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Supplementary Information

Supplementary Figure 1-6

Supplementary Table 1-3

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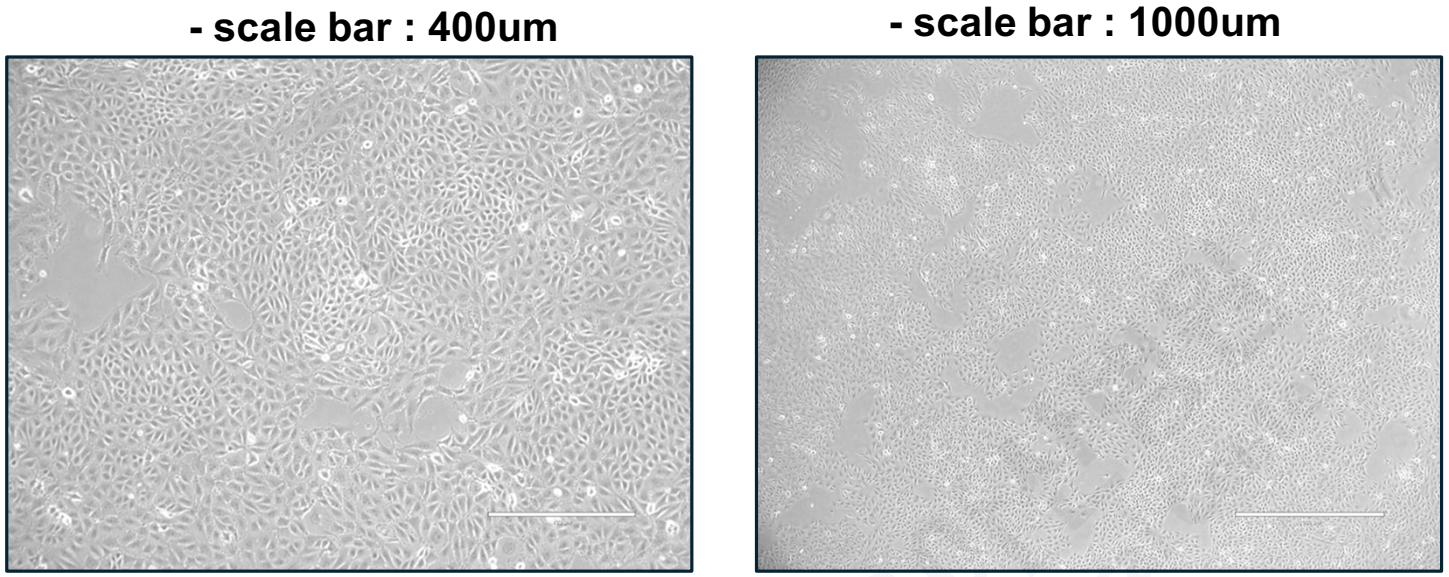
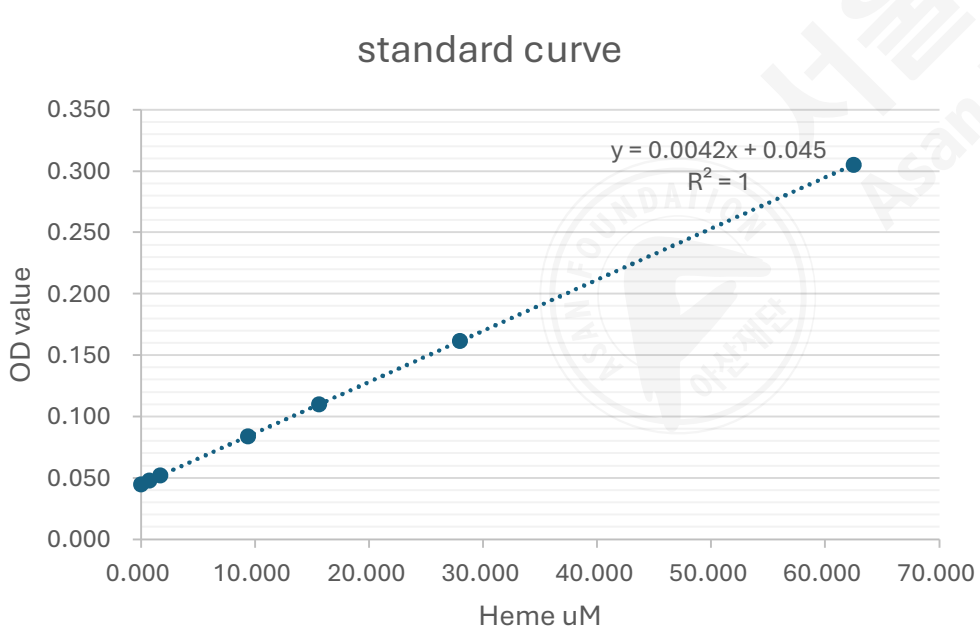
Figure S1

A



B



Supplementary Figure 1. Culture of PK15 cells and measurement of heme in bacterial extract to establish heme adaptation condition. **A.** Representative pictures of PK15 under 2% of FBS. **B.** Standard Curve for the quantitation of Heme in bacterial extract. See methods for details.

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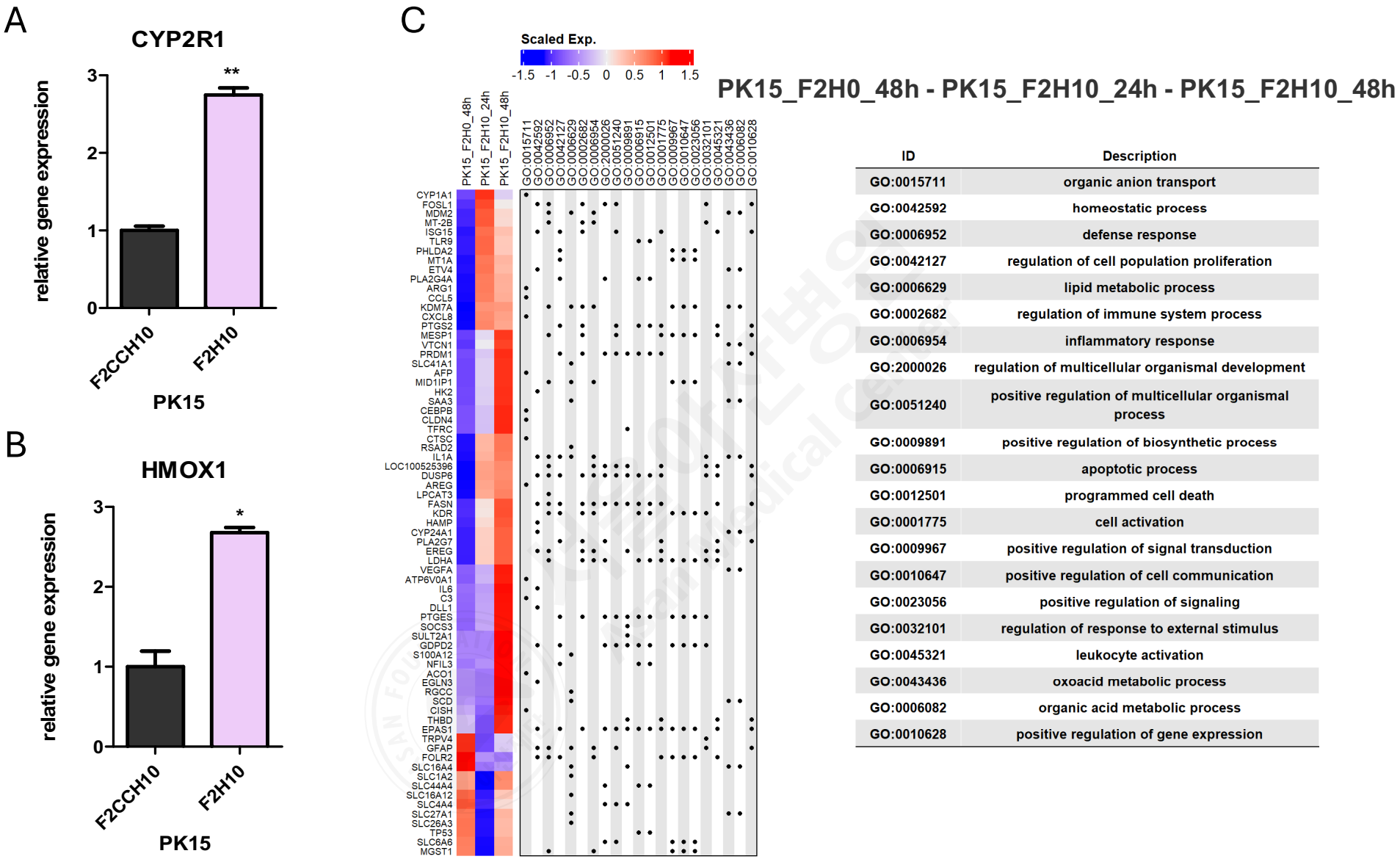
Figure S2



ID	Description
GO:0015711	organic anion transport
GO:0042592	homeostatic process
GO:0006952	defense response
GO:0042127	regulation of cell population proliferation
GO:0006629	lipid metabolic process
GO:0002682	regulation of immune system process
GO:0006954	inflammatory response
GO:2000026	regulation of multicellular organismal development
GO:0051240	positive regulation of multicellular organismal process
GO:0009891	positive regulation of biosynthetic process
GO:0006915	apoptotic process
GO:0012501	programmed cell death
GO:0001775	cell activation
GO:0009967	positive regulation of signal transduction
GO:0010647	positive regulation of cell communication
GO:0023056	positive regulation of signaling
GO:0032101	regulation of response to external stimulus
GO:0045321	leukocyte activation
GO:0043436	oxoacid metabolic process
GO:0006082	organic acid metabolic process
GO:0010628	positive regulation of gene expression

Supplementary Figure 2. RNA-seq analysis of PK15 cells treated with Heme extract show distinct gene expression alteration. **A and B**. Validation of expression changes of CYP2R1(A) or HMOX1(B) upon the treatment of Heme extracts (marked as F2H10, pink bar). As a control, the bacterial extract without heme (marked as F2CCH10) is shown (black bar). **C.** Summary of GO analysis for the time-course treatment of Heme extracts in PK15 cells. Left panel shows heat map of key genes altered during the heme treatment time (24 and 48 hrs) presented with corresponding functions in GO analysis. The description for selected GO is shown in Table on right.

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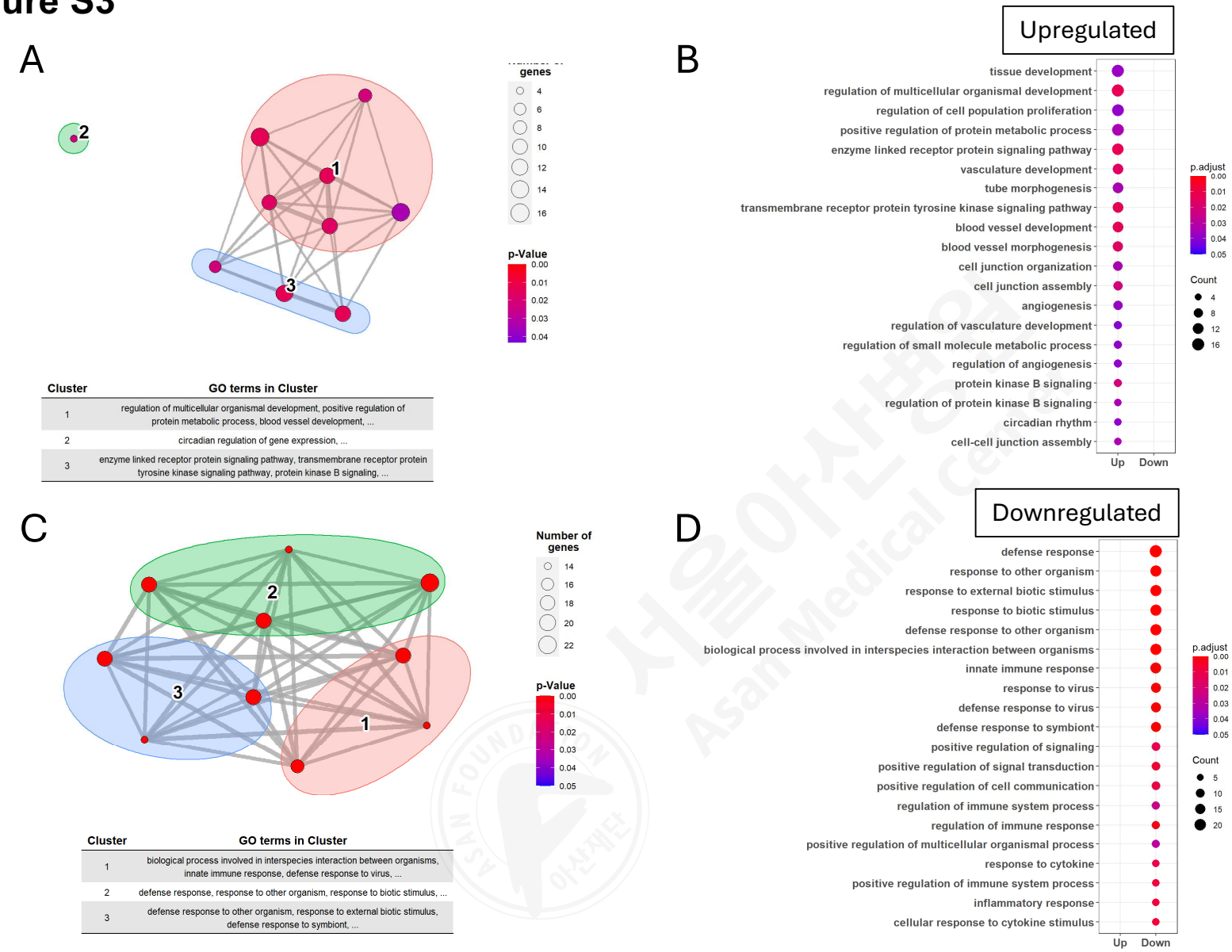
Figure S3



Supplementary Figure 3. Clustering analysis of gene expression changes under transient heme treatment in PK15 cells. **A.** Representative upregulated three clusters and its GO terms. The interaction among genes are marked as a grey lines. The size of dots indicate number of genes and color shows p-value. **B.** Upregulated biological functions enriched in the GO analysis of PK15 cells with heme treatment. The size of dots indicate the number of genes involved and color shows p-values. **C.** Representative down-regulated three clusters and its GO terms. The interaction among genes are marked as a grey lines. The size of dots indicate number of genes and color shows p-value. **D.** Down-regulated biological functions enriched in the GO analysis of PK15 cells with heme treatment. The size of dots indicate the number of genes involved and color shows p-values.

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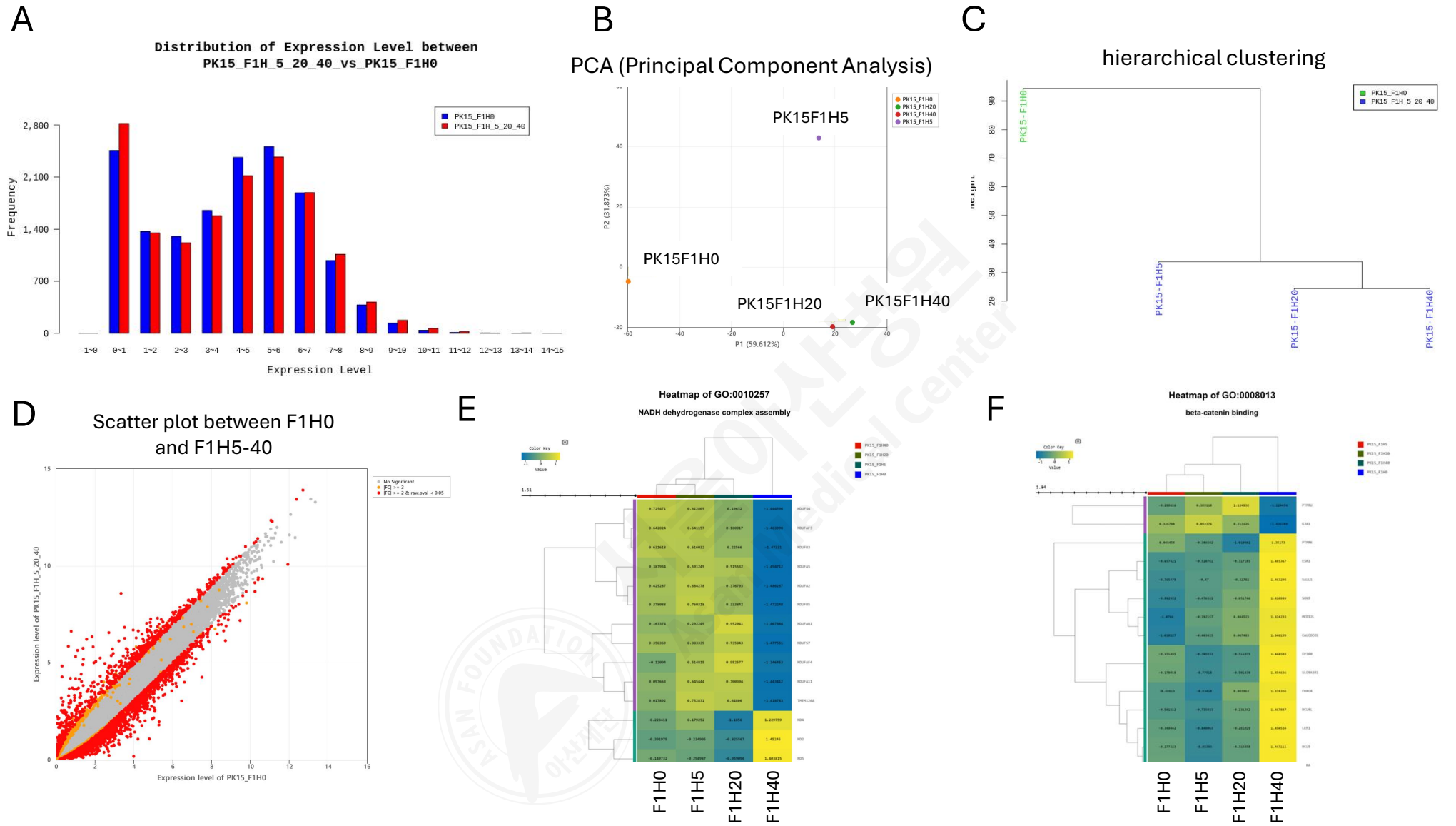
Figure S4



Supplementary Figure 4. RNA seq analysis of the heme-adapted PK15 cells (PK15H) **A**. Distribution of expression level between control (F1H10, blue bars) and Heme treated (F1H5-40, red bars) PK15H cells. **B**. PCA plot analysis of control (F1H0, Orange) and heme treated (F1H5, 20, 40 in purple, green and red dots respectively). **C**. Hierarchical clustering result of the heme treated PK15 cells. Control (F1H0, in green) and heme treated (F1H5, 20, 40, in blue) are marked. **D**. Scatter plot showing the expression correlation between control (F1H10, x axis) and Heme treated (F1H5-40, y axis) PK15H cells. Red dots are indicate significantly altered genes, **E and F.** A representative GO analysis result indicating the upregulation of NADH dehydrogenase complex assembly (E) or down-regulation of beta-catenin binding (F) in PK15H cells. The name of each gene is marked on right end and the fold change of expression is indicated by color code, with numbers in each box.

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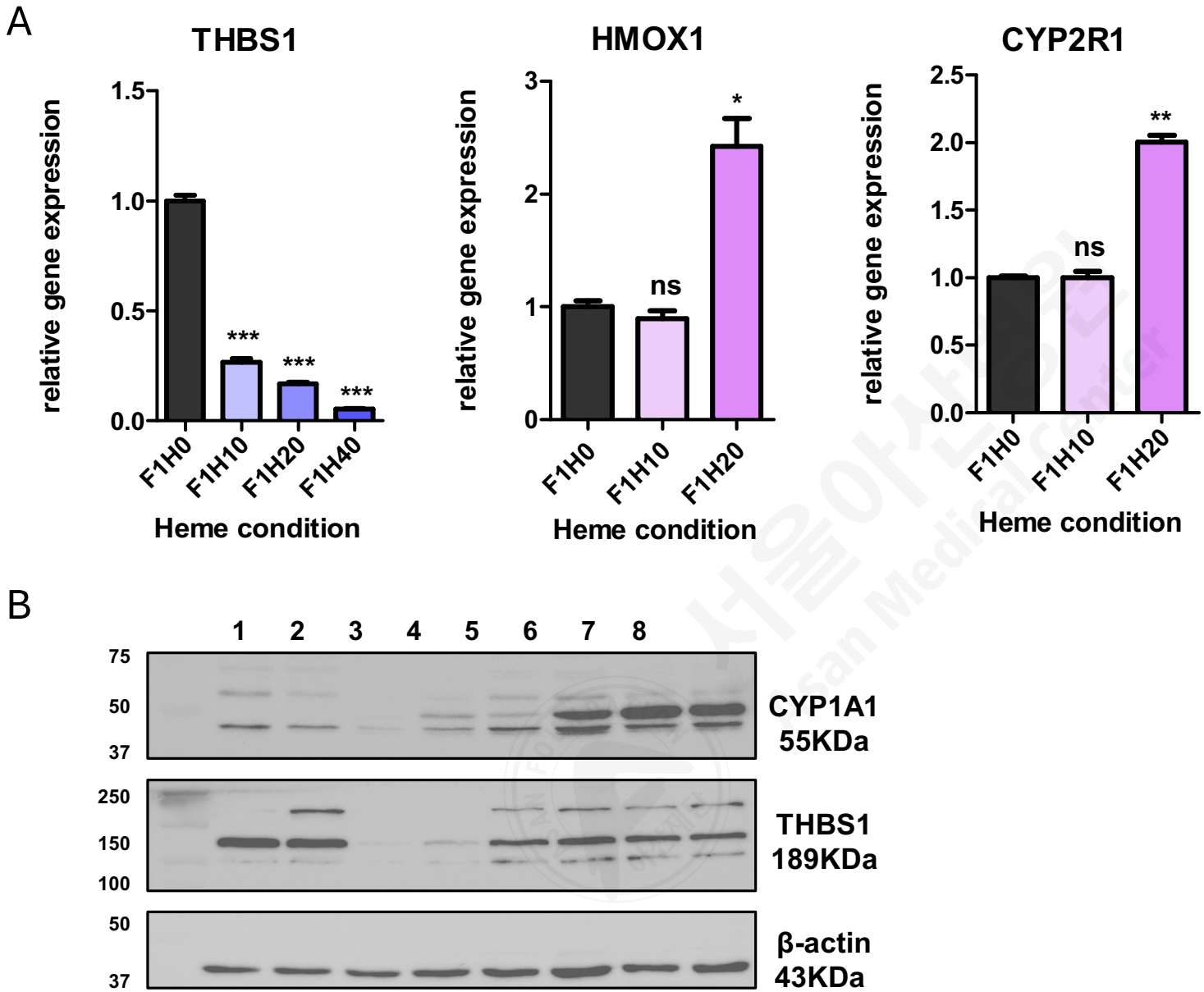
Figure S5



Supplementary Figure 5. Validation of altered gene expression in the Heme adapted PK15H cells. **A.** Real-time PCR results of THBS1, HMOX and CYP2R1 in control (F1H10, black bar) or heme adapted (F1H10, 20 40) cells. ns; not significant, * $p<0.05$, ** $p<0.005$, *** $p<0.001$. **B.** Western blot results for the CYP1A1, THBS1 and Actin. Lane information ; 1. HeLa – CYP1A1 Positive Control, 2. MDA MB 231-THBS1 Positive Control, 3. PK15 F1, 4. PK15 F1 H5, 5. PK15 F1 H20, 6. PK15 F1 H40, 7. PK15 F0 H5, 8. PK15 F0 H10.

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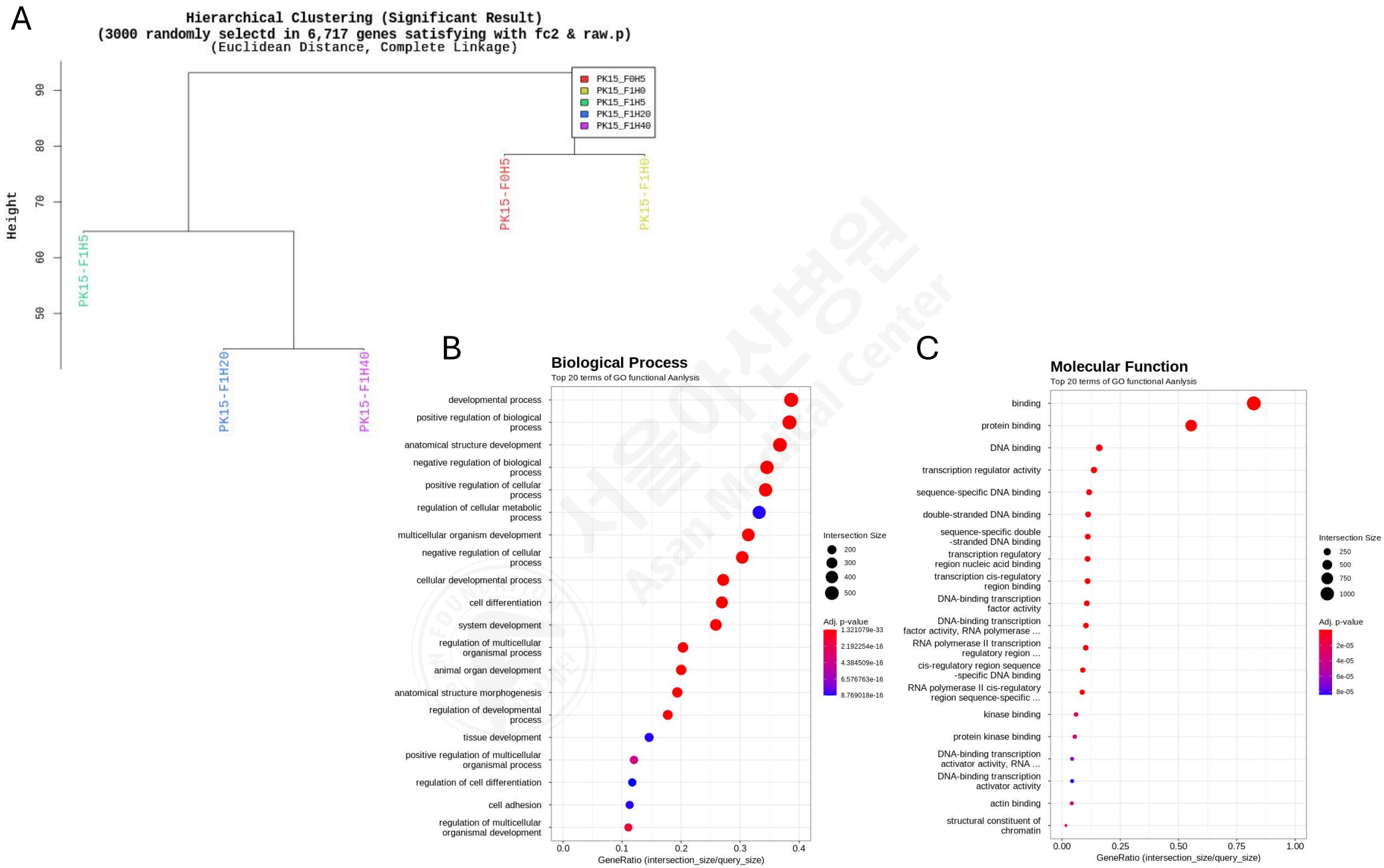
Figure S6



Supplementary Figure 6. RNA seq analysis of serum free adapted PK15H cells. **A.** Hierarchical clustering analysis of the serum free adapted PK15H cells (F0H5, in red) compared with control (F1H0, in yellow) or PK15H cells. **B and C.** A representative result of biological process (B) or Molecular function (C) that is altered during the serum free adaptation of PK15H cells. Top 20 items are listed on left of each panel and the size of dots indicate number of genes involved. The color of dots indicates p-value.

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Supplementary Table 1. List of top differentially expressed genes in PK15H cells under serum free condition supplemented with heme extracts

Transcript_ID	Gene_ Symbol	Description	PK15_F0H5/ PK15_F1H5.fc	PK15_F0H5/ PK15_F1H5.logCPM	PK15_F0H5/ PK15_F1H5.raw.pval	PK15_F0H5/ PK15_F1H5.bh.pval	PK15_F1H5. mean	PK15_F0H5. mean
NM_214009,XM_021080819	C3	complement C3	77.066497	6.171716	4.793E-118	7.241E-114	1.508482	7.163357
XM_003127647,XM_021095426	PADI1	peptidyl arginine deiminase 1, transcript variant X2	283.764993	3.621244	2.24E-101	1.6924E-97	0.117042	4.674612
NM_213774,XM_021080276,XM_021080278,XM_021080282	CD74	CD74 molecule, major histocompatibility complex, class II invariant chain	94.870023	2.267426	9.9287E-65	5.0004E-61	0.135658	3.397643
XM_021085301,XM_021085302,XM_021085303,XM_021085304,XM_021085305,XM_021085306,XM_021085307,XM_021085308,XM_021085309,XM_021085310,XM_021085311,XM_021085312,XM_021085313,XM_021085314,XM_021085315,XM_021085316	NAV2	neuron navigator 2, transcript variant X16	16.544614	5.561055	4.1524E-64	1.5685E-60	2.673643	6.492607
NM_001114062,NM_001130224,XM_005665844	SLA-DQA1	MHC class II histocompatibility antigen SLA-DQA	21.873436	3.653308	6.2962E-62	1.9026E-58	1.069435	4.647983
XM_005670100,XM_021070120,XM_021070121,XM_021070122,XM_021070123,XM_021070124,XM_021070125,XM_021070126,XM_021070127,XM_021070128,XM_021070129,XM_021070130,XM_021070131,XM_021070132,XM_021070133,XM_021070134,XM_021070135	PIGZ	phosphatidylinositol glycan anchor biosynthesis class Z, transcript variant X2	128.187241	1.911114	9.4082E-61	2.3691E-57	0.079073	3.082869
XM_001926072,XM_013989002,XM_013989003,XM_021098858,XM_021098859,XM_021098861,XM_021098862	ARNT2	aryl hydrocarbon receptor nuclear translocator 2, transcript variant X5	16.798796	4.027541	8.4217E-58	1.8178E-54	1.501322	4.990506
XM_021069364,XM_021069365	PLXND1	plexin D1, transcript variant X2	17.813055	3.495183	1.2097E-55	2.2846E-52	1.135670	4.482788
NM_001267694,XM_013991587,XM_021068574	CP	ceruloplasmin	33.858171	2.230803	1.0517E-53	1.589E-50	0.342112	3.339944
XM_013994444,XM_021083199,XM_021083200	KIAA1549L	KIAA1549 like, transcript variant X1	27.684400	2.453641	1.0174E-53	1.589E-50	0.464970	3.533655
XM_021094021,XM_021094022,XM_021094023	CDH16	cadherin 16, transcript variant X3	64.682232	1.721597	4.4556E-52	6.1199E-49	0.135658	2.907454
XR_001301430	LOC106506243	uncharacterized LOC106506243	10.289950	5.180499	1.4588E-47	1.8368E-44	2.891935	6.068394
XM_003129745,XM_003357234,XM_013979248,XM_013979249,XM_021062621,XM_021062622,XM_021062623	NOX4	NADPH oxidase 4, transcript variant X4	-18.220396	2.654489	4.7169E-46	5.4822E-43	3.680000	0.719578
XM_001925047	SERPINB7	serpin family B member 7	-16.677017	2.684923	5.3206E-45	5.7421E-42	3.702027	0.781197
gene-ND5	ND5	NADH dehydrogenase subunit 5	8.475249	7.662935	7.2039E-44	7.2562E-41	5.452050	8.505999
NM_214277,XM_005653523,XM_005653524,XM_021098304	ANPEP	alanyl aminopeptidase, membrane	31.361824	1.580816	1.6669E-43	1.5741E-40	0.242574	2.766031
XM_021091898	TSFM	Ts translation elongation factor, mitochondrial	9.938587	3.818296	3.6015E-42	3.2009E-39	1.838957	4.735223
NM_001044548,XM_021066256,XM_021066257,XM_021066258	NTN1	netrin-1	8.679659	5.128408	4.6379E-42	3.893E-39	3.040101	5.993899
NM_001244150,XM_005667370,XM_005667371	TAGLN	transgelin	10.104557	3.600623	1.0893E-41	8.6621E-39	1.670450	4.528423
XM_005671181	FAM25A	family with sequence similarity 25 member A	-8.931149	4.625820	2.5262E-41	1.9084E-38	5.501331	2.574837

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Supplementary Table 2. List of top enriched pathways in PK15H cells under serum free condition supplemented with heme extracts

term_id	term_name	adjusted_p_value	term_size	query_size	intersection_size	effective_domain_size	intersection_size_UP	intersection_size_DOWN
GO:0007275	multicellular organism development	**1.3211E-33**	3393	1363	428	19136	361	67
GO:0048856	anatomical structure development	**2.2573E-30**	4360	1363	501	19136	417	84
GO:0032502	developmental process	**7.248E-30**	4699	1363	527	19136	435	92
GO:0048731	system development	**9.7825E-26**	2827	1363	353	19136	302	51
GO:0048518	positive regulation of biological process	**1.7998E-23**	4924	1363	523	19136	428	95
GO:0048523	negative regulation of cellular process	**2.2981E-23**	3617	1363	414	19136	337	77
GO:0048869	cellular developmental process	**2.2981E-23**	3109	1363	370	19136	313	57
GO:0030154	cell differentiation	**3.5942E-23**	3084	1363	367	19136	311	56
GO:0005515	protein binding	**9.3091E-23**	7415	1313	728	17833	563	165
GO:0048522	positive regulation of cellular process	**2.0033E-22**	4306	1363	468	19136	386	82
GO:0048519	negative regulation of biological process	**1.5841E-21**	4384	1363	471	19136	372	99
GO:0051239	regulation of multicellular organismal process	**2.2398E-21**	2152	1363	277	19136	228	49
GO:0050793	regulation of developmental process	**6.3606E-21**	1796	1363	242	19136	206	36
GO:0009653	anatomical structure morphogenesis	**7.7755E-20**	2064	1363	264	19136	235	29
GO:0048513	animal organ development	**1.4796E-18**	2206	1363	273	19136	230	43
GO:2000026	regulation of multicellular organismal development	**1.1846E-16**	998	1363	151	19136	128	23
GO:0051240	positive regulation of multicellular organismal process	**3.9689E-16**	1139	1363	164	19136	136	28
GO:0031323	regulation of cellular metabolic process	**8.6713E-16**	4452	1363	453	19136	367	86
GO:0007155	cell adhesion	**8.6713E-16**	1051	1363	154	19136	136	18
GO:0009888	tissue development	**8.6713E-16**	1508	1363	199	19136	178	21

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Supplementary Table 3. List of primers used in this study

Target Gene	Sense	Sequence (5'to 3')	Annealing temperature (℃)
CYP1A1	Forward	CCATCCCTCACAGTACCACA	60
	Reverse	TTCACCCAGTGCCTTGTTGA	
RGCC	Forward	GCCACTTCCACTACGAGGAG	55
	Reverse	CGAGTTTGGCTTTCCGAGGA	
MESP1	Forward	AGTTGACGTGACGGTGGAAT	60
	Reverse	CCTGCTTGCCTCAAAGTGTT	
THBS1	Forward	CTCAGGCCTTTCTGTGAAGG	60
	Reverse	CACCACACGAATGAAACCTG	
S100A10	Forward	GCCGTCTCAAATGGAACATG	60
	Reverse	CACTGGTCCAGGTCCTTCAT	
ATP6V0A1	Forward	TAGCACTGGGCACAGCATAC	65
	Reverse	GGAAAGGTCTCCAGGTCTGC	
PCSK1N	Forward	AAACGTCTAGAGAACCCCGC	63
	Reverse	TATTGTAGGGTCCGGAGTGG	
MAFF	Forward	TCTGGTGAGATAGGAGGTTG	60
	Reverse	CACAGATGGGGCTGTATCCT	
GPX5	Forward	CTGTCCTCACCCTTCTGAGC	65
	Reverse	GAAGCAGGATGGCTTCTGTC	
CYP2R1	Forward	GTATCCATGAGGACGCAGGT	60
	Reverse	GCCAGGAGTTGAATCAGAGC	
LPO	Forward	CCACCTTGACCTCTCAGCTC	65
	Reverse	GCCTCAGCCAAGACTTGTTC	
HMOX1	Forward	CTTGCCCTCTGCATTCTTTC	60
	Reverse	AGCTGGACAGAGATGCTGGT	