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SUPPLEMENTAL DATA

S-palmitoylation-related genes in Crohn's disease: Bioinformatic identification and validation

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Full article is available at the following link: S-palmitoylation-related genes in Crohn's disease: Bioinformatic identification and validation

Table S1. qRT-PCR primers used in this study

Gene symbol	Direction	Sequence (5'→3')
ZDHHC23	Forward	AGCTGCGTTGGAGAATCAAATC
	Reverse	AAGGACAGAGCCGAGCTGTA
IFITM1	Forward	ACCCCCAAGTGCCTGAACATC
	Reverse	TCACAGAGCCGAATACCAGTAACAG
GAPDH	Forward	GCTCCGTCAAGGCTGAGAAC
	Reverse	TGGTGAAGACGCCAGTGGA

Table S2. Twenty-three SP-DEGs associated with S-palmitoylation machinery in CD

Gene symbol	logFC	Change	*p* value	Adj. *p* value	Chromosome
PP	0.70071787	Up	0.00000935	0.000265	21q21.3
IFITM1	0.34971408	Up	0.0000162	0.0004	11p15.5
JAM3	0.86852164	Up	0.0000301	0.000639	11q25
ADRA2B	0.48014511	Up	0.000122	0.00175	2p13.2
RHEB	0.45936434	Up	0.000158	0.00211	7q36.3
ZDHHC17	0.58332008	Up	0.000187	0.00239	12q21.31
LAMP2	0.41193953	Up	0.00028	0.00326	Xq24
CD81	0.68590087	Up	0.000747	0.00677	11p15.5
ESR1	0.44759294	Up	0.000823	0.00727	6q25.1
PHGDH	0.55013839	Up	0.00355	0.0218	1p12
ZDHHC1	0.36641346	Up	0.00454	0.026	16q22.1
IGF2R	0.4069581	Up	0.00461	0.0264	6q25.3
KCNMA1	0.60322702	Up	0.00615	0.0327	10q22.3
ACSL5	-0.57398959	Down	0.0000000565	0.00000703	10q25.2
BAX	-0.43345017	Down	0.000355	0.0039	19q13.33
ZDHHC12	-0.4983531	Down	0.000394	0.00422	9q31.3
LYPLA1	-0.37387787	Down	0.000598	0.00574	8q21.3
PRDX5	-0.44751259	Down	0.00245	0.0165	11q13.2
ZDHHC9	-0.54307115	Down	0.0027	0.0177	Xq26.1
TNFRSF6	-0.30684647	Down	0.00335	0.0208	10q24.1

CSNK1G3	-0.31750374	Down	0.0047	0.0267	5q31.2
LCK	-0.45662064	Down	0.0101	0.0467	1p35.2
ZDHHC23	-1.5805629	Down	0.00000188	0.0000836	3p21.31

Abbreviations: SP-DEGs: S-palmitoylation-related differentially expressed genes; CD: Crohn's disease.

Table S3. Standardized effect sizes (Hedge's g)

Gene	Cohort	Hedge's g	CI lower	CI upper	Sample size CD	Sample size control
ZDHHC23	GSE83448	1.347	0.861	1.832	39	14
ZDHHC23	GSE16879	1.777	1.019	2.535	19	6
ZDHHC23	GSE59071	1.653	0.894	2.413	11	8
IFITM1	GSE83448	0.989	0.538	1.441	39	14
IFITM1	GSE16879	1.812	1.047	2.577	19	6
IFITM1	GSE59071	1.758	0.998	2.518	11	8

Table S4. Diagnostic performance of *ZDHHC23* and *IFITM1* across independent cohorts

Gene	*ZDHHC23*	*ZDHHC23*	*ZDHHC23*	*IFITM1*	*IFITM1*	*IFITM1*
Cohort	GSE83448	GSE16879	GSE59071	GSE83448	GSE16879	GSE59071
AUC	0.912	0.956	0.943	0.842	0.965	0.955
95% CI	0.812-1.000	0.864-1.000	0.841-1.000	0.733-0.952	0.901-1.000	0.859-1.000
Cut-off	10.572	7.773	8.8448	15.597	11.426	10.009
Sensitivity	0.97436	1	0.90909	0.69231	0.89474	0.90909

Specificity	0.78571	0.83333	0.875	0.85714	1	1
Accuracy	0.92453	0.96	0.89474	0.73585	0.92	0.94737
TP	38	19	10	27	17	10
TN	11	5	7	12	6	8
FP	3	1	1	2	0	0
FN	1	0	1	12	2	1
PPV	0.92683	0.95	0.90909	0.93103	1	1
NPV	0.91667	1	0.875	0.5	0.75	0.88889
Youden's J	0.76007	0.83333	0.78409	0.54945	0.89474	0.90909

AUC: Area under the ROC curve; 95% CI: 95% confidence interval for AUC; Cut-off: Optimal threshold determined by maximizing Youden's J index; TP: True positive; TN: True negative; FP: False positive; FN: False negative; PPV/NPV: Calculated based on observed disease prevalence in each cohort; Youden's J: Youden's index (Sensitivity + Specificity - 1).

Table S5. Pairwise comparisons of AUCs between cohorts using DeLong's test

Gene	Cohort comparison	DeLong's test statistic	*p* value
ZDHHC23	GSE83448 vs GSE16879	-0.748	0.454
ZDHHC23	GSE83448 vs GSE59071	-0.497	0.619
ZDHHC23	GSE16879 vs GSE59071	0.245	0.806
IFITM1	GSE83448 vs GSE16879	1.677	0.0935
IFITM1	GSE83448 vs GSE59071	-1.704	0.0884
IFITM1	GSE16879 vs GSE59071	0.229	0.819

DeLong's test was used to compare AUC values between cohorts for each gene

No statistically significant differences were observed in diagnostic performance across cohorts for either gene (all $p > 0.05$).

Table S6. Standardized effect sizes (Hedge's g)

Gene	Immune cell	Algorithm	Correlation coefficient	CI lower	CI upper	Sample size
ZDHHC23	Tregs	CIBERSORT	0.45	0.209	0.641	53
ZDHHC23	M0 Macrophages	CIBERSORT	-0.38	-0.591	-0.13	53
ZDHHC23	Activated Dendritic Cells	CIBERSORT	-0.35	-0.567	-0.095	53
IFITM1	Tfh	CIBERSORT	0.42	0.174	0.617	53
IFITM1	Tregs	CIBERSORT	-0.51	-0.689	-0.283	53

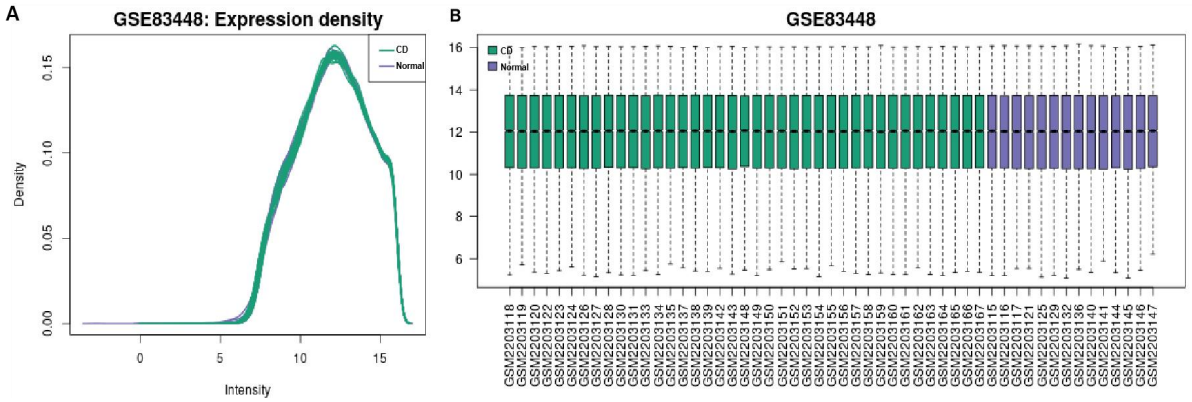

Figure S1. Evaluation of data homogeneity and absence of batch effects. (A) Density plot illustrating gene expression profiles across all samples. The overlapping curves signify a consistent and uniform distribution, indicating successful normalization. (B) Boxplot displaying expression distributions for all samples. The similar medians and interquartile ranges throughout the dataset confirm the absence of significant technical batch effects.

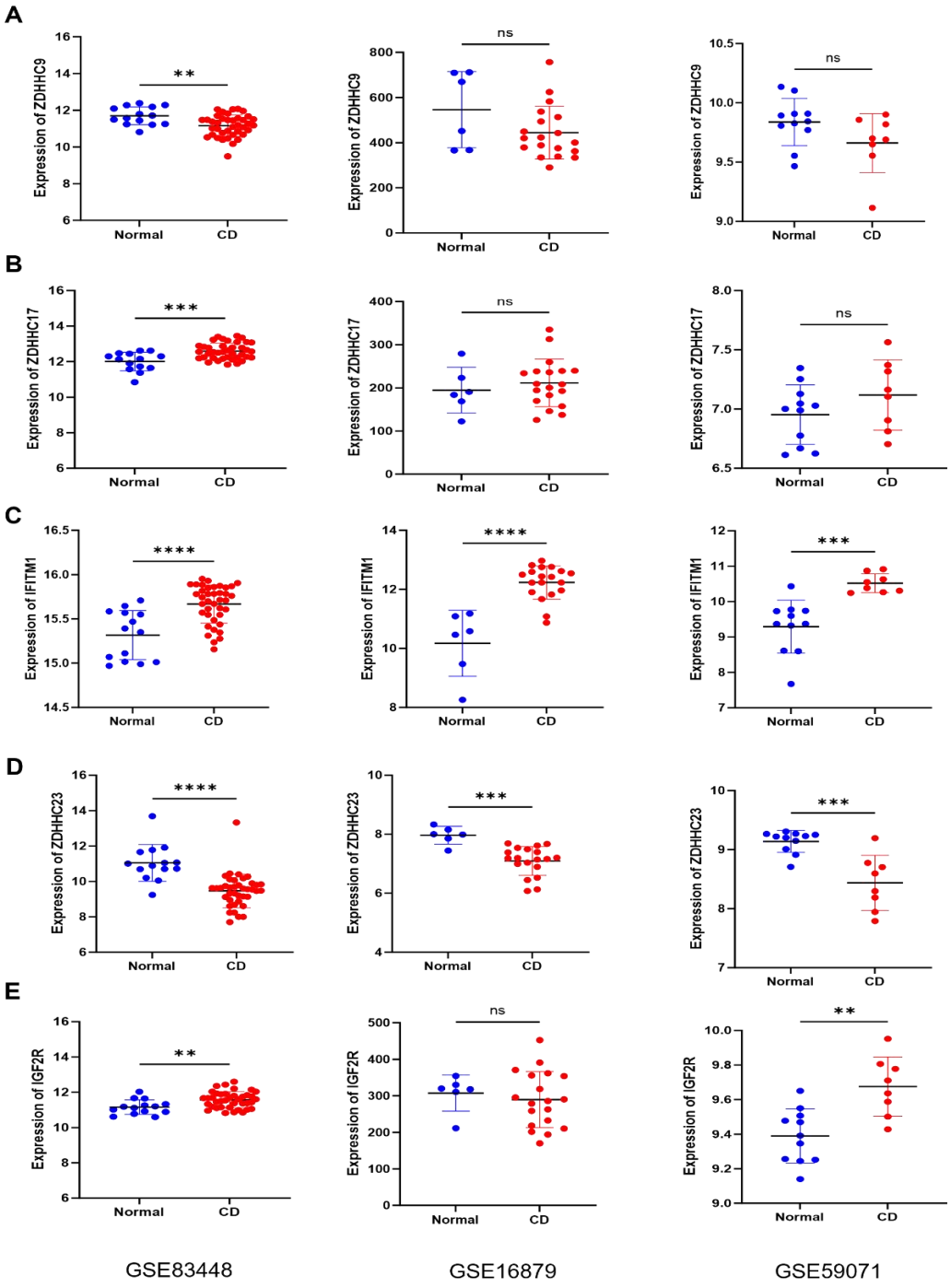

Figure S2. A multi-cohort validation of expression patterns was conducted for the top five ranked genes (*ZDHHC9, ZDHHC17, ZDHHC23, IFITM1, IGF2R*) among the nine hub candidates. Expression levels were examined in the discovery cohort (GSE83448) and validated in two independent cohorts (GSE16879 and GSE59071), confirming decreased *ZDHHC23* and increased *IFITM1* in CD compared with controls. **p*<0.05, ***p*<0.01, ****p*<0.001, *****p*<0.0001

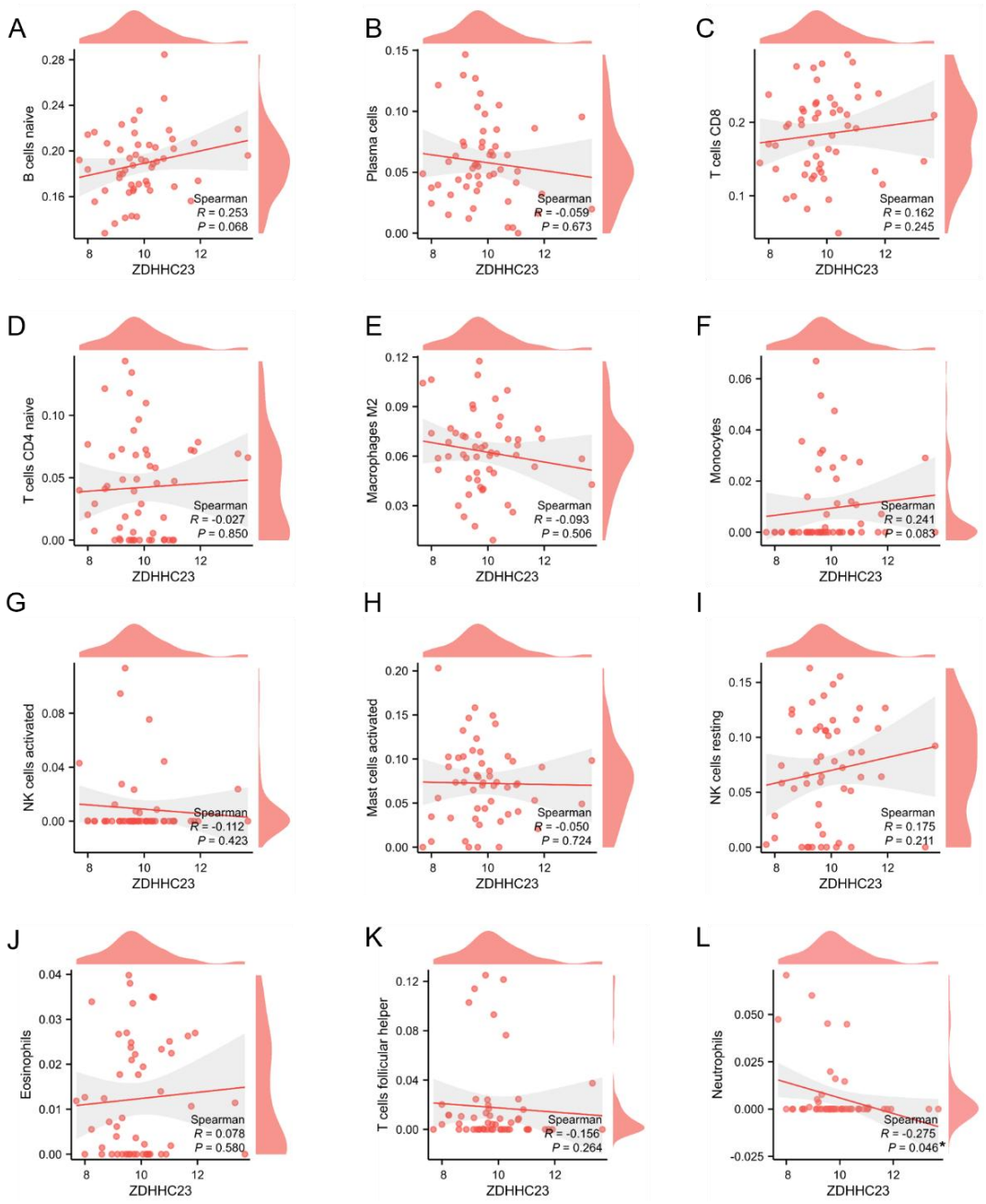

Figure S3. Correlation analysis between *ZDHHC23* expression and immune cell infiltration profiles included twelve subsets: (A) naive B cells, (B) plasma cells, (C) $CD8^+$ T cells, (D) naive $CD4^+$ T cells, (E) M2 macrophages, (F) monocytes, (G) activated NK cells, (H) activated mast cells, (I) resting NK cells, (J) eosinophils, (K) follicular helper T cells, and (L) neutrophils. Asterisks (*) indicate correlations that remained statistically significant after false discovery rate (FDR) correction for multiple testing across all gene-cell pairs within each algorithm ($q < 0.05$).

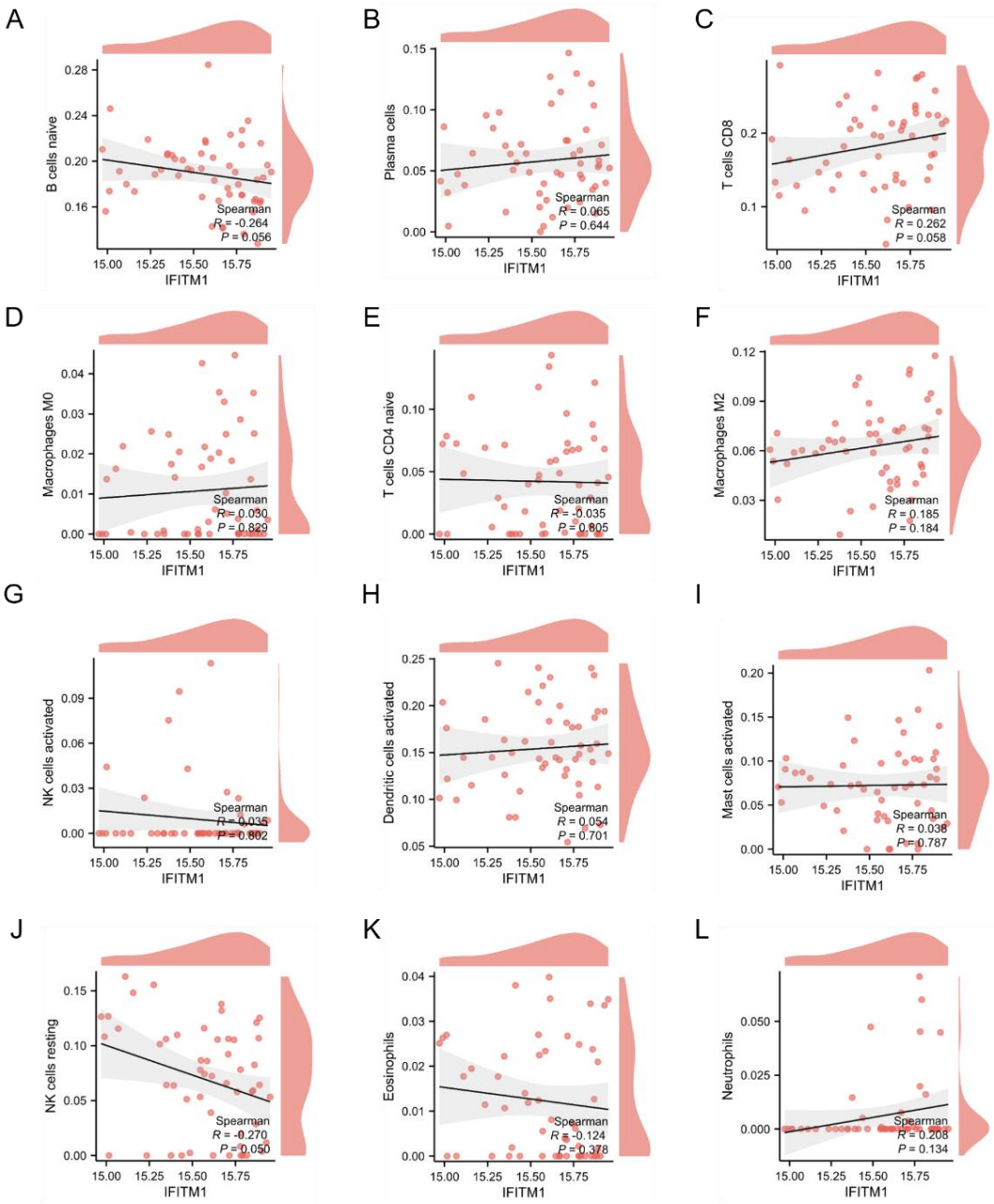

Figure S4. Correlation analysis between IFITM1 expression and immune cell infiltration profiles encompassed twelve subsets: (A) naive B cells, (B) plasma cells, (C) $CD8^+$ T cells, (D) M0 macrophages, (E) naive $CD4^+$ T cells, (F) M2 macrophages, (G) activated NK cells, (H) activated dendritic cells, (I) activated mast cells, (J) resting NK cells, (K) eosinophils, and (L) neutrophils. After applying false discovery rate (FDR) correction for multiple testing across all gene–cell pairings, none of the correlations remained statistically significant ($q < 0.05$).