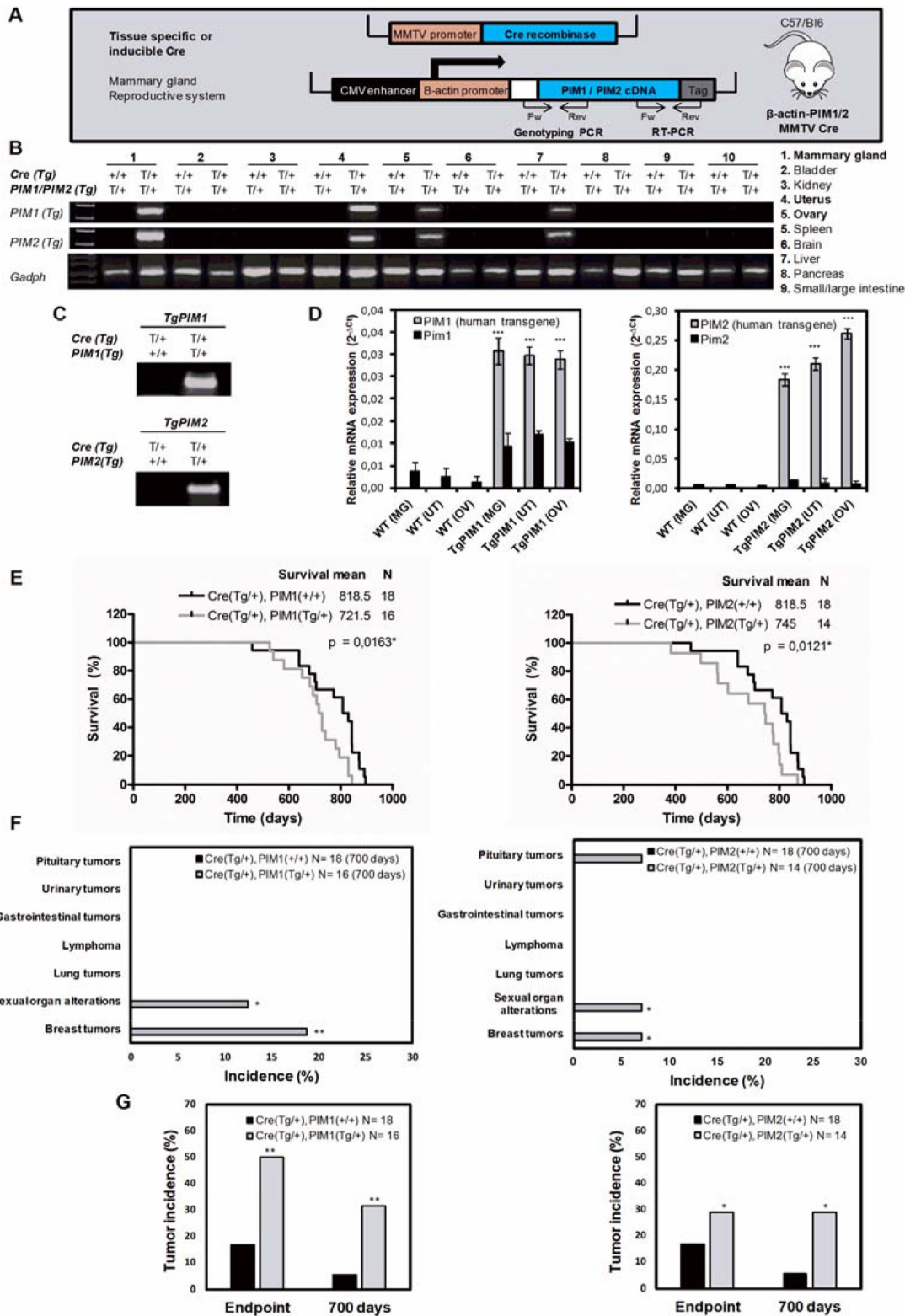
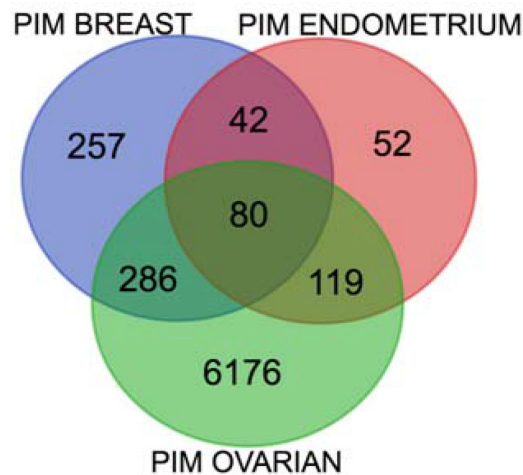


Inflammation and stem markers association to PIM1/PIM2 kinase-induced tumors in breast and uterus

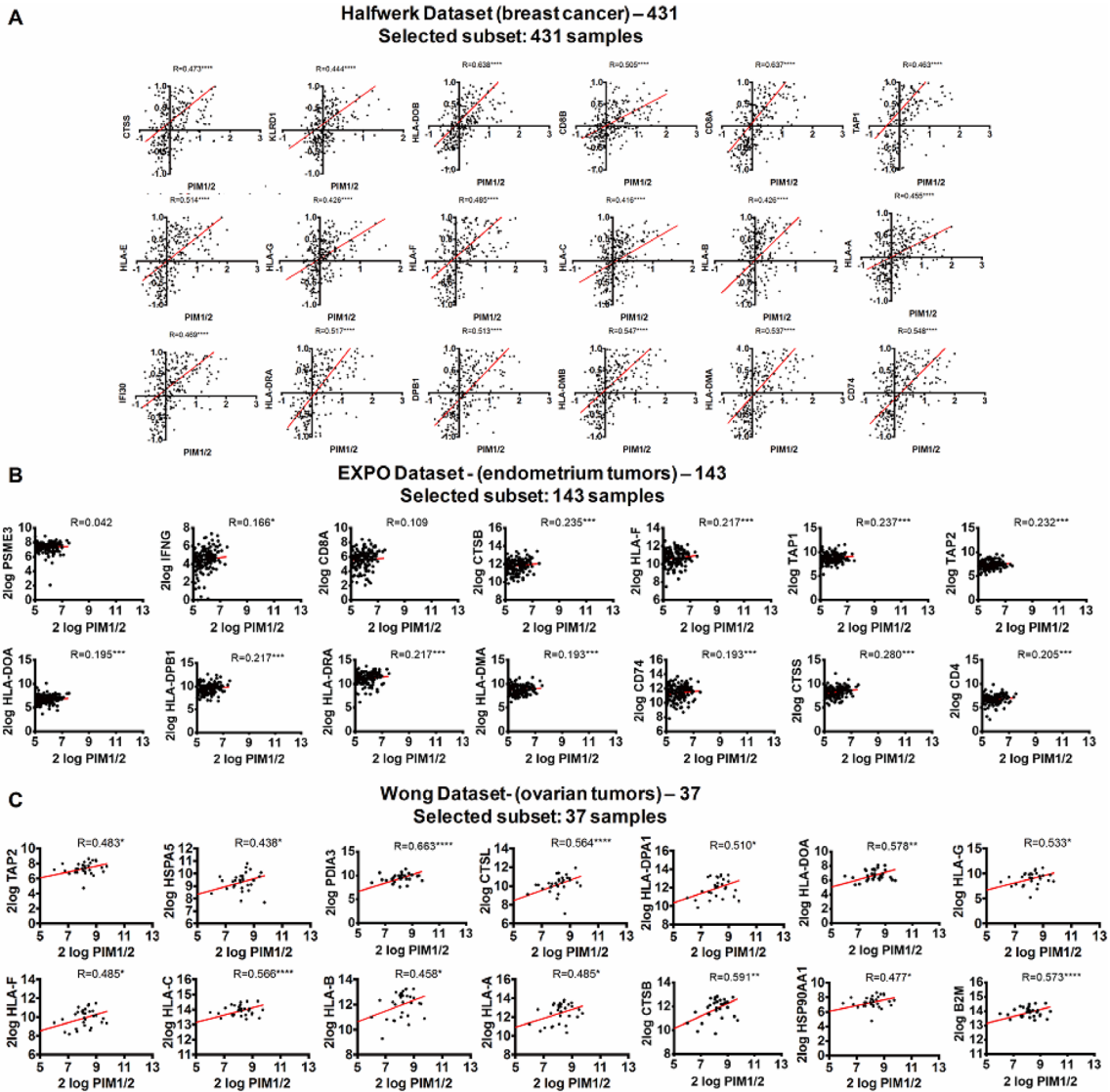
SUPPLEMENTARY MATERIALS



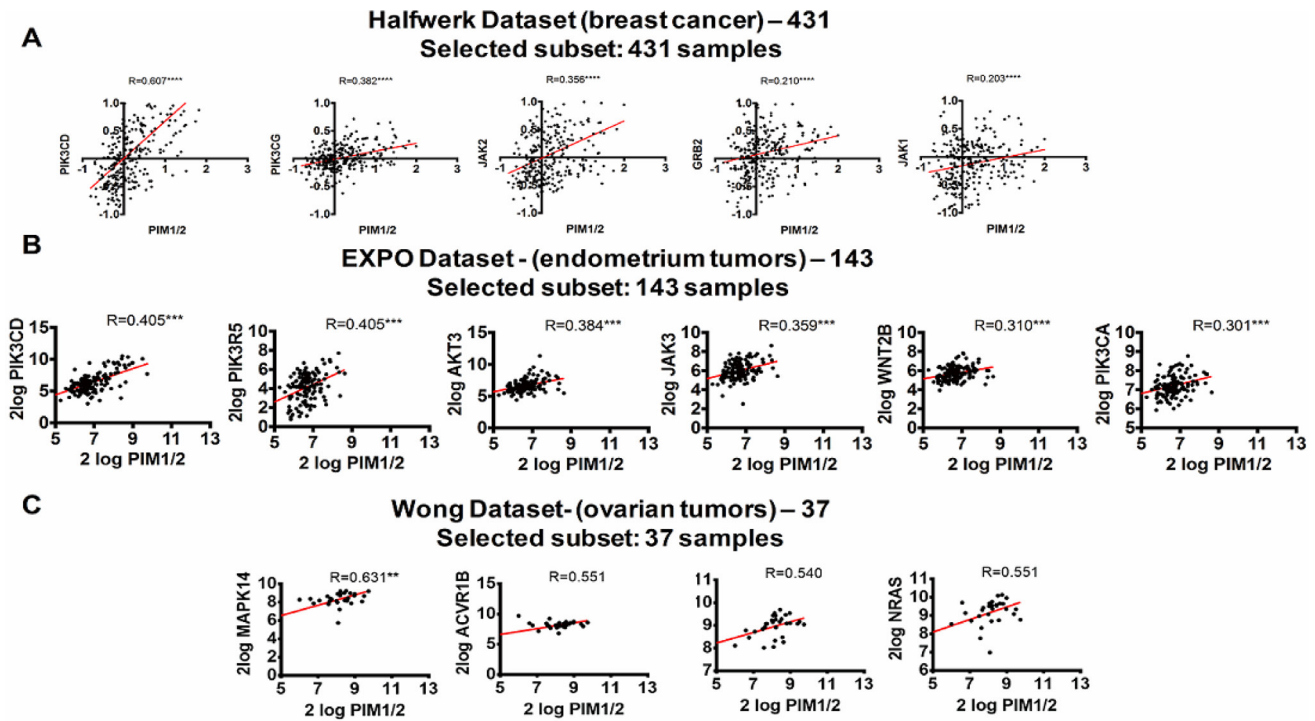
Supplementary Figure 1: Generation and validation of transgenic MMTV-Cre/PIM1 and MMTV-Cre/PIM2 conditional murine models. (A) Animals carrying respective PIM1/PIM2 transgenes silenced by a stop sequence flanked by LoxP sites were crossed with mice expressing Cre-recombinase regulated by the MMTV promoter. The result is two mouse lines that express either PIM1 or PIM2 human transgenes specifically in hormone-dependent tissues, where Cre expression excised the Lox/Stop/Lox cassette and allowed transgene expression. (B) Reverse transcriptase PCR was performed to validate tissue-specific relative expression levels of either PIM1 or PIM2 in TgPIM1/TgPIM2 models, indicating specific transgene expression in the mammary gland, female reproductive organs (uterus and ovary) and brain tissues. (C) DNA obtained from the digested tails of the mice was used to genotype the TgPIM1 or TgPIM2 models by PCR. (D) Levels of PIM1/PIM2 transgene relative to Pim1/Pim2 endogenous mRNAs were measured in mammary glands (MG), uterus (UT) and ovary (OV) of both TgPIM1/TgPIM2 and WT mice by qRT-PCR. (E) TgPIM1, TgPIM2 and WT model mouse survival as indicated by Kaplan-Meier curves. Both models exhibit a statistically significant reduction of survival rate compared with WT mice. (F) Tumor incidence in both female models at day 700. Data show the observed macroscopic pathologies in TgPIM1 and TgPIM2 mice compared with WT background, indicating a statistically significant tumor incidence increase in sexual organ alterations in both models. (G) Total tumor incidence in both models at day 700 and the clinical endpoint. This graph shows total percentage of tumors observed from a macroscopic view per model compared with WT, indicating a statistically significant increase in the percentage of total tumor types in the TgPIM1/TgPIM2 models. The *p*-value was obtained using a one-tailed Student's *t*-test in (A, B, C, D, F and G). The *p*-value was obtained using a Mantel-Cox test (also called Log rank Chi2-based test) in (E), (**p* < 0,05), (***p* < 0,01) and (***)*p* < 0,001).



Supplementary Figure 2: Venn-diagram of genes that correlate with PIM1 and PIM2 in Breast, endometrial and ovarian tumors, and genes common to combination of tumor types.



Supplementary Figure 3: One to one Pearson's correlation of the list of immune system-dependent genes signature depicted in the heatmaps displayed in Figure 5 of human breast, endometrium and ovary tumors databases analysed.



Supplementary Figure 4: One to one Pearson’s correlation of the list of stem-dependent genes signature described in the heatmaps displayed in figure 6 of human breast, endometrium and ovary tumors databases analysed.

Supplementary Table 1: Common genes correlating with PIM1 and PIM2 expression in breast, ovarian and endometrium tumors. See Supplementary_Table_1

Supplementary Table 2: GO enrichment for process of genes correlating with PIM1 and PIM2 expression in all three breast, ovarian and endometrium tumors

GoId	Term	Corrected <i>P</i> value
GO:0009617	response to bacterium	3,43E+07
GO:0002376	immune system process	7,67E+07
GO:0043207	response to external biotic stimulus	2,71E+08
GO:0051707	response to other organism	2,71E+08
GO:0009607	response to biotic stimulus	4,28E+08
GO:0032496	response to lipopolysaccharide	4,33E+07
GO:0006952	defense response	4,68E+08
GO:0006955	immune response	5,91E+07
GO:0002237	response to molecule of bacterial origin	6,40E+06
GO:0034097	response to cytokine	3,32E+09
GO:0048583	regulation of response to stimulus	5,15E+09
GO:0009605	response to external stimulus	0.000132246974172086
GO:0007165	signal transduction	0.000185186238256669
GO:0051716	cellular response to stimulus	0.000192915225072843
GO:0007166	cell surface receptor signaling pathway	0.000194146704417251
GO:0071345	cellular response to cytokine stimulus	0.000200820221676121
GO:0050896	response to stimulus	0.000219101934006273
GO:0033622	integrin activation	0.000229550239127017
GO:0045087	innate immune response	0.000371687962152313
GO:0002682	regulation of immune system process	0.000400231755999877
GO:0044700	single organism signaling	0.000571677399544299
GO:0048584	positive regulation of response to stimulus	0.000606581231880176
GO:0023052	signaling	0.000607020301368781
GO:0007154	cell communication	0.000615016495071497
GO:0002684	positive regulation of immune system process	0.00153489328532374
GO:1901700	response to oxygen-containing compound	0.00195704193579501
GO:0051704	multi-organism process	0.00253810803158615
GO:0048518	positive regulation of biological process	0.00267249757157674
GO:0010033	response to organic substance	0.00367383310482357
GO:0006950	response to stress	0.00398041612316704
GO:1901701	cellular response to oxygen-containing compound	0.00657112985458861
GO:0033993	response to lipid	0.00711211266110781
GO:0050900	leukocyte migration	0.00832471826976121
GO:0050789	regulation of biological process	0.0090034003189567
GO:0080134	regulation of response to stress	0.00925245974559772

Supplementary Table 3: Proteins of antigen processing and presentation (KEGG) (HLA and inflammation) correlating with PIM1 or PIM2 levels in breast cancer

<i>Halfwerk dataset</i>		
	<i>r-value</i>	<i>P-Value</i>
HLA-DOB	0.638	< 0.01
CD8A	0.637	< 0.01
CD74	0.548	< 0.01
HLA-DMB	0.547	< 0.01
HLA-DMA	0.537	< 0.01
HLA-DPA1	0.517	< 0.01
HLA-DRA	0.517	< 0.01
HLA-E	0.514	< 0.01
DPB1	0.513	< 0.01
CD8B	0.505	< 0.01
HLA-F	0.485	< 0.01
CTSS	0.473	< 0.01
IFI30	0.469	< 0.01
TAP1	0.463	< 0.01
HLA-A	0.455	< 0.01
KLRD1	0.444	< 0.01
HLA-G	0.435	< 0.01
HLA-B	0.426	< 0.01
HLA-C	0.416	< 0.01

Supplementary Table 4: Proteins of antigen processing and presentation (KEGG) (HLA and inflammation) correlating with PIM1 or PIM2 levels in endometrial cancer

<i>GSE2109</i>		
	<i>r-value</i>	<i>P-Value</i>
CD4	0.205	< 0.01
CTSS	0.280	< 0.01
CD74	0.193	< 0.01
HLA-DMA	0.193	< 0.01
HLA-DRA	0.217	< 0.01
HLA-DPB1	0.217	< 0.01
HLA-DOA	0.195	< 0.01
TAP2	0.232	< 0.01
TAP1	0.237	< 0.01
HLA-F	0.217	< 0.01
CTSB	0.235	< 0.01
CD8A	0.109	0.09
IFNG	0.166	0.02
PSME3	0.042	0.55

Supplementary Table 5: Proteins of antigen processing and presentation (KEGG) (HLA and inflammation) correlating with PIM1 or PIM2 levels in ovarian cancer

<i>GSE40595</i>		
	<i>r-value</i>	<i>P-Value</i>
PDIA2	0.664	< 0.01
CTSB	0.591	0.01
HLA-DOA	0.578	0.01
B2M	0.573	< 0.01
HLA-C	0.566	< 0.01
CTSL	0.564	< 0.01
HLA-G	0.533	0.02
HLA-DPA1	0.510	0.02
HLA-F	0.485	0.03
HLA-A	0.485	0.03
TAP2	0.483	0.03
HSP90AA1	0.477	0.03
HLA-B	0.458	0.04
HSPA5	0.438	0.05

Supplementary Table 6: Proteins of proteins of pathways controlling stemness and pluripotency (KEGG) correlating with PIM1 or PIM2 levels in breast cancer

<i>Halfwerk dataset</i>		
	<i>r-value</i>	<i>P-Value</i>
PIK3CD	0.607	< 0.01
PIK3CG	0.382	< 0.01
JAK2	0.356	< 0.01
GRB2	0.210	< 0.01
JAK1	0.203	< 0.01

Supplementary Table 7: Proteins of pathways controlling stemness and pluripotency (KEGG) endometrial cancer

<i>GSE2109</i>		
	<i>r-value</i>	<i>P-Value</i>
PIK3CD	0.405	< 0.01
PIK3R5	0.405	< 0.01
AKT3	0.384	< 0.01
JAK3	0.359	< 0.01
WNT2B	0.310	< 0.01
PIK3CA	0.301	< 0.01

Supplementary Table 8: Proteins of Pathways controlling stemness and pluripotency (KEGG) correlating with PIM1 or PIM2 levels in Ovarian Cancer

<i>GSE40595</i>		
	<i>r-value</i>	<i>P-Value</i>
MAPK14	0.631	0.02
ACVR1B	0.553	0.07
GRB2	0.540	0.06
NRAS	0.506	0.08