Supplemental Methods

Gene Transfer Vector

The AAVrh.10hARSA viral vector was produced under cGMP-like conditions, by co-transfection of 2 plasmids, one coding for the therapeutic gene (pAAV2-CAG-hARSA) and the other providing AAV replication and capsid functions plus the adenoviral helper functions (pPAK-MArh.10) into a stable human embryonic kidney cell line (293T) in the presence of PEI reagent (Polysciences, Warrington, PA). *pAAV2-CAG-hARSA.* The pAAV2-CAG-hARSA plasmid is comprised of an AAV2 gene transfer vector backbone (with inverted terminal repeats (ITR) of AAV2) flanking an expression cassette consisting of the human cytomegalovirus enhancer (CMV)/promoter, splice donor/left hand intron sequence from chicken β-actin, right hand intron sequence/splice acceptor from rabbit β-globin (this enhancer/promoter/intron sequence is referred to as "CAG"), the human ARSA cDNA (amino acid 507 isoform); rabbit β-globin polyA, as previously described[1] (Supplemental Figure 1). The resulting pAAV2-CAG-hARSA plasmid was characterized by restriction mapping and sequencing.

The *pPAK-MArh.10* helper plasmid for production of recombinant AAVrh.10 vectors provides all of the necessary Ad and AAV helper functions. The rep gene is derived from AAV2 and therefore it is used in pseudotyping strategies where an AAV2 derived genome is packaged in the capsid of AAVrh.10. There is a fusion of the AAV2 and AAVrh.10 genomes such that the cap genes are expressed by the p40 promoter of AAV[2]. The two plasmids (500 μg pAAV2-CAG-hARSA and 1 mg pPAK-MArh.10) were added to 293T cells in the presence of PolyFect® reagent (Qiagen Sciences, Germantown, MD) for 72 hr. The resulting AAVrh.10hARSA vector was purified by differential density using an iodixanol gradient[1, 3-5]. The final solution was sterile filtered through a 0.22 μm membrane filter dispensed at prescribed volumes into cryovials and

frozen at -80°C until day of surgery. All vector lots were tested for sterility, mycoplasma and endotoxin, as previously described[1, 3, 5].

The AAVrh.10Luc vector used for *in vitro* anti-AAVrh.10 assays was produced following the same protocol as described previously[5, 6]. The AAVrh.10Luc vector was propagated, purified, and stored as described above.

The AAVrh.10hARSA and AAVrh.10Luc vectors were titered for genome copies by quantitative polymerase chain reaction (PCR) using TaqMan-based analysis reagents (Applied Biosystems, Foster City, CA) and a CAG-specific primer–probe set to amplify the vector promoter CAG region (forward primer: GTCAATGGGTGGAGTATTTACGG and reverse primer: AGGTCATGTACTGGGCATAATGC, designed using Primer Express software (Applied Biosystems)[5]. Acceptance criteria for the assay includes specifications for the correlation coefficient (R^2) of the standard curve, the reaction efficiency as measured by the slope of the standard curve and the positive and negative controls. For the QC DNA samples, the copy numbers should have a percent relative error of <20% with a replicate coefficient of variation of ≤2%[5, 7].

Nonhuman Primate CNS Imaging Scans

All CNS imaging was performed at the Citigroup Biomedical Imaging Center (CBIC), Weill Cornell Medical College. In order to determine the precise location of burr hole placement and depth of catheter tip necessary for targeting the intraparenchymal regions with vector administration, each NHP was imaged by magnetic resonance imaging (MRI) and computerized axial tomography (CT) of the brain prior to surgery to map their brains and to obtain baseline images for safety studies. NHP were anesthetized with ketamine (5-10 mg/kg) plus dexmedetomidine (0.015–0.02 mg/kg) given intramuscular (IM, quadriceps), endotracheally intubated, and maintained on gas anesthesia (isoflurane, 1-3 %) during the procedure, and

provided with fluids through a catheter inserted into a saphenous vein. The head was mounted in a MRI-compatible stereotaxic frame (David Kopf Instruments, Tujunga, CA) using ear bars, a palette clamp, and ventral orbit clamps. The stereotaxic frame was the same device that is used during surgery. With the monkey's head mounted in the stereotaxic frame, the monkey and frame were slid together into a send/receive head MRI coil used for human imaging (Magnetom Trio, 3.0 Tesla machine; Siemens Healthcare, Malvern, PA). Once a number of volumetric scans (T1 and T2 imaging) were acquired, the NHP was removed from the MRI machine and transferred, still attached to head frame, to a CT machine for computed tomography (Biograph mCT machine; Siemens Healthcare) to generate a more complete image of the NHP brain and skull. The MRI and CT images were subsequently aligned and fused using OsiriX-MD software (Pixmeo SARL; Bernex, SWZ) for each individual NHP to generate a 3-D map of the brain[1]. Stereotaxic coordinates of target sites (in semiovale white matter) were determined prior to surgery to align the manipulator arms correctly over the NHP skulls for accurate placement of catheters used to deliver the AAV vectors.

Stereotaxic Administrations of AAVrh.10hARSA

Each animal was anesthetized as described previously[1, 5, 8]. Each monkey received continuous fluids via an intravenous line kept open with 0.9% saline, and an endotracheal tube for isoflurane (1-3%). The animals were positioned in Kopf stereotaxic frame with bilateral ear bars and mouth bite plate in the same position as recorded from the MRI session. The scalp was shaved with electric razor and area was prepped and draped in sterile fashion. Bupivacaine (0.25% solution) was injected subdermal at the intended midline incision site 5 minutes before surgical incision. A 4 to 6 cm midline incision was made and the scalp was retracted using small fishhooks to expose the craniotomy sites on the calvarium. Sterilized manipulators were attached to stereotaxic frame and burr hole sites marked using sterile marking pen at predetermined

coordinates obtained from the MRI/CT scan. Anterior/posterior (A/P) measurements were from the ear bar set at 0.0 mm. Midline (M/L) measurements were from the central coronal suture. Burr holes were made corresponding to the predetermined tract for administration sites using a sterile 3 mm drill bit. To guide the micro-catheters into the cortex, 22G x 3.5" Quincke spinal needles (Becton Dickinson, Franklin Lakes, NJ) were clamped to each stereotaxic manipulator arm. The vector pre-loaded microcapillary catheter[1] was threaded down the spinal needles to pre-determined depth coordinates and secured with surgical tape. The vector lines were flushed with small amounts of vector to ascertain the lines were clear prior to lowering the catheters into the brain. The targets were calculated by setting the visualized pia dural measurement as 0 mm. All stereotaxic arms were set at 0 degrees rotation for this study. Each NHP had two catheters inserted into the targeted regions at one time (in left and right hemispheres). Infusions began 2 min after the catheters were lowered to the calculated target site, and following the injection, a similar timed delay of 2 minutes was performed before the catheters were moved for the next deposit. Administration of AAV vectors in this study were made to brain parenchyma white matter, as two deposits per catheter track to maximize vector spread, ventrally (-10 mm) first and then the catheter raised to the overlaying white matter (-5 mm). The catheters were withdrawn at a rate of 0.5 mm/min and manipulator arms reset for the next set of targets. The target dose was delivered at a constant infusion rate of 1 μl/min to minimize infusion related damage. Stereotaxic coordinates are listed based on ear bar (A/P = 0 mm) and central coronal suture (M/L = 0). Typically, the first target site was at A/P +23 mm, M/L -10 mm, D/V at -10 & -5 mm; the second site at A/P +6 mm, M/L -10 mm, D/V at -10 and -5 mm; and the third site at A/P -6 mm, M/L -10 mm, D/V at -10 and -5 mm. Same coordinates were used for both hemispheres to deliver the vector bilaterally. As sham controls, NHP received the same surgery with PBS (15 μl per site) replacing the AAVrh.10hARSA vector; all criteria, assessments and timeline were the same as

the vector-treated animals. The NHP was then removed from stereotaxic frame and placed in the recovery position until awake. Post-operative analgesia with carprofen [4.0 mg/kg, subcutaneous (s.c.)] and buprenex (0.6 ml, s.c. into scalp around incision) was administered when breathing and reflexes are stable, and then the monkey was recovered from the anesthetic. The NHP were carefully laid back in their home cage in the recovery position (on the left side) and closely monitored while recovering from anesthesia. A neurological exam was carried out following full recovery from sedation, assessing paralysis by monitoring the NHP's muscle movements on both sides, use of all extremities for climbing, facial muscle usage (yawning, chewing), eye reactivity, and ability to stand from recovery position (on left side). All monkeys recovered well from surgery and exhibited normal behavior without any apparent adverse effects up to the time of sacrifice.

Necropsy Sample Collection

A subset of organs (brain, thymus, liver, heart, lung, kidney, adrenals, thyroid/parathyroid, pituitary, spleen, testes/epididymides, and ovaries/oviducts), and any other organs with gross abnormalities discovered during the examination, were weighed for comparisons of untreated *vs* treated animals. Sections of multiple organs (detailed in Supplemental Table II) were collected at necropsy and fixed in 10% neutral buffered formalin for histopathological analysis. In addition to those listed organs, the calvaria was retained for examination of the inferior aspect for pathology around burr holes and pia mater.

For collection of the CNS samples, the brain was excised from the skull, weighed, and divided into two hemispheres for analysis. The left hemisphere was fixed in 10% neutral-buffered formalin for 2 wk, and sectioned into 3 mm coronal sections for histopathology, with multiple sections, especially those around the administration sites, assessed for pathological pathologic findings or infiltrates. The right hemisphere was processed during necropsy by

sectioning into 1 cm coronal slabs, flash-frozen in liquid nitrogen, and stored at -80 ˚C for later assessment. The spinal cord was extracted at 3 levels from each NHP: cervical C3-C4, thoracic T6-T7, and lumbar L2-L4 and placed in 10% neutral buffered formalin for histopathology (hematoxylin and eosin [H&E] and immunohistochemistry stains).

Pathologic Examination

Sections of organs collected at necropsy were processed routinely in alcohol and xylene, embedded in paraffin, sectioned at a 5-micron thickness, stained with H&E. After heat-induced epitope retrieval in a buffer at pH 9.0 (for CD3) or 6.0 (for CD20 and CD68), the slides were stained by immunohistochemistry for CD68 (microglial cells and monocyte-derived macrophages), CD3 (T cells), and CD20 (B cells). Antibodies used for IHC included: rabbit anti-human CD3 (1:100 dilution, Vector Laboratories VP-RM01); mouse anti-human CD20 (1:1000 dilution, Dako M0755); and mouse anti-human CD68 (1:5000 dilution, Dako M0814). Secondary detection antibodies (anti-mouse, or -rabbit) were biotinylated and used at 1:500 dilution (Vector Labs, BA-2000, BA-1000), and followed by an avidin-biotin detection system (Vectastain Elite ABC-HRP Kit, Vector Labs, PK-6100). The chromogen used was 3,3'-diaminobenzidine (DAB; Sigma) and counterstain was hematoxylin. IHC slides were scanned digitally, using a Mirax scanner (Pannoramic MIDI, 3DHistech, Budapest, HUN) at 20X magnification (0.50 μm/pixel), and analyzed with the Pannoramic Viewer (version 1.15.3; 3DHistech) (Aperio/Leica Microsystems). IHC stained sections were assessed and score qualitatively as follows. For CD3 and CD201 (minimal): 0: No positive cells; 1: Rare positive cells, without perivascular cuffing; 2 (mild): Small number of positive cells, without perivascular cuffing; 3 (moderate): Moderate number of positive cells, with thin perivascular cuffs; 4 (marked): High number of positive cells, with thick perivascular cuffs. For CD68: 0: No positive cells; 1 (minimal): Small number of positive cells with a round morphology and abundant

cytoplasm (consistent with activated macrophages or microglial cells); 2 (mild): Moderate number of positive cells with a round morphology and abundant cytoplasm; 3 (moderate): Large number of positive cells with a round morphology and abundant cytoplasm, without effacement of normal brain tissue; 4 (marked): Large number of positive cells with a round morphology and abundant cytoplasm, extending over a large area and with elimination of normal brain tissue.

Supplemental References

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Supplemental Table I. Quantitative Assessment of Nonhuman Primate Behavior Following Vector Administration[1]

Summary score	Behaviors
Individual score[2]	
Anxiety score	Chewing, penile erection, scratching, yawning, threaten outside
Arousal score	Bipedal lookout, shift, tail flag, vertical climb, vocalization
Quiet behaviors score	Cage pick, self-groom, eating, drinking
Sedation score	Eyes closed, freeze (motionless), face-down
Abnormal motions score	Stereotypy, dyskinesia, dystonia
Healthy sum[3]	Sum of behaviors that relate to overall good health of the nonhuman primate which include anxiety, arousal and quiet behaviors
Abnormal sum[4]	Sum of behaviors that relate to overall poor health of the nonhuman primate which include sedation and abnormal motions

[1] Behavioral assessment were done pre-vector administration and on days 0, 7, 14, 28, 56, 91, 182, and 365 post-vector administration (± 1-3 days), prior to sedation for blood draws.

[2] Nonhuman primate behavior (normal and abnormal) described in this chart were visually assessed by observers blinded to treatment and route from the videotape sessions of primates in individual home cages in the absence of stimuli. The primates were given a score of "1" for 5 sec of each specific normal and abnormal behavior. The totals for each behavior were determined for 3 min assessment session, and assigned into the anxiety, arousal, quiet behavior, sedation, and abnormal motion categories as described above.

[3] The sum of normal typical primate behaviors (anxiety, arousal, and quiet behaviors) was calculated as the "Healthy" score for each session.

[4] The sum of abnormal behaviors (sedation and abnormal motions) was calculated as the "Abnormal" score for each session.

Supplemental Table II. Organs and Tissues Harvested from Nonhuman Primates for Gross and Histopathology Assessment[1]

Adrenal glands[2]	Ovaries[2]
Aorta (ascending)	Oviducts
Bone and bone marrow[3]	Pancreas
Brain (with olfactory bulb)[2]	Parathyroids
Calvaria (skullcap)	Pituitary gland
Cecum	Prostate gland
Cervix	Rectum
Colon	Salivary glands[2,9]
Diaphragm	Sciatic nerve
Duodenum	Seminal vesicles[2]
Epididymis[2]	Skeletal muscle[2,10]
Esophagus	Skin[2,10]
Eyes[2,4]	Spinal cord[2,12]
Facial nerves[2,5]	Spleen[2]
Gallbladder	Stomach[13]
Heart (with pericardium)[2,6]	Testes[2]
Ileum[2]	Thymus
Jejunum	Thyroid gland
Kidneys[2]	Trachea[14]
Liver[2]	Urinary bladder
Lungs (with main stem bronchi)[2,7]	Uterus
Lymph nodes[2,8]	Vagina
Mammary glands (with thoracic skin)	Any gross abnormalities

[1] All tissues assessed for gross pathologic findings
[2] Tissues weighed and samples of each to be stored frozen (unfixed)
[3] Section from sternum
[4] Both eyes, with lacrimal glands and optic nerve
[5] Glossopharyngeal and facial nerves
[6] Heart sampled in 2 locations: Right ventricular and atrial wall with tricuspid valve, and left ventricular and atrial wall with mitral valve; portion of left ventricular wall (apex) saved frozen
[7] Lung sampled from 5 lobes for fixation, 1 from each site: Right cranial, middle, and caudal lobes, Left cranial and caudal lobes; only 1 portion of right cranial lobe saved for frozen sample
[8] Tracheobronchial, mesenteric, popliteal and inguinal
[9] Parotid and submandibular glands
[10] Biceps (from biceps femoris muscle)
[11] From the thigh, with subcutis
[12] Cervical, thoracic, lumbar – 1 sample from each level
[13] Fundus and pylorus samples
[14] Anterior and carina (taken with esophagus)

Supplemental Figure Legends

Supplemental Figure 1. AAVrh.10hARSA and target sites for vector administration. Shown is the structure of the AAVrh.10hARSA vector expression cassette. The AAVrh.10hARSA vector genome consists of the two inverted terminal repeats of AAV serotype 2, encapsidation signal (Ψ) and the expression cassette. The expression cassette comprises the CAG promoter (that includes the human cytomegalovirus (CMV) enhancer; the chicken β-actin promoter / splice donor and 5' end of intron; the 3' end of the rabbit β-globin intron and splice acceptor), the normal human ARSA cDNA with a Kozak sequence, and the polyadenylation / transcription stop signal from rabbit β-globin. This was packaged in an AAVrh.10 capsid. The AAVrh.10hARSA vector was administered to the CNS of nonhuman primates. 24 African Green monkeys (13 females and 11 males) were administered AAVrh.10hARSA or controls to the brain via catheters to deliver vector to three sites at 2 depths bilaterally (6 sites, 12 locations total) in the white matter centrum ovale at 2 doses: low total dose [$2.85x10^{10}$ genome copies (gc)], equivalent to human clinical dose of $2.85x10^{11}$ gc (previously used via a similar route of administration in children[9, 10]) and high total dose ($1.5x10^{12}$ gc), a 1.7-log higher dose, equivalent to a human dose of $1.5x10^{13}$ gc, with AAVrh.10Null and PBS used as controls. Sites A and B correspond to white matter deposits in the primate frontal lobe (anterior and posterior); site C corresponds to the deposits in the parietal lobe. The schematic brain drawings indicate the approximate site of the vector deposits, site A (red line), site B (green line), and site C (blue line). NHP of both sexes were randomly assigned to the 4 cohorts. The low and high dose AAVrh.10hARSA cohorts included 4M/4F, the AAVrh.10Null cohort 3F/1M, and the PBS sham control cohort 2F/2M.

Supplemental Figure 2. Effect of CNS administration of AAVrh.10hARSA on body weight. Twenty-four African green monkeys were assessed for general health parameters at regular intervals. At indicated time, the NHP were sedated with assessment of general safety parameters

including temperature, pulse, respiratory rate and body weight (kg). NHP were assessed at 9 time points, >1 wk prior to surgery (pre), on the day of administration (day 0), and on wk 1, 2, 4, 8, 13, 26, and 52. Times for assessment up to 2 wk were ± 1 d and times over 2 wk were ± 3 d. Shown is the weight (kg) of each NHP in the study, color-coded by treatment (yellow, PBS; red, AAVrh.10Null; blue, AAVrh.10hARSA-high dose; green, AAVrh.10hARSA-low dose). **A.** Males (squares, n=11); **B.** Females (circles, n=13). Note that a subset of each group was euthanized for histopathology at 1, 13, 26 and 52 wk, and therefore the number of measurements decreased with time. The normal range, shown as the gray-shaded area, for each parameter was calculated separately per sex as the mean ± 2X standard deviation of body weight of monkeys in the study at the 2 pre-surgery time points.

Supplemental Figure 3. Effect of CNS administration of AAVrh.10hARSA on serum chemistry parameters. Twenty-four African green monkeys were assessed for multiple serum chemistries parameters at regular intervals. At indicated time, the NHP were sedated for blood sampling with assessment of serum chemistries. Health surveillance was monitored for all monkeys pre- and post-surgery by monitoring their blood to evaluate whether the brain surgeries had any long-term effect on the health of the monkeys. NHP were assessed at 9 time points, >1 wk prior to surgery (pre), on the day of administration (day 0), and on wk 1, 2, 4, 8, 13, 26, and 52. Times for assessment up to 2 wk were ± 1 d and times over 2 wk were ± 3 d. **A-DD.** Results of 30 serum chemistry parameters for each animal in the study, color-coded by treatment (yellow, PBS; red, AAVrh.10Null; blue, AAVrh.10hARSA-high dose; brown, AAVrh.10hARSA-low dose). **A.** Blood urea nitrogen (BUN); **B.** creatinine; **C.** BUN/creatinine ratio; **D.** alkaline phosphatase (ALP); **E.** alanine aminotransferase (ALT); **F.** aspartate aminotransferase (AST); **G.** lactate dehydrogenase (LDH); **H.** gamma glutamyl transferase (GGT); **I.** total bilirubin; **J.** direct bilirubin; **K.** indirect bilirubin; **L.** total protein; **M.** albumin; **N.** globulin; **O.** albumin/globulin

ratio; **P.** phosphorus; **Q.** calcium; **R.** glucose; **S.** cholesterol; **T.** triglyceride; **U.** creatine kinase; **V.** total bicarbonate; **W.** amylase; **X.** lipase; **Y.** magnesium; **Z.** sodium; **AA.** potassium; **BB.** chloride; **CC.** sodium/potassium ratio; and **DD.** anion gap. The values for each parameter were plotted for individual animals with daily mean shown by long black line. Males (right, squares, n=11); females (left, circles, n=13). Note that a subset of each group was euthanized for histopathology at 1, 13, 26 and 52 wk, and therefore the number of measurements decreased with time. The normal range, shown as the gray-shaded area, for each parameter was calculated separately per sex as the mean ± 2X standard deviation of each serum parameter of monkeys in the study at the 2 pre-surgery time points.

Supplemental Figure 4. Effect of direct CNS administration of AAVrh.10hARSA and surgical treatment on nonhuman primate hematology. Twenty-four African green monkeys were assessed for complete blood count parameters at regular intervals. At indicated time, the NHP were sedated for blood sampling with assessment of blood counts. Health surveillance was monitored for all monkeys pre- and post-surgery by monitoring their blood to evaluate whether the brain surgeries had any long-term effect on the health of the monkeys. NHP were assessed at 9 time points, >1 wk prior to surgery (pre), on the day of administration (day 0), and on wk 1, 2, 4, 8, 13, 26, and 52. Times for assessment up to 2 wk were ± 1 d and times over 2 wk were ± 3 d. **A-T.** Results of 20 hematologic parameters for each NHP in the study, color-coded by treatment (yellow, PBS; red, AAVrh.10Null; blue, AAVrh.10hARSA-high dose; brown, AAVrh.10hARSA-low dose). **A.** Red blood cells (RBC); **B.** hemoglobin; **C.** hematocrit; **D.** mean corpuscular volume (MCV); **E.** mean corpuscular hemoglobin (MCH); **F.** mean corpuscular hemoglobin concentration (MCHC); **G.** reticulocytes; **H.** reticulocytes (%); **I.** platelets; **J.** white blood cells (WBC); **K.** neutrophils; **L.** lymphocytes; **M.** monocytes; **N.** eosinophils; **O.** basophils; **P.** neutrophils (% of total cells); **Q.** lymphocytes (%); **R.** monocytes (%); **S.**

eosinophils (%); and **T.** basophils (%). The values for selected parameters are plotted for individual animals with daily mean shown by long black line. Males (right, squares, n=11); females (left, circles, n=13). Note that a subset of each group was euthanized for histopathology at 1, 13, 26 and 52 wk, and therefore the number of measurements decreased with time. The normal range, shown as the gray-shaded area, for each parameter was calculated separately per sex as the mean ± 2X standard deviation of each parameter in the study at the 2 pre-surgery time points.

Supplemental Figure 5. Assessment of nonhuman primate behavior for changes in healthy behavior attributable to direct CNS administration of AAVrh.10hARSA and/or surgery. Twenty-four African green monkeys were assessed for normal and abnormal behavior parameters at regular intervals, pre- and post-surgery. At indicated time, the NHP were videotaped for assessment of animal behaviors. Health surveillance was monitored for all monkeys pre- and post-surgery by monitoring their activity levels and actions to evaluate whether the brain surgeries had any long-term effect on the health of the monkeys. NHP were assessed at 9 time points, >1 wk prior to surgery (pre), on the day of administration (day 0), and on wk 1, 2, 4, 8, 13, 26, and 52. Times for assessment up to 2 wk were ± 1 d and times over 2 wk were ± 3 d. Shown are the results of normal behavior ("Healthy scores") for each NHP in the study, color-coded by treatment (yellow, PBS; red, AAVrh.10Null; blue, AAVrh.10hARSA-high dose; brown, AAVrh.10hARSA-low dose). **A.** Females (circles, n=13); **B.** Males (squares, n=11). Note that a subset of each group was euthanized for histopathology at 1, 13, 26 and 52 wk, and therefore the number of measurements decreased with time. Each animal was videotaped for 3 min in the absence of outside stimuli and subsequently analyzed by 2 blinded observers for 20 specific primate behaviors (normal, n=14 and abnormal, n=6), scoring "1" for 5 sec of each behavior. The sum of normal typical primate behaviors (anxiety, arousal, and quiet groups) was

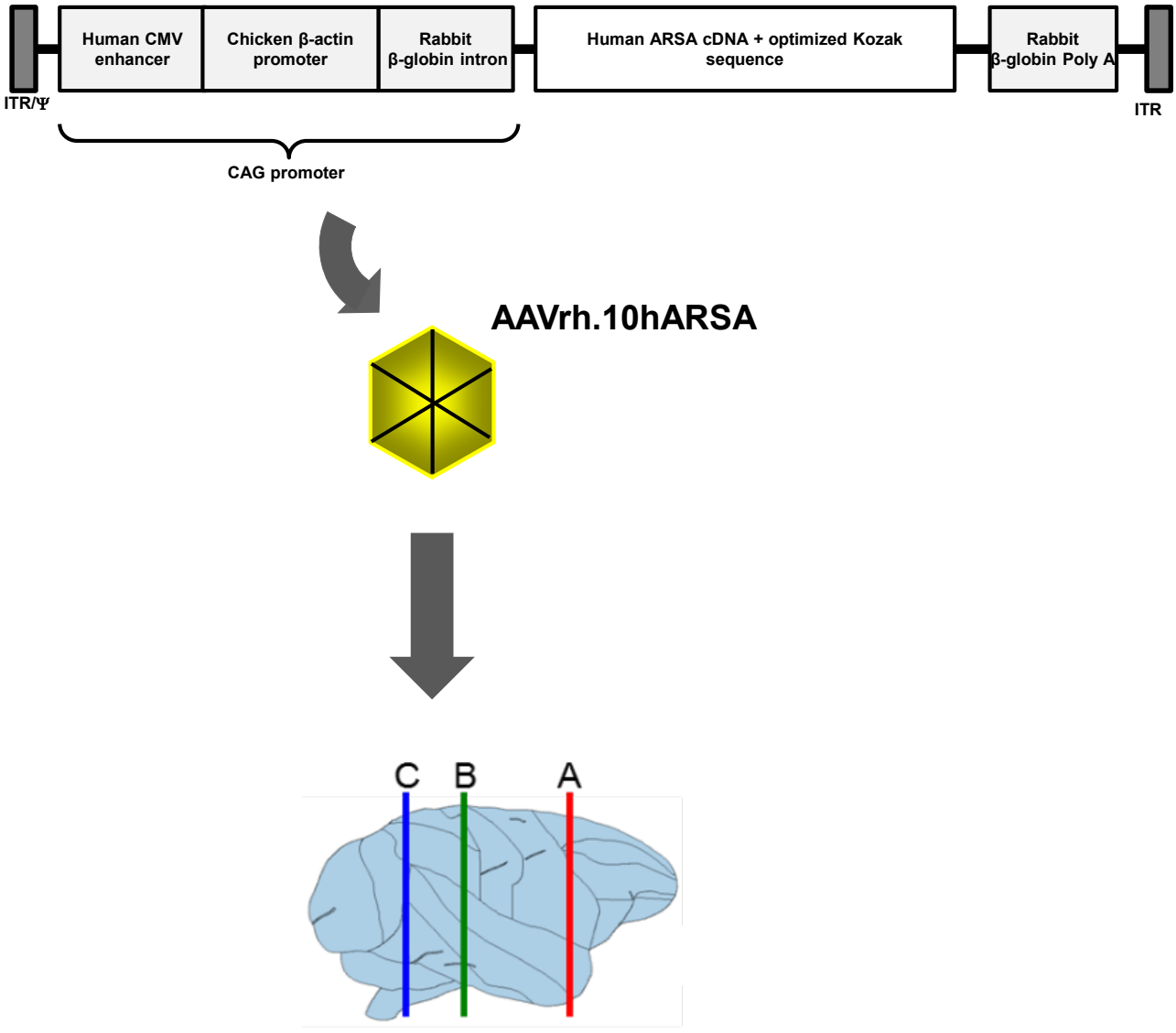
calculated as a "Healthy" score (defined in Supplemental Table I) for each session and plotted as the mean ± standard deviation as a function of time. The normal range, shown as the gray-shaded area, of Healthy scores was calculated separately per sex as the mean ± 2X standard deviation of behavior sums at the 2 pre-surgery time points. No difference was seen between the PBS *vs* AAV-treated NHP for males or females (1-way ANOVA with Bonferroni's multiple comparisons test): PBS *vs* high dose AAVrh.10hARSA, $p>0.9$; PBS *vs* low dose AAVrh.10hARSA, $p>0.3$; PBS *vs* high dose AAVrh.10Null, $p>0.9$; overall $p>0.3$.

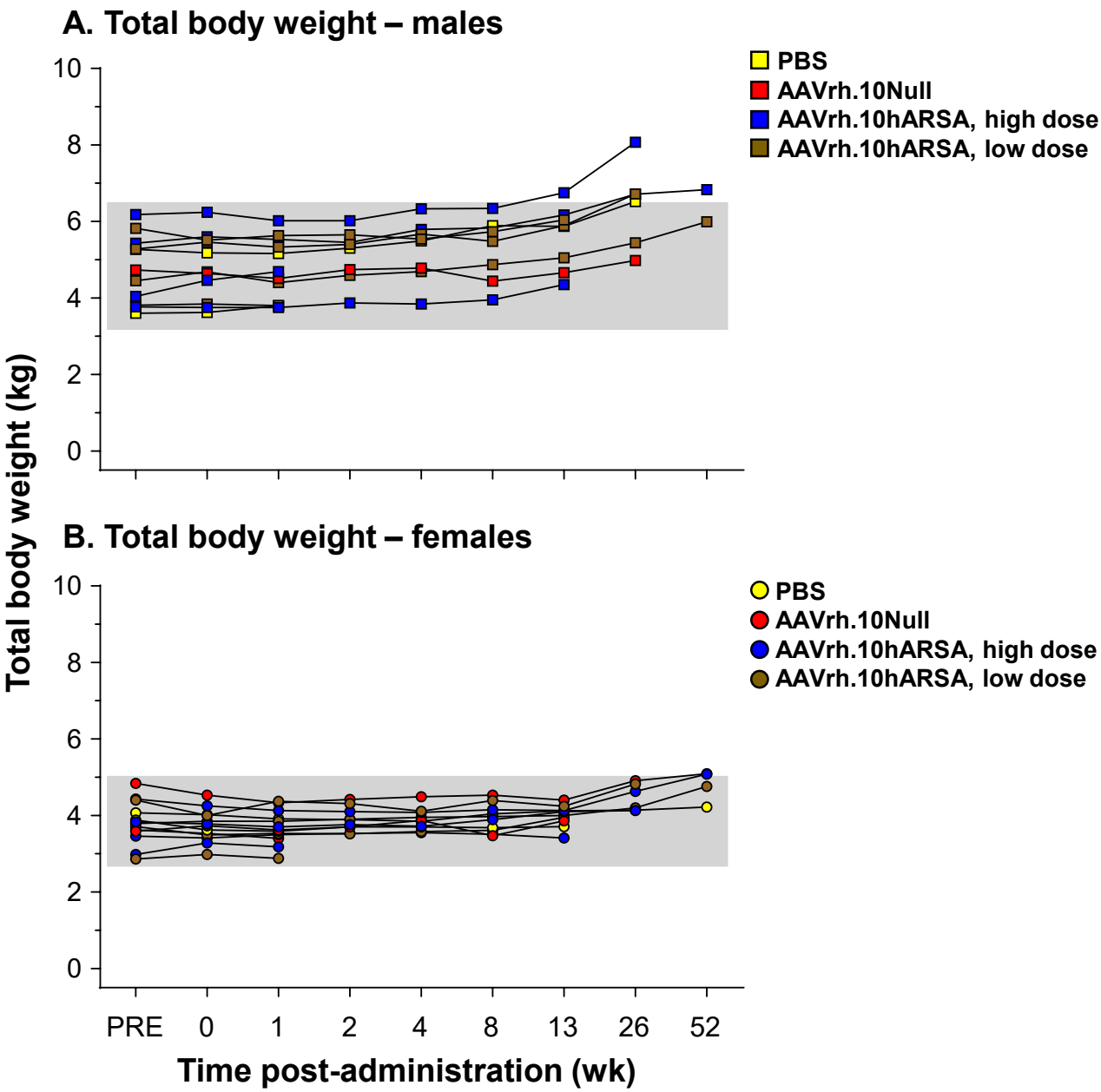
Supplemental Figure 6. Assessment of nonhuman primate brains by MRI for changes attributable to direct CNS administration of AAVrh.10hARSA and/or surgery. The abnormalities were at the sites of vector administration and catheter tip. **A.** Brain pathologic findings as percentage of total brain volume. T1 MRI scans of the long-term NHP brains (n=18 NHP) were analyzed for the size and volume of the pathologic findings or regions of interest in every coronal slice (>50) using Image J software. Each T1 coronal slice (in the MRI series) was evaluated for regions of interest, which were traced and area calculated by Image J. The volume of the region of interest was determined by multiplying the area by the number of coronal slices the regions of interest appeared and divided by total brain volume (TBV) as determined by MRI measurements to determine the region of interest % per TBV. Results are sorted by time of MRI scan (13, 26 or 52 wk, post-administration) and spread out to see individual data points. The pre-surgery data is not shown (all NHP were negative for pathologic findings). Shown are the results for each NHP in the study, color-coded by treatment (yellow, PBS; red, AAVrh.10Null; blue, AAVrh.10hARSA-high dose; and brown, AAVrh.10hARSA-low dose). The symbols represent dates of necropsy: triangles, NHP euthanized at 13 wk; squares, NHP euthanized at 26 wk; circles, and NHP euthanized at 52 wk. There was no correlation between gender and MRI ROI volume. Note that a subset of each group was euthanized for histopathology at 13, 26 and 52 wk,

and therefore the number of individual assessments decreased with time. The 13, 26, 52 wk scores have been linked by black lines for each 26 and 52 wk study NHP to show the results of repeated MRI scans. **B.** Brain pathologic findings as percentage of total brain volume. Box and whisker plots of the data in Supplemental Figure 6A. The multiple ROI scores have been combined into one set for each treatment group, with median (shown as black lines in the boxes), the upper and lower quartiles (as boxes), and highest and lowest values (as the whiskers). Shown are the results for all NHP assessed by MRIs (n=18) with males and females combined and color-coded by treatment (yellow, PBS; red, AAVrh.10Null; blue, AAVrh.10hARSA-high dose; and brown, AAVrh.10hARSA-low dose). Comparisons of the data was made by 1-way ANOVA with Dunnett's multiple comparisons test.

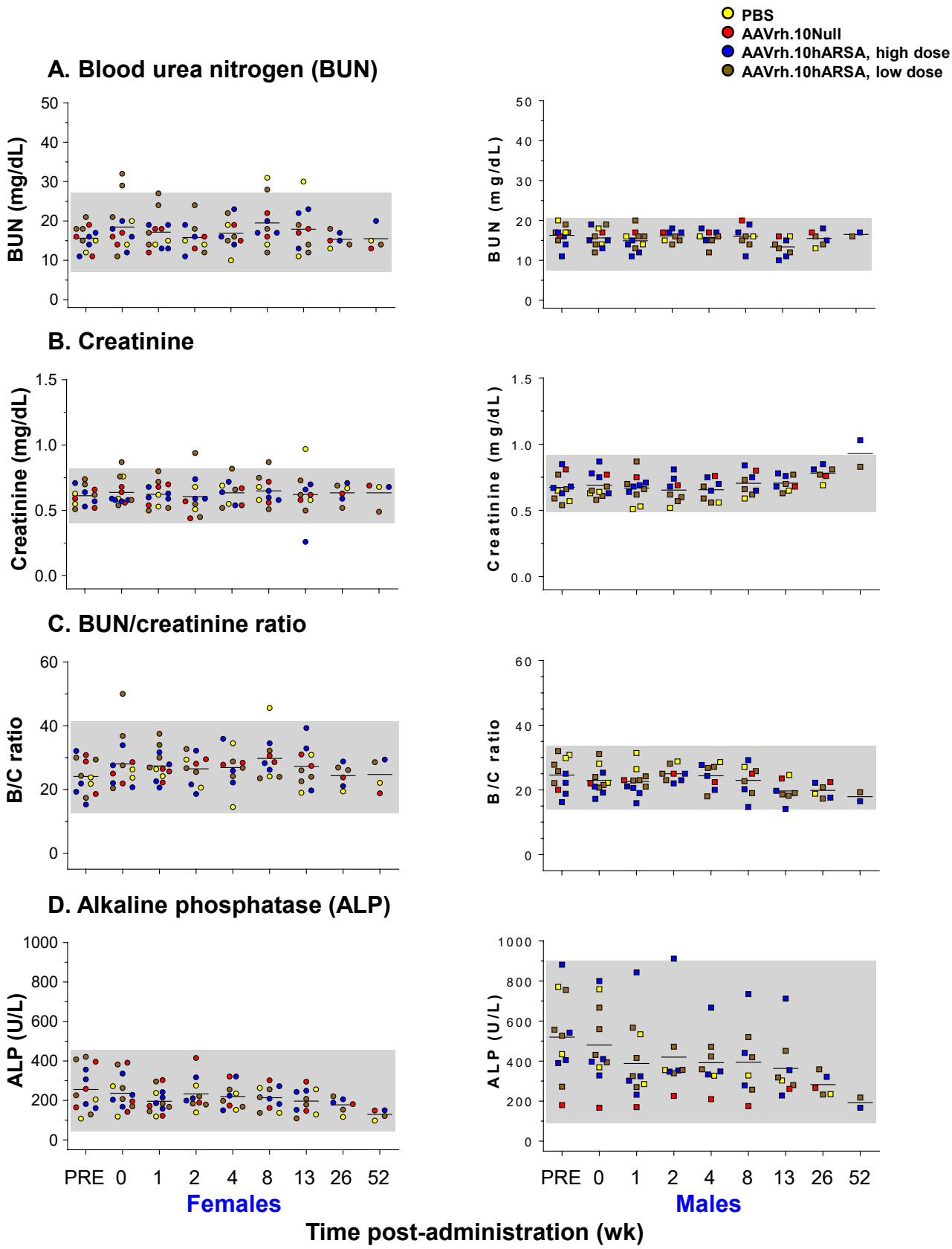
Supplemental Figure 7. Histopathology assessment of distal pathology in spinal cord following vector administration of AAVrh.10hARSA, AAVrh.10 Null, or PBS in CNS white matter. Spinal cord samples were examined from 3 regions per NHP, by histopathologic staining with hematoxylin and eosin (H&E) for abnormalities and pathology. **A-D,** examples of the cervical spinal cord (axial sections) findings from the 13 wk NHP dosage cohorts. The resulting spinal cord pathology from the lateral funiculi of the white matter of the cervical spinal cord is shown for the PBS (**A,** female), AAVrh.10Null (**B,** female; total dose $1.5x10^{12}$ gc), low dose (**C,** male; total dose $2.85x10^{11}$ gc), and high dose (**D,** female; total dose $1.5x10^{12}$ gc) AAVrh.10hARSA groups. Black arrows indicate noted pathology, dilated myelin sheaths with a macrophage inside. Black scale bar, 100 μm.

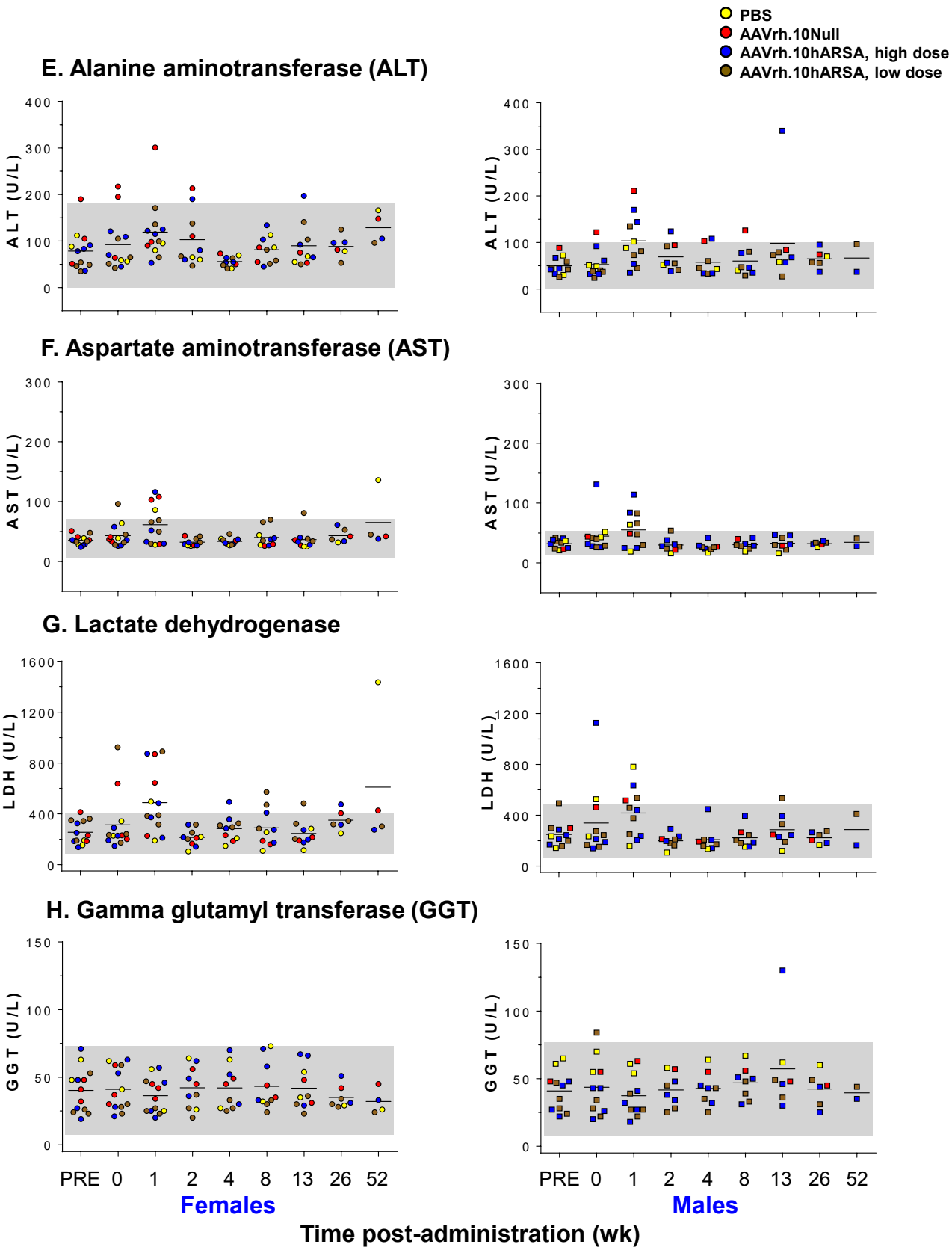
Supplemental Figure 1

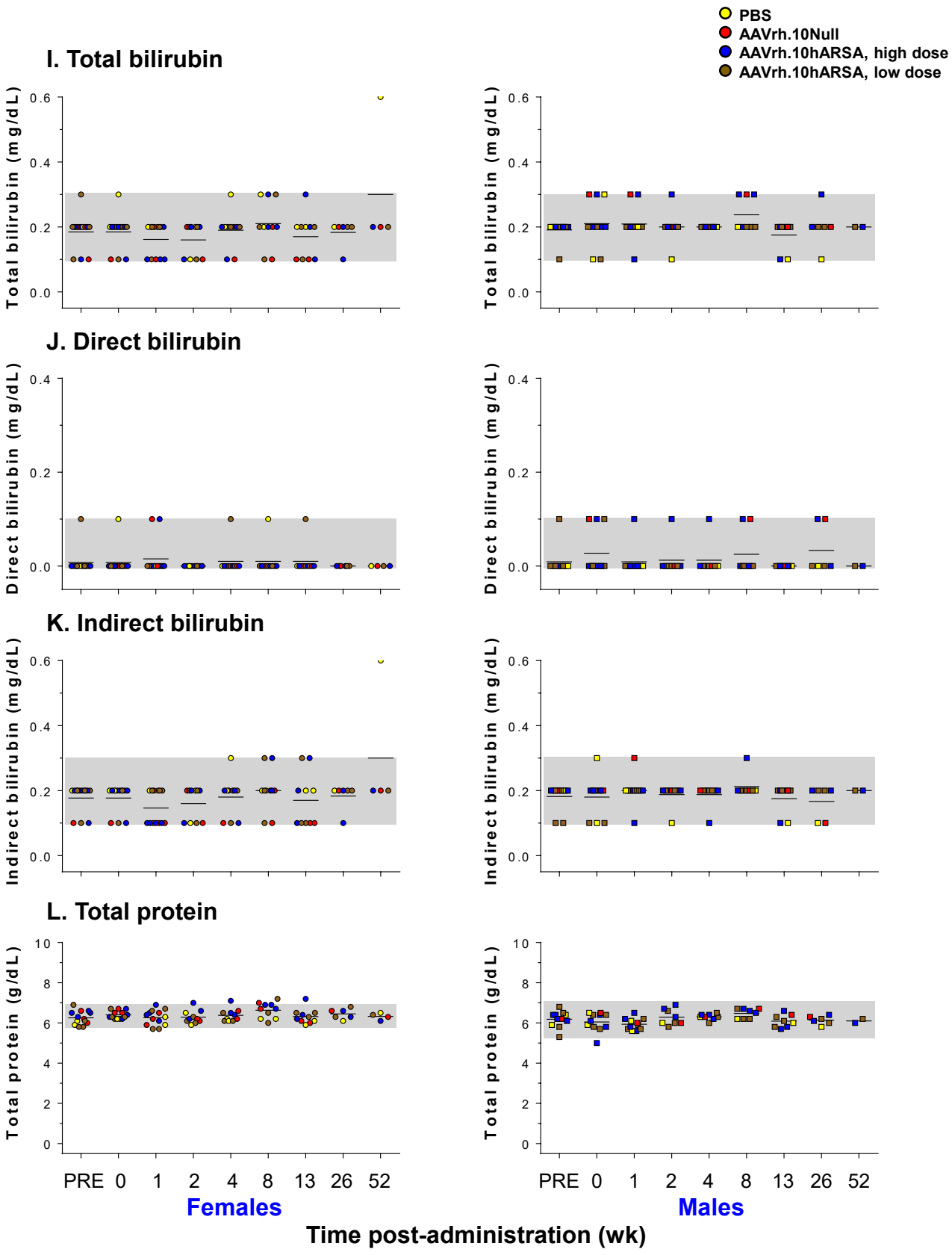


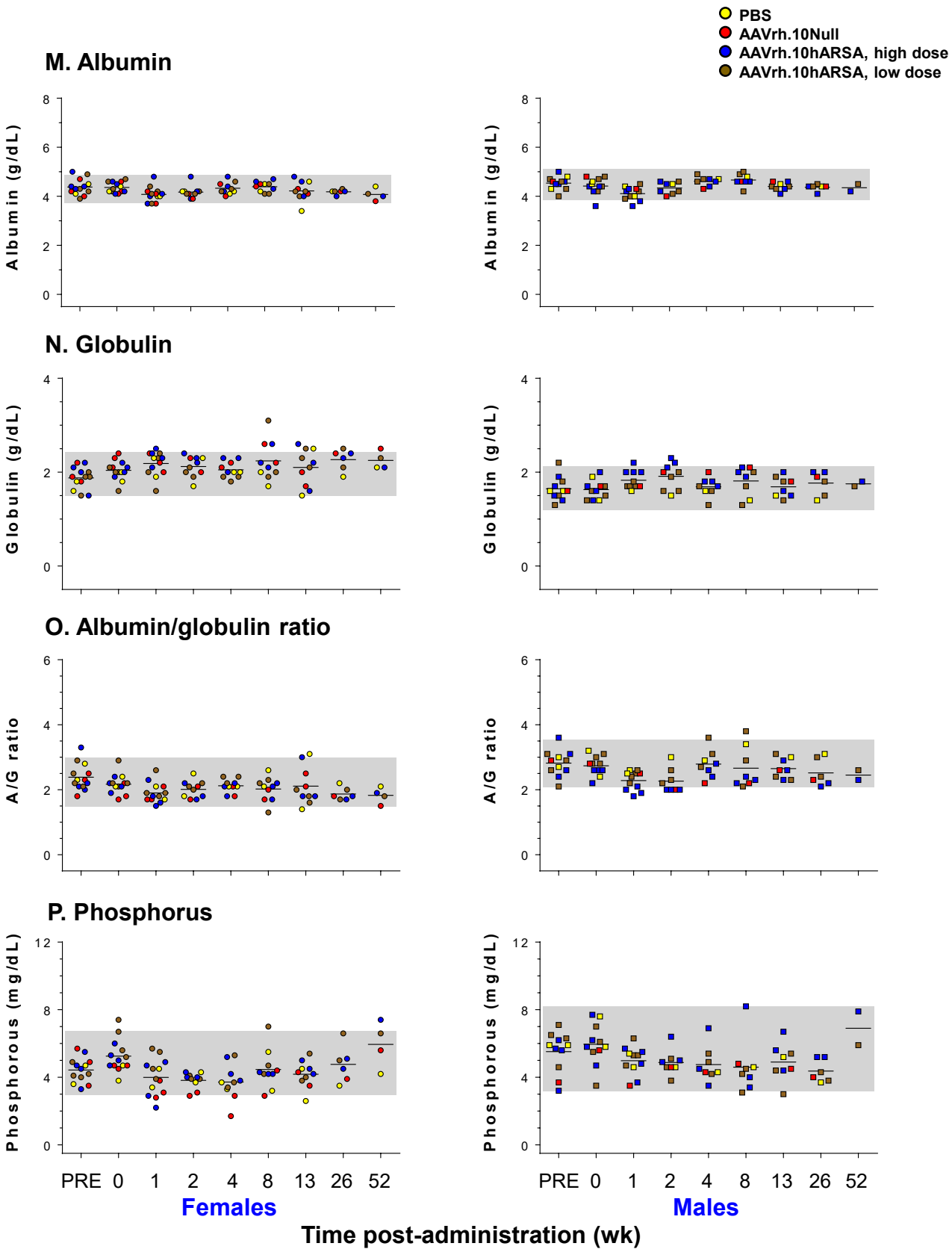

A. Total body weight – males
PBS
AAVrh.10Null
AAVrh.10hARSA, high dose
AAVrh.10hARSA, low dose
Total body weight (kg)
10
8
6
4
2
0
B. Total body weight – females
PBS
AAVrh.10Null
AAVrh.10hARSA, high dose
AAVrh.10hARSA, low dose
PRE
0
1
2
4
8
13
26
52
Time post-administration (wk)

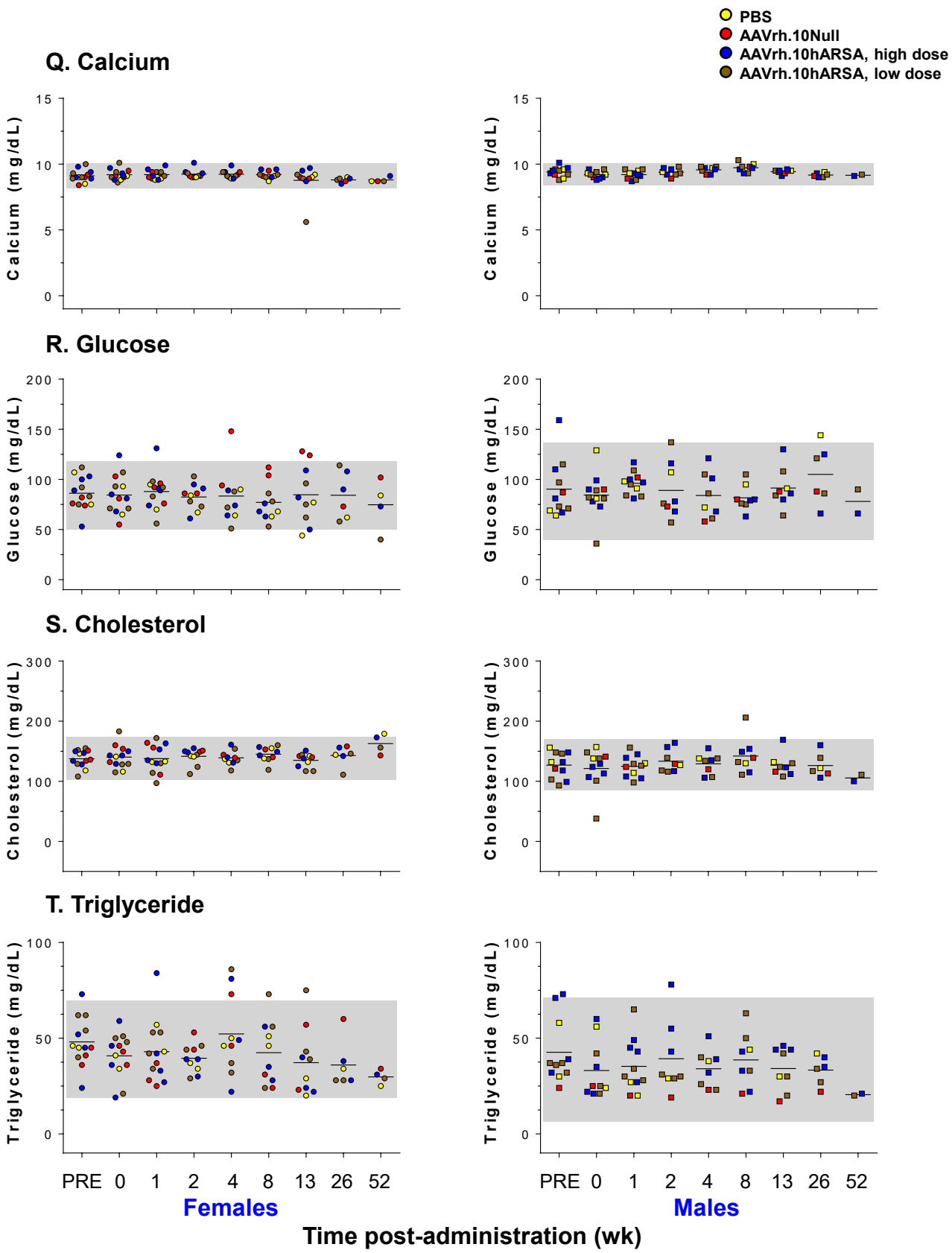
Supplemental Figure 3A-D



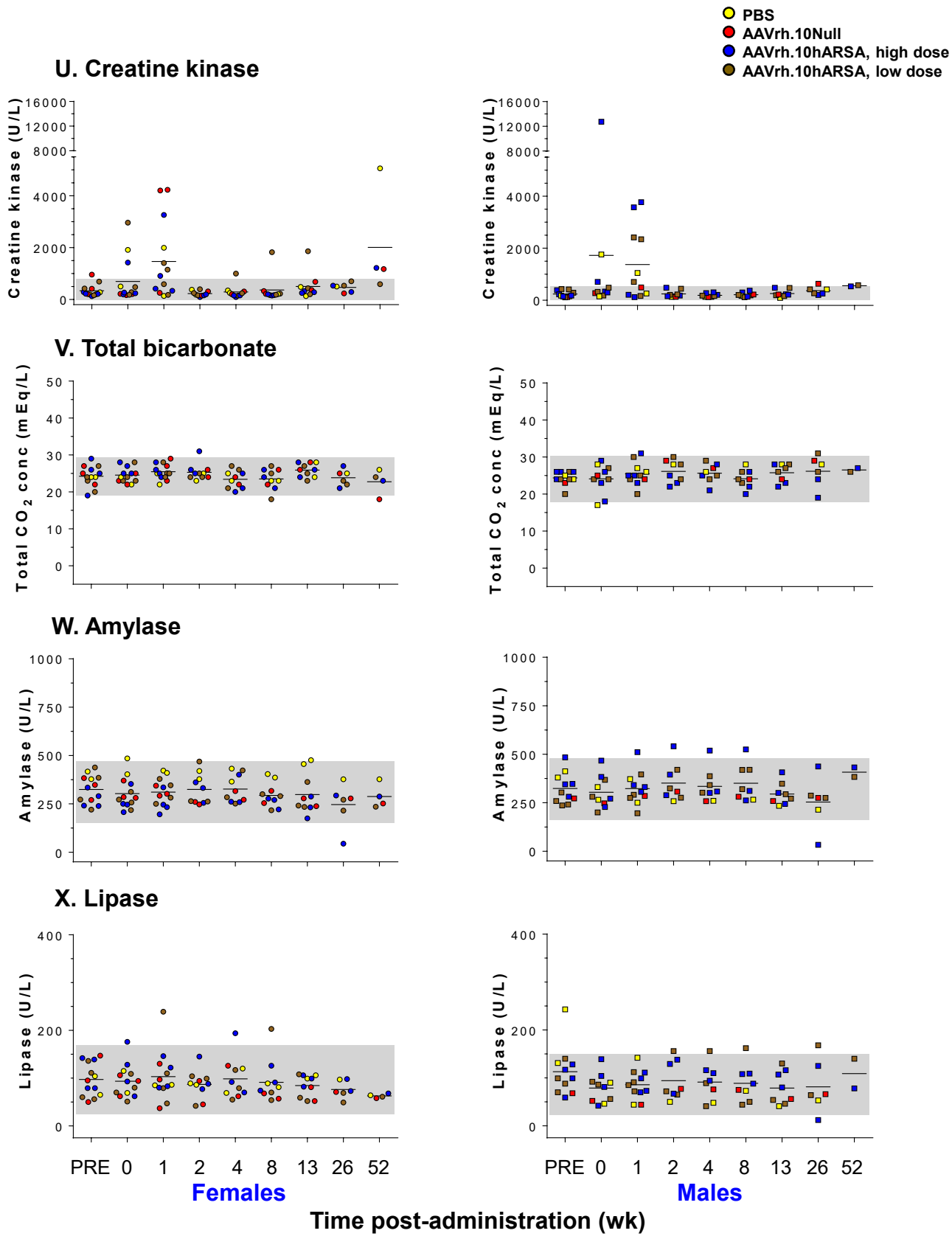

PBS
AAVrh.10Null
AAVrh.10hARSA, high dose
AAVrh.10hARSA, low dose
E. Alanine aminotransferase (ALT)
ALT (U/L)
F. Aspartate aminotransferase (AST)
AST (U/L)
G. Lactate dehydrogenase
LDH (U/L)
H. Gamma glutamyl transferase (GGT)
GGT (U/L)
PRE 0 1 2 4 8 13 26 52
Females
Males
Time post-administration (wk)

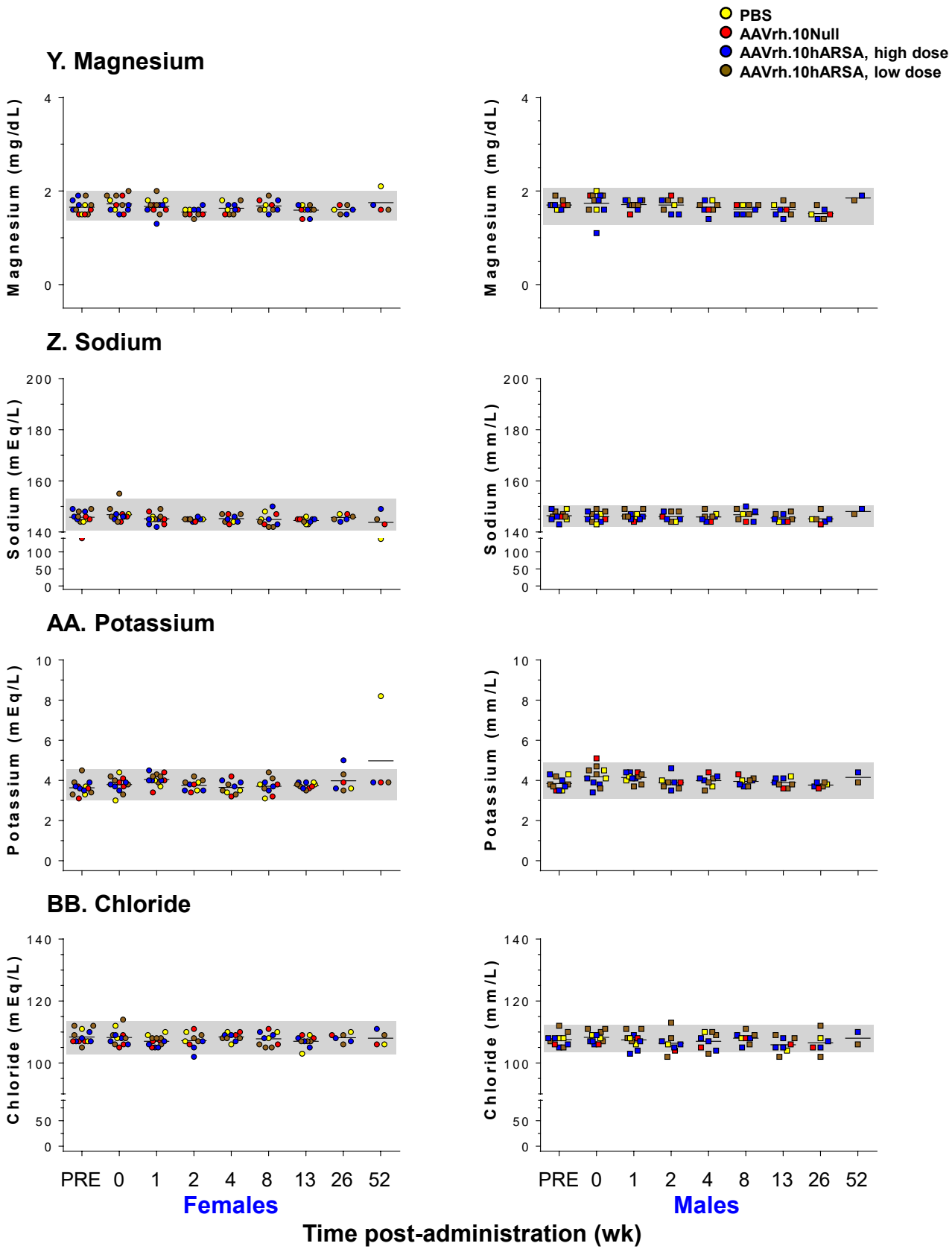

PBS
AAVrh.10Null
AAVrh.10hARSA, high dose
AAVrh.10hARSA, low dose
I. Total bilirubin
Total bilirubin (mg/dL)
J. Direct bilirubin
Direct bilirubin (mg/dL)
K. Indirect bilirubin
Indirect bilirubin (mg/dL)
L. Total protein
Total protein (g/dL)
PRE 0 1 2 4 8 13 26 52
Females
Males
Time post-administration (wk)


PBS
AAVrh.10Null
AAVrh.10hARSA, high dose
AAVrh.10hARSA, low dose
M. Albumin
Albumin (g/dL)
N. Globulin
Globulin (g/dL)
O. Albumin/globulin ratio
A/G ratio
P. Phosphorus
Phosphorous (mg/dL)
PRE 0 1 2 4 8 13 26 52
Females
Males
Time post-administration (wk)

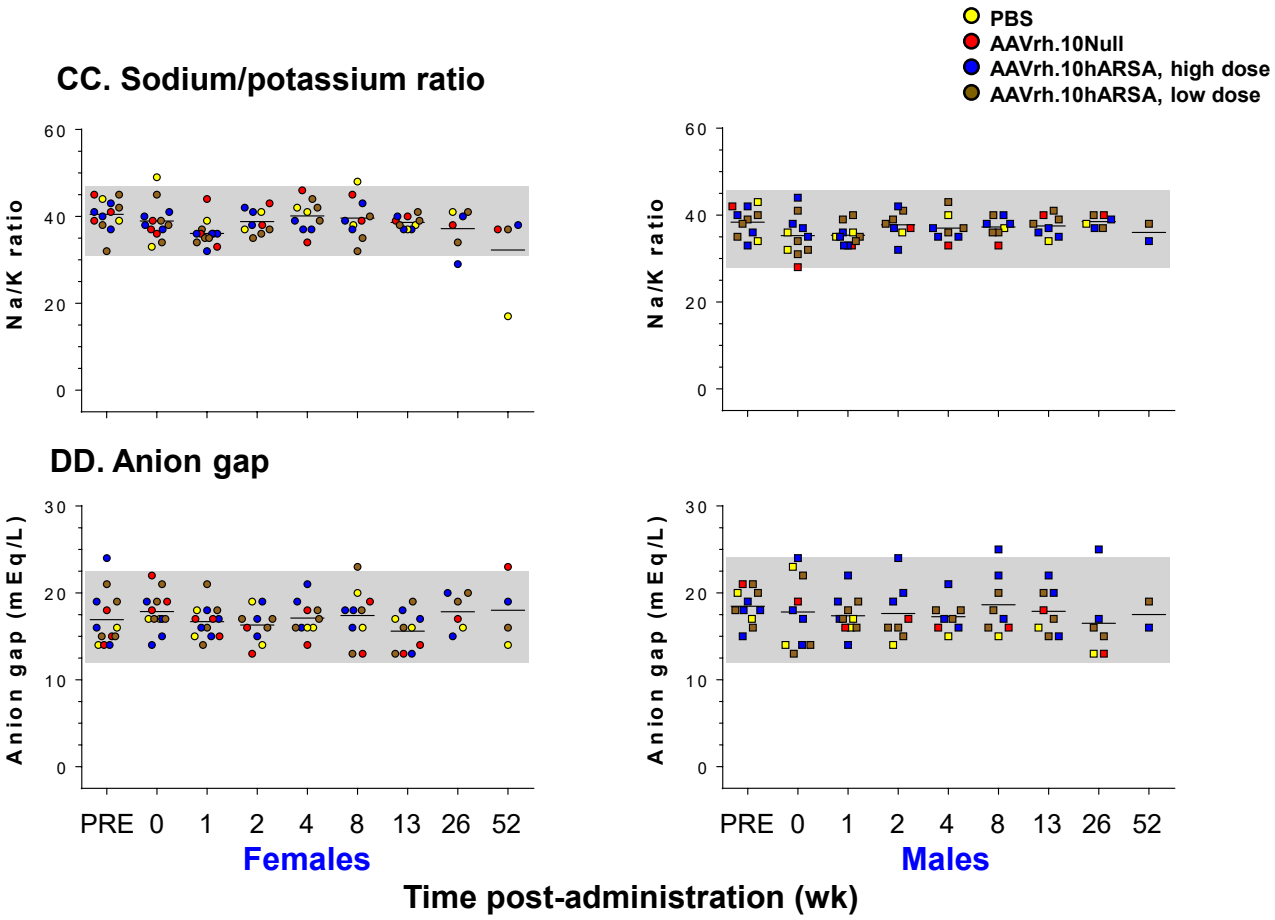

PBS
AAVrh.10Null
AAVrh.10hARSA, high dose
AAVrh.10hARSA, low dose
Q. Calcium
Calcium (mg/dL)
R. Glucose
Glucose (mg/dL)
S. Cholesterol
Cholesterol (mg/dL)
T. Triglyceride
Triglyceride (mg/dL)
PRE 0 1 2 4 8 13 26 52
Females
Males
Time post-administration (wk)

Supplemental Figure 3U-X

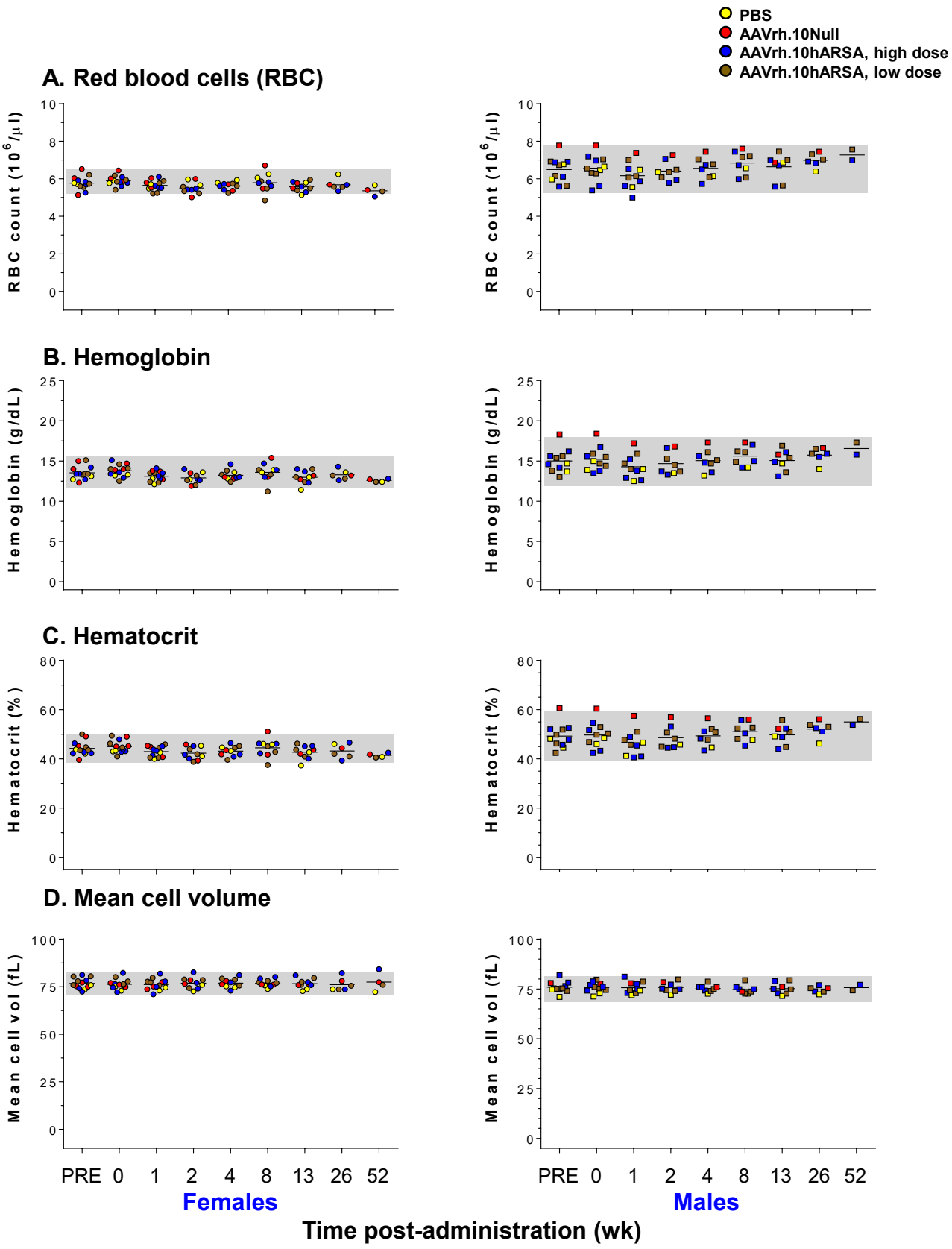


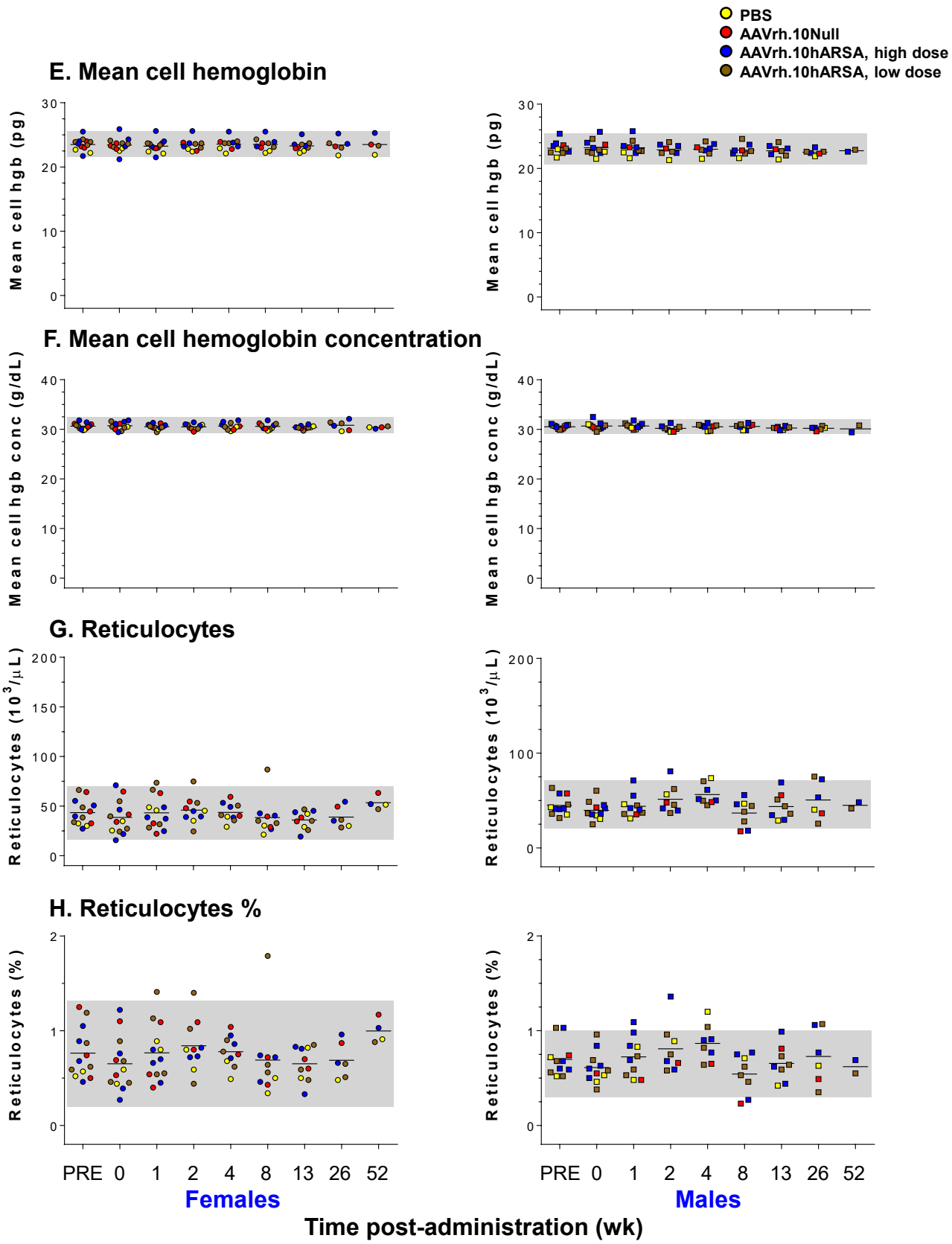

PBS
AAVrh.10Null
AAVrh.10hARSA, high dose
AAVrh.10hARSA, low dose
Y. Magnesium
Magnesium (mg/dL)
Z. Sodium
Sodium (mEq/L)
Sodium (mm/L)
AA. Potassium
Potassium (mEq/L)
Potassium (mm/L)
BB. Chloride
Chloride (mEq/L)
Chloride (mm/L)
PRE 0 1 2 4 8 13 26 52
Females
Males
Time post-administration (wk)

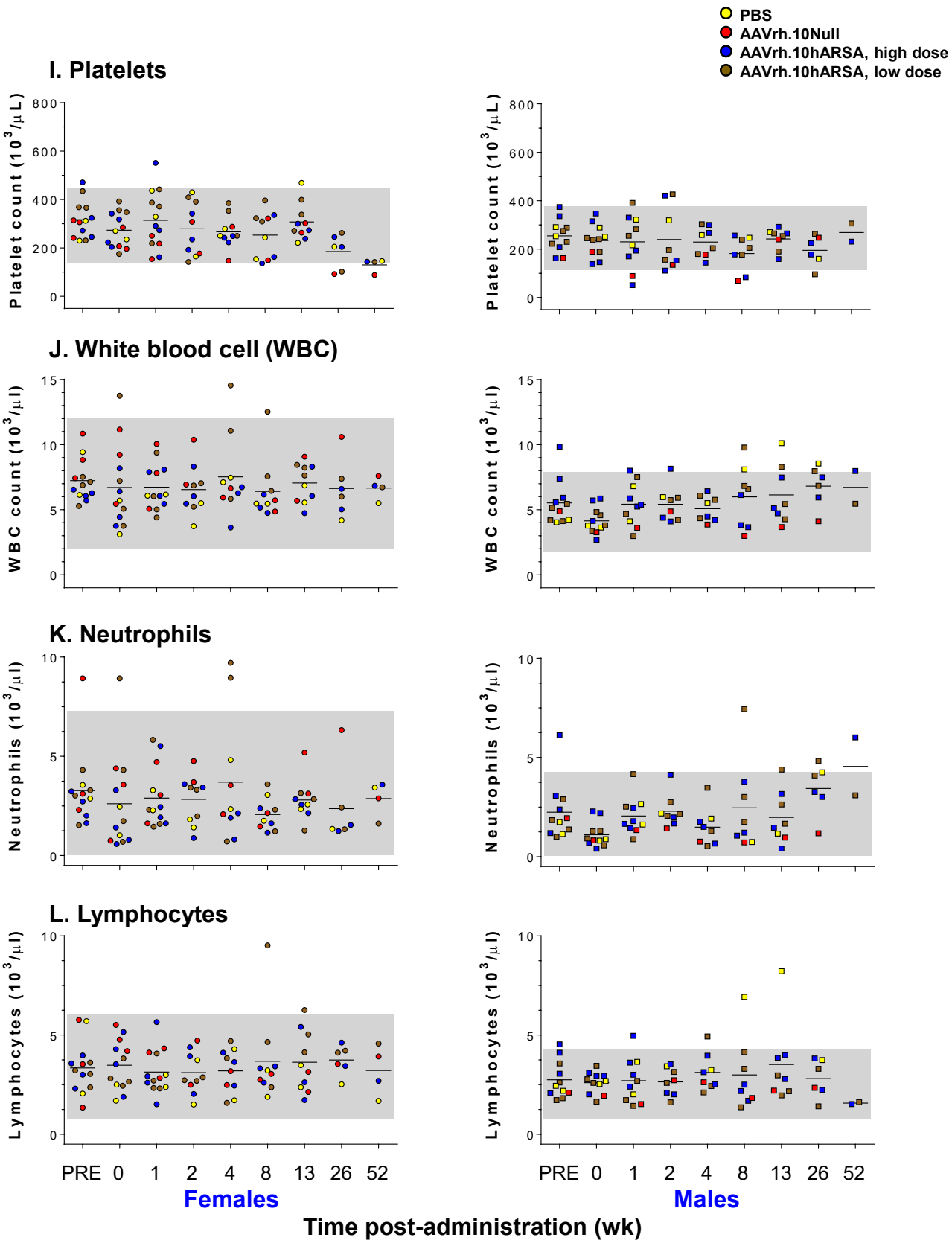
Supplemental Figure 3CC-DD

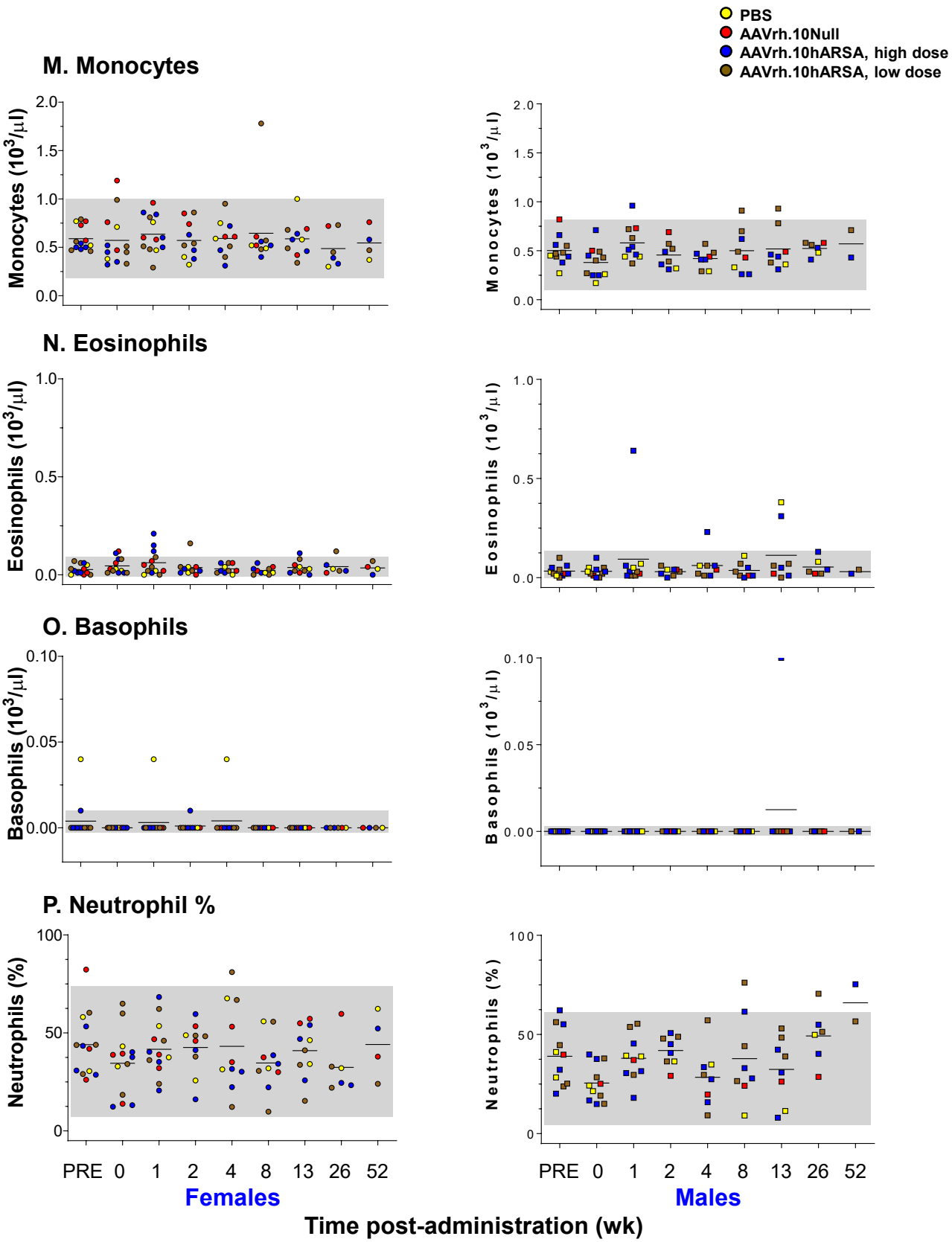


Supplemental Figure 4A-D

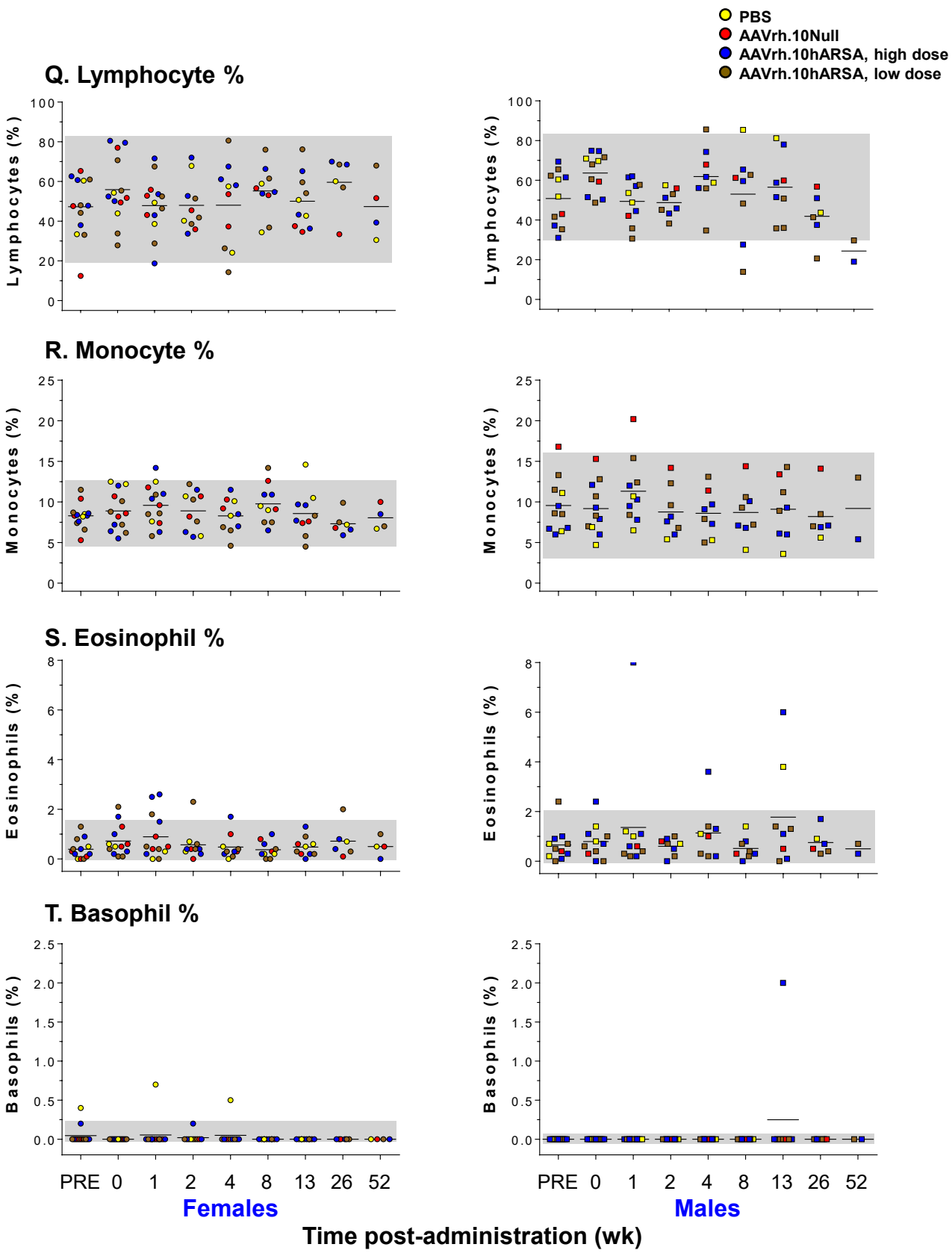


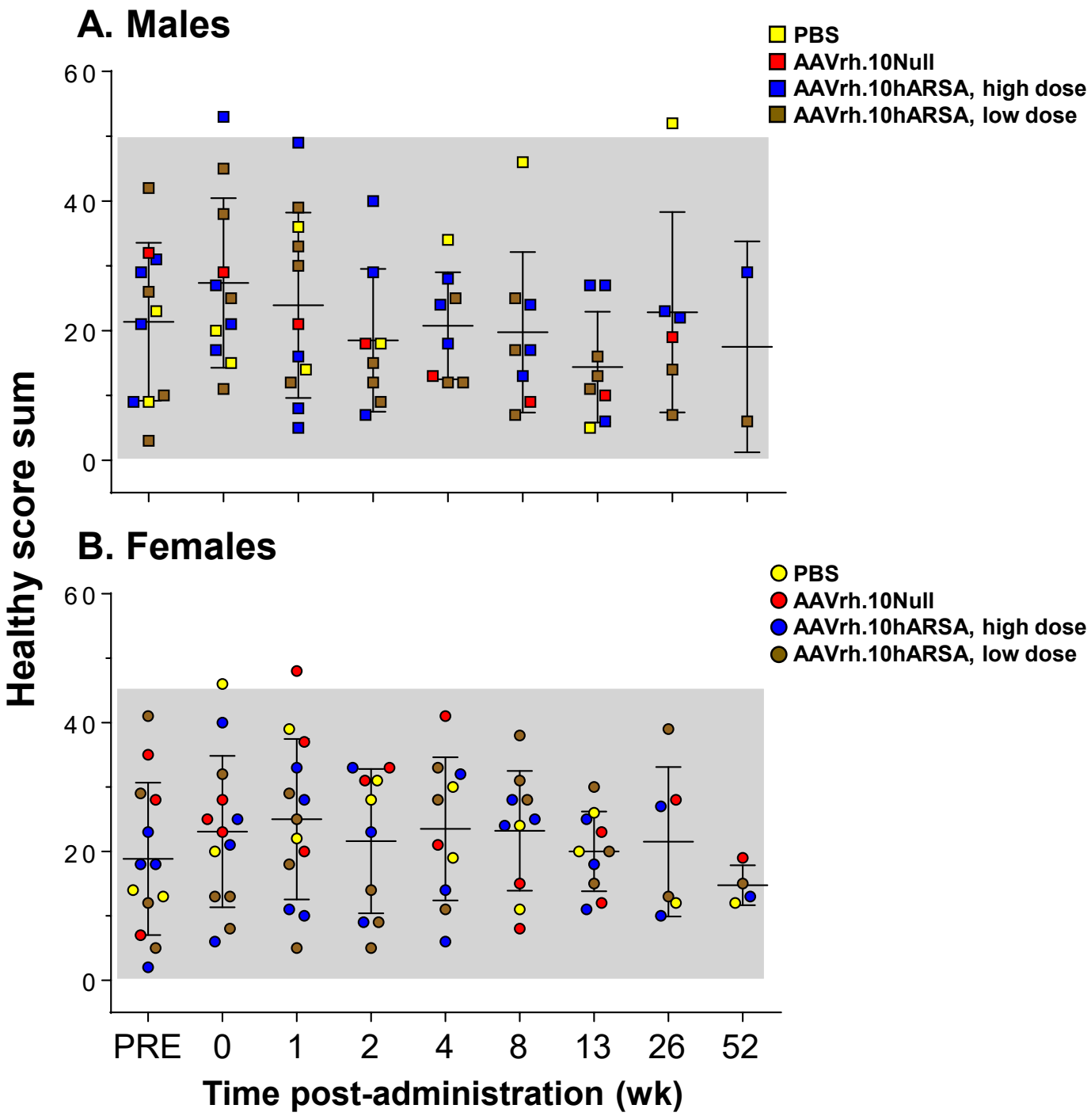

PBS
AAVrh.10Null
AAVrh.10hARSA, high dose
AAVrh.10hARSA, low dose
E. Mean cell hemoglobin
Mean cell hgb (pg)
F. Mean cell hemoglobin concentration
Mean cell hgb conc (g/dL)
G. Reticulocytes
Reticulocytes (10^3/µL)
H. Reticulocytes %
Reticulocytes (%)
PRE 0 1 2 4 8 13 26 52
Females
Males
Time post-administration (wk)

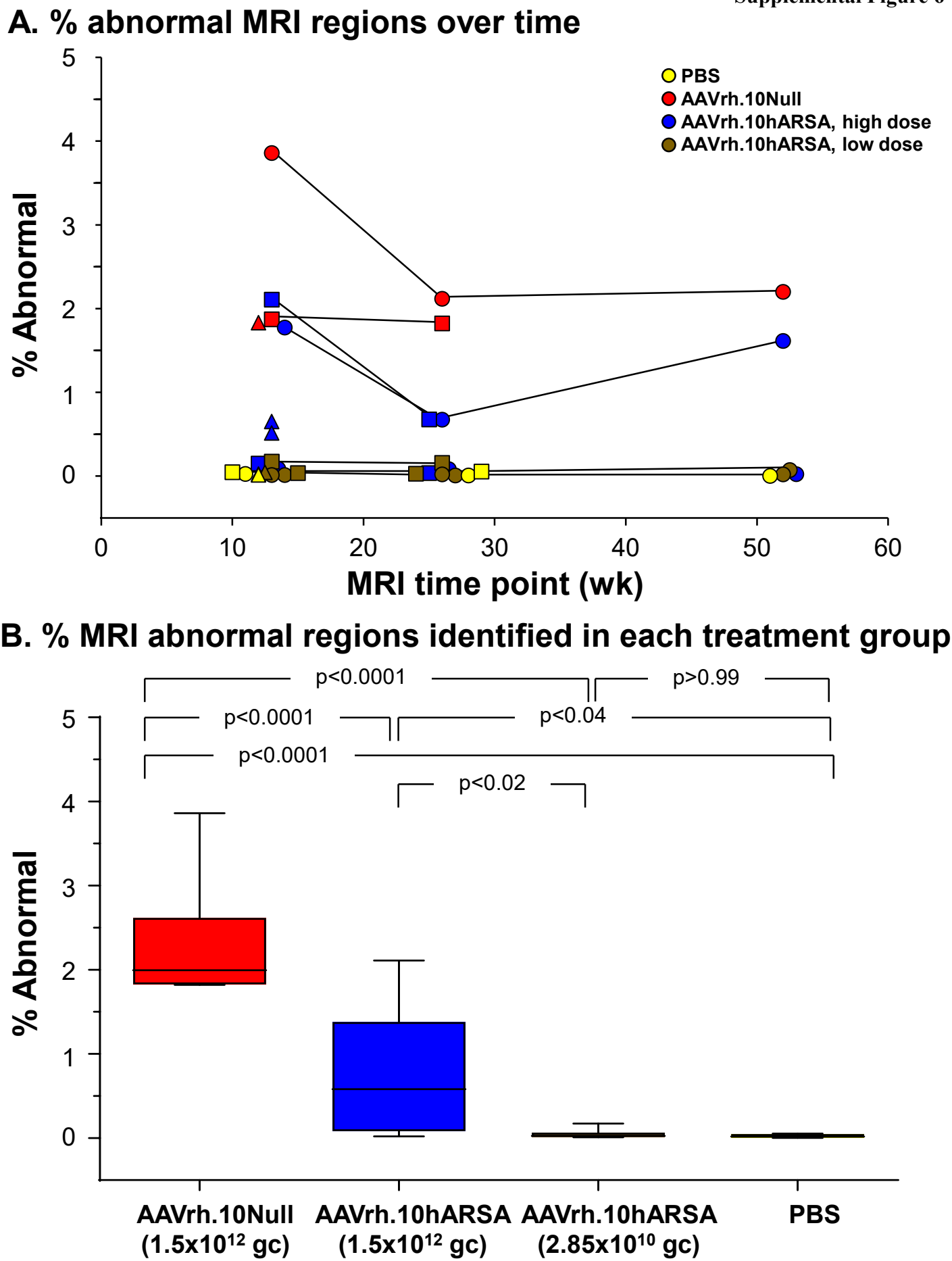

PBS
AAVrh.10Null
AAVrh.10hARSA, high dose
AAVrh.10hARSA, low dose
I. Platelets
Platelet count (10^3/μL)
J. White blood cell (WBC)
WBC count (10^3/μl)
K. Neutrophils
Neutrophils (10^3/μl)
L. Lymphocytes
Lymphocytes (10^3/μl)
PRE 0 1 2 4 8 13 26 52
Females
Males
Time post-administration (wk)

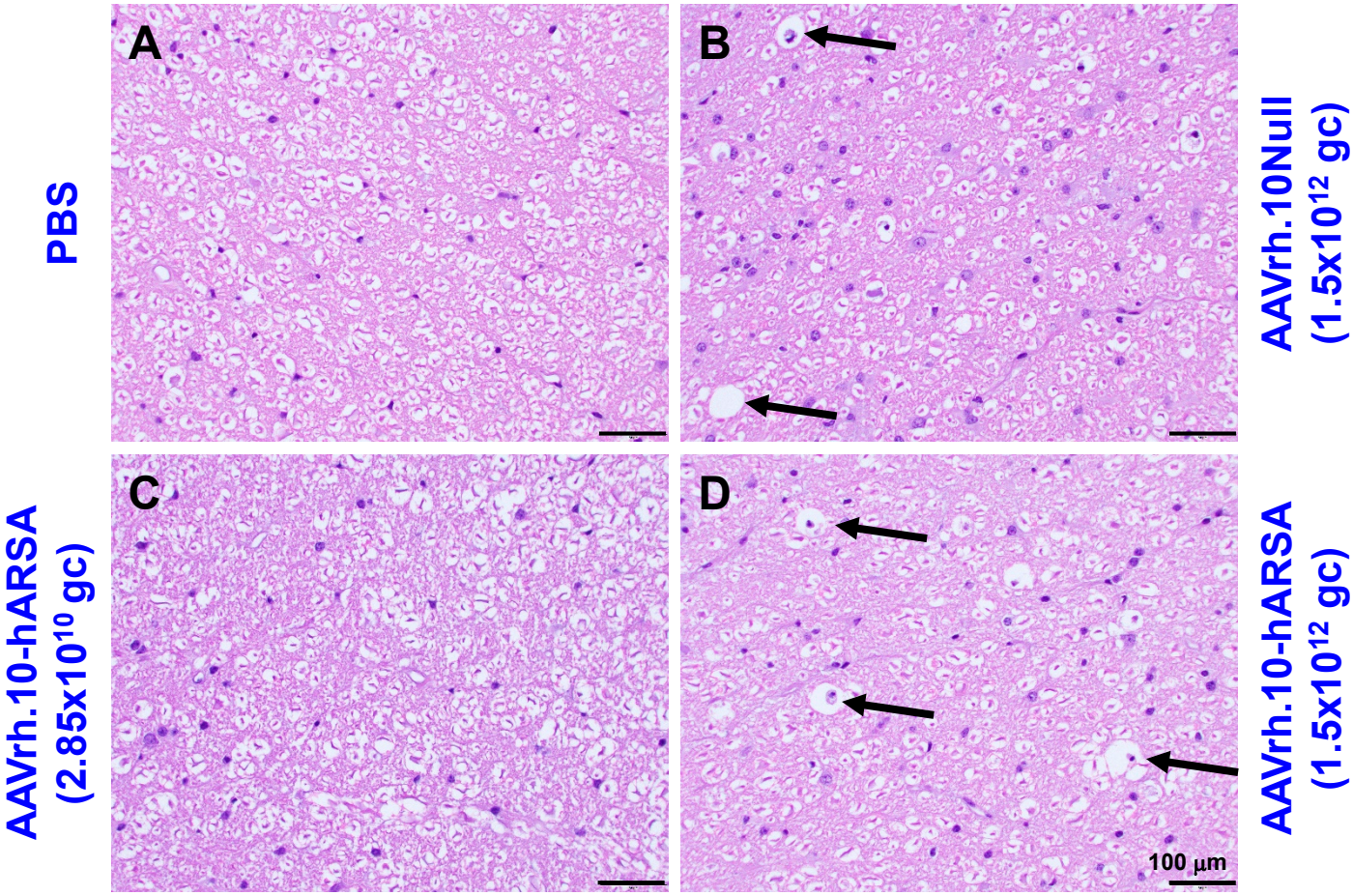

PBS
AAVrh.10Null
AAVrh.10hARSA, high dose
AAVrh.10hARSA, low dose
M. Monocytes
Monocytes (10³/μl)
N. Eosinophils
Eosinophils (10³/μl)
O. Basophils
Basophils (10³/μl)
P. Neutrophil %
Neutrophils (%)
PRE 0 1 2 4 8 13 26 52
Females
Males
Time post-administration (wk)

Supplemental Figure 4Q-T




A. Males
PBS
AAVrh.10Null
AAVrh.10hARSA, high dose
AAVrh.10hARSA, low dose
60
40
20
0
B. Females
PBS
AAVrh.10Null
AAVrh.10hARSA, high dose
AAVrh.10hARSA, low dose
60
40
20
0
Healthy score sum
PRE
0
1
2
4
8
13
26
52
Time post-administration (wk)


A. % abnormal MRI regions over time
PBS
AAVrh.10Null
AAVrh.10hARSA, high dose
AAVrh.10hARSA, low dose
% Abnormal
MRI time point (wk)
B. % MRI abnormal regions identified in each treatment group
p<0.0001
p>0.99
p<0.0001
p<0.04
p<0.0001
p<0.02
% Abnormal
AAVrh.10Null (1.5x10^12 gc)
AAVrh.10hARSA (1.5x10^12 gc)
AAVrh.10hARSA (2.85x10^10 gc)
PBS


A
B
C
D
PBS
AAVrh.10Null
(1.5x10^12 gc)
AAVrh.10-hARSA
(2.85x10^10 gc)
AAVrh.10-hARSA
(1.5x10^12 gc)
100 µm