

## Supplementary Materials for

### Durable multitransgene expression in vivo using systemic, nonviral DNA delivery

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Fig. S1. HEDGES hG-CSF cDNA injection elicits the production of endogenous mouse anti-hG-CSF antibodies.

Fig. S2. Intravenous HEDGES injection of the human TP53 cDNA is expressed predominantly in mouse VECs.

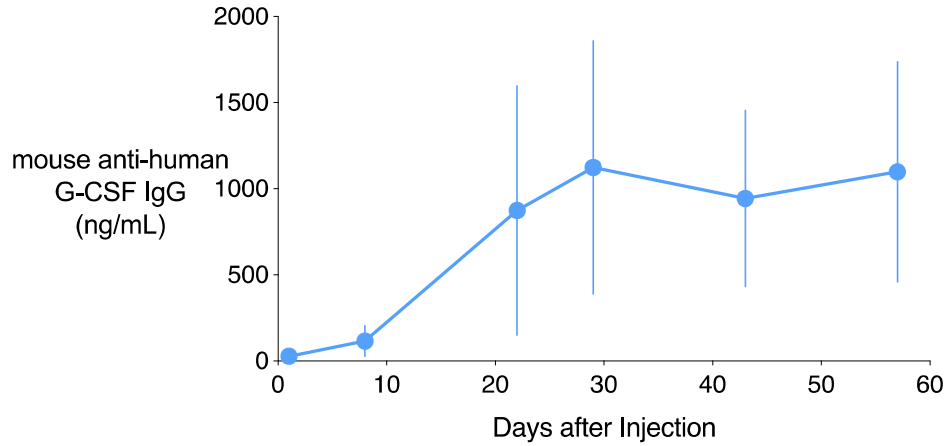
Fig. S3. Intravenous injection of HEDGES vector DNA does not detectably integrate into genomic DNA of injected mice.

Fig. S4. HEDGES treatment with a  $\beta$ -GAL reporter-containing plasmid results in expression of reporter in CD31<sup>+</sup>CD45<sup>-</sup> lung cells in mice.

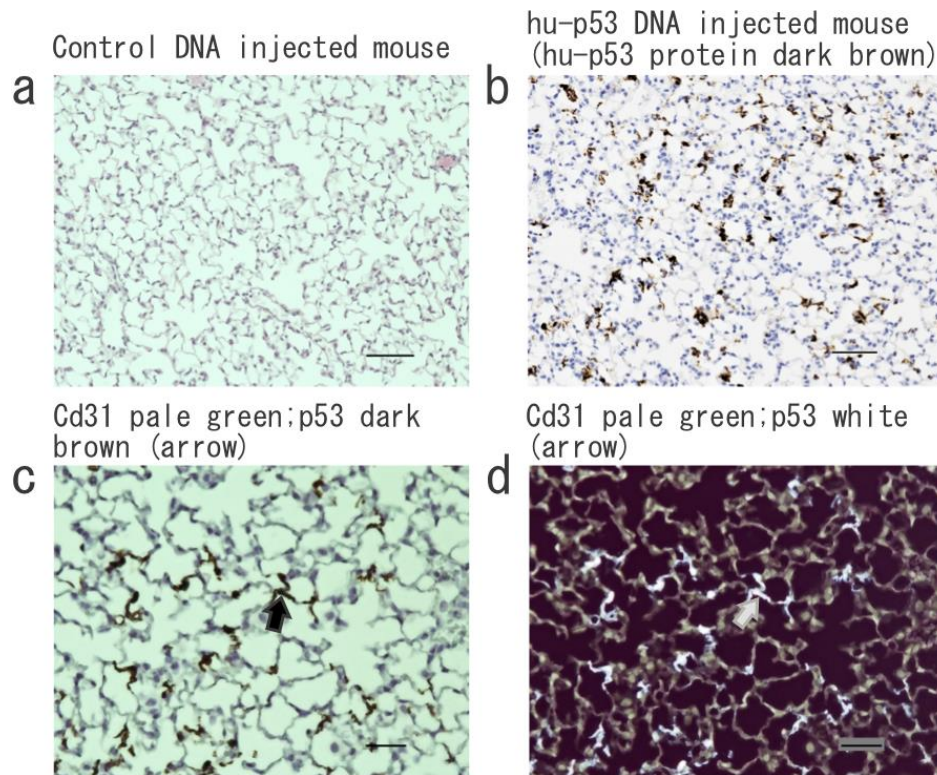
Fig. S5. Development of mouse anti-rituximab antibodies suppresses rituximab levels in HEDGES-treated mice.

Table S1. HEDGES expression plasmids.

## Supplementary Figures and Tables

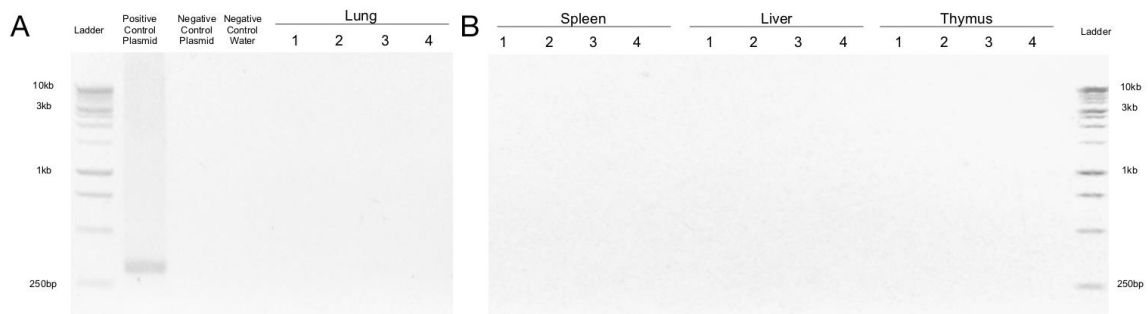


**Fig. S1. HEDGES hG-CSF cDNA injection elicits the production of endogenous mouse anti-hG-CSF antibodies.** On day 0, groups of 3 CD1 mice were IV injected with Lactated Ringer's or with 1 $\mu$ mol each of DOTAP liposomes and DMPC liposomes each incorporating 2.5% Dexamethasone 21-Palmitate, followed by 75 $\mu$ g of an hG-CSF cDNA expression vector. Mice were bled on day 1 and at weekly or bi-weekly intervals thereafter and analyzed for the presence of mouse anti-hG-CSF antibodies by ELISA.

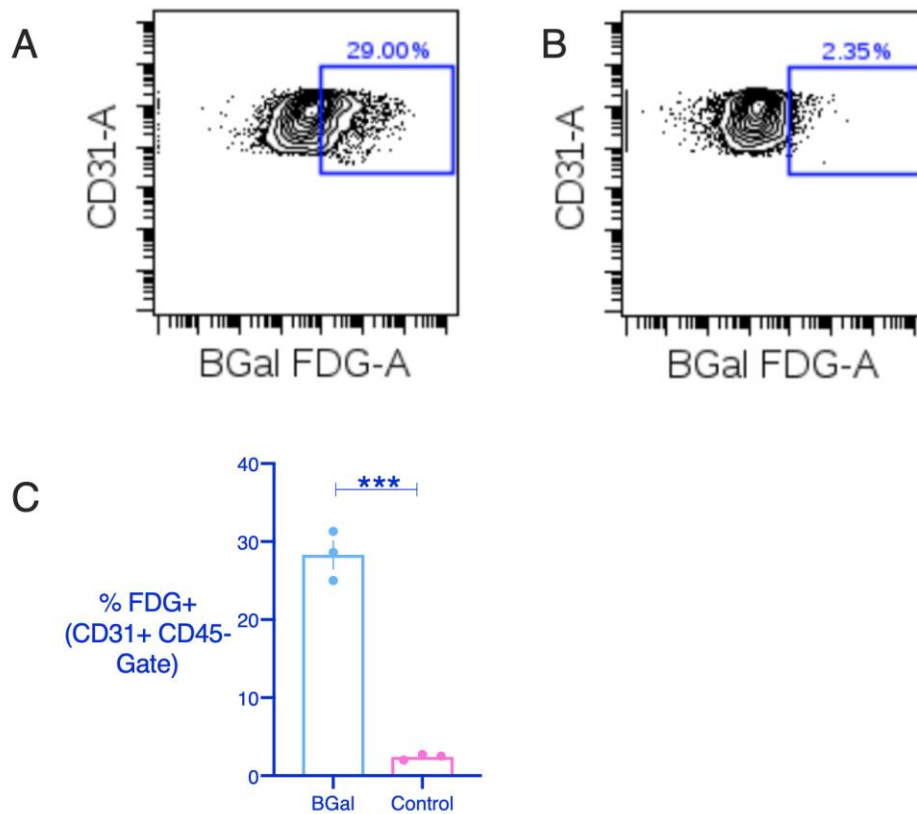


(Bar - Figure a, b-50um; c, d-25um)

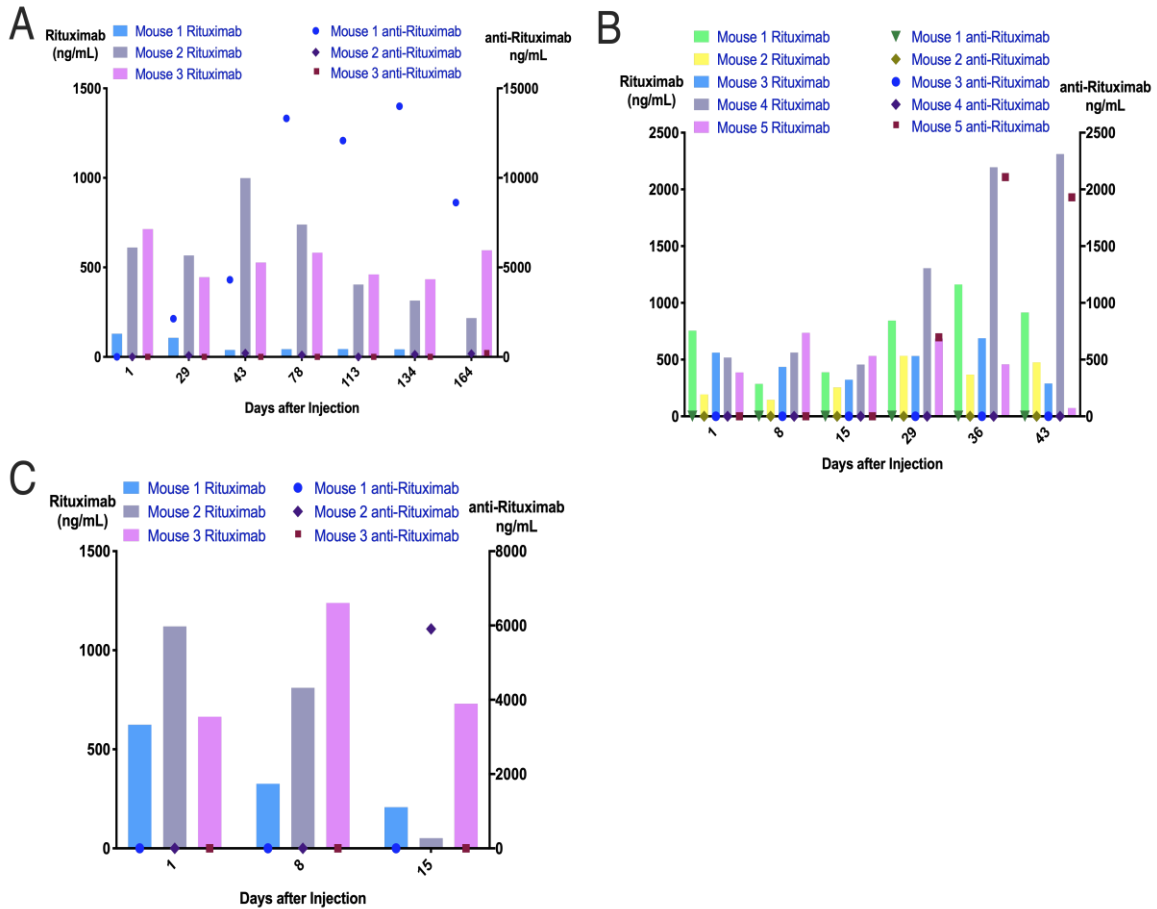
**Fig. S2. Intravenous HEDGES injection of the human TP53 cDNA is expressed predominantly in mouse VECs.** On day 0, groups of 3 CD1 mice were IV injected with Lactated Ringer's or with 1umol each of DOTAP liposomes and DMPC liposomes each incorporating 2.5% Dexamethasone 21-Palmitate, followed by 75ug of a vector encoding human TP53. Lungs were harvested 24 hours later, infused with 10% neutral buffered formalin, and processed for immunohistochemistry. Representative images from two independent experiments shown, with staining for human TP53 or dual-staining for human TP53 and mouse CD31 (PECAM-1). (a) and (b) show images of anti-hTP53-stained lung sections from a control mouse (a) or a hTP53 cDNA-injected mouse (b) where hTP53 stains brown. (c) and (d) show images of dual-stained lung sections from an hTP53 cDNA-injected mouse. Mouse CD31 stains pale green and hTP53 stains dark brown (arrow) in (c), while mCD31 stains pale green and hTP53 stains white (arrow) in (d).



**Fig. S3. Intravenous injection of HEDGES vector DNA does not detectably integrate into genomic DNA of injected mice.** On day 0, groups of 4 CD1 mice were IV injected with Ringer's or pure DOTAP liposomes followed by 60 micrograms of an EF1pro/hG-CSF expression vector. After 32 weeks, mice were euthanized and (A) lung and (B) spleen, liver, and thymus were harvested. Integration analysis by nrLAM-PCR was performed as previously described. Purified EF1pro/hG-CSF plasmid DNA was also run as a positive control.



**Fig. S4. HEDGES treatment with a  $\beta$ -GAL reporter-containing plasmid results in expression of reporter in  $CD31^+CD45^-$  lung cells in mice.** Total lungs cells from HEDGES reporter (A) and control (B) treated mice are analyzed for cell surface CD31 and intracellular  $\beta$ -galactosidase (BGal) expression by flow cytometry (events shown were initially gated on lacking cell surface CD45). Representative contour plots are shown depicting BGal expression using the fluorescent substrate FDG, along with surface CD31 expression. (C) Mean/SEM percent FDG (+) cells within a  $CD31^+ CD45^-$  gate are shown for groups of mice treated and analyzed as in A and B. All individual mice are shown. Data is representative of at least 2 independent experiments with 2-3 mice per group. \*\*\*  $P < 0.001$  by student t-test.



**Fig. S5. Development of mouse anti-rituximab antibodies suppresses rituximab levels in HEDGES-treated mice.** Mice were HEDGES-injected on day 0 with DOTAP liposomes and DMPC liposomes, followed by plasmid DNA encoding rituximab. Sera from one group of mice each from three separate experiments were assessed for presence of anti-rituximab antibodies (A-C). Sera from multiple time-points were assessed by ELISA. Serum rituximab levels of individual mice are displayed as bars (left y-axis) and corresponding levels of anti-rituximab in the same mice are displayed as symbols (right y-axis).

**Table S1. HEDGES expression plasmids.**

Supplementary Table 1: Composition and Arrangement of HEDGES Expression Plasmids

Plasmid Name	Enhancer 1	Promoter 1	Coding Sequence 1	Enhancer 2	Promoter 2	Coding Sequence 2	Enhancer 3	Promoter 3	Coding Sequence 3
EF1pro/hg-CSF	hCMV	EF-1	hg-CSF	n/a	n/a	n/a	n/a	n/a	n/a
EF1pro/Luc – EF1pro/hg-CSF	mCMV	EF-1	Luciferase	mCMV	EF-1	hg-CSF	n/a	n/a	n/a
hCMVenh:hCMVpro/hg-CSF	hCMV	hCMV	hg-CSF	n/a	n/a	n/a	n/a	n/a	n/a
mCMVenh:EF1pro/hg-CSF	mCMV	EF1	hg-CSF	n/a	n/a	n/a	n/a	n/a	n/a
mCMVenh:EF1pro/RituxHC – hCMVenh:EF1pro/RituxLC	mCMV	EF-1	Rituximab Heavy Chain	hCMV	EF-1	Rituximab Light Chain	n/a	n/a	n/a
mCMVenh:EF1pro/MepoluzHC – hCMVenh:EF1pro/MepoluzLC	mCMV	EF-1	Mepoluzimab Heavy Chain	hCMV	EF-1	Mepoluzimab Light Chain	n/a	n/a	n/a
hCMVenh:EF1pro/5J8HC – mCMVenh:EF1pro/5J8LC	hCMV	EF-1	5J8 Heavy Chain	mCMV	EF-1	5J8 Light Chain	n/a	n/a	n/a
mCMVenh:EF1pro/hg-CSF – mCMVenh:EF1pro/MepoluzHC – hCMVenh:EF1pro/MepoluzLC	mCMV	EF-1	hg-CSF	mCMV	EF-1	Mepoluzimab Heavy Chain	hCMV	EF-1	Mepoluzimab Light Chain
EF1pro/Luc	mCMV	EF-1	Luciferase	n/a	n/a	n/a	n/a	n/a	n/a