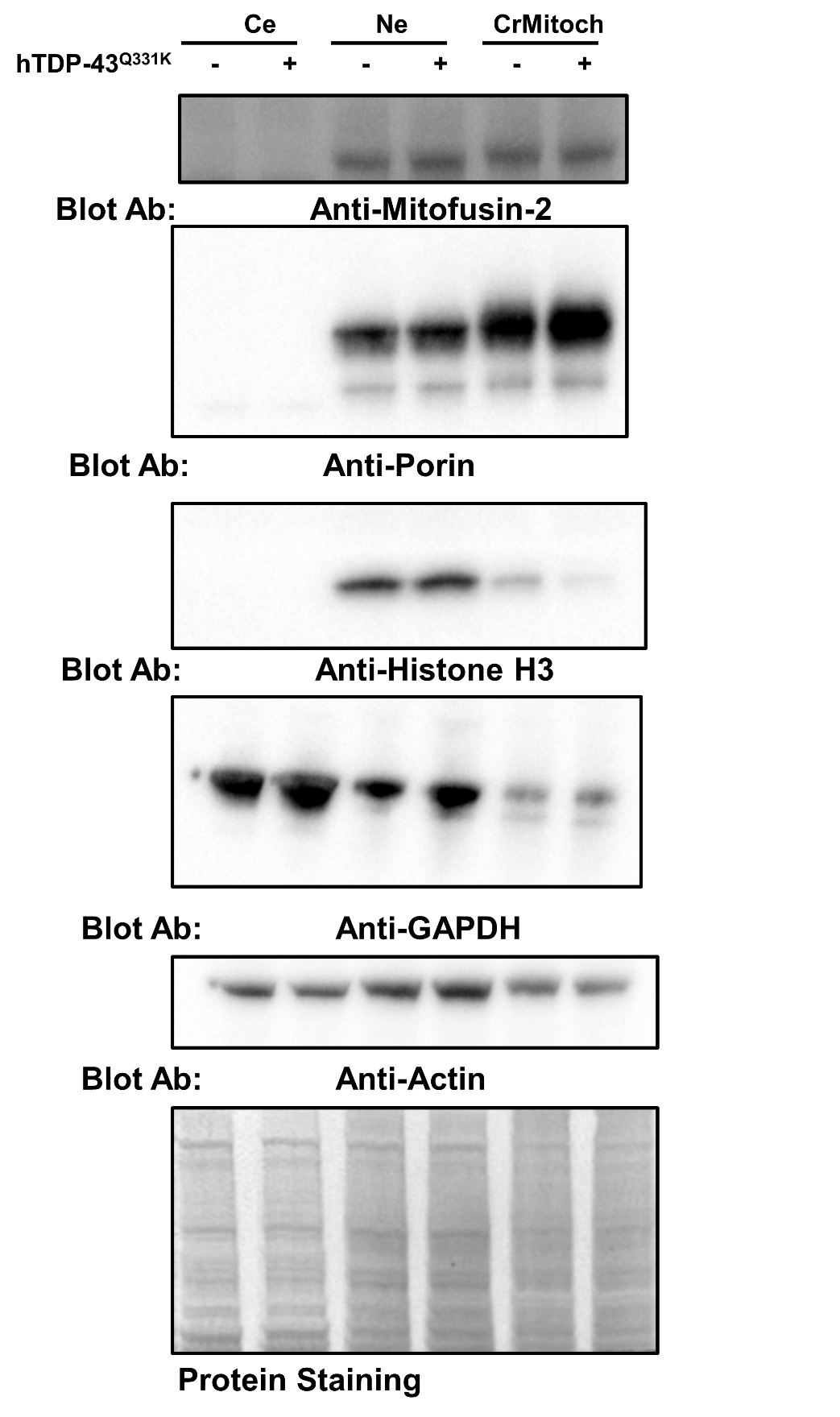
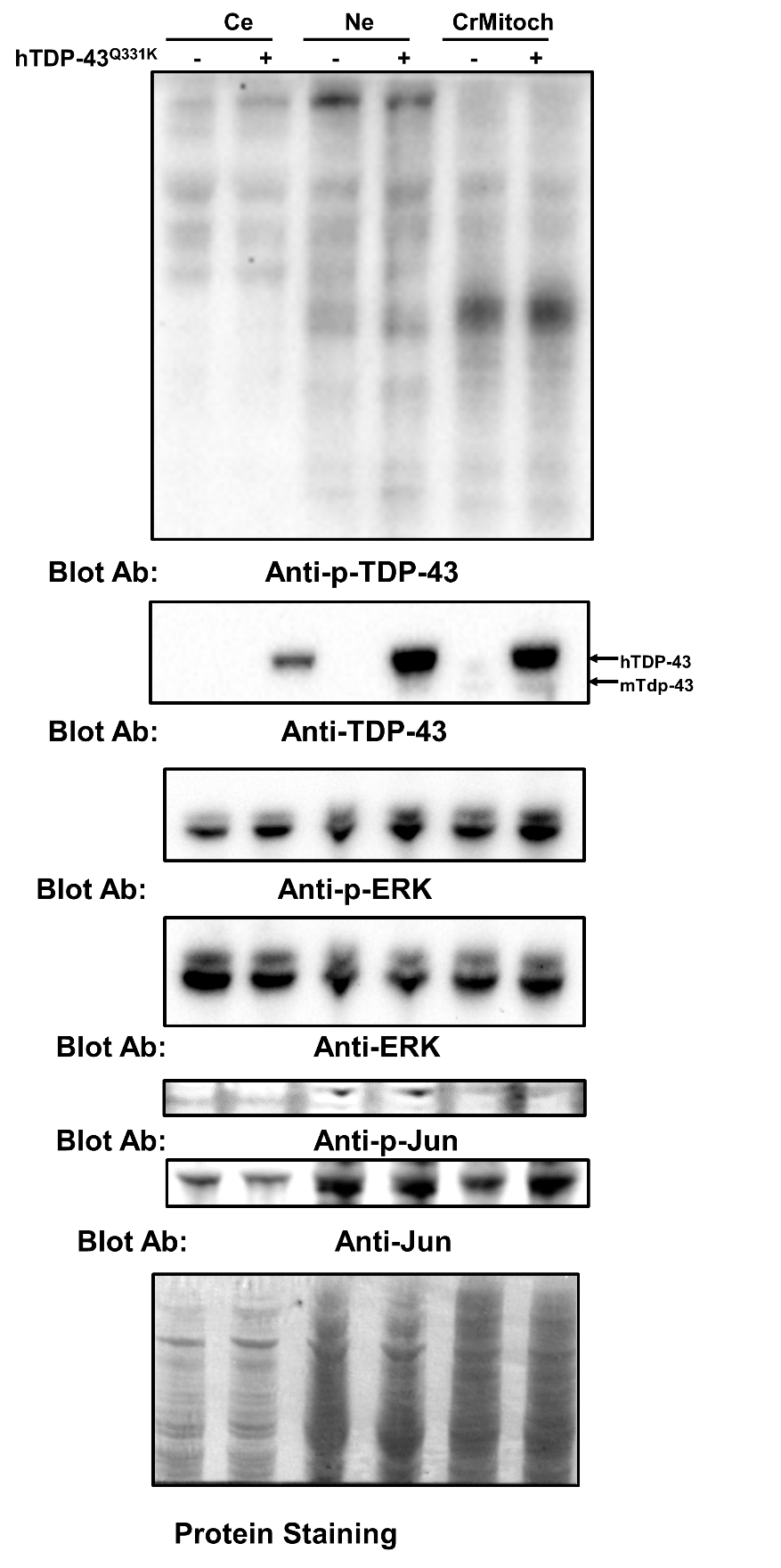


Supplemental figure 1. Oxidative stress induces changes between the nucleocytosolic relationships of proteins implicated in neurodegeneration. In all cases, total immunoreactivities found in nuclear and cytosolic compartments are related linearly significantly (in all cases p<0.001), though the slope is significantly affected by oxidative stress (shown p values for comparison of slopes).  Shown are the linear relationships between nuclear and cytosolic content, with 95% confidence intervals indicated with discontinous lines (n=200 to 296 cells for p-TDP-43; n=191-255 for p-ERK; n=234-326 for p-Jun  and n=217-415 for REST, obtained in at least 4 independent replicates). Inset of graphs show the equations of the linear relationships, separated by colors.



Supplemental Figure 2. Enrichment of protein markers in subcellullar fractionation. As shown by western-blot analyses of brain lysates after subcellular fractionation, in addition to nuclear enriched (Ne) and cytosolic enriched (Ce) compartments, crude mitochondrial fractions (CrMitoch) both non transgenic and transgenic hTDP-43 mice show the relative enrichment of Mitofusin-2 and porin in crude mitocondria, with almost the absence of histone H3 in non-nuclear fractions, and the high abundance of GAPDH in cytosolic extracts. Actin was distributed equally among the three fractions.



Supplemental Figure 3. Cellular subfractionation evidence for in vivo colocalization of proteins implicated in neurodegeneration with mitochondrial components. As shown by western-blot analyses of brain lysates after subcellular fractionation, in addition to nuclear enriched (Ne)  and cytosolic enriched (Ce)  compartments, crude mitochondrial fractions (CrMitoch) both non transgenic and transgenic hTDP-43 mice show the presence of p-TDP-43, p-ERK and Jun. Levels were quantified by densitometry in brains from 90 day old mice. Western-blot shown are for male specimens.

Supplemental Table 1. Effect of H2O2 treatment in protein subcellular distribution by confocal microscopy

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Source of Variation | % of total variation TDP-43 | % of total variation p-Jun | % of total variation p-ERK | % of total variation REST |
| Interaction | 7.862\*\*\*\* | 0.3051 | 4.401\*\*\*\* | 1.407\*\*\*\* |
| Stress | 12.23\*\*\*\* | 3.610\*\*\* | 12.31\*\*\*\* | 4.307\*\*\*\* |
| Location | 31.14\*\*\*\* | 0.04323 | 12.04\*\*\*\* | 39.41\*\*\*\* |

\*\*\*\*Indicate p<0.0001 after two way ANOVA

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Supplemental Table 2. Effect of mutated *TARDBP* overexpression in subcellular distribution of transcription factors   |  |  |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | Source of Variation | % of total variation TDP-43 | % of total variation p-TDP-43 | % of total variation p-TDP-43/TDP-43 | % of total variation  ERK | % of total variation  p-ERK | % of total variation  p-ERK/ERK | % of total variation  Jun | %of total variation p-Jun | %of total variation p-Jun/Jun | % of total variation REST | | Subcellular location | 8.455 | 47.63\*\*\*\* | 5.106 | 20.96 | 52.46\*\*\*\* | 42.86\*\*\* | 16.76\* | 63.03\*\*\*\* | 15.16 | 39.96\*\* | | Sex | 8.736 | 13.62\*\* | 4.433 | 0.8403 | 0.5431 | 1.015 | 3.633 | 9.650\*\*\* | 0.1989 | 0.6926 | | hTDP-43 overexpression | 22.51\*\* | 1.437 | 14.30\* | 0.7036 | 3.221 | 3.696 | 3.566 | 0.01932 | 5.320 | 0.8719 | | Subcellular location x Sex | 2.034 | 1.860 | 4.662 | 1.007 | 13.81\*\* | 3.210 | 9.362 | 6.350\* | 4.894 | 0.8915 | | Subcellular location x hTDP-43 overexpression | 4.407 | 7.622 | 2.430 | 0.08271 | 0.9421 | 0.8392 | 9.667 | 1.460 | 7.719 | 2.031 | | Sex x hTDP-43 overexpression | 4.588 | 2.278 | 5.143 | 0.03548 | 0.7200 | 0.6995 | 3.620 | 0.3800 | 2.074 | 0.004417 | | Subcellular location x Sex x hTDP-43 overexpression | 0.7420 | 0.5555 | 3.948 | 0.3125 | 0.2751 | 0.2329 | 8.345 | 4.474\* | 3.752 | 0.2244 |   \*\*\*\*Indicate p<0.0001, \*\*\* p<0.001, \*\*p<0.01, and p<0.05 after three way ANOVA |  |  |  |  |

Supplemental Table 3. Effect of Epoxomycin treatment in protein subcellular distribution by confocal microscopy

|  |  |  |  |
| --- | --- | --- | --- |
| Source of Variation | % of total variation p-ERK | % of total variation p-Jun | % of total variation REST |
| time | 0.02292\*\*\*\* | 0.1592\*\*\*\* | 0.1421\*\* |
| Cytosol vs nuclei | 90.56\*\*\*\* | 31.72\*\*\*\* | 32.14\*\*\*\* |
| Epoxomycin treatment | 7.383\*\*\*\* | 2.957\*\*\*\* | 0.0002409 |
| time x Cytosol vs nuclei | 0.2466\*\*\*\* | 5.703\*\*\*\* | 0.1810\*\*\* |
| time x Epoxomycin treatment | 0.3134\*\*\*\* | 1.444\*\*\*\* | 1.139\*\*\*\* |
| Cytosol vs nuclei x Epoxomycin treatment | 2.433\*\*\*\* | 51.37\*\*\*\* | 0.3210\*\*\* |
| time x Cytosol vs nuclei x Epoxomycin treatment | 0.4035\*\*\*\* | 1.042\*\*\*\* | 0.02023 |

\*\*\*\*Indicate p<0.0001 after three way ANOVA

Supplemental Table 4. Effect of thapsigargin treatment in protein subcellular distribution by confocal microscopy

|  |  |  |  |
| --- | --- | --- | --- |
| Source of Variation | % of total variation p-ERK | % of total variation p-Jun | % of total variation REST |
| Time | 0.04933\*\*\*\* | 1.269\*\*\*\* | 3.619\*\*\*\* |
| Cytosol vs nuclei | 79.41\*\*\*\* | 91.46\*\*\*\* | 22.06\*\*\*\* |
| Thapsig treatment | 12.88\*\*\*\* | 1.709\*\*\*\* | 2.153\*\*\*\* |
| Time x Cytosol vs nuclei | 0.08946\*\*\*\* | 1.512\*\*\*\* | 0.3063\*\*\*\* |
| Time x Thapsig treatment | 1.417\*\*\*\* | 2.077\*\*\*\* | 1.651\*\*\*\* |
| Cytosol vs nuclei x Thapsig treatment | 3.749\*\*\*\* | 0.5553\*\*\*\* | 0.7645\*\*\*\* |
| Time x Cytosol vs nuclei x Thapsig treatment | 1.006\*\*\*\* | 0.5563\*\*\*\* | 0.8305\*\*\*\* |

\*\*\*\*Indicate p<0.0001 after three way ANOVA

Supplemental Table 5. Primers employed for quantitation of REST transcriptional regulation.

|  |  |  |  |
| --- | --- | --- | --- |
| Gene | Accession No. | Application | Sequence (5’ 3’) |
| *BID* | NM\_197967 | RT-PCR | (F) agtgggagggctacgatgag |
| (R) gatgctacggtccatgctgt |
| *PUMA* | NM\_014417 | RT-PCR | (F) cccgtgaagagcaaatgag |
| (R) accccctgatgaaggtgag |
| *BAX* | NM\_138761 | RT-PCR | (F) tctgacggcaacttcaactg |
| (R) cgtcccaaagtaggagagga |
| *FADD* | NM\_003824 | RT-PCR | (F) ctggggaagaagacctgtg |
| (R) gcacacgctctgtcaggtt |
| *DAXX* | NM\_001350 | RT-PCR | (F) aagcctccttggattctggt |
| (R) atcatcctcctgaccctcct |
| *FAS* | NM\_000043 | RT-PCR | (F) agttggggaagctctttcactt |
| (R) cagtcttcctcaattccaatcc |
| *PSEN2* | NM\_000447 | RT-PCR | (F) cctcggggacttcatcttc |
| (R) tgaacacagcaagcagcag |
| *PESENEN* | NM\_172341 | RT-PCR | (F) tgaacctggagcgagtgtc |
| (R) taggctgggacaaggaagg |
| *P35* | NM\_003885 | RT-PCR | (F) caaaccaggagcattttgtgt |
| (R) attcctgtggcttgttctgtg |
| *P39* | NM\_003936 | RT-PCR | (F) ccttcattacgcctgcaaa |
| (R) tctcgttgcccatgtagga |
| *KCNQ2* | NM\_172106 | RT-PCR | (F) gcgcaacgccttctacc |
| (R) gacagcacgaggcagga |
| *KV2.1* | NM\_004975 | RT-PCR | (F) ggaaggcgaggagttcg |
| (R) gggcaatggtggagagg |
| *KCNJ6* | NM\_002240 | RT-PCR | (F) ctctcggtgctgatgtgaaa |
| (R) tgaaacggagcaagactgaa |
| *CAT* | NM\_001752 | RT-PCR | (F) atccagaagaaagcggtcaa |
| (R) cagatttgccttctcccttg |
| *FOXO1* | NM\_002015 | RT-PCR | (F) tggggcaacctgtcctac |
| (R) ggcacgctcttgaccatc |
| *SOD1* | NM\_000454 | RT-PCR | (F) ggcaaaggtggaaatgaaga |
| (R) gggcctcagactacatccaa |
| *1433ζ* | NM\_145690 | RT-PCR | (F) agcccgtaggtcatcttgg |
| (R) tgaagcattggggatcaag |
| *ARC* | NM\_015193 | RT-PCR | (F) cgcctggagaagaatcagag |
| (R) gggaaccttgagacctgttg |
| *BCL2* | NM\_000633 | RT-PCR | (F) ggaggattgtggccttcttt |
| (R) gccgtacagttccacaaagg |
| *CASP2* | NM\_032982 | RT-PCR | (F) ttgccgaagatgagactgc |
| (R) gcgttcaccttaaccagca |
| *ANT1* | NM\_001151 | RT-PCR | (F) gggctctaccagggtttca |
| (R) cgtcacactctgggcaatc |
| *PDCD7* | NM\_005707 | RT-PCR | (F) gcaggaggtggaggagaag |
| (R) tggaggacagacccctttc |
| *MAPK11* | NM\_002751 | RT-PCR | (F) taccggcaggagctgaac |
| (R) ttcttcaccgccaccttc |
| *MAPK12* | NM\_002969 | RT-PCR | (F) ccaccttcaccttccacct |
| (R) gcgtctgctctgatggatg |
| *GAP43* | NM\_001130064 | RT-PCR | (F) gggaggcttgaggaaaaatc |
| (R) gcagcttggacatcatcctt |
| *EGR1* | NM\_001964 | RT-PCR | (F) gttaccccagccaaaccac |
| (R) tgggttggtcatgctcact |
| *NRXN3* | NM\_004796 | RT-PCR | (F) gggaacaacacagacgacct |
| (R) ctcggctcacattcaacaaa |
| *GRIA4* | NM\_000829 | RT-PCR | (F) gcagcgccttacatatctcc |
| (R) ccaaccattttgtcctgctt |
| *MEF2C* | NM\_001193350 | RT-PCR | (F) ggggactatggggagaaaaa |
| (R) gcttgttggtgctgttgaag |
| *SANP25* | NM\_003081 | RT-PCR | (F) tcatccgcagggtaacaaa |
| (R) ttggcctcatcaattctgg |
| *SST* | NM\_001048 | RT-PCR | (F) gaccccagactccgtcagt |
| (R) gctcaagcctcatttcatcc |
| *ATP2B2* | NM\_001683 | RT-PCR | (F) ggctcacacagaaggaggag |
| (R) ggatggaggttcgagattca |
| *GAD1* | NM\_000817 | RT-PCR | (F) ttgcaccagtgtttgtcctc |
| (R) aggaccagtttaggcacagc |
| *GAD2* | NM\_000818 | RT-PCR | (F) gacctgctccagtctccaaa |
| (R) agggcgcacagtttgtttc |
| *CYCS* | NM\_018947 | RT-PCR | (F) tgaaaagggaggcaagca |
| (R) ccccagatgatgcctttg |
| *mCYCS* | NM\_007808 | RT-PCR | (F) ccaaatctccacggtctgtt |
| (R) gtctgccctttctcccttct |
| *mMAPK11* | NM\_011161 | RT-PCR | (F) ccagaaggtggctgtaaagaag |
| (R) gcctgacacttgacgatgttatt |
| *mCASP3* | NM\_00981 | RT-PCR | (F) tgtcatctcgctctggtacg |
| (R) aaatgaccccttcatcacca |
| *mβ-actin* | NM\_007393 | RT-PCR | (F) tgggacgacatggagaaga |
| (R) tggggtgttgaaggtctca |
| *mCIDEA* | NM\_007702 | RT-PCR | (F) agggacaacacgcatttca |
| (R)cattgagacagccgaggaa |
| *CALB1* | NM\_004929 | RT-PCR | (F) gaactctggaggaacgctga |
| (R) aggctgtgatgagggatgac |
| *SCN3B* | NM\_018400 | RT-PCR | (F) attgtttcccctggcttctc |
| (R) gcctccacctcctctctctt |
| *GABRB3* | NM\_021912 | RT-PCR | (F) gcttcttggcttctctggtg |
| (R) aacgagatgccattcactcc |
| *JIP1* | NM\_005456 | RT-PCR | (F) caccacgctcaacctctttc |
| (R) gtgtctgctcccctgtcttc |

p-Jun

|  |  |  |
| --- | --- | --- |
| Source of Variation | % of total variation p-Jun | P value |
| Time | 0.1592\*\*\*\* | <0.0001 |
| Cytsol vs nuclei | 31.72\*\*\*\* | <0.0001 |
| Epoxomyc | 2.957\*\*\*\* | <0.0001 |
| Time x Cytsol vs nuclei | 5.703\*\*\*\* | <0.0001 |
| Time x Epoxomyc | 1.444\*\*\*\* | <0.0001 |
| Cytsol vs nuclei x Epoxomyc | 51.37\*\*\*\* | <0.0001 |
| Time x Cytsol vs nuclei x Epoxomyc | 1.042\*\*\*\* | <0.0001 |

REST

|  |  |  |
| --- | --- | --- |
| Source of Variation | % of total variation | P value |
| Time | 0.1421\*\* | 0.0128 |
| Cytosol vs Nucli | 32.14\*\*\*\* | <0.0001 |
| Epoxom treat | 0.0002409 | 0.9183 |
| Time x Cytosol vs Nucli | 0.1810\*\*\* | 0.0050 |
| Time x Epoxom treat | 1.139\*\*\*\* | <0.0001 |
| Cytosol vs Nucli x Epoxom treat | 0.3210\*\*\* | 0.0002 |
| Time x Cytosol vs Nucli x Epoxom treat | 0.02023 | 0.3474 |

**Effect of Thapsigargin treatment in protein subcellular distribution by confocal microscopy**

|  |  |  |  |
| --- | --- | --- | --- |
| Source of Variation | % of total variation p-ERK | % of total variation p-Jun | % of total variation REST |
| Time | 0.04933\*\*\*\* | 1.269\*\*\*\* | 3.619\*\*\*\* |
| Cytosol vs nuclei | 79.41\*\*\*\* | 91.46\*\*\*\* | 22.06\*\*\*\* |
| Thapsig treatment | 12.88\*\*\*\* | 1.709\*\*\*\* | 2.153\*\*\*\* |
| Time x Cytosol vs nuclei | 0.08946\*\*\*\* | 1.512\*\*\*\* | 0.3063\*\*\*\* |
| Time x Thapsig treatment | 1.417\*\*\*\* | 2.077\*\*\*\* | 1.651\*\*\*\* |
| Cytosol vs nuclei x Thapsig treatment | 3.749\*\*\*\* | 0.5553\*\*\*\* | 0.7645\*\*\*\* |
| Time x Cytosol vs nuclei x Thapsig treatment | 1.006\*\*\*\* | 0.5563\*\*\*\* | 0.8305\*\*\*\* |

p-Jun

|  |  |  |
| --- | --- | --- |
| Source of Variation | % of total variation | P value |
| Time | 1.269\*\*\*\* | <0.0001 |
| cytosol nuclei | 91.46\*\*\*\* | <0.0001 |
| thapsi | 1.709\*\*\*\* | <0.0001 |
| time x cytosol nuclei | 1.512\*\*\*\* | <0.0001 |
| time x thapsi | 2.077\*\*\*\* | <0.0001 |
| cytosol nuclei x thapsi | 0.5553\*\*\*\* | <0.0001 |
| time x cytosol nuclei x thapsi | 0.5563\*\*\*\* | <0.0001 |

REST

|  |  |  |
| --- | --- | --- |
| Source of Variation | % of total variation | P value |
| Time | 3.619\*\*\*\* | <0.0001 |
| Nuclei vs cytosol | 22.06\*\*\*\* | <0.0001 |
| Thapsigargin treatment | 2.153\*\*\*\* | <0.0001 |
| Time x Nuclei vs cytosol | 0.3063\*\*\*\* | 0.0001 |
| Time x Thapsigargin treatment | 1.651\*\*\*\* | <0.0001 |
| Nuclei vs cytosol x Thapsigargin treatment | 0.7645\*\*\*\* | <0.0001 |
| Time x Nuclei vs cytosol x Thapsigargin treatment | 0.8305\*\*\*\* | <0.0001 |

**Effect of H2O2 treatment in protein subcellular distribution by confocal microscopy**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Source of Variation** | **% of total variation TDP-43** | **% of total variation p-Jun** | % of total variation p-ERK | % of total variation REST |
| **Interaction** | **7.862\*\*\*\*** | **0.3051** | 4.401\*\*\*\* | 1.407\*\*\*\* |
| **Stress** | **12.23\*\*\*\*** | **3.610\*\*\*** | 12.31\*\*\*\* | 4.307\*\*\*\* |
| **Location** | **31.14\*\*\*\*** | **0.04323** | 12.04\*\*\*\* | 39.41\*\*\*\* |

**Table Analyzed p-jun**

|  |  |  |
| --- | --- | --- |
| **Source of Variation p-Jun** | **% of total variation** | **P value** |
| **Interaction** | **0.3051** | **0.0634** |
| **Stress** | **3.610\*\*\*** | **<0.0001** |
| **Location** | **0.04323** | **0.3763** |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  |  |  |
| |  |  |  | | --- | --- | --- | | Source of Variation p-ERK | % of total variation | P value | | Interaction | 4.401\*\*\*\* | <0.0001 | | Stress | 12.31\*\*\*\* | <0.0001 | | Location | 12.04\*\*\*\* | <0.0001 | |  |  |  |  |
| |  |  |  | | --- | --- | --- | | Source of Variation REST | % of total variation | P value | | Interaction | 1.407\*\*\*\* | <0.0001 | | Stress | 4.307\*\*\*\* | <0.0001 | | Location | 39.41\*\*\*\* | <0.0001 |   Effect of mutated *TARDBP* overexpression in subcellular distribution of transcription factors   |  |  |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | Source of Variation | % of total variation TDP-43 | % of total variation p-TDP-43 | % of total variation p-TDP-43/TDP-43 | % of total variation  ERK | % of total variation  p-ERK | % of total variation  p-ERK/ERK | % of total variation  Jun | %of total variation p-Jun | %of total variation p-Jun/Jun | % of total variation REST | | Subcellular location | 8.455 | 47.63\*\*\*\* | 5.106 | 20.96 | 52.46\*\*\*\* | 42.86\*\*\* | 16.76\* | 63.03\*\*\*\* | 15.16 | 39.96\*\* | | Sex | 8.736 | 13.62\*\* | 4.433 | 0.8403 | 0.5431 | 1.015 | 3.633 | 9.650\*\*\* | 0.1989 | 0.6926 | | hTDP-43 overexpression | 22.51\*\* | 1.437 | 14.30\* | 0.7036 | 3.221 | 3.696 | 3.566 | 0.01932 | 5.320 | 0.8719 | | Subcellular location x Sex | 2.034 | 1.860 | 4.662 | 1.007 | 13.81\*\* | 3.210 | 9.362 | 6.350\* | 4.894 | 0.8915 | | Subcellular location x hTDP-43 overexpression | 4.407 | 7.622 | 2.430 | 0.08271 | 0.9421 | 0.8392 | 9.667 | 1.460 | 7.719 | 2.031 | | Sex x hTDP-43 overexpression | 4.588 | 2.278 | 5.143 | 0.03548 | 0.7200 | 0.6995 | 3.620 | 0.3800 | 2.074 | 0.004417 | | Subcellular location x Sex x hTDP-43 overexpression | 0.7420 | 0.5555 | 3.948 | 0.3125 | 0.2751 | 0.2329 | 8.345 | 4.474\* | 3.752 | 0.2244 | |  |  |  |  |
|  |  |  |  |  |