Supplemental Figure 1
Distribution of SILAC peptide ratios between secreted and cytosolic proteins. Proteins harvested from control (T98 unlabeled mixed with T98 labeled) conditioned media or cytosol were separated by SDS-PAGE and subjected to tryptic digestion followed by mass spectrometry analysis. Peptide ratios were obtained using the Census algorithm, then log transformed and plotted into bins of 0.08 against frequency (number of peptides in each bin).

Supplemental Table 1
Total proteins quantified in the conditioned media of glioblastoma cell lines T98, U118, LN18 and U87. Proteins collected from conditioned media of SILAC labeled T98 cells were mixed 1:1 with conditioned media from either unlabeled T98, U118, LN18 or U87 cells. A reverse labeleing experiment was performed with U87 labeled and T98 unlabeled conditioned media. Proteins were then fractionated by SDS-PAGE and bands were excised for in-gel digestion and mass spectrometry analysis. Proteins were identified by Sequest and quantified using the Census algorithm. Mean SILAC peptide ratios (unlabeled/labeled) are shown ± standard deviation, with number of peptides quantified in parentheses. Proteins identified in only one member of the SILAC pair (i.e. only labeled or only unlabeled) are not given a ratio but designated as "only" with total spectral count shown in parentheses. These singleton peptides were validated by manual inspection of the raw data. "NQ/ND" indicates proteins which were not detected or not quantified in a sample. In some cases, proteins were identified at multiple positions on the SDS-PAGE gel and these are indicated by their apparent molecular masses in the "MW" column. Subcellular localizations (either known or predicted) are summarized using UniProtKB (http://www.uniprot.org/), NCBI AceView (http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/), WoLF PSORT (http://wolfpsort.org/) and the literature. Proteins not detected in a particular sample are indicated as "ND".

Supplemental Table 2a-e
Peptides identified in the conditioned media from T98, LN18, U118 and U87 cells. Peptides quantified for each SILAC pair. 2a) LN18 unlabeled and T98 labeled; 2b) T98 unlabeled and T98 labeled control cells; 2c) U118 unlabeled and T98 labeled; 2d) U87 unlabeled and T98 labeled (forward SILAC); and 2e) U87 labeled and T98 unlabeled (reverse SILAC). R@ and K# indicate peptides labeled with heavy arginine and lysine isotopes, respectively, while M* indicates oxidized methionine.

Supplemental Table 3
Proteins found differentially secreted by U118, LN18 and U87 cell lines relative to the T98 cell line. SILAC ratios greater than three standard deviations (99% C.I.) from the mean of the control ratios (unlabeled T98/labeled T98) were considered significantly up or down regulated and are indicated by ** or *, respectively. Mean peptide ratios are reported ± standard deviation with the number of quantified peptides in parentheses. Proteins identified in only one member of the SILAC pair (i.e. only labeled or only unlabeled) are not given a ratio but designated as "only" with total spectral count shown in parentheses. These singleton peptides were validated by manual inspection of the raw

data and a spectral count of four or greater was considered significant. "NQ/ND" indicates proteins which were not detected or not quantified in a sample. In some cases, proteins were identified at multiple positions on the SDS-PAGE gel and these are indicated by their apparent molecular masses in the "MW" column. Only proteins with known or predicted extracellular, cell membrane and lysosomal/endosomal localizations are shown.

Supplemental Table 4

Correlation of glioblastoma secretome profiles with invasive phenotype. Levels of invasiveness were correlated with spectral counts of secreted, cell membrane and lysosomal proteins expressed by each cell line using Pavlidis template matching. Black template line above heat map represents relative invasion potential of each cell line, and red line indicates mean relative expression of all correlated proteins within each column. Red heat map blocks reflect relative high protein expression and green represent lower expression. Spectral counts for proteins expressed by healthy primary astrocytes (371) were not included in the analysis, but are shown as a reference.