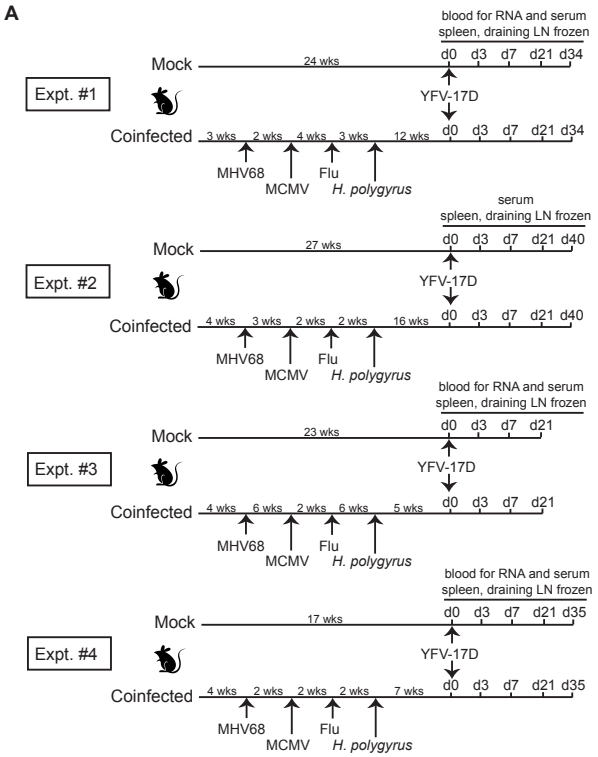
Figure S1

A

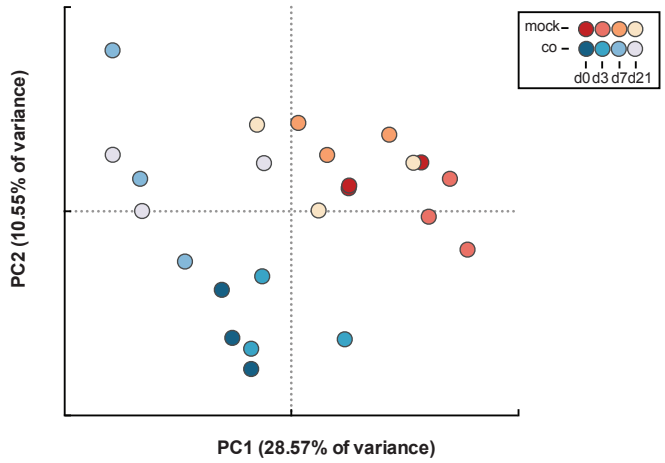


B

		Number of mice per timepoint					Total mice	
		Day 0	Day 3	Day 7	Day 21	Day 34-40	per group	per expt.
Expt. #1	Mock	5	5	5	5	5	25	50
	Coinfected	5	5	5	5	5	25	
Expt. #2	Mock	4	4	4	4	3	19	39
	Coinfected	4	4	4	4	4	20	
Expt. #3	Mock	3	4	4	4	0	15	30
	Coinfected	3	4	4	4	0	15	
Expt. #4	Mock	4	4	4	4	4	20	40
	Coinfected	4	4	4	4	4	20	

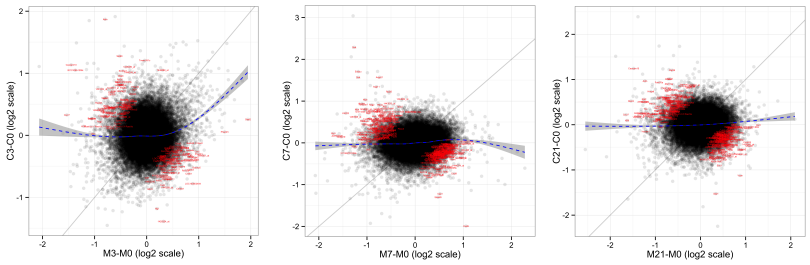
Supplemental Figure 1, related to Figure 1. Experimental design of co-infection experiments.
(A) Diagrams of experiment design including timing of infections.
(B) Table with the numbers of mice in each experimental group.

Figure S2



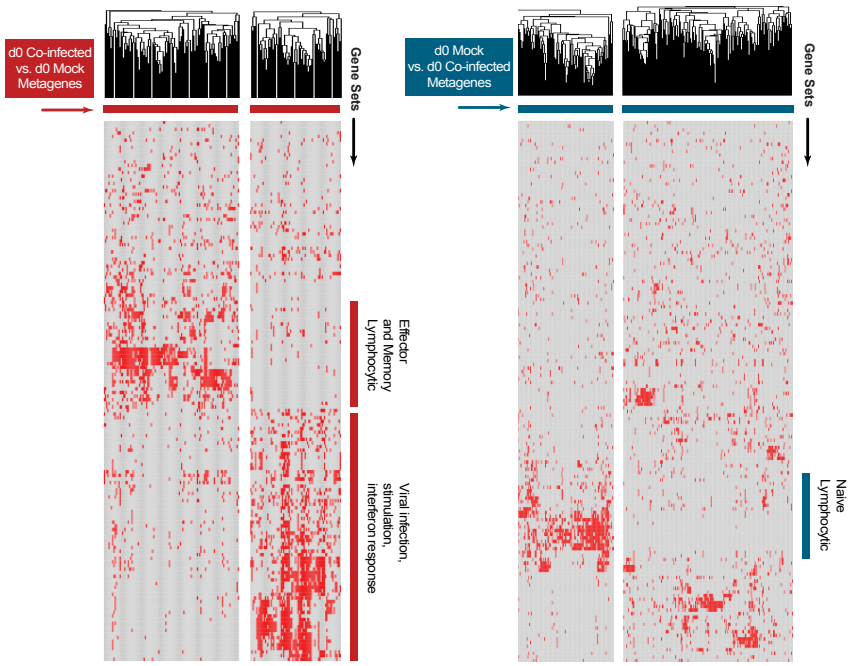
Supplemental Figure 2, related to Figure 2. Principle coordinate analysis (PCA) of overall gene expression in each experimental group. PCA plot with the samples using only the top 10% varying genes. Co indicates co-infection. Each dot represents one time point from one experiment.

Figure S3



Supplemental Figure 3, related to Figure 2. Scatter plots of fold change comparing mock and co-infected samples after vaccination. A loess curve (dashed blue line) was used to infer the local trend between the two systems. The genes falling on the identity line (dark gray line) exhibited similar levels of expression in both systems. Genes differentially expressed (|FC|>1.3) in at least one comparison are highlighted in red. Solid lines indicate absolute 2-fold changes. The x and y axes in the scatter plots show the log2(FC) in the level of gene expression 3 , 7 and 21 days post vaccination in mock mice and co-infected respectively.

Figures S4



Supplemental Figure 4, related to Figures 3 and 4, Table S2. Leading Edge Metagene (LEM) analysis reveals groups of gene sets that are enriched in co-infected and mock mice at day 0 prior to vaccination. Heatmaps depicting membership of individual genes within a LEM (columns) to top 150 significantly enriched ImmSigDB gene-sets (rows) for a given pairwise comparison. For each comparison, standard GSEA was performed using the ImmSigDB database of gene-sets (Godec et al., 2016). Genes in the top 150 enriched sets (ranked by Normalized Enrichment Score) were filtered to only leading edge genes and subsequently clustered into groups (metagenes) using an NMF algorithm. Hierarchical clustering of genes within individual metagenes was performed to obtain the final heatmap. Metagenes with qualitatively discernible 'blocks' of gene-set membership were annotated according to the identity of corresponding enriched gene-sets.

Supplemental Table 1. Identity of genes found in each of five clusters identified in Figure 2.

Supplemental Table 2. Identity of genes identified by LEM in Figure S4, and used for comparison in Figures 3 and 4.

Supplemental Experimental Procedures:

Animals, infections, and sample collection

C57BL6/J animals were ordered from Jackson or were bred in our specific pathogen-free animal facility at Washington University according to state and federal guidelines. All animal protocols were approved by IACUC. Numbers and sex of animals were as follows: expt. #1 50 females, expt. #2 39 males, expt. #3 30 females, expt. #4 40 females. The individual experiments are outlined in Figure S1. In summary, at weaning mice were divided into two groups. One was a mock group that was housed in the biohazard facility and the second group was designated the co-infected group. The co-infected mice were infected at 4 weeks of age with 10^5 plaque forming units (PFU) intranasally of murine γ-herpesvirus-68 (MHV68) under light anesthesia. At approximately 6 weeks of age co-infected mice were infected with 10^5 PFU (in phosphate buffered saline (PBS)) intraperitoneally with tissue culture passage murine cytomegalovirus (MCMV). At approx. 8 weeks of age they were infected with 10^3 PFU influenza strain WSN (in PBS) intranasally. At approx. 10 weeks of age they were infected with 50 L3 larvae of *Heligmosomoides polygyrus* by oral gavage. After the series of infections were complete, mice were housed in the biohazard facility for an additional 5-12 weeks. Both mock and co-infected groups were challenged with 10^6 PFU of yellow fever virus strain 17D (YFV-17D) subcutaneously in the footpad. Prior to challenge stool samples were collected from co-infected animals to confirm infection with *H. polygyrus* by egg counts. Mice were sacrificed on days 0, 3, 7, 21, and for experiments 1, 2, and 4 on days 34-40. Mice were selected from different cages for each time point. Blood, spleen, draining lymph node were collected. Spleen and draining lymph node were snap frozen at -80^{0}C. Blood was collected into tubes containing EDTA and spun down. Serum was frozen at -20^{0}C. Blood cell pellets were processed using RiboPure RNA purification kit from whole blood (Thermo Fisher Scientific). RNA from individual mice from each time point was pooled within an experiment for microarrays. RNA quality was confirmed by nanodrop and bioanalyzer. RNA from expt. #2 and d34-40 samples from the other three experiments was discarded due to low quality. Microarray from three individual experiments was performed to ensure reproducibility of the data based on previous experiments (Canny et al., 2014). All other RNA samples were used for microarray analysis. Samples were amplified using NuGEN Ovation RNA Amplification and hybridized to Affymetrix Mouse 430 2.0 arrays.

Pathogens and infections

MHV68 WUSM strain (ATCC VR1465) was grown in 3T12 cells (ATCC CRL-164), concentrated and titer determined as previously described (Pavlova et al., 2003). MCMV Smith strain was grown in 3T12 cells, concentrated, and titered as previously described (Heise and Virgin, 1995). Influenza WSN strain was grown and titered as previously described. *H. polygyrus* L3 larvae were harvested from fecal egg cultures as previously described and resuspended in ddH_2O (Camberis et al., 2003). YFV-17D was a kind gift from Michael Diamond and James Brien at Washington University. It was grown in Vero cells, purified and titered as described (Erickson and Pfeiffer, 2013). MCMV, influenza virus, and YFV-17D were resuspended in PBS with no serum.

ELISA for anti-YFV IgG

YFV-17D was UV-inactivated, and used to coat Immulon II flat-bottomed ELISA plates. Plates were blocked with 3% bovine serum albumin (BSA). Serum samples were heat inactivated for 30 minutes at 56^{0}C. Half-$\log_{10}$ serial dilutions were plated on plates overnight at 4^{0}C. Secondary antibody was goat-anti-mouse-HRP from Jackson Immunoresearch (#115-035-100) used at a dilution of 1:10,000. For standard curve, Abcam #3576 anti-YFV antibody was used.

Focus forming assay for antibody neutralization of YFV

Serum samples were heat inactivated for 30 minutes at 56^{0}C. Sera was serially diluted in media and mixed with 240 PFU of YFV-17D. After incubating for 1 hour at 37^{0}C, virus/serum mixture was added to plated Vero cells in 96-well plates (WHO strain, obtained from Michael Diamond and James Brien). Cells were overlayed with 1% carboxymethylcellulose (Sigma M0512-250G) in Minimum Essential Medium (MEM), and incubated for 48 hours at 37^{0}C with 5% CO_2. Cells were fixed, washed, and stained using the Trublue Peroxidase substrate (KPL, #50-78-02). Spots were counted using ImmunoSpot software.

Analysis of Cytokines

Cytokines were quantitated in serum using the ProcartaPlex Mouse 17-plex cytokine panel (Catalog # EPX170-26087-901). Values that were below the manufacturer's stated limit of detection were set to zero. Two values were excluded from the IFNγ day 7 data (one from mock and one from co-infected) for being extremely high and far outside the range of other values.

Measurement of YFV genome copies

Protocol for detection of YFV genomes was adapted from (Mantel et al., 2008). Draining lymph node was disrupted using 0.5 ml Tri reagent (Sigma), and RNA was purified according to manufacturer protocol. cDNA was synthesized using ImProm-II reverse transcriptase (Promega). Forward primer was 5'-GCACGGATGTAACAGACTGAAGA-3', reverse primer was 5'-CCAGGCCGAACCTGTCAT -3', and probe was 5'-Fam- CGACTGTGTGGTCCGGCCCATC-Tamra-3'. Actin Taqman primers and probes were: 5'- GCTCCTTCGTTGCCGGTCCA-3', 5'- TTGCACATGCCGGAGCCGTT-3', probe 5'-JOEN-CACCAGTTC/ZEN/GCCATGGATGACGA-IABkFQ-3'.

Analysis of microarray data

Prior to analysis, mouse microarray data were processed and normalized using the Affymetrix MAS5 algorithm and batch correction was performed using the ComBat algorithm. Principal components analysis was performed in R. Genes exhibiting differential kinetics between co-infected and mock samples over the d0 to d21 time course were identified using maSigPro (Conesa et al., 2006) (default FDR <0.05). Briefly, a cubic regression model was defined and then adjusted by the least-squares technique to yield significantly differentially expressed genes. A backward stepwise regression was then used to identify 1024 genes moving differentially between co-infected and mock. These genes were clustered in the space of all samples using the tightClust algorithm into five clusters (Tseng and Wong, 2005). 323 genes failed to cluster and were omitted at this stage. Overlap significance between genes of each cluster and Gene Ontology terms was determined by hypergeometric test using the Gorilla enrichment analysis tool(Eden et al., 2009). Gene set enrichment analysis (GSEA) was performed as described previously (Subramanian et al., 2005). LEM (Leading Edge Metagene) analysis was performed downstream of GSEA to yield groups of genes, termed metagenes, which are coordinately upregulated in a given phenotypic comparison and common to multiple enriched gene sets. Metagenes were identified for d0 co-infected relative to d0 mock and vice versa. Briefly, for a given phenotypic comparison, GSEA was performed using ImmuneSigDB, a curated compendium of 4872 gene sets describing a wide range of cell states and experimental perturbations from immunology literature (Godec et al., 2016). The top 150 significantly enriched gene sets, as restricted by an $FDR < 0.25$ and ranked by $P < 0.05$, were subsetted for their leading edge genes. These genes were then clustered into metagenes using non-negative matrix factorization. Enrichments of co-infected and mock metagenes in petstore vs. laboratory mouse (GSE78979), cohoused vs. laboratory mouse (GSE78979), and adult PBMC vs. cord PBMC array data (GSE27272) were determined using standard GSEA. Raw human adult and neonatal cord PBMC microarray data were obtained from a previous unaffiliated study profiling the peripheral blood of 72 smoking or non-smoking women and the cord blood of their neonates (Gene Expression Omnibus, accession code GSE27272) (Votavova et al., 2011). Human microarray data were quantile normalized using preprocessCore (Bioconductor).

Statistical methods

One-way ANOVA analysis was performed using Prism, and Brown-Forsythe test was used to compare standard deviations. When Brown-Forsythe test indicated unequal standard deviations Kruskal-Wallis test was performed.

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