Supplementary material

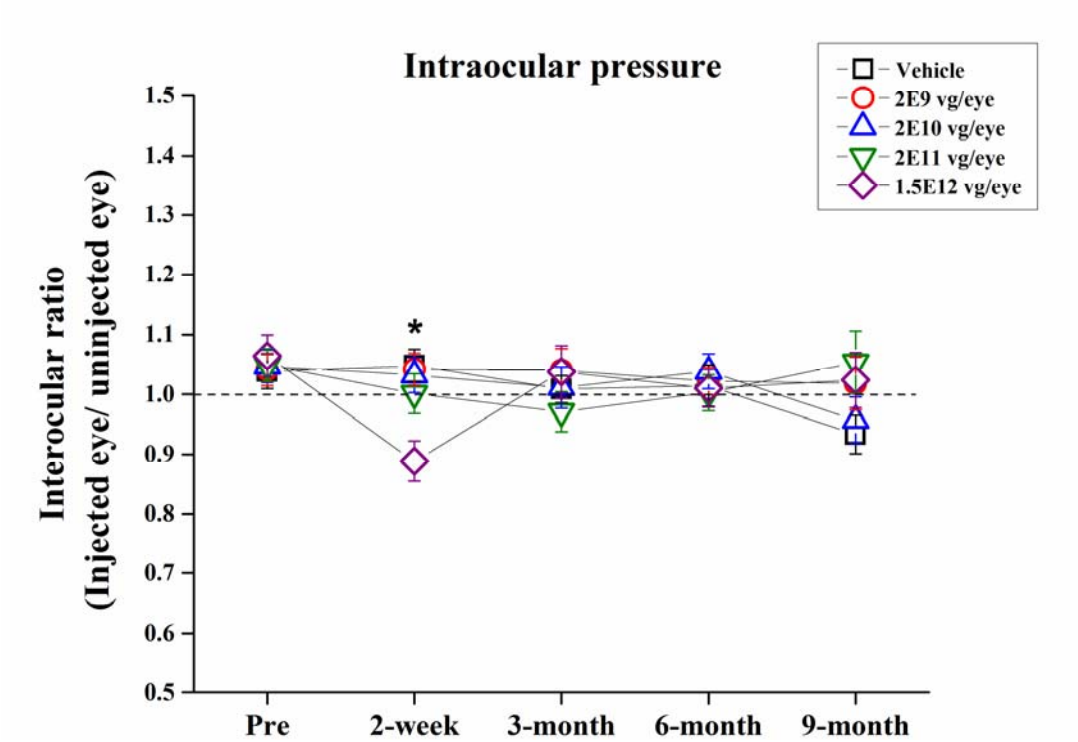

Figure S1. Interocular ratio in intraocular pressure (IOP) in New Zealand White rabbits intravitreally injected with AAV8-scRS/IRBPhRS at 4 doses and vehicle at 5 different time points.

The mean ± SE IOP interocular ratio (injected eye / uninjected eye) is shown at 5 time points (pre-treatment, 2 weeks, 3, 6 and 9 months after injection) for all vector doses and vehicle. A ratio equal to 1, evidenced by the black dotted line, indicates no difference between the IOP in the treated vs the untreated eye. At 2 weeks IOP interocular ratio was significantly decrease in the 1.5E12 vg/eye group respect to the control (p <0.01), indicating a transient reduction in IOP in the injected eye. No other significant differences in IOP interocular ratio were found at other time points. *statistically significant difference from control (< 0.05).

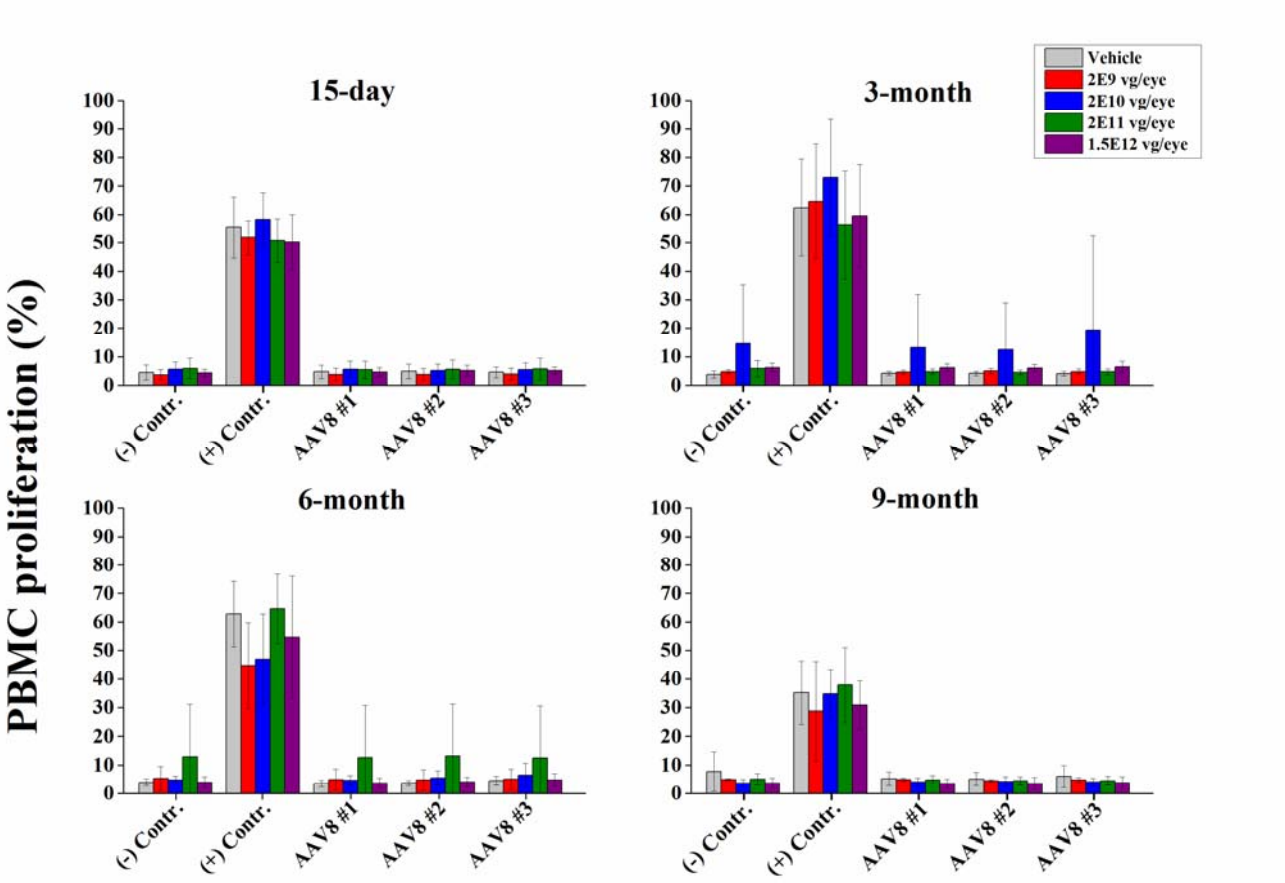

Figure S2. Measurement of AAV 8-specific peripheral blood mononuclear cell (PBMC) proliferative response in New Zealand White rabbits injected with AAV8-scRS/IRBPhRS and vehicle at 4 different time points after treatment.

PBMC isolated from blood of rabbits injected with AAV8-RS1 were labeled with the fluorescent dye CSPE to measure cell proliferation by dye dilution. Cells were cultured for 6 days in the presence of Concanavalin A (positive control) or AAV8 peptides distributed in 3 pools. Culture medium served as negative control. The cells were stained for viability with Zombie Aqua dye, fixed and analyzed on a LSR Fortessa flow cytometer for the CSPE and Zombie. Percentage stimulation above background was determined for each sample. PBMC proliferative response to AAV8 stimulation was below or similar to negative control at any tested vector dose indicating the lack of T-cell response in rabbits injected with AAV8-scRS/IRBPhRS. Each percentage value is expressed as mean ± SD.

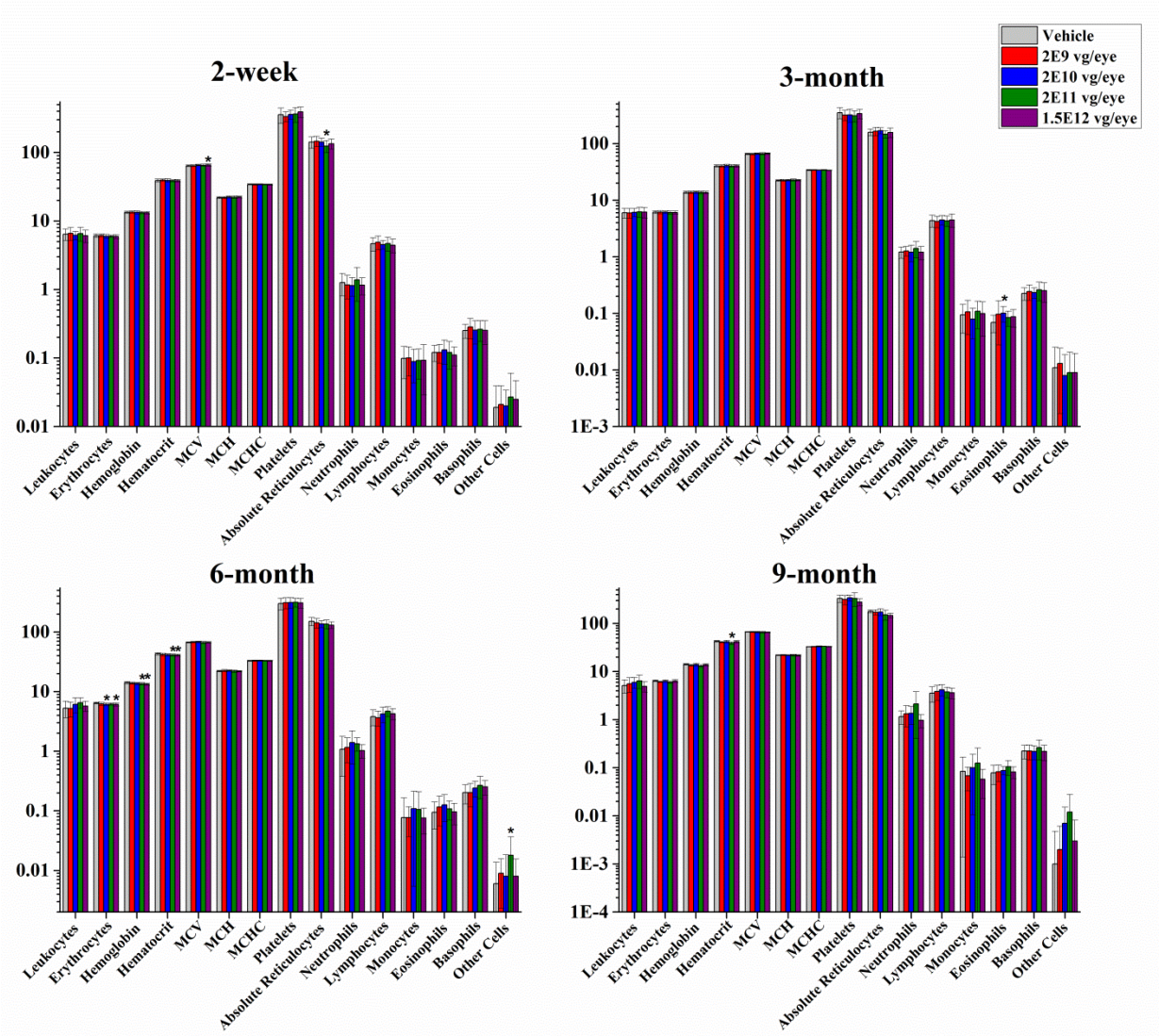

Figure S3. Hematological values in New Zealand rabbits injected with AAV8-scRS/IRBPhRS and vehicle at 4 different time points after treatment.

Each value is expressed as mean ± SD. There were no test article-related effects among hematology parameters in any treatment group. All mean and individual values were considered within expected ranges for biological and/or procedure-related variation despite occasional mean values that reached statistical significance. *statistically significant difference from control (< 0.05).

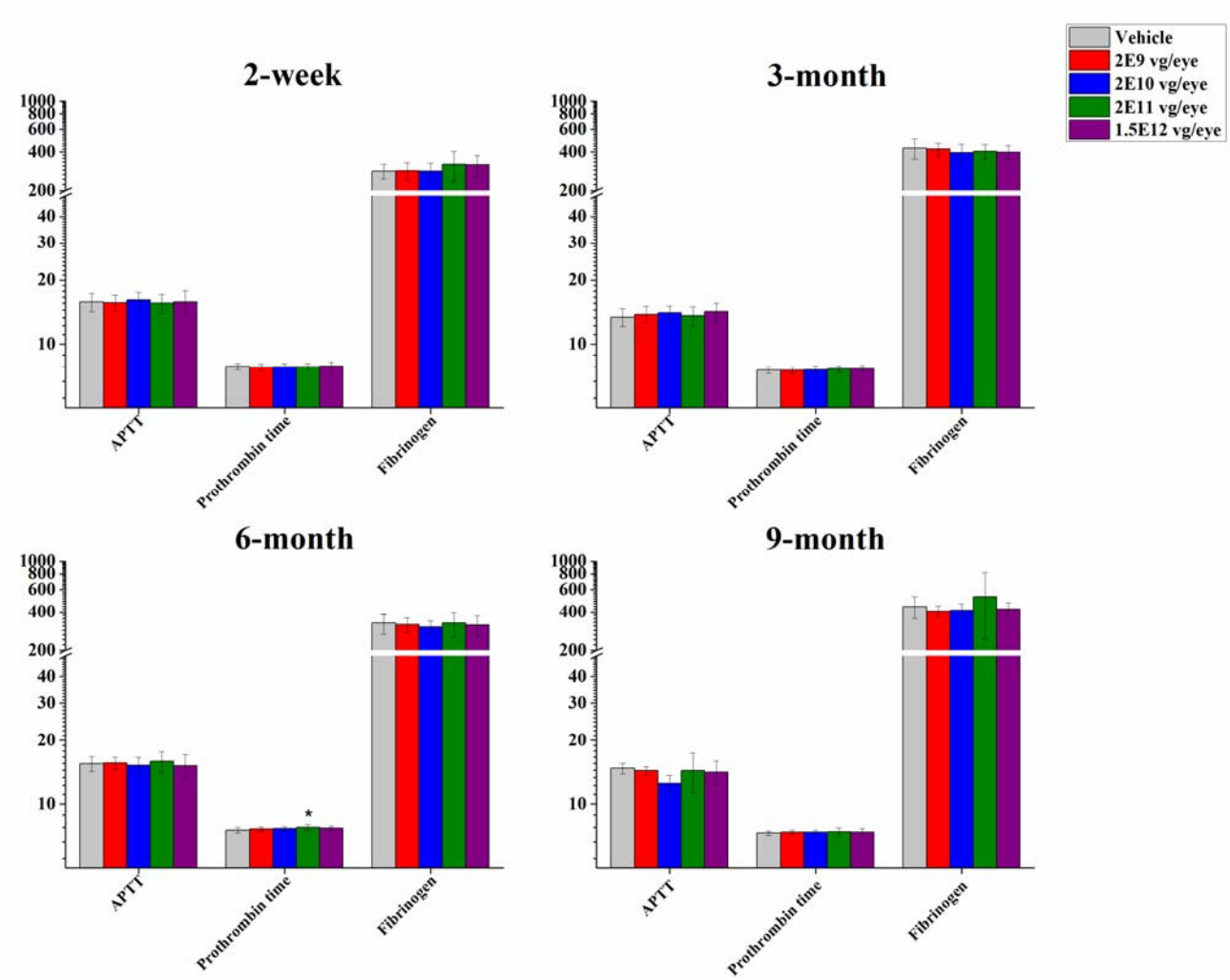

Figure S4. Coagulation parameters in New Zealand rabbits injected with AAV8-scRS/IRBPhRS and vehicle at 4 different time points after treatment.

Each value is expressed as mean ± SD. There were no test article-related effects among coagulation times or fibrinogen values in any treatment group. All mean and individual values were considered within expected ranges for biological and/or procedure-related variation despite occasional mean values that reached statistical significance. *statistically significant difference from control (< 0.05).

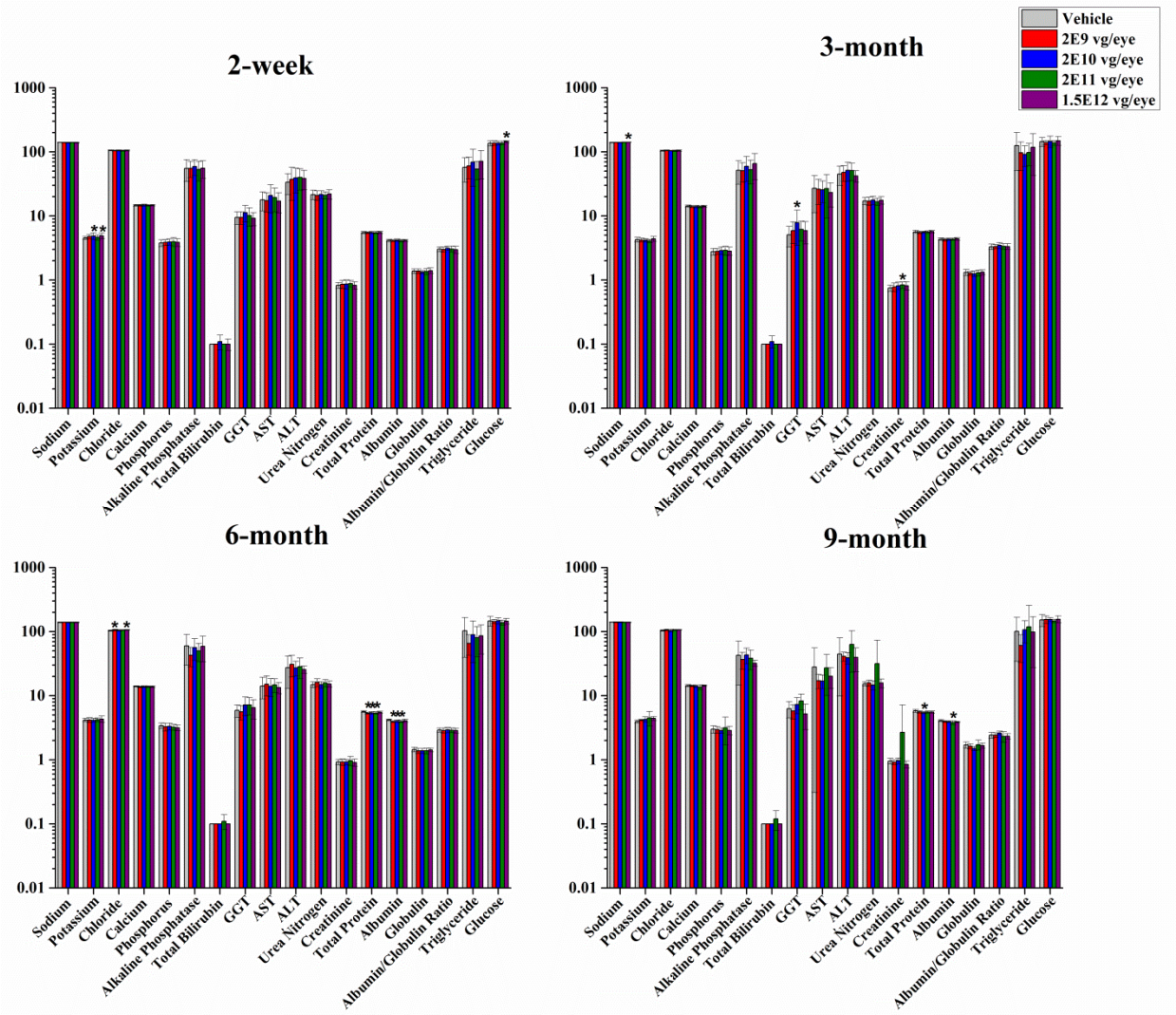

Figure S5. Clinical chemistry values in New Zealand rabbits injected with AAV8-scRS/IRBPhRS and vehicle at 4 different time points after treatment.

Each value is expressed as mean ± SD. There were no test article-related effects among clinical chemistry analytes in any treatment group. All mean and individual values were considered within expected ranges for biological and/or procedure-related variation despite occasional mean values that reached statistical significance. *statistically significant difference from control (< 0.05).

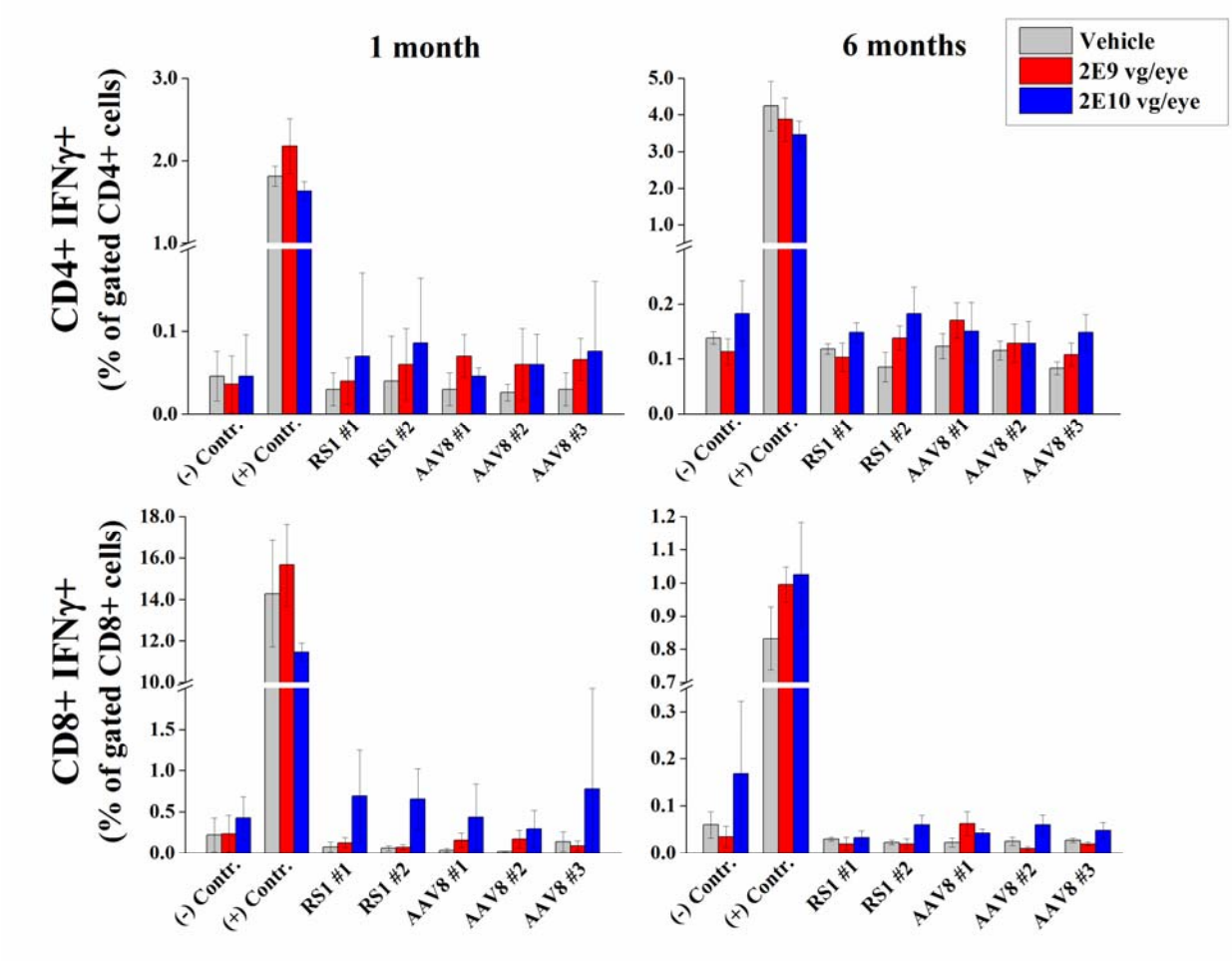

Figure S6. T-cell response assay in Rs1-Ko mice treated with AAV8-scRS/IRBPhRS and vehicle at 2 different time points after treatment.

In vitro stimulation of IFN-γ production was evaluated by incubation of mouse spleen cells with positive control (PMA and ionomycin) or AAV8 peptide library (3 pools) or RS1 peptide library (2 pools) for 6 hours. Culture medium was used as negative control. The cells were later primed with antibodies against CD3+/CD45+/CD4+/CD8+/ IFN-γ to measure the frequency of CD3 4 and CD3 8 cells that produce IFN-γ. The results show that percentage of CD4 and CD8 cells producing IFN-γ in response to AAV8 or RS1 peptides were similar to those of negative control for both vector doses thus indicating the absence of specific T cell response. Flow cytometry was performed using a BD™ LSR Fortessa Flow Cytometer System. Each percentage value is expressed as mean ± SD.

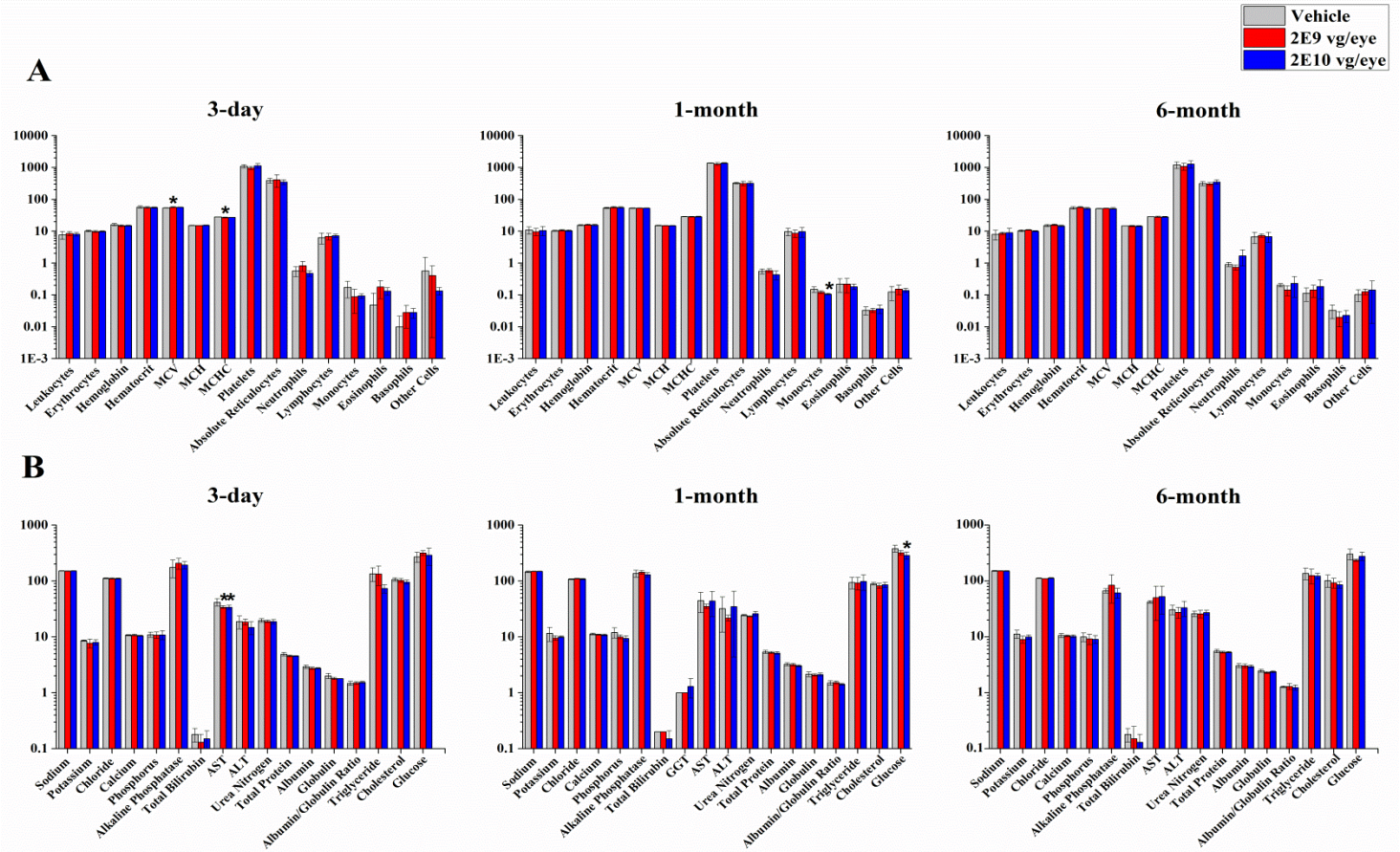

Figure S7. (a) Hematological (a) and clinical chemistry values in Rs1-KO mice injected with AAV8-scRS/IRBPhRS and vehicle at 3 different time points after treatment.

Each value is expressed as mean ± SD. There were no test article related effects among hematology parameters and clinical chemistry analytes in any treatment group. All mean and individual values were considered within expected ranges for biological and/or procedure-related variation despite occasional mean values that reached statistical significance. Statistically significant difference from control (< 0.05).

Table S1. Titer of AAV8 Neutralizing Antibodies in New Zealand White Rabbit Serum.

Rabbit #	Treatment	Baseline	2-week	3-month	6-month	9-month
1023	**Vehicle**	<50	<50	<50	<50	<50
1024	**Vehicle**	<50	<50	<50	<50	<50
1025	**Vehicle**	<50	<50	<50	<50	<50
1026	**Vehicle**	<50	<50	<50	<50	<50
1027	**Vehicle**	<50	<50	<50	<50	<50
1028	**Vehicle**	<50	<50	<50	<50	<50
2019	**Vector (2E9 vg/eye)**	<50	200	100	100	50
2020	**Vector (2E9 vg/eye)**	<50	50	50	50	<50
2021	**Vector (2E9 vg/eye)**	<50	100	200	200	100
2022	**Vector (2E9 vg/eye)**	<50	100	100	50	50
2023	**Vector (2E9 vg/eye)**	<50	100	100	<50	<50
2024	**Vector (2E9 vg/eye)**	<50	100	50	<50	<50
3019	**Vector (2E10 vg/eye)**	<50	800	>1600	>1600	>1600
3020	**Vector (2E10 vg/eye)**	<50	800	800	800	800
3021	**Vector (2E10 vg/eye)**	<50	400	200	100	200
3022	**Vector (2E10 vg/eye)**	<50	1600	400	400	400
3023	**Vector (2E10 vg/eye)**	<50	400	400	200	200
3024	**Vector (2E10 vg/eye)**	<50	1600	200	100	100
4019	**Vector (2E11 vg/eye)**	<50	400	>1600	800	800
4020	**Vector (2E11 vg/eye)**	<50	1600	400	400	400
4021	**Vector (2E11 vg/eye)**	<50	1600	800	400	400
4022	**Vector (2E11 vg/eye)**	<50	1600	>1600	1600	1600
4023	**Vector (2E11 vg/eye)**	<50	1600	>1600	>1600	>1600
4024	**Vector (2E11 vg/eye)**	<50	800	>1600	1600	1600
5019	**Vector (1.5E12 vg/eye)**	<50	>1600	>1600	>1600	>1600
5020	**Vector (1.5E12 vg/eye)**	<50	800	>1600	>1600	>1600
5021	**Vector (1.5E12 vg/eye)**	<50	>1600	>1600	>1600	>1600
5022	**Vector (1.5E12 vg/eye)**	<50	1600	>1600	>1600	>1600
5023	**Vector (1.5E12 vg/eye)**	<50	>1600	>1600	>1600	>1600
5024	**Vector (1.5E12 vg/eye)**	<50	>1600	>1600	>1600	>1600

Table S2. Ophthalmic examination findings in Rs1-KO mice.

Treatment	Baseline	3-day	1-month	6-month
Vehicle	(0/54)	**Chorioretinal lesion** (19/54) **Retinal hemorrhage** (1/54) **Vitreal hemorrhage** (1/54)	**Chorioretinal lesion** (12/37)	**Chorioretinal lesion** (11/21)
AAV8-vector 2E9 (vg/eye)	(0/54)	**Chorioretinal lesion** (26/54) **Vitreal hemorrhage** (2/54)	**Chorioretinal lesion** (18/37) **Vitreal hemorrhage** (2/37)	**Chorioretinal lesion** (13/21)
AAV8-vector 2E10 (vg/eye)	(0/54)	**Chorioretinal lesion** (29/54) **Retinal hemorrhage** (2/54)	**Chorioretinal lesion** (19/37)	**Chorioretinal lesion** (12/21)

Ophthalmic abnormalities in the injected eye are reported as observed number by total number of animals. Except for one mouse in the 2E9 vg/eye group with a diffuse corneal opacity in the untreated eye at baseline that remained unchanged throughout duration of the study, no other mouse showed any abnormality in the untreated eye during the study.

Table S3. Titer of AAV8 Neutralizing Antibodies in Rs1-Ko Mouse Plasma.

Mouse #	***Treatment***	***3-day***	***6-month***
4006	**Vehicle**	<50	-
4007	**Vehicle**	<50	-
4003	**Vehicle**	-	<50
4004	**Vehicle**	-	<50
7001	**Vehicle**	-	<50
7002	**Vehicle**	-	<50
7003	**Vehicle**	-	<50
7004	**Vehicle**	-	<50
5006	**Vector (2E9 vg/eye)**	<50	-
5007	**Vector (2E9 vg/eye)**	<50	-
5003	**Vector (2E9 vg/eye)**	-	50
8001	**Vector (2E9 vg/eye)**	-	<50
8002	**Vector (2E9 vg/eye)**	-	<50
8003	**Vector (2E9 vg/eye)**	-	<50
8004	**Vector (2E9 vg/eye)**	-	<50
6006	**Vector (2E10 vg/eye)**	50	-
6007	**Vector (2E10 vg/eye)**	<50	-
6002	**Vector (2E10 vg/eye)**	-	400
6005	**Vector (2E10 vg/eye)**	-	100
9001	**Vector (2E10 vg/eye)**	-	200
9002	**Vector (2E10 vg/eye)**	-	<50
9003	**Vector (2E10 vg/eye)**	-	50
9004	**Vector (2E10 vg/eye)**	-	100

Table S4. Organs and tissues included in pathology analysis.

- Adrenal [1,2]
- Aorta
- Bone with marrow [femur]
- Bone with marrow [rib] [3]
- Bone with marrow [sternum]
- Brain [cerebrum, midbrain, cerebellum, medulla/pons] [1]
- Brain, optic chiasm [3]
- Epididymis [1,2]
- Eye including optic nerve
- Gallbladder
- GALT [gut associated lymphoid tissue]
- Gastrointestinal tract:
  - esophagus
  - stomach [cardia, fundus, and pylorus]
  - duodenum
  - jejunum
  - ileum
  - cecum
  - colon
  - rectum
- Gonads:
  - testis [1,2]
- Gross lesions
- Harderian gland, right
- Heart [1]
- Joint tibiofemoral
- Kidney [1,2]
- Lacrimal gland, right [3], exorbital [2, 4]
- Larynx
- Liver [1]
- Lung with bronchi [1]
- Lymph nodes: mandibular, right [3] and mesenteric
- Pancreas
- Pituitary [1]
- Prostate[1] and seminal vesicle [1]
- Salivary glands:
  - mandibular [1]
  - sublingual [1, 4]
  - parotid
- Sciatic nerve
- Skeletal muscle, biceps femoris
- Skin
- Spinal cord [cervical, thoracic, and lumbar]
- Spleen [1]
- Thymus [1]
- Thyroid/parathyroid [1,2]
- Tongue
- Trachea
- Ureter
- Urinary bladder

[1] Weighed organ; [2] Paired organs; [3] Examined only in rabbits; [4] Examined only in mice. In rabbits only the right mandibular gland was weighed. In mice the combined weight of the right mandibular and sublingual salivary gland was obtained.