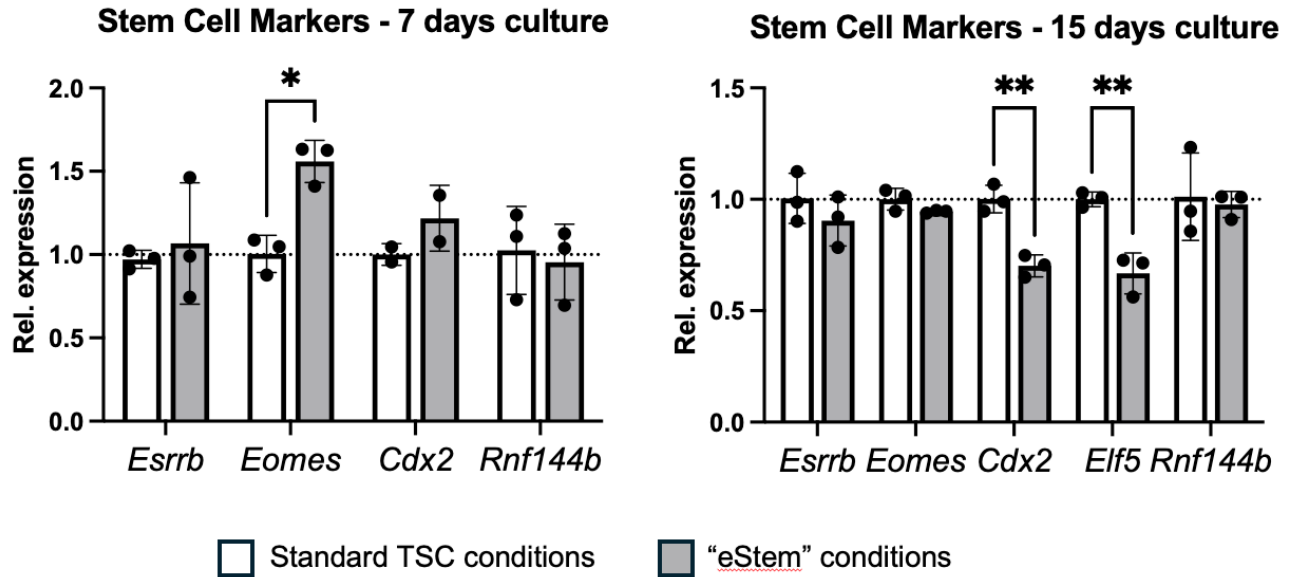


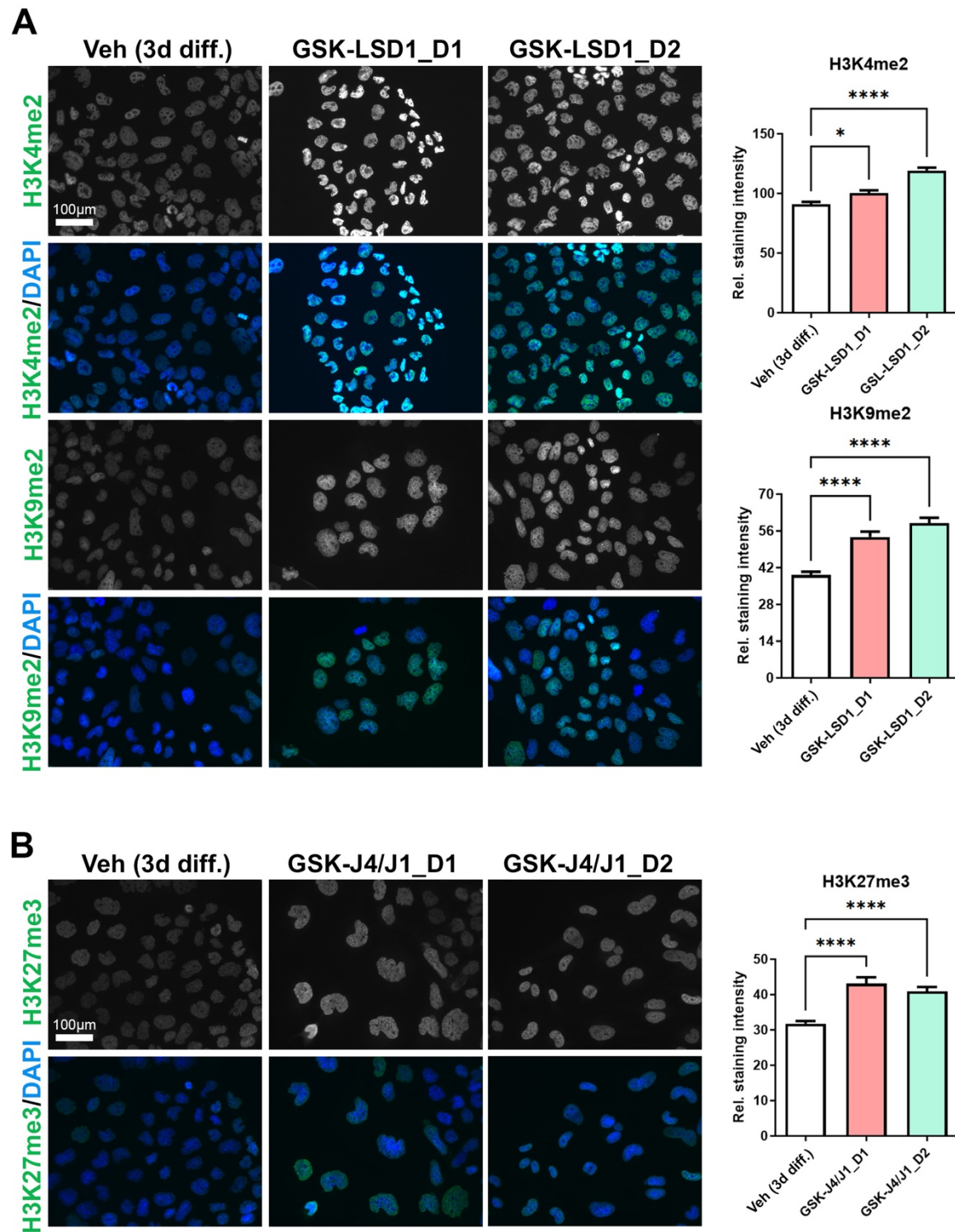
Suppl. Figure 1



Supplementary Figure 1. Test of stem cell marker enhancement using previously established "eStem" conditions.

RT-qPCR data of stem cell marker expression levels after 7 and 15 days of culture of mouse TSCs in either standard stem cell media conditions, or in standard stem cell media supplemented with "eStem" additives. In detail, standard TSC medium consisted of 20% fetal bovine serum, 1mM sodium pyruvate, 1x Antibiotic-Antimycotic, 55μM 2-mercaptoethanol, 37.5ng/ml bFGF, and 1μg/ml heparin in RPMI 1640 with L-Glutamine, with 70% of the medium pre-conditioned on mouse embryonic fibroblasts. "eStem" conditions used here consisted of the same medium but additionally supplemented with Br-cAMP (200nM), IL11 (50ng/mL), Activin A (50ng/mL), Bmp7 (25ng/mL) and LPA (5nM), with cells plated on Laminin-521 coated wells (as described by Seong *et al.*, Cell Stem Cell 2022). Statistical analysis was performed by two-way ANOVA. *p<0.05; **p<0.01 (N=3 biological replicates each performed in technical triplicate).

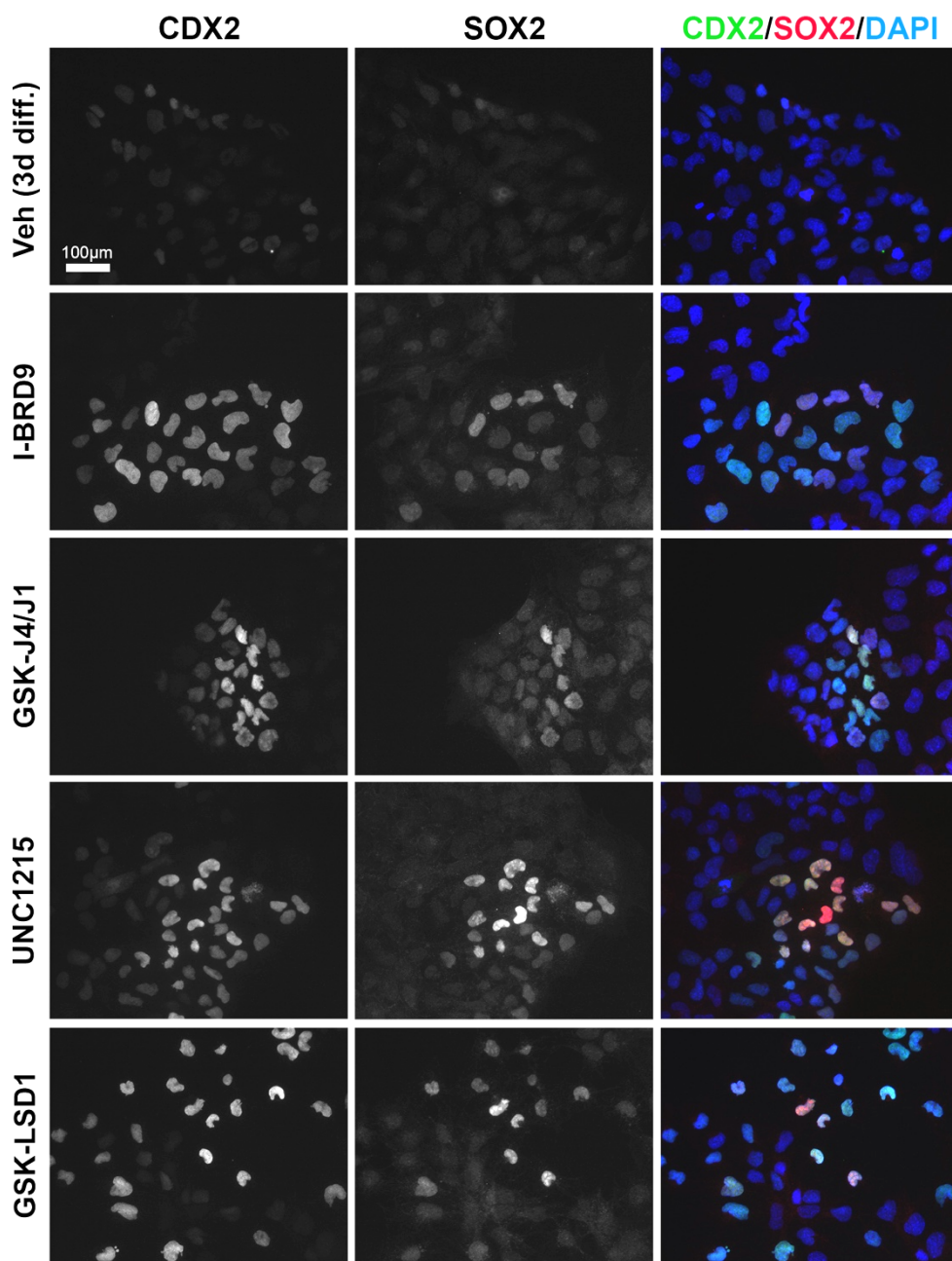
Suppl. Figure 2



Supplementary Figure 2. Validation of the effectiveness of epigenetic inhibitors.

A. Immunofluorescence staining for histone modifications H3K4me2 and H3K9me2 that are targets of the demethylase inhibitor GSK-LSD1 on TSCs differentiated for 3 days in the presence or absence of two different drug doses (Dose 1 = D1: IC50; Dose 2 = D2: 10x IC50). Quantification of immunofluorescence signals demonstrates a significant and dose-dependent increase in H3K4me2 and H3K9me2 in the presence of the inhibitor. Statistical analysis was performed by one-way ANOVA. * $p < 0.05$, **** $p < 0.0001$. **B.** Impact of H3K27-specific histone demethylase inhibitor GSK-J4/J1 on the abundance of H3K27me3 signal. Quantification of immunofluorescence signals demonstrates a significant increase in H3K27me3 staining in the presence of the inhibitor. All images were taken at identical exposure settings and are representative of 5 independent regions each. For statistical analysis, all 5 regions were analyzed. Statistical analysis was performed by one-way ANOVA. **** $p < 0.0001$.

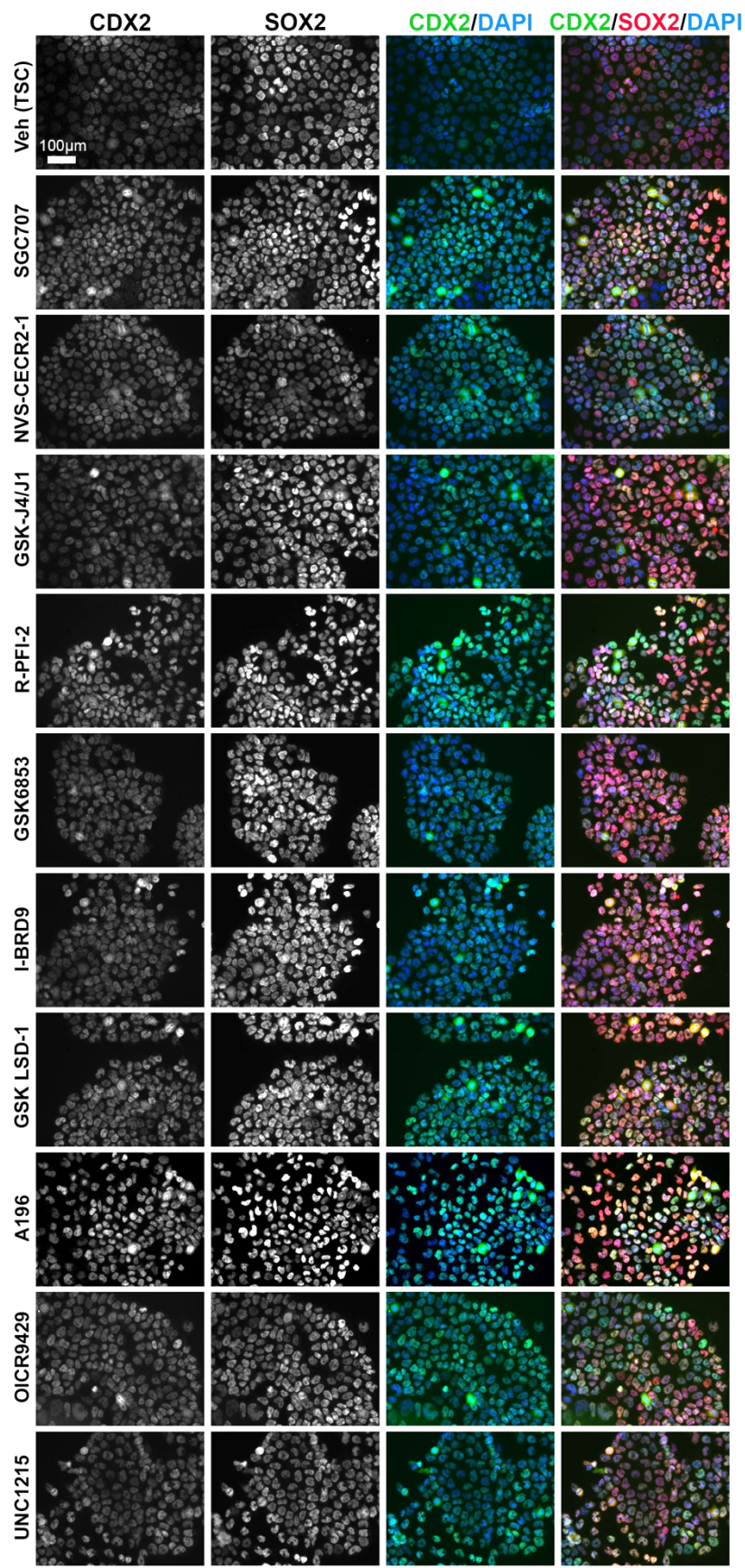
Suppl. Figure 3



Supplementary Figure 3. Delayed loss of TSC stemness markers in the presence of various epigenetic inhibitors.

CDX2 and SOX2 double immunofluorescence staining on TSCs differentiated for 3 days in the presence of vehicle (Veh) control or the indicated inhibitors at IC50 concentration. Combined presence of CDX2 and SOX2 indicates retention of stem-like characteristics. All images were taken at identical exposure settings and are representative of 5 independent regions each.

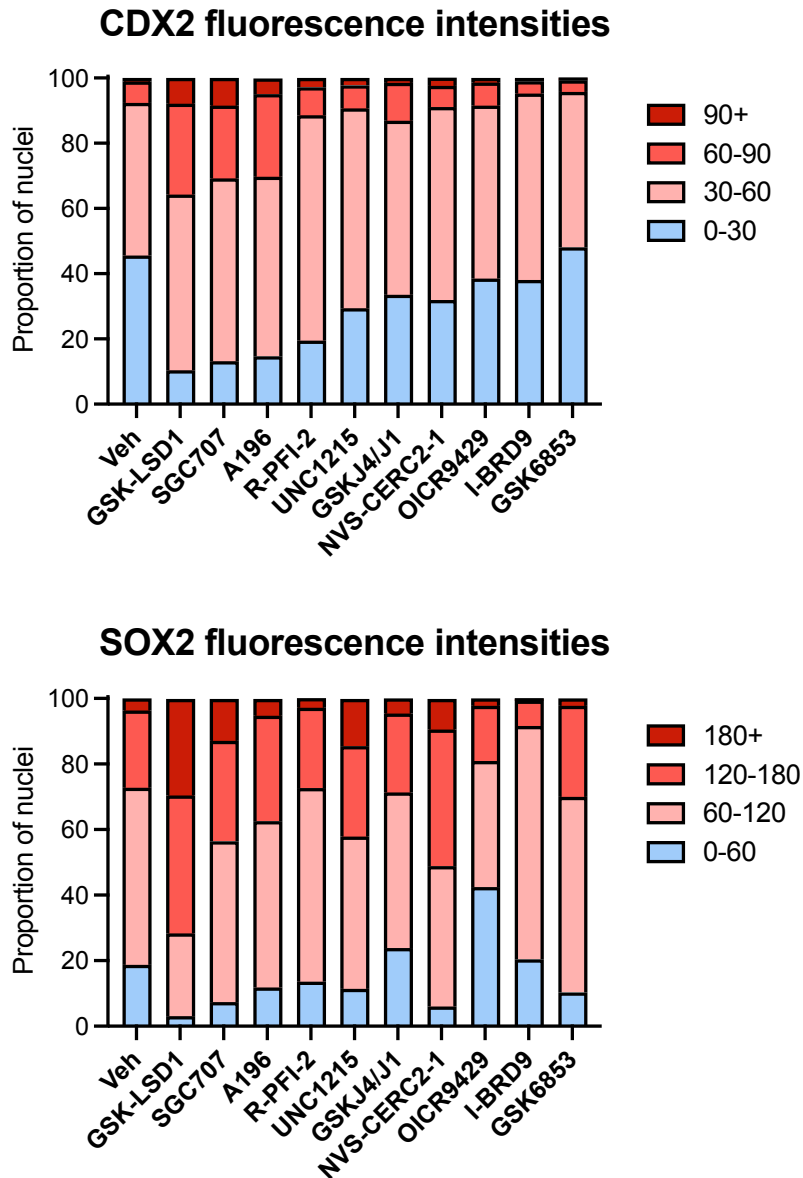
Suppl. Figure 4



Supplementary Figure 4. Enhanced stemness characteristics in the presence of various epigenetic inhibitors.

CDX2 and SOX2 double immunofluorescence staining on TSCs grown in stem cell conditions in the presence of vehicle (Veh) control or the indicated inhibitors at IC₅₀ concentration. All images were taken at identical exposure settings and are representative of 5 independent regions each.

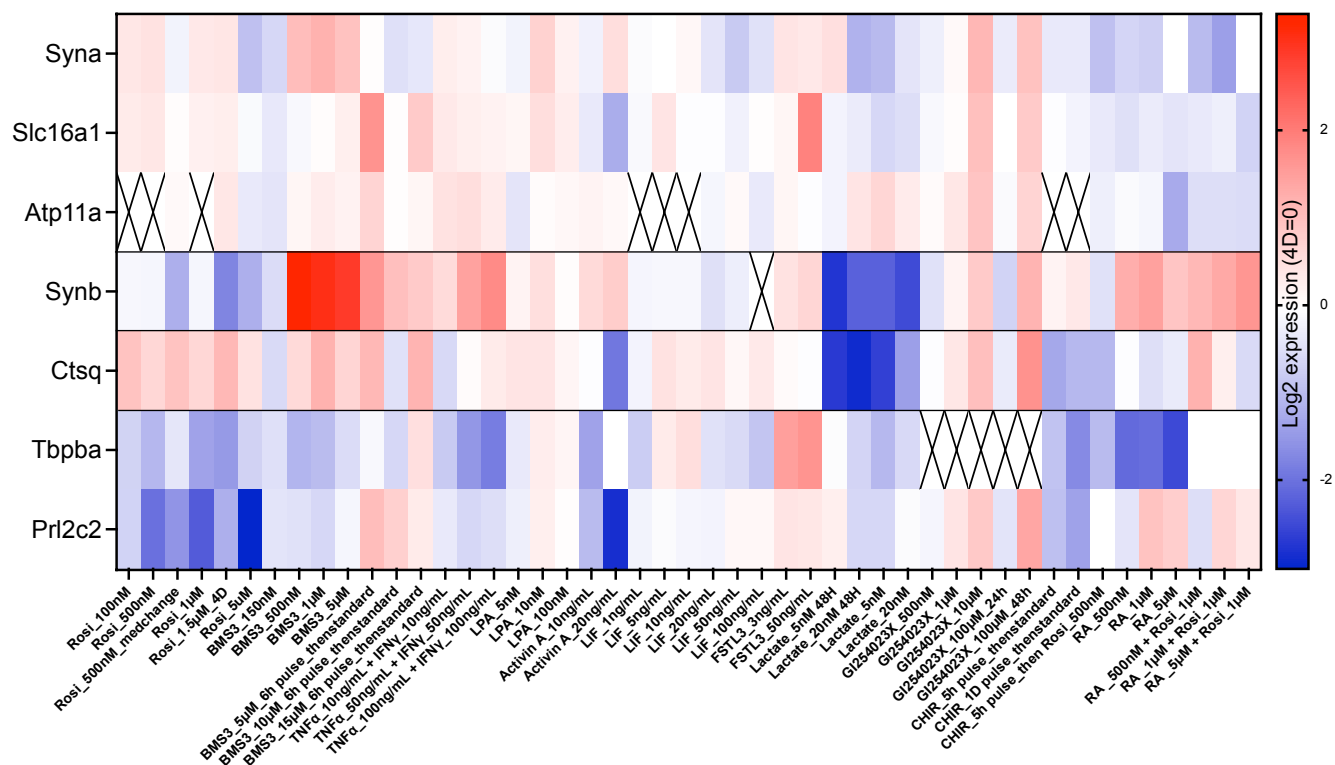
Suppl. Figure 5



Supplementary Figure 5. Quantification of CDX2 and SOX2 staining intensities.

CDX2 and SOX2 immunofluorescence staining intensities (as quantified and shown in Fig. 3C) were binned into arbitrarily defined low-, medium- and high-expressing cells to display proportions in each category. Intensity values are in arbitrary units.

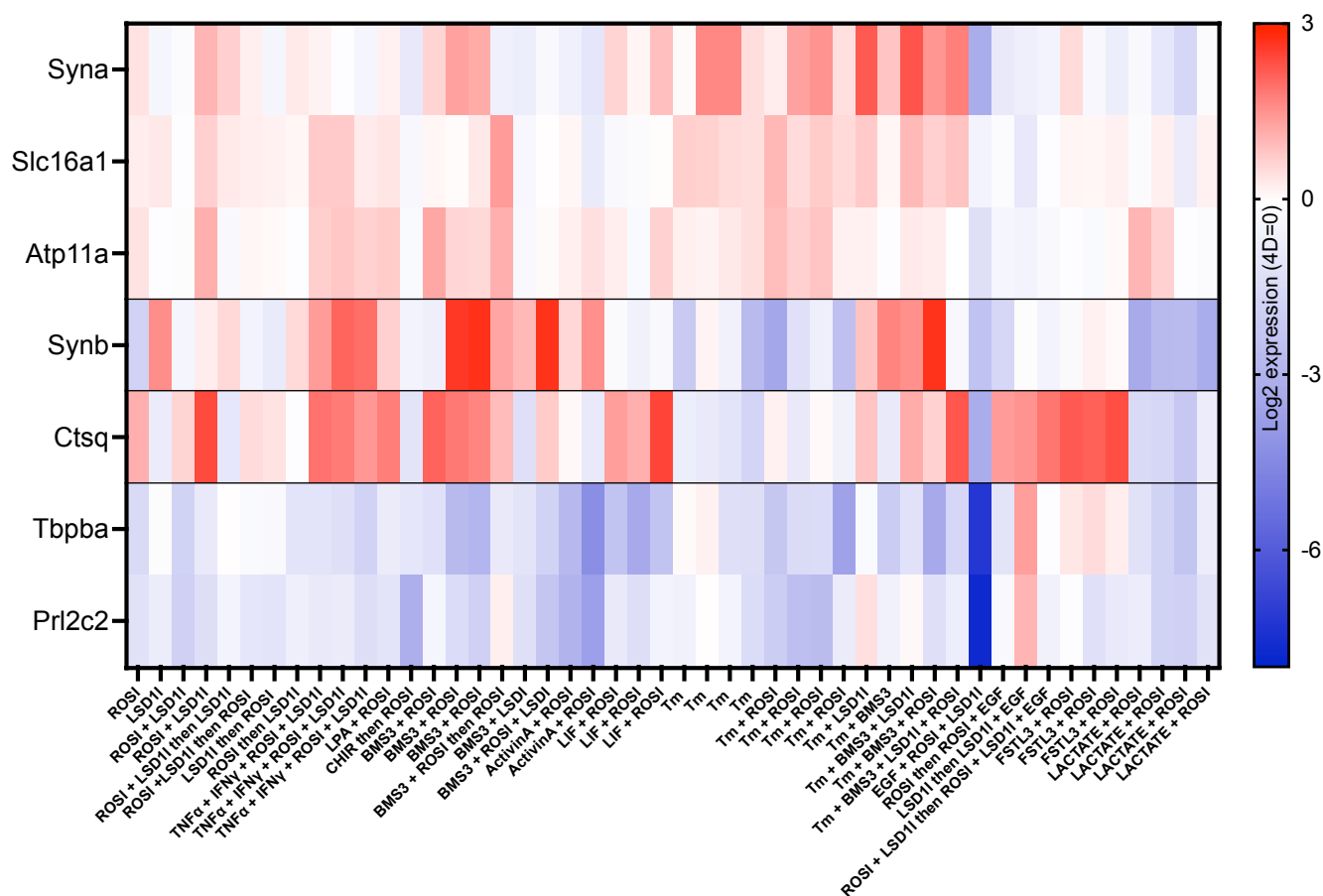
Suppl. Figure 6



Supplementary Figure 6. Screen of the effects of individual media additives on TSC differentiation.

Heatmap of trophoblast cell type-specific marker gene expression levels displayed as Log₂-transformed values after normalization to vehicle control (N=1 for all doses, except for all ROSI-only doses and all CHIR + ROSI for which N=3).

Suppl. Figure 7



Supplementary Figure 7. Broad screen of combinatorial drug treatment regimens aimed at optimizing conditions that specifically promote SynT-I differentiation.

Heatmap of trophoblast cell type-specific marker gene expression levels displayed as Log₂-transformed values after normalization to vehicle control. The precise treatment details are provided in Supplementary Table 3. LSD11 refers to the use of the KDM1A inhibitor GSK-LSD1 (N=1-2 for all doses, except ROSI-only for which N=3).

Supplementary Table 1: Drug doses

DRUG	Domain: Protein target	Alt. name	IC50 (nM)	IC90 (μM)	DOSES USED	
					Dose 1	Dose 2
A196	MT: SUV420H1/H2	KMT5B/C	24	1	24nM	48nM
A366	MT: G9A/GLP	EHMT2/1	3.3/38	1	3.3nM	38nM
A395	KME: EED		34	1	34nM	68nM
BAY-299	BRD: BRPF2/TAF1	BRD1/TAF1	67/14	1	14nM	67nM
BAY-598	MT: SMYD2	KMT3C	27	1	27nM	270nM
BAY-850	BRD: ATAD2A	ATAD2	85/753	3	85nM	753nM
BAZ2-ICR	BRD: BAZ2A/2B		130/180	1	130nM	180nM
BI-9564	BRD: BRD9/7		14/239	1	14nM	293nM
GSK-J4/J1	KDM: JMJD3/UTX	KDM6B/A	60	5	60nM	600nM
GSK-LSD1	KDM: LSD1	KDM1A	16	1	16nM	160nM
GSK2801	BRD: BAZ2A/2B		257/136	1	136nM	257nM
GSK343	MT: EZH2		4	3	4nM	40nM
GSK4027	BRD: PCAF/GCN5	KAT2B/A	60	1	60nM	120nM
GSK591	MT: PRMT5		11	1	11nM	110nM
GSK6853	BRD: BRPF1B	BRPF1	8	1	8nM	80nM
GSK864	DEHYDR: IDH1 mutant		9/15/20/17	0.3	9nM	17nM
GSK8814	BRD: ATAD2A/B	ATAD2/2B	50	1	50nM	500nM
I-BRD9	BRD: BRD9		67	1	67nM	670nM
I-CBP112	BRD: CREBBP/EP300		151/625	1	151nM	625nM
(+)-JQ1	BRD: BET Family		50/90	1	50nM	90nM
MS023	MT: PRMT type 1		39/135/93/4/5	1	4nM	135nM
MS049	MT: PRMT4/6	CARM1/PRMT6	44/63	5	65nM	130nM
NI-57	BRD: BRPRF1/2/3		31/108/408	1	31nM	408nM
NVS-CECR2-1	BRD: CECR2		8	1	8nM	80nM
OF-1	BRD: BRPRF1/2/3		100/500/2400	1	100nM	2.4μM
OIRC9429	WD40: WDR5		64	3	64nM	640nM
PFI-3	BRD: SMARCA2/4		89/48	1	48nM	89nM
R-PFI-2	MT: SETD7		2	1	2nM	20nM
SGC-CBP30	BRD: CREBBP/EP300		21/38	1	21nM	38nM
SGC0946	MT: DOT1L		0.4	1	0.4nM	4nM
SGC707	MT: PRMT3		33	1	33nM	330nM
TP-064	MT: PRMT4	CARM1	<10	1	10nM	100nM
TP472	BRD: BRD9/7		33/340	1	33nM	340nM
UNC1215	KME: L3MBTL3		40	1	40nM	400nM
UNC1999	MT: EZH2/1		10/45 ± 3	3	50nM	100nM

Supplementary Table 2: Drug treatment regimens displayed in main figures

Figure	ID	REAGENT	DOSE (in standard differentiation media)
Fig. 4B	1	ACTIVIN A	Activin A (10ng/mL) for 4 days
Fig. 4B	2	ACTIVIN A	Activin A (20ng/mL) for 4 days
Fig. 4B	3	ACTIVIN A + ROSI	Activin A (10ng/mL)+ Rosiglitazone (1 μ M) for 4 days
Fig. 4B	4	ACTIVIN A + ROSI	Activin A (20ng/mL) + Rosiglitazone (1 μ M) for 4 days
Fig. 4B	5	CHIR99021	CHIR99021 (3 μ M) for 4 days
Fig. 5B	1	ROSI	Rosiglitazone (100nM) for 4 days
Fig. 5B	2	ROSI	Rosiglitazone (500nM) for 4 days
Fig. 5B	3	ROSI	Rosiglitazone (1 μ M) for 4 days
Fig. 5B	4	ROSI	Rosiglitazone (1.5 μ M) for 4 days
Fig. 5B	5	ROSI	Rosiglitazone (5 μ M) for 4 days
Fig. 5C	1	ROSI	Rosiglitazone (1.5 μ M) for 4 days
Fig. 5C	2	GSK-LSD1	GSK-LSD1 (IC50) for 4 days
Fig. 5C	3	ROSI + GSK-LSD1	Rosiglitazone (1.5 μ M) + GSK-LSD1 (IC50) media refresh at day 2, treatment for 4 days
Fig. 5C	4	ROSI + GSK-LSD1 -> GSK-LSD1	Rosiglitazone (1.5 μ M) + GSK-LSD1 (IC50) for 2 days THEN GSK-LSD1 (IC50) for 2 days
Fig. 5C	5	ROSI + GSK-LSD1 -> ROSI	Rosiglitazone (1.5 μ M) + GSK-LSD1 (IC50) for 2 days THEN Rosiglitazone (1.5 μ M) for 2 days
Fig. 5C	6	GSK-LSD1 -> ROSI	GSK-LSD1 (IC50) for 2 days THEN Rosiglitazone (1.5 μ M) for 2 days
Fig. 5C	7	ROSI -> GSK-LSD1	Rosiglitazone (1.5 μ M) for 2 days THEN GSK-LSD1 (IC50) for 2 days
Fig. 6B	1	TM	Tunicamycin 0.05 μ g/mL for 4 days
Fig. 6B	2	TM	Tunicamycin 0.06 μ g/mL for 4 days
Fig. 6B	3	TM	Tunicamycin 0.075 μ g/mL for 4 days
Fig. 6B	4	TM	Tunicamycin 0.1 μ g/mL for 4 days
Fig. 6B	5	TM + ROSI	Tunicamycin 0.05 μ g/mL + Rosiglitazone (1 μ M) for 4 days
Fig. 6B	6	TM + ROSI	Tunicamycin 0.06 μ g/mL + Rosiglitazone (1 μ M) for 4 days
Fig. 6B	7	TM + ROSI	Tunicamycin 0.075 μ g/mL + Rosiglitazone (1 μ M) for 4 days
Fig. 6B	8	TM + ROSI	Tunicamycin 0.1 μ g/mL+ Rosiglitazone (1 μ M) for 4 days
Fig. 6B	9	TM + GSK-LSD1	Tunicamycin 0.075 μ g/mL + GSK-LSD1 (IC50) for 4 days
Fig. 7B	1	BMS3	BMS-3 (150nM) for 4 days
Fig. 7B	2	BMS3	BMS-3 (500nM) for 4 days
Fig. 7B	3	BMS3	BMS-3 (1 μ M) for 4 days
Fig. 7B	4	BMS3	BMS-3 (5 μ M)
Fig. 7B	5	BMS3	Standard base media for 24h THEN BMS-3 (5uM) for 6h THEN standard base media for the remainder of the 4 days
Fig. 7B	6	BMS3	Standard base media for 24h THEN BMS-3 (10uM) for 6h THEN standard base media for the remainder of the 4 days
Fig. 7B	7	BMS3	Standard base media for 24h THEN BMS-3 (15uM) for 6h THEN standard base media for the remainder of the 4 days
Fig. 7B	8	BMS3 + ROSI	BMS-3 (150nM) + Rosiglitazone (1 μ M) for 4 days

Fig. 7B	9	BMS3 + ROSI	BMS-3 (500nM) + Rosiglitazone (1µM) for 4 days
Fig. 7B	10	BMS3 + ROSI	BMS-3 (1µM) + Rosiglitazone (1µM) for 4 days
Fig. 7B	11	BMS3 + ROSI -> ROSI	Standard base media for 24h, THEN BMS-3 (10µM) + Rosiglitazone (1µM) for 6h THEN Rosiglitazone (1µM) for the remainder of the 4 days
Fig. 7B	12	TM + BMS3	Tunicamycin 0.075 µg/mL + BMS-3 (150nM) for 4 days
Fig. 7B	13	TM + BMS3 + GSK-LSD1	Tunicamycin 0.075 µg/mL + BMS-3 (150nM) + GSK-LSD1 (IC50) for 4 days
Fig. 7B	14	TM + BMS3 + ROSI	Tunicamycin 0.075 µg/mL + BMS-3 (150nM) + Rosiglitazone (1µM) for 4 days
Fig. 7B	15	TM + BMS3 + GSK-LSD1 + ROSI	Tunicamycin 0.075 µg/mL + BMS-3 (150nM) + GSK-LSD1 (IC50) + Rosiglitazone (1µM) for 4 days

Supplementary Table 3: Extended treatment regimens and drug combinations

CONDITION	TREATMENT (in standard differentiation media)
ROSI	Rosiglitazone (1.5µM) for 4 days
LSD1I	GSK-LSD1 (IC50) for 4 days
ROSI + LSD1I	Rosiglitazone (1.µM) + GSK-LSD1 (IC50) for 4 days
ROSI + LSD1I	Rosiglitazone (1.5µM) + GSK-LSD1 (IC50) media refresh at day 2, treatment for 4 days
ROSI + LSD1I then LSD1I	Rosiglitazone (1.5µM) + GSK-LSD1 (IC50) for 2 days THEN GSK-LSD1 (IC50) for 2 days
ROSI +LSD1I then ROSI	Rosiglitazone (1.5µM) + GSK-LSD1nhibitor (IC50) for 2 days THEN Rosiglitazone (1.5µM) for 2 days
LSD1I then ROSI	GSK-LSD1 (IC50) for 2 days THEN Rosiglitazone (1.5µM) for 2 days
ROSI then LSD1I	Rosiglitazone (1.5µM) for 2 days THEN GSK-LSD1 (IC50) for 2 days
TNFα + IFNγ + ROSI + LSD1I	TNFα (10ng/mL) + IFNγ (10ng/mL) + Rosiglitazone (1.5µM) + GSK-LSD1 (IC50) for 4 days
TNFα + IFNγ + ROSI + LSD1I	TNFα (50ng/mL) + IFNγ (50ng/mL) + Rosiglitazone (1.5µM) + GSK-LSD1 (IC50) for 4 days
TNFα + IFNγ + ROSI + LSD1I	TNFα (100ng/mL) + IFNγ (100ng/mL) + Rosiglitazone (1.5µM) + GSK-LSD1 (IC50) for 4 days
LPA + ROSI	1-Oleoyl lysophosphatidic acid (10nM) + Rosiglitazone (1.5µM) for 4 days
CHIR then ROSI	Chiron99021 (3µM) for 5hr THEN Rosiglitazone (500nM) for the remainder of the 4 days
BMS3 + ROSI	BMS-3 (150nM) + Rosiglitazone (1µM) for 4 days
BMS3 + ROSI	BMS-3 (500nM) + Rosiglitazone (1µM) for 4 days
BMS3 + ROSI	BMS-3 (1µM) + Rosiglitazone (1µM) for 4 days
BMS3 + ROSI then ROSI	Standard base media for 24h, THEN BMS-3 (10µM) + Rosiglitazone (1µM) for 6h THEN Rosiglitazone (1µM) for the remainder of the 4 days
BMS3 + LSDI	BMS-3 (150nM) + GSK-LSD1 (IC50) for 4 days
BMS3 + ROSI + LSDI	BMS-3 (150nM) + Rosiglitazone (1µM) + GSK-LSD1 (IC50) for 4 days
ActivinA + ROSI	Activin A (10ng/mL)+ Rosiglitazone (1µM) for 4 days
ActivinA + ROSI	Activin A (20ng/mL) + Rosiglitazone (1µM) for 4 days
LIF + ROSI	LIF (20ng/mL) + Rosiglitazone (1µM) for 4 days
LIF + ROSI	LIF (50ng/mL) +Rosiglitazone (1µM) for 4 days
LIF + ROSI	LIF (100gn/mL) + Rosiglitazone (1µM) for 4 days
Tm	Tunicamycin (0.05µg/mL) for 4 days
Tm	Tunicamycin (0.06µg/mL) for 4 days
Tm	Tunicamycin (0.075µg/mL) for 4 days
Tm	Tunicamycin (0.1µg/mL) for 4 days
Tm + ROSI	Tunicamycin (0.05µg/mL) + Rosiglitazone (1µM) for 4 days
Tm + ROSI	Tunicamycin (0.06µg/mL) + Rosiglitazone (1µM) for 4 days
Tm + ROSI	Tunicamycin (0.075µg/mL) + Rosiglitazone (1µM) for 4 days
Tm + ROSI	Tunicamycin (0.1µg/mL) + Rosiglitazone (1µM) for 4 days
Tm + LSD1I	Tunicamycin (0.075µg/mL) + GSK-LSD1 (IC50) for 4 days
Tm + BMS3	Tunicamycin (0.075µg/mL) + BMS-3 (150nM) for 4 days

Tm + BMS3 + LSD1I	Tunicamycin (0.075µg/mL) + BMS-3 (150nM) + GSK-LSD1 (IC50) for 4 days
Tm + BMS3 + ROSI	Tunicamycin (0.075µg/mL) + BMS-3 (150nM) + Rosiglitazone (1µM) for 4 days
Tm + BMS3 + LSD1I + ROSI	Tunicamycin (0.075µg/mL) + BMS-3 (150nM) + GSK-LSD1 (IC50) + Rosiglitazone (1µM) for 4 days
EGF + ROSI + LSD1I	EGF (100ng/mL) + Rosiglitazone (1µM) + GSK-LSD1 (IC50) for 4 days
ROSI then ROSI + EGF	Rosiglitazone (1.5µM) for 2 days THEN Rosiglitazone (1.5µM) + EGF (100ng/mL) for 2 days
LSD1I then LSD1I + EGF	GSK-LSD1 (IC50) for 2 days THEN GSK-LSD1 (IC50) + EGF (100ng/mL) for 2 days
ROSI + LSD1I then ROSI + LSD1I + EGF	Rosiglitazone (1.5µM) + GSK-LSD1 (IC50) for 2 days THEN Rosiglitazone (1.5µM) + GSK-LSD1 (IC50) + EGF (100ng/mL) for 2 days
FSTL3 + ROSI	FSTL3 (3ng/mL) + Rosiglitazone (1µM) for 4 days
FSTL3 + ROSI	FSTL3 (15ng/mL) + Rosiglitazone (1µM) for 4 days
FSTL3 + ROSI	FSTL3 (50ng/mL) + Rosiglitazone (1µM) for 4 days
LACTATE + ROSI	Lactate (5nM) + Rosiglitazone (1µM) for 2 days THEN Rosiglitazone (1µM) only for 2 days
LACTATE + ROSI	Lactate (20nM) + Rosiglitazone (1µM) for 2 days THEN Rosiglitazone (1µM) only for 2 days
LACTATE + ROSI	Lactate (5nM) + Rosiglitazone (1µM) for 4 days
LACTATE + ROSI	Lactate (20nM) + Rosiglitazone (1µM) for 4 days

Supplementary Table 4: RT-qPCR primer sequences

Marker of	Target	Primer	5'-3' sequence
Stem	<i>Cdx2</i>	FWD	AGTGAGCTGGCTGCCACACT
		REV	GCTGCTGCTGCTTCTTCTTGA
Stem	<i>Eomes</i>	FWD	TCGCTGTGACGGCCTACCAA
		REV	AGGGGAATCCGTGGGAGATGGA
Stem	<i>Esrrb</i>	FWD	AGTACAAGCGACGGCTGG
		REV	CCTAGTAGATTCTGAGACGATCTTAGTCA
Stem	<i>Spry4</i>	FWD	GACTGACCCACTCGGGTTTCG
		REV	GGCGCTCTGCTGTCAAGGAG
Stem	<i>Dusp6</i>	FWD	TGAGAATGCGGGCGAGTTCA
		REV	AGCGGCTGATACCTGCCAAG
Stem	<i>Bmp4</i>	FWD	TGGCCCTCGACCAGGTTTAT
		REV	AAGGCTCAGAGAAGCTGCGG
Stem	<i>Elf5</i>	FWD	ATTCGCTCGCAAGGTTACTCC
		REV	GGATGCCACAGTTCTCTTCAGG
Early diff.	<i>Rnf144b</i>	FWD	GCGCCCAGATGATGTGCAAGA
		REV	CCCCACTACCTGTGTTCCGT
SynT-I	<i>Syna</i>	FWD	CCTCACCTCCCAGGCCCTC
		REV	GGCAGGGAGTTTGCCACGA
SynT-I	<i>Slc16a1 (Mct1)</i>	FWD	CTCCAGTGCTGTGGGCTTGG
		REV	GCGATGATGAGGATCACGCCA
SynT-I	<i>Atp11a</i>	FWD	ACTGGACGTTTCTCGGCGTT
		REV	TGAACACCAGCGTCCCGAAG
SynT-I	<i>Bmper</i>	FWD	CAGAGAGGCGCCTGCTGTGAAC
		REV	AGCCGGGGTTTGCCACTTGAAG
SynT-II	<i>Synb</i>	FWD	GGCAACTCTCCGCAGCTGACAC
		REV	ACGAACCAGCTGTGCTTGAGCC
SynT-II	<i>Gcm1</i>	FWD	ACTTCTGGAGGCACGACGGA
		REV	TCGGGATTTACAGCAGGAAGCG
sTGCs	<i>Ctsq</i>	FWD	AATTGGCTATGGTTATGTGGGA
		REV	TCACACAGTAGGGTATTGGG
SpT	<i>Tpbpa</i>	FWD	ACTGGAGTGCCCAGCACAGC
		REV	GCAGTTCAGCATCCAAGTGGC
TGC	<i>Prl2c2 (Plf)</i>	FWD	AACGCAGTCCGGAACGGGG
		REV	TGTCTAGGCAGCTGATCATGCCA
Housekeeping	<i>Sdha</i>	FWD	TGGTGAGAACAAAGAAGGCATCA
		REV	CGCCTACAACCACAGCATCA