



SUPPLEMENTARY FIG. S5. Low density of cells alleviates HR-induced ROS accumulation and mitochondrial apoptosis. Cells were seeded at low density (25,000 cells/cm², no GJ formed) or high density (125,000 cells/cm², GJ formed) in 24-well cell culture plates and subjected to H24R4 treatment. (A–C) Effects of cell density on cell growth, relative LDH release, and apoptotic rates. (D, E) Effects of cell density on ROS generation after H24R4 treatment, detected with DHE staining (D, stained in red, scale bar 50 μm) and DCFH-DA staining (E). (F) Effects of cell density on mitochondrial superoxide formation, detected by MitoSOX Red dye staining. (G). Effects of cell density on mitochondrial membrane potential. Data are presented as mean ± SE (n=4). *p<0.05 compared with control group at high density; #p<0.05 versus H24R4 group at high density. DCFH-DA, 6-carboxy-2'-7'-dichlorodihydrofluorescein diacetate; GJ, gap junction; H24R4, hypoxia for 24 h and reoxygenation for 4 h; HR, hypoxia reoxygenation; LDH, lactate dehydrogenase.