SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

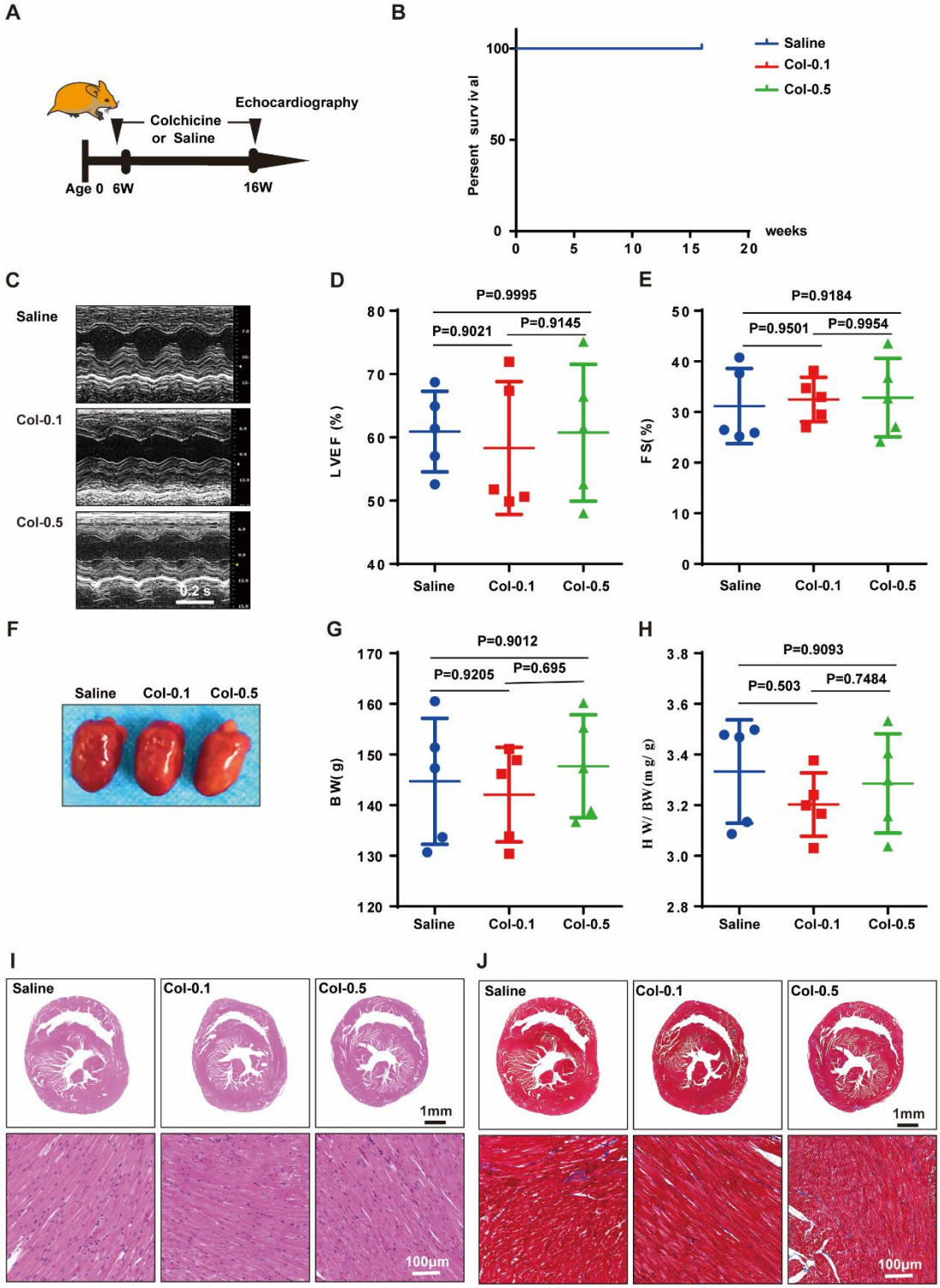
The small molecule drugs were purchased as below: Doxorubicin (S7306, Selleck), Colchicine (S2284, Selleck) and Chloroquine (S6999, Selleck). Plasmid Miniprep Kit (#DK001-01A, novoprotein, China), PrimeScript RT reagent Kit (#RR037A, TAKARA). The antibodies used in this study were as follows: anti-GAPDH (#60004-1-Ig, proteintech); anti-SQSTM1/P62(#ab56416, abcam); anti-LC3B (#ab48394, abcam); anti-phospho-mTOR (Ser2448, #ab109268, abcam); anti-phospho-AMPK (#ab133448, abcam), anti-phospho-ULK1 (Ser757, #14202, Cell Signaling Technology); anti-ATG5 (#ab118327, abcam); anti- phospho-PI3K (Try199, #4228, Cell Signaling Technology); anti-phospho-AKT (Try308, #ab105731, abcam); anti-Beclin1 (#11306, proeintech); anti-TP53 (#60283, proeintech); anti-PINK1 (#23274, proeintech); anti-Parkin (#14060, proeintech); anti-TOM20 (#11802, proeintech); anti-α-Tubulin (#ab7291, abcam); anti-β-Tubulin (#sc-5274, santa cruz); anti-rabbit IgG (#ab6721, abcam); anti-mouse IgG (#ab6728, abcam). WB Ultra-Sensitive ECL Luminescent Liquid (#ED0015-B, SparkJade，China).

Table S1. Echocardiography of hamsters in different groups.

Syrian hamster	PBS	DOX	DC0.1	DC0.5
n	6	6	6	4
FS (%)	32.76%± 54.16%	21.64%± 5.236% (*)	30.22%± 4.639% (†)	20.83%± 4.043% (‡)
LVEF (%)	61.01%± 7.18%	43.75%± 8.854% (*)	57.07± 7.271% (†)	42.32%± 7.555% (‡)
LV VOL s(μl)	35.66± 6.821	48.22± 12.39	53.79± 21.28	64.93± 34.71
LV VOL d(μl)	92.22± 13.37	87.44± 27.74	121.7± 33.18	109.1± 46.54
LVID s(mm)	3.012± 0.2459	3.401± 0.3697	3.525± 0.6136	3.781± 0.8653
LVID d(mm)	4.486± 0.2803	4.359± 0.5496	5.025± 0.6047	4.752± 0.8988
LVPW s(mm)	1.819± 0.0958	1.739± 0.085	1.695± 0.1782	1.745± 0.277
LVPW d(mm)	1.397± 0.1363	1.577± 0.1417	1.377± 0.1977	1.654± 0.297
IVS s(mm)	1.884± 0.1434	1.81± 0.1616	1.852± 0.1719	1.872± 0.2181
IVS d(mm)	1.346± 0.2096	1.415± 0.1928	1.339± 0.2345	1.37± 0.2197

Fraction shortening (FS); left ventricle ejection fraction (LVEF); left ventricle volume in the end-diastole (LV Vol;d) and end-systole period (LV Vol;s). internal dimension of the left ventricle in the end-diastole (LV ID;d) and end-systole period(LV ID;s). LV posterior wall thickness in the end-diastole (LVPW; d) and end-systole period (LVPW; s).**;** internal septal thickness in the end-diastole (IVS; d) and end-systole period (IVS; s). DOX: Doxorubicin; DC0.1: DOX + Colchicine (0.1mg/kg, daily); DC0.5: DOX + Colchicine (0.5mg/kg, daily) Means ± SD, one - way ANOVA (Tukey post- test). * <0.05 vs. PBS group; †<0.05 vs. DOX group; ‡ <0.05 vs. PBS group.

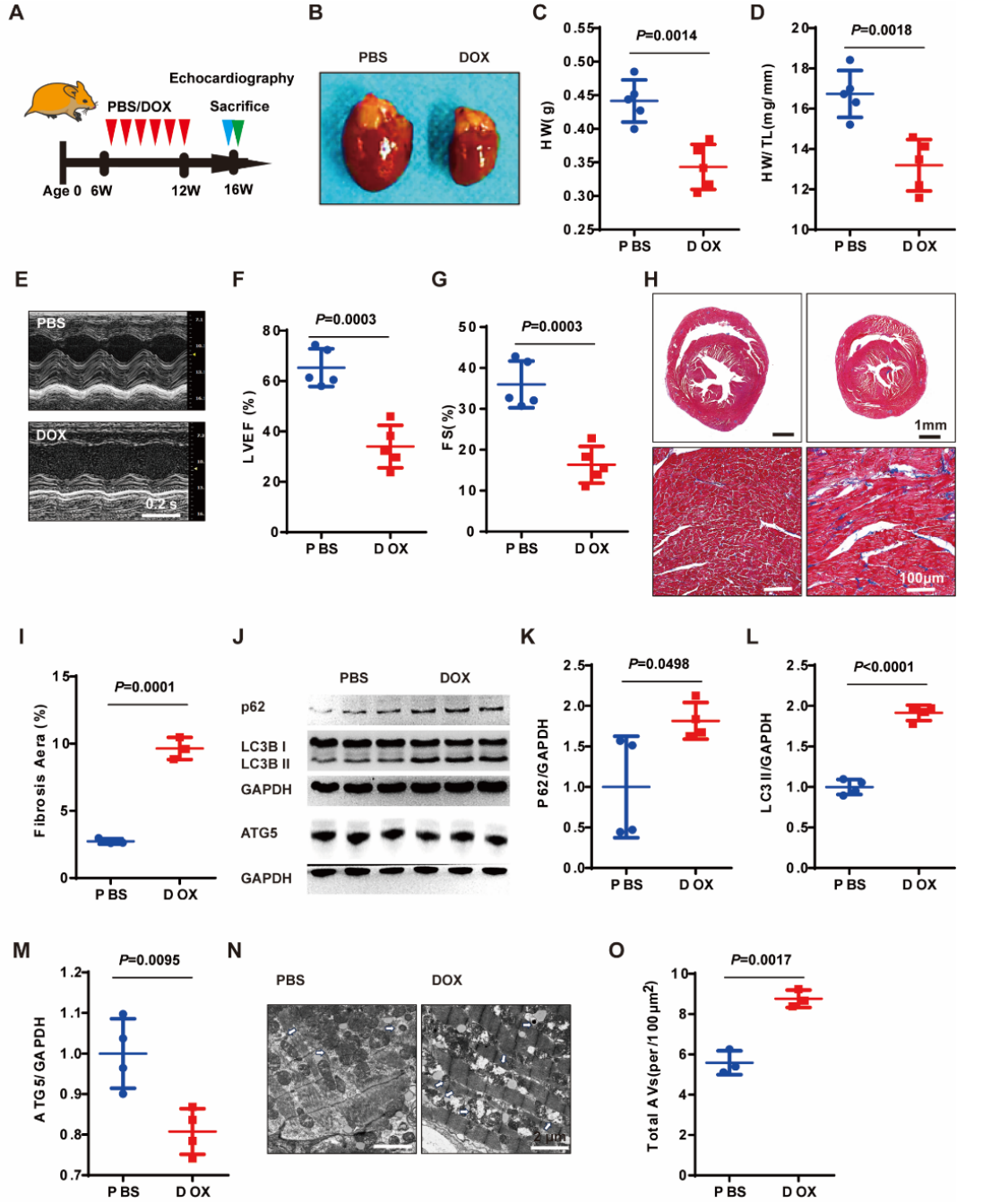
Figure S1. Low-dose colchicine alone is well tolerable for Syrian hamsters.



A. Schematic of *in vivo* colchicine administration experimental protocol. **B.** Kaplan–Meier survival analysis hamsters in different groups. (n=5 per group, none died during the performance), means ± SD, *P* value was determined by log-rank (Mantel–Cox) test. **C-E**. Representative M-mode echocardiographic images and LVEF, FS measurements of Syrian hamsters treated with PBS, 0.1mg/kg and 0.5mg/kg colchicine (Col-0.1 and Col-0.5). **F-H.** Representative

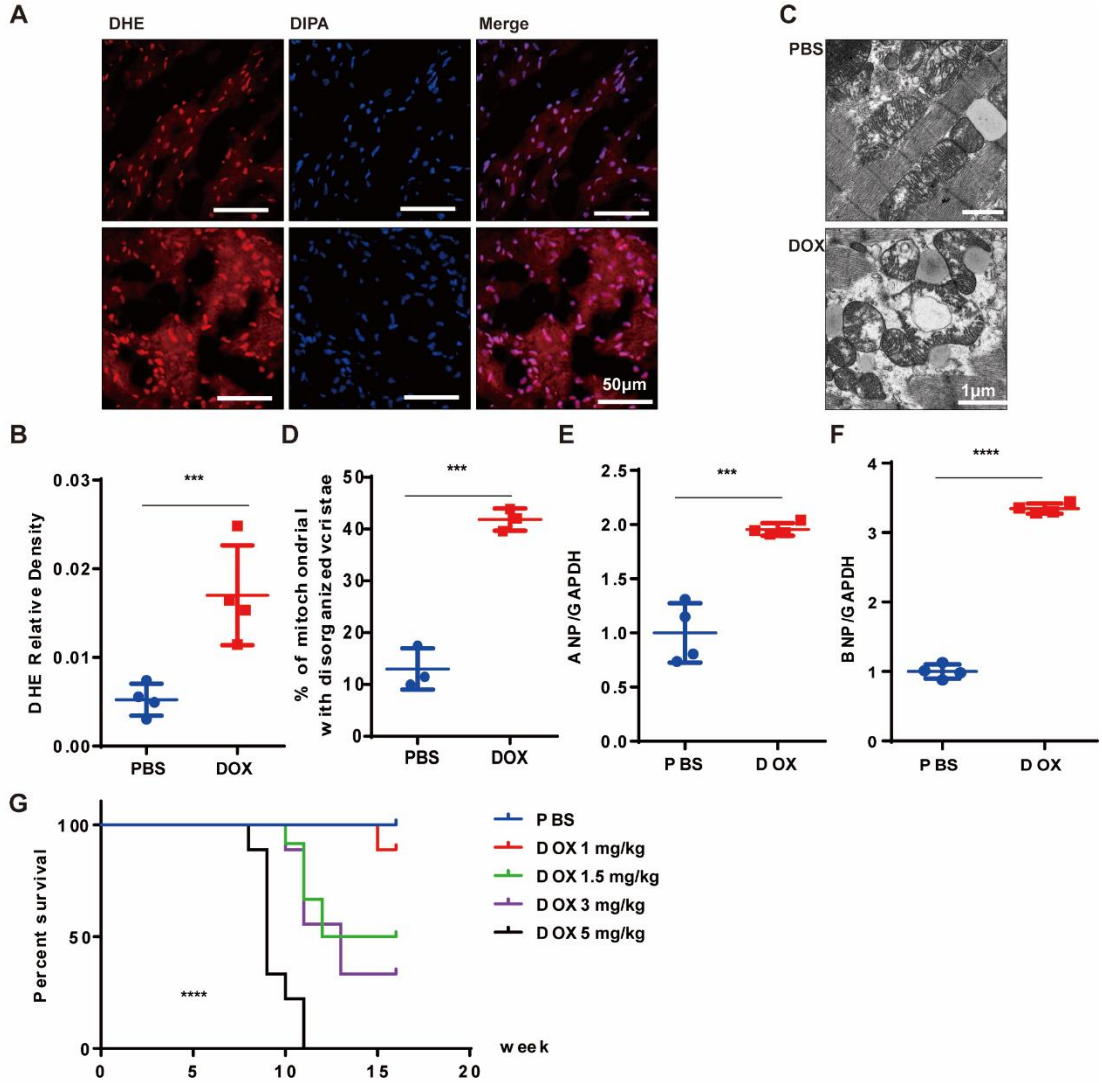
gross morphology of the hearts from different groups, and measurements of body weight (BW), heart weight/body weight (HW/BW). Data of (**D, E, G** and **H**) are analyzed by one-way ANOVA (Tukey post- test), means ± SD. **I-J.** Representative hematoxylin and eosin (H&E) and Masson's trichrome staining of heart sections from different groups, similar results were found in more than three different hamsters for each group. For **D-H,** data were presented as means ± SD，2-tailed Student *t* test.

Figure S2. DOX induced heart failure and impaired autophagy in Syrian hamsters.



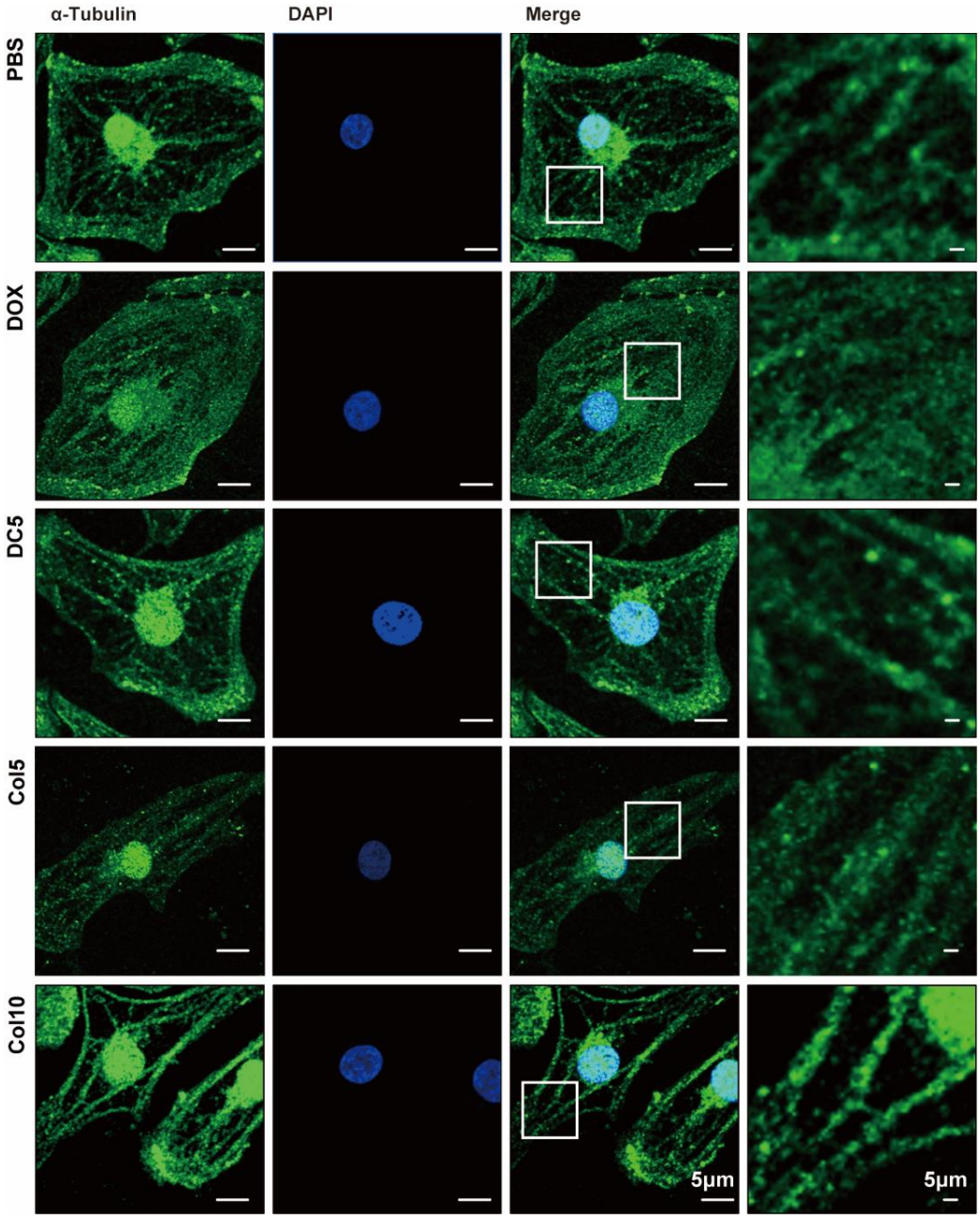
A. Schematic of *in vivo* DOX induced heart failure experimental protocol. **B.** Gross morphology of the heart from PBS and DOX treated hamsters. **C-D.** Heart weight (HW) and ratios of heart weight to tibia length (HW/TL). **E-G.** Representative M-mode echocardiographic images and LVEF, FS measurements of Syrian hamsters treated with PBS or DOX. For above data, n=5 each group. **H-I.** Representative Masson' trichrome staining and measurements of fibrosis. **J-M.** Representative WB analysis and quantification of the expression of P62, LC3 and ATG5. (n=4 per group). **N.** Representative TEM images showing AVs marked by white arrow. **O.** Measurements of the autophagic vacuoles (AVs) per100 μm^2(3 random fields per sample, n=3 per group). DOX indicates doxorubicin; AVs indicates autophagic vacuole. For all data were represented as means ± SD, 2-tailed Student t test.

Figure S3. DOX induced damaged mitochondrial and ROS overload in hamster hearts.



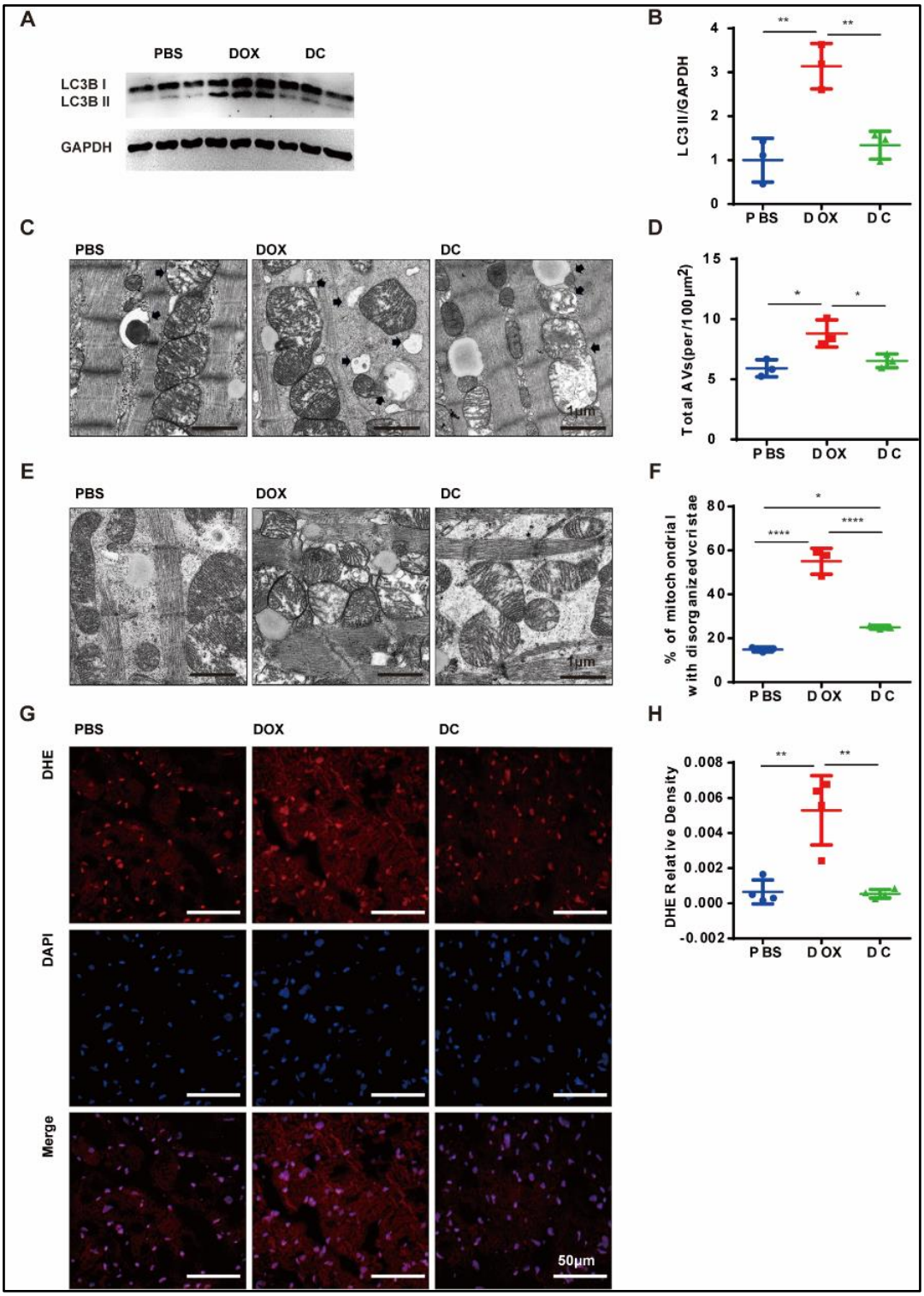
A. Representative immunofluorescence staining for labeling reactive oxygen species (ROS). Left, dihydroethidium (DHE); middle, DAPI; right, Merge. **B**. Quantification of the ROS level (n=4 per group). **C-D.** Representative TEM images for labeling mitochondrial and quantitative analyses of mitochondrial cristae (3 random fields per sample, n=3 per group). **E-F.** Quantitative q-PCR analysis of ANP and BNP mRNA expression (n=4 per group). **G.** Kaplan–Meier survival analysis of hamsters in different groups. (n=8-12 per group), means ± SD, *P* value was determined by log-rank (Mantel–Cox) test. ANP indicates atrial natriuretic peptide, BNP, brain natriuretic peptide. For all data were represented as means ± SD, 2-tailed Student t test, $P > 0.05$, non-significant (ns), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Figure S4. α-Tubulin immunocytochemistry analysis for the microtubule network in hiPSC-CMs.



DC5 indicates doxorubicin + colchicine (5nM). Col5, Col10 indicates colchicine 5nM,10nM respectively.

Figure S5. Low-dose colchicine rebalanced cellular homeostasis in Syrian hamsters.



A-B. Representative WB analysis and quantification of the LC3B-II. **C.** Representative TEM images showing AVs marked by black arrow. **D.** Measurements of the AVs per100 μm^2 (3 random fields per sample). **E-F.** Representative TEM images for labeling mitochondrial and quantitative analyses of mitochondrial cristae (3 random fields per sample). (For **B, D** and

F n=3 per group). **G**. Representative immunofluorescence staining for labeling reactive oxygen species (ROS). Upper, dihydroethidium (DHE); middle, DAPI; down, Merge. **H**. Quantification of the ROS level (n=4 per group). For above data, means ± SD, one - way ANOVA (Tukey post- test). $P > 0.05$, non-significant (ns), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. DC: DOX+ 0.1mg/kg colchicine daily.