Figure S1. Blood Meal Does Not Alter Directionally Global Protein Levels in Hemocytes

Hemocytes from sugar fed and blood fed females were analyzed for potential blood meal-induced increase in total protein production using FUNCAT. (A) Click-it chemistry can be used to assess AHA incorporation and thus metabolic labeling of newly synthesized proteins in *An. gambiae* *in vivo*. Signal levels are marginal after a ten minute labeling period (top row) and are readily detectable after four hours of exposure to AHA (bottom row). (B) Confocal images of representative AHA-labelled hemocytes isolated from sugar fed and blood fed mosquitoes 24h post blood meal. Confocal maximum intensity projections are shown in A and B, blue, DAPI; green, AHA. Scale bar is 10µm. (C) Quantification of AHA incorporation was graphed as median with interquartile range; *n* = 93, 110, 77. In two out of three replicates, blood feeding led to significant albeit very small fold changes in protein production (Rep 1: 1.48 fold increase; Mann-Whitney U test, *P* <0.0001; Rep3: 1.39 fold decrease; Mann-Whitney U test, *P* = 0.001), while no statistically significant changes were observed in the second replicate (Rep 2: 1.07 fold increase; Mann-Whitney U test, *P* = 0.522).
Figure S2. Quantification of Hemocyte Activation

Hemocytes from sugar fed and blood fed females were analyzed for blood meal-induced activation markers. \( n = 91, 69, 121 \) for WGA (A), \( n = 150, 126, \) and 109 for pERK (B), \( n = 103, 120, \) and 86 for TEP-1 (C), and \( n = 180, 136, \) and 149 for PPO6 (D). Quantification of activation markers was done with Image J analysis to obtain relative fluorescence. Hemocyte activation experiments are done with three biological replicates. All are graphed as median with interquartile range and are statistically significantly different (Mann-Whitney U test, \( P < 0.0001 \)).
Figure S3. Syringe System Setup for Hemocyte Perfusion

(A) Syringe system (Hamilton Company, Reno, NV, USA) for hemocyte perfusion includes the following: (i) dispenser, (ii) 50 µl glass syringe, (iii) PEEK tubing, (iv) pulled glass needle (1mm outer diameter, 0.5mm inner diameter), (v) coupler between tubing and needle, and compression fittings between (vi) needle and coupler [1mm], (vii) coupler and tubing [1/16 inch], and (viii) syringe and tubing [1/16 inch]. (B) Priming kit to backfill glass syringe and whole system, process demonstrated in (C).