Spatial control of in vivo CRISPR–Cas9 genome editing via nanomagnets

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The potential of clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR associated protein 9 (Cas9)-based therapeutic genome editing is hampered by difficulties in the control of the in vivo activity of CRISPR–Cas9. To minimize any genotoxicity, precise activation of CRISPR–Cas9 in the target tissue is desirable. Here, we show that, by complexing magnetic nanoparticles with recombinant baculoviral vectors (MNP-BVs), CRISPR–Cas9-mediated genome editing can be activated locally in vivo via a magnetic field. The baculoviral vector was chosen for in vivo gene delivery because of its large loading capacity and ability to locally overcome systemic inactivation by the complement system. We demonstrate that a locally applied magnetic field can enhance the cellular entry of MNP-BVs, thereby avoiding baculoviral vector inactivation and causing a transient transgene expression in the target tissue. Because baculoviral vectors are inactivated elsewhere, gene delivery and in vivo genome editing via MNP-BVs are tissue specific.

The clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR associated protein 9 (Cas9) system is a revolutionary genome editing technology that can efficiently modify target genes in mammalian cells. Preclinical studies have shown that the CRISPR–Cas9 system provides unprecedented opportunities for treating a variety of genetic and infectious diseases. Although in vitro genome editing of cultured cells has many clinical applications, to potentially cure a wide range of diseases, including muscular dystrophy, cystic fibrosis and cancer, it is necessary to perform in vivo genome editing. The CRISPR–Cas9 system targets a short stretch of DNA via the hybridization of a complementary guide RNA and binding of the Cas9 protein, which recognizes a protospacer adjacent motif. The guide RNAs can hybridize to DNA sequences containing base mismatches and DNA and RNA bulges compared with the intended target sequence. Consequently, the CRISPR–Cas9 system can have off-target activities, causing gene mutations, deletions, insertions or translocations, which may lead to tumorigenic or other deleterious events. A major challenge for clinical applications of CRISPR–Cas9-based in vivo genome editing is, therefore, to selectively activate the CRISPR–Cas9 system in the desired tissue or organ to maximize the therapeutic efficacy and minimize genotoxicity.

To improve the specificity of the CRISPR–Cas9 system, many tools have been developed for identifying potential guide RNA off-target sites, and the Cas9 nucleases have been designed with controllable nuclease activities. For example, the Cas9 nucleases have been fragmented into non-functional units, which can dimerize to form active nucleases under blue light radiation. Cas9 can also be delivered as inducible transgenes that can only be transcribed in the presence of a chemical cue; for example, doxycycline. However, in vivo applications, optical signals cannot penetrate deeply into the body owing to the strong absorption and scattering of light by biological tissues. Chemically regulated Cas9 expression relies on the biodistribution of transgenes. Alternatively, in vivo genome editing can be controlled through targeted delivery of the CRISPR–Cas9 system. In particular, the viral vectors with tissue tropism (for example, the adeno-associated viral vectors) are being explored for tissue-specific genome editing in vivo. However, most viral vectors for in vivo gene delivery are derived from the viruses originated from human or other mammals. It is difficult to control the systemic dissemination and replication of these viral vectors, which increases the risk of genotoxicity.

Recent studies have shown that magnetic nanomaterials can be used to alter molecular or cellular processes in vivo mechanically or thermally in response to a magnetic field. Compared with chemical or optical signals, a magnetic field with a strength of 1.5–3.0 Tesla, such as that in a typical magnetic resonance imaging scanner, has no evident adverse effect to the human body and the magnetic field is not attenuated by the tissue. Here, we show the development of a magnetic responsive gene delivery system consisting of a baculoviral vector complexed with magnetic iron oxide nanoparticles (MNP-BV) that enables spatial control of in vivo genome editing.

The baculoviral vector is derived from a cylindrical insect virus (Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV)); 30–60 nm in diameter and 250–300 nm in length). The large size of the baculoviral vector allows an extraordinary DNA packing capacity (>38 kilobases) compared with most other viruses, thus enabling the integration of multiple gene expression cassettes into a single viral vector. Although the baculoviral vector lacks the ability to replicate in mammalian cells, it can transduce many types of mammalian cells with high efficiency and low cytotoxicity, providing robust and transient gene expression. However, in vivo applications of baculoviral vector have been hampered by its inactivation by the complement system. Previous studies have shown that baculoviral vector injected systemically can trigger the classical pathway of the complement system and lead to a significant reduction of viral transduction. Baculoviral vector injected locally into muscle or tumour can induce moderate transduction due to reduced exposure to the complement system. It has been shown that baculoviral vectors can transduce tissues that are naturally immune privileged, such as the brain, eye or testis. The complement-induced baculoviral vector inactivation can also be circumvented to some extent by protecting the baculoviral vector...
with a surface coating or treating the animal with agents that inhibit the complement system \(^\text{30,35,36}\). In this study, we show that serum inactivation of baculoviral vector can be used as an ‘off’ switch to limit systemic activities of the baculoviral vector, and an external magnetic field can serve as an ‘on’ switch for tissue-specific genome editing by promoting margination and cell entry of the MNP-BV complex locally. This hybrid nanoparticle–viral vector system provides a unique delivery vehicle for spatial control of CRISPR–Cas9-mediated in vivo genome editing.

**Results and discussion**

**Construction of the MNP-BV vector.** Recombinant baculoviral vector was produced, purified and concentrated according to published protocols (see Methods). The titre of various baculoviral vectors was determined by a quantitative PCR (qPCR) assay and plaque assay (Supplementary Fig. 1). Magnetic iron oxide nanoparticles (MNPs) that can bind to baculoviral vectors were synthesized in three steps. First, magnetite nanocrystals were synthesized through thermodecomposition of iron acetylacetonate in benzyl ether \(^\text{37}\). As-synthesized nanocrystals were 15.5 ± 1.1 nm in diameter and had a saturation magnetization of 87.2 emu g\(^{-1}\), similar to that of bulk magnetite (Supplementary Fig. 2a,b). Water-dispersible MNPs were generated by coating the nanocrystals with copolymers of phospholipid and polyethylene glycol (PEG) using a dual solvent exchange method \(^\text{38}\). Coated MNPs (MNP-PEG) were then conjugated with TAT peptide (GRKKRRQRRRPQ) \(^\text{39}\)—a positively charged peptide generated by coating the nanocrystals with copolymers of phosphomagnetite (Supplementary Fig. 2a,b). Water-dispersible MNPs were found to have a saturation magnetization of 87.2 emu g\(^{-1}\). When MNP-TAT was mixed with baculoviral vector in phosphate buffered saline (PBS), multiple MNP-TATs could attach to a single baculoviral vector to form the MNP-BV hybrid (Fig. 1a). We found that, when mixed with MNP-TAT, more than 90% of baculoviral vectors could be pulled down by a magnetic plate, while mixing with MNP-PEG (that is, without TAT) had a negligible effect on the dispersion of baculoviral vectors (Fig. 1b). When MNP-TAT was pre-incubated with heparin—a negatively charged molecule—the pulldown efficiency was reduced to 60% (Fig. 1b). Further pulldown assays were performed, respectively, using MNPs conjugated with positively charged peptide polyarginine (MNP-polyArg) instead of TAT, and mixed with adenoviruses instead of baculoviral vector, revealing similar trends (Fig. 1b). Our results suggest that the interaction between baculoviral vector and MNP-TAT is largely due to the non-specific electrostatic interactions between the positively charged peptide and negatively charged viral surface \(^\text{40}\). Importantly, when serum was added to the sample, the pulldown efficiency of baculoviral vector mixed with MNP-TAT was only reduced by 10% (Fig. 1b), suggesting that baculoviral vector and MNP-TAT could remain associated during systemic circulation.

**Nanomagnets improve cell entry and transduction efficiency of baculoviral vector in vitro.** We further investigated the effect of nanomagnets on the interactions of baculoviral vector with cultured hepatoma cells (Hepa 1–6 cells) that have high baculoviral vector infectability (Supplementary Fig. 4) \(^\text{41}\). The cells were incubated with baculoviral vector or MNP-BV for 10 min—a much shorter time than is used in a typical in vitro viral transduction assay. Cells incubated with baculoviral vector alone had negligible intracellular baculoviral vector, as examined by immunostaining with the anti-vp39 antibody (Fig. 1c) \(^\text{42}\). In contrast, under a magnetic field, a large number of MNP-BV complexes entered the cells after a short period (10 min) of incubation (Fig. 1c and Supplementary Videos 1 and 2). Transmission electron microscopy (TEM) images of cell cross-sections show co-existence of MNPs and baculoviral vector in the lysosomes (Supplementary Fig. 5), suggesting concurrent cellular internalization of MNPs and baculoviral vector.

To examine the effect of nanomagnets on baculoviral vector-induced transgene expression in vitro, we constructed BV-LUC and BV-eGFP, baculoviral vectors containing the luciferase and enhanced green fluorescent protein (eGFP) cassette, respectively (Fig. 2a). BV-eGFP was mixed with MNP-TAT, and the mixture was incubated with Hepa 1–6 cells under a magnetic field for 30 min,
resulting in a much larger number of eGFP-positive cells compared with BV-eGFP alone, BV-eGFP under a magnetic field but without MNPs, or BV-eGFP with MNPs but without an applied magnetic field (Fig. 2b,d). When BV-eGFP was mixed with MNPs labelled with the fluorophore Dii (maximum excitation/emission wavelengths = 549/565 nm), MNPs could be observed in perinuclear vesicles in the cells that had strong eGFP expression, indicating that transgene expression occurred in the presence of MNPs (Fig. 2c). The baculoviral vector transduction efficiency was determined by quantifying eGFP fluorescence and luciferase activity in the cells incubated with BV-eGFP and BV-LUC, respectively (Fig. 2d,e). The luciferase activity shown in Fig. 2e was normalized against that in cells incubated with BV-LUC alone for 4 h, resulting in a high level of luminescence (Supplementary Fig. 4c). Clearly, having baculoviral vector mixed with MNPs or under the magnetic field alone did not affect the transgene expression (Fig. 2d,e). Having baculoviral vector mixed with MNPs and applying a magnetic field could increase the transgene expression fivefold compared with that of baculoviral vector alone (Fig. 2e). We found that with both MNPs and an applied magnetic field, BV-eGFP and BV-LUC could efficiently transduce many other cell types, such as HeLa, U87 and human umbilical vein endothelial cells (HUVECs).
Nanomagnets can help overcome serum inactivation of baculoviral vector in vitro. To determine whether MNPs can protect baculoviral vector from serum inactivation similar to that of polymer coating or ligand displaying25,27,28, we performed baculoviral vector transduction in a culture medium with 50% adult mouse serum (AMS), which contains the complement system to inactivate baculoviral vector. When the cells were incubated with baculoviral vector alone, baculoviral vector transduction was suppressed by AMS, as indicated by the low percentage of eGFP-positive cells and negligible luciferase expression (Fig. 3a,b). Neither MNPs nor the magnetic field alone could rescue BV-eGFP or BV-LUC transduction. In contrast, when associated with MNPs and under an applied magnetic field, BV-eGFP had high transduction efficiency and BV-LUC induced a high level of luciferase expression in Hepa 1–6 cells (Fig. 3a,b), suggesting that the inactivation of baculoviral vector by AMS was diminished. When MNP-BV-LUC was used under a magnetic field, the level of transgene expression in the presence of AMS was even higher than that induced by BV-LUC alone without AMS (Figs. 2e and 3b).

To further elucidate the mechanisms underlying the complement evasion, we quantified the baculoviral vector genome copy and transgene expression in baculoviral vector-transduced cells (Fig. 3c). AMS dramatically decreased the endocytosis of baculoviral vector, as indicated by the much-reduced baculoviral vector genome copies in cultured cells. MNP-TAT alone did not have a significant effect on the baculoviral vector endocytosis, nor the transgene expression. Under an applied magnetic field, the endocytosis of baculoviral vector complexed with MNP-TAT increased 20-fold in the normal medium and more than fourfold in the presence of AMS. These results suggest that baculoviral vector inactivation was mainly due to inhibition of baculoviral vector cell entry by either enzymatic degradation of baculoviral vector or masking of the baculoviral vector surface. It is likely that with MNPs-BVs the physical forces generated by MNPs under the magnetic field induce a rapid cellular uptake of baculoviral vector, which may outrace the cascade involved in serum-mediated baculoviral vector inactivation. Even when the MNPs-BVs were mixed with AMS for 30 min before applying the magnetic field, the level of baculoviral vector internalization was not reduced, confirming the high stability of the MNP-BV complex in the serum found in our pulldown study (Fig. 1b). However, having MNPs complexed with baculoviral vector reduced the levels of luciferase messenger RNA (mRNA) in Hepa 1–6 cells, possibly due to interference with the endosomal escape of baculoviral vector. The combined effects of MNPs and the magnetic field on overcoming the baculoviral vector evasion from the complement system are depicted schematically in Supplementary Fig. 10. Our results suggest that MNPs together with a magnetic field can enhance the endocytosis of baculoviral vector through enrichment of baculoviral vectors on the cell surface, and the increase in the interaction of baculoviral vector with cell membrane via positively charged TAT peptide. It is also possible that magnetic force exerted on MNPs on the cell surface and in the intracellular vesicles may induce changes in actin filaments, similar to what we reported previously23,26, which may further increase baculoviral vector endocytosis. However, this does not rule out other mechanisms, such as endocytosis mediated by microtubules and heparin sulfate.

We investigated whether serum inactivation and magnetic activation could be combined to provide spatial control of baculoviral vector transduction. Cells seeded in a chamber slide were incubated with MNP-BV-eGFP in the presence of AMS, and only half of the chamber was placed on a block magnet. We found that at 12 h post-transduction, most eGFP-positive cells were in the area above the magnet (Fig. 3d). The level of transgene expression increased with the MNP-to-baculoviral vector ratio and the strength and duration of the applied magnetic field (Supplementary Fig. 11). When a mixture of baculoviral vector and MNP-TAT was infused through silicone tubing at physiologically relevant flow rates, baculoviral vector could be captured by a block magnet placed next to the tubing (Supplementary Fig. 12). This suggests that block magnets can increase the margin of the MNP-BV complex circulating in the blood vessels and enhance the contact between MNP-BV and the vascular endothelium. In addition, an artificial vein was created by growing a layer of endothelial cells in the silicone tube. The MNP-BV-eGFP vector in culture medium containing AMS was infused into the tubing at a flow rate of 7 mm s⁻¹. A section of the tubing was placed along a block magnet during the infusion. After overnight incubation, we found that only the cells in the tubing next to the magnet showed eGFP fluorescence (Fig. 3e), further demonstrating the ability to provide spatial control of baculoviral vector transduction.

We integrated the cassettes encoding eGFP, Streptococcus pyogenes Cas9 and guide RNA targeting the mouse Vegfr2 gene into one plasmid for baculoviral vector packaging (BV-CRISPR), thanks to the baculoviral vector’s large DNA loading capacity (>38 kilobases) (Fig. 4a). When delivered as a plasmid or via the BV-CRISPR vector, respectively, into Hepa 1–6 cells, the CRISPR–Cas9 system had cutting efficiencies of 9–30% of the mouse Vegfr2 gene (Supplementary Fig. 13). When Hepa 1–6 cells were incubated with the MNP-BV vector carrying CRISPR–Cas9 (MNP-BV-CRISPR) in the medium containing 50% AMS, both the eGFP expression and CRISPR–Cas9-induced gene modification rate increased with the strength of the applied magnetic field (Fig. 4b). Without applying a magnetic field to overcome baculoviral vector inactivation by AMS, there was no eGFP expression or site-specific Vegfr2 gene modification in Hepa 1–6 cells (Fig. 4b).

In vivo baculoviral vector transduction and genome editing via local injection. It has been shown that conventional viral vectors (such as adenoviral vectors) injected into tumour tissue locally (intratumoral injection) can disseminate through the systemic circulation and induce a high level of transgene expression in the liver49. In contrast, in vivo transduction of baculoviral vector via local injection is most effective in immune privileged tissues lacking the complement system50,51; otherwise, baculoviral vector transduction is suppressed, presumably caused by partial exposure to the complement system due to local bleeding and in situ secretion of the Bcell.
complement factors. We performed intratumoral injection of baculoviral vector and found that BV-LUC could induce moderate transgene expression in subcutaneous tumours (Fig. 5a,b). If the tumour was injected with MNP-BV-LUC and subjected to a magnetic field for 1 h post-injection, the transgene expression was increased markedly (Fig. 5a,b). Ex vivo imaging of dissected tumours and vital organs showed that the transgene expression was limited to injected tumour tissue (Fig. 5c,d). Local baculoviral vector transduction and transgene expression could also be achieved in the mouse hind limb following intramuscular injection (Supplementary Fig. 14).

To examine MNP-BV-CRISPR-induced genome editing, we injected the mice intratumorally with MNP-BV-CRISPR and

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**Fig. 3 | Nanomagnets help baculoviral vector overcome serum inactivation in vitro.**

**a, b.** In vitro activation of transgene expression against AMS. Hepa 1–6 cells were incubated with BV-eGFP (a) or BV-LUC (b) for 30 min in a culture medium containing 50% AMS. The luciferase activity was normalized against that in cells incubated with BV-LUC alone for 4 h without AMS (Supplementary Fig. 4c). Data represent means ± s.e.m. ***P < 0.001 based on one-way ANOVA and a post-hoc Dunnett test. BV, baculoviral vector; MF, magnetic field.

**c.** Copies of BV genome and transgene mRNA in transduced cells. Hepa 1–6 cells were incubated with premixed BV-LUC and MNPs under designated conditions. Copies of BV genome and luciferase mRNA were quantified by qPCR. Data represent means ± s.e.m. *P < 0.05, **P < 0.01 and ***P < 0.001 based on one-way ANOVA and a post-hoc Dunnett test. NS, not significant.

**d.** Nanomagnet-induced BV transduction in a static cell culture. Cells cultured in a chamber slide were incubated with MNP-BV-eGFP in a culture medium containing 50% AMS for 30 min, while the other half of the chamber was placed on a block magnet. Most eGFP-positive cells localized in the area on top of the magnet.

**e.** Nanomagnet-induced BV transduction in an artificial vessel. HUVECs were seeded in silicone tubing (inner diameter = 0.3 mm). MNP-BV-eGFP was infused in culture medium containing 50% AMS with a syringe pump for 10 min while the tubing was aligned with the edge of a bar magnet. Top and bottom panels are the bright-field and fluorescence images, respectively. Red arrows mark the range of the magnet. The dark streak in the bright-field image (top) shows MNP-BV-eGFP captured by the magnetic force. After infusion, the cells were incubated with fresh culture medium and GFP expression was examined after 24 h. GFP expression was only found in the HUVECs next to the magnet (bottom).
It is well known that viral gene delivery and tissue-specific genome editing, overcoming the coupled with a magnetic field provides a means for efficient local in vivo activation of baculoviral vector transduction and local challenge of non-specific dissemination. Consequently, local injection of MNP-BV Vegfr2 pattern detected by NGS of the mouse CRISPR following direction injection. A representative mutation samples collected from different parts of the tumour as a result of the tumour (Fig. 5g). The indel rates (0 to 4.7%) varied among the samples (Fig. 5f). In a parallel study, at 96 h post-transduction, the mouse tumours = 100) in culture medium containing 50% AMS for 30 min in the presence of different-strength MFs. Transgene expression was examined by eGFP fluorescence at 24 h after transduction (top, fluorescence images; bottom, bright-field images). There was minimal transgene expression in the cells without the magnetic treatment. The level of transgene expression increased with the strength of the MF. Scale bar, 100 μm. After 72 h, the cells were harvested and examined by T7E1 assay (bottom). Consistent with the trend in eGFP expression, the CRISPR-mediated Vegfr2 disruption correlated with the MF strength.

placed the tumour under a magnetic field. Following the workflow illustrated in Fig. 5e, at 24 h post-MNP-BV-CRISPR delivery, the eGFP-positive cells were harvested from the mouse tumours and T7E1 assays were performed to quantify the gene modification rate. We found that MNP-BV-CRISPR induced site-specific gene modification in transduced cells with a ~30% indel rate (Fig. 5f). In a parallel study, at 96 h post-transduction, the mouse tumours and vital organs were homogenized and the modifications in the mouse Vegfr2 gene were analysed by next-generation sequencing (NGS). We found that gene modification was only detectable in the tumour (Fig. 5g). The indel rates (0 to 4.7%) varied among the samples collected from different parts of the tumour as a result of the heterogeneous distribution of the magnetic field and MNP-BV-CRISPR following direction injection. A representative mutation pattern detected by NGS of the mouse Vegfr2 locus is summarized in Supplementary Fig. 15. Therefore, local injection of MNP-BV coupled with a magnetic field provides a means for efficient local gene delivery and tissue-specific genome editing, overcoming the challenge of non-specific dissemination.

In vivo activation of baculoviral vector transduction and local genome editing via systemic delivery. It is well known that viral vectors delivered via systemic injection can reach the vascular endothelium and—in the case of fenestrated or leaky vessels—the stromal and parenchymal cells behind the vessel wall. Although baculoviral vector administrated intravenously can circulate throughout the body, circulating baculoviral vector can trigger the classical complement cascade that leads to baculoviral vector inactivation. As shown in our in vitro baculoviral vector transduction study, a magnetic field can drive MNP-BV towards the cell surface and enhance its cellular uptake with faster kinetics, which overcomes baculoviral vector inactivation by the complement system. We expect that magnetic activation of baculoviral vector can enable selective in vivo genome editing in the tissue exposed to the applied magnetic field.

First, we tested the nanomagnet-based approach in vivo with the mouse liver, which can be readily influenced by a block magnet. MNP-BV-LUC was administrated systemically through tail vein injection, and the mouse liver was positioned on top of a block magnet for 1 h (Fig. 6a). Transgene expression was evaluated by examining the luciferase activity with live animal imaging. Consistent with the results from our in vitro studies, the mice treated with MNP-BV-LUC and subjected to a magnetic field showed strong luminescence in the liver, whereas there was no luminescence in the mice treated with BV-LUC alone, or with MNP-BV-LUC but without applying a magnetic field (Fig. 6b,c). Ex vivo examination confirmed that the high luciferase expression was only in the liver tissue exposed to the magnetic field; other vital organs, including the heart, lung, spleen and kidney, did not show a luminescence signal (Fig. 6d,e). The level of luciferase expression in the liver also increased with the strength of the magnetic field (Supplementary Fig. 16). Importantly, the luciferase expression in the mouse liver lasted less than 48 h (Fig. 6f,g), indicating that nanomagnet-induced transgene expression is transient. MNP-BV-LUC did not induce significant acute liver damage (Supplementary Figs. 17 and 18).

The MNP-BV transduction activated by a magnetic field was further examined in the spleen, where a small block magnet was placed next to the spleen following the tail vein injection of MNP-BV (Supplementary Fig. 19a). At 24 h post-transduction, ex vivo imaging of dissected organs showed that the luciferase activity in the spleen was significantly higher than in the heart, lung and kidney (Supplementary Fig. 19b,c), suggesting that the nanomagnet-induced transgene expression in vivo can be switched on locally with good tissue specificity. Since the liver was exposed to the applied magnetic field as well, luciferase activity also increased in the liver.

For in vivo genome editing, the mice were injected with MNP-BV-CRISPR and subjected to a magnetic field targeting the mouse liver similar to that shown in Fig. 6a. Following the workflow illustrated in Fig. 5e, at 24 h post-MNP-BV-CRISPR delivery, the eGFP-positive cells were harvested from the mouse liver tissue and T7E1 assays were performed to quantify the gene modification rate. We found that the nanomagnets induced site-specific gene modification in the transduced mouse liver cells with a ~50% indel rate (Fig. 6h). A representative pattern of the indels at the Vegfr2 target locus is shown in Supplementary Fig. 20. Our NGS analysis suggests that ~96% of mutations may lead to a frameshift (3N + 1, 3N + 2). In a parallel experiment, mouse organs including the liver, heart, lung, spleen and kidney were harvested 4 d post-MNP-BV-CRISPR delivery and the genomic DNA was extracted for NGS analysis. Site-specific gene modification was detected in the liver but not in the off-target organs (Fig. 6i). As expected, without selecting baculoviral vector-transduced cells that are eGFP positive, the gene editing rates in the liver tissue samples collected somewhat randomly were much lower than with selection, since magnetic-field-activated baculoviral vector transduction was localized, and the liver samples for NGS were chosen by guessing where the magnetic field was effectively applied. Nevertheless, the NGS results shown in Fig. 6i confirm that there was efficient magnetic-field-driven genome editing with high tissue specificity.
To determine whether spatial control of genome editing can be realized in another tissue, we also performed genome editing in the tumour-carrying mice following systemic injection of MNP-BV-CRISPR. The tumour was placed between two block magnets for 1 h following injection, and the analysis of gene editing in the tumour was performed in eGFP-positive cells selected using the workflow illustrated in Fig. 5e. Gene modifications at the Vegfr2 locus in the transduced cells in the tumour were quantified by both T7E1 assay and NGS analysis. Indels due to specific gene editing were detected in the tumour, albeit at a lower frequency compared with those induced by intratumoral injection (Supplementary Fig. 21). The decrease in the gene editing efficiency was probably due to the limited amount of MNP-BV penetrating into the tumour stroma47.

Taken together, our results provide strong evidence of efficient magnetic-field-driven baculoviral vector transduction and transgene expression in different organs, and of spatially controlled genome

Fig. 5 | Magnetic-field-enhanced transgene expression and genome editing in subcutaneous tumours. The hybrid vehicle with the luciferase-expressing cassette (MNP-BV-LUC) was injected directly into a subcutaneous tumour. The tumour was then placed between two block magnets for 1 h. a,b. Bioluminescence analysis of transgene expression (imaged (a) and quantified (b)) in the tumour at 24 h post-transduction. Data represent means ± s.e.m. *P = 0.03 based on a one-tailed Student’s paired t-test. BV, baculoviral vector; MF, magnetic field. c,d. Bioluminescence analysis of transgene expression (imaged (c) and quantified (d)) in the dissected tumours and vital organs. Data represent means ± s.e.m. **P = 0.004 based on a one-tailed Mann-Whitney U-test. In a parallel study, the mice were injected with MNP-BV-CRISPR. e. Flow chart of the genome editing analysis of CRISPR-Cas9-targeted cells from the mouse tumour. Scale bars, 80 μm. f. Analysis of in vivo mouse Vegfr2 gene editing by T7E1 assay. CTL, control. g. Analysis of genome editing in the tumour and vital organs (n = 3 per group). In the tumour, multiple samples were collected at the regions near the block magnet. The samples with no detectable editing rate (that is, <0.1%) are plotted as having a 0.05% editing rate.
Fig. 6 | A magnetic field enables liver-specific transgene expression and genome editing in vivo via systemic injection. a, Schematic of MNP-BV-mediated transgene delivery in the mouse liver. MNP-BV-LUC was administrated to the mouse through intravenous injection. A block magnet was pressed against the mouse liver to trigger local transgene expression. A contour plot indicated the magnetic force applied to individual MNPs at a distance of 1 mm from the top of the magnet. b, c, Bioluminescence analysis of transgene expression (imaged (b) and quantified (c)). Nude mice were injected with PBS (wild type), baculoviral vector (BV) alone, MNP-BV or MNP-BV, followed by magnetic field (MF) treatment for 1 h. In the positive control, C3 knockout mice were injected with BV alone. In all groups, the dose of the virus was 10⁹ p.f.u. of virus per mouse. At 24 h post-injection, the mice were imaged using an IVIS small animal live imaging system. Data represent means ± s.e.m. ***P < 0.001 based on one-way ANOVA and a post-hoc Dunnett test. Note that the MF triggered high transgene expression in mice injected with MNP-BV, while without the magnetic treatment, the signal was negligible due to serum inactivation. d, e, Biodistribution of transgene expression (imaged (d) and quantified (e)). In the MNP-BV + MF group, the organs were isolated 24 h after injection, and bioluminescence of the vital organs was measured ex vivo. The liver showed a high level of transgene expression, while bioluminescence was undetectable in the lung, kidney, spleen and heart. All luminescence activity was normalized against the peak value in the plot. Data represent means ± s.e.m. ***P < 0.001 based on one-way ANOVA and a post-hoc Dunnett test. f, g, In vivo transgene expression (imaged (f) and quantified (g)) at 24 h and 48 h post-injection. Data represent means ± s.e.m. *P = 0.018 based on a one-tailed Student’s t-test. h, Analysis of in vivo mouse Vegfr2 gene editing by T7E1 assay. At 24 h post-MNP-BV-CRISPR delivery (under the MF), eGFP-positive cells were selected from mouse liver tissue and T7E1 assays were performed to quantify the gene modification rate. CTL, control. i, Analysis of genome editing in vital organs (n = 4 per group). Multiple samples were collected in the mouse liver at the regions near the block magnet, and gene editing was quantified using NGS. Note that the samples with no detectable editing rate (that is <0.1%) are plotted as having a 0.05% editing rate.
editing in different tissues, such as the liver and tumour. Specifically, our in vivo studies demonstrate that the nuclease activity of the CRISPR–Cas9 system packaged in MNP-BV can be induced in the target tissues/organ on demand by the applied magnetic field, and both direct and systemic injection of MNP-BV enable site-specific genome editing. The MNP-BV-based in vivo delivery system takes advantage of the ability of nanomagnets to overcome baculoviral vector inactivation locally, thus enabling spatial control of in vivo gene delivery and genome editing. Our studies also provide insight into the mechanism of binding between baculoviral vector and TAT-modified magnetic nanoparticles, and how the MNP-BV system prevents complement inactivation of baculoviral vector.

Effective transduction of the MNP-BV complex in vivo is triggered by the applied magnetic field, which serves as an activator of transgene expression and genome editing. Baculoviral vector activation and gene editing efficiency depend on the strength and spatial distribution of the magnetic field, distribution of MNP-BV in the tissue and accessibility of the target gene in somatic cells. In this proof-of-concept study, although the block magnets used for both in vitro and in vivo experiments could generate a strong magnetic field (1.48 Tesla), the resulting magnetic force applied to the MNPs attached to baculoviral vector varied with the location (distance and angle relative to the block magnet) of the MNP-BV complex. Due to the size of the magnet used (see Supplementary Fig. 19) and the challenge in positioning the block magnet very close to the mouse spleen, under systemic delivery, it was difficult to have baculoviral vector transduction only in the spleen, and not in the liver (which is next to the spleen and has a much larger size). Furthermore, when delivered systemically, it is likely that MNP-BVs could only be transduced in the vascular endothelium in the tumour and the few layers of stroma cells behind the leak vessel, resulting in a much-reduced distribution of the magnetic field, distribution of MNP-BV in the tissue and accessibility of the target gene in somatic cells. In this proof-of-concept study, although the block magnets used for both in vitro and in vivo experiments could generate a strong magnetic field (1.48 Tesla), the resulting magnetic force applied to the MNPs attached to baculoviral vector varied with the location (distance and angle relative to the block magnet) of the MNP-BV complex. Due to the size of the magnet used (see Supplementary Fig. 19) and the challenge in positioning the block magnet very close to the mouse spleen, under systemic delivery, it was difficult to have baculoviral vector transduction only in the spleen, and not in the liver (which is next to the spleen and has a much larger size). Furthermore, when delivered systemically, it is likely that MNP-BVs could only be transduced in the vascular endothelium in the tumour and the few layers of stroma cells behind the leak vessel, resulting in a much-reduced gene editing rate compared with local injection. With a large magnetic field gradient consisting of a set of coils optimally positioned, a strong magnetic field gradient could be applied anywhere in the body, thus overcoming these limitations. Efficient gene editing in specific cells in the target tissue can be achieved by optimizing the route of administration. Owing to the large DNA loading capacity of baculoviral vector, the MNP-BV-based delivery system also has the potential to facilitate multiplexed genome editing in vivo.

Methods
Production of baculoviral vector. Recombinant baculoviral vectors, including BV-LUC, BV-eGFP and BV-CRISPR, were constructed using the Bac-to-Bac baculovirus expression system (Thermo Fisher Scientific). Briefly, the expression cassette of cytomegalovirus (CMV)-luciferase, elongation factor 1α (EF1α) cassette of cytomegalovirus (CMV)-luciferase, elongation factor 1α (EF1α) promoter, small RNA (sgRNA) is a single guide RNA, and CBh is chicken β actin hybrid promoter) was inserted into the pFastBac vector and transformed into DH10Bac competent cells. The recombinant bacmids containing virus genome and the expression cassette were subsequently used to transduce insect cells in vitro and in vivo experiments. Recombinant baculoviral vector stocks were determined using both the plaque assay and the qPCR titration assay (Supplementary Fig. 1).

Synthesis of MNPs. MNPs were synthesized according to previously published protocols28–30. In brief, magnetite nanocrystals were synthesized through thermolysis compositions of FeCl3 (Sigma-Aldrich) in benzyl ether oleic acid (Sigma-Aldrich) and oleylamine (Sigma-Aldrich) as the capping molecules. As-synthesized nanocrystals were coated with DSPE–mPEG2000 (Avanti Polar Lipids) and DSPE–PEG-maleimide (Avanti Polar Lipids) at a molar ratio of 9:1 using a dual solvent exchange method31. To conjugate peptides to the surface of MNPs, freshly coated MNPs were mixed with cys-TAT peptides (GCGCGRGRRKRQRRRQ; GenScript) or (Arg)12-cys (RRRGGRRRRRRRRRR; GenScript) at a molar ratio of 1:400 in PBS and incubated overnight. Unconjugated peptides were removed by washing the nanoparticles with deionized water in centrifugal filter tubes (cut-off molecular weight = 100 kDa). The physical properties of the MNPs were characterized using TEM, dynamic light scattering (Möbus, Wyatt Technology) and a superconducting quantum interference device (MPMS; Quantum Design).

In vitro magnetic pulldown study. BV-LUC (2 × 106 plaque-forming units (p.f.u.)) or Adv-LUC (with adenovirus instead of baculovector; 2 × 106 p.f.u.; Vector Biolabs) were mixed with 5 μg iron of unconjugated MNPs (MNP-PEG), MNPs conjugated with TAT (MNP-TAT) or MNPs conjugated with (Arg)12 (MNP-polyArg) in PBS for 20 min. In the heparin group, MNP-TAT was first mixed with heparin (10 units) for 20 min before mixing with baculoviral vector. Then, the mixtures were added to 500 μl culture medium in 8-well chamber slides. In the serum group, the medium contained 50% AMS. The chamber slides were incubated on a magnetic plate for 10 min with gentle shaking. Finally, 50 μl supernatant was collected and copies of free viruses were quantified by qPCR (BacPAK qPCR Titration Kit; Takara).

Cell culture. Hepa 1–6 cells, human cervical adenocarcinoma cells (HeLa) and human glioblastoma cells (U87) were purchased from the American Type Culture Collection. HUVECs were purchased from Lonza. All cells were cultured according to the standard protocols provided by the distributors, without further authentication. The cells were tested for Mycoplasma routinely.

In vitro baculoviral vector transduction. In vitro baculoviral vector transduction was performed with either BV-eGFP, BV-LUC or BV-CRISPR. eGFP fluorescence was used to evaluate the transduction efficiency or spatial distribution of baculoviral vector transduction. Luciferase activity was used to examine the level of transgene expression. In a typical in vitro baculoviral vector transduction experiment, the cells were seeded in a chamber slide. Before baculoviral vector transduction, 2 × 105 p.f.u. baculoviral vector was mixed with 4 μg iron of MNPs for 20 min. Then, the cells were incubated with the mixture for 30 min with or without the magnet. In each group, the cells were transduced with baculoviral vector at an MOI of 100 p.f.u. per cell unless otherwise specified. After transduction, the cells were incubated with fresh medium. At 24 h post-transduction, the cells transduced by BV-eGFP were examined for eGFP fluorescence by flow cytometry or fluorescence microscopy. For the cells transduced by BV-LUC, the luciferase activity was measured using an in vitro luciferase kit (ONE-Glo Luciferase Assay System; Promega) in a microplate reader. The cells transduced with BV-CRISPR were cultured for 72 h and the genomic DNA was extracted and analysed by T7E1 assay for gene editing efficiency, as described below.

Cytotoxicity study. Hepa 1–6 cells were cultured in 96-well plates and incubated with baculoviral vector at designated MOIs with or without MNPs for 12 h. After treatment, the cells were incubated in fresh medium for 3 d and cell viability was evaluated by MTT assay.

Immunofluorescence staining. The cells were seeded in chamber slides and incubated with baculoviral vector or MNP-BV under designated conditions. After treatment, the cells were fixed in 4% paraformaldehyde for 20 min, permeabilized with PBS containing 0.1% Triton for 3 min and blocked with 5% bovine serum albumin for 1 h at room temperature. Baculoviral vector was detected by incubating the cells with an antibody against vp39 (kindly provided by L. Volkman and T. Okawa) overnight at 4 °C followed by an Alexa Fluor 647 goat anti-mouse IgG antibody (Abcam)32. Then, the cells were stained with Alexa Fluor 488 phalloidin (Thermo Fisher Scientific) and Hoechst 33342 (Thermo Fisher Scientific). Images were acquired with a confocal microscope (Nikon A1 Confocal Microscope).

Genomic DNA extraction, PCR amplification and T7E1 assay. Genomic DNA was extracted from the cell or tissue samples using the Quick-DNA Miniprep Plus Kit (Zymo Research). The amplification containing the CRISPR cutting site was amplified by PCR using Q5 High-Fidelity 2X Master Mix (forward primer: CCCCCCATTCGCGAAGGTGTGTA; reverse primer: AGGCAGGACTGATTGAGTCCC) under the following PCR conditions: 98 °C for 30 s; 34 cycles (98 °C for 10 s, 63 °C for 30 s and 72 °C for 30 s); 72 °C for 2 min; and hold at 4 °C. The PCR products were analysed by gel electrophoresis in 1% agarose and purified using a MiniElute PCR Purification Kit (Qiagen). Mismatched duplex DNA was obtained by denaturation and renaturation of 1 μg of purified PCR product. The renatured DNA was digested with T7 endonuclease I (New England Biolabs) at 37 °C for 1 h and analysed by gel electrophoresis in 2% agarose. The rate of gene modification was calculated using the following equation:

\[
\% \text{gene modification} = 100 \times \left(1 - \frac{1}{\left[\frac{1}{2}\right]}\right)
\]
Magnetically activated baculoviral vector transduction in vivo. All animal studies were approved by the Institutional Animal Care and Use Committee at Rice University. Athymic nude mice (4–5 week-old females) were purchased from Charles River Laboratories. C3 knockout mice were purchased from the Jackson Laboratories. The organs were fixed in 10% formalin solution overnight and embedded in paraffin. Histology evaluation was performed in tissue sections stained with hematoxylin and eosin.

Results

Magnetically activated baculoviral vector transduction in vivo. The organs were fixed in 10% formalin solution overnight and embedded in paraffin. Histology evaluation was performed in tissue sections stained with hematoxylin and eosin.

To investigate MNP-BV-induced transduction via systemic injection, the mice were injected with Baculoviral vector mixed with 20 µg of iron MNP 40 µl sterile PBS was injected intratumorally. The tumour was placed between two parallel grade NS2 NdFeB block magnets (L x W x H = 1” x 1/2” x 1/2”; KB Magnetics) for one hour under anesthesia. At 24 h post-injection, each mouse was injected with in vivo luciferase substrate (Promega) intraperitoneally and imaged using IVIS kinetic III live imaging system (PerkinElmer). Then, the mice were immediately euthanized and the tumour and vital organs were dissected for ex vivo imaging.

To investigate MNP-BV-induced transduction via systemic injection, the mice were injected with Baculoviral vector mixed with 0.1 mg of iron MNP) dispersed in 40 µl sterile PBS was injected intratumorally. The tumour was placed between two parallel grade NS2 NdFeB block magnets (L x W x H = 1” x 1/2” x 1/2”; KB Magnetics) for one hour under anesthesia. At 24 h post-injection, each mouse was injected with in vivo luciferase substrate (Promega) intraperitoneally and imaged using IVIS kinetic III live imaging system (PerkinElmer). Then, the mice were immediately euthanized and the tumour and vital organs were dissected for ex vivo imaging.

In vivo toxicity of MNP-BV. To examine the in vivo toxicity of MNP-BV, vital organs and blood were harvested from treated mice after 10 d post-injection. The organs were fixed in 10% formalin solution overnight and embedded in paraffin. Histology evaluation was performed in tissue sections stained with hematoxylin and eosin. Alamine transaminase and aspartate aminotransferase levels in the blood were measured using the ALT ELISA Kit (Bioc prepare.com) and AST Colorimetric Kit (BioVision), respectively, according to the manufacturers’ instructions.

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

Data availability.

The authors declare that all data supporting the findings of this study are available within this paper and its Supplementary Information. The raw datasets are available from the corresponding author upon reasonable request. The custom script used to analyse the indels in the NGS data is available at https://github.com/piyuranjan/NucleaseIndelActivityScript.

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Author contributions
H.Z., S.T. and G.B. conceived the idea, designed the study and wrote the manuscript. H.Z., S.T., L.Z. and H.D. performed the experiments and data analysis. C.M.L. helped with the CRISPR guide RNA design and performed the NGS analysis.

Competing interests
H.Z., S.T. and G.B. filed a US patent application (US20170239370A1) based on the results presented in this paper.

Additional information
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  *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

Comsol Multiphysics and Matlab were used for the simulation of the magnetic field generated by the block magnets.

Data analysis

GraphPad Prism was used for statistical analysis. The custom script used to analyse the indels in the NGS data is available at https://github.com/piyuranjan/NucleaseIndelActivityScript.

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Life sciences study design

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

| Sample size | No prior sample-size calculation was performed. In all studies, a sample size of 3–4 was used initially. Statistical analysis was performed on the collected data and the results were used to determine if more samples were needed. |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | Exclusion criteria were not used in this study. |
| Replication | All chemicals and cell lines were purchased from the vendors with sufficient quality controls. All plasmids used in this study were verified by appropriate sequencing. All experiments were recorded in a repeatable manner. |
| Randomization | All cells and animals were randomized among groups. |
| Blinding | Blinding was not used in this study. The data analyses on luminescence intensity, genome editing efficiency, etc. were directly calculated with corresponding software, without decision making by the researchers. |

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ ☒ Unique biological materials |
| ☒ ☒ Antibodies |
| ☒ ☒ Eukaryotic cell lines |
| ☒ Palaeontology |
| ☒ ☒ Animals and other organisms |
| ☒ Human research participants |

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

- TAT peptide-conjugated magnetic iron oxide nanoparticles were synthesized in the lab. The baculoviruses packaged with Sp cas9 nuclease and guide RNAs were generated in the lab. Both are available from the corresponding author upon reasonable request.

Antibodies

- Antibodies used: The antibody against vp39 was a gift from Prof. Loy Volkman and Dr. Taro Ohkawa.
- Validation: The antibody has been used in previous studies (refs. 41–43).

Eukaryotic cell lines

Policy information about cell lines

- Cell line source(s): Mouse hepatoma cells (Hepa 1-6), human cervical adenocarcinoma cells (HeLa), and human glioblastoma cells (U87) were purchased from ATCC. Human umbilical vein endothelial cells (HUVEC) were purchased from Lonza.
- Authentication: None of the cells were authenticated in the lab.
Mycoplasma contamination
The cells were tested for mycoplasma routinely.

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
Athymic nude mice (4–5 weeks old, female) were purchased from Charles River Laboratories.
C3 knockout mice (4–5 weeks, female) were purchased from the Jackson Laboratory.

Wild animals
The study did not involve wild animals.

Field-collected samples
The study did not involve samples collected from the field.

Flow Cytometry

Plots

Confirm that:
☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
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Methodology

Sample preparation
All cells were cultured according to the standard protocols from the distributors. The cells were transduced with adenovirus, baculovirus, or the complex of magnetic nanoparticles and viral particles under designated conditions. For flow cytometry analysis, the cells were detached using trypsin-EDTA, washed with PBS and analyzed using BD Accuri C6 Plus.

Instrument
BD Accuri C6 Plus.

Software
FlowJo.

Cell population abundance
10,000 events were collected in predetermined gates for each group.

Gating strategy
Gating was determined based on the distribution of the negative control (untransduced cells), which excluded 99.7% of the control cells (Supplementary Fig. S4A).

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.