SUPPLEMENTAL MATERIAL

Methods

CaMKII activity assay

CaMKII activity was measured using previously published protocols (1, 2). In brief, hearts were isolated, ventricles homogenized in lysis buffer, and immediately assayed in assay buffer containing 50 mmol/L HEPES, 10 mmol/L magnesium acetate, 1 mg/ml BSA, 20 μmol/L Syntide-2 (a synthetic CaMKII-specific peptide substrate), 1mmol/L DTT, 400 nmol/L [γ-^{32}P]ATP, pH 7.5, and 1 mmol/L EGTA (for measurement of autonomous activity) or 500 μmol/L $CaCl_2$ and 1 μmol/L calmodulin (for measurement of maximal activity). The reaction was carried out at 37°C for 10 min and blotted onto Whatman P81 phosphocellulose paper. Percent activation was calculated as the ratio of autonomous activity to maximal activity.

Nuclear fractionation

Ventricular tissue was fractionated using previously published protocols (1, 3). In brief, frozen ventricular tissue was homogenized in lysis buffer containing 70 mmol/L sucrose, 190 mmol/L mannitol, 20 mmol/L HEPES, and 200 μmol/L EDTA using a dounce tissue grinder. Lysates were centrifuged at 600g for 10 min at 4°C. Supernatants were aspirated and pellets washed three times by resuspending pellets with lysis buffer and centrifuging at 600g for 10 min at 4°C. Supernatants were aspirated, pellets resuspended in nuclear extraction buffer containing 20 mmol/L HEPES, 25% glycerol, 420 mmol/L NaCl, 1.5 mmol/L magnesium chloride, and 200 μmol/L EDTA and incubated on ice for 10 min. Nuclear lysates were then centrifuged at 600g for 10 min at 4°C and supernatants collected (nuclear fraction).

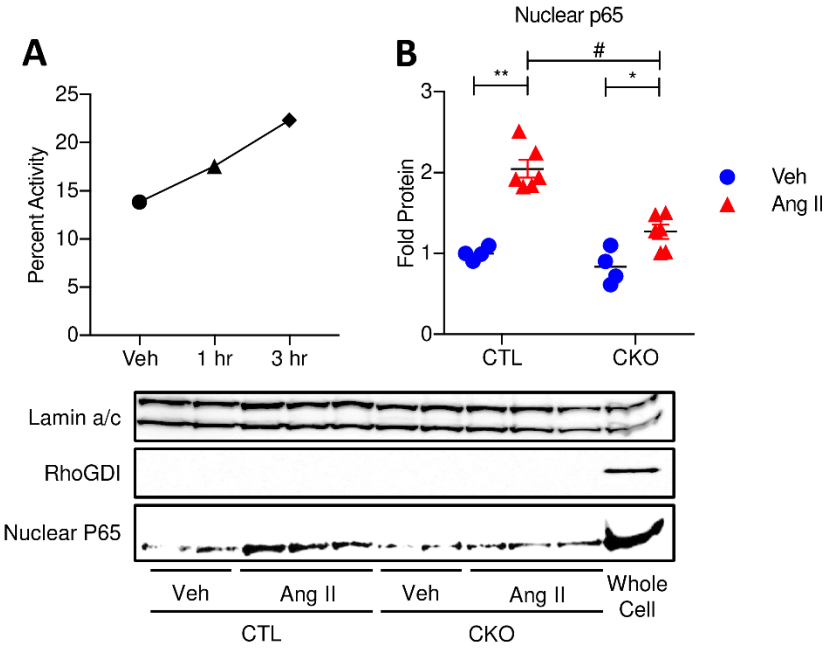
Blood pressure readings

Systolic and diastolic blood pressure was measured in un-anesthetized mice at baseline, 1, 3,

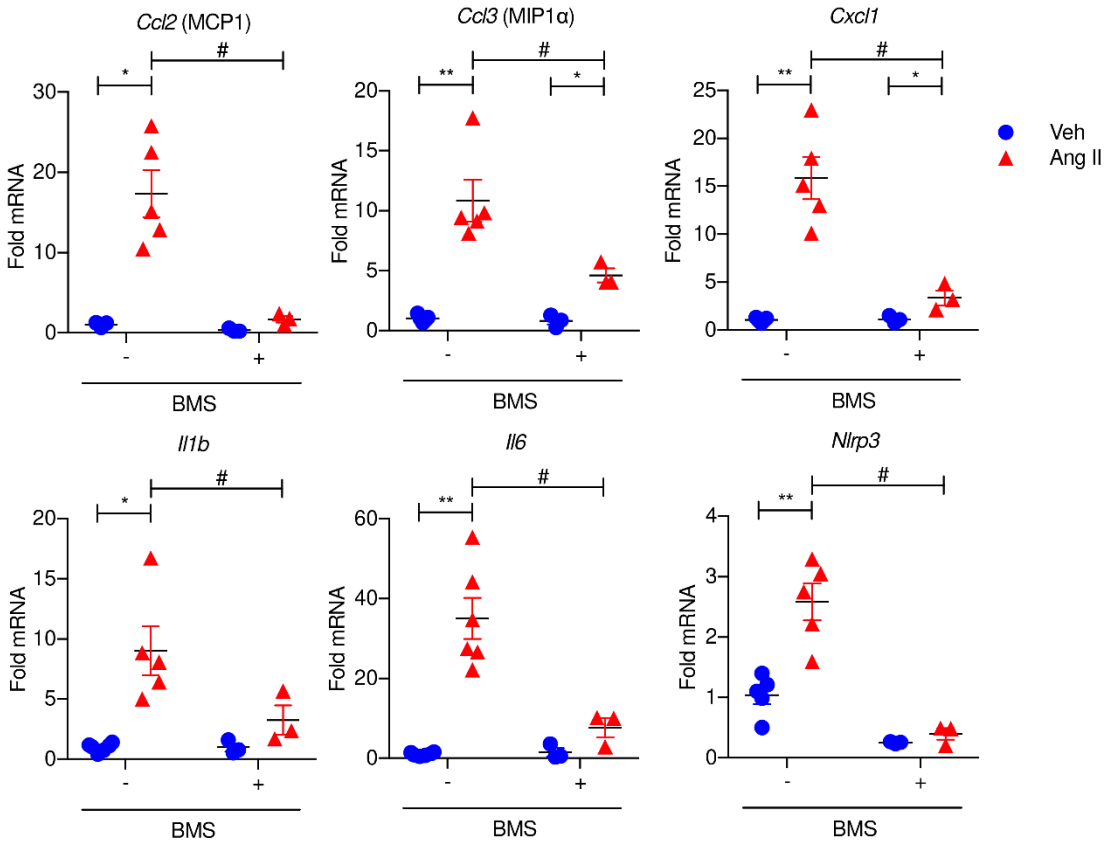
and 7 days Ang II infusion using a tail-cuff blood pressure system (CODA System, Kent Scientific). Data are presented as the average of ten cycle measurements per mouse.

Supplemental references

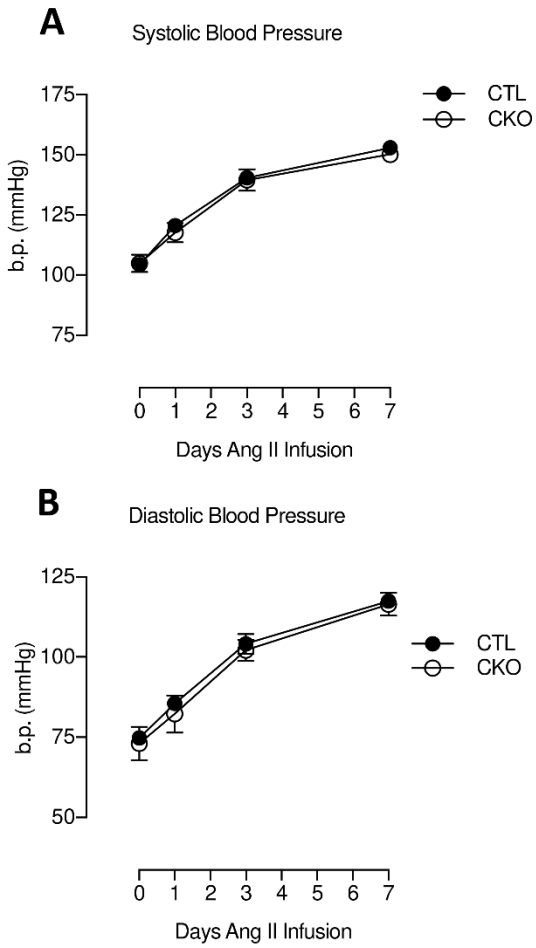
1. Gray CB, Suetomi T, Xiang S, Mishra S, Blackwood EA, Glembotski CC, et al. CaMKIIdelta subtypes differentially regulate infarct formation following ex vivo myocardial ischemia/reperfusion through NF-kappaB and TNF-alpha. *J Mol Cell Cardiol.* 2017;103:48-55.
2. Grimm M, Ling H, Willeford A, Pereira L, Gray CB, Erickson JR, et al. CaMKIIdelta mediates beta-adrenergic effects on RyR2 phosphorylation and SR Ca(2+) leak and the pathophysiological response to chronic beta-adrenergic stimulation. *J Mol Cell Cardiol.* 2015;85:282-91.
3. Miyamoto S, Purcell NH, Smith JM, Gao T, Whittaker R, Huang K, et al. PHLPP-1 negatively regulates Akt activity and survival in the heart. *Circ Res.* 2010;107(4):476-84.



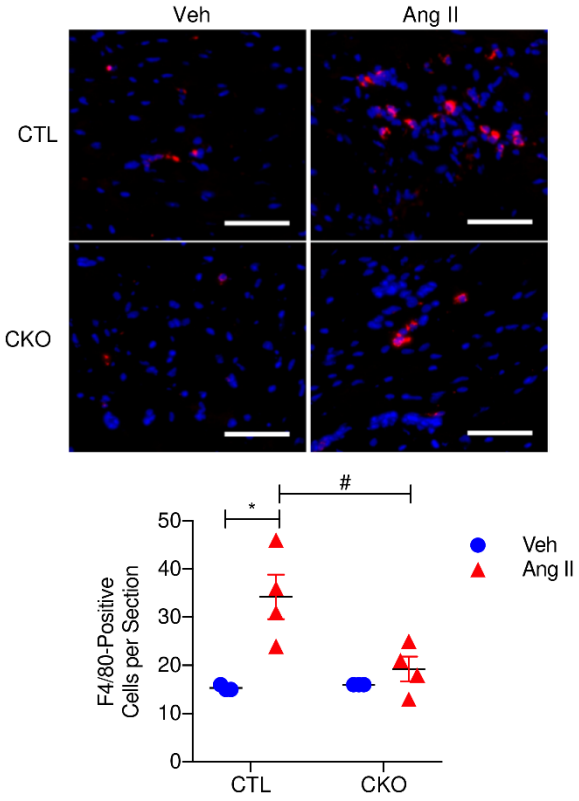
Supplemental Figure 1. Angiotensin II infusion activates CaMKIIδ and elicits NFkB signaling through CaMKIIδ. (**A**) Autonomous CaMKII activity measured in ventricular lysates of mice infused with saline (Veh) or Ang II for 1 and 3 hours using a ^{32}P enzymatic CaMKII activity assay. (**B**) Western blot and quantitation of the nuclear factor kappa B subunit P65 in nuclear fractions isolated from hearts of *Camk2d*$^{fl/fl}$ control (CTL) and cardiomyocyte-specific CaMKIIδ knockout (CKO) mice after 3 hours of saline (Veh) or Ang II (1.5 μg/kg/min) infusion (n=4-6 each group). Lamin a/c was used as a nuclear loading control and RhoGDI was used as a nuclear fraction purity control. Two-way ANOVA was used for all comparisons. **P*<0.05 vs Veh, ***P*<0.01 vs Veh, #*P*<0.05 CTL Ang II vs CKO Ang II.



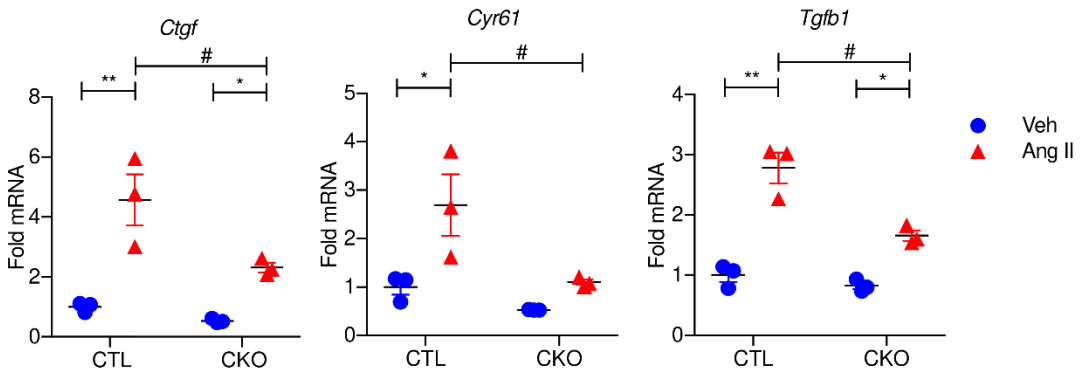
Supplemental Figure 2. Angiotensin II infusion induces inflammatory gene expression through NFkB. mRNA expression of monocyte chemoattractant protein 1 (*Ccl2*/MCP1), macrophage inflammatory protein 1α (*Ccl3*/MIP1α), C-X-C motif ligand 1 (*Cxcl1*), interleukin 1β (*Il1b*) interleukin 6 (*Il6*), and *NLRP3* in ventricular lysates of *Camk2d*$^{fl/fl}$ control mice infused with saline or Ang II (1.5 μg/kg/min) for 3 hours and injected intraperitoneally with water (-) or 45 mg/kg BMS-345541 (+) 1 hour prior to Ang II infusion (n=3-6 each group). Two-way ANOVA was used for all comparisons. **P*<0.05 vs Veh, ***P*<0.01 vs Veh, #*P*<0.05 Ang II vs Ang II + BMS.



Supplemental Figure 3. Angiotensin II infusion increases blood pressure to similar extents in *Camk2d*$^{fl/fl}$ control and cardiomyocyte-specific CaMKIIδ KO mice. Systolic (**A**) and diastolic (**B**) blood pressure measured in un-anesthetized mice at baseline, 1, 3, and 7 days Ang II (1.5 μg/kg/min) infusion (n=10 each group).

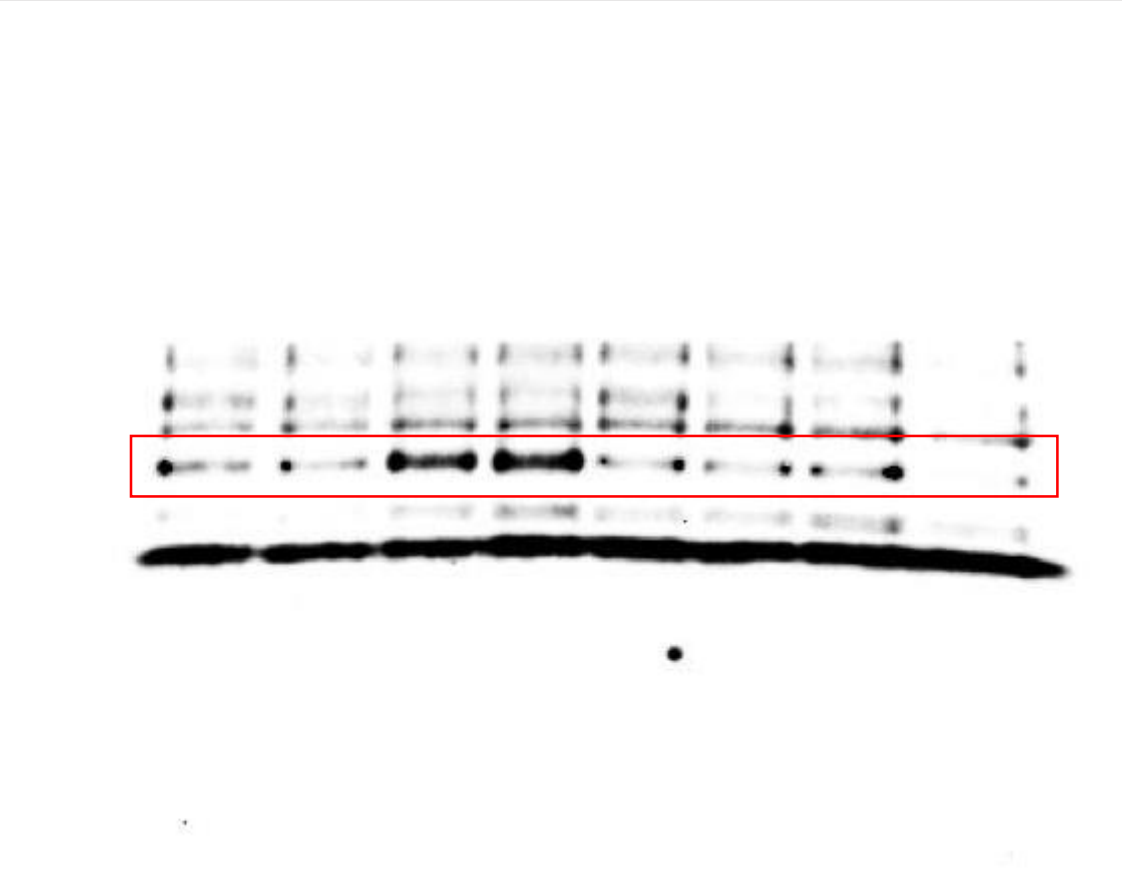


Supplemental Figure 4. Angiotensin II recruits F4/80-positive macrophages through cardiomyocyte CaMKIIδ. Representative pictures and quantitation of cardiac cryosections stained with antibody to the mature macrophage marker F4/80 taken from *Camk2d*$^{fl/fl}$ control (CTL) and cardiomyocyte-specific CaMKIIδ KO (CKO) mice infused with saline (Veh) or Ang II (1.5 μg/kg/min) for 1 day (n=3-4 each group). F4/80 is red and DAPI is blue. Two-way ANOVA was used for all comparisons. **P*<0.05 vs Veh, #*P*<0.05 CTL Ang II vs CKO Ang II. Scale bar=50 μm.



Supplemental Figure 5. Angiotensin II induces profibrotic mediator expression through CaMKIIδ signaling in the cardiomyocyte. mRNA expression of CCN family member 1/cysteine-rich angiogenic factor 61 (*Cyr61*), CCN family member 2/connective tissue growth factor (*Ctgf*), and transforming growth factor β1 (*Tgfb1*) in ventricular lysates of *Camk2d*$^{fl/fl}$ control (CTL) and cardiomyocyte-specific CaMKIIδ knockout (CKO) mice after 7 days of saline (Veh) or Ang II (1.5 μg/kg/min) infusion (n=3 each). Two-way ANOVA was used for all comparisons. **P*<0.05 vs Veh, ***P*<0.01 vs Veh, #*P*<0.05 CTL Ang II vs CKO Ang II.

Full unedited blot for Figure 1C



MCP-1

GAPDH

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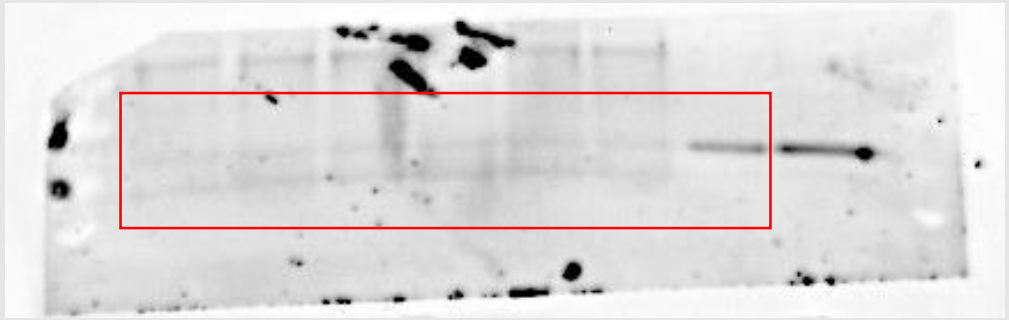
Full unedited blot for Figure 2C



Cleaved Caspase 3

GAPDH

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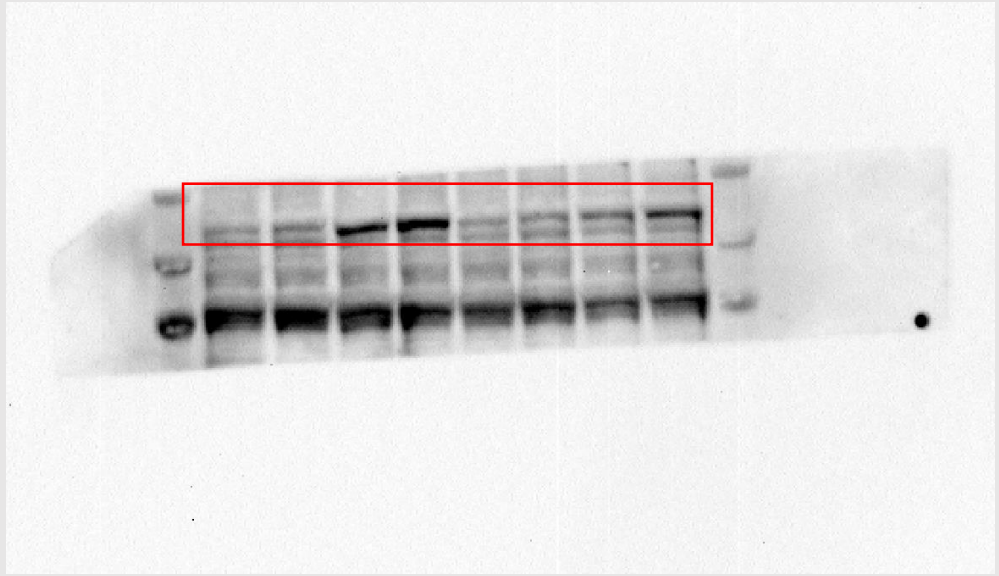
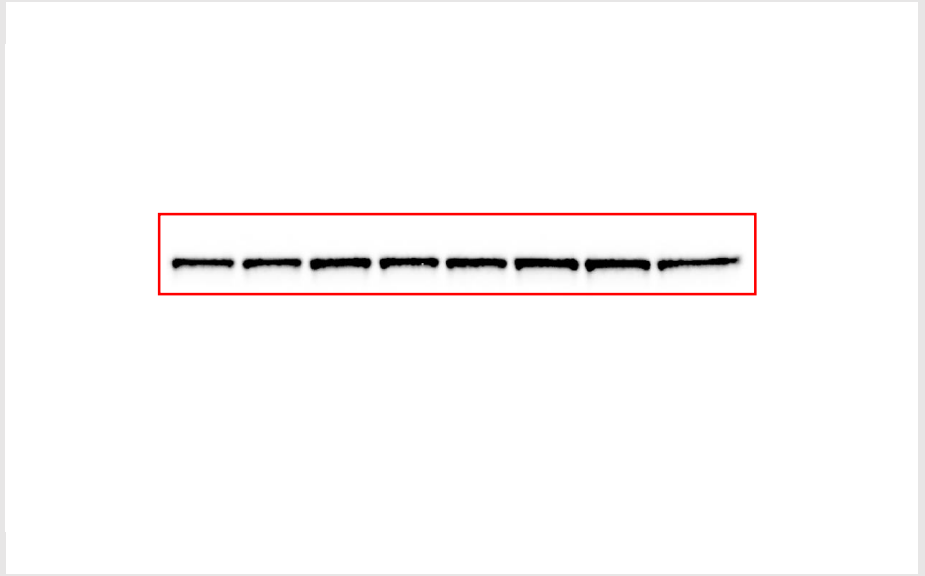
Full unedited blot for Figure 4A





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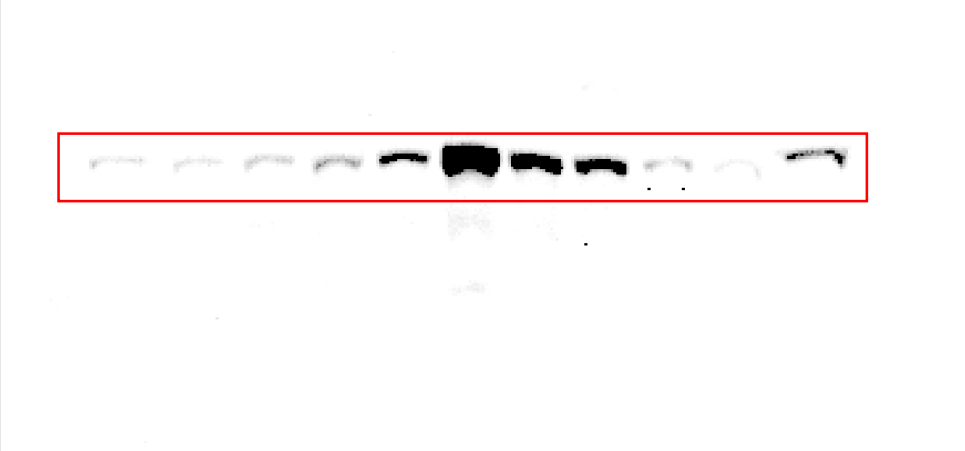
Full unedited blot for Figure 4B

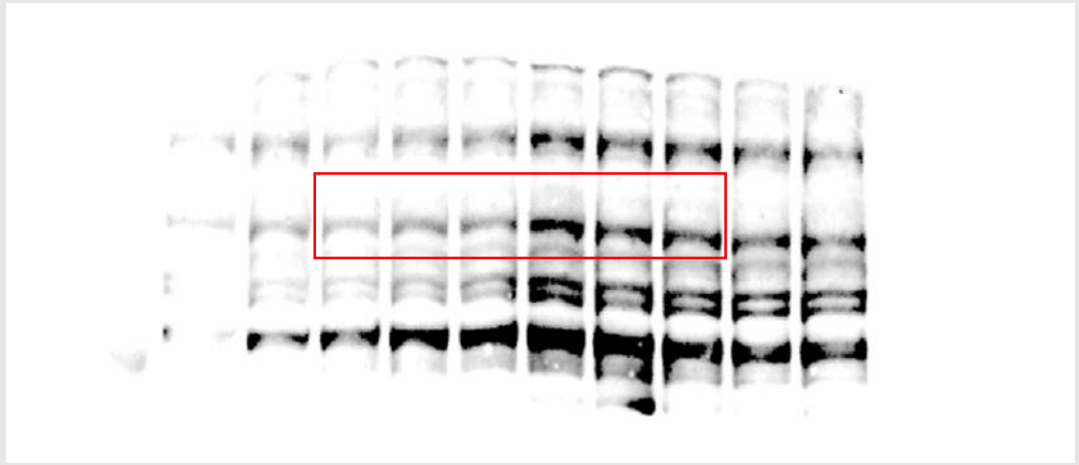


NLRP3

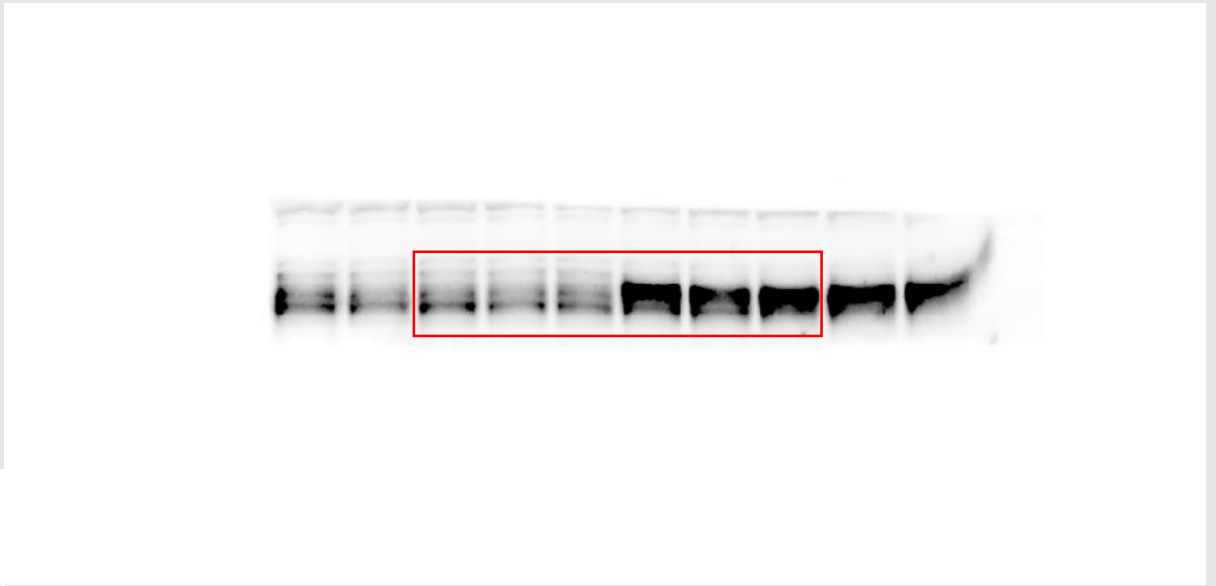
GAPDH

Lanes that are presented in the submission are highlighted by a red square

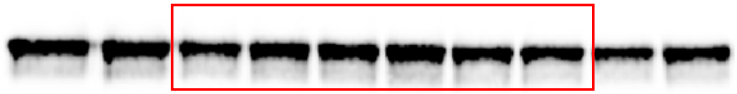
Full unedited blot for Figure 5B



NLRP3



tCaMKIIδ



GAPDH

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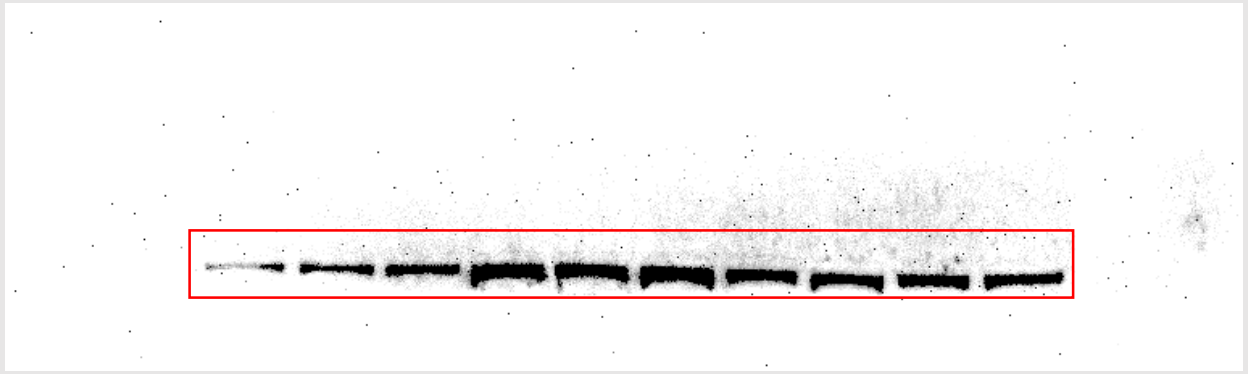
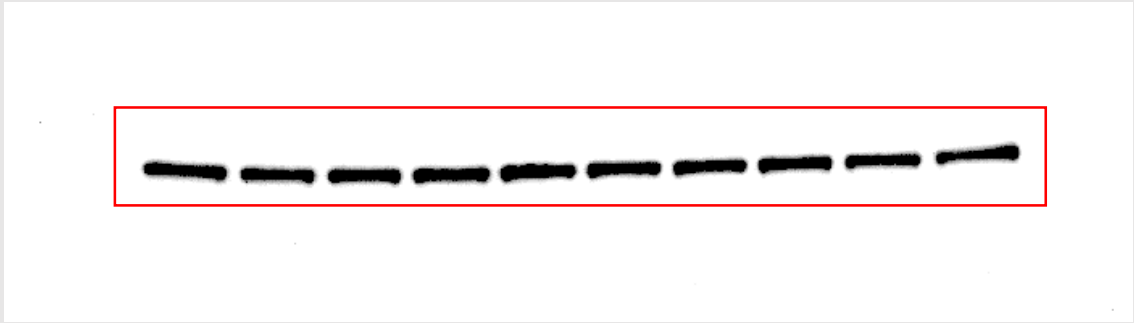
Full unedited blot for Figure 7A



NLRP3



GAPDH

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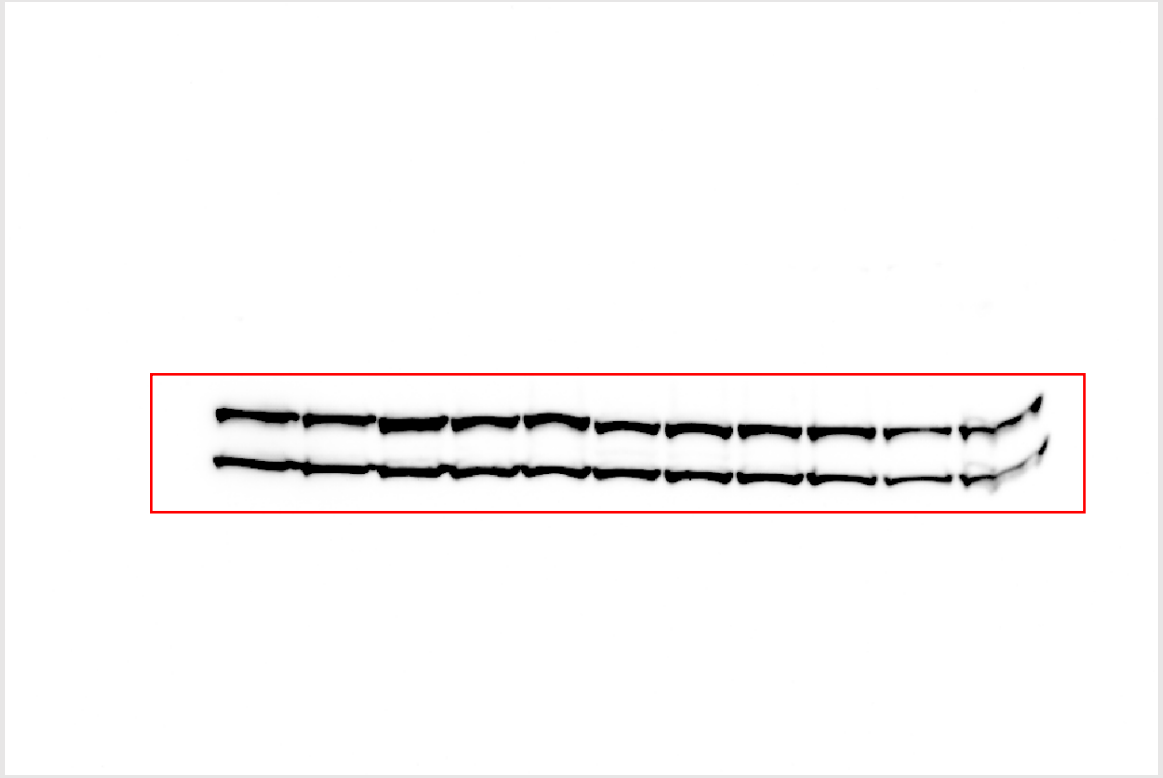
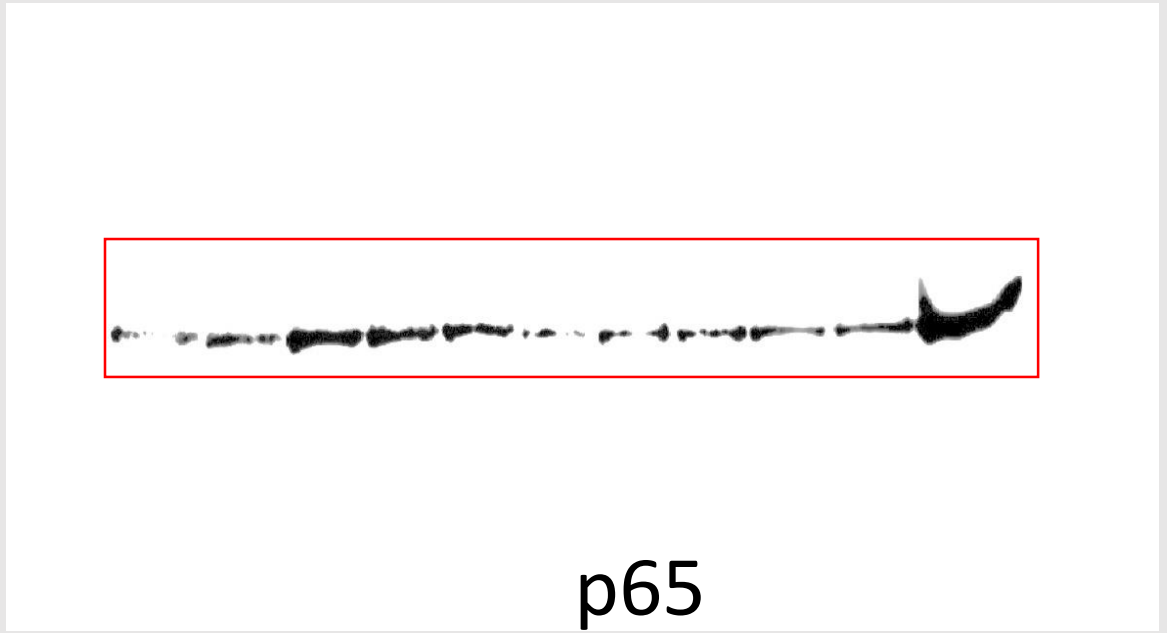
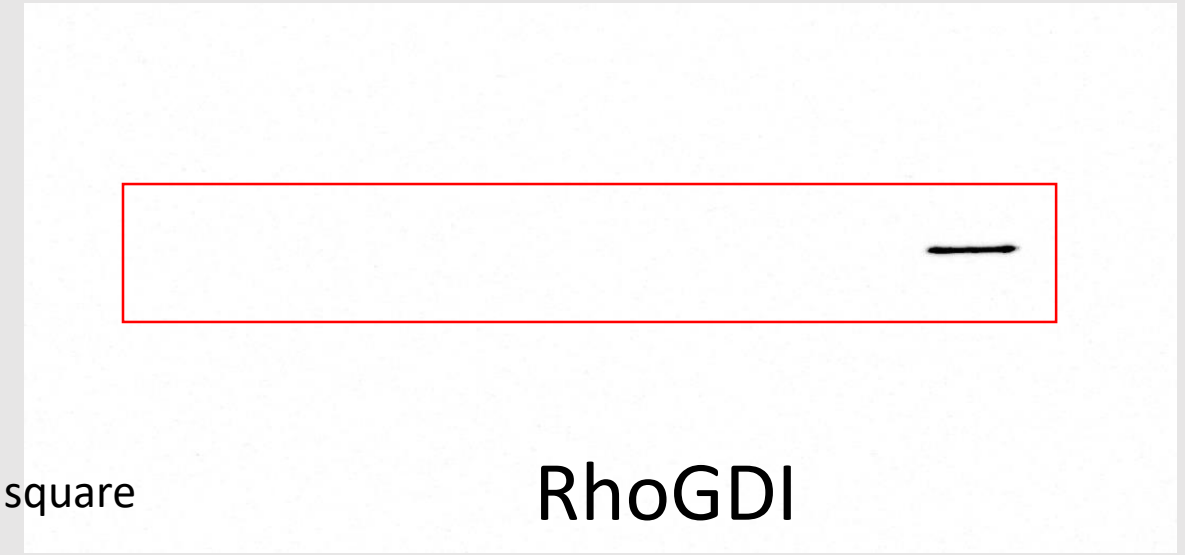
Full unedited blot for Supplemental Figure 1B



Lamin a/c



p65



RhoGDI

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