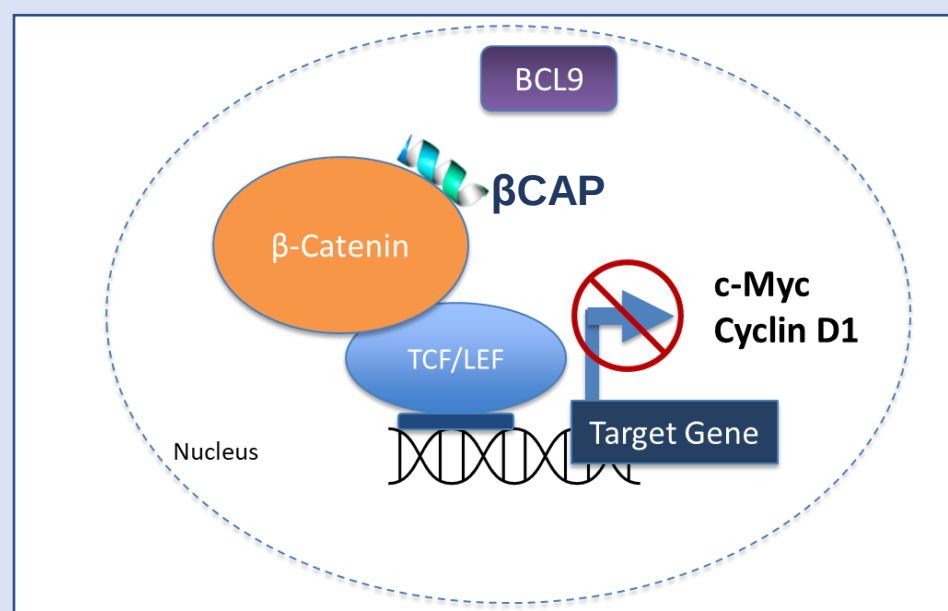


Introduction

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Canonical Wnt signaling pathway has historically been considered an “undruggable” cancer target, as small molecule approaches have proven ineffective or toxic. Activating mutations in the Wnt/ β -catenin signaling pathway drive cancer initiation and contribute to tumor growth, angiogenesis and metastasis in many solid tumors and hematological malignancies. BCL9 is a co-factor of β -catenin that promotes tumor progression by conferring enhanced proliferative, metastatic, and angiogenic properties to cancer cells.



Antagonism of the BCL9/ β -Catenin interaction suppresses oncogenic Wnt gene transactivation, while not disrupting interactions with destruction complex components such as APC or factors involved in β -catenin homeostatic functions such as TCF4 and E-cadherin. Sapience has designed β -catenin antagonist peptides (β CAPs) to inhibit β -catenin’s interaction with BCL9 and impair tumor cell proliferation and survival. Here, we report the anti-tumor activity of a β CAP candidate both in vitro and in vivo.

Abstract

β -catenin is an oncogenic transcription factor that has an essential role in cell development and biology. Increased β -catenin expression or activity has been implicated in many cancers, including breast and colorectal, and is associated with poor prognosis. Despite extensive ongoing research, β -catenin is conventionally considered ‘undruggable’, and there are no current drugs available to target Wnt/ β -catenin signaling. B-cell CLL/lymphoma 9 protein (BCL9) is a positive regulator of β -catenin transcriptional activity that is overexpressed in tumors. Disruption of β -catenin interaction with BCL9 has been shown genetically to suppress oncogenic Wnt/ β -catenin transcription and represents a powerful approach to inhibit this previously undruggable target. Here, we describe the anti-tumor activity of a β -catenin antagonist peptide (β CAP), designed to disrupt the association of β -catenin with co-activator BCL9. Target engagement of β CAP with recombinant β -catenin was demonstrated by circular dichroism spectroscopy and inhibition of the β -catenin/BCL9 interaction was demonstrated in transiently transfected RKO cells by NanoBRET assay. Administration of β CAP to HEK293 cells stably transfected with TOPFlash reporter plasmid resulted in dose-dependent inhibition with an EC_{50} of 1.2 μ M. Further, β CAP attenuated oncogenic gene transactivation in colorectal cancer (CRC) cells (HCT116 and COLO320DM) and HR^{POS} breast cancer cells (MCF7), significantly decreasing the expression of Axin2 and CDK4 ($p < 0.05$), while not impacting Axin2 or CDK4 gene expression in Wnt-independent RKO and NCI-H460 cells. Administration of β CAP to 4T1-luc triple-negative breast cancer, HCT116 and COLO320DM cells resulted in dose-dependent in vitro cytotoxicity 48 hours post exposure, with EC_{50} values of 1.4, 4.4 and 1.2 μ M, respectively. In an in vivo orthotopic 4T1-luc tumor model, administration of β CAP via subcutaneous injection resulted in dose-dependent inhibition of tumor growth and significantly reduced tumor volume compared to control tumors ($p < 0.01$). These data demonstrate the significant in vitro and in vivo anti-cancer activity of β CAP and emphasize its potential as a potent therapeutic for Wnt/ β -catenin-dependent cancers.

Results

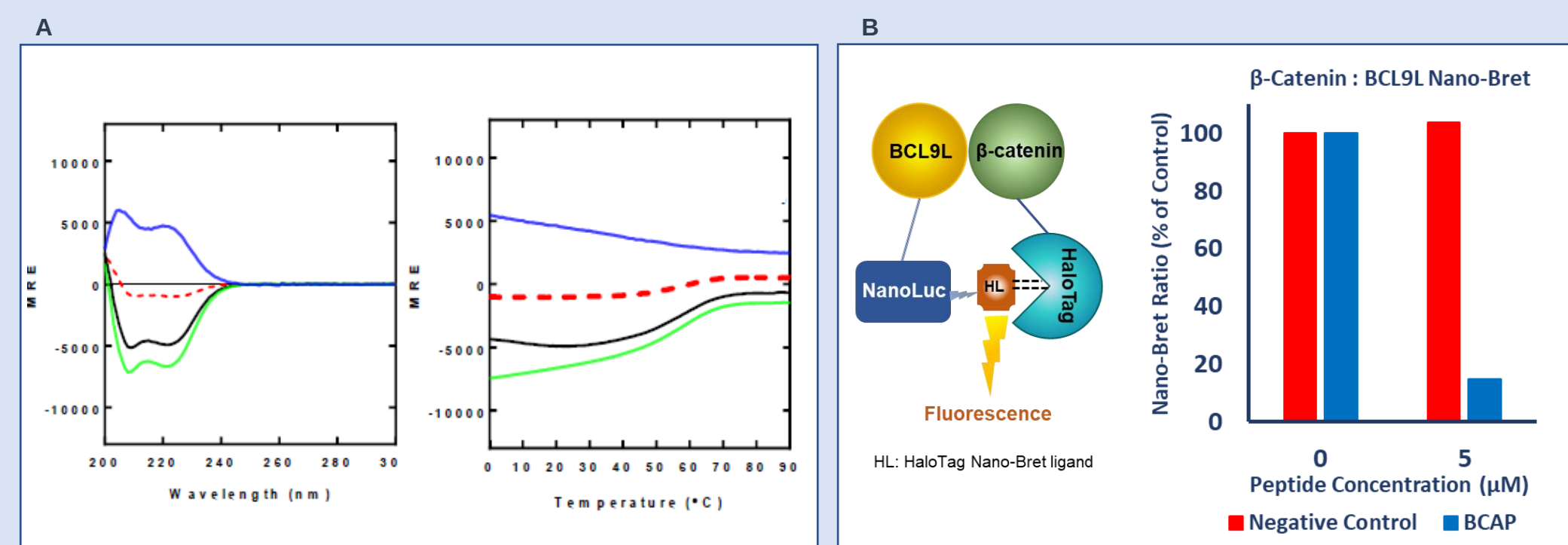


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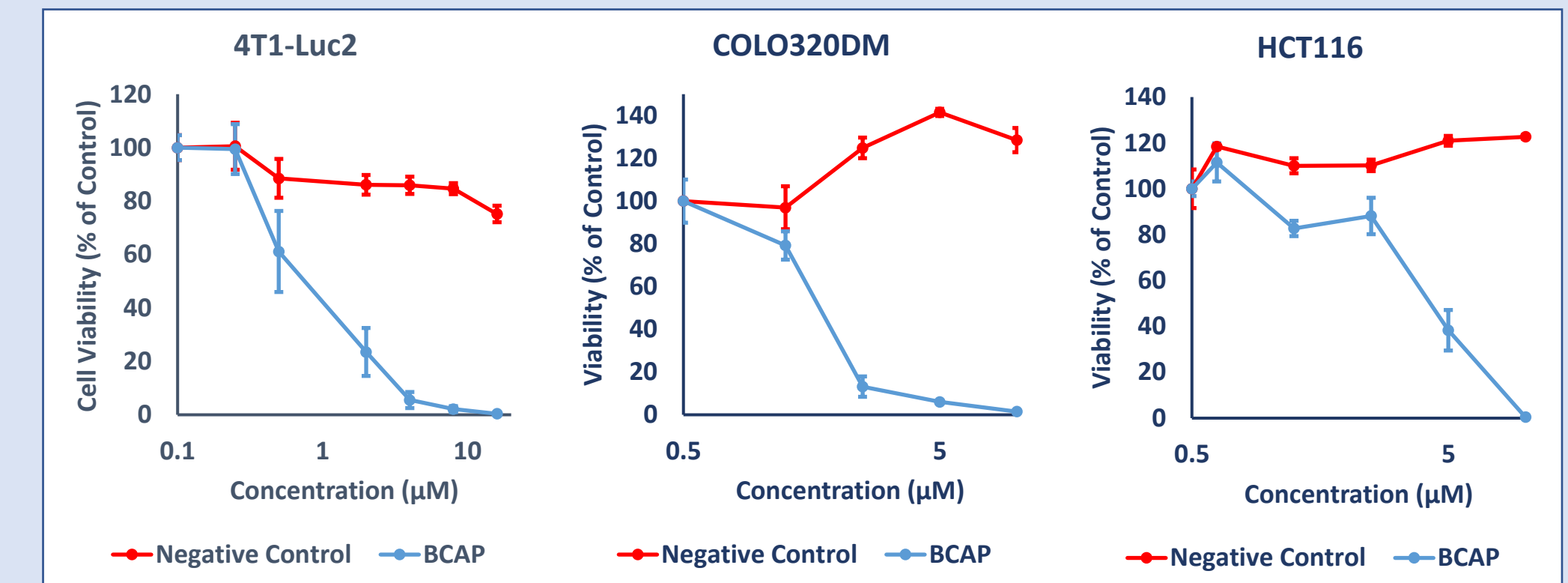


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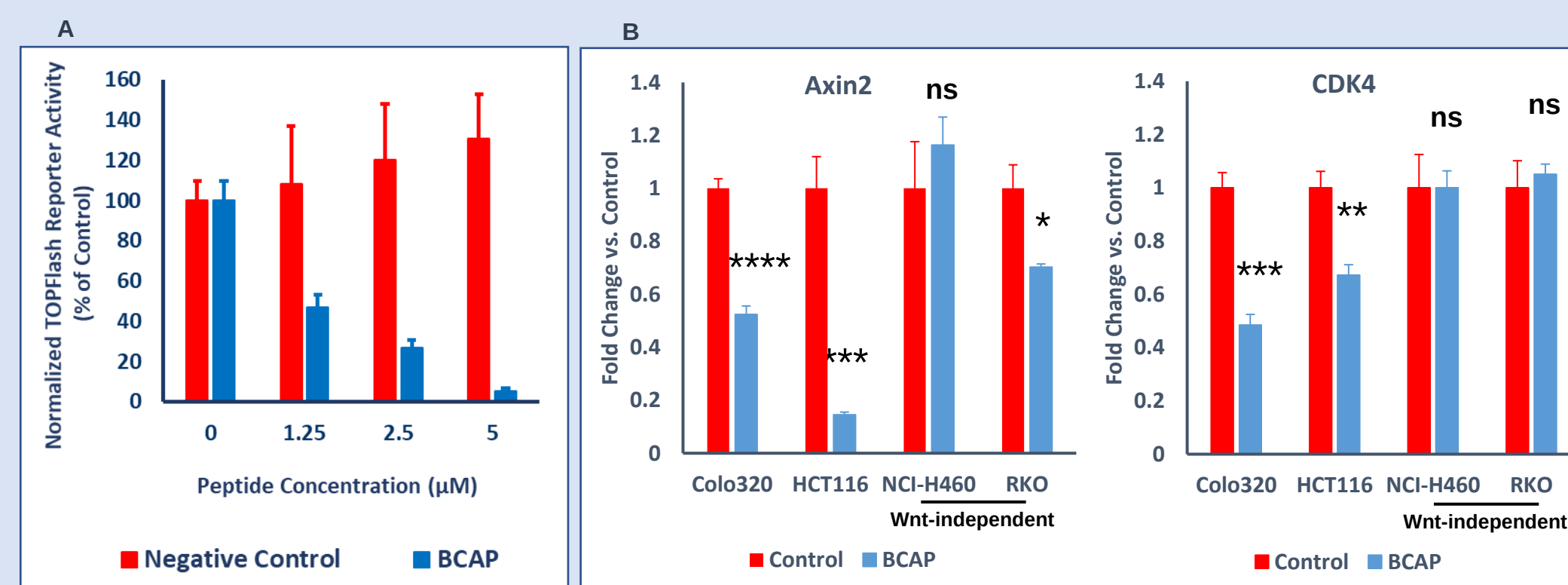


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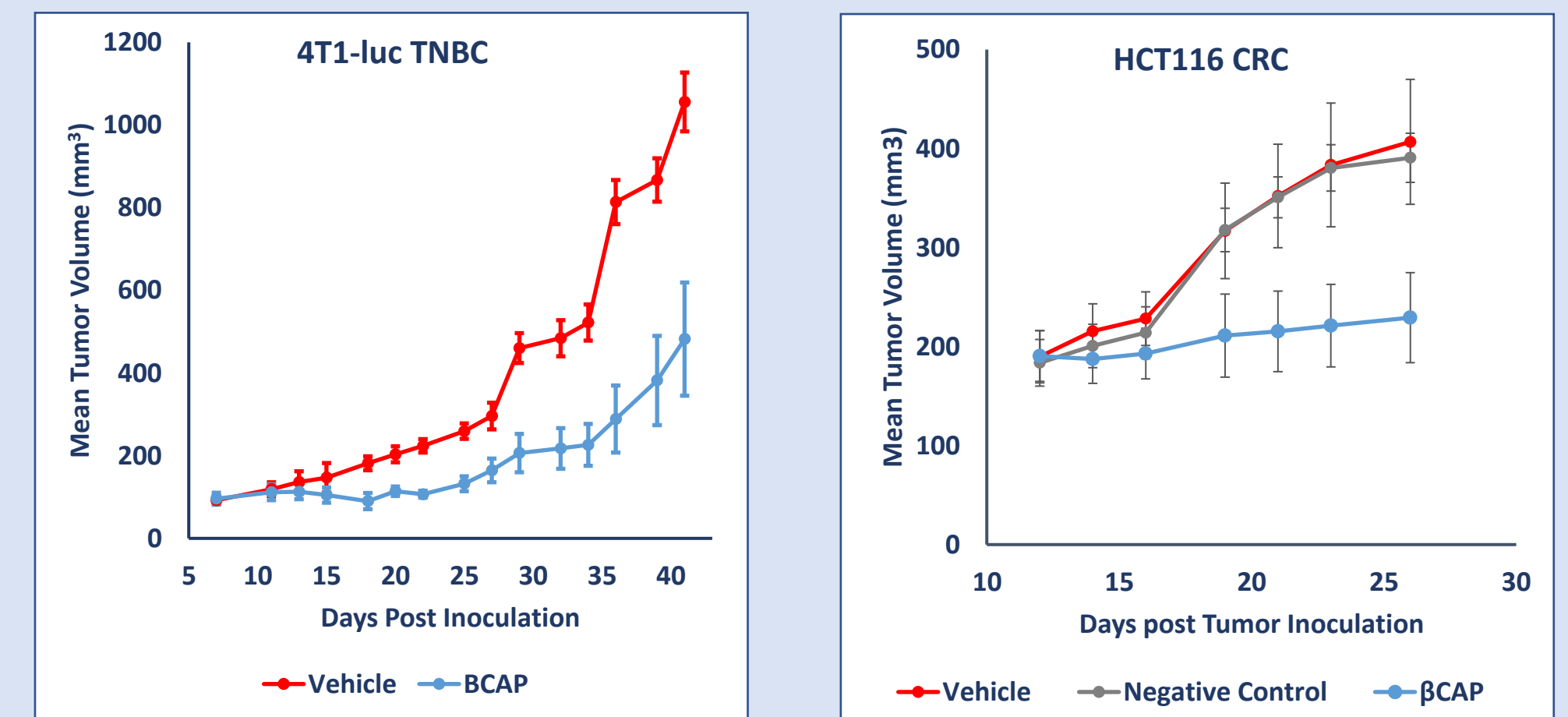


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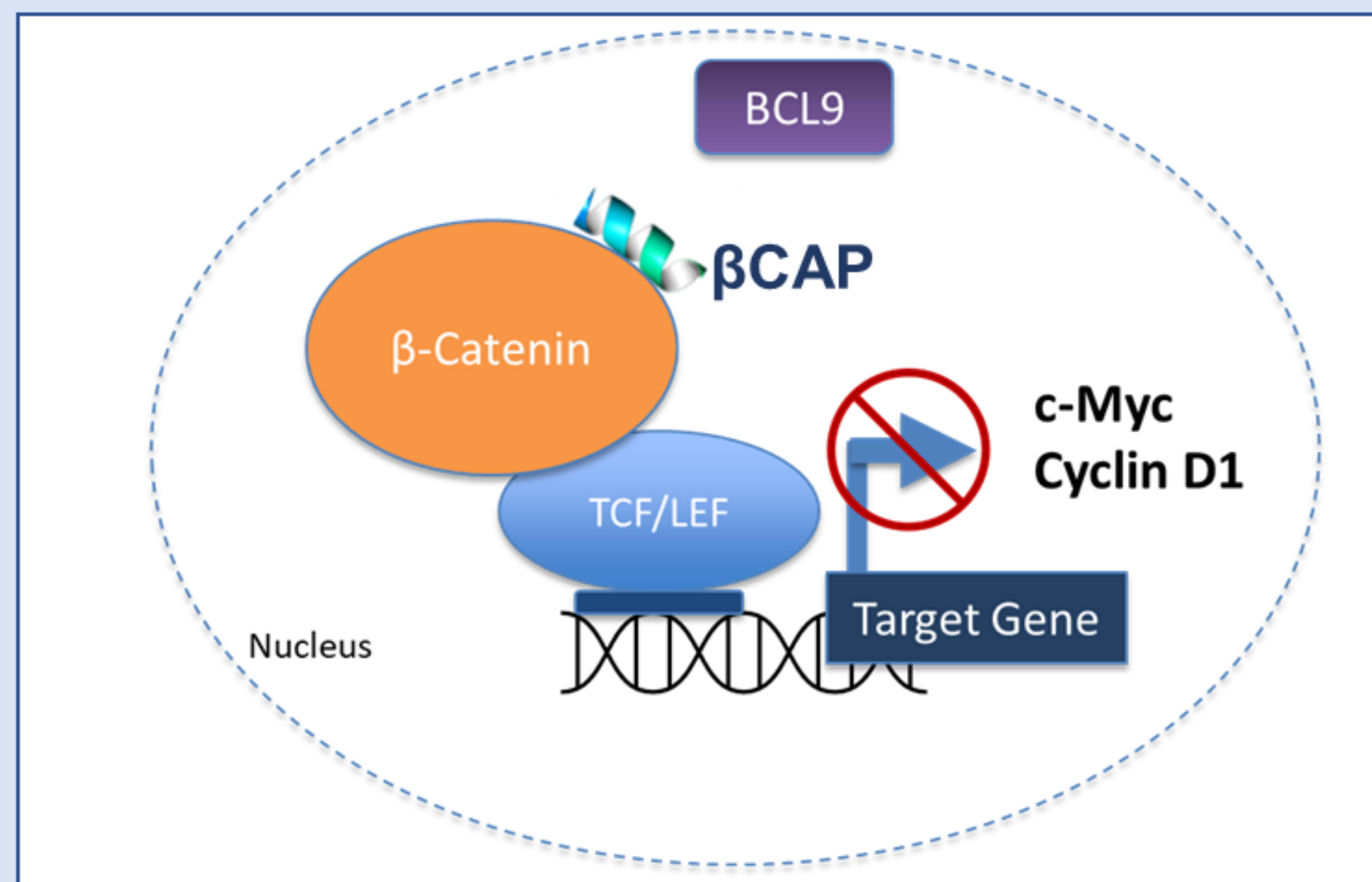
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- β -catenin antagonist peptide (β CAP) interacts with β -catenin (demonstrated by CD) and disrupts its interaction with BCL9 (evidenced by Nano-BRET signal reduction).
- β CAP inhibits the Wnt pathway and reduces Wnt-driven gene expression, as determined by TOPflash reporter assay and qPCR analysis. A statistically significant suppression of target gene transactivation in Wnt-dependent cell lines vs. Wnt-independent cell lines provides evidence of β CAP functional specificity.
- β CAP demonstrates potent cytotoxic activity against breast cancer and CRC cell lines with EC_{50} values of < 5 μ M.
- β CAP causes significant tumor growth delay in orthotopic triple-negative breast cancer and subcutaneous colorectal cancer xenograft models.
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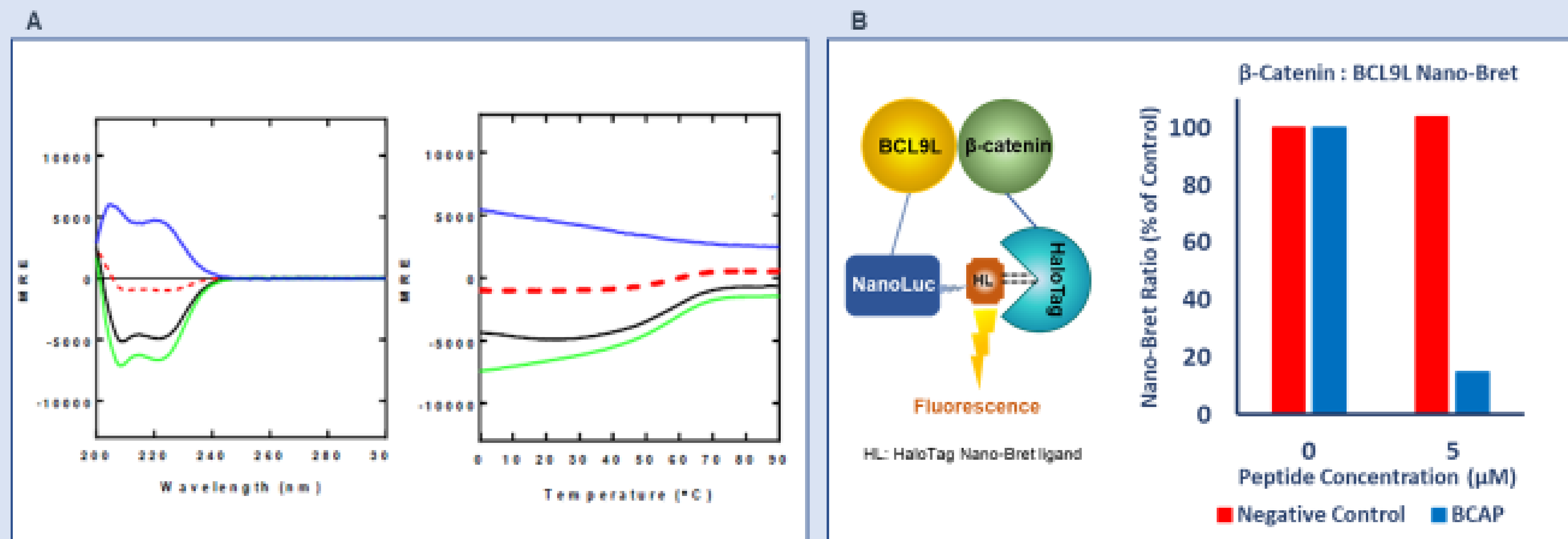


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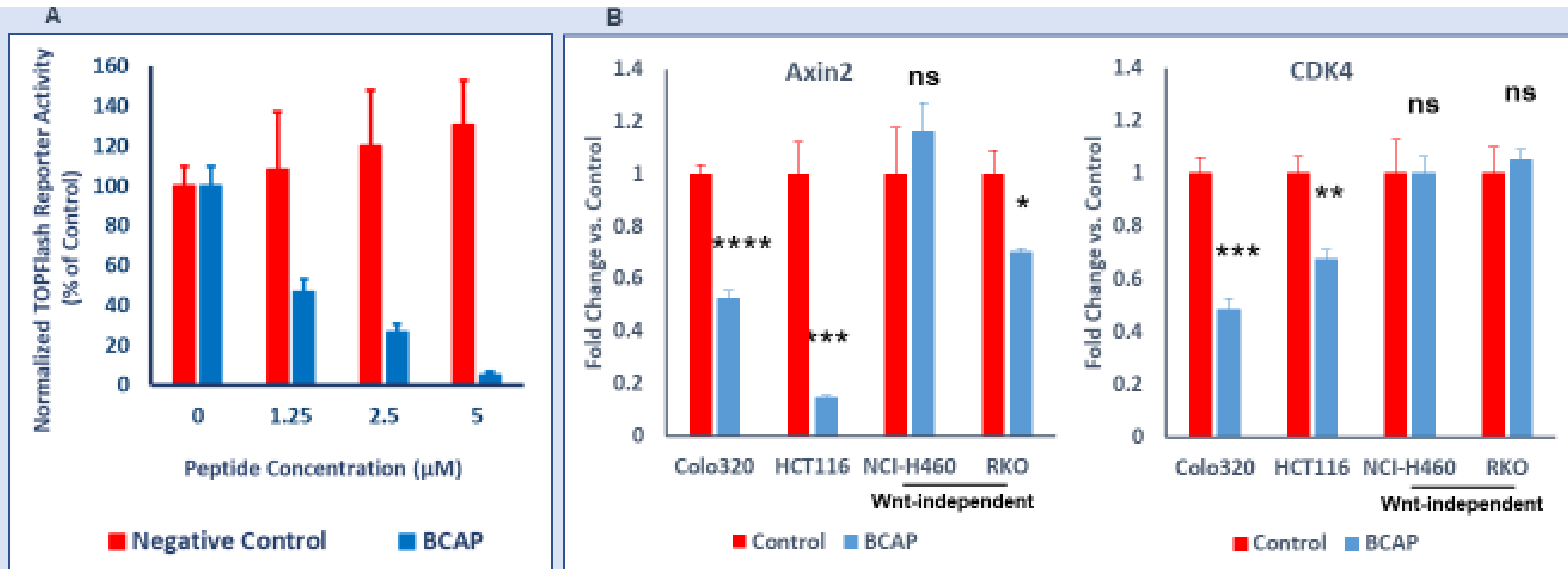


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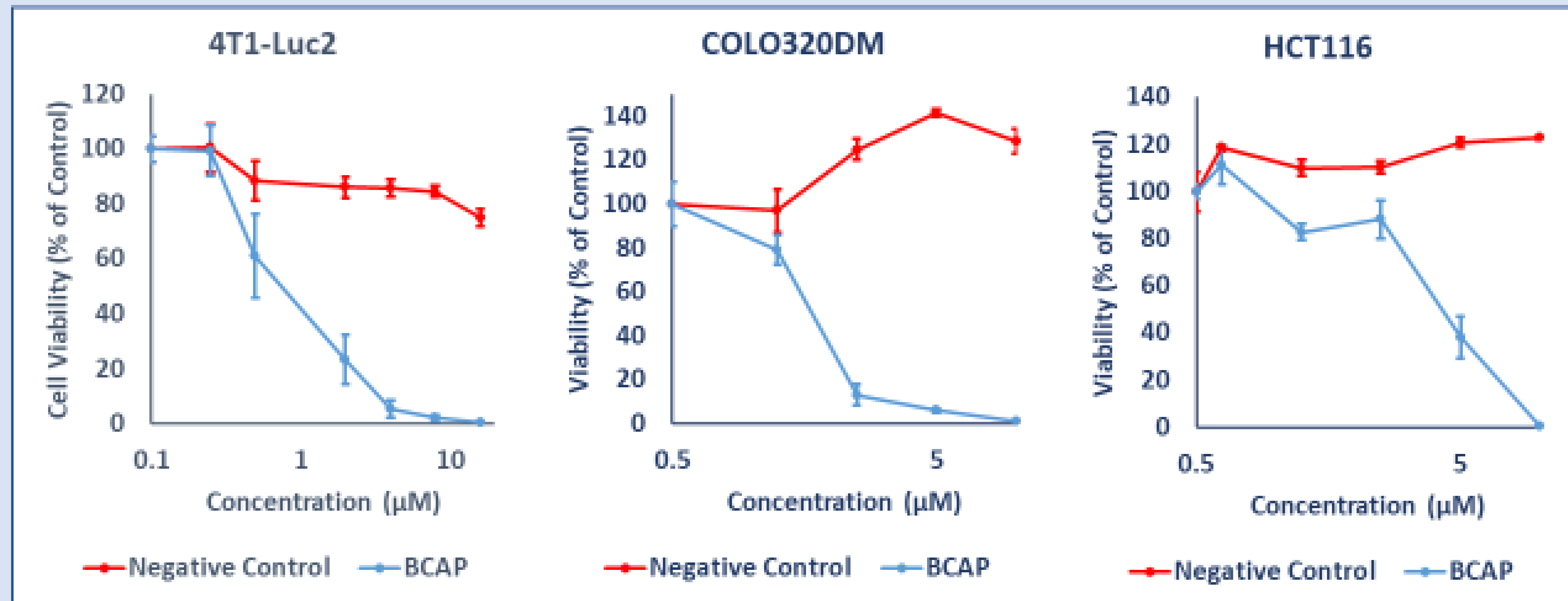


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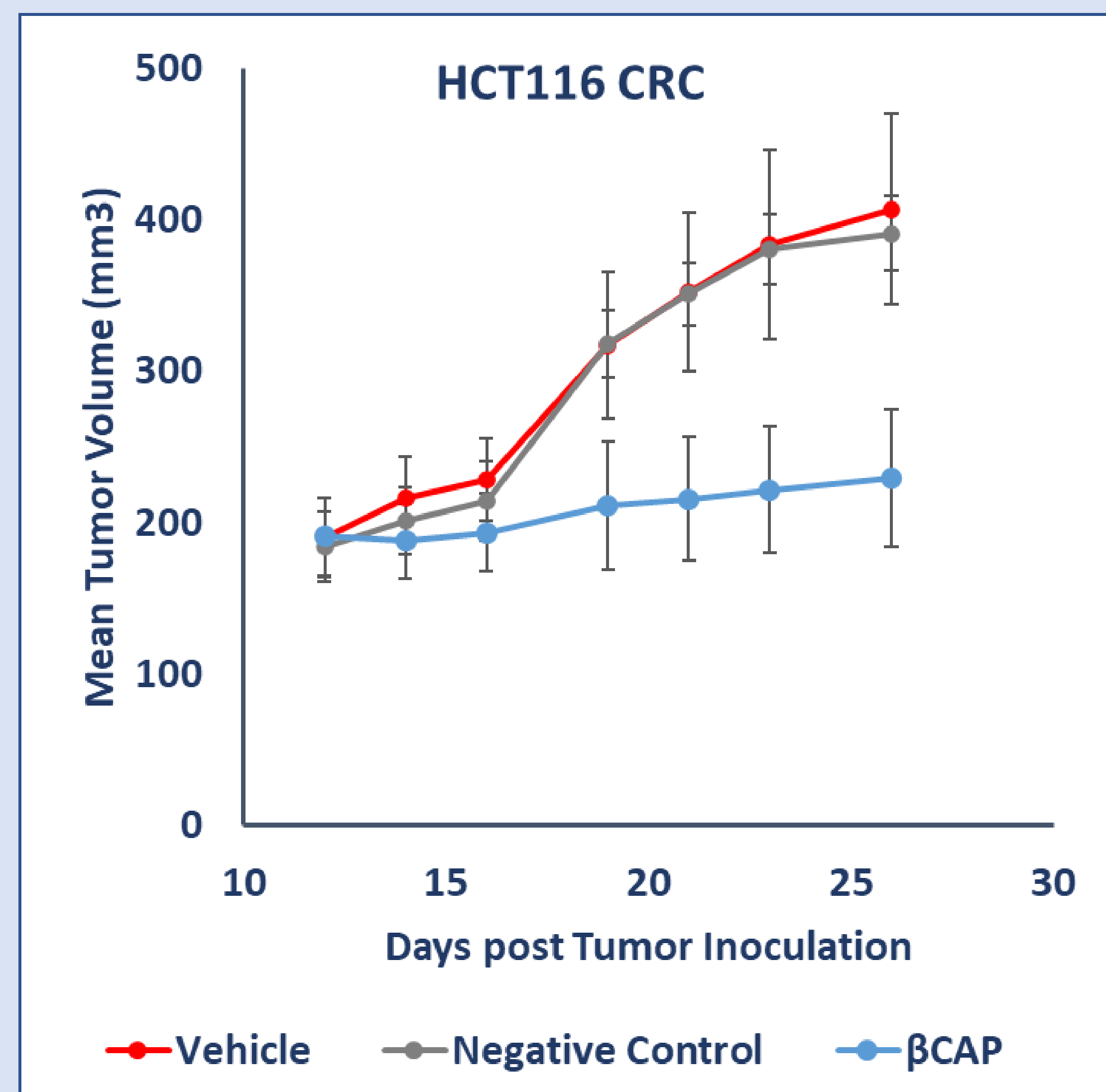
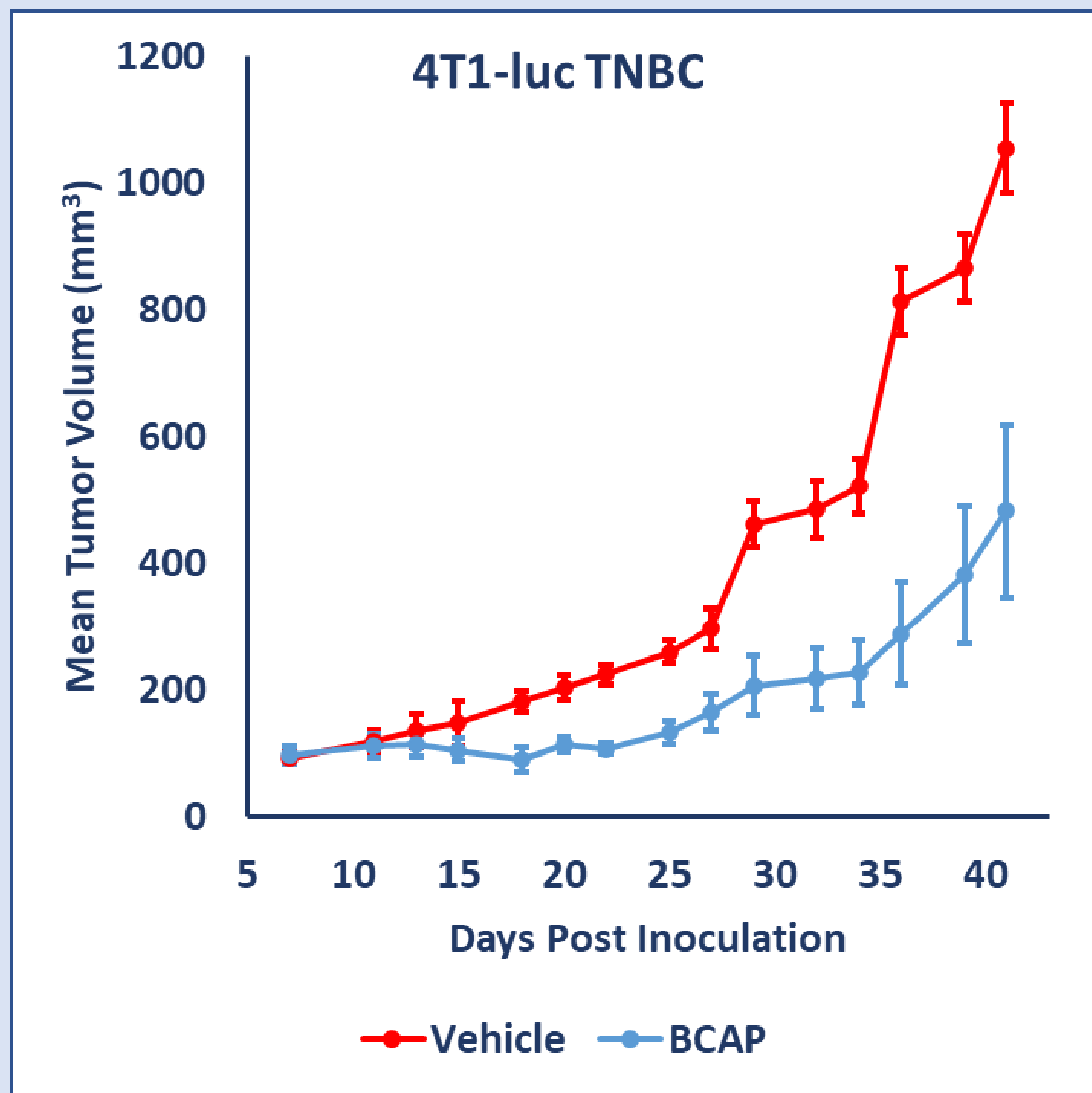
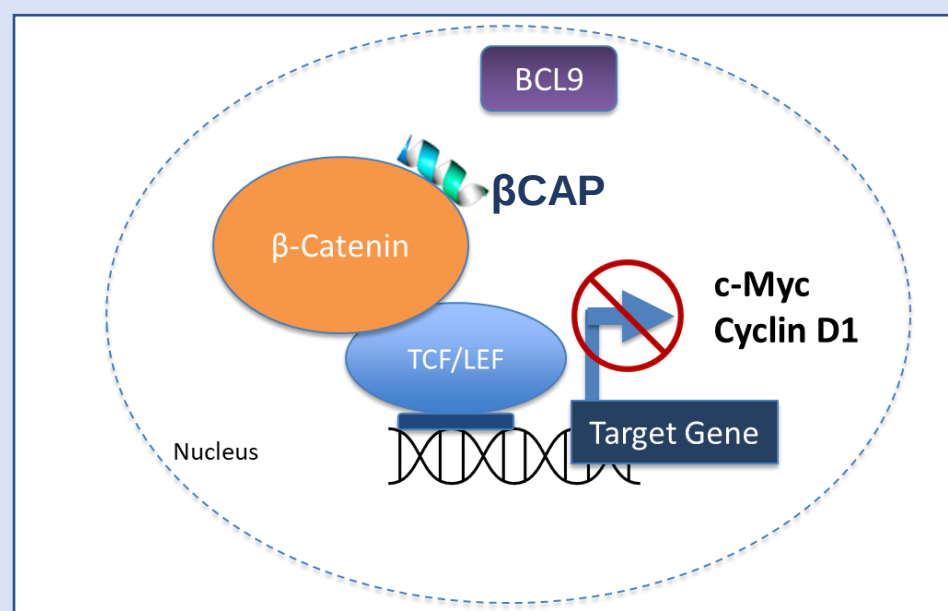


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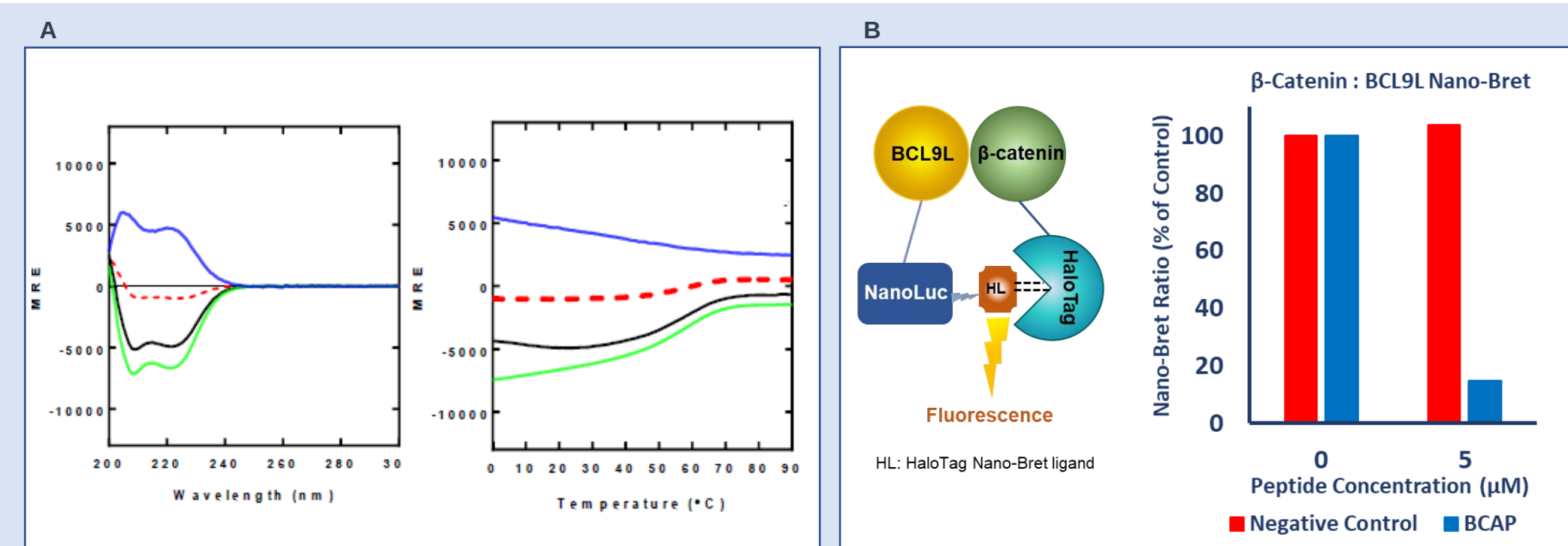


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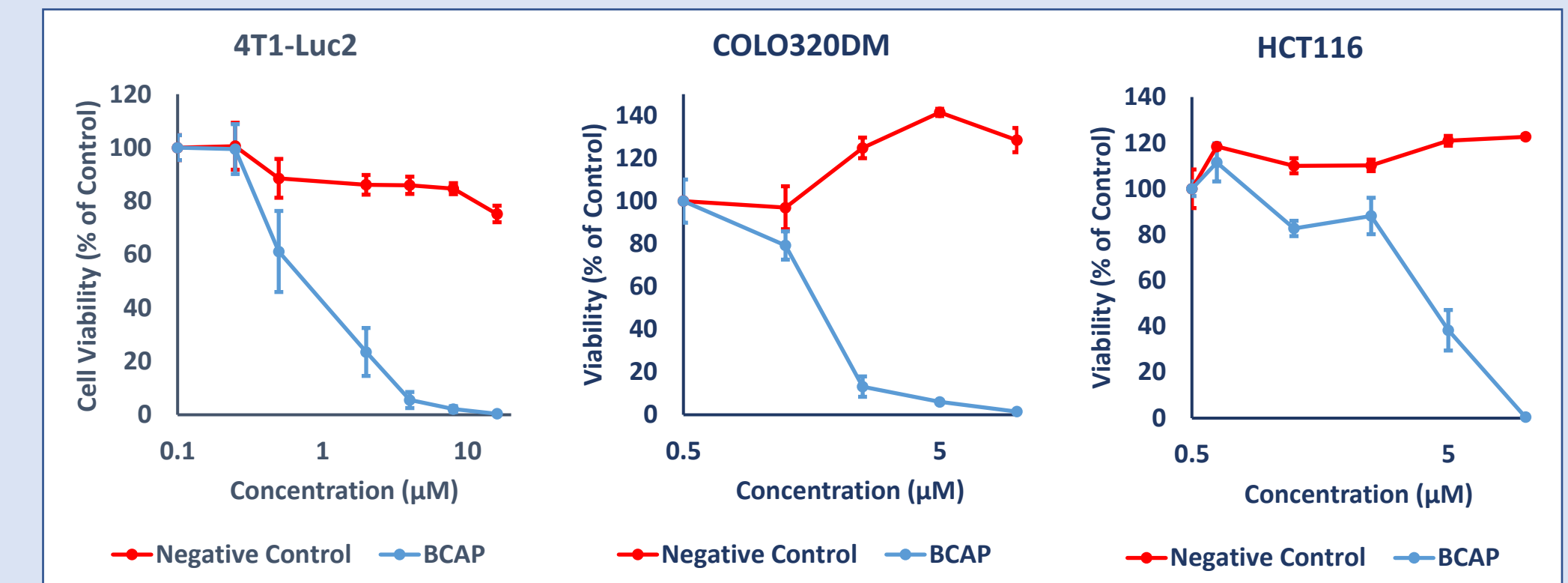


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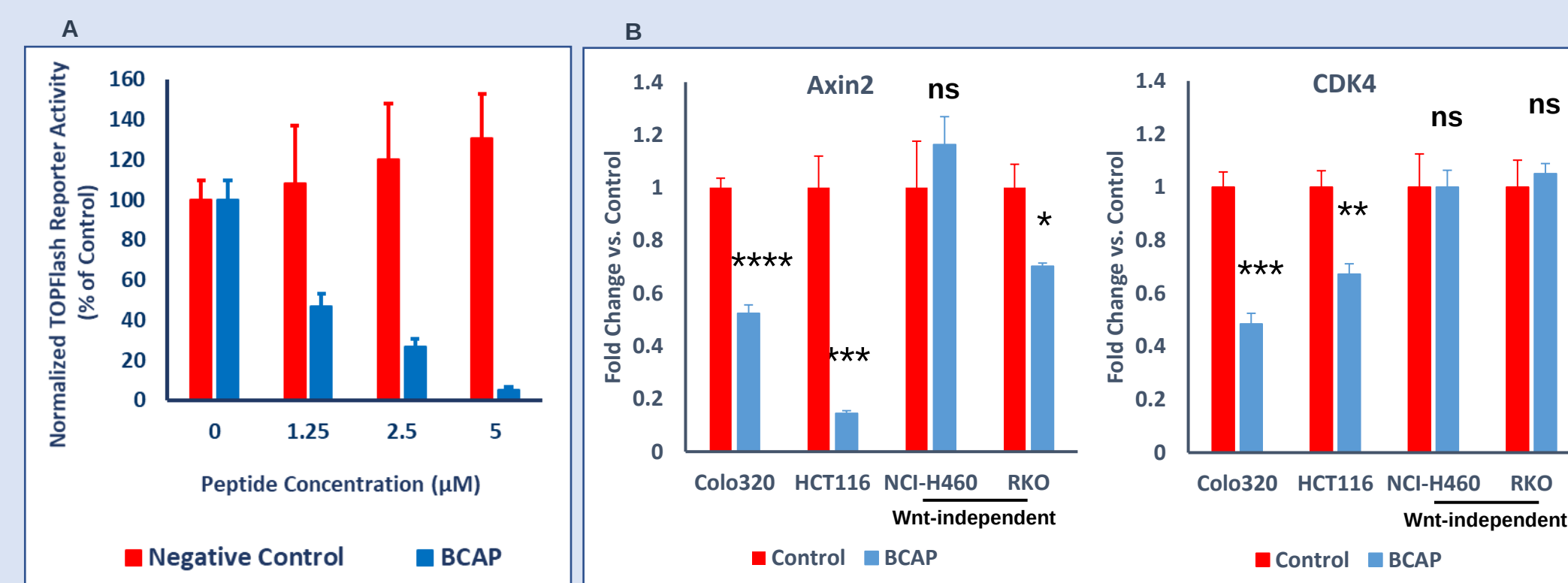


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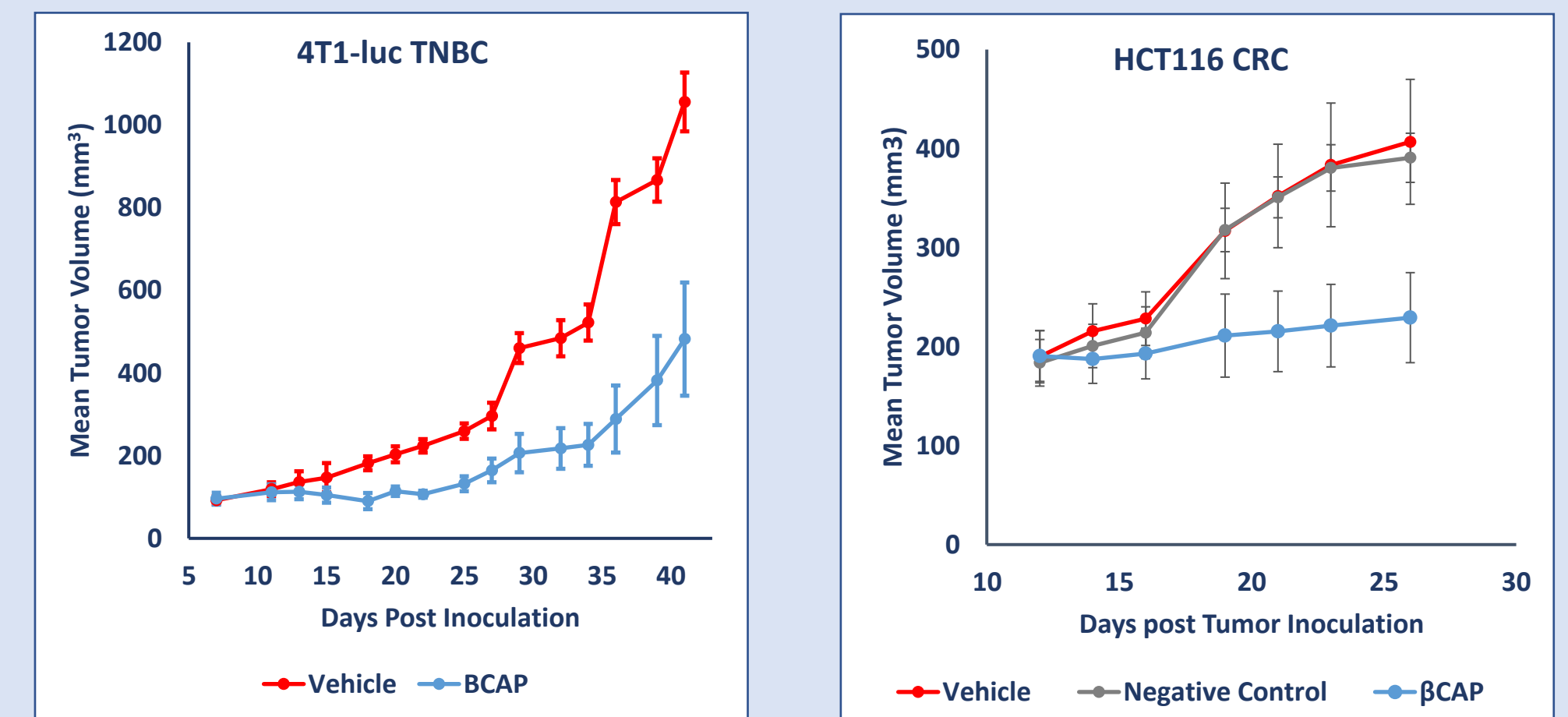


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