Supporting Information

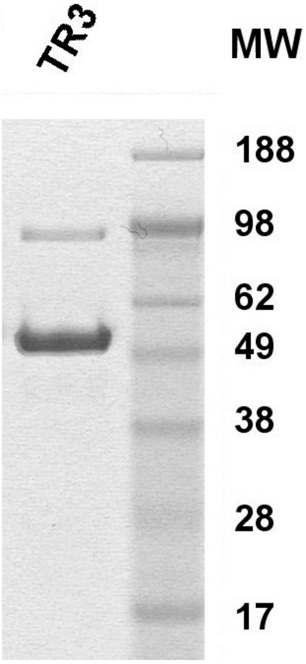
Supplemental Table 1: [a]Results of peptide mass mapping by MALDI-MS and ESI-MS for tryptic digest of mTR-3.

			Semisynthetic TR		Truncated TR	
Residue #	Sequence of Tryptic Fragments of mTR-3	$M+H^+$ (predicted m/z)	MALDI-MS (obs. m/z)	ESI-MS (obs. m/z)	MALDI-MS (obs. m/z)	ESI-MS (obs. m/z)
353-416	SSTLMDYSNVPTTVFTPLEYGCVGLSEEEAVALHGQEHVEVYHAYYKPLEFTVADRDASQCYIK	7200.4	Not obs.	Not obs.	Not obs.	Not obs.
308-340	IIVDAQEATSVPHIYAIGDVAEGRPELTPTAIK	3474.8	3475.0	3476.9	3474.7	3476.9
188-216	TLVVGASYVALECAGFLTGIGLDTTVMMR	2988.5	Not obs.	Not obs.	Not obs.	Not obs.
422-449	EPPQLVLGLHFLGPNAGEVTQGFALGIK	2902.6	2902.5	2904.3	Not obs.	2904.3
222-244	GFDQQMSSLVTEHMESHGTQFLK	2637.2	2637.3	2638.9	2637.1	2638.9
450-473	CGASYAQVMQTVGIHPTCSEEVVK	2537.2	2535.3	Not obs.	2535.1	Not obs.
76-93	DAHHYGWEVAQPVQHNWK	2202.0	2202.1	2203.3	2201.9	2203.3
1-23	MGGQQSFDLLVIGGGSGGLACAK	2166.1	Not obs.	Not obs.	Not obs.	Not obs.
253-270	KLPTNQLQVTWEDHASGK	2052.0	2052.0	2053.2	2051.9	2053.2
143-160	ATLLSAEHIVIATGGRPR	1862.1	1862.0	1863.1	1862	1863.1
167-182	GALEYGITSDDIFWLK	1827.9	1827.9	1829	1827.8	1829
271-285	EDTGTFDTVLWAIGR	1680.8	1680.8	1681.8	1680.8	1681.8
47-61	WGLGGTCVNVGCIPK	1503.7	1501.5, 1503.7	1504.8	1501.6	Not obs.
63-75	LMHQAALLGGMIR	1410.8	1410.7	1411.7	1410.7	1411.8
32-43	VAVADYVEPSPR	1302.7	1302.6	1303.4	1302.6	1303.4
94-104	TMAEAVQNHVK	1227.6	1227.6	1228.4	1227.5	1228.4
480-491	SGLEPTVTGCUG (full length)	1169.4	Not obs.	1169.3	Not applicable	Not applicable
126-135	ASFVDEHTVR	1160.6	1160.5	1161.2	1160.5	1161.2
479-488	RSGLEPTVTG (truncated)	1016.5	Not applicable	Not applicable	Not obs.	1017.1
105-111	SLNWGHR	869.4	869.3	869.9	869.3	869.9

480-488	SGLEPTVTG (truncated)	860.4	Not applicable	Not applicable	Not obs.	860.3
245-252	GCVPSHIK	840.4	840.4	841.0	Not obs.	Not obs.
297-304	AGISTNPK	787.4	787.1	787.9	Not obs.	Not obs.
112-117	VQLQDR	758.4	758.3	Not obs.	758.3	Not obs.
161-166	YPTQVK	735.4	735.3	735.8	735.3	Not obs.
291-296	TLNLEK	717.4	Not obs.	717.8	Not obs.	717.8
24-30	EAAQLGK	716.4	Not obs.	Not obs.	Not obs.	Not obs.
121-125	YFNIK	684.4	684.2	684.8	684.3	Not obs.
417-421	MVCMR	639.3	639.2	Not obs.	639.2	Not obs.
286-290	VPETR	601.3	Not obs.	Not obs.	Not obs.	Not obs.
344-348	LLAQR	600.4	Not obs.	Not obs.	Not obs.	Not obs.
474-478	LHISK	597.4	Not obs.	597.7	597.3	Not obs.
217-221	SIPLR	585.4	585.3	Not obs.	585.3	Not obs.

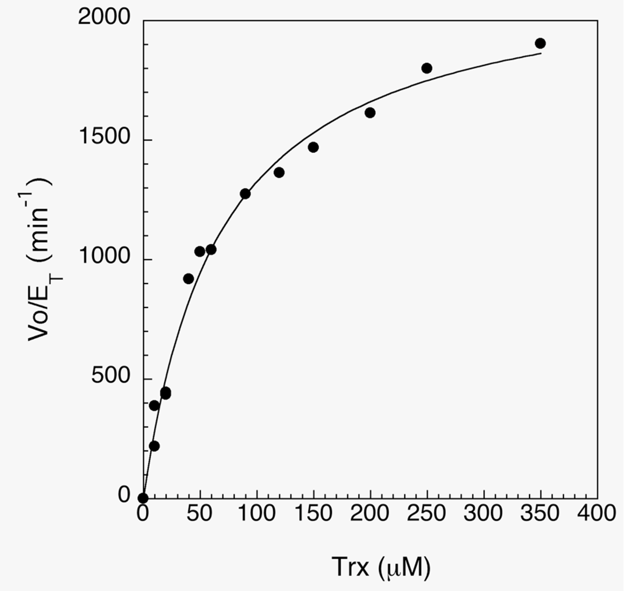
[a] The truncated and semisynthetic forms of mTR-3 were digested by trypsin as described in the Methods and then peptides were analyzed by both MALDI-MS and ESI-MS. Each peptide identified by ESI-MS was verified by MS/MS fragmentation. 70% of the total peptide sequence was verified using both forms of MS. The C-terminal fragment containing selenocysteine was verified by ESI-MS. The reduced and oxidized forms of the truncated enzyme were analyzed by MALDI-MS and at least one peptide could be identified in both oxidation states. For analysis by ESI-MS, only the oxidized form of the peptide was digested by trypsin. The C-terminus of the truncated enzyme was identified by ESI-MS in the carboxylate form.

Supplemental Figure 1



12% SDS-PAGE gel of mouse TR3 produced as full-length selenocysteine-containing enzyme using the engineered SECIS element method in *E. coli* cells. The enzyme is produced and purified as described in the Methods.

Supplemental Figure 2



Michaelis-Menten plot of the semisynthetic TR (optimized protocol) using Trx as substrate. This enzyme, which has a Se content of 91%, has a k_{cat} of 2220 min^{-1} as determined by fitting the data to the Michaelis-Menten equation.