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# Supplementary Materials for

## Chemical proteomic map of dimethyl fumarate–sensitive cysteines in primary human T cells

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**The PDF file includes:**



**Other Supplementary Material for this manuscript includes the following:**
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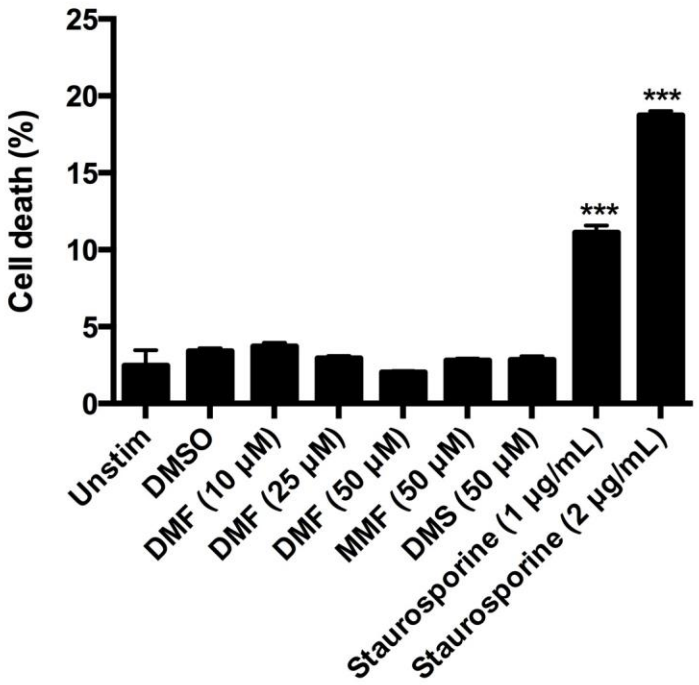


**Fig. S1. DMF does not affect T cell viability.** Primary human T cells were left unstimulated or were stimulated with anti-CD3/CD28 antibodies in the presence of the indicated compounds for 8 hours. The cells were then stained with LIVE/DEAD fixable blue stain and analyzed by flow cytometry to determine the percentages of gated $CD4^+$ T cells. The cytotoxic drug staurosporine was used as a positive control to reduce T cell viability. Data are means ± SEM of five replicates per condition. $^{**}P < 0.01$ by two-tailed, unpaired *t* test in comparison to the DMSO-treated cells.

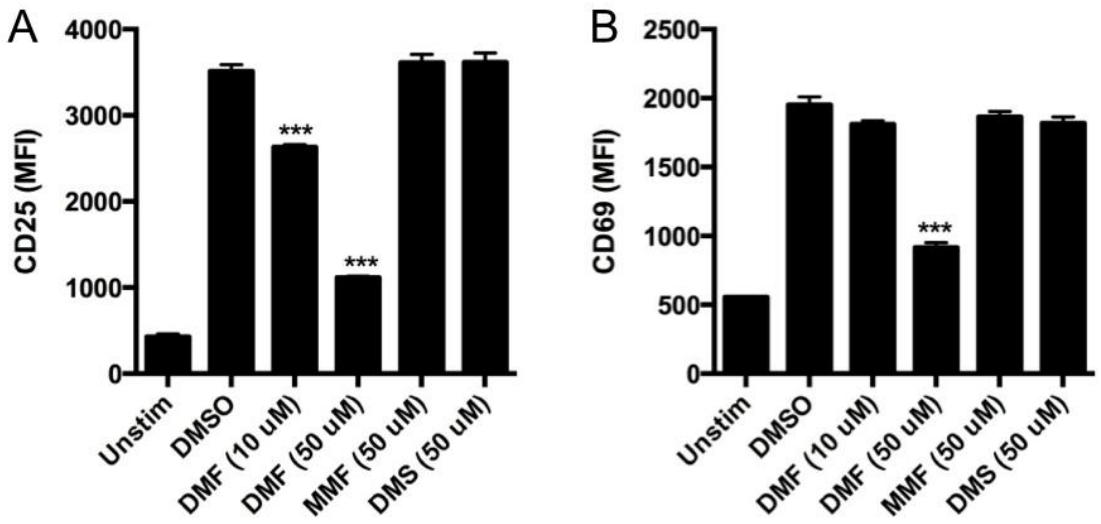


**Fig. S2. DMF, but not MMF, inhibits the activation of primary mouse T cells.** (**A** and **B**) Splenic T cells from C57BL/6 mice were left unstimulated (Unstim) or were stimulated (Stim) with anti-CD3/CD28 antibodies in the presence of DMSO or the indicated concentrations of DMF, MMF, and DMS for 8 hours. Activation was assessed by flow cytometric analysis of the cell-surface abundance of CD25 (A) and CD69 (B), which are presented as MFIs. Data are means ± SEM of four experiments per group. ***$P < 0.001$ by two-tailed, unpaired *t* test in comparison to the DMSO-treated cells.

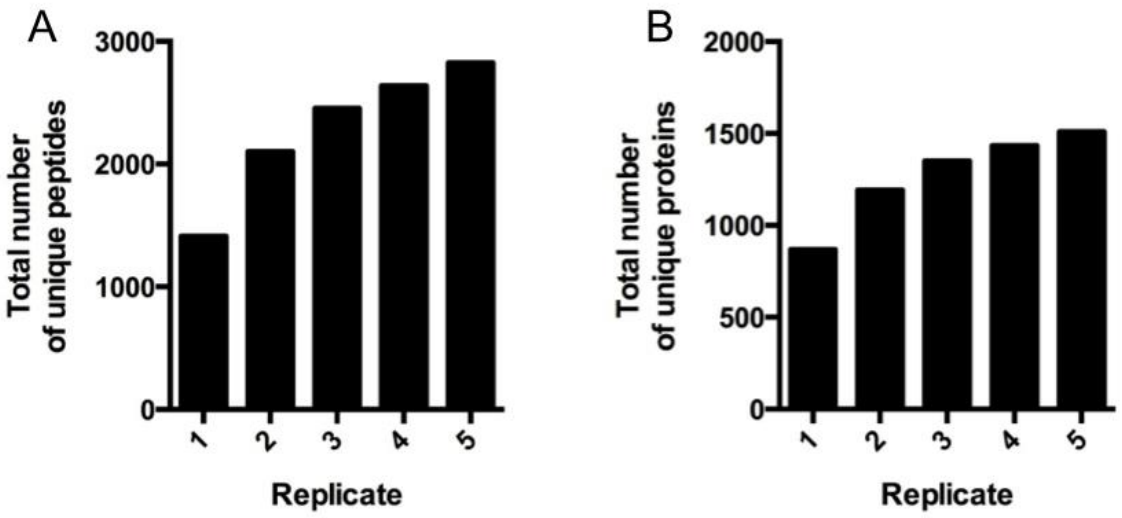


**Fig. S3. The cumulative number of unique quantified peptides and proteins after five biological replicates of isoTOP-ABPP.** (**A** and **B**) The total numbers of unique quantified peptides (A) and proteins (B) began to plateau after five biological replicates of the isoTOP-ABPP experiment in primary human T cells treated with 50 μM DMF for 4 hours.

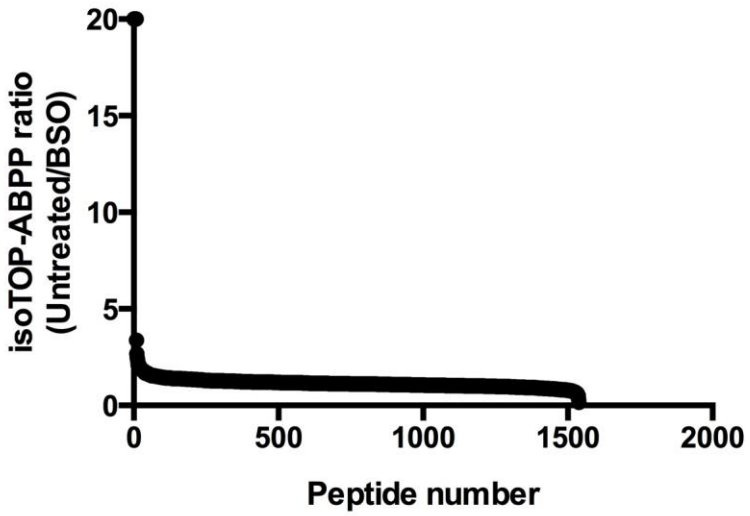


**Fig. S4. Analysis of BSO-treated primary human T cells by isoTOP-ABPP.** Primary human T cells were treated with 2.5 mM BSO for 4 hours and then were analyzed by isoTOP-ABPP. Data represent aggregate quantified cysteine residues from two isoTOP-ABPP experiments.

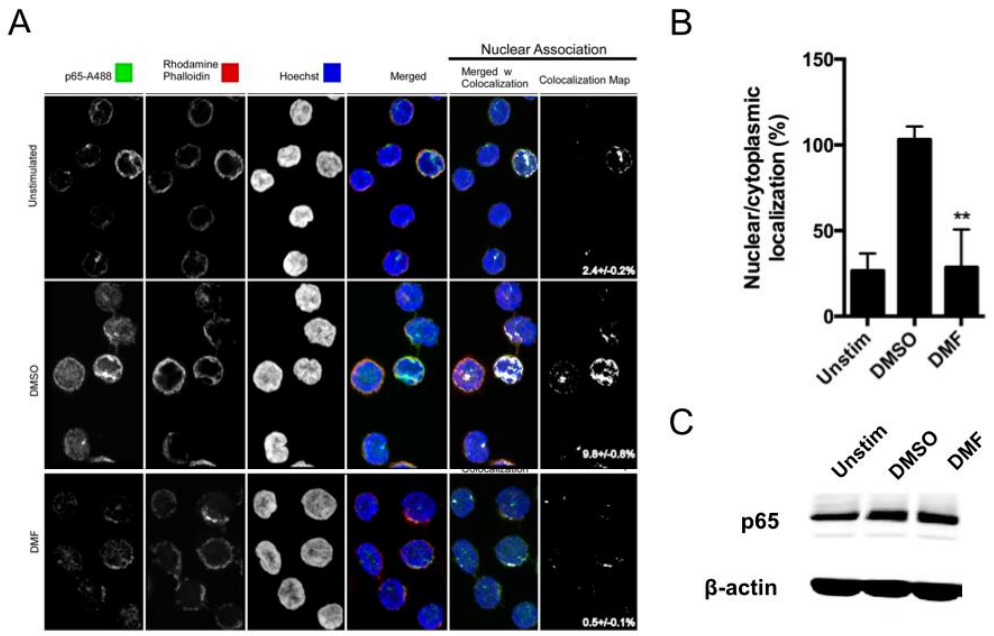


**Fig. S5. DMF inhibits the translocation of NF-κB–p65 to the nucleus in primary human T cells.** (**A**) Primary human T cells were left unstimulated or were stimulated with anti-CD3/CD28 antibodies in the presence of DMSO or 50 μM DMF for 1 hour. Shown are maximum image projections (MIPs) of z stacks from the middle of each cell. Cells were labeled with p65-488 (green), rhodamine phalloidin (red), and Hoechst (blue). Images were captured on a Zeiss 780 laser-scanning confocal microscope and further processed for Mander's correlation coefficients with Zeiss ZEN software. The colocalized signal was extracted from the MIP image and pseudo-colored in white (last panel of series). Percentages in the bottom right corners are Mander's correlation coefficients between the p65 and nuclear stains. Images are representative of three experiments. (**B**) Ratio of nuclear to cytoplasmic localization of p65 for the cells shown in (A). Data are means ± SEM of three experiments. (**C**) Primary human T cells were left unstimulated or were stimulated with anti-CD3/CD28 antibodies in the presence of DMSO or 50 μM DMF for 1 hour before being analyzed by Western blotting with antibodies against the indicated targets. Western blot is from one experiment.

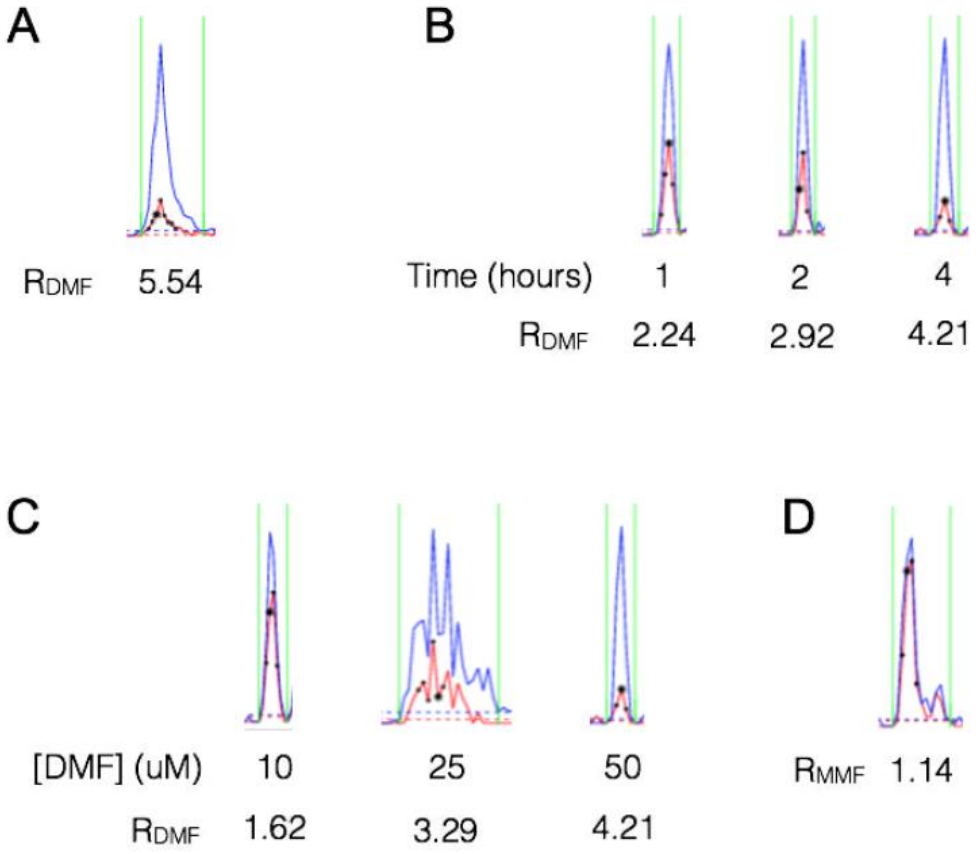


**Fig. S6. Analysis of the sensitivity of PKCθ residues Cys$^{14}$ and Cys$^{17}$ to DMF and MMF.** (**A**) Primary mouse splenic T cells treated with 50 μM DMF for 4 hours were subjected to isoTOP-ABPP analysis to determine the sensitivity of Cys$^{14}$ and Cys$^{17}$ of PKCθ. MS1 profiles are representative of three isoTOP-ABPP experiments. (**B** and **C**). Analysis of the time-dependence (B) and concentration-dependence (C) of the sensitivity of Cys$^{14}$ and Cys$^{17}$ in human PKCθ to DMF as determined by isoTOP-ABPP experiments. MS1 profiles are representative of two to five isoTOP-ABPP experiments. (**D**) Cys$^{14}$ and Cys$^{17}$ of human PKCθ are insensitive to MMF. Primary human T cells treated with 50 μM MMF for 4 hours were subjected to isoTOP-ABPP analysis to determine the sensitivity of Cys$^{14}$ and Cys$^{17}$ of PKCθ. MS1 profiles are representative of four isoTOP-ABPP experiments.

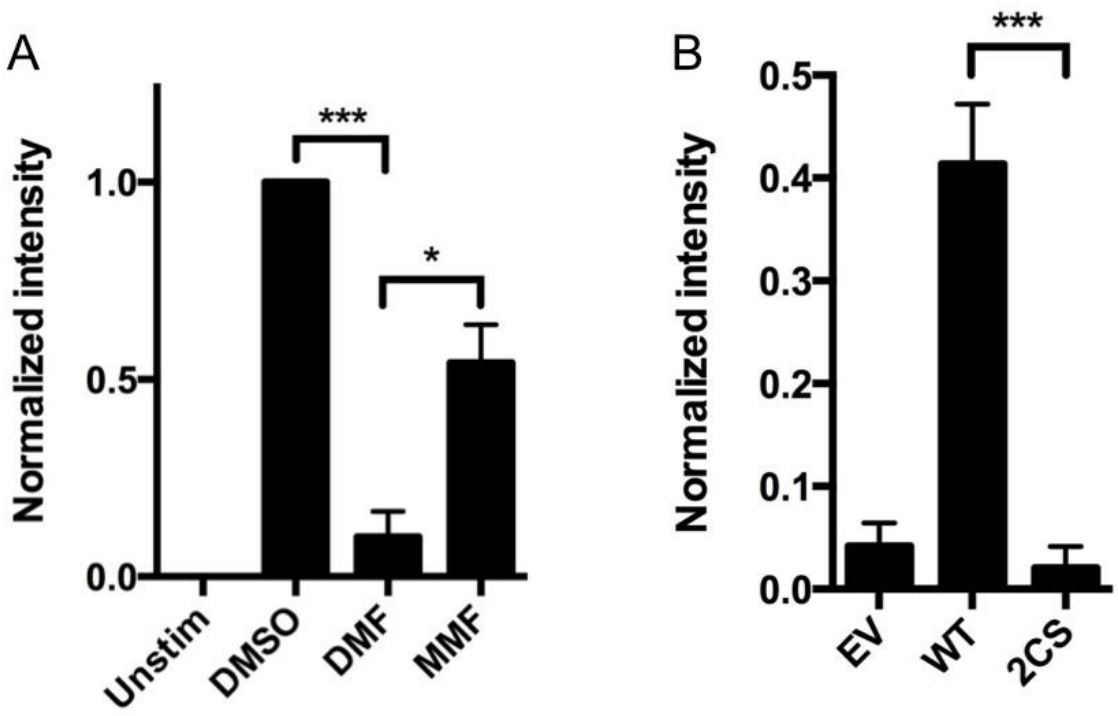


**Fig. S7. Densitometric analysis of PKCθ band intensities.** (**A**) Densitometric analysis of the ratios of PKCθ band intensities to CD28 band intensities from Fig. 5D, from which background IgG control values were subtracted. Results were normalized to DMSO control values, which were set at 1. Data are means ± SEM of three independent biological experiments. (**B**) Densitometric analysis of the ratios of PKCθ band intensities to CD28 band intensities from Fig. 5E, from which background IgG control values were subtracted. Data are means ± SEM of four independent biological experiments. **P* < 0.05, ****P* < 0.001 by two-tailed, unpaired *t* test.

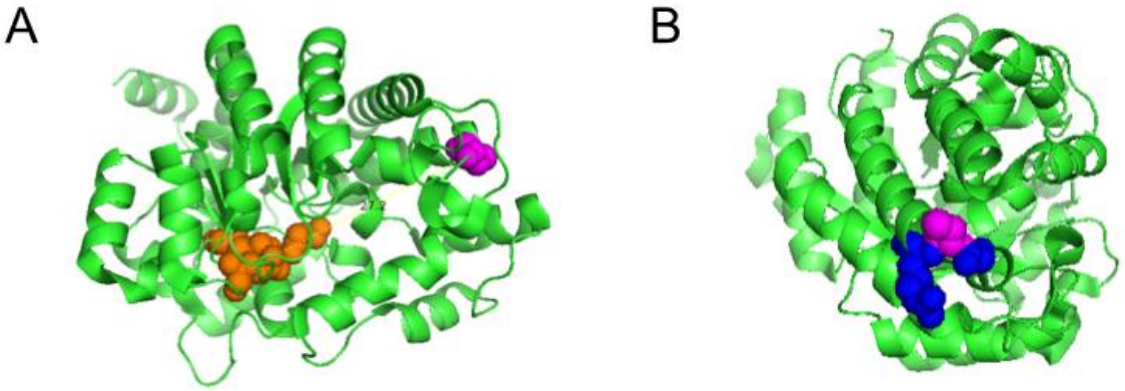


**Fig. S8. DMF-sensitive cysteine residues in ADA.** (**A**) The DMF-sensitive cysteine residue $Cys^{75}$ (magenta) is ~25 Å from the ADA active site (orange). (**B**) Mutations in both residues neighboring $Cys^{75}$, $Gly^{74}$ and $Arg^{76}$ (blue), are associated with the severe combined immunodeficiency known as ADASCID (OMIM: 608958). PDB accession number: 3IAR.

**Table S1. List of DMF-sensitive cysteine residues in human T cells.** DMF-sensitive cysteine residues are defined as those that showed R values (DMSO/DMF) > 4 in isoTOP-ABPP experiments that compared DMSO-treated T cells with DMF-treated T cells.

| **Name** | **Full name** | **Protein function** | **Residue** | **Conserved in mice** | **Role in immunology** |
|---|---|---|---|---|---|
| ADA | Adenosine deaminase | Adenosine deaminase | $Cys^{75}$ | Yes | Positive regulator of T cell co-activation (*48*) |
| AGFG2 | Arf-GAP domain and FG repeat-containing protein 2 | GTPase activator | $Cys^{39}$ | Yes | Unknown |
| AIP | AH receptor-interacting protein | Transcription factor binding | $Cys^{122}$ | Yes | Unknown |
| CRKL | Crk-like protein | Poly(A) RNA binding | $Cys^{249}$ | Yes | Unknown |
| FLII | Protein flightless-1 homolog | Actin binding | $Cys^{46}$ | Yes | Unknown |
| GAK | Cyclin-G-associated | Serine/threonine protein kinase | $Cys^{87}$ | Yes | Unknown |

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| | kinase | | | | |
| HUWE1 | E3 ubiquitin-protein ligase HUWE1 | E3 ubiquitin-protein ligase | $Cys^{3372}$ | Yes | Unknown |
| IKBKB | Inhibitor of nuclear factor κ-B kinase subunit | Serine kinase | $Cys^{464}$ | Yes | Phosphorylates IκBα in NF-κB pathway (*24*) |
| IL16 | Pro-interleukin-16 | Cytokine | $Cys^{1004}$ | Yes | Influences migration of $CD4^+$ lymphocytes (*49*) |
| IRF4 | Interferon regulatory factor 4 | DNA binding | $Cys^{194}$ | Yes | Regulates dendritic cell and B cell development, as well as T cell and B cell differentiation (*50–53*) |
| IRF8 | Interferon regulatory | DNA binding | $Cys^{306}$ | Yes | Plays a negative regulatory role in |

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| | factor 8 | | | | immune cells. Binds to upstream regulatory region of MHC class I genes (*54*, *55*). Regulates the development and differentiation of myeloid cells (*53*). |
| KIAA0528 | Uncharacterized protein | Calcium-dependent phospholipid binding | $Cys^{993}$ | Yes | Unknown |
| LAS1L | Ribosomal biogenesis protein | Poly(A) RNA binding | $Cys^{456}$ | Yes | Unknown |
| MARS2 | Methionine--tRNA ligase, mitochondrial | Methionine-tRNA ligase | $Cys^{425}$ | Yes | Unknown |
| MAT2A | S-adenosylmet | Methionine adenosyltransfer | $Cys^{56}$ | Yes | Unknown |

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| | hionine synthase isoform type-2 | ase | | | |
| MAT2A | S-adenosylmethionine synthase isoform type-2 | Methionine adenosyltransferase | $Cys^{104}$ | Yes | Unknown |
| MTCH2 | Mitochondrial carrier homolog 2 | Induces mitochondrial depolarization | $Cys^{296}$ | Yes | Unknown |
| PGP | Phosphoglycolate phosphatase | Phosphatase | $Cys^{297}$ | Yes | Unknown |
| PML | Protein Promyelocytic leukemia | RNA/DNA binding | $Cys^{479}$ | Yes | Modulates TGF-β signaling, induced by interferon to promote antiviral responses (*56–58*) |
| PRKCQ | Protein kinase C θ | Serine/threonine protein kinase | $Cys^{14}$ | Yes | Promotes TCR signaling through |

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| | type | | | | activation of NF-κB and other transcription factors (*25*) |
| PYGB | Glycogen phosphorylase, brain form | Phosphorylase | $Cys^{326}$ | Yes | Unknown |
| RARS | Arginine--tRNA ligase, cytoplasmic | tRNA binding | $Cys^{32}$ | Yes | Unknown |
| SON | Protein SON | RNA/DNA binding | $Cys^{92}$ | Yes | Unknown |
| SYNE2 | Nesprin-2 | Actin binding | $Cys^{553}$ | Yes | Unknown |
| TDRKH | Tudor and KH domain-containing protein | RNA binding | $Cys^{109}$ | Yes | Unknown |
| THNSL1 | Threonine synthase-like 1 | Threonine synthase | $Cys^{324}$ | Yes | Unknown |
| THOC1 | THO complex subunit 1 | RNA/DNA binding | $Cys^{49}$ | Yes | Unknown |

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| TNFAIP3 | Tumor necrosis factor α–induced protein 3 | Ubiquitin-specific protease | $Cys^{54}$ | Yes | Inhibits NF-κB signaling upon TCR-mediated T cell activation (*27*, *28*, *59*) |
| UBR4 | E3 ubiquitin-protein ligase | Ubiquitin ligase | $Cys^{2554}$ | Yes | Unknown |
| USP7 | Ubiquitin carboxyl-terminal hydrolase 7 | Ubiquitin-specific protease | $Cys^{315}$ | Yes | Deubiquitylates FoxP3, increasing $T_{reg}$ cell suppressive capacity (*60*, *61*) |
| VDAC3 | Voltage-dependent anion-selective channel protein | Mitochondrial outer membrane channel | $Cys^{65}$ | Yes | Unknown |
| VDAC3 | Voltage-dependent anion-selective channel | Voltage-gated anion channel | $Cys^{36}$ | Yes | Unknown |

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| | protein | | | | |
| ZC3HAV1 | Zinc finger CCCH-type antiviral protein 1 | Poly(A) RNA binding | $Cys^{645}$ | Yes | Inhibits viral replication (*62*) |
| ZNF346 | Zinc finger protein 346 | RNA binding | $Cys^{68}$ | Yes | Unknown |
| AARS | Alanine--tRNA ligase, cytoplasmic | Alanine-tRNA ligase | $Cys^{773}$ | No | Unknown |
| APOBEC3C | Probable DNA dC-dU-editing enzyme | Cytidine deaminase | $Cys^{130}$ | No | Inhibits retrovirus replication (*63*, *64*) |
| BCL2A1 | Bcl-2-related protein A1 | Scaffolding protein | $Cys^{55}$ | No | Expression induced by inflammatory cytokines (*65*) |
| BCL2A1 | Bcl-2-related protein A1 | Scaffolding protein | $Cys^{19}$ | No | Unknown |
| CHRAC1 | Chromatin accessibility complex | Chromatin remodeling | $Cys^{55}$ | No | Unknown |

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| | protein 1 | | | | |
| DCXR | L-xylulose reductase | Xylulose reductase | $Cys^{244}$ | No | Unknown |
| GHDC | GH3 domain–containing protein | Uncharacterized | $Cys^{502}$ | No | Unknown |
| IRAK4 | Interleukin-1 receptor–associated kinase 4 | Serine/threonine protein kinase | $Cys^{13}$ | No | Helps initiate innate immune response by promoting the phosphorylation of IRAK1 upon IL-1R/TLR activation. (*66*, *67*). Also implicated in T cell activation (*68*) |
| NADSYN 1 | Glutamine-dependent NAD(+) synthetase | NAD(+) synthase | $Cys^{428}$ | No | Unknown |

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| PGLS | 6-phospho-gluconolactonase | Hydrolysis of 6-phosphoglucono lactone | $Cys^{32}$ | No | Unknown |
| PRKDC | DNA-dependent protein kinase catalytic subunit | Serine/threonine protein kinase | $Cys^{4045}$ | No | Regulates DNA damage response, involved in V(D)J recombination (*69*) |
| PUSL1 | tRNA pseudouridine synthase-like 1 | Pseudouridine synthase | $Cys^{292}$ | No | Unknown |
| RIN3 | Ras and Rab interactor 3 | GTPase activator | $Cys^{942}$ | No | Unknown |
| SCLY | Selenocysteine lyase | Selenocysteine lyase | $Cys^{22}$ | No | Unknown |
| SPCS2 | Signal peptidase complex subunit 2 | Peptidase | $Cys^{17}$ | No | Unknown |
| TRNT1 | CCA tRNA nucleotidyltr | tRNA binding | $Cys^{373}$ | No | Mutations lead to B cell |

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| | ansferase 1, mitochondrial | | | | immunodeficiency as well as progressive reductions in T and NK cells (OMIM number 616084) (*70*) |
| TUBGCP3 | γ-tubulin complex component 3 | γ-tubulin binding | $Cys^{194}$ | No | Unknown |
| UBE2L6 | Ubiquitin/ISG15-conjugating enzyme E2 L6 | Ubiquitin-conjugating enzyme | $Cys^{98}$ | No | Acts as an E2 enzyme for an IFN-induced ubiquitin-like protein (*71*) |