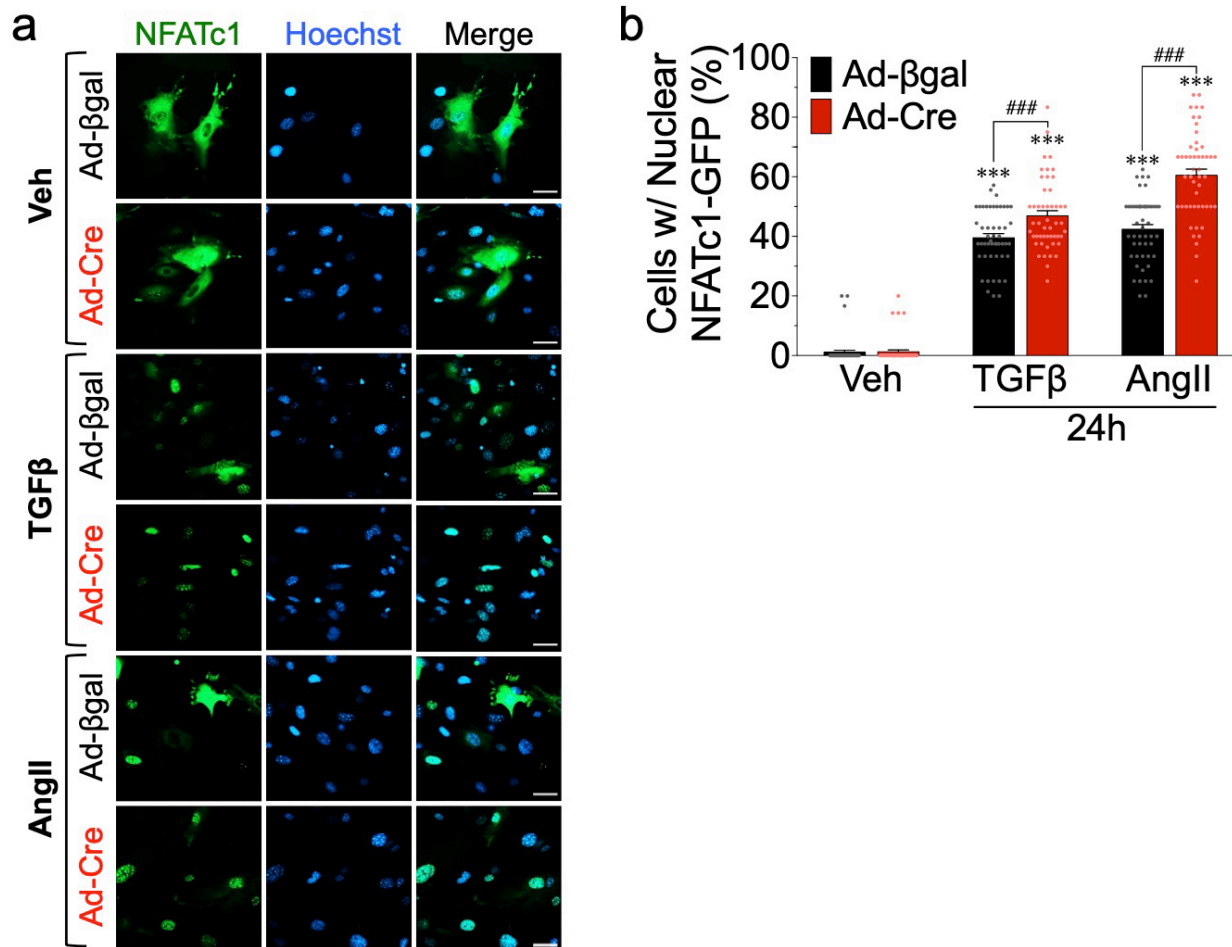


Supplemental Figures:

Mitochondrial Calcium Exchange links Metabolism with the Epigenome to Control Cellular Differentiation

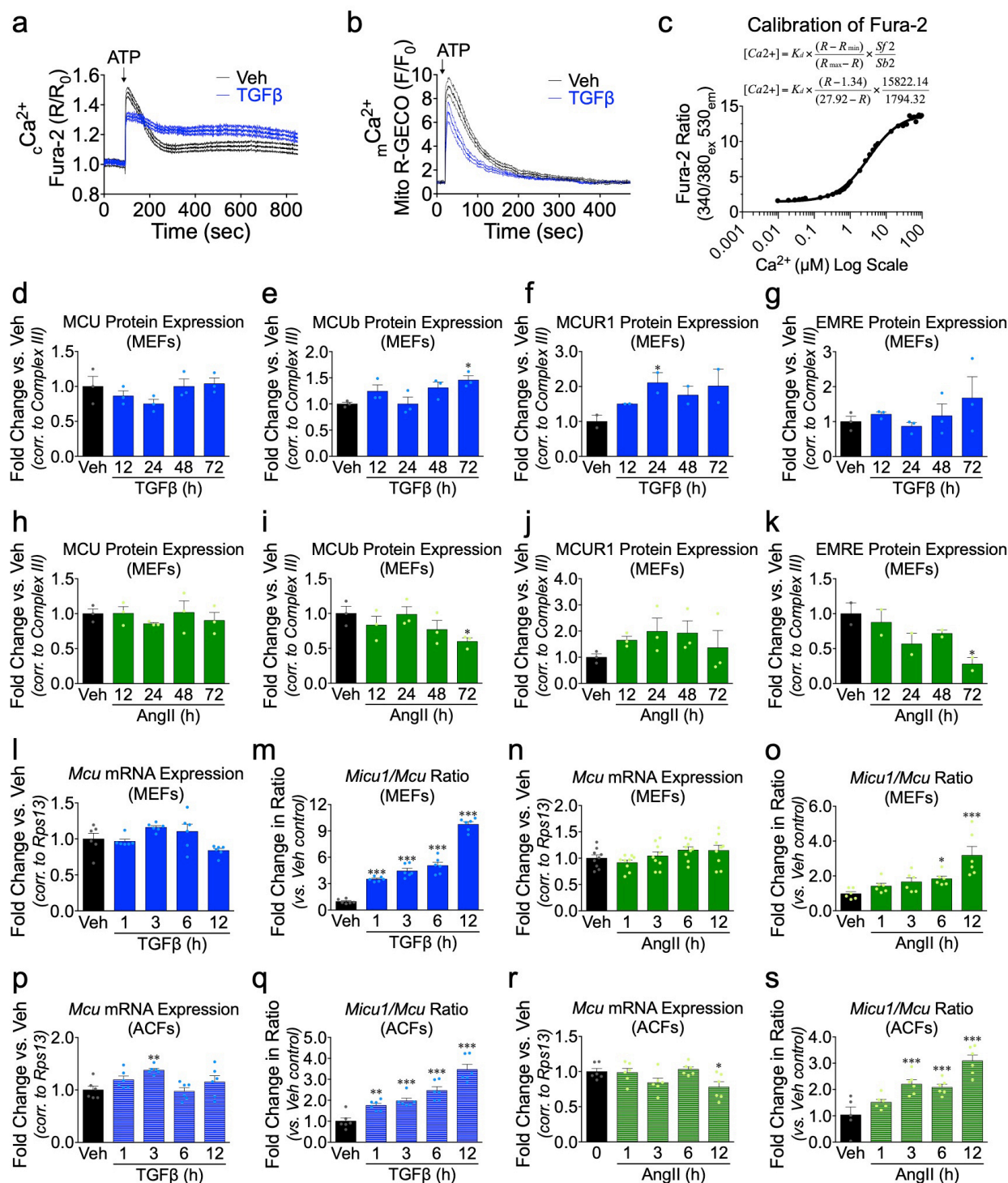
Alyssa A. Lombardi et al. *Nature Communications*. 2019

Supplemental Figure 1 – related to Figure 1.



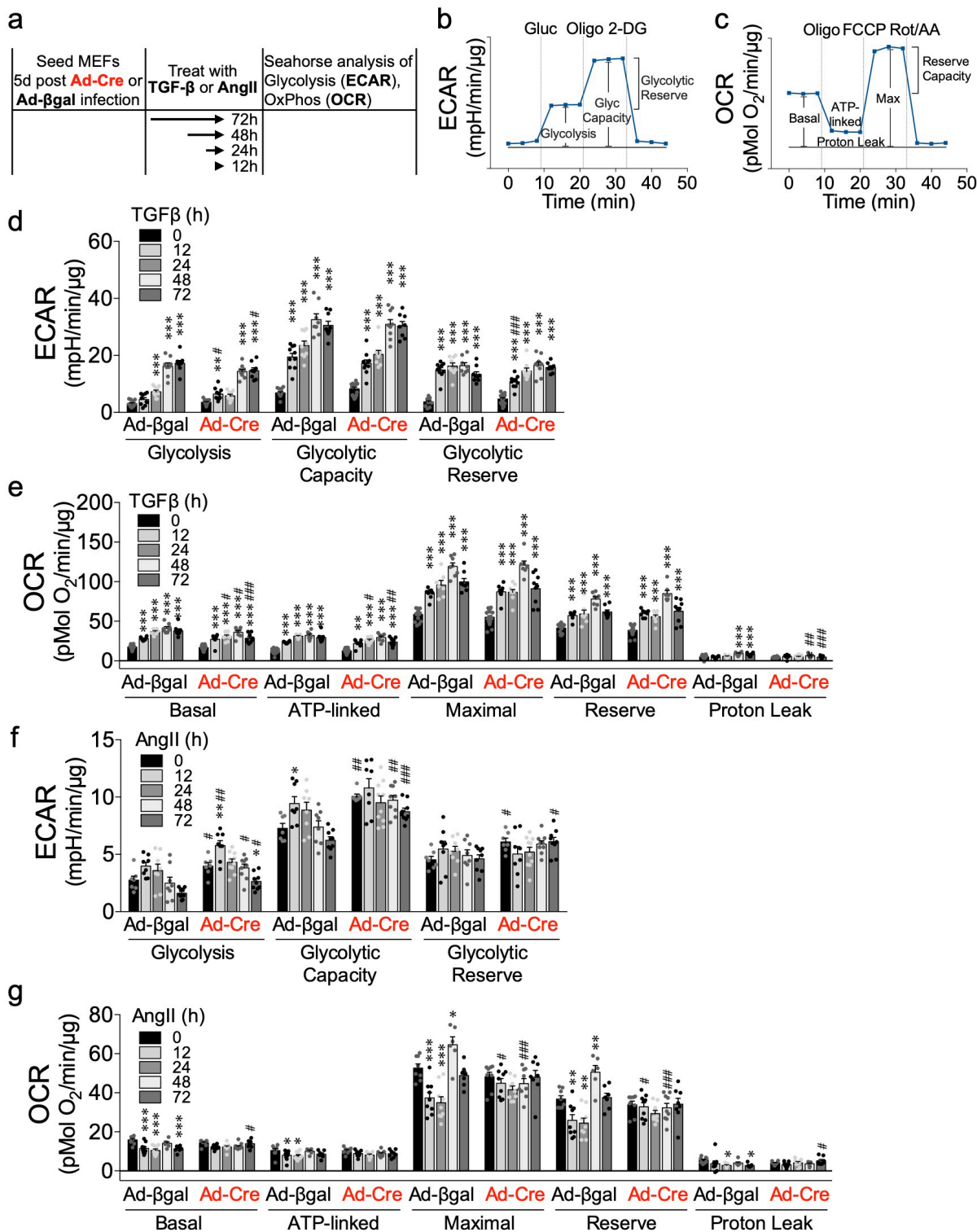
Supplemental Figure 1 – related to Figure 1. Loss of mCa^{2+} uptake enhances cytosolic signaling. **a,b)** *Mcu*^{-/-} (Ad-Cre) and control (Ad-βgal) MEFs were transduced with adenovirus-encoding NFATc1-GFP and 24h later treated with TGFβ or AngII for 24h. Cells were stained with Hoechst to demarcate nuclei and imaged on a fluorescence microscope. Representative images are shown. Percentage of cells with nuclear NFATc1 was quantified. n=3 per group each with 50 cells quantified. All data shown as mean ± SEM. ***p<0.001 vs. vehicle control analyzed by ANOVA. ###p<0.001 vs. Ad-βgal analyzed by t-test. Scale bar = 50μm.

Supplemental Figure 2 – related to Figure 2.



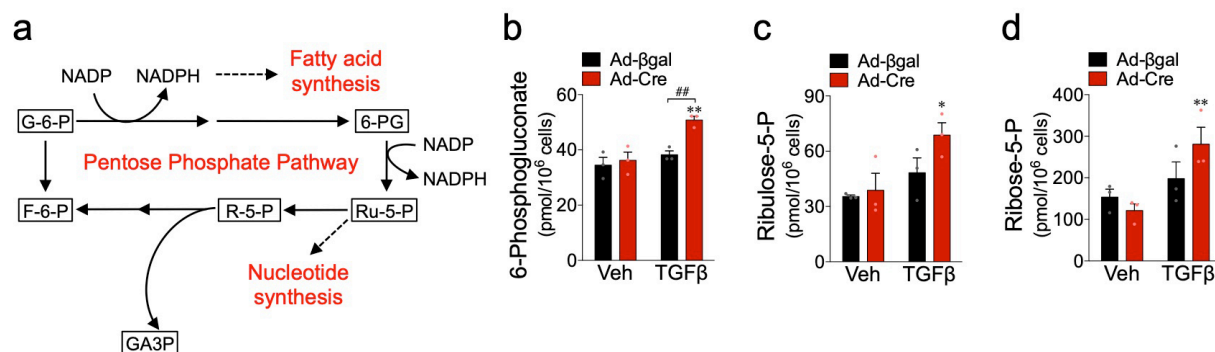
Supplemental Figure 2 – related to Figure 2. Calibration of Fura-2 Ca^{2+} reporter and quantification of expression of mtCU components post-TGF β or AngII. a) WT MEFs plus or minus (+/-) 12h TGF β were loaded with the Ca^{2+} -sensitive dye Fluo-4 AM. Fluorescence was recorded while 1mM ATP was delivered to initiate purinergic receptor-mediated IP3R Ca^{2+} release. n=28 cells. **b)** WT MEFs +/- 12h TGF β were transduced with adenovirus encoding the mitochondrial calcium sensor, Mito R-GECO and fluorescence was recorded during 1mM ATP treatment. n=24 cells. **c)** Fura-2 was calibrated by the generation of a standard curve of Ca^{2+} (0.01-100 μM) in experimental intracellular buffer to quantify actual Ca^{2+} content as shown in Figure 2c. Fura-2 fluorescence ratio was converted to $[\text{Ca}^{2+}]$ by the following equation: $[\text{Ca}^{2+}] = K_d * (R - R_{\min}) / (R_{\max} - R) * Sf2/Sb2$. ($R_{\min}$ = ratio in 0- Ca^{2+} ; $R_{\max}$ = ratio at saturation; Sf2=380/510 reading in 0- Ca^{2+} ; Sb2=380/510 reading with Ca^{2+} saturation. **d-k)** Fold change in expression of mtCU components from immunoblots in Figure 2h,m. Band density analysis was corrected to the loading control, Complex III (subunit UQCRC2). n=3 samples. **l-o)** Fold change in mRNA expression of *Micu1* and the ratio of *Micu1/Mcu* in MEFs. *Rps13* was used as the housekeeping gene; n=6. **p-s)** Fold change in mRNA expression of *Micu1* and the ratio of *Micu1/Mcu* in mouse adult cardiac fibroblasts (ACFs). *Rps13* was used as the housekeeping gene; n=6 samples. *Ca²⁺ traces*: solid line = mean, dashed line = SEM. All data shown as mean $\pm$ SEM. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ vs. vehicle control analyzed by ANOVA.

Supplemental Figure 3 – related to Figure 3.



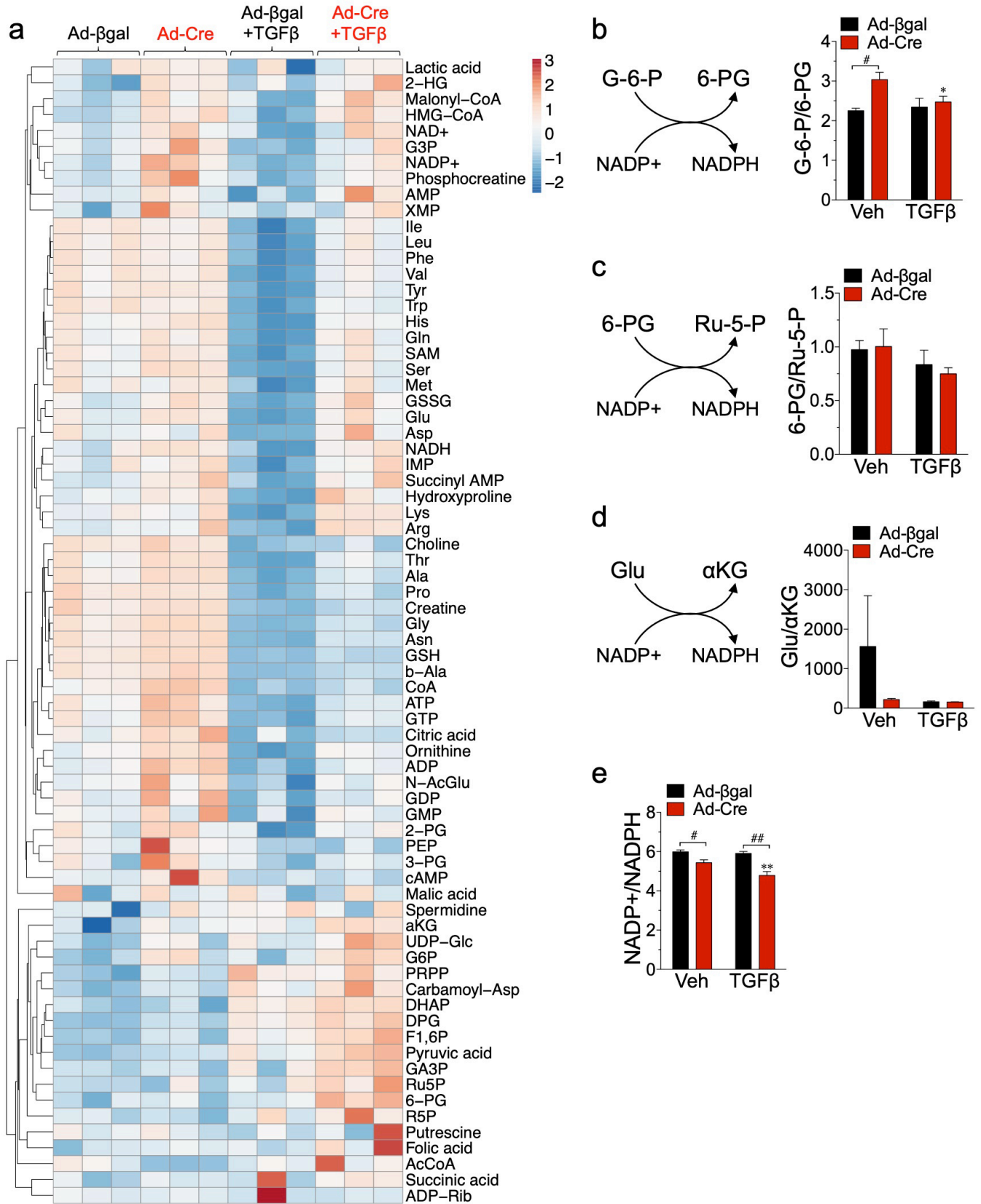
Supplemental Figure 3 – related to Figure 3. Seahorse analysis of glycolysis and oxidative phosphorylation. a-c) Schematic of experimental timeline. MEFs were treated with fibrotic stimuli for 12, 24, 48, or 72h and assayed for Glycolysis and Oxidative Phosphorylation using a Seahorse XF96 analyzer to measure extracellular acidification rates (ECAR, glycolysis) or oxygen consumption rates (OCR, OxPhos). **d)** Quantification of glycolysis, glycolytic capacity, and glycolytic reserve in *Mcu*^{-/-} (Ad-Cre) and control (Ad-βgal) MEFs post-TGFβ. For Ad-βgal, n = 14 (t0), 10 (t12), 9 (t24), 8 (t48) and 9 (t72). For Ad-Cre, n = 15 (t0), 10 (t12), 9 (t24), 9 (t48) and 8 (t72). **e)** Quantification of basal respiration, ATP-linked respiration, maximal respiration, reserve capacity, and proton leak in *Mcu*^{-/-} and control MEFs post-TGFβ. For Ad-βgal, n = 18 (t0), 8 (t12), 7 (t24), 8 (t48) and 8 (t72). For Ad-Cre, n = 18 (t0), 8 (t12), 9 (t24), 7 (t48) and 9 (t72). **f)** Quantification of glycolysis, glycolytic capacity, and glycolytic reserve in *Mcu*^{-/-} and control MEFs post-AngII. For Ad-βgal, n = 7 (t0), 8 (t12), 8 (t24), 8 (t48) and 9 (t72). For Ad-Cre, n = 7 (t0), 8 (t12), 10 (t24), 9 (t48) and 9 (t72). **g)** Quantification of basal respiration, ATP-linked respiration, maximal respiration, reserve capacity, and proton leak in *Mcu*^{-/-} and control MEFs post-AngII. For Ad-βgal, n = 8 (t0), 9 (t12), 9 (t24), 6 (t48) and 7 (t72). For Ad-Cre, n = 8 (t0), 9 (t12), 9 (t24), 9 (t48) and 8 (t72).. All data shown as mean ± SEM. *** *p*<0.001, ** *p*<0.01, * *p*<0.05 vs. vehicle control analyzed by ANOVA. ### *p*<0.001, ## *p*<0.01, # *p*<0.05 vs. Ad-βgal analyzed by *t*-test.

Supplemental Figure 4 – related to Figure 3.



Supplemental Figure 4 – related to Figure 3. Quantification of metabolites involved in the pentose phosphate pathway. **a)** Schematic of the pentose phosphate pathway: glucose-6-phosphate (G-6-P), 6-phosphogluconate (6-PG), ribulose-5-phosphate (Ru-5-P), ribose-5-phosphate (R-5-P), glyceraldehyde-3-phosphate (GA3P), fructose-6-phosphate (F-6-P). **b-d)** Absolute concentration of pentose phosphate pathway metabolites 6-phosphogluconate, ribulose-5-P phosphate, and ribose-5-phosphate; n=3/group. All data shown as mean $\pm$ SEM. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ vs. vehicle control analyzed by ANOVA. ### $p < 0.001$, ## $p < 0.01$, # $p < 0.05$ vs. Ad- β gal analyzed by t -test.

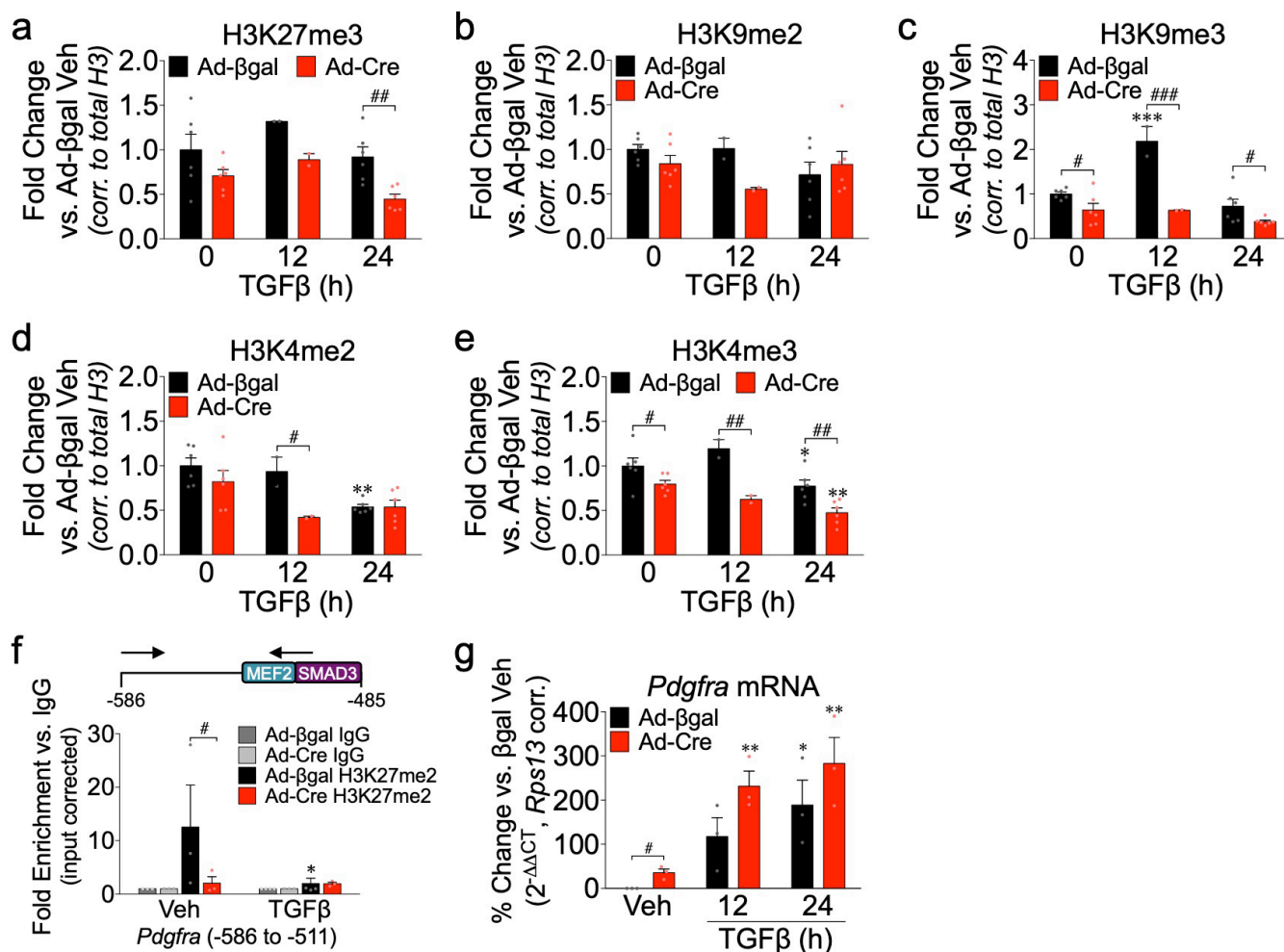
Supplemental Figure 5 – related to Figures 3 and 4.



Supplemental Figure 5 – related to Figures 3 and 4. a) Heat map of metabolites. Heat map representation of metabolome profiles of *Mcu*^{-/-} (Ad-Cre) and control (Ad-βgal) MEFs at baseline and post-TGFβ (12h). Unit variance scaling is applied to rows. Rows are clustered using Manhattan distance and average linkage. *Also see Supplemental Data File 1. b-e)* Ratios of metabolites involved in enzymatic reactions that require NADP⁺ as a cofactor, derived from absolute metabolite concentrations. An observed change in the metabolite ratio provides potential insight into the redox state of the cell, which could affect the interconversion of these metabolites; n=3. **b)** glucose-6-phosphate (G-6-P)/6-phosphogluconate (6-PG), **c)** 6-PG/ribulose-5-phosphate (Ru-5-P), **d)** Glutamate (Glu)/α-ketoglutarate (αKG), **e)** Cellular redox state (NADP⁺/NADPH ratio) examined by luciferin-based assay in WT and *Mcu*^{-/-} MEFs ± 24h TGFβ; n=5. These data are suggestive that mtCU-Ca²⁺ uptake has a greater impact on mitochondrial NADPH redox than cytosolic redox state. *All data shown as mean ± SEM. **p<0.01, *p<0.05 vs. vehicle control analyzed by ANOVA. ##p<0.01, #p<0.05 vs. Ad-βgal analyzed by t-test.*

Supplemental Data File 1 – related to Figures 3 and 4. Absolute concentration of metabolites; n=3. *All data shown as mean ± SEM. (see excel file)*

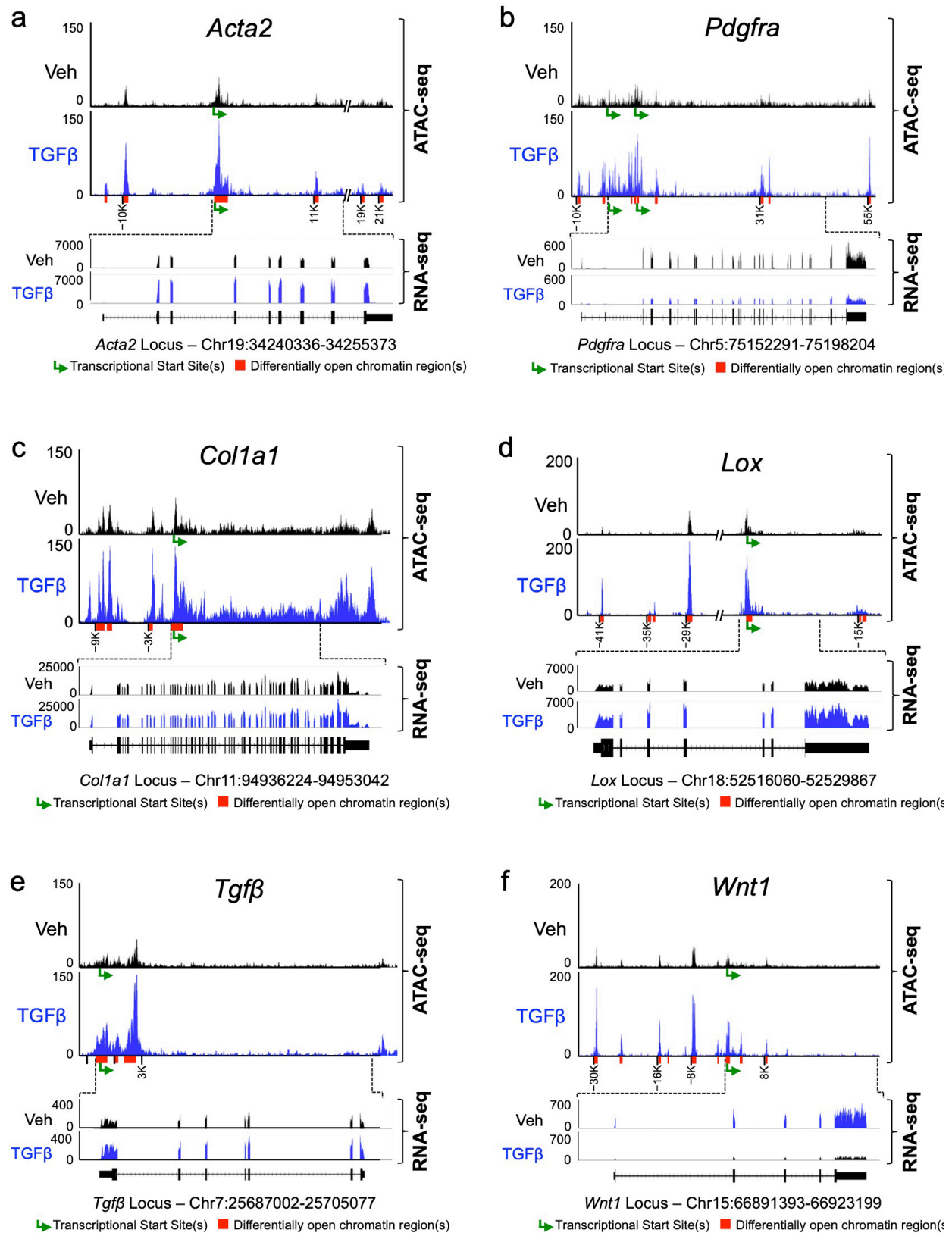
Supplemental Figure 6 – related to Figure 5.



Supplemental Figure 6 – related to Figure 5. Quantification of H3K methylation.

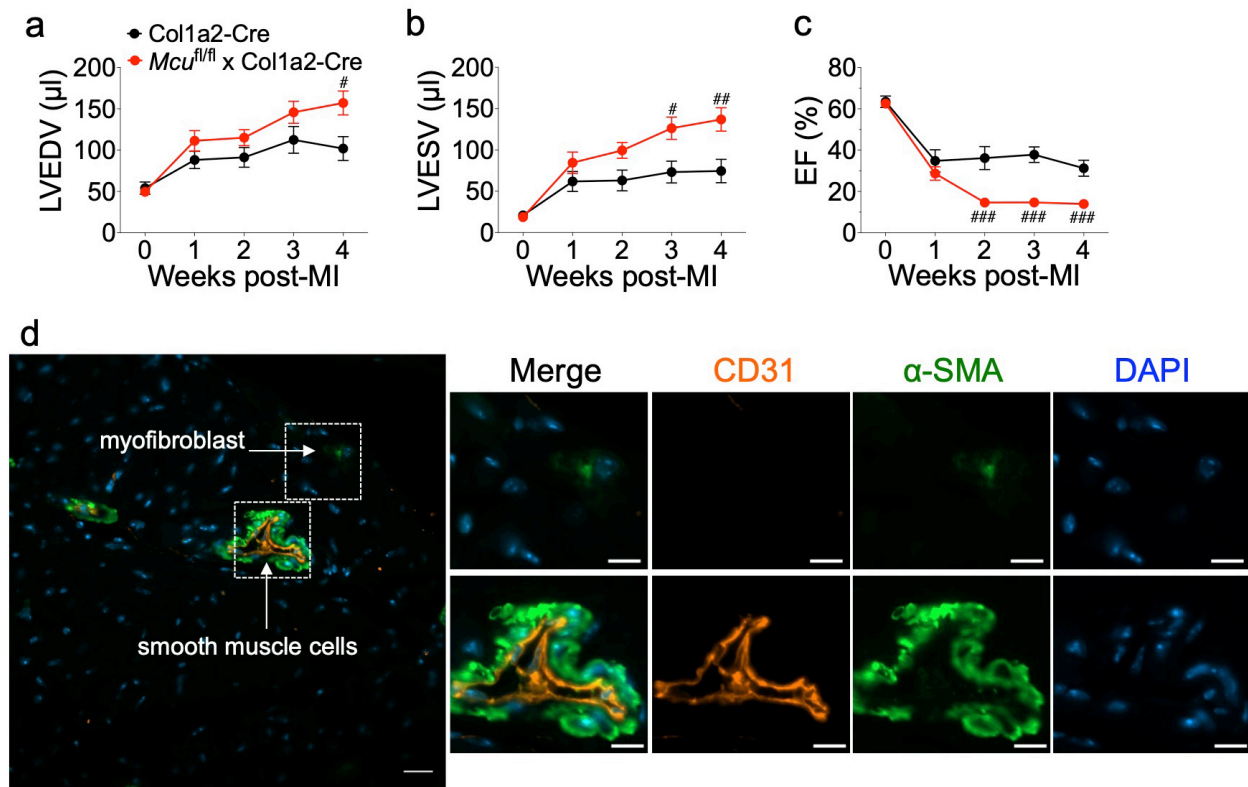
a-e) MEFs were treated with TGFβ for 0, 12 or 24h and cell lysates were immunoblotted for specific methylated histone 3 lysine (H3K) residues – H3K27me3, H3K9me2, H3K9me3, H3K4me2, H3K4me3. Total H3 was used as a nuclear loading control and tubulin served as a total lysate loading control. Band density was normalized to total H3. **f)** H3K27me2 ChIP-qPCR of *platelet-derived growth factor receptor alpha* (*Pdgfra*) in *Mcu*^{-/-} (Ad-Cre) and control (Ad-βgal) MEFs at baseline (veh) and following 12h TGFβ. Schematic shows loci of qPCR primers in relationship to myofibroblast transcription factor binding sites – MEF2 (myocyte enhancer factor 2), SMAD3 (SMAD family member 3). **g)** qPCR of *Pdgfra* mRNA in *Mcu*^{-/-} (Ad-Cre) and control (Ad-βgal) MEFs at baseline (veh) and post-TGFβ. n=3 experiments for all quantified data. All data shown as mean ± SEM. ***p<0.001, **p<0.01, *p<0.05 vs. vehicle control analyzed by ANOVA. ###p<0.001, ##p<0.01, #p<0.05 vs. Ad-βgal analyzed by t-test.

Supplemental Figure 7 – related to Figure 5.



Supplemental Figure 7 – related to Figure 5. ATAC-seq and RNA-seq data. a-e) WT MEFs were treated with vehicle or TGF β and assessed for chromatin accessibility and transcription using ATAC-seq and RNA-seq. Results of key myofibroblast gene loci are shown – α -smooth muscle actin (*Acta-2*), platelet-derived growth factor receptor alpha (*Pdgfra*), collagen type I alpha 1 chain (*Col1a1*), lysyl oxidase (*Lox*), transforming growth factor β (*Tgf β*), and wnt family member 1 (*Wnt1*). The height of the genome browser tracks shows the number of reads normalized by read depth and overall peak enrichment in the library.

Supplemental Figure 8 – related to Figure 6.



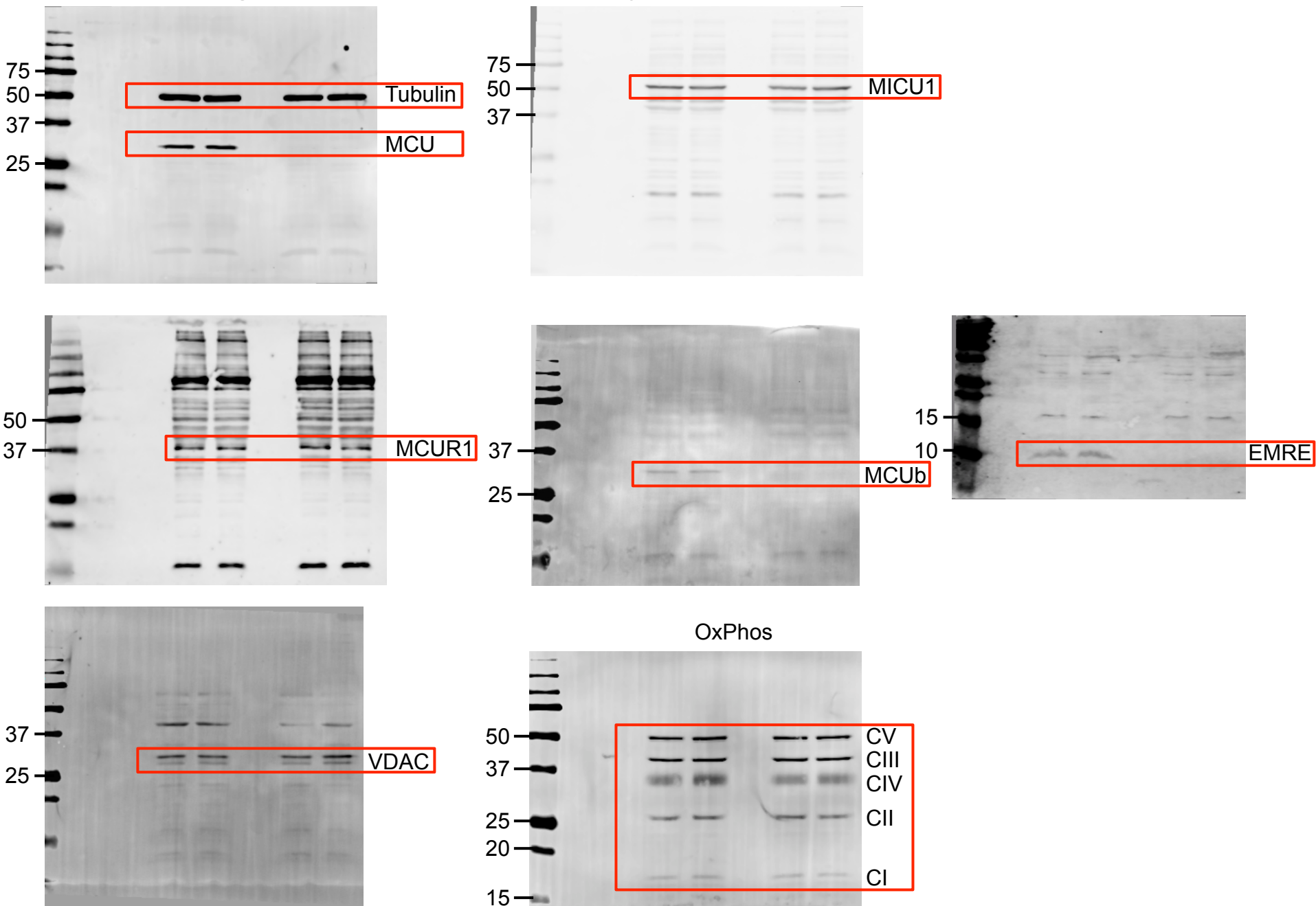
Supplemental Figure 8 – related to Figure 6. Echocardiographic parameters and representative immunohistochemistry images of myofibroblast identification.

a-c) *Mcu^{fl/fl}* x Col1a2-CreERT and control Col1a2-CreERT mice were treated with tamoxifen (40mg/kg/day) for 10d and allowed to rest 10d before permanent ligation of the left coronary artery. Cardiac function was analyzed by echocardiography 1wk prior to MI and every week thereafter. M-mode echo measurements of left ventricular end diastolic volume (LVEDV), left ventricular end systolic volume (LVESV), and ejection fraction (EF) were acquired. n=10 Col1a2-Cre, n=20 *Mcu^{fl/fl}* x Col1a2-Cre. **d)** To quantify myofibroblasts, tissue sections were stained with anti- α -SMA, anti-CD31 (endothelial cell marker), and DAPI. Representative immunohistochemistry images show identification of myofibroblasts (α -SMA+/CD31-) vs. smooth muscle cells (α -SMA+/CD31+). All data shown as mean $\pm$ SEM. ###p<0.001, ##p<0.01, #p<0.05 vs. Ad- β gal analyzed by t-test. Large image left: scale bar = 20 μ m, small inset images right = 10 μ m. Also see Supplemental Table 1.

Supplemental Table 1 – related to Figure 6. Echocardiographic results of left-ventricular (LV) function at baseline (wk 0) and post-MI. All values were collected from M-mode analysis to measure intraventricular septum thickness (IVS) during diastole (IVS;d) and systole (IVS;s), left-ventricular end-diastolic diameter (LVEDD), left-ventricular end-systolic diameter (LVESD), left-ventricular end-diastolic posterior wall thickness (LVEDPW), left-ventricular end-systolic posterior wall thickness (LVESPW), ejection fraction (EF), fractional shortening (FS), left-ventricular end-diastolic volume (LVEDV), and left-ventricular end-systolic volume (LVESV). *All data shown as mean ± SEM. ***p<0.001, **p<0.01, *p<0.05 vs. wk 0 analyzed by ANOVA. ###p<0.001, ##p<0.01, #p<0.05 vs. Ad-βgal analyzed by t-test.*

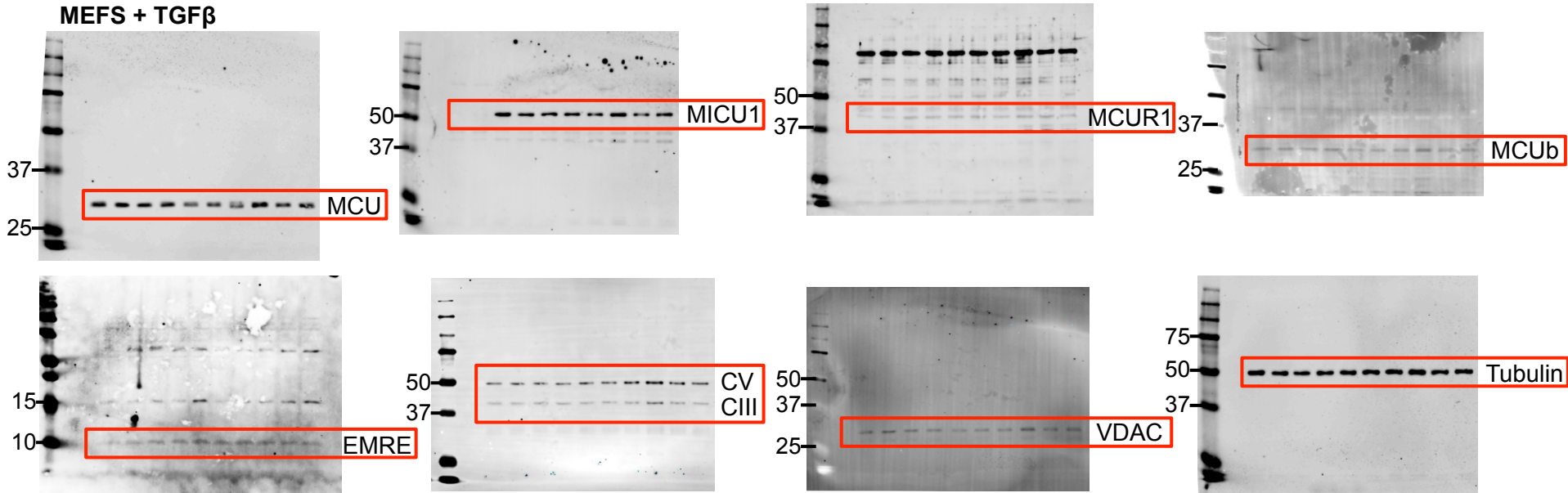
	Wks post-MI	n/group	IVS;d (mm)	IVS;s (mm)	LVEDD (mm)	LVESD (mm)	LVEDP (mm)	LVESP (mm)	EF (%)	FS (%)	LVEDV (μl)	LVESV (μl)
Col1a2-Cre	0	10	0.69±0.07	1.00±0.09	3.53±0.18	2.35±0.17	0.68±0.03	0.96±0.07	63.43±2.74	33.94±1.92	54.11±7.41	20.81±4.29
	1	10	0.70±0.04	0.90±0.06	4.35±0.22	3.66±0.30**	0.71±0.05	0.92±0.10	34.83±5.36***	16.95±2.81***	88.12±10.45	61.86±12.00
	2	10	0.69±0.04	0.94±0.08	4.40±0.24	3.67±0.32**	0.72±0.07	0.85±0.09	36.18±5.56***	17.79±3.03***	91.32±11.98	63.14±12.63
	3	10	0.67±0.06	0.91±0.09	4.80±0.29**	3.94±0.30***	0.67±0.05	0.87±0.08	37.80±3.78***	18.47±2.01***	112.43±16.19*	73.19±13.27
	4	10	0.68±0.04	1.00±0.08	4.60±0.27*	3.96±0.32***	0.78±0.08	0.87±0.08	31.25±3.87***	14.86±1.98***	101.94±14.43	74.53±14.25*
Mcu^{fl/fl} x Col1a2-Cre	0	20	0.90±0.03##	1.27±0.04	3.44±0.07	2.31±0.06	0.87±0.06	1.18±0.07	62.54±1.24	33.06±0.88	49.52±2.41	18.71±1.20
	1	20	0.66±0.02***	0.93±0.06***	4.77±0.21***	4.16±0.25***	0.76±0.05	0.90±0.06**	28.72±3.28***	13.71±1.65***	111.30±12.27***	84.58±12.95***
	2	20	0.54±0.03***	0.67±0.05*** #	4.88±0.16***	4.56±0.17***	0.60±0.06**	0.69±0.06***	14.68±1.75*** ####	6.63±0.83*** ####	115.12±9.72***	99.42±9.53***
	3	20	0.61±0.04***	0.78±0.07***	5.39±0.19***	5.04±0.21*** ##	0.64±0.06**	0.68±0.06***	14.71±2.03*** ####	6.73±0.97*** ####	145.79±13.30***	126.27±13.46*** #
	4	20	0.62±0.05***	0.72±0.07*** #	5.55±0.23*** #	5.21±0.24*** ##	0.61±0.05**	0.67±0.04***	13.96±1.95*** ####	6.39±0.94*** ##	157.11±14.40*** #	136.95±14.26*** ##

Supplemental Figure 9. Western blots related to Figure 1.

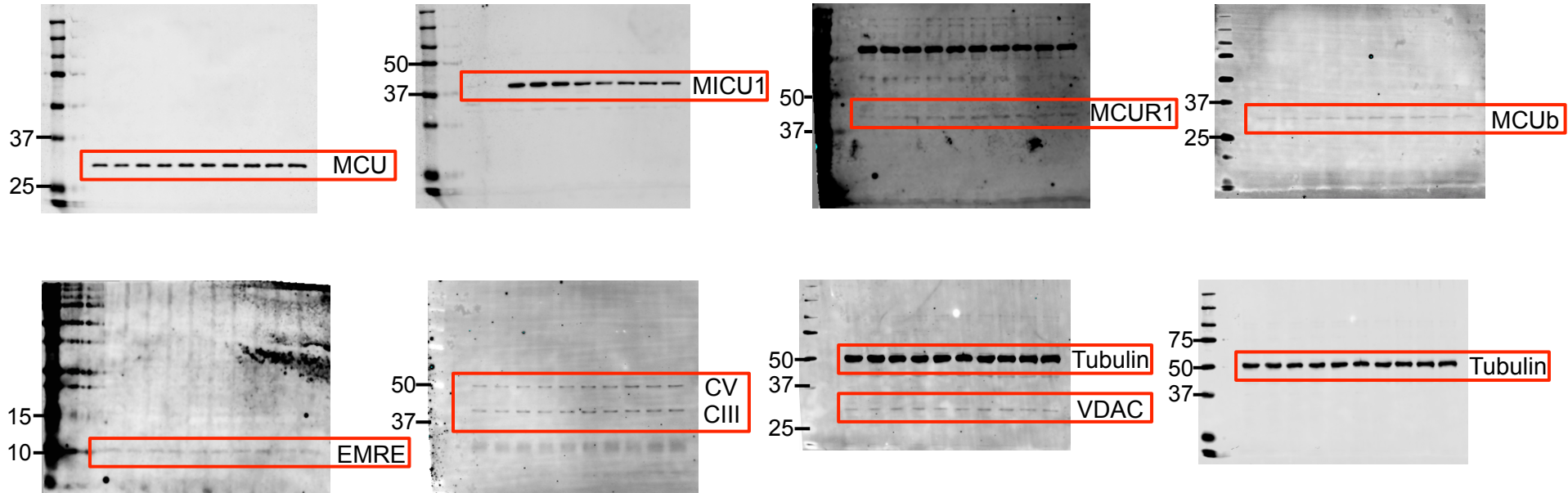


Supplemental Figure 10. Western blots related to Figure 2.

MEFS + TGFβ

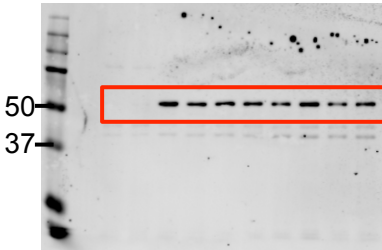


MEFS + AngII

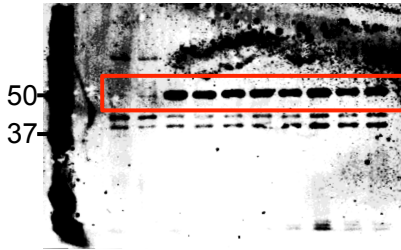
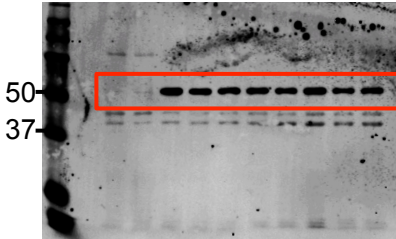


Western blots related to Figure 2 continued.

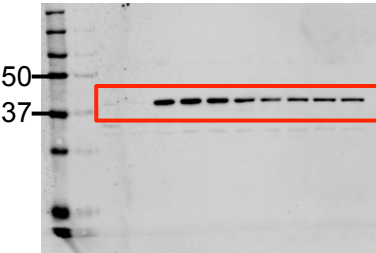
MEFS + TGFβ



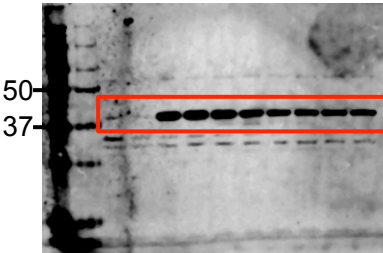
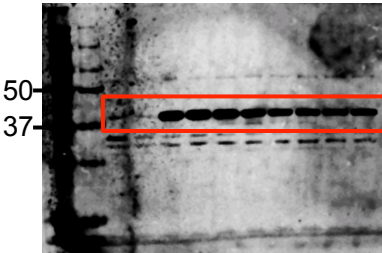
Higher exposure



MEFS + AngII

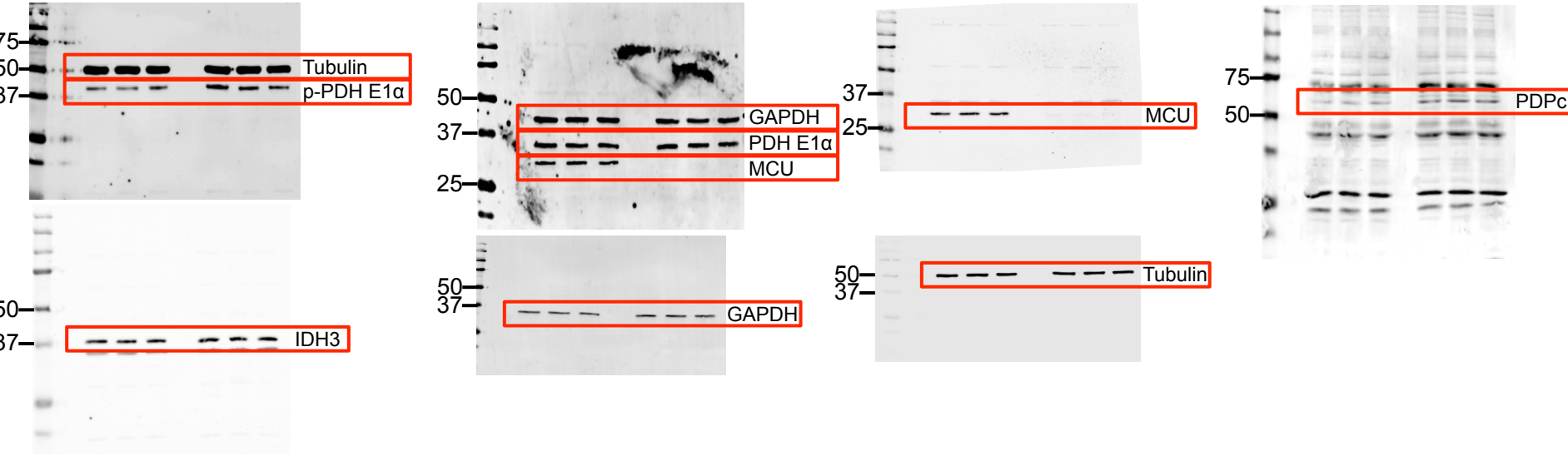


Higher exposure

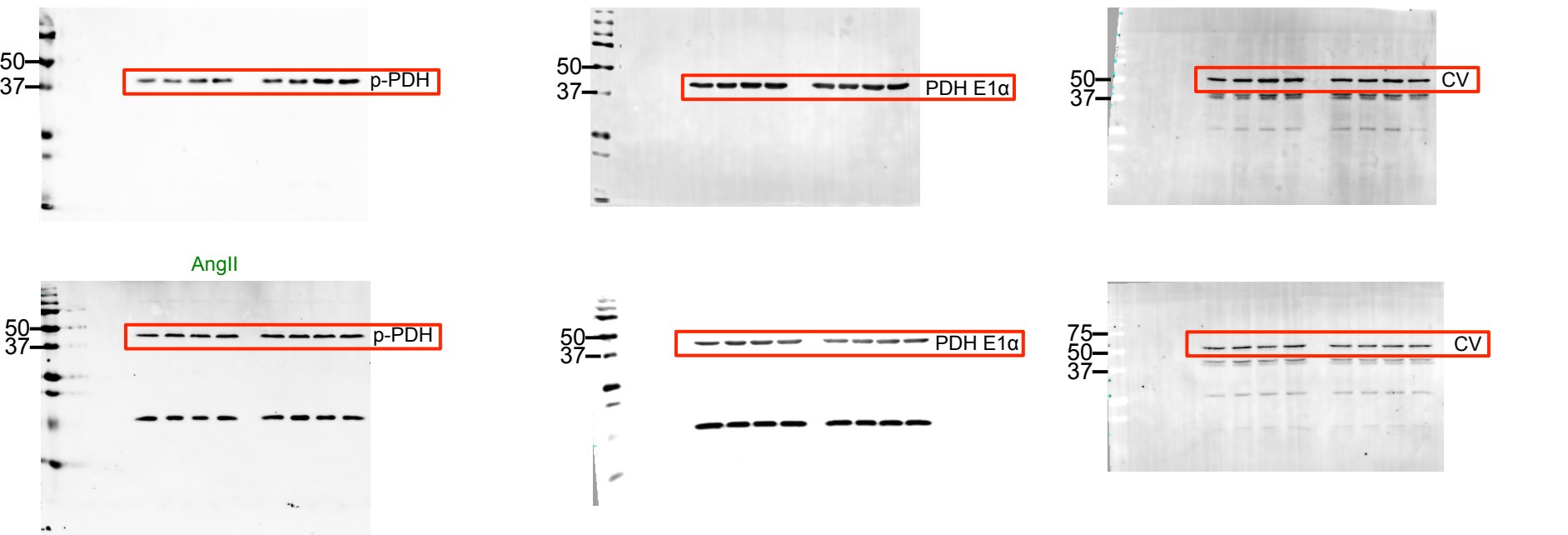


Supplemental Figure 11. Western blots related to Figure 4.

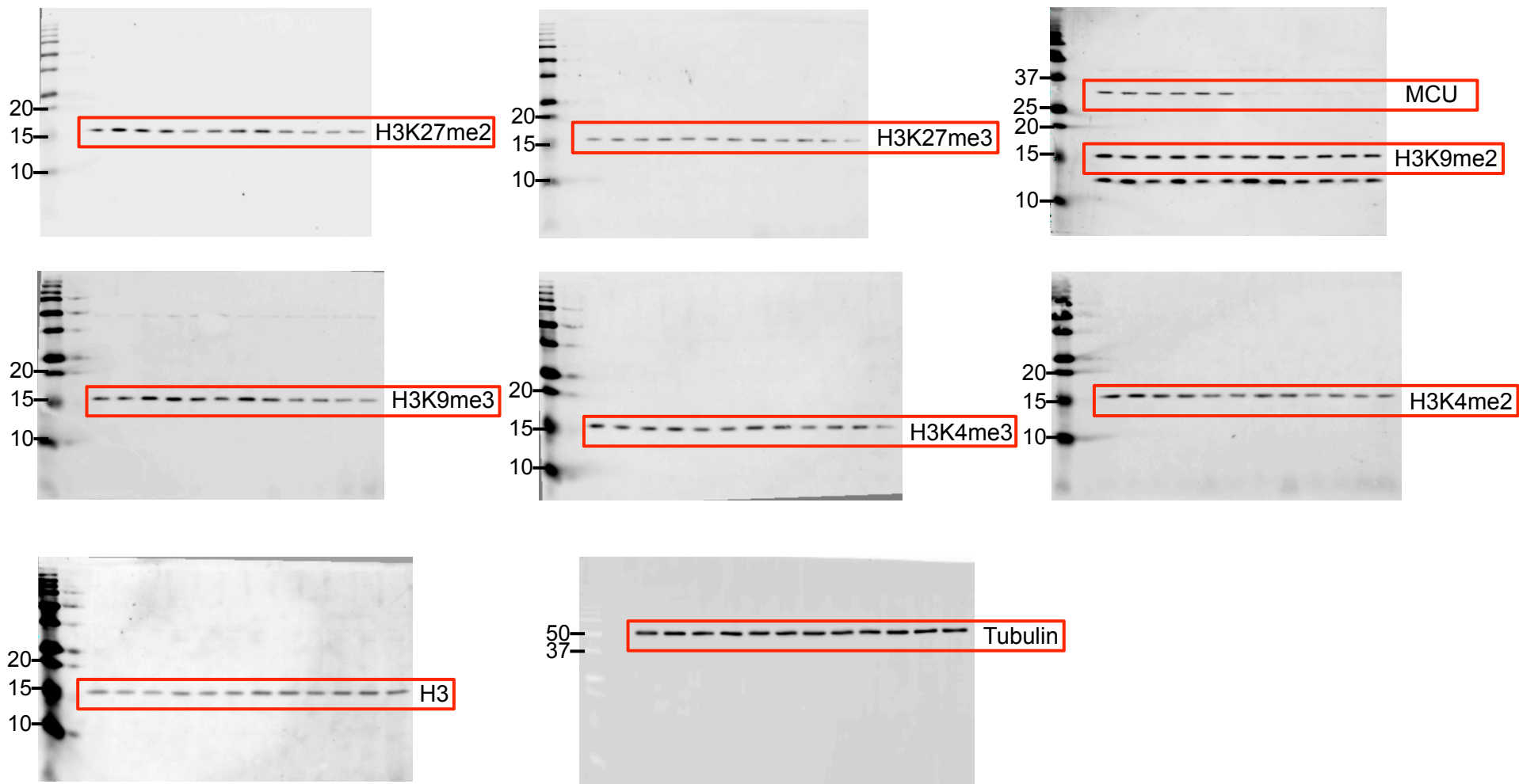
4B



4D



Supplemental Figure 12. Western blots related to Figure 5.



Supplemental Figure 13. Western blots related to Figure 6.

