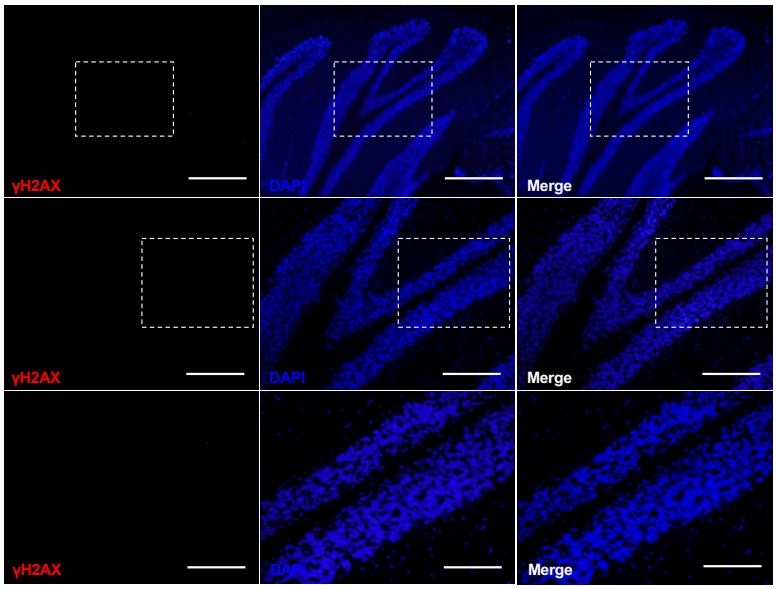
**HDAC1 Dysregulation Induces Aberrant Cell Cycle and DNA Damage in Progress of TDP-43 Proteinopathies**

Cheng-Chun Wu, Lee-Way Jin, I-Fang Wang, Wei-Yen Wei, Pei-Chuan Ho, Yu-Chih Liu and Kuen-Jer Tsai

# Appendix

**Appendix Fig. S1**




**Appendix Figure S1. Staining of γH2AX and DAPI in the cerebellum of 12-month-old FTLD-TDP Tg mice.**

Representative IF staining of γH2AX and DAPI in the cerebellum of 12-month-old FTLD-TDP Tg mice. At this time point, we cannot detect any γH2AX immunoreactive cells. Upper graph, scale bar: 500 μm. The circled area is emphasized for showing the distribution of immunoreactivity in cell subregions. Middle graph, scale bar: 200 μm, lower graph, scale bar : 100 μm. n= 4 sections per mouse, N = 5 mice per group.

## Appendix Supplementary Method

### Nuclear HDACs activity assay in cell line

For *in vitro* HDACs activity tests, SHSY5Y cells were cultured in DMEM/F12 medium (Thermo, catalog NO. 11320033) with 10% FBS (Bioindustry, 04-001-1A-US) and were induced to differentiate by neural basal medium (Thermo, catalog NO. 21103049) containing 2% of B27 supplements (Thermo, catalog NO. 17504044), 10 μM retinoid acid (Sigma, R2625), and 1% FBS for 5 days, the medium was replaced every day. For compound administration, 1, 10, 50 μM of compound 5104434 were treated into differentiated SH-SY5Y cells for 72 hour, the 5104434 contained medium medium was replaced every day. For the activity assays, the compound 5104434 treated cells were subjected to nuclear extraction following the manual instructions. The activity of HDAC1, 2, 3, 8 were evaluated by using the activity assay kit (Enzo Life Sciences, BML-AK500-0001, BML-AK512, BML-AK531, BML-AK518), total 30 μg of nuclear protein from each sample was inputted for the assay.

## Appendix Table 1. List of P value of figure 1C; 6B; 7D and EV4

### Fig. 1C

PCR

| WT V.S. Tg | | p-value | |
|---|---|---|---|
| | E2F1 | < 0.0001 | **** |
| | Cyclin E | 0.025439821 | * |
| | PCNA | 0.013657367 | * |
| | p21 | 0.000533315 | *** |
| Western blot | | | |
| WT V.S. Tg | | p-value | |
| | E2F1 | 0.004175469 | ** |
| | Cyclin A | 0.036820881 | * |
| | PCNA | 0.00323307 | ** |
| | p21 | < 0.0001 | **** |
| | γH2AX | 0.001980008 | ** |

### Fig. 6B

| | | p-value | |
|---|---|---|---|
| trial3 | Tg+Vehicle vs. WT+Vehicle | 0.0004 | *** |
| trial4 | Tg+Vehicle vs. WT+Vehicle | <0.0001 | **** |
| | Tg+Vehicle vs. Tg+5104434 | 0.0199 | # |
| trial5 | Tg+Vehicle vs. WT+Vehicle | <0.0001 | **** |
| | Tg+Vehicle vs. Tg+5104434 | < 0.0001 | #### |
| trial6 | Tg+Vehicle vs. WT+Vehicle | <0.0001 | **** |
| | Tg+Vehicle vs. Tg+5104434 | < 0.0001 | #### |

### Fig. 7D

| WT V.S. Tg | | p-value | |
|---|---|---|---|
| | E2F1 | < 0.0001 | **** |
| | PCNA | 0.0019 | ** |
| | p21 | < 0.0001 | **** |
| | γH2AX | < 0.0001 | **** |
| Tg V.S. Tg+5104434 | | | |
| | E2F1 | < 0.0001 | **** |
| | PCNA | 0.0972 | |
| | p21 | < 0.0001 | **** |
| | γH2AX | 0.0005 | *** |

### Fig. EV4B

| | | P value | |
|---|---|---|---|
| trial3 | *WT_veh vs Tg_Veh* | <0.0001 | **** |
| trial4 | *WT_veh vs Tg_Veh* | <0.0001 | **** |
| trial5 | *WT_veh vs Tg_Veh* | <0.0001 | **** |
| trial6 | *WT_veh vs Tg_Veh* | <0.0001 | **** |
| trial4 | *Tg_veh vs Tg_6mg* | 0.0438 | # |

| | | | |
|---|---|---|---|
| trial5 | *Tg_veh vs Tg_6mg* | 0.0381 | # |
| trial6 | *Tg_veh vs Tg_6mg* | 0.0001 | ### |
| trial5 | *Tg_veh vs Tg_30mg* | 0.0489 | @ |
| trial6 | *Tg_veh vs Tg_30mg* | 0.001 | @@@ |

**Fig. EV4D**

| | | | |
|---|---|---|---|
| EV4D-1 | EV4D-1 | P value | |
| | *WT_veh vs Tg_Veh* | <0.0001 | **** |
| | *Tg_veh vs Tg_6mg* | 0.0041 | ** |
| | *Tg_veh vs Tg_30mg* | 0.0006 | *** |
| EV4D-2 | EV4D-2 | P value | |
| | *WT_veh vs Tg_Veh* | <0.0001 | **** |
| | *Tg_veh vs Tg_6mg* | 0.0355 | * |
| | *Tg_veh vs Tg_30mg* | 0.0091 | ** |

**Fig. EV4E**

| | | | |
|---|---|---|---|
| | | P value | |
| | *WT_veh vs Tg_Veh* | 0.0002 | *** |
| | *Tg_veh vs Tg_6mg* | 0.0119 | * |
| | *Tg_veh vs Tg_30mg* | 0.0055 | ** |