Supplementary Figures

Supplementary Fig. 1. Elabela fragments induce β-arrestin 2 recruitment to hAPJ and its internalization and these effects are antagonized by protamine.
Confocal pictures of U2OS cells stably expressing APJ and β-arrestin 2-GFP stimulated with PBS or with Elabela 11 (1μM), Elabela 22 (100nM) and Elabela 32 (100nM) for 1 hour (left panels). Pretreatment of U2OS cells with protamine (1μM (for Elabela 22 and Elabela 32) or 10μM (for Elabela 11)) fully inhibits Elabela fragments to induce β-arrestin 2-GFP recruitment to APJ and its internalization (right panels). The green channel illustrates the localization of β-arrestin 2-GFP, the blue channel (DAPI) shows the nucleus. Scale bar, 20μm. Data are representative of three independent experiments.

Supplementary Fig. 2. Protamine does not bind to Ether-a-go-go-Related Gene potassium channel (hERG).
Protamine does not displace binding of [3]H-Dofetilide to hERG when compared to Dofetilide. Data represent the mean ± s.e.m. of three independent experiments each performed in triplicate.

Supplementary Fig. 3. Apelin induces β-arrestin 1 recruitment to hAPJ and this effect is antagonized by protamine.
Dose-response curve of apelin 13 (**a**) or protamine (**b**) for β-arrestin 1 recruitment to APJ was measured by BRET in HEK-293T cells transiently co-expressing APJ-Rluc and β-arrestin 1-YFP. (**c**) Protamine dose-dependently inhibits β-arrestin 1 recruitment to APJ induced by apelin 13 (100 nM). Data are expressed as the percentage of the maximal BRET signal obtained in cells stimulated with apelin 13 100nM (100%). Data represent the mean ± s.e.m. of nine (**a**), five (**b**), three (**c**) independent experiments each performed in duplicate.

Supplementary Fig. 4. Poly-D-Lysine does not inhibit β-arrestin 2 recruitment to hAPJ induced by apelin.
(**a**) Dose-response curve of poly-D-lysine for β-arrestin 2 recruitment to APJ measured by BRET in HEK-293T cells transiently co-expressing APJ-Rluc and β-arrestin 2-YFP. (**b**) Poly-D-lysine (1μg/well) does not modify the dose-dependent β-arrestin 2 recruitment to APJ induced by apelin 13. Data represent the mean ± s.e.m. of four (**a**) and three (**b**) independent experiments each performed in duplicate.

Supplementary Fig. 5 . Protamine does not inhibit β-arrestin 2 recruitment to AT1a receptor induced by angiotensin.
BRET experiments were performed in HEK-293T cells coexpressing AT1aR-Rluc and β-arrestin 2-YFP. (**a**) Dose-response curve of angiotensin II; (**b**) Dose-response curve of protamine; (**c**) Dose-response curve of protamine on β-arrestin 2 recruitment induced by 100nM of angiotensin II. Data represent the mean ± s.e.m. of four (**a**; **b**) and three (**c**) independent experiments each performed in duplicate.

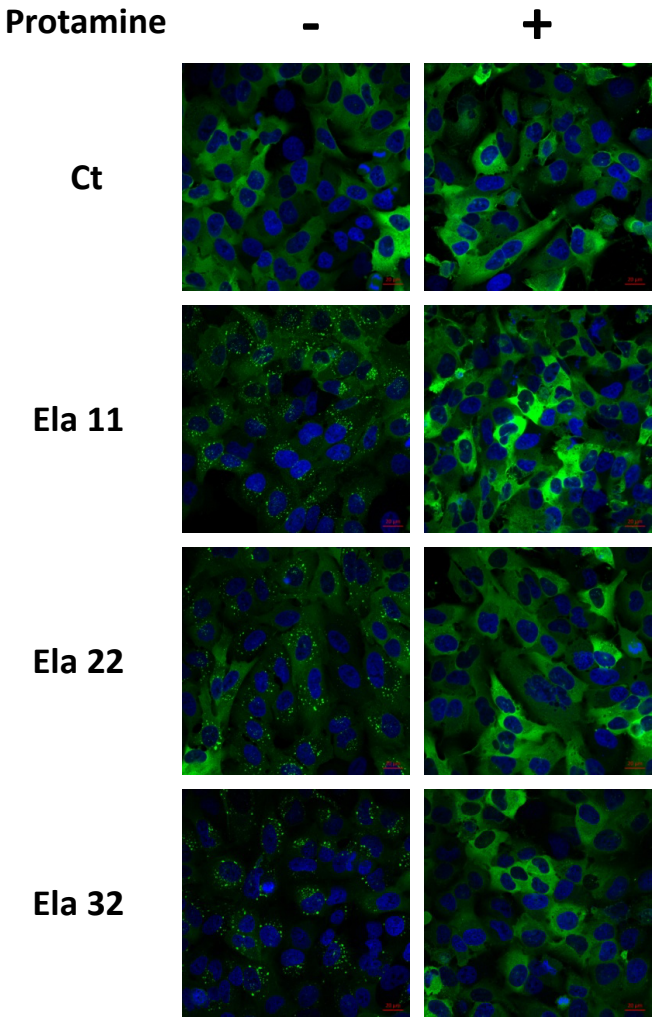
Supplementary Fig. 6. Effect of intravenous protamine injection on tumor growth.
TS/A tumor cells overexpressing apelin were injected subcutaneously in the flank of mice. Saline solution or protamine (5000 Units/kg) was injected intravenously twice a day and tumor volumes (TS/A tumor cell line overexpressing apelin) were measured. Only tumor volumes at days 10 and 21 are presented. Data are analysed by unpaired, Student's *t*-test. *, $P < 0.01$ (Protamine vs Saline solution). $n = 20$ animals per group.

Supplementary Fig. 7. Apelin and protamine do not modify insulin response to an oral glucose load.
During the Oral Glucose Tolerance Test, the blood was collected from the tail vein 15 min after the oral glucose load and insulin blood levels were measured. Mice were intravenously treated with saline (black bar, $n = 8$), apelin 13 (200 pmol/kg) (red bar, $n = 5$), protamine (10 Units/kg) and apelin 13 (200 pmol/kg) (blue bar, $n = 7$) or with protamine alone (10 Units/kg) (orange bar, $n = 5$).

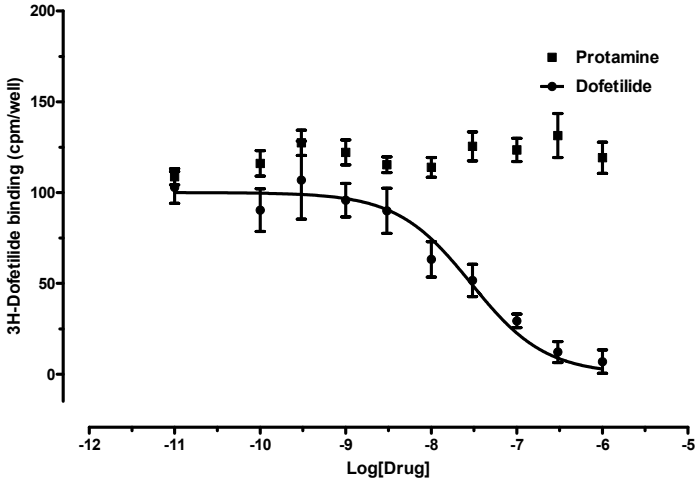
Supplementary Fig. 8. Protamine and heparin do not modify arterial blood pressure.
Time-dependent changes of arterial blood pressure after an intravenous administration at time 0 of saline (♦, black curve, $n = 5$), protamine (700 Units/kg) (●, orange curve, $n = 5$), heparin (1400 Units/kg) (■, brown curve, $n = 5$), protamine (700 Units/kg) with heparin (1400 Units/kg) (▲, grey curve, $n = 5$). Each point represents the mean ± s.e.m.

Supplementary Table 1. Screen and identification of protamine.

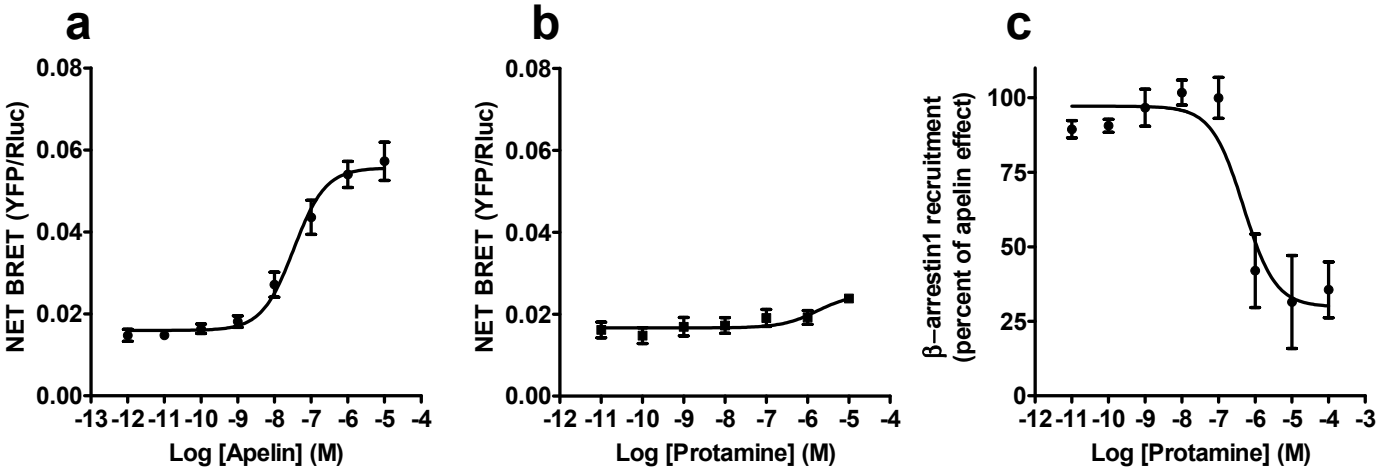
Supplementary Figure 1



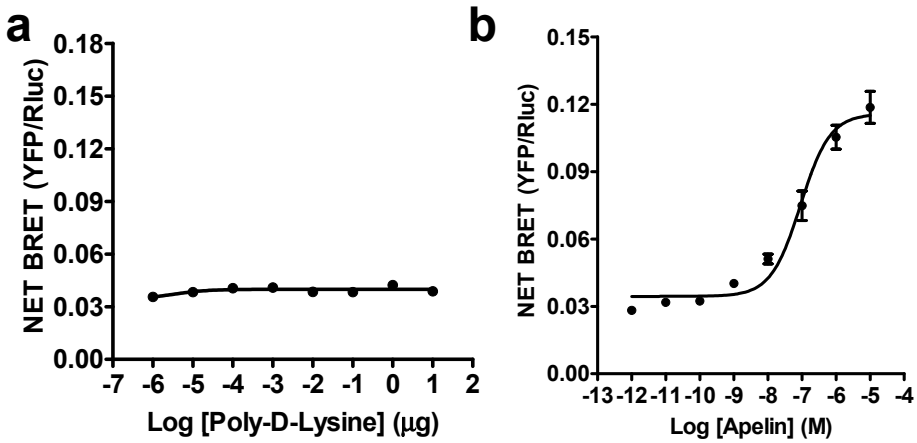
Supplementary Figure 2



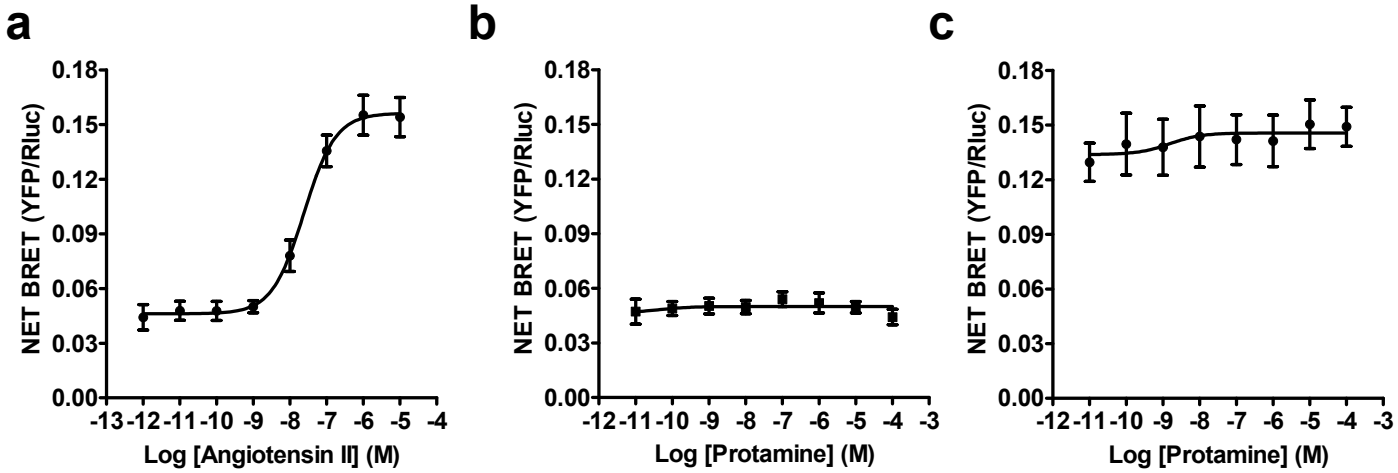
Supplementary Figure 3



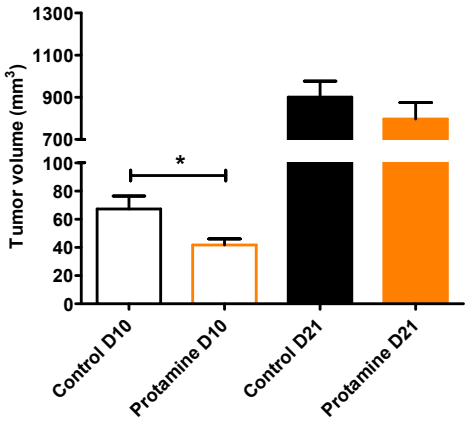
Supplementary Figure 4



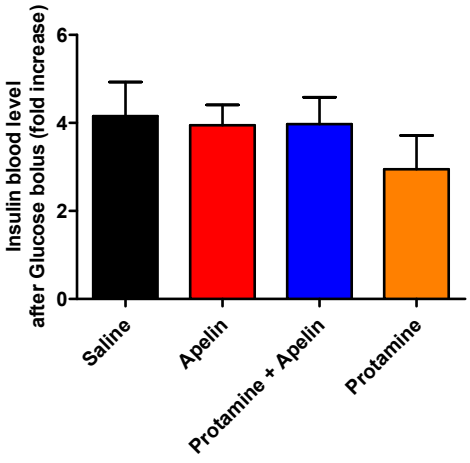
Supplementary Figure 5



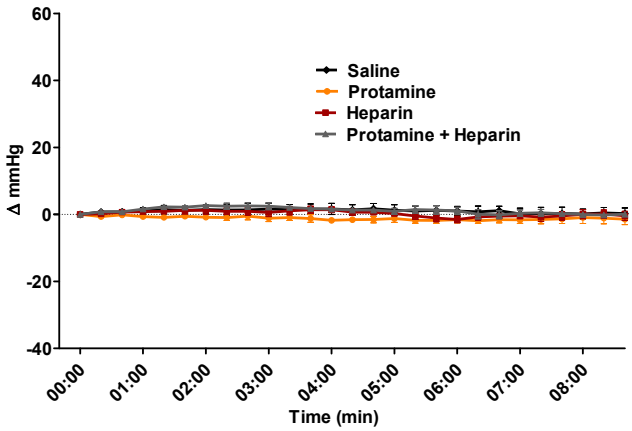
Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8



Supplementary Table 1

Protamine Chloride, grade V

Assay	
Type of assay	Refer to the online Materials and Methods Section : Fluorescence microscopy and screening
Target	Human apelin receptor (UniProtKB - P35414)
Primary measurement	Refer to the online Materials and Methods Section : Fluorescence microscopy and screening.
Key reagents	No specific reagents are required.
Assay protocol	Refer to the online Materials and Methods Section : Fluorescence microscopy and screening.

Libraries
John Hopkins University Clinical Compound Library (JHCCL): The JHCCL ver 1.0 contains 1514 compounds of which 1082 are FDA approved drugs and 432 are foreign approved drugs (7 384-well plates).
Tripos Compound Library: The Tripos Compound Library contains about 50,000 compounds (625 96-well plates)

Screen	
Format and concentration tested	10µM final of each compounds dissolved in DMSO were used per well. DMSO was used as negative control. Refer to the online Materials and Methods Section : Fluorescence microscopy and screening.
Assay validation/QC	Z factor for the assay was 0.6. The assay was performed in a similar manner as described: https://pubchem.ncbi.nlm.nih.gov/bioassay/493036

Post-HTS analysis	
Additional assays	Injectable solution of protamine sulfate (Protamine Choay®) was tested in the original assay and its antagonist activity measured using different approaches (binding, β-arrestins recruitment, cAMP measurements, ...)

Sequences of protamine fragments contained in the injectable solution of protamine sulfate (Protamine Choay®) used in the different experiments.

Protamine 1	H-Pro AAAAA Ser Ser Ser A Pro Ile AAAAA Pro A Ala Ser AAAAA Gly Gly AAAA OH
Protamine 2	H-Pro AAAA Ser Ser AA Pro Val AAAAA Pro A Val Ser AAAAA Gly Gly AAAA OH
Protamine 3	H-Pro AAAA Ser Ser Ser A Pro Val AAAAA Pro A Val Ser AAAAA Gly Gly AAAA OH
Protamine 4	H-Pro AAAA Ala Ser AA Ile AAAAA Pro A Val Ser AAAAA Gly Gly AAAA OH
Where A = Arginine, Ser = Serine, Pro = Proline, Ile = Isoleucine, Ala = Alanine, Gly = Glycine, Val = Valine	