CU Boulder OCG Note: The following example Authentication of Key Biological and/or Chemical Resources is from a K08 proposal provided as an example by NIAID (https://www.niaid.nih.gov/grants-contracts/sample-applications).

Authentication of Key Biological and/or Chemical Resources

Key biological resources in this proposal include

1: Sera from human individuals with latent or active tuberculosis.
These are collected by our collaborator Dr Blanca Restrepo. Key control experiments performed in Dr Alter's lab have validated that the functional activity of IgG isolated via our methods of preparation and storage are maintained over time and freeze thaw cycles consistent with our manner of use. This includes the function of antibody dependent cellular phagocytosis. Multiple filtration steps by ultracentrifugation are employed in the isolation of IgG from the sera of these individuals to prevent indiscriminant contamination. This is confirmed by endotoxin testing.

2: Primary human monocyte derived macrophages.
There are multiple approaches to isolating primary human monocyte derived macrophages in the literature. In this study, they are isolated from peripheral blood from healthy human donors in the Boston, MA area which carries a low prevalence of tuberculosis. These donors are screened and negative for HIV, HCV and HBV. CD14 positive cells are isolated from whole blood with EasySep CD14 Selection Kit and matured for 7 days in RPMI with 10% fetal calf serum in low adherent flasks. Monocyte derived macrophages were characterized by flow cytometry as CD14-, CD40+, HLA ABC+, CD11b+, CD16+ (FcγRIIIa) and CD32+ (FcγRIIa). Alternative methods of maturation include the use of human sera instead of fetal calf sera and GM-CSF for the purposes of macrophage polarization. We have extensively tested these methods of maturation as well as duration of maturation (7, 14 and 21 days). Our method of maturation provides the most reliable and reproducible results for our *M. tuberculosis* macrophage restriction assay.

3. CEM-NKr CCR5+ T lymphoblast cell line.
This cell line is used in the Fc effector assay for Ab dependent cellular cytotoxicity in Aim 1. These cells were obtained from NIH AIDS reagents. These are maintained per suggested protocol by NIH AIDS reagents. This includes regular image cytometer analysis based on fluorescent dyes acridine orange and DAPI to stain the total and dead cell populations, respectively via Chemometec Nucleocounter. They are monitored by Internal controls to validate their functionality include the lack of antibody and the absence of antigen to assess indiscriminate cellular cytotoxicity. Further validation of the Ab dependent cellular cytotoxicity assays lies within validation of natural killer (NK) cells as described below.

4. Primary human natural killer (NK) cells.
Primary human natural killer (NK) cells are used in the Fc effector assay for Ab dependent cellular cytotoxicity in Aim 1. These cells are isolated from peripheral blood from healthy human donors in the Boston, MA area which carries a low prevalence of tuberculosis. These donors are screened and negative for HIV, HCV and HBV. NK cells were isolated from whole blood with RosetteSep (Stem Cell Technologies). They are used on the day of isolation and the purity of their isolation is confirmed by flow cytometry as CD3- and CD16 and/or CD56+. Wells containing serve as a positive control for maximal NK cell activation to ensure that they are functionally optimatlly.

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Authentication of Key Biological and/or Chemical Resources

Growth Inhibition Assays

Type O+ human erythrocytes used in the growth inhibition assays (GIAs) will be obtained from the Indiana Blood Center. *Plasmodium falciparum* 3D7 blood stage parasites used in GIAs will be cultured from cryopreserved stocks (courtesy of Jianbing Mu, National Institute of Allergy and Infectious Diseases) that have been authenticated as 3D7 by 6-loci microsatellite genotyping.

Hepatocyte Invasion Assays

For the microscale human liver platform (also called micropatterned co-culture assay), Dr. Sangeeta Bhatia's laboratory uses commercially available cryopreserved primary human hepatocytes (BioreclamationIVT; Invitrogen) and 3T3-J2 murine embryonic fibroblasts (courtesy of Howard Green, Harvard Medical School). Sanaria (Rockville, Maryland) will supply the *P. falciparum* NF54 for this study.

Flow cytometry

Antibodies used for flow cytometry will be purchased from three commercial vendors: BioLegend, BD Biosciences and R&D systems. The following are anticipated antibodies based on our prior experience:

Panel	Marker	Fluorochrome	Clone	Catalog
Surface	CD19	Brilliant Violet 785	HIB19	302240
Surface	CD21	APC	HB5	17-0219-42
Surface	CD27	Brilliant Violet 421	M-T271	356418
Surface	CD10	Brilliant Violet 510	HI10a	312219
Surface	CD23	PE-Cy7	B3B4	101613
Surface	IgG	PE-CF594	G18-145	562538
Surface	CD95	Brilliant Violet 605	DX2	305627
Surface	CD14	Brillian Violet 785	M5E2	301840
Surface	CD16	Brilliant Violet 605	3G8	302040
Surface	anti-FcεRI, common γ subunit	FITC	2472959	FCABS400F
Surface	CD3	Horizon V500	UCHT1	561416
Surface	CD4	PerCP	RPA-T4	300527
Surface	CD8	Brilliant Violet 570	RPA-T8	301038
Surface	CD56	Brilliant Violet 711	HCD56	318335
Surface	TCR γ/δ	APC	B1	331211
Surface	FCRL5	PE	509f6	340304
Intracellular	Phospho-STAT6 (Y641)	PE		IC3717P
Intracellular	T-bet	PE-CF594	O4-46	562467
Intracellular	STAT6	APC	253906	IC2167A
Intracellular	GATA3	PE-Cy7	L50-823	560405
Intracellular	IFN-γ	APC/Cy7	4S.B3	502529
Intracellular	IL-6	PE	MQ2-13A5	501106

CU Boulder OCG Note: The following example Authentication of Key Biological and/or Chemical Resources is from a R15 proposal provided as an example by NIAID (https://www.niaid.nih.gov/grants-contracts/sample-applications).

AUTHENTIFICATION OF KEY BIOLOGICAL AND CHEMICAL RESOURCES

All key resources for this proposal will be authenticated to enhance the reproducibility of our results, as appropriate and according to NIH policy.

Key Biological Resources that will be utilized in this proposal include:

Cell lines: DH5α (*E. coli* competent cells), BL21(DE3) (*E. coli* competent cells), Vero cells (from kidney epithelial cells extracted from African green monkey).

Cell lines MAX Efficiency® DH5α™ Competent Cells will be purchased from ThermoFisher Scientific (Invitrogen™ distributer; catalog number 18258012), and come with the company's authentication certificate.

Cell lines One Shot® BL21(DE3) Chemically Competent *E. coli* will be purchased from ThermoFisher Scientific (Invitrogen™ distributer; catalog number C600003), and come with the company's authentication certificate.

Cell lines Vero Cell Line will be purchased from Sigma-Aldrich® (84113001-1VL) and come with the company's authentication certificate.