

## Supplementary Materials:

### Upregulation of cytoglobin in 3D-cultures of head and neck cancer cells: Role of hypoxia and implications for cisplatin resistance

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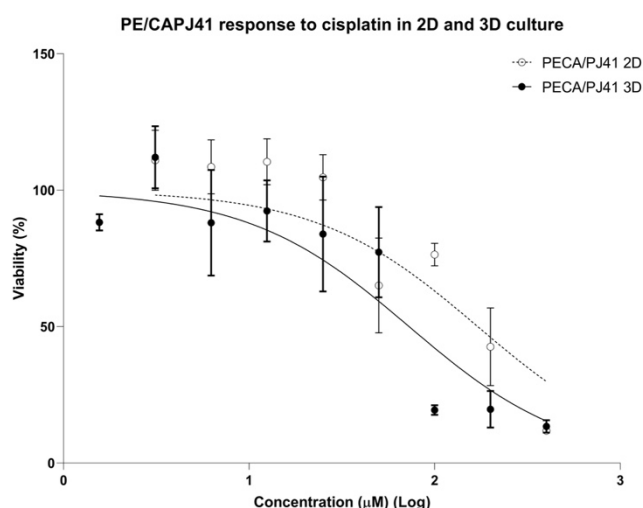
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## Supplementary Materials & Methods

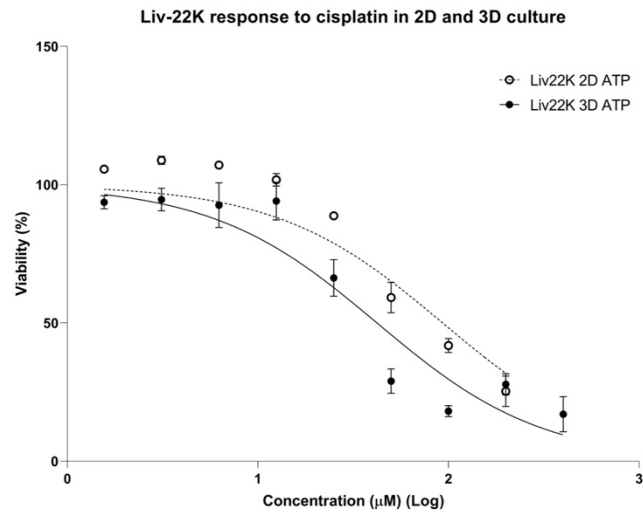
### Cobalt chloride, antimycin A and 5-azacytadine induce differential cytotoxicity in cell lines

The MTT assay measures the reductive capacity of the cell and is commonly used as a measure of cellular viability<sup>1</sup>. Cellular reductive capacity is primarily generated in active mitochondria, the greater the reduction of formazan salt, the greater the cellular viability. The IC<sub>50</sub> concentrations generated using the MTT assay were determined using non-linear least squares regression analysis (**Figure S4-S6**). Exposure of cells to cobalt chloride resulted in IC<sub>50</sub> concentrations of 173.7  $\mu$ M in PE/CAPJ41, 123.2  $\mu$ M in NOK-hTERT and 395.7  $\mu$ M in Liv-22K cells. The exposure of the cells to antimycin A resulted in IC<sub>50</sub> concentrations of 4.63  $\mu$ M in PE/CAPJ41, 5.70  $\mu$ M in NOK-hTERT and 5.52  $\mu$ M in Liv-22K cells. The exposure of the cells to 5-azacytadine resulted in IC<sub>50</sub> concentrations of 1.90  $\mu$ M in PE/CAPJ41, 1.86  $\mu$ M in NOK-hTERT and 2.22  $\mu$ M in Liv-22K cells.

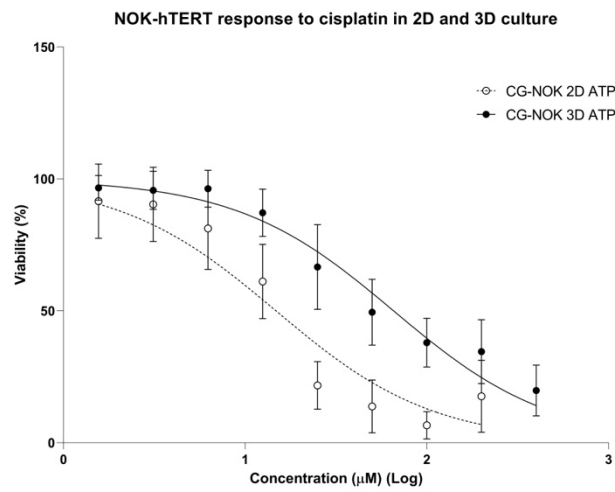
## Supplementary Figures



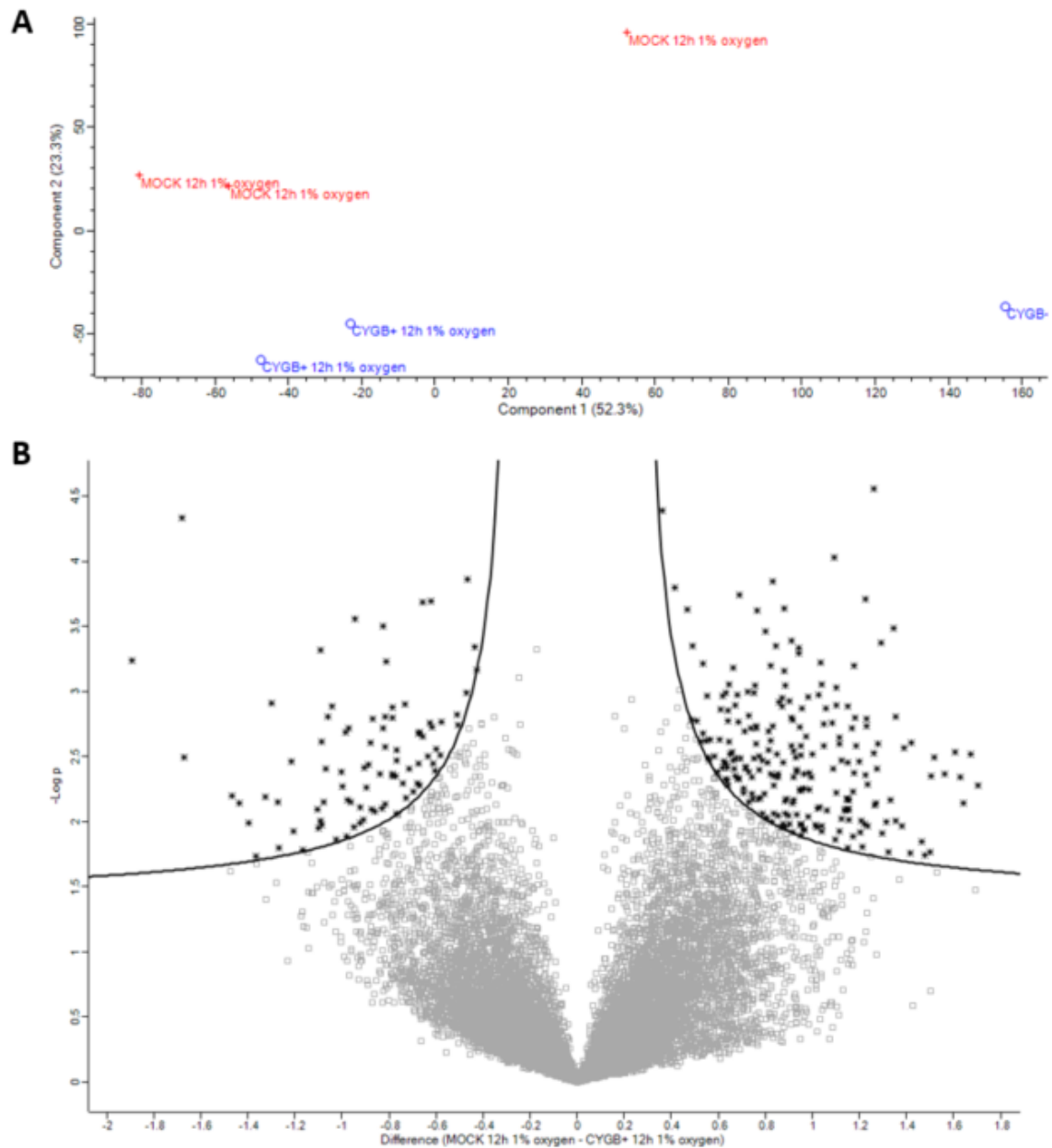
**Figure S1:** Concentration-response curve of PE/CAPJ41 in response to cisplatin treatment.



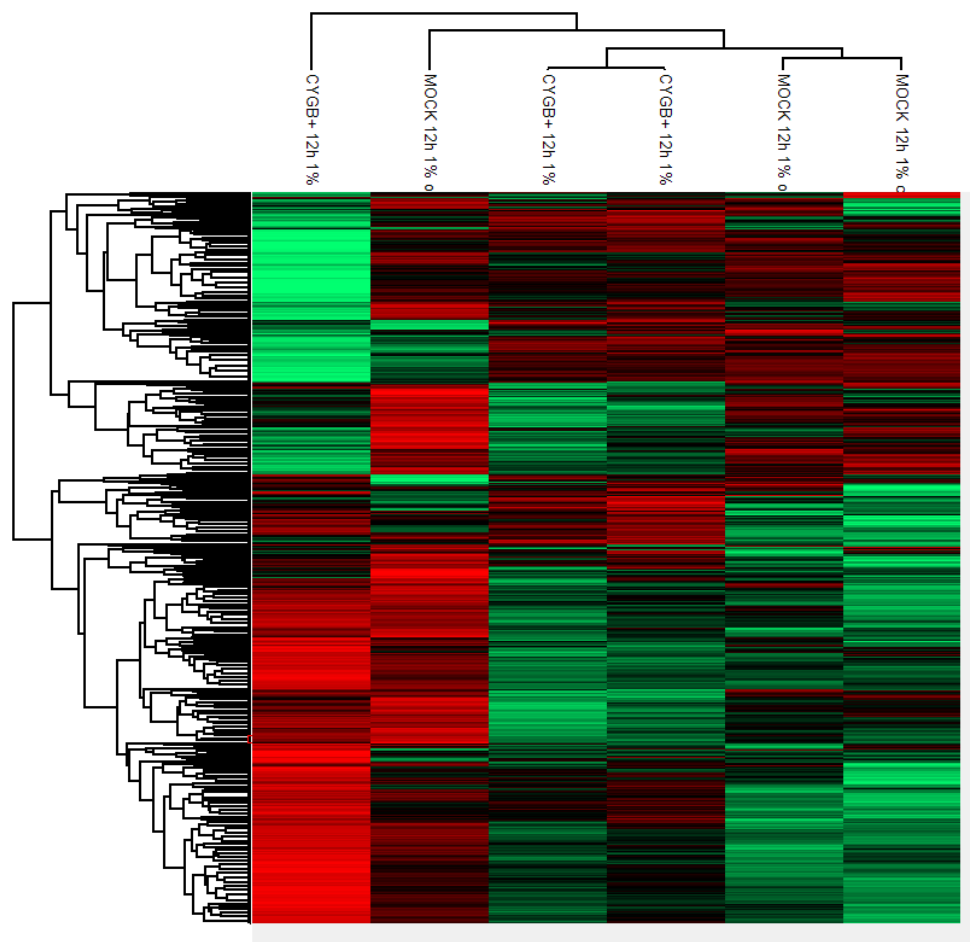
**Figure S2:** Concentration-response curve of Liv-22K in response to cisplatin treatment.



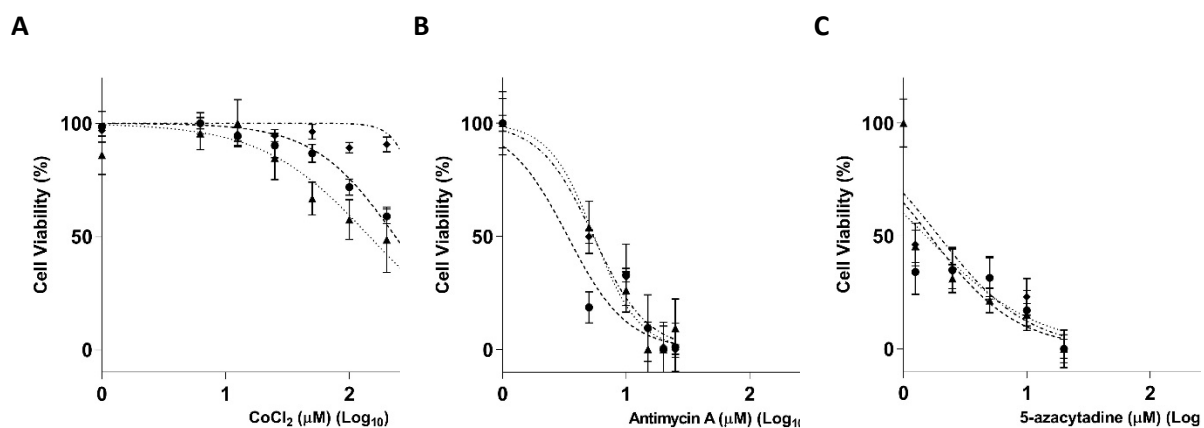
**Figure S3:** Concentration-response curve of NOK-hTERT in response to cisplatin treatment.



**Figure S4: A)** Principal component analysis of replicates of mock (un-transfected PE/CAPJ41 cells) vs CYGB (cytoglobin transfected PE/CAPJ41 cells) after 12 h cultured at 1 %  $O_2$  tension. **B)** Volcano plot represents the results of multiple  $t$ -tests with FDR correction of 0.05 applied. Stars on the right and left of significance line indicate statistically significantly differentially expressed genes (on right upregulated in CYGB expressing cells and on left upregulated in un-transfected cells) after 12 h growth in 1 %  $O_2$  tension.



**Figure S5:** Heat map represents hierarchical clustering of un-transfected PE/CAPJ41 cells (MOCK) vs cytoglobin transfected PE/CAPJ41 cells (CYGB) after 12 h cultured at 1 % O<sub>2</sub> tension. Euclidian distancing represents clusters of commonly associated genes based on their differential expression.



**Figure S6:** Dose-response curve of PE/CAPJ41, NOK-hTERT and Liv22K cells to cobalt chloride (**A**), antimycin A (**B**) and 5-azacytadine (**C**) over a 24 h period. Dose-response measured using the MTT assay and viability represented as a normalized absorbance of the control. Error bars represent standard error of the mean.

## Supplementary Tables

**Table S1A:** Description of genes used in study.

Gene	Function
<i>Actin</i>	Actin. Assembly of microfilament
<i>B2M</i>	$\beta_2$ microglobulin. Component of the class I Major Histocompatibility Complex
<i>CYGB</i>	Cytoglobin. A hexacoordinated heme containing globin
<i>NQO1</i>	NAD(P)H Quinone Dehydrogenase 1. Quinone reductase enzyme under control of NRF-2
<i>NRF-2</i>	Nuclear factor erythroid 2. Antioxidant regulator. Potentially under control of CYGB
<i>HIF1A</i>	Hypoxia inducing factor 1A. Associated with cellular response to hypoxia
<i>NFkB</i>	Nuclear Factor kappa B. Early stage inflammatory response
<i>GADD45A</i>	Growth arrest and DNA damage-inducible protein. Gene transcripts levels sensitive to growth arrest and DNA damaging agents
<i>Casp3</i>	Caspase 3. Interacts with Caspase 8 and 9 and plays vital role in execution-phase of cell apoptosis
<i>GJB6</i>	Gap junction beta-6-protein. Gap junction protein often expressed in spheroids cultures
<i>MKI</i>	KI-67. Used as marker of proliferation, commonly upregulated in spheroid culture
<i>CDH1</i>	E-cadherin. Calcium dependent cell-cell adhesion glycoprotein expressed on cell surface. Relevant in spheroid culture

**Table S1B:** List of genes and oligonucleotide sequences used in gene expression analysis. Shaded used as reference genes.

Gene	Oligonucleotides (5'-3')	Gene	Oligonucleotides (5'-3')
<i>CYGB</i>	ATCCTCATCCTCATCTTCATCCT (F) CTGGGTCTGGTTACAAACATCA	<i>NFkB</i>	CTTAGGAGGGAGAGCCAC (F) CTTCTGCCATTCTGAAGCCG (R)
<i>NQO1</i>	GGACTGCACCAGAGCCAT (F) TCCTTTCTTCTCAAAGCCG (R)	<i>Casp3</i>	GCCTCTTCCCCATTCTCAT (F) CTTCCATGTATGATCTTGGTTCC (R)
<i>HIF1A</i>	GACAAGCCACCTGAGGAGAG (F) ACGCGGAGAAGAGAAGGAAA	<i>GJB6</i>	CACGGGCATCCTGTGTTTTG (F) TGCATGAAGAGGGCGTACAA (R)
<i>GADD45A</i>	CACTGTCGGGGTGTACGAAG (F) CCTGGATCAGGGTGAAGTGG (R)	<i>MKI</i>	TTACCGGGCGGAGGTATGAA (F) GCTGGCTCCTGTTACGTAT (R)
		<i>CDH1</i>	GGGGTTAAGCACAACAGCAA (F) CAAAATCCAAGCCCGTGGTG (R)
<i>Actin</i>	ACTCTTCCAGCCTTCCTTCC (F) GTTGGCGTACAGGTCTTGC (R)	<i>B2M</i>	TTTGGCTCACAGTGTAAGGG (F) GTCACCCCACTATGCCATT (R)

**Table S2:** List of statistically significant differentially expressed genes upregulated in *CYGB* expressing PE/CAPJ41 cells after 12 h incubation in 1 % O<sub>2</sub> tension in comparison to un-transfected (Mock) PE/CAPJ41 cells.

Gene ID	Gene name	"-LOG(P-value)"
TMBIM6	transmembrane BAX inhibitor motif containing 6	3.630797
SAMD4A	sterile alpha motif domain containing 4A	2.776229
SULF2	sulfatase 2	2.611485
CALM3	calmodulin 3 (phosphorylase kinase, delta)	3.216663
ARHGAP5	Rho GTPase activating protein 5	2.624791
MID1	midline 1 (Opitz/BBB syndrome)	2.965334
DHRS4	dehydrogenase/reductase (SDR family) member 4	2.499962
DGKZ	diacylglycerol kinase, zeta	2.379752
TMEM105	transmembrane protein 105	2.633613
PKP3	plakophilin 3	2.411263
HSD17B11	hydroxysteroid (17-beta) dehydrogenase 11	2.993964
TOX4	TOX high mobility group box family member 4	2.320592
PSMB9	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)	2.411239
LPIN3	lipin 3	2.852881
BCAS4	breast carcinoma amplified sequence 4	2.772968
RCAN3	RCAN family member 3	2.518902
BNIP3L	BCL2/adenovirus E1B 19kDa interacting protein 3-like	2.894734
MIS18BP1	MIS18 binding protein 1	2.348443
IRF7	interferon regulatory factor 7	2.694847
RNF24	ring finger protein 24	2.812161
C21orf70	chromosome 21 open reading frame 70	2.379565
NGDN	neuroguidin, EIF4E binding protein	2.21599
CTHRC1	collagen triple helix repeat containing 1	2.731393
SSH2	slingshot homolog 2 (Drosophila)	2.500654
WEE1	WEE1 homolog (S. pombe)	2.181654
CPNE1	copine I	2.029758
CD163L1	CD163 molecule-like 1	2.691015
C16orf48	chromosome 16 open reading frame 48	2.624383
NEK11	NIMA (never in mitosis gene a)- related kinase 11	3.843369
ZNF395	zinc finger protein 395	2.514658
FLJ90757	hypothetical LOC440465	1.954944
HSPA13	heat shock protein 70kDa family, member 13	2.953165
FAM165B	family with sequence similarity 165, member B	1.956582
FOXL1	forkhead box L1	2.157639
LOC389831	hypothetical LOC389831	2.926221
CLCN4	chloride channel 4	3.390881
NRP1	neuropilin 1	2.050944
NDRG2	NDRG family member 2	1.984551
PARD6B	par-6 partitioning defective 6 homolog beta (C. elegans)	1.893531
SYTL1	synaptotagmin-like 1	2.652832

FANCF	Fanconi anemia, complementation group F	2.242375
LRRCC1	leucine rich repeat and coiled-coil domain containing 1	2.346
SNORD17	small nucleolar RNA, C/D box 17	4.033822
LOC441869	hypothetical protein LOC441869	2.220962
ACOT4	acyl-CoA thioesterase 4	2.59909
PTK6	PTK6 protein tyrosine kinase 6	2.082694
MAMDC2	MAM domain containing 2	2.175704
TEP1	telomerase-associated protein 1	2.093223
CFH	complement factor H	2.717929
TSPAN1	tetraspanin 1	2.798061
EPS8L2	EPS8-like 2	2.579602
LCTL	lactase-like	2.526304
ASB9	ankyrin repeat and SOCS box containing 9	2.367036
DIAPH2	diaphanous homolog 2 (Drosophila)	2.53151

\*-Log(*p*-value) represents *p*-value determined between un-transfected and *CYGB* transfected cells through *t*-test with 0.05 FDR correction.



**Table S3:** List of statistically significant differentially expressed genes downregulated in *CYGB* expressing PE/CAPJ41 cells after 12 h incubation in 1 % O<sub>2</sub> tension in comparison to un-transfected (Mock) PE/CAPJ41 cells. Data is expressed as fold-change induction in un-transfected cells

Gene ID	Gene name	"-LOG(P-value)"
HTRA1	HtrA serine peptidase 1	3.23655
MOXD1	monooxygenase, DBH-like 1	2.495421
SPP1	secreted phosphoprotein 1	2.201717
LOC728769	hypothetical LOC728769	2.141085
ELL2	elongation factor, RNA polymerase II, 2	1.732045
ADRBK2	adrenergic, beta, receptor kinase 2	2.189362
FSTL3	follistatin-like 3 (secreted glycoprotein)	2.152406
BAIAP2L2	BAI1-associated protein 2-like 2	1.798771
REEP5	receptor accessory protein 5	2.463585
FAM25A	family with sequence similarity 25, member A	1.783071
UIMC1	ubiquitin interaction motif containing 1	2.090902
RPL28	ribosomal protein L28	1.952637
TRIM37	tripartite motif containing 37	2.007681
MYO10	myosin X	1.970876
SNHG9	small nucleolar RNA host gene 9 (non-protein coding)	2.611201
CHAC1	ChaC, cation transport regulator homolog 1 (E. coli)	2.153926
METRNL	meteorin, glial cell differentiation regulator-like	2.403716
XRCC4	X-ray repair complementing defective repair in Chinese hamster cells 4	2.802982
YAF2	YY1 associated factor 2	2.380972
SQSTM1	sequestosome 1	2.272216
SLMO1	slowmo homolog 1 (Drosophila)	2.690173
MLXIP	MLX interacting protein	1.88925
TCOF1	Treacher Collins-Franceschetti syndrome 1	2.164422
HES5	hairy and enhancer of split 5 (Drosophila)	2.714889
ASNS	asparagine synthetase (glutamine-hydrolyzing)	2.14765
SEL1L3	sel-1 suppressor of lin-12-like 3 (C. elegans)	1.954379
DPM3	dolichyl-phosphate mannosyltransferase polypeptide 3	2.111932
PTPRM	protein tyrosine phosphatase, receptor type, M	1.986757
CDC23	cell division cycle 23 homolog (S. cerevisiae)	2.017137
SLC35E2	solute carrier family 35, member E2	2.410292
RALBP1	ralA binding protein 1	2.265475
KIAA0114	KIAA0114	2.440977
CAST	calpastatin	2.6029
NADK	NAD kinase	2.090557
CDC42EP1	CDC42 effector protein (Rho GTPase binding) 1	2.794323
C1orf133	chromosome 1 open reading frame 133	2.075355
RDM1	RAD52 motif 1	2.103115
PPP1R14C	protein phosphatase 1, regulatory (inhibitor) subunit 14C	3.500974
AOX1	aldehyde oxidase 1	2.574537
ABCC3	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	2.130332

C17orf37	chromosome 17 open reading frame 37	3.228043
C5orf45	chromosome 5 open reading frame 45	2.797773
PPP4R1	protein phosphatase 4, regulatory subunit 1	2.353034
RNU105A	RNA, U105A small nucleolar	2.877051
THOC3	THO complex 3	2.47027
PTPRB	protein tyrosine phosphatase, receptor type, B	2.29411
C9orf9	chromosome 9 open reading frame 9	2.901474
HIGD2A	HIG1 hypoxia inducible domain family, member 2A	2.181702
METTL12	methyltransferase like 12	2.290182
AP3B1	adaptor-related protein complex 3, beta 1 subunit	2.444963
TMEM222	transmembrane protein 222	2.674985
POC5	POC5 centriolar protein homolog (Chlamydomonas)	2.683338
FUNDC2	FUN14 domain containing 2	3.686194
ZCCHC9	zinc finger, CCHC domain containing 9	2.653828
CLPB	ClpB caseinolytic peptidase B homolog (E. coli)	2.498364
UPP1	uridine phosphorylase 1	2.762458
FAM27A	family with sequence similarity 27, member A	3.694651
C19orf24	chromosome 19 open reading frame 24	2.728574
FTH1	ferritin, heavy polypeptide 1	2.446903
SAFB2	scaffold attachment factor B2	2.393978
LOC100268168	hypothetical LOC100268168	2.560377
TUBA4A	tubulin, alpha 4a	2.510788
CCDC124	coiled-coil domain containing 124	2.762999
C7orf41	chromosome 7 open reading frame 41	2.739206
TMED3	transmembrane emp24 protein transport domain containing 3	2.98947
PHF20L1	PHD finger protein 20-like 1	3.866148
FIS1	fission 1 (mitochondrial outer membrane) homolog (S. cerevisiae)	3.164292

\*-Log(*p*-value) represents *p*-value determined between un-transfected and *CYGB* transfected cells through *t*-test with 0.05 FDR correction.

## References

1. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **65**, 55–63 (1983).