SENTI BIO™

Engineering pharmacologically relevant, FDA-approved small-molecule-regulated gene circuits for therapeutic applications in the brain

Rebecca Cottman[1], Vanessa Johnson[1], Michelle Hung[1], Yin Yin Chong[1], Andrew Banicki[1], Mengxi Tian[1], Chen-Ting Lee[1], Eren Chu[1], Assen Roguev[1], Monika Avina[1], Alex Danza[1], Brett Kiedaisch[1], Raghav Kannan[3], Chandra Verma[3], Chew-Li Soh[2], Conor McAuliffe[2], Mark Tomishima[2], Russell Gordley[1], Gary Lee[1], Tim Lu[1]

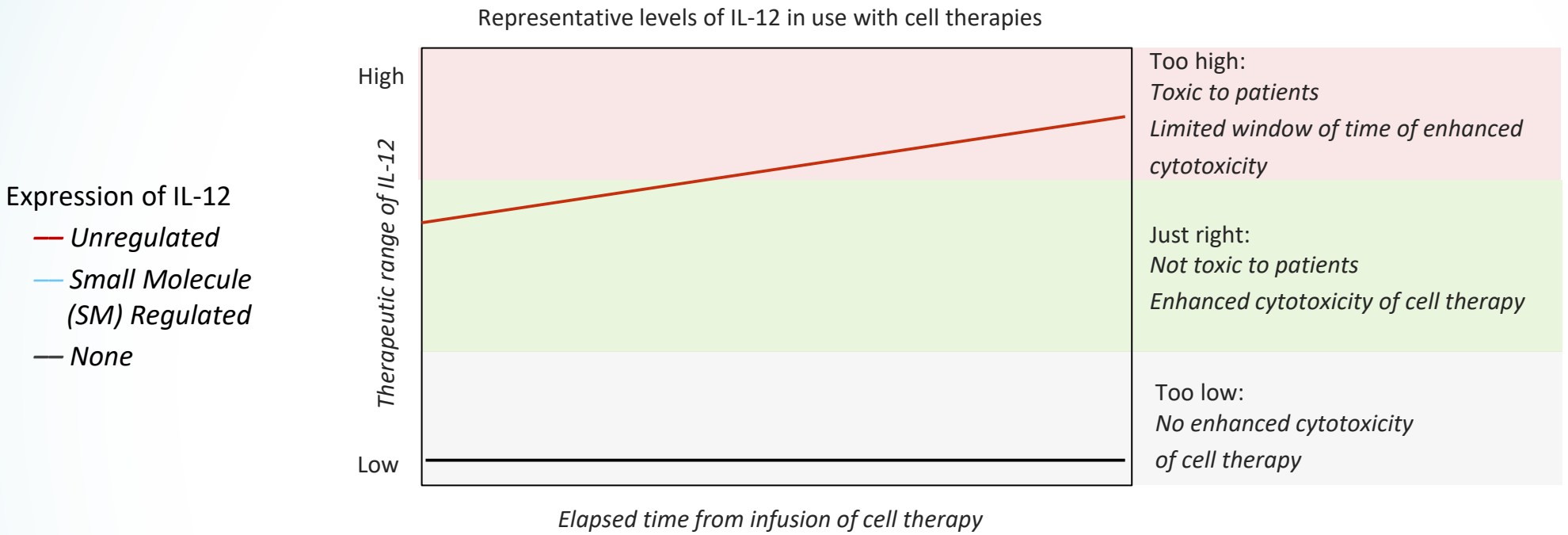
1 Senti Biosciences, Inc.
2 BlueRock Thearaputics
3 Aplomex

Presented by: Rebecca Cottman
May 18, 2023

Disclosures

Rebecca Cottman is a paid employee of Senti Biosciences, Inc

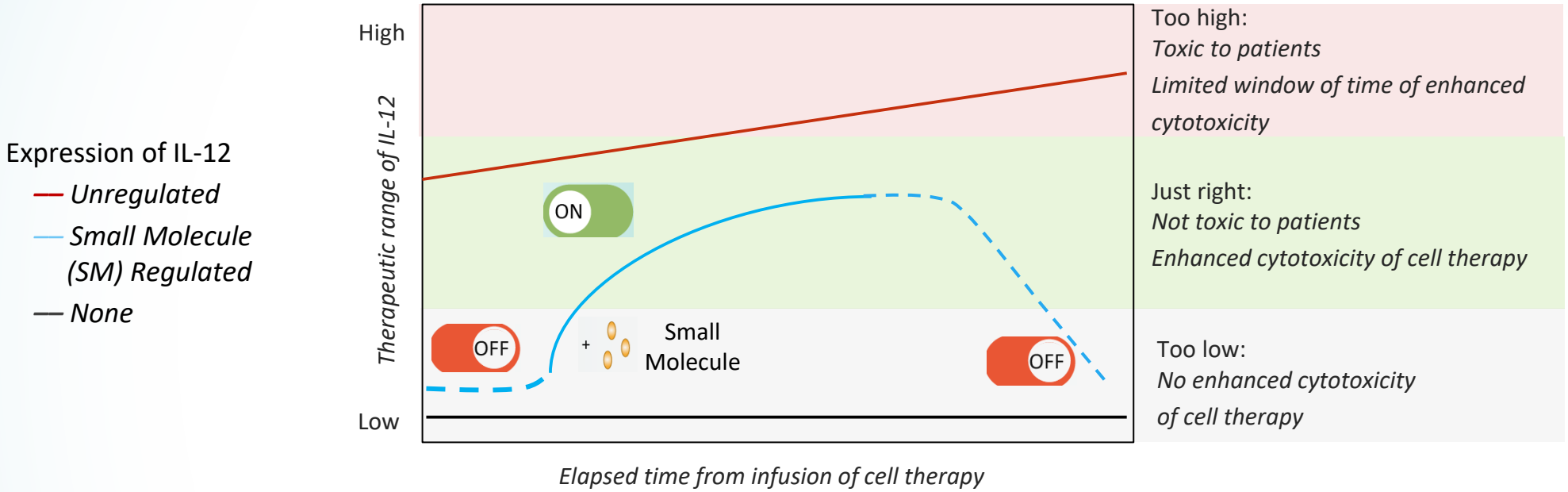
Regulated expression of IL-12 would overcome critical limitations preventing use in tumor immunotherapy applications and cancer patients



- **IL-12 is a highly potent immune activator** with the potential to **stimulate the tumor immunity cycle**
- **Unregulated IL-12 either through injection or expressing IL-12 as part of adoptive T cell therapies** using a poorly regulated promoter **has resulted in significant clinical toxicities** (Zhang et al., Clin. Can. Res. 2015; Portielje et al., Clin. Can. Res. 1999; Bajetta et al., Clin. Can. Res. 1998).
- **Narrow therapeutic window** associated with IL-12 has limited success to date

Regulated expression of IL-12 would overcome critical limitations preventing use in tumor immunotherapy applications and cancer patients

Representative levels of IL-12 in use with cell therapies



- **IL-12 is a highly potent immune activator** with the potential to **stimulate the tumor immunity cycle**
- **Unregulated IL-12 either through injection or expressing IL-12 as part of adoptive T cell therapies** using a poorly regulated promoter **has resulted in significant clinical toxicities** (Zhang et al., Clin. Can. Res. 2015; Portielje et al., Clin. Can. Res. 1999; Bajetta et al., Clin. Can. Res. 1998).
- **Narrow therapeutic window** associated with IL-12 has limited success to date

Goal: Build a SM regulated transcriptional switch for controlled IL-12 expression with the potential for therapeutic applications in the brain in collaboration with BlueRock THERAPEUTICS

Characteristics of existing small molecule regulated transcriptional switches

Available SM-based switches	FDA-approved SM & Convenient mode of delivery	Beneficial pharmacokinetics	Crosses Blood-Brain-Barrier (BBB)	
Grazoprevir	✓	✗	✗	*Tague, E, et al. Nat Methods 2018*
Rimiducid (rapamycin rapalogs)	✓	✓	✗	*Rivera VM, et al. Nat Med 1996*
Caffeine	▬	✗	✓	*Bojar, D, et al. Nat Commun 2018*
Tamoxifen	✓	✓	✓	*Gallinari, P, et al. Chem Biol 2005*

BlueRock THERAPEUTICS

Characteristics of existing small molecule regulated transcriptional switches

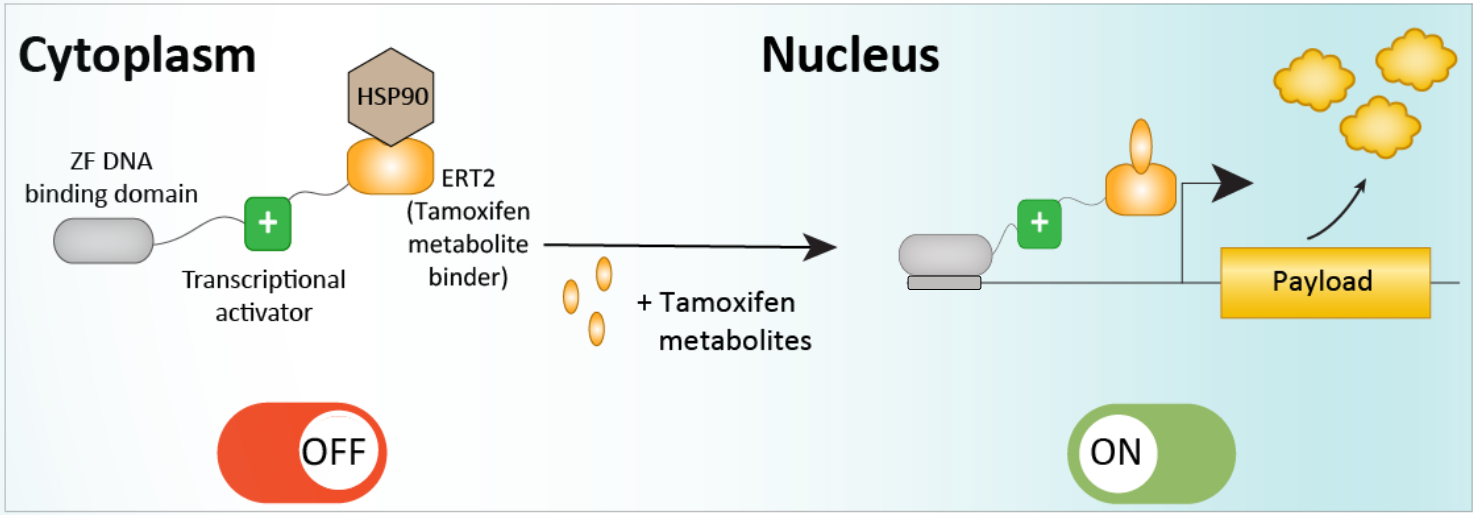
BlueRock THERAPEUTICS

Available SM-based switches	FDA-approved SM & Convenient mode of delivery	Beneficial pharmacokinetics	Crosses Blood-Brain-Barrier (BBB)	
Grazoprevir	✓	✗	✗	*Tague, E, et al. Nat Methods 2018*
Rimiducid (rapamycin rapalogs)	✓	✓	✗	*Rivera VM, et al. Nat Med 1996*
Caffeine	▬	✗	✓	*Bojar, D, et al. Nat Commun 2018*
Tamoxifen	✓	✓	✓	*Gallinari, P, et al. Chem Biol 2005*

- **Tamoxifen is an FDA-approved small molecule that can cross the blood brain barrier (BBB)** enabling this technology for potential applications in the brain.
- **Convenient mode of SM delivery with favorable pharmacokinetics** - easy drug treatment with oral dosing achieving stable drug concentrations in patients after chronic dosing with 10x concentration in tissues/tumors/organs

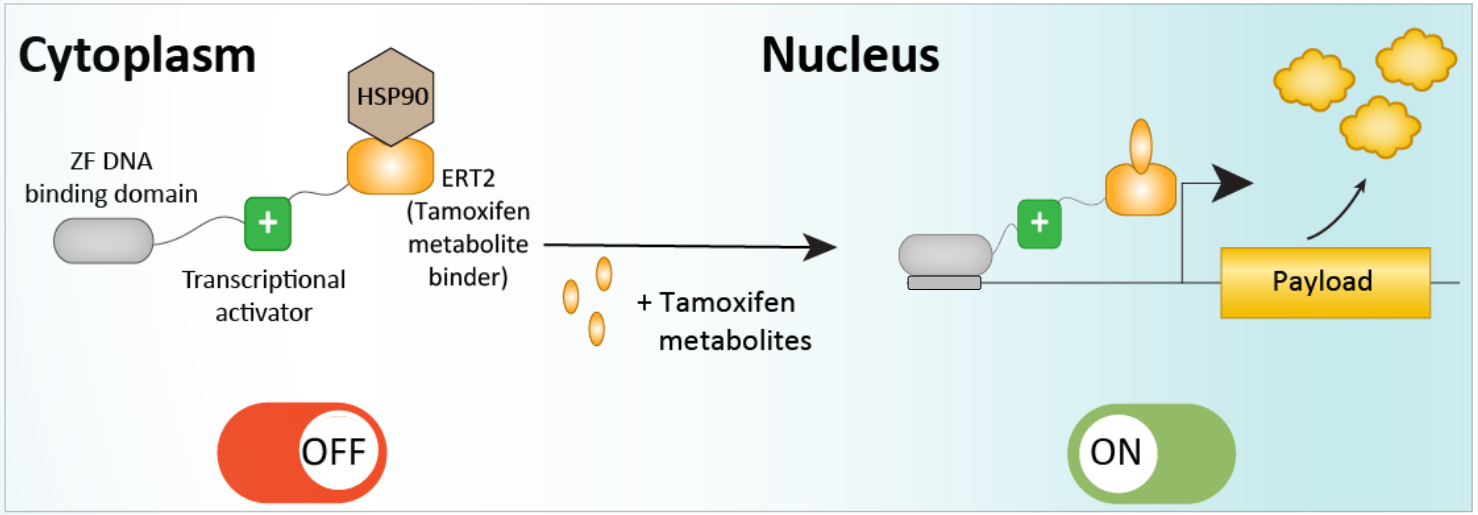
Design of Senti's Tamoxifen-regulated transcriptional switch and target performance metrics

Tamoxifen-regulated transcriptional switch



Design of Senti's Tamoxifen-regulated transcriptional switch and target performance metrics

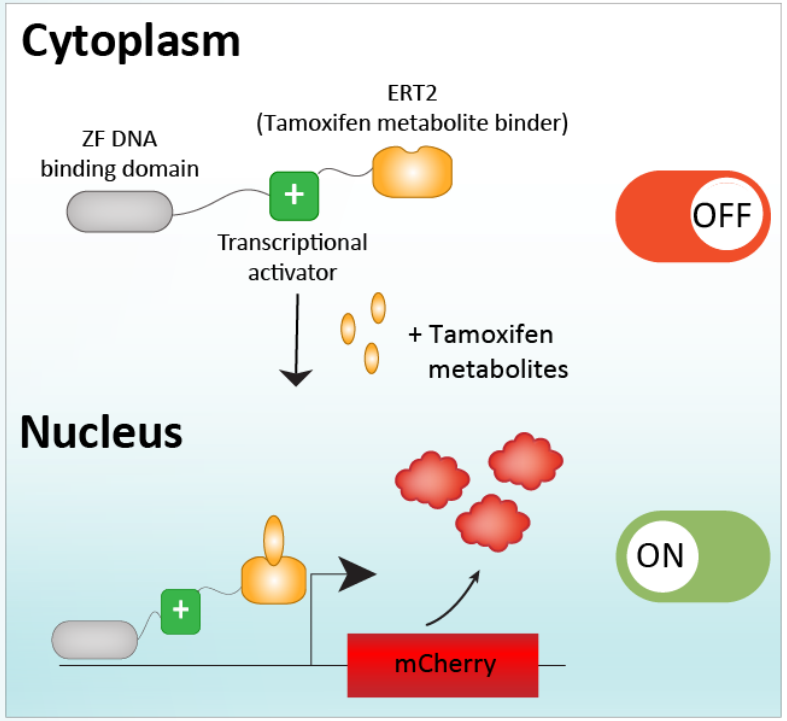
Tamoxifen-regulated transcriptional switch

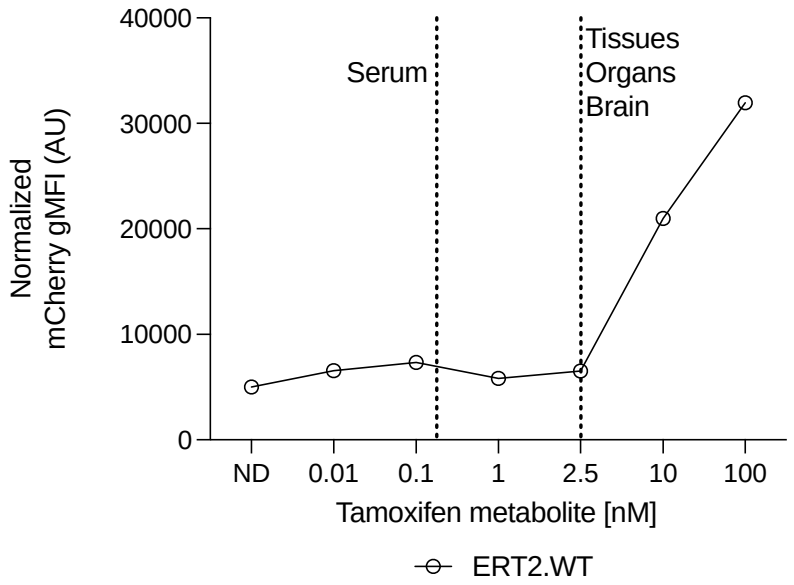


Target switch performance metrics

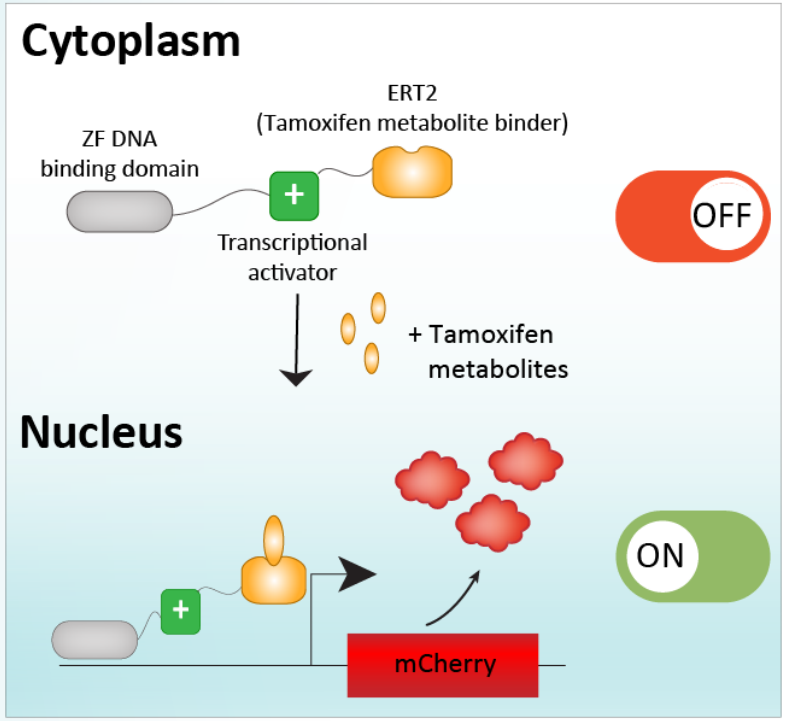
- ***Active in the Brain:*** Responsive to **2.9 nM tamoxifen metabolites** (estimated concentration in the brain)
- ***Safe:*** Expression of payload is tightly controlled by SM with low basal expression
- ***Dose Dependent:*** payload level depends on small molecule dose

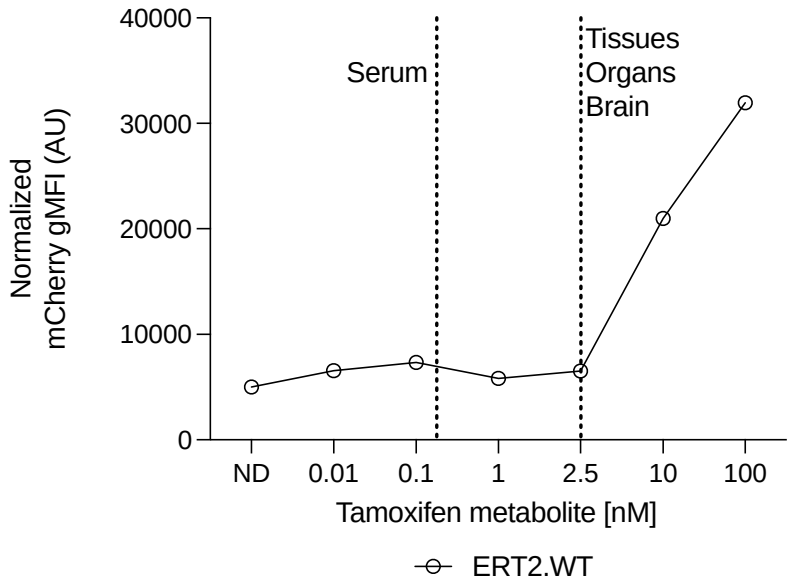
Tamoxifen-regulated transcriptional switch with wildtype ERT2 requires further engineering to perform in clinical setting





Tamoxifen-regulated transcriptional switch with wildtype ERT2 requires further engineering to perform in clinical setting



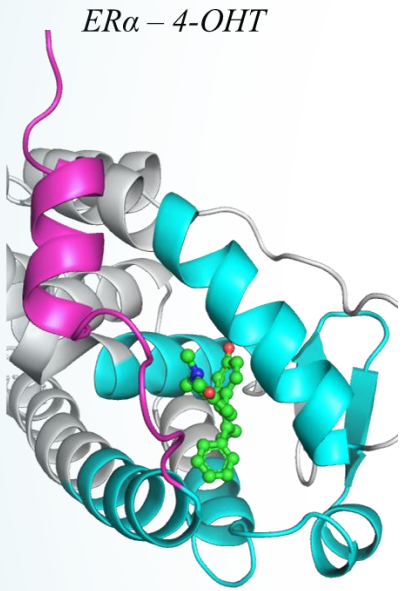


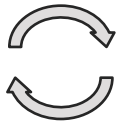
Target switch performance metrics

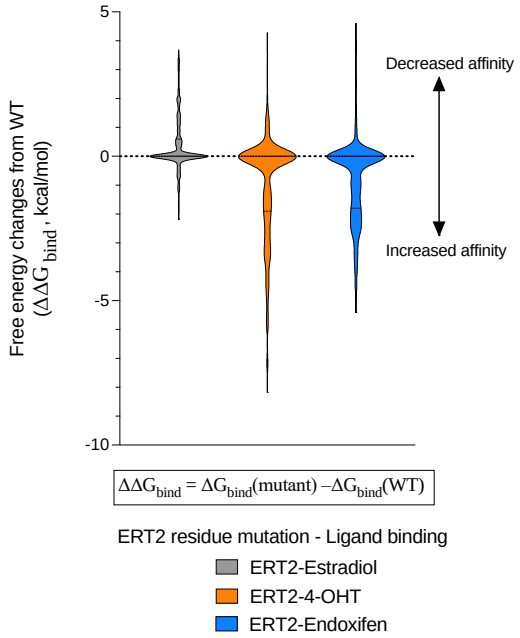
- ***Active in the Brain:*** Responsive to **2.9 nM tamoxifen metabolites** (estimated concentration in the brain)
- ***Safe:*** Expression of payload is tightly controlled by SM with low basal expression
- ***Dose Dependent:*** payload level depends on small molecule dose

The protein-SM binding interface is structurally complex but well characterized unique opportunity to utilize computational prediction methods to enable engineering a ligand binding domain with improved affinity to Tamoxifen metabolites

Engineering a better ERT2: Computational prediction of mutations with greater SM affinity

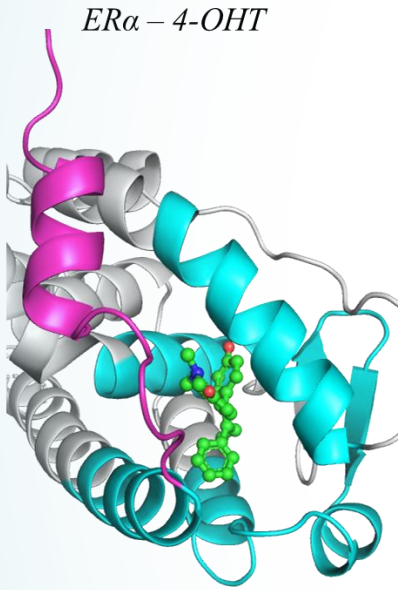


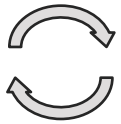


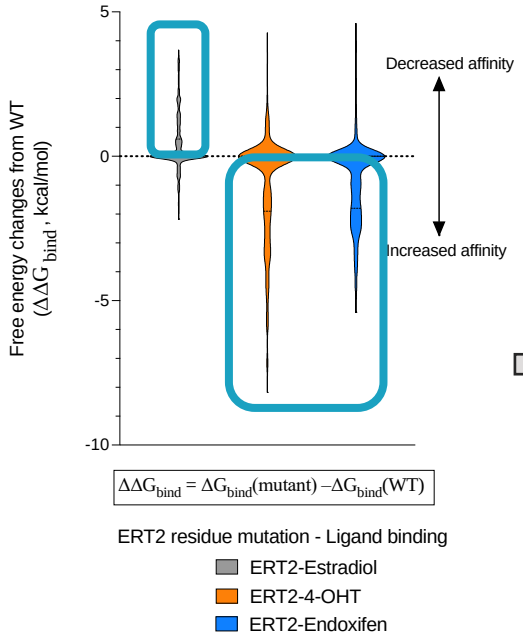


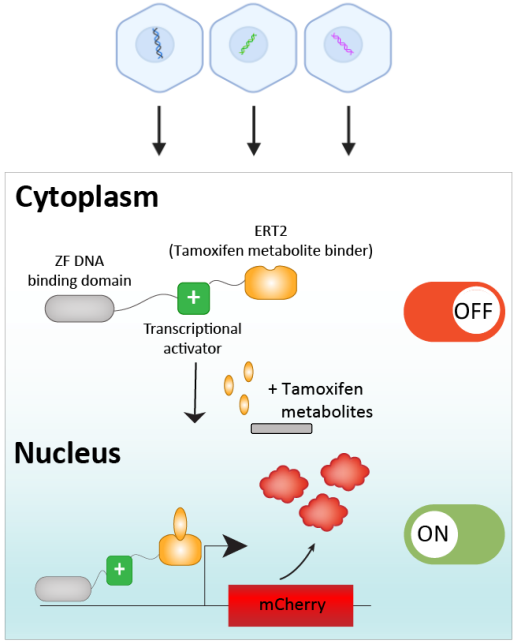
In collaboration with aplomex

Engineering a better ERT2: Computational prediction of mutations with greater SM affinity





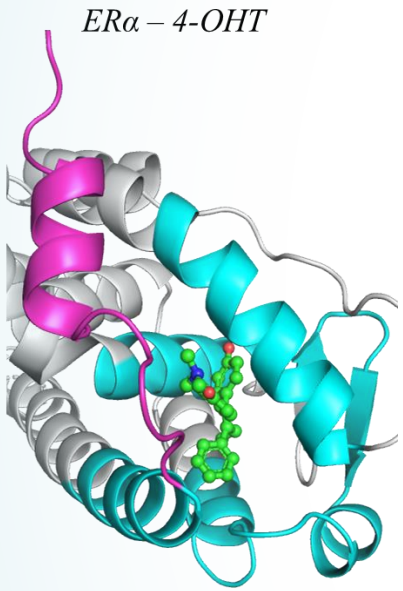


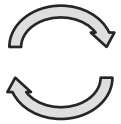


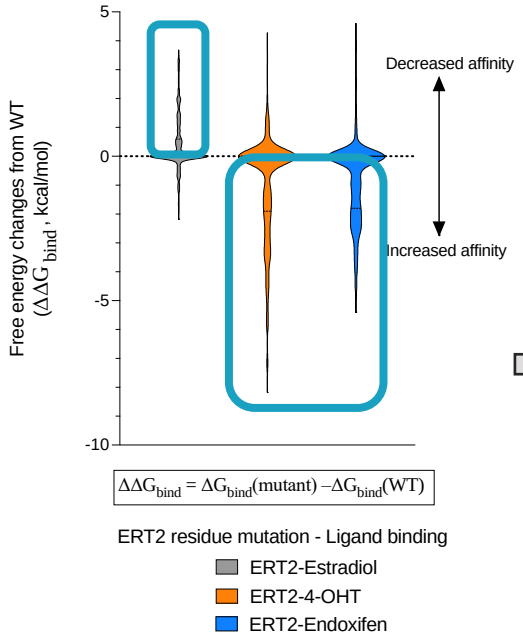
In collaboration with aplomex

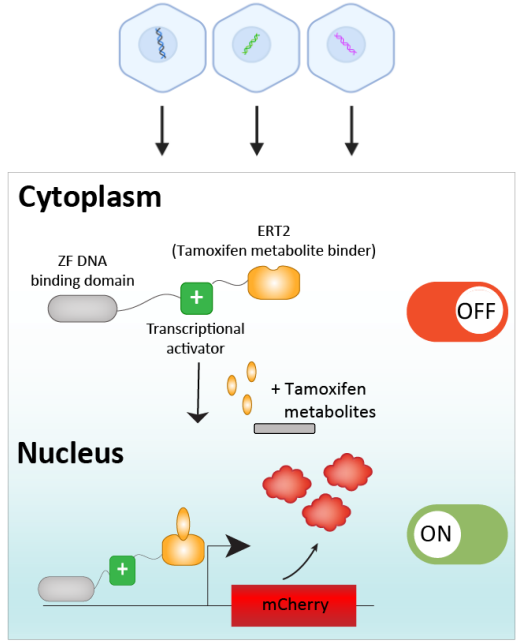
Mutations that maintained insensitivity to estradiol and increased affinity for tamoxifen metabolites

Engineering a better ERT2: Computational prediction of mutations with greater SM affinity

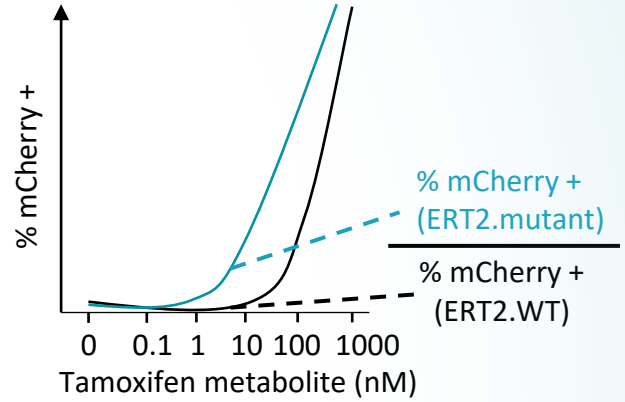








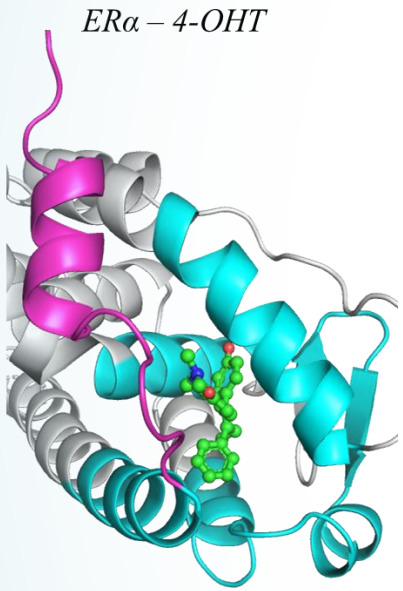
Calculating fold improved sensitivity

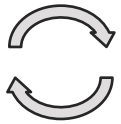


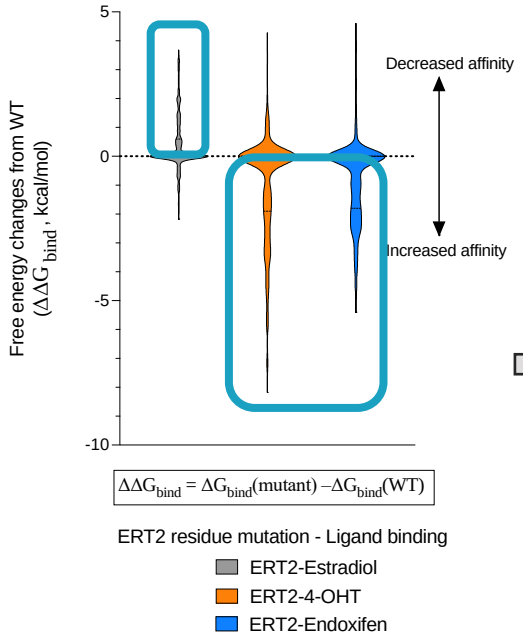
In collaboration with aplomex

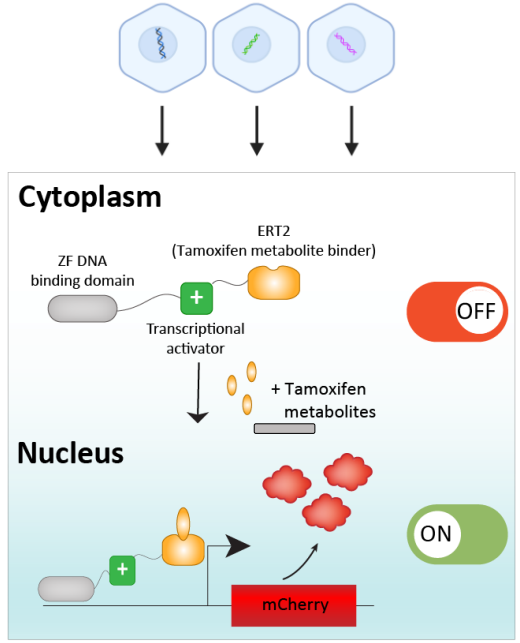
Mutations that maintained insensitivity to estradiol and increased affinity for tamoxifen metabolites

Computational prediction of mutations with greater SM affinity results in higher drug sensitivity switch

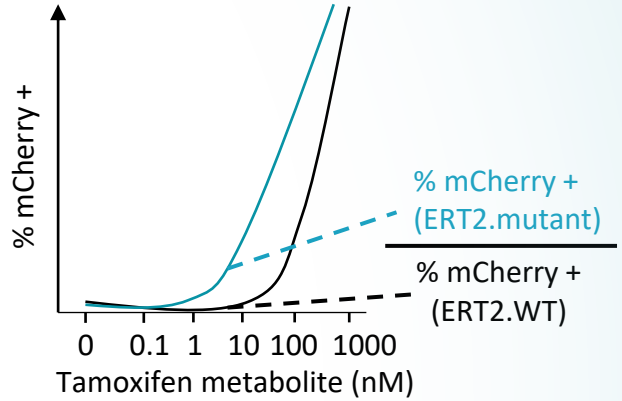


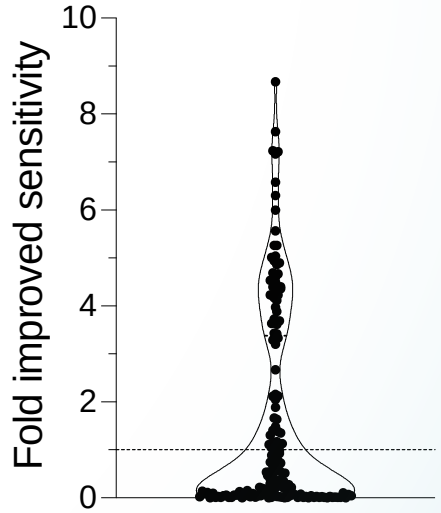






Calculating fold improved sensitivity

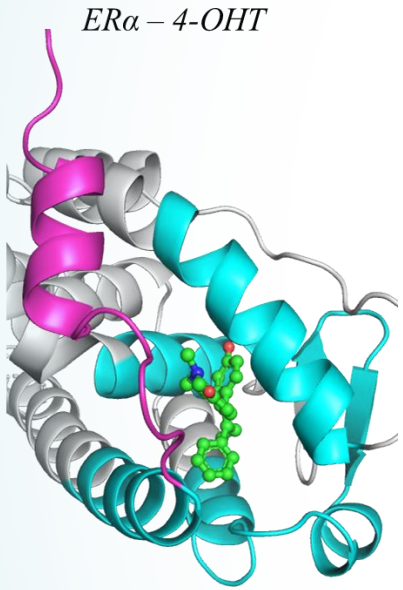


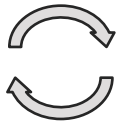


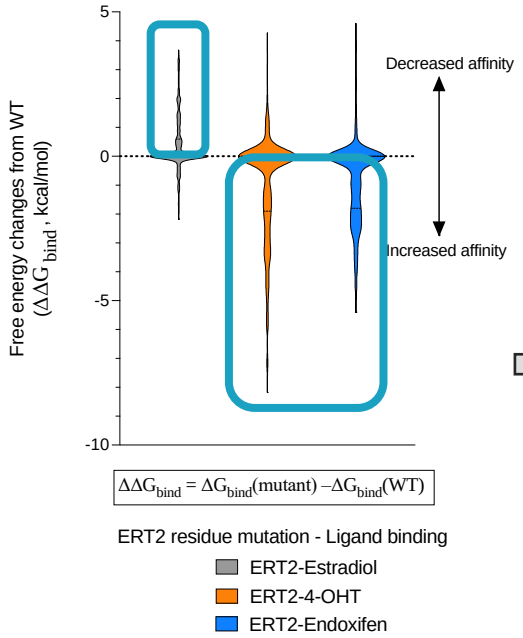
Mutations that maintained insensitivity to estradiol and increased affinity for tamoxifen metabolites

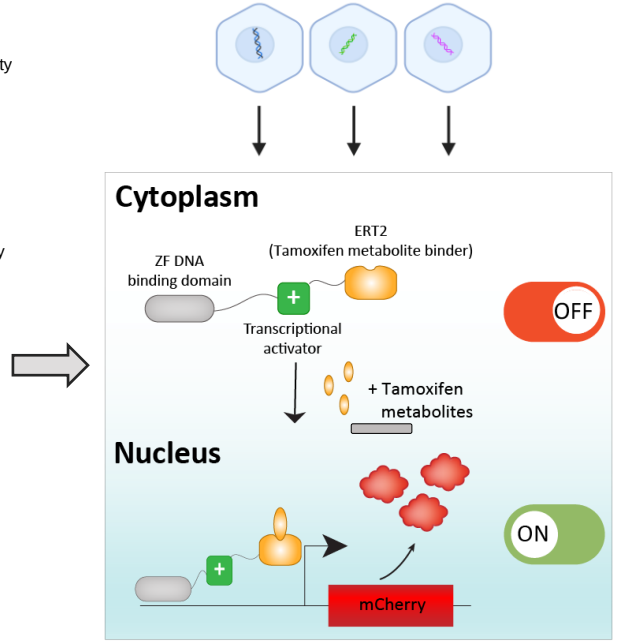
In collaboration with aplomex

Computational prediction of mutations with greater SM affinity results in higher drug sensitivity switch

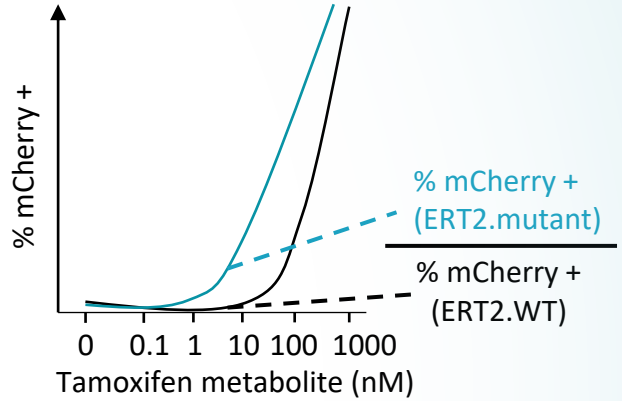


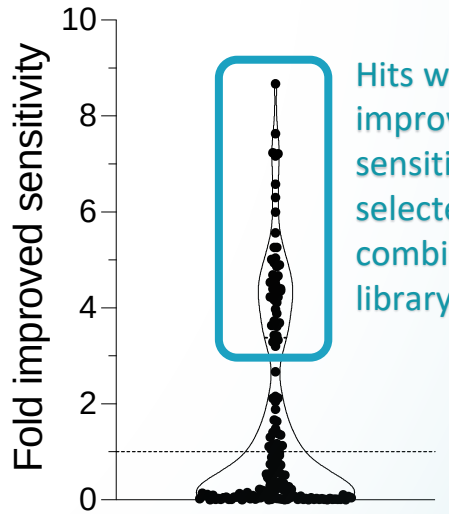






Calculating fold improved sensitivity



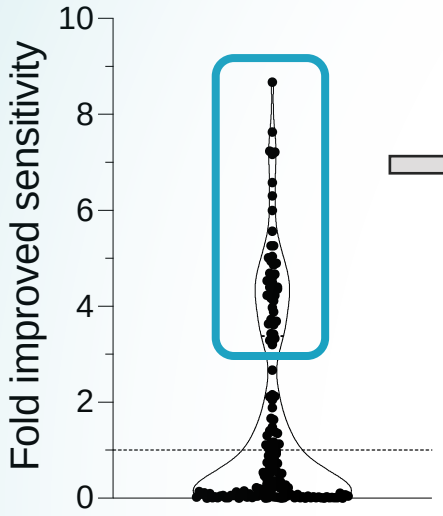


Hits with >3 fold improved sensitivity were selected to build combinatorial library

In collaboration with aplomex

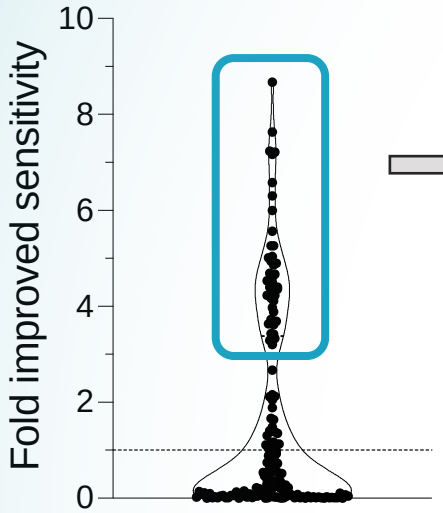
Mutations that maintained insensitivity to estradiol and increased affinity for tamoxifen metabolites

Engineering a better ERT2 SM binding domain



Combinatorial Library of selected ERT2 mutations

Engineering a better ERT2 SM binding domain

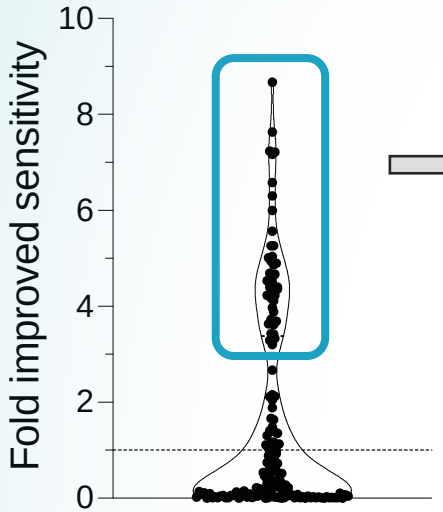


Combinatorial Library of selected ERT2 mutations

↓

Sequential sorting for library members with sensitivity to decreasing concentrations of tamoxifen metabolites using reporter cell line

Engineering a better ERT2 SM binding domain



Combinatorial Library of selected ERT2 mutations

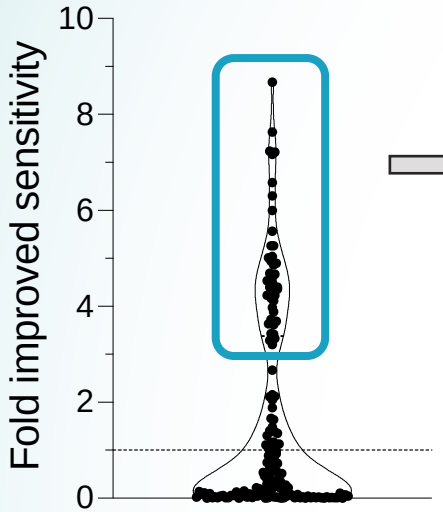
↓

Sequential sorting for library members with sensitivity to decreasing concentrations of tamoxifen metabolites using reporter cell line

↓

Identification of ERT2 mutants in sorted pools utilizing PacBio Long-read sequencing

Senti has identified ERT2 mutations with improved sensitivity to tamoxifen metabolites

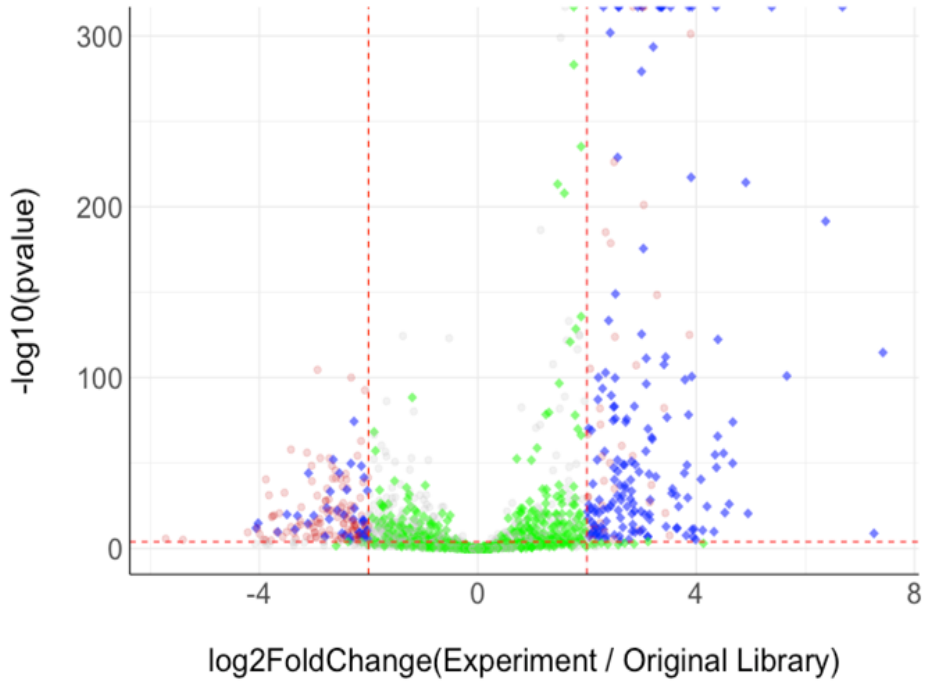


Combinatorial Library of selected ERT2 mutations

Sequential sorting for library members with sensitivity to decreasing concentrations of tamoxifen metabolites using reporter cell line

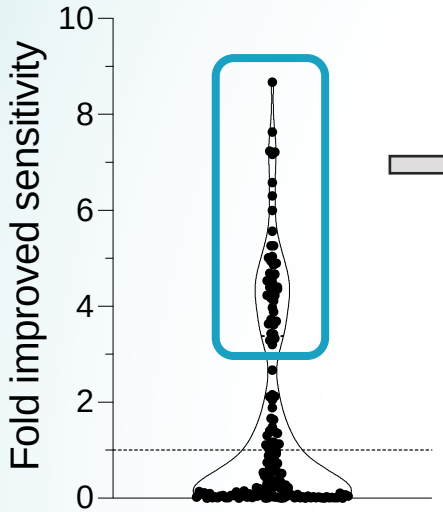
Identification of ERT2 mutants in sorted pools utilizing PacBio Long-read sequencing

Enrichment analysis of sequenced ERT2 mutations responsive to 0.1 nM tamoxifen metabolites



Senti has built and screened a combinatorial library which identified new ERT2 mutations enriched in populations of cells responsive to 0.1 nM tamoxifen metabolites

Senti has identified ERT2 mutations with improved sensitivity to tamoxifen metabolites

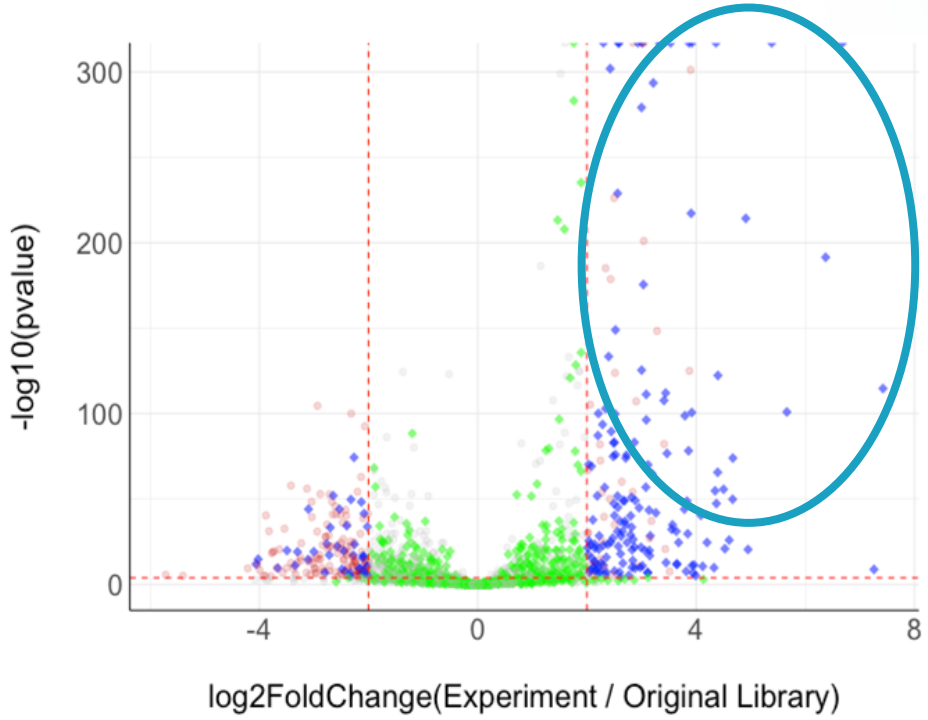


Combinatorial Library of selected ERT2 mutations

Sequential sorting for library members with sensitivity to decreasing concentrations of tamoxifen metabolites using reporter cell line

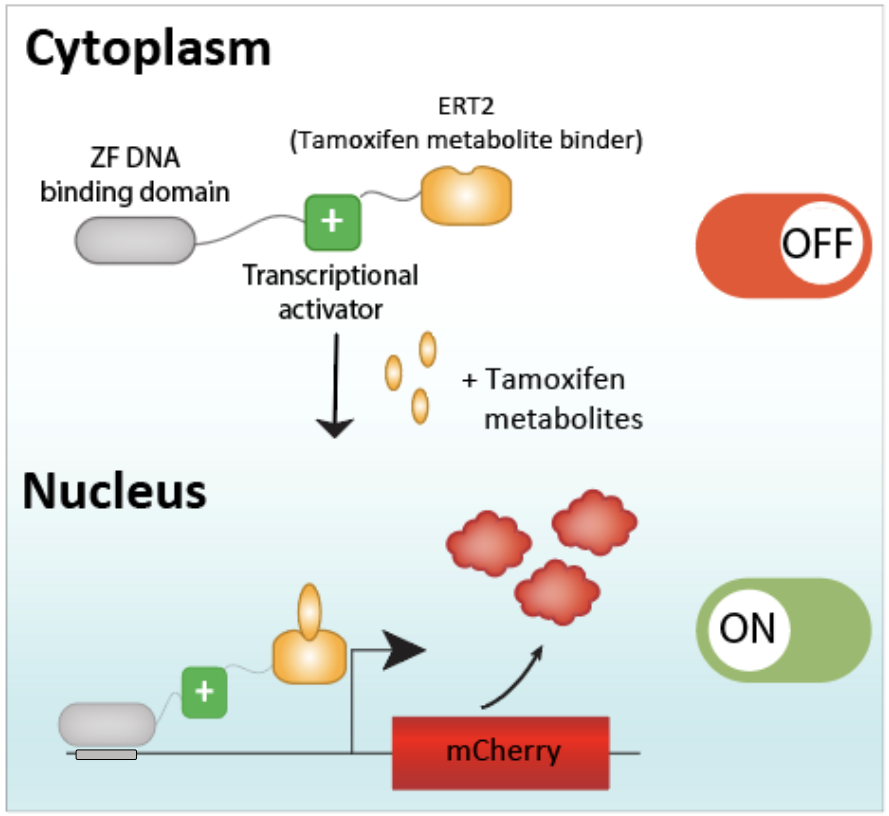
Identification of ERT2 mutants in sorted pools utilizing PacBio Long-read sequencing

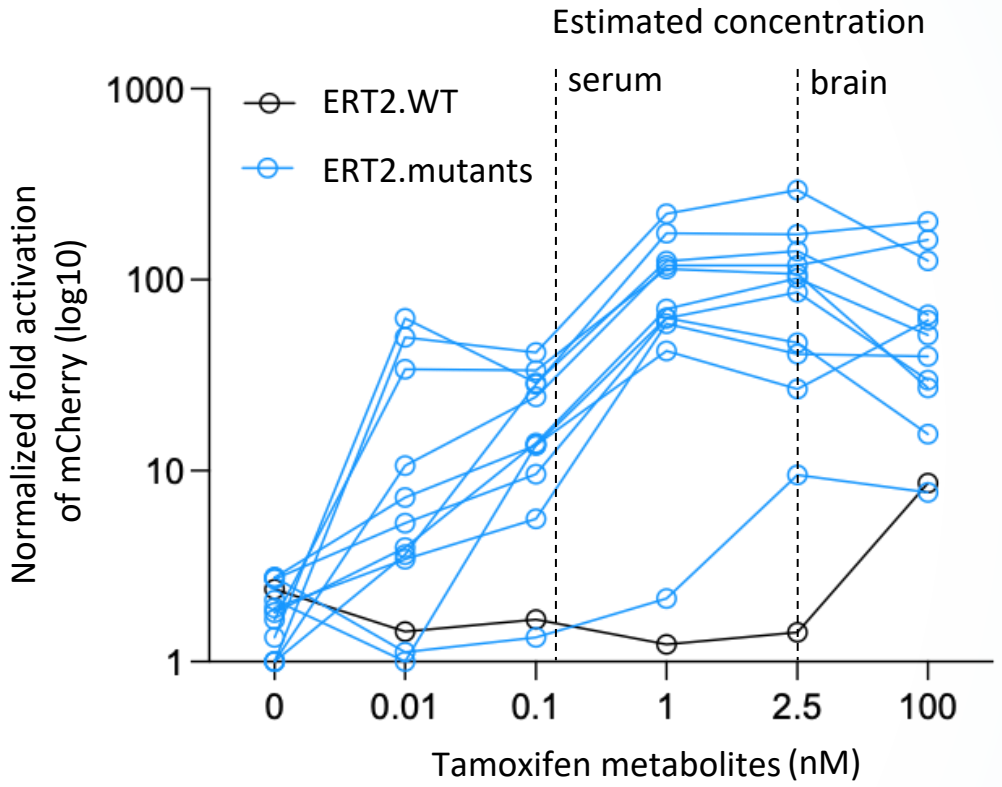
Enrichment analysis of sequenced ERT2 mutations responsive to 0.1 nM tamoxifen metabolites



Senti has built and screened a combinatorial library which identified new ERT2 mutations enriched in populations of cells responsive to 0.1 nM tamoxifen metabolites

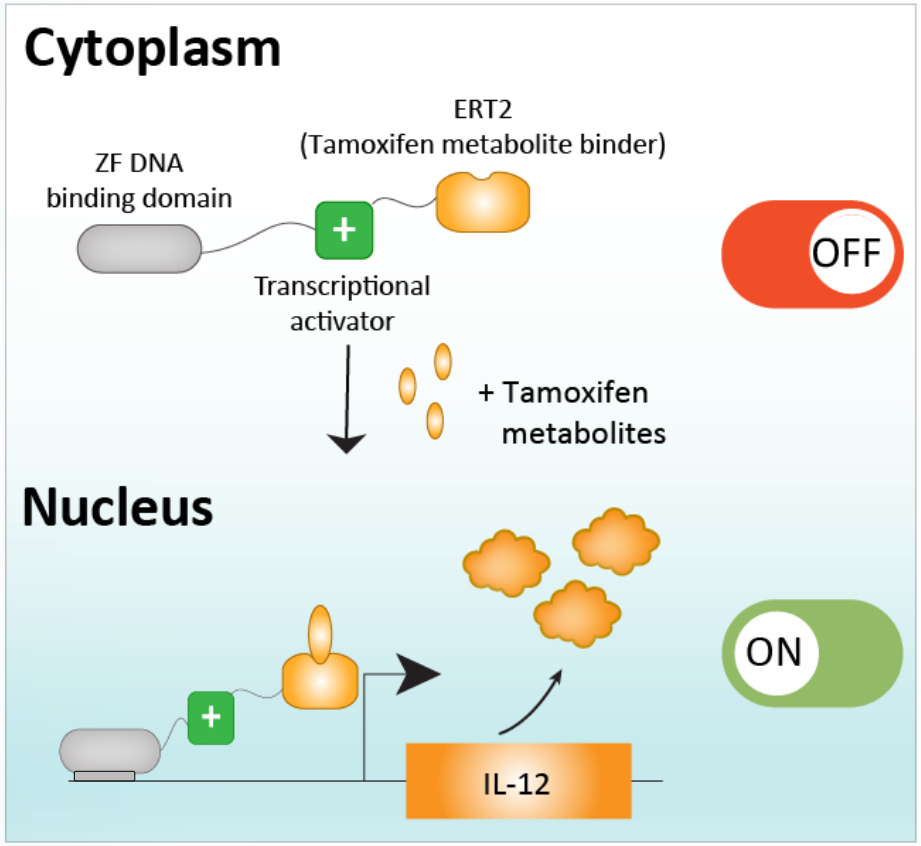
Senti has engineered a therapeutically relevant transcriptional switch



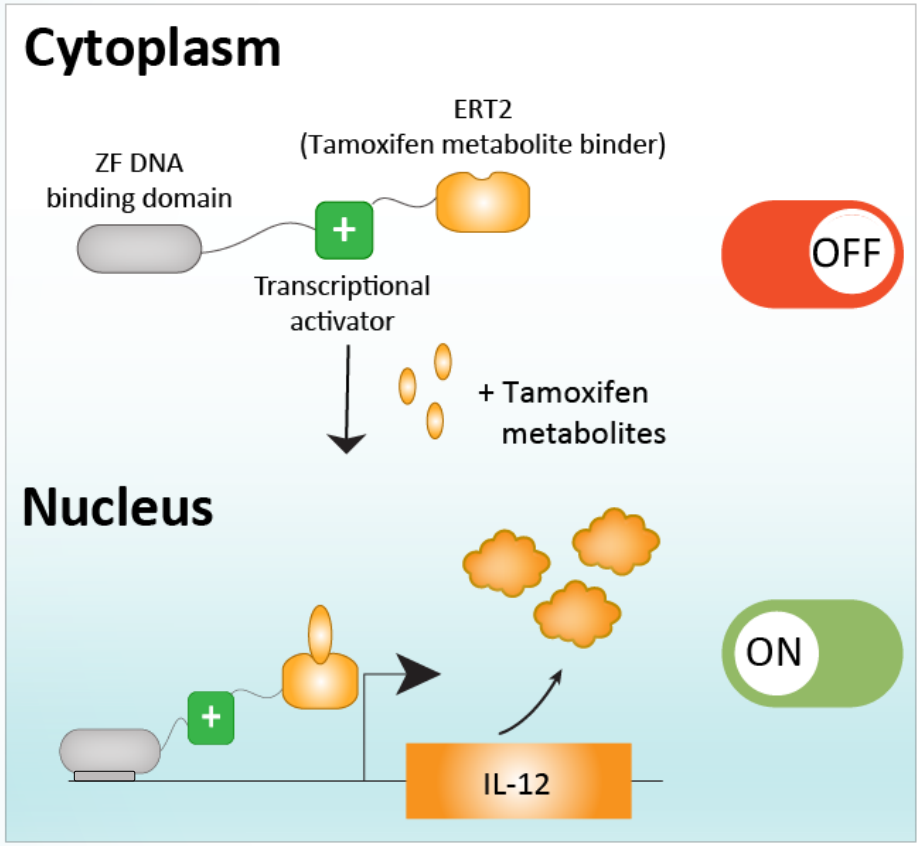


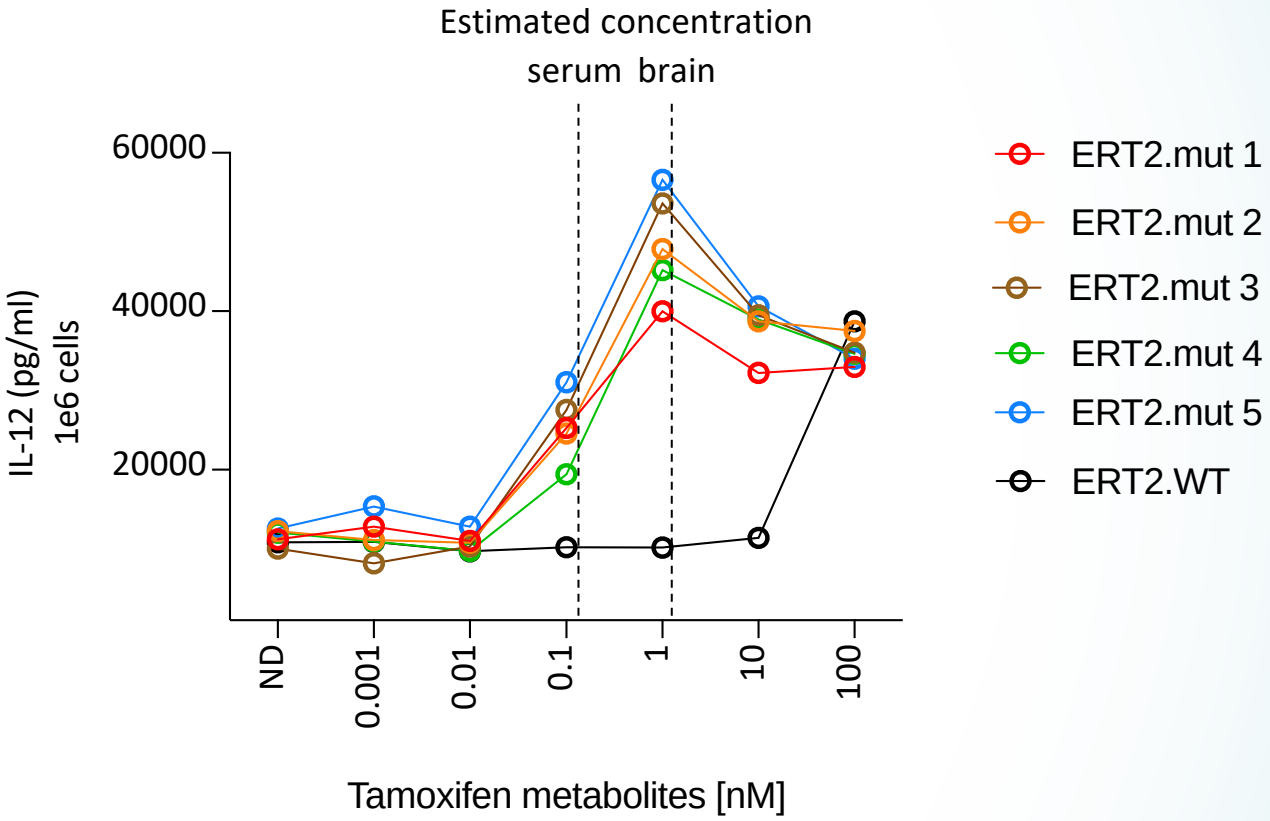
Senti has demonstrated function of 10 ERT2 mutants (blue) with improved sensitivity to tamoxifen metabolites compared to wildtype (black)

Evaluate regulation of IL-12 with Senti engineered transcriptional switch



Senti has engineered a therapeutically relevant transcriptional switch for regulation of IL-12

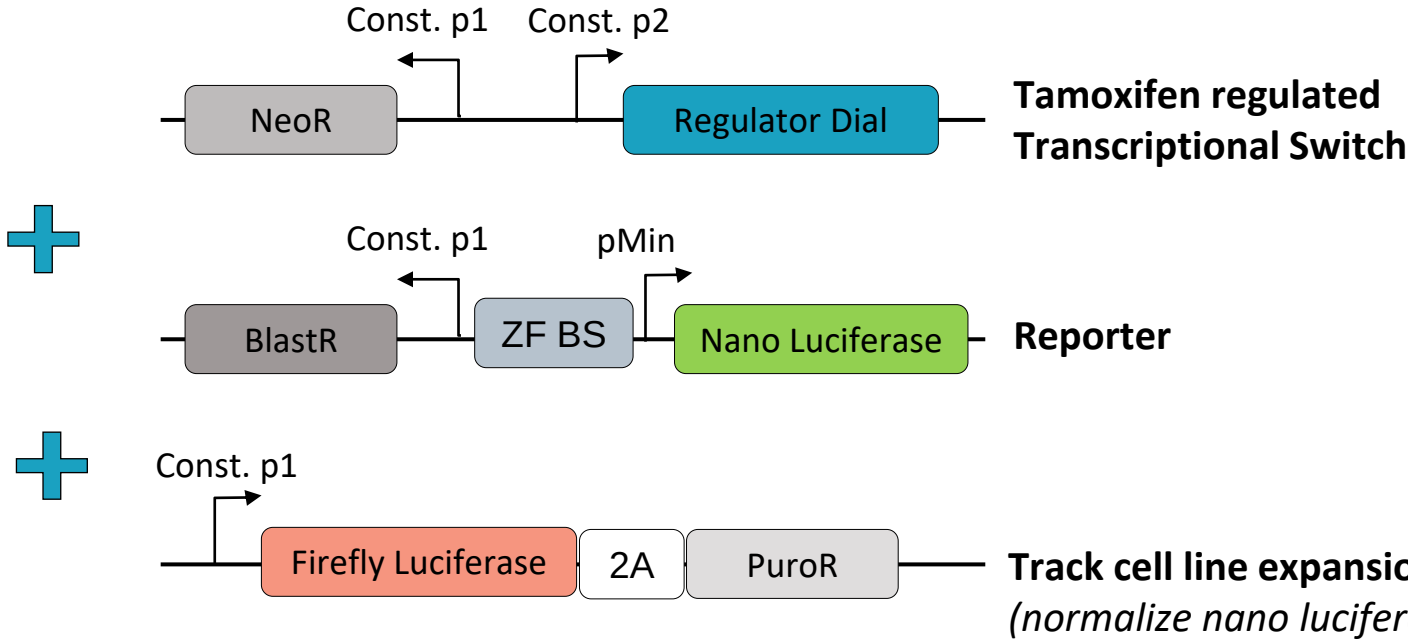




Senti engineered ERT2 mutants in context of Senti's tamoxifen-regulated transcriptional switch enable dose dependent regulation of IL-12 expression at physiologically relevant tamoxifen metabolite concentrations

in vivo evaluation of Tamoxifen-regulated transcriptional switch

Regulator Dial cell line



1. Normalization of Nanoluc across tumor burden:

$$Normalized\ NanoLuc = \frac{NanoLuc}{Firefly\ Luc}$$

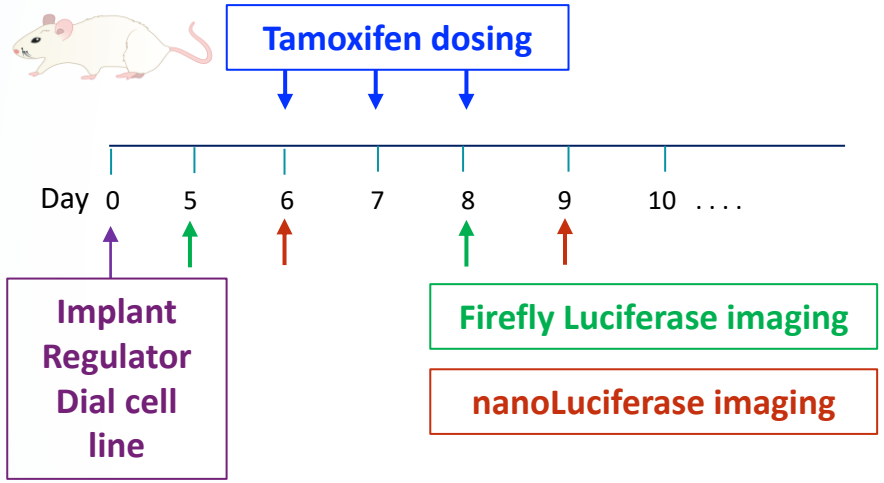
2. Fold change of Nanoluc reporter compared to average vehicle condition:

$$Fold\ activation = \frac{Normalized\ NanoLuc_{\textbf{Drug}\ treated\ mice}}{Normalized\ NanoLuc_{\textbf{Vehicle}\ treated\ mice}}$$

in vivo evaluation of Tamoxifen-regulated transcriptional switch

Study Design

In vivo

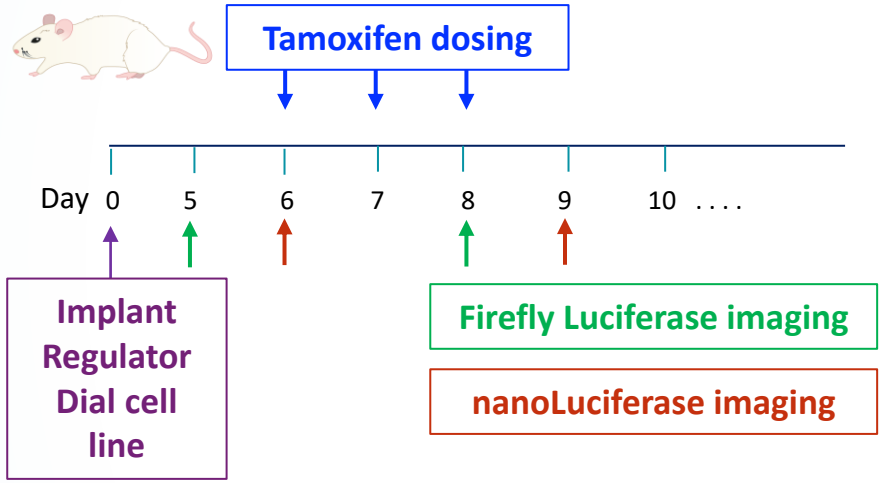


Induction of nanoLuciferase *in vivo*

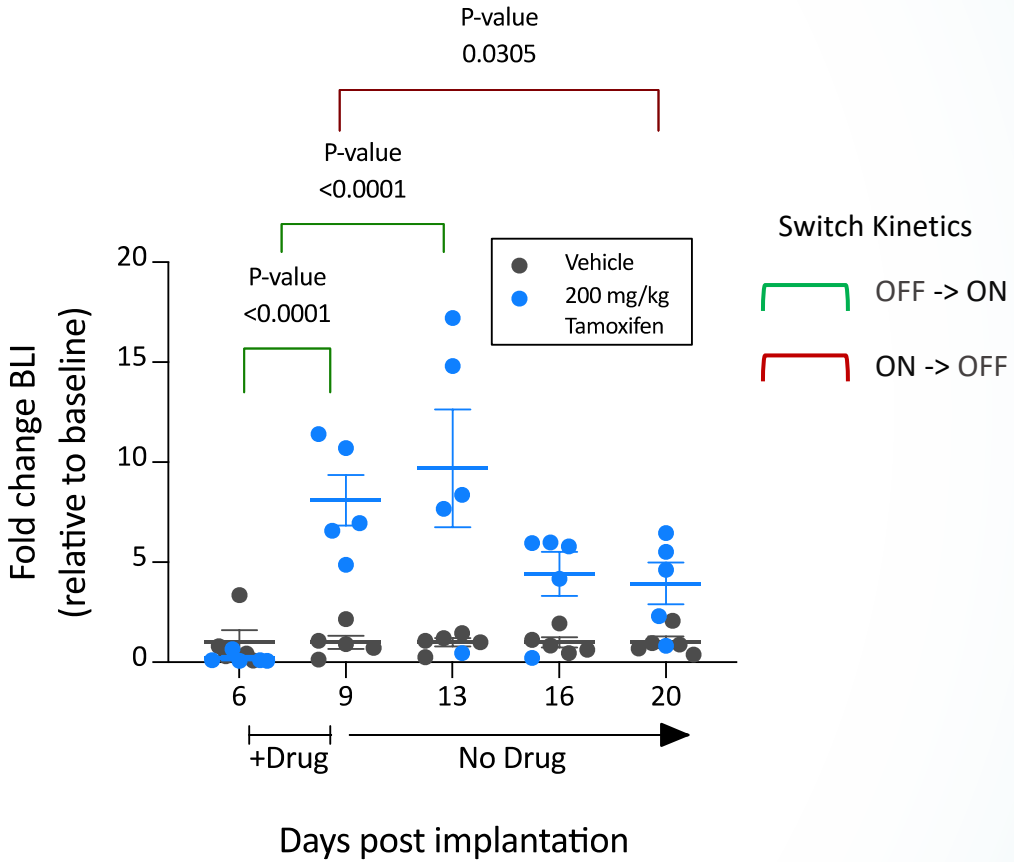
Transcriptional switch demonstrates functionality *in vivo*

Study Design

In vivo



Induction of nanoLuciferase *in vivo*



Senti's Tamoxifen-regulated transcriptional switch is dose dependent and results in robust and reversible payload expression *in vivo*

Engineering pharmacologically relevant, FDA-approved small-molecule-regulated gene circuit for therapeutic applications in the brain

Senti engineered Tamoxifen-regulated transcriptional switch has the potential to enable the following benefits:

- ***Optimized*** for ***safe***, low expression of potent cytokines in the absence of small molecule drug and ***robust, dose dependent*** induction of cytokine production at ***pharmacologically relevant concentrations*** of tamoxifen metabolites
- ***Versatile*** to regulate potentially any payload of interest
- ***Expansive*** targeting range responsive to drug concentrations seen throughout human body
- ***Convenient*** regulation by ***orally dosed, FDA-approved small molecule***

Engineering pharmacologically relevant, FDA-approved small-molecule-regulated gene circuit for therapeutic applications in the brain

Senti engineered Tamoxifen-regulated transcriptional switch has the potential to enable the following benefits:

- ***Optimized*** for ***safe***, low expression of potent cytokines in the absence of small molecule drug and ***robust, dose dependent*** induction of cytokine production at ***pharmacologically relevant concentrations*** of tamoxifen metabolites
- ***Versatile*** to regulate potentially any payload of interest
- ***Expansive*** targeting range responsive to drug concentrations seen throughout human body
- ***Convenient*** regulation by ***orally dosed, FDA-approved small molecule***

Expand catalog of available effectors for use with cell therapies

Effectors:	**New Effectors:**
IL15	IL12
	Antibodies
	Target receptors

Engineering pharmacologically relevant, FDA-approved small-molecule-regulated gene circuit for therapeutic applications in the brain

Senti engineered Tamoxifen-regulated transcriptional switch has the potential to enable the following benefits:

- ***Optimized*** for ***safe***, low expression of potent cytokines in the absence of small molecule drug and ***robust, dose dependent*** induction of cytokine production at ***pharmacologically relevant concentrations*** of tamoxifen metabolites
- ***Versatile*** to regulate potentially any payload of interest
- ***Expansive*** targeting range responsive to drug concentrations seen throughout human body
- ***Convenient*** regulation by ***orally dosed, FDA-approved small molecule***

Expand catalog of available effectors for use with cell therapies

Effectors:

IL15

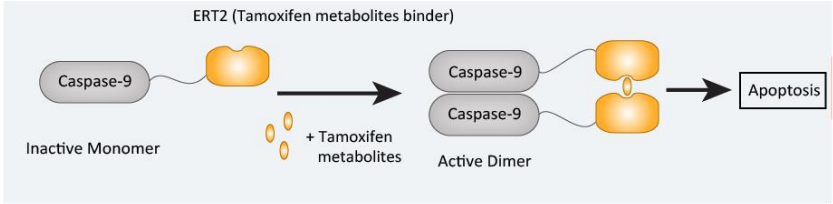
New Effectors:

IL12

Antibodies

Target receptors

Tamoxifen Regulated Safety Kill Switch



Control new cell behaviors:

See our collaborator's abstract:

Title: Engineering a Gene Circuit-Enabled Cell Therapy with a Tamoxifen Regulated Safety Switch for Inducible Cell Death in Human Pluripotent Stem Cells and their Derivatives

Soh et al. (abstract 742)

Acknowledgements

Thank you to the fantastic team at Senti Biosciences and our collaborators at BlueRock

See our other Senti Abstracts:

Title: Designing cell-state-specific synthetic promoters as Smart Sensors to control macrophage polarization
Liu et al. (abstract 1535)

Title: Massively parallel and systematic engineering platform for highly compact, cell-type specific, and potent Smart Sensor promoters for precision retinal gene therapies
Cichewicz et al. (abstract 341)

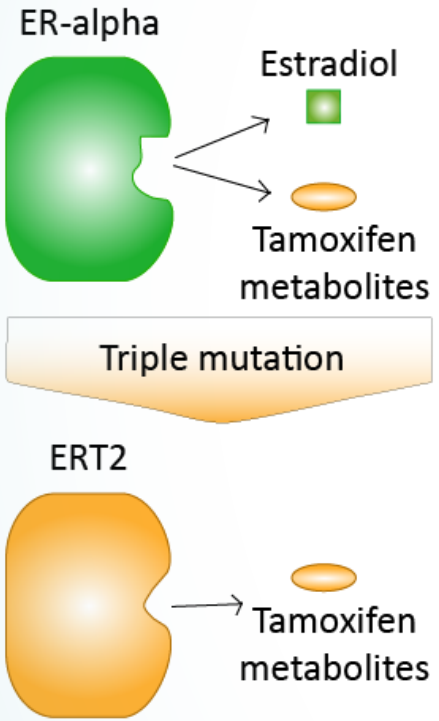
Title: High-throughput engineering of Logic Gated-gene circuits for precision CAR cell therapies
Frankel et al. (abstract 1408)

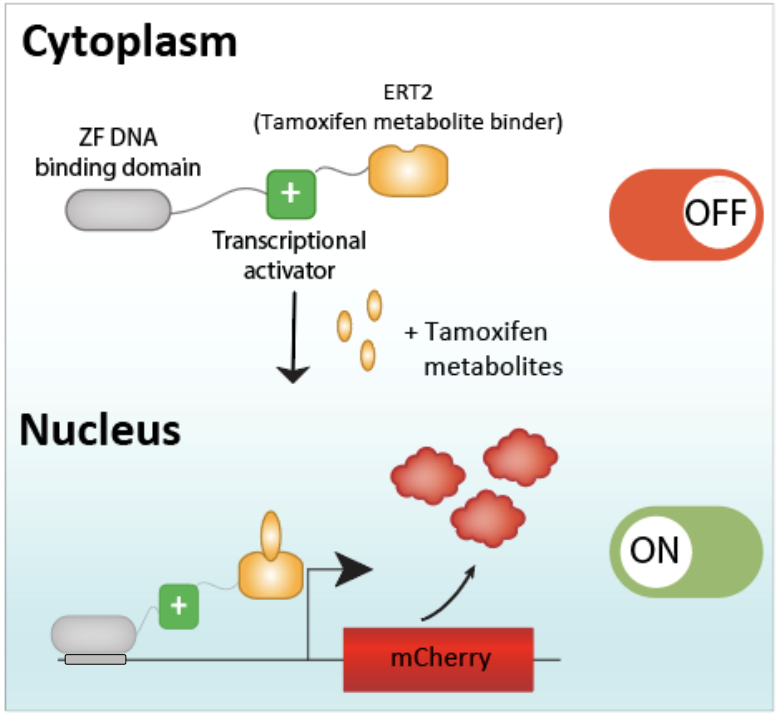
See our collaborator's Abstract:

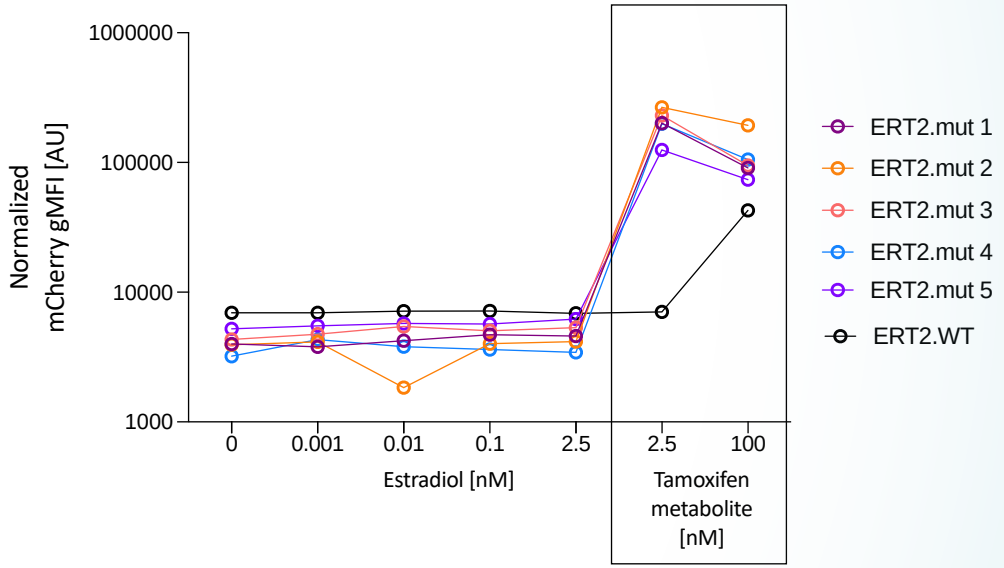
Title: Engineering a Gene Circuit-Enabled Cell Therapy with a Tamoxifen Regulated Safety Switch for Inducible Cell Death in Human Pluripotent Stem Cells and their Derivatives
Soh et al. (abstract 742)

Tamoxifen regulated gene circuits would enable regulation of cell therapies via an FDA approved, BBB permeable small molecule

Origin of the ERT2 SM binding domain







Gallinari, P, et al. Chem Biol 2005

Engineered ERT2 mutants maintain insensitivity to physiological levels of Estradiol