Engineering Synthetic Biotic Medicines™ for the Treatment of Cancer

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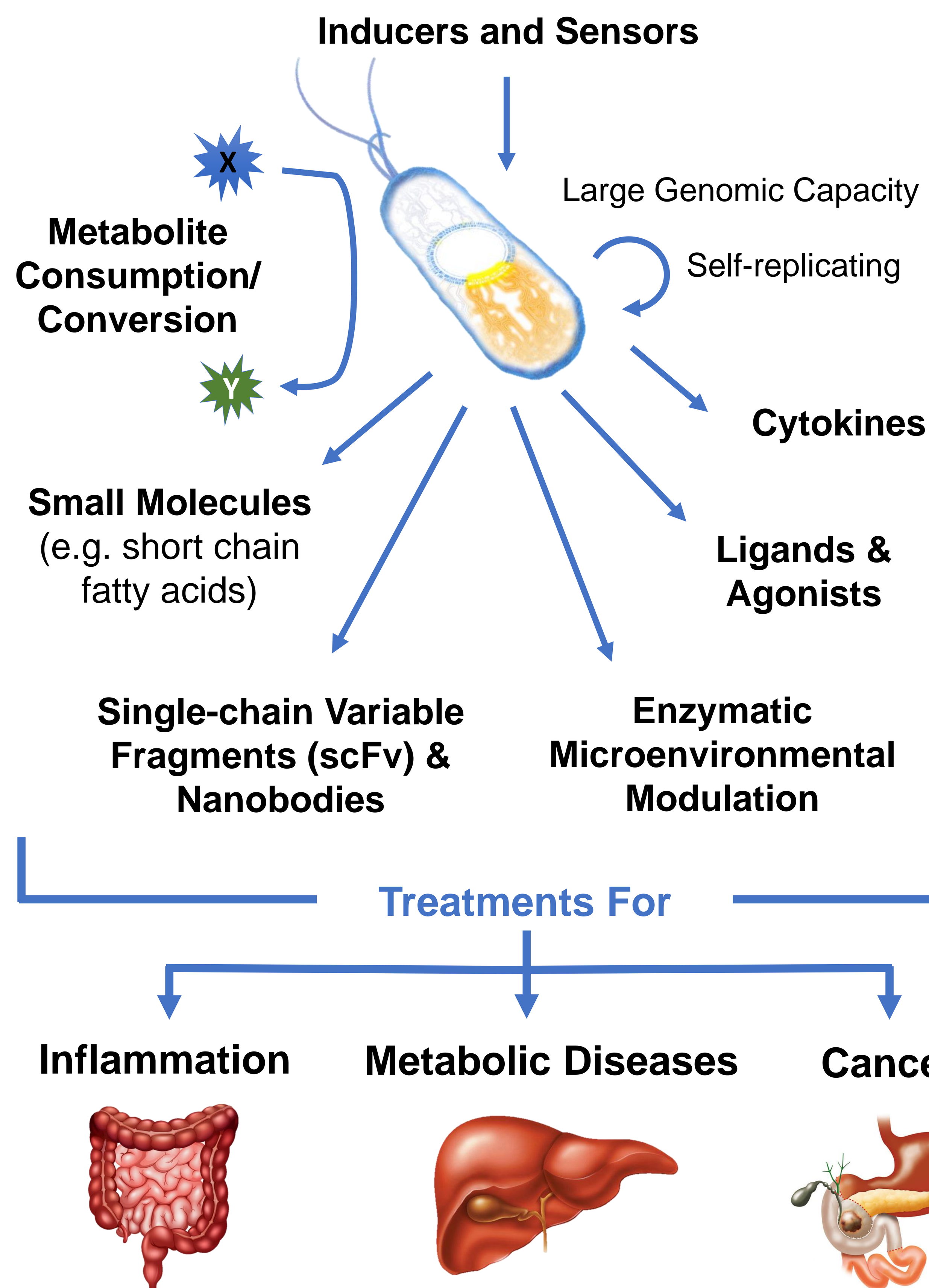
Abstract

At Synlogic we apply synthetic biology to non-pathogenic bacteria (*E. coli* Nissle) to develop "Synthetic Biotic medicines" which perform and deliver critical therapeutic functions to treat diseases throughout the body. Our synthetic biology platform allows us to design bacterial strains capable of executing metabolic conversions (production or consumption of metabolites), secretion of proteins (chemokines, cytokines, enzymes) and secretion or display of ligands and single-chain Fv (scFv) molecules to trigger or disrupt ligand-receptor interactions.

Here we demonstrate the utility of engineered *E. coli* Nissle as a multifunctional, therapeutic platform technology for the modulation of immune responses in cancer. By monitoring bacterial abundance following intratumoral injection we show that Nissle specifically colonizes syngeneic murine tumors and persists for up to 15 days in immunocompetent hosts. Using a GFP reporter system, we show the capabilities of engineered Nissle to respond to various inputs, both small molecules and environmental, for specific control of gene expression. Finally, we show three approaches for engineering additional immunomodulatory functionalities into Nissle, including the consumption of immune suppressive metabolites, the secretion of biologically active cytokines and the production of potent pathogen-associated immune agonists. These engineered strains show anti-tumor activity in a variety of syngeneic mouse models, as single agents or in combination with checkpoint inhibitors.

Taken together, these results establish our synthetic biology-based platform as a versatile system for the localized and sustained modulation of the tumor microenvironment, and support the development of Synthetic Biotic™ medicines as a novel approach for the treatment of cancer.

Synthetic Biotic Medicines for Treating Human Disease



***E. Coli.* Nissle as a platform for the development of Synthetic Biotic medicines for the treatment of human disease.** To generate living Synthetic Biotic therapies, we genetically engineer the non-pathogenic commensal bacteria *Escherichia coli* Nissle 1917 (EcN) to carry out a variety of therapeutically relevant functions. Using constitutive and inducible promoters and modular genetic circuits we drive the tunable expression of a variety of enzymes and effectors molecules. These molecules can remain within EcN, be displayed on the cell surface or be actively secreted. In the context of metabolic disease, we engineer Synthetic Biotic medicines capable of consuming or converting toxic metabolites from within the gut. Synthetic Biotic therapies also represent a useful means of local modulation of immune responses. Immune attenuation can be accomplished via the expression of suppressive metabolites or cytokines, while immune activation can be triggered via consumption of suppressive metabolites, secretion of proinflammatory cytokines or production of potent immune agonists.

Engineering Non-pathogenic *E.coli* Nissle for the Treatment of Cancer

E. coli Nissle selectively colonize and persist long term within tumors and can be engineered to respond to various inputs

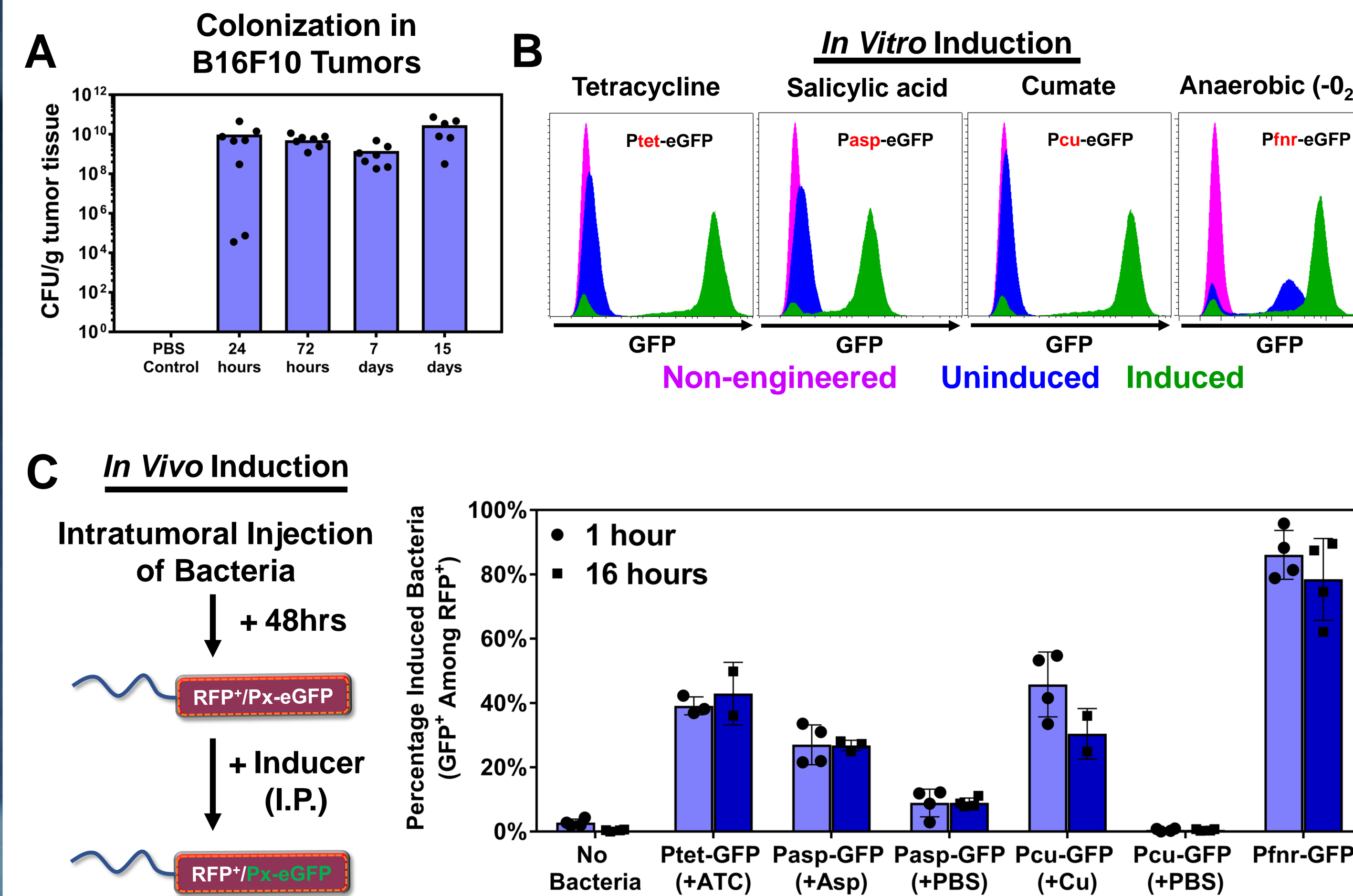


Figure 1: *E. coli* Nissle is a robust engineerable vector for the treatment of cancer. (A) 1e6 CFUs of EcN was intratumorally administered into established B16F10 tumors and presence of bacteria was measured by colony forming unit assay over time. (B) EcN strains engineered to express eGFP under the indicated promoters were exposed to various inducer agents or conditions and analyzed by flow cytometry. (C) EcN strains constitutively expressing mCherry and engineered to inducibly express eGFP as described above were injected into B16F10 tumors. Two days post injection mice were treated (I.P.) with various inducer agents or PBS control and intratumoral bacteria were harvested 1 and 16 hours post treatment. Percentage GFP induction among all RFP⁺ bacteria was measured by flow cytometry.

Consuming immuno-suppressive metabolites from the tumor microenvironment

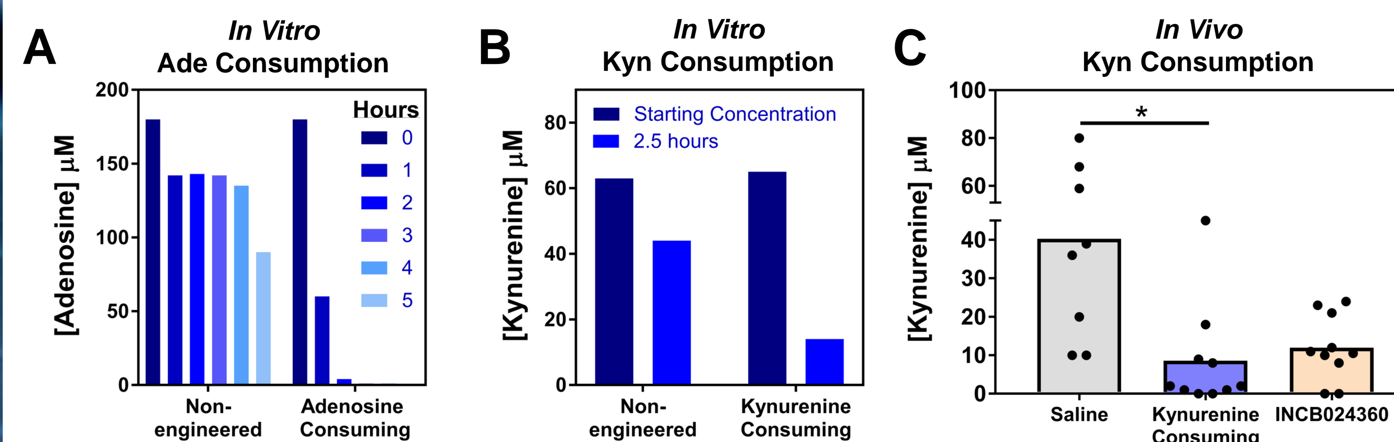


Figure 2: Engineered EcN can consume the immuno-suppressive metabolites Ade and Kyn. To assess *in vitro* metabolite consumption activity, non-engineered bacteria were compared to (A) an adenosine (Ade)-consuming strain or (B) a kynurenine (Kyn)-consuming strain. Cells were incubated with an initial concentration of 180 uM of Ade or 80 uM Kyn respectively at 37°C and then samples were analyzed via LCMS. (C) *In vivo* activity of the Kyn-consuming strain was compared to treatment with a benchmark IDO inhibitor (INCB024360) following I.T. or I.P. administration of CT26 tumor bearing mice, respectively. Statistical significance determined using unpaired t test, * P < 0.05.

Production and secretion of biologically active cytokines (IL-15, IFNγ and TNFα)

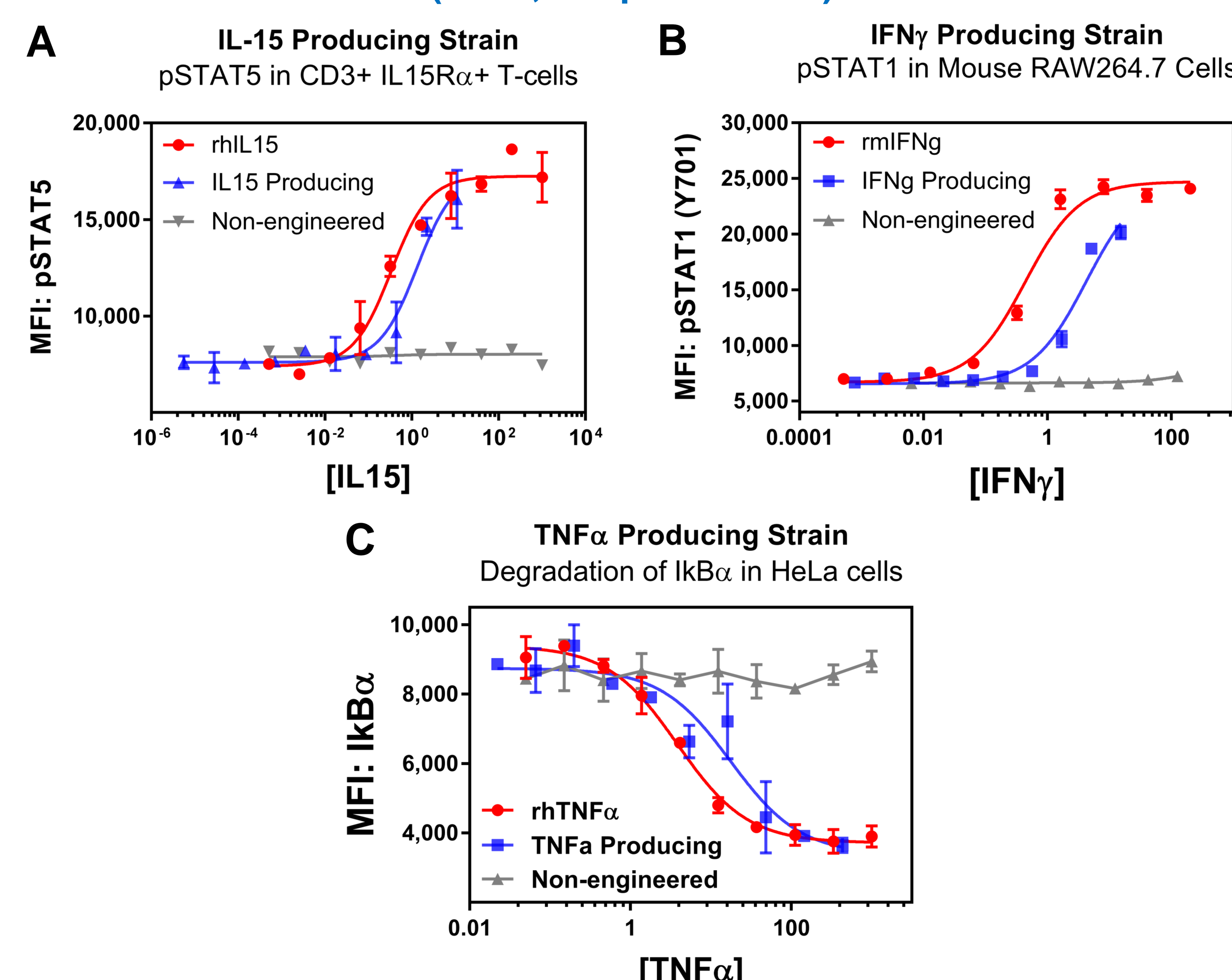


Figure 3: Activity of IL-15-, IFNγ- and TNFα-secreting strains. The *in vitro* activity of various selected cytokines was evaluated via cell-based assay utilizing serial dilutions of bacterial supernatants. (A) PHA-L activated human T cells were stimulated for 20 min at 37°C and analyzed for pSTAT5 by flow cytometry. (B) RAW264.7 cells were stimulated for 15 min at 37°C and analyzed by flow for pSTAT1. (C) HeLa cells were stimulated for 10 min at 37°C and analyzed by flow cytometry for loss of IκBα.

Production of high levels of intratumoral TNFα and the subsequent control of tumor growth in CT26 tumors

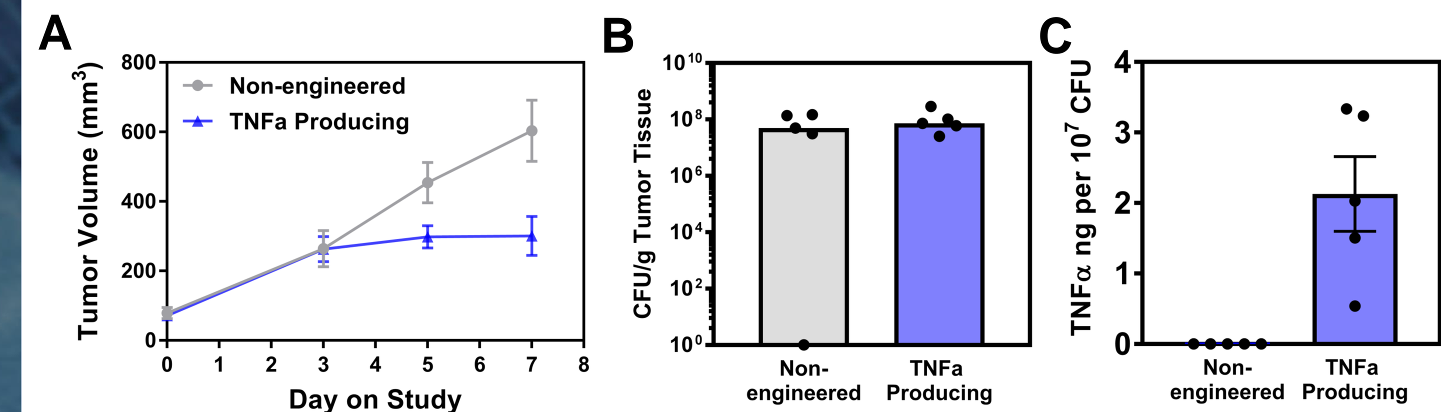


Figure 4: *In vivo* secretion of TNFα and impact on CT26 tumor growth. 1e7 CFUs of wild type EcN or EcN engineered to secrete TNFα were I.T. administered into established CT26 tumors. Mice were treated with anhydrous tetracycline I.P. on days 1, 4 and 7 to induce expression of TNFα. (A) Mean tumor volume is shown for each experimental group. (B & C) Tumors were harvested on day 8 and analyzed for bacterial presence by CFU assay and abundance of TNFα by ELISA.

Intratumoral enzymatic conversion of pro-drugs (5-FC → 5-FU)

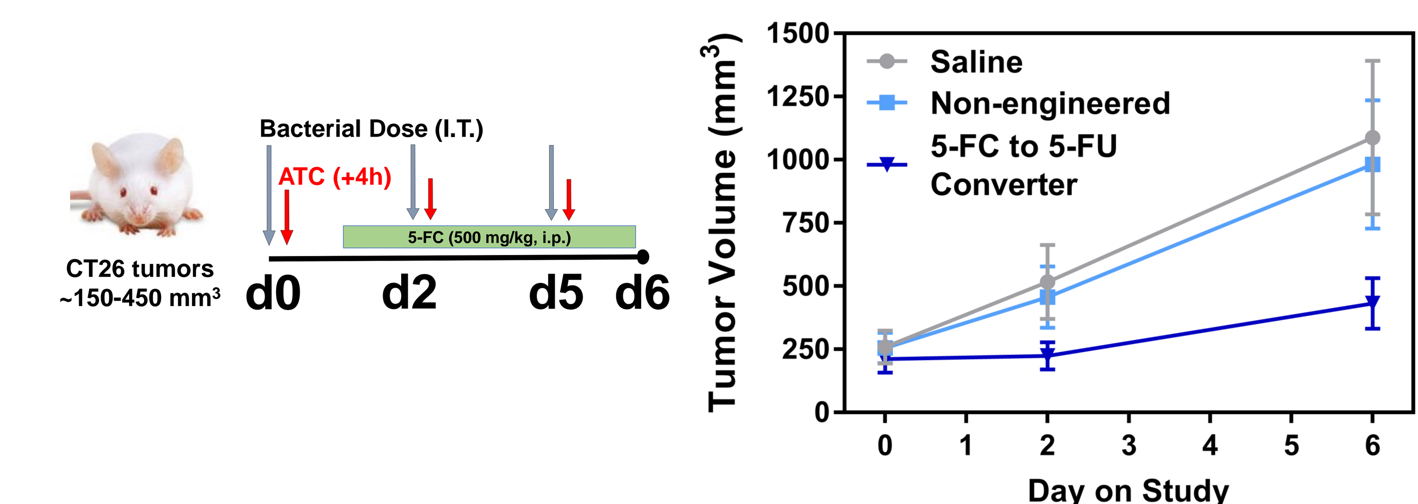


Figure 5: Conversion of the pro-drug 5-FC to 5-FU by engineered EcN. Large (150-450mm³) CT26 tumors were injected with EcN expressing cytosine deaminase (CD) under the tetracycline promoter. Mice were treated with ATC 4 hours post-injection to induce expression of CD and starting 24 hours later given daily I.P. doses of 5-FC. Mice were monitored for signs of toxicity and tumor growth.

Engineering an inducible STING agonist (ci-di-AMP) producing circuit in *E. coli* Nissle

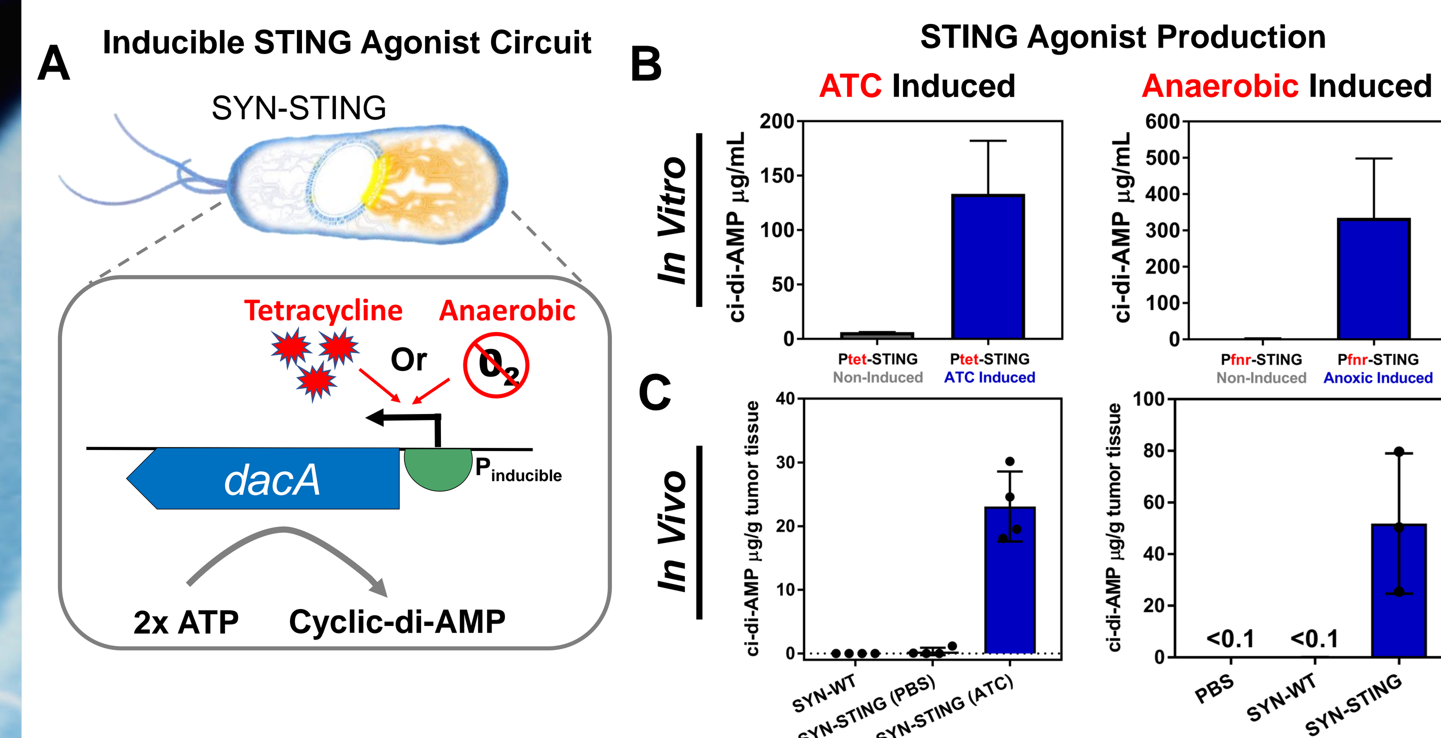


Figure 6: Engineering the inducible expression of a STING agonist in EcN. (A) To construct the STING agonist production circuit, a tetracycline (pTet) and anaerobic (pFNR) inducible diadenylate cyclase gene (*dacA*) from *Listeria monocytogenes* was transformed into EcN (referred to as SYN-STING). (B) SYN-STING was exposed to either 200 ng/mL anhydrous tetracycline (ATC) or anaerobic conditions for 4 hours *in vitro*. (C) B16F10 tumors (~40-80mm³) were injected with wildtype or ci-di-AMP producing bacteria (1e7 CFUs) via I.T. injection. Four hours post bacterial administration mice were injected with ATC I.P. to induce ci-di-AMP production or PBS. 24 hours post administration tumors were harvested and intratumor ci-di-AMP was measured. Levels of ci-di-AMP were analyzed via LCMS (B) in bacterial pellet or (C) in tumor homogenate.

SYN-STING treatment results in the generation of anti-tumor immunity

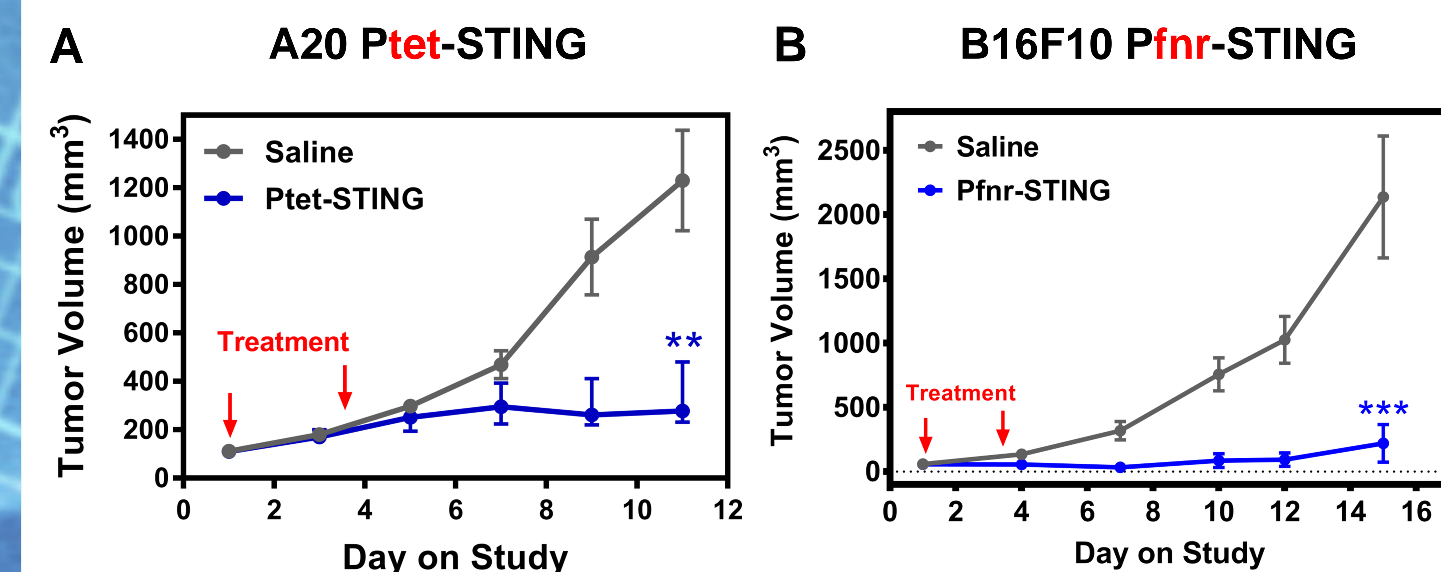


Figure 7: Anti-tumor activity of SYN-STING treatment on the B16F10 and A20 models. A20 and B16F10 tumors (~40-80mm3) received 2x doses of saline or bacteria (1e8 CFUs of indicated strain) via I.T. injection. Mice treated with Tet inducible bacteria were given ATC 4 hours post bacterial dose. Mean tumor volumes for each experimental group are shown for (A) A20 and (B) B16F10 tumors. Statistical significance determined using unpaired t test for tumor volume at the final time point. Indicated group compared to Saline; * P < 0.05, *** P = 0.0008.