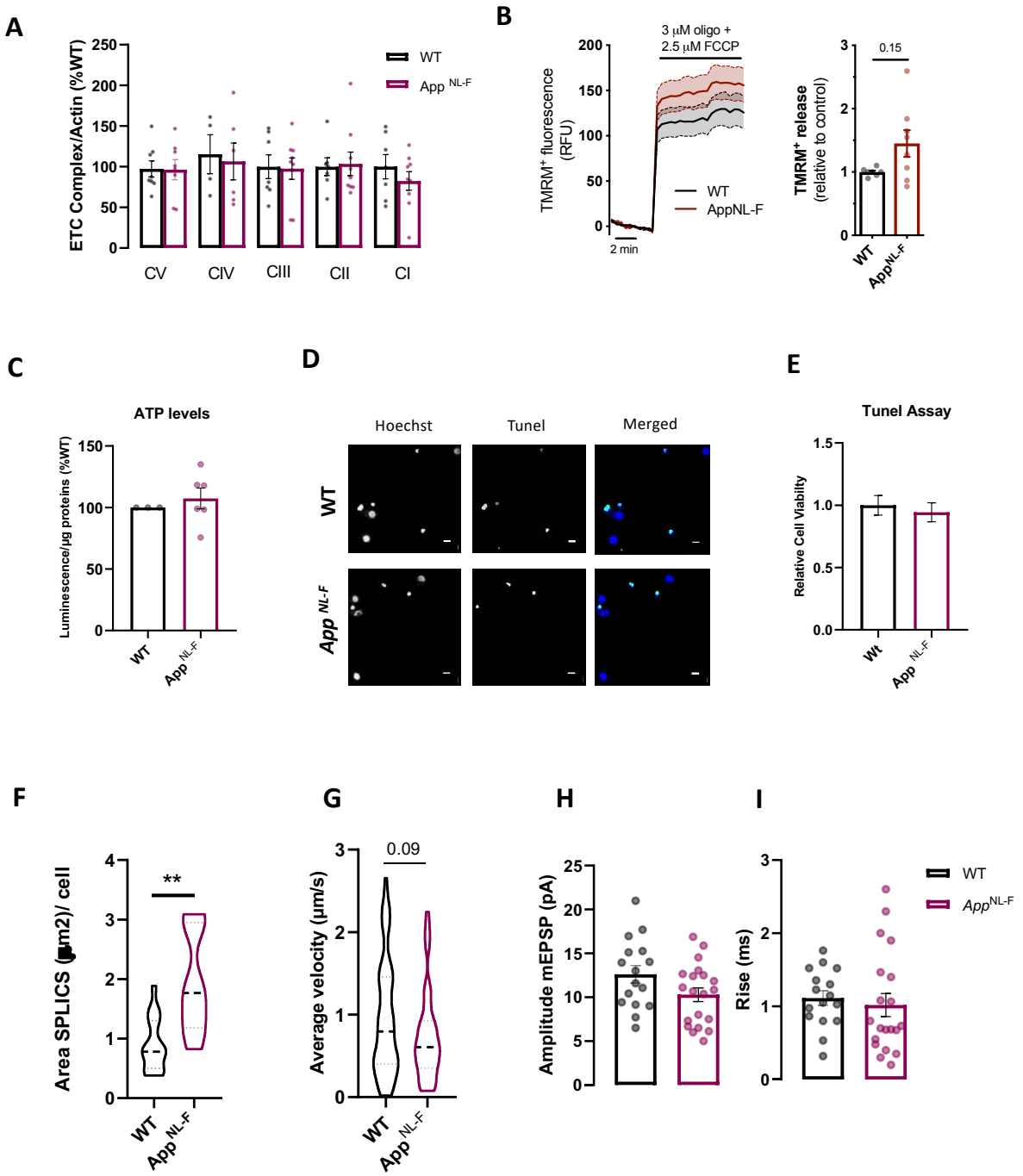
Supplementary Material

Mitochondrial Alterations in Neurons Derived from the Murine *App*$^{NL-F}$ Knock-In Model of Alzheimer's Disease

Supplementary Figure 1. A) Bar graphs show quantification of OXPHOS cocktail blots from WT and *App*$^{NL-F}$ (n=7-9 independent cultures) from Fig 2D. B) Representative traces of TMRM release upon mitochondrial depolarization with 3.2 µM oligomycin and 2.5 µM FCCP. C) Bar graph shows quantification of ATP luminescence normalized to protein content in WT or *App*$^{NL-F}$ cultures; 5 µM AA was used as a negative control by incubating non treated cells for 1 h prior to analysis (n=3-6 independent cultures) and data normalized to protein concentration per well and to respective WT culture. D) Representative confocal images of TUNNEL signal and Hoechst staining in WT or *App*$^{NL-F}$ cultures. E) Relative cell viability was measured by dividing number of TUNNEL objects per Hoechst objects (n= 3 independent cultures). Data shown as mean ± SEM. **p ≤ 0.01. F) Quantification of SPLICS area µm per cell from Fig. 3D (n=16 WT and n=9 *App*$^{NL-F}$ from 2 independent cultures). G) Mitochondrial velocity (µm/s) was calculated through the kymographs shown Fig. 4C (n=12-15 neurites from 3 independent cultures). Electrophysiological studies quantification from Fig. 4H show H) amplitude mEPSCs and I) rise time of mEPSCs (n=16-20, from 3-4 independent cultures).


A
ETC Complex/Actin (%WT)
250
200
150
100
50
0
CV
CIV
CIII
CII
CI
WT
App NL-F
B
TMRM+ fluorescence (RFU)
200
150
100
50
0
3 μM oligo +
2.5 μM FCCP
WT
AppNL-F
2 min
TMRM+ release (relative to control)
3
2
1
0
0.15
WT
AppNL-F
C
ATP levels
Luminescence/μg proteins (%WT)
150
100
50
0
WT
App NL-F
D
Hoechst
Tunel
Merged
WT
App NL-F
E
Tunel Assay
Relative Cell Viability
1.5
1.0
0.5
0.0
Wt
App NL-F
F
Area SPLICS (μm2)/ cell
4
3
2
1
0
**
WT
App NL-F
G
Average velocity (μm/s)
3
2
1
0
0.09
WT
App NL-F
H
Amplitude mEPSP (pA)
25
20
15
10
5
0
I
Rise (ms)
3
2
1
0
WT
AppNL-F

Supplementary Figure 2. Further quantification of electron micrographs shown in Fig. 3A. Violin plots show A) number of MERCS, B) length of MERCS, C) number of mitochondria per cell, and D) average mitochondrial perimeter per cell (4 independent cultures, 5 cells analyzed per culture). *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001