SUPPLEMENTARY INFORMATION

Quantitative optical trapping on single cellular organelles in cell extract

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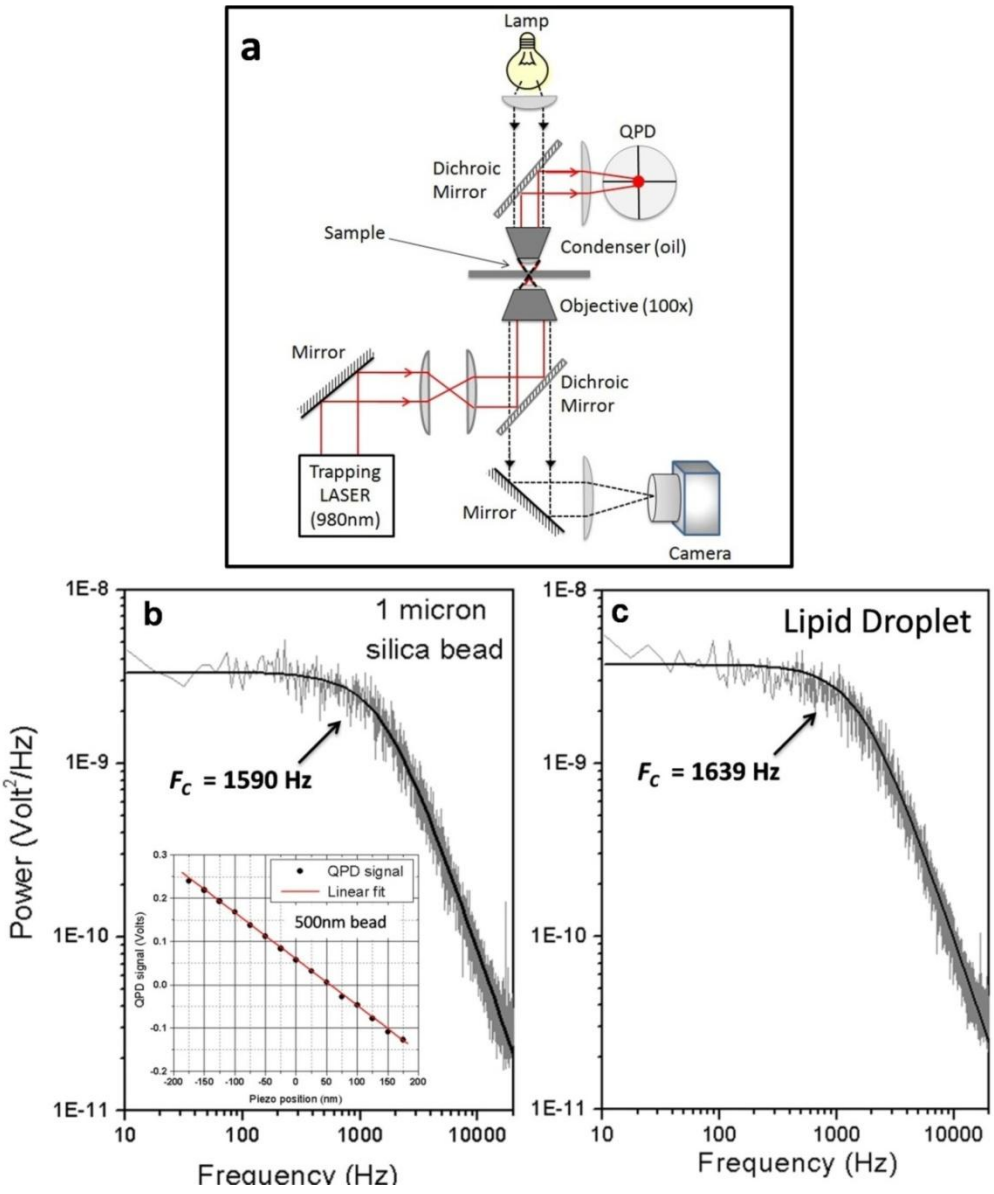
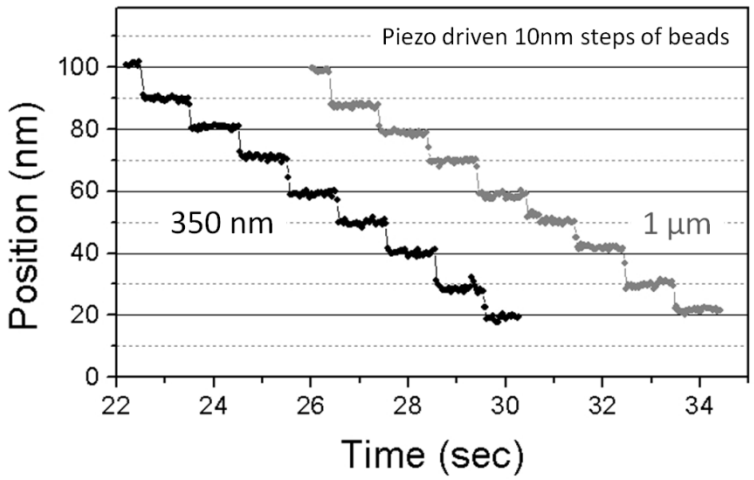
SUPPLEMENTARY FIGURE 1 The optical trapping and detection setup with typical power spectra.

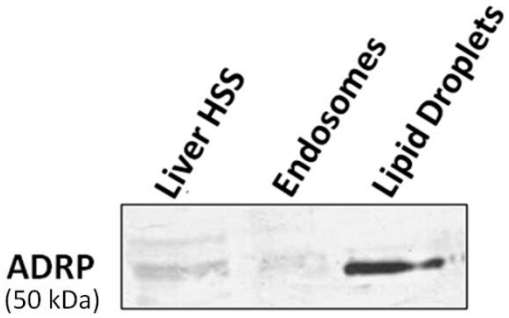
(a) Schematic of trap and quadrant photodiode detection **(b)** Power spectra for a bead and **(c)** for a lipid droplet. Both data fit well to a Lorentzian function (solid lines) showing that the trap functions as a harmonic (linear) spring for the bead and LD. The corner frequency (F_C) is indicated. **Inset** shows the quadrant photodiode (QPD) signal as a function of position of a 500nm bead that was stuck to a coverslip surface, and moved in increments of 25nm using a piezo stage. The QPD signal is linear to a distance of ±160nm from the trap centre (zero position). The linearity extends further for beads of larger size.



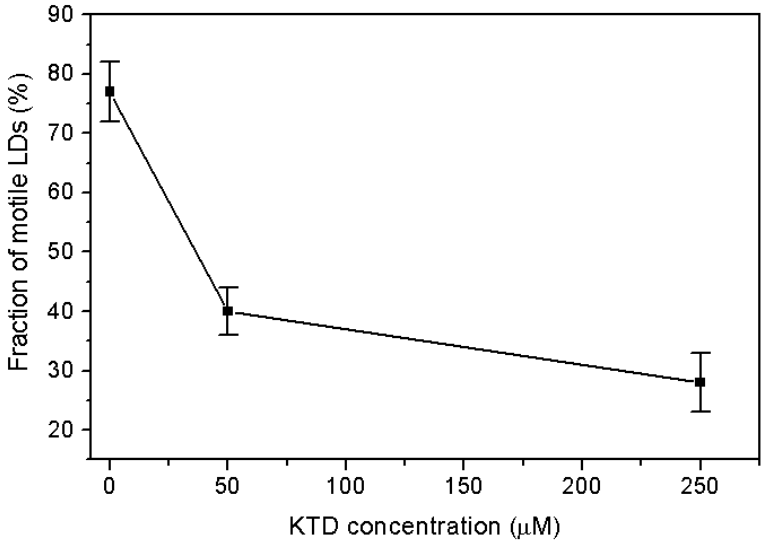
SUPPLEMENTARY FIGURE 2 **Video-tracking based position detection is insensitive to the object size.** Beads of 350nm and 1 micron diameter were stuck to a coverslip in the same field of view. The coverslip was then moved in steps of 10nm using a piezoelectric stage. The motion was recorded as a movie and video tracked. Clear 10nm steps can be observed for both the beads, establishing the accuracy and sub-pixel resolution of video tracking across this range of object size. Most LDs investigated for force measurement were visually selected to be within a size range of 400nm – 2microns.



SUPPLEMENTARY FIGURE 3 **The motile fraction containing organelles is enriched in lipid droplets.** Adipocyte differentiation related protein (ADRP; an established marker for liver LDs) is detected specifically in the buoyant top fraction after a sucrose step gradient (Supplementary information 1.6). This fraction contains numerous highly refractile spherical organelles (Upper inset, FIG 2a) which display motility on microtubules. Liver cytosol (HSS) and a vesicle fraction containing mostly endosomes did not show detectable levels of ADRP.

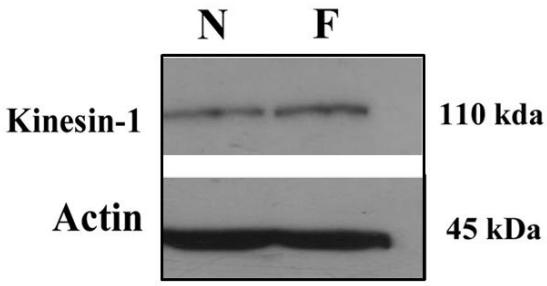


SUPPLEMENTARY FIGURE 4 A kinesin-1 specific inhibitor inhibits LD motion. Fraction of LDs moving against the optical trap in presence of kinesin tail domain (KTD) peptide, a specific inhibitor of kinesin-1 activity. About 75% LDs moved against the trap in untreated LDs. The mentioned KTD concentration is that in the final motility buffer as well as during incubation.



SUPPLEMENTARY FIGURE 5 There is no significant difference in kinesin-1 in liver upon fasting.

Liver extract from normal (N) or 16-hour fasted (F) rats was probed for kinesin-1 and actin (loading control). No significant change in kinesin-1 level could be detected upon fasting.



SUPPLEMENTARY NOTES

Caveats with optical trapping inside cells

To address the obvious limitations of optical trap assays with purified motors coated on artificial beads, optical trapping has been reported on lipid droplets inside *Drosophila melanogaster* embryos[4-5,7] and in cultured mammalian cells[6]. However, there are significant technical hurdles to such experiments. For in vitro experiments the trapped bead is initially diffusing around in solution, and gets centered in the trap automatically before it is placed on a microtubule or actin filament. In contrast, in vivo trapping requires a cargo already attached to a microtubule and moving at ~1 micron/sec along a complicated trajectory to be centered rapidly in an optical trap with a precision of ~5nm. This is a technically challenging task, which we believe has not been addressed completely in earlier studies. If the trap is not centered precisely, the assumption of a linear harmonic spring is invalid. Further, the force exerted by a trap depends on size of the trapped intracellular cargo. This size cannot be determined precisely, and trap calibration methods for variable-sized cargo (like VMatch) cannot be applied inside cells (see main text). These difficulties may have caused disagreements between reports[4-7] on the in vivo force of kinesin-1. The reported in vivo forces (1-3pN) are also significantly smaller than the vitro force of kinesin[2] (=5-8pN; depending on species). We have overcome some of these difficulties in a specific model system, namely the long-distance robust transport of latex bead phagosomes inside macrophage cells. This model system has two critical advantages which were not available in earlier in vivo studies. First, the long-distance linear transport of latex bead phagosomes inside cells which allowed us to reliably identify stalls where the phagosome was precisely centered in the trap by observing its trajectory before, during and after stall. Second, the uniform size of latex bead phagosomes circumvented problems of trap stiffness variation with cargo size. We foresee continuing technical difficulties on a wider variety of in vivo model systems unless the trap centering issue is addressed carefully.

Advantages of force-measurement on organelles in cell extract

i. *Motors existing in a native-like state on the membrane of a real cellular cargo can be probed.* A native-like state of motors is likely if properties of motion of the cargo inside cells (e.g. velocity, distance moved etc.) is similar to that observed in cell-extract. This has been demonstrated recently by us for endosomes extracted from *Dictyostelium discoideum*[8].

ii. *Problems related to trap-centering are eliminated.* It is difficult to center an optical trap on an intracellular cargo and on the filament on which it is moving. In cell extract, freely diffusing organelles in solution get automatically centered when they are trapped, and can then be lowered onto a MT or actin filament to study force generation. This procedure is similar to bead assays[2], and therefore the centering is equally precise.

iii. *Motor(s) driving a particular organelle can be identified.* It is not easy to identify the major motor(s) driving motion of a specific organelle inside cells. This is usually done by RNAi-knockdown of motors or by introducing motor-specific function blocking antibodies into cells. However, such treatment could lead to general arrest of motion with organelles of different

kinds crowding the MTs, whereupon motility of the organelle of concern could be inhibited even if it is driven by a different motor. This problem should be of lesser concern in a diluted cell extract containing largely organelles of one kind (see later).

iv. *Novel motor-associated proteins can be identified.* If the motor(s) driving motion of an organelle can be identified (see above), motor-associated proteins can be pulled down using co-immunoprecipitations (co-IPs) with an antibody against the motor. If co-IPs are done with the purified organelle as sample, background is reduced and interactions specific to the organelle membrane can be deduced.

v. *Motor-regulators can be identified and their function understood at single-cargo level*. Adding peptides that mimic the motor-binding domain of motor-associated proteins can play a dominant-negative role, thereby helping to assay the functional role of motor co-factors.

vi. *Cell extract assays could yield information that is more physiologically relevant than measurements in cultured cells.* Though counter-intuitive, this is possible if organelles extracted directly from living animals are used for motility/force-measurement. Animals subjected to specific metabolic conditions can be used to understand the connections between metabolic state and motor function in high-resolution biophysical assays.

VMatch compared to other methods of optical trap calibration

To the best of our knowledge, quantitative force-measurement at single-motor resolution has not been reported on organelles in cell extract. Several other methods that could be used to calibrate an optical trap for objects of unknown size have been published. However, all these reports have used beads of different known sizes to demonstrate their calibration. None of the methods has actually reported measurement of motor-forces on motor-coated beads or on organelles extracted from cells (as demonstrated here by VMatch). We compare some of the published methods to VMatch below.

Camera based methods CMOS video cameras have been used to directly measure (in distance units) the thermal fluctuations for calibration of optical traps[27-29]. These studies are only able to demonstrate the calibration of weak traps (low corner frequency of ~100 Hz or less). For measurement of motor forces, an optical trap is required to be quite stiff and has higher corner frequency (for example, 1.6 KHz for lipid droplets; **Supplementary Fig 1**). This is because the trap must be strong enough to measure upto ~10-15 pico Newton force generated by multiple motors such as kinesin-1. CMOS cameras will undersample the thermal fluctuations in such a situation to inflate the variance, and will not allow correct calibration of the trap. This problem does not arise in VMatch because a QPD is used at 40KHz to sample the fluctuations.

Moving-trap method It is possible to move the optical trap by a known distance across the stationary sample using acousto-optic deflectors (AODs) or galvanometer mirrors. The corresponding response on the QPD can be measured to obtain a QPD calibration factor[14]. The amplitude of trap displacement is ~1.5 microns. The trap must be typically swept across 5-6 times to complete the calibration. Thus, the optical trap effectively samples a distance of about 10 microns in the trapping solution before calibration can be completed. In our experience, moving the

trap over such a long distance in a cell extract always leads to small cytosolic particles (debris) getting trapped. These particles introduce a variable error of 20-30% in calibration of the QPD position. This is a unique problem when working with cell extract (where these particles cannot be avoided), and has not been realized by the papers which simplify the optical trap calibration by using beads in a purified (debris-free) buffer solution. VMatch does not require the trap to be moved, and therefore this problem does not arise.

Further, in the work by Vermeluen et al[14], it is observed that if the trap position is moved over a distance larger than the bead diameter then the results are less reproducible. This may be because of free diffusion of the bead during periods when the trap has moved beyond radius of the bead. Many cellular organelles (lipid droplets, pigment granules, endosomes) are typically 0.5 microns in diameter. It is difficult to use an AOD to reliably displace the laser beam over such a small distance (the required input voltage is too small). Therefore, the Vermeluen method cannot be used on most sub-micron cellular organelles, which are free to diffuse when the trap is at its extremal position, and will therefore result in wrong QPD calibration factors. This problem has been discussed by Vermeulen et al[15]. In contrast, VMatch works very well for organelles from 0.3 microns upwards (see results in the present work).

Combined power spectrum and sinusoidal motion Another method for trap calibration[15] measures the power spectrum of a trapped bead while simultaneously moving the surrounding medium in a sinusoidal pattern using a piezo stage. As mentioned earlier, for measurements in cell extract there are always small particles of varied shape and size (cytosolic debris) in the solution. This precludes the power spectra type of measurements because constructing a reliable power spectrum (that can be fitted to a Lorentzian function) requires a recording of 30-40 seconds of thermal fluctuation of a single particle. When we tried this experiment in cell extract, small particles invariably got trapped (along with the lipid droplet) as the power spectrum was being taken. The problem was compounded because of the motion of the surrounding medium which contained particles that moved into the trap. This distorted the power spectrum, indicating that the trap was now not functioning as a linear spring.

We believe that VMatch is much simpler compared to the other methods. VMatch does not require any complicated mathematics or additional instrumentation (e.g. expensive AODs, an additional set of optical elements such as laser/mirrors/lenses for a second detection laser). Since ready-made commercial optical traps are now available, VMatch brings to interested workers the possibility of functionally interrogating native-like motor complexes in a controlled in vitro environment.

REFERENCES IN SUPPLEMENTARY NOTES

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