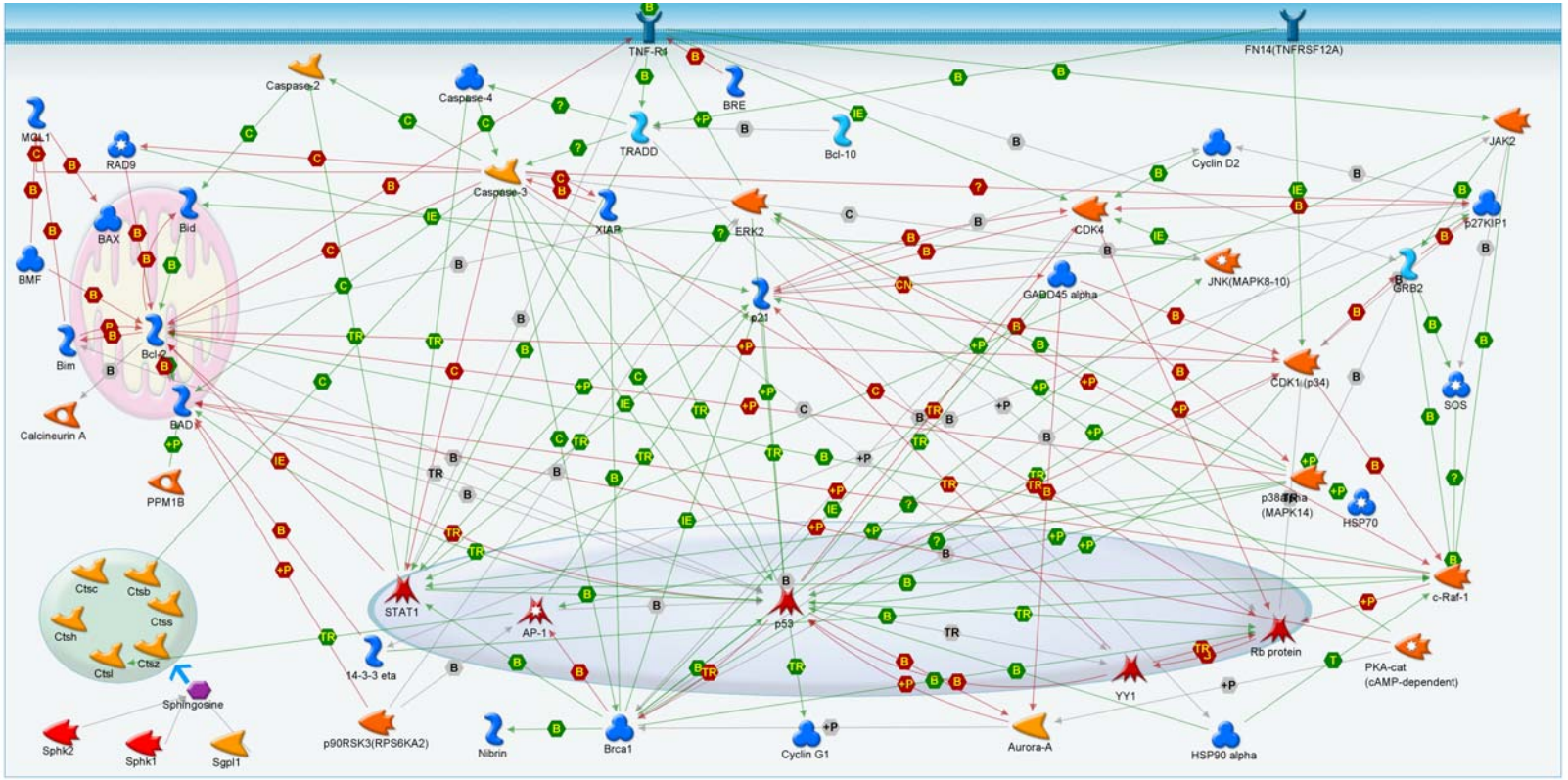
Appendix 4A: Cell-death signaling maps based on retinal gene regulation after intraorbital nerve crush and intraorbital nerve transection.



Cell-death signalling map summarizing the regulation and gene interactions of retinal-regulated genes after intraorbital nerve crush (IONC) and intraorbital nerve transection (IONT) injuries. Data from our array analyses were loaded in MetacoreTM and MapEditorTM softwares (Genego Inc.). Significantly regulated MetacoreTM cell death signalling maps were merged and a custom map was generated using curated gene interactions. Gene interactions are represented by directional colour-coded arrows: grey unknown, green: activation, red: repression or inactivation of the target. Interactions are as follow: B: binding; +P: phosphorylation; TR: transcription; C: cleavage; IE: influence on expression; ?: not specified. TNFR1a, TRADD, c-fos (AP-1 complex) and Caspase 3 protein expressionhas been analyzed by western blotting, and Stat-1, TNFR1a, and TNFR12a mRNA regulation has been validated by qRT–PCR.

Appendix 4 B: **Relation of IONT and IONC regulated genes shown in the signalling maps and their temporal regulation** . Data shown here are statistically significant net changes in gene expression (pvalue FDR limma and maSigpro <0,01 and Bvalue>0). Below 1 down-regulation, above 1 up-regulation. Empty cells refer either to control level of expression which is one or to not statistically significant changes. **IONT:** intraorbital nerve transection. **IONC:** intraorbital nerve crush.

Gene Name	Symbol in map	GENE SYMBOL	IONT 12h	IONT 24h	IONT 48h	IONT 3d	IONT 7d	IONT 15d	IONC 12h	IONC 24h	IONC 48h	IONC 3d	IONC 7d
BACULOVIRAL IAP REPEAT-CONTAINING 4	*XIAP*	*Birc4*		0.44	0.91	0.44	2.22		0.16	0.59	0.24		
B-CELL CLL/LYMPHOMA 10	*Bcl-10*	*Bcl10*		1.48	1.13	1.44	0.83						
BCL2 MODIFYING FACTOR	*BMF*	*Bmf*			0.43								
BCL2-ASSOCIATED X PROTEIN	*Bax*	*Bax*				1.62	1.25	1.19					
BRAIN AND REPRODUCTIVE ORGAN-EXPRESSED PROTEIN	*BRE*	*Bre*						1.54					
CASPASE 11	*Caspase 4*	*Casp11*	1.19	1.44	1.65	3.90	1.34		1.40	1.15	1.49	1.37	1.27
CASPASE 2	*Caspase2*	*Casp2*		0.20	0.97	0.21				0.50			
CASPASE 3, APOPTOSIS RELATED CYSTEINE PROTEASE	*Caspase3*	*Casp3*	6.47	1.62	7.83	1.23				3.02	1.25	1.32	
CATHEPSIN C	*Ctsc*	*Ctsc*	3.89	1.52	3.10	1.94	2.61	1.31	3.09				
CATHEPSIN H	*Ctsh*	*Ctsh*				1.62	1.71	1.49					
CATHEPSIN L	*Ctsl*	*Ctsl*			1.56	1.36	1.50						
CATHEPSIN S	*Ctss*	*Ctss*	1.19	1.22	1.39	2.64	2.25						
CATHEPSIN Z	*Ctsz*	*Ctsz*	2.86	1.91	6.06	4.43			4.40				
CYCLIN D2	*Cyclin D2*	*Ccnd2*	5.68	1.37	3.24	1.52							
CYCLIN G1	*Cyclin G1*	*Ccng1*		1.72	1.59	1.49				1.46			
CYCLIN-DEPENDENT KINASE INHIBITOR 1A	*p21*	*Cdkn1a*	1.40	1.41	1.62	3.05							
CYCLIN-DEPENDENT KINASE INHIBITOR 1B	*p27KIP*	*Cdkn1b*	1.37	1.14	1.16	1.10							
FBJ MURINE OSTEOSARCOMA VIRAL ONCOGENE HOMOLOG	*AP1*	*Fos*	5.79	2.22	5.73	2.61	5.88	0.81		3.89	3.08		
FOS-LIKE ANTIGEN 1	*AP1*	*Fosl1Fra-1*	9.78	1.16	11.09				12.67				
GROWTH ARREST AND DNA-DAMAGE-INDUCIBLE 45 ALPHA	*GADD45alpha*	*Gadd45a*	2.38	1.23	3.77	2.40	3.43	1.15				2.14	2.18
HEAT SHOCK 70KD PROTEIN 5	*HSP70*	*Hspa5*	1.28	1.56	1.39	1.20	1.30	0.79	1.85				
HEAT SHOCK PROTEIN 1, ALPHA	*HSP90alpha*	*Hspca*	1.14	1.22	1.14								
JUN ONCOGENE	*AP1*	*Jun*	2.41	1.16	1.13	1.28			0.38	0.39	0.35	0.34	0.36
JUN-B ONCOGENE	*AP1*	*Junb*	16.30	1.15	16.55	2.05			7.89	5.49			
LPS-INDUCED TN FACTOR	*Litaf*		8.31	5.20	9.14	3.77	2.44	3.06	6.12	4.90	5.07	2.75	4.45
MAD HOMOLOG 4	*Smad 4*	*Smad4*		0.92	0.70	0.80			0.57				
MITOGEN ACTIVATED PROTEIN KINASE 1	*ERK*	*Mapk1*	0.60	0.82	0.54	0.60	0.67	0.75	0.38	0.60	0.61	0.81	0.57
MITOGEN ACTIVATED PROTEIN KINASE 14	*p38alpha*	*Mapk14.p38*	1.69										
MITOGEN-ACTIVATED PROTEIN KINASE 9	*JNK*	*Mapk9*	1.14	0.52	0.88	0.54			0.58				
MYELOID CELL LEUKEMIA SEQUENCE 1	*Mcl1*	*Mcl1*	1.84	1.52	1.82	1.78	1.09	1.60					
NIBRIN	*nibrin*	*Nbn*	1.27	1.27									
PROTEIN PHOSPHATASE 1B, MAGNESIUM DEPENDENT, BETA ISOFORM	*PPM1B*	*Ppm1b*	0.64	0.82	0.59	0.63	0.75	0.62	0.43	0.59			
PROTEIN PHOSPHATASE 3, CATALYTIC SUBUNIT, ALPHA ISOFORM	*Calcineurin*	*Ppp3ca*	1.14	9.29	1.26	3.82							
RAD9 HOMOLOG B	*RAD9*	*Rad9b*	7.46	2.63	5.73	3.23	2.00	2.76	6.25	2.35	5.12	1.64	3.32
RETINOBLASTOMA 1	*RB protein*	*Rb1*		1.99	1.79	1.83					0.83	0.84	0.84
RIBOSOMAL PROTEIN S6 KINASE POLYPEPTIDE 2	*p90RSK3*	*Rps6ka2*	6.71	1.71	7.41	1.96							
SERINE/THREONINE KINASE 6	*Aurora*	*Stk6*					1.50						
SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 1	*Stat 1*	*Stat1*	6.52	1.69	7.33	1.65			6.56	4.48	6.51		
SPHINGOSINE KINASE 1	*Sphk1*	*Sphk1*			2.50	3.01	1.77		4.93	2.80	3.60		
SPHINGOSINE KINASE 2	*Sphk2*	*Sphk2*							1.36				
SPHINGOSINE PHOSPHATE LYASE 1	*Sgpl1*	*Sgpl1*		2.18	1.37	2.24	1.22						
SON OF SEVENLESS HOMOLOG 1	*SOS*	*Sos1*		1.17	0.66				0.52				
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 12A	*TNFRSF12A*	*Tnfrsf12a*	5.79	3.94	11.76	8.91	3.29	1.62	4.77	2.36	10.61	4.68	4.15
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 1A	*TNFR1*	*Tnfrsf1a*	7.15	2.46	6.22	3.70	1.80	1.90	8.88	5.24	5.90	4.81	3.38
TYROSINE 3-MONOOXYGENASE/TRYPTOPHAN 5-MONOOXYGENASE A(	*14.3.3*	*Ywhah*		0.85	0.88	0.56							
V-RAF-1 MURINE LEUKEMIA VIRAL ONCOGENE HOMOLOG 1	*c-Raf-1*	*Raf1*		2.60	0.83	0.86	0.79						
YY1 TRANSCRIPTION FACTOR	*YY1*	*Yy1*	0.78	0.83	0.80	0.97	0.69		0.74	0.76	0.69	0.68	0.74