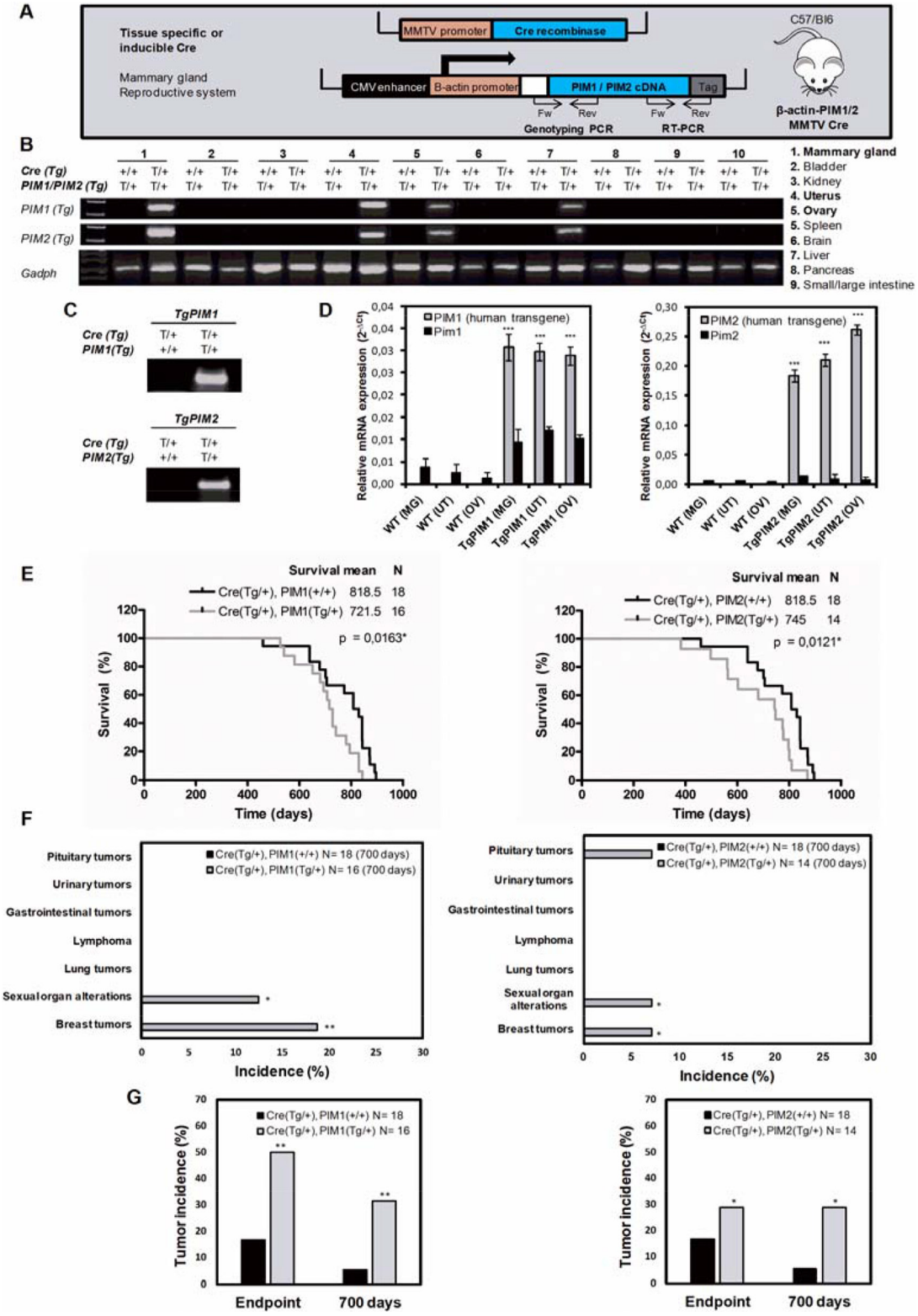
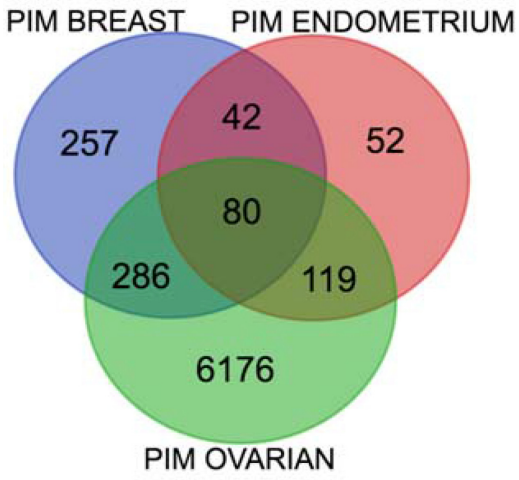
Inflammation and stem markers association to PIM1/PIM2 kinase-induced tumors in breast and uterus

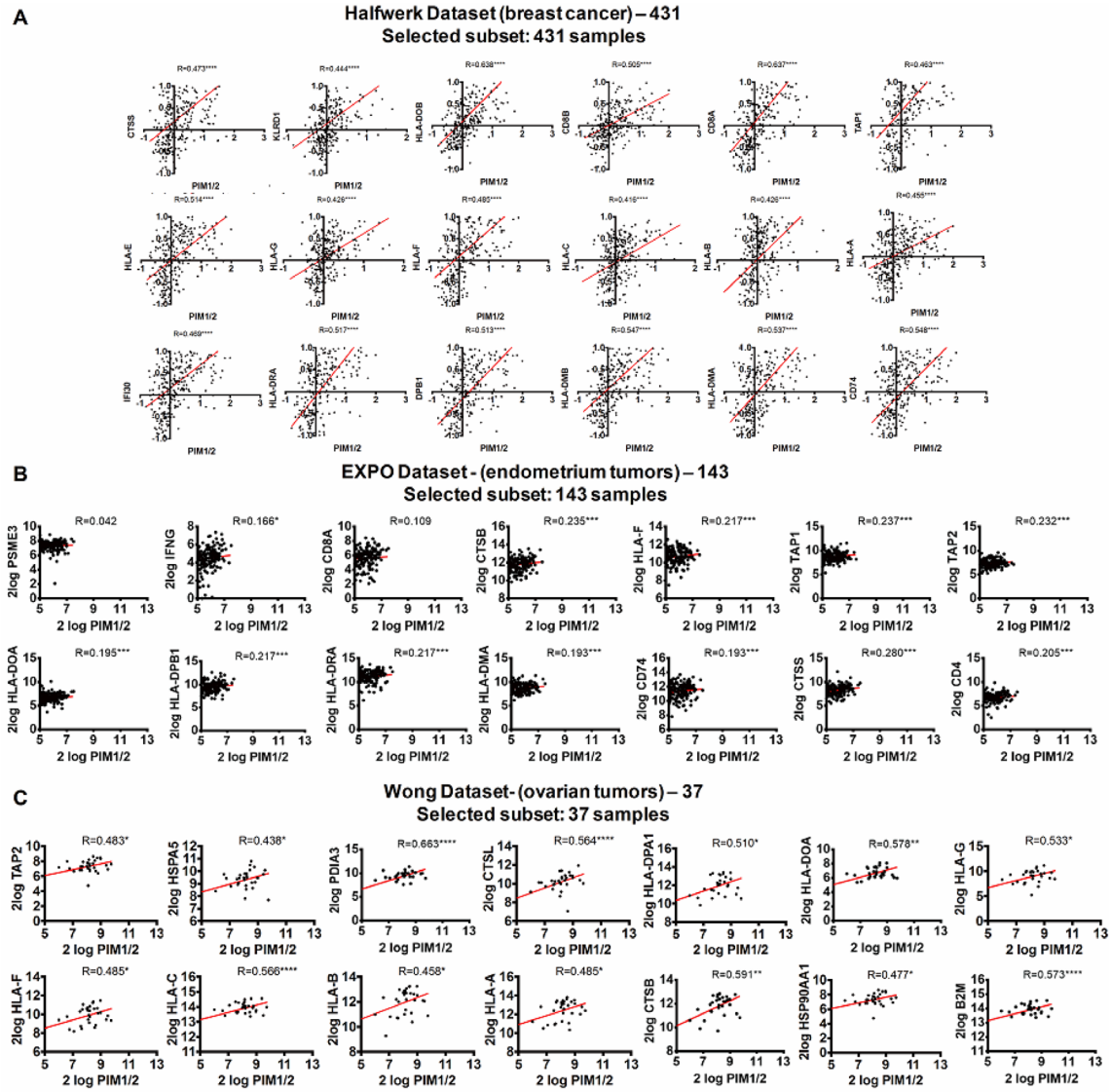
SUPPLEMENTARY MATERIALS



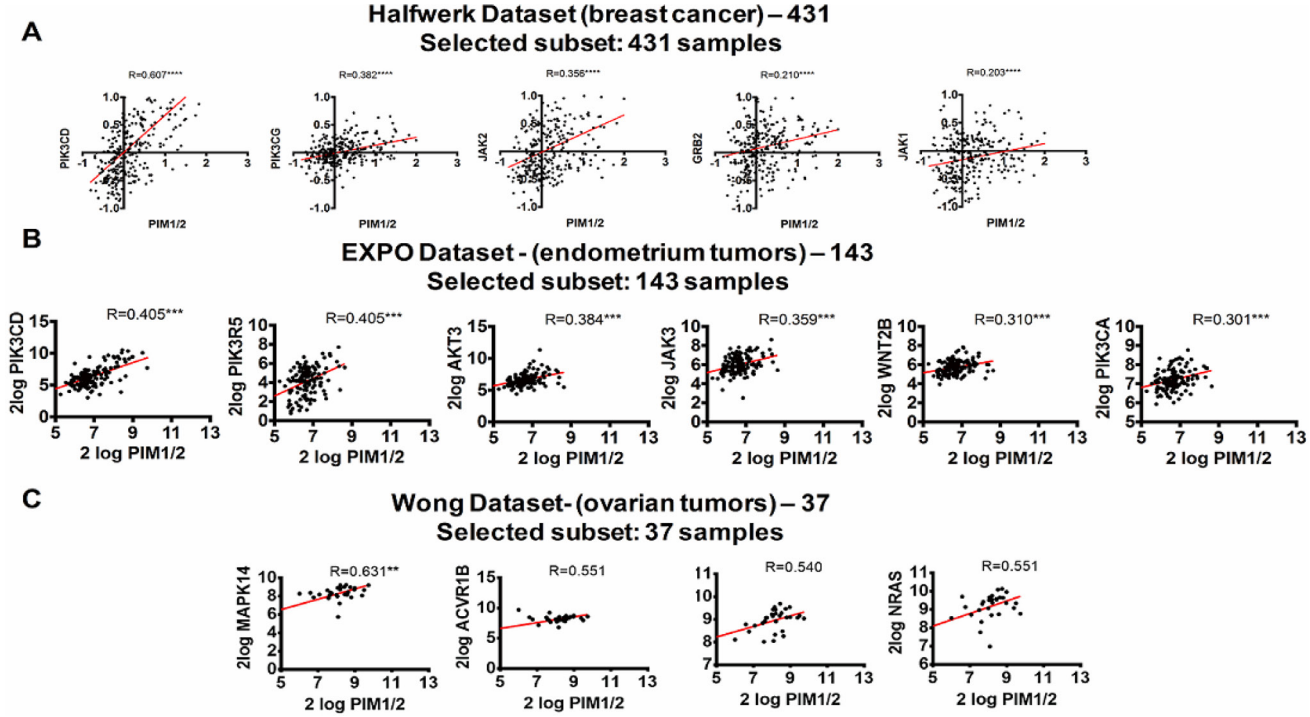
Supplementary Figure 1: Generation and validation of transgenic MMTV-Cre/PIM1 and MMTV-Cre/PIM2 conditional murine models. (**A**) Animals carrying respective PIM1/PIM2 transgenes silenced by a stop sequence flanked by LoxP sites were crossed with mice expressing Cre-recombinase regulated by the MMTV 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_*P* 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

Halfwerk dataset		
	r-value	***P-Value***
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

GSE2109		
	r-value	***P-Value***
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

GSE40595		
	r-value	***P-Value***
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

Halfwerk dataset		
	r-value	***P-Value***
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

GSE2109		
	r-value	***P-Value***
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

	GSE40595	
	r-value	***P-Value***
MAPK14	0.631	0.02
ACVR1B	0.553	0.07
GRB2	0.540	0.06
NRAS	0.506	0.08