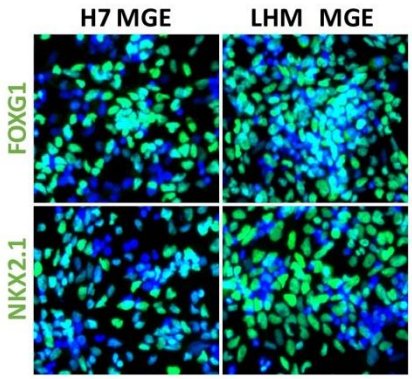

Fig S1. MGE induction of hESCs
Day 21 LHM differentiation cultures were immunostained for neural progenitor markers representing pan-forebrain (FOXG1) and MGE (NKX2.1) with DAPI counter stain (Blue). The panels shown are higher magnification of figure 1C.

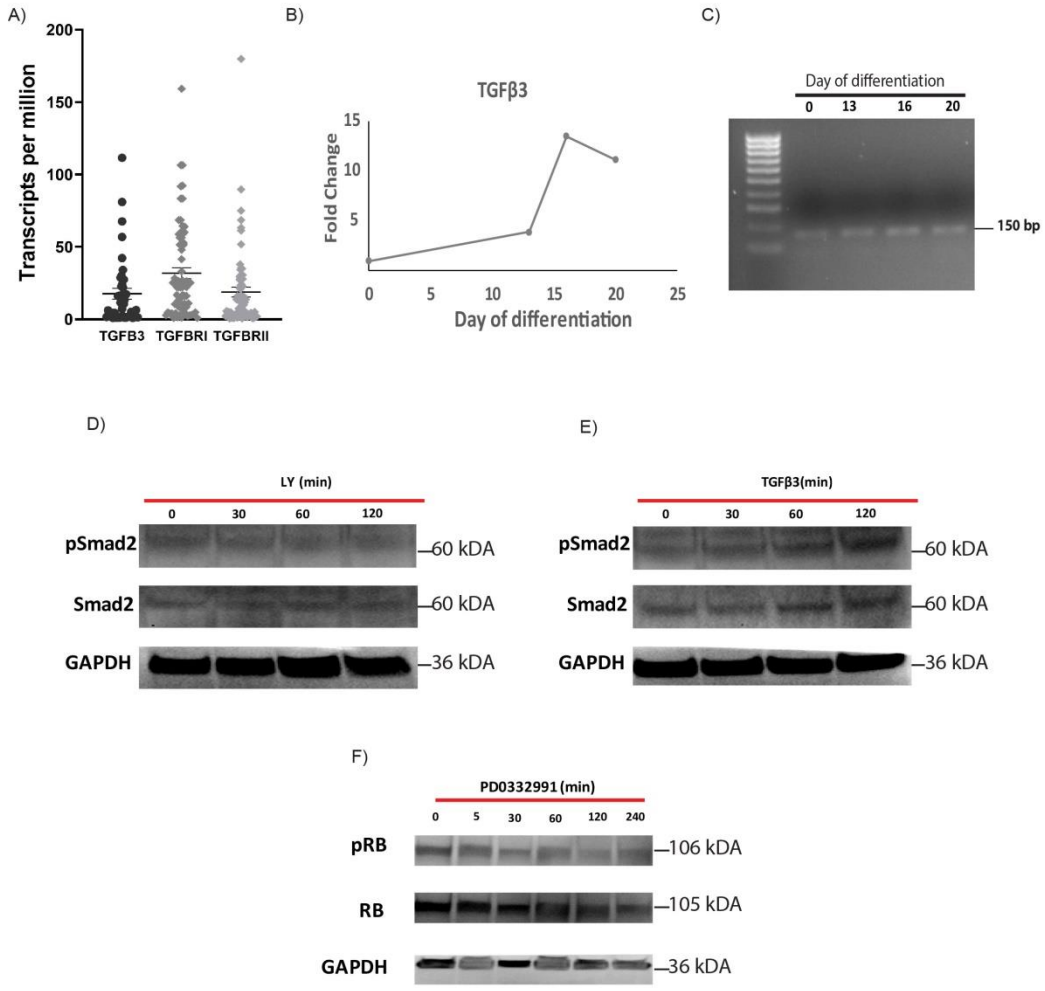

Figure S2. Validation of biological activity of TGFβ3, TGFβ inhibitor LY2109761 and CDK4/6 inhibitor PD0332991

A, Single cell RNAseq data showing the transcript levels of TGFβ3, TGFβRI and TGFβRII in a single hESC-derived MGE cells. n=42, 70 and 67, respectively; TPM: transcript per million.

B, qPCR analysis for TGFβ3 at day 13, 16 and 20 and shows its upregulation relative to undifferentiated hESCs at day 0;

C, Agarose gel electrophoresis showing the final 150bp qPCR product;

D, hESC-derived MGE cells were exposed to LY2109761 for the time indicated and processed for Western blot analysis for total Smad2 and phospho-Smad2;

E, Western blot analysis of TGFβ3 treated cells for total and phospho-Smad;.

F, Western blot analysis for total and phosphorylated RB protein on cells treated with the CDK4/6 inhibitor PD0332991.

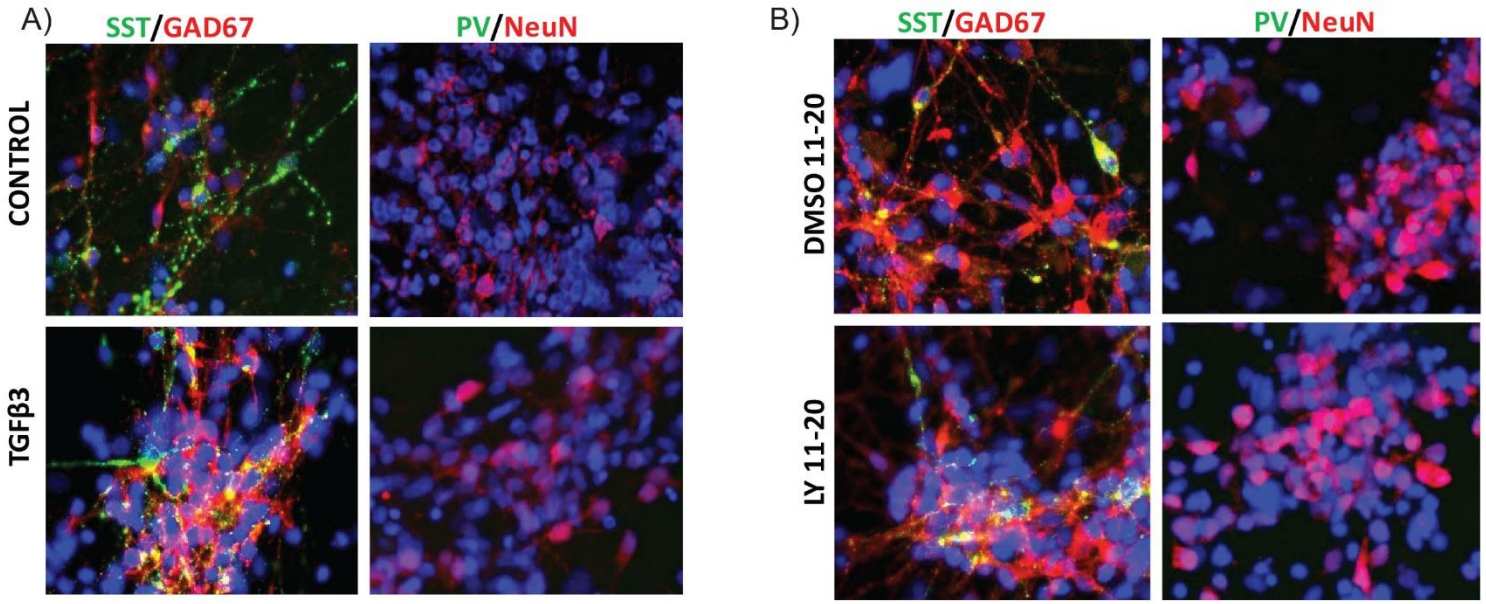

Figure S3, Higher magnification images
A, Digital magnification of Figure 2E,
B, Digital magnification of Figure 3F.

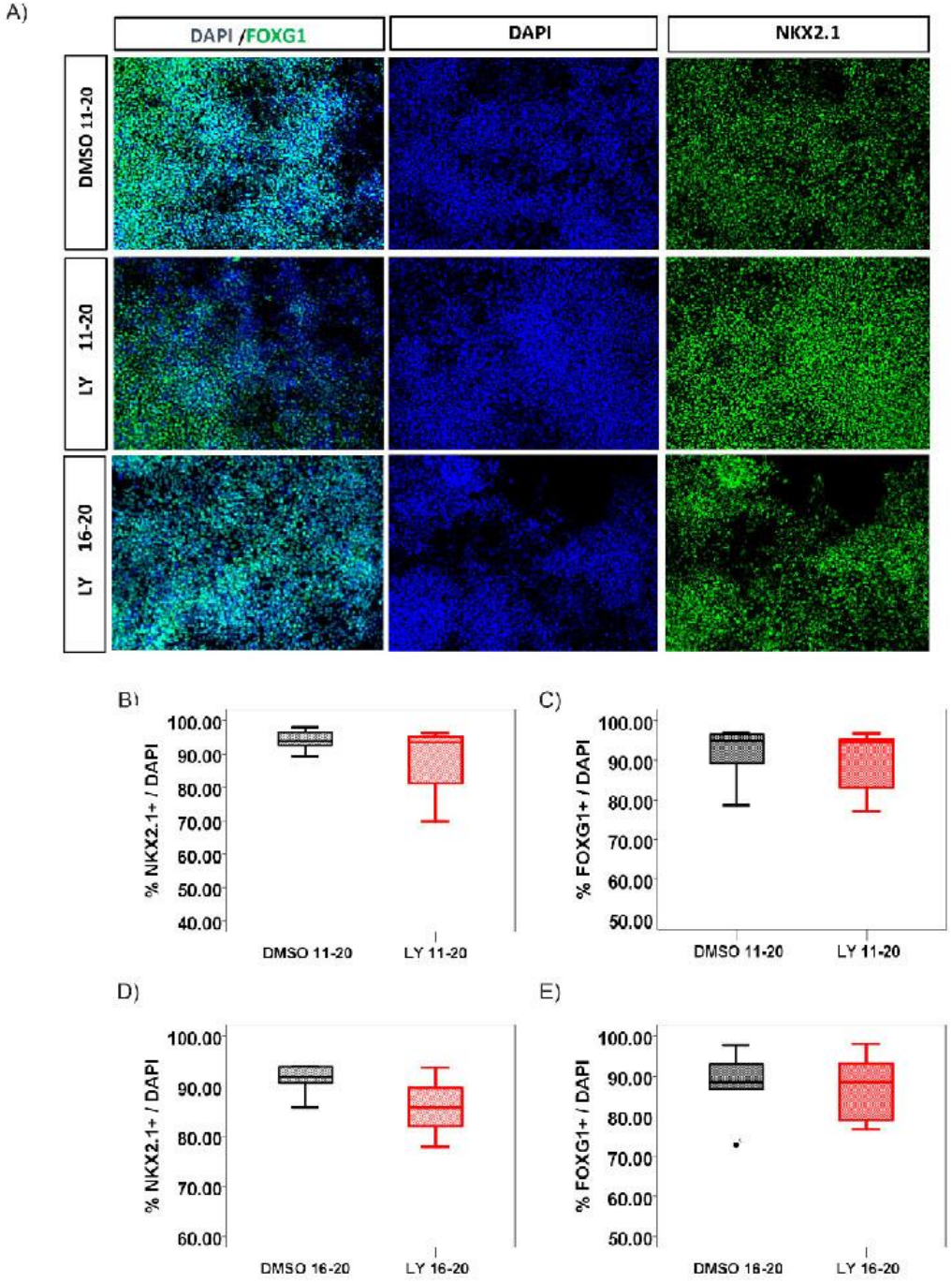
A)
DAPI /FOXG1
DAPI
NKX2.1
DMSO 11-20
LY 11-20
LY 16-20
B)
% NKX2.1+ / DAPI
100.00
90.00
80.00
70.00
60.00
50.00
40.00
DMSO 11-20
LY 11-20
C)
% FOXG1+ / DAPI
100.00
90.00
80.00
70.00
60.00
50.00
DMSO 11-20
LY 11-20
D)
% NKX2.1+ / DAPI
100.00
90.00
80.00
70.00
60.00
DMSO 16-20
LY 16-20
E)
% FOXG1+ / DAPI
100.00
90.00
80.00
70.00
60.00
50.00
DMSO 16-20
LY 16-20

Fig S4, TGFβ inhibition doesn’t affect MGE-progenitor production

A, Representative immunocytochemistry for NKX2.1 and FOXG1 in H7 parental line. B-E, Quantitative data of the marker-expressing cells in LY treated conditions and respective DMSO controls.

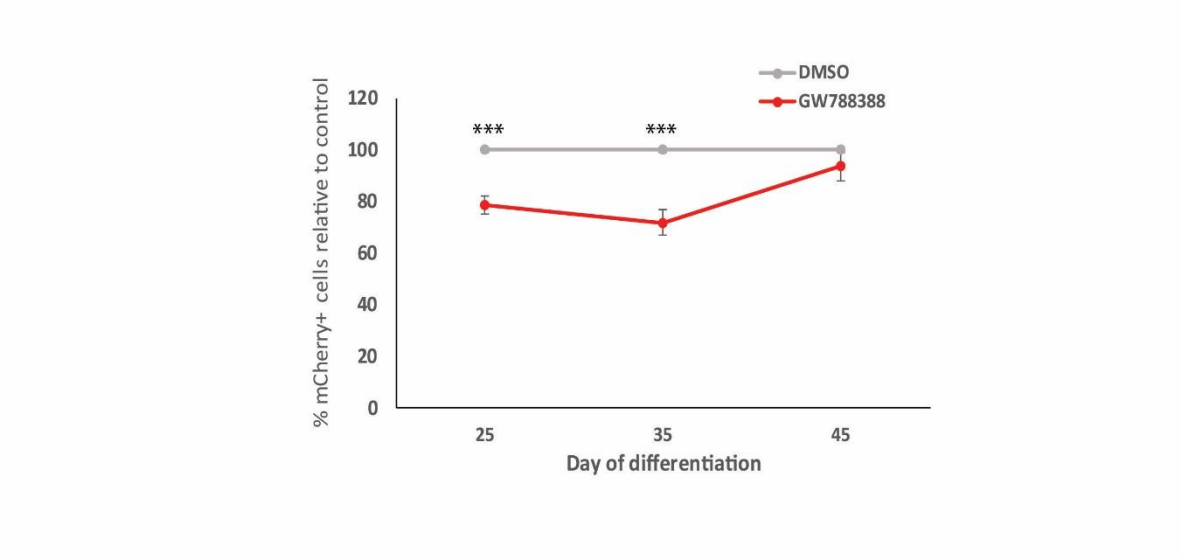

Figure S5. Effects of TGFβ inhibitor GW788388 on mCherry^{+} cell production
Day 25 and day 35 flow cytometry profile of mCherry^{+} cells of cultures treated with or without GW788388. Shown are GW788388 relative to control values where the lateral was set as 100%. *** P<0.001, univariate analysis post-hoc Bonferroni.

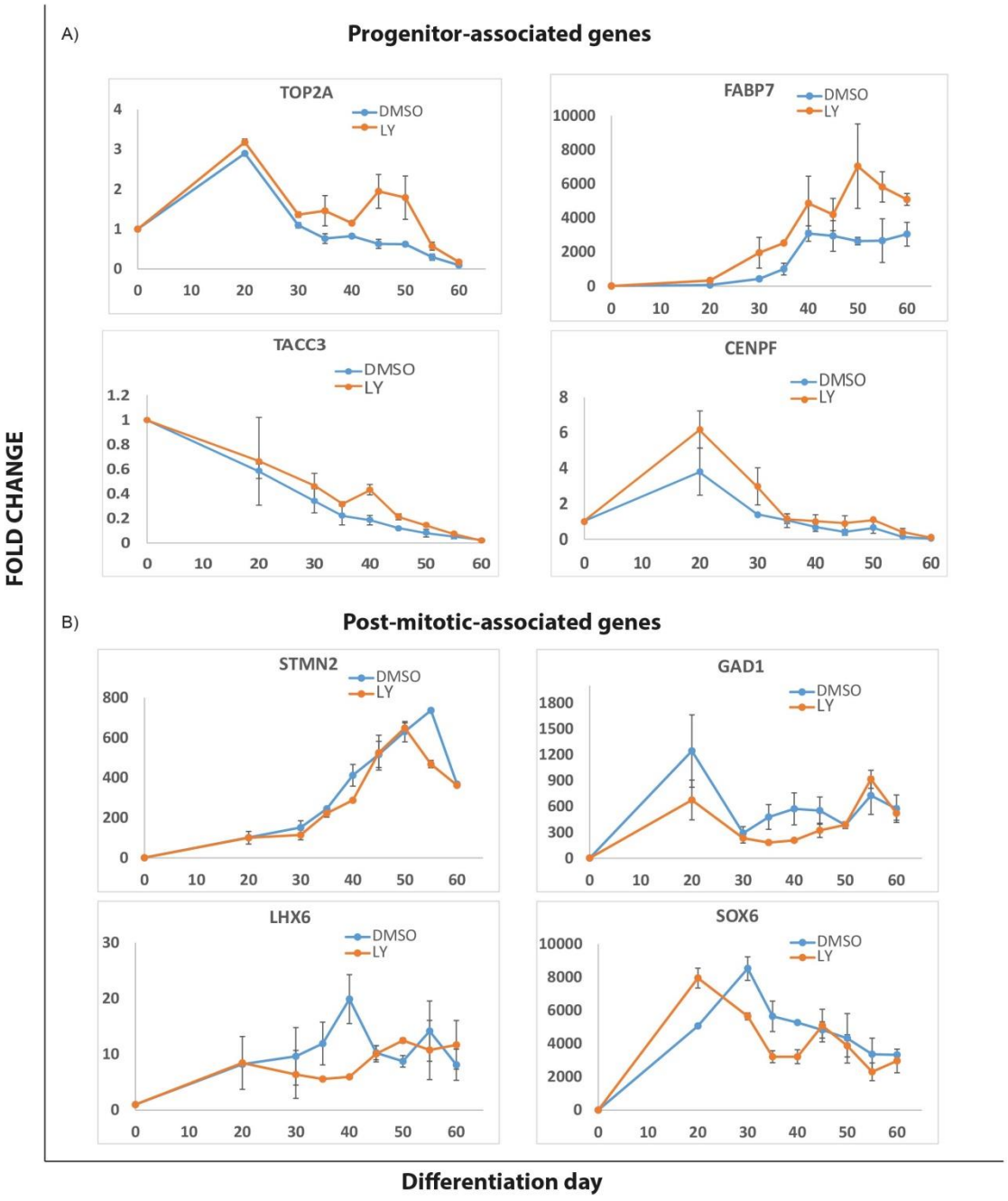
A)
Progenitor-associated genes
TOP2A
DMSO
LY
FABP7
TACC3
CENPF
B)
Post-mitotic-associated genes
STMN2
GAD1
LHX6
SOX6
FOLD CHANGE
Differentiation day

Fig S6, Effect of TGFβ inhibition on expression of genes associated with MGE progenitors and post-mitotic neurons.

A) Expression profile in LHM cells of MGE-progenitors associated genes (*TOP2, FABP7, TACC3* and *CENPF*) in control (blue) and LY-treated cultures (orange). B) Expression profile in LHM cells of genes associated to post-mitotic MGE derivatives (*STMN2, GAD1, LHX6* and *SOX6*) in control (blue) and LY-treated cultures (orange). Data is presented as the mean fold change ± SEM from triplicates of two independent experiments.

Table S1 Antibodies used for immunocytochemistry

Antigen	Species	Supplier	Code	Dilution
CALRETININ	Rabbit	Swant	CR 7697	1:500
CTIP2	Rat	Abcam	Ab18465	1:500
FOXG1	Rabbit	Abcam	ab18259	1:250
GAD67	Mouse	Millipore	mab5406	1:500
LHX6	Rabbit	Santa cruz	sc98607	1:500
mCherry	CHICKEN	ABCAM	ab205402	1:500
NeuN	RABBIT	MILLIPORE		1:500
NKX2.1	Rabbit	Abcam	Ab76013	1:1000
OCT4	Goat	Santa cruz	Sc8628	1:500
OLIG2	Goat	R&D systems	AF2418	1:200
PAX6	Mouse	DSHB		1:1000
PARVALBUMIN	Mouse	Sigma	P3088	1:100
SOMATOSTATIN	Rat	Millipore	MAB354	1:50
TBR1	Rabbit	Abcam	ab31940	1:500