Supplementary data

Supplementary Table 1: Primers used for *ESR1* molecular cloning. The full-length coding sequence (CDS) of the human *ESR1* gene was cloned into pFLAG-CMV-2 and the CDS for the 184 amino acid (184aa) N-terminus was cloned into pGEX-6P-1 and pRSFDuet™-1.

Purpose	F/R	Sequence
CDS (+)	F	ATGACCATGACCCTCCACACC
CDS (-)	R	TCAGACCGTGGCAGGGAAA
CDS (+) with EcoRI site	F	GGAATTC ATGACCATGACCCTCCACACC
CDS (-) with SalI site	R	TGCGGTCGACTCAGACCGTGGCAGGGAAA
CDS (+) with BamHI site (for pGEX-6P-1)	F	CGGGATCCATGACCATGACCCTCCACA
CDS (+) with BamHI site (for pRSFDuet™-1)	F	CGGGATCCCATGACCATGACCCTCCACA
CDS (-) 184aa	R	TCAGTAGCGAGTCTCCTTGG
CDS (-) 184aa with EcoRI site	F	CGAATTCTCAGTAGCGAGTCTCCTTGG

Supplementary Table 2: Primers used for *ESR1* mutagenesis.

Target mutation	F/R	Sequence
S167>A	F	CTTCCCTTGTCATTGGTAGCGGCCAATCTTTCTCTGCC
S167>A	R	GGCAGAGAAAGATTGGCCGCTACCAATGACAAGGGAAG
S167>E	F	CATACTTCCCTTGTCATTGGTTTCGGCCAATCTTTCTCTGCCACC
S167>E	R	GGTGGCAGAGAAAGATTGGCCGAAACCAATGACAAGGGAAGTATG

Supplementary Table 3: List of antibodies. CST, Cell Signaling Technology; SA, Sigma-Aldrich; SC, Santa Cruz Biotechnology.

Target	Type	Manu-facturer	Product code	Dilution
ACTB	1°	CST	#3700	1:5000
ACTB	1°	CST	#4970	1:5000
ERα	1°	SC	F-10	1:1000
Flag	1°	SA	F1804	1:1000
Goat anti-rabbit	2°	CST	#7074	1:5000
His-tag	1°	CST	#12698	1:1000
Horse anti-mouse	2°	CST	#7076	1:5000
Phospho-Akt Substrate (RXXS*/T*)	1°	CST	#9614	1:1000
Phospho-ERα (Ser167)	1°	CST	#64508	1:1000
PIM1	1°	SC	12H8	1:500
PIM2	1°	CST	#4730	1:1000
PIM3	1°	CST	#4165	1:1000
TFF1	1°	Abcam	ab92377	1:1000
TFF1	1°	CST	#15571	1:1000

Supplementary Table 4: Primers used for qPCR.

Gene	F/R	Sequence
ESR1	F	AACCAGTGCACCATTGATAAAA
	R	CCCTCCTCTTCGGTCTTTTC
TFF1	F	GCCCAGACAGAGACGTGTA
	R	AGCCCTTATTTGCACACTGG
XBP1	F	CCCTGGTTGCTGAAGAGG
	R	TGGAGGGGTGACAACTGG
RET	F	CCGTGAAGATGCTGAAAGAGA
	R	CCTGCTTCAGGACGTTGAA
GAPDH	F	TGCACCACCAACTGCTTAGC
	R	GGCATGGACTGTGGTCATGA

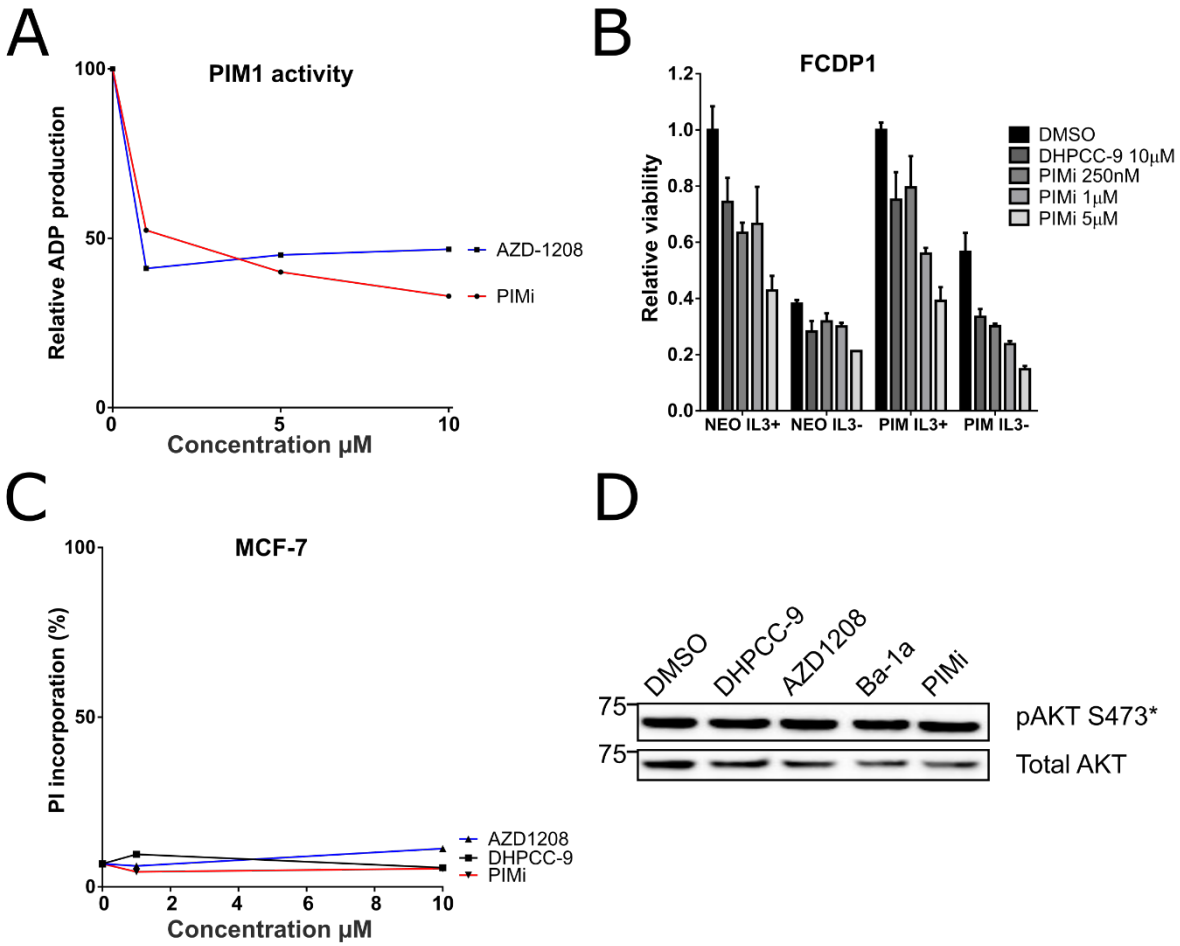
Materials, methods, and results

The inhibitory effects of the patented, but unpublished pan-PIM inhibitor PIMi were compared *in vitro* with those of the well-characterized and commercially available pan-PIM inhibitor AZD1208 (AstraZeneca), using the PIM1 enzyme system and ADP-Glo^{TM} assay (Promega) according to manufacturer´s instructions. For this purpose, 100 ng of PIM1 and 1 µg of S6 kinase substrate were incubated for 40 min with 1, 5 or 10 µM inhibitors in the presence of 10 µM ATP, after which the remaining ATP was depleted and the amount of ADP formed in the kinase reactions was quantitated by converting it first to ATP and then to luminescence, which was measured using the Synergy H1 plate reader (Agilent Technologies, Santa Clara, CA, USA). As shown in **SF. 1A**, both PIMi and AZD1208 inhibited PIM1 activity in a similar dose-dependent fashion already at 1 µM concentration..

The effects of PIMi on PIM-dependent cell survival were analysed as described for the previously characterized pan-PIM inhibitor DHPCC-9 [53]. Briefly, interleukin-3 (IL-3) - dependent FDCP1 murine myeloid cell lines stably expressing neomycin or PIM1 were cultured for 24 h with or without IL-3 in the presence of DMSO, DHPCC-9 or PIMi, after which cell viability was analysed by the MTT assay. As shown in **SF. 1B**, lack of IL-3 reduced the survival of the NEO control cells more markedly than that of PIM1-expressing cells, while the protective effects of PIM1 overexpression were completely lost in the presence of 10 µM DHPCC-9 or even smaller concentrations of PIMi.

The effects of PIM inhibitors on the viability of MCF-7 cells was determined using flow cytometry. Briefly, cells that had been treated for 24 h with increasing concentrations of PIMi, DHPCC-9 or AZD1208 were collected in PBS and then treated with a hypotonic buffer (1% Triton X-100, 0.05 mg/ml propidium iodide in PBS) for 20 minutes at 4°C. Propidium iodide incorporation into dead cells was measured using FACSCalibur flow cytometer with Cell-Quest Pro software (Becton Dickinson, Franklin Lakes, NJ, CA). As shown in **SF. 1C**, neither PIMi nor the other PIM inhibitors showed general cytotoxicity in MCF-7 cells.

To demonstrate that PIM inhibitors do not indirectly affect ERa phosphorylation by inhibiting AKT activity, western blotting was performed to analyse the expression levels of AKT and its active form phosphorylated at serine-473. For this purpose, PC-3 cells were treated with 5 µM of DHPCC-9, AZD1208, Ba-1a [52] or PIMi. As shown in **SF. 1D**, none of the PIM inhibitors used here inhibited expression or activity of AKT.



Supplementary Figure 1: Comparison between PIMi and other pan-PIM inhibitors of known structure. A) Dose-dependent effects of PIMi and AZD1208 pan-PIM inhibitors were measured from duplicate samples by the ADP-Glo™ assay. **B**) FDCP1 cell lines stably expressing neomycin (NEO) or PIM1 (PIM) were cultured for 24 h with or without IL-3 in the presence of DMSO, 10 µM DHPCC-9 or indicated concentrations of PIMi, after which cell viability was analysed from triplicate samples by the MTT assay. **C**) Propidium iodide (PI) incorporation was determined by flow cytometry from MCF-7 cells treated with different concentrations of PIM inhibitors. **D**) PC-3 cells were treated for 24 hours with 5 µM PIM inhibitors, after which expression levels of AKT and its active form phosphorylated at serine-473 (S473*) were determined by western blotting.