Table S1. **Summary of the bioinformatic analysis targeting potential new substrates for PC5/6.** Listed are the most relevant protein candidates (mouse) selected based on the following screening criteria: i) presence of a signal peptide; ii) presence of a PC5/6 specific cleavage site (R/K)-X_n-R↓**N** (n = 0, 2, 4, or 6 and X is any aa, except Cys); iii) the proteins/peptides have cardiac relevance when their gene is knocked out; iv) the cleavage site motif is conserved in both human and mouse. In bold are depicted the candidates considered for in vitro digestion experiments with the four constitutively secreted PCs: furin, PACE4, PC5/6, and PC7. The synthetic peptides designed for this purpose are also listed in bold. TM, transmembrane domains

UniProt #	Symbol	Protein	Length (aa)	Motif Studied peptide	TM
Q9R229	**BMP10**	**Bone morphogenetic protein 10**	**421**	**DSSARIRR$_{313}$NAKG**	**-**
Q62226	**Shh**	**Sonic hedgehog**	**437**	**SGRYEGKITR$_{69}$NSE**	**-**
P29319-1	**Epha3**	**Ephrin type-A receptor 3**	**983**	**TIKKDRTSR$_{449}$NSISL**	**1**
O08852	**Pkd1**	**Polycystin-1**	**4293**	**QVLMRRSQR$_{2162}$NYLE**	**> 1**
O88393	**Tgfbr3**	**Transforming growth factor, beta receptor III**	**850**	**TELKIAR$_{183}$NIYI**	**1**
Q62181	Sema3c	Semaphorin-3C	751	KAYR$_{573}$N	-
Q01279	Egfr	Epidermal growth factor receptor	1210	RELPMR$_{138}$N	1
P11276	Fn1	Fibronectin 1	2477	RLPVNR$_{957}$N RVPPSR$_{1500}$N	-
Q62009	Postn	Periostin	838	KQGR$_{483}$N	-

Supplemental Table S1

Table S2. **Comparison of the in vitro processing of 12-mer mouse BMP10 peptides, WT-mBMP10 and N315D-mBMP10, at the predicted $\underline{R}_{311}IR\underline{R}_{314}\downarrow$ cleavage site, and the kinetic constants of the furin cleavage of the WT peptide. (A)** Each synthetic peptide (200 μM) was incubated for 2h in vitro with 2U of purified soluble furin, PC5/6, PACE4, or PC7, as described in Materials and Methods. The products were separated by RP-HPLC and detected at 214 nm. The % cleavage was calculated as the ratio of the normalized peak areas (peak area/number of peptide bonds) of the C-terminal fragment (NAKG for WT-mBMP10, or DAKG for N315-DmBMP10) and of the intact 12-mer peptide (at time zero). n.d., not determined. **(B)** Calculated kinetic constants from the digestion of the 12-mer WT peptide. Fitted values ± SD to the fit.

A

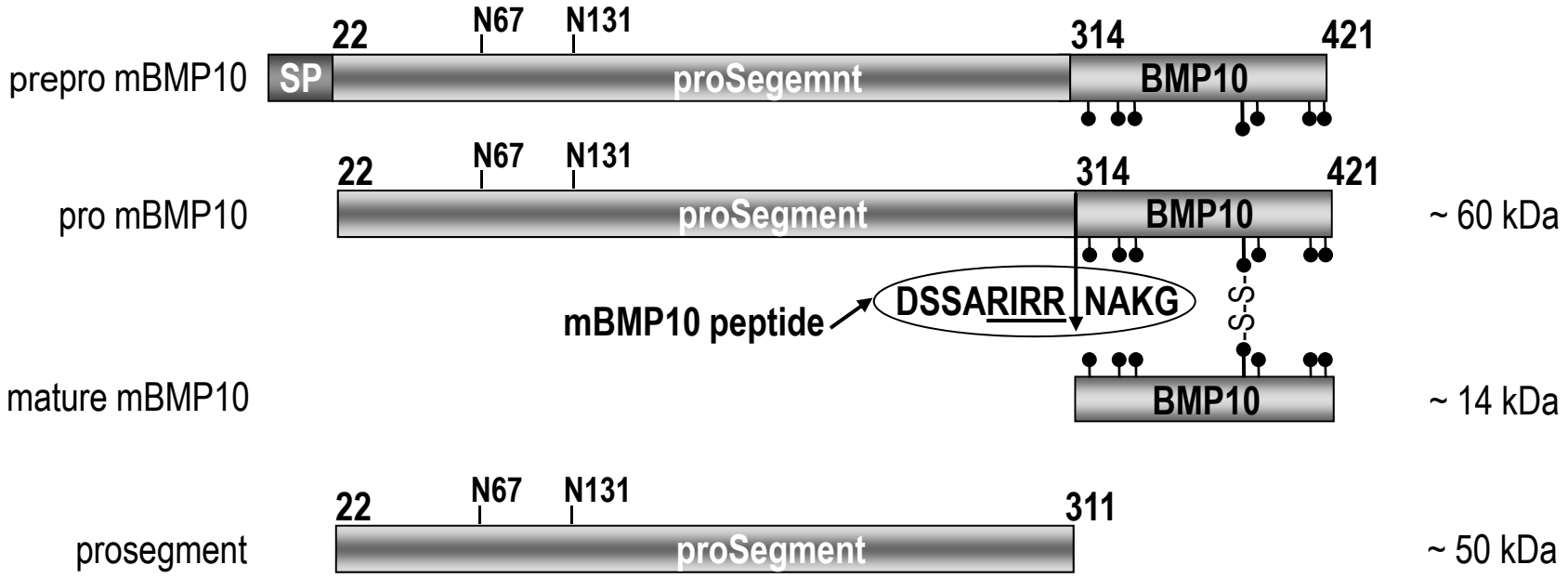
Peptide (200 μM)	Sequence	hFurin	hPACE4	hPC5	rPC7
WT-mBMP10	DSSA$\underline{R}_{310}$IR$\underline{R}_{313}$↓NAKG	86%	67%	68%	4%
N314D-mBMP10	DSSA$\underline{R}_{310}$IR$\underline{R}_{313}$↓ **D**AKG	42%	30%	23%	n.d.

B

$K_{m(app)}$ (μM)	$V_{max(app)}$ (μM·h^{-1})	$V_{max(app)}/K_{m(app)}$ (h^{-1})
34.9 ± 8.1	65.5 ± 5.4	1.9

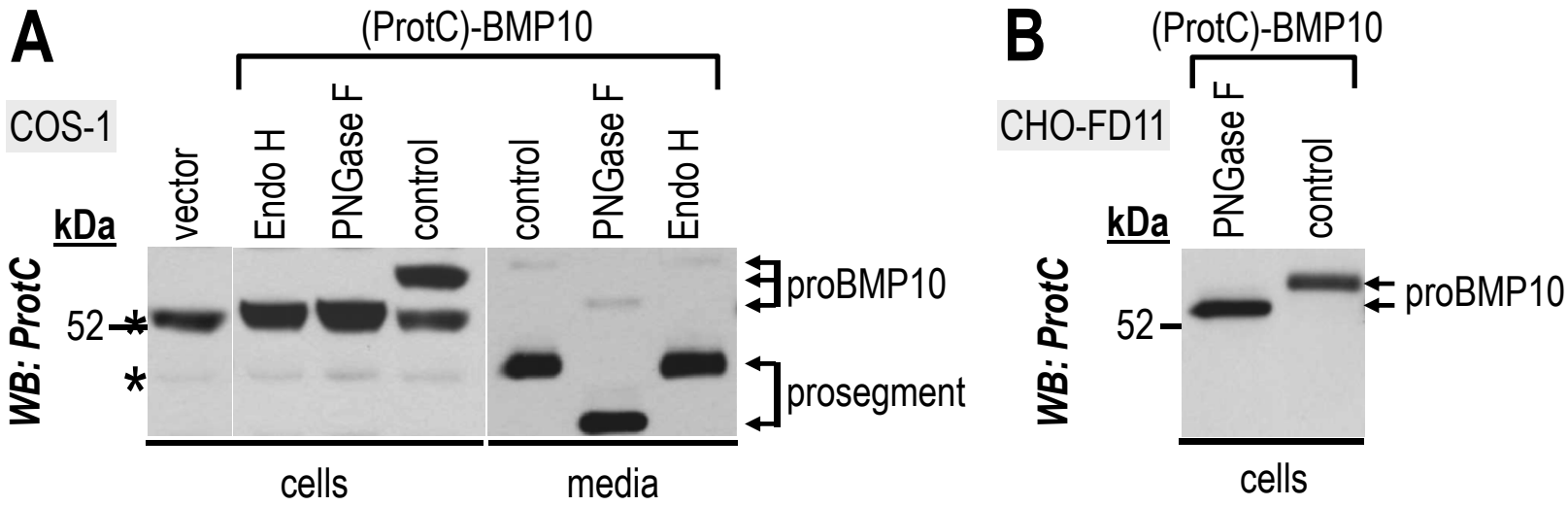
Supplemental Table S2

Fig. S1. **Schematic representation of the mouse preproBMP10 and its processed forms.** Upon removal of the signal peptide (SP), the resulting inactive BMP10 precursor (proBMP10; ~ 60 kDa) is proteolytically processed at the motif RIRR↓ into a 108 aa mature growth factor. Mature BMP10 contains the conserved pattern of 7 cysteines (depicted in ball-stick representation) out of which one is engaged in an interchain disulfide bond. Also shown are the predicted N-glycosylation sites (N67, N131) and the sequence of the 12-mer peptide (mBMP10 peptide) encompassing the predicted cleavage site of mouse proBMP10, which was used for the in vitro digestion studies.



Supplemental Figure S1

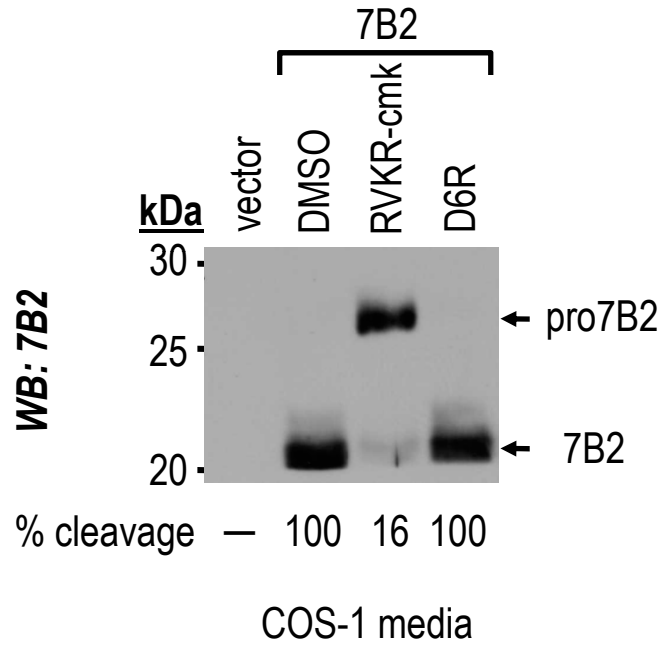
Fig. S2. **Endo H and PNGase F sensitivity of proBMP10 and processed BMP10 (prosegment)**. **(A)** Cell lysates and 20h conditioned media from COS-1 cells transiently transfected with (ProtC)-BMP10 were subjected to digestion with either PNGase F or endo H. The digestion mixtures were analyzed by Western blotting using a mouse ProtC Ab. The blot shows that both secreted proBMP10 and prosegment are PNGase F sensitive and endo H resistant. In the cells, proBMP10 is sensitive to both PNGase F and endo H. Note the presence in the cells of a non-specific band (*) just below and partially overlapping with the specific band resulting from the digestion with either of the two glycosidases. A control from cells expressing an empty vector is shown for reference. **(B)** Western blot of PNGase F digestion product of cell lysates from the furin-deficient CHO-FD11 cells expressing (ProtC)-BMP10 (proBMP10), as compared to control undigested protein. Note that in these cells the non-specific band seen in COS-1 cells (* in panel **A**) is not present.



Supplemental Figure S2

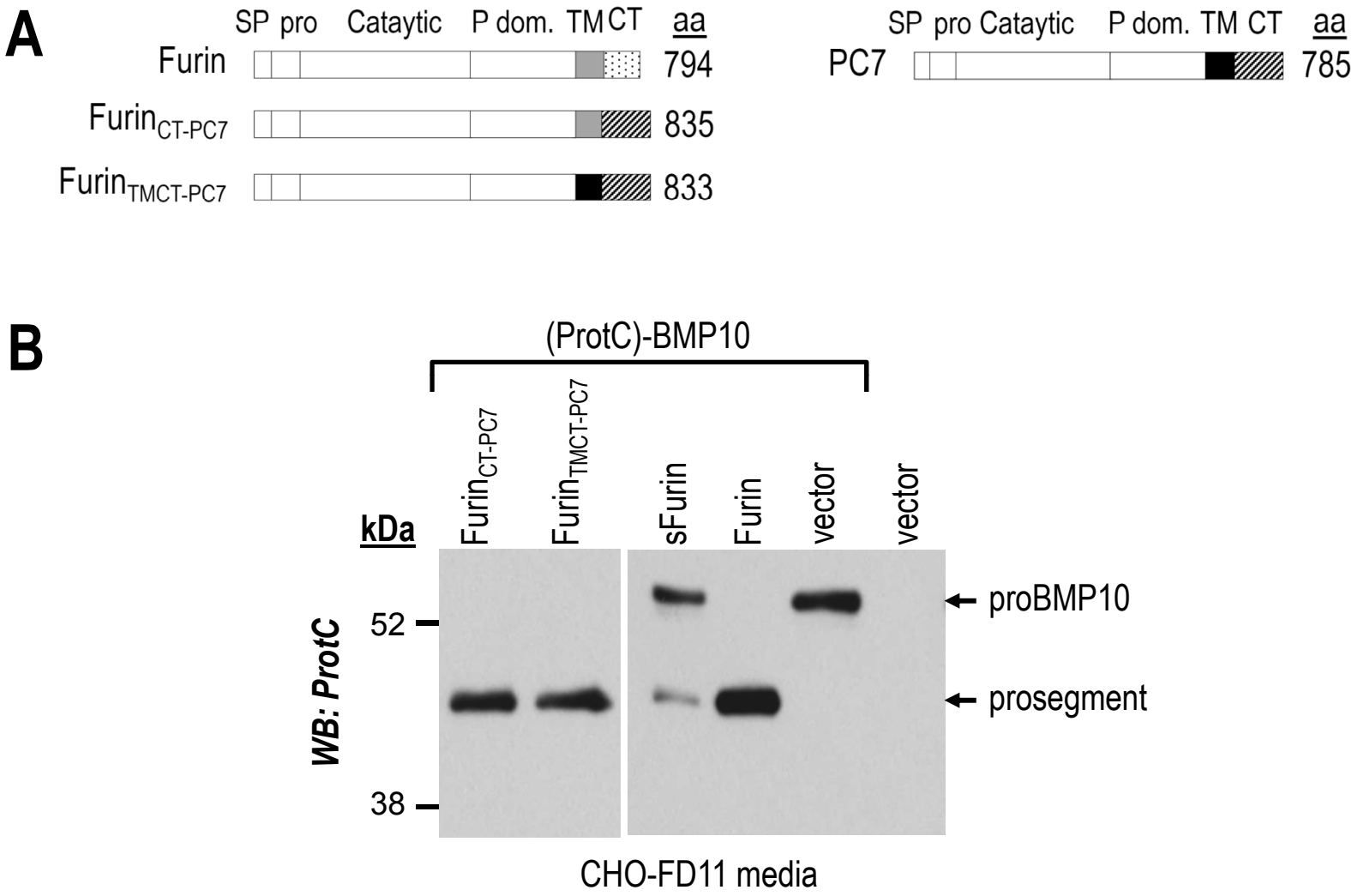
Fig. S3. **Effects of RVKR-cmk and D6R on the TGN processing of the neuroendocrine secretory precursor protein pro7B2 into 7B2.** Western blot analyses of 20h conditioned media from COS-1 cells transiently expressing either no protein (vector) or mouse pro7B2 and collected after no treatment (DMSO) or treatment with either the cell permeable convertase inhibitor RVKR-cmk (30 μM) or the cell surface inhibitor D6R (20 μM) . Proteins were revealed with a rabbit 7B2 Ab (1).

(1) Seidah, N. G., Hsi, K. L., De Serres, G., Rochemont, J., Hamelin, J., Antakly, T., Cantin, M., and Chretien, M. (1983) *Arch. Biochem. Biophys.* **225,** 525-534



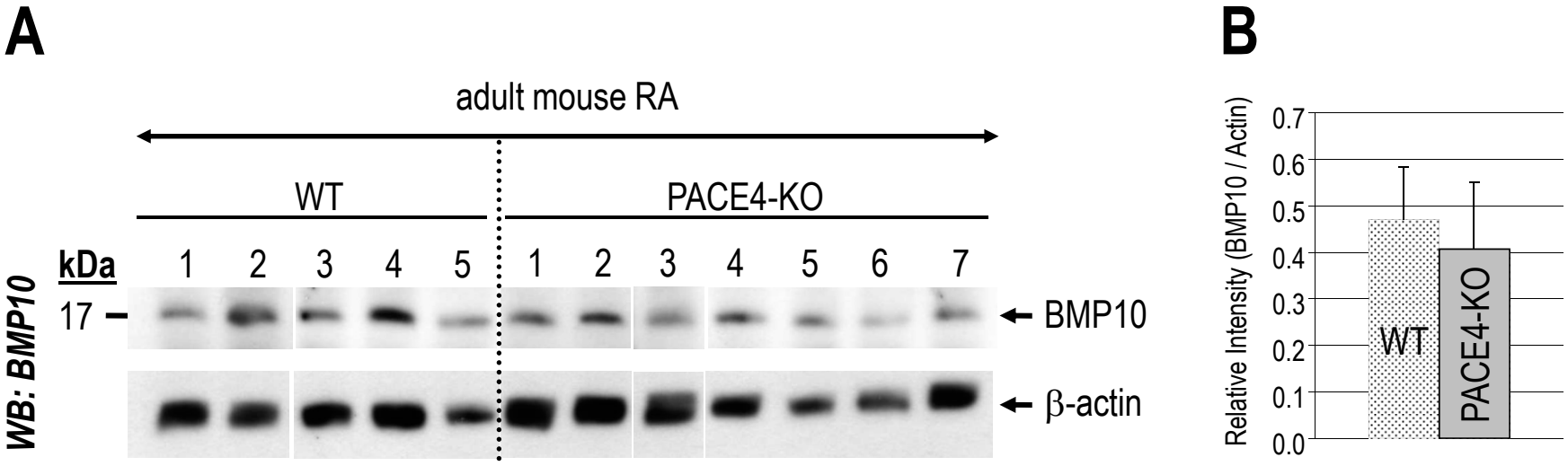
Supplemental Figure S3

Fig. S4. **The transmembrane domain of furin is required for its efficient processing of proBMP10. (A)** Schematic diagram depicting human proFurin and proPC7 and chimeras of human furin generated by the swapping of its transmembrane (TM) and/or cytopolasmic (CT) domains with the corresponding human PC7 ones ($furin_{CT\text{-}PC7}$; $furin_{TMCT\text{-}PC7}$) (33). **(B)** Western blot analyses of the 20h conditioned media from CHO-FD11 cells transiently transfected with either empty vector (vector; lane 6), or with a vector expressing (ProtC)-BMP10 and vectors expressing $furin_{CT\text{-}PC7}$ (lane 1), $furin_{TMCT\text{-}PC7}$ (lane 2), soluble furin (sFurin; lane 3), furin (lane 4), or empty vector (vector; lane 5). Proteins were revealed with a rabbit ProtC Ab. Please note that the two panels of the figure come from different parts of the same blot.



Supplemental Figure S4

Fig. S5. **In mouse heart, PACE4 does not significantly contribute to the processing of proBMP10**. **(A)** Tissue extracts from right atria isolated from adult male mice, WT and total PACE4 knockout (PACE4-KO), were analyzed by Western blotting using mouse BMP10 or β-actin antibodies. β-actin was used as a loading control. Proteins were resolved on SDS-PAGE 8% Tris-tricine gels under non-reducing conditions. The migration position of mature BMP10 is emphasized. **(B)** The bands from Western blot in (A) were quantitated using ImageJ software (National Institutes of Health) and the relative band intensities of mature BMP10 and β-actin were plotted for WT (patterned bar) and total PACE4-KO (grey bar) mice. Means ± SDEV are given and n = 5-7 mice per group. There is no significant difference between the two groups.



Supplemental Figure S5