

Profiling Survivin Isoforms During Drug Treatment of Glioblastomas

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Background

Survivin

- Survivin is a protein that promotes cancer cell survival by blocking apoptosis¹
- Salmeterol xinafoate has been shown to be able to downregulate wild-type survivin as a beta2-adrenergic receptor biased agonist favoring the G-protein pathway²
- Not much research has been down to how salmeterol xinafoate affects survivin isoforms (splice variants).

ATF5

- Activating transcription factor 5(ATF5) can upregulate survivin levels³
- Interfering with ATF5 function or expression has been shown to lead to rapid survivin depletion⁴

Questions

1. How does salmeterol xinafoate affect survivin isoforms?
2. How does the absence of a functioning ATF5 and its binding partners affect survivin isoforms?

Hypothesis

1. Salmeterol xinafoate will downregulate anti-apoptotic survivin forms-3B, wild-type and ΔEx3-while upregulating pro-apoptotic survivin isoforms-2B and 2α.
2. A non-functioning ATF5 will result in an downregulation of anti-apoptotic isoforms and an upregulation of pro-apoptotic isoforms

	Vehicle	Salmeterol Xinafoate		PBS(Control)	dn-ATF5
Wild-type survivin	Up	Down	Wild-type survivin	Up	Down
Survivin-3B	Up	Down	Survivin-3B	Up	Down
Survivin-ΔEx3	Up	Down	Survivin-ΔEx3	Up	Down
Survivin-2B	Down	Up	Survivin-2B	Down	Up
Survivin-2α	Down	Up	Survivin-2α	Down	Up

Figure 1)Hypothesis of aim 1

Figure 2)Hypothesis of aim 2

Methods

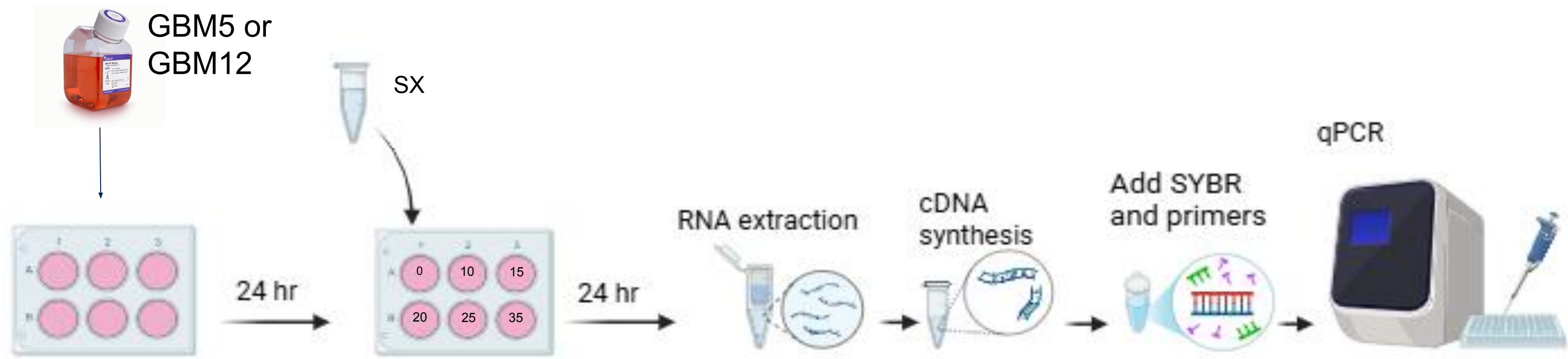


Figure 3. Illustration of experiment 1's methods. GBM12 or GBM5 is plated onto a 6-well plate. After 24 hours, a dose escalation of SX-at 0, 10, 15, 20, 25 and 35 μM-is added. For the well with 0μM, DMSO was used as a control. After 24 hours, RNA is harvested and cDNA is synthesized using High Capacity cDNA Reverse Transcription Kit. SYBR Green, cDNA and primers are added to a 96-well plate and analyzed by a Bio-Rad qPCR machine.

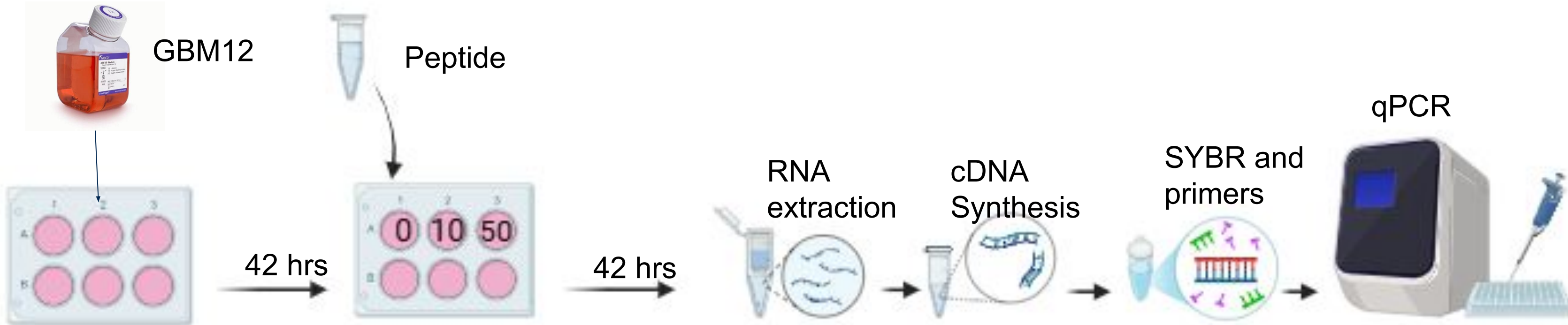


Figure 4) Illustration of experiment 2's methods. GBM12 is plated onto a 6-well plate. After 42 hours, the dn-ATF5 peptide is added in concentrations of 0, 10 and 50μM. After another 42-hours, RNA is extracted and converted to cDNA using High Capacity cDNA Reverse Transcription Kit. SYBR Green, cDNA and primers are added to a 96-well plate and analyzed by a Bio-Rad qPCR machine.

Results

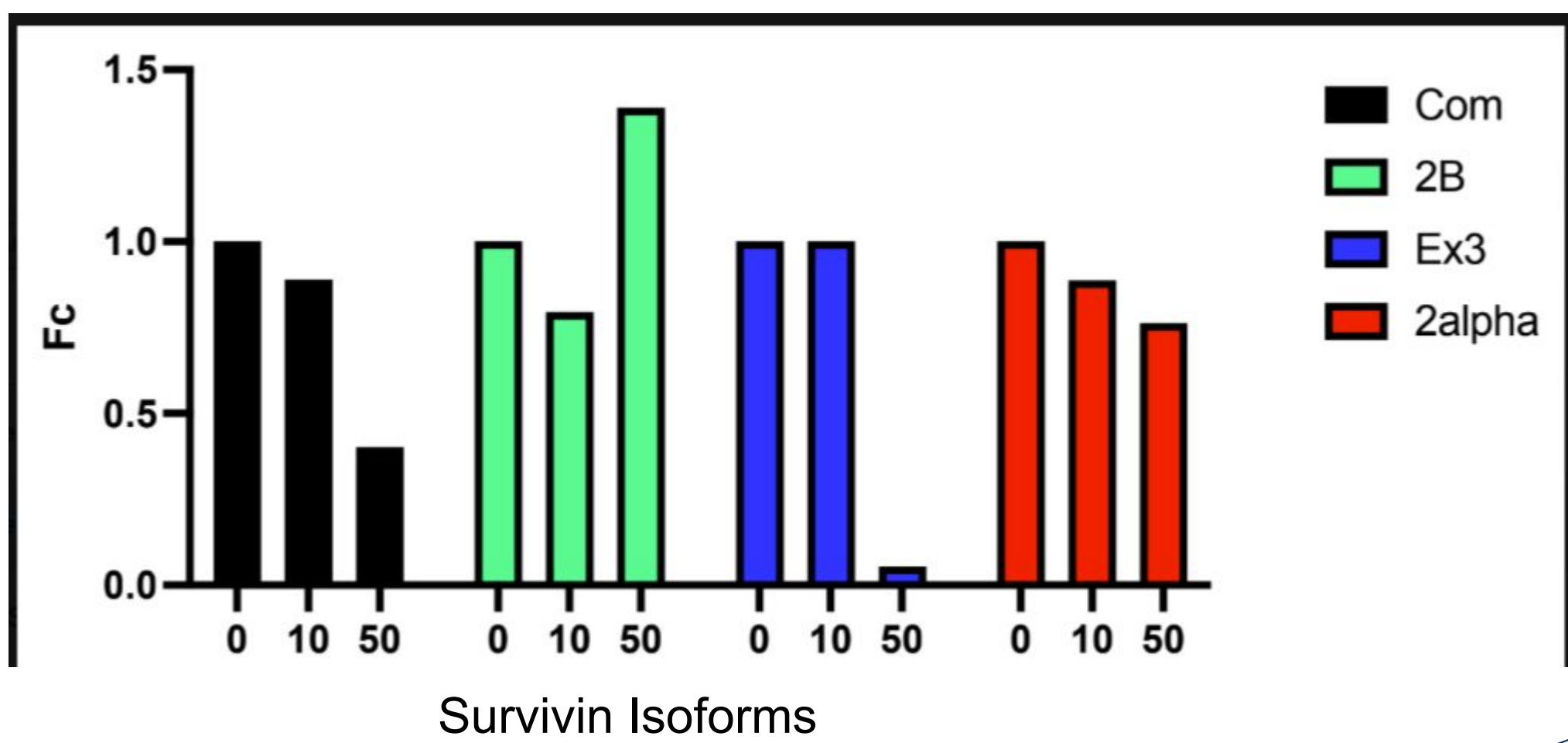


Figure 5) Experiment 2's results using GBM12. 3B was not able to be detected.

References

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- 4.Sears TK, Angelastro JM. The transcription factor ATF5: role in cellular differentiation, stress responses, and cancer. *Oncotarget*. 2017;8(48):84595-84609. doi:10.18632/oncotarget.21102

Figures 1 and 2 created in Google Docs

Figures 3 and 4 created using Biorender

Figure 5, 7 and 7 created using GraphPad Prism 11

Results

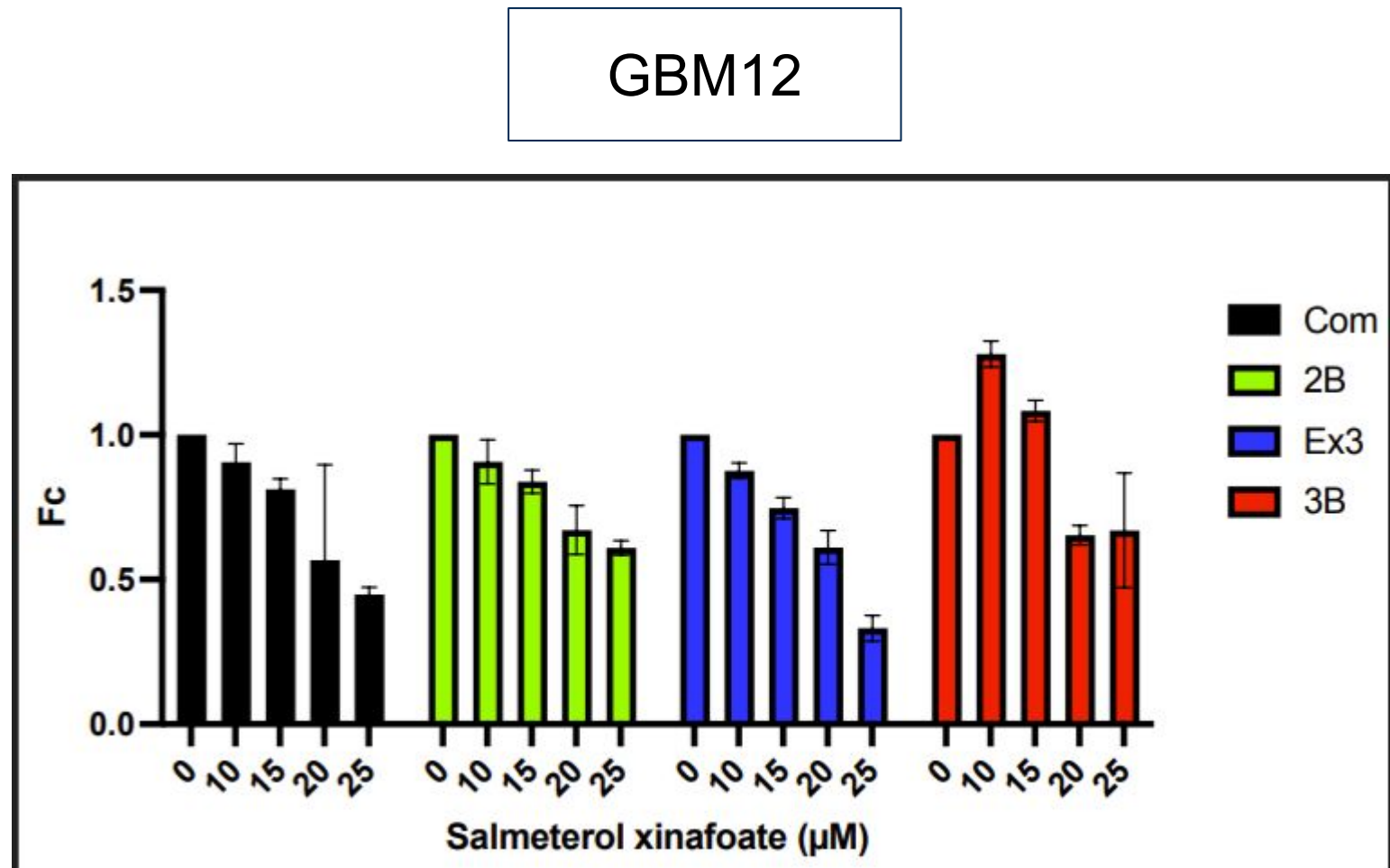


Figure 6) Experiment 1's results using GBM12. Survivin Cq is normalized against 18S Cq. Presented FC is averaged from individual results. 2α was not able to be detected

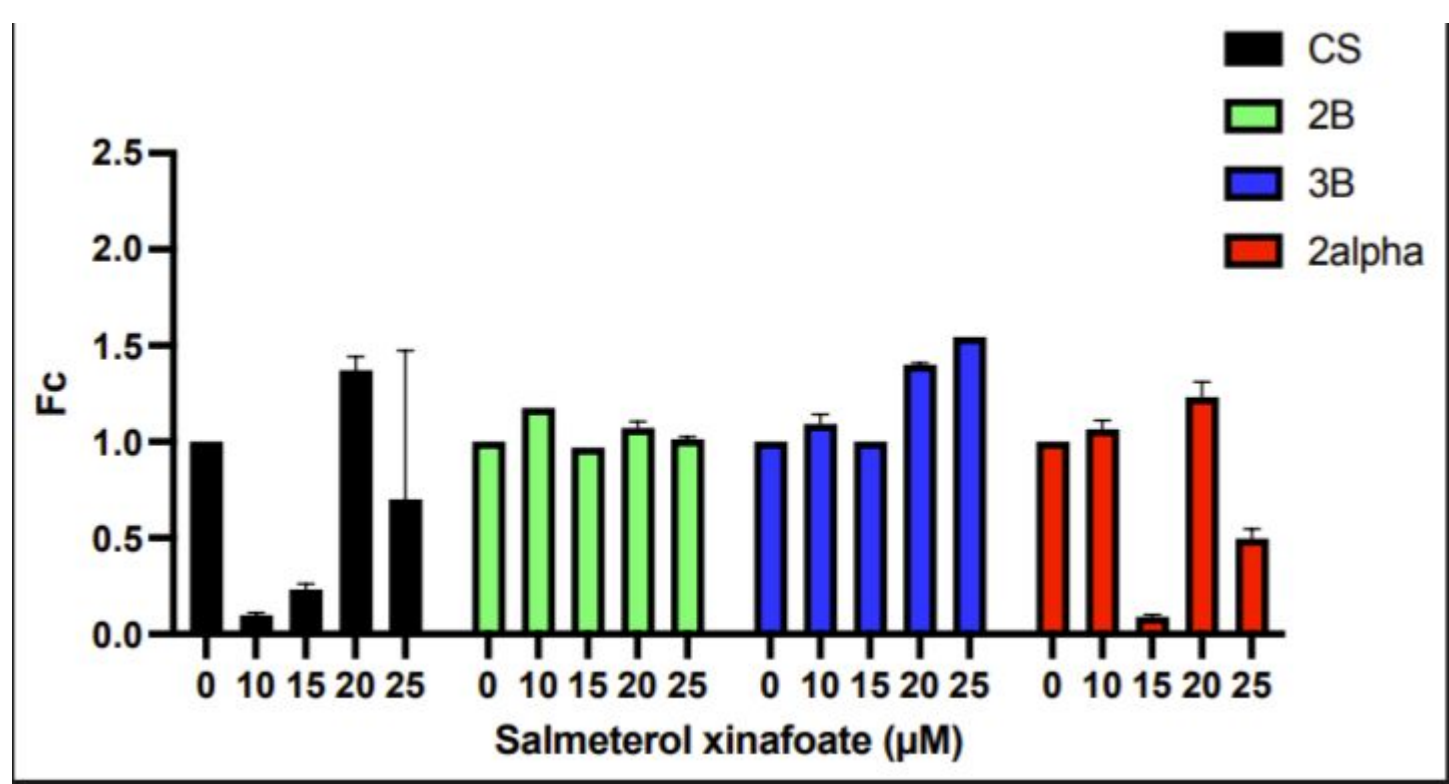


Figure 7) Experiment 1's results using GBM5. Survivin Cq is normalized against 18S Cq. Presented FC is averaged from individual results. ΔEx3 was not able to be detected

Conclusions

- Aim 1:
1. In GBM12, all isoforms decrease as SX dose increases
2. In GBM5, 2B is relatively stable while 3B increases in response to increasing SX doses. All other isoforms decrease in expression level
- Aim 2:
1. Nonfunctioning ATF5 will lead to apoptosis by downregulating pro-apoptotic survivin isoform 2B while decreasing the other isoforms

Next Steps

1. Repeat aim 1's runs with GBM12 and GBM5 more often than 2-3 times
2. For aim 1, investigate why 2B is so much higher than WT-survivin
3. Repeat aim 2's experiment with GBM5
4. Repeat aim 2 with more trials of GBM12

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