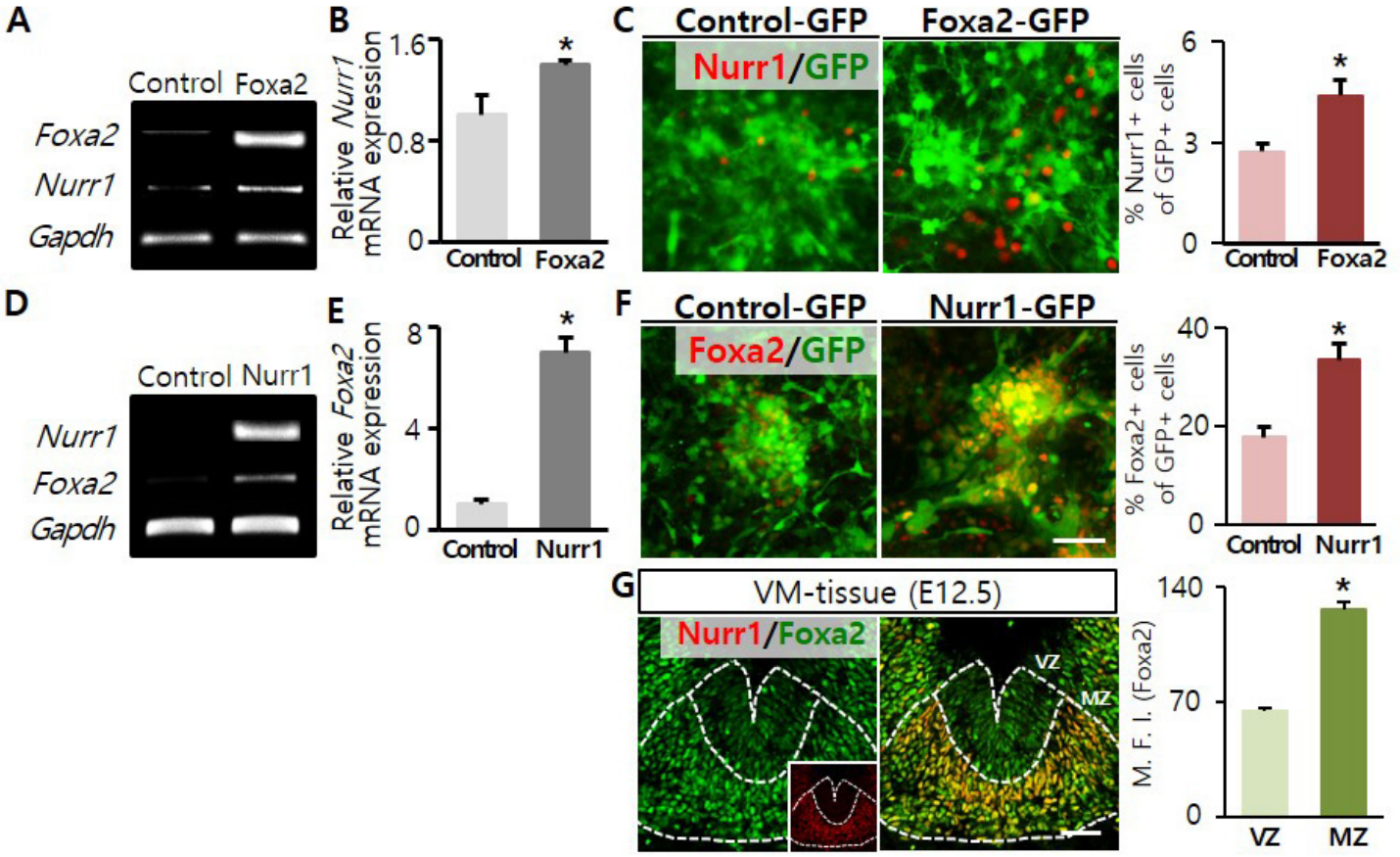
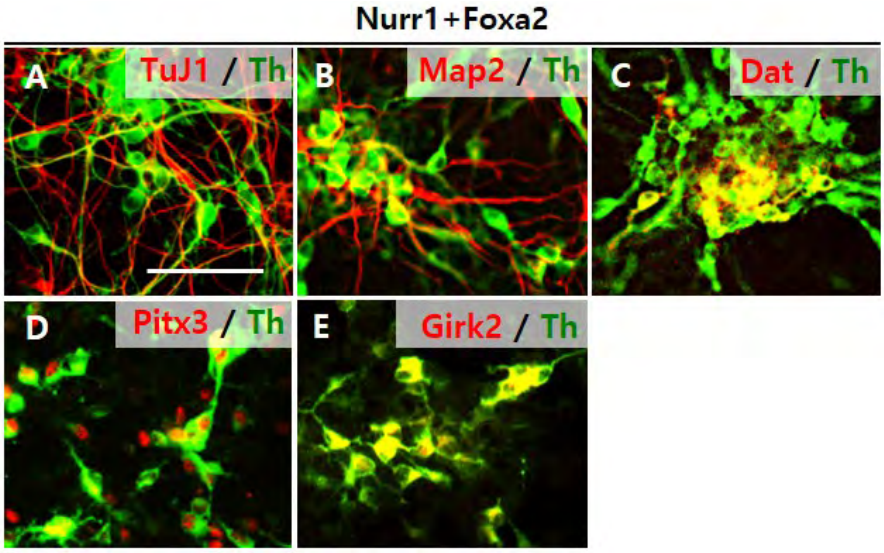
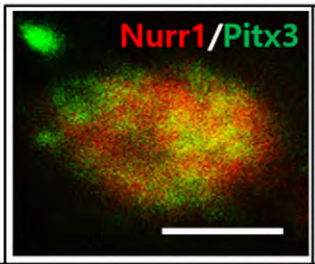
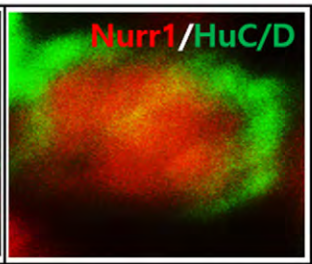


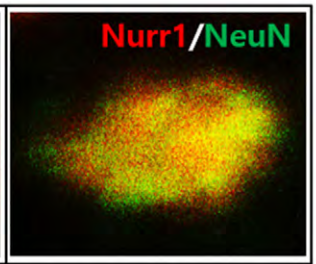
Suppl. Fig. S1. Gain-of-function analyses *in vitro* to confirm positive cross-regulatory loop between Nurr1 and Foxa2 expression. NPCs derived from VM at E11 were subcultured to examine gain-of-function effects. (A-F) The passaged VM-NPC cultures yielded fewer Nurr1$^+$ and Foxa2$^+$ cells upon differentiation than unpassaged cultures. The passaged cultures were transduced with virus expressing Foxa2-IRES-GFP (A-C) or Nurr1-IRES-GFP (D-F) and differentiated for 6 days. Control cultures were transduced with virus expressing IRES-GFP. Semi-quantitative PCR (A,D), real-time PCR (B,E), and immunocytochemistry (C,F) analyses were carried out for Nurr1 and Foxa2 expression. (C,F) are representative images for Nurr1$^+$/GFP$^+$ and Foxa2$^+$/GFP$^+$ cells, respectively. Graphs on the right depict percentages of the co-expressing cells out of total GFP$^+$ cells in 20-40 clusters randomly selected from 3 independent culture sets. *Significantly different from control at P<0.05(B), P<0.005(C), and P<0.001(E,F). (G) Comparison of Foxa2 expression levels in the Nurr1-negative ventricular zone (VZ) and Nurr1-positive mantle zone (MZ) of the embryonic mouse VM. VM tissue sections of mouse embryos at E12 were stained with anti-Foxa2 antibody (Inset, identical section Nurr1-stained). Foxa2-stained cells were randomly selected from the VZ and MZ (40 cells each) and Foxa2 expression levels were quantified as mean fluorescence intensities (MFI) of individual anti-Foxa2-stained cells. *P<0.001, Student's t-test. Scale bar, 50 μm.



Suppl. Fig. S2. Midbrain-type DA neuronal phenotypes of TH⁺ cells induced by exogenous Nurr1 and Foxa2 co-expression. Non-dopaminergic cortical NPCs were transduced with Nurr1⁺ Foxa2, and differentiated for 6 days. Immunofluorescence staining was conducted using the indicated antibodies. Scale bar, 50μm.

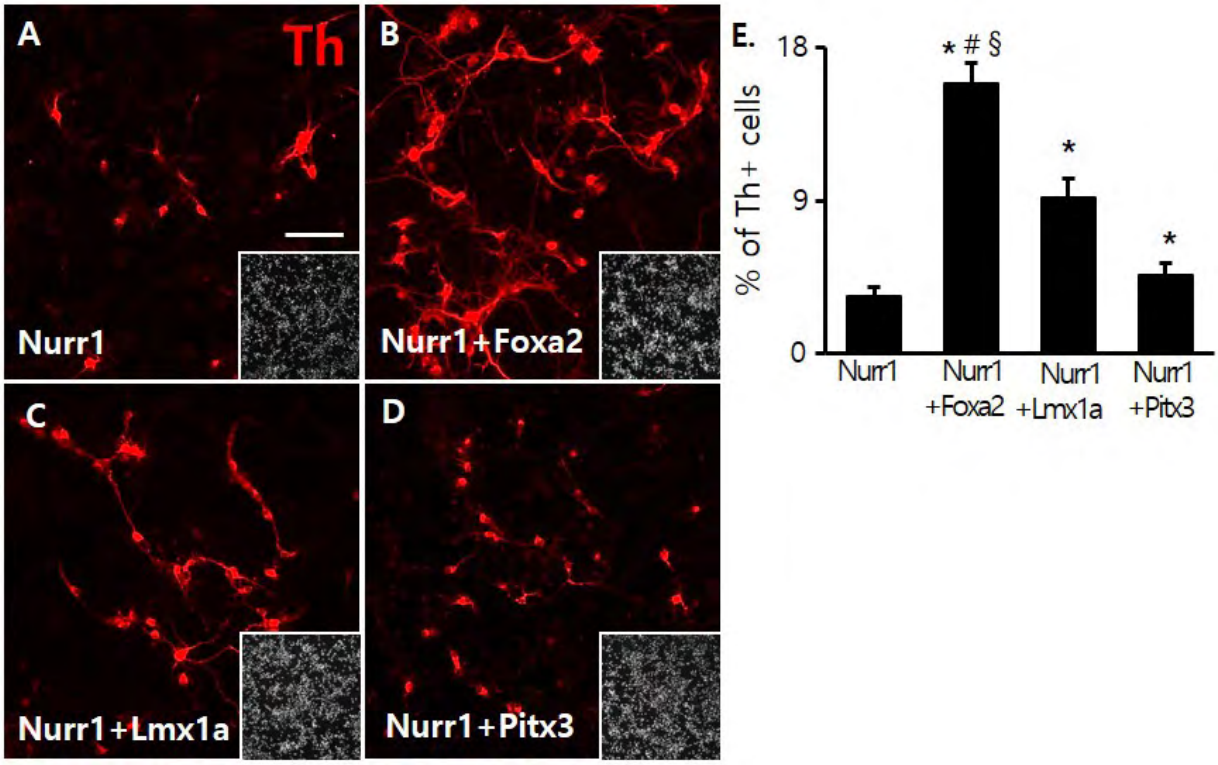






Pearson's Correlation	0.705±0.045	0.122±0.037	0.325±0.132
Overlap Coefficient	0.799±0.073	0.46±0.038	0.565±0.038

Suppl. Fig. S3. Analysis of Nurr1 colocalization with Pitx3, NeuN, and HuC/D by Pearson's correlation and overlap coefficient values. Shown are representative images of single nucleus co-stained with Nurr1/Pitx3, Nurr1/NeuN, and Nurr1/HuC/D from the VM tissue sections at E12. Scale bar, 5μm.



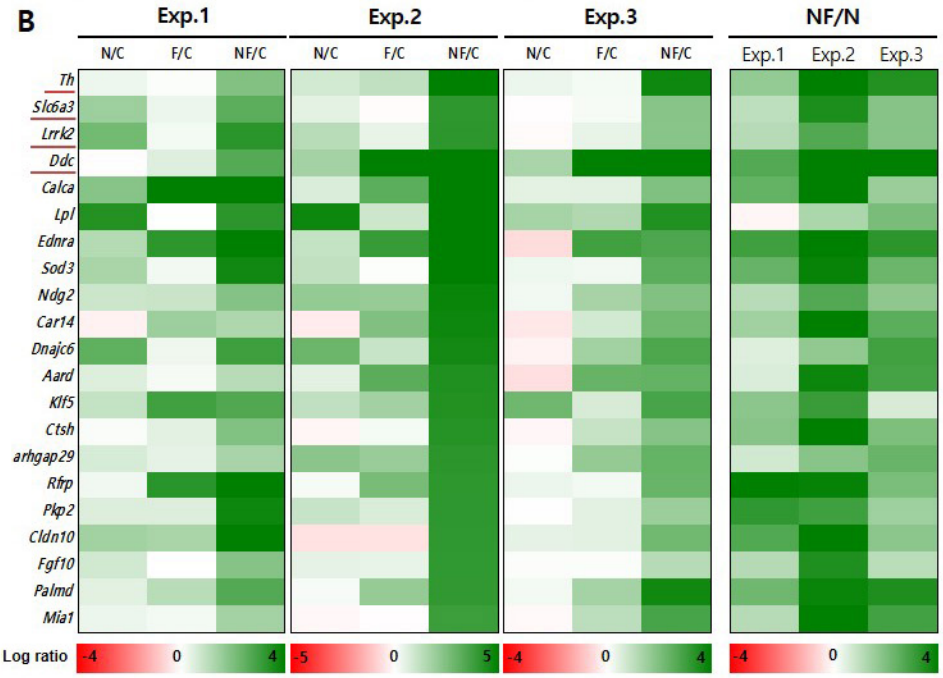
Suppl. Fig. S4. **Comparison of the activities of Nurr1 coactivators reported.** NPCs derived from mouse embryonic cortices at E12 were co-transduced with the retroviruses containing the control empty vector (A), Foxa2 (B), Lmx1a (C), or Pitx3 (D) along with Nurr1. The coactivator virus titers were carefully adjusted to $1x10^{11}$ virus particles/ml. Graph E represents the percentage of $DAPI^+$ cells that were TH^+. Significance from the control*, Nurr1+Lmx1a# and Nurr1+Pixt3§ at $P<0.001$, Scale bar, 50μm.

Suppl. Table. S1. mRNA expressions of 21 genes selected from microarray data

A

SYMBOL	DEFINITION	Accession No.	Fold Increase			
			N/C	F/C	NF/C	NF/N
Th	Tyrosine hydroxylase	NM_009377	1.71±0.02	1.52±0.43	38.47±1.18	22.513±0.41
Slc6a3	Dopamine transporter, Dat	NM_010020	1.23±0.14	-0.03±1.12	12.2±2.81	9.77±1.2
Lrrk2	Leucine-rich repeat kinase 2	NM_025730	1.96±0.6	1.22±0.02	12.01±3.63	6.13±0.01
Ddc	Dopa Decarboxylase, Aromatic L-amino acid decarboxylase, Aadc	NM_016672	1.66±0.59	26.96±4.83	39.15±6.34	14.3±1.41
Sod3	Superoxide dismutase3	NM_011435	1.96±0.22	0.04±1.12	23.36±7.23	11.67±2.37
Calca	Calcitonin/calcitonin-related polypeptide, alpha	NM_007587	1.86±0.29	4.71±3.3	28.61±14.5	17.04±10.49
Lpl	Lipoprotein lipase	NM_008509	27.16±1.77	6.41±4.7	53.7±6.31	2.0±0.36
Ednra	Endothelin receptor type A	NM_010332	1.59±0.45	9.2±3.7	24.5±9.72	14.88±1.85
Ndg2	Nur77 downstream gene 2	NM_175329	3.11±0.98	2.58±0.96	16.42±8.79	4.88±1.28
Car14	Carbonic anhydrase 14	NM_011797	-1.9±0.53	3.09±1.69	17.18±6.5	24.35±8.42
Dnajc6	DnaJ (Hsp40) homolog, subfamily C, member 6	NM_198412	4.23±2.53	2.12±0.35	18.85±2.55	6.31±3.18
Aard	Alanine and arginine rich domain containing protein	NM_175503	1.3±0.06	5.86±2.21	14.85±3.25	11.34±1.91
Klf5	Kruppel-like factor 5	NM_009769	6.66±4.48	2.2±0.83	17.67±0.17	4.8±3.26
Ctsh	Cathepsin H	NM_007801	0.16±1.34	1.3±0.24	12.67±4.31	12.76±7.19
Arhgap29	Rho GTPase activating protein 29	NM_172525	3.26±1.39	2.96±0.52	13.73±1.93	4.85±1.49
Rfrp	Neuropeptide VF precursor	NM_021892	1.39±0.36	3.15±2.05	14.23±0.09	11.0±2.95
Pkp2	Plakophilin 2	NM_026163	1.8±0.17	1.35±0.1	10.72±4.06	5.8±1.71
Cldn10	Claudin10	NM_021386	-1.65±0.09	-1.34±0.35	12.22±2.49	16.92±3.86
Palmd	Palmdelphin	NM_023245	1.31±0.24	3.26±0.43	26.49±12.01	19.19±5.65
Fgf10	Fibroblast growth factor 10	NM_008002	1.33±0.04	1.12± 0.06	10.13± 3.74	7.7±3.04
Mia1	Melanoma inhibitory activity 1	NM_019394	0.06±1.26	0.35±1.56	15.14±2.85	14.26±0.44

B



High through-put gene expression analyses were done on NPCs transduced with control (C), Nurr1 (N), Foxa2 (F), and Nurr1+Foxa2 (NF). To know co-activator role of Foxa2 in Nurr1-induced gene expression, the microarray data were analyzed for the gene expressions up-regulated (>2 folds) in NPCs expressing Nurr1+Foxa2, compared to those expressing Nurr1 alone. 21 genes fit this criterion and are listed with their expression ratios (A) and heatmaps of log2 transformed expression ratios (B). n= 3 independent microarray analyses. Each microarray analysis was done in the control-, Nurr1-, Foxa2-, Nurr1+Foxa2-transduced cultures, and interested gene expressions in the Nurr1-, Foxa2, and Nurr1+Foxa2-expressing cultures were compared with those of the control culture. Genes associated with DA neuron phenotypes are underlined.

Suppl. Table. S2. PCR primers information used in this study

Gene symbol	Sequence	Product size	Cycles & Annealing temp.
PCR primers for gene expression			
Tyrosine Hydroxylase (Th)	F : gccgtctcagagcaggatac R : agcatttccatccctctcct	196bp	30-32 cycles 60 ℃
Dopamine transporter (Dat)	F : tggcttcgttgtcttctcct R : cagctggaactcatcgacaa	221bp	26-28 cycles 58 ℃
Vesicle monoamine transporter2 (Vmat2)	F : ctttggagttggttttgc R : gcagttgtggtccatgag	300bp	26-28 cycles 58 ℃
Engrailed1 (En1)	F : tcaagactgactacagcaacccc R : ctttgtcctgaaccgtggtggtag	200bp	26-28 cycles 58 ℃
Gbx2	F : atgagcgcagcgttcccgccg R : cggcggtggcggcagcacca	200bp	26-28 cycles 58 ℃
CoREST	F : cacttggtatggacgacacg R : cagcccttaggcagaatgag	210bp	30-32 cycles 60 ℃
Forkhead box protein2 (Foxa2)	F : gacataccgacgcagctaca R : ggcaccttgagaaagcagtc	215bp	26-28 cycles 58 ℃
Nurr1	F : cggtttcagaagtgcctagc R : ttgcctggaacctggaatag	194bp	26-28 cycles 58 ℃
Gapdh	F : ctcatgaccacagtccatgc R : ttcagctctgggatgacctt	154bp	25-28 cycles 60 ℃
PCR primers for ChIP assay			
1. Primers for *Foxa2* promoter			
Foxa2 (Region1)	F : ctgcaggcagagaacacaga R : ctttctggctacccacctca	248bp	40-45 cycles 58 ℃
Foxa2 (Region2)	F : caagaccctccactccaaaa R : cagaggcaggaggatctcag	193bp	40-45 cycles 58 ℃
2. Primers for *Nurr1* promoter			
Nurr1 (Region1)	F : gcggtgggtcattgtttc R : gcgctccggttcattgtc	199bp	40-45 cycles 58 ℃
Nurr1 (Region2)	F : gggcacagtggcttaaaagt R : ctcctctgcaagttccaacc	181bp	40-45 cycles 58 ℃
Nurr1 (Region3)	F : tgaataagacacgcgtcagg R : agccccactgtcctttcttt	212bp	40-45 cycles 58 ℃
Nurr1 (Region4)	F : cagtgtcttaggggccagag R : gaagatcagctactctgctgga	221bp	40-45 cycles 58 ℃

Suppl. Table. S3. Prediction of Nurr1 and Foxa2 binding sites on promoters of DA neuronal marker genes.

TF	Gene promoter (Kb from TSS)	PWM setting		Predicted binding sites			
				Mouse		Rat	
				Sequence	Location	Sequence	Location
Nurr1	*Foxa2* (M:-1085) (R: -1069)	Conservation cutoffs	94%	AAGCTCAC	420~427	AAGCTCAA	428~435
				GTAACCTT	1041~1048	GTAACCTT	1025~1032
		Window size	50				
		Score threshold	80%				
	Th (M:-2505) (R: -2461)	Conservation Cutoffs	70%	AAGGTTAA	356-363	AAGGTTAA	336~343
				GAGGACAC	1399~1406	GAGGACAC	1346~1353
		Window size	50	AAGGTCCC	1511~1518	AAGGTCCC	1454~1461
				GAGGTCAG	1788~1795	GAGGTCAG	1747~1754
		Score threshold	80%	CTGGCCTT	2437~2444	CTGGCCTT	2392~2399
	Dat (M:-2775) (R: -2556)	Conservation Cutoffs	70%	CTGACCTA	560~567	TTGACCTA	246~253
				GTGACCAT	2045~2052	GTGACCAT	1820~1827
		Window size	50	GAGACCTG	2184~2191	GTGACCTG	1955~1962
				GTGGCCTC	2643~2650	GTGGCCTC	2423~2430
		Score thresold	75%				
Foxa2	*Nurr1* (M:-1091) (R: -1010)	Conservation Cutoffs	46%	AATGCAAATGA	212~223	ATACCAAAGAGC	129~140
				GACTGATAATTG	238~249	GAATGTGCAGGG	151~162
		Window size	50	AAATATTTACCT	370~381	CCCCGTTTCCCT	200~211
				AAGCCCCTTTAG	389~400	AAGCATCCTGTG	219~230
		Score threshold	60%				
	Th (M:-2505) (R: -2461)	Conservation Cutoffs	80%	ACACAGACAAAG	230~241	ACACAGACAAAG	211~222
				AAAGCAATATTT	320~331	AAAGCAATATTT	300~311
				CAATATTTGTGT	324~335	CAATATTTGTGT	304~315
		Window size	50	AAATCCACATTC	362~373	AAATCCACACTC	342~353
				GAGCAGGCAGTG	826~837	GAGCAGGCAGTG	783~794
				GAGTAAATAGTC	840~851	GAGTAAATAGTC	797~808
		Score threshold	85%	GAGTAGATAGTA	2000~2011	GAATAGATAGTA	1964~1975
				CTAGATTTATTT	2094~2105	CTAGATTTGTCT	2063~2074
				AATCCAGCATGG	2151~2162	AATCCAGCATGG	2109~2120
	Dat (M:-2775) (R: -2556)	Conservation Cutoffs	80%	GAATAAATGTTT	1357~1368	GAATAAATGTTT	1017~1028
				AAATGTTTGTTG	1361~1372	AAATGTTTGCTG	1021~1032
		Window size	50				
		Score threshold	85%				