

Supplementary Material For:

# Reports

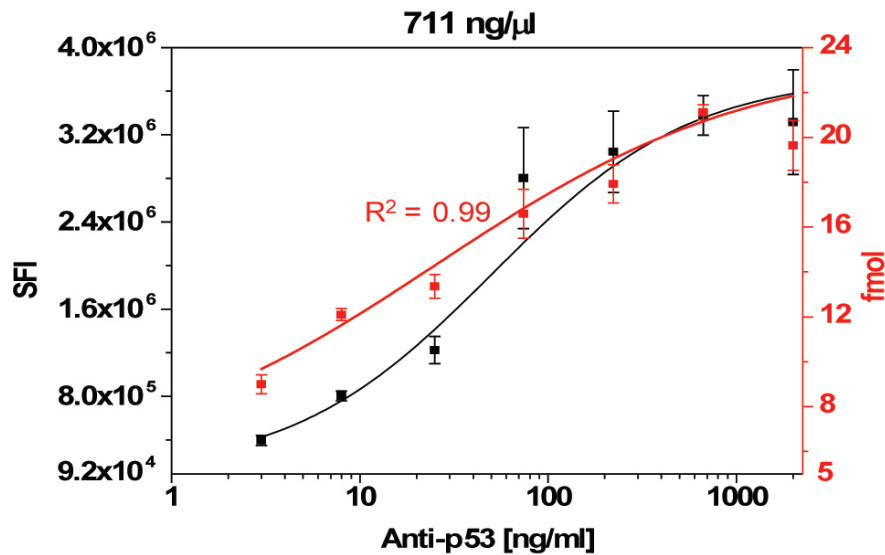
## Quantifying antibody binding on protein microarrays using microarray nonlinear calibration

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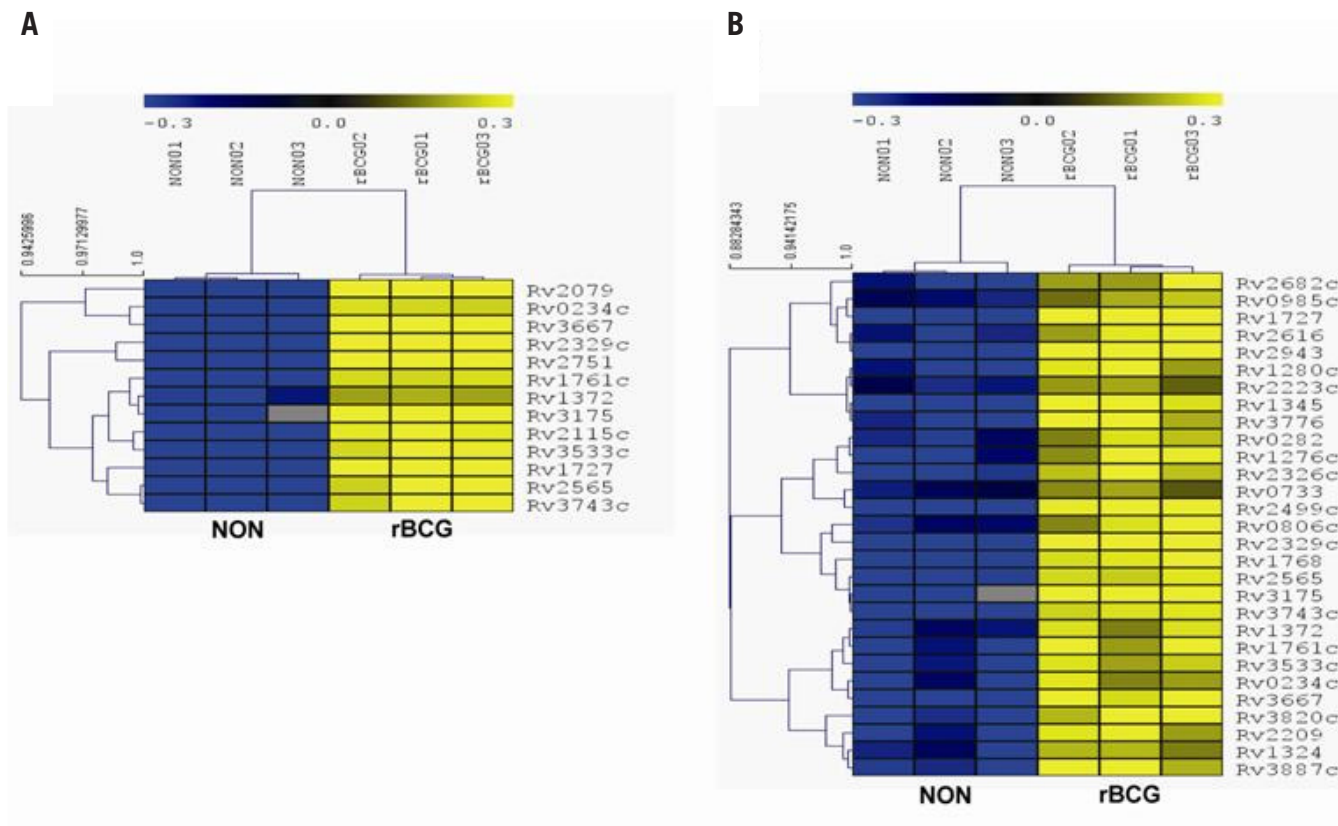
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**Keywords:** microarray nonlinear calibration; nucleic acid programmable protein arrays; in vitro transcription/translation; tuberculosis



**Supplementary Figure S1. Detection of anti-p53 antibody using a p53 DNA plasmid concentration of 711ng/μl to produce the p53 protein locally.** The values of y-axis at both sides of x-axis represent the signal intensity (black) and the fmol (red) from the blank controls, respectively.



**Supplementary Figure S2. Identification of antibodies elevated in the rBCG vaccinated guinea pigs using the Welch *t*-test.** (A) and (B) show antibodies with significantly increased levels in rBCG vaccinated compared to non-vaccinated guinea pigs analyzed without and with MiNC, respectively. The serological analysis was performed using protein microarrays displaying 849 TB proteins and probed with serum from *M. tuberculosis* challenged guinea pig TB models without (NON) and with rBCG immunization. The color from blue to black to yellow corresponds to the normalized value from low to median to high. Gray represents the signal below the 25th percentile of nonspot controls. The statistical analysis was performed using the Welch *t*-test with a P value cutoff of 0.005.

**Supplementary Table S1. Identification of antibodies elevated in rBCG vaccinated guinea pigs using Significant Analysis of Microarray.**

No.	Protein	Gene description	Annotation	Without MiNC	With MiNC
1	Rv0457c	peptidase	NC_000962.2 (547586..549607, complement)	-	+
2	Rv3509c	hypothetical protein	NC_000962.2 (3936877..3938424, complement)	-	+
3	Rv1836c	hypothetical protein	NC_000962.2 (2082603..2084636, complement)	-	+
4	Rv1280c	periplasmic oligopeptide-binding lipoprotein	NC_000962.2 (1431665..1433440, complement)	-	+
5	Rv1538c	L-aporaginase ansA	NC_000962.2 (1741212..1742192, complement)	-	+
6	Rv2616	hypothetical protein	NC_000962.2 (2945330..2945830)	-	+
7	Rv3776	hypothetical protein	NC_000962.2 (4221089..4222648)	-	+
8	Rv2499c	oxidase regulatory-like protein	NC_000962.2 (2813173..2813730, complement)	-	+
9	Rv2154c	FtsW-like protein FtsW	NC_000962.2 (2413348..2414922, complement)	-	+
10	Rv1281c	oligopeptide-transport ATP-binding protein ABC transporter	NC_000962.2 (1433433..1435271, complement)	-	+
11	Rv3801c	long-chain-fatty-acid--CoA ligase	NC_000962.2 (4261153..4263066, complement)	-	+
12	Rv0453	PPE family protein	NC_000962.2 (543174..544730)	-	+
13	Rv3820c	polyketide synthase associated protein PapA2	NC_000962.2 (4284419..4285825, complement)	-	+
14	Rv2209	integral membrane protein	NC_000962.2 (2473400..2474938)	-	+
15	Rv1092c	pantothenate kinase	NC_000962.2 (1219248..1220186, complement)	-	+
16	Rv3533c	PPE family protein	NC_000962.2 (3970705..3972453, complement)	-	+
17	Rv3555c	hypothetical protein	NC_000962.2 (3994830..3995699, complement)	-	+
18	Rv3667	acetyl-CoA synthetase	NC_000962.2 (4107792..4109747)	-	+
19	Rv2326c	transmembrane ATP-binding protein ABC transporter	NC_000962.2 (2597854..2599947, complement)	-	+
20	Rv1768	PE-PGRS family protein	NC_000962.2 (2000614..2002470)	-	+
21	Rv0782	oligopeptidase B	NC_000962.2 (874732..876390)	-	+
22	Rv1345	long-chain-fatty-acid--ACP ligase	NC_000962.2 (1509281..1510846)	-	+
23	Rv2943	IS1533 transposase	NC_000962.2 (3288464..3289705)	-	+
24	Rv2565	hypothetical protein	NC_000962.2 (2884611..2886362)	-	+
25	Rv3743c	cation transporter P-type ATPase CtpJ	NC_000962.2 (4193391..4195373, complement)	-	+
26	Rv2079	hypothetical protein	NC_000962.2 (2335355..2337325)	+	+
27	Rv2751	hypothetical protein	NC_000962.2 (3063638..3064528)	+	+
28	Rv0791c	hypothetical protein	NC_000962.2 (884797..885840, complement)	+	+
29	Rv3887c	transmembrane protein	NC_000962.2 (4370155..4371684, complement)	+	+
30	Rv3175	amidase	NC_000962.2 (3542860..3544347)	+	+
31	Rv2329c	nitrite extrusion protein 1 NarK1	NC_000962.2 (2601914..2603461, complement)	+	+
32	Rv2115c	ATPase	NC_000962.2 (2374461..2376290, complement)	+	+
33	Rv3375	amidase AmiD	NC_000962.2 (3788621..3790048)	+	+
34	Rv1727	hypothetical protein	NC_000962.2 (1953270..1953839)	+	+
35	Rv1761c	hypothetical protein	NC_000962.2 (1994671..1995054, complement)	+	+
36	Rv0436c	CDP-diacylglycerol--serine O-phosphatidyltransferase.	NC_000962.2 (524530..525390, complement)	+	-
37	Rv1576c	phiRV1 phage protein	NC_000962.2 (1780643..1782064, complement)	+	-
38	Rv1844c	6-phosphogluconate dehydrogenase	NC_000962.2 (2093731..2095188, complement)	+	-
39	Rv1188	proline dehydrogenase	NC_000962.2 (1331021..1332010)	+	-
40	Rv3667	acetyl-CoA synthetase	NC_000962.2 (4107792..4109747)	+	-
41	Rv1345	long-chain-fatty-acid--ACP ligase	NC_000962.2 (1509281..1510846)	+	-

\* The positive hits were selected with a delta value higher than 1.9 and an FDR of 0.

**Supplementary Table S2. Identification of antibodies elevated in rBCG vaccinated guinea pigs using the Welch *t*-test.**

No.	Protein	Gene description	Annotation	Without MiNC	With MiNC
1	Rv0282	hypothetical protein	NC_000962.2 (342130..344025)	-	+
2	Rv0733	adenylate kinase	NC_000962.2 (826122..826667)	-	+
3	Rv0806c	UDP-glucose-4-epimerase CpsY	NC_000962.2 (899732..901330, complement)	-	+
4	Rv0985c	large-conductance mechanosensitive channel	NC_000962.2 (1101025..1101480, complement)	-	+
5	Rv1276c	hypothetical protein	NC_000962.2 (1425438..1425914, complement)	-	+
6	Rv1280c	periplasmic oligopeptide-binding lipoprotein	NC_000962.2 (1431665..1433440, complement)	-	+
7	Rv1324	thioredoxin	NC_000962.2 (1487161..1488075)	-	+
8	Rv1345	long-chain-fatty-acid--ACP ligase	NC_000962.2 (1509281..1510846)	-	+
9	Rv1768	PE-PGRS family protein	NC_000962.2 (2000614..2002470)	-	+
10	Rv2209	integral membrane protein	NC_000962.2 (2473400..2474938)	-	+
11	Rv2223c	exported protease	NC_000962.2 (2493837..2495399, complement)	-	+
12	Rv2326c	transmembrane ATP-binding protein ABC transporter	NC_000962.2 (2597854..2599947, complement)	-	+
13	Rv2499c	oxidase regulatory-like protein	NC_000962.2 (2813173..2813730, complement)	-	+
14	Rv2616	hypothetical protein	NC_000962.2 (2945330..2945830)	-	+
15	Rv2682c	1-deoxy-D-xylulose-5-phosphate synthase	NC_000962.2 (2998052..2999968, complement)	-	+
16	Rv2943	IS1533 transposase	NC_000962.2 (3288464..3289705)	-	+
17	Rv3776	hypothetical protein	NC_000962.2 (4221089..4222648)	-	+
18	Rv3820c	polyketide synthase associated protein PapA2	NC_000962.2 (4284419..4285825, complement)	-	+
19	Rv3887c	transmembrane protein	NC_000962.2 (4370155..4371684, complement)	-	+
20	Rv2565	hypothetical protein	NC_000962.2 (2884611..2886362)	+	+
21	Rv3175	amidase	NC_000962.2 (3542860..3544347)	+	+
22	Rv3533c	PPE family protein	NC_000962.2 (3970705..3972453, complement)	+	+
23	Rv3667	acetyl-CoA synthetase	NC_000962.2 (4107792..4109747)	+	+
24	Rv3743c	cation transporter P-type ATPase CtpJ	NC_000962.2 (4193391..4195373, complement)	+	+
25	Rv2329c	nitrite extrusion protein 1 NarK1	NC_000962.2 (2601914..2603461, complement)	+	+
26	Rv1372	hypothetical protein	NC_000962.2 (1544825..1546006)	+	+
27	Rv1727	hypothetical protein	NC_000962.2 (1953270..1953839)	+	+
28	Rv1761c	hypothetical protein	NC_000962.2 (1994671..1995054, complement)	+	+
29	Rv0234c	succinic semialdehyde dehydrogenase	NC_000962.2 (279605..281140, complement)	+	+
30	Rv2079	hypothetical protein	NC_000962.2 (2335355..2337325)	+	-
31	Rv2751	hypothetical protein	NC_000962.2 (3063638..3064528)	+	-
32	Rv2115c	ATPase	NC_000962.2 (2374461..2376290, complement)	+	-

\*The positive hits were identified using the Welch *t*-test with a *P* value cutoff of 0.005.