Supplementary Figure S1. Evaluation of UPARANT toxic effects. (A-E) Maximum tolerated dose (MTD) was evaluated by histological examination of hematoxylin/eosin stained
sections of retina/choroids of 15 mice without CNV lesions. UPARANT was dissolved in PBS and serially diluted using a half-log interval to obtain four different concentrations: 108 (E), 36 (D), 12 (C) and 4 (B) mg mL\(^{-1}\). Each solution was injected (1 µL) in the left eye of 3 mice, while the right eyes were injected with vehicle (PBS; A). For clarity, UPARANT concentrations in succinate salt are equivalent to 84.86, 28.29, 9.43 and 3.14 mg mL\(^{-1}\) of active UPARANT peptide. To mimic the drug administration schedule used in the CNV model, mice received 3 subsequent injections of UPARANT, spaced 4 days apart from each other. The mice were sacrificed 3 days after the last injection. No evidence of abnormalities in the choroid, RPE or retina were observed after vehicle or UPARANT at 4, 12, or 36 mg mL\(^{-1}\). RPE swelling and pigmented cell migration toward the photoreceptor layer (arrows), as well as ruptures and deficiencies in the Bruch’s membrane (asterisks), were observed at 108 mg mL\(^{-1}\). Scale bar, 100 µm. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; RPE, retinal pigment epithelium. (F-I) Transcript levels of apoptotic markers were evaluated by qPCR in RPE-choroid complexes of mice untreated, or treated with vehicle or with UPARANT at 4 or 12 mg mL\(^{-1}\). Data were analyzed by the formula \(2^{\Delta\Delta CT}\) using RpL13a as internal standard. In particular, laser treatment increased transcript levels of Bax (F), cytochrome c (H) and caspase-3 (I) without affecting Bcl-2 (G). After laser treatment, transcript levels of Bax, cytochrome c and caspase-3 were about 2.2- \((P < 0.01)\), 1.9- \((P < 0.001)\) and 2.0-fold \((P < 0.001)\) higher than in controls, respectively. UPARANT had no affect on transcript levels of apoptotic markers \(*P < 0.01\) and **\(P < 0.001\) versus control; ANOVA). Data are presented as scatter plots. Each plot represents the mean ± SEM of data from 3 independent samples, each containing 1 RPE-choroid complex.
Supplementary Figure S2. Comparison of the effects of 4 mg mL\(^{-1}\) UPARANT on VEGF transcripts with those of systemic UPARANT. Transcript levels of VEGF were evaluated by qPCR in RPE-choroid complexes of mice untreated, or treated with vehicle or with UPARANT administered either intravitreally as described at 4 mg mL\(^{-1}\), or subcutaneous (sc), at doses of 0.3 or 1 mg, administered every second day starting at day 2 after laser-induction. Data were analyzed by the formula \(2^{-\Delta\Delta CT}\) using RpL13a as internal standard. Systemic UPARANT was effective in reducing VEGF transcripts that increased by about 1.8-fold \((P < 0.001)\) after laser treatment. In particular, intravitreal UPARANT reduced levels of VEGF by approximately 23% \((P < 0.001)\), while 0.3 and 1 mg UPARANT reduced VEGF by about 11% \((P < 0.01)\) and 30% \((P < 0.001)\), respectively. VEGF levels after 0.3 mg UPARANT ranged 1.2-fold higher \((P < 0.01)\) than after intravitreal UPARANT. VEGF levels after 1 mg UPARANT were no different than after intravitreal UPARANT \((^*P < 0.001 \text{ versus control;} \ $P < 0.01 \text{ and } ^{**}P < 0.001 \text{ versus vehicle-treated;} \ ^{§}P < 0.01 \text{ versus } 4 \text{ mg mL}^{-1} \text{ UPARANT; ANOVA})\). Data are presented as scatter plots. Each plot represents the mean ± SEM of data from 3 independent samples, each containing 1 RPE-choroid complex.
Supplementary Figure S3. Effects of UPARANT on angiogenic factors. Protein levels of VEGF (A, B), Ang-2 (A, C) and FGF-2 (A, D) were evaluated by Western blot and densitometric analysis in RPE-choroid complexes of mice untreated or treated with vehicle or with UPARANT at 4 or 12 mg mL⁻¹. Protein expression was relative to the loading control β-actin. After laser treatment, protein levels of VEGF, Ang-2 and FGF-2 were about 2.6-, 4.2- and 3.1-fold higher than in controls, respectively. Upon 4 mg mL⁻¹ UPARANT, the levels of VEGF, Ang-2 and FGF-2 were about 1.9-, 2.1- and 1.8-fold higher than in controls, respectively. Upon 12 mg mL⁻¹ UPARANT, the levels of VEGF, Ang-2 and FGF-2 did not statistically differ from those measured in controls (*P < 0.01 and **P < 0.001 versus control; §P < 0.001 versus vehicle-treated; ANOVA). Data are presented as scatter plots. Each plot represents the mean ± SEM of data from 3 independent samples, each containing 2 RPE-choroid complexes.