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Supplemental Information

Human GBP1 Differentially Targets *Salmonella* and *Toxoplasma* to License Recognition of Microbial Ligands and Caspase-Mediated Death

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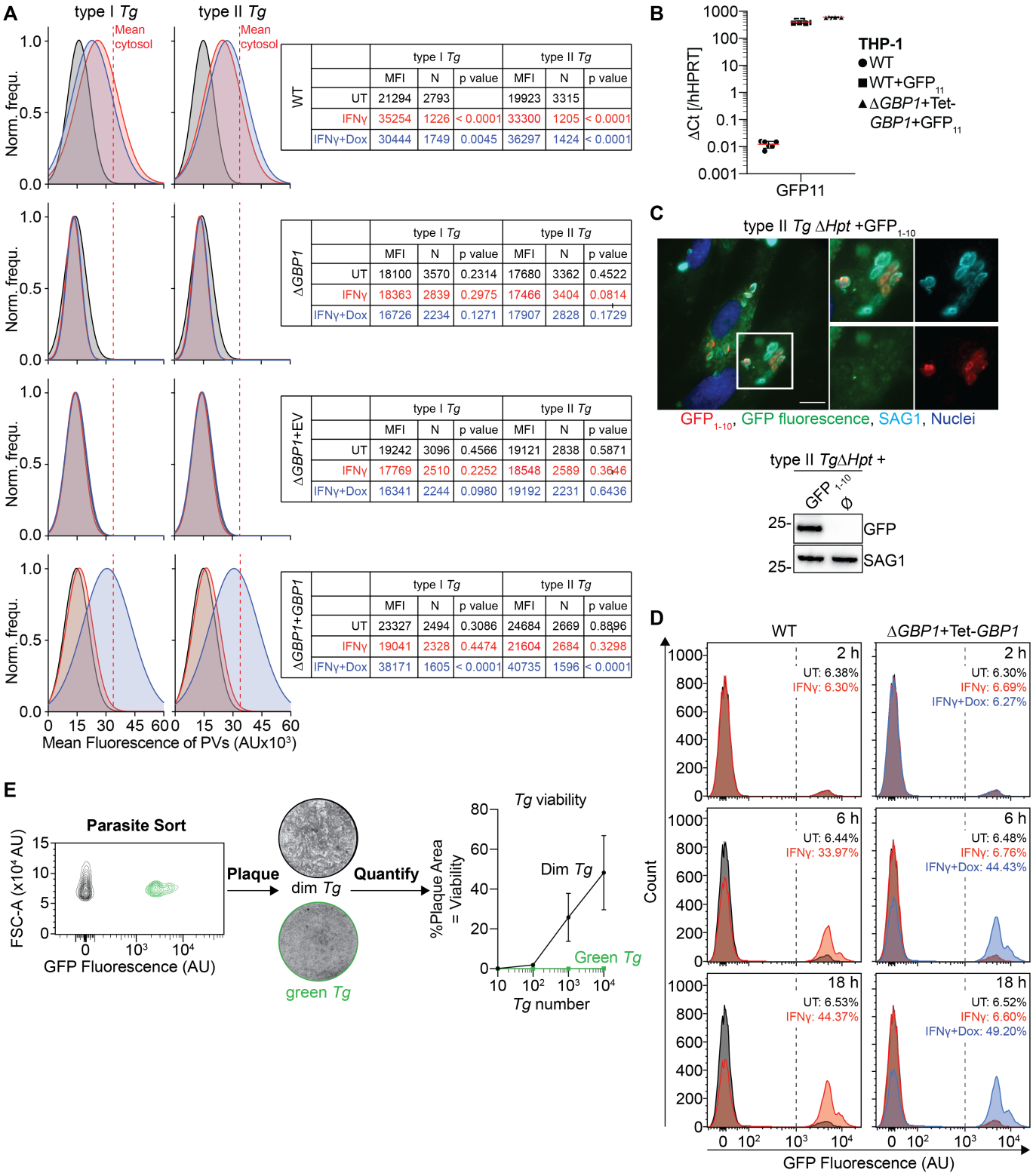

		type I *Tg*			type II *Tg*		
		MFI	N	p value	MFI	N	p value
WT	UT	21294	2793		19923	3315	
	IFNγ	35254	1226	< 0.0001	33300	1205	< 0.0001
	IFNγ+Dox	30444	1749	0.0045	36297	1424	< 0.0001

		type I *Tg*			type II *Tg*		
		MFI	N	p value	MFI	N	p value
ΔGBP1	UT	18100	3570	0.2314	17680	3362	0.4522
	IFNγ	18363	2839	0.2975	17466	3404	0.0814
	IFNγ+Dox	16726	2234	0.1271	17907	2828	0.1729

		type I *Tg*			type II *Tg*		
		MFI	N	p value	MFI	N	p value
ΔGBP1+EV	UT	19242	3096	0.4566	19121	2838	0.5871
	IFNγ	17769	2510	0.2252	18548	2589	0.3646
	IFNγ+Dox	16341	2244	0.0980	19192	2231	0.6436

		type I *Tg*			type II *Tg*		
		MFI	N	p value	MFI	N	p value
ΔGBP1+GBP1	UT	23327	2494	0.3086	24684	2669	0.8896
	IFNγ	19041	2328	0.4474	21604	2684	0.3298
	IFNγ+Dox	38171	1605	< 0.0001	40735	1596	< 0.0001

Figure S1: Novel dye influx assay and split-GFP system allows quantitation of GBP1 contribution to *Toxoplasma* vacuole and parasite disruption (Related to Figure 1).
(A) Representative normalized frequency plots (Norm. frequ.) and data tables of fluorescence intensities of vacuoles in type I or type II *Tg*-infected THP-1 WT, THP-1 Δ*GBP1*, THP-1 Δ*GBP1*+Tet-empty vector (EV) or THP-1 Δ*GBP1*+Tet-*GBP1* cells treated with IFNγ, Doxycycline (Dox) or left untreated and stained with CellMask. Mean fluorescence signal of the cytosol indicated by dashed red line and mean fluorescence intensity of the vacuoles (MFI) shown in table. N = number of vacuoles.
(B) RT-qPCR to confirm expression of GFP_{11} fragment in the cytosol of THP-1 WT and THP-1 Δ*GBP1*+Tet-*GBP1* cells transduced with GFP_{11}-Lentiviral particles.
(C) Representative immunofluorescence images (top) and immunoblot (bottom) from type II *Tg* Δ*Hpt*+GFP_{1-10} or type II *Tg* Δ*Hpt* to confirm expression of the GFP fragment and absence of GFP fluorescence. Red: anti-GFP for GFP_{1-10}; Green: GFP fluorescence; White: *Tg* surface antigen 1 (SAG1); Blue: Nuclei. Scale bar 20 µm.
(D) Representative flow cytometry analysis of proportion of GFP-fluorescing and thus disrupted parasites harvested from untreated (UT) or IFNγ-primed THP-1 WT+GFP_{11} or from untreated, IFNγ-only or IFNγ- and Doxycycline (Dox)-treated THP-1 Δ*GBP1*+Tet-*GBP1*+GFP_{11} cells at 2, 6 or 18 hours post infection. Proportion of fluorescing parasites above the threshold of 10^3 AU indicated in the figure.
(E) Viability determination of Pru Δ*HpT*+GFP_{1-10} parasites harvested from IFNγ-primed THP-1 WT+GFP_{11} cells at 18 hours p.i, sorted based on their fluorescence (left), plaqued onto HFF cells (middle) and quantification of plaque area depending on number of parasites used for plaque formation (right).
Data information: Graphs show mean ± SEM in **(B)** from n = 6 and in **(E)** from n = 3 independent experiments. Graphs in **(A)** representative of n = 3 independent experiments. *P* values in **(A)** from nested one-way ANOVA comparing means of n = 3 independent experiments from indicated condition to untreated WT cells.

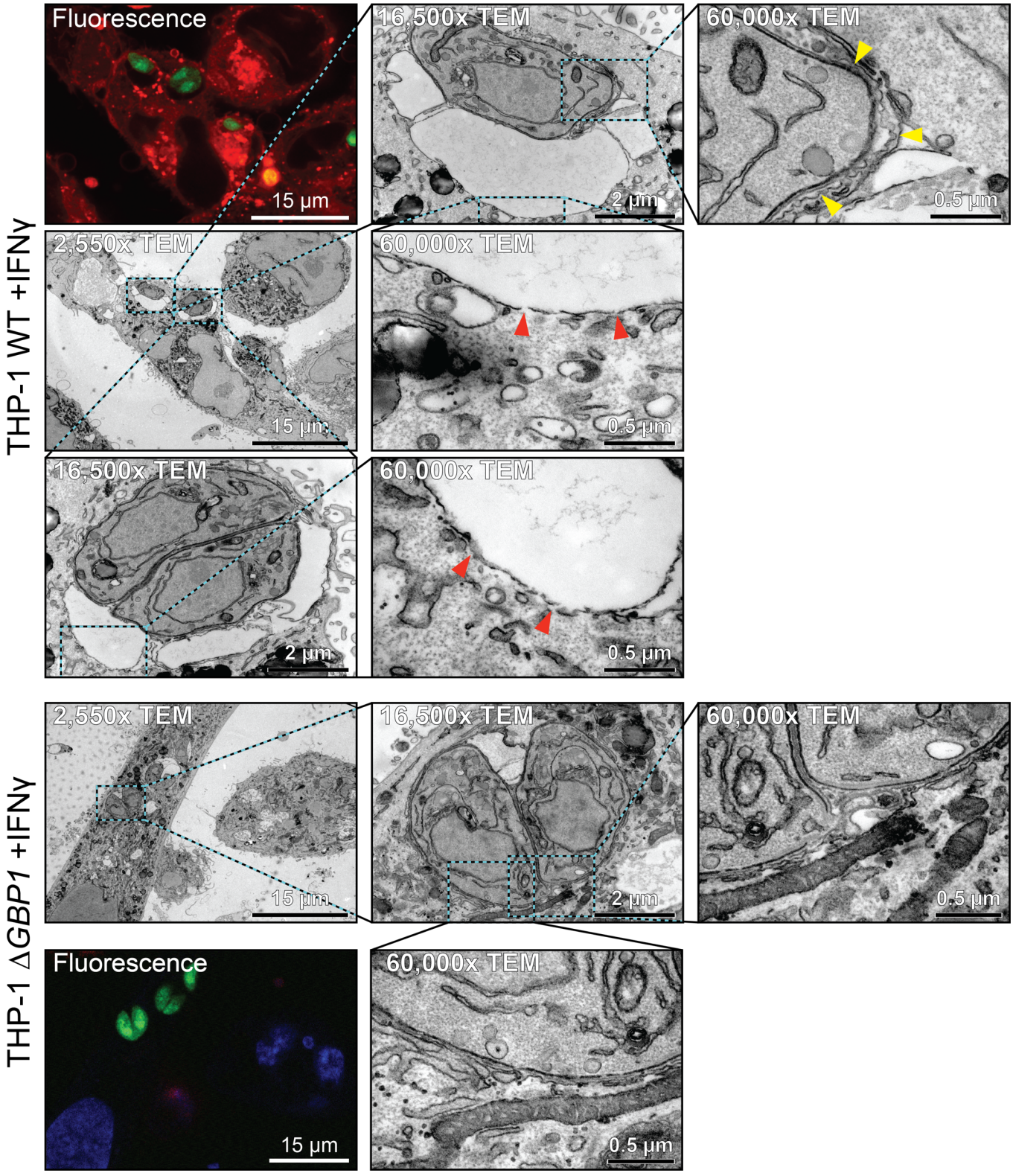
THP-1 WT +IFNγ
Fluorescence
15 µm
16,500x TEM
2 µm
60,000x TEM
0.5 µm
2,550x TEM
15 µm
60,000x TEM
0.5 µm
16,500x TEM
2 µm
60,000x TEM
0.5 µm
THP-1 ΔGBP1 +IFNγ
2,550x TEM
15 µm
16,500x TEM
2 µm
60,000x TEM
0.5 µm
Fluorescence
15 µm
60,000x TEM
0.5 µm

Figure S2: Correlative light and electron microscopy reveals ultrastructural defects of GBP1-targeted *Toxoplasma* vacuole membranes (Related to Figure 1).
Representative images of correlative light and electron microscopy of THP-1 WT or Δ*GBP1* cells (flooded with CellMask for fluorescence imaging), pre-treated with IFNγ to induce GBP1 expression and infected with type I (RH) *Tg* for 6 hours. Parasites indicated boxes are shown at higher magnifications as indicated (TEM, transmission electron microscopy). Yellow arrowheads mark areas of vacuole membrane ruffling and red arrowheads mark areas of vacuole membrane disruption. Red: CellMask; Green: *Tg*; Blue: Nuclei. Scale bars as indicated.

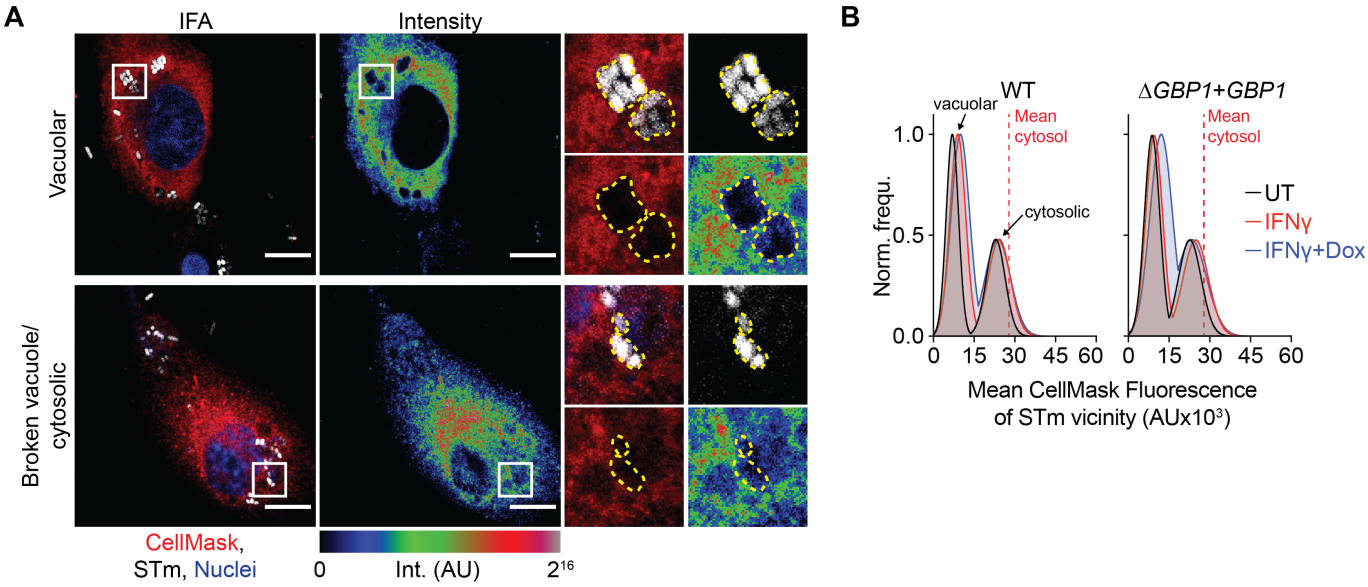

Figure S3: CellMask dye influx assay for measuring *Salmonella* vacuole escape (Related to Figure 2).

(A) Representative immunofluorescence images and fluorescence intensity map of intact *Salmonella* Typhimurium (STm)-containing vacuoles (SCVs) or broken SCV/STm in the cytosol of IFNγ-primed and CellMask-flooded THP-1 WT macrophages at 4 hours p.i. Area of interest for fluorescence intensity measurement as automatically determined by HRMAn indicated by the yellow, dashed line. Red: CellMask; Grey: STm; Blue: Nuclei. Scale bars 10 µm.

(B) Representative normalized frequency plots (Norm. frequ.) of fluorescence intensities of vicinity of STm in infected THP-1 WT or THP-1 Δ*GBP1*+Tet-*GBP1* cells treated with IFNγ, Doxycycline (Dox) or left untreated and stained with CellMask. Mean fluorescence signal of the cytosol indicated by dashed red line.

Data information: Graphs in **(B)** representative of n = 2 independent experiments.

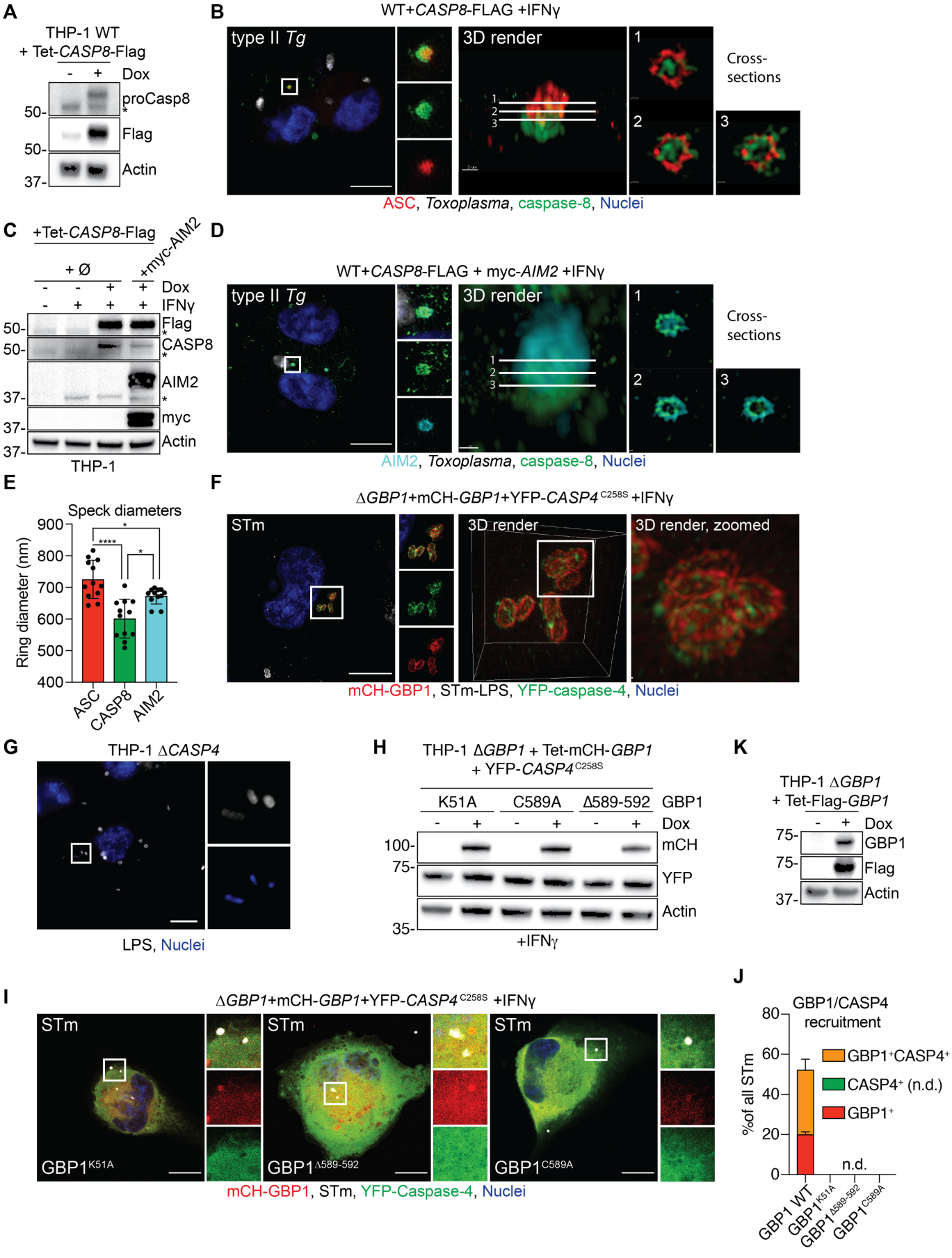
A
THP-1 WT
+ Tet-CASP8-Flag
- + Dox
50-
proCasp8
*
50-
Flag
37-
Actin
B
WT+CASP8-FLAG +IFNγ
type II Tg
3D render
1
2
3
Cross-sections
ASC, Toxoplasma, caspase-8, Nuclei
C
+Tet-CASP8-Flag
+ Ø
+myc-AIM2
Dox
IFNγ
50-
Flag
*
50-
CASP8
*
37-
AIM2
*
37-
myc
37-
Actin
THP-1
D
WT+CASP8-FLAG + myc-AIM2 +IFNγ
type II Tg
3D render
Cross-sections
AIM2, Toxoplasma, caspase-8, Nuclei
E
Speck diameters
Ring diameter (nm)
900
800
700
600
500
400
****
*
*
ASC
CASP8
AIM2
F
ΔGBP1+mCH-GBP1+YFP-CASP4 C258S +IFNγ
STm
3D render
3D render, zoomed
mCH-GBP1, STm-LPS, YFP-caspase-4, Nuclei
G
THP-1 ΔCASP4
LPS, Nuclei
H
THP-1 ΔGBP1 + Tet-mCH-GBP1
+ YFP-CASP4 C258S
K51A
C589A
Δ589-592
GBP1
- + - + - + Dox
100-
mCH
75-
YFP
Actin
35-
+IFNγ
K
THP-1 ΔGBP1
+ Tet-Flag-GBP1
- + Dox
75-
GBP1
75-
Flag
37-
Actin
I
ΔGBP1+mCH-GBP1+YFP-CASP4 C258S +IFNγ
STm
GBP1 K51A
STm
GBP1 Δ589-592
STm
GBP1 C589A
mCH-GBP1, STm, YFP-Caspase-4, Nuclei
J
GBP1/CASP4
recruitment
%of all STm
80
60
40
20
0
GBP1+CASP4+
CASP4+ (n.d.)
GBP1+
n.d.
GBP1 WT
GBP1 K51A
GBP1 Δ589-592
GBP1 C589A

Figure S4: SIM of caspase activation platforms, molecular determinants of GBP1 and caspase-4 recruitment to cytosolic *Salmonella* and cell line verification immunoblots (Related to Figure 3).

(A) Representative immunoblots for proCaspase-8, Flag and β-actin from THP-1 +Tet-*CASP8*-Flag cells showing Doxycycline (Dox)-inducible caspase-8-Flag expression. Cells were treated with Dox as indicated or left untreated. * endogenous caspase-8.

(B) Left: Representative structured illumination immunofluorescence microscopy images from THP-1 WT+Tet-*CASP8*-Flag cells treated with IFNγ and Dox to induce caspase-8 expression and infected with type II *Toxoplasma gondii* (*Tg*) for 4 hours. Right: 3D reconstruction and slices through the ASC-caspase-8 speck. Red: ASC; Grey: *Tg*; Green: caspase-8; Blue: Nuclei. Scale bar 10 µm.

(C) Representative immunoblots for Flag, caspase-8 (CASP8), AIM2, myc and β-actin from THP-1 WT, THP-1 +Tet-*CASP8*-Flag and THP-1 +Tet-*CASP8*-Flag+myc-*AIM2* cells showing Dox-inducible caspase-8-Flag expression and constitutive expression of myc-AIM2. Cells were treated with IFNγ, Dox or left untreated as indicated. * endogenous proteins.

(D) Left: Representative structured illumination immunofluorescence microscopy images from THP-1+Tet-*CASP8*-Flag+myc-*AIM2* cells treated with IFNγ and Dox and infected with type II *Tg* for 4 hours. Right: 3D reconstruction and slices through the AIM2-caspase-8 speck. Cyan: AIM2; Grey: *Tg*; Green: caspase-8; Blue: Nuclei. Scale bar 10 µm.

(E) Ring diameters of the indicated proteins within an inflammasome speck of cells shown in **(B)** and **(D)**.

(F) Left: Representative structured illumination immunofluorescence microscopy images from THP-1 Δ*GBP1*+Tet-mCH-*GBP1*+YFP-*CASP4*^{C258S} cell treated with IFNγ and Dox and infected with *Salmonella* Typhimurium (STm) SL1344 (MOI = 30) for 2 hours. Right: 3D reconstruction of the GBP1-caspase-4 signaling platform on the cytosolic STm. Red: mCH-GBP1; Grey: STm-LPS; Green: YFP-caspase-4; Blue: Nuclei. Scale bar 10 µm.

(G) Representative immunofluorescence image of THP-1 Δ*CASP4* cells infected with STm SL1344 (MOI = 30) for 1 hour and stained with monoclonal anti-*Salmonella*-LPS antibody. Grey: STm-LPS; Blue: Nuclei. Scale bar: 5 µm.

(H) Representative immunoblots for mCherry, YFP and β-actin from THP-1 Δ*GBP1*+Tet-mCH-*GBP1* cells expressing the indicated mutant of GBP1 and also stably expressing YFP-*CASP4*^{C258S}. Cells were primed with IFNγ and treated with Doxycycline (Dox) as indicated.

(I) Representative immunofluorescence images and **(J)** quantification of GBP1 and caspase-4 recruitment to STm in IFNγ-primed and Dox-treated THP-1 Δ*GBP1*+Tet-mCH-*GBP1*+YFP-*CASP4*^{C258S} cells infected with STm SL1344 (MOI = 30) for 2 hours. Cells expressed the indicated mutants of GBP1. Red: mCH-GBP1; Grey: STm; Green: YFP-caspase-4; Blue: Nuclei. Scale bar 10 µm; n.d. not detected.

(K) Representative immunoblots for GBP1, Flag and β-actin from THP-1 Δ*GBP1*+Tet- Flag-*GBP1* cells showing Dox-inducible Flag-GBP1 expression. Cells were treated with Doxycycline (Dox) as indicated or left untreated.

Data information: Graph in **(E)** shows quantification from n = 12 inflammasome specks and mean ± SEM. Graph in **(J)** shows mean ± SEM of n = 3 independent experiments. * *P ≤ 0.05;* **** *P ≤ 0.0001* for indicated comparisons in **(E)** from one-way ANOVA following adjustment for multiple comparisons.

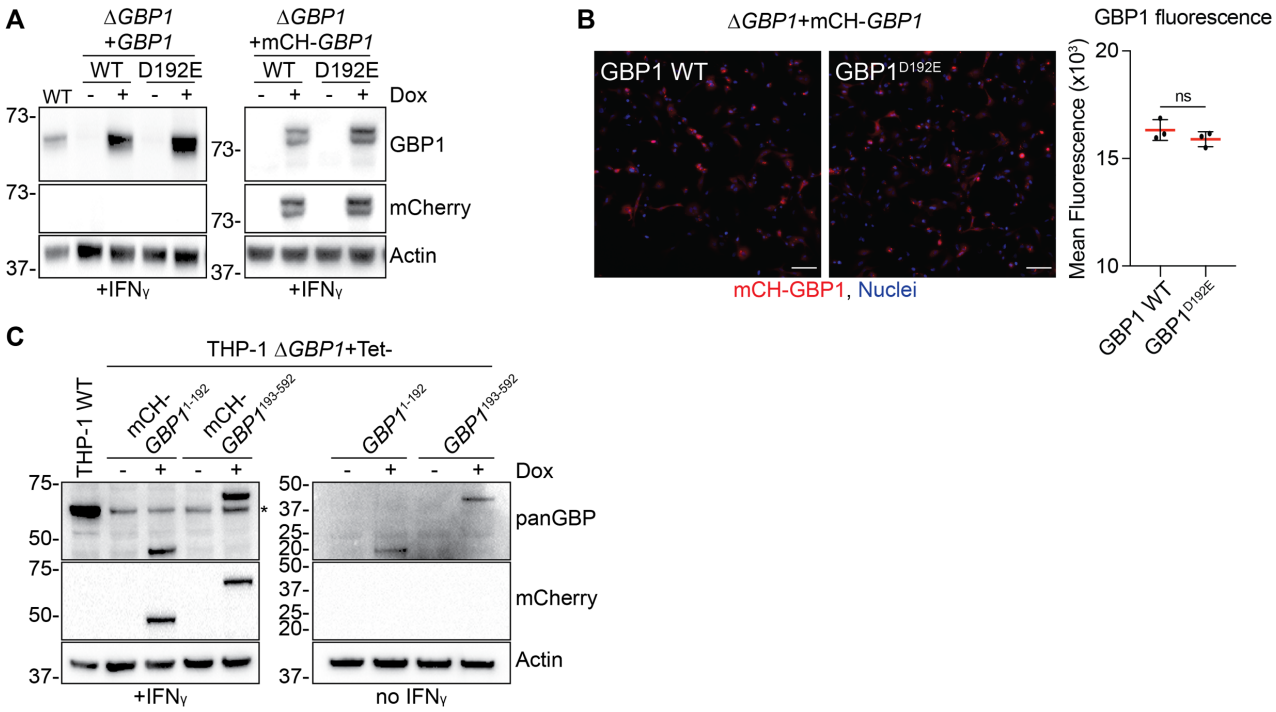

Figure S5: Verification and quality control of GBP1^{D192} mutant and fragment cell lines (Related to Figure 4 and 5).

(A) Representative immunoblots of GBP1, mCherry (mCH) and β-actin from IFNγ-primed THP-1 Δ*GBP1*+Tet-*GBP1* or *GBP1*^{D192E} with and without mCH-tag for Doxycycline (Dox)-inducible expression. Cells were treated with Dox as indicated.

(B) Representative images and quantification of mean fluorescence per cell from 100 fields of view from THP-1 Δ*GBP1*+Tet-mCH-*GBP1* or +Tet-mCH-*GBP1*^{D192E} cells treated with IFNγ and Dox to induce mCH-GBP1 expression. Red: mCH-GBP1; Blue: Nuclei. Scale bars 100 µm.

(C) Immunoblot for human GBPs (panGBP), mCherry and Actin to confirm Dox-inducible expression of GBP1 fragments 1-192 or 193-592 with and without mCherry-tag in THP-1 Δ*GBP1*+Tet cells. * Marks endogenous, other GBP family members detected by the panGBP antibody in IFNγ-treated cells.

Data information: Graph in **(B)** shows mean ± SEM from n = 3 independent experiments. *P* values in **(B)** from t-test. ns, not significant.

Table S1: Oligonucleotide primer.

Overview of oligonucleotide primers used in this study for molecular cloning of new plasmids and RT-qPCR. (Related to STAR methods section)

Cloning primer		
Name	**Sequence 5'-3'**	**Purpose**
pTet_*CASP8*-fwd	TGCGGCCGCACCATGGGCGGTAGGCGTGTAC	Amplify *CASP8* ORF for cloning into pTet backbone
Flag_*CASP8*-rev	GTGGTCCTTATAGTCATCAGAAGGGAAGACAAGTTTTTTTCTTAGTGTGAAAG	
Casp-8 _Flag-fwd	GTCTTCCCTTCTGATGACTATAAGGACCACGACGG	Amplify Flag-tag for cloning into pTet backbone with *CASP8* ORF
pTet-Flag-rev	GATCGATCAGGGATCCTACTTATCGTCATCGTCTTTGTAATCAATATC	
Flag_*GBP1*-fwd	GATATTGATTACAAAGACGATGACGATAAGATGGCATCAGAGATCCACATGACAGG	Amplify *GBP1* ORF for cloning into pTet backbone
pTet_*GBP1*-rev	CTAGCGAATTCGGCCGATCGATCAGGGATCTTAGCTTATGGTACATGCCTTTCGTCGTC	
pTet_Flag-fwd	AATTAGCGCTACCGGTGCGGCCGCACCATGGACTATAAGGACCACGACGGAG	Amplify Flag-tag for cloning into pTet backbone with *GBP1* ORF
GBP1 _Flag-rev	TGGGCCTGTCATGTGGATCTCTGATGCCATCTTATCGTCATCGTCTTTGTAATCAATATCATGATCCTTG	
GBP1-D192E-fwd	CCCTGGACTTGGAAGCAGAGGGACAACCCC	Mutate *GBP1* ORF
GBP1-D192E-rev	GGGACCTGAACCTTCGTCTCCCTGTTGGGG	
pLEX-*AIM2*-fwd	CTCGAGCTCAAGCTTGCCACCATGGAACAGAAACTCATCTCTGAAGAGGATCTG	Amplify myc-*AIM2* ORF for cloning into pLEX backbone
pLEX-*AIM2*-rev	CCGCTTTACTTGTACCCTAGAATAGGGCCCTCTAGATGCATGCTC	
pLenti-repair-1-fwd	CGGGAGTATCCG	Repair digested lentiCRISPR-V2
pLenti-repair-1-frev	AATTCGGATACTCCCGGTAC	
pLenti-repair-2-fwd	CTAGACTCGAGGATCG	Repair digested lentiCRISPR-V2 and add multiple cloning site
pLenti-repair-2-rev	GATCCGATCCTCGAGT	
GFP11-PCR-fwd	AACACAGGACCGGTTGCCACCATGCGCGATCACATGGTCCTGCT	Amplify GFP11 ORF for cloning into pLenti-P2A-Puro backbone
GFP11-PCR-rev	TTGTTGCGCCGGATCCCTTGTACAGCTCGTCCATGCCG	
pGRA-GFP1-10-fwd	ATCAAGCAAGATGCAAATGTTCGCCGTAAAACATTGTTTGCTGG	Amplify GFP1-10 ORF for cloning into pGRA-HA-HPT backbone
pGRA-GFP1-10-rev	TTCGTCGTAGTCTTATTATTTTTCATTTGGATCTTTGCTCAGGACTGTTTGT	
pTet-GBP1_1-192-fwd	AATTAGCGCTACCGGTGCGGCCGCACCATGGCATCAGAGATCCACATGACAGG	Amplify GBP1 fragment 1-192 for cloning into pLenti-Tet
pTet-GBP1_1-192-rev	CTAGCGAATTCGGCCGATCGATCAGGGATCTTAATCTGCTTCCAAGTCCAGGGAGAAAT	
pTet-GBP1_193-593-fwd	AATTAGCGCTACCGGTGCGGCCGCACCATGGGACAACCCCTCACACCAGATG	Amplify GBP1 fragment 193-592 for cloning into pLenti-Tet
pTet-GBP1_193-593-rev	CTAGCGAATTCGGCCGATCGATCAGGGATCTTAGCTTATGGTACATGCCTTTCGTCGTC	
pTet-mCH-fwd	AATTAGCGCTACCGGTGCGGCCGCACCATGGTGAGCAAGGGCGAGG	Amplify mCherry for cloning into pLenti-Tet and tagging of the two GBP1 fragments
GBP1_1-192-mCH-rev	TGGGCCTGTCATGTGGATCTCTGATGCCATCTTGTACAGCTCGTCCATGCC	
GBP1_193-593-mCH-rev	TGTGAGGGGTTGTCCCATCTTGTACAGCTCGTCCATGCC	
mCH-GBP1_1-192-fwd	TCCACCGGCGGCATGGACGAGCTGTACAAGATGGCATCAGAGATCCACATGACAGG	Amplify GBP1 fragment 1-192 for cloning into pLenti-Tet and tagging with mCherry
pTet-GBP1_1-192-rev	CTAGCGAATTCGGCCGATCGATCAGGGATCTTAATCTGCTTCCAAGTCCAGGGAGAAAT	
mCH-GBP1_193-593-fwd	TCCACCGGCGGCATGGACGAGCTGTACAAGATGGGACAACCCCTCACACCAGATG	Amplify GBP1 fragment 193-592 for cloning into pLenti-Tet and tagging with mCherry
pTet-GBP1_193-593-rev	CTAGCGAATTCGGCCGATCGATCAGGGATCTTAGCTTATGGTACATGCCTTTCGTCGTC	
qCPR primer		
Name	**Sequence 5'-3'**	**Purpose**
GFP11-fwd	AATCCTGGACCGACCGAGTA	qPCR for GFP11
GFP11-rev	GAGTTCTTGCAGCTCGGTGA	
HPRT-fwd	ACCAGTCAACAGGGGACATAA	qPCR for HPRT
HPRT-rev	CTTCGTGGGGTCCTTTTCACC	

Table S2. Electron Microscopy sample preparation protocol.

Full BioWave program details for preparation of samples for electron microscopy. (Related to STAR methods section)

Description	Step#	Time (min)	Time (sec)	Power (Watts)	SteadyTemp temperature (ºC)	Vacuum cycle vent time (sec)	Vacuum cycle vacuum time (sec)	Vacuum set point (inch Hg)	User Prompt (1 = YES, 0 = NO)	Vacuum OFF (1 = no vacuum, 0 = vacuum)	Vacuum cycle (1 = ON, 0 = OFF)	Vacuum ON (1 = vacuum, 0 = no vacuum)
BENCH STEP Rinse in 0.1M PB	1	0	0	0	21	0	0	0	1	1	0	0
BENCH STEP Rinse in 0.1M PB	2	0	0	0	21	0	0	0	1	1	0	0
Rinse in 0.1M PB	3	0	40	250	21	0	0	0	1	1	0	0
Rinse in 0.1M PB	4	0	40	250	21	0	0	0	1	1	0	0
Osmium ON	5	2	0	100	21	0	0	20	1	0	0	1
Osmium OFF	6	2	0	0	21	0	0	20	0	0	0	1
Osmium ON	7	2	0	100	21	0	0	20	0	0	0	1
Osmium OFF	8	2	0	0	21	0	0	20	0	0	0	1
Osmium ON	9	2	0	100	21	0	0	20	0	0	0	1
Osmium OFF	10	2	0	0	21	0	0	20	0	0	0	1
Osmium ON	11	2	0	100	21	0	0	20	0	0	0	1
BENCH STEP Rinse in water	12	0	0	0	21	0	0	0	1	1	0	0
BENCH STEP Rinse in water	13	0	0	0	21	0	0	0	1	1	0	0
Rinse in water	14	0	40	250	21	0	0	0	1	1	0	0
Rinse in water	15	0	40	250	21	0	0	0	1	1	0	0
Thiocarbohydrazide ON	16	2	0	100	40	0	0	20	1	0	0	1
Thiocarbohydrazide OFF	17	2	0	0	40	0	0	20	0	0	0	1
Thiocarbohydrazide ON	18	2	0	100	40	0	0	20	0	0	0	1
Thiocarbohydrazide OFF	19	2	0	0	40	0	0	20	0	0	0	1
Thiocarbohydrazide ON	20	2	0	100	40	0	0	20	0	0	0	1
Thiocarbohydrazide OFF	21	2	0	0	40	0	0	20	0	0	0	1
Thiocarbohydrazide ON	22	2	0	100	40	0	0	20	0	0	0	1
BENCH STEP Rinse in water	23	0	0	0	21	0	0	0	1	1	0	0
BENCH STEP Rinse in water	24	0	0	0	21	0	0	0	1	1	0	0
Rinse in water	25	0	40	250	21	0	0	0	1	1	0	0
Rinse in water	26	0	40	250	21	0	0	0	1	1	0	0
Osmium ON	27	2	0	100	21	0	0	20	1	0	0	1
Osmium OFF	28	2	0	0	21	0	0	20	0	0	0	1
Osmium ON	29	2	0	100	21	0	0	20	0	0	0	1
Osmium OFF	30	2	0	0	21	0	0	20	0	0	0	1
Osmium ON	31	2	0	100	21	0	0	20	0	0	0	1
Osmium OFF	32	2	0	0	21	0	0	20	0	0	0	1
Osmium ON	33	2	0	100	21	0	0	20	0	0	0	1
BENCH STEP Rinse in water	34	0	0	0	21	0	0	0	1	1	0	0
BENCH STEP Rinse in water	35	0	0	0	21	0	0	0	1	1	0	0
Rinse in water	36	0	40	250	21	0	0	0	1	1	0	0
Rinse in water	37	0	40	250	21	0	0	0	1	1	0	0
Uranyl acetate ON	38	2	0	100	40	0	0	20	1	0	0	1
Uranyl acetate OFF	39	2	0	0	40	0	0	20	0	0	0	1
Uranyl acetate ON	40	2	0	100	40	0	0	20	0	0	0	1
Uranyl acetate OFF	41	2	0	0	40	0	0	20	0	0	0	1
Uranlyl acetate ON	42	2	0	100	40	0	0	20	0	0	0	1
Uranyl acetate OFF	43	2	0	0	40	0	0	20	0	0	0	1
Uranyl acetate ON	44	2	0	100	40	0	0	20	0	0	0	1
BENCH STEP Rinse in water	45	0	0	0	40	0	0	0	1	1	0	0
BENCH STEP Rinse in water	46	0	0	0	40	0	0	0	1	1	0	0
Rinse in water	47	0	45	250	40	0	0	0	1	1	0	0
Rinse in water	48	0	45	250	40	0	0	0	1	1	0	0
Lead aspartate ON	49	2	0	100	50	0	0	20	1	0	0	1
Lead aspartate OFF	50	2	0	0	50	0	0	20	0	0	0	1
Lead aspartate ON	51	2	0	100	50	0	0	20	0	0	0	1
Lead aspartate OFF	52	2	0	0	50	0	0	20	0	0	0	1
Lead aspartate ON	53	2	0	100	50	0	0	20	0	0	0	1
Lead aspartate OFF	54	2	0	0	50	0	0	20	0	0	0	1
Lead aspartate ON	55	2	0	100	50	0	0	20	0	0	0	1
BENCH STEP Rinse in water	56	0	0	0	21	0	0	0	1	1	0	0
BENCH STEP Rinse in water	57	0	0	0	21	0	0	0	1	1	0	0
Rinse in water	58	0	45	250	21	0	0	0	1	1	0	0
Rinse in water	59	0	45	250	21	0	0	0	1	1	0	0
70% Ethanol ON	60	0	40	250	21	0	0	0	1	1	0	0
70% Ethanol ON	61	0	40	250	21	0	0	0	1	1	0	0
90% Ethanol ON	62	0	40	250	21	0	0	0	1	1	0	0
90% Ethanol ON	63	0	40	250	21	0	0	0	1	1	0	0
100% Ethanol ON	64	0	40	250	21	0	0	0	1	1	0	0
100% Ethanol ON	65	0	40	250	21	0	0	0	1	1	0	0
50% Resin ON	66	3	0	250	21	30	30	20	1	0	1	0
100% Resin ON	67	3	0	250	21	30	30	20	1	0	1	0
100% Resin ON	68	3	0	250	21	30	30	20	1	0	1	0
100% Resin ON	69	3	0	250	21	30	30	20	1	0	1	0
100% Resin ON	70	3	0	250	21	30	30	20	1	0	1	0
TURN SYSTEM OFF	71	0	0	0	21	0	0	0	0	1	0	0