

Supplemental Figure A

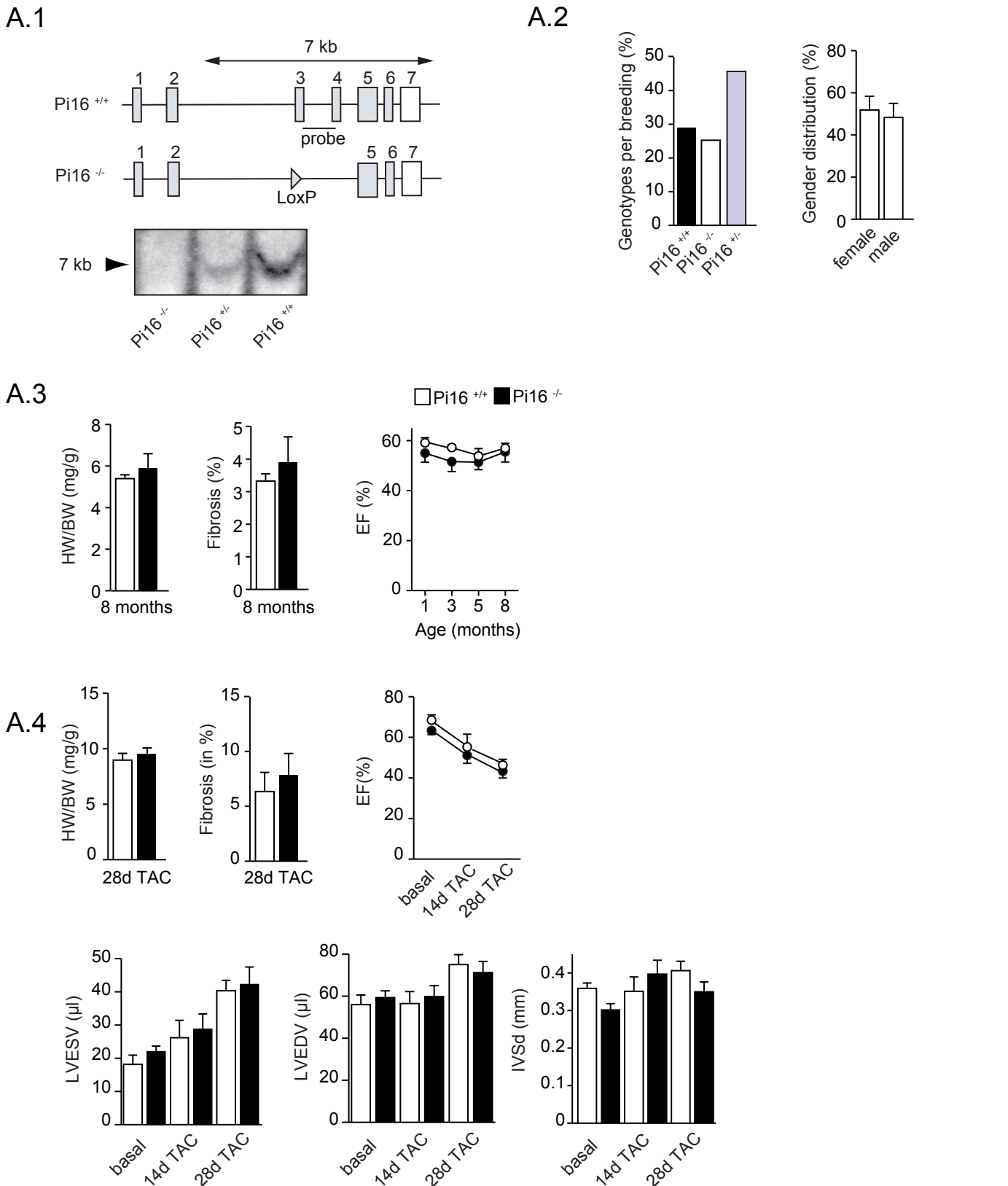
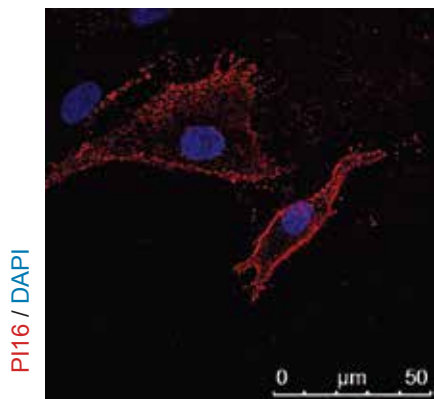


Fig. A: Generation and phenotyping of *Pi16*-deficient mice. (A.1) Targeting strategy for the generation of a murine floxed *Pi16* allele, affecting exons 3 and 4 of the *Pi16* locus. Southern Blot analysis of tail biopsies from homozygous and heterozygous *Pi16*-deficient mice, after crossing with a nestin-Cre transgenic mouse line. (A.2) Genotype and gender distribution in breedings of *Pi16*^{+/-} mice. (A.3) Basal analysis of cardiac parameters in *Pi16*^{+/+} and *Pi16*^{-/-} mice. Ejection fraction measured by echocardiography 1, 3, 5 and 8 months after birth (n = 5-7). HW/BW ratio (n = 4-6) and interstitial fibrosis quantification (n = 5-7) were determined at 8 months of age. Data are mean ± s.e.m. (A.4) Comparison of echocardiographic data from *Pi16*^{+/+} (open bars) and *Pi16*^{-/-} littermate mice (black bars) analyzed under basal conditions or after TAC surgery (n = 7-14). Diagrams show recordings of the following parameters: ejection fraction (EF), left ventricular end-systolic volume (LVESV), left ventricular end-diastolic volume (LVEDV) and intraventricular septum in diastole (IVSd). As a parameter for cardiac remodelling, the ratios of heart weight to body weight (HW/BW) and the extent of left ventricular fibrosis (n = 6-14) were determined in 28 d after TAC surgery. Data are mean ± s.e.m.

Supplemental Figure B

B.1



B.2

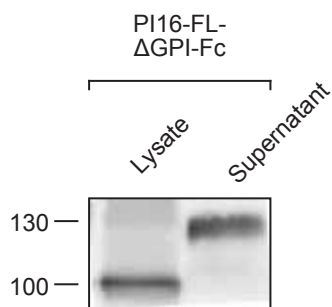
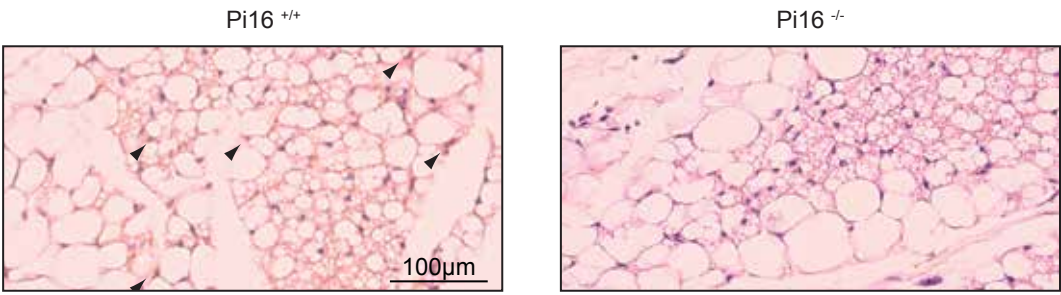


Fig. B: PI16 localizes to the membrane via a GPI anchor. (B.1) Confocal microscopy of CHO cells after stable transfection with PI16, showing membrane association of the protein (red). PI16 was detected using a specific anti-PI16 antibody (scale bar represents 20 μ m). (B.2) Immunoblot analysis of PI16- Δ GPI-Fc in cell culture supernatant and in lysates from transiently transfected HEK293 cells, confirming data shown in Fig. 1E.

Supplemental Figure C

C.1



C.2

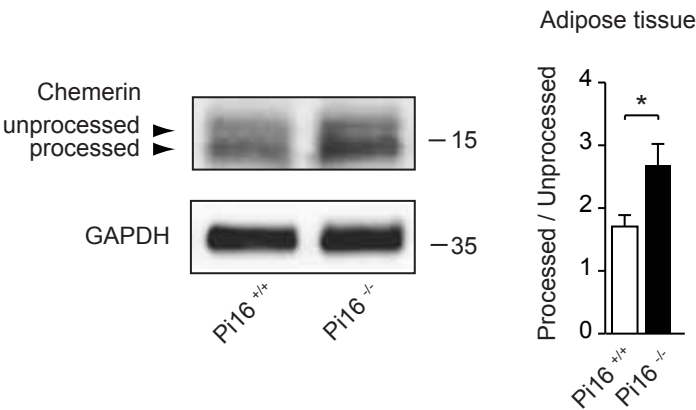


Fig. C: Chemerin processing phenotype in adipose tissue of $\text{Pi16}^{-/-}$ mice. (C.1) Immunohistochemical detection of PI16 in adipose tissue from $\text{Pi16}^{+/+}$ or $\text{Pi16}^{-/-}$ mice. (C.2) Western blot analysis of processed and unprocessed chemerin in retroperitoneal adipose tissue from $\text{Pi16}^{+/+}$ and $\text{Pi16}^{-/-}$ mice. Quantification is shown as ratio of both ($n = 6-9$). Data are mean \pm s.e.m. and are analysed using unpaired t-test.

Supplemental Table A

	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	MPV
Pi16 ^{+/+}	3.52	9.56	16.1	0.47	49.4	16.83	34.07	470.3	6.77
	±0.63	±0.29	±0.87	±0.01	±0.78	±0.42	±1.25	±63.57	±0.12
Pi16 ^{-/-}	4.46	9.33	15.47	0.46	49.1	16.6	33.8	512.3	6.87
	±0.53	±0.51	±0.55	±0.01	±2.33	±0.36	±1.04	±97	±0.21

Table A: Blood parameters of Pi16^{+/+} and Pi16^{-/-} mice. Basal blood parameters for Pi16^{+/+} and Pi16^{-/-} mice. WBC = white blood cell count, RBC = red blood cell count, HGB = hemoglobin, HCT = hematocrit, MCV = mean erythrocyte volume, MCH = mean amount of hemoglobin per erythrocyte, MCHC = mean corpuscular hemoglobin, PLT = platelets, MPV = mean platelet volume (n = 3) (data are mean ± SD).