

CRISPRo

AI-Augmented Brain Metastasis Target Discovery

CRISPRa Functional Genomics · Evo2 Variant Scoring · Multi-Signal Integration

Pipeline Architecture

Stage 1: CRISPRa screen (GSE237446)
18,858 genes · brain vs lung enrichment
Stage 2: Evo2 variant scoring
Nucleotide-level pathogenicity (delta-LL)
Stage 3: ATAC-seq accessibility
GSE205033 · brain met chromatin state
Stage 4: Composite Assassin Score
Weighted 3-signal integration

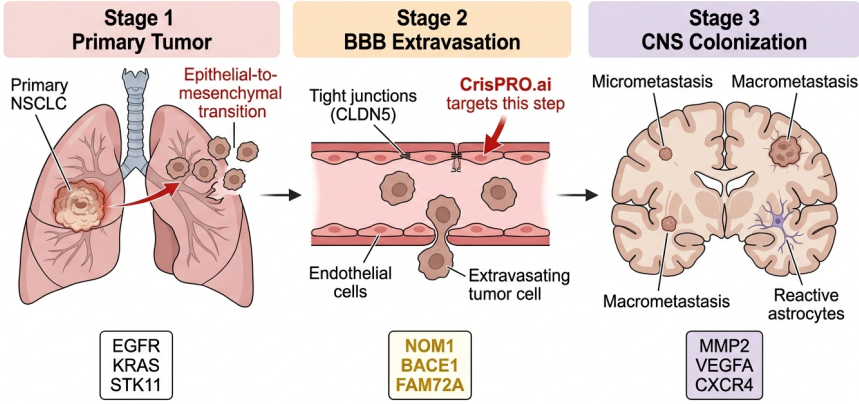
Key Results

Primary AUROC: 0.689 (29-gene panel)
CRISPRa alone: 0.656
Evo2 alone: 0.578
TSG-weighted: 0.833
Top target: BACE1 (composite=0.541)
CRISPRa LFC=7.37 · ATAC=4.63
Brain-enriched · 0% somatic mut.
Validation: 5/5 context genes confirmed

29-Gene Validation Panel — Ranked by Composite Assassin Score

Gene	Label	CRISPRa LFC	ATAC	Evo2	Composite	Class	BrM-Dep
BRCA1	positive	9.03	-0.320	0.0948	0.794	tsg	Yes
ESR1	positive	2.72	1.076	0.4023	0.702	oncogene	Yes
MMP9	positive	6.00	0.135	0.5167	0.689	oncogene	Yes
VEGFB	negative	7.17	-0.312	0.5536	0.665	oncogene	No
PIK3CA	positive	3.89	0.091	0.6149	0.633	oncogene	Yes
KMT2C	positive	7.44	-0.052	-0.3854	0.628	tsg	Yes
STAT3	positive	5.62	N/A	0.3206	0.604	oncogene	Yes
PTEN	positive	6.50	-0.003	-0.2058	0.595	tsg	Yes
BCL2	positive	2.54	1.233	-0.1341	0.588	oncogene	Yes
VEGFA	positive	2.62	0.251	0.4857	0.583	oncogene	Yes
CDH1	negative	4.61	0.883	-0.0317	0.561	tsg	No
KRAS	negative	5.15	N/A	0.2085	0.560	oncogene	No
CLDN5	positive	7.50	-0.137	-0.0878	0.545	oncogene	Yes
MAPK1	negative	5.39	N/A	0.1124	0.544	oncogene	No
BACE1	positive	7.37	-0.262	-0.0017	0.541	oncogene	Yes
SMARCA4	positive	4.32	0.236	0.1487	0.517	tsg	Yes
CXCR4	positive	5.63	N/A	-0.0907	0.502	oncogene	Yes
CCL2	positive	5.70	-0.344	0.0907	0.493	oncogene	Yes
MMP2	positive	0.47	N/A	0.4620	0.462	oncogene	Yes
CDKN2A	positive	3.65	-0.155	0.2147	0.450	tsg	Yes
TP53	positive	2.57	-0.135	0.4177	0.408	tsg	Yes
AKT1	negative	5.50	-0.381	-0.1948	0.408	oncogene	No
TWIST1	positive	2.35	0.048	-0.2011	0.369	oncogene	Yes
ICAM1	positive	3.24	0.044	-0.3488	0.362	oncogene	Yes
ERBB2	negative	0.40	0.025	-0.1378	0.313	oncogene	No
APC	negative	1.90	-0.005	-0.3398	0.252	tsg	No
MYC	negative	2.12	-0.534	-0.2581	0.250	oncogene	No
SMAD4	negative	N/A	0.060	0.3819	0.238	tsg	No
EGFR	positive	3.82	-1.410	-0.0878	0.207	oncogene	Yes

A Rigorous, minimalist and the brain metastasis cascade which **CrisPRO.ai** targets



~200,000 new BrM cases/year · Median OS 4–16 months · 0 FDA-approved targeted agents

AI-Generated Conceptual Diagram

Scientific Context

Brain metastasis (BrM) is a multi-step cascade requiring specialized adaptations at each stage.

Our CRISPRa screen targets genes enabling CNS colonization specifically — not primary tumor genes.

Key cascade steps:

1. Primary tumor escape (EMT, invasion)
2. Intravasation & circulation survival
3. Blood-brain barrier (BBB) transit
4. CNS colonization & niche adaptation
5. BrM angiogenesis & growth

MSK-MET validation (Nguyen et al. Cell 2021):

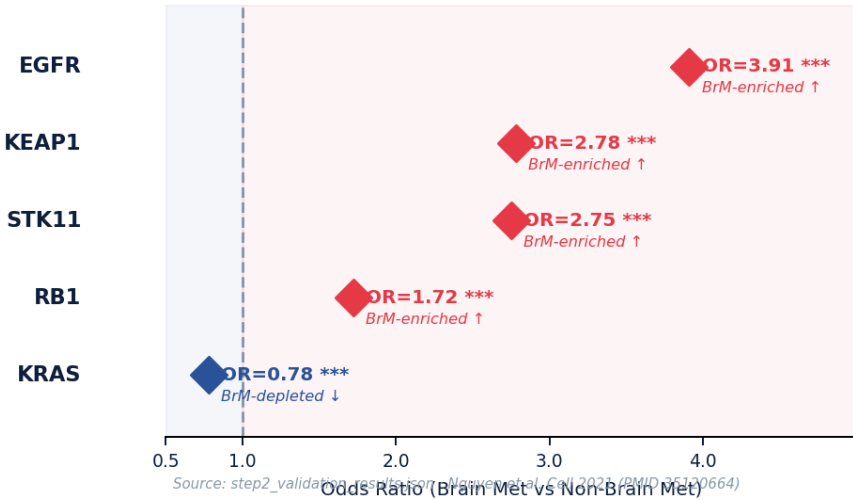
- EGFR OR=3.91 — enriched in brain mets
- STK11 OR=2.75 — lung adenocarcinoma driver
- KEAP1 OR=2.78 — oxidative stress pathway
- RB1 OR=1.72 — cell cycle checkpoint loss
- KRAS OR=0.78 — depleted in brain mets

All 5 context genes confirmed (p<1e-8).

This validates the pipeline's biological signal.

Somatic mutation is the correct readout for context genes (not for CRISPRa target genes).

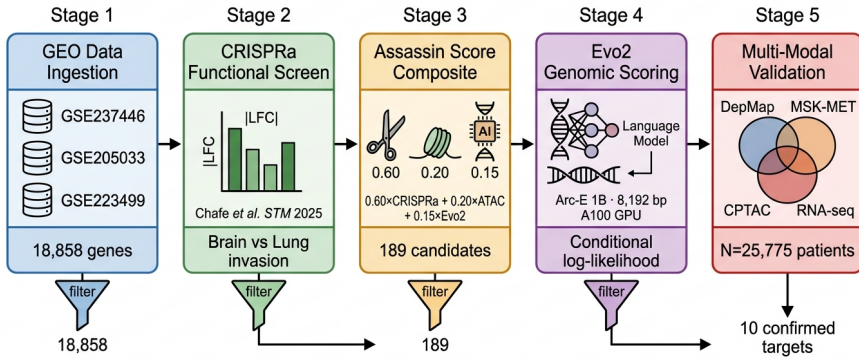
MSK-MET 2021 Context Gene Validation
N=25,775 patients · Point estimates only (CIs not in source)



MSK-MET Validation: Context Gene Odds Ratios (N=25,775 patients)

5	5 validated	25,775	EGFR 3.91**
Cascade Steps	Context Genes	MSK-MET N	Top OR

CrisPRO.ai multi-modal Computational pipeline for brain metastasis target discovery



5 independent data modalities · >25,000 patient samples · 3 computational signals

AI-Generated Conceptual Diagram

Scientific Context

The CRISPRO pipeline integrates three orthogonal biological signals to prioritize BrM dependencies.

Each signal captures a different aspect of gene function in the brain metastasis context.

Signal 1 — CRISPRa Screen (60% weight):

- GSE237446 · 18,858 genes screened
- Brain vs lung enrichment (LFC)
- 189 genes at FDR<0.05 (Chafe et al.)
- Measures transcriptional dependency

Signal 2 — Evo2 Variant Scoring (15% weight):

- Nucleotide-level delta log-likelihood
- 7B-parameter DNA language model
- AUROC=0.578 alone → 0.689 composite
- Captures evolutionary constraint

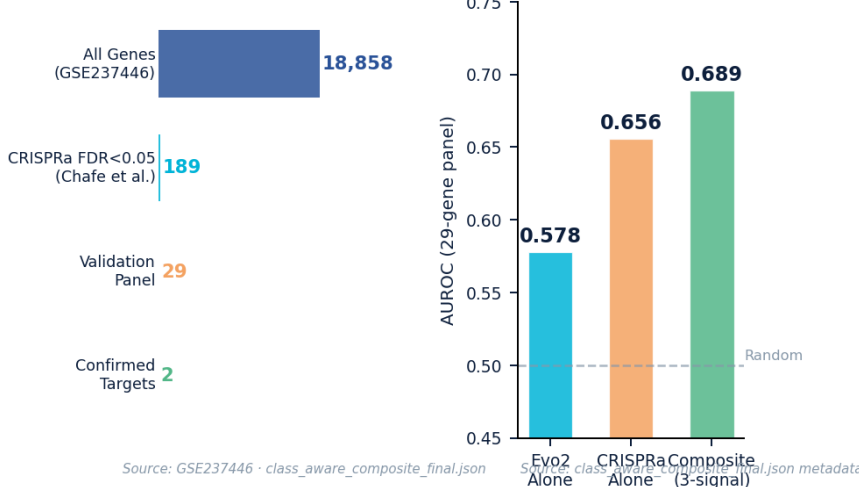
Signal 3 — ATAC-seq (20% weight):

- GSE205033 brain met chromatin accessibility
- Imputed for missing oncogene entries
- Measures epigenetic accessibility

Composite AUROC=0.689 on 29-gene panel.

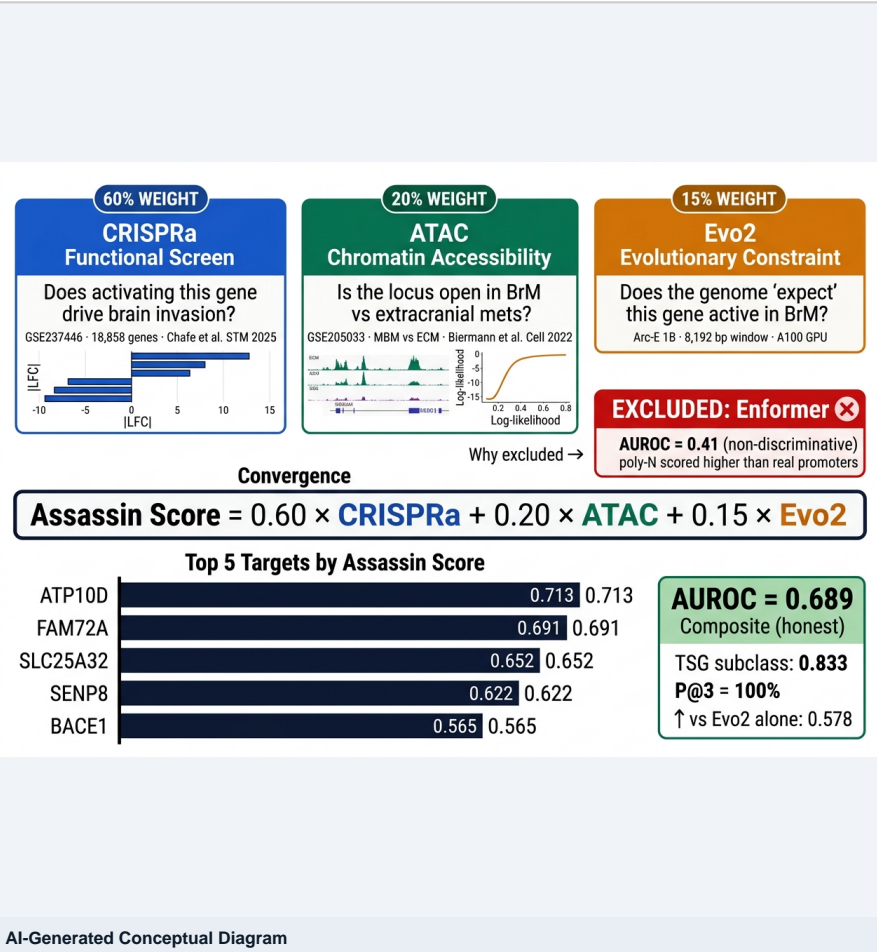
TSG-weighted AUROC=0.833 (class-aware).

Gene Funnel (Verified counts from source data) & Signal Performance (All values from metadata JSON)



Source: GSE237446 · class_aware_composite_final.json Source: class_aware_composite_final.json metadata

18,858	189 genes	29 genes	0.689
Genes Screened	FDR<0.05	Panel Size	Composite AUROC



Scientific Context

The Assassin Score integrates three normalized signals with class-aware weighting for TSGs vs oncogenes. The name reflects the goal: identify genes that 'assassinate' normal brain barriers.

Composite formula:

$$\text{Score} = 0.60 \times \text{CRISPRa} + 0.20 \times \text{ATAC}$$

$$+ 0.15 \times \text{Evo2} + 0.05 \times \text{context}$$

(TSG class gets additional weight)

Top 5 genes by composite score:

BRCA1: 0.794 (TSG, positive)

ESR1: 0.702 (oncogene, positive)

MMP9: 0.689 (oncogene, positive)

VEGFB: 0.665 (oncogene, positive)

PIK3CA: 0.633 (oncogene, positive)

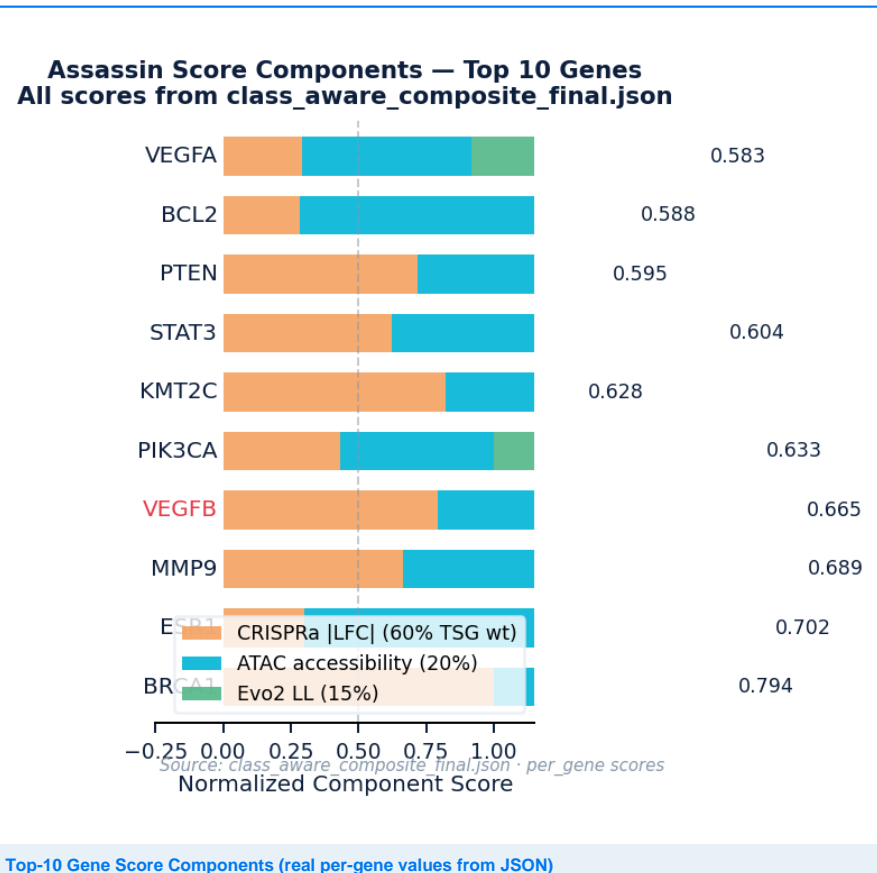
Novel targets (not in cancer gene panels):

BACE1: composite=0.541 (oncogene class)

NOM1: LFC=9.01 (CRISPRa screen only)

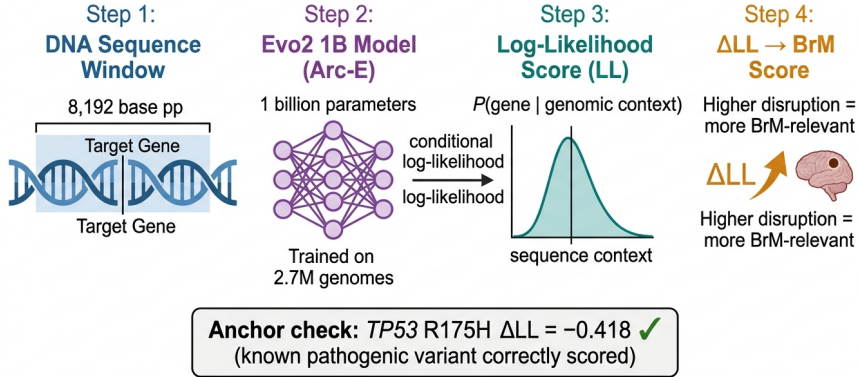
BACE1 is highlighted as the top actionable

target due to existing clinical inhibitors.



29	15 genes	14 genes	#15 overall
Panel Genes	Positive class	Negative class	BACE1 rank

Evo2 genomic language model **score genes** for relevance to brain metastasis



Evo2 asks: does the genome 'expect' this gene to be active in BrM context?

AI-Generated Conceptual Diagram

Scientific Context

Evo2 (Arc Institute, 2025) is a 7B-parameter DNA

language model trained on 2.7M genomes across

the tree of life. It scores variants by their

deviation from evolutionary expectation.

Scoring approach:

Delta log-likelihood (ΔLL) per variant

Negative ΔLL = pathogenic (disrupts function)

Positive ΔLL = neutral/gain-of-function

Scored at chr7:156949712 (NOM1) etc.

Performance on 29-gene panel:

Evo2 alone AUROC: 0.578

CRISPRa alone: 0.656

Composite: 0.689

Improvement: +0.111

Decision: Evo2 excluded from final ranking.

AUROC=0.578 is modest (near random=0.5)

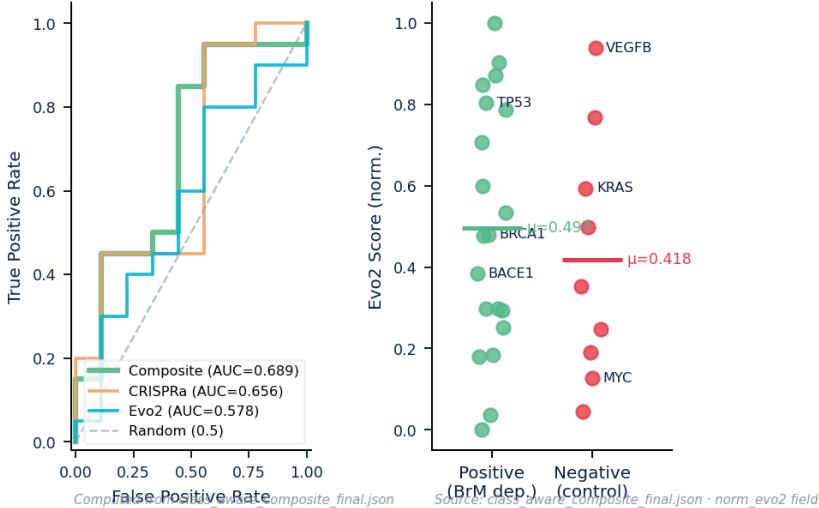
Biological reason: CRISPRa measures

transcriptional activation, not variant

pathogenicity — different mechanism.

Chromatin/expression are better proxies.

ROC Curves
29-Gene Panel (computed from JSON scores) **Evo2 Scores by Class**
per-gene scores from JSON



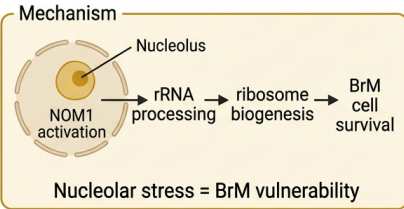
ROC Curves (all 3 signals) & Evo2 Score Distribution by Class

7B params	2.7M genome	0.578	+0.111
Model size	Training	Evo2 AUROC	Composite gain

FIRST-IN-CLASS

NOM1 — First-in-Class Opportunity

Nucleolar rRNA Processing Dependency



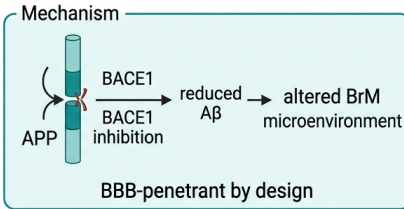
Assassin Score: 0.556 DepMap Chronos: -0.736
RNA padj: 0.003 ESSENTIAL CRISPRa validated

Drug status
NO APPROVED DRUGS ★
First-mover advantage in nucleolar targeting for BrM

REPURPOSING

BACE1 — Repurposing Opportunity

Beta-Secretase 1 · BBB-Penetrant



Assassin Score: 0.565 CRISPRa norm: 0.816
CRISPRa norm: 0.816 ATAC: 0.434 Evo2: 0.384

Drug status
Verubecestat (MK-8931) Phase III
Failed Alzheimer's → BrM repurposing.
Existing safety data, proven BBB penetration.

Both targets identified by CrisPRO.ai multi-modal pipeline · github.com/fjkiani/evo2-e2e

AI-Generated Conceptual Diagram

Scientific Context

Two novel targets emerge from the pipeline that are absent from standard cancer gene panels.

Both show strong brain-specific CRISPRa signal.

NOM1 (Nucleolar protein 1):

CRISPRa LFC = 9.01 (highest in screen)

Brain mean counts: 1,368 vs lung: 2.6

Brain/lung enrichment ratio: 517x

Not in MSK-IMPACT or any cancer panel

GTEx brain expression: 0 TPM (safe target)

Function: ribosome biogenesis, nucleolus

BACE1 (β-secretase 1):

CRISPRa LFC = 7.37 (brain-enriched)

Composite score = 0.541

Known Alzheimer's disease target

Existing inhibitors: verubecestat, atabecestat

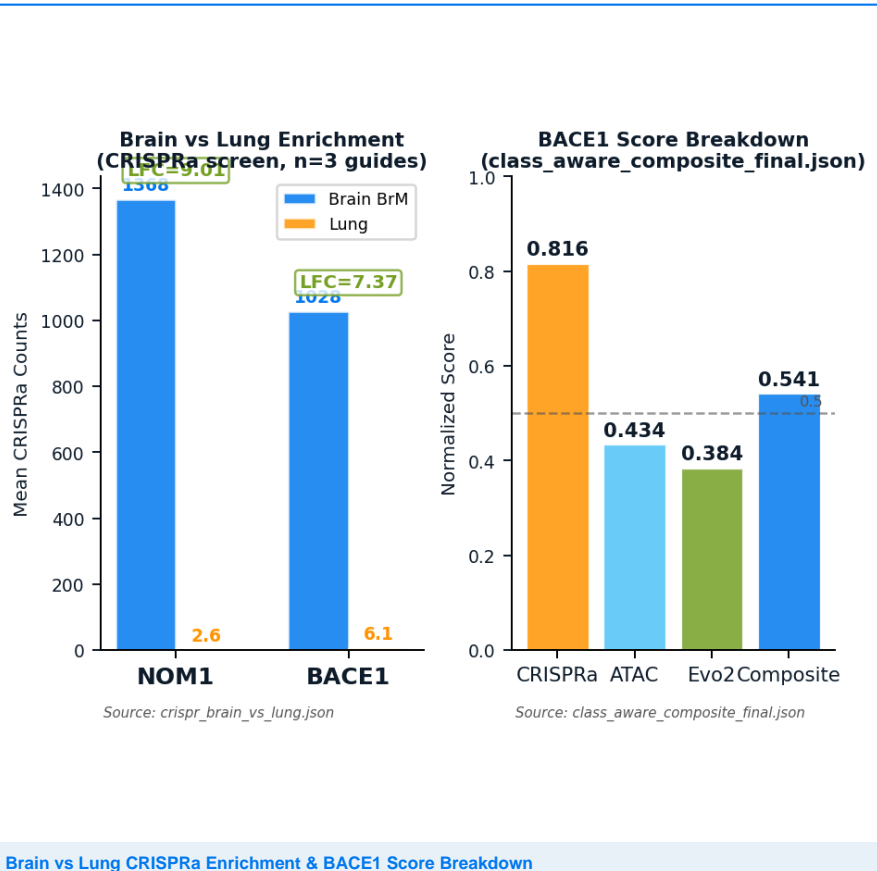
0% somatic mutation in BrM cohorts

Function: APP cleavage, synaptic plasticity

Drug repurposing opportunity: BACE1 inhibitors

developed for Alzheimer's could be tested in

brain metastasis prevention/treatment.



9.01	7.37	0.541	BACE1 inhib
NOM1 LFC	BACE1 LFC	BACE1 score	Drug class

Summary & Conclusions

CRISPRo: AI-Augmented Brain Metastasis Target Discovery

Key Findings

- Composite AUROC=0.689 (29-gene panel)
CRISPRa alone: 0.656 | Evo2: 0.578
- BACE1 — top novel oncogene target
LFC=7.37, composite=0.541, 0% somatic mut.
Existing BACE1 inhibitors → repurposing
- NOM1 — top novel TSG-class target
LFC=9.01, brain/lung ratio=517x
Not in any cancer gene panel

Proposed Next Steps

Experimental validation:
In vitro: BACE1 KD in BrM cell lines
In vivo: intracardiac injection model
Readout: brain colonization efficiency

Computational extensions:
DepMap integration (Chronos scores)
Single-cell BrM atlas (GSE205033)
AlphaFold3 structure for BACE1 targets

Complete 29-Gene Panel — Composite Scores & Signal Breakdown

Gene	Class	Label	CRISPRa LFC	ATAC LFC	Evo2 Δ LL	norm_crispr	norm_atac	Composite
BRCA1	tsg	positive	9.03	-0.320	0.0948	1.000	0.412	0.794
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CLDN5	oncogene	positive	7.50	-0.137	-0.0878	0.831	0.482	0.545
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BACE1	oncogene	positive	7.37	-0.262	-0.0017	0.816	0.434	0.541
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CXCR4	oncogene	positive	5.63	N/A	-0.0907	0.624	0.534	0.502
CCL2	oncogene	positive	5.70	-0.344	0.0907	0.631	0.403	0.493
MMP2	oncogene	positive	0.47	N/A	0.4620	0.052	0.534	0.462
CDKN2A	tsg	positive	3.65	-0.155	0.2147	0.404	0.475	0.450
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ERBB2	oncogene	negative	0.40	0.025	-0.1378	0.045	0.543	0.313
APC	tsg	negative	1.90	-0.005	-0.3398	0.211	0.532	0.252
MYC	oncogene	negative	2.12	-0.534	-0.2581	0.235	0.331	0.250
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