

Introduction

Sapience Therapeutics is focused on discovering and developing peptide-based therapeutics to previously 'undruggable' targets for major unmet medical needs, particularly high mortality cancers.

ST101 is a peptide antagonist of the oncogenic transcription factor CCAAT/Enhancer Binding Protein Beta (C/EBP β). ST101 is currently being evaluated in a Phase 1/2 clinical study in patients with advanced unresectable and metastatic solid tumors, with planned expansion cohorts in breast cancer, melanoma, prostate cancer and GBM. A description of the ongoing clinical trial can be found on poster #LB114.

Abstract

Breast cancer (BC) is the most common invasive cancer in women, with more than 2.1 million cases and over 626,000 deaths occurring worldwide annually. Although age-adjusted mortality from BC has been declining since 1990, the median survival for patients with metastatic disease remains at 2–3 years, and 5-year survival approximates 25%. Thus, the medical need for more effective agents in this clinical setting remains very high. C/EBP β is a transcription factor overexpressed in many cancers that regulates expression of factors that promote tumor survival, proliferation and inhibit differentiation. C/EBP β dimers, such as with Jun/Fos and CREB/ATF family members, form via basic leucine zipper (bZIP)-driven coiled-coil interactions and are necessary for its association with DNA and transcriptional activity. Due in part to the difficulty in therapeutically targeting transcription factors, no effective C/EBP β inhibitors are clinically available. ST101 is a novel peptide antagonist of C/EBP β currently being evaluated in a Phase 1/2 clinical study in patients with advanced unresectable and metastatic solid tumors. ST101 was designed to bind the C/EBP β bZIP domain, thereby preventing dimer formation and inhibiting its transcriptional activity. Here we describe ST101 non-clinical anti-tumor activity to support further development as a therapeutic approach in BC. In vitro studies in 4T1-luc triple-negative breast cancer (TNBC) and MCF7 HRPOS breast adenocarcinoma cells demonstrate ST101 dose-dependent impact of cell viability (EC50 of 4.8 and 2.1 μ M, respectively), accompanied by significant impact on C/EBP β -mediated gene expression as determined by qPCR analysis. In contrast, normal human mammary epithelial cells were not sensitive to ST101 (EC50 >100 μ M). In vivo experiments indicate dose-dependent tumor growth inhibition in an orthotopic 4T1-luc model following ST101 exposure, with significant tumor growth delays observed following 25 mg/kg ST101 (p <0.05 vs. controls). Mice treated with 25 mg/kg ST101 via subcutaneous injection demonstrated significant tumor growth delay and decrease in tumor volume vs. controls. These data emphasize the potential of ST101 as a potent peptide therapeutic for HRPOS and TN BC.

ST101 Mechanism of Action

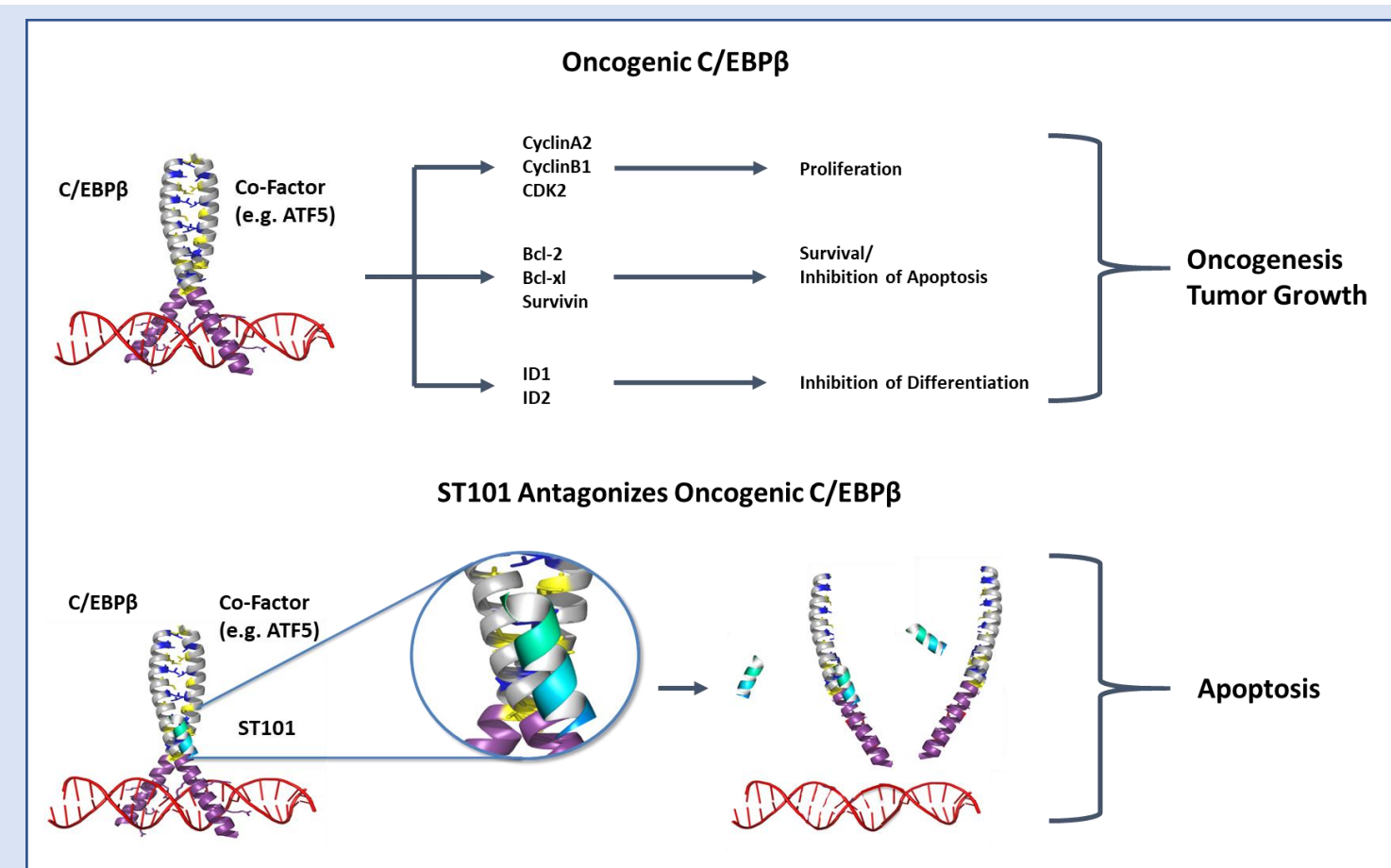


Figure 1: Mechanism of action of ST101. C/EBP β overactivation in breast cancer drives tumor cell proliferation, survival and inhibits differentiation. ST101 disrupts the interaction of C/EBP β with cofactors such as ATF5, antagonizing oncogenic gene transactivation that deprives tumor cells of the survival signals they are dependent upon, and resulting in selective tumor cell death.

ST101 In Vitro Activity

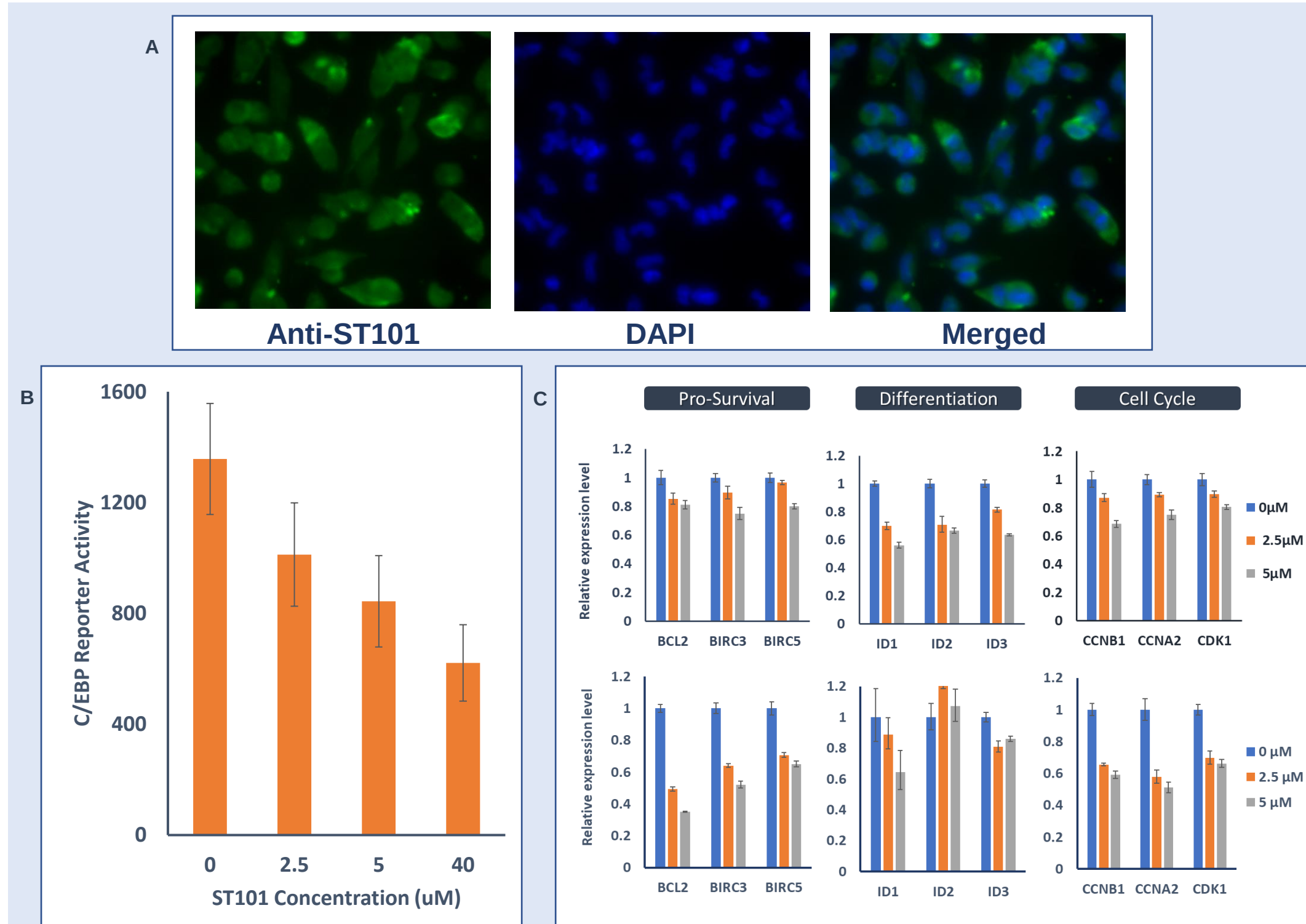


Figure 2: ST101 Rapidly Enters Cells and Engages C/EBP β . A) U251 cells were exposed to 0.1 to 10 μ M ST101 for 5 minutes and washed extensively with PBS to remove excess ST101. ST101 cell penetration was assessed using a CellInsight CX7 High-Content Screening Platform following immunofluorescence staining with a rabbit polyclonal antibody generated against the ST101 modified bZIP domain and anti-rabbit secondary reagent. Representative images for cells exposed to 0.3 μ M ST101 are shown. B) Renilla luciferase gene was transiently expressed from a C/EBP β responsive element in the presence and absence of ST101. ST101 induces a dose-dependent reduction in the expression of the luciferase gene, indicative of C/EBP β transcriptional activity inhibition. C) Quantitative PCR analysis of genes involved in survival (BCL2, BIRC3, BIRC5); differentiation (ID1, ID2 and ID3); and cell cycle (cyclin B1, cyclin A2 and cyclin-dependent kinase 1). Top panels, MCF7 cells were exposed to ST101 (0, 2.5, or 5 μ M) for 24 hrs, bottom panels 4T1-luc TNBC cells were exposed to ST101 (0, 2.5, or 5 μ M) for 48 hrs. Following RNA extraction, qPCR analysis was performed. Data represents log2 normalized expression ($2^{\Delta\Delta Ct}$) and standard error of mean.

ST101 In Vivo Activity

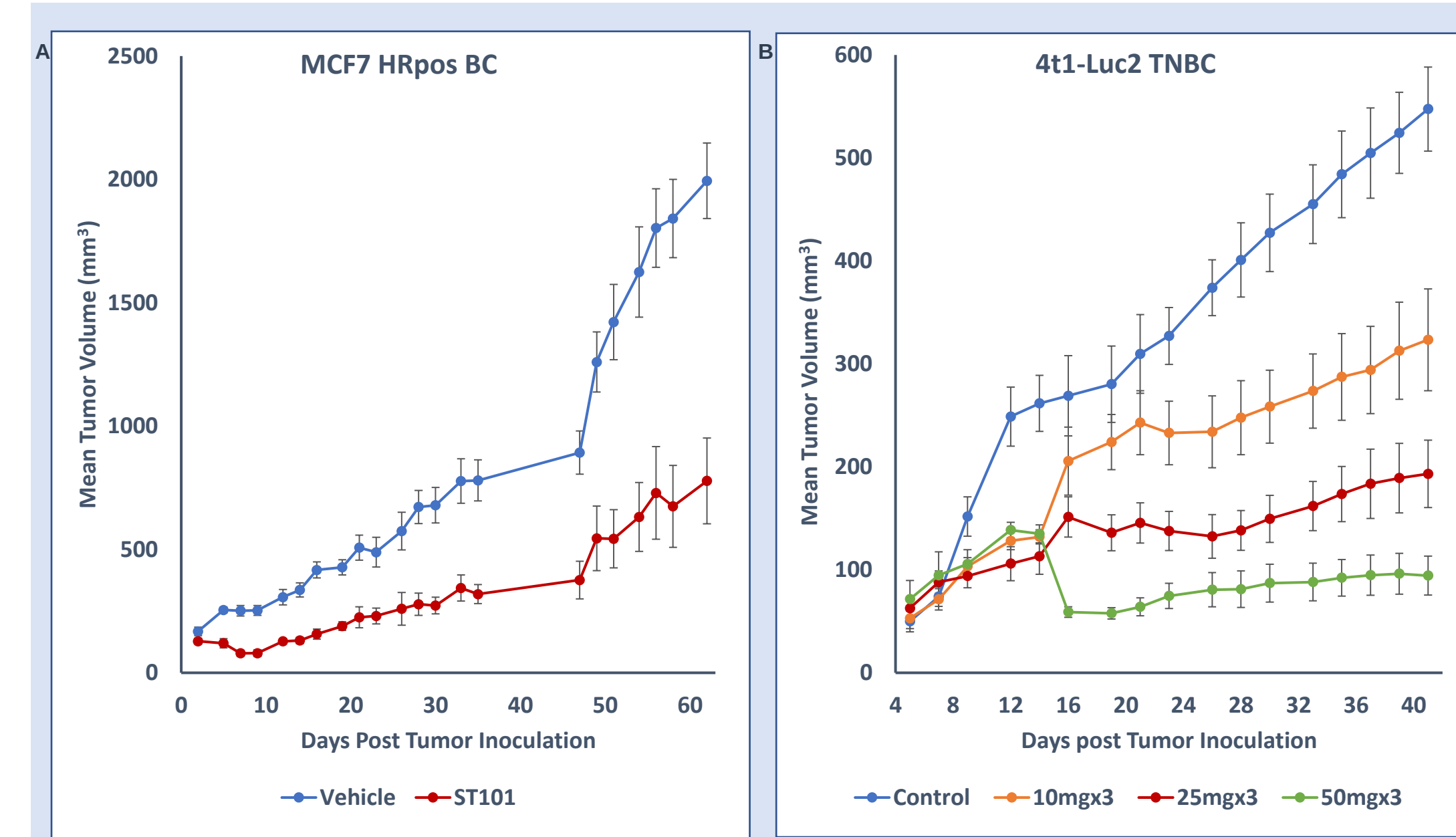


Figure 4: ST101 Displays Significant Anti-breast Cancer Activity in vivo. A) Nude mice received a 0.5 mg 60-day slow-release 17 β -estradiol pellet between the skin and muscle on the lateral side of the neck between the ear and shoulder one day prior to subcutaneous flank MCF7 HR^{positive} breast cancer xenograft implant. Starting on day 2, ST101 was administered at a dose of 25 mg/kg three days per week for three weeks. Wilcoxon matched-pairs signed rank test indicated significant difference in tumor growth between ST101 and control (p <0.001; n=8 mice in control and n=4 mice in ST101 group). B) Balb/c mice were inoculated with 1x10⁵ 4T1-luc TNBC cell into the 4th mammary fatpad. Starting on day 7, ST101 was administered at the indicated dose three days per week by subcutaneous injection. Wilcoxon matched-pairs signed rank test indicated significant difference in tumor growth between ST101 and control (p <0.001; n=6/group).

Conclusions

- ST101 rapidly enters cells and the nucleus within minutes of exposure.
- Exposure to ST101 impacts C/EBP β target gene transactivation in vitro.
- ST101 exposure results in significant decreases in gene expression of pro-survival factors (BCL2, BIRC3 and BIRC5), inhibition of differentiation factors (ID1, ID2, and ID3) and proliferative factors (cyclin B1, cyclin A2 and cyclin-dependent kinase 1).
- Significant tumor growth delay (TGD) was observed following ST101 administration in MCF7 HR^{pos} subcutaneous xenograft tumors (p <0.001) and 4T1-luc TNBC orthotopic tumors (p <0.001).
- ST101 represents a promising therapeutic approach for breast cancer.
- ST101 is currently being evaluated in a Phase 1/2 clinical study in patients with advanced unresectable and metastatic solid tumors. A Phase 2 expansion cohort in HR^{pos} BC is planned.

ST101 Mechanism of Action

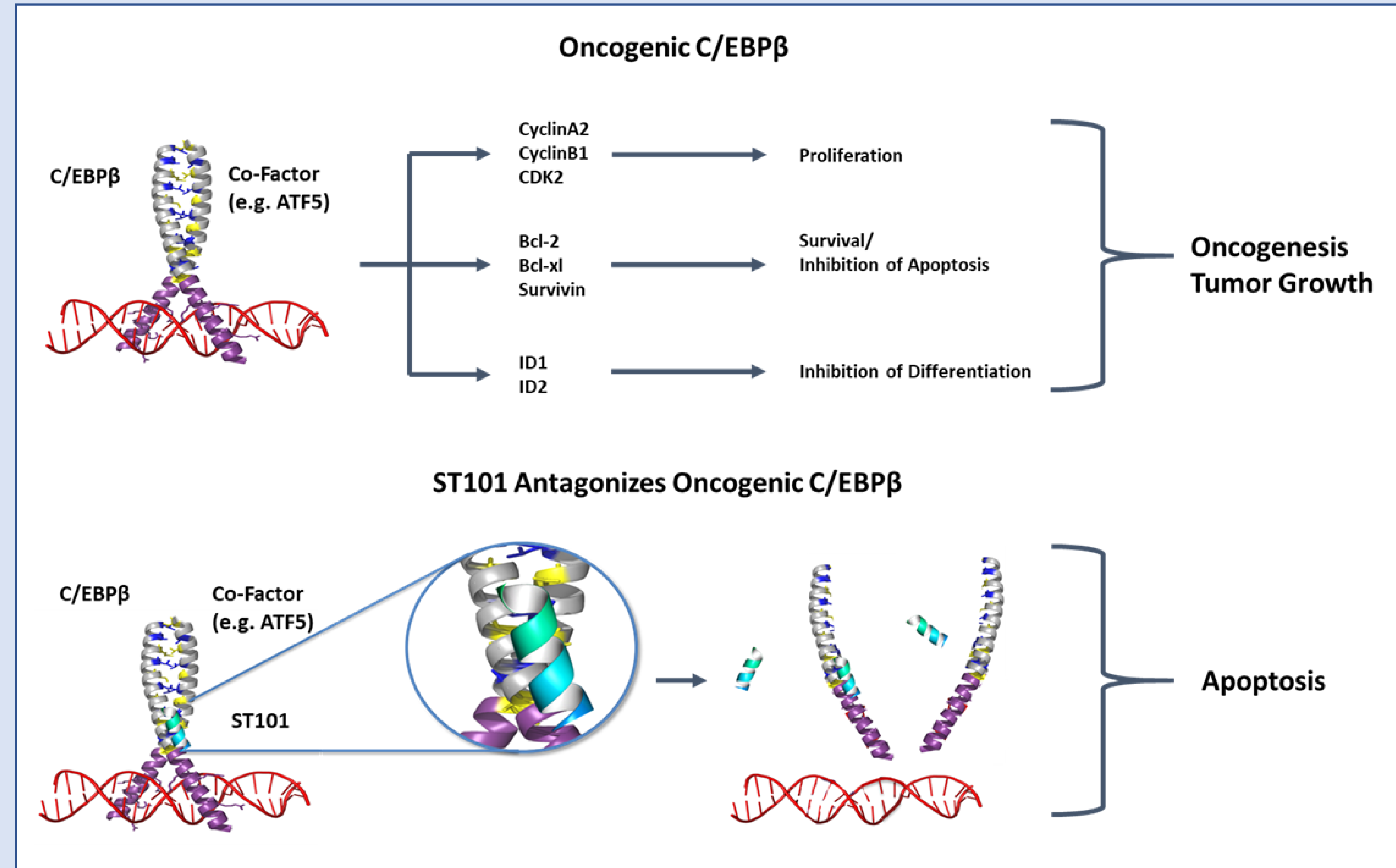


Figure 1: Mechanism of action of ST101. C/EBP β overactivation in breast cancer drives tumor cell proliferation, survival and inhibits differentiation. ST101 disrupts the interaction of C/EBP β with cofactors such as ATF5, antagonizing oncogenic gene transactivation that deprives tumor cells of the survival signals they are dependent upon, and resulting in selective tumor cell death.

ST101 In Vitro Activity

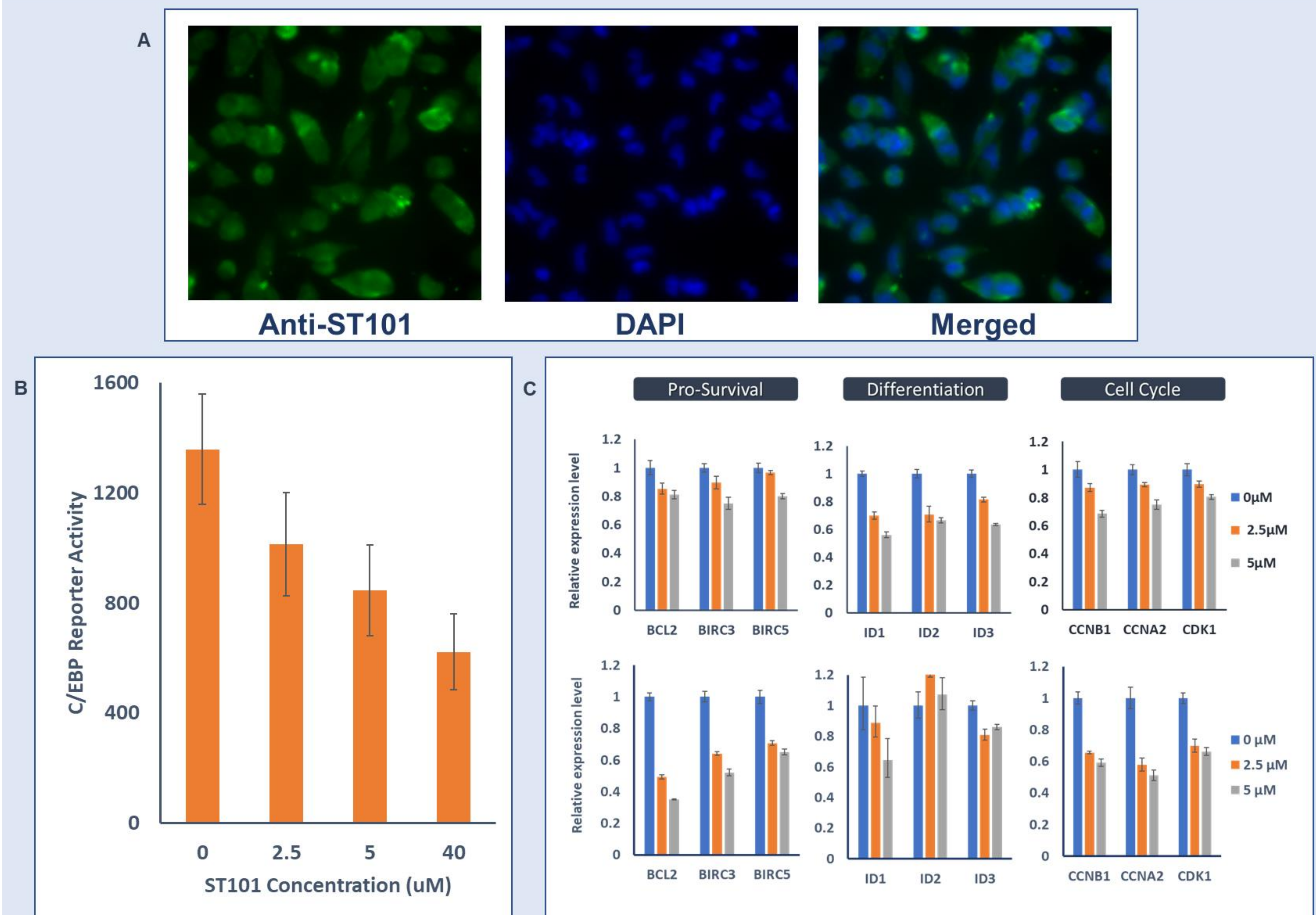


Figure 2: ST101 Rapidly Enters Cells and Engages C/EBP β . A) U251 cells were exposed to 0.1 to 10 μ M ST101 for 5 minutes and washed extensively with PBS to remove excess ST101. ST101 cell penetration was assessed using a CellInsight CX7 High-Content Screening Platform following immunofluorescence staining with a rabbit polyclonal antibody generated against the ST101 modified bZIP domain and anti-rabbit secondary reagent. Representative images for cells exposed to 0.3 μ M ST101 are shown. B) Renilla luciferase gene was transiently expressed from a C/EBP β responsive element in the presence and absence of ST101. ST101 induces a dose-dependent reduction in the expression of the luciferase gene, indicative of C/EBP β transcriptional activity inhibition. C) Quantitative PCR analysis of genes involved in survival (BCL2, BIRC3, BIRC5); differentiation (ID1, ID2 and ID3); and cell cycle (cyclin B1, cyclin A2 and cyclin-dependent kinase 1). Top panels, MCF7 cells were exposed to ST101 (0, 2.5, or 5 μ M) for 24 hrs, bottom panels 4T1-luc TNBC cells were exposed to ST101 (0, 2.5, or 5 μ M) for 48 hrs. Following RNA extraction, qPCR analysis was performed. Data represents log2 normalized expression ($2^{\Delta\Delta Ct}$) and standard error of mean.

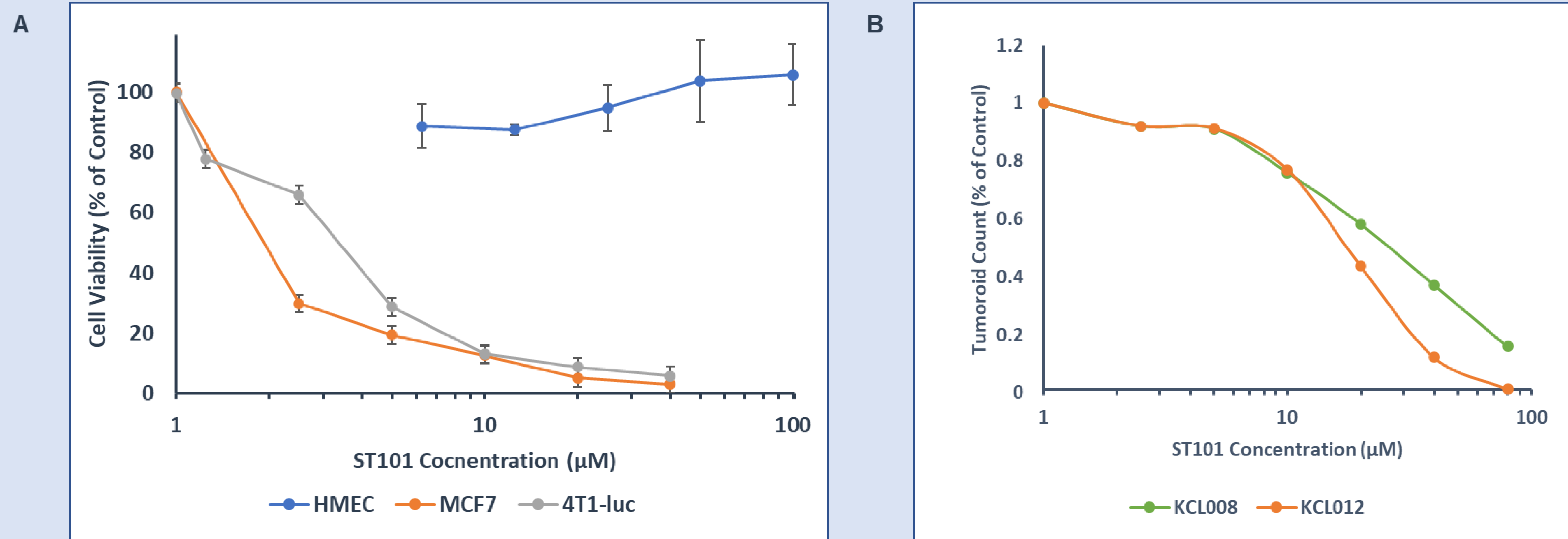


Figure 3: ST101 Selectively Induced Tumor Cell Death in vitro. A) Cell viability of MCF7 HR^{positive} breast cancer and normal human mammary epithelial cells (HMECs) were determined following exposure to ST101 for 48 hrs (MCF7 exposed to 2.5 to 40 μM ; HMEC exposed to 6.25 to 100 μM). Viability was analyzed following annexin V/PI staining by high-content imaging and is presented as percent of vehicle-treated control. Data represents mean $\pm$ SEM for a minimum of 3 replicates for each data point. B) Patient-derived breast cancer tumoroids were grown in semi-solid medium for 24 hrs prior to 7-day exposure to ST101 (0-100 μM). Tumoroid cell viability and overall tumoroid count were assessed following Hoechst and Rhodamine-Phalloidin staining to label DNA and actin cytoskeleton, respectively. Each data point represents mean $\pm$ SEM for 4 replicates.

ST101 In Vivo Activity

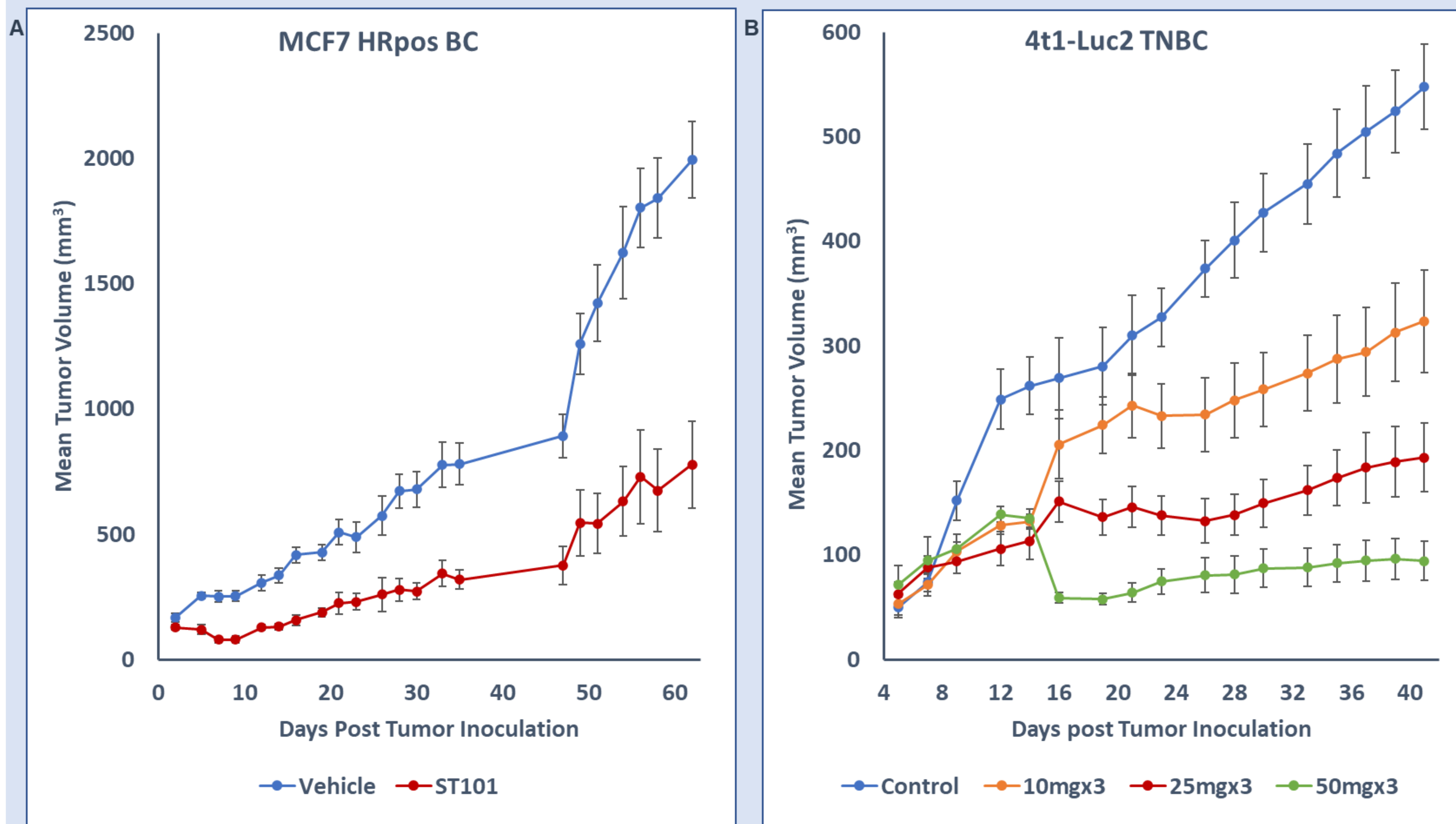


Figure 4: ST101 Displays Significant Anti-breast Cancer Activity in vivo. A) Nude mice received a 0.5 mg 60-day slow-release 17β -estradiol pellet between the skin and muscle on the lateral side of the neck between the ear and shoulder one day prior to subcutaneous flank MCF7 HR^{positive} breast cancer xenograft implant. Starting on day 2, ST101 was administered at a dose of 25 mg/kg three days per week for three weeks. Wilcoxon matched-pairs signed rank test indicated significant difference in tumor growth between ST101 and control ($p < 0.001$; $n = 8$ mice in control and $n = 4$ mice in ST101 group). B) Balb/c mice were inoculated with 1×10^5 4T1-luc TNBC cell into the 4th mammary fatpad. Starting on day 7, ST101 was administered at the indicated dose three days per week by subcutaneous injection. Wilcoxon matched-pairs signed rank test indicated significant difference in tumor growth between ST101 and control ($p < 0.001$; $n = 6$ /group).

Introduction

Sapience Therapeutics is focused on discovering and developing peptide-based therapeutics to previously 'undruggable' targets for major unmet medical needs, particularly high mortality cancers.

ST101 is a peptide antagonist of the oncogenic transcription factor CCAAT/Enhancer Binding Protein Beta (C/EBP β). ST101 is currently being evaluated in a Phase 1/2 clinical study in patients with advanced unresectable and metastatic solid tumors, with planned expansion cohorts in breast cancer, melanoma, prostate cancer and GBM. A description of the ongoing clinical trial can be found on poster #LB114.

Abstract

Breast cancer (BC) is the most common invasive cancer in women, with more than 2.1 million cases and over 626,000 deaths occurring worldwide annually. Although age-adjusted mortality from BC has been declining since 1990, the median survival for patients with metastatic disease remains at 2–3 years, and 5-year survival approximates 25%. Thus, the medical need for more effective agents in this clinical setting remains very high. C/EBP β is a transcription factor overexpressed in many cancers that regulates expression of factors that promote tumor survival, proliferation and inhibit differentiation. C/EBP β dimers, such as with Jun/Fos and CREB/ATF family members, form via basic leucine zipper (bZIP)-driven coiled-coil interactions and are necessary for its association with DNA and transcriptional activity. Due in part to the difficulty in therapeutically targeting transcription factors, no effective C/EBP β inhibitors are clinically available. ST101 is a novel peptide antagonist of C/EBP β currently being evaluated in a Phase 1/2 clinical study in patients with advanced unresectable and metastatic solid tumors. ST101 was designed to bind the C/EBP β bZIP domain, thereby preventing dimer formation and inhibiting its transcriptional activity. Here we describe ST101 non-clinical anti-tumor activity to support further development as a therapeutic approach in BC. In vitro studies in 4T1-luc triple-negative breast cancer (TNBC) and MCF7 HRPOS breast adenocarcinoma cells demonstrate ST101 dose-dependent impact of cell viability (EC50 of 4.8 and 2.1 μ M, respectively), accompanied by significant impact on C/EBP β -mediated gene expression as determined by qPCR analysis. In contrast, normal human mammary epithelial cells were not sensitive to ST101 (EC50 >100 μ M). In vivo experiments indicate dose-dependent tumor growth inhibition in an orthotopic 4T1-luc model following ST101 exposure, with significant tumor growth delays observed following 25 mg/kg ST101 (p <0.05 vs. controls). Mice treated with 25 mg/kg ST101 via subcutaneous injection demonstrated significant tumor growth delay and decrease in tumor volume vs. controls. These data emphasize the potential of ST101 as a potent peptide therapeutic for HRPOS and TN BC.

ST101 Mechanism of Action

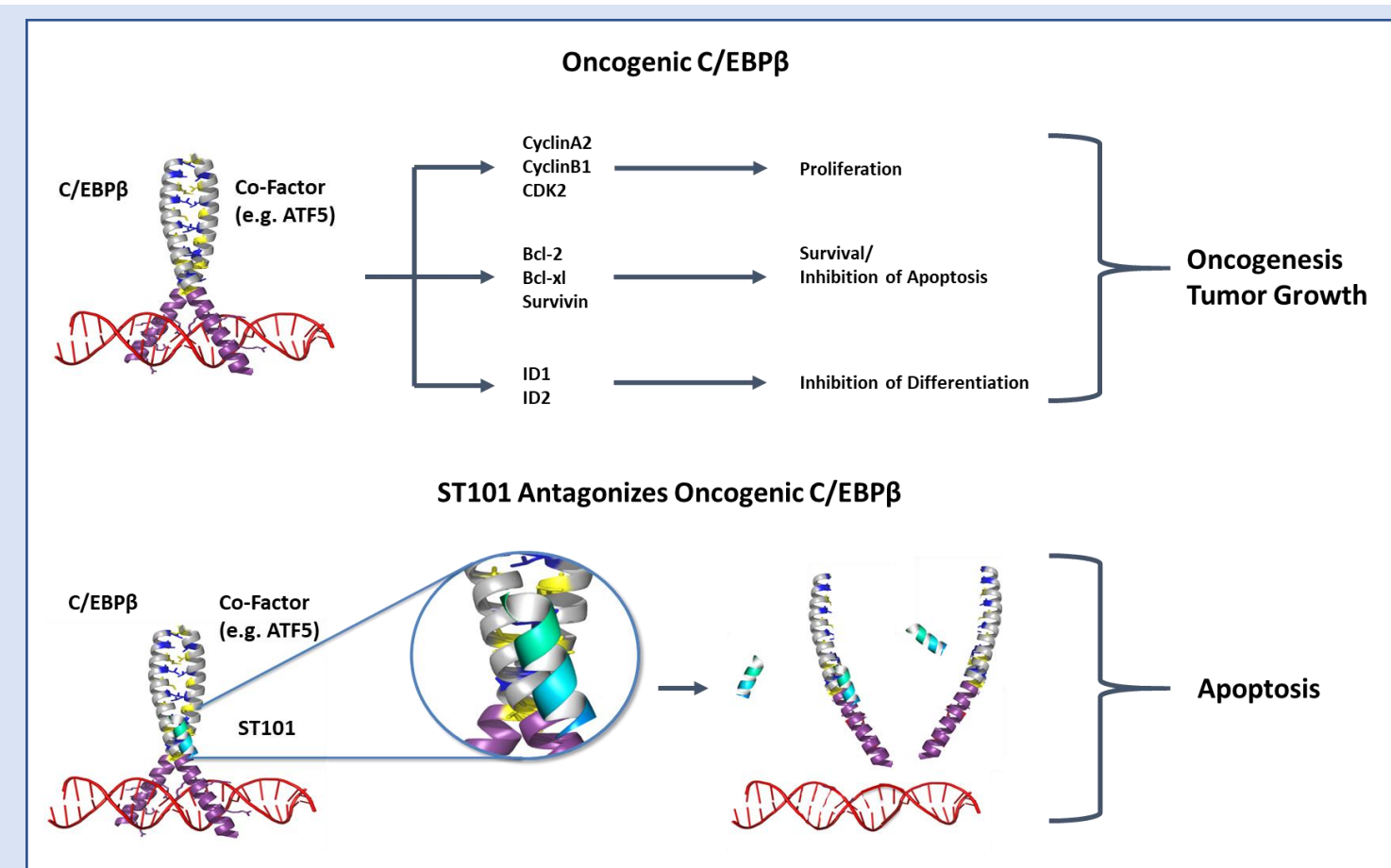


Figure 1: Mechanism of action of ST101. C/EBP β overactivation in breast cancer drives tumor cell proliferation, survival and inhibits differentiation. ST101 disrupts the interaction of C/EBP β with cofactors such as ATF5, antagonizing oncogenic gene transactivation that deprives tumor cells of the survival signals they are dependent upon, and resulting in selective tumor cell death.

ST101 In Vitro Activity

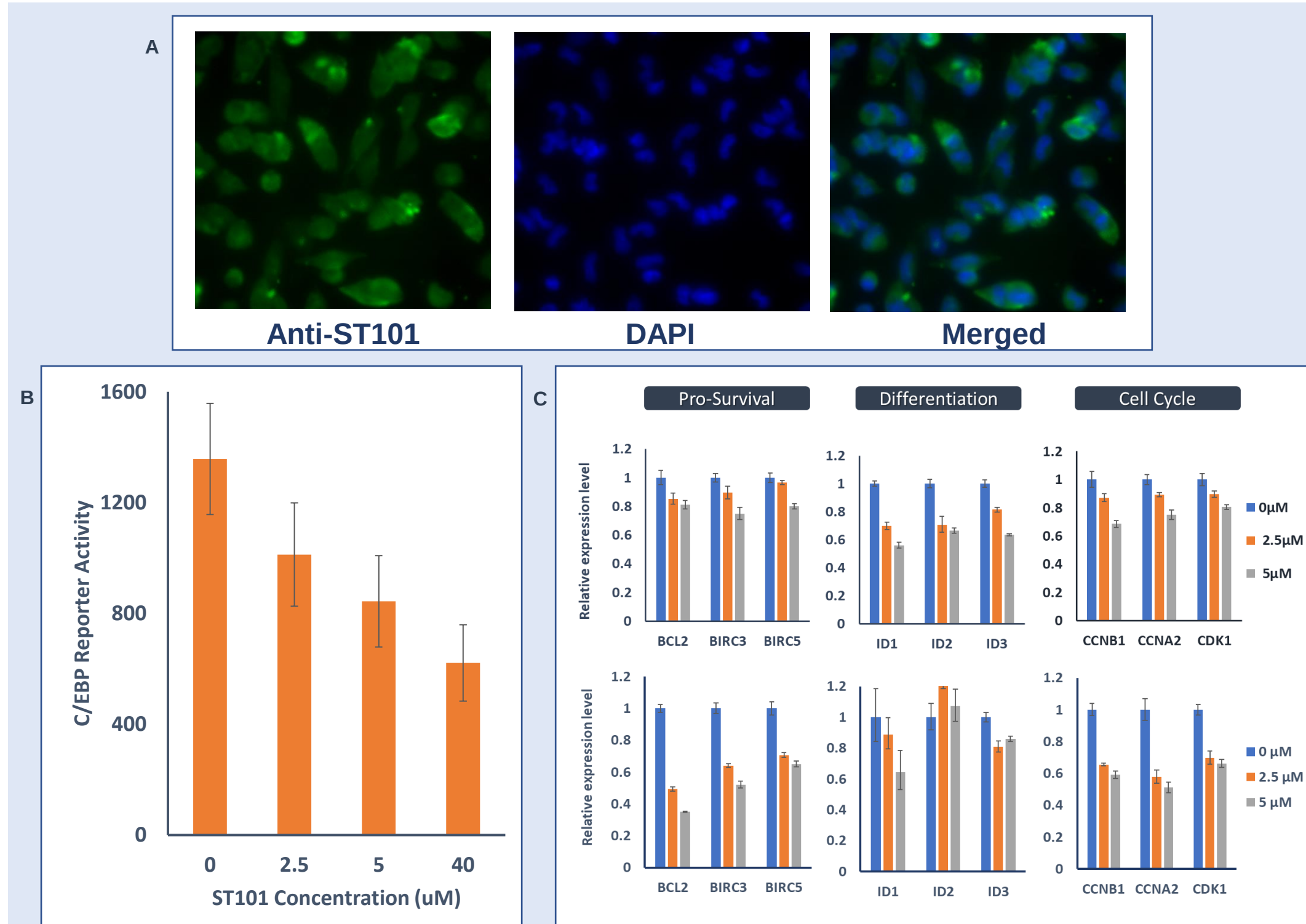


Figure 2: ST101 Rapidly Enters Cells and Engages C/EBP β . A) U251 cells were exposed to 0.1 to 10 μ M ST101 for 5 minutes and washed extensively with PBS to remove excess ST101. ST101 cell penetration was assessed using a CellInsight CX7 High-Content Screening Platform following immunofluorescence staining with a rabbit polyclonal antibody generated against the ST101 modified bZIP domain and anti-rabbit secondary reagent. Representative images for cells exposed to 0.3 μ M ST101 are shown. B) Renilla luciferase gene was transiently expressed from a C/EBP β responsive element in the presence and absence of ST101. ST101 induces a dose-dependent reduction in the expression of the luciferase gene, indicative of C/EBP β transcriptional activity inhibition. C) Quantitative PCR analysis of genes involved in survival (BCL2, BIRC3, BIRC5); differentiation (ID1, ID2 and ID3); and cell cycle (cyclin B1, cyclin A2 and cyclin-dependent kinase 1). Top panels, MCF7 cells were exposed to ST101 (0, 2.5, or 5 μ M) for 24 hrs, bottom panels 4T1-luc TNBC cells were exposed to ST101 (0, 2.5, or 5 μ M) for 48 hrs. Following RNA extraction, qPCR analysis was performed. Data represents log2 normalized expression ($2^{\Delta\Delta Ct}$) and standard error of mean.

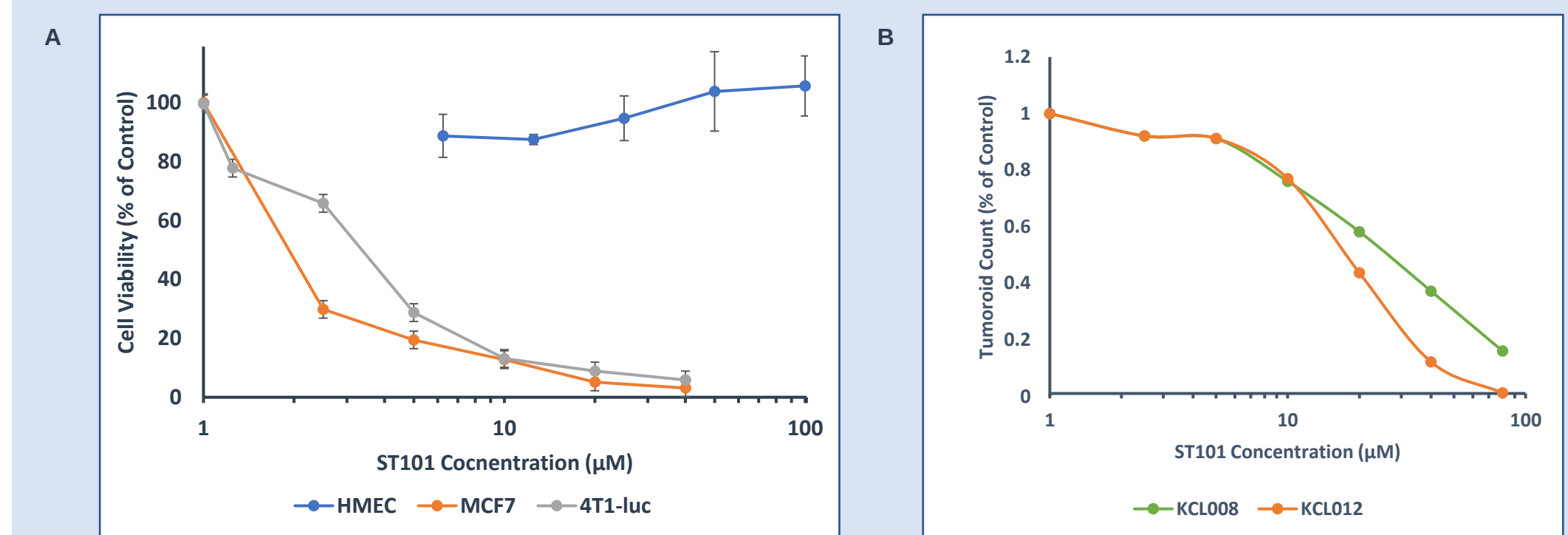


Figure 3: ST101 Selectively Induced Tumor Cell Death in vitro. A) Cell viability of MCF7 HR^{positive} breast cancer and normal human mammary epithelial cells (HMECs) were determined following exposure to ST101 for 48 hrs (MCF7 exposed to 2.5 to 40 μ M; HMEC exposed to 6.25 to 100 μ M). Viability was analyzed following annexin V/PI staining by high-content imaging and is presented as percent of vehicle-treated control. Data represents mean $\pm$ SEM for a minimum of 3 replicates for each data point. B) Patient-derived breast cancer tumouroids were grown in semi-solid medium for 24 hrs prior to 7-day exposure to ST101 (0-100 μ M). Tumouroid cell viability and overall tumouroid count were assessed following Hoechst and Rhodamine-Phalloidin staining to label DNA and actin cytoskeleton, respectively. Each data point represents mean $\pm$ SEM for 4 replicates.

ST101 In Vivo Activity

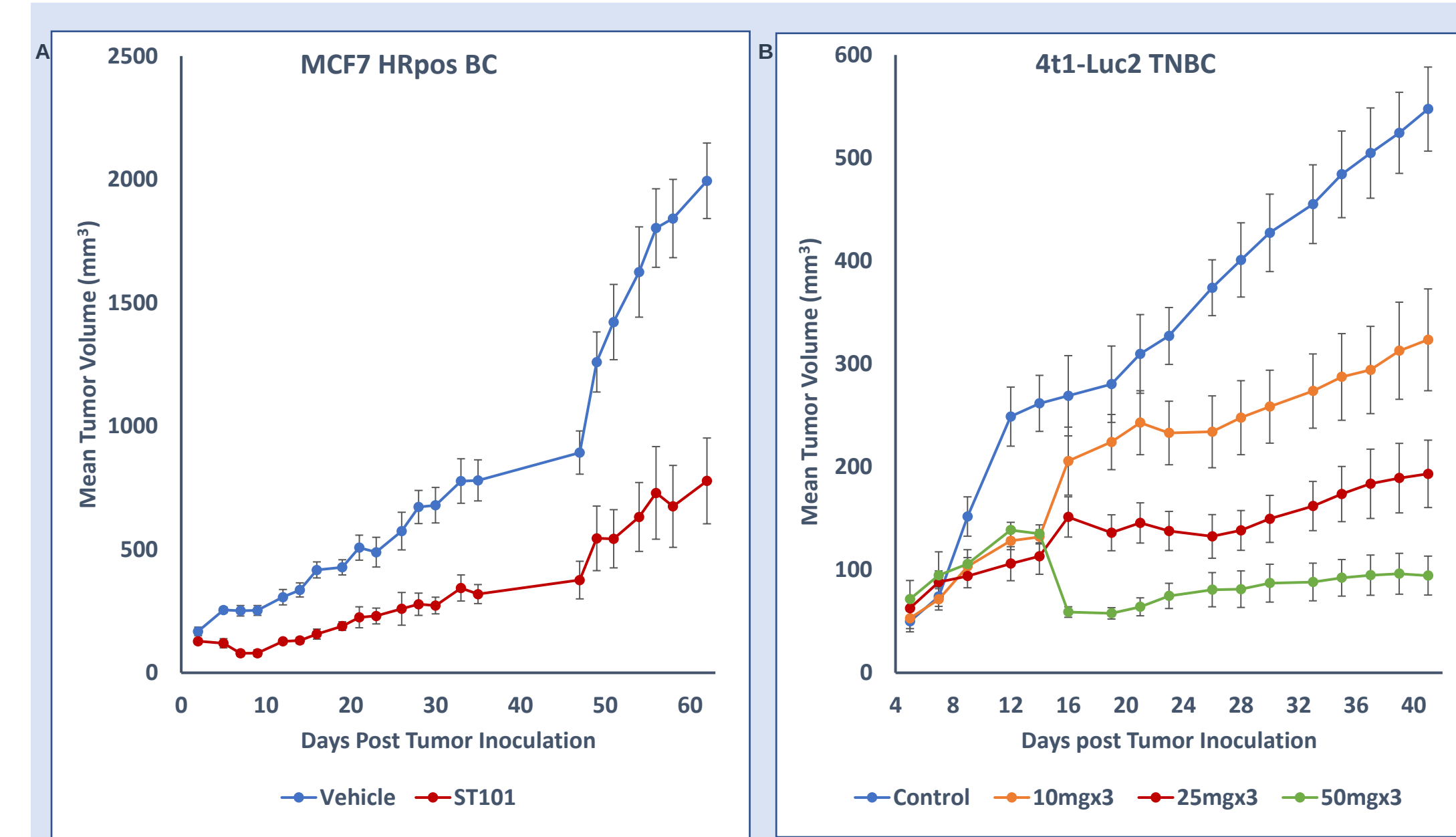


Figure 4: ST101 Displays Significant Anti-breast Cancer Activity in vivo. A) Nude mice received a 0.5 mg 60-day slow-release 17 β -estradiol pellet between the skin and muscle on the lateral side of the neck between the ear and shoulder one day prior to subcutaneous flank MCF7 HR^{positive} breast cancer xenograft implant. Starting on day 2, ST101 was administered at a dose of 25 mg/kg three days per week for three weeks. Wilcoxon matched-pairs signed rank test indicated significant difference in tumor growth between ST101 and control (p <0.001; n=8 mice in control and n=4 mice in ST101 group). B) Balb/c mice were inoculated with 1×10^5 4T1-luc TNBC cell into the 4th mammary fatpad. Starting on day 7, ST101 was administered at the indicated dose three days per week by subcutaneous injection. Wilcoxon matched-pairs signed rank test indicated significant difference in tumor growth between ST101 and control (p <0.001; n=6/group).

Conclusions

- ST101 rapidly enters cells and the nucleus within minutes of exposure.
- Exposure to ST101 impacts C/EBP β target gene transactivation in vitro.
- ST101 exposure results in significant decreases in gene expression of pro-survival factors (BCL2, BIRC3 and BIRC5), inhibition of differentiation factors (ID1, ID2, and ID3) and proliferative factors (cyclin B1, cyclin A2 and cyclin-dependent kinase 1).
- Significant tumor growth delay (TGD) was observed following ST101 administration in MCF7 HR^{pos} subcutaneous xenograft tumors (p <0.001) and 4T1-luc TNBC orthotopic tumors (p <0.001).
- ST101 represents a promising therapeutic approach for breast cancer.
- ST101 is currently being evaluated in a Phase 1/2 clinical study in patients with advanced unresectable and metastatic solid tumors. A Phase 2 expansion cohort in HR^{pos} BC is planned.