

Supplementary Information for

Autophagy in rat Müller glia cell is modulated by Sirtuin4/AMPK/mTOR pathway and induce apoptosis under oxidative stress

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This PDF file includes:

FIGURE S1-S15

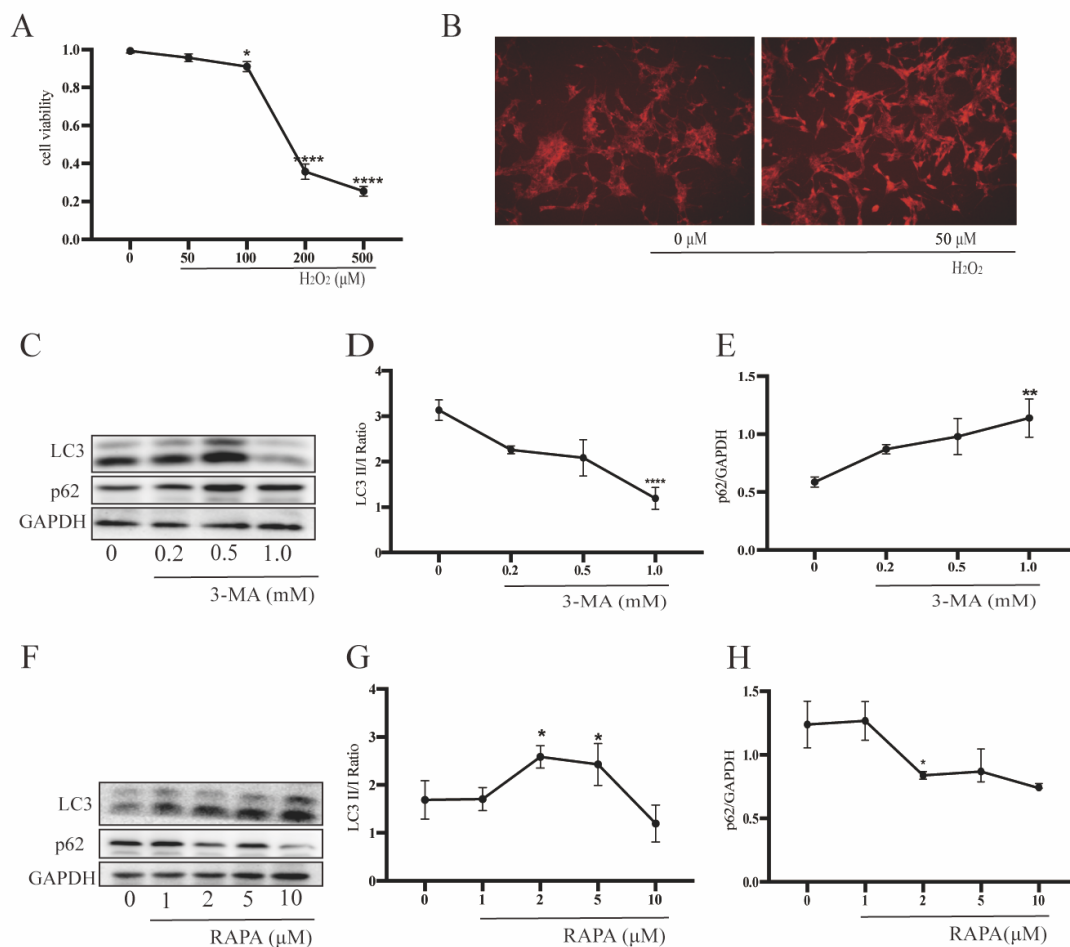


FIGURE S1: The subtoxic level of H₂O₂ and optimal concentration of 3-MA and rapamycin. (Sup.1 A) CCK8 were performed to detect cell viability under different H₂O₂ doses. The MGC were exposed to the H₂O₂ at 0, 50, 100, 150, 200μM respectively for 24h, and found that exposure of MGC to 50μM did not lead to loss of cell viability. (Sup.1 B) the ROS generation in r-MC under normal condition or 50μM H₂O₂ was detected by DHE kit via immunofluorescence. (Sup.1 C-E) The quantitative analysis of LC3 and SQSTM1/p62 were performed to detect optimal concentration of 3-MA. Data are shown as mean ± SEM. (n=3 per group, ***P*<0.01, *****P*<0.0001). The autophagic response which is determined by the change in expression of LC3 and SQSTM1/p62. Western blot analysis showed that 3-MA can significantly repress autophagy function at concentration of 1mM. (Sup.1 F-H) The quantitative analysis of LC3 and SQSTM1/p62 were performed to detect optimal concentration of rapamycin. Data are shown as mean ± SEM. (n=3 per group, **P*<0.05) The autophagic response which is determined by the change in expression of LC3 and SQSTM1/p62. Western blot analysis showed that rapamycin can significantly enhance autophagy function at concentration of 2μM. (p62, SQSTM1/p62)

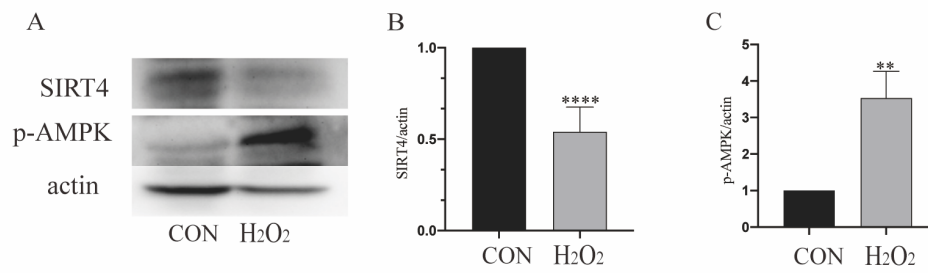
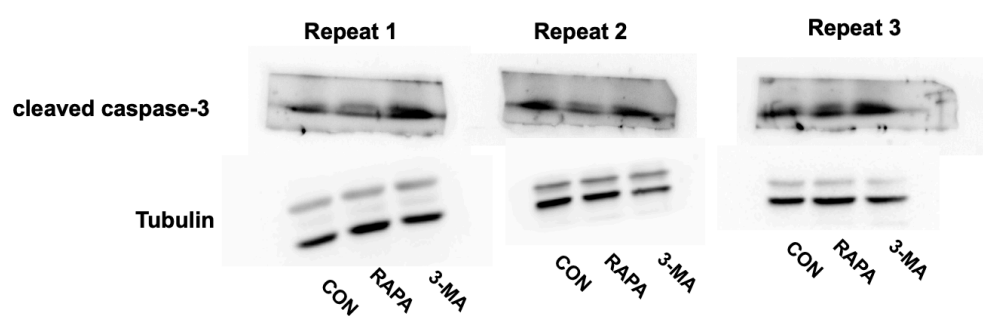


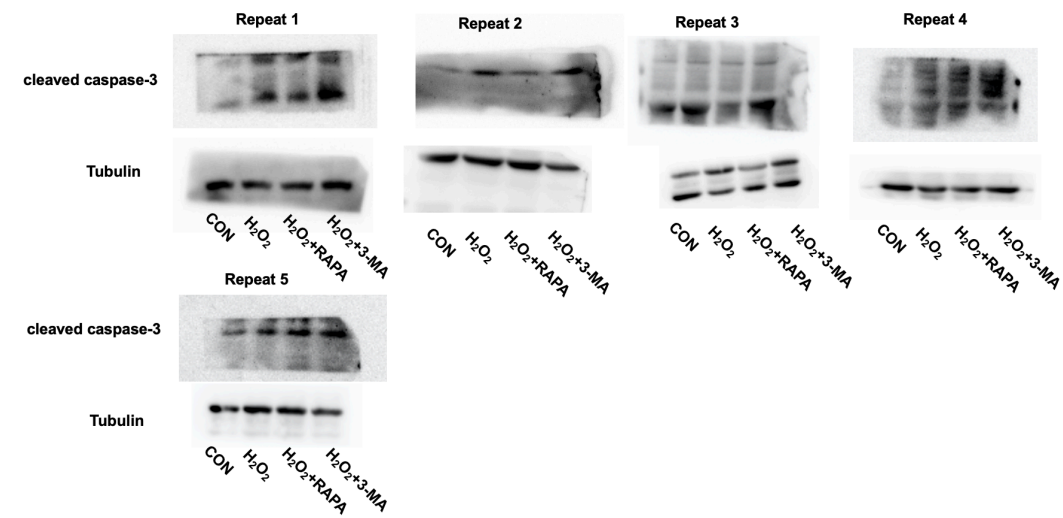
FIGURE S2: 50 μ M H₂O₂ can inhibit SIRT4 and activate p-AMPK expression in Müller glial cells. (A) The expressional level of SIRT4 and p-AMPK with or without 50 μ M H₂O₂ treatment in Müller glial cells. (B) SIRT4 is downregulated under 50 μ M H₂O₂. Data are shown as mean \pm SEM. (n=3 per group, **** P <0.0001). (C) p-AMPK is activated under 50 μ M H₂O₂. Data are shown as mean \pm SEM. (n=3 per group, ** P <0.01).

FIGURE S3



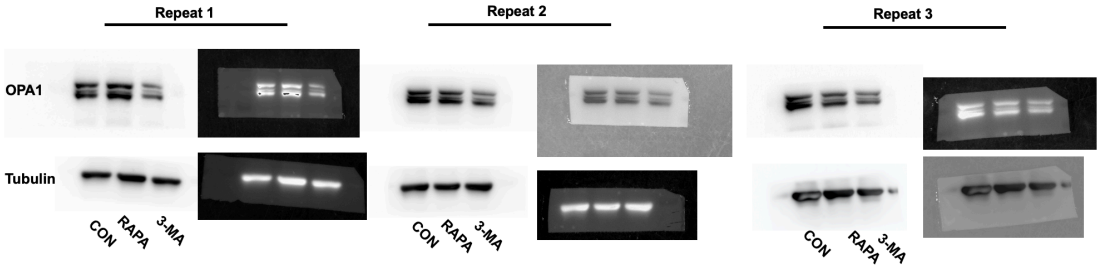
Original western images used for preparing FIGURE 1B

FIGURE S4



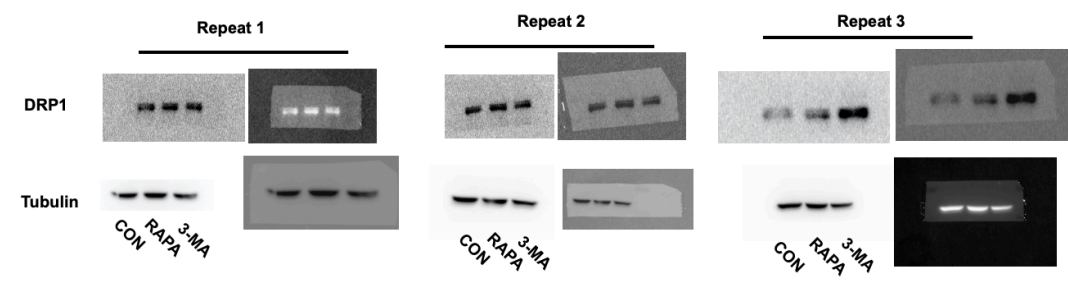
Original western images used for preparing FIGURE 1C

FIGURE S5



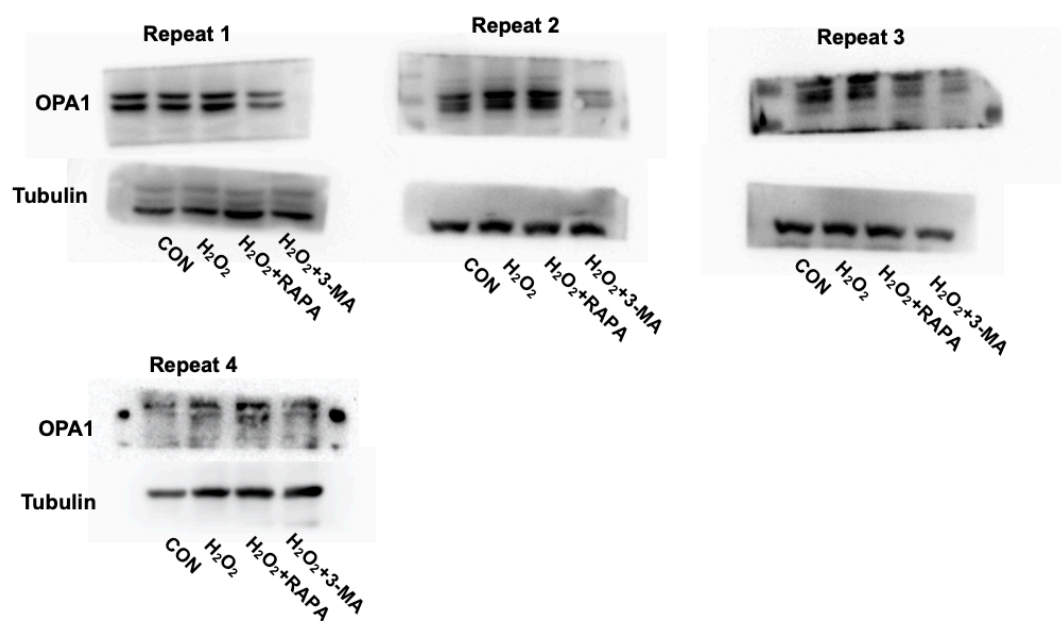
Original western images used for preparing FIGURE 2E

FIGURE S6



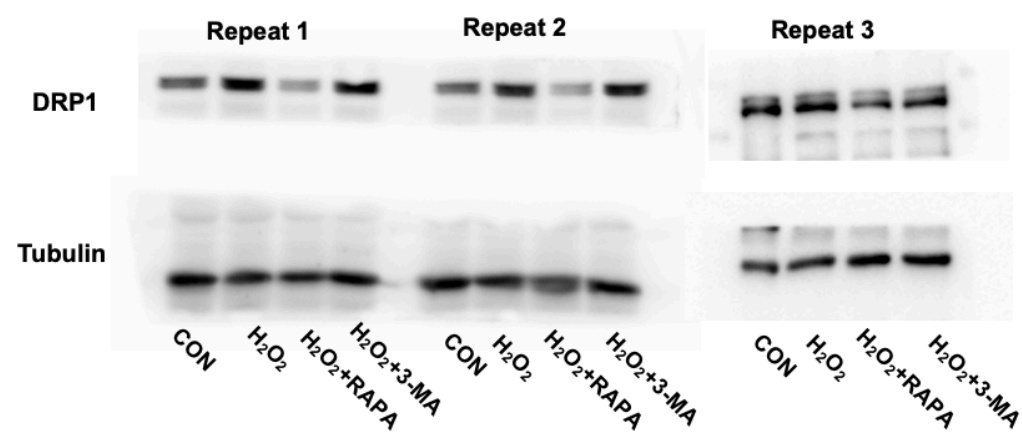
Original western images used for preparing FIGURE 2F

FIGURE S7



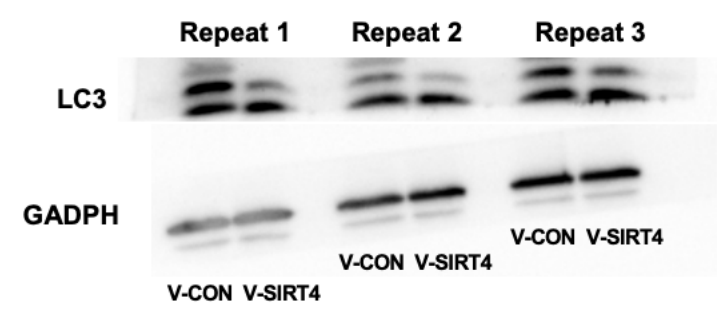
Original western images used for preparing FIGURE 2G

FIGURE S8



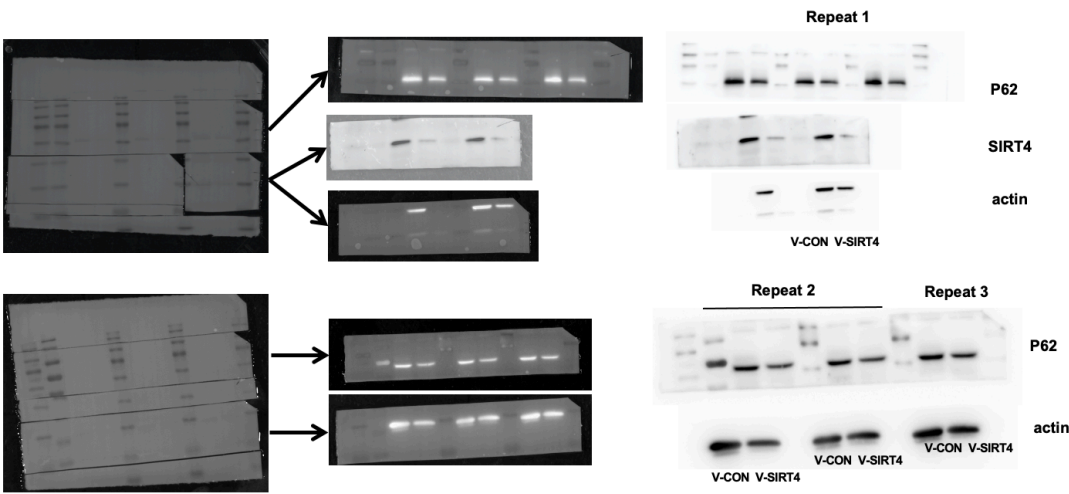
Original western images used for preparing FIGURE 2H

FIGURE S9



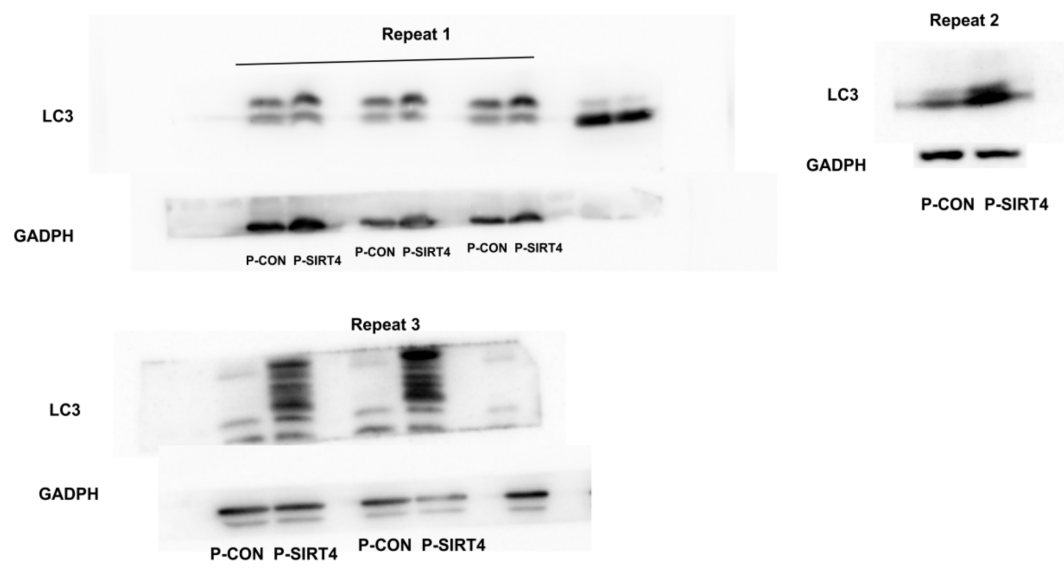
Original western images used for preparing Figure 6 H

FIGURE S10



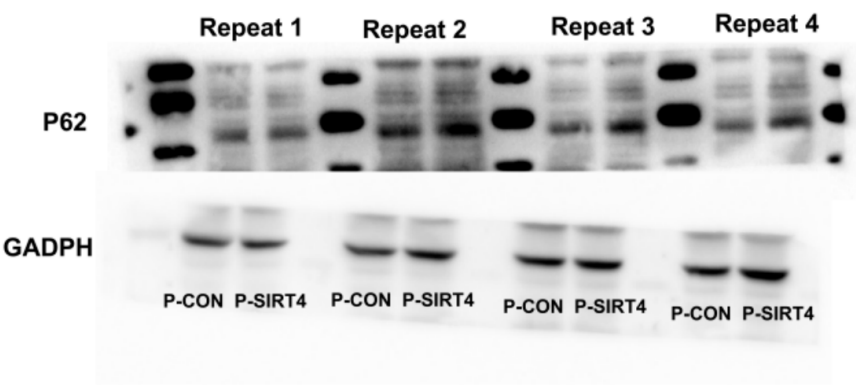
Original western images used for preparing FIGURE 6 I

FIGURE S11



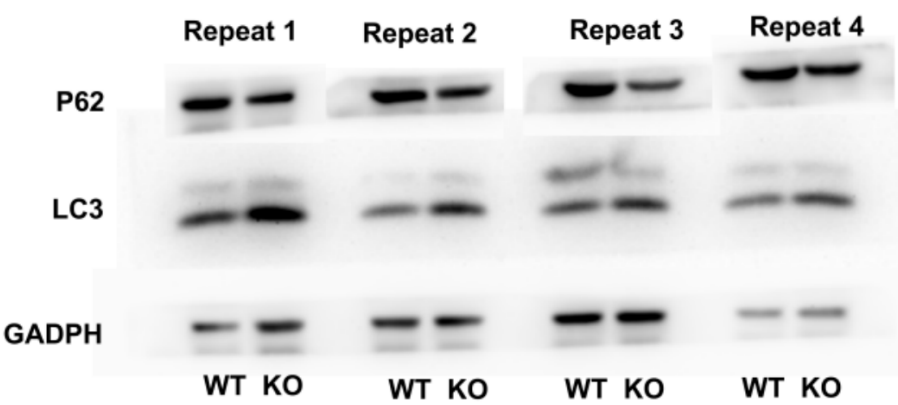
Original western images used for preparing Figure 6E

FIGURE S12



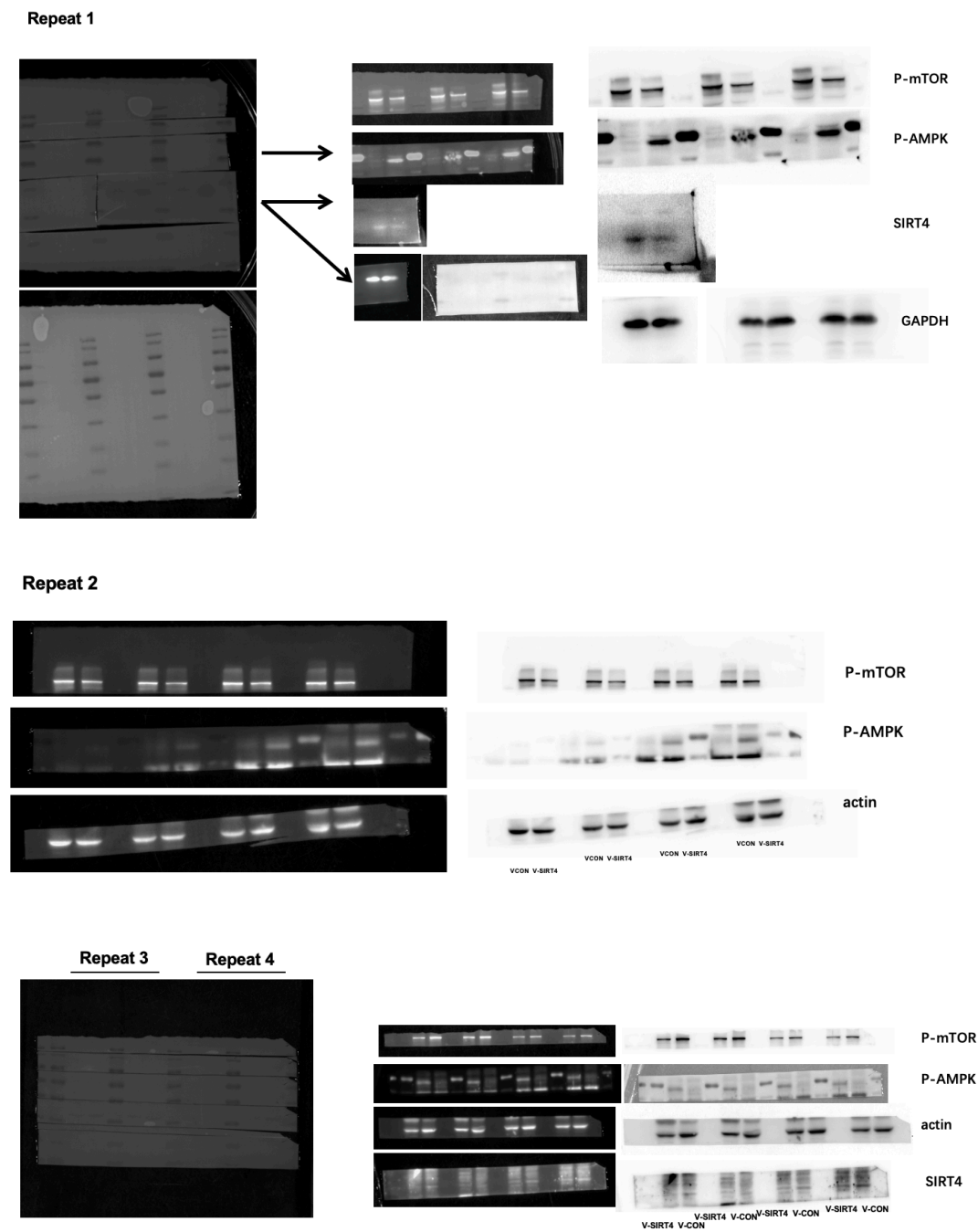
Original western images used for preparing Figure 6F

FIGURE S13



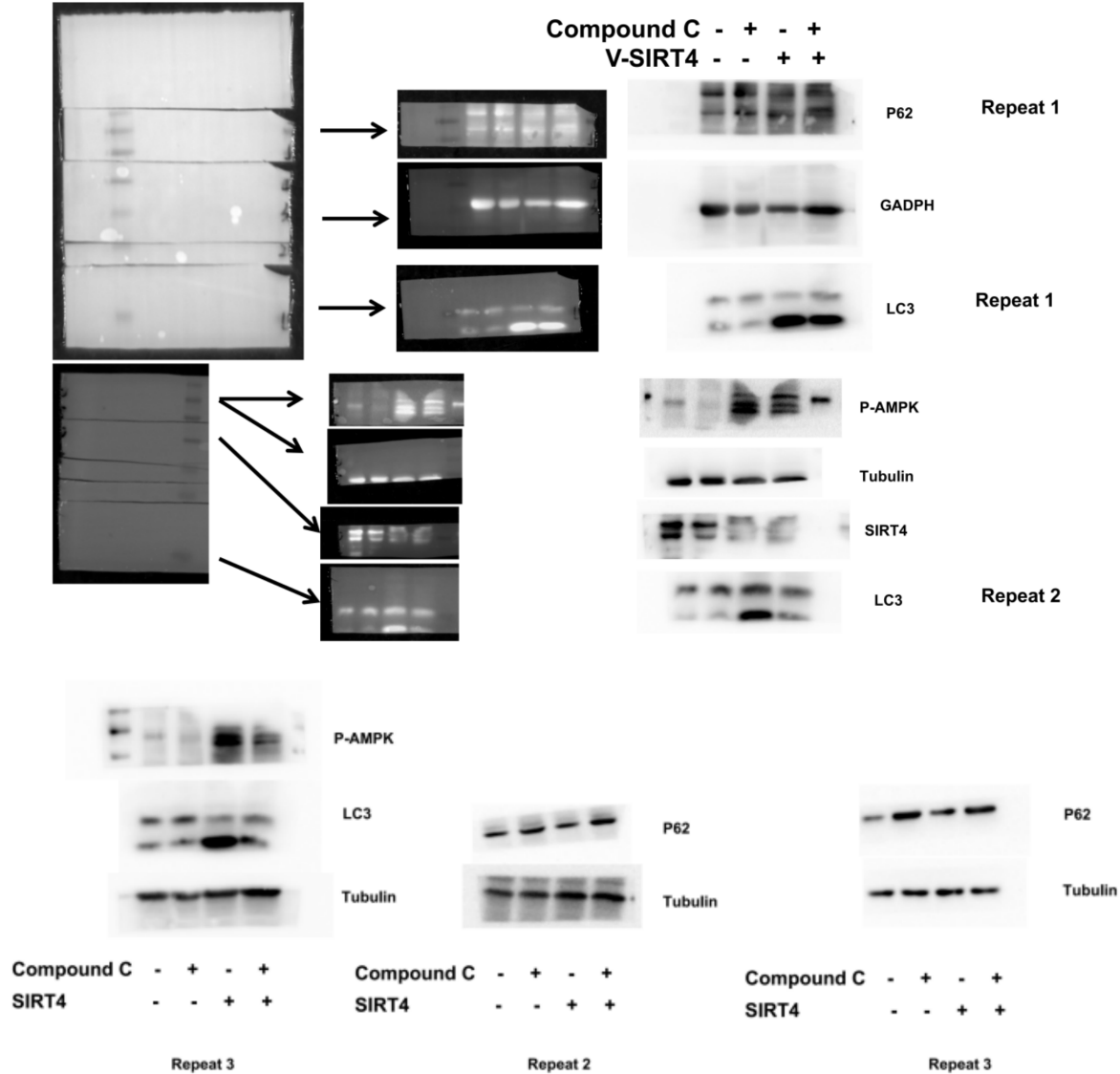
Original western images used for preparing FIGURE 6 B - C

FIGURE S14



Original western images used for preparing FIGURE 7A-B

FIGURE S15



Original western images used for preparing Figure 7 D -F