

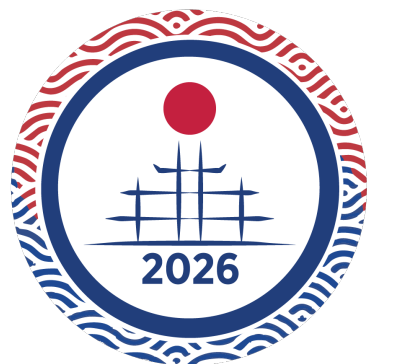


P2.047: Non-Neuronal Cholinergic Signaling Drives Aggressive Small Cell Lung Cancer via Targetable Choline Transport

Masato Inazu^{1,2}, Maki Kimura², Naoko Tajima², Tsuyoshi Yamanaka²

¹Institute of Medical Science, ²Department of Preventive Medicine, Tokyo Medical University, Tokyo JAPAN.

Correspondence: inazu@tokyo-med.ac.jp



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INTRODUCTION

Small cell lung cancer (SCLC) is an aggressive neuroendocrine malignancy characterized by rapid tumor growth, early dissemination, and poor clinical outcomes. Although initial responses to platinum-based chemotherapy are common, most patients eventually develop treatment-resistant disease, highlighting the need for novel therapeutic strategies (Kim et al., 2025). Recent evidence indicates that SCLC harbors a functional non-neuronal cholinergic system (NNCS), in which acetylcholine acts as an autocrine growth factor (Inazu et al., 2013). However, the biological significance of choline transport, the rate-limiting step for acetylcholine synthesis, remains poorly understood in SCLC.

Choline transporter-like protein 1 (CTL1/SLC44A1) and FLVCR choline and heme transporter 1 (FLVCR1) are major choline transporters that may coordinate cholinergic signaling, tumor proliferation, and metabolic adaptation. We hypothesized that CTL1 and FLVCR1 establish a targetable metabolic dependency that promotes SCLC progression.

Objective: To determine the biological, clinical, and therapeutic significance of CTL1/FLVCR1-mediated choline transport in SCLC through functional studies, single-cell transcriptomics, survival analyses, independent cohort validation, and pharmacological inhibition.

Hypothesis: CTL1/FLVCR1-dependent choline transport drives a poor-prognosis, neuroendocrine-proliferative SCLC subtype and represents a therapeutically actionable vulnerability.

METHODS

Experimental Analyses:

Choline uptake kinetics were evaluated in NCI-H69 and NCI-H82 cells using [³H]choline uptake assays.

Expression of NNCS-related genes (CTL1/SLC44A1, FLVCR1, ChAT, BuChE, and VACHT) was quantified by qRT-PCR and immunofluorescence. Cell viability and caspase-3/7 activity were assessed following cholinergic stimulation or treatment with the choline transport inhibitors Amb4269951 and Amb4269675.

Computational and Clinical Analyses:

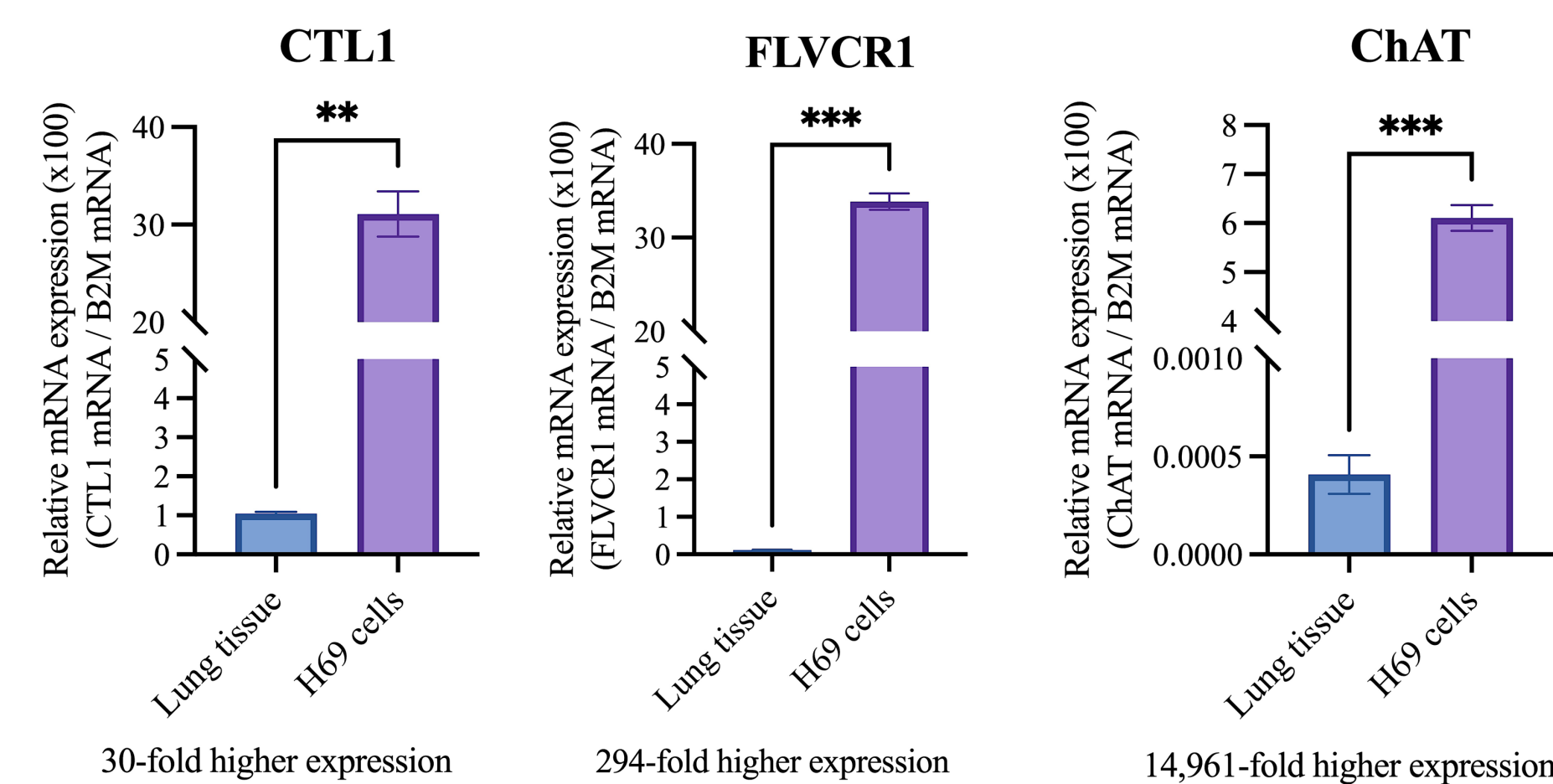
Single-cell RNA-seq datasets were analyzed using Seurat to identify transporter-expressing tumor populations and lineage associations.

Prognostic significance was evaluated in the SCLC UCologne 2015 cohort (n=77) using Kaplan-Meier and multivariable Cox regression analyses.

Key findings were independently validated in the GSE149507 cohort (n=18).

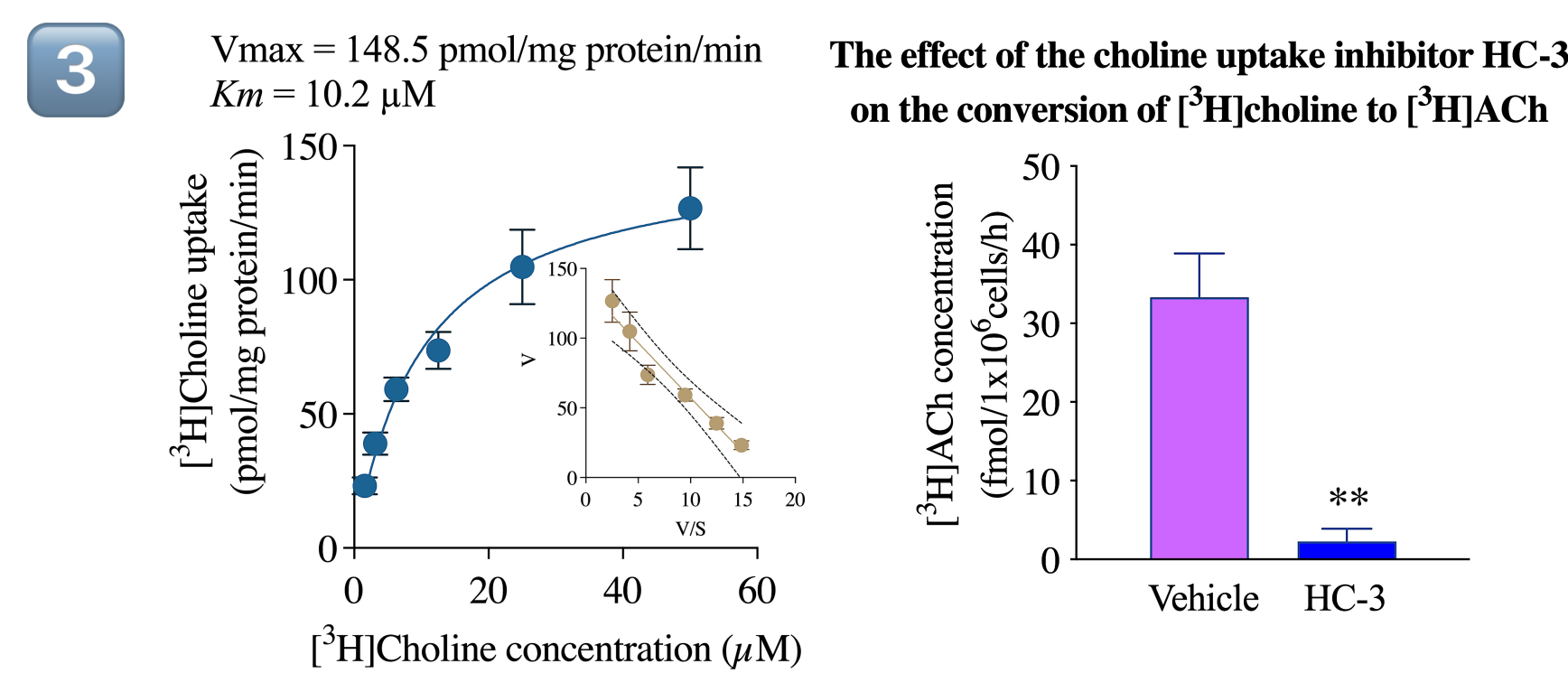
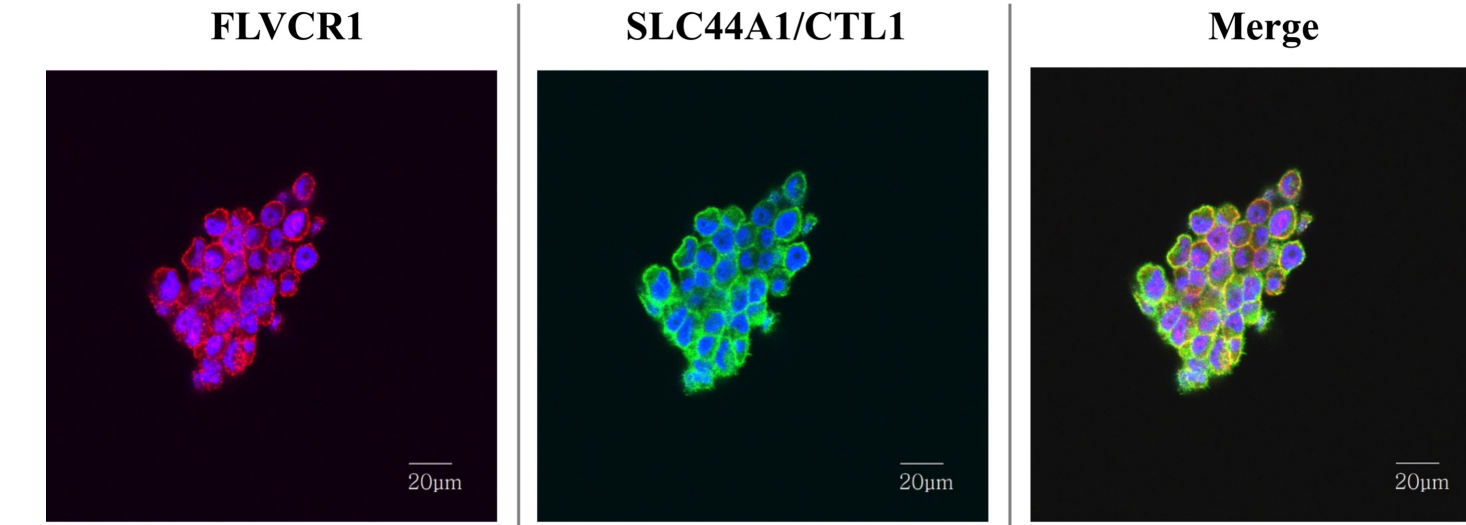
RESULTS

1 Expression of CTL1, FLVCR1 and ChAT mRNA in normal lung tissue and H69 cells



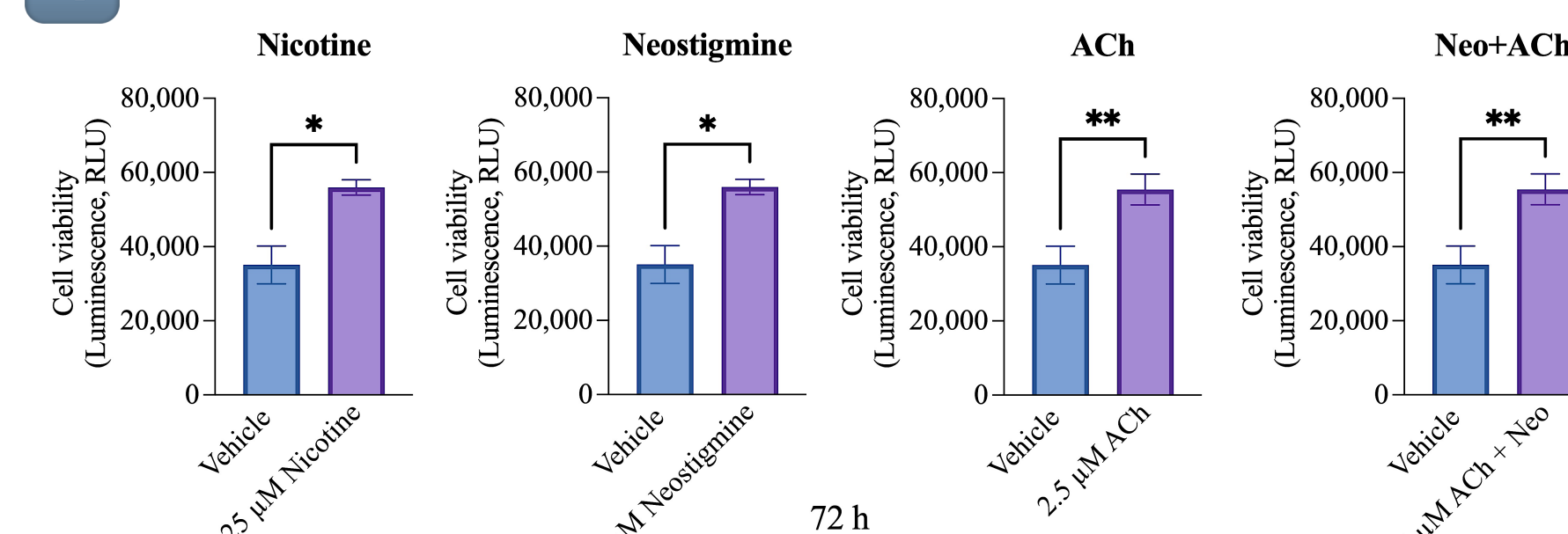
RESULTS

2 Expression of FLVCR1 and SLC44A1 (CTL1) in H69 cells



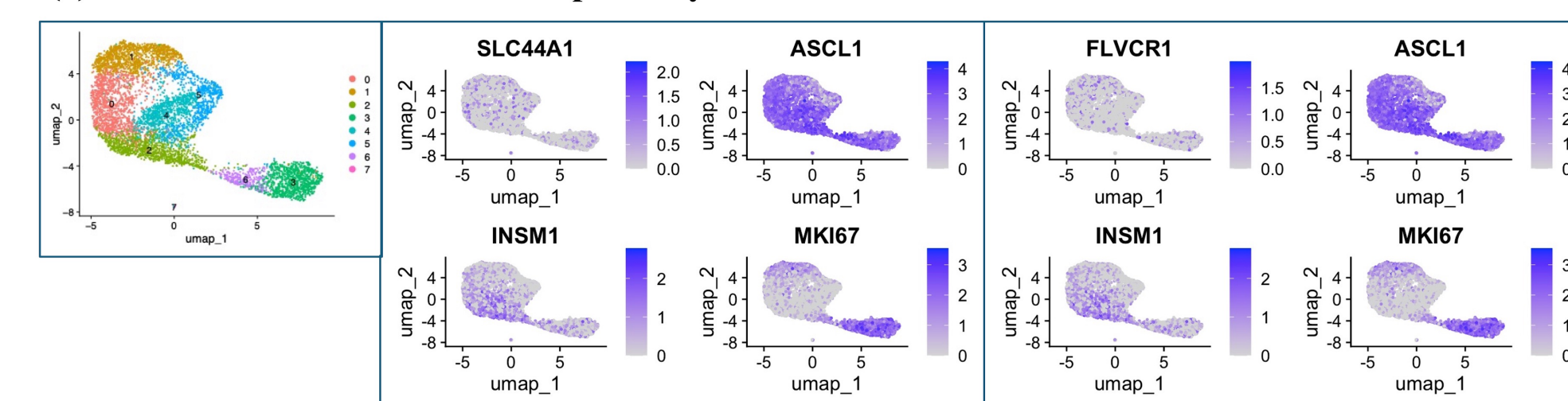
Functional cholinergic machinery is highly active in SCLC.

4 Effect of cholinergic systems on cell viability in H69 cells

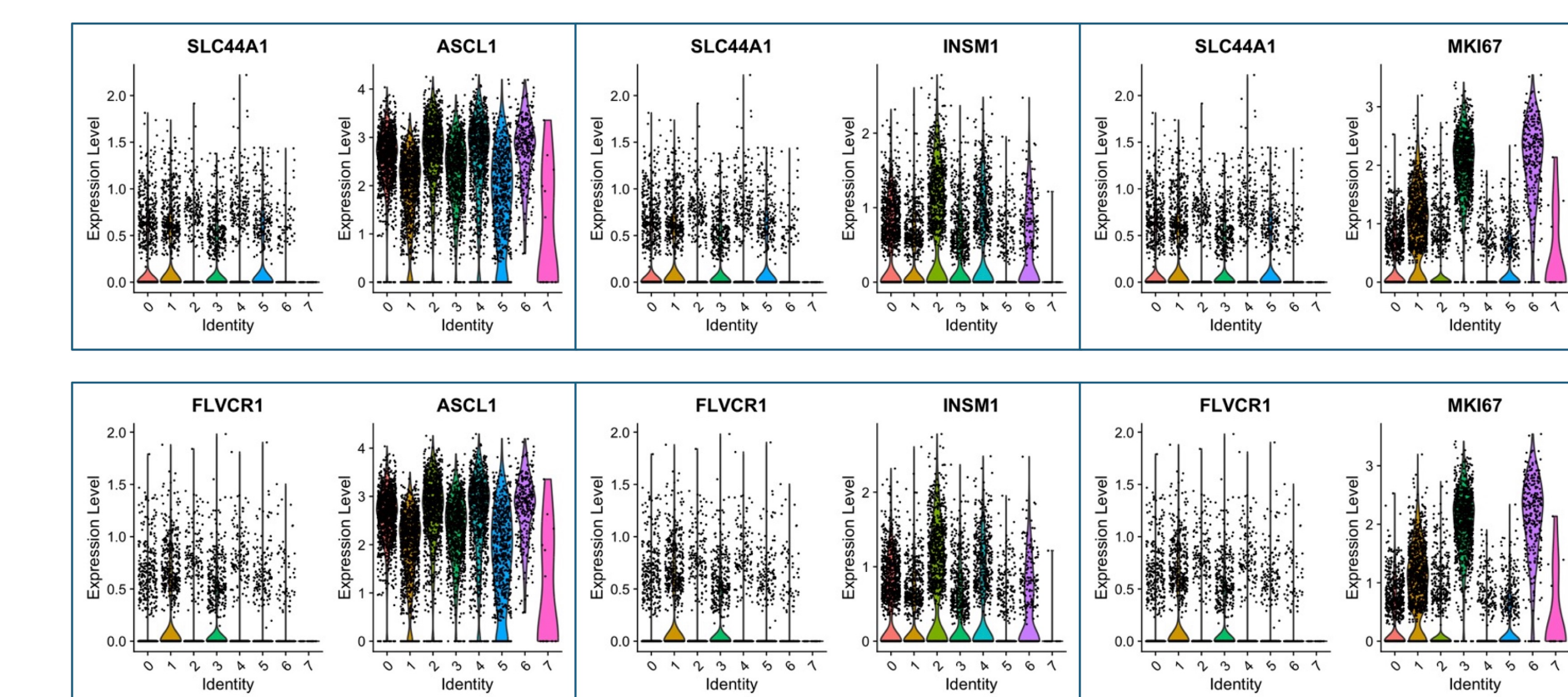


5 Cluster-restricted transporter expression revealed by single-cell RNA-seq

(1) UMAP visualization of transcriptionally distinct SCLC tumor cell clusters.



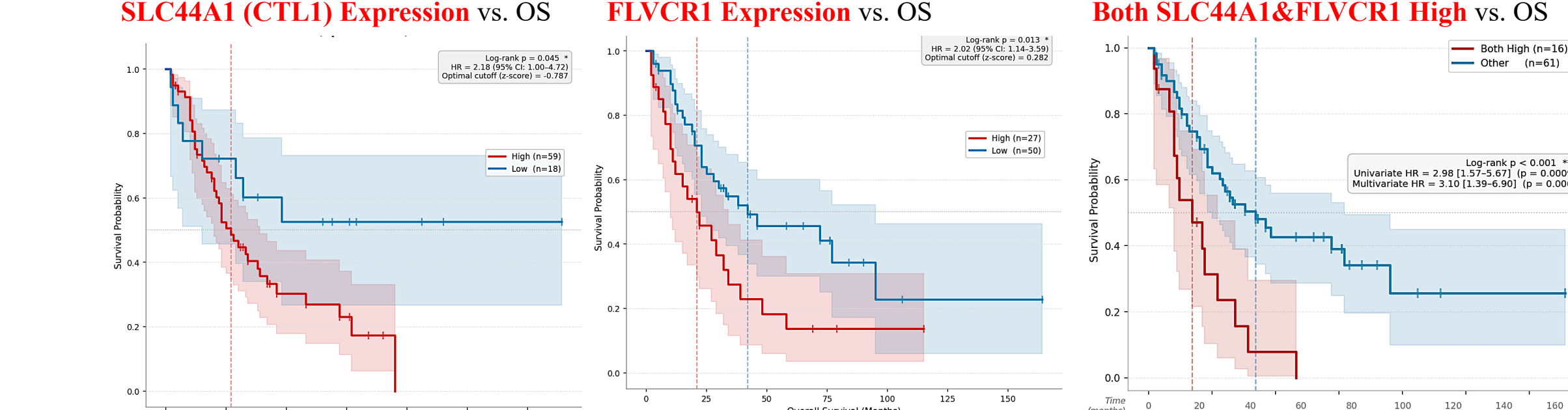
(2) Cluster-specific gene expression patterns showing enrichment of SLC44A1 and FLVCR1 in an ASCL1/INSM1- and MKI67-positive neuroendocrine-proliferative subpopulation.



Single-cell RNA-seq analysis revealed transcriptional heterogeneity within SCLC tumors. SLC44A1 and FLVCR1 expression was enriched within a discrete neuroendocrine-proliferative subpopulation marked by ASCL1/INSM1 and MKI67 expression, indicating spatial restriction of transporter co-dependency.

CTL1 and FLVCR1 are enriched in a neuroendocrine-proliferative tumor compartment.

6 Kaplan-Meier Survival Analysis – Small Cell Lung Cancer (sclc_ucologne_2015)

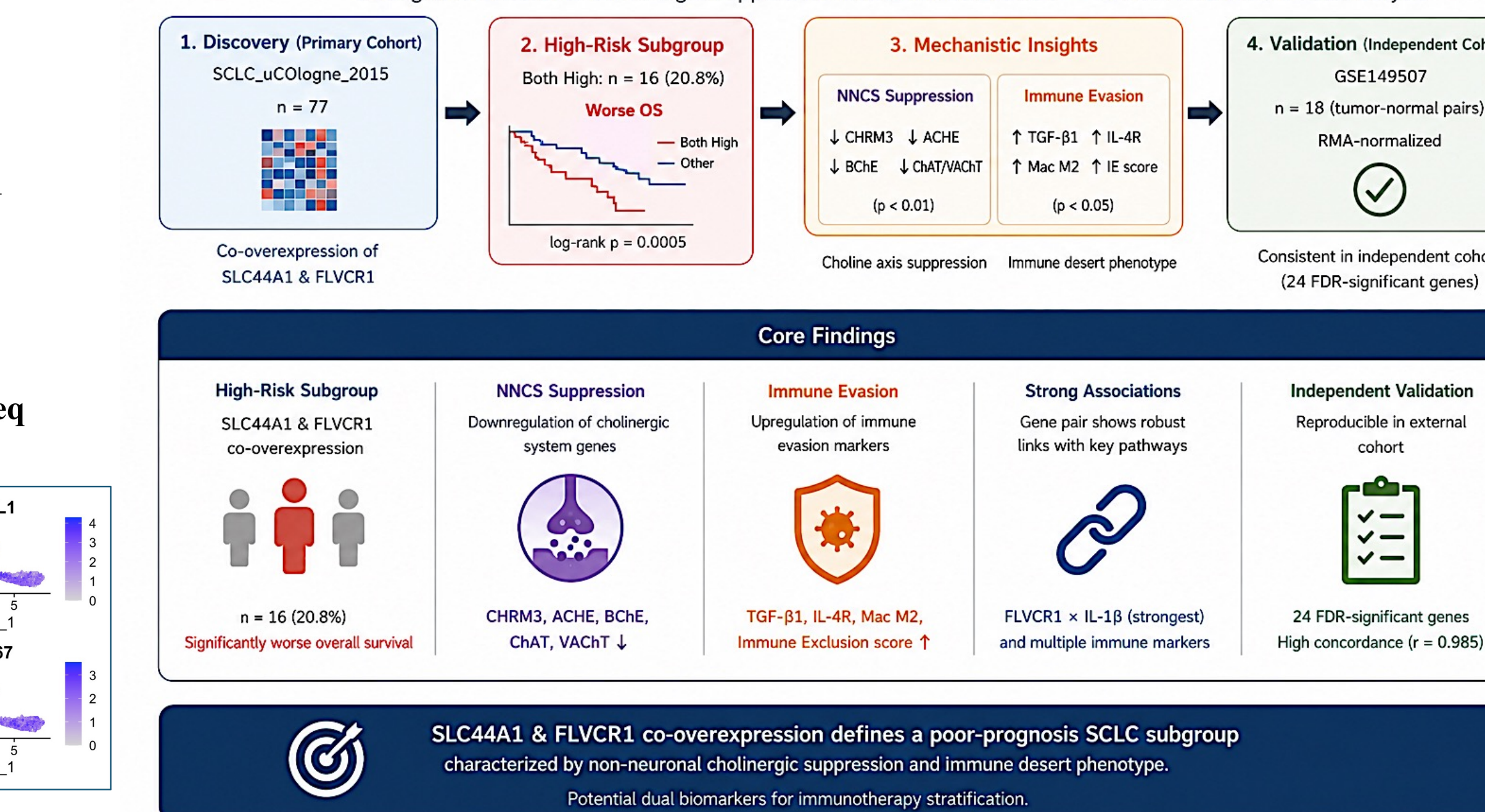


7 All primary-cohort findings were directionally and statistically replicated in GSE149507

Finding	Primary cohort (n = 77)	GSE149507 (n = 18)	Replicated
FLVCR1 tumor overexpression	Adverse prognosis, HR = 2.81	log ₂ FC = +1.31, p < 0.001†	✓
CHRM3 downregulation	Both High, p = 0.008	Tumor ↓, log ₂ FC = −0.89, p = 0.003†	✓
TGFB1 downregulation	Both High, p = 0.003	Tumor ↓, log ₂ FC = −0.51, p < 0.001†	✓
M2-macrophage score reduction	Both High, p = 0.020	CD163 ↓ MRC1 ↓ (each FDR†)	✓
FLVCR1 × TGF-β1	r = −0.45, FDR†	r = −0.56, p = 0.015	✓
SLC44A1 × PD-1	r = −0.29, ns	r = −0.76, p < 0.001†	✓ (amplified)
FLVCR1 × PD-L1	r = −0.49, ns	r = −0.79, p < 0.001†	✓ (amplified)

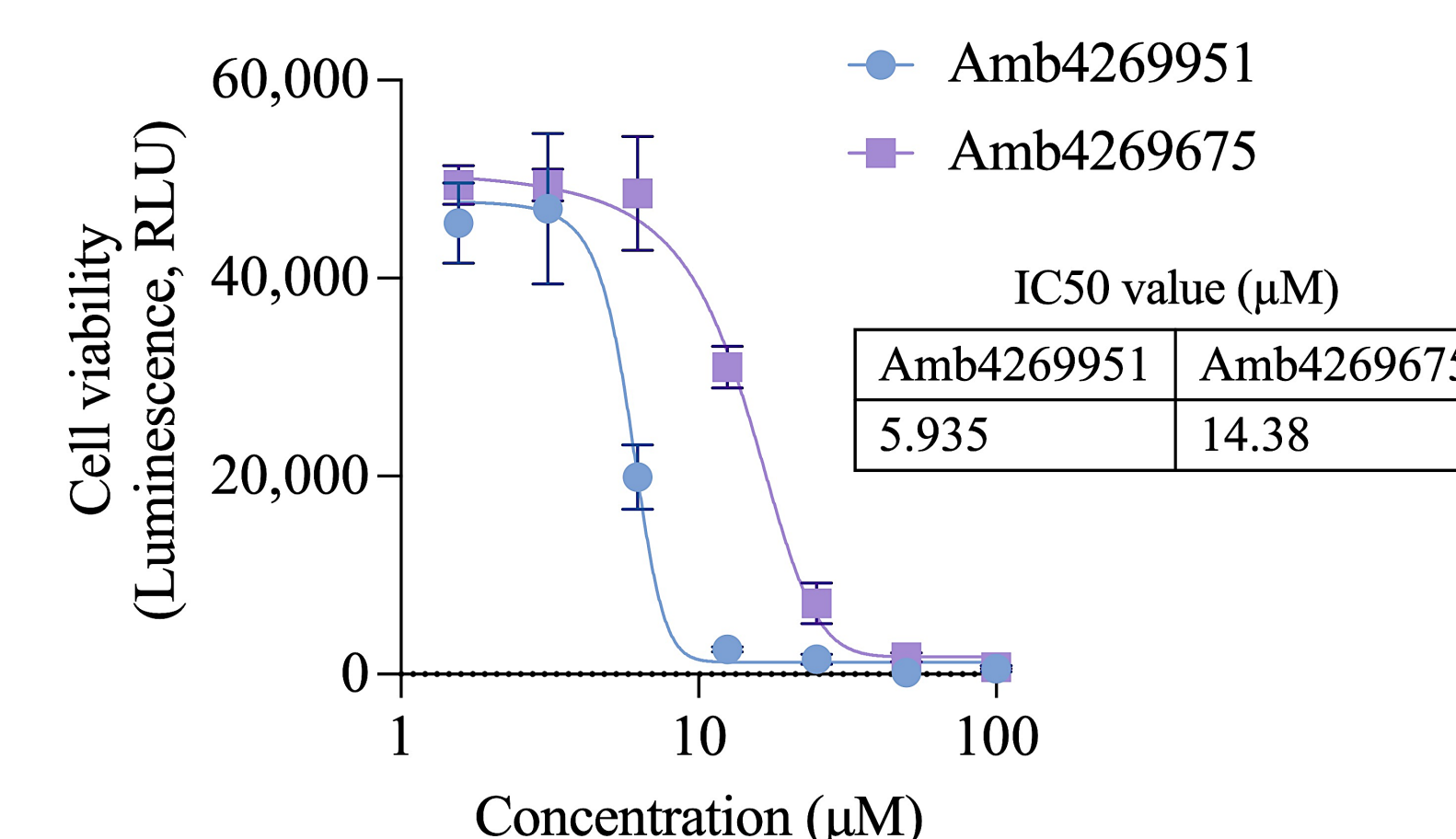
SLC44A1/FLVCR1-PD-1/PD-L1 negative correlations were substantially stronger in the validation cohort (r = −0.76 to −0.79 vs. non-significant in the primary cohort), reinforcing the **immune-desert hypothesis** with independent evidence from a distinct platform and patient population.

8 SLC44A1 & FLVCR1 Co-overexpression Defines a High-Risk SCLC Subgroup



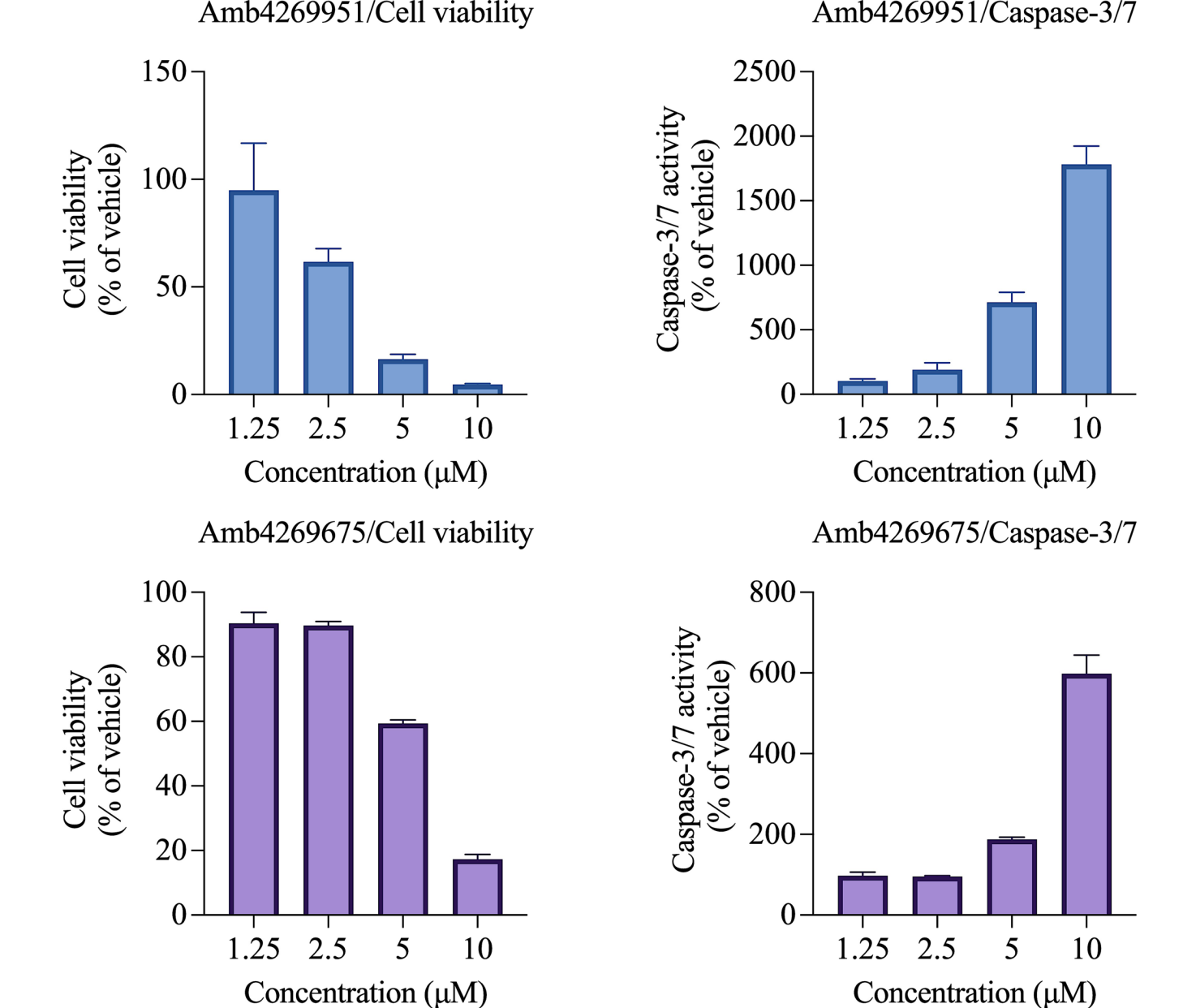
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9 Effect of choline uptake inhibitors on cell viability in H82 cells



RESULTS

10 Effect of choline uptake inhibitors on cell viability and Caspase-3/7 activity in H69 cells



DISCUSSION AND CONCLUSION

The non-neuronal cholinergic system represents a lineage-associated metabolic dependency in SCLC. CTL1 and FLVCR1 are highly expressed, co-localized, and functionally active in neuroendocrine-proliferative tumor cells, where they support cholinergic signaling and tumor growth. Their co-overexpression identifies a clinically aggressive subgroup characterized by poor survival, immune-desert features, and reproducible transcriptomic alterations across independent cohorts. Pharmacologic inhibition of choline transport suppresses cell viability and induces apoptosis, highlighting CTL1/FLVCR1-mediated choline transport as a promising therapeutic target for refractory SCLC.

TAKE-HOME MESSAGE

CTL1 and FLVCR1 define a biologically distinct, poor-prognosis SCLC subtype characterized by neuroendocrine proliferation, immune exclusion, and dependence on choline transport.

Targeting CTL1/FLVCR1-mediated choline transport represents a novel and actionable therapeutic strategy for refractory SCLC.

REFERENCES & ACKNOWLEDGEMENTS

References:

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- Inazu M, Yamada T, Kubota N, Yamanaka T. Functional expression of choline transporter-like protein 1 (CTL1) in small cell lung carcinoma cells: a target molecule for lung cancer therapy. Pharmacol Res. 2013, 76:119-31.

AI use disclosure:

Generative AI (Claude Anthropic and ChatGPT) assisted with bioinformatic code generation. All analyses, interpretations, and conclusions are the sole responsibility of the authors.