Supplementary Information

A bidirectional fluorescent two-hybrid system for monitoring protein-protein interactions

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Table S1. Primers used for construction of tdTomato reporter strain.

Primer	Sequence
tdTomato Forward	GTTGTTGGATCCTTGAAAAACAAGATATCTTAAATGAAAATACAA GAAAATCTTAAATTAGAGCTCAGGAGGTGCAAAAATGGTGAGCAA GGGCGAGGGGTC
tdTomato Reverse	GTTGTTGAATTCTTACTTGTACAGCTCGTCCATG

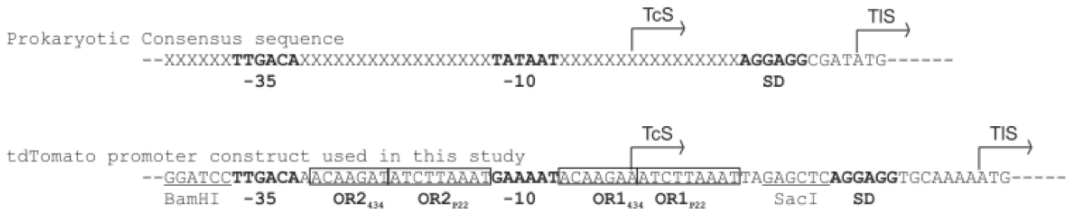

Figure S1. Promoter sequence, transcription (TcS) and translation start (TlS) of the tdTomato FTHS construct.

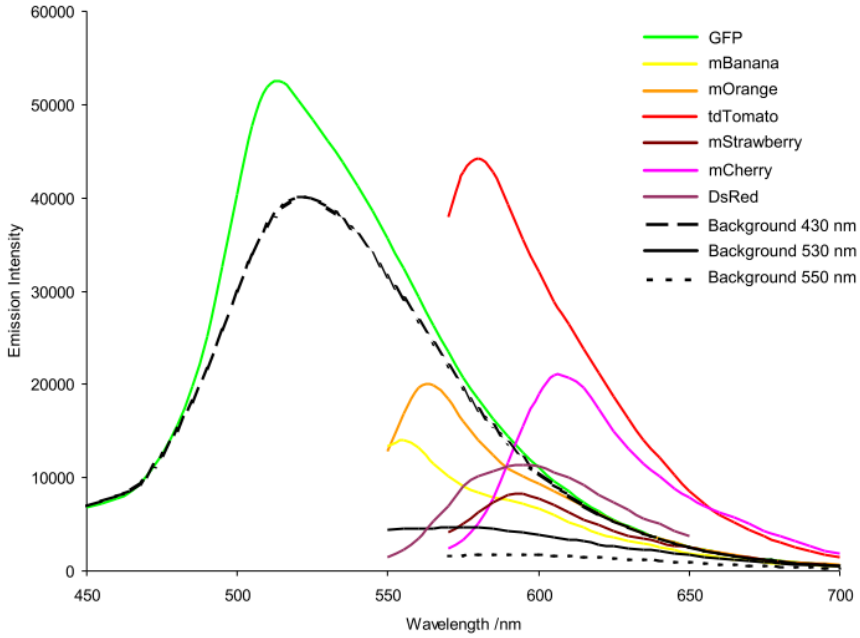

Figure S2. Excitation of fluorescent proteins expressed from pRSET vector in BL21(DE3). GFP excited at 430 nm; mOrange and mBanana excited at 530 nm; tdTomato, mCherry and mStrawberry excited at 550 nm and DsRed exited at 557 nm. Background excitation of BL21(DE3) also measured at the same wavelengths used for fluorescent protein excitation. Each produced from an average of four readings.

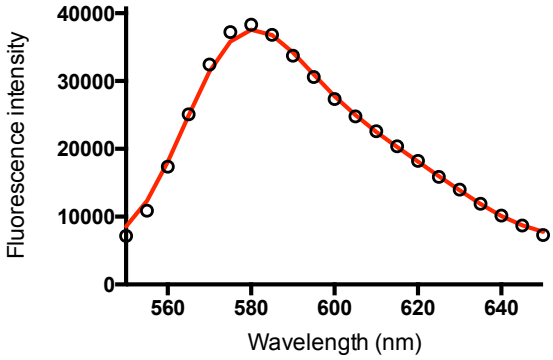

Figure S3. Fluorescence-emission scan of the FTHS strain excited at 514 nm.

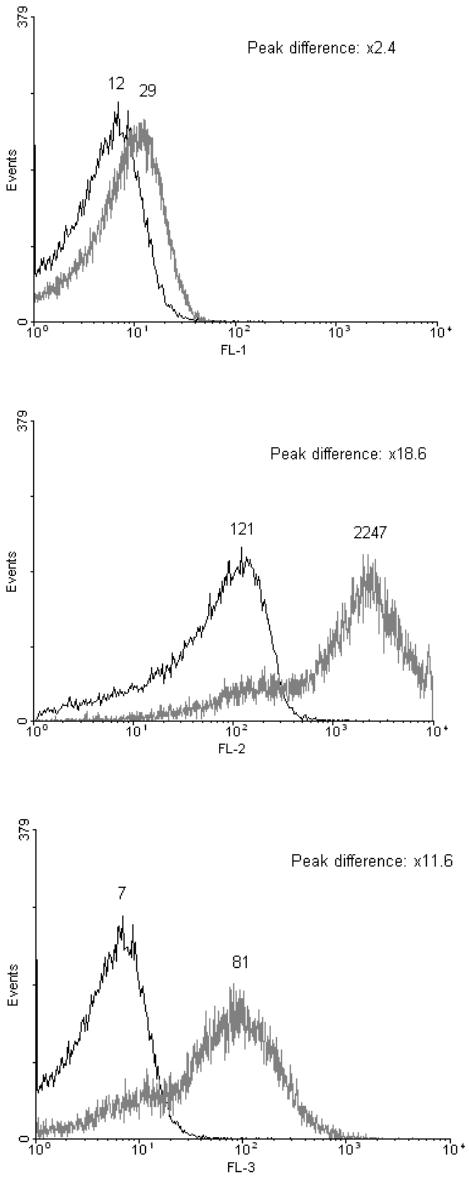

Figure S4. FACS histograms illustrating the fluorescence of cell strains expressing tdTomato (grey line) and strain where tdTomato reporter construct has been repressed (black line).

Where filters used are: top panel: FL-1 (530 ±40), middle panel: FL-2 (600 ±30) and bottom panel: FL-3 (670 ±30).

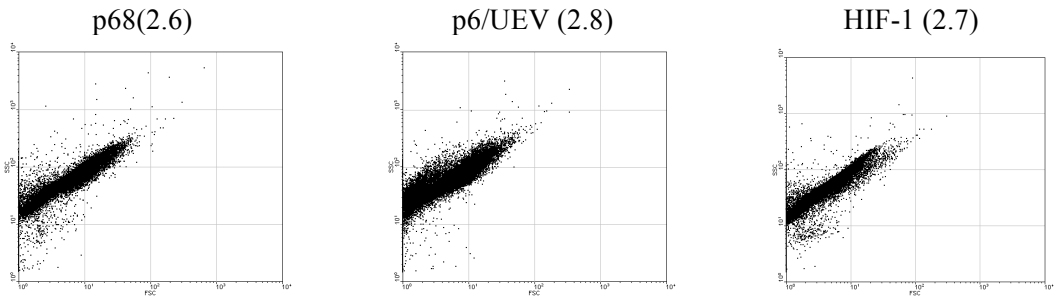

Figure S5. FSC (y-axis) against side scatter (SSC) (x-axis) different levels of tdTomato expression. All strains induced at 100 μM IPTG, therefore p68 shows uninhibited expression of tdTomato whilst the p6/UEV, HIF-1 and HIF-2 strains had fully repressed tdTomato expression. Value presented in brackets indicates the mean forward scatter value of 45000 events. No obvious change in *E. coli* size was observed.