SELECTIVE DUAL INHIBITION OF β-CATENIN IN WNT-DRIVEN CANCERS VIA Gid8-ASSISTED TRANSLOCATION AND A PRECISION PEPTIDE-BASED GENE THERAPY

INTRODUCTION

- Hyperactive Wnt signaling drives ~90% of hepatocellular carcinoma (HCC) cases, yet remains "undruggable"[1-3] (Fig. 1A)
  - The hallmark mechanism, mutant β-catenin (ArmS10 in *Drosophila*) nuclear translocation, remains unclear[4, 5]
- Gid8 has been shown to affect normal Wnt signaling, but its role in cancerous, hyperactive Wnt signaling is unstudied[6] (Fig. 1B)
- **What role does Gid8 play in regulating mutant β-catenin nuclear translocation in cancers?**
- In liver cells, β-catenin acts at the **membrane to maintain adherens junctions/polarity** and in the **nucleus to drive proliferation/cancer**[7]
- A β-catenin$^{24\text{-}79}$ peptide has been shown to suppress Wnt signaling[8] (Fig. 1C)
- The full-length peptide is too large for selective targeting of nuclear β-catenin while preserving wild-type β-catenin, a major roadblock in cancer therapy[8]
- **Does truncating the β-catenin$^{24\text{-}79}$ peptide enable selective inhibition?**

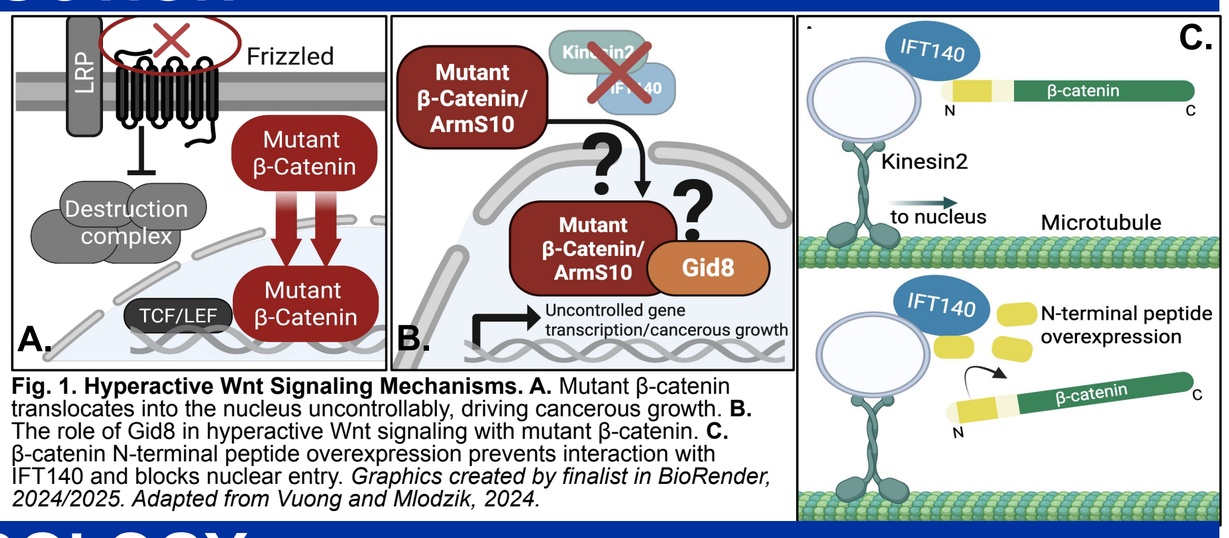

Fig. 1. Hyperactive Wnt Signaling Mechanisms. A. Mutant β-catenin translocates into the nucleus uncontrollably, driving cancerous growth. **B.** The role of Gid8 in hyperactive Wnt signaling with mutant β-catenin. **C.** β-catenin N-terminal peptide overexpression prevents interaction with IFT140 and blocks nuclear entry. *Graphics created by finalist in BioRender, 2024/2025. Adapted from Vuong and Mlodzik, 2024.*

METHODOLOGY

Phase 1:

1. Gal4/UAS Drosophila crosses were performed (4 experimental groups)
2. Offspring's adult wings, wing discs, and salivary glands were dissected
3. Immunofluorescence staining was conducted
4. Co-IP western blot between Gid8 and Arm/ArmS10 was performed[9]
5. HEK293 cells were transfected with Gid8 and Gid8* (with C-terminal 3 mutations)

Phase 2:

1. β-catenin$^{24\text{-}79}$ peptide and 3 truncated fragments within the peptide were cloned
2. Huh7, HepG2, and Hep3B cells were cultured and transfected with each peptide construct separately[10]
3. MTT assays, immunofluorescence staining, and Western blots were performed to quantify hyperactive Wnt activity

RESULTS AND DISCUSSION

Gid8 REGULATES HYPERACTIVE WNT SIGNALING

- Adult wings: Knockdown or underexpression of Gid8 **rescued** ArmS10-induced excessive hair growth, an indicator of hyperactive Wnt ($p<0.0001$; Fig. 2)
- **Gid8 amplifies hyperactive Wnt**
- Wing discs: Knockdown Gid8 + ArmS10 showed less Senseless expression than ArmS10 alone ($p<0.0001$)
- **Gid8 promotes Wnt hyperactivation, linking Gid8 to cell proliferation not degradation**
- Salivary glands: Knockdown Gid8 + ArmS10 had less Arm in the nucleus (less Wnt; $p<0.0001$)
- **First evidence linking Gid8 to hyperactive Wnt signaling**

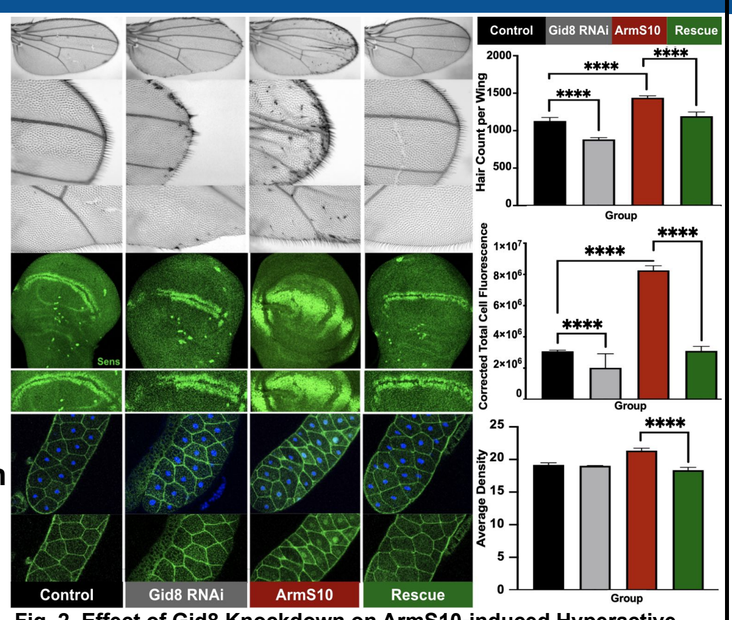

Fig. 2. Effect of Gid8 Knockdown on ArmS10-induced Hyperactive Wnt. GFP stained for Senseless, a Wnt target gene, in wing discs. GFP stained for Arm in salivary glands. ***** p<0.0001. Images taken by finalist on microscope, 2024. Graphs created by finalist in GraphPad, 2024.*

Identifies Gid8 as a **novel regulator** of hyperactive Wnt with **implications for cancer regulation**

TARGETABLE REGION IN Gid8

- In HEK293 cells with active Wnt, expression of Gid8* (contains mutated residues 167, 168, and 172) nearly abolished Gid8's nuclear localization ($p<0.0001$; Fig. 3)
- **Identifies these 3 residues as critical for Gid8 nuclear entry**

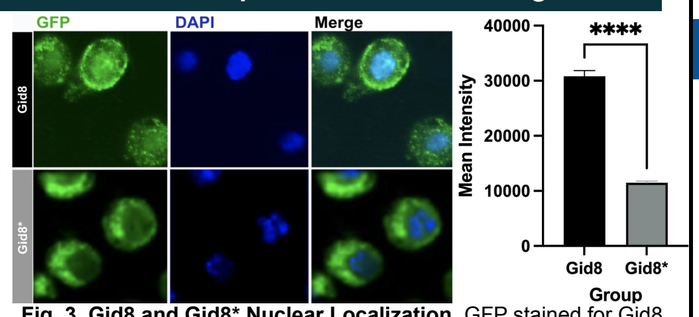

Fig. 3. Gid8 and Gid8* Nuclear Localization. GFP stained for Gid8. ***** p<0.0001. Images taken by finalist on microscope, 2024. Graph created by finalist in GraphPad, 2024.*

Defines **precise mutations** in Gid8's nuclear localization sequence (C-terminal) that can be **targeted to limit hyperactive Wnt signaling**

Gid8 GUIDES NUCLEAR TRANSLOCATION

- Wing discs: Gid8 colocalized with Kinesin2 and IFT140, proteins that guide wild type β-catenin to the nucleus ($p<0.01$; 0.001; Fig. 4)
  - Links Gid8 to intracellular transport
- Mutant β-catenin nuclear entry is mediated by Gid8-dependent engagement of Kinesin2/IFT140 transport pathways
- Co-IP assays **confirmed interactions between Gid8 and mutant ArmS10** ($p<0.01$; Fig. 5)
  - Transport-dependent mechanism of Gid8 controlling nuclear entry during hyperactive Wnt signaling

Mutant β-catenin enters the nucleus with Gid8, a previously unclear mechanism

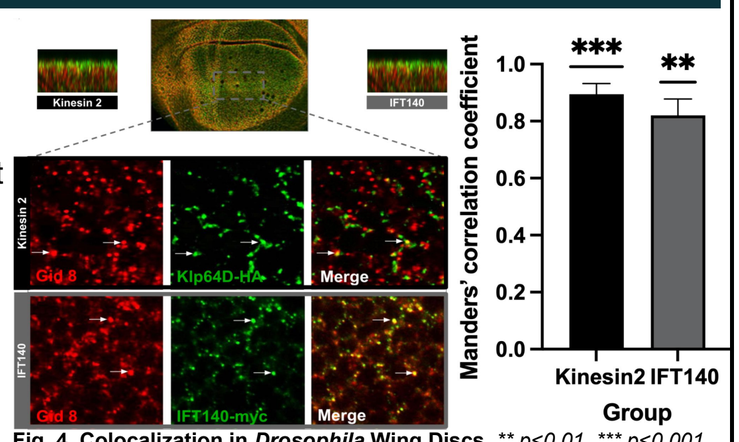

Fig. 4. Colocalization in *Drosophila* Wing Discs. *** p<0.01. *** p<0.001. Images taken by finalist on microscope, 2024. Graph created by finalist in GraphPad, 2024.*

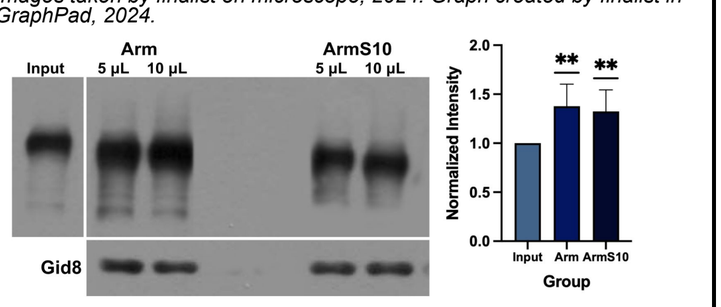

Fig. 5. Co-IP Assay. Arm and ArmS10 bound to Gid8. *** p<0.01. Images taken by finalist on microscope, 2024. Graph created by finalist in GraphPad, 2024.*

FRAGMENT 2 MITIGATES HCC PROLIFERATION

- Overexpression of **residues 41-58** in β-catenin **(fragment 2)** consistently produced the strongest reduction in HCC proliferation across Huh7, Hep3B, and HepG2 cells ($p<0.0001$; Fig. 6)
- Fragment 2 defines the essential inhibitory region within the β-catenin$^{24\text{-}79}$ peptide

Fragment 2 **overcomes delivery and stability limitations of the full-length β-catenin peptide** by defining an 18-amino-acid inhibitory region

- Makes selective β-catenin inhibition clinically achievable due to small size

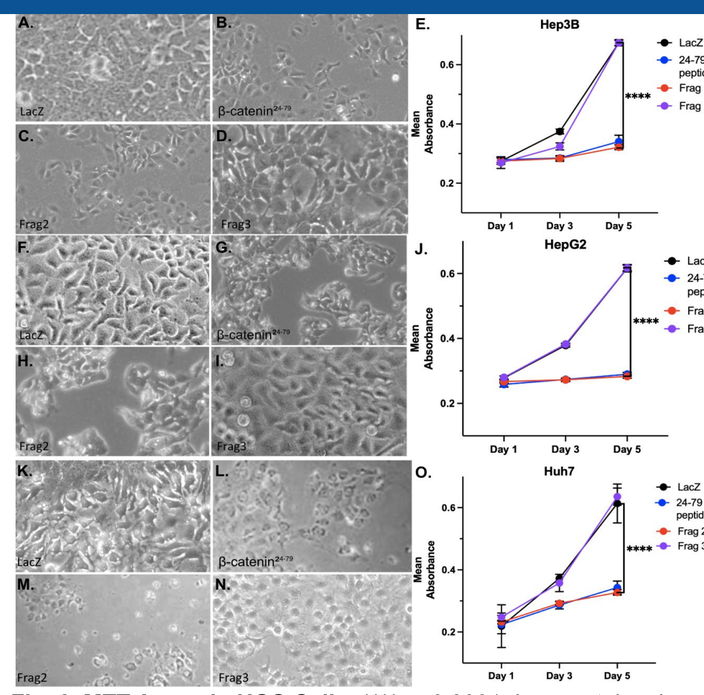

Fig. 6. MTT Assay in HCC Cells. ***** p<0.0001. Images taken by finalist on microscope, 2025. Graph created by finalist in GraphPad, 2025.*

FRAGMENT 2 ACHIEVES SELECTIVE INHIBITION

- In Hep3B and Huh7 cells, Fragment 2 produced the largest reduction in cancerous, nuclear β-catenin ($p<0.0001$; Fig. 7D, 8D, 9A, 10A)
- Membrane β-catenin showed smaller changes, indicating retention at adherens junctions and preservation of normal β-catenin functions (ns; $p<0.01$; Fig. 9B, 10B)
- Previously unachievable to block nuclear entry without disrupting membrane β-catenin

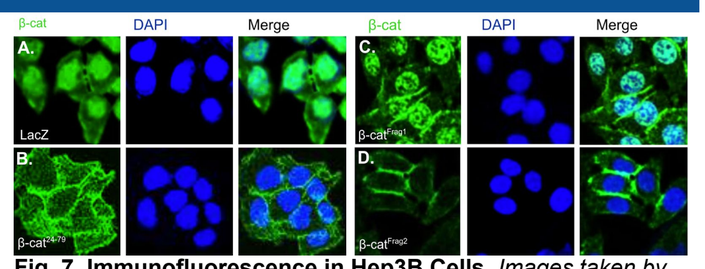

Fig. 7. Immunofluorescence in Hep3B Cells. *Images taken by finalist on microscope, 2025.*

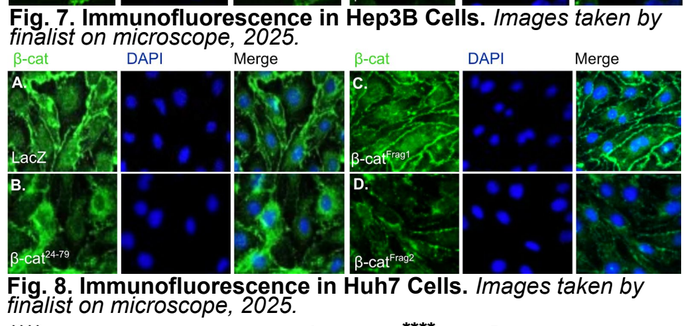

Fig. 8. Immunofluorescence in Huh7 Cells. *Images taken by finalist on microscope, 2025.*

Fragment 2 resolves the central Wnt targeting problem by **separating β-catenin's cancerous nuclear function** from its **essential membrane role**

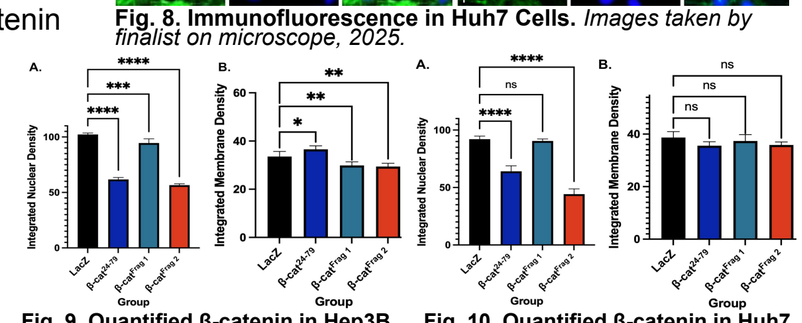

Fig. 9. Quantified β-catenin in Hep3B cells. **p<0.05. **p<0.01. ***p<0.001. ****p<0.0001. Graph created by finalist in GraphPad, 2025.*

Fig. 10. Quantified β-catenin in Huh7 cells. *ns, not significant. ****p<0.0001. Graph created by finalist in GraphPad, 2025.*

- In HepG2 cells, Fragment 2 significantly reduced nuclear β-catenin ($p<0.01$; Fig. 11)
- Cytoplasmic levels of β-catenin remained unchanged

Shows that restricting nuclear access alone is **sufficient to suppress hyperactive Wnt signaling** without disrupting cytoplasmic pools

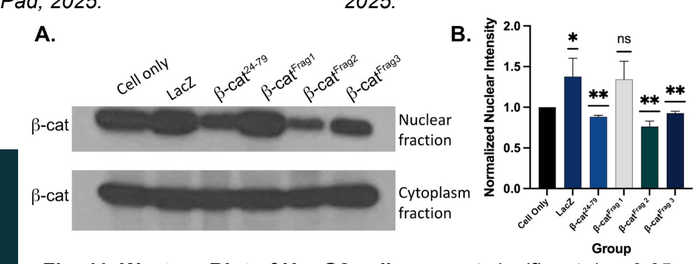

Fig. 11. Western Blot of HepG2 cells. *ns, not significant. * p<0.05. ** p<0.01. Images taken by finalist on microscope, 2025. Graph created by finalist in GraphPad, 2025.*

CONCLUSION

- Resolves **two key gaps in Wnt-driven cancers:** how mutant β-catenin enters the nucleus and how cancerous Wnt signaling can be inhibited without disrupting normal β-catenin
- Gid8 was identified as a transport-specific regulator that controls mutant translocation, and an inhibitory region (residues 41-58) was defined that selectively blocks nuclear entry
  - Upstream and downstream Wnt inhibitors failed: they disrupt essential β-catenin membrane functions, causing on-target toxicity and limiting therapeutic efficacy[11]
- **Initiates a novel baseline for precision peptide-based gene therapy**

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[1] L.-J. Liu, S.-X. Xie, Y.-T. Chen, J.-L. Xue, C.-J. Zhang, and F. Zhu, "Aberrant regulation of Wnt signaling in hepatocellular carcinoma," *World J Gastroenterol*, vol. 22, no. 33, pp. 7486–7499, Sep. 2016, doi: 10.3748/wjg.v22.i33.7486. [2] Z. Zhao *et al.*, "Wnt/β-Catenin signaling pathway in hepatocellular carcinoma: pathogenic role and therapeutic target," *Front. Oncol.*, vol. 14, Apr. 2024, doi: 10.3389/fonc.2024.1367364. [3] W.-J. Park and M. J. Kim, "A New Wave of Targeting 'Undruggable' Wnt Signaling for Cancer Therapy: Challenges and Opportunities," *Cells*, vol. 12, no. 8, p. 1110, Apr. 2023, doi: 10.3390/cells12081110. [4] W. Hwang, "Beta-Catenin Nuclear Transport In Wnt Signaling: Kap-Beta2/transportin Mediates Nuclear Import Of Beta-Catenin Via A Py-Nls Motif In A Ran Gtpase Dependent Manner," *Yale Medicine Thesis Digital Library*, Jan. 2023, [Online]. Available: https://elischolar.library.yale.edu/ymtdl/4182 [5] C. C. Anthony, D. J. Robbins, Y. Ahmed, and E. Lee, "Nuclear Regulation of Wnt/β-Catenin Signaling: It's a Complex Situation," *Genes*, vol. 11, no. 8, p. 886, Aug. 2020, doi: 10.3390/genes11080886. [6] Y. Lu *et al.*, "Twa1/Gid8 is a β-catenin nuclear retention factor in Wnt signaling and colorectal tumorigenesis," *Cell Res*, vol. 27, no. 12, pp. 1422–1440, Dec. 2017, doi: 10.1038/cr.2017.107. [7] T. Pradhan-Sundd *et al.*, "Dual β-Catenin and γ-Catenin Loss in Hepatocytes Impacts Their Polarity through Altered Transforming Growth Factor-β and Hepatocyte Nuclear Factor 4α Signaling," *Am J Pathol*, vol. 191, no. 5, pp. 885–901, May 2021, doi: 10.1016/j.ajpath.2021.02.008. [8] L. T. Vuong and M. Mlodzik, "Wg/Wnt-signaling-induced nuclear translocation of β-catenin is attenuated by a β-catenin peptide through its interference with the IFT-A complex," *Cell Rep*, vol. 43, no. 6, p. 114362, Jun. 2024, doi: 10.1016/j.celrep.2024.114362. [9] "Co-Immunoprecipitation (Co-IP) and Pull-Down - US." Accessed: Feb. 09, 2026. [Online]. Available: https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-assays-analysis/protein-interaction-analysis/co-immunoprecipitation-co-ip-pull-down.html [10] "QIAprep Spin Miniprep Kit | Plasmid DNA Isolation | QIAGEN." Accessed: Feb. 09, 2026. [Online]. Available: https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/plasmid-dna/qiaprep-spin-miniprep-kit [11] S. Mao *et al.*, "Targeted protein degradation of Wnt/β-catenin signaling pathway: an effective strategy for cancer therapy," *Journal of Translational Medicine*, vol. 23, no. 1, p. 1233, Nov. 2025, doi: 10.1186/s12967-025-07333-1.

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