Supplementary text:

Haploid sperm hypothesis formulated by Thomas Morgan (Morgan, 1944):

The haploid sperm hypothesis is based on the statistical evaluation of thousands of crossings performed by Morgan and colleagues. From these experiments it concludes the following five assumptions:

(i) Self-sterility is defined by two to five independently inheriting genomic loci.

(ii) All self-sterility loci are polymorphic leading to many alleles within the population.

(iii) All alleles of all loci have to be identical between parents to prevent fertilization.

(iv) Sperm express only one allele of each locus.

(v) Oocytes express both alleles of every locus.

Thus, assuming only one genomic locus is controlling self-sterility and both parents are heterozygous in this locus having different alleles, four different allelic combinations are possible among F1 offspring (Fig. 3A). If siblings would be crossed to each other, 16 different combinations would be possible of which four would be cross-sterile. So, 25% of siblings in one batch will be cross-sterile given the “one locus self-sterility model”. By increasing the number of self-sterility loci, the percentage of cross-sterile combinations within the F1 generation will drastically decrease. The expected probability of cross-sterility in F1 siblings can be calculated according to the formula shown in Figure 3A. For example, if both parents are heterozygous in all self-sterility loci, percentages of cross-sterility within F1 generation of 6.25%, 1.6% and 0.4% would be expected assuming two, three and four self-sterility loci respectively.

References:

Morgan, T. H. (1944). "The genetic and the physiological problems of self-sterility in Ciona. VI. Theoretical Discussion of Genetic Data." J. Exp. Zool. **95**: 37-59.

Fig. S1: Northern European *Ciona intestinalis* belong to subspecies B. PCR-based markers 1 (A), 2 (B) and 4 (C) from Suzuki et al., 2005 were used for amplification from genomic DNA of randomly sampled Norwegian (NOR), German (Ger) or Japanese (Jap) *Ciona intestinalis* individuals. Numbers indicate the size in base pairs of amplified fragments.

Fig. S2: vCRL1 proteins are highly variable among animals. Multiple alignment of 15 ovarian vCRL1 protein sequences from 11 animals. All sequences originate from *Ciona intestinalis* ssp. B animals except those of databank sequences of Japanese animal J (species A, marked with a diamond). Bars underneath sequence alignment represent domains of the vCRL1 proteins. Red - signal peptide, blue - CCP domain, grey - transmembrane domain. For graphical representation by Neigbor-joining method see Fig. 1C.

Fig. S3: Isolation of the vCRL1 genomic locus. (A) Schematic overview of six sequenced BAC clones representing both haplotypes of a single individual (grey) as well as two haplotypes from different animals (black and white) compared to their approximate localization in the JGI *Ciona intestinalis* genome v2.0. Blue arrow - *vCRL1* gene, blue triangle - repetitive *vCRL1* 5' sequence, yellow arrow - Ig/CCP domain containing genes. (B) Identity plot of indicated 40 kb sequence marked by dashed red line in (A) comparing either clone 30c04 or 4H2 with 11F21. (C) Putative gene map of BAC 11F21 by gene prediction. Arrows represent genes and their location on + or - strand. *vCRL1* gene (#5) is colored in blue. (D) Domain structures of predicted proteins encoded by 11F21. SP - signal peptide, CCP - complement control protein domain, Ig - immunoglobulin-like domain, TM - transmembrane domain, Cad - cadherin domain, ZnF - zinc finger domain, AdE3 - adenovirus E3 domain; (E) Southern blot analysis using genomic DNA of five unrelated *Ciona* individuals digested with *Hind*III and probe directed against sequence between CCP and IG domains encoded by predicted gene #8. Note that all animals show different hybridization patterns as well as varying numbers of signals indicating presence of a gene cluster and high interindividual polymorphism of this genomic locus. (F) Expression analysis for predicted genes of BAC 11F21. Grey/white boxes indicate presence/absence of ESTs respectively.

Fig. S4: Production of recombinant vCRL1 protein and polyclonal α-vCRL1 sera. (A) Domain structures of expressed vCRL1 variants and graphical representation of the produced his-tagged vCRL1 proteins. Only the extracellular portion (dashed red line) was used. His - His-tag. (B) Coomassie stained SDS-PAGE of Ni-NTA chromatography fraction for construct vCRL1-o. L - whole bacterial lysate, F - flow through. Eluted vCRL1-o protein bands are marked by asterisk. (C) Purified recombinant vCRL1 protein variants. CB - Coomassie stained SDS-PAGE. (D) Analysis of polyclonal α-vCRL1 mouse serum. WB - Western blot using either monoclonal α-his-tag antibody or polyclonal α-vCRL1 sera N12, N13 or N28. N12, N13 and N28 were generated using purified vCRL1-h, vCRL1-o and vCRL2-o as antigen respectively. Note the different specificity of α-vCRL1 sera depending on the used antigen. vCRL1 protein bands are marked by asterisk. 1h, 1o and 2o - protein variants vCRL1-h, vCRL1-o or vCRL2-o; c - proteins purified under same conditions from *E. coli* cells not transfected with expression construct.

Fig. S5: Polyclonal α-vCRL1 serum inhibits heterologous fertilization in *Ciona*. (A) Fertilization rate of heterologous inseminations of oocytes for ten different unrelated *Ciona* animals in the presence of either control or α-vCRL1 serum. Addition of α-vCRL1 serum drastically decreases fertilization rate compared to controls. Note the differential response among the ten animals tested indicating variable antibody specificity resulting from high interindividual polymorphism of vCRL1 protein. (B) Concentration dependency of the inhibitory effect of α-vCRL1 serum. Black - control serum, red - α-vCRL1 serum. The data represent the mean of three replicates carried out for each experiment ± standard deviation.

Fig. S6: Cross-sterility among F1 siblings. Offspring of a single cross were tested for pairwise cross-sterility by *in vitro* fertilization. Numbers indicate a single offspring individual and letters represent parental animals. Black - autologous controls (sterile), white - successful fertilization, grey - cross-sterile combination. Note, that some oocyte preparations were slightly contaminated with heterologous sperm (indicated by asterisk). Thus, a small percentage of eggs were fertilized. For summarized data see Tab. 1.

Fig. S7: Injection of siRNA into adult *Ciona intestinalis*. (A) Experimental overview of siRNA production and injection into adult *Ciona intestinalis* specimen. Each animal was dosed with three injections of 15 µg siRNAs every second day. (B) Semi-quantitative RT-PCR analysis of siRNA injected animals.

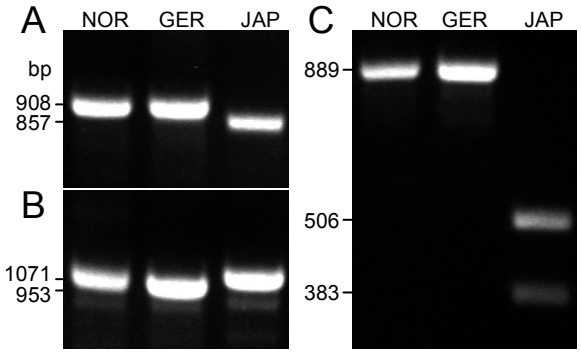

Fig. S1
Sommer et al., 2012

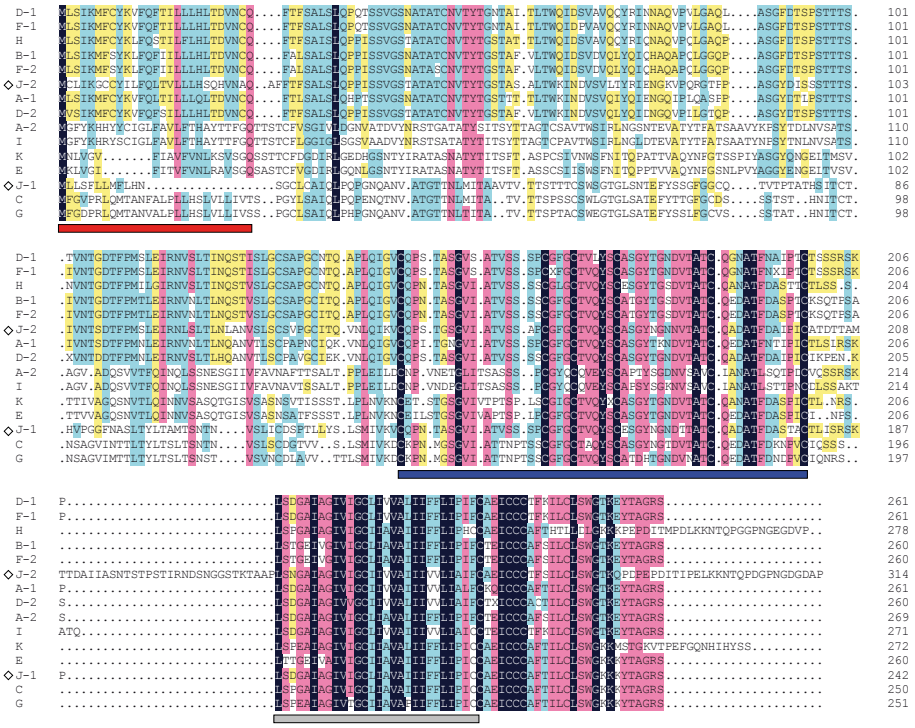

Fig. S2
Sommer et al., 2012

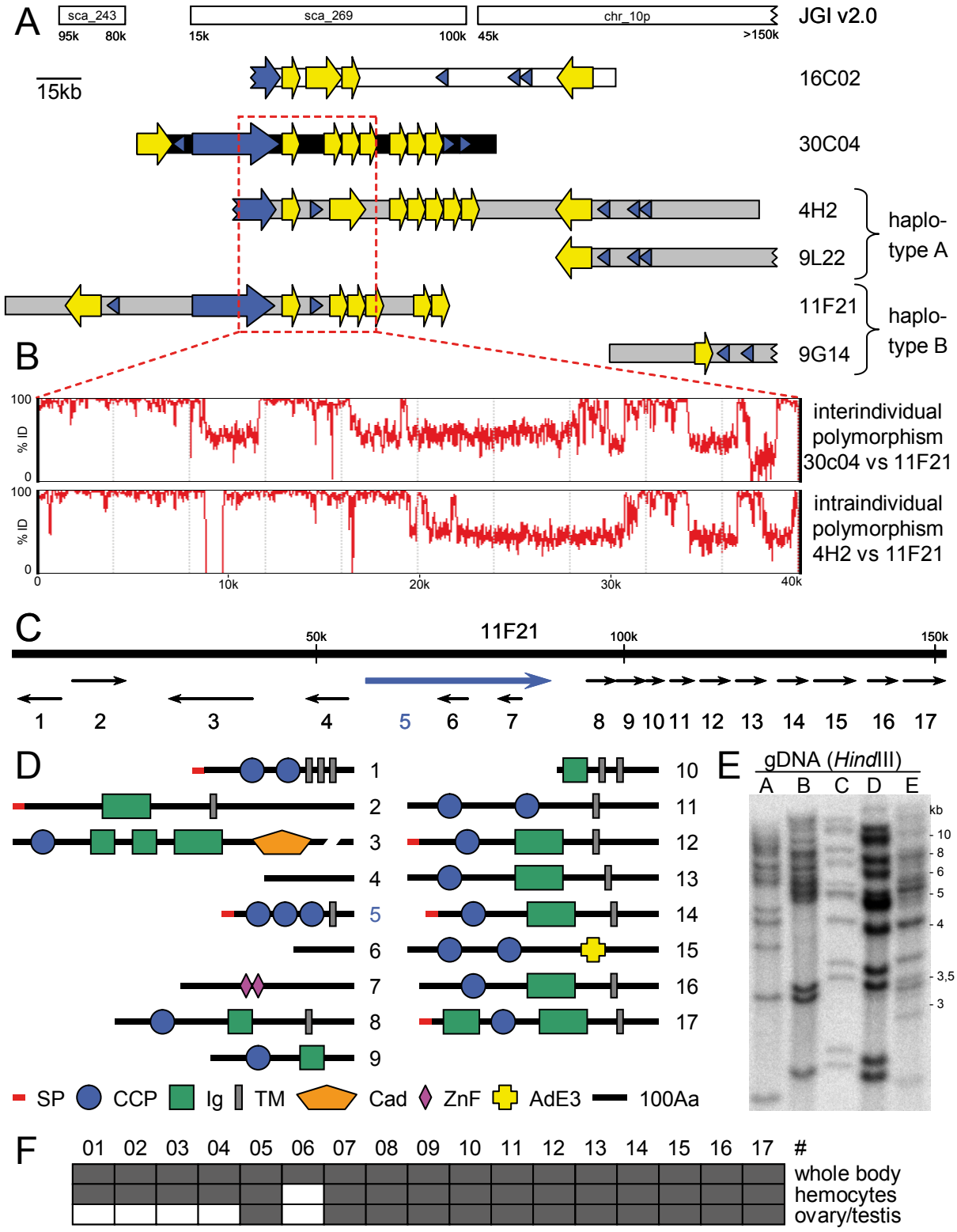

Fig. S3
Sommer et al., 2012

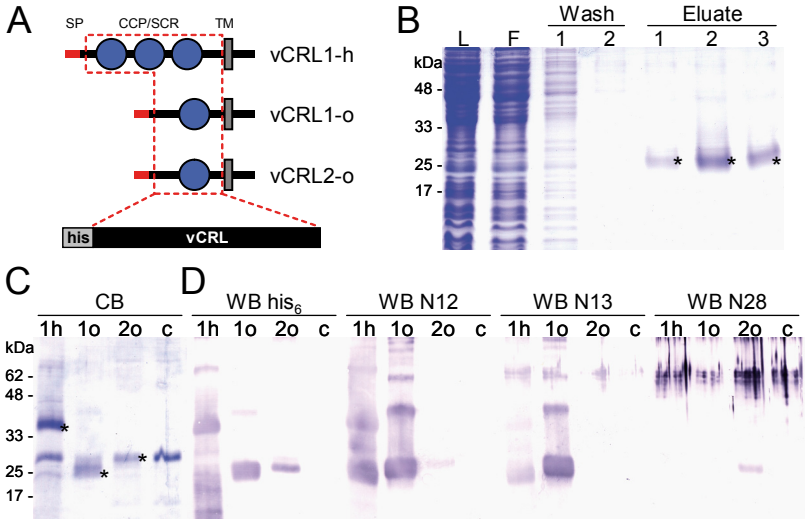

Fig. S4
Sommer et al., 2012

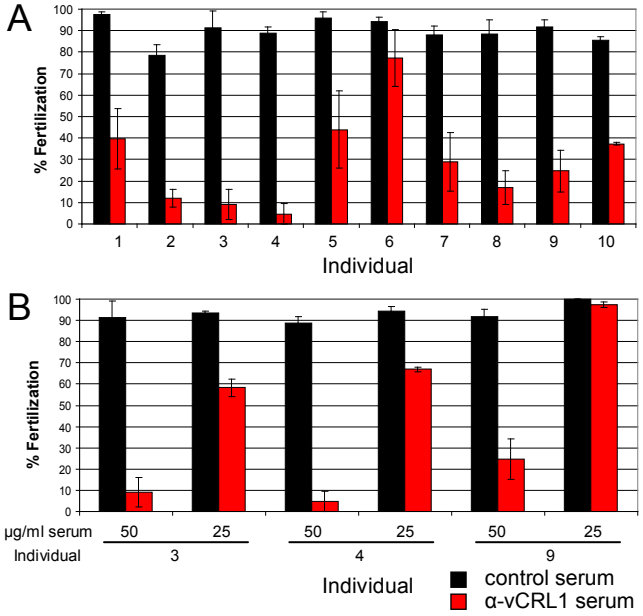

Fig. S5
Sommer et al., 2012

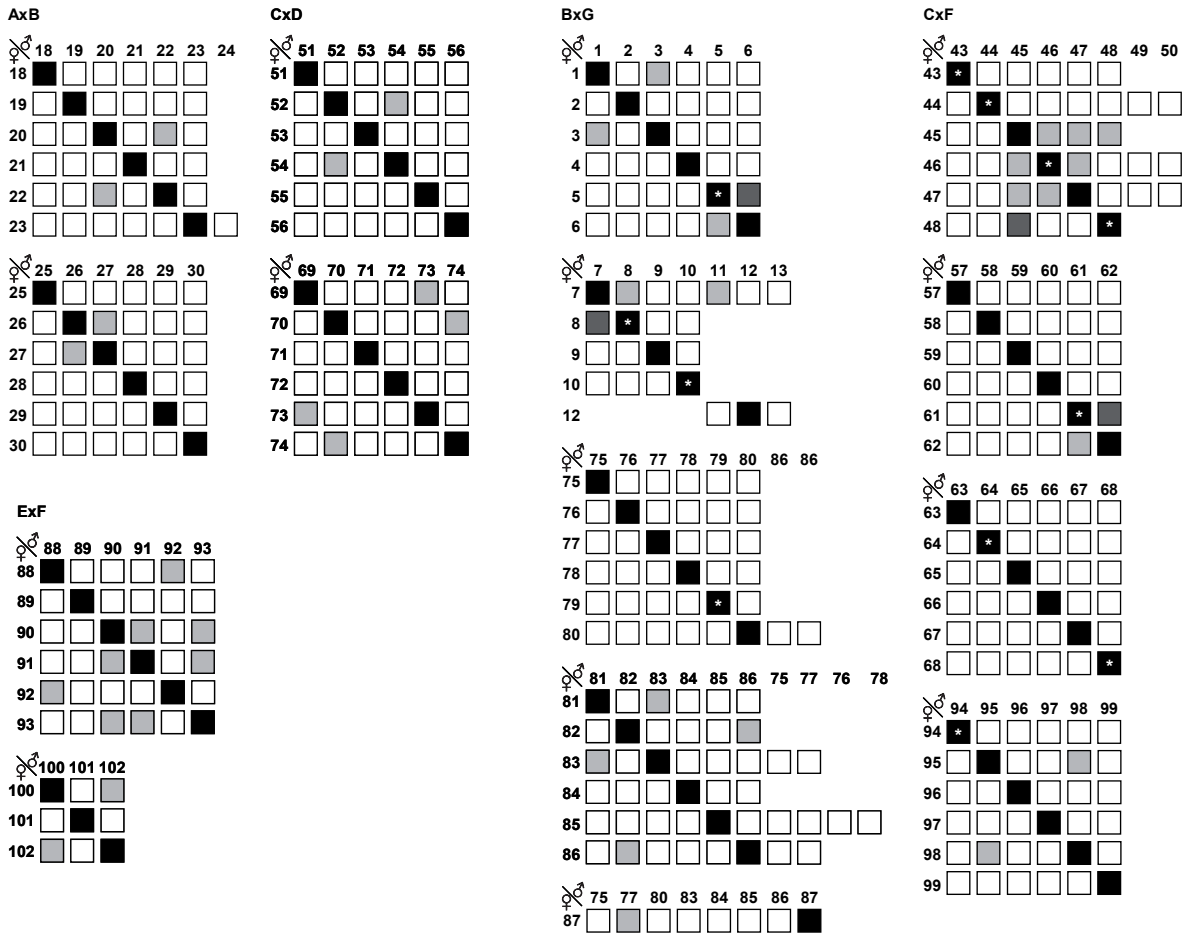

Fig. S6
Sommer et al., 2012

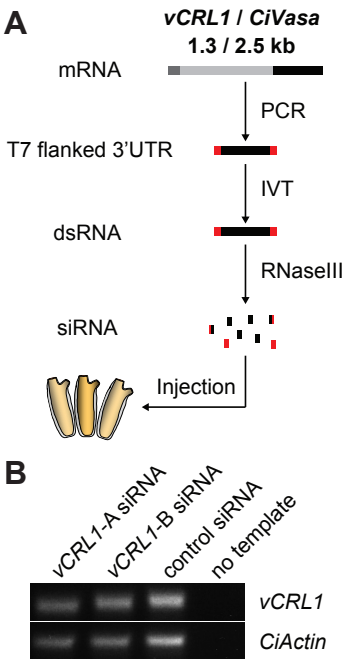

Fig. S7
Sommer et al., 2012