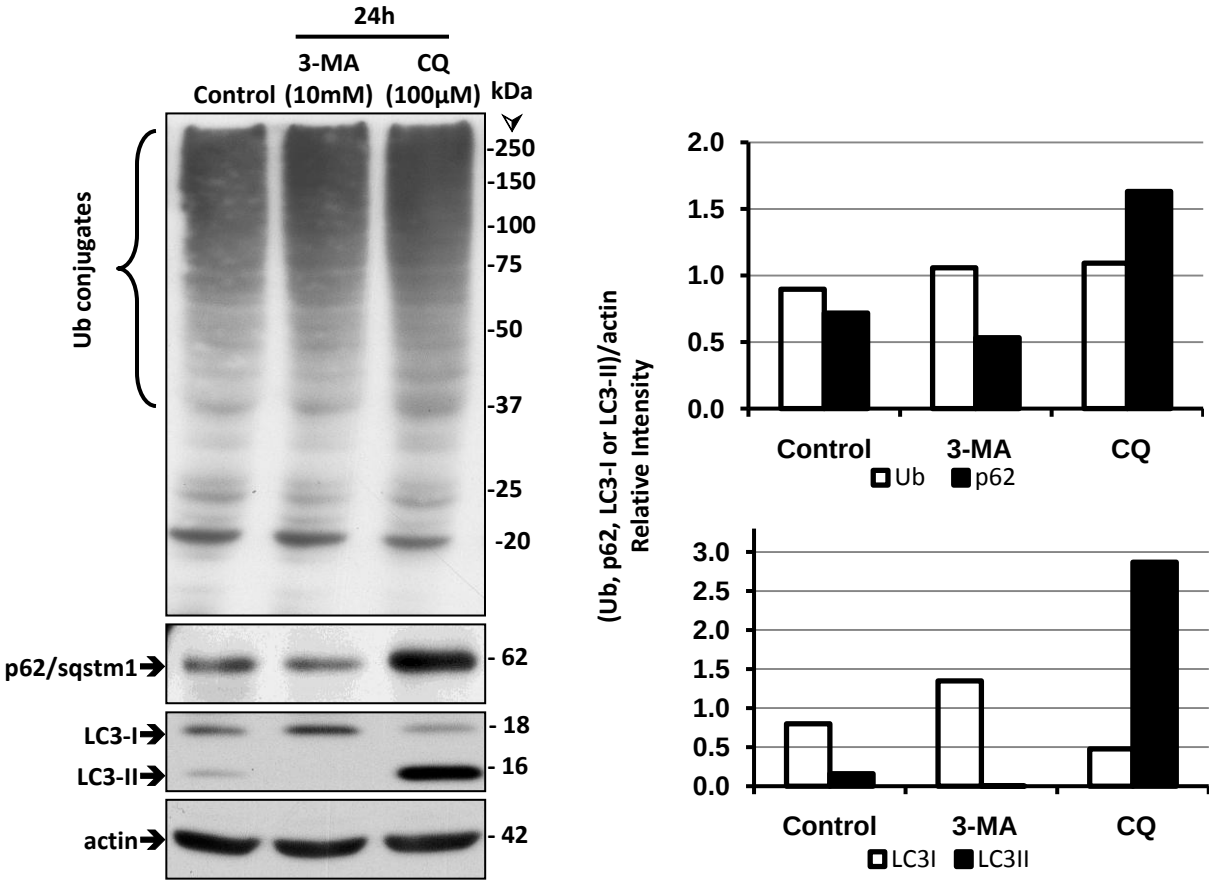
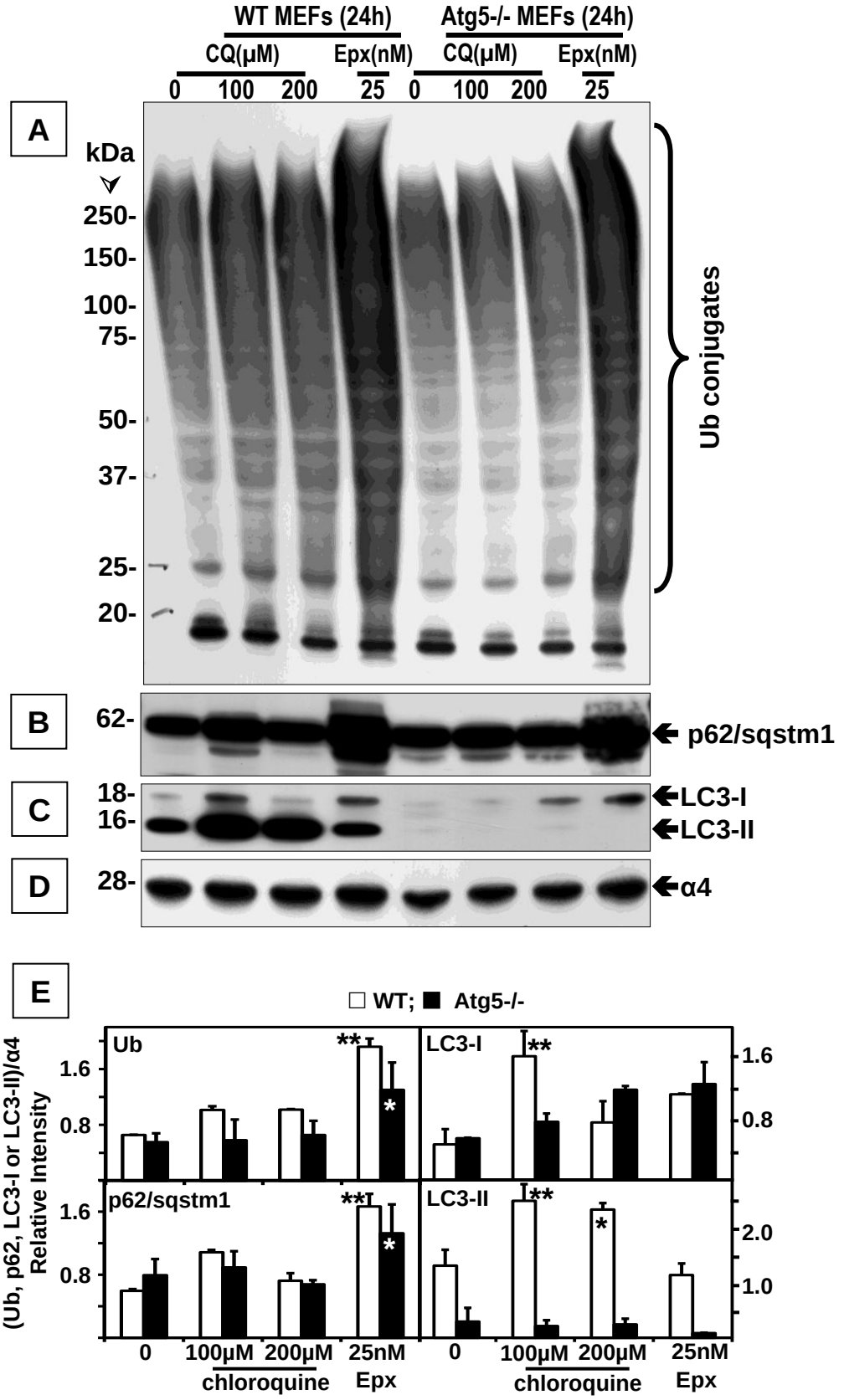
Supplemental Fig. 1 – Comparison of the effects of the autophagy inhibitors 3-MA and chloroquine on ubiquitinated proteins, p62/sqstm1, LC3-I and II in SK-N-SH cells. Western blot analyses (8% or 10% gels) to detect ubiquitinated proteins, p62/sqstm1, LC3-I, LC3-II and actin as loading control in total extracts of human SK-N-SH neuroblastoma cells (40 µg of protein/lane). Cells were treated with the different inhibitors for 24h. Molecular mass markers in kDa are shown on the right. Similar results were obtained in duplicate experiments. 3-MA, 3-Methyladenine; CQ, chloroquine. The levels of ubiquitinated proteins (Ub), p62/sqstm1 (p62), LC3-I and LC3-II were semi-quantified by densitometry (*graph on the right*).

Supplemental Fig. 2 – Effect of chloroquine (CQ) and epoxomicin (Epx) on ubiquitinated protein, p62/sqstm1, LC3-I and LC3-II levels in wild type (WT) and Atg5-/- mouse embryonic fibroblasts (MEF). Western blot analyses (8% or 10% gels) to detect ubiquitinated proteins (**A**), p62/sqstm1 (**B**), LC3-I and –II (**C**) and the proteasome α4 subunit (**D**, loading control) in total cell extracts (40 µg of protein/lane) treated with each inhibitor for 24h. Molecular mass markers in kDa are shown on the left. The protein levels were semi-quantified by densitometry (**E**). Data represent the relative intensity and means and s.d. from duplicate experiments. The *asterisks* identify values that are significantly different (**p at least <0.05; **p<0.01*) from control conditions (0).

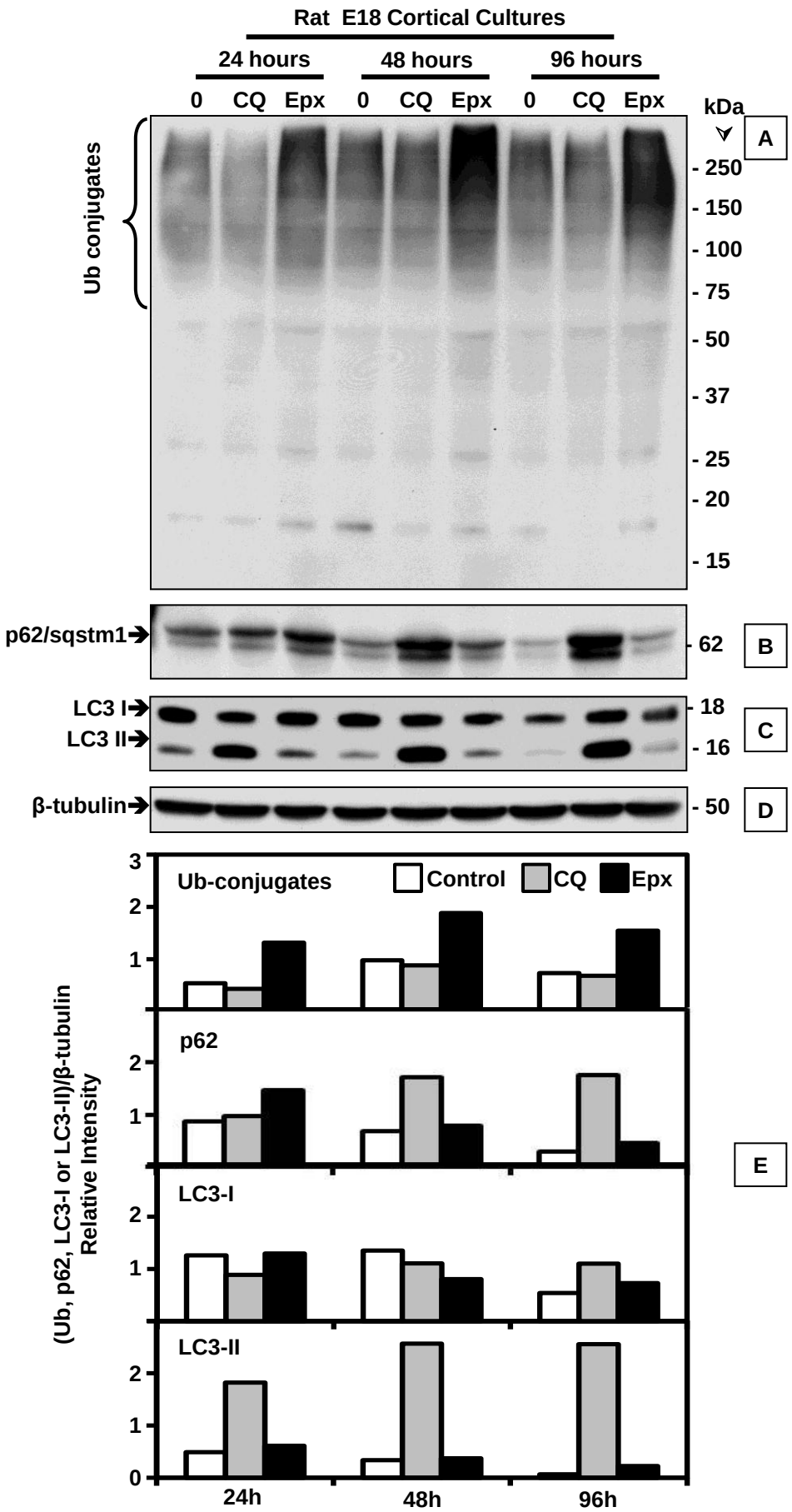
Supplemental Fig. 3 – Effect of chloroquine (CQ, 10µM) and epoxomicin (Epx, 5nM) on ubiquitinated protein, p62/sqstm1, LC3-I and LC3-II levels in rat primary neuronal cortical cultures. Western blot analyses (10% gels) to detect ubiquitinated proteins (**A**), p62/sqstm1 (p62, **B**), LC3-I and –II (**C**) and β-tubulin (**D**, loading control) in total cell extracts (40 µg of protein/lane) treated with each inhibitor for 24h, 48h, and 96h. Molecular mass markers in kDa are shown on the right. The protein levels were semi-quantified by densitometry (**E**). Data represent relative intensity obtained from at least three cell culture dishes per condition.



Supplemental Figure 1– Myeku & Figueiredo-Pereira



Supplemental Figure 2– Myeku & Figueiredo-Pereira



Supplemental Figure 3– Myeku & Figueiredo-Pereira