncogenesis, it is also possible that lymphomas of mature T cells might be influenced by symmetric cell division. A deeper understanding of asymmetric T-cell division as it applies to mTORC1 and c-Myc, may help to explain these observations.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**Supplementary Information** is available in the online version of the paper.

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**Author Contributions** K.C.V. conceived the project, designed and performed most experiments, interpreted results, and co-wrote the manuscript. C.S.G. guided imaging, performed live imaging experiments, and performed experiments with TSC1 flox/flox animals. S.M. provided technical assistance with Seahorse XF24 assays and helped with several in vivo studies. S.L. performed some imaging experiments with amino acid transports, performed experiments with high IL-2, and helped with several in vivo studies. M.M.K. performed and analysed all gene expression analyses. R.W. provided intellectual contributions. D.R.G. conceived the project, supervised experimental designs, interpreted results, and co-wrote the manuscript.

**Author Information** Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to D.R.G. (douglas.green@stjude.org).
Animals. c-Myc–GFP fusion knock-in mice were generated and provided by B. Sleckman.1 OT-1 Tg (C57BL/6-TcraTcrb1000Mjb/J) mice were acquired from The Jackson Laboratory (Bar Harbour, Maine), and TSC1 flox (Tsc1tm1Kdoj) mice were a gift from H. Chi (St. Jude Children’s Research Hospital). Mice heterozygous for c-Myc were generated from c-Myc flox mice, a gift from F. Alt (Boston Children’s Hospital) (see Supplementary Methods). The experiments were not randomized, all animal experiments were conducted with either male or female sex and were performed on littermate controls (6–16 weeks old). No statistical methods were used to predetermine sample size; rather, sample sizes were selected based on animal availability and experience with variation within the immune system. Experimental analyses were not blinded. St. Jude Institutional Animal Care and Use Committee approved all procedures in accordance with the Guide for the Care and Use of Animals.

**T-cell stimulations.** For polyclonal T cells, cells were isolated from the spleens and lymph nodes of c-Myc–GFP mice by mechanical passage through a Falcon 70 µM cell strainer (Thermo Fisher Scientific) and lysis of red blood cells in hypotonic solution. Single-cell suspensions were enriched for T cells using CD45R (B220) microbeads and MACS separation (Miltenyi). Enriched cells were then stimulated on plate-bound anti-CD3 (1 µg ml⁻¹), anti-CD28 (1 µg ml⁻¹) (BioXCell), and recombinant human CD54/ICAM (0.5 µg ml⁻¹) produced in insect cells for 30–36 h. OT-1 Tg T cells were similarly isolated and enriched to ~96% purity and were either stimulated as above or overlain onto BMDCs previously pulsed with 100 nM of SIN1FEKEL peptide (60193-1, Anaspec Inc.) at 37°C for 1 h. Co-cultures were incubated at 37°C for 36–40 h. For Extended Data Fig. 5c, OT-1 Tg T cells stimulated with CD3, CD28, and ICAM for 2 h in #1.5 polymer coverslips (80426, ibidi). All stimulations for microscopy experiments, nocodazole (100 ng ml⁻¹) (Sigma-Aldrich) was added to culture for 4 h and washed off before subsequent imaging. All T cells were cultured in RPMI 1640 (Gibco) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 0.05 mM 2-mercaptoethanol, 100 U ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin at 37°C in 5% CO₂.

**T-cell imaging.** Antibodies against the following antigens were used: CD8 (13-0081-85; eBioscience), β-tubulin (T8328; Sigma-Aldrich), Numbrab (ab14140; Abcam), β2m (ab154067; Abcam), PCK1, (IM-90589-2; ImageX), c-Myc (9402; Cell Signaling), p53 (3364; Cell Signaling), p38MAPK (9234; Cell Signaling), p38 MAPK (9279; Cell Signaling), p53 (2562; Fox01 (9461; Cell Signaling), CD28-PE (12-0981-81; eBioscience), SLCA1A5 (ARPA2247; T100; AVIVA), and SLCA1A3 (ABIN377559; antibodioonline). Additionally, DAPI (D9542; Sigma-Aldrich) or #1640 (Gibco) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 0.05 mM 2-mercaptoethanol, 100 U ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin at 37°C in 5% CO₂.

**Flow cytometry.** Antibodies against eBioscience included anti-CD8-APC-eFluor780 (ab7081; anti-CD44-APC (17-0441-81), anti-CD69-PE/Cy5.5 (45-0691), anti-CD98-PE (12-0981-81), eBioscience), and anti-CD62L-PE/Cy7 (25-0621). Anti-CD4-DB40NC was acquired from BD Biosciences. Cells were stained for 20 min at 4°C in PBS, 5% BSA, 0.1% NaN₃. Cell proliferation was assessed using CellTrace Violet (C34557; Life Technologies) according to the manufacturer’s recommendations. Samples were acquired on a BD LSRII flow cytometer and analysed with Treestar FlowJo software. Cell cycle analysis was performed using BD APC BrdU Flow kit as per the manufacturer’s instructions (552398; BD Biosciences). Cells were sorted on a MoFlow (Beckman-Coulter) or Reflection (I-Cyte).

**Compounds.** Chemical inhibitors used included 1 µM MG132 (S6219; Selleckchem), 1 µM Chir911, 1 µM rapamycin (S1039; Selleckchem), 1 µM Torin2 (S2553; Selleckchem), 1 µg ml⁻¹ DQON (D2141; Sigma-Aldrich), 1 µg ml⁻¹ 3-0-ketoglutарат (K2008; Sigma-Aldrich), 0.1 µM actinomycin D (114666; Calbiochem), 100 µg ml⁻¹ cyclohexamide (C7698; Sigma-Aldrich), 1 µM 2-deoxy-o-glucose (2DG) (D6134; Sigma-Aldrich), and 1 or 5 µM IQ1 (A1910; Apexbio). Cytokines included recombinant murine IL-7 (5 ng ml⁻¹) (217-17; Peprotech), recombinant murine IL-2 (5 ng ml⁻¹) (212-12; Peprotech), and recombinant murine granulocyte macrophage colony-stimulating factor (GMCSF) for BMDC generation (1000 U ml⁻¹) (31503; Peprotech).

**Metabolic assays.** Metabolic flux assays were performed as described in ref. 3. T cells were activated by 1 µg ml⁻¹ plate-bound anti-CD3 and anti-CD28 plus ICAM for 36 h, and first division cells were sorted on c-Myc expression. We then plated 5 × 10⁵ cells per replicate with different labelled compounds overnight. Glycolytic flux was determined by measuring the detritiation of [3-3H]glucose, and fatty acid β-oxidation flux was determined by measuring the detritiation of [9,10-³H]palmitic acid. Glutamine uptake was determined by measuring the detritiation of [³H]glutamine in lysed cells for 3 h.

Respiration was measured in intact T cells using the Seahorse XF24 analyser. Primary mouse T lymphocytes were either maintained in resting state by 5 ng ml⁻¹ IL-7 or activated by 1 µg ml⁻¹ plate-bound anti-CD3 and anti-CD28 plus ICAM. After 36 h, 1 × 10⁵ resting or active T cells were seeded in plates coated with Cell-Tak (354240; Corning). After 1 h, the plate was loaded into the instrument to determine the oxygen consumption rate and extracellular acidification rate. For glycolytic stress tests, T cells sorted on c-Myc GFP were plated in glucose-free complete DMEM medium. During the course of the assay, cultures were injected with 10 mM glucose (67021; Sigma-Aldrich), 1 µM oligomycin (04876; Sigma-Aldrich), and 20 mM 2DG.

Unbiased metabolomic profiling was performed by Metabolon, Inc. Primary mouse T lymphocytes were either maintained in a resting state by 5 ng ml⁻¹ IL-7 or activated by 1 µg ml⁻¹ plate-bound anti-CD3 and anti-CD28 plus ICAM. At the end of culture, activated T cells in the first division were sorted on the basis of c-Myc expression, and 1.6 million T cells were spun down, washed once with cold PBS, and snap-frozen in liquid nitrogen. All the samples were extracted and analysed through ultra-high-pressure liquid chromatography mass-spectrometry (UPLC/MS/MS), as described in Supplementary Methods and Supplementary references 2–4.

**Infections.** For analysis of c-Myc asymmetry in vivo, 5 × 10⁵ naïve CFSE-labelled OT-1 T cells were transferred by intravenous (i.v.) injection into the tail vein of congenic wild-type recipients. Twenty-four hours after transfer, recipients were infected i.v. with 5 × 10⁶ colony-forming units of Listeria monocytogenes expressing SIN1FEKEL peptide. On day 3 after infection, spleens of recipient animals were collected and immediately flash-frozen before cryosectioning. Unfixed sections were blocked in TBS containing 2% BSA and 0.05% Tween-20 before incubation with anti-c-Myc and AF405-labelled phallolidin overnight at 4°C. C-Myc was finally detected with Cy3-labelled secondary antibody, and sections were imaged using laser scanning confocal microscopy.

For analysis of the functional consequences of c-Myc asymmetry in vivo, CellTrace-Violet-labelled, naïve c-Myc–GFP, OT-1 cells were activated on plate-bound anti-CD3/CD28/ICAM or SIN1FEKEL-pulsed BMDCs for 36 h (as described above). CellTrace-Violet dilution was used to identify T cells in the first division, which were then sorted on the basis of c-Myc–GFP fluorescence into high and low populations. 2.5 × 10⁵, 5 × 10⁵, or 7.5 × 10⁵ CD45.1/CD45.2 c-Myc–GFP high or low cells were transferred i.v. into CD45.1 recipient mice. Recipient animals were either treated with rapamycin, recipient mice received daily intraperitoneal (i.p.) injections of rapamycin at 75 µg kg⁻¹ (ref. 30) from days 0–10 after infection.

**Statistics.** Indicated statistical analyses were performed using Graphpad Prism software. Four different tests were employed: chi-square goodness of fit was used for imaging experiments with BMDMs, assuming 25% of data points expected in each graph quadrant (representative of four possible staining patterns for the two different proteins). Similarly, for imaging experiments with antibody-activated T cells, a two-tailed binomial test was employed with an expected even distribution (50%) of data points into two quadrants (representative of two possible staining patterns). Linear regression was used on the imaging data to determine whether there was a correlation between the two proteins analysed.

Unpaired Student’s t-test was used to test whether two means were significantly different from one another when the data are not linked. Paired Student’s t-test was used to analyse the fluorescence intensity in each daughter of a conjoined pair as in Fig. 1j.

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Extended Data Figure 1 | Characterization of c-Myc asymmetry.

**a**, Gating strategy for CD8/high and CD8/low cells. CellTrace-Violet-labelled naive T cells were activated with anti-CD3, anti-CD28, and ICAM for 36 h. CD8 high or low cells were identified as activated, undivided (U), first division (1), or second division (2) on the basis of dilution of CellTrace Violet. Frequencies of cells in each population are presented in table below.

**b**, Time course of CellTrace Violet dilution in naive, T cells activated with anti-CD3, anti-CD28, and ICAM for the indicated time points.

**c**, Schematic representation of staining patterns for interpretation of data represented in Fig. 1c–h. Black lines indicate axes on graphs.

**d**, Frequencies of asymmetric cell division were determined by analysing 92 conjoined daughter cells from cultures activated with anti-CD3, anti-CD28, and ICAM and 123 conjoined daughter cells from cultures activated with SIINFEKL-pulsed BMDCs. T cells were analysed for c-Myc–GFP intensity in both daughter cells. Using 1.5-times brightness in one daughter versus the other as a cut off, each pair was assigned either asymmetric or not, and the frequency of asymmetric pairs is plotted.
Extended Data Figure 2 | Representative confocal images and single stains. For data in Fig. 1. a, Fig. 1b, c; b, Fig. 1d, e; c, Fig. 1f; d, Fig. 1g; e, Fig. 1h.
Extended Data Figure 3 | c-Myc expression becomes asymmetric in late telophase. Overlay, β-tubulin (blue), Hoechst 33258 (grey), c-Myc–GFP (green), and phalloidin (red) for each mitotic phase indicated on the left as identified by chromatin and tubulin staining patterns.
Extended Data Figure 4 | Asymmetric assortment of mRNA for c-Myc target genes in activated T cells. CellTrace-Violet-labelled T cells were stimulated for 36 h with anti-CD3, anti-CD28, and ICAM. a–c, First division (a, c) or undivided (a–c) c-Myc\textsuperscript{high} (H) and c-Myc\textsuperscript{low} (L) T cells were sorted on the basis of c-Myc–GFP expression (b is undivided cells only), and RNA was extracted from each population. Fold change in gene expression for the indicated primers is quantified using the $2^{-\Delta\Delta C_t}$ method relative to $\beta$-2-microglobulin across three (a, c) or four (b) independent experiments (mean ± s.d.).
Extended Data Figure 5 | Cytokine signalling does not influence c-Myc asymmetry and asymmetric assortment of CD98. 

**a**, Mean fluorescent intensity (MFI) of c-Myc–GFP (left panel) or CD98 (right panel) in CD8^{high} (shaded bars) and CD8^{low} (open bars) in the first division after 35 h of activation by anti-CD3, anti-CD8, and ICAM and the indicated treatment for 1 h. All differences were determined significant (\(P < 0.05\)) by unpaired Student’s t-test. 

**b**, Flow cytometric analysis of c-Myc GFP and CD98 in CD8^{high} (green histograms), CD8^{low} (grey histograms), or IL-7 (5 ng ml \(^{-1}\)) rested unactivated (gold histograms) T cells in control conditions or activated in the presence of 10 ng ml \(^{-1}\) IL-2 (0–36 h after activation). Representative flow plots are on the left, and quantification of the mean fluorescence intensities of c-Myc and CD98-APC are on the right. Experiment representative of three independent experiments. 

**c**, Representative confocal image and single stain images for Fig. 2d, e. OT-I T cells stimulated on SIINFEKL-pulsed BMDCs. 

**d**, Quantification of CD98 and c-Myc asymmetry in T cells stimulated with anti-CD3, anti-CD28, and ICAM for 36 h. 100% concordance of markers (\(P = 0.0039\) Two-Tailed Binomial Test); \(R^2 = 0.2304\), \(P = 0.2304\) linear regression. 

**e**, Polarization of SLC3A2 in two representative confocal images in antibody-stimulated OT-I Tg T cells (see methods). 

**f**, Asymmetric index (difference in RFP Intensity in proximal (P) and distal (D) sides of cell) for SLC3A2 staining in activated, undivided CD8 T cells (each point represents an activated T cell). Error bars, mean ± s.d.
Extended Data Figure 6  |  Amino acid transporter SLC1A5, but not SLC1A3, asymmetrically assorts in activated T cells. a, b. Representative confocal image, quantification, and single-stain images for SLC1A5; 88.9% both bright in proximal daughter ($\chi^2 = 19.89$, DF = 3, $P = 0.0002$, chi-square goodness of fit test); $R^2 = 0.2961$, $P = 0.1299$, linear regression (a); and SLC1A3 100% c-Myc bright in proximal daughter, 62.5% SLC1A3 bright in distal daughter ($\chi^2 = 9$, DF = 3, $P = 0.0293$ chi-square goodness of fit test); $R^2 = 0.07944$, $P = 0.4989$ linear regression (b) for OT-I CD8 T cells co-cultured with SIINFEKL-pulsed BMDCs for 36h.
Extended Data Figure 7 | Regulation of p-mTOR and mTORC1 signalling by c-Myc. a, b, Quantification of p-mTOR staining and c-Myc–GFP for OT-I CD8 T cells co-cultured with SIINFEKL-pulsed BMDCs 80.9% both bright in proximal daughter ($x^2 = 25.67, \text{DF} = 3, P < 0.0001$ chi-square goodness of fit test); $R^2 = 0.2307, P = 0.0275$, linear regression (a); or T cells stimulated for 36 h with anti-CD3, anti-CD28, and ICAM 82.4% concordance of markers ($P = 0.0013$, two-tailed binomial test); $R^2 = 0.1204, P = 0.1725$, linear regression (b). Asymmetry as assessed by fluorescence intensity is expressed as (proximal − distal)/total (a), or values from (c-Myc$^{\text{high}}$ − c-Myc$^{\text{low}}$)/total (b). c, Western blot analysis of CD8 T cells activated with anti-CD3, anti-CD28, and ICAM for 6 h without treatment (ctrl) or with 1 μM or 5 μM of the bromodomain inhibitor JQ1. Data are representative of three independent experiments.
Extended Data Figure 8 | Asymmetric assortment of mTORC1 activity with c-Myc–GFP. a, b, Representative confocal images for quantifications of c-Myc–GFP and pS6 staining corresponding to Fig. 3a, b (a) or c-Myc–GFP and p70S6K corresponding to Fig. 3c (b) in OT-I T cells co-cultured with SIINFEKL-pulsed BMDCs. c, d, Quantifications for c-Myc–GFP and pS6 100% both bright in proximal daughter ($x^2 = 19.89$, DF = 3, $P = 0.0002$, chi-square goodness of fit test); $R^2 = 0.9457$, $P = 0.0055$, linear regression (c); or p70S6K 100% both bright in proximal daughter ($x^2 = 14.14$, DF = 3, $P = 0.0027$, chi-square goodness of fit test); $R^2 = 0.4875$, $P = 0.0026$, linear regression (d) staining in T cells stimulated on anti-CD3, anti-CD28, and ICAM for 36 h. e, Representative confocal images for quantifications of c-Myc–GFP and pFOXO1 staining corresponding to Fig. 3d, e. T cells stimulated with anti-CD3, anti-CD28, and ICAM for 36 h.
Extended Data Figure 9 | First-division CD8<sup>high</sup> c-Myc–GFP<sup>high</sup> CD8 T cells are more glycolytic and exhibit more glutaminolysis and pentose phosphate pathway activity than first division CD8<sup>low</sup> c-Myc–GFP<sup>low</sup> CD8 T cells but have decreased FAO. Undivided or first-division antibody-activated T cells were sorted on c-Myc expression. 1.6 million T cells from each group were analysed via UHPLC/MS/MS for unbiased metabolomic profiling performed by Metabolon, Inc. a–d, Metabolic pathway schematic (a) was generated by Metabolon Inc., as were graphs (b) for select metabolites in pathway, representing data for DNA-normalized data. Metabolic pathway schematics and graphs were also generated by Metabolon Inc. for select metabolites in the pathway, representing data for DNA-normalized data for nucleotide biosynthesis (c) and fatty acid oxidation (d).
Extended Data Figure 10 | c-Myc\textsuperscript{high} CD8 T cells are more glycolytic than CD8\textsuperscript{low} CD8 T cells without asymmetric distribution of mitochondria. 
a. The extracellular acidification rate (ECAR) for sorted c-Myc\textsuperscript{high} (circles) and c-Myc\textsuperscript{low} (squares) T cells from the first division after 36 h of activation with anti-CD3, anti-CD28, and ICAM as measured by a Seahorse Bioflux analyser during exposure to the indicated compounds are represented. Values are paired across three independent experiments.

b. The oxygen consumption rate (top panels) and ECAR (bottom panels) for basal respiration in complete RPMI with glucose of sorted c-Myc\textsuperscript{high} (shaded bars) and c-Myc\textsuperscript{low} (open bars) CD8 T cells from the first-division after 36 h of activation by anti-CD3, anti-CD28, and ICAM, as measured by a Seahorse Bioflux analyser across three independent experiments.

c. Quantification of mitochondrial DNA in sorted first-division c-Myc low and high OT-I CD8 T cells activated on anti-CD3, anti-CD28 and ICAM for 36 h. Mean ± s.d. are plotted for three technical replicates of $n = 2$ mice per group.

d. Overlay and individual channel images of β-tubulin (blue), DAPI (grey), c-Myc–GFP (green), and TOM20 (red) of an OT-I CD8 T cell activated with anti-CD3, anti-CD28 and ICAM for 36 h.