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Article:
Desroses, M., Busker, S., Astorga-Wells, J. et al. (2018) STAT3 differential scanning fluorimetry and differential scanning light scattering assays: Addressing a missing link in the characterization of STAT3 inhibitor interactions. Journal of Pharmaceutical and Biomedical Analysis , 160. pp. 80-88. ISSN: 0731-7085

https://doi.org/10.1016/j.jpba.2018.07.018

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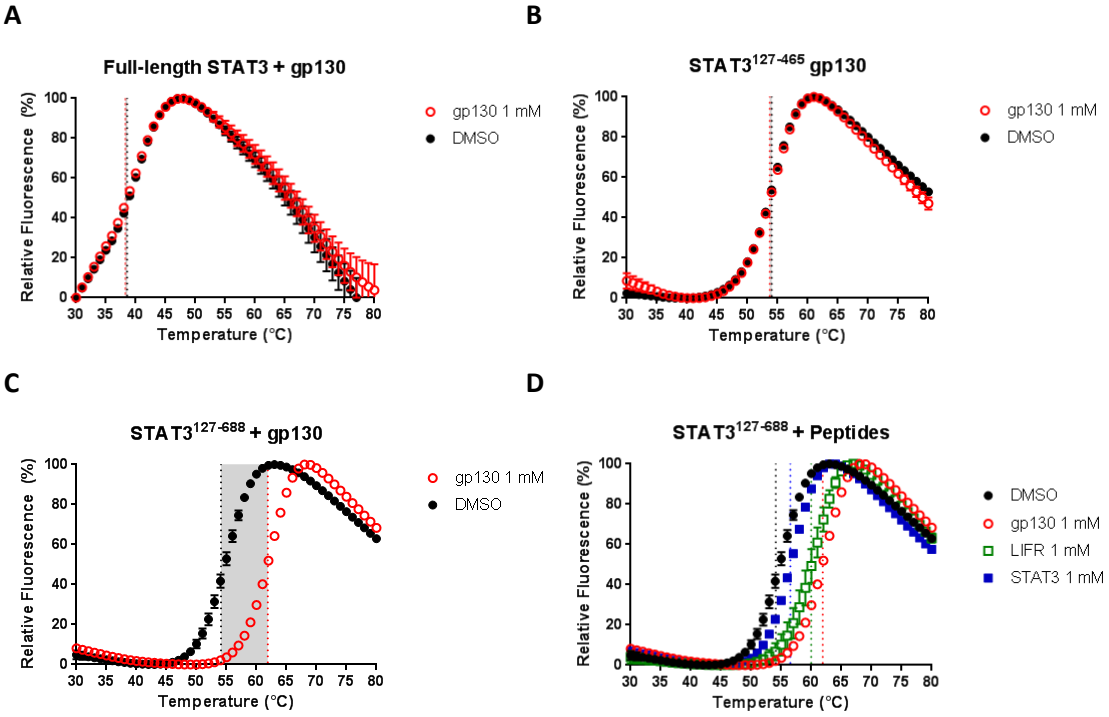
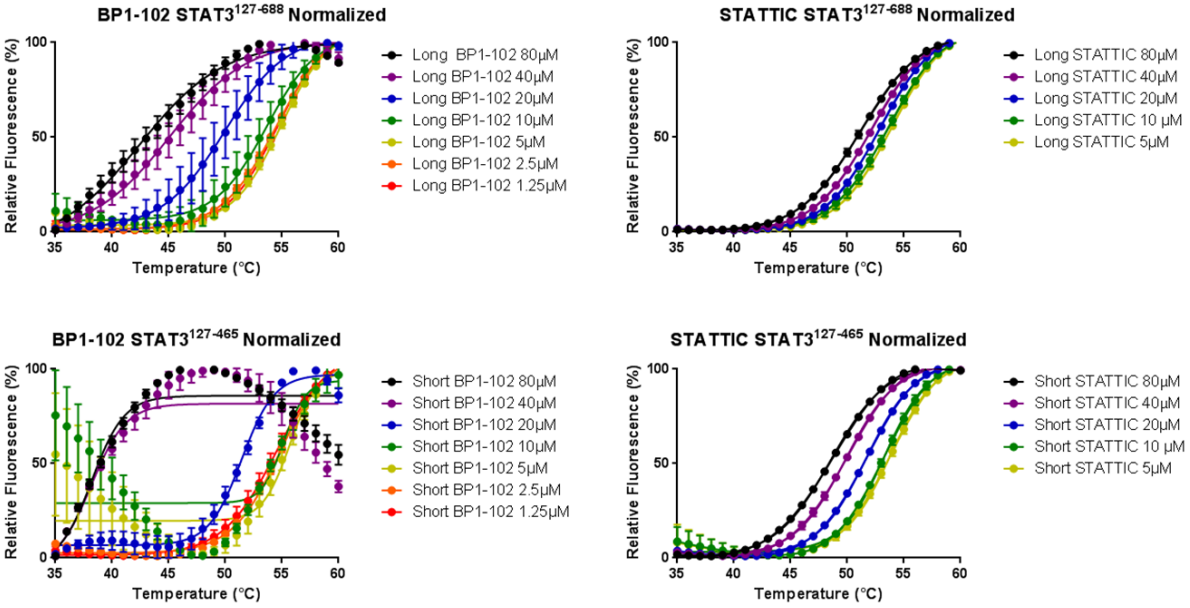
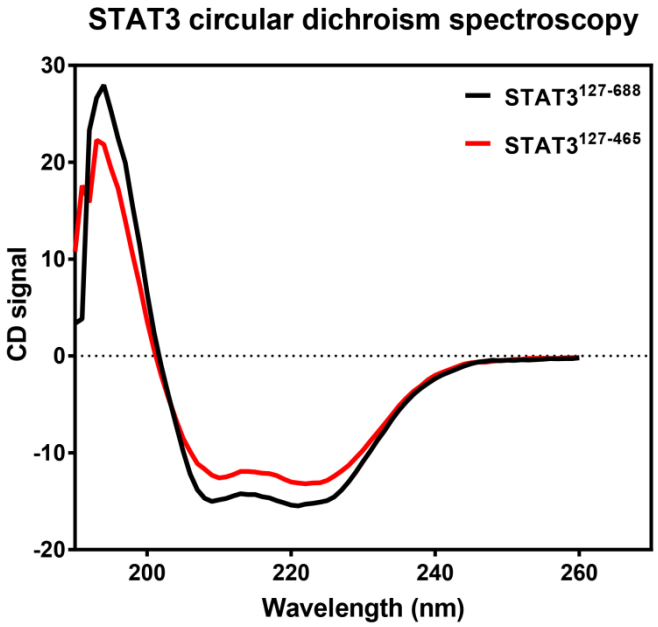
Supplementary Figures



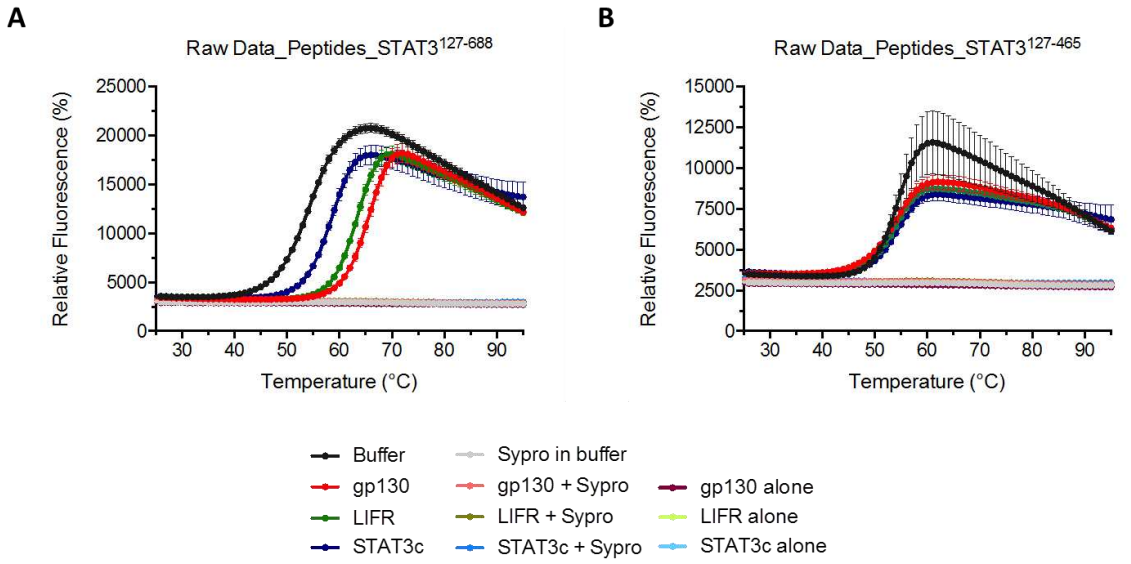
Supplementary Figure 1. Full DSF melting curves for the plots shown in **Figure 3A-C and E**. The plots as shown in **Figure 3** were used to calculate the T_m using a Boltzman sigmoidal curve fit using GraphPad and trimming the data set to 3 points beyond the minimal and maximal fluorescence.



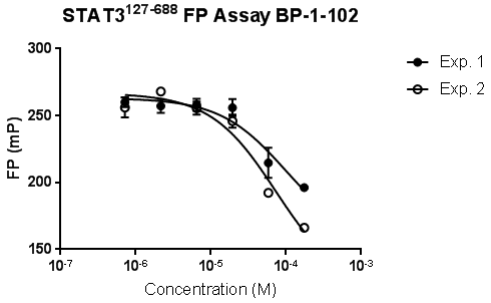
Supplementary Figure 2. DSF melting curves for $STAT3^{127-688}$ (top) and $STAT3^{127-465}$ (bottom) with increasing concentrations of BP1-102 and STATTIC. Dose-dependent decreases in T_m are observed, with more pronounced shifts for $STAT3^{127-465}$.



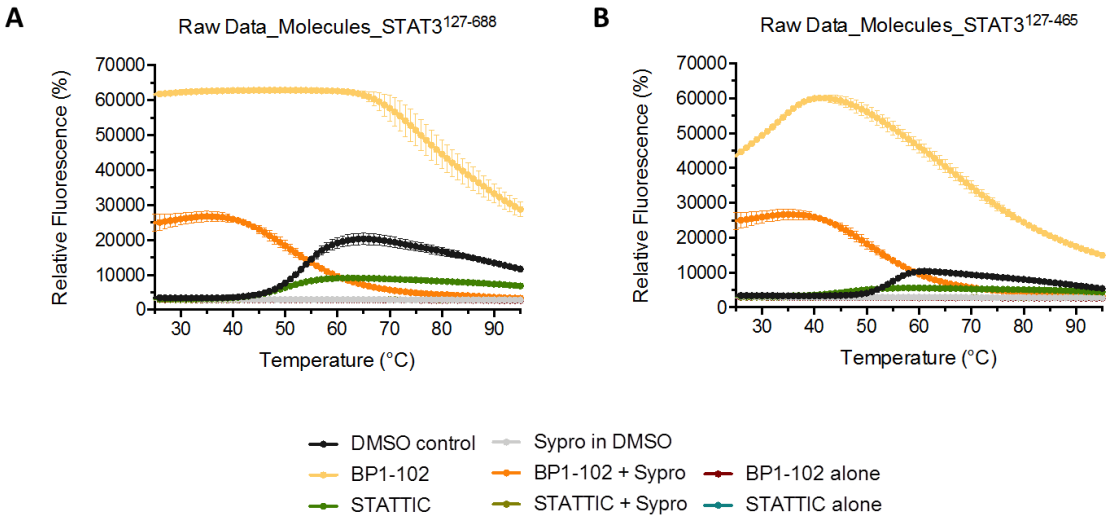
Supplementary Figure 3. Circular dichroism spectra with $STAT3^{127-465}$ and $STAT3^{127-688}$. Both truncated STAT3 variants showed mostly alpha helical secondary structures supporting that these truncations have produced folded proteins and not disordered polypeptides.



Supplementary Figure 4. DSF analysis of STAT3 inhibitor peptides with and without STAT3 variants present. Base-line levels of fluorescence were detected with Sypro Orange™ (sypro) and peptide inhibitors alone which did not increase with temperature. The presence of $STAT3^{127-688}$ (**A**) or $STAT3^{127-465}$ (**B**) is required to generate melting curves. The lack of a temperature-dependent increase in fluorescence without STAT3 present indicates no conflicting interactions should exist between the peptide inhibitors and Sypro Orange™.



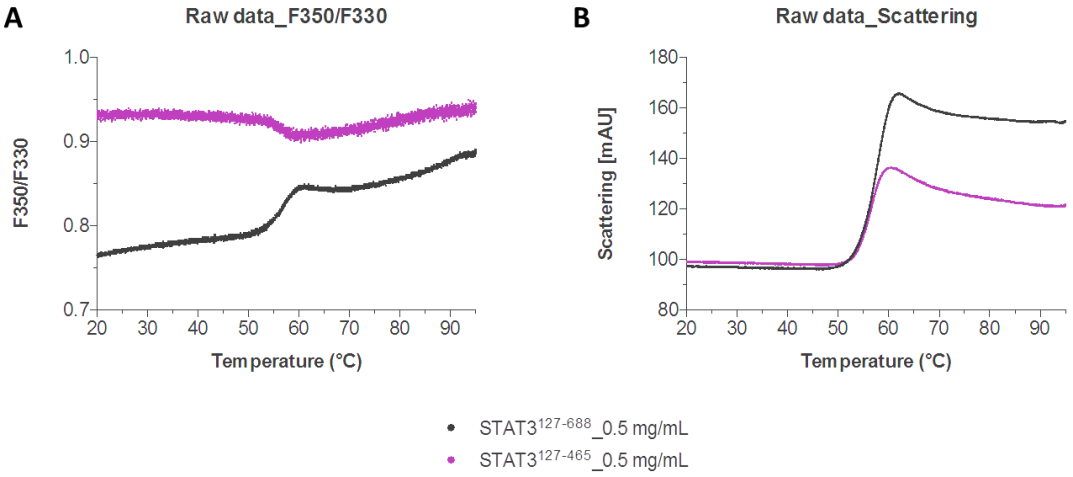
Supplementary Figure 5. FP assay with STAT3$^{127-688}$ and BP1-102, two independent experiments are shown. Treatment with increasing concentrations of BP1-102 led to a dose-dependent decrease in FP signal indicating that STAT3$^{127-688}$ interactions with 5-FAM-gp130 can be disrupted by BP1-102.



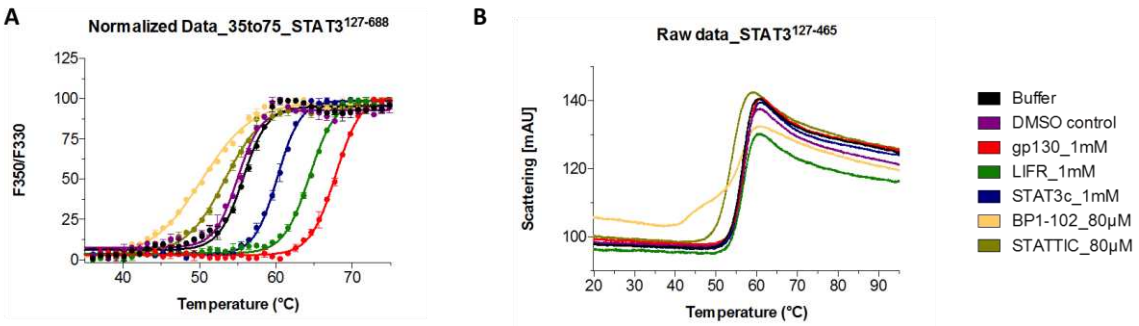
Supplementary Figure 6. DSF analysis of small molecule STAT3 inhibitors with and without STAT3 protein variants. Surprisingly, BP1-102 (80 µM), affected the fluorescence of Sypro Orange™ (Sypro) in a temperature dependent manner. Increased fluorescence was observed when BP1-102 was incubated with Sypro Orange™ which diminished at higher temperatures (shown in orange). When $STAT3^{127-688}$ (**A**) or $STAT3^{127-465}$ (**B**) was added, the fluorescence output was further increased, however, the raw data values show much higher raw fluorescence values than what is typically seen in this assay. STATTIC did not show any interaction when incubated with Sypro Orange™.

A. Melting curve of $STAT3^{127-688}$ measured by DSF upon inhibitor binding. Controls (negative and blank) contained respectively the different inhibitor + Sypro Orange or only the inhibitors or the Sypro Orange. A representative DSF profile from two independent experiments performed in triplicate is shown.

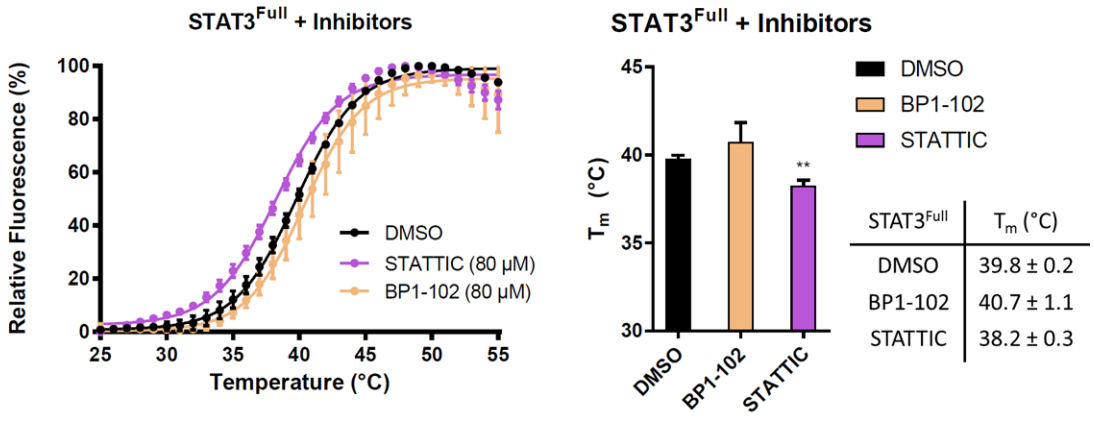
B. Melting curve of $STAT3^{127-465}$ measured by DSF after exposure to the mixtures of the inhibitors + Sypro Orange. Controls (negative and blank) contained respectively the mixtures inhibitor + Sypro Orange or only the inhibitors or the Sypro Orange. A representative DSF profile from two independent experiments performed in triplicate is shown.



Supplementary Figure 7. NanoDSF and nanoDSLS experiments with $STAT3^{127-688}$ and $STAT3^{127-465}$. **A**) Using the ratio of fluorescence at 350/330 nm to track thermal denaturing of truncated STAT3 proteins, $STAT3^{127-688}$ generated the expected melting curve, however, $STAT3^{127-465}$ did not. It is believed that this is an artifact of the low number of Trp residues contained within $STAT3^{127-465}$ which may limit the applicability of this detection method for $STAT3^{127-465}$. **B**) Measuring the thermal aggregation of STAT3 protein variants by scattering afforded the appropriate melting curves for $STAT3^{127-688}$ and $STAT3^{127-465}$. Representative experiments are shown from two independent experiments performed in duplicates.



Supplementary Figure 8. Using nanoDSF and nanoDSLS to track T_m shifts of truncated STAT3 proteins induced by inhibitors. **A**) Confirming the results from **Figures 3** and **4**, nanoDSF experiments confirm $STAT3^{127-688}$ stabilization by peptide STAT3 inhibitors (gp130, LIFR and STAT3 consensus sequence), and that small molecule STAT3 inhibtors BP1-102 and STATTIC destabilize $STAT3^{127-688}$ towards thermal degradation. **B**) Peptide STAT3 inhibitors did not affect the T_m of $STAT3^{127-465}$ measured by nanoDSLS which does not contain the SH2 domain. However, small molecule STAT3 inhibitors also destabilize this shorter truncation suggesting that BP1-102 and Stattic may interact with STAT3 at areas beyond the SH2 domain. Representative experiments are shown from two independent experiments performed in duplicates.



Supplementary Figure 9. Small molecule STAT3 inhibitors BP1-102 and STATTIC fail to stabilize $STAT3^{Full}$ towards thermal degradation in DSF experiments. **A)** A representative melting curve for $STAT3^{Full}$ incubated with small molecule inhibitors. **B)** Summary of two independent DSF experiments showing minor effects of STAT3 inhibitors on the T_m of $STAT3^{Full}$. Interestingly, STATTIC induced statistically significant destabilization of $STAT3^{Full}$, however BP1-102 did not, and instead induced a small stabilization compared to the DMSO control. Similar to STAT3127-688 experiments a high degree of variability was seen with BP1-102 perhaps due to its interaction with Sypro Orange™.