

## SUPPORTING INFORMATION

**Dense granule biogenesis, secretion and function in *Toxoplasma gondii*** by Michael B. Griffith, Camille S. Pearce, Aoife T. Heaslip

**Table S1.** List of known GRA proteins. \* indicates proteins shown to be cleaved by ASP5. Accession numbers and CRISPR scores were obtained from ToxoDB ([www.ToxoDB.org](http://www.ToxoDB.org)) (Amos et al. 2022).

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Name	Accession Number	Function	Conserved Domains	Crispr Score	Reference
Nutrient Acquisition/Associated within the IVN					
GRA2	TGME49_227620	Located at the IVN. Needed for IVN formation, suppression of antigen presentation, and uptake of host lipid droplets	Signal peptide, no transmembrane domain	2.47	(Nolan et al. 2017)
GRA6	TgME49_213067	IVN formation and uptake of host lipid droplets. Located at the IVN and PV.	transmembrane domain, no signal peptide	1.82	(Ma et al. 2014; Nolan et al. 2017; Rommereim et al. 2019)
GRA7	TGME49_203310	induces the tubulation of artificial liposomes and has been implicated in nutrient acquisition. Located in the PVM and PVMP	transmembrane domain, no signal peptide	1.9	(Coppens et al. 2006; Dunn et al. 2020)
GRA14	TGGT1_239740	Uptake of host cytosolic proteins by co-op of ESCRT. Located at the PVM.	transmembrane domain, no signal peptide	2	(Bai et al. 2018; Rivera-Cuevas et al. 2021)
GRA17	TGME49_222170	Required for PVM permeability and host nutrient access. Located at the PVM	no signal peptide or transmembrane domain	-2.93	(Gold et al. 2015)
GRA23	TGME49_297880	Required for PVM permeability and host nutrient access. Located at the PVM	signal peptide and transmembrane domain	1.88	(Gold et al. 2015)
GRA64	TGME49_202620	organizing the recruitment of ESCRT proteins and subsequent intracyclic vesicle formation. Located in the PVM	signal peptide, transmembrane domains	0.94	(Mayoral unpubl. data)
LCAT	TGME49_272420*	metabolic functions in parasite growth, virulence, and egress of tachyzoites. Located in PVM	Alpha-Beta hydrolase fold, no signal peptide or	0.65	(Pszenny et al. 2016; Coffey et al. 2018)

			transmembrane domain		
NTPaseI	TGME49_277240	Nucleoside-triphosphatase, Possible role in parasite metabolism in the PV lumen, required for parasite proliferation. Found in Type I strains	signal peptide, no transmembrane domain	ND	(Asai et al. 1995; Krug et al. 2012; Nakaar et al. 1999; Sibley et al. 1994)
NTPaseII	TGME49_277270	Nucleoside-triphosphatase , Possible role in parasite metabolism in the PV lumen, required for parasite proliferation	signal peptide, no transmembrane domain	ND	(Asai et al. 1995; Nakaar et al. 1999)
PSD1	TGME49_269920	Decarboxylates phosphatidylserine and needed for membrane biogenesis. Located at the PV	Phosphatidylserine decarboxylase domain, signal peptide and transmembrane domain	0.52	(Gupta et al. 2012)
MYR complex for Effector Translocation					
MYR1	TGME49_254470*	Translocon complex for export of GRA proteins, located in PVM space	transmembrane domain, no signal peptide	1.04	(Franco et al. 2016; Marino et al. 2018)
MYR2	TGME49_270700	Translocon complex for export of GRA proteins, located in PVM space	transmembrane domain, no signal peptide	2.39	(Franco et al. 2016; Marino et al. 2018)
MYR3	TGME49_237230	Translocon complex for export of GRA proteins, located in PVM space	No signal peptide, transmembrane domain	2.83	(Franco et al. 2016; Marino et al. 2018)
MYR4	TGME49_211460*	Translocon complex for export of GRA proteins, located in PVM space	No signal peptide, transmembrane domain	0.25	(Cygan et al. 2020)
PPMC3	TGME49_270320	Dephosphorylates secreted effector proteins within the PV	PP2C-class S/T phosphatase domain, no signal peptide, transmembrane domain	1.24	(Mayoral et al. 2020)
GRA44	TGME49_228170*	Putative phosphatase, associates with the translocon complex. Located in the PVM	acid-phosphatase domain, no signal peptide,	-3.28	(Panas et al. 2019; Blakely et al. 2020)

			transmembrane domain		
Organelle Recruitment					
MAF1	TGME49_020950*	Mediates Recruitment of Host Mitochondria and Impacts the Host Response at the PVM	signal peptide, transmembrane domain,	2.13	(Pernas et al. 2014)
GRA3	TGME49_227280	interacts with host Golgi and dysregulates anterograde transport from the ER to plasma membrane. Located in the PVM and IVN	transmembrane domain, no signal peptide	2.14	(Craver and Knoll 2007; Deffieu et al. 2019)
Immune Response and Cell cycle regulators					
GRA4	TGME49_310780	elicits both mucosal and systemic immune responses following oral infection of mice with cysts. Maintains the IVN. Located at the IVN	transmembrane domain, no signal peptide	2.34	(Labruyere et al. 1999; Nam 2009)
GRA8	TGME49_254720	regulates host immune responses by mediating mitochondrial and metabolic changes. Located in the PV.	transmembrane domain, no signal peptide	1.56	(Carey et al. 2000; Kim et al. 2018)
GRA12	TGGT1_275850	potential immune-related function. Located at the IVN.	transmembrane domain, no signal peptide	1.3	(Fox et al. 2019; Michelin et al. 2009; Wang et al. 2020)
GRA15	TGME49_275470	activates STING (the interferon stimulator). Located in host cytosol	transmembrane domain, no signal peptide	2.33	(Rosowski et al. 2011; Wang et al. 2019)
GRA16	TGME49_208830*	interfering with the cell cycle by regulating p53 to avoid premature immune detection. Located in the host cell nucleus	Signal peptide, predicted nuclear localization signal, no transmembrane domain	2.28	(Bougdoor et al. 2013; Panas and Boothroyd 2020)
GRA18	TGME49_288840	induces the expression of genes associated with an anti-inflammatory response	signal peptide no transmembrane domain	1.66	(He et al. 2018)

GRA20	TGGT1_200010	may participate in the manipulation of the host immunity. Located at the PVM	no signal peptide or transmembrane domain	2.54	(Hsiao et al. 2013; Ning et al. 2015)
GRA24	TGME49_230180*	Activates the mitogen-activated protein kinase, promotes p38 MAPK activation during host immunity. Located at the host nucleus. Negative regulation of bradyzoite differentiation.	no signal peptide, transmembrane domain, NLS	2.86	(Braun et al. 2013; Mercer et al. 2020; Odell et al. 2015)
GRA25	TGME49_290700	Modulates host immune responses. Located in the PV	signal peptide, no transmembrane domain	1.31	(Shastri et al. 2014)
GRA28	TGGT1_231960	Required for Placenta-Specific Induction of the Regulatory Chemokine CCL22 in Human and Mouse. Localized to the host nucleus	signal peptide, no transmembrane domain	1.48	(Rudzki et al. 2021)
GRA35	TGGT1_226380	induces Lewis rat macrophage pyroptosis and establishes a chronic infection in vivo. Located at the PV.	signal peptide, transmembrane, coiled-coil domains	1.98	(Bai et al. 2018; Wang et al. 2019)
GRA39	TGME49_289380	virulence factor that can interfere with host cell signaling pathways	signal peptide no transmembrane domain	-4.8	(Nadipuram et al. 2016)
GRA42	TgME49_236870	required for induction of Lewis rat macrophage pyroptosis, chaperone for GRA protein into PVM. Located in the PV lumen	signal peptide, transmembrane domains	2.8	(Wang et al. 2019)
GRA43	TgME49_237015	required for induction of Lewis rat macrophage pyroptosis, chaperone for GRA protein into PVM. Located in the PV lumen	signal peptide, transmembrane domains	2.34	(Wang et al. 2019)
GRA60	TGME49_204270	modulates host cell immunity and contributes to parasite virulence. Located at PVM.	signal peptide, transmembrane domains	-0.59	(Nyonda et al. 2021)
Cyclophilin	TGME49_221210	Identified in multiple BioID pulldowns using known PV proteins, DG localization	Has signal peptide, no	-1.6	(Nadipuram et al. 2016;

		not verified by microscopy. Possible role in suppressing host immune response	transmembrane domain		Nadipuram et al. 2020)
HCE1/ TEERG	TGGT1_239010	modulates E2F transcription factor target genes to co-opt human cells for proper parasite growth. Located in host nucleus	transmembrane domain no signal peptide	1.46	(Panas et al. 2019)
IST1	TGME49_240060*	inhibits STAT1-dependent transcription in host cell. Localized to host cell nucleus	no signal peptide or transmembrane domain	1.44	(Gay et al. 2016)
NSM	TGME49_235140	Blocks IFN-driven expression of PKR and MLKL allowing for parasite survival in the host. Located in the cytosol.	No signal peptide or transmembrane domain	1.41	(Rosenberg and Sibley 2021)
Regulation of GRA protein function and trafficking					
WNG1	TGME49_304740*	Localized to the PV lumen, phosphorylates GRA proteins, required for GRA6 insertion into the IVN	no signal peptide or transmembrane domain	1.1	(Coffey et al. 2018; Beraki et al. 2019)
WNG2	TGME49_240090*	Localized to the PV lumen, knockout reduces virulence in mice	transmembrane domain, so signal peptide	0.69	(Coffey et al. 2018)
GRA45	TGME49_316250*	ASP5 substrate that acts as chaperone for effector proteins to the PVM	signal peptide no transmembrane domain	1.8	(Cygan et al. 2020; Y. Wang et al. 2020)
Cyst Wall Proteins					
GRA31	TGGT1_220240	hypothetical protein found in cyst wall	transmembrane domain, no signal peptide	0.9	(Bai et al. 2018)
GRA50/ CST2	TGME49_203600	establishes chronic infection	transmembrane domain, no signal peptide	2.29	(Tu et al. 2019)
GRA51/ CST3	TGME49_230705	cyst wall proteins	signal peptide, transmembrane domains	1.34	(Tu et al. 2019)
GRA52/ CST5	TGME49_319340	cyst wall proteins	signal peptide, transmembrane domains	-3.96	(Tu et al. 2019)
GRA53/ CST6	TGME49_260520	cyst wall proteins	no signal peptide or transmembrane domain	1.9	(Tu et al. 2019)

GRA55	TgME49_309760	establishment or maintenance of cysts in the mouse brain. localizes to the PV and cyst wall	no signal peptide or transmembrane domain	2.18	(Nadipuram et al. 2020)
GRA56	TgME49_309930	hydrolase activity, may catabolize melibiose for energy after uptake into the PV	no signal peptide or transmembrane domain	-1.13	(Nadipuram et al. 2020)
CST1	TGME49_064660	maintains cyst wall integrity and promotes bradyzoite persistence	Transmembrane domain, no signal peptide	0.85	(Guevara et al. 2020; Tomita et al. 2013; Zhang et al. 2020)
CST4	TGME49_261650	cyst wall proteins	WD40 repeat-like domain, no signal peptide or transmembrane domain	1.61	(Tu et al. 2020)
CST7	TgME49_258870	cyst wall proteins	transmembrane domain, no signal peptide	1.32	(Tu et al. 2020)
CST8	TgME49_204340	cyst wall proteins	signal peptide, transmembrane domains	1.6	(Tu et al. 2020)
CST9	TgME49_310790	cyst wall proteins	signal peptide, no transmembrane domain	-3.1	(Tu et al. 2020)
MAG1	TGME49_270240	Expressed in Bradyzoites and modulates IL-1 $\beta$ secretion in the PV	signal peptide, transmembrane domains	1.23	(Tomita et al. 2021)
BPK1	TGME49_253330	Needed for the development and infectivity of cysts. Located at the cyst wall.	signal peptide, no transmembrane domain	-1.78	(Buchholz et al. 2011; 2013)
Proteins with unknown function					
GRA1	TGME49_270250	Potential function in suppression of host apoptosis. Localizes to the PV lumen	signal peptide, calcium binding domain, no transmembrane domain	-5.27	(Cesbron-Delauw et al. 1989; Wu et al. 2017)
GRA5	TGME49_286450	may have a critical function in parasite-host interaction. Located in PV and PVM	transmembrane domain, no signal peptide	2.58	(Chen et al. 2012; Lecordier et al. 1999)

GRA9	TGME49_251540	May be involved in parasite egress and virulence. Located at the PVM and IVN	hydrophobic alpha-helices, signal peptide, no transmembrane domain		(Guo et al. 2019; Mercier et al. 2005)
GRA10	TGME49_268900	Required for parasite growth. Located in the PV lumen	transmembrane domain, no signal peptide	-4.45	(Nam 2009; Mercier and Cesbron-Delauw 2015; Witola et al. 2014)
GRA19	TgME49_087740*	Interacts with and may have a role at the PVM	no signal peptide or transmembrane domain	-1.66	(Hsiao et al. 2013; Mercier and Cesbron-Delauw 2015)
GRA21	TgME49_041610*	Localized at the PV and PVM	signal peptide no transmembrane domain	ND	(Hsiao et al. 2013; Mercier and Cesbron-Delauw 2015)
GRA30	TGGT1_232000	Not essential in RH strain	transmembrane domain, no signal peptide	2.29	(Bai et al. 2018)
GRA33	TGGT1_247440	hypothetical protein found in cyst wall	signal peptide, transmembrane domains	1.72	(Bai et al. 2018; Tu et al. 2019)
GRA34	TGGT1_203290	hypothetical protein found in cyst wall	signal peptide, transmembrane domains	2.26	(Bai et al. 2018; Tu et al. 2019)
GRA36	TGGT1_213067	Not essential in RH strain	signal peptide, transmembrane domains	-0.21	(Bai et al. 2018)
GRA37	TGGT1_236890	Not essential in RH strain	transmembrane domain, no signal peptide	2.1	(Bai et al. 2018)
GRA38	TGGT1_312420	hypothetical protein possibly located in the PV	signal peptide, no transmembrane domain	-1.15	(Bai et al. 2018; Nadipuram et al. 2016)
GRA40	TGGT1_219810	hypothetical protein possibly in the PV	transmembrane domain, no signal peptide	0.69	(Bai et al. 2018)



GRA46	TGME49_208370*	Not essential for ME49 strain, localized to the DG membrane, not secreted into the PV	signal peptide, transmembrane domains	1.79	(Coffey et al. 2018)
GRA47	TGME49_254000	hypothetical protein important for parasite survival in ME49 strain	no signal peptide or transmembrane domain	-3.49	(Beraki et al. 2019; Tu et al. 2019)
GRA48	TgME49_267740	Not essential for ME49 strain	transmembrane domain, no signal peptide	1.89	(Tu et al. 2019)
GRA49	TgME49_244530	Not essential in ME49 strain	no signal peptide or transmembrane domain	2.2	(Tu et al. 2019)
GRA57	TGME49_217680	Not essential in ME49 strain	no signal peptide or transmembrane domain	0.99	(Nadipuram et al. 2020)
GRA58	TGME49_268790	Not essential in ME49 strain	no signal peptide or transmembrane domain	1.63	(Nadipuram et al. 2020)
GRA59	TGME49_313440	Not essential in ME49 strain	signal peptide, no transmembrane domain	1.63	(Nadipuram et al. 2020)
<b>Strand Forming Proteins</b>					
SFP1	TGGT1_289540	stranded structure formation within the PV	four coiled-coil domains, no signal peptide or transmembrane domain	0.33	(Young et al. 2020)
GRA29	TGME49_269690	regulates SFP1 and cyst formation and forms stranded structures in the PV	signal peptide, transmembrane domains	1.54	(Young et al. 2020)
<b>Egress</b>					
DGK2	TGME49_259830	Makes phosphatidic acid, required for natural parasite egress	diacylglycerol kinase catalytic domain, signal peptide, no transmembrane domain	0.05	(Bisio et al. 2019)

GRA22	TgME49_215220	required for regulating parasite egress. Located at the PV	no signal peptide or transmembrane domain	1.07	(Mercier and Cesbron-Delauw 2015; Okada et al. 2013)
GRA41	TGGT1_069070	Regulates calcium levels and the timing of egress. Secreted in the PV and associates with the TVN	N-terminal signal sequence	ND	(LaFavers et al. 2017)