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Supplementary Materials for

PIM1 controls GBP1 activity to limit self-damage and to guard against pathogen infection

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SUPPLEMENTARY FIGURES:

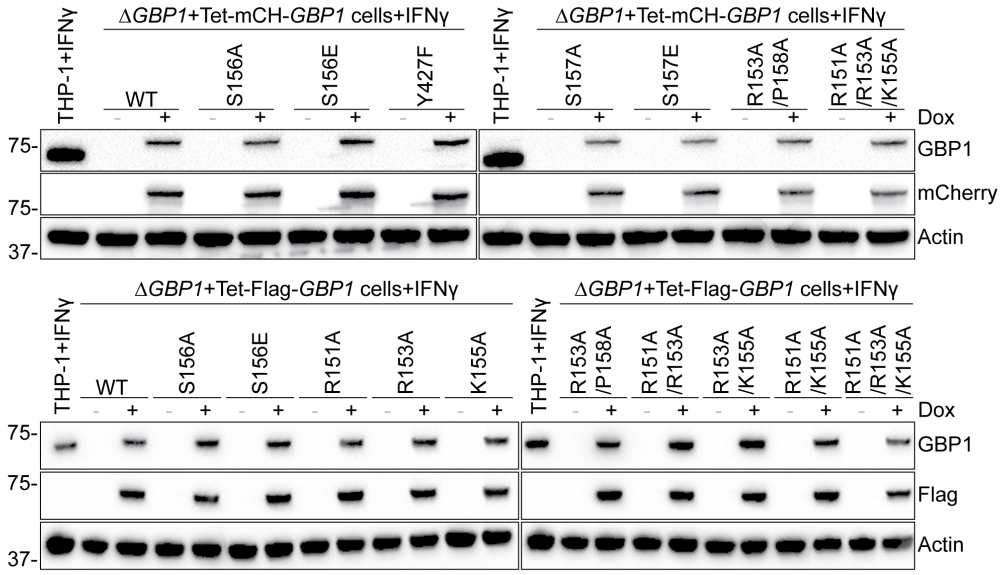

Fig. S1: Validation of GBP1-expressing THP-1 cell lines.

(A) Immunoblots of IFNγ-primed THP-1 WT, THP-1Δ*GBP1*+Tet-mCH-*GBP1* or THP-1Δ*GBP1*+Tet-Flag-*GBP1* cells expressing the indicated mutant of GBP1 under a Dox-inducible promoter. For gel source data, see **Data S5**.

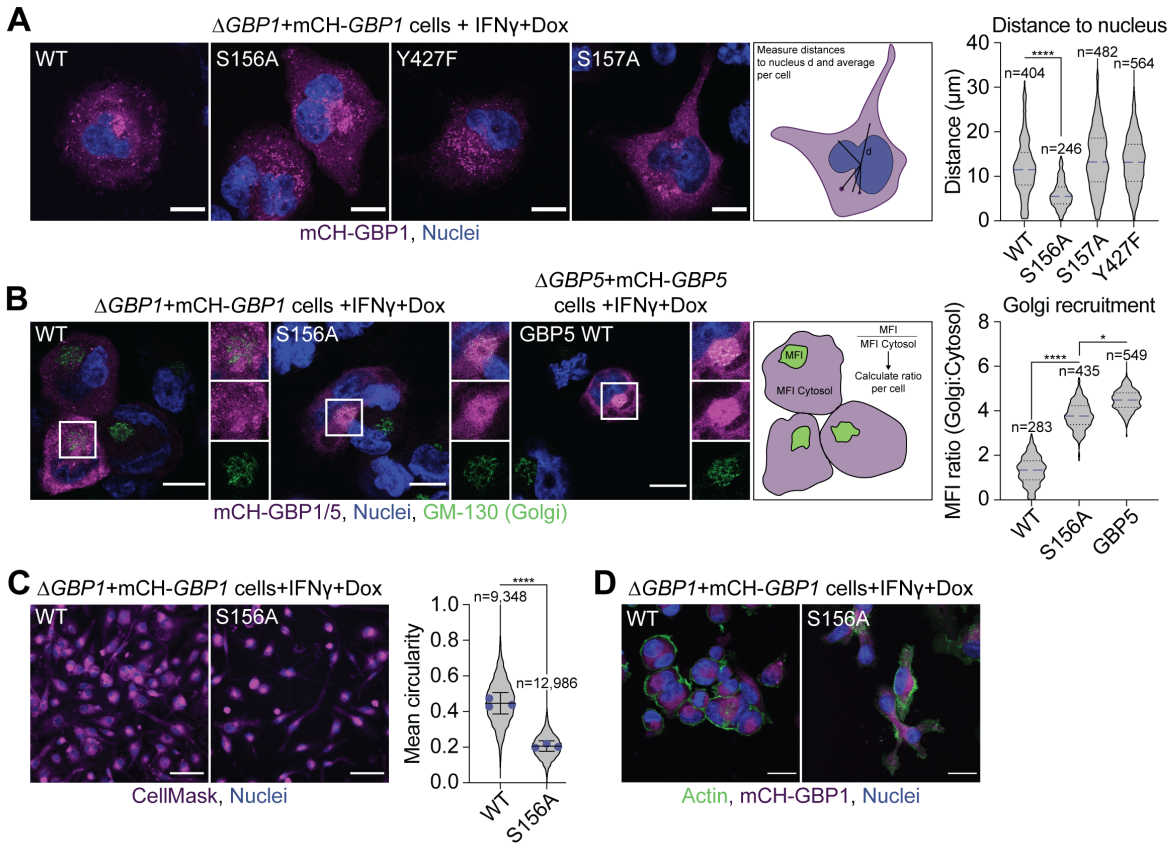

Fig. S2: Ser156 phosphorylation changes GBP1 localization.

(A) Immunofluorescence images showing localization of indicated, mCherry-tagged mutants of GBP1 or GBP1 WT, expressed in human THP-1Δ*GBP1* macrophages treated with IFNγ+Dox (left), cartoon of quantification method (middle) and quantification of average distance of GBP1 aggregates to the nucleus (right). Magenta: mCH-GBP1; Blue: Nuclei; Scale bar 10 µm. **(B)** Immunofluorescence images showing localization of mCH-GBP1 WT or S156A mutant expressed in IFNγ+Dox-treated THP-1Δ*GBP1* or mCH-GBP5 in THP-1Δ*GBP5* cells and stained for the Golgi marker GM-130 (left), cartoon of quantification method (middle) and quantification of mean fluorescence intensity (MFI) ratio of GBPs in the cytosol and the Golgi (right). Green: GM-130 (Golgi); Magenta: mCH-GBP1; Blue: Nuclei; Scale bar 10 µm. **(C)** Immunofluorescence images showing appearance of IFNγ+Dox-treated THP-1Δ*GBP1* cells expressing mCH-GBP1 WT or S156A mutant (left) and cell circularity quantification (right). Magenta: CellMask; Blue: Nuclei; Scale bar 100 µm. **(D)** Immunofluorescence images showing cortical actin cytoskeleton of IFNγ+Dox-treated THP-1Δ*GBP1* cells expressing mCH-GBP1 WT or S156A mutant. Green: Phalloidin; Magenta: mCH-GBP1; Blue: Nuclei; Scale bar 20 µm. **Data information:** Images in **(A-D)** representative of n = 3 experiments. Graph in **(A-C)** shows distribution of datapoints across n = 3 experiments (grey area) as well as mean of the repeats ± SD. Total number of quantified cells indicated in the figure. *** $P \leq 0.001$; **** $P < 0.0001$ for indicated comparisons in **(A-C)** from nested t-tests.

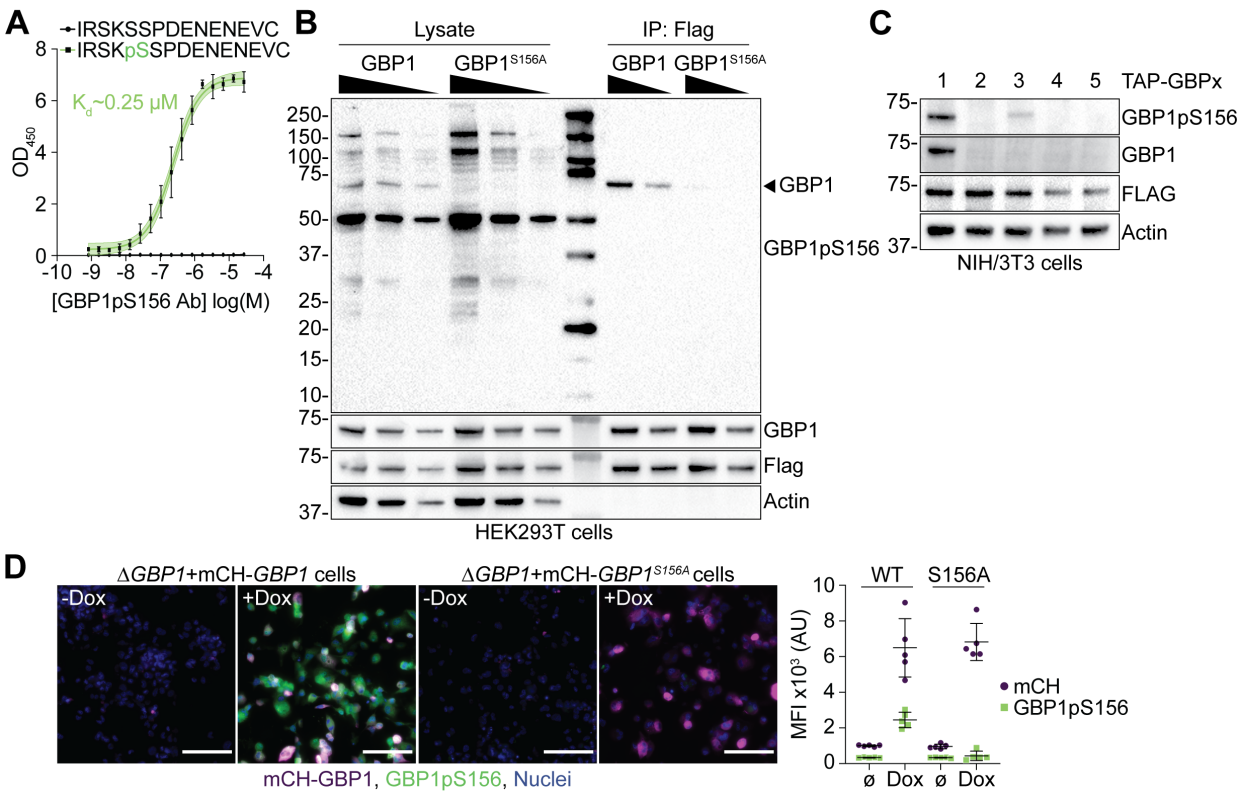

Fig. S3: Characterization of the GBP1pS156-specific antibody.
(A) In vitro binding assay determining affinity and specificity of the new GBP1pS156 antibody. **(B)** Immunoblots testing the GBP1pS156 Ab with an immunoprecipitation of Flag-GBP1 WT or Flag-GBP1^{S156A} from transfected 293T cells. Arrowhead marks the bands for phosphorylated GBP1. **(C)** Representative immunoblots for GBP1 Ab or GBP1pS156 antibodies with lysates from naïve, murine NIH3T3 cells transfected with vectors for expression of tandem-affinity-purification (TAP)-tagged human GBPs. **(D)** Immunofluorescence images and quantification of mCherry (mCH) or GBP1pS156-stain fluorescence intensity per cell of IFNγ-primed THP-1Δ*GBP1*+mCH-*GBP1* WT or mCH-*GBP1*^{S156A} cells treated with Dox as indicated. Magenta: mCH-GBP1; Green: GBP1pS156; Blue: nuclei. Scale bar, 100 µm. **Data information:** Graph in **(A)** shows mean ± SD from n = 3 experiments. Images in **(B)** representative of n = 2 and in **(C-D)** from n = 3 experiments. Graph in **(D)** shows mean ± SD from n = 5 experiments. For gel source data, see **Data S5**.

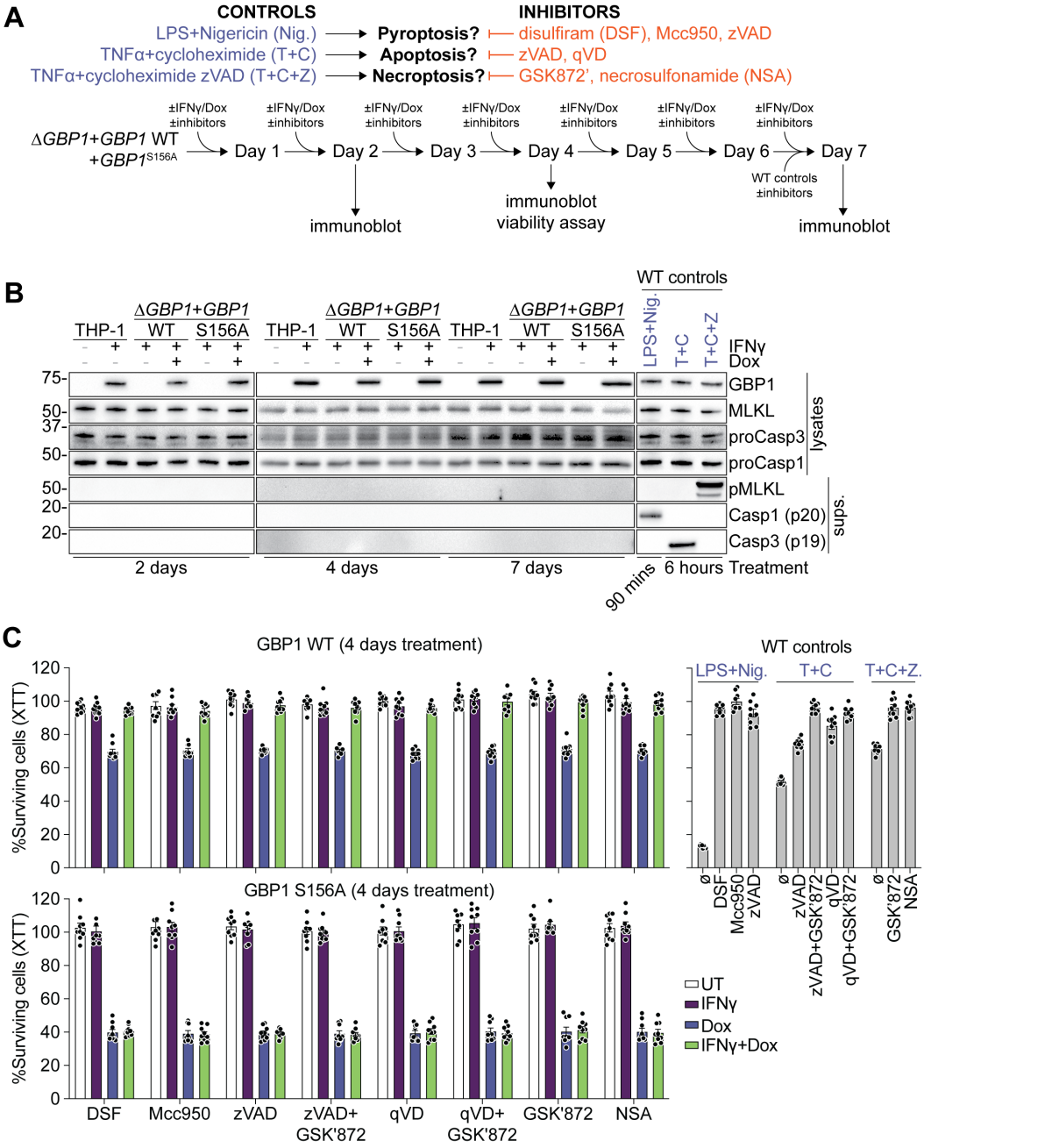

Fig. S4: Ectopic GBP1 expression does not induce regulated cell death.

(A) Overview of experimental conditions and treatments/inhibitors used to induce/block programmed cell death. **(B)** Immunoblots for markers of regulated cell death. THP-1 WT or THP-1Δ*GBP1*+*GBP1* WT or S156A expressing cells were treated with IFNγ+Dox as indicated for 2, 4 or 7 days. Regulated cell death was chemically induced by treatment with TNFα+cycloheximide (T+C) for apoptosis, Nigericin (Nig.) for pyroptosis or TNFα+cycloheximide+zVAD (T+C+Z) for necroptosis as control. **(C)** XTT cell survival assay of THP-1Δ*GBP1*+*GBP1* WT or S156A mutant expressing cells treated with IFNγ±Dox and inhibitors of programmed cell death for 4 days. **Data information:** Images in **(B)** representative of n = 3 experiments and graphs in **(C)** show mean ± SEM from n = 3 experiment. For gel source data, see **Data S5**.

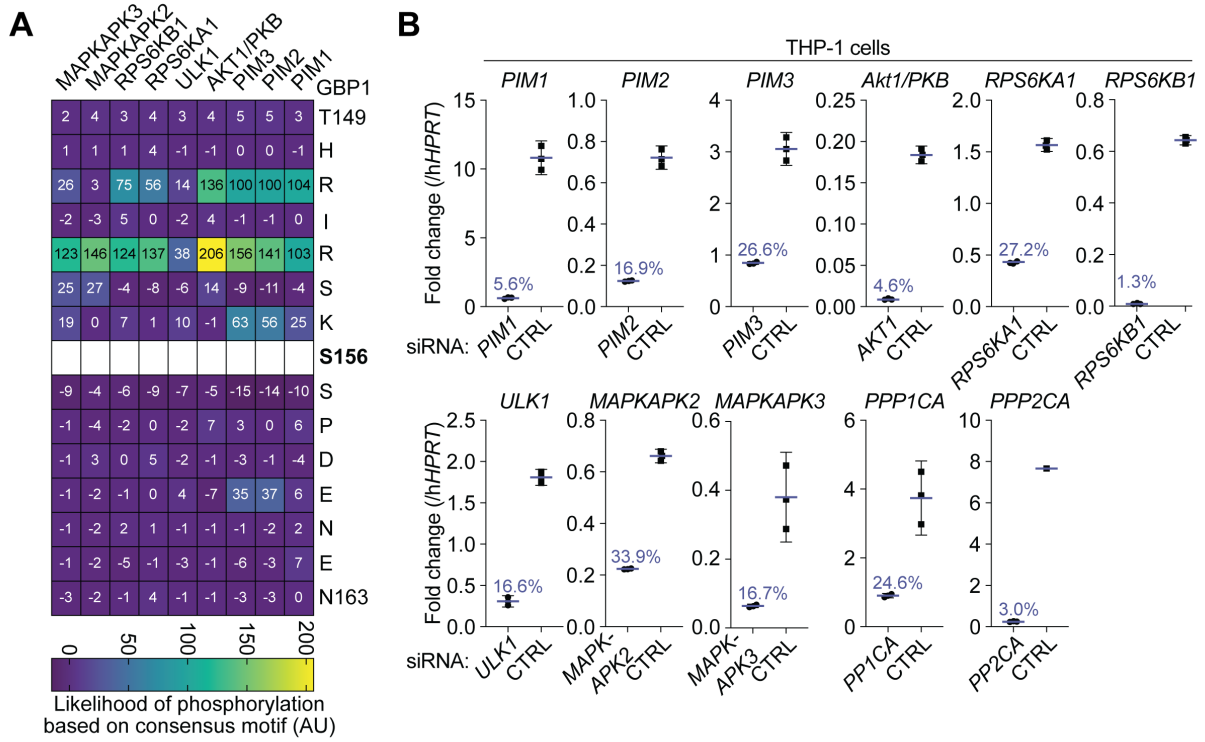

Fig. S5: Identification of GBP1-targeting candidate kinases.

(A) GBP1 Ser156 phosphorylation in silico analysis for identification of potential kinases. Diagram illustrates likelihood of motif recognition for individual kinases based on the GBP1 primary sequence. **(B)** Control of gene knockdowns using siRNA interference. Graphs showing mRNA levels (normalized to *HPRT1*) from IFNγ-primed THP-1 WT cells. Percentage mRNA levels as compared to cells transfected with non-targeting control siRNA (CTRL) are indicated. **Data information:** Graphs in **(B)** show mean ± SD from n = 3 experiments.

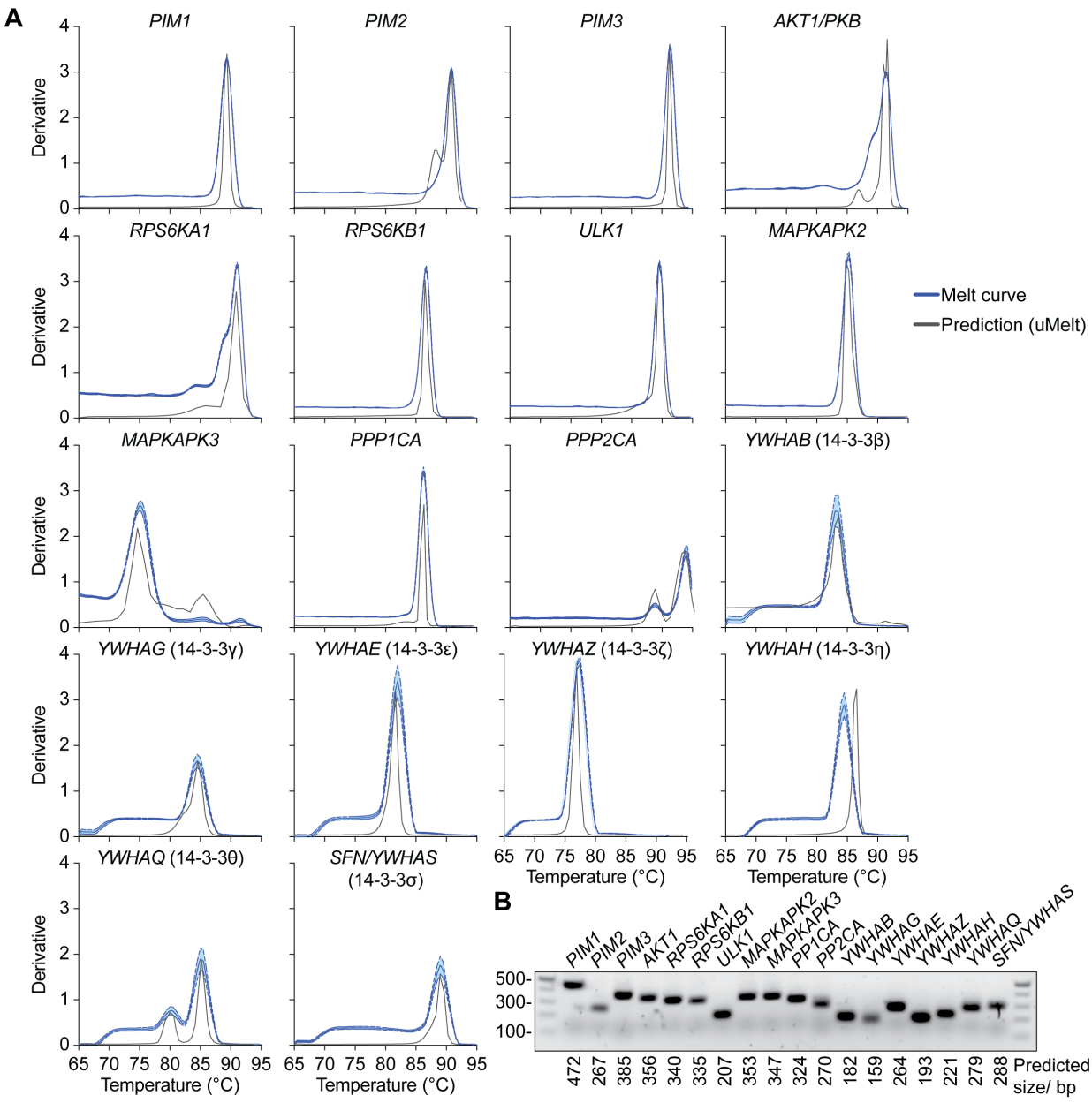

Fig. S6: Validation of RT-qPCR primers.

(A) Graphs showing measured and predicted melt curves for RT-qPCR amplicons using primer pairs for the indicated genes. **(B)** DNA gel with amplicons of the RT-qPCR primers showing specific amplification. Predicted amplicon size for the respective primer pair shown below. For gel source data, see **Data S5**.

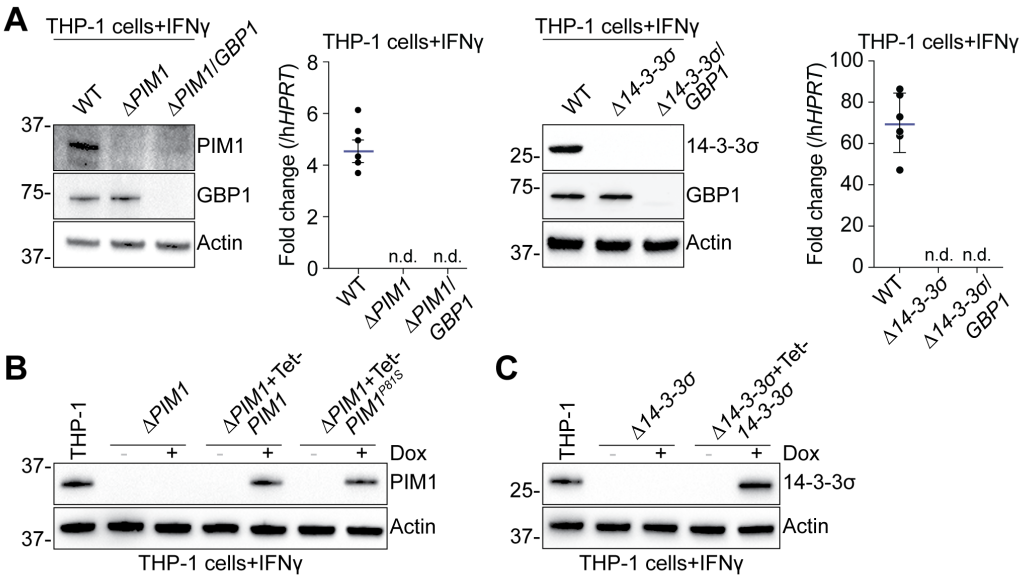

Fig. S7: Creation of THP-1 *PIM1* and *14-3-3σ* knockout cell lines and reconstitution.
(A) Immunoblots and RT-qPCR analysis of PIM1 or 14-3-3σ expression in newly created and IFNγ-primed THP WT, Δ*PIM1*, Δ*PIM1*/*GBP1*, Δ*14-3-3σ* or Δ*14-3-3σ*/*GBP1* cells; n.d. not detected. **(B)** Immunoblots of IFNγ-primed THP-1 WT or THP-1Δ*PIM1*+Tet-*PIM1* WT or kinase dead *PIM1*^{P81S} mutant cells treated with Dox or left untreated as indicated. **(C)** Immunoblots of IFNγ-primed THP-1 WT or THP-1Δ*14-3-3σ*+Tet-*14-3-3σ* cells treated with Dox or left untreated as indicated. For gel source data, see **Data S5**.

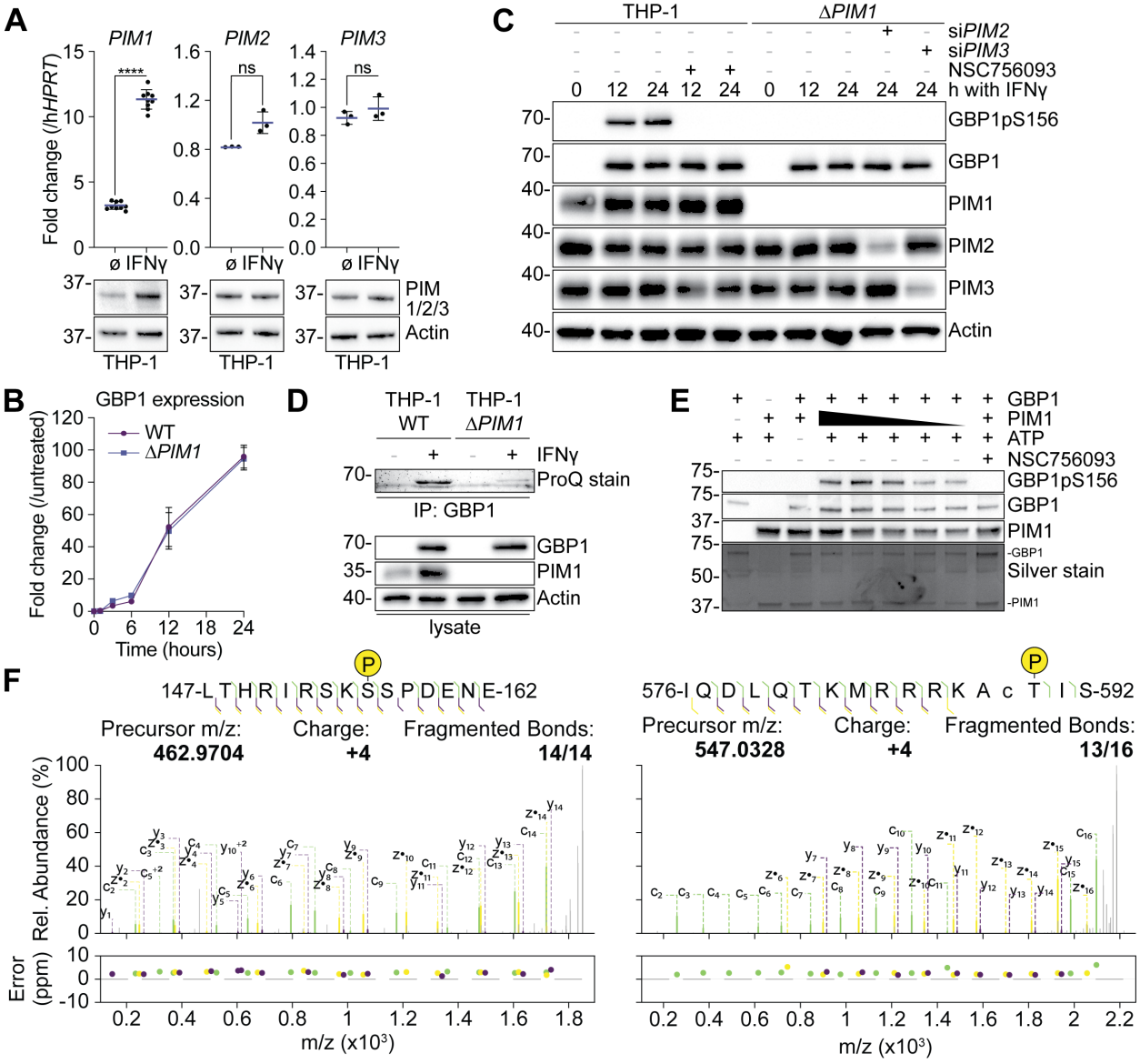

Fig. S8: PIM1 phosphorylates GBP1 in IFNγ-primed human macrophages.
(A) RT-qPCR analysis and immunoblots for PIM1/2/3 expression in naïve or IFNγ-primed THP-1 WT cells. **(B)** RT-qPCR analysis of *GBP1* mRNA expression following IFNγ-priming of THP-1 WT or Δ*PIM1* cells. **(C)** Immunoblots testing phosphorylation of endogenous GBP1 in THP-1 WT or Δ*PIM1* macrophages, primed with IFNγ and treated with NSC756093 or transfected with *PIM2* or *PIM3* siRNA. **(D)** Immunoblots and ProQ phosphoprotein stain of immunoprecipitated endogenous GBP1 in IFNγ-primed THP-1 WT or Δ*PIM1* cells. **(E)** Immunoblots and silver stain of in vitro kinase assays. **(F)** ETD-activated MS/MS spectra of peptides containing Ser156 (left) or Thr590 (right). Spectra display fragment ions used to identify the monophosphorylated peptides and localize the phosphorylation (probability > 0.9999). **Data information:** Images in **(A)** and **(C-E)** representative of n = 3 experiments. Graphs in **(A)** show mean ± SD from n = 9 experiments (PIM1) or n = 3 experiments (PIM2/3). Graph in **(B)** shows mean ± SD from n = 3 experiments. For gel source data, see **Data S5**.

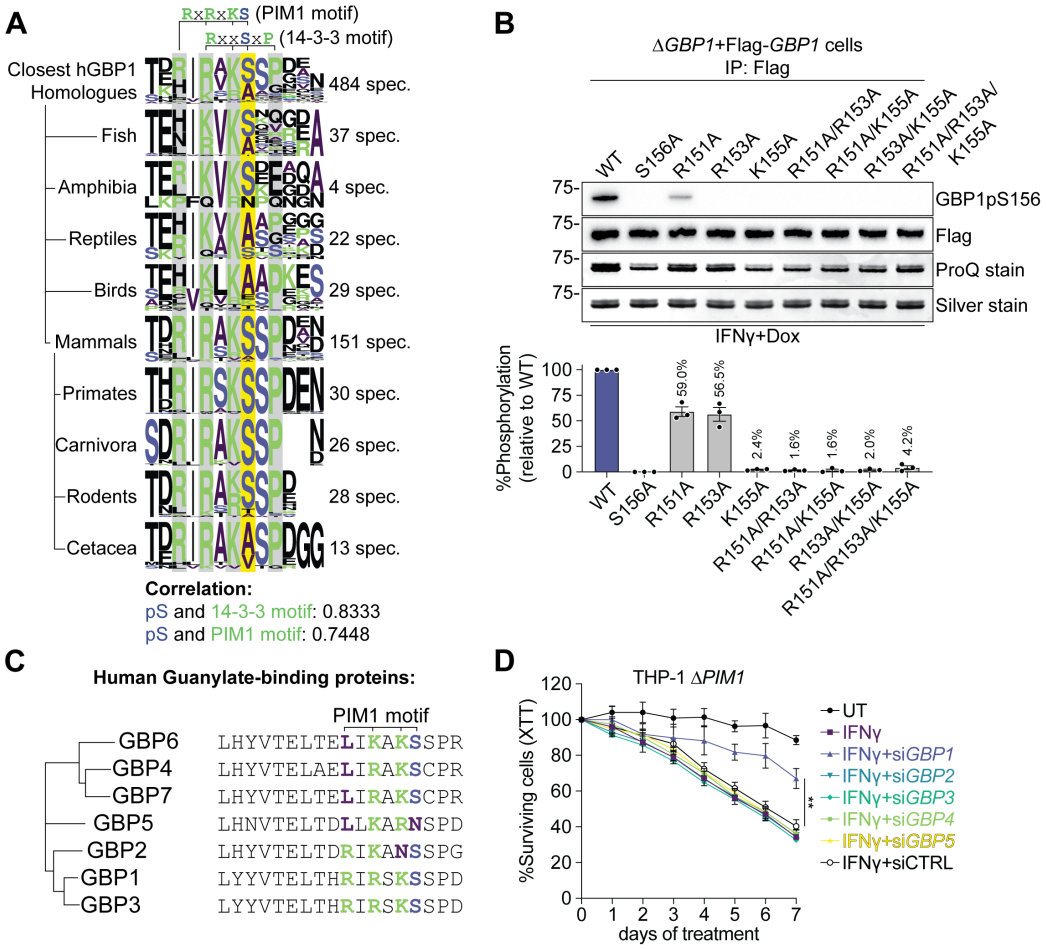

Fig. S9: Investigation of the GBP1 kinase recognition motif.

(A) Sequence conservation analysis of the closest human GBP1 homologue from 484 species. Sequence motifs show amino acid composition of the site surrounding Ser156 for the indicated class/order of species. Overall correlation between occurrence of the corresponding phosphorylation site to human Ser156 and either the 14-3-3 protein binding motif or the PIM1 recognition motif indicated below. **(B)** Immunoblots and silver/ProQ phosphoprotein gel stain of immunoprecipitated Flag-GBP1 from IFNγ+Dox-treated THP-1Δ*GBP1*+Flag-*GBP1* cells expressing the indicated mutant of GBP1 or GBP1 WT and quantification of phosphorylation level at Ser156. **(C)** Sequence alignment and phylogenetic tree of human GBP1-7. Residue equivalent to Ser156 of GBP1 highlighted in blue and basophilic kinase recognition motif in green. Disruptive amino acid replacements shown in magenta. **(D)** XTT cell survival assay of IFNγ-treated THP-1Δ*PIM1* cells transfected with the indicated siRNAs against human GBPs or non-targeting siCTRL. **Data information:** Images and graph in **(B+D)** representative of n = 3 experiments. Graph in **(B)** shows normalized ProQ signal relative to GBP1 WT (=100%) and GBP1 S156A mutant (=0%). Graph in **(D)** shows mean ± SEM from n = 3 experiments, normalized to untreated cells. ** $P \leq 0.01$ for indicated comparisons in **(D)** from one-way ANOVA For gel source data, see **Data S5**.

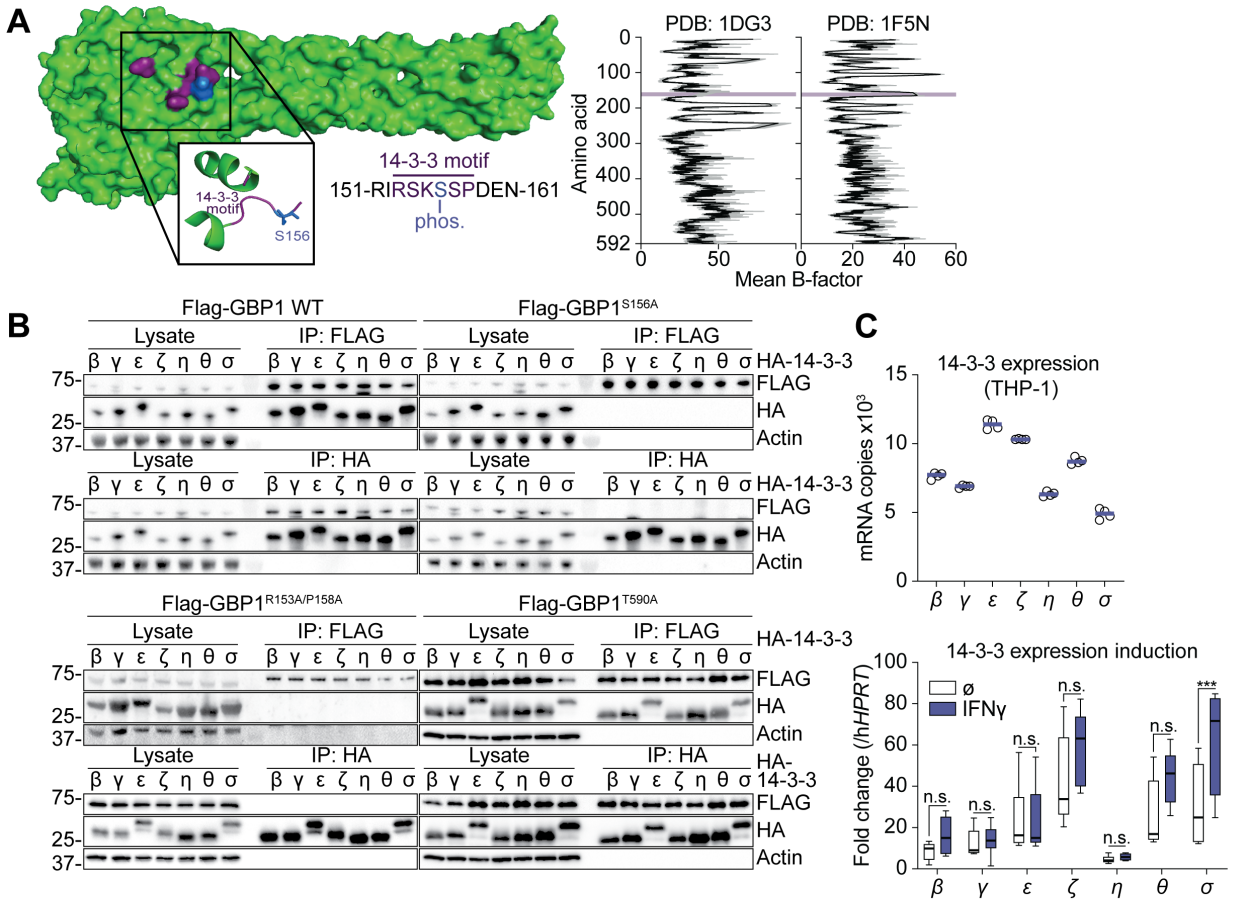

Fig. S10: Characterization of GBP1 interaction with 14-3-3 proteins.
(A) Crystal structure of human GBP1 with predicted 14-3-3-binding-site highlighted in purple and Ser156 phosphorylation site in blue and graphs showing B-factor of two GBP1 crystal structures with the phosphorylation-motif-containing loop highlighted in purple. **(B)** Immunoblots for immunoprecipitations of Flag-GBP1 WT, Flag-GBP1^{S156A}, Flag-GBP1$^{R153A/P158A}$ or Flag-GBP1^{T590A} and reverse IP of HA-14-3-3 proteins from HEK293T cells co-transfected with vectors expressing the respective proteins. **(C)** RT-qPCR analysis of absolute 14-3-3 protein expression in naïve THP-1 WT cells as compared to plasmid standard and expression induction following IFNγ-stimulation. **Data information:** Images in **(B)** representative of n = 3 experiments. Graphs in **(C)** show mean of n = 4 experiments (14-3-3 expression) or box plot with median and interquartile range (relative expression induction; n = 3). *** $P \leq 0.001$ in **(C)** comparing IFNγ-treated to naïve cells from 2-way ANOVA following adjustment for multiple comparisons; n.s. not significant. For gel source data, see **Data S5**.

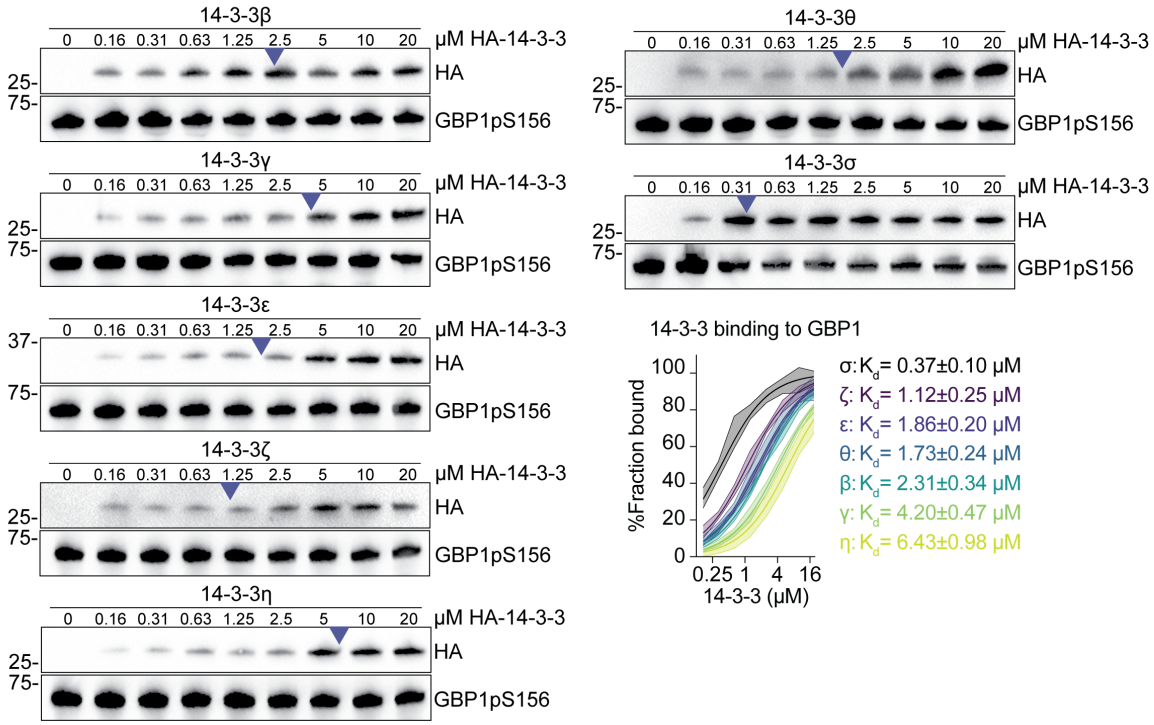

Fig. S11: Determination of GBP1:14-3-3 protein interaction affinities.
Immunoblots for semi-quantitative co-IP using immobilized GBP1pS156 to determine affinity of the respective 14-3-3 protein added in the indicated concentrations. Blue arrowheads mark approximate K_d of the interaction. Graph showing result of semi-quantitative co-immunoprecipitations, plotting percentage of bound 14-3-3 protein to immobilized GBP1pS156 bait against added concentration of respective 14-3-3 protein.
Data information: Images and graph representative of n = 3 experiments. Graph shows mean ± SD (error bands). For gel source data, see **Data S5**.

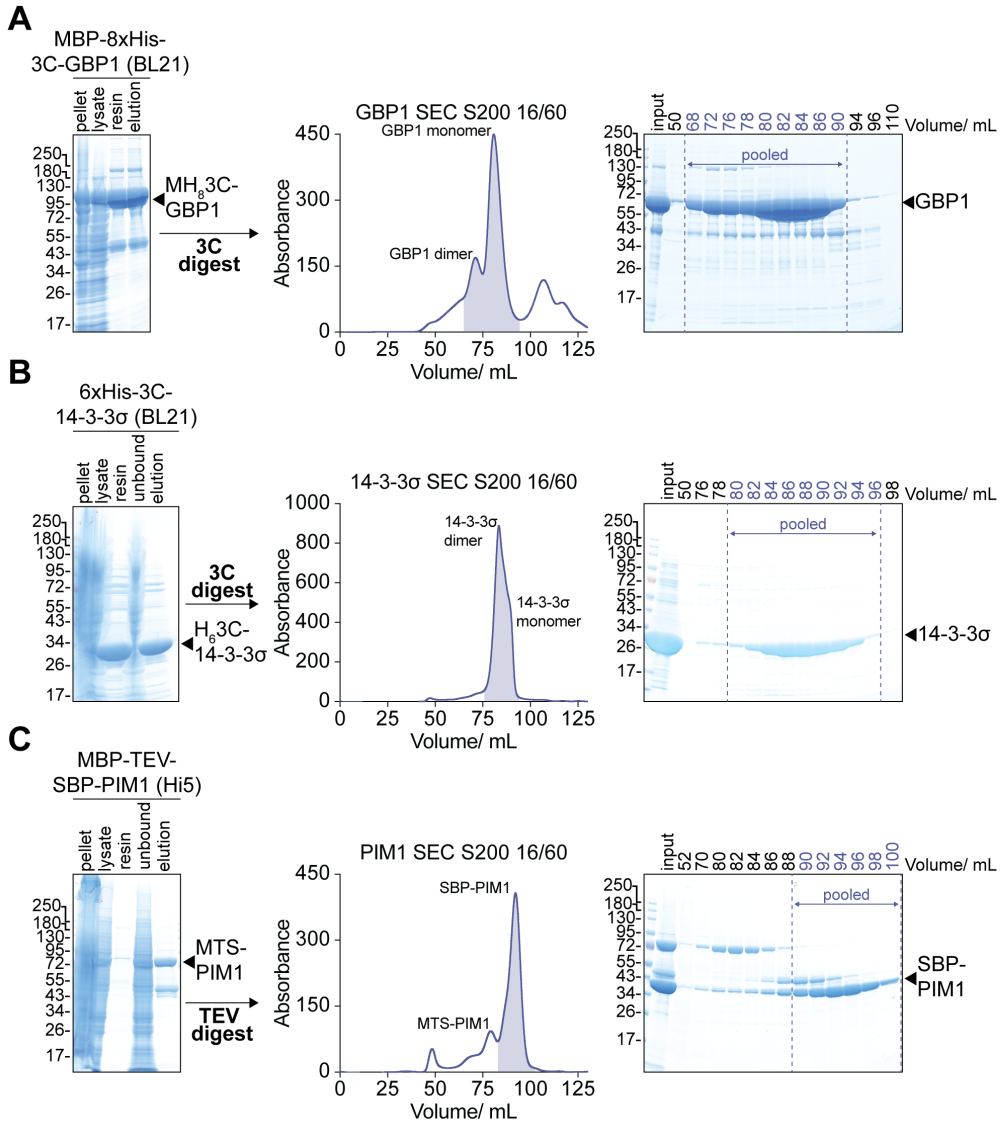

Fig. S12: Production of recombinant human GBP1, 14-3-3σ and PIM1.
Figure showing expression and purification of recombinant GBP1 **(A)**, 14-3-3σ **(B)** and PIM1 **(C)**. Coomassie stain of bulk purification using Ni-NTA affinity pulldown of MBP-8xHis-3C-GBP1 or 6xHis-3C-14-3-3σ from BL21 bacteria or amylose affinity purification of MBP-TEV-SBP (MTS)-PIM1 from Hi5 insect cells (left). Following digest with 3C- or TEV-protease, proteins were purified using size exclusion chromatography (SEC). Representative chromatograms and Coomassie-stained SDS-PAGE gels shown on the right, with indicated fractions that were pooled to obtain the purified proteins. For gel source data, see **Data S5**.

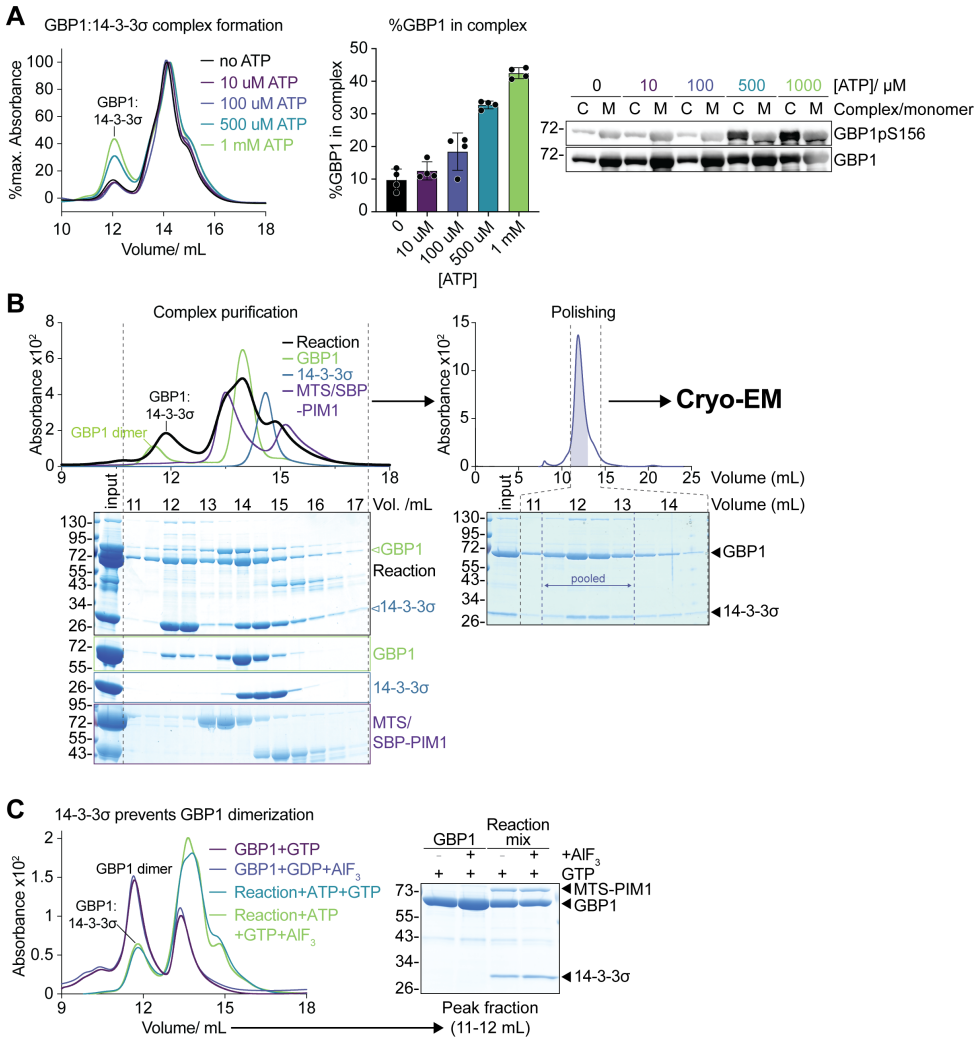

Fig. S13: Biochemical characterization of the GBP1:14-3-3σ complex.

(A) Size exclusion chromatography (SECs) of GBP1 phosphorylation by PIM1 and binding to 14-3-3σ (left). Peak quantification for GBP1:14-3-3σ complex and free GBP1 monomer, plotted as proportion of GBP1 contained within the complex (middle). Immunoblots for GBP1 phosphorylation in complex (C) or monomer (M) containing fractions from depicted chromatography. **(B)** Chromatograms and SDS-PAGE gels of indicated fractions of GBP1, PIM1 and 14-3-3σ alone or from an in vitro complex formation reaction (left). GBP1:14-3-3σ complex containing peak fractions were pooled and re-purified for polishing samples before structure determination by cryo-electron microscopy. Chromatogram and SDS-PAGE gel with indicated fractions used for structure determination (right). **(C)** SEC chromatograms and SDS-PAGE gels of peak fractions illustrating GBP1 dimerization. Samples contained GBP1 or GBP1+PIM1+14-3-3σ+ATP (Reaction mix) with addition of GTP, GDP+AlF_3. **Data information:** Chromatograms, graphs and blots shown in **(A)** representative of n = 4 and in **(B-C)** from n = 3 experiment. For gel source data, see **Data S5**.

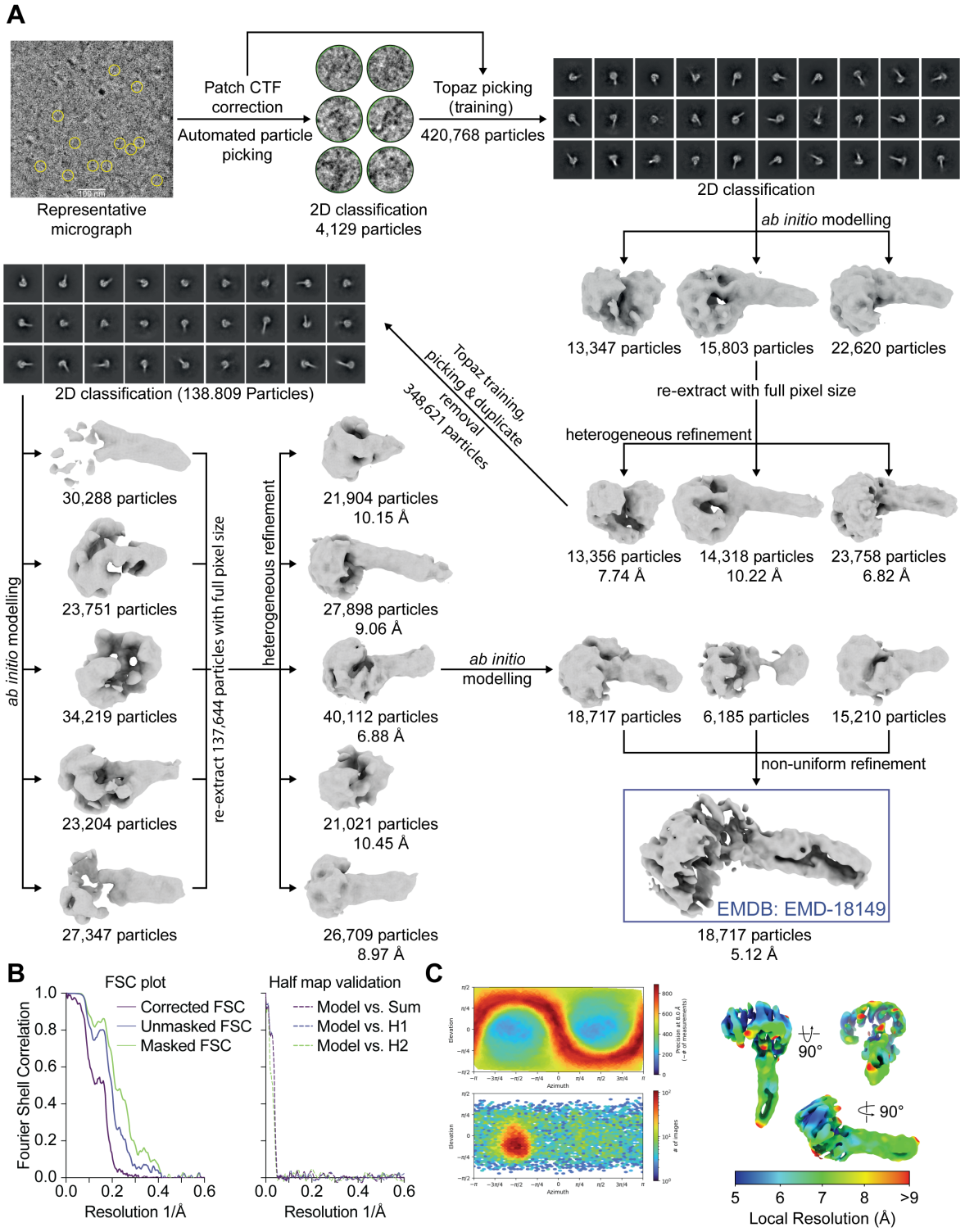

Fig. S14: Supporting cryo-electron microscopy data.

(A) Overview of cryo-EM data analysis strategy to obtain a 5.1 Å map of the trimeric GBP1:14-3-3σ complex. The final model was obtained using rigid body docking of previously described structures and without refining to the map. **(B)** Fourier shell correlation (FSC) and half map validation plots of the GBP1:14-3-3σ complex. **(C)** Angular distribution coverage (left) and EM density colored by local resolution (right).

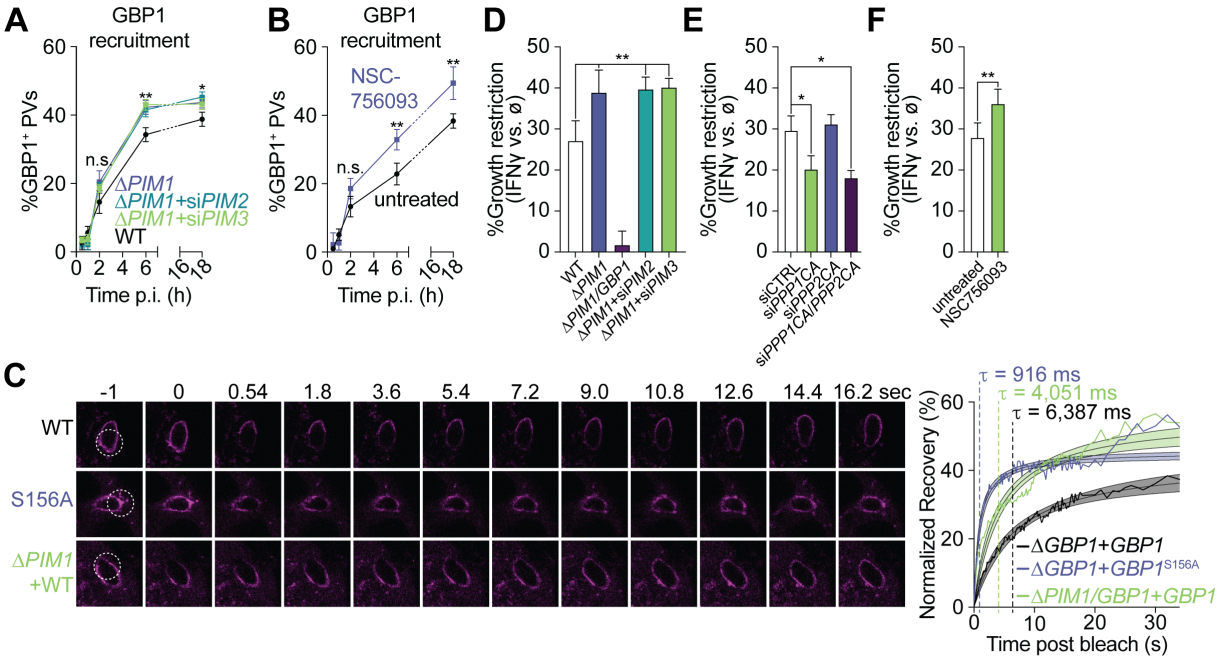

Fig. S15: Effect of external factors on *Toxoplasma gondii* vacuole targeting and growth restriction by GBP1.

(A) GBP1 recruitment quantification to type II *Toxoplasma gondii* (Tg) vacuoles at indicated times post infection in IFNγ-primed THP-1 WT or IFNγ+Dox-treated THP-1Δ*PIM1*/*GBP1*+mCH-*GBP1* cells transfected with siRNA for *PIM2* or *PIM3*. **(B)** mCH-GBP1 recruitment quantification to type II Tg vacuoles at indicated times post infection in IFNγ+Dox-primed THP-1Δ*GBP1*+mCH-*GBP1* cells treated with NSC756093. **(C)** Image sequence of fluorescence recovery after photobleaching (FRAP) experiments bleaching GBP1 on type II Tg vacuoles in IFNγ+Dox-primed cells at 2 hours p.i. Bleach area indicated by dotted circle. Graphs showing analysis of mCH-GBP1 fluorescence intensity to obtain recovery times ($\tau_{1/2}$) using hyperbolic curve. **(D-F)** Analysis of type II Tg-growth restriction at 18 hours p.i. in indicated cell lines treated with IFNγ or left untreated and transfected with siRNA for *PIM2* or *PIM3* **(D)**, transfected with indicated siRNAs **(E)** or treated with GBP1:PIM1 interaction inhibitor NSC756093 **(F)**. **Data information:** Graphs in **(A+B)** show mean ± SD from n = 3 experiments. Images in **(C)** representative of n = 10 bleached vacuoles per cell line. Graphs in **(C)** show mean and curve fit ± SD (error bands). Graphs in **(D-F)** show mean ± SD of n = 3 experiments plotted as relative growth restriction comparing to untreated cells. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ for comparison to WT cells in **(A)** or between untreated and NSC756093-treated cells in **(B)** and for indicated comparisons in **(D-F)** from 2-way ANOVA following adjustment for multiple comparisons; n.s. not significant.

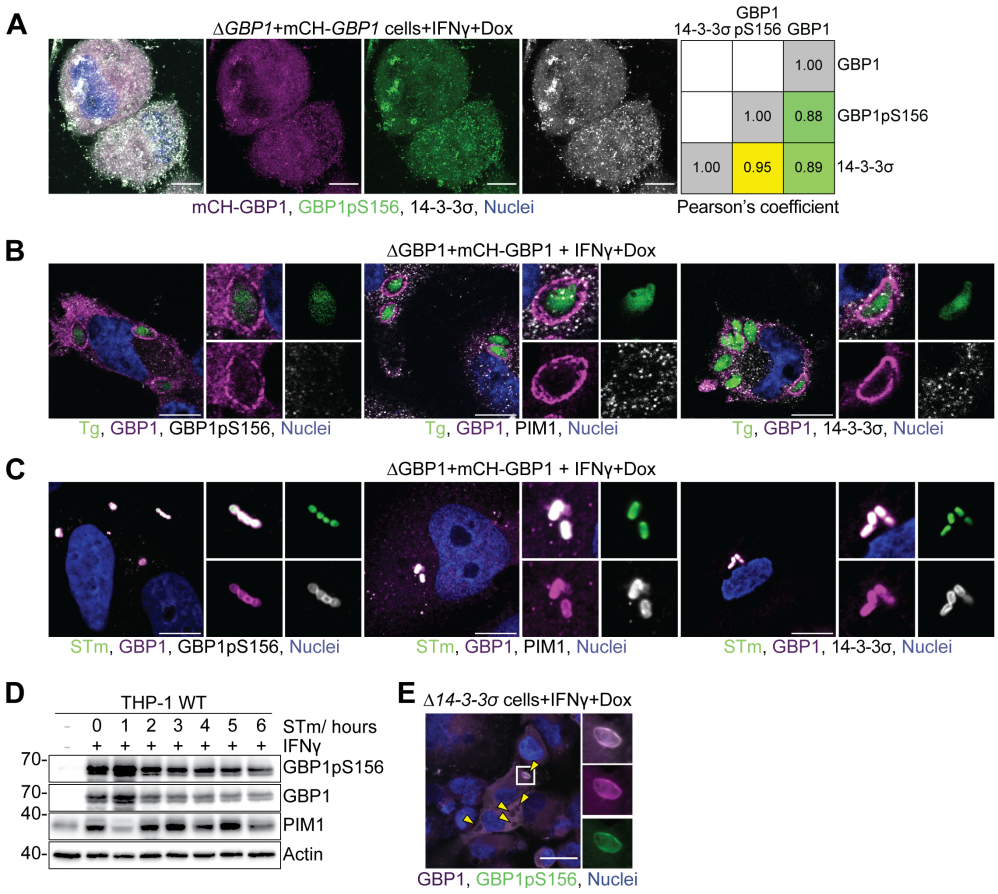

Fig. S16: Protein localization and pathogen targeting by GBP1 upon infection.
(A) Immunofluorescence images from IFNγ+Dox-treated THP-1Δ*GBP1*+mCH-*GBP1* cells stained for 14-3-3σ and GBP1pS156 and analysis of fluorescence signal colocalization analysis using Pearson's colocalization coefficient. Magenta: mCH-GBP1; Grey: 14-3-3σ; Green: GBP1pS156; Blue: nuclei. Scale bar, 10 µm. **(B)** Immunofluorescence images of IFNγ+Dox-treated THP-1 Δ*GBP1*+mCH-*GBP1* cells infected with type II *Toxoplasma gondii* (Tg) for 6 hours and stained for GBP1pS156, PIM1 or 14-3-3s. Magenta: mCH-GBP1; Grey: GBP1pS156/PIM1/14-3-3σ; Green: Tg; Blue: nuclei. Scale bar, 10 µm. **(C)** Immunofluorescence images of IFNγ+Dox-treated THP-1 Δ*GBP1*+mCH-*GBP1* cells infected with *Salmonella Typhimurium* (STm, MOI = 10) for 2 hours and stained for GBP1pS156, endogenous PIM1 or 14-3-3 σ. Magenta: mCH-GBP1; Grey: GBP1pS156/PIM1/14-3-3σ; Green: STm; Blue: nuclei. Scale bar, 10 µm. **(D)** Immunoblot analysis for GBP1 phosphorylation and PIM1 protein levels following STm infection (MOI = 10) of IFNγ-primed THP-1 WT cells at the indicated time points post infection. **(E)** Immunofluorescence images from IFNγ+Dox-treated THP-1Δ*14-3-3σ* cells infected with type II Tg for 6 hours and stained for GBP1 and GBP1pS156. Arrowheads: Tg position based on DNA stain. Magenta: GBP1; Green: GBP1pS156; Blue: nuclei. Scale bar, 20 µm. **Data information:** Images in **(A-E)** representative of n = 3 experiments. Heat map in **(A)** shows mean from n = 12 cells per condition imaged by 3D confocal microscopy. For gel source data, see **Data S5**.

SUPPLEMENTARY TABLES

Table S1: Cryo-EM data collection and processing.

	GBP1:14-3-3σ complex (EMDB: EMD-18149)
Data collection and processing	
Magnification	215,000
Voltage (kV)	300
Electron exposure (e–/Å^2)	65.5
Defocus range (μm)	-0.8 to -2
Pixel size (Å)	0.638
Symmetry imposed	C1
Initial particle images (no.)	8,689,007
Final particle images (no.)	18,717
Map resolution (Å) FSC threshold	5.12 0.143
Map resolution range (Å)	5.12 - 10

Table S2: Overview of cell lines and *Toxoplasma gondii* parasite lines.

Cell line	Source
HEK293T	The Francis Crick Institute, Cell Services
HFF	The Francis Crick Institute, Cell Services
NIH3T3	The Francis Crick Institute, Cell Services
Organoid 389	Wanigasooriya *et al*, 2022 (*52*)
THP-1 WT	ATCC, TIB-202
THP-1Δ*GBP1*	Fisch *et al*, 2019 (*11*)
THP-1Δ*GBP1*+Tet	Fisch *et al*, 2019 (*11*)
THP-1Δ*GBP1*+Tet-EV	Fisch *et al*, 2019 (*11*)
THP-1Δ*GBP1*+Tet-Flag-*GBP1*^{K155A}	This study
THP-1ΔGBP1+Tet-Flag-*GBP1*^{R151A}	This study
THP-1Δ*GBP1*+Tet-Flag-*GBP1*$^{R151A/K155A}$	This study
THP-1Δ*GBP1*+Tet-Flag-*GBP1*$^{R151A/R153A}$	This study
THP-1Δ*GBP1*+Tet-Flag-*GBP1*$^{R151A/R153A/K155A}$	This study
THP-1Δ*GBP1*+Tet-Flag-*GBP1*^{R153A}	This study
THP-1Δ*GBP1*+Tet-Flag-*GBP1*$^{R153A/K155A}$	This study
THP-1Δ*GBP1*+Tet-Flag-*GBP1*$^{R153A/P158A}$	This study
THP-1Δ*GBP1*+Tet-Flag-*GBP1*^{S156A}	This study
THP-1Δ*GBP1*+Tet-Flag-*GBP1*^{S156E}	This study
THP-1Δ*GBP1*+Tet-Flag-*GBP1* WT	Fisch *et al*, 2020 (*13*)
THP-1Δ*GBP1*+Tet-*GBP1* WT	Fisch *et al*, 2019 (*11*)
THP-1Δ*GBP1*+Tet-mCH-*GBP1*$^{R151A/R153A/K155A}$	This study
THP-1Δ*GBP1*+Tet-mCH-*GBP1*$^{R153A/P158A}$	This study
THP-1Δ*GBP1*+Tet-mCH-*GBP1*^{S156A}	This study
THP-1Δ*GBP1*+Tet-mCH-*GBP1*^{S156E}	This study
THP-1Δ*GBP1*+Tet-mCH-*GBP1*^{S157A}	This study
THP-1Δ*GBP1*+Tet-mCH-*GBP1*^{S157E}	This study
THP-1Δ*GBP1*+Tet-mCH-*GBP1* WT	Fisch *et al*, 2019 (*11*)
THP-1Δ*GBP1*+Tet-mCH-*GBP1*^{Y427F}	This study
THP-1Δ*PIM1*	This study

THP-1Δ*PIM1*/*GBP1*	This study
THP-1Δ*PIM1*/*GBP1*+Tet	This study
THP-1Δ*PIM1*/*GBP1*+Tet-EV	This study
THP-1Δ*PIM1*+Tet	This study
THP-1Δ*PIM1*+Tet-EV	This study
THP-1Δ*PIM1*+Tet-*PIM1*^{P81S}	This study
THP-1Δ*PIM1*+Tet-*PIM1* WT	This study
THP-1Δ*14-3-3σ*	This study
THP-1Δ*14-3-3σ*/*GBP1*	This study
THP-1Δ*14-3-3σ*/*GBP1*+Tet	This study
THP-1Δ*14-3-3σ*/*GBP1*+Tet-EV	This study
THP-1Δ*14-3-3σ*+Tet	This study
THP-1Δ*14-3-3σ*+Tet-EV	This study
THP-1Δ*14-3-3σ*+Tet-*14-3-3σ*	This study
Parasite line	**Source**
Type II (Prugniaud) Tg-GFP-Luc	Kim *et al*, 2007 (*81*)
Type II (Prugniaud) TgΔ*TgIST*	Moritz Treeck, The Francis Crick Institute
Type II (Prugniaud) TgΔ*Hpt*	Moritz Treeck, The Francis Crick Institute
Type II (Prugniaud) TgΔ*Hpt*+GFP$_{1-10}$	Fisch *et al*, 2020 (*13*)
Bacteria strain	**Source**
Salmonella Typhimurium SL1344-GFP	Fisch *et al*, 2019 (*11*)

Table S3: List of siRNAs.

Target gene	Cat. number	Target gene	Cat. number
PIM1	L-003923	*PPP1CA*	L-008927
PIM2	L-005359	*PPP2CA*	L-003598
PIM3	L-032287	*GBP1*	L-005153
AKT1/PKB	L-003000	*GBP2*	L-011867
RPS6KA1	L-003025	*GBP3*	L-031864
RPS6KB1	L-003616	*GBP4*	L-018177
ULK1	L-005049	*GBP5*	L-018178
MAPKAPK2	L-003516	Neg. CTRL	D-001810
MAPKAPK3	L-005014		

Table S4: List of CRISPR guide RNA sequences.

Guide	Sequence 5' à 3'
PIM1-gRNA1	GGAGGCCCGAGAGGAGTCGG
PIM1-gRNA2	GTTCTGCTGAATGCCGCGAT
14-3-3σ-gRNA1	ATGCCAGCCCCGAACAAGAG
14-3-3σ-gRNA2	GGAGCAAGAACAGCGCCTAG

Table S5: List of qPCR primer sequences.

qPCR primer	Sequence 5' à 3'
PIM1	GCTCGGTCTACTCAGGCATC
	TACACTCGGGTCCCATCGAA
PIM2	TTCAGTGGGCTCAATCTGCG
	GACTGAGTCTGACAAGGGGG
PIM3	TACACCGACTTCGACGGCAC
	CTCCTCACAAGCTCTCGCTG
AKT1	TCCTGGTCCTGTCTTCCTCAT
	GTACTCCCCTCGTTTGTGCAG
RPS6KA1	AATGGACAGACCTCAGGGGA
	CCTCGGTCTGGAAGGCATAG
RPS6KB1	CCCTGTACGCATGCTCCTAC
	CCATGCCAAGTTCATATGGTCC
ULK1	GACCGCATTCACAGCATCAC
	AATGCACAGCTTGCACTTGG
MAPKAPK2	GTACATCCTGCTGTGTGGGTA
	ACAACCTGGTCAGCCAAGTG
MAPKAPK3	TCTTCCTAGAACCGCGGGC
	CCTCAGTTTTCTCACCCTGCC
PPP1CA	CCTGCTGGAAGTGCAGGG
	CCACGGAGCAGGAAGAAGTT
PPP2CA	CGAGTACTGCGGTGAGAGC
	CAGGATTTCTTTAGCCTTCTCGC
YWHAB	GGGCATGAACTCTCCAACGA
	ATGTCCTGCAGTTCTGCCTC
YWHAG	AAGAATTGCAGCGAGACCCA
	TTTGCTGATCTCGTGGGCTT
YWHAE	CACCTCATTCCAGCAGCTAACAC
	CAACCTGCAGGCACGGTC
YWHAZ	AGGAGCCCGTAGGTCATCTT
	GACTTTGCTCTCTGCTTGTGAAGC
YWHAH	CCGCTACTTAGCAGAGGTCG
	TGGCATCATCGAAGGCTTGT
YWHAQ	TGGCCTACAAGAACGTGGTC
	TTCGATCATCACCACACGCA
YWHAS (14-3-3σ)	TATAAGAACGTGGTGGGCGG
	GATGCGCTTCTTGTCGTCAC
HPRT1	ACCAGTCAACAGGGGACATAA
	CTTCGTGGGGTCCTTTTCACC

Table S6: List of primary antibodies.

IB: immunoblot, IF: immunofluorescence, IP: immunoprecipitation. Ms: mouse, Gt: goat, Rb: rabbit

primary Antibody	IB	IF	IP		Source	RRID
14-3-3σ	x	x		Ms	ab14123, abcam	RRID:AB_300927
β-actin	x			Gt	ab8229, abcam	RRID:AB_306374
caspase-1	x			Ms	Bally-1, Adipogen	RRID:AB_2490257
cleaved caspase-3	x			Rb	#9661, CST	RRID:AB_2341188
E-cadherin	x			Rb	#3195, CST	RRID:AB_2291471
Flag-M2	x		x	Ms	F3165, Sigma	RRID:AB_259529
GAPDH	x			Rb	#2118, CST	RRID:AB_561053
GBP1 (mAb)	x	x	x	Ms	Frickel lab	-
GBP1 (pAb)	x			Rb	Frickel lab	-
GBP1pS156	x	x	x	Rb	Frickel lab	-
GM130		x		Rb	ab52649, abcam	RRID:AB_880266
HA	x		x	Rb	#3724, CST	RRID:AB_1549585
Histone H3	x			Rb	#4499, CST	RRID:AB_10544537
mCherry	x			Rb	ab167453, abcam	RRID:AB_2571870
MLKL	x			Rb	#14993, CST	RRID:AB_2721822
pan14-3-3	x			Rb	#8312, CST	RRID:AB_10860606
PIM1	x	x		Ms	ab54503, abcam	RRID:AB_882031
PIM2	x			Rb	#4730, CST	RRID:AB_2163921
PIM3	x			Rb	#4165, CST	RRID:AB_1904094
pMLKL	x			Rb	#91689, CST	RRID:AB_2732034
pro-caspase-3	x			Rb	#9662, CST	RRID:AB_331439
TgSAG1		x		Ms	Frickel lab	-

DATA LEGENDS:

Data S1: MS/MS spectrum of GBP1-Ser156 and Thr590 phosphorylation (Separate Excel file). Table showing experimental, and respective theoretical values of fragments in MS/MS spectrum of GBP1 peptide L^{148}THRIRSKpSSPDENE^{162} containing the Ser156 phosphorylation site and I^{576}QDLQTKMRRRKACTIS^{592} containing the Thr590 phosphorylation site, used for mapping of the sites to the GBP1 sequence. Extracted from Interactive Peptide Spectral Annotator.

Data S2: Evolutionary analysis of GBP1 homologues (Separate Excel file).
Table containing the information on the closest human GBP1 homologue from 484 species used for evolutionary analysis of occurrence of corresponding GBP1 Ser156 residue, the 14-3-3 binding motif and the PIM1 recognition motif.

Data S3: GBP1 interactome in uninfected THP-1 cells (Separate Excel file).
Table containing the mass spectrometry analysis for identification of GBP1 interacting proteins in IFNγ-primed THP-1 cells. Annotation: orange: significant interactor; green: GBP1; dark green: 14-3-3 proteins; red: common contaminant as identified using the CRAPome database.

Data S4: GBP1 interactome in *Toxoplasma gondii*-infected THP-1 cells (Separate Excel file).
Table containing the mass spectrometry analysis for identification of GBP1 interacting proteins in IFNγ-primed and type II *Toxoplasma gondii*-infected THP-1 cells. Annotation: orange: significant interactor upon infection, as compared to uninfected cells; green: highlighted significant hits; red: common contaminant as identified using the CRAPome database.

Data S5: Uncropped gels and immunoblots (Separate pdf file).
Uncropped immunoblots and gels.

Data S6: Source data (Separate Excel file).
Excel file containing all underlying source data.