Uncovering Protein Prenylation in Th1 Cells: Novel Prenylation Sites and Insights into Statin and Farnesyltransferase Inhibition

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Supplementary Material

Supplementary Tables

Supplementary Table S1: Summary of the identified proteins in the experiment with activated and non-activated Th1 cells, and in activated Th1 cells pretreated or not with statin, together with information on the sites of prenylation. Provided as separate Supplementary Material.

Supplementary Table S2: Proteins with a discrepancy in the annotated and the experimental prenylation moiety.

ID	name	annotated_moiety	position	evidenceCode	publication	experimental_moiety	motif
O15498	YKT6	farnesyl cys	-4	ECO_0000269	doi:10.1073/pnas.0401183101	farnesyl cys, geranylgeranyl cys	CAAX
P09543	CN37,CNP	farnesyl cys	-4	ECO_0000250		farnesyl cys, geranylgeranyl cys	CXXX
P04899	GNAI2	farnesyl cys	-4	extended_search	doi:10.1039/c3mb70593e	geranylgeranyl cys	CAAX
O95164	UBL3	geranylgeranyl cys	-4	ECO_0000305	doi:10.1074/jbc.m602283200	farnesyl cys	CAAX
P20340	RAB6A	geranylgeranyl cys	[-3, -1]	ECO_0000250		farnesyl cys	CXC
P50151	GBG10	geranylgeranyl cys	-4	ECO_0000269	doi:10.1074/jbc.270.37.21765	farnesyl cys	CAAX
Q15669	RHOH	geranylgeranyl cys	-4	ECO_0000250		farnesyl cys	CXXX
Q8WV99	ZFN2B	geranylgeranyl cys	-4	ECO_0000250		farnesyl cys	CXXX
Q96S21	RB40C	geranylgeranyl cys	-4	ECO_0000250		farnesyl cys	CXXX
Q9BRT3	MIEN1	geranylgeranyl cys	-4	ECO_0000269	doi:10.1074/jbc.m111.254599, doi:10.1038/onc.2009.145	farnesyl cys	CAAX
Q9UK08	GBG8	geranylgeranyl cys	-4	ECO_0000250		farnesyl cys	CAAX
Q9Y3L5	RAP2C	geranylgeranyl cys	-4	ECO_0000250		farnesyl cys	CAAX
P08134	RHOC	geranylgeranyl cys	-4	ECO_0000250		farnesyl cys, geranylgeranyl cys	CXXX
P11233	RALA	geranylgeranyl cys	-4	ECO_0000269	doi:10.1128/mcb.00057-07, doi:10.1016/s0021-9258(18)92889-9	farnesyl cys, geranylgeranyl cys	CXXX
P11234	RALB	geranylgeranyl cys	-4	ECO_0000269	doi:10.1128/mcb.00057-07	farnesyl cys, geranylgeranyl cys	CXXX
P15153	RAC2	geranylgeranyl cys	-4	ECO_0000269	doi:10.1016/s0021-9258(18)92889-9	farnesyl cys, geranylgeranyl cys	CXXX
P32456	GBP2	geranylgeranyl cys	-4	ECO_0000269	doi:10.1371/journal.pone.0014246	farnesyl cys, geranylgeranyl cys	CXXX
P51159	RB27A	geranylgeranyl cys	[-3, -1]	ECO_0000250		farnesyl cys, geranylgeranyl cys	CXC
P59768	GBG2	geranylgeranyl cys	-4	ECO_0000250		farnesyl cys, geranylgeranyl cys	CAAX
P60953	CDC42	geranylgeranyl cys	-4	ECO_0007744, ECO_0000269	doi:10.1016/s0092-8674(00)80670-4	farnesyl cys, geranylgeranyl cys	CAAX
P61006	RAB8A	geranylgeranyl cys	-4	ECO_0000269	doi:10.1016/0014-5793(93)80897-4	farnesyl cys, geranylgeranyl cys	CAAX
P61026	RAB10	geranylgeranyl cys	[-2, -1]	ECO_0000250		farnesyl cys, geranylgeranyl cys	CC
P61224	RAP1B	geranylgeranyl cys	-4	ECO_0000269	doi:10.1073/pnas.87.22.8960	farnesyl cys, geranylgeranyl cys	CXXX
P61225	RAP2B	geranylgeranyl cys	-4	ECO_0000269	doi:10.1042/bj2890349	farnesyl cys, geranylgeranyl cys	CAAX
P61586	RHOA	geranylgeranyl cys	-4	ECO_0000250		farnesyl cys, geranylgeranyl cys	CAAX
P63000	RAC1	geranylgeranyl cys	-4	ECO_0000269	doi:10.1016/s0021-9258(18)92889-9	farnesyl cys, geranylgeranyl cys	CAAX
P63218	GBG5	geranylgeranyl cys	-4	ECO_0000250		farnesyl cys, geranylgeranyl cys	CXXX
P84095	RHOG	geranylgeranyl cys	-4	ECO_0000250		farnesyl cys, geranylgeranyl cys	CAAX
Q14088	RB33A	geranylgeranyl cys	[-3, -1]	ECO_0000250		farnesyl cys, geranylgeranyl cys	CXC
Q86YS6	RAB43	geranylgeranyl cys	[-3, -1]	ECO_0000250		farnesyl cys, geranylgeranyl cys	CXC
Q92930	RAB8B	geranylgeranyl cys	-4	ECO_0000250		farnesyl cys, geranylgeranyl cys	CXXX
Q96PP8	GBP5	geranylgeranyl cys	-4	ECO_0000269	doi:10.1371/journal.pone.0014246	farnesyl cys, geranylgeranyl cys	CAAX
Q9H0U4	RAB1B	geranylgeranyl cys	[-2, -1]	ECO_0000269	doi:10.1042/bj3181007	farnesyl cys, geranylgeranyl cys	CC
P62745	RHOB	geranylgeranyl cys in endosomal form, farnesyl cys in plasma membrane form	-4	ECO_0000269	doi:10.1074/jbc.270.14.7864, doi:10.1016/s0021-9258(19)88661-1	farnesyl cys	CXXX

Supplementary Table S3: Proteins with the new prenylation motifs CXXX, CXX, CX, and CCC

Protein_ID	Protein_Name	Experimental moiety	non_canonical isoform	PositionC New_Motif CXXXX	PositionC New_Motif CXX	PositionC New_Motif CX	PositionCs New_Motif CCC
P27487	DPP4	F-Az & GG-Az		-5			
Q9NZC3	GDE1	GG-Az		-5			
Q69YN4	KIAA1429	F-Az & GG-Az	4	-5			
Q68DE3	KIAA2018	F-Az		-5			
Q8N4C8	MINK1	GG-Az		-5			
Q9NZJ7	MTCH1	F-Az & GG-Az		-5			
Q9UBB6	NCDN	GG-Az		-5			
Q8TD19	NEK9	F-Az		-5			
Q8WXH0	SYNE2	F-Az & GG-Az	8	-5			
Q93038	TNFRSF25	GG-Az	4	-5			
Q9C0C9	UBE2O	F-Az		-5			
A6NN14	ZNF729	GG-Az		-5			
Q8N6S5	ARL6IP6	GG-Az			-3		
Q07812	BAX	F-Az & GG-Az	5		-3		
P01024	C3	F-Az			-3		
Q9BSY4	CHCHD5	F-Az	2		-3		
Q6NVY1	HIBCH	GG-Az	2		-3		
P10321	HLA-C	F-Az			-3		
P35914	HMGCL	F-Az			-3		
P22234	PAICS	F-Az			-3		
P15151	PVR	F-Az & GG-Az	4		-3		
Q00765	REEP5	F-Az & GG-Az	2		-3		
P23921	RRM1	F-Az			-3		
O95562	SFT2D2	F-Az & GG-Az			-3		
P41440	SLC19A1	GG-Az	3		-3		
O75558	STX11	F-Az & GG-Az			-3		
Q8NBK3	SUMF1	F-Az	3		-3		
Q7Z403	TMC6	F-Az	4		-3		
Q9Y294	ASF1A	F-Az				-2	
Q92556	ELMO1	F-Az				-2	
Q9BUT9	FAM195A	F-Az				-2	
Q14331	FRG1	F-Az				-2	
O94927	HAUS5	F-Az				-2	
Q15365	PCBP1	F-Az				-2	
P22307	SCP2	F-Az	3			-2	
Q9UHD2	TBK1	F-Az				-2	
Q86T03	TMEM55B	F-Az & GG-Az	3			-2	
O95985	TOP3B	F-Az & GG-Az	3			-2	
P67936	TPM4	F-Az				-2	
Q96CW5	TUBGCP3	F-Az & GG-Az	2			-2	
Q8N806	UBR7	F-Az				-2	
O14980	XPO1	F-Az & GG-Az				-2	
P43403	ZAP70	F-Az				-2	
Q8IZ83	ALDH16A1	F-Az	2				[-3, -2, -1]

Supplementary Table S4: Proteins with exactly one internal, potentially prenylated cysteine.

Protein_ID	**Protein_Name**	**Experimental moiety**	**non_canonical isoform**	**Position C Internal C_1**	**Sequence Internal_Motif**
Q9BQE5	APOL2	F-Az		-172	VAGITCSVVEL
P53367	ARFIP1	GG-Az		-236	LNTYKCTRQII
P36542	ATP5C1	F-Az		-196	SDRGLCGAIHS
Q16836	HADH	F-Az		-104	KHPVSCKDTPG
Q8N4V1	MMGT1	GG-Az		-75	AFAVTCYGIVH
P00395	MT-CO1	F-Az		-16	EWLYGCPPPYH
P00414	MT-CO3	F-Az & GG-Az		-44	TFLTICFIRQL
O00483	NDUFA4	F-Az & GG-Az		-38	FNPDVCWDRNN
O75438	NDUFB1	F-Az		-34	GFVIGCYLDRK
Q92968	PEX13	GG-Az		-184	EGTVACLGAED
Q96M27	PRRC1	F-Az		-98	WFDIGCLVVED
Q5XKP0	QIL1	F-Az		-59	FSQYVCQQTGL
P60468	SEC61B	F-Az & GG-Az		-58	RKNASCGTRSA
P60059	SEC61G	F-Az		-44	RLVKRCTKPDR
Q99720	SIGMAR1	F-Az & GG-Az		-130	WMGAMCLLHAS
P0DJ93	SMIM13	F-Az & GG-Az		-67	LLLMVCGWYFV
Q8N131	TMEM123	F-Az & GG-Az		-22	ILYIGCKMYYS
Q9BTV4	TMEM43	F-Az & GG-Az		-47	KAFAFCVATSL
Q9UMX0	UBQLN1	F-Az	4	-28	
Q9UDW1	UQCR10	F-Az	2	-9	
P63027	VAMP2	GG-Az		-14	ILGVICAIILI
Q9BV40	VAMP8	F-Az & GG-Az		-20	MIVLICVIVFI

Supplementary Table S5: The 49 proteins that were significantly less farnesylated upon FTI treatment with the information on their prenylation motif, whether they have been known to be prenylated (0 = not known; 1 = known), and whether they have been identified before as significantly enriched in samples treated with F-Az (0 = not identified, 1 = identified in any F-Az treated samples in Th1 cells activated versus non-activated and activated Th1 cells pretreated or not with statin).

Protein_ID	**Protein_Name**	**Motif**	**Known prenylated**	**Identified in F-Az before**
Q9Y3P9	RABGAP1	C	0	1
Q8NDI1	EHBP1	CaaX	0	0
Q8N3D4	EHBP1L1	CaaX	0	1
O14610	GNGT2	CaaX	1	1
P01112	HRAS	CaaX	1	1
Q27J81	INF2	CaaX	0	1
P01116	KRAS	CaaX	1	1
P20700	LMNB1	CaaX	1	1
P01111	NRAS	CaaX	1	1
P40855	PEX19	CaaX	1	1
O43900	PRICKLE3	CaaX	0	1
P51157	RAB28	CaaX	1	1
Q9Y3L5	RAP2C	CaaX	1	1
P62070	RRAS2	CaaX	1	1
O15498	YKT6	CaaX	1	1
O95236	APOL3	CXXX	0	1
Q5VW32	BROX	CXXX	1	1
P49454	CENPF	CXXX	1	1
Q6P2H3	CEP85	CXXX	1	1
Q5SZL2	CEP85L	CXXX	0	1
Q5TAQ9	DCAF8	CXXX	1	1
P31689	DNAJA1	CXXX	1	1
O60884	DNAJA2	CXXX	1	1
Q8WW22	DNAJA4	CXXX	1	1
Q9BVM2	DPCD	CXXX	1	1
Q8IW50	FAM219A	CXXX	0	1
P32455	GBP1	CXXX	1	1
P02545	LMNA	CXXX	1	0
Q03252	LMNB2	CXXX	1	1
Q32MZ4	LRRFIP1	CXXX	1	1
Q16644	MAPKAPK3	CXXX	0	1
P55209	NAP1L1	CXXX	1	1
Q99733	NAP1L4	CXXX	1	1
Q93096	PTP4A1	CXXX	1	1
Q12974	PTP4A2	CXXX	1	1
P10114	RAP2A	CXXX	1	1
Q15382	RHEB	CXXX	1	1
Q15669	RHOH	CXXX	1	1
Q4ADV7	RIC1	CXXX	0	0
Q96EA4	SPDL1	CXXX	0	1
Q6PHR2	ULK3	CXXX	1	1
Q8N5D0	WDTC1	CXXX	0	1
Q8WV99	ZFAND2B	CXXX	1	1
Q9UI42	CBPA4	Internal	0	0
P41567	EIF1	Internal	0	0
P52292	IMA1	Internal	0	0
Q8IUC0	KR131	Internal	0	0
Q8N0X7	SPART	Internal	0	0
Q969T9	WBP2	Internal	0	0

Supplementary Table S6: Differentially expressed proteins in total extracts comparing activated and non-activated Th1 cells. Provided as separate Supplementary Material.

Supplementary Table S7: The 63 proteins that were significantly differentially prenylated comparing activated and non-activated Th1 cells; see Supplementary Table S8 for the full list of 179 differentially prenylated proteins and the filtering.

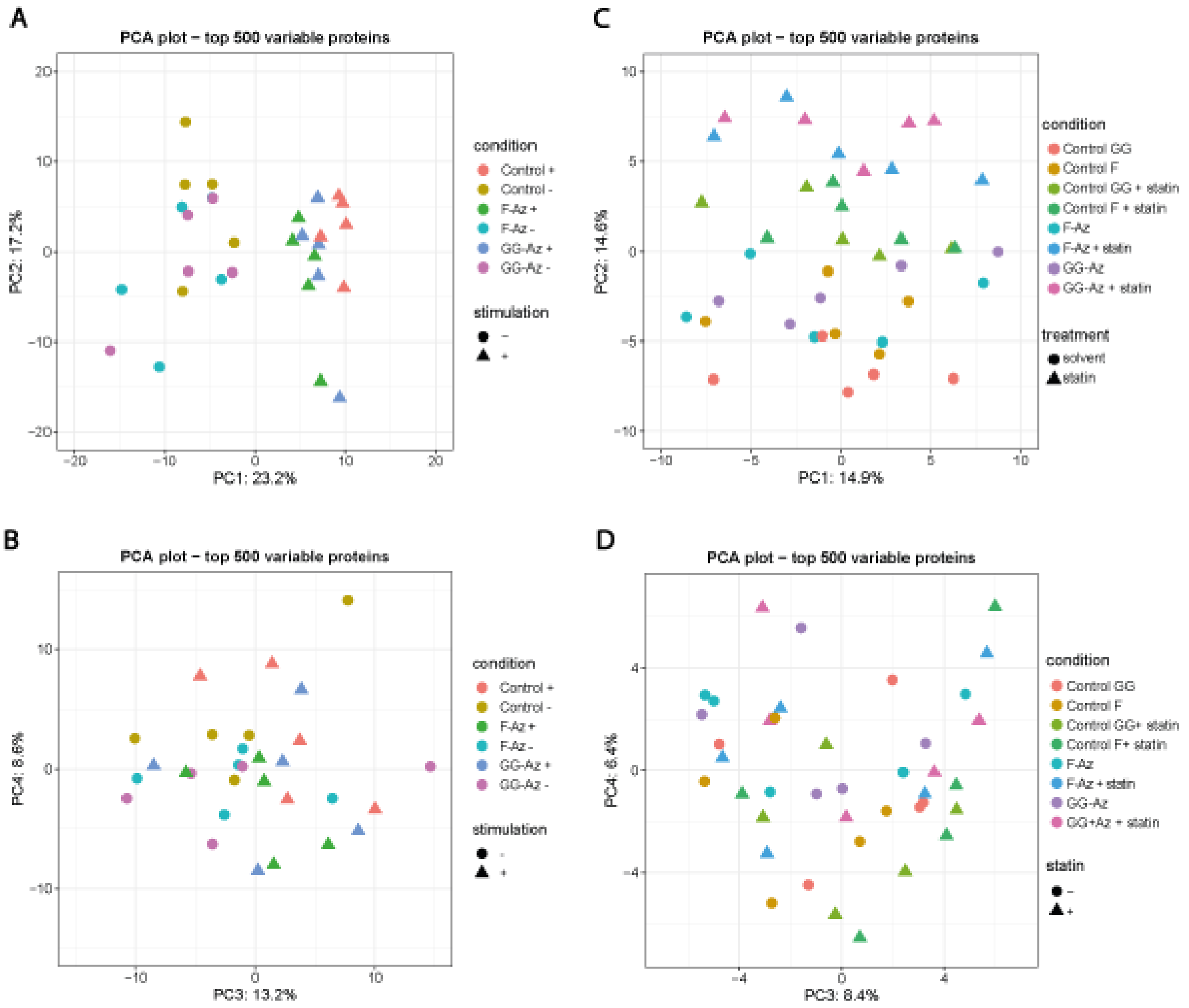
Protein Name	Protein ID	Known prenylated	Average FC	FAz_Act vs FAz_non-act p.adj	FAz_Act vs FAz_non-act log_fc	GGAz_Act vs GGAz_non-act p.adj	GGAz_Act vs GGAz_non-act log_fc
GNG4	P50150	GNG4	5.38			6.89E-03	5.380
NDFIP2	Q9NV92		5.01			1.14E-13	5.010
KDM6B	O15054		4.95	6.28E-14	4.950		
CD9	P21926		4.12			5.79E-04	4.120
CD69	Q07108		4.065	6.28E-14	3.450	1.48E-09	4.680
RAD21L1	Q9H4I0		3.67	3.00E-03	3.670		
ZNF697	Q5TEC3		3.415	1.10E-02	3.730	4.87E-02	3.100
TNFRSF25	Q93038		3.01			1.56E-04	3.010
RBM19	Q9Y4C8		2.72			1.14E-13	2.720
VDR	P11473		2.545	3.39E-02	2.170	3.21E-10	2.920
GNG12	Q9UBI6	GNG12	2.5	3.19E-09	2.500		
NDFIP1	Q9BT67		2.27			4.41E-03	2.270
NCOA2	Q15596		2.1			1.10E-07	2.100
CD82	P27701		2.035	6.28E-14	2.400	2.35E-09	1.670
FNDC3B	Q53EP0		2.03	2.36E-11	2.030		
PVR	P15151		2.01	1.05E-03	2.010		
SLC39A14	Q15043		1.935	6.65E-03	1.810	7.03E-03	2.060
TRPS1	Q9UHF7		1.83	3.12E-03	1.830		
CD63	P08962		1.67	1.14E-02	1.670		
ARID5A	Q03989		1.61			5.95E-03	1.610
GNAQ	P50148		1.49			8.35E-05	1.490
DPAGT1	Q9H3H5		1.48	6.28E-14	1.480		
PNO1	Q9NRX1		1.46			8.03E-03	1.460
SYNGAP1	Q96PV0		1.41			2.09E-04	1.410
TYSND1	Q2T9J0		1.39	6.40E-03	1.390		
SLC38A1	Q9H2H9		1.36	3.83E-05	1.360		
IL2RA	P01589		1.33			3.91E-02	1.330
CD44	P16070		1.28	6.80E-03	1.110	9.84E-11	1.450
NAF1	Q96HR8		1.23			2.57E-05	1.230
CYP51A1	Q16850		1.21	2.43E-02	1.210		
NSDHL	Q15738		1.09	1.79E-03	1.090		
GFI1	Q99684		1.08	1.35E-02	1.080		
ZNF267	Q14586		1.08			2.47E-02	1.080
FAM60A	Q9NP50		1.04	2.15E-03	1.040		
BZW2	Q9Y6E2		0.923			2.28E-02	0.923
BMS1	Q14692		0.868	4.83E-02	0.868		
RFTN1	Q14699		0.859	1.49E-02	0.859		
ATN1	P54259		0.843			4.88E-02	0.843
ZNF16	P17020		0.833			3.78E-03	0.833
SRA1	Q9HD15		0.769	7.52E-03	0.769		
VASP	P50552		0.683	7.07E-03	0.683		
ZMYND11	Q15326		-0.611			4.49E-02	-0.611
VEZF1	Q14119		-0.626			4.94E-02	-0.626
ZMYM3	Q14202		-0.667	1.06E-02	-0.667		
ZNF44	P15621		-0.724			4.43E-02	-0.724
ZBTB14	O43829		-0.745	1.67E-02	-0.745		
BTBD11	A6QL63		-0.785	3.63E-02	-0.785		
ZNF792	Q3KQV3		-0.88	7.44E-03	-0.880		
PRKCQ	Q04759		-0.935	2.13E-03	-0.935		
CD3D	P04234		-0.94			1.12E-03	-0.940
CD3E	P07766		-1			4.13E-02	-1.000
ZNF565	Q8N9K5		-1.05	3.52E-06	-1.050		
GBP1	P32455	GBP1	-1.39	3.34E-02	-1.390		
CRACR2A	Q9BSW2	CRACR2A	-1.54			1.60E-02	-1.540
RAB28	P51157	RAB28	-1.63	4.93E-03	-1.630		
SELPLG	Q14242		-1.84			1.45E-08	-1.840
S1PR4	O95977		-2.04	1.19E-03	-1.930	7.58E-03	-2.150
PTP4A1	Q93096	PTP4A1	-2.24	6.55E-05	-2.240		
ZNF518B	Q9C0D4		-2.35	2.41E-03	-2.350		
RHOH	Q15669	RHOH	-2.4	4.08E-03	-2.400		
CXCR3	P49682		-2.45			1.98E-02	-2.450
ICAM2	P13598		-2.5			1.14E-13	-2.500
INF2	Q27J81		-2.85	3.68E-02	-2.850		

Supplementary Table S8: Differentially prenylated proteins comparing activated and non-activated Th1 cells. Provided as separate Supplementary Material.

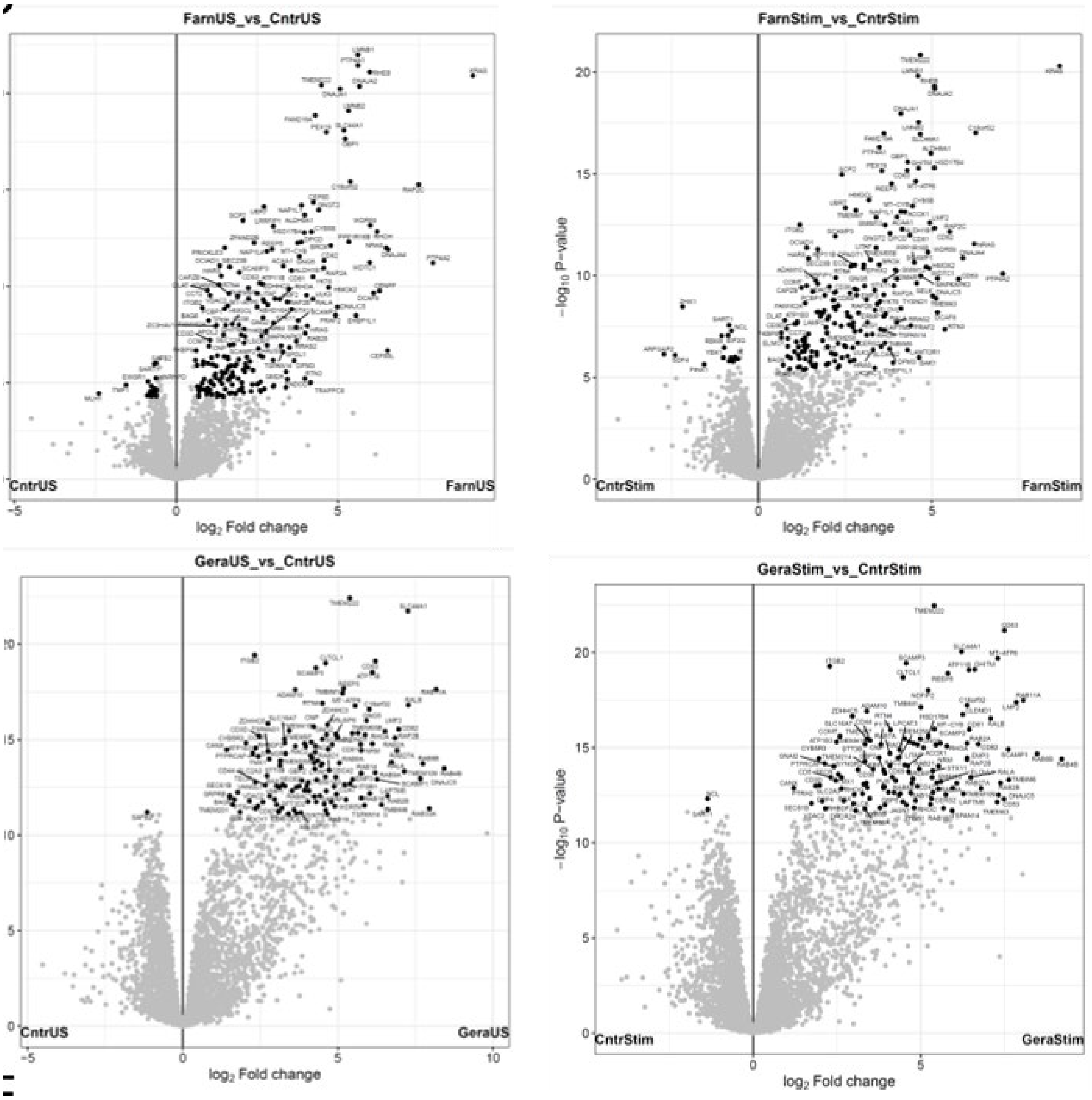
Supplementary Table S9: Differentially expressed proteins in total extracts comparing activated Th1 cells pretreated or not with statin. Provided as separate Supplementary Material.

Supplementary Table S10: Differentially prenylated proteins comparing activated Th1 cells pretreated or not with statin. Provided as separate Supplementary Material.

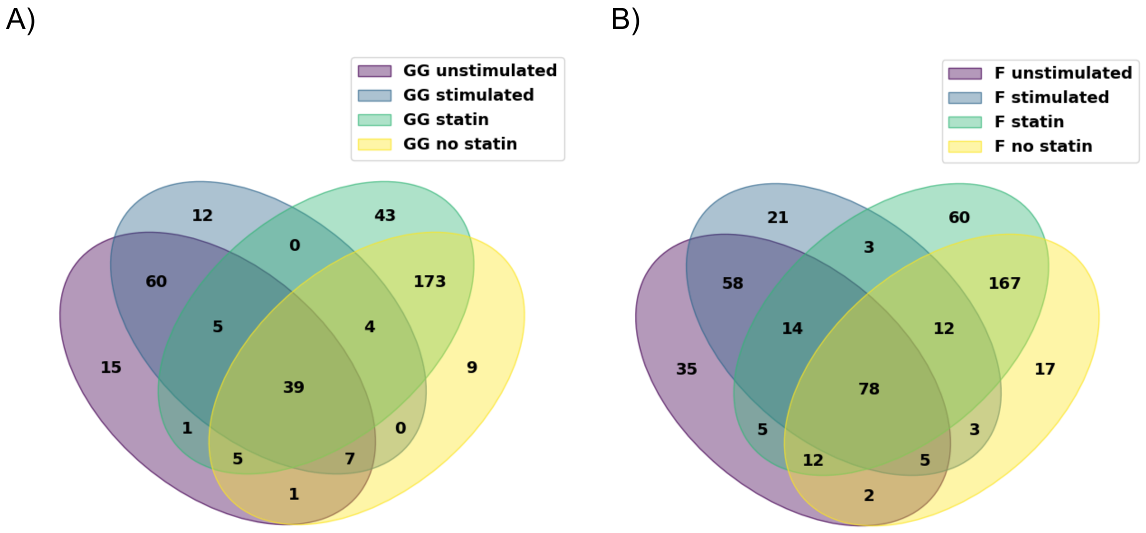
Supplementary Figures



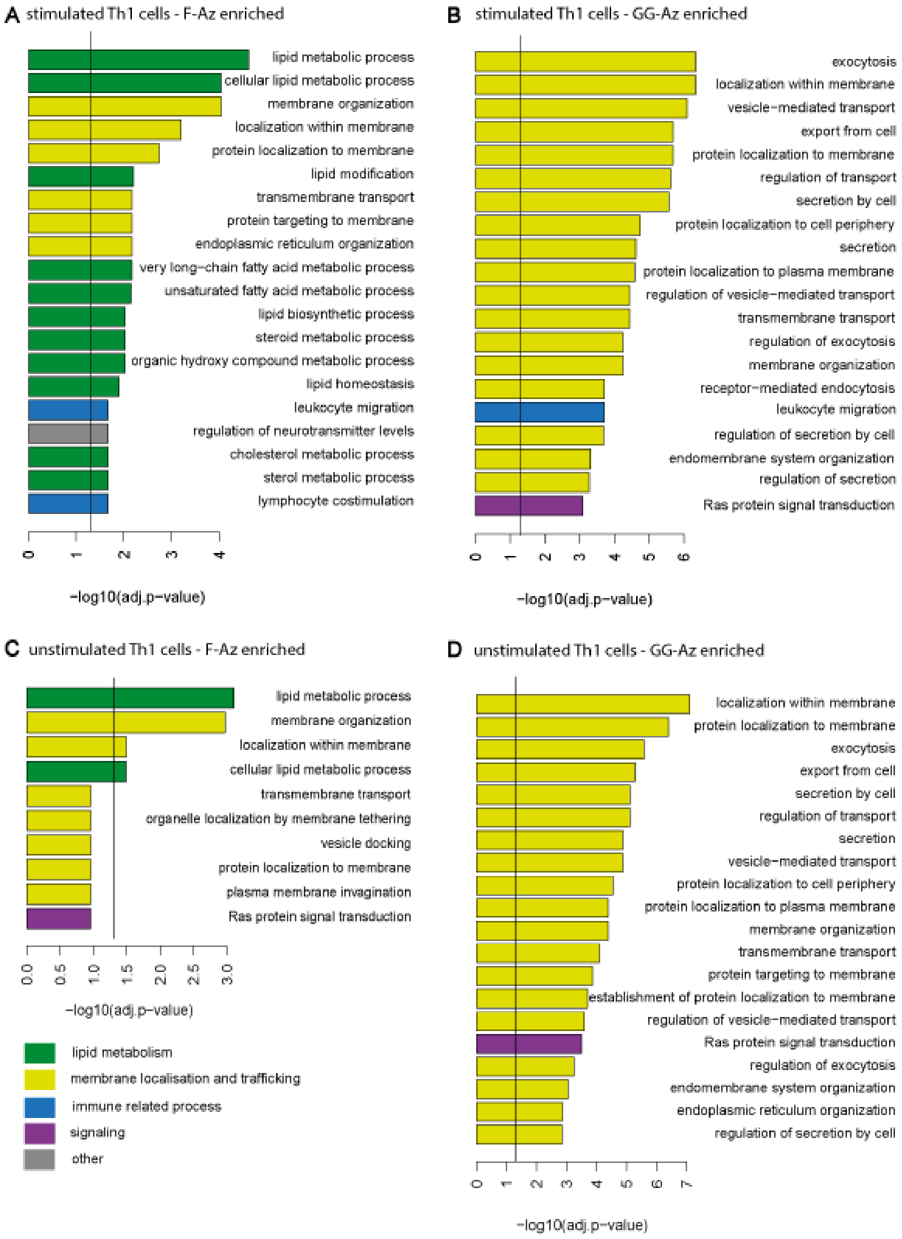
Supplementary Figure S1: PCA plots of the top 500 variable proteins in total extract samples. A) PC1 against PC2, and B) PC3 against PC4 from the PCA of the top 500 variable proteins in the total extract samples of activated and non-activated Th1 cells treated with GG-Az, F-Az or mock treatment. C) PC1 against PC2, and D) PC3 against PC4 from the PCA of the top 500 variable proteins in the total extract samples of activated Th1 cells pretreated or not with statin and treated with GG-Az, F-Az or mock treatment.



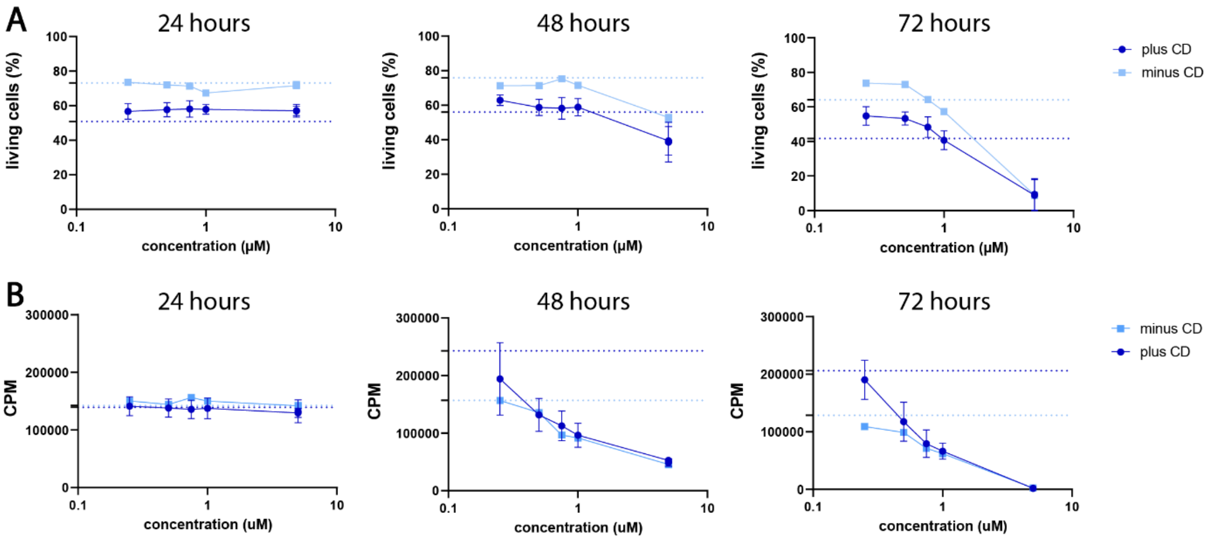
Supplementary Figure S2: Volcano plots of the enrichment samples comparing farnesylated (top) and geranylgeranylated proteins (bottom) to the respective background controls in non-stimulated (left) and stimulated (right) Th1 cells.



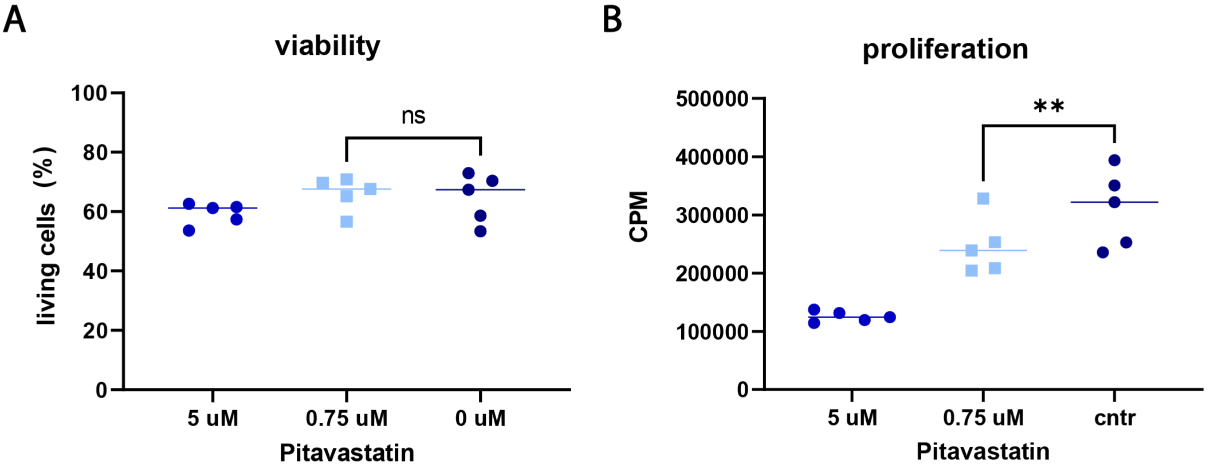
Supplementary Figure S3: Venn diagrams showing the overlaps between the significantly enriched proteins in: A) GG-Az-treated and B) F-Az-treated activated and non-activated Th1 cells, as well as in activated Th1 cells pretreated or not pretreated with statin, compared to their respective mock-treated background controls.



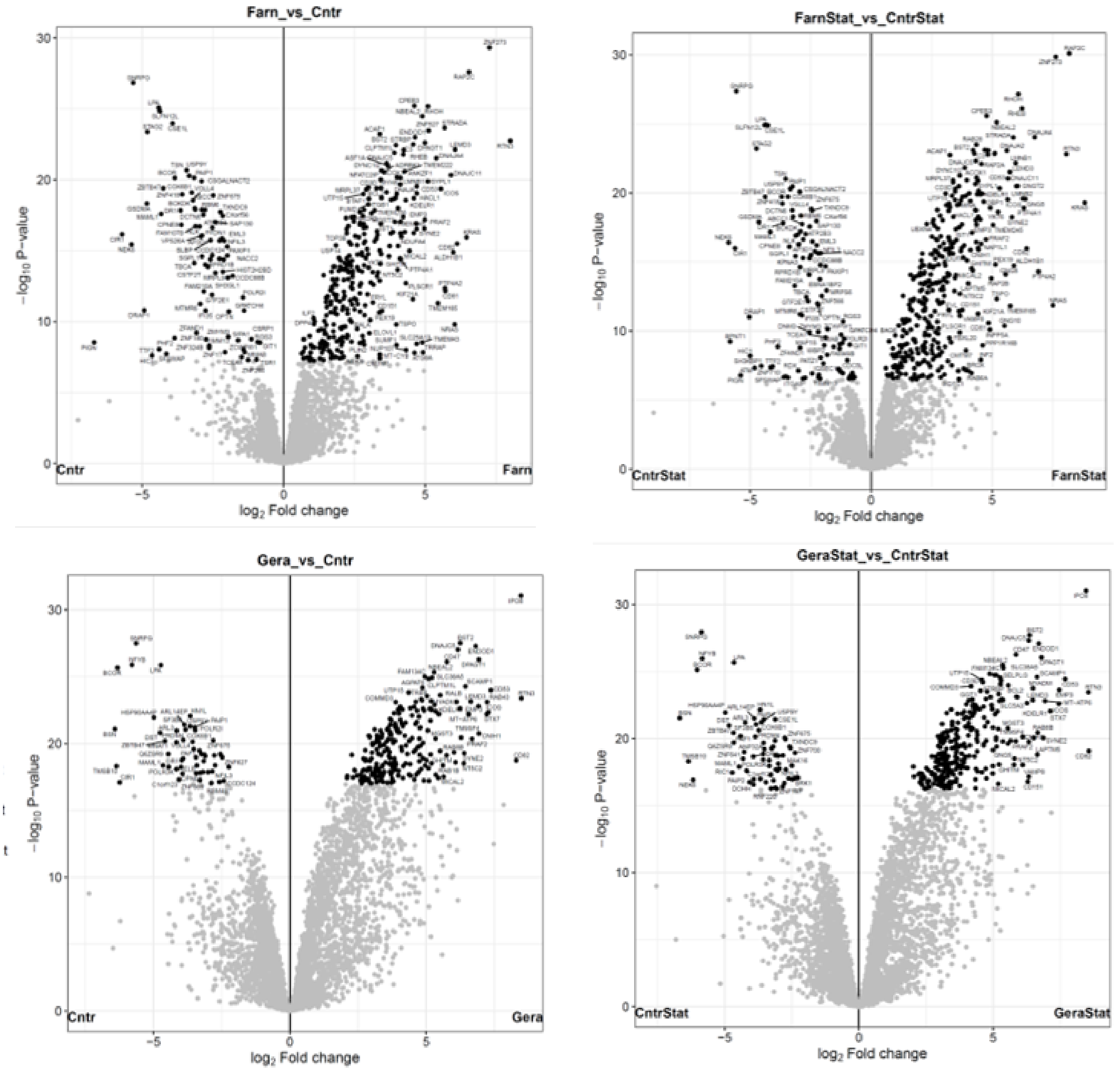
Supplementary Figure S4: Enriched Gene Ontology Biological Process (GO-BP) terms in the significantly enriched prenylated proteins in A) activated Th1 cells treated with F-Az, B) activated Th1 cells treated with GG-Az, (C) non-activated Th1 cells treated with F-Az, and D) non-activated Th1 cells treated with GG-Az.



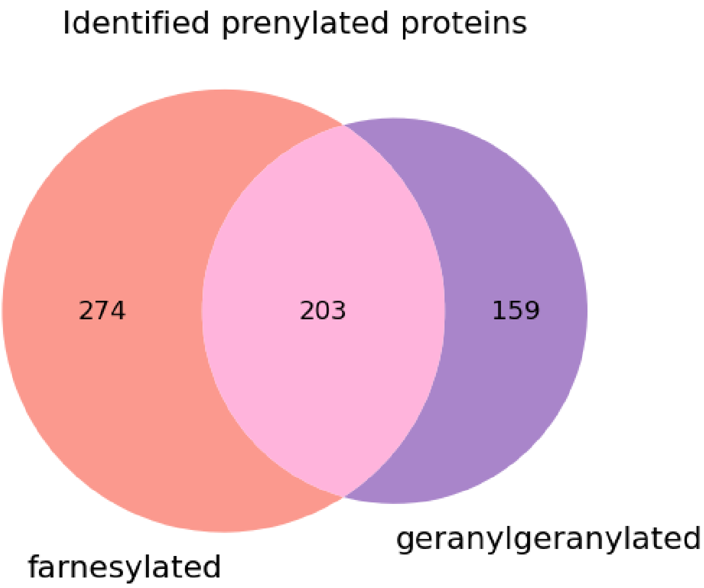
Supplementary Figure S5: Pitavastatin kinetics and optimal concentrations were assessed over 1–3 days using inhibition of proliferation with minimal cell death as the readout using *in vitro*-differentiated Th1 cells that were either activated with anti-CD3/anti-CD8 antibodies (dark blue) or left non-activated (light blue). Analysis of A) cell proliferation with the H3-thymidine incorporation assay and of B) cell viability with the live dead staining using propidium iodide in cells treated with Pitavastatin at the indicated concentrations for 24, 48, and 72 hours. The mean CPMs and percentages of living cells in activated and non-activated Th1 cells without statin treatment are indicated with dotted lines. CPM: counts per minute.



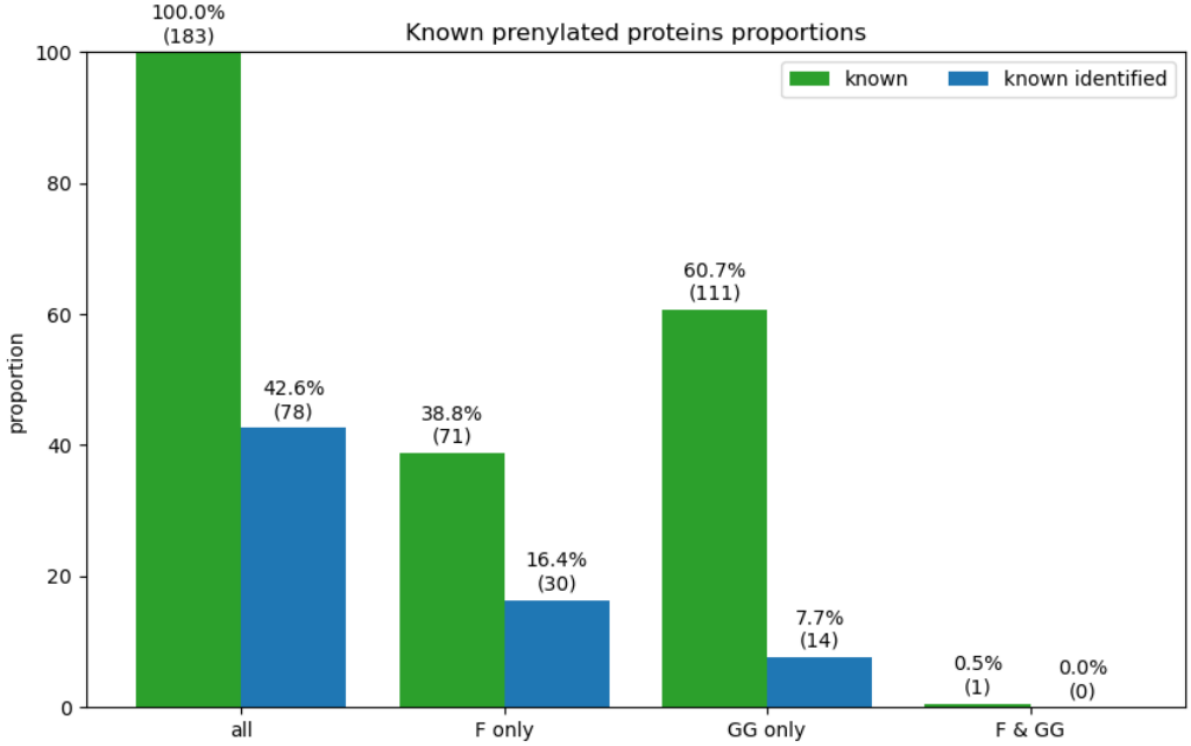
Supplementary Figure S6: Analysis of cell viability using live/dead staining (A) and cell proliferation using the H3-thymidine incorporation assay (B) of the Th1 cells used in the experiment, confirming that 0.75 µM Pitavastatin significantly reduced cell proliferation without causing a significant change in the percentage of living cells. PI: propidium iodide, CPM: counts per minute.



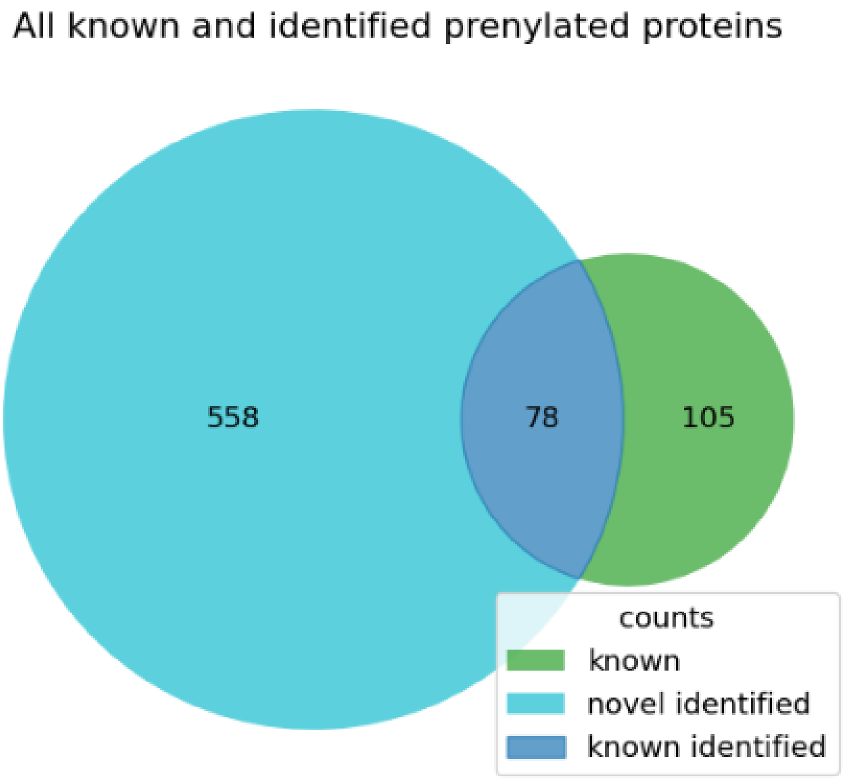
Supplementary Figure S7: Volcano plots of the enrichment samples comparing proteins identified in samples treated with F-Az (top) or GG-Az (bottom) to the respective background controls without isoprenoid addition, in activated Th1 cells either not pretreated with statin (left) or pretreated with statin (right).



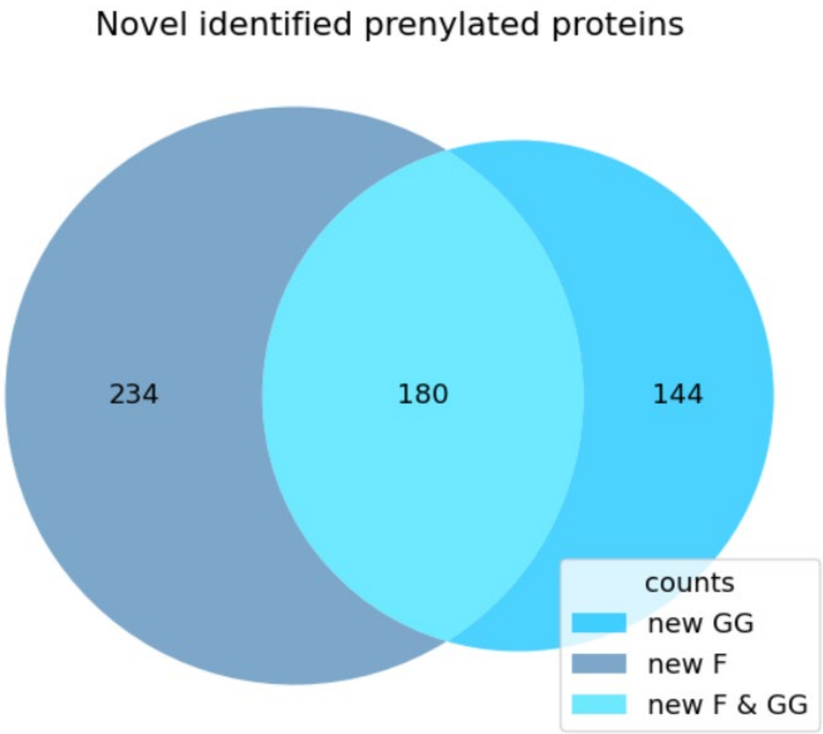
Supplementary Figure S8: Venn diagram illustrating the significantly enriched farnesylated and geranylgeranylated proteins identified through statistical analyses comparing proteins enriched in F-Az or GG-Az-treated samples to their respective mock-treated background controls. All significantly enriched proteins containing a cysteine in any of their isoforms were included. The results encompass experiments with both activated and non-activated Th1 cells, as well as activated Th1 cells pretreated or not pretreated with statin.



Supplementary Figure S9: Proportion of identified known prenylated (78 proteins, 42.6%), farnesylated (30), geranylgeranylated (14), and both geranylgeranylated and farnesylated proteins (0) compared to all annotated prenylated (183), farnesylated (71), geranylgeranylated (111), and both geranylgeranylated and farnesylated proteins (1) in UniProt.



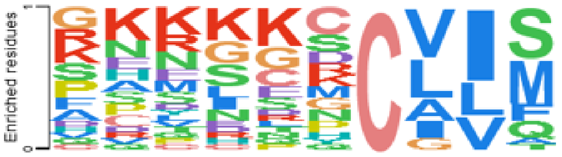
Supplementary Figure S10: Of all the 636 identified prenylated proteins, 78 (12.3%) are annotated as prenylated in UniProt, whereas 558 are novel that have not previously been known to be prenylated.



Supplementary Figure S11: Venn diagram with the number of newly identified prenylated proteins in cells treated with GG-Az, F-Az, or both.

Sequence Logos: Known prenylated proteins from UniProt

F known CAAX (25)



GG known CAAX (27)

F known CXXX (46)

GG known CXXX (44)

GG known CXC (20)

GG known CCX (4)

GG known CC (16)

GG known C (1)
FEESGNRSYC

Supplementary Figure S12: Sequence logos of the 10 most C-terminal amino acids of the proteins known to be farnesylated (F) or geranylgeranylated (GG), separated by the different canonical prenylation motifs.

KNOWN identified prenylated proteins with canonical motif

F identified CAAX (12)

GG identified CAAX (13)

F identified CXXX (22)

GG identified CXXX (16)

GG identified CXC (10)

GG identified CC (5)

Supplementary Figure S13: Sequence logos of the 10 most C-terminal amino acids of the proteins known to be prenylated and identified here as significantly enriched in samples treated with F-Az (F) or or GG-Az (GG), separated by the different canonical prenylation motifs.

NEW identified prenylated proteins with canonical motif

F identified CAAX (5)

F identified CXXX (15)

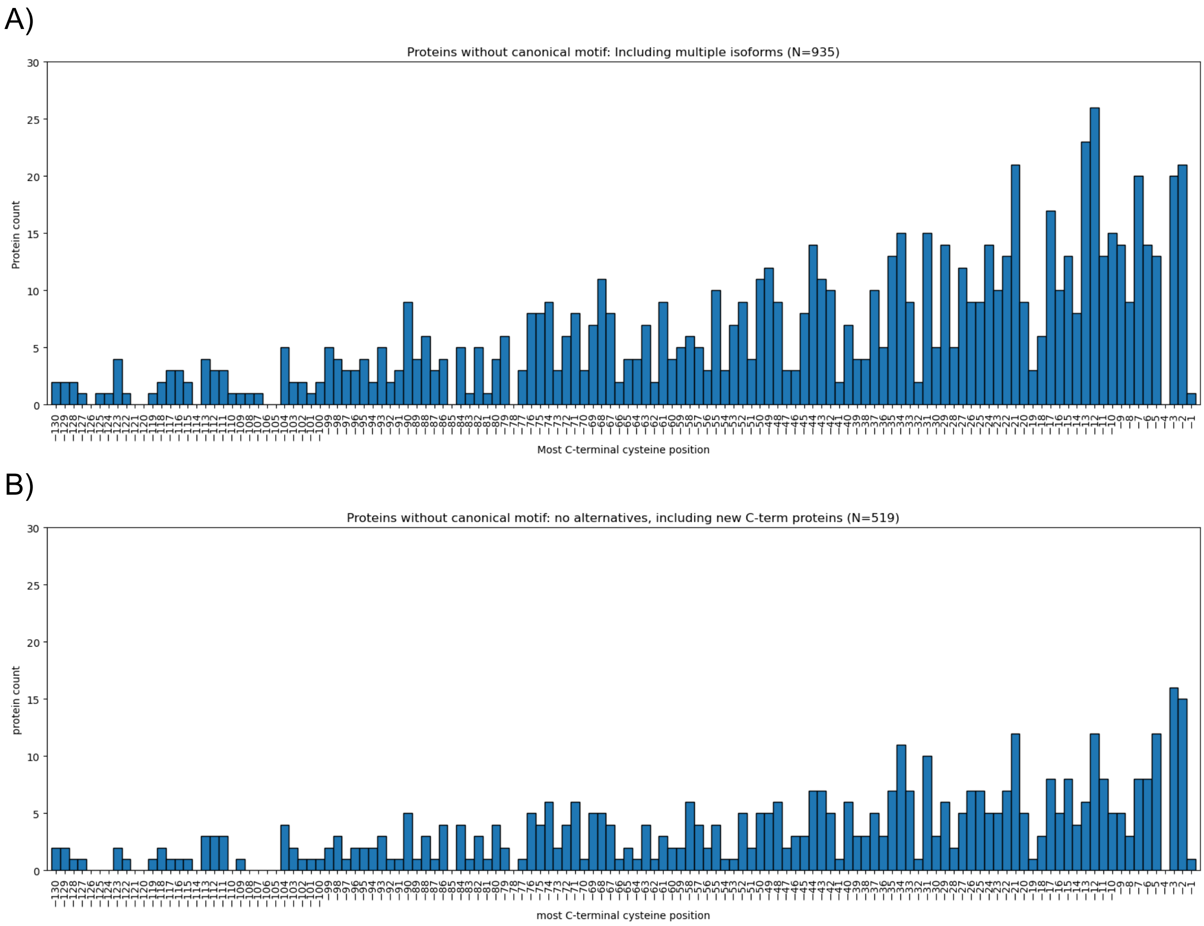
GG identified CXXX (6)

F identified CXC (3)

F identified C (12)

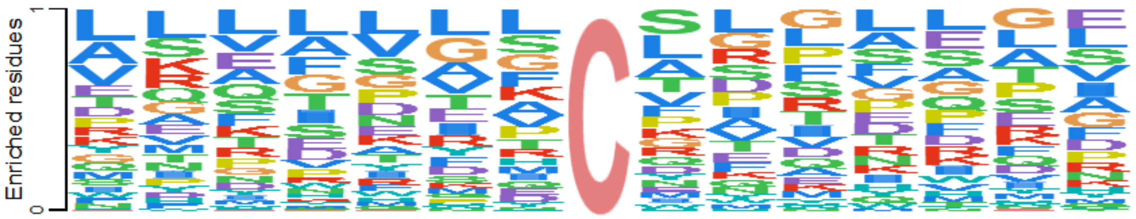
GG identified C (4)

Supplementary Figure S14: Sequence logos of the 10 most C-terminal amino acids of the proteins not previously known to be prenylated and identified here as significantly enriched in samples treated with F-Az (F) or GG-Az (GG), separated by the different canonical prenylation motifs.

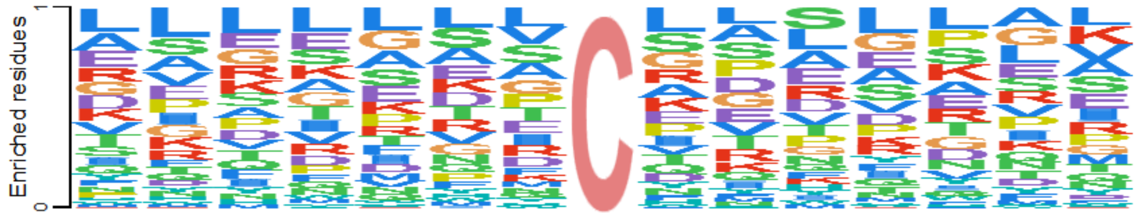


Supplementary Figure S15: A) Histogram of the most C-terminal cysteine positions of all the 935 distinct isoforms of the 519 proteins, which did not contain any canonical prenylation motif in any of their isoforms. B) Histogram of the most C-terminal cysteine positions in 519 proteins without a canonical C-terminal prenylation motif, considering only the canonical isoform or, if it did not contain a cysteine, the isoform with the highest number of cysteines. These consist of 44 proteins with a newly identified C-terminal prenylation motif, 241 proteins with an internal prenylation motif and no alternative isoform, and 234 proteins with an internal prenylation motif and multiple isoforms,

A) pren_all_C1 (N=194)



B) back_all_C1 (N=296)



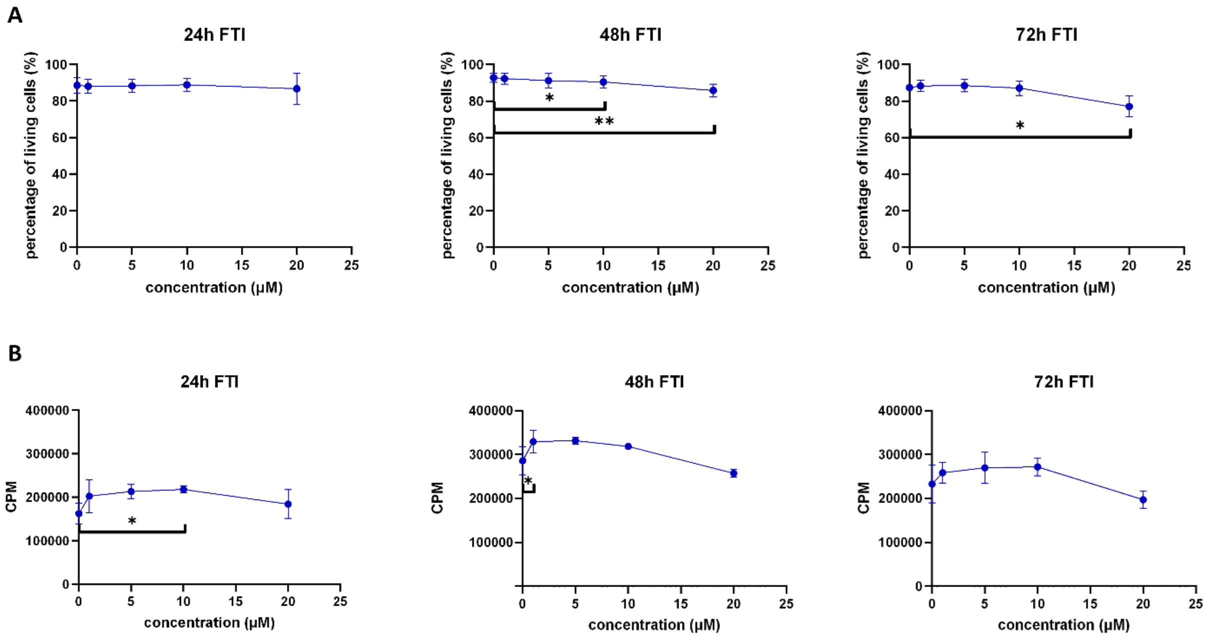
Supplementary Figure S16: A) Sequence logo showing the distribution of the 15 amino acids with the potentially prenylated cysteine in the central position for the 195 internally prenylated proteins for which the potentially prenylated cysteine was neither within the first seven N-terminal amino acids of the protein nor part of a CC, CXC, or CCC motif. B) Same as in A, but for the background proteins that were not identified as prenylated.

A) Farnesylated: 70

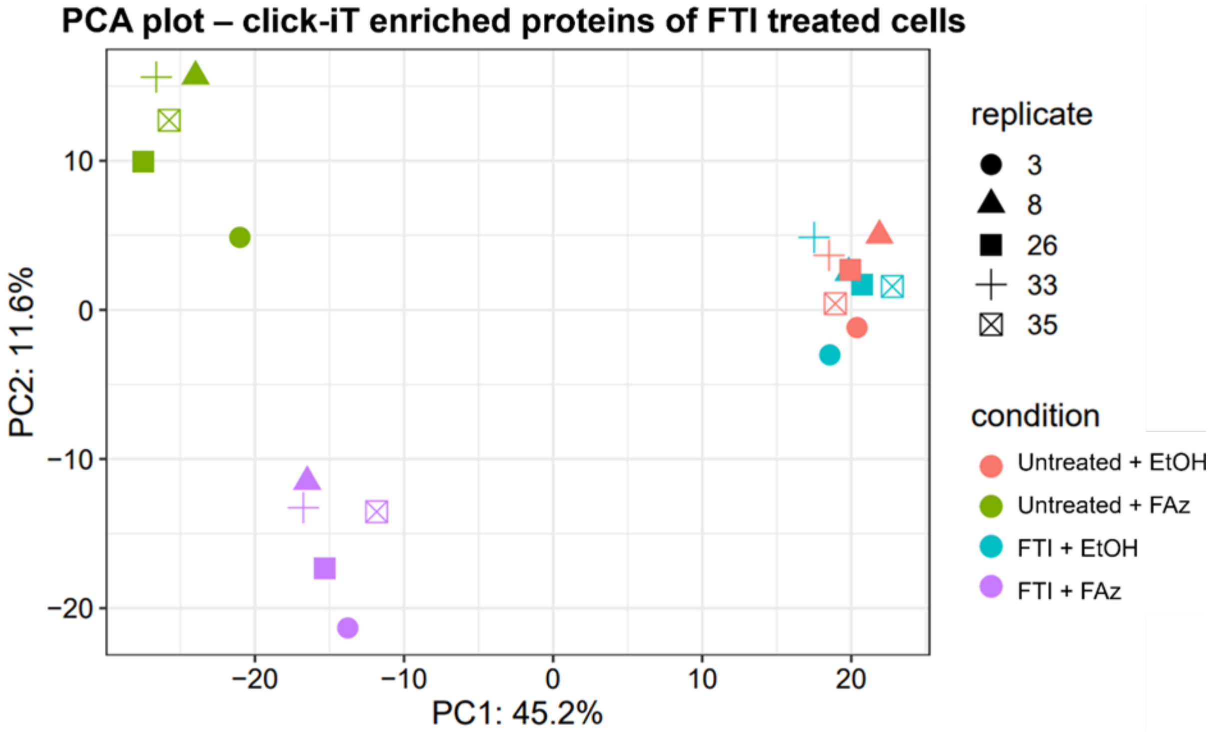
B) Geranylgeranylated: 52

C) Farnesylated & Geranylgeranylated: 73

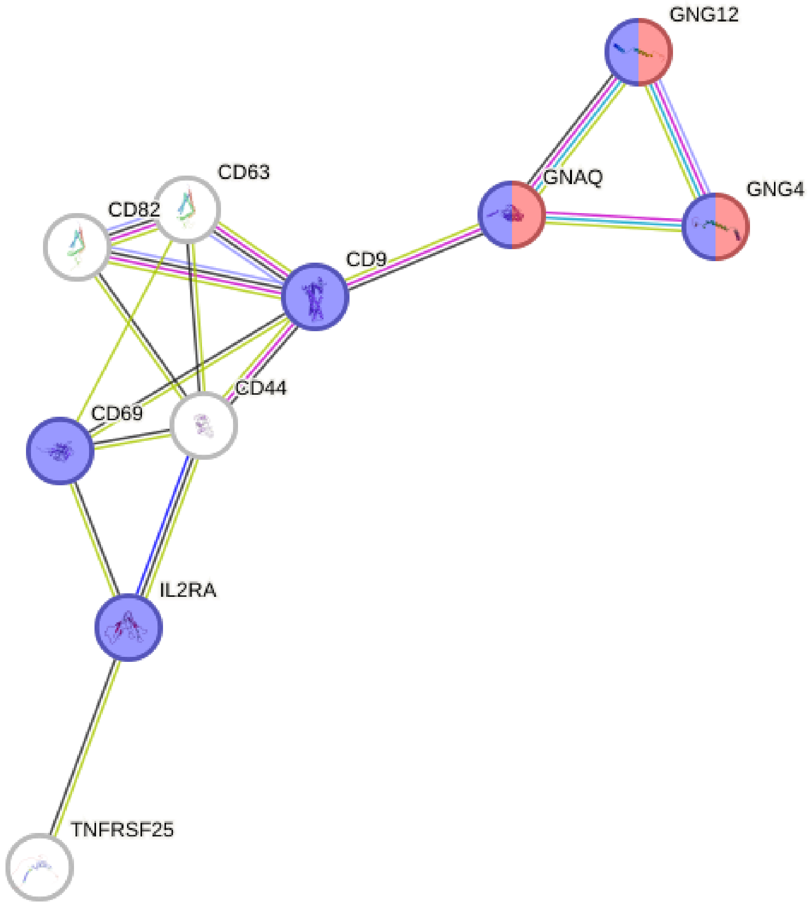
Supplementary Figure S17: Sequence logo showing the distribution of the 15 amino acids with the potentially prenylated cysteine in the central position for the 195 internally prenylated proteins for which the potentially prenylated cysteine was neither within the first seven N-terminal amino acids of the protein nor part of a CC, CXC, or CCC motif, for the prenylated proteins identified A) only in samples treated with F-Az, B) only in samples treated with GG-Az, and C) both in samples treated F-Az or GG-Az.



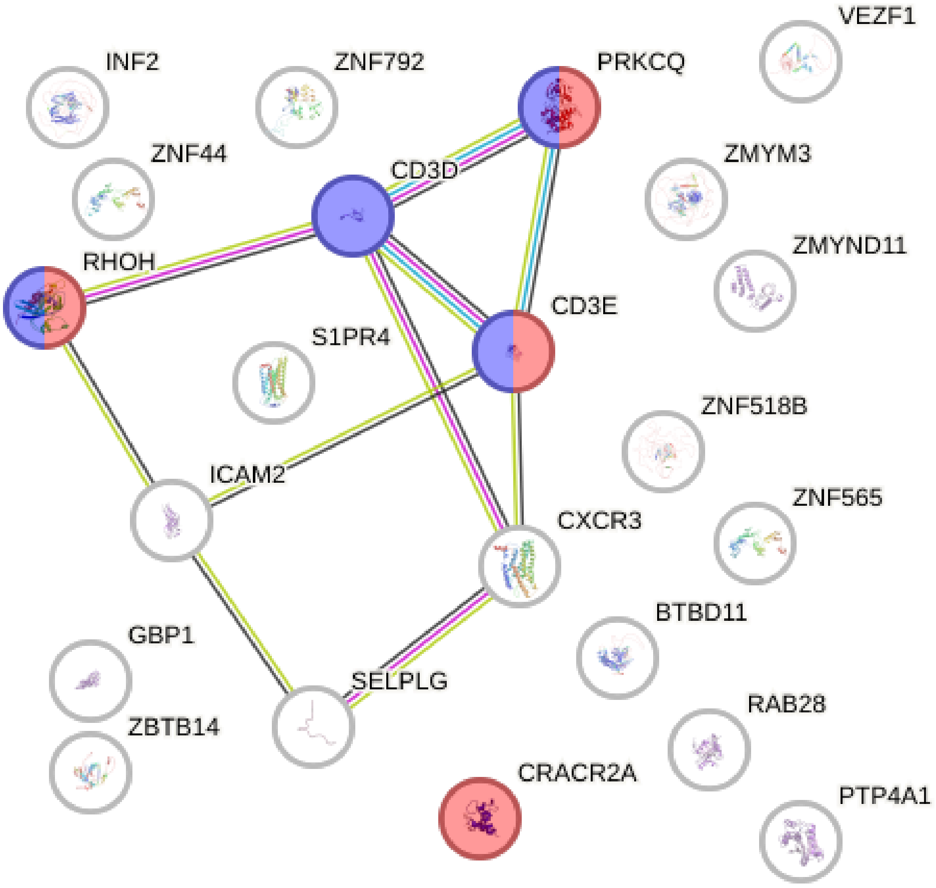
Supplementary Figure S18: Optimal concentrations of the farnesyl transferase inhibitor FTI-277 were assessed over 1–3 days using inhibition of proliferation with minimal cell death as the readout using *in vitro*-differentiated Th1 cells. Analysis of A) cell proliferation with the H3-thymidine incorporation assay and of B) cell viability with the live dead staining using propidium iodide in cells treated with FTI-277 at the indicated concentrations for 24, 48, and 72 hours. For each concentration, samples from each donor were measured in three technical replicates, and the mean of three donors ± standard deviation is shown (** = p-value < 0.01, * = p-value < 0.05); CPM: counts per minute.



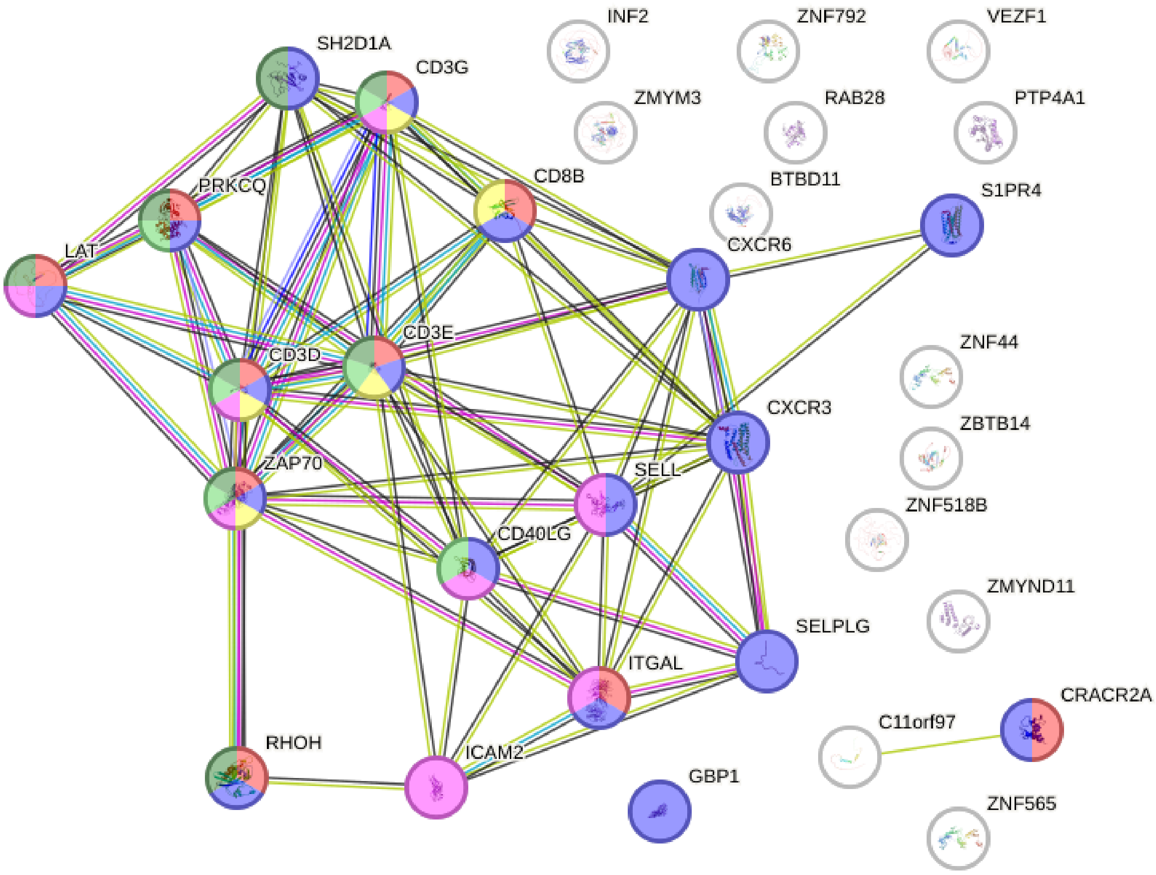
Supplementary Figure S19: Principal Component Analysis (PCA) of prenylation enrichment samples from five different donors, showing PC1 and PC2. In vitro-differentiated Th1 cells were either pre-treated or not with 20 µM farnesyl transferase inhibitor (FTI-277) for 1 hour, followed by incubation with F-Az or ethanol (mock treatment) for an additional 23 hours.



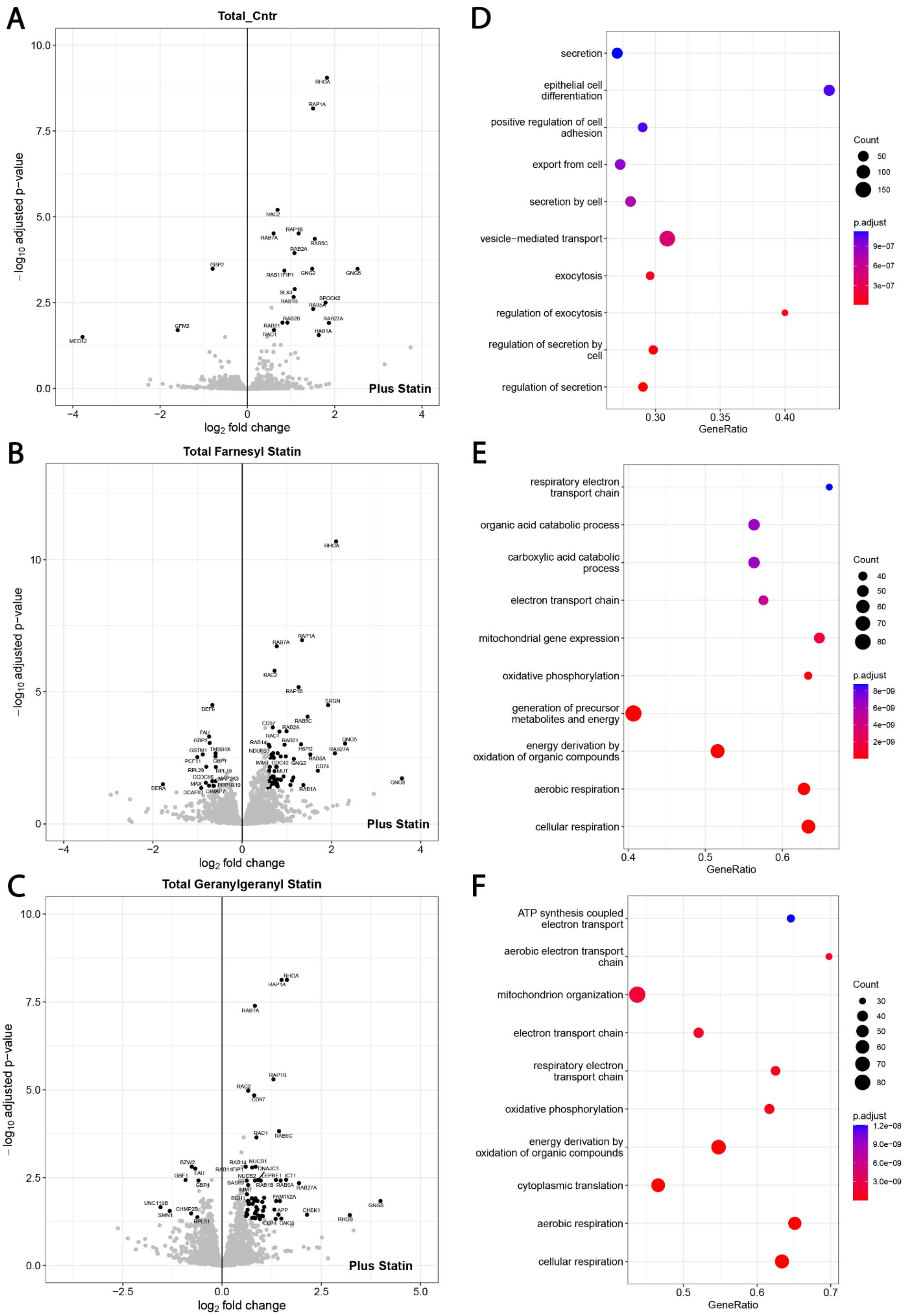
Supplementary Figure S20: Cluster 3 from the STRING analysis of the proteins showing increased prenylation in activated Th1 cells compared to non-activated Th1 cells. Proteins annotated to the GO Cellular Component terms “Heterotrimeric G-protein complex” and “Side of membrane” are shown in red and purple, respectively.



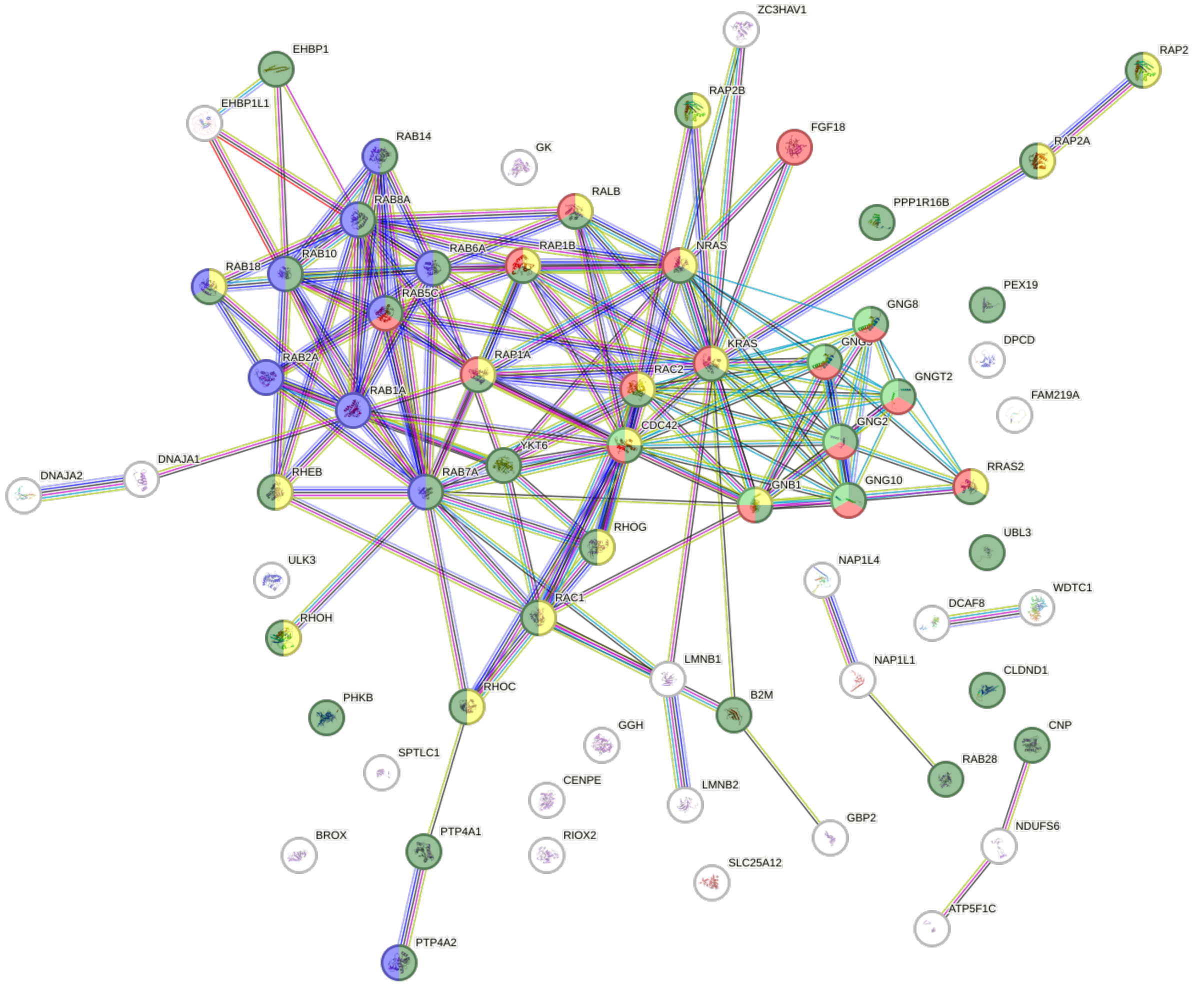
Supplementary Figure S21: STRING network analysis of the 22 proteins with increased prenylation in non-activated Th1 cells compared to activated Th1 cells. Proteins annotated with the overrepresented GO Biological Process term “Immunological synapse” are shown in red, and those associated with the WikiPathway “Modulators of TCR signalling and T cell activation” in purple.



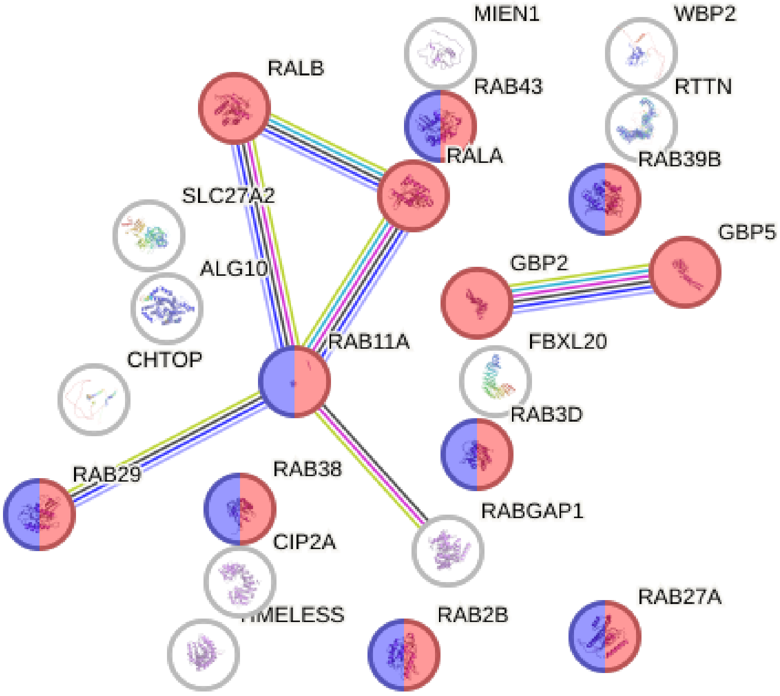
Supplementary Figure S22: STRING network analysis of the 22 proteins with increased prenylation in non-activated Th1 cells compared to activated Th1 cells plus 10 interactors. Proteins annotated with the overrepresented GO Biological Process terms “T cell activation” and “Immune system process” are shown in red and purple, respectively, those associated with the GO Cellular Component term “T cell receptor complex” in yellow, those in the WikiPathway “Modulators of TCR signalling and T cell activation” in dark green, and those in the KEGG Pathway “T cell receptor signalling pathway” in light green.



Supplementary Figure S23: Proteome changes in response to statin treatment. (A–C) Volcano plots of total extracts comparing protein levels in (A) mock-treated Th1 cells and in cells treated with (B) F-Az or (C) GG-Az, in activated Th1 cells with or without statin pretreatment. (D–F) Dot plots of GSEA results analysing the proteins from (A–C).



Supplementary Figure S24: STRING network analysis of the 67 significantly enriched proteins in statin-pretreated, F-Az-treated samples. Proteins annotated with the most overrepresented GO Biological Process term “Small GTPase mediated signal transduction” are shown in yellow, those associated with the most overrepresented GO Cellular Component term “Plasma membrane” in dark green, those in the most overrepresented KEGG Pathway “Ras signalling pathway” in red, and those in the two most overrepresented Reactome Pathways “RAB geranylation” and “G beta:gamma signalling through CDC42” in purple and green, respectively.



Supplementary Figure S25: STRING network analysis of the 22 significantly enriched proteins in non-statin-pretreated, GG-Az-treated samples. Proteins annotated with the most overrepresented GO Molecular Function term “GTPase activity” are shown in red, and those associated with the most Reactome Pathway “RAB geranylation” in purple.