

Supplementary Information

Calpain Inhibition Attenuates Adipose Tissue Inflammation and Fibrosis in Diet-induced Obese Mice

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[^] Deceased

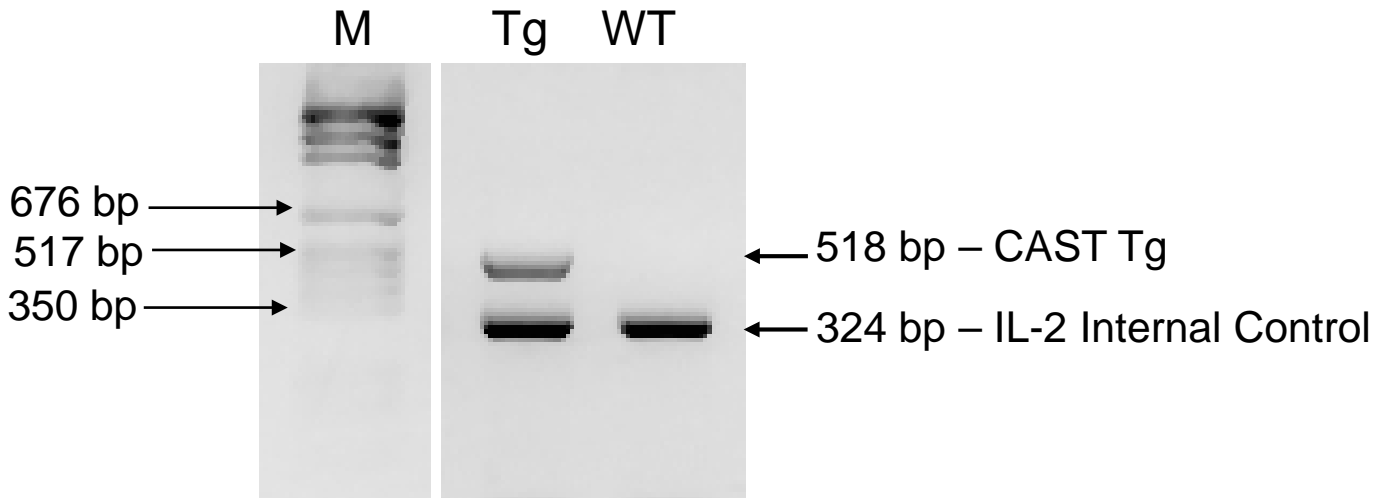
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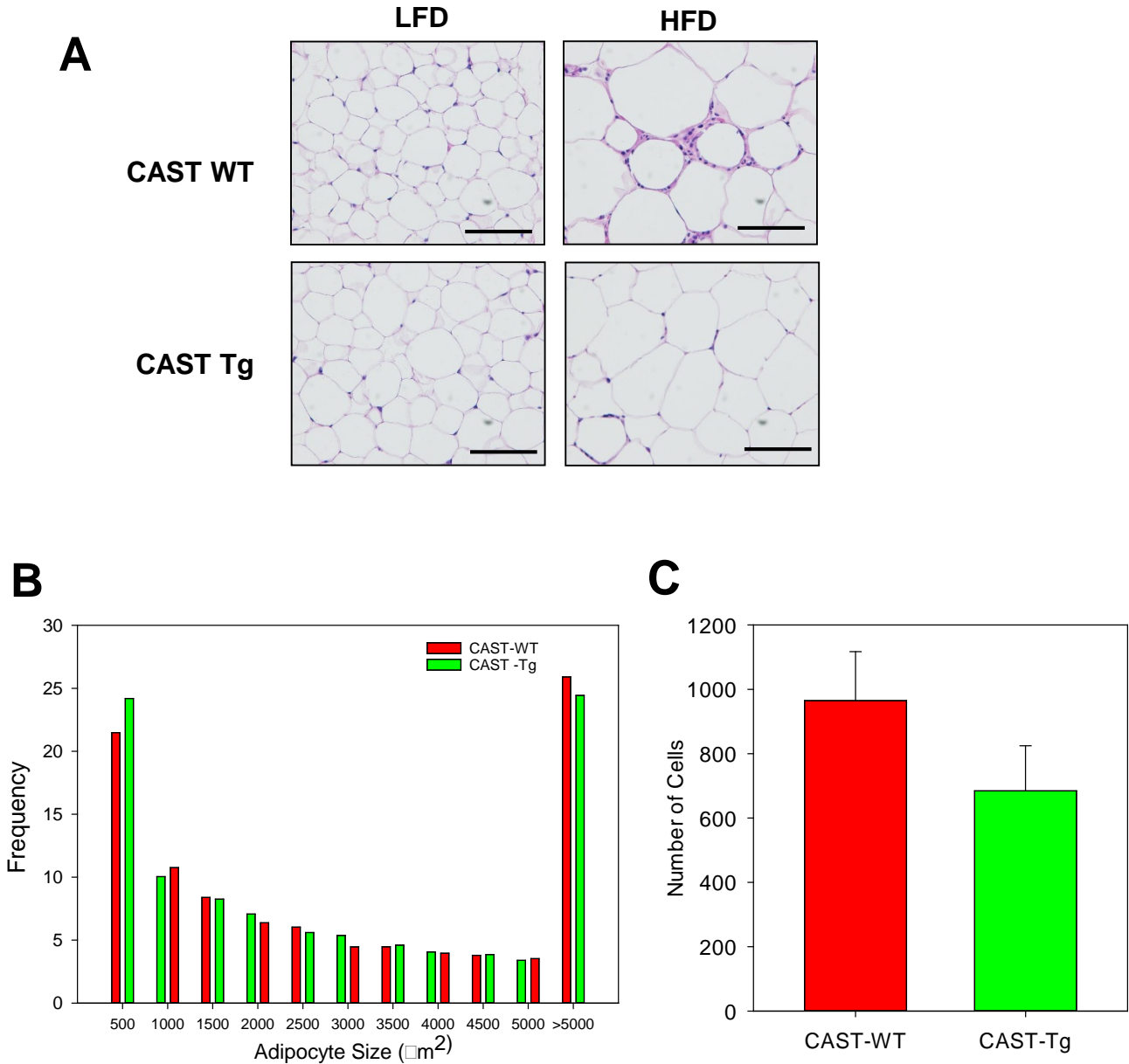
Number of Figures	:	8
Number of Tables	:	2

Calpastatin Tg genotyping

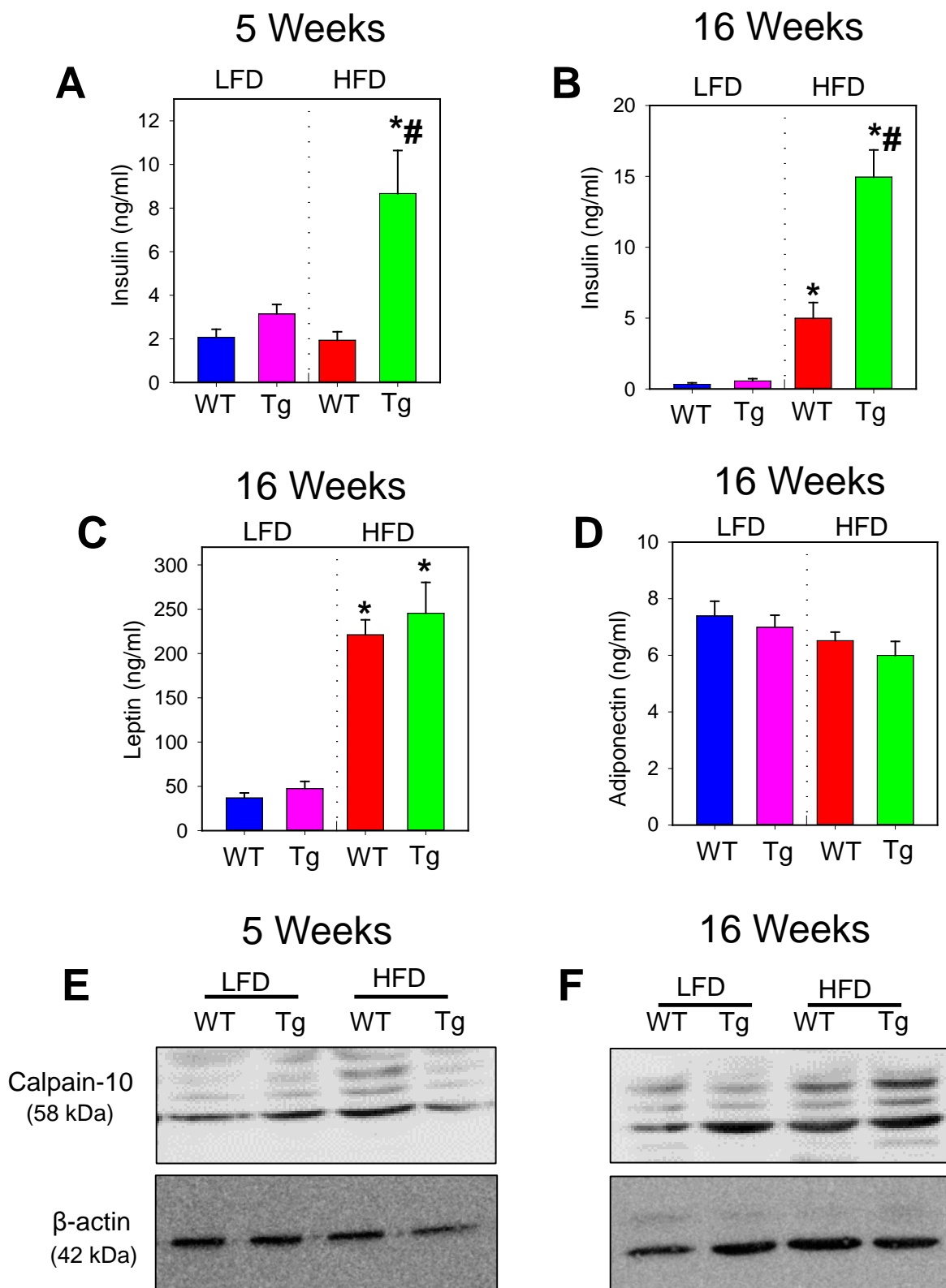


Supplementary Figure I. Genotyping of experimental mice for the CAST transgene (Tg) by PCR.

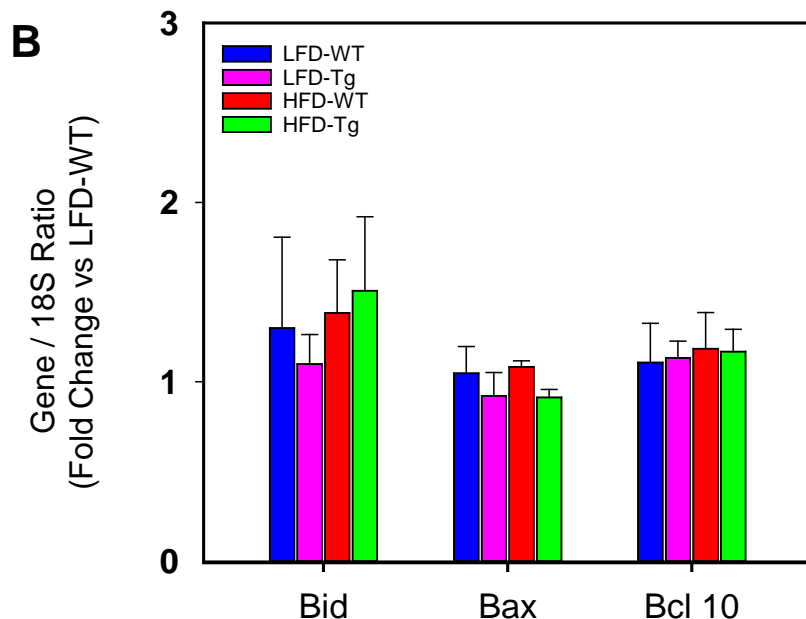
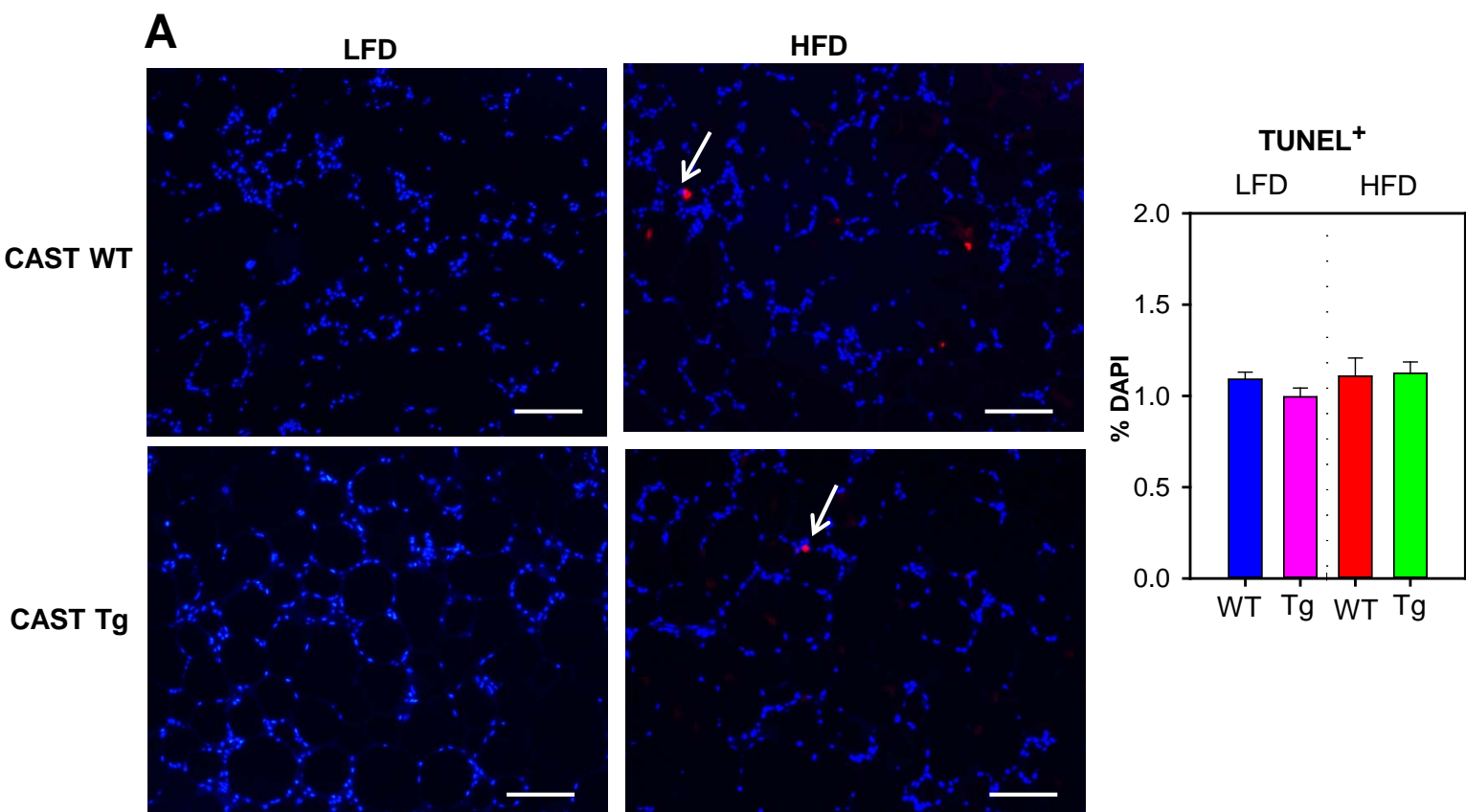
A. Genomic DNA from tail was isolated and screened by PCR for the CAST-Tg allele. IL-2 was used as an internal control. PCR on tail DNA yielded amplicons of 518 and 324 bp for CAST-Tg and IL-2 alleles, respectively. (M= Molecular weight ladder)



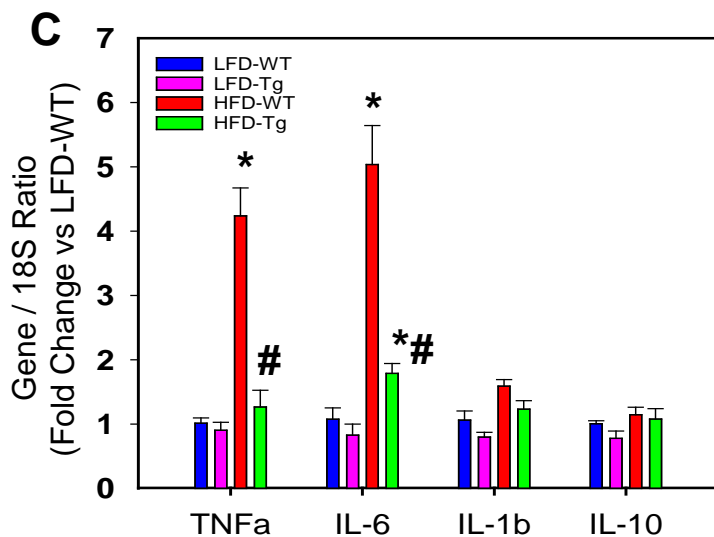
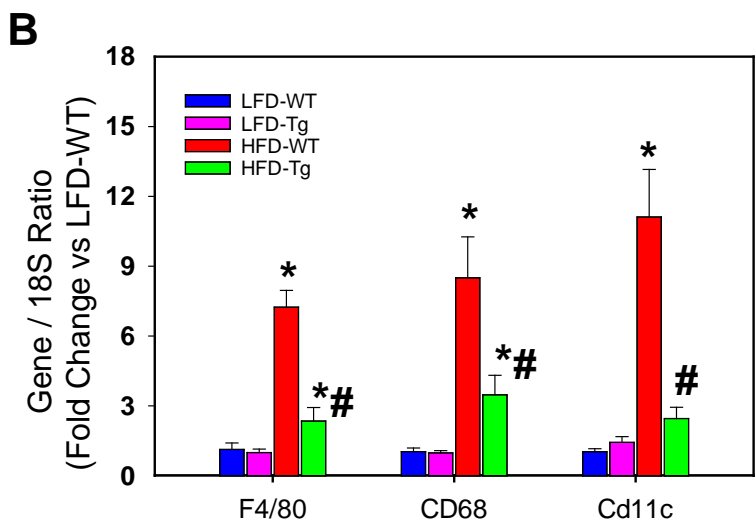
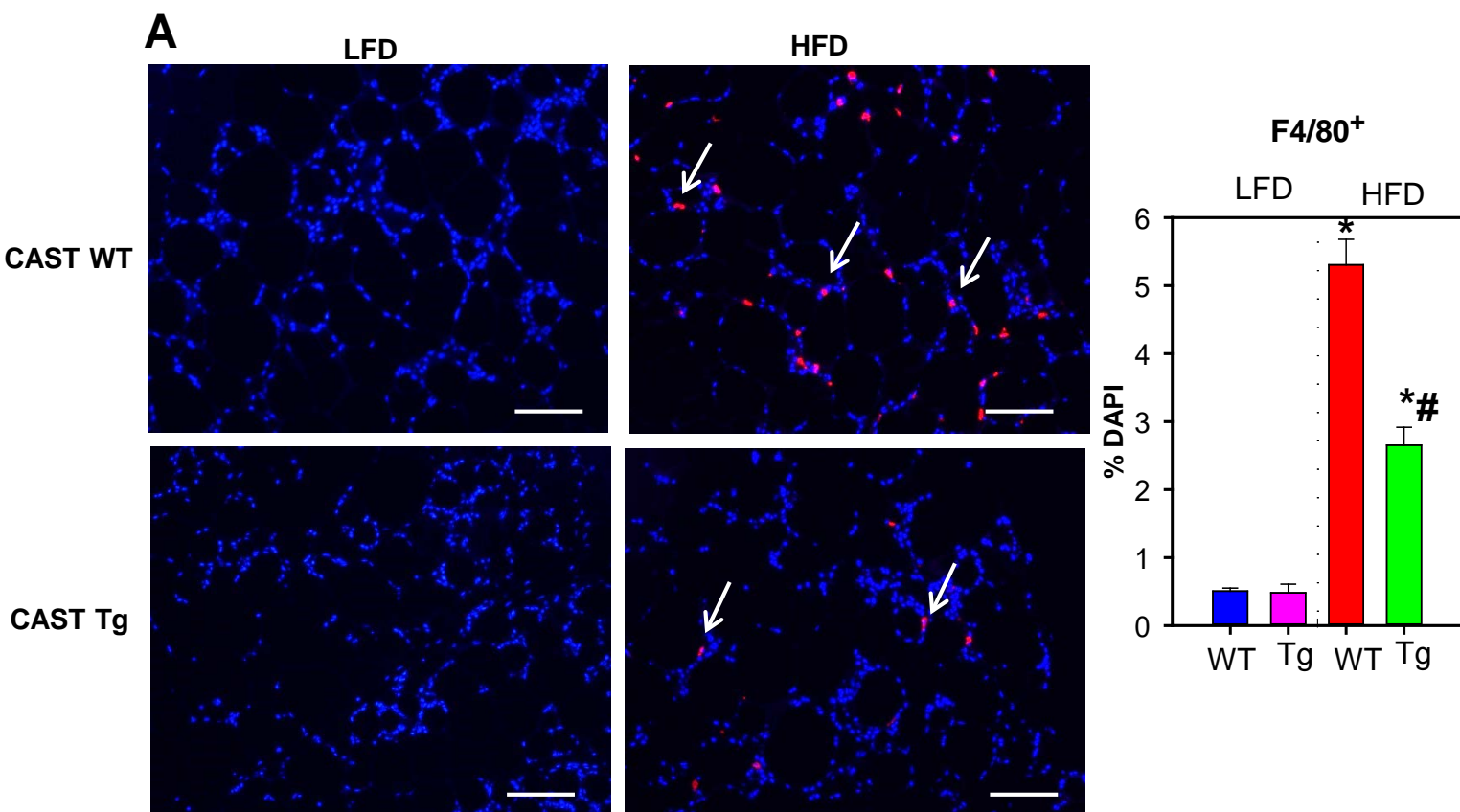
Supplementary Figure II. CAST overexpression had no effect on obesity-induced adipocyte expansion. **A.** Representative hematoxylin and eosin staining of EpiWAT cross-sections from LFD and HFD fed CAST WT and Tg mice. **B.** Histogram of adipocyte diameters in EpiWAT from HFD fed CAST WT and Tg mice. **C.** Quantification of adipocyte number per measurement frame (n=5-6 mice). Values are represented as mean \pm SEM.



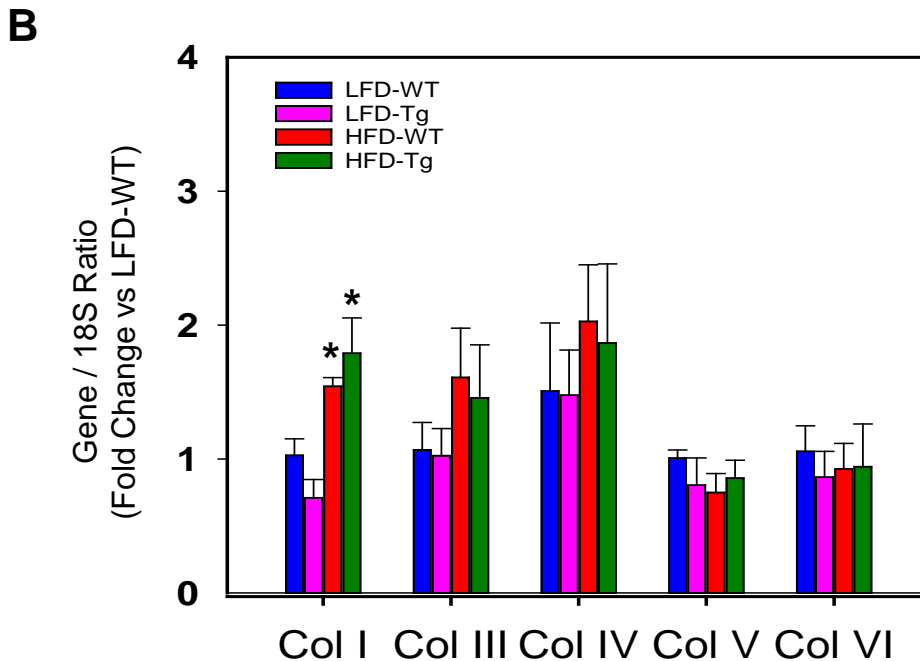
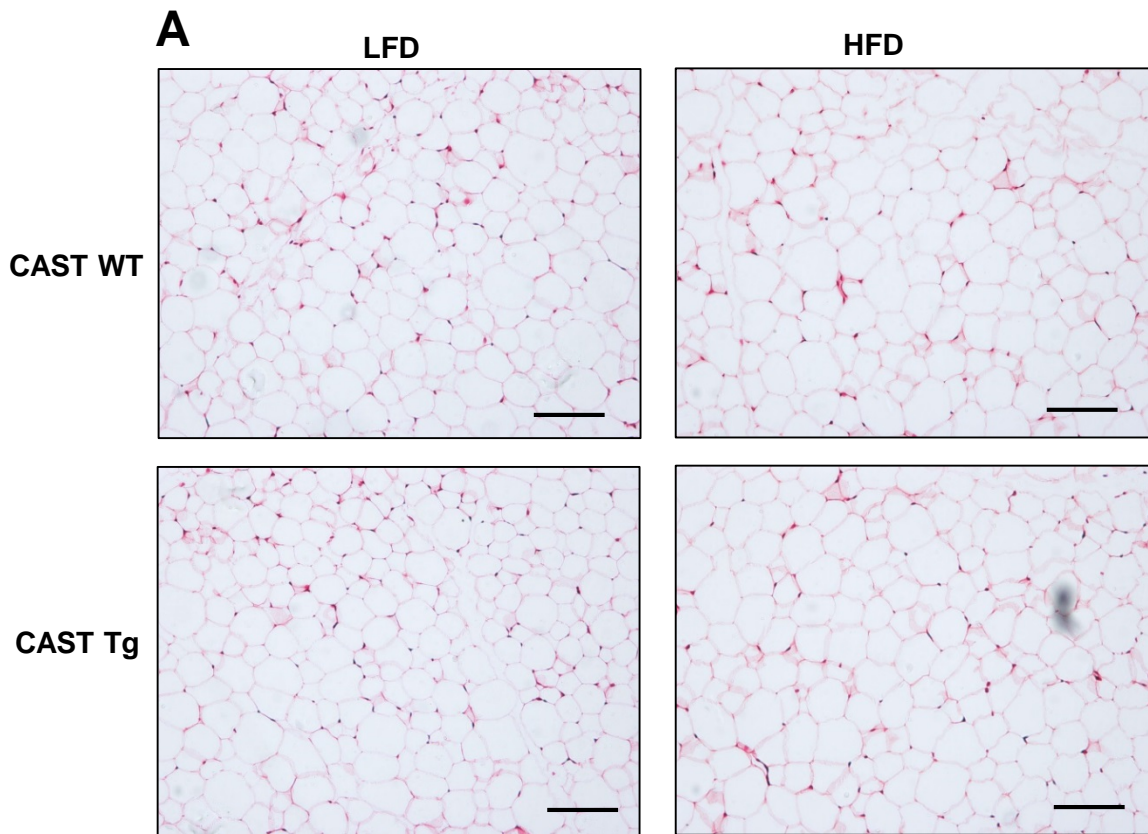
Supplementary Figure III. CAST overexpression increased plasma insulin levels upon HFD feeding. Plasma Insulin (A,B), leptin (C) and adiponectin (D) were measured by ELISA (n=12-14). Values are represented as mean \pm SEM. * denotes $P < 0.05$ HFD vs LFD; # $P < 0.05$ CAST WT vs CAST Tg (Two-way ANOVA with Holm-Sidak post hoc analysis). E, F. Calpain-10 protein was detected by Western blotting of EpiWAT extracts from WT and CAST Tg mice fed a LFD or HFD diet for 5 weeks or 16 weeks (n=4).



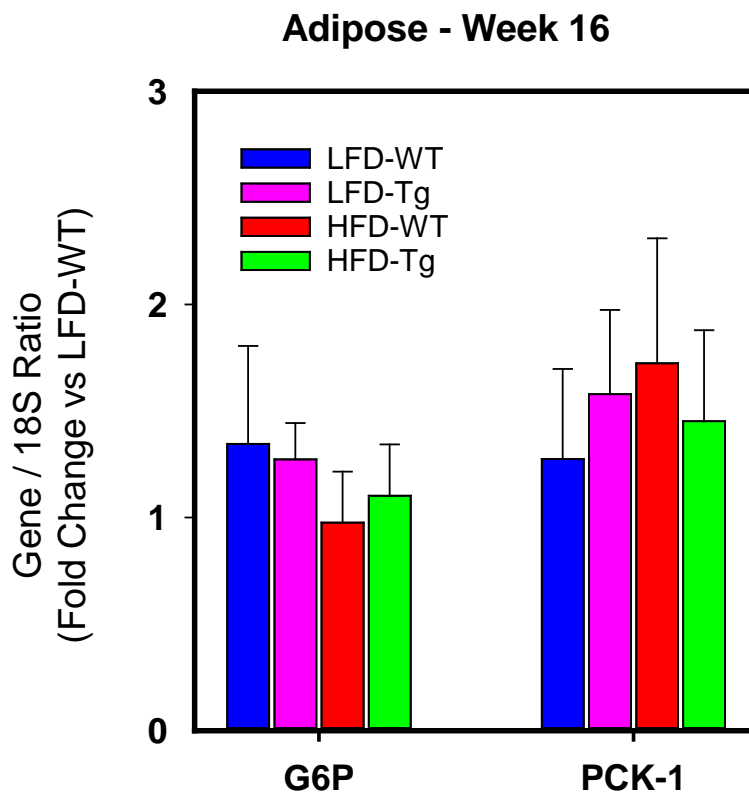
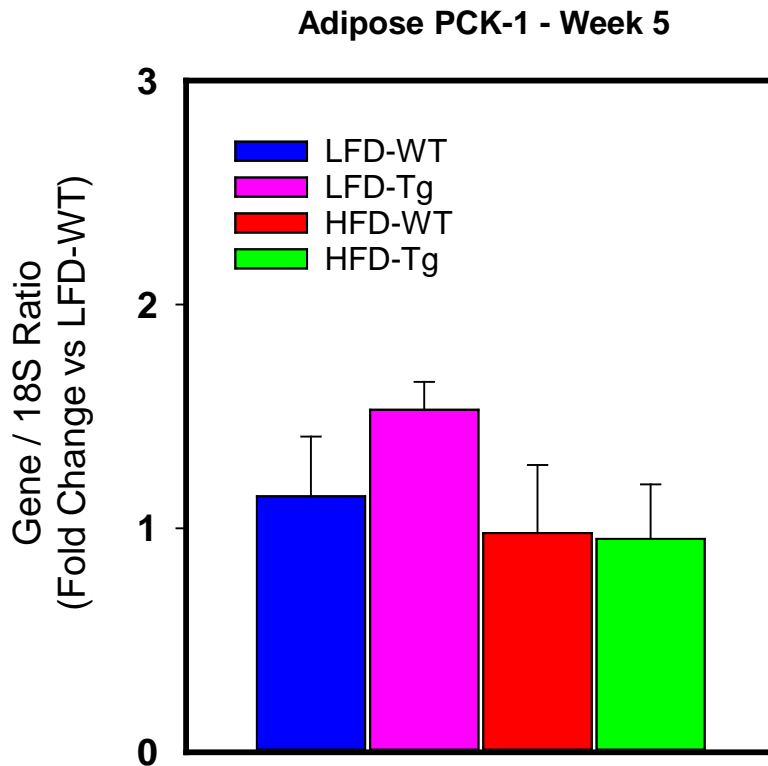
Supplementary Figure IV. Early stage obesity does not promote adipocyte cell death. **A.** Representative TUNEL staining of EpiWAT cross-sections from 5 weeks LFD and HFD fed CAST WT and Tg mice. The nuclei were stained with DAPI (blue) and the TUNEL-positive cells (red) were indicated by arrows. Under fluorescent microscopy, TUNEL-positive cells were counted from 10 fields at the power of 100x magnification (n=5). **B.** mRNA abundance of Bid, Bax and Bcl 10 genes in EpiWAT from LFD and HFD fed CAST WT and Tg mice were analyzed by qPCR (n=4-6). Values are represented as mean \pm SEM (Two-way ANOVA with Holm-Sidak post hoc analysis).



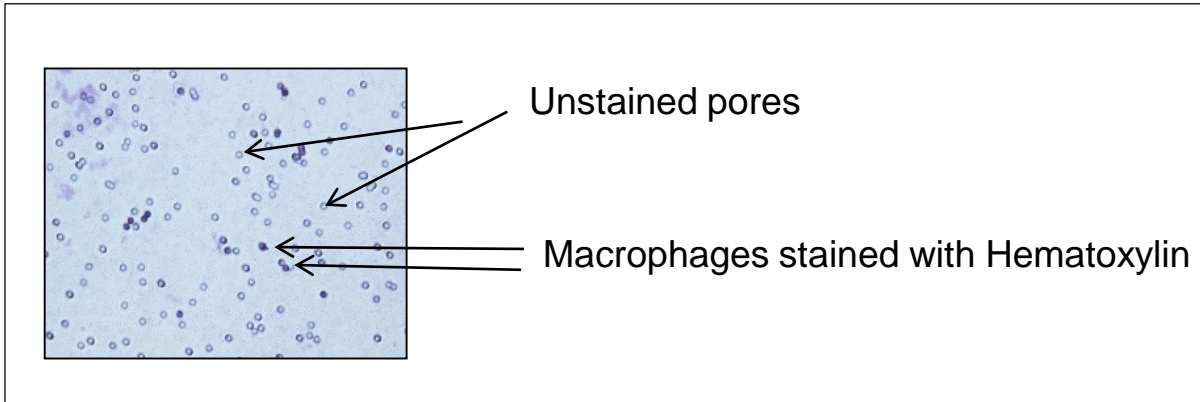
Supplementary Figure V. CAST overexpression significantly reduced macrophage accumulation and inflammatory genes during the early stage of obesity. **A.** Representative immunofluorescent staining of F4/80 in EpiWAT cross-sections from 5 weeks LFD and HFD fed CAST WT and Tg mice. The nuclei were stained with DAPI (blue) and the F4/80-positive cells (red) were indicated by arrows. Using fluorescent microscopy, F4/80-positive cells were counted from 10 fields at the power of 100x magnification (n=5). **B.** mRNA abundance of F4/80, CD68, and CD11c genes in EpiWAT from LFD and HFD fed CAST WT and Tg mice were analyzed by qPCR (n=5). **C.** mRNA abundance of TNF α , IL-6, IL-1 β , and IL-10 genes in EpiWAT from LFD and HFD fed CAST WT and Tg mice (n=5). * denotes $P < 0.05$ when comparing LFD vs HFD (Two-way ANOVA with Holm-Sidak post hoc analysis).



Supplementary Figure VI. Early stage obesity showed no change in interstitial fibrosis in adipose tissue. **A.** Representative Sirius red staining of EpiWAT cross-sections from 5 weeks LFD and HFD fed CAST WT and Tg mice. **B.** mRNA abundance of Col I, Col III, Col IV, Col V and Col VI genes in EpiWAT from LFD and HFD fed CAST WT and Tg mice were analyzed by qPCR (n=5). Values are represented as mean \pm SEM. * denotes $P < 0.05$ when comparing LFD vs HFD (Two-way ANOVA with Holm-Sidak post hoc analysis).

A**B**

Supplementary Figure VII. CAST overexpression had no effect on adipose tissue PCK-1 mRNA abundance. mRNA abundance of G6P and PCK-1 in adipose tissue from 16 week (**A**) and PCK-1 in adipose tissue from 5 week (**B**) LFD and HFD fed CAST WT and Tg mice were analyzed by qPCR (n=5). Values are represented as mean \pm SEM. (Two-way ANOVA with Holm-Sidak post hoc analysis).



Supplementary Figure VIII. An example of the transwell membrane migrated macrophages stained with hematoxylin.

Supplementary Table I. Effects of calpastatin overexpression on food and water intake in LFD and HFD fed mice

Groups	CAST WT		CAST Tg	
Diet	LFD	HFD	LFD	HFD
N	10	12	10	13
Food Intake (g/day)	4.3 ± 0.18	3.5 ± 0.08	4.1 ± 0.13	3.1 ± 0.15
Water Intake (ml/day)	4.2 ± 0.88	3.0 ± 0.49	3.9 ± 0.72	3.2 ± 0.53

Values are represented as means ± SEMs.

Supplementary Table II. Primers used for real-time PCR

Gene	Primers	Product size (bp)
Bid	5'-CTGCCTGTGCAAGCTTACTG-3' 5'-GTCTGGCAATGTTGTGGATG-3'	142
Bax	5'-TGTTTGCTGATGGCAACTTC-3' 5'-GATCAGCTCGGGCACTTTAG-3'	104
Bcl 10	5'-GCAGGGAGAAAACACAGAGC-3' 5'-CGGAATTGCACCTAGAGAGG-3'	156
Herpud 1	5'-CCTGGCTTCTCTGGCTACAC-3' 5'-GTCGGGACAAAAGTTCCTGA-3'	104
F4/80	5'-CTGTAACCGGATGGCAAAC-3' 5'-ATGGCCAAGGCAAGACATAC-3'	123
CD68	5'-CCAATTCAGGGTGAAGAAA-3' 5'-ATGGGTACCGTCACAACCTC-3'	114
CD11c	5'-CAAAATCTCCAACCCATGCT-3' 5'-TCTGGGAAGCCAAATACGAC-3'	128
CD206	5'-CAAGGAAGGTTGGCATTGT-3' 5'-CCTTTCAGTCCTTTCGAAGC-3'	111
MCP-1	5'-CAGCCAGATGCAGTTAACGC -3' 5'-TCTGGACCCATTCTTCTTG-3'	175
CCR2	5'-ACACCCTGTTTCGCTGTAGG-3' 5'-GATTCCTGGAAGGTGGTCAA-3'	133
TNF α	5'-CCCACTCTGACCCCTTACTC-3' 5'-TCACTGTCCCAGCATCTTGT-3'	114
IL-6	5'-GGGAAATCGTGGAAATGAGAAA-3' 5'-AAGTGCATCATCGTTGTTTCATACA-3'	167
IL-1 β	5'-TCGTGCTGTCGGACCCATAT-3' 5'-GTCGTTGCTTGGTTCTCCTTGT-3'	110
IL-10	5'-CCAAGCCTTATCGGAAATGA-3' 5'-TCTCACCCAGGGAATTCAA-3'	190
Col I	5'-TGGCAACAAAGGAGACACTG-3' 5'-GGCTCCTCGTTTTCTTCTT-3'	97
Col III	5'-ACCAAAAGGTGATGCTGGAC-3' 5'-GACCTCGTGCTCCAGTTAGC-3'	110
Col IV	5'-CCAAAGGATCAGTTGGAGGA-3' 5'-CTCTCCTTTGGCTCCCTTCT-3'	119
Col V	5'-GGTCCCTGACACACCTCAGT-3' 5'-TGCTCCTCAGGAACCTCTGT-3'	183

Col VI	5'-GGGACACGACACCTCTCAGT-3' 5'-TTGGCAGGAAATGACATTGA-3'	119
ABCA1	5'-CTGGTTTGGTGAGGAAATTCA-3' 5'-ACCTTCATGCCATCTCGGTA-3'	150
ABCG1	5'-GCTGGGAAGTCCACACTCAT-3' 5'-ATCATGGGTCTCTGAAGAGT-3'	173
FAS	5'-CCAGTGTCACCACCAAGCG-3' 5'-GGAGCGCAGGATAGACTCAC-3'	111
FABP	5'-CAATAGGTCTGCCCCGAGGAC-3' 5'-AGCTTGACGACTGCCTTGAC-3'	193
G6P	5'-TGGATTCCGGTGTGTTGAACG-3' 5'-GCAAGGTAGATCCGGGACAG-3'	84
PCK-1	5'-ATGAAAGGCCCGCACCATGTA-3' 5'-AGGCCAGTTGTTGACCAAA-3'	235
PPAR γ	5'- AGCATCAGGCTTCCACTATG -3' 5'- ATCCGGCAGTTAAGATCACA-3'	111
Adiponectin	5'-GGAACTTGTGCAGGTTGGAT-3' 5'-CGAATGGGTACATTGGGAAC-3'	293
CEBP α	5'-TGGACAAGAACAGCAACGAG-3' 5'-TCACTGGTCAACTCCAGCAC-3'	127
CEBP β	5'-CAAGCTGAGCGACGAGTACA-3' 5'-AGCTGCTCCACCTTCTTCTG-3'	156
PREF-1	5'-GGCAGTGCATCTGCAAGGAT-3' 5'-CAGGTCCACGCAAGTTCCAT-3'	104
UCP-1	5'-TGGAAAGGGACGACCCCTAA-3' 5'-CAGGAGTGTGGTGCAAAACC-3'	168
18S	5'-CTCTGTTCCGCCTAGTCCTG 5'-AATGAGCCATTTCGAGTTTC	170

Full-length blot of representative Western Blot images of Figure 1A, 1D, 1E, 1F and Figure 5B and 5E.

Figure 1

A

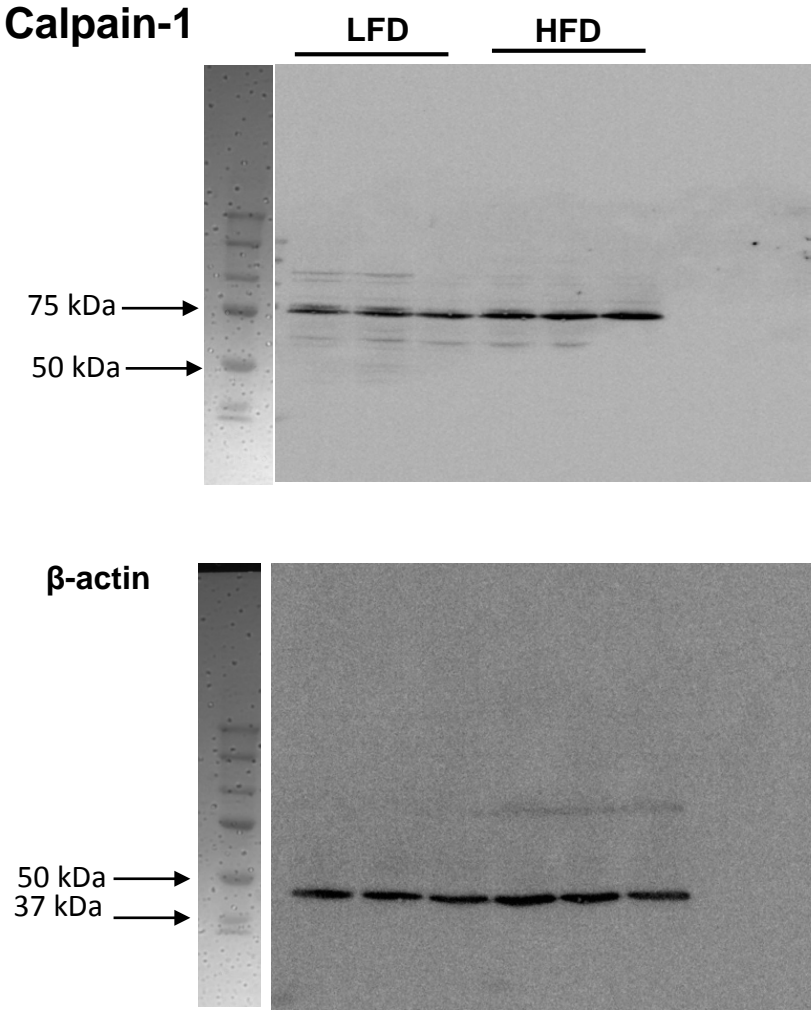


Figure 1

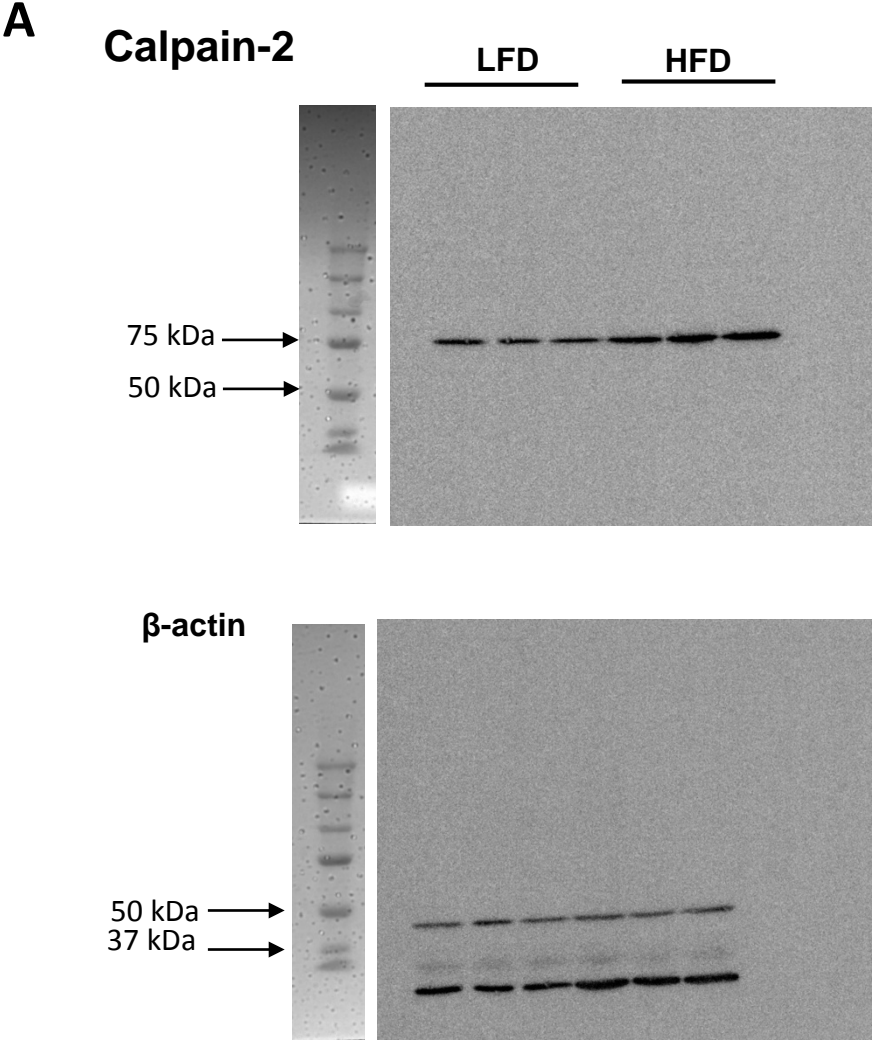


Figure 1

A

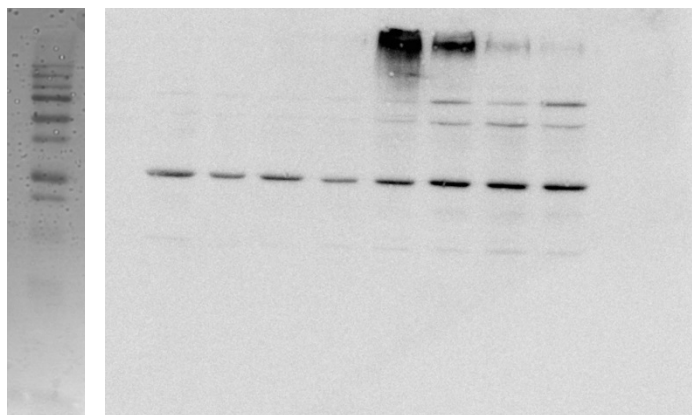
Calpain-4

LFD

HFD

37 kDa →

25 kDa →



β -actin

50 kDa →

37 kDa →

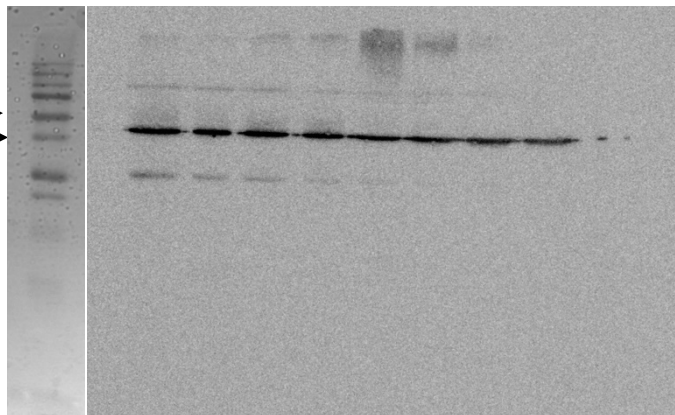


Figure 1

A

CAST

LFD

HFD

100 kDa →
75 kDa →



β-actin

50 kDa →
37 kDa →

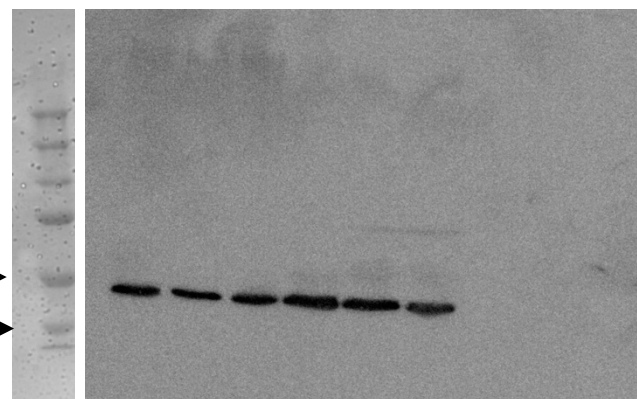
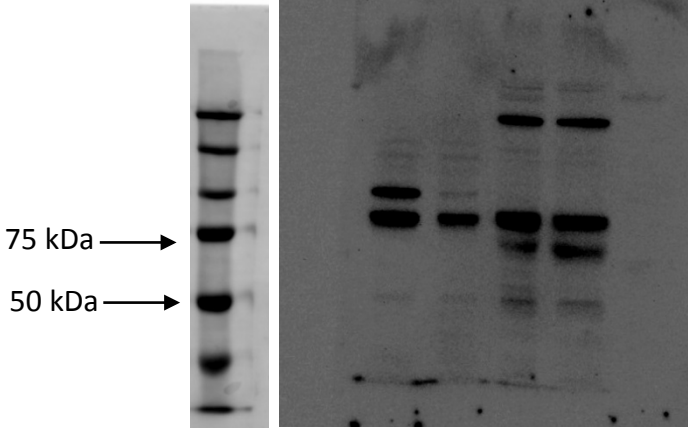


Figure 1

D **Calpain-1** Adip SVF
Epi SC Epi SC



β -actin

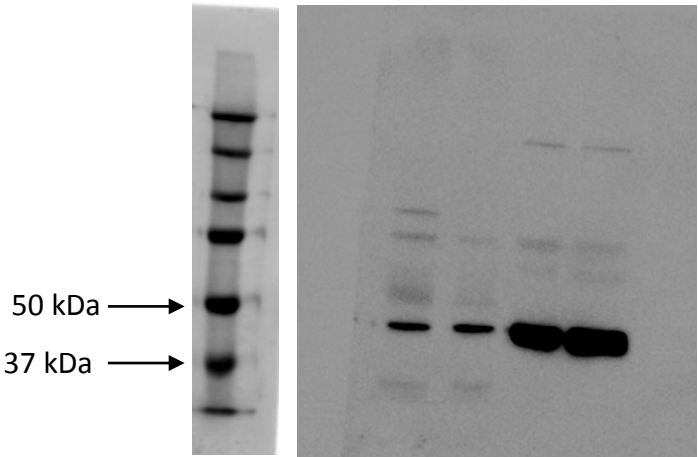
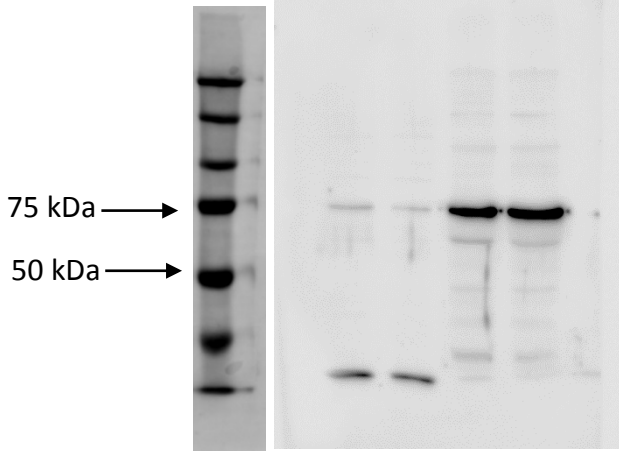


Figure 1

D Calpain-2

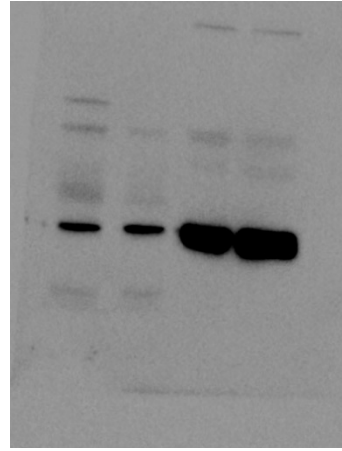
1 Minute Exposure

Adip SVF
Epi SC Epi SC



3 Minute Exposure

Adip SVF
Epi SC Epi SC



β -actin

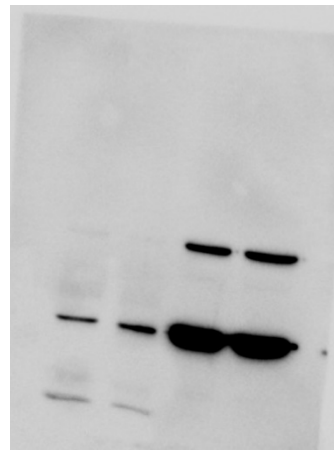
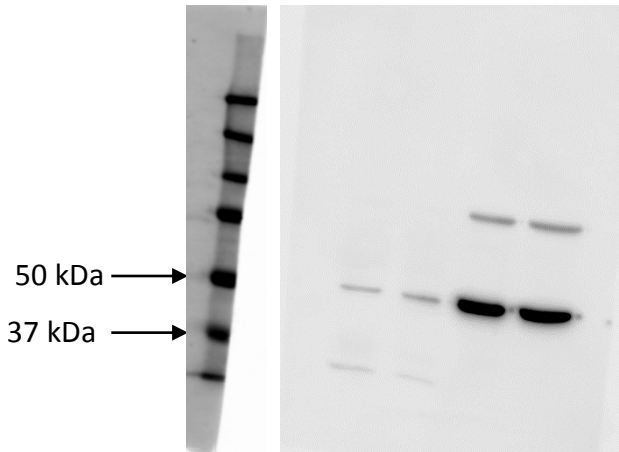
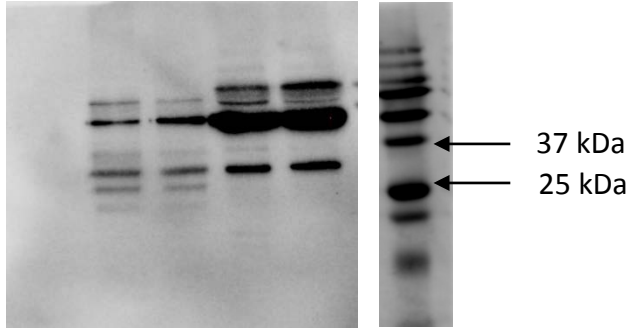


Figure 1

D Calpain-4

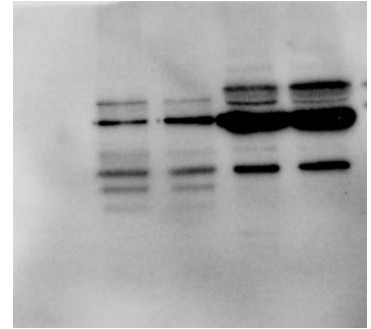
1 Minute Exposure

Adip SVF
Epi SC Epi SC



3 Minute Exposure

Adip SVF
Epi SC Epi SC



β -actin

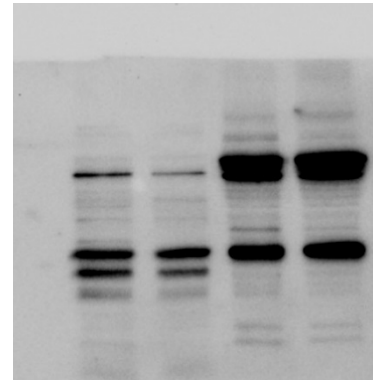
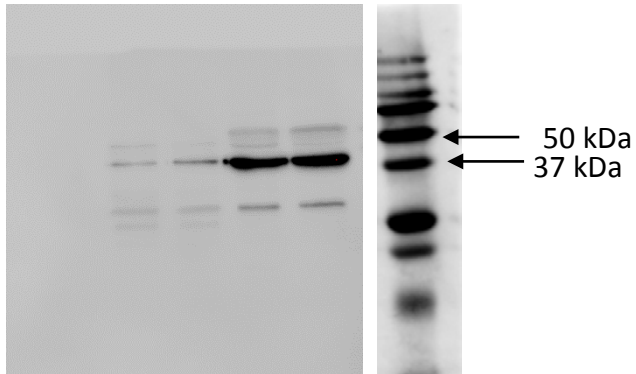


Figure 1

E

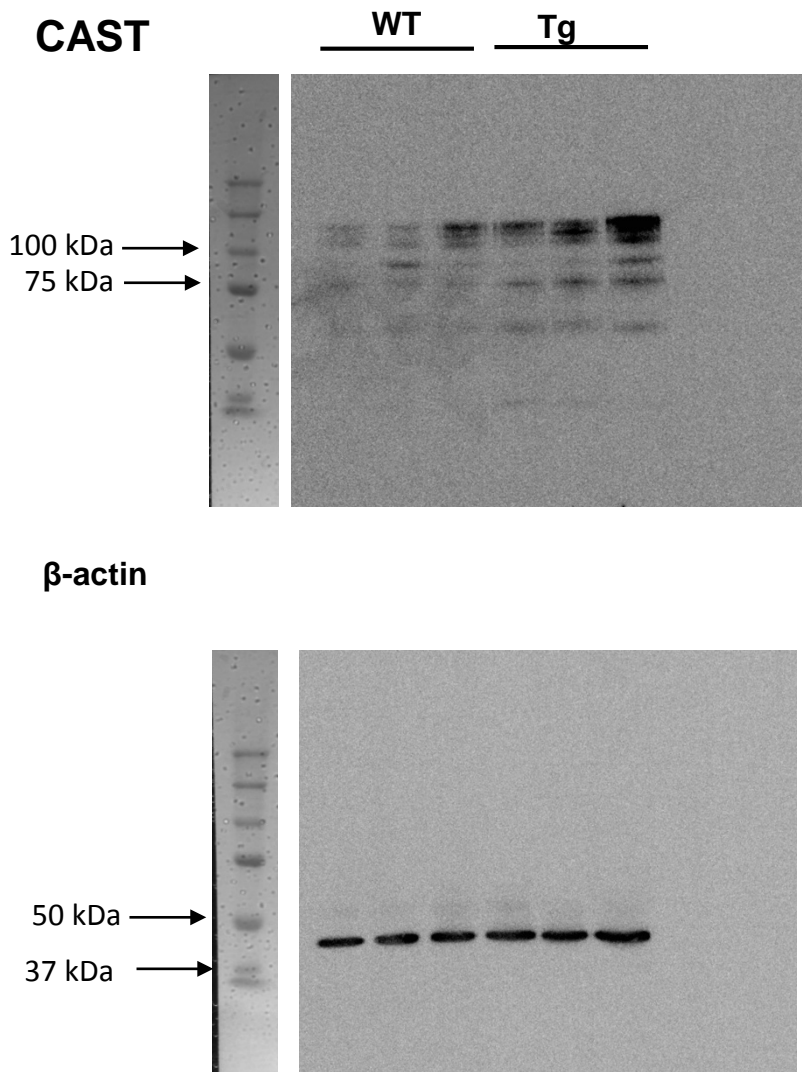
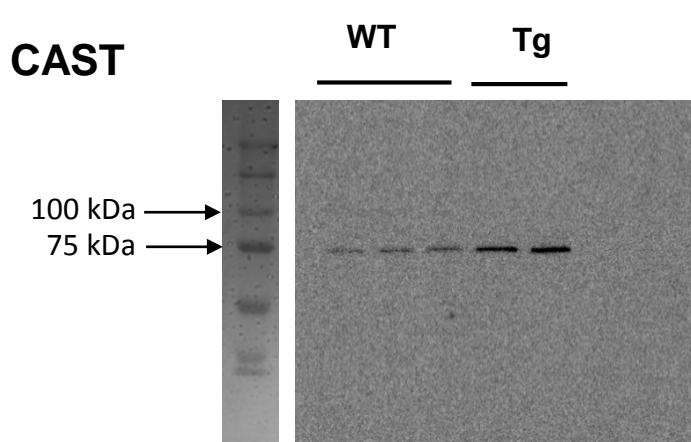


Figure 1

F



β-actin

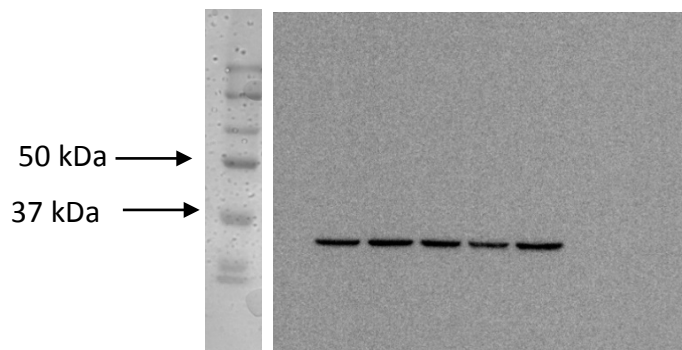


Figure 5

B

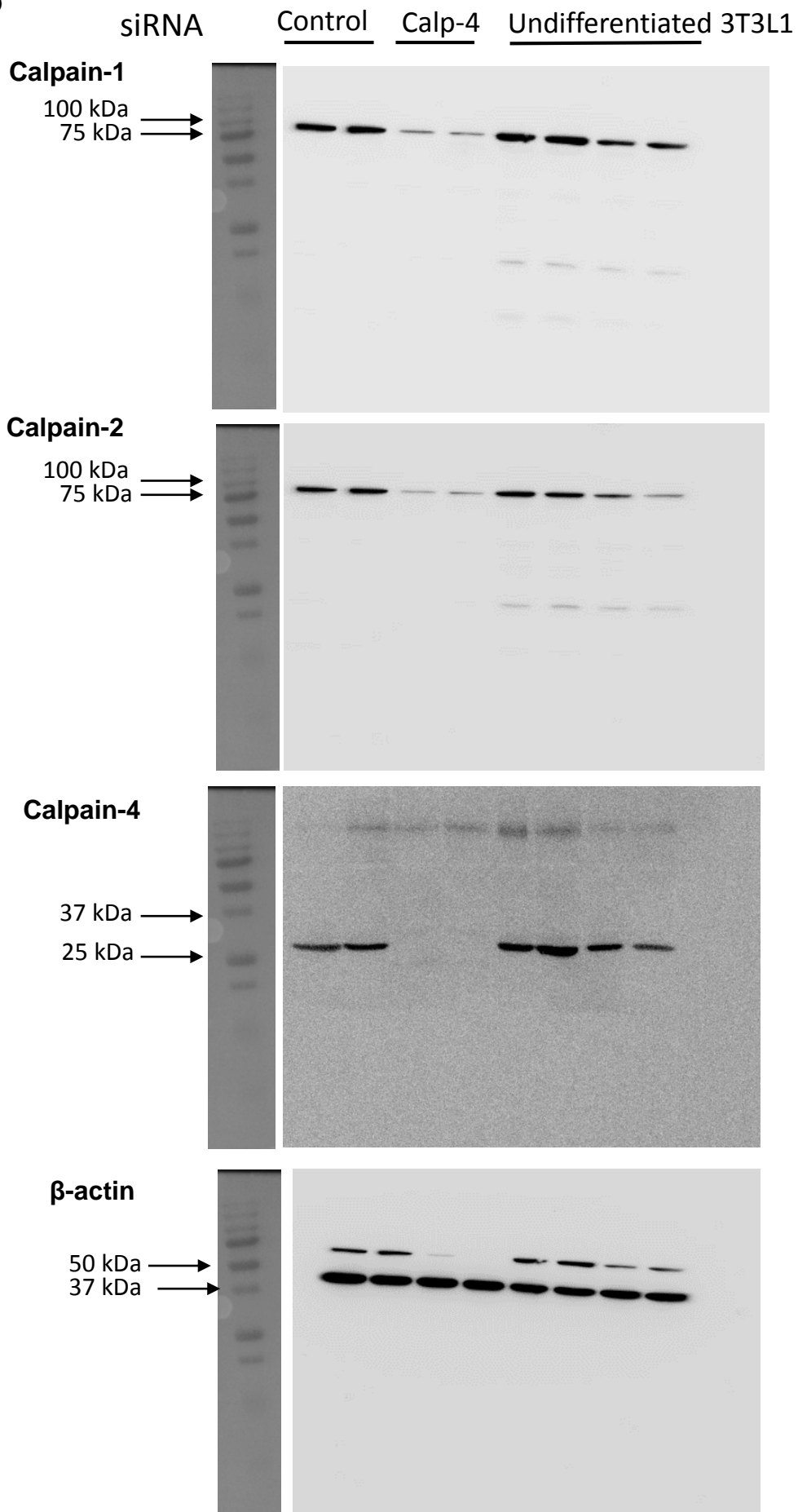


Figure 5

E

