## Supplemental Fig. 1.

Phylogeny of Ciona savignyi alpha laminin genes

(A) Neighbor-joining tree depicting the relationships of *C. savignyi* alpha laminin genes. Bootstrap values are indicated at the appropriate nodes. Branch lengths are drawn to scale; scale bar indicates changes per amino acid position.

Clustal W was used to make an amino acid alignment of the known or predicted alpha laminins from *C. savignyi*, *D. melanogaster*, *M. musculus* and *H. sapiens*. A phylogenetic analysis was performed using the neighbor-joining (Saitou and Nei, 1987) option of PAUP 4.0 beta 10 (Swofford, 2002), using the midpoint rooting function. A 1000-replicate bootstrap of the dataset was performed using the neighbor joining/upgma function with bootstrap values greater than 50% indicated on the neighbor-joining tree.

## Supplemental Fig. 2.

Cs-lam $\alpha$ 3/4/5 expression and linkage in chm<sup>35</sup>

(A) RT-PCR for *Cs*-lam $\alpha$ 3/4/5 and cellulose synthase (control), showing decreased *Cs*-lam $\alpha$ 3/4/5 expression in *chm*<sup>35</sup>.

(B) *Cs*-lam $\alpha$ 3/4/5 SNP mapping in *chm*<sup>35</sup>. A region of *Cs*-lam $\alpha$ 3/4/5 was PCR amplified and directly sequenced from pools of *chm*<sup>35</sup> tadpole larvae and their wildtype siblings. Only a single allele is seen in the mutant pool at two sites that are polymorphic in the wildtype pool, indicating tight linkage between *chm*<sup>35</sup> and *Cs*-lam $\alpha$ 3/4/5.

## Supplemental Fig. 3.

Confirmatory genotyping of the phenotypes segregating in a aim/+;chm/+ intercross (A) Single larva genotyping of the progeny from a cross between aim/+;chm/+ parents. 5 larvae from each of the phenotypic classes shown in Fig. 5C were genotyped to confirm that the most severe phenotype is indeed the aim/aim;chm/chm double mutant. chm was genotyped by a SNP in an intron of Cs-lam $\alpha$ 3/4/5 (lam). aim was genotyped by an intronic amplification length polymorphism in Cs-pk (pk). Embryos from the most severe phenotypic class always showed the mutant allele for both genes, confirming that they were the double homozygotes. No obvious dominant suppression or enhancement was observed. (oligos, Cs-pk: TACCACAAATCGCAGGACTACTCAT and CCAAACACTTCCGCAACCGGGACCGAGGTT; Cs-lam $\alpha$ 3/4/5: GTGCGCGGTAGAGTGTCAGATGGGTGCAAC and CGGAGTGAGCAGCAGCAGCTGCACCCCAACACAGT.

suppl 1 A



suppl 2





