Supplementary Data

Materials and Methods

Mice. *TNT-iCre* was constructed using the rat cardiac troponin T promoter (1) to drive the reverse tet activator (Clontech) This was coinjected into oocytes along with Cre driven from a tet activator-dependent promoter. Mice were on a mixed strain background. Doxycycline (Dox) was provided in the drinking water at 1 mg/ml to induce the Cre recombinase in TNT-iCre mice. *Fog2*$^{fl/fl}$ (2), *Fog2*$^{Lz/+}$ (3), Fog2$^{+/-}$ (4), *Nkx2-5*^{Cre} (5), *Myh6-Cre* (6), *Tie2Cre* (7), *Rosa26*^{fsLz} (8), *Gata4*^{Ki} (9), *Wnt1Cre* (10), *Wt1*$^{GFPCre/+}$ (11), and *Tie2-Lz* (12) mice were described previously.

Histology. Tissues were fixed in 4% PFA and embedded in paraffin. 5 μm sections were cut and stained with either H.E. or Masson's trichrome stain.

Perfusion assays. Mice were administered 2 mg/kg biotinylated Bs-1 lectin (Vector) or 60 mg/kg hypoxyprobe-1 (NPI, Inc). Following injection, excised hearts were fixed with 4% PFA/PBS/0.1 M KCl, and processed for immunohistochemistry. MIBI uptake assay was measured by injecting 10 μCi ^{99m}Tc-MIBI via the tail vein. Sixty minutes following injection, cardiac uptake was measured with a scintillation counter. Cardiac uptake was expressed as the fraction of the injected dose that was taken up in the heart per mg heart weight. For microfil (Flow Tech) injection, microfil was prepared as recommended by the manufacturer and injected into the LV after ligation of the ascending aorta. Injections were done blinded to genotype. Hearts were cleared in methyl-salicylate, as recommended (Flow Tech).

Antibodies. Antibodies were used at 1:100 dilution except as indicated, were from the following sources: FOG2 and HSP47 (Santa Cruz), activated caspase-3 (Cell Signaling), FLK1 (BD Biosciences), GFP (Invitrogen), Hypoxyprobe-1 (NPI), Collagen III (Southern Biotech), SMA (Sigma), Bs-1 lectin (Vector Labs), β-gal (MP Biochemicals, 1:5000), and RALDH2 (1:1000; gift from Peter McCaffery, University of Aberdeen, U.K.).

Wholemount staining. For coronary staining, E13.5-14.5 embryonic hearts were fixed in Zinc fixative (BD Pharmingen) overnight at 4°C. Tissues were bleached in 3% hydrogen peroxide for 1 hour, followed by blocking with 5% normal donkey serum (Jackson Immunoresearch) and 0.1% Triton X-100 (Sigma) in PBS. Tissues were incubated with 1:100 biotinylated PECAM antibody (BD Pharmingen) overnight at 4˚C. After washing, samples were developed with the ABC method using DAB chromagen (Vector Labs). Coronary coverage was measured on whole mount images as the ratio of area covered by coronary vessels to ventricular area. Measurements were made by a blinded observed.

Electronic microscopy. Embryos were fixed overnight (2.5% glutaraldehyde, 2% paraformaldehyde in 0.1 M Phosphate buffer at pH 7.4), postfixed (1% OsO4 in 0.1 M phosphate buffer), washed, dehydrated, and infiltrated with epon-araldite (13). Ultrathin sections were imaged by transmission electron microscopy.

In vitro EMT assay. Collagen gel was made in 1 mg/ml collagen in M199, followed by equilibration with Optimem containing 1% insulin-transferrin-selenium (ITS, Invitrogen), 1% rat serum and antibiotics (Invitrogen). Embryonic E12.5 hearts apexes were dissected in sterile HBSS (Invitrogen) and plated on drained collagen gels for 24 hours. Subsequently, the explants were incubated in M199 supplemented with 5% FBS for 5 days, with medium changed every other day.

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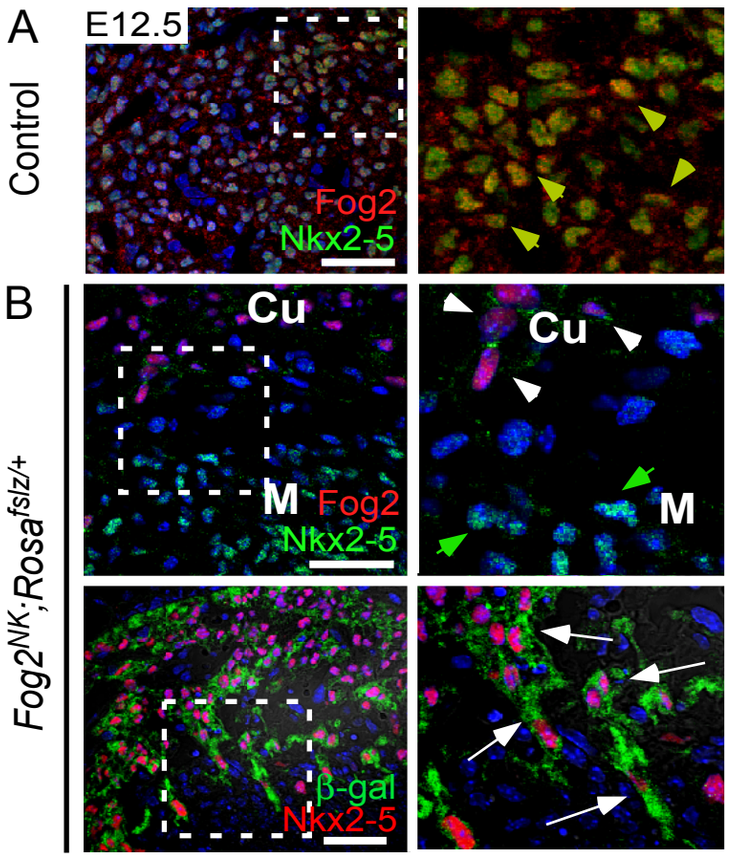
Supplementary Table 1. EKG of Fog2 Knockout hearts

	RR	PR	QRS	QTC
Fog2^{MC}	0.25 ± 0.03	0.05 ± 0.00	0.02 ± 0.00	1.32 ± 0.14
Controls	0.24 ± 0.02	0.04 ± 0.00	0.02 ± 0.00	1.22 ± 0.11

Note: *Fog2*^{MC}: *Fog2*$^{flox/flox}$*;Myh6-CCre*; Controls: *Fog2*$^{flox/+}$*;Myh6-Cre* or *Fog2*$^{flox/-}$*;Myh6-Cre*; n=3-5 for each group.

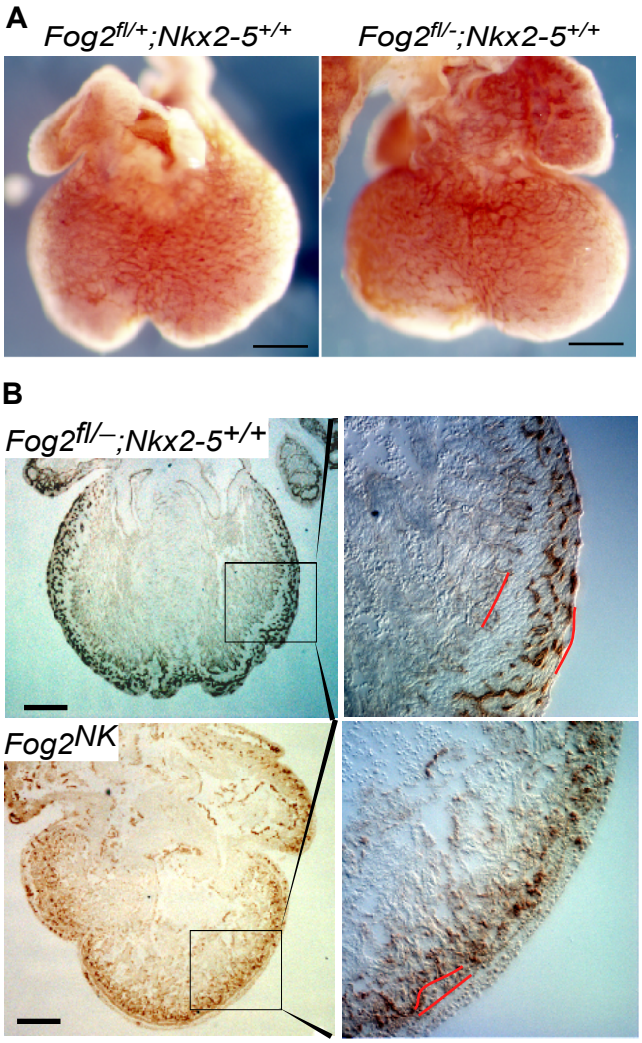
Supplemental Table 2

GENES	Forward	Backward
ANF	GGCCATATTGGAGCAAATCCTGTG	CATGACCTCATCTTCTACCGGCAT
BNP	TGGGAAGTCCTAGCCAGTCTC	CTGTCTCTGGGCCATTTCCT
SERCA2	AATCTCCTTGCCTGTGATCC	CGGGTTGTTCCAGGTAGTTT
GATA4	CCCTGGAAGACACCCCAAT	TGGACATGGCCCCACAAT
FOG2	TGGGTCAGCTTGATGGTAAA	GGAGATGGTT AGC
CTF1	CCACCAGACTGACTCCTCAA	CTGTTGCTGCACGTATTCCT
KCNE1	CCCTGTCCTGGAAAGATTGT	GGACAGGGAAGACAGAGAGC
FOXC1	TTTCCTGCTCATTCGTCTTG	CTTTCCCGTTCTTTCGACAT
VEGFA	GAGGATGTCCTCACTCGGAT	TCTCAGACCACACTGAAGCC
VEGFB	AGCCACCAGAAGAAAGTGGT	GCTGGGCACTAGTTGTTTGA
VEGFC	CCCAAACCAGTCACAATCAG	GGTAATGTTGCTGGCAGAGA
FGF1	AGCTTTCTCCCAAGAGACCA	TCATGGCGTTTGTGTCCTAT
FGF2	GCTGCTGGCTTCTAAGTGTG	TACTGCCCAGTTCGTTTCAG
FGF9	GCGGTGGGTTCTTATTGATT	AAATTGGCAAGTCCTCATCC
FGF12	ATTCCTCAACCCTGTATCGC	ATGAGATGAGGGCTTGGTTT
FGF16	CCATGACTCAAGGGAGCTTT	CTATGCCCAATCCTGAAGGT
THBS	CCCTGATGGTAGCTGGAAAT	CTCATCGACGTCTTTGCACT
TIMP1	ATATCCGGTACGCCTACACC	GCCCGTGATGAGAAACTCTT
TIMP2	CAACGAGACCCTGCAGTCTA	GAAATCAGCAGAAACAGGCA
HPSE	CGATGTCTGTAGTGCTGGCT	TCTGATTGCTGCTGGATCTC
COL4A3	TTAAGTTCAGGCTGGTGCTG	TTAAGTTCAGGCTGGTGCTG
COL15A1	GCAATGAGGATCTGCTGAGA	CATCAGTTCCTGGTTTCCCT
COL18A1	CATGAGGGCAGTCCATACAC	GGTGCACATAGGAACTGTGG
CTGF	TGTGTGACGAGCCCAAGGA	TTGGGTCTGGGCCAAATGT
FN1	GTTGTCTGACGCTGGCTTTA	AATCGCATCTGAAATGACCA
GAPDH	detected with a Taqman based Assay (Applied Biosystems)	



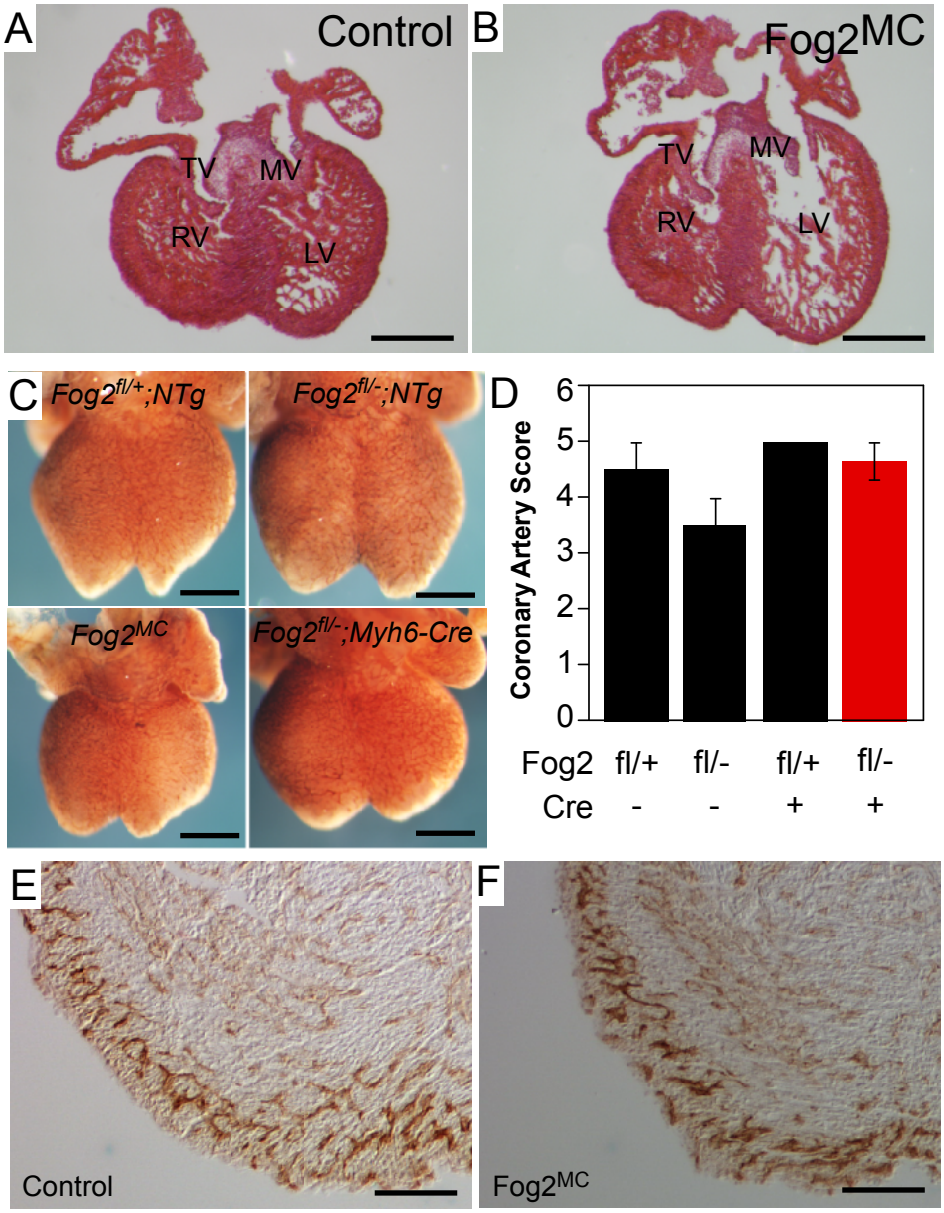
Supplemental Figure 1, Zhou et al.

Loss of FOG2 in *Fog2*^{NK} heart at E12.5. (**A**) FOG2 (red) was expressed in cardiomyocytes (yellow arrowheads) in control heart (*Fog2*$^{fl/-}$;*Nkx2-5*$^{+/+}$). (**B**) FOG2 was not detected in myocytes of *Fog2*^{NK} heart (green arrowheads), while it was still expressed in endocardial cushion mesenchymal cells (Cu, white arrowheads). The Cre-activated reporter *Rosa*^{fsLz} showed robust expression in NKX2-5^{+} cardiomyocytes (white arrows). Bar= 50 μm.

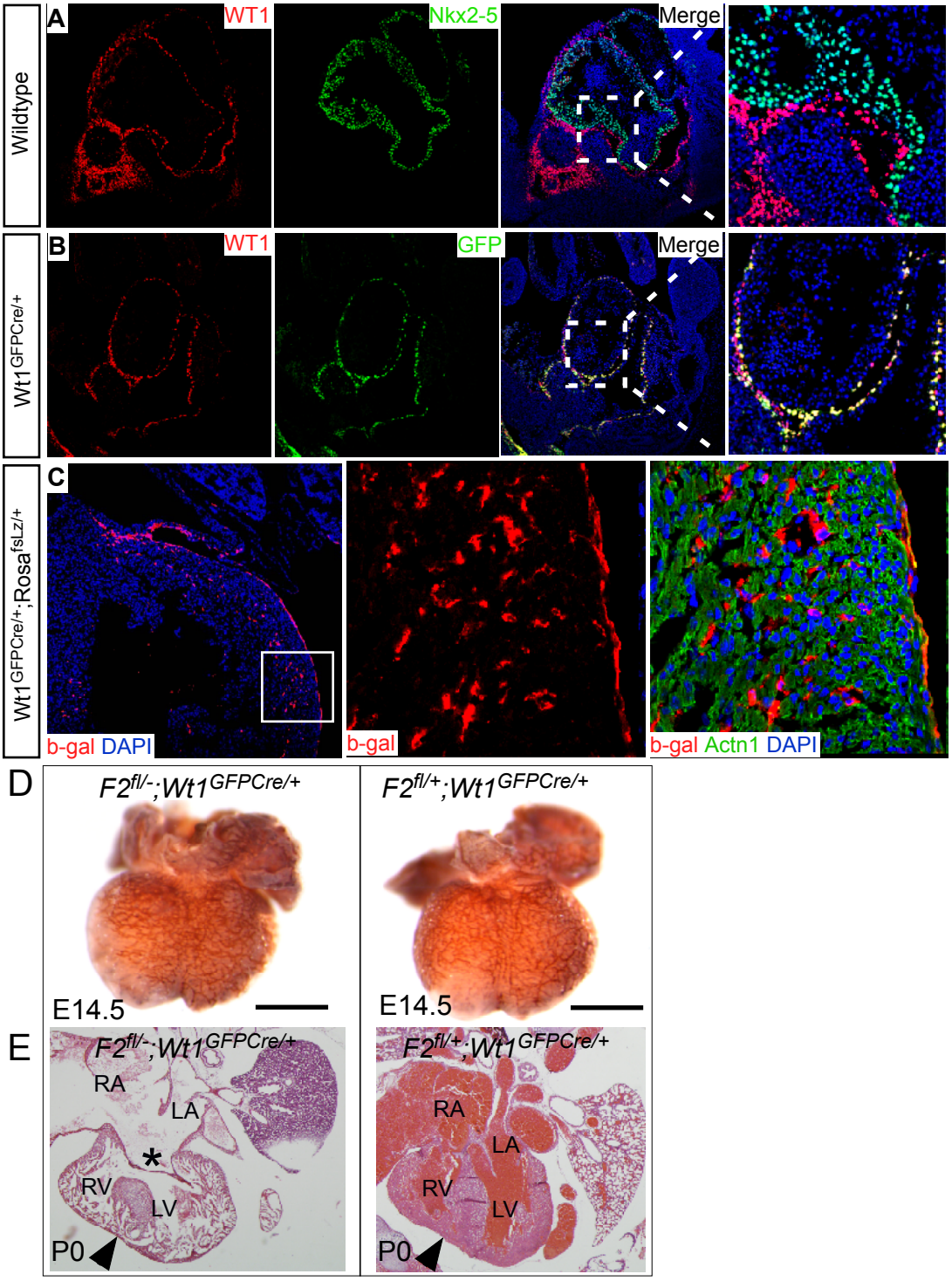


Supplemental Figure 2, Zhou et al.

Coronary plexus in *Fog2*^{NK} and littermate controls. (**A**) PECAM wholemount staining of two control genotypes. (**B**) Section of PECAM wholemount stained hearts demonstrating a paucity of coronary vessels in *Fog2*^{NK} compared with littermate control. The boundaries of the compact myocardium are indicated by red lines. Bar = 200 μm.

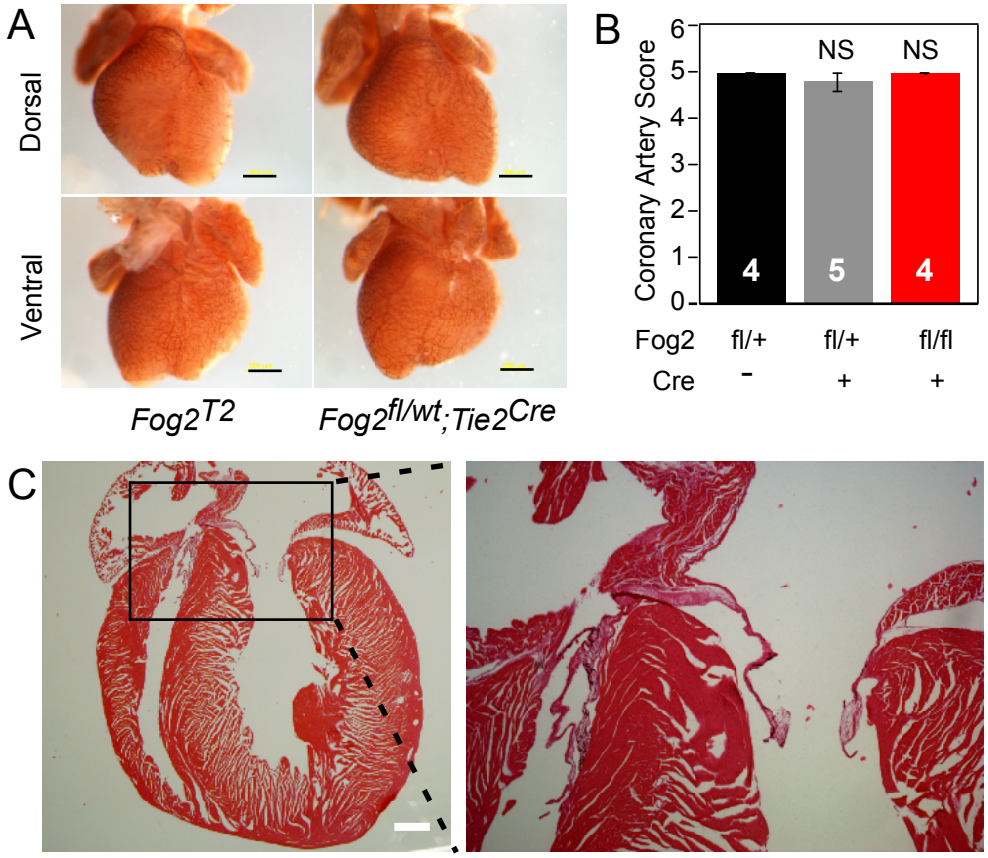


Supplemental Figure 3, Zhou et al.
Late deletion of *Fog2* in cardiomyocytes results in normal heart development. (**A-B**) H.E. staining of *Fog2*^{MC} and littermate control *Fog2*$^{fl/-}$*;NTg*. Bar=200 μm. (**C-D**) PECAM wholemount staining of *Fog2*^{MC} hearts and littermate controls. There was no difference between groups. n=3-5. Bar=200 μm. (**E-F**) Sections of wholemount stained *Fog2*^{MC} heart showed no defect in intramyocardial coronary vessels compared with littermate control *Fog2*$^{fl/-}$*;NTg*. Bar=50 mm.

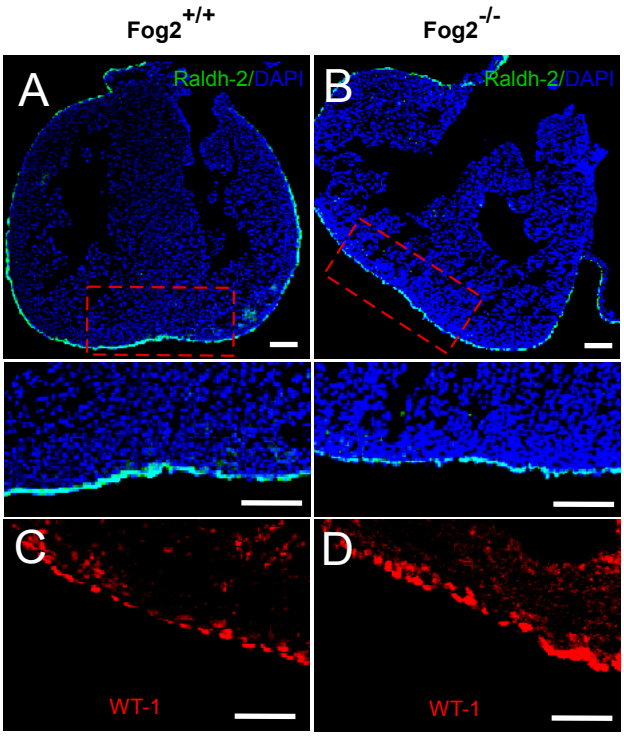


Supplemental Figure 4, Zhou et al.

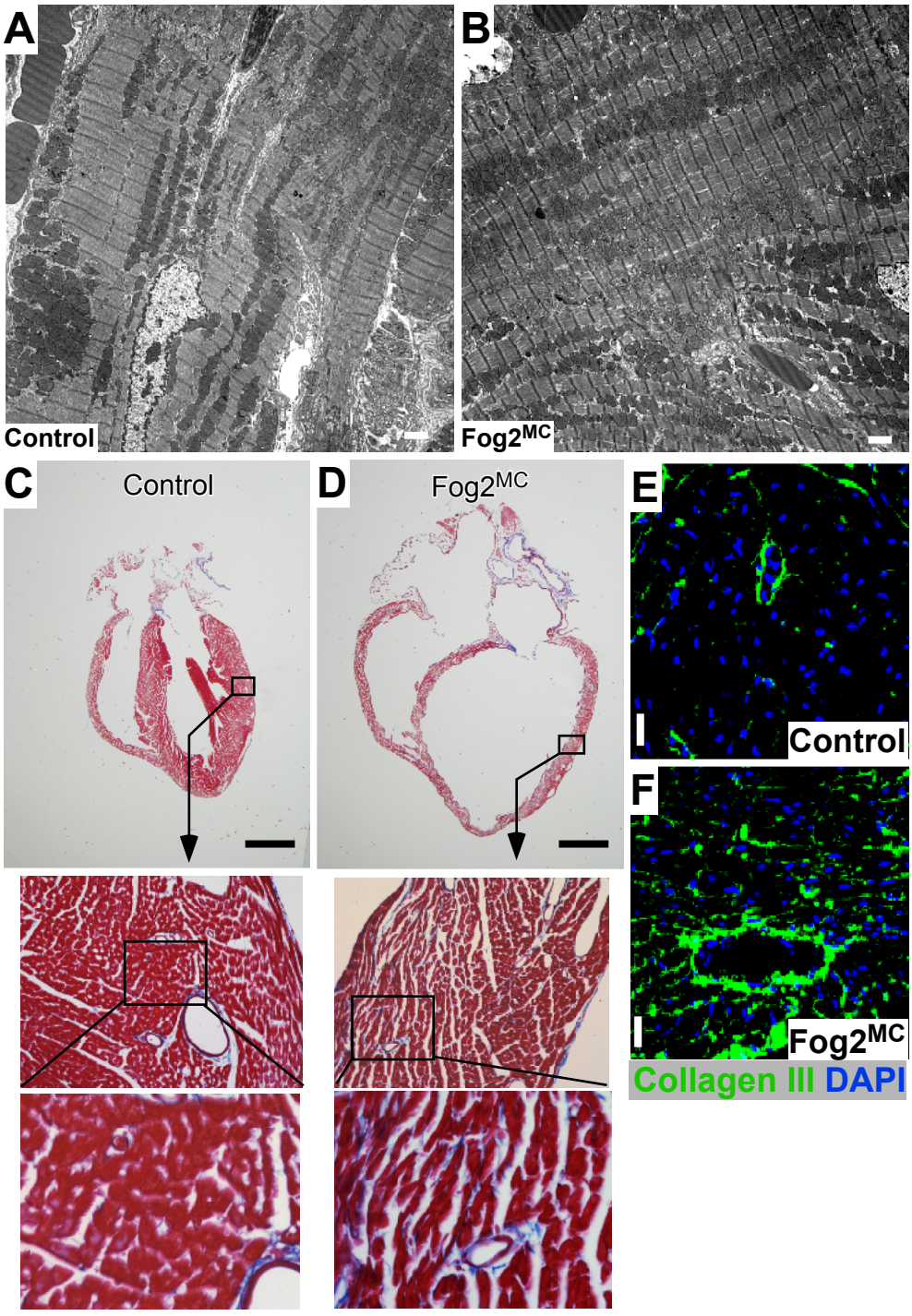
Epicardial recombination by *Wt1*^{GFPCre} and epicardial inactivation of Fog2 (**A**) WT1 (red) was expressed in epicardium, but not in NKX2-5^{+} myocardium (Green). (**B**) In *Wt1*$^{GFPCre/+}$ heart, GFP and WT1 were colocalized in epicardium, demonstrating that GFPCre expressed from the WT1 locus recapitulated endogenous WT1 expression. (**C**) EPDC, labeled by b-gal in *Wt1*$^{GFPCre/+}$; *Rosa26*$^{fsLz/+}$ mice, were found within the myocardium. (**D-E**). PECAM whole mount and H.E. section staining of hearts after inactivation of Fog2 by Wt1GFPCre (left), or control (right). Coronary vasculogenesis was normal, but pups died perinatally with thin myocardium (black arrowhead) and common atrioventricular canal (asterisk). D, E14.5; E, postmortum of P0 pups.



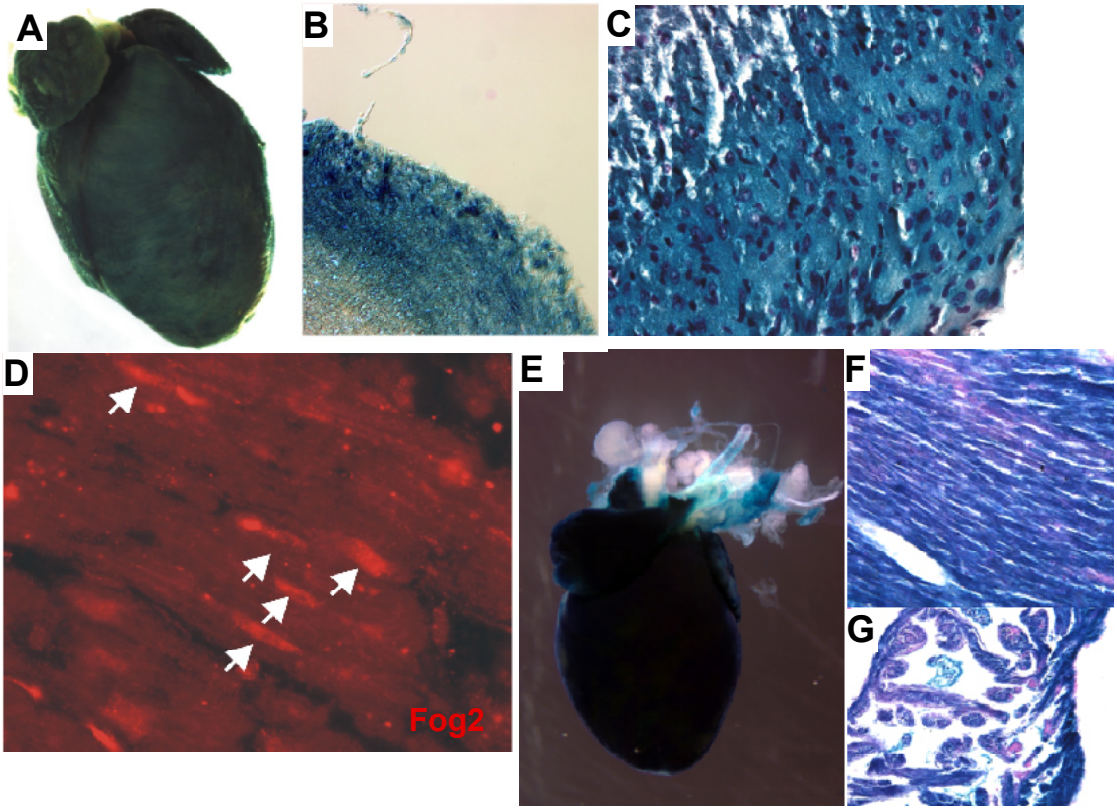
Supplemental Figure 5, Zhou et al.
Endothelial deletion of Fog2. (**A-B**) PECAM whole mount staining and quantitation of them showed coronary vasculogenesis was unperturbed in *Fog2*^{T2} mutant hearts. Bar= 300 μm. (**C**) H.E. staining of adult *Fog2*^{T2} heart. No defects were observed. Bar=500 μm.



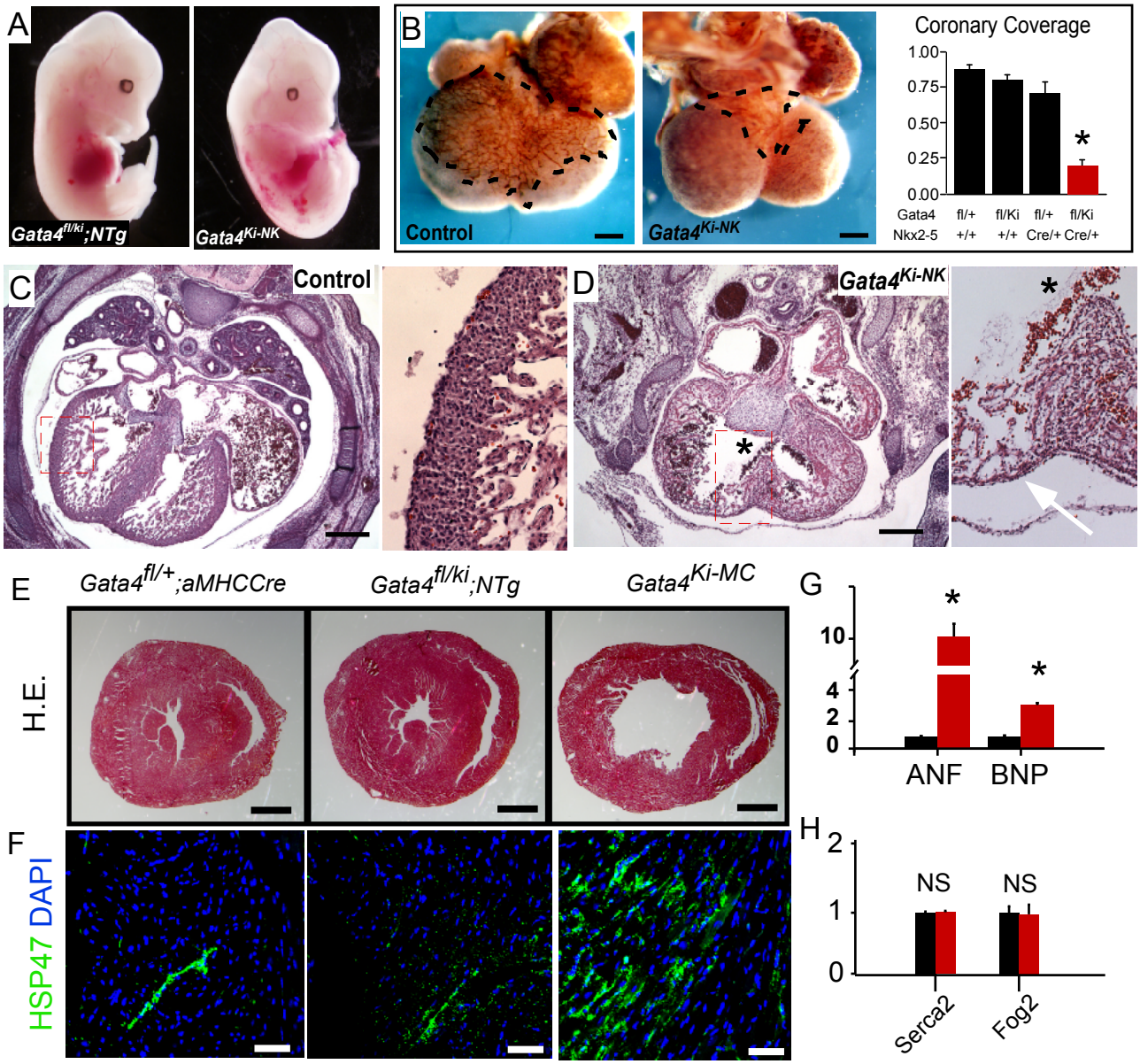
Supplemental Figure 6, Zhou et al.
Intact epicardium in *Fog2* null heart. RALDH2 (green; **A-B**) and WT1 (red, **C-D**) expression in $Fog2^{-/-}$ and $Fog2^{+/+}$ epicardium was indistinguishable. Bar = 50 μm.



Supplemental Figure 7, Zhou et al.
Characterization of *Fog2*^{MC} myocardium. (**A-B**) Electronic microscopy showed no detectable ultrastructural change in *Fog2*^{MC} compared with littermate control *Fog2*$^{fl/-}$*;NTg*. Bar=2 μm. (**C-D**) Trichrome staining of *Fog2*^{MC} and control hearts demonstrated increased fibrosis in mutants. (**E-F**) Increased collagen III deposition in *Fog2*^{MC} hearts. (**J**) Quantitation of activated caspase3^{+} endothelial cells. n=3, **P*<0.05.

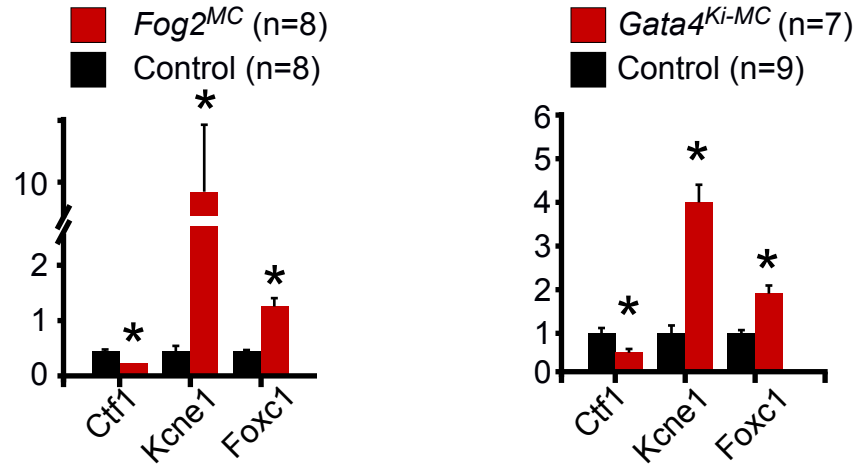


Supplemental Figure 8, Zhou et al.
(**A**) Wholemount X-gal staining of *Fog2-Lz* adult heart. (**B-C**) Sections of *Fog2-LZ* adult heart after wholemount X-gal staining. (**D**) Fog2 (red) was expressed in nucleus of cardiomyocytes (white arrows). (**E**) Wholemount X-gal staining of *iTNT-Cre;Rosa26*^{fsLz} Dox-treated postnatal heart. (**F-G**) Sections of heart in (E) showed X-gal^{+} cardiomyocytes.



Supplemental Figure 9, Zhou et al.

Early loss of FOG2-GATA4 interaction. (**A**) At E12.5, *Gata4*$^{Ki\text{-}NK}$ embryos showed edema and peripheral hemorrhage. (**B**) Reduced coronary plexus in *Gata4*$^{Ki\text{-}NK}$ mutants, demonstrated by wholemount PECAM staining of E13.5 embryo hearts. n=3-5 per group. Bar=100 μm. (**C-D**) H.E. staining of E14.5 embryos showed AV cushion defect, VSD (asterisk) and thin myocardium (white arrows). Bar=500 μm. (**E**) Short axis adult heart sections stained with H.E. *Gata4*$^{Ki\text{-}MC}$ mice had enlarged and dilated ventricles compared with controls. Bar=1 mm. (**F**) Fibrosis marker HSP47 staining was significantly increased in *Gata4*$^{Ki\text{-}MC}$ hearts compared with controls. Bar=50 μm. (**G-H**) Expression of heart failure-related genes in *Gata4*$^{Ki\text{-}MC}$ heart apexes (n=7) compared with controls (n=9). *, $P<0.05$



Supplementary Figure 10. Zhou et al.
Differential expression of genes downstream of FOG2-GATA4. A subset of genes differentially expressed in Fog2MC hearts and of interest to cardiomyocyte function were validated by qRTPCR. *, $P < 0.05$.