SUPPORTING INFORMATION

Designing Flavoprotein-GFP fusion Probes for Analyte-specific Ratiometric Fluorescence Imaging

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DNA Sequences of TrxR-mCherry

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Primers for G154V Mutation of TrxR

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G154V_Rev: AAC CGC GGT ATT GAC GCC GCC GAT GAC

Protein Sequence of TrxR-mCherry

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DNA Sequences of LipDH-mCherry (for bacterial expression)

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Protein Sequences of LipDH-mCherry (for bacterial expression)

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DNA Sequence of $TrxR_{GV}$-mRuby2

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Protein Sequence of $TrxR_{GV}$-mRuby2

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DNA Sequence of LipDH-mCherry (for mammalian expression)

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Protein Sequences of LipDH-mCherry (for mammalian expression)

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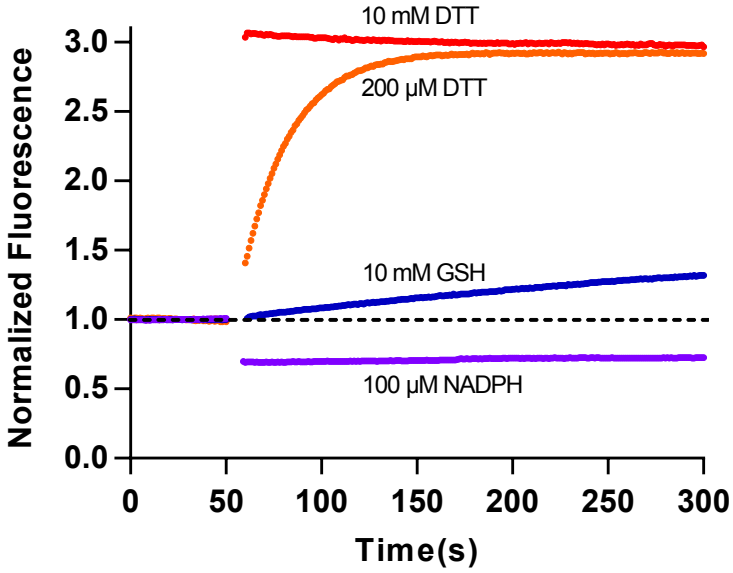

Figure S1. Reduction of *Ec*Trx1 followed by tryptophan fluorescence. Solutions of 5 μM Trx1 (in 200 μL of 50 mM phosphate buffer, pH 7.5, 25 °C) were monitored by fluorescence (excitation 290 nm with emission at 350 nm). Potential reductants (100 µM NADPH, 10 mM GSH, 200 μM or 10 mM DTT) were added at 50 s. The lines represent an average of 3 experiments normalized relative to unit initial fluorescence.

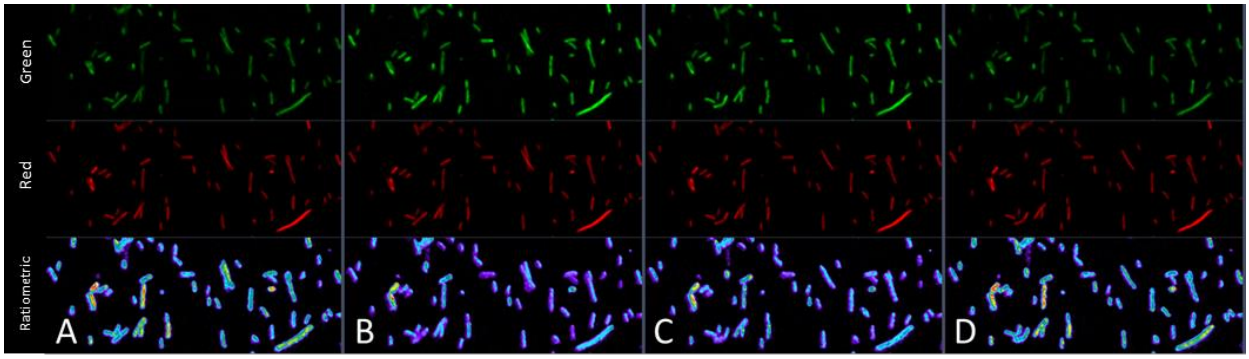

Figure S2. Confocal field of view of *E. coli* cells expressing $TrxR_{GV}$-mCherry sensor. Cells were allowed to adhere to the base of poly-L-lysine treated ibidi flow cells. The wells were serially infused with M9 media containing 5 mM DTT, 5 mM diamide, medium alone, and finally 5 mM DTT (Panels A-D, respectively).

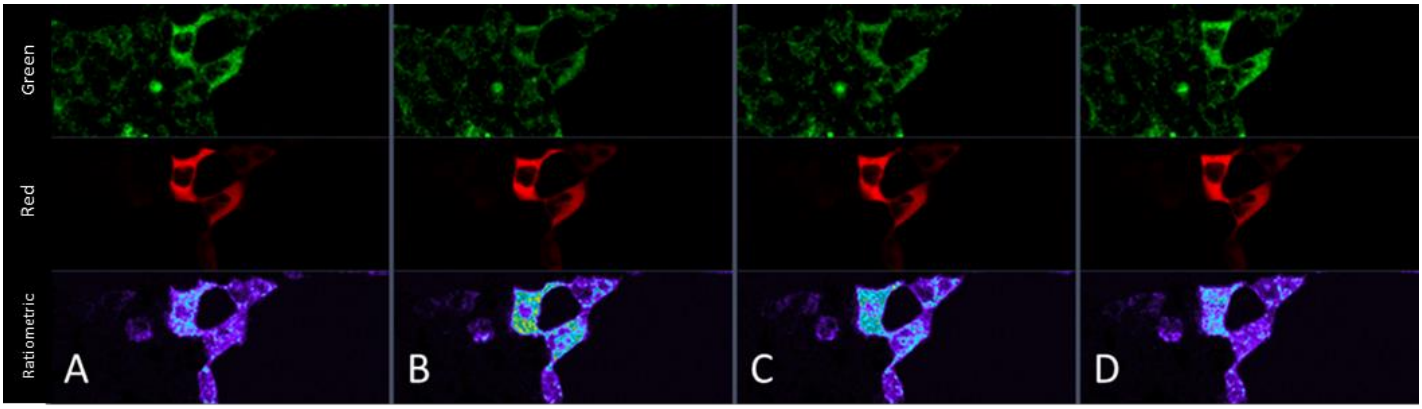

Figure S3. Confocal field of view for one of the NAD^+/NADH sensor flow cell experiments. HEK293T cells over-expressing LipDH-mCherry are attached to the bottom face of an ibidi flow cells. Solutions flowing over the cells were as described in the Text. Panel A-D contain, respectively, 10 mM pyruvate, 10 mM lactate, media alone, and 10 mM pyruvate.