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Supplementary Data

The Transcriptomic Response of Mixed Neuron-Glial Cell Cultures to 1,25-Dihydroxyvitamin D3 Includes Genes Limiting the Progression of Neurodegenerative Diseases

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Supplementary Table 1
Primers used for RT-qPCR experiments

Gene	Forward	Reverse
ACTB	ACCAGAGGCATACAGGGACA	CTAAGGCCAACCGTGAAAAG
CAR14	GTTTCCCAGCCGCTTCTT	TGGGTCTAGGTGTGGGAATC
CBS	GCAGTGACAACCCCAAACA	GCCTGGTCAGGAGTGGTG
CRABP1	TGTGCAGTGAATCTTGTTCTCA	AAGGTCGGAGAGGGCTTC
CYP24a1	TCTTGATTTGGGGGTGAAAA	CTGCCATTGCGTTCTGT
DIO2	GGAATTGGGAGCATCTTCAC	CTGCGCTGTGTCTGGAAC
FBLN1	CGGCACTGCTGCTTACAG	CAGGACCAGCTAAGATTCCT
GRPR	CTTCCGGGATTCGATCTG	TGATTCAGAGTGCCTACAATCTTC
ITGA8	AGTTCTGTGCTCCTCTTGGAA	TGGAGAATTCACTGGGGACT
ITIH3	CTCTGGGAGGCTCCGTTT	CTCTGGCTTGGAGACCTCTG
LCAT	GAGGGGGAGAAACAAGTTGA	ACACGGCCTGTCATCCTC
NUPR1	GTGTGGTGTCTGTGGTCTGG	GAAGCTGCTGACCAAGTTCC
4833424O15Rik	GGAGCTAGGTTTCCTGTAACCAC	AACCGTCCGATTTCTTGGA
SLC7a3	GAGGAACAGCAGGCACCTT	AATTTCTGGGGTCATCTGGA
VDR	CTTCTCTGGGGACTCCTCCT	TGGACGAGTCCATCATGTCT
Tesc	AGGGTTTCCCGAGAGCAG	TCACGAAAGGAGAAGCTGAAAT
Cyp24a1	TCTTGATTTGGGGGTGAAAA	CTGCCATTGCGTTCTGT
SDHA	CAGTTCCACCCCACAGGTA	TCTCCACGACACCCTTCTGT

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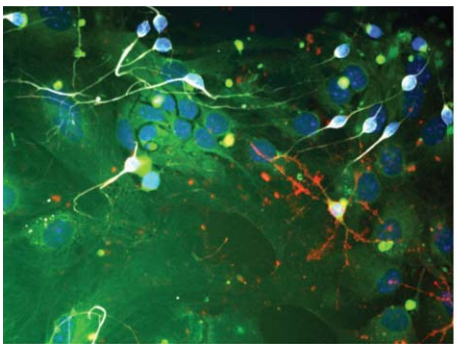
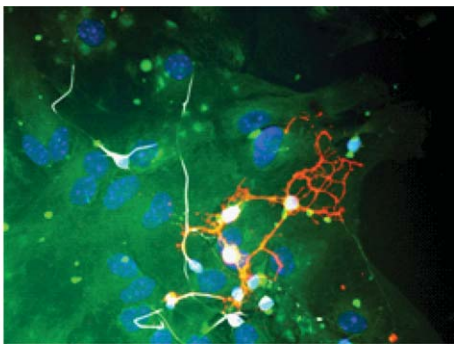
A



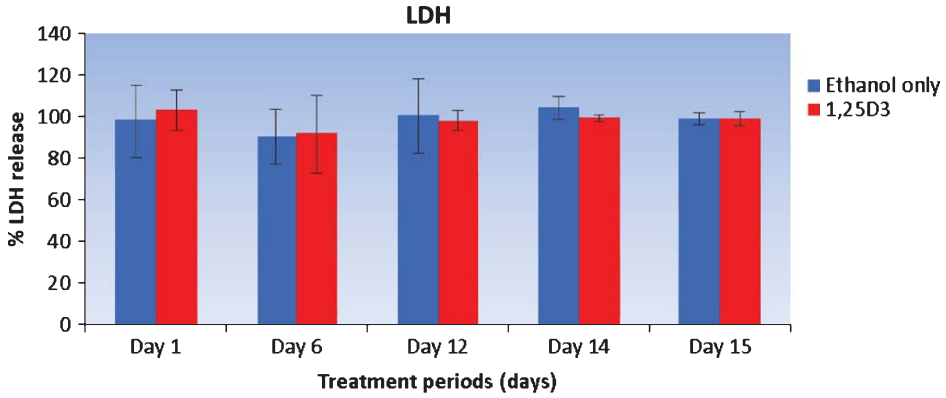
B



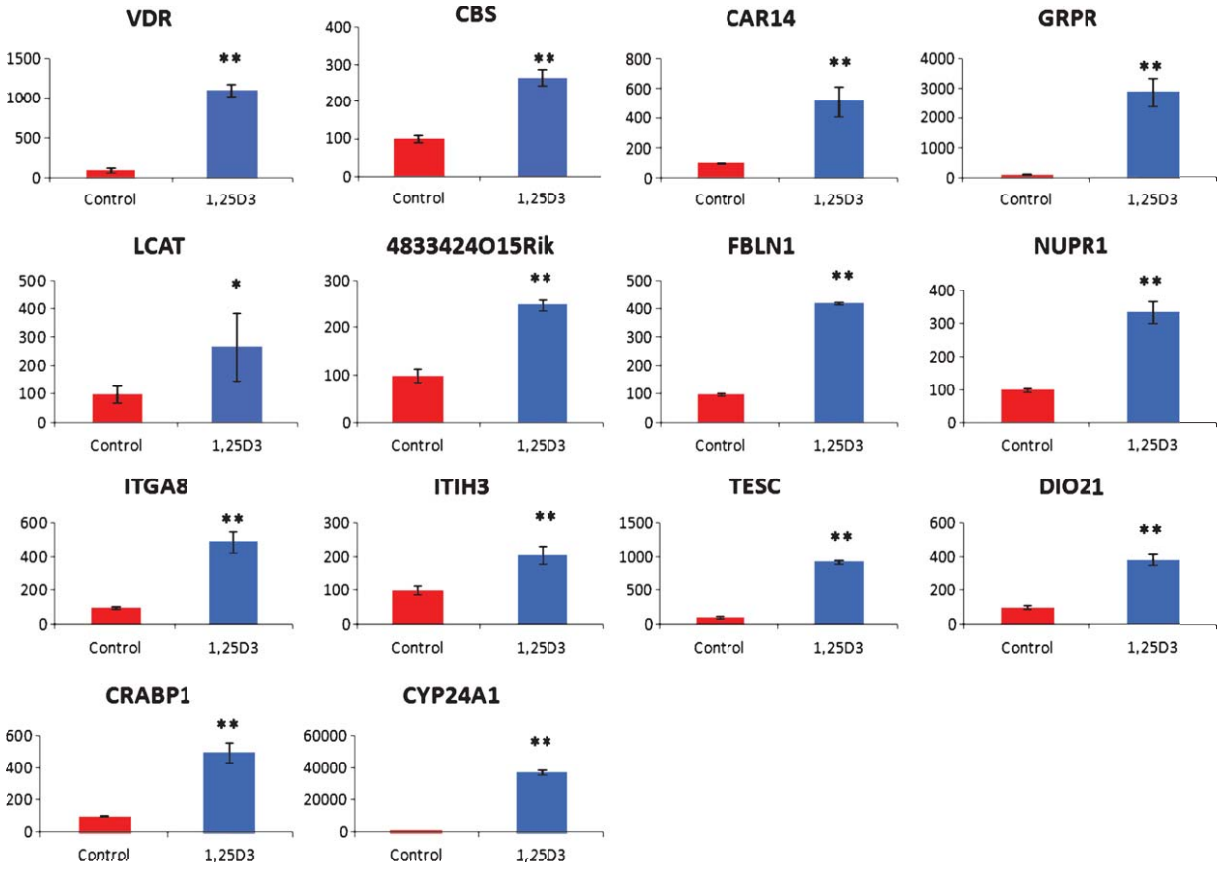
C

Probe set ID	Gene name	Gene symbol	Variation fold
10576332	tubulin, beta 3	Tubb3	1.0
10547807	enolase 2, gamma neuronal	Eno2	1.1
10383920	neurofilament, heavy polypeptide	Nefh	1.0
10421100	neurofilament, medium polypeptide	Nefm	1.0
10416175	neurofilament, light polypeptide	Nefl	1.0
10593293	neural cell adhesion molecule	Ncam1	1.1
10389047	amiloride-sensitive cation channel 1, neuronal	Accn1	0.9
10426782	amiloride-sensitive cation channel 2, neuronal	Accn2	1.0
10391798	glial fibrillary acidic protein	GFAP	0.9
10360227	phosphoprotein enriched in astrocytes 15A	Pea15a	0.9
10530847	phosphoprotein enriched in astrocytes 15B	Pea15b	1.1
10596575	mesencephalic astrocyte-derived neurotrophic factor	Manf	1.1
10381154	2',3'-cyclic nucleotide 3' phosphodiesterase	Cnp	1.0
10457022	myelin basic protein	Mbp	1.0
10436828	oligodendrocyte transcription factor 1	Olig1	0.8
10436823	oligodendrocyte transcription factor 2	Olig2	0.8
10361895	oligodendrocyte transcription factor 3	Olig3	1.0
10405058	oligodendrocyte myelin glycoprotein	Omg	1.0
10450845	myelin oligodendrocyte glycoprotein	Mog	1.1

Supplementary Figure 1. Immunophenotyping of mixed neuron-glial cell cultures. Mixed neuron-glial cell cultures prepared as described in the material and methods section were simultaneously stained with GFAP (astrocytes, green), βIII-tubulin (neuron, white), and O4 (oligodendrocytes, red) antibodies. Nuclei were stained with DAPI (blue). A) Control culture (79.2% astrocytes ($GFAP^+$), 18.2% neurons ($\beta III\text{-tubulin}^+$), and 2.6% oligodendrocytes ($O4^+$); number of counted cells = 1909. B) 1,25D3 treated culture (76.1% astrocytes ($GFAP^+$), 21.4% neurons ($\beta III\text{-tubulin}^+$), and 2.5% oligodendrocytes ($O4^+$), number of counted cells = 1221). C) 1,25D3 treatment did not affect the expression of genes coding for specific markers for neurons (yellow), astrocytes (green), or oligodendrocytes (blue). Tubb3, βIII-tubulin; Nef-H, neurofilament Heavy subunit; Nef-M, neurofilament medium subunit; Nef-L, neurofilament Light subunit; ENO2, enolase 2; Accn1, brain sodium channel 1; Accn2, brain sodium channel 2; Ncam1, neural cell adhesion molecule 1; GFAP, glial fibrillary acidic protein; Pea-15a, phosphoprotein enriched in astrocytes; Pea-15b, phosphoprotein enriched in astrocytes; Manf, mesencephalic astrocyte-derived neurotrophic factor; CNP, 2′,3′-cyclic nucleotide 3′ phosphodiesterase; MBP, myelin basic protein; Olig1, oligodendrocyte transcription factor 1; Olig2, oligodendrocyte transcription factor 2; Olig3, oligodendrocyte transcription factor 3; Omg, oligodendrocyte myelin glycoprotein; Mog, myelin oligodendrocyte glycoprotein



Supplementary Figure 2. Lactate dehydrogenase (LDH) release of mixed neuron-glial cells during the experiment. LDH release of the untreated group was considered 100%. The ethanol and 1,25D3 groups were not significantly changed compared to the untreated group ($p > 0.2$ for all cases).



Supplementary Figure 3. Confirmatory RT-qPCR of the transcriptomic data for the genes newly identified as up-regulated by 1,25D3 and putatively involved in neurodegenerative or psychiatric diseases, or brain morphogenesis. Results are depicted relative to control and normalized to β-actin and succinate dehydrogenase complex, subunit A (SDHA) mRNA. (Student's *t* test $*p < 0.05$; $**p < 0.01$). VDR, vitamin D receptor; CBS, cystathionine-beta-synthase; CAR14, carbonic anhydrase XIV; GRPR, gastrin-releasing peptide receptor; LCAT, lecithin-cholesterol acyltransferase; FBLN1, RIKEN cDNA 4833424O15, fibulin 1; NUPR1, nuclear protein, transcriptional regulator 1; ITGA8, integrin alpha 8; ITIH3, inter-alpha-trypsin inhibitor heavy chain 3; TECS, tescalcin; DIO2, deiodinase, iodothyronine, type II; CRABP1, cellular retinoic acid binding protein 1; Cyp24A1, 1,25-dihydroxyvitamin D3 24-hydroxylase.