Supplementary material

Figures S1 to S9 and Table S1.

Figure S1: Real-time recording of circadian oscillation in human mammary epithelial cells upon circadian clock resetting by three different methods. (A) MCF10A were transduced with pLV7-Bsd-p(Bmal1)-dLuc and synchronised using either serum shock (redline), dexamethasone (DEX, black line) or forskolin (FSK, grey line). Bioluminescence was recorded every 10 minutes for 90h. (B) Similar experimental design on MCF12A, C) on HME-1 cells and (D) on HMECs. (E) Summary of oscillation properties for each cell line and synchronisation protocol.

Figure S2: Ranking score of reference genes. Reference gene rankings for each cell line and for each of the four methods used by RefFinder software were compiled to obtain a ranking score for each reference gene. The reference genes were ranked according to their ranking score from left to right. The ranking score for each reference gene is the sum of the rank values for each cell line for a given method.

Figure S3: Distribution of Cq values of candidate reference genes after dexamethasone or forskolin synchronisation. (A) Expression is represented as the qPCR quantification cycle value (Cq) obtained from qPCR in MCF10A (green), MCF12A (blue), HME-1 (red) and HMEC (white) cell lines after synchronisation by dexamethasone (DEX)**.** Variability is displayed as medians (line in the box), 25th and 75th percentiles (box) and min to max (whiskers). A total of 13 different samples were used for MCF10A, 11 for MCF12A, 11 for HME-1 and 14 for HMEC (Table S1). Gene symbols are detailed in Table 1. (B) Similar experiment using forskolin (FSK) to synchronise cells for the circadian rhythm. A total of 15 different samples were used for MCF10A, 13 for MCF12A, 12 for HME-1 and 16 for HMEC (Table S1).

Figure S4: Expression stabilities and ranking of reference genes according to delta Ct method, GeNorm, NormFinder and BestKeeper in dexamethasone treated cells. The Y- axis represents the

stability value as calculated by each of the four methods (SD for delta Ct method, M-value for GeNorm, σ-value for NormFinder and SD for BestKeeper). Green, blue, red and black colours are specific of each cell lines respectively MCF10A, MCF12A, HME-1 and HMEC. To calculate the stability value, a total of 13 different samples were used for MCF10A, 11 for MCF12A, 11 for HME-1 and 14 for HMEC.

Figure S5: Expression stabilities and ranking of reference genes according to delta Ct method, GeNorm, NormFinder and BestKeeper in forskolin treated cells. The Y- axis represents the stability value as calculated by each of the four methods (SD for delta Ct method, M-value for GeNorm, σ-value for NormFinder and SD for BestKeeper). Green, blue, red and black colours are specific of each cell lines respectively MCF10A, MCF12A, HME-1 and HMEC. To calculate the stability value, a total of 15 different samples were used for MCF10A, 13 for MCF12A, 12 for HME-1 and 16 for HMEC.

Figure S6: Analysis of the oscillatory profile of each of the nine tested reference genes in MCF10A. For each gene, gene expression was normalised using geometric mean of the four most stable reference genes (RPLP0, HSPCP, RPL4, TBP). The dotted lines represent the modelled curves derived from the normalised expression data using cosine algorithm. The coefficient of determination r^2 and the p-value were calculated for each cell line for each gene.

Figure S7: Analysis of the oscillatory profile of each of the nine tested reference genes in MCF12A.

For each gene, gene expression was normalised using geometric mean of the four most stable reference genes (RPLP0, HSPCP, RPL4, TBP). The dotted lines represent the modelled curves derived from the normalised expression data using cosine algorithm. The coefficient of determination r^2 and the p-value were calculated for each cell line for each gene.

Figure S8: Analysis of the oscillatory profile of each of the nine tested reference genes in HME1.

For each gene, gene expression was normalised using geometric mean of the four most stable reference genes (RPLP0, HSPCP, RPL4, TBP). The dotted lines represent the modelled curves derived

from the normalised expression data using cosine algorithm. The coefficient of determination r^2 and the p-value were calculated for each cell line for each gene.

Figure S9: Analysis of the oscillatory profile of each of the nine tested reference genes in HMEC.

For each gene, gene expression was normalised using geometric mean of the four most stable reference genes (RPLP0, HSPCP, RPL4, TBP). The dotted lines represent the modelled curves derived from the normalised expression data using cosine algorithm. The coefficient of determination r^2 and the p-value were calculated for each cell line for each gene.

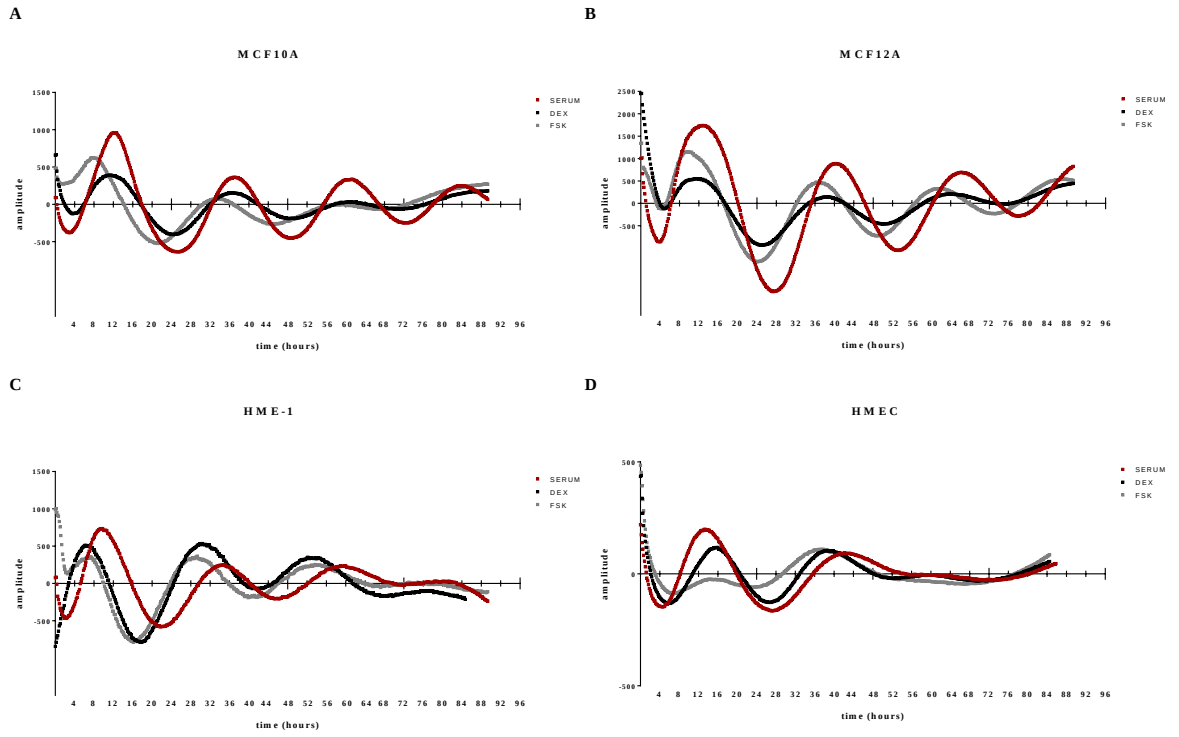
Figure S10: Analysis of the oscillatory profile of BMAL1 and PER3 in MCF10A, MCF12A, HME1 and HMECs using normalisation with GAPDH, ACTB and the geometric mean of the four most stable genes. BMAL1 and PER3 exhibit circadian transcription and their expression were normalised for each cell line with GAPDH (blue line with triangles), ACTB (red line with diamonds) and the geometric mean of the four most stable reference genes (GM of 4 genes (RPLP0, HSPCP, RPL4 and TBP), black line with circles). The dotted lines represent the modelled curves derived from the normalised expression data using cosine algorithm. The coefficient of determination r^2 and the p-value were calculated for each cell line for each gene.

Table S1: Number of biological replicates by conditions and cell lines. CONT: control condition (unsynchronised cells). CTs: circadian times after synchronisation. DEX: dexamethasone. FSK: forskolin.

Table S1

		Number of samples			
Synchronization	**Conditions**	**MCF10A**	**MCF12A**	**HME-1**	**HMEC**
SERUM SHOCK	CONT	7	7	7	1
	CT0	1	1	1	1
	CT3	1	1	1	1
	CT6	1	1	1	1
	CT9	1	1	1	1
	CT12	1	1	1	1
	CT15	1	1	1	1
	CT18	1	1	1	1
	CT21	1	1	1	1
	CT24	1	1	1	1
	CT27	1	1	1	1
	CT30	1	1	1	1
	CT33	1	1	1	1
	CT36	1	1	1	1
	CT39	1	1	1	1
	CT42	1	1	0	1
	CT45	1	1	0	0
	CT48	1	1	1	1
	SUM (SERUM)	24	24	22	17
- DEXAMETHASONE	CONT	5	3	6	6
	CT0	4	4	4	4
	CT24	4	4	4	4
	SUM (DEX)	13	11	14	14
FORSKOLIN	CONT	5	5	6	8
	CT0	5	4	3	4
	CT24	5	4	3	4
	SUM (FSK)	15	13	12	16
	SUM	**52**	**48**	**50**	**47**

Figure S1



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	SERUM			DEX			FSK		
	Period	Amplitude	Goodness of Fit %	Period	Amplitude	Goodness of Fit %	Period	Amplitude	Goodness of Fit %
MCF10A	24.9	1174.8	85.3	23.9	660.0	75.5	27.0	496.3	68.8
MCF12A	25.9	1872.7	86.4	25.4	1019.6	77.8	25.4	1027.7	60.5
HME-1	24.3	927.5	74.5	22.45	862.14	69.35	24.85	587.745	87.65
HMEC	25.3	148.3	72.5	22.5	168.7	65.0	19.6	89.5	67.8

Figure S2

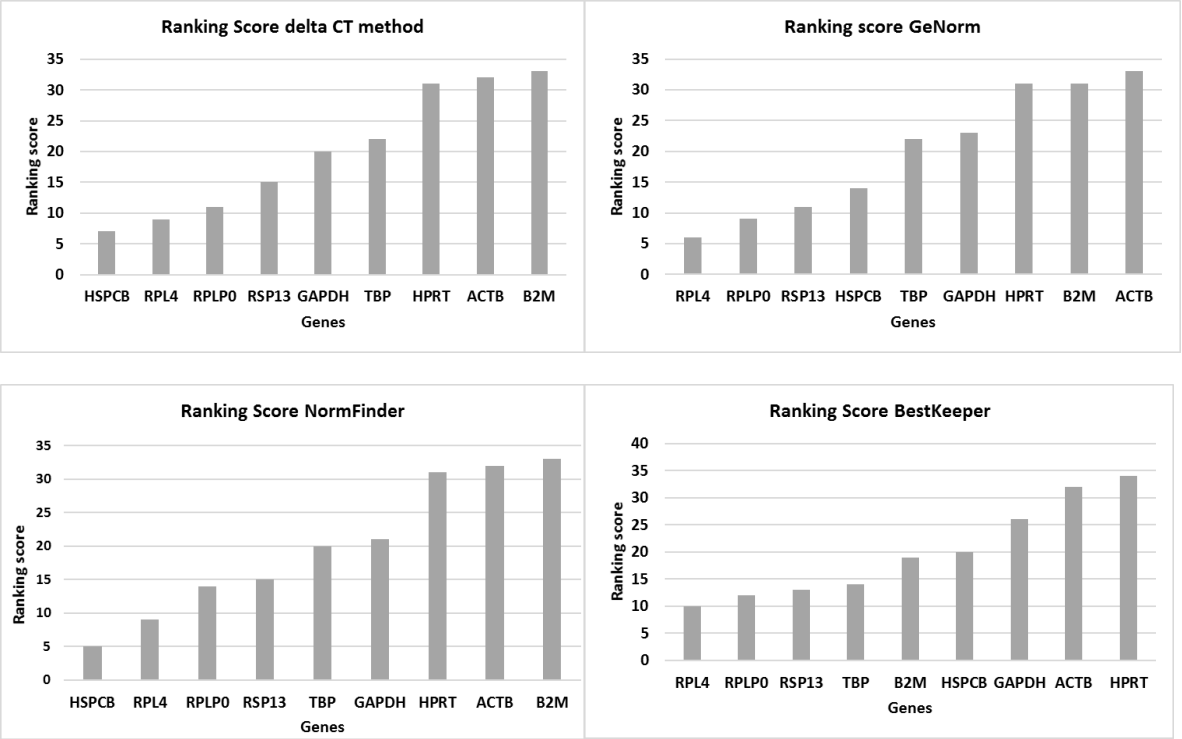

Ranking Score delta CT method
Ranking score
35
30
25
20
15
10
5
0
HSPCB RPL4 RPLP0 RSP13 GAPDH TBP HPRT ACTB B2M
Genes
Ranking score GeNorm
Ranking score
35
30
25
20
15
10
5
0
RPL4 RPLP0 RSP13 HSPCB TBP GAPDH HPRT B2M ACTB
Genes
Ranking Score NormFinder
Ranking score
35
30
25
20
15
10
5
0
HSPCB RPL4 RPLP0 RSP13 TBP GAPDH HPRT ACTB B2M
Genes
Ranking Score BestKeeper
Ranking score
40
35
30
25
20
15
10
5
0
RPL4 RPLP0 RSP13 TBP B2M HSPCB GAPDH ACTB HPRT
Genes

Figure S3

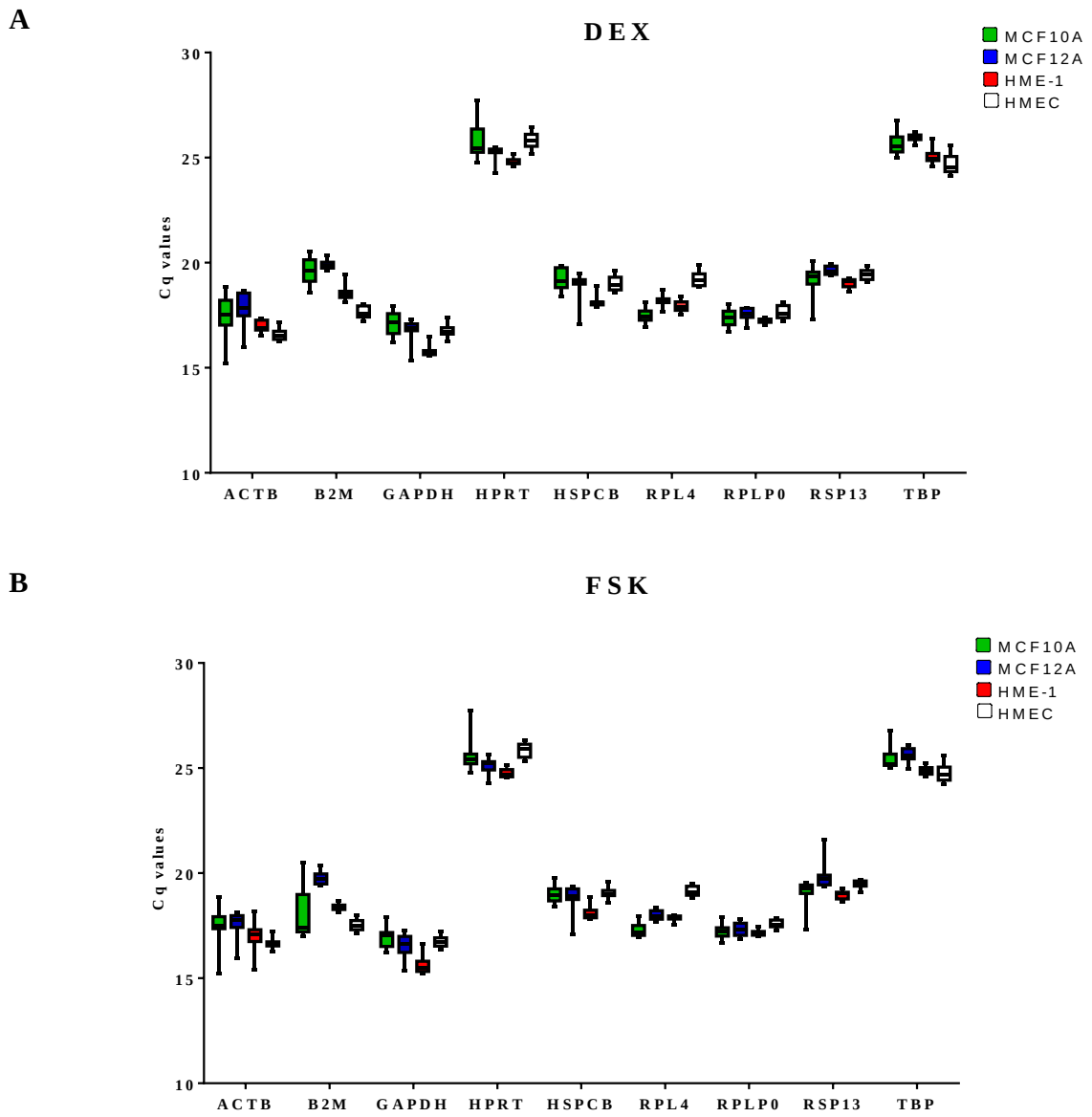

A
DEX
MCF10A
MCF12A
HME-1
HMEC
Cq values
30
25
20
15
10
ACTB
B2M
GAPDH
HPRT
HSPCB
RPL4
RPLP0
RSP13
TBP
B
FSK
MCF10A
MCF12A
HME-1
HMEC
Cq values
30
25
20
15
10
ACTB
B2M
GAPDH
HPRT
HSPCB
RPL4
RPLP0
RSP13
TBP

Figure S4

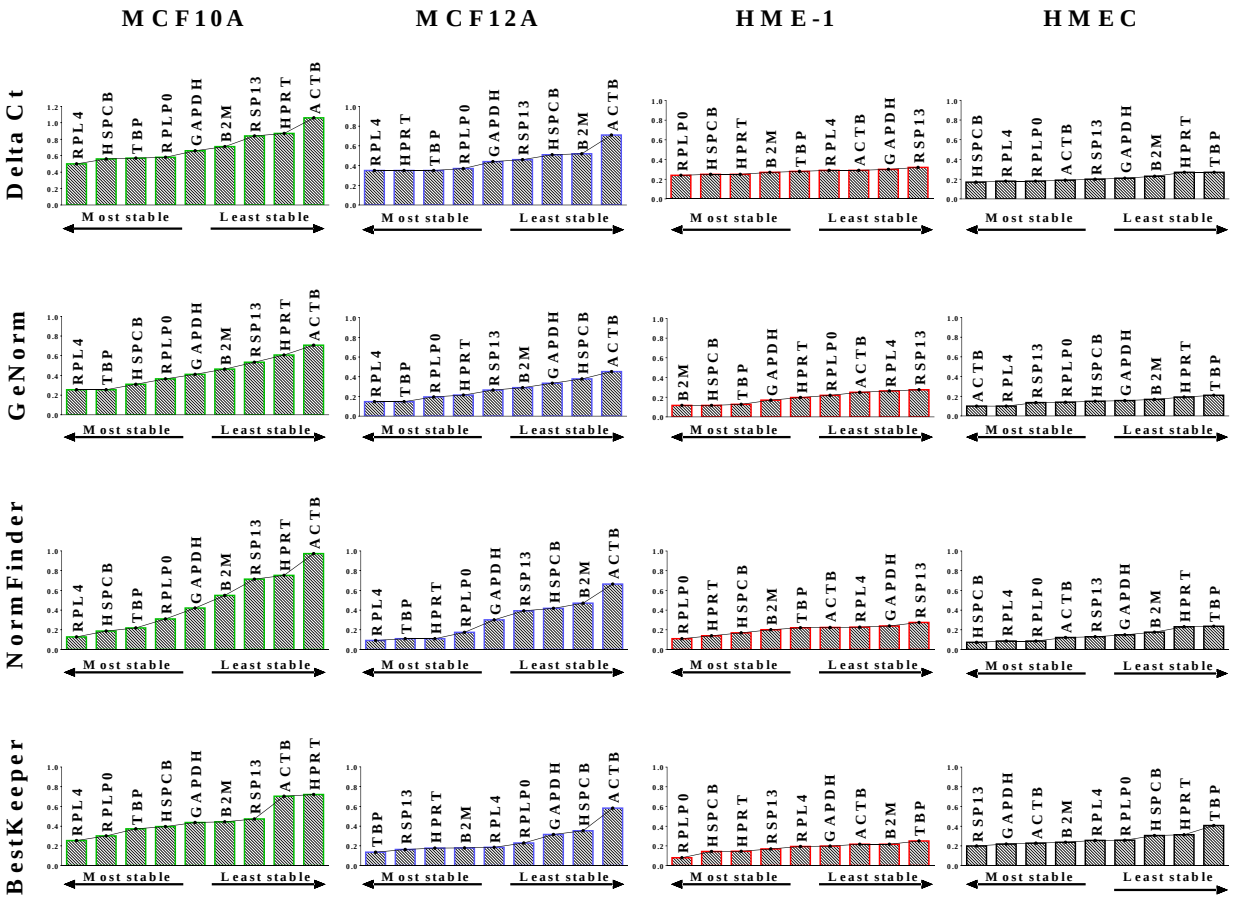
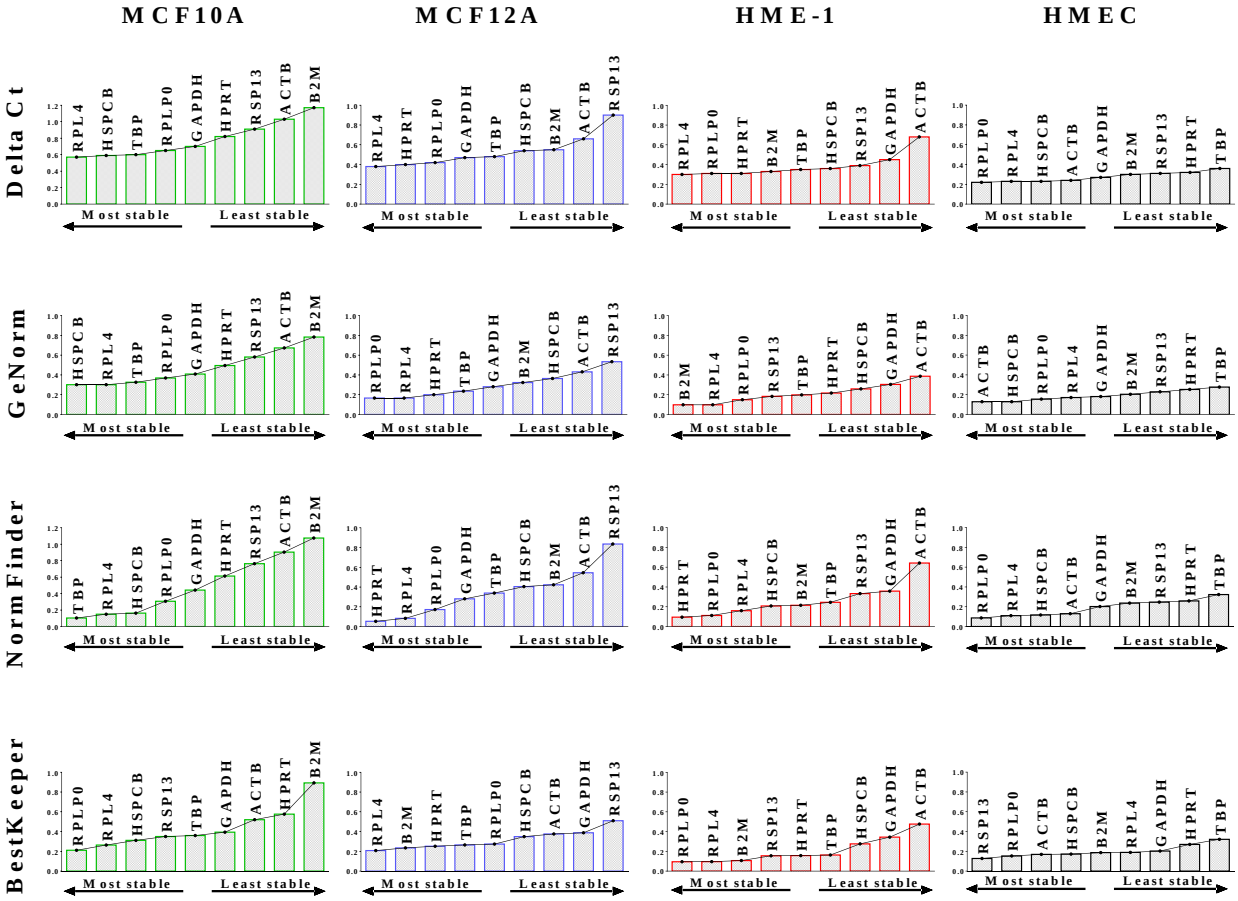

MCF10A
MCF12A
HME-1
HMEC
Delta Ct
GeNorm
NormFinder
BestKeeper
Most stable
Least stable

Figure S5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MCF12A
HME-1
HMEC
Delta Ct
GeNorm
NormFinder
BestKeeper
Most stable
Least stable

Figure S6

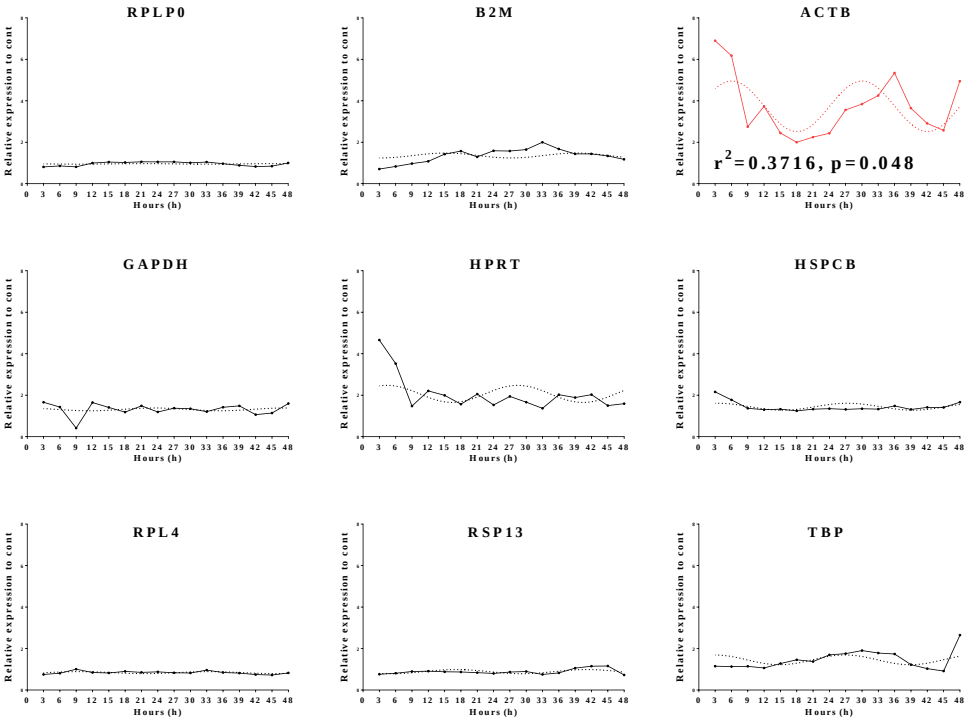

RPLP0
B2M
ACTB
r²=0.3716, p=0.048
GAPDH
HPRT
HSPCB
RPL4
RSP13
TBP
Relative expression to cont
Hours (h)

Figure S7

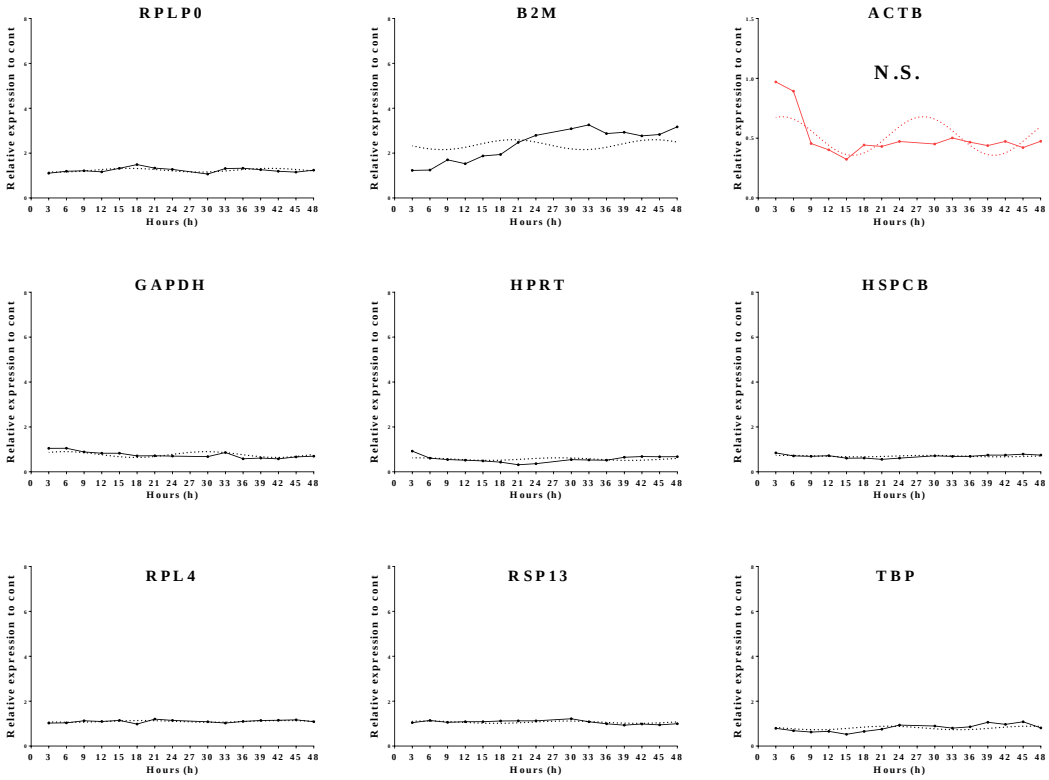

RPLP0
B2M
ACTB
N.S.
GAPDH
HPRT
HSPCB
RPL4
RSP13
TBP
Relative expression to cont
Hours (h)

Figure S8

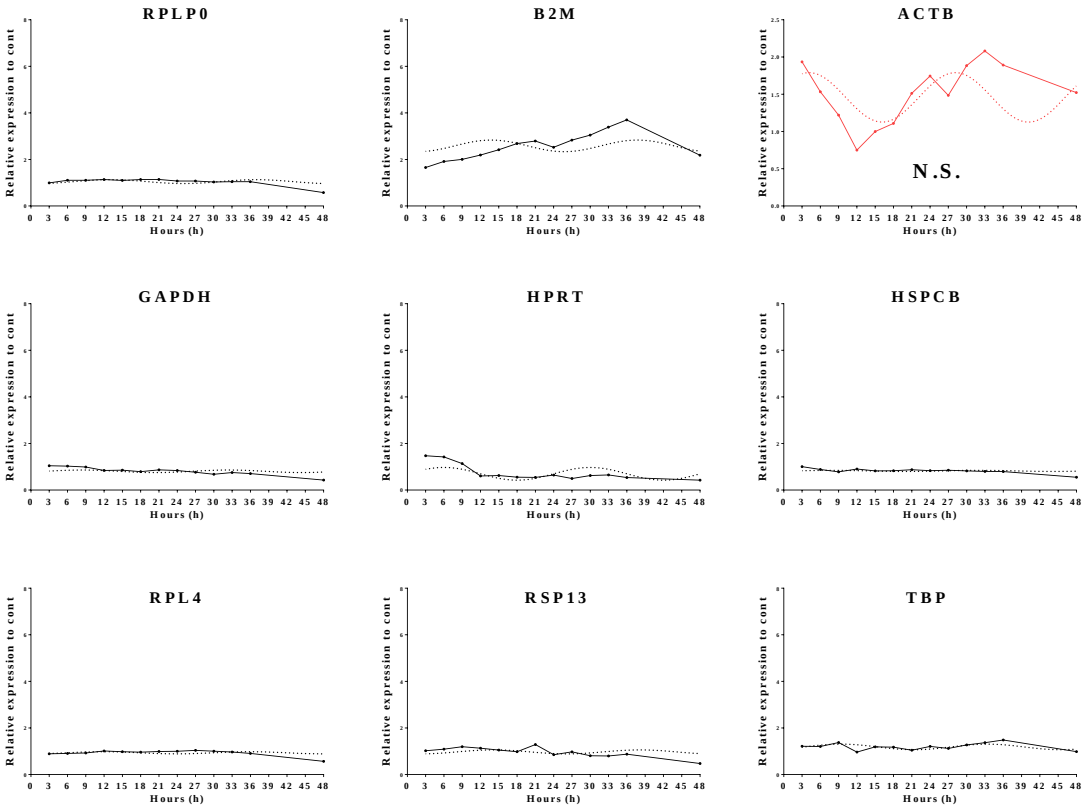

RPLP0
B2M
ACTB
N.S.
GAPDH
HPRT
HSPCB
RPL4
RSP13
TBP
Relative expression to cont
Hours (h)

Figure S9

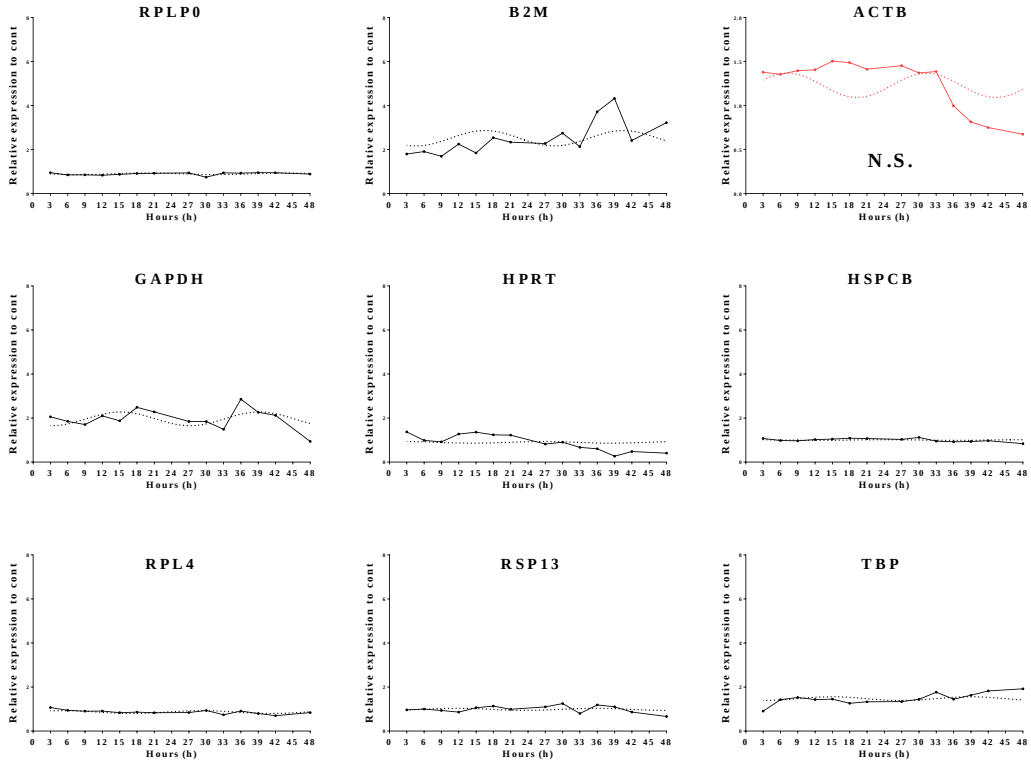
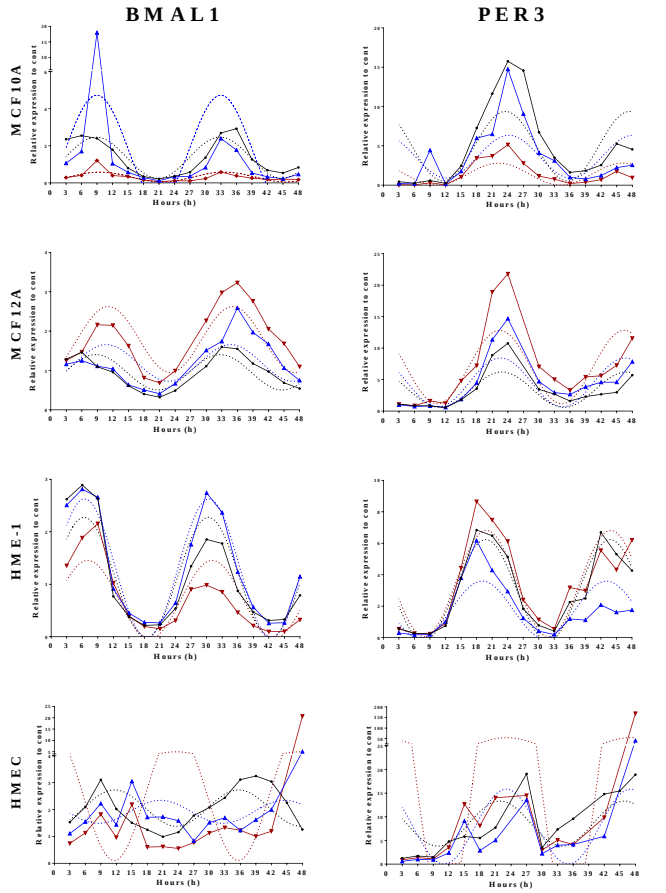

RPLP0
B2M
ACTB
N.S.
GAPDH
HPRT
HSPCB
RPL4
RSP13
TBP
Relative expression to cont
Hours (h)

Figure S10



MCF10A	BMAL1		PER3	
	r^2	p-value	r^2	p-value
GAPDH	0.23	N.S.	0.256	N.S.
ACTB	0.5054	0.0102	0.432	0.0253
GM of 4 Genes	**0.7259**	**0.0002**	**0.4837**	**0.0189**

MCF12A	BMAL1		PER3	
	r^2	p-value	r^2	p-value
GAPDH	0.3516	N.S.	0.5105	N.S.
ACTB	0.6287	0.0016	0.4899	0.0118
GM of 4 Genes	**0.6382**	**0.0022**	**0.6117**	**0.0055**

HME-1	BMAL1		PER3	
	r^2	p-value	r^2	p-value
GAPDH	0.9213	0	0.5921	0.0029
ACTB	0.7126	0.0003	0.7947	0.0176
GM of 4 Genes	**0.7992**	**3*10^5**	**0.9014**	**3*10^7**

HMEC	BMAL1		PER3	
	r^2	p-value	r^2	p-value
GAPDH	0.1149	N.S.	0.2646	N.S.
ACTB	0.1775	N.S.	0.255	N.S.
GM of 4 Genes	**0.419**	**0.0293**	**0.5821**	**0.0127**