Supplementary File 1

Confirmation of Antibody Specificity

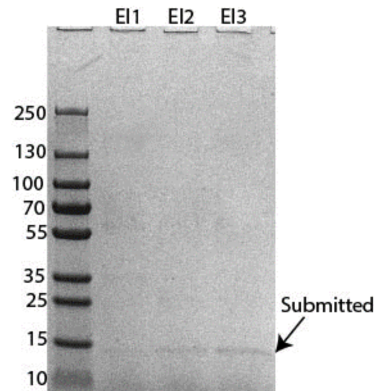
We confirmed the specificity of the commercial anti-histone H3 (citrulline R2+R8+R17) antibody (Abcam 5103, Abcam, Cambridge, MA) utilized in western blot methodology as follows: The commercial antibody was attached to Affigel-10 resin (Biorad, Hercules, CA) which was then utilized to immunoprecipitate abdominal fluid from a dog with septic peritonitis. Septic canine abdominal fluid has been previously employed as a source of citrullinated H3.[1] The antibody-coupled-affigel resin was extensively washed, then any bound protein was successively eluted (3 times) in 0.1 M glycine pH 3.0, and immediately neutralized in Tris pH 8.0. The elutions were boiled in Laemmli buffer and subjected to SDS-PAGE on a TGX 4-20% gel. The gel was stained with Gel code blue (see Supplementary Figure 1). The visible band from the third elution was excised, and submitted to the University of Michigan Proteomics and Peptide Synthesis Core. The core facility performed in-gel digestion with trypsin with a ProGest robot (DigiLab, Hopkinton, MA) by first washing with 25mM ammonium bicarbonate followed by acetonitrile, then reducing with 10mMN dithiotreitol at 60°C. Reduction was followed by alkylation with 50mM iodoacetamide and then digestion with trypsin (Promega, Madison, WI) at 37°C. Following digestion, the sample was quenched with formic acid and analyzed. Analysis was performed by nano liquid chromatography with tandem mass spectrometry (LC-MS/MS) with a Waters NanoAcquity HPLC system (Milford, MA) interfaced to a ThermoFisher Q Exactive mass spectrometer. Peptides were loaded on a trapping column and eluted over a 75µm analytical column at 350nL/min; both columns were packed with Luna C18 resin (Phenomenex, Torrance, CA). The mass spectrometer was operated in data-dependent mode, with the Orbitrap operating at 70,000 FWHM and 17,500 FWHM for MS and MS/MS respectively. The fifteen most abundant ions were selected for MS/MS. Data were searched using a local copy of Mascot (Matrix Science) with the following parameters:

Enzyme: Trypsin/P
Database: UniProt Canis lupus familiaris (concatenated forward and reverse plus common contaminants)
Fixed modification: Carbamidomethyl (C)
Variable modifications: Oxidation (M), Acetyl (N-term), Pyro-Glu (N-term Q), Deamidation (N/Q)
Mass values: Monoisotopic
Peptide Mass Tolerance: 10 ppm
Fragment Mass Tolerance: 0.02 Da
Max. Missed cleavages: 2

Mascot DAT files were parsed into Scaffold (Proteome Software) for validation, filtering and to create a non-redundant list per sample. Data were filtered using at 1% protein and peptide FDR and requiring at least two unique peptides per protein.

Canine histone H3 (uniprot ID E2R6K5) was unambiguously identified. Data uploaded to the Scaffold software version 4.8.7 (Proteome Software Inc) showed 5 unique peptides for this protein, with two peptides having a Prophet probability score of greater 95%.

We did not repeat manufacturer confirmation of specificity for citrullination as the manufacturer has previously shown that it does not cross-react with histone H3 unless it has been citrullinated by the enzyme Peptidyl Arginine Deiminase 4.[2]



Supplementary Figure 1. Western blot demonstrating the eluents (EL1-3) obtained after immunoprecipitating septic canine abdominal fluid with anti-histone H3 (citrulline R2 + R8 + R17) antibody (Abcam 5013, Abcam, Cambridge, MA) attached to Affigel-10 resin and then washing with 0.1 M glycine, pH 3.0. EL3 was submitted for nano liquid chromatography with tandem mass spectrometry for identification of the protein immunoprecipitated by the antibody.

References:

1. Lawson C, Smith SA, O'Brien M, et al. Neutrophil Extracellular Traps in Plasma from Dogs with Immune-mediated Hemolytic Anemia. J Vet Intern Med 2018;32:128-134.
2. Abcam. Anti-Histone H3 (citrulline R2 + R8 + R17) antibody - ChIP Grade (ab5103) product monograph. In: 2020.