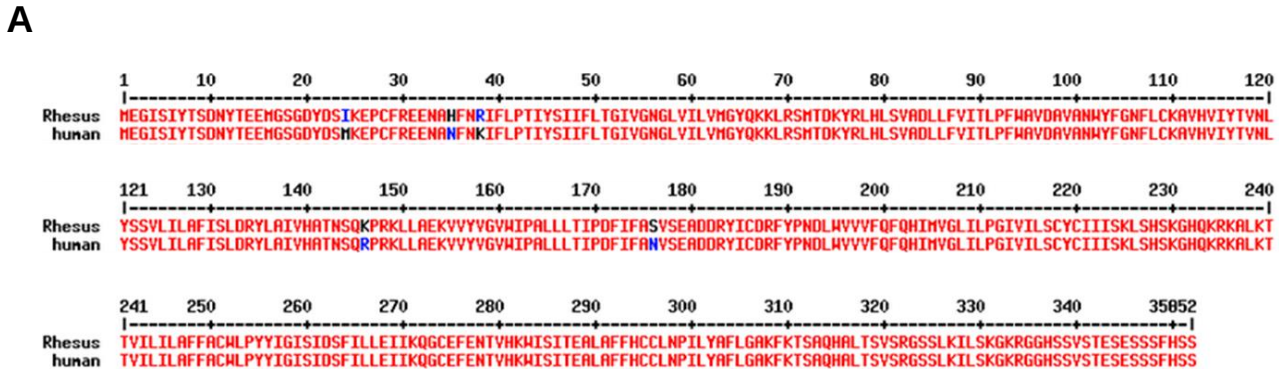


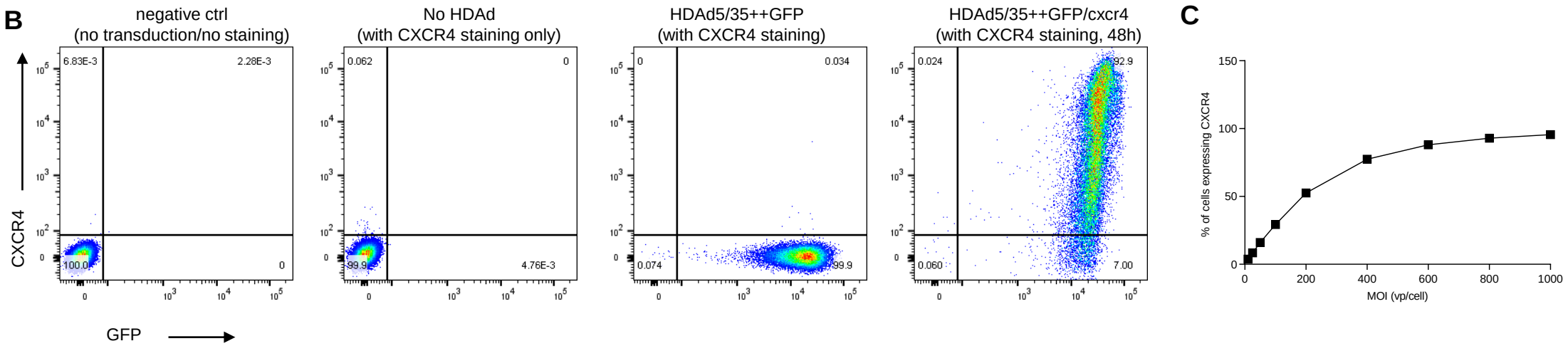




**Fig.S1. Validation of cxcr4 expression. A)** Amino acid alignment of human CXCR4 (accession# EAX11616) and rhesus CXCR4 (accession# NP_001036110). **B)** Transduction of K562 cells with HDAd5/35++GFP/cxcr4 at an MOI of 500 vp/cell. Shown are GFP/CXCR4 flow cytometry plots after staining with a human CXCR4-specific mAb at 48 hours after transduction. **C)** Percentage of $CXCR4^{+}$ cells after transduction of K562 cells with different MOIs of HDAd5/35++GFP/cxcr4.

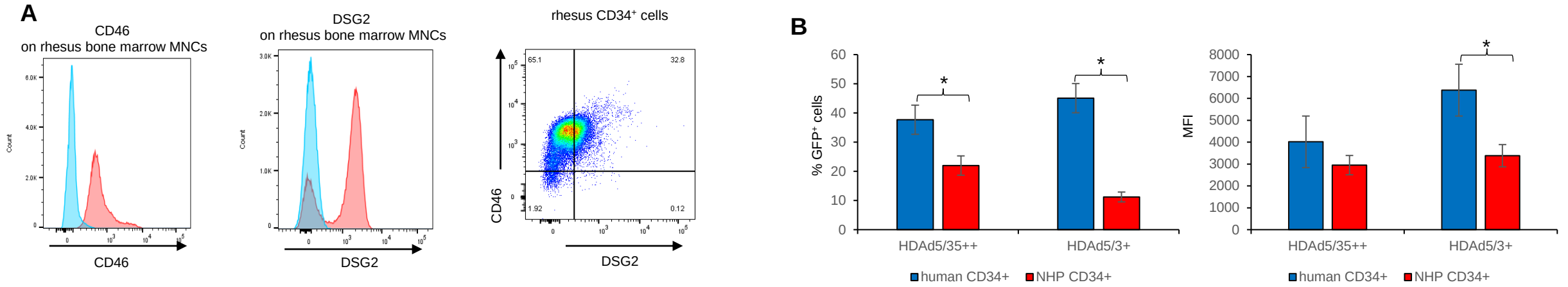
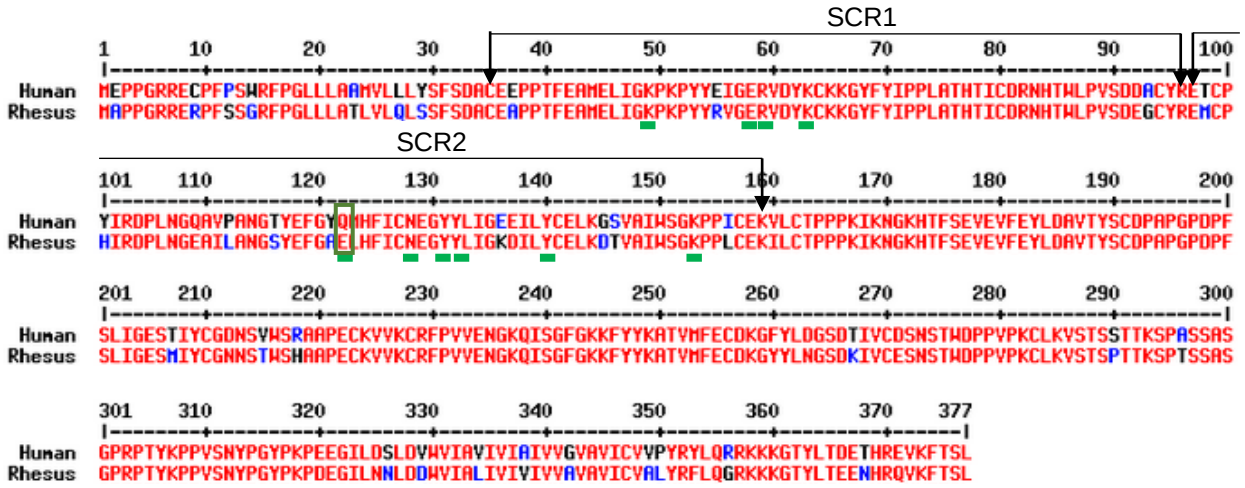



**Fig.S2. CD46 and DSG2 expression as well as *in vitro* transduction of rhesus HSCs. A)** Flow cytometry analyses. *Left panel:* Staining of NHP bone marrow mononuclear cells (MNCs) with CD46mAb M177-FITC (red curve). The blue curve shows unstained cells. *Middle panel:* Staining of NHP BM MNCs with polyclonal anti-human DSG2 antibody followed by a PE-labeled secondary antibody. The blue curve shows staining with the secondary antibody only. *Right panel*: Staining of NHP BM CD34$^+$ cells (isolated using the human CD34 MicroBead kit) with CD46 and DSG2 antibodies. **B)** Transduction of human and NHP CD34$^+$ cells with HDAd5/35++GFP and HDAd5/3+GFP at an MOI of 2000 vp/cell. GFP was analyzed 24 hours after adding the viruses. *: $p<0.05$

**CD46**




**DSG2**

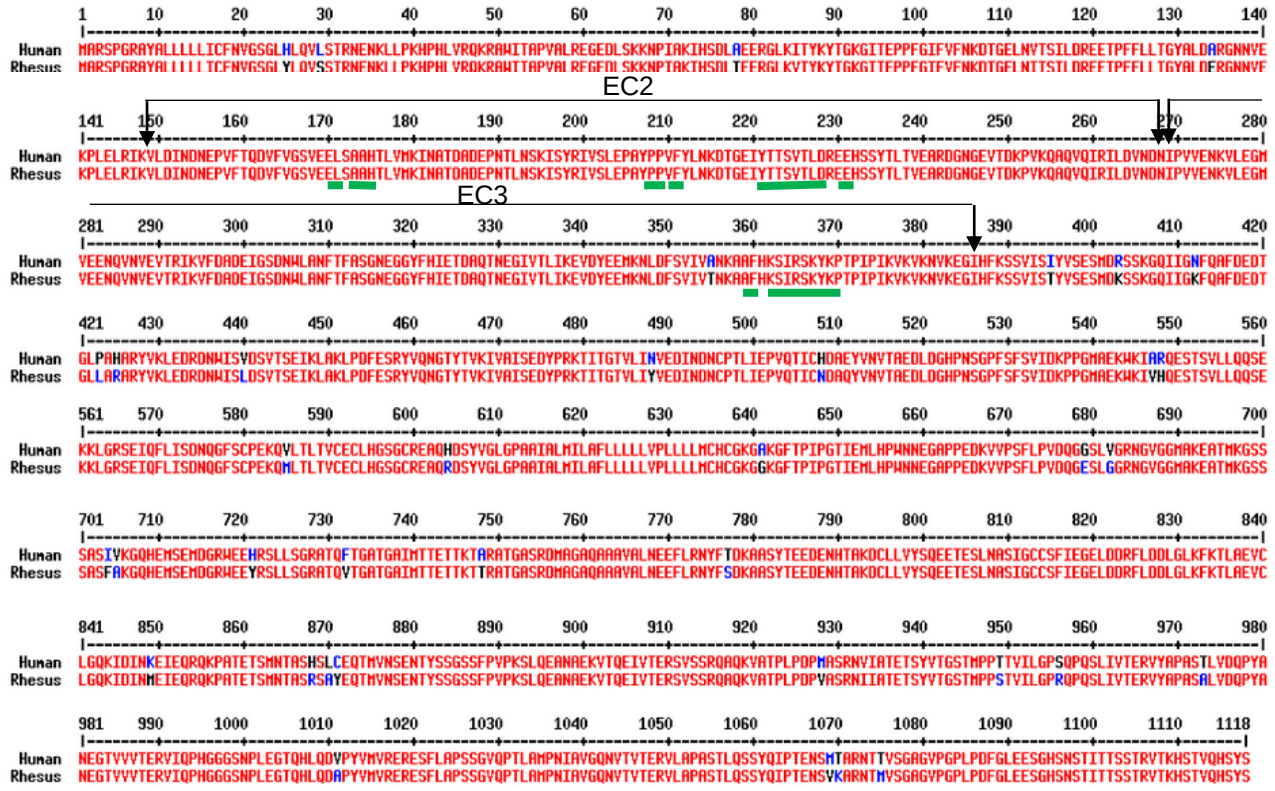



**Fig.S3 Amino acid sequence alignment of human and rhesus CD46 and DSG2**. The accession numbers were NP_758861 and NP_00182675 for human and rhesus CD46, respectively. The homology between the two proteins is 86%. CD46 residues that are critical in binding to the Ad35 fiber knob are underlined green 61. Notably, one of these critical residues is different between the species (see green box). The accession numbers were NP-001934 and XP_001098597 for human and rhesus DSG2, respectively. The homology between the two proteins is 96%. The Ad3 fiber interacting residues within DSG2 are underlined green. SCR1 and SCR2: extracellular short consensus repeats 1 and 2; EC2, EC3: extracellular domains 2 and 3.

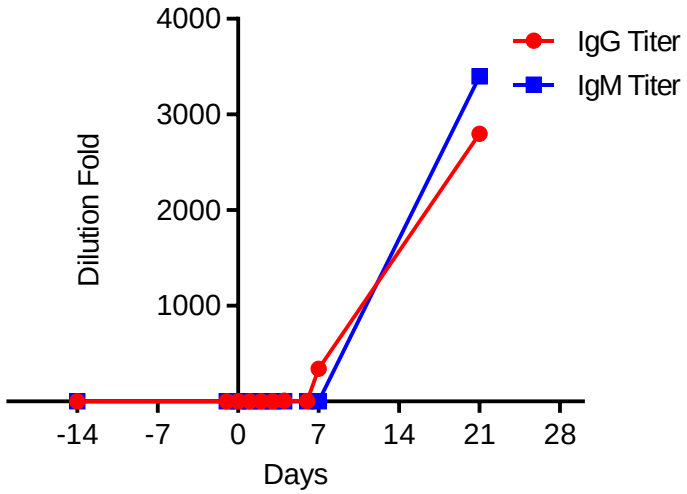



**Fig.S4. Anti-GFP binding IgM and IgG antibody titers in the animal injected with HDAd5/35++/GFP/cxcr4.** Shown are $IC_{50}$ values.

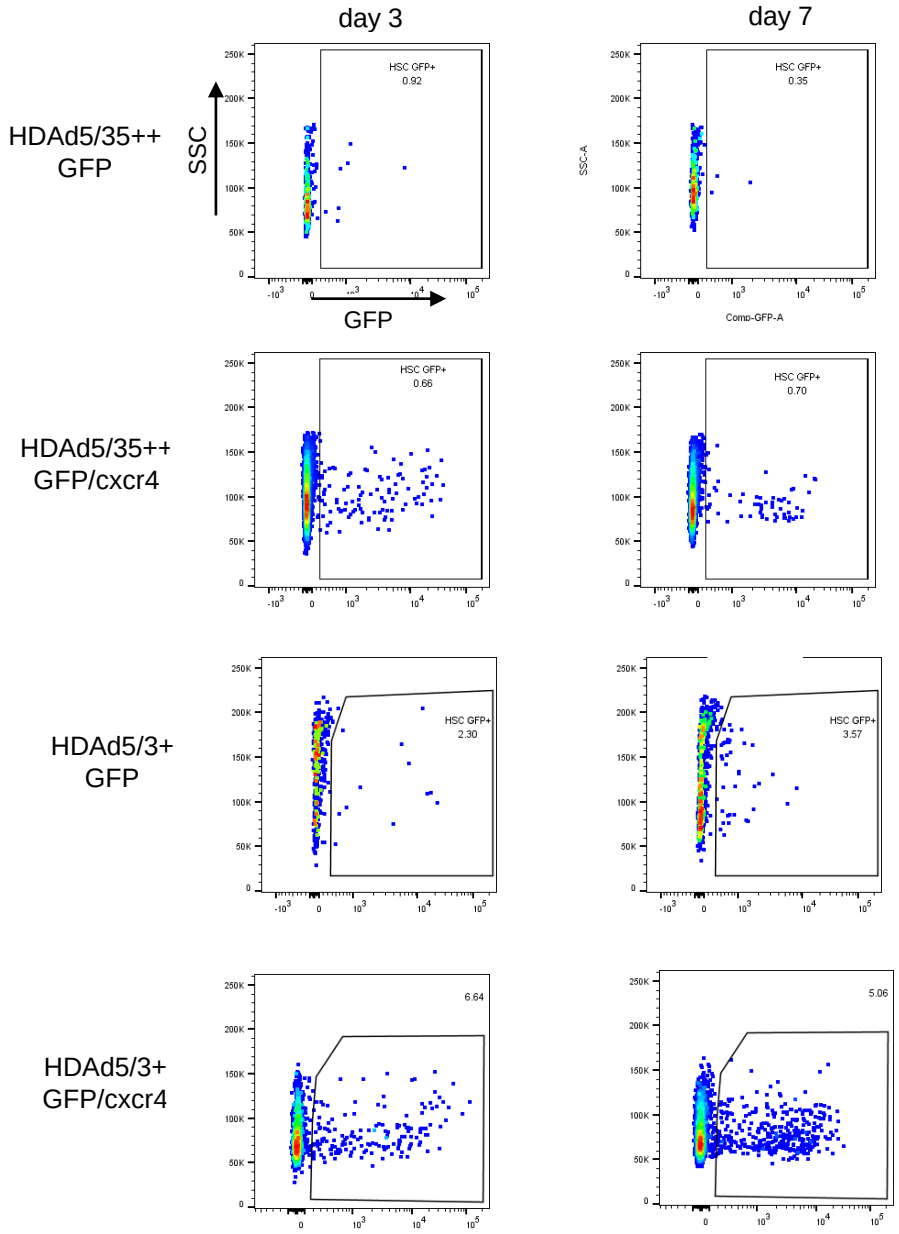



**Fig.S5. Representative GFP flow cytometry plots of data shown in Fig.5B** (after gating of $CD34^{+}/CD45RA^{-}/CD90^{+}$ cells).

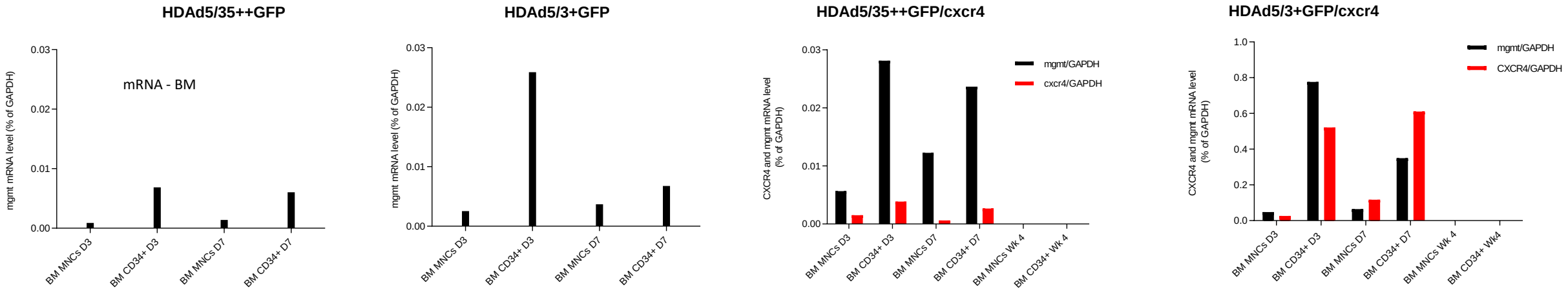
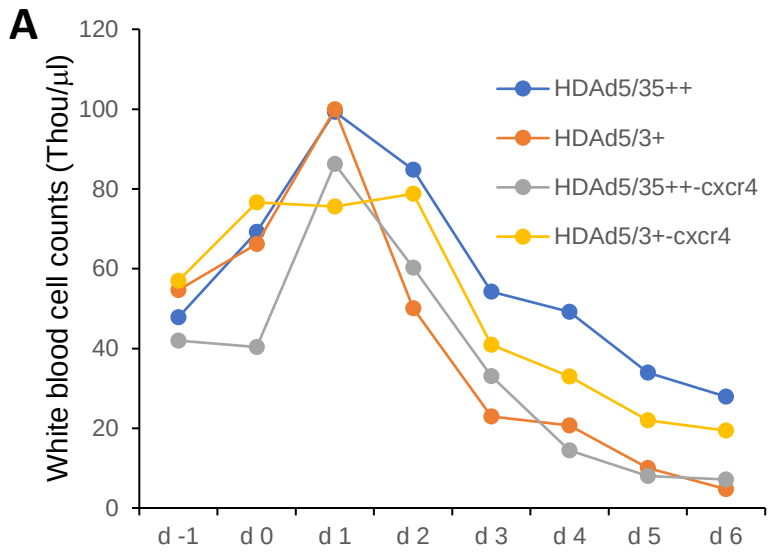



**Fig.S6. Analysis of transgene mRNA levels in BM MNCs and BM CD34⁺ cells.** mgmt$^{P140}$ and cxcr4 mRNA levels are shown relative to mRNA levels of the house-keeping gene GAPDH. Transgene mRNAs were not detectable in week 4 and 8 samples.



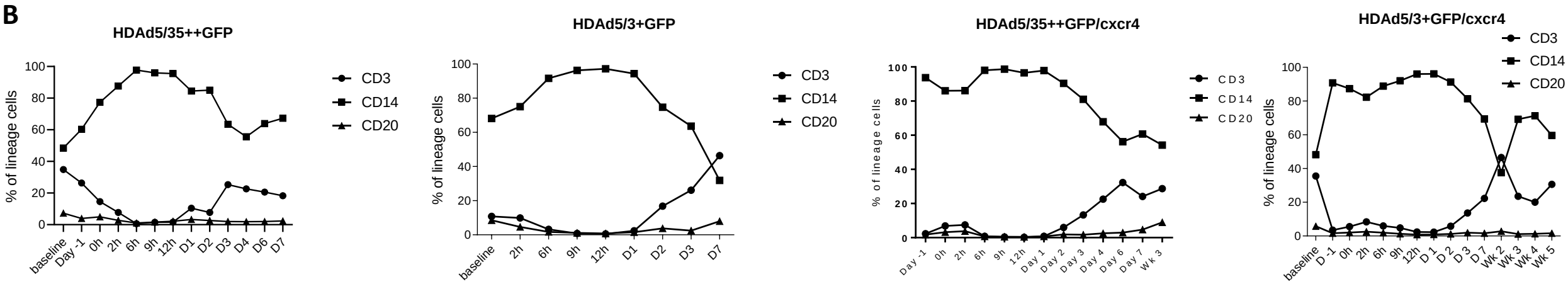




**Fig.S7. Effect of mobilization on white blood cells and blood cell lineages. A)** White blood cell counts. **B)** Percentage of $CD3^+$, $CD14^+$, and $CD20^+$ lineage-positive cells in peripheral blood. $CD3^+$, $CD14^+$, and $CD20^+$ cells are mostly T-cells, monocytes, and B-cells, respectively.

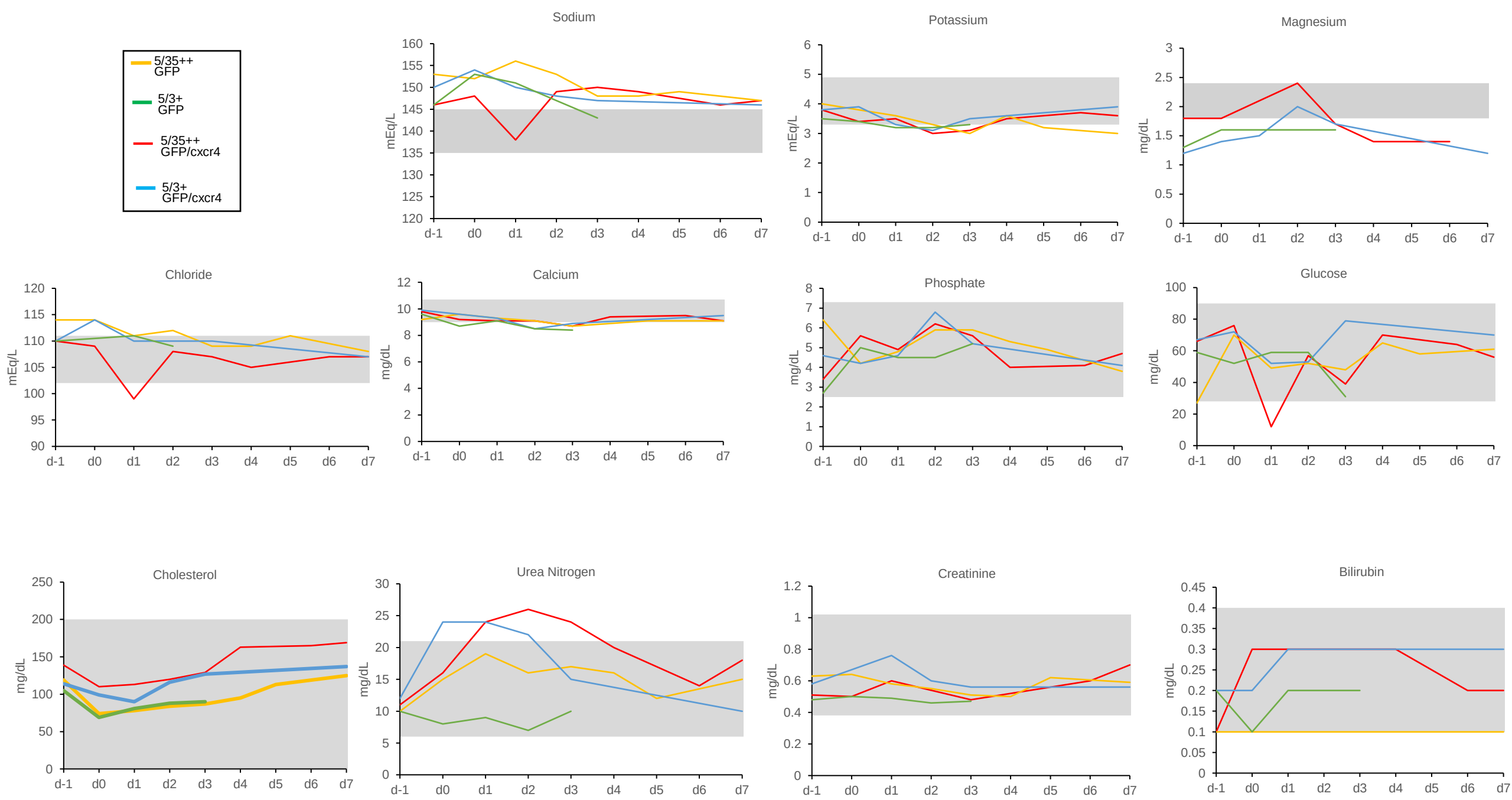



**Fig.S8. Hematological parameters.** The normal range is shown in grey shade.

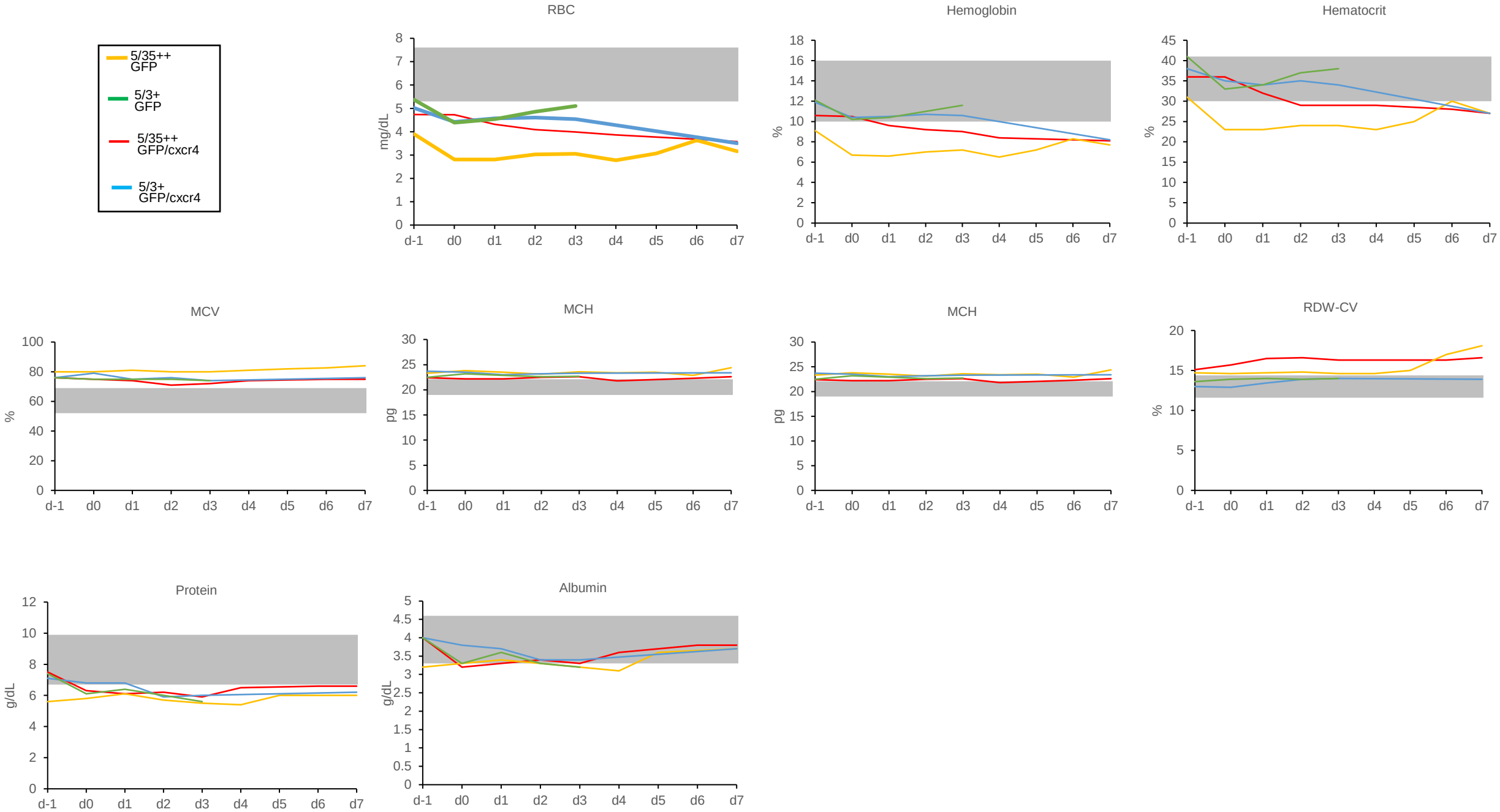



**Fig.S9. Hematological parameters.** The normal range is shown in grey shade.

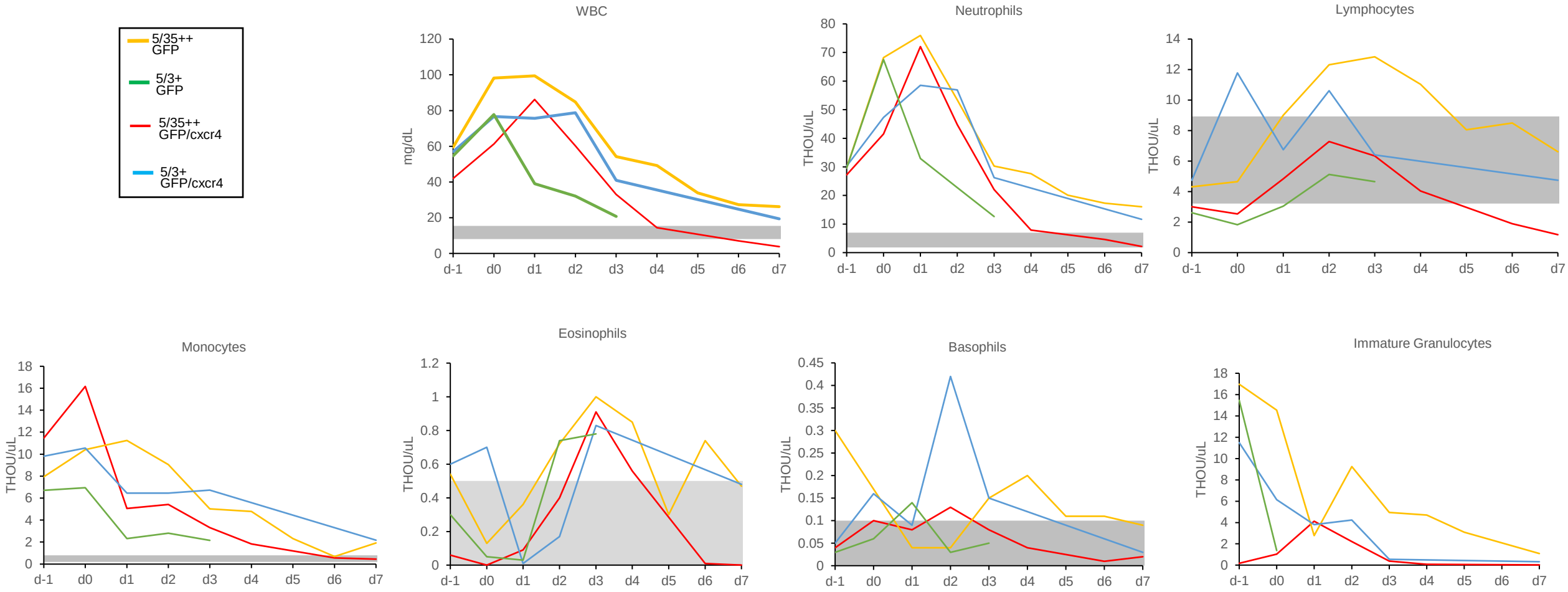



**Fig.S10. Hematological parameters.** Blood cell counts. The normal range is shown in grey shade.

**Suppl. Methods.**

***Adenovirus vectors****:* The Ad5/3+ helper vector was based on pHPBG [1], the sequence corresponding to the Ad5 fiber knob domain was replaced with the sequence encoding the affinity-enhanced Ad3 fiber knob domain [2] by homologous recombination in *Escherichia coli.* The resulting plasmid pHPBG(Ad5/3+) was digested by PacI and transfected into 293 cells. The helper virus Ad5/3+ was rescued, amplified, and purified by using standard Ad propagation and purification techniques. For the generation of the HDAd-PT4-mgmt/GFP vector, the DNA fragment containing the enhanced *Sleeping Beauty* transposon terminal inverted repeats (PT4 IRs) (sequence were based on the plasmid PT4/HB (Addgene, #108352)) flanked by FRT sites was synthesized by Genscript (Nanjing, PRC) and inserted into plasmid pUC57-Mini (Genscript) to generate PUC57-PT4. The EF1α-MGMT$^{p140k}$/GFP-SV40pA cassette was PCR amplified from vector HDAd-GFP/mgmt [3] and inserted into pUC57-PT4 between the two PT4 IRs to generate the corresponding plasmid PT4-EF1α-MGMT$^{p140k}$/GFP. The transposon cassette flanked by PT4 IRs and FRT sites was then PCR amplified and inserted into NheI-NheI site of pHCA using the In-Fusion HD cloning kit (Takara) to generate pHCA-PT4-mgmt/GFP vector. The CMV-CXCR4-pA fragment was PCR amplified from pcDNA3-CXCR4 (Addgene, #98942) and inserted into BstBI-SwaI sites of pHCA-PT4-mgmt/GFP (outside of transposon) by Gibson assembly (New England Biolabs) to generate pHCA-PT4-mgmt/GFP-CXCR4. The vector HDAd-PT0-mgmt/GFP was described previously [3].

***CD34$^+$ cell isolation and culture:*** Human CD34$^+$ cells from healthy G-CSF-mobilized adult donors were provided by the Fred Hutch Research Cell Bank Core. Rhesus CD34$^+$ cells were isolated using a human CD34 MicroBead kit from Miltenyi Biotech. Flow cytometry demonstrated that >98% of cells were CD34-positive. Cytokines and growth factors were from Peprotech (Rocky Hill, NJ). For *in vitro* studies, CD34$^+$ cells were cultured in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% heat-inactivated FCS, 1% BSA 0.1 mmol/l 2-mercaptoethanol, 4 mmol/l glutamine and penicillin/streptomycin, Flt3 ligand (Flt3L, 25 ng/ml), interleukin 3 (10 ng/ml), thrombopoietin (TPO) (2 ng/ml), and stem cell factor (SCF) (25 ng/ml).

***Red Blood Cell (RBC) competition:*** 3x10$^5$ 293 cells were plated per well. RBCs from 100μl human or rhesus EDTA-blood were washed with PBS and resuspended in 600μl DMEM/FCS. 100μl were mixed with HDAd vectors (MOI 500vp/cell) and added to cells for one hour with shaking. The supernatant was then aspirated, 293 cells were washed and incubated for 2 days. Transduction was measured by GFP flow cytometry.

***Gating of GFP-positive CD34$^+$/CD45RA$^-$/CD90$^+$ cells:*** The following antibodies were used. CD34APC (563 Clone): Cat# 561209 from BD; CD90 PE (5e10 Clone): Cat # 328110 from BioLegend; CD45RA APCH7 (5H9 Clone): Cat# 561212 from BDCD45NHP BV421 (D0581283 Clone): Cat# 561291 from BD.

***GFP immunofluorescence analysis on CD34$^+$ cells:*** CD34$^+$ cells were isolated from bone marrow mononuclear cells using MACS. After cytospin, 1x10$^5$ CD34$^+$ cells were airdried and fixed with 4% PFA/PBS for 15 mins, and after washing with PBS/0.1% Tween 20), permeabilized with 0.1% Triton-X100/PBS for 10 mins. Slides were then blocked with 2% non-fat dry milk /PBS for one hour followed by overnight incubation with anti-GFP-AF488 at a dilution of 1:500 (Thermo Fisher, A-21311) at 4°C. Washed cells were mounted with Vectashield + DAPI and photographs were taken with an EVOS M5000 system (Invitrogen).

***Measurement of vector copy number:*** Total DNA from bone marrow cells, PBMCs, or 10-20mg tissue was extracted using the Quick-DNA miniprep kit (Zymo Research). Viral DNA extracted from purified HDAd virus stocks was serially diluted and used for a standard curve. qPCR was conducted in triplicate using the power SYBR™ Green PCR master mix on a StepOnePlus real-time PCR system (Applied Biosystems). 9.6 ng DNA (9600 pg/6 pg/cell = ~1600 cells) was used for a 10 µL reaction. The following primer pairs were used: rhesus macaque GAPDH forward, 5'- aaggcagtgggagaaatgtg-3', and reverse, 5'- ttgattttggagggatctcg-3'; human $mgmt^{p140k}$ forward, 5'- tgagaggcaatcctgtcaag-3', and reverse, 5'- CAACCGGTGGCCTTCATGGG-3'.

***Real-time reverse transcription PCR:*** Total RNA was extracted from $5x10^6$ cells or 100µl blood by using TrIzol™ reagent (Thermo Fisher Scientific) following the manufacture's phenol-chloroform extraction method. Quantitect reverse transcription kit (Qiagen) and power SYBR™ green PCR master mix (Thermo Fisher Scientific) were used. Real time quantitative PCR was performed on a StepOnePlus real-time PCR system (AB Applied Biosystems). The following primer pairs were used: rhesus macaque GAPDH forward, 5'- atgttcgtcatgggtgtgaa-3', and reverse, 5'- gtcttctgggtggcagtgat-3'; human $mgmt^{p140k}$ forward, 5'- tgagaggcaatcctgtcaag-3', and reverse, 5'- CAACCGGTGGCCTTCATGGG-3'; Human cxcr4 forward, 5'- aagcgaggtggacattcatc-3', and reverse, 5'- caacagatggctggcaacta -3'.

***Cytometric Bead Array:*** The NHP Th1/Th2 cytokine CBA kit (BD Biosciences 557800) was used to measure serum levels for IL-2, IL-4, IL-5, IL-6, TNF, IFNγ.

***Anti-HDAd and anti-GFP serum antibodies:*** Plates were coated with recombinant GFP (0.3µg per well in 0.1M Na-bicarbonate buffer pH9.6, o/n at 4°C) or $5x10^8$ vp/well of the corresponding HDAd5/35++ or HDAd5/3+ vector. Serial serum dilutions (starting at 1:50, 3x subsequent dilutions) were added for 1 hour. After washing, HRP-conjugated secondary antibodies against NHP IgG or IgM (Invitrogen PA1-84631 and 62-6820, respectively) were used at 1:10,000 dilution for 1 hour. After washing, Thermo 1-Step Ultra TMB Solution (ThermoFisher Scientific) was added, and the color was allowed to develop for 7 minutes before stopping with 2N sulfuric acid. Absorbance readings at 450 nm were taken with a plate reader. Antibody reactivity curves were plotted with Graphpad Prism with a 4-parameter curve, and EC50 values were accordingly calculated.

1. Shayakhmetov DM, Li ZY, Gaggar A, et al. Genome size and structure determine efficiency of postinternalization steps and gene transfer of capsid-modified adenovirus vectors in a cell-type-specific manner. *J Virol*. 2004;78(18):10009-10022.
2. Wang H, Yumul R, Cao H, et al. Structural and functional studies on the interaction of adenovirus fiber knobs and desmoglein 2. *J Virol*. 2013;87(21):11346-11362.
3. Wang H, Richter M, Psatha N, et al. A Combined In Vivo HSC Transduction/Selection Approach Results in Efficient and Stable Gene Expression in Peripheral Blood Cells in Mice. *Mol Ther Methods Clin Dev*. 2018;8:52-64.