Synthetic immunomodulation with a CRISPR super-repressor in vivo

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Transient modulation of the genes involved in immunity, without exerting a permanent change in the DNA code, can be an effective strategy to modulate the course of many inflammatory conditions. CRISPR-Cas9 technology represents a promising platform for achieving this goal. Truncation of guide RNA (gRNA) from the 5’ end enables the application of a nuclease competent Cas9 protein for transcriptional modulation of genes, allowing multifunctionality of CRISPR. Here, we introduce an enhanced CRISPR-based transcriptional repressor to reprogram immune homeostasis in vivo. In this repressor system, two transcriptional repressors—heterochromatin protein 1 (HP1a) and Krüppel-associated box (KRAB)—are fused to the MS2 coat protein and subsequently recruited by gRNA aptamer binding to a nuclease competent CRISPR complex containing truncated gRNAs. With the enhanced repressor, we demonstrate transcriptional repression of the Myeloid differentiation primary response 88 (Myd88) gene in vitro and in vivo. We demonstrate that this strategy can efficiently downregulate Myd88 expression in lung, blood and bone marrow of Cas9 transgenic mice that receive systemic injection of adeno-associated virus (AAV)2/1-carrying truncated gRNAs targeting Myd88 and the MS2-HP1a-KRAB cassette. This downregulation is accompanied by changes in downstream signalling elements such as TNF-α and ICAM-1. Myd88 repression leads to a decrease in immunoglobulin G (IgG) production against AAV2/1 and AAV2/9 and this strategy modulates the IgG response against AAV cargos. It improves the efficiency of a subsequent AAV9/CRISPR treatment for repression of proprotein convertase subtilisin/kexin type 9 (PCSK9), a gene that, when repressed, can lower blood cholesterol levels. We also demonstrate that CRISPR-mediated Myd88 repression can act as a prophylactic measure against septicaemia in both Cas9 transgenic and C57BL/6J mice. When delivered by nanoparticles, this repressor can serve as a therapeutic modality to influence the course of septicaemia. Collectively, we report that CRISPR-mediated repression of endogenous Myd88 can effectively modulate the host immune response against AAV-mediated gene therapy and influence the course of septicaemia. The ability to control Myd88 transcript levels using a CRISPR-based synthetic repressor can be an effective strategy for AAV-based CRISPR therapies, as this pathway serves as a key node in the induction of humoral immunity against AAV serotypes.

A useful genetic engineering platform should employ both transcriptional control and gene editing on demand to allow a high level of control at both the DNA and RNA levels (for example, to simultaneously modulate immune responses), a goal achievable by changing the length of guide RNAs (gRNAs) from the 5’ end when using Cas9 nuclease10. However, it is not known if truncated gRNAs can provide an effective means for synthetic repression of transcription in vivo, giving rise to physiologically relevant phenotypes.

Here, we set out to determine whether we can achieve synthetic immunomodulation in vivo using a CRISPR-based enhanced transcriptional repressor. Myeloid differentiation primary response 88 (Myd88) is a key node in innate and adaptive immune responses that acts as an essential adaptor molecule for a number of signalling pathways including the Toll-like receptor (TLR), the response to septicaemia and the formation of adaptive immunity against viruses such as the adeno-associated virus (AAV)20-23. MYD88 activating mutations are implicated in a number of lymphoid malignancies, in particular Waldenström macroglobulinemia and activated B-cell diffuse large B-cell lymphomas24. However, it is not clear whether we can achieve

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control over its transcription in vivo. Given the central role of MyD88 signalling in innate and adaptive immunity we sought to examine synthetic transcriptional modulation over this locus in vivo.

Results
CRISPR-mediated repression with MS2-HP1a-KRAB is superior to MS2-KRAB in vitro. We previously reported ‘enhanced’ CRISPR-based transcriptional repressors in vitro, developed by direct fusion of a set of modulators to catalytically dead Cas9 protein (MeCP2, MBD2 or heterochromatin protein 1 [HP1a])13. We first devised an experiment to determine which transcriptional repression domain from our previously published candidates can lead to efficient transcriptional repression when fused to the MS2 coat protein (referred to here as MS2) and recruited to the CRISPR complex by gRNA aptamer binding (Fig. 1a)13. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of a set of target genes in human embryonic kidney 293 (HEK293FT) cells established that MS2-HP1a-KRAB enabled efficient repression across the genes we tested (Fig. 1b).

To translate these findings in vivo, we set out to utilize nuclease competent Streptococcus pyogenes (Sp)-Cas9 transgenic mice, as they enable us to eliminate potential confounding effects associated with delivery of Cas9. As such, we devised a pair of truncated gRNAs that target Cas9 nuclease and MS2-HP1a-KRAB to the Myd88 promoter (Fig. 1c). This strategy allows Cas9 nuclease to be repurposed to a nucleuse null protein for transcriptional repression. We first compared the functionality of the truncated gRNA compared to the full-length gRNA in mouse neuroblastoma (N2A) cells (Fig. 1c). RNA sequencing showed that transcriptional repression using truncated gRNA is as efficient and specific as traditional 20-nt gRNA-based repression in vitro (Fig. 1d,e). Moreover, this strategy yielded a similar efficacy in vitro in repressing the Myd88 locus as when a dCas9-HP1a-KRAB fusion protein is used with comparable levels of dCas9 and HP1a-KRAB (Extended Data Fig. 1a,b). Next, we set out to examine MS2-HP1a-KRAB-mediated repression of endogenous mouse Myd88 levels in vitro and compared the efficiency with commonly used KRAB-based transcriptional repression. We used a previously reported non-targeting Mock gRNA as a control23, qRT-PCR for Myd88 demonstrated the in vitro functionality of the gRNAs and superiority of MS2-HP1a-KRAB in the repression of endogenous Myd88 (Fig. 1f).

CRISPR-mediated repression of the Myd88 locus can be achieved efficiently in vivo by recruitment of MS2-HP1a-KRAB to gRNA. To test this repressor in vivo, we pursued delivery through packaging gRNAs and MS2-repression cassettes within AAVs. Different AAV serotypes have been used to deliver CRISPR in vivo. The most common serotype has been AAV9, which has high affinity to parenchymal cell populations24,25. Here, we employed a hybrid AAV2/1 serotype, which is a recombinant AAV consisting of AAV2 inverted terminal repeats, and AAV1 Rep and Capi genes (referred to, here on, as AAV1 for simplicity). AAV1 has been shown to be effective in the transduction of components of the immune system and non-parenchymal cells such as dendritic and endothelial cells26–30. Moreover, AAV1 capsid can induce MyD88 signalling as part of the pathways of immunity against AAVs in the host31,32. Our assessment of AAV1 tissue affinity revealed the highest expression in blood, lung and bone marrow (Extended Data Fig. 2). Subsequently, we performed systemic delivery of AAV1/Myd88 gRNA or control AAV1/Mock gRNA with MS2-HP1a-KRAB or MS2-KRAB cassettes to Cas9 nuclease transgenic mice (Fig. 2a). Three weeks after injections, blood, lung and bone marrow were collected and Myd88 expression was assessed by qRT-PCR (Fig. 2b). Compared to un.injected controls, AAV delivery led to an increase in Myd88 across the different tissues we tested. Treatment with CRISPR to repress endogenous Myd88 with HP1a-KRAB led to a significant reduction in the level of Myd88 in blood (~84%), lung (~75%) and bone marrow (~63%) as compared to the Mock gRNA-treated group, in agreement with the high affinity of AAV1 for these tissues. Administration of the KRAB domain alone led to a less pronounced repression of Myd88 in lung (~52%), blood (~59%) and bone marrow (~34%), with slightly higher variation among the animals tested (Fig. 2b).

To assess the potency of repression in rewiring the downstream gene regulatory network, we evaluated the levels of tumour necrosis factor-α (TNF-α) and intercellular adhesion molecule-1 (ICAM-1), two signalling elements directly modulated by the Myd88 signalling pathway33–35. Myd88 targeting with MS2-HP1a-KRAB led to a significant reduction in icam-1 and Tnfa expression across multiple tissues, whereas targeting with MS2-KRAB did not lead to a similar consistent effect (Fig. 2c,d and Supplementary Table 1).

To perform a systematic assessment of the repression efficiency of the MS2-HP1a-KRAB system as compared to MS2-KRAB, we performed next-generation RNA sequencing on the bone marrow of mice treated with these constructs. MS2-HP1a-KRAB-treated mice expressed lower Myd88 levels compared to MS2-KRAB-treated ones (Extended Data Fig. 3a), which was accompanied with changes in downstream signalling pathways such as Il1β. Of note, gene ontology (GO) enrichment analysis revealed that Myd88–MS2-HP1a-KRAB-treated mice had significant downregulation of signalling pathways implicated in the immune and defence response against foreign organisms and bacteria, which are pathways associated with MyD88 function (Extended Data Fig. 3b). Similarly, the Reactome database revealed the TLR pathway as one of the substantial downregulated pathways in the presence of MS2-HP1a-KRAB (Extended Data Fig. 3c). This evidence suggests that modulation of Myd88 and its downstream immune pathways is most effective with the MS2-HP1a-KRAB repressor in vivo.

Interestingly, volcano plotting of differentially expressed genes revealed the constant region of the heavy chain of immunoglobulin G1 and G2 (Ighg1 and Ighg2b) and other immunoglobulin-related heavy and light chain genes as most downregulated with

Fig. 1 | Aptamer-mediated CRISPR repression in vitro. a, Schematic of aptamer-mediated recruitment of repressor domains to the CRISPR complex. b, mRNA expression of targeted genes following aptamer-mediated recruitment of repressor domains to the CRISPR complex in HEK293FT cells. Fold changes were quantified relative to the dCas9-only control group (N = 3 biologically independent samples). c, Top: schematic representation of the gRNA binding sites targeted to the promoter of Myd88. Bottom: schematic of the experiment design. Mouse neuroblastoma (N2A) cells were transfected with either 14-nucleotide or 20-nucleotide Myd88 gRNA pairs together with dCas9 plasmid and the MS2-HP1a-KRAB cassette. Expression levels of Myd88 mRNA were analysed using qRT-PCR three days post transfection. d, Fold changes of mRNA of Myd88 were quantified relative to the ‘No guide’ group (N = 3 biologically independent samples). Data are presented as mean ± s.e.m. e, Mean expression levels of 24,476 protein-coding and 16,648 non-coding RNA genes following the targeting Myd88 gene. For visualization purposes, the values were transformed to a log (TPM + 1) scale (TPM, transcripts per million). R denotes the Pearson correlation coefficient between two groups (N = 3 biologically independent samples). Bars represent mean ± s.e.m. f, qRT-PCR analysis of Myd88 mRNA expression levels post lipopolysaccharide (LPS) treatment in N2A cells. Fold changes were quantified relative to the expression level of cells receiving non-targeting Mock gRNA (N = 4 biologically independent samples). Data are presented as mean ± s.e.m. Statistical analysis was performed using the non-parametric one-tailed Mann-Whitney U test. *P < 0.05 was considered significant. Statistical source data are provided in Source Data Fig. 1.
HP1a-KRAB relative to KRAB (Fig. 2c). This is an interesting finding in light of the mouse genetic background (C57BL/6), which has been shown to produce high level of IgGs. CRISPR-mediated repression of Myd88 leads to modulation of humoral response against AAV-mediated gene therapy and the efficacy of its function. Previous studies have demonstrated that viral
DNA stimulates TLR (that is, TLR9), which in turn activates Myd88 and initiates downstream signalling events leading to adaptive immunity and antibody production against AAVs\(^8,16\). In light of earlier evidence and the observed repression of the immunoglobulin pathway, we asked whether there was a decrease in the AAV-specific humoral response following treatment with AAV1 carrying Myd88-targeting gRNAs and MS2-HP1a-KRAB cassettes (here on referred to as AAV1/Myd88, for simplicity) as compared to control viruses carrying Mock gRNAs (AAV1/Mock). Three weeks after injection, we measured the IgG response against the AAV1 capsid. We detected a 50% decrease in IgG response following treatment with AAV1 carrying Myd88 repressors to Cas9 nuclease transgenic mice.

Antibody formation against the AAV capsid is an important barrier to re-administration of AAV-based gene therapies, often leading to rapid clearance of the virus and other deleterious effects related to destruction of the virus or transduced cells by the immune system. To further probe the prophylactic effect of Myd88 repression on modulating humoral immunity upon AAV1 re-administration, we asked whether pre-treatment with AAV1/Myd88 can influence IgG levels against AAV1 upon re-administration of AAV1/Mock. Analysis of IgG1 and IgG2A in the plasma demonstrated lower levels after initial Myd88 repression, hinting to the potential of this strategy in modulating the humoral response to AAV1 re-administration (Fig. 3b and Extended Data Fig. 4a,b).
To examine the extensibility of this strategy to modulate the response against other AAV serotypes, we pre-treated mice with AAV1/Myd88 or AAV1/Mock and, seven days later, injected them systemically with AAV9 that carried a LacZ or *Staphylococcus aureus* Cas9 (SaCas9) cassette. In both instances, analysis of total IgG levels against AAV9 demonstrated that Myd88 repression led to a lower antibody response against AAV9. Moreover, this was accompanied by significantly lower antibodies against SaCas9 and higher transcript levels of LacZ in the blood (Fig. 3c,d). These data demonstrate that modulation of immunoglobulin production through Myd88 repression can influence the humoral response against more than one AAV serotype and its cargo. Higher LacZ expression in blood in this context hints to potentially higher efficiency of the gene therapy using this approach.

To further explore this notion in the context of CRISPR therapies, we pre-treated the mice with AAV1/Myd88 or AAV1/Mock and then subjected them to two rounds of AAV9-based gene therapies 7 and 14 days apart (Fig. 3e). In this case, AAV9 carries a cassette for CRISPR-mediated repression of proprotein convertase subtilisin/kexin type 9 (PCSK9), similar to the strategy we employed for Myd88 repression. PCSK9 is an enzyme encoded by the PCSK9 gene. This enzyme binds to the low-density lipoprotein (LDL) receptor at the surface of hepatocytes and initiates ingestion of the LDL receptor. Accordingly, when PCSK9 is blocked or repressed, more LDL receptors are present to remove LDL from the blood, which lowers blood LDL-cholesterol levels. This enzyme has been the target of previous in vivo CRISPR applications.

Our data show that the AAV1/Myd88 pre-treated group has decreased Myd88 expression (Extended Data Fig. 4c,d) as well as lower IgG1 and total IgG levels against AAV9 compared to the control (Fig. 3f). This observation was accompanied by better PCSK9 repression and lower plasma cholesterol levels, suggesting increased efficiency of the gene therapies (Fig. 3g,h). Altogether, these data present an exciting opportunity to modulate humoral immunity against AAV, possibly through prophylactic repression of Myd88 with a tool inherently suited to perform both gene editing and epigenetic modulation (nuclease competent CRISPR).

CRISPR-mediated Myd88 repression does not create visible adverse effects in the long term. Next, we probed the long-term efficacy of AAV1/Myd88 repression in vivo to further assess its durability and possible negative consequences. Analysis of Myd88 transcripts in lung, blood, and bone marrow 23 weeks after injection showed Myd88 repression in the AAV1/Myd88 group (Fig. 4a). To assess possible negative consequences of the long-term reduction of Myd88 levels, we analysed some key indicators of major internal organ function, including blood urea nitrogen (BUN) for kidney, alanine transaminase (ALT) and albumin for liver, lipase for pancreas, and lactic acid dehydrogenase (LDH) as a marker of tissue damage. None of these markers were significantly different from the Mock-treated groups (Fig. 4b). Moreover, tracking the weight of the mice suggested that there were no detectable deleterious effects on the general health and well-being as all animals demonstrated comparable weights (Fig. 4c).

**CRISPR-mediated Myd88 repression in vivo can act as a prophylactic measure against septicemia in Cas9 transgenic and C57BL/6 mice.** We next asked whether this strategy could act as a prophylactic modality during septicemia, when there is an augmented systemic immune response. Septicaemia is a pressing medical issue due to the emergence of antibiotic resistance and the rising longevity of patients suffering from chronic diseases. Moreover, high mortality rates due to septicemia remain a medical challenge following trauma in the battlefield, highlighting the need for novel prevention strategies.

We pre-treated Cas9 mice with AAV1/Myd88 or AAV1/Mock and after three weeks subjected them to systemic LPS (from *Escherichia coli* O127:B8) treatment. Six hours following LPS, we collected lung, blood and bone marrow and assessed the transcript levels of Myd88 and major inflammatory cytokines (Fig. 5a). We observed significant repression of Myd88 in lung (61%), blood (80%) and bone marrow (76%) compared to AAV1/Mock-treated mice (Fig. 5b). In response to LPS, plasma lactate level, a systemic marker associated with septicemia and tissue damage, was significantly lower when mice were pre-treated with the AAV1/Myd88 repression cassette before LPS exposure, indicating reduced systemic injury (Fig. 5c). Additionally, Myd88 repression prevented upregulation of a wide range of inflammatory and immune-related cytokines that are directly or indirectly downstream of Myd88 signalling, such as *Icam-1*, *Ifta*, *Ncf*, *Ifn-a*, *Ifn-b*, *Ifn-g* and *Stat4* (Fig. 5d and Extended Data Figs. 5 and 6). Analysis of plasma and lung cytokine levels using a quantitative ELISA-based chemiluminescent assay revealed lower level of cytokines in *Myd88*-repressed mice (Fig. 5e).

To explore whether we can achieve similar outcomes by simultaneous delivery of Cas9 and gRNA-MS2-HP1a-KRAB cassettes to wild-type animals, we examined a dual AAV1 system in which a second virus carries the Sp-Cas9 nuclease cassette (Fig. 6a). This strategy was capable of decreasing *Myd88* transcripts in C57BL/6 mice, both in the presence and absence of septicemia, leading to a phenotypically relevant response similar to what we observed in Cas9 transgenic animals (Fig. 6b–d and Extended Data Fig. 7).

**Nanoparticle-mediated delivery of Myd88 targeting CRISPR super-repressors after exposure to LPS can serve as a therapeutic modality against septicemia.** To examine the therapeutic potential of this approach after exposure to LPS, we sought to deliver CRISPR plasmids to C57BL/6 through a nanoparticle-based approach, as
they enable faster and more feasible delivery for CRISPR-based gene modulation as compared to the AAV-based system. Given the significance of liver damage after i.p. LPS exposure and the notion that the majority of the nanoparticles delivered systematically accumulate in the liver and lungs, we focused on studying the liver. We first examined whether AAV1/Myd88 can repress \textit{Myd88} expression in liver. Having difficulty repressing \textit{Myd88} in the liver with our current pair of gRNAs, we designed another pair targeting a different region of the \textit{Myd88} promoter. The new gRNAs led to \textit{Myd88} repression in the liver upon AAV-mediated delivery to
Cas9 transgenic mice. This was also observed following LPS injury (Extended Data Fig. 8a,b). Next, we set out to examine the therapeutic effect of this system in C57BL/6 mice 2 h post exposure to LPS. We injected the mice with nanoparticles carrying Cas9, gRNAs and MS2-HP1a-KRAB cassettes and examined the systemic inflammatory response against LPS (Fig. 7a). Seventy-two hours post CRISPR delivery, blood, lung, liver and bone marrow were collected and Myd88 expression was assessed by qRT-PCR. Treatment led to a reduction in the levels of Myd88 in the blood, lung, bone marrow and liver as compared to the Mock-treated group (Fig. 7b). This Myd88 repression prevented upregulation of a wide range of inflammatory markers followed by LPS exposure (Fig. 7c). Analysis of plasma markers of tissue damage of the liver showed that we could modulate the detrimental effects of LPS injection (Fig. 7d). In particular, high-density lipoprotein (HDL) has been shown to increase following LPS treatment to eliminate systemic LPS so as to protect tissues from damage and has been associated with MyD88 signalling. In accordance with this, we found Myd88 repression decreased HDL, LDL and cholesterol when compared to Mock-treated groups. In addition, Myd88 repression decreased ALT and aspartate aminotransferase (AST), two markers of hepatocyte damage, which further suggests that this approach can be effective therapeutically (Fig. 7d).

Discussion

In summary, we provide a potent transcriptional therapeutic modality for synthetic control of the immune response in vivo using a newly developed CRISPR-based transcriptional super-repressor against endogenous Myd88. We show that this system is effective in modulating downstream immune signalling and can create a visible protective phenotype in vivo. This notion is especially attractive in the case of delivery using a less common AAV serotype (AAV2/1) known to target smaller cellular populations in vivo (for example, non-parenchymal cells).

We demonstrate that targeting the Myd88 locus with AAV1/CRISPR generates less immunoglobulin against AAV1 and AAV9
and modulates general immunoglobulin expression patterns, consistent with previous reports on the failure of generation of an antigen-specific IgG2a response in MyD88−/− animals.45 The ability to control Myd88 transcript levels using a CRISPR-based synthetic repressor is of significance in light of the common challenges involved with AAV-based clinical gene therapies, as this pathway has

Fig. 5 | CRISPR-based modulation of host inflammatory response can be a prophylactic measure against LPS-mediated sepsis in Cas9 transgenic and WT mice. a, Schematic of the experimental design to assess the protective effect of CRISPR-mediated Myd88 repression in sepsis. A total of 1012 GCs of AAV vectors were injected into Cas9-expressing mice via retro-orbital injection. Approximately three weeks later they were treated intraperitoneally (i.p.) with LPS (5 mg kg−1). The mice were euthanized 6 h post LPS injection. b, qRT-PCR analysis of in vivo Myd88 expression relative to the universal control following LPS injection (N = 6 mice for injected groups, except the bone marrow of Myd88 group (N = 5 mice) and N = 2 mice for the not injected group). Data are presented as mean ± s.e.m. c, Circulating l-lactate in plasma samples collected from mice 6 h post LPS injection (N = 3 mice). Data are presented as mean ± s.e.m. d, qRT-PCR analysis of Icam-1 and Tnfα mRNA expression in lung, blood and bone marrow quantified relative to the universal control following LPS injection (N = 6 mice for injected groups, except the bone marrow of Myd88 group (N = 5 mice) and N = 2 mice for the not injected group). Data are presented as mean ± s.e.m. e, Measurement of a panel of inflammatory cytokines in lung and plasma using a multiplex-ELISA assay; values are displayed in the heatmaps as log10 of the measured concentration (N = 3 mice). IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; MIP, macrophage inflammatory protein; MCP, monocyte chemoattractant protein; RANTES, regulated on activation, normal T cell expressed and secreted; IFNγ, interferon gamma. Statistical analysis was performed using the non-parametric one-tailed Mann–Whitney U test. P ≤ 0.05 was considered significant (* P ≤ 0.05, ** P ≤ 0.01). Statistical source data are provided in Source Data Fig. 5.
Fig. 6 | Developing protection following LPS-mediated septicaemia using a dual AAV CRISPR/Cas9 strategy with AAV1/Cas9 and AAV1 carrying gRNA-MS2-HP1a-KRAB. a, Schematic of the experiments. C57BL/6 mice received a total of 2x10^{12} GCs of AAV1/Cas9 and AAV1/Myd88 or AAV1/Mock vectors via retro-orbital injection and approximately three weeks later they were treated i.p. with LPS (5 mg kg\(^{-1}\)). Mice were euthanized 6 h post LPS injection. b, qRT-PCR for Myd88 expression was performed on blood, bone marrow and lung samples collected from mice (N=4 mice for all groups except for blood of the Mock treated group (N=3 mice)). Data are presented as mean ± s.e.m. c, Six hours post LPS injection, plasma samples were collected from mice and the concentration of L-lactate was assessed in different groups (N=3). Data are presented as mean ± s.e.m. d, qRT-PCR analysis of Icam-1, Tnfα, Ncf, Il6 and Il1β mRNA expression in blood, bone marrow and lung. Fold change expression levels were quantified relative to the universal control (N=4 mice). Data are presented as mean ± s.e.m. (Mock, Mock-HP1a-KRAB; Myd88, Myd88-HP1a-KRAB). Universal control is a blood sample collected from an uninjected Cas9 transgenic mouse. Statistical analysis was performed using the non-parametric one-tailed Mann-Whitney U test. *P ≤ 0.05 was considered significant. Statistical source data are provided in Source Data Fig. 6.
**Fig. 7 | Therapeutic delivery of nanoparticles carrying DNA encoding Myd88-targeting CRISPR confers protection against LPS-mediated septicemia.**

a. Schematic of the experiment. C57BL/6 mice were treated i.p. with LPS (2.5 mg kg\(^{-1}\)). After 2 h, mice received Cas9 and Myd88 or Mock vectors via retro-orbital injection using nanoparticles. Seventy-two hours post retro-orbital injection, mice were euthanized.

b. Lung, blood, and bone marrow samples were collected from mice. The expression levels of Myd88 and a panel of immune-related genes were assessed by qRT-PCR. qRT-PCR analysis of Myd88 repression following LPS injection and CRISPR-mediated therapy (N = 10 mice). Data are presented as mean ± s.e.m.

c. qRT-PCR analysis of Icam-1, Tnfα, Ncf, Il6 and Il1β mRNA expression in different tissues. Fold changes were quantified relative to the universal control (N = 10 mice). Data are presented as mean ± s.e.m.

d. Plasma concentration of cholesterol (N = 11 mice for Cas9+Mock+LPS, N = 9 mice for Cas9+Myd88+LPS), plasma concentration of HDL (N = 11 mice for Cas9+Mock+LPS, N = 8 mice for Cas9+Myd88+LPS), plasma concentration of LDL (N = 10 mice for Cas9+Mock+LPS, N = 8 mice for Cas9+Myd88+LPS), plasma concentration of ALT (N = 7 mice for Cas9+Mock+LPS, N = 4 mice for Cas9+Myd88+LPS) and plasma concentration of AST (N = 8 mice for Cas9+Mock+LPS, N = 5 mice for Cas9+Myd88+LPS). Data are presented as mean ± s.e.m. (Mock, Mock-HP1α-KRAB; Myd88, Myd88-HP1α-KRAB). Universal control is a blood sample collected from an uninjected Cas9 transgenic mouse. Statistical analysis was performed using the non-parametric one-tailed Mann–Whitney U test. P ≤ 0.05 was considered significant (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001). Statistical source data are provided in Source Data Fig. 7.
been shown to be a key node in the induction of humoral immunity against many AAV serotypes and not just AAV2/1 in vivo. Moreover, we argue that this method can be a powerful tool to dissect biological questions at the level of Myd88 transcription. Here, we demonstrate that a prophylactic regime that represses Myd88 can be used to increase the efficiency of subsequent viral-based gene delivery by preventing a surge in humoral response.

This strategy was also effective in modulating the systemic inflammatory response against LPS-induced endotoxaemia both prophylactically and therapeutically. CRISPR-mediated endogenous repression of Myd88 prevented upregulation of a wide range of inflammatory markers and conferred a protective phenotype. Further studies are needed to address the extensibility of CRISPR super-repressors to other endogenous genes of the immune system and to define target tissues and cellular players, as well as to characterize applicability to other infectious diseases. However, the ability to modulate the host immune response using this strategy is a promising step towards generating a universal yet targeted tool to prevent an exaggerated inflammatory response and severe tissue damage in the context of emerging infectious diseases.

HP1a protein contains a chromodomain (CD) and a chromoshadow domain (CSD), which interact with methylated H3K9 and H3K9-specific histone methylases, including SetDB1 and Suv39h1/2, respectively\(^{11,12}\). In this study, to minimize the potential non-specific effects of ectopic HP1a expression, we used a truncated form of HP1a containing only the CSD. Several questions remain about how and if this truncated version still leads to the spread of chromatin repression marks beyond the targeted loci. HP1 proteins can undergo CSD-mediated dimerization, but such homodimerization alone cannot explain the ability of HP1a proteins to spread along chromatin\(^{13-15}\). Further analysis is needed to look at the genome-wide effects of using truncated HP1a protein.

Taken together, we demonstrate the promise of CRISPR-based transcriptional regulation as a readily programmable tool for modulating inflammatory conditions and protecting against an infectious condition. Employment of a nuclease competent Cas9 and a truncated gRNA in this study (the step-by-step protocol can be found at the Nature Protocol Exchange\(^{16}\)) opens up an opportunity for simultaneous application of CRISPR for targeted gene editing while modulating the immune response, which makes CRISPR-mediated gene repression superior to other systems such as shRNA-mediated repression.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41556-020-0563-3.

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Methods

MS2 fusion constructs. To construct the MS2-fused transcriptional reporters, the specific domains of interest were amplified from vectors previously published[^4] in our group and subsequently cloned into the pCDNA3-MS2-pVP64 backbone (Addgene plasmid ID: 6142453) at the BbsI site via golden-gate-based reaction. All the gRNA sequences are listed in Supplementary Table 2.

AAV vectors. Following cloning of the gRNAs into a U6-sgRNA-MS2 backbone, the U6-gRNA encoding region was amplified from this vector and inserted within the gateway entry vectors using golden gate reaction. Using the same method, the repressor or activator domain and a truncated human EF1a promoter a gift from N. Davidsohn (Dr. Church lab, Wyss Institute, Harvard Medical School, Boston, MA, USA, currently CSO of Rejuvenate Bio) were cloned into gateway entry vectors. Further subcloning of all these components into AAV backbone via gateway reaction (Invitrogen) generated final AAV vectors. Cas9 plasmids were purchased from Addgene (AAV-CMV-Cas9 #106431 and pAAV-RSV-SpCas9 #85450[^5]).

AAV packaging and purification. AAV vectors were digested by Smal digest to test the presence of the gRNAs before virus production. Verified AAV vectors were used to generate AAV2/1-Mdy88, AAV2/1 MockRNA and AAV2/1-GFP and AAV2/9-PCSK9 by PackGene Biotech, LLC. The virus titers were quantified via Real-time SYBR Green PCR at 1.5 × 10^6 GC/ml against standard curves using linearized parental AAV vectors.

Cell culture. HEK293FT and Neuro-2a cell lines (purchased from ATCC) were maintained in Dulbecco's modified Eagle's medium (DMEM; Life Technologies) with 10% fetal bovine serum (FBS; Life Technologies), 2 mM glutamine, 1.0 mM sodium pyruvate (Life Technologies) and 1% streptomycin–penicillin mix (Gibco) in incubators at 37°C and 5% CO2.

Transfection of in vitro cultured cells. HEK293FT cells were seeded approximately 50,000 cells per well in 24-well plates and transfected the next day. HEK293FT cells were co-transfected with plasmids encoding gRNA (10 ng), dCas9 or dCas9-H1a-KRAB (200 ng), MS2-fused repressor (100 ng), purumycin-resistant gene (50 ng), and Enhanced Blue Fluorescent Protein (EBFP) as a transfection control (25 ng). Polylethyleneimine (PEI; Polysciences) was used to transfect HEK293FT cells. Transfection complexes were prepared according to the manufacturer's instructions. Cells were treated with 0.5 μg/ml puromycin (Gibco-life tech) at 24h post-transfection. Cells were collected 72 h post-transfection, and total RNA was collected from cells using RNAeasy Plus Mini Kit (Qiagen).

Neuro-2a cells were seeded approximately 50,000 cells per well in 24-well plates and transfected the next day. Cells were co-transfected with plasmids encoding gRNA (10–100 ng), Cas9 nuclease (70 ng), dCas9 or dCas9-H1a-KRAB (200 ng), and EBFP as a transfection control (25 ng), and a puromycin-resistance gene (50 ng). Plasmids were delivered to Neuro-2a cells with Lipofectamine LTX. Cells were treated with 0.5 μg/ml puromycin (Gibco-life tech) at 24h post-transfection. For the experiment shown in Figs. 11 and 12 later cells were treated with LPS at the concentration of 10 μg/ml (LPS was added to induce Myd88 expression) and after 5h total RNA was collected from cells using RNAeasy Plus Mini Kit (Qiagen).

Quantitative RT-PCR (qRT-PCR) analysis. Cells or tissues were lysed, and RNA was extracted using RNAeasy Plus Mini Kit (Qiagen) or Trizol (Life Technologies) followed by cDNA synthesis using the High-Capacity RNA-to-cDNA Kit (Thermo Fisher). qRT-PCR was performed using SYBR Green PCR Master Mix (Thermo Fisher). All analyses were normalized to 18S RNA, and fold-changes were calculated against the reference controls for in vitro transfection experiments and a universal control for in vivo experiments (2). Universal controls were a blood sample collected from an uninjected Cas9 transgenic mouse, which did not receive any AAV injection and was kept as the reference throughout all analyses for comparison of values among different organs. Primer sequences for qPCR are listed in Supplementary Table 3.

Phytoimmunology. After harvesting mice, plasma samples were aliquoted and stored at −80°C. Plasma levels of cholesterol were measured via a colorimetric assay performed according to the manufacturer's instructions (Thermo Scientific Total Cholesterol Reagents #TR13421). Plasma PCSK9 protein levels were quantified by ELISA according to the manufacturer's instructions (R&D Systems #MPC900).

ELISA-based chemiluminescent assay. Lung samples were lysed using 1x cell lysis buffer (Cell Signaling) (ratio of 100 mg of tissue to 1 ml of buffer), which was followed by homogenization and sonication of the lysed tissue. The assay was performed using the Q-Plex Mouse Cytokine – Screen (16-Plex) kit (Quansys Biosciences) following the manufacturer's protocol. Briefly, samples or calibrators were added into wells of a 96-well plate arrayed with antibody specific antibodies that capture GMCSF, IL-1α, IL-1β, IL-4, IL-5, IL-6, IL-12/70, IL-17, MCP-1, MIP-1α, RANTES, and TNFα. Plates were then coated and biotinylated antibodies were added. After washing, streptavidin–horseradish peroxidase (SHRP) was added. Following an additional wash, the amount of SHRP remaining on each location of the array was measured with the addition of a chemiluminescent substrate.

Antibody ELISA. Anti-AAV antibody assay. Fifty microliters of AAV particles diluted in 1x coating buffer (13 mM sodium carbonate, 35 mM sodium bicarbonate buffer, pH 9.2) containing 2× 10^6 viral particles were added to each well in a Microlon high protein binding 96-well plate (Greiner) and incubated overnight. Wells were washed three times with 1× Tris Buffered Saline + Tween-20 (TBST, Bethyl) and blocked with 1× Tris Buffered Saline + 1% BSA (Bethyl) for 1h at room temperature (RT). Wells were washed three times with TBST. The standard curve for Fig. 3B was generated using purified mouse antibody (Mouse host IgG2a anti-AAV1 (Fitzgerald-MBS8331111), Mouse IgG1 unlabelled – Southern Biotech clone 15H6,Mouse IgG2a unlabelled) in twofold dilutions in TBST + 1% BSA + 1× 500 negative control mouse plasma, beginning from a concentration of 10,000 ng/ml AAV1 antibody. The standards were added to the plate followed by diluting the plasma samples (samples were diluted 1:500 for Fig. 3B and no dilution for Fig. 3c–f) and incubating for 1 h at RT. Wells were washed four times with TBST and then goat anti-mouse HRP antibody was added at a concentration of 1:500 and incubated for 1 h at RT. Wells were washed four times with wash buffer + 1% BSA + 1× BSA with the well plate covered. Reactions were terminated by adding 0.18 M H2SO4 after the development of the standard curve (15 min). Finally, absorbance was measured at 450 wavelengths using a plate reader (BioTek).

Absorbance results were exported and analysed in Excel.

Anti-SaCas9 antibody assay. Microplates were coated with 50 μl per well at 1 μg/ml Sacs9 protein diluted in 50 mM carbonate buffer at pH 9.0. Plates were incubated overnight at 4°C. Wells were washed three times with 1× TBST (Bethyl). Wells were blocked with 200 μl/well of blocking buffer (PBS containing 1% BSA and 0.02% azide) and incubated overnight at 4°C. Wells were washed three times with 1× PBS + 0.02% azide. Plasma samples and control were added to the wells at 50 μl/well diluted in blocking buffer and incubated 1 h at RT. Antibodies may be serially diluted for determining titer or diluted to previously determined working concentration for screening assays or antigen quantification. Wells were washed three times with PBS containing 0.05% Tween-20. Goat anti-mouse HRP antibody was added at a concentration of 1:500 and incubated for 1 h at RT. Wells were washed. Reactions were terminated by adding 0.18 M H2SO4 after 15 min. Finally, absorbance was measured at 450 wavelengths using a plate reader (BioTek).

Absorbance results were exported and analysed in Excel.

Lactate assay. Blood samples were collected using EDTA coated tubes. Samples were centrifuged at 1,000 × g for 10 min. Plasma was collected and stored at −80°C. Lactate assay was performed following the manufacturer's protocol (1–Lactate Assay Kit, Cayman Chemicals). Briefly, samples were deproteinized by adding 0.5 M MPA. After pelleting the protein, supernatant was added to potassium carbonate and centrifuged at 10,000 × g for 5 min at 4°C. Samples were diluted four-fold and added to the designated wells. Next, assay buffer cofactor mixture, Fluorometric Substrate, and Enzyme Mixture were added to each well. The plate was incubated for 20 min at RT, and the fluorescence was measured using an excitation wavelength of 530–540 nm and an emission wavelength of 585–595 nm. Absorbance results were exported and analyzed in Excel according to the manufacturer's protocol.

Examination of liver injury after LPS injection. Plasma samples were sent to IDEXX Laboratories to measure a panel of tissue injury markers including ALT, cholesterol, LDL, HDL, BUN, albumin, and LDH, and lipase.

Animals. Animal studies were conducted with adherence to the guideline for the care and use of laboratory animals of the NIH. All the experiments with animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Arizona State University and have been performed according to institutional guidelines. All the experiments were performed on at least three mice of 6–8 weeks old per group. Both male and female were included in experiments. The sample size in each group is indicated in each figure legend.

Both male and female Rosa26-Cas9 knockin mice (JAX Stock number 026179) and male C278L6/mice (JAX Stock number: 000664) were used for AAV/Crispr reprogramming experiments.

Retro-orbital injections. AAV particles were delivered to mice through retro-orbital injection of the venous sinus. Animals were anesthetized with 3% isoflurane, and virus particles were injected into the left eye with 100 microliters of AAV solution (1 × 10^11 to 1 × 10^12 genome copy per mouse).

Tissue harvest. Mice were euthanized via CO2 inhalation. Tissue samples taken from liver, lung, bone marrow and blood were collected in RLT Plus buffer (Qiagen) and frozen or snap frozen for RNA analysis.
In vivo LPS Administration. Mice were given intraperitoneal (i.p.) injection of lipopolysaccharides (from Escherichia coli 0127:B8 (Sigma-Aldrich, St. Louis, MO, USA) dissolved in phosphate-buffered saline (PBS) at a concentration of 2–5 mg/ml. Mice were euthanized 6–72 h post LPS injection (timeline is included in schematics) via CO2 inhalation.

In vivo PepJet administration. Mice received 60 μg of DNA containing 10 μg Cas9 and 50 μg Myd88-HP1a-KRAB or Mock-HP1a-KRAB via retro-orbital injection. PepJet reagent (SignaGen Laboratories, Catalog #: SL100501) was used for in vivo transfection. DNA was mixed with PepJet at the ratio of PepJet:DNA (μg) 2:1 and prepared according to the manufacturer’s protocol.

RNA sequencing and data analysis. In vitro experiments. N2A cells were co-transfected with 10 ng gRNA targeting N2A cells were RNA sequencing and data analysis. In vitro experiments. 2:1 and prepared according to the manufacturer’s protocol. L): DNA (μg) PepJet reagent (SignaGen Laboratories, Catalog #: SL100501) was used for in vivo and 50 ng MS2-HP1a-KRAB, 50 ng puromycin-resistant gene, and 25 ng transfection control. Cells were treated with 0.5 μg/ml puromycin (Gibco-life tech) at 24 h post-transfection. Total RNA was extracted 72 h post transfection using RNeasy Plus Mini Kit (Qiagen) and sent to UCLA TGGB core on dry ice. Ribosomal RNA depletion and paired-end read library preparation were performed at UCLA core followed by RNA sequencing using NextSeq500. Coverage was 20 million reads per sample. FASTQ files with pair-ended 75 bp reads were then aligned to the mouse GRCm38 reference genome sequence (Ensembl release 90) with STAR, and uniquely mapped read counts (an average of 1.48 million reads per sample) were obtained with Cufflink. The read counts for each sample were then normalized for the library size to CPM (counts per million reads) with edgeR. Custom R scripts were then used to generate plots.

In vivo experiments. RNA was extracted from mice bone marrow samples using RNeasy Plus Mini Kit (Qiagen) followed by globin mRNA depletion using GLOBinClear Kit, mouse/rat kit (Thermo fisher). Non-directional library preparation was performed at Novogene Corporation Inc. followed by RNA sequencing using Illumina Nova Platform with paired-end 150 run (2x 150 bases). Coverage was at minimum 25 million reads per sample. FASTQ files were then aligned to mouse genome sequence using STAR software, and uniquely mapped read counts were visualized with Integrative Genomics Viewer (IGV). Gene expression level was calculated by the number of mapped reads. According to all gene expression level (RPKM or FPKM) of each sample, correlation coefficient of sample between groups was calculated. Read counts obtained from Gene Expression Analysis were used for differential expression analysis and differential expression analysis of different groups was performed using the DESeq2 R package. Hierarchical clustering analysis was carried out of log2 (FPKM +1) of union differential expression genes, within all comparison groups. ClusterProfiler software was used for enrichment analysis, including GO Enrichment, DO Enrichment, KEGG Enrichment and Reactome Enrichment.

Statistical analysis and reproducibility. All in vitro experiments shown were done in triplicates with similar results obtained. All in vivo experiments were repeated in at least three biological replicates with similar results obtained. Mice were randomly allocated to control or experimental conditions. Experimenters were not blinded to conditions during data collection or analyses. Statistical analyses are included in the figure legends. Data are presented as the mean ± s.e.m. **N = number of individual transfections for in vitro experiments and N = number of animals for in vivo experiments. Statistical analyses were performed using Prism 7 Software (GraphPad) using the non-parametric one-tailed Mann-Whitney U test. *P ≤ 0.05 was considered significant (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this Article.

Data availability. RNA-seq data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession code GSE152412. Source data are provided with this paper. All other data supporting the findings are available upon reasonable request. All materials are available upon completion of a material transfer agreement.

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Author contributions
F.M., M.R.E. and S.K. designed the study and the associated experiments. F.M., N.C.Y. and A.C. generated the constructs. F.M. and R.L. performed in vitro experiments. F.M., M.R.E. and S.K. designed the study and the associated experiments. F.M., N.C.Y. and A.C. generated the constructs. F.M. and R.L. performed in vitro experiments. F.M., M.R.E. and S.K. designed the study and the associated experiments. F.M., N.C.Y. and A.C. generated the constructs. F.M. and R.L. performed in vitro experiments. F.M., M.R.E. and S.K. designed the study and the associated experiments. F.M., N.C.Y. and A.C. generated the constructs. F.M. and R.L. performed in vitro experiments. F.M., M.R.E. and S.K. designed the study and the associated experiments. F.M., N.C.Y. and A.C. generated the constructs. F.M. and R.L. performed in vitro experiments. F.M., M.R.E. and S.K. designed the study and the associated experiments. F.M., N.C.Y. and A.C. generated the constructs. F.M. and R.L. performed in vitro experiments. F.M., M.R.E. and S.K. designed the study and the associated experiments. F.M., N.C.Y. and A.C. generated the constructs. F.M. and R.L. performed in vitro experiments. F.M., M.R.E. and S.K. designed the study and the associated experiments. F.M., N.C.Y. and A.C. generated the constructs. F.M. and R.L. performed in vitro experiments. F.M., M.R.E. and S.K. designed the study and the associated experiments. F.M., N.C.Y. and A.C. generated the constructs. F.M. and R.L. performed in vitro experiments. F.M., M.R.E. and S.K. designed the study and the associated experiments. F.M., N.C.Y. and A.C. generated the constructs. F.M. and R.L. performed in vitro experiments.

Competing interests
S.K. is a co-founder of SafeGen Therapeutics. An international patent application has been filed for this work (PCT/US19/60285).

Additional information
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Correspondence and requests for materials should be addressed to M.R.E. or S.K.
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Extended Data Fig. 1 | Evaluation of endogenous Myd88 gene expression using different CRISPR-mediated repressor circuits. (a-b) N2A cells were transfected with Myd88 gRNA pairs along with either dCas9 plasmid fused to HP1α-KRAB or dCas9 and MS2-HP1α-KRAB on two separate cassettes. Expression levels of (a) Myd88, (b) dCas9, and HP1α-KRAB are quantified relative to No-Guide group (N=3 independent samples) The bars represent the mean ± S.E.M. Statistical analysis was performed using the non-parametric one-tailed Mann-Whitney test. A p value ≤ 0.05 was considered significant (*P ≤ 0.05). Statistical source data are provided in Source data extended data fig. 1.
Extended Data Fig. 2 | In vivo analysis of AAV1 tropism towards different tissues. AAV1-GFP was delivered to C57BL/6 mice via retro-orbital injection. GFP expression was assessed in different tissues by qRT-PCR. Average fold change expression levels are indicated above each group and are quantified relative to not injected mice (N=3 for not injected group, N=4 for AAV-GFP group, N=5 for AAV-GFP group in spleen, and N=6 for AAV-GFP group in liver). Statistical source data are provided in Source data extended data fig. 2.
Extended Data Fig. 3 | RNA-seq analyses of bone marrow samples collected from mice treated with AAV1/Myd88-MS2-HP1aKr ab versus AAV1/Myd88-MS2-Krab. (a) Scatter plot comparing expression of genes (Fragments Per Kilobase of transcript per Million mapped reads FPKM) in two replicates of bone marrow from Myd88-MS2-HP1a-KRAB versus Myd88-MS2-KRAB. Myd88, Il1β, Icam-1, Tnfa and Il6 are highlighted in red and the most downregulated genes in Myd88-MS2-HP1a-KRAB groups as compared to Myd88-MS2-KRAB are highlighted in Cyan (N=2 mice). (b) GO enrichment bar graph comparing bone marrow samples collected from mice treated with AAV1/Myd88-MS2-HP1a-KRAB versus AAV1/Myd88-MS2-KRAB. The top 20 significantly enriched terms in the GO enrichment analysis are displayed. Note that pathways such as defense response to bacteria, which are associated with Myd88 signaling are mostly down regulated when HP1a-KRAB was used (N=2 mice). (c) Reactome Enrichment bar graph displaying the top 20 enriched genes in the Reactome database comparing in the BM samples of Myd88-MS2-HP1a-KRAB versus Myd88-MS2-KRAB (N=2). Statistical analysis was performed using the two-tailed t test and the method of multiple comparisons adjustments was Benjamini-Hochberg. A p value ≤ 0.05 was considered significant (*P ≤ 0.05). p values are provided in supplementary information table 4. [AU: please define the “n” in above panels].
Extended Data Fig. 4 | Evaluation of endogenous Myd88 gene expression following multiple AAV administration. (a) Schematic of experiments demonstrating Cas9 transgenic mice treated with AAV1/Myd88 or AAV1/Mock at day 1, followed by a second administration of AAV1/Mock on day 21. (b) qRT-PCR analysis of Myd88 expression level in lung, blood, and bone marrow of Cas9 transgenic mice (N = 4 mice). Fold changes are relative to universal control. The bars represent the mean + S.E.M. (c) Schematic of the experiment. Cas9 nuclease transgenic mice were treated with AAV1-Myd88 or AAV1-Mock vectors via retro-orbital injection followed by a second and third injection of AAV9-PCSK9 vectors on day 7 and 21. (d) qRT-PCR analysis of Myd88 expression level in lung, blood, and bone marrow of Cas9 transgenic mice (N = 4 mice). The bars represent the mean + S.E.M. (Mock, Mock-HP1a-KRAB; Myd88, Myd88-HP1a-KRAB; PCSK9, PCSK9-HP1a-KRAB). Fold changes are relative to universal control. Universal control is a blood sample collected from an uninjected Cas9 transgenic mouse. Statistical analysis was performed using the non-parametric one-tailed Mann-Whitney U test. A p value ≤ 0.05 was considered significant (*P ≤ 0.05). Statistical source data are provided in Source data extended data fig. 4.
Extended Data Fig. 5 | Analysis of a set of immune-related transcripts following LPS injury. qRT-PCR analysis of Ncf, Il6, Ifnγ, and Il1β mRNA expression in lung, blood, and bone marrow quantified relative to the universal control following LPS injection (N = 6 mice for injected groups except for Blood and Bone marrow of Myd88+LPS N=5, and N = 2 mice for Not Injected group). The bars represent the mean + S.E.M. (Mock, Mock-HP1a-KRAB; Myd88, Myd88-HP1a-KRAB). Universal control is a blood sample collected from an uninjected Cas9 transgenic mouse. Statistical analysis was performed using the non-parametric one-tailed Mann-Whitney U test. A p value ≤ 0.05 was considered significant (*P ≤ 0.05 and **P ≤ 0.01). Statistical source data are provided in Source data extended data fig. 5.
Extended Data Fig. 6 | Assessing the level of a panel of immune related genes in lung and bone marrow following LPS injection. qRT-PCR analysis of in vivo CD68, Infα, Infβ, CD4, Cxcl1, and Stat4 relative to the universal control following LPS injection in lung and bone marrow. (N = 6 mice for injected groups except for Bone Marrow of Myd88+LPS group, Lung of Mock+LPS, and Lung of Myd88+LPS for Cxcl1 N=5 mice, and Lung of Mock+LPS group for Cxcl1 N=4 mice, and N = 2 mice for Not Injected group). The bars represent the mean ± S.E.M. (Mock, Mock-HP1a-KRAB; Myd88, Myd88-HP1a-KRAB). Universal control is a blood sample collected from an uninjected Cas9 transgenic mouse. Statistical analysis was performed using the non-parametric one-tailed Mann-Whitney U test A p value ≤ 0.05 was considered significant (*P ≤ 0.05 and **P ≤ 0.01). Statistical source data are provided in Source data extended data fig. 6.
Extended Data Fig. 7 | Targeted gene silencing in wild-type mice using a dual CRISPR/Cas9 system with AAV1/Cas9 and AAV1 carrying gRNA-MS2-HP1a-KRAB. AAV1 viruses were delivered to wild-type mice via retro-orbital injection. qRT-PCR analysis was performed to assess Myd88, Icam-1, Tnfα, Ncf, Il6, and Il1β mRNA expression in blood, bone marrow, and lung. Fold change expression levels were quantified relative to the universal control (N = 4 mice). The bars represent the mean ± S.E.M. (Mock, Mock-HP1a-KRAB; Myd88, Myd88-HP1a-KRAB). Universal control is a blood sample collected from an uninjected Cas9 transgenic mouse. Statistical analysis was performed using the non-parametric one-tailed Mann-Whitney U test. A p value ≤ 0.05 was considered significant (*P ≤ 0.05 and **P ≤ 0.01). Statistical source data are provided in Source data extended data fig. 7.
Extended Data Fig. 8 | Assessing the repression efficiency of AAV1-Myd88 targeting a different region of Myd88 in liver. (a) qRT-PCR analysis of in vivo Myd88 expression in liver samples 3 weeks post retro-orbital injection of AAV1 in Cas9 transgenic animals. Gene expression fold-change was quantified relative to the universal control (N=2 mice for Not Injected group, N=4 mice for Mock, N=7 mice for Myd88Guideset1, N=3 mice for Myd88Guideset2). The bars represent the mean ± S.E.M. (b) qRT-PCR analysis of in vivo Myd88 expression in liver samples 6 hours post LPS injection. Fold change expression levels were quantified relative to the universal control (N=2 mice for Not Injected group, N=6 mice for Mock, N=4 mice for Myd88Guideset2). The bars represent the mean ± S.E.M. Universal control is a blood sample collected from an uninjected Cas9 transgenic mouse. Statistical analysis was performed using the non-parametric one-tailed Mann-Whitney U test. A p value ≤ 0.05 was considered significant (*P ≤ 0.05 and **P ≤ 0.01). Statistical source data are provided in Source data extended data fig. 8.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

☐ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement

☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

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☐ Only common tests should be described solely by name; describe more complex techniques in the Methods section.

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☐ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted

Give P values as exact values whenever suitable.

☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

☐ Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Sequencing data was collected by illumina sequencing.

Data analysis

Statistical analyses were performed using prims version 7 Software (GraphPad). Integrative Genomics Viewer (IGV) version 2.7.2, DESeq2 Rpackage26 V2.1.6.3, and ClusterProfiler27 software v2.4.3 were used for RNA sequencing visualization and analysis. The Partek flow (Partek® Flow® software, version 7.0 Copyright ©; 2019 Partek Inc., St. Louis, MO, USA) and Ingenuity Pathway Analysis (IPA) (IPA: Causal analysis approaches in Ingenuity Pathway Analysis. Bioinformatics. 2014 Feb 15;30(4):523-30) were used for bioinformatics methods and data analysis, respectively. The reads were mapped to the transcript set (Ensembl GRCm38.97 for mouse) using STAR - 2.6.1d (STAR: ultrafast universal RNA-seq aligner, A Dobin, CA Davis, F Schlesinger, J Drenkow, C Zaleski, S Jha, P Batut, Bioinformatics 29 (1), 15-21) and RSEM(RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. Li B et al. BMC Bioinformatics. (2011)). After obtaining gene counts, the counts were normalized by TMM. The Principal Component Analysis (PCA) was applied to the transcript counts. The differential gene expressions were examined by edgeR (edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Robinson MD1, McCarthy DJ, Smyth GK. Bioinformatics. (2010)). For all results of differential gene expression analysis, the p-values, and fold changes (FC) filtered were applied. The filter was p >= 0.05, and |FC| >= 1.5 for all differential gene expression results.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
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RAW sequencing data will be available at NCBI’s Geo database. All other raw and interpreted data is available.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☑ Life sciences   ☐ Behavioural & social sciences   ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
Sample sizes were chosen based on a priori power calculation and validated by comparing with commonly accepted sample size in the relevant literature. All sample sizes are described in the individual figure legends.

Data exclusions
Multiple independent rounds of AAV injections were performed. All the attempts at replication were successful. If the animal did not receive comparable level of AAV virus as control group due to an unexpected technical issue at the time of retro-orbital injection or their age did not match the control group, they were marked and excluded from downstream assays. This criteria was predicted and predefined before the down stream assays and those mice were excluded from the analysis at the time of termination of the experiment.

Replication
All attempts at replication were successful. Multiple mice were examined for each endpoint. All findings can be reproduced with the same methodology in the multiple mice. Methods and materials used in our experiments were described in the manuscripts to allow reliable replication of our studies. We have provided details of mice age, dosage of the virus, sequences of the vectors, injection method and details of data analysis for making sure that the data is reproducible.

Randomization
Randomization was not used in this project. Both Male and Female mice were used in our studies. Experimental and Control mice were age matched. Mice were allocated into groups by cage. To help control for variation, all samples for each individual assay were processed by the same person.

Blinding
Person performing the injections was blinded on the groups and outcomes. Raw data is presented.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☐   | Antibodies            |
| ☑   | Eukaryotic cell lines |
| ☑   | Palaeontology         |
| ☑   | Animals and other organisms |
| ☑   | Human research participants |
| ☑   | Clinical data         |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChIP-seq              |
| ☑   | Flow cytometry        |
| ☑   | MRI-based neuroimaging |

Antibodies

Antibodies used
For AAV ELISA assays we used purified mouse host IgG2a anti-AAV1 (Fitzgerald) antibody in twofold dilutions (MBS30111), Mouse IgG1 unlabelled in twofold dilutions - Southern Biotech, catalog number O102-01, clone number 15H6, lot number K3218-XB19; Fritz JH, Rojas OL, Simard N, McCarthy DD, Hapfelmeier S, Rubino S, et al. Acquisition of a multifunctional IgA+ plasma cell phenotype in the gut. Nature. 2012;481:199-203. (ELISA), Mouse IgG2a unlabelled in twofold dilutions, catalog number 0103-01, clone number HBOC-1, lot number D2619-Z45; (Roopenian DC, Christianoon GJ, Sproule TJ, Brown AC, Akilesh S, Jung N, et al.)
Validation

They have all been used in prior publications and verified by their respective companies and come with specification sheets. Antibodies were originally chosen based on their use in published papers with similar applications to our studies. References: (Roopenian DC, Christianson GJ, Sproule TJ, Brown AC, Akilesh S, Jung N, et al. The MHC class I-like IgG receptor controls perinatal IgG transport, IgG homeostasis, and fate of IgG-Fc-coupled drugs. J Immunol. 2003;170:3528-33. (ELISA)). DiLillo DJ, Tan GS, Palese P, Ravetch JV. Broadly neutralizing hemagglutinin stalk-specific antibodies require FcγR interactions for protection against influenza virus in vivo. Nat Med. 2014;20:143-51. (ELISA).

Eukaryotic cell lines

Policy information about cell lines

| Cell line source(s) | HEK293FT and Neuro-2acell lines used in the in vitro assays were purchased from ATCC. |
| Authentication       | The cell line is used in multiple previous publications and has been validated by the company. ATCC uses methods including an assay to detect species specific variants of the cytochrome C oxidase I gene (COI analysis) to rule out inter-species contamination and short tandem repeat (STR) profiling to distinguish between individual human cell lines and rule out intra-species contamination. Additionally we examine cell morphology and periodically use sequencing to verify the identity of the cells. |
| Mycoplasma contamination | Cells were tested for mycoplasma and results are negative. |
| Commonly misidentified lines (See ICLAC register) | No commonly misidentified cell lines were used. |

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | The study involved laboratory animals. Male C57Bl/6 mice (JAXStock number: 000664) and both male and female Rosa26-Cas9 knockin mice (JAX Stocknumber 026179) were used. All the experiments were performed on at least 3 mice of 6-8 weeks old per group. |
| Wild animals       | No wild animal was used in our studies. |
| Field-collected samples | No field collected sample was used in our studies. |
| Ethics oversight   | All the experiments with animals were approved by the Institutional Animal Care and Use Committee (IACUC) at Arizona State University and have been performed according to institutional guidelines. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.