Review Dawson et al. *The relationship between cancer progression and social environment in Drosophila*

We are grateful for the productive and interesting comments made by both reviewers. Please find our responses and corrections below and in the manuscript.

Sincerely,

Fred Mery

This is an original manuscript looking at the effects of sociality on non-infection disease progression and vice-versa. Please see below for a detail list of comments and suggestions which I hope will increase the clarity of the manuscript (because the lack of line numbers I found it easier to list them in order of appearance, rather than importance). The short format makes for a slightly frustrating read, as I found myself wanting to know much more about these tumours, and about the potential mechanistic and adaptive explanations behind these rather sophisticated behaviours (cancerous Drosophila flies that prefer to mix with other cancerous flies rather than healthy ones - wow). Other than that, and as you will see, my main comments relate to the statistical analyses, which I think could be clarified pretty much throughout.

We apologize for the missing line numbers…they have now been added.

More details are now provided concerning the nature of the tumours.

We also provide more details concerning the stats as suggested by both reviewers

Introduction

Page 5 (first para.), (1) Previous examples of the correlation between cancer progression and social isolation have been described in intrinsically social mammals such as humans and mice. I have a hard time thinking about Drosophila as a stereotypically social animal, perhaps a few lines about adult Drosophila social behaviour would help to better understand why this is a good model for addressing this issue. (2) To understand the adaptive nature of Drosophila faced with cancerous vs non cancerous flies I also feel the need to know more about these "intestinal-like cancers" (which is a peculiar way to call them – do you mean "intestinal cancer-like tumours?"). Do they occur naturally or are they a laboratory construct? What are their fitness effects on the flies (if any?). It seems surprising that these tumours have no effect on fly performance or longevity (page 24)– so what do they do? And why would you expect Drosophila to have evolved adaptive strategies to deal with them?

1) While Drosophila is more traditionally considered a model organism from a genetic perspective, there is now a substantial number of studies and evidence that demonstrates the importance and prevalence of social interactions in Drosophila (Battesti et al, Sarin & Dukas, 2009, Mery et al 2009). We have now included a sentence in the introduction which elaborates on this point.

2) While we use mutant flies with induced tumours in our study, it has been shown that cancer occurs in natural populations of Drosophila making them a good model to investigate adaptive strategies against cancer. While these tumours are shown not to affect locomotor behaviour or survival of flies, cancer may affect other fitness traits. A recently published study showed that the presence of cancer affects the reproductive strategies of female flies. This has now been highlighted in the manuscript (intro and discussion).

Results
Page 5 (Biological model) (3) What is MARCM and what does it mean that "The flies contained […] MARCM clones"?
This has been corrected. We now provide further details about these clones

Page 6 (first para.) (4) Substitute "bred in tubes" for "kept in tubes" (there was no breeding involved) (5) The last couple of sentences "After 21 days…" and "More surprisingly…" require a statistical test (in the form of a post-hoc contrast – you can do this by e.g. lumping together the alone and heterogeneous treatment and checking whether there's a significant change in deviance in the model)
Bred has been changed to kept now throughout the manuscript.

Figure 1 initially contained results of a Tukey's post hoc classification (mentioned in the figure legend). They have now been added to the figure.

Page 6 (Social interactions para.) (6) A general comment I have throughout the paper is that there is very little information about the statistical analyses. It would help if the Methods had a Statistical Analysis section detailing how the analyses were made (what program? How were the models built? Were they simplified? that kind of thing). Here, for example, you have 3 different F and p values associated to the analyses in figures 2B. Are these 3 different statistical analyses (I hope not) or post-hoc contrasts after fitting a full mixed model (to account for the tube effect) with group composition and fly state (and their interaction) as fixed explanatory variables? This seems to be the appropriate way to do this analysis. (7) Same goes for analyses of data in 2C. (8) I'm also wandering how was the data handled, particularly in the heterogeneous group, where you have a higher replication for the control flies (n=7 per tube) than for the cancerous flies (n=1). Unless I'm mistaken, you do not seem to mention how many true replications (tubes) of each there are.
We have now expanded and explained in more detail how we performed the statistical analyses. In particular in the previous version we forgot to mention that, to avoid the problem of unequal sample size, before analysis, that we averaged the values measured to obtain, for each group, one value for cancerous state and/or control state

Page 7 (Social environment choice) (9) (second para.) I see how what you are saying in this paragraph fits well with what I see in Figure 3, however I got confused by the stats. For example, it looks like the target fly effect is only significant 7 days post induction, so I find it surprising that you have a significant target fly main effect but a not significant target fly*age interaction. As I read on, I'm not even sure whether what is being tested is the difference between control and cancerous targets, or between observed vs expected (random) choice. Where does this second chi-square value for age come from? A bit more info about how was this analysis done would help understand.
We now provide more information on the way the analyses were done. We apologize but found an error in the statistical analysis of the dual choice (age is in fact strongly significant, no change in the results of the other factors). We observed a general decrease in preference for the cancerous stimulus group, but we could detect such decrease only in cancerous target flies. This may explain the lack of significant interaction target fly*age.

(10) Figure 4 (there is no Figure 5) could be clearer: I would change X axis label to "Stimulus

flies (days post-induction)" and add "Target flies" to the cancerous and control labels. I would also call these latter ones "empty" rather than control. (11) The exact same comments regarding the stats as for the dual experiment apply here.
Figure 5 has been changed to figure 4. Figure 3 & 4 have now been changed.

Discussion
Page 8 (12) (second para.) I do not understand why, if social isolation is the sole issue, cancerous flies do better in the presence of other cancerous flies rather than in the presence of healthy flies. I find this result very interesting but also very perplexing. Any thoughts? (13) You also suggest (page 9) that the attraction of cancerous flies to other cancerous flies may be an adaptive strategy aimed at reducing cancer progression. To be convinced by these adaptive explanations, I would need to know more about the conditions under which these tumours originated (see comments on Intro).
The point we tried to make is that cancerous flies get more social contact with other cancerous individuals than with healthy ones. Healthy (non-cancerous) flies avoid cancerous flies which may lead to a perceived social isolation in cancerous flies, whereas cancerous flies significantly choose to be close to each other leading to more social contact.

We have added explanation about tumour induction in the introduction and in the M&Ms.

Methods
Page 12 (last para) (14) The people that've named these Drosophila lines seem to have some serious psychological issues (!). Because of all the colons and semicolons it took me a while to understand this. You may want to cater for people like me by modifying the initial sentence like thus: "We used two different Drosophila lines: a cancerous line (yw, HS-flp….) and a control line (yw, HS-flp….) which were balanced over co-segregating….". Please also explain what does this last part of the sentence mean ("balanced over…"). (15) "In all experiments flies were…" this seems to be a repeat of what has already been said (or else the subtlety was lost on me).
We apologize for the genetic jargon, and we fully understand the difficulty for a non-Drosophila researcher to follow. The point is that we have to respect the genetic code so that Drosophila geneticists know the exact genetics of the lines used. There are no cancerous and none-cancerous line. Cancerous and control flies are offspring from crosses of stable lines. Because of homozygous lethality the parental lines are stabilized using the so-called balancer chromosomes; due to length limitation, we cannot describe all these genetic tools. However to make it clearer for none- Drosophila people we have added references and explanation of the clonal strategy.

Page 13 (first para) (16) This would indeed have been the perfect control for your experiment, as otherwise the temperature treatment is confounded with the Apc-Ras mutation. To what extent could your results be simply explained by the heat shock instead of the tumour? A quick check of the literature shows that hsps can have multiple pleiotropic effects in the organism, including on fly behavior. This is not discussed in the manuscript, but I think it should. Would an alternative have been to have a heat-shocked control line in the behavioural experiments?
We agree that heat shock can have multiple pleiotropic effects. However, in our case it is a consequence of leaky expression of the HS promoter that may direct very low levels of flipase, potentially inducing clones at low but unpredictable frequency. Although, we have observed a low rate of none heat shocked flies exhibiting tumors of rather smaller size, it was obvious for us that these flies might strongly interfere and bias the results, when analysing the effect of

social interactions. This is why in all experiments control and cancerous flies were heat shock at 3 days post emergence. We tried to make this clear.

Page 13 (social breeding) (17) It may be a good idea to call this "social environment" as per the Results section
This has been changed

(18) How many food tubes were there for each of the 3 treatments?
This has been added

(19) Was the "tube" effect taken into account in the model? i.e. is this a mixed model with treatment as a fixed factor and tube as a random one?
Because in the heterogeneous environment only one cancerous fly was present, we grouped fly guts randomly from different tubes (this is now mentioned in the manuscript). Thus tube was not a random factor and only treatment was included as a fixed factor.

Page 14 (Social environment choice) (20) I had to read the experimental set up a few times to understand it. To avoid confusion between the different types of cages, it may be a good idea to refer to the outer cage as a "plastic box".
This has been corrected

Supplementary Information
Page 24 (21) "and locomotor" …activity?
This has been corrected

(22) How many groups of cancerous / control females were used for each of the 3 post-induction treatments?
This is now mentioned in the figure legend

(23) Please tell us what was used as the random variable (tube?) in the mixed model.
This is now clarified, tube is our level of replication

(24) You seem to have three fixed parameters (instead of 2): fly state, time and age. Not sure what you mean by "age" though (is this the post-induction treatment? i.e. 7, 14 and 21 days? – if so it may be a good idea to keep terminology constant)
Age is days post induction. This is now clarified

(25) "no difference between cancerous or control flies could be observed at any age ($p>0.5$ for each age tested)" – how were these multiple tests done? Are these post-hoc contrasts? This sentence is misleading (as it sounds like you have done separate statistical tests for each age, which would not be a good idea). Stating that you do not have a significant interaction between fly state and age (if this indeed the case), would obviate the need to do either multiple tests or contrasts.
We agree that presenting the full model (which include age and state) is a better option. it is now presented this way.

Reviewer 2:

This is an interesting study using the drosophila model system to study the effects of social interactions on cancer progression; a study which would be extremely difficult if not impossible to conduct in the human population. The experiments are well-thought-out, however, I do have doubts regarding some statistical analyses.

Major comments:

Statistical analysis:

We now provide more analyses (especially concerning treatment comparisons) and present a more detailed explanation of how we performed our statistical analyses.

- I am confused with the statistical analysis in the choice experiments. The authors show the results of a logistic regression in the text, where they ask the questions whether age, cancerous state/stimulus, or the interaction between them affects choice. They are thus comparing different groups in their choice, but they do not directly test for one group whether they are more attracted to a certain cage over the other (just differences between groups). This is more or less tested in individual tests for deviation of random choice (0.5) with asterisks in figures 3-4, however, the method of this testing is not described but seems to be performed for each individual point separately and p-values should thus be adjusted for multiple testing. Was this done? I suggest the authors to have the principle analysis be done on whether cancerous flies are attracted to a certain social group, a secondary analysis would be whether there are differences between cancerous vs control and age of the fly.

Stars on figure 3 and 4 represent significant deviation from random choice calculated for each line and age. We disagree with the necessity of doing the adjustment for multiple testing as we are not comparing the treatments among them in this analysis (compared to figure 1 and 2). We believe that this representation allows the reader to see at which age and state there is a significant effect and can conclude that there is for example aversion of the cancerous flies by the control ones when tumours are well developed.

We clarified each analysis done and detail the statistics.

- P7, paragr3: “This was especially pronounced when flies were young i.e. at the very beginning of the tumor development”. However, the interaction between age and target fly is not significant so this is not a significant effect

We made this clear that here we were not comparing cancerous vs control

- P7, paragr3: “However, at later … P<10-3).” I believe this p-value must be based on the individual datapoint analysis that is not described. This becomes confusing because you first report a non-significant interaction (see point above), but here you don´t talk about a difference between groups, but a difference from random choice. Please rewrite the results so these distinctions become clearer.

We hope this is now clarified

- P7, paragr4: “Cancerous flies showed … P=0.44”, same point as above, you report the non-significant result but mention a significant effect. Report statistical analysis.

We apologize, the significance of attraction towards the social stimulus could only be understood by mentioning the p value of the intercept. It has been added.

- P6, paragr1: “More surprisingly, we … together (Fig. 1).”: report statistical results backing up this result.
This is now clearly shown in figure 1 with the post-hoc analysis

In the concluding paragraph of the discussion there is a referral to the contribution of this study to the evolutionary ecology of cancer, it would be great if the authors could expand a few sentences on this. What are the evolutionary benefits? Could such behavior be adaptive for cancer or is it an unintended consequence of a non-specific infection avoiding behavior?
The tumor cells in this study do not impact fitness, could this bias any conclusions drawn from this study?
We modified the discussion accordingly to this comment

Minor comments:
P4, paragr1: remove comma behind “it is therefore,”P4, paragr1: social overcrowding has been found to induce psychiatric and metabolic disorders. How about communicable diseases? That would be important to include since this study specifically highlights non-communicable diseases as opposed to communicable diseases.
Correction done. We had initially the feeling that entering into a ‘transmissible’ vs ‘non transmissible’ discussion would be out of the scope of the manuscript and preferred not mentioning it too much

P4, paragr2: remove comma after “non-transmissible ones),”
This has been corrected

P5,paragr3: rephrase first sentence: “biological model: … progenitor cells”
This has been corrected

P6, paragr2, social interactions (fig 2): Contact duration and number of contracts seem very correlated, is there an explanation for this? Is this correlated in individual flies as well? I trail length and number of contacts was similar that would make sense (the more they move, the more likely they interact), but if contact duration is longer, it seems there would be less time for meeting other flies. Thus, this suggests a double effect? Would it be possible to see this extra strong effect by analyzing amount of time spent alone?
This correlation between number of contact and contact duration suggest that flies in an homogeneous group of cancerous flies are more aggregated than flies of an heterogeneous group or a group of control individuals. We clarified this in the manuscript

P7,paragr1: The small size of the arena did not allow the authors to disentangle the direction of social contact. Could the size of the arena thus also have affected the conclusions in this study?
We agree that the size of the arena might be a constrained to disentangle the direction of social contact. This is mainly a technical and logistic constrain. We do not really see however how it could have bias the conclusion of the study (for breeding the flies were kept in even smaller tube). We found clear variation in contact duration and number depending on group composition.

P7,paragr4: Fig 5 = Fig 4
This has been corrected

P8,paragr4 “Even if not… with being sick”: if this is a general response, shouldn´t cancerous flies also avoid other cancerous flies (which they don´t)? If it is a general response, they may want to avoid flies with contagious infection despite themselves having cancerous cells. Please discuss.
Even if we have not observed clear ‘avoidance’ of the cancerous flies by other cancerous ones we still see a decrease in preference. We believe that there could be a balance between avoidance of the potentially contagious individual and attraction of individual of the same type. This balance may vary with cancer progression.

P13, paragr3: Do the authors have data that wing-clipping (left or right) does not affect behavior?
In previous studies we showed that wing clipping does not affect the interactive behaviour of the flies (Battesti et al 2015). Note that in the present study all flies are wing clipped (left or right) which should also eliminate any potential variation in wing clipping effect

Figure 1: adding asterisks showing statistical differences between groups would be helpful
In the previous version we forgot to present the results of a post-hoc classification. This has now been corrected

Figure 3: remove “28” from x-axis
This has been corrected

Figure 4: A header would be helpful. Interaction plotting such as figure 3 for consistency would also help the reader.
This has been corrected

***Title*:**

The relationship between cancer progression and social environment in Drosophila

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Summary:

The ecological benefits of sociality in gregarious species are widely acknowledged. However, only limited data is available on how the social environment influences non-communicable disease outcomes. For instance, despite extensive research over the past decades, the role of the social environment on cancer progression remains unclear and controversial. This is mainly because epidemiological studies suffer from the complexity of inter-correlated factors and it is still unknown whether distinct social group composition can also differentially affect tumor growth. Here, we exposed adult *Drosophila* with colorectal-like tumors to different social environments. We show that both cancerous flies bred in complete isolation, or in a group with non-cancerous individuals, exhibit increased tumor progression compared to those bred with other cancerous conspecifics. Based on video-tracking and social interaction analyses, we propose that this dramatic effect may be a consequence of perceived social isolation due to differential social interaction rates. We found that flies can discriminate between individuals at different stages of tumor growth; control flies actively avoid flies with cancer but only at the later stages of tumor development, whereas cancerous flies display strong social interactions with cancerous flies in the early stages of tumor growth. Our study demonstrates the reciprocal links between cancer and social interactions, as well as highlighting how sociality impacts health and fitness in animals and its potential implications for disease ecology and ecosystem dynamics.

Keywords: Social interaction, group composition, cancer, drosophila, ecology of non-transmissible disease.

Highlights:

- While it is well established that social life offers many fitness benefits, the influence of social environment on non-transmissible diseases has rarely been considered.
- Here we used a Drosophila model to explore the reciprocal links between social environment, tumor development and behavior in individual flies.

- We found that flies kept in isolation developed tumors at a faster rate than those kept in groups and more surprisingly that the identity of group members can significantly affect tumor progression.
- Flies can discriminate between individual states at different stages of tumor growth; control flies actively avoid flies with cancer but only at the later stages of tumor development, whereas cancerous flies display strong social interactions with flies in the early stages of tumor growth
- Our findings bring new perspectives to the importance of social structure on non-transmissible disease progression which may have important consequences for animal health.

Introduction

In gregarious species sociality not only offers important positive benefits associated with reducing predation risk [1] and increasing foraging efficiency [2], but also provides additional adaptive benefits by reducing overall metabolic demand [3], providing thermal advantages [4], decreasing stress responses [5] and increasing disease avoidance [6]. It is therefore generally accepted that an individual's social environment affects a large range of behavioral, psychosocial, and physiological pathways. Limited empirical evidence (mostly based on human studies) suggests that extreme social environments such as complete isolation or overcrowding of conspecifics in a group can potentially induce and accelerate pathological disorders. For example, in mammals, social isolation has been associated with faster progression of type 2 diabetes [7], cardiovascular or cerebrovascular disorders [8], and, notably, early and faster mammary cancer development [9, 10]. Moreover, social overcrowding has been found to induce psychiatric and metabolic disorders [11]. Few human studies have attempted to explore the role of social interactions on cancer progression, and the topic remains controversial. Adverse psycho-social factors, including traumatic life events, high levels of depressive symptoms, or low levels of social support, have been related to higher rates of, for example, breast and colon cancers [12, 13]. However, these community based studies or meta-analyses often suffer from the complexity of inter-correlated factors. For example, low sample sizes, high risk behaviors associated with stress (e.g. smoking), and the heterogeneity and retrospective origins of these studies make it difficult to find a conclusive causal relationship between cancer progression and social conditions.

In addition to human studies, laboratory based experiments on gregarious species (e.g. Sprague-Dawley rats) have demonstrated an association between persistent social isolation and inflammatory responses linked to numerous disease processes, including cancer [9, 10, 14]. Despite cancer (both transmissible and non-transmissible) being an emerging important factor influencing life history traits even at early stage [15-18] , little is known regarding the reciprocal links between the social environment and the development and progression of this illness. Increasing evidence demonstrates that oncogenic phenomena are extremely prevalent in host populations, and not just in post-reproductive individuals as

previously believed [19]. It is still largely unclear for both animals and humans how specific social group composition can directly affect tumor progression, and vice versa.

Drosophila has proven to be a powerful model system to address these issues. Social interactions are an important life history trait, particularly in female flies, who use social information to make fitness enhancing decisions [20-22]. More importantly behavioral and physiological processes have been found to be influenced by the degree of social interaction while eliminating all other confounding variables. In *Drosophila*, social isolation leads to a reduced lifespan [23], increased aggression [24-26], reduced need for sleep [27, 28] and a decrease in the fiber number of the mushroom bodies in the integrative nervous center [29]. Furthermore, tumor-like overproliferation of tissues has been found to occur naturally in Drosophila [30, 31] and induced tumors have also been found to influence fitness traits in individuals [16, 18] .

Here, to explore the reciprocal relationship between social environment and cancer progression, we made use of a colorectal-like tumor model [32]. Thanks to genetic tools the tumors can be induced at a precise developmental stage and followed over fly lifespan. The tumors are generated by inducing clones in intestinal progenitor cells that are homozygous mutants for the two *Drosophila Apc* (*Adenomatous polyposis coli*) genes and that express an oncogenic form of the proto-oncogene Ras. Interestingly, loss-of-function of the APC tumor suppressor and expression of oncogenic Ras are critical steps to malignancy in the human colorectal track [33]. First, we exposed tumor-bearing *Drosophila* females to various social environments for 21 days and measured tumor growth and social interactions. Subsequently, we tested control and cancerous flies for their social environment preferences predicting that cancer flies should presumably prefer the social environment which limits cancer progression.

Results

Biological model: The flies used contained heat shock-induced MARCM (Mosaic analysis with a repressible cell marker) clones [34] induced in 3-day old adult virgin females intestinal progenitor cells.

The clones were mutant for both *Drosophila APC* genes, *Apc* and *Apc2*, and expressed the oncogenic form of Ras, Ras^{V12} and the GFP marker (Apc-Ras clones) [32]. It has been shown that these compound Apc-Ras clones, but not clones either expressing Ras^{V12} or mutated for the *APC* genes, expand as aggressive intestinal tumor-like overgrowths that reproduce many hallmarks of human colorectal cancer [32]. The number of GFP-positive gut cells was monitored with age every 7-days by flow cytometry from flies bearing either Apc-Ras or neutral clones, hereafter referred to as cancerous and control flies, respectively. As expected, a clear increase in the number of GFP-positive tumor cells was observed three weeks after clone induction (Supplementary Fig. 1 $F_{1,33}=8.6$; $P=0.006$). Gut dissections confirmed the presence of tumors in the gut (Supplementary Fig. 2). The presence of tumor cells (Apc-Ras clones) had little impact on fly performance and survival over the three weeks of the experimental study [32] (Supplementary Fig. 3)

Cancer progression and social environment: To investigate the impact of social environment on tumor progression we exposed adult cancerous females for 21 days, post induction, to various social environments in 40ml food tubes. Individual virgin cancerous females were either kept in tubes alone (social isolation), in groups composed of seven other cancerous flies (homogeneous groups) or in groups with seven non-cancerous control females (heterogeneous groups). Groups of eight control flies were used as a reference (homogeneous group). Tumor growth was significantly affected by the social environment (Wald $\chi^2_2=6.7$, $P=0.031$). After 21 days we observed that tumor growth was dramatically higher in cancerous flies kept in isolation than in cancerous flies kept in homogeneous groups (Fig. 1). More surprisingly, we also observed that cancerous individual flies kept within a group of control flies showed an increased number of tumor cells compared to cancerous flies grouped together (Fig. 1).

Social interactions: we then analyzed how social interactions were affected by tumor progression and group composition. Using a video tracking setup, we followed the locomotion and interactions of groups of flies (3 weeks post induction) placed in an arena for 1 hour. For social interaction measures we used homogenous groups of eight control or eight cancerous flies, and a heterogeneous group consisting of seven control and one cancerous fly which were kept together for 21 days post induction. Social interaction analyses confirmed that control and cancerous flies had similar locomotor activity

independent of their social environment (Fig. 2A; log (trail length): group composition: $F_{1,84} = 2.64$, P = 0.1; fly state: $F_{1,84} = 0.13$, P = 0.7; fly state x group composition: $F_{1,84} = 3.8$, P = 0.061). However, the length of interaction a fly had with another strongly diverged according to group composition and fly state (contact duration: group composition: $F_{1,84} = 14.8$, $P < 10^{-3}$; fly state: $F_{1,84} = 26.8$, $P < 10^{-3}$; fly state x group composition: $F_{1,84} = 22.9$, $P < 10^{-3}$). In homogeneous groups, cancerous flies had longer interactions compared to homogenous control groups (Fig. 2B). Control flies showed the same contact duration whether in homogeneous or heterogeneous groups, whereas cancerous flies showed a strong decrease in contact duration in the presence of control flies (Fig. 2B). Similarly the average number of contact per fly also differed depending on the social context and the state of the flies (number of contact: group composition: $F_{1,84} = 17.5$, $P < 10^{-3}$; fly state: $F_{1,84} = 11.4$, P = 0.001; fly state x group composition: $F_{1,84} = 4.4$, P = 0.038) Flies in the control group had the same number of contacts when in the homogenous and heterogeneous groups, while cancerous flies, once again, showed a decrease in the number of contacts when placed with control flies (heterogeneous group) compared to when in a group with other cancerous flies (Fig. 2C). Taken together this would suggest that, in a homogeneous group of cancerous flies, individuals are more aggregated than in a heterogeneous group or a homogeneous group of control flies. We thus concluded that, for a cancerous fly, the composition of the social group strongly affects the level of social interactions. However, our measure of social contact was constrained by the small size of the arena and therefore did not allow us to disentangle the direction of the social contact i.e. which fly showed avoidance and which fly showed attraction.

Cancer progression and social environment choice: Based on the results described above we tested whether cancerous and/or control flies would show variation in social environment choice depending on the level of tumor progression. Using a similar protocol to Saltz [35], we assessed social preference by putting two small mesh cages, each containing 8 “stimulus flies” (cancerous or control) in a plastic, transparent box. The small mesh cages were placed on top of a small petri-dish containing standard food. We introduced a “target fly” (cancerous or control) in the enclosed box and recorded their position over 7h i.e. whether the fly was found on one of the two mesh cages. Target and stimulus flies were tested at different ages post heat-shock induction.

When target flies were given the choice between a cancerous and a control stimulus group of the same age, cancerous and control flies showed on average a decrease preference for the cancerous stimulus group with age. Cancerous flies appeared, on average, more attracted than control flies to other cancerous individuals (Fig 3; target fly: Wald $\chi^2_1=4.1$, $P=0.04$; age: Wald $\chi^2_1=17.6$, $P<10^{-3}$; age x target fly: Wald $\chi^2_1=2.7$, $P=0.1$). This attraction was especially pronounced when flies were young i.e. at the very beginning of the tumor development. However, at later stages of tumor growth, this preference decreased (Fig 3; intercept: Wald $\chi^2_1=17.63$, $P<10^{-3}$; age effect on preference: Wald $\chi^2_1=21.2$, $P<10^{-3}$). On the other hand, control flies showed no preference for either group until the third week of cancer growth, where a strong preference for other control flies was observed (Fig 3; intercept: Wald $\chi^2_1=0.46$, $P=0.49$; age effect on preference Wald $\chi^2_1=2.7$, $P=0.09$).

To understand whether these preferences, in a dual choice, were due to avoidance or attraction, young (7 days post heat shock) target flies were given a choice between a stimulus group in a mesh cage (8 flies) and an empty mesh cage using a similar experimental design. Cancerous flies showed, on average, attraction for the social group, independent, of the age or the state of the stimulus flies (Fig 4; intercept: Wald $\chi^2_1=8.1$, $P<10^{-3}$; stimulus: Wald $\chi^2_1=0.06$, $P=0.79$; stimulus age: Wald $\chi^2_1=1.4$, $P=0.23$; stimulus x stimulus age: Wald $\chi^2_1=0.6$, $P=0.44$). While control flies showed, on average, no clear attraction for the social group, they clearly avoided 3-week-old cancer flies (Fig 4; intercept: Wald $\chi^2_1=4.4$, $P=0.036$; stimulus: Wald $\chi^2_1=2.6$, $P=0.1$; stimulus age: Wald $\chi^2_1=3.37$, $P=0.066$; stimulus x stimulus age: Wald $\chi^2_1=6.61$, $P=0.01$).

Discussion

Here, we show that social environment can significantly shape the development of intestinal-like cancer in Drosophila. Consistent with previous studies on mammals [10, 36], cancerous flies kept in isolation exhibit faster tumor progression than flies kept in groups of other cancerous individuals. However, more importantly we find that variation in group composition also leads to increased proliferation of tumor cells, thus highlighting how subtle variations in social structure may have dramatic effects on the progression of non-transmissible diseases.

Despite the opportunity to interact with others, individual cancerous flies, kept in groups with control flies, developed tumors at similar rates to when cancerous flies were bred in isolation. Social interaction analyses revealed that despite similar locomotor activities, cancerous flies interact considerably less with control flies compared to when they are housed with other cancerous conspecifics. This reduction of social contact may potentially be perceived as a form of social isolation by cancerous flies, which could result in increased tumor growth, analogous to when flies are kept in true isolation. In humans, it has been proposed that the subjective perceived feeling of social isolation may impact psychological and physiological traits as much as real social isolation [37]. Furthermore, the social environment choice experiment suggests that control flies may actively recognize and avoid cancerous individuals, especially when tumor growth is significant. Potentially, this may be a result of tumor-induced changes in cuticular hydrocarbons, pheromone profiles or even gut biome. Alternatively, it could also simply reflect common infection avoidance [38, 39], a behavior that has also recently been observed in Drosophila [40]. Even if not contagious, cancerous flies may show particular behaviors, or produce chemical cues, which are generally associated with being sick.

These findings offer new perspectives on the reciprocal relationship between disease and social behavior. While we observe that social structure has profound effects on disease progression, our study also suggest that disease might play a fundamental role in influencing group composition. We found that cancer developed at a slower rate when flies were with other cancerous flies. Moreover, we observed that cancerous flies, instead of showing avoidance behavior towards other cancerous flies as non-cancerous flies did, exhibit strong social attraction towards each other, especially at the beginning of tumor development. This raises questions on the very early impact of internal oncogenic process on individual behavior and natural selection pressures on this process [41]. Previous research shows that female Drosophila, bearing colorectal tumors, bring forward their peak oviposition period suggesting that flies are adapted to minimize the costs of cancer on fitness [18]. The social behavior of cancerous flies found in this study could also be interpreted as an adaptive process, which reduces cancer progression. Further studies would be necessary to determine the exact proximate factors responsible

for the effect found in this study, as well as the extent to which generalizations can be made across other cancer types (or indeed other illnesses) and animal species.

Our findings highlight the importance of social structure on disease progression, beyond the context of transmission. This is the first time that a direct link between social environment, specifically group composition, and cancer progression has been shown, while removing all other confounding psycho-sociological parameters that are frequently encountered in human studies. More generally, this study brings new light to how sociality impacts health and fitness in animals and its potential implication in human disease therapy. Moreover, we provide essential data to the emerging topic of evolutionary ecology of cancer, and demonstrate the importance of neoplasia as a fitness limiting factor that potentially influences life history adaptations and strategies.

Author Contributions:

E.H.D, T.B, C.M., F.T, J.M and F.M designed the experiments. E.H.D., T.B., J.D.S., C.M., M.S. and J.M. performed the experiments. C.S, J.M and F.M analyzed the data. E.H.D., J.M.., F.T., A.C., C.S., B.U. and F.M. wrote the manuscript.

Acknowledgements

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References

1. Beauchamp, G. (2016). Function and structure of vigilance in a gregarious species exposed to threats from predators and conspecifics. Anim. Behav. *116*, 195-201.
2. Dalerum, F., Lange, H., Skarpe, C., Rooke, T., Inga, B., and Bateman, P.W. (2008). Foraging competition, vigilance and group size in two species of gregarious antelope. S. Afr. J. Wildl. Res. *38*, 138-145.
3. Nadler, L.E., Killen, S.S., McClure, E.C., Munday, P.L., and McCormick, M.I. (2016). Shoaling reduces metabolic rate in a gregarious coral reef fish species. J. Exp. Biol. *219*, 2802-2805.
4. McFarland, R., Fuller, A., Hetem, R.S., Mitchell, D., Maloney, S.K., Henzi, S.P., and Barrett, L. (2015). Social integration confers thermal benefits in a gregarious primate. J. Anim. Ecol. *84*, 871-878.
5. Apfelbeck, B., and Raess, M. (2008). Behavioural and hormonal effects of social isolation and neophobia in a gregarious bird species, the European starling (Sturnus vulgaris). Horm. Behav. *54*, 435-441.
6. Behringer, D.C., Butler, M.J., and Shields, J.D. (2006). Ecology: avoidance of disease by social lobsters. Nature *441*, 421-421.
7. Nonogaki, K., Nozue, K., and Oka, Y. (2007). Social isolation affects the development of obesity and type 2 diabetes in mice. Endocrinology *148*, 4658-4666.
8. Venna, V., Xu, Y., Doran, S., Patrizz, A., and McCullough, L. (2014). Social interaction plays a critical role in neurogenesis and recovery after stroke. Translational psychiatry *4*, e351.
9. McClintock, M.K., Conzen, S.D., Gehlert, S., Masi, C., and Olopade, F. (2005). Mammary cancer and social interactions: identifying multiple environments that regulate gene expression throughout the life span. The Journals of Gerontology Series B: Psychological Sciences and Social Sciences *60*, 32-41.
10. Hermes, G.L., Delgado, B., Tretiakova, M., Cavigelli, S.A., Krausz, T., Conzen, S.D., and McClintock, M.K. (2009). Social isolation dysregulates endocrine and behavioral stress while increasing malignant burden of spontaneous mammary tumors. Proceedings of the National Academy of Sciences *106*, 22393-22398.
11. Lin, E.-J.D., Sun, M., Choi, E.Y., Magee, D., Stets, C.W., and During, M.J. (2015). Social overcrowding as a chronic stress model that increases adiposity in mice. Psychoneuroendocrinology *51*, 318-330.
12. Chida, Y., Hamer, M., Wardle, J., and Steptoe, A. (2008). Do stress-related psychosocial factors contribute to cancer incidence and survival? Nat. Clin. Pract. Oncol. *5*, 466-475.
13. Lin, Y., Wang, C., Zhong, Y., Huang, X., Peng, L., Shan, G., Wang, K., and Sun, Q. (2013). Striking life events associated with primary breast cancer susceptibility in women: a meta-analysis study. J. Exp. Clin. Cancer Res. *32*, 1.
14. Hermes, G.L., Rosenthal, L., Montag, A., and McClintock, M.K. (2006). Social isolation and the inflammatory response: sex differences in the enduring effects of a prior stressor. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology *290*, R273-R282.
15. Vittecoq, M., Ducasse, H., Arnal, A., Møller, A.P., Ujvari, B., Jacqueline, C.B., Tissot, T., Missé, D., Bernex, F., Pirot, N., et al. (2015). Animal behaviour and cancer. Anim. Behav. *101*, 19-26.
16. Ujvari, B., Gatenby, R.A., and Thomas, F. (2016). The evolutionary ecology of transmissible cancers. Infect., Genet. Evol. *39*, 293-303.
17. Ujvari, B., Beckmann, C., Biro, P.A., Arnal, A., Tasiemski, A., Massol, F., Salzet, M., Mery, F., Boidin-Wichlacz, C., and Missé, D. (2016). Cancer and life-history traits: lessons from host–parasite interactions. Parasitology *143*, 533-541.
18. Arnal, A., Jacqueline, C., Ujvari, B., Leger, L., Moreno, C., Faugere, D., Tasiemski, A., Boidin-Wichlacz, C., Misse, D., and Renaud, F. (2016). Cancer brings forward oviposition in the fly Drosophila melanogaster. Ecology and Evolution.
19. Madsen, T., Arnal, A., Vittecoq, M., Bernex, F., Abadie, J., Labrut, S., Garcia, D., Faugère, D., Lemberger, K., and Beckmann, C. (2017). Cancer Prevalence and Etiology in Wild and Captive Animals. Ecology and Evolution of Cancer, 11.

20. Sarin, S., and Dukas, R. (2009). Social learning about egg-laying substrates in fruitflies. Proc. R. Soc. B. *276*, 4323-4328.
21. Battesti, M., Moreno, C., Joly, D., and Mery, F. (2012). Spread of Social Information and Dynamics of Social Transmission within Drosophila Groups. Curr. Biol. *22*, 309-313.
22. Mery, F., Varela, S.A.M., Danchin, E., Blanchet, S., Parejo, D., Coolen, I., and Wagner, R.H. (2009). Public Versus Personal Information for Mate Copying in an Invertebrate. Curr. Biol. *19*, 730-734.
23. Ruan, H., and Wu, C.F. (2008). Social interaction-mediated lifespan extension of Drosophila Cu/Zn superoxide dismutase mutants. Proceedings of the National Academy of Sciences *105*, 7506-7510.
24. Hoffmann, A.A. (1987). A laboratory study of male territoriality in the sibling species Drosophila melanogaster and D. simulans. Anim. Behav. *35*, 807-818.
25. Hoffmann, A.A. (1990). The influence of age and experience with conspecifics on territorial behavior inDrosophila melanogaster. J. Insect Behav. *3*, 1-12.
26. Zhou, C., Rao, Y., and Rao, Y. (2008). A subset of octopaminergic neurons are important for Drosophila aggression. Nat. Neurosci. *11*, 1059-1067.
27. Ganguly-Fitzgerald, I., Donlea, J., and Shaw, P.J. (2006). Waking experience affects sleep need in Drosophila. Science *313*, 1775-1781.
28. Donlea, J.M., Ramanan, N., and Shaw, P.J. (2009). Use-dependent plasticity in clock neurons regulates sleep need in Drosophila. Science *324*, 105-108.
29. Technau, G.M., and Technau, G.M. (2007). Fiber number in the mushroom bodies of adult Drosophila melanogaster depends on age, sex and experience. J. Neurogenet. *21*, 183-196.
30. Stark, M.B. (1918). An hereditary tumor in the fruit fly, Drosophila. The Journal of Cancer Research *3*, 279-301.
31. Gateff, E. (1978). Malignant neoplasms of genetic origin in Drosophila melanogaster. Science *200*, 1448-1459.
32. Martorell, Ò., Merlos-Suárez, A., Campbell, K., Barriga, F.M., Christov, C.P., Miguel-Aliaga, I., Batlle, E., Casanova, J., and Casali, A. (2014). Conserved mechanisms of tumorigenesis in the Drosophila adult midgut. PLoS One *9*, e88413.
33. Smith, G., Carey, F.A., Beattie, J., Wilkie, M.J., Lightfoot, T.J., Coxhead, J., Garner, R.C., Steele, R.J., and Wolf, C.R. (2002). Mutations in APC, Kirsten-ras, and p53—alternative genetic pathways to colorectal cancer. Proceedings of the National Academy of Sciences *99*, 9433-9438.
34. Lee, T., and Luo, L. (2001). Mosaic analysis with a repressible cell marker (MARCM) for Drosophila neural development. Trends Neurosci. *24*, 251-254.
35. Saltz, J.B. (2011). Natural genetic variation in social environment choice: context-dependent gene–environment correlation in *Drosophila melanogaster*. Evolution *65*, 2325-2334.
36. Moreno-Smith, M., Lutgendorf, S.K., and Sood, A.K. (2010). Impact of stress on cancer metastasis. Future oncology *6*, 1863-1881.
37. Cacioppo, J.T., and Cacioppo, S. (2014). Social relationships and health: The toxic effects of perceived social isolation. Social and personality psychology compass *8*, 58-72.
38. Kiesecker, J.M., Skelly, D.K., Beard, K.H., and Preisser, E. (1999). Behavioral reduction of infection risk. Proceedings of the National Academy of Sciences *96*, 9165-9168.
39. Loehle, C. (1995). Social barriers to pathogen transmission in wild animal populations. Ecology *76*, 326-335.
40. Vale, P.F., and Jardine, M.D. (2017). Infection avoidance behavior: Viral exposure reduces the motivation to forage in female Drosophila melanogaster. Fly *11*, 3-9.
41. Tissot, T., Arnal, A., Jacqueline, C., Poulin, R., Lefèvre, T., Mery, F., Renaud, F., Roche, B., Massol, F., and Salzet, M. (2016). Host manipulation by cancer cells: expectations, facts, and therapeutic implications. Bioessays.

42. Battesti, M., Pasquaretta, C., Moreno, C., Teseo, S., Joly, D., Klensch, E., Petit, O., Sueur, C., and Mery, F. (2015). Ecology of information: social transmission dynamics within groups of non-social insects. Proceedings of the Royal Society of London B: Biological Sciences *282*, 20142480.
43. Branson, K., Robie, A.A., Bender, J., Perona, P., and Dickinson, M.H. (2009). High-throughput ethomics in large groups of Drosophila. Nat Meth *6*, 451-457.
44. Gargano, J.W., Martin, I., Bhandari, P., and Grotewiel, M.S. (2005). Rapid iterative negative geotaxis (RING): a new method for assessing age-related locomotor decline in Drosophila. Exp. Gerontol. *40*, 386-395.
45. Tinkerhess, M.J., Ginzberg, S., Piazza, N., and Wessells, R.J. (2012). Endurance training protocol and longitudinal performance assays for Drosophila melanogaster. JoVE (Journal of Visualized Experiments), e3786-e3786.

Figure Legend:

Figure 1: *Gut tumor progression as a function of social environment.* FACS analysis of GFP-positive cells in guts dissected from 21 days old control or cancerous females as a function of social environment. Error bars: standard error of the mean. N=15 measures for each treatment. Letters are Tukey's post hoc classification

Figure 2: *Social interactions for 21 days old females in homogeneous (8 cancerous flies or 8 control flies) or heterogeneous groups (1 cancerous and 7 control flies)*. 2A: total foraging trail. 2B: averaged cumulated social contact duration one individual has with another individual of the group. 2C: averaged number of contacts one individual has with another individual of the group. Error bars: standard error of the mean. NS: $P>0.05$; ***: $P<10^{-3}$. N=27 heterogeneous groups and N=18 homogeneous group for each fly state. Letters are Tukey's post hoc classification

Figure 3: *Dual choice experiment*: proportion of target flies choosing to land on the mesh cage containing cancerous flies as a function of age. N=12-21 per treatment. Stars indicate deviation from random choice (binomial test per state and age): ns: $P>0.05$; *: $P<0.05$, **: $P<0.01$; Error bars: standard error of the mean

Figure 4: *Attraction vs aversion experiment*. Proportion of flies choosing to land on the mesh cage containing stimulus flies (4A: cancerous ones; 4B: control ones) as a function of age of the stimulus flies. Stars indicate deviation from random choice (binomial test per state and age) ns: $P>0.05$; *: $P<0.05$, **: $P<0.0$. N=16 per treatment.

Methods:

Drosophila stocks and genetics: *yw,HS-flp;esg-gal4,UAS-GFP;FRT82B,Tub-Gal80* (line 1), *yw,HS-flp;UAS-Ras^{V12};FRT82B,Apc2^{N175K},Apc^{Q8}* (line 2) and *yw,HS-flp;;FRT82B* (line 3) flies [32] were balanced over co-segregating *SM5-TM6B* balancers. In all experiments, cancerous flies were *HS-flp;esg-*

391 *gal4*,*UAS-GFP/UAS-Ras*^{V12};*FRT82B*,*Tub-Gal80/FRT82B*,*Apc2*^{N175K},*Apc*^{Q8} (offspring 1 of line 1 crossed
392 to line 2), whereas controls were *HS-flp*;*esg-gal4*,*UAS-GFP*;*FRT82B*,*Tub-Gal80/FRT82B* (offspring 2
393 of line 1 crossed to line 3). In this study, MARCM clones [32] were randomly generated in
394 heterozygous flies by flipase-induced exchange of pairing chromosome arms, resulting in mosaic
395 individuals where homozygous *Apc2*^{N175K}, *Apc*^{Q8} mutant cells lacked the Gal80 repressor, thereby
396 allowing Gal4 activity and the subsequent expression of GFP and Ras^{V12} for clones located in intestinal
397 progenitor cells. Conversely, MARCM control clones are wild type for both Apc genes and do not
398 express Ras^{V12}. MARCM clones were generated by a 1 hr heat shock at 37°C of 3 days old females [32].
399 Although several attempts were made to use non-induced (no heat shock) offspring 1 flies as controls,
400 due to unpredictable tumor appearance (a few of flies developing tumor without heat shock) the lineage
401 was declared not suitable as reliable control.

402 ***Flow cytometry***: Prior to the quantification of GFP-positive cells as an estimate of tumor progression,
403 flies were starved overnight, provided only with water. The entire midgut and the Malpighian tubules,
404 that also exhibit tumor-like structures, were dissected in PBS (Phosphate buffer saline). Samples of 5
405 guts (originating from different tubes of the same treatment) were digested by collagenase (125µg in
406 60µl PBS) (Sigma-Aldrich) for 2h at 27°C with gentle agitation. Samples were then complemented with
407 10 µl Trypsin 10X (Sigma-Aldrich) and nuclei were stained by Hoechst 33342 (0.5µg/ml) for at least
408 1hr. Samples were filtered and analyzed on a Partec PAS III (Figure S1).

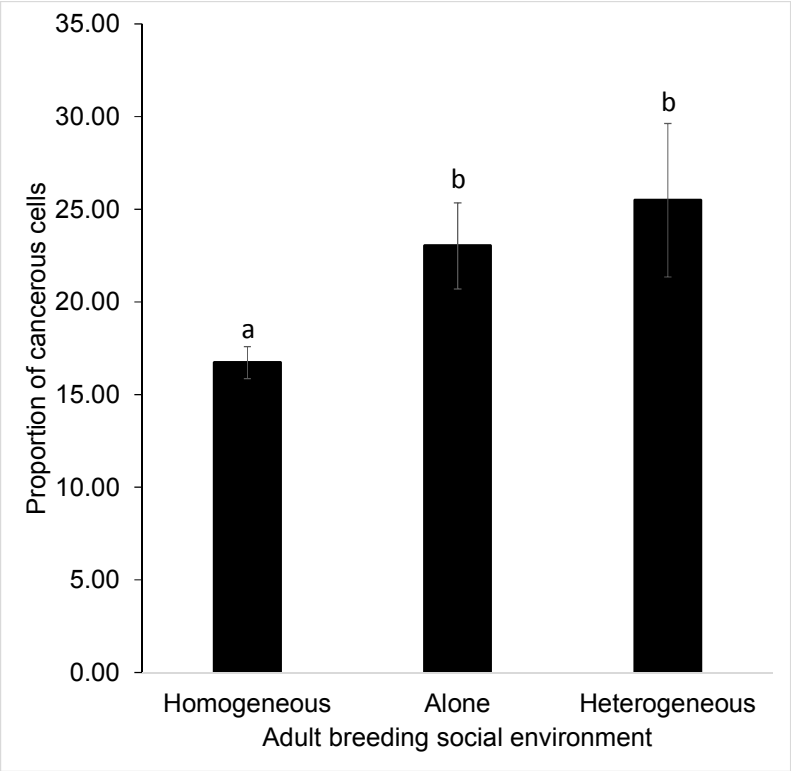
409 ***Social environment***: flies were sexed at emergence and control or cancerous females were kept in
410 groups until the third day post emergence. Control and cancerous virgin females were heat shocked at
411 37°C during 1h. Flies were then introduced into new 40ml food tubes according to their treatment.
412 Control and cancerous flies were partially wing-clipped on the right or left wing to distinguish their
413 genotype. Previous behavioural studies have shown that wing clipping has no effect on social
414 interactions[42] Flies were then kept at 25°C on standard food (renewed every 3 days) until day 21 post
415 induction. Note that the size of the tubes was small (40ml) enough to limit any effect of complete social
416 isolation. Tumour size was estimated with flow cytometry. Data were analyzed with generalized linear

model (binomial distribution, Pearson correction for over-dispersion) on the number of tumor cells vs the total number of cells counted.

Social interactions: Each group (composed of flies taken from different food tubes i.e. had never previously interacted together) was introduced into a semi opaque white polyoxymethylene (Delrin) arena (diameter 100mm; height 5mm) covered with a transparent Plexiglas for 1h. Our experimental design allowed us to simultaneously track 4 groups of 8 flies over the 4h. The tracking apparatus consisted of four synchronized firewire cameras (Guppy pro, Allied vision technologies), each filming one interaction arena that was backlighted by a 150X150 mm IR backlight (R&D vision). We used Vision software to analyze spatial data (open-source C-trax 0.3.7 [43] that allowed us to collect 10 positions per second for each fly over 1h video experiments. Tracking corrections were made post C-trax analysis with fixerrors Matlab toolbox (Ctrax-allmatlab version 0.2.11) using Matlab software 7.11.0 to suppress swaps between individuals. We then calculated, for each fly, the total length of the path, the distance to other flies, the number of contacts with other flies (a contact was considered when the distance between the centers of two individuals was smaller than, or equal to, one mean body length of the individuals for 1s or more) and the duration of each contact. We then averaged these value to obtain, for each group, one value for cancerous state and/or control state. For each measure we performed a general linear model including as fixed explanatory variables group composition (homogeneous vs heterogeneous), fly state (cancerous or control) and the interaction group composition x fly state. Post-hoc contrast

Social environment choice: flies were sexed at emergence and control or cancerous females were kept in groups until the third day post emergence. Control and cancerous virgin females were heat shocked at 37°C during 1h and kept in group until the day of the experiment. The experimental setup consisted of a 17x12x5cm plastic box in which 2 small 2x2x2cm mesh cages were introduced and each placed on a 3cm diameter petri dish containing standard food. The two cages were positioned at opposite ends of the box.Groups of eight flies were placed in the mesh cages. In the dual choice experiment one mesh cages contained control flies whereas the other contained cancerous flies. In the attraction vs avoidance experiment only one of the two cages contained stimulus flies. A target fly (control or cancerous) was

introduced in the box 15h before starting the experiment. The position of the fly was then visually recorded every 30min between 10am and 5pm. We only considered the cases where the fly was on a mesh cage or the associated petri dish. Data (number of times a target fly was observed on a cancerous stimulus cage (for the dual choice experiment) or the stimulus cage (for the attraction vs aversion experiment) compared to the total number of cage landing over 7h) were then analyzed with a general linear model and a binary logistic regression. We first compared the behavior of cancerous vs control flies: State of the target fly was included as a fixed factor and Age of the fly was included as a covariate. For the dual choice experiment, target and stimulus flies were of the same age (7, 14 or 21 days post induction) whereas for the attraction vs aversion experiment the target fly was always 7 days old post induction and the stimulus flies were 7 or 21 days post induction. We then analyzed the behavior of each fly state including age as a covariate. Significance of the intercept in the model provide information on the preference of the flies (random choice vs preference for a stimulus).



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458 Figure 1

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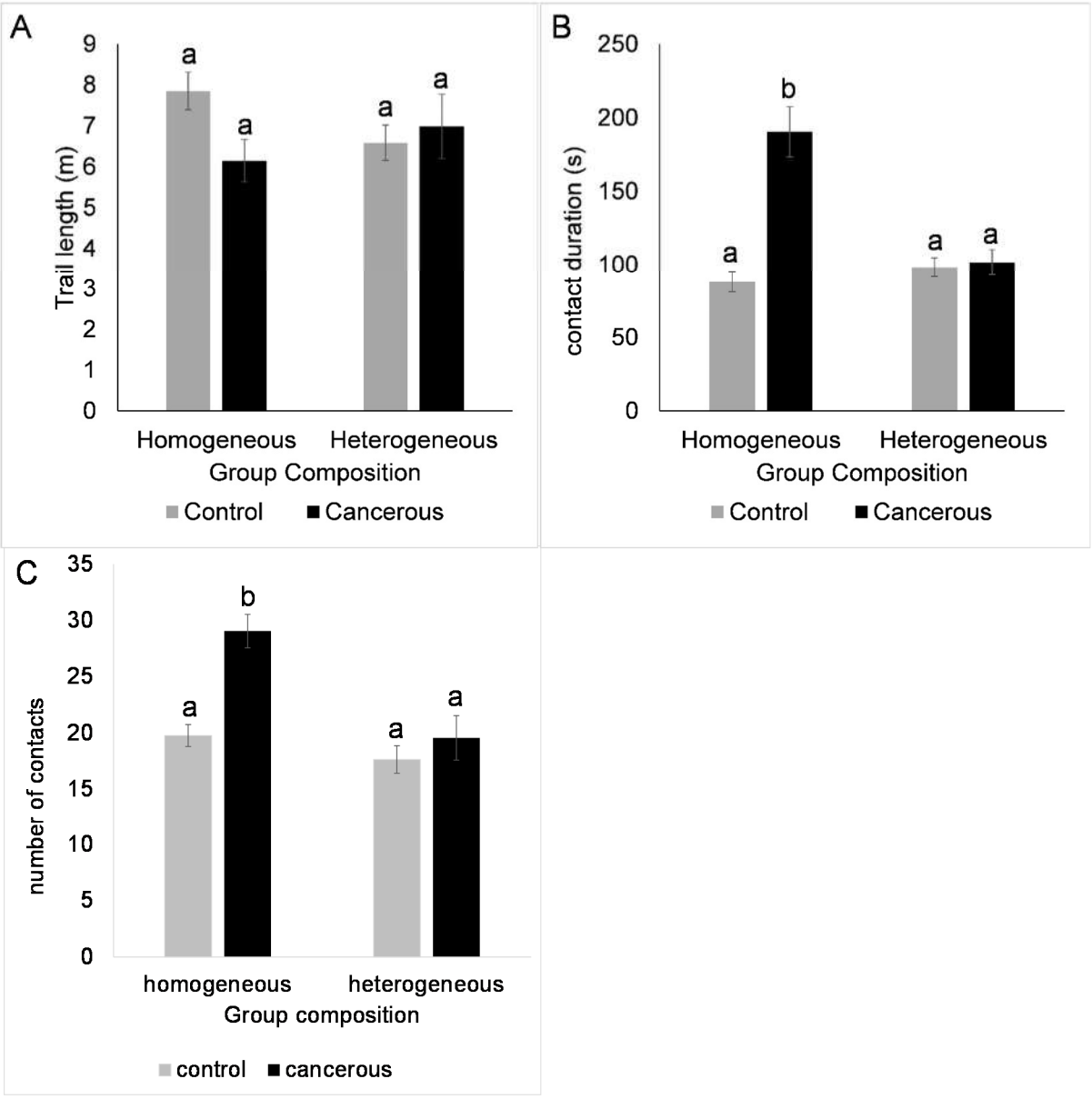

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463 Figure 2

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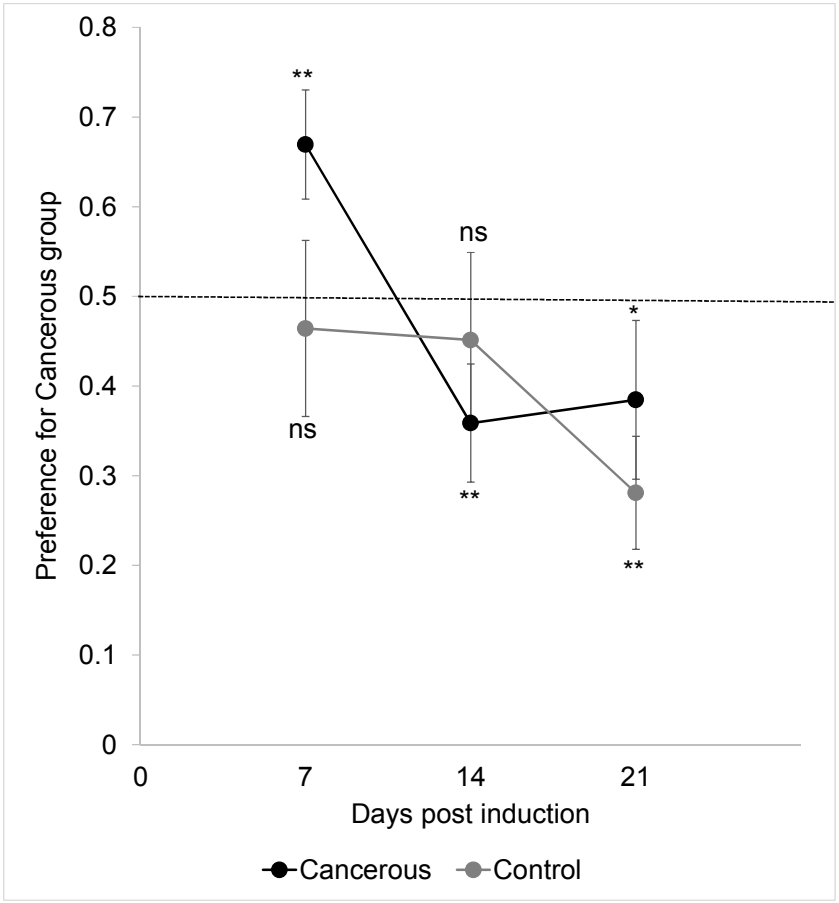
0.8
0.7
0.6
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0.2
0.1
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Preference for Cancerous group
**
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ns
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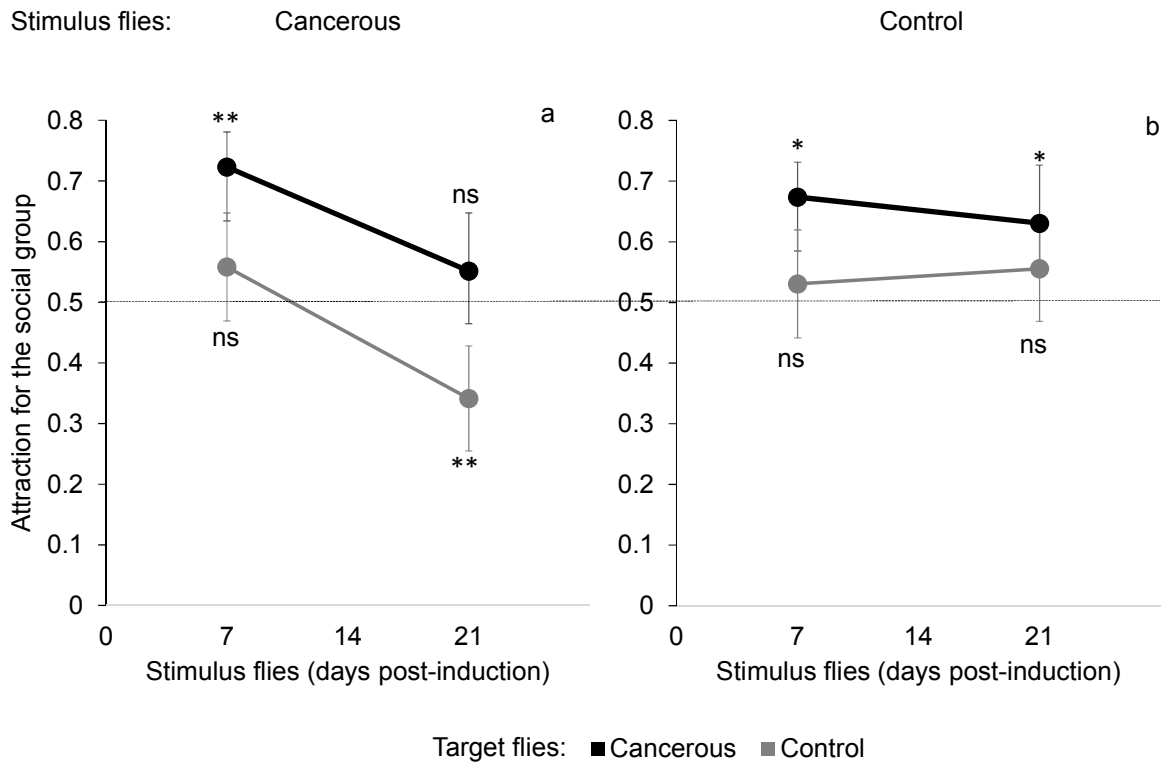
*
**
0
7
14
21
Days post induction
Cancerous
Control

Figure 3


Stimulus flies:
Cancerous
Control
a
b
Attraction for the social group
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0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
**
ns
ns
**
*
*
ns
ns
0
7
14
21
Stimulus flies (days post-induction)
Target flies:
Cancerous
Control

Figure 4

SUPPLEMENTARY INFORMATION

The relationship between cancer progression and social environment in Drosophila

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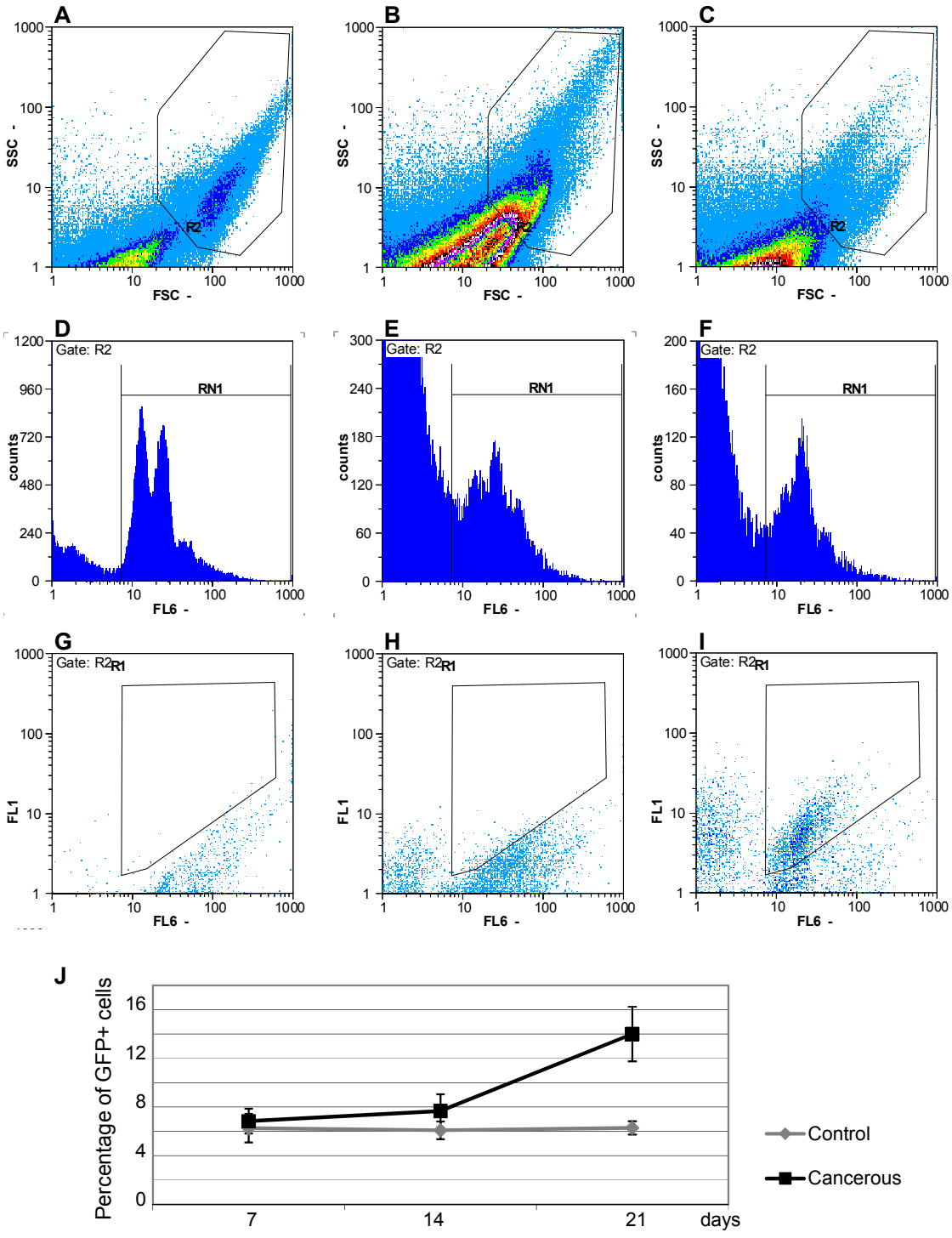

Figure S1

Figure S1*: FACS analysis of intestinal cells*. (**A-C**) SSC versus FSC Dotplots to gate (R2) the cells of interest from dissected imaginal discs (A) or guts of w^{1118} (B) and cancerous (C) flies. (**D-F**) Selection

505 of the *Drosophila* cells within the R2 gate, with respect to their DNA content (RN1); imaginal disc cells
506 allow to identify diploid G1 and G2 cells (2 major pics in D); the intestinal cells of w^{1118} (E) and
507 cancerous (F) flies, which undergo DNA endoreplication contain at least the DNA content of G1
508 imaginal disc cells. (**G-I**) FL6 (DNA) versus FL1 (GFP) Dotplots to select the GFP-positive cells from
509 gut of cancerous flies (R1 in **I**), whereas the R1 gate is empty when analyzing imaginal disc cells (G)
510 and gut cells (H) of w^{1118} flies. (**J**) FACS quantification of GFP-positive cells in cancerous (black line)
511 and control (grey line) guts dissected from adult females at 7, 14 and 21 days past heat shock-induced
512 clonal recombination. Genotype of cancerous and control flies are *HS-flp*;*esg-gal4*,*UAS-GFP/UAS-*
513 Ras^{V12};*FRT82B*,*Tub-Gal80/FRT82B*,$Apc2^{N175K}$,Apc^{Q8} and *HS-flp*;*esg-gal4*,*UAS-GFP*;*FRT82B*,*Tub-*
514 *Gal80/FRT82B*, respectively. Error bars: standard error of the mean. N=8 measures for each treatment.

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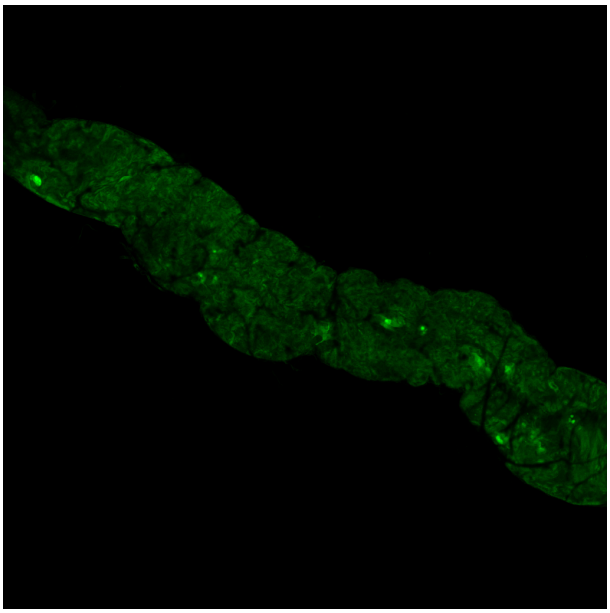
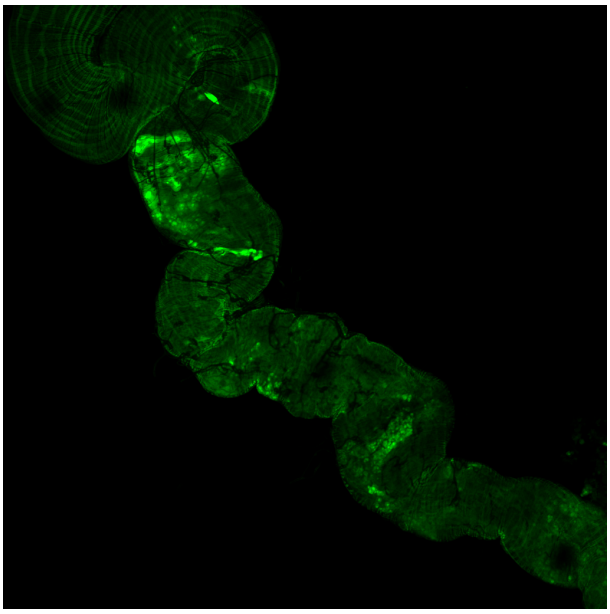
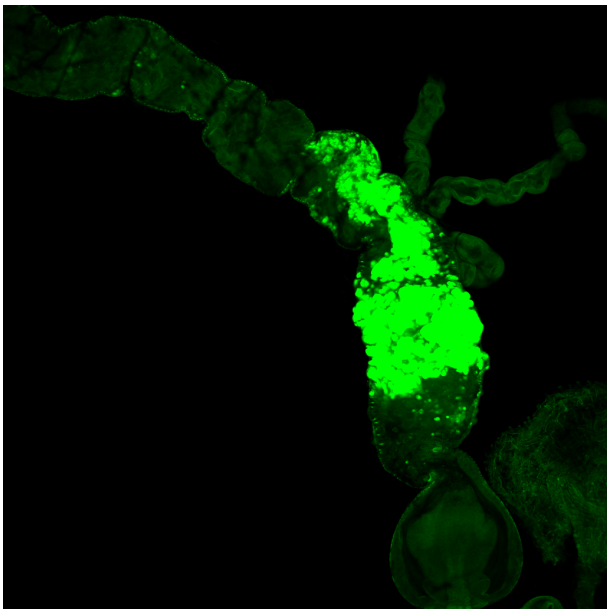


(A) 7 days post induction



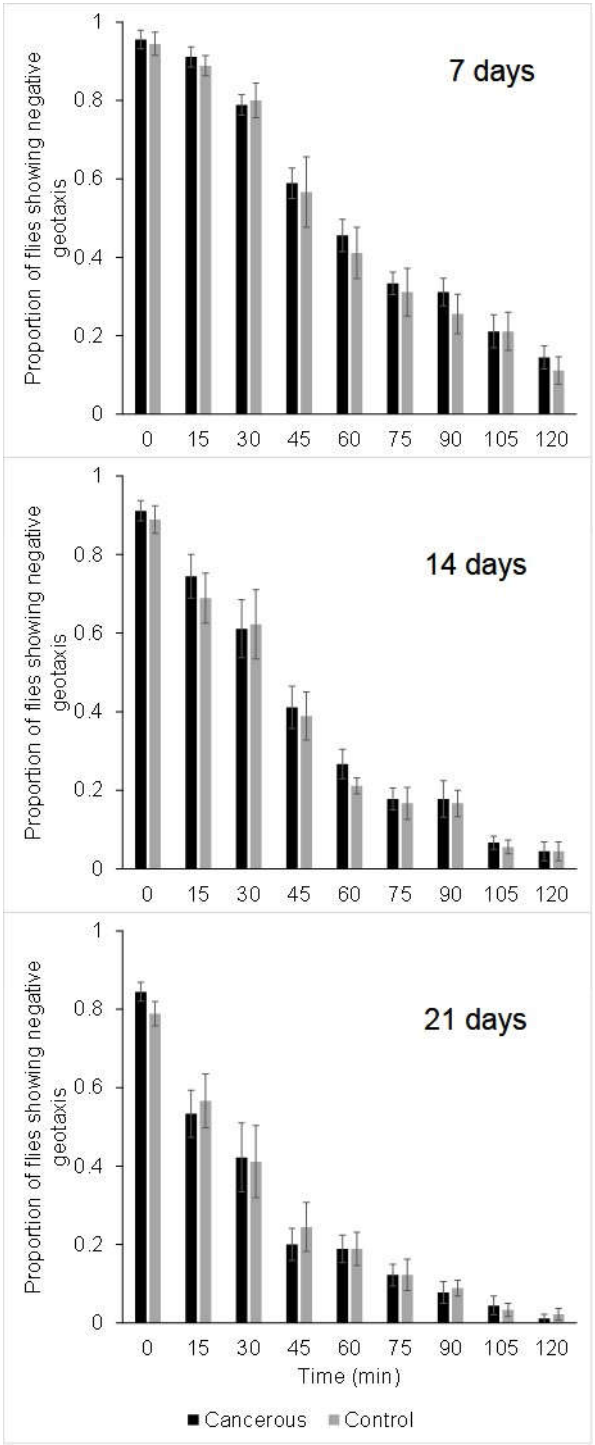
(B) 14 days post induction



(C) 21 days post induction

Figure S2*: Progression of tumor growth in the Midgut.* (**A-C**) APC-Ras clones in the midgut labeled by GFP (green) at 7 days (A), 14 days (B) and 21 days (C) after clonal-induced recombination. Note that 7-day old clones mostly appear as isolated cells, whereas 14-day old clones appear as groups of few cells; 21-day old clones invade large portions of the midgut. Guts from overnight starved flies were dissected in PBS and fixed with 3,7% formaldehyde in PBT (PBS, 0,1% Tween 20) for 20 mn at room temperature; guts were then extensively washed and mounted in DABCO (sigma). Image acquisitions were obtained using laser scanning confocal microscope (LSM700; Carl Zeiss, Jena, Germany) and a solid-state 488-nm laser for exciting GFP. Image were acquired at a resolution of 1024x1024 pixels using a water immersion objective (x10 achroplan 0.3 NA). .

Fly physical performance: We compared physical and behavioral performances between control and cancerous flies at different ages by exposing them to a 'negative geotaxis' behavior test. This involved repeatedly tapping a tube of flies, which causes them to fall, and then measuring the number of individuals that show an escape response (ascending the walls of the tube) over time. When repeatedly performed, this escape response tends to diminish due to exhaustion. This test has been extensively used as a robust proxy to estimate physical and behavioral performance and locomotor activity. We followed a protocol based on the one initially developed by JW Gargano and co-workers [44] and modified by MJ Tinkerhess [45]. Groups of 10 cancerous or control females (7, 14 or 21 days post induction) were introduced into 40ml tubes and placed vertically on a platform which automatically taps the tubes every 4 seconds causing all flies to fall to the bottom of the tube. Every 15 min we visually recorded the number of flies in each tube showing negative geotaxis (climbing at least 2/3 of the tube between two taps). The experiment lasted 2 hours. The data were analyzed with repeated measures ANOVA including time (repeated measure), fly state (cancerous or control) and age (covariate 7,14 or 21 days post induction) as factors. The presence of tumor cells (Apc-Ras clones) has little impact on fly performance and survival over the three weeks of the experimental study. Cancerous and control flies subjected to repeated taps over two hours showed a progressive decline in negative geotaxis response (Repeated measure ANOVA: time: $F_{1,50} = 319{,}9$ $P<10^{-3}$; Fig. S3) which was stronger as flies got older (age: $F_{1,50} = 63.1$ $P<10^{-3}$; time x age: $F_{1,50} = 8.8$; $P = 0.004$). However, no difference between cancerous or control flies could be observed at any age post-induction (state: $F_{1,50} = 0.73$; $P = 0.39$; state x age: $F_{1,50} = 0.17$; $P = 0.68$) suggesting that despite the general observation of age related impairments, Apc-Ras-induced tumors do not affect physical performance and locomotion 3 weeks after induction.



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557 Figure S3: *Repeated 'negative geotaxis' behaviour test performed at different ages for cancerous and*
558 *control flies*. Proportion of flies ascending the walls of the tube after it has been repeatedly tapped.
559 Measures were taken every 15min over 2h at different ages (7, 14 or 21 days post induction). Error bars:
560 standard error of the mean. N=18 groups followed during 2h for each treatment and age

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