Loss of *Gata4* in Sertoli cells impairs the spermatogonial stem cell niche and causes germ cell exhaustion by attenuating chemokine signaling

Supplementary Material

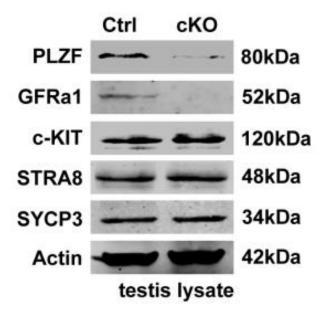


Figure S1: Western blot analysis of undifferentiated spermatogonia markers (PLZF and GFRa1), the differentiated spermatogonia marker c-KIT, and meiosis markers (STRA8 and SYCP3) in testis lysate from control and *Gata4* cKO mice. Actin served as a protein loading control.

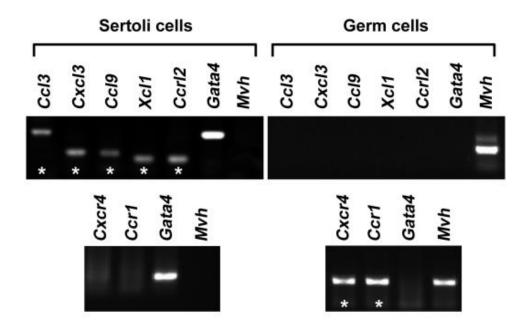


Figure S2: RT-PCR amplification of *Cxcl12*, *Ccl3*, *Ccl9*, *Xcl1*, *Ccrl2*, *Cxcr4* and *Ccr1* in cDNA samples from Sertoli cells and germ cells from P5 mice. *Gata4* is a Sertoli cell marker, and *Mvh* is a germ cell marker. A representative image from three independent experiments is shown.

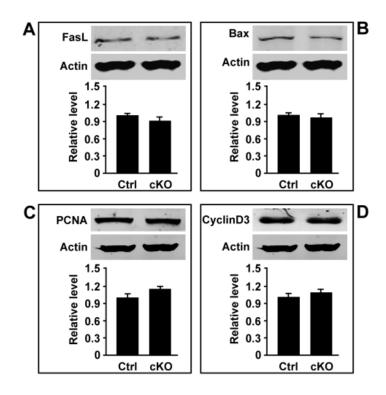


Figure S3: Western blot analysis of apoptotic markers (FasL and Bax) and proliferative markers (PCNA and Cyclin D3) in Sertoli cell lysates from control and *Gata4* cKO testes. Actin served as a protein loading control.

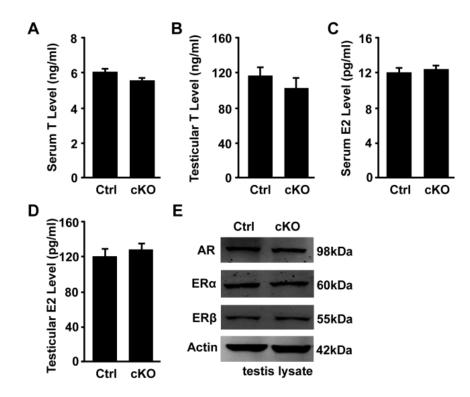


Figure S4: Serum and testicular testosterone (T) levels and estradiol (E2) levels in pubertal control and *Gata4* cKO mice (n=3 each group). Western blot analysis of AR, ER α and ER β in testis lysates from control and *Gata4* cKO mice. Actin served as a protein loading control.

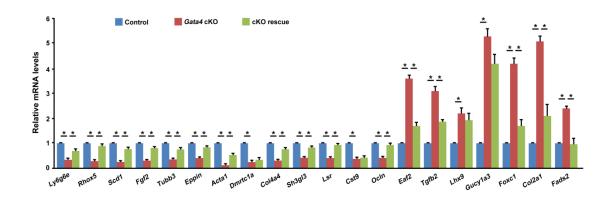


Figure S5: q-PCR analysis of twenty genes in cDNA samples from control, *Gata4* cKO and rescue testes (after CXCL12 and CCL9 treatment for 4 days). *Gapdh* served as the internal control gene. The data are expressed as the mean±S.D. *p<0.05, **p<0.01.

Supplemental Table 1: Primers used in this study.

Name		Primer sequence	Size
Cre	Forward:	TCCAATTTACTGACCGTACACCAA	550bp
Cie	Reverse:	CCTGTACCTGGCAATTTCGGCTA	·
flox	Forward:	CCC AGT AAA GAA GTC AGC ACA AGG AAA C	flox/flox= ~455 bp
	Reverse:	AGA CTA TTG ATC CCG GAG TGA ACA TT	+/flox = 355 bp and ~455 bp +/+ = 355 bp
Wt1	Forward:	AATGACCTCCCAGCTTGAATG	100hn
	Reverse:	CCGTGGGTGTGTATTCTGTACT	192bp
Mark	Forward:	TGTGGTCATATATGGAGGAAC	172bp
Mvh	Reverse:	ATCCAACATTCGATCAGCTTC	17200
Fgf2	Forward:	GCGACCCACACGTCAAACTA	108bp
' giz	Reverse:	CCGTCCATCTTCCTTCATAGC	
Cxcl12	Forward:	TGCATCAGTGACGGTAAACCA	146bp
	Reverse:	TTCTTCAGCCGTGCAACAATC	
Ccl3	Forward:	TGTACCATGACACTCTGCAAC	109bp
00.0	Reverse:	CAACGATGAATTGGCGTGGAA	·
Cxcr4	Forward:	GAGGCCAAGGAAACTGCTG	145bp
	Reverse:	GCGGTCACAGATGTACCTGTC	
Ccr1	Forward:	CTCATGCAGCATAGGAGGCTT	142bp
	Reverse:	ACATGGCATCACCAAAAATCCA	
XcI1	Forward:	TTTGTCACCAAACGAGGACTAAA	164bp
	Reverse:	CCAGTCAGGGTTATCGCTGTG	•
Ccrl2	Forward:	CCCCGGACGATGAATATGATG	170bp
	Reverse:	CACCAAGATAAACACCGCCAG	
Gata4	Forward:	CCCTACCCAGCCTACATGG	139bp
	Reverse:	ACATATCGAGATTGGGGTGTCT	
Dmrt1	Forward: Reverse:	GACCCCGCCTACTACAGCA GTCTGAGCAGGCACGTAAGG	191bp
	Forward:	ACTCGGAAGAACAGCATGATG	
Rhox5	Reverse:	CCCTGGTGCCACTATCCTT	204bp
Codd	Forward:	TTCTTGCGATACACTCTGGTGC	104hn
Scd1	Reverse:	CGGGATTGAATGTTCTTGTCGT	104bp
Tubb3	Forward:	TAGACCCCAGCGGCAACTAT	127bp
	Reverse:	GTTCCAGGTTCCAAGTCCACC	
Eppin	Forward:	CCAGGGACCTAGTCTAGCTGA	219bp
	Reverse:	CGACGAAAGTAAGCCATACAGT	
Acta1	Forward:	CCCAAAGCTAACCGGGAGAAG	134bp
	Reverse:	CCAGAATCCAACACGATGCC	
Dmrtc1a	Forward:	GGAACCTCGTAAGGACTTTTCTC	123bp
	Reverse:	TGCATGTGATGGATGAGCA	
Col4a4	Forward:	GCCTGGTGTCGGGATCAAA	211bp
	Reverse:	AGCTGGAGTCAACAAAATGCC	
Tgfb2	Forward:	TCGACATGGATCACATGGA	147bp
	Reverse: Forward:	CCCTGGTACTGTTGTAGATGGA CCCCGGACAAGAAGATCACTC	
Foxc1	Reverse:	AGGTTGTGCCGTATGCTGTTC	109bp
	Forward:	CCAGCGGACTTGCATACC	
Eaf2	Reverse:	GCCAACCTCAAGATTTCCTTCAC	153bp
	Forward:	AAGGAGGTAACCAGGGAGAG	
Fads2	Reverse:	CCGCTGGGACCATTTGGTAA	150bp
	Forward:	TGGATTTGGACGCATTTGGTA	
Gapdh	Reverse:	TTTGCACTGGTACGTGTTGAT	211bp
	NOVEISE.	THOSAGIAGGIATORI	

Supplemental Table 2: Primary and secondary antibodies used in this study.

Primary Antibody	Source	Code	Application
Goat anti-GATA4	Santa Cruz	sc-1237	IHC/WB
Rabbit anti-WT1	Epitomics	2797-1	IHC
Rabbit anti-MVH	Abcam	ab13840	IHC
Goat anti-GFRa1	R&D Systems	AF560	IF/WB
Mouse anti-PLZF	Santa Cruz	sc-28319	IF/WB
Rabbit anti-STRA8	Abcam	ab49602	IF/WB
Rabbit anti-c-KIT	Cell Signaling	#3074	IF/WB
Mouse anti-γ-H2AX	Abcam	ab26350	IF
Rabbit anti-SYCP3	Abcam	ab15093	IF/WB
Goat anti-CXCL12	Santa Cruz	sc-6193	IF
Rabbit anti-CXCR4	Abcam	ab124824	IF
Rabbit anti-CCL9	R&D Systems	AF463	IF
Mouse anti-CCR1	R&D Systems	MAB5986	IF
Rabbit anti-TRS4	Our lab		IF
Rabbit anti-AR	Abcam	ab133273	WB
Mouse anti-ERα	Abcam	ab16460	WB
Rabbit anti-ERβ	Abcam	ab3577	WB
Rabbit anti-FasL	Abcam	ab68338	WB
Rabbit anti-Bax	Abcam	ab7977	WB
Rabbit anti-PCNA	Abcam	ab18197	WB
Rabbit anti-Cyclin D3	Abcam	ab52598	WB
Secondary Antibody	Source	Code	Application
Anti Rabbit IgG/biotin	Zhong Shan	ZB-2010	IHC
Anti Goat IgG/biotin	Zhong Shan	ZB-2050	IHC
Anti Goat IgG/TRITC	Invitrogen	A11058	IF
Anti Mouse IgG/FITC	Cell Signaling	#4408	IF
Anti Mouse IgG/TRITC	Cell Signaling	#8890	IF
Anti Rabbit IgG/FITC	Cell Signaling	#4412	IF
Anti Rabbit IgG/TRITC	Cell Signaling	#8889	IF
Anti Goat Dye 800CW	LI-COR	926-32214	WB

Supplemental Table 3: Down-regulated genes in *Gata4* cKO compared to control testes at P5.

Spink8 Serine peptidase inhibitor, Kazal type 8 78709 Protease inhibitors 27,9 1,65×10* Pop4 Puxinje cell protein 4 18546 Cell migration/chemotaxis 1.40 1.38+0* Cxcz4 Chemokine (CX-Cm dolf) receptor 4 12767 Chemokine receptor 1.35 4.30+0* Enp2 Ectionacides pryphophosphatase/hosphopidesterase 2 18060 Lipid signaliam molecule 5.0 5.01-0* Mybpc1 Myosin bindring protein C, Swo-type 109272 Cel movement 4.6 2.24+10* Cd3 Chemokine (C-C motif) ligand 3 20302 Chemokines 3.6 1.79+10* Mota 10 Homeobox A10 19395 Testicular descent 4.3 3.38+10* Clock12 C-type lectin domain family 12, member B 7.0187 Serofil transcription factor 4.3 3.48+10* Clock34 Collage MpP IV, alpha 4 12398 Chemokine C-C multi ligand 9 2038 Chemokines 4.1 2.94+10* Collage Chemokine C-C multi ligand 9 2038 Chemokine C-O-C multi ligand 1 2.21-10* 2.21-10*	Gene	Description	NCBI gene ID	Function	Log fold change	P-value
Pcp-Increal Purknipe cell protein 4 1846b Cell migration/chemicaxis 1.4.0 1.38-10-10 Cocard Chemokine (PC-K one) (Proceptor) 1.5.9 A. 30-10-10 A. 30-10-10 Enpig2 Ectorundecides pyriphosphotalesides (Policy one) 1802 Lipid signaling molecula 5.0 6.89-10-10 Enpig2 Ectorundecides pyriphosphotalesides (Policy one) 19227 Cell movement 4.8 2.24-10-10 GCG Chemokine (PC-motif) ligand 3 2002 Chemokines 3.8 1.79-10-10 Final Saff In Right protein (3.1) Saper (Policy one) 1.50-10-10 4.3 3.35-10-10 Cloci 2D Chyps lectin domain family 12, member 18 7.118-30 Cell surface receptor 4.1 7.011-01-01 Colled B Chemokine (PC-motif) ligand 3 2.00 Cell surface receptor 4.1 7.011-01-01 Colled C Chemokine (PC-motif) ligand 3 2.00 Cell surface receptor 4.1 7.011-01-01 Colled C Chemokine (PC-motif) ligand 1 2.00 Chemokine CC-motif ligand 2 2.00-01 Employer Autor					WT to cKO	WT to cKO
Actart Chemokine (CX-C molf) incorplor 4 21767 Chemokine recorptor 13.5 4.00-10 ¹ Actor 1.00 Actart Actin alphin 1, Selectal muscle 11869 Lipid signaling molecule 5.0 5.10-10 ¹ Actor GO3 Chemokine (C- chem) ligand 3 2002 Cle movement 4.6 2.24×10 ¹ Co. GO3 Chemokine (C- chem) ligand 3 2003 Chemokine (C- chem) ligand 3 2003 Chemokine (C- chem) ligand 3 3.33-10 ¹ Testicular descort 4.3 3.33-10 ¹ Testicular descort 4.0 3.35-10 ¹ Testicular descort 4.0 4.70-10 ¹ Testicular descort 4.1 7.01-10 ¹ Actor 4.0 5.91-10 ¹ Testicular descort 4.1 7.01-10 ¹ Actor 4.0 5.91-10 ¹ Testicular descort 4.0 1.91-10 ¹ Testicular descort 4.1 7.0<	Spink8	Serine peptidase inhibitor, Kazal type 8	78709	Protease inhibitors	27.9	1.65×10 ⁻⁵
Acata Acatin, alpha 1, skeletal muscle 11459 Cytoskeletal organization 8.0 6.89-10* Engige Ectonucledictide preprincipates alphosphodisterises of 2 2808 5.00-10* 5.00-10* Mybport Myosan binding protein C. slow-type 1992/2 Cell movement 4.6 2.24×10* Art 138rt1 Ring finger protein als By abudgen 74244 Ring finger protein als 3 3.35×10* Cloci 2D Chypie lectin domain family 12* member B 77887 Serticular descent 4.3 3.35×10* Cloci 2D Cypie lectin domain family 12* member B 7183 Cell surface receptor 4.1 7.08×10* Cloci 2D Chypie lectin domain family 1, member B 2008 Extracellular matrix 4.0 2.91×10* Codial Chemokine (CA- crofid) ilgand 4 2209 Extracellular matrix 4.0 2.91×10* Aktr 18 Aldo-keto reductase family 1, member C18 1.05×10* Speniture 3.8 7.04×10* Mapa Microtabule-associated protein 7 2.01×10* Stable Alexandor Chemokine (CA- crofid) ilgand 12 2.03×10*	Pcp4	Purkinje cell protein 4	18546	Cell migration/chemotaxis	14.0	1.38×10 ⁻⁵
Exprage 2 Ectoroulecloides proxymosphatisae/phosphodiselerase 2 189027 Updit signaling molecule 5.0 5.10-10 ⁻¹⁰ Affect Mykport Mysean binding protein C, slow-lype 108272 Cell movement 4.8 2.24+10 ⁻¹⁰ Col3 Chemokine (C-C molf) ligand 3 20002 Chemokine (C-C molf) ligand 3 3.30-10 ⁻¹⁰ Monard Homeobox A10 15396 Teatboul released 4.3 3.30-10 ⁻¹⁰ Drink 1a MNF1/Bart Rink Farrily C1a 7.018 Sertoli transcription factor 4.3 3.58-10 ⁻¹⁰ Col622b C-Sype ledicil domain faming 12, member B 7.118 2.018 Sertoli transcription factor 4.1 7.011-10 ⁻¹⁰ Col64a Colligaen, type RV, alpha 4 12839 Extracellular mark 4.0 1.59-10 ⁻¹⁰ Kct48 K-Channel letramerisation domain containing 8 1.4078 1.4078 1.008-10 ⁻¹⁰ Kct28 K-Channel letramerisation domain containing 8 1.408-10 ⁻¹⁰ 5.948-10 ⁻¹⁰ Kct28 K-Channel letramerisation domain containing 8 1.409-10 ⁻¹⁰ 5.948-10 ⁻¹⁰ Cox12 <	Cxcr4	Chemokine (C-X-C motif) receptor 4	12767	Chemokine receptor	13.5	4.30×10 ⁻⁴
Mybgsc1 Mybosin binding protein C. slow-type 108272 Cell movement 4.6 2.24-10¹ Col3 Chemokines (C.C. molf) ligand 3 200302 Chemokines 3.6 1.79×10¹ Art13eH1 Rung flager protein in 18 peaudogene 7.424 Ring flager protein 4.5 3.38×10¹ DmrtT-Ia DMRT-Ike finnily C1a 70887 Testicular descent 4.3 3.38×10¹ Cloci 2D Chype lectin domain family 12, member 8 71183 Cell surface receptor 4.1 7.01×10¹ Codel 4 Chlidgen, type IV, alpha 4 1239 Extracellular matrix 4.0 1.59×10¹ Codel Chemokine (C.C. Crodif) ligand 1 2008 Chemokine Combil ligand 1 24043 Subunit of GABA-B receptor 3.8 7.54×10² Akr 18 Akr 21 Alpha Seto reductase family 1, member 0:18 105349 Spermatogenia metabolism 3.7 2.51×10² Cod212 Chemokine (C.C. Arcell ligand 1 20034 Spermatogenia metabolism 3.5 2.64×10² Alpsyl Muchosia Selectin maceria 3.5 3.64×10² 3.5<	Acta1	Actin, alpha 1, skeletal muscle	11459	Cytoskeletal organization	8.0	6.69×10 ⁻³
Cold Chemokines (C-C mortl) igand 3 2002 Chemokines 3.6 1.79-10-10-10-10-10-10-10-10-10-10-10-10-10-	Enpp2	Ectonucleotide pyrophosphatase/phosphodiesterase 2	18606	Lipid signaling molecule	5.0	5.10×10⁴
Ref	Mybpc1	Myosin binding protein C, slow-type	109272	Cell movement	4.6	2.24×10 ⁻³
Damzt		Chemokine (C-C motif) ligand 3		Chemokines		1.79×10 ⁻³
Control to College C						
Coloration Col						
MW6t To WAP Four-disultifice core domain 10 628756 Protease inhibitories 4.1 6.38-010** Cot494 Collagen, type IV, alpha 4 12339 Extracellular matrix 4.0 15.99-10** Cot29 Chemokine (C-C motif) Igand 9 20308 Protein kinase 3.8 7.24-10** Kct/BI K: channel tetramerisation domain containing 8 24043 Submit of GABA-B receptor 3.8 7.84-10** Akr1c18 Aldo-keto reductase family 1, member C18 105349 Sepmratogonia metabolism 3.7 2.51+10** Map7 Microbuble-associated protein 7 3.7 17761 Subbitting microtrubules 3.5 18.40+10** Rod Security Security Alexandrase 1 20249 Falty acid metabolism 3.5 18.40+10** Rogs N-acetylglutamate synthase 217214 Mitochondrial matrix 3.5 2.249-10** Fig2 Fibrobal signowth factor 2 14173 SSC self-renew 3.4 1.32*-10** Shgjs SH-G-bamain RRB2-like 3 20096 Cytoskelelar protein 3.2 1.71*-10** Co		-		•		
Code/a Collagen, type IV, alpha 4 12839 Estracellular matrix 4,0 1,59-10° Bmp2k BMP2 inducible kinase 140780 Protein kinase 3.8 1,32×10° Kctd8 K** channel tetramerisation domain ontaining 8 243943 Subunit of GABA-B receptor 3.8 7,64×10° Akt-161 Akd-kect preductate family 1, member C18 16549 Spermatogonia metabolism 3.7 254-14° Cx212 Chemokine (C-X-C motif) ligand 12 20315 Chemokines 4.6 6,80×10° Map7 Microbubila-associated protein 7 17761 Stabilizing microtubules 3.5 8,04×10° Rhox5 Reproductive homeobox 5 18817 Serotic cell metabolism 3.5 2,49×10° Rhox5 Reproductive homeobox 5 18817 Serotic cell metabolism 3.5 2,49×10° Fig/2 Fibroblast growth factor 2 14173 SScelf-renew 3.4 1,32×10° Sh3g3 SH3-domain GRB2-like 3 20400 Cytoskeletal protein 3.4 1,37×10° Sh3g3 SH3-domain GRB2-like 3				·		
Ceg8 Chemoskine (C-C motf) ligand 9 20308 Chemoskines 4.0 2.10-10-10 Rmp2k BMP2 inducible kinase 140760 Protein kinase 3.8 1.32-10-10 Kcrlaff Kr 'channel latramerisation domain containing 8 243943 Subunit of SABA-B receptor 3.8 7.64-10-10 Cxcrl12 Chemokine (C-C motf) ligand 12 2015 Chemokines 4.6 6.00-10 Cxcrl21 Chemokine (C-C motf) ligand 12 2015 Chemokines 4.6 6.00-10 Scd1 Stearoyl-Conzory A-C desaturase 1 20249 Fally add metabolism 3.5 8.04-10 Rhox5 Reproductive homeobox 5 18617 Sertoli cell metabolism 3.5 2.49-10 Rbg3 Naga Nacestypitulariate synthase 217214 Mitochondrial matrix 3.5 2.198-10 Fig2 Fibrobast growth factor 2 14173 SSC self-renew 3.4 14.39-10 Sh3g3 Sh3-domain GRD2-like 3 2008 Cyctoskeletal protein 3.1 1.71-10 Cc13 Chemokine (C-C motf) receptor 1						
Bringalx BMP2 inducible kinase 140780 Protein kinase 3.8 1.32-101 KcrldB K* Channel letramerisation domain containing 8 243043 Subunit of GABA-B receptor 3.8 7.84-101 Auto-fatB Addo-keto reductase family 1, member C18 105349 Spermatogonia metabolism 3.7 2.51-101 Mag7 Microtubule-associated profein 7 17761 Statising microtubules 3.5 0.44-101 Scat1 Staroy-Coenzyme A desaturase 1 20249 Fatty acid metabolism 3.5 1.84-107 Roso Reproductive homebox 5 1817 Serticic cil metabolism 3.5 2.49-101 Naga N-acetylglutamate synthase 217214 Mitchchordrial matrix 3.5 2.249-101 Ly6ge Ly6ge Ly76ge						
Kctd8 Kr. channel tetramerisation domain containing 8 243043 Subunit GABA-B receptor 3.8 7.64+107 Cxr112 Chemokine (C-X-C motif) ligand 12 20315 Chemokines 4.6 680×10° Cxr12 Chemokine (C-X-C motif) ligand 12 20315 Chemokines 4.6 680×10° Scd1 Stararyl-Conzyme A desaturase 1 2029 Faty add metabolism 3.5 8.04+10° Scd1 Staroryl-Conzyme A desaturase 1 2029 Faty add metabolism 3.5 8.04+10° Roproductive homeobox 5 18817 Sortoi cell metabolism 3.5 2.49+10° Nags N-acetylejutamate synthase 217214 Michondrain matrix 3.5 2.249+10° Ly6g6e Lymphocyte antigen 6 complex, locus G6E 70274 Signal transduction 3.4 1.432+10° Fg12 Fibrobiast growth factor 2 14173 SSC self-renew 3.4 1.432+10° Sh3gi3 SH3-domain GRE2-like 3 2008 Cystoskeletal protein 3.4 1.432+10° Ziz Chemokine C-Cornotify receptor 1 1278						1.32×10 ⁻³
Cox12 Chemokinea (C-X-C motif) ligand 12 20315 Chemokines 4.8 6.80-019 Map7 Microtubule-associated protein 7 17761 Stabilizing microtubules 3.5 8.04×10* Scot1 Stearoy-Coenzyme A desaturase 1 20249 Fatty acid metabolism 3.5 1.84×10* Rhox5 Reproductive homeobox 5 18817 Sertoli cell metabolism 3.5 2.49×10* Lygg6e Lymphocyte antigen 6 complex, locus G8E 70274 Signal transduction 3.4 1.92×10* Fg12 Fibrobiast growth factor 2 14173 SSC self-renew 3.4 1.43×10* Sh3dg18 SH3-domain GRE2-likes 2008 Cytoskeletal protein 3.4 1.43×10* Zcg1 Chemokine (C-C motif) receptor 1 12768 Chemokine receptor 3.2 1.71×10* Ccr1 Chemokine (C-C motif) receptor 1 12768 Chemokine receptor 3.0 1.85×10* Act Chemokine (C-C motif) receptor 1 12148 Self transcription affactor 3.0 1.85×10* Galar GATA binking protein						7.64×10 ⁻³
Map7 Microtubule-associated protein 7 17761 Stabilizing microtubules 3.5 8.044107 Sod1 Staeroyl-Coenzyme A desaturase 1 2024 Fatty acid metabolism 3.5 1.84×10* Rhox5 Reproductive homeobox 5 18817 Sertoli cell metabolism 3.5 2.49×10* Naga N-acetylglutamate synthase 2.17214 Mitochondrial matrix 3.5 2.29×10* LyRg6e Lymphocyte antigon 6 complex, locus G8E 70274 Signal transduction 3.4 1.37×10* Sh3g3 ST3-domain GRB2-like 3 2040e Cytoskeletal protein 3.4 1.37×10* Zic3 Zic6 finegror protein of the cerebellum 3 22773 Pluroptoency maintenance 3.2 7.71×10* Ccr1 Chemokine (C-C motif) ligand 1 16963 Chemokine Co-C motify leoptor 1 12768 Chemokine Co-C motify leoptor 1 14460 Sertoli transcriptional factor 3.1 1.58×10* Galat 1 GATA binding protein 1 14460 Sertoli transcription factor 3.0 4.19×10* Elovi2 ELovi L stray acid elongated 3.	Akr1c18	Aldo-keto reductase family 1, member C18	105349	Spermatogonia metabolism	3.7	2.51×10 ⁻³
Scd1 Stearoyl-Coenzyme A desaturase 1 20249 Fatty acid metabolism 3.5 1.84100 Rhox5 Reproductive homeobox 5 18617 Sertotic cell metabolism 3.5 2.49×10 Nags N-acetylgulturantes synthase 217214 Mitochondrial martix 3.5 2.19×10³ Fg/Z Eirobalast growth factor 2 14173 SSC self-renew 3.4 1.92×10³ Sh3g/3 SH3-domain GRB2-like 3 20408 Cytoskeletal protein 3.4 1.37×10³ Zic3 Zinc finger protein of the cerebellum 3 22773 Pluripotency maintenance 3.2 1.71×10³ Ccr1 Chemokine (C-C mottl) report 12788 Chemokine receptor 3.2 1.71×10³ Xcl1 Chemokine (C-C mottl) report 14460 Serbic transport 3.0 1.85×10³ Gata1 GATA binding protein 1 14460 Serbic transport 2.9 4.98×10³ Eliviz ELOVL fatty acid elongase 2 54326 Fatty acid elongation 3.0 3.59×10° Cot2 Eliviz Serbic microscopic like 2	Cxcl12	Chemokine (C-X-C motif) ligand 12	20315	Chemokines	4.6	6.80×10 ⁻³
Rhox5 Reproductive homeobox 5 18817 Sertoli cell metabolism 3.5 2.49+10¹ Nags Nacetylgultamate synthase 217214 Mitochondrial matrix 3.5 2.19+10¹ Lydigge Lymphocyte antigen 6 complex, locus GRE 70724 Signal transduction 3.4 1.92+10¹ Fir2 Fibroblast growth factor 2 14173 SSC self-renew 3.4 1.73×10¹ Sh3gl3 SH3-domain GRB2-like 3 20406 Cytoskeletal protein 3.4 1.73×10¹ Zic3 Zinc finger protein of the cerebellum 3 22773 Pluripotency maintenance 3.2 1.71×10² Ccr1 Chemokine (C-C motif) ligand 1 16863 Chemokines 3.1 1.56×10³ Gata 1 GATA binding protein 1 14460 Sertoli transcriptional factor 3.0 4.19×10³ Elovi2 ELOVL taffy acid elongase 2 4326 Faty acid elongation 3.0 4.98×10³ Vinka WNRk lysine deficient protein kinase 3 69847 Transcellular Ca¹* transport 2.9 3.88×10³ Cott Chemokine (C-C motif)	Map7	Microtubule-associated protein 7	17761	Stabilizing microtubules	3.5	8.04×10 ⁻⁴
Nages	Scd1	Stearoyl-Coenzyme A desaturase 1	20249	Fatty acid metabolism	3.5	1.84×10 ⁻³
Lyefg6e Lymphocyte antigen 6 complex, locus G6E 70274 Signal transduction 3.4 1.92±10³ Fgf2 Fibroblast growth factor 2 14173 SSC self-renew 3.4 1.43±10³ Sh3gl3 SH3-domain GRB2-like 3 20408 Cyfoskeletal protein 3.4 1.33±10³ Zic3 Zinc finger protein of the cerebellum 3 22773 Pluripotency maintenance 3.2 1.71±10³ Ccr1 Chemokine (C-C motif) receptor 1 12768 Chemokine receptor 3.2 7.67±10³ Cr1 Chemokine (C-C motif) groeptor it 1 12768 Chemokine receptor 3.2 7.67±10³ Gata1 GATA binding protein 1 14460 Sertoli transcriptional factor 3.0 1.85±10³ Gata1 GATA binding protein 1 21432 Att associate 2.9 4.98±10³ Wink4 WIK Wk Jusine deficient protein kinase 3 69847 Transcellular Ca³* transport 2.9 3.88±10³ Ceri2 Chemokine (C-C motif) receptor-like 2 54199 Chemokine receptor 2.9 2.28±10³ Rarest Ret		•				2.49×10 ⁻³
Fig. Fibroblast growth factor 2	Nags	N-acetylglutamate synthase		Mitochondrial matrix		2.19×10 ⁻³
Sh3gl3 SH3-domain GRB2-like 3 20408 Cytoskeletal protein 3.4 1.37×10³ Zic3 Zinf Inger protein of the cerebellum 3 22773 Pluripotency maintenance 3.2 1.71×10³ Ccr1 Chemokine (C motif) ligand 1 16963 Chemokine cereptor 3.2 7.67×10³ Xcl1 Chemokine (C motif) ligand 1 16963 Chemokines 3.1 1.56×10³ Qpct Glutaminyl-peptide cyclotransferase 70536 Glutaminyl cyclase 3.0 1.88×10³ Gata1 GAT A Indiring protein 1 1.4460 Secritol transcriptional factor 3.0 4.98×10³ Elovi2 ELOVL fatty acid elongase 2 54326 Fatty acid elongation 3.0 3.59×10³ Tot1 Toell lymphoma breakpoint 1 21432 Akt associate 2.9 4.98×10³ Vinct WMK Lysine deficient protein kinase 3 69847 Transcellular Ca² transport 2.9 2.28×10³ Ccr12 Chemokine C-C motif) receptor like 2 54199 Chemokine receptor 2.9 2.28×10³ Cst2 Phace Passocial prote	Ly6g6e	Lymphocyte antigen 6 complex, locus G6E		Signal transduction	3.4	1.92×10 ⁻³
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Supplemental Table 4: Up-regulated gene transcripts in P5 *Gata4* cKO compared to control testes.

Gene	Description	NCBI gene I	D Function L	cKO to WT og fold change	cKO to WT P-value
Gpnmb	Glycoprotein (transmembrane) nmb	78709	Macrophage activation & adhesion	n 18.3	8.19×10⁴
Mia1	Melanoma inhibitory activity 1	12587	Extracellular matrix	16.1	5.92×10 ⁻⁶
Lrrtm4	Leucine rich repeat transmembrane neuronal 4	243499	Membrane protein	14.5	7.73×10 ⁻³
Ucma	Upper zone of cartilage matrix associated	68527	Matrix-associated protein	13.9	6.05×10 ⁻⁴
Tmem26	Transmembrane protein 26	327766	Transmembrane protein	13.3	8.08×10 ⁻⁴
Col2a1	Collagen, type II, alpha 1	12824	Extracellular matrix	8.1	1.00×10 ⁻⁴
Bhlhe22	Basic helix-loop-helix family, member e22	59058	Transcriptional factor	8.1	6.74×10 ⁻⁴
Pcsk6	Proprotein convertase subtilisin/kexin type 6	18553	Follicular proprotein convertase	7.7	1.88×10 ⁻⁵
Gucy1a3	Guanylate cyclase 1, soluble, alpha 3	60596	Signal transduction	7.6	1.74×10 ⁻⁵
Mapt	Microtubule-associated protein tau	17762	Stablilizing microtubules	7.5	2.15×10⁴
Gabrb2	(GABA) A receptor, subunit beta 2	14401	Ligand-gated ion channel	7.4	7.06×10 ⁻⁴
Svs6	Seminal vesicle secretory protein 6	20945	Secretion protein	7.4	2.52×10 ⁻³
kcnk3	Potassium channel, subfamily K, member 3	16527	K ⁺ channel	7.2	5.78×10 ⁻⁵
Naip5	NLR family, apoptosis inhibitory protein 5	17951	Apoptosis	6.9	6.72×10 ⁻³
Crispld2	Cysteine-rich secretory protein LCCL domain 2	78892	Cell polarity complex	6.8	4.34×10 ⁻⁵
Eaf2	ELL associated factor 2	106389	Androgen-regulated tumor suppre	ssor 6.6	1.32×10 ⁻³
Kcnj10	Potassium channel, subfamily J, member 10	16513	K⁺ channel	6.5	7.28×10 ⁻³
Ntsr1	Neurotensin receptor 1	18216	Tumorigenesis	6.3	1.80×10⁴
Oxtr	Oxytocin receptor	18430	Lipid metabolism	6.2	2.00×10 ⁻⁴
Xdh	Xanthine dehydrogenase	22436	Oxidative metabolism	6.1	2.13×10⁴
Cdhr5	Cadherin-related family member 5	72040	Cellular junctions	6.1	4.18×10 ⁻⁴
Svs5	Seminal vesicle secretory protein 5	20944	Unknown function	6.0	8.06×10 ⁻⁵
Col27a1	Collagen, type XXVII, alpha 1	373864	Extracellular matrix	5.9	4.87×10 ⁻³
Adh1	Alcohol dehydrogenase 1 (class I)	56734	Vitamin A metabolism	5.8	4.81×10 ⁻⁵
Hsd3b1	Hydroxy-delta-steroid dehydrogenase, 3b-1	15492	Steroidogenesis	5.5	5.85×10 ⁻⁵
Star	Steroidogenic acute regulatory protein	20845	Steroidogenesis	5.5	7.64×10 ⁻⁴
Gria4	Glutamate receptor, ionotropic, AMPA4	14802	Gated ion channels	5.5	1.74×10 ⁻³
Муос	Myocilin	70536	Glucocorticoid response	5.2	2.61×10 ⁻³
Tmem90b	Transmembrane protein 90B	433485	Transmembrane protein	5.0	3.41×10 ⁻³
Col4a6	Collagen, type IV, alpha 6	94216	Extracellular matrix	5.0	2.08×10⁴
Bhmt	Betaine-homocysteine methyltransferase	12116	Folate metabolism	5.0	6.45×10 ⁻⁴
	·	16000		4.8	7.00×10 ⁻⁴
Lgf1	Insulin-like growth factor 1		Insulin/IGF signaling		
Uhrf2	Ubiquitin-like, containing PHD and RING finger 2		E3 ubiquitin-protein ligase	4.7	1.20×10 ⁻³
Tgfb2	Transforming growth factor, beta 2	21808	TGFβ/SMAD pathway, RA	4.3	3.89×10 ⁻⁴
Pxt1	Peroxisomal, testis specific 1	69307	Germ cell apoptosis	4.3	3.20×10 ⁻⁴
Foxc1	Forkhead box C1	17300	Granulosa transcription factor	4.2 4.2	2.51×10 ⁻³
Cst8 Apoc1	Cystatin 8 Apolipoprotein C-I	13012 11812	Degradation Lipid metabolism	3.9	3.74×10 ⁻⁴ 1.07×10 ⁻³
Car12	Carbonic anyhydrase 12	76459	Zinc metalloenzyme	3.7	7.19×10 ⁻⁴
Vldlr		22359	Lipoprotein metabolism	3.7	8.69×10⁴
	Very low density lipoprotein receptor				
Sftpc	Surfactant associated protein C	20389	Retinoic Acid-regulated gene	3.4	2.79×10 ⁻³
Phlda1	Pleckstrin homology-like domain, A,1	21664	Apoptosis	3.3	7.67×10 ⁻³
Rai2	Retinoic acid induced 2	24004	Retinoic Acid-regulated gene	3.2	1.06×10 ⁻³
Npvf	Neuropeptide VF precursor	60531	Apoptosis	3.2	2.24×10 ⁻³
Amer2	APC membrane recruitment 2	72125	Wnt/β-catenin signaling	3.1	1.52×10 ⁻³
Hmgcs2	Hydroxy-methylglutaryl-Coenzyme A synthase 2		Cholesterol biosynthesis	3.1	1.65×10 ⁻³
Tspan15	Tetraspanin 15	70423	Extracellular matrix	3.0	1.83×10 ⁻³
Lhx9	LIM homeobox protein 9	16876	Sertoli transcrpition factor	2.8	5.37×10 ⁻³
Pik3r1	PI3K, regulatory subunit, polypeptide 1	18708	PI3K signaling	2.7	5.47×10 ⁻³
Pde4d	Phosphodiesterase 4D, cAMP specific	238871	cAMP-catabolizing enzyme	2.6	3.00×10 ⁻³
Igfbp3	Insulin-like growth factor binding protein 3	16009	Apoptosis	2.5	6.34×10 ⁻³
Cpxm1	Carboxypeptidase X 1 (M14 family)	56264	Rhox5-negative regulated gene	2.5	6.72×10 ⁻³
Col4a6	Collagen, type IV, alpha 6	94216	Extracellular matrix	2.4	1.92×10 ⁻³
Tbx3	T-box 3	21386	Apoptosis	2.3	2.41×10 ⁻³
Trim9	Tripartite motif-containing 9	94090	E3 ubiquitin-protein ligase	2.3	1.91×10 ⁻³
Col9a2	Collagen, type IX, alpha 2	12840	Extracellular matrix	2.3	2.13×10 ⁻³
Ctsd	Cathepsin D	13033	Lysosomal apoptosis	2.3	1.08×10 ⁻³
	Low density lipoprotein receptor protein 1	16971	Sertoli endocytosis	2.2	1.28×10 ⁻³
Lrp1			-		
Fads2	Fatty acid desaturase 2	56473	Fatty acid synthesis	2.2	1.64×10 ⁻³
Prom1	Prominin 1	19126	Testicular seminoma	2.2	2.71×10 ⁻³
Smoc1	SPARC related modular calcium binding 1	64075	Sertoli matricellular protein	2.2	1.59×10 ⁻³
Fads1	Fatty acid desaturase 1	76267	Fatty acid synthesis	2.1	1.81×10 ⁻³
Ppp1r9b	Protein phosphatase 1, regulatory subunit 9B	217124	Retinoic Acid-regulated gene	2.1	2.78×10 ⁻³
Aifm2	Apoptosis-inducing factor, mitochondrion 2	71361	Apoptosis	2.0	3.78×10 ⁻³