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Article

Differential Response Following Infection of Mouse CNS with Virulent and Attenuated Vaccinia Virus Strains

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Supplementary Materials

Table S1. Selected early (2 d.p.i.) and late (4-5 d.p.i.) differentiating genes used for quantitative real-time RT-PCR.

Gene#	Early (2 d.p.i.)	Gene #	Late (4-5 d.p.i.)
1	Ifi44-Mm00505670_m1	1	Cd3d-Mm00442746_m1
2	Irf7-Mm00516793_g1	2	Cd3e-Mm01179194_m1
3	Ifi27l2a-Mm01329883_gH	3	Cd8a-Mm01182107_g1
4	Ifit1-Mm00515153_m1	4	Cd8b1-Mm00438116_m1
5	Isg15-Mm01705338_s1	5	Cd27-Mm01185212_g1
6	Slfn1-Mm00488306_m1	6	Cxcr3-Mm99999054_s1
7	Rtp4-Mm00469759_m1	7	Foxp3-Mm00475162_m1
8	Ifit3-Mm01704846_s1	8	Gimap7-Mm00525315_m1
9	Zbp1-Mm01247052_m1	9	Ncr1-Mm01337324_g1
10	Cxcl10-Mm00445235_m1	10	Ptprcap-Mm01236556_m1
11	Ms4a6c-Mm00459296_m1	11	Sh2d1a-Mm01316997_m1

12	Slfn4-Mm01298330_m1	12	Il11-Mm00434162_m1
13	18S-Hs99999901_s1	13	Mmp3-Mm00440295_m1
		14	Mmp8-Mm00439509_m1
		15	Slc10a6-Mm00512730_m1
		16	Timp1-Mm01341360_g1
		17	Trem1-Mm01278455_m1
		18	Cxcl2-Mm00436450_m1
		19	Cxcr2-Mm99999117_s1
		20	18S-Hs99999901_s1

Table S2. Significant brain gene expression level following VACV infection (correspomding to Table 3).

Gene	*p* value			
	Day 4 p.i.		Day 5 p.i.	
	Wyeth	WR	Wyeth	WR
C1ra	6.5E-58	6.6E-07	1.2E-19	1.1E-46
C1rb	1.1E-07	6.9E-03	3.3E-05	5.7E-05
C1s	1.6E-87	6.0E-07	6.6E-102	4.8E-65
C2	1.4E-51	2.3E-03	8.7E-13	1.8E-26
C3	7.6E-30	3.6E-09	5.4E-32	1.2E-139
C6	1.6E-05	4.7E-01	1.1E-02	7.4E-02
H2-Aa	6.7E-67	4.0E-04	6.6E-80	5.4E-65
H2-Eb1	3.3E-140	3.2E-03	5.1E-57	2.7E-61
H2-K1	2.1E-284	2.4E-14	1.7E-130	6.7E-199
Cd3d	2.7E-17	2.1E-01	1.3E-27	5.0E-05
Cd3e	2.9E-22	1.0E-02	4.8E-36	4.6E-09
Cd3g	4.1E-17	2.4E-01	3.4E-32	8.4E-06
Cd8a	4.6E-25	9.3E-02	1.1E-44	3.1E-09
Cd8b1	7.2E-14	1.6E-01	1.2E-40	1.1E-04
Cd2	1.4E-17	7.0E-01	1.3E-28	9.0E-07
Cd27	5.5E-16	8.0E-01	2.4E-22	1.7E-03
Cd5	9.6E-05	2.1E-01	9.8E-21	4.6E-01
Ccl2	3.3E-68	3.8E-21	5.1E-14	8.5E-80
Ccl4	3.0E-19	NA	5.6E-10	1.4E-32
Ccl5	3.6E-41	2.3E-09	4.7E-74	3.4E-23
Ccl7	4.1E-37	2.4E-08	2.2E-22	3.5E-42

Ccl8	9.5E-21	1.7E-02	3.0E-28	1.4E-09
Ccl12	1.2E-64	6.1E-19	1.2E-15	5.9E-18
Ccl19	2.7E-11	6.4E-01	1.8E-11	2.0E-03
Mki67	7.3E-27	9.0E-02	8.8E-83	6.1E-19
CD40	3.9E-11	6.1E-01	3.0E-12	9.7E-06
Tmem119	4.4E-22	8.7E-01	9.7E-17	5.2E-01
Cx3cr1	1.8E-01	1.1E-02	7.4E-02	5.6E-25
Prf1	5.2E-21	6.3E-01	6.92E-22	4.07E-19
Gzma	2.3E-42	2.2E-06	1.07E-45	8.17E-29
Gzmb	2.0E-55	3.6E-04	2.39E-48	1.25E-55
Mmp3	2.6E-03	1.1E-17	5.0E-01	8.7E-22
Mmp8	1.0E-01	1.3E-04	4.2E-02	1.3E-21
Mmp13	5.0E-01	8.2E-04	1.0E-01	8.5E-06
Mmp19	1.5E-05	2.0E-09	2.8E-06	1.5E-42
Timp1	4.1E-18	1.1E-22	3.1E-15	1.1E-95

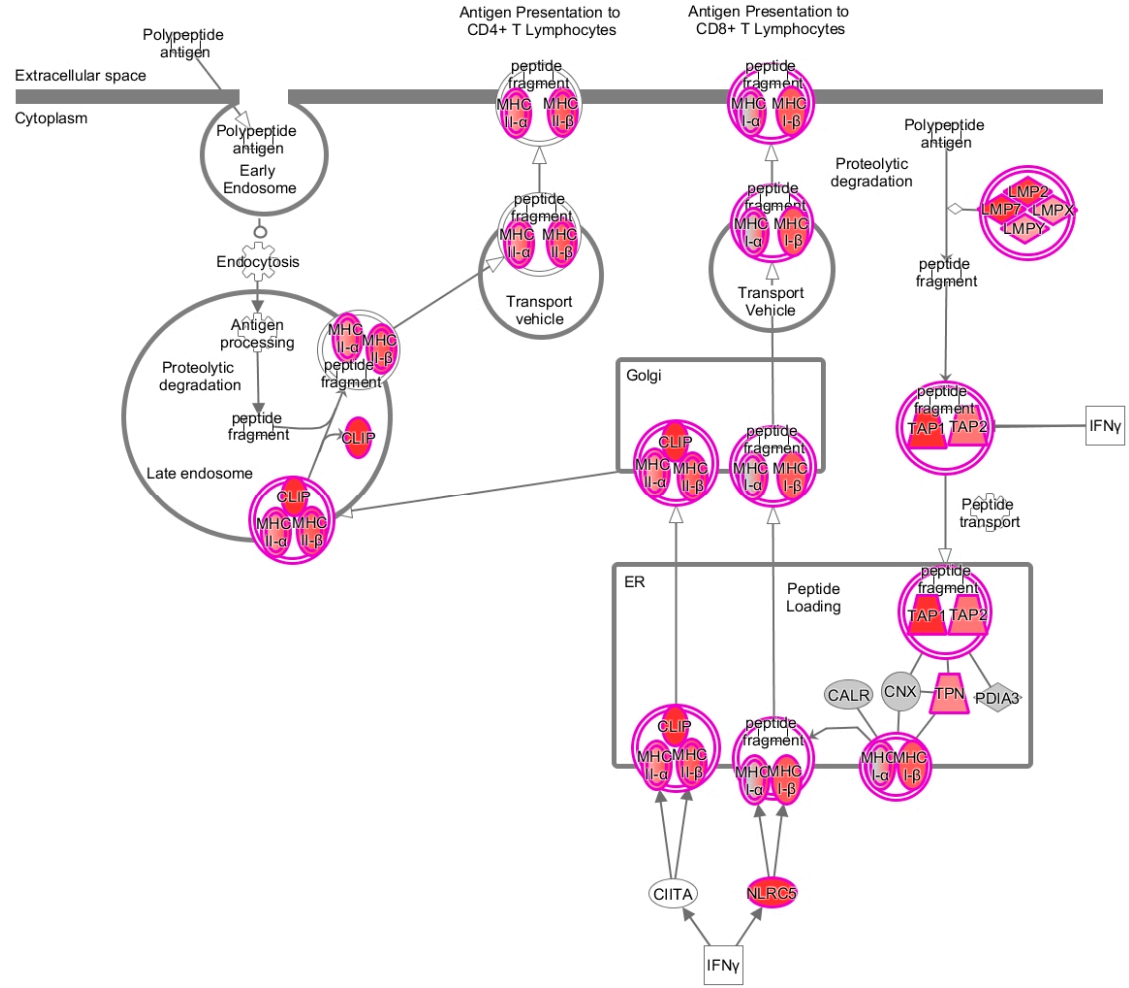
Figure S1. Top canonical pathways following infection with VACV-Wyeth and VACV-WR. IPA analysis of brain gene expression 2, 4 and 5 d.p.i with VACV-Wyeth (106 pfu) and VACV-WR (100pfu). Shared pathways are color coded respectively.

No.	**VACV-Wyeth (p-values)**				**No.**	**VACV-WR (p-values)**			
#	**Canonical Pathway**	**Day2**	**Day4**	**Day5**	**#**	**Canonical Pathway**	**Day2**	**Day4**	**Day5**
1	Th1 and Th2 Activation Pathway	0.5	23.0	34.4	1	Hepatic Fibrosis / Hepatic Stellate Cell Activation	0.5	19.6	23.0
2	Th1 Pathway	0.5	16.7	30.2	2	Granulocyte Adhesion and Diapedesis	0.0	13.9	19.2
3	T Helper Cell Differentiation	1.9	16.3	28.5	3	Role of Macrophages Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	1.4	11.4	20.0
4	Type I Diabetes Mellitus Signaling	2.3	15.2	24.1	4	Atherosclerosis Signaling	0.0	12.6	19.5
5	Th2 Pathway	0.5	16.3	24.3	5	Acute Phase Response Signaling	1.3	14.0	16.4
6	TREM1 Signaling	0.0	14.4	24.7	6	Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	0.5	9.4	19.0
7	Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	2.4	16.2	20.5	7	Agranulocyte Adhesion and Diapedesis	0.0	14.2	14.4
8	Role of Macrophages Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	2.7	13.1	22.3	8	Dendritic Cell Maturation	0.6	8.1	17.1
9	Altered T Cell and B Cell Signaling in Rheumatoid Arthritis	0.5	13.2	24.2	9	Type I Diabetes Mellitus Signaling	0.0	7.0	17.8
10	iCOS-iCOSL Signaling in T Helper Cells	1.1	14.5	21.6	10	Th1 Pathway	0.0	7.1	16.5

11	Dendritic Cell Maturation	2.7	13.1	20.3	11	Colorectal Cancer Metastasis Signaling	2.8	6.6	13.9
12	Acute Phase Response Signaling	2.5	14.9	16.4	12	Th1 and Th2 Activation Pathway	0.0	5.8	17.3
13	Hepatic Fibrosis / Hepatic Stellate Cell Activation	2.4	11.2	18.4	13	Glucocorticoid Receptor Signaling	7.5	6.2	9.2
14	EIF2 Signaling	18.8	12.8	0.0	14	Role of Osteoblasts Osteoclasts and Chondrocytes in Rheumatoid Arthritis	1.0	6.4	14.4
15	CD28 Signaling in T Helper Cells	1.4	12.4	17.5	15	Axonal Guidance Signaling	5.9	6.0	9.6
16	Role of NFAT in Regulation of the Immune Response	2.6	13.1	15.4	16	LXR/RXR Activation	0.0	8.7	12.8
17	Antigen Presentation Pathway	6.3	9.7	14.9	17	TREM1 Signaling	0.0	5.1	15.9
18	PKCθ Signaling in T Lymphocytes	1.8	11.7	15.8	18	IL-8 Signaling	4.6	3.5	12.7
19	Crosstalk between Dendritic Cells and Natural Killer Cells	0.5	11.6	17.1	19	IL-10 Signaling	1.0	7.1	12.0
20	Death Receptor Signaling	2.3	9.5	16.3	20	Molecular Mechanisms of Cancer	6.7	3.0	10.4
21	Communication between Innate and Adaptive Immune Cells	0.0	10.7	17.0	21	T Helper Cell Differentiation	0.0	4.8	15.1
22	IL-10 Signaling	2.1	12.5	12.1	22	NF-κB Signaling	2.5	4.1	12.7
23	Tec Kinase Signaling	1.4	9.4	15.1	23	Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	1.8	6.3	11.2
24	Granulocyte Adhesion and Diapedesis	0.0	10.5	15.3	24	Osteoarthritis Pathway	1.7	6.5	10.7
25	Atherosclerosis Signaling	0.3	10.1	15.1	25	Altered T Cell and B Cell Signaling in Rheumatoid Arthritis	0.0	3.1	15.6
26	T Cell Receptor Signaling	1.5	11.0	12.9	26	Antigen Presentation Pathway	0.3	7.8	10.5
27	IL-6 Signaling	2.0	8.6	14.0	27	Th2 Pathway	0.3	3.7	14.2
28	Phagosome Formation	1.4	9.7	13.2	28	EIF2 Signaling	18.1	0.0	0.0
29	NF-κB Signaling	2.0	7.9	13.9	29	IL-6 Signaling	0.9	6.3	10.5
30	PI3K Signaling in B Lymphocytes	2.7	10.6	10.3	30	Leukocyte Extravasation Signaling	1.5	6.9	9.2
31	Interferon Signaling	5.3	7.5	10.9	31	Phagosome Formation	0.9	3.9	11.8
32	Graft-versus-Host Disease Signaling	1.1	9.4	12.4	32	Death Receptor Signaling	0.3	5.7	10.1
33	Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	1.8	8.5	11.9	33	Communication between Innate and Adaptive Immune Cells	0.0	2.4	13.6
34	Toll-like Receptor Signaling	1.4	8.2	12.6	34	iCOS-iCOSL Signaling in T Helper Cells	0.7	1.5	13.5

Figure S2. Antigen Presentation Pathway. Genes associated with antigen presentation following infection with (A) VACV-Wyeth (-log(*p*-values): 6.3) and (B) VACV-WR 2 d.p.i. (-log(*p*-values): 0.3). Differentially expressed genes are highlighted in color. Color intensity indicates the degree of up regulation (red) or down-regulation (green) relative to the day 2 mock infected brains. Solid lines represent direct interactions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

A



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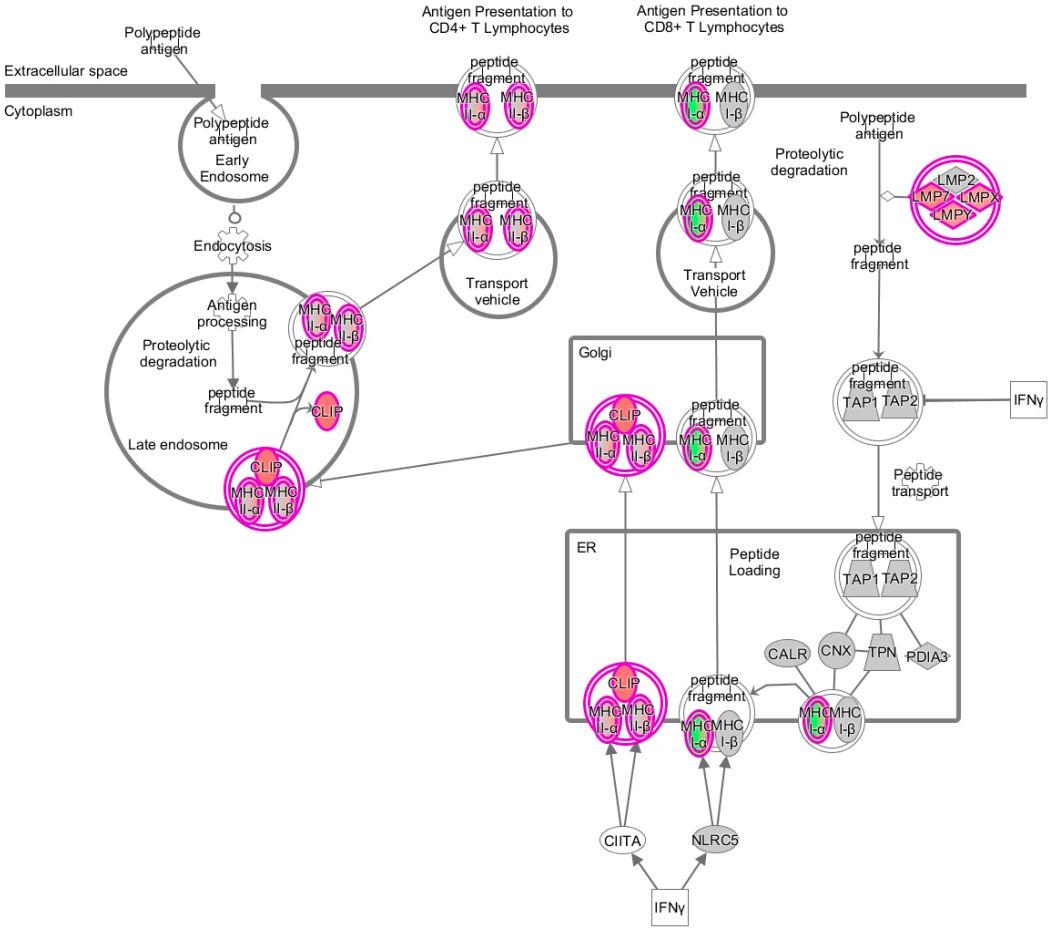
Antigen Presentation to CD4+ T Lymphocytes
Antigen Presentation to CD8+ T Lymphocytes
Polypeptide antigen
Extracellular space
Cytoplasm
Polypeptide antigen
Early Endosome
Endocytosis
Antigen processing
Proteolytic degradation
peptide fragment
CLIP
Late endosome
MHC II-α
MHC II-β
peptide fragment
Transport vehicle
Transport Vehicle
Golgi
Proteolytic degradation
LMP2
LMP7
LMPX
LMPY
TAP1
TAP2
IFNγ
Peptide transport
ER
Peptide Loading
CALR
CNX
TPN
RDIA3
MHC I-α
MHC I-β
CIITA
NLRC5

Figure S3. Mitochondrial Dysfunction Pathway. Genes associated with mitochondrial dysfunction following infection with (A) VACV-Wyeth (-log(*p*-values): 6.6) and (B) VACV-WR 2 d.p.i. (-log(*p*-values): 13.7). Differentially expressed genes are highlighted in color. Color intensity indicates the degree of up regulation (red) or down-regulation (green) relative to the day 2 mock infected brains. Solid lines represent direct interactions and dashed lines represent indirect interactions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

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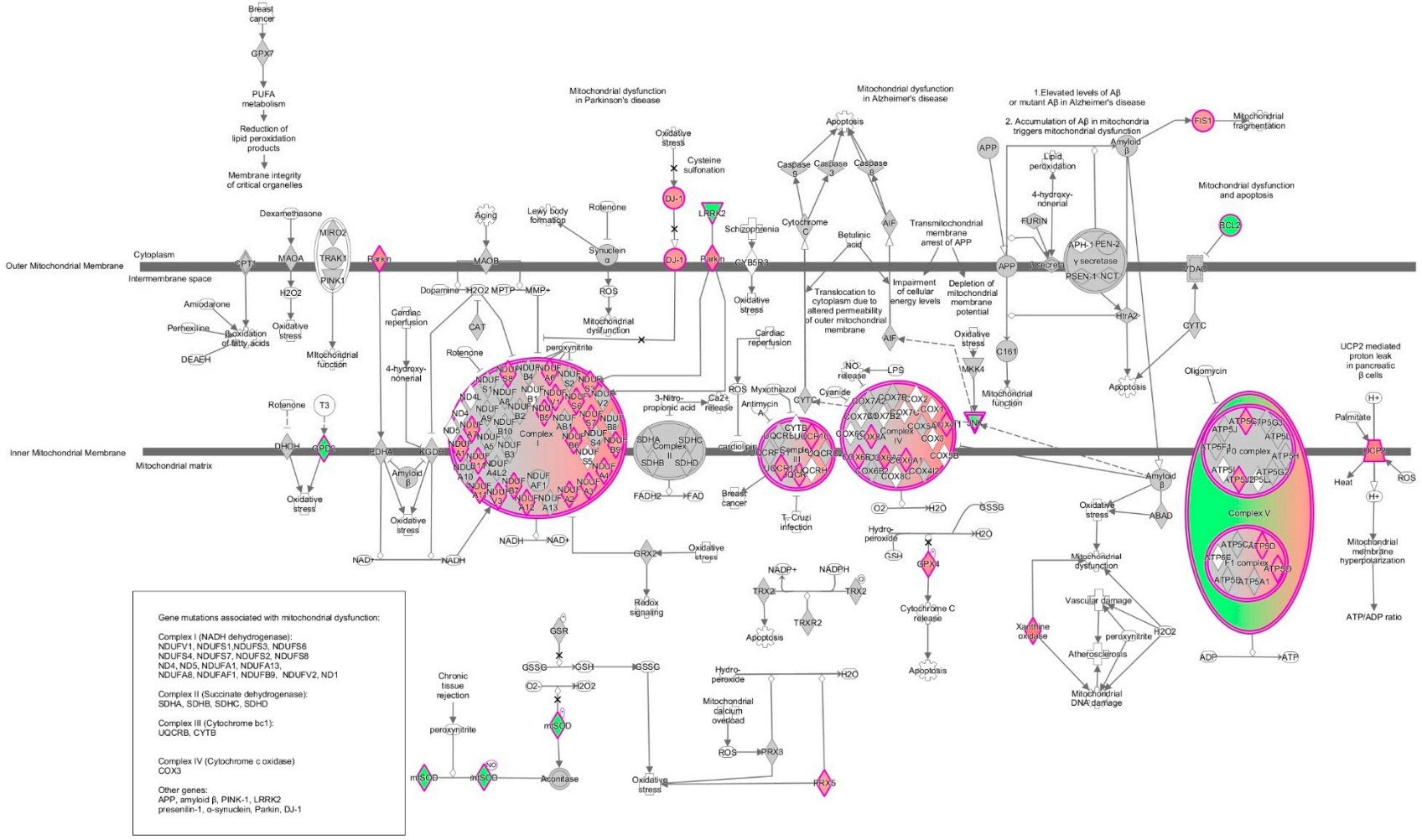

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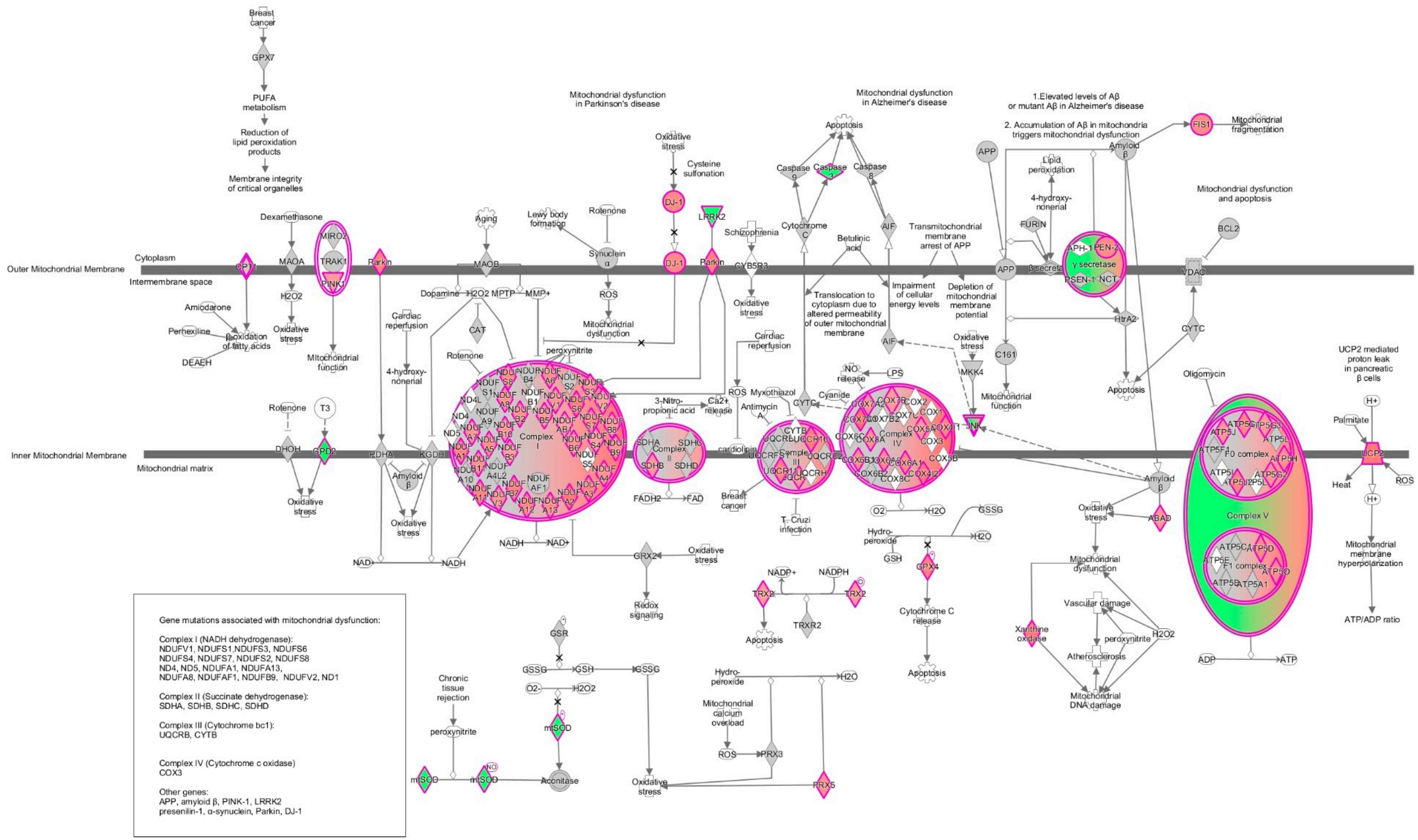
Breast cancer
GPX7
PUFA metabolism
Reduction of lipid peroxidation products
Membrane integrity of critical organelles
Mitochondrial dysfunction in Parkinson's disease
Mitochondrial dysfunction in Alzheimer's disease
1.Elevated levels of Aβ or mutant Aβ in Alzheimer's disease
2. Accumulation of Aβ in mitochondria triggers mitochondrial dysfunction
FIS1
Mitochondrial fragmentation
Mitochondrial dysfunction and apoptosis
Outer Mitochondrial Membrane
Cytoplasm
Intermembrane space
Inner Mitochondrial Membrane
Mitochondrial matrix
Dexamethasone
MAOA
MIRO2
TRAK1
PINK1
Parkin
Aging
Lewy body formation
Rotenone
Synuclein α
Oxidative stress
Cysteine sulfonation
DJ-1
LRRK2
Schizophrenia
CYB5R3
Caspase 9
Caspase 3
Caspase 8
Apoptosis
Cytochrome C
Betulinic acid
AIF
Transmitochondrial membrane arrest of APP
APP
Lipid peroxidation
4-hydroxy-nonenal
FURIN
Amyloid β
APH-1
PEN-2
γ secretase
PSEN-1
NCT
BCL2
VDAC
Amiodarone
Perhexiline
β oxidation of fatty acids
DEAEH
H2O2
Mitochondrial function
Dopamine
MPTP
MMP+
CAT
ROS
Mitochondrial dysfunction
peroxynitrite
Cardiac reperfusion
Translocation to cytoplasm due to altered permeability of outer mitochondrial membrane
Impairment of cellular energy levels
Depletion of mitochondrial membrane potential
HtrA2
CYTC
C161
MKK4
Oligomycin
UCP2 mediated proton leak in pancreatic β cells
H+
Palmitate
UCP2
Heat
ROS
Rotenone
T3
DHOH
GPD2
PDHA
KGDH
Complex I
3-Nitro-propionic acid
Ca2+ release
Myxothiazol
Antimycin A
cardiolipin
Complex II
SDHA
SDHB
SDHC
SDHD
Complex III
CYTB
UQCRB
UQCRH
UQCRFS
Cyanide
NO release
LPS
Complex IV
COX1
COX2
COX3
COX5B
F0 complex
F1 complex
Complex V
Oxidative stress
ABAD
Mitochondrial dysfunction
Vascular damage
Atherosclerosis
Mitochondrial DNA damage
Xanthine oxidase
Mitochondrial membrane hyperpolarization
ATP/ADP ratio
ADP
ATP
FADH2
FAD
Breast cancer
T. Cruzi infection
O2
H2O
GSSG
Hydro-peroxide
GSH
GPX4
Cytochrome C release
NAD+
NADH
GRX2
Redox signaling
NADP+
NADPH
TRX2
TRXR2
GSR
GSH
O2-
mtSOD
Aconitase
Chronic tissue rejection
Mitochondrial calcium overload
PRX3
PRX5
Gene mutations associated with mitochondrial dysfunction:
Complex I (NADH dehydrogenase):
NDUFV1, NDUFS1,NDUFS3, NDUFS6
NDUFS4, NDUFS7, NDUFS2, NDUFS8
ND4, ND5, NDUFA1, NDUFA13,
NDUFA8, NDUFAF1, NDUFB9, NDUFV2, ND1
Complex II (Succinate dehydrogenase):
SDHA, SDHB, SDHC, SDHD
Complex III (Cytochrome bc1):
UQCRB, CYTB
Complex IV (Cytochrome c oxidase)
COX3
Other genes:
APP, amyloid β, PINK-1, LRRK2
presenilin-1, α-synuclein, Parkin, DJ-1

Figure S4. Oxidative Phosphorylation Pathway. Genes associated with oxidative phosphorylation following infection with (A) VACV-Wyeth (-log(*p*-values): 7.0)and (B) VACV-WR 2 d.p.i. (-log(*p*-values): 14.2). Differentially expressed genes are highlighted in color. Color intensity indicates the degree of up regulation (red) or down-regulation (green) relative to the day 2 mock infected brains. Solid lines represent direct interactions and dashed lines represent indirect interactions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

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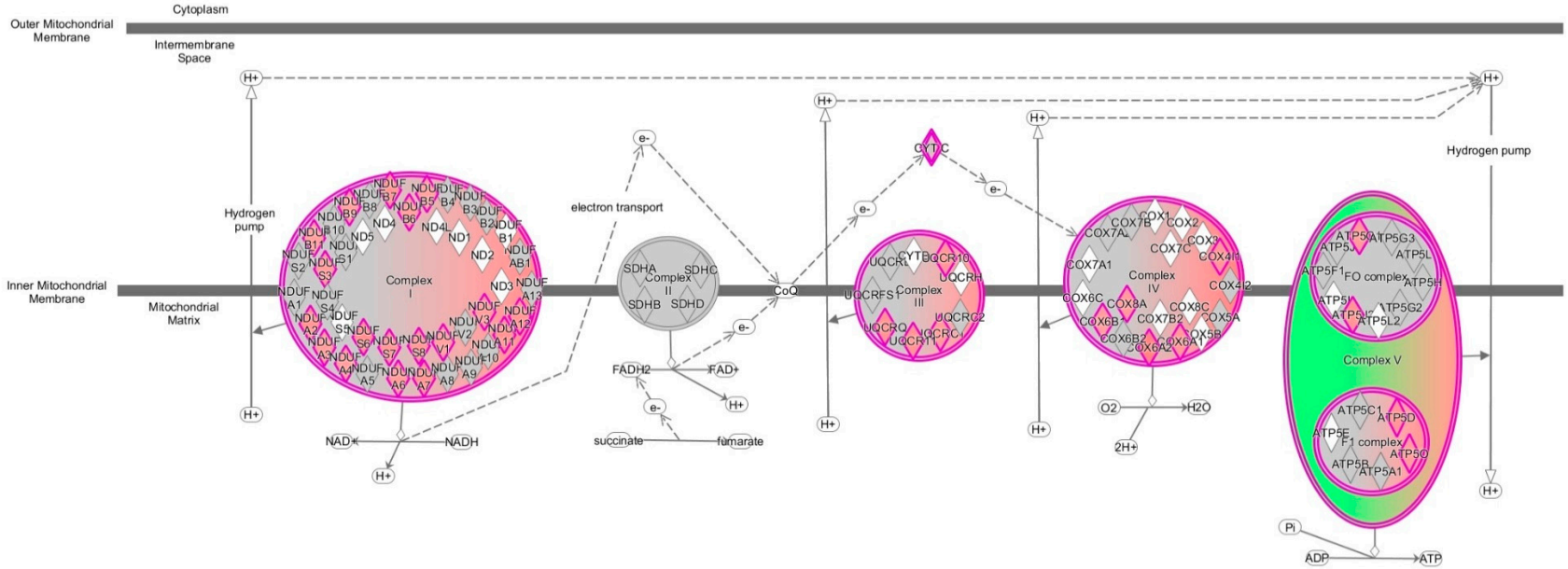

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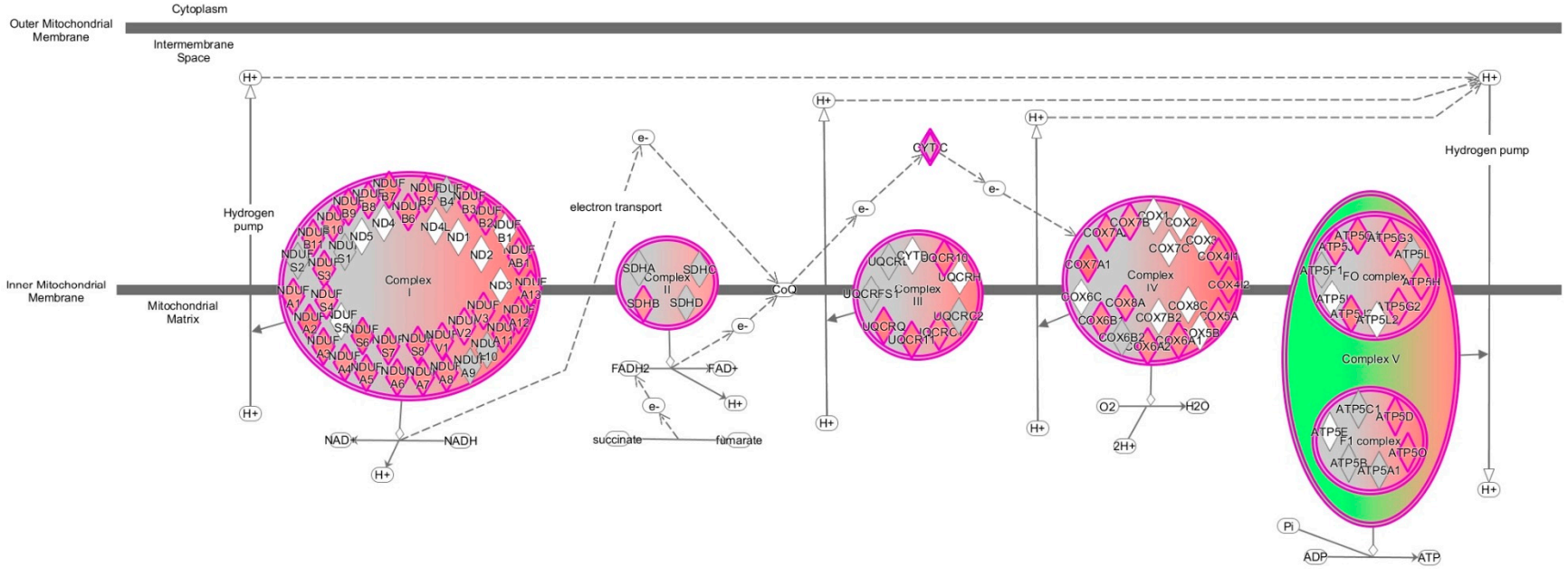
Cytoplasm
Outer Mitochondrial Membrane
Intermembrane Space
Inner Mitochondrial Membrane
Mitochondrial Matrix
Hydrogen pump
Hydrogen pump
electron transport
Complex I
Complex II
SDHA
SDHB
SDHC
SDHD
CoQ
Complex III
CYTC
UQCRC1
UQCRC2
UQCRH
UQCRB
UQCRQ
UQCRFS1
UQCRC1
Complex IV
COX1
COX2
COX3
COX4I1
COX4I2
COX5A
COX5B
COX6A1
COX6A2
COX6B1
COX6B2
COX6C
COX7A1
COX7A2
COX7B
COX7C
COX8A
COX8C
Complex V
FO complex
F1 complex
ATP5A1
ATP5B
ATP5C1
ATP5D
ATP5E
ATP5F1
ATP5H
ATP5I
ATP5J
ATP5J2
ATP5L
ATP5L2
ATP5O
NADH
NAD
FADH2
FAD+
succinate
fumarate
O2
H2O
2H+
Pi
ADP
ATP
H+
e-