**Supplemental Methods:**

ST101 is a 38 amino acid synthetic peptide composed entirely of D-amino acids. It has a CAS# of 2407100-74-5 and its chemical name is H-D-valyl-D-alanyl-D-glutamyl-D-alanyl-D-arginyl-D-glutamyl-D-glutamyl-D-leucyl-D-glutamyl-D-arginyl-D-leucyl-D-glutamyl-D-alanyl-D-arginyl-D-leucyl-glycyl-D-glutaminyl-D-alanyl-D-arginyl-glycyl-D-glutamyl-D-leucyl-D-lysyl-D-lysyl-D-tryptophyl-D-lysyl-D-methionyl-D-arginyl-D-arginyl-D-asparaginyl-D-glutaminyl-D-phenylalanyl-D-tryptophyl-D-leucyl-D-lysyl-D-leucyl-D-glutaminyl-D-arginine.

The synthesis of ST101 is carried out following the general solid-phase procedure first described by Merrifield (1) and well-known procedures that have been utilized for over 30 years. The alpha-amino group of each amino acid starting material is protected with a 9-Fluorenylmethoxycarbonyl (Fmoc-) group, while sidechain functional groups used in the synthesis of ST101 are protected as follows: Arginine [Pbf (Nω-(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl))], Asparagine [Trt (Trityl)], Glutamic Acid [OtBu (tert-Butyl ester)], Glutamine [Trt (Trityl)], Lysine [Boc (tert-Butoxycarbonyl)], Tryptophan [Boc (tert-Butoxycarbonyl)].

The peptide resin is swelled and washed with dimethylformamide (DMF) and drained. The resin is then treated twice with a solution of 20% piperidine in DMF to remove the Fmoc group protecting the N-terminal amine of the peptide resin. The resin is washed multiple times to remove residual piperidine and a solution containing diisopropylcarbodiimide (DIC), Oxymapure®, and the next amino acid is added. Progress of the coupling reaction is monitored by using the ninhydrin test (2). If the ninhydrin test is found to be positive, indicating the presence of unreacted amine, the coupling is repeated, using a lesser amount of the amino acid derivative and coupling reagents. When the ninhydrin test is negative, the resin is washed multiple times with DMF, and the remaining amino acid derivatives in the sequence are coupled sequentially using the same cycle (Fmoc removal, washing, coupling, washing). Repetition of the coupling cycle with the remaining amino acid derivatives in the sequence results in the fully protected peptide-resin. After coupling the last amino acid group (D-Val) to the peptide resin, the resin-peptide is treated twice with 20% piperidine to remove the Fmoc group and subsequently washed with DMF, isopropanol, and methyl tert-butyl ether (MTBE), and dried to constant weight.

The peptide is cleaved from the resin and sidechain protection groups are removed by treatment of the resin-peptide from the first stage with trifluoracetic acid (TFA), in the presence of a scavengers (water, triisopropylsilane (TIPS), dithiothreitol (DTT), ammonium iodide), to give the crude product. The crude peptide is precipitated with spent resin using cold MTBE, collected by filtration, then dried under reduced pressure.

The crude product is purified by a multi-step, preparative, reverse-phase HPLC process. The HPLC chromatography stationary phase (C18 Daiso Gel, 120 Å, 10 μ). The crude peptide with the spent resin is stirred with a solution of water, CH3CN, and acetic acid and filtered. This filtered solution is loaded onto the C18 column pre-equilibrated with a dilute solution of acetonitrile and H3PO4 in water. The first step is accomplished by eluting the adsorbed peptide from the column using a gradient consisting of mobile phases 0.1% H3PO4 in water and acetonitrile. Fractions are analyzed for peptide purity by analytical HPLC. Fractions meeting the specified in-process control acceptance criteria for purity are pooled for the second step in the purification process that also converts the peptide to its acetate form.

For the second RP HPLC purification step the pools from the first purification step are diluted with USP Purified Water and applied to the same prepared column used in the first purification. The column is washed sequentially with an aqueous solution of CH3CN/ NH4OAc followed by an aqueous solution of CH3CN/acetic acid. This second purification step converts the salt form to acetate while also giving additional purification of process-related impurities. The adsorbed peptide is eluted from the column by applying a gradient of aqueous CH3CN/acetic acid. Fractions are collected and analyzed for peptide purity. Fractions meeting the acceptance criteria for purity are pooled for lyophilization. The resultant ST101 peptide is an acetate salt with a purity by analytical HPLC of >95%.

Structure of ST101 was confirmed by mass spectroscopy. Electrospray ionization mass spectrometry (ESI-MS) was used to verify the molecular mass. Samples were analyzed on an Orbitrap Fusion™ Tribrid™ mass spectrometer (FT-MS). Full mass range scans were gathered with the Orbitrap FT-MS detector, showing the +5, +6, +7, +8, and +9 charged ions. Data from the Orbitrap detector was used to determine the monoisotopic and the average mass of the ST101 peptide and to verify that each was a signal of ST101. Table 1 shows the observed monoisotopic and average mass for the +5, +6, +7, +8, and +9 charged molecular ions (Table 1). The observed monoisotopic mass as calculated from the [M+7H]7+ ion is 4721.61. The observed average mass as calculated from the [M+7H]7+ ion is 4725.21. The results confirm the expected monoisotopic and average mass of the ST101.

The amino acid sequence of ST101 was confirmed by performing collision-induced dissociation (CID) MS-MS. This technique uses deliberate fragmentation of the charged states to obtain structural information from the ion spectra created. The fragment ions observed from both the N- and C-terminus confirmed the amino acid sequence of the ST101 (Supplemental Tables 2 and 3).

The enantiomeric purity was determined by hydrolysis of ST101 followed by GC-MS analysis. The data indicate that the level of L-enantiomer for each amino acid was ≤ 0.2 %, confirming that ST101 is comprised of D-amino acids (Supplemental Table 3).

**Supplemental Tables and Figures**

| **Molecular ion** | **Observed Monoisotopic Mass (Da)** | **Observed Averaged Mass (Da)** |
| --- | --- | --- |
| [M+5H]5+ | 945.328 | 945.97 |
| [M+6H]6+ | 787.943 | 788.50 |
| [M+7H]7+ | 675.524\* | 676.13\*\* |
| [M+8H]8+ | 591.208 | 591.66 |
| [M+9H]9+ | 525.630 | 526.03 |

\*This ion was used for the monoisotopic mass calculation. \*\*This ion was used for the average mass calculation.

**Supplemental Table 1: Observed MS Molecular ions of ST101**

|  |  | **N-terminal Sequence ions** | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| n | aa | **Expected *b1+*** | **Observed *b1+*** | **Expected *b2+*** | **Observed *b2+*** | **Expected *b3+*** | **Observed *b3+*** | **Expected *b4+*** | **Observed *b4+*** | **Expected *b5+*** | **Observed *b5+*** |
| 0 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 1 | V | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 2 | A | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 3 | E | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 4 | A | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 5 | R | 527.29 | 527.29 | --- | --- | --- | --- | --- | --- | --- | --- |
| 6 | E | 656.34 | 656.34 | --- | --- | --- | --- | --- | --- | --- | --- |
| 7 | E | 785.38 | 785.38 | --- | --- | --- | --- | --- | --- | --- | --- |
| 8 | L | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 9 | E | 1027.51 | 1027.51 | --- | --- | --- | --- | --- | --- | --- | --- |
| 10 | R | --- | --- | 592.31 | 592.31 | --- | --- | --- | --- | --- | --- |
| 11 | L | 1296.69 | 1296.69 | 648.85 | 648.85 | --- | --- | --- | --- | --- | --- |
| 12 | E | 1425.73 | 1425.73 | 713.37 | 713.37 | --- | --- | --- | --- | --- | --- |
| 13 | A | --- | --- | 748.89 | 748.89 | --- | --- | --- | --- | --- | --- |
| 14 | R | --- | --- | 826.94 | 826.94 | --- | --- | --- | --- | --- | --- |
| 15 | L | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 16 | G | --- | --- | --- | --- | 608.33 | 608.33 | --- | --- | --- | --- |
| 17 | Q | --- | --- | --- | --- | 651.02 | 651.02 | --- | --- | --- | --- |
| 18 | A | --- | --- | 1011.54 | 1011.54 | 674.70 | 674.70 | --- | --- | --- | --- |
| 19 | R | --- | --- | 1089.59 | 1089.59 | 726.73 | 726.73 | --- | --- | --- | --- |
| 20 | G | --- | --- | 1118.10 | 1118.10 | 745.74 | 745.74 | --- | --- | --- | --- |
| 21 | E | --- | --- | --- | --- | 788.75 | 788.75 | --- | --- | --- | --- |
| 22 | L | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 23 | K | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 24 | K | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 25 | W | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 26 | K | --- | --- | --- | --- | 1016.57 | 1016.57 | --- | --- | --- | --- |
| 27 | M | --- | --- | --- | --- | 1060.25 | 1060.25 | --- | --- | --- | --- |
| 28 | R | --- | --- | --- | --- | 1112.28 | 1112.28 | 834.46 | 834.46 | --- | --- |
| 29 | R | --- | --- | --- | --- | 1164.31 | 1164.32 | 873.49 | 873.49 | --- | --- |
| 30 | N | --- | --- | --- | --- | --- | --- | 902.00 | 902.00 | --- | --- |
| 31 | Q | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 32 | F | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 33 | W | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 34 | L | --- | --- | --- | --- | --- | --- | 1045.57 | 1045.58 | 836.66 | 836.67 |
| 35 | K | --- | --- | --- | --- | --- | --- | 1077.59 | 1077.60 | 862.28 | 416.26 |
| 36 | L | --- | --- | --- | --- | --- | --- | 1105.87 | 1105.87 | --- | --- |
| 37 | Q | --- | --- | --- | --- | --- | --- | 1137.88 | 1137.88 | 910.51 | 910.51 |
| 38 | R | --- | --- | --- | --- | --- | --- | --- | --- | 941.73 | 941.73 |

All ions are numbered from the N-terminus

**Supplemental Table 2: N-terminal Sequence Ions Detected in CID MS-MS Spectra of ST101 [M+5H]5+**

|  |  | **C-terminal Sequence ions** | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| n | aa | **Expected *y1+*** | **Observed *y1+*** | **Expected *y2+*** | **Observed *y2+*** | **Expected *y3+*** | **Observed *y3+*** | **Expected *y4+*** | **Observed *y4+*** | **Expected *y5+*** | **Observed *y5+*** |
| 0 | V | --- | --- | --- | --- | --- | --- | --- | --- | 945.33 | 945.33 |
| 1 | A | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 2 | E | --- | --- | --- | --- | --- | --- | 1138.88 | 1138.88 | 911.31 | 911.31 |
| 3 | A | --- | --- | --- | --- | --- | --- | --- | --- | 885.50 | 885.50 |
| 4 | R | --- | --- | --- | --- | --- | --- | --- | --- | 871.29 | 871.29 |
| 5 | E | --- | --- | --- | --- | --- | --- | 1049.84 | 1049.84 | --- | --- |
| 6 | E | --- | --- | --- | --- | --- | --- | 1017.58 | 1017.58 | --- | --- |
| 7 | L | --- | --- | --- | --- | --- | --- | 985.32 | 985.32 | --- | --- |
| 8 | E | --- | --- | --- | --- | --- | --- | 957.04 | 957.04 | --- | --- |
| 9 | R | --- | --- | --- | --- | --- | --- | 924.78 | 924.79 | --- | --- |
| 10 | L | --- | --- | --- | --- | 1180.68 | 1180.70 | --- | --- | --- | --- |
| 11 | E | --- | --- | --- | --- | --- | --- | 857.49 | 857.49 | --- | --- |
| 12 | A | --- | --- | --- | --- | 1099.97 | 1099.97 | 825.23 | 825.23 | --- | --- |
| 13 | R | --- | --- | --- | --- | 1076.29 | 1076.29 | 807.47 | 807.47 | --- | --- |
| 14 | L | --- | --- | --- | --- | 1024.25 | 1024.25 | --- | --- | --- | --- |
| 15 | G | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 16 | Q | --- | --- | --- | --- | 967.55 | 967.55 | --- | --- | --- | --- |
| 17 | A | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 18 | R | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 19 | G | --- | --- | 1273.23 | 1273.23 | 849.15 | 849.15 | --- | --- | --- | --- |
| 20 | E | --- | --- | 1244.72 | 1244.71 | --- | --- | --- | --- | --- | --- |
| 21 | L | --- | --- | 1180.19 | 1180.19 | 787.13 | 787.13 | --- | --- | --- | --- |
| 22 | K | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 23 | K | --- | --- | 1059.60 | 1059.61 | 706.74 | 706.72 | --- | --- | --- | --- |
| 24 | W | --- | --- | 995.56 | 995.56 | 664.04 | 664.04 | --- | --- | --- | --- |
| 25 | K | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 26 | M | --- | --- | 838.47 | 838.47 | --- | --- | --- | --- | --- | --- |
| 27 | R | --- | --- | 772.95 | 772.95 | --- | --- | --- | --- | --- | --- |
| 28 | R | 1388.79 | 1388.79 | 694.90 | 694.90 | --- | --- | --- | --- | --- | --- |
| 29 | N | 1232.69 | 1232.69 | 616.85 | 616.85 | --- | --- | --- | --- | --- | --- |
| 30 | Q | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 31 | F | 990.59 | 990.59 | --- | --- | --- | --- | --- | --- | --- | --- |
| 32 | W | 843.52 | 843.52 | --- | --- | --- | --- | --- | --- | --- | --- |
| 33 | L | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 34 | K | 544.36 | 544.36 | --- | --- | --- | --- | --- | --- | --- | --- |
| 35 | L | 416.26 | 416.26 | --- | --- | --- | --- | --- | --- | --- | --- |
| 36 | Q | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 37 | R | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 38 |  | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |

Supplemental Table 3: C-terminal Sequence Ions Detected in CID MS-MS Spectra of ST101 [M+5H]5+. All ions are numbered from the N-terminus. All ions are numbered from the N-terminus.

|  |  |
| --- | --- |
| Amino Acid | Result |
| Alanine | <0.10 % L-Enantiomer |
| Arginine | <0.10 % L-Enantiomer |
| Aspartic acid | 0.21 % L-Enantiomer |
| Glutamic acid | 0.18 % L-Enantiomer |
| Leucine | <0.10 % L-Enantiomer |
| Lysine | <0.10 % L-Enantiomer |
| Methionine | 0.19 % L-Enantiomer |
| Phenylalanine | 0.17 % L-Enantiomer |
| Tryptophan | <0.10 % L-Enantiomer |
| Valine | 0.10 % L-Enantiomer |

**Supplemental Table 4: L-Amino acid content of ST101**.



**Supplemental Table 5: Sequences of bZIP domain peptides used for circular dichroism.** Helical position (a-g) of amino acids indicated on top line. Underline region indicates putative binding domains on each peptide.

See excel file included with submission

**Supplemental Table 6: Fold change expression level of differentially expressed CEBPβ target genes in A549 cells**. A unique list of 489 CEBPβ target genes were compiled from MSigDB CEBPb\_01, CEBPB\_02 and TTGCWCAAY\_CEBPB\_02 gene sets. Among them, 413 genes were differentially regulated by ST101 in A549 cells (1<expression fold change>1).

**Supplemental Figures:**

**A picture containing table

Description automatically generatedA B**

**C **

**Supplemental Fig. 1: U251 cells display differential sensitivity to ST101.** (A) Cell viability studies using Annexin V/PI stain indicate that U251-LS cells display reduced sensitivity to ST101 compared to parent U251 cells. (B) ST101 induces proteasomal degradation of C/EBPβ in U251 (parent) cells at ST101 exposures consistent with biologic activity. Cells were exposed to the indicated ST101 concentration for 24 hrs, and Western blot analysis was performed as described in the methods. (C) ATCC Short Tandem Repeat (STR) Profile analysis suggests that both U251 and U251-LS cultures obtained from ECACC via Millipore Sigma contain a mix of cell lines, including U251 GBM and HCT116 colon carcinoma cells. Reduced ST101 sensitivity observed in U251-LS is likely due to increased HCT116 content.

**Diagram

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**Supplemental Fig. 2: ST101 associates with C/EBPγ but not ATF5.** Circular dichroism (CD) spectra data for the interaction of ST101 with C/EBPγ or ATF5. A, Spectra for ST101 and C/EBPγ bZIP peptide were measured at 20°C at a total peptide concentration of 150 μM and presented as mean residue ellipticity (MRE). The minima at 208 and 222 nm are indicative of a helical structure. Experiments were performed in 10 mM potassium phosphate and 100 mM potassium fluoride (pH 7.0). The helical signature of ST101 interaction with C/EBPγ (solid black line) exceeds the average generated from the component peptides (red dashed line), indicating that a heterodimeric complex is formed. Van’t Hoff analysis indicates the KD of this complex as 555 µM (approximately 3000-fold weaker than the observed ST101-C/EBPβ interaction). B, The thermal stability of ST101 and C/EBPγ measured using the temperature dependence of the CD signal at 222 nm. C, Spectra for ST101 and ATF5 bZIP peptide were measured as in (A). The spectra generated by a solution containing both ST101 with ATF5 matches the average spectra generated from the component peptides, indicating that ST101 does not interact with ATF5 and no heteromeric complex is formed. Red-dashed line indicates the theoretical curve derived from the mean of component curves for ST101 and ATF5. D, The thermal stability of peptide pairs measured using temperature dependence of the CD signal at 222 nm confirms that ST101 does not interact with the ATF5 leucine zipper domain.

**Chart, line chart

Description automatically generatedSupplemental Fig. 3: ELISA assay quantifying the association of recombinant full length ATF5 to plate-bound recombinant C/EBPβ.** ATF5 was detected using rabbit-anti-ATF5 antibody followed by goat anti-rabbit HRP conjugated secondary reagent.

Chart

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**Supplemental Fig. 4:** (A) Circular dichroism (CD) spectra data measured at 20°C at a total peptide concentration of 150 μM as in Fig. 1. Data presented as mean residue ellipticity (MRE). Resultant spectra of dimer exchange experiment in which C/EBPβ was added to a solution of ST101 and ATF5 overlays spectra of ST101 in solution with C/EBPβ.

**Chart, bar chart

Description automatically generatedSupplemental Fig. 5: ST101 does not impact non-specific luciferase reporter.** A549 cells were transfected with (A) LightSwitch™ C/EBPβ Promoter Reporter (Active Motif) or (B) a negative control construct (R04, Active Motif), using Xtremegene transfection reagent. The day after transfection, cells were exposed to ST101 concentrations and luminescence was measured 16h post-peptide treatment. ST101 did not impact luciferase expression in either LightSwitch™ C/EBPβ Promoter Reporter or RO4 controls, indicating that ST101 activity is specific to C/EBPβ target gene expression.

**Chart

Description automatically generatedSupplemental Fig. 6: ST101 exposure specifically reduces C/EBPβ protein expression.** HCT116 cells were treated with increasing concentrations of ST101 for 24 hours, and total cell lysate were probed with antibodies against (A) C/EBPβ, (B) C/EBPγ and β-Actin. Densitometric analysis of the immunoblots were done using ImageJ software and total C/EBPβ protein levels normalized to β-Actin as loading control. Results are the means ± SD pooled from at least two independent experiments, *\*\* P* < 0.01, *\*\*\* P* < 0.001 versus untreated control. (C) Real-time RT-PCR was used to determine the transcript levels of C/EBPβ in HCT116 cells in the presence and absence of the indicated concentrations of ST101 for 24 hours.

**Diagram

Description automatically generatedSupplemental Fig. 7: Inhibition of ubiquitination pathway reversed ST101-mediated C/EBPβ degradation.** (A) U251-LS cells were treated with the proteasome inhibitor, MG-132 (10 μM) with and without ST101 (20 μM) and total C/EBPβ protein levels were assessed by Western blot and reprobed for β-Actin as loading control. Similar results were observed in parent U251 cells. (B) HCT116 cells were treated with ST101 (20 µM) for 24 hours in the absence and presence of MG-132 (2, 5 and 10 μm) and total C/EBPβ protein levels were assessed by Western blot and re-probed for β-Actin as loading control. The graph represents the ratio of total C/EBPβ to β-Actin. (C) A549 cells were treated with ST101 (0.2, 2 and 5 µM) for 24 hours in the absence and presence of MG-132 (2, 5 and 10 μm) and total C/EBPβ protein levels were assessed by Western blot and reprobed for β-Actin as loading control. (D) U251 cells were treated with increasing concentrations of TAK-243 (5, 10, 50 nM) for 24 hours in the presence and absence of ST101 (20 µM). After treatment, whole cell lysates were prepared and levels of global mono- and poly- ubiquitylated proteins (anti-ubiquitin antibody) were measured by immunoblotting. (E) U251-LS cells were treated with ST101 (20 µM) for 24 hours in the absence and presence of a selective small molecule NEDD8-activating enzyme (NAE) inhibitor, MLN4924 (10, 100, 1000 nM). Total C/EBPβ protein levels were then assessed by immunoblotting and reprobed for β-Actin to ensure equal loading. The graph represents the ratio of total C/EBPβ to β-Actin., *\* P* < 0.05.

**Chart, bar chart

Description automatically generatedSupplemental Fig. 8: ST101 impacts C/EBPβ gene transactivation in vitro**

Quantitative PCR analysis of cell cycle factors cyclin D3 and cyclin-dependent kinase 2 in A549 cells exposed to ST101 (0, 2.5, 5 or 10 µM) for 24 h. Following RNA extraction, qPCR analysis was performed. Data represents log2 normalized expression (2^ΔΔCt) and standard error of mean.

**Diagram

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**Supplemental Fig. 9: Flow cytometric analysis of ST101 impact on A549 cell proliferation**

Cells were treated as described in Fig. 6B. DNA content quantification and distribution was assessed by flow cytometry analysis following propidium iodide (PI) staining. Live cell gating was performed (left panels), single cells were gated via PI width and area signals, and the area histogram for PI was used to determine the percentage of cells in G1, S and G2M phases (right panels).

**Chart

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**Supplemental Fig. 10: ST101 does not impact body weight following repeat-dose administration.** A, U251 cells were implanted into subcutaneous flank of NOD-scid mice as described in Figure 7. ST101 (50 mg/kg) was administered three times per week for 3x/week by subcutaneous injection. Body weights were monitored 3x/week for the duration of the experiment. No significant change in animal body weight following ST101 exposure was observed (p=n.s.). Similar results were observed following ST101 administration in: B, U251 tumor-bearing mice following subtherapeutic ST101 administration in combination with TMZ, as described in Figure 7D (p=n.s.); C, MCF7 tumor-bearing mice following ST101 administration described in Figure 7E (p=n.s.); D, A375 tumor-bearing mice following ST101 administration described in Figure 7F (p=n.s.); E, DU145 tumor-bearing mice following ST101 administration described in Figure 7G (p=n.s.); and F, A549 tumor-bearing mice following ST101 administration described in Figure 7H (p=n.s.).

Chart

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**Supplemental Fig. 11: ST101 displays potent anti-tumor activity in combination with temozolomide in T98G glioblastoma mouse subcutaneous xenograft model.** T98G subcutaneous xenograft tumors were grown to an average volume of 210 mm3 prior to mice receiving ST101 (25 mg/kg administered three days per week for three weeks by subcutaneous injection), temozolomide (100 mg/kg administered 3 days per week for 1 week by oral gavage) or combination of ST101 and temozolomide. Wilcoxon matched-pairs signed rank test indicated significant difference in tumor growth between combination group and vehicle or TMZ (p<0.001; n=5-6 mice/group) and combination group and ST101 (p<0.05, n=6 mice/group).

A picture containing text, different, several

Description automatically generated **Control ST101**

**Supplemental Fig. 12: ST101 penetrates the blood brain barrier of naïve C57BL/6 mice.** ST101 (50 mg/kg) or vehicle was administered by intravenous injection into the lateral tail-vein of naïve C57BL/6 (n=3/group). Two h post injection, mice were euthanized and brain were harvested and stored in 4% paraformaldehyde. Tissues were processed, and immunohistochemistry was performed on 2 µM sections using a rabbit polyclonal antibody generated against the ST101 bZIP domain and anti-rabbit secondary reagent. Representative images indicate ST101 (identified by brown DAB stain) in microvascular and glial cells, with no stain present in vehicle-treated controls.

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**Supplemental Fig. 13: ST101 does not elicit an antibody response in C57BL/6 mice.** ST101 (10 mg/kg) was administered once weekly for 6 weeks by intravenous injection into the lateral tail-vein of naïve C57BL/6 (n=5/group). Sera was collected prior to ST101 exposure and 24 hrs post each ST101 boost. Anti-drug antibody response (10-fold dilutions from 1:10 to 1:10,000) was determined by ELISA. No significant increase in signal was observed in mouse sera post ST101 exposure compared to pre-immune sera. Rabbit polyclonal antibody generated against the ST101 bZIP domain in the presence of adjuvant was used as positive control in this experiment.

**References**

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2. Kaiser E, Colescott RL, Bossinger CD, Cook PI. Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides. Anal Biochem **1970**;34:595-8