

microRNA Blockade in Triple Negative Breast Cancer Cells and Non-Small Cell Lung Cancer Cells without Passenger Strand Side Effects

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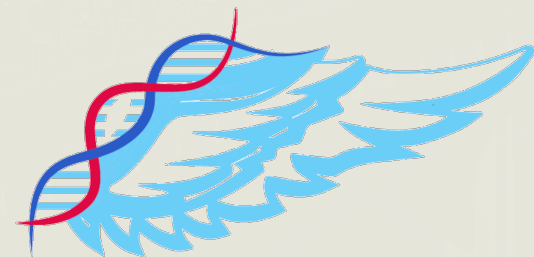
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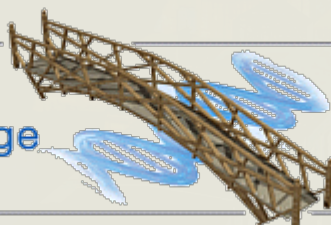
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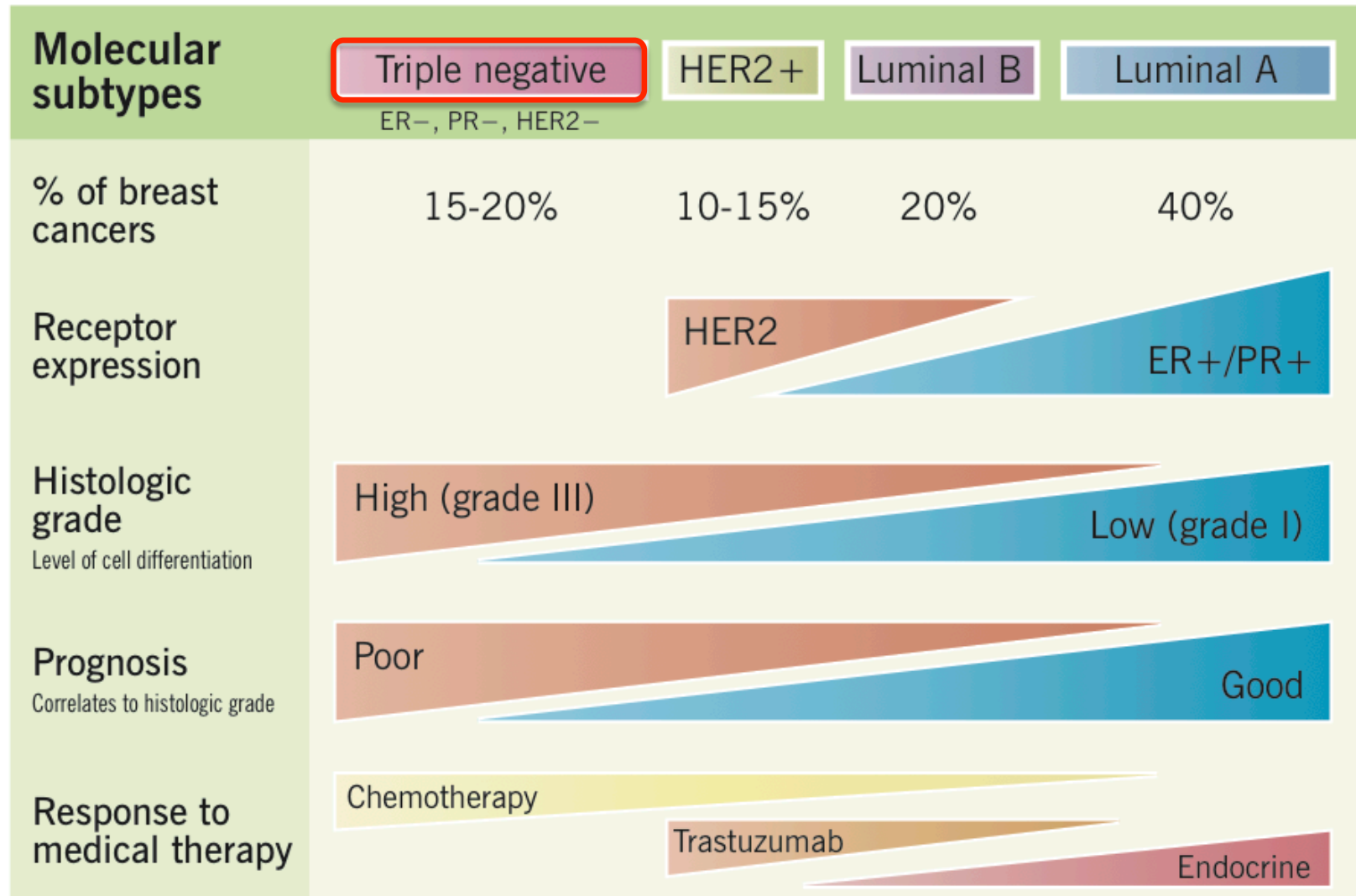
Nucleic Acids
Chemistry & Biology
7th Cambridge
Symposium



3rd-6th
September
2017



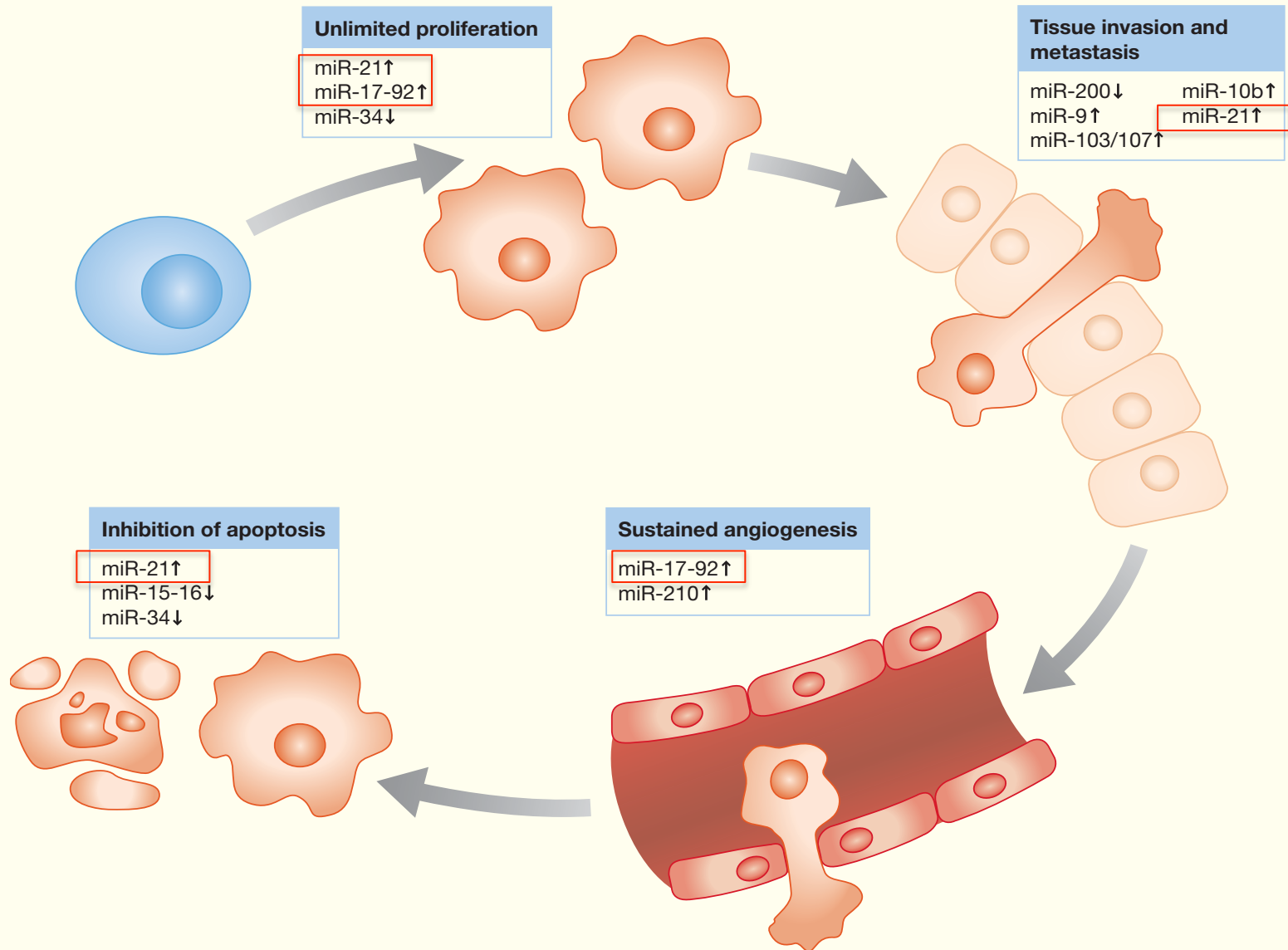
Breast cancer subtypes



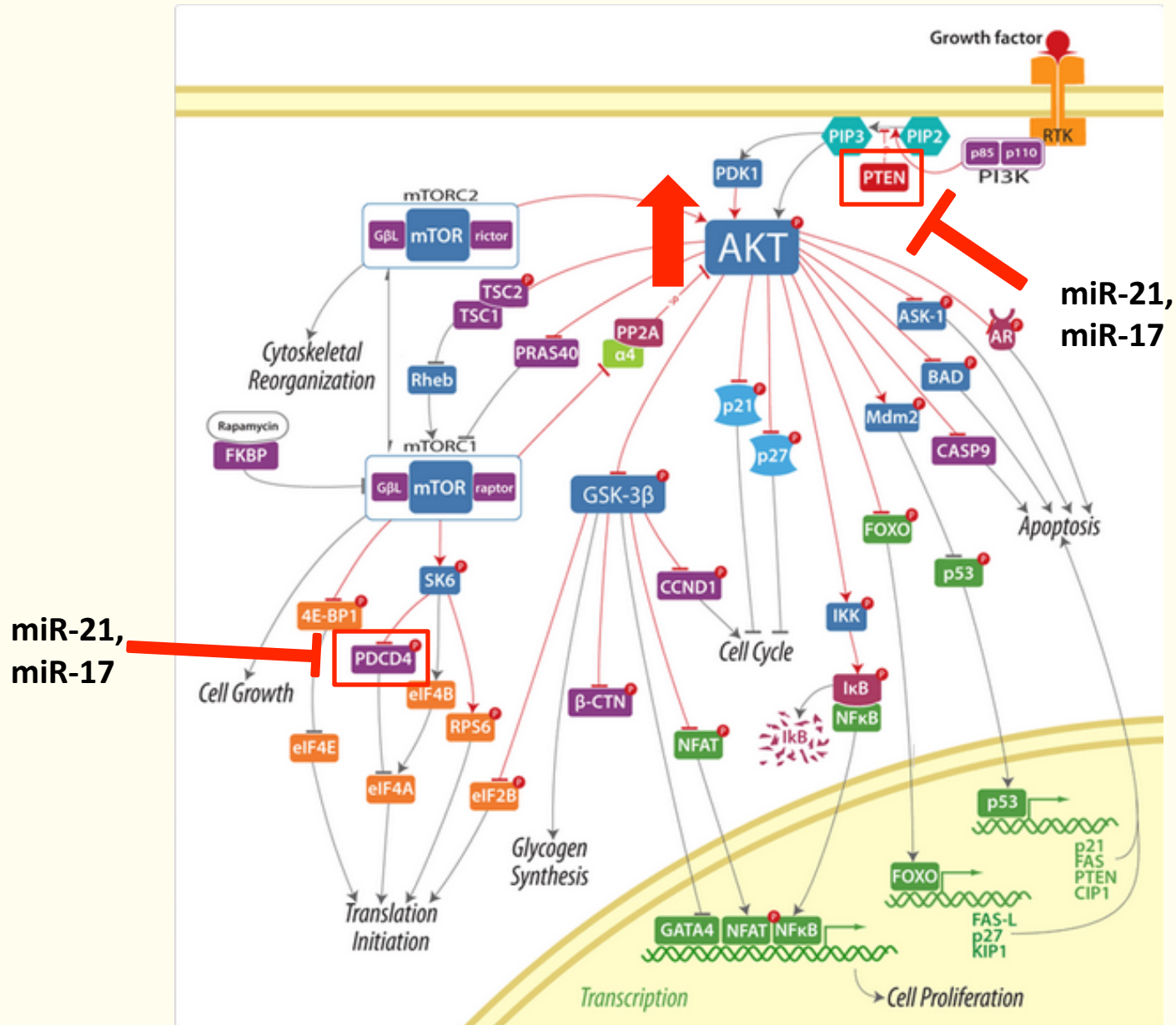
Triple negative tumours respond best to chemotherapy, similar to other aggressive cancers.

Luminal A tumours respond best to endocrine therapy, e.g. antiestrogen or aromatase inhibitor.

microRNAs in cancer

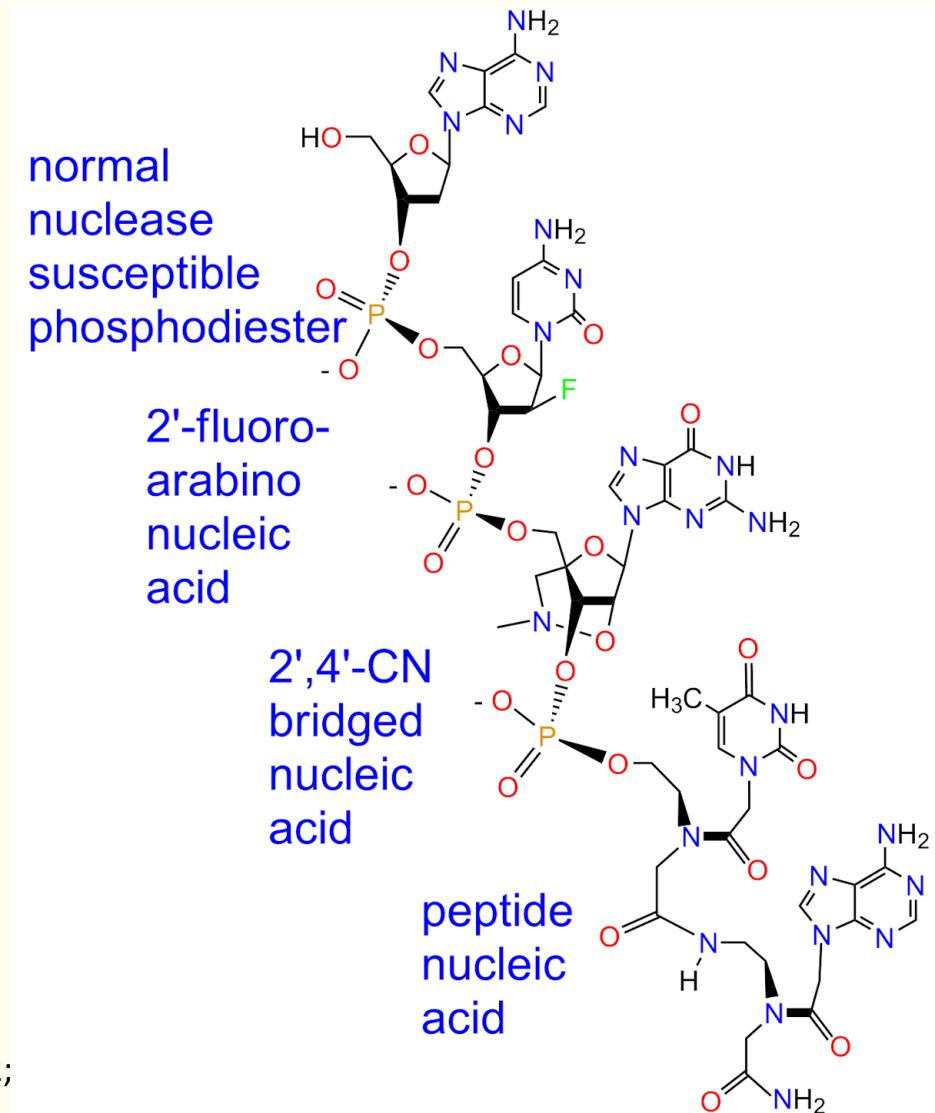


AKT activation is an interplay between miR-21 and miR-17



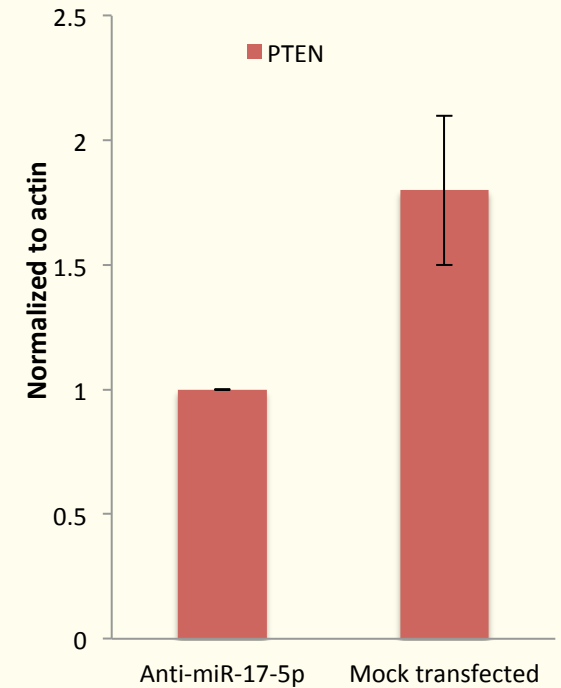
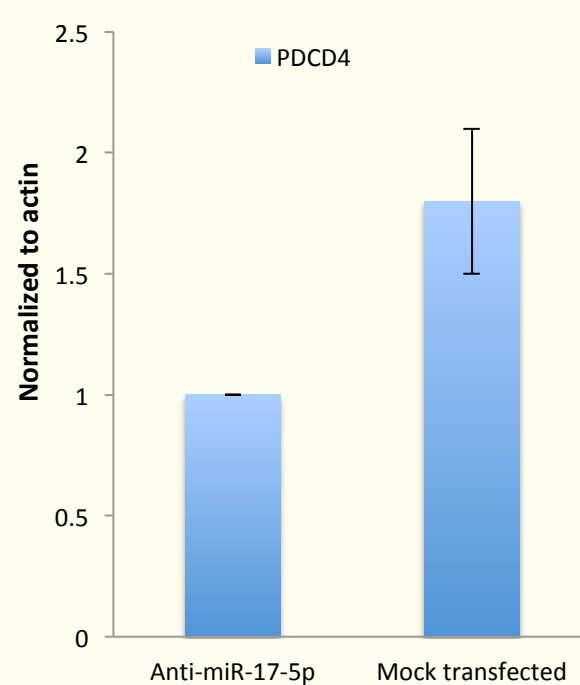
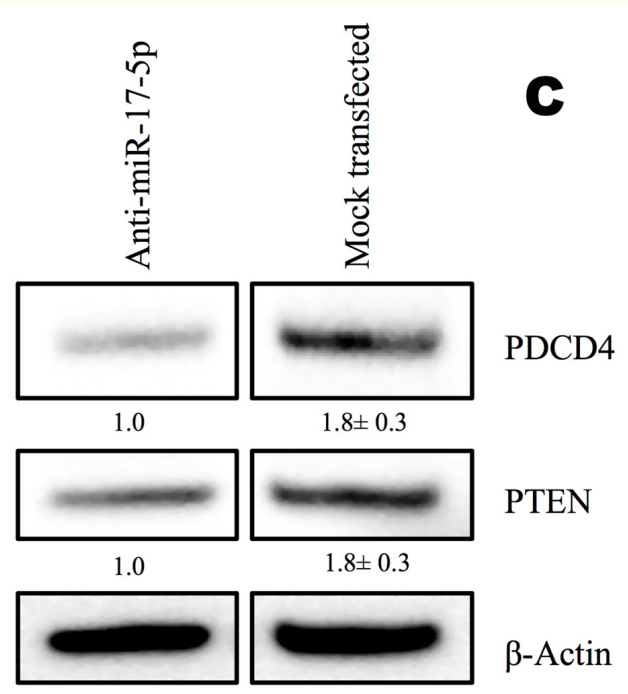
DNA/RNA analogs of increasing stability, binding affinity and specificity

backbone schematics of
normal phosphodiester (PO),
2'-fluoro-arabino nucleic acid
(FANA),
2'-NC-bridged nucleic acid
(NC-BNA),
and peptide nucleic acid
(PNA)

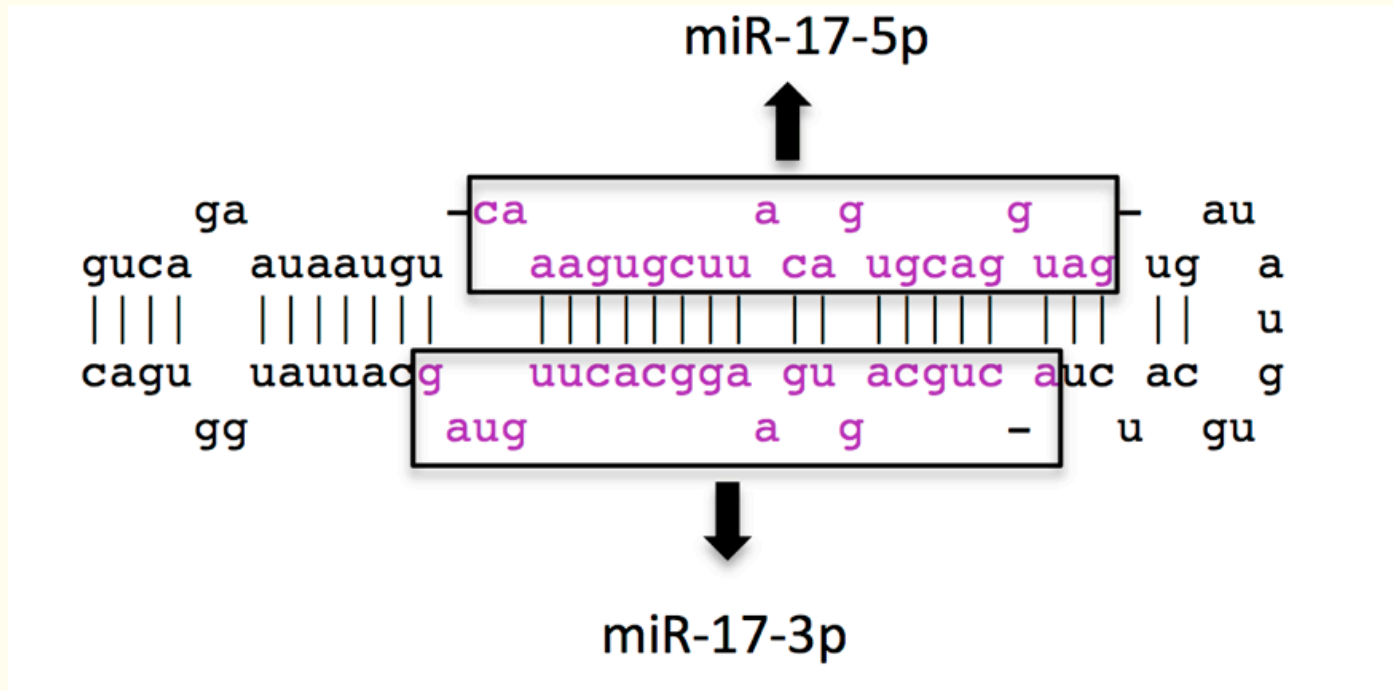


Yamamoto, T., et al. (2012), *Mol Ther Nucleic Acids* **1**: e22;
Kalota, A., et al. (2006), *Nucleic Acids Res* **34**(2): 451-461;
Chaubey, B., et al. (2008), *Oligonucleotides* **18**(1): 9-20

miR-17-5p knockdown by DNA-LNA chimera unexpectedly decreased PDCD4 and PTEN protein in MDA-MB-231 triple negative breast cancer cells

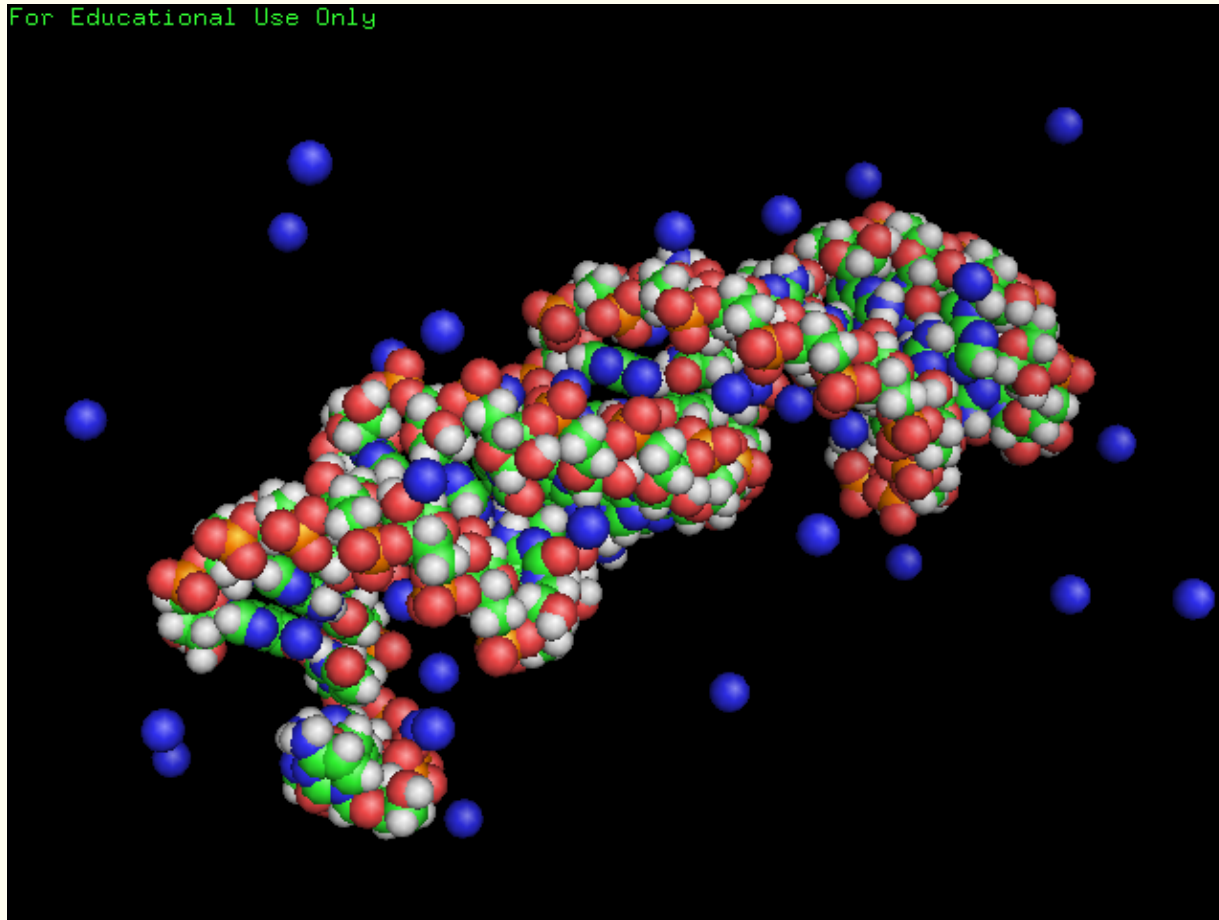


pre-miRNA structure of miR-17 illustrates sequence similarity between DNA-LNA chimera and miR-17-3p passenger strand



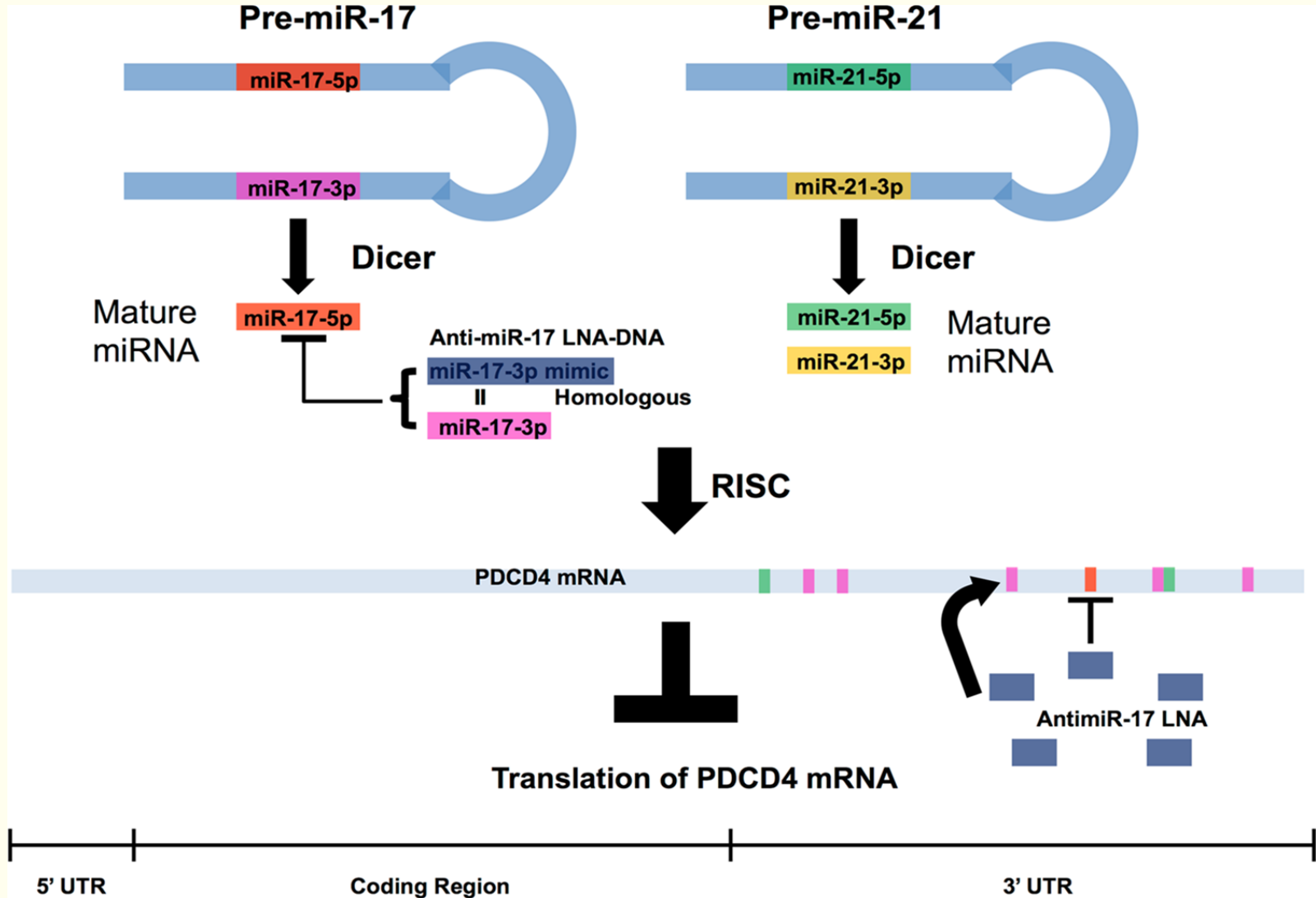
5' A-CUGCAGUG-AAGGCAC-UUGUAG 3' miR-17-3p
 5' ACCTGCACCTGTAAG-CACTTTG 3' Anti-miR-17-5p LNA

Molecular dynamics imply that miR-17-3p passenger strand can form stable A-form helix with mRNA 3'-UTR targets

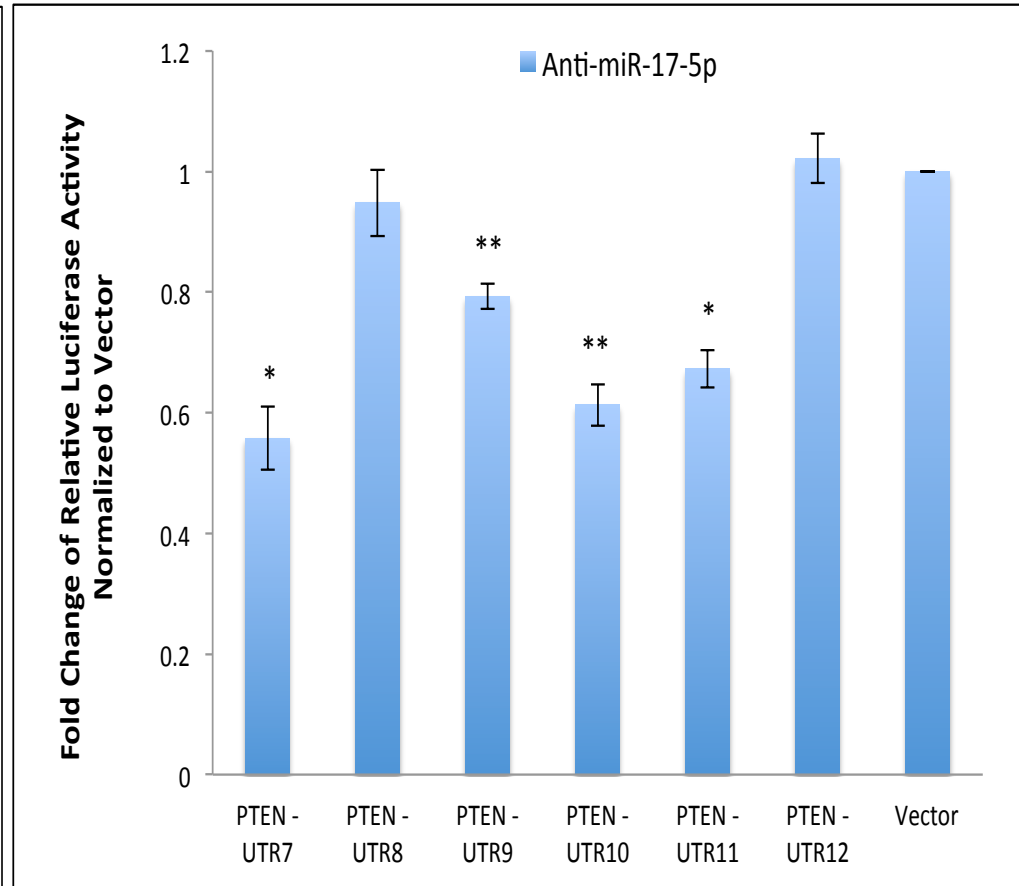
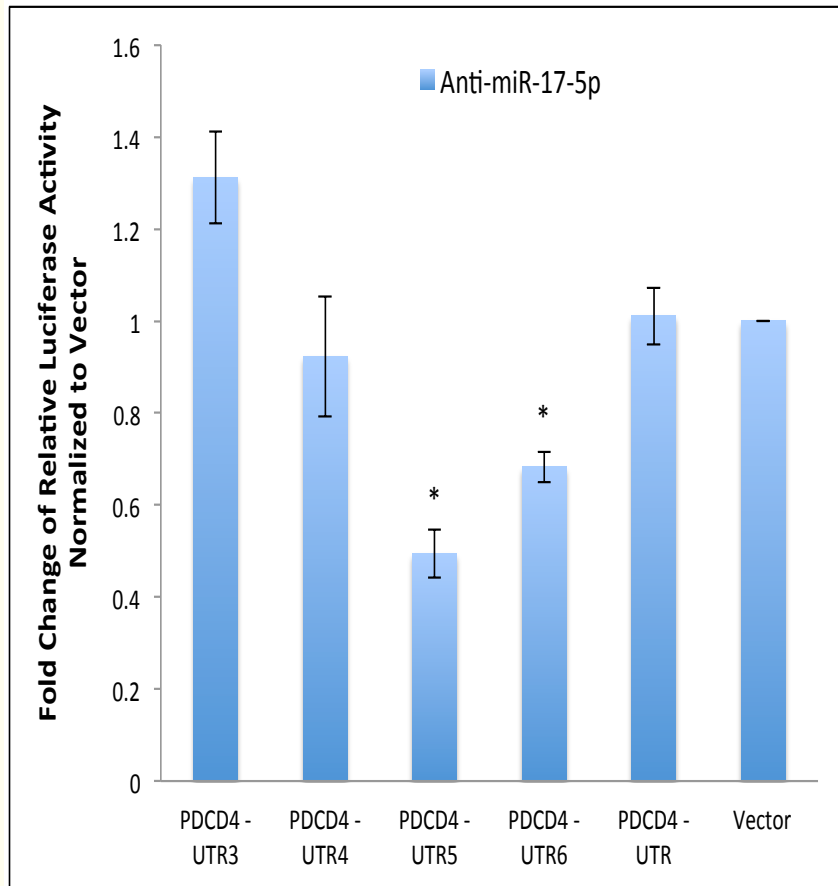


AMBER 12 accelerated molecular dynamics of *PTEN* mRNA 3'-UTR target with miR-17-3p in explicit H₂O with 100 mM NaCl at 300°K, implying that mRNA 3'-UTR: oncomiR duplexes can be accommodated in the substrate groove of Ago2.

Competition between anti-miR-17-5p and miR-17-5p for inhibition of *PDCD4* mRNA

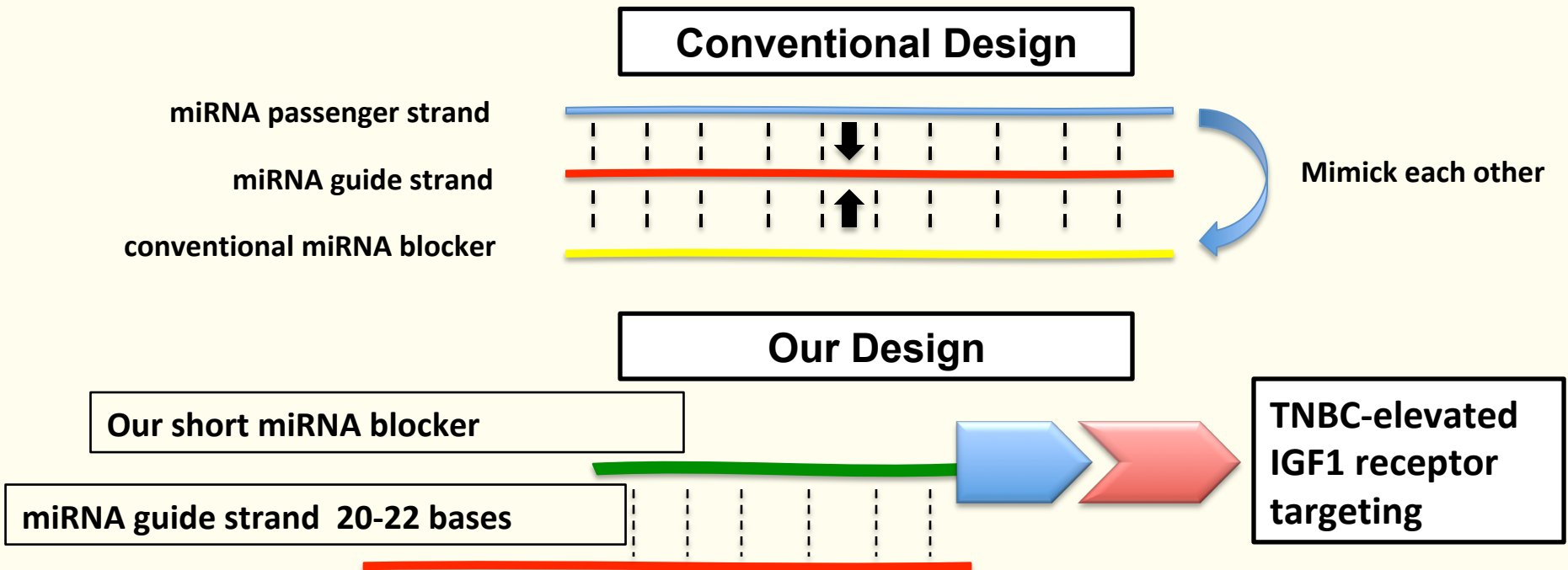


Anti-miR-17-5p DNA-LNA lowered the expression of luciferase vectors containing predicted *PDCD4* and *PTEN* 3'UTR target sites for miR-17-3p

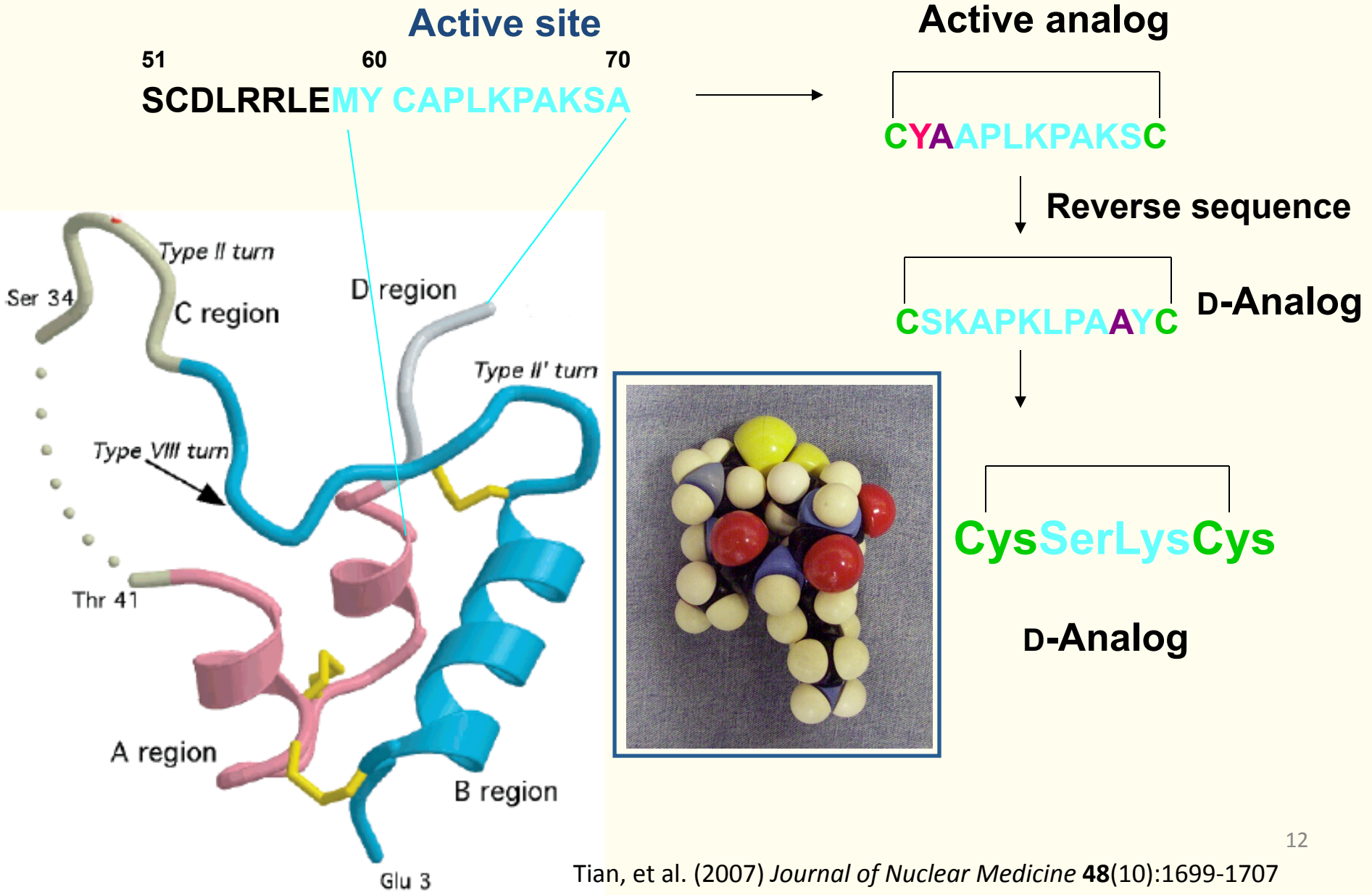


miRNA blocker design strategy

- Eliminate side effects of conventional microRNA blockers
- Next generation RNA backbone (NC-BNA) to elevate potency
- TNBC cell-specific delivery method
- No complicated formulation, soluble in saline, intravenous route

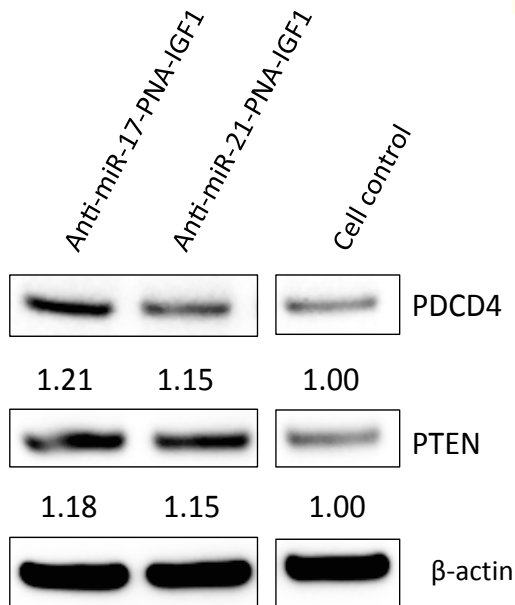


Delivery - IGF1 retro-inverso analog

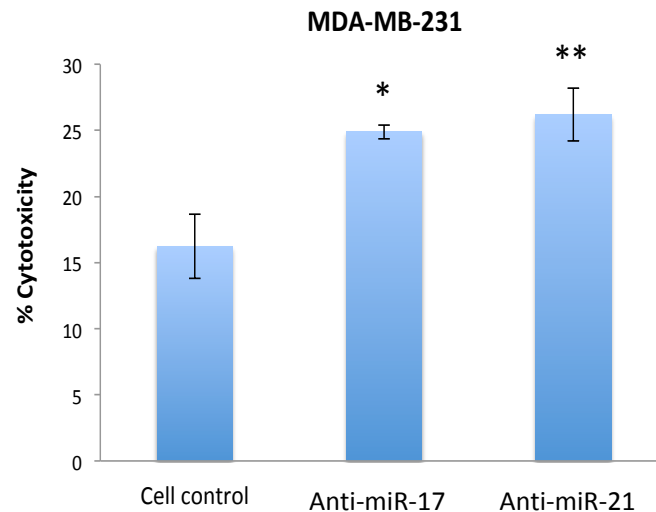


PNA-peptide IC50 $\approx 1 \mu\text{M}$ in TNBC cells

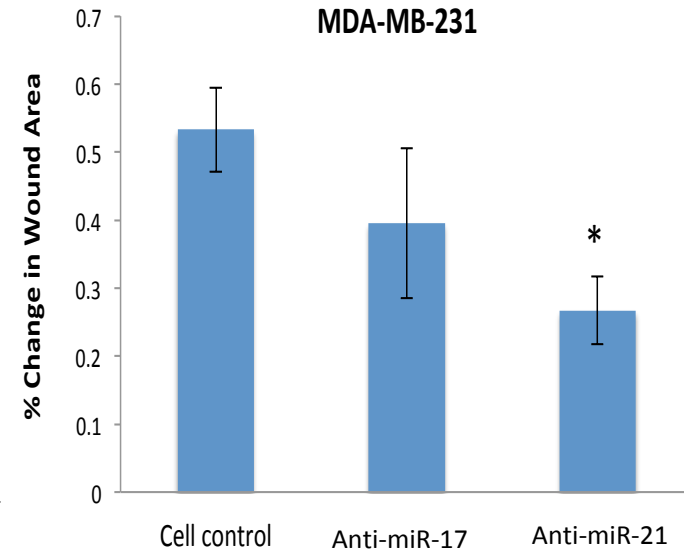
Western blot



Apoptosis



Migration



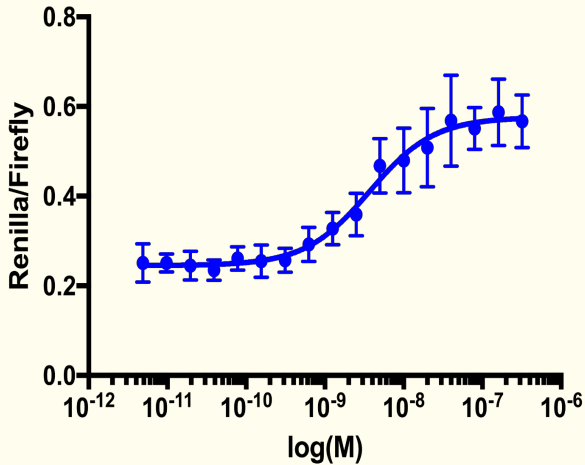
miR-17-5p blocker: Ac-GTAAGCACTTTG-AEEA-cyclo-D(CSKC)

miR-21-5p blocker: Ac-TCTGATAAGCTA-AEEA-cyclo-D(CSKC)

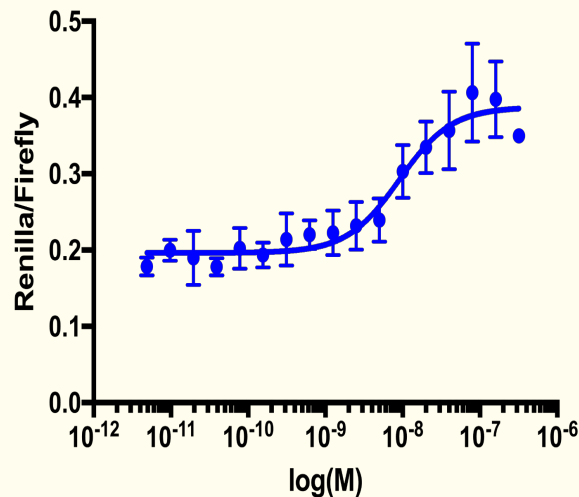
MDA-MB-231 cells were incubated 48 h at 37°C with 1 μM agent before analysis.

BNA-DNA-BNA gapmers IC50 = 4 nM for luciferase activation in TNBC cells

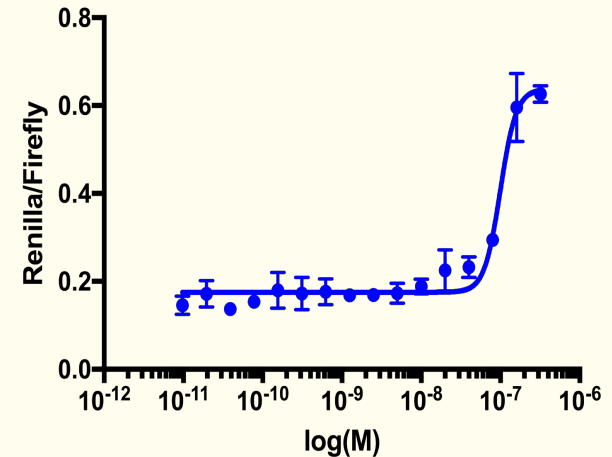
BNA IC50 = 4 nM



LNA IC50 = 9 nM

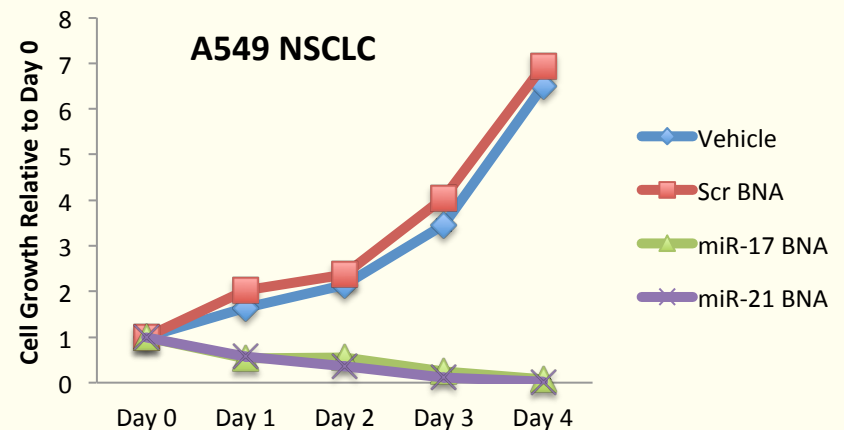
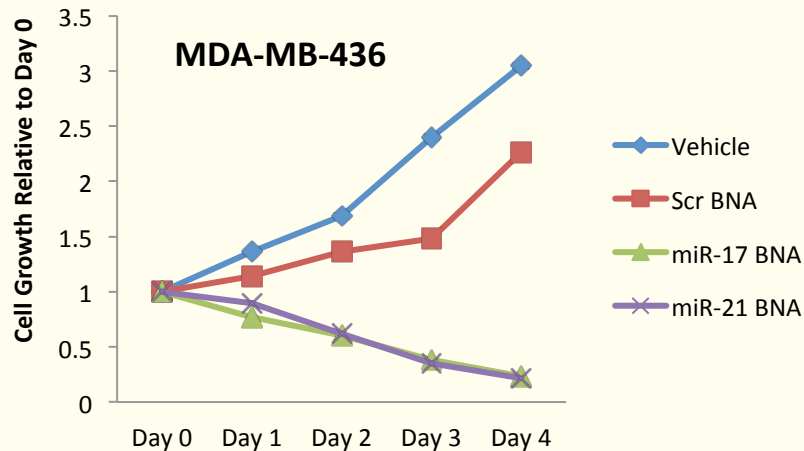
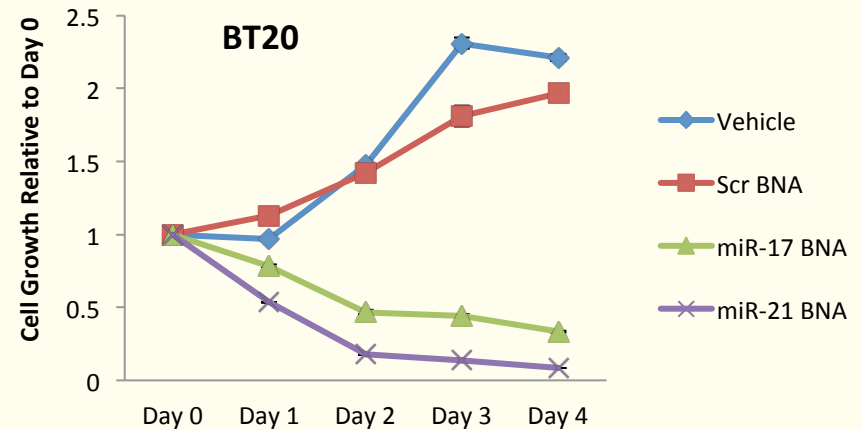
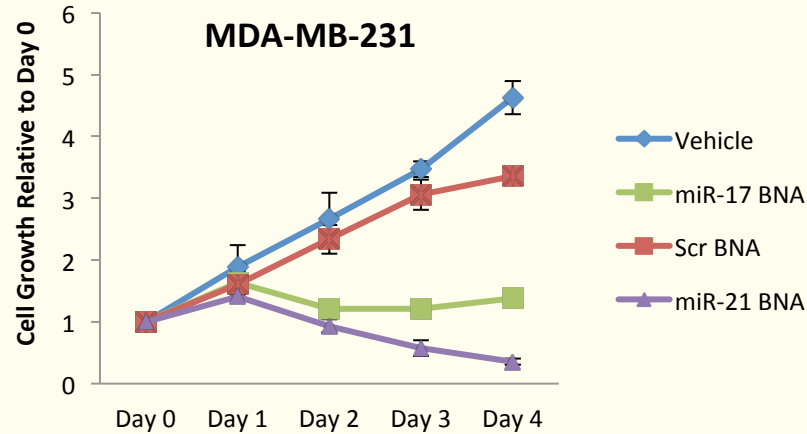


FANA IC50 = 100 nM



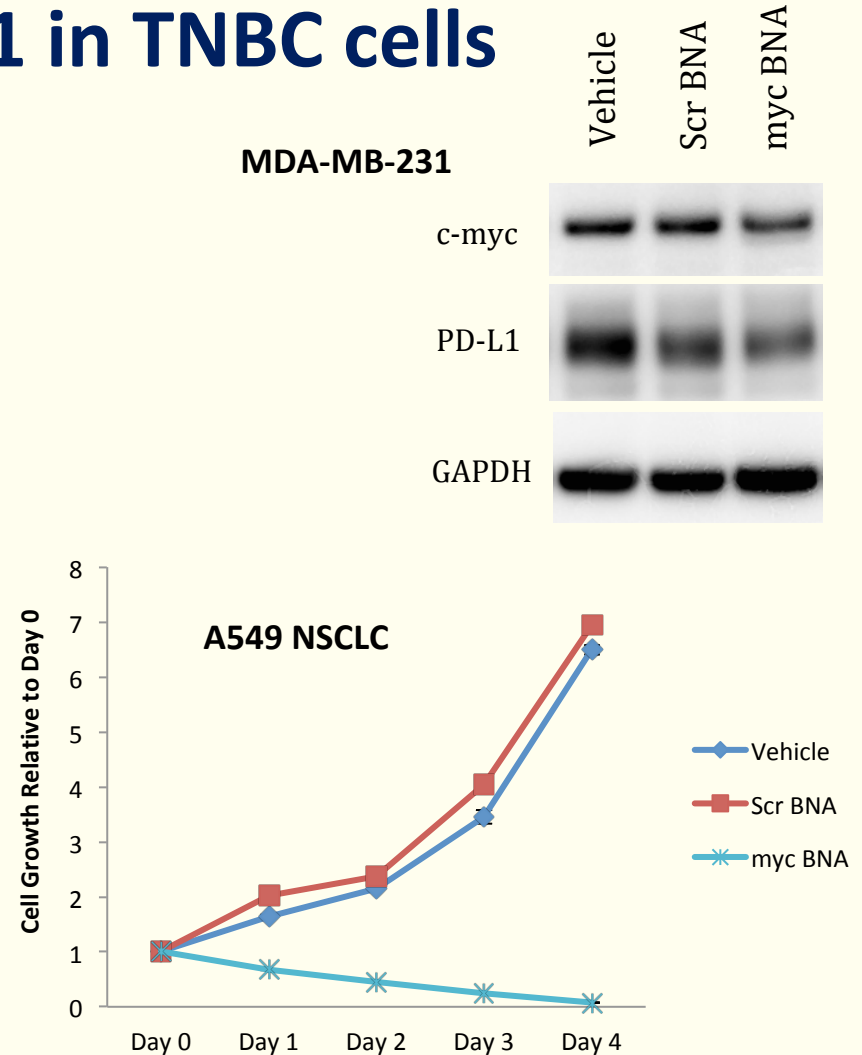
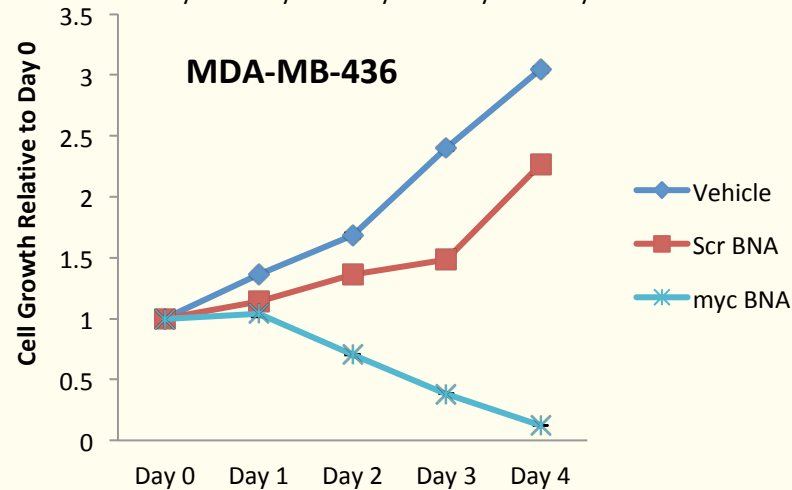
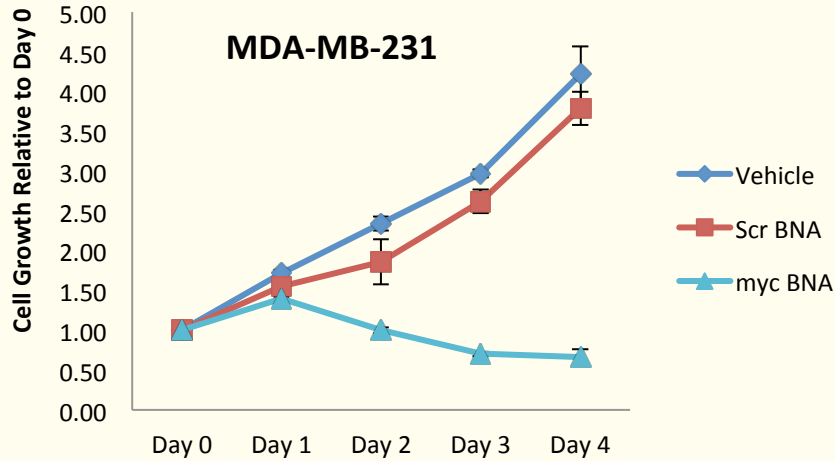
MDA-MB-231 cells were incubated 24 h at 37°C with co-transfected luciferase vector and miR-17-5p ACTGTAAGCACTTTG gapmer before analysis.

BNA-DNA-BNA gapmers slowed proliferation in TNBC cells and NSCLC cells



Cells were incubated 48 h at 37°C with 50 nM miR-17-5p ACTGTAAGCACTTTG gapmer or miR-21-5p CAGTCTGATAAGCTA gapmer before analysis.

BNA-DNA-BNA gapmers against unique *MYCC* mRNA target also slowed proliferation and reduced PD-L1 in TNBC cells



Cells were incubated 48 h at 37°C with 50 nM *MYCC* gapmer before analysis.

Summary

- The functional changes in TNBC cells treated with 1 μ M PNA-peptide 12mer were modest, indicating low efficacy.
- TNBC cells and NSCLC cells slowed proliferation dramatically upon transfection with 50 nM microRNA and *MYCC* mRNA BNA-DNA-BNA 15mer gapmers.
- ?? And *MYCC* BNA-DNA-BNA gapmers reduced PD-L1 expression in TNBC cells.
- Future blocking experiments will utilize BNA-DNA-BNA-peptide conjugates.

Acknowledgements

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