Supplementary Methods

TMT-Mass Spectrometry Methods

Each biological replicate (i.e. All 6 samples - M, L, H in 21% and 1% O_2) was performed in a single run where each sample was tagged with a separate label. Corresponding isobaric labels of each sample can be found in cells BO2:CF2 of Source Data for Figure 1 - Sheet "All". The proteins were reduced in 1 mM DTT for 1 hour at 56 °C and the free cysteine residues were alkylated using iodoacetamide. The proteins were precipitated with 5 volumes of acetone overnight at 20 °C and centrifugation. The proteins were digested according to the TMT 10-plex manual, but using Trypsin/LysC (Promega). 50 µg of protein from each condition was labeled using 0.4 mg of TMT 10-plex (ThermoFisher) by incubating at room temperature for 1 h. The labeling reaction was stopped using 5% hydroxylamine. The peptides were mixed and the solvent removed under vacuum. The pellet was resuspended and were analyzed on an Orbitrap analyzer (Q-Exactive, ThermoFisher, San Jose, CA) outfitted with a nanospray source and EASY-nLC nano-LC system (ThermoFisher, San Jose, CA). Lyophilized peptide mixtures were dissolved in 0.1% formic acid and loaded onto a 75 µm x 50 cm PepMax RSLC EASY-Spray column filled with 2 µM C18 beads (ThermoFisher San, Jose CA) at a pressure of 800 Bar. Peptides were eluted over 240 min at a rate of 250 nl/min using a stepwise gradient (0%-4% Acetonitrile containing 0.1% Formic Acid over 2 min; 4%-28% Acetonitrile containing 0.1% Formic Acid over 226 min, 28%-95% Acetonitrile containing 0.1% Formic Acid over 2 minutes, constant 95% Acetonitrile containing 0.1% Formic Acid for 10 min). Peptides were introduced by nano-electrospray into the Q-Exactive mass spectrometer (Thermo-Fisher). The instrument method consisted of one MS full scan (525–1600 m/z) in the Orbitrap mass analyzer with an automatic gain control (AGC) target of 1e6, maximum ion injection time of 120 ms and a

resolution of 35 000 followed by 15 data-dependent MS/MS scans with a resolution of 35,000, an AGC target of 1e6, maximum ion time of 120 ms, and one microscan. The intensity threshold to trigger a MS/MS scan was set to an underfill ratio of 0.2%. Fragmentation occurred in the HCD trap with normalized collision energy set to 30 V. The dynamic exclusion was applied using a setting of 40 s. The raw data was searched against the Uniprot database using Proteome Discoverer (Version 2.2.0.388 ThermoFisher Scientific) which also extracted the quantitation data from the 10 TMT tags.

Quantification was carried out using SequestHT and Amanda 2.0 search engines. Unique and razor peptides were used, with a minimum peptide length of 6 amino acids, minimum 1 unique peptide, and average reporter signal to noise threshold of 10. False discovery rates of 0.01 (strict) and 0.05 (relaxed) for PSMs and peptides were used. Abundances were normalized to the same total peptide amount per channel and scaled so that the average abundance per protein and peptide was 100. The mean abundance was calculated for each protein from the three biological replicates, followed by the ratio between L/M and H/M in both normoxia and hypoxia. Two-tailed unpaired t-tests were performed on the L/M and H/M ratios within each oxygen concentration. The M fraction was used as reference in each condition because: 1) There should be stoichiometric amounts of 40S and 60S subunits; 2) To account for differences in total ribosome numbers/translation rates between conditions, we measured the distribution of RPs in L and H versus M (L/M and H/M) rather than the absolute amounts between hypoxia and normoxia (i.e. $H_{hypoxia}/H_{normoxia}$).