Global proteome and ubiquitinome changes in the soluble and insoluble fractions of Q175 Huntington mice brains

Karen A. Sap[1], Arzu Tugce Guler[1], Karel Bezstarosti[2], Aleksandra E. Bury[1], Katrin Juenemann[3,4], Jeroen A.A. Demmers[2], Eric A. Reits[1]

Material included:

Figure S1: Arg-C and chymotrypsin digestion
Figure S2: Distribution of protein and peptide intensities
Figure S3: Correlation between global proteome and ubiquitinome for diGly sites identified exclusively in mutant or exclusively in wild-type samples
Figure S4: Increased relative abundance of full length mHtt after proteasome inhibition and autophagy inhibition in StHdh Q111/Q111 cells

Table S1. List of all identified and quantified proteins in soluble fraction. Information derived from MQ protein groups file and from Student's t-test in Perseus.
Table S2. List of all identified and quantified proteins in insoluble fraction. Information derived from MQ protein groups file and from Student's t-test in Perseus.
Table S3. List of all identified and quantified diGly sites in the soluble fraction. Information derived from MQ GlyGly(K)sites table and from Student's t-test in Perseus.
Table S4. List of all identified and quantified diGly sites in the insoluble fraction. Information derived from MQ GlyGly(K)sites table and from Student's t-test in Perseus.
Table S5. Proteins and diGly sites exclusively identified in one group. Information derived from MQ protein groups file and MQ GlyGly(K)sites table. List of proteins and diGly sites identified in 4 out of 4 replicates in one group and in none or 1 in the other group
Table S6. Overlap protein expression and gene expression. Gene expression data from Langfelder 2016 *Nat. Neurosci.* 19, 623–633 doi:10.1038/nn.4256.

Figure S1

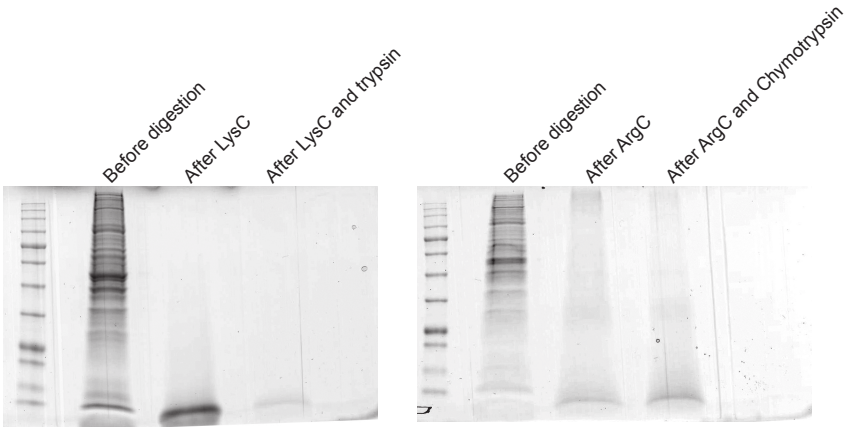
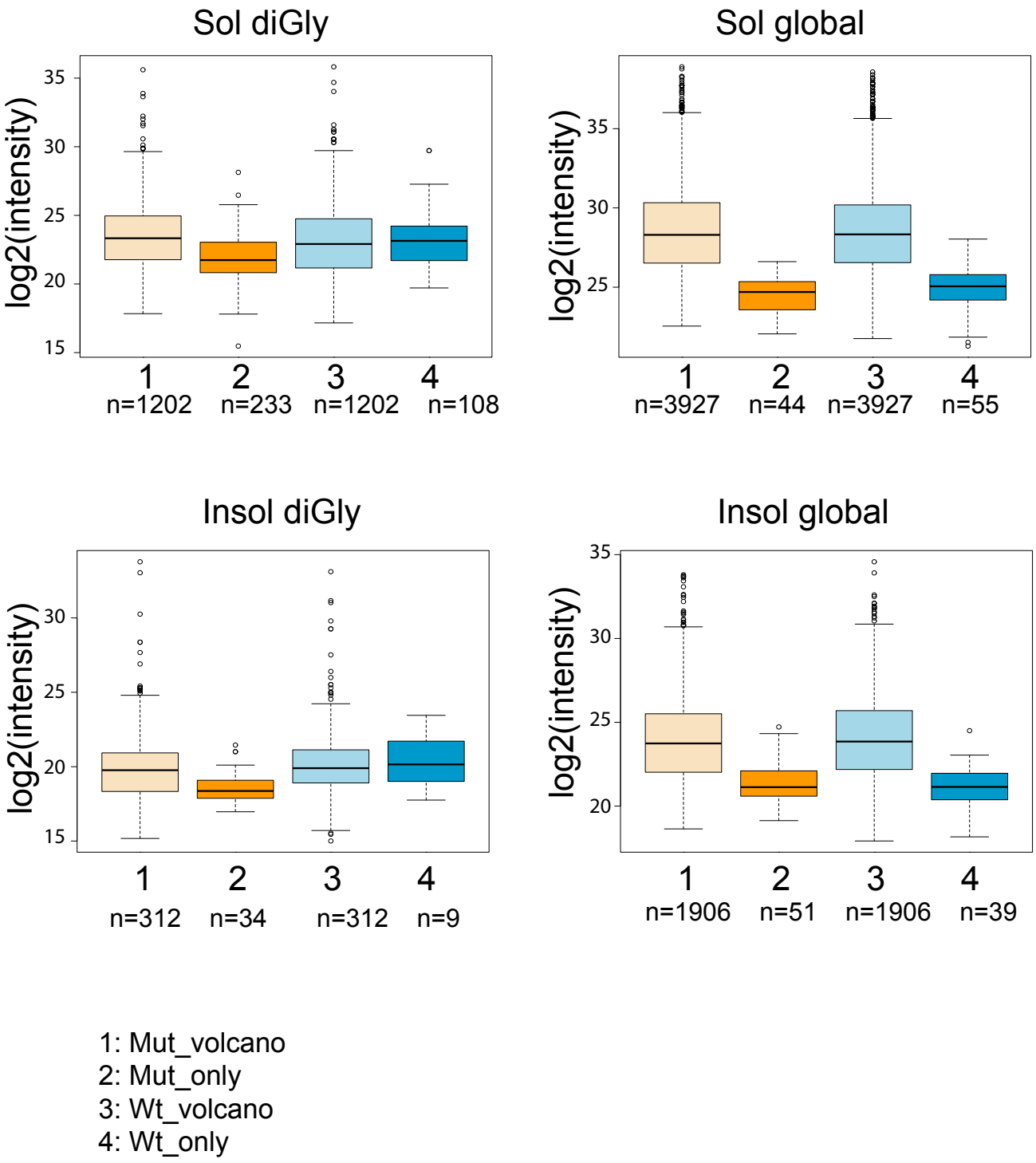


Figure S1. Arg-C and chymotrypsin digestion

Coomassie staining before and after digestion shows that digestion with all enzymes resulted in decreased protein molecular weight, suggesting that digestion was effective.

Figure S2



1: Mut_volcano
2: Mut_only
3: Wt_volcano
4: Wt_only

Figure S2. Distribution of protein and peptide intensities

Boxplots of log2 transformed intensities of diGly sites and global proteins in insoluble and soluble. Each figure shows four boxplots that are plotted from the distribution of the intensities of sites/proteins that have a positive fold change in the volcano plot (up in mutant), identified in all mutant but either in none or only one of the wildtype replicates (only in mutant), have a negative fold change in the volcano plot (down in mutant), identified in all wildtype but either in none or only one of the mutant replicates (only in wildtype).

Figure S3

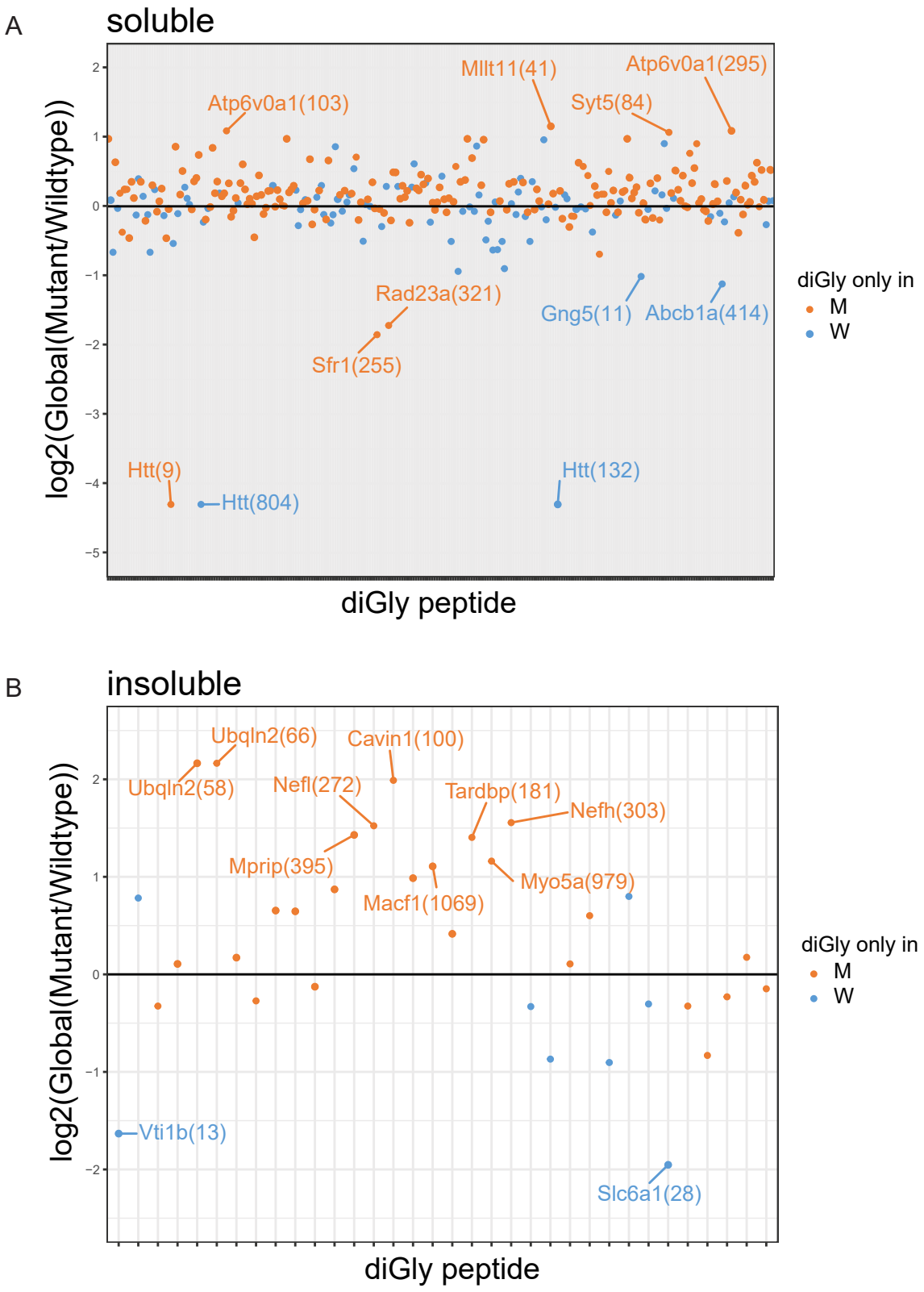


Figure S3. Correlation between global proteome and ubiquitinome for diGly sites identified exclusively in mutant or exclusively in wild-type samples

Log2 fold change in global protein levels are shown for diGly sites that are only identified either in mutant or wild type. Each diGly site identified in all mutant but either in none or only one of the wild-type replicates are denoted as 'only in mutant' and shown in orange. The ones identified in all wild-type but either in none or only one of the mutant replicates are denoted as 'only in wild type' and shown in blue. Peptides which have their proteins differentially expressed in global with a fold change either higher than 1 or lower than -1 are labeled.

Figure S4

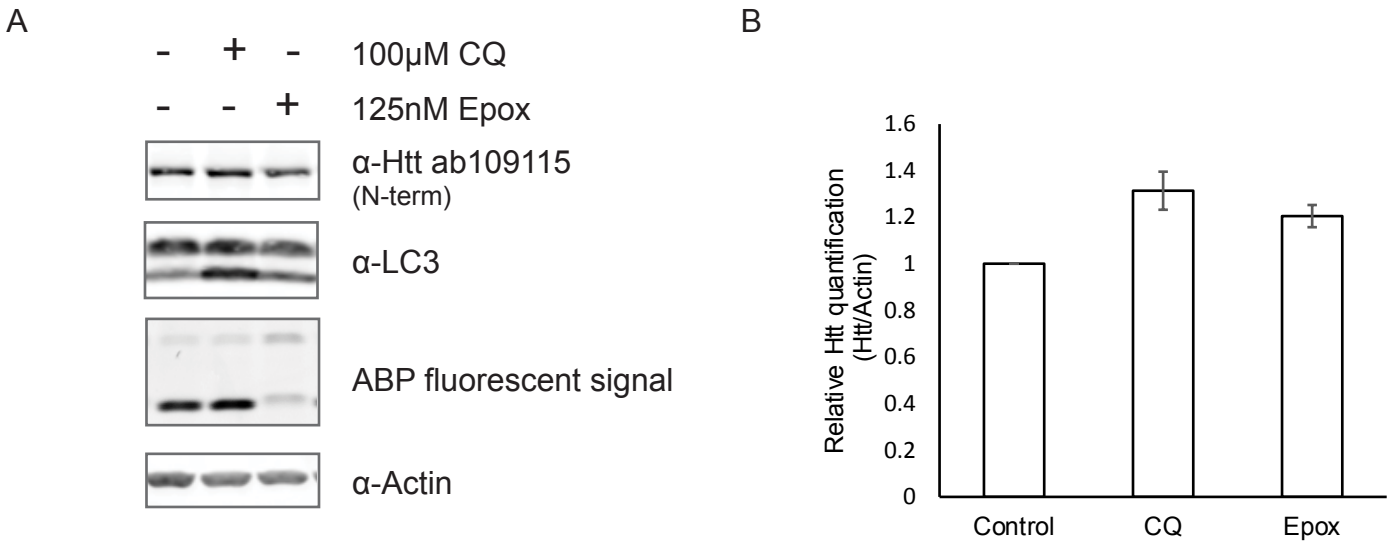


Figure S4. Increased relative abundance of full length mHtt after proteasome inhibition and autophagy inhibition in StHdh Q111/111 cells

A) SDS-PAGE westernblot of full length mHtt from StHdh Q111/111 cells treated for 16h with autophagy inhibitor (100µM Chloroquine), or proteasome inhibitor (125 nM Epoxomicin). Three replicates are shown. WB with Ab directed against LC3 was done to evaluate autophagy inhibition. Proteasome activity was evaluated by the use of Activity-Based Probe and scanning on Typhoon. Actin was used as a loading control. B) Relative quantification of mHtt protein level based in intensity of corrected for actin intensity. Error bars show standard error of the mean of three replicates.