Mapping and Exploring the Collagen-I Proteostasis Network

Andrew S. DiChiara, Rebecca J. Taylor, Madeline Y. Wong, Ngoc-Duc Doan, Amanda M. Del Rosario, Matthew D. Shoulders

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SUPPORTING EXPERIMENTAL PROCEDURES

Materials and Reagents. Antibodies used were obtained from the following suppliers: Santa Cruz: HA probe (sc-7392), PDI (sc-20132), CRTAP (sc-99367), CyPB (sc-20361); ProteinTech: DNAJB11 (15484-1-AP); GeneTex: GFP (GTX113617), HYOU1 (GTX102255); Sigma: Colα2(I) (SAB4500363), LAMP-1 (SAB3500285), GM130 (G7295), actin (A1978); MyBioSource: Colα1(I) (MBS502153); Cedarlane: Human Collagen Type I (CL50111AP-1); Agilent Technologies: Rat Anti-DYKDDDDK (200474); Enzo Life Sciences: HSP47 (ADI-SPA-470), KDEL (ADI-SPA-827), Calreticulin (ADI-SPA-601), Golim4 (ALX-804-603-C100); IBL America: Reticulocalbin-1 (10367); Abcam: Erp29 (ab11420); Abgent: FKBP10 (AP7383b); Cell Signaling: Ero1L (3264), Erp44 (3798), Erp57 (2881), Erp72 (5033), Calumenin (11991). Antibody beads were obtained from Sigma: FLAG M1 Agarose Affinity Gel (A4596), FLAG M2 Affinity Gel (A2220), and HA-Agarose beads (A2095). Secondary antibodies were obtained from LiCor Biosciences: 800CW Goat Anti-Rabbit, 800CW Goat Anti-Mouse, 800CW Goat Anti-Rat, 800 CW Donkey Anti-Goat, 800CW Streptavidin, 680LT Goat Anti-Rabbit, 680LT Goat Anti-Mouse, 680Lt Goat Anti-Rat. Hygromycin and G-418 were obtained from Enzo Life Sciences. Puromycin was obtained from Corning. All media and cell culture reagents were obtained from Corning/Cellgro, unless otherwise noted. Restriction enzymes, ligases and polymerases were obtained from New England BioLabs. DNA/RNA preparation kits were obtained from Omega BioTech. *Col1A1* and *Col1A2* genes were obtained from the Origene True Clone Repository, Accession Numbers SC112997 and SC126717, respectively.

Vector Construction and Cell Line Generation. Collagen-I genes were PCR-amplified to insert into the pTRE-Tight vectors. Q5 Polymerase (New England BioLabs)

was used according to the manufacturer's protocol, with the addition of the High GC Enhancer. Collagen-I genes were PCR-amplified using the following primers: *Col1A1:* Forward – 5′-ACATCAGCGGCCGCACAAGAGGAAGGCCAAGTCGAG-3′, Reverse – 5′- AAAAAAGTCGACTTACAGGAAGCAGACAGGG-3′; *Col1A2*: Forward = 5′-AAAAAAGCGGCCGCAACATGCCAATCTTTACAAGAGGAAAC-3′, Reverse – 5′-AAAAAAGATATCTTATTTGAAACAGACTGGGCCAATG-3′. After 35–40 PCR cycles with a 2 min extension time each, samples were gel purified using Omega Gel Purification Kits. Preprotrypsin signal sequences with either HA or FLAG epitope tags were inserted into pTRE-Tight first, using BamHI and EcoRV, and the collagen-I genes were then inserted into the respective vectors. Collagen-I vector propagation was always performed in *recB* and *recJ*-deficient Sure2 *E. coli*, which we found to be essential to prevent recombination of the GC-rich collagen genes.

Once sequenced, vectors were transfected with XFect (ClonTech) into HT-1080 TetOff cells (ClonTech) and co-transfected with either a puromycin or hygromycin linear selection marker (ClonTech). 48 h post-transfection, cells were treated with the appropriate antibiotic (0.25 μg/mL puromycin, or 150 μg/mL hygromycin) for 10–12 d for stable selection. Heterostable colonies were amplified, and then split to establish genetically homogenous single colonies for the collagen-I genes. The process was then repeated to create cell lines that express both *Col1A1* and *Col1A2* gene transcripts.

Quantitative RT-PCR. Relative mRNA expression levels of genes of interest were assessed by quantitative RT-PCR. Cells were harvested by trypsinization, washed with PBS, and total RNA was extracted using the Omega RNA Purification Kit according to the manufacturer's instructions. RNA concentrations were quantified and normalized to 1 μg total RNA for cDNA reverse transcription. Using the Applied Biosystems Reverse Transcriptase cDNA Kit, cDNA was synthesized in a BioRad Thermocycler. LightCycler

480 Sybr Green Master Mix (Roche), appropriate primers (purchased from Integrated DNA Technologies, Life Technologies and Sigma Aldrich, **Table S4**) and cDNA were used for amplification in a Light Cycler 480 II Real Time PCR Instrument in the MIT BioMicro Center. Primer integrity was assessed by thermal melt to ensure homogeneity. Transcripts were normalized to the housekeeping gene *Rplp2,* and all measurements were performed in triplicate.

Lysis, Protein Preparation, and Immunoprecipitations. Collagen-I expression by HT-1080 cells, unless noted otherwise, was induced by removing dox and adding 50 μM sodium ascorbate for 48 h, with fresh sodium ascorbate supplementation at the 24 h mark, then cells were harvested and washed three times with 1x-phosphate buffered saline (PBS). Samples for stable (no covalent crosslinking) IPs were lysed using the following lysis buffer (LB): 50 mM Tris-HCl, pH 7.5, 150 mM sodium chloride, 1 mM EDTA, 1.5 mM $MgCl_2$, 1% Triton X-100, protease inhibitor tablets (Thermo Fisher Scientific) and 1.5 mM phenylmethylsulfonyl fluoride (PMSF; Amresco). Cells used for cross-linking IPs were cross-linked in 1x-PBS in the presence of 0.2 mM dithiobis(succinimidyl propionate) (DSP; Lomant's Reagent), rotating at rt for 30 min. Cross-linking was quenched by addition of 0.1 M Tris at pH 8.0 (final concentration), rotating for 15 min at rt. Cells were then lysed in RIPA: 150 mM sodium chloride, 50 mM Tris-HCl, pH 7.5, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), protease inhibitor tablets and 1.5 mM PMSF. All lysed samples were centrifuged at 21,100 × g for 15 min at 4 °C. Supernatants were collected, quantified for protein content, normalized, and incubated for 16 h with the appropriate antibody-conjugated beads. Samples were then washed with the corresponding lysis buffer (LB or RIPA) and eluted using 300 mM Tris at pH 7.5 with 6% SDS by boiling for 10 minutes. Eluates were then subjected to SDS-PAGE and Western blotting, or prepared for mass spectrometry analysis.

Mass Spectrometry Sample Preparation and Analysis. Labeled cells were analyzed by mass spectrometry to ensure >95% incorporation of isotopically labeled amino acids into the proteome. After elution from the beads, samples were then treated with 100 μM dithiothreitol (DTT) to release the interactome from the collagen-I bait protein (cleaving the crosslinker, DSP). Each sample was then centrifuged over a 100 kDa molecular weight cut off filter (100 MWCO, Millipore) to ensure deeper coverage of the interactome by removing high levels of collagen-I proteins. The flow-through (i.e., the interactome) samples were precipitated by adding 450 μL of MeOH to a lysate sample of <150 μL and vortexed. 150 μL of chloroform was then added and vortexed. Finally, 450 μL of water was added and samples were vortexed and then centrifuged at 10,000 × g for 3 min. The upper aqueous phase was removed while the precipitate at the solvent interface was preserved. The collected precipitate was then washed 3 × 1 mL with MeOH, centrifuging between washes. The washed pellet was dried and resuspended in 8 M urea, 50 mM ammonium bicarbonate. 10 mM DTT was added, samples were mixed, and then incubated in a 56 °C water bath for 45 min. Samples were then cooled and incubated with 55 mM iodoacetamide for 1 h in the dark while rotating. Samples were next incubated with 1 μg of sequencing-grade trypsin (Promega) overnight at rt, while rotating. Proteolyzed samples were acidified to a final concentration of 5% formic acid and subjected to C18 Stage Tips for desalting. Prior to sample addition, Stage Tips were washed with 0.1% TFA, then 0.1% TFA with 90% acetonitrile, then again with 0.1% TFA. Samples were then loaded, ensuring all the volume passed through the column. Each column was then washed with 0.1% formic acid, and eluted with 0.1% formic acid with 80% acetonitrile. Elutions were dried by speedvac, resuspended up to 20 μL of 0.1% formic acid, and injected onto a nanoflow HPLC, with MS data acquired on a Thermo QExactive mass spectrometer (LC-MS/MS). Protein identification was carried out using

the Mascot database search software. Database search results were assembled using Proteome Discoverer.[1] Mascot search parameters were as follows: mass tolerance for precursor ions was 10 ppm; fragment ion mass tolerance was 0.8 Da; 2 missed cleavages of trypsin; fixed modifications were carbamidomethylation of cysteine; variable modifications were methionine oxidation, and hydroxylation of proline, lysine, aspartate, or asparagine. Peptides with Mascot scores greater than 25 and isolation inference less than 30 were considered identified, resulting in an average false discovery rate of 0.0077. SILAC quantitation was calculated by integrating the area under the curve of the light, medium, and heavy MS peaks. A fraction of the IP supernatant was analyzed and used to normalize the quantitation from the collagen IP to account for potential variability, similar to a loading control.

We later searched our MS data for evidence of aspartyl hydroxylation. In the HT1080$^{Col\text{-}I}$ heavy-labeled sample, we identified 4 peptides with the sequence VLCDDVICDETK, of which one was modified as shown in **Figure 5D**, across two replicates. For collagen-I secreted from Saos-2 cells, we used an inclusion list to better enrich peptides with appropriate m/z ratios (searching for both the unmodified and hydroxylated VLCDDVICDETK peptides), and found that 1/25 peptides were modified as shown in **Figure 5E**. The complete search results are not provided in **Tables S1** or **S2** because our analysis of MS2 fragmentation patterns for all other individual peptides labeled by the Proteome Discoverer search parameters as having aspartyl hydroxylation indicated that the mass difference resulting from hydroxylation could at least theoretically be explained by a proximal proline or lysine being hydroxylated. Thus, such MS2 scans provided insufficient data to definitively assign hydroxylation to one residue or the other.

^{35}S Metabolic Labeling of Cells and Pulse-Chase Experiments. HT-1080$^{Col\text{-}I}$ cells were plated on poly-D-lysine-coated plates 36 h before initiating the pulse-chase, under the appropriate conditions (+/- dox, + ascorbate). Before the pulse with ^{35}S-

methionine/cysteine, cells were starved of methionine and cysteine by treating for 30 min with Cys/Met-free DMEM (FBS was dialyzed for 16–18 h against PBS prior to making the media). After starving, cells were pulsed for 30 min with ^{35}S-containing media. Pulsing media was removed, and full DMEM replaced it for the indicated chase times. At the conclusion of each time point, cells and media were harvested and immunoprecipitated for HA-tagged collagen-I overnight, using HA-antibody beads. The next day, samples were washed with RIPA buffer, eluted from the beads using 6x-loading buffer, and separated by SDS-PAGE. Gels were then dried using a gel slab dryer for 1 h at 80 °C. Dried gels were applied to the mounting screen, developed using a GE Healthcare Phospho Screen, and imaged on a Typhoon 3–4 d later.

Confocal Microscopy. HT-1080$^{Col\text{-}I}$ cells, suspended in complete DMEM with doxycycline (dox; 1 ng/mL), were plated on a 24-well plate with coverslips (Chemglass Life Sciences, NJ) and incubated for 24 h at 37 °C in a humidified 5% CO_2 atmosphere. The media was changed to Tet-approved media (ClonTech) and the cells were incubated in the 37 °C incubator for 48 h without dox or ascorbate. Media was removed and coverslips were washed three times with PBS. The cells were then fixed with 4% formaldehyde for 30 min and permeabilized with 0.1% Triton in PBS for another 30 min. Coverslips were incubated for 1 h at rt in a blocking buffer containing 1% BSA in TBS (pH 7.5). Double-labeling was performed by incubating coverslips in TBS (5% BSA, 0.01% sodium azide) containing mouse anti-HA (1:200) and then rabbit anti-PDI (1:200), anti-LAMP1 (1:500), or anti-GM130 (1:500) for 2 h at rt or overnight at 4 ˚C. Secondary antibodies (Alexa Fluor 488-conjugated anti-mouse or Alexa Fluor 568-conjugated anti-rabbit) were diluted (1:1000) in TBS (5% BSA, 0.01% sodium azide) and then applied to the coverslips for 1 h at rt. After each incubation, cells were rinsed with TBS at least three times. Nuclei were stained for 15 min at rt with DRAQ5 (1 μM). After the final washing with PBS, the sections were then mounted with ProLong to prevent

photobleaching. Negative controls for non-specific binding of the secondary antibodies obtained by omitting primary antibodies in the staining protocol were included for each experiment. The subcellular localization was directly analyzed with an HCX PL APO 63X/1.40-0.60 oil objective mounted onto a Leica TCS-SP2 confocal microscope (Buffalo Grove, IL, USA) equipped with a Leica microsystem. Co-localization quantification was performed using ImageJ software, and calculated for seven different cells per slide in biological triplicate.

Collagen-I Encoding Lentivirus Production and Saos-2 Stable Cell Line Development. A Col1A1.pENTR1A vector was constructed by ligating *COL1A1* cut from the Col1A1.pTRE.Tight vector using the NotI and EcoRV restriction sites into a pENTR1A vector already containing an ER-targeting preprotrypsin signal sequence and an HA epitope tag. The resulting construct was inserted into the pLenti.CMV/TO.DEST Gateway destination vector (Life Technologies) via LR Clonase-mediated recombination (Life Technologies). pLenti vectors were sequence-confirmed before proceeding with lentivirus production. Lentiviral production was performed by co-transfecting 293FT cells with the lentiviral plasmids and packaging vectors encoding RRE, REV, and VSVG using Lipofectamine 2000 (Life Technologies). Briefly, the plasmid mixture (pLenti vector (15 μg), RRE (15 μg), REV (6 μg), and VSVG (3 μg)) was incubated with 60 μL of Lipofectamine 2000 in 3 mL of Opti-MEM media for 45 min at rt. The mixture was then added dropwise to a 10 cm dish of 293FT cells (8×10^6 cells) and incubated at 37 °C for 12 h. The media was then removed and replaced with DMEM (6 mL) and the plates were incubated for another 36 h. 48 h post-transfection, viral supernatant was collected and used immediately for transductions of Saos-2-TREx cells (see below).

To create Saos-2T-REx cells, Saos-2 cells (125,000 cells/well in a 12-well plate) were transduced with a range of volumes of lentivirus encoding a constitutively expressed tetracycline repressor protein prepared as described above. Polybrene (4

μg/mL) was added to increase the efficiency of viral infection. 12 h post-transduction, the media was removed and replaced by fresh DMEM media for another 24 h before selection. Cells were selected for using 2 μg/mL blasticidin, and single colonies were isolated and assayed for the colony most responsive to dox treatment (using Tet-responsive CFP to visualize activity of the tetracycline repressor protein). Once a genetically homogenous single colony was isolated, Saos-2T-REx cells were transduced as above with a range of volumes of crude viral supernatants of Tet-Responsive HA-collagen-α1(I), selected using 250 μg/mL hygromycin, and assayed for expression at the heterogenous cell population level. The resulting heterogenous cell line was used for IP analysis of the collagen interactome and for shRNA knockdowns. For shRNA knockdowns, cells were transduced with commercially available lentiviruses (Sigma) at an MOI of 2, or with homemade lentiviruses produced as described above. Stable cells were selected using 2 μg/mL puromycin. The resulting heterogenous cell populations were used for further experiments.

Saos-2 Differentiation and Collagen-I Production. Cells were treated as previously described to induce Saos-2 differentiation into osteoblasts.[2] Briefly, cells were treated with McCoy's 5A media, supplemented with 10% heat inactivated FBS, penicillin/streptomycin, L-glutamine, 50 μM sodium ascorbate and 5 mM β-glycerophosphate for three days prior to soluble collagen-I secretion analysis.

SUPPORTING REFERENCES

(1) Perkins, D.N.; Pappin, D.J.C.; Creasy, D.M.; Cottrell, J.S. Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis* **1999**, *20*, 3551–3567.

(2) Cheng, S.; Lai, C.; Blystone, S.D.; Avioli, L.V. Bone mineralization and osteoblast differentiation are negatively modulated by integrin αvβ3. *J. Bone. Miner. Res.* **2001**, *16*, 277–288.

Table S1. Quantitative profiling of hydroxylated collagen-I peptides obtained from HT-1080$^{Col\text{-}I}$ cells in the presence or absence of ascorbate (see Excel file).

Table S2. Quantitative proteomic profiling of the collagen-I interactome (see Excel file).

Table S3. Compilation of shRNA constructs used in **Figures 5** and **6**.

Gene Name	Oligo Sequence (5′ to 3′)
Non-Mammalian	CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGTTTTT
Erp29	CCGGCAAGTTCGTCTTGGTGAAGTTCTCGAGAACTTCACCAAGACGAACTTGTTTTT
P4HB	CCGGAGGTGAAATCAAGACTCACATCTCGAGATGTGAGTCTTGATTTCACCTTTTTTG
SerpinH1	CCGGCCTCTACAACTACTACGACGACTCGAGTCGTCGTAGTAGTTGTAGAGGTTTTT
AnxA2	CCGGCGGGATGCTTTGAACATTGAACTCGAGTTCAATGTTCAAAGCATCCCGTTTTTG
CRTAP	CCGGCTTTACTCTCCAAAGTGAAAGCTCGAGCTTTCACTTTGGAGAGTAAAGTTTTTTG
Ero1L	CCGGCCTCATAAATGACCCATAATTCTCGAGAATTATGGGTCATTTATGAGGTTTTTG
Golim4	CCGGGAAGATATAAACCCAGCAGATCTCGAGATCTGCTGGGTTTATATCTTCTTTTTTG
RCN1	CCGGCCGCAGAGTTTCATGATTCTTCTCGAGAAGAATCATGAAACTCTGCGGTTTTT
Calu	CCGGGTTAGAGATGAGCGGAGGTTTCTCGAGAAACCTCCGCTCATCTCTAACTTTTTG
ASPH	CCGGCGTGGTTTATGGTGATTGCATCTCGAGATGCAATCACCATAAACCACGTTTTTG
CKAP4	CCGGGCAGGATTTGAAAGCCTTAAACTCGAGTTTAAGGCTTTCAAATCCTGCTTTTTG
DNAJB11	CCGGGTTGGCTTTGAGATGGATATTCTCGAGAATATCCATCTCAAAGCCAACTTTTT
PRKCSH	CCGGCAGCCTTCAAAGATGGGTAAACTCGAGTTTACCCATCTTTGAAGGCTGTTTTTG
SPARC	CCGGCGGTTGTTCTTTCCTCACATTCTCGAGAATGTGAGGAAAGAACAACCGTTTTT
TGM2	CCGGACAGCAACCTTCTCATCGAGTCTCGAGACTCGATGAGAAGGTTGCTGTTTTTTG
PDIA3	CCGGGCTTACTATGATGTGGACTATCTCGAGATAGTCCACATCATAGTAAGCTTTTTTG
PDIA4	CCGGCCTGAGAGAAGATTACAAATTCTCGAGAATTTGTAATCTTCTCTCAGGTTTTTG
Erp44	CCGGGCACCCAGTGAATATAGGTATCTCGAGATACCTATATTCACTGGGTGCTTTTTG
TXNDC5	CCGGGCCAAGCGAAAGACGAACTTTCTCGAGAAAGTTCGTCTTTCGCTTGGCTTTTTG

Table S4. Compilation of primers for qPCR used in **Figure 1** and **Figure S3**.

Transcript	Forward	Reverse
RPLP2	5′-CCATTCAGCTCACTGATAACCTT-3′	5′-CGTCGCCTCCTACCTGCT-3′
COL1A1 (triple helix)	5′-TGGTAGCCGTGGTTTCCCTG-3′	5′-TCCAGTCAGACCCTTGGCAC-3′
COL1A2 (triple helix)	5′-TGGCTCGAGAGGTGAACGTG-3′	5′-AGCACCGTTGACTCCAGGAC-3′
COL1A1 (c-propeptide)	5′-GCAACAGCCGCTTCACCTAC-3′	5′-AGCCGAATTCCTGGTCTGGG-3′
COL1A2 (c-propeptide)	5′-TCGCTCAGCACCTTCTCTCAG-3′	5′-TGGGTGGCTGAGTCTCAAGTC-3′
CHOP	5′-GGAGCTGGAAGCCTGGTATG-3′	5′-GCCAGAGAAGCAGGGTCAAG-3′
BiP	5′-GCCTGTATTTCTAGACCTGCC-3′	5′-TTCATCTTGCCAGCCAGTTG-3′
Erdj4	5′-GGAAGGAGGAGCGCTAGGTC-3′	5′-ATCCTGCACCCTCCGACTAC-3′
ANXA2	5′-CCTTATCTGGCCACCTGGAG-3′	5′-GCTCCTGGTTGGTTCTGGAG-3′
ASPH	5′-GGTTCCTGTGGAGGCAGAAC-3′	5′-GGTTCTCCTGTGGGTCCATC-3′
CKAP4	5′-ACGTGGAGGAGCTGAAGAGG-3′	5′-AAGTCCTGAGGAGGCAGACG-3′
CRTAP	5′-TCCGGTTGAGAAATTTGTGG-3′	5′-GGTTCTGCTGCATGACCTTG-3′
DNAJB11	5′-GAACCCCTCGTCAGCAAGAC-3′	5′-TCTCTTGCCGACAATTGCAC-3′
ERO1L	5′-AAGAGGCCGTGTCCTTTCTG-3′	5′-TCCACTGCTCCAAGTCGTTC-3′
ERP29	5′-CTTCCCCTGGATACGGTCAC-3′	5′-CTGCCACCAAGAGATCATCG-3′
P4HB	5′-AAATCAAGCCCCACCTGATG-3′	5′-TATCCCAAATGGGAGCCAAC-3′
RCN1	5′-CCACTGGATCCTCCCTCAAG-3′	5′-CCCCGTAATTGGTAGCTTGG-3′
SERPINH1	5′-CAGCCTCATCATCCTCATGC-3′	5′-TTCTGCAGGTCATGGGTCAC-3′
SPARC	5′-AGAAGCTGCGGGTGAAGAAG-3′	5′-GGAGAGGTACCCGTCAATGG-3′
TGM2	5′-TGGCATGGTCAACTGCAACG-3′	5′-GCACTGGCCATACTTGACGC-3′
GOLIM4	5′-AACGAGAAGCAGCCAACCTC-3′	5′-GCAAAGCTTCCTGGTGTTCC-3′
CALU	5′-TTATGTGCCTGTCCCTGTGC-3′	5′-TTGCTTCTTCAGCACCCAAG-3′
PRKCSH	5′-CGACTGCAAAGATGGCTCTG-3′	5′-GCCGCTGTTGTACTCGTCTG-3′

SUPPORTING FIGURE LEGENDS

Figure S1, Related to Figure 2. Immunoblots of Protease-Treated Collagen-I and MS2 Scans of Hydroxylated Collagen-I Peptides

(A) Complete images for the immunoblots shown in **Figure 2B**.

(B) Top Panel: MS2 data showing hydroxylation on proline 986 of collagen-α1(I) upon ascorbate treatment of collagen-I expression induced HT-1080$^{Col\text{-}I}$ cells. The internal fragment labeled as PIGP*-28 and y_6 together provide clear evidence for the Xaa-position Pro being hydroxylated.

Bottom Panel: MS2 data showing hydroxylation on proline 707 of collagen-α2(I) upon ascorbate treatment of collagen-I expression induced HT-1080$^{Col\text{-}I}$ cells. The a_6 peak provides clear evidence for the Xaa-position Pro being hydroxylated. For both spectra, numbering of amino acids begin at the first Gly-Xaa-Yaa repeat.

Asterisks (*) indicate the site of modification. In the case of prolyl modification, the * indicates hydroxylation. In the case of cysteine modification, the * indicates alkylation during the mass spectrometry workflow to prevent re-oxidation of cysteines. For labeling of spectra, the b-ion is termed a fragment of the parent ion, originating at the N-terminus of the parent peptide, and extending the number of amino acids into the peptide indicated. y-Ions are the same, but originating at the C-terminus of the peptide. An a-ion is a b-ion that has lost a carboxyl group during the fragmentation. For clarity, not all y and b ions are labeled on the peptide sequence, but all ions identified are labeled in each spectrum.

Figure S2, Related to Figure 3. Co-Immunoprecipitation of Known Collagen-I Interactors from HT-1080$^{Col\text{-}I}$ Cells Upon Covalent Crosslinking

Immunoblot showing the ability to co-IP an array of known components of the collagen-I proteostasis network using the covalent crosslinking protocol employed in **Figure 3**. The control sample is HT-1080$^{Col\text{-}I}$ cells that produce collagen-I that is not HA-tagged.

Figure S3, Related to Figures 5 and 6. Stable shRNA Knockdown Saos-2 Cell Lines Analyzed By qPCR

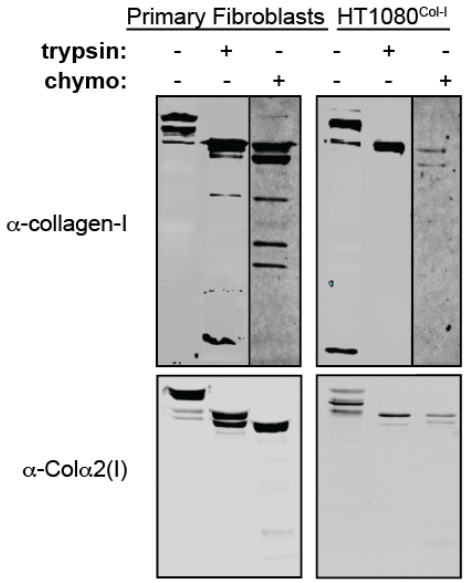
(A) Bar charts showing the extent of knockdown for each individual stable Saos-2 cell line assayed for collagen-I secretion in **Figures 5** and **6**. Samples were normalized to the given gene's expression in a corresponding control sample. The shRNA construct is listed along the x-axis, and the corresponding gene analyzed is listed as the title of the graph. Results are presented as the mean ±95% confidence interval.

(B) Replicate data relating to **Figure 5C**, with corresponding average fold change in collagen-I secretion compared to control cells (n = 2).

(C) Relative *BiP* and *Erdj4* mRNA levels upon stable Erp29 knockdown in Saos-2 cells compared to control Saos-2 cells showing no detectable UPR activation upon Erp29 knockdown.

Figure S1

A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chymo: - - + - - +
α-collagen-I
α-Colα2(I)

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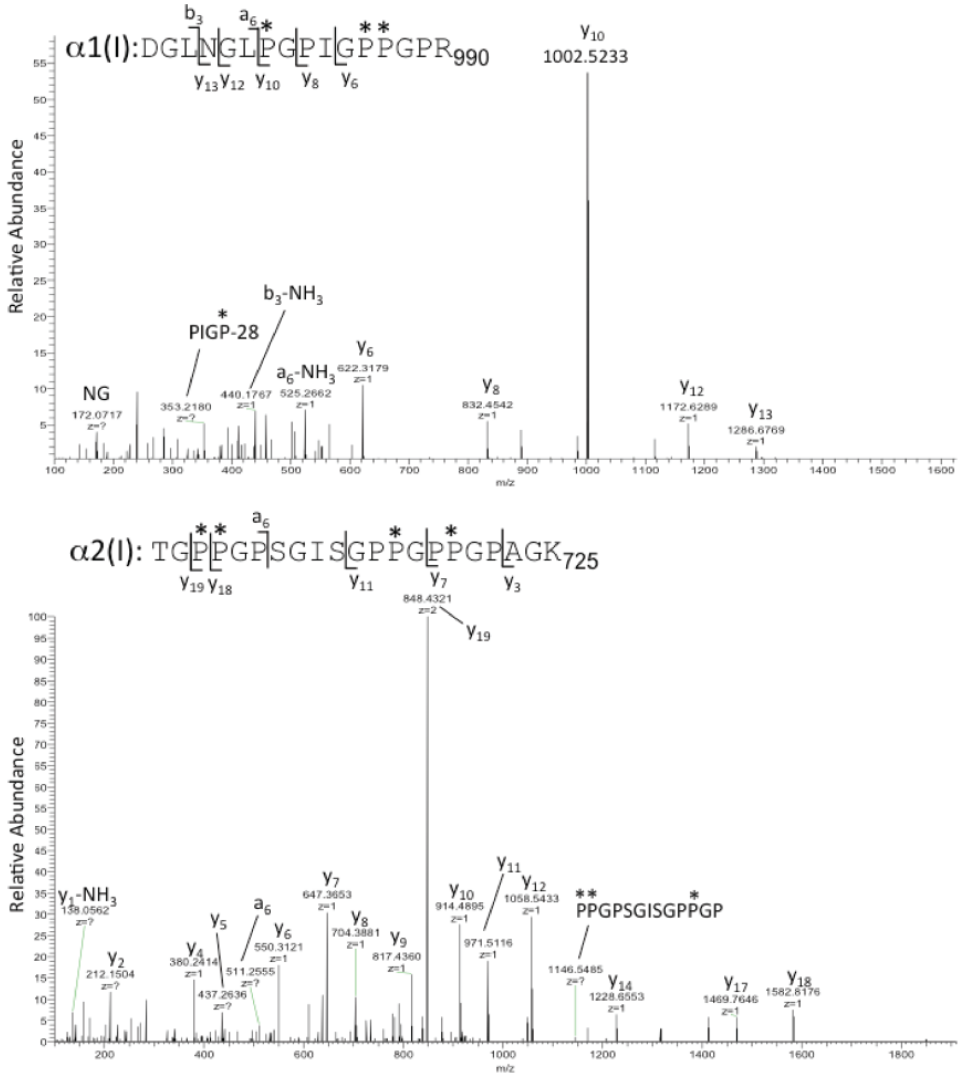
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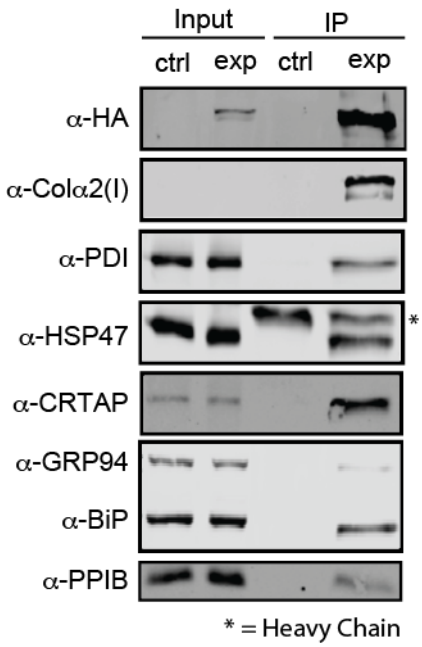
Figure S2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exp
ctrl
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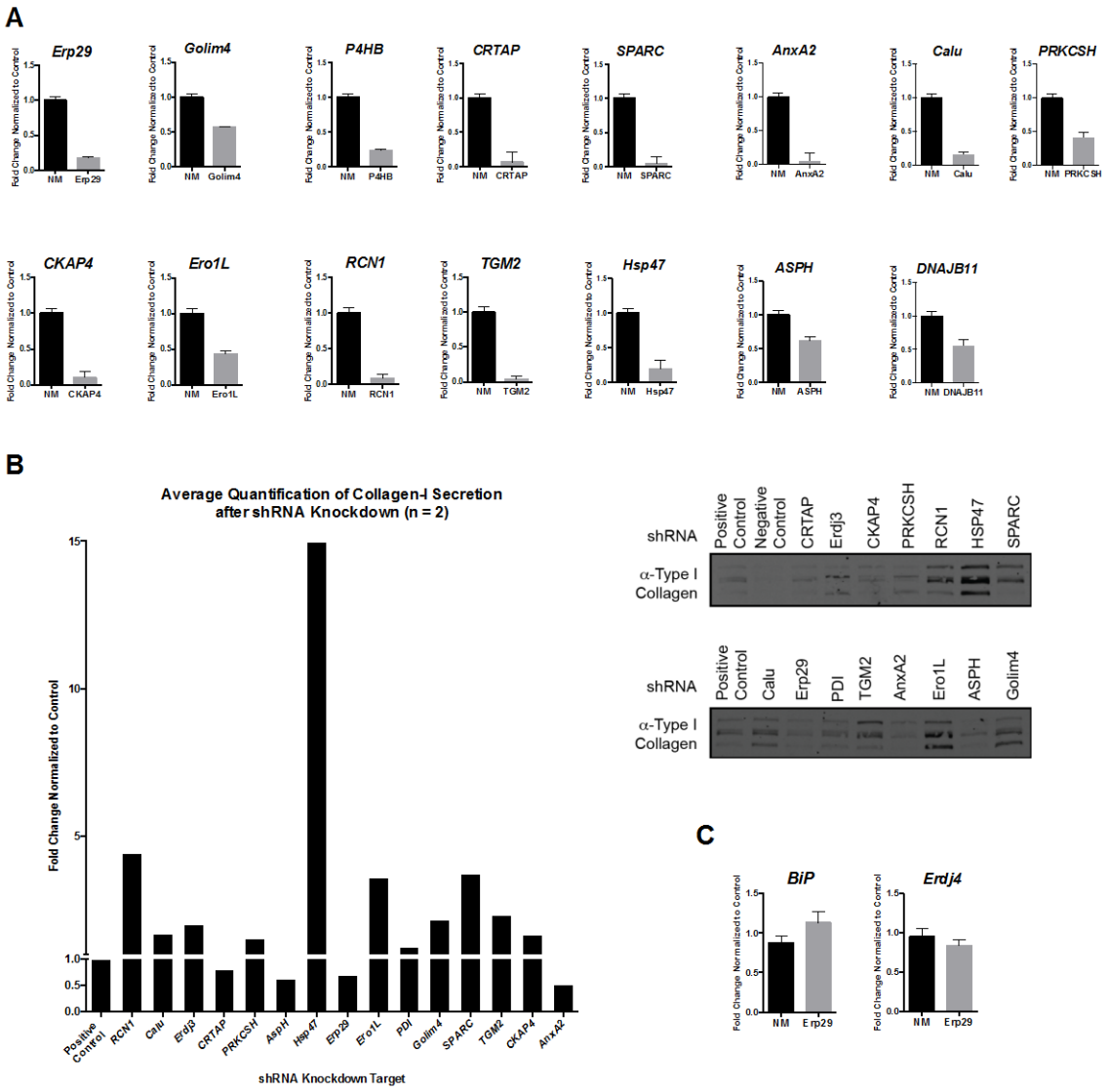
α-BiP
α-PPIB
* = Heavy Chain

Figure S3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CRTAP
SPARC
AnxA2
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PRKCSH
CKAP4
Ero1L
RCN1
TGM2
Hsp47
ASPH
DNAJB11
Fold Change Normalized to Control
NM
B
Average Quantification of Collagen-I Secretion after shRNA Knockdown (n = 2)
Fold Change Normalized to Control
shRNA Knockdown Target
Positive Control
RCN1
Calu
Erdj3
CRTAP
PRKCSH
AspH
Hsp47
Erp29
Ero1L
PDI
Golim4
SPARC
TGM2
CKAP4
AnxA2
shRNA
Positive Control
Negative Control
CRTAP
Erdj3
CKAP4
PRKCSH
RCN1
HSP47
SPARC
α-Type I Collagen
Calu
Erp29
PDI
TGM2
AnxA2
Ero1L
ASPH
Golim4
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