Figure Legends

Fig. S1: Biological reproducibility and linearity between lysate arrays and western blotting, related to Figures 1 and 2. Data obtained from 2 biological replicates of infected and uninfected cells on protein lysate microarrays. Signal from antibodies that recognize total p53 (A) or Bad pS112 (B) are shown. (C) Graph representing the ratio of infected (GFP-positive) cells to uninfected (GFP-negative) cells for signals obtained, plotted against the log of the p-value obtained. Each point represents a single antibody of the Akt/mTor pathway. Only antibodies which demonstrate significant differences in signal between infected and uninfected cells are represented. (D) Graph representing the ratio of infected (GFP-positive) cells to uninfected (GFP-negative) cells for signals obtained, plotted against the log of the p-value obtained. Each point represents a single antibody against total or phosphorylated p53. Only antibodies which demonstrate significant differences in signal between infected and uninfected cells are represented. (E) Graph representing the ratio of infected (GFP-positive) cells to uninfected (GFP-negative) cells for signals obtained, plotted against the log of the p-value obtained. Each point represents a single antibody of the Bcl-2 family. Only antibodies which demonstrate significant differences in signal between infected and uninfected cells are represented.

Fig. S2: Nutlin-3 targets host hepatocyte to remove liver stage parasites, related to Figure 3. (A) Asexual *P. falciparum* parasites diluted to 0.5% parasitemia, synchronized to >95% ring stages then cultured with DMSO or 20μM Nutlin-3 for 48

hours. Parasitemia of treated and untreated cultures is similar. (B) HepG2-CD81 cells were treated with Nutlin-3 or DMSO alone for 24h then infected with *P. yoelii* sporozoites. Liver stages were visualized by immunofluorescence and counted manually. (C) HepG2-CD81 cells were treated with either DMSO or Nutlin-3 for 24 then incubated with *P. yoelii* sporozoites. Sporozoite traversal rates were monitored by addition of 1mM FITC dextran during incubation. Cells, which suffer wounded membranes take-up the FITC dextran and can be quantitated by FACS. Sporozoite traversal rates were similar between Nutlin-3 and DMSO-treated preparations, suggesting that Nutlin-3 does not inhibit cell traversal activity of sporozoites. (D) Cells were treated and infected with sporozoites as described in 4B. Rather than total number of liver stages, the percentage of hepatocytes infected with sporozoites was monitored after 48 hours of Nutlin-3 treatment. A significantly lower percentage of hepatocytes are infected with sporozoites at this time point, suggesting Nutlin-3 treatment does not remove parasites by non-specific killing of hepatocytes. (E) A lower percentage of sporozoites are found inside hepatocytes after Nutlin-3 treatment than in control-treated cells 90 minutes after invasion. (F) Nutlin-3 treatment increases p21 in vivo. Mice were treated with Nutlin-3 daily for two days. 24 or 44 h after the final treatment, RNA was monitored for p21 and GAPDH transcript levels by qPCR. Error bars represent standard deviation of analytical replicates. (G) Nutlin-3 decreases size of Plasmodium liver stages that remain after treatment. Representative image of liver stage size in untreated (left) or Nutlin-3 treatment (middle) HepG2-CD81 cells after 24h of Nutlin-3 treatment. PVM marker UIS4 is shown in red. Liver stages in Nutlin-3 treated cultures are approximately half the size of untreated liver stages (right).

Table S1: Signaling changes observed in *P. yoelii*-infected HepG2-CD81 cells, related to Table 1 and Figure 1.

Protein of Interest	product #	Species	Company	I/U	Std	T-Test	H-B value	H-B pass?
Significantly Upregulated, passes Holm Benferroni								
p-Rb (S807/S811)	9308	R	CST	1.39	0.15	0.0022	0.0024	Yes
p-BCL-2 (S70)	2827	R	CST	1.26	0.05	0.0010	0.0018	Yes
p-Akt1/2/3 (S473)	9271	R	CST	1.24	0.06	0.0009	0.0017	Yes
p-mTOR (S2448)	2971	R	CST	1.21	0.03	0.0000	0.0011	Yes
p-Akt1/2/3 (S473)	4058P	R	CST	1.20	0.03	0.0000	0.0011	Yes
p-p53 (pS37)	9289	R	CST	1.19	0.04	0.0001	0.0013	Yes
p-BCL-2 (T56)	2875	R	CST	1.19	0.03	0.0004	0.0015	Yes
Atg7	2631	R	CST	1.17	0.02	0.0000	0.0012	Yes
p-p53 (pS9)	9288	R	CST	1.16	0.05	0.0013	0.0020	Yes
p-mTOR (S2481)	2974	R	CST	1.16	0.06	0.0017	0.0023	Yes
p-cdc2 (Y15)	9111	R	CST	1.16	0.03	0.0000	0.0012	Yes
p-p53 (S392)	9281	R	CST	1.15	0.03	0.0006	0.0017	Yes
Atg3	3415	R	CST	1.15	0.03	0.0003	0.0014	Yes
p-c-Cbl (Y774)	3555	R	CST	1.15	0.03	0.0004	0.0015	Yes
Cl- Caspase 3 (D175)	9661	R	CST	1.14	0.03	0.0000	0.0012	Yes
p-BAD (S112)	5284	R	CST	1.14	0.03	0.0001	0.0013	Yes
p-cdc2 (Y15)	4539	R	CST	1.13	0.04	0.0005	0.0016	Yes
p53	2527	R	CST	1.11	0.01	0.0000	0.0011	Yes
FADD	2782	R	CST	1.09	0.02	0.0015	0.0022	Yes
p-NF-κB p65 (S536)	3033P	R	CST	1.08	0.02	0.0005	0.0016	Yes
GYS2 pS641	3891	R	CST	1.07	0.02	0.0015	0.0021	Yes
Significantly Downregulated, passes Holm Benferroni								
p-BAD (S112)	06-853	R	Upstate	0.91	0.03	0.0012	0.0019	Yes
p53	9282	R	CST	0.82	0.04	0.0010	0.0019	Yes
p53	2524	M	CST	0.74	0.05	0.0003	0.0014	Yes
p-p53 (S15)	9286	M	CST	0.73	0.09	0.0004	0.0014	Yes
p-BAD (S136)	06-846	R	Upstate	0.73	0.04	0.0002	0.0013	Yes
Non-statistically significant data and/or data does not pass Holm Benferroni								
p-p53 (S15)	9284	R	CST	1.33	0.40	0.1061	0.0050	No
Atg12	4180	R	CST	1.15	0.04	0.0043	0.0029	No
p-p90RSK (S380)	9341S	R	CST	1.14	0.07	0.0145	0.0038	No
Fas	4233	R	CST	1.12	0.06	0.0063	0.0033	No
Cl- Caspase 3 (D175)	9664	R	CST	1.11	0.03	0.0042	0.0028	No
p-p53 (T81)	2676	R	CST	1.10	0.03	0.0029	0.0025	No
p-p53 (S46)	2521	R	CST	1.08	0.03	0.0040	0.0026	No
Beclin-1	3495	R	CST	1.08	0.05	0.0328	0.0042	No

p-c-Cbl (Y731)	3554	R	CST	1.07	0.05	0.0566	0.0045	No
Atg5	8540	R	CST	1.07	0.08	0.1697	0.0071	No
p-NF-κB p105 (S933)	4806P	R	CST	1.06	0.06	0.1504	0.0063	No
Cl- PARP (Asp214)	9541	R	CST	1.06	0.02	0.0094	0.0036	No
p-MDM2 (S166)	3521	R	CST	1.05	0.02	0.0050	0.0031	No
p-p53 (pS6)	9285	R	CST	1.05	0.05	0.1222	0.0056	No
FLIP	3210	R	CST	1.05	0.06	0.2028	0.0083	No
p-Rb (S795)	9301	R	CST	1.04	0.17	0.6595	0.0167	No
p-p53 (pS20)	9287	R	CST	1.03	0.23	0.7903	0.0250	No
LC3B	3868	R	CST	1.02	0.08	0.6591	0.0125	No
LC3A	4599	R	CST	1.01	0.03	0.2894	0.0100	No
p-BAD (S136)	5286	R	CST	1.00	0.03	0.8868	0.0500	No

Table S1: Protein microarrays were printed using whole cell extracts from FACS sorted infected and uninfected hepatoma cells, and probed using a number of antibodies targeted to signaling molecules in the host cell. Quantified fluorescent signal was calculated as a ratio of the signals from infected cells to uninfected cells, normalized for actin for spotting variation. Significance was calculated by t-test and then the Holm-Benferroni method for multiple hypothesis testing using the Infected/Uninfected (I/U) ratio and propagated error. Reasons for which an antibody may give a non-significant signal include no change in signaling, off-target antibody interaction with other proteins, and weak total signal resulting in proportionally larger error. Data shown is representative of three independent experiments.

Supplemental Methods

Cell Lines and Culture: HepG2 CD81 hepatoma cells were a kind gift from INSERM-TRANSFERT. Cells were maintained in DMEM complete media (Dulbecco's Modified Eagle Medium (Cellgro, Manassas, VA), supplemented with 10% FBS (Sigma-Aldrich, St. Louis, MO), 100 IU/mL penicillin (Cellgro), 100µg/mL streptomycin (Cellgro), 2.5 µg/mL fungizone (HyClone/ Thermo Fisher, Waltham, MA) and 5 µg/mL gentamicin (BioWhittaker/ Lonza, Basel, Switzerland)), and split 1-2 times weekly. Where indicated, cells were treated with 20 µM Nutlin-3 in DMSO.

Mosquito Rearing and Sporozoite Production: For *P. berghei* and *P. yoelii* sporozoite production, female 6–8-week-old Swiss Webster (SW) mice (Harlan, Indianapolis, IN) were injected with blood stage *P. yoelii* (17XNL) or *P. berghei* (ANKA) parasites to begin the growth cycle. Animal handling was conducted according to Institutional Animal Care and Use Committee-approved protocols. We used infected mice to feed female *A. stephensi* mosquitoes after gametocyte exflagellation was observed. For all *P. yoelii* experiments we isolated salivary gland sporozoites according to standard procedures at days 14 or 15 post blood meal. We isolated *P. berghei* sporozoites 20 days post-infection. For each experiment, salivary glands were isolated in parallel in order to ensure sporozoites were extracted from salivary glands in the under the same conditions.

Analysis of Microarray Data: Signal intensities from target proteins were normalized using the β-actin signal intensities from the same spot to normalize for spotting variation. Data from quadruplicate spots were averaged, and the ratio of signal from

GFP-positive samples to GFP-negative samples computed for each target protein. For each antibody specific to a hepatocyte protein measured, we calculated a value according to equation (1).

$$x = \frac{\mathrm{signal(i)}/\beta\mathrm{actin(i)}}{\mathrm{signal(u)}/\beta\mathrm{actin(u)}} \qquad (1)$$

where signal refers to the signal derived from a particular antibody, *i* refers to signals derived from infected cells and *u* refers to signals for uninfected lysates. By this calculation, antibodies with the largest *x* value represent proteins highly expressed or modified in infected cells, whereas the antibody with the lowest value of x represent proteins or post-translational states more commonly found in uninfected cells. Statistical significance was calculated using a 2-tailed t-test with the standard error against a ratio of 1 (unchanged from GFP-positive to GFP-negative).

Differences observed between antibodies against the same target protein can occur due to a number of factors. Most frequently, this occurs because of differences in background signal between antibodies. For antibodies where background signal is greater, the magnitude of changes appears smaller. When background signal is smaller, the magnitude of changes appears larger. However, by directly comparing signal obtained via western blot to signal obtained by lysate array (Figure 2B) or comparing trends that exist within pathways (Figure S1C-E) we can determine which antibodies produce reliable data and which pathways are robustly perturbed.

Quantification of liver burden by real-time qRT-PCR: Total RNA was extracted using TRIzol reagent (Invitrogen). cDNA synthesis and qRT-PCR was was performed

using the Super Script III Platinum two-step qRT-PCR kit according to the manufacturer's instructions (Invitrogen). All PCR amplification cycles were performed at 95°C for 30 s for DNA denaturation, and 60°C for 4 min for primer annealing and DNA strands extension. Parasite ribosomal 18S RNA was amplified using primers with sequences: 5'GGGGATTGGTTTTGACGTTTTTGCG3' and 5'AAGCATTAAATAAAGCGAATACATCCTTAT3'. Mouse GAPDH was amplified using sequences 5'CCTCAACTACATGGTTTACAT3' and 5'GCTCCTGGAAGATGGTGATG3'.