SUPPLEMENTARY MATERIAL 1

**Gamma-glutamylcysteine synthetase and tryparedoxin 1 exert high control on the antioxidant system in *Trypanosoma cruzi* contributing to drug resistance and infectivity.**

by

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Redox Biology, 2019.

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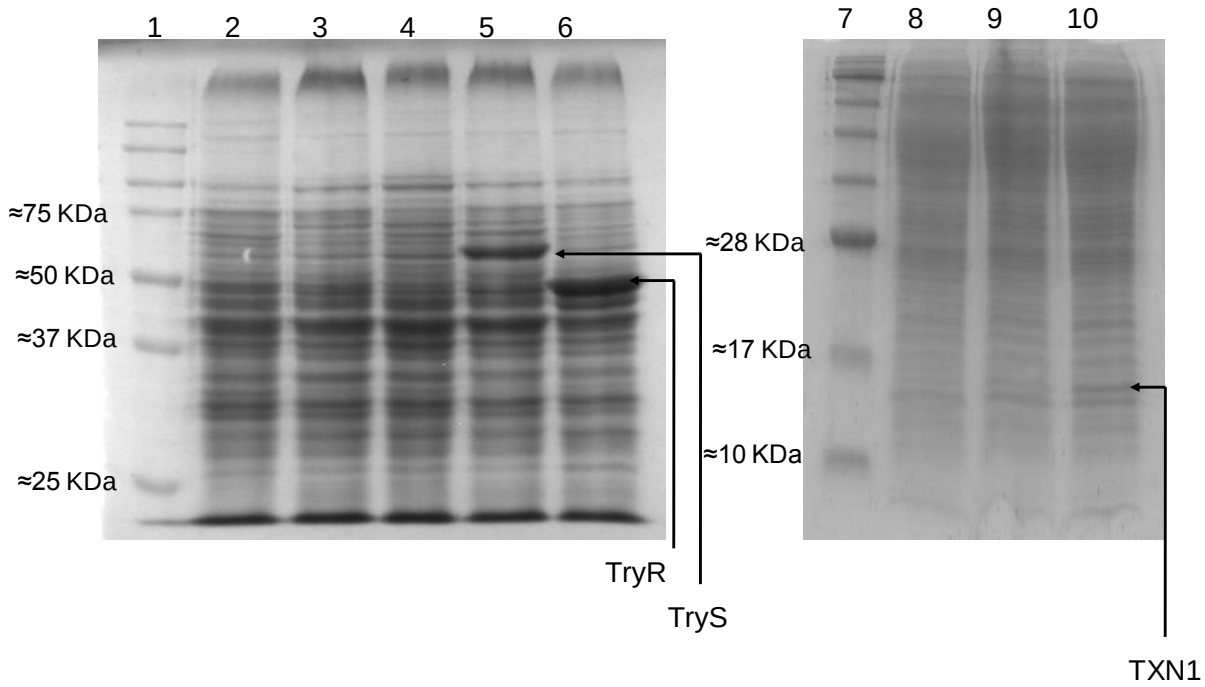


**Figure S1.1. SDS-PAGE of soluble cell protein of controls and OE-parasites.** Proteins of soluble enriched fractions were separated by SDS-PAGE. A) Lanes correspond to 1) molecular weight marker, 2) Wt, 3) mock, 4) OE-γECS3 (79 kDa), 5) OE-TryS1 (74 kDa), and 6) OE-TryR3 (54 kDa) soluble-enriched fractions. B) Lanes correspond to 7) molecular weight marker, 8) Wt, 9) mock, 10) OE-TXN1-2 (16 kDa) soluble-enriched fractions. The arrows indicate overexpression of the respective enzymes, although γECS overexpression was not distinguishable by Coomassie-blue staining. It is worth mentioning that in A, the protein bands always run below the respective protein markers.

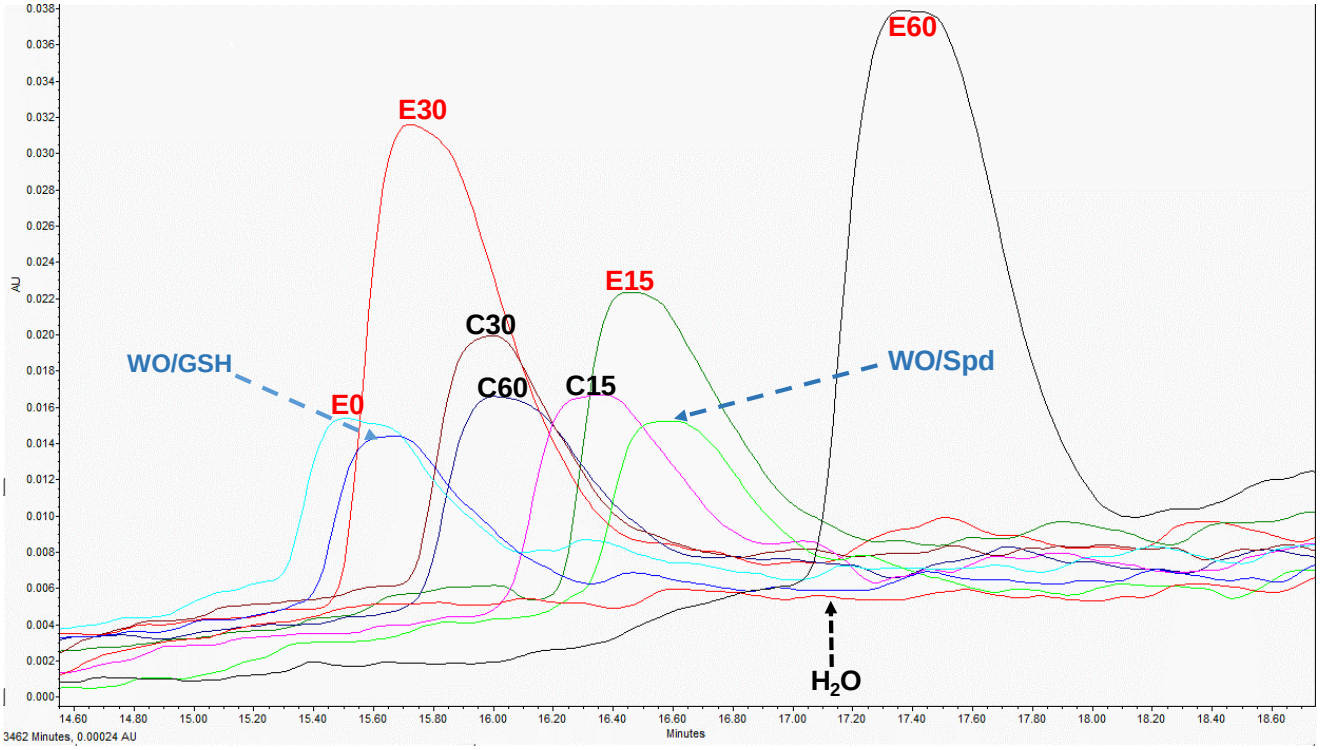


**Figure S1.2. HPLC profile showing the time dependent $T(SH)_2$ production by TryS activity in soluble cell protein fraction of Wt cells.**
TryS activity was determined as described in section 2.6. C and E mean control reaction (absence of GSH) and full reaction with GSH and Spd, respectively. The number besides the letter means the reaction time in min. WO, reactions lacking the indicated substrate.

**Table S1.1. TryS activity in OE-TryS clones**

| clone | [GSH] at which maximum rate value (*v*) was observed (mM) | Maximum *v* observed (mU/mg) | *Vmax** obtained from fitting (mU/mg) | ratio *v/Vmax* (%) |
|---|---|---|---|---|
| TryS 1 | 4.2 | 84 | 170 | 49% |
| | 3.8 | 123 | 220 | 56% |
| TryS 2 | 4.2 | 80 | 154 | 52% |
| | 3.8 | 163 | 304 | 54% |
| TryS 3 | 2.1 | 88 | 172 | 51% |
| | 3.8 | 103 | 182 | 57% |
| | 2.8 | 113 | 244 | 46% |

*Values obtained from GSH saturation curve, using soluble cell protein fractions from OE-TryS epimastigotes and the PyK/LDH enzymatic coupled assay. Each row corresponds to independent parasite extracts from the different TryS clones. The experimental data were fitted by the equation of substrate inhibition $v = \frac{Vmax \cdot S}{Km + S + \frac{S^2}{Ki}}$.

**Table S1.2. Enzyme activities and $T(SH)_2$ synthesis fluxes in clones of OE-γECS parasites**

| **Cell type** | **γECS activity** (nmol/min x mg cell protein**)** | **Ratio vs Wt** | **$T(SH)_2$ synthesis flux (J)** (nmol/ min x mg cell protein) | **% J** |
|---|---|---|---|---|
| Wt | < 3 | 1 | 0.6 ± 0.2 | 100 |
| mock | < 3 | 1 | 1 | 172 |
| γECS A | 3 | 1 | | |
| γECS B | 10 ± 2$^{\#}$ | 3 | | |
| γECS C | 12.7 ± 4$^{\#}$ | 4 | 1.3 | 217 |
| γECS 1 | 12.4 ± 2.6$^{\#}$ | 4 | 0.8 ± 0.1 | 129 |
| γECS D | 17.4 ± 4$^{\#}$ | 6 | | |
| γECS E | 18.2 ± 6$^{\#}$ | 6 | | |
| γECS 2 | 18.1 ± 3.5$^{\#}$* | 6 | 1.2 ± 0.1$^{\&}$ | 196 |
| γECS 3 | 20.2 ± 3.7$^{\#}$* | 7 | 2 ± 0.4$^{\&}$ | 340 |
| γECS pop | 22.1 ± 5.3$^{\#}$* | 7 | 1.6 | 267 |

Values are mean ± SD of at least three independent cultures of the parental strain or the indicated clone. $^{\#}$p<0.01 *versus* control (Wt or mock) and γECS A clone, *p<0.01 vs. OE-γECS B, C and 1. $^{\&}$p<0.01 *versus* Wt and OE-γECS 1 clones.

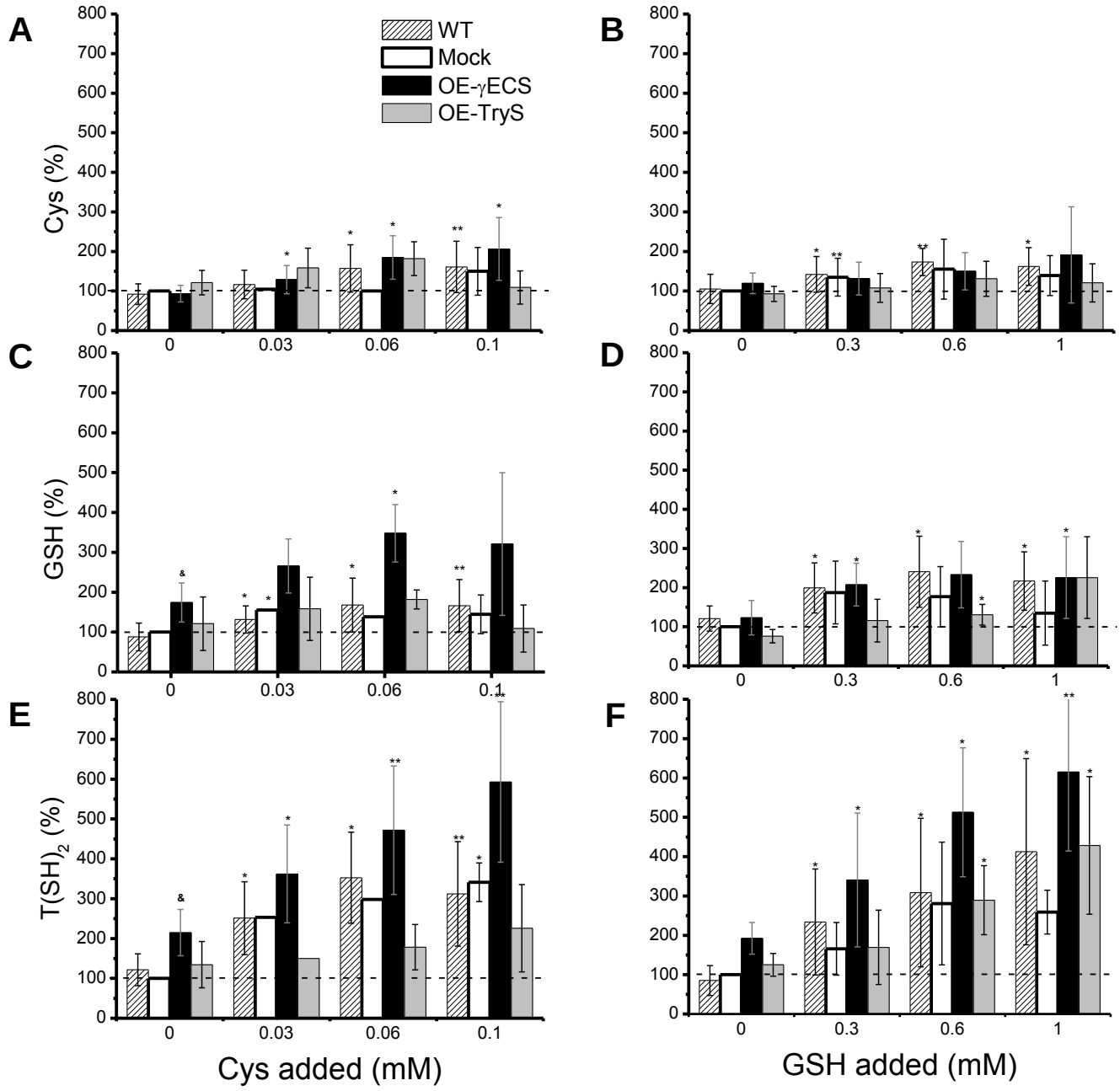


**Figure S1.3. Thiol contents in OE- γECS and OE-TryS parasites supplemented with Cys or GSH.**

Cys, GSH or $T(SH)_2$ contents in Wt, mock, OE-γECS parasites and OE-TryS parasites at different external Cys (A, C and E) or GSH (B, D and F) concentrations. Values are expressed as % of thiol content *versus* mock parasites which corresponds to: Cys1.1 ± 0.1 mM, 1.0 ± 0.1 mM (A and B); GSH 1.6 ± 0.6 mM, 1.4 ± 0.4 mM (C and D) and $T(SH)_2$ 0.8 ± 0.3 mM and 0.9 ± 0.4 (E and F), respectively. *$p < 0.05$, **$p < 0.01$ *vs*. mock.

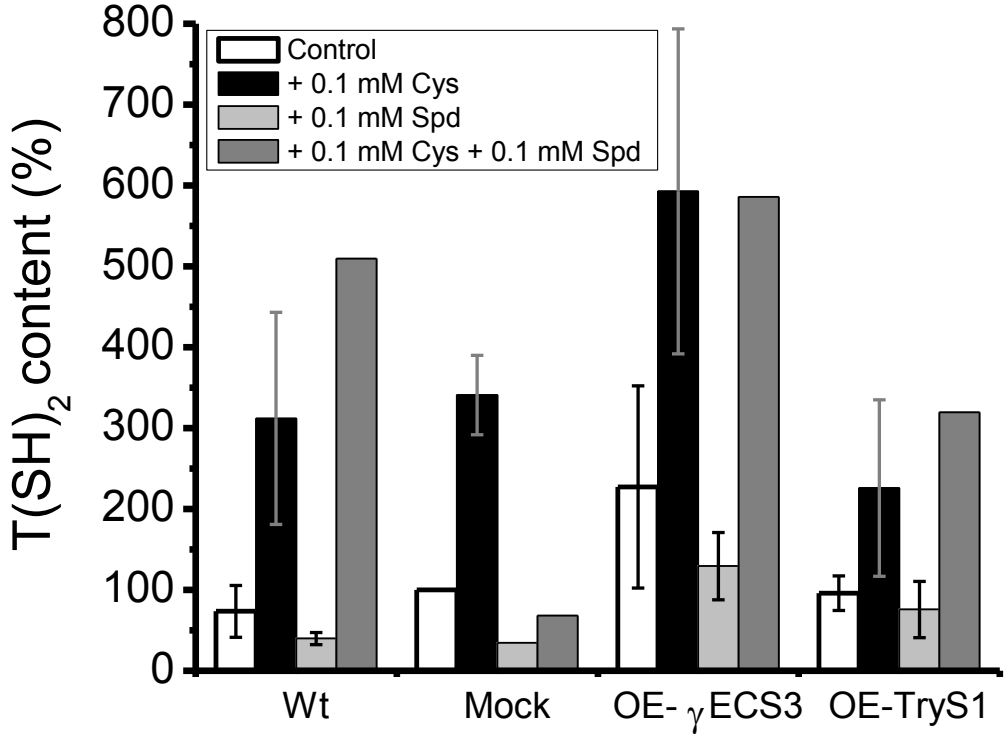


**Figure S1.4. $T(SH)_2$ content in epimastigotes supplemented with pathway synthesis precursors.**
$T(SH)_2$ contents in epimastigotes non-supplemented (control) and supplemented with 0.1 mM Cys, 0.1 mM Spd or their combination. Values are expressed as % of $T(SH)_2$ content *vs.* mock parasites without any supplementation; 100% corresponds to 1.4 ± 0.4 mM. n=3 except for Cys + Spd supplementation which was n=2.

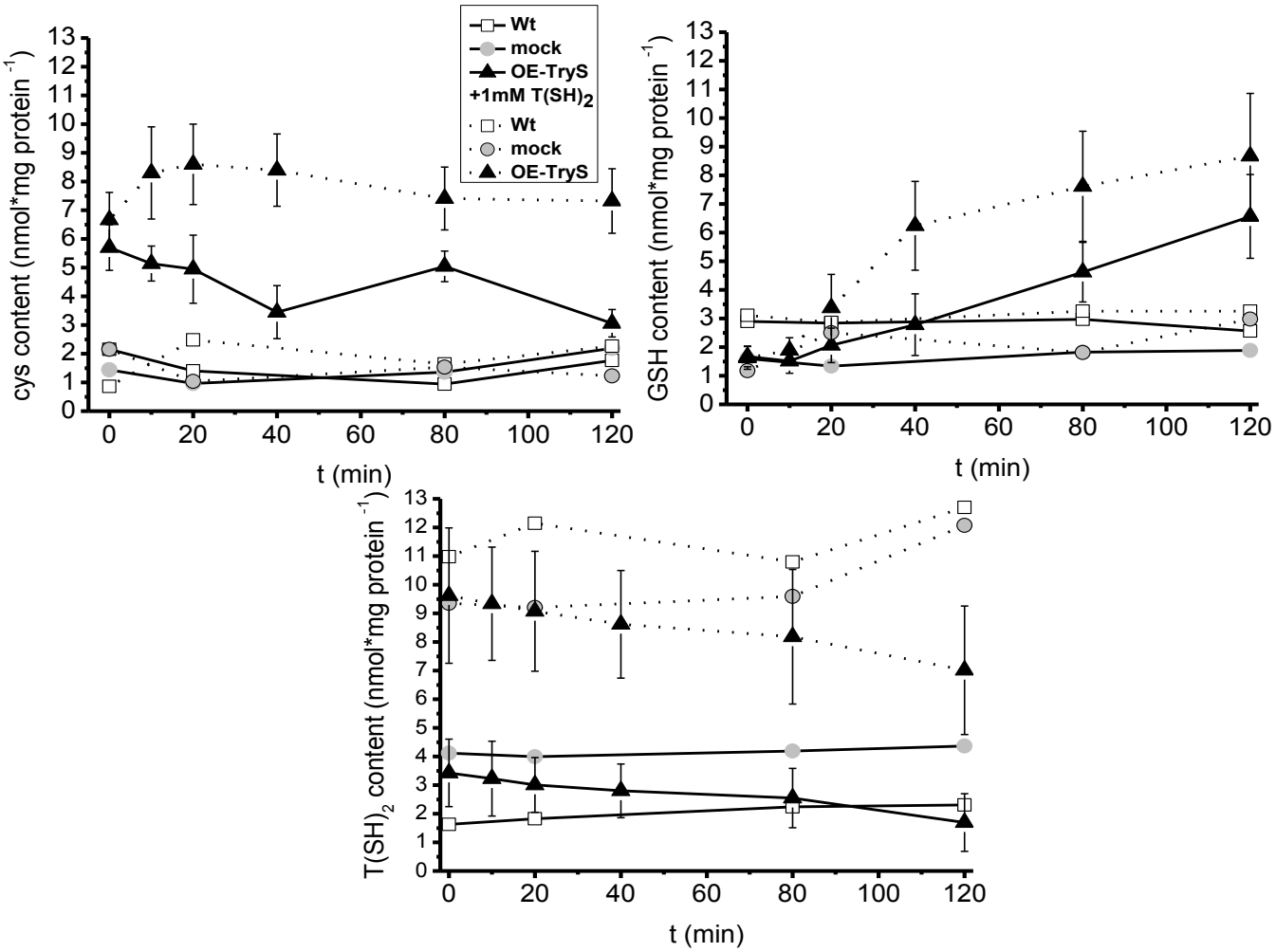


**Figure S.1.5. Thiol content in $T(SH)_2$ degradation assays using OE-TryS parasites soluble cell protein.**

Cys, GSH and $T(SH)_2$ variations through time, when $T(SH)_2$ degradation was evaluated in OE-TryS1 clone soluble cell protein fractions in the absence or presence of 1 mM $T(SH)_2$ added. The calculated $T(SH)_2$ degradation rates were $13 \pm 5$ and $21 \pm 9$ nmol/min x mg cell soluble protein in the absence and presence of added $T(SH)_2$, respectively.

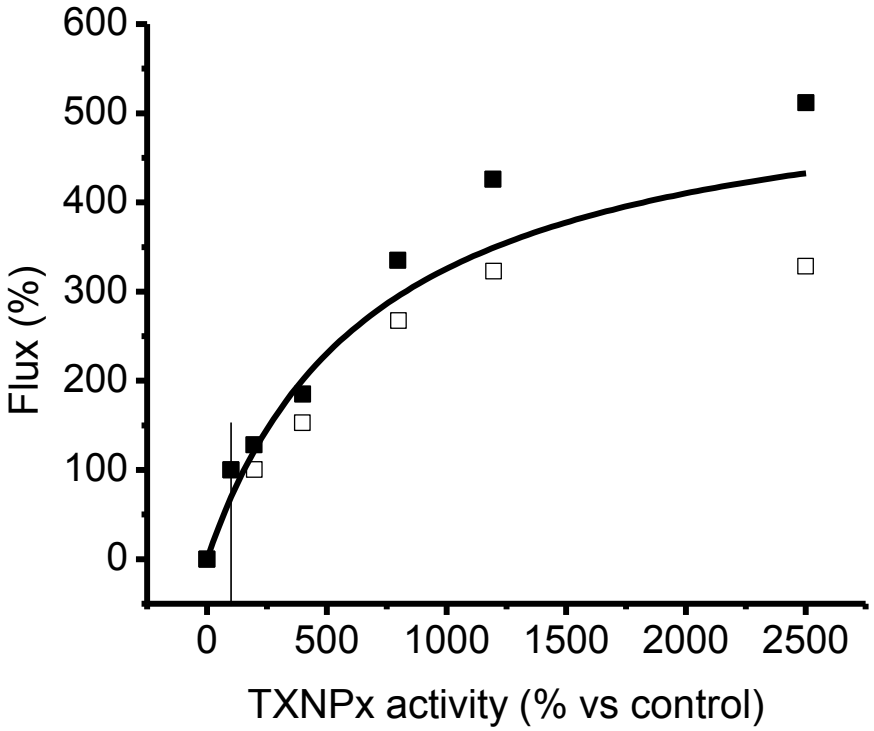


**Fig S1.6. TXNPx flux control coefficient determination in a mixed, reconstituted pathway.**

Variations in the *ex vivo* pathway fluxes of Wt (empty) and mock (black) cell soluble protein by adding increasing activities of recombinant TXNPx above the wild-type activity (100%). The $C^{J}_{ai}$ was determined at the enzyme activity and flux value attained in the absence of recombinant TXNPx addition.