Supplementary Information

"Pathogenic Role of Cytoplasmic IKKα in Meibomian Gland Dysfunction and Dry Eye Disease". Verónica A. García-García V. A. et al.

Supplementary Figures and Tables

Supplementary Fig. S1. Progressive acinar cell hyperplasia, hyperproliferation, and inflammation in the meibomian glands of C-IKKα mice. A MGs from 2-month-old C-IKKα mice show structural disorganization, with inter-acinar (blue/white arrowhead) and intra-acinar (orange arrowhead) inflammation, as well as basal cell hyperplasia (red/white arrow). At 12 months, most acini consist primarily of basal acinar cells (red/white arrow) with few differentiated meibocytes (black arrows), abundant inflammatory foci (blue/white arrowheads), and evident fibrosis (asterisk). **B** Quantification of BrdU-positive basal acinar cells reveals a significant increase in C-IKKα mice older than 2 months. Data were collected from 3–4 fields per sample (20×), and quantified from microscope images using Fiji, an open-source image analysis software, and are expressed as $BrdU^+$ cells/mm^2. $^{**}p < 0.01$; $^{***}p < 0.005$. Three mice per genotype and age were analyzed; and analyzed by three independent researchers. **C** CD45 immunostaining of MGs from 24-month-old control and C-IKKα mice shows abundant cellular infiltration in the transgenic mice, whereas in controls, inflammation is much lower and primarily confined to the conjunctiva (star). Scale bar: 50 μm.

Supplementary Fig. S2. Apoptosis and necrosis in the meibomian glands of C-IKKα mice. Representative histological images 9-month-old transgenic mice showing: **A** apoptotic basal acinar cells (arrowheads) in the MGs; **B** necrosis (arrows) associated with inflammation. Scale bars: A: 30 μm; B: 60 μm.

Supplementary Fig. S3. Expression pattern of keratin 5 and endogenous IKKα in control mice. **A**, **B** K5 immunostaining in Control (**A**) and C-IKKα (**B**) mice shows keratin expression in basal acinar cells (red arrows) and ducts (d; black arrow) of the meibomian glands. **C** Representative immunostaining with the sc-7182 antibody showing endogenous IKKα expression in basal acinar cells (red arrows) and ducts (black arrow) of control mice. Four to five mice per genotype, aged 1-9 months, were analyzed. Images shown correspond to 1-month-old control mice. Scale bars: A, C, 60 μm; B, 80 μm.

Supplementary Fig. S4. Enrichment of inflammatory pathway–associated gene signatures in C-IKKα mice. GSEA/GOPB of meibomian glands from control and C-IKKα transgenic mice show enrichment of genes involved in inflammatory signaling pathways in transgenic mice. Gene sets were considered significantly dysregulated when *p-adjust* < 0.05 and FDR< 0.25.

Supplementary Fig. S5. Enrichment of gene signatures associated with both innate and adaptive immunity in C-IKKα mice. GOBP analysis of meibomian glands from control and C-IKKα transgenic mice reveals enrichment of genes involved in innate/adaptive immunity pathways, as well as immune activation processes, in the transgenic mice. Gene sets were considered significantly dysregulated when *p-adjust* < 0.05 and FDR< 0.25.

Supplementary Fig. S6. Hallmarks of lipid-related genes are significantly downregulated in the meibomian glands of C-IKKα mice. GOBP analysis reveals downregulation of genes encoding enzymes involved in lipid metabolism in the meibomian glands of C-IKKα transgenic mice. Gene sets were considered significantly dysregulated when *p-adjaust* < 0.05 and FDR< 0.25.

Supplementary Fig. S7. qPCR validation of dysregulated key genes involved in inflammation, immune response, lipid metabolism, and meibocyte differentiation in the meibomian glands of C-IKKα mice. mRNA levels were measured in 9-month-old control and C-IKKα mice (each symbol represents one animal; circle: control mice; squares: transgenic mice); normalized to *Tbp*. **A** Increased central cytokine genes linked to dry eye disease. **B** Elevated H2-Ea/Eb complex components in C-IKKα mice. **C** Upregulation in C-IKKα mice of a 4-gene diagnostic signature distinguishing dry eye patients from healthy subjects. **D** Decreased expression of the key enzymes for meibum production in C-IKKα mice. **E** Increased basal acinar cell marker *Slc1a3* in the MGs of transgenic mice. **F** Reduced expression of early and late meibocyte differentiation markers in C-IKKα mice. **G** Induction of *Itgal*, encoding CD11a, the alpha subunit of the functional LFA-1 integrin, and its ligand *Icam1* in transgenic mice. **H** Upregulation of *Nlrp3* in C-IKKα mice. *p*-values <0.05 were considered significant: ** *p*-value<0.01; *** *p*-value<0.005; **** *p*-value<0.001; ns: not significant. Data are mean ± SD.

Supplementary Fig. S8. Upregulation of additional signaling pathways and functions related to innate and adaptive immune responses in C-IKKα mice. GOBP analysis reveals upregulation of signaling pathways and functions related to innate and adaptive immune responses -including cell adhesion and matrix remodeling- in the meibomian glands and conjunctival tissues of C-IKKα mice. These changes have not been described in human studies of Sjögren's syndrome (SS) or non-SS patients used for comparison with our C-IKKα murine model. Gene were considered significantly upregulated in C-IKKα vs. Control when FC > 2 and *p-adjust* < 0.05.

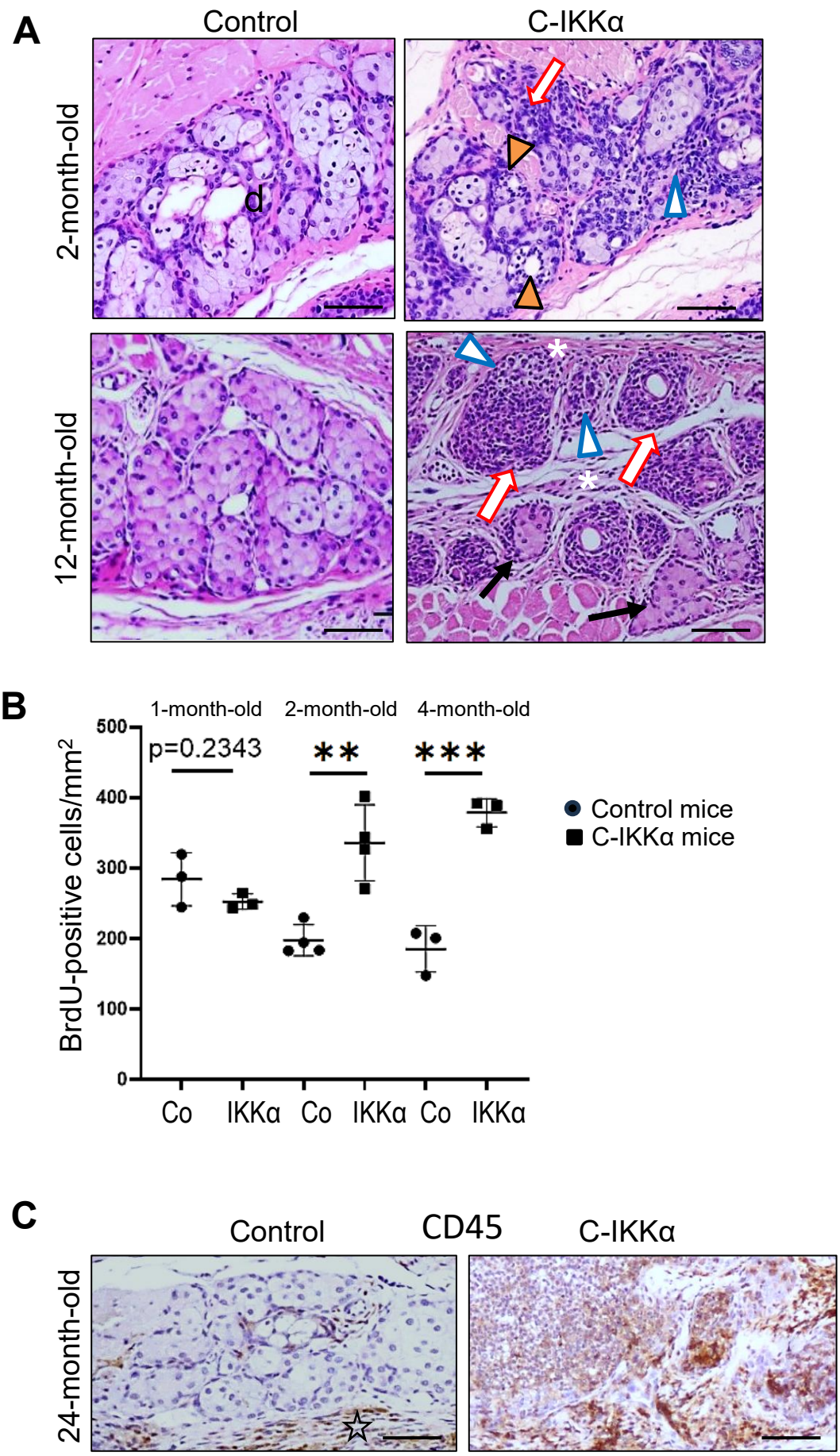
Supplementary Fig. S9. Decreased expression of the desmosomal proteins Desmoglein1 and Plakoglobin in the meibomian glands of C-IKKα mice. Immunohistochemical staining of MG sections from control and C-IKKα mice shows Desmoglein 1 (Dsg1) and Plakoglobin (Pkg) expression at the plasma membrane of all differentiated meibocytes in the acini of control mice. In contrast, in transgenic mice, these proteins are detected in only a few acini containing differentiated meibocytes (arrows). Four to five mice per genotype, aged 2-6 months, were analyzed. Images shown are from 4-month-old mice. Scale bar: 50 µm.

Supplementary Table of primers. The sequences of the primers used in the qPCRs are shown.

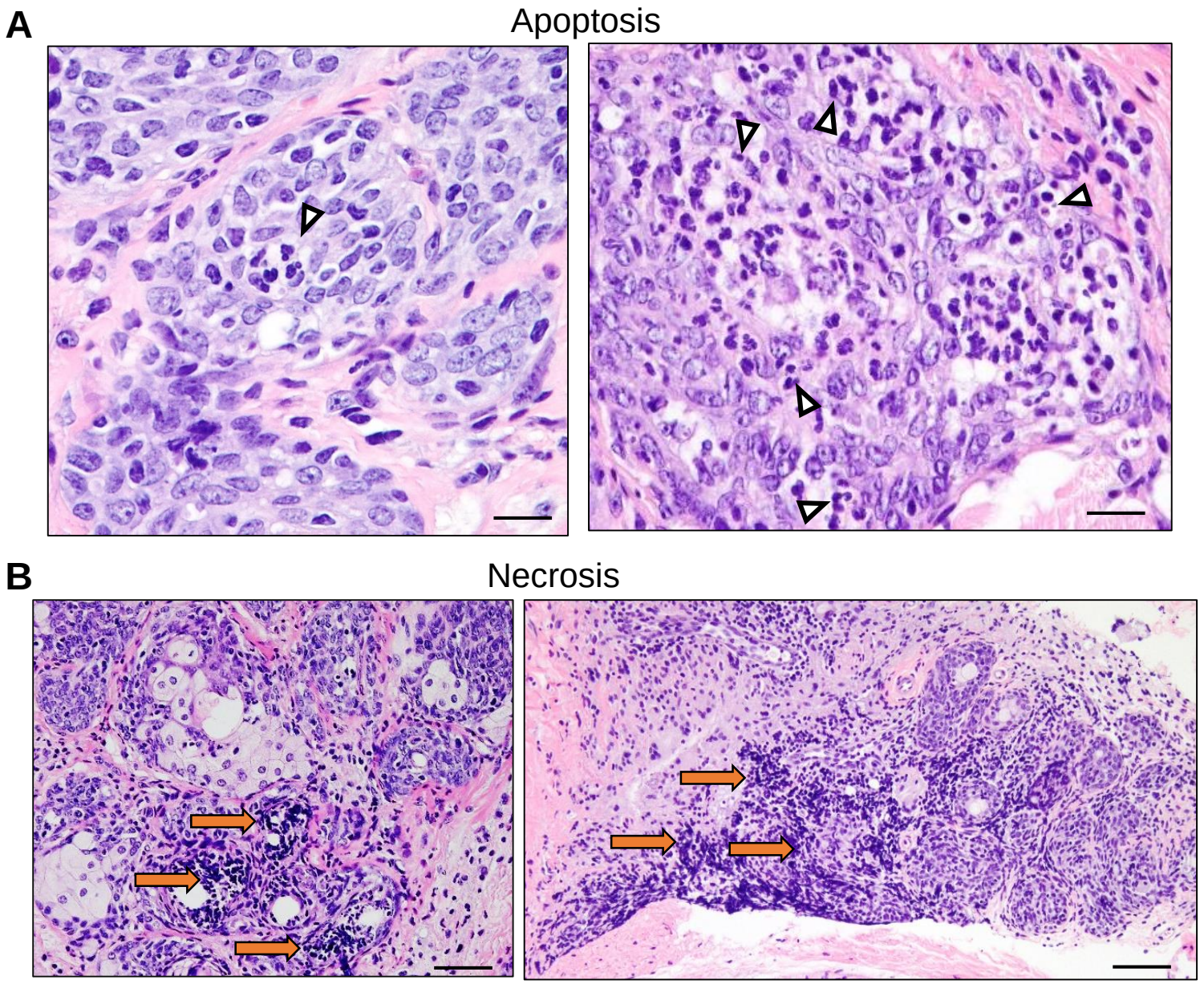
Table S1. Additional upregulated inflammatory/immune cluster genes in the MGs and conjunctiva of C-IKKα mice. Genes highlighted in black are upregulated in C-IKKα mice but were not detected in the SS and non-SS studies used for comparison with our murine model. Genes highlighted in green are shared with at least one of the studies of DED/MGD patients, with or without Sjögren's syndrome (see Table 1). Genes highlighted in blue (*Il17c, Tbx21, Rorc*) are examples mentioned in the text as immunoregulatory genes that are upregulated in C-IKKα mice but were not detected in the referenced human DED samples. Gene were considered significantly upregulated when FC > 2 and p-adjust < 0.05 (C-IKKα vs. Control).

Table S2. Downregulation of genes encoding lipid metabolism-related enzymes in the meibomian glands of C-IKKα mice. Transgenic mice show downregulation of genes involved in processes related to meibum synthesis, including cholesterol and cholesterol esters metabolism, fatty acid biosynthesis and desaturation, fatty acid

elongation, metabolism of odd-chain and branched fatty acids, metabolism of WEs and O-acylated ω-hydroxy fatty acids (OAHFAs), and the regulation of energy balance, lipid storage and mobilization. Underexpressed genes in C-IKKα mice: FC > 2 in Control vs IKKα, *p-adjust* < 0.05.



Supplementary Fig. S1



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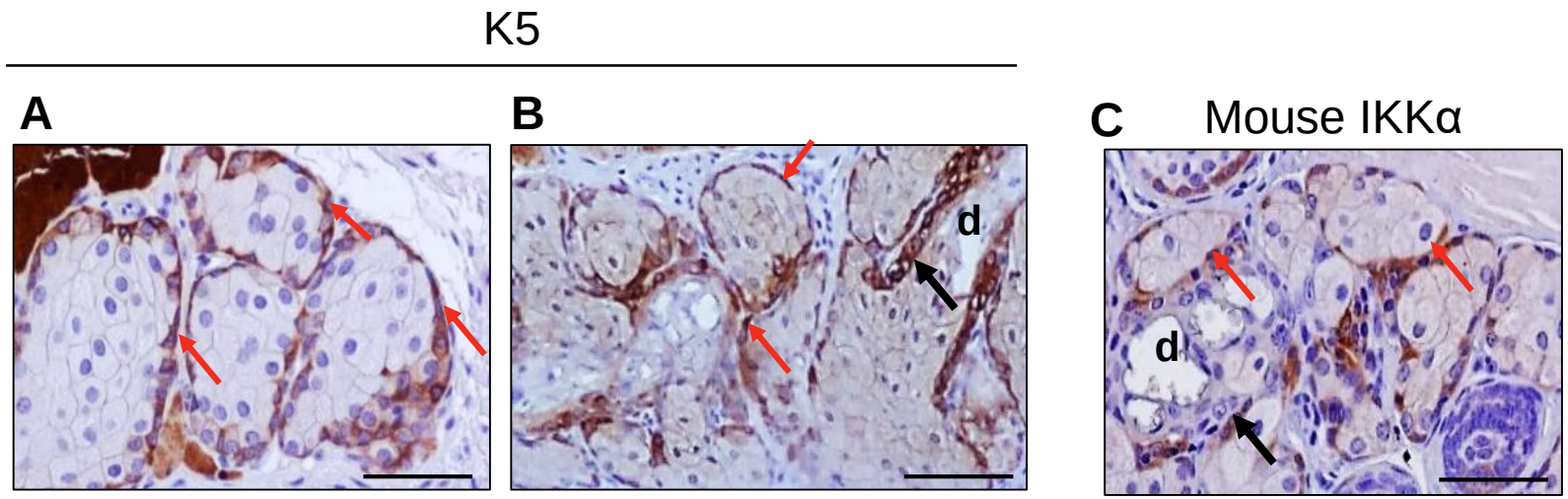

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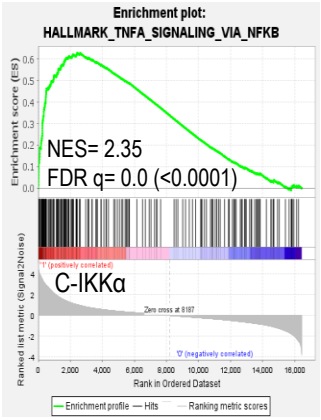
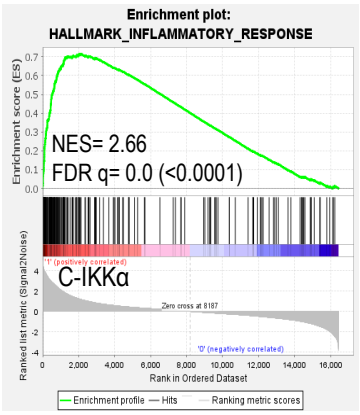
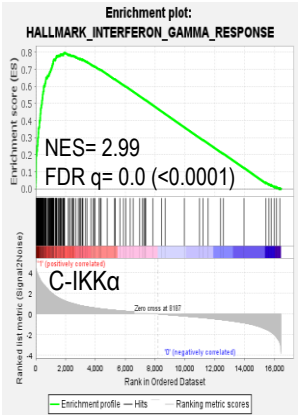
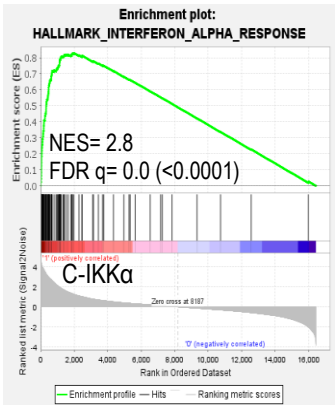
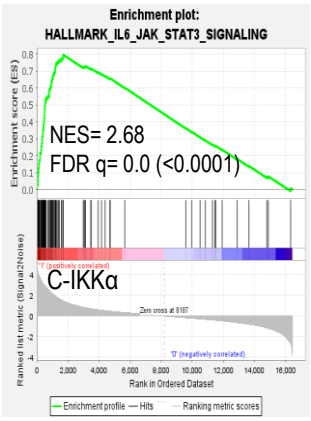
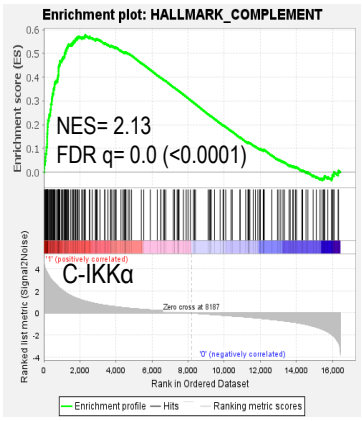


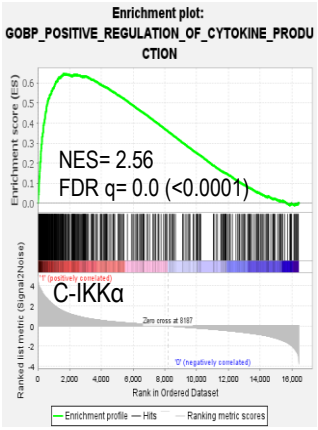


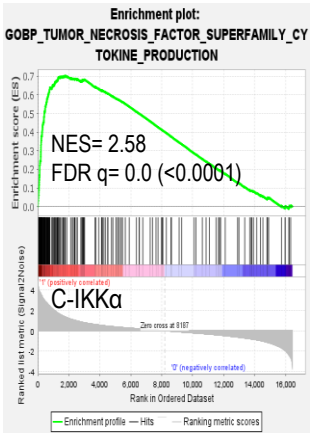


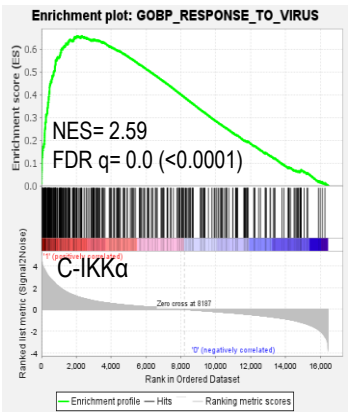


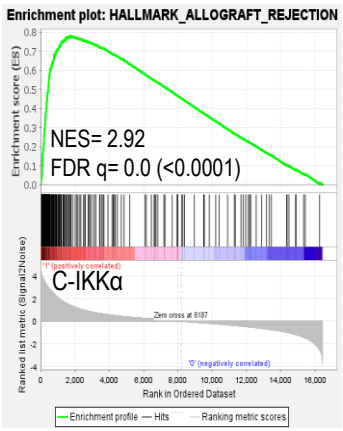


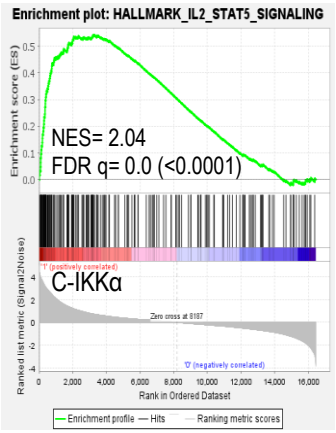




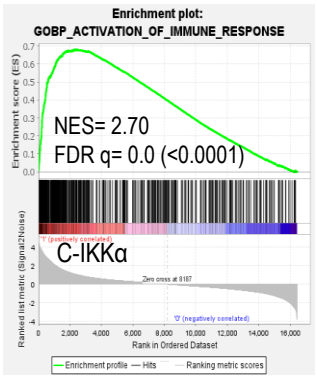


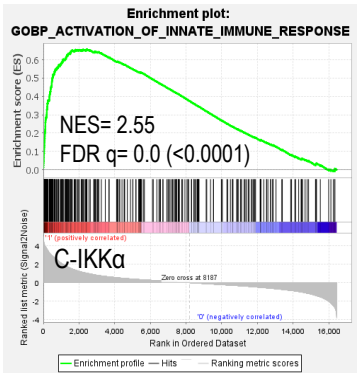


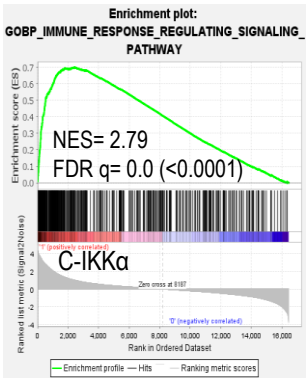


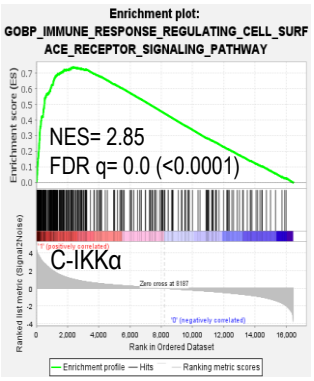


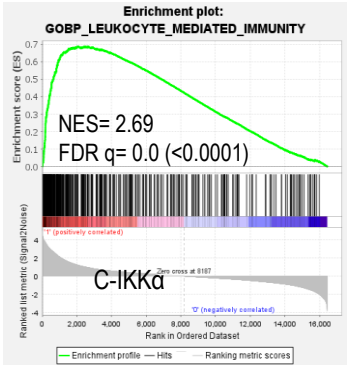
Supplementary Figure S4

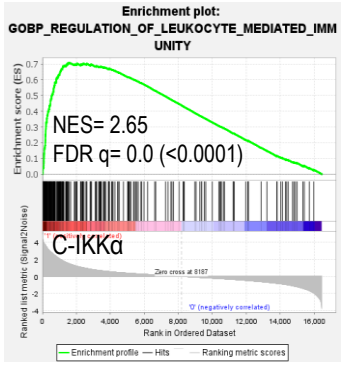


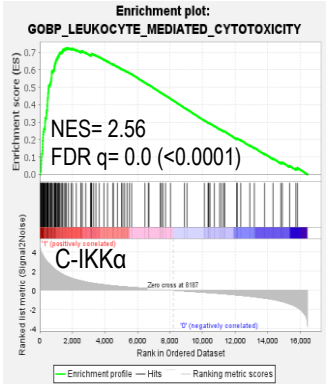


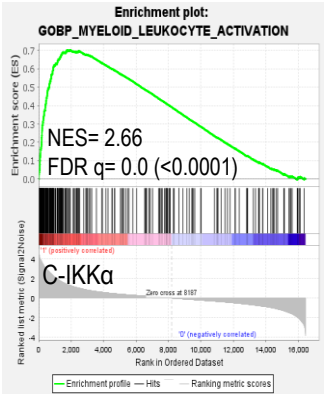


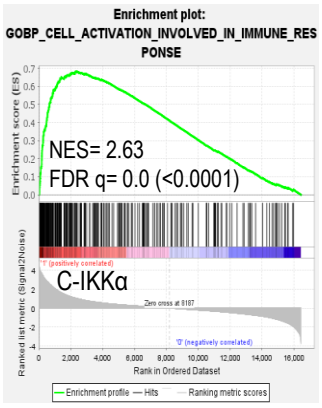


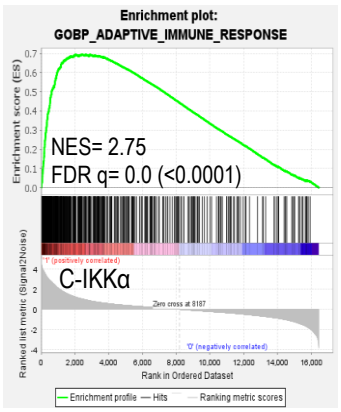


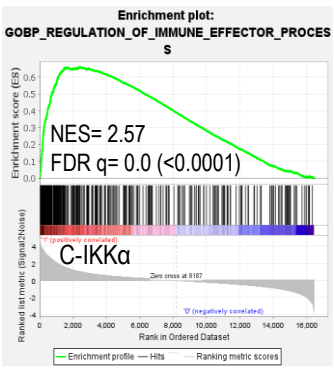


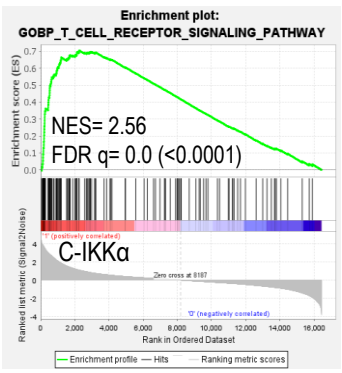


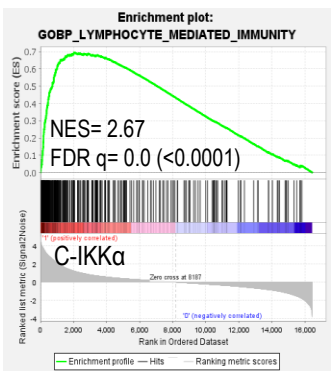


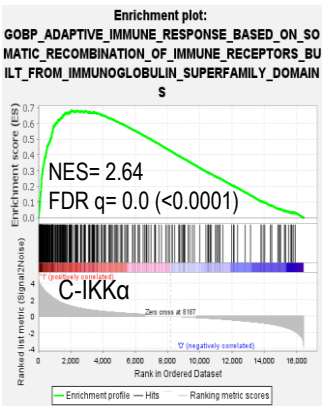


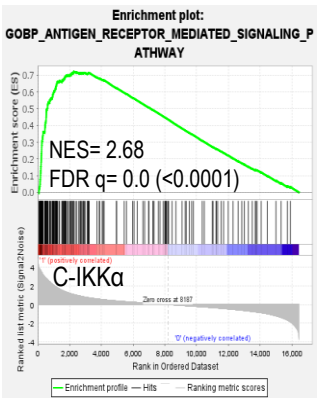




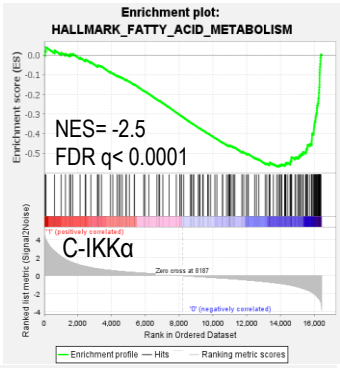


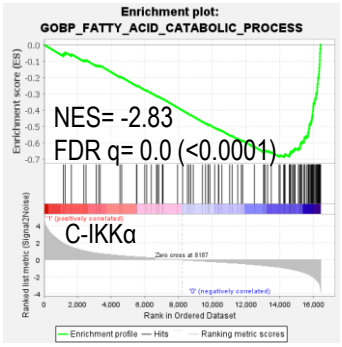


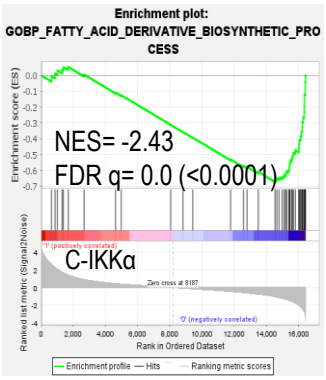


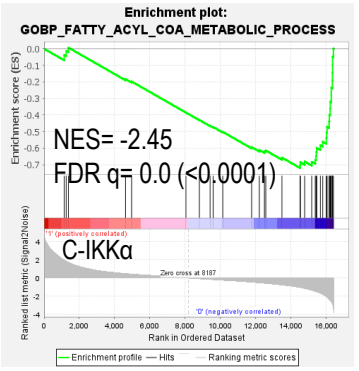


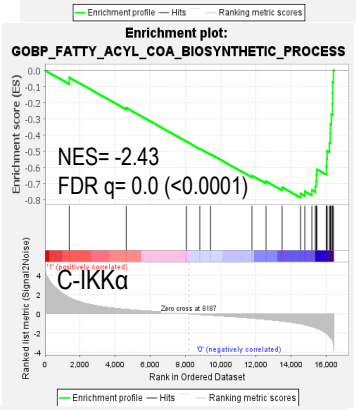
Supplementary Figure S5

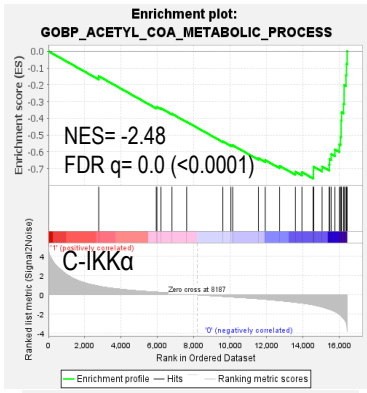


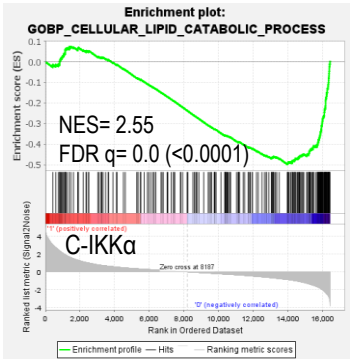


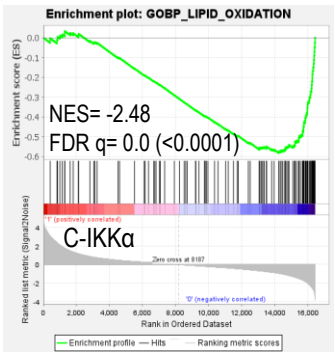


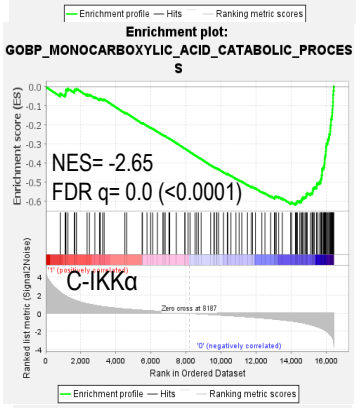


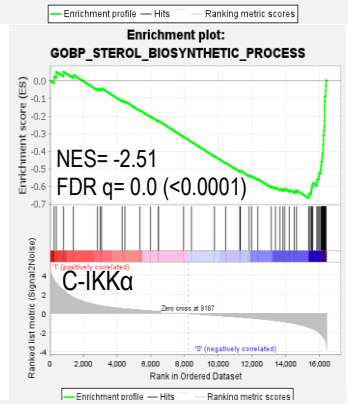


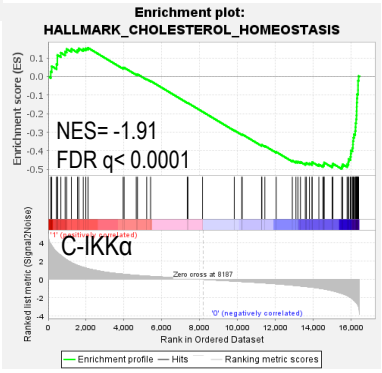


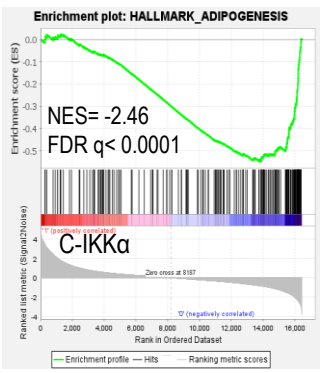


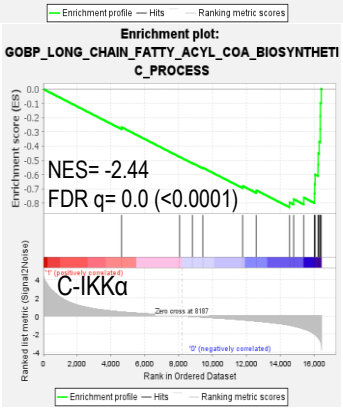


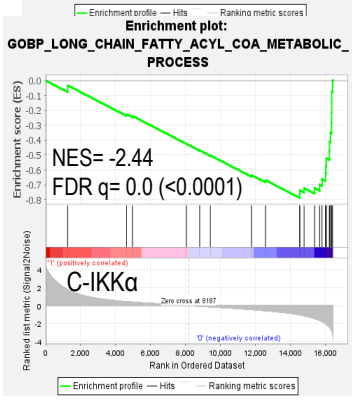


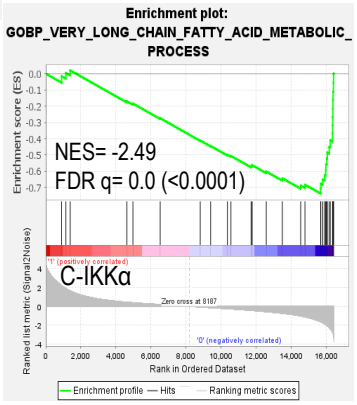


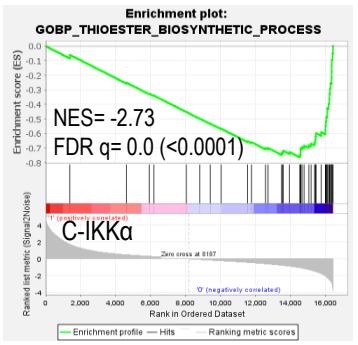


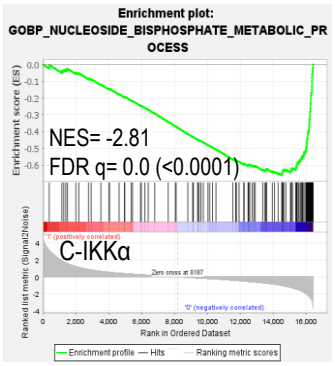


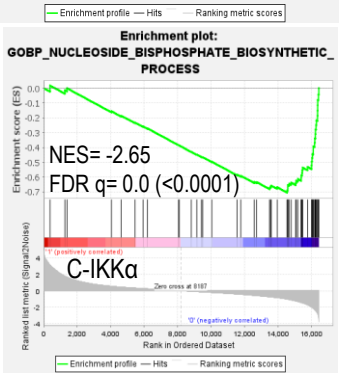




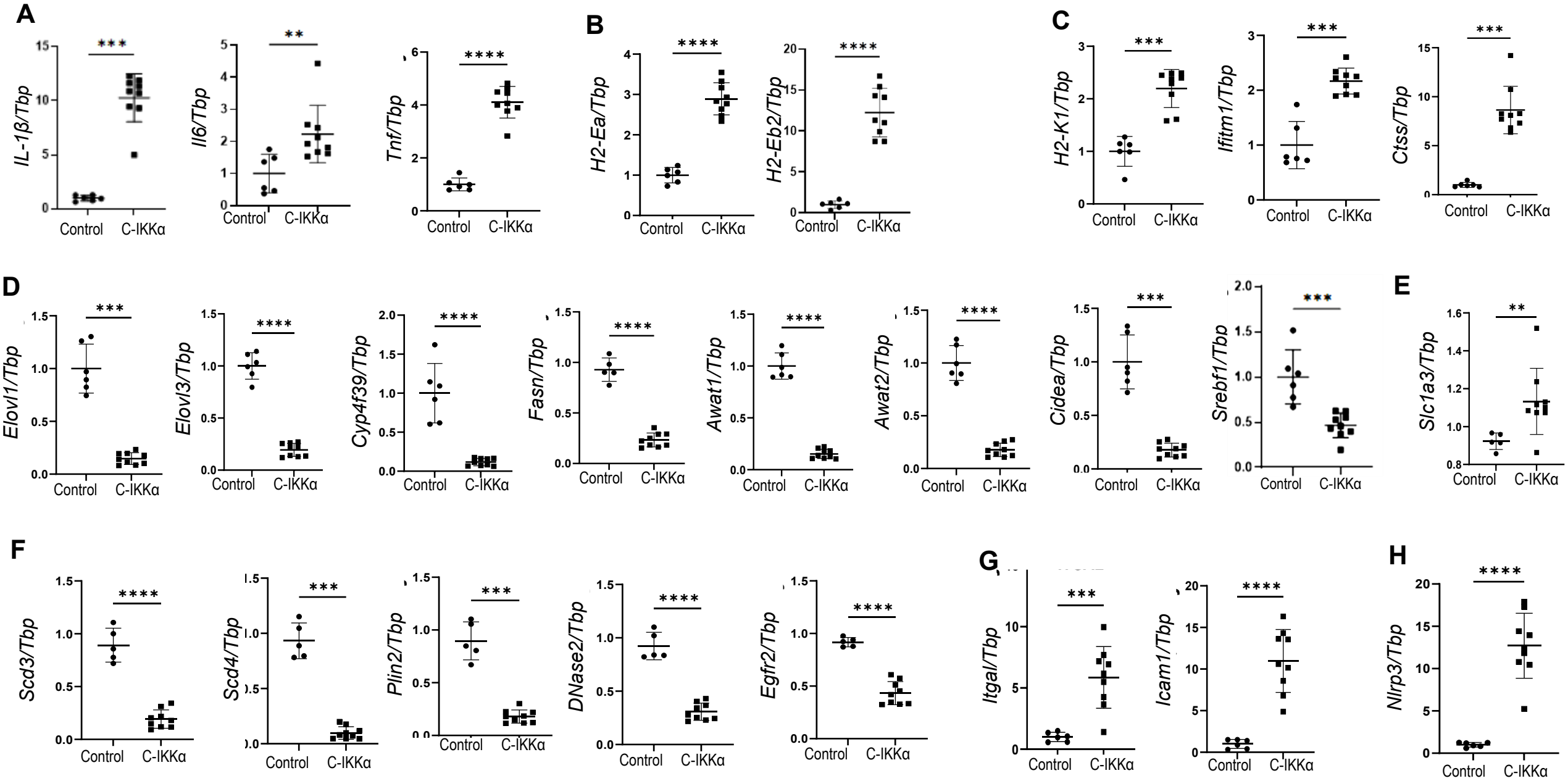






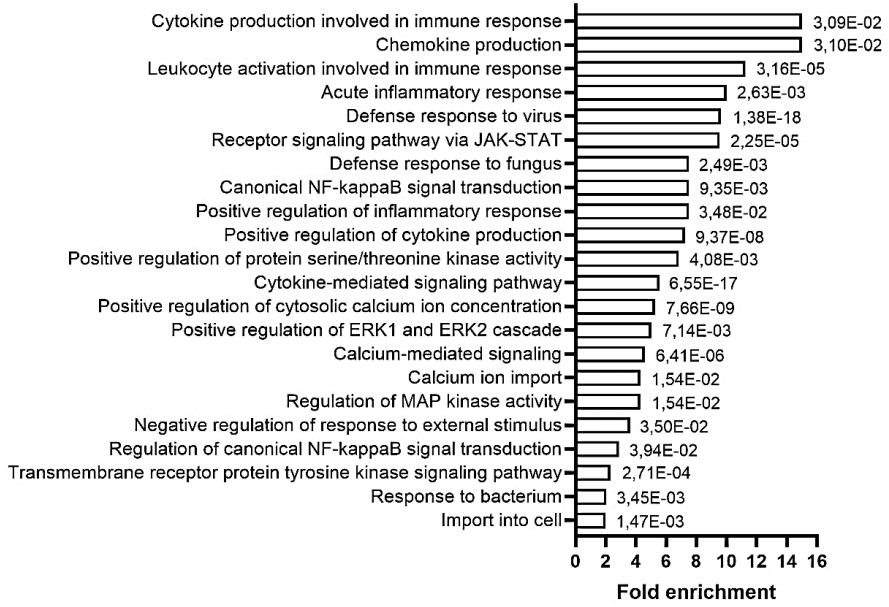


Supplementary Figure S6

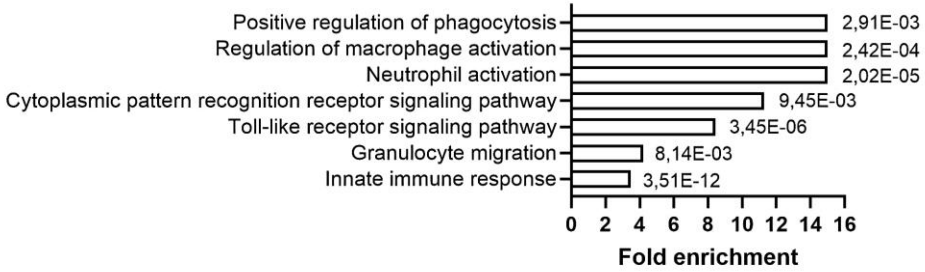


Suppl Figure S7

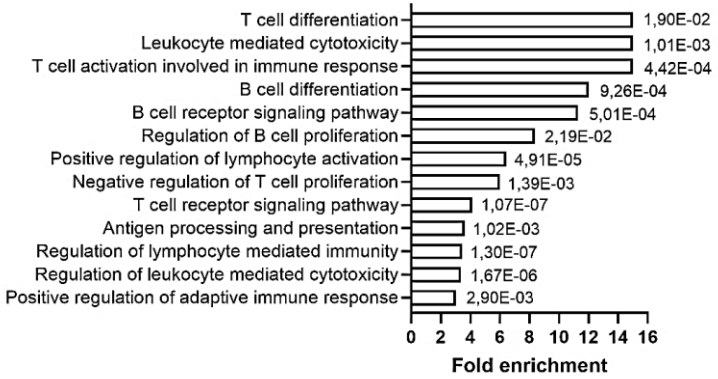
General immune response



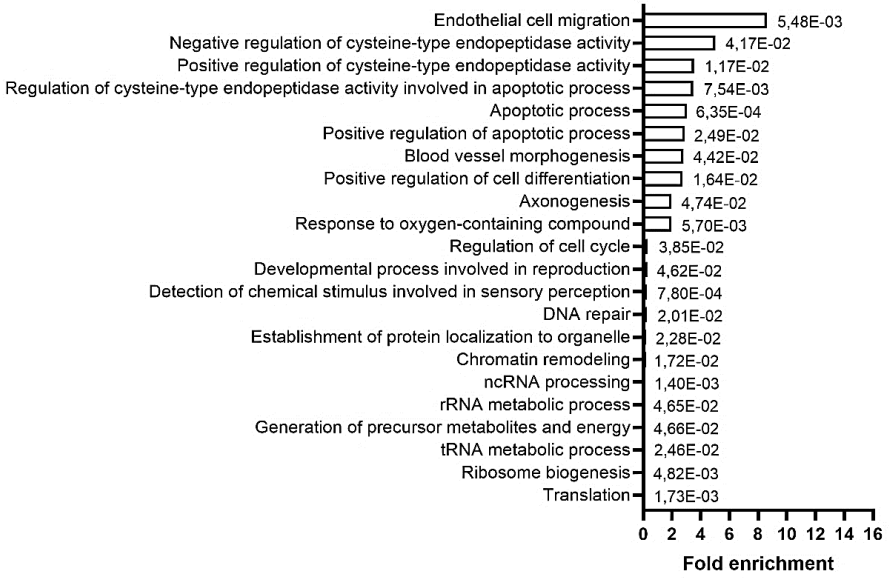
Innate immune response



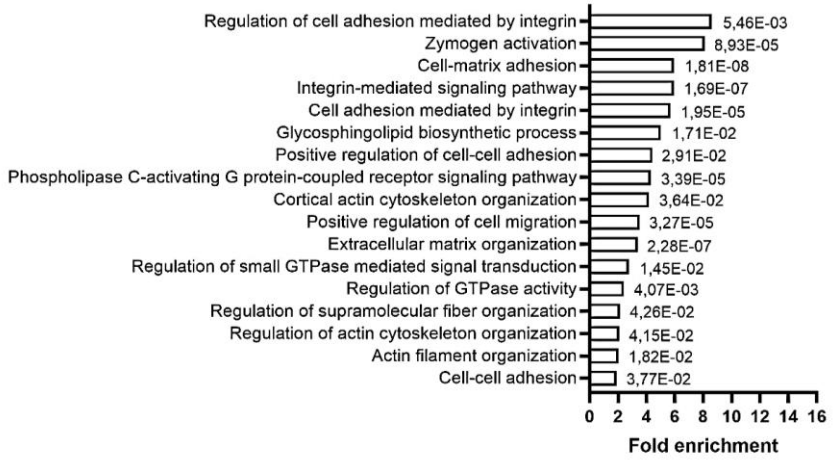
Adaptive immune response



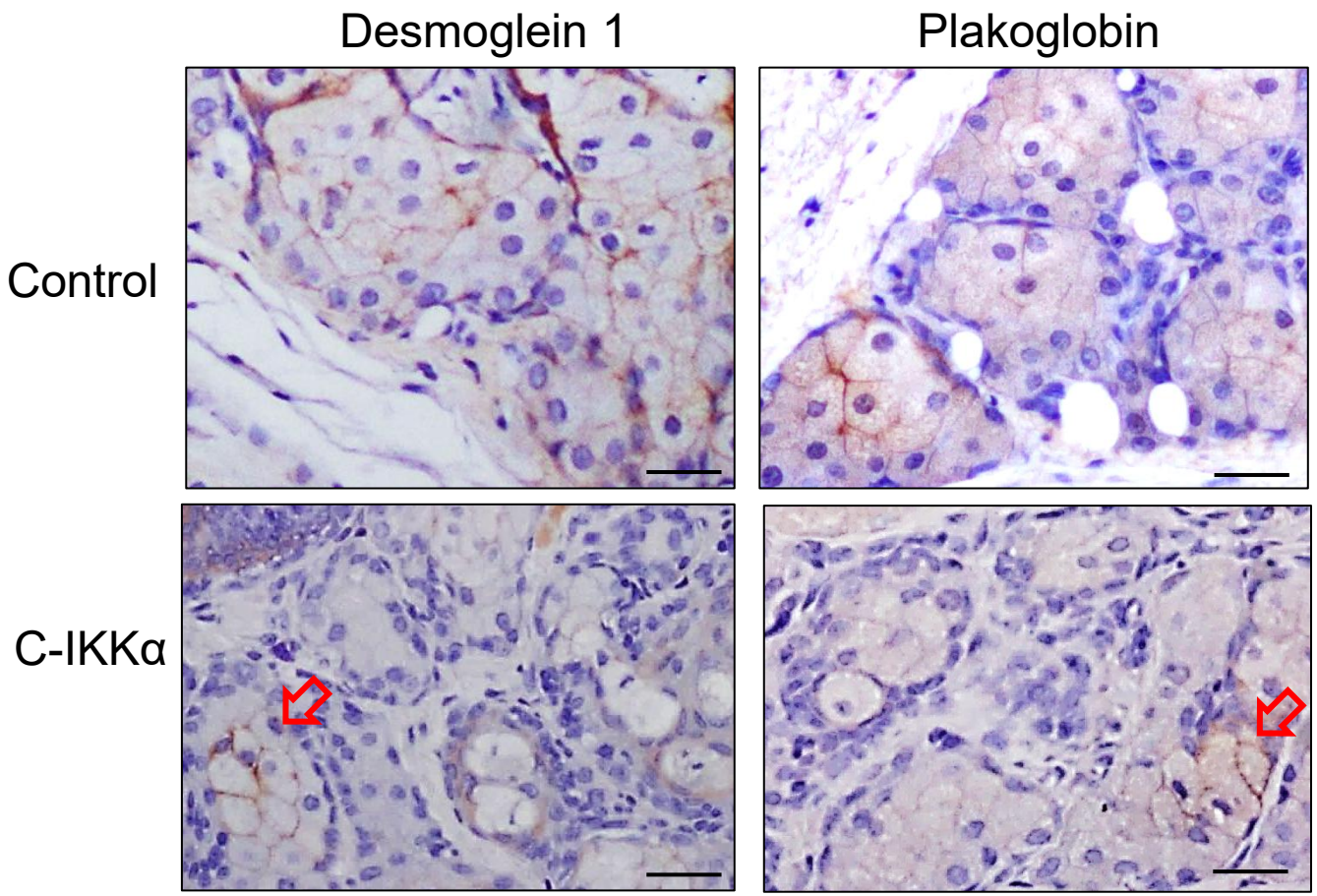
Other processes



Cell adhesión and matrix remodelling



Suppl Fig. S8



Suppl Figure S9

Supplementary Table of Primers. Oligonucleotide sequences used in qPCR analyses.

Gene	Oligonucleotides	
	Forward (5'→3')	Reverse (5'→3')
Tbp	GGGAGAATCATGGACCAGAA	GATGGGAATTCCAGGAGTCA
Pparg	GCTCCAAGAATACCAAAGTGCG	ACAGACTCGGCACTCAATGG
Il1b	TGCCACCTTTTGACAGTGATG	AGCCCTTCATCTTTTGGGGT
Il6	GCTACCAAACTGGATATAATCAGGA	CCAGGTAGCTATGGTACTCCAGAA
Tnf	CAGCCGATGGGTTGTACCTT	TCTTGACGGCAGAGAGGAGG
H2-ea	ACTGTGAGGTGGATCACTGG	AGGGTTTTCTCTTCAAACTCCCA
H2-eb2	CGCTAGGTCTGTTCATCCTCG	GACACTGATCCTGCTCTCAG
H2-k1	TGGAGTGGCTCCGCAGATA	TGGGCCTTTGGGGAATCTGT
Ifitm1	ACTCTTCATGAACTTCTGCTGC	CTTCCTGTCCCTAGACTTCACG
Ctss	CCACGCTGCCATCAGAAG	AGTCCAGGGTAGGGTCTCTC
Ccl4	AACCTAACCCCGAGCAACAC	AGGGTCAGAGCCCATTGGTG
Elovl1	GGTTGGCTGAGTACCTACACC	GGCCACTCGAACCATCCGAA
Elovl3	GCAAGGTTGTTGAACTGGGAGA	CATGAACCAGCCACCCGAAG
Cyp4f39	GTGACTGCCAGGAGAGAATGA	AGGTAGTGATGCAAGCGGTA
Fasn	GGAAGATGCCTTCCGCTACA	CTCCTCCCGTACCTGGACAA
Awat1	GGCCGCTACCAACAGTTTAC	AACGCCTGCCACCTTTATCT
Awat2	CAGCCCTTAAACATGGGGTGT	GGAAGCGATTGACAAAGCCC
Cidea	ACAGAAATGGACACCGGGTA	GACATTGAGACAGCCGAGGA
Slc1a3	TGGACTGGTTTCTGGACCG	GTGCTCGACAATCCCTGCTC
Scd3	CCTGCTGCAAGAAGAGATGAC	CGTCTTCACCTTCTCTCGTCC
Scd4	CTCAACGCTTCACCGATCCT	ACCTGCTGGAGATCTCTTGTG
Plin2	TCTATGTCTCGTGGGTGGAGT	GTGACTCGATGTGCTCAACA
Dnase2a	CGCAGCTTGCAGCCATTGTA	AGGAGCAGGACACCCTTCGTA
Egfr	TGGGTGGCCTCCTCTTCATA	AGGTTCCACGAGCTCTCTCT
Itgal	AAGGGACCAAAAGGTGGGCA	AGATCTGGCAGGGGATGACA
Icam1	GTGGGTCGAAGGTGGTTCTT	GCAGTTCCAGGGTCTGGTTT
Nlrp3	AAGCAATGCCCTTGGAGACA	AGGCTGCAGTTGTCTAATTCCA

Table S1. Additional upregulated inflammatory/immune cluster genes in the MGs and conjunctiva of C-IKKα mice.

Signaling pathway Gene family	Genes
Chemokines/ cytokines and their receptors	Il1b, Il2, Il5, IL6, Il7, Il9, Il10, Il12b, Il15, Il17c, Il17d, Il23a, Il31a, Il36b, Il36a, Il6st, Il1rn, Il1r1, Il1r2, Il2rg, Il4ra, Il7r, Il9r, Il10ra, **Il13ra1**, Il15ra, Il18rap, Il23r, Il36rn, Ccl1, Ccl3, Ccl4, Ccl5, Ccl7, Ccl9, Ccl12, Ccl20, Ccl22, Ccl27a, Cxcl1, Cxcl2, Cxcl3, Cxcl5, Cxcl9, Cxcl10, Cxcl13, Cxcl14, Cxcl16, Cxcr3, Cxcr4, Cxcr5, Ccr1, Ccr2, Ccr4, Ccr5, Ccr6, Ccr7, Ccr8, Ccrl2, Icam1
TNF superfamily	Tnf, Lta, Ltb, Cd40, Cd40lg, Faslg, Tnfs4, Tnfs8, Tnfs10, Tnfs11, Tnfs13b, Tnfsf14, Tnfs15, Traf2, Tnfaip3, Birc2
Apoptosis	Fas, Fasl, Bax, Rela, Casp1, Casp3, Casp4, Casp6, Casp7, Casp8, Casp12, Trp53, Ly96, Ctsc, Psmb8, Psmb9, Tnf, Clu, Cd69, Tnfsf10, Birc3, Cd38, Bmf, Ifngr1, Irf1, Bcl2l11, Bid, Prf1, Psen2, Ppt1, Sod2, Isg20, Gadd45b, Ifnb1, Gadd45a, Tspo
MAPK activation	Mapkapk2, Mapkapk3, Map2k5, Map3k1, Map3k4, Map3k5, Map3k13, Map3k14, Mapk7, Mapk9, Mknk1
Metalloproteases	Mmp3, Mmp9, Mmp7, Mmp12, Mmp13, Mmp14, Mmp15, Mmp16, Mmp23, Mmp25, Mmp27
STAT family	Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b, Stat6
NF-kB signaling pathway	Nfkb2, Mpa3k14 (NIK), Relb, Rela, Ikbkb (Ikkb), Nfkb1, Rel, Nfkbia (IKKa), Nfkbie, Nfkbib, Nfkbid, Ldoc1
Complement signaling pathway and related proteins	C3, C4a, C4b, C8a, C1qa, C1qb, C1qc, C1ra, C1rb, C1ri, C1rl, C1s1, C1s2, Cd55, C3ar1, C5ar1, C5ar2, Cr2, Fpr1, Clu, Trem2, Cmklr1, Itgb2, Serping1, Masp1
Prostaglandin signaling	Ptgir, Ptgs2, Ptges, Ptgs2os, Ptgfrn, Ptger3, Ptger4, Ptgdr2
Class I MHC	H2-K1, H2-K2, Ly96, Psmb7, Psmb8, Psmb9, Tap1, Tap2, Tapbp, Cd14, Ctss, H2-Q1, H2-Q2, H2-Q3, H2-Q4, H2-Q5, H2-Q6, H2-Q7, H2-Q10, H2-M2, H2-Aa, H2-Ab1, H2-Bl, H2-DMa, H2-DMb1, H2-Ea, H2-Eb1, H2-M2, H2-M3, H2-M5, H2-T3, H2-T22
Class II MHC	H2-Aa, H2-Ab1, H2-Ea, H2-Eb1, H2-Eb2, H2-DMa, H2-DMb1, H2-DMb2, H2-Ob, Cd4, Cd74, Cd86, Ctss, Ctsc, Trem2, Mrc1, Tyrobp
B cell receptor signaling	Cd200R1, Blk, Nfkb1, Btk, Nfkbia, Cd22, Cd38, Cd79a, Cd79b, Cmtm3, Blnk, Pik3cd, Lpxn, Prkcb, Ptpn22, Stap1, Ptpn6, Ptprc, Bank1, Sh2b2, Rftn1, Plcl2, Syk, Nfam1, Nckap1l, Cd300a, Gcsam, Lat2, Itk, Klhl6, Lck, Lyn, Iftm1, Psmb8, Psmb9
Lymphocyte activation	Glyrp2, Adam8, Eomes, Ccr2, Adora2a, Mfng, Slamf6, Zc3h12d, Aif1, Mmp14, Prex1, Aire, Btla, Fas, Cd200r1, Msn, Rhoh, Socs1, Slamf7, Myb, Tnfsf14, Axl, Tnfrsf14, Bcl3, Ccdc88b, Il18r1, Bid, Cd84, Cxcr5, Bst1, Cd7, Btk, Scart1, Nkg7, Skap2, Nlrc3, Casp8, Sh2d2a, Tlr9, Runx1, Runx2, Runx3, Ccnd3, Cd2, Cd3d, Cd3e,Cd3g, Cd247, Cd4, Cd5, Cd6, Cd8a, Cd8b, Tigit, Cd22, Cd28, Cd86, Tnfsf8, Cd37, Cd38, Cd40, Cd40lg, Cd44, Cd47, Cd48, Cd70, Cd74, Cd79a, Cd79b, Pax5, Cftr, Blnk, Cd83, Ticam2, Icos, Sash3, Pik3cd, Pik3cg, Ccr6, Ccr7, Ncr1, Apbb1ip, Il27ra, Themis2, Adgrg3, Tbx21, Pou2af1, Pou2f2, Cr2, Rsad2, Csf1r, Rorc, Prf1, Il21r, Prkcb, Pkn1, Cxadr, Ripor2, Tox, Psen2, Ptpn22, Sirpa, Jaml, Tespa1, Lax1, Clec4e, Ptpn6, Ptprc, Clnk, Bank1, Il23r, Rac2, Dock2, Tnfaip8l2, Dtx1, Parp3, Relb, Tspan32, Arpc1b, Elf4, Cmtm7, Treml2, Tcirg1, Ikzf1, F2rl1, Fcer1g, Sla2, Ccl5, Gpnmb, Ccl20, Fgr, Foxj1, Flt3, Flt3lg, Zc3h12a, Ikzf3, Themis, Batf, Dock10, St3gal1, Slamf1, Il23a, Tbl3, Klrk1, Slc11a1, Lat, Clec4d, Tnfsf13b, Card11, Fut7, Cd276, Ildr2, Spi1, Cd244, Spib, Spn, Lrrc32, Sit1, Stat3, Stat4, Stat5A, Nod2, Plcl2, Syk, Tacr1, Loxl3, Carmil2, Cracr2A, Nfkbid, Nfam1, Tgfb1, Havcr2, Coro1a, Thy1, Crtam, Tnfrsf13b, Hsh2d, Tnfaip3, Tnfrsf1b, Nckap1l, Hhex, H2-DMa, H2-DMb, H2-O, H2-Aa, H2-K, H2-D, H2-Eb2, Patz1, Cd300a, Clec7a, Hlx, Tnfrsf4, Txk, Ttrobp, Vav, Vcam1, Was, Lat2, Icam1, Irf8, Zap70, Ifnar1, Ifnar2, Rasal3, Pik3r6, Il1b, Il2ra, Il2rb, IL2rg, Il4r, Il6st, Il7, Il7r, Il9r, Slamf8, Il12b, Il15, Il15ra, Inpp5d, Irf1, Irf7, Itga4, Itgal, Itgax, Itgb2, Itk, Laptm5, Jak2, Jak3, Cyria, Cxcr4, Kit, Lst1, Slamf9, Lag3, Lck, Lcp1, Lgals1, Nlrp3, Lgals9, Lipa, Cd180, Lyn
Type I Interferon and related proteins	Ifi35, Ifi44, Ifit1, Ifit2, Ifit3, Irf1, Irf2, Irf3, Irf4, Irf5, Irf7, Irf8, Irf9, Mx1, Mx2, Oasl2, Oas1a, Oas1b, Oas1c, Oas1g, Oas2, Oas3, Psmb8, Psmb9, Stat1, Stat2, Sting1, Adar, Mmp12, Trim65, Samhd1, Isg15, Ikbke, Tbkbp1 ,Ptpn6, Nlrc5, Sp100, Ifih1, Usp18, Traf3, Rigi, Zbp1, Ifnar1, Ifnar2
Type II Interferon and related proteins	H2-Eb2, Icam1, Stat1, Ifng, Irf1, Socs1, Parp14, Parp9, Nr1h3 ,Nlrc5, Sp100, Stat1, Nod2, Hck, Rigi, Txk, Ifngr1, Ifngr2, Jak2
TLR and associated factors	Ly96, Nod2, S100a8, S100a9, Pik3ap1, Birc2, Arrb2, Btk, Tlr9, Cd86, Cd40, Ticam2, Ctss, Tnip3, TnipP1, Tlr1, Tlr2, Tlr3, Tlr4, Tlr5, Tlr6, Tlr7, Tlr8, Tlr9, Tlr11, Tlr12, Tlr13, Tasl, Smpdl3b, Chadl, Irak3, Traf3, Cd300a, Scimp, Irf1, Irf7, Unc93b1, Cd180
Leukotriene receptors and related enzymes	Alox5ap, Cysltr1, Cysltr2, Alox12, Alox15, Alox5, Pla2g4a, Ggt5

Table S2. Downregulation of genes encoding lipid metabolism-related enzymes in the meibomian glands of C-IKKα mice.

Cholesterol and Cholesterol esters metabolism		
Gene	**Gene name**	***p-adjust***
Acat2	*Acetyl-Coenzyme A acetyltransferase2*	2.66E-35
Dhcr7	*7-Dehydrocholesterol reductase*	8.87E-32
Mvd	*Mevalonate diphospho decarboxylase*	2.92E-32
Acat1	*Acetyl-Coenzyme A acetyltransferase1*	4.28E-28
Pmvk	*Phosphomevalonate kinase*	4.56E-26
Hmgcr	*3-Hydroxy-3-methylglutaryl-Coenzyme A reductase*	2.78E-25
Msmo1	*Methylsterol monoxygenase 1*	1.04E-25
Hmgcs2	*3-Hydroxy-3-methylglutaryl-Coenzyme A synthase2*	5.82E-24
Insig1	*Insulin induced gene 1*	1.17E-24
Hmgcs1	*3-Hydroxy-3-methylglutaryl-Coenzyme A synthase1*	1.78E-23
Lss	*Lanosterol synthase*	3.68E-21
Soat1	*Sterol O-acyltransferase 1*	1.89E-21
Sc5d	*Sterol-C5-desaturase*	6.49E-17
Sqle	*Squalene epoxidase*	3.17E-15
Stard4	*StAR-related lipid transfer (START) domain containing 4*	9.72-15
Idi1	*Isopentenyl-diphosphate delta isomerase*	2.79E-15
Mvk	*Mevalonate kinase*	2.33E-14
Dhcr24	*24-dehydrocholesterol reductase*	2.30E-13
Fdft1	*Farnesyl diphosphate farnesyl transferase 1*	1.65E-11
Vnn1	*Vanin 1*	1.68E-08
Idi2	*Isopentenyl-diphosphate delta isomerase 2*	0.00044

Fatty acid biosynthesis and desaturation genes		
Gene	**Gene name**	***p-adjust***
Fads1	*Fatty acid desaturase 1*	1.21E-44
Phyh	*Phytanoyl-CoA hydroxylase*	1.37E-36
Fasn	*Fatty acid synthase*	4.96E-28
Scd2	*Stearoyl-Coenzyme A desaturase 2*	9.17E-23
Fads6	*Fatty acid desaturase 6*	2.19E-19
Scd3	*Stearoyl-Coenzyme A desaturase 3*	5.08E-17
Scd4	*Stearoyl-Coenzyme A desaturase 4*	3.41E-17
Fads2	*Fatty acid desaturase 2*	3.03E-12
Scd1	*Stearoyl-Coenzyme A desaturase 1*	1.15E-12
Fads3	*Fatty acid desaturase 1*	0.00083
Fatty acid elongation-related genes		
Acsbg1	*Acyl-CoA synthetase bubblegum family member 1*	1.56E-42
Elovl7	*ELOVL Fatty Acid Elongase 7*	5.40E-36
Hacd2	*3-hydroxyacyl-CoA dehydratase 2*	4.90E-33
Elovl4	*ELOVL Fatty Acid Elongase 4*	4.51E-33
Hsd17b12	*Hydroxysteroid (17-beta) dehydrogenase12*	8.45E-25
Elovl3	*ELOVL Fatty Acid Elongase 3*	9.35E-20
Hacd3	*3-hydroxyacyl-CoA dehydratase 3*	5.49E-19
Elovl6	*ELOVL Fatty Acid Elongase 6*	9.59E-18
Elovl1	*ELOVL family member 1, elongation of long chain fatty acids*	2.65E-18
Hacd4	*3-Hydroxyacyl-CoA dehydratase 4*	2.65E-18
Hsd17b10	*Hydroxysteroid (17-beta) dehydrogenase10*	2.27E-10
Hacl1	*2-hydroxyacyl-CoA lyase 1*	0.0001

Metabolism of odd-chain and branched fatty acids		
Gene	**Gene name**	***p-adjust***
Acox2	*Acyl-Coenzyme A oxidase 2, branched chain*	8.08E-34
Dbt	*Dihydrolipoamide branched chain transacylase E2*	1.69E-24
Echdc1	*Enoyl Coenzyme A hydratase domain containing 1*	1.13E-17
Bckdhb	*Branched chain ketoacid dehydrogenase E1, beta polypeptide*	2.38E-15
Pcca	*Propionyl-Coenzyme A carboxylase, alpha polypeptide*	8.12E-06
Bckdk	*Branched chain ketoacid dehydrogenase kinase*	0.00356
Bcat2	*Branched chain aminotransferase 2, mitochondrial*	0.00246
Metabolism of WEs and O-acylated ω-hydroxy fatty acids (OAHFAs)		
Gene	**Gene name**	***p-adjust***
Awat1	*Acyl-CoA wax alcohol acyltransferase 1*	2.61E-37
Cyp4f39	*Cytochrome P450, family 4, subfamily f, polypeptide 39*	*3.80E-31*
Far2	*Fatty acyl CoA reductase 2*	4.43E-19
Awat2	*Acyl-CoA wax alcohol acyltransferase 1*	1.24E-17
Far1	*Fatty acyl CoA reductase 2*	3.66E-10
Regulation of energy balance, lipid storage and mobilization		
Plin4	*Perilipin 4*	6.81E-45
Plin5	*Perilipin 5*	1.81E-44
Ces1d	*Carboxylesterase 1D*	8.66E-38
Plin2	*Perilipin 2*	8.25E-34
Mgll	*Monoglyceride lipase*	1.35E-29
Gpat4	*Glycerol-3-phosphate acyltransferase 4*	5.43E-26
Cidea	*Cell death-inducing DNA fragmentation factor, alpha subunit-like effector A*	2.66E-23
Gpam	*Glycerol-3-phosphate acyltransferase mitochondrial*	7.15E-22
Plin1	*Perilipin 1*	6.85E-14
Dgat2	*Diacylglycerol O-acyltransferase 2*	1.87E-14