A purine metabolic checkpoint that prevents autoimmunity and autoinflammation

Graphical abstract

Highlights

- A FAMIN-enabled purine metabolon in dendritic cells restrains T cell priming
- FAMIN prevents cytoplasmic NADH/NAD⁺ reductive stress that enhances priming
- Inosine generated from hypoxanthine by FAMIN inhibits T cell priming
- FAMIN ameliorates immunopathology in influenza but dampens tumor surveillance

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In brief

Saveljeva et al. identify a biochemical mechanism in dendritic cells that restrains T cell priming and prevents immunopathology but dampens tumor surveillance. FAMIN enables a purine nucleotide cycle, which prevents cytoplasmic NADH/NAD⁺ reductive stress that augments antigen presentation, and it generates inosine, which inhibits T cell activation.

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A purine metabolic checkpoint that prevents autoimmunity and autoinflammation

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SUMMARY

Still’s disease, the paradigm of autoinflammation-cum-autoimmunity, predisposes for a cytokine storm with excessive T lymphocyte activation upon viral infection. Loss of function of the purine nucleoside enzyme FAMIN is the sole known cause for monogenic Still’s disease. Here we discovered that a FAMIN-enabled purine metabolon in dendritic cells (DCs) restrains CD4+ and CD8+ T cell priming. DCs with absent FAMIN activity prime for enhanced antigen-specific cytotoxicity, IFNγ secretion, and T cell expansion, resulting in excessive influenza A virus-specific responses. Enhanced priming is already manifest with hypomorphic FAMIN-I254V, for which ~6% of mankind is homozygous. FAMIN controls membrane trafficking and restrains antigen presentation in an NADH/NAD+-dependent manner by balancing flux through adenine-guanine nucleotide interconversion cycles. FAMIN additionally converts hypoxanthine into inosine, which DCs release to dampen T cell activation. Compromised FAMIN consequently enhances immunosurveillance of syngeneic tumors. FAMIN is a biochemical checkpoint that protects against excessive antiviral T cell responses, autoimmunity, and autoinflammation.

INTRODUCTION

Deorphaning an autoimmunity risk gene product unearthed an unprecedented function at the heart of cellular metabolism, conserved from bacteria to man. This risk gene encodes FAMIN (also known as LACC1, C13orf31), an enzyme that unifies in a single protein the activities of adenosine deaminase (ADA; adenosine + H2O → inosine + NH3), purine nucleoside phosphorylase (PNP; inosine + phosphate [Pi] # hypoxanthine + ribose-1-phosphate [R1P]; guanosine + Ppi # guanine + R1P), and methylthioadenosine phosphorylase (MTAP; methylthioadenosine [MTA] + Ppi # adenine + methyl-thioribose-1-phosphate [MTR1P]). FAMIN’s fourth catalytic activity is that of adenosine phosphorylase (adenosine + Pi # adenine + R1P), previously considered absent from eukaryotic metabolism (Cader et al., 2020). Adenine and ribose are primordial metabolites from which life is thought to have emerged from prebiotic biochemistry (Ralser, 2018). They are defining constituents of the genetic code, the energy currency, and the major cofactors of a cell. Since purine nucleotide de novo synthesis yields straight to nucleotides (i.e., purine monophosphates; IMP, AMP, and GMP), ADA, PNP, and MTAP had been thought to be the sole enzymes to supply purine nucleobases (adenine, guanine, and hypoxanthine) from nucleosides (adenosine, guanosine, inosine, and MTA) (Bzowska et al., 2000). ADA and PNP deficiency causes severe combined immunodeficiency (SCID) with loss of T and B lymphocytes (Giblett et al., 1972, 1975). Loss of function of FAMIN, in sharp contrast, is linked to autoimmunity and autoinflammation, specifically to Still’s disease (also known as systemic juvenile idiopathic arthritis, sJIA), juvenile idiopathic arthritis (JIA), and early-onset Crohn’s disease (Al-Mayouf et al., 2020; Patel et al., 2014; Rabionet et al., 2019; Wakil et al., 2015; Yasin and Schulert, 2018). These very rare loss-of-function mutations aside, partial loss of activity, caused
by a SNP that leads to a valine-for-isoleucine substitution at amino acid 254 (I254V), for which ~6% of humans are homozygous, increases risk of Crohn’s disease and leprosy (Barrett et al., 2008; Zhang et al., 2009).

FAMIN loss of function is the sole known cause of autosomal-recessive (i.e., monogenic) forms of Still’s disease. Still’s disease affects young children; starts with daily recurring fever, rash, and lymph node enlargement; and morphs over weeks into a debilitating arthritis (Yasin and Schulert, 2018). The initial phase resembles periodic fever syndromes with inflammasome activation and IL-1β and IL-18 release; the later arthritic phase is thought to be driven by pathogenic T lymphocytes. About 20% of children with Still’s disease develop “secondary hemophagocytic lymphohistiocytosis” (HLH; also known as “macrophage activation syndrome,” MAS) (Brisse et al., 2016b; Grom et al., 2016). HLH/MAS is typically triggered by viral infections and can occur even when Still’s disease is in remission while on IL-1/IL-6-blocking therapeutics (Grom et al., 2016). HLH features a cytokine storm accompanied by excessive expansion and activation of CD4+ and CD8+ T lymphocytes and hemophagocytic, IFNγ-activated macrophages. It manifests with disseminated intravascular coagulation (DIC), acute respiratory distress syndrome, and multi-organ failure, and is often fatal (Brisse et al., 2016a, 2016b). HLH/MAS is not restricted to Still’s disease and children. For example, virus-induced HLH/MAS is caused by Epstein-Barr virus and many other pathogens and has been implicated in fatality from seasonal (H3N2), avian (H5N1), and swine (H1N1/2009) influenza A virus (IAV) infections (Beutel et al., 2011; Henter et al., 2010).

Mice with germline deletion of Famin, or genome-edited to express one of the Still’s disease-linked loss-of-function mutations (C284R; “FaminP284R” mice), develop normally under specific pathogen-free conditions. Similarly, mice genome-edited to express fully active (254I; “FaminP254I” or partially active FAMIN (254V; “FaminP254V”) are indistinguishable (Cader et al., 2016). FaminP254I and FaminP284R mice, however, do develop more severe lipopolysaccharide (LPS)-induced sepsis, evidence of DIC, and increased plasma IL-1β levels, compared to mice expressing fully active FAMIN (Cader et al., 2016). Compromised FAMIN activity also leads to lower reactive oxygen species (ROS) production, decreased bacterial killing, altered NLRP3 inflammasome activation, and cytokine secretion in macrophages (Cader et al., 2016; Lahiri et al., 2017), and FaminP254I mice develop more severe experimental arthritis and colitis (Kang et al., 2020; Skon-Hegg et al., 2019). How loss of FAMIN activity, which is abundantly expressed in macrophages and dendritic cells (DCs) while largely absent from T cells (Heng et al., 2008), predisposes to autoimmunity remains unknown. Particularly elusive is via what mechanism altered core purine metabolism due to the absence of multifunctional FAMIN, which is tethered to the cytoplasmic surface of peroxisomes (Cader et al., 2016), could affect immune function, since monofunctional ADA, PNP, and MTAP are ubiquitously present.

Here we report a purine metabolon in DCs that potently restrains T cell priming by dampening membrane trafficking and hence the pace of antigen uptake and presentation, and by releasing inosine that provides an inhibitory signal via the adenosine A2A receptor (A2AR). Impaired FAMIN catalysis results in excessive IAV-specific T cell responses and lung immunopathology, but also in enhanced tumor immune surveillance. We describe a purely biochemical mechanism within DCs that exerts fundamental control over T lymphocyte priming.

RESULTS

FAMIN activity in DCs restrains the influenza A virus-specific T cell response

Originally aiming to gain clues into HLH predisposition, we infected Famin mutant mice with a murine-adapted H3N2 IAV strain (AX-31) (Everitt et al., 2012). FaminP254I and FaminP284R mice, which endogenously express disease-linked hypomorphic and loss-of-function variants, respectively, developed more severe disease compared to FaminP254I mice, which express fully active FAMIN (Figures 1A and S1A). This was associated with more apoptosis, reflecting lung damage (Figure 1B), and elevated plasma IFNγ and IL-10 levels (Figure 1C). Anti-inflammasory IL-10 is produced by IFNγ+ IAV-specific CD8+ T cytotoxic type 1 cells (Tc1) in IAV-infected lungs (Sun et al., 2009), prompting us to quantify CD8+ T cells specific for NP366-374, an immunodominant IAV nucleoprotein epitope. On day 7 of infection, numbers of NP366-374-reactive CD8+ T cells in bronchoalveolar lavage (BAL) were 4-fold higher even in FaminP254I compared to FaminP254I, and higher still in FaminP284R mice (Figures 1D and S1B). Cytokine production by Tc1 cells is dependent on their interaction with, and costimulation by, CD11c+ DCs infiltrating the infected lung (Hufford et al., 2011). Deletion of Famin solely in CD11c+ DCs (FaminP254I;Cre;‘‘FaminADEC’’; Figure S1C) increased the numbers of NP366-374-specific and PA224-233 (an IAV polymerase acidic protein epitope)-specific CD8+ T cells in BAL compared to littermate FaminP254I/lox/lox mice (‘‘FaminWT’’; Figure 1E). In contrast to germ-line variation, selective deletion in FaminADEC mice did not increase immunopathology (Figure S1D). Interestingly, the excessive IAV-specific T lymphocyte response in FaminADEC lungs was associated with somewhat higher expression of IAV M protein compared to their respective controls (Figures 1F and S1E). We concluded that reduced or absent FAMIN activity in DCs resulted in exaggerated hyperinflammatory IAV-specific CD8+ T cell responses that did not augment control of the viral infection.

FAMIN in DCs restrains priming of class I and class II-restricted antigens

Intrigued by the selective increase in T cell responses emanating from FAMIN deficiency in DCs, we focused our study on whether and how FAMIN controls T cell priming and turned to ovalbumin (OVA) as a model antigen. Baseline percentages of splenic and lymph node CD4+ and CD8+ T lymphocytes, and splenic cDC1s and cDC2s, were indistinguishable between FaminP254I, FaminP254I, and FaminP284R mice (Table S1). Splenic CD11c+ DCs from FaminADEC mice primed naive OVA257-264-specific OT-I T lymphocytes for increased expansion and IFNγ secretion compared to those primed by FaminWT DCs, irrespective of whether they were pulsed with OVA257-264 peptide, OVA protein, or necrotic fibroblasts expressing a non-secreted OVA (bm1T-OVA; Sancho et al., 2009) requiring cross-presentation (Figures 2A–2C). Antigen-specific cytotoxicity, IFNγ, and granzyme B release were higher when naive OT-I T cells had been primed
by Famin<sup>ADC</sup> than by Famin<sup>WT</sup> splenic DCs (Figures 2D and 2E). CD8<sup>+</sup> conventional DCs type 1 (cDC1) preferentially prime naive CD8<sup>+</sup> T cells, and CD11b<sup>+</sup> cDC2 preferentially CD4<sup>+</sup> T cells (Durairaj and Murphy, 2016). Exaggerated cytotoxic T lymphocyte (CTL) responses were similarly observed when primed by bone marrow (BM)-derived Famin<sup>-/-</sup> compared to Famin<sup>WT</sup> cDC1 (Figure S2A), hence extending to DCs immunologically distinct from splenic DCs (Naik et al., 2005). BM-derived cDC1 from Famin<sup>284R</sup> and Famin<sup>-/-</sup> mice also primed OT-I T cells for CTL activity when infected with influenza A virus (IAV) (Figure S2B). Restimulation of cDC1-OT-I T cell co-cultures after 72 h (Figure 2F), or after further differentiation over 6 days into antigen-specific T effector (T<sub>E</sub>) and T effector memory (T<sub>EM</sub>) cells via IL-2 and IL-15 (Figure 2G), respectively, resulted in highest IFN<sub>γ</sub> secretion when priming was provided by Famin<sup>284R</sup> and Famin<sup>-/-</sup> cDC1, intermediate by Famin<sup>254V</sup>, and lowest by Famin<sup>254I</sup> cDC1. Hence, CD8<sup>+</sup> T cell responses increased with decreasing FAMIN activity in DCs, and the enhanced priming effect persisted when further differentiated into T<sub>E</sub> and T<sub>EM</sub> cells.

To investigate this in vivo, OT-I T cells were adoptively transferred into Famin<sup>ADC</sup> and Famin<sup>WT</sup> mice followed by intraperitoneal immunization with ovalbumin, and CTL activity was assessed 4 days later. OVA<sup>257-264</sup>-specific cytotoxicity, IFN<sub>γ</sub>, and granzyme B release of splenic T cells were strikingly higher in Famin<sup>ADC</sup> compared to Famin<sup>WT</sup> mice (Figure 2H). Increased CTL activity was intrinsic, as OT-I proliferation in vivo was similar between genotypes upon adjuvant-free priming (Figure S2C). Hence, lack of FAMIN activity in DCs enhanced their ability to prime CD8<sup>+</sup> T cell responses to a model antigen in vivo.

The ability of FAMIN-impaired DCs to prime exaggerated antigen-specific T cell responses extended to MHC II-restricted
Figure 2. DC FAMIN restrains CD4+ and CD8+ T cell responses

(A–C) IFNγ release and OT-I T cell proliferation indices after 72 h of co-culture with splenic Famin<sup>WT</sup> or Famin<sup>−/−</sup> DC11c<sup>+</sup> DCs pulsed with OVA<sup>257-264</sup> peptide (A), ovalbumin (B), or UV-irradiated bm1 T OVA mouse embryonic fibroblasts (C) (n = 3).

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CD4+ T cells. Famin−/− splenic DC11c+ DCs, pulsed with OVA253-339, primed for increased proliferation of syngeneic naive OVA253-339-specific OT-II T cells when compared to Famin+/+ DCs (Figure 2I). The levels of IFNγ and IL-2 were 4- and 8-fold higher, respectively, in supernatants from co-cultures with Famin−/− compared to Famin+/+ DCs (Figures 2J and 2K). The proportion of intracellular IFNγ+ OT-II T cells upon restimulation increased from 40% ± 8% to 75% ± 6% when they had been primed by OVA253-339-pulsed Famin−/− compared to Famin+/+ BM-derived cDC2 (Figure 2L). The proportion of IL-4+, IL-17+, and Foxp3+ OT-II T cells remained below 1% (Figure S2D). Naive CD4+ OT-II T cells adaptively transferred into FaminDKO mice exhibited increased proliferation upon intraportal cDC1 vaccination compared to those transferred into FaminWT mice (Figure 2M).

Altogether, impaired FAMIN in DCs increased antigen-specific T cell priming via both class I and II in vitro and in vivo.

**FAMIN controls DC metabolism and tunes antigen uptake and presentation without a transcriptional signature**

Despite the profound differences in their priming activity, Famin itself and genomically adjacent Ccdc122 were the sole differentially expressed genes (DEGs) in Famin−/− compared to Famin+/+ BM-derived cDC1 analyzed by RNA sequencing (RNA-seq; Figure 3A). A comparison of cDC1 from Faminp.254I and Faminp.254V mice did not reveal a single DEG (Figure S3A), and only 56 up- and 32 downregulated transcripts between Faminp.254I and Faminp.254V cDC1 (Figure S3B; Table S2). Among those DEGs were only four (Fgfr1, Tlr7, Ikbg, and Lcn2) encoding immune mediators, and no enrichment for gene ontology processes indicative of FAMIN controlling the pace of the process. Famin genotype did not affect protein expression of ADA, PNP, and MTAP (Figure S3C), which share catalytic activities with genotype did not affect protein expression of ADA, PNP, and MTAP (Figure S3C), raising the possibility of a bona fide biochemical mechanism controlling T cell priming.

Priming of naive T cells entails T cell receptor (TCR) binding to a peptide-MHC complex on a professional antigen-presenting cell, which is then fine-tuned by costimulatory molecules and antigen uptake was accelerated and available for presentation via MHC class I for CD8+ T cells, the latter requiring endosome-to-cytosol transfer (Blander, 2018). This can be measured using endocyted β-lactamase in DCs that are pre-loaded with a cytosolic probe that loses its FRET signal upon β-lactamase cleavage, when the latter gains access to the cytosol (Cebrian et al., 2011). Compared to Faminp.254I cDCs, Faminp.284R splenic DCs exhibited increased probe cleavage, especially at the earliest time point, demonstrating increased endosome-to-cytosol transfer (Figure 3E). The peptide repertoire presented on surface MHC I is continuously optimized by peptide exchange in the endoplasmic reticulum (ER) (Williams et al., 2002). Increased staining with monoclonal antibody 25-D1.16, which recognizes OVA257-264 bound to H-2Kb (Porgador et al., 1997), directly demonstrated increased peptide presentation on Faminp.284R and Faminp.254I compared to Faminp.254V splenic DCs after a pulse with OVA257-264 (Figures 3F and S3F). The difference between Famin genotypes in peptide:MHC I complexes was again most pronounced early after the OVA257-264 pulse, indicative of FAMIN controlling the pace of the process. Famin genotype did not affect total surface MHC I and II expression (Table S4; Figure S3G). Hence, loss of FAMIN activity led to faster-paced endosomal antigen uptake, transfer to cytosol, and peptide exchange and presentation on MHC I.

FAMIN can promote flux through a cycle that interconverts IMP to succinyl-AMP (S-AMP), AMP, and back to IMP via sequential activities of adenylosuccinate synthase (ADSS), adenylosuccinate lyase (ADSL), and AMP deaminase (AMPD) (Figure 3G) (Cader et al., 2020). In skeletal muscle and macrophages, the IMP-S-AMP cycle promotes energy metabolism and is referred to as purine nucleotide cycle (PNC) (Cader et al., 2020; Lowenstein and Tormheim, 1971). Cellular levels of IMP, S-AMP, and AMP decreased from Faminp.254V to Faminp.254I BM-derived cDC1 (Figures 3B and S3H). Tracing [13C16]palmitate, we observed decreased flux into Krebs cycle metabolites α-ketoglutarate, succinate, and malate in Faminp.254V and Faminp.284R compared to Faminp.254I BM-derived DC1s (Figure S3I). We also detected decreased onward flux into aspartate (which enters the IMP-S-AMP cycle) in Faminp.284R DCs. A similar pattern

(D and E) Specific cytotoxicity against OVA257-264-pulsed wild-type splenocytes (D), and IFNγ and granzyme B release (E) of OT-I T cells that had been primed with FaminWT or FaminDKO splenic DCs pulsed with OVA257-264 (n = 3).

(F) IFNγ secretion from re-stimulated (OVA257-264 for 5 h) OT-I T cells after 72 h of priming with Faminp.254I, Faminp.254V, Faminp.284R, or Famin−/− BM-derived cDC1 pulsed with OVA257-264 (n = 3).

(G) IFNγ secretion after 5 h anti-CD3/CD28 re-stimulation of OT-I T cells that had been differentiated into T E and T EM cells, following 72 h of priming by Faminp.254I, Faminp.254V, Faminp.284R, and Famin−/− BM-derived cDC1 (Figures 3B and S3C; Table S3), raising the possibility of a bona fide biochemical mechanism controlling T cell priming.

(H) OVA257-264-specific cytotoxicity, granzyme B, and IFNγ secretion of splenocytes of Faminp.254I and Faminp.284R mice that had been adoptively transferred with naive OT-I T cells and immunized with ovalbumin 72 h earlier (n = 3).

(I) Proliferation indices of OT-I T cells 96 h after priming with OVA253-339-pulsed splenic Faminp.254I and Famin−/− DCs (n = 3).

(J and K) IFNγ (J) and IL-2 (K) in supernatants of OT-I cells 96 h after priming with OVA253-339-pulsed splenic Faminp.254I and Famin−/− DCs (n = 3).

(L) Percentage IFNγ+ OT-II T cells after restimulation with anti-CD3/CD28, following priming with OVA253-339-pulsed BM-derived cDC2 7 days earlier (n = 3).

(M) Proliferation indices of OT-II T cells adoptively transferred into Faminp.254V and FaminWT mice 72 h after immunization with ovalbumin (n = 3).

Data represented as mean ± SEM. *p < 0.05, **p < 0.01, and ***p < 0.001 (one-way ANOVA or unpaired two-tailed Student’s t test where appropriate). See also Figure S2.
Figure 3. FAMIN controls DC metabolism and tunes antigen uptake and presentation without a transcriptional signature
(A) Differentially expressed genes between Famin<sup>−/−</sup> and Famin<sup>+/+</sup> BM-derived cDC1s (n = 4; GEO: GSE126473).
(B) Heatmap of purine nucleotide levels in Famin<sup>p.254I</sup>, Famin<sup>p.254V</sup>, and Famin<sup>p.284R</sup> cDC1s (n = 6/5/6); for details, see Table S3.
in labeled Krebs cycle metabolites was present after a pulse with \([^{13}C_4]\) glucose, where onward flux (via pyruvate dehydrogenase) into \([^{13}C_2]\) aspartate decreased, and onward flux (via pyruvate carboxylase or malic enzyme) into \([^{13}C_6]\) glucose, where onward flux (via pyruvate dehydrogenase) into \([^{13}C_2]\) aspartate increased in FAMIN-impaired DCs (Figure S3I). In contrast, flux from \([^{13}C_3,^{15}N_2]\) glutamine into Krebs cycle metabolites and aspartate increased in FAMIN-impaired cDC1s (Figure S3J). Overall, this was consistent with perturbed fatty acid oxidation (FAO) and lipid carbon channeling into the PNC, as well as compensatory changes in glutamine metabolism, mirroring key observations in FAMIN-deficient macrophages (Cader et al., 2020). Consequently, cDC1s’ energy metabolism and prevents cytoplasmic acidification. The extra-cellular acidification rate (ECAR) was also lower in Famin\(^{-/-}\) compared to Famin\(^{+/+}\) BM-derived cDC1s (Figure 3I), and secretion of lactate, with which protons (H\(^+\)) are co-exported, correspondingly declined from Famin\(^{-/-}\) to Famin\(^{+/+}\) (Figures 3J and 3K). Corresponding observations were made in splenic DCs (Figure S3J), in which cDC2 predominate over cDC1 (Table S1). The cytoplasmic pH (pH\(_c\)) of cDC1 (data not shown) and splenic DCs became more acidic as FAMIN activity decreased (Figure 3K). This demonstrated that FAMIN promotes DCs’ energy metabolism and prevents cytoplasmic acidification. By consuming aspartate and releasing its carbons as fumarate, which can be hydrated to malate, the IMP–S-AMP–AMP cycle can affect electron (e\(^-\)) transfer between cytoplasm and mitochondria, which ensues via the malate-aspartate shuttle (Borst, 2020; Cader et al., 2020). The aspartate pool supplying the IMP–S-AMP–AMP cycle was inaccessible by exogenously supplied \([^{13}C_5,^{15}N_2]\) malate (Figure 3L), similar to most cells in culture (Brsou et al., 2013). Fractional incorporation of exogenously provided \([^{13}C_5]\) malate was strikingly higher in Famin\(^{-/-}\) compared to Famin\(^{+/+}\) and Famin\(^{-/-}\) BM-derived DCs, as levels of unlabeled malate were conversely lowest in Famin\(^{-/-}\) and highest in Famin\(^{+/+}\) cells (Figures 3M and S3K). These differences in cellular malate, encompassing cytoplasmic and mitochondrial pools, corroborated that energy metabolism is pervasively perturbed in FAMIN-impaired DCs. Exogenous malate resulted in marked differences in levels of aspartate, IMP, S-AMP, and AMP between Famin genotypes (Figure 3N). Altogether this pointed, in analogy to macrophages (Cader et al., 2020), to the IMP–S-AMP–AMP cycle as an immediate biochemical effector of FAMIN catalysis.

Adenine-guanine nucleotide interconversion paces antigen uptake and T cell priming
We therefore asked whether IMP–S-AMP–AMP cycling restrains DC antigen uptake. Halting the IMP–S-AMP–AMP cycle with L-alanosine and hadacidin, IMP- and aspartate-analog inhibitors of ADSS (Guicherit et al., 1994), respectively, indeed increased AF647-OVA uptake in Famin\(^{-/-}\) BM-derived cDC1 to levels observed in Famin\(^{-/-}\) cells (Figures 4A and 4B). In contrast, blocking ADSS in Famin\(^{-/-}\) cDC1 did not further increase AF647-OVA uptake (Figures 4A and 4B). Increased antigen uptake by L-alanosine, conditional on Famin genotype, was similarly observed in BM-derived cDC2 (Figure S4A). 6-thio-IMP, a metabolite of clinically used immunomodulators 6-mercaptopurine (6-MP) and azathioprine (Hanauer et al., 2019; Tiede et al., 2003), has been reported to inhibit ADSS (Atkinson et al., 1964). 6-MP phenocopied L-alanosine and hadacidin on Famin-dependent AF647-OVA uptake in splenic DCs (Figure 4C), affecting cDC1 and cDC2 subsets equally (Figure S4B). Transfection of splenic DCs with Adss small interfering RNA (siRNA) increased AF647-OVA uptake in Famin\(^{-/-}\) cells to levels observed in control-transfected Famin\(^{-/-}\) cells, while not further increasing uptake in the latter (Figure S4C). Inhibition of AMPD with Cpd3 (Admyre et al., 2014) recapitulated effects of ADSS inhibition (Figure 4D), corroborating that FAMIN-enabled IMP–S-AMP–AMP cycling restrains antigen uptake. We next assessed whether increased antigen uptake upon blocking the IMP–S-AMP–AMP cycle translates into enhanced T cell priming. Compared to control-silenced OVA-pulsed splenic Famin\(^{-/-}\) DCs, those silenced for Adss, Adsl, or Ampd2/Ampd3 primed
Figure 4. Adenine-guanine nucleotide interconversion paces antigen uptake and T cell priming

(A–D) Percentage AF647-OVA+ cDC1s (A, B, and D) or splenic DCs (C) following incubation with AF647-OVA for 30 min in the presence of L-alanosine (A), hadacidin (B), 6-mercaptopurine (6-MP) (C), or Cpd3 (D) (n = 3–6, 3 mice per genotype; none of the treatments affected cell viability; please note control panels are shared between B and D).

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naive OT-I T cells for increased proliferation, with IFNγ elevated upon Adss knockdown (Figures 4E, 4F, S4D, and S4E). Consistent with fatty acid carbon entering the IMP–S–AMP–AMP cycle (Cader et al., 2020; Figure S3), silencing the rate-limiting enzyme of FAO, Cpt1α, in Famin−/− DCs elevated their OT-I priming potency to levels observed in Famin+/+ cells, while not augmenting it further in the latter (Figure S4F). Hence, knockdown of IMP–S–AMP–AMP cycle enzymes in FAMIN-sufficient DCs, or blocking the upstream supply of carbon entering the cycle, phenocopied enhanced priming due to compromised FAMIN.

Membrane trafficking, including endocytosis and processes leading to antigen presentation, is controlled by proteins regulated by GTP/GDP binding (Kirschner and Mitchison, 1986; Stenmark, 2009). Guanine nucleotides are synthesized from IMP via xanthosine monophosphate (XMP), catalyzed by IMP dehydrogenase (IMPDH) and GMP synthase (GMPS; Figure 3G). GMP reductase (GMPR) converts GMP back to IMP (Figure 3G) (Hedstrom, 2012), which, together with the IMP–S–AMP–AMP cycle, interconverts adenine and guanine nucleotides. Cellular purine nucleotide levels all declined with compromised FAMIN activity, except XMP, which trended higher (Figure S4G). XMP also increased, and AMP and AMP decreased, upon AMPD inhibition in Faminp.254I cDC1s (Figure 4G; Table S5). This suggested increased flux through IMPDH consequent to impaired FAMIN or IMP–S–AMP–AMP blockade. The IMPDH inhibitor methylamine (NAP), an immunosuppressant effective in arthritis and transplantation (Broen and van Laar, 2020), reduced AF647-OVA uptake in Faminp.254I and Faminp.254V splenic DCs (Figure 4H), as did the GMPS inhibitor psicofuranine (Udaka and Moyer, 1963) (Figure 4I), suggesting increased flux through GMPS, too. Increased flux through GMPS was evident by [15N1] GMP labeling increasing from Faminp.254I to Faminp.254V DCs after a pulse with [13C6,15N2] glutamine (Figure 4J), from which the amide nitrogen is transferred to form GMP (Figures 3G and S4H) (Tesmer et al., 1996). No GMP isopomerophers with [15N2] or higher were observed, which would have indicated de novo purine nucleotide synthesis (Figure S4I). We therefore tested whether increased flux through IMPDH and GMPS mediated enhanced priming. OVA-pulsed splenic DCs with Impdh1/Impdh2 knockdown (Figures S4D and S4E) primed naive OT-I T cells for lower IFNγ secretion compared to control-silenced DCs (Figures 4K–4M), and lower proliferation, which declined from the different levels primed by Faminp.254I and Faminp.254V DCs (Figures 4N–4P). OT-I T cell IFNγ secretion and proliferation also trended lower when primed by Gmps-silenced splenic DCs (Figures 4K–4P). This demonstrated that increased flux via IMPDH and GMPS in FAMIN-impaired DCs was responsible for their increased antigen uptake and T cell priming.

**IMPDH-dependent NADH/NAD+ redox state controls the pace of antigen uptake and MHC I recycling**

Bypassing IMPDH and GMPS with exogenous guanine increased AF647-OVA uptake in Faminp.254I and Faminp.254V splenic DCs, phenocopying enhanced antigen uptake of Faminp.254I or Faminp.254V DCs (Figure 5A). In the latter, guanine did not further augment uptake (Figure 5A). Prima vista, this suggested that guanine nucleotide pools may control antigen uptake. Increased antigen uptake, however, was at odds with decreased GTP and GDP levels in FAMIN-impaired DCs (Figure 3B). This implied a byproduct of the IMP–XMP–GMP cycle, rather than guanine nucleotide pool size, may be responsible for increased membrane trafficking. IMPDH reduces NAD+ to NADH + H+ (Figure 3G). Inhibition of IMPDH rescued cytoplasmic acidification in Faminp.254I and Faminp.254V DCs (Figure 5B), adding further evidence for enhanced flux through IMPDH. An altered pHc can affect vesicular trafficking (Heuser, 1989; Korolchuk et al., 2011; Walton et al., 2018). ADSS inhibition in Faminp.254I and Faminp.254V DCs enhanced AF647-OVA uptake (Figure 4A) without causing cytoplasmic acidification (Figure 5C), arguing against changes accounting for altered membrane trafficking. IMPDH inhibition did not rescue OCR or ECAR deficits in Faminp.254I and Faminp.254V DCs (Figures 5D and 5E), confirming that compromised OXPHOS and glycolysis are not directly responsible for exaggerated antigen uptake. As total cellular NAD(+) integrates protein-bound and free forms across cytoplasmic and mitochondrial pools with their distinct redox states, we measured the secreted lactate/pyruvate ratio to deduce the cytosolic free NADH/NAD+ ratio (Goodman et al., 2020; Krebs, 1967; Williamson et al., 1967). Faminp.254I splenic DCs exhibited a markedly higher lactate/pyruvate ratio than Faminp.254V cells (Figure 5F), implying increased cytosolic NADH/NAD+. In contrast, the secreted β-hydroxybutyrate/acetocetate ratio, reflecting mitochondrial free NADH/NAD+, remained unchanged (Figure S5A). To investigate whether re-oxidation of cytoplasmic NADH rescues exaggerated antigen uptake, we provided pyruvate as external acceptor that regenerates NAD+ via lactate dehydrogenase (LDH) (Figure 5G). Pyruvate indeed rescued increased AF647-OVA uptake in Faminp.254I splenic DCs (Figure 5H). Four-carbon α-ketobutyrate (AKB) is an alternative substrate for regenerating NAD+ from NADH via LDH (Figure 5G) (Sullivan et al., 2015). AKB is primarily used as an acceptor and not as carbon substrate in other...
Figure 5. IMPDH-dependent NADH/NAD⁺ redox state controls the pace of antigen uptake and MHC I recycling

(A) Percentage AF647-OVA⁺ splenic DCs of indicated genotypes following incubation with guanine (n = 6, 3 mice per genotype).

(B) pH of Famin⁻/⁻, Famin²/², and Famin⁻/⁻ BM-derived cDC1s in the presence of MPA (n = 8–12, 3 mice per genotype).

(C) pH of Famin⁻/⁻, Famin²/², and Famin⁻/⁻ splenic DCs in the presence of L-alanosine (n = 9, 3 mice per genotype).

(D) OCR of Famin⁻/⁻ and Famin²/² BM-derived cDC1s in the presence of MPA or vehicle control. Basal OCR and OCR following addition of oligomycin A (Oligo), FCCP, and Rot + Ant (n = 7–14, 3 mice per genotype).

(E) Basal ECAR of Famin⁻/⁻ and Famin²/² BM-derived cDC1s in the presence of MPA or vehicle control (n = 16–18, 3 mice per genotype).

(F) Ratio of secreted lactate to pyruvate, reflective of free cytosolic NADH/NAD⁺, in supernatants of Famin⁻/⁻ and Famin⁻/⁻ splenic DCs matured overnight in RPMI-1640/10% FBS (n = 9, from 3 mice per genotype).

(G) Schematic depicting the lactate dehydrogenase (LDH) reaction, in which pyruvate is converted to lactate with regeneration of NAD⁺; α-ketobutyrate acts as an alternative electron acceptor and is converted to α-hydroxybutyrate.

(H) Percentage AF647-OVA⁺ splenic DCs of indicated genotypes following incubation with pyruvate or α-ketobutyrate (AKB) overnight and replenished for the time of the assay (n = 6, 3 mice per genotype).

(I) Percentage AF647-OVA⁺ Famin⁻/⁻ splenic DCs following overnight incubation with pyruvate or AKB alone or in presence of Cpd3 (n = 6, 3 mice per genotype).

(J) Percentage of MHC I recycled in Famin⁻/⁻ and Famin⁻/⁻ BM-derived cDC1s over time (n = 6, 3 mice per genotype).

(K) Percentage of MHC I recycled in Famin⁻/⁻ BM-derived cDC1s at indicated times following overnight incubation with AKB or control (n = 3–5, 3 mice per genotype).

Data represented as mean ± SEM. *p < 0.05, **p < 0.01, and ***p < 0.001 (one-way ANOVA or unpaired two-tailed Student’s t test where appropriate). See also Figure S5.
Figure 6. The FAMIN catalytic product inosine released from DCs dampens T cell activation during priming
(A) IFNγ secretion from naive OT-I T cells activated by anti-CD3/CD28 in presence of 24 h supernatants of Famin−/− or Famin+/+ CD11c+ splenic DCs (n = 3).
(B and C) Gene set enrichment analysis (GSEA) of RNA-seq dataset of naive OT-I T cells activated by anti-CD3/CD28 for 24 h in the presence of Famin−/− or Famin+/+ splenic DC supernatant; data depict enrichment of the Hallmark gene sets “oxidative phosphorylation” (B) and “Myc Targets V2” (C).

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metabolic pathways. AKB precisely phenocopied the rescue of antigen uptake achieved by pyruvate, decreasing AF647-OVA uptake to equally low levels in Famin+/− and Famin+/+ splenic DCs (Figure 5H). AKB reduced the secreted lactate/pyruvate ratio as expected (Figure S5B), while the pH$_c$ difference was retained between Famin+/− and Famin+/+ splenic DCs (Figure S5C), consistent with cytoplasmic acidification not accounting for increased antigen uptake. Augmentation of baseline OCR was markedly different between pyruvate and AKB (Figures SSD and SSE). This was consistent with only pyruvate entering mitochondrial oxidation (Figure 5G), which we confirmed by tracing [13C]$_{p.254V}$ pyruvate and [13C]$_{p.254I}$ AKB into Krebs cycle metabolites in Famin+/− and Famin+/+ cDC1s (Figures S5F–S5J). Importantly, pyruvate and AKB also rescued increased AF647-OVA uptake in Famin+/− splenic DCs, in which the IMP–S-AMP–AMP cycle was halted by Cpd3 (Figure 5I). AF647-OVA uptake serves as proxy of one specialized endocytic pathway, but membrane trafficking is involved in all the different routes of antigen uptake, processing, and (cross-)presentation (Alloatti et al., 2016; Blander, 2018). Considering whether FAMIN-mediated redox control of membrane trafficking is a more general principle, we asked whether FAMIN affects MHC I recycling, required for loading of cross-presented peptides (Belabed et al., 2020; Joffre et al., 2012). MHC I recycling was higher in Famin+/− compared to Famin+/+ cDC1 (Figure 5J). AKB markedly reduced MHC I recycling in Famin+/− and barely in Famin+/+ cDC1 (Figures S5K and S5J), revealing that cytoplasmic NADH/NAD$^+$ affects the pace of MHC I recycling, too. This provided strong evidence that enhanced antigen uptake and presentation in FAMIN-impaired DCs is caused by increased reduction of cytoplasmic NAD$^+$ to NADH by IMPDH due to an imbalance in adenine-guanine nucleotide interconversion cycles.

**The FAMIN product inosine dampens T cell activation during priming**

Fixing splenic DCs with glutaeraldehyde after pulsing with OVA257–264 retained the ability of Famin+/− compared to Famin+/+ DCs to prime for increased OT-I T cell proliferation (Figure S6A), but the capacity to prime for enhanced IFNγ secretion by Famin+/− DCs, however, was lost (Figure S6B). This indicated that optimal priming requires mutual dynamic transmembrane signaling. It also raised the possibility that soluble factors released from DCs might be involved, too. Famin+/− and Famin+/+ DCs, and Famin+/−, Famin+/+, and Famin+/− cDC1s, were indistinguishable in their expression of co-stimulatory and co-inhibitory molecules (Table S4; Figure S6C). Cell-free supernatants of Famin−/− DCs primed naive anti-CD3/CD28-activated OT-I T cells to secrete 2-fold more IFNγ compared to supernatants of Famin+/+ DCs (Figure 6A). Transcriptsome of OT-I T cells activated in the presence of Famin−/− compared to Famin+/+ DC supernatants were enriched for hallmark gene sets (Leone et al., 2019) indicative of elevated effector function (Figures 6B, 6C, S6D, and S6E; Table S6). IL-12p70 and IFNγ secretion by Famin−/− and Famin+/+ DCs in co-culture with naive OT-I T cells was indistinguishable (Figure S6F). Freeze-thaw cycles did not affect Famin−/− DC supernatants’ enhanced stimulatory capacity (data not shown), which was retained after passing through a 3 kDa filter (Figure 6D), pointing to a small molecule. To enable its identification, we switched to serum-free OptiMEM media to reduce complexity. OptiMEM supernatants of Famin−/− and Famin+/+ DCs retained differences in IFNγ induction in anti-CD3/CD28-activated CD8+ T cells (Figure S6G). They were particularly stark between those elicited by Famin+/− compared to Famin+/+ DC supernatants (Figure 6E), CD4+ OT-II T cells were also primed for heightened IFNγ secretion by Famin+/− and Famin+/+ compared to Famin+/− DC supernatants (Figure 6F). This suggested that DCs secrete a small molecule in a FAMIN-dependent manner that inhibits priming of naive CD4+ and CD8+ T cells.

Unbiased high-resolution LC-MS of supernatants of Famin+/+ and Famin−/− splenic DCs resolved ~1,100 features, revealing inosine as the top-ranking identifiable LC-MS feature of differential abundance (Figures 6G and 6H). A second unbiased LC-MS screen comparing Famin+/− with Famin+/+ splenic
Figure 7. FAMIN-dependent conversion of extracellular hypoxanthine into inosine

(A) FAMIN-catalyzed enzymatic conversion of \([^{13}C_5^{15}N_4]\) hypoxanthine to \([^{13}C_5^{15}N_4]\) inosine.

(B) Cellular \([^{13}C_5^{15}N_4]\) inosine in Famin\(^{p.254I}\), Famin\(^{p.254V}\), and Famin\(^{p.284R}\) splenic DCs pre-equilibrated in OptiMEM for 3 h before a 3 h pulse with \([^{13}C_5^{15}N_4]\) hypoxanthine in OptiMEM (n = 6, 3 mice per genotype).

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DC supernatants identified two metabolites, inosine and pro-pionyl-carnitine, overlapping with the first screen (Figure 6I). As a catalytic product of FAMIN, inosine, whose levels were highest with fully active FAMINp254I (Figures 6J and 6K), was a plausible candidate. Pure inosine dose-dependently reduced OT-I T cell priming. Among the four adenosine receptors, direct binding of, and activation by, inosine has been shown for A2AR (Welihinda et al., 2018), the only adenosine receptor expressed on T cells (Cekic et al., 2013). In OT-I T cells activated by anti-CD3/CD28 in the presence of DC supernatants, the A2AR agonist CGS21680 (Hutchison et al., 1989) inhibited proliferation and IFNγ secretion and abrogated Famin genotype-related differences (Figures 6P and 6Q). A2AR antagonism with SCH58261 (Zocchi et al., 1996) had the converse effect (Figures 6P and 6Q). This demonstrated that FAMIN-dependent release of inosine from DCs inhibited activation of naive T cells.

FAMIN-dependent conversion of extracellular hypoxanthine into inosine

Nucleobases and nucleosides equilibrate across the plasma membrane via purine/pyrimidine transporters (Bowser-Casteel and Hays, 2017), prompting us to consider whether external nucleobases may supply the substrate for the synthesis of inosine in DCs (Figure 7A). A 3 h pulse of splenic DCs with 25 μM [15C5,15N4] hypoxanthine labeled over half of extracellular and cellular hypoxanthine (Figures S7A–S7D). Labeled hypoxanthine was converted to cellular [15C5,15N4] inosine, which was highest in Faminp254I and lowest in Faminp254V DCs (Figures 7B and S7E). This resulted in [15C5,15N4] inosine released into supernatants, whose levels were, in relative terms, ∼35% and ∼22% higher in Faminp254I and Faminp254V respectively, than in Faminp254R DCs (Figure 7C). Even fractional [15C5,15N4] inosine labeling increased from Faminp254R to Faminp254V to Faminp254I supernatants, averaging at one-fifth of total (Figure 7C), despite unlabeled inosine levels increased alongside, too (Figure 7D). Since OptiMEM is inosine-free and levels in RPMI-1640/10% FBS negligible (<100 pM, below the lowest detectable standard; Figure 7E), media change for labeling studies prompt an immediate equilibrative efflux; hence, these marked differences likely underestimate the contribution of FAMIN to inosine release during priming in situ. Plasma levels of inosine were similar across Famin germline mutants and DC-selective deletion (Figures S7F and S7G), consistent with a model of localized release. Perturbation in adenosine-guanine nucleotide interconversion revealed increased inosine release upon IMPDH inhibition and a slight decrease upon ADSS and AMPD inhibition, with differential release across Famin genotypes remaining intact (Figures 7F–7H). Altogether these studies demonstrated that elevated inosine release by DCs with active FAMIN amplifies an inhibitory signal during T cell priming, generated by phosphoribosylation of largely extracellularly derived hypoxanthine.

Compromised FAMIN catalysis enhances tumor immune surveillance

We finally turned to tumor immunosurveillance, a model not confounded by increased viral replication associated with IMPDH activity (To et al., 2016), to assess endogenous CTL function primed in the setting of polymorphic Famin variants. Increased DC antigen presentation enhances tumor-specific immunity by inducing Th1 and CTL responses (Xia et al., 2018). A2AR signaling prevents T cell anti-tumor immunity by inhibiting CTL activation and maintaining naive T cells quiescent (Cekic et al., 2013; Ohta et al., 2006). Faminp254V and Faminp254R mice developed markedly larger tumors compared to Faminp284R mice (Figures 7I and 7J) when subcutaneously injected with a syngeneic Lewis lung carcinoma cell line expressing ovalbumin (LL2-OVA) (Kraman et al., 2010). Protection was associated with nominally higher OVA257-264-specific CD8+ T cell numbers in peripheral blood in Faminp284R compared to Faminp254V and Faminp254R mice (Figure S7H). These results were consistent with augmented priming that translated into increased CTL activity and tumor immunosurveillance when FAMIN activity is compromised.

DISCUSSION

Here we discovered that purine nucleotide and nucleoside turnover in DCs represses T cell immunity, with FAMIN acting as a purely biochemical immune checkpoint. FAMIN achieves this via two main routes. First, FAMIN restrains endocytosis, antigen processing, and presentation via cytoplasmic NADH/NAD+ through balancing adenine-guanine nucleotide interconversion. Second, it amplifies an inhibitory signal through the generation of locally released inosine. The relative contribution of altered
antigen presentation versus altered inosine release to T cell priming is impossible to disentangle, since both are directly catalytically controlled by FAMIN. ADA, PNP, and MTAP do not compensate for FAMIN’s absence, although they share three of four of FAMIN’s catalytic activities. This suggests that FAMIN is at the center of a dedicated purine metabolon that biochemically restrains DCs’ priming activity.

Pacing membrane trafficking via adenine-guanine nucleotide interconversion cycles through an NADH/NAD⁺-sensitive mechanism, consequent to hyperactive IMPDH that reduces NAD⁺ to NADH, may represent a general principle. Vectorial physical membrane displacements, which can be energized by transmembrane e⁻ transport, occur upon plasma membrane internalization and, in the opposite direction, during membrane budding and vesicle formation (Morré and Morré, 2011). NADH can activate vectorial membrane transfer, elegantly demonstrated in cell-free systems for the transfer from the trans Golgi apparatus to the plasma membrane (Rodríguez et al., 1992). Aside from IMPDH, NAD⁺ is reduced to NADH by several cytoplasmic reactions, foremost by glyceroldehyde dehydrogenase of glycolysis, and re-oxidized by LDH and the malate-aspartate shuttle via mitochondria. NADH/NAD⁺ reductive stress due to perturbation in any of those reactions might therefore also impact membrane trafficking and antigen presentation. NADH/NAD⁺ reductive stress in the liver emerges as the causal mechanism for features of the metabolic syndrome associated with hypomorphous GCKR, such as hepatic insulin resistance and increased triglyceride release (Goodman et al., 2020). GCKR localizes at the probably most pleiotropic genome-wide association study (GWAS) locus and encodes liver-specific glucokinase regulatory protein, which helps prevent a futile metabolic cycle with glycolysis during gluconeogenesis (Goodman et al., 2020). Whether obesity, which increases risk for autoimmunity (Versini et al., 2014), causes NADH/NAD⁺ reductive stress in DCs is unknown.

Via equilibration of purine nucleobases and nucleosides across the plasma membrane, DCs may survey their vicinity. They respond to hypoxanthine by converting it to inosine, damping T cell activation. Since phosphorylation is reversible, DCs might bidirectionally respond to local hypoxanthine and inosine availability during immunological synapse formation. T cells, in particular naive CD4⁺ T cells, can release hypoxanthine (Fan et al., 2019), which may enable a dynamic interaction with FAMIN catalysis in DCs affecting the priming threshold. Systemic hypoxanthine and inosine levels closely track each other, consistent with their rapid interconversion via PNP (Sun et al., 2019). The intestinal microbiota may also affect inosine plasma levels (Mager et al., 2020). Inosine activation of the A₂AR on T cells is itself complex: inosine either prevents Th1 differentiation and blunted anti-tumor immunity in anti-CTLA4-treated mice or, conversely, enhanced both, when co-supplied with IFNγ in vivo and a TLR9 agonist in vivo (Mager et al., 2020). The mechanism underlying this switch remained unclear. Inosine can also serve as an alternative carbon source for CD8⁺ T cells when glucose is unavailable (Wang et al., 2020). The complete (254I versus 254V) and almost-complete (254I versus 284I and Famin-+/+ versus Famin−/−) absence of transcriptional changes excludes that autocrine inosine-triggered receptor signaling in DCs dampens their priming capacity.

The biochemical mechanism reported herein sheds new light on predisposition for HLH/MAS. Hyperactivated CTLs and their IFNγ activate macrophages, triggering hemophagocytosis and a cytokine storm (Brisse et al., 2016b). Persistence of antigen-presenting DCs resulting in uncontrolled CTL priming occurs in primary, genetic HLH, consequent to impaired perforin-mediated antigen-selective removal of DCs (Lykens et al., 2011). However, most patients with secondary, acquired HLH have unimpaired cytotoxicity (Bryceson et al., 2012). Acquired HLH/MAS complicates diverse, mostly viral, infections, malignancies, autoimmune and autoinflammatory disorders (Brisse et al., 2016a), and treatment with chimeric antigen receptor (CAR) T cells (Nellapu et al., 2018). Five of six candidate genes at the major 3p21.31 risk locus for severe COVID-19 (Ellinghaus et al., 2020; Nakanishi et al., 2021; Pairo-Castineira et al., 2021) point toward DC-T cell interactions (Kaser, 2020), remarkable as that hyperinflammation shares features with HLH/MAS, including hemophagocytosis (Lucas et al., 2020; Prieto-Pérez et al., 2020). It remains unclear why excessive T cell activation by FAMIN-impaired DCs results in enhanced immunosurveillance of tumors but fails to control IAV infection.

A final point is that experiments in wild-type mice may grossly over-estimate anti-viral and anti-tumor T cell immunity that can be expected in the majority of humans, since mice naturally express hypomorphic FAMIN-254V. As we show here, the single amino acid change to −254I (for which −89% of humans are homozygous or heterozygous) results in 4-fold lower numbers of nucleoprotein-specific CTLs upon experimental IAV infection and a profound reduction in T cell effector function. This polymorphism (rs3764147) has been linked to a possible founder effect (Rivas et al., 2018), hinting that excessive priming may have afforded evolutionary benefits.

Limitations of study
The IMP-S-AMP–AMP cycle operates at the center of energy metabolism, directly and instantaneously affecting glycolysis, electron transfer, FAO, Krebs cycle activity, glutamine oxidation, and the urea cycle (Cader et al., 2020; Lowenstein, 1972, 1990). The lack of technology with tempo-spatial resolution to resolve metabolites across cellular compartments is a particularly acute limitation, compounded by the unparalleled degree of interconnectedness, fast substrate cycles, and redundancies within central purine metabolism. This poses challenges, e.g., for directly measuring flux through IMPDH, and for determining inosine levels at the immunological synapse in situ.

STAR METHODS
Detailed methods are provided in the online version of this paper and include the following:

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- EXPERIMENTAL MODEL AND SUBJECT DETAILS
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  - Dendritic cell and T cell isolation
C.B., G.W.S., and S.S. performed RNA-seq; A.B. generated CRISPR/Cas9 reagents; critical reagents; L.M.H. provided experimental support; J.C.L., S.C., L.-M.H., T.D.L., and G.D. contributed to the design and development of ultra-high-performance liquid chromatography and mass spectrometry; A.B.I.-R., M.N.M.-I., J.O.J., and N.C.K. performed experiments; J.A.W. provided funding for the Bridge BRC Cell Phenotyping Hub for flow cytometry support, the MRC Metabolic Diseases Unit; S.G. conducted experiments; K.R. and S.S. contributed to the design and development of the study; and input from all authors, coordinated the project, designed experiments, interpreted data, and prepared the manuscript.

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AUTHOR CONTRIBUTIONS

S.S., G.W.S., and K.R. together with M.Z.C., L.-M.H., R.P.d.A.R., L.W.U., A.B.I.-R., M.N.M.-I., J.O.J., and N.C.K. performed experiments; J.A.W. provided ultra-high-performance liquid chromatography and mass spectrometry; S.C., L.-M.H., T.D.L., and G.D. contributed to in vivo experimentation; R.S.B. contributed critical reagents; L.M.H. provided experimental support; J.C.L., C.B., G.W.S., and S.S. performed RNA-seq; A.B. generated CRISPR/Cas9 mice lines; A.K. devised the study and, together with S.S., G.W.S., and K.R. and input from all authors, coordinated the project, designed experiments, interpreted data, and prepared the manuscript.

DECLARATION OF INTERESTS

The University of Cambridge has filed patent applications relating to this work. The authors declare no other competing financial interests.

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REFERENCES

Admyre, T., Amrot-Fors, L., Andersson, M., Bauer, M., Bjursell, M., Drmota, T., Hallen, S., Hartleib-Geschwinder, J., Lindmark, B., Liu, J., et al. (2014). Inhibition of AMP deaminase activity does not improve glucose control in rodent models of insulin resistance or diabetes. Chem. Biol. 21, 1486–1496.
Al-Mayouf, S.M., Almutairi, A., Albrawi, S., Fathalla, B.M., Attyouf, R., Aienazi, A., Abu-Shukair, M., Alwahedneh, A., Alsonbul, A., Zienti, M., et al.; for Pediatric Arab Rheumatology Group (PARAG) (2020). Pattern and diagnostic evaluation of systemic autoinflammatory diseases other than familial Mediterranean fever among Arab children: a multicenter study from the Pediatric Rheumatology Arab Group (PARAG). Rheumatol. Int. 40, 49–56.
Alloatti, A., Kotsias, F., Magalhaes, J.G., and Amigorena, S. (2016). Dendritic cell maturation and cross-presentation: timing matters! Immunol. Rev. 272, 97–108.
Atkinson, M.R., Morton, R.K., and Murray, A.W. (1964). Inhibition of adenylosuccinate synthetase and adenylosuccinate lyase by 6-thiopurine 5’-phosphate. Biochem. J. 92, 398–404.
Birsoy, K., Wang, T., Chen, W.W., Freinkman, E., Abu-Remaileh, M., and Biran, J.D., Fischer, A., de Saint Basile, G., van Endert, P., Sepulveda, F.E., and Kahn, C. (2010). Kinesin-1 regulates antigen cross-presentation through the scission of tubulations from early endosomes in dendritic cells. Nat. Commun. 11, 1817.
Beutel, G., Wiezen, O., Eder, M., Hafer, C., Schneider, A.S., Kielstein, J.T., Kuhn, C., Heim, A., Ganzmuller, T., Kreipe, H.H., et al. (2011). Virus-associated hemophagocytic syndrome as a major contributor to death in patients with 2009 influenza A (H1N1) infection. Crit. Care 15, R80.
Birsoy, K., Wang, T., Chen, W.W., Freinkman, E., Abu-Ramah, M., and Sabatini, D.M. (2015). An essential role of the mitochondrial electron transport chain in cell proliferation is to enable aspartate synthesis. Cell 162, 540–551.
Blander, J.M. (2018). Regulation of the cell biology of antigen cross-presentation. Annu. Rev. Immunol. 36, 717–753.
Blum, J.S., Wearsch, P.A., and Cresswell, P. (2013). Pathways of antigen transduction in dendritic cells by 6-thioinosine 5’-phosphate. Biochem. J. 454, 2241–2259.
Brant, S.R., Silverberg, M.S., Taylor, K.D., Barnadad, M.M., et al.; NIDDK IBD Genetics Consortium; Belgian-French IBD Consortium; Welcome Trust Case Control Consortium (2008). Genome-wide association defines more than 30 distinct susceptibility loci for Crohn’s disease. Nat. Genet. 40, 955–962.
Belabeled, M., Mauvais, F.X., Maschialdi, S., Kurowska, M., Goudin, N., Huang, J.D., Fischer, A., de Saint Basile, G., van Endert, P., Sepulveda, F.E., and Ménasché, G. (2020). Kinesin-1 regulates antigen cross-presentation through the scission of tubulations from early endosomes in dendritic cells. Nat. Commun. 11, 1817.
Brisse, E., Wouters, C.H., and Matthys, P. (2016b). Advances in the pathogenesis of primary and secondary haemophagocytic lymphohistiocytosis: differences and similarities. Br. J. Haematol. 174, 203–217.

Broen, J.C.A., and van Laar, J.M. (2020). Mucopolysaccharidosis, ataxiopathine and tacrolimus: mechanisms in rheumatology. Nat. Rev. Rheumatol. 16, 167–178.

Bryceson, Y.T., Pende, D., Maui-Pavlic, A., Gilmour, K.C., Ufheil, H., Vraetz, T., Chiang, S.C., Marcearno, S., Meazza, R., Bondzio, I., et al. (2012). A prospective evaluation of degranulation assays in the rapid diagnosis of familial hemophagocytic syndromes. Blood 119, 2754–2763.

Bzowska, A., Kulkowska, E., and Shugar, D. (2000). Purine nucleoside phosphorylases: properties, functions, and clinical aspects. Pharmacol. Thér. 88, 349–425.

Cader, M.Z., Borovik, K., Zhang, Q., Assadi, G., Kempest, S.L., Sewell, G.W., Saveleva, S., Ashcroft, J.W., Clare, S., Mukhopadhyay, S., et al. (2016). C13orf31 (FAMIN) is a central regulator of immunometabolic function. Nat. Immunol. 17, 1046–1056.

Cader, M.Z., de Almeida Rodrigues, R.P., West, J.A., Sewell, G.W., Mdl-Ibrahim, M.N., Rekine, S., Sirago, G., Unger, L.W., Iglesias-Romerio, A.B., Ramsbom, K., et al. (2020). FAMIN is a multifunctional purine enzyme enabling the purine nucleotide cycle. Cell 180, 278–295.e23.

Cantrill, D. (2015). Signaling in lymphocyte activation. Cold Spring Harb. Perspect. Biol. 7, a016788.

Cebranı, İ., Visentin, G., Blanchard, N., Jouve, M., Bobard, A., Moita, C., Enninga, J., Moita, L.F., Amigorena, S., and Savina, A. (2011). Sec22b regulates phagosomal maturation and antigen crosspresentation by dendritic cells. Nat. Immunol. 12, 167–178.

Cebrian, I., Visentin, G., Blanchard, N., Jouve, M., Bobard, A., Moita, C., Enninga, J., Moita, L.F., Amigorena, S., and Savina, A. (2011). Sec22b regulates phagosomal maturation and antigen crosspresentation by dendritic cells. Nat. Immunol. 12, 167–178.

Chen, W., Chiang, S.C., Marcenaro, S., Meazza, R., Bondzio, I., et al. (2012). A prospective evaluation of degranulation assays in the rapid diagnosis of familial hemophagocytic syndromes. Blood 119, 2754–2763.

Cibotti, G., Visentin, G., Blanchard, N., Jouve, M., Bobard, A., Moita, C., Enninga, J., Moita, L.F., Amigorena, S., and Savina, A. (2011). Sec22b regulates phagosomal maturation and antigen crosspresentation by dendritic cells. Nat. Immunol. 12, 167–178.

Cukovic, I., Visentin, G., Blanchard, N., Jouve, M., Bobard, A., Moita, C., Enninga, J., Moita, L.F., Amigorena, S., and Savina, A. (2011). Sec22b regulates phagosomal maturation and antigen crosspresentation by dendritic cells. Nat. Immunol. 12, 167–178.

Dobin, A., and Gingeras, T.R. (2015). Mapping RNA-seq reads with STAR. Nat. Protoc. 10, 1612–1629.

Dori, V., and Murphy, K.M. (2016). Functions of murine dendritic cells. Immunity 45, 719–736.

Ellinghaus, D., Degenhardt, F., Bujanda, L., Buti, M., Alibios, A., Invernizzi, P., Fernández, J., Pratti, D., Baselli, G., Asselta, R., et al.; Severe Covid-19 GWAS Group (2020). Genome-wide association study of severe Covid-19 with low memory requirements. Nat. Engl. J. Med. 383, 1522–1534.

Everitt, A.R., Clare, S., Pertel, T., John, S.P., Wash, R.S., Smith, S.E., Chin, C.R., Feeley, E.M., Sims, J.S., Adams, D.J., et al.; GeniGIS Investigators; MOSAIC Investigators (2012). IFITM3 restricts the morbidity and mortality associated with influenza. Nature 484, 519–523.

Fan, K., Li, Y., Yang, H., Mao, X., Guo, J., Wang, F., Huang, L., Li, Y., and Abraham, C. (2020). Myeloid cell expression of LACC1 is required for bacterial clearance and control of intestinal inflammation. Gastroenterology 159, 1051–1067.

Kasar, A. (2020). Genetic risk of severe Covid-19. N. Engl. J. Med. 383, 1590–1591.

Kim, D., Langmead, B., and Salzberg, S.L. (2015). HISAT: a fast spliced aligner with low memory requirements. Nat. Methods 12, 329–342.

Korolchuk, V.I., Saiki, S., Lichtenberg, M., Siddiqi, F.H., Roberts, E.A., Imarisio, S., Jehovah, L., Sarkar, S., Futter, M., Menzies, F.M., et al. (2011). Lysosomal positioning coordinates cellular nutrient responses. Nat. Cell Biol. 13, 453–460.

Kraman, M., Bambour, P.J., Arnold, J.N., Roberts, E.W., Magiera, L., Jones, J.O., Gopinathan, A., Tuveson, D.A., and Fearon, D.T. (2010). Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha. Science 330, 827–830.

Krebs, H.A. (1967). The redox state of nicotinamide adenine dinucleotide in the cytoplasm and mitochondria of rat liver. Adv. Enzyme Regul. 5, 409–434.

Lahiri, A., Hedl, M., Yan, J., and Abraham, C. (2017). Human LACC1 increases innate receptor-induced responses and a LACC1 disease-risk variant modulates these outcomes. Nat. Commun. 8, 15614.

Leone, R.D., Zhao, L., Engiert, J.M., Sun, I.M., Oh, M.H., Sun, I.H., Arwood, M.L., Bettencourt, I.A., Patel, C.H., Wen, J., et al. (2019). Glutamine blockade induces divergent metabolic programs to overcome tumor immune evasion. Science 366, 1013–1021.

Lowenstein, J.M. (1972). Ammonia production in muscle and other tissues: the purine nucleotide cycle. Physiol. Rev. 52, 382–414.

Lowenstein, J.M. (1990). The purine nucleotide cycle revisited [corrected]. Int. J. Sports Med. 11 (Suppl 2), S37–S46.

Lowenstein, J.M., and Tornheim, K. (1971). Ammonia production in muscle: the purine nucleotide cycle. Science 177, 397–400.

Lucas, C., Wong, P., Klein, J., Castro, T.B.R., Silva, J., Sundaram, M., Ellington, M.K., Mao, T., Oh, J.E., Israelow, B., et al.; Yale IMPACT Team (2020). Longitudinal analyses reveal immunological misfiring in severe COVID-19. Nature 584, 463–469.
Lykens, J.E., Terrell, C.E., Zoller, E.E., Risma, K., and Jordan, M.B. (2011). Perforin is a critical physiologic regulator of T-cell activation. Blood 118, 618-626.

Mager, L.F., Burkhard, R., Pett, N., Cooke, N.C.A., Brown, K., Ramay, H., Paik, S., Stagg, J., Groves, R.A., Gallo, M., et al. (2020). Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. Science 369, 1481-1489.

Manjunath, N., Shankar, P., Wan, J., Weninger, W., Crowley, M.A., Hieshima, K., Springer, T.A., Fan, X., Shen, H., Lieberman, J., and von Andrian, U.H. (2001). Effector differentiation is not prerequisite for generation of memory cytotoxic T lymphocytes. J. Clin. Invest. 108, 871-878.

Morré, D.J., and Morré, D.M. (2011). Non-mitochondrial coenzyme Q. Biofactors 37, 355-360.

Naik, S.H., Prieto-Pérez, L., Fortes, J., Soto, C., Vidal-González, A., Alonso-Rián, M., Lafarga, M., Cortí, M.J., Lazaro-García, A., Pérez-Tanoira, R., Trascasa, A., et al. (2020). Histiocytic hyperplasia with hemophagocytosis and acute alveolar damage in COVID-19 infection. Mod. Pathol. 33, 2139-2146.

Rabinot, R., Remesal, A., Mensa-Vilaró, A., Murias, S., Alocibranas, R., González-Roca, E., Ruiz-Ortiz, E., Antón, J., Iglesias, E., Modesto, C., et al. (2019). Biallelic loss-of-function LACC1/FAMIN mutations presenting as rheumatoid factor-negative polyarticular juvenile idiopathic arthritis. Sci. Rep. 9, 4579.

Raiser, M. (2018). An appeal to magic? The discovery of a non-enzymatic metabolism and its role in the origins of life. Biochem. J. 475, 2577-2592.

Rodriguez, M., Moreau, P., Paulik, M., Lawrence, J., Morré, D.J., and Morré, D. (1992). NADH-activated cell-free transfer between Golgi apparatus and plasma membranes of rat liver. Biochim. Biophys. Acta 1170, 131-188.

Sancho, D., Joffre, O.P., Keller, A.M., Rogers, N.C., Martínez, D., Hernanz-Falcón, P., Rosewell, I., and Reis e Sousa, C. (2009). Identification of a dendritic cell receptor that couples sensing of necrosis to immunity. Nature 458, 899-903.

Skon-Hegg, C., Zhang, J., Wu, X., Sagolla, M., Ota, N., Wuster, A., Tom, J., Doran, E., Ramamoorthi, N., Capizzi, P., et al. (2019). LACC1 regulates TNF and IL-17 in mouse models of arthritis and inflammation. J. Immunol. 202, 183-193.

Stenmark, H. (2009). Rap GTPases as coordinators of vesicle traffic. Nat. Rev. Mol. Cell Biol. 10, 513-525.

Sullivan, L.B., Gui, D.Y., Hosios, A.M., Bush, L.N., Freinkman, E., and Vander Heiden, M.G. (2015). Supporting asparagine biosynthesis is an essential function of respiration in proliferating cells. Cell 162, 552-563.

Sun, J., Madan, R., Karp, C.L., and Braciale, T.J. (2009). Effector T cells control lung inflammation during acute influenza virus infection by producing IL-10. Nat. Med. 15, 277-284.

Taggart, M., Lahoud, M.H., O‘Keeffe, M., Shao, Q.X., Chen, W.F., et al. (2005). Cutting edge: generation of splenic CD8+ and CD8- dendritic cell equivalents in Fms-like tyrosine kinase 3 ligand bone marrow cultures. J. Immunol. 174, 6592-6597.

Nakanishi, T., Papageorgiou, S., Degenhardt, F., Cordié, M., Butler-Laporte, G., Maya-Miles, D., Bujanda, L., Bousrey, Y., Nieri, M.E.K., Palom, A., et al. (2021). Age-dependent impact of the major common genetic risk factor for COVID-19 on severity and mortality. J. Clin. Invest. 131, e152386.

Neelapu, S.S., Tummala, S., Leclerc, W., Gnanaprakasam, J.N.R., Chen, X., Kang, S., Xu, X., Wang, G., Ye, H., and Asa, J. (2019). Purine catabolism shows a dampened circadian rhythmicity in a high-fat diet-induced mouse model of obesity. Molecules 24, 4524.

Tesmer, J.J., Klem, T.J., Deras, M.L., Davisson, V.J., and Smith, J.L. (1996). The crystal structure of GMP synthetase reveals a novel catalytic triad and is a structural paradigm for two enzyme families. Nat. Struct. Biol. 3, 74-86.

Tiede, I., Fritz, G., Strand, S., Poppe, D., Dvorsky, R., Strand, D., Lehr, H.A., Wirtz, S., Becker, C., Atrey, R., et al. (2003). CD28-dependent Rac1 activation is the molecular target of azathioprine in human CD4+ T lymphocytes. J. Clin. Invest. 111, 1133-1145.

To, K.K.W., Mok, K.Y., Chan, A.S.F., Cheung, N.N., Wang, P., Lui, Y.M., Chan, J.F.W., Chan, H., Chan, K.H., Kao, R.Y.T., and Yuen, K.Y. (2016). Mycophenolic acid, an immunomodulator, has potent and broad-spectrum in vitro antiviral activity against pandemic, seasonal and avian influenza viruses affecting humans. J. Gen. Virol. 97, 1807-1817.

Versini, M., Jeandel, P.Y., Rosenthal, E., and Shoenfeld, Y. (2014). Obesity in autoimmune diseases: not a passive bystander. Autoimmun. Rev. 13, 981-1000.

Wakil, S.M., Monies, D.M., Abouelhoda, M., Al-Tassan, N., Al-Dusery, H., Naim, E.A., Al-Younes, B., Shao, Q.X., Chen, W.F., et al. (2015). Association of a mutation in LACC1 with a monogenic rare diseases in the Ashkenazi Jewish population. PLoS Genet. 11, e1005329.

Wakil, S.M., Monies, D.M., Abouelhoda, M., Al-Tassan, N., Al-Dusery, H., Naim, E.A., Al-Younes, B., Shao, Q.X., Chen, W.F., et al. (2015). Association of a mutation in LACC1 with a monogenic rare diseases in the Ashkenazi Jewish population. PLoS Genet. 11, e1007329.

Williamson, D.H., Lund, P., and Krebs, H.A. (1967). The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver. Biochem. J. 103, 514-527.

Xia, Y., Xie, Y., Yu, Z., Xiao, H., Jiang, G., Zhou, X., Yang, Y., Li, X., Zhao, M., Li, L., et al. (2018). The mevalonate pathway is a druggable target for vaccine adjuvant discovery. Cell 175, 1059-1073.e21.
Yasin, S., and Schulert, G.S. (2018). Systemic juvenile idiopathic arthritis and macrophage activation syndrome: update on pathogenesis and treatment. Curr. Opin. Rheumatol. 30, 514–520.

Zelenay, S., Keller, A.M., Whitney, P.G., Schraml, B.U., Deddouche, S., Rogers, N.C., Schulz, O., Sancho, D., and Reis e Sousa, C. (2012). The dendritic cell receptor DNGR-1 controls endocytic handling of necrotic cell antigens to favor cross-priming of CTLs in virus-infected mice. J. Clin. Invest. 122, 1615–1627.

Zhang, F.R., Huang, W., Chen, S.M., Sun, L.D., Liu, H., Li, Y., Cui, Y., Yan, X.X., Yang, H.T., Yang, R.D., et al. (2009). Genomewide association study of leprosy. N. Engl. J. Med. 361, 2609–2618.

Zocchi, C., Orgini, E., Conti, A., Monopoli, A., Negretti, A., Baraldi, P.G., and Dionisotti, S. (1996). The non-xanthine heterocyclic compound SCH 58261 is a new potent and selective A2a adenosine receptor antagonist. J. Pharmacol. Exp. Ther. 276, 398–404.
**STAR METHODS**

**KEY RESOURCES TABLE**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| B220-PE             | Biolegend | RRID: AB_312992 |
| CD11b-Pe/Cy7        | Biolegend | RRID: AB_312799 |
| CD11c-APC           | Biolegend | RRID: AB_313778 |
| CD11c-PE            | Biolegend | RRID: AB_313777 |
| CD16/CD32           | Biolegend | RRID: AB_312801 |
| CD172a (SIRPα)-PE/Dazzle 594 | Biolegend | RRID: AB_2565279 |
| CD24-AF647          | Biolegend | RRID: AB_493485 |
| CD252-Pe/Cy7        | Biolegend | RRID: AB_2565744 |
| CD274-BV785         | Biolegend | RRID: AB_2629659 |
| CD275-PE            | Biolegend | RRID: AB_2248797 |
| CD28 (37.51)        | Thermo Fisher Scientific | RRID: AB_468921 |
| CD3-AF647           | Biolegend | RRID: AB_389323 |
| CD3-PE/Cy5          | Biolegend | RRID: AB_312674 |
| CD3-PECy7           | Biolegend | RRID: AB_312675 |
| CD3ε (145-2C11)     | Thermo Fisher Scientific | RRID: AB_468848 |
| CD4-BV605           | Biolegend | RRID: AB_2564591 |
| CD4-FITC            | Biolegend | RRID: AB_312691 |
| CD40-PE/Cy7         | Biolegend | RRID: AB_10933422 |
| CD44-FITC           | Biolegend | RRID: AB_493684 |
| CD45-APC/Cy7        | Biolegend | RRID: AB_312980 |
| CD45-Pe/Cy7         | Biolegend | RRID: AB_312978 |
| CD45.1-APC          | Biolegend | RRID: AB_313503 |
| CD45R/B220-FITC     | Biolegend | RRID: AB_312990 |
| CD64-BV605          | Biolegend | RRID: AB_2629778 |
| CD8α-BV605          | Biolegend | RRID: AB_2561352 |
| CD8α-BV650          | Biolegend | RRID: AB_11124344 |
| CD80-AF647          | Biolegend | RRID: AB_492824 |
| CD86-FITC           | Biolegend | RRID: AB_313149 |
| FoXP3-PerCP/Cy5.5   | Thermo Fisher Scientific | RRID: AB_914349 |
| H-2Kb-PerCP/Cy5.5   | Biolegend | RRID: AB_1967107 |
| H-2Kb-SIINFEKL (25-D1.16)-APC | Biolegend | RRID: AB_11219402 |
| H-2Kb/H-2D1-FITC    | Biolegend | RRID: AB_313507 |
| I-AI-E-APC/Cy7      | Biolegend | RRID: AB_1659252 |
| I-AI-E-APC/Fire™ 750 | Biolegend | RRID: AB_2616728 |
| I-AI-E-BV510        | Biolegend | RRID: AB_2561397 |
| IFNγ-PE/Cy7         | Biolegend | RRID: AB_2295770 |
| IL-17α-PerCP/Cy5.5  | BD Horizon | RRID: AB_2738642 |
| IL-4-FITC           | Thermo Fisher Scientific | RRID: AB_465387 |
| Influenza A Virus Nucleoprotein-FITC | Abcam | RRID: AB_445914 |
| SIRPα-Pe            | Biolegend | RRID: AB_2563549 |
| TCR Vα2-Pe/Cy7      | Thermo Fisher Scientific | RRID: AB_2573472 |
| TCRVβ5.1 5.2-PE     | Biolegend | RRID: AB_10612761 |
| Anti-mouse IgG, HRP-linked (polyclonal) | Cell Signaling Technology | RRID: AB_330924 |
| Anti-rabbit IgG, HRP-linked (polyclonal) | Cell Signaling Technology | RRID: AB_2099233 |
Continued

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Beta-actin (13-E4) (monoclonal) | Cell Signaling Technology | RRID: AB_2223172 |
| ADA (polyclonal) | Abcam | Cat# ab217846 |
| PNP (H-7) (monoclonal) | Santa Cruz Biotechnology | RRID: AB_10845931 |
| MTAP (42-T) (monoclonal) | Santa Cruz Biotechnology | RRID: AB_2147095 |

**Bacterial and virus strains**

| H3N2 IAV strain (A/X-31) | (Everitt et al., 2012) | N/A |

**Chemicals, peptides, and recombinant proteins**

| 20X LumiGLO Reagent and 20X Peroxide | Cell Signaling Technology | Cat# 7003 |
| 3-(hydroxynitrosoamino)-L-alanine (L-alanosine) | Cayman Chemicals | Cat# 19545, CAS: 5854-93-3 |
| 3-Nitrophenylhydrazine hydrochloride | Sigma-Aldrich | Cat# N21804-5G |
| 4X Laemmli sample buffer | Bio-Rad | Cat# 1610747 |
| 5(6)-CFDA, SE. | Thermo Fisher Scientific | Cat# C1157, CAS: 150347-59-4 |
| 6-Mercaptopurine monohydrate | Sigma-Aldrich | Cat# 852678-1G, CAS: 6112-76-1 |
| Acetic acid, glacial | Thermo Fisher Scientific | Cat# 695092, CAS: 64-09-7 |
| AMP Deaminase inhibitor, Cpd3 | Sigma-Aldrich | Cat# 533642 |
| Antimycin A | Sigma-Aldrich | Cat# A687 |
| BSA-Palmitate saturated fatty acid complex (5 mM) | Cayman Chemicals | Cat# 29558 |
| CGS-21680 hydrochloride hydrate | Sigma-Aldrich | Cat# C141, CAS: 124182-57-6 |
| Collagenase D | Sigma-Aldrich | Cat# 11088858001 |
| Complete Protease Inhibitor Cocktail | Roche | Cat# 11836170001 |
| D-Glucose-13C6 | Sigma-Aldrich | Cat# 389374, CAS: 110187-42-3 |
| ExtrAvidin-R-Phycerythrin | Sigma-Aldrich | Cat# E4011 |
| FCCP | Sigma-Aldrich | Cat# C2920 |
| Fixable viability dye eFluor450 | Thermo Fisher Scientific | Cat# 65-0863-14 |
| Glutaraldehyde solution | Sigma-Aldrich | Cat# G7651, CAS 111-30-8 |
| Guanine | Sigma-Aldrich | Cat# 51030, CAS: 635-39-2 |
| H-2D(b) Influenza A NP366-374 | NIH Tetramer Facility | N/A |
| CGS-21680 hydrochloride hydrate | Sigma-Aldrich | Cat# C141, CAS: 124182-57-6 |
| Collagenase D | Sigma-Aldrich | Cat# 11088858001 |
| Complete Protease Inhibitor Cocktail | Roche | Cat# 11836170001 |
| D-Glucose-13C6 | Sigma-Aldrich | Cat# 389374, CAS: 110187-42-3 |
| ExtrAvidin-R-Phycerythrin | Sigma-Aldrich | Cat# E4011 |
| FCCP | Sigma-Aldrich | Cat# C2920 |
| Fixable viability dye eFluor450 | Thermo Fisher Scientific | Cat# 65-0863-14 |
| Glutaraldehyde solution | Sigma-Aldrich | Cat# G7651, CAS 111-30-8 |
| Guanine | Sigma-Aldrich | Cat# 51030, CAS: 635-39-2 |
| H-2D(b) Influenza A NP366-374 | NIH Tetramer Facility | N/A |
| H-2D(b) Influenza A PA224-233 | NIH Tetramer Facility | N/A |
| H-2K(b) chicken OVA257-264 SIINFEKL | NIH Tetramer Facility | N/A |
| Hadacidin | SantaCruz Biotechnology | Cat# sc-490177, CAS: 689-13-4 |
| Hypoxanthine-13C5,15N4 | Cambridge Isotope Laboratories | Cat# CNLM-7894-0 |
| Inosine | Sigma-Aldrich | Cat# I4625, CAS: 58-63-9 |
| Inosine, 13C5 | Omicron Biochemicals | Cat# NUC-072 |
| Inosine, 15N4 | Cambridge Isotope Laboratories | Cat# NLM-4264-PK |
| L-aspartic acid-13C5,15N4 | Sigma-Aldrich | Cat# 607835, CAS 202468-27-7 |
| L-Glutamine-13C5,15N2 | Sigma-Aldrich | Cat# 607683 |
| L-malic acid-13C4 | Cambridge Isotope Laboratories | Cat# CLM-8065-0 |
| LPS (O111:B4) | Sigma-Aldrich | Cat# LPS25 |
| mFlt3L | Miltenyi Biotec | Cat# 130-097-372 |
| miL-15 | Miltenyi Biotec | Cat# 130-094-072 |
| miL-2 | Miltenyi Biotec | Cat# 130-094-054 |
| Mycophenolic acid | Sigma-Aldrich | Cat# MS255-50MG, CAS: 24280-93-1 |
| N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride | Sigma-Aldrich | Cat# E6383-5G |
| Oligomycin | Sigma-Aldrich | Cat# 75371, CAS: 579-13-5 |
| OVA257-264 SIINFEKL | Invivogen | Cat# vac-sin |

(Continued on next page)
| REAGENT or RESOURCE SOURCE | IDENTIFIER |
|-----------------------------|------------|
| OVA<sub>323-339</sub> ISQAVHAAHAEINEAGR Invivogen | Cat# vac-isq |
| Ovalbumin Sigma-Aldrich | Cat# A5503 |
| Ovalbumin, Alexa Fluor 488 Conjugate Thermo Fisher Scientific | Cat# O34781 |
| Ovalbumin, Alexa Fluor 647 Conjugate Thermo Fisher Scientific | Cat# O34784 |
| Palmitic acid Sigma-Aldrich | Cat# P0500, CAS 57-10-3 |
| Palmitic acid-<sup>13</sup>C<sub>16</sub> Sigma-Aldrich | Cat# 605573, CAS: 56599-85-0 |
| Paraformaldehyde Thermo Fisher Scientific | Cat# 28908, CAS: 3025-89-4 |
| Pierce BCA Protein Assay Kit Thermo Fisher Scientific | Cat#23225 |
| Poly-L-Lysine Sigma-Aldrich | Cat# P8920-100ML, CAS: 25988-63-0 |
| Psicofuranine Cayman Chemicals | Cat# 19574, CAS: 1874-54-0 |
| Pyridine Sigma-Aldrich | Cat# 270970-100ML |
| Rotenone Sigma-Aldrich | Cat# R8875, CAS: 83-79-4 |
| SCH 58261 Sigma-Aldrich | Cat# 54588, CAS: 160098-96-4 |
| Sodium α-ketobutyrate Sigma-Aldrich | Cat# K0875, CAS 2013-26-5 |
| Sodium α-ketobutyrate-<sup>13</sup>C<sub>4</sub> Cambridge Isotope Laboratories | Cat# CLM-6164-0.5, CAS: 2483736-24-7 |
| Sodium chloride Sigma-Aldrich | Cat# S9888, CAS 7647-14-5 |
| Sodium pyruvate Sigma-Aldrich | Cat# P5280, CAS 113-24-6 |
| Sodium pyruvate-<sup>13</sup>C<sub>3</sub> Sigma-Aldrich | Cat# 490717, CAS 142014-11-7 |
| SYTOX Blue Dead Cell Stain Thermo Fisher Scientific | Cat# S34857 |
| SYTOX Green Nucleic Acid Stain Thermo Fisher Scientific | Cat# S7020 |
| β-lactamase Sigma-Aldrich | Cat# P0389, CAS: 9073-60-3 |

**Critical commercial assays**

| Anti-PE MicroBeads Miltenyi Biotec | Cat# 130-048-80 |
| CD11c MicroBeads UltraPure Miltenyi Biotec | Cat# 130-108-338 |
| CD4<sup>+</sup> T Cell Isolation Kit, mouse Miltenyi Biotec | Cat# 130-104-454 |
| CD8<sup>+</sup> T Cell Isolation Kit, mouse Miltenyi Biotec | Cat# 130-104-075 |
| Foxp3 / Transcription Factor Staining Buffer Set Thermo Fisher Scientific | Cat# 00-5523-00 |
| IFNγ gamma Mouse ELISA kit Thermo Fisher Scientific | Cat# 88-7314-22; RRID: AB_2575066 |
| In situ Cell Death detection Kit, POD Sigma-Aldrich | Cat# 11684819710 |
| LEGENDplex Mouse Inflammation Panel Biolegend | Cat# 740150 |
| LiveBLAzer FRET-B/G Loading Kit with CCF4-AM Thermo Fisher Scientific | Cat# K1095 |
| Mouse Dendritic Cell Nucleofection Kit Lonza | Cat# VPA-1011 |
| Mouse Granzyme B DuoSet ELISA R&D Systems | Cat# DY1865 |
| Mouse IFN-α ELISA kit (TCM) PBL Assay Science | Cat# 42120-1 |
| Mouse IL-2 ELISA kit Thermo Fisher Scientific | Cat# 15530997 |
| pHrodo Red AM Intracellular pH Indicator Thermo Fisher Scientific | Cat# P53572 |
| Red Blood Cell Lysis Solution (10 x ) Miltenyi Biotec | Cat# 130-094-183 |
| RNeasy Mini Kit QIAGEN | Cat# 74104 |
| RNeasy Plus Micro Kit QIAGEN | Cat# 74034 |
| Thermo Fisher Scientific IL-12 p70 Mouse Uncoated ELISA Kit Thermo Fisher Scientific | Cat# 12384003 |
| TruSeq stranded mRNA library prep kit Illumina | Cat# 20020594 |

**Deposted data**

| RNA-Seq (Dendritic cell dataset) This paper | GEO: GSE126473 |
| RNA-Seq (T cell dataset) This paper | GEO: GSE147370 |

**Experimental models: Cell lines**

| LL2-ovalbumin (Kraman et al., 2010) | N/A |
| MEFs, bm1 T OVA (Sancho et al., 2009) | N/A |
## Continued

### REAGENT or RESOURCE SOURCE IDENTIFIER

| Experimental models: Organisms/strains | 
|----------------------------------------|
| **Mouse: Famin**<sup>−/−</sup> | (Cader et al., 2016) N/A |
| **Mouse: Famin**<sup>+/+</sup> | (Cader et al., 2016) N/A |
| **Mouse: Famin**<sup>p.254I</sup> | (Cader et al., 2016) N/A |
| **Mouse: Famin**<sup>p.254V</sup> | (Cader et al., 2016) N/A |
| **Mouse: Famin**<sup>p.284R</sup> | (Cader et al., 2016) N/A |
| **Mouse: Famin**<sup>WT</sup> (<sup>Famin</sup>fl/fl) | N/A N/A |
| **Mouse: Famin**<sup>D<sub>DC</sub></sup> (<sup>Famin</sup>fl/fl; Cd11c-Cre) | N/A N/A |

### Oligonucleotides

| ON-TARGETplus Mouse Adsl siRNA | Horizon Cat# L-064380-01 |
|---------------------------------|--------------------------|
| ON-TARGETplus Mouse Adss siRNA | Horizon Cat# L-060265-01 |
| ON-TARGETplus Mouse Ampd2 siRNA | Horizon Cat# L-063716-01 |
| ON-TARGETplus Mouse Ampd3 siRNA | Horizon Cat# L-042904-01 |
| ON-TARGETplus Mouse Gmpr siRNA | Horizon Cat# L-046519-01 |
| ON-TARGETplus Mouse Gmps siRNA | Horizon Cat# L-049796-01 |
| ON-TARGETplus Mouse Impdh1 siRNA | Horizon Cat# L-042235-01 |
| ON-TARGETplus Mouse Impdh2 siRNA | Horizon Cat# L-062809-01 |
| ON-TARGETplus Mouse Cpt1 siRNA | Horizon Cat# L-042456-01 |
| ON-TARGETplus Non-targeting Control Pool | Horizon Cat# D-001810-10 |
| Murine Adss F5′-CTGGCCACACAGTTGCGTA-3′; R5′-AAGCCTTTTTCGCCAGC-3′ | Thermo Fisher Scientific N/A |
| Murine Adss F5′-GGATCACACAGGTGGAGC-3′; R5′-TGTCACCCAGTGCTCTAA-3′ | Thermo Fisher Scientific N/A |
| Murine Ampd2 F5′-CTTCCCTGATTGGCCATCC-3′; R5′-CTTCTGGCAGTCTCCTGC-3′ | Thermo Fisher Scientific N/A |
| Murine Ampd3 F5′-CTGGCAACGGAATCTTGGAA –3′; R5′-GTGGCGGGAAGGTGTGTTG –3′ | Thermo Fisher Scientific N/A |
| Murine Cpt1a F5′-TGGCATTACACTCGTGTGTT-3′; R5′-GCTCAGGGCTCCAGTTT-3′ | Thermo Fisher Scientific N/A |
| Murine Gmrpr2 F5′-CAGCATCCACTAGTGGCAAGAG-3′; R5′-CGGTTAGGCCACATCAGG-3′ | Thermo Fisher Scientific N/A |
| Murine Gmps F5′-CTTGTGGCCAGTGTTGAGC-3′; R5′-CTTCTGGCAGTGAAGCTG-3′ | Thermo Fisher Scientific N/A |
| Murine Impdh1 F5′-GGCTACGTTCCCGAGGATG-3′; R5′-GGCTGATGTCAGGTCCACT-3′ | Thermo Fisher Scientific N/A |
| Murine Impdh2 F5′-CTTCTGTTGGGGATGAGGAG-3′; R5′-GCTCAGGGCTCCAGTTT-3′ | Thermo Fisher Scientific N/A |
| Murine Famin F5′-TGGGTTGCTCACTCCGCTG-3′; R5′-GGAGACTGCTGATTCTTGAGA-3′ | Thermo Fisher Scientific N/A |

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests for materials should be directed to and will be fulfilled by the Lead Contact, Arthur Kaser (ak729@cam.ac.uk).

Materials availability
Unique resources generated in this study are available on reasonable request, although may require completion of a Materials Transfer Agreement.

Data and code availability
- RNA Sequencing datasets generated in this study have been deposited at the Gene Expression Omnibus (GSE126473 and GSE147370) and are publicly available as of the date of publication.
- This paper does not report original code.
- Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon reasonable request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice
Age-, gender-, and, whenever possible, littermate-matched 6- to 11-week-old mice were used for all experiments. Mice were housed in a 19-21°C environment on a 12 h light/ dark cycle. Health status screening was performed every three months using sentinel mice. Mice were fed on a universal maintenance chow diet purchased from Safe (Safe 105). Famin+/+, Famin−/−, Faminp.254i, Faminp.254V
and Famin\textsuperscript{p.284R} mice, generated on a C57BL/6NTac background, have previously been described (Cader et al., 2016). The loxp-flanked conditional allele (Famin\textsuperscript{tm1a(KOMP)}) allele was generated by converting mice with homologous recombination of the tm1a(KOMP) Wtsi construct (Cader et al., 2016) with a FipO recombinase-transgenic mouse. Famin\textsuperscript{lox/lox} mice with Itgax-Cre (CD11c-Cre) mice (B6.Cg-Tg(Itgax-cre)1-1Reiz/J, on a C57BL/6 background). Itgax-Cre:Famin\textsuperscript{tm1a(KOMP)} mice and their controls were born at Mendelian ratio and developed normally, consistent with what we previously reported for the other Famin mutant mice used in this study (Cader et al., 2016). None of these mice develop spontaneous autoimmunity or autoinflammation under specific pathogen-free conditions. OT-I;Rag\textsuperscript{-/-} (‘OT-I’) and OT-II;Ptprca\textsuperscript{-/-};Ptprcb\textsuperscript{-/-} (‘OT-II’) mice on a C57BL/6 background have previously been described (Barden et al., 1998; Hogquist et al., 1994). Maintenance and breeding under specific pathogen-free conditions was performed at the Central Biomedical Services facility or Phenomics Laboratory, University of Cambridge. UK Home Office and local ethics approval has been granted for all experimental procedures.

**Dendritic cell and T cell isolation**

DCs were isolated from spleens digested in 1 mg/mL collagenase D (Sigma, 11088858001) in complete media in the presence of DNase (Sigma, D4263). Positive selection for CD11c\textsuperscript{+} DCs was performed using CD11c UltraPure beads following the manufacturer’s protocol (Miltenyi, 130-100-875). BM-derived DCs were isolated from murine tibias and femurs by flushing in complete RPMI-1640 medium, filtering through a 70 \( \mu \)m cell strainer, lysing red blood cells using red blood cell lysis solution (Miltenyi, 130-094-183) in accordance with manufacturer’s instructions, and subsequent resuspension in complete RPMI-1640 medium (containing 100 U/mL of penicillin-streptomycin, 10 mM HEPES buffer and 10% FBS) followed by culture for 9 days in mFlt3L (100 ng/mL, Miltenyi, 130-097-372), replenished on days 3 and 6. After 9 days in culture, isolation of BM-derived DCs into cDC1 and cDC2 subsets was performed using a protocol adapted from (Zelenay et al., 2012). Briefly, 9-day Flt3L-expanded BM-derived cDC2s were isolated by positive selection using SIRP\textsuperscript{α}-PE antibody (Biolegend, 144011) and anti-PE microbeads (Miltenyi, 130-048-80), followed by negative selection of cDC1 through depletion of plasmacytoid DCs with anti-B220-PE antibody (Biolegend, 103207) and anti-PE microbeads (Miltenyi, 130-048-80). For maturation prior to all subsequent analysis, splenic DCs were rested overnight, while BM-derived cDC1s or cDC2s were treated with 1 \( \mu \)M LPS (O111:B4, Sigma, LPS25) for 18 h prior to pulsing with antigen. Mature DCs were pulsed for 30 min with 1 \( \mu \)g/mL LPS (O111:B4, Sigma, LPS25) for 18 h. All treatments of splenic DCs or BM-derived cDC1s were performed during this maturation step unless specified otherwise. Inhibitors were used at the following final concentrations: 25 \( \mu \)M (Figures 4A and S4A) or 100 \( \mu \)M (Figure S5C)-L-alanosine (Cayman Chemicals, 19545), 50 \( \mu \)M hadacardin (SantaCruz, sc-490177), 5 \( \mu \)M 6-mercaptopurine (Sigma, 852678-1G-A), 5 \( \mu \)M Cpd3 (Sigma, 533642), 0.8 (Figures 4H and 5B) or 1 \( \mu \)M (Figures 5D, 5E, and 7F) mycophenolic acid (Sigma, M5255-50MG), 100 \( \mu \)M psicofuranine (Cayman Chemicals, 19574), Guanine was used at 100 \( \mu \)M (Sigma, 51030); pyruvate (Sigma, PS280) and \( \alpha \)-ketobutyrate (Sigma, K0875) were used at 1 mM.

T cells isolated from spleens and lymph nodes of OT-I;Rag\textsuperscript{-/-};Rag2\textsuperscript{-/-} and OT-II;Ptprca\textsuperscript{-/-};Ptprcb\textsuperscript{-/-} mice were purified using negative selection for CD8\textsuperscript{+} or CD4\textsuperscript{+} T cells (Miltenyi, 130-104-075 and 130-104-454). Purity (80%–90%) was confirmed by flow cytometry.

Either male or female mice were taken for DC/ T cell isolation – each individual experiment was gender-matched. All cells were cultured at 37°C, 5% CO\textsubscript{2}.

**METHOD DETAILS**

**Influenza infection**

Isoflurane anesthetized 6-8 week-old female mice were infected by intranasal inoculation with 10\textsuperscript{4} plaque forming units (PFU) Influenza A virus H3N2 A/X-31 strain in 50 \( \mu \)L of sterile PBS. Disease activity was scored in a blinded to genotype and group allocation, using the following scoring system: 5 – healthy mouse; 4 – mouse is hunched, looks hypothermic; 3 – ruffled fur, squinting eyes; 2 – mouse is hunched; 1 – mouse looks lethargic. LEGENDplex Mouse Inflammation Panel (Biolegend, 740150) was used to determine cytokine levels in plasma according to manufacturer’s protocol and fluorescent signals assessed by flow cytometry. Absolute quantification was performed using standard curves generated in the assay. For analysis of influenza-specific T cells bronchoalveolar lavage with cold PBS was performed. Influenza viral load was determined in total RNA extracted from lungs (RNeasy kit, QIAGEN, 74104), via qRT-PCR using primers for influenza M protein transcript, 5′-GGACTGCAGCTTAGACGCTT-3′ and 5′-CATCCTGTGTTATATGAGGCCCAT-3′ (Prabhu et al., 2013). Influenza M protein mRNA levels were normalized to mActb (5′-GATGCTCCCGGGGTGTATT-3′ and 5′-GGGTACTCGGGTCAGG-3′). Terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) was performed on formalin-fixed paraffin embedded sections of lungs using the In Situ Cell Death Detection Kit, POD (Roche, 11684817910) following manufacturer’s instructions. An Olympus CX31 microscope with Nikon DS-Fi2 camera attachment was used to analyze the sections.

**In vitro T cell priming and restimulation**

Spleenic DCs were rested overnight in normal media, and BM-derived cDC1 or cDC2s were activated with 1 \( \mu \)g/mL LPS (O111:B4, Sigma, LPS25) for 24 h to pulse with antigen. Mature DCs were pulsed for 30 min with 1 \( \mu \)g/mL (spleen DC11c\textsuperscript{+} DCs) or 0.5 \( \mu \)g/mL (cDC1 and cDC2 BM-derived DCs) OVA\textsuperscript{257-264} peptide (SIINFEKIL) (Invivogen, vac-sin), 1 \( \mu \)g/mL OVA\textsuperscript{323-339} peptide (ISOAVAHAAHAEINEAQR) (Invivogen, vac-isoq), 1 mg/mL of ovalbumin (Sigma, A5503), or UV-irradiated bm1T-ova MEFs (Sancho et al., 2009) as indicated. OT-I and OT-II cells were labeled with carboxyfluorescein succinimidyl ester (CFSE; Molecular Probes, C1157) according to manufacturer’s protocol to co-culture with antigen-pulsed DCs. OT-I cells and DCs were co-cultured for 72 h at a ratio of 5:1 for cDC1 and splenic DCs; OT-II cells and DCs were co-cultured at 2:1 for 96 h. For differentiation into T\textsubscript{E}...
and T<sub>EM</sub> cells 72 h after priming, OT-I cells were passaged for 5 days with mIL-2 (5,000 IU/mL, Miltenyi, 130-094-055) or mIL-15 (100 IU/mL, Miltenyi, 130-094-072), respectively (Manjunath et al., 2001). Primed OT-I cells were re-seeded at the indicated time-points at 10<sup>6</sup> cells/mL, restimulated for 5 h with plate-bound anti-CD3 (5 µg/mL, Thermo Fisher Scientific, 16-0037-81) and soluble anti-CD28 (2 µg/mL, Thermo Fisher Scientific, 16-0289-81), and supernatants were then analyzed for IFN<sub>γ</sub> release. For analysis of intracellular cytokines, OT-II cells were rested for 3 days following 96 h co-culture with DCs prior to restimulation for 5 h with 1 µg/mL OVA<sub>257-264</sub> or anti-CD3/CD28. For co-culture experiments using fixed dendritic cells, mature splenic DCs were pre-loaded with 1 µg/mL of OVA<sub>257-264</sub> for 30 min and fixed for 10 min in 0.01 or 0.05% of glutaraldehyde solution (Sigma, G7651). Following 2 washes in PBS, CFSE-labeled OT-I T cells were added at 5:1 ratio and co-cultured for 72 h.

**Cytotoxicity assays**

Cytotoxicity was measured as previously described (Noto et al., 2013). Briefly, after 72 h of priming, OT-I T cells were passaged twice at 48 h intervals in media containing 5,000 IU/mL mIL-2. Effector T cells were then combined with target cells at the indicated ratios for 5 h at 37°C. Target cells were splenocytes from wild-type mice pulsed with 1 µg/mL of OVA<sub>257-264</sub> for 30 min and labeled with 5 µM of CFSE (CFSE<sub>high</sub>), which were combined at 1:1 ratio with control splenocytes labeled with 0.5 µM CFSE (CFSE<sub>low</sub>). Specific lysis was equal to 100 – ((CFSE<sub>high</sub>/CFSE<sub>low</sub>) in the presence of cytotoxic CD8 T cells / (CFSE<sub>high</sub>/CFSE<sub>low</sub>) in the absence of cytotoxic CD8 T cells) x 100. Supernatants were harvested for cytokine analysis by ELISA.

**In vivo T cell priming**

CD<sup>8</sup> T cells and CD<sup>4</sup> T cells were isolated from spleens and lymph nodes of OT-I;Rag<sup>−/−</sup> and OT-II;Ptprca;Rag2<sup>−/−</sup> mice, respectively. Following labeling with 5 µM of CFSE, 5 x 10<sup>6</sup> T cells/mouse were intravenously injected into Famin<sup>ADC</sup> mice and their controls. 24 h later, mice were immunized with 25 µg ovalbumin intraperitoneally. Three days later, proliferation indices of splenic T cells were calculated based on CFSE dilution and, in the case of OT-I cells, CTL activity of total splenocytes analyzed as described above.

**Flow cytometry**

Cells were blocked with purified anti-CD16/CD32 antibody (Biolegend, 101302) for 30 min on ice and stained with corresponding antibodies for cell surface molecules. For intracellular staining, samples were fixed with 2% paraformaldehyde (Thermo Fisher Scientific, 28908), permeabilized using Wash/Perm buffer (BD Biosciences, 554723), and stained for intracellular cytokines using standard protocols; FOXP3 was stained with Mouse Foxp3 buffer set (Biolegend, 560409) per manufacturer’s protocol. Peptide-tetramer staining was analyzed in whole blood obtained through tail bleeds, with antibodies and peptide-loaded tetramers added directly to blood, and samples diluted with buffer prior to analysis by flow cytometry. Monomers for H-2D<sup>β</sup> Influenza A NP<sub>366-374</sub> (ASNENMETM), H-2D<sup>β</sup> Influenza A PA<sub>224-233</sub> (SSLENFRAYV), and H-2K<sup>β</sup> OVA<sub>257-264</sub> (SIINFEKL) were obtained from the NIH Tetramer Core Facility and tetramers prepared using Extravidin–PE (Sigma, E4011) as per standard protocol. Single cell fluorescence was analyzed on BD LSRFortessa or Attune NxT flow cytometers. Data analysis was performed using FlowJo software v8/v10; gating strategies for experiments are listed in Table S7.

**Cytokine measurement by ELISA**

Supernatants from experiments were analyzed using ELISA according to the manufacturer’s instructions (IFN<sub>γ</sub>, Thermo Fisher Scientific, 15501107; IFNα, PBL Assay Science, 42120-2; IL-2, Thermo Fisher Scientific, 15133787; IL-12p70, Thermo Fisher Scientific, 12384003; granzyme B, R&D Systems, DY1865).

**RNA extraction and sequencing**

RNeasy Mini Kit (QIAGEN, 74104) was used to extract RNA samples and were quantified with a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific). RNA quality was assessed using the Agilent 2200 or 2100 TapeStation system (Agilent Technologies). Libraries were prepared using TruSeq stranded mRNA library prep kit (Illumina, 20020594) in accordance with the manufacturer’s instructions. Sequencing of libraries was performed using an Illumina NextSeq 500 platform with NextSeq 500-Mid Output kit generating 1x75 bp end reads (T cell dataset GSE126473) or 2x150bp end reads (dendritic cell dataset GSE147370). FastQ files were quality-checked (FastQC; http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and any residual adaptor sequences were removed (TrimGalore; http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). Reads were subsequently aligned to the appropriate reference genome (mm10, UCSC for cDCs, Ensembl Mus_musculus.GRCm38 for T cells) using HISAT2 (Kim et al., 2015). RNA-Seq data generated as part of this study can be accessed at the Gene Expression Omnibus (GEO: GSE126473, GSE147370).

**Immunoblot**

6 x 10<sup>6</sup> BM-derived cDC1s were washed once in ice-cold PBS and lysed in ice-cold RIPA buffer supplemented with protease inhibitors (Complete Protease Inhibitor Cocktail, Roche). After lysing for 15 min on ice, cell debris was removed by centrifugation for 15 min at 4°C. Protein levels were quantified using the Pierce BCA Protein Assay Kit (Thermo Scientific) and samples were normalized.
to protein content before addition of 4X laemml buffer (Bio-Rad) and boiling at 95°C for 5 min. Samples were run on a 10% SDS-PAGE gel. Proteins were transferred to a nitrocellulose membrane using a Trans-Blot Turbo transfer system before blocking for 1 h at room temperature in 5% milk in TBS-T. Membranes were incubated with primary antibodies overnight at 4°C in 5% milk in TBS-T. These were detected by incubation with HRP-conjugated secondary antibodies for 1 h at room temperature and visualized using 20X LumiGlo reagent (Cell Signaling).

Metabolic tracing experiments

For metabolic tracing experiments using BM-derived cDC1s, cells were isolated and stimulated for 18 h with 1 μg/mL LPS as described above. Following this, cells were incubated for 3 h with 300 μM [13C4] malic acid (Cambridge Isotope Laboratories, CLM-8065-0), 100 μM [13C14] palmitic acid (Sigma-Aldrich, P0500), 1 mM [13C3] sodium pyruvate (Sigma-Aldrich, 490717), 1 mM [13C4] α-ketobutyrate (Cambridge Isotope Laboratories, CLM-6164) or 150 μM L-aspartic acid-[13C4, 15N1] (Sigma-Aldrich, 207244) supplemented into complete RPMI-1640 medium as applicable. In glutamine labeling experiments, BM-derived cDC1s were incubated for 3 h with 2 mM [15N2, 13C6] glutamine (Sigma-Aldrich, 607983), added into RPMI glutamine free medium. For glutamine labeling experiments, BM-derived cDC1s were pulsed for 1 h with 2 g/L [13C6] glucose (Sigma-Aldrich, 389374) supplemented into RPMI glucose free medium. For metabolic tracing experiments utilizing splenic CD11c+ DCs, cells were isolated and rested overnight before pre-equilibration in OptiMEM for 3 h and subsequent addition of [13C6, 15N4] hypoxanthine (Cambridge Isotope Laboratories, CNLM-7894-0) to a final concentration of 25 μM for 3 h prior to harvesting of supernatants and cell extracts.

For measurements of systemic inosine levels plasma was collected by cardiac puncture followed by centrifugation in EDTA-coated tubes for 15 min at 4°C at 2000 g. 20 μL aliquots were taken and prepared for LC-MS by addition of 100 μL 4:1 methanol:water followed by vortexing and centrifugation at 20 000 g. The supernatants were then dried using a centrifugal evaporator (Savant, ThermoFisher). Samples were reconstituted in 100 μL ammonium acetate containing 2 μM [13C10, 15N6] adenosine monophosphate and adenosine triphosphate, 10 μM [13C4] succinic acid, 1 in 5000 diluted [U-13C, U-15N] mixture of amino acids (all purchased from Sigma Aldrich) and 50 nM [13C6] inosine (Cambridge Isotope Laboratories) as internal standards. Faminp.254e, Faminp.254v and Faminp.284r mice were fasted for 18 h prior to harvesting.

Extraction of aqueous metabolites

After washing with PBS or 162 mM ammonium acetate adjusted to pH 7.4 (as appropriate), cell pellets were then extracted using the 2:1 chloroform:methanol method described by Folch (Folch et al., 1957) with modifications to the method as previously detailed (Cader et al., 2020). All solvents used were HPLC or LC-MS grade and obtained from Fisher Scientific. Aqueous extracts were stored at −80°C prior to analysis.

LC-MS sample preparation

Aqueous extracts of cells were dried using a centrifugal evaporator (Savant, ThermoFisher) and reconstituted in 10 mM ammonium acetate containing 2 μM [13C10, 15N6] adenosine monophosphate and adenosine triphosphate, 10 μM [13C4] succinic acid, and a 1 in 5000 diluted [U-13C, U-15N] mixture of amino acids (all purchased from Sigma Aldrich) and internal standards. Where appropriate, internal standards were omitted during isotopic labeling experiments to prevent contamination with labeled substrates. The samples were then vortexed and sonicated for 5 min, followed by brief pulsed centrifugation to recover maximum volume.

Molecular formula determination using accurate mass and isotopic mass distribution, confirmed by authentic standard, were used to validate identification of inosine as the top-ranking identifiable LC-MS feature of differential abundance between supernatants of Famin+/− and Famin−/− splenic DCs. For analysis of cell culture supernatants in subsequent experiments, 20 μL of supernatant was aliquoted directly onto a styrene 96 well plate (Corning) followed by dilution with 100 μL of 10 mM ammonium acetate containing 50 nM [13C6] inosine (Omicron Biochemicals) or 50 nM [15N4] inosine (Cambridge Isotope Laboratories) as an internal standard. Where appropriate, the internal standard was omitted. For absolute quantitation of both labeled and unlabeled inosine, an inosine calibration line was prepared in the appropriate cell culture medium in the following concentrations: 100 pM, 1 nM, 5 nM, 10 nM, 50 nM, 100 nM and 1 μM. These calibrators were then subjected to the same dilution and preparation described above. All plates were sealed with a pre-slit silicone sealing mat prior to injection (Thermo Fisher Scientific).

LC-MS analysis of aqueous metabolites

A Q Exactive Plus orbitrap coupled to a Vanquish Horizon ultra high performance liquid chromatography system was used for all the analysis. LC-MS methodology used corresponds to the ACE C18-PFP and the Phenomenex Gemini-NX protocols described previously (Cader et al., 2020), utilizing identical chromatographic and MS parameters. The majority of analyses (for example detection of nucleotides, nucleosides and organic acids etc.) was carried out using the ACE C18-PFP column and, where appropriate, nucleoside phosphates were measured on a BEH amide HILIC column as detailed in Cader et al. (2020). For analysis of supernatants, where sensitivity was critical, 10 μL was injected with the first minute of chromatography being switched to waste to prevent build-up of matrix containing contaminants in the source of the mass spectrometer.

All solvents and additives used were LC-MS or Optima grade and obtained from Fisher Scientific or Merck.
Hydrazone derivatization of keto acids and hydroxy carboxylic acids in cell culture supernatants and subsequent LC-MS analysis

An internal standard solution was prepared by extracting 100 mg of U^{13}C lyophilized algae (Sigma) using the Folch extraction described above. Supernatants were dried using a centrifugal evaporator (Savant, Thermo Fisher) and derivatised according to a modified version of the protocol previously described (Han et al., 2013). Briefly, 50 μL of 75% aqueous methanol was added to the dried culture medium followed by 10 μL of the internal standard mix. To this mixture, 30 μL of 250 mM 3-nitrophenylhydrazine (in 50% aqueous methanol), 30 μL of 150 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (in methanol) and 30 μL of 7.5% pyridine (in 75% aqueous methanol) were added sequentially. The resulting mixture was vortexed and samples allowed to derivatise for 1 h on ice. Samples were subsequently quenched with 5 mg/mL butylated hydroxytoluene and 420 μL of water and centrifuged to pellet any salts from the media.

The LC gradient employed for the separation of the hydrazone derivatives utilized a binary solvent mixture consisting of mobile phase A, 0.1% formic acid in water and B, 0.1% formic acid in methanol and the column was an Acquity CSH C18 (100 x 2.1mm, 1.7 μm). The gradient program was as follows: 18% B was increased to 90% B in a linear gradient over 6.75 min, held at 90% for a further minute followed by re-equilibration for 1 min to give a total run time of 9 min. The flow rate was 400 μL/min and the column oven temperature was 40 °C. The injection volume was 5 μL. To prevent derivatisation reagents from entering the ion source, a switch was employed for the first 2 min of the gradient program. Samples were run in negative ion mode using MS parameters previously described (Cader et al., 2020).

LC-MS data processing

All data were acquired using Xcalibur (Version 4.1, Thermo Fisher Scientific). Targeted processing was carried out using Xcalibur and unbiased analysis using Compound Discoverer (Version 2.1 or Version 3.1, Thermo Fisher Scientific). Untargeted analysis utilized data from both positive and negative ionization modes. Chromatogram peaks for each differential metabolite were manually verified using Xcalibur (Version 4.1, Thermo Fisher Scientific) and identities validated using the high-resolution m/z METLIN database (Scripps Research Institute). To confirm identification of inosine and in cases of ambiguity, compound retention times were validated against known external standard solutions. For all cellular and serum metabolite analysis, target peak areas corresponding to metabolites were normalized to total ion content unless otherwise indicated. For absolute quantitation of inosine in supernatants, normalization of target peaks was performed with reference to internal standards, and quantitation performed with reference to a calibration line between 10 pM and 1 μM prepared in the appropriate sample matrix. Relative quantitation of metabolite levels in supernatant tracing studies were not normalized.

All sample data were processed using Compound Discoverer (Version 2.1 and Version 3.1, Thermo Fisher Scientific) to accurately calculate total ion content for use as a normalization factor. For labeling studies, incorporation into specific compounds was determined by accurate mass shift of +1.0034 and +0.9970 for 13C and 15N respectively. Endogenous levels of 13C and 15N compounds of interest were determined by reference to control samples pulsed with unlabelled compounds of investigation, and endogenous levels subtracted from quantified isotopomers in the labeled samples as applicable.

Determination of extracellular acidification rate and oxygen consumption rate

Bone marrow-derived cDC1 stimulated overnight with LPS (O111:B4, Sigma-Aldrich, LPS25), or isolated splenic DCs matured overnight, were seeded prior to analysis at 3 × 10^5 cells per well on Poly-L-lysine-coated plates (Sigma-Aldrich, P8920), as indicated. Cells were then washed twice and incubated for 1 h in XF assay medium (unbuffered DMEM pH 7.4 with 10 mM glucose, 100 μM sodium palmitate and 2 mM L-glutamine) in a non-CO2 incubator at 37 °C as per manufacturer’s instructions (Agilent Technologies). Measurements of extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were determined using an XF-96 Extracellular Flux Analyzer (Agilent Technologies). Serial measurements were obtained under basal conditions and following addition of 1 μM oligomycin (Sigma-Aldrich, 75371), 1.5 μM FCCP (Sigma-Aldrich, C2920) and 100 nM rotenone with 1 μM antimycin A (Sigma-Aldrich, R8875 and A867). For determination of glycolysis, ECAR measurements were obtained under basal conditions.

Cytoplasmic pH assay

Intracellular pH was compared using pHrodo Red AM (Thermo Fisher Scientific, P35372) fluorogenic probe for measurement of cytoplasmic pH according to manufacturer’s protocol. In brief, splenic DCs were matured overnight and incubated with 5 μM pHrodo for 30 min at 37 °C in a non-CO2 incubator in Hank’s Balanced Salt Solution (HBSS) and washed once before fluorescence signal was measured using a microplate reader (Tecan infinite M1000, Tecan Group or CLARIOstar plus, BMG Labtech) with an excitation/emission of 560/580nm.

Antigen uptake assay

LPS-treated cDC1s or splenic DCs were incubated with 0.05 mg/mL of OVA-AF647 (Thermo Fisher Scientific O34784) in HBSS containing HEPES at 37 °C in a non-CO2 incubator for 30 min, while control cells were kept on ice to account for passive diffusion. Following washes in ice cold HBSS, samples were prepared for analysis by flow cytometry as described above. For analysis of splenic cDC1s and cDC2s cells were gated on CD11c+MHC II+CD8+CD11b+ and CD11c+MHC II+CD11b+CD64-, respectively.
Endosome-to-cytosol uptake assay
Assay was performed as previously described (Cebrian et al., 2011), with some modifications using the LiveBLAzer FRET-B/G Loading Kit with CCF4-AM (Thermo Fisher Scientific, K1095). LPS-treated BM-derived DCs were loaded with 1 μM of CCF4-AM at room temperature, followed by incubation with 2 mg/mL β-lactamase (Sigma, P0389) for the indicated time. Cells were subsequently analyzed by flow cytometry, and blue-to-green (excitation 405 nm, emission 450 nm/525 nm). FRET ratio was used as an indicator for efficiency of antigen export into the cytosol. Response ratios were calculated as per manufacturer’s instructions and normalized to the signal intensity of control cells, which had been incubated on ice.

MHC I recycling assay
The rate of MHC I recycling was assessed as previously described (Belabed et al., 2020). LPS-treated cDC1s were incubated in the presence of 1 mM sodium α-ketobutyrate (Sigma-Aldrich, K0875), or control for 18 h. Cells were blocked with anti-CD16/CD32 antibody (Biolegend, 101302) and subsequently incubated with anti-H-2Kβ-FITC (Biolegend, 114605) for 30 min on ice. To enable internalisation, cells were incubated for 30 min at 37°C in complete RPMI-1640 medium with 1 mM sodium α-ketobutyrate replenished as applicable. After washing in 1% BSA-PBS, cells were subsequently incubated in stripping buffer (0.5 M NaCl, 0.5% acetic acid, pH 3.0) for 10 min on ice. After washing with ice cold PBS, cells were fixed in 2% paraformaldehyde (to determine basal MHC I staining after internalisation step) or re-incubated in complete pre-warmed RPMI-1640 medium (with 1 mM sodium α-ketobutyrate replenished if applicable) for 15 min or 30 min to allow MHC I recycling. After re-incubation, cells were incubated in stripping buffer for 10 min on ice, washed in ice cold PBS and fixed in 2% paraformaldehyde followed by analysis by flow cytometry. The difference in mean fluorescence intensity after re-incubation was determined to calculate the % MHC I recycled at each time point as described previously.

siRNA transfection
Freshly isolated splenic CD11c+ DCs were transfected with 30 pmol/sample of siRNA (purchased from Dharmacon, Horizon Discovery) using a Mouse Dendritic Cell Nucleofector Kit (Lonza, VVPA-1011) and Nucleofector 2b. Cells were rested for 36-48 h prior to assays (antigen uptake or co-culture). RNA for knockdown validation was extracted using RNeasy Plus Micro Kit (QIAGEN), reverse transcribed using M-MLV Reverse Transcriptase (Thermo Fisher) and SYBR Green Q-PCR (Eurogentec) was performed using QuantStudio 7 Flex (Thermo Fisher). For primer sequences see Key resources table.

T cell activation assays in presence of DC-secreted soluble factor
Splenic CD11c+ DCs were rested overnight in RPMI, and on the next day cultured in OptiMEM or RPMI for 3 h. Cell-free supernatants were harvested and frozen immediately or centrifuged using 3 kDa cut-off spin columns. OT-I or OT-II T cells isolated from spleens and LNs were seeded at 10⁵ cells/mL and stimulated for 72 h with plate-bound anti-CD3 (5 μg/mL, 16-0037-81, Thermo Fisher Scientific) and soluble anti-CD28 (2 μg/mL, 16-0289-81, Thermo Fisher Scientific) in presence of supernatants harvested from DCs, soluble inosine with or without 100 nM SCH 58261 (Sigma Aldrich, S4568) or 0.5 μM CGS-21680 (Sigma Aldrich, C141). For T cell RNA-seq experiments, naive OT-I T cells were cultured in the presence of 2 h splenic DC supernatant and stimulated for 24 h with anti-CD3/CD28 prior to RNA extraction.

Tumor xenograft model
2.5 × 10⁴ LL2-OVA cells (for experiments comparing Famin^{p.254I}, Famin^{p.254V}, Famin^{p.284R} mice) or 2 × 10⁴ LL2-OVA cells (for experiment comparing Famin^{p.254V} and Famin^{h.284R} mice) in PBS were subcutaneously injected into the left flank of gender- and age-matched mice of 6-10 weeks of age. Tumor growth was assessed at least every other day in a fully blinded fashion, using a caliper for both the long (L) and short (S) dimensions, and tumor volume calculated using the equation volume = (L × S²)/2.

QUANTIFICATION AND STATISTICAL ANALYSIS
Statistical analyses were performed using Graphpad Prism 6.0 /8 /9 or, and as described in LC-MS analysis methods, Compound Discoverer 2.1 / Compound Discoverer 3.1 (Thermo Scientific). Unless otherwise stated, statistical significance was calculated as appropriate using unpaired, two-tailed Student’s t test or ordinary one-way ANOVA and Tukey post hoc test as described in the figure legends. Formal statistical determination of whether the data met assumptions of the approach was not undertaken. Grubbs’ test was used to identify outliers within datasets. Where indicated, FDR-adjusted p values were calculated using the Benjamini-Hochberg procedure. All in vivo experiments were performed in a blinded manner. Data are represented as mean and standard error of the mean (SEM). A P value of < 0.05 was considered significant.