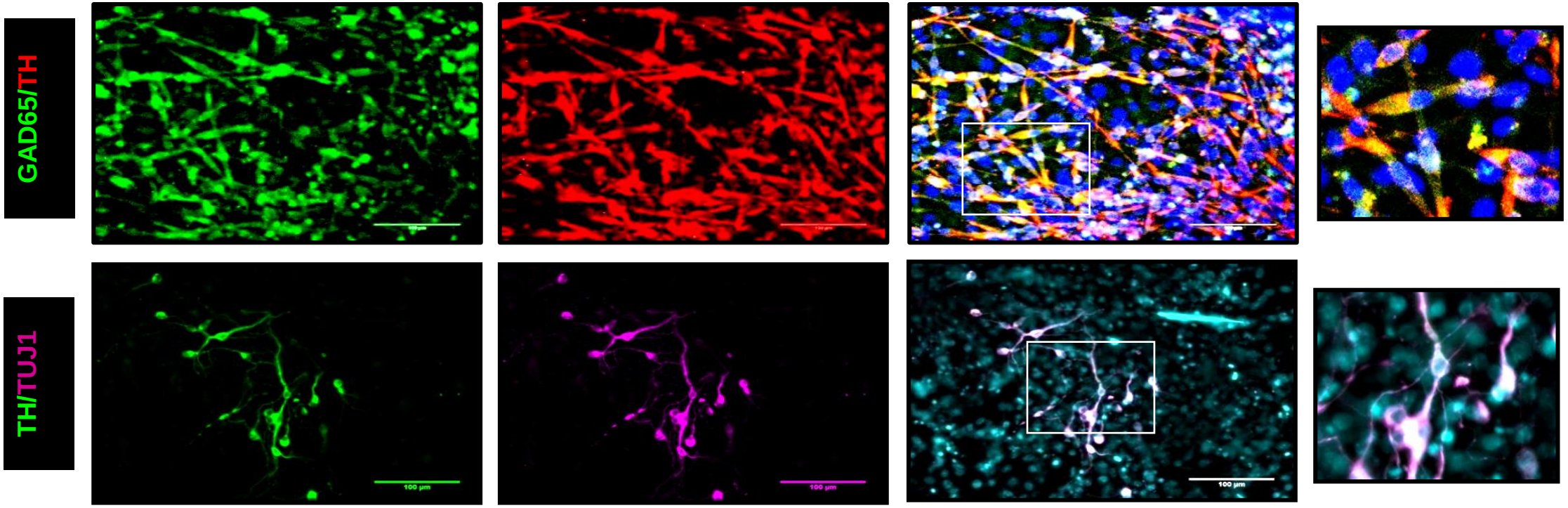
a)




b)

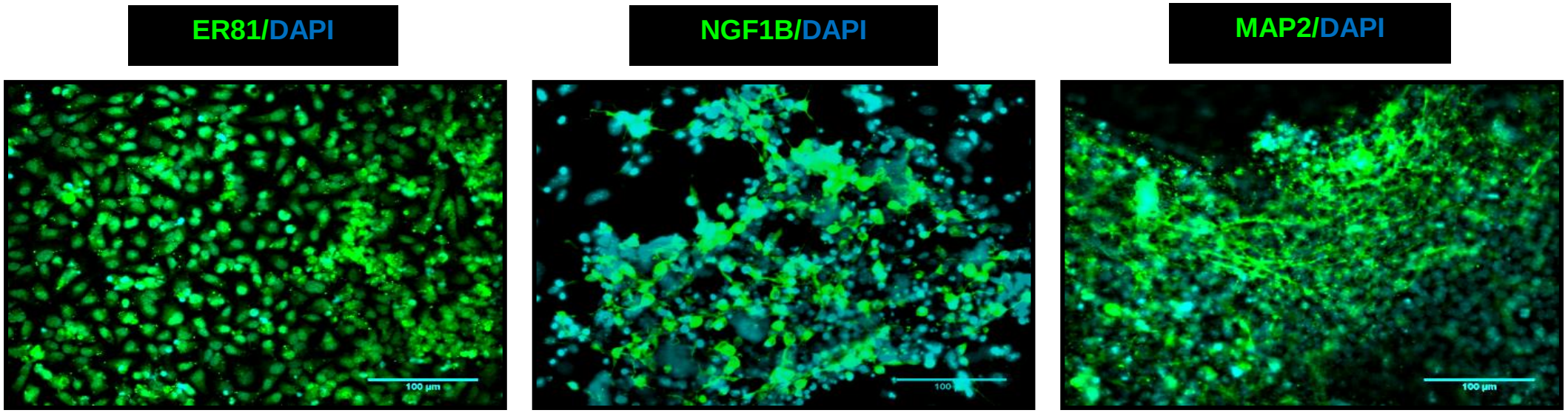
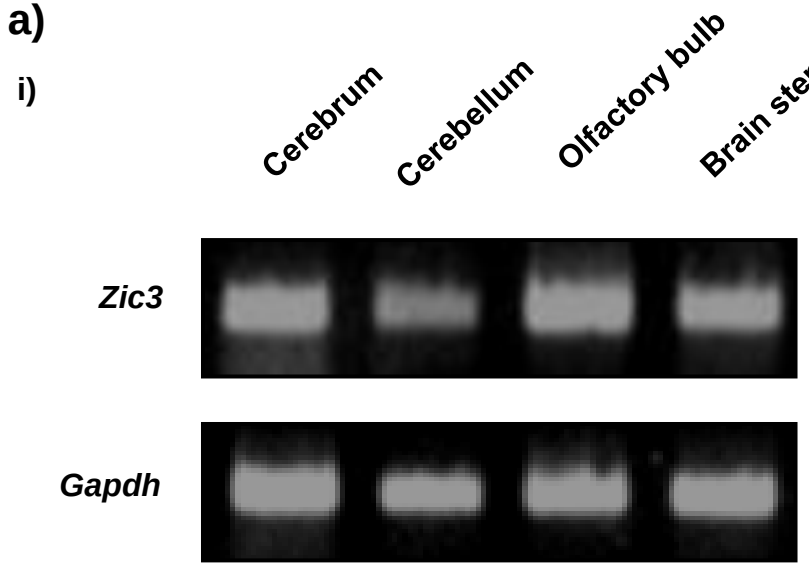


**Figure S1: mESCs efficiently differentiate to OB DA like neurons *in vitro*:**
(a) Co-expression analysis of TH with GAD65 and TUJ1, b) staining for ER81, NGF1B and MAP2 in OB DA like neurons differentiated from mESCs. Scale bar represents 100 µm.

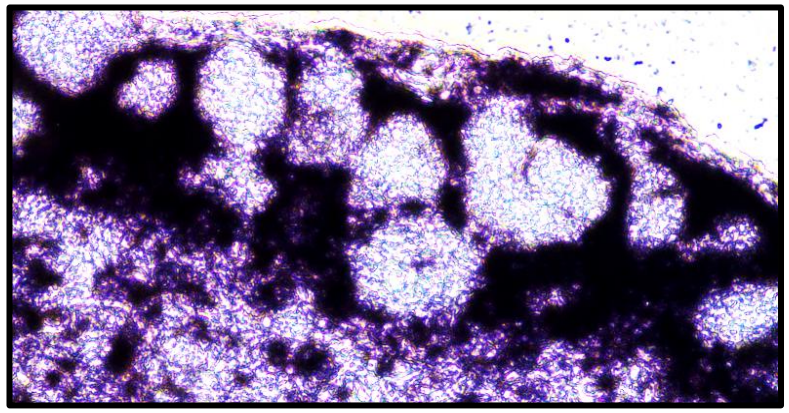
a)

i)



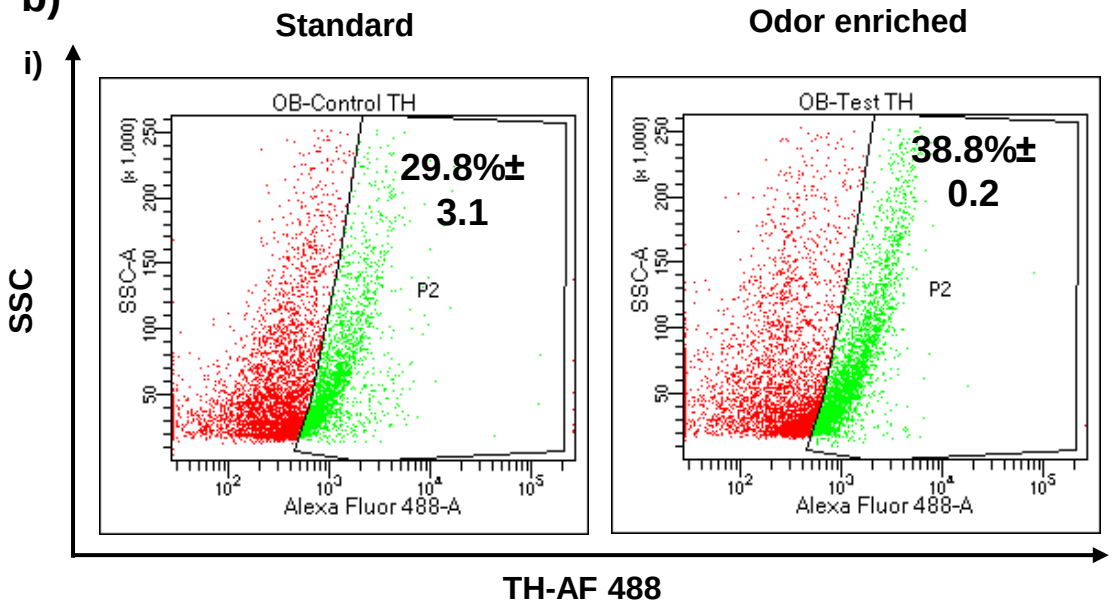

ii)

*Zic3* mRNA



b)

i)




ii)

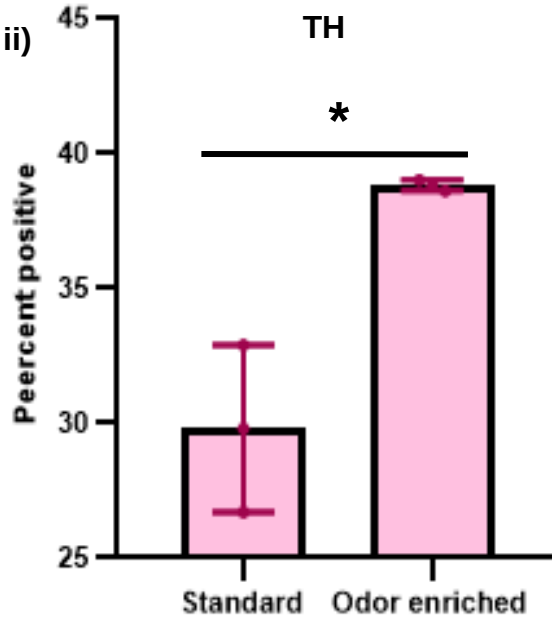


**Figure S2: ZIC3 is expressed in various regions of brain including olfactory bulb**

(a) Transcript analysis of *Zic3* in various regions of brain (i), *In situ* hybridization showing Zic3 mRNA expression majorly in PGL of mouse OB (ii), (b) flow cytometry analysis (i) and quantification (ii) of ZIC3 in control and odor enriched mouse OB tissue. Mean+/-SE of three independent biological triplicates, * $p \leq 0.05$.

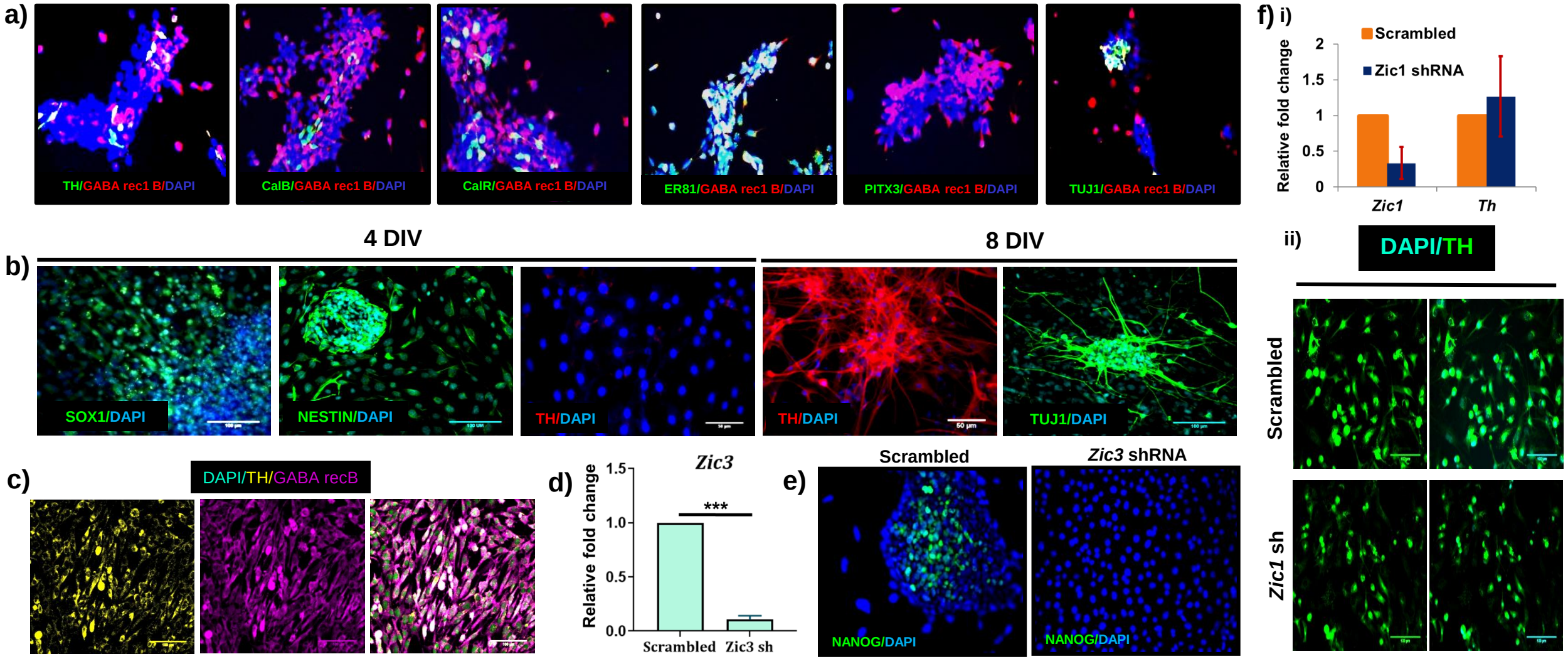

a)
TH/GABA rec1 B/DAPI
CalB/GABA rec1 B/DAPI
CalR/GABA rec1 B/DAPI
ER81/GABA rec1 B/DAPI
PITX3/GABA rec1 B/DAPI
TUJ1/GABA rec1 B/DAPI
b)
4 DIV
8 DIV
SOX1/DAPI
NESTIN/DAPI
TH/DAPI
TH/DAPI
TUJ1/DAPI
c)
DAPI/TH/GABA recB
d)
Zic3
***
Relative fold change
1.5
1.0
0.5
0.0
Scrambled
Zic3 sh
e)
Scrambled
Zic3 shRNA
NANOG/DAPI
NANOG/DAPI
f) i)
Scrambled
Zic1 shRNA
Relative fold change
2
1.5
1
0.5
0
Zic1
Th
ii)
DAPI/TH
Scrambled
Zic1 sh

**Figure S3: Characterization of Olfactory bulb primary neurons and analysis of effect of ZIC family member ZIC1 on OB differentiation**

a) Immunofluorescence analysis of different OB genes along with demonstrating the absence of MB DA specific gene PITX3 which confirms the OB identity b) Expression analysis of progenitor genes SOX1 and Nestin and matured gene TH at the end of 4 DIV and co-expression analysis of TH and TUJ1 at the end of 8 DIV, c) Co-expression analysis of GABA rec1B and TH, a hallmark of OB DA neurons at the end of 8 DIV. d) Transcript analysis of ZIC3 in OB primary neurons cultured in presence of either scrambled or ZIC3 shRNA construct, e) Expression of Nanog, the downstream target of ZIC3 in mESCs cultured in presence of either scrambled or Zic3 shRNA construct, f) Transcript analysis *Zic1* and *Th* and protein analysis of TH in primary OB neurons cultured in presence of either Scrambled or Zic1 shRNA. Scale bar represents 100 µm. Mean+/-SE of biological triplicates, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

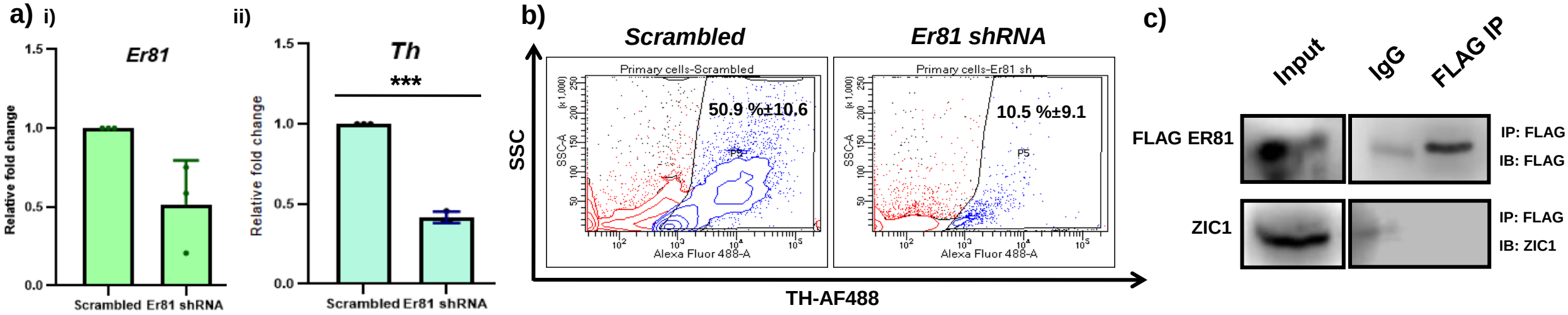

a) i)
Er81
Relative fold change
1.5
1.0
0.5
0.0
Scrambled
Er81 shRNA
ii)
Th
***
Relative fold change
1.5
1.0
0.5
0.0
Scrambled
Er81 shRNA
b)
Scrambled
Er81 shRNA
SSC
Primary cells-Scrambled
50.9 %±10.6
P5
SSC-A
(x 1,000)
Alexa Fluor 488-A
Primary cells-Er81 sh
10.5 %±9.1
P5
TH-AF488
c)
Input
IgG
FLAG IP
FLAG ER81
IP: FLAG
IB: FLAG
ZIC1
IP: FLAG
IB: ZIC1

**Figure S4: ER81 regulates TH expression and fails to interact with ZIC1**

a) Transcript analysis of *Er81* and *Th* and protein analysis of TH (b) in primary Ob neurons transduced with either scrambled or *Er81* shRNA. c) Co-immunoprecipitation analysis in HEK-293T cells overexpressing immunoreactive tags FLAG-ER81 and immunoprecipitation was performed using FLAG antibody and immunoblot performed using ZIC1 antibody. Mean+/-SE of biological triplicates, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

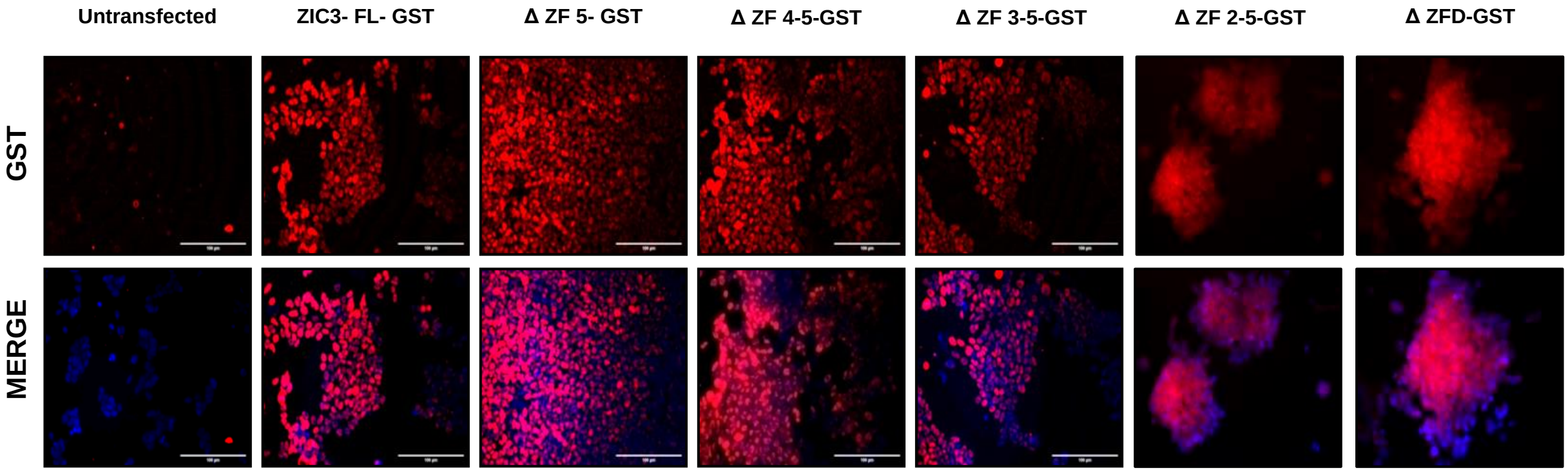



**Figure S5: Zinc finger domain 1-2 is essential for nuclear localization of ZIC3**

Immunofluorescence analysis of ZIC3 in cells overexpressing different deletion constructs of ZIC3 tagged to GST. Immunofluorescence was performed using GST antibody. Scale bar represents 100 µm

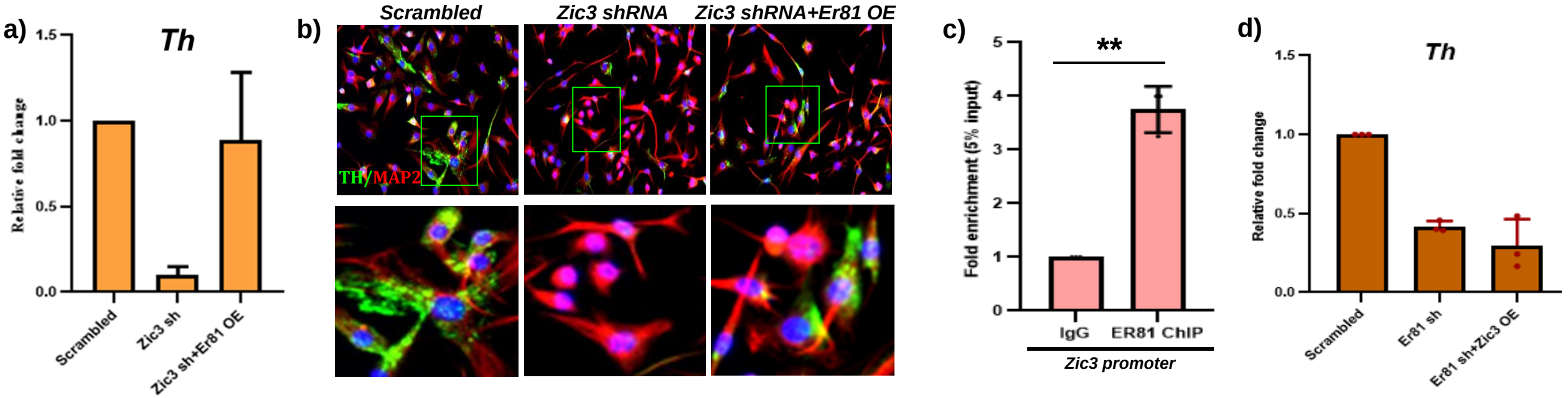
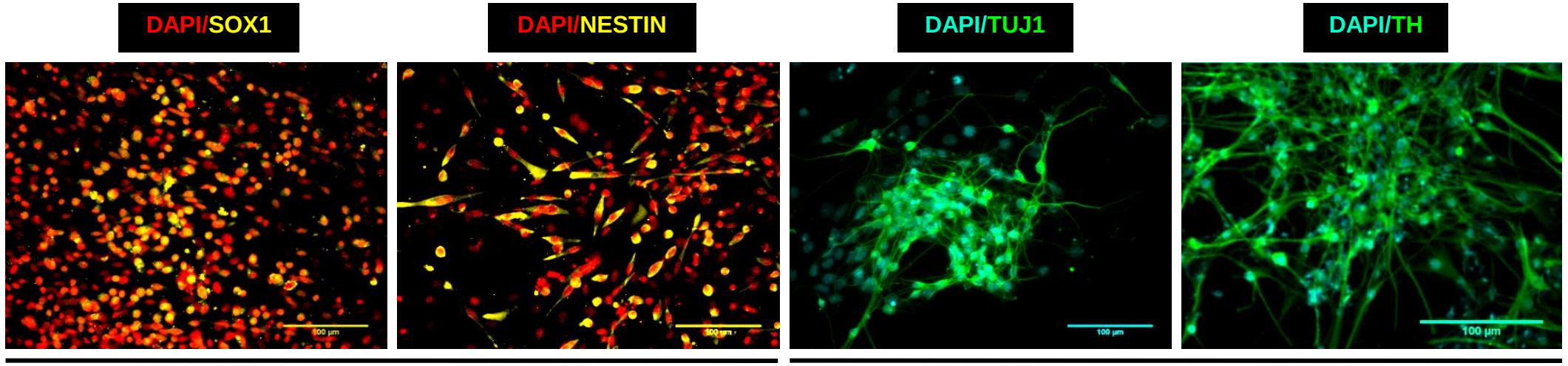

a)
Th
Relative fold change
1.5
1.0
0.5
0.0
Scrambled
Zic3 sh
Zic3 sh+Er81 OE
b)
Scrambled
Zic3 shRNA
Zic3 shRNA+Er81 OE
TH/MAP2
c)
**
Fold enrichment (5% input)
5
4
3
2
1
0
IgG
ER81 ChIP
Zic3 promoter
d)
Th
Relative fold change
1.5
1.0
0.5
0.0
Scrambled
Er81 sh
Er81 sh+Zic3 OE

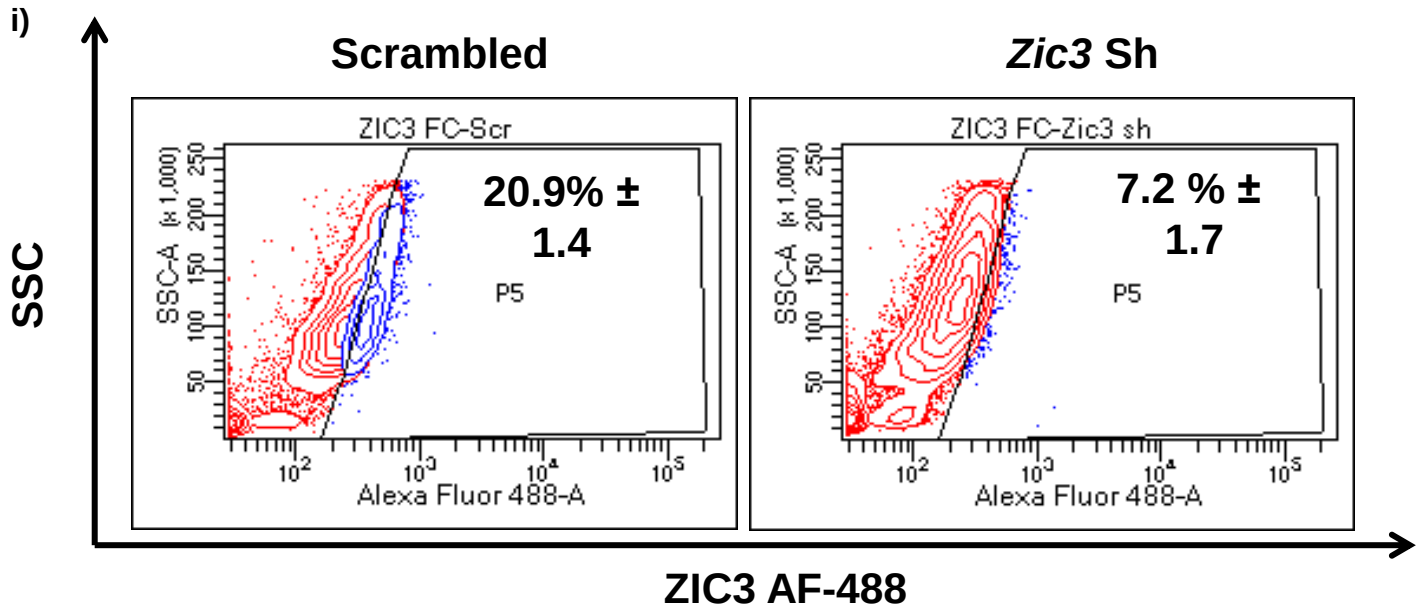
**Figure S6: ZIC3 fails to rescue *Th* expression in *Er81* knockdown condition**

Effect of *Er81* over-expression on *Th* mRNA (a) and TH protein (b) levels in OB DA neurons with *Zic3* loss of function, (c) ChIP PCR with ER81 antibody showing binding of ER81 to *Zic3* promoter, (d) expression of *Th* in OB primary neurons with *Er81* shRNA and *Zic3* over-expression . Scale bar represents 100 µm. Mean+/-SE of biological triplicates, ** $p \leq 0.01$.

a)


DAPI/SOX1
DAPI/NESTIN
DAPI/TUJ1
DAPI/TH
100 µm
4 DIV
8 DIV

b)


i)
Scrambled
Zic3 Sh
ZIC3 FC-Scr
ZIC3 FC-Zic3 sh
20.9% ± 1.4
7.2 % ± 1.7
P5
SSC-A (x 1,000)
Alexa Fluor 488-A
SSC
ZIC3 AF-488

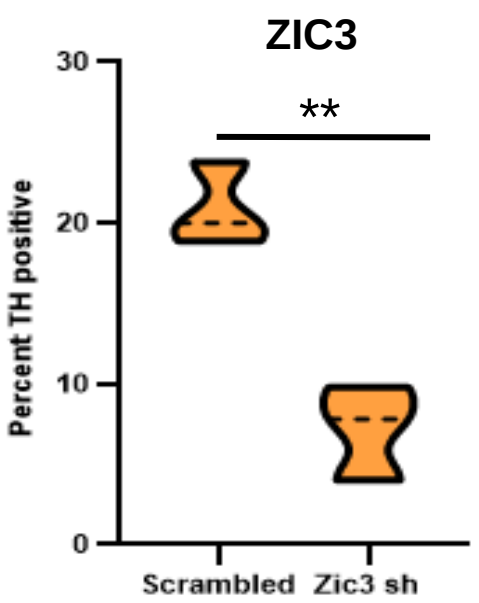

ii)
ZIC3
**
Percent TH positive
Scrambled
Zic3 sh

**Figure S7: Derivation of MB DA like neurons from primary neurospheres**

(a) Immunofluorescence showing the expression of neural progenitor markers SOX1 and NESTIN in MB primary cells 4 DIV and matured neural markers TUJ1 and DA marker TH in 8 DIV, (b) knockdown efficiency of ZIC3 in MB DA neurons as shown flow cytometric staining (i) and quantification (ii). Scale bar represents 100 µm. Mean+/-SE of biological triplicates, ** $p \leq 0.01$.