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SUPPLEMENTARY MATERIAL

corresponding to:

Characterization of proteolytic activities during intestinal regeneration of the sea cucumber, *Holothuria glaberrima*

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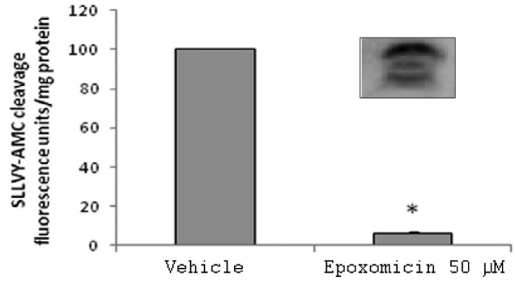
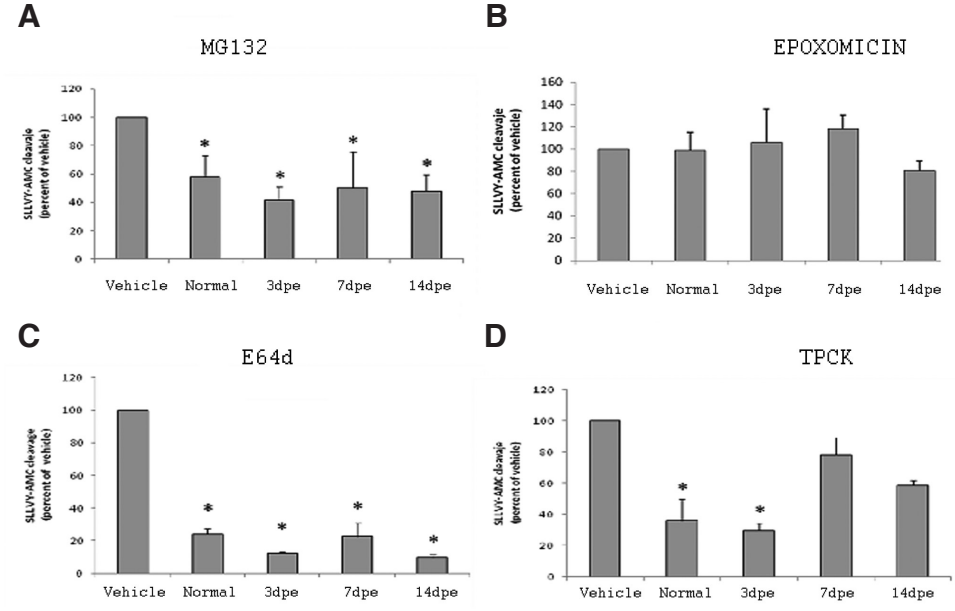
SUPPLEMENTARY TABLE S1

PCR PRIMERS FOR CALPAINS 5 AND 7

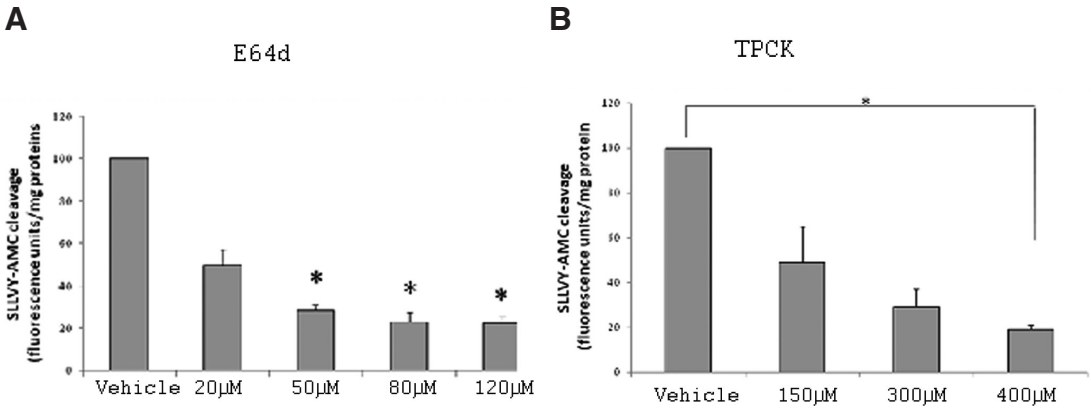
Gene	Sequence	Product length (base pairs)
Calpain 5	F 5' GTTTACACCCACTCACAG R 3' CTCCGTTCCATTCCTTTT	520
Calpain 7	F 5' GTTTGGCGGTATCCTGAA R 3' ATCGCTTTTTACGGTGGTTG	189
NADH	F 5' CGGCTACTTCTGCGTTCTTC R 5' ATAGGCGCTGTCTCACTGGT	241
NADH	F5' CAATGGTTGTTGCTGGAGTCTTT R5' CGCAGAAGTAGCCGCGAATAT	125



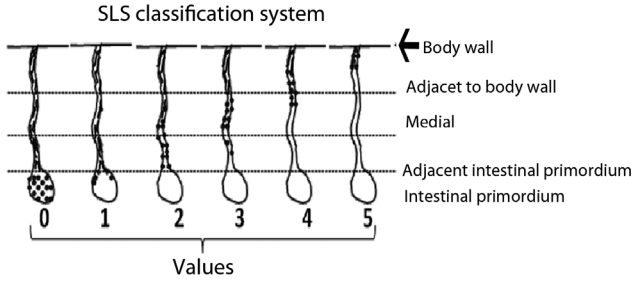
Supplementary Fig. S1. Effect of epoxomicin on SLLVY-AMC cleavage by *H. glaberrima* sperm extracts. *Gonad (spermatozoa) extracts were prepared as described in methods. Aliquots of these extracts were incubated with epoxomicin (50μM) for 15 min and enzymatic activity was measured for 30 min. Results are expressed as percent of activity compared to vehicle which was considered 100% and represent the fluorescence units/mg protein at 30 min of assay ± SE. In the insert, the result of a Western blot showing the presence of proteasome α-subunits is shown. *p<0.05 significantly different. t-test. n=3.*



Supplementary Fig. S2. Effect of proteasome and protease inhibitors on SLLVY-AMC cleavage on high molecular weight (HMW) protein fractions. *Intestinal extracts were prepared as described in methods. Aliquots of these extracts were incubated with MG132 10 μM* **(A),** *Epoxomicin 50 μM* **(B),** *E64d 80 μM* **(C)** *or TPCK 80 μM* **(D)** *by 15 min and the enzymatic activity toward the substrate was measured for 30 min. Results are expressed as percent of activity compared to vehicle (100%). Each bar represents the mean fluorescence units/mg proteins at 30 min of assay for each stage of intestinal regeneration. *Different from their respective vehicle. p<0.05, TWO WAY ANOVA and t-test. n=5 experiments for MG132 and n=3 experiments for epoxomicin, E64d and TPCK.*



Supplementary Fig. S3. Dose response effect of E64d and TPCK on SLLVY-AMC cleavage. *Intestinal extracts at 7 dpe were prepared as described in methods (HMW) protein fractions. Aliquots of these extracts were incubated with E64d* **(A)** *or TPCK* **(B)** *by 15 min and the enzymatic activity to the substrate was measured for 30 min. Results are expressed as percent of activity with respect to vehicle (100%). Each bar represents the mean fluorescence units/mg proteins at 30 min of assay for each stage of intestinal regeneration. *Different from vehicle. p<0.05, ONE WAY ANOVA and Tukey's test. n=3 different experiments.*



Supplementary Fig. S4. Classification system of spindle-like structure (SLS) distribution in regenerating intestine. *SLS distribution was calculated using the following qualitative classification system: 0 = strong SLS labeling in intestinal primordium; 1 = medium labeling of SLS in intestinal primordium; 2 = SLS in mesentery near the proximal regenerating area; 3 = SLS labeling at medium mesenteric portion; 4 = SLS labeling at mid distance between body wall and intestinal primordium; 5 = SLS labeling at area closer to the ventral body wall area.*