Small molecule inhibition of β-catenin nuclear entry: A novel cancer target

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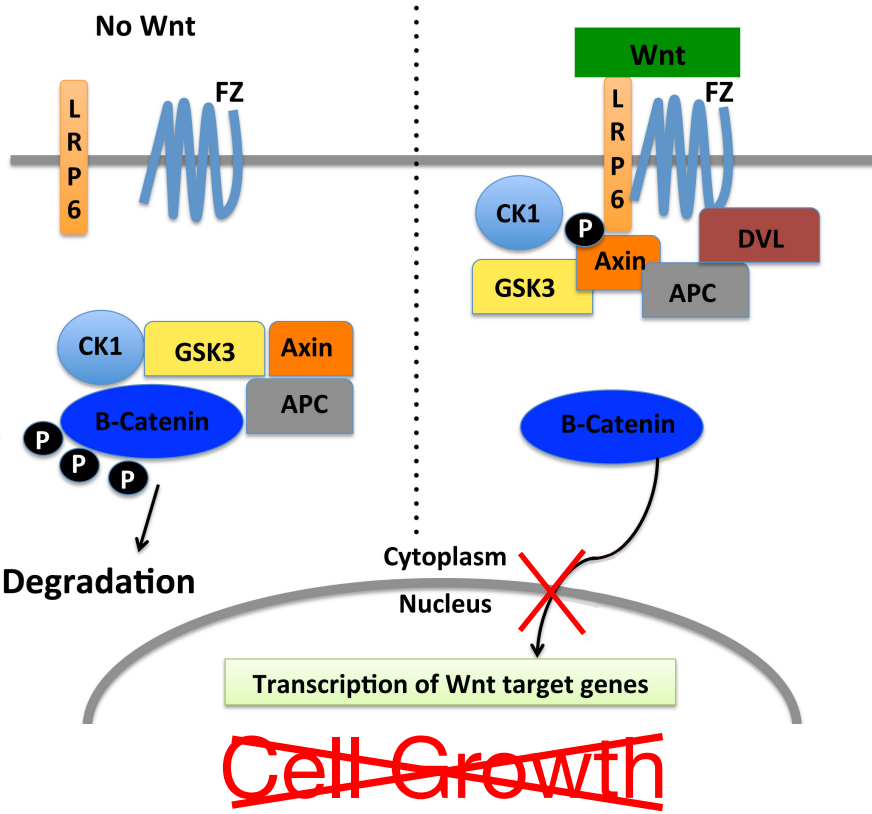
Targeting Wnt – a therapeutic gap

- Wnt/β-catenin signaling orchestrates numerous biological processes including cell proliferation and regeneration
- Wnt pathway overactivation has been observed in colon, breast, lung, and hematopoietic malignancies
- Lead Indication: Colon Cancer ($10B therapeutic market, 832,000 deaths/year)
  - Wnt – 90% colorectal cancers
  - Mutations common in colon cancer (APC, Axin1/2) stabilize β-catenin so it goes into nucleus and activates growth
- Therapeutic approach: prevent β-catenin from entering nucleus **– our lab has discovered this**

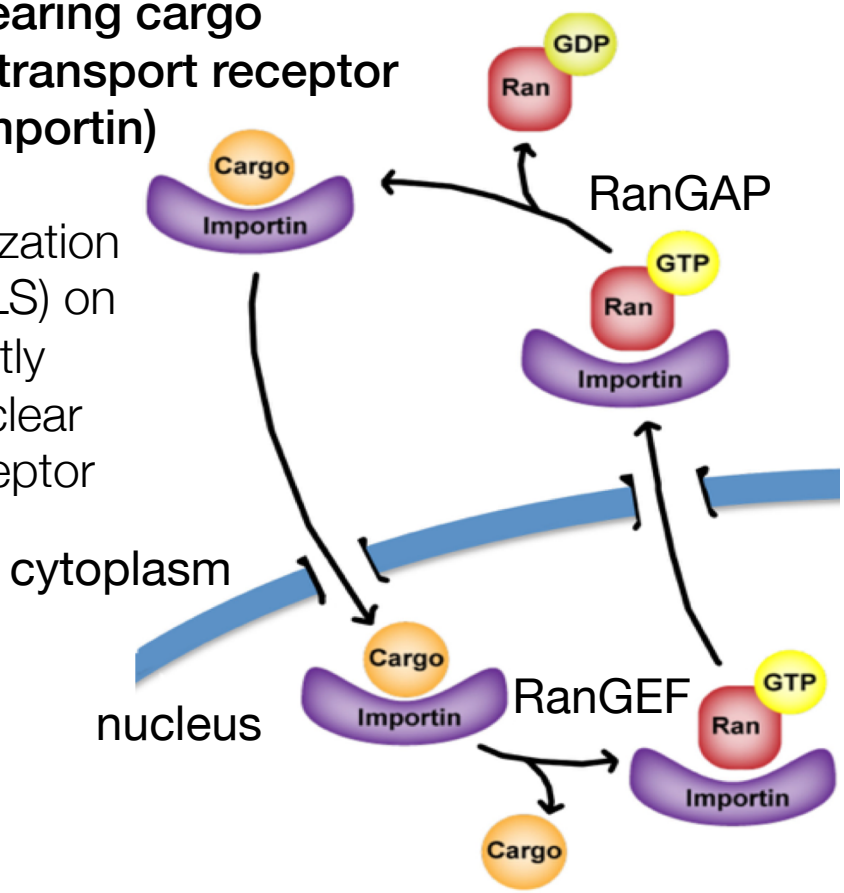


Current Understanding of β-catenin Nuclear Transport - Published

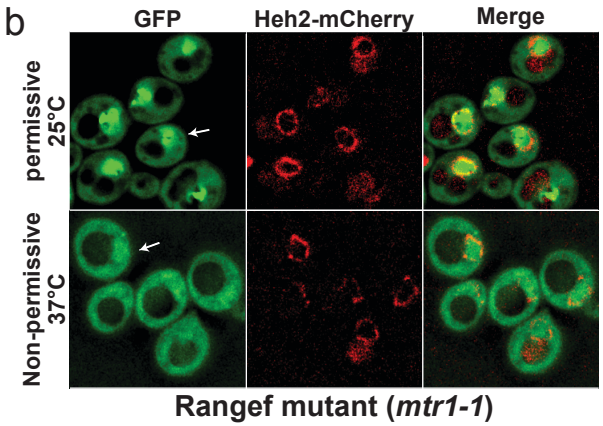
Three Key Elements of Nuclear Transport

1. Ran Dependence
2. NLS – bearing cargo
3. Nuclear transport receptor (NTR) (importin)

Nuclear localization sequence (NLS) on cargo is directly bound by nuclear transport receptor

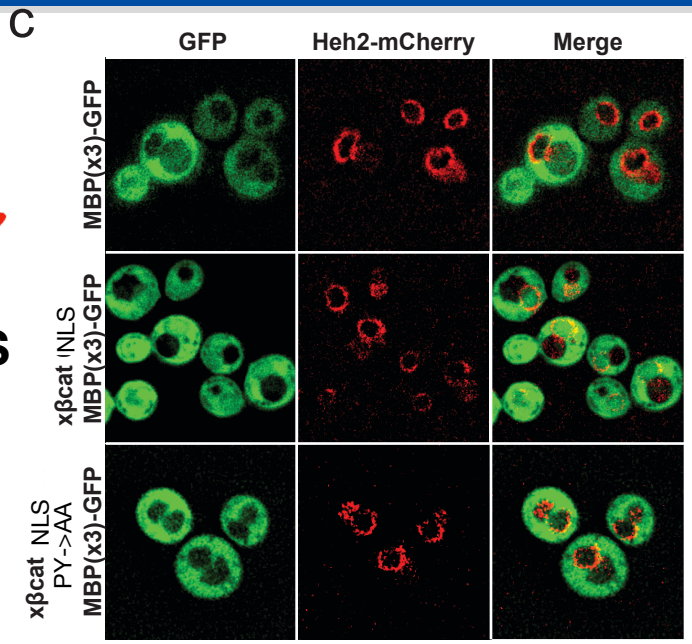


DISCOVERY: Identification of Key Elements Required for β-catenin Nuclear Transport (Unpublished)

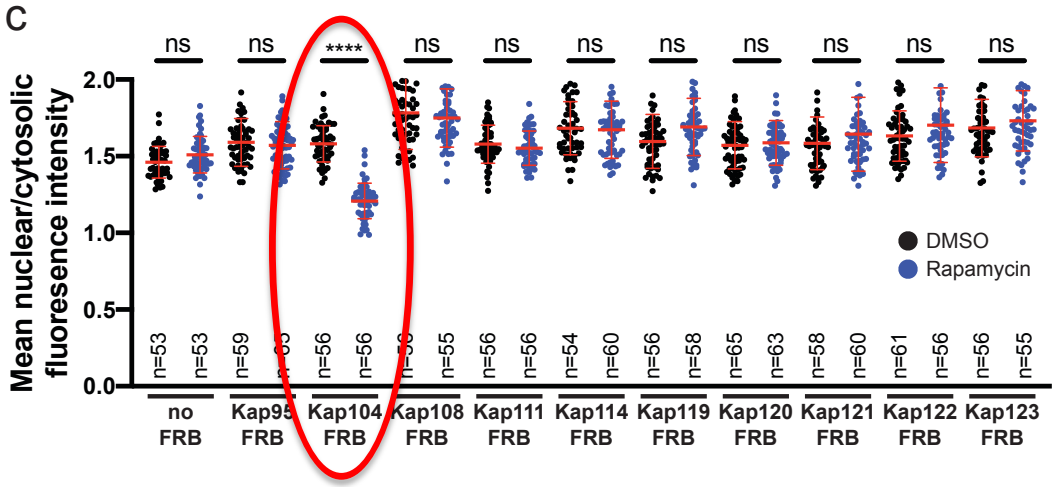


1. Ran Dependence ✓

2. PY-NLS ✓



3. NTR- TNPO1/2 ✓



PY-NLS

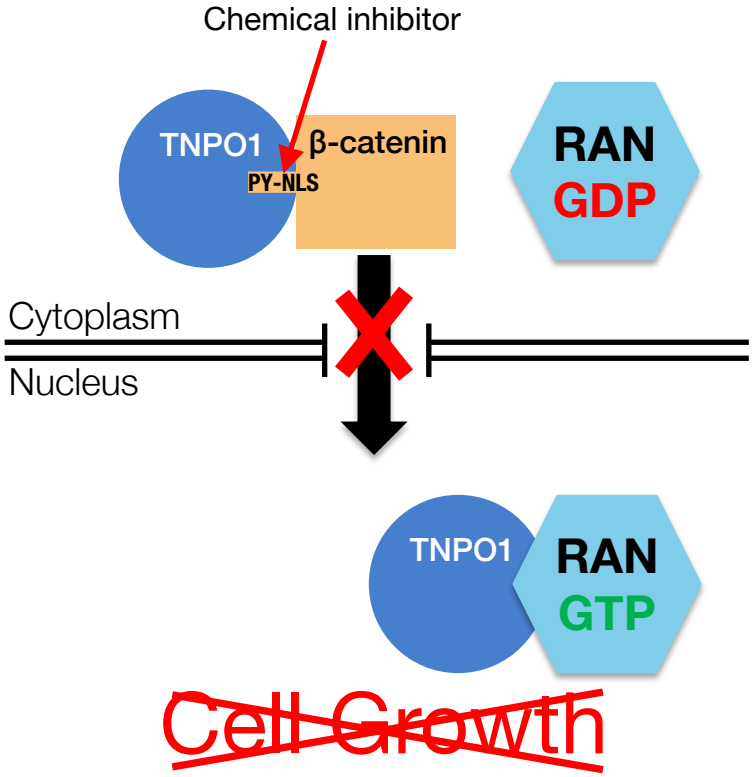
Hydrophobic/Basic motif—R-X2-5—PY

↓

AA

Inhibiting PY-NLS Prevents β-catenin Nuclear Entry

1. Ran dependence
2. β-catenin NLS – precise sequence
3. NTR – TNPO1/2
4. Direct binding – 2 critical AAs



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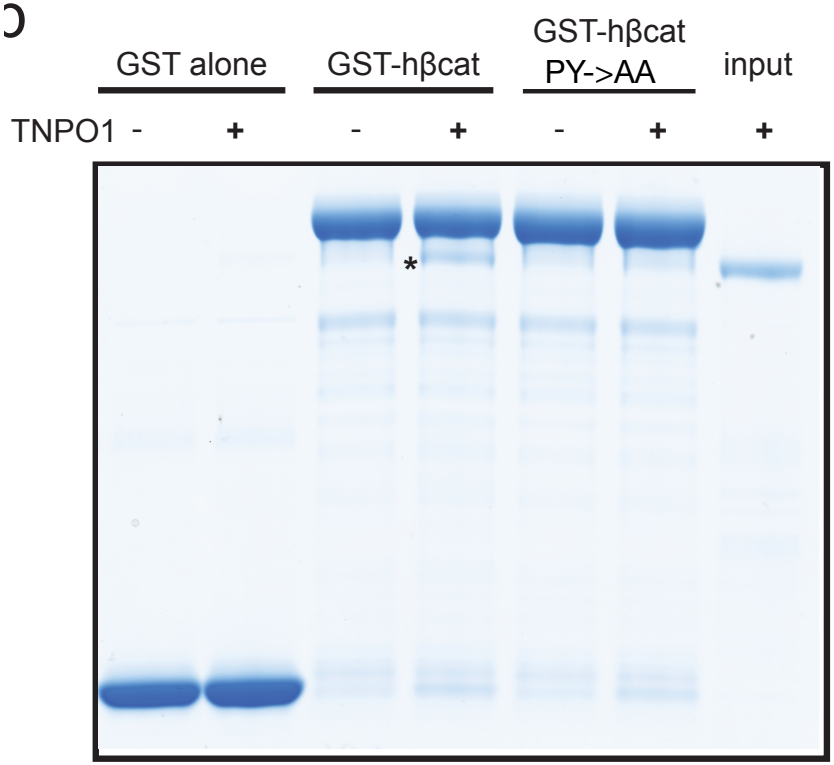
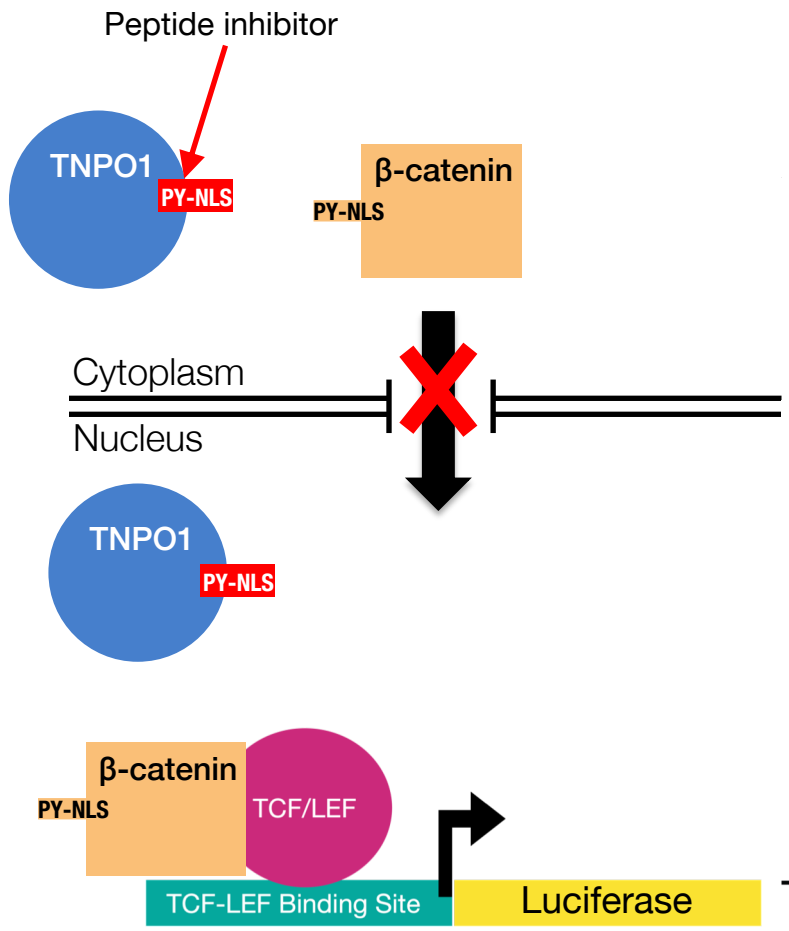
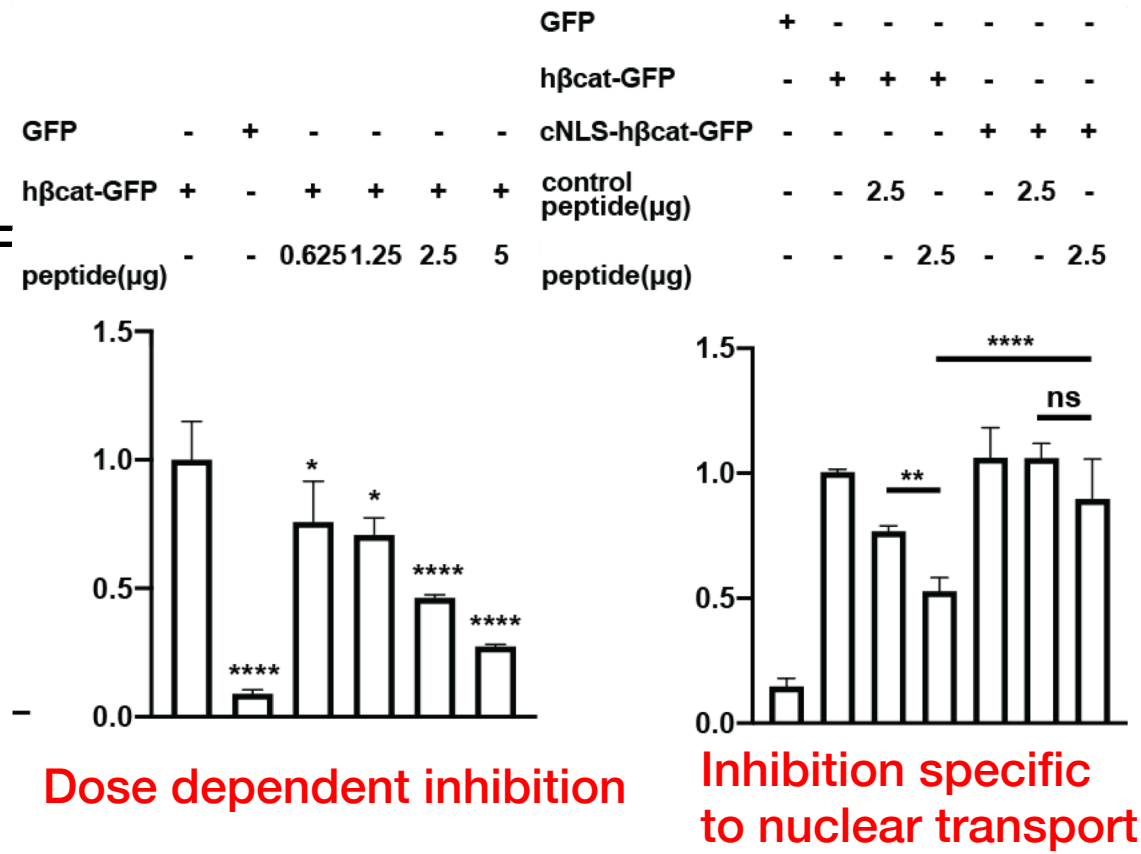


Proof of Concept: PY-NLS peptide blocks β-catenin Nuclear Entry





Dose dependent inhibition

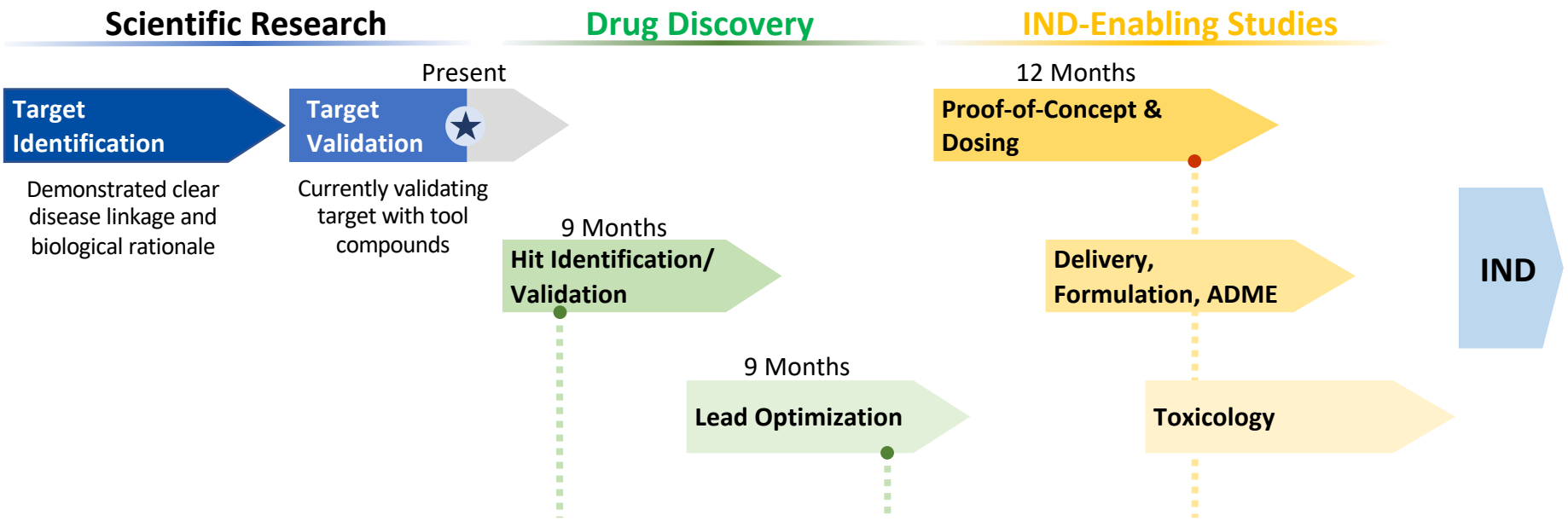
Inhibition specific to nuclear transport

Future Studies

- Phase I – Drug Discovery/Validation
  - Crystal structure - β-catenin PY-NLS <-> TNPO1
  - Screen for small molecule inhibitors (protein-protein interaction)
  - Peptide inhibitor modulation/delivery
  - In vitro validation of PPI - Mouse (TOPFLASH)

- Phase II – Lead optimization/IND enabling studies
  - Dosing/Optimize chemistry for delivery
  - In vitro efficacy
  - in vivo efficacy
  - Additional medicinal chemistry
  - Toxicology

Timeline & Funding Proposal



Project Team

- **Mustafa Khokha, MD – PI (mustafa.khokha@yale.edu)**
  - Pediatric Critical Care
  - Developmental Biology expertise – cellular signaling (*Xenopus*, mouse)
  - Current Scientific Approach – patient driven gene discovery in patients with birth defects -> basic developmental mechanism discovery
- **Patrick Lusk, PhD – PI (Patrick.lusk@yale.edu)**
  - Cell Biology
  - Nuclear Transport expertise
  - Yeast model
- **Woong Hwang – MD/PhD student**
  - Basic science training in Developmental Biology – mouse cell lines, human cell lines, yeast, *Xenopus*
  - Identified molecules in β-catenin nuclear transport
- **Valentyna Kostiuk – MD/PhD student**
  - Knockout TPO1/2 in human cell lines

PY-NLS – TNPO1 Stucture

- Structure of TNPO1 is known
- Crystal structure solved
- PY-NLS structures have some variability
- Could be exploited by small molecular to specifically block β-catenin PY-NLS – TNPO1 interaction specifically