Partial loss of interleukin 2 receptor gamma function in pigs provides mechanistic insights for the study of human immunodeficiency syndrome

Supplementary Data

Supplementary Table S1: Production of IL2RG pig

No of Experiments	Numbers of embryos transferred	No of IL2RG Piglets
#1	216	1
#2	48 Blastocysts	4
#3	187	2
#4	179	4

Supplementary Table S2: Primer set lists for IL2RG KO pig analysis

Gene		Primer sequences
IL2RG	Forward	CTGGACTATTAGAAGGATGTGGGC
	Reverse	ATATAGTGGGAAGCCTGGGATGGCT

*PCR product size: 225bp and 192bp

Supplementary Table S3: Primer set lists for off-targeting analysis of IL2RG KO pig

Gene		Primer sequences
LRRIQ1	Forward	CGTTTGTTAAAACTGCAGCATA
	Reverse	TTTTGCTTCCCTTTCCTTCC
BNC2	Forward	AGCCAGAGGAAGGGGTTTTA
	Reverse	GTTAACCAGCTCAGGCAAC
SLC17A5	Forward	GTCTGGTTGCAGCTCAAGGT
	Reverse	GCCACTGTGGACTCTAGAGGAT
ZNF334	Forward	ATTCACACAGGGGAGAAACG
	Reverse	GTGGAAATTTTTCCCCCATT
TTN	Forward	CTTTGGACCTGCCCACTTT
	Reverse	GGATGTGTGATCGGTTCCAT
PGRMC2	Forward	TGAGGGAGAGAGGAGACCTG
	Reverse	CTAGGGGAAGGAAAGGGATG
AVPR2	Forward	GGCGTACATGCCTACCATCT
	Reverse	CTGTCCACGGTCTTTGTGG
CCDC18	Forward	TTCTCCCAACCCCATTTACA
	Reverse	CCTGAGTTGAACCAGCACCT
ZSWIM2	Forward	AAAAAGTTCTTCCTGTTTTGACAGA
	Reverse	TGGTTATTCCACCAATGCAA

Supplementary Table S4: Antibody list for immunohistochemistry

Primary antibodies	Host	Dilution	Source
IL-2Rα	Mouse	1/100	Santa Cruz Biotechnology,
IL2Rγ	Rabbit	1/200	Bioss
CD3	Mouse (LN10)	1/50	Leica Biosystems
CD4	Rabbit	1/100	LSbio
CD8	Goat	1/100	LSbio
CD20	Mouse(MJ1)	Predilution	Leica Biosystems
CD21	Mouse(2G9)	Predilution	Leica Biosystems
CD79a	Mouse(11E3)	Predilution	Leica Biosystems
CD56	Mouse(1B6)	1/100	Novocastra
CD25	Mouse(4C9)	predilution	Leica Biosystems
CD1d	Mouse	1/100	Santa Cruz Biotechnology,
CD44	Mouse	1/100	LSbio
CD335	Rabbit	1/100	Bioss
FOXP3	Rabbit	1/500	Abcam

Supplementary Table S5: Primer list for Real-Time RT-PCR

For Table S5, please see the attached Excel file

Supplementary Table S6: KEGG in thymus

KEGG	Genes
Cytokine-cytokine receptor interaction	CCL2, IL6ST, IL18, CXCL2, CXCL9, CCL8, IL15, CCL5, CCL4, CXCL12, IL10, CXCL10, IL12RB1, IL10RA, IFNG, CXCR6, TPO, IL2RG, FAS, EGF, IL13RA1, IFNGR2, IL1A, GHR, IL4, EGFR, MET, LIFR, CCL19, CD40, KDR, CCR9, ACVR2A, TNFSF10, CCR6, TNFSF13B, VEGFA, BMP7, BMPR1A
Jak-STAT signaling pathway	IL4, IL6ST, LIFR, BCL2L1, IL15, STAT1, IL10, IL12RB1, CCND3, IL10RA, IFNG, TPO, IL2RG, IL13RA1, IFNGR2, GHR
Leukocyte transendothelial migration	F11R, CLDN7, OCLN, CLDN4, MMP9, MYLPF, CLDN10, CXCL12, CDH5, VCL, MYL9, VCAM1, MAPK12, MAPK13, MLLT4
Complement and coagulation cascades	PLAT, A2M, C3, CFB, SERPING1, C1S, C1QC, C1QA, VWF, C1QB, THBD, SERPINE1, CFH, C2
Hematopoietic cell lineage	IL4, CD9, CD36, CD3G, CD3D, CD8A, CD3E, CD2, TPO, ANPEP, CD4, ITGB3, IL1A
Systemic lupus erythematosus	C1QA, C1QB, CD80, FCGR2B, C3, IFNG, H2AFZ, C1S, CD40, C2, C1QC, IL10
T cell receptor signaling pathway	IL4, CD3G, MAPK12, CD8A, CD3D, CD3E, MAPK13, LCK, IFNG, CD247, CD4, IL10

TGF-beta signaling pathway	ACVR2A, ID1, SMAD7, SMAD6, IFNG, ID4, DCN, BMP7, THBS1, BMP5, BMPR1A
Hypertrophic cardiomyopathy (HCM)	SLC8A1, ACTC1, CACNA2D1, TNNC1, CACNB1, IGF1, SGCD, ITGB3, PRKAA2, TPM1
Aldosterone-regulated sodium reabsorption	ATP1B1, NR3C2, HSD11B2, IGF1, IGF2, SFN, IRS1, SLC9A3R2
Intestinal immune network for IgA production	CCR9, IL4, TNFSF13B, CD80, IL15, CD40, CXCL12, IL10
Primary immunodeficiency	CD8A, CD3D, CD3E, LCK, IL2RG, CD4, CD40
Allograft rejection	IL4, PRF1, CD80, IFNG, FAS, CD40, IL10
Glycosphingolipid biosynthesis	B3GALNT1, HEXB, FUT1, FUT2

Supplementary Table S7: Cellular component in thymus

For Table S7, please see the attached Excel file

Supplementary Table S8: Molecular function in thymus

Molecular function	Genes
Identical protein binding	CLDN7, CLDN4, CD8A, TNNC1, IL6ST, HEXB, SNCA, E2F8, TPD52, HOOK1, APP, APOA1, PLOD1, FAP, FAS, GHR, EGFR, MLXIPL, C1QB, VEGFA, COL1A2, NPPC, COL1A1, CAV2, CAV1, ENPP1, CD247, CLDN10, BCL2L1, ITGB3, CD74, SLC11A1, IGF1R, CD2, CD4, THBS1, CEBPA, S100A16, SMAD6, EPHX2, MUL1, CIDEA, CENPF, BIRC5, ADIPOQ, VWF, FAAH, HPGD, BMPR1A
Protein kinase activity	FGFR2, CAV2, CCL2, NEK2, PKMYT1, AURKA, AURKB, PRKG1, CCL4, IGF1R, SBK1, TEK, MASTL, PRKAA2, SYK, SRPK3, EGFR, CDK1, BCR, MET, PDK4, AXL, NPR1, PBK, IRS1, WEE1, KDR, EPHA4, ACVR2A, CAMK4, MAPK12, CCND3, MAPK13, PLK1, NTRK2, LCK, GRK5, CIT, MELK, CDC42BPB, BMPR1A
Protein homodimerization activity	CAV2, CD8A, ENPP1, TNNC1, IL6ST, HEXB, CD247, E2F8, TPD52, SLC11A1, PLOD1, FAP, CD2, CD4, GHR, CEBPA, S100A16, SMAD6, EPHX2, MLXIPL, CIDEA, CENPF, BIRC5, ADIPOQ, VWF, C1QB, FAAH, VEGFA, NPPC, HPGD, BMPR1A

Cytokine activity	IL4, CCL2, IL18, CXCL2, CXCL9, CCL8, CCL19, IL15, CCL5, ADIPOQ, CXCL12, CCL4, IL10, CXCL10, TNFSF10, TNFSF13B, VEGFA, IFNG, BMP7, IL1A, BMP5, CMTM5
Cytokine binding	A2M, IL6ST, LIFR, CD74, CCR9, CCR6, IL12RB1, CD36, DARC, IL10RA, CXCR6, IL2RG, IL13RA1, THBS1, IFNGR2, GHR, CCRL1
Polysaccharide binding	FGFR2, SELP, CCL2, ENPP1, TLR2, PGLYRP1, CCL8, FGF10, POSTN, DCN, COL5A3, TINAGL1, APP, VEGFA, ADAMTS1, THBS1, BMP7
Glycosaminoglycan binding	FGFR2, SELP, CCL2, TLR2, PGLYRP1, CCL8, FGF10, POSTN, DCN, COL5A3, APP, VEGFA, ADAMTS1, THBS1, BMP7
Extracellular matrix structural constituent	AMBN, LAMA4, COL27A1, LUM, COL1A2, COL15A1, MGP, CD4, COL1A1, TINAGL1, COL5A3, LAMB1
Chemokine activity	CCL2, CXCL2, CXCL9, CCL8, CCL19, CCL5, CCL4, CXCL12, CXCL10
Insulin-like growth factor binding	IGF1R, WISP3, IGFBP7, KAZALD1, IGF2, IGFBP3, IGFBP5

Supplementary Table S9: Biological function in thymus

Biological function	Genes
Immune response	CD8A, IL18, SNCA, PPARG, TLR2, PGLYRP1, TLR3, TLR4, IL15, CXCL12, C1QC, IL10, CXCL10, CFP, IFNG, CFH, LTF, MS4A2, FAS, IL1A, CCRL1, CRISP3, SIT1, SERPING1, PDCD1LG2, CCR9, C1QA, C1QB, CCR6, TNFSF13B, VEGFA, SEMA4D, GBP1, CCL2, ENPP1, C3, CYSLTR2, CXCL2, CLU, CXCL9, CCL8, C1S, CCL5, CCL4, CD74, IGF1R, SLC11A1, IL2RG, CD4, C2, THBS1, IL4, OLR1, CFB, SMAD6, MYLPF, CCL19, SAMHD1, TINAGL1, CD180, TNFSF10, FCGR2B, CD274, DMBT1, BMPR1A
Inflammatory response	A2M, ADORA3, CCL2, S100A8, AIF1, C3, CLU, CXCL2, S100A9, TLR2, CXCL9, TLR3, CCL8, TLR4, IL15, C1S, CCL5, C1QC, CCL4, IL10, CXCL10, CFP, SLC11A1, CFH, ITIH4, MS4A2, C2, THBS1, IL1A, F11R, SELP, OLR1, CFB, EPHX2, CCL19, IGF2, SERPING1, CD40, CDO1, CD180, CD163, C1QA, C1QB, DARC
Response to hormone stimulus	ME1, CAV2, RBP4, HMGB2, A2M, CAV1, CCL2, ENPP1, IGFBP7, PTGS1, PPARG, AURKA, BCL2L1, GNG12, CCL5, AQP1, IL10, IGF1R, FAS, THBS1, AGRP, GHR, ACTA1, GATM, CRYAB, MGP, IGF2, NPY1R, CDO1, STAT1, ADIPOQ, IRS1, ABCB4, RERG, C1QB, RETN, CDKN1A, BTG2, FABP3, FABP4, HSD11B2, SORT1, COL1A1, BMP7
Positive regulation of immune system process	EDN3, BLM, IL6ST, C3, IL18, CLU, CD247, TLR2, KLRK1, TLR3, TLR4, IL15, C1S, C1QC, CXCL12, CD74, VCAM1, CFP, SLC11A1, IFNG, CD2, CFH, MS4A2, CD4, IL2RG, C2, IL13RA1, THBS1, LAG3, SYK, IL4, CD3E, CFB, SERPING1, CD40, PDCD1LG2, C1QA, C1QB, CDKN1A, TNFSF13B, CD80, VEGFA, LCK

Regulation of cell activation	BLM, IL6ST, IL18, SNCA, KLRK1, TLR4, IL15, CD74, IL10, VCAM1, IFNG, CD2, MS4A2, CD4, IL2RG, FAS, THBS1, IL13RA1, LAG3, SYK, IL4, SELP, SIT1, CD3E, CD40, PDCD1LG2, CDKN1A, TNFSF13B, CD80, CD274, LCK
Blood circulation	CAV2, EDN3, CAV1, TNNC1, MYL1, PPARG, PTGS1, CXCL12, TPM1, CXCL10, EDNRA, EDNRB, CALCB, GUCY1A3, ACTC1, OLR1, ACTA2, EPHX2, NPR1, SERPING1, NPY1R, STAT1, ADIPOQ, NPY, PLN, VEGFA, COL1A2, NPPC, ACE2, HSD11B2, GUCY1B3
Regulation of leukocyte activation	BLM, IL6ST, IL18, SNCA, KLRK1, IL15, CD74, IL10, VCAM1, IFNG, CD2, MS4A2, CD4, IL2RG, FAS, THBS1, IL13RA1, LAG3, SYK, IL4, SIT1, CD3E, CD40, PDCD1LG2, CDKN1A, TNFSF13B, CD80, CD274, LCK
Positive regulation of leukocyte activation	IL4, BLM, IL6ST, CD3E, IL18, KLRK1, IL15, CD40, PDCD1LG2, CD74, VCAM1, CDKN1A, TNFSF13B, CD80, LCK, IFNG, CD2, MS4A2, CD4, IL2RG, THBS1, IL13RA1, SYK
Positive regulation of phosphorylation	EGFR, IL4, CAV1, IL6ST, CD3E, IGF1, IGF2, TLR4, ADIPOQ, EDNRA, EDNRB, ACVR2A, CD80, CCND3, IFNG, CD4, BMP7, THBS1, EGF, SYK, BMPR1A, GHR

Supplementary Table S10: KEGG in spleen

KEGG	Genes
Cytokine-cytokine receptor interaction	TNF, IL8, CXCL9, CD70, IL15, CXCL11, CCL4, CXCL10, TNFSF10, TNFSF13B, CCR5, IL10RB, SR-PSOX, IFNG, IL1B, EPOR, IL12B
Chemokine signaling pathway	CCL2, CCR5, IL8, SR-PSOX, CCL21, CXCL9, STAT1, CXCL11, LOC780409, CCL4, CXCL10
Chemokine signaling pathway	CCL2, CCR5, IL8, SR-PSOX, CCL21, CXCL9, STAT1, CXCL11, LOC780409, CCL4, CXCL10
NOD-like receptor signaling pathway	NOD2, CCL2, TNF, NOD1, IL8, IL1B, CASP1
Systemic lupus erythematosus	TNF, C3, FCGR1A, IFNG, LOC396781, C1S, C2
Allograft rejection	TNF, SLA-3, IFNG, LOC396781, IL12B, SLA-5
Type I diabetes mellitus	TNF, SLA-3, IFNG, IL1B, IL12B, SLA-5
Allograft rejection	TNF, SLA-3, IFNG, LOC396781, IL12B, SLA-5
Jak-STAT signaling pathway	IL10RB, IFNG, EPOR, IL15, IL12B, STAT1
Graft-versus-host disease	TNF, SLA-3, IFNG, IL1B, SLA-5
Viral myocarditis	ITGAL, SLA-3, LOC396781, SLA-5
Complement and coagulation cascades	C3, BDKRB1, C1S, C2
Autoimmune thyroid disease	SLA-3, LOC396781, SLA-5

Supplementary Table S11: Cellular component in spleen

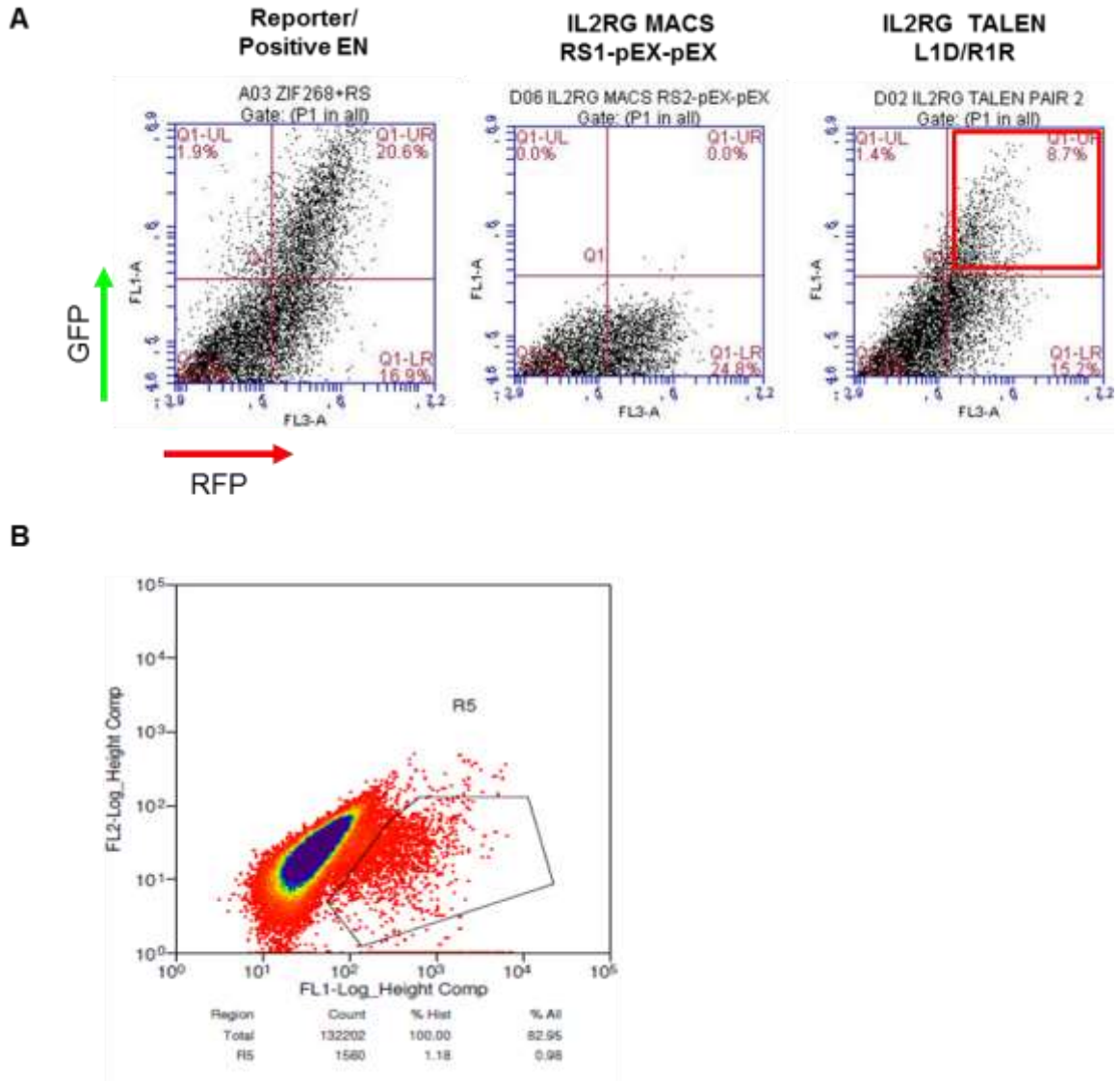
Cellular component	Genes
extracellular region	CCL2, TNF, C3, CXCL9, CD70, LOC733603, IL15, CXCL11, CCL4, CXCL10, TIMP1, NPG4, CCL21, IFNG, IL1B, TGFA, C2, LOC396596, IL8, IL27, PMAP-23, CD163, VWF, AMBN, ADM, NPY, SR-PSOX, SLPI, EGFL8, IL12B, IGFBP3, LOC780409
extracellular region part	CCL2, TNF, IL8, C3, IL27, LOC733603, CD70, IL15, CXCL11, CCL4, TIMP1, VWF, AMBN, NPY, CCL21, SR-PSOX, IFNG, TGFA, IL1B, IL12B, LOC780409
intrinsic to membrane	ITGAL, TLR10, TNF, TBXAS1, SLC15A2, SLC7A9, BDKRB1, CD70, GP91-PHOX, AQP1, SLA-5, CD163, SLA-3, CCR5, TFRC, SR-PSOX, TAP1, CD274, TGFA, EPOR, TREM1
extracellular space	CCL2, TNF, IL8, C3, IL27, LOC733603, CD70, IL15, CXCL11, CCL4, NPY, CCL21, SR-PSOX, IFNG, IL1B, TGFA, IL12B, LOC780409
plasma membrane	ITGAL, TNF, SLA-3, TFRC, CCR5, IRG6, TGFA, BDKRB1, SLA-5, CD163
membrane-bounded vesicle	VWF, TFRC, ESD, CTSB
cytoplasmic membrane-bounded vesicle	VWF, TFRC, ESD, CTSB
vesicle	VWF, TFRC, ESD, CTSB
cytoplasmic vesicle	VWF, TFRC, ESD, CTSB

Supplementary Table S12: Molecular function in spleen

Molecular function	Genes
cytokine activity	CCL2, TNF, IL8, IL27, CXCL9, CD70, IL15, CXCL11, CCL4, CXCL10, CCL21, SR-PSOX, IFNG, IL1B, IL12B, LOC780409
cation binding	ADSSL1, TBXAS1, CYP3A39, C1S, STAT1, GP91-PHOX, CAPN3, SOD2, DDX58, IRG6, EGFL8, KLF1, GBA, ALOX12
ion binding	ADSSL1, TBXAS1, CYP3A39, C1S, STAT1, GP91-PHOX, CAPN3, SOD2, DDX58, IRG6, EGFL8, KLF1, GBA, ALOX12
metal ion binding	DDX58, ADSSL1, TBXAS1, CYP3A39, IRG6, EGFL8, C1S, GP91-PHOX, STAT1, CAPN3, KLF1, ALOX12, SOD2
nucleotide binding	DDX58, IFIH1, ADSSL1, CKM, TAP1, RBM38, OAS1, OAS2, GP91-PHOX, MX1
purine nucleotide binding	DDX58, IFIH1, ADSSL1, CKM, TAP1, OAS1, OAS2, GP91-PHOX, MX1
chemokine activity	CCL2, IL8, CCL21, CXCL9, CXCL11, LOC780409, CCL4, CXCL10
chemokine receptor binding	CCL2, IL8, CCL21, CXCL9, CXCL11, LOC780409, CCL4, CXCL10
transition metal ion binding	DDX58, TBXAS1, CYP3A39, IRG6, GP91-PHOX, KLF1, ALOX12, SOD2
ribonucleotide binding	DDX58, IFIH1, ADSSL1, CKM, TAP1, OAS1, OAS2, MX1

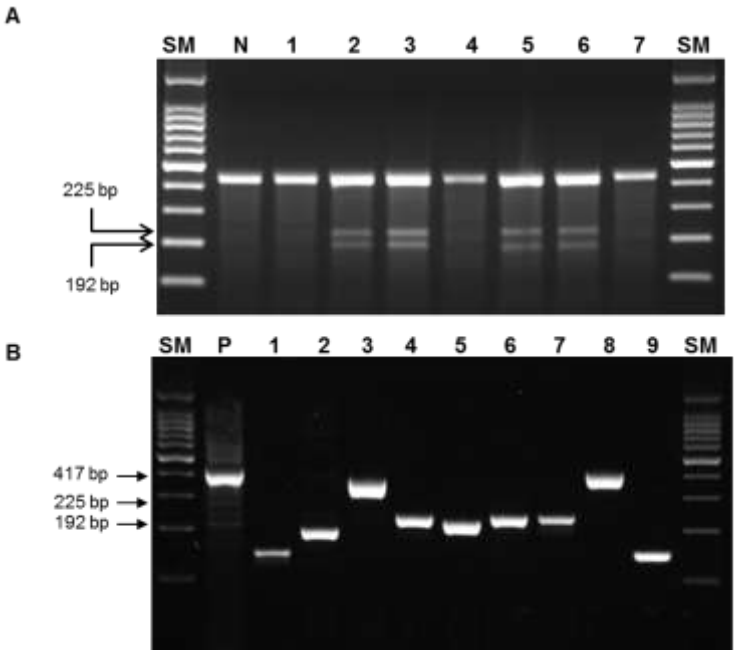
Supplementary Table S13: Biological function in spleen

Biological function	Genes
immune response	CCL2, TNF, C3, CXCL9, CD70, OAS1, IL15, C1S, OAS2, CXCL11, SLA-5, CCL4, CXCL10, SLA-3, CCL21, IRG6, IFNG, IL1B, C2, TLR10, IL8, IL27, DDX58, TNFSF10, TNFSF13B, LOC780409
defense response	TLR10, CCL2, IL8, C3, IL27, LYZ, PMAP-23, LOC733603, C1S, CCL4, CD163, DDX58, NPG4, IRG6, IL1B, C2
response to wounding	VWF, CCL2, IL8, C3, IL27, IL1B, LOC733603, C1S, C2, CCL4, CD163
inflammatory response	CCL2, IL8, C3, IL27, IL1B, LOC733603, C1S, C2, CCL4, CD163
proteolysis	C3, IL1B, LOC733603, C1S, C2, CD163
proteolysis	C3, IL1B, LOC733603, C1S, C2, CD163
oxidation reduction	CCL2, NPY, IL8, SR-PSOX, CCL4
acute inflammatory response	NOD2, IFIH1, NOD1, CASP1, IGFBP3
behavior	NOD2, IFIH1, NOD1, CASP1, IGFBP3
regulation of cell death	CCL2, IL8, SR-PSOX, CCL4

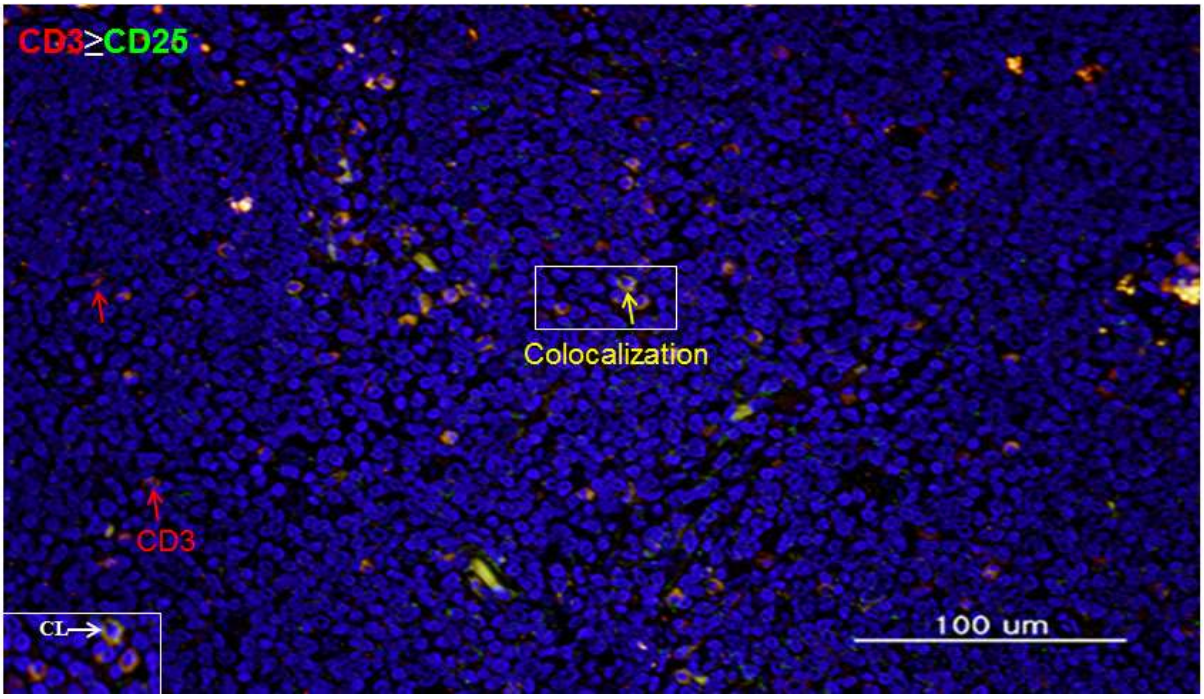


Supplementary Figure S1. Cell sorting analysis harboring IL2RG mutated gene. (A) The activity of the designed TALENs was pre-validated by introducing the TALENs with a reporter into HEK 293T cells. Enrichment of cells expressing RFP/GFP, compared to controls of RFP/GFP positive cells, was detected when TALEN sets were introduced with a reporter. After validation, the constructs coding for TALENs and a reporter for IL2RG were introduced into pig fibroblast cells by electroporation (B). After 48h post-transfection, the

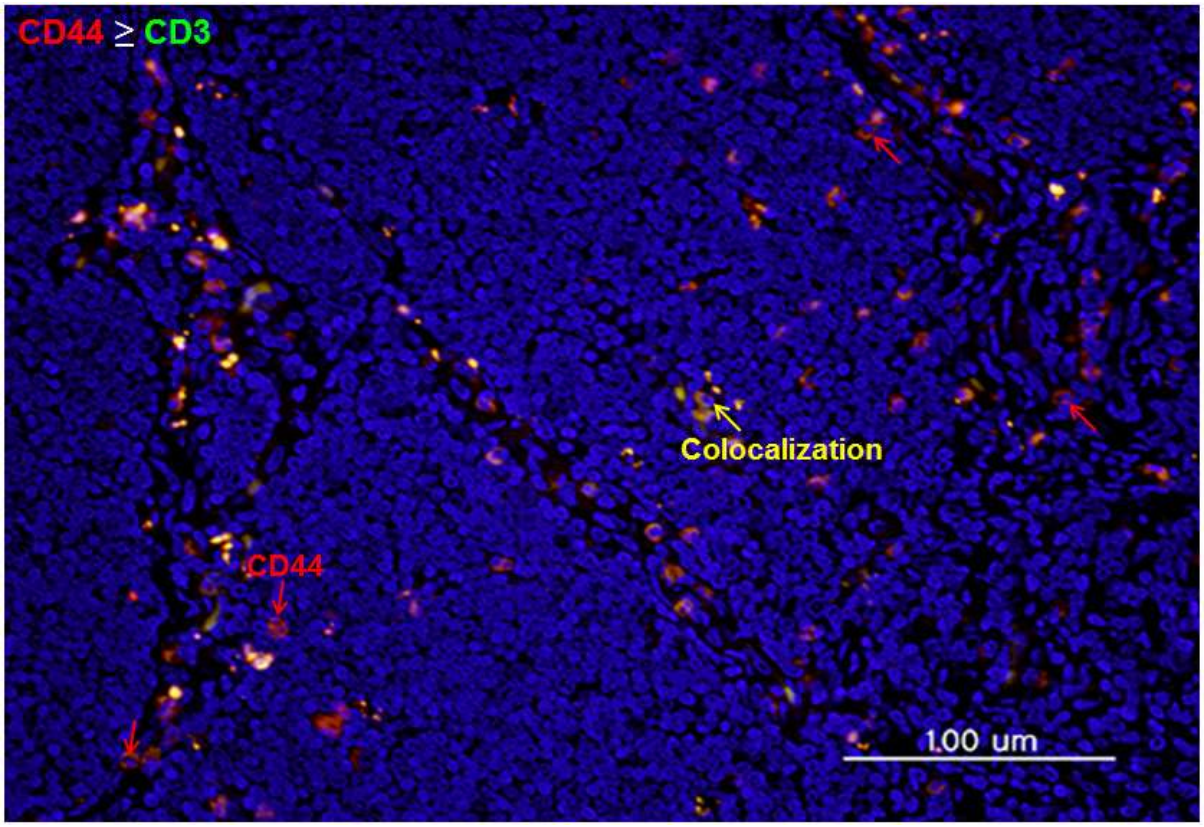
Cells were sorted for GFP+ cells by FACS and individually plated into 96-well plates. The frequency of GFP + cells from each transfection ranged from 23.0% to 38.0%. The box blew the arrow indicates the gate used to sort GFP+ cells.



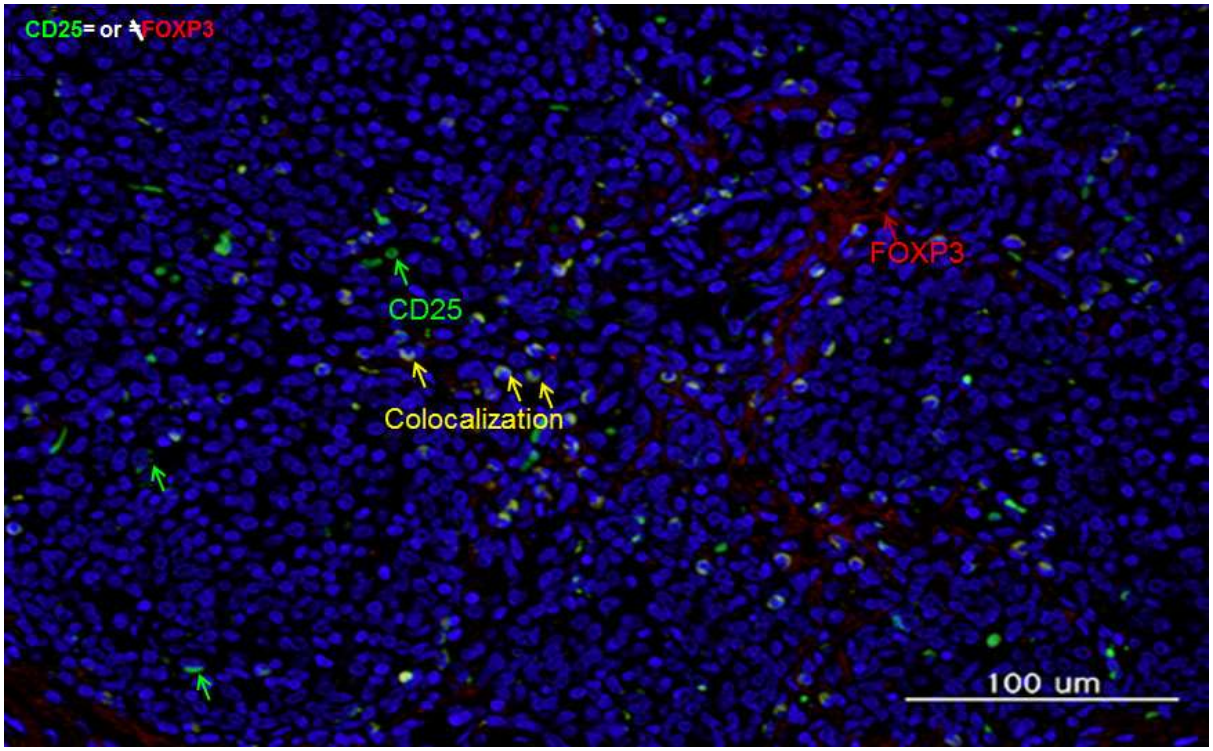
Supplementary Figure S2. Off-site targeting analysis of IL2RG genes from IL2RG mutated pig. (A) Genotyping of IL2RG KO piglets (B) Surveyor nuclease digest of heteroduplex DNA revealed no additional off-targetng mutations at the 9 loci with highest homology to pig IL2RG gene; SM: size marker, lane 1:LRRIQ1, 2:BNC2, 3:SLC17A5, 4:ZNF334, 5:TTN, 6:PGRMC2, 7:AVPR2, 8:CCDC18, 9:ZSWIM2



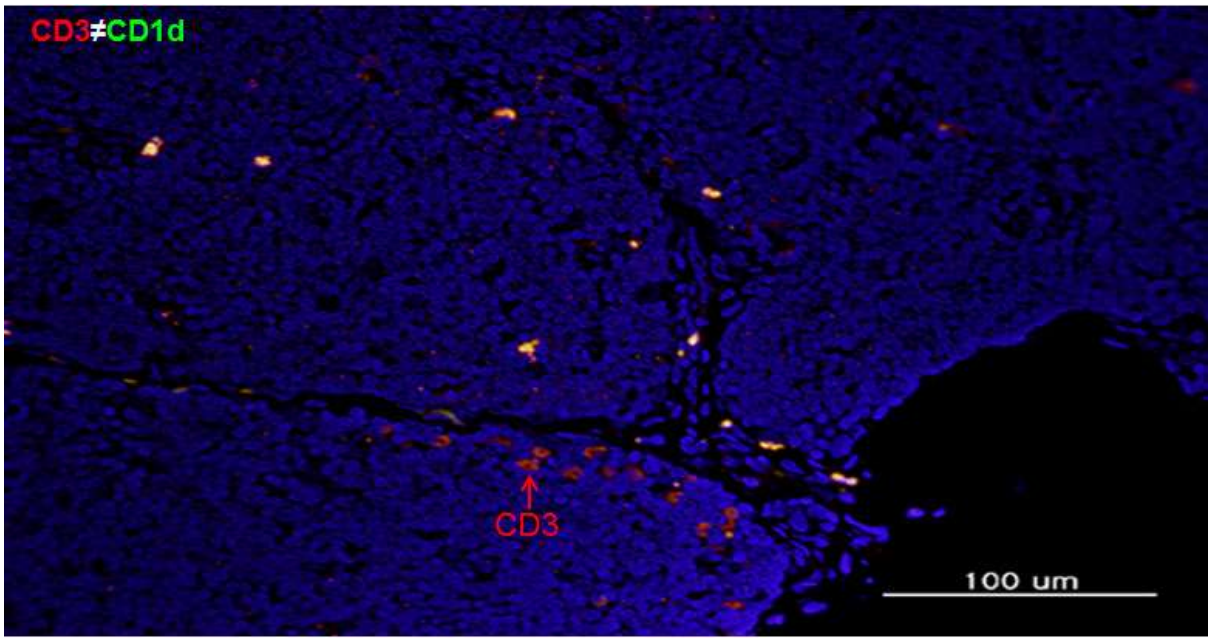
Supplementary Figure S3.1. Immunohistochemistry analysis of CD3 and CD25 positive cells in thymus. For co-localization of CD25 and CD3, thymic paraffin sections were stained with CD3 (red) and CD25 (green) antibodies where the DP cells appear yellow in merged images due to the co-expression of CD3 and CD25 proteins. CD3 staining indicates maturing T lymphocytes, whereas CD25 staining shows DN2-3 T cells or Treg cells.



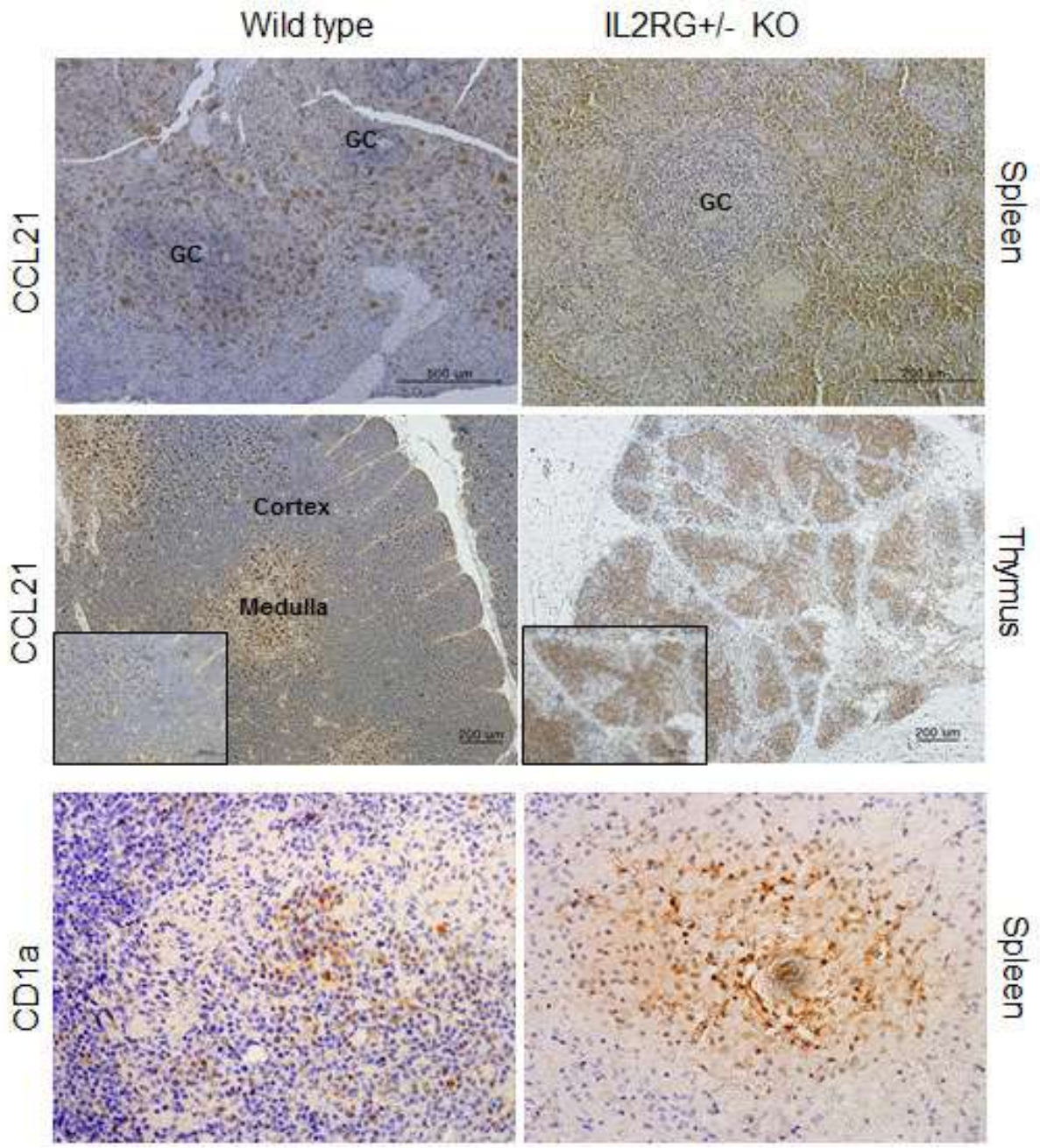
Supplementary Figure S3.2. Immunohistochemistry analysis of CD3 and CD44 positive cells in thymus. For co-localization of CD3 and CD44, thymic paraffin sections were stained with CD3 (green) and CD44 (red) antibodies. CD44 staining indicate memory T helper 1 (Th1) cells and DN1-2 stage of T cell development.



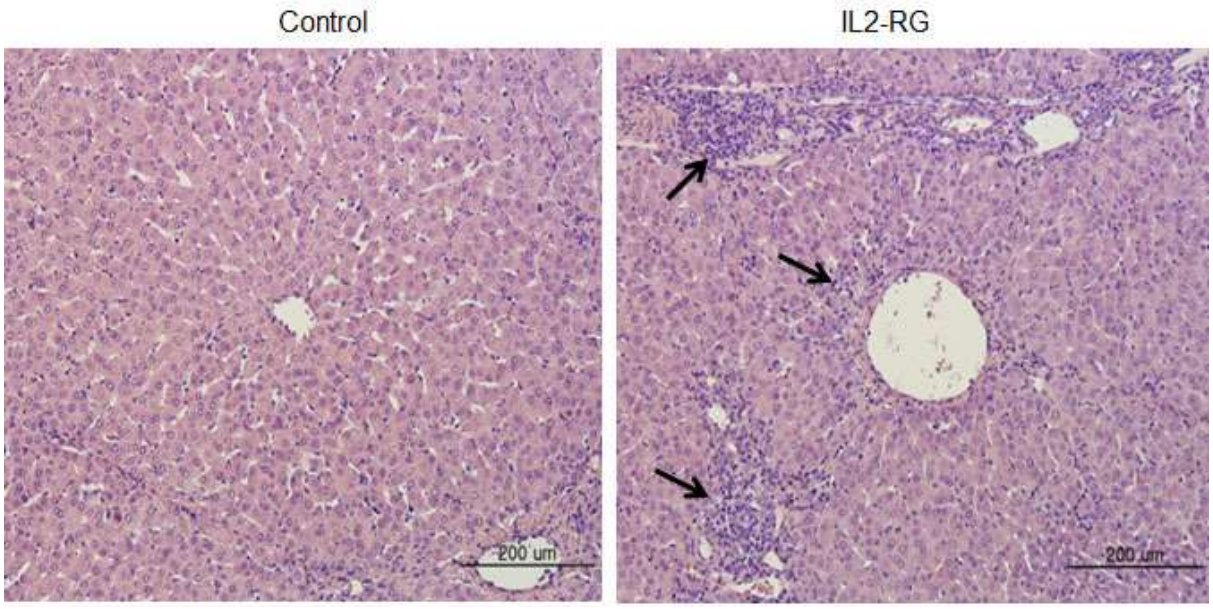
Supplementary Figure S3.3. Immunohistochemistry analysis of CD25 and FOXP3 positive cells in thymus. For co-localization of CD25 and FOXP3 expression, thymic paraffin sections were stained with CD25 (green) and FOXP3 (red) antibodies. FOXP3 staining indicates medullary mature Treg.



Supplementary Figure S3.4. Immunohistochemistry analysis of CD3 and CD1d positive cells in thymus. For co-localization of CD3 and CD1d staining, thymic paraffin sections were stained with CD3 (red) and CD1d (green) antibodies. CD1d-presented lipid antigens indicate natural killer T (NKT) cells.

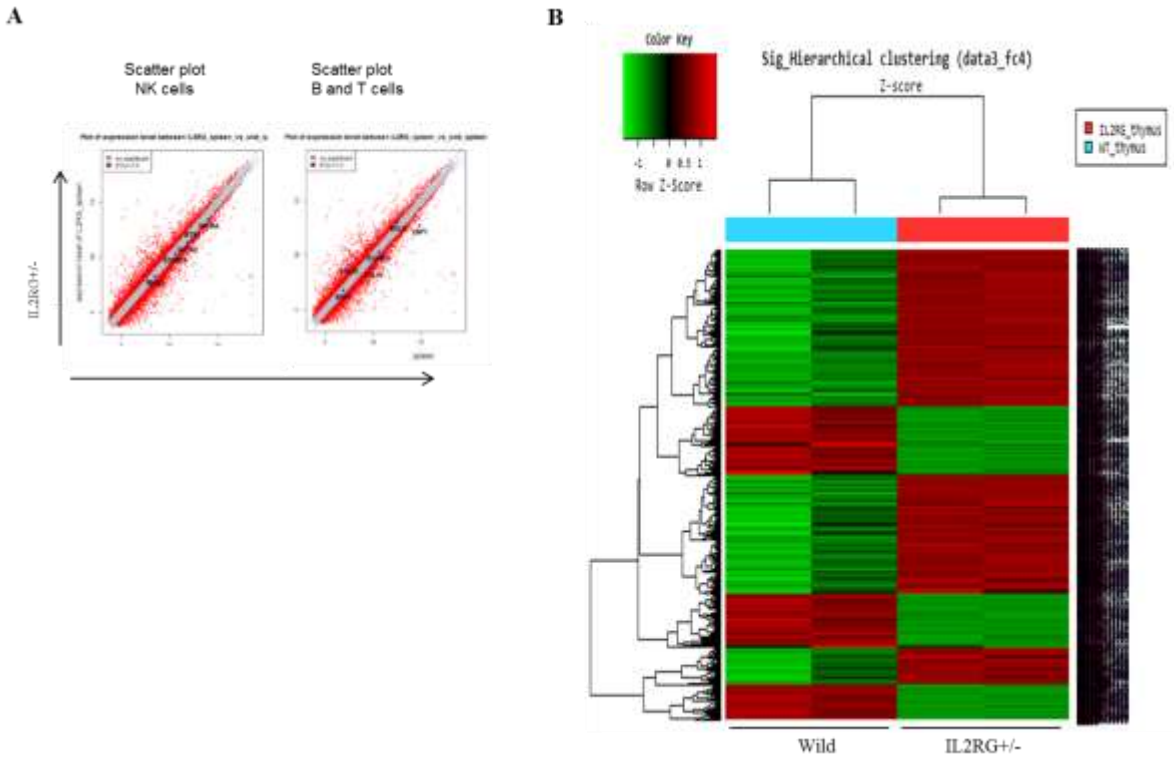


Supplementary Figure S3.5. Immunohistochemistry analysis of CCL21 and CD1a positive cells in spleen and thymus. CCL21 and CD1a indicate a maker of immature DC cells.

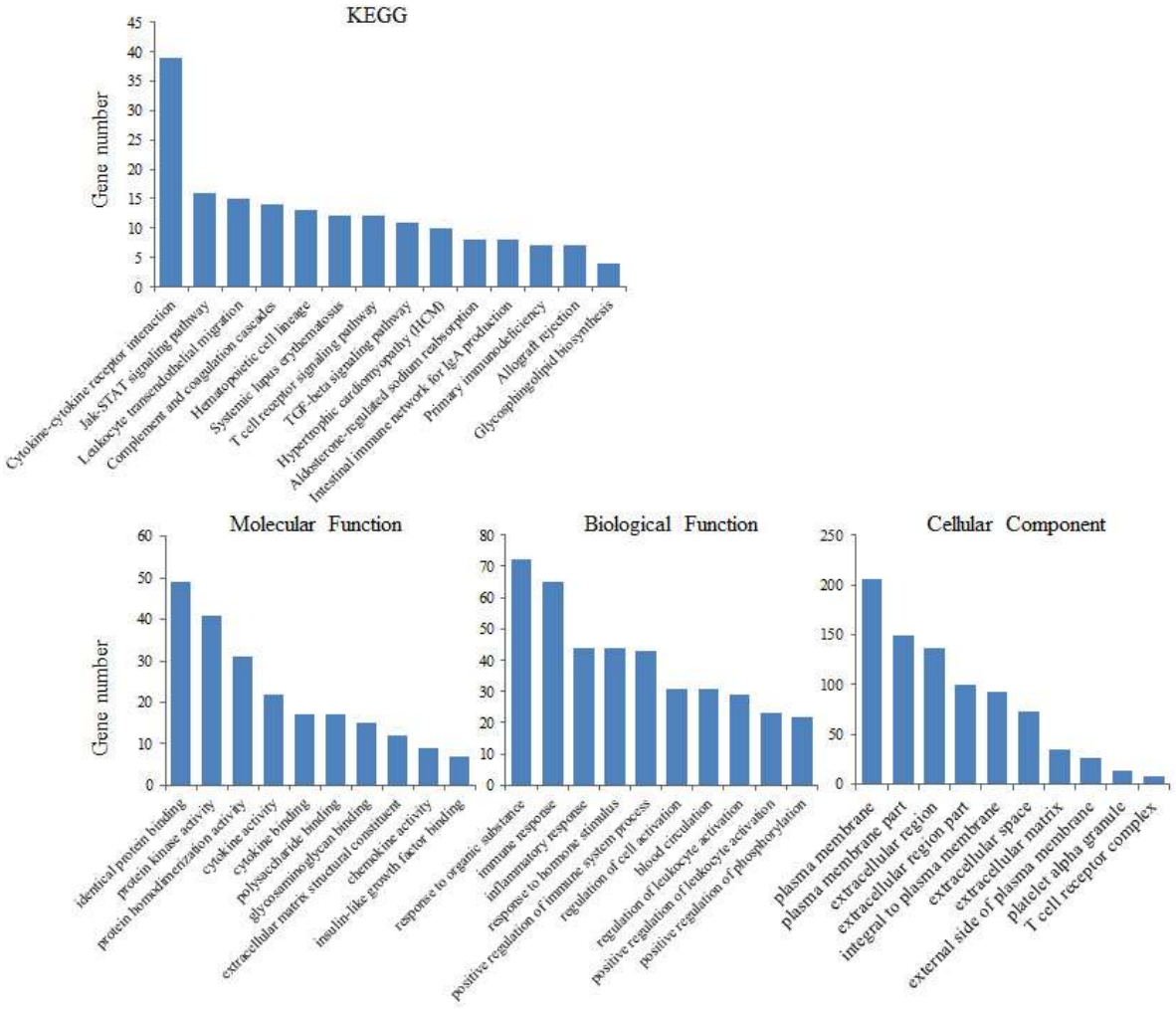


Supplementary Figure S4. Macrophages infiltration in liver of *mIL2RG*$^{+/\Delta 69\text{-}368}$ KO pig.

Arrow indicate abnormal lymphocyte infiltration into liver of *mIL2RG*$^{+/\Delta 69\text{-}368}$ KO pig.

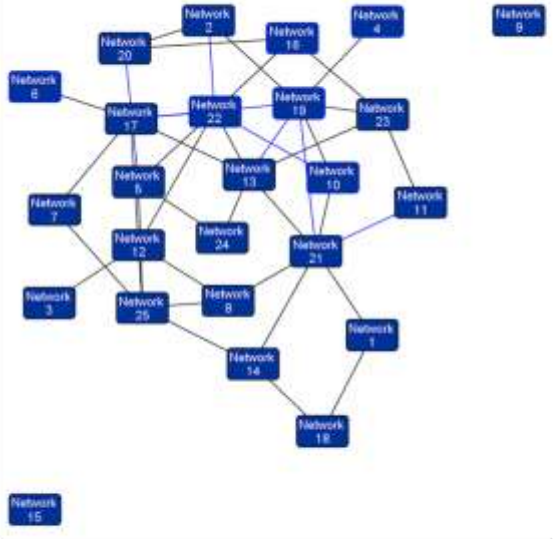


Supplementary Figure S5. Hierarchical clustering in WT and *mIL2RG*$^{+/\Delta 69\text{-}368}$ KO pig. Pairwise scatter plot analysis of the global gene expression profiles of NK (left) or B/T cell (right) from IL2RG KO pig and WT. Black lines indicate boundaries of 2-fold difference in gene expression levels. The bar to the right indicates the scattering density; the higher the scattering density, the darker the grey color. Gene expression levels are depicted in $\log_2$ scale. The number of differentially expressed genes is indicated under each scatter plot(A). Hierarchical clustering of WT and IL2RG thymus(B). Color represents the log intensities: Red: upregulated genes; green: downregulated genes. The heat map was plotted using "Heatplus" package in R program.



Supplementary Figure S6. **KEGG pathway enrichment analysis, GO biology process,** molecular function, and cellular function analysis in WT and mIL2RG$^{+/\Delta 69\text{-}368}$ KO pig thymus. Using DAVID program, up- and down- regulated gene expressions were divided into 3 subgroups such as Biological process, Cellular component, and Molecular function. Each subgroup shows the top 10 of the gene function group.

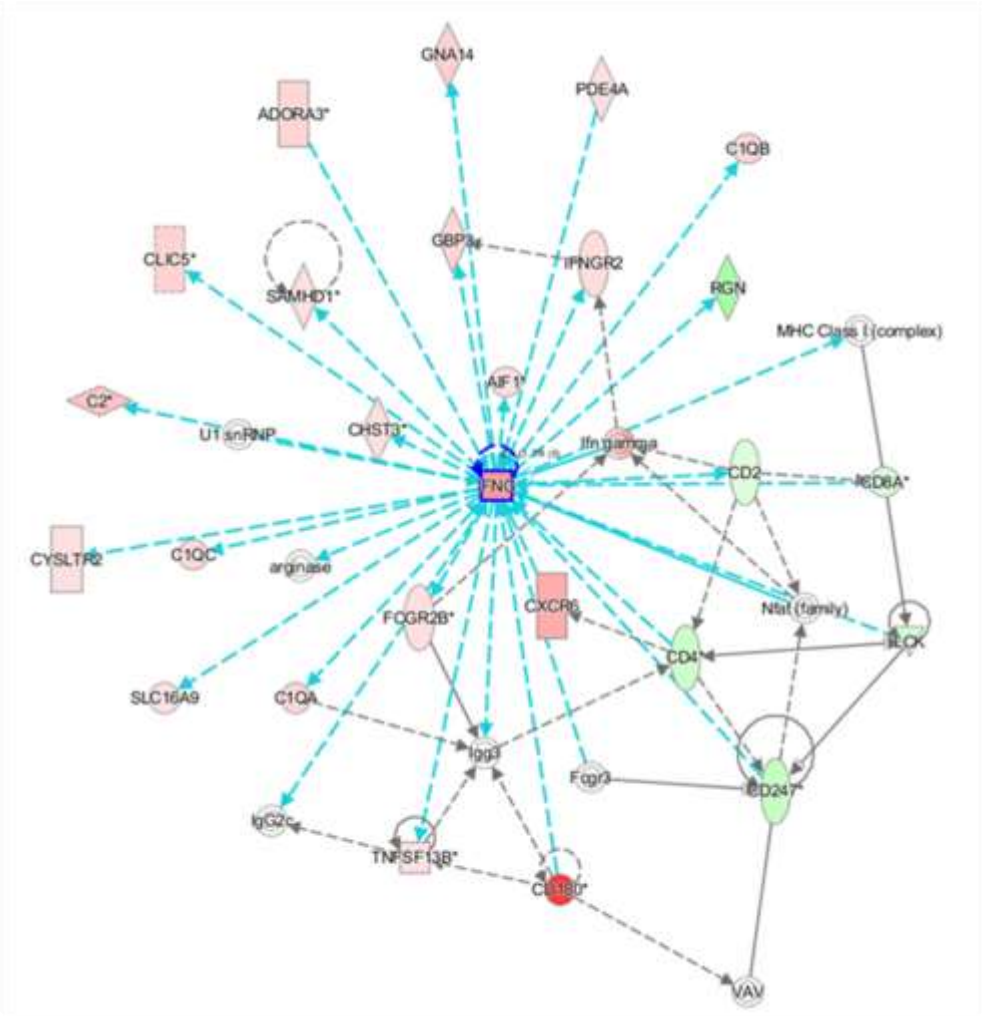
A



B

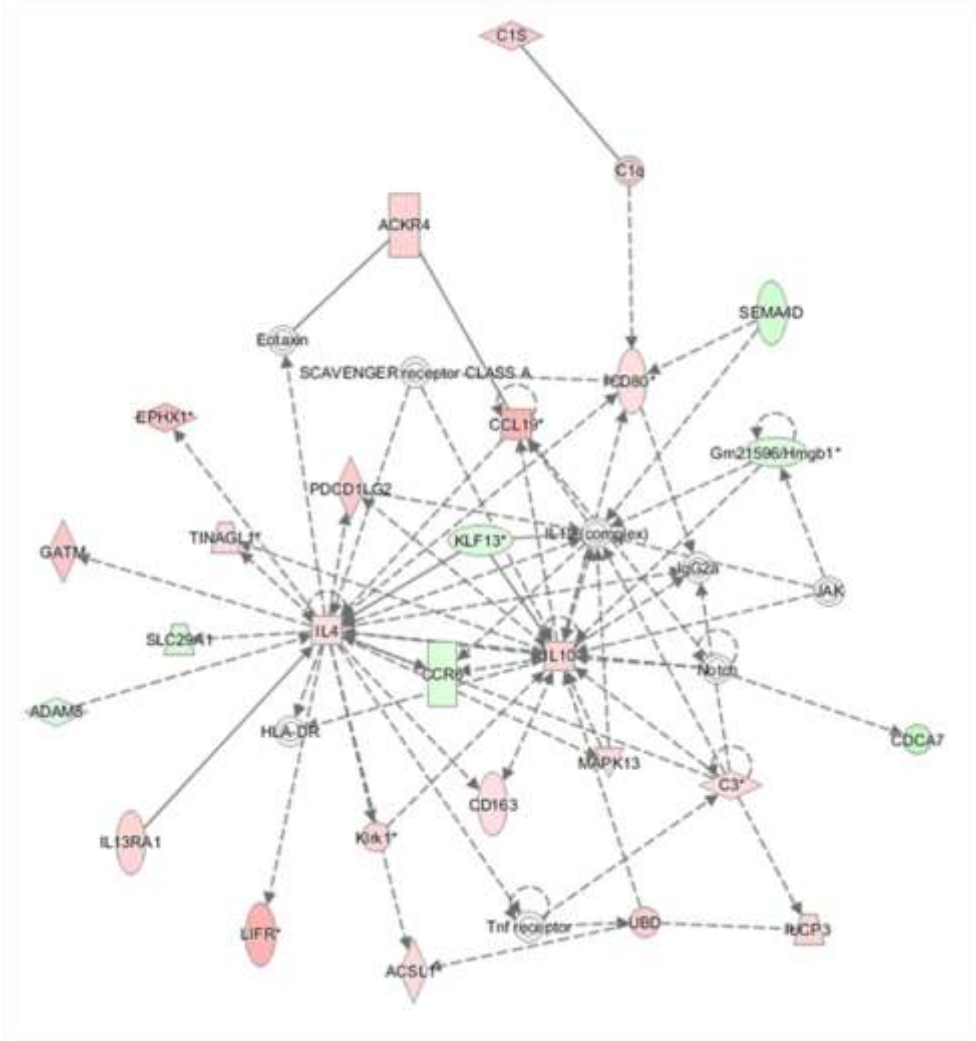
ID network	Focus molecule	Score	Top disease and functions
1	29	36	Cardiovascular system development and function, Organismal defvelopment, Organ morphology
2	29	36	Cancer, Organismal injury and abnormalities, dermatological disease and conditions
3	27	32	Cellular development, Tissue development, Skelectal and muscular system development and function
4	26	30	Connective tissue disorders, **immunological disease, inflammaroty disease**
5	26	30	Cell-to-cell signaling and interaction, **Hematological system development and function, Immune cell trafficking**
6	26	30	Cancer, Organismal injury and abnormalities, Reproductive system disease
7	25	28	Cell cycle, DNA replicaion, Recombination and repair, Cell death and survival
8	25	28	Cancer, Organismal injury and abnormalities, Reproductive system disease
9	23	25	Cell-to-cell signaling and interaction, Cellular function and maintenance, **Hematological system development and function**
10	23	25	Gastrointestinal disease, Hepatic system disease, Liver fibrosis
11	23	25	Endocrine system Disorders, Gastrointestinal disease, Immunogical disease
12	23	23	Carbohydrate metabolism, Lipid metabolism, Small molecular biochemistry
13	22	21	Digestive system development and function, Organ morphology, Mervous system development and function
14	21	21	Cancer, **Hematological disease, Immunological disease**
15	21	20	Connective tissue disorder, **Immunological disease, Inflammatory disease**
16	20	17	Cardiac hypertrophy, Cardiovascular disease, Developmental disorder
17	18	17	Cellular development, Cancer, hematological disease
18	18	17	Cellular assembly and organization, Prgan morphology, Cancer
19	18	17	Connective tissue disorders, Dermatological disease and conditions, Developmental disorder
20	18	17	Cell death and survival, Cellualr assembly and organization, DNA replication, Recombination and repair
21	17	15	Cellular movement, Carbohydrate metabolism, Molecualr transport
22	17	15	Cardiovascular system development and function, Organ morphology, Behavior
23	16	14	Developmental disorder, Hereditary disorder, Opthalm c disease
24	16	14	Cellular compromise, Cellular function and maintenance, cell death and survival
25	16	14	Cellular Assembly and Organization, Cell cycle, Reproductive System Development and function

Supplementary Figure S7. 1. Network diagrams of ingenuity pathway analysis. Twenty-five clusters were identified including their enrichment score (ES). Each network in a box is displayed according to genes central to the pathway. The top 5 networks (Figure 7.2, 7.3, 7.4, 7.5, and 7.6) for the IL2RG KO pig-derived thymus by IPA analysis. The genes (up-regulated genes indicate red color, but down-regulated genes indicate green color) are networked based on the evidence in the IPA. For further details, see the Materials and Methods section. Network 7.2, 7.3, 7.4, 7.5, and 7.6 are mainly related with immune-related diseases and function.



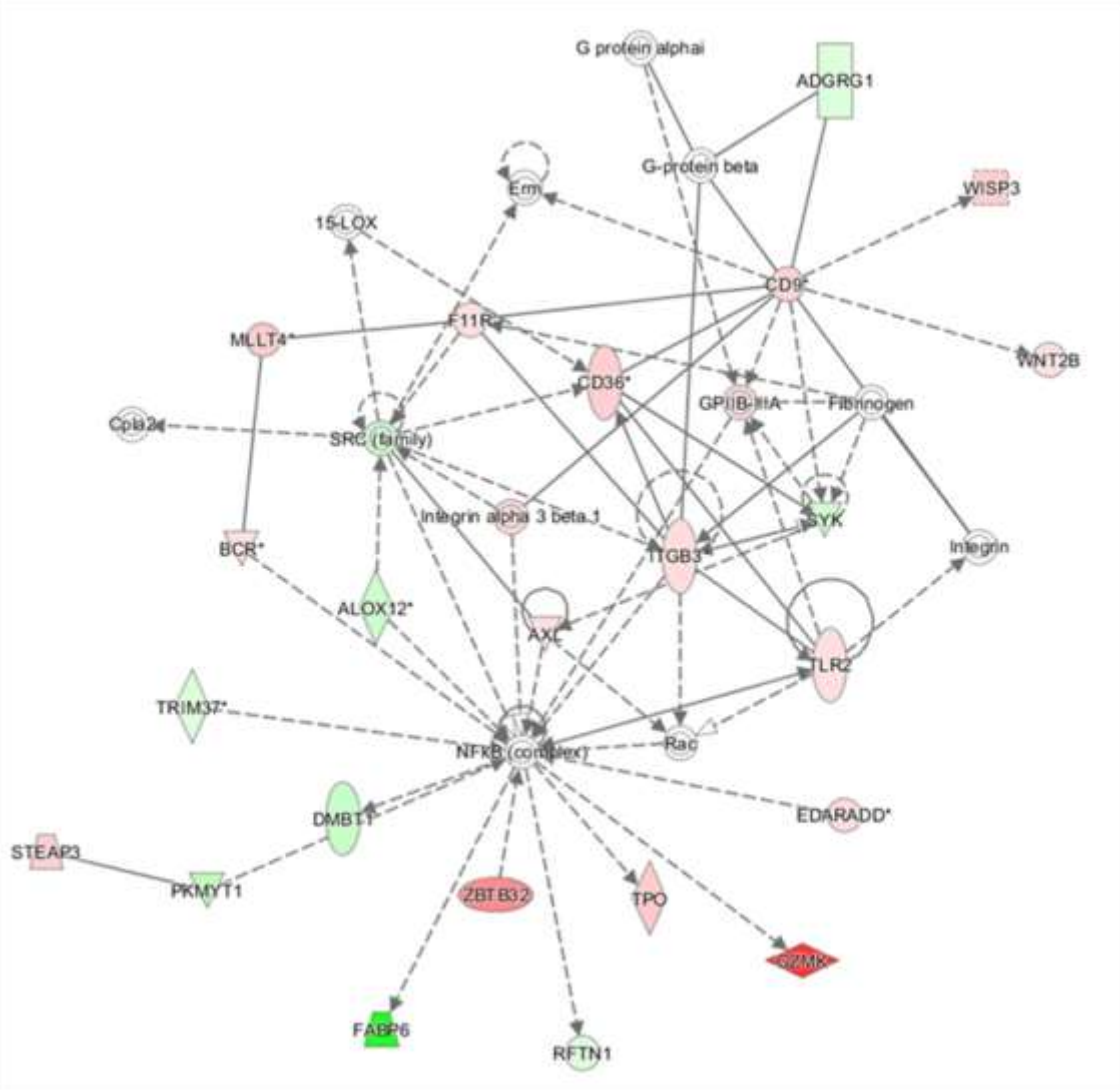
Supplementary Figure S7.2. Network 4 shows immune-related disease such as immunological disease and inflammatory disease. The core gene is IFNG and most of related genes are slightly up-regulated than WT. Abbreviations: Guanine nucleotide binding protein Alpha 14 (GNA14), Adenosine A3 receptor (ADORA3), Phosphodiesterase 4A CAMP-Specific (PDE4A), Complement component 1 Q subcomponent B Chain (C1QB), Guanylate binding protein 3 (GBP3), Interferon gamma receptor2 (IFNGR2), RGN, Chloride intracellular channel 5 (CLIC5), SAM domain and HD domain 1 (SAMHD1), Allograft inflammatory factor 1 (AIF1), Carbohydrate sulfotransferase 3 (CHST3), C2, U1SNRNP, Interferon gamma (IFNG), Ifn gamma, MHC class I (complex), Cysteinyl leukotriene receptor 2 (CYSLTR2), Complement component 1 C chain (C1QC), arginase, Fc fragment of

IgG (FCGR2B), Solute carrier family 16 member 9 (SLC16A9), C1QA, Chemokine receptor 6 (CXCR6), Cluster of differentiation 4 (CD4), Cluster of differentiation 2 (CD2), Cluster of differentiation 8a (CD8a), Nuclear factor of activated T-cells (Nfat), Lymphocyte-specific protein tyrosine kinase (LCK), Cluster of differentiation 247 (CD247), Vav 1 oncogene (VAV), Igg3, Fc gamma receptor 3 (Fcgr3), IgG2c, Tumor necrosis factor superfamily member 13b (TNFSF13B), Cluster of differentiation 180 (CD180).



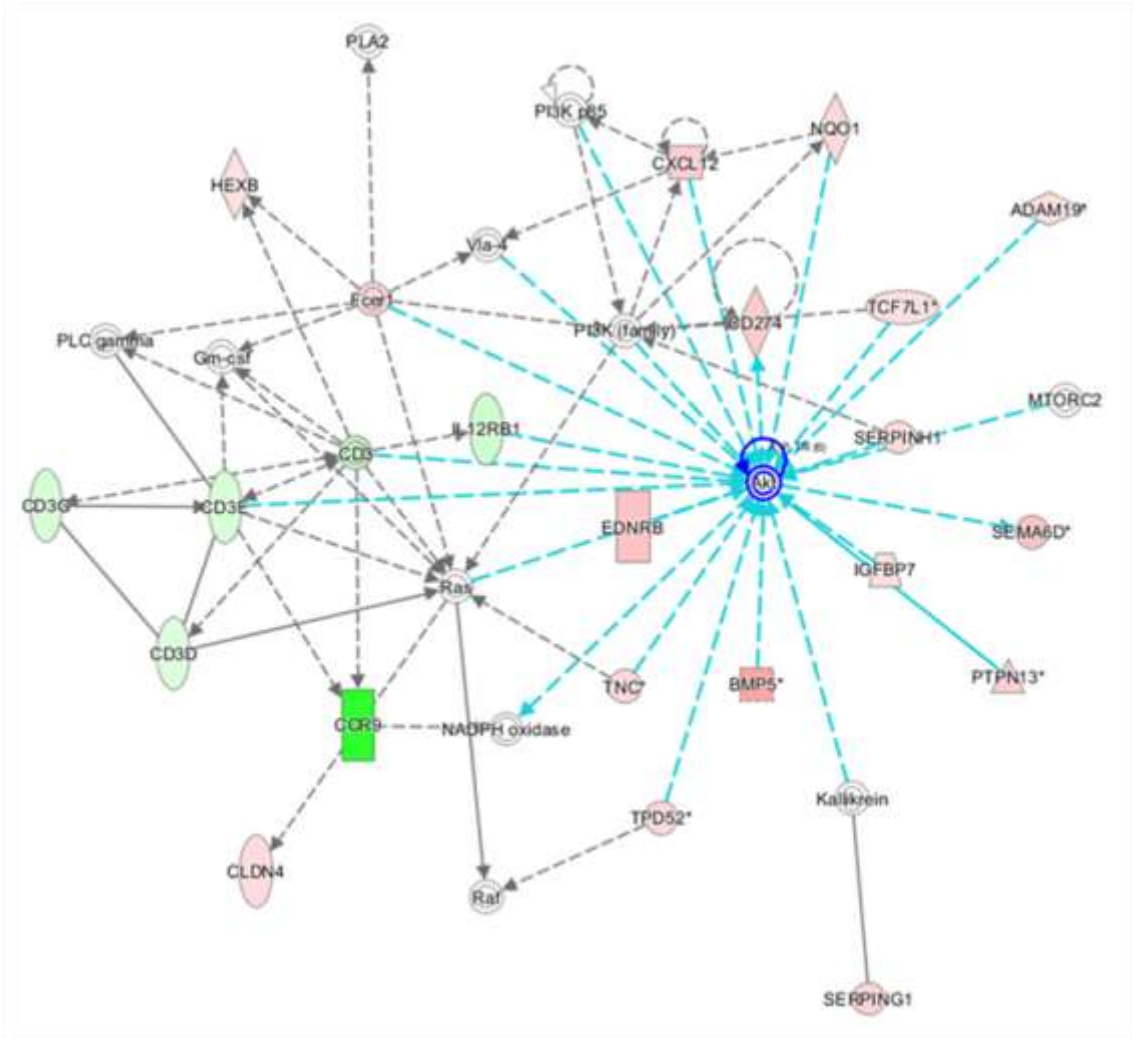
Supplementary Figure S7.3. Network 5 shows hematological system development and function and immune cell trafficking. Core genes are IL4 and IL10. Abbreviations: C1S, Atypical chemokine receptor 4 (ACKR4), Semaphorin-4D (SEMA4), Cluster of differentiation 80 (CD80), Chemokine ligand 19 (CCL19), Gm21596/Hmgb1, Eotaxin, Epoxide hydrolase 1 (EPHX1), Programmed cell death 1 ligand 2 (PDCD1LG2), Kruppel-like factor 13 (KLF13), IgG2a, Janus kinase (JAK), Notch, Cell Division cycle associated 7 (CDCA7), Interleukin-10 (IL-10), Mitogen-activated protein kinase 13 (MAPK 13), Complement component 3 (C3), Uncoupling protein 3 ubiquitin D (UBD), Cluster of differentiation 163 (CD163), Chemokine receptor 6 (CCR6), Interleukin 4 (Il4), HLA-DR, KLRK1, Tnl receptor, acyl-CoA synthetase long-chain family member 1 (ACSL1),

Tubulointerstitial nephritis antigen-like 1 (Tinagl1), Solute carrier family 29 member 1 (Slc29a1), Glycine amidinotransferase (GATM), ADAM metallopeptidase domain 8 (ADAM8), Interleukin 13 receptor subunit alpha 1 (IL13RA1), Leukemia inhibitory factor receptor alpha (LIFR).



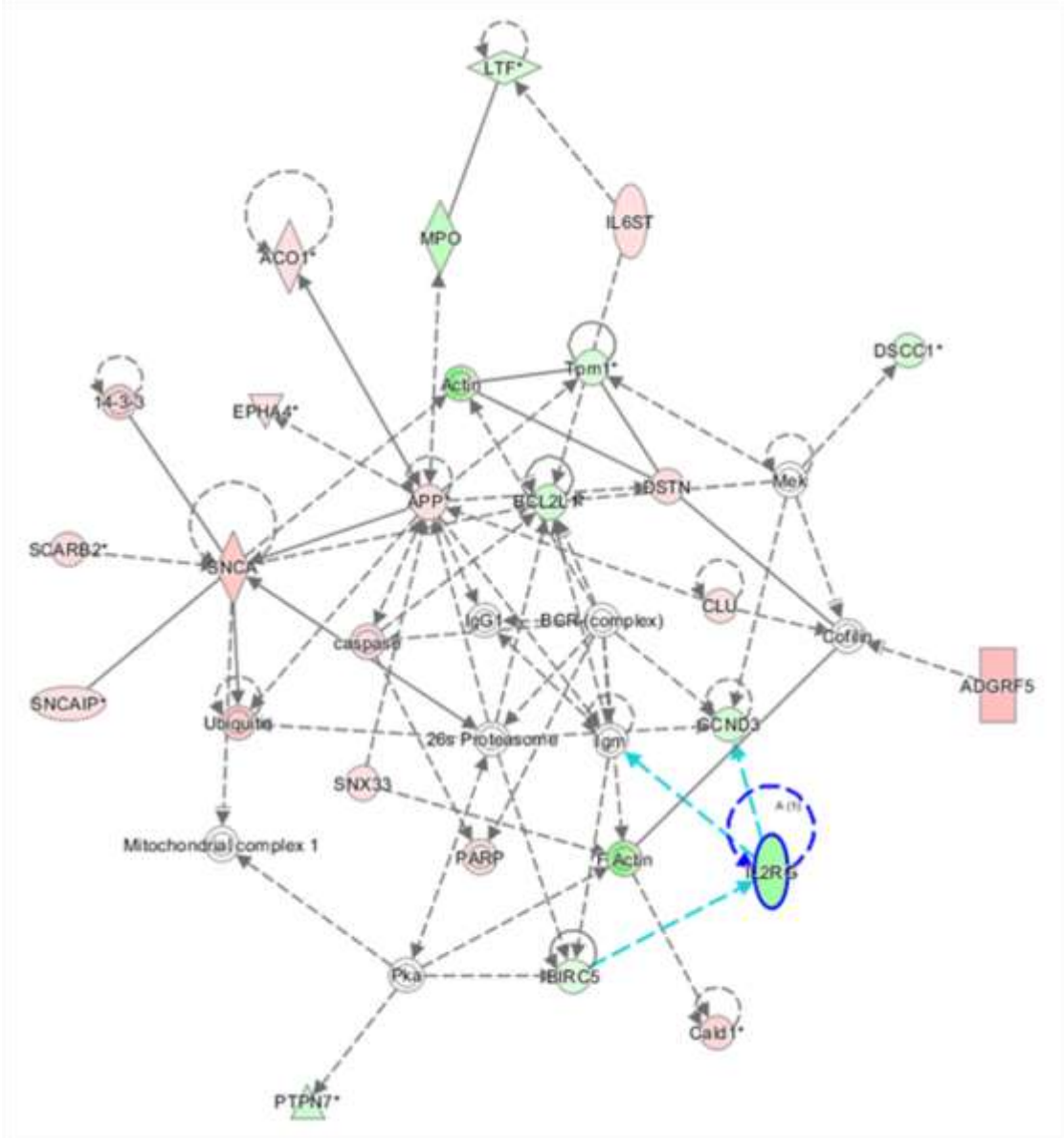
Supplementary Figure S7.4. Network 9 shows Cell-to-cell signaling and interaction, Cellular function and maintenance, Hematological system development and function. Core gene is NFkB but it was not detected in microarray data. Abbreviations: G protein alpha i subunit (Galphai), Adhesion G protein-coupled receptor G1 (ADGRG1), WNT1 inducible signaling pathway protein 3 (WISP3), Wingless-type MMTV integration site family member 2B (Wnt2b), Cluster of differentiation 9 (CD9), Fibrinogen C domain-containing protein 1-like (Fibrinogen), Guanine nucleotide binding proteins (G Protein beta), Earmuff (Erm), F11 receptor (F11R), Cluster of differentiation 36 (CD36), GPIIB IIIA, Spleen tyrosine kinase (SYK), Toll-like receptor 2 (TLR2), Integrin subunit beta 3 (ITGB3), RAC, AXL receptor

tyrosine kinase (AXL), Integrin alpha 3 beta1, 15LOX, Myeloid/lymphoid or mixed-lineage leukemia (MLLT4), SRC (family), Nuclear factor kappa B (Nfkb), Arachidonate 12-lipoxygenase (ALOX12), Breakpoint cluster region (BCR), Cytosolic phospholipases A2 (cPLA2), Tripartite motif containing 37 (TRIM37), Deleted in malignant brain tumors 1 (DMBT1), Protein kinase, membrane associated tyrosine/threonine 1 (PKMYT1), STEAP Family Member 3 (STEAP3), Zinc finger and BTB domain containing 32 (ZBTB32), Thyroid peroxidase (TPO), EDAR (ectodysplasin-A receptor)-associated death domain (EDARADD), granzyme K (GZMK), RFN1, Fatty acid binding protein 6 (FABP6).



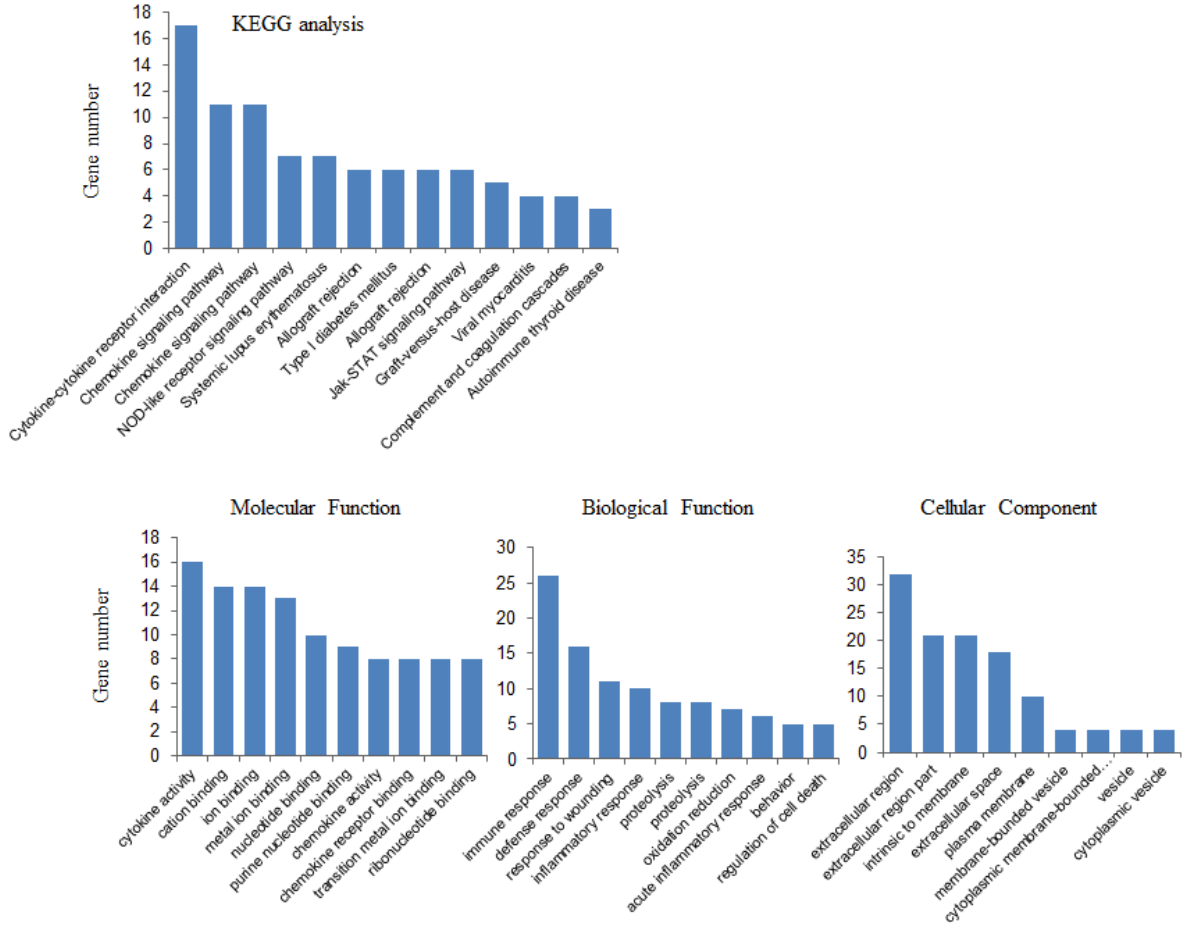
Supplementary Figure S7.5. Network 14 shows hematological disease and immunological disease. Core gene is AKT, it was not detected in microarray data. Abbreviations: phospholipase A2 (PLA2), phosphatidylinositol 3-kinase (PI3K) p85 ,chemokine (C-X-C motif) ligand 12 (CXCL12), NAD(P)H dehydrogenase, quinone 1(NQO1), ADAM metallopeptidase domain 19 (ADAM19), transcription factor 7 like 1 (TCF7L1), Cluster of Differentiation 274 (CD274), serpin peptidase inhibitor, clade H (heat shock protein 47), member 1 (SERPINH1), MTORC2, Sema domain 6D (SEMA6D), Insulin like growth factor binding protein (IGFBP7), Protein tyrosine phosphatase, non-receptor type 13 (PTPN13), Bone morphogenetic protein 5 (BMP5), Kallikrein (KLK), Serpin peptidase inhibitor, clade G member 1 (SERPING1), Tumor protein D52 (TPD52), Tenascin C (TNC),

Endothelin receptor type B (EDNRB), Ras, NADPH oxidase, Raf, interleukin 12 receptor subunit beta 1 (IL12RB1), Via4, Phosphatidylinositol 3-kinase (PI3K), Fcer1, Hexosaminidase B (HEXB), Phosphoinositide-specific phospholipase C (PLC), Gm-csl, Cluster of Differentiation 3 (CD3), Cluster of Differentiation 3e (CD3E), Cluster of Differentiation 3g (CD3G), Cluster of Differentiation 3d (CD3D), CDR9, Chemokine (C-C Motif) Receptor 9 (CCR9), Claudin 4 (CLDN4).



Supplementary Figure S7.6. Network 15 shows immunological disease and inflammatory disease. Abbreviations: Lactotransferrin (LTF), Interleukin 6 signal transducer (IL6ST), Myeloperoxidase (MPO), Aconitase 1 (ACO1), DNA replication and sister chromatid cohesion 1 (DSCC1), Tropomyosin 1 (Tpm1), Actin, BCL2-like 1 (BCL2L1), Destrin (DSTN), MAP kinase-ERK kinase (Mek), ClSusterin (CLU), Cofilin, Adhesion G protein-coupled receptor F5 (ADGRF5), GCND3, Interleukin 2 receptor gamma chain (IL2RG), Breakpoint cluster region (BCR), Lgm, F actin, Baculoviral IAP repeat containing 5 (BIRC5), Protein lysine acetyltransferase (Pka), Protein tyrosine phosphatase,

non-receptor type 7 (PTPN7), Sorting nexin 33 (SNX33), Caspase, Ubiquitin, synuclein alpha (SNCA), Eph receptor A4 (EPHA4), 14-3-3, Scavenger receptor class B member 2 (SCARB2), Synuclein alpha interacting protein (SNCAIP), 26s proteasome, IgG1, Ayloid beta precursor protein (APP), Mitochondria complex 1, Poly- polymerase (PARP).

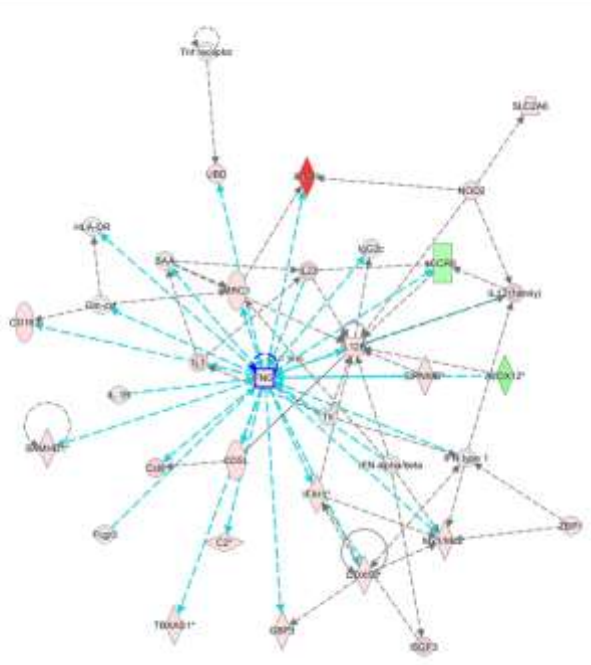


Supplementary Figure S8. KEGG pathway enrichment analysis, GO biology process, molecular function, and cellular function analysis in WT and mIL2RG$^{+/\Delta 69\text{-}368}$ KO pig spleen. Using DAVID program, up- and down- regulated gene expressions were divided into 3 subgroups such as Biological process, Cellular component, and Molecular function. Each subgroup shows top 10 of gene function group.

A

ID network	# molecules	Score	Disease and function
1	22	36	Cell-to-cell signaling and interaction, cellular movement, immune cell trafficking
2	21	33	Infectious disease, connective tissue disorders, inflammatory disease
3	20	31	Hematological system development and function, tissue morphology, cellular function and maintenance
4	19	29	Infectious disease, cell morphology, cell-to-cell signaling and interaction
5	16	23	Cellular movement, Hematological system development and function, immune cell trafficking
6	13	17	Dermatological diseases and conditions, reproductive system development and function, immunological disease
7	12	15	Cellular development, connective tissue development and function, skeletal and muscular system development and function
8	11	14	Cell morphology, cellular compromise, cellular function and maintenance
9	11	14	Embryonic development, hair and skin development and function, organ development
10	8	9	Drug metabolism, molecular transport, small molecule biochemistry
11	1	2	Cancer, cardiovascular disease, cell cycle
12	1	2	Development disorder, ophthalmic disease, organismal injury and abnormalities
13	1	2	RNA post-transcriptional modification, DNA replication, recombination, and repair, cell death and survival

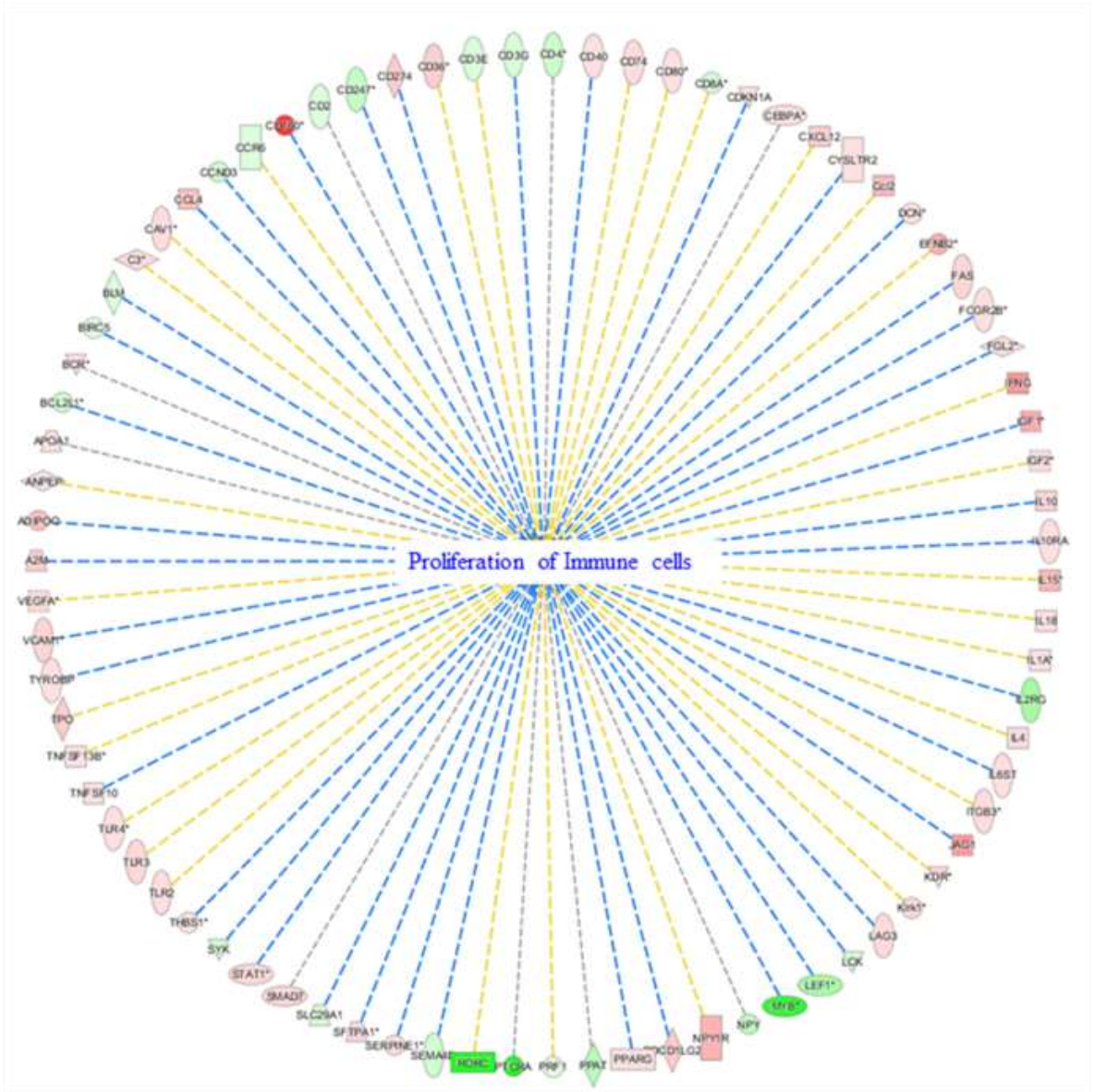
B



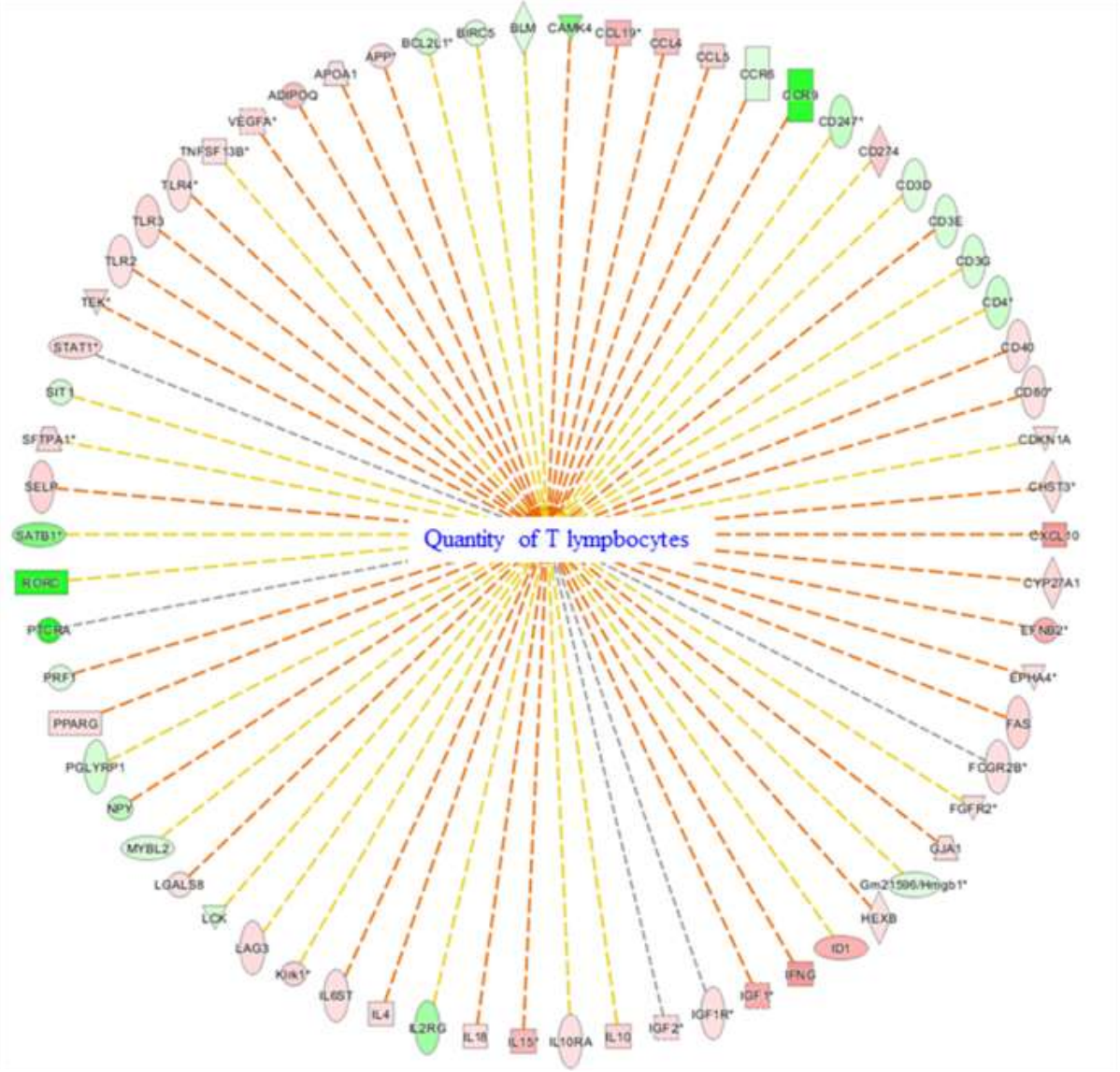
Supplementary Figure S9. Network analysis by IPA program in spleen. (a) Summary of each network. Network 1-6 have immune-related diseases and function. (b) In Network 4, core gene is IFNG and most of related genes are slightly up-regulated than WT. Abbreviations: Tnf receptor, Ubiquitin D (UBD), Major histocompatibility complex (HLA-DR), Cluster of differentiation 163 (CD163), Granulocyte-macrophage colony-stimulating factor (Gm-csf), Mannose receptor C type 1 (MRC1), Immunoresponsive gene 1 (IRG1), Interleukin 23 (IL23), IgG2c, Solute carrier family 2 member 6 (SLC2A6), Nucleotide-binding oligomerization domain-containing protein 2 (NOD2), Chemokine receptor 6 (CCR6), Interleukin 12 (IL12) family, Interleukin 12 beta (IL12B), Interleukin 1 (IL1), Interleukin 1 receptor (IL1R), SAM domain and HD domain-containing protein 1 (SAMHD1), Chemokine (C-C Motif) ligand 8 (Ccl8), CD5 Molecule-Like (CD5L), Interferon gamma (IFNG), Toll-like receptors (Tlr), Glycoprotein nmb (GPNMb), Arachidonate 12-lipoxygenase (ALOX12), Fcg receptor III (Fcgr3), Complement component 2 (C2), Thromboxane A synthase 1 (TBXAS1), Guanylate binding protein 3 (GBP3),

Interferon-induced helicase (IFIH1), DEAD box polypeptide 58 (DDX58), Interferon alpha/beta (IFN alpha/beta), Type I interferons (IFN type1), MX dynamin-Like GTPase 1/2 (Mx1/Mx2), Z-DNA binding protein 1 (ZBP1), IFN-stimulated gene factor 3 (ISGF3).

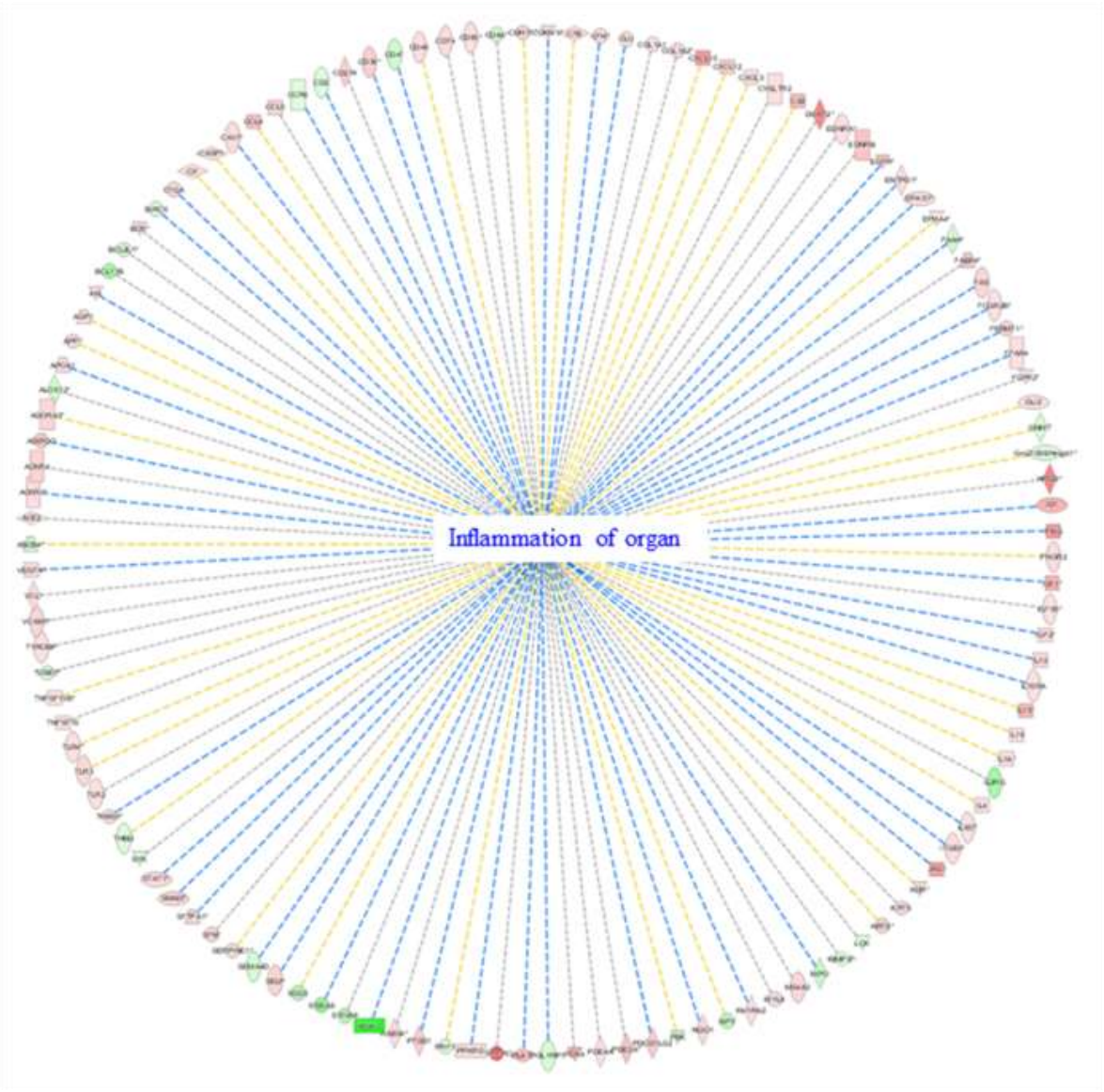
10.1Proliferation of immune cell



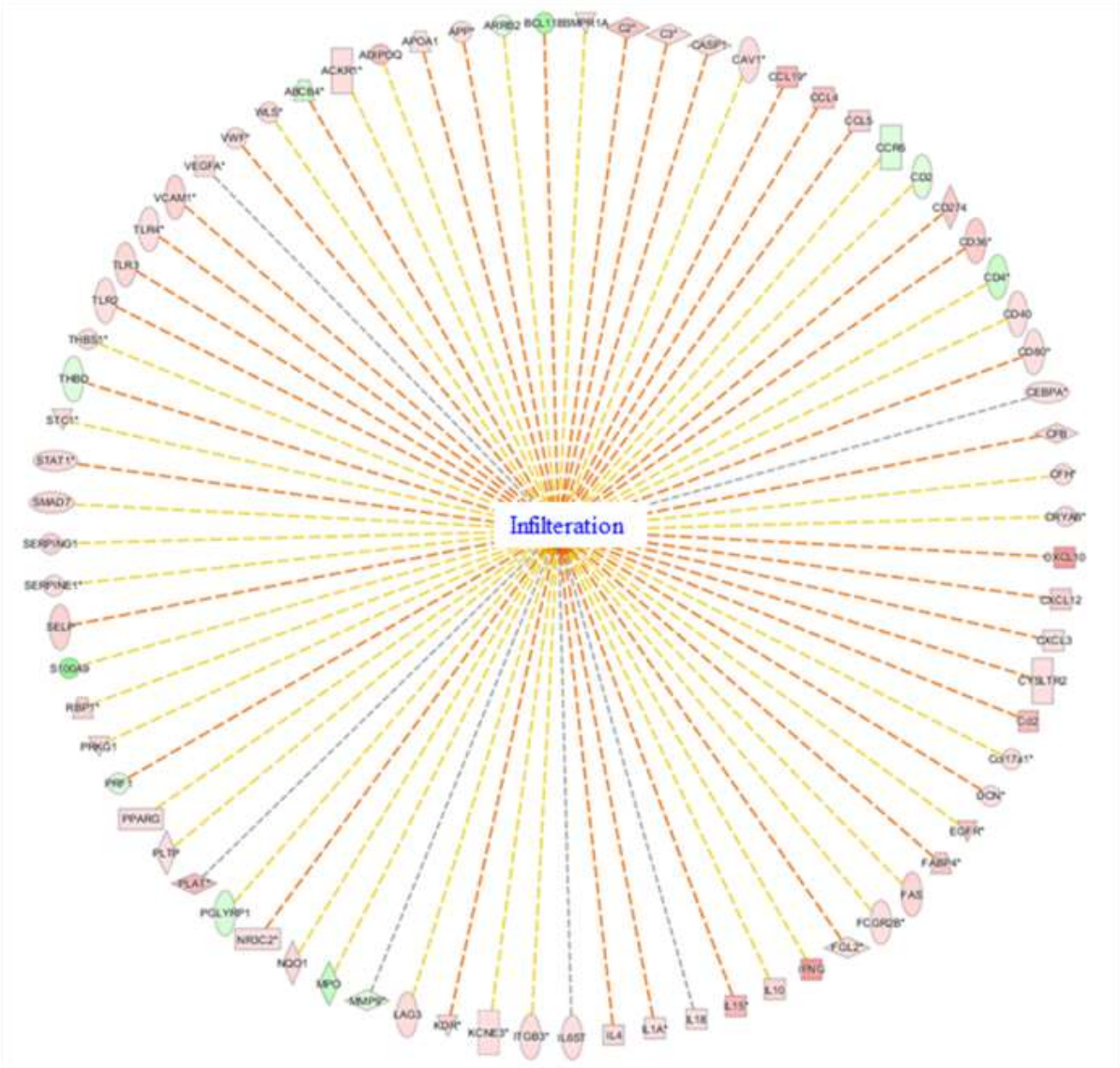
10.2 Quantity of T lymphocytes



10.3 Inflammation of organ



10.4 Infilteration



Supplementary Figure S10. Network 10.1 to 10.4 displays the relationship between proliferation of immune cells, quantity of T lymphocytes, inflammation of organ, infiltration and their target molecules in IL2RG KO pig-derived thymus. The colors indicate the level of mRNA expression: upregulated genes are represented in red and downregulated genes in green. Most immune-related genes were up-regulated compared to WT.