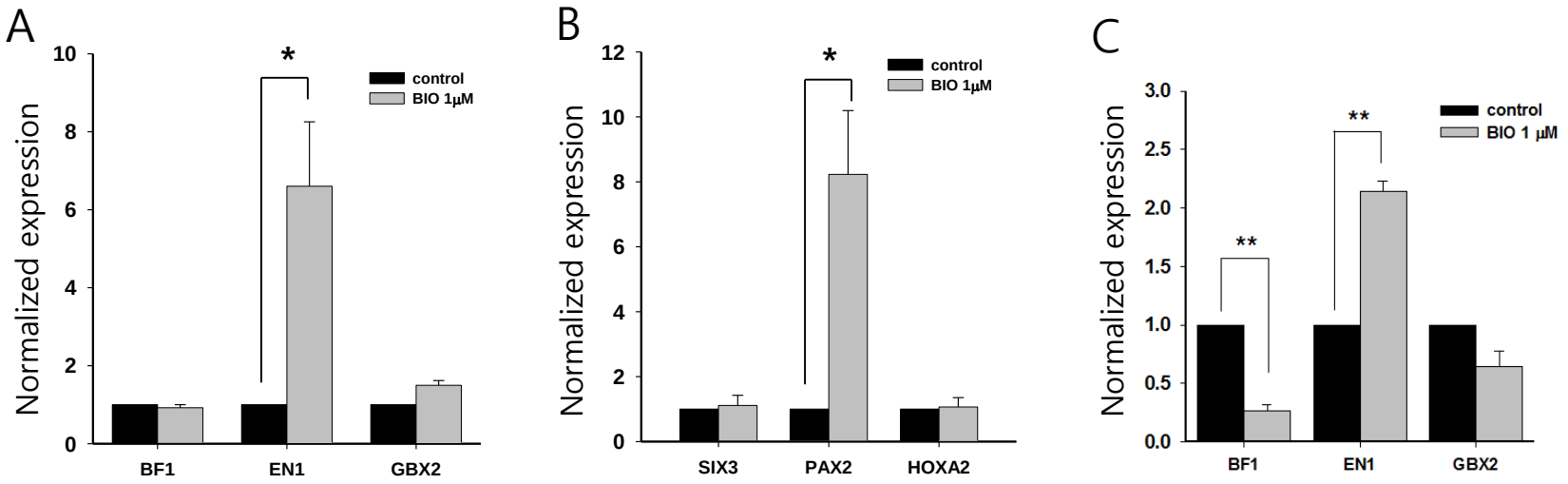
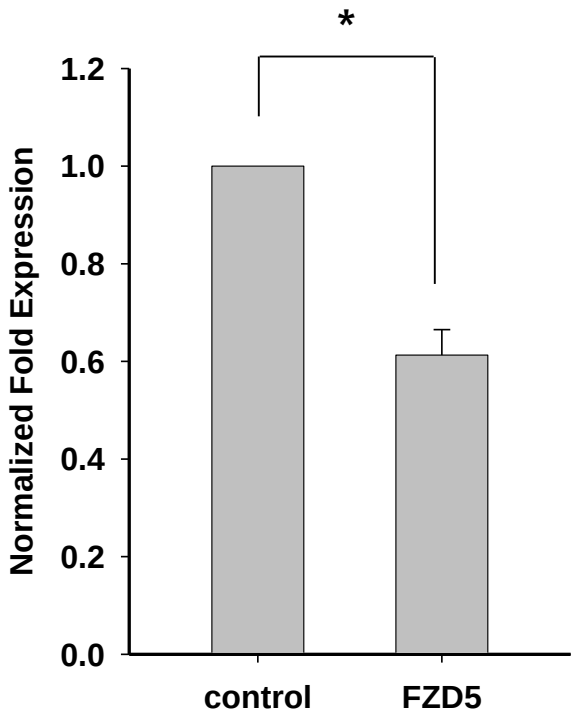
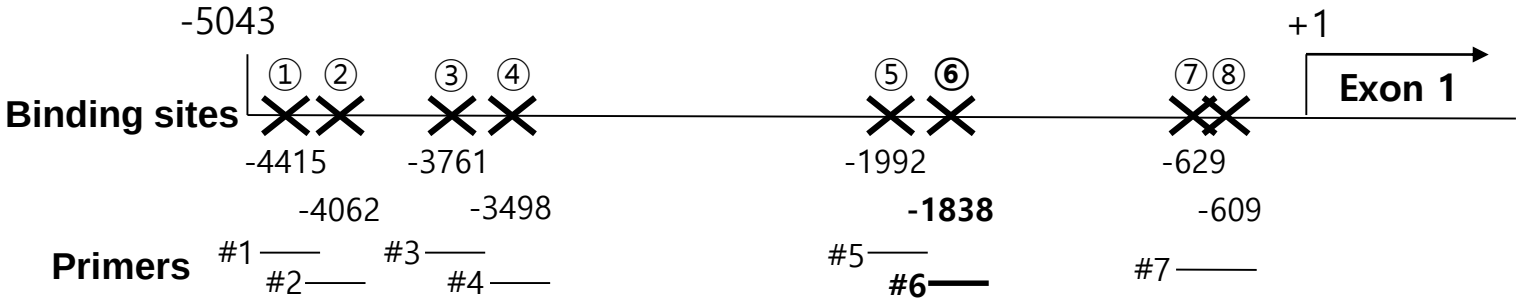


Supplementary Figure 1. (A-B) Effects of BIO on regionalization of iPS-WT3-derived NPCs. EN1 and PAX2 expression increased prominently with 1μM BIO. (C) Midbrain specification by BIO treatment in NPC suspension culture. Data are expressed as mean±SEM. *p<0.05, **p<0.01 compared with control group by Student's *t* test.



Supplementary Figure 2. Effects on endogenous EN1 expression after treatment with Wnt receptor antagonist FZD5 compared with control in iPS-Epi3-derived NPCs. Data are expressed as mean±SEM. *$p<0.05$ compared with control group by Student's *t* test.

A



B

Primer #	Position	Sequence
1	-4415(-)	TTTTGTT
2	-4062(+)	CTTTGCT
3	-3761(-)	TTTTGTT
4	-3498(+)	CTTTGCT
5	-1992(+)	AGTAAAG
6	**-1838(+)**	**CTTTGAT**
7	-629(-)	ATGAAAG
-	-609(-)	TGCAAAG

Supplementary Figure 3. (A) Schematic representation of the human EN1 promoter with the eight putative TCF4 binding sites identified by Patch 1.0 (Transfac®). (B) The sequences and positions of the putative TCF4 binding site. Two out of the eight putative binding motifs (⑦ and ⑧) are in close proximity to each other, and thus we checked seven sites (①-⑦) in TCF4-immunoprecipitated chromatin. Note that only one position (-1838(+), site 6) was a consensus sequence for TCF4.

Supplementary Table 1

Gene name	Primer sequence
Bf1-F	5’- AGAGCAGCACATCCATGAG -3’ 5’- TTGAGTTACAACGGCACCAC -3’
Bf1-R	5’- AAAACTTGGCAAAGAGGGTCT -3’ 5’- GCGAGTTCTGAGTCAACACG -3’
En1-F	5’- GCCCGTGGTCAAAACTGA -3’
En1-R	5’- TTCTTCTTCAGCTTCCTGGTG -3’
Gbx2-F	5’- CCAAAGAGGGCTCGCTGCTC -3’ 5’- GGAGGACGGCAAAGGCTTC -3’
Gbx2-R	5’- TCGTCTTCCACCTTTGACTCGTC -3’ 5’- TCTTCCACCTTTGACTCGTCTTTC -3’
Six3-F	5’- CTCCTCCCCCACTCCTTC -3’
Six3-R	5’- GGGTATCCTGATTTCGGTTTG -3’
Pax2-F	5’- GCACACAGACATACCCAGTTG -3’
Pax2-R	5’- AGGGTGGAGGTGGGGTAG -3’
HoxA2-F	5’- CAAGAAAACCGCACTTCTGC -3’
HoxA2-R	5’- CGATTTCCAGGGATTCTTTG -3’
beta-actin-F	5’- GCTCTTTTCCAGCCTTCCTT -3’
beta-actin-R	5’- CTTCTGCATCCTGTCAGCAA -3’
Wnt1-F	5’- GCGTCTGATACGCCAAAATC -3’
Wnt1-R	5’- GGATTCGATGGAACCTTCTG -3’
Wnt3a-F	5’- TGTTGGGCCACAGTATTCCT -3’
Wnt3a-R	5’- ATGAGCGTGTCACTGCAAAG -3’
Wnt5b-F	5’- GTGCAGAGACCCGAGATGTT -3’
Wnt5b-R	5’- CAGGCTACGTCTGCCATCTT -3’
Wnt7a-F	5’- AGTACAACGAGGCCGTTCAC -3’
Wnt7a-R	5’- GCACGTGTTGCACTTGACAT -3’
FGF2-F	5’- TCGAACCGCTGTGTCTCCTACG -3’
FGF2-R	5’- GCCTCCAAGTAGCAGCCAAAGAAA -3’
FGF5-F	5’- CCCTCGGGGCGCCGGACCGGCA -3’
FGF5-R	5’- AAGTTCTGGCTGCTCCGACTGCTT -3’
FGF8-F	5’- TGCAGAATGCCAAGTACGAG -3’
FGF8-R	5’- TTGAGTTTTGGGTGCCCTAC -3’
GAPDH-F	5’- ACCACAGTCCATGCCATCAC -3’
GAPDH-R	5’- TCCACCACCCTGTTGCTGTA -3’

Supplementary Table 2

Site	Primer sequence	
#1	sense	5'- GAGAGAGAGGAGAGAGAG -3'
	anti-sense	5'- TCCCTCTTATCTAAGGCAGT -3'
#2	sense	5'- TTTTCCTTTGCTGCCTCC -3'
	anti-sense	5'- ATCCTAAGCAATTCTGTCCAA -3'
#3	sense	5'- CGGCACTTGTAGCAGAAAT -3'
	anti-sense	5'- GATGACCAGCGGTTTTGT -3'
#4	sense	5'- ACCCTGGCATTTGTCTTTG -3'
	anti-sense	5'- CCTGTGTGTCCATAGAATTTTG -3'
#5	sense	5'- GCATTTATCATCCCATTACTGTAAC -3'
	anti-sense	5'- TTTACCTTTCATGTAGTGGAGC -3'
#6	sense	5'- CTCACTTTGATTAAGCGTCTTG -3'
	anti-sense	5'- CTGGTCAAAAGCTCTGTCA -3'
#7	sense	5'- ACTCCCTGTTCACAACTTCA -3'
	anti-sense	5'- CGCTGCACCCCAAGTATT -3'

Supplementary Table 3

Name	Primer sequence
En1-pro-full.FOR	5'- CTAGCCCGGGCTCGAGattcgggtctgggttctgg -3'
En1-pro-full.REV	5'- GATCGCAGATCTCGAGtgtctctggttgctgttattgaaggatgg -3'