Chapter 5 : Supplementary Material Figure Legends

Supplementary Table 5a: Initial analysis of the data generated from the first SILAC experiment using the filter parameters optimized in table 4.2.

The raw files generated from the 40 samples prepared in figure 5.2 were searched against the IPI human v3.14 database and the results were filtered using the multiple threshold (Match = 4, Delta Cn = 0.100, Xcorr = 1.00, Sp = 350, RSp = 5, Percent ions = 50%) parameters optimised in table 4.2. The false positive rate was calculated as 0.89% when the acquired ms/ms spectra were searched against a database containing the decoy database also containing the randomized sequences as described in chapter 4. There were a total of 485 proteins identified of which 168 had peptides that were suitable to calculate the ratios between the [$^{13}C_6$] Arg RG/C2 and [$^{12}C_6$] Arg PRG/C2 MV proteins. The Xpress column highlighted in pink indicate the proteins with >1.5-fold increase in secretion via MVs in the PRG/C2 cell line and the Xpress column highlighted in blue indicate the proteins with >1.5-fold decrease in the PRG/C2 cell line.

* - the peptides identified in these 3 proteins were identified from the same MS/MS scans and the software couldn't decide which of these proteins these peptides belong to.

(All protein Xpress ratios indicated represent the average of all peptide Xpress ratios calculated by Bioworks).

Supplementary Table 5b: Comparison of the TPP results for all 4 SILAC experiments.

The raw, dta and out files for all 40 samples in each experiment were converted to the relevant file formats and each experiment was analysed separately using the TPP. The results from the TPP analysis for all 4 experiments were then compared using Microsoft Excel. The table shows the results of this comparison. Proteins in the green, purple, yellow and white cells are those identified in 4, 3, 2 and 1 experiment. The ASAPRatios highlighted in pink are those that demonstrated >1.5fold increase in secretion by the PRG/C2 cell line. The ASAPRatios highlighted in blue are those that demonstrated >1.5fold decrease in secretion by the PRG/C2 cell line.

Supplementary Table 5c: Proteins identified in these SILAC surveys that were previously reported in proteomic profiles of CM and MVs derived from various cancer cell lines.

The proteins identified in this SILAC survey that were found in at least 2 experiments (564 proteins) were compared with those previously reported in other proteomic profiles of CM derived from various cancer types surverys (Gronborg et al., 2006, Kulasingam and Diamandis, 2007, Mbeunkui et al., 2006, Mbeunkui et al., 2007, Mears et al., 2004, Ratajczak et al., 2006b, Sardana et al., 2008, C. C. Wu et al., 2008, Choi et al., 2007)). The table lists the 243 proteins that were identified in this survey that have been previously reported to be secreted by cancer cell lines.

Supplementary Table 5d: Raw data from the TPP analysis for experiment C13 ARG A.

Table to show the raw data from the TPP analysis for experiment C13 ARG A using a filter of protein probability >0.70.

Supplementary Table 5e: Raw data from the TPP analysis for experiment C13 ARG B.

Table to show the raw data from the TPP analysis for experiment C13 ARG B using a filter of protein probability >0.70.

Supplementary Table 5f: Raw data from the TPP analysis for experiment C13 LYS A.

Table to show the raw data from the TPP analysis for experiment C13 LYS A using a filter of protein probability >0.70.

Supplementary Table 5g: Raw data from the TPP analysis for experiment C13 LYS B.

Table to show the raw data from the TPP analysis for experiment C13 LYS B using a filter of protein probability >0.70.

Supplementary Table 5h: Proteins identified in these SILAC surveys that were previously reported in a proteomic profile of MVs derived from the HT29 cell line.

The proteins identified in this SILAC survey that were found in at least 2 experiments (564 proteins) were compared with those previously reported in a proteomic profile of MVs derived from a CRC cell line HT29 (Choi et al., 2007)). The table lists 101 proteins that Were common to both studies.

Supplementary Table 5i: Proteins identified that were unique to this study when compared to other secreted protein proteomic surveys.

The proteins identified in this SILAC survey that were found in at least 2 experiments (564 proteins) were compared with those previously reported in other proteomic profiles of CM derived from various cancer types surverys (Gronborg et al., 2006, Kulasingam and Diamandis, 2007, Mbeunkui et al., 2006, Mbeunkui et al., 2007, Mears et al., 2004, Ratajczak et al., 2006b, Sardana et al., 2008, C. C. Wu et al., 2008, Choi et al., 2007)). The table lists the 321 proteins identified that were unique to this study.