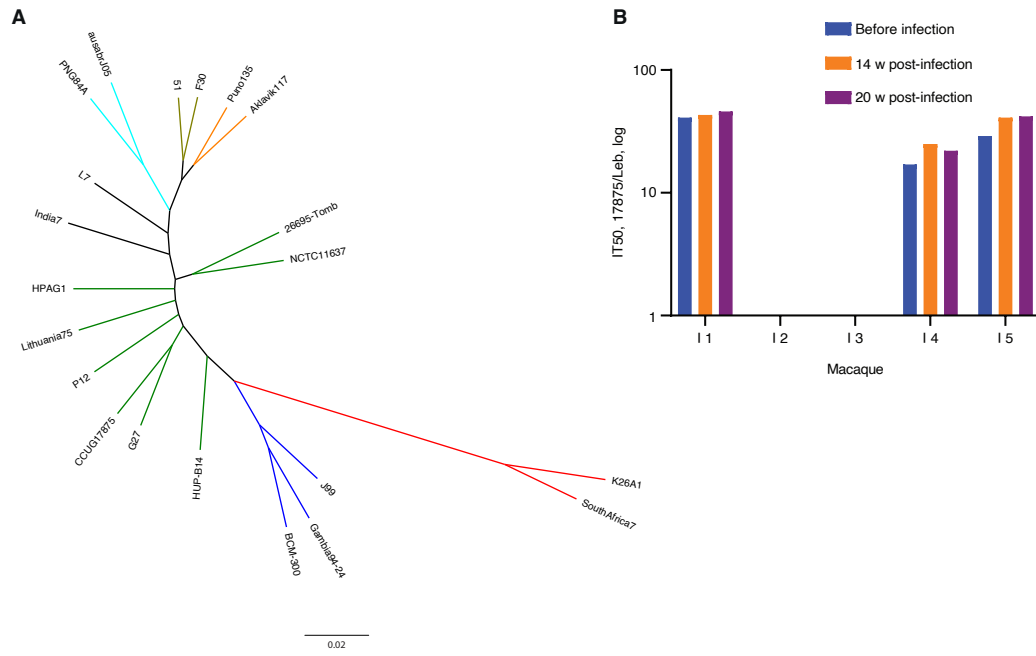


1 Supplemental figures

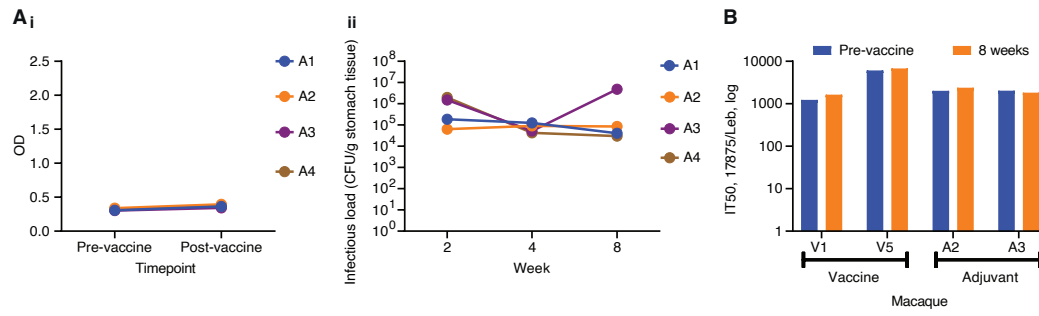
2



1 **Figure S1. The BCM300 strain phylogeny and induction of IT50 responses by**
2 **challenge infection in rhesus macaques.**

3 **(A)** Maximum likelihood (PhyML) phylogeny relating CCUG 17875 (17875/Leb, GenBank
4 CP090367) and BCM-300 (GenBank assembly GCA_900149805.1) to a reference set of
5 worldwide *H. pylori* genomes. Branch colors denote the *H. pylori* phylogeographic
6 populations hpAfrica2 (Africa, red), hpAfrica1 (Africa blue, including strains BCM-300 and
7 reference strain J99), hpEurope (Europe, green, including reference strains CCUG17875
8 and 26695-Tomb), hpAsia2 (India, black), hpSahul (Australia and Papua New Guinea,
9 turquoise), hspEAsia (South Korea and Japan, olive), and hspIndigenousAmerica (Peru
10 and Canada, orange).

11 **(B)** Five SPF rhesus macaques were challenge infected with *H. pylori* J166 to test for the
12 induction of IT50 responses. However, three of the animals, no. 1, 4, and 5, demonstrated
13 pre-challenge IT50 titers (in blue) as tested with 17875, suggesting that they carried *H.*
14 *pylori* before the start of the test.
15



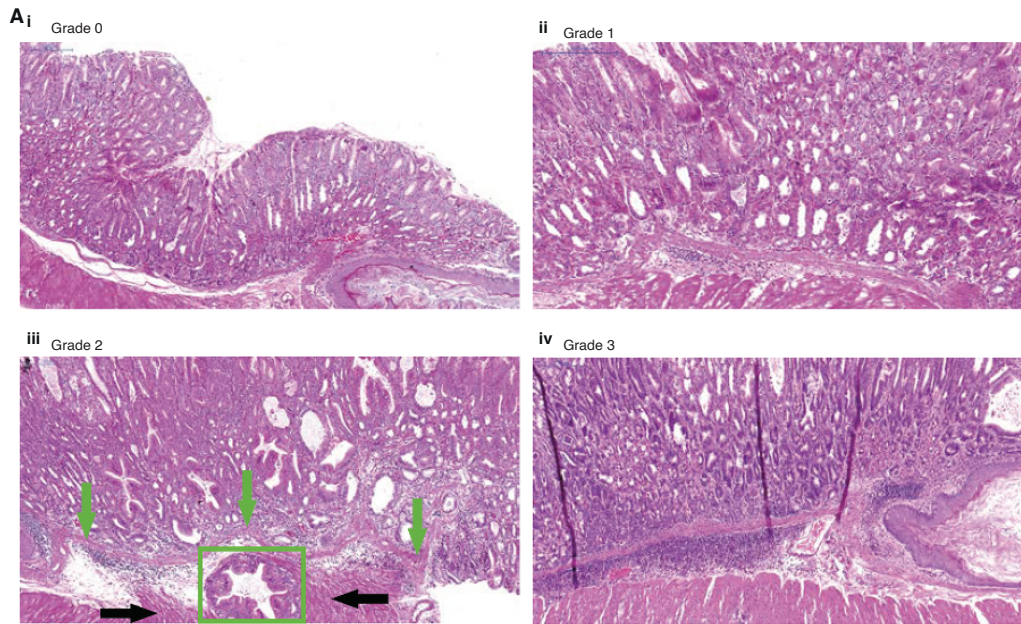
1 **Figure S2. Vaccination and IT50 responses in rhesus macaques.**

2 **(A \bar{i})** ELISA detection of serum BabA antibodies from adjuvant-only control animals, where
3 all four animals were BabA ELISA-negative. **(A \bar{i})** The four adjuvant-only control animals
4 demonstrated stable high infection loads during the 8 weeks of infection.

5 **(B)** The vaccinated animals V1 and V5 and the adjuvant-only control animals A2 and A3
6 demonstrated high pre-challenge IT50 titers when tested with strain 17875.

7

1



2

1 **Figure S3. The Leb-mouse gastric cancer animal model. The gastric mucosal**
2 **inflammatory infiltration scores**

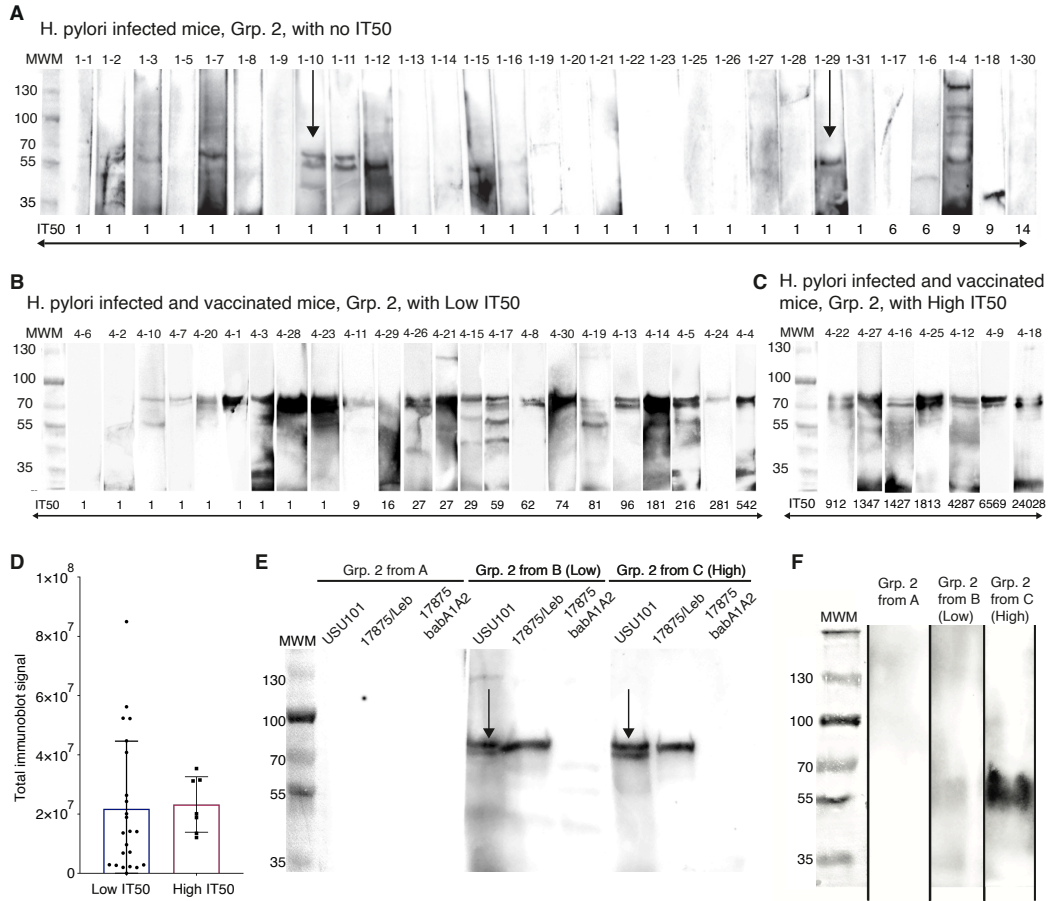
3 **(Ai)**: Grade 0: The mucous membrane and submucous plate contain rare inflammatory
4 cells, which are diffused throughout the tissue. Inflammatory cells do not form piles
5 (infiltrations) or groups. There are fewer than 20 inflammatory cells (neutrophils,
6 lymphocytes, macrophages, and plasmocytes) visible in the field of view (FOV) (diameter:
7 1000 μm).

8 **(Aii)**: Grade 1: The inflammatory cells are located in the basal part of the mucous
9 membrane and submucous plate where they can form small groups or piles. There are
10 20–100 inflammatory cells (neutrophils, lymphocytes, macrophages, and plasmocytes)
11 visible in the FOV.

12 **(Aiii)**: Grade 2: Inflammatory cells are seen in all parts of the mucous membrane and
13 submucous plate. Inflammatory infiltrates (like small lymph nodules) are seen in the basal
14 part of the mucous membrane and submucous plate. The gastric tissue contains 100–
15 300 inflammatory cells per FOV. In addition, the tissue exhibits fulminant gastric
16 adenocarcinoma with growth and penetration through the submucosal layer (lamina
17 propria indicated by green arrows) and with the invasion of cancer tissue (green box) into
18 the muscular layer (indicated by the black arrows).

19 **(Aiv)**: Grade 3: The inflammatory infiltrates are present in all layers of the gastric tissue
20 and form large inflammatory infiltrates (similar to lymphatic nodes) in the submucous plate
21 (between the muscular and mucus layers). The gastric tissue contains more than 300
22 inflammatory cells in the FOV.

23



1 **Figure S4. The first vaccination experiment induced protection against**
2 **inflammation and cancer.**

3 The sera samples from the 30 non-vaccinated mice and the 30 vaccinated mice were
4 analyzed by immunoblot detection of whole bacterial protein extracts from *H. pylori* strain
5 USU101, i.e., the strain used for the 12-month infections. The *H. pylori* protein extract
6 was separated on an SDS gel under denaturing (in **A**, **B**, **C**, and **E**) or semi-native (**F**)
7 conditions. After immunoblot transfer, the membranes were cut into strips. The
8 immunoblot signals were detected with mouse sera diluted 1:250 and goat-anti-mouse
9 HRP-Ab. The strips in **A**, **B**, and **C** were arranged according to IT50s. Under reducing
10 conditions, the 78 kDa BabA protein band migrated with a molecular mass of ~75 kDa;
11 (**A**) with no immuno-detected bands present and in (**B**) and (**C**) where both the BabA and
12 the BabB (migrating slightly faster) bands are present (also in (**E**)).

13 (**A**) The 30 infected but not vaccinated mice all lacked BabA/BabB immunoblot signals,
14 with a median IT50 of 9 ± 5 (similar to the non-infected mice with a median IT50 of 6 ± 5)
15 (**Table 3B** and **3C**). The blot was cut into strips and arranged according to IT50s.

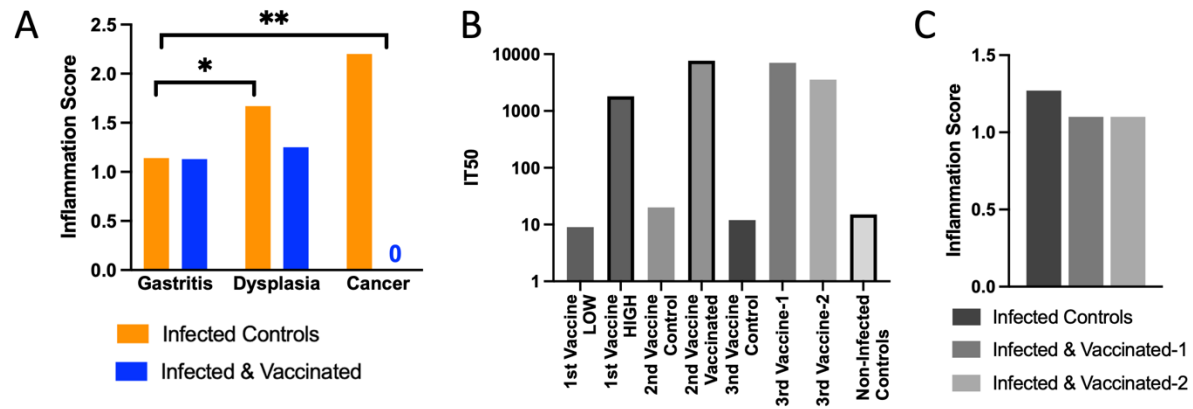
16 (**B**) The 23 infected and vaccinated mice that displayed LOW IT50s with median IT50 =
17 27 and mean IT50 = 74 (**Table S4A**). The blot was cut into strips and arranged according
18 to IT50s.

19 (**C**) The seven infected and immunized mice that displayed HIGH IT50s, with median IT50
20 = 1813 and mean IT50 = 5769 (**Table S4A**). The blot was cut into strips and arranged
21 according to IT50s.

22 (**D**) Scanning of the immunoblot chemiluminescence signal from all immunoblot strips
23 from immunized mice, i.e., from (**B**) with LOW IT50 and from (**C**) with HIGH IT50, showed
24 that the two groups demonstrated no significant difference in intensity in immune
25 response signals against the BabA and BabB bands. Thus, the vaccinated HIGH IT50
26 mice did not show a stronger signal for linear BabA and BabB epitopes compared to the
27 LOW IT50 mice suggesting that the HIGH IT50 group of mice were not hyper-responding
28 *per se* but rather displayed additional and different immune responses.

29 (**E**) Seven sera samples were pooled from A, from B (Low IT50) and from C (High
30 IT50) and used to probe three immunoblots with whole bacterial protein extracts from
31 strain *H. pylori* USU101 (SabA⁻, BabA⁺, BabB⁺), 17875/Leb (SabA⁻, BabA⁺, BabB⁻), and
32 17875bab1babA2 (SabA⁺, BabA⁻, BabB⁻). The *H. pylori* protein extracts were separated
33 on SDS gels under denaturing and reducing conditions. Sera from the infected but non-
34 immunized animals did not display the ~70–75 kDa BabA/BabB-bands (from **A**), but the
35 bands were visible in sera from immunized animals (from **B** and from **C**) and separated
36 protein extracts from *H. pylori* strains USU101 and 17875/Leb. Two bands were seen on
37 the immunoblot of strain USU101 that expresses both BabA and BabB. In comparison,
38 only the upper ~75 kDa band was seen for 17875/Leb that expresses BabA but not BabB.
39 In support of this, the ~70–75 kDa bands were not seen on immunoblot of sera from
40 immunized animals using protein extracts from the *babA*-deletion mutant
41 17875bab1babA2 that does not express BabA or BabB. In contrast, the SabA band was
42 not detected in the 17875bab1babA2 strain, which suggests that SabA is less
43 immunogenic. These results showed that vaccination with BabA and BabB protein raised
44 a humoral immune response against BabA and BabB in almost every vaccinated mouse
45 i.e., the animals in (**B**) and (**C**) with LOW vs. HIGH IT50s. In contrast, the infected but not
46 vaccinated animals (**A**) did not demonstrate natural immune responses towards

1 BabA/BabB. Blots with several dilution series were cut out and aligned for presentation.
2 (F) Seven sera samples were pooled from A, from B (Low IT50) and from C (High IT50)
3 and were used to probe three immunoblots with purified BabA protein from strain
4 17875/Leb. Different from (E), in this test the BabA protein was separated by SDS gel
5 electrophoresis under semi-native and non-reducing conditions, similar to **Figures 1I** and
6 **S1H** in *Bugaytsova et al., ms 1*. The BabA protein was detected only using sera from
7 mice in (C), i.e., vaccinated mice with High IT50 responses, whereas mice from groups **A**
8 and **B**, i.e., the infected vs. the infected and vaccinated mice with Low IT50s, provided no
9 immuno-signal with semi-native BabA protein. For this test, the sera samples were 2-fold
10 more dilute (1:500) in order to minimize the (weaker) immune detection of linear epitopes
11 and to preferentially display the (stronger) immune signal of bbAbs that bind to structural
12 and folded BabA epitopes. Thus, the antibodies that were common for both the
13 vaccinated mice with Low (**B**) or High (**C**) IT50s recognize and bind linear BabA epitopes
14 on denaturing immunoblots. In contrast, the bbAbs that were only found in the sera of
15 mice with High IT50 (**C**) can bind to structural BabA epitopes under semi-native
16 conditions. Strips were cut out and aligned because the denatured vs. semi-native
17 samples needed to be separated.
18



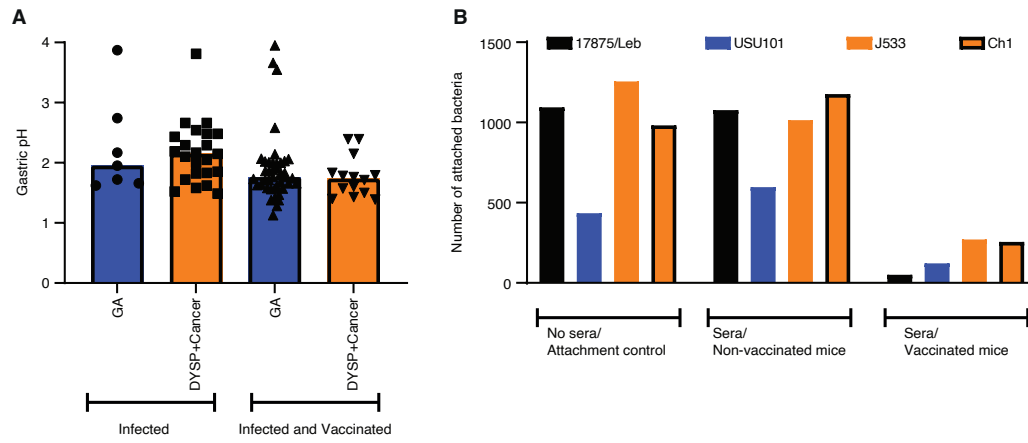
1
2

1 **Figure S5. Both the second and third vaccination experiments induced protection**
2 **against inflammation and cancer.**

3 **(A)** Similar to the first vaccination experiment, also in the second experimental series the
4 non-vaccinated mice had higher inflammation scores compared to the vaccinated mice.
5 The non-vaccinated mice with dysplasia scored 1.67, Dunn's test $p = 0.039^*$, and those
6 with cancer scored 2.2, $p < 0.001^{**}$, compared to the mice with gastritis that scored 1.1.

7 **(B)** The distribution of median IT50s for the first, second, and third vaccine experiments.
8 In the first vaccination experiment, the LOW IT50 group demonstrated a median
9 background IT50 = 16, which was in contrast to the several log-fold higher IT50s, median
10 IT50 = 1813, in the HIGH IT50 group (**Figure 4**). In the second vaccination experiment
11 (**Figures 5 A-C**), the 16 non-vaccinated control mice demonstrated similar low IT50 = 20,
12 in contrast to the several log-fold higher IT50s, median IT50 = 7318, in the 21 vaccinated
13 mice. Also, in the third vaccination experiment (**Figure 5D**) the 30 non-vaccinated control
14 mice demonstrated similar low IT50, median IT50 = 15, in contrast to the several log-fold
15 higher IT50s, median IT50 = 7026, in the 27 vaccinated mice in the Vaccine-1 group and
16 the several log-fold higher IT50s, median IT50 = 3557, in the 28 vaccinated mice in the
17 Vaccine-2 group. The 4 non-infected mice demonstrated no or low IT50, with a median
18 IT50 = 15.

19 **(C)** The lower gastric cancer incidence in the third vaccine experiment might be a
20 consequence of the lower mucosal inflammation score of 1.25 in the non-vaccinated
21 animals and 1.1 in the vaccinated animals (Vaccine-1 and Vaccine-2) compared to the
22 higher inflammation score of 1.5 in the non-vaccinated mice in the second vaccine
23 experiment.
24



1 **Figure S6. Vaccination preserved gastric acidity and induced bbAb responses that**
2 **reduced *H. pylori* gastric mucosal attachment.**

3 **(A).** In the third vaccination series, the non-vaccinated mice demonstrated elevated
4 gastric pH, whereas the vaccinated groups preserved the gastric acidity over the 12-
5 month period, $p = 0.00073^{***}$.

6 **(B)** Sera from the second vaccinated group of mice reduced attachment to the human
7 gastric mucosa of *H. pylori* 17875/Leb, USU101, J533 (Japan), and Ch1 (China)
8 compared to the sera of the non-vaccinated controls.
9

1 Supplemental Tables

2

3 Table S1 related to Figure 1.

4

5 Table S2. related to Figure 2.

6

7 Table S3. related to Figure 3.

8

9 Table S4. related to Figures 4 and 5

10

11 Table S5. related to Figures 5 and 6.

12

13

1 **Table S1**

2

3 **Table S1A related to Fig. 1A.**

4

The sera IT50s of healthy volunteers from placebo-controlled phase 1/2 study.

Serum Code	IT50, <i>H. pylori</i> BCM300		IT50, <i>H. pylori</i> 17875/Leb	
	Pre-infected	Post-12 weeks	Pre-infected	Post-12 weeks
1001	1	297	1	1
1002	1	139	1	1
1003	1	267	1	1
1004	1	1823	1	1
1006	1	111	1	1
1007	1	437	1	30 ^a
1008	1	54	1	1
1010	1	246	1	1
1016	1	181	1	1
1018	18	368	1	85 ^a
1019	1	1039	1	1
1021	1	285	1	1
1022	1	880	1	1
1023	1	370	1	1
1024	1	1492	1	51 ^a
1027	1	1	1	1
1028	1	3467	1	1
1029	1	54	1	16
1030	1	2772	1	27 ^a
1031	1	212	1	1

1034	1	70	1	1
1038	1	128	1	1
1039	25	68	1	1
1040	1	62	1	1
1042	1	58	1	1
1047	1	23	1	1
1048	1	134	1	50 ^a
1049	30	41	1	1
1053	1025	438	1	6

^a - Immune response above the critical IT50>30 is indicated by colored boxes.

Table S1B related to Fig. 1Bi.

The sera IT50s of SPF macaques challenge infected with *H. pylori* J166 and tested with J166 and 17875/Leb.

Macaque	IT50 <i>H. pylori</i> , J166 (Fig. 1Bi)			IT50 <i>H. pylori</i> , 17875/Leb (fig. S1A)		
	Before infection	14 w post-infection	20 w post-infection	Before infection	14 w post-infection	20 w post-infection
I 1	13546	12424	11629	41	43	46
I 2	12	195	228	1	1	1
I 3	52	167	174	1	1	1
I 4	9346	12789	11191	17	25	22
I 5	7364	13945	9452	29	41	42

Table S1C related to Fig. 1Bii.

The sera IT50s of SPF macaques challenge infected with *H. pylori* J166 and tested with J166 and I9.

Macaque	IT50 <i>H. pylori</i> , J166		IT50 <i>H. pylori</i> , I9	
	Before infection	20 w post-infection	Before infection	20 w post-infection
I 2	20	231	16	45
I 3	65	223	32	50

1 **Table S2 related to Fig. 2.**

2

3 **Table S2A related to Fig. 2Ai and fig. S2 Ai.**

4 **The sera ELISA of vaccinated and control macaques.**

Time-point	ELISA								
	V1 ^a	V2	V3	V4	V5	A1 ^b	A2	A3	A4
Pre-vaccine	0.336	0.351	0.361	0.366	0.282	0.307	0.339	0.303	0.318
Postvaccine	0.501	1.950	2.371	0.950	1.382	0.363	0.395	0.343	0.375

5 V^a – vaccinated macaque

6 A^b – control macaque with adjuvant only

7

8 **Table S2B related to Fig. 2Aii and Fig. S2Aii.**

9 ***H. pylori* infectious load in vaccinated and control macaques.**

Time after vaccination	Infectious load (CFU/g of stomach tissue)									
	V1 ^a	V2	V3	V4	V5	A1 ^b	A2	A3	A4	
Week 2	95.500	140	665	2.750.000	132.000	187.000	63.900	1.500.000	1.970.000	
Week 4	36.100	1	1	2.880.000	383.000	125.000	90.900	52.400	42.900	
Week 8	154.000	205	6.290	40.600.000	2.410.000	40.500	85.300	4.860.000	29.900	

10 V^a – vaccinated macaque

11 A^b – control macaque with adjuvant only

12

13 **Table S2C related to Fig. 2B.**

14 **The sera IT50s of vaccinated and control macaques.**

Time-point	IT50, <i>H. pylori</i> 17875/Leb and I9									
	V2 ^a		V3		V4		A1 ^b		A4	
	17875/Leb	I9	17875/Leb	I9	17875/Leb	I9	17875/Leb	I9	17875/Leb	I9
Pre-serum	4	18	1	38	1	7	1	30	1	17
Week 2	8	63	30	102	17	58	1	30	1	25
Week 4	25	79	28	131	18	52	1	-	1	-
Week 8	46	89	17	171	12	56	1	-	1	-

15 V^a – vaccinated macaque

16 A^b – control macaque with adjuvant only

17

1 Table S3 related to Fig. 3 and 4.

2

3 Table S3A related to Fig 3B.

4 Sera IT50 for *H. pylori* 17875/Leb and Histological Scoring of *H. pylori* infected and vaccinated Leb-mice.

5 Group I, Infected Mice (34 mice).

Mouse	CFU/g	Leb binding, %	Chronic Gastritis	Dysplasia	Cancer	Inflammation Score	Final Diagnosis	IT50
#1-1	13333	0	+	+	+	2	Cancer	1
#1-2	0	-	+	-	-	1	GA	1
#1-4	1600	10	+	+	-	1	DYSP	1
#1-5	1278	0	+	-	-	2	GA	1
#1-8	0	-	+	+	+	3	Cancer	1
#1-10	0	-	+	+	+	3	Cancer	1
#1-12	0	-	+	-	-	2	GA	1
#1-13	0	-	+	-	-	1	GA	1
#1-14	6321	0	+	+	+	3	Cancer	1
#1-15	438	0	+	+	+	2	Cancer	1
#1-16	0	-	+	-	-	1	GA	1
#1-18	469	0	+	+	+	3	Cancer	1
#1-19	0	-	+	+	+	2	Cancer	1
#1-21	125	0	+	+	+	3	Cancer	1
#1-24	0	-	+	+	-	1	DYSP	1
#1-25	3950	0	+	+	+	3	Cancer	1
#1-27	0	-	+	-	-	1	GA	1
#1-28	39	0	+	+	+	3	Cancer	1
#1-29	563	0	+	+	+	3	Cancer	1
#1-30	0	-	+	-	-	2	GA	1
#1-31	145	0	+	+	+	3	Cancer	1
#1-32	886	0	+	+	+	3	Cancer	1
#1-33	283	0	+	+	+	3	Cancer	1
#1-34	0	-	+	+	+	3	Cancer	1
#1-35	205	0	+	+	-	2	DYSP	2
#1-17	2200	0	+	-	-	2	GA	3
#1-6	0	-	+	+	+	3	Cancer	3
#1-7	0	--	+	-	-	1	GA	4

#1-20	0	-	+	+	+	3	Cancer	6
#1-26	0	-	+	+	+	3	Cancer	7
#1-3	0	-	+	+	-	2	DYSP	7
#1-23	0	-	+	+	-	2	DYSP	7
#1-22	0	-	+	+	-	2	DYSP	7
#1-9	4556	0	+	+	+	3	Cancer	10
16 CFU positive/34 mice						77/34 animals=2.26		
Mean, 2274								
Median, 725								

1
2
3
4

**Table S3B related to Fig 3B.
Group II, Infected Mice (30 mice).**

Mouse	CFU/g	Leb binding, %	Chronic Gastritis	Dysplasia	Cancer	Inflammation Score	Final Diagnosis	IT50
#1-1	4222	0	+	-	-	1	GA	1
#1-2	0	-	+	-	-	0	GA	1
#1-3	0	-	+	-	-	0	GA	1
#1-5	0	-	+	+	+	3	Cancer	1
#1-7	1000	0	+	+	+	2	Cancer	1
#1-8	0	-	+	+	-	0	DYSP	1
#1-9	200	3	+	+	-	1	DYSP	1
#1-10	263	0	+	+	+	3	Cancer	1
#1-11	8499	0	+	+	+	3	Cancer	1
#1-12	10500	0	+	+	+	3	Cancer	1
#1-13	0	-	+	-	-	1	GA	1
#1-14	0	-	+	-	-	0	GA	1
#1-15	0	-	+	-	-	1	GA	1
#1-16	0	-	+	+	+	2	Cancer	1
#1-19	0	-	+	+	+	3	Cancer	1
#1-20	0	-	+	+	-	2	DYSP	1
#1-21	80	0	+	+	-	1	DYSP	1
#1-22	0	-	+	-	-	1	GA	1
#1-23	0	-	+	-	-	1	GA	1
#1-25	0	-	+	-	-	0	GA	1

#1-26	0	-	+	+	-	0	DYSP	1
#1-27	0	-	+	+	-	1	DYSP	1
#1-28	0	-	+	+	-	1	DYSP	1
#1-29	947	0	+	+	+	2	Cancer	1
#1-31	0	-	+	+	+	1	Cancer	1
#1-6	0	-	+	+	+	1	Cancer	6
#1-17	0	-	+	+	-	2	DYSP	6
#1-4	57629	0	+	+	+	3	Cancer	9
#1-18	0	-	+	-	-	1	GA	9
#1-30	0	-	+	+	+	2	Cancer	14
	9 CFU positive/30 mice					42/30 animals=1.4		
	Mean, 9260							
	Median, 1000							

1
2
3
4

**Table S3C related to Fig 3B.
Group III, Non- infected Mice (12 mice).**

Mouse	CFU/g	Leb binding, %	Chronic Gastritis	Dysplasia	Cancer	Inflammation Score	Final Diagnosis	IT50
#2-1	0	-	-	-	-	0		1
#2-2	0	-	-	-	-	0		1
#2-3	0	-	-	-	-	0		1
#2-5	0	-	-	-	-	0		1
#2-6	0	-	-	-	-	0		1
#2-7	0	-	-	-	-	0		1
#2-9	0	-	-	-	-	0		1
#2-10	0	-	-	-	-	0		1
#2-11	0	-	-	-	-	0		1
#2-12	0	-	-	-	-	0		1
#2-13	0	-	-	-	-	0		1
#2-14	0	-	-	-	-	0		1
	Mean, 0					0/12 animals=0		
	Median, 0							

5
6

1 **Table S3D related to Fig. 3C.**
 2 **Histological Scoring of long-term (2-12 months) *H. pylori* infected Leb-mice.**

Group	Mouse ID	CFU/g	Leb binding, %	Gastritis	Dysplasia	Cancer	Final Diagnosis	Inflammation Score
2 month post-infection 10 mice	B140	300	11	+	-	-	GA	1
	B141	0	-	+	-	-	GA	1
	B165	250	12	+	-	-	GA	1
	B172	1833	10	+	+	-	DYSP	2
	B177	750	18	+	-	-	GA	1
	B178	375	19	+	-	-	GA	1
	B212	6833	0	+	-	-	GA	2
	B213	1750	9	+	-	-	GA	2
	B216	4917	8	+	-	-	GA	1
	B217	37000	0	+	-	-	GA	1
		Mean, 6001						
	Median,1750							
6 month post-infection 29 mice	B80	0	0	+	-	-	GA	1
	B82	167	9	+	-	-	GA	1
	B84	0	-	+	+	-	DYSP	2
	B86	3750	5	+	-	-	GA	1
	B87	1000	11	+	+	-	DYSP	2
	B88	250	11	+	-	-	GA	1
	B92	188	0	+	-	-	GA	2
	B105	50	16	+	-	-	GA	1
	B106	3917	0	+	+	-	DYSP	2
	B111	10400	14	+	+	-	DYSP	2
	AB112	0	-	+	-	-	GA	1
	AB113	464	5	+	+	-	DYSP	2
	AB115	800	11	+	-	-	GA	2
	AB116	500	9	+	-	-	GA	1
	AB117	125	0	+	-	-	GA	1
	AB119	50	11	+	-	-	GA	1
	AB120	542	8	+	-	-	GA	2
AB121	0	-	+	+	-	DYSP	2	
B144	36	11	+	+	-	DYSP	1	

	B145	333	10	+	+	-	DYSP	1
	B146	1125	-	+	-	-	GA	1
	B154	281	10	+	-	-	GA	1
	B159	156	13	+	+	-	DYSP	2
	B161	225	12	+	+	-	DYSP	2
	B162	313	12	+	+	-	DYSP	1
	B167	1500	9	+	-	-	GA	1
	B169	464	8	+	+	-	DYSP	1
	B196	0	-	+	-	-	GA	2
	B197	1075	8	+	-	-	GA	2
		Mean, 1112						
		Median, 333						
9 month post-infection 34 mice	B83	125	8	+	-	-	GA	1
	B85	0	-	+	-	-	GA	1
	B89	0	-	+	-	-	GA	1
	B116	0	-	+	-	-	GA	1
	B119	0	-	+	+	-	DYSP	2
	B120	0	-	+	+	-	DYSP	2
	B121	0	-	+	-	-	GA	2
	B122	143	4	+	+	-	DYSP	1
	B123	781	5	+	+	-	DYSP	1
	B125	0	-	+	-	-	GA	1
	B126	156	6	+	+	-	DYSP	1
	B138	0	-	+	-	-	GA	1
	B139	0	-	+	-	-	GA	1
	B148	143	5	+	-	-	GA	1
	B150	150	6	+	-	-	GA	1
	B157	0	-	+	-	-	GA	1
	B160	0	-	+	-	-	GA	1
	B163	222	8	+	-	-	GA	2
	B168	50	9	+	+	-	DYSP	1
	B170	0	-	+	+	+	Cancer	2
B171	500	5	+	+	+	Cancer	2	
B180	107	4	+	-	-	GA	1	
B183	2500	0	+	+	-	DYSP	1	

	B184	0	-	+	+	-	DYSP	1	1	
	B185	313	4	+	+	+	Cancer	2	2	
	B186	156	4	+	+	-	DYSP	2	3	
	B187	281	3	+	+	-	DYSP	1	4	
	B188	417	11	+	+	-	DYSP	2	5	
	B189	1750	0	+	-	-	DYSP	1	6	
	B190	2568	0	+	-	-	GA	1	7	
	B191	28	0	+	+	-	GA	1	8	
	B192	333	8	+	+	-	DYSP	1	9	
	B193	0	-	+	+	-	GA	2	10	
	AB110	292	3	+	-	-	GA	2	11	
		Mean, 551							12	
		Median, 251							13	
12 month post- infection 10 mice	B133	0	-	+	-	-	GA	1	14	
	B134	0	-	+	-	-	GA	1	15	
	B135	167	19	+	+	-	DYSP	1	16	
	B136	0	-	+	-	-	GA	1	17	
	B151	250	17	+	+	+	Cancer	2	18	
	B152	0	-	+	+	-	DYSP	2	19	
	B203	500	7	+	+	+	Cancer	2	20	
	B204	726	10	+	+	-	DYSP	1	21	
	B205	0	-	+	+	-	DYSP	1	22	
	B206	0	-	+	+	+	Cancer	1	23	
			Mean, 410							24
			Median, 375							

25

26

1 **Table S3E related to Fig. 3D.**
 2 **Sera IT50 for *H. pylori* 17875/Leb and Histological Scoring of *H. pylori* infected Leb-mice after eradiction of**
 3 **infection by antibiotics.**

Group	Mouse ID	Dysplasia	Cancer	Final Diagnosis
Non-infected (9 mice)	B740	-	-	
	B741	-	-	
	B742	-	-	
	B743	-	-	
	B745	-	-	
	B746	-	-	
	B747	-	-	
	B748	-	-	
	B749	-	-	
12 week post-infection (7 mice)	B758	-	-	
	B759	-	-	
	B767	-	-	
	B773	-	-	
	B774	-	-	
	B776	+	-	DYSP
	B788	-	-	
22 week post-infection (10 mice)	B777	+	+	Cancer
	B778	+	-	DYSP
	B779	-	-	Cancer
	B780	-	-	Cancer
	B782	+	-	DYSP
	B783	+	-	DYSP
	B784	+	+	Cancer
	B785	+	+	Cancer
	B786	+	-	DYSP
	B787	-	-	-
48 week post-infection (10 mice)	B800	-	-	
	B801	+	+	Cancer
	B802	+	+	Cancer
	B803	-	-	

	B804	+	-	DYSP	1
	B805	+	+	Cancer	2
	B807	-	-		3
	B808	+	+	Cancer	4
	B809	+	-	DYSP	5
	B810	+	-	DYSP	6

7
8
9
10

Table S3F related to Figure 3E and 3F.

Prevalence of CFU-positive *H. pylori* Leb-mice and their Leb-binding activity at the 12 months end-point.

Group	Treatment	CFU-positive mice, %	Leb-binders among outputs, %
Group I, Fig. 3B	<i>H. pylori</i> infected Leb mice	47	6
Group II, Fig. 3B	<i>H. pylori</i> infected Leb mice	30	11
Group Infected/Controls, Fig. 5 ABC	<i>H. pylori</i> infected Leb mice	38	16
Group Infected/Controls, Fig.5 D	<i>H. pylori</i> infected Leb mice	20	66
Group 12m, Fig. 3C	<i>H. pylori</i> infected Leb mice	40	40
	Median	38	16
	Mean	35	28

11
12

1 Table S4

2

3 Table S4A. related to Fig. 4B.

4 Group II, Infected and Vaccinated LOW and HIGH IT50 Mice (30 mice).

Mouse	CFU/g	Leb binding, %	Chronic Gastritis	Dysplasia	Cancer	Inflammation Score	Final Diagnosis	IT50
Low IT50 (23 mice)								
#4-1	0	-	+	+	-	0	DYSP	1
#4-2	1571	0	+	+	+	3	Cancer	1
#4-3	0	-	+	+	+	3	Cancer	1
#4-6	0	-	+	+	-	1	DYSP	1
#4-7	0	-	+	+	-	1	DYSP	1
#4-10	0	-	+	-	-	0	GA	1
#4-20	441	5	+	+	-	2	DYSP	1
#4-23	0	-	+	-	-	1	GA	1
#4-28	200	0	+	+	-	0	DYSP	1
#4-11	0	-	+	-	-	1	GA	9
#4-29	800	0	+	+	+	2	Cancer	16
#4-21	1312	0	+	+	+	3	Cancer	27
#4-26	0	-	+	+	+	2	Cancer	27
#4-15	100	10	+	+	+	2	Cancer	29
#4-17	0	-	+	+	+	2	Cancer	59
#4-8	0	-	+	+	+	1	Cancer	62
#4-30	400	9	+	+	+	1	Cancer	74
#4-19	3583	0	+	+	+	2	Cancer	81
#4-13	0	-	+	+	+	3	Cancer	96
#4-14	0	-	+	+	+	3	Cancer	181
#4-24	0	-	+	+	+	1	Cancer	281
#4-5	4317	0	+	+	+	3	Cancer	216
#4-4	0	-	+	+	+	2	Cancer	542
						39/23 animals=1.7		Median, 27
High IT50 (7 mice)								
#4-22	0	-	+	-	-	0	GA	912
#4-27	1500	0	+	+	+	2	Cancer	1347

#4-16	0	-	+	-	-	1	GA	1427
#4-25	0	-	+	-	-	0	GA	1813
#4-12	1466	0	+	+	-	2	DYSP	4287
#4-9	0	-	+	+	+	2	Cancer	6569
#4-18	0	-	+	-	-	0	GA	24028
						46/30 animals=1.5		Median, 1813

1
2
3
4
5
6
7
8
9
10
11
12

Table S4B related to Fig. 4C.
The 1st Vaccine Experiment. Summary of incidence of disease

Titer	GAstritis	DYSPlasia	Cancer	<u>Cancer</u> GAstritis
Low IT50 (L)	3	5	15	3
High IT50 (H)	4	1	2	0.5
Ratio Low IT50 / High IT50				6
Fisher's Test				0.038

The panel shows the number of animals in the group of “Infected and vaccinated” with a 3.0 vs. 0.5 ratio of Cancer/Gastritis for the Low (23 animals) vs. High (7 animals) IT50 groups, respectively. The difference in ratios demonstrated a 6-fold reduced risk for gastric cancer in the High IT50 group ($p < 0.038$).

Table S4C related to Fig. 3 and Fig. 4.
The Cancer model Summary of disease and inflammation scores.

Group	Disease/ Score	0	1	2	3	Total Score	No. Mice	Mean Infl. Scores/ Disease	Mean Infl. Scores/ Group	No. mice/ Group	Disease
Exp. 1, Grp. 1, Infected	Gastritis		5	4		13	9	1.4			9/34=26%
	Dysplasia		2	4		10	6	1.7			6/34=18%
	Cancer			3	16	54	19	2.8	2.25 (Fig. 3B)	34	19/34=56%
Exp. 2, Grp. 2, Infected	Gastritis	4	6			6	10	0.6			10/30=33%
	Dysplasia	2	4	2		8	8	1.0			8/30=27%
	Cancer		2	4	6	28	12	2.3	1.4 (Fig. 3B, 4A)	30	12/30=40%
Exp. 2, Grp. 2,	Gastritis	1	2			2	3	0.7			3/23=13%

Infected, Vaccine, Low IT50	Dysplasia	2	2	1		4	5	0.8			5/23=22%
	Cancer		3	6	6	33	15	2.2	1.7	23	15/23=65%
Exp. 2, Grp. 2, Infected, Vaccine, High IT50	Gastritis	3	1			1	4	0.25			4/7=57%
	Dysplasia			1		2	1	2.0			1/7=14%
	Cancer			2		4	2	2.0	1.0 (Fig. 4C)	7	2/7=28%
							94			94	In Figure 4C

1

Summary Inflammation score / disease	Disease	Total Scores	No. Mice	Scores/cases	Mean Scores
	Gastritis	13+6+2+1	9+10+4+4	22 / 27	0.8
	Dysplasia	10+8+4+2	6+8+5+1	24 / 20	1.2
	Cancer	54+28+33+4	19+12+15+2	119 / 48	2.5

2

3

1 Table S4D related to Fig. 5.

2

3 The 2nd Vaccine Experiment related to Fig. 5ABC.

Mouse	CFU/g	Leb binding, %	Gastric pH	Chronic Gastritis	Dysplasia	Cancer	Inflammation Score	Final Diagnosis	IT50, 17875/Leb
Infected mice (16 mice)									
B891			1.5	+	-	-	1	GA	20
B892			2.37	+	+	-	2	DYSP	16
B893	7700	3	1.77	+	+	+	3	Cancer	22
B894	56	X	2.8	+	+	+	2	Cancer	10
B895			1.54	+	+	-	2	DYSP	6
B896			4.44	+	-	-	1	GA	24
B897	750	0	3.19	+	+	-	1	DYSP	24
B898			1.92	-	-	-	0	Healthy	20
B912			3.04	+	+	+	1	Cancer	17
B922			1.82	+	+	+	2	Cancer	10
B935	813	X	1.27	+	-	-	2	GA	22
B936	929	0	3.26	+	+	+	3	Cancer	24
B937			1.52	+	-	-	1	GA	13
B938			1.35	+	-	-	1	GA	30
B940			1.33	+	-	-	1	GA	8
B942	167	X	1.34	+	-	-	1	GA	21
							Mean, 1.5		Median, 20
Infected and Vaccinated mice (21 mice)									
B899			2.4	+	-	-	1	GA	7667
B900			4.96	+	-	-	1	GA	8539
B901			3.53	-	-	-	0	Healthy	8698
B903			1.82	+	-	-	1	DYSP	33622
B904	150	16	3.83	+	-	-	1	GA	3959
B905			1.77	+	-	-	1	GA	4146
B906	2107	3	1.66	+	-	-	1	GA	3969
B907	143	0	1.47	+	-	-	2	GA	6163
B908	458	7	1.79	+	-	-	1	GA	2270
B909			1.7	+	-	-	1	GA	7318
B910			2.18	+	-	-	1	GA	764

B917	286	1	1.86	+	-	-	0	Healthy	12035
B918			1.64	+	-	-	1	GA	11284
B924			2.48	+	+	-	1	DYSP	5798
B927			1.33	+	-	-	1	GA	5663
B929			1.49	+	+	-	2	DYSP	10934
B930			1.68	+	-	-	2	GA	13338
B931	333	X	1.87	+	-	-	1	GA	33113
B932			1.43	+	+	-	1	DYSP	40035
B933	50	X	1.45	+	-	-	1	GA	12999
B934	83	X	1.34	+	-	-	1	GA	5893
							Mean, 1.05		Median, 7667

1 X – slow growing outputs

2

1 **Table S4E**

2

3 **The 3rd Vaccine Experiment related to Fig. 5D.**

4 **Infected (30 mice) and Vaccinated w High antigen dose (27 mice) and,**

5 **Infected and Vaccinated with Low antigen dose (28 mice)**

6

Mouse	CFU/g	Leb binding, %	Gastric pH	Chronic Gastritis	Dysplasia	Cancer	Inflammation Score	Final Diagnosis
Infected control mice (30 mice)								
B988			2.19	+	+	+	2	Cancer
B989	267	15	2.17	+	+	-	2	DYSP
B990			2.54	+	+	+	3	Cancer
B991			3.87	+	-	-	1	GA
B992	154	0	1.62	+	+	-	1	DYSP
B993			1.62	+	-	-	1	GA
B994			1.82	+	+	-	1	DYSP
B995			1.72	+	-	-	1	GA
B1013			2.17	+	-	-	1	GA
B1014			1.95	+	-	-	1	GA
B1015			1.49	+	+	-	2	DYSP
B1016			2.29	+	-	-	1	GA
B1017			2.05	+	+	+	1	Cancer
B1018			2.66	+	-	-	2	GA
B1019			2.48	+	+	+	1	Cancer
B1036	71	0	1.58	+	+	-	1	DYSP
B1037	286	34	2.48	+	+	-	1	DYSP
B1039			1.52	+	+	-	1	DYSP
B1040			1.66	+	+	-	1	DYSP
B1041			2.66	+	+	-	1	DYSP
B1042			2.15	+	+	-	1	DYSP
B1043			3.81	+	+	-	1	DYSP
B1060			1.85	+	+	-	1	DYSP
B1061			2.43	+	+	-	1	DYSP
B1062	295	22	2.1	+	+	+	2	Cancer

B1063			2.74	+	-	-	1	GA
B1064			1.83	+	+	-	1	DYSP
B1065			2.29	+	+	-	2	DYSP
B1066			1.72	+	-	-	1	GA
B1067	62	20	2.0	+	+	-	1	DYSP
Vaccine-1 (27 mice)								
B996	28	X	1.6	+	-	-	1	GA
B997			1.5	+	-	-	1	GA
B999			1.68	+	-	-	1	GA
B1000	159	21	1.78	+	+	-	1	DYSP
B1001	100	20	1.4	+	-	-	2	DYSP
B1002			1.76	+	-	-	1	GA
B1003	83	X	1.71	+	-	-	1	GA
B1020	188	23	2.14	+	-	-	1	GA
B1021	125	X	1.86	+	-	-	1	GA
B1022			1.66	+	-	-	1	GA
B1023			1.57	+	-	-	1	GA
B1024			1.64	+	-	-	1	GA
B1025	42	X	2.03	+	-	-	2	GA
B1026	3050	31	2.58	+	-	-	1	GA
B1044			1.73	+	-	-	1	GA
B1045			1.28	+	-	-	1	GA
B1048	105	21	2.39	+	+	-	1	DYSP
B1049			1.91	+	-	-	1	GA
B1050	175	X	3.55	+	-	-	1	GA
B1051			2.07	+	-	-	1	GA
B1068	2156	0	2.02	+	-	-	1	GA
B1070			1.86	+	+	-	1	DYSP
B1071	167	0	3.95	+	-	-	1	GA
B1072			1.71	+	-	-	1	GA
B1073	31	X	2.15	+	+	-	2	DYSP
B1074			1.79	+	+	-	1	DYSP
B1075	333	34	1.83	+	-	-	1	GA
Vaccine-2 (28 mice)								
B1004			1.13	+	-	-	1	GA

B1005			2.39	+	-	-	1	GA
B1006			1.56	+	-	-	1	GA
B1009			3.66	+	-	-	2	GA
B1010			1.43	+	+	-	2	DYSP
B1011			1.57	+	+	-	1	DYSP
B1028			1.39	+	-	-	1	GA
B1029			1.61	+	-	-	1	GA
B1030			1.76	+	-	-	1	GA
B1031			1.76	+	-	-	1	GA
B1032			2.0	+	-	-	1	GA
B1033			1.38	+	-	-	1	GA
B1034			1.42	+	-	-	1	GA
B1052			1.47	+	-	-	1	GA
B1054			1.38	+	-	-	1	GA
B1055	1000	5	1.99	+	-	-	2	GA
B1056			1.83	+	-	-	1	GA
B1057			2.01	+	-	-	1	GA
B1058	375	19	1.63	+	-	-	1	GA
B1059			1.71	+	-	-	1	GA
B1076	94	X	1.66	+	-	-	1	GA
B1077	188	1	1.56	+	-	-	1	GA
B1078			2.06	+	-	-	1	GA
B1079	125	21	1.74	+	-	-	1	GA
B1080			1.88	+	-	-	1	GA
B1081			1.58	+	-	-	1	GA
B1082	917	0	1.83	+	-	-	1	GA
B1083	1096	0	2.0	+	-	-	1	GA
Non-infected Control (4 mice)								
B1084			1.38	+	-	-		GA
B1115			1.49	-	-	-		Healthy
B1116			4.82	-	-	-		Healthy
B1117			1.46	-	-	-		Healthy

1 X – slow growing outputs

1 **Table S5.**

2

3 **Table S5A related to Fig. S5B.**

4 **IT50 of pooled sera of infected (control) and infected and vaccinated mice,**
5 **from 3rd Vaccine Experiment. .**

6

Group	Pool	Mouse Serum ID	IT50 of the Pool, tested with 17875/Leb
Infected/Control	Ctrl	B991	12
		B992	
		B993	
		B1016	
Infected and Vaccinated-1	V1-1	B996	11709
		B997	
		B999	
		B1044	
		B1045	
	V1-2	B1022	5823
		B1068	
		B1070	
		B1073	
		B1074	
	V1-3	B1023	7788
		B1024	
		B1025	
		B1026	
	V1-4	B1048	3882
		B1071	
		B1072	
		B1075	
	V1-5	B1000	6263
		B1020	

		B1021	
		B1049	
	V1-6	B1001	9941
		B1002	
		B1003	
		B1050	
		B1051	
			Median, 7026
Infected and Vaccinated-2	V2-1	B1004	2517
		B1005	
		B1009	
		B1010	
		B1055	
	V2--2	B1030	2828
		B1054	
		B1059	
		B1076	
		B1079	
	V2-3	B1028	7679
		B1029	
		B1031	
		B1032	
	V2-4	B1006	3645
		B1011	
		B1033	
		B1034	
	V2-5	B1052	3468
		B1056	
		B1057	
		B1058	
	V2-6	B1077	5207
B1078			

		B1080	
		B1081	
		B1082	
		B1083	
			Median, 3557
Non-Infected Control	NIC	B1084	15
		B1117	
		B1115	
		B1116	

1

2

Table S5B, related to Figure 6B.

3

The IT50 of pooled sera from the 2nd Vaccine Experiment of Infected Control mice and Vaccinated mice.

Origin	Strain	IT50 of Sera pool	
		Control mice	Vaccinated mice
Europe	17875/Leb	1	11732
	J166	1	353
	Sw44	22	165
	Sw103	1	117
	S864	56	160
	USU101	43	271
Asia	I9	81	235
	Ch1	1	345
	J533	26	602
North America/Alaska	A714	1	90
	A723	13	120
South America	P330	34	145
	P436	19	113

4

5