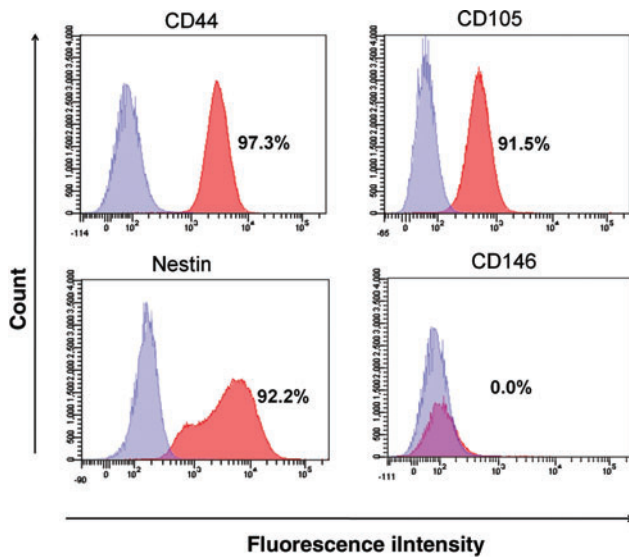
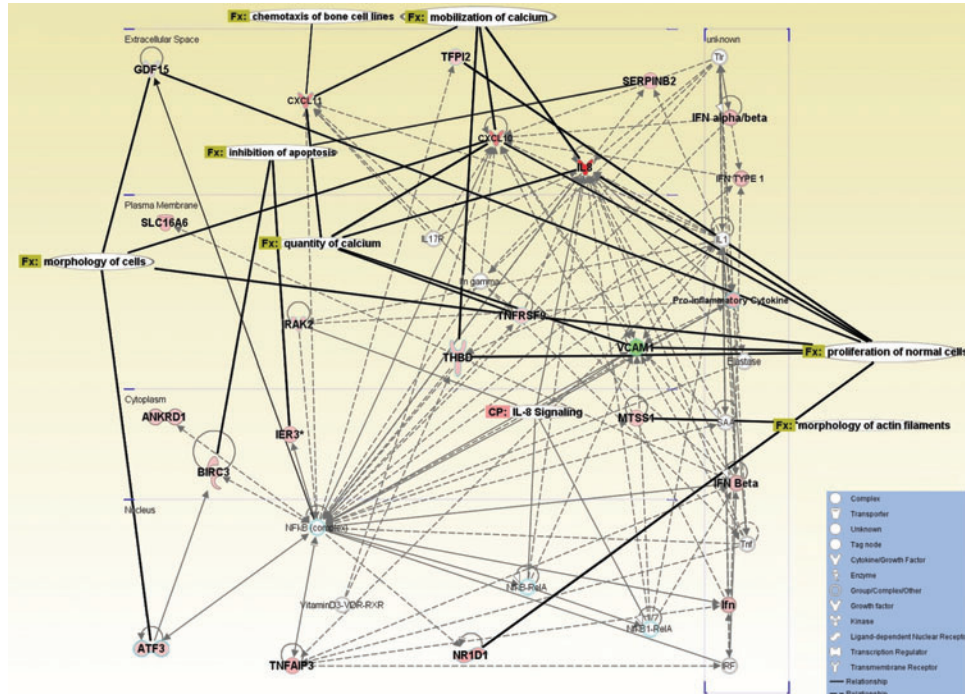


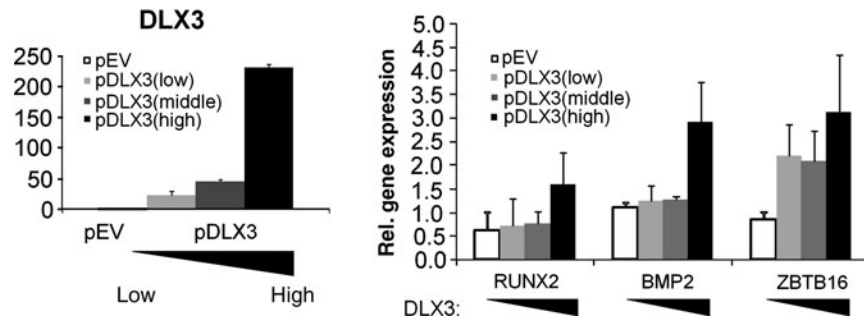
Supplementary Data



SUPPLEMENTARY FIG. S1. Flow cytometry analyses of DFCs. DFCs were positive for cell-surface markers CD 44, CD 105, and Nestin and negative for CD 146. The relative levels of cell surface expression are shown by the shift of the red histograms to the right compared with the fluorescence of the isotype-matched antibodies used as controls (blue histograms). The percentage of positive cells is indicated.



SUPPLEMENTARY FIG. S2. Ingenuity analysis. Network of pathways and biological processes induced after DLX3 over-expression in DFCs, generated through the use of Ingenuity Pathways Analysis (IPA; Ingenuity® Systems, <http://www.ingenuity.com>) using direct and indirect relationships between molecules. Direct interactions appear in the network diagram as a solid line (edge), whereas indirect interactions as a dashed line. All edges are supported by at least 1 reference from the literature, from a textbook, or from canonical information stored in the Ingenuity Pathways Knowledge Base. Molecules are represented as nodes. The intensity of the node color indicates the degree of up- (red) or down- (green) regulation. Nodes are displayed using various shapes that represent the functional class of the gene product. Fx: function. CP: canonical pathway.



SUPPLEMENTARY FIG. S3. DLX3 influences the gene expression of osteogenic markers in a dose-dependent manner in DFCs. The gene expression of DLX3, RUNX2, BMP2, and ZBTB16 was determined by qRT-PCR analysis after 48 hours of transfection. Three different dilutions of the pDLX3 plasmid, for a low, middle, and high expression of DLX3, and the empty vector (pEV) were used for transfections.

SUPPLEMENTARY TABLE S1. REAL-TIME PCR PRIMERS

<i>Gene</i>	<i>Forward 5' → 3'</i>	<i>Reverse 5' → 3'</i>	<i>Accession number</i>	<i>Probe number</i>
Distal-less homeobox 3 (<i>DLX3</i>)	GAGCCTCCTACCCGGCAATAC	TCCTCCTTCACCCGACACTG	NM_005220.2	26
Collagen 3 (<i>COL3A1</i>)	CCGTGGTCCACAAGGATTAC	GCCTGGTTCTCCAGGTTTT	NM_000090.3	SYBR green
Hairy and enhancer of split 1, (<i>Drosophila</i>) (<i>HES1</i>)	AGTGAAGCACCTCCGGAAC	CGTTCATGCACICCGCTGA	NM_005524.3	SYBR green
Jun proto-oncogene (<i>JUN</i>)	CCAAAAGGATAGTGGGATGTTT	CTGTCCCTCTCCCACTGCAAC	NM_002228.3	SYBR green
Early growth response 1 (<i>EGR1</i>)	AGCCCTACGACCTGAC	GGTTTGGCTGGGGTAACTG	NM_001964.2	SYBR green
Interleukin 8 (<i>IL8</i>)	CTTCCAAAGCTGGCCGTGGCTCT	TTCTCCACAACCCTCTGCACCCAG	NM_000584.2	SYBR green
Vascular cell adhesion molecule 1 (<i>VCAM1</i>)	TGGAGGCTGGGAAGCCGATCAC	GGGTCCAGGGGAGATCTCAACAG	NM_001078.2	SYBR green
Integrin, alpha 8 (<i>ITGA8</i>)	AGGAGAAAAGCAGACGGAAGT	CCCCAGCAGCAACTGAGTA	NM_003638.1	78
Integrin, beta 8 (<i>ITGB8</i>)	CCATGGAAAATCTGTGTGCTG	GGCCCTTTGAAATTGACACAG	NM_002214.2	26
Zinc finger and BTB domain containing 16 (<i>ZBTB16</i>)	CAAGAAGTTCAGCCTCAAGCA	CAC TCAAAGGGCTTCTCACC	NM_006006.4	78
Bone sialoprotein (<i>BSP</i>)	ACTGCCAGAGGCTCACTCC	TCATTTTGGTGATTGCTTCCCT	NM_004967.2	SYBR green
Alkaline phosphatase (<i>ALP</i>)	CAACCCCTGGGAGGAGAC	GCAITGGTGTGTACCGTCTTG	NM_000478.3	19
Bone morphogenetic protein 2 (<i>BMP2</i>)	CGGACTGCGGTCTCCTAA	GGAAGCAGCAACGCTAGAAG	NM_001200.2	SYBR green
Glyceraldehyde-3-phosphate-dehydrogenase (<i>GAPDH</i>)	AGCCACATCGCTCAGACAC	GCCCAATACGACCAAATCC	NM_002046	SYBR green
Runt-related transcription factor 2 (<i>RUNX2</i>)	CACCATGTCAAGCAAAACTTCTT	TCACGTCGCTCATTTTGC	NM_001015051.3	SYBR green

SUPPLEMENTARY TABLE S2. GENES REGULATED AFTER DLX3 OVEREXPRESSION IN DFCs

<i>Gene symbol</i>	<i>Gene name</i>	<i>Log2 Ratio</i>
ODZ2	Odz, odd Oz/ten-m homolog 2 (<i>Drosophila</i>)	-5.48
PSAT1	Phosphoserine aminotransferase 1	-3.70
LOC100130876	Hypothetical LOC100130876	-3.56
C20orf103	Chromosome 20 open reading frame 103	-3.43
HAPLN1	Hyaluronan and proteoglycan link protein 1	-3.34
EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1	-3.01
C4orf31	Chromosome 4 open reading frame 31	-2.87
SLC38A4	Solute carrier family 38, member 4	-2.80
PHGDH	Phosphoglycerate dehydrogenase	-2.79
SVEP1	Sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1	-2.78
ALDH1L2	Aldehyde dehydrogenase 1 family, member L2	-2.74
SLC7A11	Solute carrier family 7, (cationic amino acid transporter, y+ system) member 11	-2.71
C3	Complement component 3	-2.70
SFRP4	Secreted frizzled-related protein 4	-2.68
SULF2	Sulfatase 2	-2.63
FBN2	Fibrillin 2	-2.60
ADRA2A	Adrenergic, alpha-2A-, receptor	-2.59
SLITRK6	SLIT and NTRK-like family, member 6	-2.58
PDE5A	Phosphodiesterase 5A, cgmp-specific	-2.47
FAM38B2	Family with sequence similarity 38, member B2	-2.45
MFAP4	Microfibrillar-associated protein 4	-2.45
ASPEN	Asporin	-2.44
TRIM16L	Tripartite motif-containing 16-like	-2.43
OR4F15	Olfactory receptor, family 4, subfamily F, member 15	-2.41
PCK2	Phosphoenolpyruvate carboxykinase 2 (mitochondrial)	-2.39
CHRM2	Cholinergic receptor, muscarinic 2	-2.35
ACSS3	Acyl-coa synthetase short-chain family member 3	-2.34
PTGIS	Prostaglandin I2 (prostacyclin) synthase	-2.31
C4orf49	Chromosome 4 open reading frame 49	-2.29
GPRC5B	G protein-coupled receptor, family C, group 5, member B	-2.27
OLFML1	Olfactomedin-like 1	-2.27
TOX	Thymocyte selection-associated high mobility group box	-2.26
GLT8D4	Glycosyltransferase 8 domain containing 4	-2.25
C5orf36	Chromosome 5 open reading frame 36	-2.24
PLXNC1	Plexin C1	-2.23
ITGB8	Integrin, beta 8	-2.23
C5orf23	Chromosome 5 open reading frame 23	-2.23
ELN	Elastin	-2.21
ASNS	Asparagine synthetase	-2.20
UNQ3028	Tssp3028	-2.20
VCAM1	Vascular cell adhesion molecule 1	-2.14
EFHD1	EF-hand domain family, member D1	-2.12
MATN2	Matrilin 2	-2.10
FNDC1	Fibronectin type III domain containing 1	-2.09
SORBS2	Sorbin and SH3 domain containing 2	-2.08
WISP1	WNT1 inducible signaling pathway protein 1	-2.08
OMD	Osteomodulin	-2.07
F2RL2	Coagulation factor II (thrombin) receptor-like 2	-2.07
ANGPT1	Angiopoietin 1	-2.07
EFNA5	Ephrin-A5	-2.07
CBS	Cystathionine-beta-synthase	-2.05
MNS1	Meiosis-specific nuclear structural 1	-2.05
ITGA8	Integrin, alpha 8	-2.03
SLC46A3	Solute carrier family 46, member 3	-2.01
COL3A1	Collagen, type III, alpha 1	-2.00
EGR1	Early growth response 1	2.02
NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	2.02
KRTAP2-4	Keratin associated protein 2-4	2.03
KRTAP2-4	Keratin associated protein 2-4	2.03
HES1	Hairy and enhancer of split 1, (<i>Drosophila</i>)	2.04
BMP2	Bone morphogenetic protein 2	2.05
NR4A2	Nuclear receptor subfamily 4, group A, member 2	2.06

(continued)

SUPPLEMENTARY TABLE S2. (CONTINUED)

<i>Gene symbol</i>	<i>Gene name</i>	<i>Log2 Ratio</i>
GDF15	Growth differentiation factor 15	2.08
CHST2	Carbohydrate (N-acetylglucosamine-6-O) sulfotransferase 2	2.09
SPRY4	Sprouty homolog 4 (<i>Drosophila</i>)	2.12
KRTAP4-9	Keratin associated protein 4-9	2.15
VEGFA	Vascular endothelial growth factor A	2.16
HIST2H4A	Histone cluster 2, H4a	2.16
HIST2H4A	Histone cluster 2, H4a	2.16
GPRC5A	G protein-coupled receptor, family C, group 5, member A	2.17
SLC20A1	Solute carrier family 20 (phosphate transporter), member 1	2.18
PLAT	Plasminogen activator, tissue	2.19
TM4SF1	Transmembrane 4 L six family member 1	2.21
TNFRSF9	Tumor necrosis factor receptor superfamily, member 9	2.21
TIPARP	TCDD-inducible poly(ADP-ribose) polymerase	2.21
NPTX1	Neuronal pentraxin I	2.21
LOC554202	Hypothetical LOC554202	2.26
BHLHE41	Basic helix-loop-helix family, member e41	2.26
TFPI2	Tissue factor pathway inhibitor 2	2.27
KRTAP4-11	Keratin associated protein 4-11	2.31
KRTAP2-1	Keratin associated protein 2-1	2.32
KRTAP2-1	Keratin associated protein 2-1	2.33
PLAU	Plasminogen activator, urokinase	2.33
KRTAP2-4	Keratin associated protein 2-4	2.33
KRTAP2-4	Keratin associated protein 2-4	2.33
SCG2	Secretogranin II (chromogranin C)	2.33
C3orf59	Chromosome 3 open reading frame 59	2.34
ATF3	Activating transcription factor 3	2.36
GADD45A	Growth arrest and DNA-damage-inducible, alpha	2.36
FZD8	Frizzled homolog 8 (<i>Drosophila</i>)	2.37
ANKRD1	Ankyrin repeat domain 1 (cardiac muscle)	2.37
DUSP4	Dual specificity phosphatase 4	2.39
MTSS1	Metastasis suppressor 1	2.41
GPR56	G protein-coupled receptor 56	2.42
STC1	Stanniocalcin 1	2.47
PRSS35	Protease, serine, 35	2.47
BIRC3	Baculoviral IAP repeat-containing 3	2.50
JUN	Jun oncogene	2.52
CDCP1	CUB domain containing protein 1	2.53
KRTAP4-8	Keratin associated protein 4-8	2.57
IER3	Immediate early response 3	2.57
PRDM1	PR domain containing 1, with ZNF domain	2.58
SIK1	Salt-inducible kinase 1	2.58
SERPINB2	Serpin peptidase inhibitor, clade B (ovalbumin), member 2	2.61
IER3	Immediate early response 3	2.61
IER3	Immediate early response 3	2.61
ABCA13	ATP-binding cassette, sub-family A (ABC1), member 13	2.63
DUSP6	Dual specificity phosphatase 6	2.65
IFNB1	Interferon, beta 1, fibroblast	2.68
KRTAP4-11	Keratin associated protein 4-11	2.68
ETS2	V-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	2.70
SPRY2	Sprouty homolog 2 (<i>Drosophila</i>)	2.75
THBD	Thrombomodulin	2.77
SLC16A6	Solute carrier family 16, member 6 (monocarboxylic acid transporter 7)	2.87
IRAK2	Interleukin-1 receptor-associated kinase 2	2.88
EREG	Epiregulin	2.94
TMEM158	Transmembrane protein 158	2.99
RSPO3	R-spondin 3 homolog (<i>Xenopus laevis</i>)	3.04
CLDN1	Claudin 1	3.12
TNFAIP3	Tumor necrosis factor, alpha-induced protein 3	3.15
GEM	GTP binding protein overexpressed in skeletal muscle	3.25
CCRN4L	CCR4 carbon catabolite repression 4-like (<i>S. Cerevisiae</i>)	3.25
ACTN2	Actinin, alpha 2	3.29
LIF	Leukemia inhibitory factor (cholinergic differentiation factor)	3.33

(continued)

SUPPLEMENTARY TABLE S2. (CONTINUED)

<i>Gene symbol</i>	<i>Gene name</i>	<i>Log2 Ratio</i>
<i>ESM1</i>	Endothelial cell-specific molecule 1	3.54
<i>NR1D1</i>	Nuclear receptor subfamily 1, group D, member 1	3.62
<i>ANGPTL4</i>	Angiopoietin-like 4	3.67
<i>HBEGF</i>	Heparin-binding EGF-like growth factor	4.05
<i>MMP1</i>	Matrix metalloproteinase 1 (interstitial collagenase)	4.47
<i>CXCL11</i>	Chemokine (C-X-C motif) ligand 11	4.94
<i>CXCL10</i>	Chemokine (C-X-C motif) ligand 10	5.47
<i>DLX3</i>	Distal-less homeobox 3	8.63
<i>IL8</i>	Interleukin 8	8.78

ADP, adenosine diphosphate; ATP, adenosine-5'-triphosphate; EGF, epidermal growth factor; NTRK, nonreceptor tyrosine kinase; TCDD, 2,3,7,8-tetrachlorodibenzodioxin; ZNF, zinc finger.

SUPPLEMENTARY TABLE S3. TOP MOLECULAR AND CELLULAR FUNCTIONS AFTER DLX3 OVEREXPRESSION IN DFCs

<i>Name</i>	<i>P value</i>	<i>No. Molecules</i>
Cellular growth and proliferation	$2.18 \times 10^{-12} - 6.08 \times 10^{-14}$	50
Cellular development	$1.79 \times 10^{-11} - 7.47 \times 10^{-4}$	51
Cellular movement	$7.48 \times 10^{-11} - 7.55 \times 10^{-4}$	45
Cell death	$1.90 \times 10^{-9} - 7.73 \times 10^{-4}$	53
Cell-to-cell signaling and interaction	$7.30 \times 10^{-9} - 7.55 \times 10^{-4}$	40

SUPPLEMENTARY TABLE S4. TOP PHYSIOLOGICAL SYSTEM DEVELOPMENT AND FUNCTIONS AFTER DLX3 OVEREXPRESSION IN DFCs

<i>Name</i>	<i>P value</i>	<i>No. Molecules</i>
Tissue development	$5.40 \times 10^{-9} - 7.47 \times 10^{-4}$	41
Cardiovascular system development and function	$1.54 \times 10^{-8} - 7.55 \times 10^{-4}$	31
Organismal development	$1.54 \times 10^{-8} - 7.55 \times 10^{-4}$	35
Organismal survival	$2.38 \times 10^{-8} - 2.01 \times 10^{-5}$	31
Skeletal and muscular system development and function	$4.04 \times 10^{-8} - 6.70 \times 10^{-4}$	18