

Figure S1. Expression of LCN2 in the utero-tubular junction (UTJ). UTJ tissues were prepared from females in estrous without mating ($\times (-)$), estrous mated with vasectomized males (\times VM) or estrous mated with normal males (\times NM), homogenized and 10 mg of protein per sample was subjected to SDS-PAGE. Protein-transferred membranes were probed with a rabbit polyclonal antibody against mouse LCN2. The blot transfer efficiency was checked by staining with CBB after immunoblotting. Densities of LCN2 or albumin bands of UTJ samples were quantified with a densitometer. LCN2 quantity was adjusted with albumin density and relative amounts are indicated (amount of $\times (-)$ was considered 1.0).

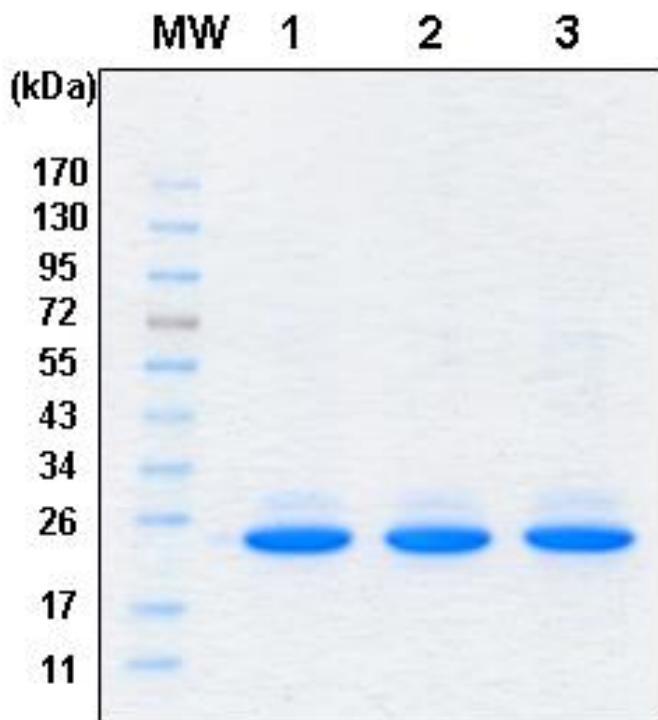


Figure S2. Preparation of recombinant LCN2 protein.

The C-terminal FLAG-tagged version of LCN2 protein (LCN2-FLAG) was purified from culture supernatant of CAAG-Lcn2-FLAG-transfected CHO cells using an anti-FLAG agarose affinity column. Here, three independent samples containing 1 µg of protein were applied to SDS-PAGE and stained with GelCode Blue.

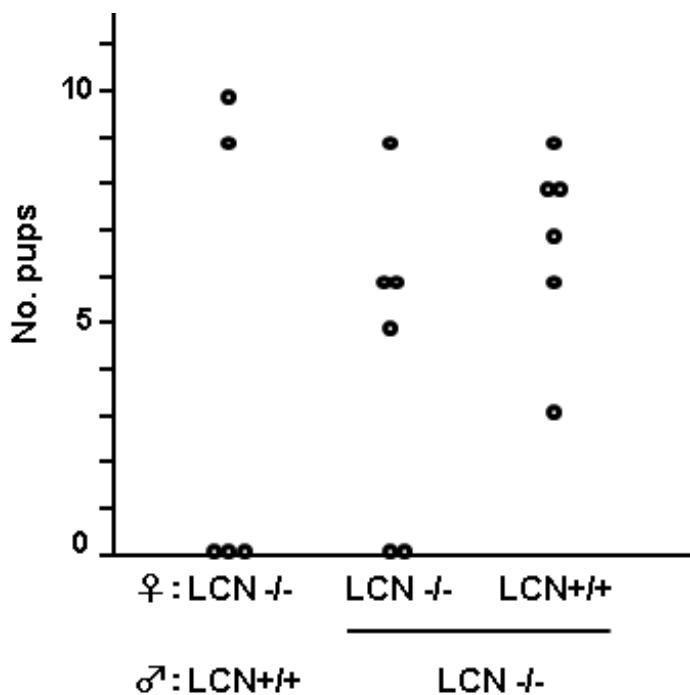


Figure S3.Pregnancy of *Lcn2*^{-/-} female. *Lcn2*^{-/-} or *Lcn2*^{+/+} females were mated with males indicated for two month and number of pups from individual females at first delivery was indicated. Animals with no pup showed no sign of pregnancy during this period.

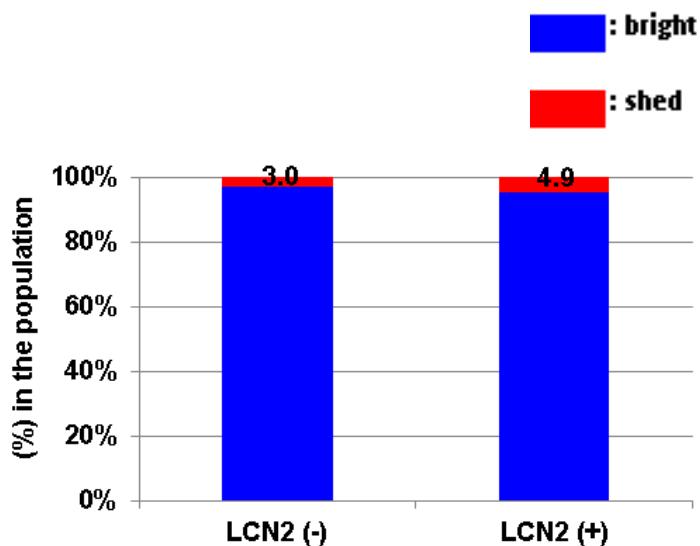


Figure S4. Fate of EGFP (K268Q)-GPI upon LCN2 treatment. Sperm collected from the epididymis of *Lcn2*^{-/-} were incubated as in (Fig. 2A) and observed for EGFP fluorescence. Sperm classified with regard to the dual patterns are indicated by their proportion in the population examined. Number of sperm examined: LCN2 (-), n=166; LCN2 (+), n=184. Data from three independent experiments were accumulated. (%) shed sperm is indicated.

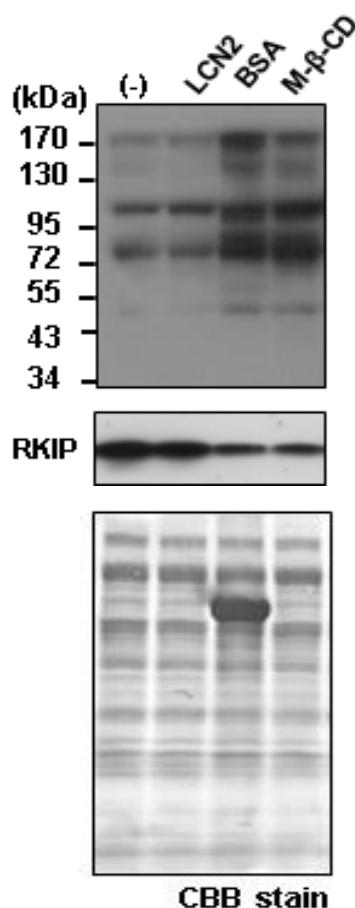


Figure S5. LCN2 does not induce protein tyrosine phosphorylation or RKIP down regulation. 2.5×10^5 sperm incubated in HTF-PVA, HTF-PVA-LCN2, HTF-BSA or HTF-PVA-M- β -CD for 120 min were boiled in SDS-PAGE sample buffer and subjected to immunoblotting. Protein-blotted membranes were probed with anti-phosphotyrosine antibodies or anti-RKIP antibody. The transfer efficiency was checked by membrane staining with Coomassie Brilliant Blue (CBB). Residual BSA can be seen in the BSA lane.

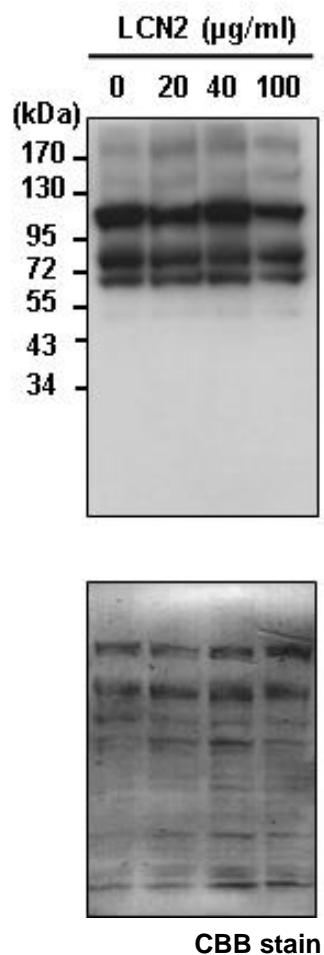


Figure S6. LCN2 cannot induce protein tyrosine phosphorylation even with an excess dose. 2.5×10^5 sperm incubated with the indicated dose of LCN2 for 120 min were boiled in SDS-PAGE sample buffer and subjected to immunoblotting. Protein-blotted membranes were probed with anti-phosphotyrosine antibodies. The transfer efficiency was checked by membrane staining with CBB.

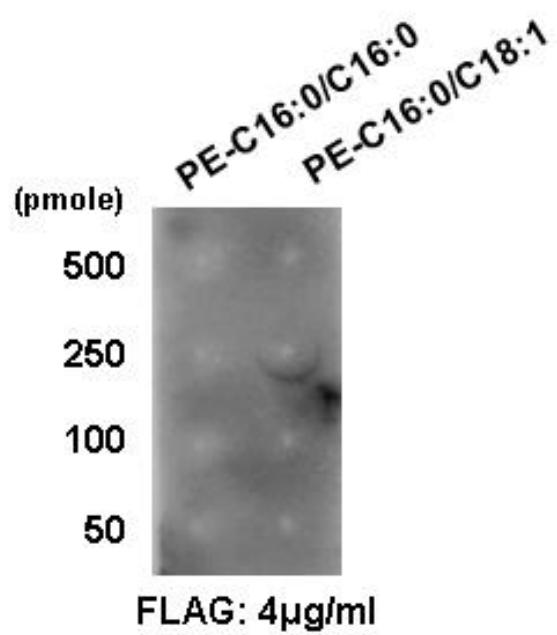
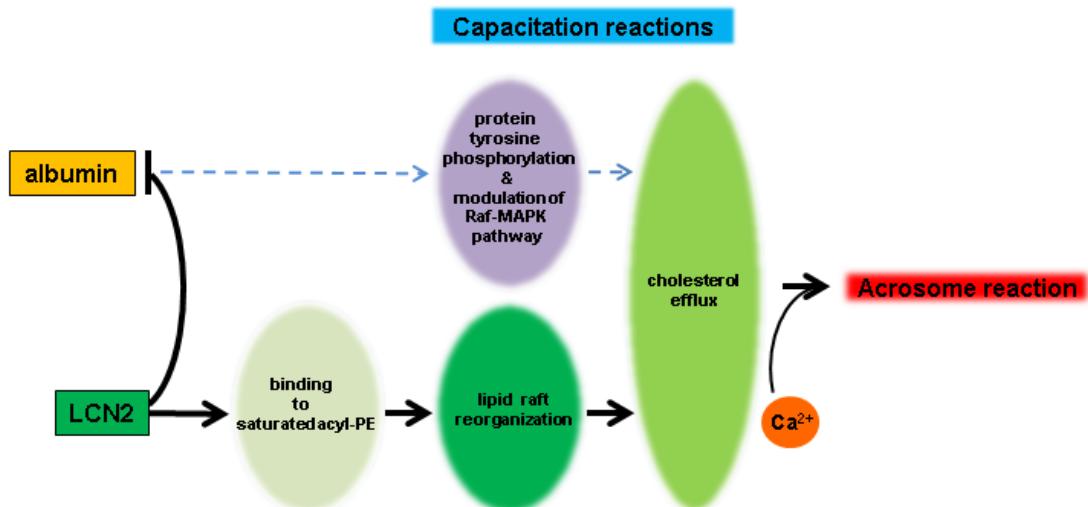


Figure S7. FLAG peptide does not bind to PE. To exclude the possibility that recombinant LCN2 binds to PE via FLAG sequence, membranes blotted with PE were contacted with 4 μ g/ml FLAG peptide and binding was examined as in Fig 4B. Binding of FLAG peptide was not detected at all.

LCN2(+): normal state



LCN2(-): alternative state

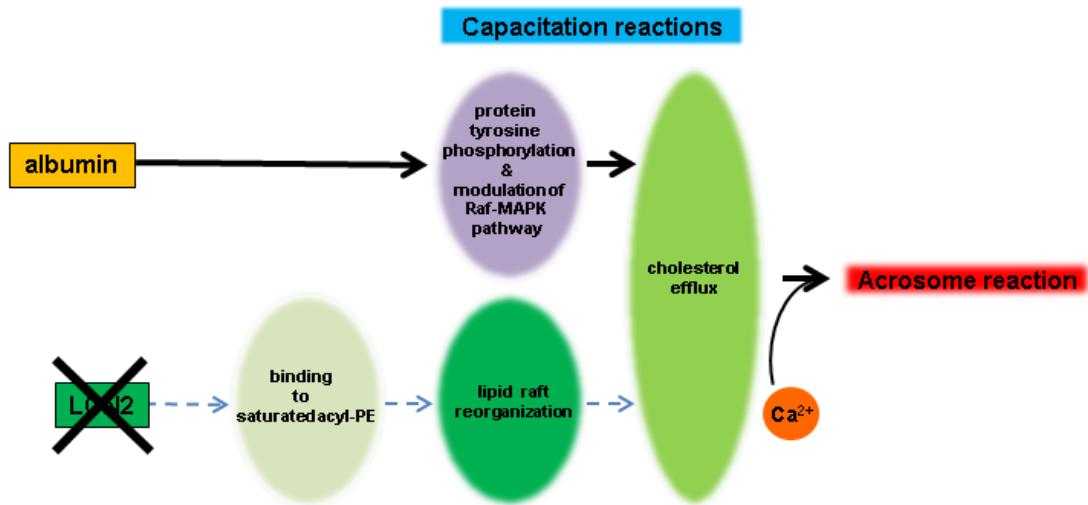


Figure S8. Possible model for sperm maturation in mammals.

In the normal state, LCN2 binds to PE followed by lipid raft reorganization and linkage to the common machinery, such as cholesterol efflux and acrosome reaction. At the same time, LCN2 suppresses the albumin-mediated capacitation pathway. In contrast, when LCN2 is absent, the albumin-mediated pathway becomes active, followed by protein tyrosine phosphorylation or Raf-MAP kinase pathway modulation, and then linkage to the common machinery.

Table S1. Results of microarray analyses**1: females in estrous no mating****2: females in estrous mated with vasectomized male****3: females in estrous mated with normal male**

2 vs. 1	3 vs. 1	3 vs. 2	Description
	1.84516 3	3.30127	Mus musculus laminin gamma2 chain mRNA, partial cds. [AF106279]
	1.08707 1	2.34522 2	Mus musculus RIKEN cDNA 5830467P10 gene (5830467P10Rik), mRNA [NM_198029], Fermt1
	1.29856 7	2.53707 5	Mus musculus laminin, beta 3 (Lamb3), mRNA [NM_008484]
	0.58466	2.30033 1	Mus musculus RAS-related C3 botulinum substrate 1 (Rac1), mRNA [NM_009007]
	0.97253 5	2.52444 4	Mus musculus selectin, platelet (p-selectin) ligand (Selplg), mRNA [NM_009151]
	0.54366 9	2.22442 2	Mus musculus plakophilin 3 (Pkp3), mRNA [NM_019762]
	1.71328 8	2.03860 6	Mus musculus intercellular adhesion molecule (Icam1), mRNA [NM_010493]
	1.42315 4	2.40242 7	Mus musculus desmoglein 2 (Dsg2), mRNA [NM_007883]
	0.62521	2.87553	Mus musculus BTB (POZ) domain containing 9 (Btbd9), mRNA [NM_172618]
0.53956 3	2.78453 4	2.24497	Mus musculus 18 days pregnant adult female placenta and extra embryonic tissue cDNA, RIKEN full-length enriched library, clone:3830421F03 product:hypothetical protein, full insert sequence. [AK014446]
2.82408 4	5.25656 6	2.43248 2	Mus musculus mucin 4 (Muc4), mRNA [NM_080457]
1.28899 6	5.15555 7	3.86656 1	Mus musculus mucin 4 (Muc4), mRNA [NM_080457]
	0.72260 3	2.59686 8	Mus musculus uroplakin 1A (Upk1a), mRNA [NM_026815]
	0.87118 3	2.13659	Mus musculus attractin like 1 (Attnl1), mRNA [NM_181415]
	1.84516 3	3.30127	Mus musculus laminin gamma2 chain mRNA, partial cds. [AF106279]
0.20401	3.11051	2.90650	Mus musculus UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 6 (Galnt6), mRNA

5	6	1	[NM_172451]
	0.97253	2.52444	Mus musculus selectin, platelet (p-selectin) ligand (Selplg), mRNA [NM_009151]
	5	4	
	2.24132	3.11304	Mus musculus lectin, galactose binding, soluble 9 (Lgals9), mRNA [NM_010708]
	3	3	
	1.17228	2.01953	Mus musculus F-box protein 2 (Fbxo2), mRNA [NM_176848]
	5	9	
	1.92438	2.67239	Mus musculus glucokinase (Gck), mRNA [NM_010292]
	7	4	
	0.96717	2.60541	Mus musculus calreticulin (Calr), mRNA [NM_007591]
	2		
	1.26986	2.52766	Mus musculus glutamine fructose-6-phosphate transaminase 1 (Gfpt1), mRNA [NM_013528]
	3	6	
2.09211	4.42134	2.32922	Mus musculus carbonic anhydrase 9 (Car9), mRNA [NM_139305]
3	1	8	
1.18436	4.05363	2.86927	Mus musculus leucine-rich alpha-2-glycoprotein 1 (Lrg1), mRNA [NM_029796]
1	3	1	
0.6087	2.80516	2.19646	Mus musculus deleted in colorectal carcinoma (Dcc), mRNA [NM_007831]
2	3	3	
3.34574	6.26374	2.91800	Mus musculus lipocalin 2 (Lcn2), mRNA [NM_008491]
3	7	4	
3.40217	5.58162	2.17944	PREDICTED: Mus musculus similar to interferon-induced protein with tetratricopeptide repeats 1, transcript variant 1 (LOC667373), mRNA [XM_001000875]
2	1	9	
0.36758	2.40130	2.03371	Mus musculus stomatin (Epb7.2)-like 3 (Stoml3), mRNA [NM_153156]
4	3	9	
0.19640	3.48694	3.29054	Mus musculus MAX dimerization protein 1 (Mxd1), mRNA [NM_010751]
1	2	1	
0.74462	2.59780	1.85317	Mus musculus plakophilin 1 (Pkp1), mRNA [NM_019645]
5	4	9	
0.84706	2.79775	1.95069	Mus musculus NLR family, apoptosis inhibitory protein 1 (Naip1), mRNA [NM_008670]
3	5	2	
0.31507	2.04586	1.73079	Mus musculus ubiquitin-conjugating enzyme E2L 6 (Ube2l6), mRNA [NM_019949]
1	9	8	
0.43152	2.15080	1.71928	Mus musculus adult male colon cDNA, RIKEN full-length enriched library, clone:9030414N23 product:similar to Tripartite motif protein 30 (Down regulatory protein of interleukin 2 receptor) [Mus musculus], full insert sequence. [AK136456]
3	4	1	
0.07733	1.67761	1.60027	Mus musculus CDC42 effector protein (Rho GTPase binding) 2 (Cdc42ep2), mRNA [NM_026772]

	9	7	8	
1.05079	2.92015	1.86936		Mus musculus hepatocyte growth factor activator (Hgfac), mRNA [NM_019447]
6	7	2		
0.13128	1.87823	1.74695		Mus musculus signal transducer and activator of transcription 1 (Stat1), mRNA [NM_009283]
3	3			
0.20397	2.24944	2.04547		Mus musculus gap junction membrane channel protein beta 2 (Gjb2), mRNA [NM_008125]
2	8	6		
0.84614	2.77939	1.93325		Mus musculus chimerin (chimaerin) 2 (Chn2), mRNA [NM_023543]
2	5	2		
0.18121	1.80377	1.62255		Mus musculus HIV-1 tat interactive protein 2, homolog (human) (Htatip2), mRNA [NM_016865]
9	5	6		
0.50373	2.28493	1.78120		Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4933431K05 product:hypothetical ARM repeat structure containing protein, full insert sequence. [AK017008]
3	4	1		
1.27769	3.20614	1.92845		Mus musculus serine (or cysteine) peptidase inhibitor, clade B (ovalbumin), member 12 (Serpib12), mRNA [NM_027971]
2	6	4		
4.85485	6.51264	1.65778		Mus musculus EGL nine homolog 3 (C. elegans) (Egln3), mRNA [NM_028133]
8	2	4		
1.94003	3.71139	1.77136		Mus musculus peptidoglycan recognition protein 1 (Pglyrp1), mRNA [NM_009402]
1	8	7		
0.11155	2.05827	1.94671		Mus musculus tetratricopeptide repeat domain 29 (Ttc29), mRNA [NM_183096]
7		3		
0.22081	1.98479	1.76397		Mus musculus ChaC, cation transport regulator-like 1 (E. coli) (Chac1), mRNA [NM_026929]
8	1	3		
1.68478	3.64755	1.96276		Mus musculus interferon-induced protein with tetratricopeptide repeats 1 (Ifit1), mRNA [NM_008331]
8	4	7		
0.18439	3.42337	3.23898		Mus musculus 14-3-3 protein sigma mRNA, complete cds. [AF058798]
5	7	1		
0.53074	2.1976	1.66685		Mus musculus leucine rich repeat containing 46 (Lrrc46), mRNA [NM_027026]
9		1		
0.42902	2.19616	1.76713		Mus musculus mal, T-cell differentiation protein-like (Mall), mRNA [NM_145532]
3	2	9		
0.10547	1.82275	1.71728		Mus musculus myosin, heavy polypeptide 6, cardiac muscle, alpha (Myh6), mRNA [NM_010856]
8	9			
0.28667	1.36473	1.07806		Mus musculus SAM pointed domain containing ets transcription factor (Spdef), mRNA [NM_013891]
4	5			
0.82019	1.82493	1.00473		Mus musculus RAS-like, family 10, member A (Rasl10a), mRNA [NM_145216]

	1		9	
0.07204	1.64478	1.57274		Mus musculus breast carcinoma amplified sequence 1 (Bcas1), mRNA [NM_029815]
1	1	1		
0.48883	1.52025	1.03141		Mus musculus SEC14-like 2 (<i>S. cerevisiae</i>) (Sec14l2), mRNA [NM_144520]
9	4	5		
0.01096	1.31112	1.30016		Mus musculus acireductone dioxygenase 1 (Adi1), mRNA [NM_134052]
3	3	3		
1.34124	2.51124	1.17000		Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4930534K13 product:hypothetical protein, full insert sequence. [AK015966]
3	7	3		
1.97264	3.93040	1.95775		Mus musculus six transmembrane epithelial antigen of the prostate 1 (Stear1), mRNA [NM_027399]
4	3	9		
1.01893	2.81768	1.79875		Mus musculus mixed lineage kinase domain-like (Mlk1), mRNA [NM_029005]
2	4	2		
1.59453	2.65556	1.06102		Mus musculus RIKEN cDNA 1500005I02 gene (1500005I02Rik), mRNA [NM_028055]
7	5	8		
0.11485	1.47629	1.36143		Mus musculus armadillo repeat containing 3 (Armc3), mRNA [NM_001081083]
9	1	2		
0.83934	2.26314	1.42379		Mus musculus leucine rich repeat containing 18 (Lrrc18), mRNA [NM_026253]
7	3	6		
0.75656	2.09312	1.33656		Mus musculus leucine rich repeat containing 18 (Lrrc18), mRNA [NM_026253]
4	4			
0.45662	2.53981	2.08318		PREDICTED: Mus musculus proprotein convertase subtilisin [XM_129214]
5	1	6		
0.87868	2.39545	1.51677		Mus musculus expressed sequence Al451617 (Al451617), mRNA [NM_199146]
5	7	2		
0.41499	4.56220	4.14720		Mus musculus 2'-5' oligoadenylate synthetase 3 (Oas3), mRNA [NM_145226]
9	8	8		
0.67072	1.80037	1.12964		Mus musculus phospholipase C, eta 1 (Plch1), mRNA [NM_183191]
9	2	3		
0.89609	2.04512	1.14903		Mus musculus leucine rich repeat containing 48 (Lrrc48), mRNA [NM_029044]
1	5	4		
0.56889	1.81237	1.24348		Mus musculus tripartite motif protein 6 (Trim6), mRNA [NM_001013616]
1	4	3		
0.07868	1.53048	1.45179		Mus musculus cytochrome P450, family 2, subfamily s, polypeptide 1 (Cyp2s1), mRNA [NM_028775]
4	2	8		
0.01388	1.20871	1.19482		Mus musculus prostaglandin F2 receptor negative regulator (Ptgfrn), mRNA [NM_011197]

	9	5	7	
0.56833	1.71878	1.15044		Mus musculus translin-associated factor X (Tsnax) interacting protein 1 (Tsnaxip1), mRNA [NM_024445]
6	4	9		
0.84706	2.79775	1.95069		Mus musculus NLR family, apoptosis inhibitory protein 1 (Naip1), mRNA [NM_008670]
3	5	2		
0.25484	1.43852	1.18368		Mus musculus vascular endothelial growth factor A (Vegfa), transcript variant 2, mRNA [NM_009505]
1	1	1		
0.23763	1.44637	1.20874		Mus musculus RAB, member of RAS oncogene family-like 5 (Rabl5), mRNA [NM_026073]
1	1	1		
1.47928	2.79829	1.31900		Mus musculus DNA segment, Chr 11, Lothar Hennighausen 2, expressed (D11Lgp2e), mRNA [NM_030150]
4	2	8		
0.80129	1.93275	1.13145		Mus musculus protein phosphatase 1J (Ppm1j), mRNA [NM_027982]
5		5		
3.25885	5.48829	2.22944		Mus musculus Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1 (Cited1), mRNA [NM_007709]
1	8	7		
1.74946	3.05325	1.30378		Mus musculus myxovirus (influenza virus) resistance 1 (Mx1), mRNA [NM_010846]
5		5		
1.52018	3.62854	2.10836		Mus musculus apolipoprotein B editing complex 2 (Apobec2), mRNA [NM_009694]
3	5	2		
0.01358	1.47054	1.45695		Mus musculus tumor necrosis factor receptor superfamily, member 10b (Tnfrsf10b), mRNA [NM_020275]
9	6	7		
0.92743	2.33786	1.41043		Mus musculus a disintegrin and metalloproteinase domain 28 (Adam28), transcript variant 2, mRNA [NM_183366]
6	9	3		
7.73527	9.62623	1.89096		Mus musculus chitinase 3-like 1 (Chi3l1), mRNA [NM_007695]
2	4	2		
0.93588	2.47958	1.54369		Mus musculus glutamic-oxaloacetic transaminase 1-like 1 (Got1l1), mRNA [NM_029674]
6	2	6		
0.42941	1.52609	1.09668		Mus musculus ribonuclease P 25 subunit (human) (Rpp25), mRNA [NM_133982]
4	4	4		
0.78934	1.79931	1.00997		Mus musculus argininosuccinate synthetase 1 (Ass1), mRNA [NM_007494]
3	4	2		
0.28133	1.41906	1.13773		Mus musculus kinase non-catalytic C-lobe domain (KIND) containing 1 (Kndc1), mRNA [NM_177261]
6	5			
0.33233	1.67093	1.33860		Mus musculus Eph receptor A5 (Epha5), mRNA [NM_007937]
5	7	2		
0.39123	1.92110	1.52986		Mus musculus amyloid beta (A4) precursor protein-binding, family A, member 1 binding protein (Apba2bp), mRNA

4	2	8	[NM_021546]
0.68035 5	1.75696 5	1.07660 6	Mus musculus keratin 14 (Krt14), mRNA [NM_016958]
1.92662 4	3.11476 1	1.18813 7	Mus musculus NADPH oxidase organizer 1 (Noxo1), mRNA [NM_027988]
1.45701 5	2.67562 9	1.21861 5	Mus musculus developing brain homeobox 2 (Dbx2), mRNA [NM_207533]
1.14497 5	2.34236 2	1.19738 7	Mus musculus sperm associated antigen 16 (Spag16), transcript variant 2, mRNA [NM_025728]
0.44291 8	1.75314 6	1.31022 8	Mus musculus DnaJ (Hsp40) homolog, subfamily A, member 4 (Dnaja4), mRNA [NM_021422]
2.20769 5	3.43960 9	1.23191 4	Mus musculus transient receptor potential cation channel, subfamily V, member 6 (Trpv6), mRNA [NM_022413]
0.34248 2	2.25218 2	1.9097	Mus musculus cytochrome P450, family 2, subfamily a, polypeptide 4 (Cyp2a4), mRNA [NM_009997]
0.43087 6	1.48818 9	1.05731 2	Mus musculus alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide (Adh7), mRNA [NM_009626]
0.55610 8	1.68230 5	1.12619 7	Mus musculus maternal embryonic leucine zipper kinase (Mekk), mRNA [NM_010790]
0.43152 3	2.15080 4	1.71928 1	Mus musculus adult male colon cDNA, RIKEN full-length enriched library, clone:9030414N23 product:similar to Tripartite motif protein 30 (Down regulatory protein of interleukin 2 receptor) [Mus musculus], full insert sequence. [AK136456]
1.05079 6	2.92015 7	1.86936 2	Mus musculus hepatocyte growth factor activator (Hgfac), mRNA [NM_019447]
0.54385 7	1.86123 2	1.31737 5	Mus musculus HIV-1 tat interactive protein 2, homolog (human) (Htatip2), mRNA [NM_016865]
0.12859 8	2.05653 8	1.92794 8	Mus musculus cytochrome P450, family 2, subfamily b, polypeptide 9 (Cyp2b9), mRNA [NM_010000]
2.07596 6	3.40052 2	1.32455 6	Mus musculus ceruloplasmin (Cp), transcript variant 2, mRNA [NM_007752]
1.97623 6	5.13348 8	3.15725 2	Mus musculus 2'-5' oligoadenylate synthetase-like 1 (Oasl1), mRNA [NM_145209]
0.74363 9	1.82346 2	1.07982 2	Mus musculus RAB27b, member RAS oncogene family (Rab27b), transcript variant 1, mRNA [NM_030554]
0.20397 2	2.24944 8	2.04547 6	Mus musculus gap junction membrane channel protein beta 2 (Gjb2), mRNA [NM_008125]
3.40217	5.58162	2.17944	PREDICTED: Mus musculus similar to interferon-induced protein with tetratricopeptide repeats 1, transcript variant 1

2	1	9	(LOC667373), mRNA [XM_001000875]
1.31888	2.61461	1.29572	Mus musculus plasminogen activator, urokinase receptor (Plaur), mRNA [NM_011113]
8	4	7	
1.35561	2.75789	1.40227	Mus musculus hematopoietic SH2 domain containing (Hsh2d), mRNA [NM_197944]
6	1	6	
0.26253	2.10565	1.84311	RIKEN cDNA 1190002A17 gene [Source:MarkerSymbol;Acc:MGI:1916120] [ENSMUST00000074156]
8	7	9	
3.38325	4.55255	1.16929	Mus musculus CUB and zona pellucida-like domains 1 (Cuzd1), mRNA [NM_008411]
7	4	7	
1.32284	2.35394	1.0311	Mus musculus SKI-like (Skil), transcript variant 1, mRNA [NM_011386]
9	9		
0.20244	1.63462	1.43217	Mus musculus mitogen activated protein kinase 13 (Mapk13), mRNA [NM_011950]
9	5	7	
0.19661	1.24394	1.04732	Mus musculus guanine nucleotide binding protein, alpha 14 (Gna14), mRNA [NM_008137]
2	1	9	
1.08245	2.39977	1.31731	Mus musculus solute carrier family 34 (sodium phosphate), member 2 (Slc34a2), mRNA [NM_011402]
4	2	8	
0.22932	1.27147	1.04214	Mus musculus CCAAT/enhancer binding protein (C/EBP), delta (Cebpd), mRNA [NM_007679]
8	2	4	
0.84614	2.77939	1.93325	Mus musculus chimerin (chimaerin) 2 (Chn2), mRNA [NM_023543]
2	5	2	
0.76876	1.82501	1.05624	Mus musculus peroxisome proliferator activated receptor gamma (Pparg), mRNA [NM_011146]
8	6	8	
1.20630	2.48884	1.28254	Mus musculus aryl hydrocarbon receptor-interacting protein-like 1, mRNA (cDNA clone MGC:25485 IMAGE:4501401), complete cds. [BC028285]
3	8	5	
0.36758	2.40130	2.03371	Mus musculus stomatin (Epb7.2)-like 3 (Stoml3), mRNA [NM_153156]
4	3	9	
0.14722	1.16268	1.01546	Mus musculus schlafen 5 (Slfn5), mRNA [NM_183201]
3	8	5	
3.54891	5.12314	1.57423	Mus musculus Z-DNA binding protein 1 (Zbp1), mRNA [NM_021394]
1	1		
0.19640	3.48694	3.29054	Mus musculus MAX dimerization protein 1 (Mxd1), mRNA [NM_010751]
1	2	1	
0.08257	1.11772	1.03515	Mus musculus caspase 1 (Casp1), mRNA [NM_009807]
4	4	1	
0.81731	2.23435	1.41704	Mus musculus phospholipase A2, group IVF (Pla2g4f), mRNA [NM_001024145]

	4	6	3	
0.18121	1.80377	1.62255		Mus musculus HIV-1 tat interactive protein 2, homolog (human) (Htatip2), mRNA [NM_016865]
9	5	6		
0.42113	1.74078	1.31965		Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4921528H16 product:hypothetical RNI-like structure containing protein, full insert sequence. [AK014976]
5	8	4		
0.63513	1.74356	1.10843		Mus musculus FBJ osteosarcoma oncogene (Fos), mRNA [NM_010234]
3	2			
1.27769	3.20614	1.92845		Mus musculus serine (or cysteine) peptidase inhibitor, clade B (ovalbumin), member 12 (Serpinc12), mRNA [NM_027971]
2	6	4		
0.17990	1.29867	1.11877		Mus musculus ATPase type 13A5 (Atp13a5), mRNA [NM_175650]
7	8	2		
0.66220	1.86205	1.19984		Mus musculus complement component 3 (C3), mRNA [NM_009778]
9	1	2		
4.85485	6.51264	1.65778		Mus musculus EGL nine homolog 3 (C. elegans) (Egln3), mRNA [NM_028133]
8	2	4		
1.94003	3.71139	1.77136		Mus musculus peptidoglycan recognition protein 1 (Pglyrp1), mRNA [NM_009402]
1	8	7		
0.11155	2.05827	1.94671		Mus musculus tetratricopeptide repeat domain 29 (Ttc29), mRNA [NM_183096]
7		3		
0.12734	1.28794	1.16060		Mus musculus cyclin-dependent kinase-like 3 (Cdkl3), mRNA [NM_153785]
3	8	5		
3.65983	4.97076	1.31093		Mus musculus mucin 4 (Muc4), mRNA [NM_080457]
1	3	2		
0.10292	1.53416	1.43123		Mus musculus GIPC PDZ domain containing family, member 2 (Gipc2), mRNA [NM_016867]
7	5	9		
0.46343	1.56099	1.09756		Mus musculus a disintegrin and metalloproteinase domain 8 (Adam8), mRNA [NM_007403]
9		9		
3.74710	5.75337	2.00627		Mus musculus lipase, gastric (Lipf), mRNA [NM_026334]
4	5			
0.58254	2.62837	2.04582		Mus musculus plasminogen activator, urokinase receptor (Plaur), mRNA [NM_011113]
8	1	3		
0.06424	1.18624	1.12200		Mus musculus insulin-like 3 (Ins3), mRNA [NM_013564]
9		9		
1.68478	3.64755	1.96276		Mus musculus interferon-induced protein with tetratricopeptide repeats 1 (Ifit1), mRNA [NM_008331]
8	4	7		
0.12455	1.26093	1.13638		Mus musculus spire homolog 2 (Drosophila) (Spire2), mRNA [NM_172287]

4	5	2	
4.11912	5.36780	1.24867	Mus musculus ring finger protein 183 (Rnf183), mRNA [NM_153504]
5	3	7	
0.75926	1.87531	1.11605	Mus musculus acyltransferase like 1 (Ayt1), mRNA [NM_173014]
1	7	6	
0.88542	3.44185	2.55642	Mus musculus 2'-5' oligoadenylate synthetase 3 (Oas3), mRNA [NM_145226]
9		1	
1.24721	2.42560	1.17839	Mus musculus phorbol-12-myristate-13-acetate-induced protein 1 (Pmaip1), mRNA [NM_021451]
1	7	5	
0.00041	3.07149	3.07108	Mus musculus radical S-adenosyl methionine domain containing 2 (Rsad2), mRNA [NM_021384]
2	8	7	
2.19280	3.69013	1.49733	Mus musculus interferon regulatory factor 7 (Irf7), mRNA [NM_016850]
1	8	7	
0.18439	3.42337	3.23898	Mus musculus 14-3-3 protein sigma mRNA, complete cds. [AF058798]
5	7	1	
4.88698	8.08068	3.19369	Mus musculus phospholipase A2, group IIE (Pla2g2e), mRNA [NM_012044]
3	1	8	
0.13807	1.61952	1.48144	Mus musculus carbonyl reductase 2 (Cbr2), mRNA [NM_007621]
3		7	
2.09211	4.42134	2.32922	Mus musculus carbonic anhydrase 9 (Car9), mRNA [NM_139305]
3	1	8	
0.54221	2.02126	1.47904	Mus musculus phosphoinositide-3-kinase, catalytic, gamma polypeptide (Pik3cg), mRNA [NM_020272]
7	1	3	
0.98795	2.11994	1.13198	Mus musculus Max dimerization protein 3 (Mxd3), mRNA [NM_016662]
4		6	
1.39462	2.85514	1.46051	Mus musculus mevalonate (diphospho) decarboxylase (Mvd), mRNA [NM_138656]
9	3	4	
0.32899	2.45308	2.12409	Mus musculus E74-like factor 3 (Elf3), mRNA [NM_007921]
5	6	1	
0.15375	1.50070	1.34695	Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4930502N02 product:hypothetical Adenylate kinase/Glutamic acid-rich region containing protein, full insert sequence. [AK019664]
7	7		
0.42902	2.19616	1.76713	Mus musculus mal, T-cell differentiation protein-like (Mall), mRNA [NM_145532]
3	2	9	
0.89532	1.97865	1.08332	Mouse argininosuccinate synthetase (Ass) mRNA, complete cds. [M31690]
2		8	
0.53956	2.78453	2.24497	Mus musculus 18 days pregnant adult female placenta and extra embryonic tissue cDNA, RIKEN full-length enriched

	3	4		library, clone:3830421F03 product:hypothetical protein, full insert sequence. [AK014446]
0.39691	2.23221		1.8353	Mus musculus cytochrome P450, family 2, subfamily a, polypeptide 5 (Cyp2a5), mRNA [NM_007812]
4	5			
0.31935	2.26455		1.9452	Mus musculus cytochrome P450, family 2, subfamily a, polypeptide 5 (Cyp2a5), mRNA [NM_007812]
2	2			
1.83558	2.96838		1.1328	Mus musculus radical S-adenosyl methionine domain containing 2 (Rsdad2), mRNA [NM_021384]
6	6			
2.82408	5.25656		2.43248	Mus musculus mucin 4 (Muc4), mRNA [NM_080457]
4	6		2	
1.28899	5.15555		3.86656	Mus musculus mucin 4 (Muc4), mRNA [NM_080457]
6	7		1	
1.65971	2.99954		1.33982	Mus musculus basic helix-loop-helix domain containing, class B2 (Bhlhb2), mRNA [NM_011498]
6	2		7	
1.14474	2.34142		1.19668	Mus musculus latent transforming growth factor beta binding protein 2 (Ltpb2), mRNA [NM_013589]
9	9			
0.07204	1.64478		1.57274	Mus musculus breast carcinoma amplified sequence 1 (Bcas1), mRNA [NM_029815]
1	1		1	
0.48883	1.52025		1.03141	Mus musculus SEC14-like 2 (S. cerevisiae) (Sec14l2), mRNA [NM_144520]
9	4		5	
0.01096	1.31112		1.30016	Mus musculus acireductone dioxygenase 1 (Adt1), mRNA [NM_134052]
3	3			
1.34124	2.51124		1.17000	Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4930534K13 product:hypothetical protein, full insert sequence. [AK015966]
3	7		3	
0.10547	1.82275		1.71728	Mus musculus myosin, heavy polypeptide 6, cardiac muscle, alpha (Myh6), mRNA [NM_010856]
8	9			
1.59453	2.65556		1.06102	Mus musculus RIKEN cDNA 1500005I02 gene (1500005I02Rik), mRNA [NM_028055]
7	5		8	
0.83934	2.26314		1.42379	Mus musculus leucine rich repeat containing 18 (Lrrc18), mRNA [NM_026253]
7	3		6	
0.75656	2.09312		1.33656	Mus musculus leucine rich repeat containing 18 (Lrrc18), mRNA [NM_026253]
4	4			
0.74462	2.59780		1.85317	Mus musculus plakophilin 1 (Pkp1), mRNA [NM_019645]
5	4		9	
0.87868	2.39545		1.51677	Mus musculus expressed sequence Al451617 (Al451617), mRNA [NM_199146]
5	7		2	
0.89609	2.04512		1.14903	Mus musculus leucine rich repeat containing 48 (Lrrc48), mRNA [NM_029044]

1	5	4	
0.56889	1.81237	1.24348	Mus musculus tripartite motif protein 6 (Trim6), mRNA [NM_001013616]
1	4	3	
0.01388	1.20871	1.19482	Mus musculus prostaglandin F2 receptor negative regulator (Ptgfrn), mRNA [NM_011197]
9	5	7	
0.56833	1.71878	1.15044	Mus musculus translin-associated factor X (Tsnax) interacting protein 1 (Tsnaxip1), mRNA [NM_024445]
6	4	9	
0.25484	1.43852	1.18368	Mus musculus vascular endothelial growth factor A (Vegfa), transcript variant 2, mRNA [NM_009505]
1	1		
1.18436	4.05363	2.86927	Mus musculus leucine-rich alpha-2-glycoprotein 1 (Lrg1), mRNA [NM_029796]
1	3	1	
0.80129	1.93275	1.13145	Mus musculus protein phosphatase 1J (Ppm1j), mRNA [NM_027982]
5		5	
1.92251	3.04164	1.11913	Mus musculus 2'-5' oligoadenylate synthetase 1A (Oas1a), mRNA [NM_145211]
5	8	3	
1.74946	3.05325	1.30378	Mus musculus myxovirus (influenza virus) resistance 1 (Mx1), mRNA [NM_010846]
5		5	
0.40164	1.69384	1.29219	Mus musculus myosin, heavy polypeptide 7, cardiac muscle, beta (Myh7), mRNA [NM_080728]
9	8	8	
0.6087	2.80516	2.19646	Mus musculus deleted in colorectal carcinoma (Dcc), mRNA [NM_007831]
2		3	
0.42941	1.52609	1.09668	Mus musculus ribonuclease P 25 subunit (human) (Rpp25), mRNA [NM_133982]
4		4	
0.54398	1.78167	1.23769	Mus musculus myosin, heavy polypeptide 7, cardiac muscle, beta (Myh7), mRNA [NM_080728]
2	3	2	
0.67032	1.84290	1.17257	Mus musculus wingless-related MMTV integration site 4 (Wnt4), mRNA [NM_009523]
9	1	2	
0.39123	1.92110	1.52986	Mus musculus amyloid beta (A4) precursor protein-binding, family A, member 1 binding protein (Apba2bp), mRNA [NM_021546]
4	2	8	
1.92662	3.11476	1.18813	Mus musculus NADPH oxidase organizer 1 (Noxo1), mRNA [NM_027988]
4	1	7	
1.14497	2.34236	1.19738	Mus musculus sperm associated antigen 16 (Spag16), transcript variant 2, mRNA [NM_025728]
5	2	7	
0.44291	1.75314	1.31022	Mus musculus DnaJ (Hsp40) homolog, subfamily A, member 4 (Dnaja4), mRNA [NM_021422]
8	6	8	
2.20769	3.43960	1.23191	Mus musculus transient receptor potential cation channel, subfamily V, member 6 (Trpv6), mRNA [NM_022413]

	5	9	4	
0.31507	2.04586	1.73079		Mus musculus ubiquitin-conjugating enzyme E2L 6 (Ube2l6), mRNA [NM_019949]
1	9	8		
0.43152	2.15080	1.71928		Mus musculus adult male colon cDNA, RIKEN full-length enriched library, clone:9030414N23 product:similar to Tripartite motif protein 30 (Down regulatory protein of interleukin 2 receptor) [Mus musculus], full insert sequence. [AK136456]
3	4	1		
0.07733	1.67761	1.60027		Mus musculus CDC42 effector protein (Rho GTPase binding) 2 (Cdc42ep2), mRNA [NM_026772]
9	7	8		
1.05079	2.92015	1.86936		Mus musculus hepatocyte growth factor activator (Hgfac), mRNA [NM_019447]
6	7	2		
0.54385	1.86123	1.31737		Mus musculus HIV-1 tat interactive protein 2, homolog (human) (Htatip2), mRNA [NM_016865]
7	2	5		
1.32548	2.51218	1.18669		Mus musculus tripartite motif protein 25 (Trim25), mRNA [NM_009546]
3	2	9		
0.13128	1.87823	1.74695		Mus musculus signal transducer and activator of transcription 1 (Stat1), mRNA [NM_009283]
3	3			
0.20397	2.24944	2.04547		Mus musculus gap junction membrane channel protein beta 2 (Gjb2), mRNA [NM_008125]
2	8	6		
1.31888	2.61461	1.29572		Mus musculus plasminogen activator, urokinase receptor (Plaur), mRNA [NM_011113]
8	4	7		
1.35561	2.75789	1.40227		Mus musculus hematopoietic SH2 domain containing (Hsh2d), mRNA [NM_197944]
6	1	6		
3.38325	4.55255	1.16929		Mus musculus CUB and zona pellucida-like domains 1 (Cuzd1), mRNA [NM_008411]
7	4	7		
0.20244	1.63462	1.43217		Mus musculus mitogen activated protein kinase 13 (Mapk13), mRNA [NM_011950]
9	5	7		
0.22932	1.27147	1.04214		Mus musculus CCAAT/enhancer binding protein (C/EBP), delta (Cebpd), mRNA [NM_007679]
8	2	4		
1.20630	2.48884	1.28254		Mus musculus aryl hydrocarbon receptor-interacting protein-like 1, mRNA (cDNA clone MGC:25485 IMAGE:4501401), complete cds. [BC028285]
3	8	5		

Table S2. Statistical analyses of data in this study**Fig 1C****GM1**

Exp. 1	Lcn2 ^{-/-}	Lcn2 ^{+/+}
hazy	31	4
apical ridge	5	4
entire head	1	23
total	37	31
(%) eh	2.7	76.6

Exp. 2	Lcn2 ^{-/-}	Lcn2 ^{+/+}
hazy	11	5
apical ridge	17	2
entire head	2	24
total	30	31
(%) eh	2.7	77.4

Exp. 3	Lcn2 ^{-/-}	Lcn2 ^{+/+}
hazy	22	2
apical ridge	10	0
entire head	0	30
total	32	32
(%) eh	0	93.7

Exp. 4	Lcn2 ^{-/-}	Lcn2 ^{+/+}
hazy	22	8
apical ridge	10	9
entire head	0	17
total	32	34
(%) eh	0	50

Exp. 5	Lcn2 ^{-/-}	Lcn2 ^{+/+}
hazy	11	5
apical ridge	13	11
entire head	6	14
total	30	30

(%) eh	20	46.6
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	Lcn2-/-	Lcn2+/+
total		
hazy	97	24
apical ridge	55	26
entire head	9	108
total	161	158
(%) eh	5.5	68.3

	Lcn2-/-	Lcn2+/+
Exp. 1	2.7	76.6
Exp. 2	2.7	77.4
Exp. 3	0	93.7
Exp. 4	0	50
Exp. 5	20	46.6
average	5.1	68.8
±SD	5.3	11.5
T-TEST		0.000922727

Fig 1D

EGFP(K268Q)-GPI

Exp. 1	Lcn2-/-	Lcn2+/+
bright	29	8
shed	1	22
total	30	30
(%) shed	3.3	73.3

Exp. 2	Lcn2-/-	Lcn2+/+
bright	27	1
shed	5	27
total	32	28
(%) shed	15.6	96.4

Exp. 3	Lcn2-/-	Lcn2+/+
bright	31	2
shed	1	30
total	32	32

(%) shed	3.1	93.7
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Exp. 4	Lcn2-/-	Lcn2+/+
bright	32	17
shed	5	17
total	37	34
(%) shed	13.5	50

Exp. 5	Lcn2-/-	Lcn2+/+
bright	24	15
shed	6	15
total	30	30
(%) shed	20	50

total	Lcn2-/-	Lcn2+/+
bright	143	43
shed	18	111
total	161	154
(%) shed	11.1	72

	Lcn2-/-	Lcn2+/+
Exp. 1	3.3	73.3
Exp. 2	15.6	96.4
Exp. 3	3.1	93.7
Exp. 4	13.5	50
Exp. 5	20	50
average	11.1	72.6
±SD	7.5	12.6
T-TEST		0.002326718

Fig 2A

GM1

Total sperm examined	LCN2 (-)	LCN2 (+)
hazy	97	6
apical ridge	106	71
entire head	7	161
total	210	238

%entire head	3.3	67.6
%entire head	LCN2 (-)	LCN2 (+)
Exp. 1	1.8	68.9
Exp. 2	1.9	67.4
Exp. 3	5.9	65.5
average	3.2	67.26666667
±SD	2.338803113	1.703917056
T-TEST		2.76192E-06

Fig 2B

Izumo1

Total sperm examined	LCN2 (-)	LCN2 (+)
apical ridge	192	195
entire head	26	38
total	218	233
%entire head	11.9	16.3

%entire head	LCN2 (-)	LCN2 (+)
Exp. 1	17.4	18.9
Exp. 2	17.8	18.3
Exp. 3	3.6	19.5
Exp. 4	9.8	7
average	12.15	15.925
±SD	6.785032547	5.970134002
T-TEST	P=	0.435492625

Fig 2C

GM1

Total sperm examined	(-)	A23187	LCN2	LCN2+A23187
hazy	57	9	44	3
apical ridge	116	146	100	73
entire head	23	36	58	133
total	196	191	202	209
%entire head	11.7	18.8	28.7	63.6
%entire head	(-)	A23187	LCN2	LCN2+A23187

Exp. 1	13.1	29.7	29.2	86.6
Exp. 2	11.1	13.4	24.6	51.9
Exp. 3	11.1	13.3	32.3	53.8
average	11.76666667	18.8	28.7	64.1
±SD	1.154700538	9.43980932	3.874274126	19.508716
T-TEST	P=	0.26943512	0.001916553	0.009747019
ANOVA				P<0.05

Fig 2D

Izumo1

Total sperm examined	(-)	A23187	LCN2	LCN2+A23187
apical ridge	162	161	166	159
entire head	52	42	39	114
total	214	203	205	273
%entire head	24.3	20.7	19	41.8

%entire head	(-)	A23187	LCN2	LCN2+A23187
Exp. 1	21.7	22	23.1	42.3
Exp. 2	24.7	19.3	18	35.6
Exp. 3	26.4	20.3	16	47.1
average	24.26666667	20.53333333	19.03333333	41.66666667
±SD	2.3797759	1.365039682	3.661056314	5.776100184
T-TEST	P=	0.077919172	0.106518839	0.00849676
ANOVA				P<0.003

Fig 2E

Total sperm examined	LCN2 (-)	LCN2 (+)	LCN2 (+) +PKAi	LCN2 (+) + PI3-Ki
hazy	107	18	174	42
apical ridge	108	91	42	59
entire head	14	116	22	138
total	229	225	238	239
%entire head	6.1	51.6	9.2	57.7

% entire head	LCN2 (-)	LCN2 (+)	LCN2 (+) +PKAi	LCN2 (+) + PI3-Ki
Exp. 1	8.5	62.9	8.5	68.6
Exp.2	4.3	57.7	11.4	48.8
Exp. 3	5.7	36.9	8	57.3

average	6.166666667	52.5	9.3	58.233333333
± SD	2.138535324	13.75790682	1.835755975	9.932941827
T-TEST	P=	0.004495697		0.000890124
		P=	0.005726893	
ANOVA				P<0.001

Fig 2F

cholesterol in the sup

mg/ml	(-)	LCN2	BSA	M-β -CD
Exp. 1	0.069	0.227	0.344	1.049
Exp. 2	0.086	0.368	0.316	0.475
Exp. 3	0.069	0.25	0.312	0.497
average	0.074666667	0.281666667	0.324	0.673666667
± SD	0.009814955	0.075646106	0.017435596	0.325234275
T-TEST	P=	0.009306808	2.72547E-05	0.033262197
ANOVA				P<0.0001

Fig 3A

		LCN2 (-)	LCN2 (+)
Exp. 1	No. of 2 cell embryos	2	15
	No. of total eggs	24	34
	(%) fertilization	8.3	44.1
Exp. 2	No. of 2 cell embryos	4	33
	No. of total eggs	37	42
	(%) fertilization	10.8	78.6
Exp. 3	No. of 2 cell embryos	8	20
	No. of total eggs	56	36
	(%) fertilization	14.3	55.6
Exp. 4	No. of 2 cell embryos	6	15
	No. of total eggs	20	29
	(%) fertilization	30	51.7
Exp. 5	No. of 2 cell embryos	5	12
	No. of total eggs	23	30
	(%) fertilization	21.7	40

		BSA	M- β -CD
Exp. 1	No. of 2 cell embryos	48	37
	No. of total eggs	49	37
	(%) fertilization	98	100
Exp. 2	No. of 2 cell embryos	29	25
	No. of total eggs	31	25
	(%) fertilization	93.6	100
Exp. 3	No. of 2 cell embryos	25	30
	No. of total eggs	28	34
	(%) fertilization	89.3	88.2
Exp. 4	No. of 2 cell embryos	22	27
	No. of total eggs	23	32
	(%) fertilization	95.7	84.4

(%) fertilization	LCN2 (-)	LCN2 (+)	BSA	M- β -CD
Exp. 1	8.3	44.1	98	100
Exp. 2	10.8	78.6	93.6	100
Exp. 3	14.3	55.6	89.3	88.2
Exp. 4	30	51.7	95.7	84.4
Exp. 5	21.7	40	ND	ND
average	17.02	54	94.15	93.15
$\pm SD$	8.838947901	15.05838637	3.699098989	8.060397013
T-TEST	P=	0.001472043	8.38601E-07	3.1321E-06
ANOVA				P<0.0001

Fig 4B

Total sperm examined	LCN2 binding (-)	LCN2 binding (+)
hazy	152	0
apical ridge	14	27
entire head	0	130
total	166	157
%entire head	0	82.8
% entire head	LCN2 binding (-)	LCN2 binding (+)

Exp. 1	0	87.5
Exp. 2	0	82.4
Exp. 3	0	78
average	0	82.63333333
±SD	0	4.754296303
T-TEST	P=	7.25178E-06

Fig 4C

% LCN2-bound sperm	10min	30min	60min	90min	120min
Exp. 1	0	0	23.1	30.7	43.5
Exp. 2	0	2	29.4	42.9	50.2
Exp. 3	0	4.4	27.9	48.1	51.3
average	0	2.133333333	26.8	40.56666667	48.33333333
±SD	0	2.203028219	3.290896534	8.93159187	4.221768982
T-TEST	P=	0.168797546	0.000146627	0.001411088	3.81568E-05
Total sperm examined	210	223	234	287	271

Fig 4D

Total sperm examined	10min	30min	60min	90min	120min
hazy	164	98	49	11	6
apical ridge	16	86	111	96	71
entire head	1	6	43	114	161
total	181	190	203	221	238
%entire head	0.55	3.2	21.2	51.2	67.6

% entire head	10min	30min	60min	90min	120min
Exp. 1	0	3.8	22.4	36.7	68.9
Exp. 2	0	2.9	31.9	56.1	67.4
Exp. 3	1.6	2.9	9	58.2	65.5
average	0.533333333	3.2	21.1	50.33333333	67.26666667
±SD	0.923760431	0.519615242	11.50521621	11.8534102	1.703917056
T-TEST	P=	0.01208221	0.036706994	0.001916569	4.7351E-07

Fig 5F

cinnamycin binding

Total sperm examined	LCN2 (-)	LCN2 (+)
cinnamycin (+)	136	55

cinnamycin (-)	43	130
total	179	185
%cinnamycin (-) sperm	24	70.3

%cinnamycin (-) sperm	LCN2 (-)	LCN2 (+)
Exp. 1	30.9	55.7
Exp. 2	20	82
Exp. 3	21.9	73
average	24.26666667	70.23333333
± SD	5.822656896	13.36650041
T-TEST	P=	0.005467361

Supplementary Fig S4

EGFP(K268Q)-GPI

Total sperm examined	LCN2 (-)	LCN2 (+)
bright	161	175
shed	5	9
total	166	184
%shed	3	4.9

%shed	LCN2 (-)	LCN2 (+)
Exp. 1	1.9	1.5
Exp. 2	1.9	4.8
Exp. 3	5.1	8.9
average	2.966666667	5.066666667
± SD	1.847520861	3.707200201
T-TEST	P=	0.429451158