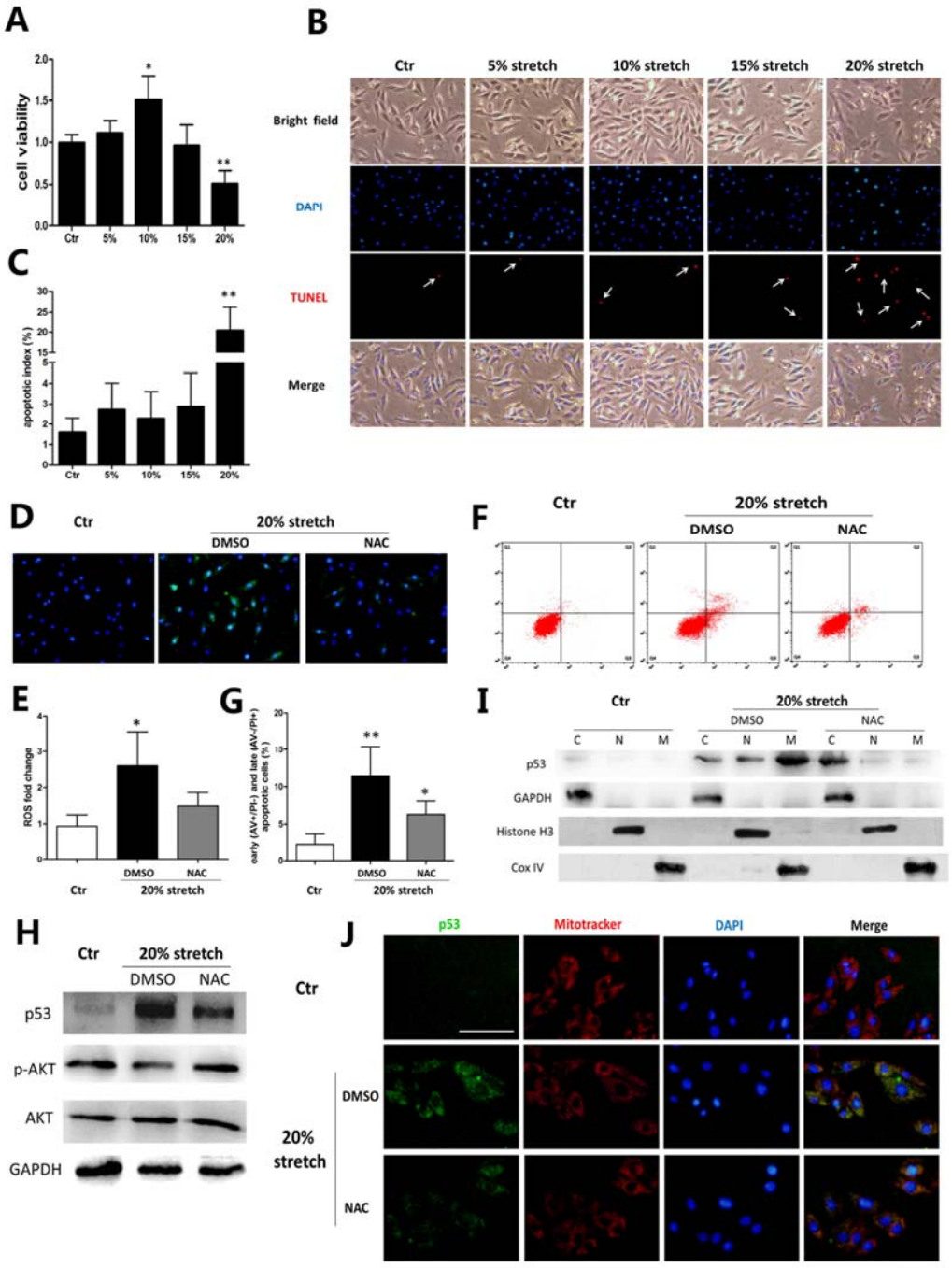
Supplemental Materials

Molecular Biology of the Cell

Jing et al.



Supplementary Figure 1. 20% stretch-induced ROS led to mitochondrial translocation of p53 and apoptosis in L6 myoblasts

(a) L6 cells were subjected to stretches of 5%, 10%, 15% and 20% magnitudes for 24 h, and control cells were cultured under same condition for 24h. Cell viability was tested by MTT assay. (b) Cells were stretched with 5%, 10%, 15% and 20% magnitudes for 24h, followed by TUNNEL and DAPI staining. The apoptotic nuclei were recognized by TUNNEL staining (white arrow). (c) The number of apoptotic nuclei and total nuclei were counted, and apoptotic index was calculated as the percentage of

apoptotic nuclei in total nuclei number per field. Three different fields are included, and data are shown as mean ± standard deviation (S.D.). (d) 2',7'-dichlorodihydrofluorescein diacetate (DCFDA) and DAPI staining of myoblasts subjected to 20% stretch for 24h, pretreated with either DMSO (control) or NAC. ROS generation was observed under the fluorescence microscope. (e) ROS levels per 3,000 cells of each group was calculated by Microplate Fluorescence Reader FL600, and the data were normalized to values obtained from the control group. (f) AV/PI staining and flow cytometry analysis displayed elevated apoptosis of 20% stretched myoblasts and attenuated apoptosis when ROS generation was suppressed by NAC. (g) Statistical analysis of the percentage of early (AV+/PI−) and late (AV−/PI+) apoptotic cells. Data combined from 3 independent experiments are presented as mean ± standard deviation (S.D.). Significant differences were shown by *P < 0.05 and **P < 0.01, compared with static control cells. (h) Representive WB result of p53, p-AKT and AKT protein level in myoblasts 20% stretch stimuli for 24h, with or without NAC pretreatment. GAPDH was used as loading control. (i) Protein samples from the control, 20% stretch with DMSO and 20% stretch with NAC groups were seperated for nuclear, cytoplasmic and mitochondrial fractions, and WB experiments were applied to detect p53 subcellular localization. H3 Histone, GAPDH and Cox IV were used as loading controls of nuclear, cytoplasmic and mitochondrial fractions. (j) Immunofluorescence results of p53 subcellular localization in the above groups. Pictures of p53 staining were taken under same exposure time. Scale bar, 50μm.