Materials List for:

Long-term *In Vivo* Tracking of Inflammatory Cell Dynamics Within *Drosophila* Pupae

Helen Weavers[1,2], Anna Franz[1], Will Wood[3], Paul Martin[1,4]

[1]School of Biochemistry, Biomedical Sciences, University of Bristol

[2]School of Cellular and Molecular Medicine, Biomedical Sciences, University of Bristol

[3]MRC Centre for Inflammation Research, University of Edinburgh, Queens Medical Research Institute

[4]School of Physiology, Pharmacology, and Neuroscience, Biomedical Sciences, University of Bristol

Correspondence to: Helen Weavers at helen.weavers@bristol.ac.uk

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Materials

Name	Company	Catalog Number	Comments
***Drosophila* stocks**			
ubiquitous GFP-tagged E-cadherin ;Ubi-p63E-shg.GFP; (chrII)	Kyoto Stock Center, DGRC	#109007	Ubi-p63E promoter sequences drive expression of *Drosophila* E-cadherin (*shotgun*) tagged at the C-terminal end with GFP.
ubiquitous GFP-tagged E-cadherin ;;Ubi-p63E-shg.GFP (III)	Bloomington *Drosophila* Stock Centre (Indiana University)	#58742	Ubi-p63E promoter sequences drive expression of *Drosophila* E-cadherin (*shotgun*) tagged at the C-terminal end with GFP.
ubiquitous GFP-tagged Moesin P{sGMCA}3.1	Bloomington *Drosophila* Stock Centre (Indiana University)	#59023	The ubiquitously expressed sqh promoter/enhancer drives expression of a fragment of Moesin (that includes the actin binding sequences) tagged with GFPS65T.
hemocyte specific serpent-Gal4 driver ;srp-Gal4;	Generated by Katja Bruckner	Generated by Katja Bruckner	Expression of Scer\GAL4 fused to a polyA tail is under the control of 2 genomic sequences from upstream of *Drosophila* serpent. Ref: Brückner, K., Kockel, L., Duchek, P., Luque, C.M., Rørth, P., Perrimon, N. The PDGF/VEGF receptor controls blood cell survival in Drosophila. Dev Cell. 7 (1), 73–84, doi: 10.1016/j.devcel.2004.06.007 (2004).
UAS-nuclearRFP w1118;;P{UAS-RedStinger}6	Bloomington *Drosophila* Stock Centre (Indiana University)	#8545 or #8547	UAS regulatory sequences drive expression of the DsRed.T4 form of RFP which is tagged at the C-terminal end with a nuclear localisation signal
UAS-cytoplasmicGFP ;;P{UAS-GFP.S65T}	Bloomington *Drosophila* Stock Centre (Indiana University)	Multiple stocks available (e.g. #1522)	Expression of the S65T version of GFP by UAS regulatory sequences; the S65T variant exhibits increased brightness.
UAS-photoconvertibleKaede w1118;; P{UAS-Kaede.A}3	Bloomington *Drosophila* Stock Centre (Indiana University)	#26161	Kaede protein emits bright green fluorescence after synthesis, but changes efficiently to a bright stable red fluorescence on irradiation with UV.
GFP-tagged spaghetti squash w1118;;P{sqh-GFP.RLC}	Bloomington *Drosophila* Stock Centre (Indiana University)	#57145	The sqh coding region, which is tagged at the C-terminal end with a T:Avic\GFP^{S65T} tag, is expressed

			under the control of the natural sqh promoter.
Name	**Company**	**Catalog Number**	**Comments**
Ingredients for fly food media			Fly food media is made according to standard procedures (see Greenspan, R. 1997. Fly Pushing: The Theory and Practice of Drosophila Genetics. Cold Spring Harbor Press. 1-191 pp.)
maize	Wild Oats, Bristol, UK (or equivalent supplier)	Contact supplier direct	organic
soya flour	Wild Oats, Bristol, UK (or equivalent supplier)	Contact supplier direct	organic
malt extract	Wild Oats, Bristol, UK (or equivalent supplier)	Contact supplier direct	organic
molasses	Wild Oats, Bristol, UK (or equivalent supplier)	Contact supplier direct	organic
Difco agar	BD Biosciences, Fisher Scientific	DF0142-15-2	For preparation of fly food
Propionic acid	Sigma	402907	For preparation of fly food
Nipagen	Sigma	79721	For preparation of fly food
Dried baker's yeast	Redstar, Dutscher Scientific, UK LTD	Redstar, Dutscher Scientific, UK LTD	For preparation of fly food
Name	**Company**	**Catalog Number**	**Comments**
Sample preparation and mounting			
Parafilm	Sigma	P7793-1EA	For preparation of heptane glue
Fine sable paintbrush	Daler-Rowney (or equivalent)	#0 or 1	
Forceps	Fisher Scientific (or Fine Science Tools)	NC9404145	Dumont #5
Glass bottomed dishes for imaging	MatTek	P35G-0-10-C	We suggest using 35mm petri dishes, with at least a 10mm Microwell, 0.085-0.13mm cover glass, uncoated. Dishes with larger microwells will enable increasing numbers of pupae to be mounted and imaged in a single experiment.
Heptane	Sigma	51730-5ML	For preparation of heptane glue
Double sided sticky tape (e.g. Scotch)	Agar Scientific	AGG263	For preparation of heptane glue
50ml tube (for heptane glue)	Falcon tubes from Fisher Scientific	14-432-22	For preparation of heptane glue
Glass microscope slides	Agar Scientific	AGL4244	For dissection of *Drosophila* pupae
Dissecting stereo microscope with brightfield	Leica (or equivalent)	M50	For dissection of *Drosophila* pupae
Microscissors	John Weiss International	103123	Miniature Research Scissors (straight)
Name	**Company**	**Catalog Number**	**Comments**
Laser ablation and imaging			
Nitogen ablation laser	Spectra-Physics (or Andor equivalent)	Model VSL-337ND-S	For wounding, this should be attached to a widefield imaging system
Multilaser confocal laser-scanning microscope (CLSM)	Leica (or equivalent)	TCS AOBS SP8 or SP5-II attached to a Leica DMi8 inverted epifluorescence microscope (or equivalent)	Ideally including a motorised stage for multi-site and 'mosaic' scanning, plus ‘hybrid’ GaAsP detectors (that offer much greater sensitivity and boosting of low signal)

Environmental chamber	Life Imaging Services (or equivalent)	"Microscope Temperature Control System"	Attached to Confocal microscope for temperature control during imaging
Name	**Company**	**Catalog Number**	**Comments**
Image Analysis Software			
FRAP software module	Leica (or equivalent)	CLSM FRAP software module	For performing photoconversion of photoconvertible fluorophores such as Kaede
ImageJ (image analysis software)	National Institutes of Health (NIH)	https://imagej.nih.gov/ij/	Schneider, C.A., Rasband, W.S., Eliceiri, K.W. "NIH Image to ImageJ: 25 years of image analysis". Nature Methods 9, 671-675, 2012.
ImageJ plugin "Manual Tracking"	National Institutes of Health (NIH)	https://imagej.net/Manual_Tracking	
ImageJ plugin "TrackMate"	ImageJ, NIH	https://imagej.net/TrackMate	Tinevez, JY.; Perry, N. & Schindelin, J. et al. (2016), "TrackMate: An open and extensible platform for single-particle tracking.", Methods 115: 80-90, PMID 27713081
Volocity (high performance 3D imaging software)	Perkin Elmer	Volocity 6.3	For image analysis
IMARIS (image analysis software)	Bitplane	IMARIS for Cell Biologists	For image analysis