

# ***Per1/Per2* Knockout Affects Spleen Immune Function in Elderly Mice via Inducing Spleen Lymphocyte Ferroptosis**

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**Table S1.** Changes in the ratio of DKO/ WT-related differential mRNAs  
in ferroptosis pathways

<b>Gene Symbol</b>	<b>Description</b>	<b>logFC</b>	<b>P.Value</b>	<b>change</b>
<i>Atr</i>	Ataxia telangiectasia and Rad3 related	0.772927	2.94E-06	UP
<i>Atm</i>	Ataxia telangiectasia-mutated gene	0.392001	0.006306	UP
<i>Prkdc</i>	Protein Kinase, DNA-Activated, Catalytic Subunit	0.473959	0.000732	UP
<i>Chek1</i>	Checkpoint Kinase 1	1.801378	4.35E-16	UP
<i>Chek2</i>	Checkpoint Kinase 2	0.976704	1.37E-09	UP
<i>Apex2</i>	Apurinic/Apyrimidinic Endodeoxyribonuclease 2	0.333752	0.030491	UP
<i>Msh6</i>	MutS Homolog 6	0.635101	3.82E-06	UP
<i>Pms2</i>	PMS1 Homolog 2, Mismatch Repair System Component	0.440204	0.00027	UP
<i>Dnmt1</i>	DNA Methyltransferase 1	0.432902	0.015051	UP
<i>H2afx</i>	H2A histone family, member X	0.805355	0.000103	UP
<i>Msh5</i>	MutS Homolog 5	0.417796	0.030714	UP
<i>Msh3</i>	MutS Homolog 3	0.842693	9.58E-10	UP
<i>Bcl6</i>	BCL6 Transcription Repressor	0.036382	0.794541	NOT
<i>Gadd45b</i>	Growth Arrest And DNA Damage Inducible Beta	-0.37955	0.008765	DOWN
<i>Sod3</i>	Superoxide Dismutase 3	-0.73053	8.73E-05	DOWN
<i>Smad4</i>	SMAD Family Member 4	-0.2693	0.034612	DOWN
<i>Smad3</i>	SMAD Family Member 3	-0.28746	0.019942	DOWN
<i>Foxo1</i>	Forkhead Box O1	-0.4173	0.012844	DOWN
<i>Klf2</i>	Kruppel Like Factor 2	-0.4301	0.013976	DOWN
<i>Sirt1</i>	Sirtuin 1	0.89069	7.00E-08	UP
<i>Foxo4</i>	Forkhead Box O4	0.337196	0.007948	UP
<i>Foxo3</i>	Forkhead Box O3	0.788951	0.000103	UP
<i>Cd36</i>	CD36 Molecule	0.844657	2.27E-06	UP
<i>Acs14</i>	Acyl-CoA Synthetase Long Chain Family Member 4	0.500407	0.002451	UP
<i>Lox</i>	Lysyl Oxidase	0.569453	0.039129	UP
<i>Lpcat3</i>	Lysophosphatidylcholine Acyltransferase 3	0.887262	3.15E-07	UP
<i>Steap3</i>	STEAP3 Metalloreductase	1.077301	2.53E-06	UP
<i>Slc11a2</i>	Solute Carrier Family 11 Member 2	0.763997	2.73E-06	UP
<i>Ireb2</i>	Iron Responsive Element Binding Protein 2	0.741732	4.40E-06	UP
<i>Tulp4</i>	TUB Like Protein 4	-0.25114	0.032295	DOWN
<i>Slc39a8</i>	Solute Carrier Family 39 Member 8	1.586668	2.93E-09	UP
<i>Tfrc</i>	Transferrin Receptor	2.454485	9.12E-10	UP
<i>Prnp</i>	Prion Protein	0.472854	0.005242	UP
<i>Ftl1</i>	Fms related tyrosine kinase 1	-0.2649	0.036538	DOWN
<i>Rorc</i>	RAR Related Orphan Receptor C	-0.92871	8.70E-05	DOWN

<i>Srebf2</i>	Sterol Regulatory Element Binding Transcription Factor 2	-0.43092	0.015567	DOWN
<i>Gpx4</i>	Glutathione Peroxidase 4	0.132068	0.487136	NOT
<i>Sepsecs</i>	Sep (O-Phosphoserine) TRNA:Sec (Selenocysteine) TRNA Synthase	0.407574	0.044122	UP
<i>Secisbp2</i>	SECIS Binding Protein 2	0.042339	0.730102	NOT
<i>Trit1</i>	TRNA Isopentenyltransferase 1	-0.17204	0.169288	NOT
<i>Hmgcr</i>	3-Hydroxy-3-Methylglutaryl-CoA Reductase	0.149581	0.212724	NOT
<i>Mvd</i>	Mevalonate Diphosphate Decarboxylase	-0.31105	0.051668	NOT
<i>Sumo1</i>	Small ubiquitin like modifier 1	0.451489	0.0005123	UP
<i>Sumo2</i>	Small ubiquitin like modifier 2	0.478137	0.0070311	UP
<i>Ube2o</i>	ubiquitin conjugating enzyme E2 O	0.698306	0.0150987	UP
<i>Ube3a</i>	Ubiquitin Protein Ligase E3A	0.671174	1.66E-05	UP
<i>Arntl</i>	Aryl Hydrocarbon Receptor Nuclear Translocator Like	-0.51523	0.000585	DOWN
<i>Sqstm1</i>	Sequestosome 1	-0.34414	0.049441	DOWN
<i>Egln2</i>	Egl-9 Family Hypoxia Inducible Factor 2	-0.32685	0.005514	DOWN
<i>Hif1a</i>	Hypoxia Inducible Factor 1 Subunit Alpha	0.414608	0.002703	UP

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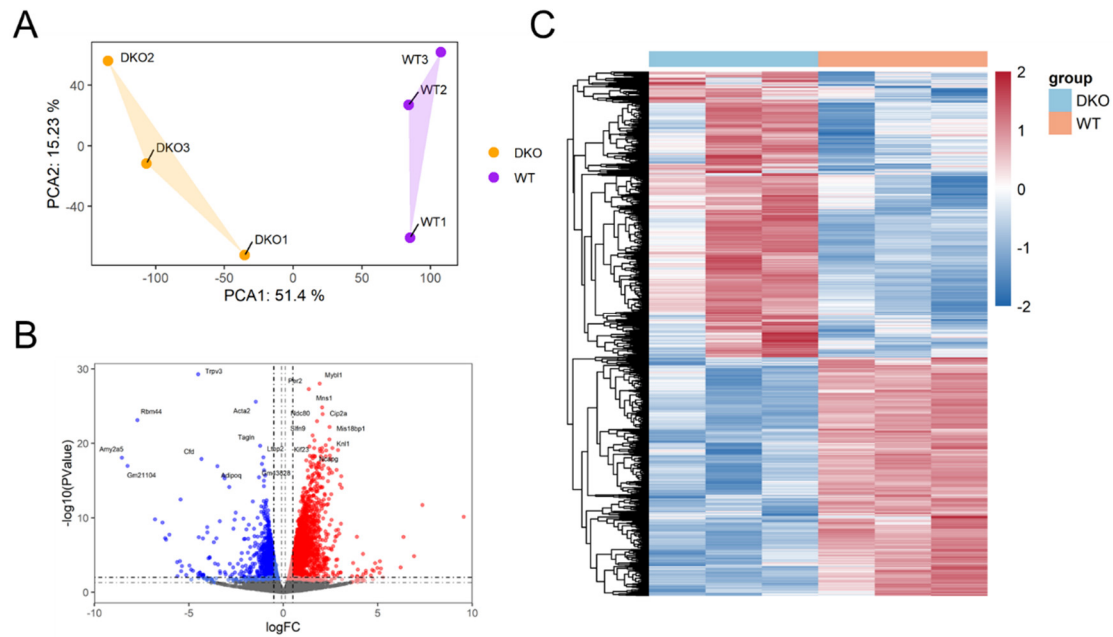
**Table S2.** Antibodies used in this study

Protein	Manufacturer	LOT.NO
GPX4	proteintech	67763-1-Ig
SLA	Proteintech	11551-1-AP
SBP2	Proteintech	12798-1-AP
IPT	BIOSS	bs-17134R
HMGCR	abcam	ab174830
MVD	Proteintech	15331-1-AP
BMAL1	Santa	SC-373955
SQSTM1	Proteintech	18420-1-AP
EGLN2	Affinity	DF7918
HIF1A	Santa	SC-13515
$\beta$ -actin	Rabbit	abcam

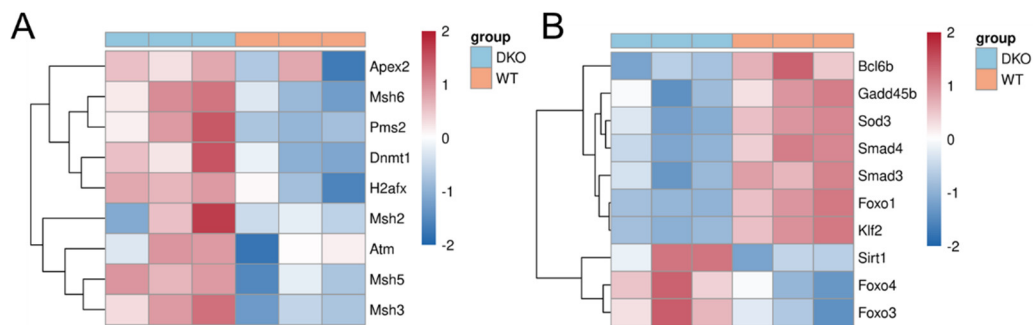
**Table S3.** qPCR primers used in this study

Gene	Forward Primer (5-3)	Reverse Primer (3-5)
<i><math>\beta</math>-actin</i>	CGTTGACATCCGTAAAGACC	AACAGTCCGCCTAGAAGCAC
<i>Rory</i>	TGCGACTGGAGGACCTTCTACG	GTTGTTGGACGGGCTGAGGATG
<i>Srebp2</i>	CACAAGTCTGGCGTTCTGAGGAAG	CCAGCTTCAGCACCATGTTCTCC

SUPPLEMENTARY FIGURES



**Figure S1. Differentially expressed spleen RNA between 14-month-old DKO and WT mice ( $|\log_2 \text{fold change}| > 0.1$ ,  $p\text{-value} < 0.05$ ).** Compared with normal samples, 3583 mRNA were upregulated and 2290 mRNA were downregulated in DKO mice. (A) Principal component analysis (PCA) of DKO samples and WT samples; (B) Volcano map for differentially expressed mRNAs. Red stands for up-regulation, blue stands for down-regulations, and gray stands for intermediate in volcanoes. Each point represents a gene. (C) Heatmap for differentially expressed mRNAs. Red stands for DKO male mice, and blue stands for WT male mice.



**Figure S2. Heatmap for DEMRNAs involved in (A) DNA repair pathways, (B) FoxO signaling pathway. Blue: DKO mice; Red: WT mice.**

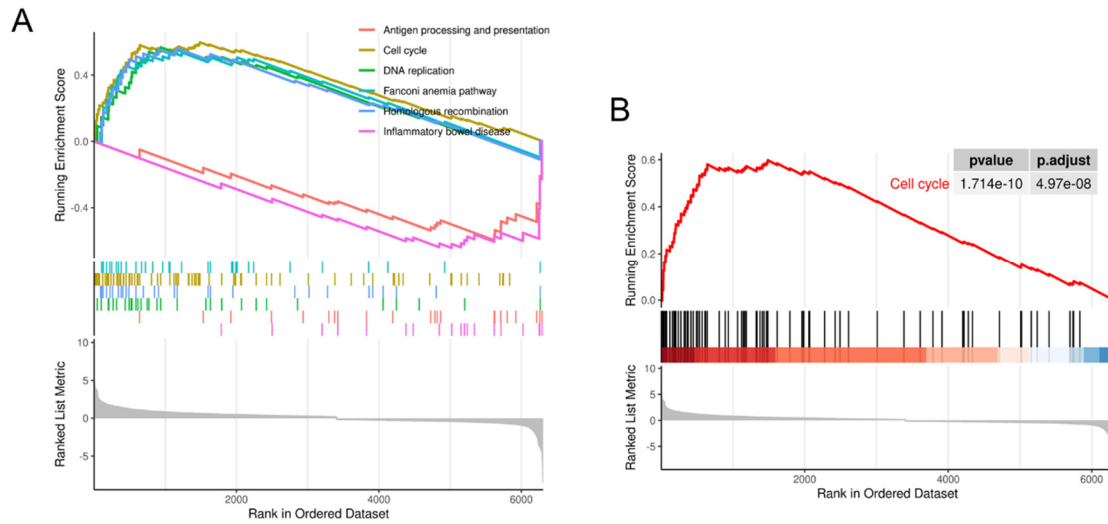


Figure S3. Gene set enrichment analysis (GSEA) for DEmRNAs

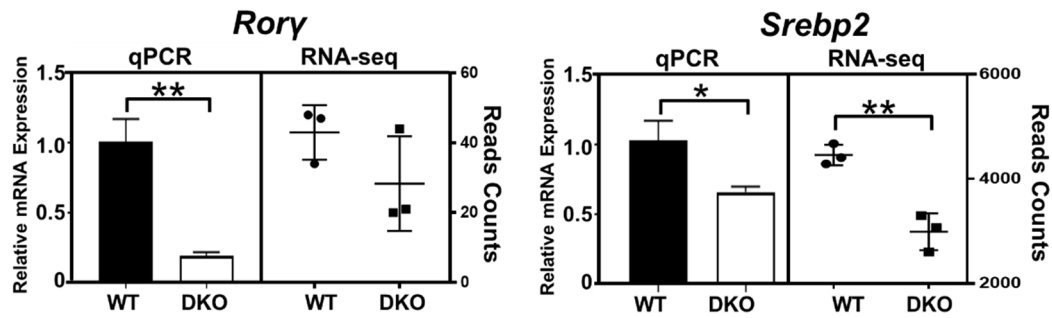
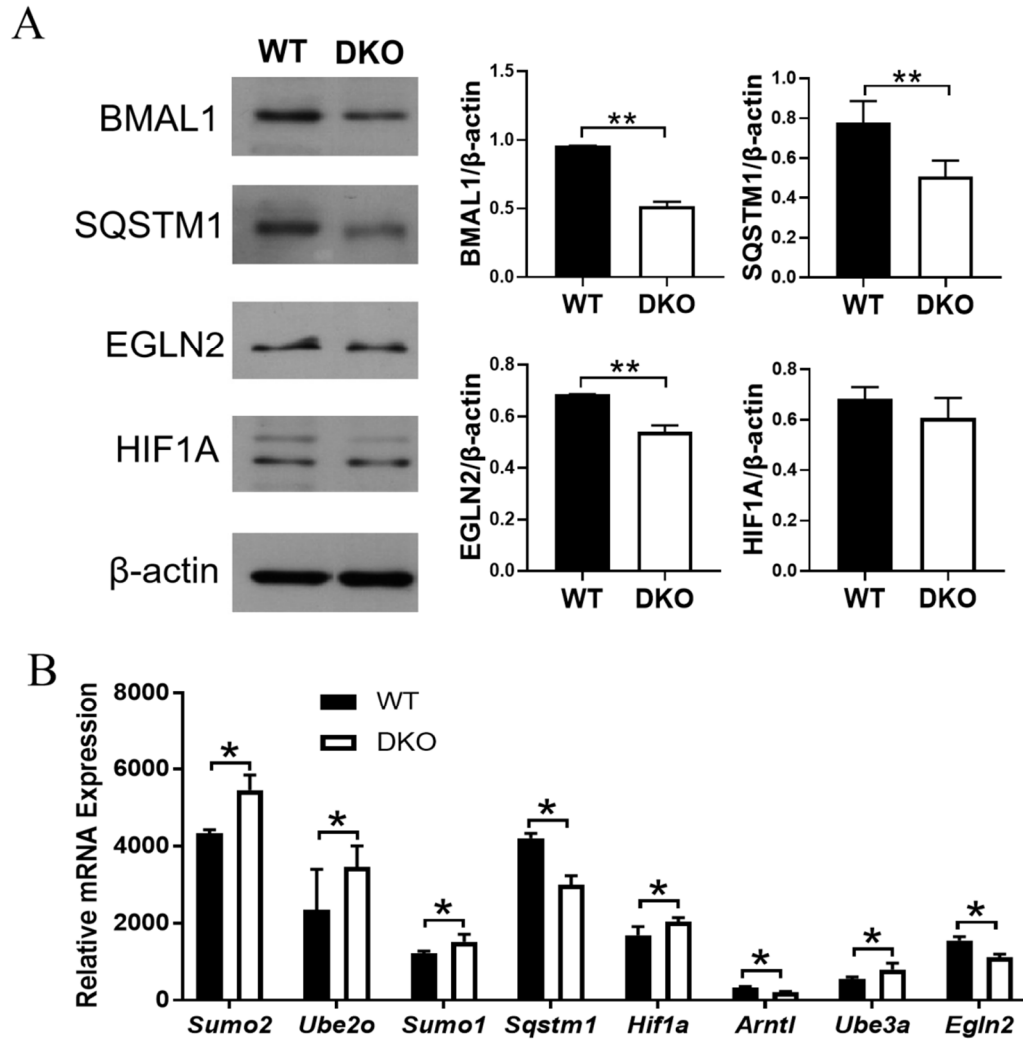


Figure S4. RT-qPCR results (left side of each image) and mRNA relative expression in Reads counts (right side of each image) of *Rory* and *Srebp2*. Data are presented as mean  $\pm$  SD,  $n = 3/\text{group}$ . \*  $p < 0.05$ , \*\*  $p < 0.01$ .



**Figure S5. Expression of selective autophagic degradation related genes.** (A) Western blot results of Bmal1, SQSTM1, EGLN2, and HIF1A. (B) Relative mRNA expression of *Sumo2*, *Ube2o*, *Sumo1*, *Sqstm1*, *Hif1a*, *Arntl*, *Ube3a*, *Egl2*. Data are presented as mean  $\pm$  SD, n = 2/group.\*  $p < 0.05$ , \*\*  $p < 0.01$ .