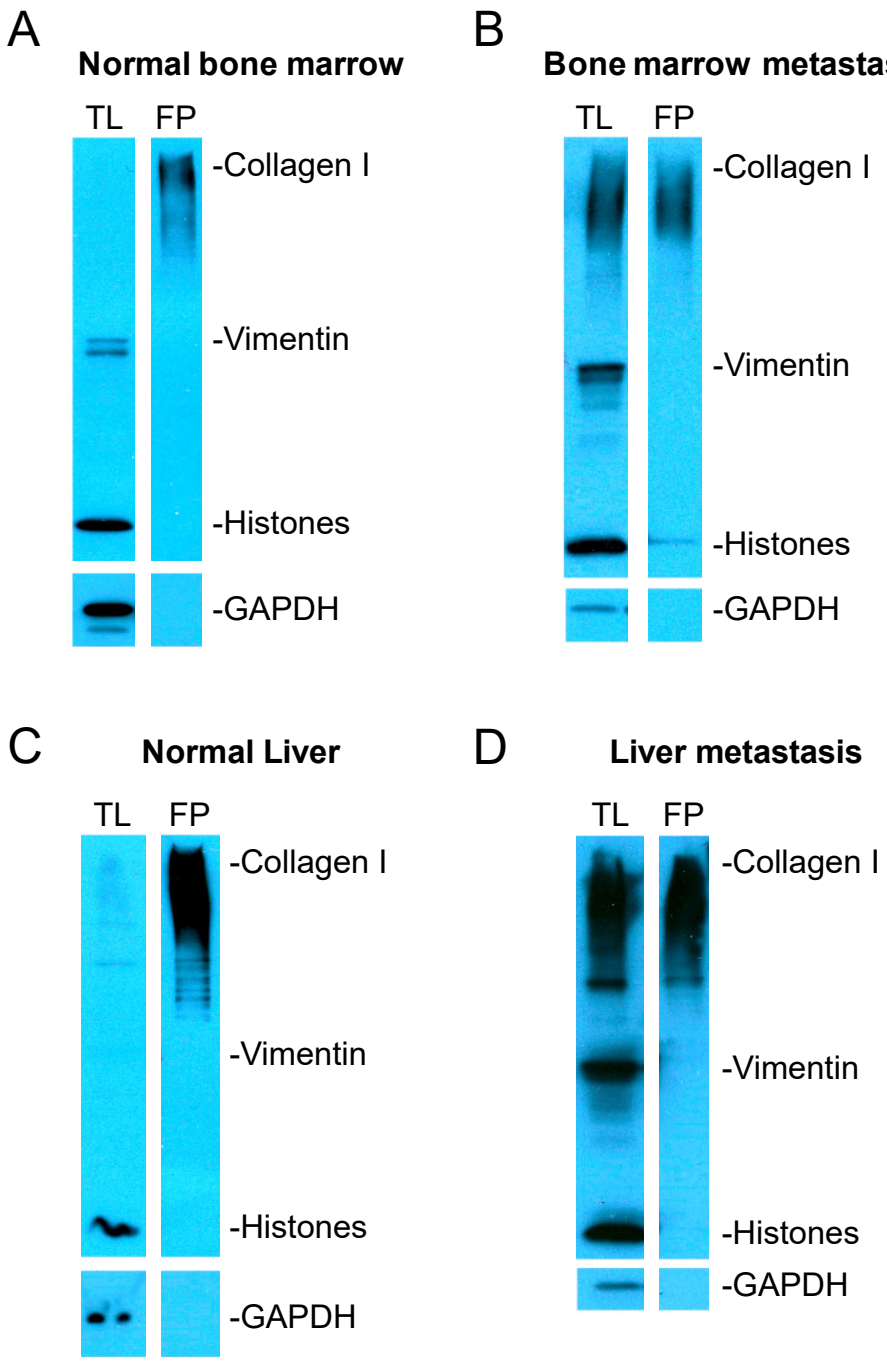
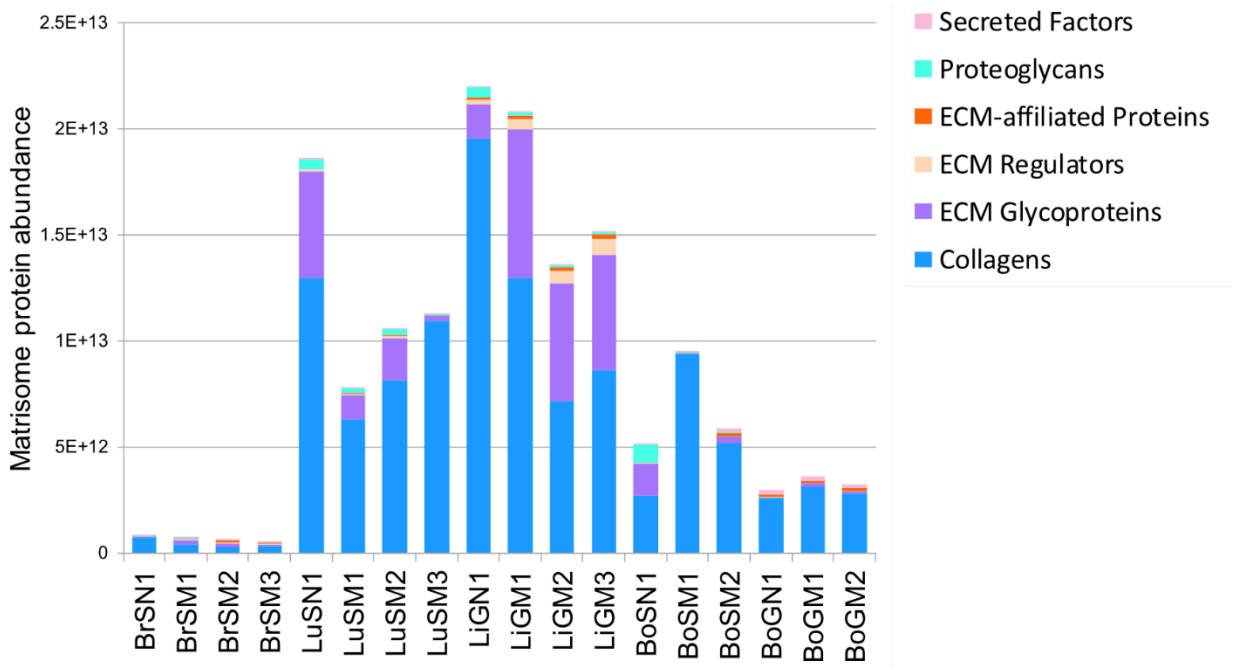
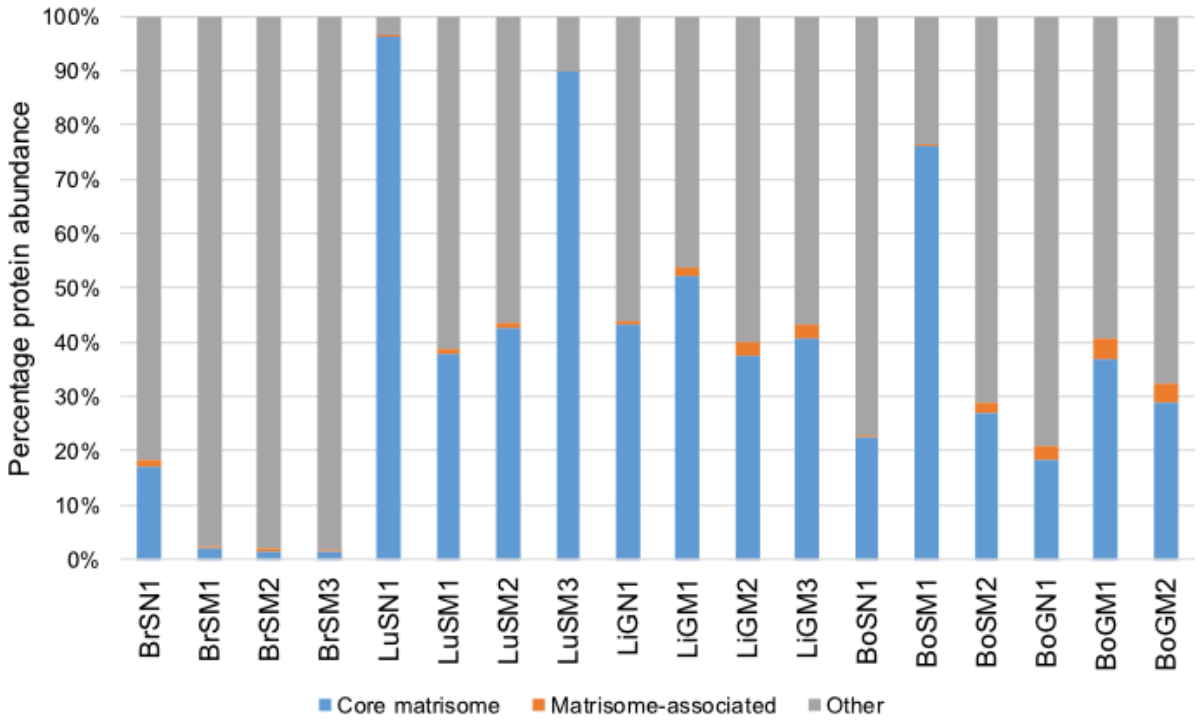


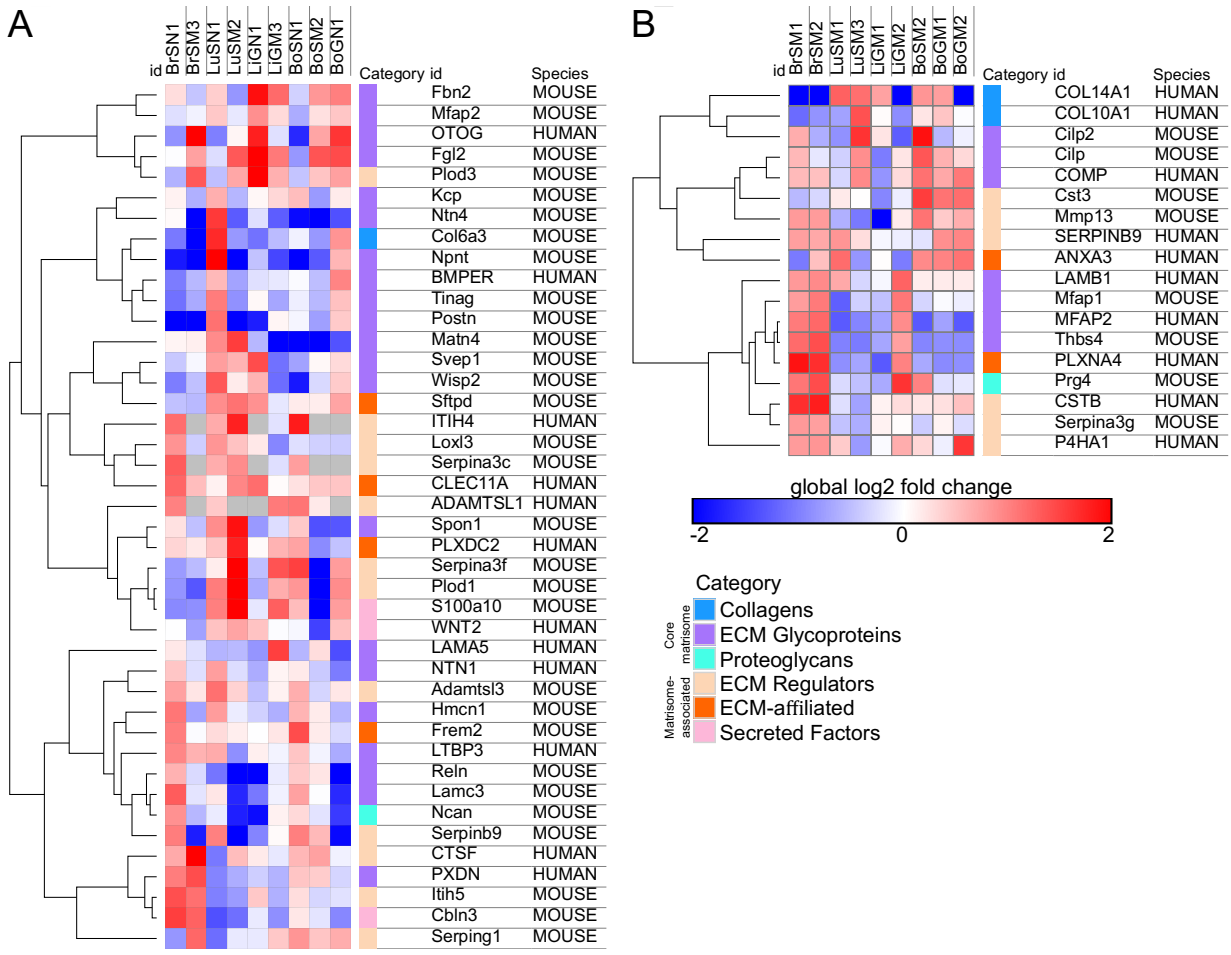
Supplementary Figure S1. Quality control western blots.
Example quality control western blots from ECM enrichment to monitor extraction of contaminant proteins, including the original total lysate (TL) and final pellet (FP). Shown are samples of normal mouse bone marrow (A), bone marrow metastasis (B), normal mouse liver (C) and liver metastasis (D).



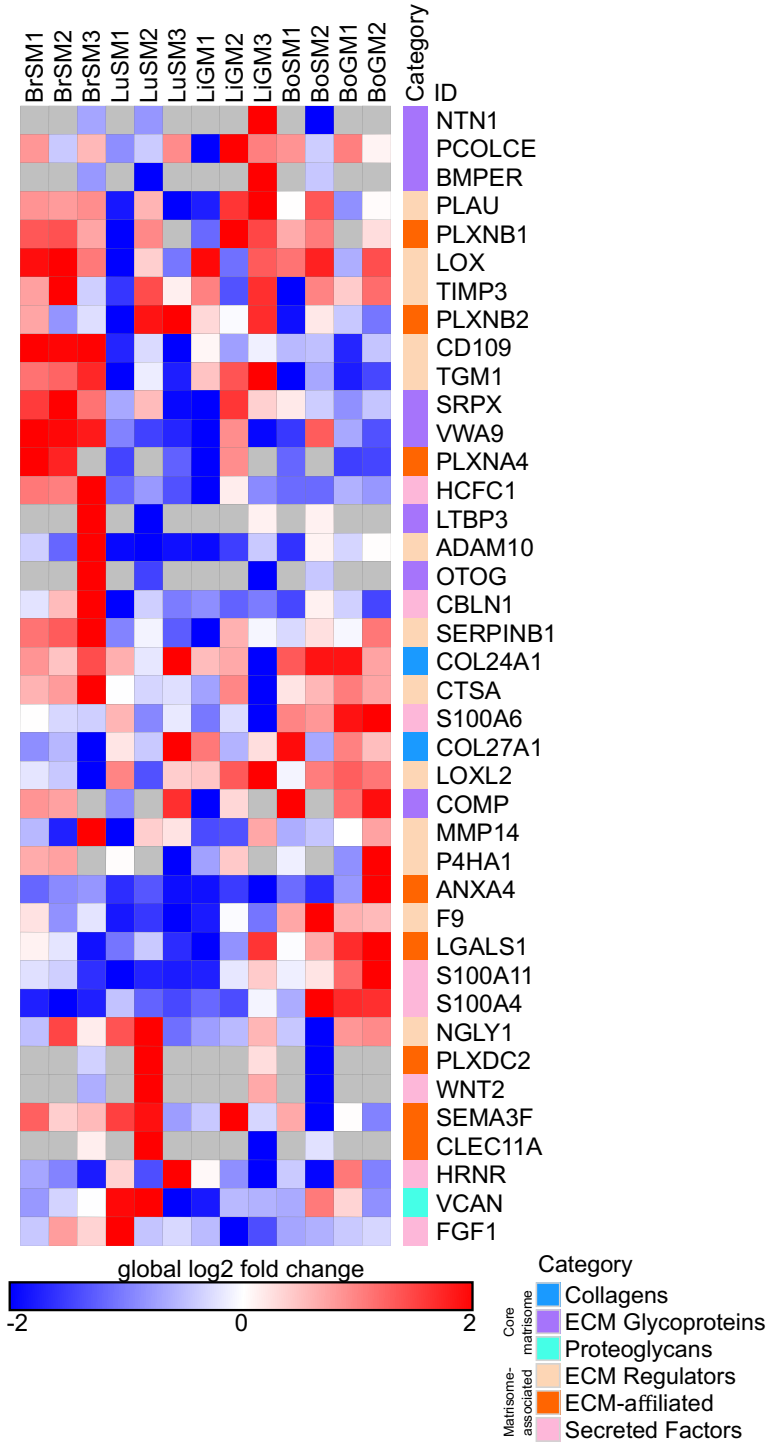
Supplementary Figure S2. Label-free matrisome protein abundance.
Quality control pre-fractionation mass spectrometry showing total intensity of matrisome proteins observed in each sample, broken down by matrisome category.



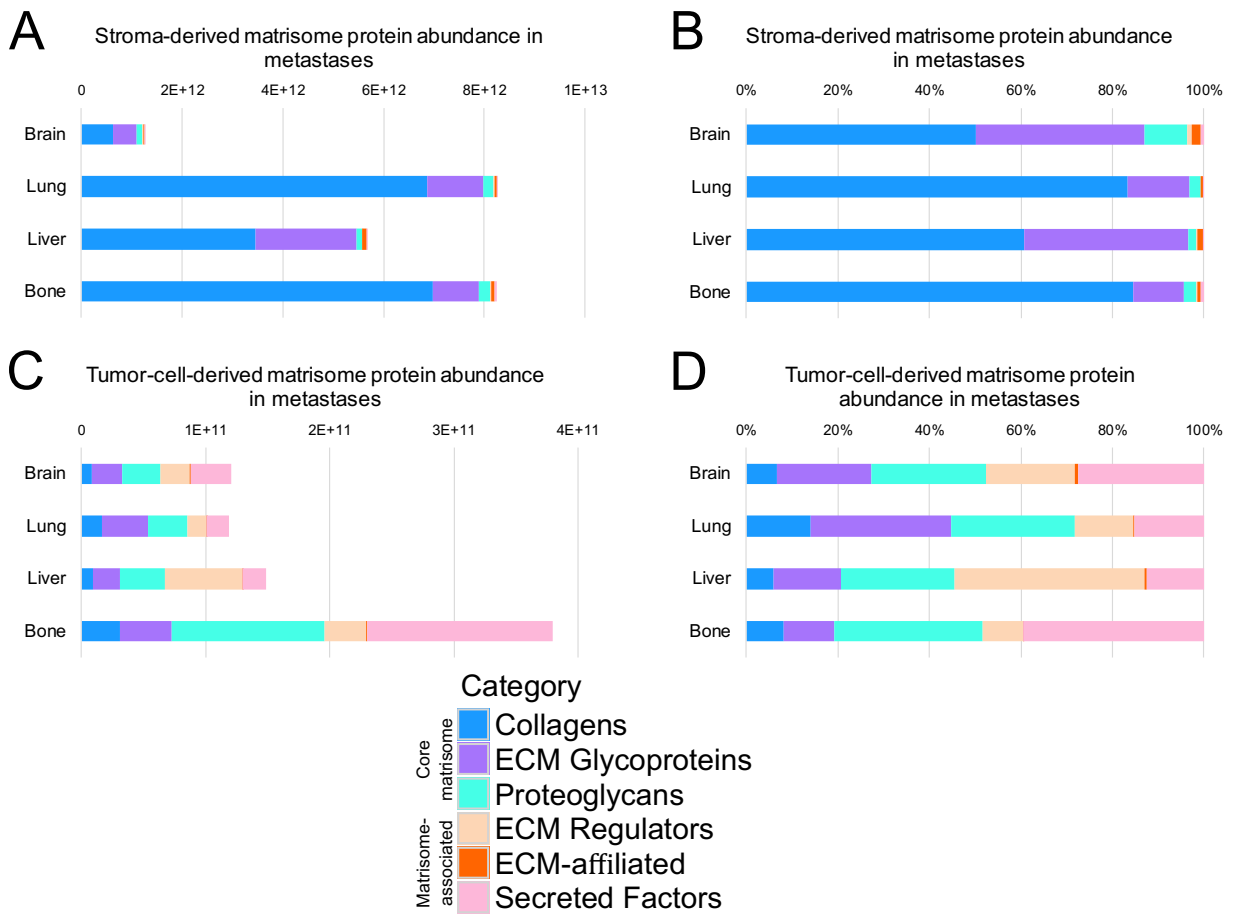
Supplementary Figure S3. Label-free percentage protein abundance.
Quality control pre-fractionation mass spectrometry showing total intensity of core matrisome, matrisome-associated and other proteins as a percentage of total protein abundance.



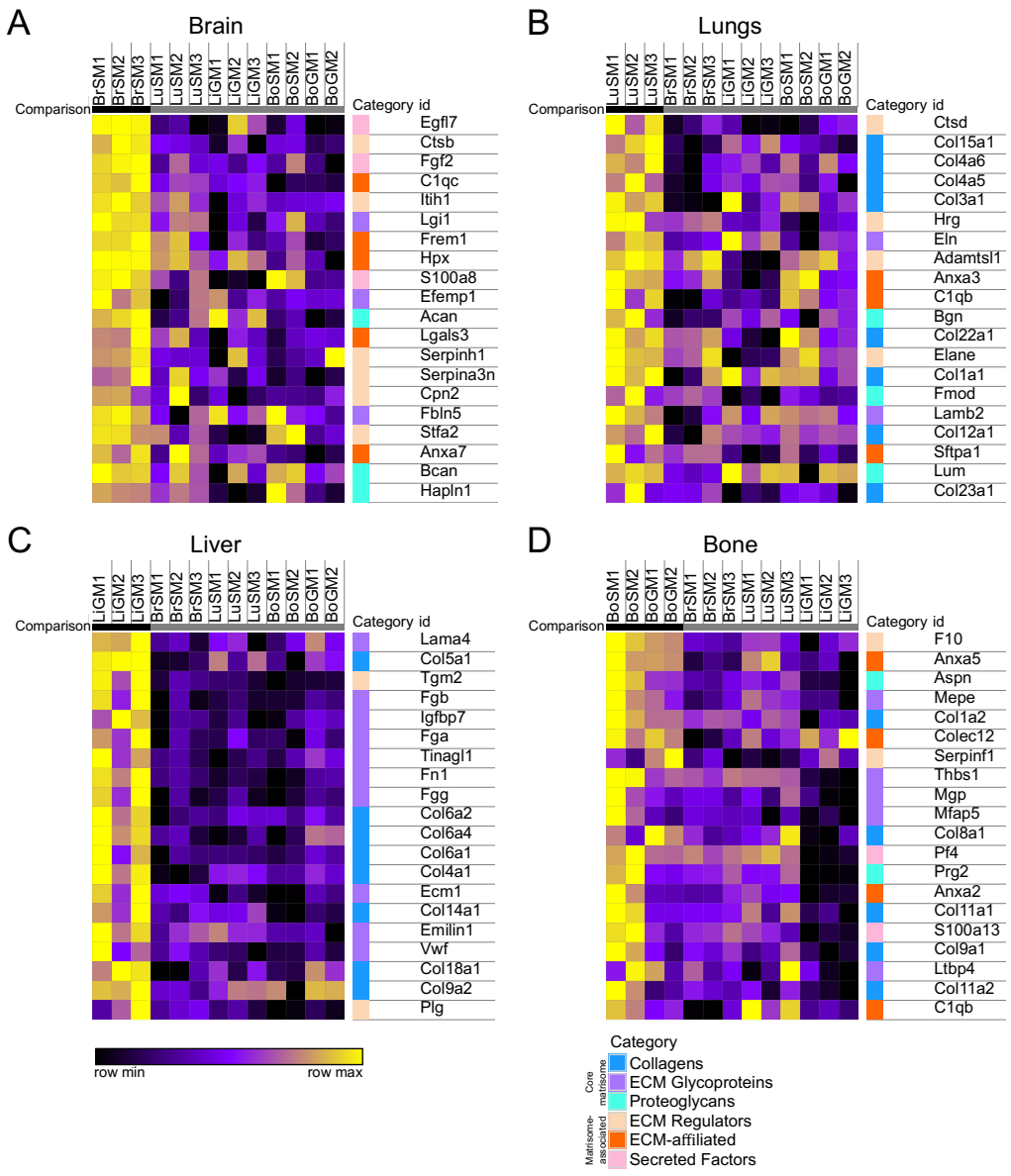
Supplementary Figure S4. Proteins identified in only one TMT 10-plex.
Heatmaps of global log2-fold change values for the 60 proteins quantified that were observed in only TMT Plex A (A) or TMT Plex B (B). Gray boxes indicate proteins not detected in a given sample.



Supplementary Figure S5. Tumor-cell-only proteins.
Heatmap of global log2-fold change values for the 40 proteins quantified that were produced only by tumor cells. Gray boxes indicate proteins not detected in a given sample.

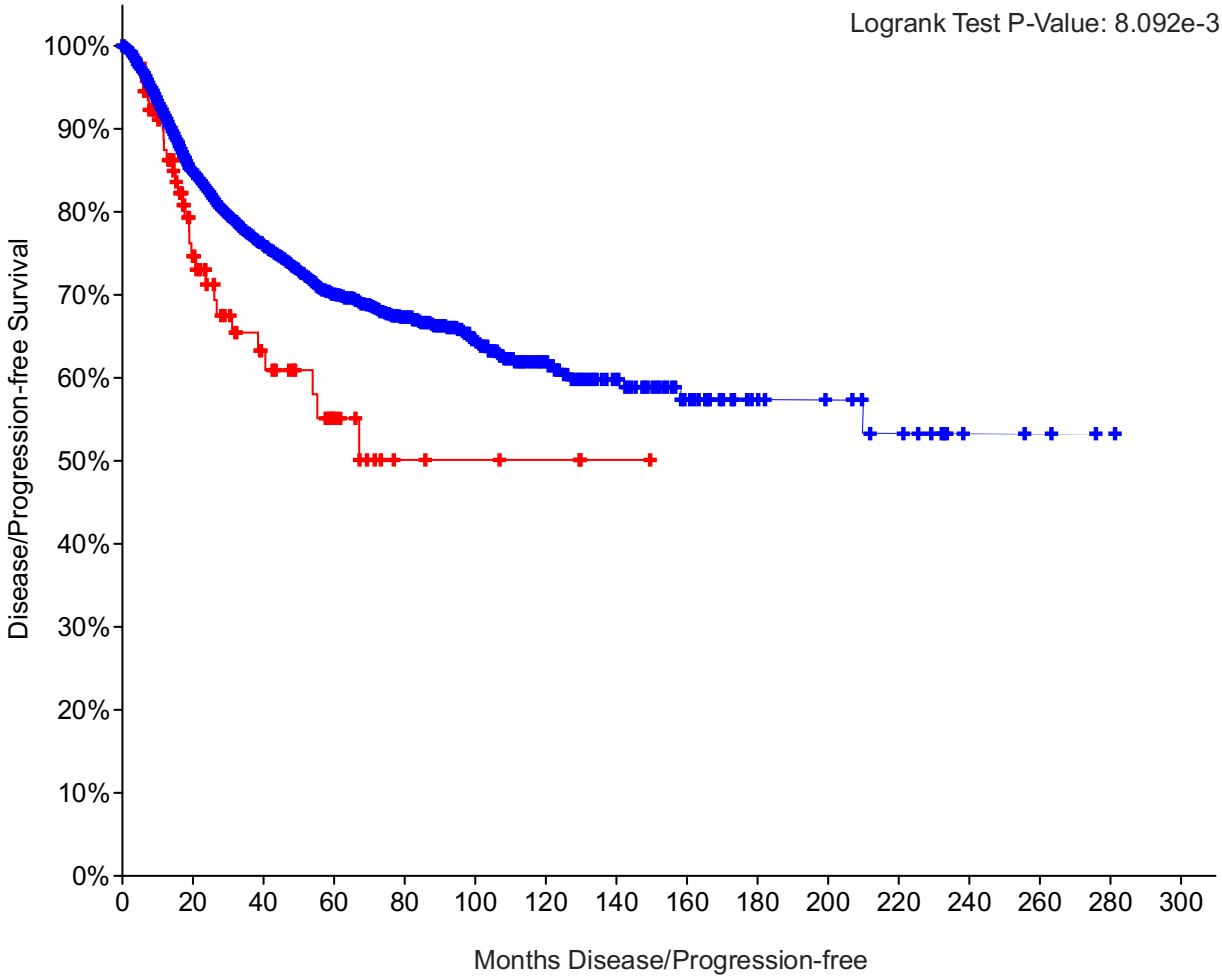


Supplementary Figure S6. Abundance of tumor-cell-derived and stroma-derived matrisome proteins in metastases to different tissues.
Abundance of different categories of matrisome proteins in metastases to different tissues, derived from fractional intensities of TMT channels from all metastatic samples. (A) Total abundance of stroma-derived matrisome proteins per metastatic sample from each tissue. (B) Relative abundance of stroma-derived matrisome protein categories in all metastases to each tissue. (C) Total abundance of tumor-cell-derived matrisome proteins per metastatic sample from each tissue. (D) Relative abundance of tumor-cell-derived matrisome protein categories in all metastases to each tissue.

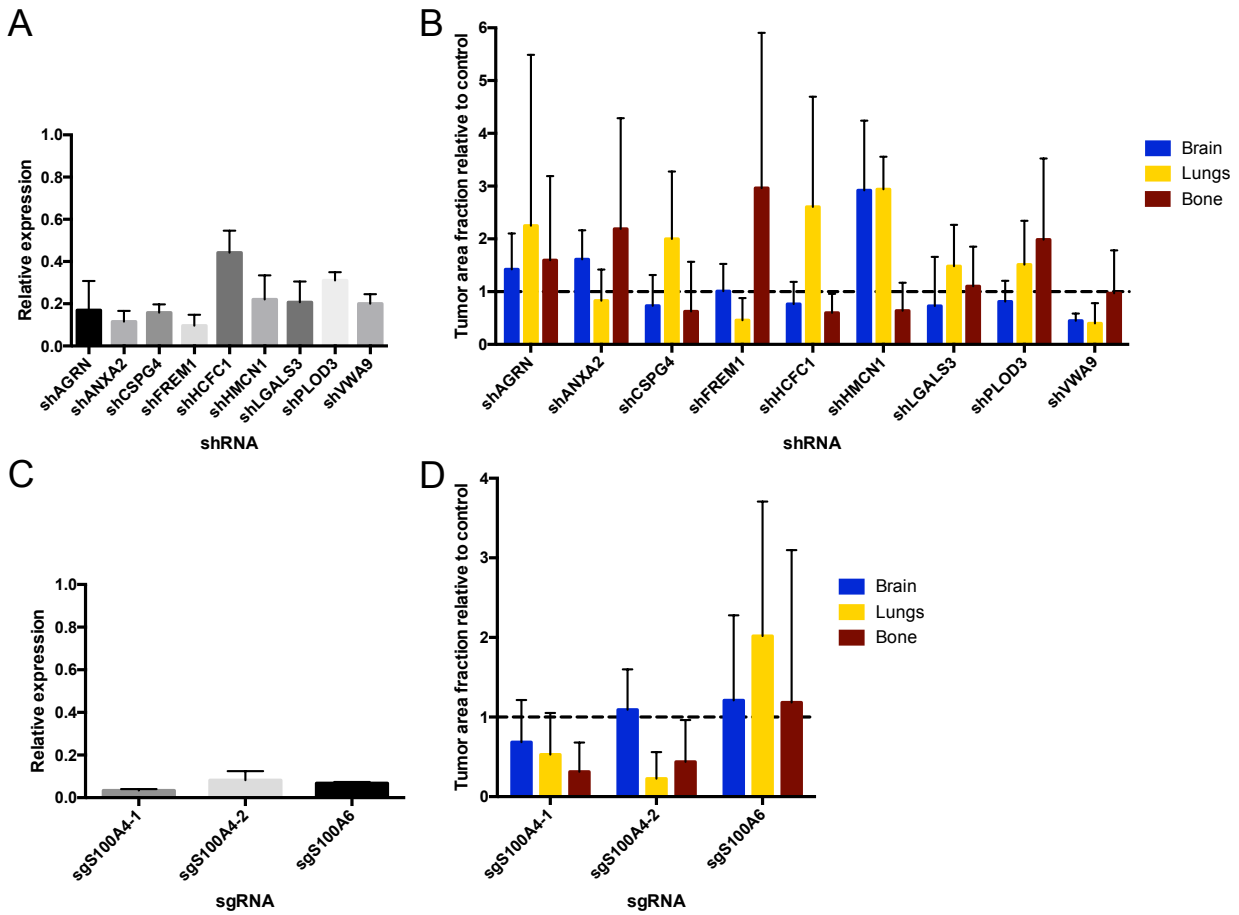


Supplementary Figure S7. Stromal proteins elevated in metastases relative to normal tissue.

Comparison of stroma-derived proteins among different metastatic sites. The value shown for each protein has been normalized by subtracting the value of the same protein from the corresponding normal tissue. All values shown thus represent changes in protein abundance in a given metastatic sample compared to normal tissue. A marker selection comparison has then been performed to distinguish stroma-derived proteins elevated in each particular metastatic tissue (comparisons identified above) relative to all other metastatic tissues, with the top 20 proteins shown in rank order for brain (A), lung (B), liver (C) and bone (D) metastases.

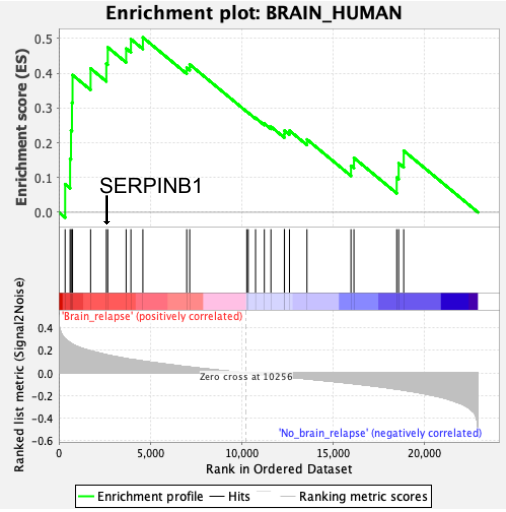


Supplementary Figure S8. Alterations in SERPINB1 are associated with lower progression-free survival.
Kaplan-Meier estimate of disease/progression-free survival with or without alterations in SERPINB1. Data are from The Cancer Genome Atlas (TCGA) PanCancer Atlas Studies (10,967 samples from 32 studies), via cBioPortal (www.cbioportal.org).

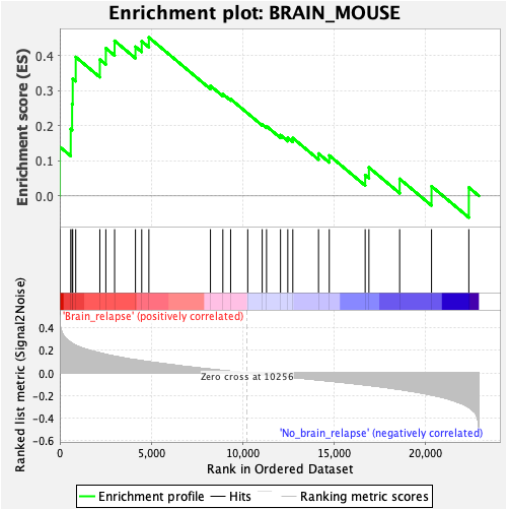


Supplementary Figure S9. Additional proteins evaluated for altered metastatic tropism. (A) qPCR of expression in MDA-MB-231 of proteins targeted for shRNA-mediated knockdown, relative to cells expressing a control shRNA (against Firefly luciferase). (B) Fraction of tissue surface area occupied by tumors in mice that received intracardiac injections of cells expressing the shRNAs shown. ANXA2 was identified as a broad marker of metastasis, while the rest were markers of brain metastasis (see Fig. 4A). All results are displayed compared to the average tumor burden in each tissue caused by cells expressing a control shRNA. n=3-7 mice per group. (C) qPCR of expression in MDA-MB-231 of proteins targeted for sgRNA-mediated knockdown, relative to cells expressing a control sgRNA (against mouse Timp1). (D) Fraction of tissue surface area occupied by tumors in mice that received intracardiac injections of cells expressing the sgRNAs shown. S100A4 was identified as marker of overall metastasis and S100A6 was identified as a marker of bone metastasis (see Fig. 4A). All results are displayed compared to the average tumor burden in each tissue caused by cells expressing a control sgRNA. n=6-8 mice per group.

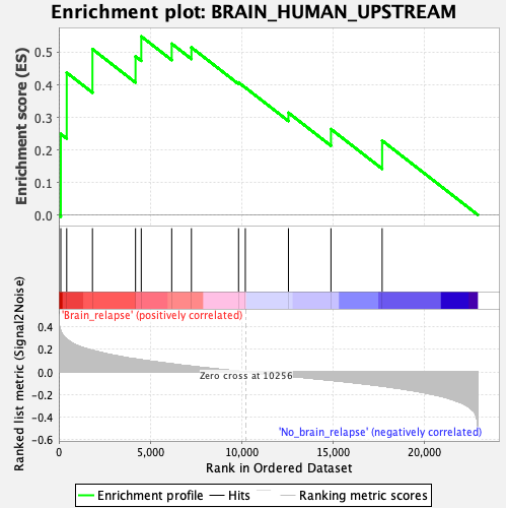
A



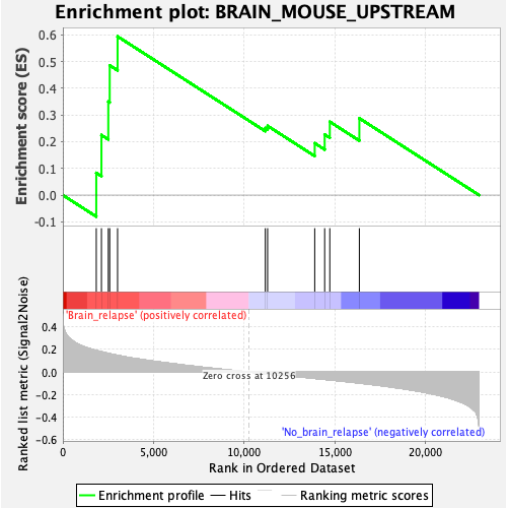
B



C



D



E

Name	Size	ES	NES	NOM p-val	FDR q-val	FWER p-val	Rank at max	Leading edge
BRAIN_MOUSE_UPSTREAM	11	0.594291	1.597841	0.029613	0.118376	0.059	3015	tags=45%, list=13%, signal=52%
BRAIN_MOUSE	27	0.451151	1.595303	0.022293	0.060043	0.06	4874	tags=41%, list=21%, signal=52%
BRAIN_HUMAN_UPSTREAM	12	0.548746	1.468620	0.073232	0.092450	0.112	4512	tags=42%, list=20%, signal=52%
BRAIN_HUMAN	27	0.503241	1.413609	0.135714	0.091132	0.143	4600	tags=41%, list=20%, signal=51%

Supplementary figure S10. Gene Set Enrichment Analysis of ECM proteins and predicted upstream regulators in brain metastases.

Comparison of gene sets based on data from this paper against ranked expression data from human patient primary tumors (GSE12276) with or without brain relapse ("Brain_relapse" vs "No_brain_relapse"). The following gene sets were tested: (A) tumor-cell-derived or (B) stroma-derived ECM proteins elevated in brain metastases ("BRAIN_HUMAN" and "BRAIN_MOUSE", respectively), or the top 12 predicted upstream regulators of those (C)

tumor-cell-derived or (D) stroma-derived ECM proteins (“BRAIN_HUMAN_UPSTREAM” and “BRAIN_MOUSE_UPSTREAM”, respectively). The ranked position of SERPINB1 in (A) is noted. (E) Detailed enrichment results, including enrichment score (ES), normalized enrichment score (NES), nominal p-value (NOM p-val), false discovery rate q-value (FDR q-val), and family-wise error rate p-value (FWER p-val). All gene sets were significantly enriched in primary tumors with brain relapse, as determined by FDR q-value<.25.