LEGENDS TO SUPPLEMENTARY INFORMATION

Supplementary Table 1. Rates of false positive peptide identifications determined by decoy database searches. Between the brackets are the number decoy database hits over hits in the true database.

Supplementary Table 2. Altered protein levels upon monocular deprivation. Proteins that are significantly (p<0.05, t-test) different between P30 and P30-MD (Table 2) are shown. All proteins that are quantified with less stringent criteria are marked in grey and are not considered significantly affected in our analysis. Of these proteins, those with an FDR>=15% are under the black line. Proteins quantified with less than 2 peptides in more than one set are indicated in italics.

Supplementary Table 3. Altered protein levels with age or dark rearing. Proteins that are significantly (p<0.05, t-test) different between P30 and P46 (Table 3A), P46 and P46-DR (Table 3B) and P30 and P46-DR (Table 3C) are shown. All proteins that are quantified with less stringent criteria are marked in grey and are not considered significantly affected in our analysis. Of these proteins, those with an FDR>=15% are under the black line. Proteins quantified with less than 2 peptides in more than one set are indicated in italics. Proteins quantified with less than 2 peptides in more than one set are indicated in italics.

Supplementary Table 4. Quantitation data of all proteins quantified in all four sets and all four experimental conditions identified with a confidence of >85%. Shown are protein identification confidence intervals (those below 95% are indicated in grey), the average normalized expression levels for the four sets under the four experimental conditions (p30, MD, p46 and DR), their standard deviations, the ratio’s of p30-MD:p30, P46:p30, p46-DR:p30 and p46-DR:p46, the corresponding t-tests and FDRs and the numbers of peptides used for quantitation in each set. If quantitation occurred with only one peptide in more than one set the values are marked in grey.
Supplementary Table 5. Categorization of proteins. A list of all 467 proteins identified with stringent criteria are shown in the list. The proteins are categorized as mitochondrial proteins or as regulating the cytoskeleton (A=Actin cytoskeleton, NTS=Neurofilament, Tubulin or Septin cytoskeleton), synaptic efficacy or signal transduction.

Supplementary Table 6. List of mitochondrial proteins shown depicted in figure 4. Mitochondrial proteins whose levels were significantly (p<0.05 t-test, FDR<15%) altered with age or DR are shown at the top. In grey the mitochondrial proteins are listed that did not show significantly altered levels with age or DR.

Supplementary Table 7A-D. Peptide identification and iTRAQ quantification information of the four sets (A-D) of experimental data.

Supplementary Table 8. Proteins found to be significantly regulated at the synaptic protein level and at the mRNA level upon MD (8A) or DR (8B). Proteins quantified with less stringent criteria (less than 2 peptides in more than 1 set, in italics, FDR>=15% lower part of lists) are marked in grey. Relative expression levels are shown, at the left for the synaptic protein level, at the right for the mRNA level. P-values (t-test) are listed.

Supplementary Figure 1. Quantitative assessments of Western blot analyses performed on proteins significantly regulated with iTRAQ proteomics. Despite the modest changes in protein expression under the different experimental conditions, we confirmed changes in levels for most of the tested proteins (9/14) when assessed with Western Blot analysis (P<0.05, one tailed Student's t-test of independent samples). Two more proteins showed a trend in the same direction (P<0.06).
**Supplementary Figure 2.** Correlation between mRNA and synaptic protein expression of those proteins significantly regulated at both levels during MD (A) or DR (B). With DR, if proteins are significantly regulated at the mRNA and protein level, there is a strong correlation between these levels (closed circles, corr=0.59). The correlation remains the same if less stringently quantified proteins are included (open quadrants, corr=0.59). For MD the situation is different and significantly altered mRNA and proteins do not correlated (0.05 for stringently quantified proteins, closed circles, or -0.06 for less stringently quantified proteins).