Supplemental Table 1. i3Neuron CAG>mtTagGFP2 lentivirus MOI titration day 7 results for 7,500 cells per well cell density

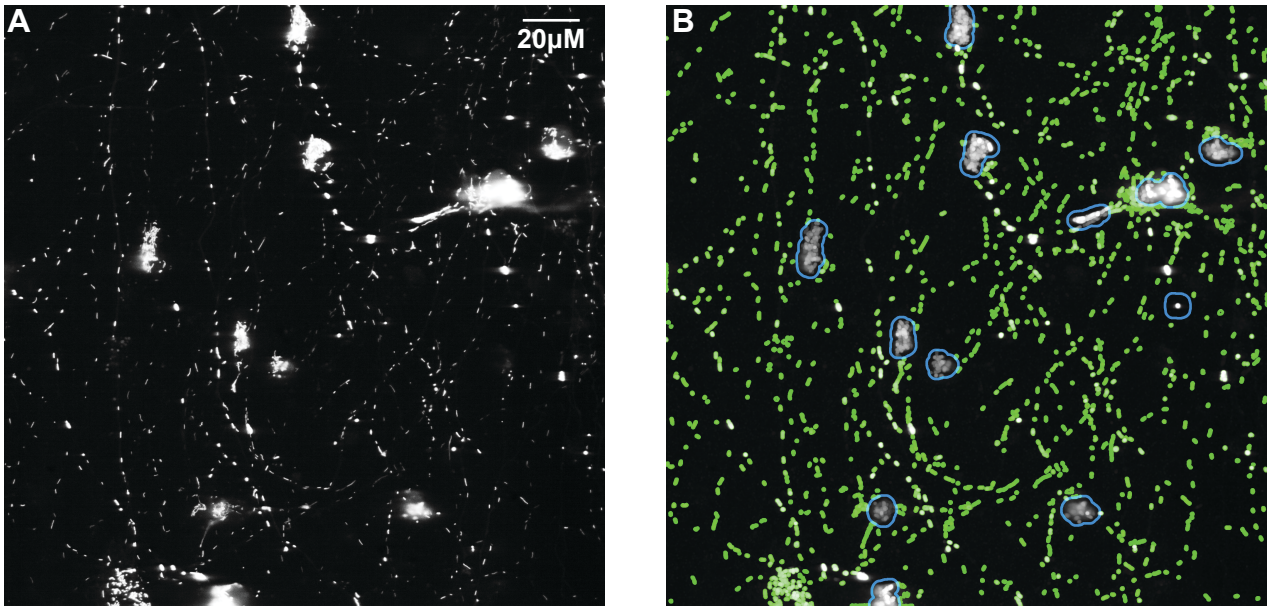
parameter	MOI	day	average*	SEM	ANOVA**
cell body count	1	7	1.0	0.4	NS
cell body count	3	7	2.3	0.2	NS
cell body count	5	7	2.3	0.4	
cell body count	7	7	3.9	0.3	p value = 0.0149
cell body count	10	7	4.6	0.4	p value = 0.0005
CA	1	7	28	8	p value < 0.0001
CA	3	7	118	8	NS
CA	5	7	172	16	
CA	7	7	231	24	NS
CA	10	7	308	22	p value < 0.0001
mito count	1	7	27	8	p value = 0.0137
mito count	3	7	108	7	NS
mito count	5	7	158	15	
mito count	7	7	214	24	NS
mito count	10	7	281	21	p value = 0.0001
median circularity	1	7	0.63	0.162	NS
median circularity	3	7	0.81	0.009	
median circularity	5	7	0.79	0.011	NS
median circularity	7	7	0.80	0.009	NS
median circularity	10	7	0.79	0.007	NS
median length	1	7	0.94	0.24	p value = 0.0050
median length	3	7	1.51	0.02	NS
median length	5	7	1.55	0.04	
median length	7	7	1.51	0.03	NS
median length	10	7	1.55	0.02	NS

CA = sum of the total mitochondrial area; NS = not significant; MOI = multiplicity of infection. *4 fields were imaged per well and 7 independent wells were tested per MOI; field data was collapsed into an average well value for each variable tested; the 7 well values were averaged together to provide the data in the table above. **ANOVA results illustrate whether the parameter value at a particular MOI was significantly different from that same parameter value at a MOI=5.

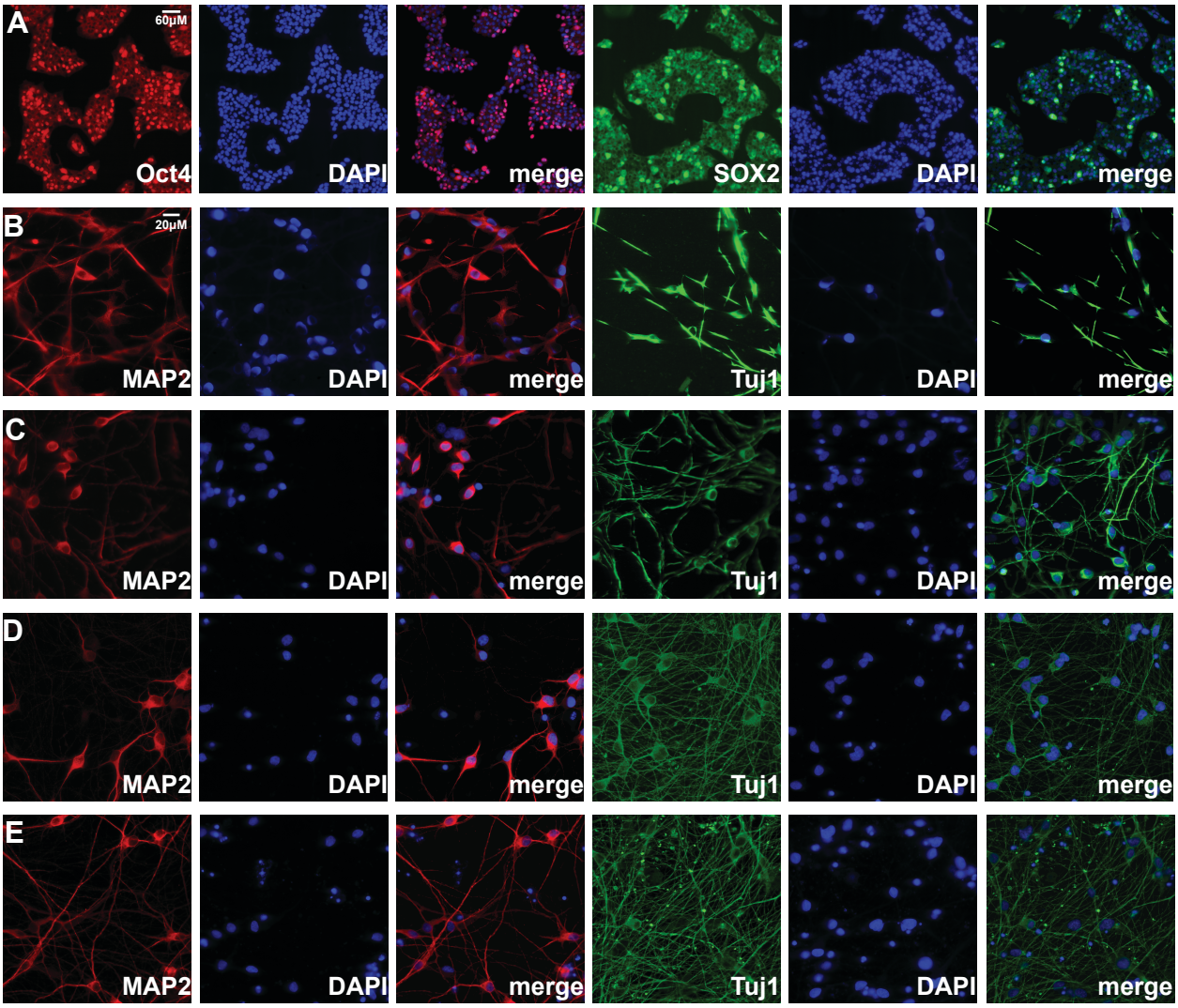
Supplemental Table 2. i3Neuron CAG>mtTagGFP2 lentivirus MOI titration day 8 results for 7,500 cells per well cell density

parameter	MOI	day	average*	SEM	ANOVA**
cell body count	1	8	0.9	0.4	p value = 0.0386
cell body count	3	8	2.7	0.2	NS
cell body count	5	8	2.8	0.6	
cell body count	7	8	3.7	0.3	NS
cell body count	10	8	5.2	0.6	p value = 0.0050
CA	1	8	30	9	p value < 0.0001
CA	3	8	147	7	NS
CA	5	8	204	12	
CA	7	8	298	32	p value = 0.0264
CA	10	8	411	30	p value < 0.0001
mito count	1	8	29	9	p value = 0.0050
mito count	3	8	138	6	NS
mito count	5	8	199	16	
mito count	7	8	289	32	p value = 0.0421
mito count	10	8	386	30	p value < 0.0001
median circularity	1	8	0.58	0.15	NS
median circularity	3	8	0.80	0.01	NS
median circularity	5	8	0.81	0.01	
median circularity	7	8	0.80	0.01	NS
median circularity	10	8	0.79	0.01	NS
median length	1	8	1.02	0.27	NS
median length	3	8	1.52	0.04	NS
median length	5	8	1.52	0.04	
median length	7	8	1.51	0.03	NS
median length	10	8	1.54	0.03	NS

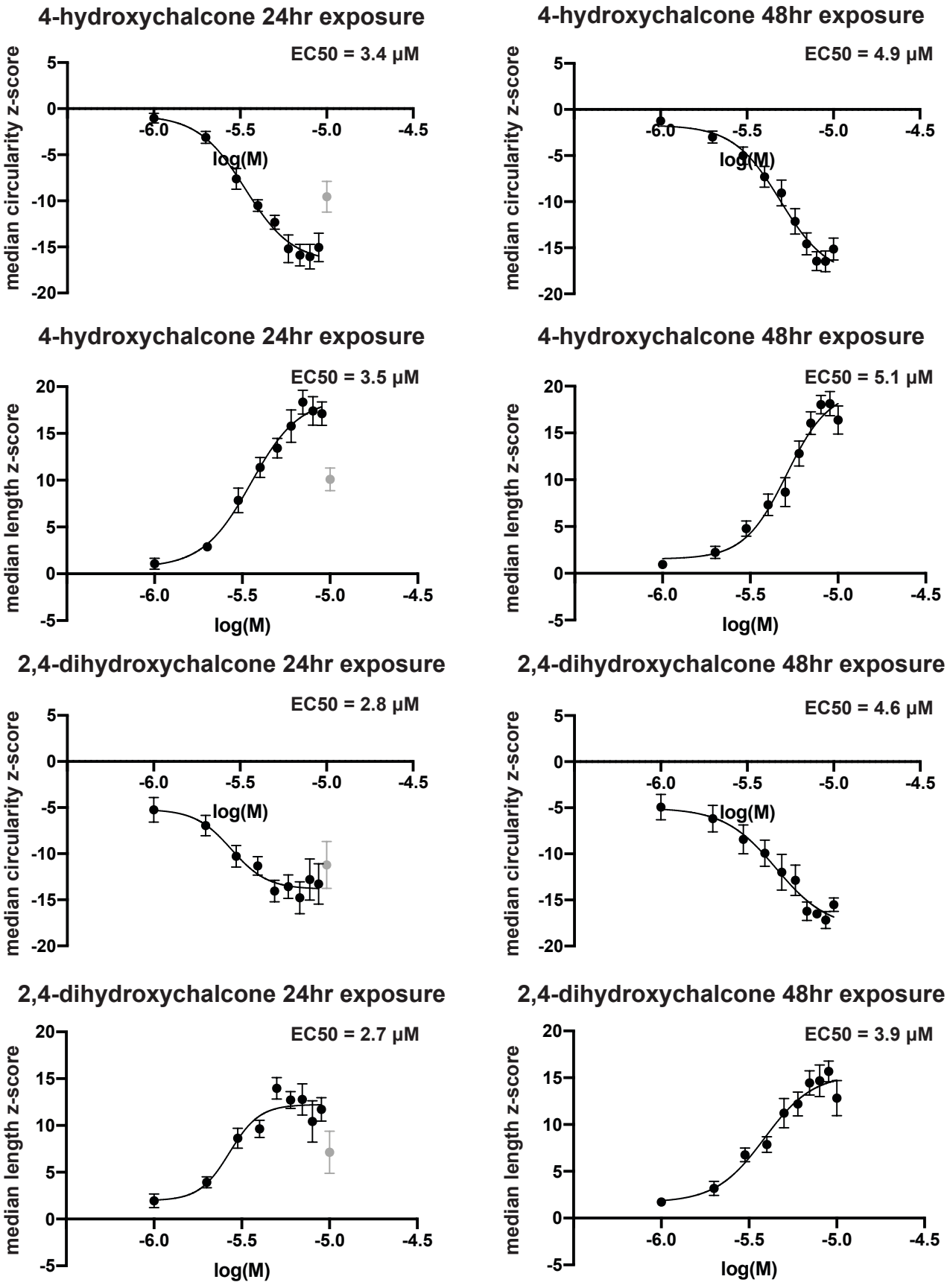
CA = sum of the total mitochondrial area; NS = not significant; MOI = multiplicity of infection. *4 fields were imaged per well and 7 independent wells were tested per MOI; field data was collapsed into an average well value for each variable tested; the 7 well values were averaged together to provide the data in the table above. **ANOVA results illustrate whether the parameter value at a particular MOI was significantly different from that same parameter value at a MOI=5.



supplemental figure 1



supplemental figure 2



supplemental figure 3

Supplemental figure 1. I3Neuron projection image segmentation**. A)** 60X projection image of i3Neurons plated at a density of 15,000 cells per well and transduced at a MOI=5 with lentivirus to label mitochondria with a fluorescent marker. These i3Neurons were not subjected to any type of compound treatment. The projection image was made by collapsing images of three z-dimensional slices. **B)** The same 60X projection image after segmentation for mitochondria contained within i3Neuron neuritic projections. Mitochondria within the somas of the i3Neurons (surrounded by blue circles) are excluded from analysis since accurate calls regarding length and circularity cannot be made from these overlapping organelles. However, the number of blue circles is counted for each field as the parameter: cell body count. Green highlighted mitochondria indicate those mitochondria that are included in the analysis pipeline. From these green highlighted mitochondria, the following parameters are calculated for each field: mitochondrial count, sum of the total mitochondria area (CA), the median circularity of the mitochondria, and the median length of the mitochondria.

Supplemental figure 2. Immunocytochemistry of iPSC and i3Neurons. **A)** iPSCs were dissociated into single cells and plated at a density of 24,000 cells per well of a 96 well plate. Two days later, pluripotency markers Oct4 and Sox2 primary antibodies were added and images were captured using a 20X objective. Pluripotent markers are visible in the iPSC cultures. Neuronal markers of MAP2 and Tuj1 (beta-III tubulin) were also used during this analysis; however, staining for these markers was not obviously different from background (not shown). **B)** iPSCs were dissociated into single cells and exposed to doxycycline for 3 days to promote differentiation. At this point (cell day 3), neuronal markers MAP2 and Tuj1 were added to the i3Neurons and images were captured using a 60X objective. After only 3 days of differentiation, the i3Neurons express typical neuronal makers of MAP2 and Tuj1. **C)** i3Neurons that were previously differentiated for 3 days and cryopreserved were revived and plated on PLO laminin coated 384 well plates. After 4 days of growth (cell day 7), the cells were subjected to ICC experiments using neuronal markers. **D)** Images of i3Neurons at cell day 10 using the same markers. **E)** i3Neurons were co-cultured with human astrocytes for 25 days (cell day 28) and then subjected to ICC experiments as described. Neuronal markers MAP2 and Tuj1 continue to be expressed at i3Neuron cell days 7, 10 and 28.

Supplemental figure 3. Dose response curves for mitochondrial median circularity and length using 4-hydroxychalcone and 2,4-dihydroxychalcone. Each compound was assayed over a concentration range from 1 to 10 μM on two duplicate plates as described in the Materials and Methods; however, a different batch of i3Neurons was used for these experiments than what was used in the main manuscript. Robust z-scores were calculated for mitochondrial median circularity and length values of the neuritic mitochondria for each well and were plotted against the $\log_{10}$ of the molar concentrations of the compounds. Each point on the plot represents the robust z-score average$\pm$SEM of 8 independent wells between the two independent plates. As can been seen, different batches of i3Neurons can tolerate high concentrations of each compound differently. In the i3Neuron batch used in the main manuscript, high concentrations of compound caused toxicity in the wells as illustrated by increased and/or decreased z-scores (light grey points) where appropriate. Whereas, in this batch of i3Neurons, few points had to be

excluded from the dose response curves. However, whether or not toxicity occurred from high concentrations of compound, the EC_{50} values for the compounds at each time point and parameter remained fairly constant illustrating the reproducibility of this assay from batch to batch.