Supplemental Figures

Identification of potential mediators of retinotopic mapping: a comparative proteomic analysis of optic nerve from WT and Phr1 retinal knock-out mice

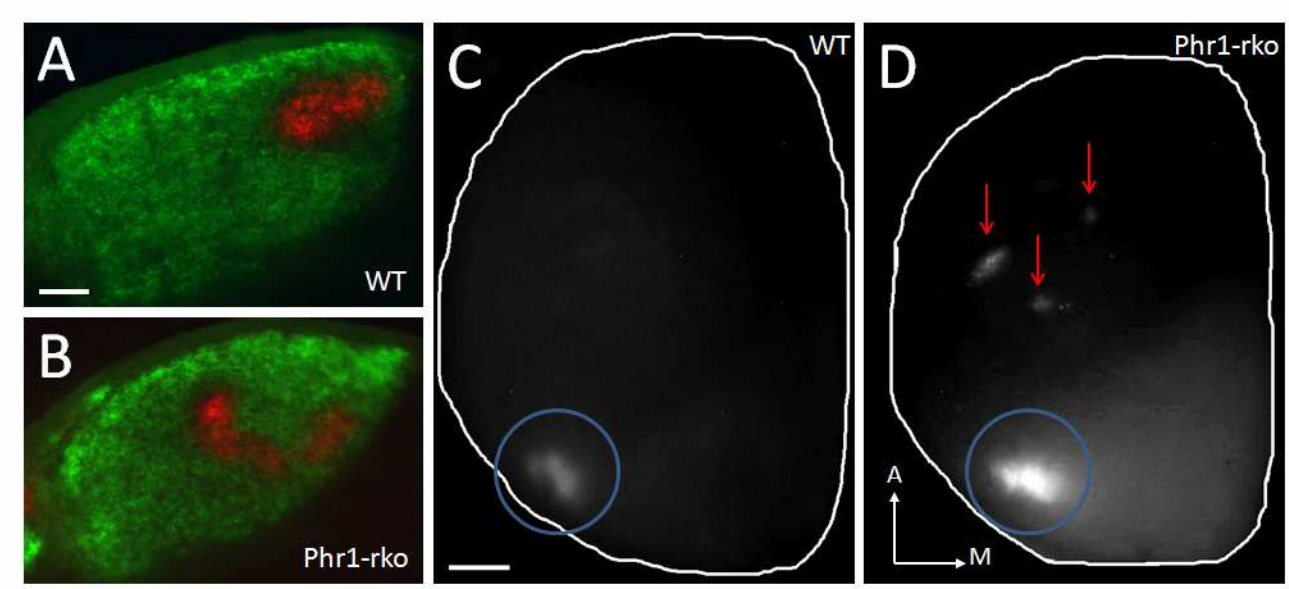


Figure S1. Presynaptic deletion of the E-3 ubiquitin ligase *Phr1* results in mistargeting of retinofugal projections. (A, B) RGC axons from the ipsilateral (red) and contralateral eye (green) are anterogradely labeled with fluorescently tagged cholera toxin B (Alexa-488 and Alexa-594, respectively). In WT animals the ipsilateral projection occupies a small region in the medial aspect of the dLGN (A). The ipsilateral projection in the Phr1-rko is interrupted and shifted within the nucleus (B), reflecting disrupted retinogeniculate topographic mapping.[1] (C, D) Retinocollicular projections are similarly affected, mapping inappropriately in the Phr1-rko. Termination zones of neighboring RGCs labeled in the nasal-dorsal quadrant of the retina with a localized injection of the lipophilic tracer DiI are usually focal and compact in the postero-lateral superior colliculus (C; blue circle). Following Cre-mediated conditional deletion of *Phr1* from RGCs multiple ectopic terminal zones are apparent with locations anterior to their normal position (D; red arrows).[2] Scale bar =10 µm in A, B; 400 µm in C, D; a, anterior; m, medial.

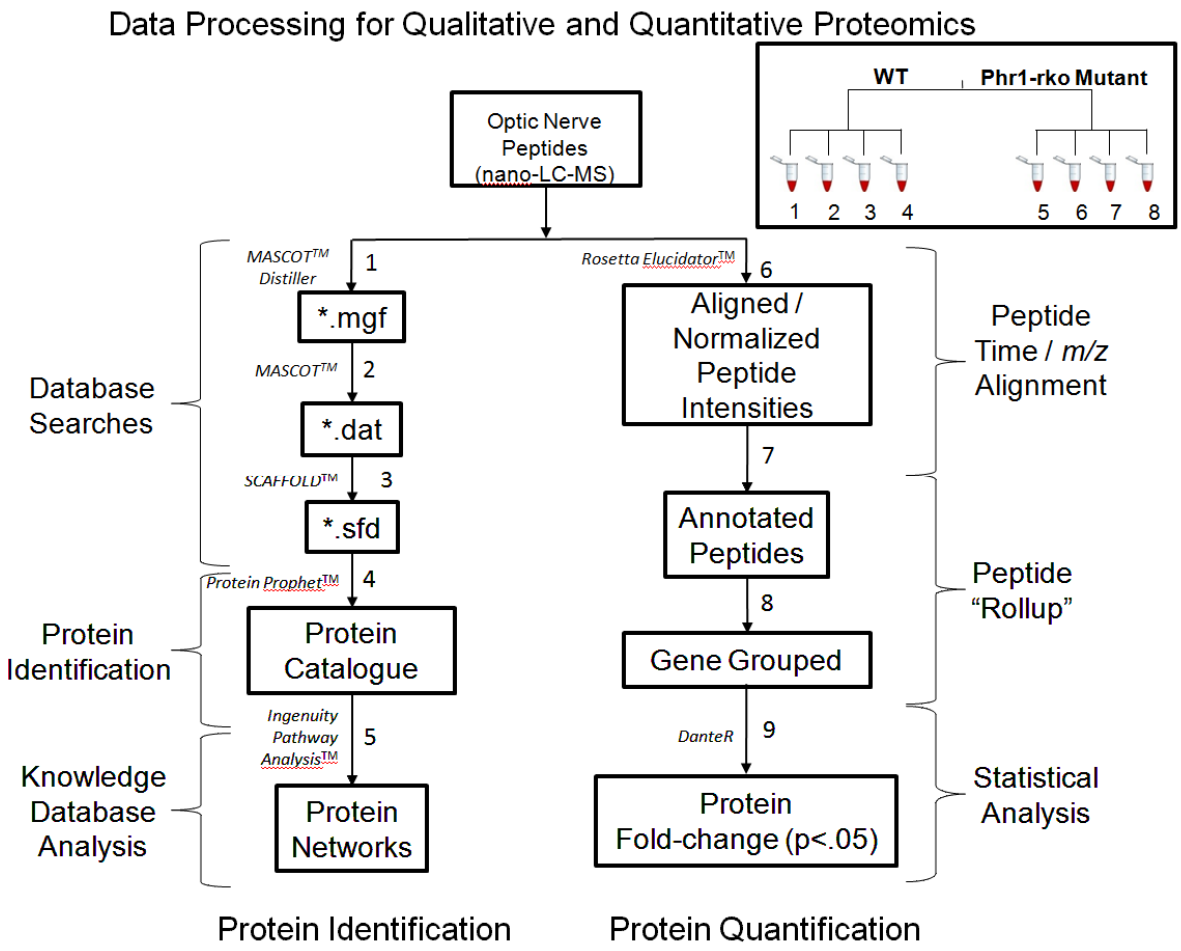


Figure S2. Data processing for quantitative, label-free proteomics analysis of mouse optic nerves. In step 1, the unprocessed LC-MS/MS files that were acquired using X-calibur (Thermofisher, ver. 2.0.7) were processed using Mascot Distiller software (ver 2.3.0.2) for database searching using the MS2 spectra. After creating the *.mgf files, the MS2 data were searched using MASCOT (ver. 2.2.04)[1] against a UniProt Mouse protein database downloaded 20110502 (72,503 entries) with an added bovine serum albumin sequence (Uniprot acession No., P02769) (Step 4). The MS1 and MS2 mass tolerances were set at 50 ppm and 0.8 Da, respectively. Carbamidomethyl was set as a fixed modification for Cys residues and Met residue oxidation was allowed as a variable modification. The database search results were imported into Scaffold software (ver. 3.00.03) (Step 6) and the proteins were qualified using the Prophet algorithm[2] with protein and peptide thresholds of 95% and 50%, respectively (Step 4). The identified proteins and supporting mass spectrometric data are given in Table S1. For knowledge-based analysis, the combined protein annotations were uploaded into the Ingenuity Pathways (IPA) (Ingenuity Systems, Redwood City, CA) (Step 5). Biological networks were generated from the imported proteins as gene object in the Ingenuity Pathways Knowledge Base (Step 5). (Figure S2 legend continued)

The same set of unprocessed LC-MS files were imported into Rosetta Elucidator™ (Rosetta Biosoftware, ver 3.3) and the peptide ion chromatograms were aligned and mean normalized using the following modification of the previously described parameters:[3] "Peak time score minimum = 0.5; peak m/z score minimum = 0.5; Scan width of m/z = 350-1400; LC time range of 30 -140 min; intensity scaling based on the mean intensity of all features (Step 6). The aligned peptide ion currents were annotated within the software by generating *.dta files (Step 4) and searching the UNIPROT human database using MASCOT as described above (Step 5). For relative protein quantification, the peptide ion current signals from all charge states for each peptide were concatenated unique using a visual script within the Rosetta™ software. In order to group the peptides from the protein database searches as individual genes, the mouse gene symbols were extracted from the UniProt protein descriptors for each peptide. This was performed using the following formula in Excel:
=LEFT(MID(AJ2,FIND("GN=",AJ2)+LEN("GN="),999),FIND("PE=",MID(AJ2,FIND("GN=",AJ2)+LEN("GN="),999))-1) where AJ2 is the cell where the protein name exists. The spreadsheet was then sorted according to Gene Name. The gene grouped peptides and normalized peptide intensities were exported in Excel *.xls format (Step 10) (Table S2). The gene-grouped peptide intensity data were imported into DAnTE-R for statistical analysis[4,5] (Step 9).

References for Figure S2 legend.

1. Perkins, D. N.; Pappin, D. J. C.; Creasy, D. M.; Cottrell, J. S., Probability-based protein identification by searching sequence databases using mass spectrometry data. ELECTROPHORESIS 1999, 20, (18), 3551-3567.
2. Keller, A.; Nesvizhskii, A.; Kolker, E.; Aebersold, R., Empirical statistical model to estimate the accuracy of peptide identifications made by MS/MS and database search. Analytical Chemistry 2002, 74, (20), 5383-92.
3. Neubert, H.; Bonnert, T. P.; Rumpel, K.; Hunt, B. T.; Henle, E. S.; James, I. T., Label-Free Detection of Differential Protein Expression by LC/MALDI Mass Spectrometry. Journal of Proteome Research 2008, 7, (6), 2270-2279.
4. Karpievitch, Y.; Stanley, J.; Taverner, T.; Huang, J.; Adkins, J. N.; Ansong, C.; Heffron, F.; Metz, T. O.; Qian, W.-J.; Yoon, H.; Smith, R. D.; Dabney, A. R., A statistical framework for protein quantitation in bottom-up MS-based proteomics. Bioinformatics 2009, 25, (16), 2028-2034.
5. Polpitiya, A. D.; Qian, W.-J.; Jaitly, N.; Petyuk, V. A.; Adkins, J. N.; Camp, D. G.; Anderson, G. A.; Smith, R. D., DAnTE: a statistical tool for quantitative analysis of -omics data. Bioinformatics 2008, 24, (13), 1556-1558.

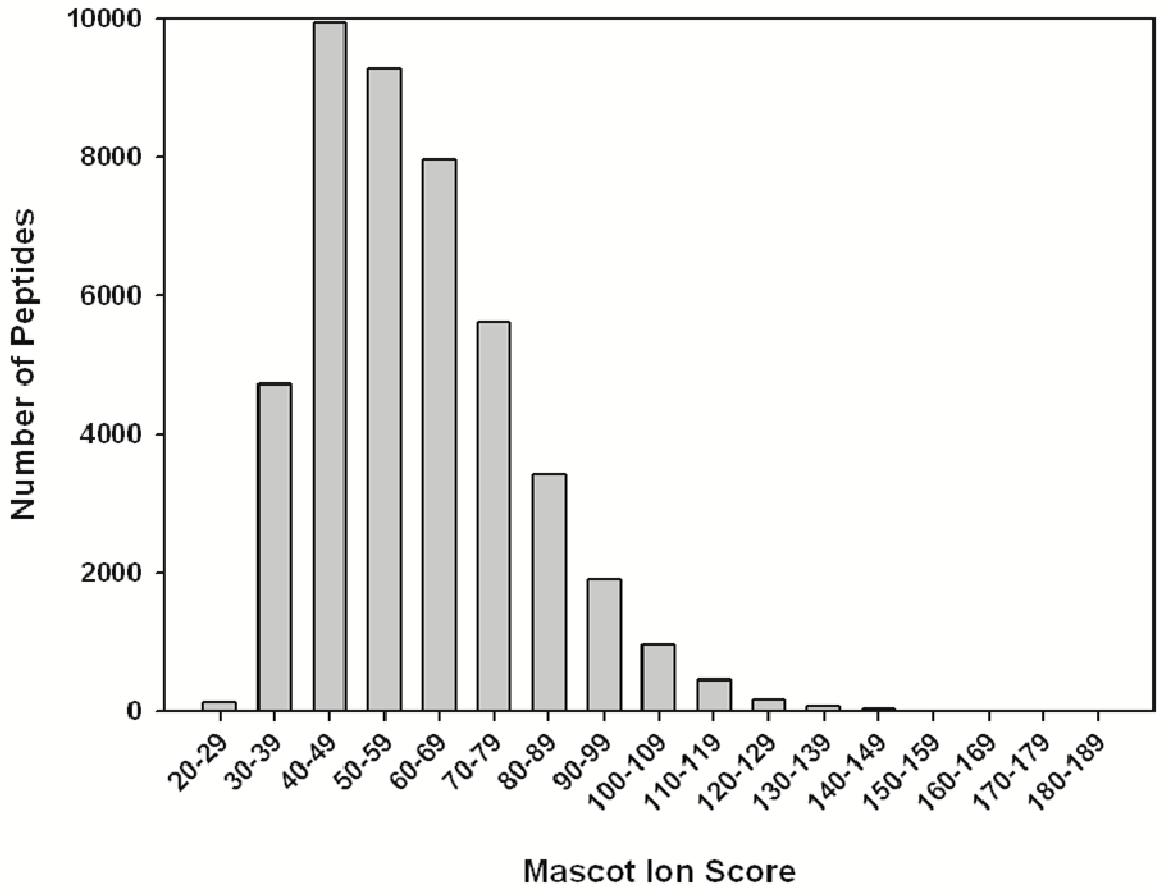


Figure S3. Distribution of Mascot Ion Scores for all 44,676 peptides identified. >90% of peptides had scores >40. Most peptides with scores <40 were associated with proteins identified with additional higher scoring peptides.

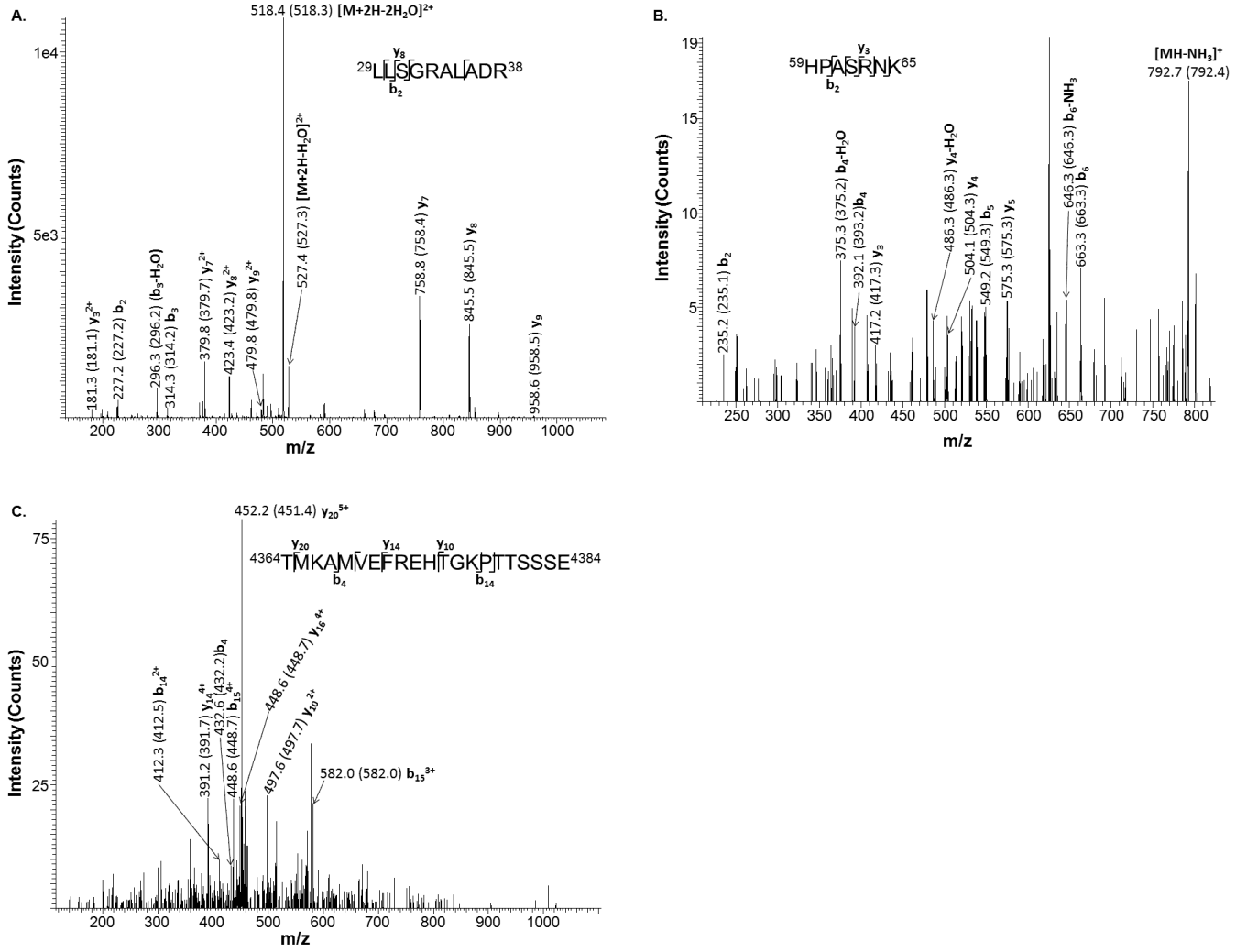
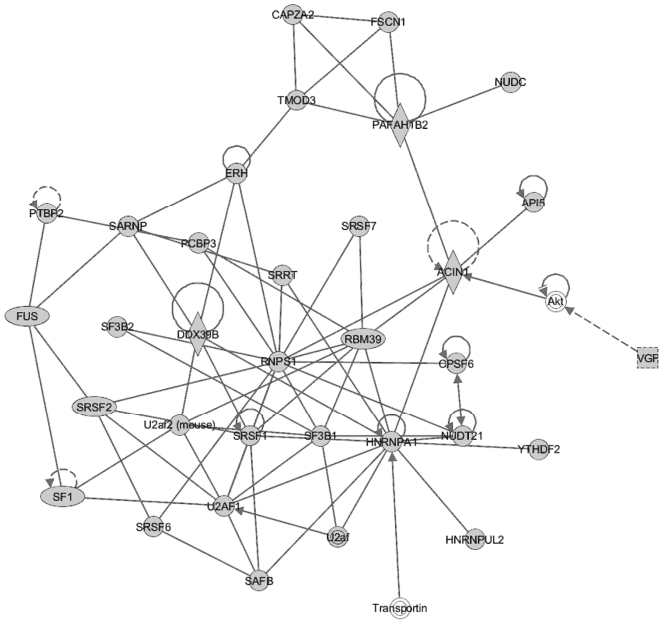


Figure S4. Tandem mass spectra from differential gel band (MW ~ 130kDa, Figure 1) of peptides consistent with the indicated sequences from mouse Phr1. (A) LLSGRALADR, encompassing residues 29-38 (theoretical $[M+2H]^{2+}$ = 536.317; observed, 536.293), (B) HPASRNK, encompassing residues 59-65 (theoretical $[M+H]^{+}$ = 809.438; observed, 809.440), and (C) TMKAMVEFREHTGKPTTSSSE (theoretical $[M+5H]^{5+}$ = 471.628; observed, 471.631), encompassing residues 4364-4384 of the *Phr1* sequence.

Figure S5. Ingenuity Interaction Networks. For complete listings of ID, molecules in network, score, focus molecules, and top functions, see Table S5: Ingenuity Interaction Networks.

Network #1



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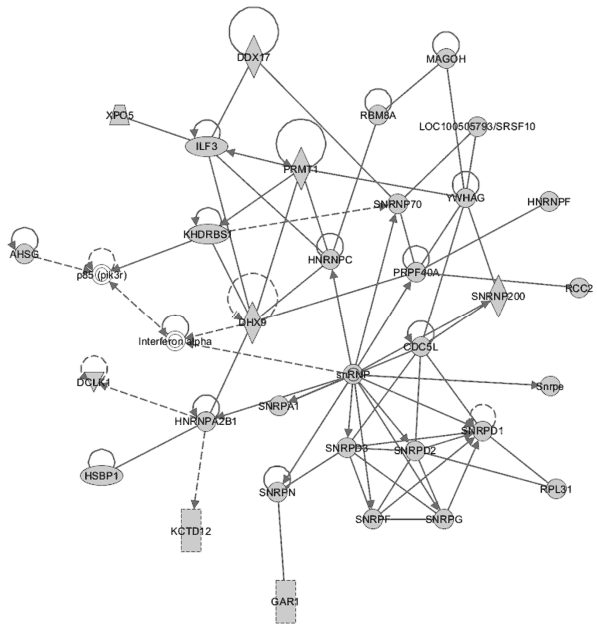

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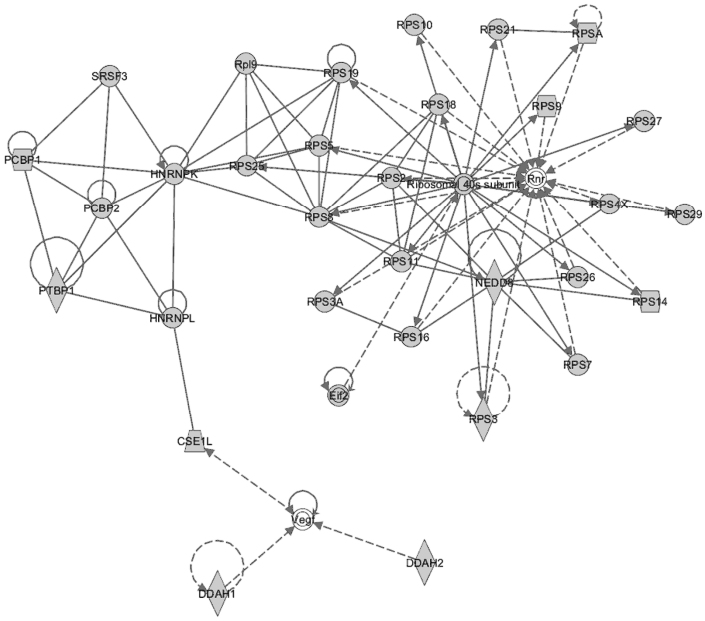

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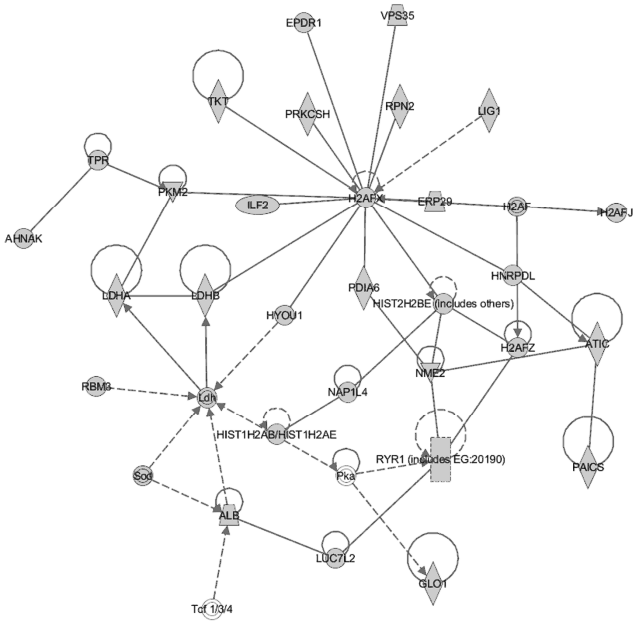

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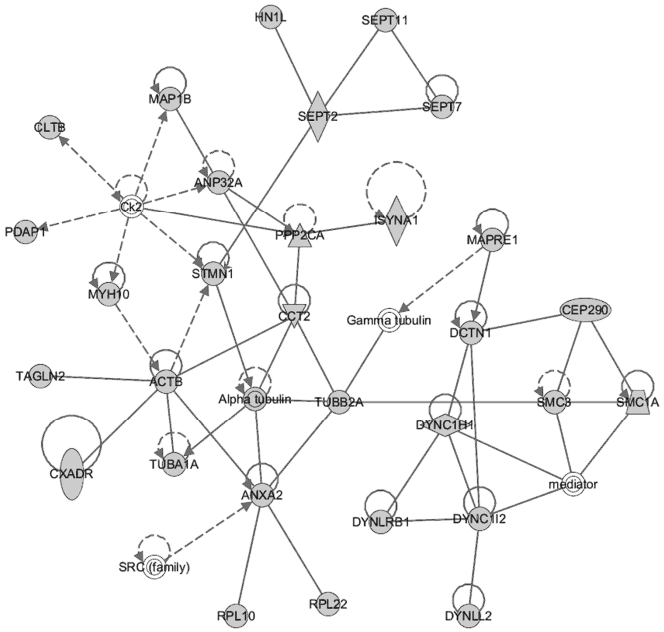

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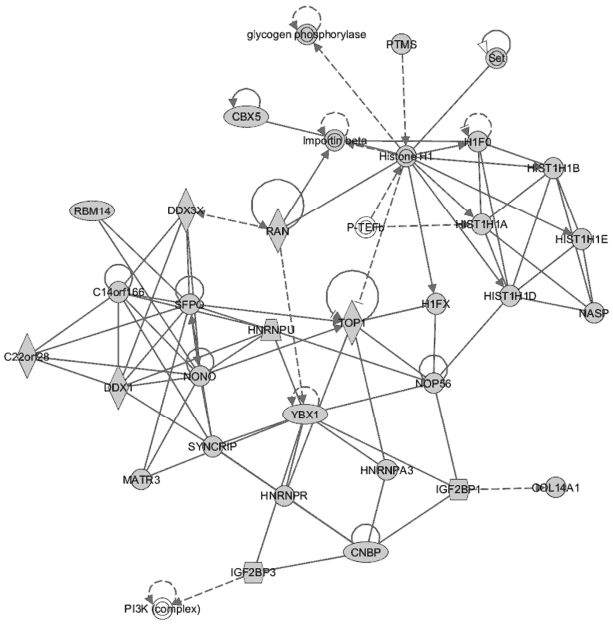

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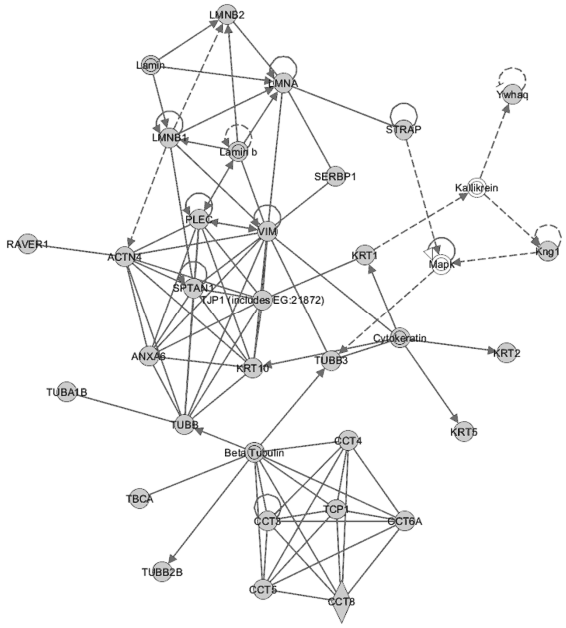

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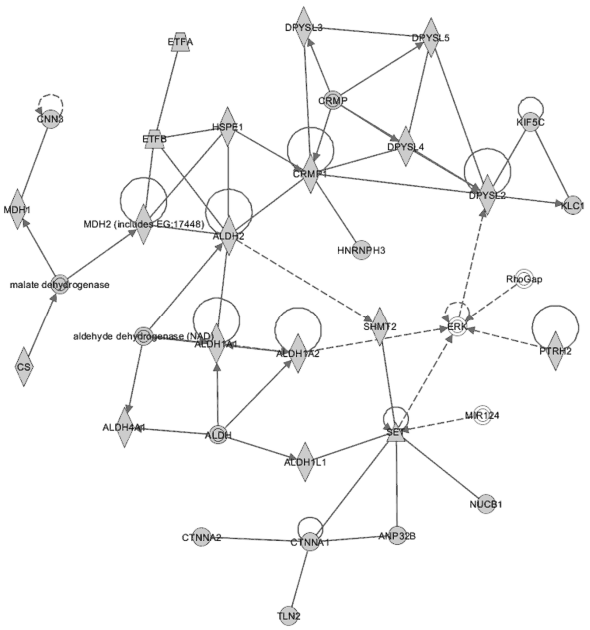

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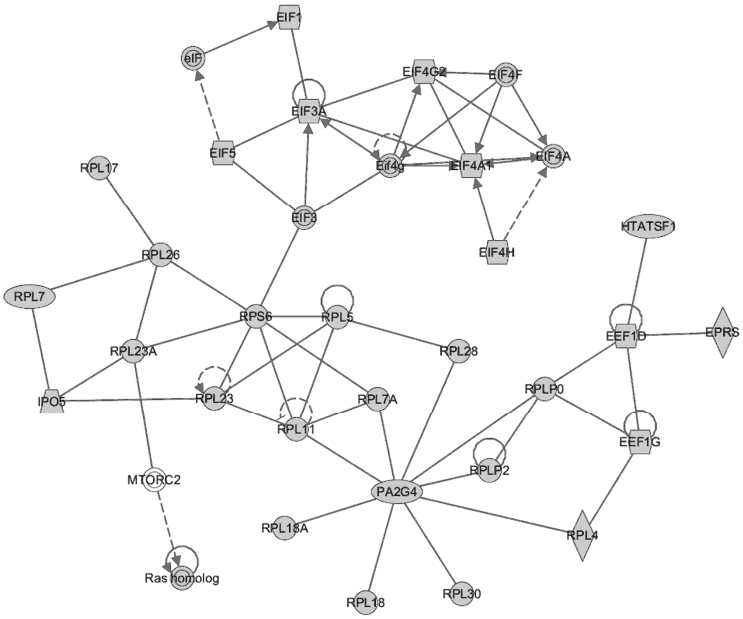

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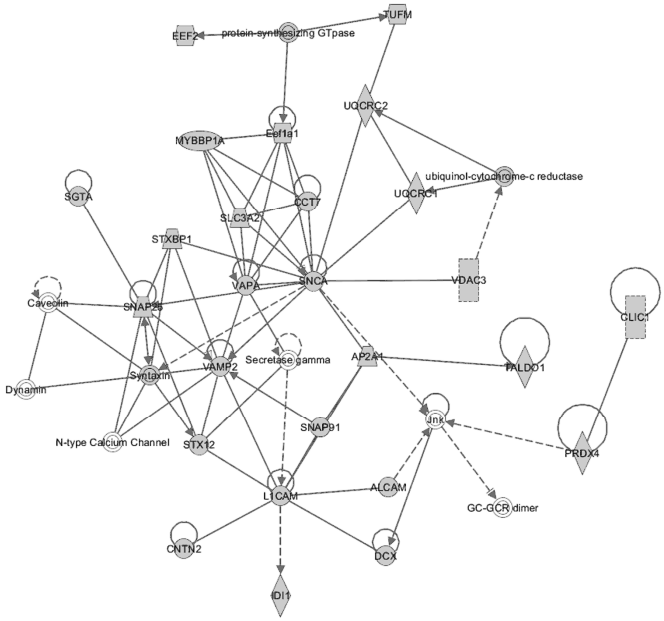

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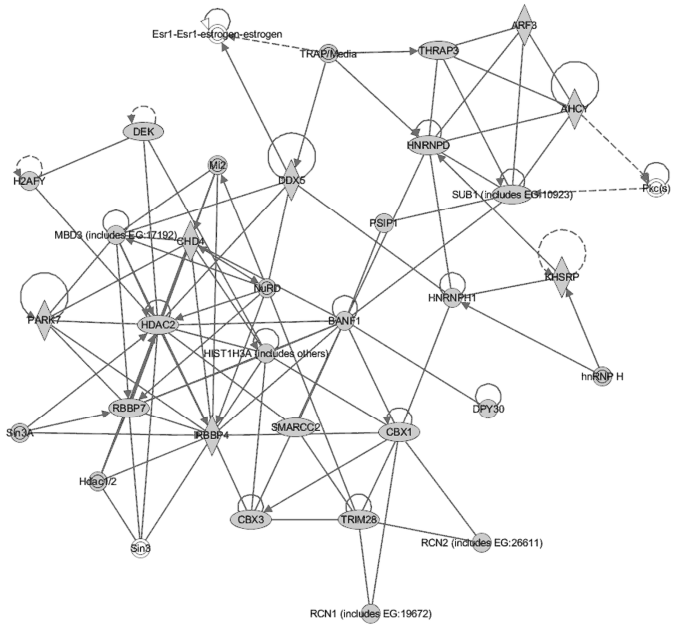

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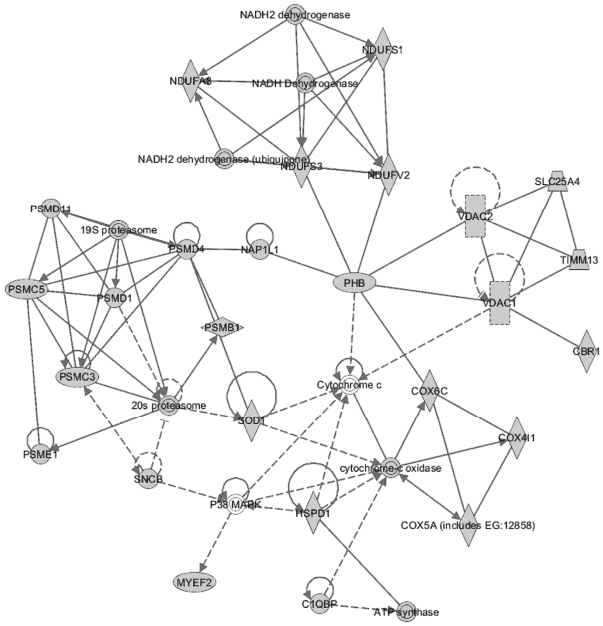

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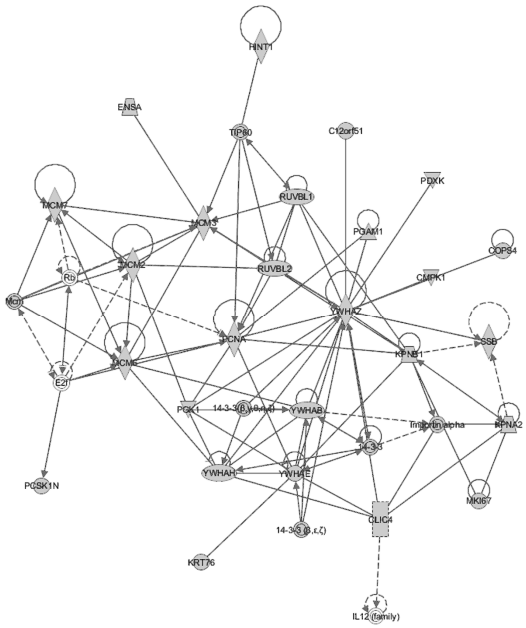

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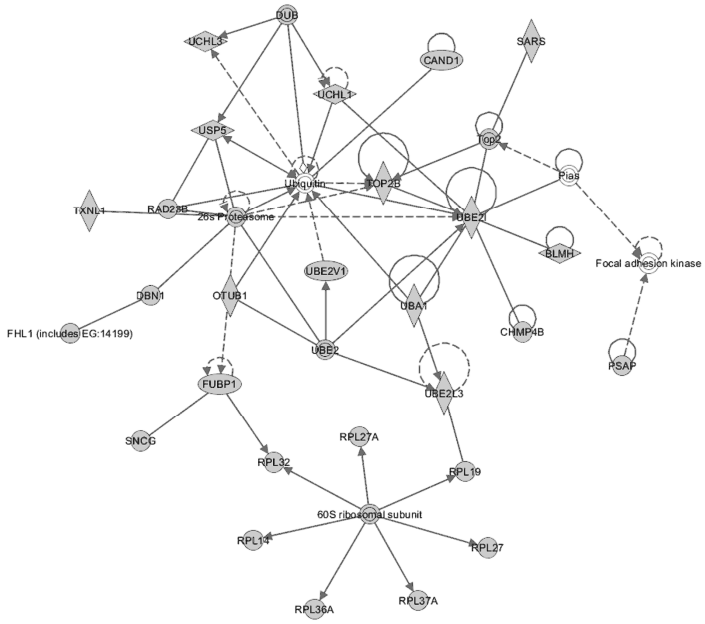

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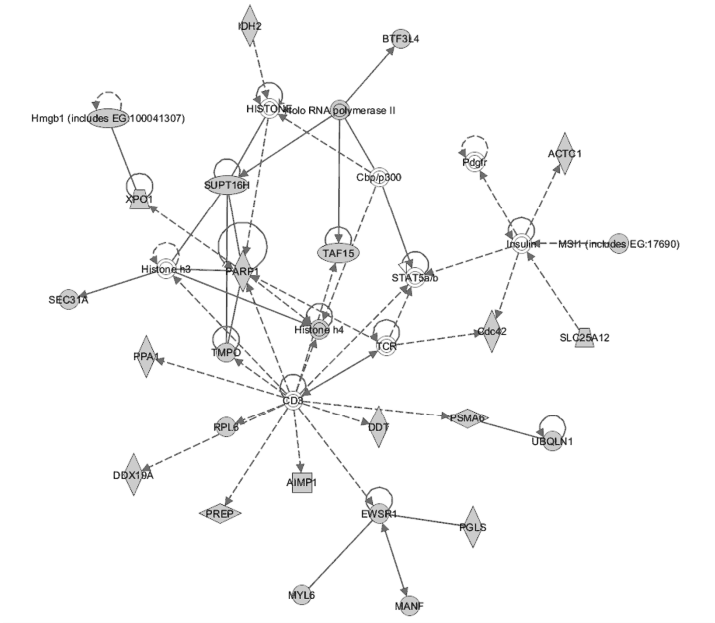

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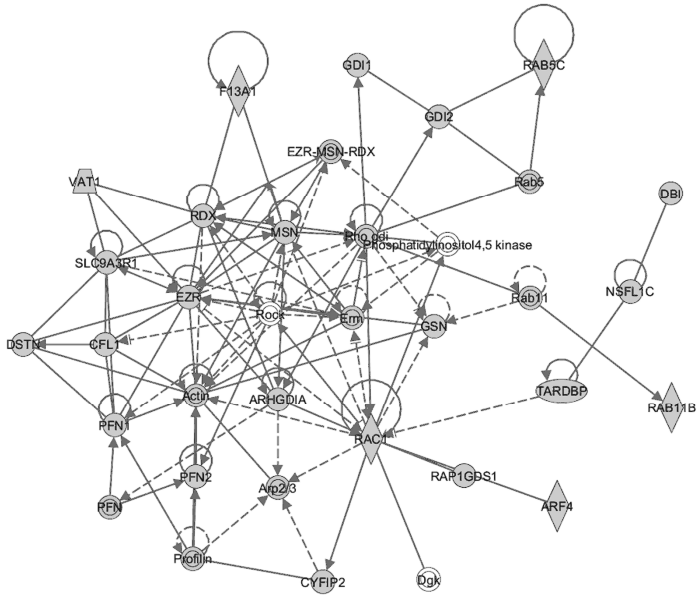

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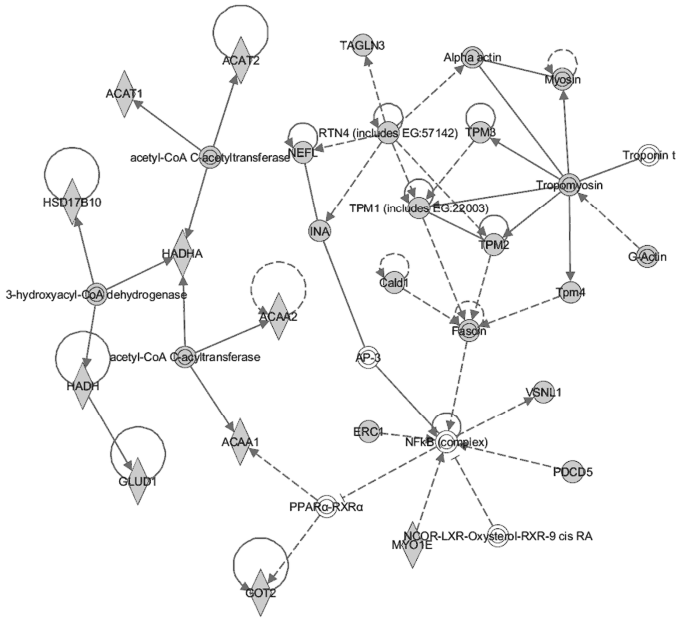

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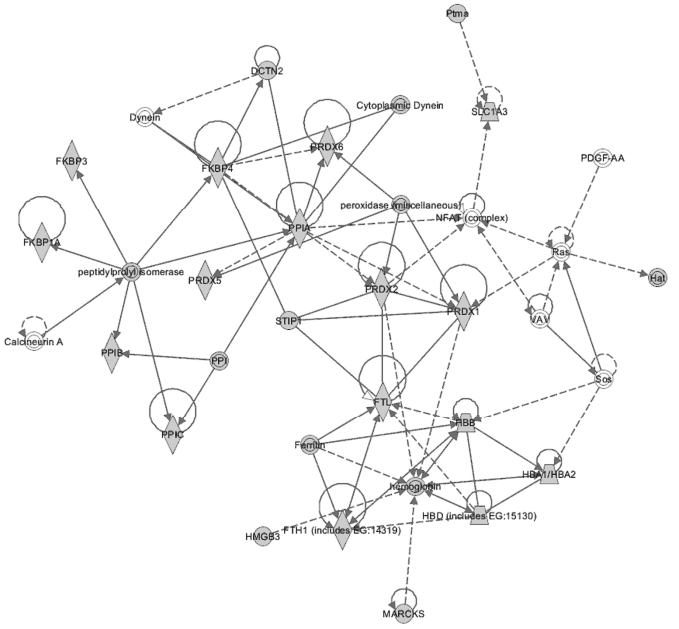

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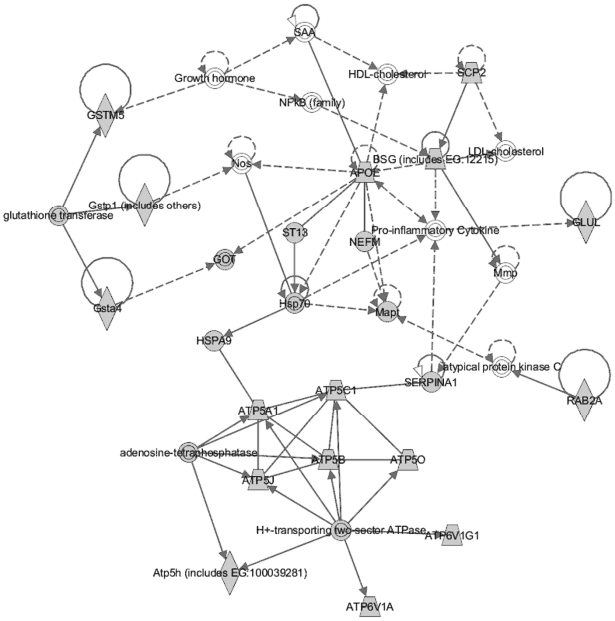

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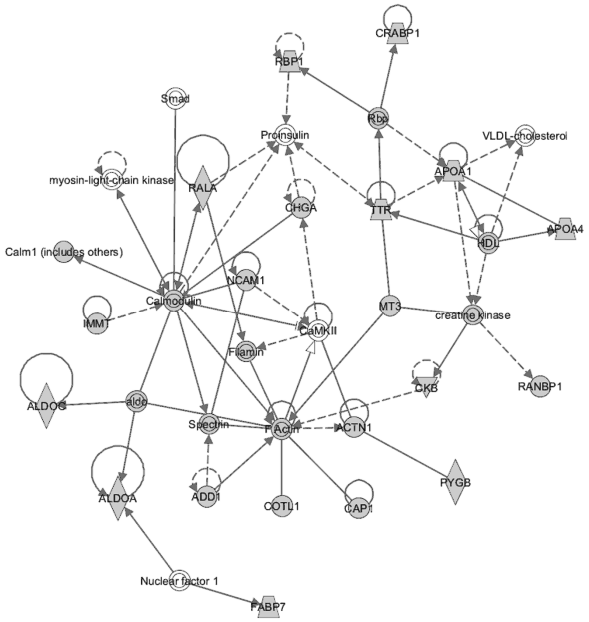

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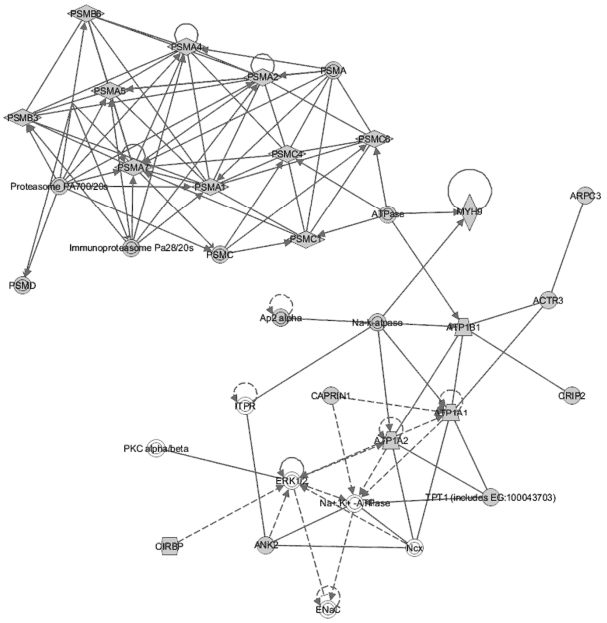

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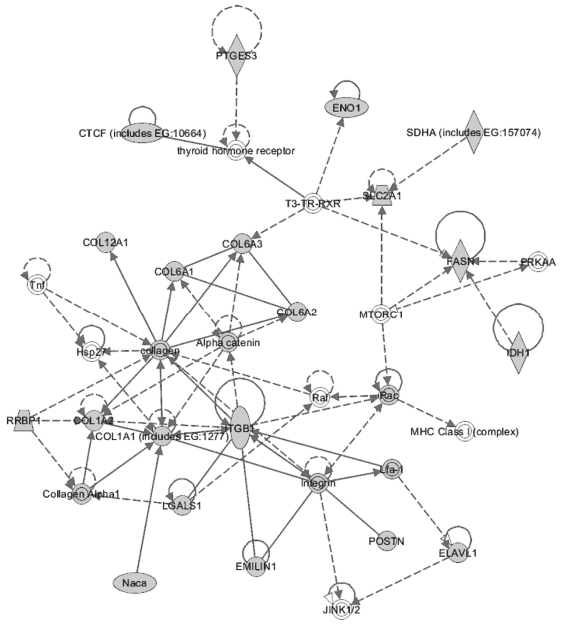

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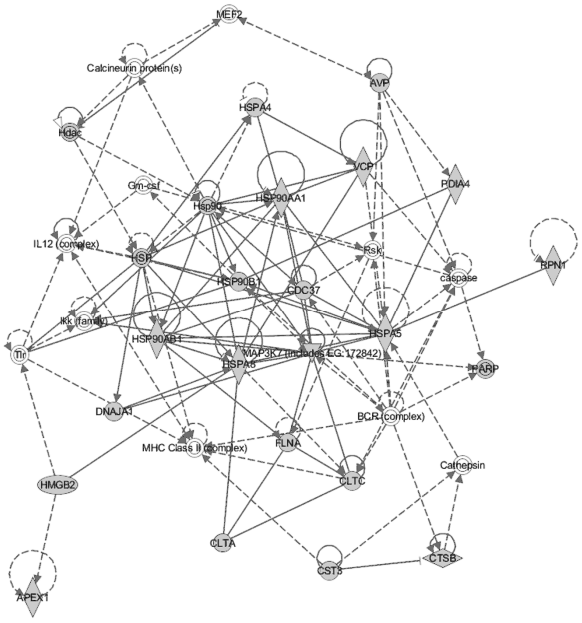

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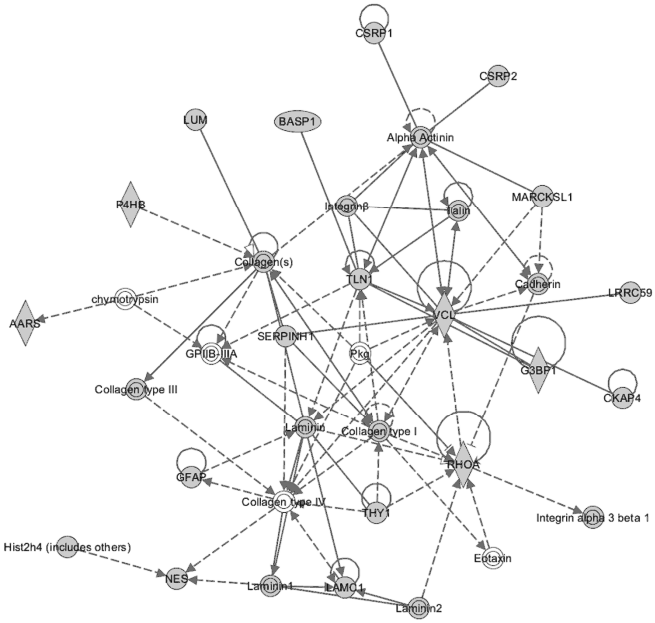

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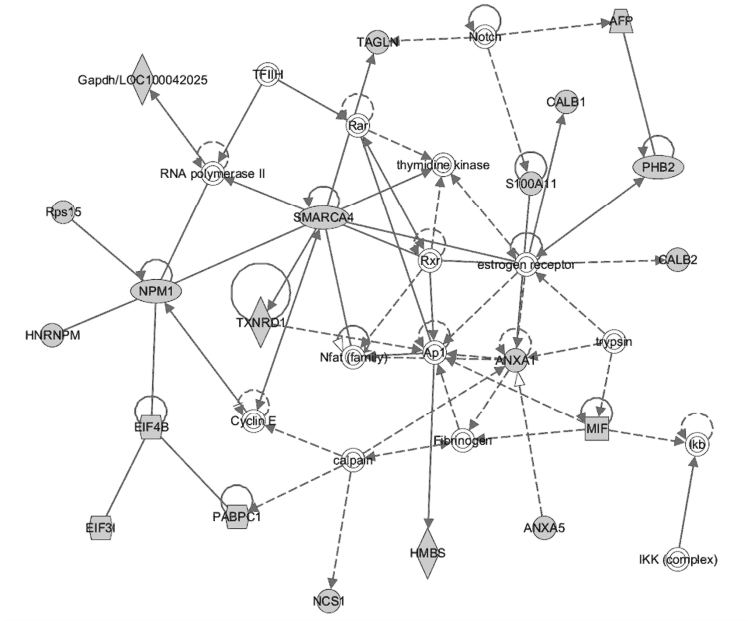
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estrogen receptor
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TXNRD1
HNRNPM
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Ap1
ANXA1
trypsin
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Cyclin E
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