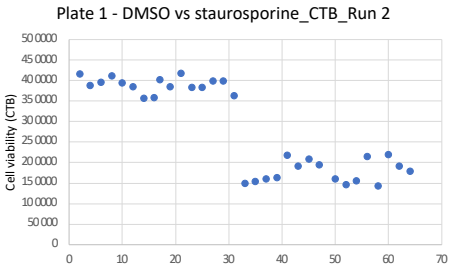
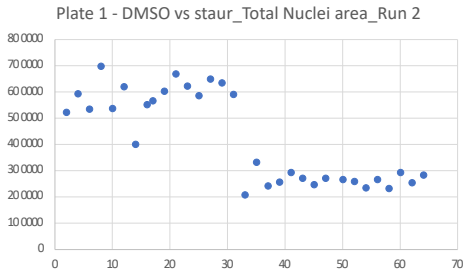
Results/Run 2/ Plate 1 (dmso + library at 0.2µM)
QC Control wells





Zfactor	0.34994241

Zfactor	0.07936598

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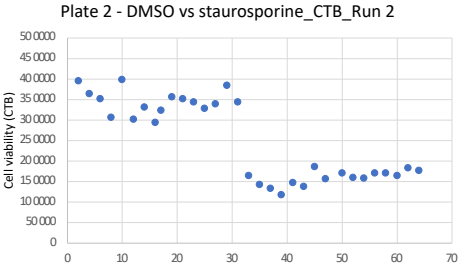
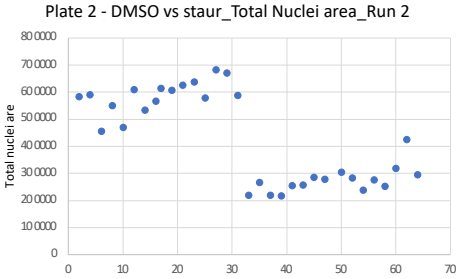
Results/Run 2/ Plate 2 (dmso + library at 2µM)
QC Control wells





Zfactor	0.198704529

Zfactor	-0.08355616

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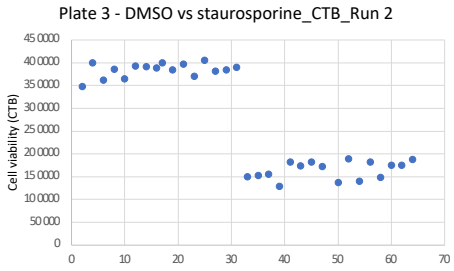
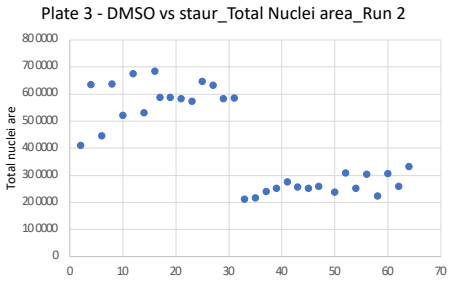
Results/Run 2/ Plate 3 (CBD + library at 0.2µM)
QC Control wells



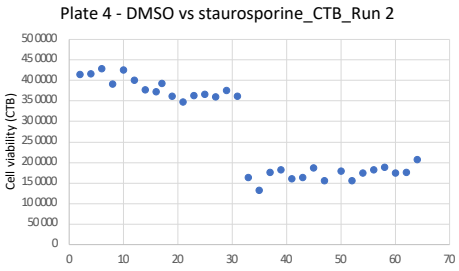


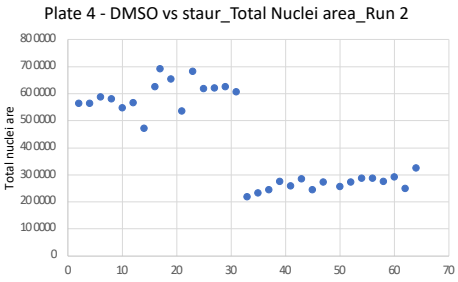
Zfactor	0.51979796

Zfactor	-0.0396623

18

Results/Run 2/ Plate 4 (CBD + library at 2µM)
QC Control wells





Zfactor	0.39309778

Zfactor	0.24674941

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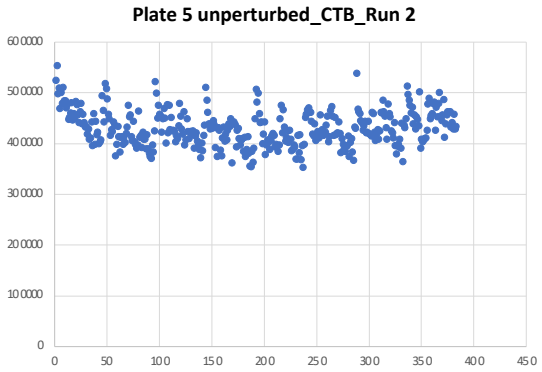
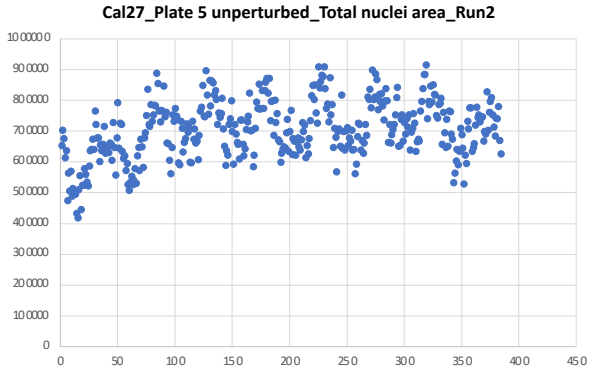
Results/Run 2/ Plate 5(Unperturbed)
QC Control wells

1st plate to add CTB



AVG	430677.125
STD DEV	34643.7958
%CV	8.04402969



AVG	703781.427
STD DEV	93563.0908
%CV	13.2943393

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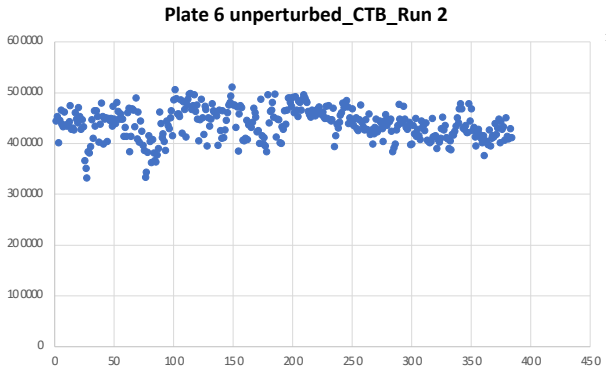
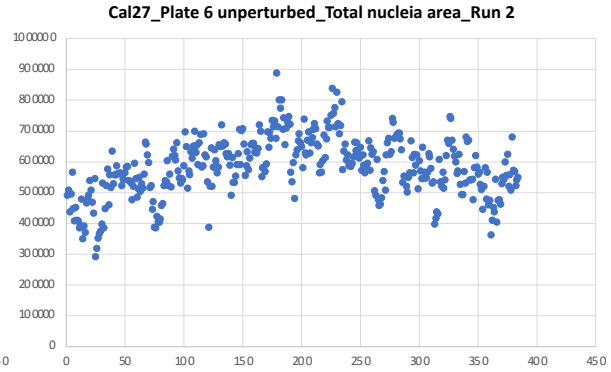
Results/Run 2/ Plate 9 (Unperturbed)
QC Control wells

Last plate to add CTB

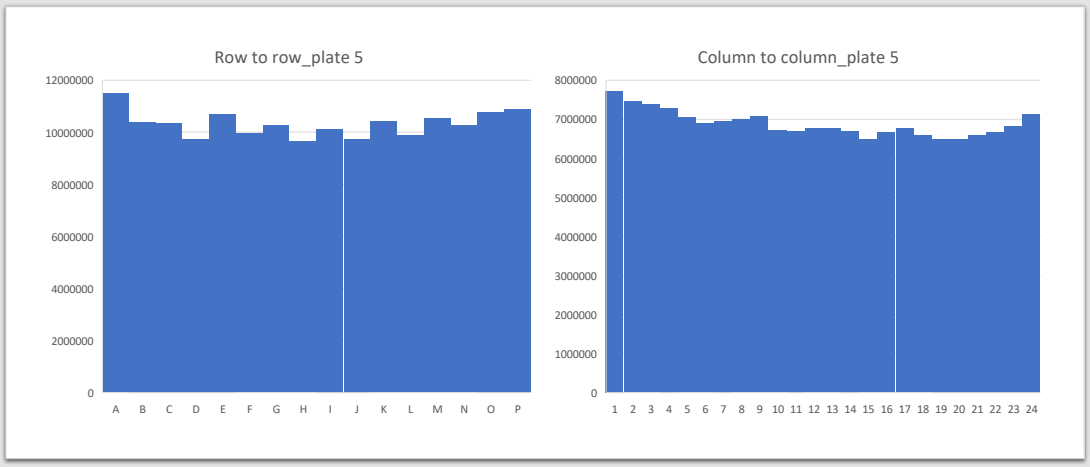


AVG	437723.19
STD DEV	30927.8108
%CV	7.06560939



AVG	581847.201
STD DEV	95858.1036
%CV	16.4747899

21



Row/column variation on unperturbed plates from CTB readout_plate 5

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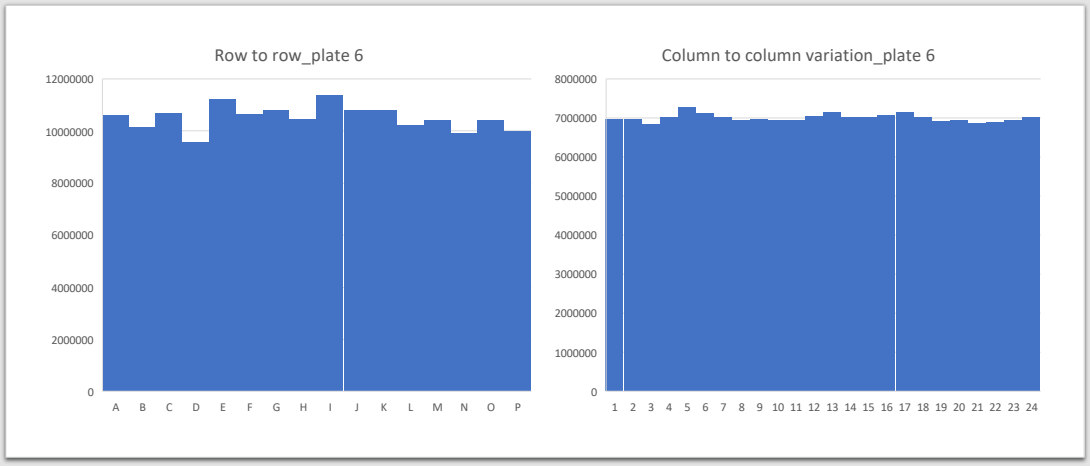

Row/column variation on unperturbed plates from CTB readout_plate 6

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Conclusions

- Z score_variation= we have high variability within the DMSO wells and staurosporine. Possible reasons for this variability:
  - Performance of wellmate = liquid being added on the wall/edges of the wells
  - New 384-well plate = we used Corning 3712 for run 1, and Corning 3764 for run 2 (3764 is supposed to be the replacement for 3712, they have overall the same characteristics, but they look quite different by "eye").
- Z scores_overall= the reason why the separation is not large might be because Cal27 is not as sensitive to staurosporine, as Fadu is. Furthermore, we have drugs within the library that are consistently killing more cells than staurosporine (Bortezomib and dinaciclib).
- Wellmate= the possibility for most of the variability coming from the wellmate are also due:
  - Unperturbed plates showed a clear row-to-row variation
  - Because I noticed liquid in the wall/edges/top of the well after seeding the cells and adding the CTB, I had to spin the plates. This spinning could possibly explain the higher variability in the outter wells
  - To possinly address this, Margret will **replace the tubing cassete for the wellmate**

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New 384-well plates

	Corning® 3712 | 384-Well – RUN 1	Corning® 3764 | 384-Well – RUN 2
Well Bottom	Flat and clear	Flat and clear
Well Volume	112µL	112µL
Recommended Working Volume	20 to 80µL	20 to 80µL
Cell Growth Area	0.06cm2	0.06cm2
Required pin tool adjustment?	N/A	No
Required wellmate adjustment?	N/A	YES

Link for the plates

https://www.capitolscientific.com/Corning-3712-384-Well-x-112L-Clear-Flat-Bottom-Cell-Culture-Microplates-with-Lid-TC-Treated-Bl#:~:text=This%20Corning%C2%AE%20384%2Dwell,volume%20of%2025%20to%2050%C2%B5L.

https://ecatalog.corning.com/life-sciences/b2c/US/en/Microplates/Assay-Microplates/384-Well-Microplates/Corning%C2%AE-384-well-Black-Clear-and-White-Clear-Bottom-Polystyrene-Microplates/p/3764

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