Supplemental Methods Section

Cell culture

The mammary carcinoma cell lines 168FARN and 4T1 were provided by Dr J. Yang (University of California, San Diego). Wild type and LRP-1 knockout MEFs were provided by Dr J. Herz (University of Texas, Dallas). These cell lines were maintained in DMEM supplemented with 10% FCS. PN-1

LRP-1 RNA interference:

168FARN cells were transiently transfected by Optifect reagent (Invitrogen), using a ratio 5/ 1 of Optifect/siRNA, with siRNA against LRP-1 (5'-CUCUCAGAACAUCCUAGCUdTdT-3') or scramble-control siRNA (5'-UGACACGCCUACUACUUACdTdT-3'). Transfections were performed in serum free medium containing 0.1% stripped BSA. The day following transfection, LRP-1 siRNA-transfected and control siRNA -transfected cells were incubated in fresh transfection media containing tPA/PN-1 complex for 24 hours and CM was subjected to Gelatin zymography

SYBR-Green Real-Time RT-PCR and Semi quantitative RT-PCR

Mouse *mmp-9* RT-PCR forward and reverse primers sequences were the following: 5'-GCATACTTGTACCGCTATGGT-3' and 5'-TGTGATGTTATGATGGTCCC-3', respectively. Mouse *actin* RT-PCR forward and reverse primers sequences were the following 5'-TGCGTGACATCAAAGAGAAG-3' and the RT-PCR reverse primer was 5'-TTCATTGACCTCAACTACATG-3', respectively.

Total RNA was extracted using RNeasy Mini kit (Qiagen) and reverse transcribed with oligo(d)T primers with "Ready-To-Go You-Prime First-Strand" beads (GE Healthcare). cDNAs were used for PCR using SYBR-Green Master PCR mix (Thermo Scientific) in triplicates. PCR and data collection were performed on the ABI Prism 7000 (Applied Biosystems). All quantitations were normalized to an endogenous control actin. The relative quantitation value for MMP-9 compared to Actin is expressed as $2^{-(Ct-Cc)}$ (Ct and Cc are the mean threshold cycle differences after normalizing to actin).

For semi-quantitative RT-PCR, primers used for cDNA amplification are reported in Table S1. PCR consisted of a first step of denaturation (5 minutes at 95°) followed by cycles of three steps: 1min at 95°, 1 min at 55°, 1 min at 72° and a final step of polymerization at 72° for 5min. Number of cycles varied for individual mRNAs and are reported in Table S1.

Supplemental figure legends:

Supplemental figure S1: PN-1 induces an increase of secreted MMP-9 protein in 168FARN cells.

168FARN cells were starved in DMEM for 12 hours and incubated with DMEM containing 0.2μM PN-1 or not. The CM was left on the cells for the indicated times before collecting for an analysis of MMP-9 by immunoblotting to show the accumulation of MMP-9 over time. After 6 hours of PN-1 treatment there was a visible increase in secreted MMP-9.

Supplemental figure S2: Growth curve of MMP-9-rescued-PN-1-knock-down 4T1 tumors and control tumors in Balb/c mice.

Five Balb/c mice were each injected with 5.10^5 cells: PN-1-shRNA1 or PN-1-shRNA2 4T1 cells stably transfected with either pcDNA vector (PN-1-shRNAs-ctrl) or pcDNA-MMP-9 (PN-1-shRNAs-MMP-9) and control mock transfected 4T1 cells. When tumors became palpable, tumor size was recorded twice a week during the 26 days following injection. Each point is mean +/-SD of tumor size in each group.

Supplemental figure S3: PN-1 expression correlates with estrogen receptor-α status in breast cancers.

Normalized PN-1 RNA expression from (A) Minn's data-set (1'), (B) Richardson's data-set (2'), (C) Chin's data-set (3') and (D) Yu's data-set (4'). The tumor numbers in each category (ER+α positive and ER-α negative) are indicated in parentheses and p values are for A-D, 4.30E-07, 1.5E-04, 1.7E-04 and 0.003, respectively.

1'. Minn, A. J., Gupta, G. P., Siegel, P. M., Bos, P. D., Shu, W., Giri, D. D., et al. Genes that mediate breast cancer metastasis to lung. Nature, 436: 518-524, 2005.

2'. Richardson, A. L., Wang, Z. C., De Nicolo, A., et al. X chromosomal abnormalities in basal-like human breast cancer. Cancer Cell, 9: 121-132, 2006.

3'. Chin, K., DeVries, S., Fridlyand, J., et al. Genomic and transcriptional aberrations linked to breast cancer pathophysiologies. Cancer Cell, 10: 529-541, 2006.

4'. Yu, A. E., Hewitt, R. E., Connor, E. W., and Stetler-Stevenson, W. G. Matrix metalloproteinases. Novel targets for directed cancer therapy. Drugs Aging, 11: 229-244, 1997.

Supplemental figure S4: Model for PN-1 function as a mediator of metastasis.

Based on the data presented here we propose a model whereby 4T1 tumor cells secrete PN-1, which forms complexes with secreted targeted proteases (e.g. PN-1/uPA, PN-1/thrombin…). The PN-1/protease complex binds to LRP-1 causing an induction of Erk signaling. The expression of MMP-9, and potentially other genes, is controlled by this pathway. Importantly, we show here by a rescue experiment performed with PN-1 KD 4T1 cells that MMP-9 is essential for the metastatic phenotype.

Supplemental figure S5: PAI-1/tPA complex, but not uncomplexed PAI-1, promotes an increase in MMP-9 gelatinase activity in 168FARN cells.

(A) Gelatin zymography performed on conditioned media (CM) from 168FARN cells treated for 24 hours with the indicated concentrations of PAI-1 (left panel) or PN-1 (right panel). (B) Gelatin zymography performed on CM from 168FARN cells treated with tPA (0.2 μM), PAI-1 (0.2 μM), PN-1 (0.2 μM), tPA/PAI-1 complex (0.2 μM) and tPA/PN-1 complex (0.2 μM) for 24 hours.

Supplemental figure S6: Twist expression is unchanged in PN-1-shRNA 4T1 cells.

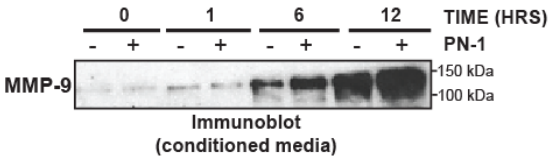
Semi-quantitative RT-PCR analysis to determined the level of Twist, PN-1 and Actin (as loading control) in PN-1 shRNA1, PN-1 shRNA2, mock and parental 4T1 cells.

Supplemental figure S7: Growth curve and metastatic behavior of 4T1 tumors in wild type and PN-1 null mice

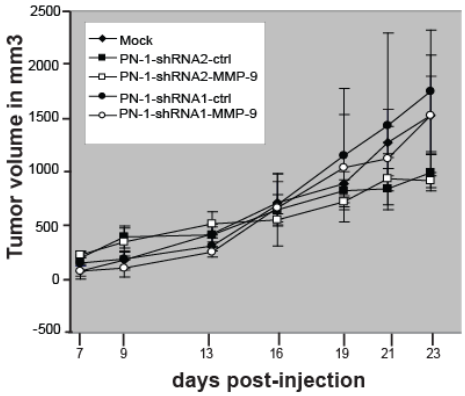
(A) Nine wild type and 9 PN-1 null mice were injected with parental 4T1 cells (5.10^5 cells) When tumors became palpable, tumor size was recorded twice a week during the 26 days following injection. Each point is mean +/-SD of tumor size in each group. (B) Quantification of lung metastases in wild type and PN-1 null mice day 26 post-injection.

Table S1: primers used for MMP-9, PN-1, LRP-1, PAI-1 and Actin cDNA amplification

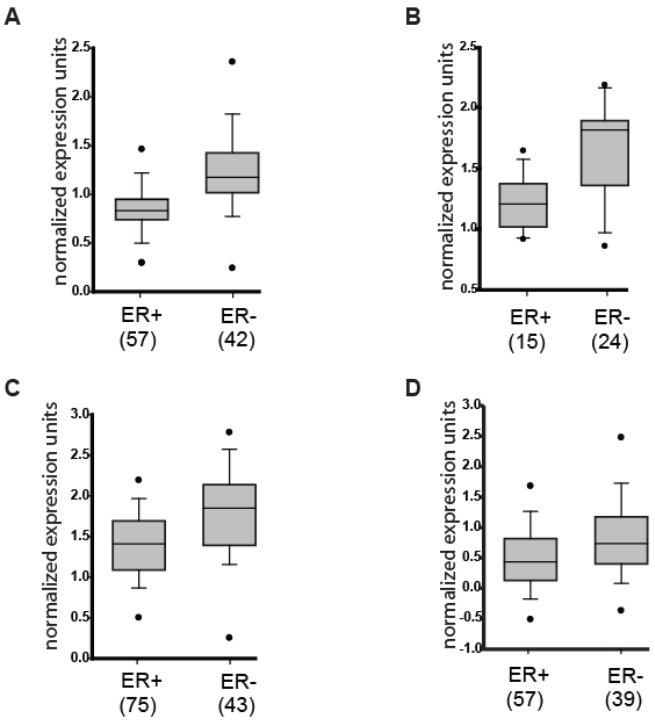
Gene	Primers sequence (5'-3') Forward Reverse	Product length (pb)	Number of cycles
Mouse MMP-9	CCTAGTGAGAGACTCTACAC AGAGCCACGACCATACAGATAC	507	30
Mouse LRP-1	CAAAGGTGGAGCGCTGTGAC ACCTGCAGGTCCTTGCCTTG	479	32
Mouse PN-1	GCGATATAATGTAAACGGAG CAAAAATTGATGGACTCAGAG	224	30
Mouse PAI-1	ATGAGATCAGTACTGCGGATGCCATCT GCACAGAGACGGTGCTGCCATCAGACT	322	30
Mouse Twist	ACATCGACTTCCTGTACCAGGTC AACAATGACATCTAGGTCTCCGG	196	29
Mouse Actin	GTGGGCCGCTCTAGGCACAA CTCTTTGATGTCACGCACGATTTC	539	27



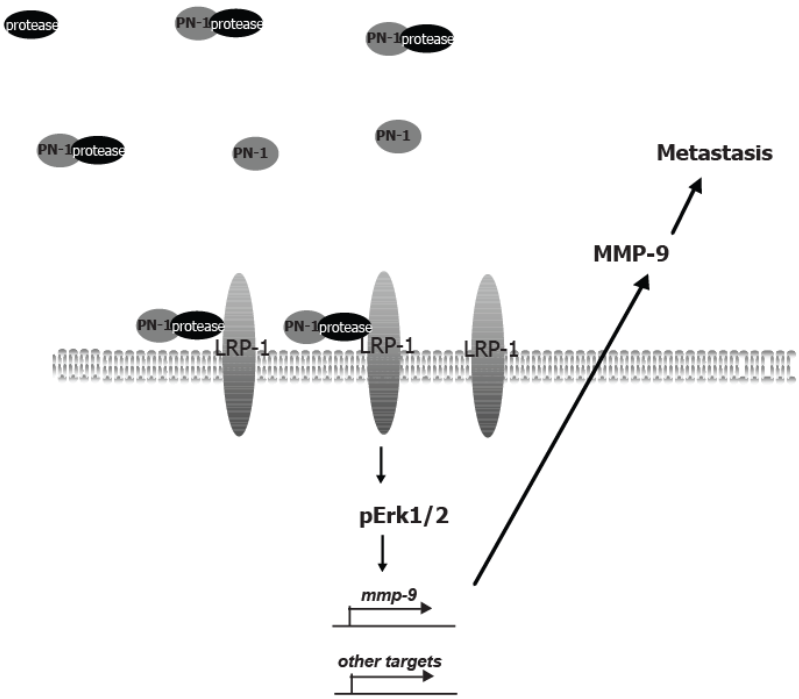
Fayard et al. **supplementary Figure. S1**



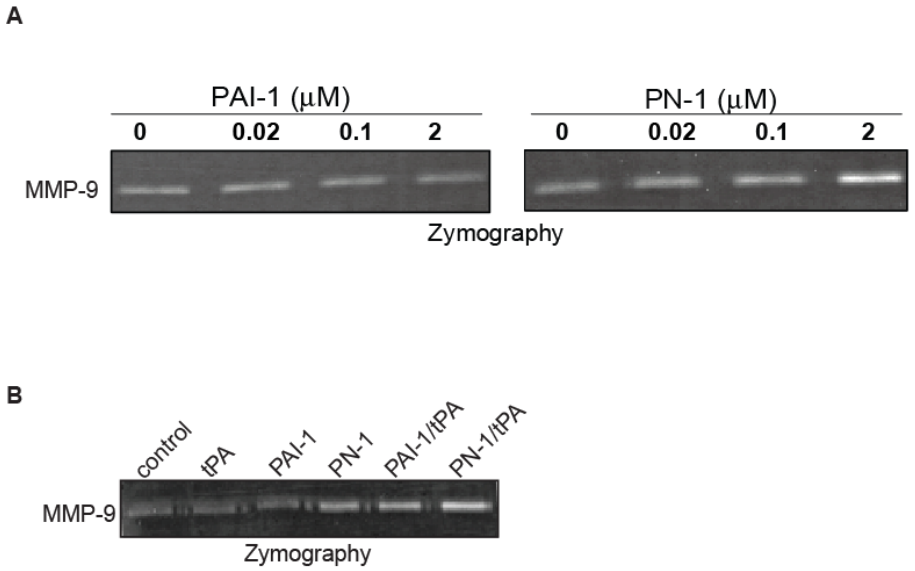
Fayard et al. **supplementary Figure. S2**



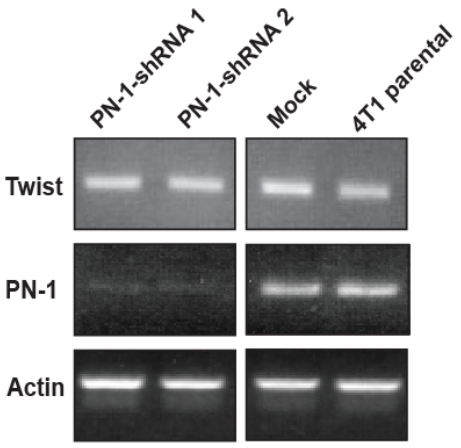
Fayard et al. **supplementary Figure. S3**



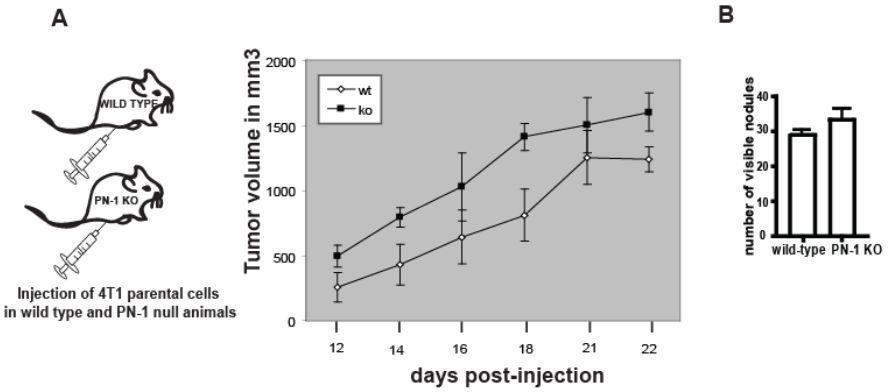
Fayard et al. **supplementary Figure. S4**



Fayard et al. **supplementary Figure. S5**



Fayard et al. **supplementary Figure. S6**



Fayard et al. **supplementary Figure. S7**