Supplementary Information

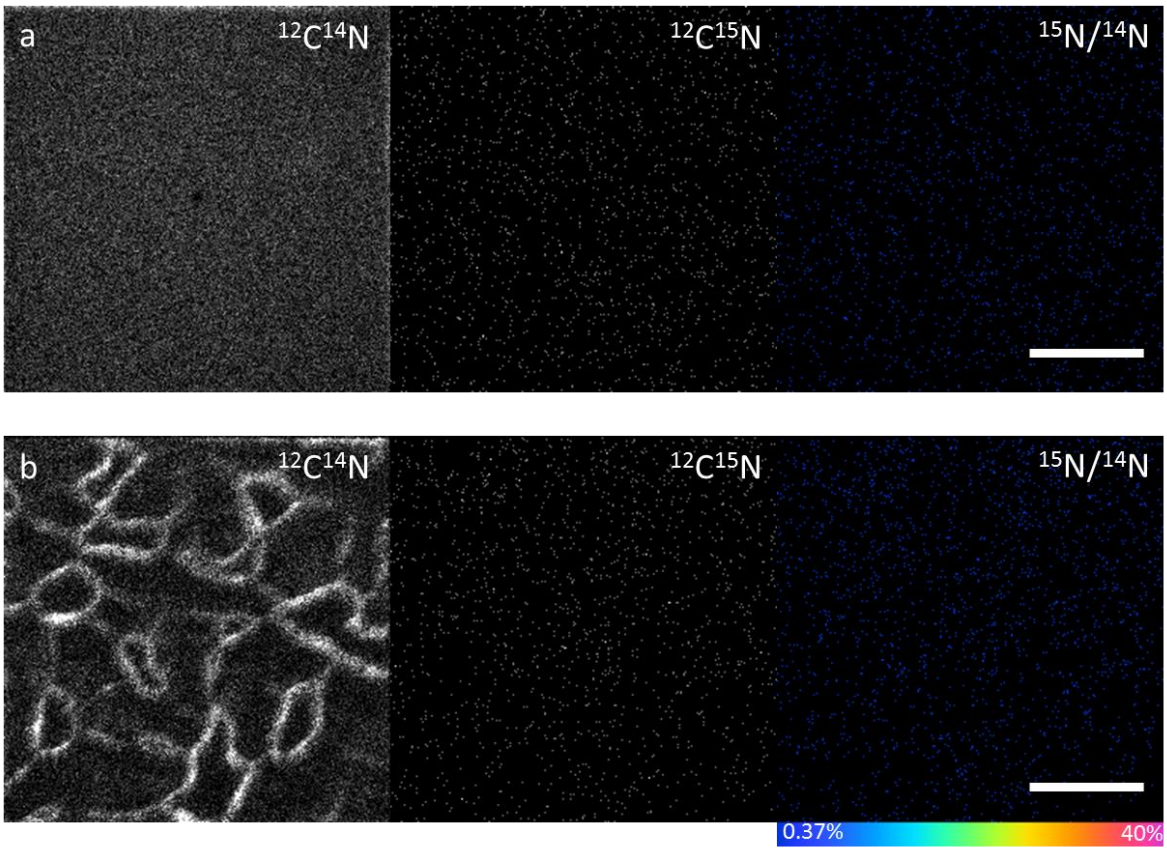

Figure S1. NanoSIMS images of 2 control samples; bare lipid bilayers and unlabelled amhelin treated lipid bilayers. (a) $^{12}C^{14}N^-$, $^{12}C^{15}N^-$ and $^{15}N/^{14}N$ HSI images of a 10 µm × 10 µm area of bare lipid bilayers showing a featureless surface and very low signal from contamination on the surface. (b) $^{12}C^{14}N^-$, $^{12}C^{15}N^-$ and $^{15}N/^{14}N$ HSI images of a 10 µm × 10 µm area of unlabelled amhelin treated lipid bilayers showing pore-like features and low $^{12}C^{15}N^-$ signals. Both $^{15}N/^{14}N$ HSI images show a natural abundance of the ^{15}N, 0.37%. Scale of $^{12}C^{15}N^-$ images: 0 – 1. Scale bar: 3 µm. ratio image: 0.37% – 40%.

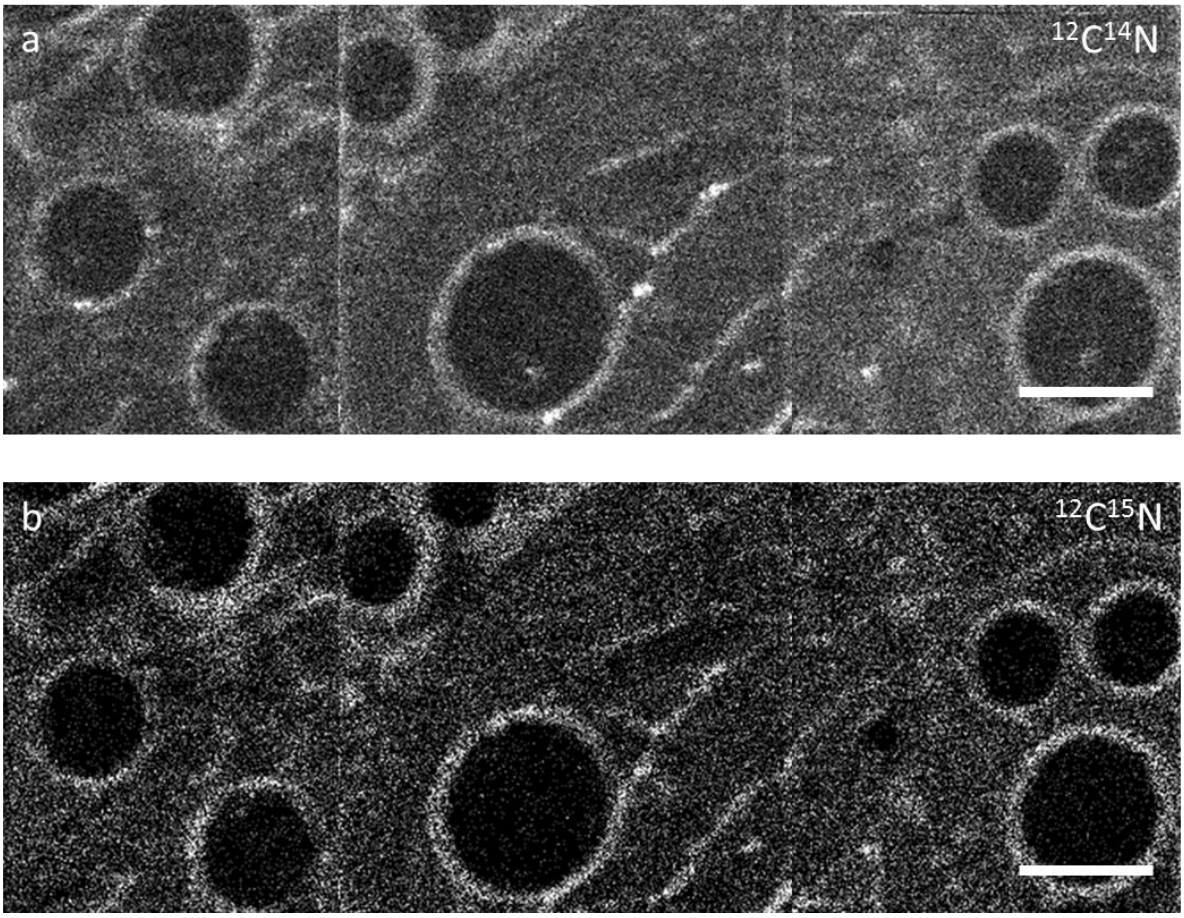

Figure S2. NanoSIMS images of ^{15}N-amhelin treated lipid bilayers. (a, b) $^{12}C^{14}N^-$ and $^{12}C^{15}N^-$ NanoSIMS images from the same area as Fig 2 showing high signals from the pore edges and filament-like features, and low signals inside the pores. Scale of $^{12}C^{14}N^-$ image: 0 – 15. Scale of $^{12}C^{15}N^-$ image: 0 – 3. Scale bar: 3 µm.

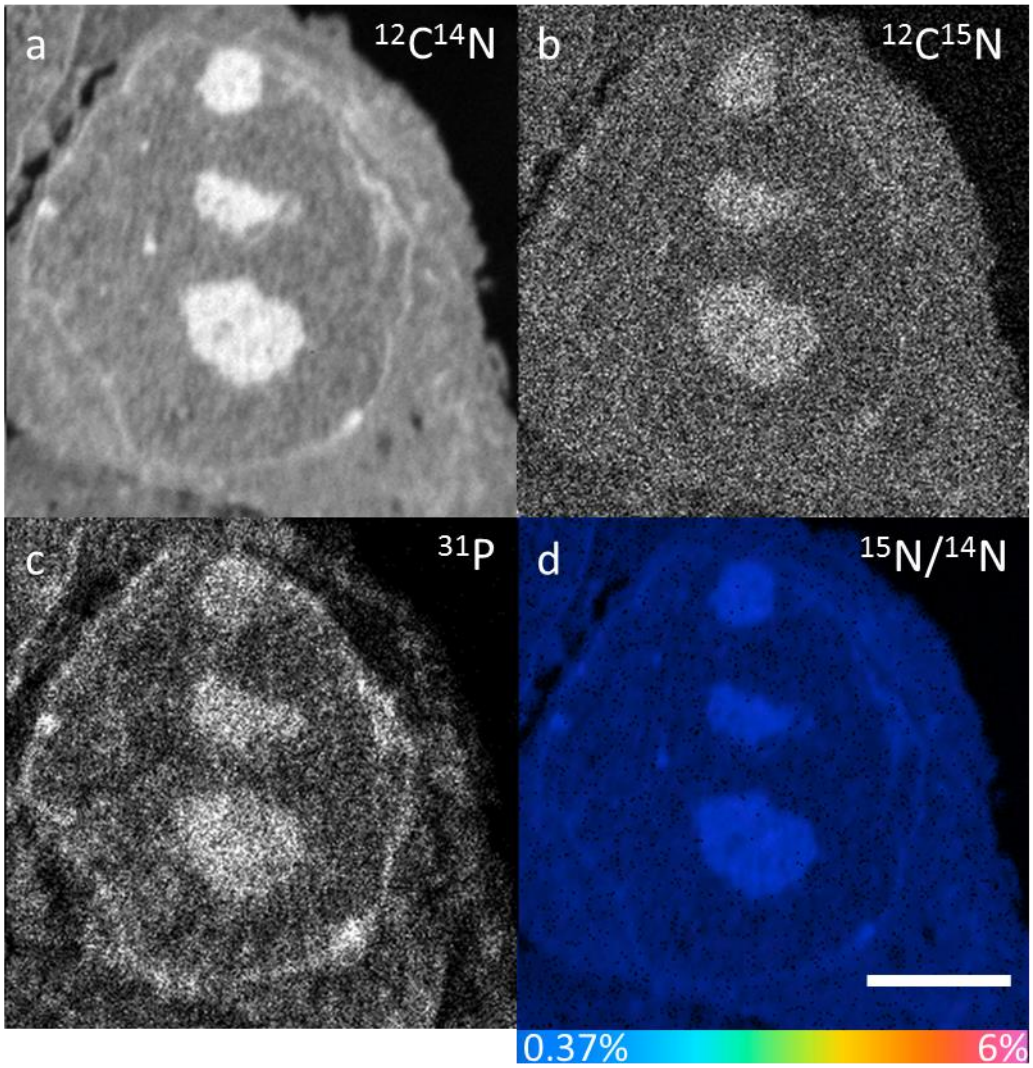

Figure S3. NanoSIMS images on a control MCF7 cell. (a, b, c, d) $^{12}C^{14}N^{-}$, $^{12}C^{15}N^{-}$, $^{31}P^{-}$ and $^{15}N/^{14}N$ HSI images of a cell cultured without isotope labelling. The nucleoli, nucleus membrane and cytoplasm are shown in the images. $^{15}N/^{14}N$ ratio image shows the colour characteristic of the natural background concentration of ^{15}N. Scale bar: 4 µm. Colour scale of $^{15}N/^{14}N$ image: 0.37% – 6%.

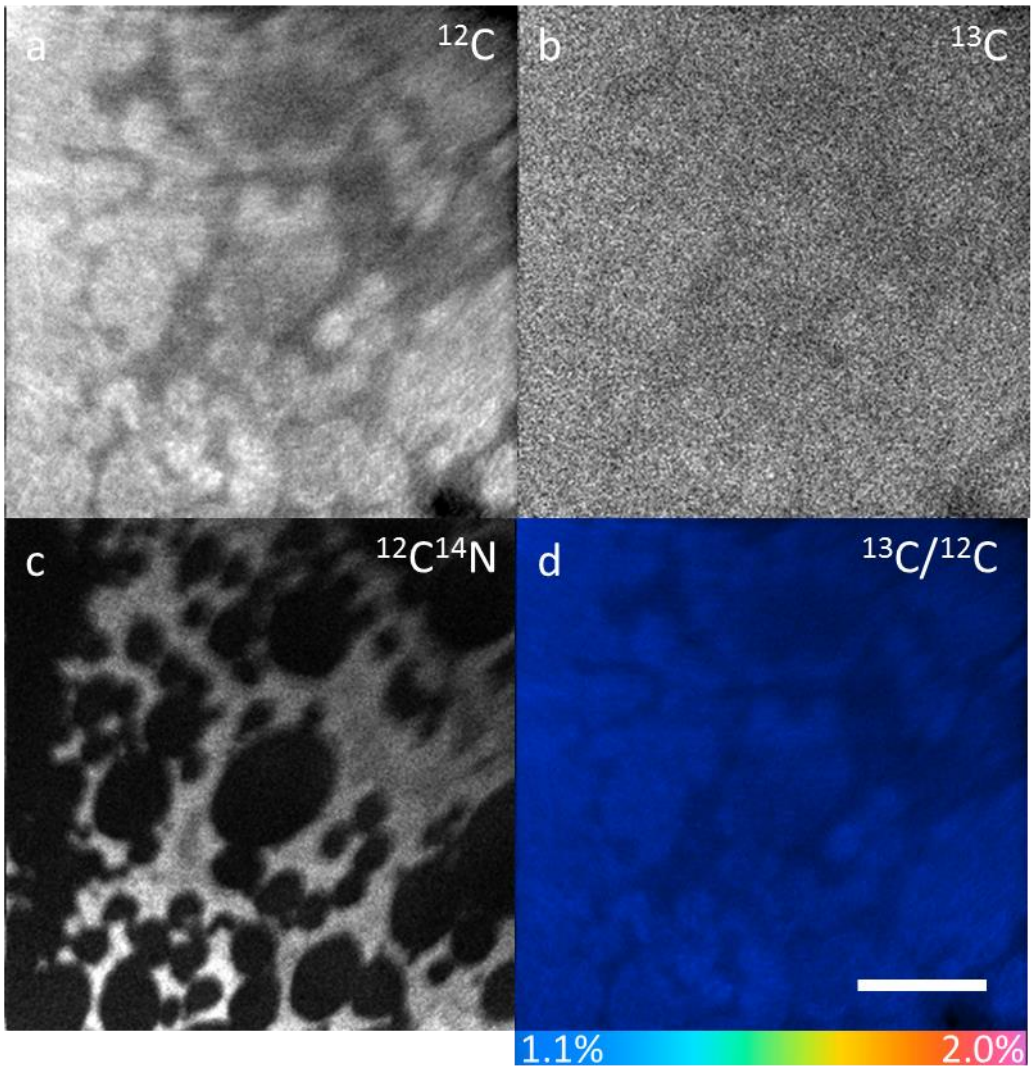

Figure S4. NanoSIMS images on control BAT sample. (a, b, c, d) Low-magnification $^{12}C^-$, $^{13}C^-$, $^{12}C^{14}N^-$ and $^{13}C/^{12}C$ HSI images of a 40 µm × 40 µm area of brown adipose tissue from a wild-type mouse after 4 days of feeding with a diet containing no extra ^{13}C. The $^{13}C/^{12}C$ HSI image shows the natural abundance of ^{13}C over the whole area (1.1%). Scale bar: 10 µm. Colour scale of $^{13}C/^{12}C$ ratio images: 1.1% – 2.0%.

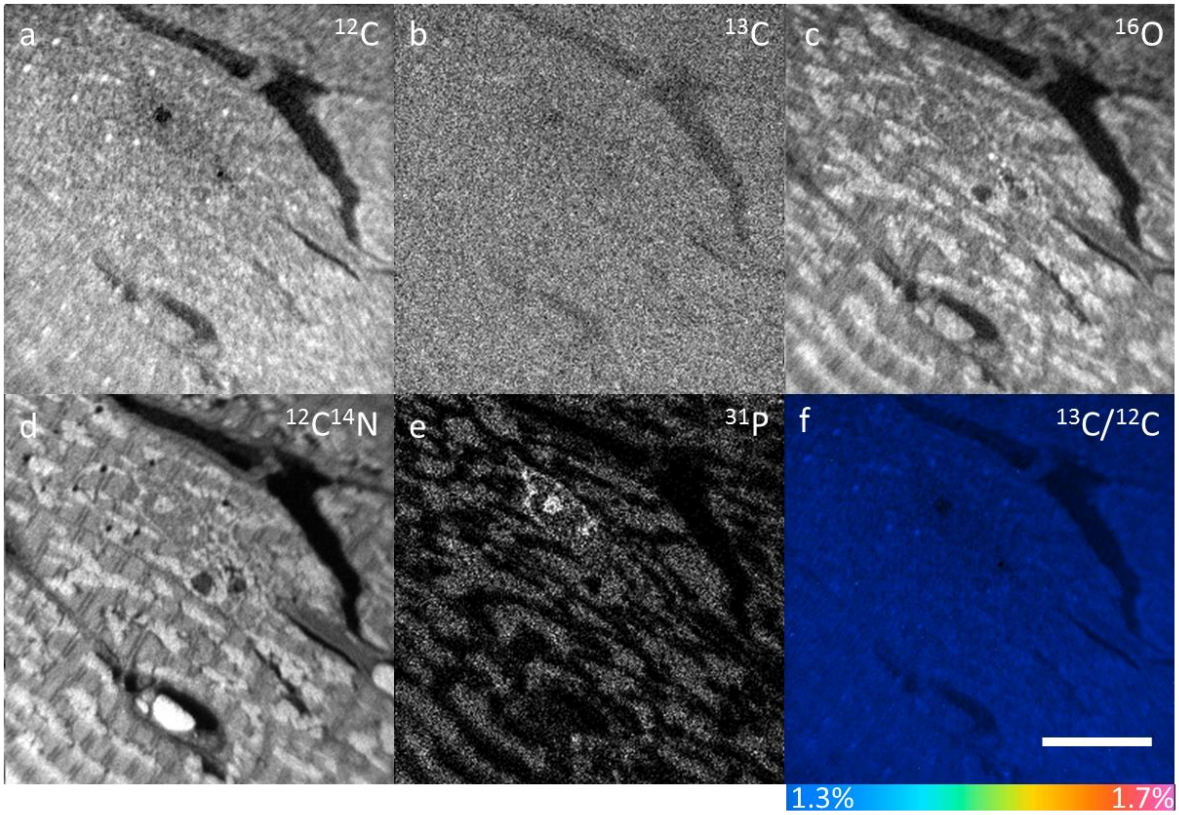

Figure S5. NanoSIMS images on control mouse heart sample. (a, b, c, d, e, f) Low-magnification $^{12}C^-$, $^{13}C^-$, $^{16}O^-$, $^{12}C^{14}N^-$, $^{31}P^-$ and $^{13}C/^{12}C$ HSI images of a 35 µm × 35 µm area of heart tissue from a wild-type mouse after 4 days of feeding with a diet containing no extra ^{13}C. The $^{13}C/^{12}C$ HSI image shows the natural abundance of ^{13}C over the whole area (1.1%). Scale bar: 10 µm. Colour scale of $^{13}C/^{12}C$ ratio images: 1.3% – 1.7%.