

Online Research @ Cardiff

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <http://orca.cf.ac.uk/124154/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Bradley, Josephine, Pope, Iestyn, Wang, Yisu, Langbein, Wolfgang, Borri, Paola and Swann, Karl 2019. Dynamic label-free imaging of lipid droplets and their link to fatty acid and pyruvate oxidation in mouse eggs. *Journal of Cell Science* 132 (13) , jcs228999. 10.1242/jcs.228999 filefile

Publishers page: <http://dx.doi.org/10.1242/jcs.228999> <<http://dx.doi.org/10.1242/jcs.228999>>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



Fig.1

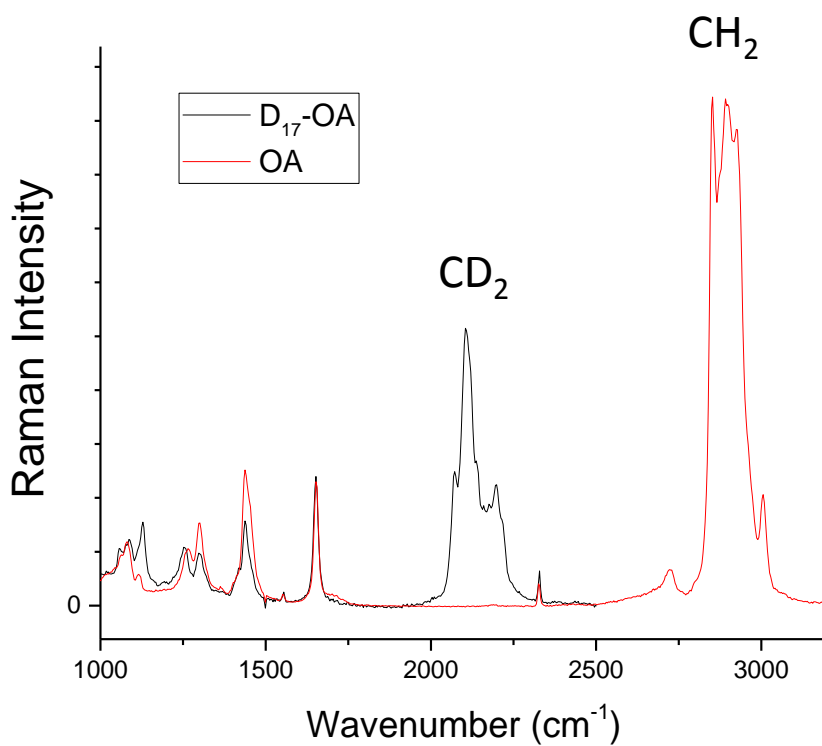
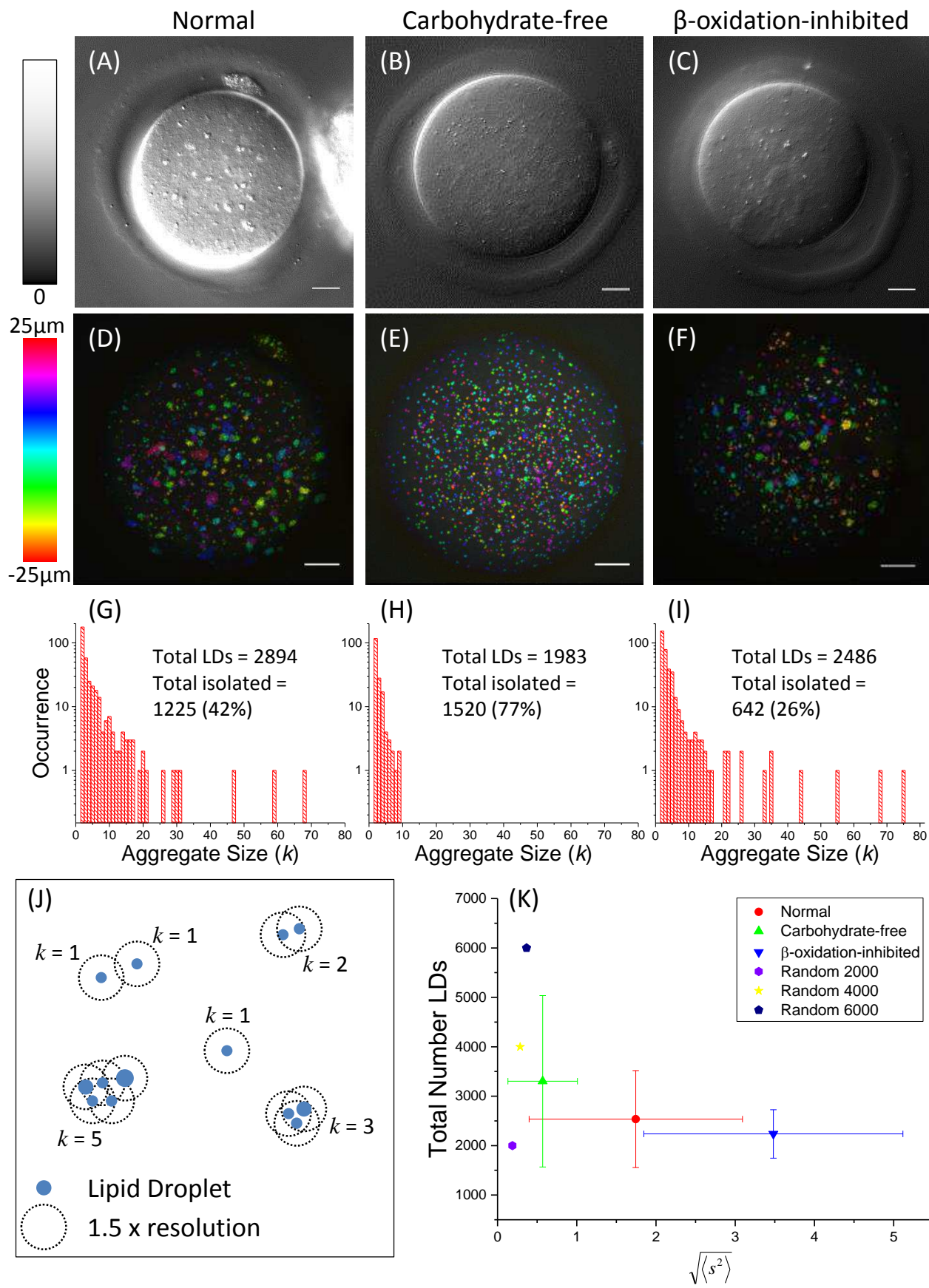
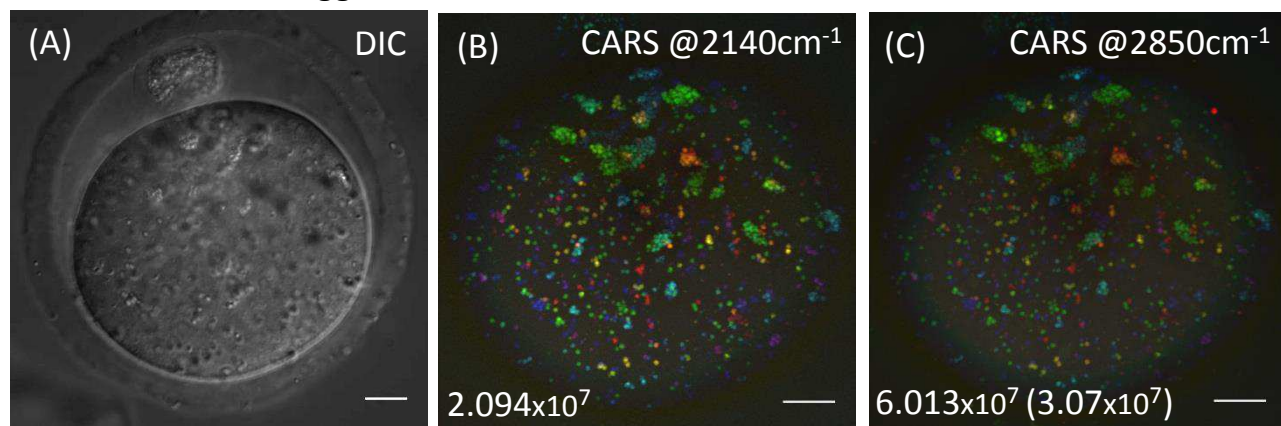


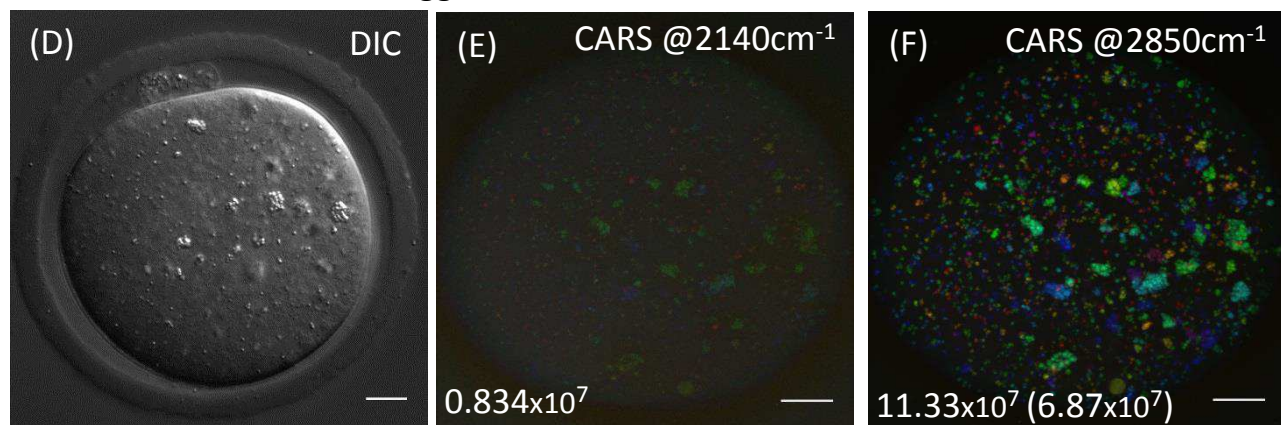
Fig.2



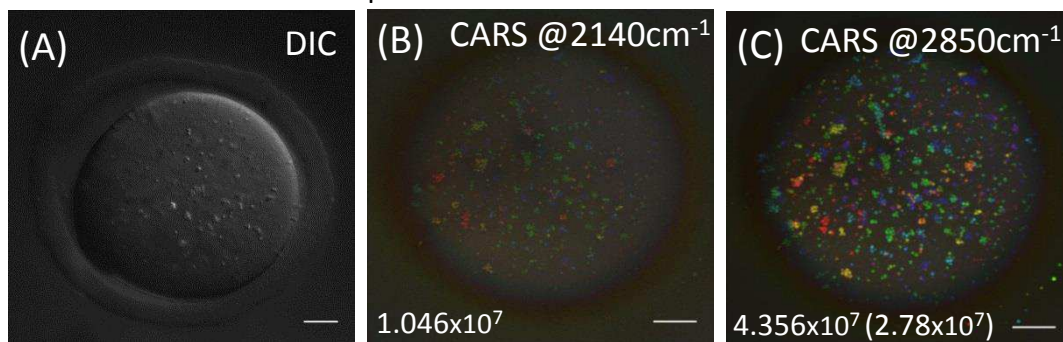
D-OA-matured MII Egg at time 0



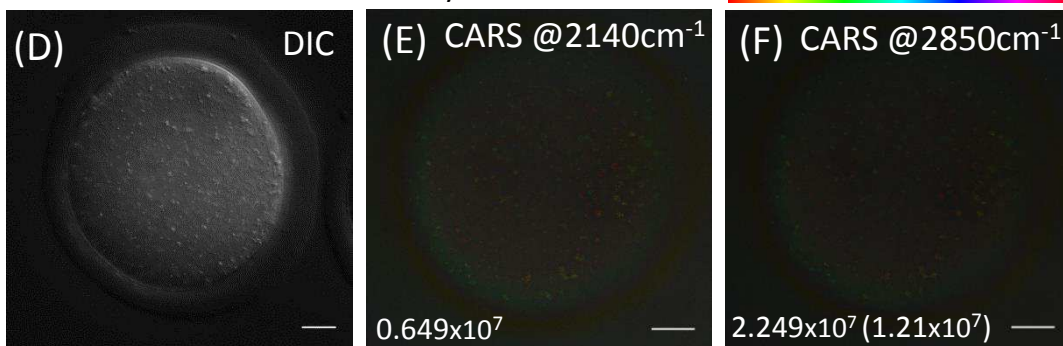
No D-OA IVM Control MII Egg



D-OA loss after ~6hrs in simple M2 medium



D-OA loss after ~6hrs with carbohydrate starvation



No D-OA loss after ~6hrs with β -oxidation-inhibition

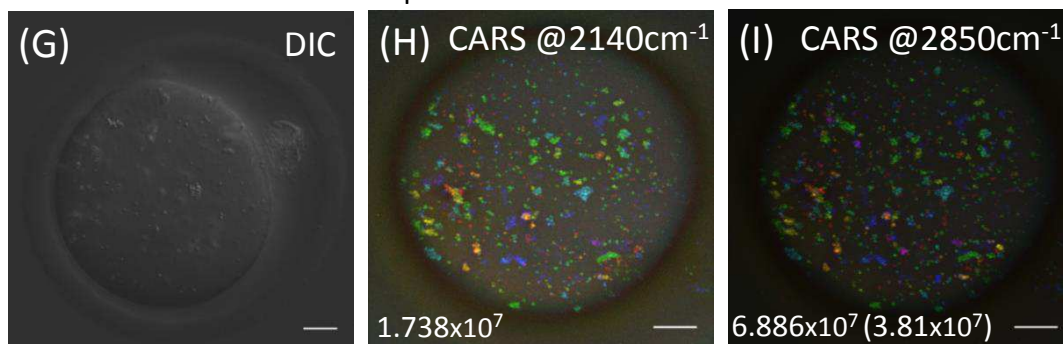


Fig.5

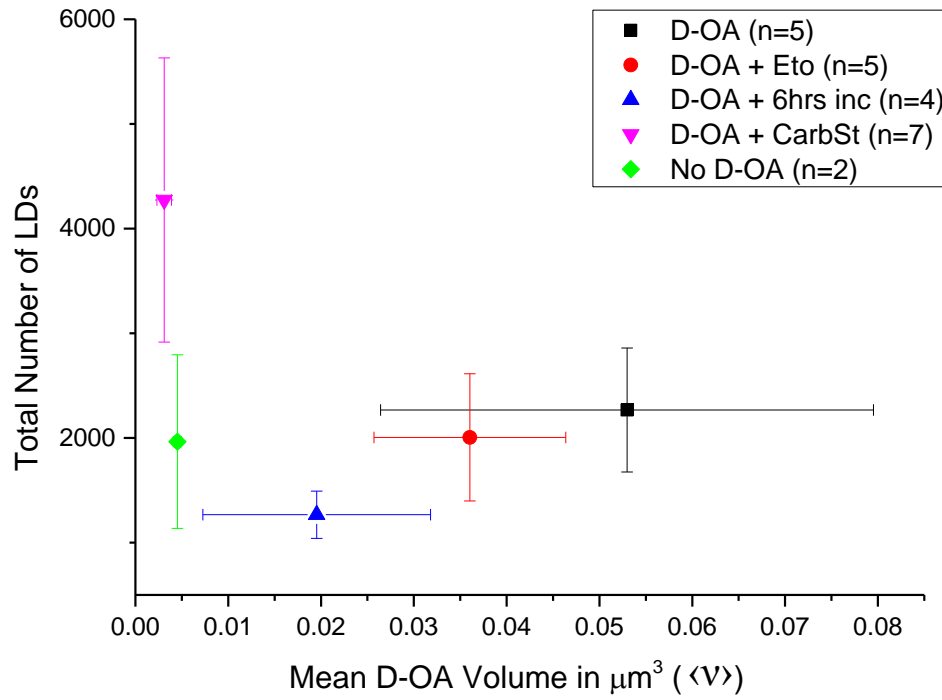
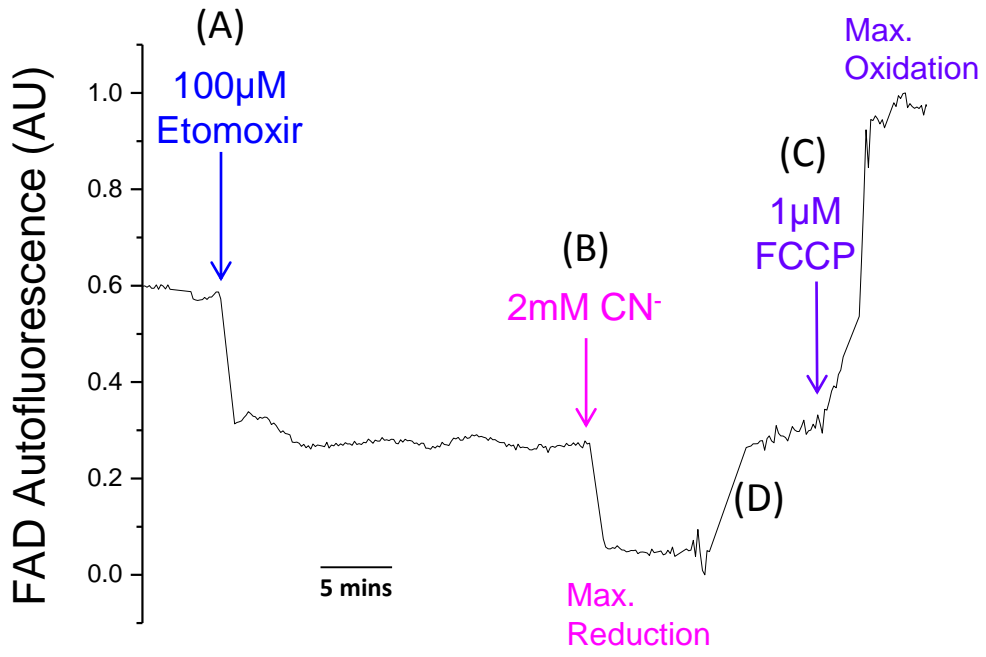
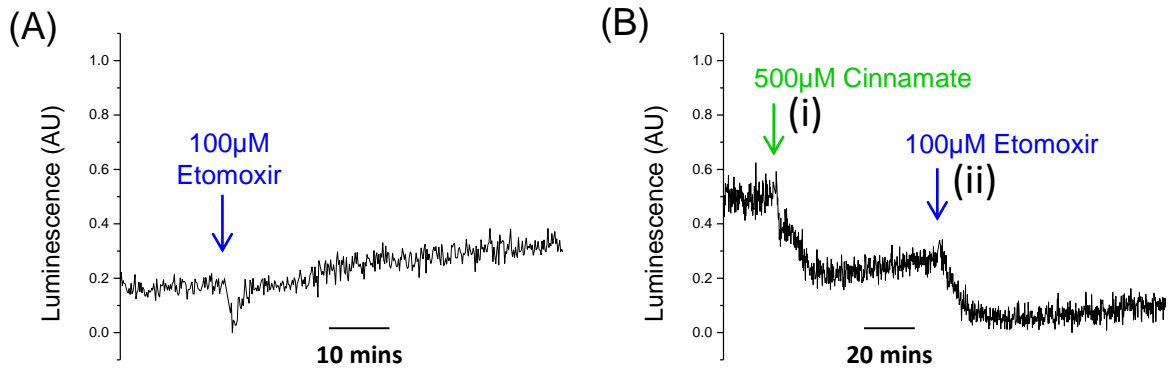
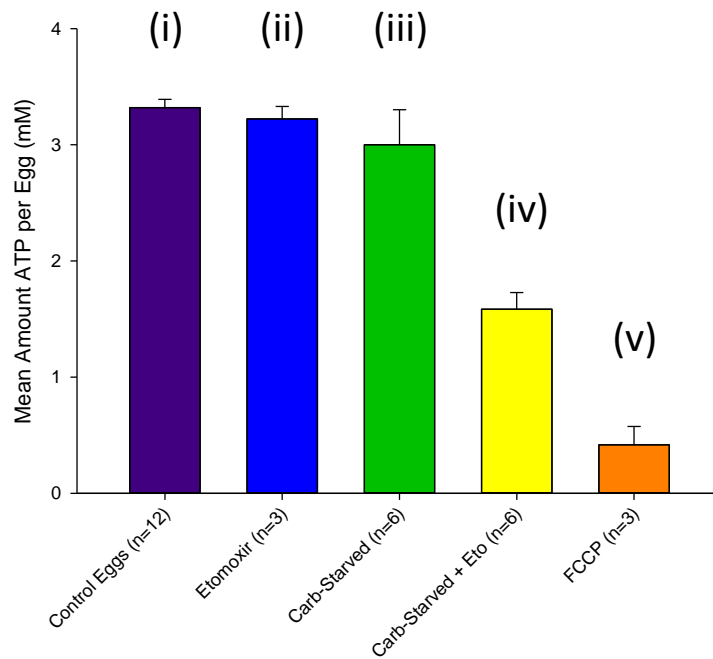


Fig.6





(C) Luminescence Assay of Whole Cell ATP



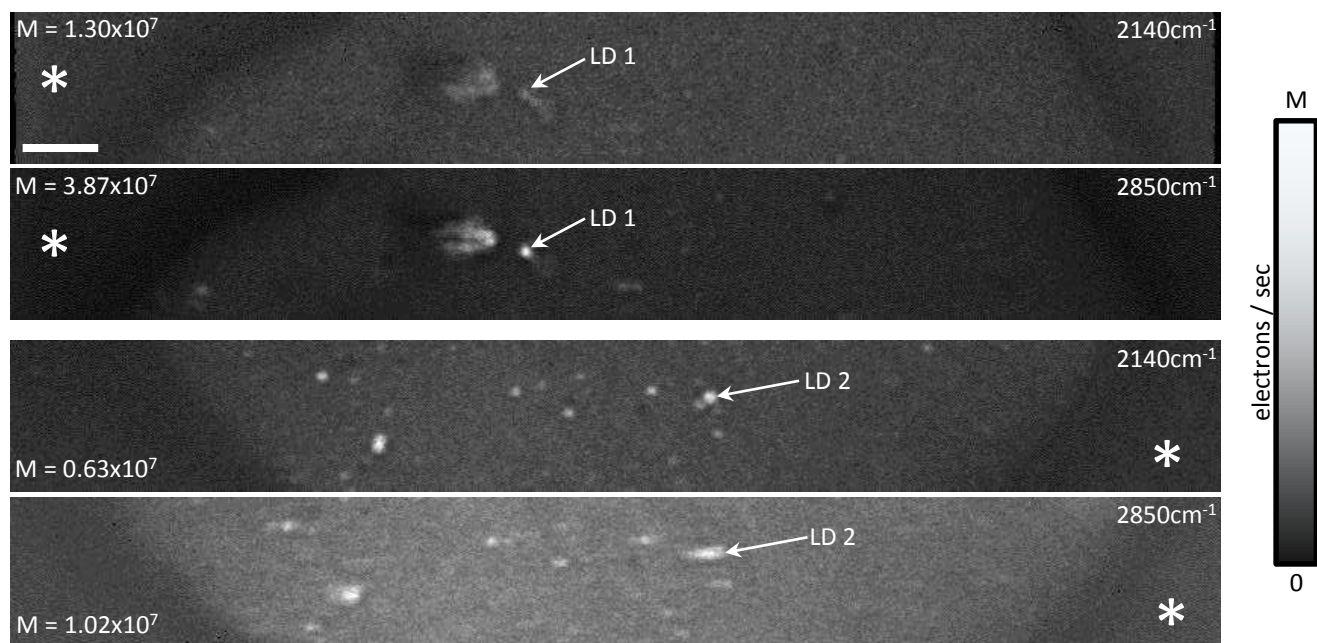
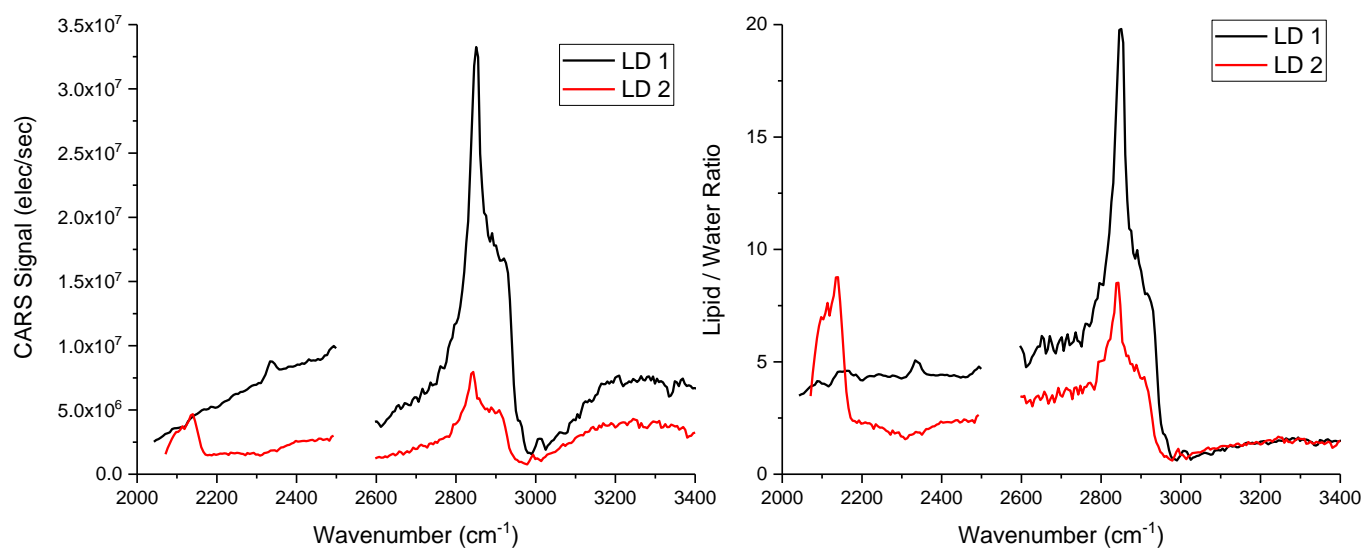


Figure S1. Lipid droplet spectrum (averaged over a single droplet, as indicated in images) taken from two different MII eggs incubated in etomoxir for 6hrs after incubation with D-OA. Graph top left, CARS signal averaged across lipid droplet. Graph top right, lipid/water ratio. Water signal was averaged in the region identified by the * in images. CARS images at the frequencies 2140 cm⁻¹ and 2850 cm⁻¹ showing the location of the lipid droplets used to plot the spectra in the above graphs. M indicates the grey scale range, in electrons per second, over which the images are plotted. Scale bar equals 5 microns.

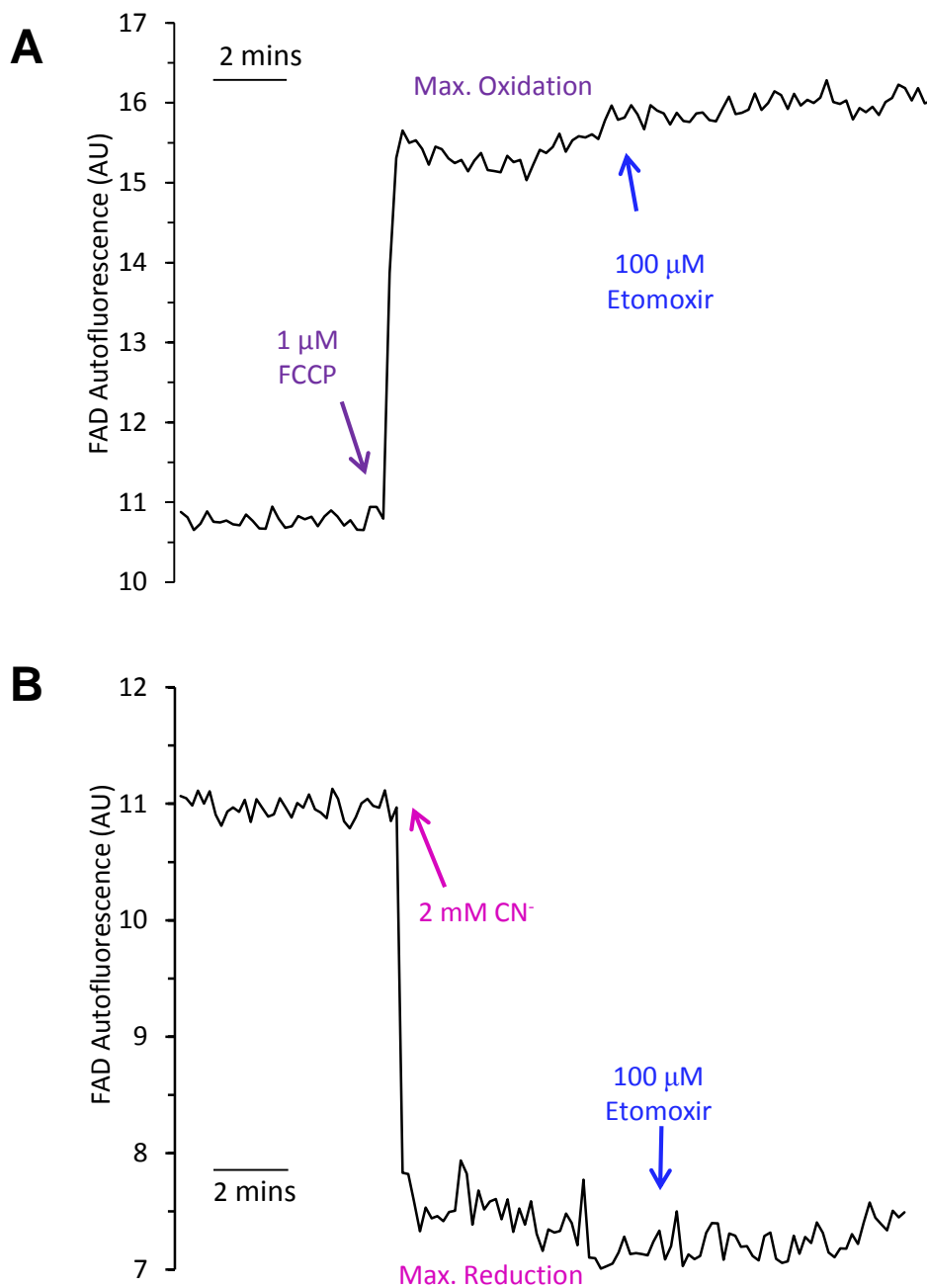


Figure S2. Etomoxir on FAD autofluorescence in mouse eggs.

Mitochondrial FAD autofluorescence signals in MII eggs incubated and imaged in standard HKSOM media. In **A** the eggs were subjected to exposed 1 μ M FCCP to cause maximum oxidation, followed by addition of 100 μ M etomoxir ($n = 9$). In **B** the addition of 2mM cyanide was added to cause maximum reduction, followed by addition of 100 μ M etomoxir ($n=9$). All data is representative of multiple trials, using 2-3 mice for each. In neither type of experiment did etomoxir cause a change in autofluorescence.

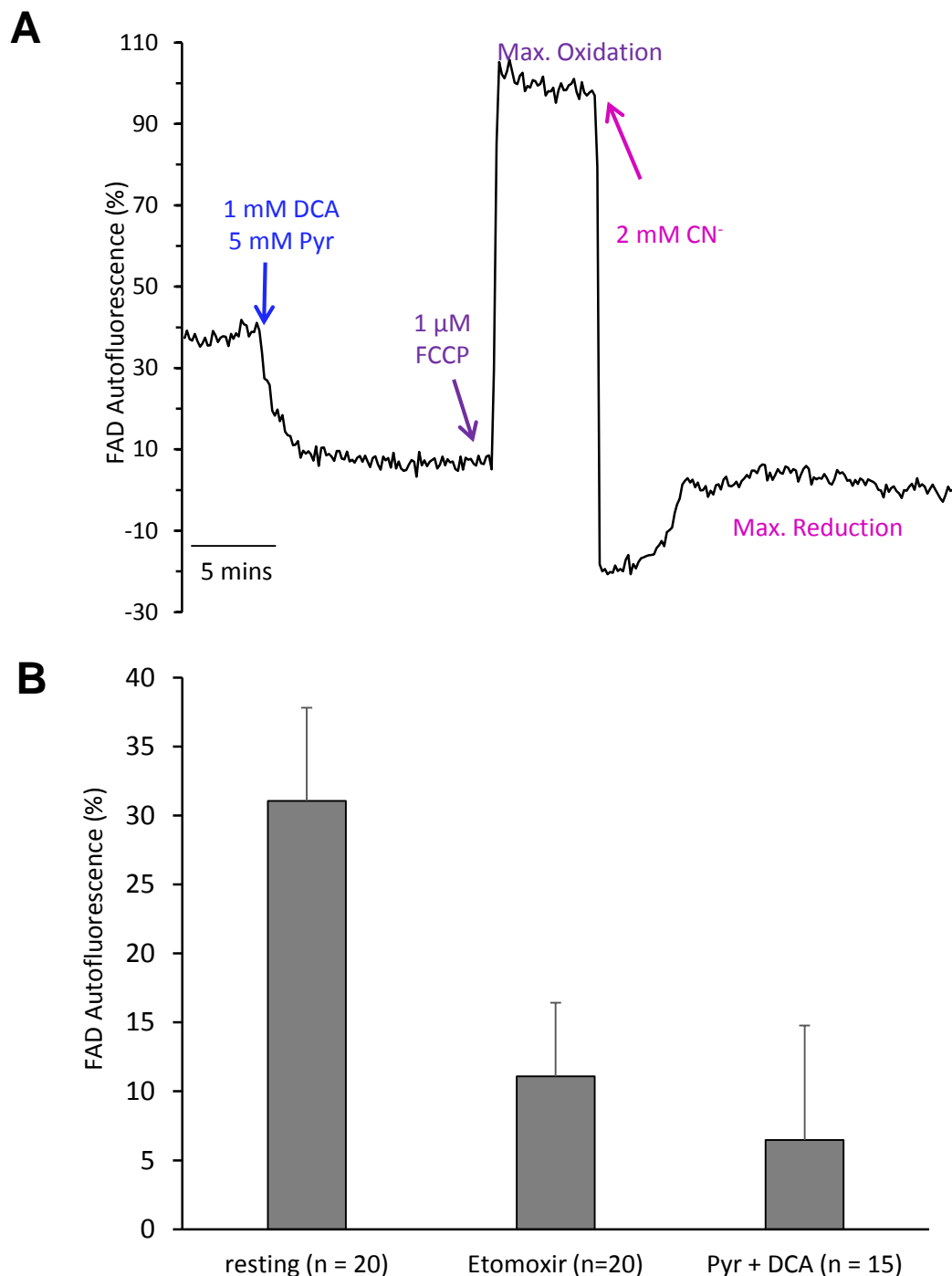


Figure S3. Effects of Pyruvate Intake On Mitochondrial Redox Potential of FAD in MII eggs.

In **A** the mitochondrial FAD autofluorescence signal was measured from MII eggs in standard HKSOM media. Then eggs were exposed to 1 mM dichloroacetate (DCA) plus 5 mM pyruvate which caused a decrease in FAD autofluorescence. This was followed by maximum oxidation (1 μ M FCCP) and induction of maximum reduction (2 mM cyanide) (n=15). Data is representative of multiple trials, using 2-3 mice for each. In **B** the average mitochondrial redox potentials are shown based upon FAD auto-fluorescence measurements, all eggs were incubated and imaged in standard HKSOM media. The resting state is that seen at the start of the experimeny, then the average is shown for eggs treated with 100 μ M etomoxir, and finally the level is shown for that seen after adding 1 mM DCA and 5 mM pyruvate.