Supplemental data

Novel roles of chloroquine and hydroxychloroquine in Graves' orbitopathy therapy by targeting orbital fibroblasts

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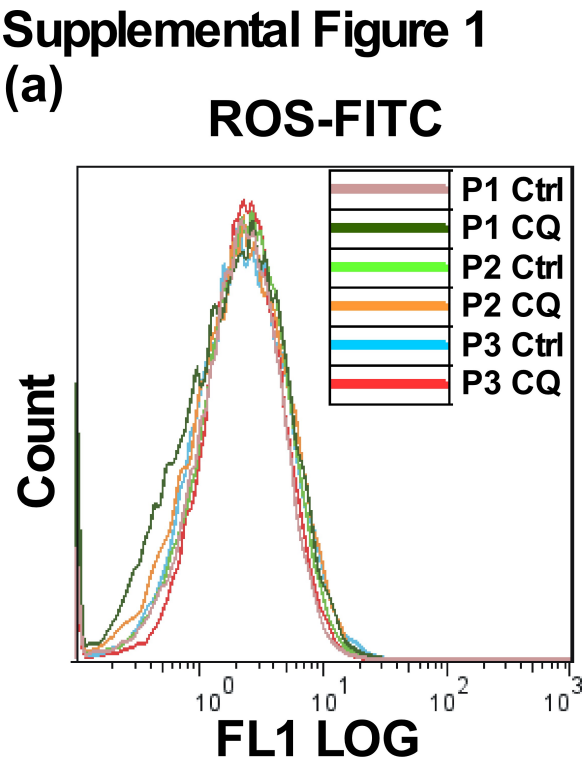
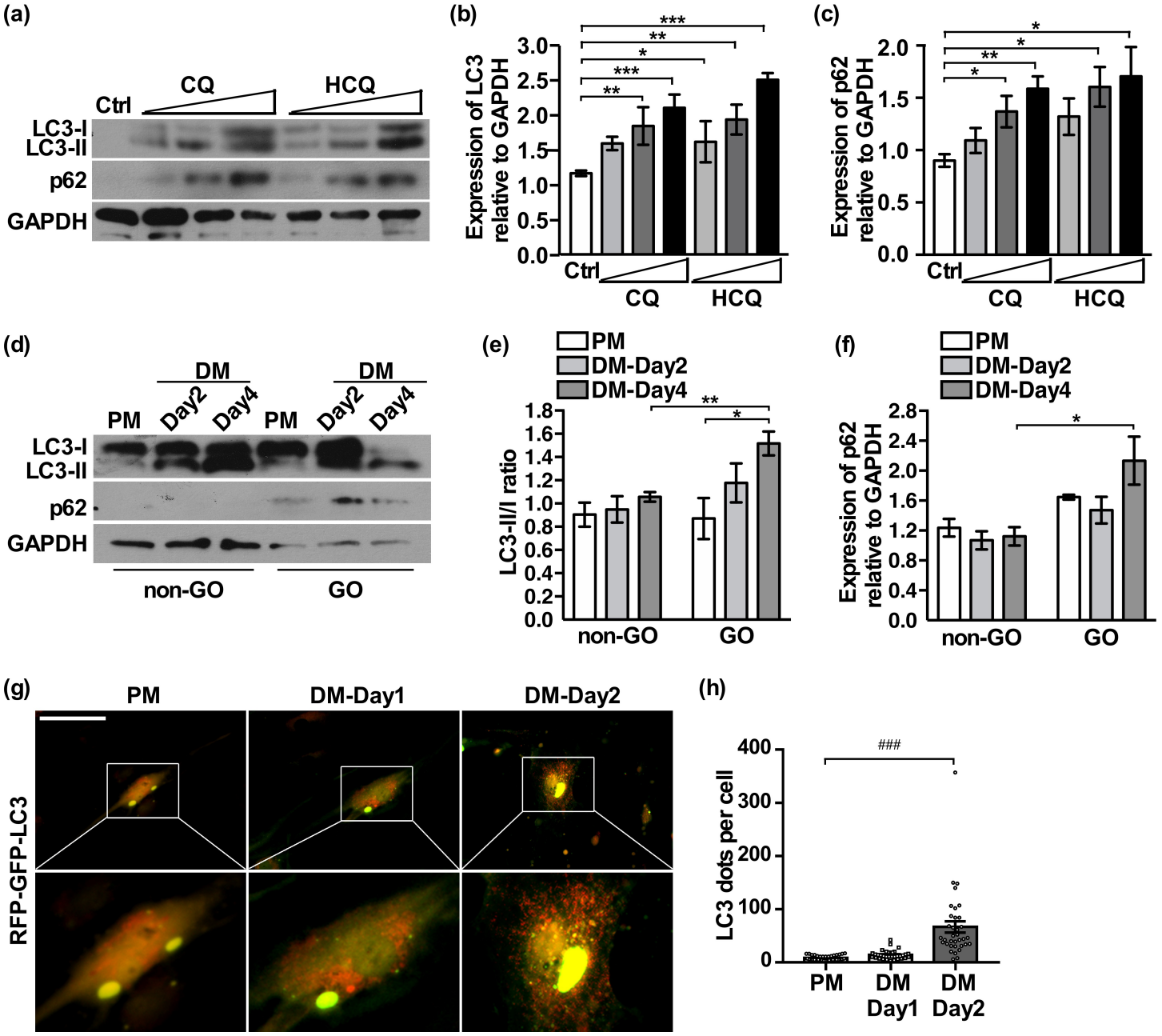


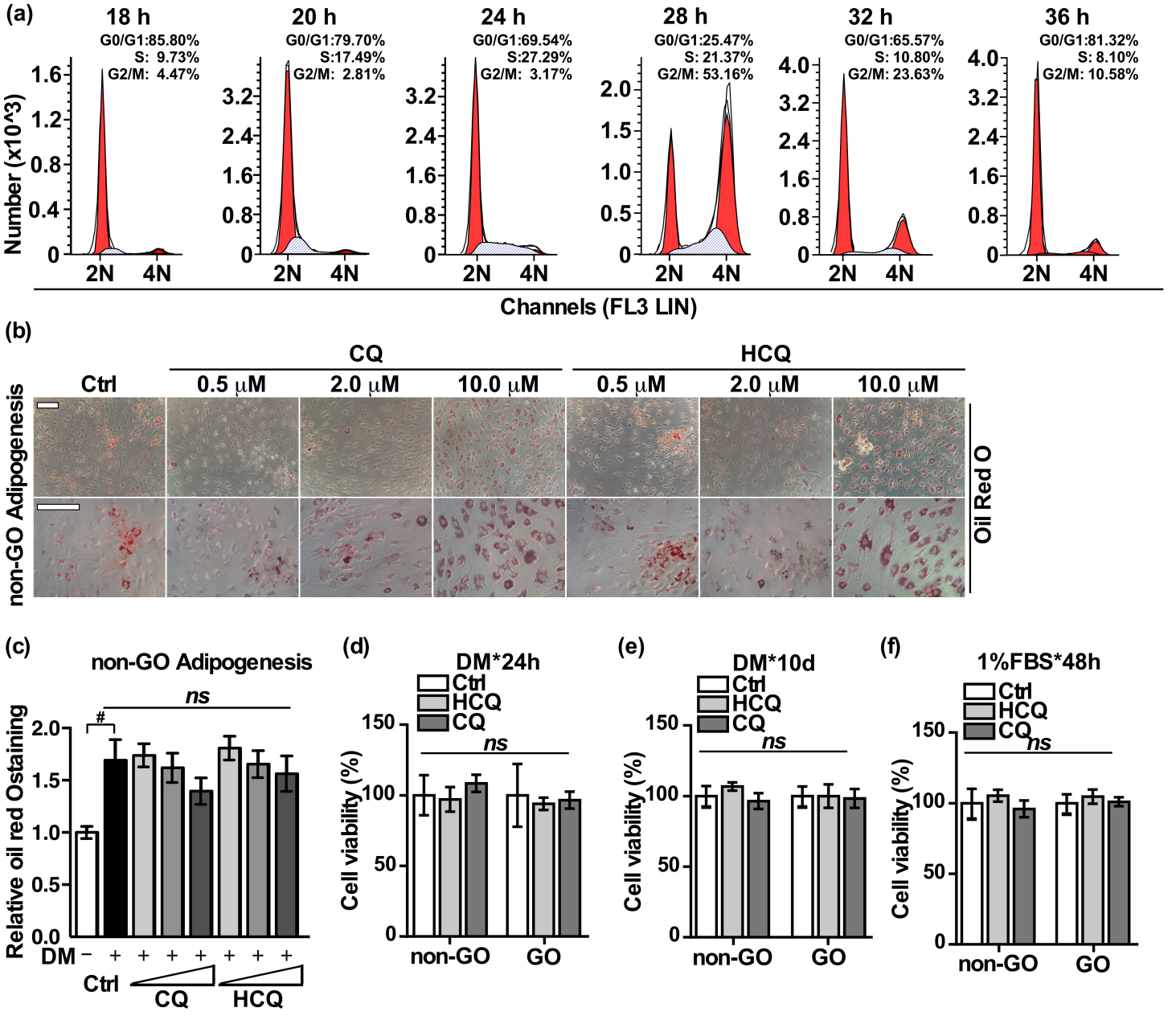
Supplemental Figure 1. ROS level of GO-OFs treated by CQ. (**a**) GO-OFs were incubated with or without 10 μM CQ in PM for 72 h before DCFH-DA (10 μM) was added and the fluorescence intensities were analyzed using a flow cytometer, n = 3.

Supplemental Figure 2



Supplemental Figure 2. Autophagy flux in the OFs. (a) GO-OFs in DM were treated with CQ/HCQ (0, 0.5, 2, 10 μM) for 2 days, and Western blot analysis was conducted to detect the expression of LC3 and p62. **(b)** The LC3 expression of differentiated cells in each group were determined by analysis of the band intensities, normalized to GAPDH, n = 3. **(c)** The p62 expression of differentiated cells in each group were determined by analysis of the band intensities, normalized to GAPDH, n = 3. **(d)** LC3 and p62 expression in GO-OFs versus non-GO-OFs in PM, DM for 2 days, and DM for 4 days. **(e)** The LC3-II/LC3-I ratios of GO- and non-GO-OFs were determined by the intensity, n = 3. **(f)** Relative p62 expression of GO- and non-GO-OFs were quantified by densitometry and normalized to GAPDH, n=3. **(g)** Fluorescence microscopy analysis of GO-OFs expressing REP-GFP-LC3 in the PM and DM. (the red dots represent autolysosomes, and the yellow dots represent autophagosomes, scale bars= 200 μm). **(h)** Total number of LC3 puncta (red dots) per cell in each group, n= 31 to 40. For **(b)**, **(c)**, **(e)**, **(f)** and **(h)**, the bar graph data are shown as the mean ± SEM. *$P < 0.05$, ***$P < 0.001$, *ns* $P \geq 0.05$ versus the Ctrl group; #$P < 0.05$, ###$P < 0.001$ versus the PM Ctrl group.

Supplemental Figure 3



Supplemental Figure 3. (a) OFs from a patient chosen at random. The cells were cultured to reach confluence before being subjected to DM. At different time points, GO-OFs were digested and fixed for detection of cell cycle using flow cytometry. Time (h) refers to the elapsed time after induction of differentiation. **(b)** Microscopic detection was performed to assess Oil Red O staining in each group (non-GO-OFs) treated with or without CQ/HCQ (0.5, 2, 10 μM), scale bars= 200 μm. **(c)** Relative quantification of the Oil Red O staining in the DM-induced group of non-GO-OFs treated with or without CQ/HCQ (0.5, 2, 10 μM), via detection of the OD values at 450 nm after the stained cells were solubilized, n= 5. **(d)** OFs obtained from 5 non-GO patients and 5 GO patients were treated with 10 μM CQ/HCQ or not in DM for 24 h. Cell viability is presented as the percentage relative to that in the untreated well. **(e)** OFs obtained from 4 non-GO patients and 4 GO patients were treated with 10 μM CQ/HCQ or not in the DM for 10 days. Cell viability is presented as the percentage relative to that in the untreated well. **(f)** OFs obtained from 5 non-GO patients and 9 GO patients were treated with 10 μM CQ/HCQ or not in the DMEM-F12 (1% FBS) for 48 h after culture in the serum-free medium for 24 h. Cell viability is presented as the percentage relative to that in the untreated well. For **(c)**, **(d)**, **(e)** and **(f)**, the data are shown as the mean ± SEM, #$P < 0.05$ versus the PM Ctrl group; *ns* $P \geq 0.05$ versus the Ctrl group.