Supporting information

1. Dihydrolipoamide interferes with the peroxide assay

The reaction catalyzed by dihydrolipoamide dehydrogenase (E3) is:
dihydrolipoamide + $NAD^+ \Leftrightarrow$ lipoamide + NADH + H^+

The peroxide assay was performed as described in the main text, replacing *E. coli* E1 for dihydrolipoyl dehydrogenase (E3). Buffer containing horseradish peroxidase (HRP), Amplex Red and E3 was incubated for 10 minutes before the addition of substrate, 1 mM dihydrolipoamide. After addition of dihydrolipoamide, peroxide was rapidly detected (Figure S1, red curve labelled 'E3 + HH-lip). Control samples were prepared in which no E3 was added; 1 mM dihydrolipoamide is added to a buffered sample containing HRP and Amplex Red. This also resulted in the generation of resorufin that indicates the presence of peroxide (Figure S1, cyan curve labelled 'HH-lip').

It is apparent in Figure S1 that the HRP-Amplex Red assay is able to detect low potential electron carriers including dihydrolipoamide. NADH at high concentrations has a similar effect [Votyakova T. V.; Reynolds I. J., *Archives of Biochemistry and Biophysics* **2004**, *432*, 138-44]. Thus, this assay is not appropriate to test if E3 produces peroxide because the substrate of E3 interferes with the assay.

2. Cytochrome c is reduced by dihydrolipoamide

Arabidopsis thaliana cytochrome c6a (a generous gift from Dr Jonathan Worrall) was prepared in 50mM phosphate pH7 and oxidized with ferricyanide. The absorbance spectrum was then recorded (Figure S2, black curve). An excess of dihydrolipoamide was added to the oxidised cytochrome c6a and a second absorbance spectrum was immediately recorded. It is apparent in Figure S2 that cytochrome c6a has the characteristic absorbance of the ferrous form (peak at 550 nm), from which it may be inferred that it has become reduced by a single electron given by dihydrolipoamide.

3. Complete reference 42

Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J., J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.;

Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A.; Revision B.04 ed.; Gaussian, Inc: Pittsburgh PA, **2003**.

Figure S1

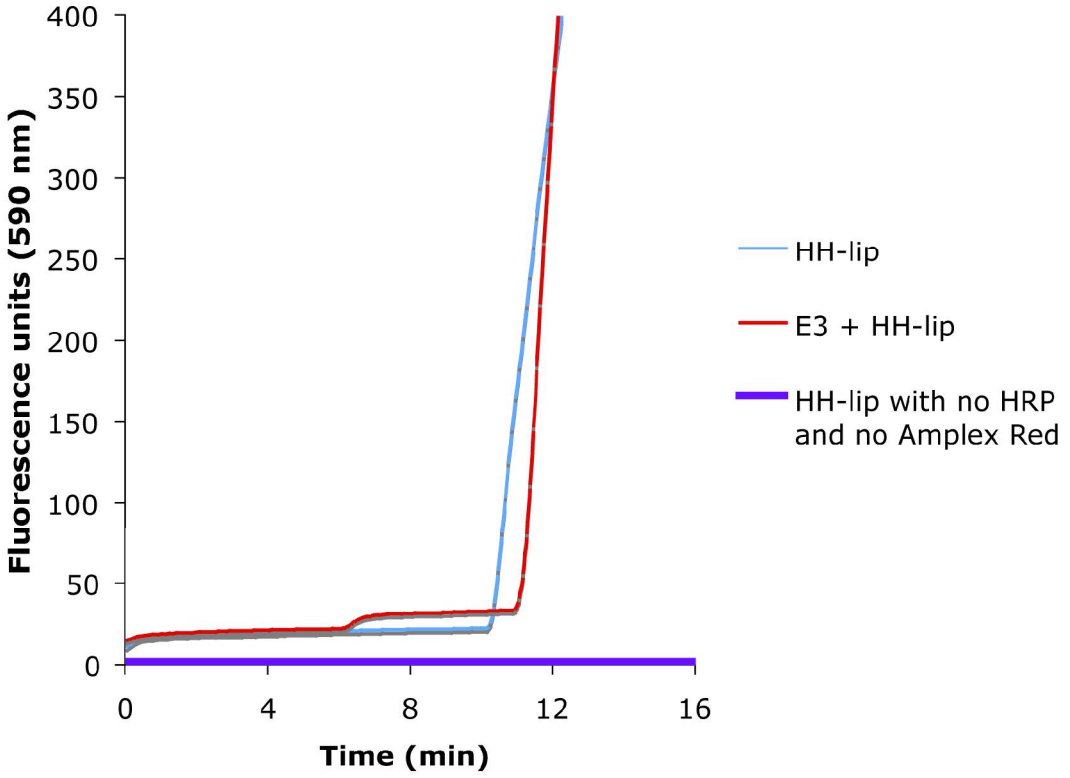
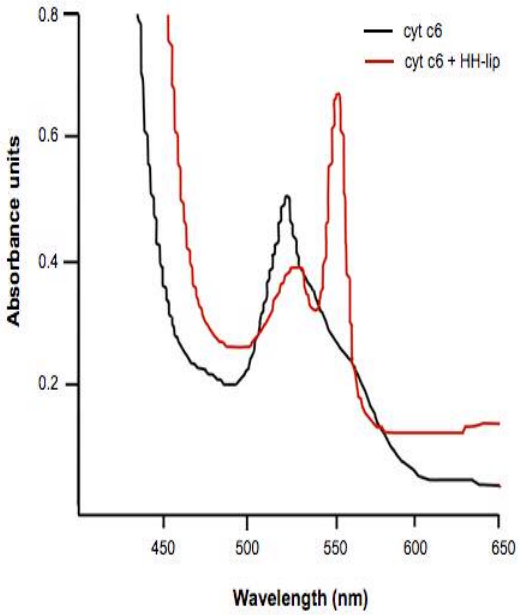


Figure S2


cyt c6
cyt c6 + HH-lip
Absorbance units
0.8
0.6
0.4
0.2
450
500
550
600
650
Wavelength (nm)