Roddie et al. Supplementary Information

Supplementary methods

Analysis of macrophage morphology

Macrophage morphology was assessed using Fiji by manually selecting (polygon selection tool) individual macrophages on the ventral midline that could be easily discriminated from their neighbours and were fully in the field of view. Area, perimeter and shape descriptor measurements were selected for analysis. Circularity is defined by the equation $4\pi \times(\text{area}/\text{perimeter}^2)$. A value of 1.0 indicates a perfect circle, with cells becoming increasingly long as this ratio approaches zero.

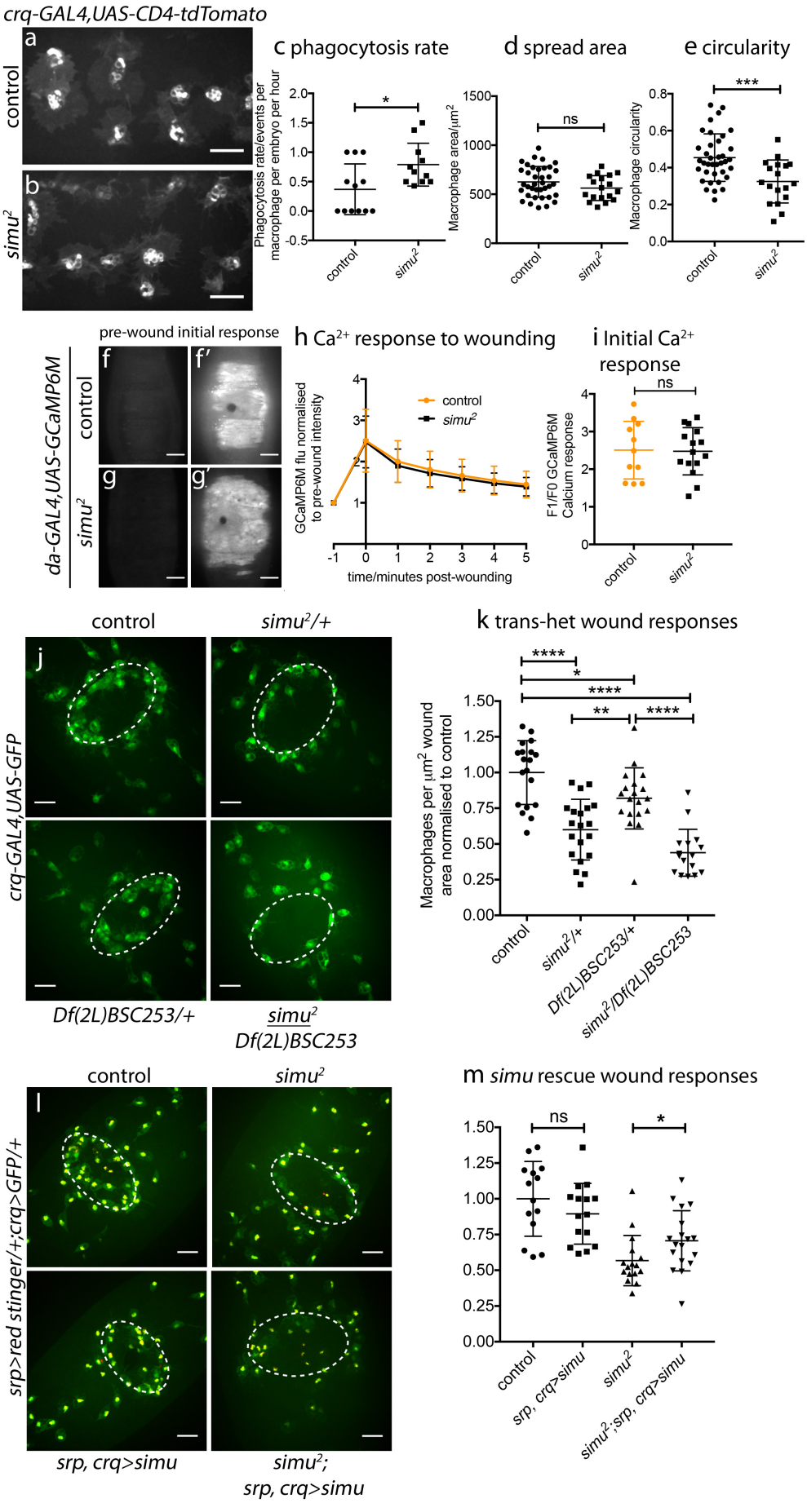
Imaging and analysis of wounding-induced calcium waves

The epithelia of embryos expressing the cytosolic calcium reporter GCaMP6M were imaged before and after wounding. Z-stacks were taken every 15 seconds for 5 minutes to show calcium responses to wounding and subsequent calcium dynamics. Pre-wound and wounding movies were concatenated in Fiji and maximum projections made. The mean gray value (MGV) of GCaMP6M fluorescence was measured within the area bounded by the vitelline membrane (selected using the polygon selection tool) for each projection time-point and the F1/F0 ratio calculated (F1 is the fluorescence at a particular time-point; F0 is the pre-wound fluorescence).

Quantification of caspase reporter activity

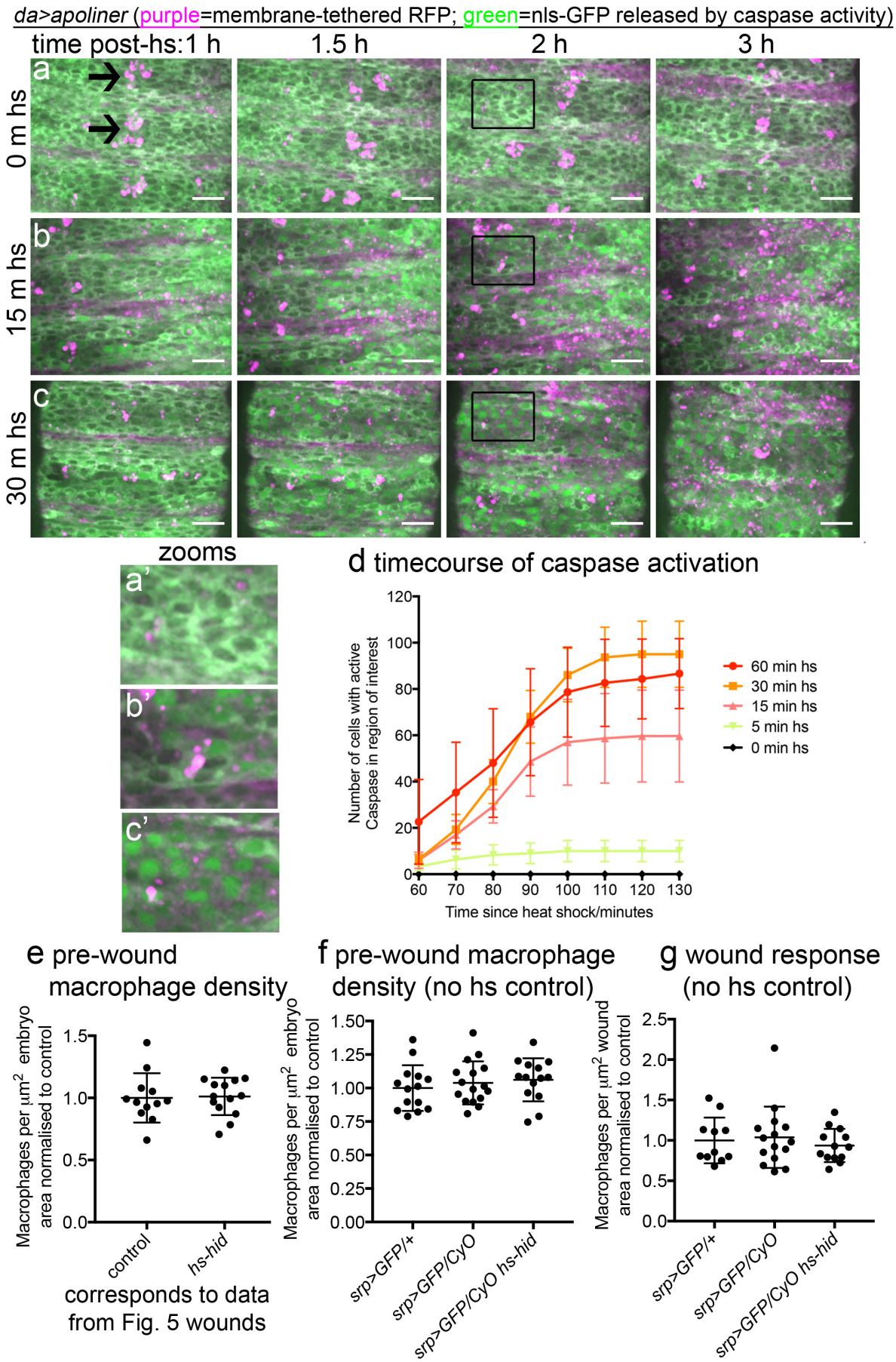
Embryos expressing a caspase reporter (apoliner) were imaged live on their ventral surface 60-minutes after heat-shock treatment (0, 15 or 30 minutes). Maximum projections were assembled in Fiji and a registration plugin (stack reg) used to minimise drift. Images were cropped to a 100μm x 90μm region of interest, corresponding to the two most-medial abdominal segments. The cumulative numbers of GFP-positive nuclei per field of view was assessed (n=3 per condition). These experiments formed the basis for selecting the 15-minute heat-shock condition and wounding at 60-minutes post-heat shock. Under these conditions and at this timepoint, cells exhibit caspase activity and are destined to undergo apoptosis, but have yet to delaminate and be removed by efferocytosis.

Supplementary Figures


crq-GAL4,UAS-CD4-tdTomato
a
b
control
simu2
c phagocytosis rate
Phagocytosis rate/events per macrophage per embryo per hour
d spread area
Macrophage area/µm2
e circularity
Macrophage circularity
control
simu2
pre-wound initial response
da-GAL4,UAS-GCaMP6M
f
f'
g
g'
h Ca2+ response to wounding
GCaMP6M flu normalised to pre-wound intensity
time/minutes post-wounding
i Initial Ca2+ response
F1/F0 GCaMP6M Calcium response
control
simu2/+
j
crq-GAL4,UAS-GFP
Df(2L)BSC253/+
simu2
Df(2L)BSC253
k trans-het wound responses
Macrophages per µm2 wound area normalised to control
control
simu2/+
Df(2L)BSC253/+
simu2/Df(2L)BSC253
l
srp>red stinger/+;crq>GFP/+
srp, crq>simu
simu2;
srp, crq>simu
m simu rescue wound responses
control
srp, crq>simu
simu2
simu2;srp, crq>simu

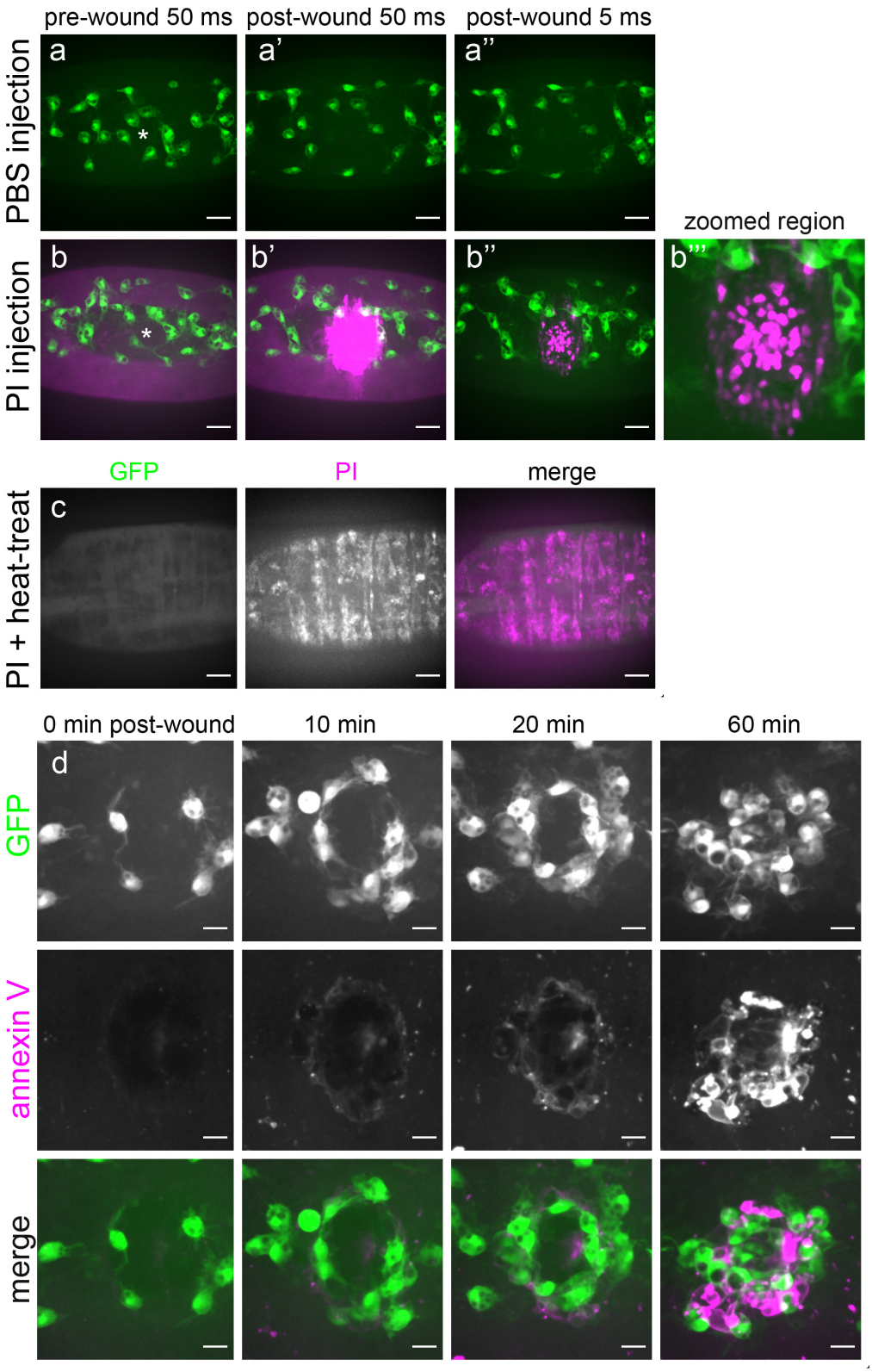
Supplementary Figure 1 - *simu* has a partially cell autonomous function in macrophages and generation of wound signals are normal in the absence of *simu*

(**a**) ventral images showing macrophage morphology in control (*w;;crq-GAL4,UAS-CD4-tdTomato*) and *simu* mutant embryos (*w;simu^2;crq-GAL4,UAS-CD4-tdTomato*) at stage 15. (**c-e**) scatterplots showing rates of phagocytosis (phagocytic events per hour, per macrophage, per embryo, **c**), macrophage spread area (μm^2 per macrophage, **d**) and macrophage circularity (**e**). Statistical comparisons made via Mann-Whitney tests; n=12 control and 11 *simu^2* embryos (**c**) and n=37 control and 18 *simu^2* macrophages taken from 11 embryos (**d-e**). p=0.028, 0.179, 0.0009 (**c-e**). (**f-g**) images of the calcium flash (visualised using GCamP6M) in the epithelium prior to (**f-g**) and immediately after wounding (**f'-g'**) in control (*w;;da-GAL4,UAS-GCamP6M*, **f-f'**) and *simu* mutant embryos (*w;simu^2;da-GAL4,UAS-GCamP6M*, **g-g'**). (**h**) line graph showing timecourses of GCamP6M response (GCamP6M mean gray value (MGV) of the entire embryonic field of view at each timepoint normalised to the pre-wound MGV) in control and *simu* mutant embryos (same genotypes as **f-g**). (**i**) scatterplot of the ratio of initial response (F1) and pre-wound (F0) MGV in control and *simu* mutant embryos (n=11 and 15, respectively; p=0.80, Mann-Whitney test). (**j**) ventral images showing wound responses in control (*w;;crq-GAL4,UAS-GFP*), *simu/+* heterozygous (*w;simu^2/+;crq-GAL4,UAS-GFP*), *Df(2L)BSC253/+* heterozygous (*w;Df(2L)BSC253/+;crq-GAL4,UAS-GFP*) and *simu/Df(2L)BSC253* trans-heterozygous embryos (*w;simu^2/Df(2L)BSC253;crq-GAL4,UAS-GFP*) at 60-minutes post-wounding. (**k**) scatterplot showing wound responses corresponding to genotypes shown in (**j**). N numbers (left-right) are 19, 20, 19, 16; ns, *, **, *** and **** denote not significant (p=0.10), p=0.043, 0.0079 and p<0.0001, respectively, via one-way ANOVA with Dunn's multiple comparisons post-test. (**l**) ventral images showing wound responses (macrophages per wound area, normalised to control) at 60-minutes post-wounding in controls (*w;srp-GAL4,UAS-red stinger/+;crq-GAL4,UAS-GFP/+*), *simu* mutants (*w;simu^2,srp-GAL4,UAS-red stinger/simu^2;crq-GAL4,UAS-GFP/+*), embryos in which *simu* was re-expressed in macrophages (*w;srp-GAL4,UAS-red stinger/+;crq-GAL4,UAS-GFP/UAS-simu*) and *simu* mutants in which *simu* was re-expressed in macrophages (*w;simu^2,srp-GAL4,UAS-red stinger/simu^2;crq-GAL4,UAS-GFP/UAS-simu*). (**m**) scatterplot showing wound responses corresponding to genotypes in (**l**). N numbers (left-right) are 14, 15, 16, 18; ns and * denote not significant (p=0.27) and p=0.017, via Mann-Whitney tests. Lines and error bars represent mean and standard deviation on scatterplots; scale bars indicate 20μm.


da>apoliner (purple=membrane-tethered RFP; green=nls-GFP released by caspase activity)
time post-hs:1 h
1.5 h
2 h
3 h
0 m hs
15 m hs
30 m hs
a
b
c
zooms
a'
b'
c'
d timecourse of caspase activation
Number of cells with active Caspase in region of interest
Time since heat shock/minutes
60 min hs
30 min hs
15 min hs
5 min hs
0 min hs
e pre-wound macrophage density
Macrophages per µm² embryo area normalised to control
control
hs-hid
corresponds to data from Fig. 5 wounds
f pre-wound macrophage density (no hs control)
srp>GFP/+
srp>GFP/CyO
srp>GFP/CyO hs-hid
g wound response (no hs control)
Macrophages per µm² wound area normalised to control

Supplementary Figure 2 - Characterisation of induction of apoptosis using *hs-hid* and heat-shock of embryos

(**a-c**) embryos ubiquitously expressing a caspase reporter and carrying the *hs-hid* transgene (*w;CyO hs-hid/+;da-GAL4,UAS-apoliner/+*) were heat-shocked for 0, 15 or 30 minutes and imaged from 60-minutes post heat-shock. Relocalisation of nls-GFP (green) from membranes (via a CD8-RFP tether, purple) to the nucleus following caspase-dependent cleavage between these two fluorophores denotes cells in which caspases are active. Projections show ventral side of embryos at indicated times after heat-shock; arrows indicate examples of macrophages with RFP-positive "historical" phagosomes; boxes show regions shown at higher magnification in (**a'-c'**). (**d**) quantification of rate of generation of caspase-positive cells (cumulative numbers of GFP-positive nuclei over time) following heat-shock (shows mean of n=3 per condition ± standard error of the mean). (**e**) scatterplot showing density of macrophages (macrophages per μm^2 embryo area, normalised to the mean of the control) on the ventral midline immediately prior to wounding in control and *hs-hid* embryos (n=12 and 14, respectively; p=0.527, Mann-Whitney test); this pre-wound data corresponds to wounded embryo data set shown in Fig. 5. (**g-f**) scatterplots of data from control experiments to address whether genetic background, rather than induction of apoptosis, accounted for the impairment of wound responses seen in Fig. 5: stage 15 *w;srp-GAL4,UAS-GFP/+*, *w;srp-GAL4,UAS-GFP/CyO* and *w;srp-GAL4,UAS-GFP/CyO hs-hid* embryos were wounded without heat-shock treatment. There was no difference in pre-wound macrophage density (n=14, 16, 14, respectively, **g**) or wound responses between genotypes (n=11, 15, 13, respectively, **f**), indicating that neither the presence of a balancer chromosome (*CyO*) nor the *hs-hid* transgenic insertion affected either developmental dispersal or recruitment to wounds. Statistical analysis via one-way ANOVA with Tukey's multiple comparison test (**f-g**); p values for (**f-g**) are as follows: *srp>GFP/+* vs. *srp>GFP/CyO* p=0.80 (**f**); p=0.95 (**g**); *srp>GFP/+* vs. *srp>GFP/CyO hs-hid* p=0.59 (**f**); p=0.87 (**g**); *srp>GFP/CyO* vs. *srp>GFP/CyO hs-hid* p=0.92 (**f**); p=0.66 (**g**). Lines and error bars represent mean and standard deviation in scatterplots; scale bars indicate 20μm in image panels.


pre-wound 50 ms
post-wound 50 ms
post-wound 5 ms
PBS injection
a
a'
a''
zoomed region
PI injection
b
b'
b''
b'''
GFP
PI
merge
PI + heat-treat
c
0 min post-wound
10 min
20 min
60 min
GFP
d
annexin V
merge

Supplementary Fig. 3 - Laser-induced injury results in presentation of phosphatidylserine and membrane rupture with large amounts of non-apoptotic debris persisting at wound sites

(**a-b**) PBS (**a**) or propidium iodide (PI) injected (**b**) stage 15 embryos with GFP-labelled macrophages (*w;;crq-GAL4,UAS-GFP*) were imaged before and immediately after wounding, showing a dramatic accumulation of PI staining at the wound site immediately after laser-mediated ablation. Timings refer to exposure duration during image capture - a shorter exposure time (5 ms) reveals detail of nuclear localisation of PI, suggestive of necrotic cell death (**b''**); zoomed panel (**b'''**) shows close up of wound region in (**b''**). (**c**) as a positive control, embryos of the same genotype were heat treated for 15 minutes at 60°C, resulting in widespread entry of PI in to cells and leakage of GFP from macrophages. (**d-d'**) injection of texas red-labelled annexin V (as a marker for phosphatidylserine (PS)) into the vitelline space of stage 15 embryos with GFP-labelled macrophages (*w;;crq-GAL4,UAS-GFP*) reveals a rapid and sustained accumulation of annexin V at the wound edge and within the wound, including on cells in the process of being engulfed by those macrophages that have migrated to that site. Scale bars represent 20μm (**a-c**) and 10μm (**d**).

Supplementary Tables

Supplementary Table 1 - Fly genotypes used in this study

Figure	Abbreviation used	Full genotype	Source (indicated for first panel in which allele/transgene used)
Fig. 1a	control	*w[1118];srp-GAL4,UAS-GFP*	*srp-GAL4,UAS-GFP* obtained from Will Wood, University of Edinburgh
Fig. 1b	*Df(3L)H99*	*w[1118];srp-GAL4,UAS-GFP;Df(3L)H99*	*Df(3L)H99* obtained from Bloomington Stock Centre and recombined to remove mapping markers
Fig. 1c, g-h	control	*w[1118];;crq-GAL4,UAS-GFP*	*crq-GAL4,UAS-GFP* obtained from Will Wood, University of Edinburgh
Fig. 1e, g-h	*Df(3L)H99*	*w[1118];;Df(3L)H99,crq-GAL4,UAS-GFP*	
Fig. 1i-j, l, n	control	*w[1118];srp-GAL4,UAS-2xFYVE-GFP;crq-GAL4,UAS-CD4-tdTomato*	*UAS-2xFYVE-GFP* and *UAS-CD4-tdTomato* obtained from Bloomington Stock Centre
Fig. 1k, m-n	*Df(3L)H99*	*w[1118];srp-GAL4,UAS-2xFYVE-GFP;Df(3L)H99,crq-GAL4,UAS-CD4-tdTomato*	
Fig. 2a-d	control	*w[1118];srp-GAL4,UAS-GFP/+;crq-GAL4,UAS-GFP/+*	
Fig. 2e, g-h	control	*w[1118];;crq-GAL4,UAS-GFP*	
Fig. 2f-h	*simu[2]*	*w[1118];simu[2];crq-GAL4,UAS-GFP*	*simu[2]* obtained from Estee Kurant, University of Haifa
Fig. 3	control	*w[1118];;crq-GAL4,UAS-GFP*	
Fig. 3	*simu[2]*	*w[1118];simu[2];crq-GAL4,UAS-GFP*	
Fig. 4	control	*w[1118];;crq-GAL4,UAS-GFP*	
Fig. 4	*simu[2]*	*w[1118];simu[2];crq-GAL4,UAS-GFP*	
Fig. 5a	apoliner	*w[1118];+/CyO hs-hid;+/da-GAL4,UAS-apoliner*	*UAS-apoliner* obtained from Jean-Paul Vincent, Crick Insitute
Fig. 5b	cDCP-1	*w[1118];+/CyO hs-hid*	*CyO hs-hid* obtained from Martin Zeidler, University of Sheffield
Fig. 5c-f	control	*w[1118];srp-GAL4,UAS-GFP/CyO*	
Fig. 5c-f	*hs-hid*	*w[1118];srp-GAL4,UAS-GFP/CyO hs-hid*	
Fig. 6	control	*w[1118];;crq-GAL4,UAS-GFP*	
Fig. 6	*Df(3L)H99*	*w[1118];;Df(3L)H99,crq-GAL4,UAS-GFP*	
Fig. 6	*simu[2]*	*w[1118];simu[2];crq-GAL4,UAS-GFP*	
Fig. 6	*simu[2];Df(3L)H99*	*w[1118];simu[2];Df(3L)H99,crq-GAL4,UAS-GFP*	
Fig. 7a-d	control	*w[1118];;crq-GAL4,UAS-GFP*	
Fig. 7a-b, e-f	*simu[2];Df(3L)H99*	*w[1118];simu[2];Df(3L)H99,crq-GAL4,UAS-GFP*	
Fig. 7b, e-f	*Df(3L)H99*	*w[1118];;Df(3L)H99,crq-GAL4,UAS-GFP*	
Fig. 7b	*simu[2]*	*w[1118];simu[2];crq-GAL4,UAS-GFP*	
Supplementary Fig. 1a, c-e	control	*w[1118];;crq-GAL4,UAS-CD4-tdTomato*	
Supplementary Fig. 1b-e	*simu[2]*	*w[1118];simu[2];crq-GAL4,UAS-CD4-tdTomato*	
Supplementary Fig. 1f, h-i	control	*w[1118];;da-GAL4,UAS-GCamP6M*	*da-GAL4* and *UAS-GCaMP6M* obtained from Alex Whitworth, University of Cambridge and Bloomington, respectively
Supplementary Fig. 1g-i	*simu[2]*	*w[1118];simu[2];da-GAL4,UAS-GCamP6M*	
Supplementary Fig. 1j-k	control	*w[1118];;crq-GAL4,UAS-GFP*	
Supplementary Fig. 1j-k	*simu[2]/+*	*w[1118];simu[2]/+;crq-GAL4,UAS-GFP*	
Supplementary Fig. 1j-k	*Df(2L)BSC253/+*	*w[1118];Df(2L)BSC253/+;crq-GAL4,UAS-GFP*	*Df(2L)BSC253* obtained from Bloomington Stock Centre
Supplementary Fig. 1j-k	*simu[2]/Df(2L)BSC253*	*w[1118];Df(2L)BSC253/simu[2];crq-GAL4,UAS-GFP*	
Supplementary Fig. 1l-m	control	*w[1118];srp-GAL4,UAS-red stinger/+;crq-GAL4,UAS-GFP/+*	*UAS-red stinger* obtained from Brian Stramer, KCL
Supplementary Fig. 1l-m	*simu[2]*	*w[1118];simu[2],srp-GAL4,UAS-red stinger/simu[2];crq-GAL4,UAS-GFP/UAS-simu*	*UAS-simu* obtained from Estee Kurant, University of Haifa
Supplementary Fig. 1l-m	*srp, crq>simu*	*w[1118];srp-GAL4,UAS-red stinger/+;crq-GAL4,UAS-GFP/UAS-simu*	
Supplementary Fig. 1l-m	*simu[2];srp, crq>simu*	*w[1118];simu[2],srp-GAL4,UAS-red stinger/simu[2];crq-GAL4,UAS-GFP/UAS-simu*	
Supplementary Fig. S2a-d	control	*w[1118];+/CyO hs-hid;+/da-GAL4,UAS-apoliner*	
Supplementary Fig. S2e	control	*w[1118];srp-GAL4,UAS-GFP/CyO*	
Supplementary Fig. S2e	*hs-hid*	*w[1118];srp-GAL4,UAS-GFP/CyO hs-hid*	
Supplementary Fig. S2f-g	*srp-GAL4,UAS-GFP/+*	*w[1118];srp-GAL4,UAS-GFP/+*	
Supplementary Fig. S2f-g	*srp-GAL4,UAS-GFP/CyO*	*w[1118];srp-GAL4,UAS-GFP/CyO*	
Supplementary Fig. S2f-g	*srp-GAL4,UAS-GFP/CyO hs-hid*	*w[1118];srp-GAL4,UAS-GFP/CyO hs-hid*	

Supplementary Fig. 3a-d	control	*w[1118];;crq-GAL4,UAS-GFP*	
Supplementary movie 1	control	*w[1118];;crq-GAL4,UAS-GFP*	
Supplementary movie 1	*simu[2]*	*w[1118];simu[2];crq-GAL4,UAS-GFP*	
Supplementary movie 2	control	*w[1118];;crq-GAL4,UAS-GFP*	
Supplementary movie 2	*simu[2]*	*w[1118];simu[2];crq-GAL4,UAS-GFP*	
Supplementary movie 3	control	*w[1118];+/CyO hs-hid;+/da-GAL4,UAS-apoliner*	
Supplementary movie 4	control	*w[1118];srp-GAL4,UAS-GFP/CyO*	
Supplementary movie 4	*hs-hid*	*w[1118];srp-GAL4,UAS-GFP/CyO hs-hid*	
Supplementary movie 5 and 6	control	*w[1118];;crq-GAL4,UAS-GFP*	
Supplementary movie 5 and 6	*Df(3L)H99*	*w[1118];;Df(3L)H99,crq-GAL4,UAS-GFP*	
Supplementary movie 5 and 6	*simu[2]*	*w[1118];simu[2];crq-GAL4,UAS-GFP*	
Supplementary movie 5 and 6	*simu[2];Df(3L)H99*	*w[1118];simu[2];Df(3L)H99,crq-GAL4,UAS-GFP*	
Supplementary movie 7	control	*w[1118];;crq-GAL4,UAS-GFP*	
Supplementary movie 7	*simu[2];Df(3L)H99*	*w[1118];simu[2];Df(3L)H99,crq-GAL4,UAS-GFP*	
Supplementary movie 8	control	*w[1118];;crq-GAL4,UAS-GFP*	

Supplementary movies

Supplementary movie 1 – Macrophages migrate at slower speeds in *simu* mutants compared to controls

30-minute movies of GFP-labelled macrophages at stage 15 on the ventral midline showing their random migration in control (*w;;crq-GAL4,UAS-GFP*, upper embryo) and *simu* mutant embryos (*w;simu^{2};crq-GAL4,UAS-GFP*, lower embryo). Movies repeat with second movie showing tracks of macrophage migration. Scale bar represents 20μm.

Supplementary movie 2 – Macrophage responses to wounds are perturbed in *simu* mutant embryos

Movies of the inflammatory migration of GFP-labelled macrophages to epithelial wounds in control (*w;;crq-GAL4,UAS-GFP*, embryo on left) and *simu* mutant embryos (*w;simu^{2};crq-GAL4,UAS-GFP*, embryo on right) at stage 15 of development on the ventral midline. Embryos wounded in the centre of the field of view. Scale bar represents 25μm.

Supplementary movie 3 - Induction of apoptosis visualised using the apoliner caspase reporter

Movie of *w;CyO hs-hid/+;da-GAL4,UAS-apoliner/+* embryos heat-shocked for 0 minutes (negative control), 5 minutes, 15 minutes, 30 minutes and 60 minutes; time-lapse movies begin 1-hour post-heat shock. Caspase activity results in cleavage of apoliner, enabling its nls-GFP to translocate to the nucleus, whereas CD8-RFP remains on membranes. Caspase activity increases rapidly from 1-hour post-heat shock, with the majority of caspase-positive cells remaining in an intact epithelium beyond the period of time in which inflammatory responses are typically followed. Movie shows green channel in first instance; second cycle shows merged image with GFP in green and RFP in purple. Scale bar represents 50μm.

Supplementary movie 4 - Exogenous apoptosis impairs inflammatory responses of macrophages

Movies of GFP-labelled macrophage migration to wounds in a control embryo (*w;srp-GAL4,UAS-GFP/CyO*) and an embryo in which exogenous apoptosis has been introduced through Hid expression (*w;srp-GAL4,UAS-GFP/CyO hs-hid*). Both embryos were heat-shocked for 15 minutes, with laser-wounding taking place 1-hour post heat-shock at a time-point when caspase activity is dramatically increasing, but when engulfment of cells destined to die has yet to take place. Fewer macrophages migrate to wounds in the presence of apoptotic cells, suggesting that apoptosis, but not necessarily phagocytosis of dying cells,

can dampen inflammatory responses. Dotted lines show wound sites; scale bar represents 20μm.

Supplementary movie 5 - Rescue of wandering migration in *simu* mutants with no apoptosis

Hour-long movies of GFP-labelled macrophages in control (*w;;crq-GAL4,UAS-GFP*), apoptosis-null *Df(3L)H99* mutant (*w;;Df(3L)H99,crq-GAL4,UAS-GFP*), *simu* mutant (*w;simu^2;crq-GAL4,UAS-GFP*) and apoptosis-null *simu* mutant embryos (*w;simu^2;Df(3L)H99,crq-GAL4,UAS-GFP*) at stage 15. Removal of apoptotic cell death rescues macrophage speeds in a *simu* mutant background. Note the absence of vacuoles in *Df(3L)H99* and *simu^2;Df(3L)H99* embryos due to the lack of apoptotic cell death. Scale bar represents 20μm.

Supplementary movie 6 - Rescued initial responses to wounds in the absence of apoptosis in *simu* mutants

Hour-long movies of GFP-labelled macrophages in control (*w;;crq-GAL4,UAS-GFP*), apoptosis-null *Df(3L)H99* mutant (*w;;Df(3L)H99,crq-GAL4,UAS-GFP*), *simu* mutant (*w;simu^2;crq-GAL4,UAS-GFP*) and apoptosis-null *simu* mutant embryos (*w;simu^2;Df(3L)H99,crq-GAL4,UAS-GFP*) at stage 15 following wounding; initial image shows pre-wound image. Removal of apoptotic cell death rescues the percentage of macrophages responding to wounds in a *simu* mutant background. Note the absence of vacuoles in *Df(3L)H99* and *simu^2;Df(3L)H99* embryos due to the lack of apoptotic cell death. Scale bar represents 20μm.

Supplementary movie 7 - Enhanced macrophage resolution from wounds in the absence of *simu*

Movies of GFP-labelled macrophages during wound responses to laser-induced epithelial wounds on the ventral midline in a control embryo (*w;;crq-GAL4,UAS-GFP*; embryo on left) and *simu* mutant that lacks apoptosis (*w;simu^2;Df(3L)H99,crq-GAL4,UAS-GFP*; embryo on right). First repeat of wound response shows GFP channel only; second repetition shows all tracks of those cells present at 0-minutes post-wounding superimposed over GFP channel; third repetition shows tracks of macrophages that leave the wound (1 cell in control embryo, 5 cells in the *simu^2;Df(3L)H99* embryo). Scale bar represents 20μm.

Supplementary movie 8 - Macrophages interact with annexin V-labelled debris at wounds

Movie showing accumulation of annexin V to label exposed phosphatidylserine (PS) at wounds during an inflammatory response in a control embryo (*w;;crq-GAL4,UAS-GFP*). Initial run of time-lapse movie shows texas red-labelled annexin V alone, second repeat

shows GFP-labelled macrophages (green) and annexin V (purple). Injection of fluorescent annexin V into the vitelline space labels the wound edge, suggesting that PS decorates stressed or damaged cells at these sites. Scale bar represents 20μm.