Tech Session 4: Gene Delivery

Emily Day

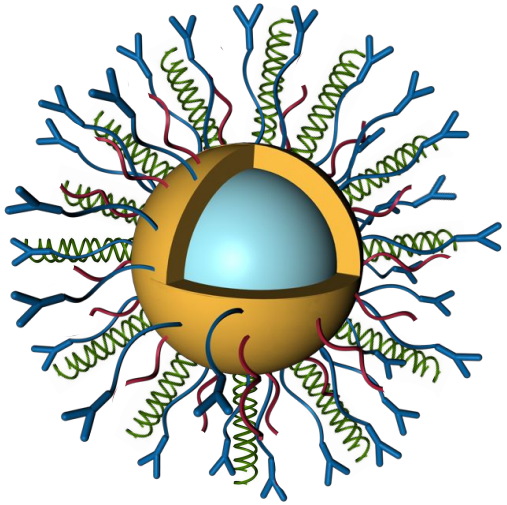
University of Delaware

CRS 2022 Annual Meeting & Expo

July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

Advanced Delivery Science

Developing Antibody/siRNA Nanocarriers to Regulate Wnt Signaling in Triple-Negative Breast Cancer

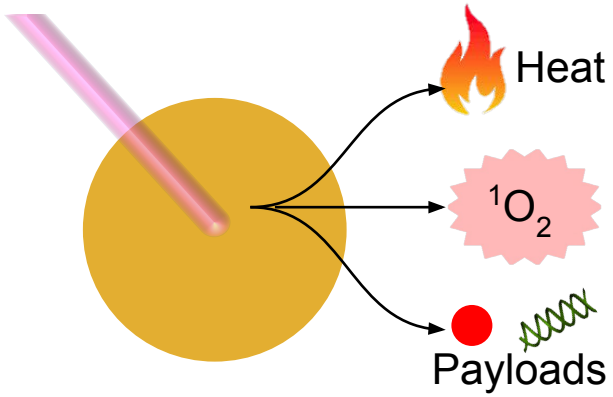


Emily S. Day

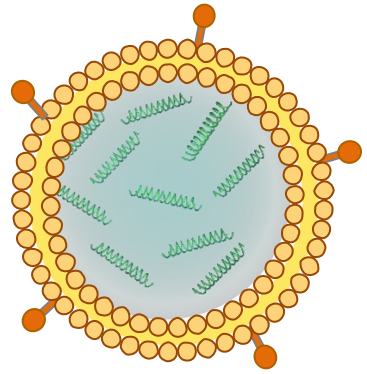
Day Lab: Engineering Nanoparticles for High Precision Therapy

Gene Regulation

Photoresponsive Therapies



Biomimetic Nanocarriers



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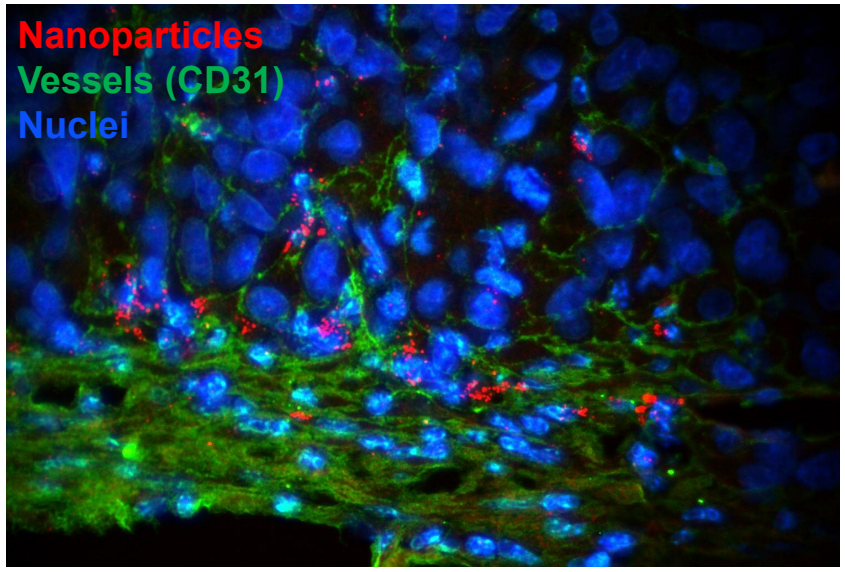
Why Nanoparticles?
They are Tailorable Tools for Medicine!

Nanoparticles are ***small enough*** to enter disease sites through circulation…



…and ***big enough*** to create unique functionality

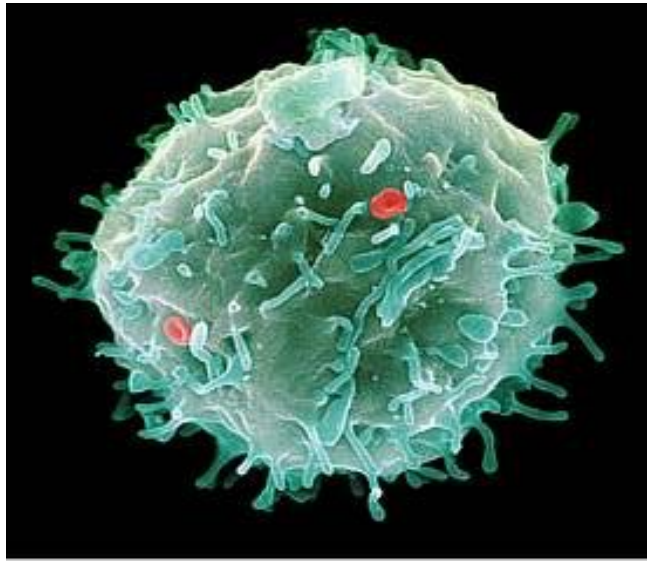
This Enables New Ways to Treat Disease That Are:

- ✔ **More precise**
- ✔ **More effective**
- ✔ **Better tolerated**

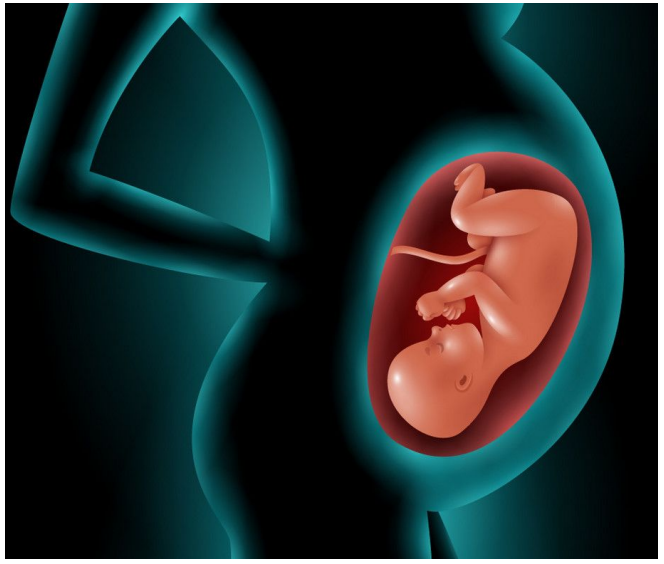
Challenges the Day Lab Seeks to Address using Nanomedicine

Aggressive Cancers

Hematologic Disorders



Maternal-Fetal Health Conditions



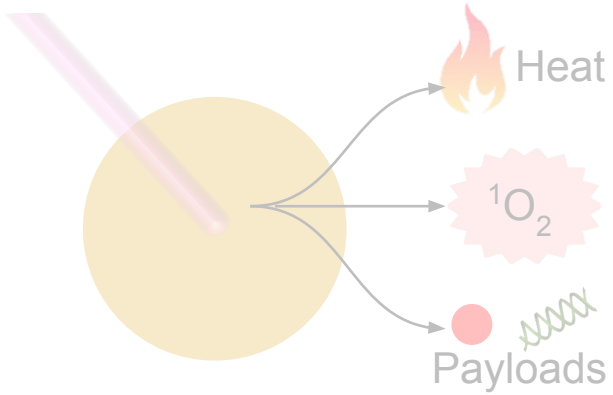
Shameless Plug: We're Hiring a Postdoc!

Interested?
Send CV to emilyday@udel.edu

Day Lab: Engineering Nanoparticles for High Precision Therapy

Gene Regulation

Photoresponsive Therapies



Biomimetic Nanocarriers

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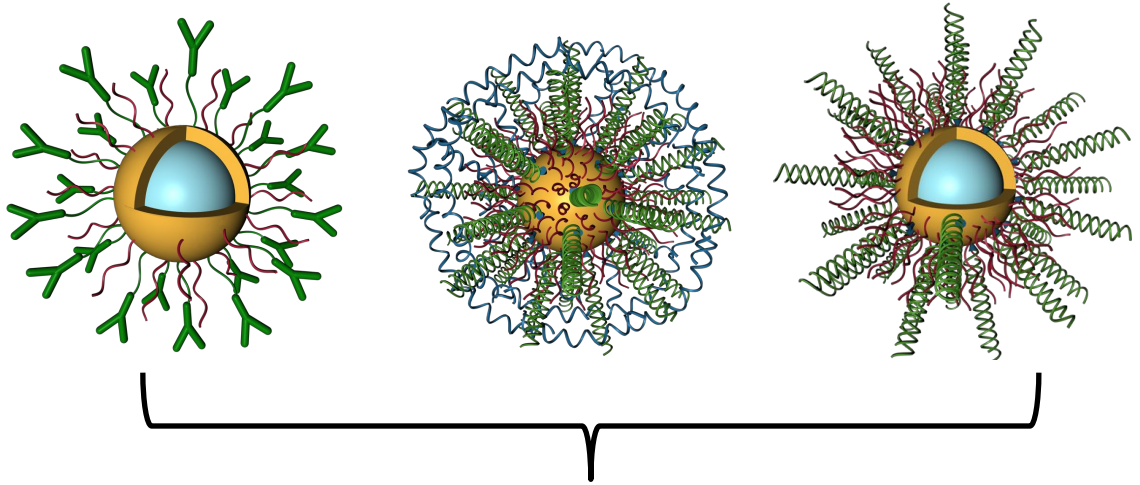
RNA and Antagonistic Antibodies: Two Pillars of Modern Medicine

RNA Duplex

Antibody

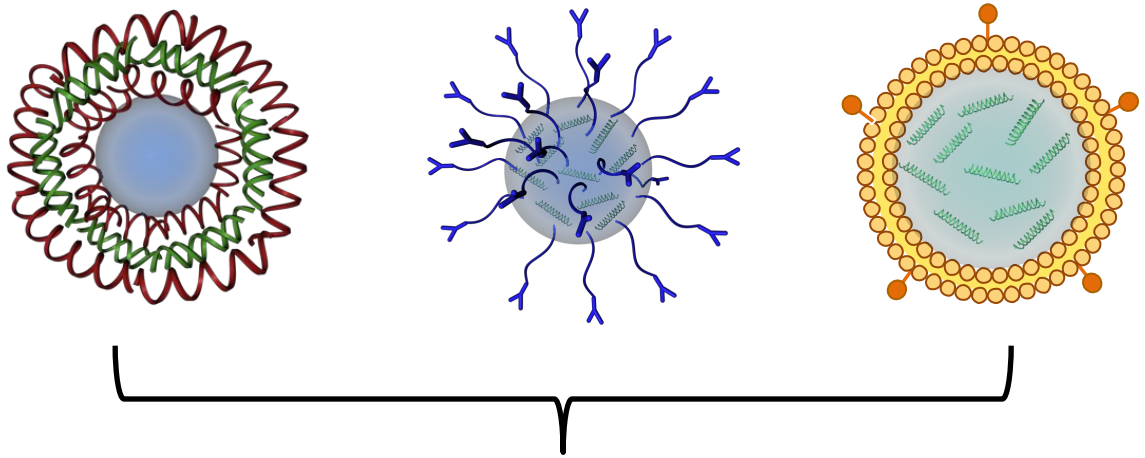
Controlling the nanoscale presentation of these materials to cells vastly improves their performance

Examples of RNA and Antibody Delivery Systems Developed in the Day Lab



Gold-Based

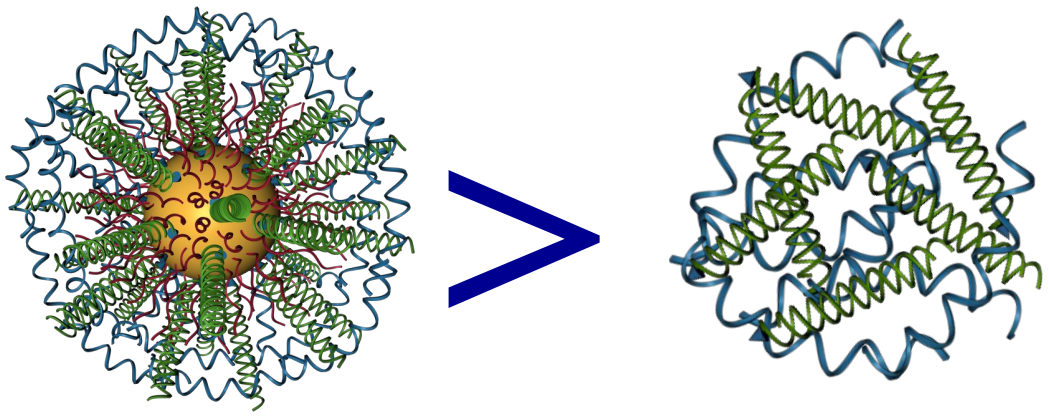
Riley & Day. *Small.* 2017.
Melamed, *et al. Molecular Therapy-Nucleic Acids*. 2018.
Melamed, *et al. Molecular Pharmaceutics*. 2018.
Riley, *et al*. *Nano Letters*. 2018.
Goyal, *et al. Cellular and Molecular Bioengineering*. 2018.
Wang, *et al. Nano Research.* 2020.
Dang, *et al. Nano Letters.* 2021.



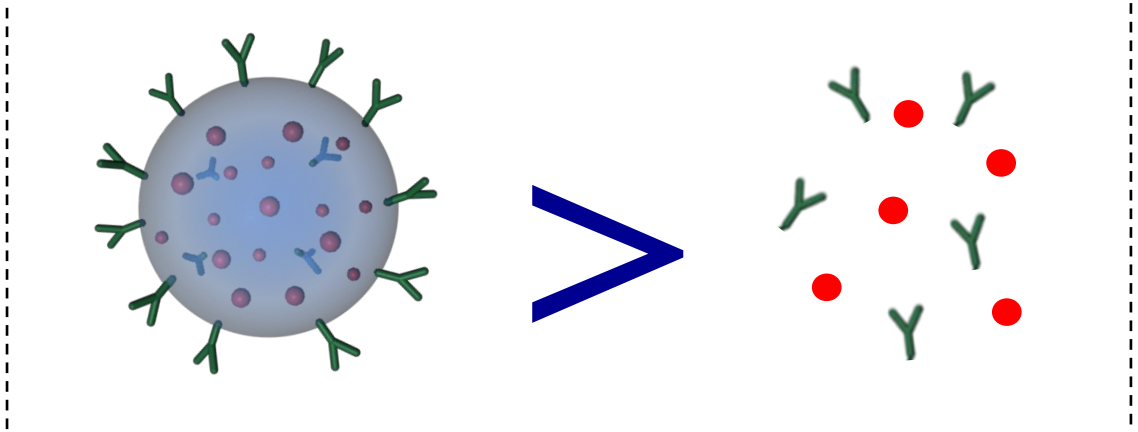
Polymer-Based

Kapadia, *et al. J Biomed Mater Res Part A.* 2019.
Valcourt, *et al. ACS Nano.* 2020.
Valcourt & Day. *Molecular Therapy-Nucleic Acids.* 2020.

Nanoscale Architecture Matters!



Melamed, *et al. Molecular Therapy-Nucleic Acids*. 2018.

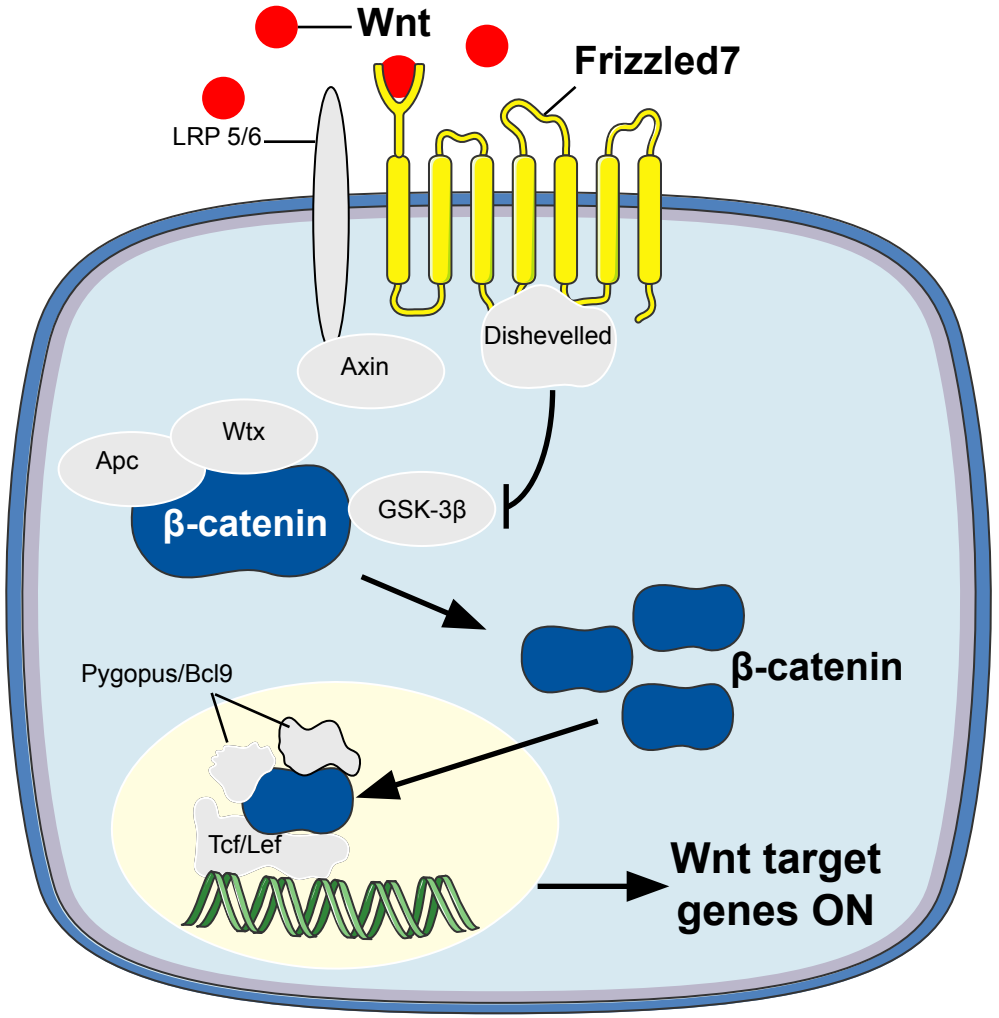


Valcourt, *et al. ACS Nano*. 2020.

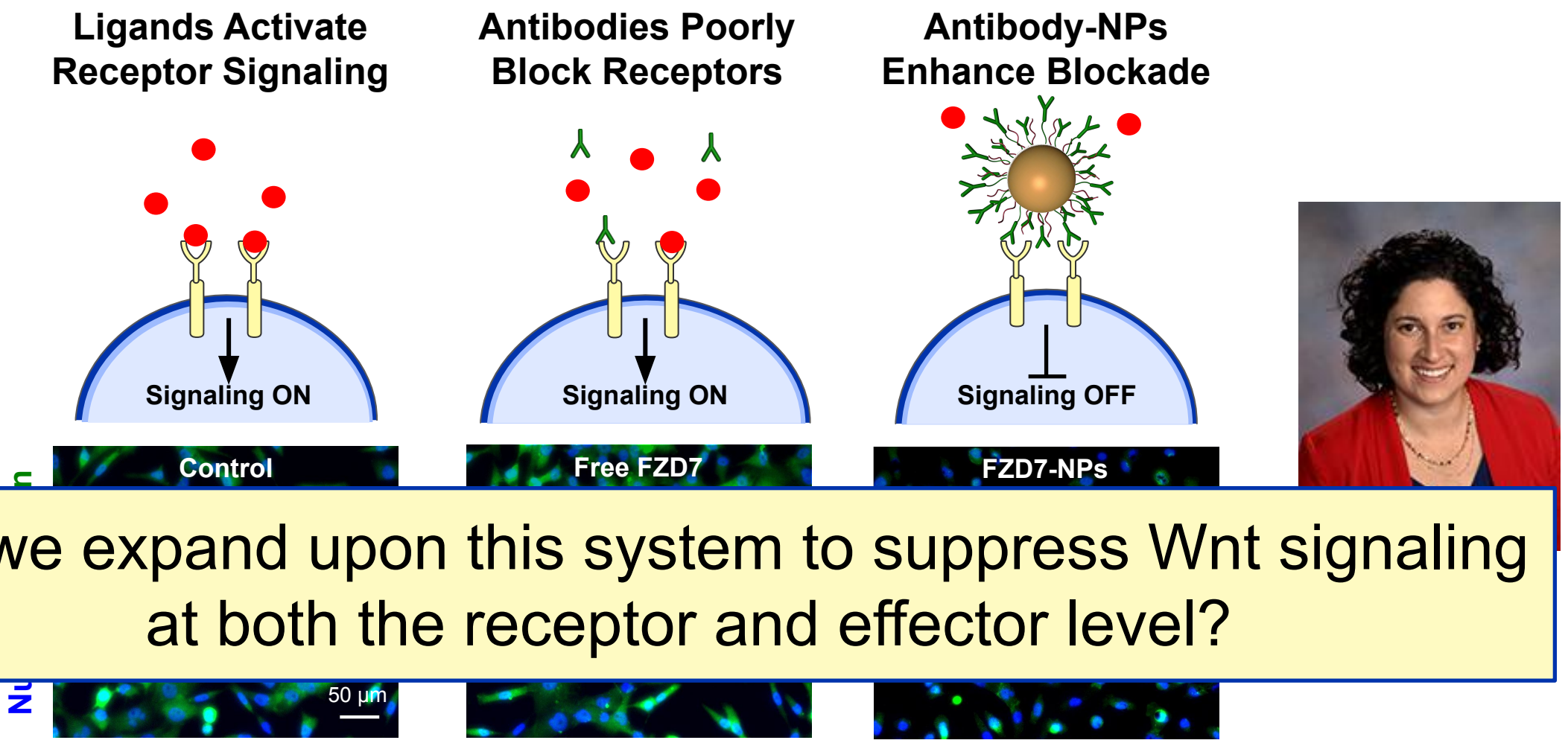
Both **what** you deliver and **how** you deliver it are important for therapeutic potency

Regulating Oncogenic Wnt Signaling in Triple-Negative Breast Cancer

- Accounts for ~20% of breast cancers
- Unsusceptible to conventional targeted or hormonal therapies
- Driven by hyperactive Wnt signaling



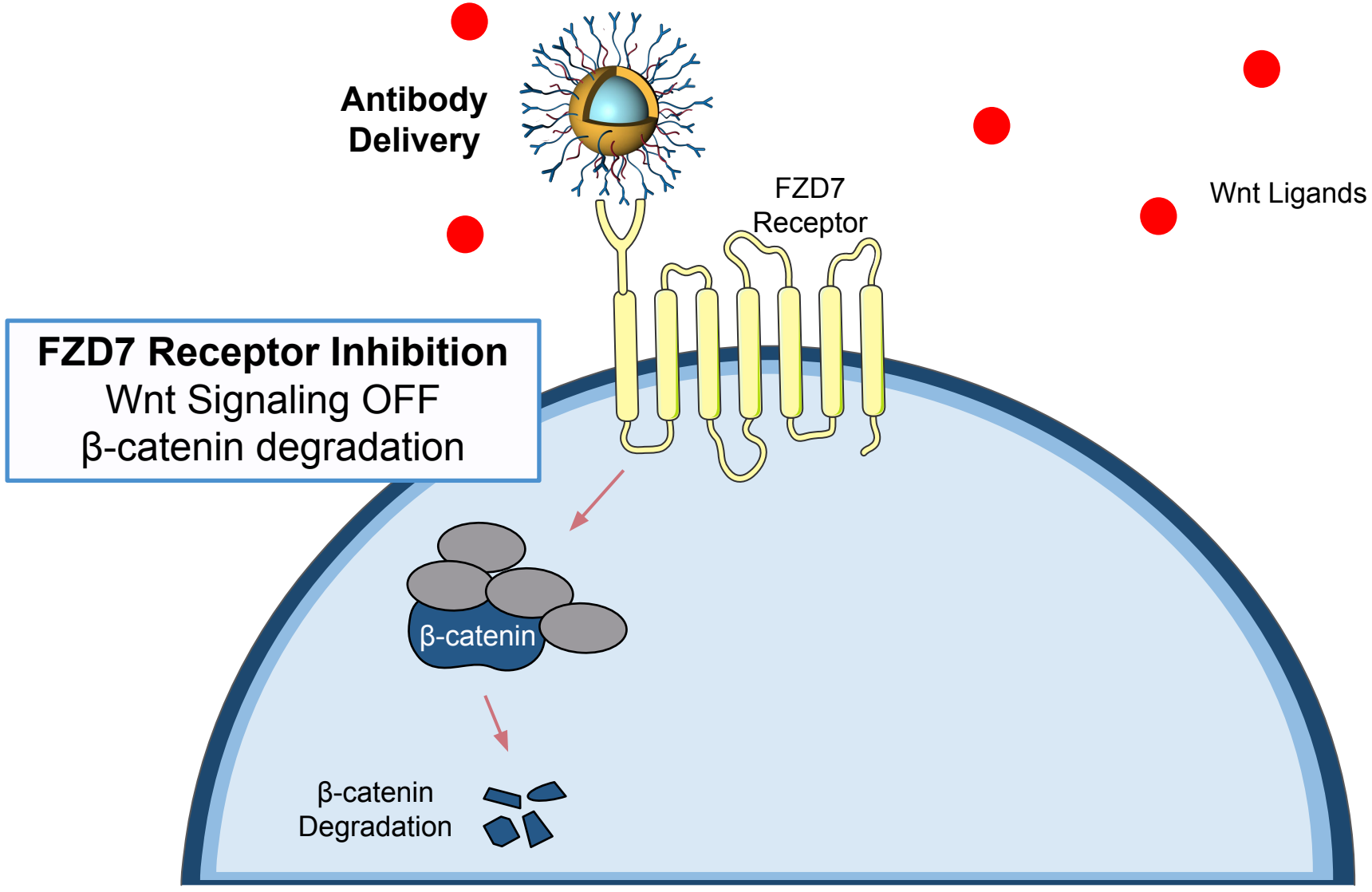
FZD7 Antibody Nanocarriers Exploit Multivalency to Enhance Wnt Signaling Blockade

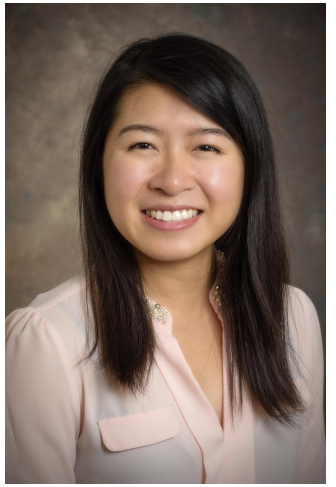


Can we expand upon this system to suppress Wnt signaling at both the receptor and effector level?

Riley RS, Day ES. *Small.* 2017.

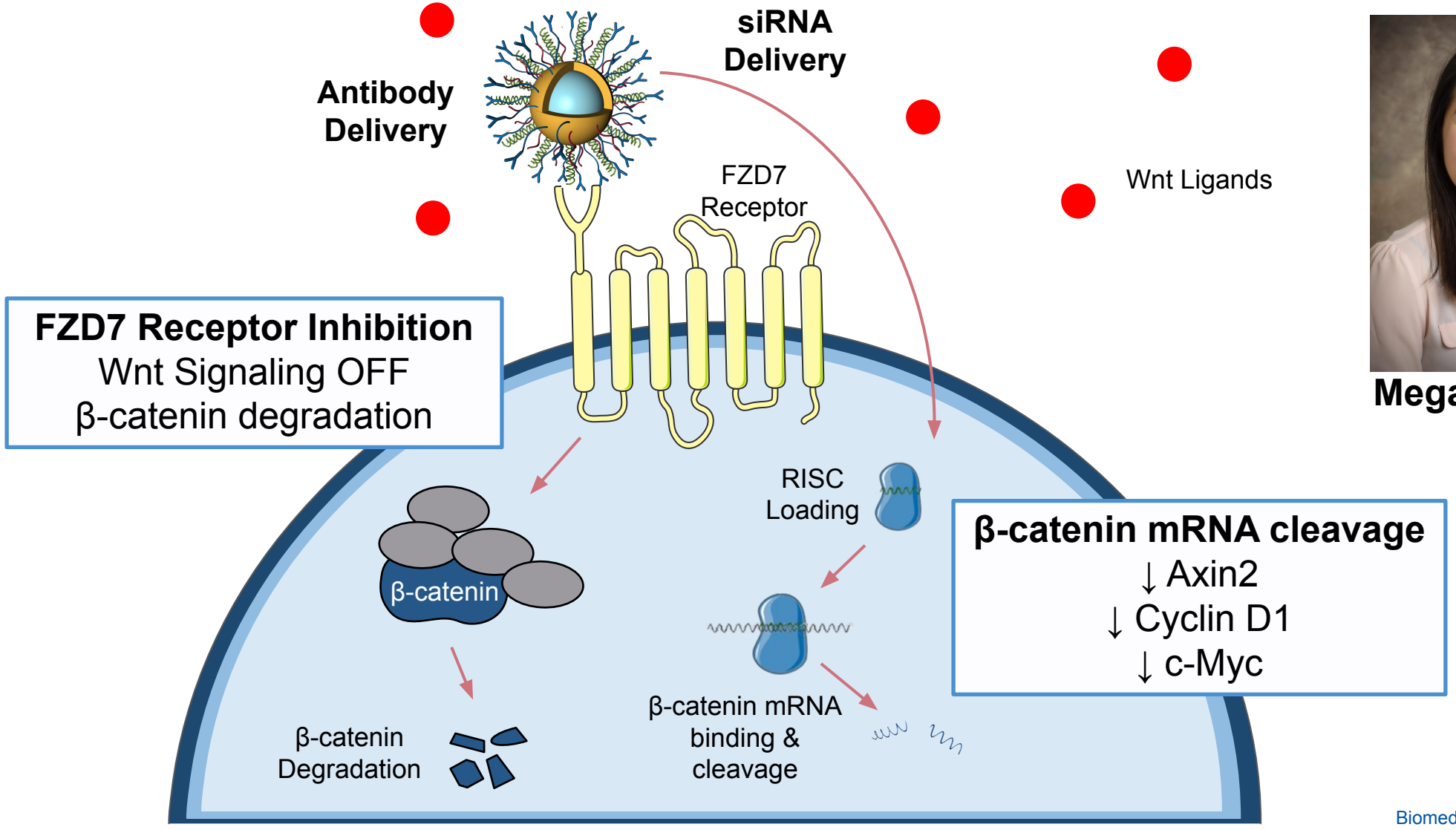
Developing NPs to Inhibit Wnt Signaling at Multiple Levels

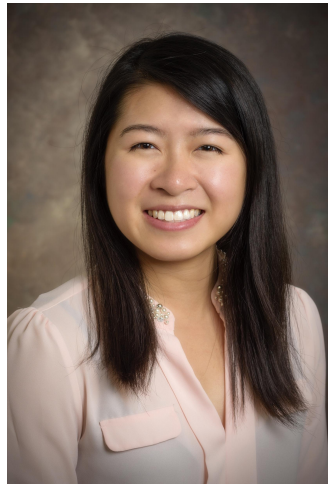




Megan Dang

Developing NPs to Inhibit Wnt Signaling at Multiple Levels





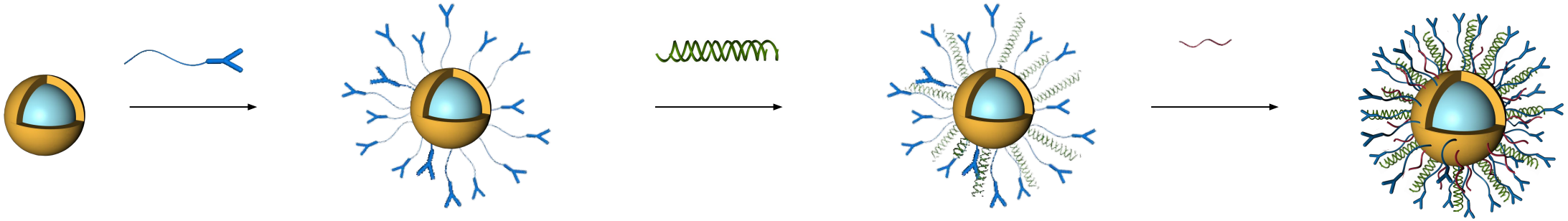
Megan Dang

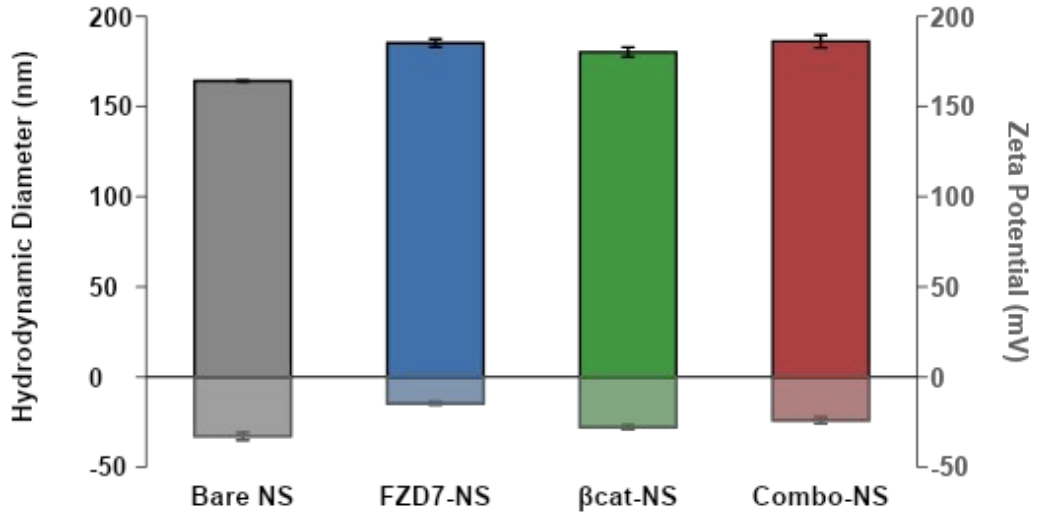
Synthesis of Nanoshell Conjugates

FZD7 Antibody
OPSS-PEG-SVA Linker

Thiolated
β-catenin siRNA

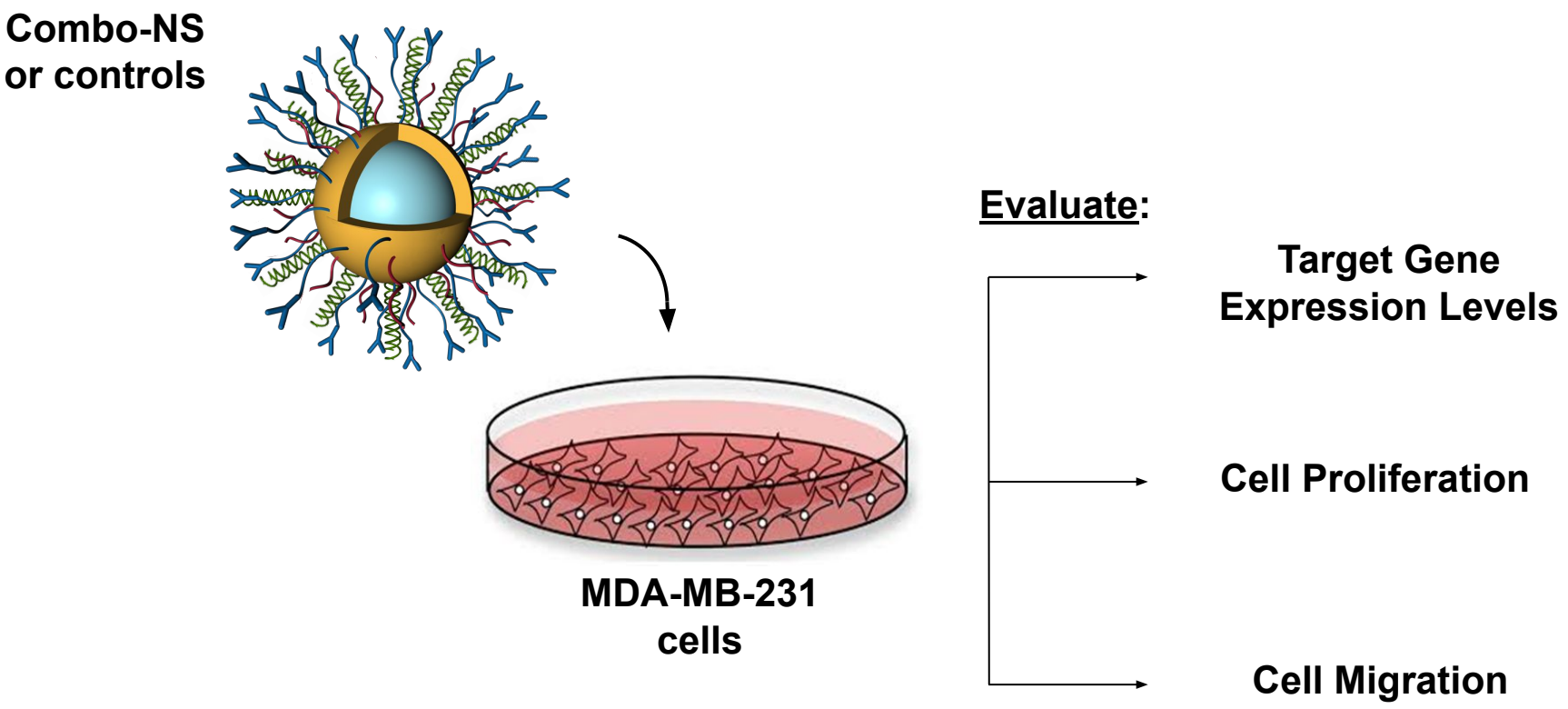
mPEG-SH



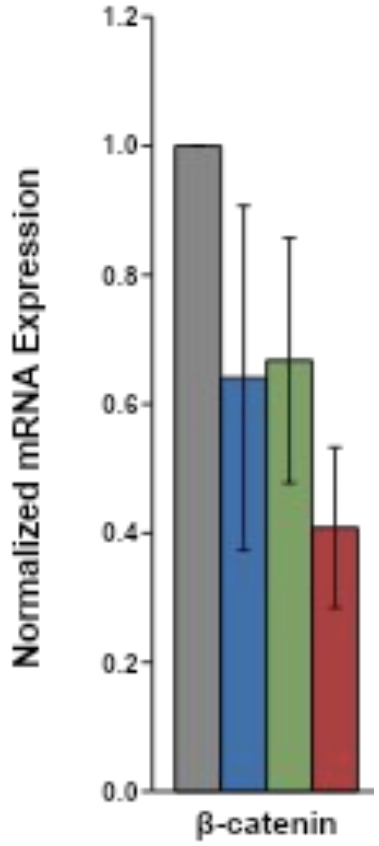


Loading Density (Molecules/NS)		
Conjugate	FZD7 Antibody	β-catenin siRNA
FZD7-NS	149 ± 12	--
βcat-NS	--	1680 ± 285
Combo-NS	157 ± 12	1610 ± 122

Evaluating the Impact of Wnt Inhibitory NS on TNBC *In Vitro*



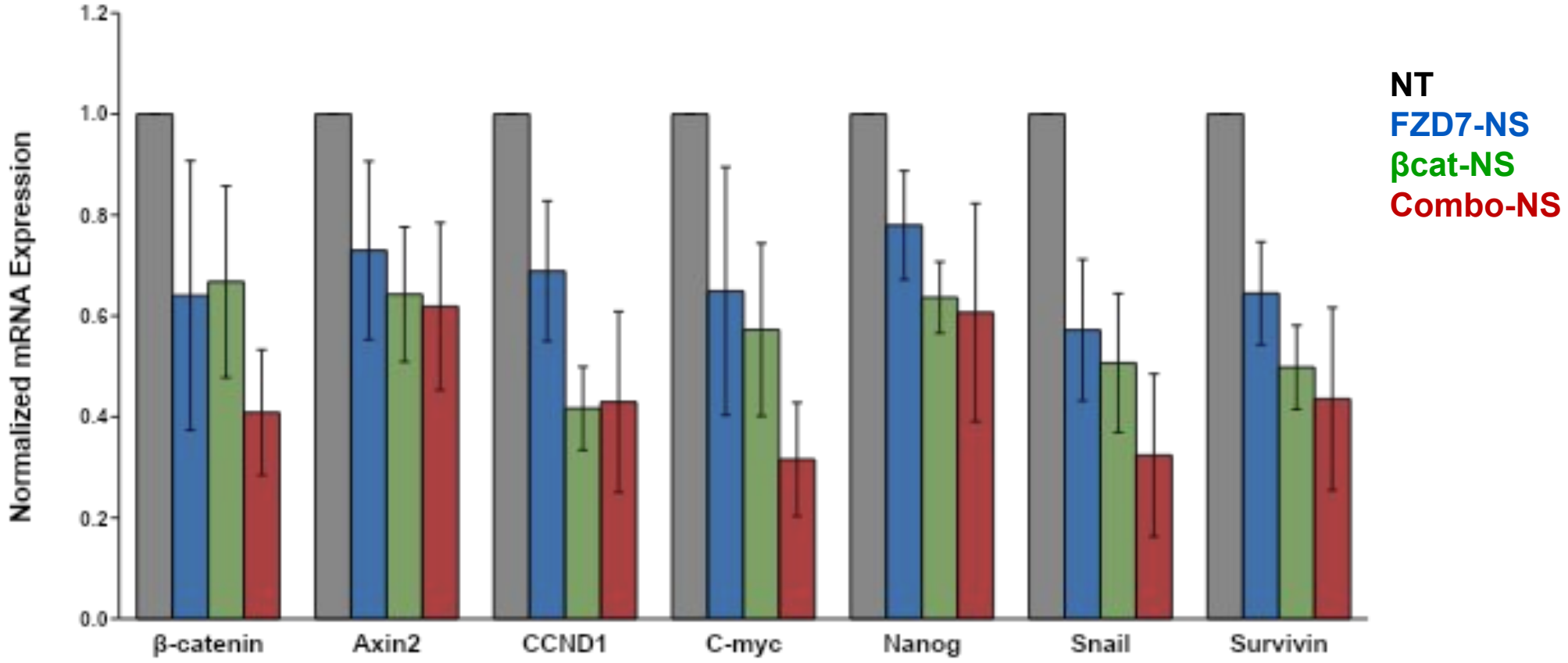
Combo-NS Suppress Wnt Target Genes More Effectively than Monotherapies



NT
FZD7-NS
βcat-NS
Combo-NS

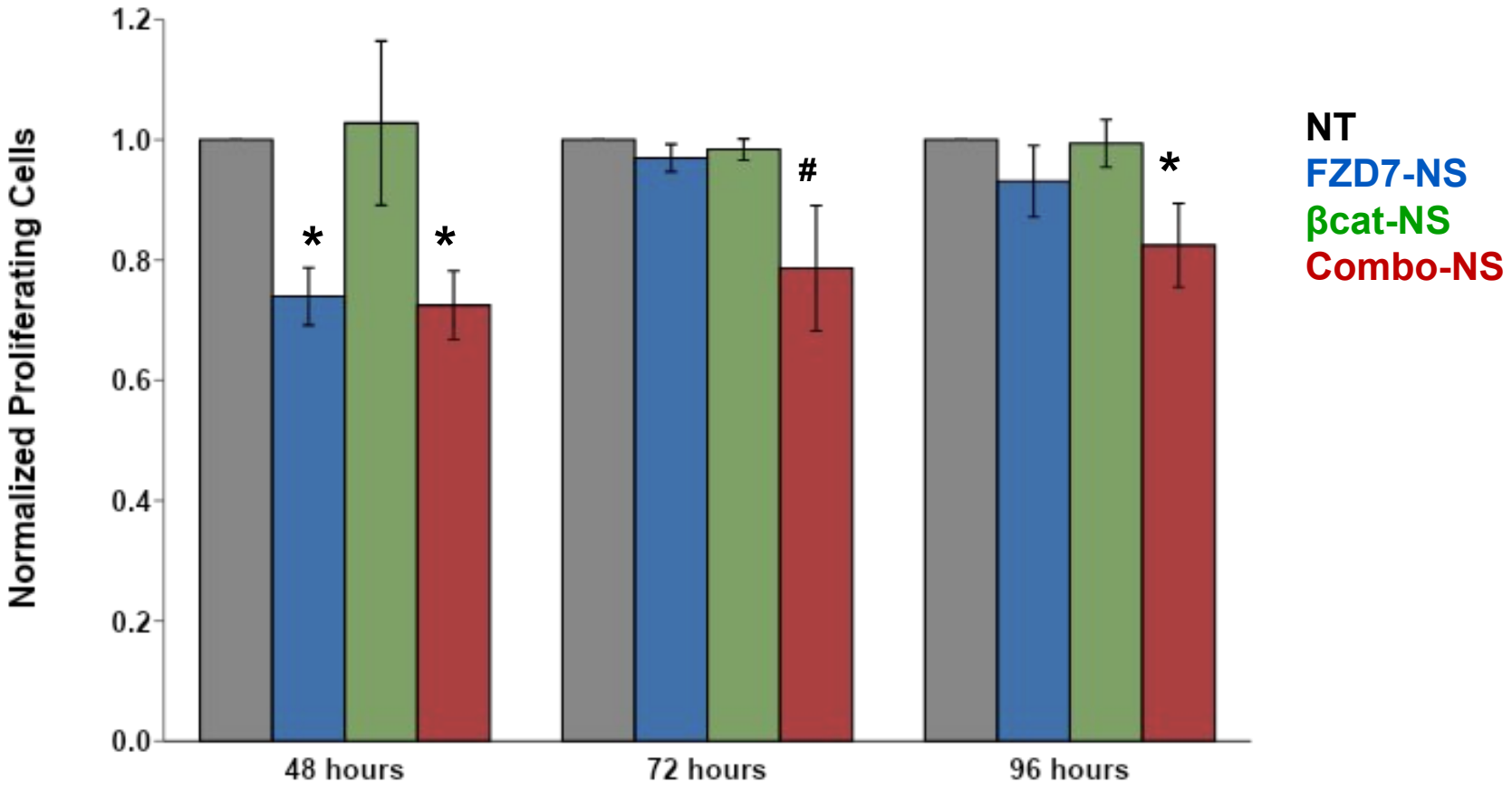
Dose=3.2E9 NS/mL; 0.5 nM antibody; 5 nM siRNA
n = 3; Measured 72 hr post-NS treatment

Combo-NS Suppress Wnt Target Genes More Effectively than Monotherapies



Dose=3.2E9 NS/mL; 0.5 nM antibody; 5 nM siRNA
n = 3; Measured 72 hr post-NS treatment

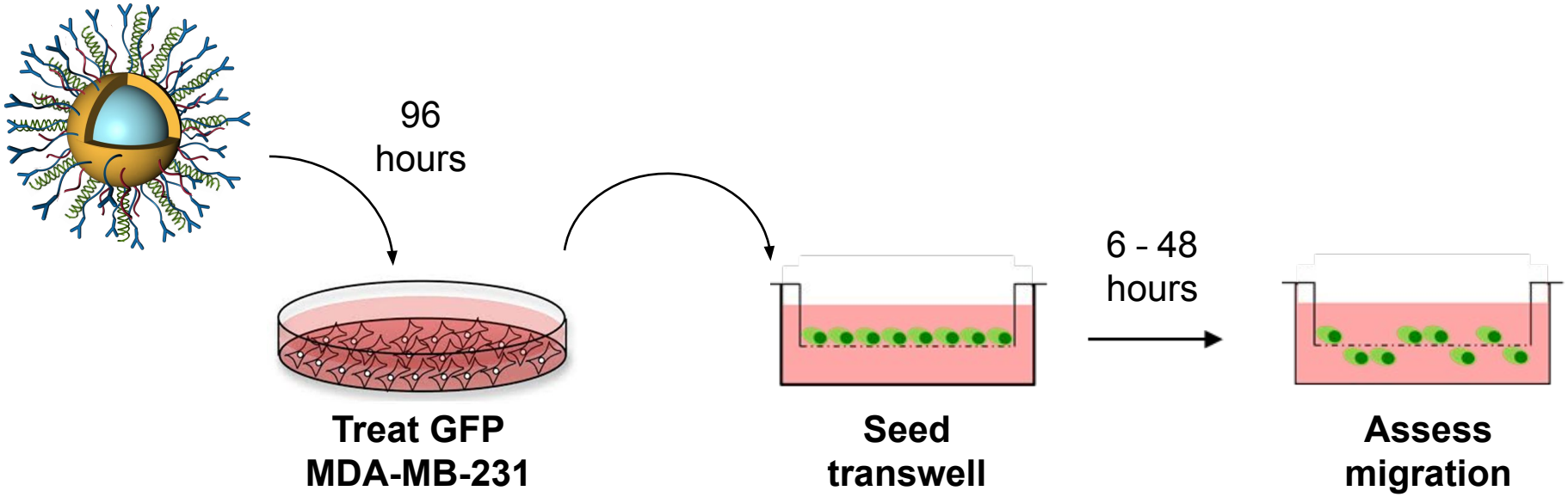
Combo-NS Sustain Repression of Cell Proliferation Through 4 Days Post-Treatment



Dose=1.6E10 NS/mL; 2.5 nM antibody; 25 nM siRNA

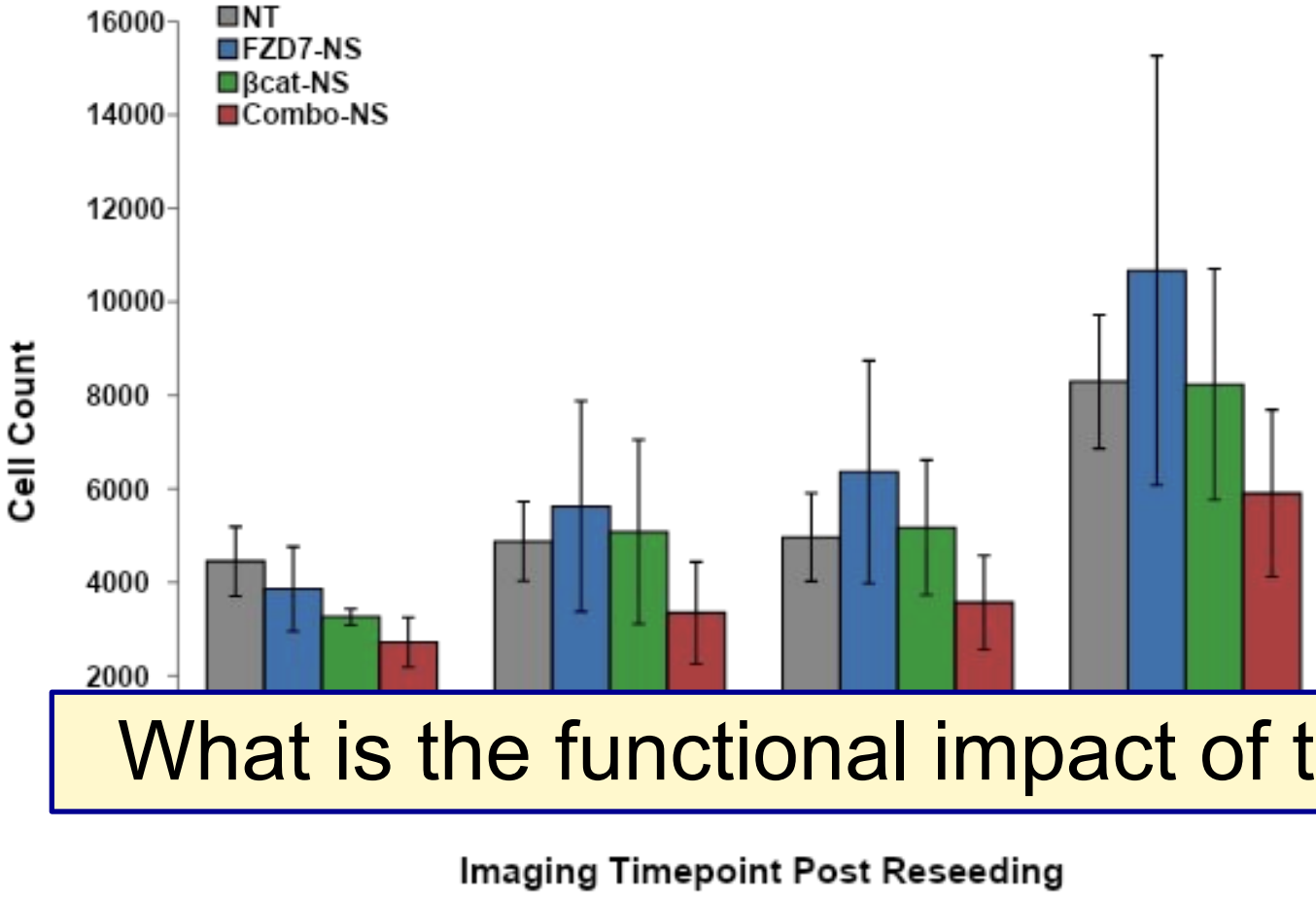
n = 3, #p<0.1, *p<0.05 versus NT

Evaluating Cell Migration in Response to NS-Mediated Wnt Inhibition

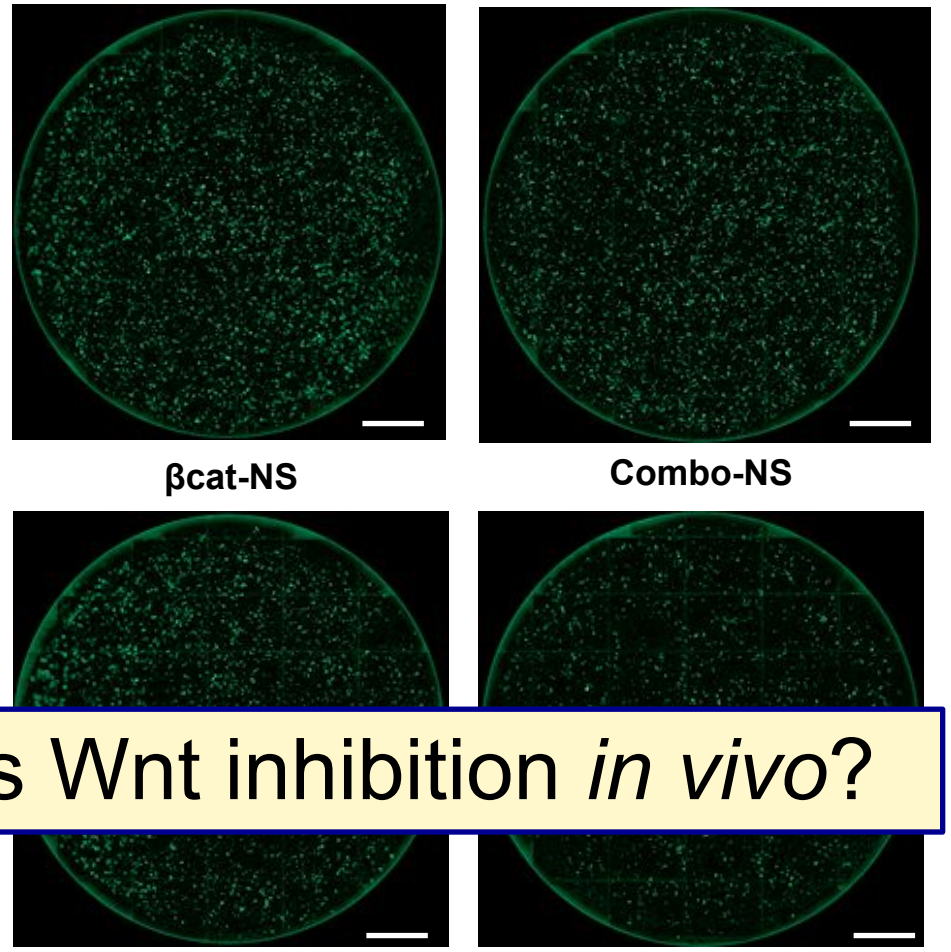


Dose=1.6E10 NS/mL; 2.5 nM antibody; 25 nM siRNA

Combo-NS Reduce Cell Migration to a Greater Extent than Monotherapies



Representative Images at 24 hours



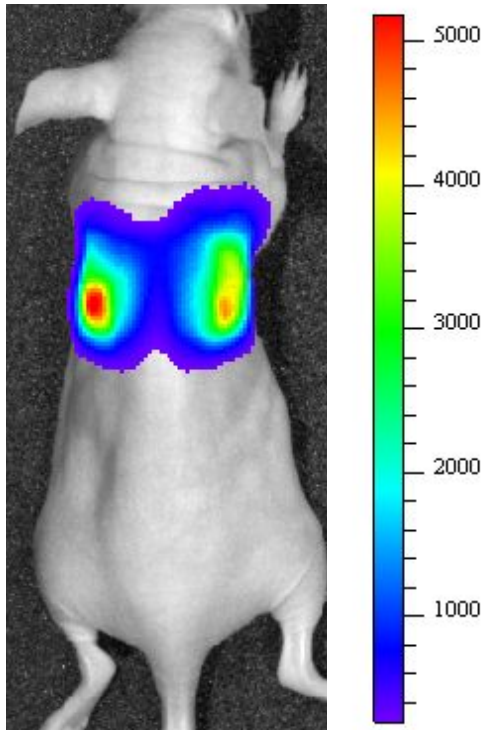
What is the functional impact of this Wnt inhibition *in vivo*?

Scale = 1 mm
n = 3

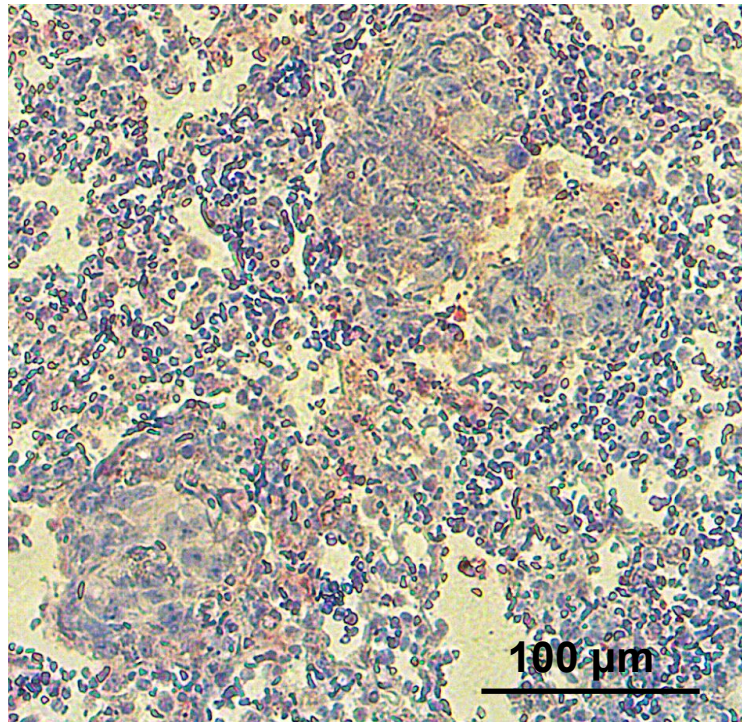
Analyzing the Impact of Wnt Inhibitory NS in an Experimental TNBC Lung Metastasis Model

I.V. Inject
MDA-MB-231 Cells

Luminescence

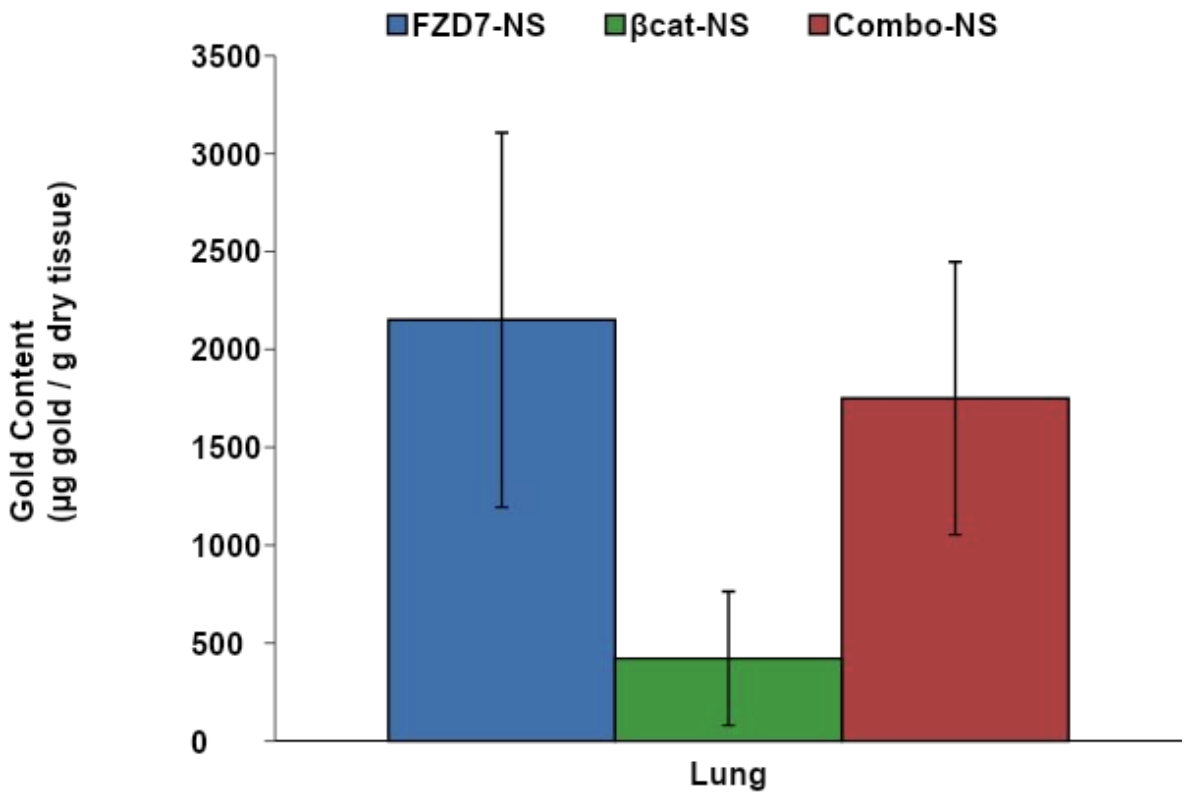


FZD7 Expression

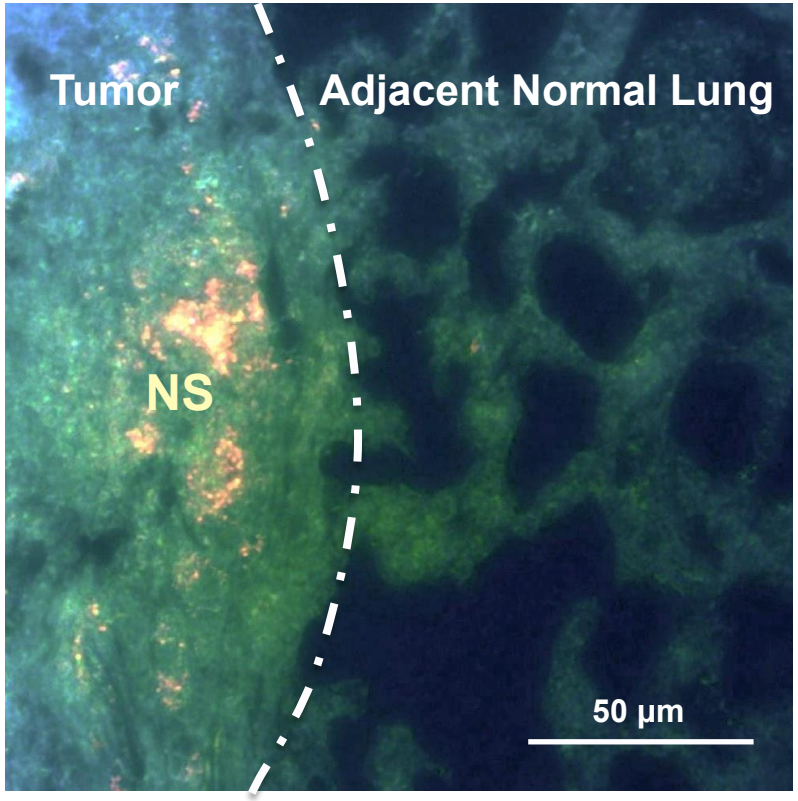


FZD7 Antibodies Facilitate NS Accumulation in TNBC Lung Metastases

ICP-MS



Darkfield Microscopy



Combo-NS May Hinder the Growth of TNBC Lung Metastases

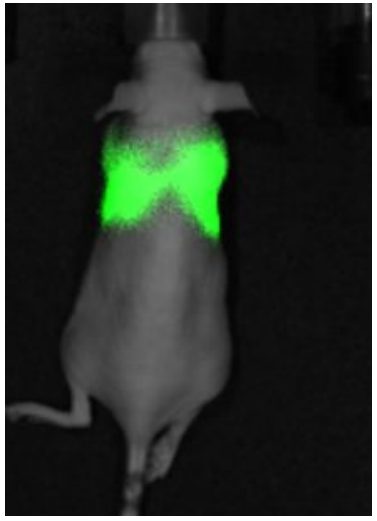
Day 0
Cell
Inoculation

Day 17, 20, 25, 39, 42, 45
Inject Combo-NS, FZD7-NS,
βcat-NS, or Saline

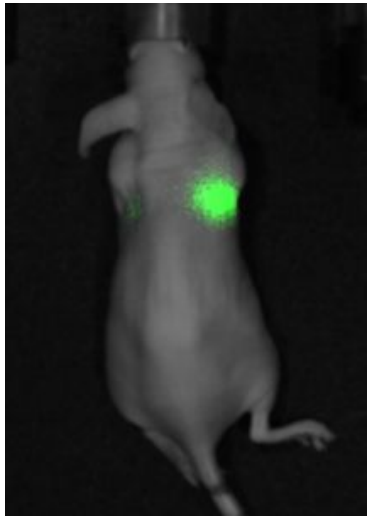
Day 59
Study
End

Day 55
(after 6 txt)

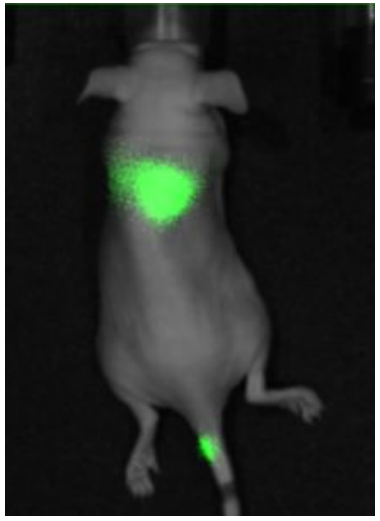
Saline



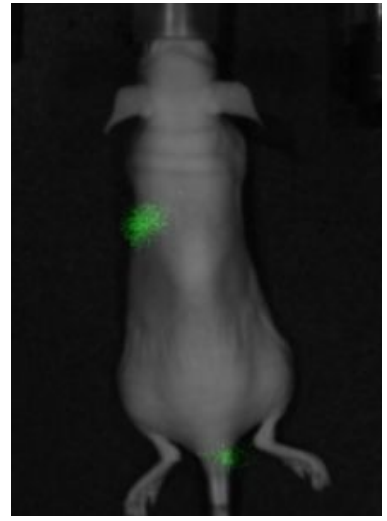
FZD7-NS



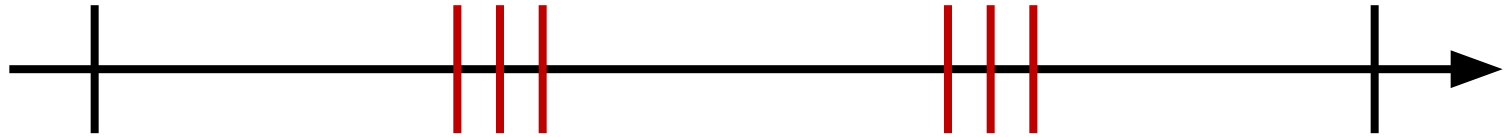
βcat-NS



Combo-NS



Combo-NS May Hinder the Growth of TNBC Lung Metastases



Day 0
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Inoculation

Day 17, 20, 25, 39, 42, 45
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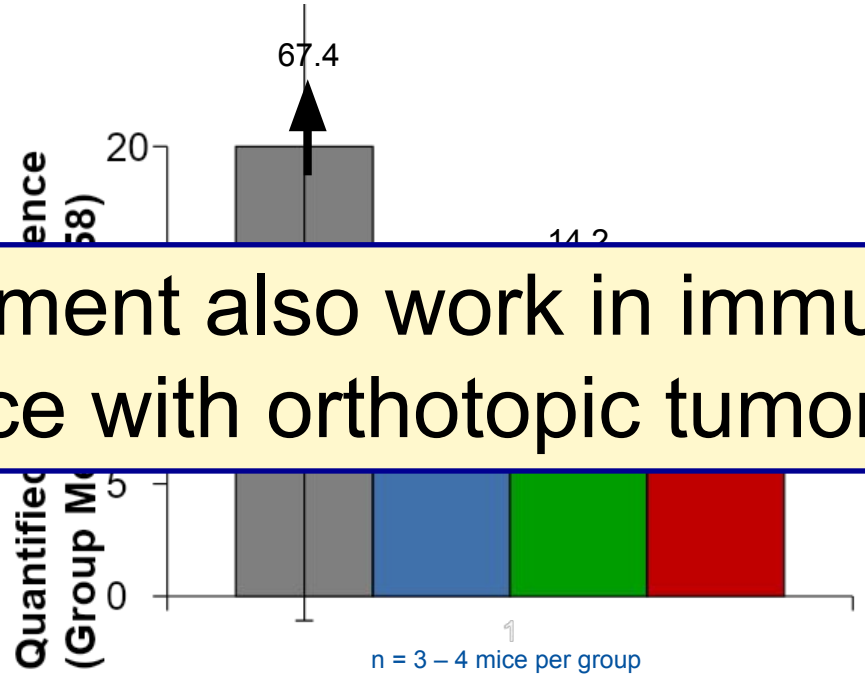
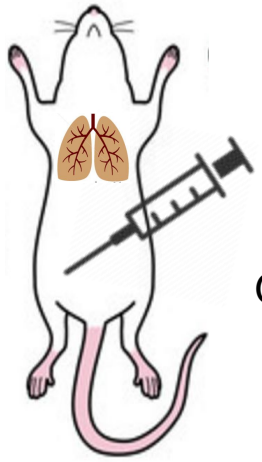


Does the treatment also work in immune competent mice with orthotopic tumors?

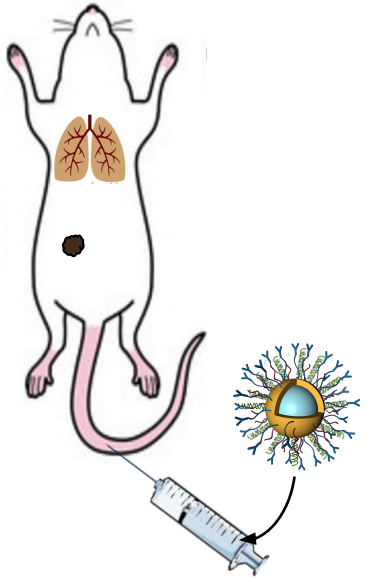
Analysis of Orthotopic 4T1 Tumor Growth in Balb/c Mice Treated with Wnt Inhibitory NPs

Inject cells



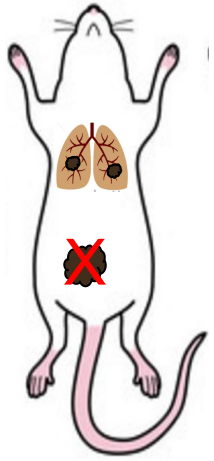
Grow tumors to ~100 mm^3

Inject NPs or saline 2X (Day 0 and 7)

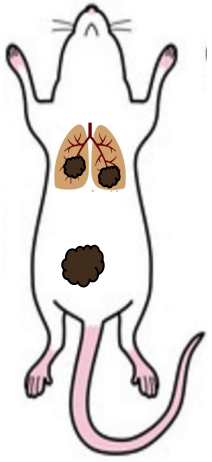


Monitor tumor growth & metastasis

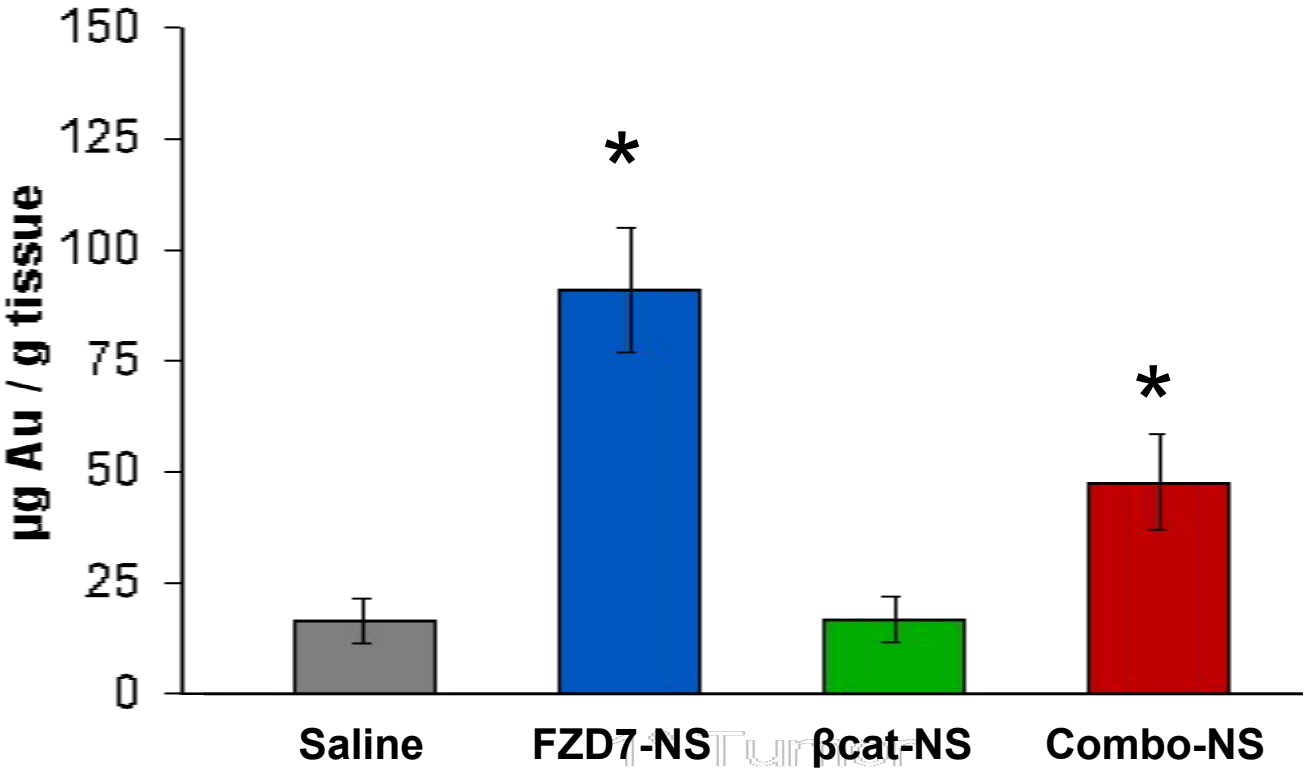
Excise tumors for ICP-MS (Day 13)



Continue treatment (Days 14, 21, 28) and assess recurrence & metastasis

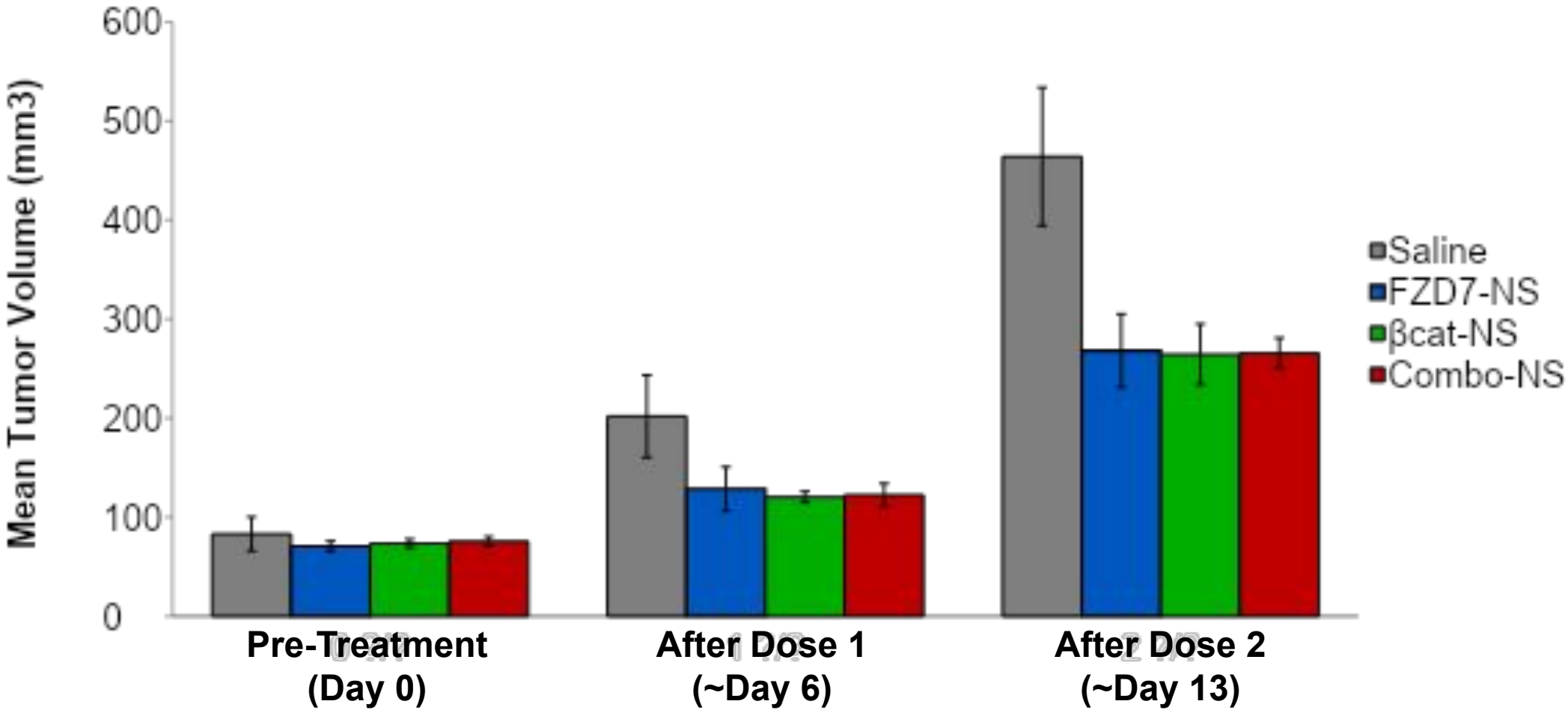


FZD7 Antibody Modification Enhances NP Accumulation in Primary Tumors



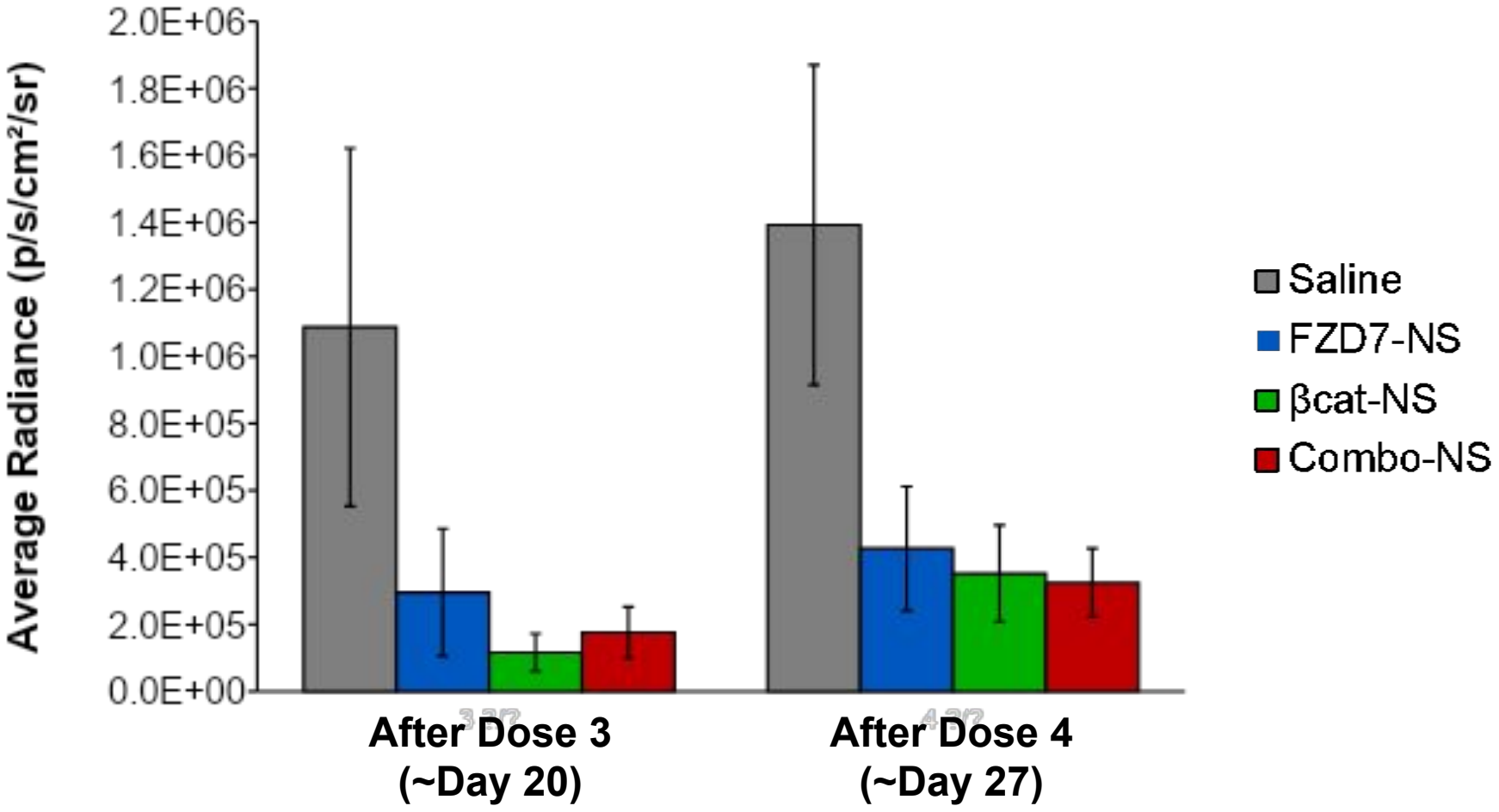
n = 6 – 7 mice / treatment; *p<0.05 vs saline control

Combo- and Mono-Therapies Inhibit Primary Tumor Growth



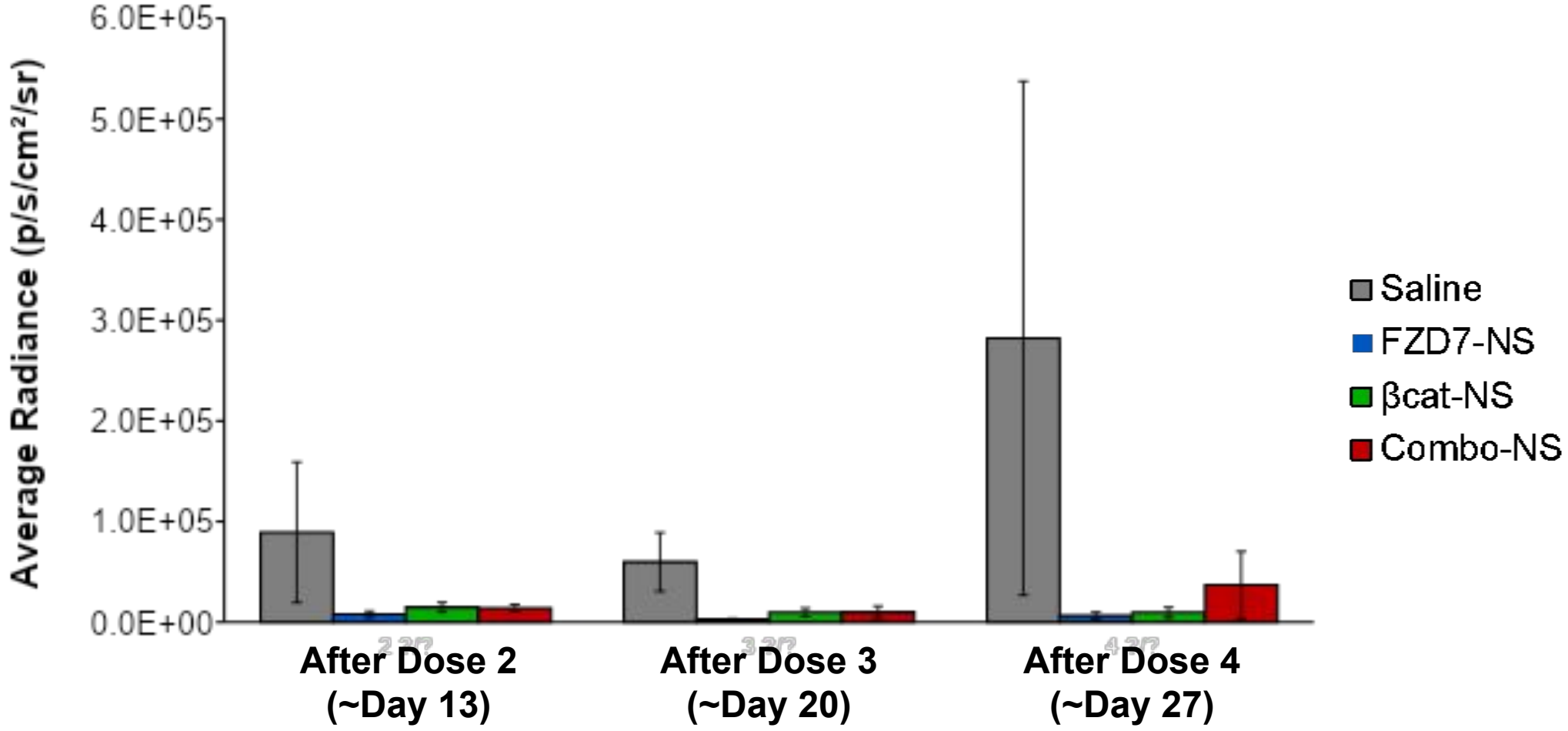
n = 6 – 7 mice / treatment

Combo- and Mono-Therapies Delay Growth of Recurrent Tumors



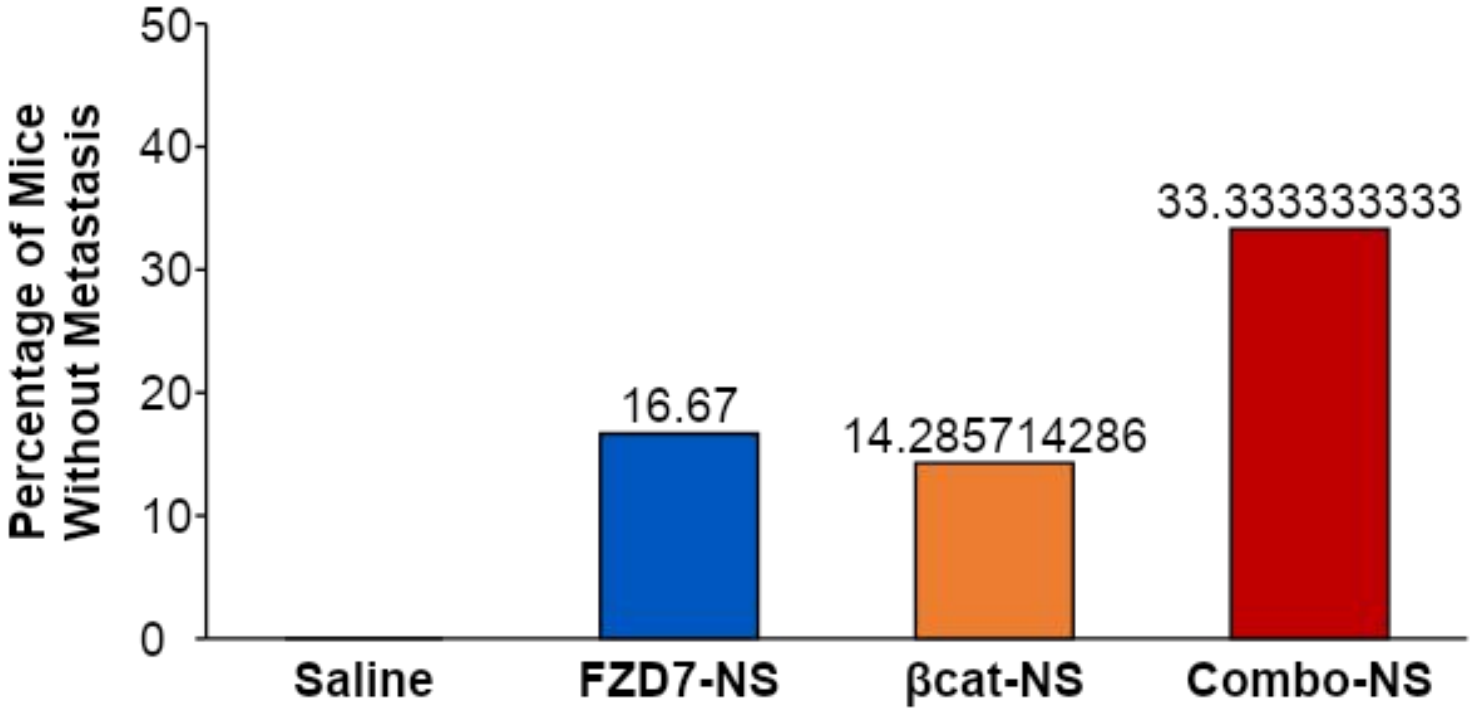
n = 6 – 7 mice / treatment

Combo- and Mono-Therapies Reduce Lung Metastasis



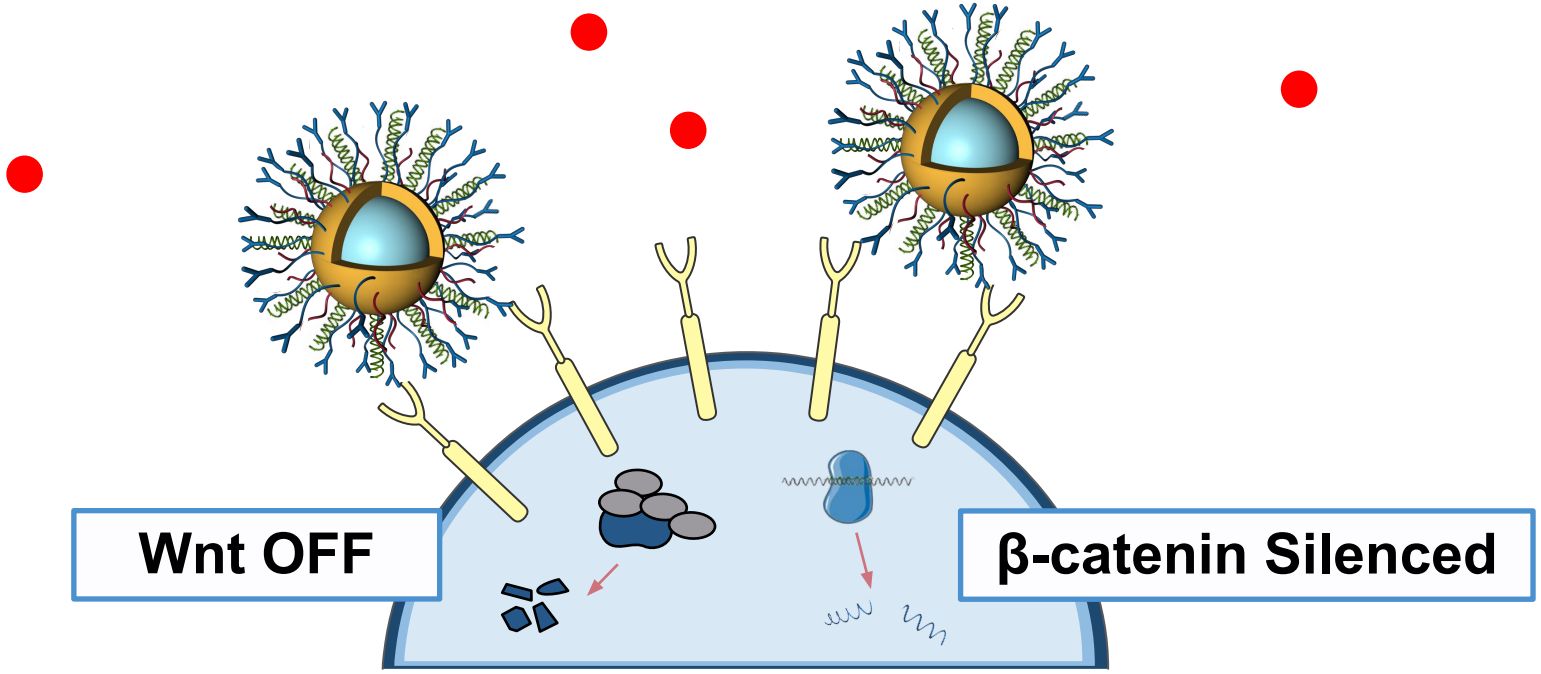
n = 6 – 7 mice / treatment

Fewer Mice Formed Metastases Following Treatment with Combo-NS



n = 6-7 mice / treatment

Conclusion: Combo-NS are Promising Tools to Combat TNBC via Wnt Inhibition



Combo- and Mono-NS can inhibit Wnt signaling in TNBC cells to impair cell function

Combo- and Mono-NS can enter primary tumors and lung metastases to reduce their growth

Co-delivery of antibodies and siRNAs is a promising strategy for multilevel gene regulation

Acknowledgments

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R01CA211925
1P20GM139760-1
U54GM104941

CAREER
DMR1752009

University of Delaware
Research Foundation

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Thank You!

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