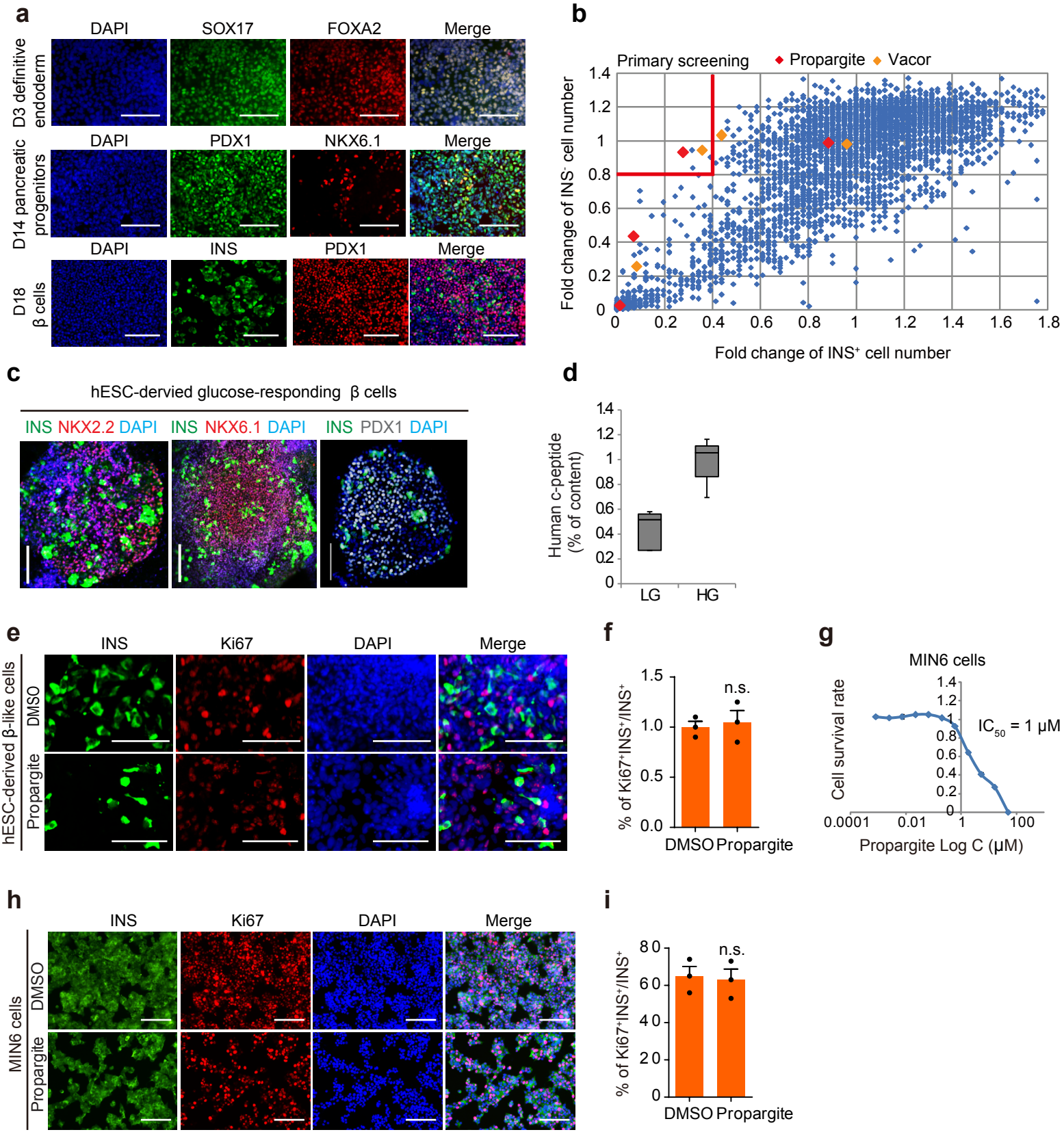
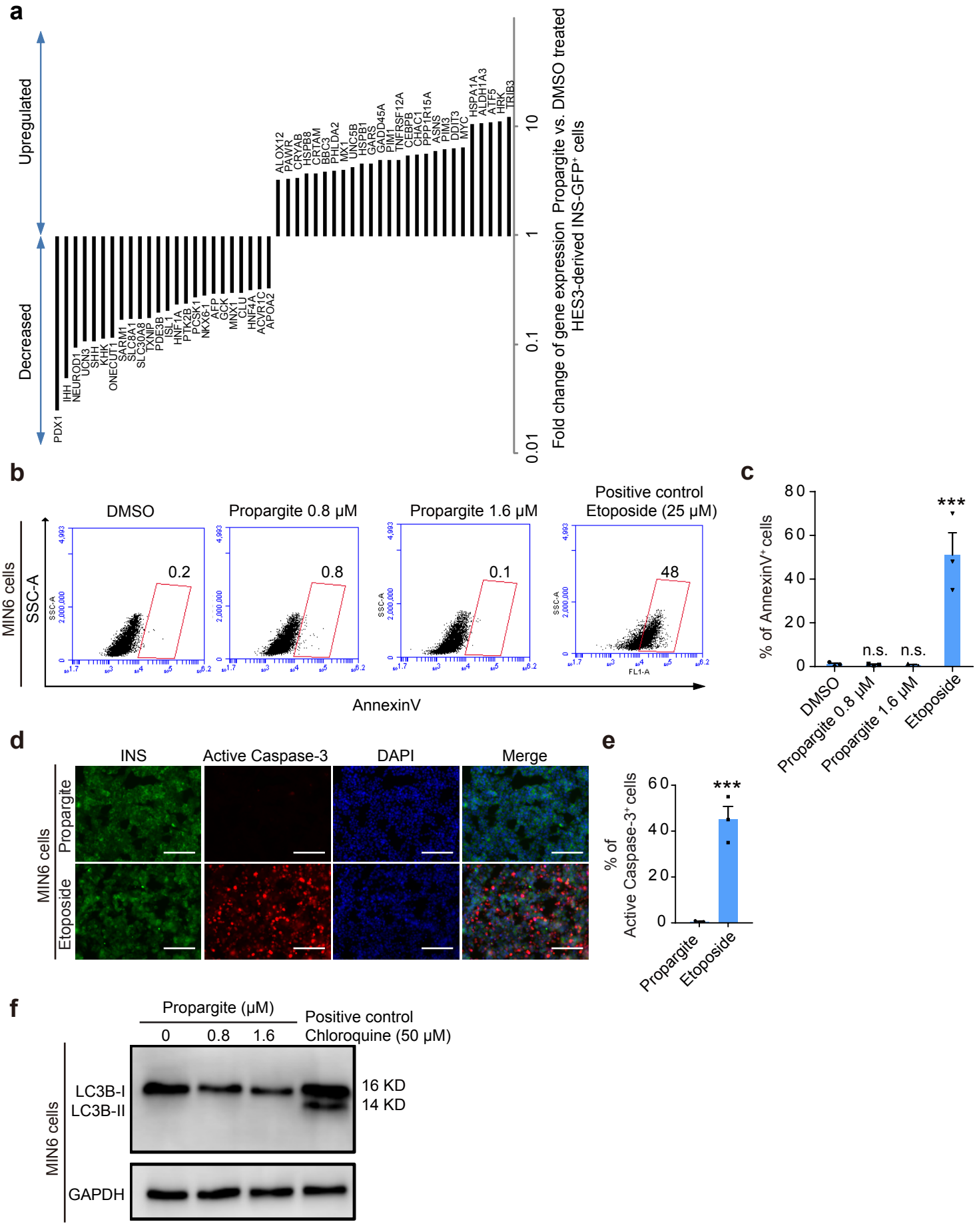
Supplementary Figure 1



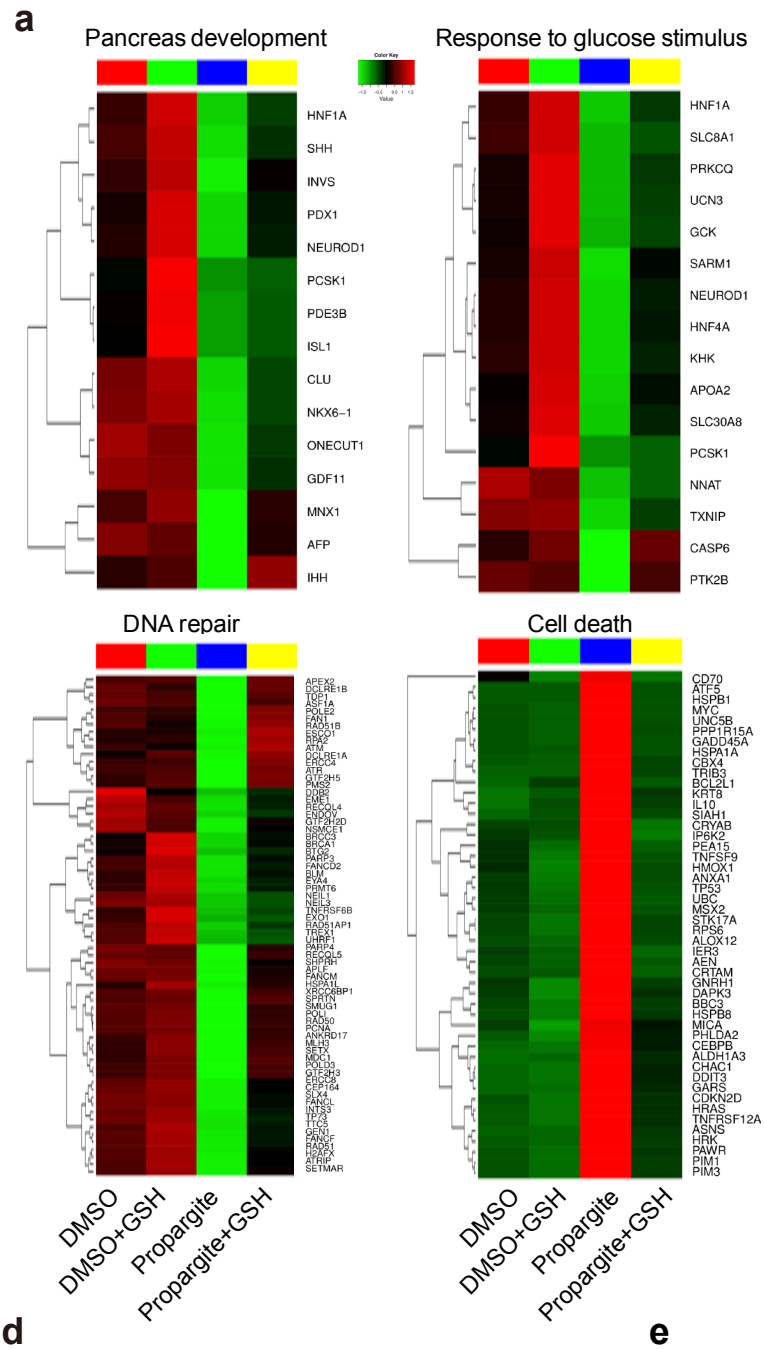
Supplementary information.

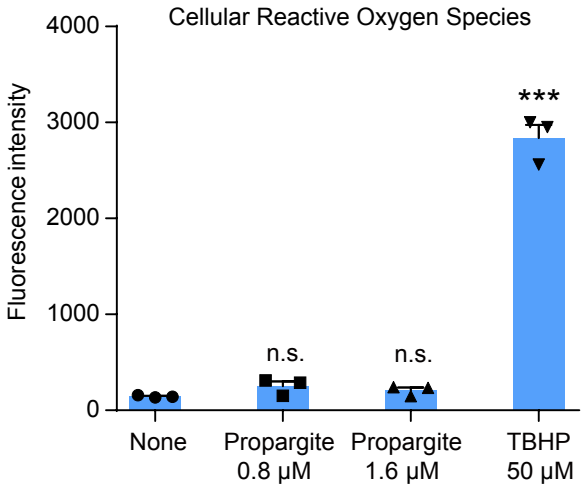
Supplementary Figure 1. Primary screen and validation of hit compounds.
(**a**) Immunocytochemistry analysis of hESC-derived definitive endoderm, pancreatic progenitors, endocrine cells and INS^+ cells using the β-cell differentiation protocol 1.
(**b**) Results of ToxCast library screening using the β-cell differentiation protocol 1. The red or orange squares highlight the positive hit compounds. (**c, d**) Confocal imaging analysis (c) and glucose stimulated insulin section (d) to characterize hESC-derived glucose-responding β-like cells generated using β-cell differentiation protocol 2. Scale bar = 150 μm. Values presented as the box plot. Centre line, the bounds of box and whiskers present the median, the first, third quartile, minimal and maximum, repsectively. (**e, f**) Representative images (e) and cell proliferation rate (f) of DMSO or 1.6 μM propargite treated β-like cells (n=3). Pancreatic β-like cells were stained for INS (green), proliferating cells were stained for Ki67 (red) and all the cells were counterstained by DAPI (blue). Scale bars, 200 μm. Cell proliferation rates were defined as the percentage of $Ki67^+INS^+$ cells/INS^+ cells. Values presented as mean±S.D. (**g**) IC_{50} of propargite on MIN6 cells. (**h, i**) Representative images (h) and cell proliferation rates (i) of DMSO or 1.6 μM propargite treated MIN6 cells. MIN6 cells were stained for INS (green), proliferating cells were stained for Ki67 (red) and all the cells were counterstained by DAPI (blue). Scale bars, 200 μm. Values presented as mean±S.D. n.s. indicates a non-significant difference.

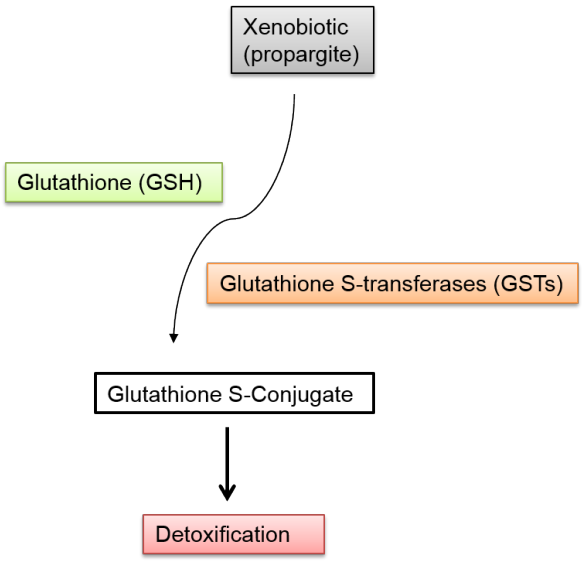

a
Upregulated
Decreased
PDX1
IHH
NEUROD1
UCN3
SHH
KHK
ONECUT1
SARM1
SLC8A1
SLC30A8
TXNIP
PDE3B
ISL1
HNF1A
PTK2B
PCSK1
NKX6-1
AFP
GCK
MNX1
CLU
HNF4A
ACVR1C
APOA2
ALOX12
PAWR
CRYAB
HSPB8
CRTAM
BBC3
PHLDA2
MX1
UNC5B
HSPB1
GARS
GADD45A
PIM1
TNFRSF12A
CEBPB
CHAC1
PPP1R15A
ASNS
PIM3
DDIT3
MYC
HSPA1A
ALDH1A3
ATF5
HRK
TRIB3
0.01
0.1
1
10
Fold change of gene expression Propargite vs. DMSO treated HES3-derived INS-GFP+ cells
b
DMSO
Propargite 0.8 μM
Propargite 1.6 μM
Positive control Etoposide (25 μM)
MIN6 cells
SSC-A
AnnexinV
0.2
0.8
0.1
48
c
% of AnnexinV+ cells
n.s.
n.s.
***
DMSO
Propargite 0.8 μM
Propargite 1.6 μM
Etoposide
d
INS
Active Caspase-3
DAPI
Merge
MIN6 cells
Propargite
Etoposide
e
% of Active Caspase-3+ cells
***
Propargite
Etoposide
f
Propargite (μM)
0
0.8
1.6
Positive control Chloroquine (50 μM)
MIN6 cells
LC3B-I
LC3B-II
16 KD
14 KD
GAPDH

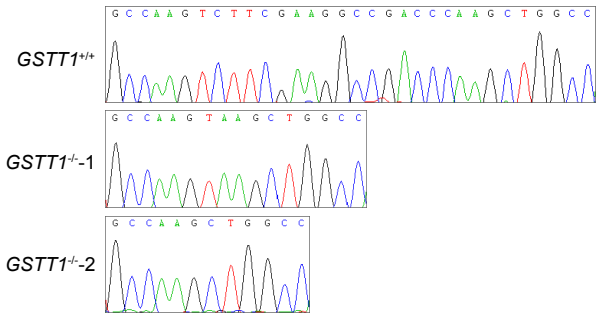
Supplementary Figure 2. Propargite-induced β-cell death is not due to cell apoptosis or autophagy.

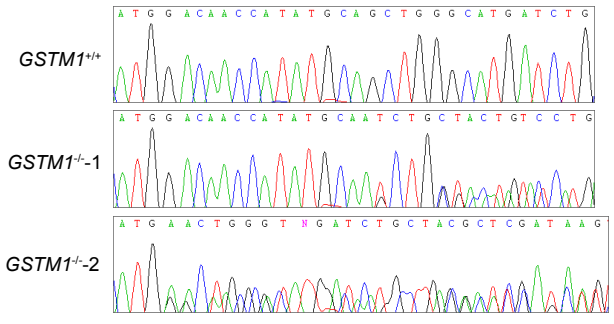
(**a**) Up/down-regulated genes (fold change >3) in the 1.6 μM propargite-treated *INS*-GFP$^+$ cells. (**b, c**) Flow cytometry analysis (b) and quantification (c) of the percentage of AnnexinV$^+$ cells for different doses of propargite-treated MIN6 cells (n=3). 25 μM Etoposide was used as a positive control. Values presented as mean±S.D. (**d, e**) Immunocytochemistry analysis (d) and quantification (e) of the percentage of Active Caspase-3$^+$ cells in 1.6 μM propargite treated MIN6 cells (n=3). 25 μM Etoposide used as a positive control. Scale bars, 200 μm. Values presented as mean±S.D. (**f**) Western blot analysis of autophagy marker (LC3B-II) after 0.8 μM or 1.6 μM propargite exposure in MIN6 cells. 50 μM Chloroquine was used as a positive control. GAPDH was used as a loading control. n.s. indicates a non-significant difference. *p* values calculated by unpaired two-tailed Student's t-test was ***$p<0.001$.

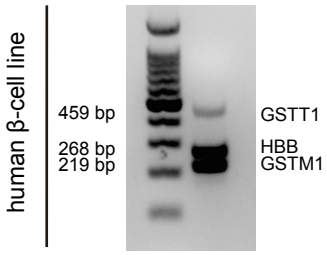

a
Pancreas development
HNF1A
SHH
INVS
PDX1
NEUROD1
PCSK1
PDE3B
ISL1
CLU
NKX6–1
ONECUT1
GDF11
MNX1
AFP
IHH
Response to glucose stimulus
HNF1A
SLC8A1
PRKCQ
UCN3
GCK
SARM1
NEUROD1
HNF4A
KHK
APOA2
SLC30A8
PCSK1
NNAT
TXNIP
CASP6
PTK2B
DNA repair
Cell death
CD70
ATF5
HSPB1
MYC
UNC5B
PPP1R15A
GADD45A
HSPA1A
CBX4
TRIB3
BCL2L1
KRT8
IL10
SIAH1
CRYAB
IP6K2
PEA15
TNFSF9
HMOX1
ANXA1
TP53
UBC
MSX2
STK17A
RPS6
ALOX12
IER3
AEN
CRTAM
GNPH1
DAPK3
BBC3
HSPB8
MICA
PHLDA2
CEBPB
ALDH1A3
CHAC1
DDIT3
GARS
CDKN2D
HRAS
TNFRSF12A
ASNS
HRK
PAWR
PIM1
PIM3
DMSO
DMSO+GSH
Propargite
Propargite+GSH
DMSO
DMSO+GSH
Propargite
Propargite+GSH


b
Cellular Reactive Oxygen Species
Fluorescence intensity
4000
3000
2000
1000
0
***
n.s.
n.s.
None
Propargite 0.8 μM
Propargite 1.6 μM
TBHP 50 μM

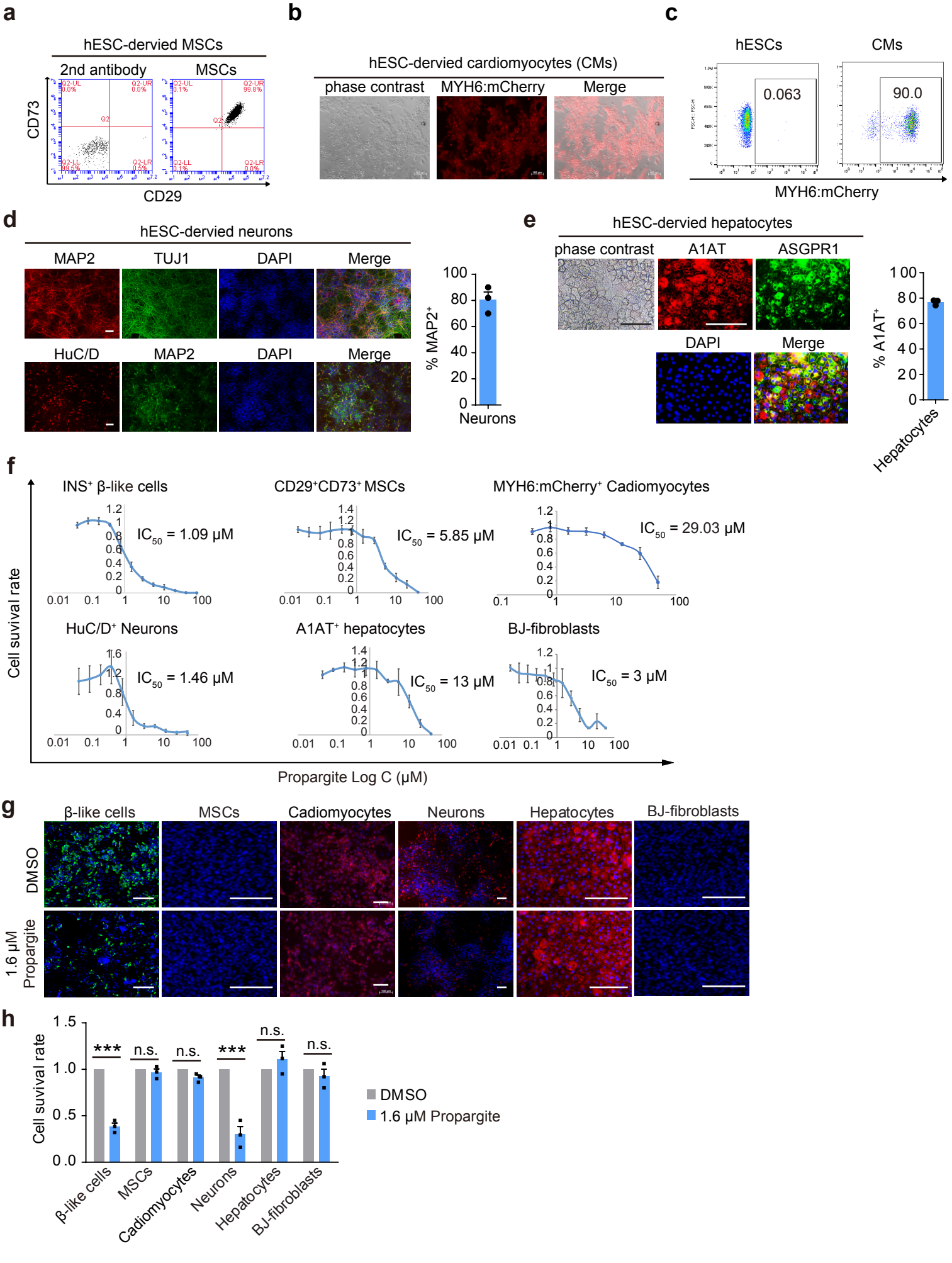

c
Xenobiotic (propargite)
Glutathione (GSH)
Glutathione S-transferases (GSTs)
Glutathione S-Conjugate
Detoxification


d
GSTT1+/+
GSTT1-/--1
GSTT1-/--2
GSTT1+/+ GCCAAGTCTTCGAAGGCCGACCCAAGCTGGCC
H176fs/H176fs
GSTT1-/--1 GCCAAGT----------AAGCTGGCC
M175fs/M175fs
GSTT1-/--2 GCCAAG----------CTGGCC


e
GSTM1+/+
GSTM1-/--1
GSTM1-/--2
GSTM1+/+ ATGGACAACCATATGCAGCTGGGCATGATCTG
M109fs/M109fs
GSTM1-/--1 ATGGACAACCATATGCAAGCTGGGCATGATCTG
ATGGACAACCATATGCA----------ATCTG
M109fs/M105fs
GSTM1-/--2 ATGGACAACCATATGCAAGCTGGGCATGATCTG
ATG----------AGCTGGGCATGATCTG


f
human β-cell line
459 bp
268 bp
219 bp
GSTT1
HBB
GSTM1

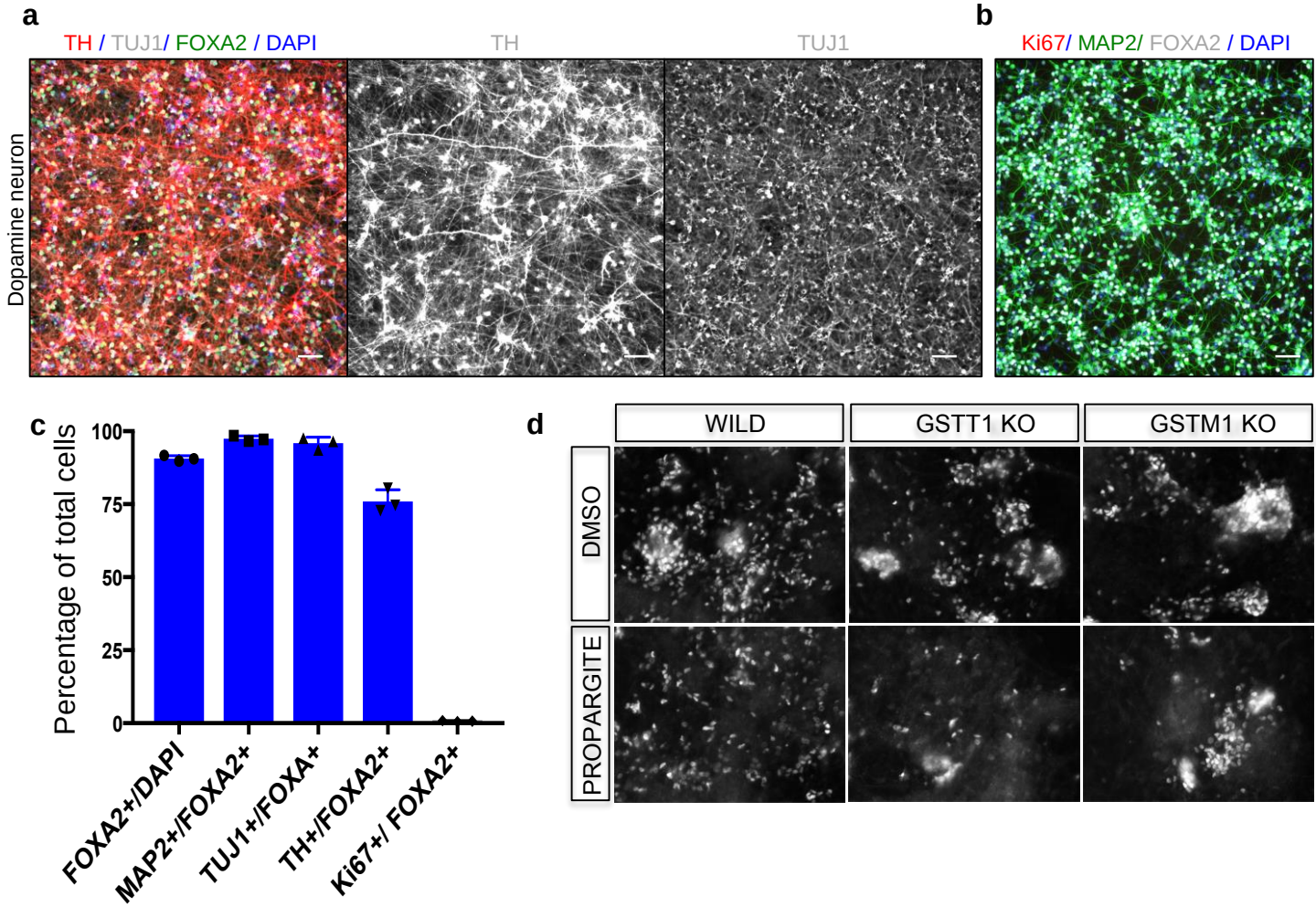
Supplementary Figure 3. GSH rescues the transcriptional alteration of propargite treated INS$^+$ cells, through an anti-oxidant independent manner.
(**a**) Transcript profiling showed that the transcriptional alteration in propargite-treated *INS*-GFP$^+$ cells were largely reversed by GSH, including gene expression related to pancreas development, pancreas function, DNA repair, and cell death. (**b**) Cellular Reactive Oxygen Species detection for different doses of propargite-treated MIN6 cells (n=3). 50 μM TBHP was used as a positive control. (**c**) Suggested mechanism for the GSH-dependent propargite detoxification pathway in rats. (**d, e**) Sequences of two representative biallelic mutants (*GSTT1*$^{-/-}$-1 and *GSTT1*$^{-/-}$-2) with frameshift mutations on *GSTT1* (d) and two representative biallelic mutants (*GSTM1*$^{-/-}$-1 and *GSTM1*$^{-/-}$-2) with frameshift mutations on *GSTM1* (e). (**f**) Genotype analysis of *GSTM1* and *GSTT1* in EndoC-βH1 cells. Values presented as mean±S.D. n.s. indicates a non-significant difference. *p* values calculated by unpaired two-tailed Student's t-test was ***$p<0.001$.


a
hESC-dervied MSCs
2nd antibody
MSCs
CD73
CD29
b
hESC-dervied cardiomyocytes (CMs)
phase contrast
MYH6:mCherry
Merge
c
hESCs
CMs
0.063
90.0
MYH6:mCherry
d
hESC-dervied neurons
MAP2
TUJ1
DAPI
Merge
HuC/D
MAP2
DAPI
Merge
% MAP2+
Neurons
e
hESC-dervied hepatocytes
phase contrast
A1AT
ASGPR1
DAPI
Merge
% A1AT+
Hepatocytes
f
INS+ β-like cells
IC50 = 1.09 μM
CD29+CD73+ MSCs
IC50 = 5.85 μM
MYH6:mCherry+ Cadiomyocytes
IC50 = 29.03 μM
HuC/D+ Neurons
IC50 = 1.46 μM
A1AT+ hepatocytes
IC50 = 13 μM
BJ-fibroblasts
IC50 = 3 μM
Cell suvival rate
Propargite Log C (μM)
g
β-like cells
MSCs
Cadiomyocytes
Neurons
Hepatocytes
BJ-fibroblasts
DMSO
1.6 μM Propargite
h
Cell suvival rate
***
n.s.
n.s.
***
n.s.
n.s.
β-like cells
MSCs
Cadiomyocytes
Neurons
Hepatocytes
BJ-fibroblasts
DMSO
1.6 μM Propargite

Supplementary Figure 4. Assessment of the toxic effect of propargite on different type of cells derived from hESCs, showing that HuC/D$^+$ neurons are highly susceptible to propargite.

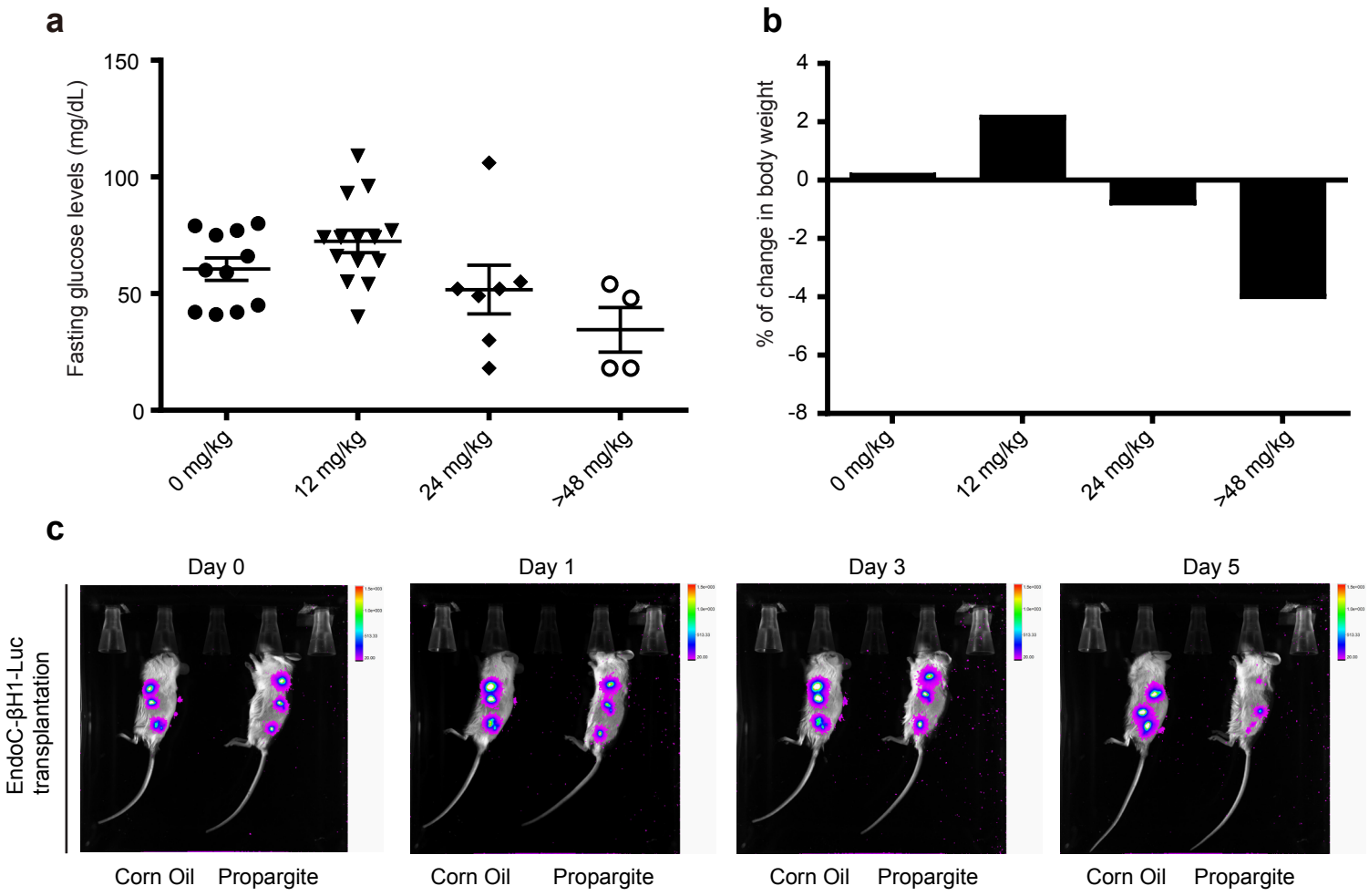
(**a**) Flow cytometry characterization of CD29$^+$ and CD73$^+$ mesenchymal stem cells (MSCs) derived from H1 hESCs. (**b-c**) microscope image (b) and flow cytometry (c) characterization of cardiomyocytes expressing MYH6+ derived from a MYH6:mCherry reporter hESC line. (**d-e**) Immunocytochemistry characterization of MAP2$^+$/TUJ1$^+$ and HuC/D$^+$/MAP2$^+$ neurons (d) and A1AT$^+$/ASGPR1$^+$ hepatocytes (e) derived from H1 hESCs. All the cells were counterstained by DAPI (blue). Scale bars, 100 μm. (**f**) Inhibition curve of propargite on pancreatic β-like cells, MSCs, cardiomyocytes, neurons, and hepatocytes derived from hESCs, and BJ-fibroblasts (n=3). Values presented as mean±S.D. (**g, h**) Representative images (g), and survival rate (h) of different types of cells derived from hESCs and BJ-fibroblasts in response to 1.6 μM propargite (n=3). All the cells were counterstained by DAPI (blue). Scale bars, 100 μm. Values presented as mean±S.D. n.s. indicates a non-significant difference. *p* values calculated by unpaired two-tailed Student's t-test was ***$p<0.001$.

Supplementary Figure 5



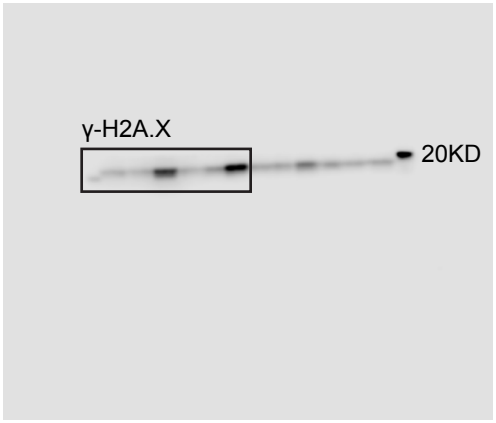
Supplementary Figure 5. Characterization of hESC-derived midbrain dopamine (mDA) neurons treated with/without propargite.
(**a-c**) More than 90% of hESC-derived mDA differentiated cells are FOXA2 positive. Among FOXA2 positive cells, more than 95% are MAP2 (a pan-neuronal marker) positive and 75% are TH (a mature DA marker which encodes late limit enzyme for dopamine production) positive, but less than 1% are Ki-67 (a marker for cell proliferation) positive. Values presented as mean±S.D. (**d**) Representative images of mDA differentiated $FOXA2^{+}$ cells treated with DMSO or propargite (3 μM) derived from isogenic wild type, *GSTT1*$^{-/-}$, and *GSTM1*$^{-/-}$ H1 hESC.

Supplementary Figure 6



Supplementary Figure 6. Propargite induces β-cell toxicity *in vivo*.
(**a**, **b**) Fasting mouse blood glucose level levels (a) and % of change in body weight (b) in corn oil (control) or different doses of propargite treated mice. Values presented as mean±S.D. (c) Representative images of *in vivo* imaging for luciferase signals from EndoC-βH1-Luc cells transplanted mice administrated with corn oil (vehicle) or propargite during the 5-day course.

Fig.2c



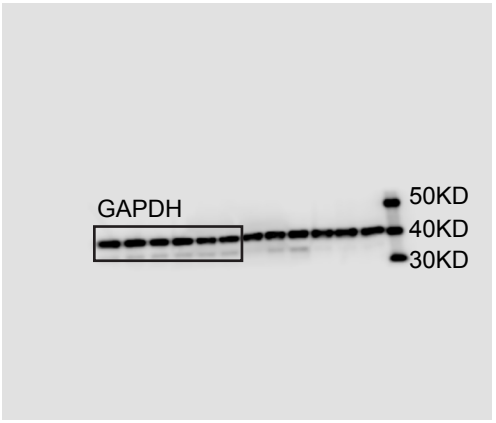
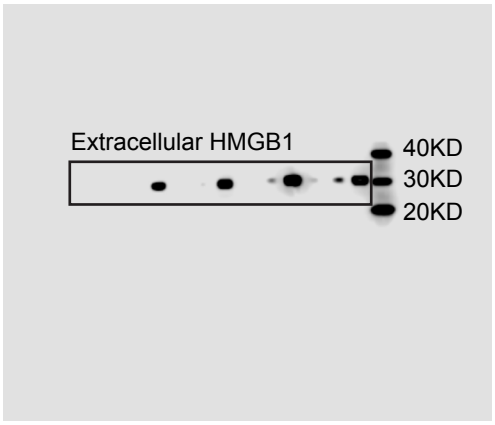


Fig.2d



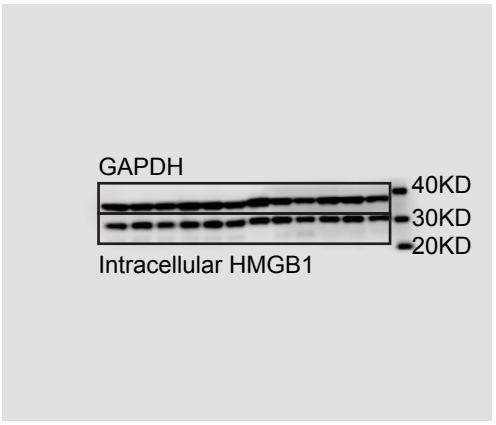
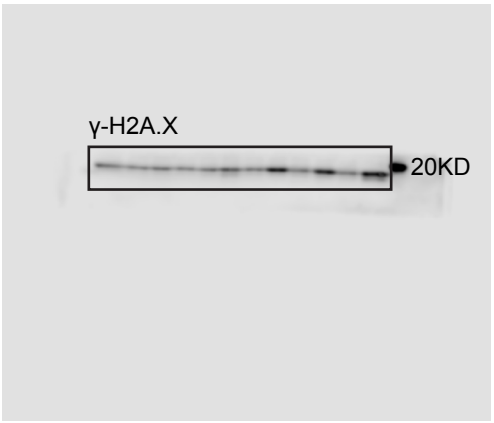
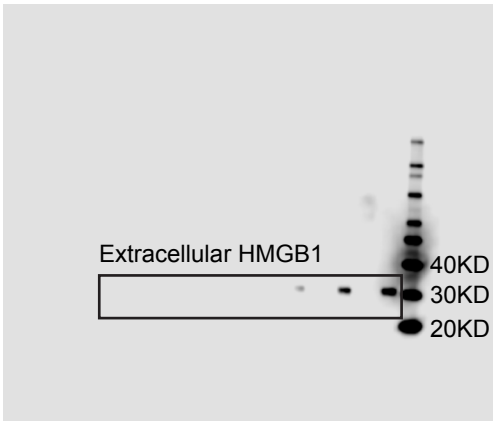


Fig.2e





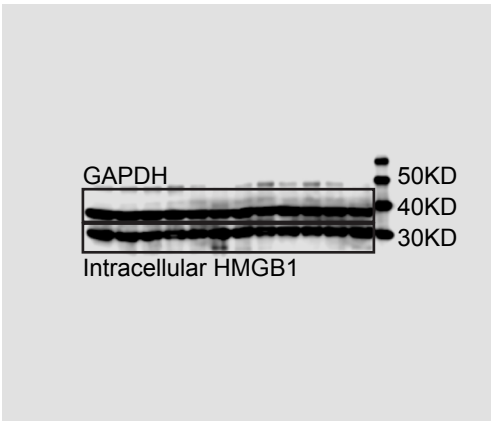
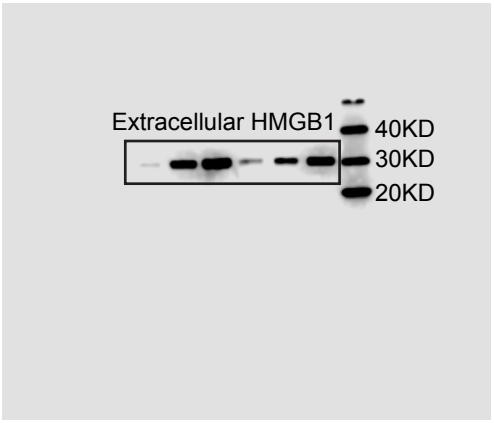
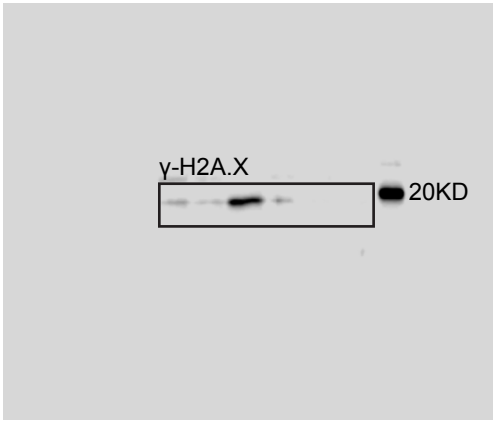


Fig.2m





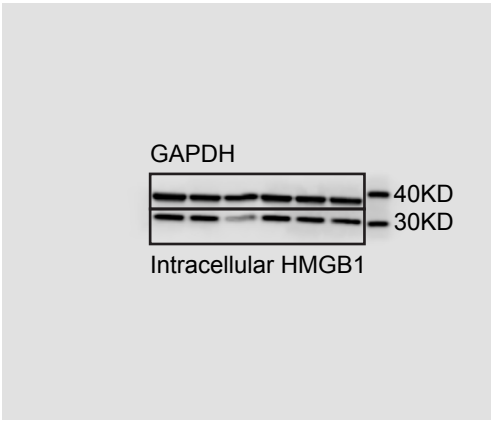
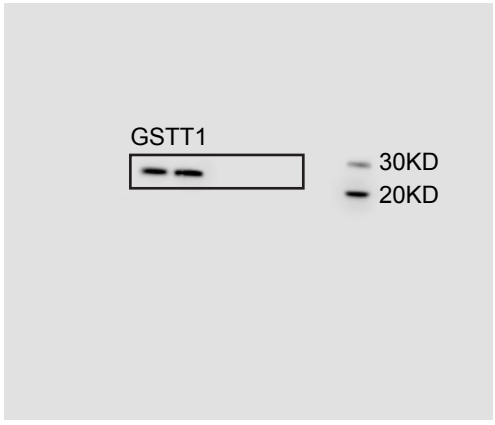
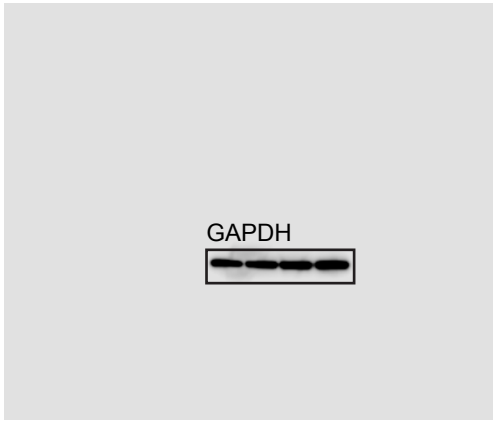
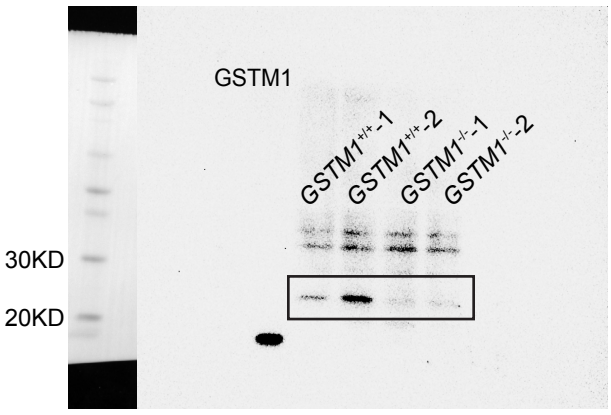


Fig. 3d


GSTT1
30KD
20KD


GAPDH


GSTM1
GSTM1+/+-1
GSTM1+/+-2
GSTM1-/--1
GSTM1-/--2
30KD
20KD

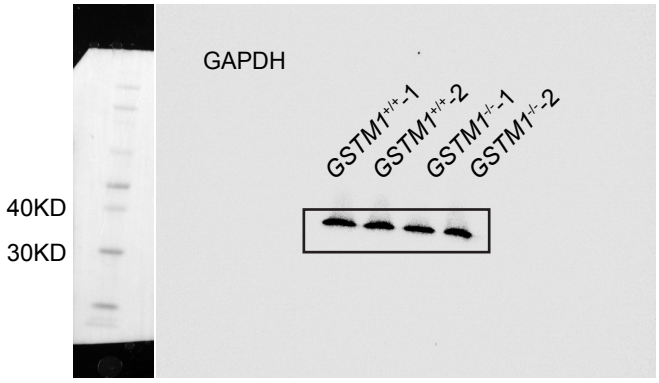

GAPDH
GSTM1+/+-1
GSTM1+/+-2
GSTM1-/--1
GSTM1-/--2
40KD
30KD

Fig. 3i


GSTT1
30KD
20KD

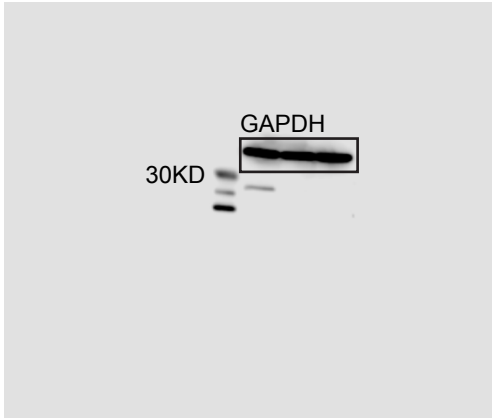
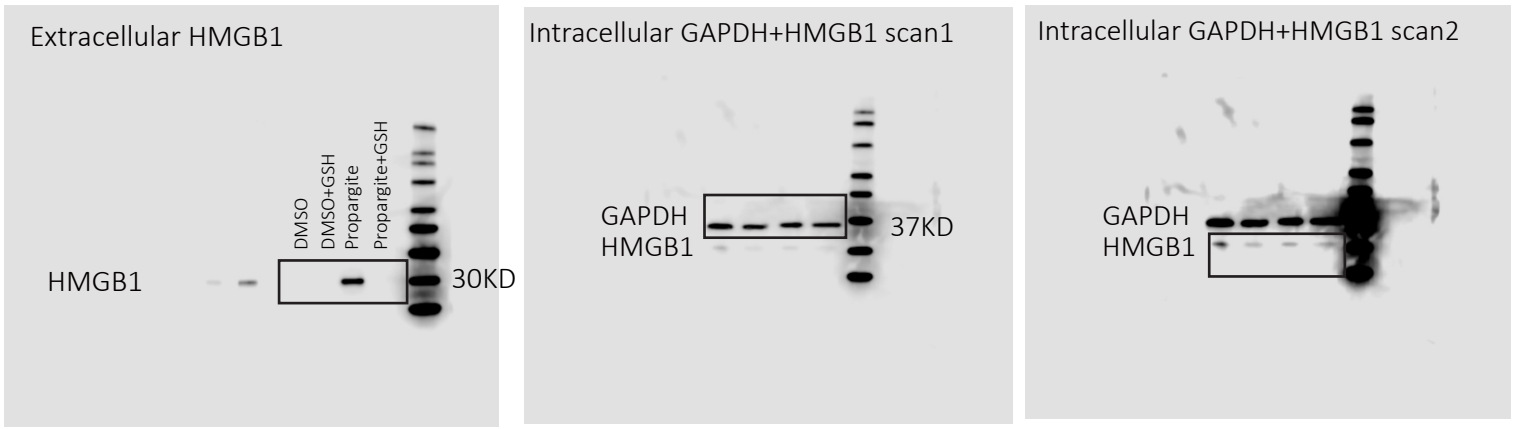
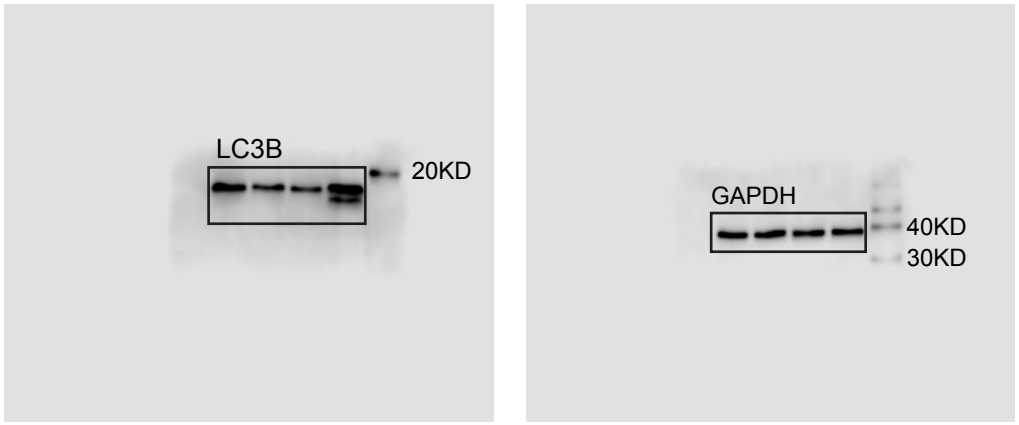

GAPDH
30KD

Fig. 4f



Supplementary Fig. 2f



Supplementary Figure 7. Uncropped blot images for Figure 2c, 2d, 2e, 2m, 3d, 3i, 4f and Supplementary Fig. 2f

Supplementary Table 1. qRT-PCR primers.

Gene	Forward (5' to 3')	Reverse (5' to 3')
DDIT3	GGAAACAGAGTGGTCATTCCC	CTGCTTGAGCCGTTCATTCTC
GADD45A	CAATTAGTGTCGTGCGGCC	TTCTGCACTCACTCACAGGC

Supplementary Table 2. sgRNA sequences for gene targeting.

Gene	CRISPR	sgRNA sequence (5' of PAM)	Targeted cells
GSTM1	sg*GSTM1*	ATGGACAACCATATGCAGCT	hESCs
GSTT1	sg*GSTT1*-1	TGGGTCGGCCTTCGAAGACT	hESCs/ EndoC-βH1 cells
	sg*GSTT1*-2	CTGGCCATGATCCGGTGAGC	EndoC-βH1 cells

Supplementary Table 3. PCR and sequencing primers (Forward) used for genotyping the knockout hESC lines.

Gene	Forward (5' to 3')	Reverse (5' to 3')
GSTM1	CAACGCCATCTTGTGCTACA	GTGTCCTAAGGTTGGTGGGA
GSTT1	TGGTCCCCAAATCAGATGCT	AAAGGACACAAGGCCTCAGT

Supplementary Table 4. Efficiency for the creation of biallelic knockout hESC lines.

Gene	Sequenced sub-clone	Monoallelic knockout	Biallelic knockout	Biallelic knockout Efficiency
GSTM1	22	2	15	68%
GSTT1	14	0	10	71%

Supplementary Table 5. PCR primers for genomic DNA amplification.

	PCR primers (5' to 3')
HBB-F	GAAGAGCCAAGGACAGGTAC
HBB-R	CAACTTCATCCACGTTCACC
GSTM1-F	GAACTCCCTGAAAAGCTAAAGC
GSTM1-R	CTTGGGCTCAAATATACGGTGG
GSTT1-F	TTCCTTACTGGTCCTCACATCTC
GSTT1-R	TCACCGGATCATGGCCAGCA

Supplementary Table 1. qRT-PCR primers.

Gene	Forward (5' to 3')	Reverse (5' to 3')
DDIT3	GGAAACAGAGTGGTCATTCCC	CTGCTTGAGCCGTTCATTCTC
GADD45A	CAATTAGTGTCGTGCGGCC	TTCTGCACTCACTCACAGGC

Supplementary Table 2. sgRNA sequences for gene targeting.

Gene	CRISPR	sgRNA sequence (5' of PAM)	Targeted cells
GSTM1	sg*GSTM1*	ATGGACAACCATATGCAGCT	hESCs
GSTT1	sg*GSTT1*-1	TGGGTCGGCCTTCGAAGACT	hESCs/ EndoC-βH1 cells
	sg*GSTT1*-2	CTGGCCATGATCCGGTGAGC	EndoC-βH1 cells

Supplementary Table 3. PCR and sequencing primers (Forward) used for genotyping the knockout hESC lines.

Gene	Forward (5' to 3')	Reverse (5' to 3')
GSTM1	CAACGCCATCTTGTGCTACA	GTGTCCTAAGGTTGGTGGGA
GSTT1	TGGTCCCCAAATCAGATGCT	AAAGGACACAAGGCCTCAGT

Supplementary Table 4. Efficiency for the creation of biallelic knockout hESC lines.

Gene	Sequenced sub-clone	Monoallelic knockout	Biallelic knockout	Biallelic knockout Efficiency
GSTM1	22	2	15	68%
GSTT1	14	0	10	71%

Supplementary Table 5. PCR primers for genomic DNA amplification.

	PCR primers (5' to 3')
HBB-F	GAAGAGCCAAGGACAGGTAC
HBB-R	CAACTTCATCCACGTTCACC
GSTM1-F	GAACTCCCTGAAAAGCTAAAGC
GSTM1-R	CTTGGGCTCAAATATACGGTGG
GSTT1-F	TTCCTTACTGGTCCTCACATCTC
GSTT1-R	TCACCGGATCATGGCCAGCA