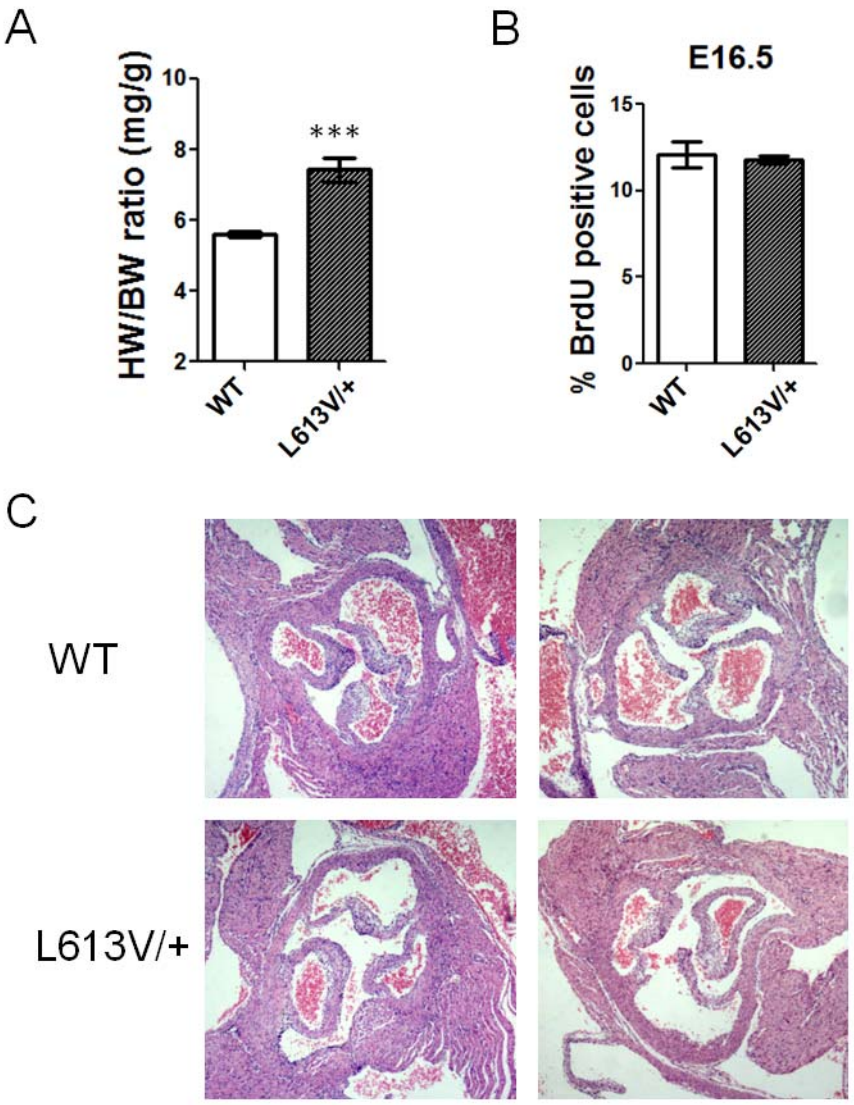
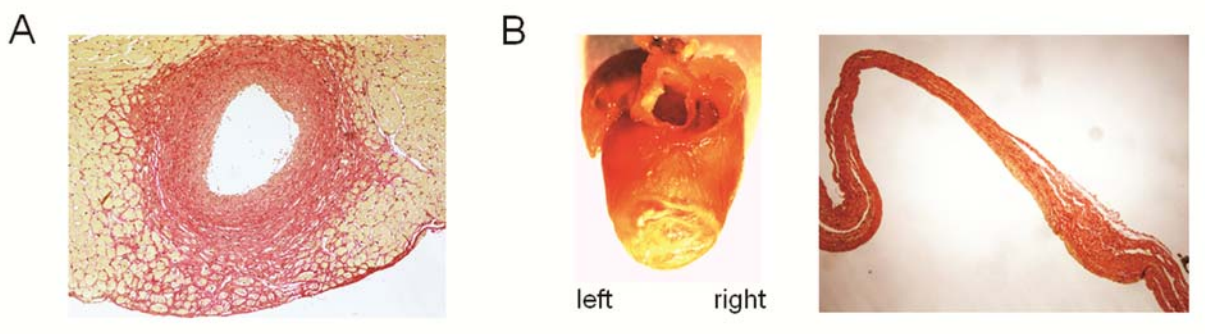
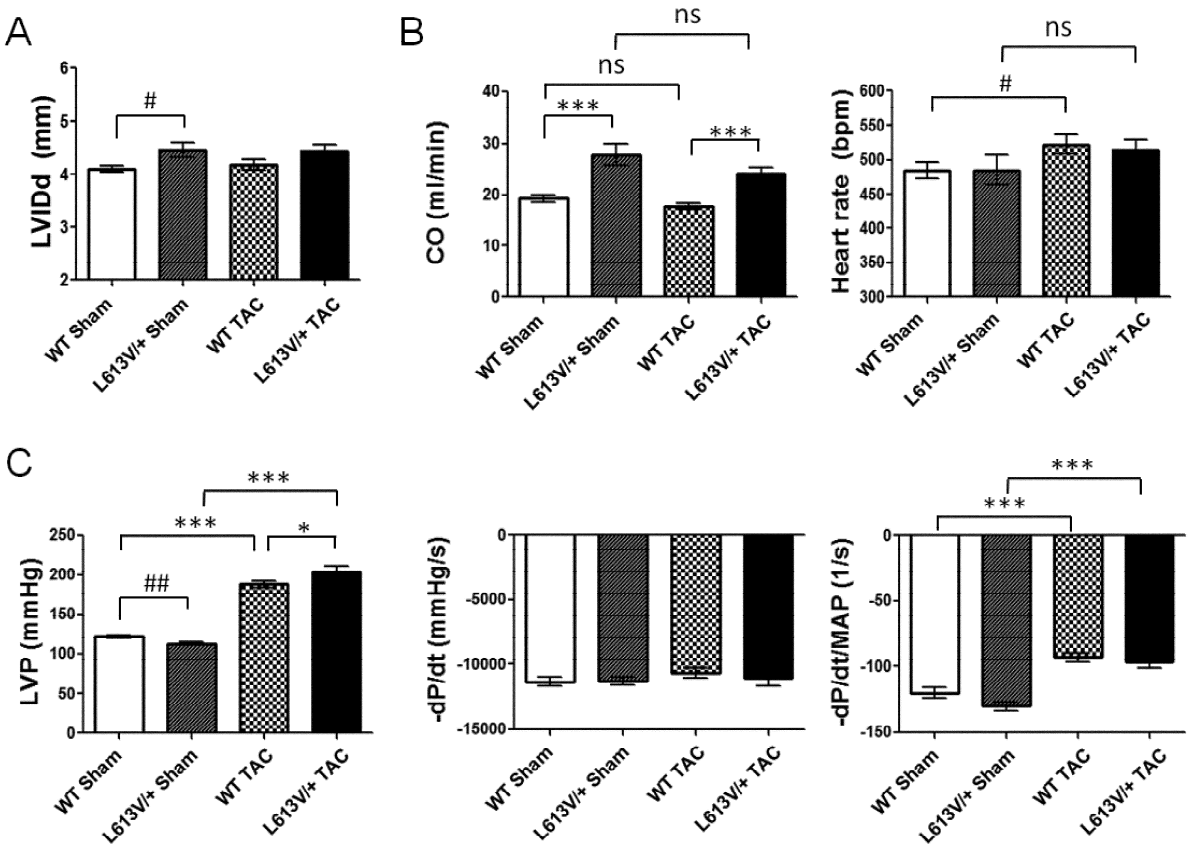


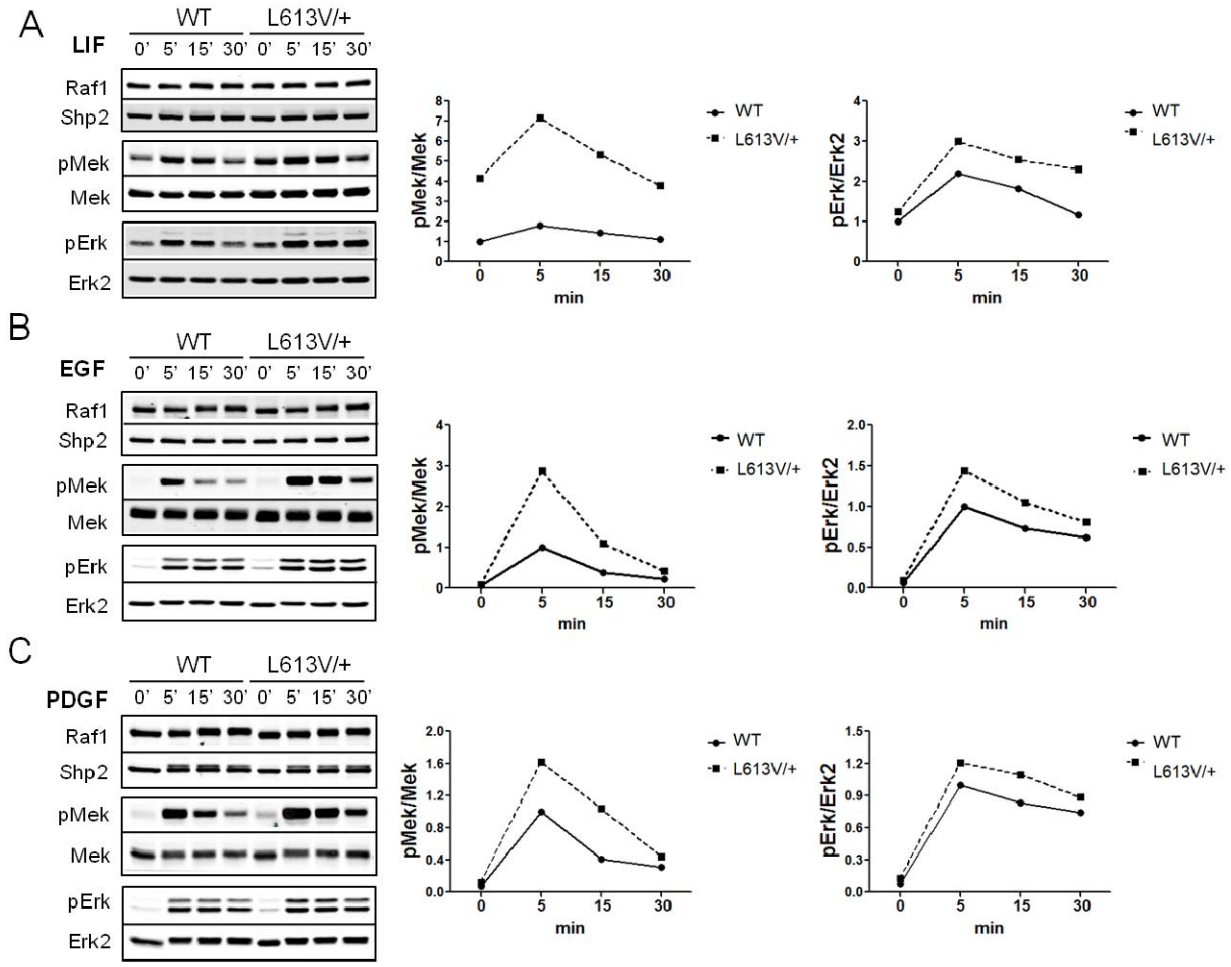
Supplemental Figure 1. Normal cardiomyocyte proliferation and valve development in L613V/+ mice. (A) L613V/+ mice show cardiac hypertrophy, as indicated by heart weight/body weight ratio (mg/g) as early as 2 weeks after birth (n=14 for each genotype). *** $p<0.0001$, 2-tailed Student's t-test. (B) No difference in BrdU incorporation in E16.5 WT (n=4) and L613V/+ (n=3) hearts. (C) Representative H&E-stained cross-sections of aortic valves in 1 week-old mice (original magnification, 40X; two individual samples are shown for each genotype). No obvious abnormalities were noted in other cardiac valves either.



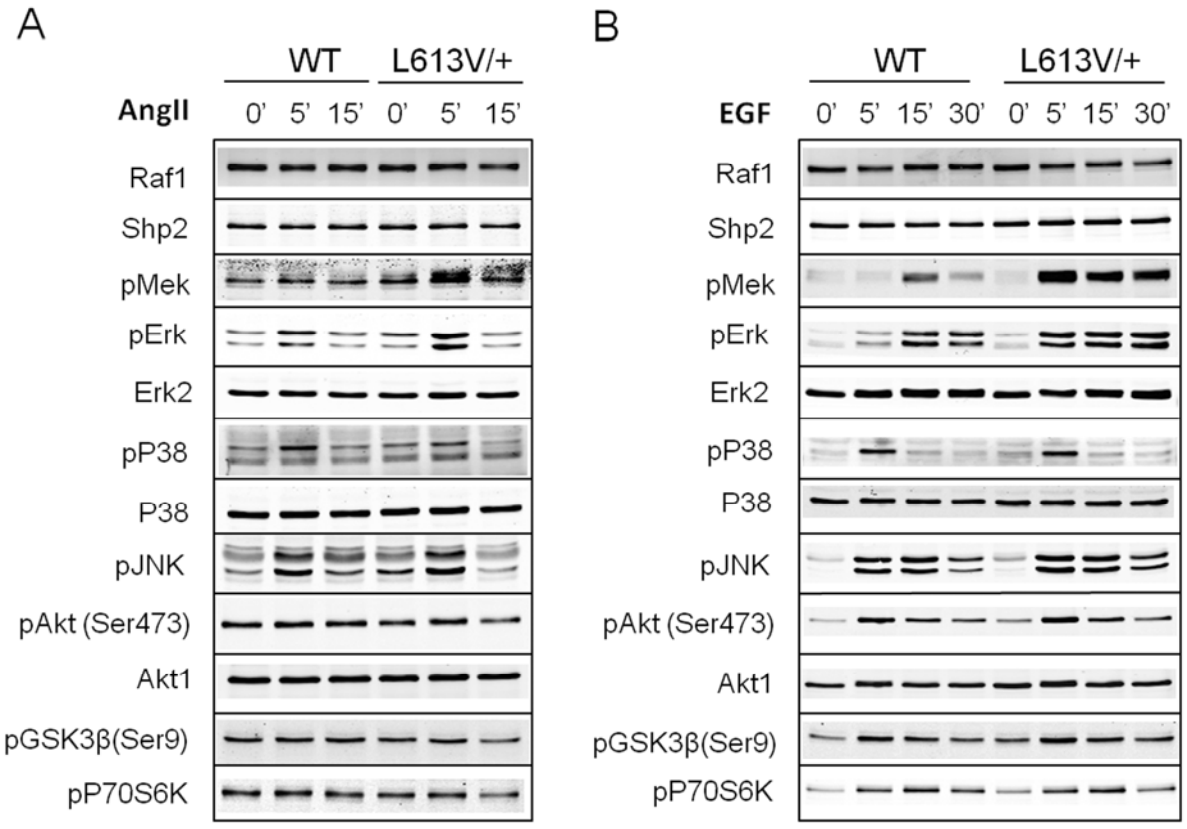
Supplemental Figure 2. Severe perivascular fibrosis and infarct in L613V/+ mice after TAC (A) Severe perivascular fibrosis in L613V/+ heart after TAC (PSR staining; original magnification, 200X). (B) Gross appearance of an LV/+ heart (left) with a severe infarct after TAC, and severe fibrosis (right) in the infarcted region (PSR staining; original magnification, 100X).



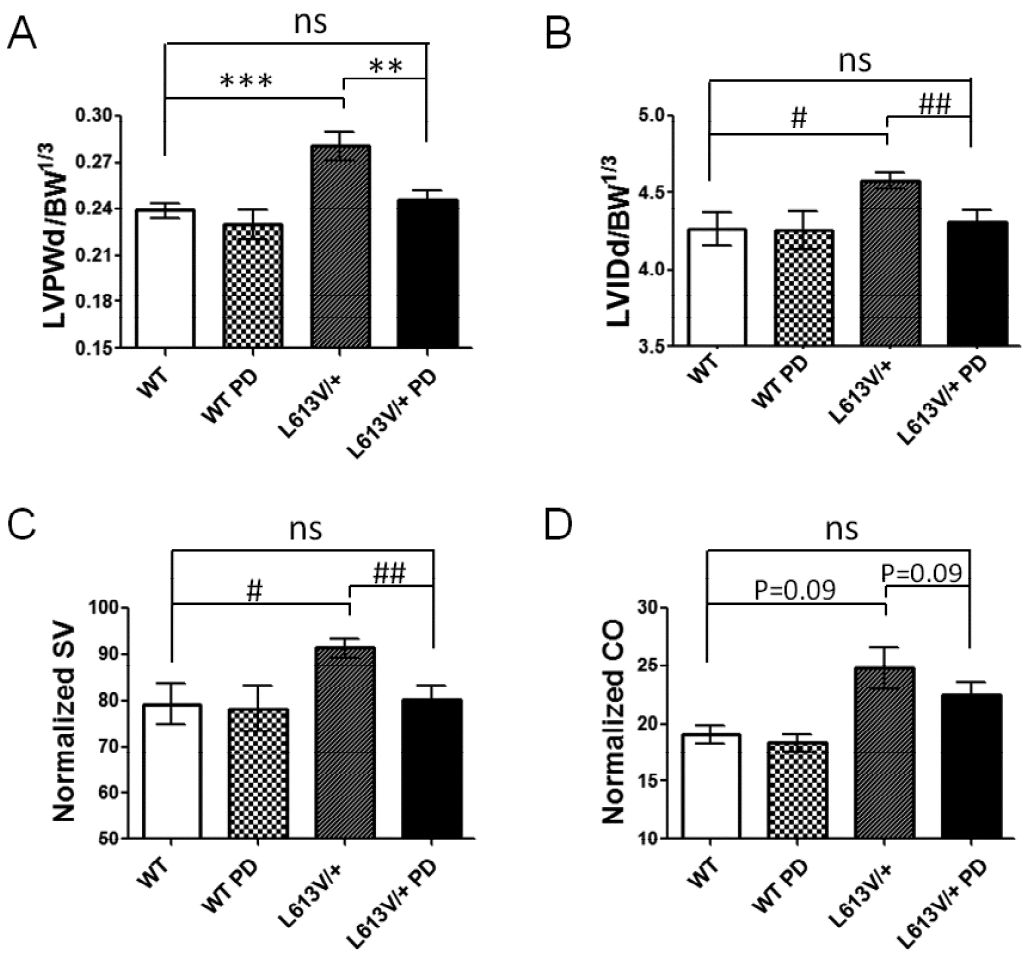
Supplemental Figure 3. Hypertrophic response after TAC (A, B) Additional echocardiographic parameters of hearts after TAC. LVIDd, left ventricular internal end-diastolic dimension; CO, cardiac output. (C) Additional invasive hemodynamic parameters of hearts after TAC. LVP, left ventricular systolic pressure; MAP, mean arterial pressure. * $p<0.05$; *** $p<0.0001$ (Bonferroni post-test when ANOVA is significant); # $p<0.05$; ## $p<0.005$ (1-tailed Student's t-test); ns=not significant.



Supplemental Figure 4. Raf1^{L613V} mutant causes increased Mek and Erk activation in multiple cell types. (A) WT and L613V/+ ES cells were removed from feeders, starved for 6 hr, and then stimulated with LIF (10^3U/ml) or left unstimulated (0'). Cell lysates (20µg protein) were resolved by SDS-PAGE, and analyzed by immunoblotting with the indicated antibodies. One of two experiments with comparable results is shown. (B, C) Primary mouse embryo fibroblasts (MEFs) from WT and L613V/+ mice were starved for 16 hr, and then either stimulated with 10 ng/ml EGF (n=4; two independent experiments in two different MEF strains) or 50 ng/ml PDGF (n=2; one experiment in each of two different MEF strains). Quantification of blots from all experiments is shown at right.



Supplemental Figure 5. Other signaling pathways are unaffected in neonatal cardiac myocytes and fibroblasts. (A) Cardiomyocytes from neonatal WT and L613V/+ mice were starved for 24 hr, and then either left unstimulated (0') or stimulated with AngII (1µg/ml). (B) Cardiac fibroblasts from neonatal WT and L613V/+ mice were starved for 16 hr, and then either left unstimulated (0') or stimulated with EGF (10ng/ml). Cell lysates (15-20µg protein) were analyzed by immunoblotting with the indicated phospho-specific antibodies for other pathways implicated in cardiac hypertrophy.



Supplemental Figure 6. Normalized cardiac morphology and function after MEK inhibitor treatment. (A) Left ventricular diastolic posterior wall thickness (LVPWd) normalized by $BW^{1/3}$. (B) Left ventricular internal end-diastolic dimension (LVIDd) normalized by $BW^{1/3}$. (C) Normalized stroke volume (SV). End-diastolic volume (EDV) = (4.5 x normalized $LVIDd^2$); End-systolic volume (ESV) = (3.72 x normalized $LVIDd^2$); SV=EDV-ESV. (D) Normalized cardiac output (CO). CO=Normalized SV X Heart rate. ** $p<0.005$; *** $p<0.0001$ (Bonferroni post-test when ANOVA is significant); # $p<0.05$; ## $p<0.005$ (1-tailed Student's t-test); ns=not significant. n=14 for WT control; n=10 for LV/+ control; n=6 for WT treatment (WT PD); n=14 for L613V/+ treatment (L613V/+ PD).

PCR Screening	sense	5’-TCCAGCTAATTGACATTGCCCGACAGACAGCTCAG-3’
	antisense	5’-GAACGGGTTGTCATCCTGCATCCGGATTACTTCTG-3’
Neo probe	sense	5’-GGA TTG CAC GCA GGT TCT CCG-3’
	antisense	5’-CGC CGC CAA GCT CTT CAG CAA-3’
5’ probe	sense	5’-TGC TCT GGA GCT CAA ACC CTC AGT GTA G -3’
	antisense	5’-CAT GGC TGA GTG GAC GGT CAG GCT G-3’
3’ probe	sense	5’-GAG ACG GCA GAT CCT CAG TAG TAC TTG-3’
	antisense	5’-ACG GTG GTA GTT GTG TCT TTG GCC ATG-3’
RT-PCR for Raf1 mRNA	sense	5’-TCT CCA TGA AGG CCT CAC GGTG-3’
	antisense	5’-AGA CTG GTA GCC TTG GGG ATG TAG-3’
Genotyping for $Raf1^{L613V}$	sense	5’-ATC CCC TGA TCT CAG CAG GCT CTAC-3’
	antisense	5’-AGT AGT CTA GGT CCT TAG CAG CAGC-3’
TaqMan probe Cat. No	*Myh6*	Mm00440359_m1
	Myh7	Mm00600555_m1
	Nppa	Mm01255748_g1
	Nppb	Mm00435304_g1
	Gapdh	4352932E

Supplemental Table 1. PCR primers.