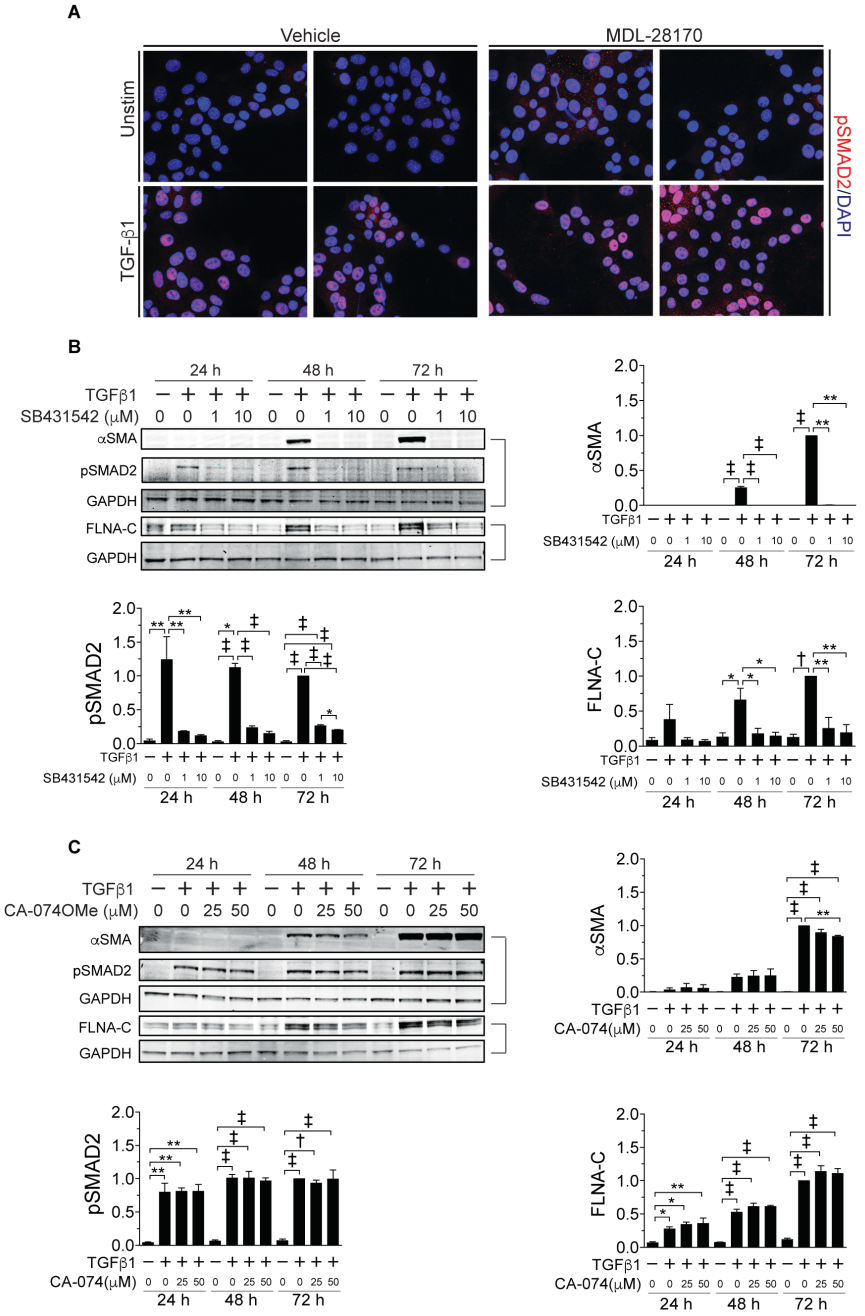
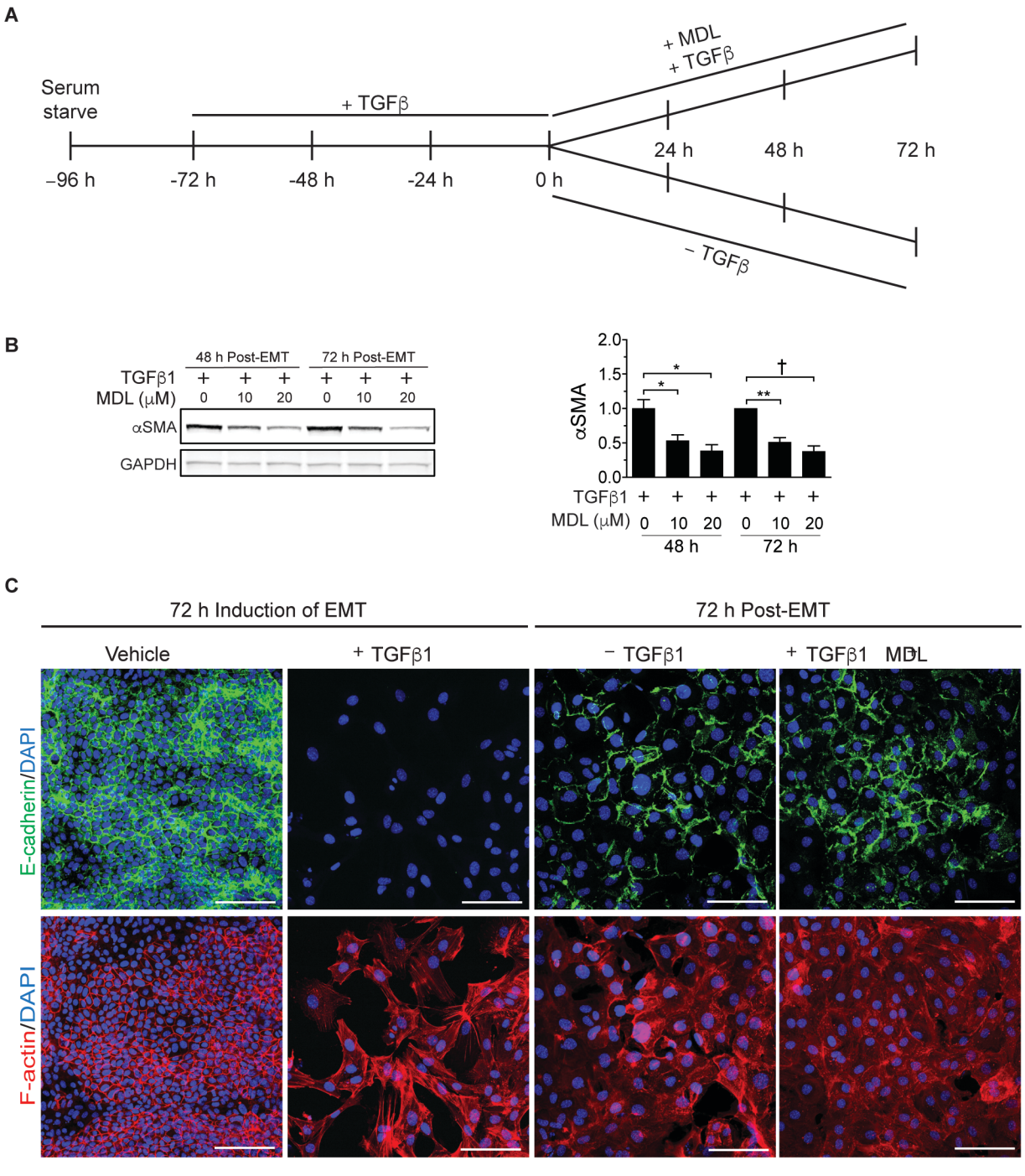
Supplementary Materials:



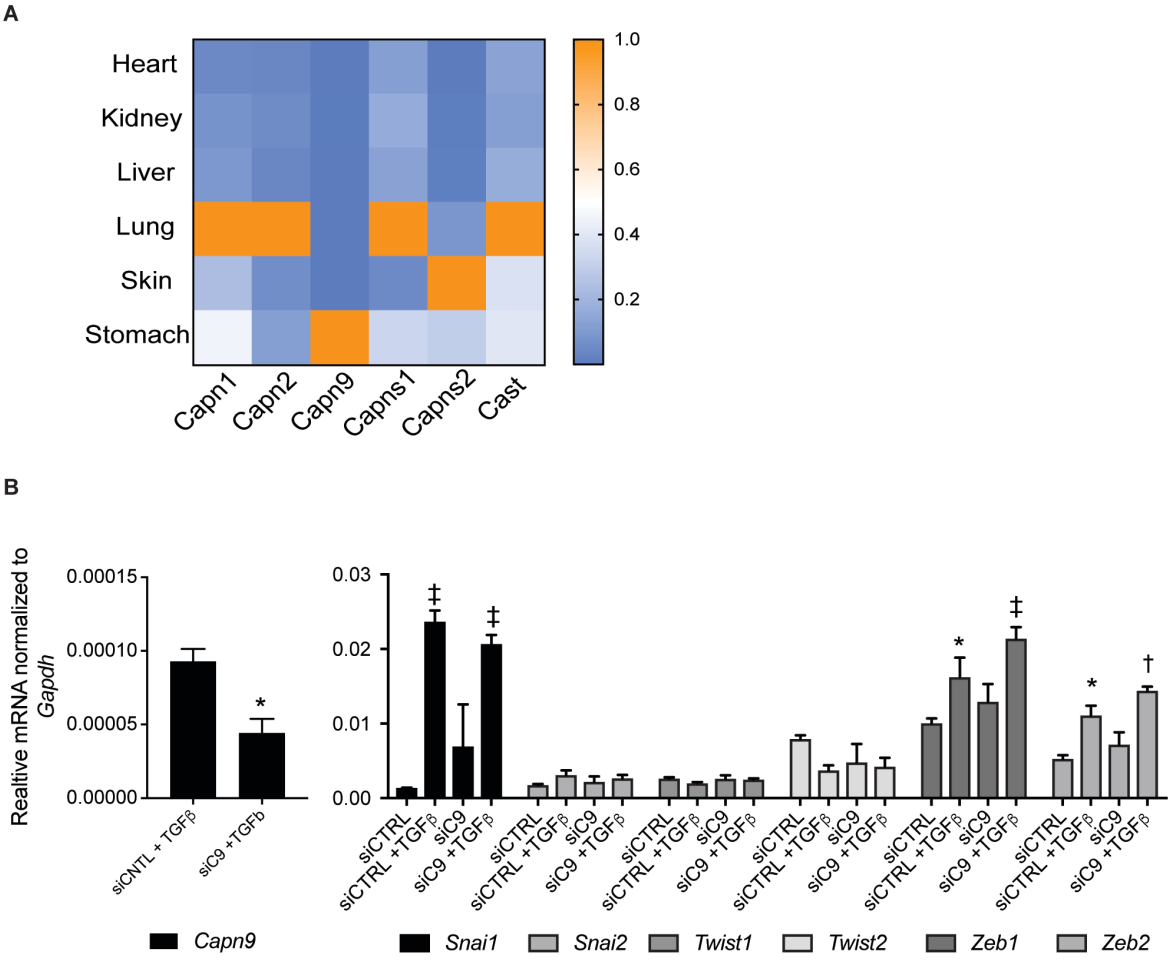
Supplementary Fig. 1. TGFβ stimulation induced pSMAD2 nuclear localization, Alk5 inhibition prevented EMT, and cathepsin B and L inhibition did not alter EMT. (**A**) Representative pSMAD2 (red) IF of NMuMG cells with and without TGFβ stimulation

(10 ng/mL) and MDL-28170 treatment (20 μM). (**B**) Representative immunoblots (bracket indicates identical gel) and quantification ($n = 3$, normalized to GAPDH) for the indicated proteins, time points after TGFβ stimulation, and concentrations of SB431542. (**C**) Representative immunoblots (bracket indicates identical gel) and quantification ($n = 3$, normalized to GAPDH) for the indicated proteins, time points after TGFβ stimulation, and concentrations of CA-074-OMe. Data are expressed as mean ± s.e.m. *$P < 0.05$, **$P < 0.01$, †$P < 0.005$, ‡$P < 0.001$ by one-way ANOVA with Tukey's *post hoc* test.



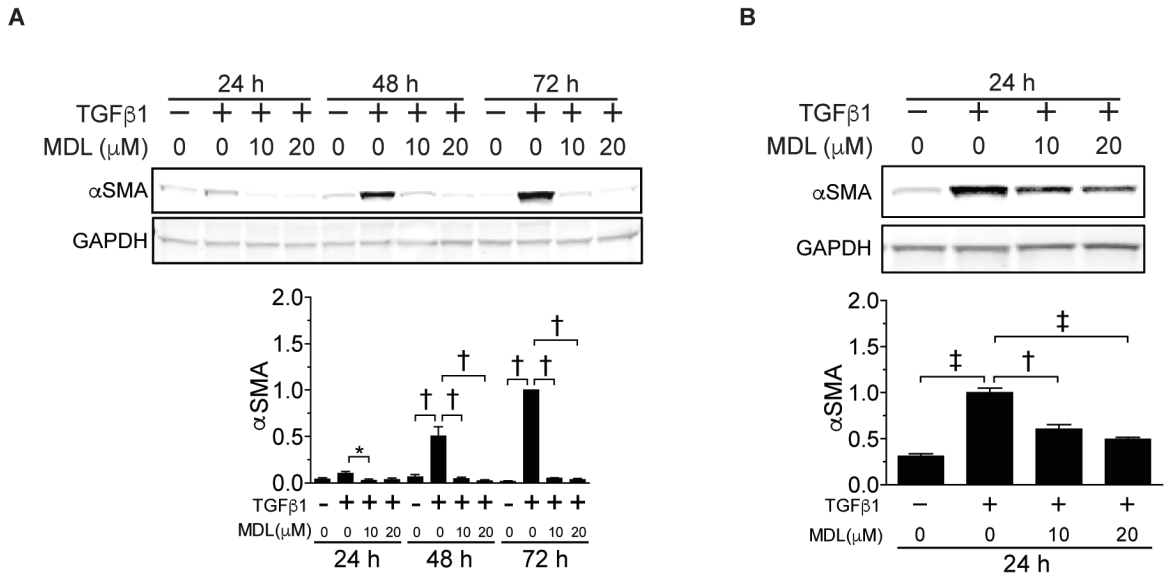
Supplementary Fig. 2. Broad spectrum calpain inhibition induced mesenchymal-to-epithelial transition (MET) in NMuMG cells. (**A**) Serum starved NMuMG cells were maintained in TGFβ for 72 h then the calpain inhibitor MDL-28170 was added for an additional 48 or 72 h with continued TGFβ exposure. (**B**) Representative immunoblot for αSMA

following the induction of mesenchymal transition with indicated concentrations of MDL-28170 (left), and (right) quantification normalized to GAPDH ($n = 3$). (**C**) Representative IF of MET in NMuMG stained for E-cadherin (green), F-actin (red), and DAPI (blue) Scale bar: 100 μm. Data are expressed as mean ± s.e.m. *$P < 0.05$, **$P < 0.01$, †$P < 0.005$, ‡$P < 0.001$ by one-way ANOVA with Tukey's *post hoc* test.

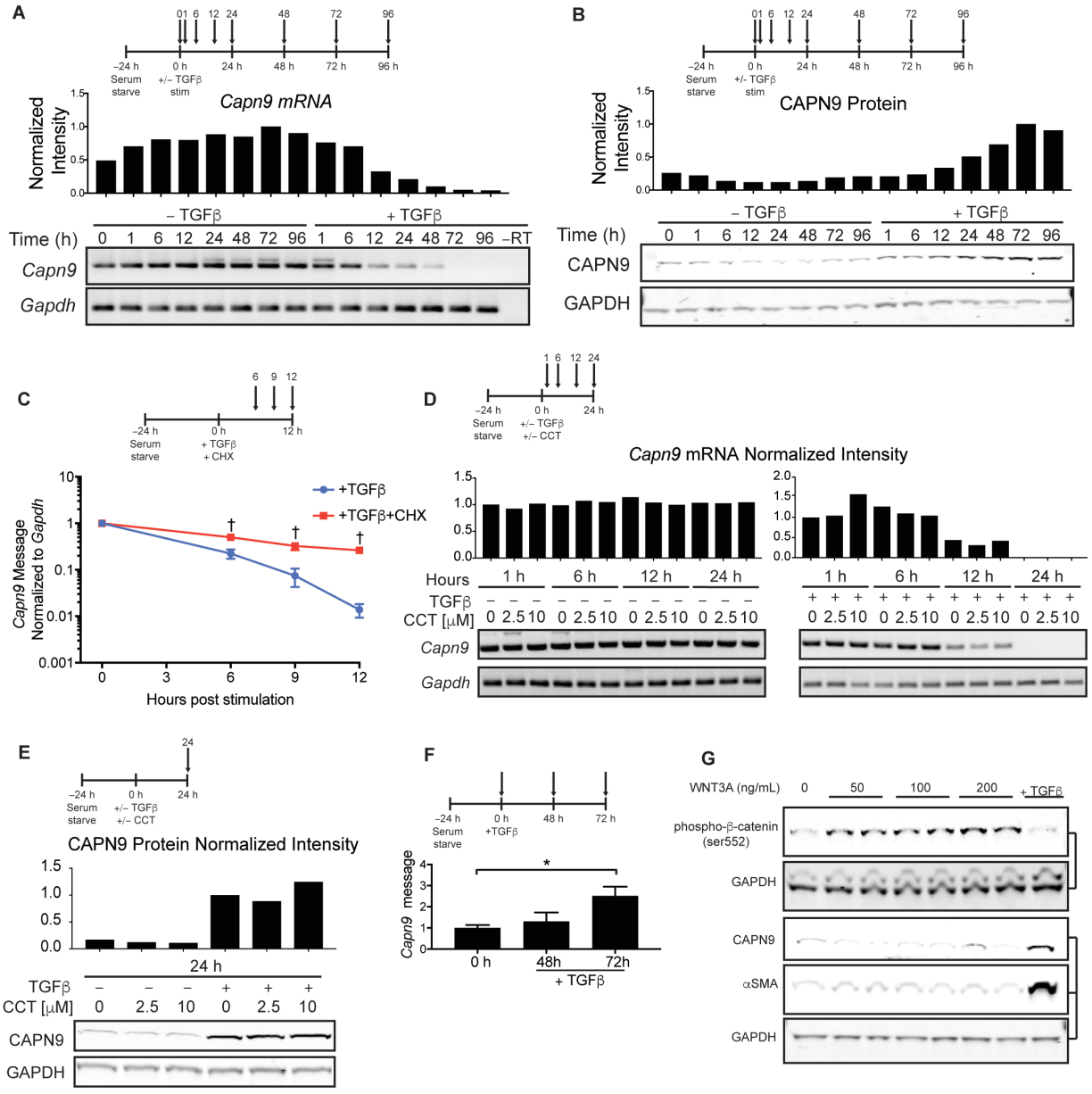


Supplementary Fig. 3. Expression of dimeric calpain message in mouse tissue and expression of EMT transcription factors in NMuMG cells. (**A**) Heat map of gene expression of dimeric calpains in mouse tissues normalized to *Gapdh* ($n = 3$). For each gene, expression is normalized to 1 in the highest expressing tissue. (**B**) Expression of *Capn9* mRNA (left)

and EMT transcription factors (right) in NMuMG cells 24 h after stimulation with TGFβ and siRNA knockdown of *Capn9* (siC9 +TGFβ) compared to controls. For each EMT transcription factor (right), statistically significant comparisons are made to unstimulated cells without knockdown (siCTRL). Data are expressed as mean ± s.e.m. **P* < 0.05, ***P* < 0.01, †*P* < 0.005, ‡*P* < 0.001 by student's t-test (right) and two-way ANOVA with Dunnett's *post hoc* test.

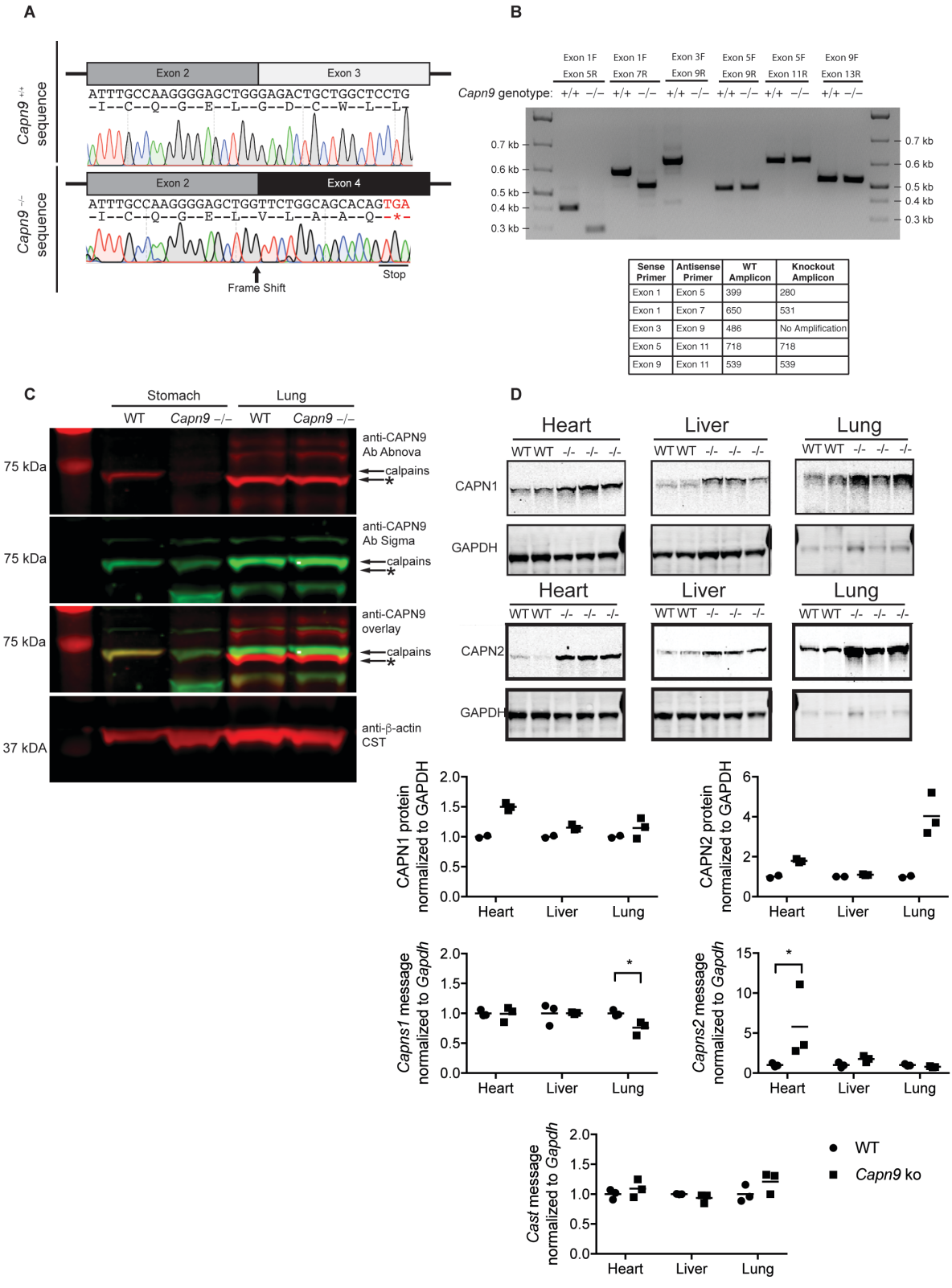


Supplementary Fig. 4. Calpain inhibition suppressed mesenchymal transition in multiple cell types. (**A**) Representative immunoblot blot and quantification of αSMA in serum starved, TGFβ1-stimulated MDCK (*n* = 4) at indicated time points with or without calpain inhibition, and (**B**) representative immunoblots and quantification of αSMA in serum starved, TGFβ1-stimulated primary NHLF (*n* = 3) at 24 h with calpain inhibition. Data are expressed as mean ± s.e.m. **P* < 0.05, ***P* < 0.01, †*P* < 0.005, ‡*P* < 0.001 by one-way ANOVA with Tukey's *post hoc* test.

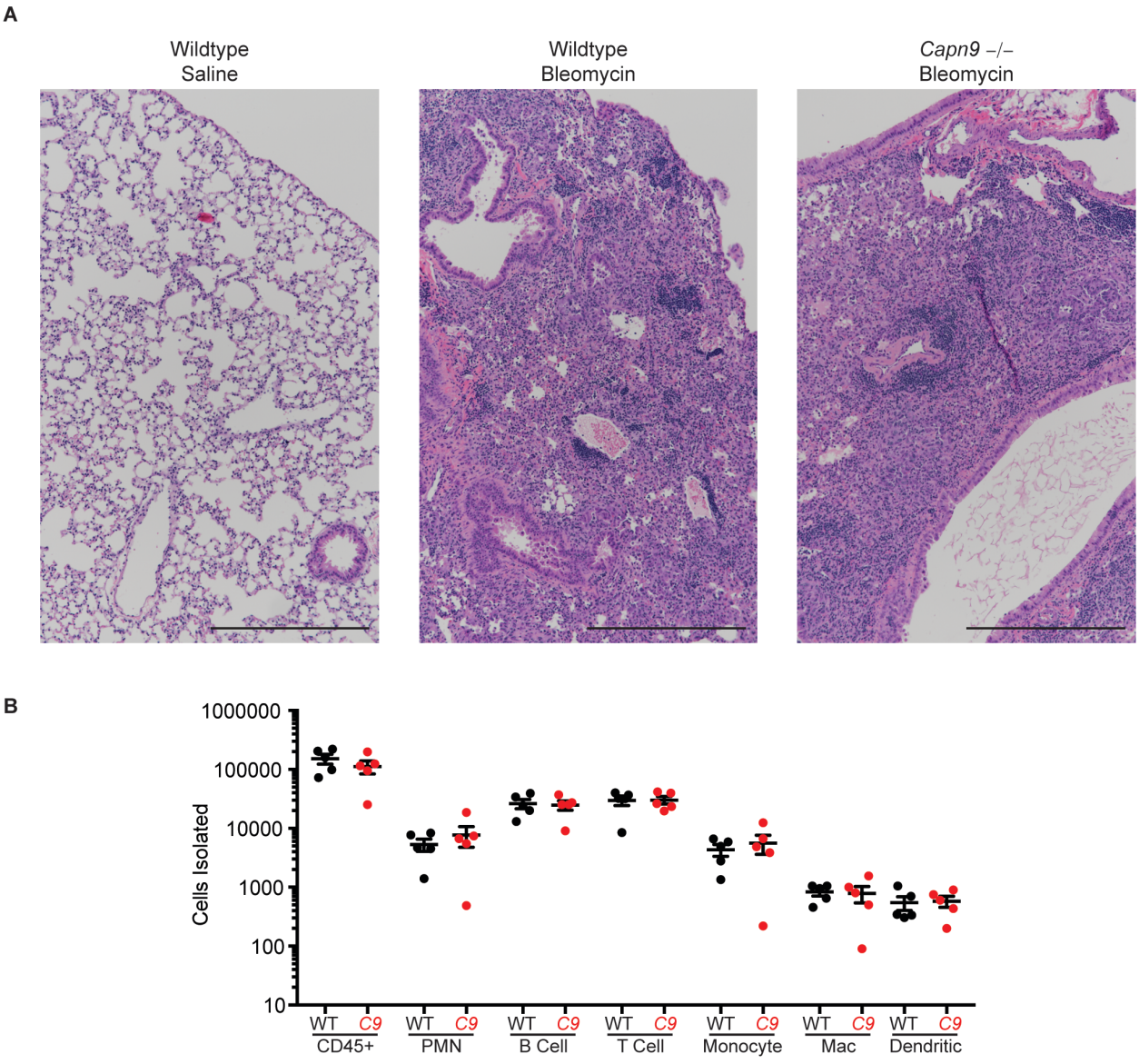


Supplementary Fig. 5. TGFβ induced expression of CAPN9 protein with coordinated translation-dependent mRNA decay in NMuMG cells. (**A**) Time course of the experiment with arrows indicating points at which samples were collected. Amplified cDNA of *Capn9* message normalized to *Gapdh* at 24 h of starvation (time point 0 h) and at

indicated time points during continued starvation (–TGFβ) or with stimulation (+TGFβ). No reverse transcriptase control indicated by "–RT". (**B**) Protein quantities of CAPN9 normalized to GAPDH with serum starvation (0 h), and in the setting of TGFβ stimulation. (**C**) Following starvation, NMuMG cells were stimulated with TGFβ and translation was inhibited with cycloheximide (CHX, 5 μg/mL). *Capn9* message at 6, 9, and 12 h after treatment was quantified by RT-qPCR and normalized to *Gapdh*. †P < 0.005 by two-way ANOVA with Sidak's post hoc test ($n = 3$). (**D**) Amplified cDNA of *Capn9* message normalized to *Gapdh* and (**E**) CAPN9 protein normalized to GAPDH following starvation with or without TGFβ stimulation in the presence of the AKT2 inhibitor CCT128930 at indicated concentrations. (**F**) *Capn9* mRNA normalized to GAPDH following TGFβ stimulation in MDCK cells. Data are expressed as mean ± s.e.m. *$P < 0.05$ by one-way ANOVA with Dunnett's *post hoc* test. (**G**) Protein abundance of CAPN9 and phospho-β-catenin in NMuMG cells 48 h after stimulation with WNT3a or TGFβ.


A
Capn9 +/+ sequence
Exon 2
Exon 3
ATTTGCCAAGGGGAGCTGGGAGACTGCTGGCTCCTG
-I--C--Q--G--E--L--G--D--C--W--L--L-
Capn9 −/− sequence
Exon 2
Exon 4
ATTTGCCAAGGGGAGCTGGTTCTGGCAGCACAGTGA
-I--C--Q--G--E--L--V--L--A--A--Q--*-
Frame Shift
Stop
B
Exon 1F Exon 5R
Exon 1F Exon 7R
Exon 3F Exon 9R
Exon 5F Exon 9R
Exon 5F Exon 11R
Exon 9F Exon 13R
Capn9 genotype: +/+ −/− +/+ −/− +/+ −/− +/+ −/− +/+ −/− +/+ −/−
0.7 kb
0.6 kb
0.5 kb
0.4 kb
0.3 kb
Sense Primer | Antisense Primer | WT Amplicon | Knockout Amplicon
Exon 1 | Exon 5 | 399 | 280
Exon 1 | Exon 7 | 650 | 531
Exon 3 | Exon 9 | 486 | No Amplification
Exon 5 | Exon 11 | 718 | 718
Exon 9 | Exon 11 | 539 | 539
C
Stomach
Lung
WT
Capn9 −/−
WT
Capn9 −/−
75 kDa
anti-CAPN9 Ab Abnova
calpains
*
75 kDa
anti-CAPN9 Ab Sigma
calpains
*
75 kDa
anti-CAPN9 overlay
calpains
*
37 kDA
anti-β-actin CST
D
Heart
Liver
Lung
WT WT -/- -/- -/-
CAPN1
GAPDH
CAPN2
GAPDH
CAPN1 protein normalized to GAPDH
CAPN2 protein normalized to GAPDH
Capns1 message normalized to Gapdh
Capns2 message normalized to Gapdh
Cast message normalized to Gapdh
Heart
Liver
Lung
*
WT
Capn9 ko

Supplementary Fig. 6. *Capn9*$^{-/-}$ mice expressed stable *Capn9* message that lack exon 3. (**A**) Sanger sequencing of cDNA isolated from wildtype and *Capn9*$^{-/-}$ mice. Knockout amplicons show skipping of exon 3 and frame shift producing a stop codon (TGA) 6 triplet codons downstream of the exon-exon junction. (**B**) PCR amplification of cDNA isolated from the stomach of wildtype and *Capn9*$^{-/-}$ mice. Amplicons including exon 3 from *Capn9*-targeted mice are predicted to be 119 bp smaller than wildtype amplicons. PCR reactions with a primer complementary to exon 3 did not amplify. (**C**) Western blot analysis of CAPN9 protein expression in wildtype and *Capn9*$^{-/-}$ mouse stomach and lung. Antibodies to CAPN9 are Abnova clone 3A6 (H00010753-M02) and Sigma (HPA020398). Asterisk indicates a nonspecific band near the expected weight of CAPN9. (**D**) Protein expression of CAPN1 and CAPN2 in mouse heart, liver, and lung tissue in wildtype and *Capn9*$^{-/-}$ (n = 2, 3 respectively). Messenger RNA expression of *Capns1/s2* and *Cast* in mouse heart, liver, and lung tissue (n = 3). Data are expressed as mean *P < 0.05 by one-way ANOVA with Sidak's *post hoc* test.



Supplemental Fig. 7. Bleomycin induced an equal inflammatory response in wildtype and *Capn9*$^{-/-}$ animals. (**A**) Representative lung H&E sections of mouse lung. Scale bar: 400 µM. (**B**) Flow cytometry of right lung lobes from wildtype and *Capn9*$^{-/-}$ receiving bleomycin (n = 5). Cells gated on live singlets, CD45+, PMN (neutrophils), B cells, T cells, macrophages and dendritic cells.