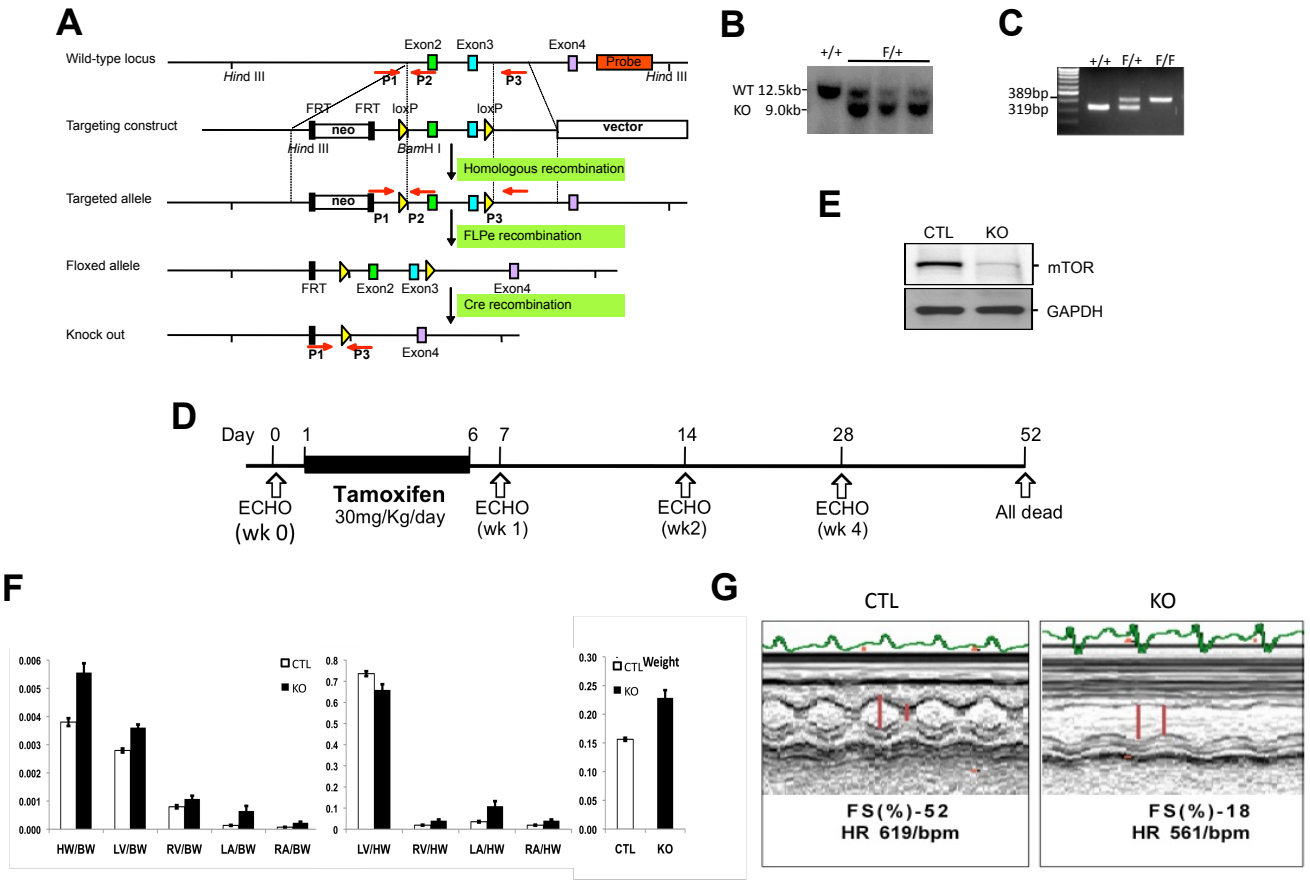
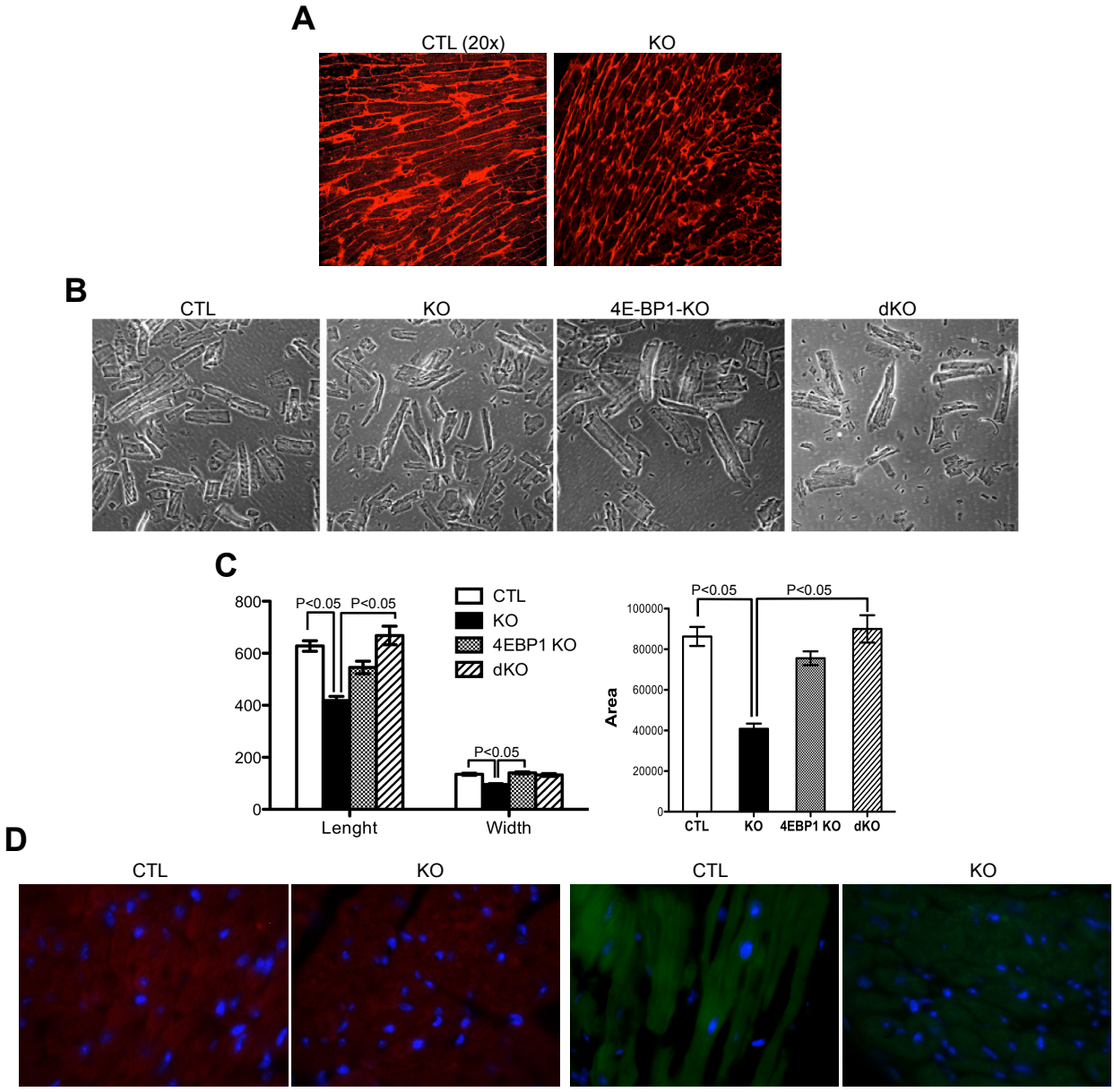
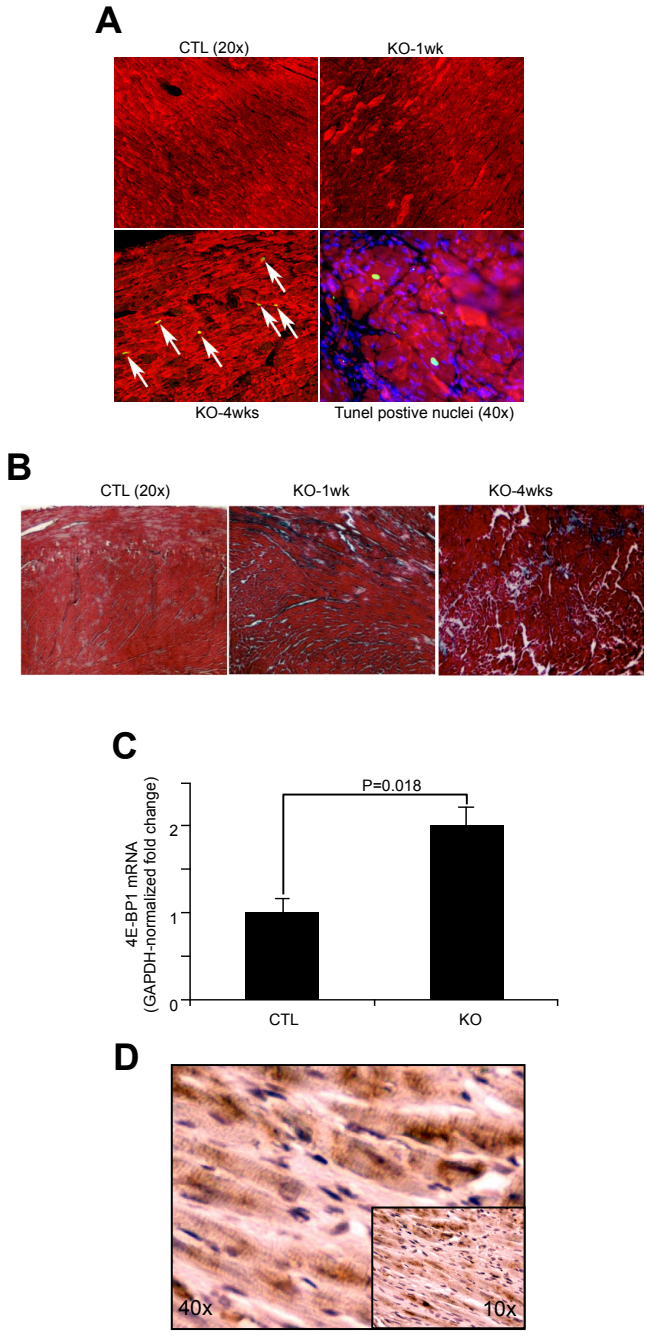


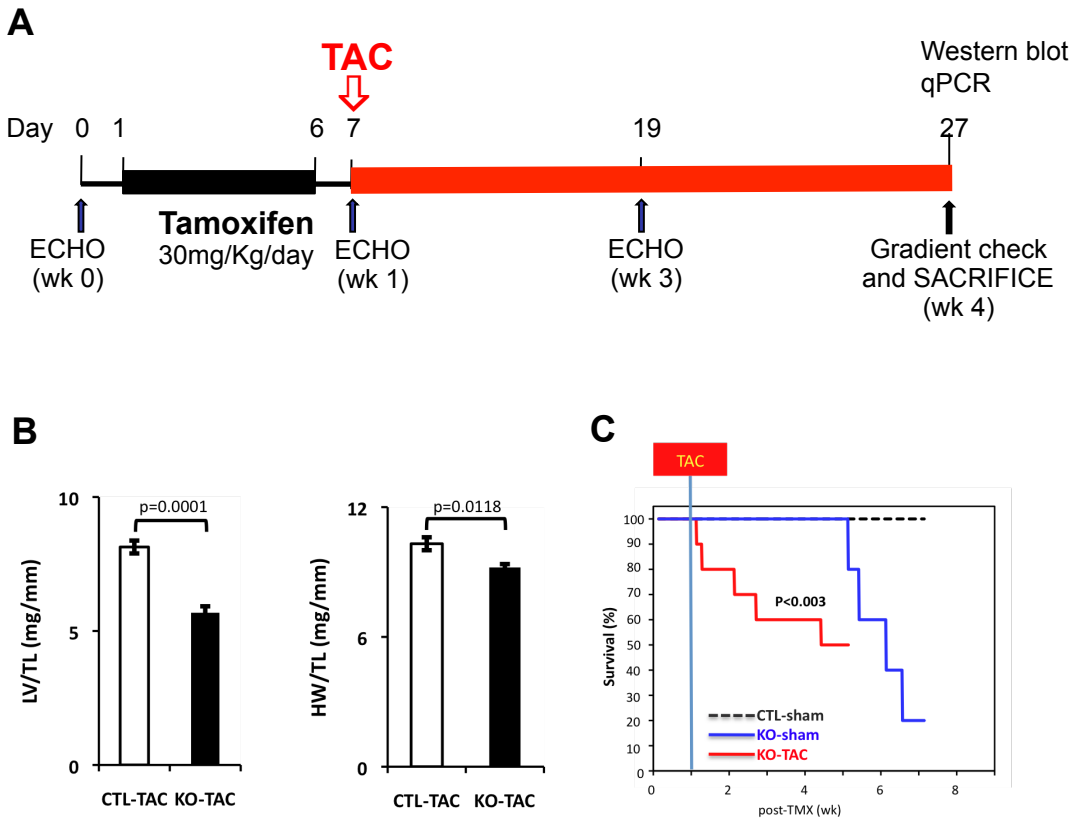
Supplemental Figure 1. Generation and assessment of an inducible, cardiac-specific *mTOR* knockout mice model. (**A**) Strategy used to knock out *mTOR* in mouse cardiomyocytes and generate the mTOR-cKO model. Exon 2 and 3 of the *mTOR* gene was flanked by loxP sites, and a neomycin (Neo) resistance cassette, flanked by FRT sites, inserted upstream. Maps of the wild-type *mTOR* locus, the targeting vector, the floxed allele, and the excised allele are shown. Exons are shown as boxes. The position of the 3' probes used for Southern blot analysis in **B** are indicated. Arrows indicate primer positions for PCR. Arrowheads indicate the loxP sites. (**B**) Southern blot of DNA obtained from wild-type (+/+) and 3 heterozygous (F/+) embryonic stem cell clones using probe shown in **A** after digestion with HindIII. The 12.5 kb and 8 kb bands represent respectively the WT and the mutant allele. (**C**) Successful recombination of *mTOR* confirmed by PCR on genomic DNA isolated from aMHC-MCM/mTOR$^{F/F}$ (F/F) mouse, wild-type mouse (+/+), and heterozygous mouse (F/+) characterized by PCR with primers P1 and P2 allowing the amplification of the loxP-containing deletion region shown in **A**; a 389-bp fragment for the floxed mTOR and 319-bp fragments for the wild-type allele. (**D**) Experimental schedule of tamoxifen administration and echocardiographic (ECHO) analyses. (**E**) Western blot demonstrating significantly reduced mTOR protein in heart homogenate from an mTOR-cKO (KO) mouse at 4 weeks post-TMX. CTL, WT-Cre control; GAPDH, glyceraldehyde 3-phosphate dehydrogenase. (**F**) Gravitometric data analyses (mean±SD, n=6 each group). White bars, control, Black bars, mTOR-cKO. HW, heart weight. BW, body weight. LV, left ventricle. RV, right ventricle. LA, left atrium. RA, right atrium. (**G**) Representative 2D-echocardiograms of CTL and KO mice at 4 wks post-TMX. FS, fractional shortening; HR, heart rate.



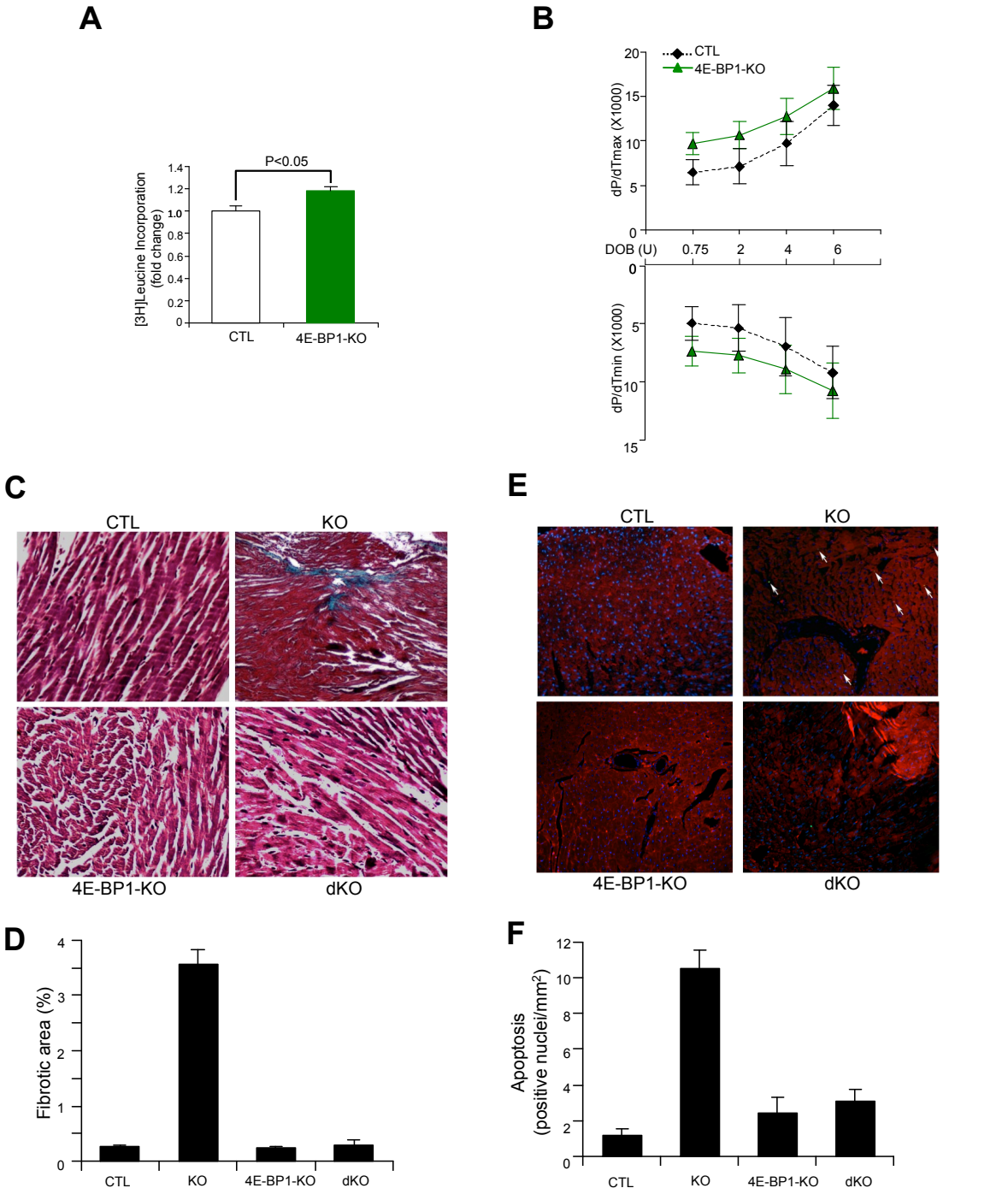
Supplemental Figure 2. (**A**) Representative fluorescence micrographs of CTL and mTOR-cKO myocardium sections stained with wheat-germ agglutinin to visualize the cell membrane (red). (**B**) Representative photo-micrographs of isolated cardiomyocytes from the various mice lines (original magnification, 20X). (**C**) Measurement (mean±SEM) of length and width (left) and calculation of cell area (right) of isolated cardiomyocytes. (**D**) Immunofluorescence for KI67 (left) and phosphorylated histone H3 (right) did not reveal a difference in cellular proliferation in the myocardium of control and mTOR-cKO mice.



Supplemental Figure 3. (**A**) Representative immunofluorescence images of heart sections processed with TUNEL to reveal apoptotic nuclei (arrows). Cardiomyocytes were decorated for α-sarcomeric actin (red) and nuclei were stained with DAPI (blue). (**B**) Representative heart sections stained with Malloy's trichrome to reveal collagen deposition. Increased interstitial fibrosis (turquoise) is evident in mTOR-cKO myocardium at 4 weeks (wks) post-TMX. (**C**) *4E-BP1* mRNA expression, by qRT-PCR, in mTOR-cKO and control myocardium at 4 weeks post-TMX (mean±SD, n=3-4 per group). (**D**) Immunohistochemical staining for 4E-BP1 in WT myocardium.



Supplemental Figure 4. (**A**) Experimental schedule of tamoxifen administration and transverse aortic constriction (TAC). ECHO, echocardiographic analyses. The pressure gradients were checked at week 4 post-TAC, before sacrifice. (**B**) Weights of the left ventricle (LV) and of hearts (HW) given as a ratio with tibial length (TL) after 2 weeks of TAC (3 weeks post-TMX) (n=5 per group). (**C**) Kaplan-Meier survival curves of mTOR-cKO subjected or not to transverse aortic banding (KO-TAC n=10 or KO-sham n=5, respectively) and of sham-operated WT-Cre (CTL-sham n=5) mice. Mortality of mTOR-cKO mice is significantly increased by pressure overload, with a 40% death rate after the first week of TAC. Time shown as weeks post-TMX.



Supplemental Figure 5. Analysis of 4E-BP1 knockout and mTOR/4E-BP1 double knockout mice. (**A**) [^{3}H]Leucine incorporation is enhanced in mice harboring deletion of *4E-BP1*. (**B**) 4E-BP1 knockout mice have improved inotropy and lusitropy at baseline and improved reactivity to dobutamine (DOB) (means±SD, n=6 for each group). (**C**) Representative photomicrographs of heart sections stained with Malloy's trichrome to evidence collagen deposition (turquoise) (original magnification, 20X). (**D**) Analysis of fibrosis at 4 weeks post-TMX (means±SD). (**E**) Representative photomicrographs of heart sections stained with TUNEL to evidence apoptotic nuclei (arrows) and decorated for α-sarcomeric actin (Red) (original magnification, 20X). (**F**) Analysis of apoptosis at 4 weeks post-TMX (means±SD).