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Supplementary information

Agonist antibody to guanylate cyclase receptor NPR1 regulates vascular tone

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Agonist antibody to guanylate cyclase receptor NPR1 regulates vascular tone

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Supplementary Table 1. Number of individuals include in genetic association analyses by cohort and ancestry group

	Ancestry groups				
Cohort	**AFR**	**AMR**	**EAS**	**EUR**	**SAS**
Geisinger Health System MyCode	4821	1967	436	157129	-
Indiana University School of Medicine	1312	-	-	3424	-
Malmö Diet and Cancer Study	-	-	-	28935	-
Mount Sinai BioMe Biobank	11746	3899	-	10390	-
U.K. Biobank	9083	611	2213	430825	10340
Penn Medicine Biobank	10869	682	656	28515	533

AFR, African; AMR, Admixed American; EAS, East Asian; EUR, European; SAS, South Asia.

Supplementary Table 2. NPR1 protein-altering variants identified as presumed loss- or gain-of-function based on their directional association with systolic or diastolic blood pressure

Mask	Variant	Systolic blood pressure			Diastolic blood pressure		
		N (Ref/Het/Alt)	Beta SD (95% CI)	P-value	N (Ref/Het/Alt)	Beta SD (95% CI)	P-value
LOF	1:153679356:G:A	277309/11/0	0.80 (0.28 to 1.31)	0.0024	277123/11/0	0.49 (−0.06 to 1.05)	0.082
	1:153686154:G:A	208328/18/0	0.55 (0.14 to 0.95)	0.0084	208328/18/0	0.31 (−0.12 to 0.7)]	0.157
	1:153686175:G:A	297370/35/0	0.35 (0.06 to 0.64)	0.016	297184/35/0	0.41 (0.10 to 0.72)	0.0088
	1:153688056:T:A	293712/147/0	0.37 (0.23 to 0.51)	3.10E-07	293526/147/0	0.31 (0.16 to 0.46)	5.30E-05
	1:153688208:C:T	288459/64/0	0.34 (0.13 to 0.55)	0.0019	288273/64/0	0.27 (0.04 to 0.50)	0.02
	1:153689947:G:A*	308442/9891/97	0.06 (0.05 to 0.08)	1.2E-13	308252/9880/97	0.05 (0.04 to 0.07)	9.8e-09
	1:153690280:C:T	277308/12/0	0.58 (0.09 to 1.07)	0.021	277122/12/0	0.59 (0.06 to 1.12)	0.028
	1:153693174:C:T*	307977/477/0	0.12 (0.05 to 0.20)	0.0016	307775/478/0	0.12 (0.03 to 0.20)	0.0064
	1:153693367:C:T	292857/42/0	0.34 (0.08 to 0.61)	0.011	292671/42/0	0.19 (−0.09 to 0.47)	0.188

GOF	1:153683428:G:A	11962/11/0	−0.60 (−1.11 to −0.10)	0.02	11948/11/0	−0.70 (−1.24 to −0.16)	0.011
	1:153685067:C:T	300752/23/0	−0.39 (−0.74 to −0.03)	0.032	300564/23/0	−0.28 (−0.66 to 0.10)	0.151
	1:153685821:G:A**	315707/2169/2	−0.07 (−0.10 to −0.03)	0.00031	315507/2168/2	−0.07 (−0.11 to −0.03)	0.00035
	1:153686205:T:C	288466/60/0	−0.23 (−0.45 to −0.01)	0.041	288280/60/0	−0.12 (−0.35 to 0.12)	0.323
	1:153686649:C:T	292706/30/0	−0.32 (−0.63 to −0.01)	0.046	292520/30/0	−0.33 (−0.67 to 0.00)	0.051

*Previously identified as LOF[15]. **Previously identified as GOF[15].

Alt, alternative allele; CI, confidence interval; Freq, frequency; GOF, gain-of-function; Het, heterozygous allele; LOF, loss-of-function; Ref, reference allele; SD, standard deviation.

Supplementary Table 3. Summary of kinetic binding parameters for the interaction of REGN5381 with NPR1 at pH 7.4

Protein injected over surface-captured REGN5381	Kinetic binding parameters (37°C)			
	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	$t_{½}$ (min)
hNPR1.mmH	5.92E+04	4.15E-04	7.00E-09	28
hNPR1.mmH + 10xANP	5.19E+04	1.74E-04	3.35E-09	66
hNPR1.mmH + 10xBNP	5.65E+04	3.13E-04	5.53E-09	37
mfNPR1.mmH	6.08E+04	3.87E-04	6.36E-09	30
cNPR1.mmH	4.11E+04	2.40E-04	5.85E-07	0.5
mNPR1.mmH	NB			

Surface plasmon resonance-binding assays were performed by injecting varying concentrations of NPR1 proteins over captured REGN5381 sensor surfaces at pH 7.4. hNPR1 was tested alone and following preincubation with 10xANP and 10xBNP.

ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; cNPR1, canine NPR1; hNPR1, human NPR1; k_a, association rate constant; k_d, dissociation rate constant; K_D, equilibrium dissociation constant; mfNPR1, cynomolgus monkey NPR1; mmH, C-terminal myc-myc-hexahistidine tag; mNPR1, mouse NPR1; NB, no detectable binding observed; NPR1, natriuretic peptide receptor 1; NT, not tested; $t_{½}$, dissociative half-life.

Supplementary Table 4. Binding specificity for the interaction of REGN5381 with NPR1, NPR2, NPR3, and ANP

			Binding kinetics (−25°C)						
Injected analyte	**Association time**	**Dissociation time**	**Highest Ag concentration injected**	**REGN5381 capture level (RU)**	**NPR1 bound at highest concentration (RU)**	**k_a ($M^{-1}s^{-1}$)**	**k_d (s^{-1})**	**K_D (M)**	**t½ (min)**
hNPR1.mmH	5 minutes	10 minutes	100 nM	106 ± 0.2	51	4.16E+04	2.16E−04	5.19E−09	53.4
hNPR1.mmH + 10xANP				106 ± 0.2	48	4.80E+04	6.19E−05	1.29E−09	186.5
hNPR2.mmH				105 ± 0.1	−1	NB	NB	NB	NB
hNPR3.mm				105 ± 0.3	0	NB	NB	NB	NB
ANP			1000 nM	685 ± 4.0	0	NB	NB	NB	NB

Surface plasmon resonance-binding assays were performed by injecting varying concentrations of NPR1, NPR1 + 10xANP, hNPR2, hNPR3 protein and ANP over captured REGN5381 sensor surfaces at pH 7.4.

ANP, atrial natriuretic peptide; hNPR1, human NPR1; hNPR2, human NPR2; hNPR3, human NPR3; ka, association rate constant; kd, dissociation rate constant; KD, equilibrium dissociation constant; mmH, C-terminal myc-myc-hexahistidine tag; mNPR1, mouse NPR1; NB, no detectable binding observed; NPR1, natriuretic peptide receptor 1; t½, dissociative half-life.

Supplementary Table 5. Summary of REGN5381 Fab residues interacting with NPR1 residues in the REGN5381+NPR1+ANP complex

NPR1 residue	Antibody residue(s) interacting with indicated NPR1 residue	
	Heavy chain	Light chain
Gly1	Tyr108	-
Asn2	-	Asp30, Try32
Gly43	Tyr106	-
Trp44	Asn104, Tyr107, Tyr108	-
Trp74	-	Lys69
Glu75	-	Ser28
Asn77	-	Gln27, Ser28
Leu336	Tyr105	-
Ala337	Ser55	-
Gly339	Trp50	-
Thr341	Tyr107	-

Thr343	-	Tyr92, Ser93
NAG1011 (linked to Asn2)	-	Ser31

Calculations are based on monomer B of NPR1. REGN5381 does not make interactions with the bound ANP, or with the opposite monomer of NPR1 from its primary binding site. Contacts are defined as two atoms (excluding hydrogens) within 3.5 Å for polar interactions, or within 4.0 Å for hydrophobic interactions. Antibody numbering is sequential, not Kabat or any other convention. NPR1 numbering is based on the mature protein after signal peptide cleavage. Density is visible for an N-glycan linked to NPR1 Asn347, and this glycan may contact REGN5381, but the EM density is too fragmentary to permit modeling of the glycan.

Ala, alanine; ANP, atrial natriuretic peptide; Asn, asparagine; Asp, aspartic acid; Gln, glutamine; Gly, glycine; Glu, glutamic acid; EM, electron microscopy; Leu, leucine; Lys, lysine; NAG, N-Acetyl-D-Glucosamine; NPR1, natriuretic peptide receptor; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine.

Supplementary Table 6. Definition of heart failure for genetic association analyses

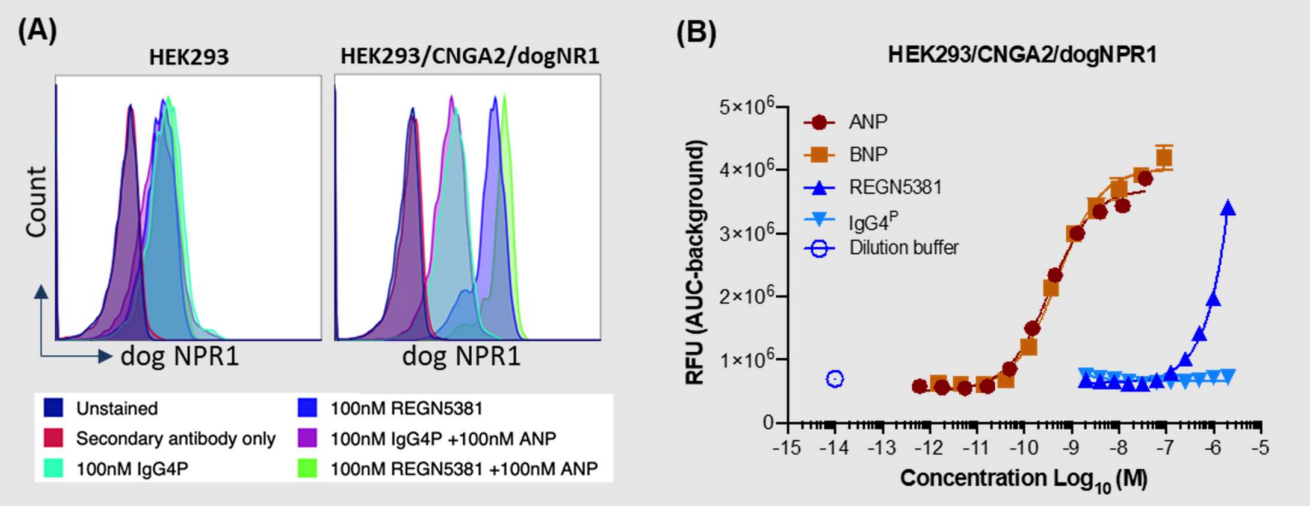
Data source	Definition of heart failure cases
ICD-9 (GHS only)	428 (Heart failure)
	40201 (Malignant hypertensive heart disease with heart failure)
	40211 (Benign hypertensive heart disease with heart failure)
	40291 (Unspecified hypertensive heart disease with heart failure)
ICD-10 codes (all cohorts)	I50 (Heart failure)
	I110 (Hypertensive heart disease with heart failure)
	I130 (Hypertensive heart and chronic kidney disease with heart failure and stage 1 through stage 4 chronic kidney disease, or unspecified chronic kidney disease)
	I132 (Hypertensive heart and chronic kidney disease with heart failure and with stage 5 chronic kidney disease, or end stage renal disease)
OPCS4 procedure codes (UKB only)	K733 (Renewal of intravenous biventricular cardiac pacemaker)
	K607 (Implantation of intravenous biventricular cardiac pacemaker system)
	K617 (Implantation of biventricular cardiac pacemaker system)
	K596 (Implantation of cardioverter defibrillator using three electrode leads)
	K597 (Renewal of cardioverter defibrillator using three electrode leads)
Self-report by interview (UKB only)	1076 (heart failure/pulmonary oedema)

	FQA (Transplantation of heart)
NOMESCO v.1 (MDCS only)	FPE26 (Implantation of transvenous cardiac pacemaker with biventricular electrodes)
	FPG36 (Implantation of transvenous cardioverter-defibrillator with generator and with biventricular electrodes)
NOMESCO v.6 (MDCS only)	3085 (Heart transplant)
	0034 (Left ventricle assist device or right ventricle assist device)

ICD10 indicates the 10th revision of the International Statistical Classification of Diseases and Related Health Problems, corresponding ICD9 codes were mapped to ICD10 when possible; OPCS4 indicates Office of Population Censuses and Surveys (OPCS) Classification of Interventions and Procedures version 4; NOMESCO indicates Nordic Medico-Statistical Committee procedure codes.

GHS, Geisinger Health System MyCode; ICD, International Classification of Diseases; MDCS, Malmö Diet and Cancer Study; UKB, U.K. Biobank.

Supplementary Figure 1. REGN5381 bound and activated canine cNPR1-mediated calcium mobilization.

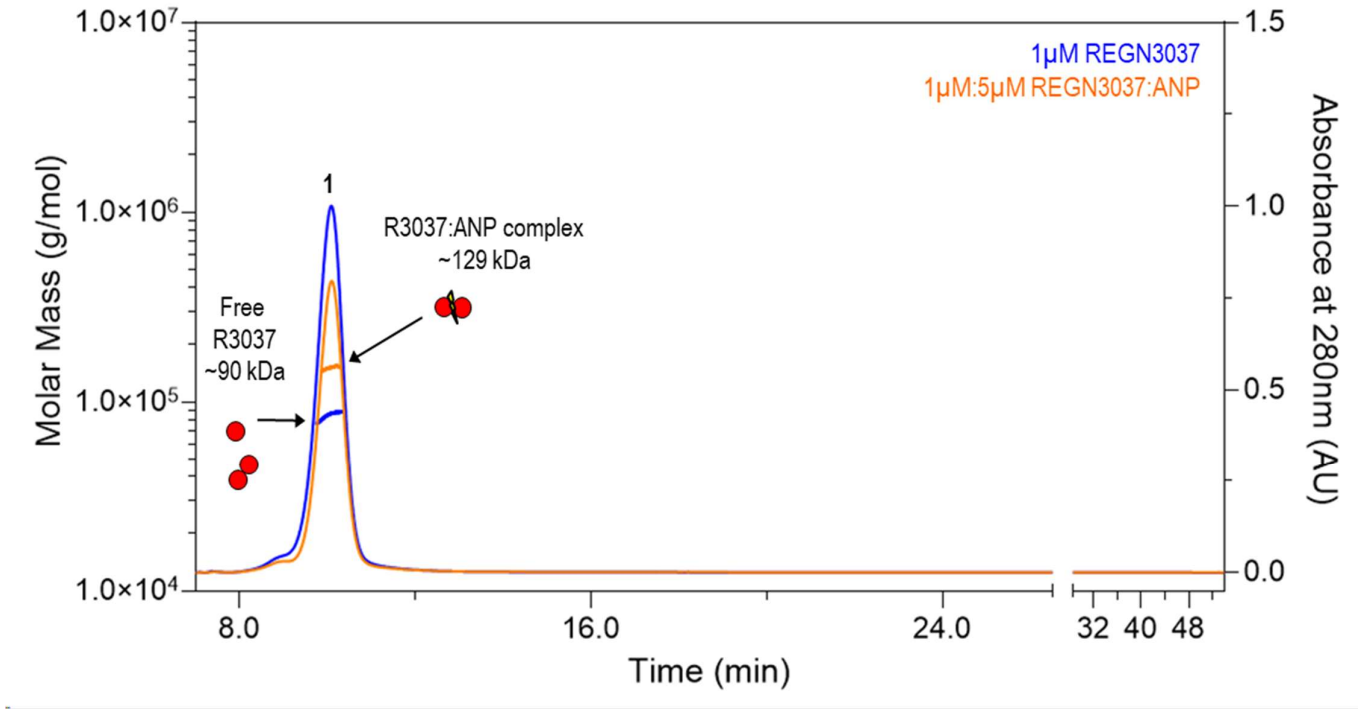


A, Specific binding of REGN5381 to cNPR1 was tested with HEK293 or HEK293/CNGA2/cNPR1 cells at 100 nM in the absence or presence of 100 nM ANP by flow cytometry. **B**, Canine cNPR1 activity was evaluated with HEK293/CNGA2/cNPR1 cells that were treated with ANP (dark brown solid circles), BNP (brown solid squares), REGN5381 (blue solid triangles), $IgG4^P$ (light blue triangles) or control (dilution buffer, blue open circles) by calcium flux assay. Open symbols indicate assay conditions when no test article was added, and closed symbols indicate assay conditions when the test article was added in a range of concentrations.

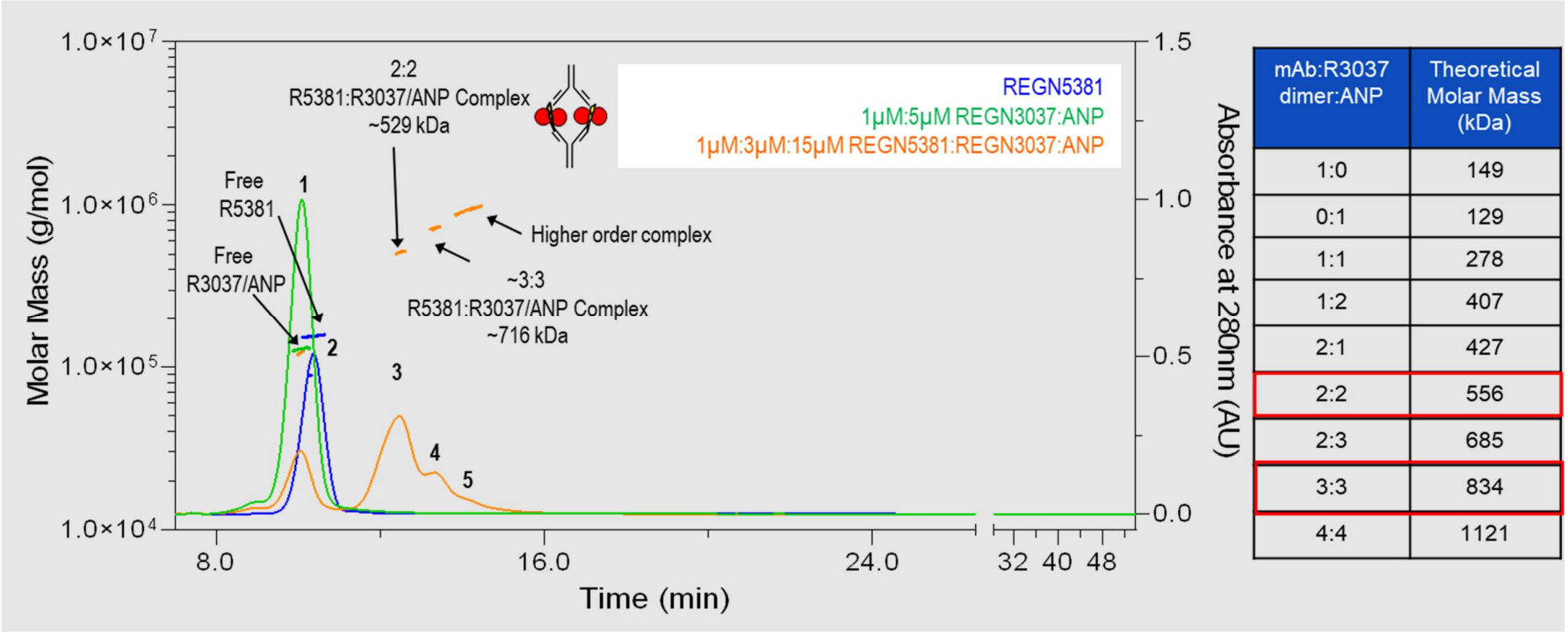
ANP, atrial natriuretic peptide; AUC, area under the curve; BNP, brain natriuretic peptide; CNGA2, cyclic nucleotide gated channel alpha 2; cNPR1, canine NPR1; $IgG4^P$, non-binding immunoglobulin G control; NPR1, natriuretic peptide receptor 1; RFU, relative fluorescence units.

Supplementary Figure 2. Molar mass and distribution of REGN5381:NPR1:ANP complexes analyzed by asymmetric flow field-flow fractionation coupled to multi-angle light scattering (A4F-MALS).

A.



B.



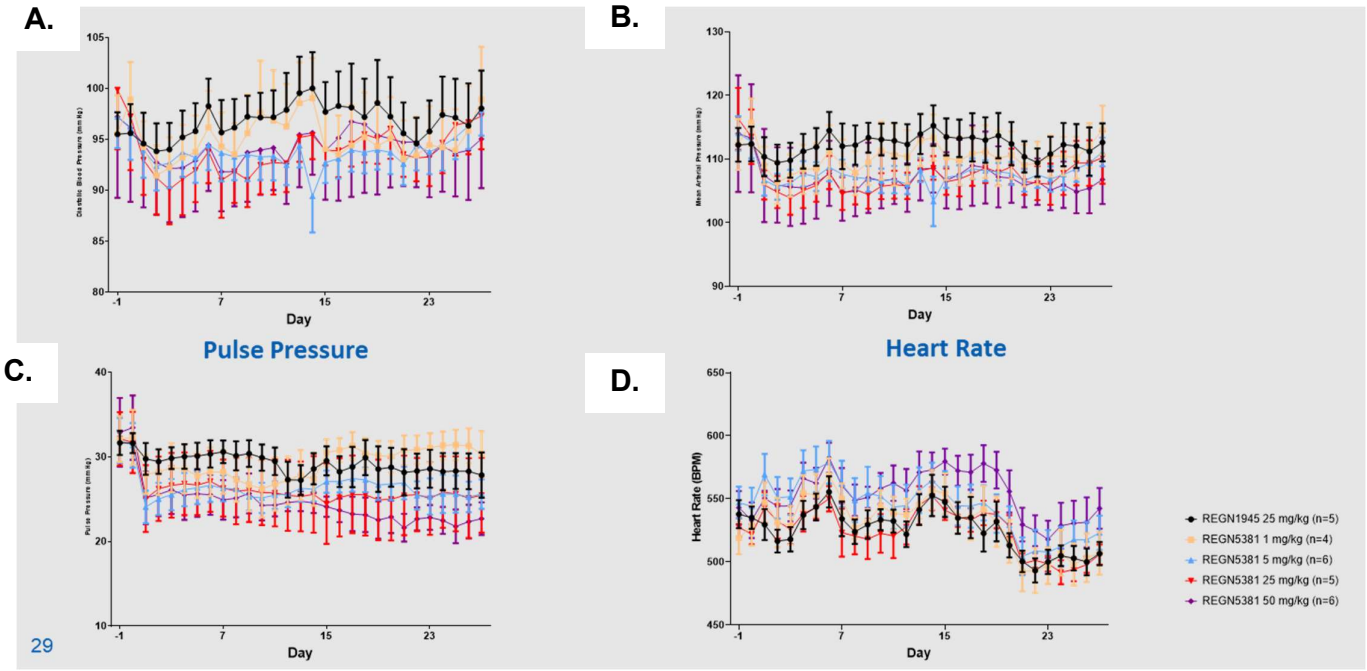
mAb:R3037 dimer:ANP	Theoretical Molar Mass (kDa)
1:0	149
0:1	129
1:1	278
1:2	407
2:1	427
2:2	556
2:3	685
3:3	834
4:4	1121

Relative UV absorbance at 215 nm as a function of retention time is shown for each sample and the measured molar masses of resolved peaks are indicated. **A**, The A4F-MALS chromatograph of NPR1 (blue), and 1 μM:5 μM NPR1:ANP molar ratio complex (orange). It demonstrates that NPR1 might be weakly associated, ANP binding caused dimerization of the hNPR1. **B**, Representative fractograms of free REGN5381 (blue), free hNPR1/ANP dimer (green), and complexes of REGN5381, hNPR1, and ANP combined in a 1 μM:3 μM:15 μM molar ratio (orange) are overlaid. Peaks 1 and 2 correspond to free hNPR1/ANP dimer (~129 kDa) and free REGN5381 (~149 kDa), while peaks 3–5 correspond to REGN5381:NPR1/ANP complexes. Based on the calculated molar masses of the individual components, peak 3 likely corresponds to a 2:2

REGN5381:NPR1/ANP dimer complex, whereas peaks 4 and 5 likely represent higher order complexes comprised of at least 3 molecules of REGN5381 bound to three or more hNPR1/ANP dimers.

ANP, atrial natriuretic peptide; hNPR1, human natriuretic peptide receptor; NPR1, natriuretic peptide receptor.

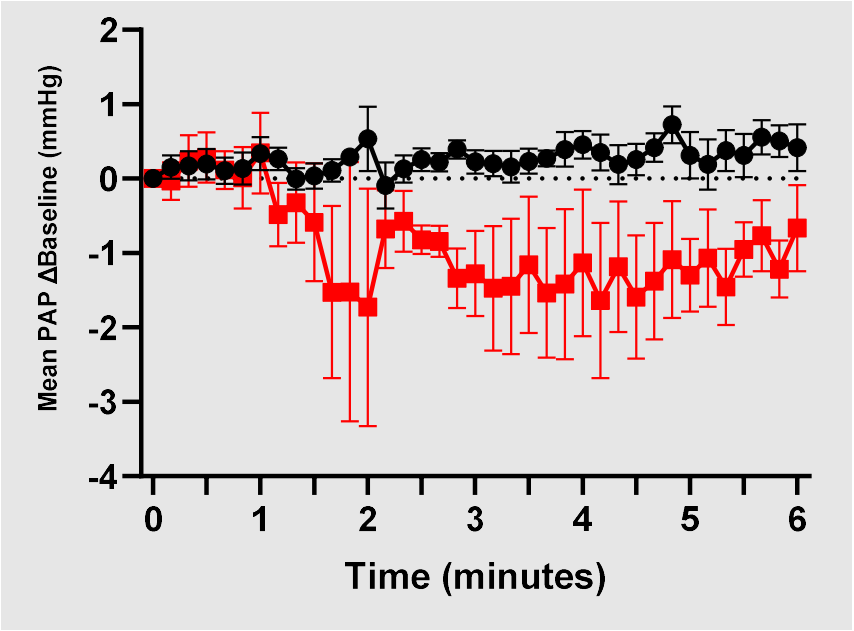
Supplementary Figure 3. Effect of REGN5381 on blood pressure, pulse pressure, and heart rate in normotensive NPR1$^{hu/hu}$ mice.



A, change in diastolic blood pressure. B, change in mean arterial blood pressure. C, change in pulse pressure. D, change in heart rate. Key: black circles, REGN1945 25 mg/kg (IgG4 isotype control; *n* = 5); yellow squares, REGN5381 1 mg/kg (*n* = 4); light blue triangles, REGN5381 5 mg/kg (*n* = 6); red triangles, REGN5381 25 mg/kg (*n* = 5); purple diamonds, REGN5381 50 mg/kg (*n* = 6).

Ig, immunoglobulin; NPR1, natriuretic peptide receptor; NPR1$^{hu/hu}$, NPR1 humanized.

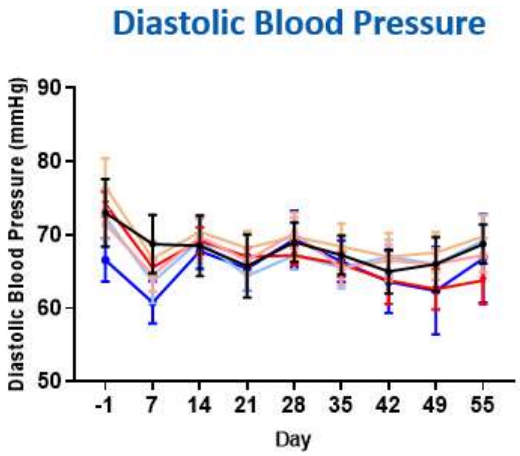
Supplementary Figure 4. Pulmonary arterial pressure in anesthetized Beagle canines administered REGN5381.



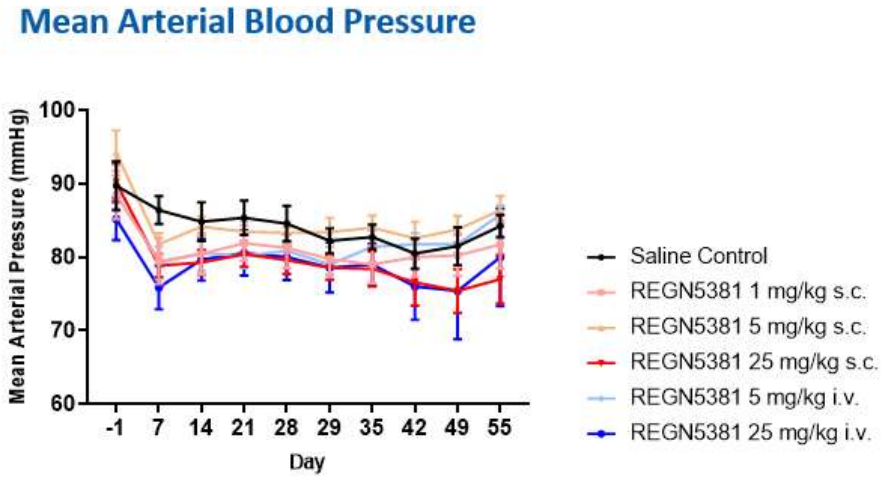
Beagle canines were instrumented with a pressure catheter. Pulmonary artery pressure (PAP) measurements were collected for each animal pre-dose (for baseline measurements) and acutely following administration of REGN5381 (red circles) or control (saline - black circles). Date are presented as mean ± standard error.

Supplementary Figure 5. A single dose of REGN5381 induced sustained blood pressure lowering in normotensive cynomolgus monkeys with decreased mean arterial pressure.

A.



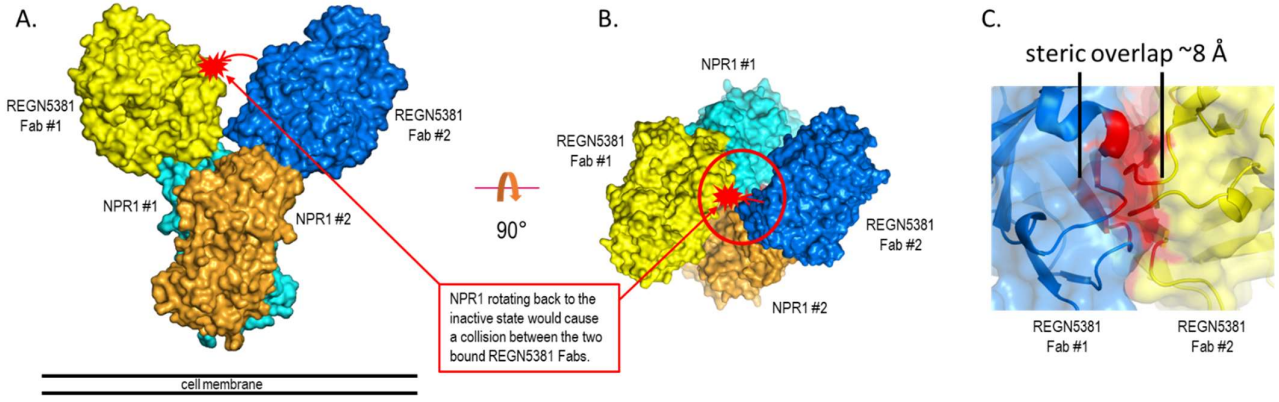
B.



A, Change in diastolic blood pressure following administration of single-dose REGN5381. **B**, Change in mean arterial blood pressure following administration of single-dose REGN5381 in normotensive cynomolgus monkeys. Key: black line, saline control; pink line, subcutaneous REGN5381 1 mg/kg; red line, subcutaneous REGN5381 25 mg/kg; light blue line, intravenous REGN5381 5 mg/kg; dark blue link, intravenous REGN5381 25 mg/kg.

IV, intravenous; SC, subcutaneous.

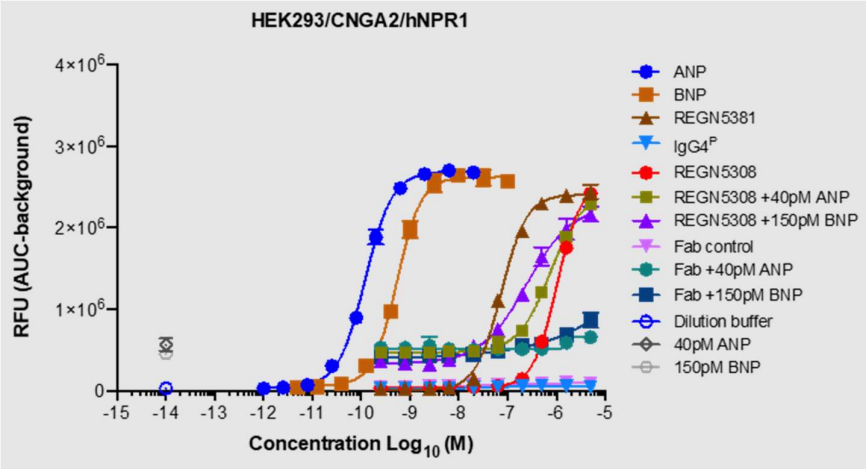
Supplementary Figure 6. Allosteric activation mechanism of REGN5381.



The active-like conformation of NPR1 ectodomain dimer bound to two REGN5381 Fab molecules is depicted in molecular surface representation, from a side view similar to Fig. 3B and from a top-down view (**A**), looking down at the cell membrane (**B**).The red arrow and "explosion" denote the steric clash that would occur if NPR1 monomer #2 tried to rotate back to the parallel, inactive conformation (see Fig. 3A) while REGN5381 Fab was still attached. **C**. A close up view of the modeled clashed conformation is shown in a semi-transparent surface and cartoon representation, with sterically overlapping regions (residues 16–25 and 61–85 from both REGN5381 Fab heavy chains) colored red. The approximate amount by which the two molecules interpenetrate is indicated.

ANP, atrial natriuretic peptide; Fab, antigen-binding fragment; NPR1, natriuretic peptide receptor.

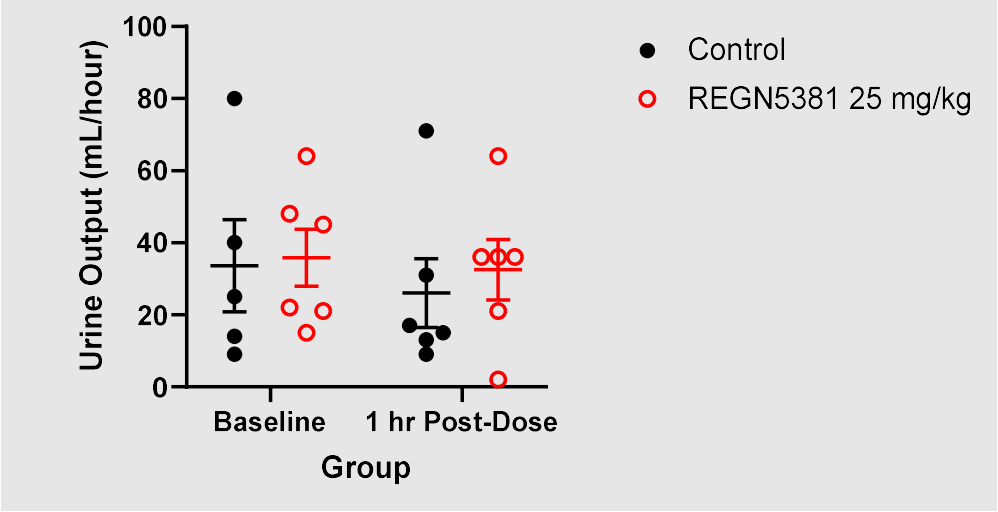
Supplementary Figure 7. Both REGN5381 full-length antibody and Fab fragment activated human NPR1-mediated calcium mobilization.



The ability of anti-NPR1 full antibody, REGN5381, or Fab fragment, REGN5308, to activate human NPR1 was tested with HEK293/CNGA2/hNPR1 cells as measured by calcium flux through CNGA2 channels. Open symbols indicate conditions when no test article was added, and closed symbols indicate conditions when the test article was added in a range of concentrations (blue solid circles, ANP; brown solid squares, BNP; dark brown solid triangles, REGN5381; light blue solid triangles, IgG4^{P}; red solid circles, REGN5308; green solid squares, REGN5308 + 40 pM ANP; purple solid triangles, REGN5308 + 150 pM BNP; pink solid triangles, Fab control; green solid circles, Fab + 40 pM ANP; dark blue solid squares, Fab + 150 pM BNP; open blue circles, dilution buffer; open dark grey diamonds, 40 pM ANP; open light grey circles, 150 pM BNP).

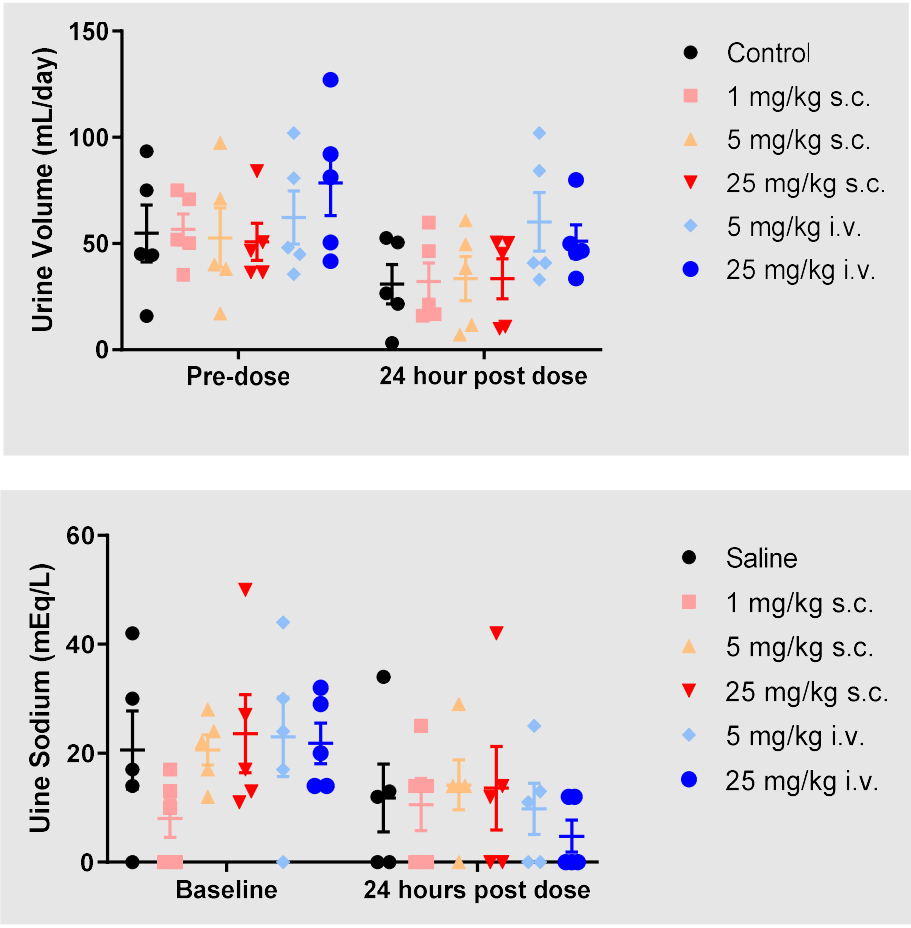
ANP, atrial natriuretic peptide; AUC, area under curve; BNP, brain natriuretic peptide; CNGA2, cyclic nucleotide gated channel alpha 2; IgG4^{P}, non-binding immunoglobulin G control; NPR1, natriuretic peptide receptor 1; RFU, relative fluorescence units.

Supplementary Figure 8. REGN5381 does not alter urine output in anesthetized Beagle canines.



Beagle canines were instrumented with a urine catheter. Urine volume measurements were collected for each animal pre-dose (for baseline measurements) and for 1-hour post-dose monitoring period following administration of REGN5381 (red circles) or control (saline - black circles). Date are presented as mean ± standard error.

Supplementary Figure 9. REGN5381 does not alter urine output or urine sodium in normotensive non-human primates.



Normotensive cynomolgus monkeys received a single intravenous bolus of saline/vehicle (*n* = 5; black circles) or REGN5381 subcutaneous (*n* = 5; 1 mg/kg [pink line]), (*n*=5 5 mg/kg [pale yellow line]), (*n* = 5;25 mg/kg [red line]) or intravenous (*n* = 5, 5 mg/kg [light blue line]) or (*n* = 5, 25 mg/kg [dark blue line]). Urine was collected via a pan placed in each cage for each animal pre-dose (for baseline measurements) and 24 hours post-dose. Urine volumes and urine sodium were assessed at baseline and 24 hours post-dose. Each symbol represents one individual animal, with the group mean ± SEM indicated.

IV, intravenous; SC, subcutaneous; SEM, standard error of the mean.