SUPPLEMENTARY DATA

1. Supplementary Table 1: Protein Summary report generated by ProteinPilot for Replicate A for the cytoplasmic fraction.

2. Supplementary Table 2: Protein Summary report generated by ProteinPilot for Replicate A for the nuclear fraction.

3. Supplementary Table 3: Protein Summary report generated by ProteinPilot for Replicate B for the cytoplasmic fraction.

4. Supplementary Table 4: Protein Summary report generated by ProteinPilot for Replicate B for the nuclear fraction.

Supplementary Tables 1 to 4 include N, Unused (ProtScore), Total (ProtScore), %Cov, %Cov(50), %Cov(95), Accession Name, Species, abundance ratios, *P*-values and Error Factors (EF) for the identified proteins.

The definitions of the table fields are described as follows: **N** is the rank of the specified protein relative to all other proteins in the list of detected proteins. **Unused (ProtScore)** is a measure of the protein confidence for a detected protein calculated from the peptide confidence for peptides from spectra that have not already been completely "used" by higher scoring winning proteins. **Total (ProtScore)** a measure of the total amount of evidence for a detected protein. The Total ProtScore is calculated using all of the peptides detected for the proteins, and does not indicate the percent confidence of the identification of a protein. **%Cov** is the percentage of matching amino acids from identified peptides having confidence greater than 0 divided by the total number of amino acids in the sequence. **%Cov(50)** and **%Cov(95)** are the percentage of matching amino acids from identified peptides having confidence greater than or equal to 50 and 95 respectively divided by the total number of amino acids in the sequence. **Ratio**: the average ratio for the protein, corrected for experimental bias. ***P*-value**: a measure of the certainty that the average ratio differs from one; the smaller the *P*-value, the more likely any differential expression is real. **EF**: measure of the error in the average ratio, expressing the 95% confidence interval (95% CI) of the average iTRAQ ratio = (ratio x EF) – (ratio / EF)].

5. Supplementary Table 5: Peptide Summary report generated by ProteinPilot for Replicate A for the cytoplasmic fraction.

6. Supplementary Table 6: Peptide Summary report generated by ProteinPilot for Replicate A for the nuclear fraction.

7. Supplementary Table 7: Peptide Summary report generated by ProteinPilot for Replicate B for the cytoplasmic fraction.

8. Supplementary Table 8: Peptide Summary report generated by ProteinPilot for Replicate B for the nuclear fraction.

Supplementary Tables 5 to 8 include N, Unused (ProtScore), Total (ProtScore), %Cov, %Cov(50), %Cov(95), Accessions, Used, Annotation, Contrib, Conf, Sequence, Modifications, Cleavages, dMass, Prec MW, Prec *m/z*, Theor MW, Theor *m/z*, Theor z, Sc, Spectrum, Time, abundance ratios, %Err, Area and Err.

The definitions of these table fields that have not been described above are: **Used:** displays whether the ratio for this peptide has been used for quantization (1) or not (0). **Annotation**: displays how the Used status is determined: “auto” when the program sets the status, “manual” when a user sets the status. **Contrib:** the contribution of the peptide, in ProtSocre Units, to the Unused ProtScore for a protein in a group. **Conf (Confidence):** the highest confidence for this peptide identification, for all the proteins identified as containing this peptide, expressed as a percentage. **Sequence:** the sequence of the peptide identified by the search. **Modifications:** modifications found by the search. **Cleavages:** indicates any atypical or missed cleavages sites. Missed cleavages are shown as “Missed Residue1-Residue2@position”. Atypical cleavages are shown as “Cleaved Residue1-Residue2@position”, where “Position” is either “N-term” or “C-term”. **ΔMass (Delta Mass):** the difference in mass between the precursor MW and the theoretical MW of the matching peptide sequence. **Prec MW (Precursor Molecular Wegth):** the monoisotopic mass for the ion fragmented in this cycle and experiment. **Prec *m/z* (Precursor mass-to-charge ratio):** the monoisotopic *m/z* for the ion fragmented in this cycle and experiment, as determined by the instrument. **Theor MW (theoretical Molecular Weight):** the molecular weight of the

peptide calculated from the sequence and any modifications. **Theor *m/z* (theoretical mass-to-charge) ratio:** theoretical MW / theoretical z. **Theor z (theoretical z):** for those spectra for which the charge state could not be determined form the data, this is the charge state for the highest scoring peptide. **Sc (Score):** the score for the peptide based on matching ions of various charge states. **Spectrum:** denotes a particular MS/MS spectrum in this processing run. For 4000 Series Explorer Software data, Spectrum is: Data Set Chromatogram Name, MS/MS Job Run, Fraction, MS/MS Job Spectrum. The Data Set number is the order in which the individual data sets were processed. **Time:** the retention time for this spectrum. **Ratio:** the ratio for the peptide, corrected for experimental bias. Specifically this is the ratio for the peak area of the iTRAQ reagent to the peak area of the iTRAQ reagent control. **%Err (Error):** a measure of the error in the calculated ratio, calculated from the error for each of the peaks in the ratio. **Area (label):** the area of the peak for the specified label. **Err (label):** a measure of the error in the peak area for the specified label.

9. Supplementary Table 9. Proteins identified in 4 sample fractions.

10. Supplementary Table 10. Proteins identified in 3 sample fractions.

Supplementary Tables 9 and 10 include the list of all the proteins identified in 4 and 3 sample fractions respectively after KiSS-1 transiently transfection. Data include gene name, accession number, %Cov(95), abundance ratios, *P*-values (PV) and Error Factors (EF).

In **Supplementary Tables 9 and 10,** information from Replicate A and Replicate B are shown in green and brown respectively. In addition, data for nuclear and cytoplasmic fractions are shown as normal and italics respectively. Ratios deemed to signify differential expression (*P*-value < 0.05) are highlighted; highlights are dark blue or dark yellow when expression ratios are <0.77 or >1.3 respectively. Equally, data are highlighted in light blue or light yellow when expression ratios are >0.77 or <1.3 respectively.

Supplementary Figure 1: Reproducibility assessment of iTRAQ experiments: (A) Venn diagram showing the number of proteins identified by ProteinPilot in nuclear fractions from both experimental replicates; a total number of 1529 proteins were identified (1157 in Replicate A and 1173 in Replicate B) of which 801 were detected in both data sets.

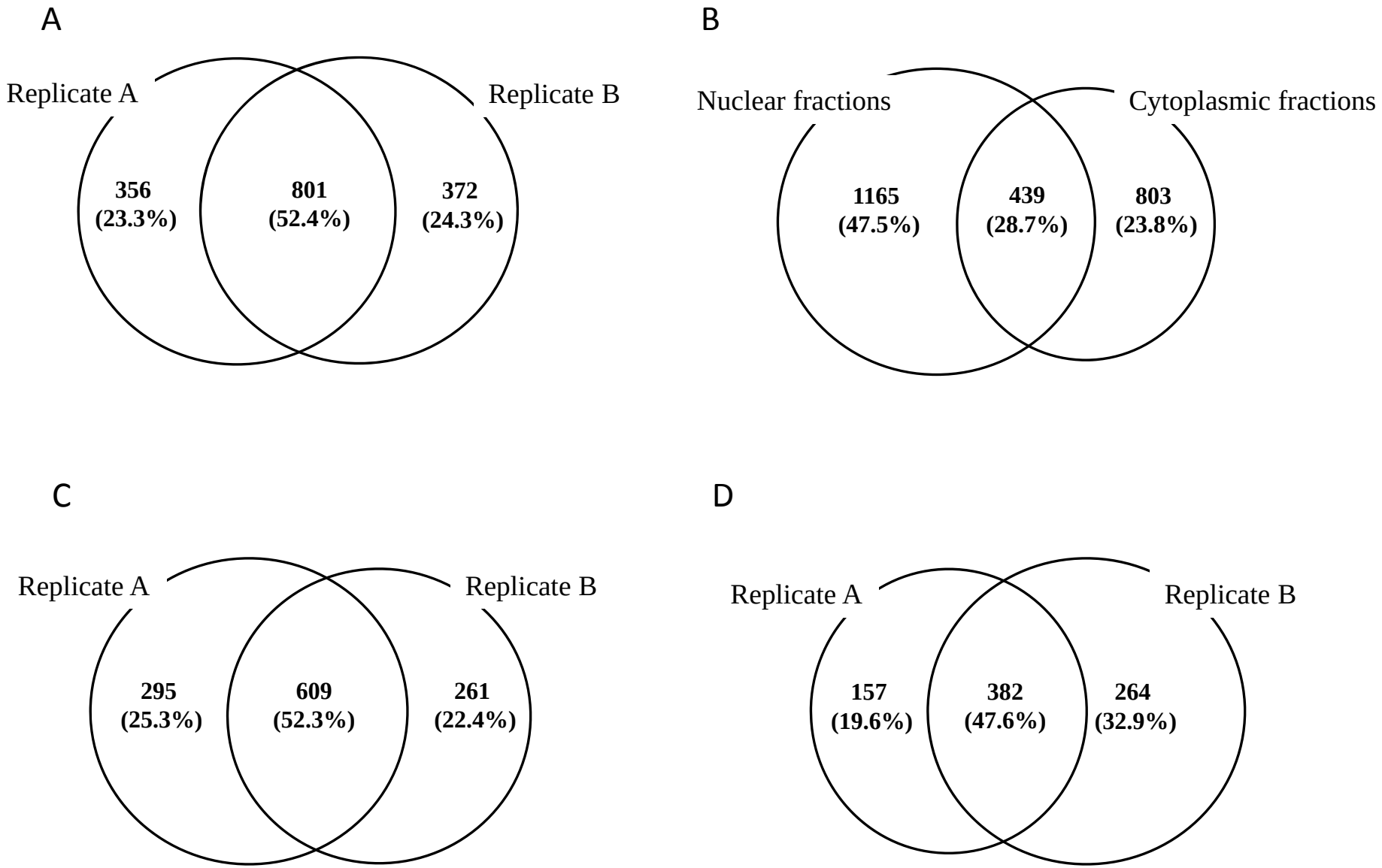
(B) Venn diagram showing the number of proteins identified by ProteinPilot in nuclear and cytoplasmic fractions; a total number of 1529 proteins were identified (1165 in nuclear fractions and 803 in cytoplasmic fractions) of which 439 were detected in both. **(C)** Venn diagram showing the number of proteins identified by ProteinPilot in nuclear fractions in both experimental replicates; a total number of 1165 proteins were identified (904 in Replicate A and 870 in Replicate B) of which 609 were detected in both data sets. **(D)** Venn diagram showing the number of proteins identified by ProteinPilot in cytoplasmic fractions from both experimental replicates; a total number of 803 proteins were identified (539 in Replicate A and 646 in Replicate B) of which 382 were detected in both data sets.

Supplementary Figure 2: Protein identification and relative quantification of Filamin A. Detection and relative quantification of Filamin A in EJ138 cells transiently transfected with a vector encompassing the full length *KiSS-1*. Proteins from MOCK, Empty vector, *KiSS-1* 24h and *KiSS-1* 48h transfectants were labelled with iTRAQ tags 114, 115, 116 and 117 respectively. Following MS^2 analysis, iTRAQ reporter and b and y ions of various peptides were detected. As representative, the MALDI-based MS^2 spectra for the singly charged peptide FNEEHIPDSPFVVPVASPSGDAR, at *m/z* 2611.2909 is shown for Filamin A, indicating amino acid sequence, annotated b-ion and y-ion series, iTRAQ ratios and an expanded view of the low-*m/z* reporter ion region showing representative relative quantification of Filamin A across the four transfectants.

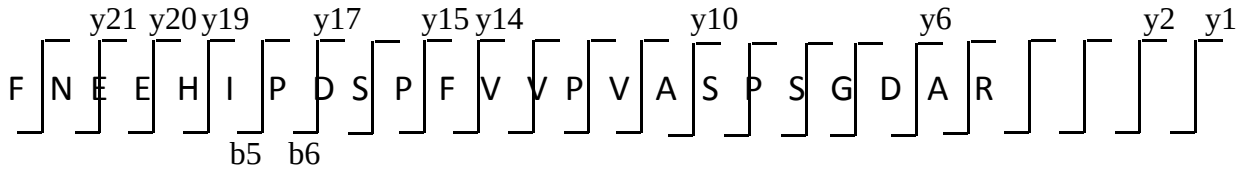
Supplementary Figure 3: Protein identification and relative quantification of Ezrin. Detection and relative quantification of Ezrin in EJ138 cells transiently transfected with a vector encompassing the full length *KiSS-1*. Proteins from MOCK, Empty vector, *KiSS-1* 24h and *KiSS-1* 48h transfectants were labelled with iTRAQ tags 114, 115, 116 and 117 respectively. Following MS^2 analysis, iTRAQ reporter and b and y ions of various peptides were detected. As representative, the MALDI-based MS^2 spectra for the singly charged peptide VTTMDAELEFAIQPNTTGK, at *m/z* 2354.2152 is shown for Ezrin, indicating amino acid sequence, annotated b-ion and y-ion series, iTRAQ ratios and an

expanded view of the low-*m/z* reporter ion region showing representative relative quantification of Ezrin across the four transfectants.

Supplementary Figure 1

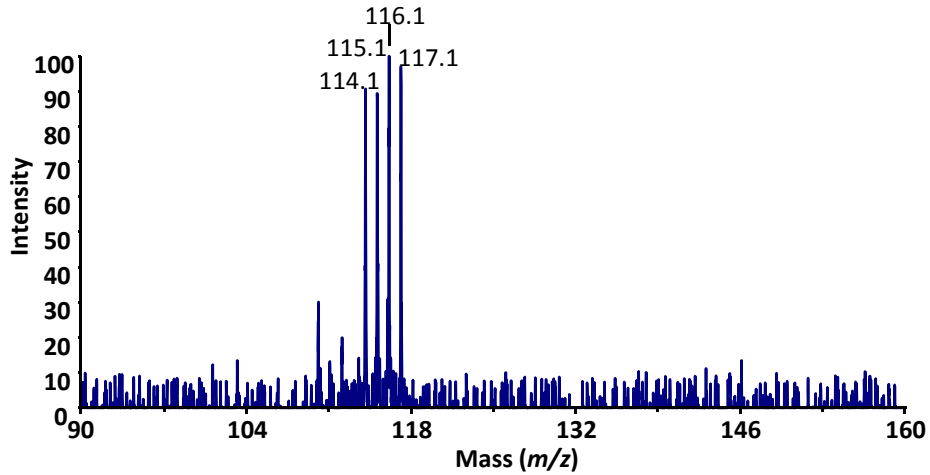


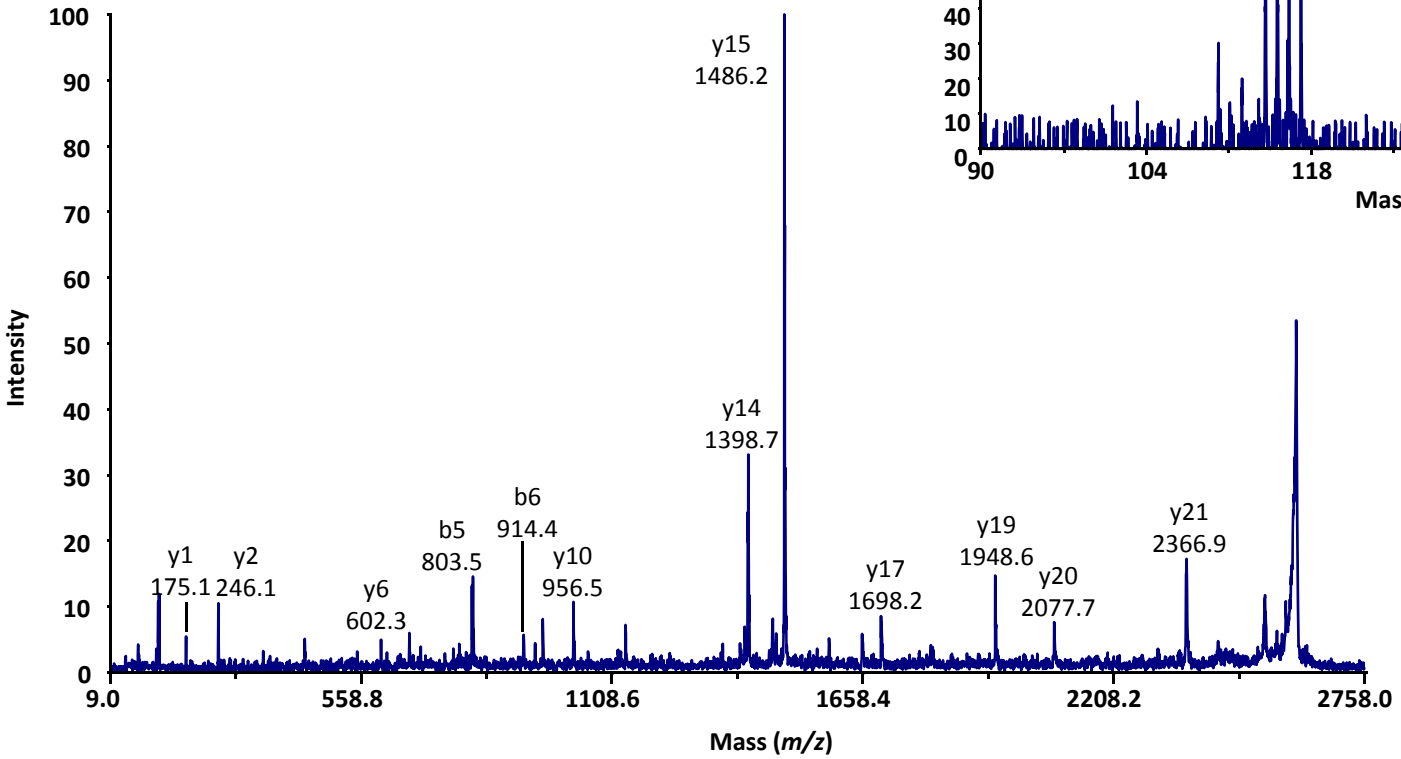
Supplementary Figure 2



Prec. *m/z* 2611.2909

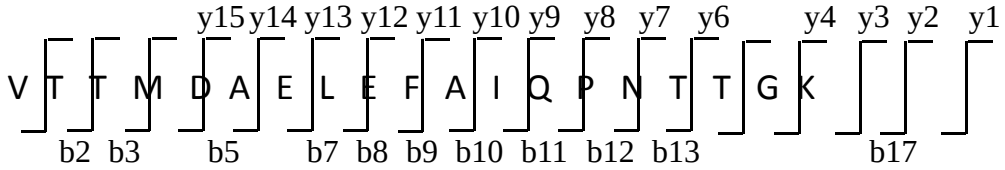
iTRAQ ratio 116:115 1.20
iTRAQ ratio 117:115 0.84





Supplementary Figure 3

A



Prec. *m/z* 2354.2152

iTRAQ ratio 116:115 1.21

iTRAQ ratio 117:115 1.50