Mass spectrometry-based proteomics to study gastric carcinogenesis: pathophysiological molecular characterization.

Nayra Felípez [1], Sheyla Montori [1], Alba Valero [1], Enrique Santamaría [2], Karina Ausin [2], Erika Peral [2], Joan Llach [3,4], Pedro Delgado [5], Pablo Florez-Diez [6], Eva Barreiro [6], Elena Arruebo [7], Raquel Vicente [7], M Teresa Soria [7], Alain Huerta [8], Silvia Patricia Ortega [9], Henar Núñez [10], Pilar Díez [10], Alberto Herreros [11], Gadea Hontoria [12], Rosa María Saíz [12], Luis Hernández [13], Carolina Mangas-Sanjuan [13], Oliver Patrón [14], Gonzalo Hijos-Mallada [15], María José Domper [15], Sara Zarraquiños [16], Astrid Irene Díez-Martín [16], Patricia Gonçalves [17], Diego de Frutos [11], José Santiago [11], Adelina García [18], Alicia Martín-Lagos [18], Fermín Estremera-Arévalo [19], Marta Gómez Alonso [19], Anabella Cuestas [3,4], Francesc Balaguer [3,4], Sergi Castellví-Bel [3,4], Glòria Fernandez-Esparrach [3,4], Miriam Cuatrecasas [20], Leticia Moreira [3,4], Joaquín Fernández-Irigoyen [2], Eduardo Albéniz [19], EpiGASTRIC EDGAR Consortium.

Corresponding authors:

Joaquín Fernández-Irigoyen (Email: joaquin.fernandez.irigoyen@navarra.es) and Eduardo Albéniz (Email: eduardo.albeniz.arbizu@navarra.es)

This file includes:

Supplementary Figures S1-S8.

Supplementary information:

Figure S1. A. Volcano plots representing the Log2 fold-change of identified protein with associated *p* values from the comparisons of benign/malignancy stages vs control group. **B.** Volcano plots representing the Log2 fold-change of identified protein with associated *p* values from the comparisons of adjacent tissues from malignancy stages vs control group. Red and green, up- and down-regulated protein, respectively.

Figure S2. **A.** Venn diagram of unique and common proteins in malignancy stages. **B.** Heatmap of unique and shared pathways between malignancy stages. **C.** Venn diagram of proteins involved in neutrophil degranulation in lesional and non-lesional tissue.

Figure S3. Mass spectrometry-based quantitation boxplots of common protein deregulation **A.** rRNA processing, **B.** lysine catabolism and **C.** phase II: conjugation of compounds in EGC-L vs control and GC-NL vs control. Unpaired t-test for equal variances with/without Welch correction, or Wilcoxon rank-sum test. *p < 0.05; **p < 0.01, ***p < 0.001 vs. control group and between groups (EGC = early gastric cancer, and GC = gastric cancer, NL = nonlesional, L = lesion).

Figure S4. Mass spectrometry-based quantitation boxplots of common protein deregulation **A.** aflatoxin activation and detoxification in malignant tissues and **B.** formation of fibrin clot/antimicrobial peptides across almost all tissue types (except CG). Unpaired t-test for equal variances with/without Welch correction, or Wilcoxon rank-sum test. *p < 0.05; **p < 0.01, ***p < 0.001 vs. control group and between groups (IM-LGD = intestinal metaplasia-low grade dysplasia, EGC = early gastric cancer, and GC = gastric cancer, NL = nonlesional, L = lesion).

Figure S5. Mass spectrometry-based quantitation boxplots of common protein deregulation **A.** vpr-mediated induction of apoptosis, **B.** signaling by high kinase activity BRAF mutants, **C.** hyaluronan uptake and degradation and **D.** regulation of insulin-like growth factor in IM-LGD-L vs control, EGC-L vs control and GC-NL vs control. Unpaired t-test for equal variances with/without Welch correction, or Wilcoxon rank-sum test. *p < 0.05; **p < 0.01, ***p < 0.001 vs. control group and between groups (IM-LGD = intestinal metaplasia-low grade dysplasia, EGC = early gastric cancer, and GC = gastric cancer, NL = nonlesional, L = lesion).

Figure S6. Mass spectrometry-based quantitation boxplots of common protein deregulation **A.** aerobic respiration and respiratory electron transport in almost all tissues (except EGC-NL). Unpaired t-test for equal variances with/without Welch correction, or Wilcoxon rank-sum test. *p < 0.05; **p < 0.01, ***p < 0.001 vs. control group and between groups (IM-LGD = intestinal metaplasia-low grade dysplasia, EGC = early gastric cancer, and GC = gastric cancer, NL = nonlesional, L = lesion).

Figure S7. Mass spectrometry-based quantitation boxplots of common protein deregulation **A.** neutrophil degranulation in all tissues. Unpaired t-test for equal variances with/without Welch correction, or Wilcoxon rank-sum test. *p < 0.05; **p < 0.01, ***p < 0.001 vs. control group and between groups (IM-LGD = intestinal metaplasia-low grade dysplasia, EGC = early gastric cancer, and GC = gastric cancer, NL = nonlesional, L = lesion).

Figure S8. Mass spectrometry-based quantitation boxplots of specific comparisons of protein deregulation in neutrophil degranulation **A.** EGC-L vs control, GC-L vs control, GC-NL vs control. **B.** IM-LGD-L vs control EGC-L vs control. Unpaired t-test for equal variances with/without Welch correction, or Wilcoxon rank-sum test. *p < 0.05; **p < 0.01, ***p < 0.001 vs. control group and between groups (IM-LGD = intestinal metaplasia-low grade dysplasia, EGC = early gastric cancer, and GC = gastric cancer, NL = nonlesional, L = lesion).

Figure S1.

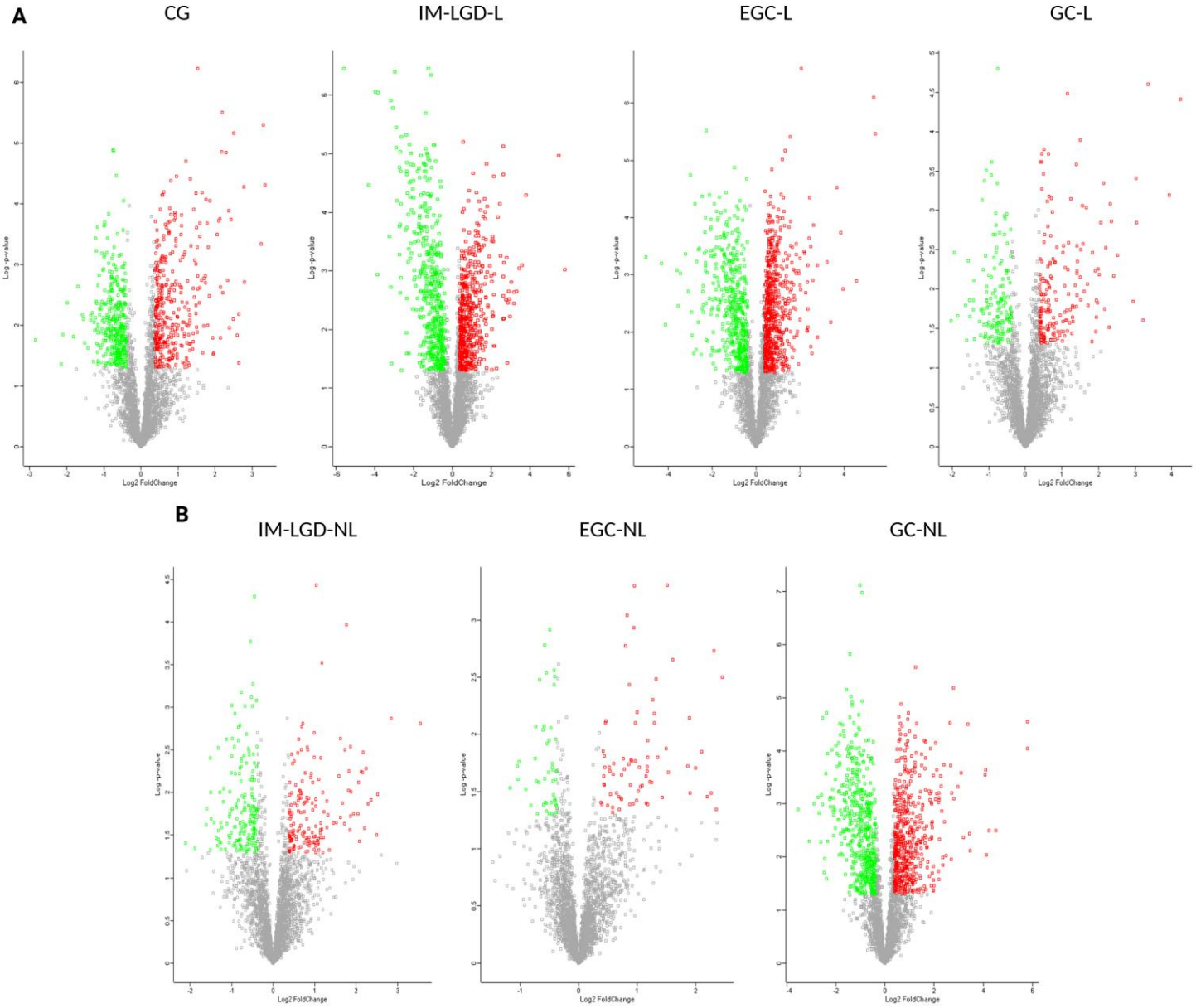

A
CG
IM-LGD-L
EGC-L
GC-L
B
IM-LGD-NL
EGC-NL
GC-NL
Log2 FoldChange
Log q-value

Figure S2.

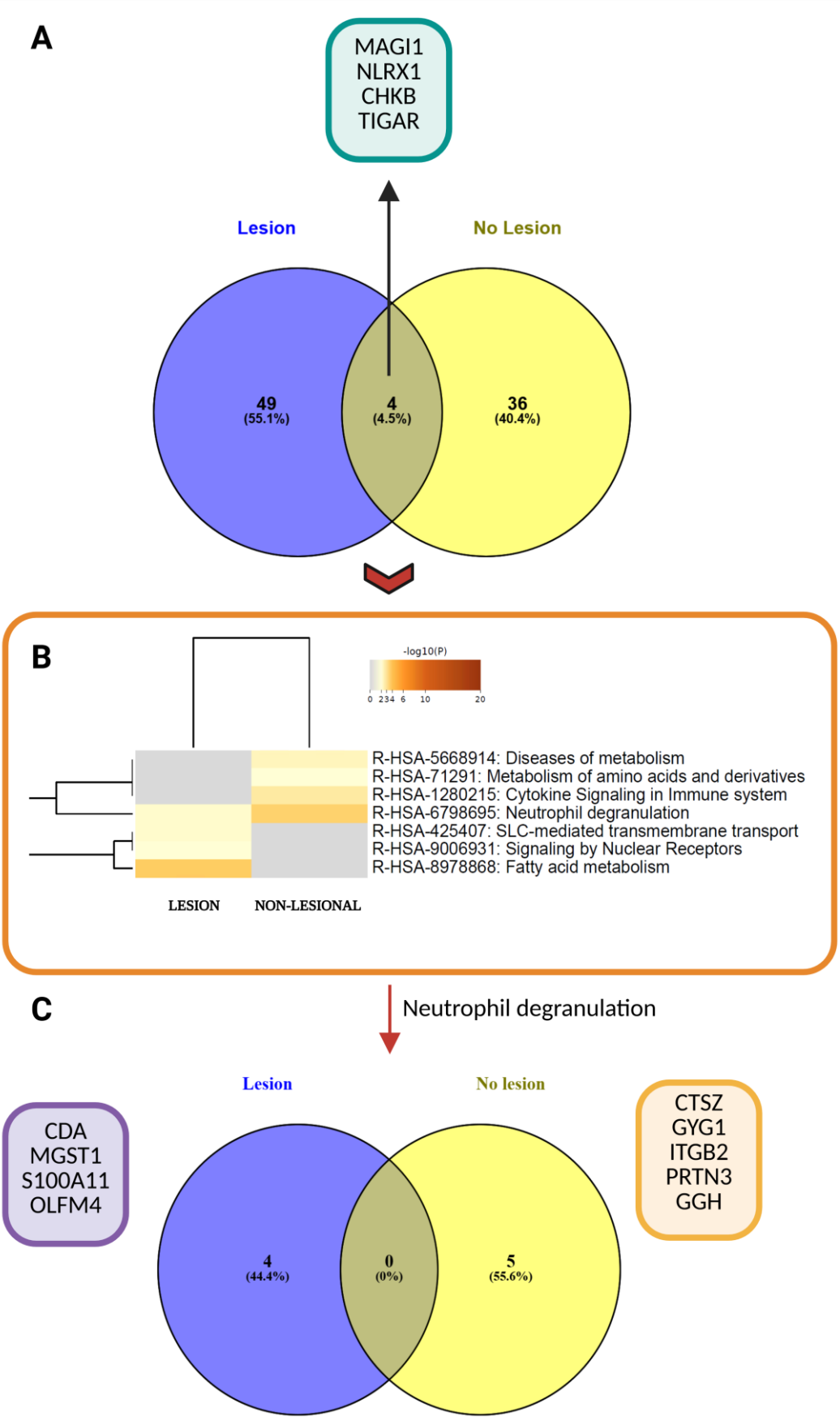
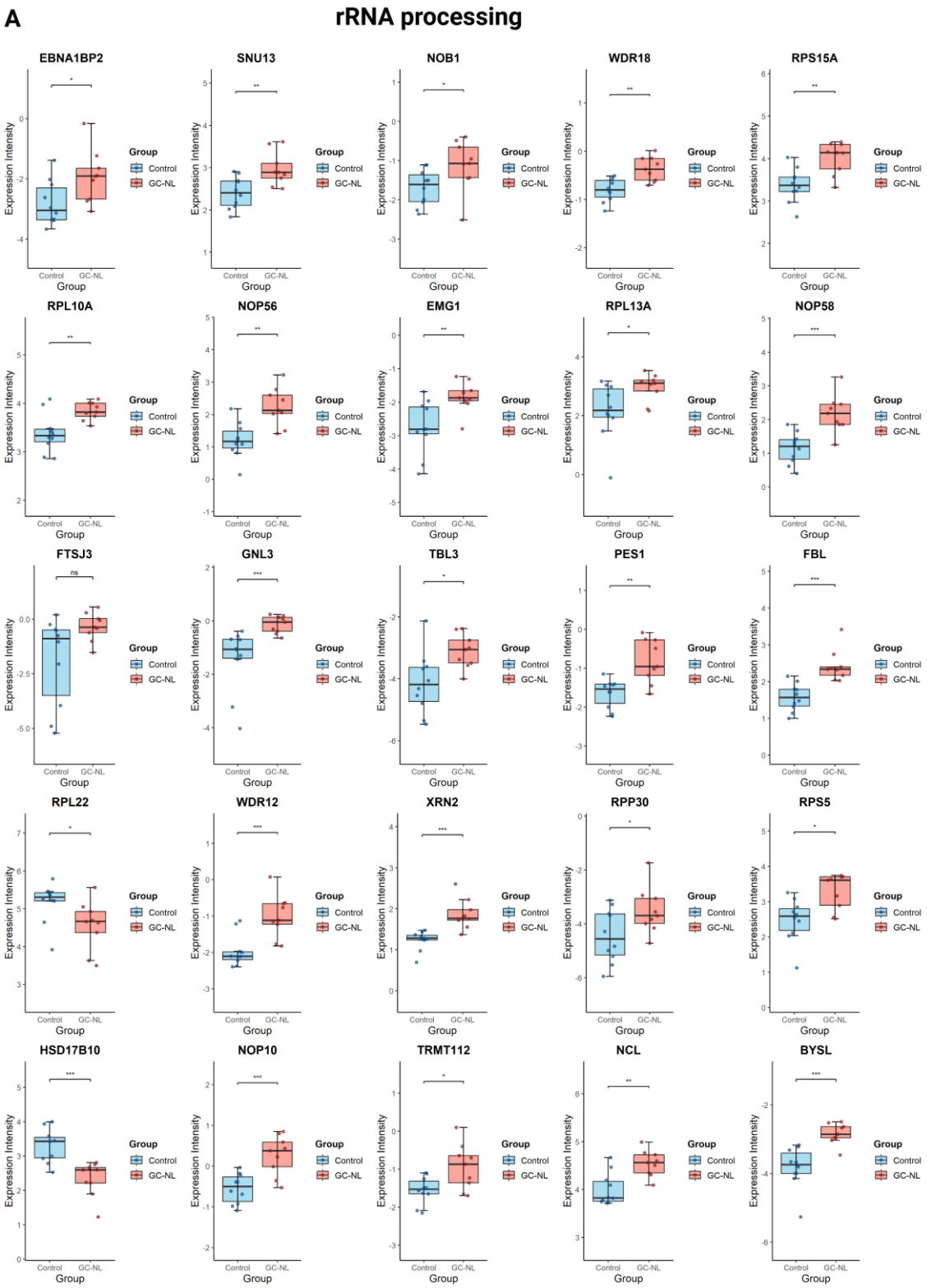

A
MAGI1
NLRX1
CHKB
TIGAR
Lesion
No Lesion
49
(55.1%)
4
(4.5%)
36
(40.4%)
B
-log10(P)
0 2 3 4 6 10 20
R-HSA-5668914: Diseases of metabolism
R-HSA-71291: Metabolism of amino acids and derivatives
R-HSA-1280215: Cytokine Signaling in Immune system
R-HSA-6798695: Neutrophil degranulation
R-HSA-425407: SLC-mediated transmembrane transport
R-HSA-9006931: Signaling by Nuclear Receptors
R-HSA-8978868: Fatty acid metabolism
LESION
NON-LESIONAL
C
Neutrophil degranulation
Lesion
No lesion
CDA
MGST1
S100A11
OLFM4
CTSZ
GYG1
ITGB2
PRTN3
GGH
4
(44.4%)
0
(0%)
5
(55.6%)

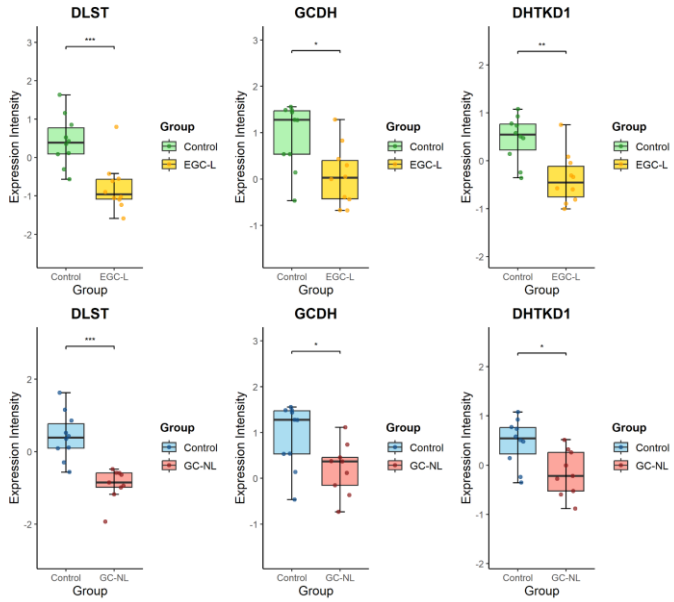
Figure S3.


A
rRNA processing
EBNA1BP2
SNU13
NOB1
WDR18
RPS15A
RPL10A
NOP56
EMG1
RPL13A
NOP58
FTSJ3
GNL3
TBL3
PES1
FBL
RPL22
WDR12
XRN2
RPP30
RPS5
HSD17B10
NOP10
TRMT112
NCL
BYSL
Expression Intensity
Group
Control
EGC-L


A
rRNA processing
EBNA1BP2
SNU13
NOB1
WDR18
RPS15A
RPL10A
NOP56
EMG1
RPL13A
NOP58
FTSJ3
GNL3
TBL3
PES1
FBL
RPL22
WDR12
XRN2
RPP30
RPS5
HSD17B10
NOP10
TRMT112
NCL
BYSL
Expression Intensity
Group
Control
GC-NL

B

Lysine catabolism



C

Phase II - Conjugation of compounds

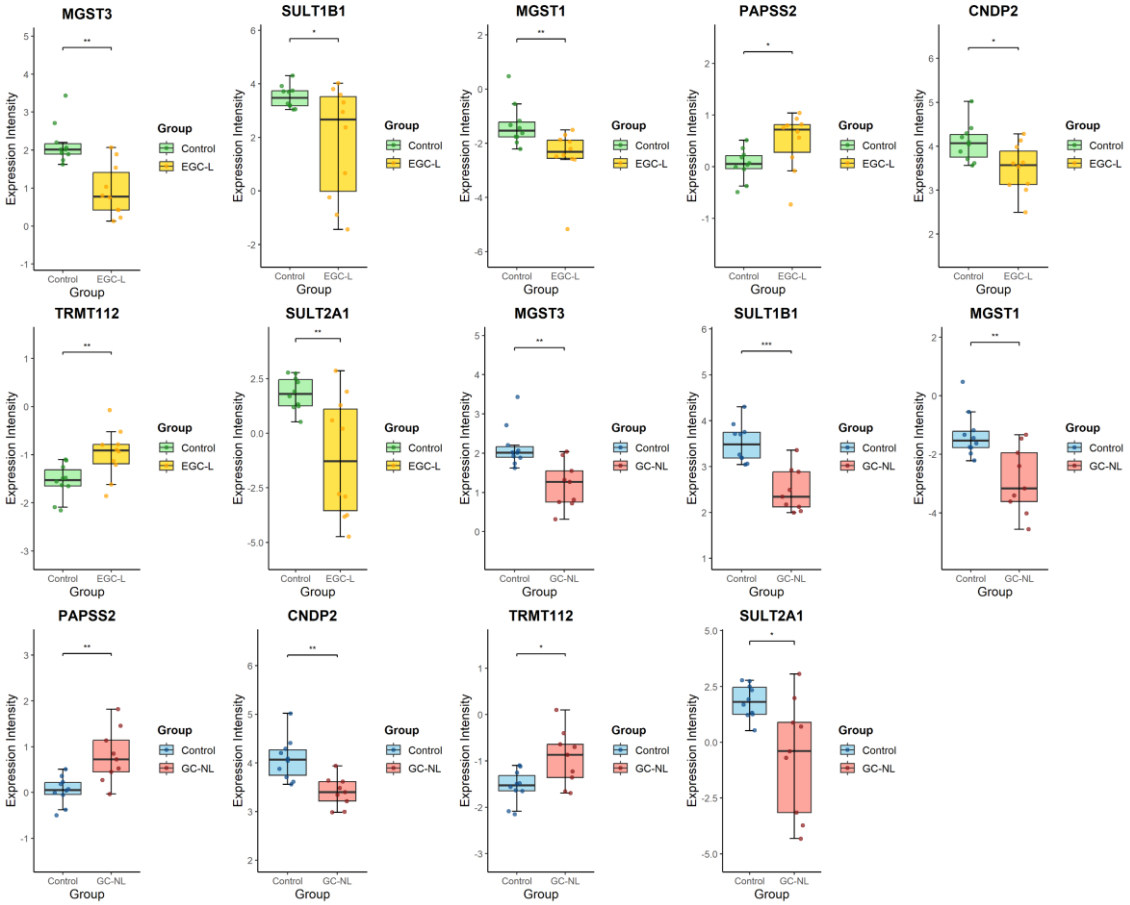


Figure S4.

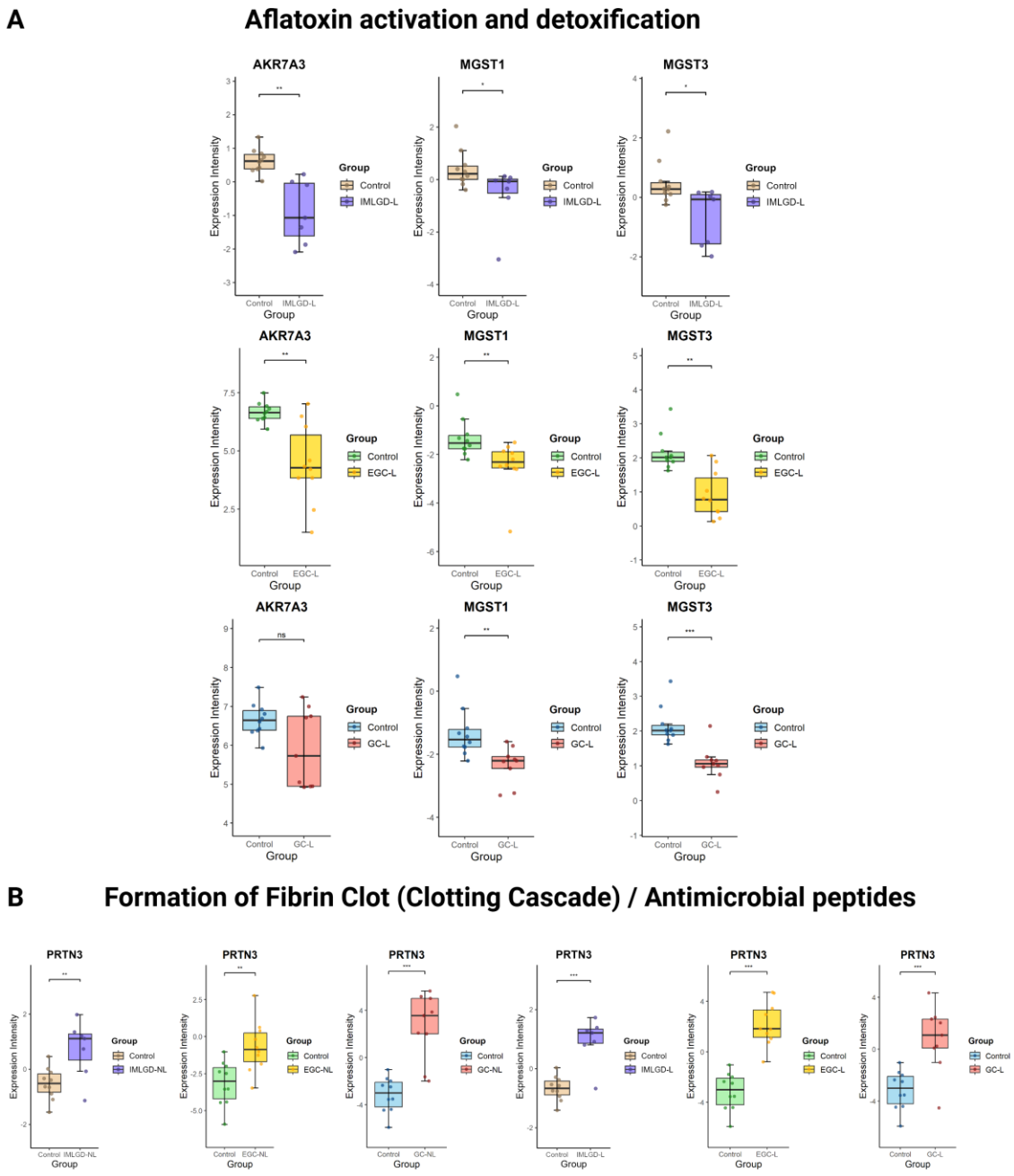
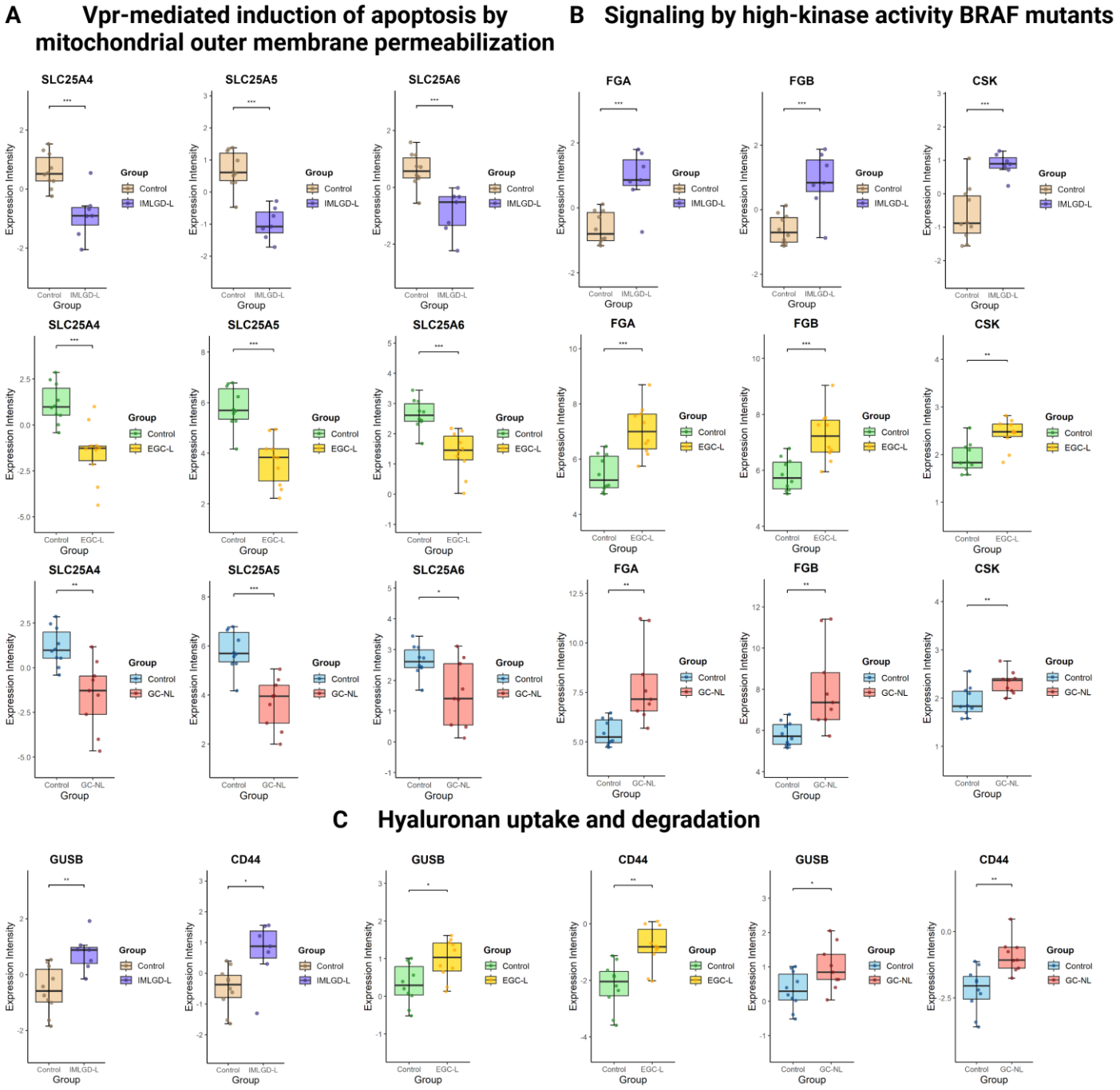

A
Aflatoxin activation and detoxification
AKR7A3
MGST1
MGST3
Expression Intensity
Group
Control
IMLGD-L
EGC-L
GC-L
ns
B
Formation of Fibrin Clot (Clotting Cascade) / Antimicrobial peptides
PRTN3
IMLGD-NL
EGC-NL
GC-NL

Figure S5.


A Vpr-mediated induction of apoptosis by mitochondrial outer membrane permeabilization
B Signaling by high-kinase activity BRAF mutants
C Hyaluronan uptake and degradation
SLC25A4
SLC25A5
SLC25A6
FGA
FGB
CSK
GUSB
CD44
Expression Intensity
Group
Control
IMLGD-L
EGC-L
GC-NL

D **Regulation of Insulin-like Growth Factor (IGF) transport and uptake by Insulin-like Growth Factor Binding Proteins (IGFBPs)**

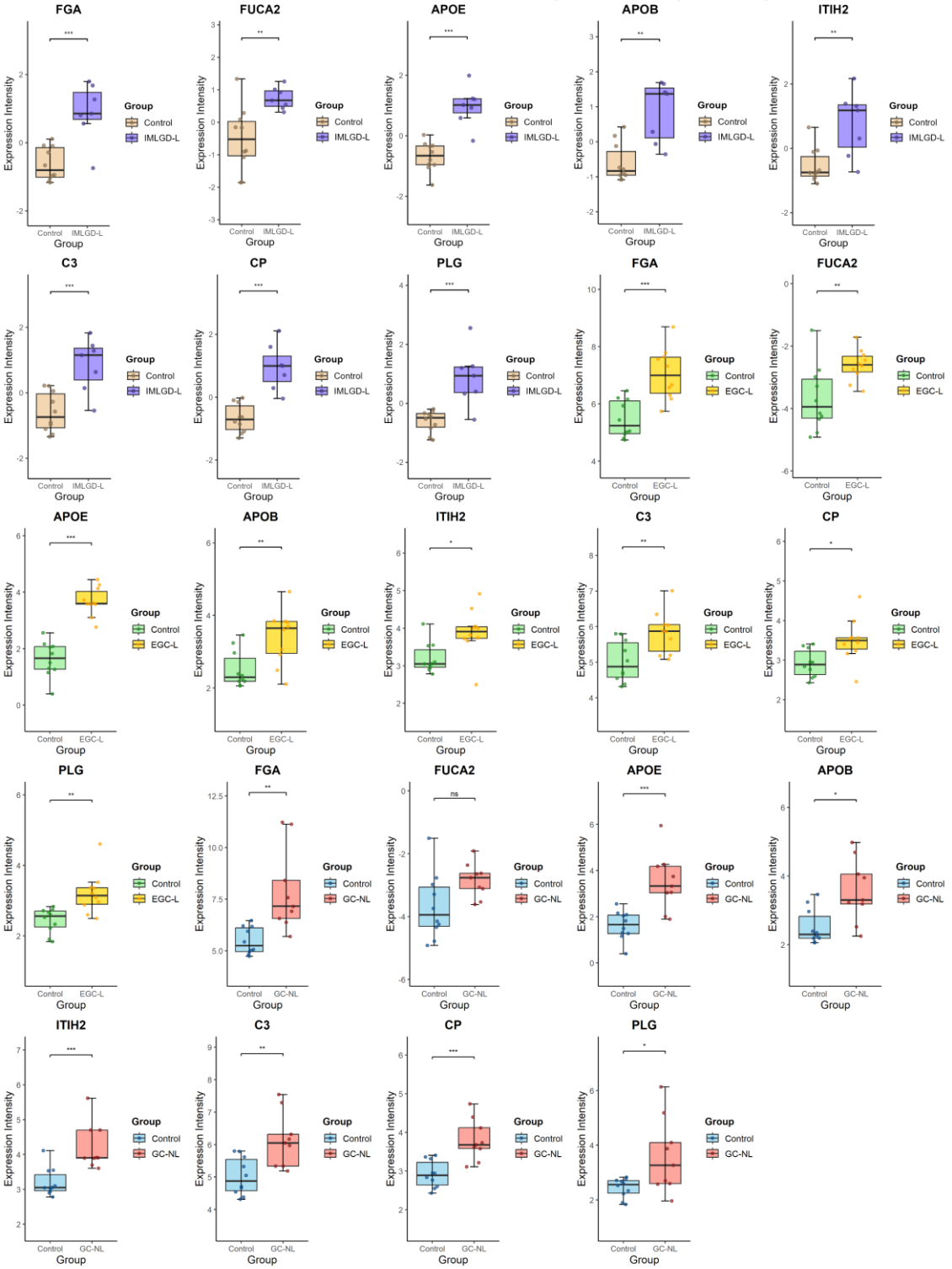
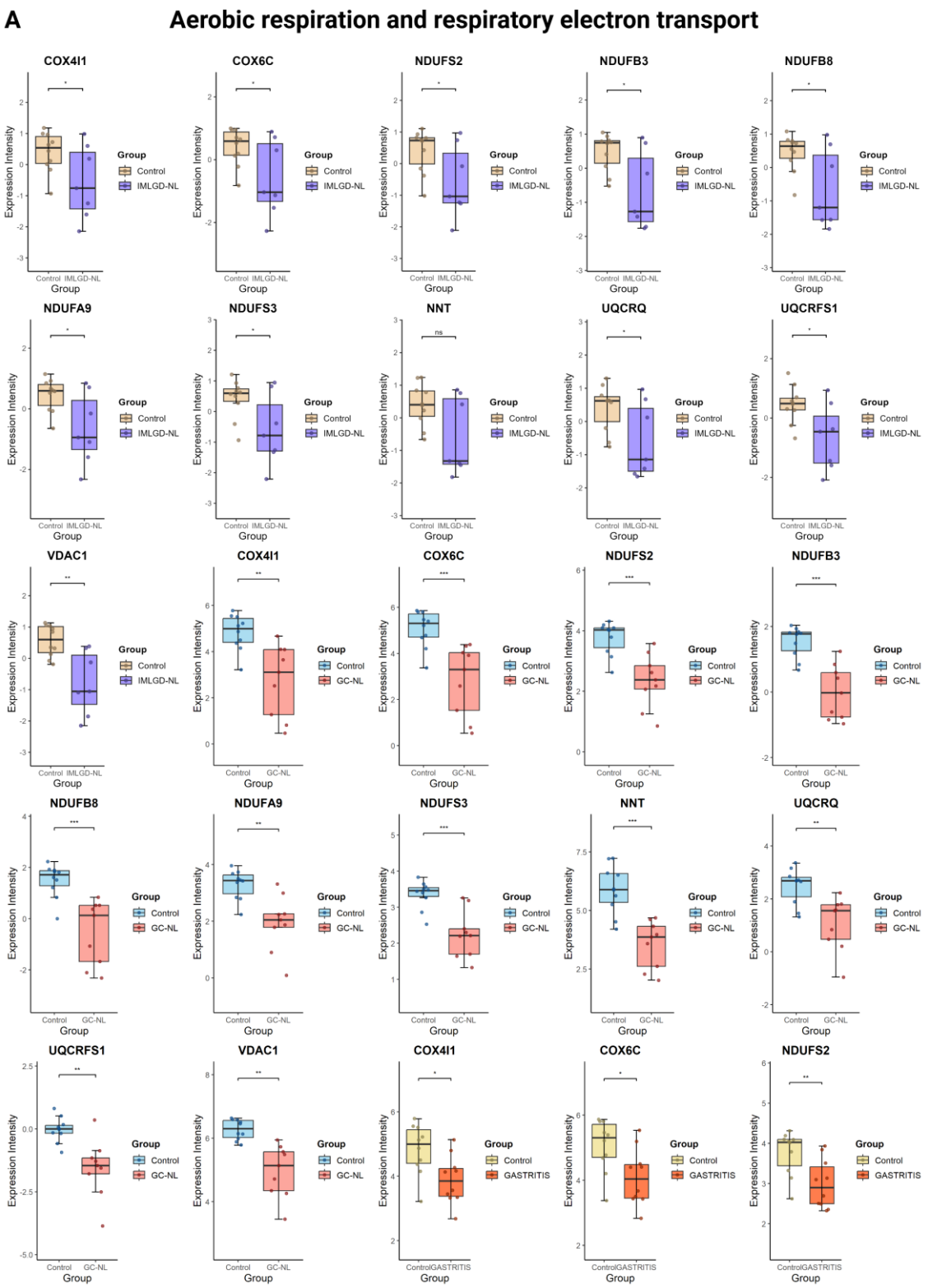
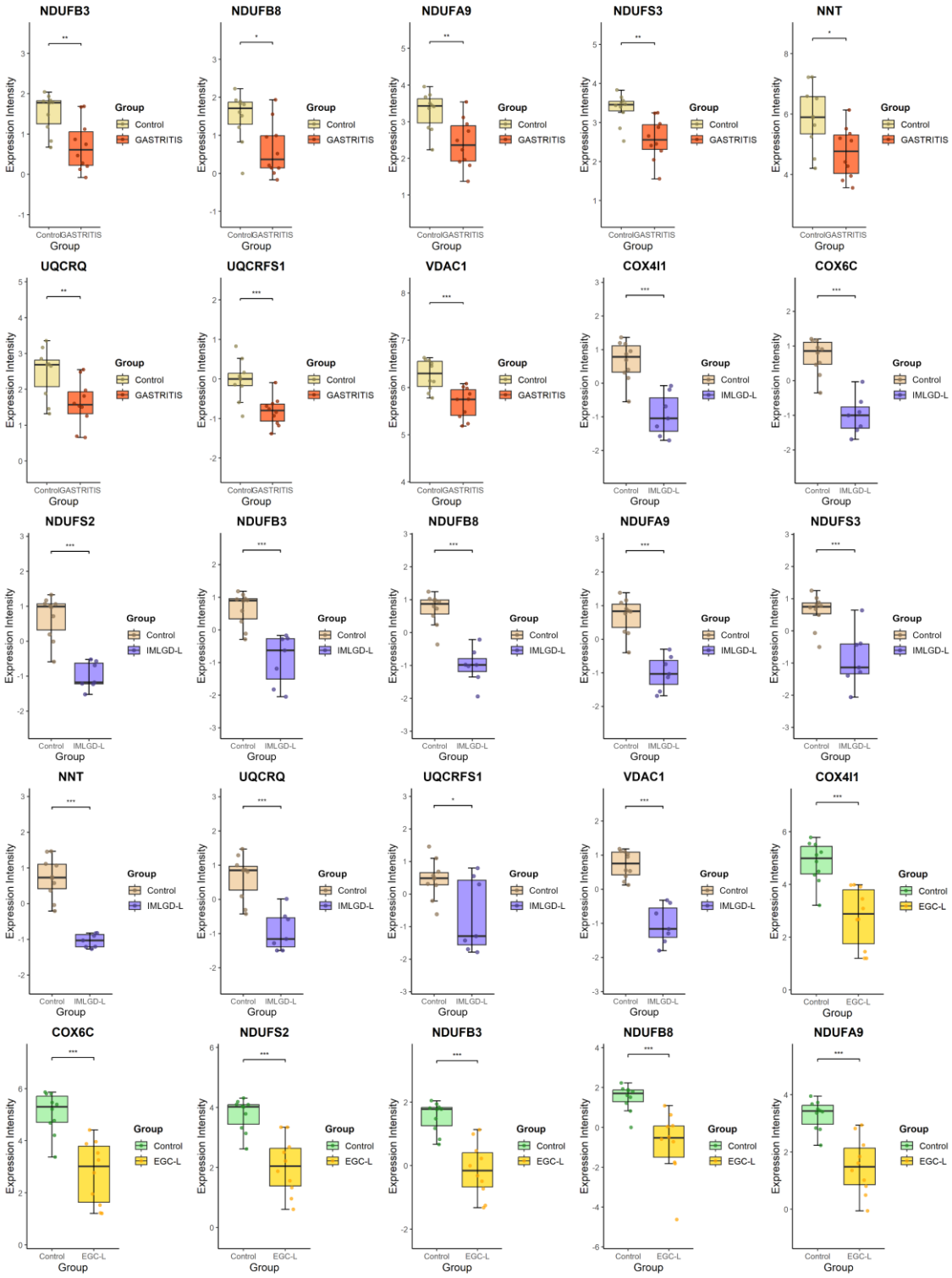


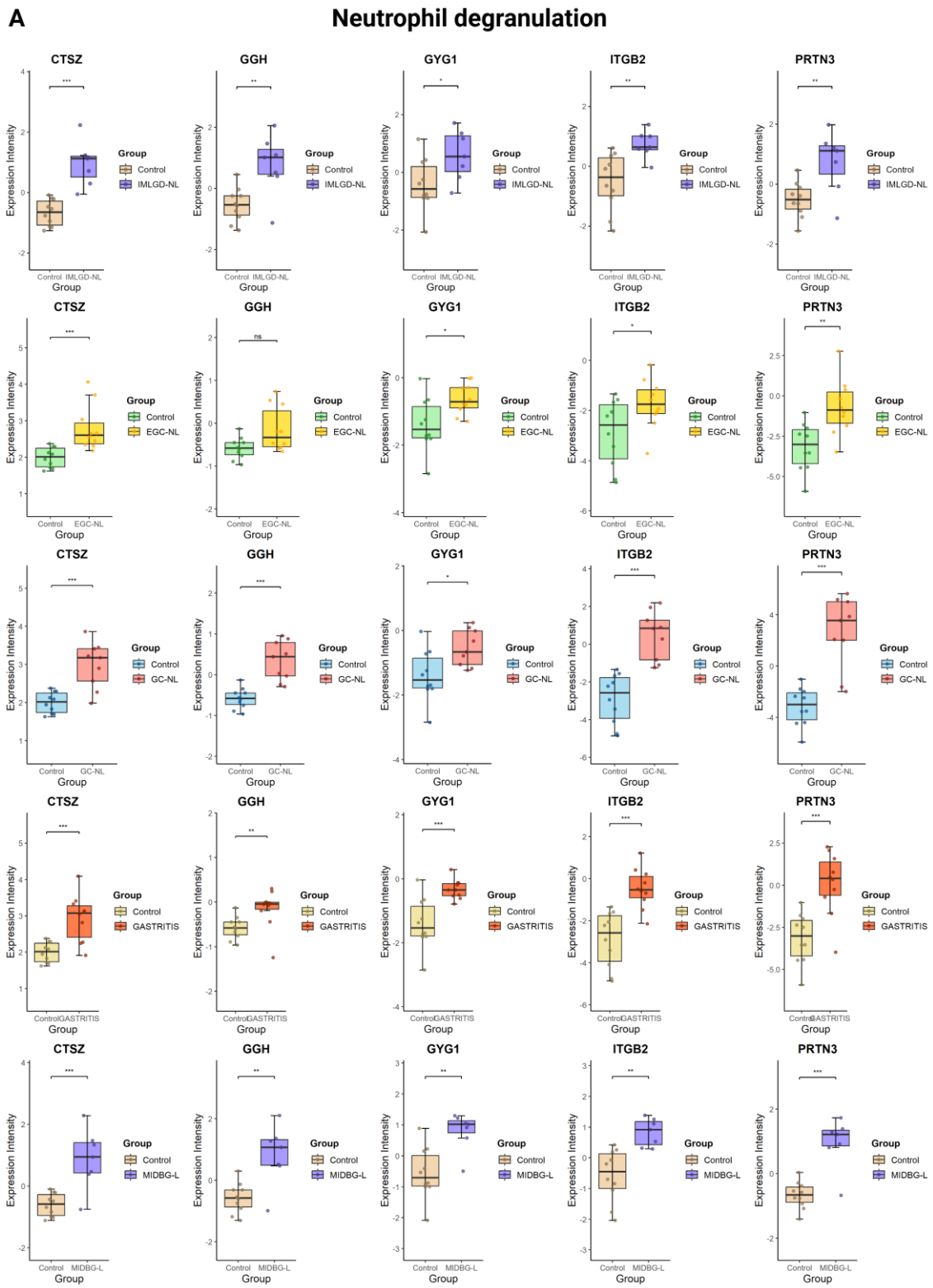
Figure S6.


A
Aerobic respiration and respiratory electron transport
COX4I1
COX6C
NDUFS2
NDUFB3
NDUFB8
NDUFA9
NDUFS3
NNT
UQCRQ
UQCRFS1
VDAC1
COX4I1
COX6C
NDUFS2
NDUFB3
NDUFB8
NDUFA9
NDUFS3
NNT
UQCRQ
UQCRFS1
VDAC1
COX4I1
COX6C
NDUFS2
Expression Intensity
Group
Control
IMLGD-NL
GC-NL
GASTRITIS


A
Aerobic respiration and respiratory electron transport
NDUFB3
NDUFB8
NDUFA9
NDUFS3
NNT
UQCRQ
UQCRFS1
VDAC1
COX4I1
COX6C
NDUFS2
NDUFB3
NDUFB8
NDUFA9
NDUFS3
NNT
UQCRQ
UQCRFS1
VDAC1
COX4I1
COX6C
NDUFS2
NDUFB3
NDUFB8
NDUFA9
Expression Intensity
Group
Control
GASTRITIS
IMLGD-L
EGC-L


A
Aerobic respiration and respiratory electron transport
NDUFS3
UQCRQ
UQCRFS1
NNT
VDAC1
COX4I1
COX6C
NDUFS2
NDUFB3
NDUFB8
NDUFA9
NDUFS3
NNT
UQCRQ
UQCRFS1
VDAC1
Expression Intensity
Group
Control
EGC-L
GC-L

Figure S7.


A
Neutrophil degranulation
CTSZ
GGH
GYG1
ITGB2
PRTN3
Expression Intensity
Group
Control
IMLGD-NL
EGC-NL
GC-NL
GASTRITIS
MIDBG-L

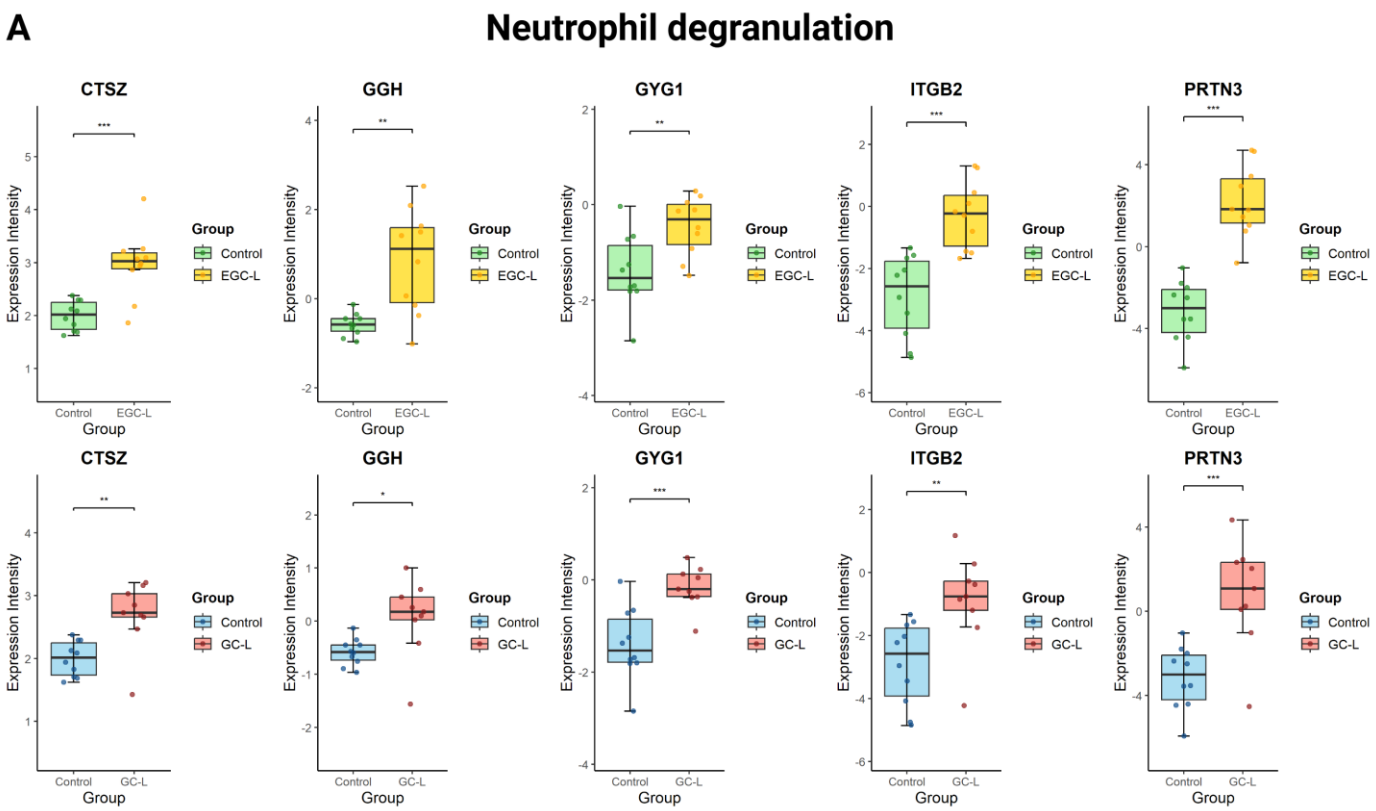
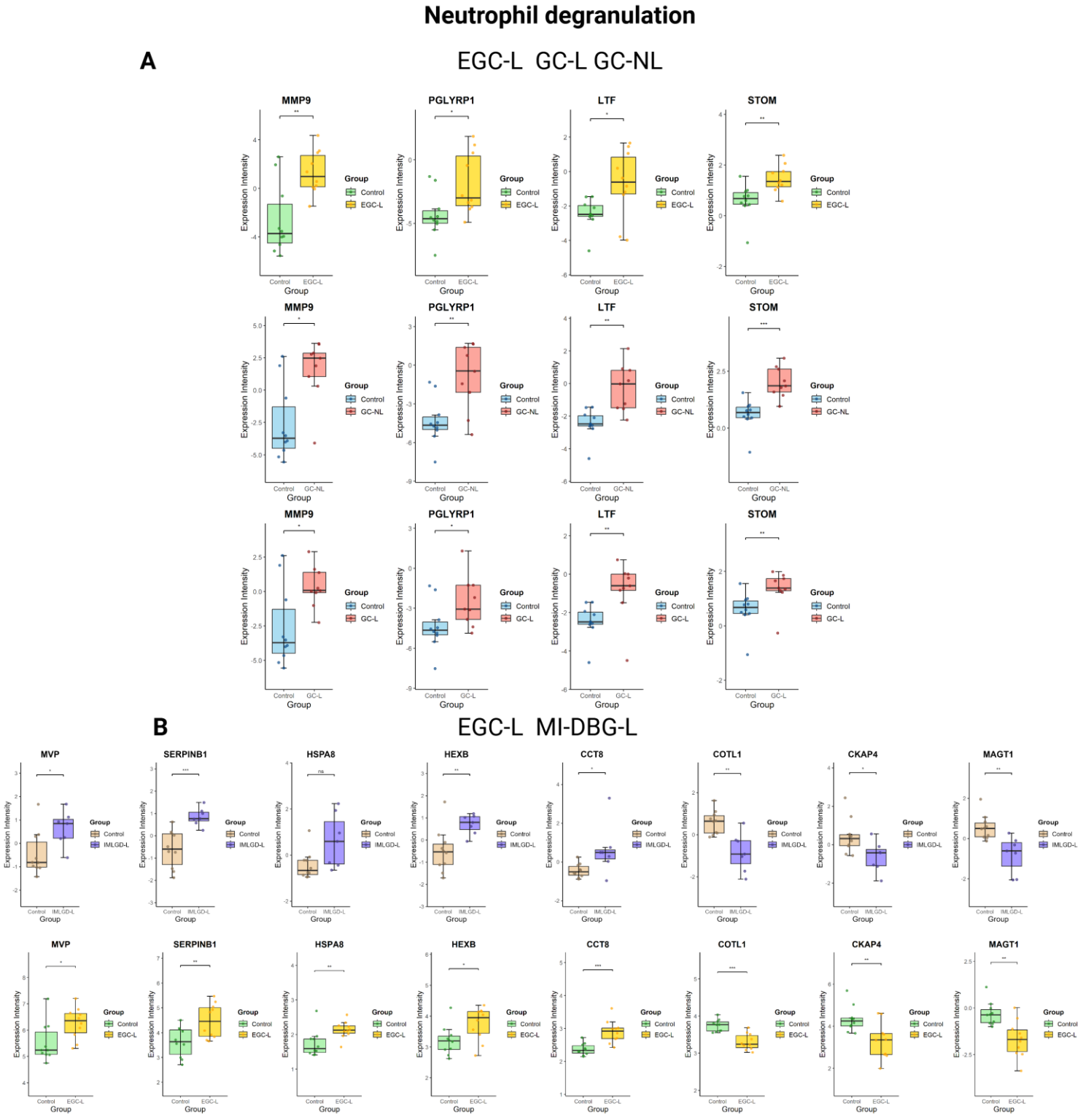

A
Neutrophil degranulation
CTSZ
GGH
GYG1
ITGB2
PRTN3
Expression Intensity
Group
Control
EGC-L
GC-L
***
**
*

Figure S8.


Neutrophil degranulation
A
EGC-L GC-L GC-NL
MMP9
PGLYRP1
LTF
STOM
Expression Intensity
Group
Control
EGC-L
GC-NL
GC-L
B
EGC-L MI-DBG-L
MVP
SERPINB1
HSPA8
HEXB
CCT8
COTL1
CKAP4
MAGT1
IMLGD-L