

Supporting Information

Paraoxon-Induced Protein Expression Changes to SH-SY5Y Cells

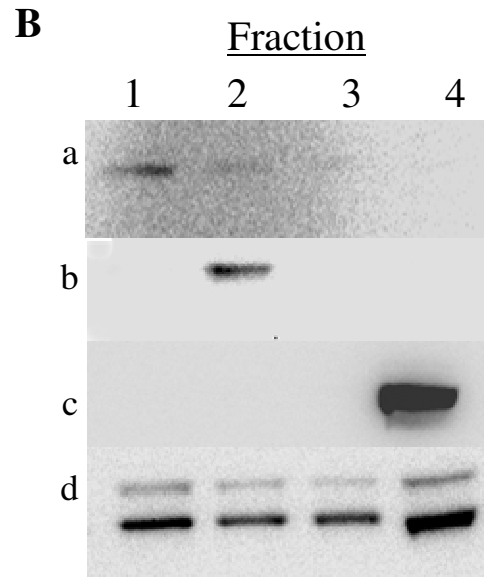
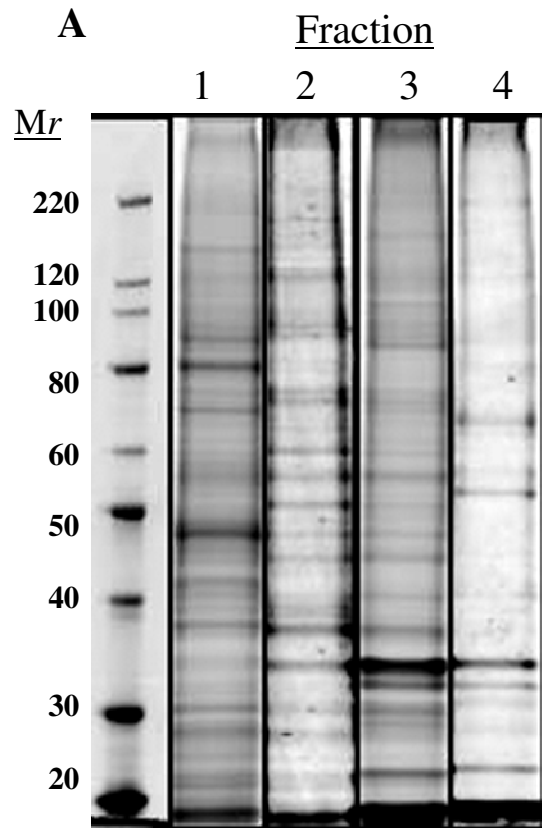
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Supplemental Figure S-1: Efficiency of the Calbiochem Subproteome extraction kit on SH-SY5Y cells. A) Coomassie stained 1-D SDS PAGE gel demonstrates changes in protein bands in different cellular fraction and % of protein in each fraction as determined by Bradford protein assay. B) Results from the western blot analysis demonstrates that the sub-proteome extraction can efficiently isolate proteins according to their localization within the cell. a) cytosolic protein phosphatase (PP2A/C) is primarily a cytosolic protein and was largely restricted to the cytosolic fraction. b) Synaptophysin is a membrane bound protein and was found in the membrane fraction. c) Neurofilament medium and was isolated in the cytosolic fraction. Map Kinase (ERK) is an enzyme that translocates from the nucleus to the membrane and was found in all fractions.

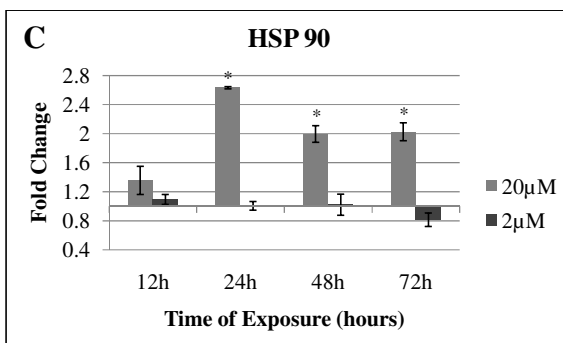
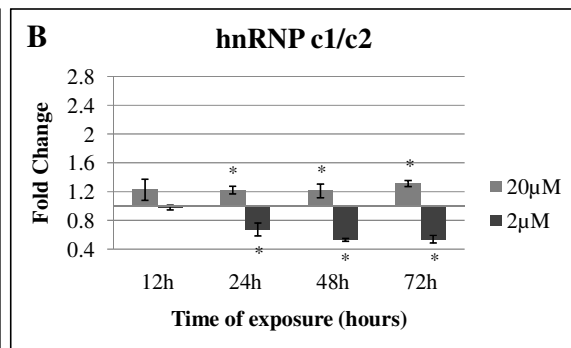
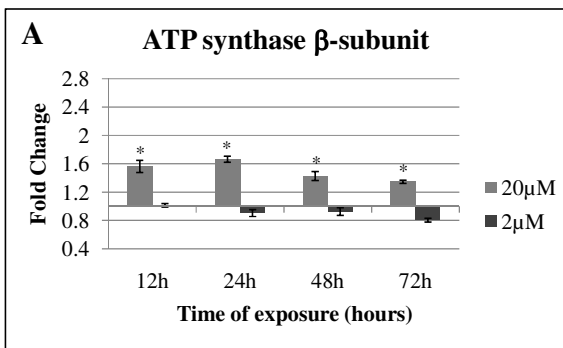
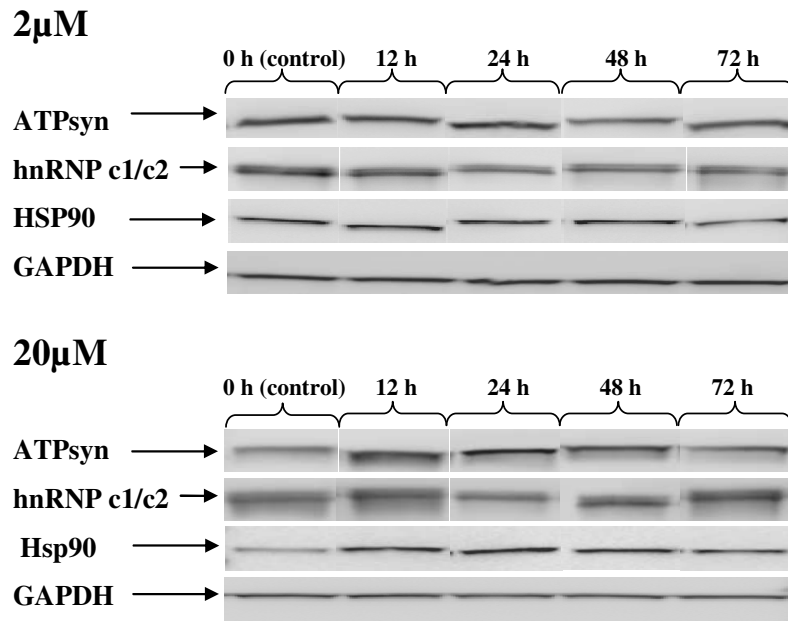


- a) Cytosolic protein phosphatase (2A/C)
- b) Synaptophysin
- c) Neurofilament
- d) Map Kinase

Protein Concentration %

- 1. 34%
- 2. 36%
- 3. 16%
- 4. 14%

Supplemental Figure S-2: Western blot analysis of SH-SY5Y cells exposed to A) 2 μ M paraoxon and B) 20 μ M paraoxon for 0-72 hours using antibodies against ATP synthase β -subunit, hnRNP c1/c2, HSP 90 and GAPDH followed by appropriate HRP-conjugated secondary antibodies. Shown above the graphs are examples of blots developed by enhanced chemiluminescence following incubation with antibodies against ATP synthase β -subunit, hnRNP c1/c2, and HSP 90, 30 μ g of total protein were loaded in each sample well. Band density measured for ATP synthase β -subunit, hnRNP c1/c2, and HSP 90 was normalized to GAPDH prior to analysis. Significance determined by one-way ANOVA followed by Dunnet's post-hoc test ($p < 0.05$; $n = 3$).



Supplemental Figure S-3:

20 μ M paraoxon does not cause cytotoxicity SH-SY5Y cells 48 hours post-exposure. After 48 hours, cells were trypsinized and counted with trypan blue to quantitate cytotoxicity. Shown is percent dead in triplicate cultures with at least 100 cells counted/sample. Unpaired, two-tailed t-test indicated that the values for control and paraoxon-exposed were not significantly different.

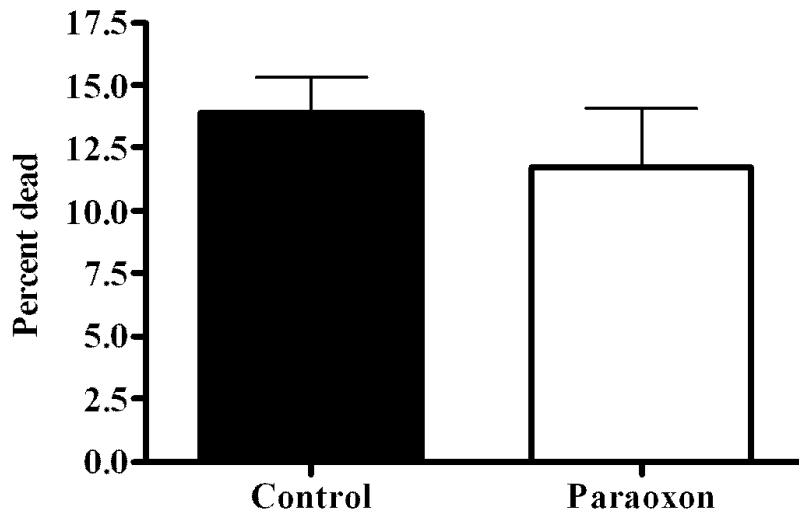


Table S-3. List of proteins found in sub-cellular fractions

Cytosolic Fraction

Match	Mascot Score	Protein Name	Sequence Coverage	Species	Subcellular Location	Theoretical Mr/pI	Calculated Mr/pI	Fold Change
TPM4_HUMAN	76	Tropomyosin alpha-4 chain (Tropomyosin-4) (TM30p1)	27%	Homo Sapien	Cytosolic	Mr: 28373 pI: 4.67	Mr: 32093 pI: 4.86	2.5
AAH21092	73	zinc finger, FYVE domain containing 19 (BC021092 NID)	24%	Homo Sapien	Cytosolic	Mr: 36365 pI: 5.29	Mr: 37170 pI: 4.88	2.6
GDIR_HUMAN	71	Rho GDP-dissociation inhibitor 1 (Rho GDI 1) (Rho-GDI alpha)	28%	Homo Sapien	Cytosolic	Mr: 23062 pI: 5.03	Mr: 29470 pI: 5.01	1.5
HHHU84	89	heat shock protein 90-beta	22%	Homo Sapien	Cytosolic	Mr: 83242 pI: 4.97	Mr: 84415 pI: 5.07	-1.7
AAH00987	104	heat shock protein 90kDa alpha (cytosolic)	30%	Homo Sapien	Cytosolic	Mr: 84350 pI: 5.10	Mr: 85430 pI: 4.95	-1.3
Mixture 1: Q96IH1_HUMAN; Q3ZCU9_HUMAN	130	FSCN1 protein; STIP1 protein	18%, 18%	Homo Sapien	Cytosolic	Mr: 55101 pI: 6.83; Mr: 68003 pI: 7.81	Mr: 55975 pI: 7.05	-1.5
ENOA_HUMAN	80	Alpha-enolase (2-phospho-D-glycerate hydro-lyase) (Non-neural enolase)	26%	Homo Sapien	Cytosolic	Mr: 47008 pI: 6.99	Mr: 57339 pI: 7.84	-2.3

Membrane Fraction

Match	Mascot Score	Protein Name	Sequence Coverage	Species	Subcellular Location	Theoretical Mr/pI	Calculated Mr/pI	Fold Change
AAH89438	89	heterogeneous nuclear ribonucleoprotein C (C1/C2)	38%	Homo Sapien	Membrane	Mr: 27804 pI: 4.55	Mr: 37775 pI: 4.97	2.8
AAH08364	113	heterogeneous nuclear ribonucleoprotein C (C1/C2),	38%	Homo Sapien	Membrane	Mr: 32374 pI: 5.00	Mr: 38828 pI: 4.98	2.8
AAH12854	116	actin, beta	38%	Homo Sapien	Membrane	Mr: 40194 pI: 5.55	Mr: 42084 pI: 5.41	1.7
A33370	211	H ⁺ -transporting two-sector ATPase beta chain precursor, mitochondrial	51%	Homo Sapien	Membrane	Mr: 56525 pI: 5.26	Mr: 48974 pI: 5.04	-3
A26885	93	heterogeneous nuclear ribonucleoprotein C	37%	Homo Sapien	Membrane	Mr: 31947 pI: 5.10	Mr: 41614 pI: 4.04	1.6
Q53FG3_HUMAN	80	Interleukin enhancer binding factor 2 variant (Fragment)	30%	Homo Sapien	Membrane	Mr: 43021 pI: 5.19	Mr: 44445 pI: 5.59	2.1
AAH12854	74	actin, beta	29%	Homo Sapien	Membrane	Mr: 40194 pI: 5.55	Mr: 42458 pI: 5.79	-3.2
S62767	122	translation elongation factor EF-Tu precursor	35%	Homo Sapien	Membrane	Mr: 49509 pI: 7.70	Mr: 47300 pI: 7.70	1.5

Nuclear Fraction

Match	Mascot Score	Protein Name	Sequence Coverage	Species	Subcellular Location	Theoretical Mr/pI	Calculated Mr/pI	Fold Change
I52690	76	prohibitin	30%	Homo Sapien	Nuclear	Mr: 29786 pI: 5.57	Mr: 31765 pI: 5.80	-1.6
AAA63192	70	histone H2B.1 (HUMHISH2BANID)	53%	Homo Sapien	Nuclear	Mr: 11324 pI: 10.14	Mr: 13983 pI: 9.85	-3.3
H2A1H_HUMAN	69	Histone H2A type 1-H	33%	Homo Sapien	Nuclear	Mr: 13767 pI: 10.88	Mr: 12120 pI: 9.46	-2.2

Cytoskeletal Fraction

Match	Mascot Score	Protein Name	Sequence Coverage	Species	Subcellular Location	Theoretical Mr/pI	Calculated Mr/pI	Fold Change
AAH06551	77	lamin B2	24%	Homo Sapien	Cytoskeletal	Mr: 67647 pI: 5.29	Mr: 67443 pI: 5.51	1.4