# Supplementary Materials

# HIF1A signaling selectively supports proliferation of breast cancer in the brain.

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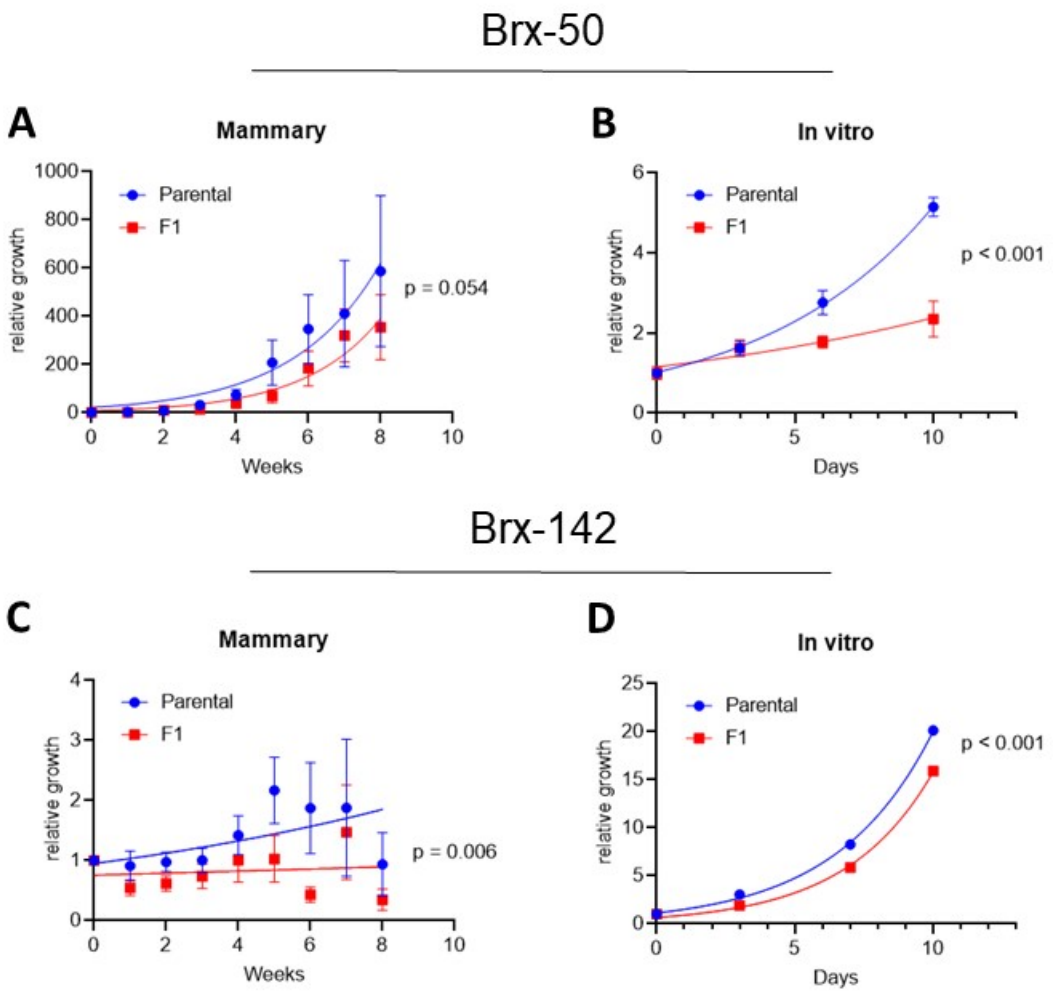
Supplementary Figures 1 to 16
Supplementary Data 1 to 7
Supplementary References

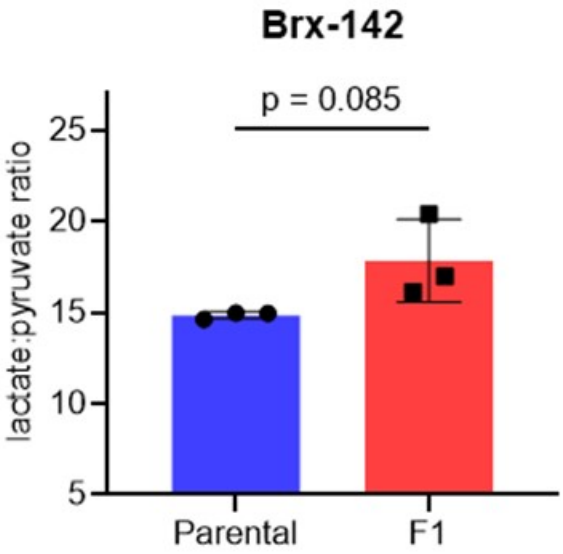
## Supplementary Figures

### Supplementary Figure 1



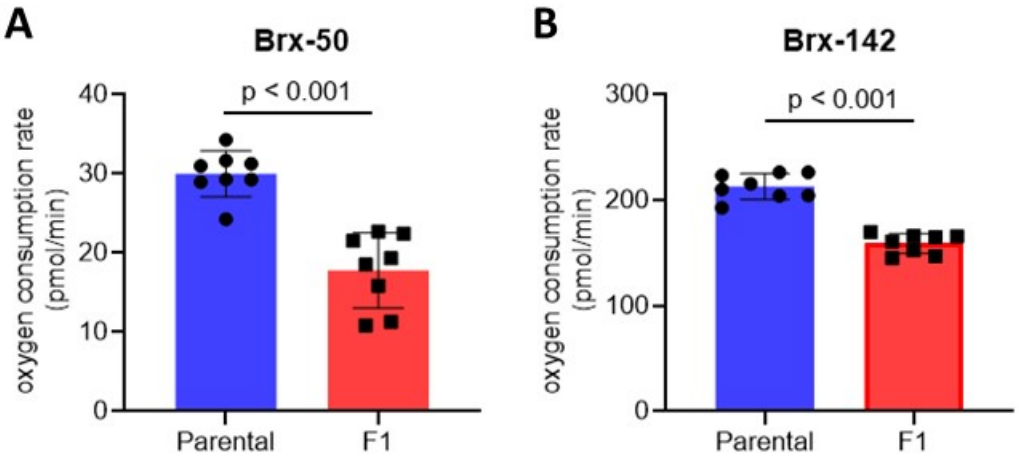

**F1 cell lines demonstrate unchanged or decreased mammary and *in vitro* growth:** Mammary and *in vitro* growth for Brx-50 (A-B) and Brx-142 (C-D) F1 cell lines (Brx-50: mammary: n = 8; *in vitro*: n = 5 | Brx-142: mammary: n = 10; *in vitro*: n = 5). P values calculated by the extra sum-of-squares F test. Data represent mean values +/- SEM. Source data are provided as a Source Data file.

**Supplementary Figure 2**



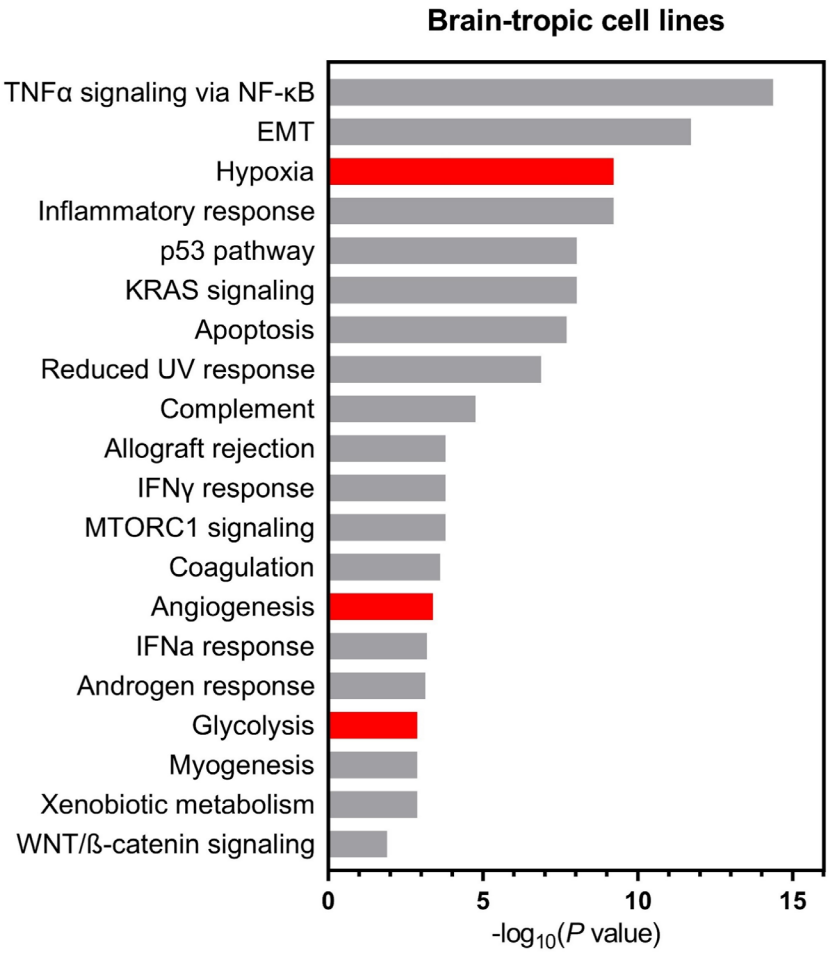

**F1 cell lines demonstrate increased glycolysis:** Lactate-to-pyruvate ratio of Brx-142 parental and F1 cells, as determined by metabolomic studies for relative levels of polar metabolites (n = 3). P value calculated by two-tailed unpaired t test. Data represent mean values +/- SD. Source data are provided as a Source Data file.

**Supplementary Figure 3**



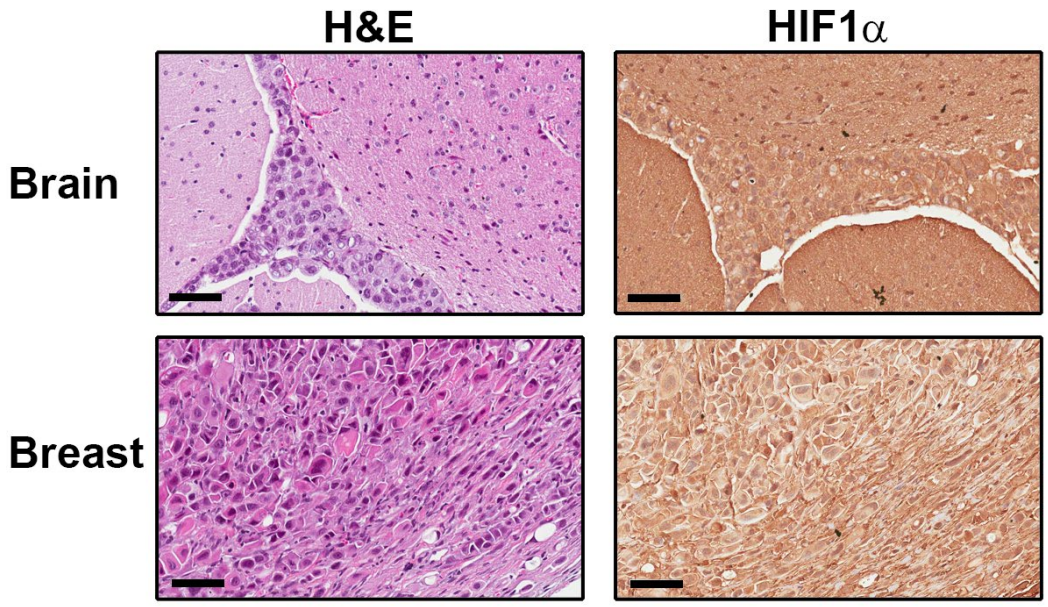

**F1 cell lines demonstrate decreased oxidative phosphorylation:** Oxygen consumption rate of Brx-50 (A) or Brx-142 (B) parental and F1 cells, as determined by live-cell Seahorse assays (n = 8). P values (Brx-50: $2.28 \times 10^{-5}$; Brx-142: $1.11 \times 10^{-7}$) calculated by two-tailed unpaired t test. Data represent mean values +/- SD. Source data are provided as a Source Data file.

**Supplementary Figure 4**



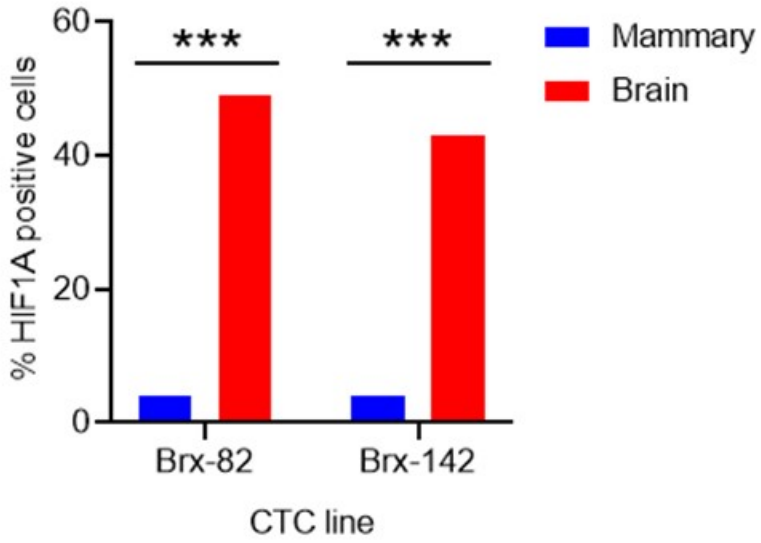

**Hypoxic signaling is increased in brain-tropic isogenic ATCC cell lines:** GSEA for Hallmarks of Cancer genesets for genes upregulated in brain-tropic MDA-MBA-231 and CN34 cells compared with parental cells (*1*). Hypoxia and downstream pathways of hypoxia (Angiogenesis and Glycolysis) are highlighted.

**Supplementary Figure 5**



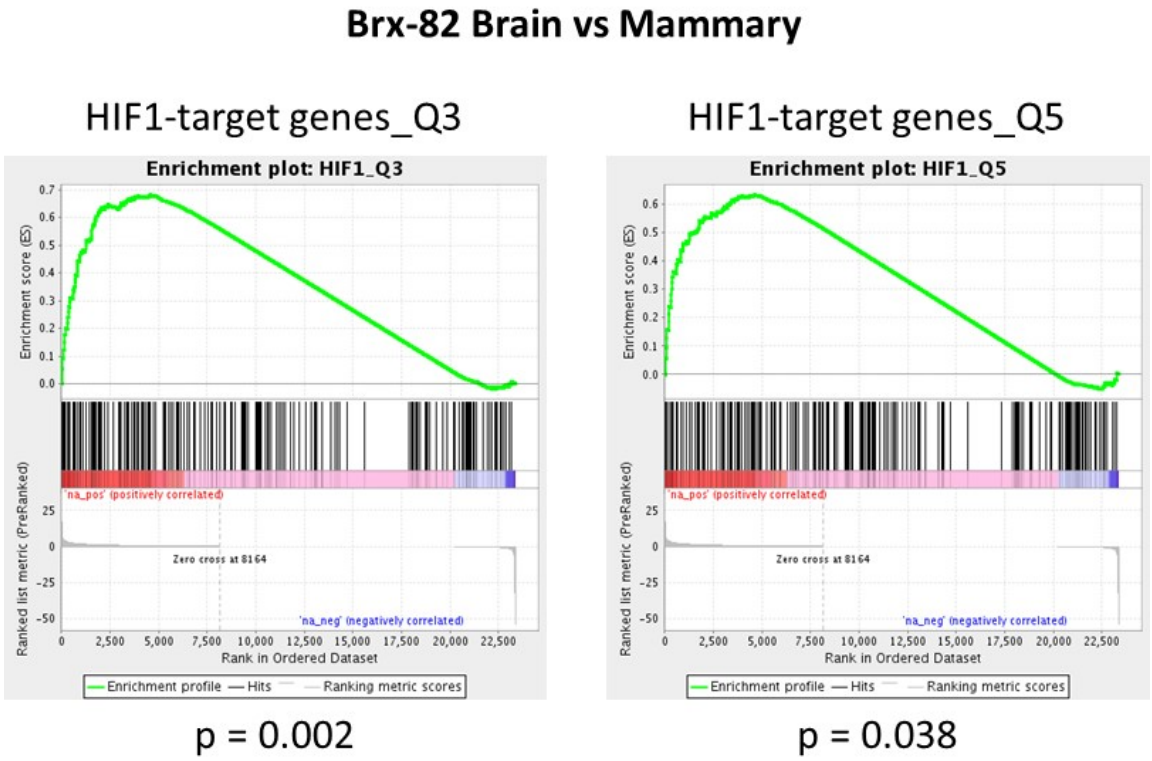

**Levels of HIF1A are increased in mouse brain metastases compared with primary breast tumors:** Representative sections of Brx-142 brain or breast tumor histology after staining with hematoxylin and eosin; or with anti-HIF1α antibody (brown) and counter-stained with hematoxylin. Scale bars: 70 μm. Images are representative of 4 tumor samples.

**Supplementary Figure 6**



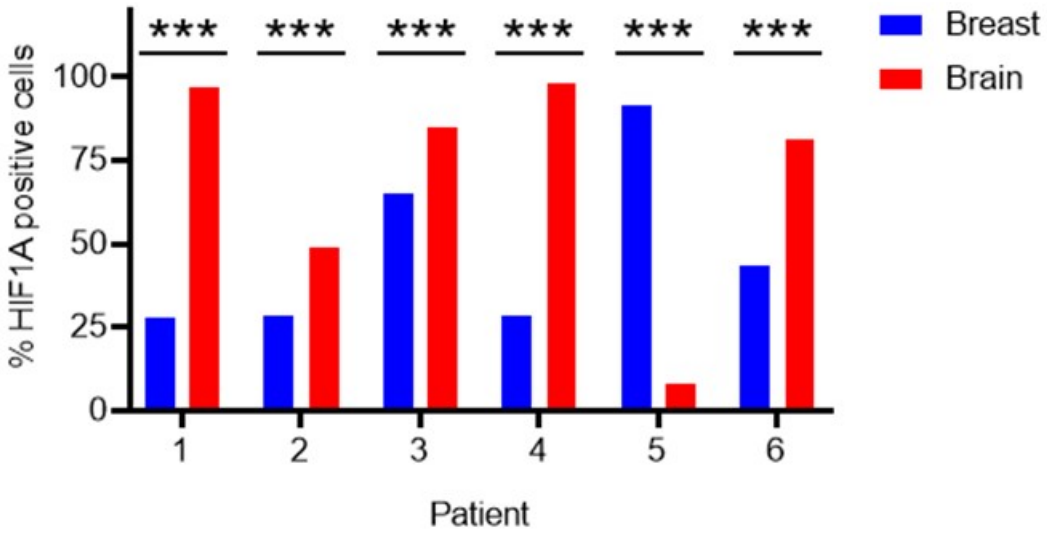

**Mouse brain tumors have increased percentages of HIF1A+ cells compared with mammary tumors:** Percentage of HIF1A+ cells in Brx-82 and Brx-142 brain and mammary tumors, as determined by automated immunohistochemical staining scoring (n = 1000). P values (Brx-82: $1.05 \times 10^{-115}$; Brx-142: $2.16 \times 10^{-93}$) calculated by two-tailed two population proportion z test. ***: $p<0.001$. Source data are provided as a Source Data file.

**Supplementary Figure 7**



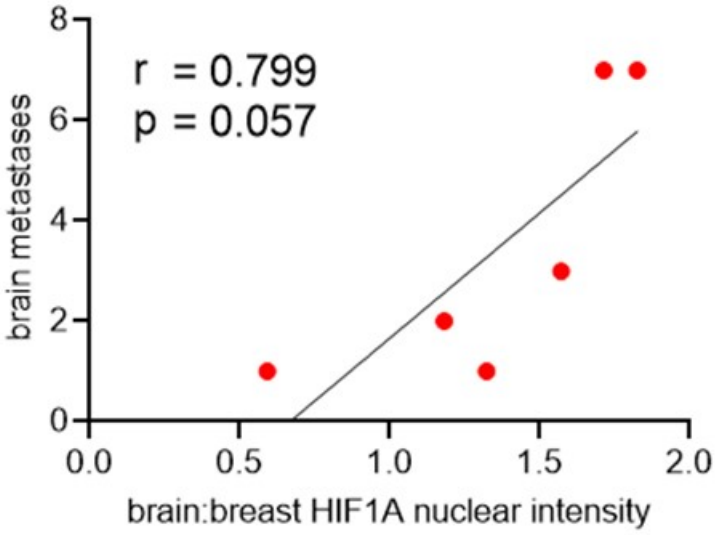

**Mouse brain tumors have enrichment for HIF1A target genes compared with mammary tumors:** Enrichment plots of the Transcription Factor Targets HIF1_Q3 and HIF1_Q5 genesets for genes enriched in Brx-82 brain tumors versus mammary tumors. Positive enrichment scores in enrichment plots indicate more enrichment of the geneset in brain tumors.

**Supplementary Figure 8**



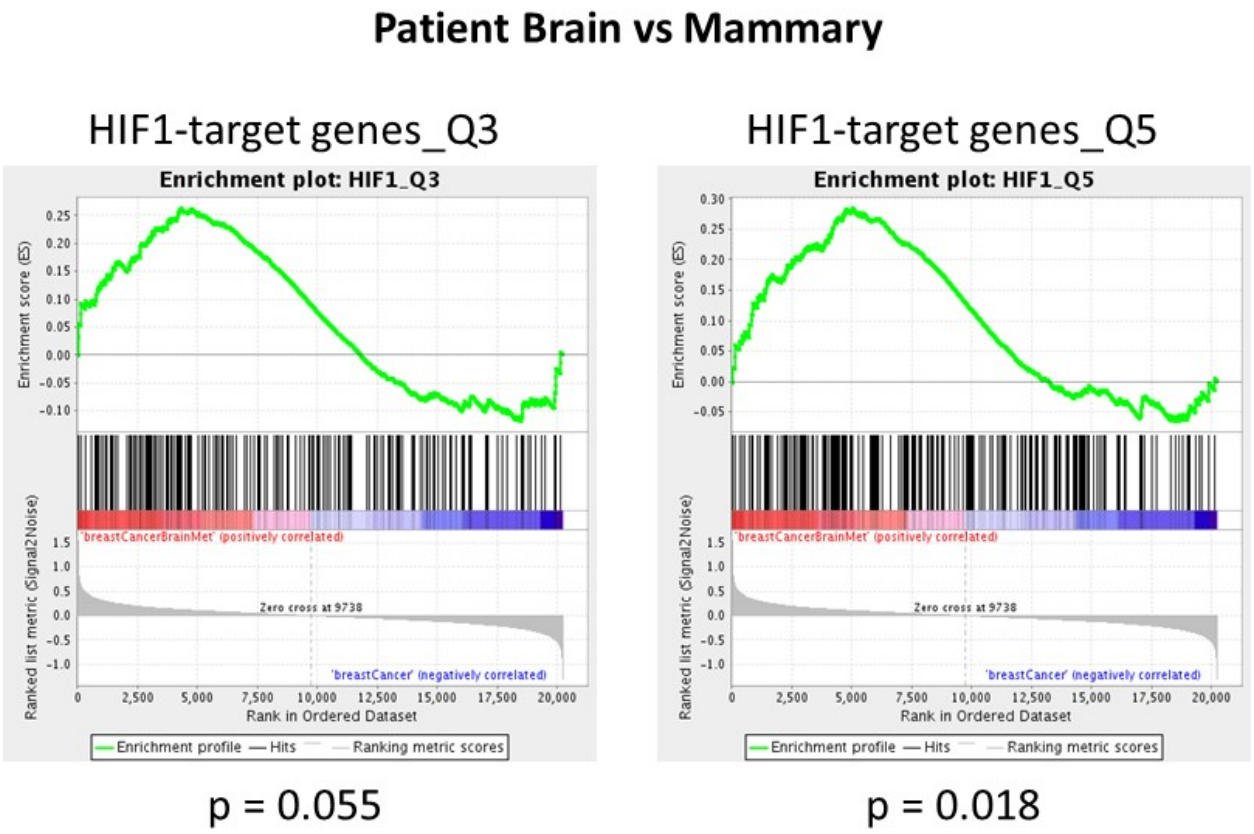

**Patient brain metastases have increased percentages of HIF1A+ cells compared with primary breast tumors:** Percentage of HIF1A+ cells in matched patient brain metastases and primary breast tumors, as determined by automated immunohistochemical staining scoring (Patient #3 breast: n = 262; all other samples: n = 1000). P values (Patient 1: $<1x10^{-300}$; Patient 2: $4.52x10^{-122}$; Patient 3: $7.02x10^{-24}$; Patient 4: $3.59x10^{-227}$; Patient 5: $<1x10^{-300}$; Patient 6: $6.83x10^{-69}$) calculated by two-tailed two population proportion z test. ***: $p<0.001$. Source data are provided as a Source Data file.

**Supplementary Figure 9**



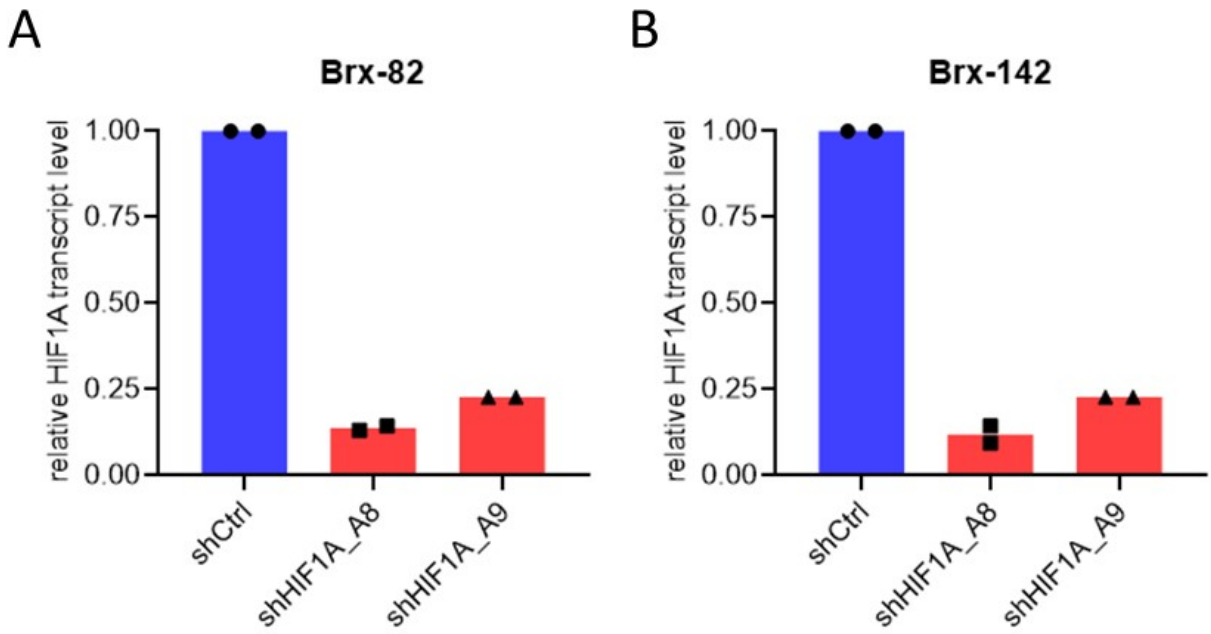

**Increased patient brain metastasis versus breast primary HIF1A staining predicts increased number of brain metastases:** Ratio of matched patient brain-to-breast HIF1A nuclear intensity positively correlates to number of detected brain metastases. Line of best fit displayed. Correlation calculated as Pearson's correlation coefficient. P value calculated by two-tailed unpaired t test.

**Supplementary Figure 10**



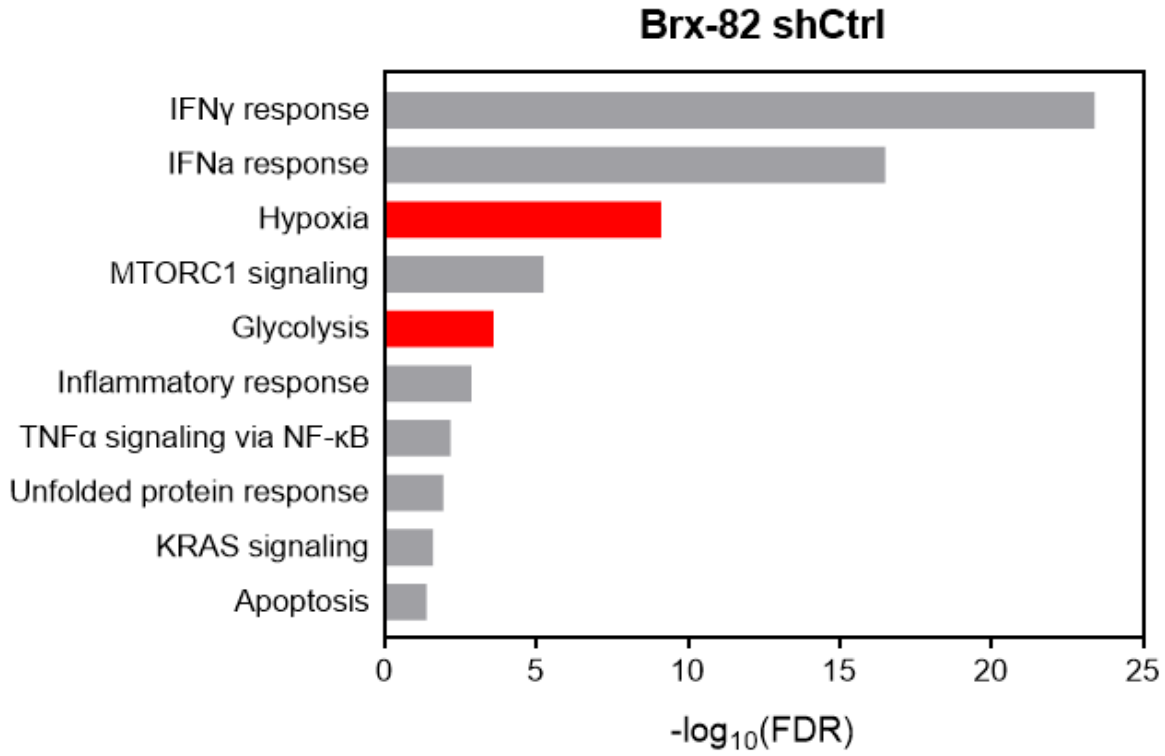

**Patient brain metastases have enrichment for HIF1A target genes compared with primary breast tumors:** Enrichment plots of the Transcription Factor Targets HIF1_Q3 and HIF1_Q5 genesets for genes enriched in patient brain metastases versus unmatched primary breast tumors (*2*). Positive enrichment scores in enrichment plots indicate more enrichment of the geneset in brain metastases.

**Supplementary Figure 11**



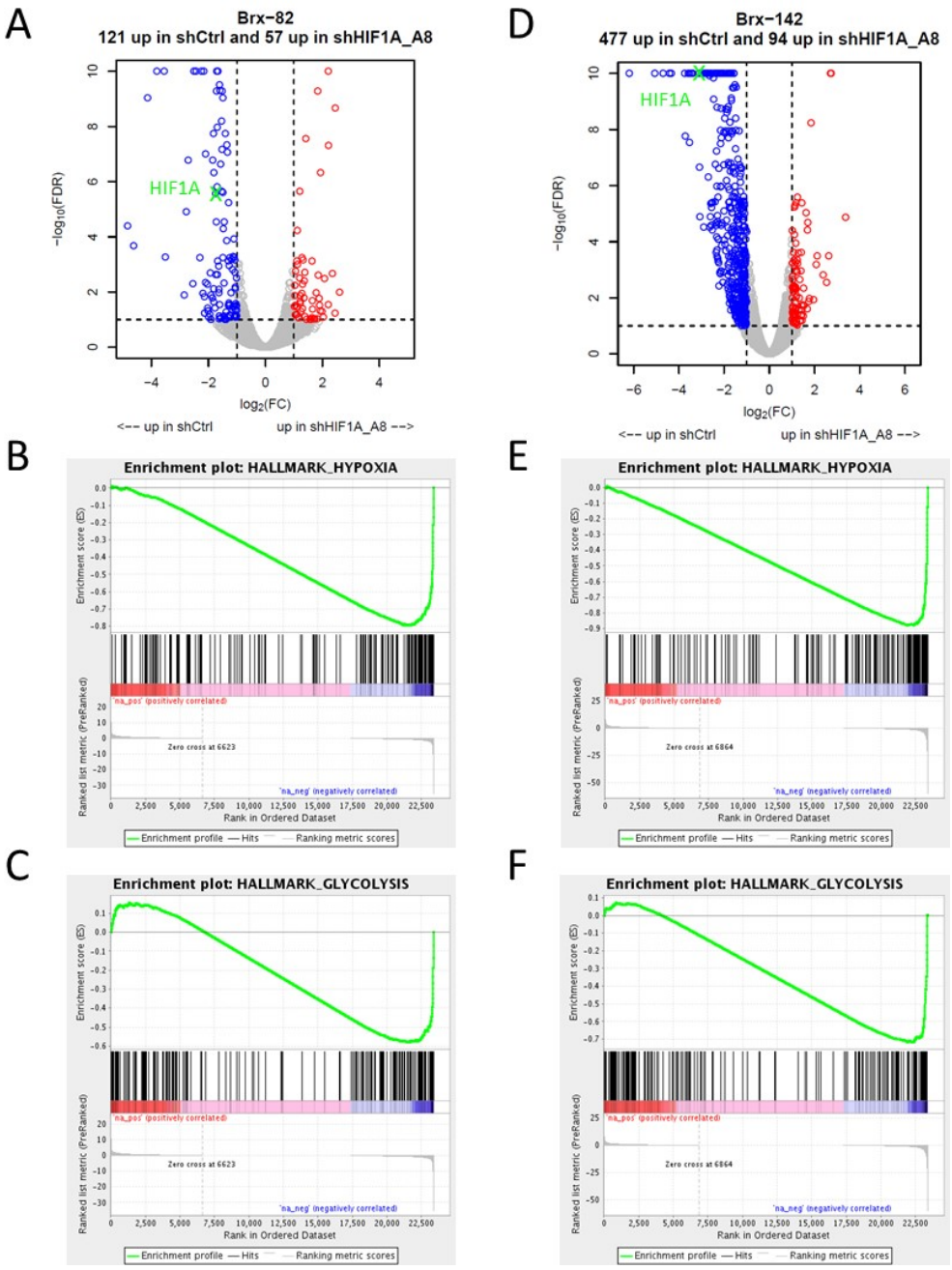

**Knockdown of HIF1A using shRNAs:** qPCR for *HIF1A* in Brx-82 (A) and Brx-142 (B) cells following small hairpin knockdown of shHIF1A (using either hairpin A8 or A9) (n = 2). Source data are provided as a Source Data file.

**Supplementary Figure 12**



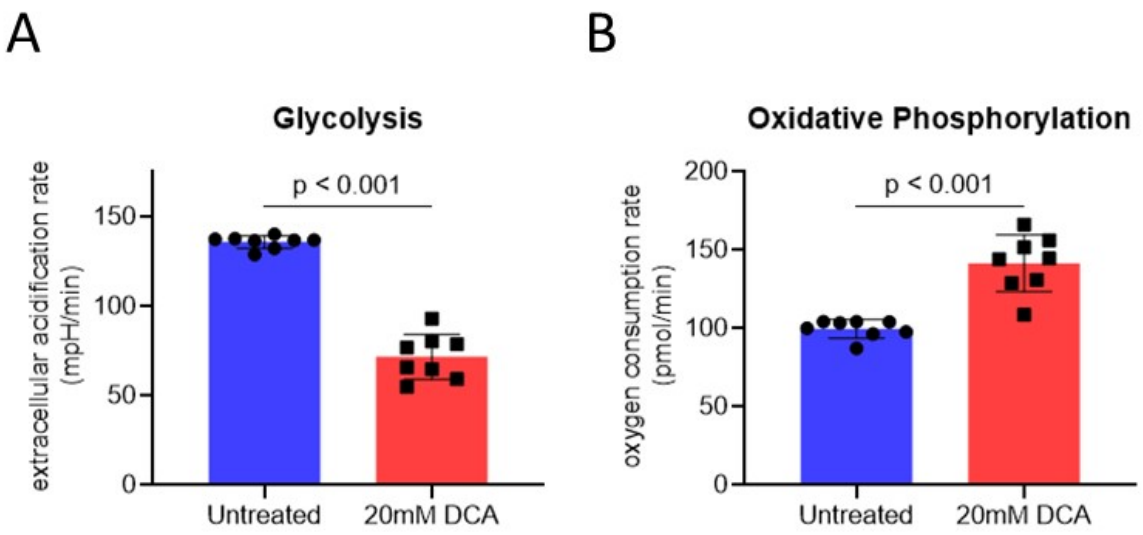

**HIF1A knockdown decreases hypoxia and glycolytic signaling:** GSEA of transcripts differentially expressed in Brx-82 shCtrl compared with shHIF1A_A8 cells (fold change > 2; FDR < 0.25). The most enriched Hallmarks of Cancer gene sets from the Broad Molecular Signatures Database and associated FDR values are shown. Hypoxia and Glycolysis genesets are highlighted.

**Supplementary Figure 13**



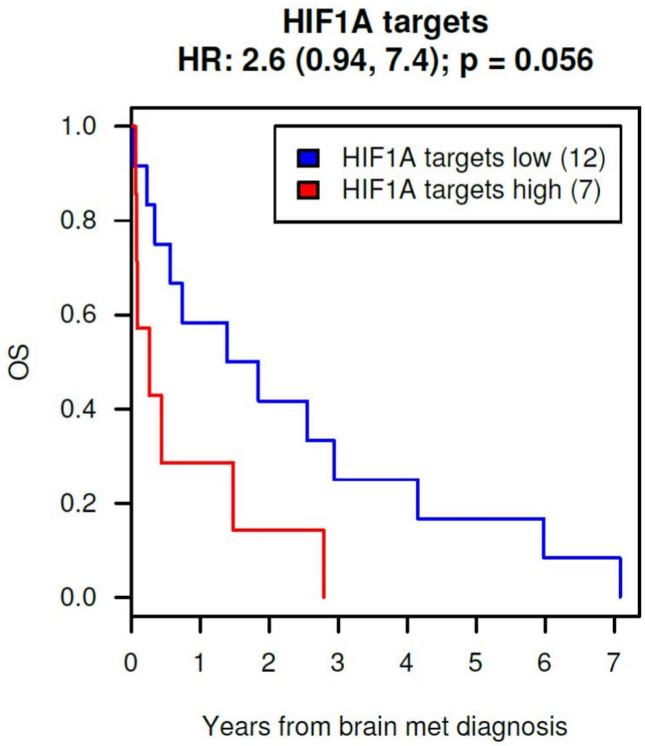

**HIF1A knockdown decreases hypoxia and glycolytic signaling:** Knockdown of HIF1A in Brx-82 (A-C) and Brx-142 (D-F) cells leads to significant changes in the transcriptome and decreased hypoxic and glycolytic signaling. Blue indicates higher expression in shCtrl cells and red indicates higher expression in shHIF1A_8 cells (FC > 2; FDR < 0.25). Genes with -$\log_{10}$(FDR) > 10 are displayed as -$\log_{10}$(FDR) = 10. Negative enrichment scores in enrichment plots indicate more enrichment of the geneset in shCtrl cells.

**Supplementary Figure 14**



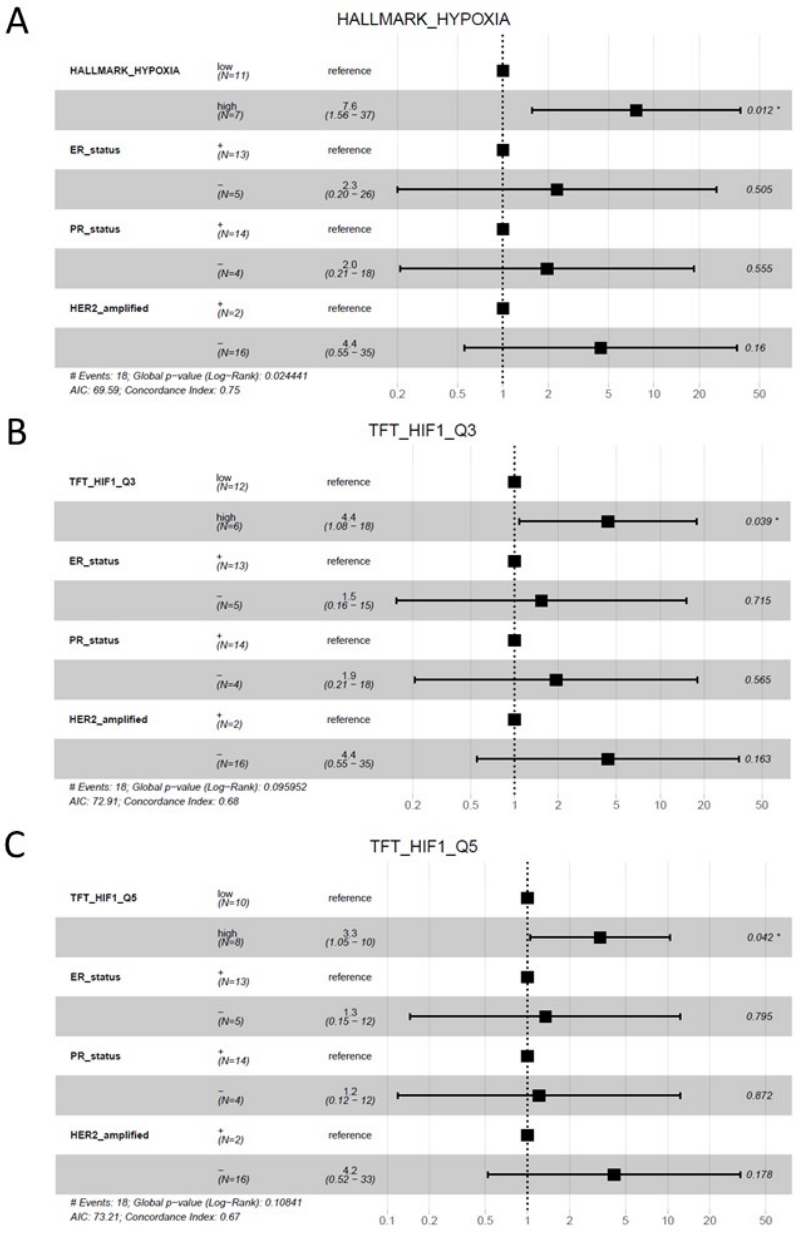

**Dichloroacetic acid treatment inhibits glycolysis:** Extracellular acidification rate (A) and oxygen consumption rate (B) of Brx-82 cells treated with 20mM dichloroacetic acid (DCA) or with vehicle, as determined by live-cell Seahorse assays (n = 8). P values (Glycolysis: $1.54x10^{-9}$; Oxidative phosphorylation: $2.31x10^{-5}$) calculated by two-tailed unpaired t test. Data represent mean values +/- SD. Source data are provided as a Source Data file.

**Supplementary Figure 15**




**HIF1A signaling in CTCs predicts poor outcome in brain metastasis patients:** Kaplan-Meier analysis of the OS following brain metastasis diagnosis for patients with high average HIF1A target gene expression in CTCs versus those with low average HIF1A target gene expression (Transcription Factor Targets HIF1_Q3 geneset). The HIF1A targets-high and HIF1A targets-low subgroups were determined on the basis of average HIF1A target gene expression across all CTCs isolated for each patient. P value calculated by log rank test.

**Supplementary Figure 16**




**Correlation of CTC hypoxic signaling to overall survival is independent of breast cancer subtype:** Multivariate Cox proportional hazards modeling of OS after brain metastasis diagnosis by ER, PR, and HER2 status together with Hallmark Hypoxia (A), Transcription Factor Targets HIF1_Q3 (B) and Transcription Factor Targets HIF1_Q5 (C) geneset expression levels. Brx-206 was excluded due to unknown HER2 status.

## Supplementary Data

### Supplementary Data 1

| CTC patient | Histology | ER status | PR status | HER2 amplified | Intracranial metastasis |
|---|---|---|---|---|---|
| **Brx-7** | Lobular | + | + | - | - |
| **Brx-29** | Lobular | + | + | - | + |
| **Brx-42** | Ductal/Lobular | + | + | - | + |
| **Brx-50** | Ductal | + | + | - | - |
| **Brx-68** | Ductal | + | - | - | - |
| **Brx-82** | Lobular | + | + | - | + |
| **Brx-142** | Lobular | + | + | - | - |

**Patient clinical characteristics for seven parental CTC lines. No patients overlap with patients shown in Supplementary Data 4. Patients Brx-42 and Brx-82 overlap with patients shown in Supplementary Data 6.**

## Supplementary Data 2

| CTC line | Gene | Type | Effect | Result | Allele_freq |
|---|---|---|---|---|---|
| Brx-50 P | CDH1 | deletion | frameshift_variant | p.Asp288ArgfsTer2 | 0.862 |
| Brx-50 P | ESR1 | SNV | missense | p.Leu536Pro | 0.423 |
| Brx-50 P | JAK3 | SNV | missense | p.Arg403Cys | 0.385 |
| Brx-50 F1 | CDH1 | deletion | frameshift_variant | p.Asp288ArgfsTer2 | 0.912 |
| Brx-50 F1 | ESR1 | SNV | missense | p.Leu536Pro | 0.493 |
| Brx-50 F1 | JAK3 | SNV | missense | p.Arg403Cys | 0.609 |
| Brx-82 P | ERBB2 | SNV | missense | p.Ser310Tyr | 1 |
| Brx-82 P | TP53 | SNV | missense | p.Arg248Gln | 0.725 |
| Brx-82 P | KEAP1 | SNV | missense | p.Ala321Thr | 0.688 |
| Brx-82 P | ERBB2 | SNV | missense | p.Glu238Lys | 0.719 |
| Brx-82 P | PIK3R1 | insertion | frameshift_variant | p.Leu347ThrfsTer17 | 0.188 |
| Brx-82 F1 | ERBB2 | SNV | missense | p.Ser310Tyr | 1 |
| Brx-82 F1 | TP53 | SNV | missense | p.Arg248Gln | 0.712 |
| Brx-82 F1 | KEAP1 | SNV | missense | p.Ala321Thr | 0.728 |
| Brx-82 F1 | ERBB2 | SNV | missense | p.Glu238Lys | 0.772 |
| Brx-82 F1 | ALK * | SNV | missense | p.Gln39Pro | 0.518 |
| Brx-82 F1 | PIK3R1 | insertion | frameshift_variant | p.Leu347ThrfsTer17 | 0.22 |
| Brx-82 F2 | ERBB2 | SNV | missense | p.Ser310Tyr | 1 |
| Brx-82 F2 | TP53 | SNV | missense | p.Arg248Gln | 0.649 |
| Brx-82 F2 | KEAP1 | SNV | missense | p.Ala321Thr | 0.668 |
| Brx-82 F2 | ERBB2 | SNV | missense | p.Glu238Lys | 0.595 |
| Brx-82 F2 | PIK3R1 | insertion | frameshift_variant | p.Leu347ThrfsTer17 | 0.211 |
| Brx-142 P | PIK3CA | SNV | missense | p.His1047Arg | 0.347 |
| Brx-142 P | TP53 | SNV | stop_gained | p.Glu349Ter | 1 |
| Brx-142 P | TERT | SNV | upstream_gene_variant | null | 0.389 |
| Brx-142 P | MAP2K1 | SNV | stop_gained | p.Glu69Ter | 0.448 |
| Brx-142 P | CDH1 | SNV | splice_donor_variant | null | 0.991 |
| Brx-142 P | ARID1A | SNV | stop_gained | p.Gln1066Ter | 0.631 |
| Brx-142 P | ATM | SNV | missense | p.Phe627Cys | 0.496 |
| Brx-142 F1 | PIK3CA | SNV | missense | p.His1047Arg | 0.293 |
| Brx-142 F1 | TP53 | SNV | stop_gained | p.Glu349Ter | 0.995 |
| Brx-142 F1 | TERT | SNV | upstream_gene_variant | null | 0.399 |
| Brx-142 F1 | MAP2K1 | SNV | stop_gained | p.Glu69Ter | 0.442 |
| Brx-142 F1 | CDH1 | SNV | splice_donor_variant | null | 1 |
| Brx-142 F1 | ARID1A | SNV | stop_gained | p.Gln1066Ter | 0.57 |
| Brx-142 F1 | ATM | SNV | missense | p.Phe627Cys | 0.497 |

**DNA-sequencing of Brx-50, Brx-82, and Brx-142 parental (P), F1 and F2 cell lines.**

*** indicates a mutation in Brx-82 F1 cells not observed in Brx-82 parental or F2 cells.**

## Supplementary Data 3

| Enriched in brain tumors | |
|---|---|
| **TFT geneset** | **NOM p-val** |
| E2F1DP2_01 | < 0.001 |
| E2F_02 | < 0.001 |
| TGASTMAGC_NFE2_01 | < 0.001 |
| E2F1DP1_01 | < 0.001 |
| E2F4DP2_01 | < 0.001 |
| E2F1_Q6 | < 0.001 |
| E2F_Q6 | < 0.001 |
| E2F_Q4 | < 0.001 |
| E2F4DP1_01 | < 0.001 |
| E2F1_Q4_01 | < 0.001 |
| E2F1_Q3 | < 0.001 |
| E2F_Q3_01 | < 0.001 |
| E2F1_Q6_01 | < 0.001 |
| E2F_Q4_01 | < 0.001 |
| E2F1DP1RB_01 | < 0.001 |
| E2F1_Q3_01 | < 0.001 |
| **HIF1_Q3** | **0.002** |
| E2F_03 | 0.002 |
| E2F_Q3 | 0.003 |
| E2F_Q6_01 | 0.003 |
| E2F1_Q4 | 0.005 |
| RAAGNYNNCTTY_UNKNOWN | 0.007 |
| ALPHACP1_01 | 0.007 |
| SCGGAAGY_ELK1_02 | 0.009 |
| NFY_Q6 | 0.010 |
| ELK1_02 | 0.010 |
| ACCTGTTG_UNKNOWN | 0.011 |
| GCTNWTTGK_UNKNOWN | 0.023 |
| CDPCR3_01 | 0.033 |
| **HIF1_Q5** | **0.038** |
| CTTTGT_LEF1_Q2 | 0.043 |
| ARNT_01 | 0.048 |
| NRF1_Q6 | 0.048 |
| USF_01 | 0.049 |
| AACYNNNNTTCCS_UNKNOWN | 0.050 |
| NFY_01 | 0.058 |
| AHR_Q5 | 0.060 |
| PAX_Q6 | 0.060 |
| SREBP1_01 | 0.062 |
| AP2_Q6 | 0.069 |
| AACTTT_UNKNOWN | 0.071 |
| RTTTNNNYTGGM_UNKNOWN | 0.083 |
| NMYC_01 | 0.087 |
| CGTSACG_PAX3_B | 0.089 |
| GCGNNANTTCC_UNKNOWN | 0.092 |
| WHN_B | 0.092 |
| EFC_Q6 | 0.096 |
| TTANTCA_UNKNOWN | 0.097 |

**GSEA of RNA-seq from Brx-82 brain and mammary tumors for pathways enriched within the Molecular Signatures Database Transcription Factor Targets genesets, showing genesets enriched in brain tumors. HIF1A genesets highlighted.**

**Supplementary Data 4**

| BCBM patient | Years between primary and brain metastasis diagnoses | ER status | PR status | HER2 amplified | Number intracranial metastases |
|---|---|---|---|---|---|
| 1 | 2.6 | + | + | - | 7 |
| 2 | 10.8 | + | + | + | 2 |
| 3 | 4.2 | + | + | - | 3 |
| 4 | 2.6 | + | - | + | 7 |
| 5 | 2.1 | - | - | + | 1 |
| 6 | 15.2 | - | + | - | 1 |

**Patient clinical characteristics for six matched primary breast and brain metastasis samples. No patients overlap with patients shown in Supplementary Data 1 or Supplementary Data 6. BCBM: breast cancer brain metastasis.**

**Supplementary Data 5**

| Enriched in brain metastases | |
|---|---|
| **TFT geneset** | **NOM p-val** |
| AP2_Q3 | 0.006 |
| E2F_Q3 | 0.007 |
| E2F_Q4_01 | 0.015 |
| **HIF1_Q5** | **0.018** |
| GGARNTKYCCA_UNKNOWN | 0.021 |
| GNCF_01 | 0.021 |
| SREBP1_02 | 0.023 |
| E2F1_Q6_01 | 0.025 |
| AACWWCAANK_UNKNOWN | 0.025 |
| SF1_Q6 | 0.027 |
| YNTTTNNNANGCARM_UNKNOWN | 0.027 |
| E2F_Q3_01 | 0.028 |
| ETF_Q6 | 0.031 |
| GGATTA_PITX2_Q2 | 0.042 |
| E2F1_Q4_01 | 0.042 |
| E2F1_Q6 | 0.054 |
| **HIF1_Q3** | **0.055** |
| NFY_Q6_01 | 0.056 |
| CDPCR3_01 | 0.057 |
| E2F1_Q3 | 0.072 |
| GGAMTNNNNNTCCY_UNKNOWN | 0.074 |
| GATA1_01 | 0.082 |
| E2F4DP1_01 | 0.087 |
| ER_Q6_02 | 0.089 |
| E2F1DP2_01 | 0.092 |
| E2F_Q6_01 | 0.096 |

**GSEA of RNA-seq from patient brain metastasis and unmatched primary breast tumors (*2*) for pathways enriched within the Molecular Signatures Database Transcription Factor Targets genesets, showing genesets enriched in brain metastases. HIF1A genesets highlighted.**

## Supplementary Data 6

| patient | CTC ID | ER status | PR status | HER2 amplified | Patient draw number | OS after brain met dx |
|---|---|---|---|---|---|---|
| BR29 | BR29_1_012913 | + | + | - | 1 | 9 |
| BR29 | BR29_2_012913 | + | + | - | 1 | 9 |
| BR29 | BR29_3_BYL_012913 | + | + | - | 1 | 9 |
| Brx-10 | Brx10_1_SC1_041113 | + | + | - | 1 | 1071 |
| Brx-10 | Brx10_1_SC2_041113 | + | + | - | 1 | 1071 |
| Brx-10 | Brx10_1_SC3_041113 | + | + | - | 1 | 1071 |
| Brx-110 | BRx-110-3--06-29-15 | + | + | - | 1 | 206 |
| Brx-110 | BRx-110-2--9-8 | + | + | - | 2 | 206 |
| Brx-110 | BRx-110-3--9-8 | + | + | - | 2 | 206 |
| Brx-110 | BRx-110-1--09-17-15 | + | + | - | 3 | 206 |
| Brx-110 | BRx-110-2--09-17-15 | + | + | - | 3 | 206 |
| Brx-110 | BRx-110-1--10-13-15 | + | + | - | 4 | 206 |
| Brx-110 | BRx-110-2--10-13-15 | + | + | - | 4 | 206 |
| Brx-111 | BRx-111-1--10-08-15 | - | - | - | 1 | 672 |
| Brx-111 | BRx-111-2--10-08-15 | - | - | - | 1 | 672 |
| Brx-111 | BRx-111-3--10-08-15 | - | - | - | 1 | 672 |
| Brx-111 | BRx-111-1--10-19-15 | - | - | - | 2 | 672 |
| Brx-122 | Brx122_1_SC1_121213 | + | + | - | 1 | 157 |
| Brx-122 | Brx122_1_SC2_121213 | + | + | - | 1 | 157 |
| Brx-122 | Brx122_1_SC3_121213 | + | + | - | 1 | 157 |
| Brx-129 | BRx-129-2--9-29 | - | - | - | 1 | 267 |
| Brx-146 | Brx146_2_relapse_SC1_082114 | - | + | - | 1 | 930 |
| Brx-146 | Brx146_2_relapse_SC2_082114 | - | + | - | 1 | 930 |
| Brx-146 | Brx146_2_relapse_SC3_082114 | - | + | - | 1 | 930 |
| Brx-156 | BRx-156-2--12-01-15 | + | + | - | 1 | 124 |
| Brx-172 | BRx-172_2_111114 | - | - | - | 1 | 506 |
| Brx-172 | BRx-172-5--11-11-14 | - | - | - | 1 | 506 |
| Brx-172 | BRx-172-1--02-01-15 | - | - | - | 2 | 506 |
| Brx-172 | BRx-172-1--4-16-15 | - | - | - | 3 | 506 |
| Brx-172 | BRx-172-2--07-07-15 | - | - | - | 4 | 506 |
| Brx-172 | BRx-172-3--07-07-15 | - | - | - | 4 | 506 |
| Brx-172 | BRx-172-1-08-04-15 | - | - | - | 5 | 506 |
| Brx-172 | BRx-172-1--08-18-15 | - | - | - | 6 | 506 |
| Brx-172 | BRx-172-1--08-25-15 | - | - | - | 7 | 506 |
| Brx-172 | BRx-172-2--08-25-15 | - | - | - | 7 | 506 |
| Brx-172 | BRx-172-1--09-09-15 | - | - | - | 8 | 506 |
| Brx-172 | BRx-172-1--9-30 | - | - | - | 9 | 506 |
| Brx-18 | BRx-18du42 | + | + | + | 1 | 2587 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Brx-206 | BRx-206-1--10-23-15 | + | + | unknown | 1 | 29 |
| Brx-206 | BRx-206-2--10-23-15 | + | + | unknown | 1 | 29 |
| Brx-206 | BRx-206-1--01-15-16 | + | + | unknown | 2 | 29 |
| Brx-206 | BRx-206-2--01-15-16 | + | + | unknown | 2 | 29 |
| Brx-206 | BRx-206-3--01-15-16 | + | + | unknown | 2 | 29 |
| Brx-206 | BRx-206-4--01-15-16 | + | + | unknown | 2 | 29 |
| Brx-221 | BRx-221-1--10-13-15 | + | + | - | 1 | 2184 |
| Brx-221 | BRx-221-2--10-13-15 | + | + | - | 1 | 2184 |
| Brx-245 | BRx-245-2--09-04-15 | - | - | - | 1 | 80 |
| Brx-42 | BRx-42-1--9-14 | + | + | - | 1 | 28 |
| Brx-42 | BRx-42-2--9-14 | + | + | - | 1 | 28 |
| Brx-42 | BRx-42-1--9-22 | + | + | - | 2 | 28 |
| Brx-42 | BRx-42-1--9-29 | + | + | - | 3 | 28 |
| Brx-42 | BRx-42-2--9-29 | + | + | - | 3 | 28 |
| Brx-42 | BRx-42-3--9-29 | + | + | - | 3 | 28 |
| Brx-42 | BRx-42-1--10-20 | + | + | - | 4 | 28 |
| Brx-42 | BRx-42_2_110314 | + | + | - | 5 | 28 |
| Brx-74 | Brx74_1_SC2_032813 | + | + | - | 1 | 21 |
| Brx-82 | Brx82_1_SC1_051613 | + | + | - | 1 | 94 |
| Brx-82 | BRx-82-2--9-15 | + | + | - | 2 | 94 |
| Brx-82 | BRx-82-1--9-29 | + | + | - | 3 | 94 |
| Brx-82 | BRx-82-2--9-29 | + | + | - | 3 | 94 |
| Brx-82 | BRx-82-1--11-13-14 | + | + | - | 4 | 94 |
| Brx-82 | BRx-82-2--11-13-14 | + | + | - | 4 | 94 |
| Brx-82 | BRx-82_1_012015 | + | + | - | 5 | 94 |
| Brx-82 | BRx-82-1--1-12-15 | + | + | - | 5 | 94 |
| Brx-82 | BRx-82-3--01-20-15 | + | + | - | 5 | 94 |
| Brx-82 | BRx-82-4--01-20-15 | + | + | - | 5 | 94 |
| Brx-82 | BRx-82_1_030915 | + | + | - | 6 | 94 |
| Brx-82 | BRx-82_2_030915 | + | + | - | 6 | 94 |
| Brx-82 | BRx-82_3_030915 | + | + | - | 6 | 94 |
| Brx-82 | BRx-82_5_030915 | + | + | - | 6 | 94 |
| Brx-82 | BRx-82_6_030915 | + | + | - | 6 | 94 |
| Brx-82 | BRx-82_7_030915 | + | + | - | 6 | 94 |
| Brx-82 | BRx-82-8--3-9-15 | + | + | - | 6 | 94 |
| Brx-86 | Brx86_1_CL1_052313 | + | + | + | 1 | 539 |
| Brx-86 | Brx86_1_SC2_052313 | + | + | + | 1 | 539 |
| Brx-86 | Brx86_1_SC3_052313 | + | + | + | 1 | 539 |
| Brx-86 | Brx86_2_BYL_SC1_052413 | + | + | + | 2 | 539 |
| Brx-86 | Brx86_2_BYL_SC2_052413 | + | + | + | 2 | 539 |
| Brx-86 | Brx86_2_BYL_SC3_052413 | + | + | + | 2 | 539 |
| Brx-87 | Brx87_1_SC1_062113 | + | + | - | 1 | 1016 |
| Brx-87 | Brx87_1_SC2_062113 | + | + | - | 1 | 1016 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Brx-95 | Brx95_1_SC1_071713 | + | + | - | 1 | 1517 |
| Brx-95 | Brx95_2_BYL_CL1_071813 | + | + | - | 2 | 1517 |

**Patient clinical characteristics for 83 patient-derived CTCs from 19 patients with brain metastases. Patients Brx-42 and Brx-82 overlap with patients shown in Supplementary Data 1. No patients overlap with patients shown in Supplementary Data 4. All patients were deceased at last follow-up.**

## Supplementary Data 7

| Primer | Sequence/Assay ID |
|---|---|
| shCtrl_hairpin | CAACAAGATGAAGAGCACCAA |
| shHIF1A_A8_hairpin | GTGATGAAAGAATTACCGAAT |
| shHIF1A_A9_hairpin | CGGCGAAGTAAAGAATCTGAA |
| shCtrl_sequencing | CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGTTTTT |
| shHIF1A_A8_sequencing | CCGGGTGATGAAAGAATTACCGAATCTCGAGATTCGGTAATTCTTTCATCACTTTTT |
| shHIF1A_A9_sequencing | CCGGCGGCGAAGTAAAGAATCTGAACTCGAGTTCAGATTCTTTACTTCGCCGTTTTT |
| ACTB_TaqMan | Hs01060665_g1 |
| HIF1A_TaqMan | Hs00153153_m1 |

**Primers used for shRNA knockdown and sequencing.**

## Supplementary References


1. P. D. Bos *et al.*, Genes that mediate breast cancer metastasis to the brain. *Nature* **459**, 1005-1009 (2009).
2. H. J. Schulten *et al.*, Comprehensive molecular biomarker identification in breast cancer brain metastases. *J Transl Med* **15**, 269 (2017).