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Supplementary Data

Changes in Brain Transcripts Related to Alzheimer's Disease in a Model of HFE Hemochromatosis are not Consistent with Increased Alzheimer's Disease Risk

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Supplementary Table 1
Primer sequences for real-time RT-PCR

Gene name and symbol	Forward primer	Reverse primer
Beta-actin *Actb*	CTGGCACCACACCTTCTA	GGTGGTGAAGCTGTAGCC
Ribosomal protein L13A *Rpl13a*	GGTGGAAGTACCAGGCAGTGA	TTCCGTAACCTCAAGATCTGCTT
Amyloid-β protein precursor *AβPP*	CCGTTGCCTAGTTGGTGAGTTT	CGACGGTGTGCCAGTGAA
Presenilin 1 *Psen1*	CTGATCGGCCTGTGCCTTA	AATCCGTGGCGAAGTAGAACA
Microtubule-associated protein tau *Mapt*	GTGGCAAGGTGCAGATAATTAATAAG	TCCACCGGCTTGTAGACTATTTG
Hairy and enhancer of split 1 *Hes1*	CACGACACCGGACAAACCA	CCTTCGCCTCTTCTCCATGATA
Hairy and enhancer of split 5 *Hes5*	GCCTCACCTCAAGGTCCACAT	GCACCCACCCATACAAAGGA

Supplementary Table 2
Significantly enriched pathways in the Average/BeadStudio gene list

DAVID		PANTHER	
Pathway	*p* value	Pathway	*p* value
MAPK signaling pathway	2.5×10^{-5}	FGF signaling pathway	4.1×10^{-5}
Long-term potentiation	1.9×10^{-4}	Huntington disease	8.4×10^{-5}
Long-term depression	3.9×10^{-4}	Ras Pathway	6.5×10^{-4}
Ubiquitin mediated proteolysis	4.1×10^{-4}	Interferon-γ signaling pathway	1.0×10^{-3}
Neurodegenerative diseases	0.003	Circadian clock system	0.001
Regulation of actin cytoskeleton	0.003	Notch signaling pathway	0.003
Purine metabolism	0.004	Ionotropic glutamate receptor pathway	0.003
Gap junction	0.004	EGF receptor signaling pathway	0.004
GnRH signaling pathway	0.004	Cytoskeletal regulation by Rho GTPase	0.004
Axon guidance	0.005	AD-amyloid secretase pathway	0.005
Colorectal cancer	0.005	Oxidative stress response	0.006
Proteasome	0.007	p38 MAPK pathway	0.007
Pancreatic cancer	0.010	Parkinson disease	0.007
Circadian rhythm	0.011	Ubiquitin proteasome pathway	0.008
Citrate cycle (TCA cycle)	0.011	General transcription regulation	0.012
Tight junction	0.013	Pyrimidine metabolism	0.014
RNA polymerase	0.014	TCA cycle	0.021
Bladder cancer	0.024	Wnt signaling pathway	0.022
Endometrial cancer	0.024	Integrin signalling pathway	0.024
Wnt signaling pathway	0.027	Endothelin signaling pathway	0.027
Aminophosphonate metabolism	0.031	Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway	0.027
Reductive carboxylate cycle	0.032	Angiogenesis	0.028
Pyrimidine metabolism	0.032	JAK/STAT signaling pathway	0.032
N-Glycan biosynthesis	0.037	B cell activation	0.034
Non-small cell lung cancer	0.037	Valine biosynthesis	0.036
Glycerophospholipid metabolism	0.037	Isoleucine biosynthesis	0.036
Valine, leucine and isoleucine degradation	0.038	DNA replication	0.039
Renal cell carcinoma	0.041	Toll receptor signaling pathway	0.040
Alzheimer's disease	0.044	Angiotensin II-stimulated signaling through G proteins and beta-arrestin	0.041
Notch signaling pathway	0.048	Cortocotropin releasing factor receptor signaling pathway	0.047
Prostate cancer	0.049	p53 pathway	0.048
		Axon guidance mediated by semaphorins	0.049
		Alpha adrenergic receptor signaling pathway	0.049

Supplementary Table 3
Enrichment of neurodegenerative disease pathways in different gene lists

Pathway	Fold enrichment	p value
PANTHER		
Alzheimer's disease	1.68	*0.005*
Parkinson's disease	1.59	*0.007*
Huntington's disease	1.74	8×10^{-5}
DAVID		
Neurodegenerative disease*	1.91	*0.003*
Alzheimer's disease	1.74	*0.044*
Parkinson's disease	–	NS
Huntington's disease	–	NS
Amyotrophic lateral sclerosis*	–	NS

Data are presented as fold enrichment and associated p value. NS, not significantly enriched. * "Neurodegenerative Disease" and "Amyotrophic Lateral Sclerosis" are not present in the PANTHER pathway database.

Supplementary Table 4
Significantly enriched pathways as determined by gene set enrichment analysis

Pathway	NES	Nominal p value	FDR q value
Average-normalized data			
Notch signaling pathway	1.98	0	0.0118
Type II diabetes mellitus	1.83	0.0014	0.0522
MAPK signaling pathway	1.78	0	0.0605
Wnt signaling pathway	1.73	0	0.0819
Axon guidance	1.68	0	0.1054
Melanogenesis	1.67	0	0.0961
Regulation of actin cytoskeleton	1.64	0.0011	0.1195
Neurodegenerative diseases	1.61	0.0085	0.1424
Taste transduction	1.59	0.0264	0.1505
Alzheimers disease	1.59	0.0201	0.1422
Insulin signaling pathway	1.58	0.0012	0.1373
Long term depression	1.57	0.0103	0.1371
Calcium signaling pathway	1.57	0.0023	0.1316
Adherens junction	1.49	0.0270	0.2311
Prostate cancer	1.49	0.0126	0.2243
Erbb signaling pathway	1.49	0.0231	0.2105
Hedgehog signaling pathway	1.47	0.0305	0.2231
Neuroactive ligand receptor interaction	1.47	0.0044	0.2168
p53 signaling pathway	1.47	0.0295	0.2071
Colorectal cancer	1.44	0.0368	0.2429
Ribosome	−1.68	0.0044	0.1451
Cubic spline-normalized data			
Type II diabetes mellitus	1.89	0	0.0882
Notch signaling pathway	1.88	0.0041	0.0457
Long term depression	1.88	0	0.0317
Alzheimer's disease	1.83	0.0034	0.0380
MAPK signaling pathway	1.77	0	0.0505
Wnt signaling pathway	1.67	0	0.0877
Neurodegenerative diseases	1.64	0.0146	0.0931
Taste transduction	1.63	0.0182	0.0899
1 and 2 methylnaphthalene degradation	1.56	0.0426	0.1237
Regulation of actin cytoskeleton	1.50	0	0.1730

A positive enrichment score indicates a tendency towards higher expression values in the wildtype control, a negative enrichment score indicates a tendency towards higher expression values in the *Hfe*$^{-/-}$. Pathways shown here passed a filter of FDR q value <0.25. NES, normalized enrichment score.