**Project:** ROS-specific Huntingtin Interactions

**Experiment:** GFP reactivation assay in wild type and HD cells

**Purpose:** To compare DNA repair rates in wildtype versus HD cells using method by Xu et al (1), second attempt

**Date:** 2017-09-07

Seeding

Seeded and transfected simultaneously.

Seeded 1 x 6-well plate each with STHdh Q7/Q7 (500 uL/10 mL from 80% 10-cm) or STHdh Q111/Q111 (500 uL/10 mL from 100% 10-cm). Next day cells were 75% confluent.

Plasmid Oxidation

Combined 40 uL (40 ug) of pmaxGFP (fresh maxi prep) with 356 uL H2O and 4 uL of 1 mM methylene blue for a final MB concentration of 10 uM.

Aliquoted 200 uL into two 35-mm dish, irradiated one with visible light by placing 18 cm from a 100 W bulb for 45 min (+VL). Wrapped the other in foil to protect from light (-VL). Column purified using 2 columns each (PCR clean up protocol) and eluted in 60 uL warm TE. Combined two elutions and measured concentration; 90 ng/uL (total of 10.8 ug, therefore 50% recovery of original 20 ug) for both samples.

Transfection

Prepared enough for 2 transfections per condition:

|  |  |  |  |
| --- | --- | --- | --- |
|  | **DNA 1X** | **DNA 2X** | **Turbofect 2X** |
| **No transfection** | 1 ug sonicated salmon sperm DNA | 2 ug SSS DNA | 4 uL |
| **GFP only** | 0.5 ug -VL GFP  0.5 ug SSS DNA | 1 ug -VL GFP  1 ug SSS DNA | 4 uL |
| **RFP only** | 0.5 ug miRFP-670  0.5 ug SSS DNS | 1 ug miRFP-670  1 ug SSS DNA | 4 uL |
| **No VL** | 0.5 ug -VL GFP  0.5 ug miRFP-670 | 1 ug -VL GFP  1 ug miRFP-670 | 4 uL |
| **VL** | 0.5 ug +VL GFP  0.5 ug miRFP-670 | 1 ug +VL GFP  1 ug miRFP-670 | 4 uL |
| **VL+Vel** | 0.5 ug +VL GFP  0.5 ug miRFP-670 | 1 ug +VL GFP  1 ug miRFP-670 | 4 uL |

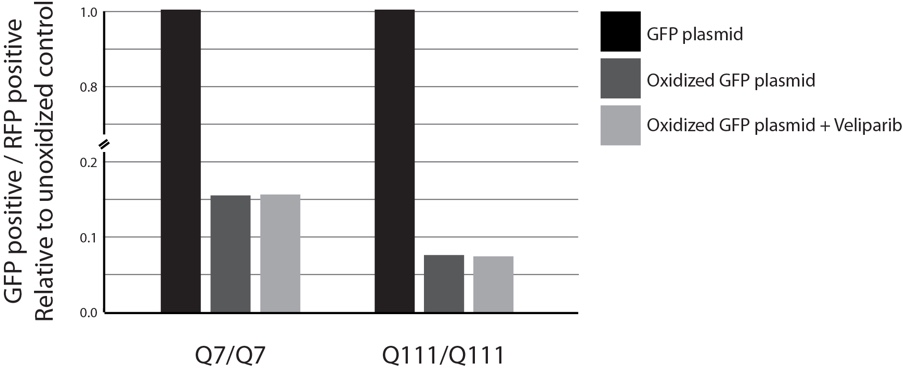
Vortexed, incubated 15 minutes. Added 100 uL dropwise to each well. Included 50 uM Veliparib in one + VL well. Incubated at 33deg.

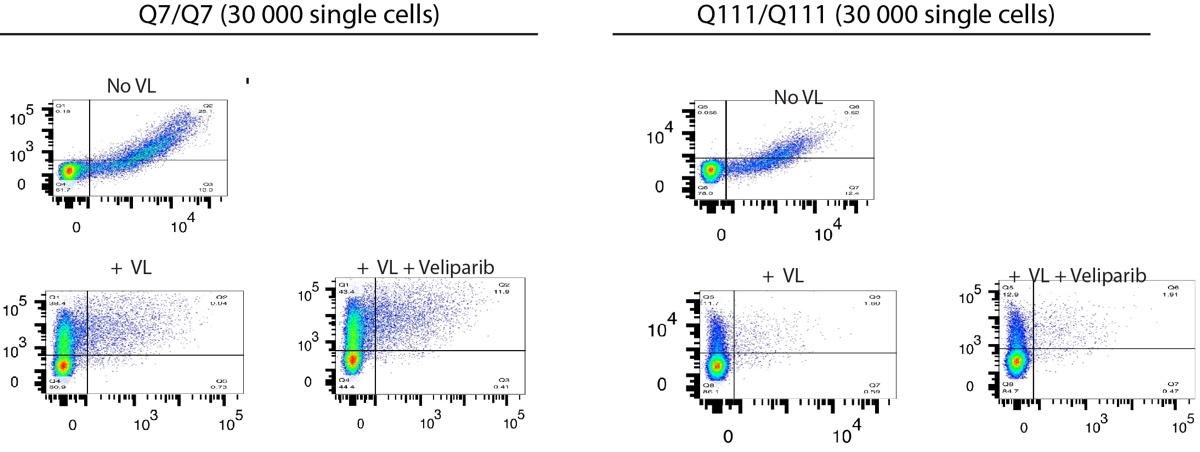
Replaced media after 24 and 48 hours -/+ 50 uM veliparib.

Results 72 hours

Harvested cells by trypsinization, washed with cold PBS, and resuspended in 500 uL FACS buffer.

Scored 30 000 cells per condition.





Conclusion

* Repair rate in Q111s is approximately half that of Q7s
* 50 uM veliparib has no effect

References

1. Xu, Zhu, Lei Zhang, Wenjun Zhang, Du Meng, Hongxia Zhang, Ying Jiang, Xiaojun Xu, et al. 2015. “SIRT6 Rescues the Age Related Decline in Base Excision Repair in a PARP1-Dependent Manner.” Cell Cycle 14 (2): 269–76. doi:10.4161/15384101.2014.980641.