

SUPPLEMENTAL MATERIAL

GENOME-WIDE TRANSCRIPTOME STUDY IN SKIN BIOPSIES REVEALS AN ASSOCIATION OF *E2F4* WITH CADASIL AND COGNITIVE IMPAIRMENT

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1. Supplemental Tables

Supplemental Table I. TaqMan probes used in the qRT-PCR.

Gene	Probe
BANP	Hs0099999904_m1
PDCD6IP	Hs00994345_m1
CAMK2G	Hs00968809_mH
E2F4	Hs00608098_m1
PPIA	Hs0099999904_m1

Supplemental Table II. Proportion of negative Z-score regarding the five neuropsychological domains adjusted by age and educational level. The neuropsychological tests were performed in five CADASIL patients from the validation cohort.

Percentage of negative Z-score

Executive Function	80% (4/5)
Attention and Information Processing Speed	60% (3/5)
Motor Speed	60% (3/5)
Visuoconstructional Skills	20% (1/5)
Verbal Memory	60% (3/5)
Working Memory	60% (3/5)

Supplemental Table III. List of the GO terms enriched in the category of Biological Process.

ID	Description	Set Size	NES	p-value	p.adjust
GO:0006082	organic acid metabolic process	482	-1.47	1.01×10^{-3}	8.49×10^{-2}
GO:0007155	cell adhesion	480	-1.41	1.01×10^{-3}	8.49×10^{-2}
GO:0009968	negative regulation of signal transduction	461	-1.39	1.01×10^{-3}	8.49×10^{-2}
GO:0010648	negative regulation of cell communication	497	-1.37	1.01×10^{-3}	8.49×10^{-2}
GO:0023057	negative regulation of signaling	497	-1.37	1.01×10^{-3}	8.49×10^{-2}
GO:1901698	response to nitrogen compound	424	-1.49	1.01×10^{-3}	8.49×10^{-2}
GO:0009628	response to abiotic stimulus	462	-1.40	1.01×10^{-3}	8.49×10^{-2}
GO:0022610	biological adhesion	484	-1.41	1.01×10^{-3}	8.49×10^{-2}
GO:0030182	neuron differentiation	472	-1.37	1.01×10^{-3}	8.49×10^{-2}
GO:1901135	carbohydrate derivative metabolic process	484	-1.49	1.01×10^{-3}	8.49×10^{-2}
GO:0019752	carboxylic acid metabolic process	449	-1.45	1.01×10^{-3}	8.49×10^{-2}
GO:0043436	oxoacid metabolic process	476	-1.49	1.01×10^{-3}	8.49×10^{-2}
GO:0010243	response to organonitrogen compound	398	-1.47	1.01×10^{-3}	8.49×10^{-2}
GO:0040012	regulation of locomotion	393	-1.47	1.01×10^{-3}	8.49×10^{-2}
GO:0051270	regulation of cellular component movement	401	-1.5	1.01×10^{-3}	8.49×10^{-2}
GO:0072359	circulatory system development	385	-1.55	1.01×10^{-3}	8.49×10^{-2}
GO:0009894	regulation of catabolic process	370	-1.59	1.01×10^{-3}	8.49×10^{-2}
GO:0034097	response to cytokine	381	-1.44	1.01×10^{-3}	8.49×10^{-2}
GO:0035295	tube development	381	-1.56	1.01×10^{-3}	8.49×10^{-2}
GO:0048878	chemical homeostasis	409	-1.43	1.01×10^{-3}	8.49×10^{-2}
GO:0060548	negative regulation of cell death	380	-1.43	1.01×10^{-3}	8.49×10^{-2}
GO:0030334	regulation of cell migration	354	-1.55	1.01×10^{-3}	8.49×10^{-2}
GO:0032787	monocarboxylic acid metabolic process	279	-1.6	1.02×10^{-3}	8.49×10^{-2}
GO:1901137	carbohydrate derivative biosynthetic process	279	-1.53	1.02×10^{-3}	8.49×10^{-2}
GO:0035239	tube morphogenesis	309	-1.54	1.02×10^{-3}	8.49×10^{-2}
GO:0090407	organophosphate biosynthetic process	280	-1.52	1.02×10^{-3}	8.49×10^{-2}

GO:0031329	regulation of cellular catabolic process	320	-1.53	1.03×10^{-3}	8.49×10^{-2}
GO:0032870	cellular response to hormone stimulus	277	-1.54	1.03×10^{-3}	8.49×10^{-2}
GO:0072358	cardiovascular system development	264	-1.55	1.03×10^{-3}	8.49×10^{-2}
GO:0071417	cellular response to organonitrogen compound	236	-1.55	1.03×10^{-3}	8.49×10^{-2}
GO:1901699	cellular response to nitrogen compound	254	-1.54	1.03×10^{-3}	8.49×10^{-2}
GO:0001944	vasculature development	260	-1.56	1.03×10^{-3}	8.49×10^{-2}
GO:0001568	blood vessel development	252	-1.54	1.03×10^{-3}	8.49×10^{-2}
GO:0048514	blood vessel morphogenesis	221	-1.53	1.04×10^{-3}	8.49×10^{-2}
GO:0030335	positive regulation of cell migration	207	-1.6	1.05×10^{-3}	8.49×10^{-2}
GO:0006979	response to oxidative stress	196	-1.6	1.05×10^{-3}	8.49×10^{-2}
GO:0044057	regulation of system process	175	-1.61	1.05×10^{-3}	8.49×10^{-2}
GO:0009896	positive regulation of catabolic process	185	-1.65	1.06×10^{-3}	8.49×10^{-2}
GO:0048193	Golgi vesicle transport	169	-1.65	1.06×10^{-3}	8.49×10^{-2}
GO:0001667	ameboidal-type cell migration	162	-1.64	1.06×10^{-3}	8.49×10^{-2}
GO:0035690	cellular response to drug	145	-1.67	1.07×10^{-3}	8.49×10^{-2}
GO:0031331	positive regulation of cellular catabolic process	158	-1.56	1.07×10^{-3}	8.49×10^{-2}
GO:0071496	cellular response to external stimulus	158	-1.57	1.07×10^{-3}	8.49×10^{-2}
GO:1901293	nucleoside phosphate biosynthetic process	144	-1.61	1.07×10^{-3}	8.49×10^{-2}
GO:0036293	response to decreased oxygen levels	140	-1.57	1.07×10^{-3}	8.49×10^{-2}
GO:0040013	negative regulation of locomotion	139	-1.57	1.07×10^{-3}	8.49×10^{-2}
GO:0050900	leukocyte migration	140	-1.56	1.07×10^{-3}	8.49×10^{-2}
GO:0051271	negative regulation of cellular component movement	139	-1.57	1.07×10^{-3}	8.49×10^{-2}
GO:0001101	response to acid chemical	148	-1.66	1.07×10^{-3}	8.49×10^{-2}
GO:0070482	response to oxygen levels	146	-1.59	1.07×10^{-3}	8.49×10^{-2}
GO:0010038	response to metal ion	149	-1.57	1.07×10^{-3}	8.49×10^{-2}
GO:0009165	nucleotide biosynthetic process	143	-1.61	1.07×10^{-3}	8.49×10^{-2}
GO:0070997	neuron death	137	-1.7	1.07×10^{-3}	8.49×10^{-2}
GO:0016236	macroautophagy	136	-1.52	1.08×10^{-3}	8.49×10^{-2}
GO:0001655	urogenital system development	133	-1.55	1.08×10^{-3}	8.49×10^{-2}
GO:0001666	response to hypoxia	133	-1.57	1.08×10^{-3}	8.49×10^{-2}

GO:0010506	regulation of autophagy	135	-1.67	1.08×10^{-3}	8.49×10^{-2}
GO:0072330	monocarboxylic acid biosynthetic process	131	-1.53	1.08×10^{-3}	8.49×10^{-2}
GO:0031668	cellular response to extracellular stimulus	129	-1.56	1.08×10^{-3}	8.49×10^{-2}
GO:0034976	response to endoplasmic reticulum stress	128	-1.63	1.08×10^{-3}	8.49×10^{-2}

NES: normalized enrichment score; p.adjust: adjusted p-value.

Supplemental Table IV. List of the GO terms enriched in the category of Cellular Components.

ID	Description	Set Size	NES	p-value	p.adjust
GO:0005789	endoplasmic reticulum membrane	472	-1.38	1.00×10^{-3}	5.03×10^{-2}
GO:0030054	cell junction	472	-1.58	1.00×10^{-3}	5.03×10^{-2}
GO:0031226	intrinsic component of plasma membrane	470	-1.42	1.00×10^{-3}	5.03×10^{-2}
GO:0005768	endosome	368	-1.45	1.01×10^{-3}	5.03×10^{-2}
GO:0005773	vacuole	338	-1.48	1.01×10^{-3}	5.03×10^{-2}
GO:0005887	integral component of plasma membrane	445	-1.47	1.01×10^{-3}	5.03×10^{-2}
GO:0098827	endoplasmic reticulum subcompartment	475	-1.38	1.01×10^{-3}	5.03×10^{-2}
GO:0044431	Golgi apparatus part	403	-1.5	1.01×10^{-3}	5.03×10^{-2}
GO:0098791	Golgi subcompartment	365	-1.55	1.01×10^{-3}	5.03×10^{-2}
GO:0098590	plasma membrane region	396	-1.54	1.01×10^{-3}	5.03×10^{-2}
GO:0098796	membrane protein complex	428	-1.41	1.01×10^{-3}	5.03×10^{-2}
GO:0012506	vesicle membrane	318	-1.6	1.01×10^{-3}	5.03×10^{-2}
GO:0030659	cytoplasmic vesicle membrane	307	-1.61	1.01×10^{-3}	5.03×10^{-2}
GO:0000139	Golgi membrane	310	-1.49	1.01×10^{-3}	5.03×10^{-2}
GO:0044437	vacuolar part	257	-1.59	1.02×10^{-3}	5.03×10^{-2}
GO:0070161	anchoring junction	227	-1.62	1.04×10^{-3}	5.03×10^{-2}
GO:0005912	adherens junction	220	-1.66	1.04×10^{-3}	5.03×10^{-2}
GO:0005911	cell-cell junction	173	-1.7	1.06×10^{-3}	5.03×10^{-2}
GO:0030055	cell-substrate junction	175	-1.63	1.06×10^{-3}	5.03×10^{-2}
GO:0005924	cell-substrate adherens junction	172	-1.67	1.06×10^{-3}	5.03×10^{-2}
GO:0005925	focal adhesion	172	-1.67	1.06×10^{-3}	5.03×10^{-2}
GO:0030139	endocytic vesicle	128	-1.64	1.08×10^{-3}	5.03×10^{-2}
GO:003066	endocytic vesicle membrane	80	-1.73	1.15×10^{-3}	5.14×10^{-2}
GO:0001739	sex chromatin	3	-1.73	1.77×10^{-3}	7.54×10^{-2}
GO:0042175	nuclear outer membrane-endoplasmic reticulum membrane network	482	-1.38	2.01×10^{-3}	7.69×10^{-2}

GO:0000323	lytic vacuole	297	-1.45	2.03×10^{-3}	7.69×10^{-2}
GO:0005764	lysosome	297	-1.45	2.03×10^{-3}	7.69×10^{-2}
GO:0005774	vacuolar membrane	181	-1.54	2.12×10^{-3}	7.75×10^{-2}
GO:0005802	trans-Golgi network	108	-1.6	2.20×10^{-3}	7.76×10^{-2}
GO:0030134	COPII-coated ER to Golgi transport vesicle	48	-1.82	2.45×10^{-3}	8.36×10^{-2}
GO:0033176	proton-transporting V-type ATPase complex	10	-1.79	2.93×10^{-3}	9.67×10^{-2}
GO:0030133	transport vesicle	155	-1.52	3.21×10^{-3}	1.03×10^{-1}
GO:0044853	plasma membrane raft	40	-1.77	3.71×10^{-3}	1.15×10^{-1}
GO:0005901	caveola	32	-1.87	3.92×10^{-3}	1.15×10^{-1}
GO:0016328	lateral plasma membrane	31	-1.78	3.92×10^{-3}	1.15×10^{-1}
GO:0001533	cornified envelope	35	1.96	4.29×10^{-3}	1.19×10^{-1}
GO:0062023	collagen-containing extracellular matrix	131	-1.57	4.30×10^{-3}	1.19×10^{-1}
GO:0005798	Golgi-associated vesicle	80	-1.59	4.62×10^{-3}	1.24×10^{-1}
GO:0031012	extracellular matrix	173	-1.51	5.29×10^{-3}	1.24×10^{-1}
GO:0043235	receptor complex	134	-1.52	5.39×10^{-3}	1.33×10^{-1}
GO:0016324	apical plasma membrane	115	-1.55	5.49×10^{-3}	1.33×10^{-1}
GO:0030061	mitochondrial crista	6	1.78	5.71×10^{-3}	1.33×10^{-1}
GO:0031314	extrinsic component of mitochondrial inner membrane	6	1.8	5.71×10^{-3}	1.33×10^{-1}
GO:0031414	N-terminal protein acetyltransferase complex	6	1.78	5.71×10^{-3}	1.33×10^{-1}
GO:0016471	vacuolar proton-transporting V-type ATPase complex	7	-1.81	5.90×10^{-3}	1.34×10^{-1}
GO:0031092	platelet alpha granule membrane	10	1.79	6.27×10^{-3}	1.36×10^{-1}
GO:0031252	cell leading edge	168	-1.47	6.36×10^{-3}	1.36×10^{-1}
GO:0097060	synaptic membrane	122	-1.55	6.49×10^{-3}	1.36×10^{-1}
GO:0009986	cell surface	261	-1.4	7.16×10^{-3}	1.36×10^{-1}
GO:0044440	endosomal part	229	-1.4	7.24×10^{-3}	1.36×10^{-1}
GO:0098797	plasma membrane protein complex	185	-1.48	7.38×10^{-3}	1.36×10^{-1}
GO:0098794	postsynapse	188	-1.47	7.40×10^{-3}	1.36×10^{-1}
GO:0044291	cell-cell contact zone	32	-1.64	7.83×10^{-3}	1.36×10^{-1}

GO:0016323	basolateral plasma membrane	95	-1.52	7.88×10^{-3}	1.36×10^{-1}
GO:0031093	platelet alpha granule lumen	26	-1.71	7.96×10^{-3}	1.36×10^{-1}
GO:0014704	intercalated disc	23	-1.74	7.16×10^{-3}	1.36×10^{-1}
GO:0005766	primary lysosome	69	-1.57	7.16×10^{-3}	1.36×10^{-1}
GO:0042582	azurophil granule	69	-1.57	7.16×10^{-3}	1.36×10^{-1}
GO:0098552	side of membrane	178	-1.44	7.16×10^{-3}	1.36×10^{-1}
GO:0060076	excitatory synapse	15	-1.72	7.16×10^{-3}	1.36×10^{-1}

NES: normalized enrichment score; p.adjust: adjusted p-value.

2. Supplemental Figures

Supplemental Figures I: Z-score regarding the five neuropsychological domains adjusted by age and educational level, evaluated in the five patients with available cognitive information.

EF: executive function; IPS: information processing speed; VCF: visuoconstructional function.

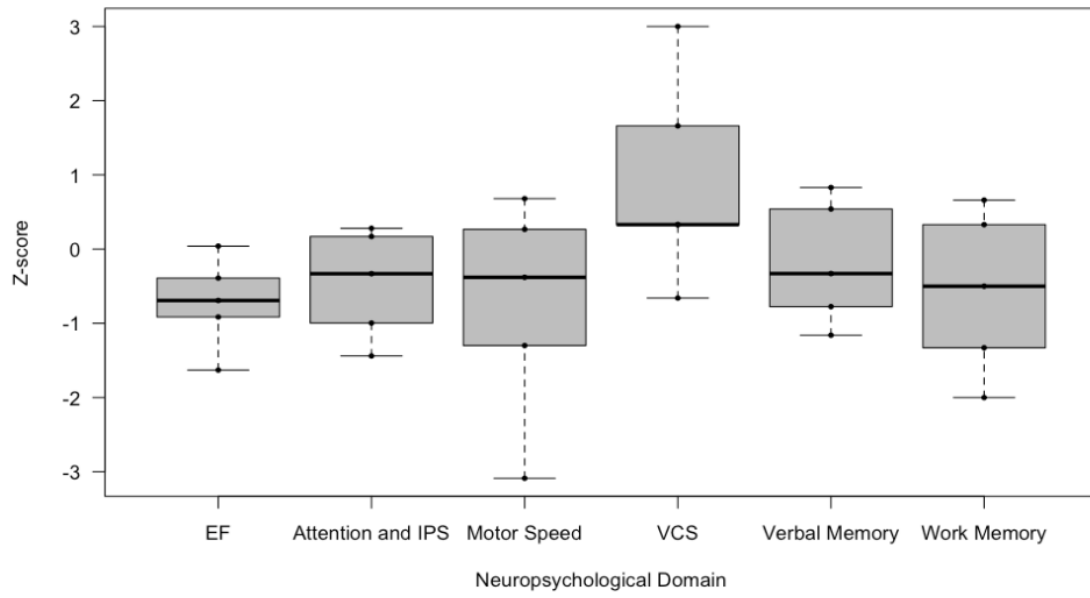
Supplemental Figure II. *E2F4* gene expression profile in decreasing order (GTEx Portal, <https://gtexportal.org/home/gene/E2F4>).

Supplemental Figure III. Single-Nuclei Brain RNA-seq expression of *E2F4*.

Supplemental Figure IV. Enrichment map (biological process) of the top 60 terms. This map groups gene ontology (GO) terms by similarity. Nodes are colored by p-value and their size reflects the number of genes found in that term.

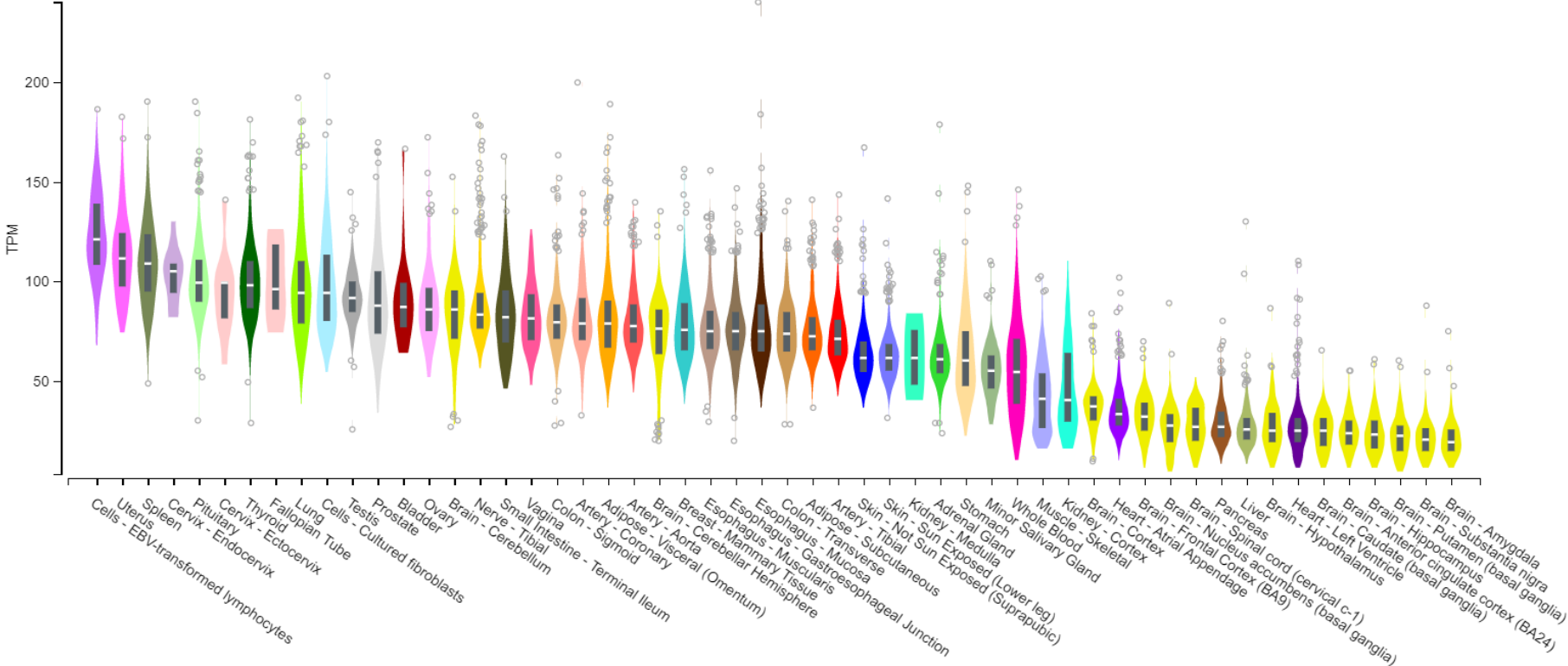
Supplemental Figure V. Enrichment map (cellular components) of the top 60 terms. This map groups gene ontology (GO) terms by similarity. Nodes are colored by p-value and their size reflects the number of genes found in that term.

Supplemental Figures I

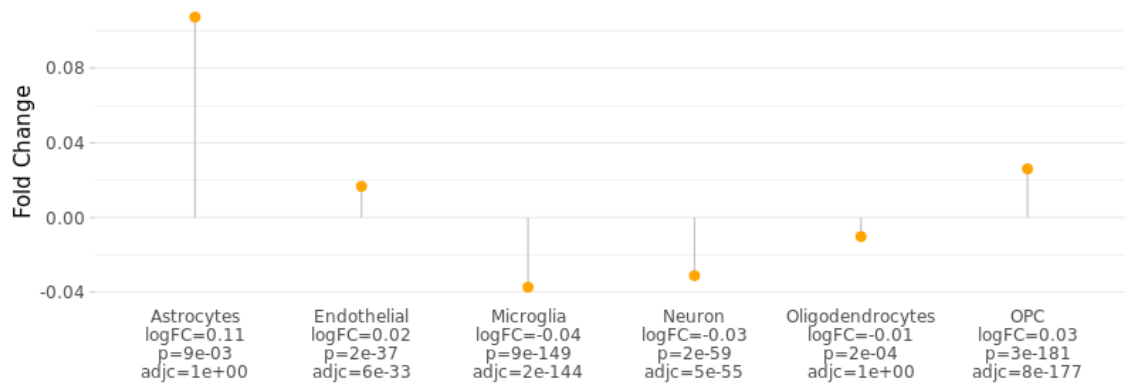


Supplemental Figure II

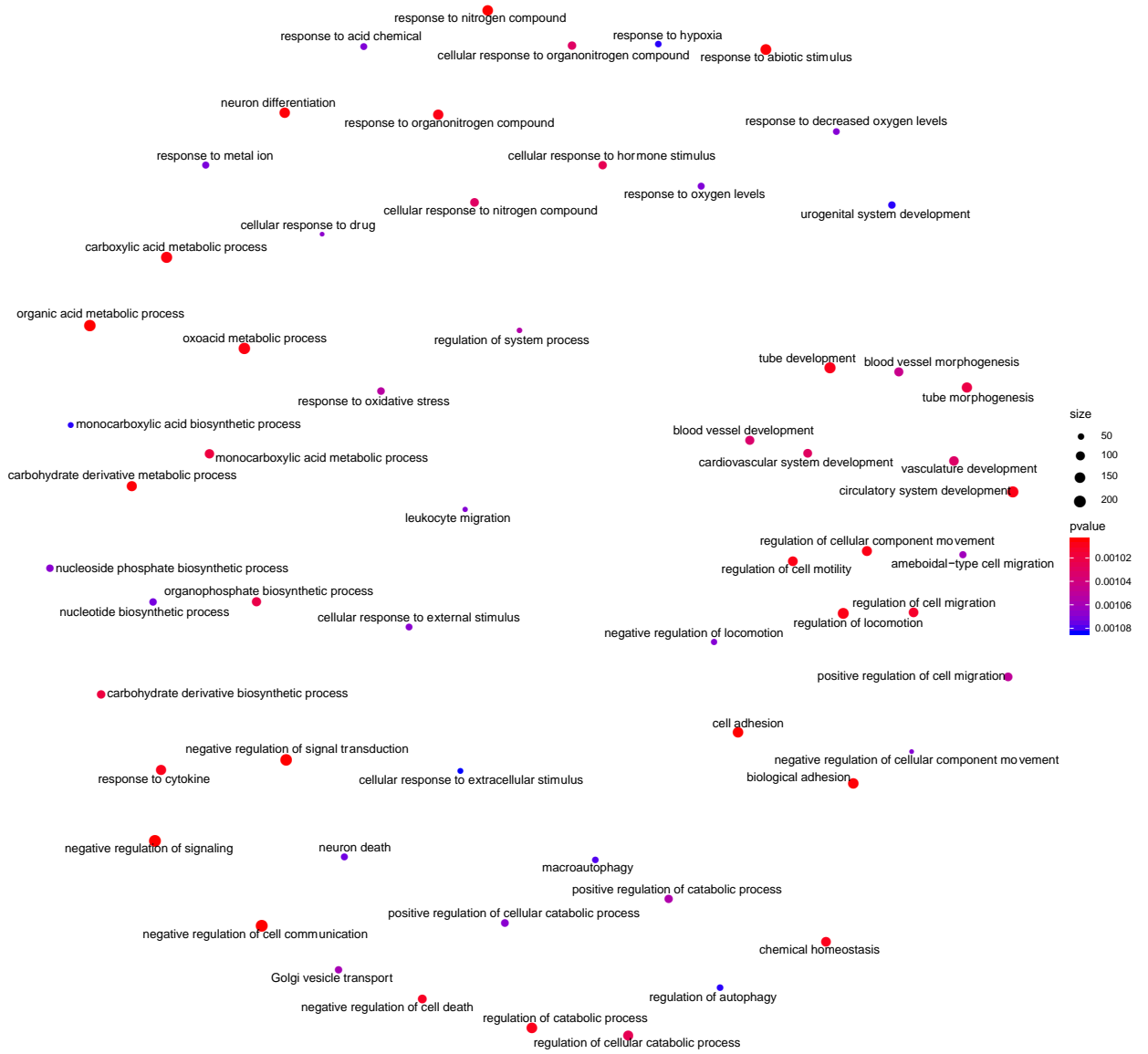
Gene expression for E2F4 (ENSG00000205250.8)



Supplemental Figure III



Supplemental Figure IV



Supplemental Figure V

