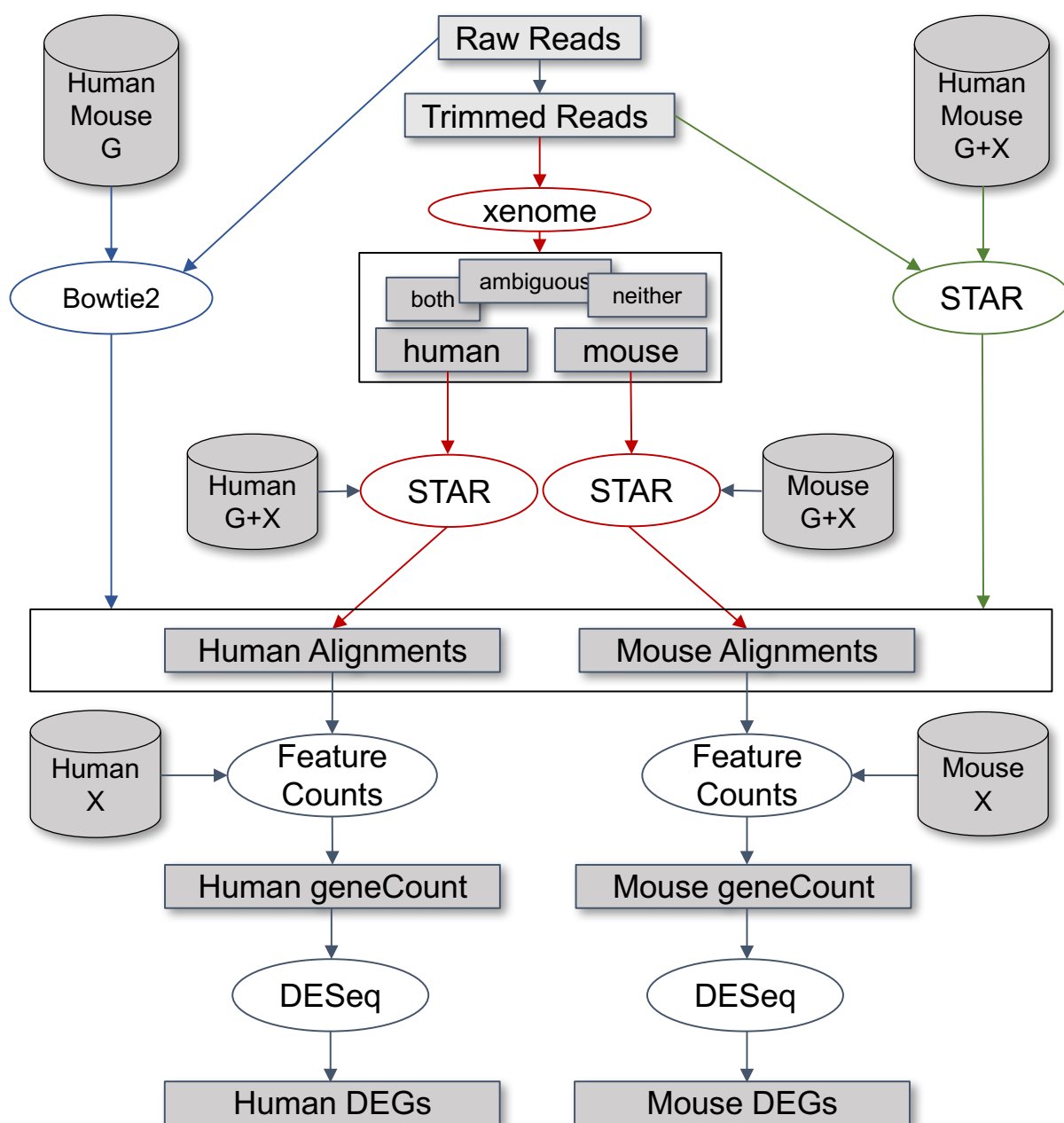


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Supplemental Information

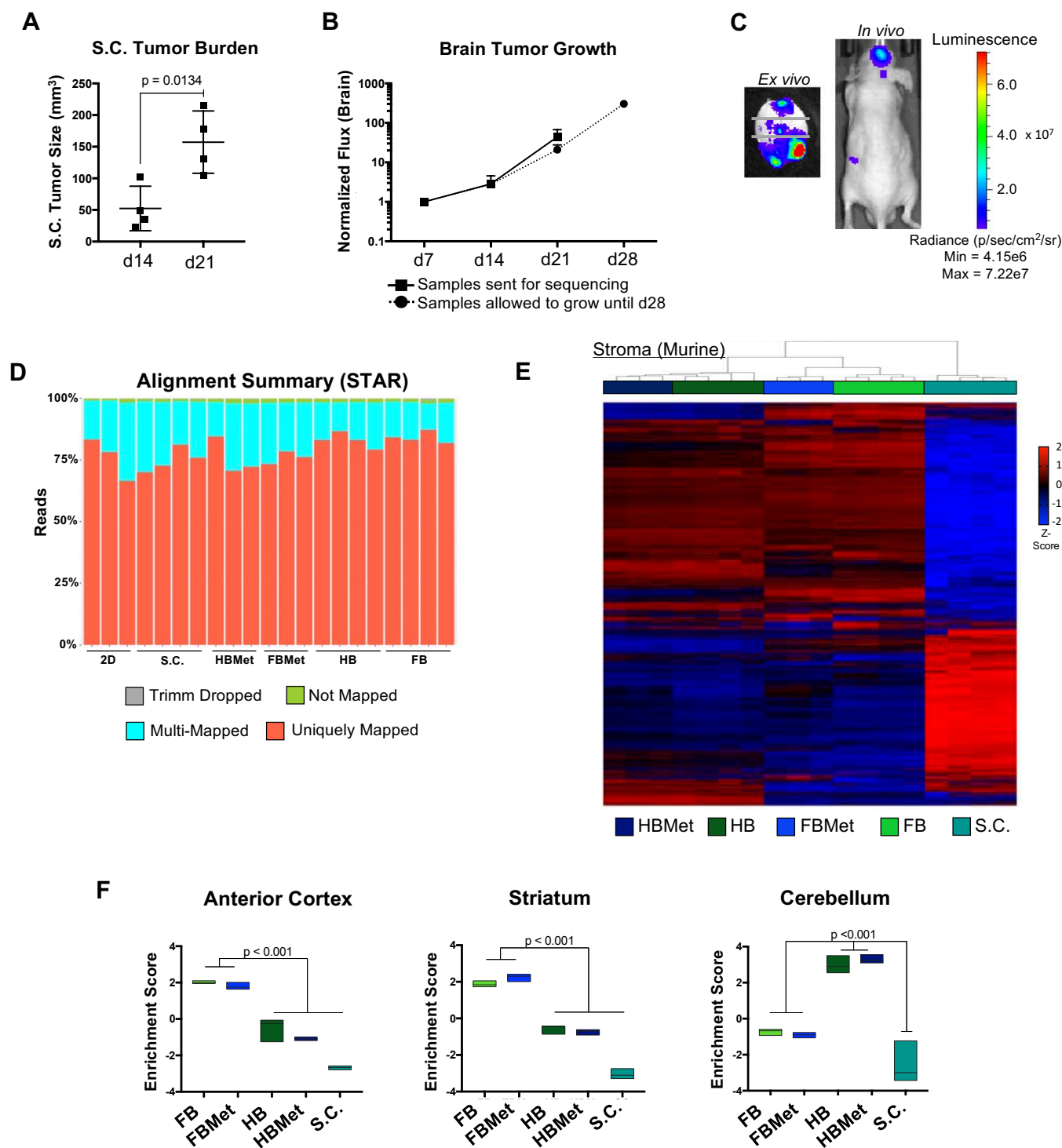
Transcriptomic Hallmarks of Tumor Plasticity and Stromal Interactions in Brain Metastasis

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Supplemental Figure 1: BMX-seq Pipeline Workflow. Related to Figure 1.

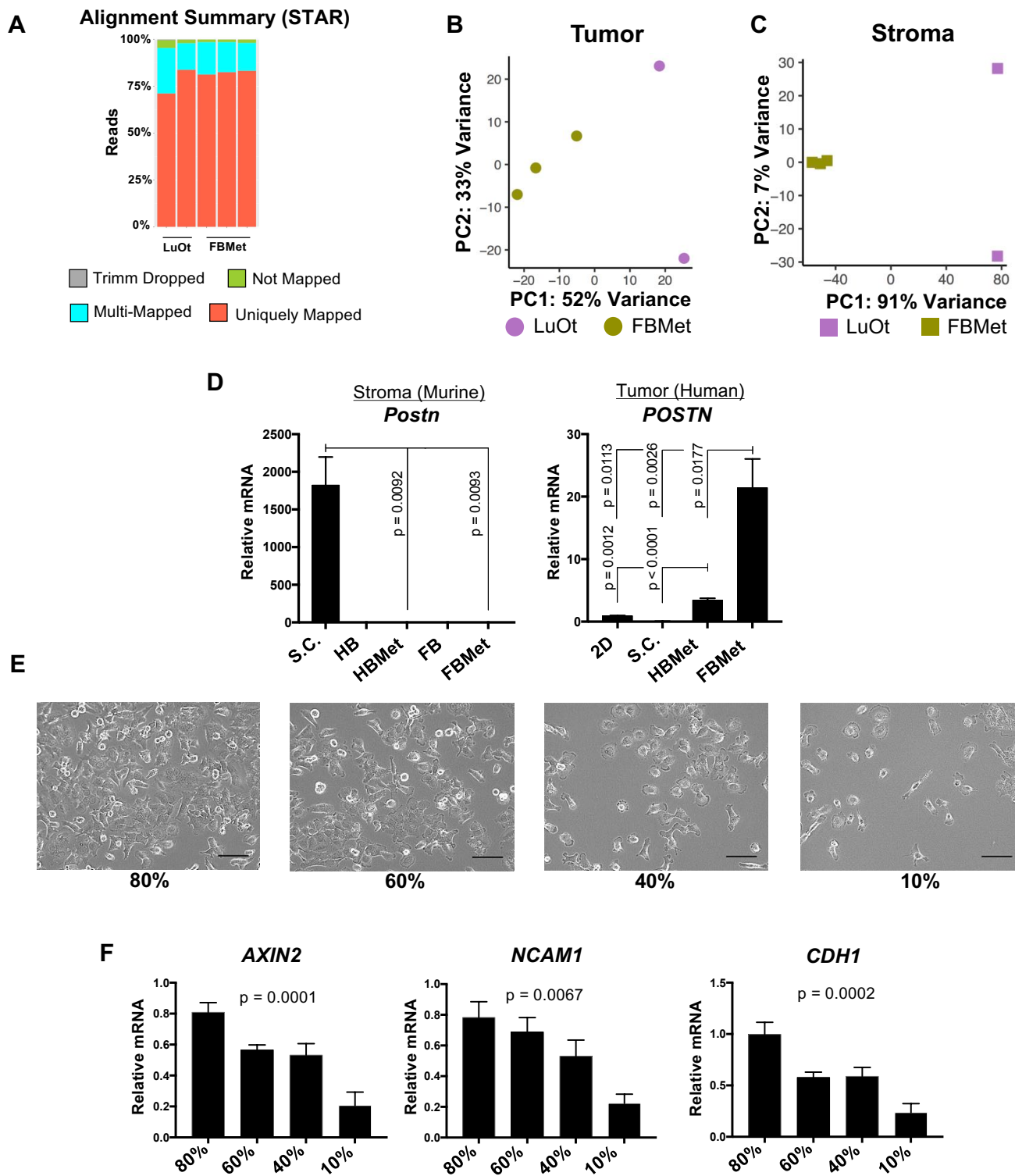
Diagram of RNA-seq workflow for BMX-seq (green), ConBowtie (blue), and Xenome (red) pipelines. The steps shared among pipelines are in gray. Rectangles indicate input or output files. Cylinders indicate the genome of interest (G) and/or transcriptome data (X). Ellipses indicate the software used in each case. A detailed description is provided in the *STAR Methods*.



Supplemental Figure 2: Growth of H2030-BrM3 cells *in vivo* and BMX-seq Analysis. Related to Figure 1 and Figure 2.

(A) Subcutaneous tumor burden (mm^3) of H2030-BrM3 cells. Samples were harvested 14-21 days post-injection for RNA sequencing. P value is computed by unpaired, Student's t-test. (B) Kinetics of brain tumor growth of H2030-BrM3 cells as measured by bioluminescence imaging (BLI). Samples were harvested at day 21 post intra-arterial injection for RNA sequencing. (C) BLI image of a representative animal at day 21 post intra-arterial injection of H2030-BrM3 cells and an *ex vivo* image of the brain showing forebrain and hindbrain tumor lesions (left). Grey lines indicate incision sites for macrodissection of hindbrain and forebrain samples.

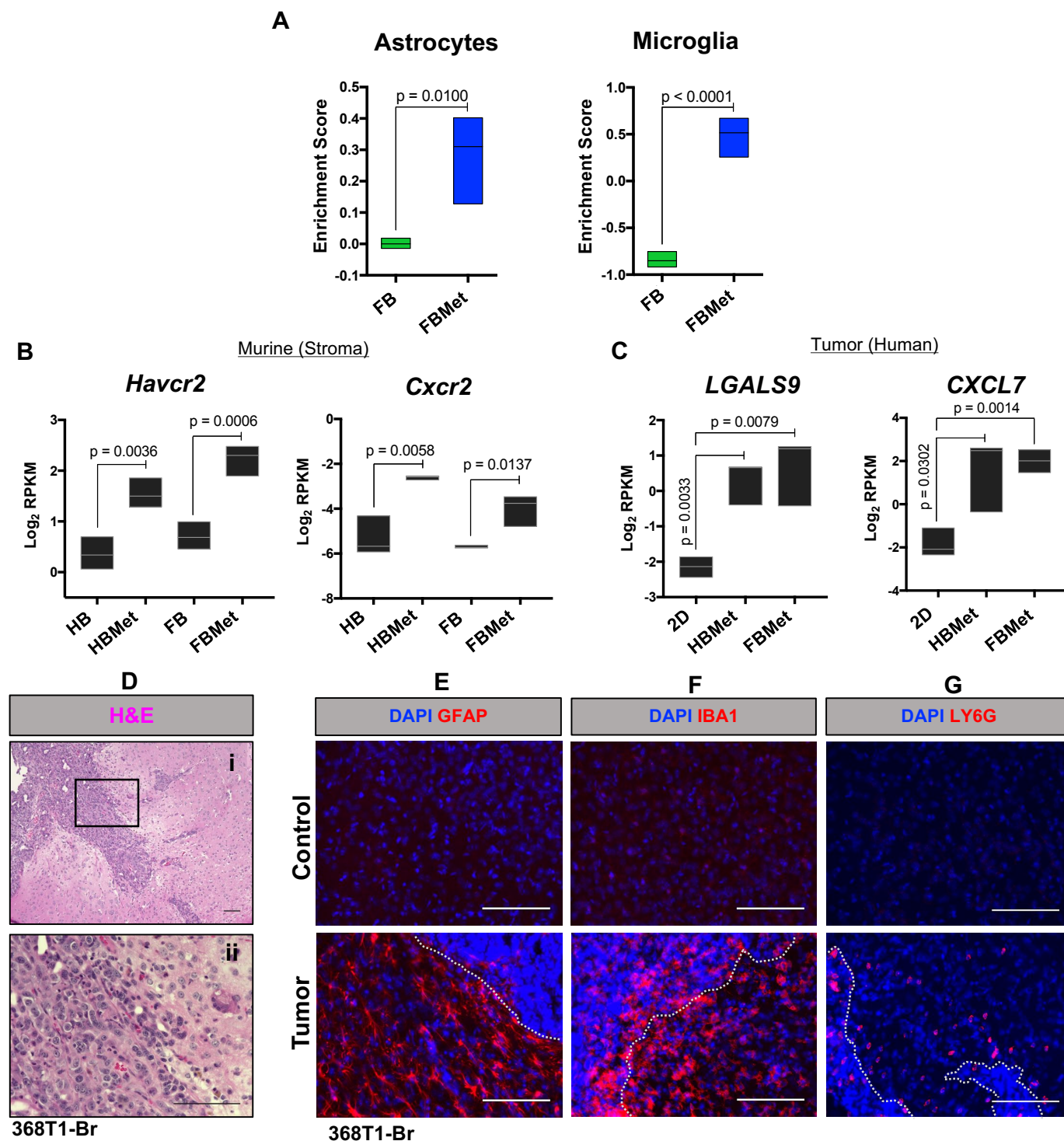
(D) Alignment summary of reads from forebrain (FB), forebrain metastasis (FBMet), hindbrain (HB), hindbrain metastasis (HBMet), S.C., and 2D samples post intra-arterial injection. Read summary includes reads from both species where applicable. Raw reads were trimmed off adapter sequences and reads shorter than 20 base pairs were excluded (Trimm Dropped). Reads were aligned to a concatenated human-mouse genome and transcriptome with STAR. Reads that did not map to the genome (Not Mapped), reads that mapped to more than one location in the concatenated genome (Multi-Mapped), and reads that mapped to a unique position (Uniquely Mapped) are plotted as a percentage of total reads. 1 sample is indicated by one bar graph. (E) Heatmap depicts hierarchical clustering of the 13, 415 genes significantly deregulated and with an average RPKM value >1.0 across stromal samples. Significance was defined by an adjusted p value <0.05. (F) Expression of signatures for the murine anterior cortex, striatum, or cerebellum were compared across forebrain (n=4), forebrain metastasis (n=3), hindbrain (n=4), hindbrain metastasis (n=4) and S.C. (n=4) samples. Gene signatures from Strand et al., 2007 were used. P values are computed by unpaired, Student's t-test.



Supplemental Figure 3: Transcriptome of H2030-BrM3 tumors grown orthotopically in the lungs (LuOt) compared to brain metastasis and validation experiments. Related to Figure 2 and Figure 3.

(A) Alignment summary of reads from forebrain metastasis (FBMet) and tumors grown orthotopically in the lungs (LuOt) as in Figure S2D. (B) Principle component analysis (PCA) comparing the gene expression profiles of H2030-BrM3 cells post intra-arterial and post intra-tracheal injection. Forebrain metastasis (n=3); lung orthotopic tumor (n=2). (C) PCA of mouse gene expression profiles across the same samples in (B). (D) mRNA expression of stromal *Postn* (left) and tumor *POSTN* (right) as measured by species specific Taqman primers. Stromal *Postn* is normalized to stromal *Hprt* and tumor *POSTN* is normalized to *HPRT1*. Hindbrain metastasis (HBMet), forebrain metastasis, 2D (n=3); hindbrain (HB), forebrain (FB), S.C. (n=4). Data are presented as mean \pm SEM. P values are computed by unpaired, Student's t-test. (E) Representative images of H2030-BrM3 cells grown in culture at 80%, 60%, 40%, and 10% confluence. Scale bars are 100 μ m.

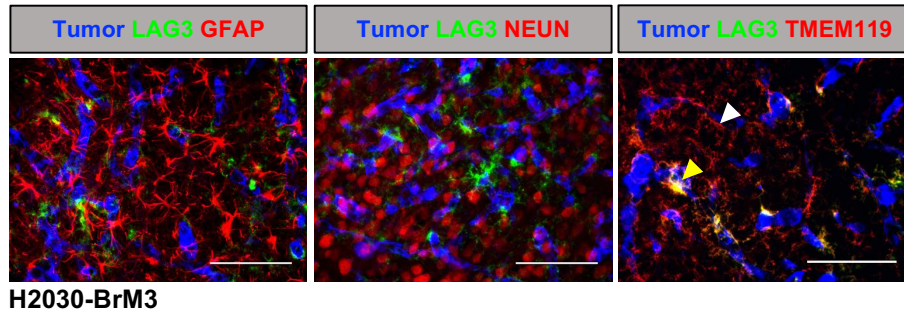
(F) mRNA expression of *AXIN2*, *NCAM1*, and *CDH1*. H2030-BrM3 cells were grown in monolayer as in (E) and RNA was extracted under each condition. Data represents the average of five biological replicates. P values are computed by one-way ANOVA. Data are presented as mean \pm SEM.



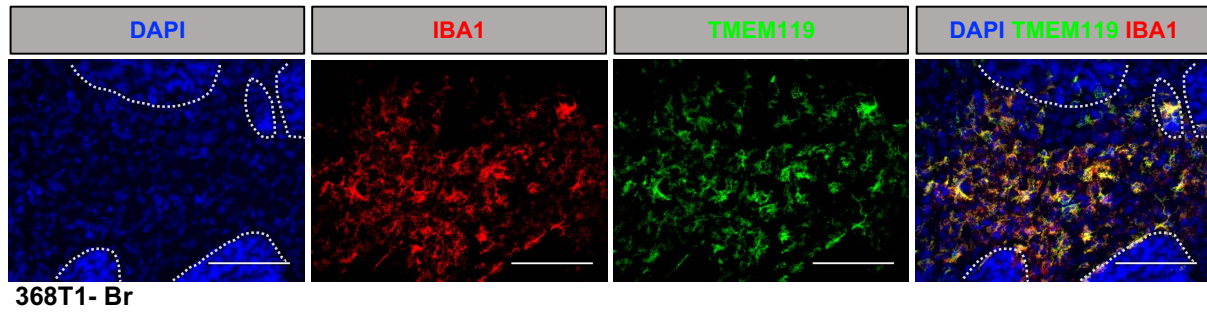
Supplemental Figure 4: Innate immune response to brain metastases in immune-compromised and immune-competent models. Related to Figure 4.

(A) Enrichment scores as calculated by the mean expression of genes known to be enriched in astrocytes (left) or microglia (right) in the stroma of forebrain metastasis (FBMet) (n=3) as compared to forebrain (FB) tissue (n=4). (B) Expression of stromal *Havcr2* and *Cxcr2* across the indicated samples. Forebrain metastasis, hindbrain metastasis (HBMet) (n= 3); hindbrain (HB), and forebrain (n=4). (C) Expression of tumor *LGALS9* and *CXCL7* (a.k.a *PPBP*) in H2030-BrM3 cells grown as forebrain metastasis or hindbrain metastasis tumors (n=3) or in monolayer (n=3). (D) H&E stains of forebrain metastasis formed 21 days after intra-cranial injection of 368T1-Br cells into B6129SF1/J recipient mice. i) 10X images. ii) 40X magnification of box denoted in i). (E-G) Representative images of immunofluorescent (IF) staining of astrocytes (GFAP;red), TAMs (IBA1;red), neutrophils (LY6G;red), and nuclei (DAPI;blue). 368T1-Br tumor cells are outlined with a discontinuous white line (bottom panels) while top panels denote control regions. P values are computed by unpaired, Student's t-test. Scale bars indicate 100 μ m.

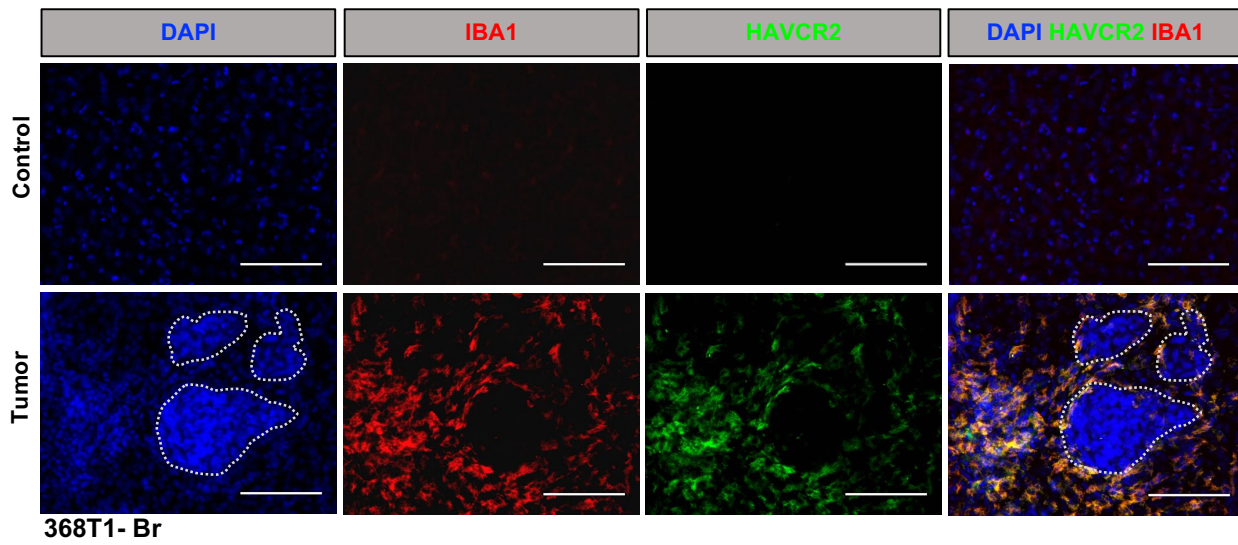
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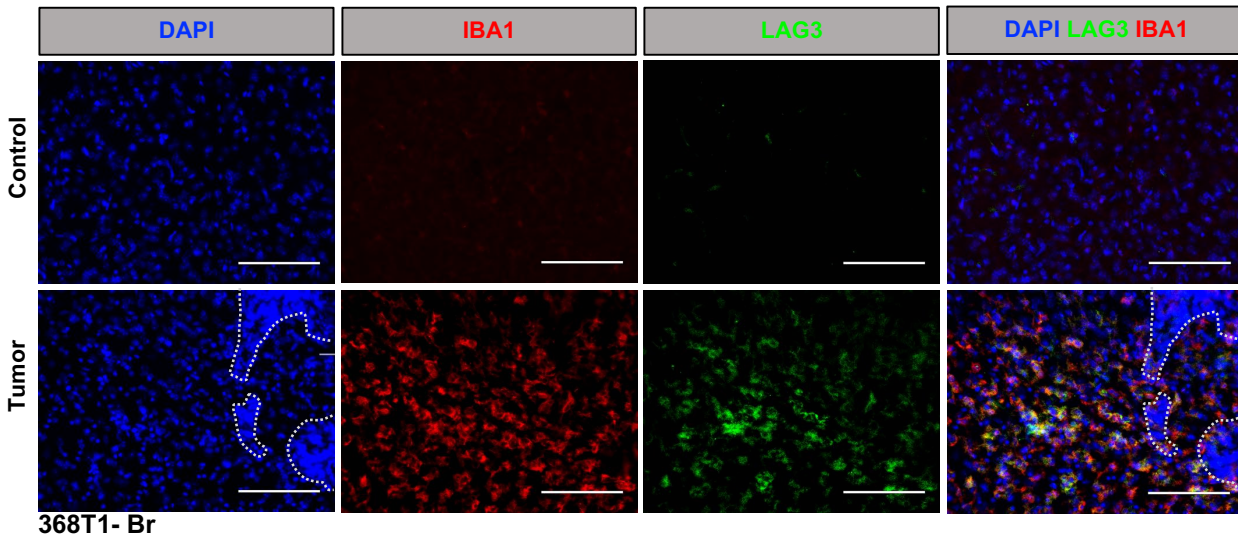
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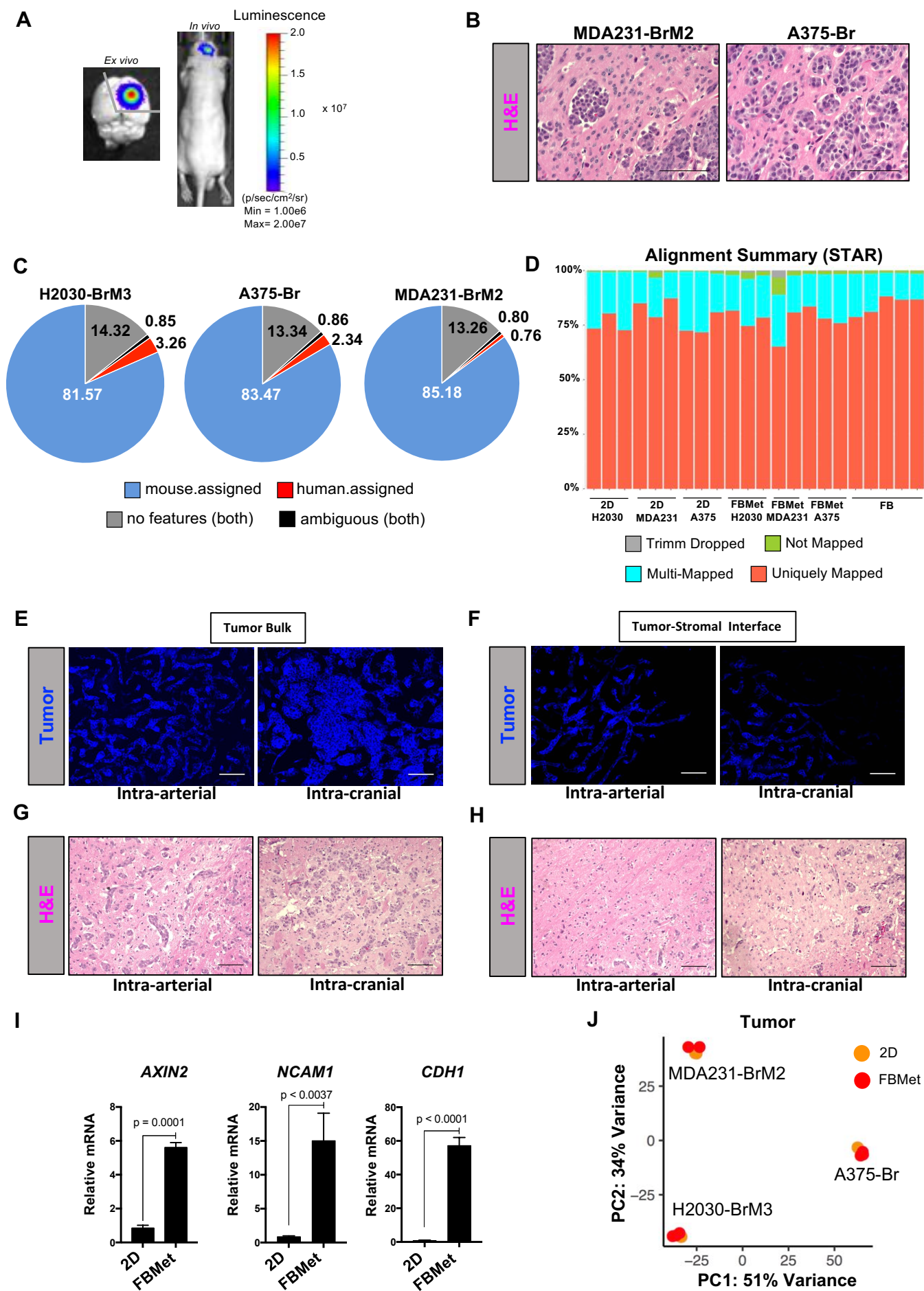


D



Supplemental Figure 5: Characterizing TAMs in the brain metastatic niche. Related to Figure 5.

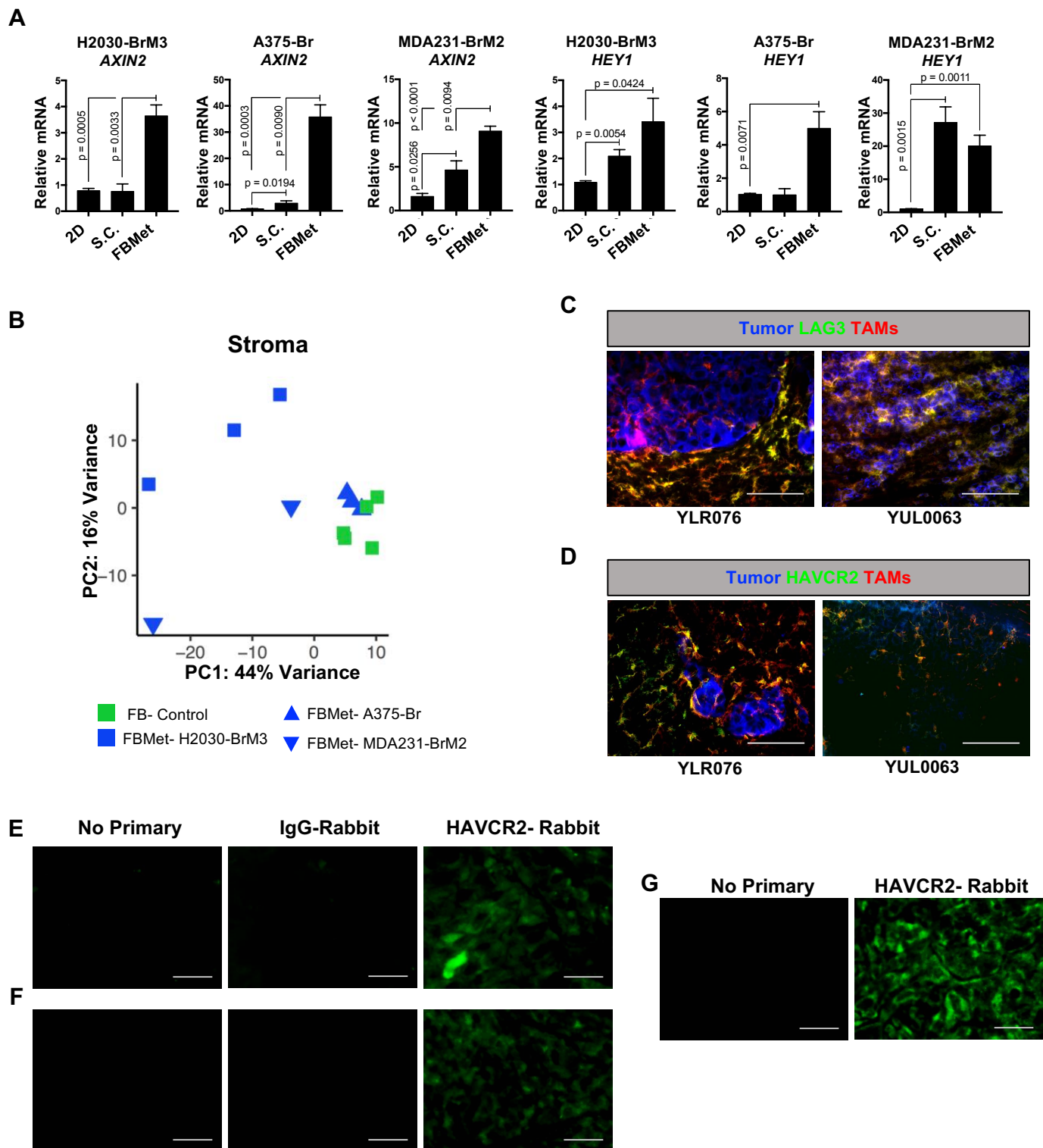
(A) Representative IF staining for LAG3 (green), co-stained with markers for astrocytes (GFAP;red), neurons (NEUN;red), microglia (TMEM119;red), and GFP positive tumor cells (blue). Yellow arrow indicates TMEM119-positive, LAG3-positive cell. White arrow indicates TMEM119-positive, LAG3-negative cell. (B) Representative IF staining of TAMs (IBA1;red), TMEM119 (green), and nuclei (DAPI;blue) in metastatic brain post intra-cranial injection of 368T1-Br cells into B6129SF1/J mice. (C) Representative IF staining of TAMs (IBA1;red), HAVCR2 (green), and tumor (DAPI;blue) in control regions (top panel) or metastatic brain (bottom panel) post intra-cranial injection of 368T1-Br cells in B6129SF1/J mice. (D) Representative IF staining of TAMs (IBA1;red), LAG3 (green), and tumor (DAPI;blue) in control regions (top panel) or metastatic brain (bottom panel) post intra-cranial injection of 368T1-Br cells in B6129SF1/J mice. 368T1-Br tumor cells are outlined with a discontinuous white line. All scale bars are 100 μ m.



Supplemental Figure 6

Supplemental Figure 6: Conserved transcriptomic responses across disease models of brain metastasis. Related to Figure 6.

(A) BLI image of representative animal at day 21 post intra-cranial injection of H2030-BrM3 cells and an *ex vivo* image of the brain showing forebrain metastasis (FBMet) only (left). Grey lines indicate incision site for macrodissection of forebrain metastasis sample. (B) H&E stains of forebrain metastasis formed 14-21 days post intra-cranial injection of MDA231-BrM2 (left) and A375-BrM cells (right). (C) The categories of reads mapped in the indicated models were plotted as in Figure 1D. (D) Alignment summary of reads from forebrain metastasis, forebrain (FB), and 2D samples across disease models as in Figure S2D. (E) Representative IF images of GFP positive H2030 BrM3 cells 21 post intra-arterial (left) or intra-cranial (right) injections. Images are of tumor bulk regions. (F) Representative IF images of GFP positive H2030-BrM3 cells 21 post intra-arterial (left) or intra-cranial (right) injections. Images of tumor-stromal interface are shown. (G) H&E stains of forebrain metastasis formed 21 days post intra-arterial (left) or intra-cranial (right) injection of H2030-BrM3 cells. Images in regions of tumor bulk are shown. (H) H&E stains of forebrain metastasis formed 21 days post intra-arterial (left) or intra-cranial (right) injection of H2030-BrM3 cells. Images in regions at tumor-stromal interface are shown. (I) mRNA expression of *AXIN2*, *NCAM1*, and *CDH1* as measured by species specific Taqman primers across the indicated samples. H2030-BrM3 cells were grown in monolayer (2D; n=3) or collected post intra-cranial injection (forebrain metastasis; n=3). P values are computed by unpaired, Student's t-test. Data are presented as mean +/- SEM. (J) Principle component analysis (PCA) comparing the tumor gene expression profiles of forebrain metastasis from H2030-BrM3 (n=3), MDA231-BrM2 (n=2), and A375-BrM (n=3) models. 2D = tumor cells grown in monolayer (n=3 replicates for each cell line).



Supplemental Figure 7: Validation of conserved tumor and stromal responses across disease models and controls for human tissue. Related to Figure 6 and Figure 7.

(A) mRNA expression of *AXIN2* and *HEY1* as measured by species specific Taqman primers across forebrain metastasis (FBMet) (n=4), 2D (n=3) and S.C. (n=2-4) samples. Housekeeping gene was *HPRT1*. Samples in this experiment were collected independently from those in Figure S6J. Forebrain metastasis samples were collected post intra-cranial injection. P values are computed by unpaired, Student's t-test. Data are presented as mean \pm SEM. (B) PCA of stromal gene expression profiles across the same forebrain metastasis samples as in Figure S6J as well as their corresponding control forebrain (FB) samples (n=5). (C) Representative IF staining of LAG3 (green), TAMs (IBA1; red), and tumor cells (YLR076=GFP, YUL0063=human specific Pan-Cytokeratin; blue) in PDX tumor bearing brains. Scale bars are 100 μ m. (D) Representative IF staining of HAVCR2 (green), TAMs (IBA1; red), and tumor cells (YLR076=GFP, YUL0063=human specific Pan-Cytokeratin; blue) in PDX tumor bearing brains. Scale bars are 100 μ m.

(E) Representative IF staining of a melanoma brain metastasis biopsy (BMTP6), using a HAVCR2 (green) antibody and the corresponding controls (no primary antibody and IgG treatment). Scale bars are 25 μ m. (F) Representative IF staining of a NSCLC brain metastasis biopsy (VC004), using a HAVCR2 (green) antibody and the corresponding controls (no primary antibody and IgG treatment). Scale bars are 25 μ m. (G) Representative IF staining of a squamous cell carcinoma biopsy (YLR076), using a HAVCR2 (green) antibody and the corresponding control (no primary antibody). Scale bars are 25 μ m.

Supplemental Table S1: Performance of BMX-seq versus pipelines ConBowtie and Xenome. Related to Figure 1.

method	sample	recovered	xmapped	missed	recoverRate	xmappingRate
Xenome	mixed.1	1916378	17204	66418	95.82%	0.86%
Xenome	mixed.2	1916477	17092	66431	95.82%	0.85%
Xenome	mixed.3	1916500	17186	66314	95.83%	0.86%
Xenome	mixed.4	1916129	17074	66797	95.81%	0.85%
Xenome	mixed.5	1916382	17158	66460	95.82%	0.86%
Xenome	mixed.6	1916778	17176	66046	95.84%	0.86%
Xenome	mixed.7	1916536	17154	66310	95.83%	0.86%
Xenome	mixed.8	1916443	17211	66346	95.82%	0.86%
Xenome	mixed.9	1916366	17209	66425	95.82%	0.86%
Xenome	mixed.10	1916269	17150	66581	95.81%	0.86%
ConBowtie	mixed.1	1929142	6474	64384	96.46%	0.32%
ConBowtie	mixed.2	1929236	6469	64295	96.46%	0.32%
ConBowtie	mixed.3	1929119	6466	64415	96.46%	0.32%
ConBowtie	mixed.4	1929168	6492	64340	96.46%	0.32%
ConBowtie	mixed.5	1929146	6505	64349	96.46%	0.33%
ConBowtie	mixed.6	1929137	6457	64406	96.46%	0.32%
ConBowtie	mixed.7	1929103	6459	64438	96.46%	0.32%
ConBowtie	mixed.8	1929085	6484	64431	96.45%	0.32%
ConBowtie	mixed.9	1929144	6481	64375	96.46%	0.32%
ConBowtie	mixed.10	1929192	6471	64337	96.46%	0.32%
BMX-seq	mixed.1	1919376	936	79688	95.97%	0.05%
BMX-seq	mixed.2	1919453	964	79583	95.97%	0.05%
BMX-seq	mixed.3	1919403	909	79688	95.97%	0.05%
BMX-seq	mixed.4	1919391	891	79718	95.97%	0.04%
BMX-seq	mixed.5	1919192	890	79918	95.96%	0.04%
BMX-seq	mixed.6	1919806	905	79289	95.99%	0.05%
BMX-seq	mixed.7	1919418	905	79677	95.97%	0.05%
BMX-seq	mixed.8	1919304	913	79783	95.97%	0.05%
BMX-seq	mixed.9	1919189	948	79863	95.96%	0.05%
BMX-seq	mixed.10	1919500	902	79598	95.98%	0.05%

Supplemental Table S2: Gene set enrichment analysis of commonly upregulated pathways when LUAD cells are grown as FBMet vs. S.C. tumors. Related to Figure 2.

GO Gene Set			
	NES	FDR q-value	Rank
GO_Chromatin_Modification	6.27	<0.001	19
GO_Regulation_of_Neuron_Differentiation	3.52	<0.001	178
GO_Neuron_Projection_Guidance	2.6	0.106	472
GO_Cell_Morphogenesis_Involved_in_Differentiation	4.33	<0.001	82

Supplemental Table S3: Lists of pathways upregulated in the brain TME as compared to the subcutaneous TME. Related to Figure 3.

<u>Pathway</u>	<u>p-value</u>
Hippo signaling pathway	8.02E-11
WNT_Signaling	8.05E-07
Wnt signaling pathway	1.04E-06
Pathways in cancer	2.88E-06
Basal cell carcinoma	1.65E-05
Melanogenesis	5.40E-05
Breast cancer	8.00E-05
Signaling pathways regulating pluripotency of stem cells	0.002801544
Prostate cancer	0.002913325
Proteoglycans in cancer	0.00386266
Rap1 signaling pathway	0.003971436
TGF-beta signaling pathway	0.004768896
HTLV-I infection	0.045205949
Oxytocin signaling pathway	0.080600269
Focal adhesion	0.115976434
Pancreatic cancer	0.230380371
PI3K-Akt signaling pathway	0.290120134
Ras signaling pathway	0.290120134
EGFR tyrosine kinase inhibitor resistance	0.467027187
ErbB signaling pathway	0.539307521

Supplemental Table S6: Gene set enrichment analysis of commonly upregulated pathways across models of brain metastatic disease. Related to Figure 6.

GO Gene Set	H2030-BrM3 FBMet vs. 2D		
	NES	FDR q-value	Rank
GO_Chromatin_Modification	4.67	<0.001	3
GO_Neuron_Projection_Guidance	2.28	0.01	88
GO_Cell_Morphogenesis_Involved_in_Neuron_Differentiation	2.57	0.002	44
GO_Cell_Projection_Assembly	2.94	<0.001	17

GO Gene Set	MDA231-BrM2 FBMet vs. 2D		
	NES	FDR q-value	Rank
GO_Homophilic_Cell_Adhesion_via_Plasma_Membrane_Adhesion_Molecules	3.03	<0.001	21
GO_Cell_Morphogenesis_Involved_in_Neuron_Differentiation	3.42	<0.001	6
GO_Neuron_Projection_Guidance	3.09	<0.001	15
GO_Cell_Projection_Assembly	2.71	0.001	40

GO Gene Set	A375-Br FBMet vs. 2D		
	NES	FDR q-value	Rank
GO_Neuroepithelial_Cell_Differentiation	2.00	0.042	74
GO_Glial_Cell_Migration	1.94	0.049	88
GO_Membrane_Docking	2.01	0.043	71
GO_Cell_Projection_Assembly	3.74	<0.001	4

Supplemental Table S7: Species-specific taqman primers. Related to Key Resources Table.

<u>Reagent (Oligonucleotides)</u>	<u>Identifier</u>
<i>ACTB</i> Taqman gene expression assay	Hs00357333_g1
<i>AXIN2</i> Taqman gene expression assay	Hs01063170_m1
<i>CDH1</i> Taqman gene expression assay	Hs00170423_m1
<i>CHGB</i> Taqman gene expression assay	Hs01084631_m1
<i>DCLK1</i> Taqman gene expression assay	Hs00973869_g1
<i>HEY1</i> Taqman gene expression assay	Hs05047713_m1
<i>HPRT1</i> Taqman gene expression assay	Hs99999909_m1
<i>ID2</i> Taqman gene expression assay	Hs04187239_m1
<i>NCAM1</i> Taqman gene expression assay	Hs00941831_m1
<i>POSTN</i> Taqman gene expression assay	Hs01566750_m1
<i>C1qb</i> Taqman gene expression assay	Mm00437836_m1
<i>Gfap</i> Taqman gene expression assay	Mm01253033_m1
<i>Havcr2 (Tim3)</i> Taqman gene expression assay	Mm00454540_m1
<i>Hprt</i> Taqman gene expression assay	Mm00446968_m1
<i>Lag3</i> Taqman gene expression assay	Mm01185093_g1
<i>Postn</i> Taqman gene expression assay	Mm01284919_m1

All Taqman Probes were purchased from Applied Biosystems.