Figure 5. Detection of deiminated proteins and PAD3 in injured E11 and E15 spinal cords. F95 is red and nuclear counterstain is blue **A-C)** Double-labelling for F95 (red) and NeuN (A), GFAP (B) and MBP (C) (all green) in E15 spinal cords 24 hours after injury. **D)** Double-labelling for PAD3 (red) and MBP (green) in E15 spinal cords 24 hours after injury. **E)** Double-labelling for F95 (red) and TUNEL (green) in E11 spinal cord 24 hors post-injury. **F)** Double-labelling for PAD3 (red) and TUNEL (green) in E15 24 hours post-injury. Scale bars = 20 µm (A, C, E,F); 10 µm (D).

Figure 6. Effect of Cl-amidine treatment on E15 chick spinal cords 24 hours after injury assessed by hematoxylin and eosin staining (A-B), co-labelling for deiminated proteins with the F95 antibody and for apoptosis by TUNEL (D-E), and staining for deiminated H3 (H-I'). **A)** Control spinal cord injury; note large cavity. **B)** Injured spinal cord treated with 80 mg/Kg Cl-amidine; note the reduction in cavity size. **C)** Quantitative analysis of cavity size (n=6; m±sd); note significant reduction (ANOVA, $p \leq 0.001$) following Cl-amidine treatment. **D)** F95 (red) and TUNEL (green) labelling of injured control spinal cord; note extensive deimination and apoptosis. **E)** F95 (red) and TUNEL (green) labelling of injured Cl-amidine-treated spinal cord. **E'**) Higher magnification of the dotted box in (E); note the reduction in both deimination and apoptosis in the treated spinal cord; the white arrowhead points to an apoptotic cell and the yellow arrow to a cell double-labelled for TUNEL and F95. **F)** Density of TUNEL-positive cells in control and Cl-amidine-treated animals scored at different distances from the injury (n=4; m±sd). The boxes indicate the scoring criteria used to evaluate the extent of apoptosis from very low/negative (1) to high (4). Note significant reduction (* $p \leq 0.04$; Student's t-test) following Cl-amidine treatment close to the injury side and absence of TUNEL-positive cells from 2 mm from the injury site in Cl-amidine-treated spinal cords. **G)** Western blot detecting histone 3 (H3) in immunoprecipitated deiminated protein fractions from 1) E11 sham-operated, 2) E15 sham-operated and E15 injured spinal cords. **H)** Control injured spinal cord; deiminated H3 (CitH3, green) is detected in the injured region in E15 spinal cords (arrows) and commonly associated with nuclei of apoptotic morphology (see insert). **I-I')** Cl-amidine-treated injured spinal cord; note greatly decreased expression of CitH3 around the injury site (box enlarged in I') compared to (H-H'). Scale bars = 260 µm (A,B,D,H,I); 130 µm (E); 65 µm (E', I'), 20 µm (insert in H).

Supplementary Figure 1. Deiminated proteins detected by immunohistochemistry with F95 antibody (red) in E11 and E15 spinal cords and co-labelling for F95 and TUNEL (green) in E15 spinal cord treated with the Ca++ ionophore BAPTA at the time of injury. F95 is red and nuclear counterstain is blue. **A)** No F95 reactivity is observed in E11 sham-operated spinal cord. **B)** F95 staining in E15 sham-operated spinal cord; no F95 reactivity is observed. **C)** F95 and TUNEL detection in an E15 spinal cord 24 hours after injury (DMSO control). **D)**

F95 and TUNEL detection in a BAPTA-treated E15 spinal cord 24 hours after injury. Scale bars = 130 µm (A-B); 65 µm (C-D).

Supplementary Figure 2. Western blot of proteins from individual E11 sham-operated and injured spinal cords 2 hours after surgery. Deiminated proteins (F95) are shown in the upper blot and the loading control (actin) in the lower panel.

Supplementary Figure 3. Staining for deiminated proteins (F95, red) and TUNEL (green) in E15 spinal cords 8 hours (A) and 4 days (B-C") post injury. **A**) Eight hours after injury TUNEL-positive cells start to be detected and some co-localzation with F95 is observed. The arrowhead points at a double-labelled cell magnified in the insert. **B)** H & E staining of a section from an E15 spinal cord 4 days after injury. **C)** F95 and TUNEL staining of a section from the same spinal cord shown in (B). Deiminated proteins and TUNEL positive cells are mainly found in the islands of tissue debris in the cavity and at the edges of the cavity. **C'-C")** High magnification of some double-labelled cells found in tissue islands (arrows). Scale bars = 20 µm (A); 250 µm (B, C).

Supplementary Table 1. Mass spectrometry-based identification of proteins immunoprecipitated by the anti-PAD3 antibody

Gel Band	Accession Number	Protein Name	Species	mW (Da)	pI (pH)	PLGS Score	Peptides	Coverage (%)
1	P84229 P70081 P62801 P70082	Histone H32	Gallus gallus	15378	11.7	229	81- TDLR -84 85- FQSSAVMALQEASEAYLVGLFEDTNLCAIHAK -116 118- VTIMPKDIQLAR -129 124- DIQLAR -129 124- DIQLARR -130	36.0294
	P62801 P70082	Histone H4 type VIII		11432	11.7	97	25- DNIQGITKPAIR -36 47- ISGLIYEETRGVLKVFLENVIR -68	24.2718
	P70082	Histone H4		11360	11.8	97	25- DNIQGITKPAIR-36 81-TVTAMDVVYALKR-93	33.0097
	P02272 P84229	Histone H2AJ		14007	11.3	597	44– VGAGAPVYMAAVLEYLTAEILELAGNAAR -72	22.4806
	P02552	Histone H2AJ	Gallus gallus	14007	11.3	619	44– VGAGAPVYMAAVLEYLTAEILELAGNAAR -72	22.4806
	P09642	Histone H2A V		13500	11.0	565	47- VGATAAVYSAAILEYLTAEVLELAGNASK -75	22.6563
2	P32882 P09206 P09244	Histone H32		15378	11.7	204	44–VGAGAPVYMAAVLEYLTAEILELAGNAAR -72	23.5294
	P84229 P62801 P70082	Tubulin alpha 1 chain Fragment		45871	4.8	630	26– AVFVDLEPTVIDEVR -40 33- PTVIDEVR -40 46- QLFHPEQLITGKEDAANNYAR -66 177- NLDIERPTYTNLNR -190 191- LIGQIVSSITASLR -204 288- DVNAAIATIK -297 301- TIQFVDWCPTGFK -313 314- VGINYQPPTVVPGGDLAK -331	25.4854
3		Tubulin alpha 3 chain Fragment	Gallus gallus	36064	5.0	267	91- NLDIERPTYTNLNR -104 156- AYHEQLSVPEITNACFEFSNQMVK -179 228- VGINYQPPTVVPGGDLAK -245 215- SIQFVDWCPTGFK -227	21.4286

4		Tubulin beta 2 chain		49920	4.6	332	3- EIVHIQAGQCGNQIGAK -19 47- INVYYNEATGNK -58 155- IREEYPDR -162 337- NSSYFVEWIPNNVK -350	11.4607
		Tubulin beta 3 chain		49829	4.6	198	47- INVYYNEATGGK -58 155- IREEYPDR -162 337- NSSYFVEWIPNNVK -350	7.6404
		Tubulin beta 7 chain		49638	4.6	252	3- EIVHIQAGQCGNQIGAK -19 63- AILVDLEPGTMDSVR -77 155- IREEYPDR -162 337- NSSYFVEWIPNNVK -350	12.1622
		Histone H32		15378	11.7	165	85- FQSSAVMALQEASEAYLVGLFEDTNLCAIHAK -116 118- VTIMPKDIQLAR -129 124- DIQLAR -129	32.3529
		Histone H4		11360	11.7	151	25- DNIQGITKPAIR -36 81- TVTAMDVVYALK -92	23.301
		Histone H2A J OS		14007	11.3	545	44- VGAGAPVYMAAVLEYLTAEILELAGNAAR -72	22.4806

Immunopreciptated samples were run on SDS-PAGE gels and the most prominent 4 bands (on silver staining) were excised from the gel and processed for identification by mass spectrometry (LC-MS/MS). The presence of several proteins within a 1D gel band results from co-migration of immunoprecipitated proteins with similar molecular weight.