GAB functions as a bioenergetic and signalling gatekeeper to control T cell inflammation
Supplementary information

Materials and Methods

Western Blot Analysis
For protein extraction, cells were lysed and sonicated at 4°C in a lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.5% SDS, 5 mM sodium pyrophosphate, protease, and phosphatase inhibitor tablet), then centrifuged at 13,000 × g for 15 min for recovering. The samples were boiled in the mixture of LDS Sample Buffer (NuPAGE) and Reducing solution (Thermo Fisher Scientific) for 5 min, after transferring to PVDF membranes by using the iBlot Gel Transfer Device (Thermo Fisher Scientific), then incubated with primary anti-ABAT (clone B-12, Santa Cruz Biotechnology) followed by incubating with the secondary antibodies conjugated with horseradish peroxidase. Immunoblots were developed on films using the enhanced chemiluminescence technique.

RNA Extraction, qPCR, and RNAseq
Total RNA was isolated by using the RNeasy Mini Kit (Qiagen). The cDNA synthesis was processed using Random hexamers and M-MLV Reverse Transcriptase (Invitrogen). BIO-RAD CFX284™ Real-Time PCR Detection System was used for SYBR green-based quantitative PCR. The relative gene expression was determined by the comparative CT method, also referred to as the 2^{−ΔΔCT} method. The data were presented as the fold change in gene expression normalized to an internal reference gene (beta2-microglobulin) and relative to the control (the first sample in the group). Fold change = 2^{−ΔΔCT} = [(CT_{gene of interest} − CT_{internal reference})]_{sample A} − [(CT_{gene of interest} − CT_{internal reference})]_{sample B}. Samples for each experimental condition were run in triplicated PCR reactions. Primer sequences were obtained from Primer Bank to detect target genes (Supplementary Table 5).

For RNA sequencing analysis, total RNA was extracted using RNeasy Mini Kit (Qiagen) and treated with DNase I according to the manufacturer's instructions. After assessing the quality of total RNA using an Agilent 2100 Bioanalyzer and RNA Nanochip (Agilent Technologies), 150 ng total RNA was treated to deplete the levels of ribosomal RNA (rRNA) using target-specific oligos combined with rRNA removal beads. Following rRNA removal, mRNA was fragmented and converted into double-stranded cDNA. Adaptor-ligated cDNA was amplified by limit cycle PCR.
After library quality was determined via Agilent 4200 TapeStation and quantified by KAPA qPCR, approximately 60 million paired-end 150 bp sequence reads were generated on the Illumina HiSeq 4000 platform. Quality control and adapter trimming were accomplished using the FastQC (version 0.11.3) and Trim Galore (version 0.4.0) software packages. Trimmed reads were mapped to the Genome Reference Consortium GRCh38 (mm10) murine genome assembly using TopHat2 (version 2.1.0), and feature counts were generated using HTSeq (version 0.6.1). Statistical analysis for differential expression was performed using the DESeq2 package (version 1.16.1) in R, with the default Benjamini-Hochberg p-value adjustment method. The Ingenuity Pathway Analysis (IPA) software (QIAGEN), the Gene Set Enrichment Analysis (GSEA) software (UC San Diego, BROAD Ins.), and the R Programming Language software were used to analyze gene signature and pathway enrichment.

NMR analysis of medium
Naïve CD4+ T cells isolated from WT mice were polarized for 72 hrs under T_{H17} or iT_{reg} culture conditions. Cells were harvested, washed by PBS, and then re-seeded at a 2 \times 10^6 cells /mL density in a conditional medium (RPMI-1640) containing 4 mM $^{13}$C5-Glutamine. Medium samples were taken at 0 hr and after 6 hrs incubation and were extracted and lyophilized. The dried samples were reconstituted in deuterated 50 mM phosphate buffer pH 8 containing 8.81 nmole DSS-d$_6$. NMR spectra were recorded on a Bruker Avance III spectrometer at 16.45 T at 15 °C in a 1.7 mm HCN inverse triple resonance cryoprobe. Presat spectra were recorded with an acquisition time of 2 s with weak irradiation at the HOD frequency during the relaxation delay of 4 s (pulse program: ZGPR). $^1$H{$^{13}$C} HSQC spectra were recorded with an acquisition time of 0.25 s and a relaxation delay of 1.75 using adiabatic decoupling of $^{13}$C (pulse program: hsqcetgpprsisp2.2). The raw data were apodized with a 1 Hz exponential line broadening and linear predicted once (Presat) or a 4-Hz line broadening exponential (HSQC), phased, and baseline corrected using third-order Bernstein polynomials and referenced to the internal DSS at 0 ppm. Peaks were deconvoluted by line fitting using MNOVA and normalized to the DSS resonance at 0 ppm. The HCCH-TOCSY 2D spectrum was acquired by recording the first plane of the 3D experiment in the carbon dimension (pulse program: hcchdigp3d). The acquisition times in the direct and indirect dimensions ($f_2$ and $f_1$) are 0.25 s and 0.03 s, respectively. The C-C mixing time was set to 10.9 ms using a DIPSI-3 spin lock scheme with $B_1$ field strength of 40 kHz. The data were processed with
1 Hz exponential apodization in $f_2$ and 5 Hz exponential with cosine squared function in the $f_1$ dimension, and further linear predicted and zero-filled to 8 k and 2k data points, respectively. The spectrum was phased and baseplane corrected before referencing glutamine peaks based on the standard spectrum. Spectral assignments were made by reference to authentic standards of GAB recorded under the same conditions.
## Table 1. Cell culture related antibodies, cytokines, and chemicals

| Description                          | Manufacturer      | Catalog Number |
|--------------------------------------|-------------------|----------------|
| InVivoMAb anti-mouse CD3, Bio X cell, Clone 145-2C11 | Bio X cell        | Cat# BE0001-1 |
| InVivoMAb anti-mouse CD28, Bio X cell, Clone 37.51 | Bio X cell        | Cat# BE0015-1 |
| InVivoMAb anti-mouse IL-2, Bio X cell, Clone JES-1A12 | Bio X cell        | Cat# BE0043   |
| InVivoMAb anti-mouse IL-4, Bio X cell, Clone 11B11 | Bio X cell        | Cat# BE0045   |
| InVivoMAb anti-mouse IFNγ, Bio X cell, Clone XMG1.2 | Bio X cell        | Cat# BE0055   |
| Recombinant Murine IL-12 p70, PeproTech | Cat# 210-12      |
| Recombinant Human TGF-β1, PeproTech   | Cat# 100-21C      |
| Recombinant Murine IL-2, PeproTech    | Cat# 212-12       |
| Recombinant Murine IL-7, PeproTech    | Cat# 217-17       |
| Recombinant Murine IL-4, PeproTech    | Cat# 214-14       |
| Recombinant Murine IL-6, PeproTech    | Cat# 216-16       |
| γ-Aminobutyric acid, Sigma            | Cat# A2129        |
| FCCP, Sigma                           | Cat# C2520        |
| Aminoguanidine; AG, Sigma-Aldrich     | Cat# A7009        |
| GDH1 Inhibitor, R162 – Calbiochem     | Cat# 5.38098      |
| Bleuculline, Cayman                   | Cat# 11727        |
| Picrotoxin, Cayman                    | Cat# 20771        |
| Flumazenil, Cayman                    | Cat# 14252        |
| (R,S)-4-Amino-5-hexenoic acid; Vigabatrin | Cat# X-1501      |
| Polyketides NV118, Isomerase therapeutics Ltd. Cambridge | Cat# 01-118-s3 |
Table 2. Cell staining antibodies and dyes

| Antibody/Fluorophore | Company | Catalog # | Clone |
|-----------------------|---------|-----------|-------|
| FITC anti-mouse CD4. | BioLegend | 100510 | RM4-5 |
| PE/Cyanine7 anti-mouse CD4 | BioLegend | 100422 | GK1.5 |
| Pacific Blue™ anti-mouse CD4 | BioLegend | 100428 | GK1.5 |
| APC/Cyanine7 anti-mouse CD8a | BioLegend | 100714 | 53-6.7 |
| PE/Cy7 anti-mouse CD69 | BioLegend | 104512 | H1.2F3 |
| PE anti-mouse CD25 | BioLegend | 102008 | PC61 |
| APC/Cy7 anti-mouse CD90.1 (Thy1.1) | BioLegend | 202520 | OX-7 |
| APC anti-mouse CD90.1 (Thy1.1) | BioLegend | 202526 | OX-7 |
| PE anti-mouse CD90.2 (Thy1.2) | BioLegend | 105308 | 30-H12 |
| APC anti-mouse TCR β chain | BioLegend | 109211 | GL3 |
| PE/Cyanine7 anti-mouse IFN-γ | BioLegend | 505826 | XMG1.2 |
| APC anti-mouse IFN-γ | BioLegend | 505810 | XMG1.2 |
| PE/Cyanine7 anti-mouse IL-17A | BioLegend | 506922 | TC11-18H10.1 |
| APC anti-mouse IL-17A | BioLegend | 506916 | TC11-18H10.1 |
| APC anti-mouse IL-4 | BioLegend | 504105 | 11B11 |
| Alexa Fluor® 647 anti-mouse FOXP3 | BioLegend | 126407 | MF-14 |
| APC anti-mouse CD62L | BioLegend | 104412 | MEL-14 |
| FITC anti-mouse/human CD44 | BioLegend | 103006 | IM7 |
| PerCP anti-mouse CD45.1 | BioLegend | 110726 | A20 |
| PerCP anti-mouse CD45.2 | BioLegend | 109826 | Clone104 |
| FITC anti-mouse/human/rat ABAT | Santa Cruz Biotechnology | sc-393769 | B-12 |
| APC anti-Hu/Mo ROR gamma (i) | eBioscience | 17-6988-82 | AFKJS-9 |
| PE anti-Hu/Mo Phospho-STAT3 (Tyr705) | eBioscience | 12-9033-42 | LUVNKLA |
| PE anti-Hu/Mo Phospho-STAT5 (Tyr694) | eBioscience | 12-9010-42 | SRBCZX |
| Pacific Blue-P-S6Ribosomal Protein (S235/236) | Cell Signaling | 8520S | D57.2.2E |
| APC anti-BrdU | BioLegend | 364114 |
| 7-AAD Viability Staining Solution | BioLegend | 420404 |
| Pyronin Y | Sigma-Aldrich | 92-32-0 |
Table 3. pMIC-ABAT sequencing

| Host | Constructs: pMIC(MSCV-IRES-mCherry) |
|------|-------------------------------------|
| MCS  | EcoRI/Bgl II/SnaBI/BamHI/MfeI/XhoI   |
| Inserts | No tag; include start codon (ATG) and stop codon |

**Sequence ID:** ref|XM_011522401.1|**Length:** 5115|**Number of Matches:** 1

**Gene-associated gene details**

**Range 1:** 466 to 1333

**GenBank Graphics Next Match Previous Match**

| **Alignment statistics for match #1** |
|--------------------------------------|
| Query | 91 |
| TCA|GCT|CCT|CAG|CTT|GTC|GCC|CCG|GCT|GCT|GGC|AGT|TCC|AGG|CAT|AGG|CTG|AAT|
| 150 |
| Subject | 466 |

| Query | 151 |
| TGG|TCG|GGT|GTC|CTT|GGA|ATC|CA|G|AC|T|GCC|AGG|CCG|ATG|T|CT|G|AG|G|
| 210 |
| Subject | 526 |

| Query | 211 |
| ATTA|GAT|GG|G|CC|T|G|T|A|G|A|G|A|G|
| 270 |
| Subject | 586 |

| Query | 271 |
| AAC|G|C|T|G|A|T|A|T|A|G|T|A|G|G|G|
| 330 |
| Subject | 645 |

| Query | 331 |
| GCC|G|G|G|G|G|G|G|C|T|T|C|T|C|T|
| 390 |
| Subject | 706 |

| Query | 391 |
| TCT|C|C|T|G|C|T|C|G|T|G|G|C|T|G|T|G|G|
| 450 |
| Subject | 766 |

| Query | 511 |
| AAA|A|T|G|G|G|G|G|C|A|T|T|T|G|C|A|G|
| 510 |
| Subject | 826 |

| Query | 551 |
| TGG|G|A|A|G|A|C|C|G|A|T|C|T|G|C|T|G|
| 570 |
| Subject | 886 |

| Query | 591 |
| CCA|T|G|C|C|G|G|C|T|C|T|C|A|T|G|
| 630 |
| Subject | 946 |

| Query | 631 |
| GAG|C|A|G|A|A|G|G|G|G|C|G|C|T|C|T|
| 690 |
| Subject | 1006 |

| Query | 691 |
| ACC|G|G|C|C|T|C|G|C|G|C|G|A|A|G|
| 750 |
| Subject | 1066 |

| Query | 731 |
| GGA|C|A|T|G|T|G|G|G|G|G|G|C|G|A|
| 810 |
| Subject | 1126 |

| Query | 791 |
| CCA|T|G|C|T|G|G|G|C|
| 870 |
| Subject | 1186 |

| Query | 811 |
| CCA|T|A|G|C|T|G|A|T|G|G|G|G|
| 930 |
| Subject | 1306 |

| Query | 871 |
| GAG|C|A|G|A|A|G|G|G|G|C|G|A|
| 990 |
| Subject | 1366 |

| Query | 931 |
| ATG|G|A|A|A|G|G|G|G|
| 1050 |
| Subject | 1404 |
Table 4. Stable isotope tracers

| Product                                      | Supplier                                      | Catalog Number |
|----------------------------------------------|-----------------------------------------------|----------------|
| U13C5-Arginine                              | Cambridge Isotope Laboratories, Inc.          | CLM2265        |
| U13C6-Glucose                               | Cambridge Isotope Laboratories, Inc.          | CLM1396        |
| U13C5-Glutamine                             | Cambridge Isotope Laboratories, Inc.          | CLM1822        |
| 4-Aminobutyric acid (GABA) (13C4, 97-99%)   | Cambridge Isotope Laboratories, Inc.          | CLM-8666       |
| 1,4-BUTANEDIAMINE (PUTRECINE) (13C4, 98%)   | Cambridge Isotope Laboratories, Inc.          | CLM-6574       |
| Gene       | primer sequences forward | primer sequences reverse | NCBI Gene ID | Primer Bank ID | Vendor (Sequences of oligonucleotides) |
|------------|--------------------------|--------------------------|--------------|----------------|----------------------------------------|
| GAD1       | AACGTATGATACTTGGTGTGGC   | CCAGGCTATTGGTCTTGTGAAG   | Mouse, 14415 | 145301579c1    | Sigma-Aldrich                          |
| GAD2       | TCCGGCTTTTGCTCTTCG       | ATGCCGCCGCTGAACCTTTT     | Mouse, 14417 | 124517708c1    | Sigma-Aldrich                          |
| ABAT       | CTGAAACAAATCCAGAATGCCGA | GGTGTGAACCTATGGGACAG     | Mouse, 268860 | 27370474a1     | Sigma-Aldrich                          |
| Aldh5a1    | CGTGCAGAAGAGAGGCTTAC     | GAACTGCCCCTGCTATATTTT    | Mouse, 214579 | 27369748a1     | Sigma-Aldrich                          |
| Akr7a5     | CGGCCAGTCCGAAACATC       | TCTAGTGTACCTTCCTCCAG     | Mouse, 110198 | 27659728a1     | Sigma-Aldrich                          |
| Glvr1      | GAAACTGGCCGGTGATCTCCT   | GGTAAGGTTTATGTTGCAAG     | Mouse, 74022 | 21312000a1     | Sigma-Aldrich                          |
| Slec5a2a   | GGCTCTCCATCTGTAGCAGC    | GCACAGGACCATAGAGATA      | Mouse, 52710 | 21313230a1     | Sigma-Aldrich                          |
| Slec6a1    | GAAAGCTGCTTGATCTGAGGTTG | AGCAAAAGATGATGAGTGTCGCC | Mouse, 232333 | 30520131a1     | Sigma-Aldrich                          |
| Slec6a1II  | TTGGTGACCCTGCTGAGGAGA   | AGCAGATGAAAGAACCCGGTTA  | Mouse, 243616 | 27370360a1     | Sigma-Aldrich                          |
| Slec6a12   | GGTCTCTGAGGAAGAGAGAT    | GGGATGAGAAAGAGTCCACC    | Mouse, 14411 | 19526806a1     | Sigma-Aldrich                          |
| Slec6a13   | CAGTACACCAAGACGAGGAG    | GCCAGCAACAGATGAGTAGA    | Mouse, 14412 | 21362295a1     | Sigma-Aldrich                          |
| Slec3a1    | ACCCTCCGTTCGCAAACAGTC   | CAAAGTCCAGATCGTGCCAGT   | Mouse, 22348 | 6678569a1      | Sigma-Aldrich                          |
| Gabra1     | AAAAAGTCGGGCTCTCTCTGAC  | CAGTCCGTCGCAAATCTCTGGA  | Mouse, 14394 | 6753936a1      | Sigma-Aldrich                          |
| Gabra2     | GCACCGTCCAAGTGGTTGTTG  | TCTGGTCTAACAGGTACATTGAT | Mouse, 14395 | 6679901a1      | Sigma-Aldrich                          |
| Gabra3     | ATGTTGGACATTCTGTAGCCCA  | CCCCAGGCTCTGTTGGCTTGG  | Mouse, 14396 | 31560695a1     | Sigma-Aldrich                          |
| Gabra4     | ACAATGAGACTCATCCATAGTCG | GCCCTTCCGTGCTGGTGAAG    | Mouse, 14397 | 33468895a1     | Sigma-Aldrich                          |
| Gabra5     | TGACCCCAACCCTCTCTTCTG   | GTGATGTTGCTATGGCTTCT  | Mouse, 110886 | 30578386a1     | Sigma-Aldrich                          |
| Gabra6     | TGCCCAAGCTCAACTTGAGA    | GCCGTAGACGGTGTACATGC    | Mouse, 14399 | 6679905a1      | Sigma-Aldrich                          |
| Gabarap    | AAGAGGAGGACCTGGTCTTGCAGA| GCTTTGGGCTCTGCTGGTTGG | Mouse, 56486 | 9789961a1      | Sigma-Aldrich                          |
| Gabarapl1  | GGACCACCCCTCGAGATCTC   | CCTCTTATCCGAGATCGGCCGAC | Mouse, 57436 | 10181206a1     | Sigma-Aldrich                          |
| Gabarapl2  | TCCGGCTCTCGATTTGTGAC    | ATGGCTCCTCCAGGAGGAGGA  | Mouse, 93739 | 31542873a1     | Sigma-Aldrich                          |
| jaknip1    | ACCGCTACATCATCGGAACCT   | GCAGCTACATCTCGGATCCTTT  | Mouse, 76071 | 30409980a1     | Sigma-Aldrich                          |
| Gabrb1     | TCCGCTGATGGTGCTATGAG    | CCGAAGCGAATGTCATATCC    | Mouse, 14400 | 6679907a1      | Sigma-Aldrich                          |
| Gabrb2     | ATGTCGGCTGTGTAAGAGGACG  | CTGGACCATCGTTGTGCAAAA   | Mouse, 14401 | 6679909a1      | Sigma-Aldrich                          |
| Gabrb3     | CTGGTCGCAAACTCGGCTTCTC | CGTGACCCCTAAGCTGGCTTGTC | Mouse, 14402 | 26350247a1     | Sigma-Aldrich                          |
| Gabrd      | ATGGGGGACTACGTGGGCTT   | CCACATTCCAGAGAGACC    | Mouse, 14403 | 6679913a1      | Sigma-Aldrich                          |
| Gabre      | CCTTCAGGGAGGTGGTGGAC    | ATCCAGGGGAGGAGTCAGGT    | Mouse, 14404 | 8393396a1      | Sigma-Aldrich                          |
| Gabrg1     | TGGTGAGTAAGAGGCTATGG    | TCCGGAGATCGAGGTTAGTA    | Mouse, 14405 | 26332763a1     | Sigma-Aldrich                          |
| Gabrg2     | ATGGTGTCGCCAAATACAGGAGG | GGGAGCAATGTTGAGCTCGT   | Mouse, 14406 | 28916679a1     | Sigma-Aldrich                          |
| Gabrg3     | GAGCTGGCCCTGCCATTCAAC   | AGGCTCCTGCTGGTTTTAGAATTT | Mouse, 14407 | 6679917a1     | Sigma-Aldrich                          |
| Gabrp      | CAGACCCAGGGCTAGTGTTTC  | AGAGGCGGATGAGCCTGTGTT  | Mouse, 216643 | 20379971a1     | Sigma-Aldrich                          |
| Gabrq      | ATGGGCACTCCAGGATATGCT   | ATCCAGAATGCTTCCAGGGCT  | Mouse, 57249 | 10048422a1     | Sigma-Aldrich                          |
| Gabrr1     | CGAGGAGCACACAGCAGAGT   | GCTGAGTCCATCGCACCTTCTG  | Mouse, 14408 | 6679919a1      | Sigma-Aldrich                          |
| Gabrr2     | ATGCCCTATTTGTAGAGACTCGC | CCACACCTACAGGATGAGCC  | Mouse, 14409 | 6679921a1      | Sigma-Aldrich                          |
| Gene   | Forward primer sequence | Reverse primer sequence | Species | Catalog number | Supplier     |
|--------|-------------------------|-------------------------|---------|----------------|--------------|
| Gabrr3 | CACCCTAAACGTGAAACAACCTGT | TCCAATAGTGCTGGAGGTAAAAC | Mouse, 328699 | 124487130c2 | Sigma-Aldrich |
| Gabbr1 | GCACAGGACACAATGAAAACAG | AGCAAATGTAATCGACTTCCA | Mouse, 54393 | 5051395a1 | Sigma-Aldrich |
| Gabbr2 | AAGACCCCATAGAGGACATCAA | GGGTGGTACGTGTCCTGG | Mouse, 242425 | 29611612a1 | Sigma-Aldrich |
| DBI    | GAATTTGACAAAGCCGCTGAG | CCCACAGTAGCTGTTCGAA | Mouse, 13167 | 6681137a1 | Sigma-Aldrich |
| Arg1   | CTCCAAAGCCAAAGCTCTTAGAG | AGGAGCTGTCATTAGGGACATC | Mouse, 11847 | 15896684a1 | Sigma-Aldrich |
| Arg2   | TCCTCCACGGGCAAATTCC | GCTGGACCATATCCACTCTTA | Mouse, 11847 | 6753110a1 | Sigma-Aldrich |
| ODC1   | GGTCTCAGAGGCCAAACAA | CAGCGTGCCATCATCCT | Mouse, 111148 | N/A | Sigma-Aldrich |
| Aldh1a1| ATACTTGTCCAGATTAGGAGGCT | GGGCTATCTTCATAATGAACA | Mouse, 11668 | 7304881a1 | Sigma-Aldrich |
| DAO    | GTGGCAAGAGGAGTGGATG | TGGAAGAGATGATACGGGAGAGTTG | Mouse, 13142 | 15929683a1 | Sigma-Aldrich |
| Aldh4a1(PDH) | CGATGGAAAGCAACACTCTTCTT | GGCGAACAGCTCGACTGTATATC | Mouse, 212647 | 34328415a1 | Sigma-Aldrich |
| Tubulin(beta-2 microglobulin) | TTCTGGTGCTTGTCCTCACTGA | CAGTAGTGCCTGGGCTTCCATT | Mouse, 12010 | 144227219c1 | Sigma-Aldrich |
**Supplemental Figure 1.** For analysis of CD4+ T cell proliferation, infiltrating and intracellular cytokines (Fig 4c, Fig 4g, Fig 4i, Fig 4k, Fig 5f, Fig 5h, Extended Data Fig 2b, Fig 4a-d, Fig 5, Fig 6b, Fig 8b, Fig 9c, and Fig 10): FSC-SSC-H gating was used as preliminary gating for lymphocyte population followed by analysis of CD4+ T cells, then checked the intracellular cytokines expression.

**Supplemental Figure 2.** For *in vivo* adaptive transfer experiment, gating strategy for flow cytometry analysis was preliminarily performed by gating for CD45.2 stain marker (Extended Data Fig 6a, OVA antigen-specific).