

## Supplemental Information

**S1 Table. List of all oxidative stress and antioxidant PCR primer targets on the PCR array.**  
Red genes represent targets that are not expressed in CD8+ T cells.

<b>ALB</b>	Albumin	<b>GLRX2</b>	Glutaredoxin 2	<b>MTL5</b>	Metallothionein-like 5, testis-specific (tesmin)	<b>PTGS1</b>	Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)
<b>ALOX12</b>	Arachidonate 12-lipoxygenase	<b>GPR156</b>	G protein-coupled receptor 156	<b>NCF1</b>	Neutrophil cytosolic factor 1	<b>PTGS2</b>	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
<b>ANGPTL7</b>	Angiotensin-like 7	<b>GPX1</b>	Glutathione peroxidase 1	<b>NCF2</b>	Neutrophil cytosolic factor 2	<b>PXDN</b>	Peroxidasin homolog (Drosophila)
<b>AOX1</b>	Aldehyde oxidase 1	<b>GPX2</b>	Glutathione peroxidase 2 (gastrointestinal)	<b>NME5</b>	Non-metastatic cells 5, protein expressed in (nucleoside-diphosphate kinase)	<b>PXDNL</b>	Peroxidasin homolog (Drosophila)-like
<b>APOE</b>	Apolipoprotein E	<b>GPX3</b>	Glutathione peroxidase 3 (plasma)	<b>NOS2</b>	Nitric oxide synthase 2, inducible	<b>RNF7</b>	Ring finger protein 7
<b>ATOX1</b>	ATX1 antioxidant protein 1 homolog (yeast)	<b>GPX4</b>	Glutathione peroxidase 4 (phospholipid hydroperoxidase)	<b>NOX5</b>	NADPH oxidase, EF-hand calcium binding domain 5	<b>SCARA3</b>	Scavenger receptor class A, member 3
<b>BNIP3</b>	BCL2/adenovirus E1B 19kDa interacting protein 3	<b>GPX5</b>	Glutathione peroxidase 5 (epididymal androgen-related protein)	<b>NUDT1</b>	Nudix (nucleoside diphosphate linked moiety X)-type motif 1	<b>SELS</b>	Selenoprotein S
<b>CAT</b>	Catalase	<b>GPX6</b>	Glutathione peroxidase 6 (olfactory)	<b>OXR1</b>	Oxidation resistance 1	<b>SEPP1</b>	Selenoprotein P, plasma, 1
<b>CCL5</b>	Chemokine (C-C motif) ligand 5	<b>GPX7</b>	Glutathione peroxidase 7	<b>OXSR1</b>	Oxidative-stress responsive 1	<b>SFTPD</b>	Surfactant protein D
<b>CCS</b>	Copper chaperone for superoxide dismutase	<b>GSR</b>	Glutathione reductase	<b>PDLIM1</b>	PDZ and LIM domain 1	<b>SGK2</b>	Serum/glucocorticoid regulated kinase 2
<b>CSDE1</b>	Cold shock domain containing E1, RNA-binding	<b>GSS</b>	Glutathione synthetase	<b>IPCEF1</b>	Interaction protein for cytohesin exchange factors 1	<b>SIRT2</b>	Sirtuin 2
<b>CYBA</b>	Cytochrome b-245, alpha	<b>GSTZ1</b>	Glutathione transferase zeta 1	<b>PNKP</b>	Polynucleotide kinase 3'-phosphatase	<b>SOD1</b>	Superoxide dismutase 1, soluble

	polypeptide						
<b>CYGB</b>	Cytoglobin	<b>GTF2I</b>	General transcription factor III	<b>PRDX1</b>	Peroxiredoxin 1	<b>SOD2</b>	Superoxide dismutase 2, mitochondrial
<b>DGKK</b>	Diacylglycerol kinase, kappa	<b>KRT1</b>	Keratin 1	<b>PRDX2</b>	Peroxiredoxin 2	<b>SOD3</b>	Superoxide dismutase 3, extracellular
<b>DHCR24</b>	24-dehydrocholesterol reductase	<b>LPO</b>	Lactoperoxidase	<b>PRDX3</b>	Peroxiredoxin 3	<b>SRXN1</b>	Sulfiredoxin 1
<b>DUOX1</b>	Dual oxidase 1	<b>MBL2</b>	Mannose-binding lectin (protein C) 2, soluble	<b>PRDX4</b>	Peroxiredoxin 4	<b>STK25</b>	Serine/threonine kinase 25
<b>DUOX2</b>	Dual oxidase 2	<b>MGST3</b>	Microsomal glutathione S-transferase 3	<b>PRDX5</b>	Peroxiredoxin 5	<b>TPO</b>	Thyroid peroxidase
<b>DUSP1</b>	Dual specificity phosphatase 1	<b>MPO</b>	Myeloperoxidase	<b>PRDX6</b>	Peroxiredoxin 6	<b>TTN</b>	Titin
<b>EPHX2</b>	Epoxide hydrolase 2, cytoplasmic	<b>MPV17</b>	MpV17 mitochondrial inner membrane protein	<b>PREX1</b>	Phosphatidylinositol -3,4,5-trisphosphate-dependent Rac exchange factor 1	<b>TXNDC2</b>	Thioredoxin domain containing 2 (spermatozoa)
<b>EPX</b>	Eosinophil peroxidase	<b>MSRA</b>	Methionine sulfoxide reductase A	<b>PRG3</b>	Proteoglycan 3	<b>TXNRD1</b>	Thioredoxin reductase 1
<b>FOXM1</b>	Forkhead box M1	<b>MT3</b>	Metallothionein 3	<b>PRNP</b>	Prion protein	<b>TXNRD2</b>	Thioredoxin reductase 2

**S2 Table. Exhaustive list of fold changes and their corresponding p-values in targets expressed in CD8+ T cells. A fold change below 1 corresponds to a downregulation ( $2^{-\Delta\text{CT}}$ ).**

Symbol	Protein Name	Fold Change Old/Young	p-value
<b>PDLIM1</b>	PDZ and LIM domain 1	0.17	0.01
<b>ANGPTL7</b>	Angiopoietin-like 7	2.93	0.01
<b>MTL5</b>	Metallothionein-like 5, testis-specific (tesmin)	0.45	0.01
<b>NUDT1</b>	Nudix (nucleoside diphosphate linked moiety X)-type motif 1	0.99	0.01
<b>TTN</b>	Titin	2.14	0.01
<b>DUOX1</b>	Dual oxidase 1	1.68	0.03
<b>GPX3</b>	Glutathione peroxidase 3 (plasma)	2.55	0.04
<b>GLRX2</b>	Glutaredoxin 2	0.47	0.06
<b>PRNP</b>	Prion protein	1.45	0.07
<b>TXNRD1</b>	Thioredoxin reductase 1	0.31	0.08
<b>PRDX1</b>	Peroxiredoxin 1	0.74	0.13
<b>CCL5</b>	Chemokine (C-C motif) ligand 5	3.81	0.15
<b>TXNRD2</b>	Thioredoxin reductase 2	1.15	0.18
<b>GPX2</b>	Glutathione peroxidase 2 (gastrointestinal)	2.15	0.20
<b>SEPP1</b>	Selenoprotein P, plasma, 1	0.34	0.21
<b>ATOX1</b>	ATX1 antioxidant protein 1 homolog (yeast)	0.50	0.22
<b>BNIP3</b>	BCL2/adenovirus E1B 19kDa interacting protein 3	1.12	0.23
<b>CCS</b>	Copper chaperone for superoxide dismutase	0.22	0.23
<b>PX1</b>	Glutathione peroxidase 1	0.18	0.23
<b>PREX1</b>	Phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 1	0.90	0.24
<b>PRDX5</b>	Peroxiredoxin 5	0.80	0.29
<b>CSDE1</b>	Cold shock domain containing E1, RNA-binding	0.49	0.30
<b>SIRT2</b>	Sirtuin 2	1.25	0.32
<b>FOXM1</b>	Forkhead box M1	1.78	0.33
<b>GTF2I</b>	General transcription factor Iii	0.52	0.34
<b>GSS</b>	Glutathione synthetase	0.26	0.37
<b>NCF1</b>	Neutrophil cytosolic factor 1	9.32	0.42
<b>ALOX12</b>	Arachidonate 12-lipoxygenase	0.60	0.46
<b>STK25</b>	Serine/threonine kinase 25	4.65	0.46
<b>GPX4</b>	Glutathione peroxidase 4 (phospholipid hydroperoxidase)	0.02	0.47
<b>SELS</b>	Selenoprotein S	0.10	0.48
<b>MPV17</b>	MpV17 mitochondrial inner membrane protein	0.57	0.49
<b>MGST3</b>	Microsomal glutathione S-transferase 3	0.49	0.50
<b>GSR</b>	Glutathione reductase	0.73	0.51
<b>PRDX2</b>	Peroxiredoxin 2	0.75	0.51
<b>SOD1</b>	Superoxide dismutase 1, soluble	6.52	0.51
<b>SRXN1</b>	Sulfiredoxin 1	0.12	0.52
<b>CAT</b>	Catalase	122.49	0.56
<b>MSRA</b>	Methionine sulfoxide reductase A	0.79	0.57
<b>MT3</b>	Metallothionein 3	0.28	0.59
<b>NCF2</b>	Neutrophil cytosolic factor 2	1.49	0.59

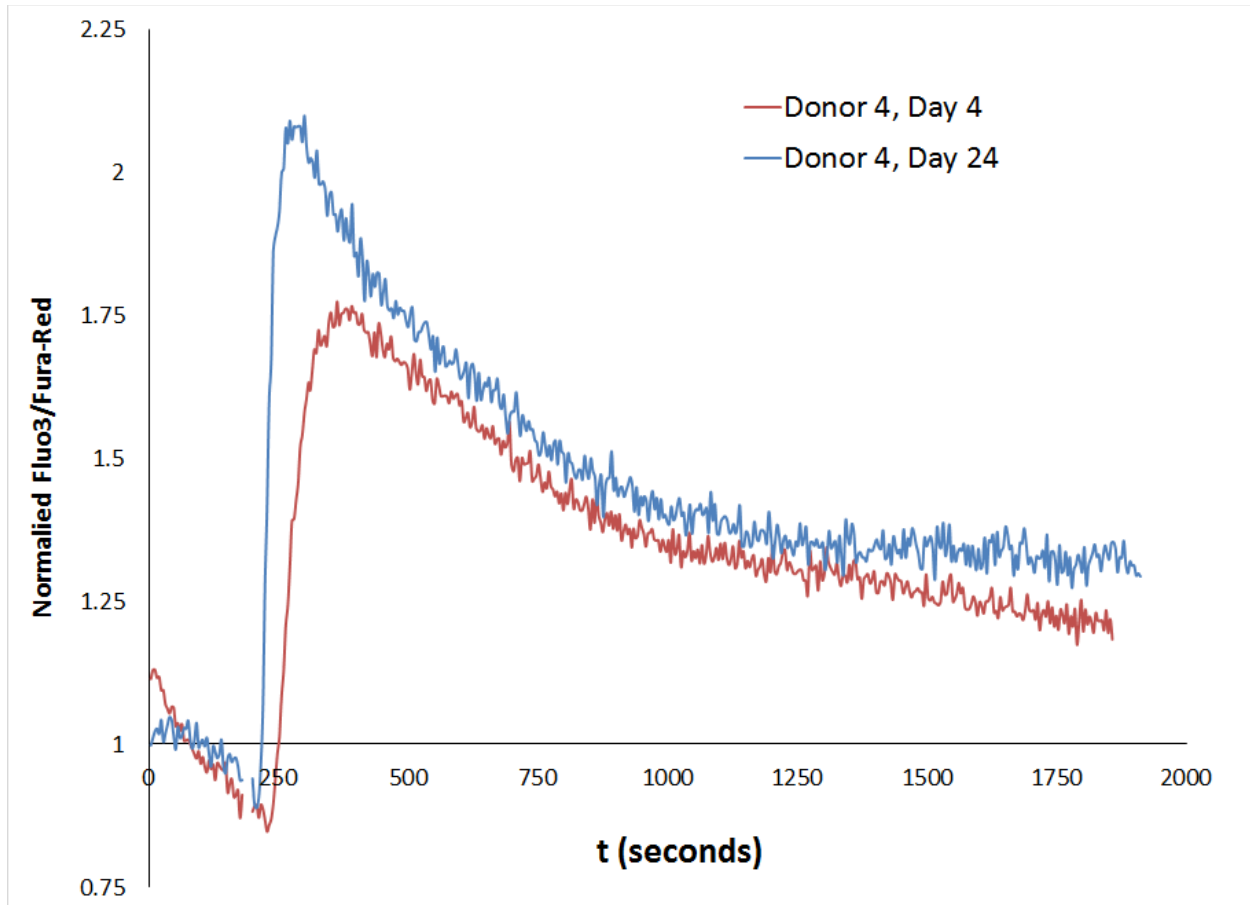
<b>OXR1</b>	Oxidation resistance 1	0.02	0.59
<b>GPX7</b>	Glutathione peroxidase 7	0.17	0.60
<b>EPX</b>	Eosinophil peroxidase	0.83	0.64
<b>CYBA</b>	P22-phox	0.41	0.65
<b>GSTZ1</b>	Glutathione transferase zeta 1	1.89	0.65
<b>PRDX6</b>	Peroxiredoxin 6	3.93	0.65
<b>IPCEF1</b>	Interaction protein for cytohesin exchange factors 1	0.65	0.66
<b>PRDX4</b>	Peroxiredoxin 4	2.87	0.70
<b>SOD2</b>	Superoxide dismutase 2, mitochondrial	0.33	0.70
<b>DUSP1</b>	Dual specificity phosphatase 1	4.56	0.71
<b>RNF7</b>	Ring finger protein 7	6.55	0.71
<b>SFTPD</b>	Surfactant protein D	3.35	0.72
<b>DHCR24</b>	24-dehydrocholesterol reductase	0.57	0.82
<b>PRDX3</b>	Peroxiredoxin 3	1.60	0.84
<b>OXSR1</b>	Oxidation resistance 1	2.20	0.95
<b>PNKP</b>	Polynucleotide kinase 3'-phosphatase	1.59	0.98

**S3 Table S3. Normalized mRNA levels of individual genes expressed in young CD8+ T cells, ranked in descending order of expression (n=6).**

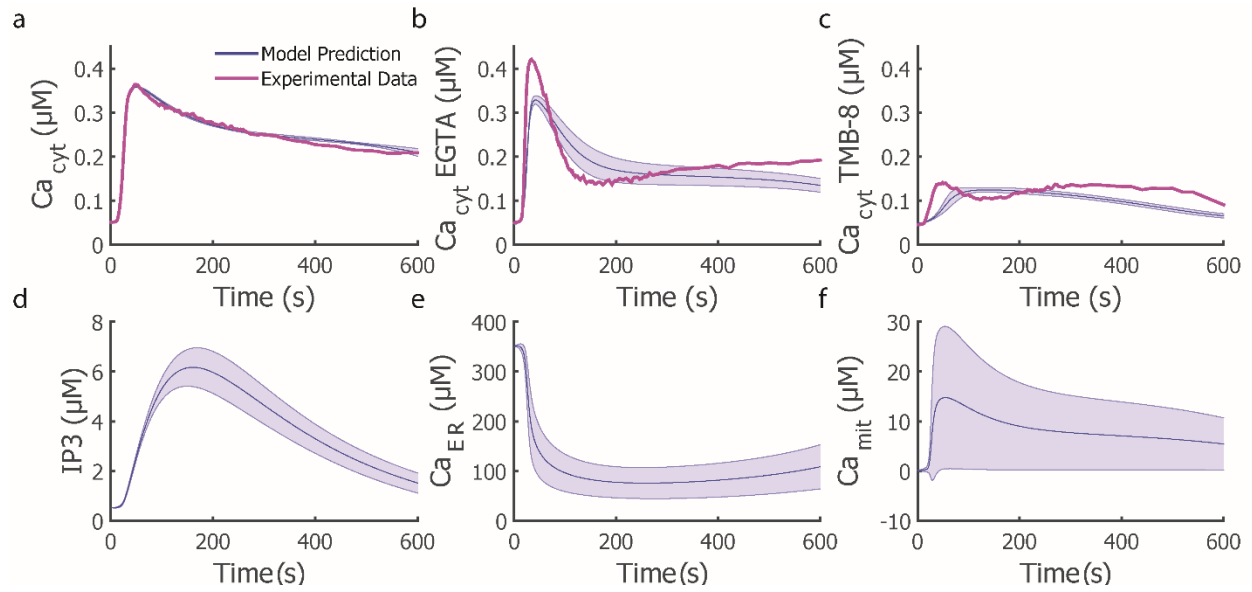
<b>Symbol</b>	<b>Protein Name</b>	<b>Mean <math>\Delta</math>Ct <math>\pm</math> SEM</b>	<b>% CV</b>
<b>CYBA</b>	P22-phox	3.77 $\pm$ 0.4	10.64
<b>CCL5</b>	Chemokine (C-C motif) ligand 5	3.58 $\pm$ 0.68	19.06
<b>SOD1</b>	Superoxide dismutase 1, soluble	4.19 $\pm$ 0.23	5.56
<b>GPX4</b>	Glutathione peroxidase 4 (phospholipid hydroperoxidase)	4.38 $\pm$ 0.40	9.24
<b>PRDX6</b>	Peroxiredoxin 6	4.60 $\pm$ 0.30	6.48
<b>GTF2I</b>	General transcription factor Iii	4.79 $\pm$ 0.28	5.93
<b>PRDX3</b>	Peroxiredoxin 3	4.61 $\pm$ 0.46	9.98
<b>PRDX2</b>	Peroxiredoxin 2	4.78 $\pm$ 0.35	7.24
<b>GPX1</b>	Glutathione peroxidase 1	5.17 $\pm$ 0.23	4.48
<b>CSDE1</b>	Cold shock domain containing E1, RNA-binding	5.19 $\pm$ 0.23	4.48
<b>FOXM1</b>	Forkhead box M1	5.39 $\pm$ 0.18	3.44
<b>PRDX5</b>	Peroxiredoxin 5	5.38 $\pm$ 0.28	5.25
<b>ATOX1</b>	ATX1 antioxidant protein 1 homolog (yeast)	5.60 $\pm$ 0.21	3.70
<b>TXNRD1</b>	Thioredoxin reductase 1	6.17 $\pm$ 0.08	1.33
<b>DHCR24</b>	24-dehydrocholesterol reductase	6.38 $\pm$ 0.18	2.83
<b>RNF7</b>	Ring finger protein 7	6.58 $\pm$ 0.21	3.13
<b>SOD2</b>	Superoxide dismutase 2, mitochondrial	6.59 $\pm$ 0.21	3.12
<b>OXSRI</b>	Oxidative-stress responsive 1	6.98 $\pm$ 0.10	1.47
<b>CAT</b>	Catalase	6.78 $\pm$ 0.4	5.92
<b>PRDX4</b>	Peroxiredoxin 4	6.99 $\pm$ 0.36	5.18
<b>MGST3</b>	Microsomal glutathione S-transferase 3	7.18 $\pm$ 0.23	3.22
<b>MPV17</b>	MpV17 mitochondrial inner membrane protein	7.18 $\pm$ 0.23	3.21
<b>GLRX2</b>	Glutaredoxin 2	7.39 $\pm$ 0.18	2.49
<b>GSS</b>	Glutathione synthetase	7.37 $\pm$ 0.28	3.81
<b>BNIP3</b>	BCL2/adenovirus E1B 19kDa interacting protein 3	7.38 $\pm$ 0.34	4.62
<b>SELS</b>	Selenoprotein S	7.78 $\pm$ 0.28	3.62
<b>STK25</b>	Serine/threonine kinase 25	7.98 $\pm$ 0.10	1.24
<b>TXNRD2</b>	Thioredoxin reductase 2	7.79 $\pm$ 0.34	4.43
<b>NUDT1</b>	Nudix (nucleoside diphosphate linked moiety X)-type motif 1	8.18 $\pm$ 0.09	1.04
<b>PNKP</b>	Polynucleotide kinase 3'-phosphatase	7.99 $\pm$ 0.31	3.89
<b>GPX7</b>	Glutathione peroxidase 7	8.00 $\pm$ 0.31	3.89
<b>OXR1</b>	Oxidation resistance 1	8.19 $\pm$ 0.23	2.85
<b>PRDX1</b>	Peroxiredoxin 1	8.19 $\pm$ 0.30	3.70
<b>CCS</b>	Copper chaperone for superoxide dismutase	8.80 $\pm$ 0.19	2.13
<b>IPCEF1</b>	Interaction protein for cytohesin exchange factors 1	8.57 $\pm$ 0.58	6.78
<b>MTL5</b>	Metallothionein-like 5, testis-specific (tesmin)	8.97 $\pm$ 0.31	3.46
<b>SIRT2</b>	Sirtuin 2	9.01 $\pm$ 0.38	4.20
<b>PREX1</b>	Phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 1	9.18 $\pm$ 0.23	2.49
<b>GSTZ1</b>	Glutathione transferase zeta 1	9.19 $\pm$ 0.37	4.07
<b>NCF2</b>	Neutrophil cytosolic factor 2	9.38 $\pm$ 0.39	4.20
<b>NCF1</b>	Neutrophil cytosolic factor 1	9.00 $\pm$ 0.89	9.91

<b>SRXN1</b>	Sulfiredoxin 1	10.18 ± 0.23	2.26
<b>MSRA</b>	Methionine sulfoxide reductase A	10.18 ± 0.3	2.93
<b>DUSP1</b>	Dual specificity phosphatase 1	10.19 ± 0.43	4.19
<b>GPX3</b>	Glutathione peroxidase 3 (plasma)	10.37 ± 0.49	4.76
<b>SEPP1</b>	Selenoprotein P, plasma, 1	10.57 ± 0.29	2.80
<b>PRNP</b>	Prion protein	11.01 ± 0.10	0.90
<b>GSR</b>	Glutathione reductase	10.78 ± 0.34	3.17
<b>PDLIM1</b>	PDZ and LIM domain 1	10.98 ± 0.3	2.79
<b>TTN</b>	Titin	11.19 ± 0.42	3.79
<b>MT3</b>	Metallothionein 3	11.4 ± 0.73	6.40
<b>ANGPTL7</b>	Angiopoietin-like 7	12.17 ± 0.23	1.87
<b>ALOX12</b>	Arachidonate 12-lipoxygenase	12.17 ± 0.42	3.48
<b>EPX</b>	Eosinophil peroxidase	12.58 ± 0.41	3.29
<b>DUOX1</b>	Dual oxidase 1	12.78 ± 0.34	2.68
<b>SFTPD</b>	Surfactant protein D	12.57 ± 0.76	6.07
<b>PXDN</b>	Peroxidasin homolog (Drosophila)	13.21 ± 0.62	4.70
<b>GPX2</b>	Glutathione peroxidase 2 (gastrointestinal)	13.99 ± 0.47	3.36

**S1 Fig. Example flow cytometry median calcium traces from a donor obtained at Day 4 and Day 24.** After cold-binding with anti-CD3 and anti-CD28 as described in Materials and Methods, cells were sampled for 3 minutes by flow cytometry before addition of anti-mouse IgG. The average of the fluorescence ratio was calculated at 4 second intervals and then normalized to the average baseline value to provide a fold-change value.

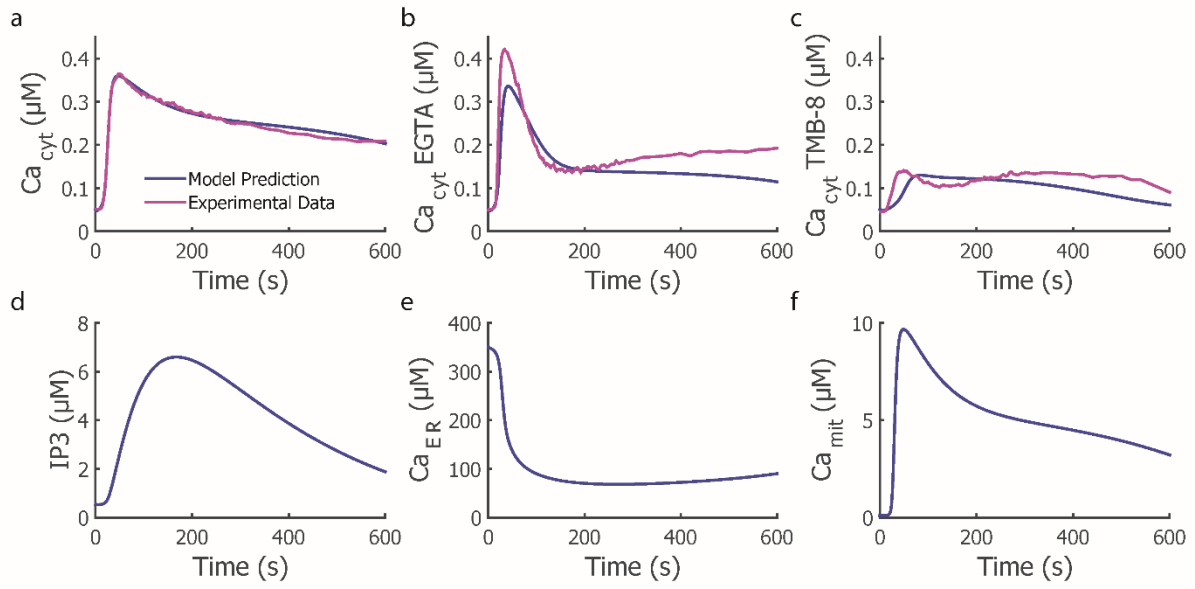


**S2 Fig. Optimization of Jurkat T Cell Model using data obtained by no inhibitor, TMB-8, and EGTA conditions.** Plots represent 17 different optimized parameter sets that were obtained from comparing the model prediction to experimental data.





**S3 Fig. Jurkat T Cell model behavior by parameter set used to fit Young CD8+ model**



**S4 Table. Optimized parameter set obtained from the Jurkat T Cell Model fitting used for the seeding the initial population of parameter values for the genetic algorithm optimization of the Young CD8<sup>+</sup> T Cell Model to experimental data.**

<b>Parameter</b>	<b>Jurkat T cells</b>
$\beta_i^*$	0.056
$\beta_{er}^*$	0.049
$\beta_{mit}$	0.033
$\rho_{er}$	0.015
$\rho_{mit}$	0.08
$k_{PLCact}$	0.0033 s <sup>-1</sup>
$k_{PLCdeact}$	0.042 s <sup>-1</sup>
$k_{IP3prod}$	0.48 μM <sup>-1</sup> s <sup>-1</sup>
$k_{IP3deg}^*$	0.010 s <sup>-1</sup>
$V_{IP3}$	4.0 s <sup>-1</sup>
$K_{IP3}$	0.57 μM
$K_{act}$	0.13 μM
$A$	0.079
$K_{inh}$	1 μM
$K_{IP3inh}^*$	0.82 μM
$K_{ERleak}^*$	0.0043 s <sup>-1</sup>
$V_{serca}^*$	112.75 μM s <sup>-1</sup>
$K_{serca}$	0.43 μM
$V_{mitin}$	388.6 μM s <sup>-1</sup>
$K_{mitin}$	0.81 μM
$V_{mitout}^*$	188.9 μM s <sup>-1</sup>
$K_{mitout}^*$	4.03 μM
$V_{crac}$	2.4 μM s <sup>-1</sup>
$K_{soc}^*$	363.5 μM
$K_{stim}$	178.1 μM

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$K_{PMleak}$	$1.1e-6 \text{ s}^{-1}$
$V_{pmca}^*$	$2.14 \text{ } \mu\text{M s}^{-1}$
$K_{pmca}$	$0.11 \text{ } \mu\text{M}$

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**S5 Table. Optimized parameter set obtained from fitting the Young CD8<sup>+</sup> T Cell Model to experimental data.** This parameter set was used for all sensitivity analysis performed on the Young CD8<sup>+</sup> T Cell Model.

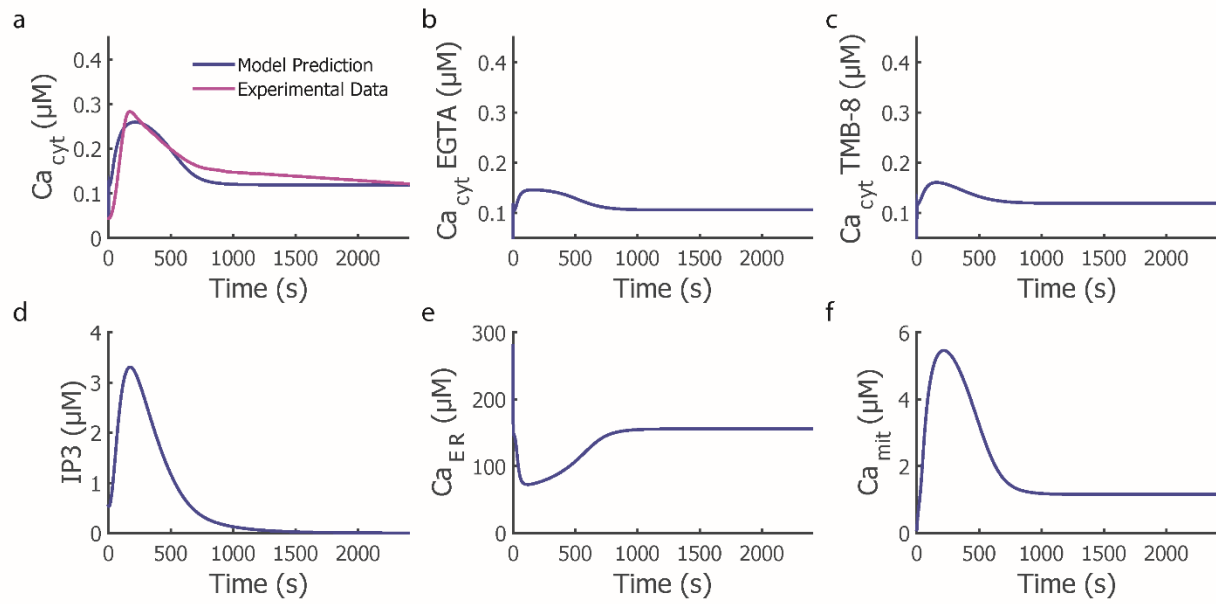
Parameter	Primary CD8 <sup>+</sup> T cells
$\beta_i^*$	0.047
$\beta_{er}^*$	0.98
$\beta_{mit}$	0.033
$\rho_{er}$	0.015
$\rho_{mit}$	0.08
$k_{PLCact}$	0.0033 s <sup>-1</sup>
$k_{PLCdeact}$	0.042 s <sup>-1</sup>
$k_{IP3prod}$	0.48 μM <sup>-1</sup> s <sup>-1</sup>
$k_{IP3deg}^*$	0.018 μs <sup>-1</sup>
$V_{IP3}$	4.0 μs <sup>-1</sup>
$K_{IP3}$	0.57 μM
$K_{act}$	0.13 μM
$A$	0.079
$K_{inh}$	1 μM
$K_{IP3inh}^*$	1.5 μM
$K_{ERleak}^*$	0.048 s <sup>-1</sup>
$V_{serca}^*$	103.88 μM s <sup>-1</sup>
$K_{serca}$	0.43 μM
$V_{mitin}$	388.6 μM s <sup>-1</sup>
$K_{mitin}$	0.81 μM
$V_{mitout}^*$	244.7 μM s <sup>-1</sup>
$K_{mitout}^*$	4.7 μM
$V_{crac}$	2.4 μM s <sup>-1</sup>

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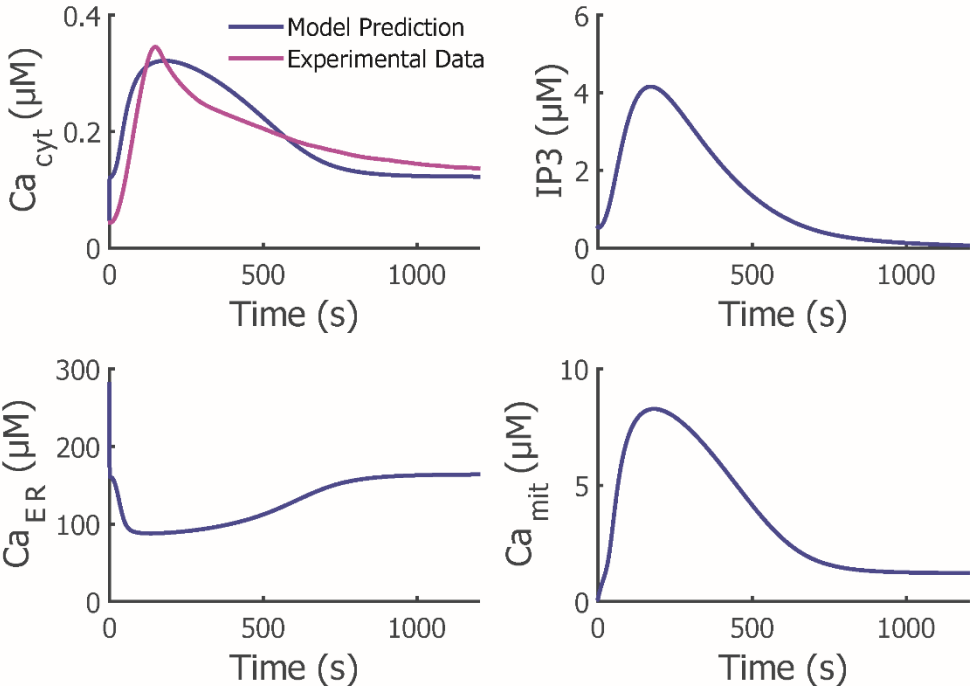
$K_{soc}^*$	358.8 $\mu\text{M}$
$K_{stim}$	178.1 $\mu\text{M}$
$K_{PMleak}$	1.1e-6 $\text{s}^{-1}$
$V_{pmca}^*$	2.08 $\mu\text{M s}^{-1}$
$K_{pmca}$	0.11 $\mu\text{M}$

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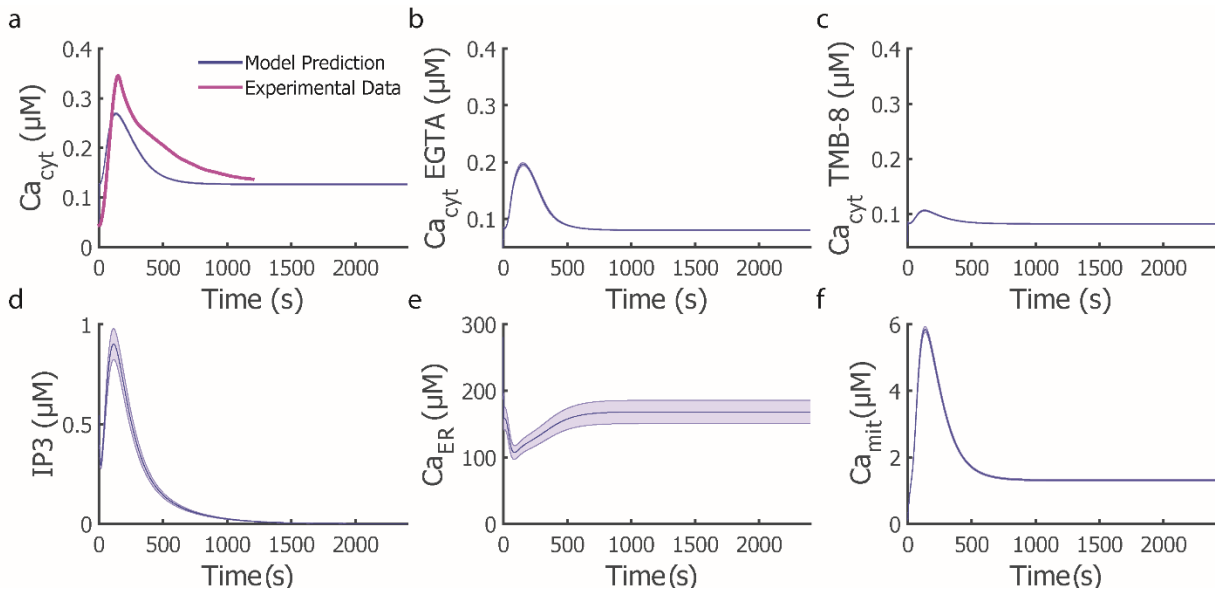
**S4 Fig. Optimized Young CD8<sup>+</sup> T Cell Model.**



S5 Fig. Best fit of Old CD8<sup>+</sup> T Cell Model varying only two parameters,  $V_{crac}$  and  $V_{pmca}$ .

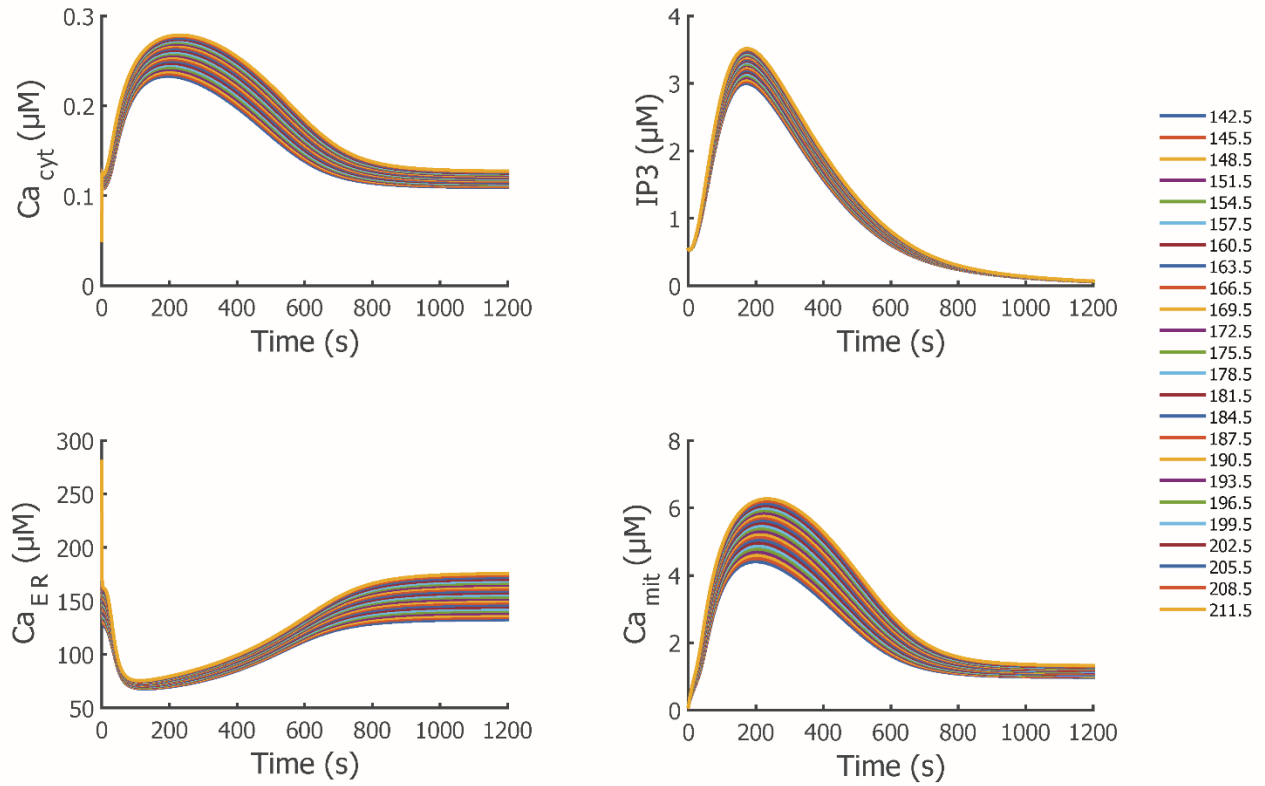


**S6 Fig. Best fit of Old CD8<sup>+</sup> Young T Cell Model varying the seven parameters as identified in the sensitivity analysis of the Young CD8<sup>+</sup> Young T Cell Model.**

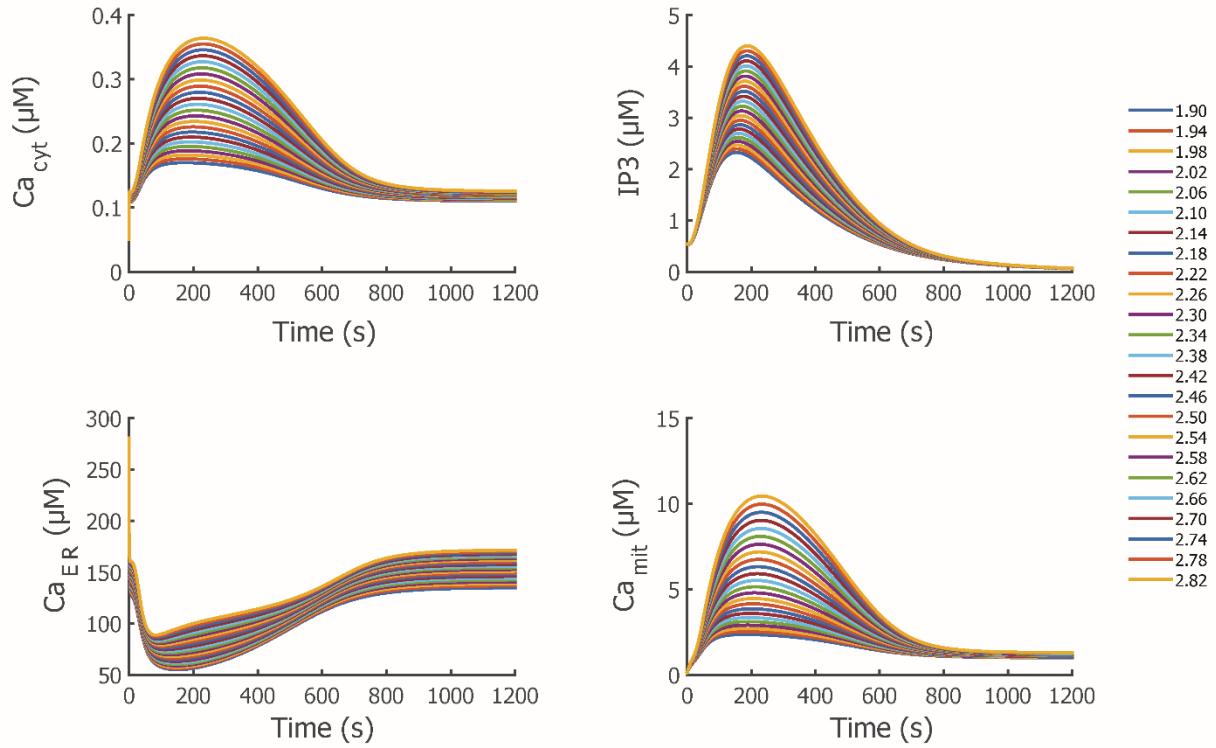




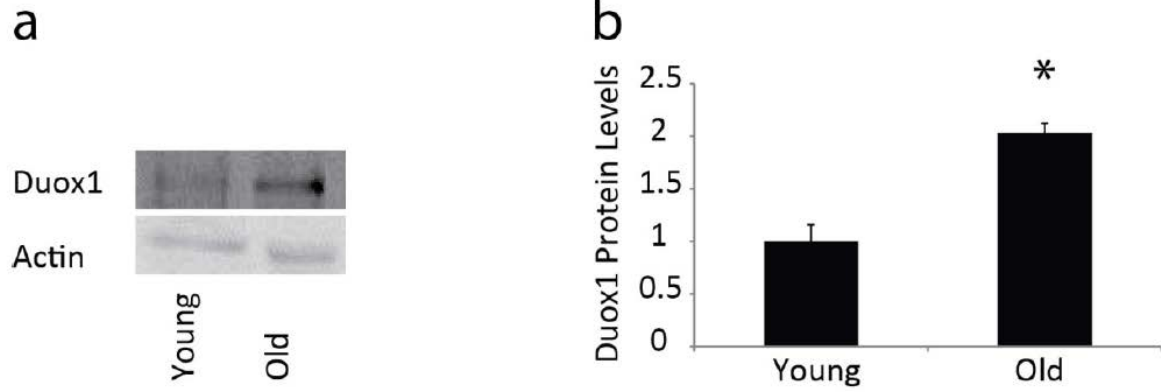
**S7 Fig. Varying  $K_{stim}$  from the Young CD8<sup>+</sup> T Cell Model fit to investigate the effects on calcium traces.  $K_{stim}$  was varied +/- 20% the fit value of 178.**



**S8 Fig. Varying  $V_{crac}$  from the Young CD8<sup>+</sup> T Cell Model to investigate the effects on calcium traces.  $V_{crac}$  was varied +/- 20% the fit value of 2.37.**



**S9 Fig. Validation of RT-PCR results with Duox 1 expression.** a) Representative Western Blot. b) Quantification of the Western Blots. Protein levels are normalized to the young cells protein expression level. \*  $p < 0.05$  (paired 2-tail t-test).



**S10 Fig. Expression of STIM1 in young and old primary human CD8+ T cells.**

