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**Identification of novel TMPRSS2:ERG mechanisms in prostate cancer metastasis:
involvement of MMP9 and PLXNA2**

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ABSTRACT

Prostate cancer (PCa) is one of the major public health problems in Western countries. Recently, the *TMPRSS2:ERG* gene fusion, which results in the aberrant expression of the transcription factor ERG, has been shown to be the most common gene rearrangement in PCa. Previous studies have determined the contributions of this fusion in PCa disease initiation and/or progression *in vitro* and *in vivo*. In this study on *TMPRSS2:ERG* regulation in PCa, we used an androgen receptor (AR) and *TMPRSS2:ERG* fusion double-negative prostate cancer cell model: PC3c. In three cell clones with different *TMPRSS2:ERG* expression levels, ectopic expression of the fusion resulted in significant induction of cell migration and invasion in a dose-dependent manner. In agreement with this phenotype, high-throughput microarray analysis revealed that a set of genes, functionally associated with cell motility and invasiveness, were deregulated in a dose-dependent manner in *TMPRSS2:ERG*-expressing cells. Importantly, we identified increased *MMP9* and *PLXNA2* expression in *TMPRSS2:ERG*-positive PCa samples, and their expression levels were significantly correlated with *ERG* expression in a PCa cohort. In line with these findings, there was evidence that *TMPRSS2:ERG* directly and positively regulates *MMP9* and *PLXNA2* expression in PC3c cells. Moreover, *PLXNA2* upregulation contributed to *TMPRSS2:ERG*-mediated enhancements of PC3c cell migration and invasion. Furthermore, and importantly, *PLXNA2* expression was upregulated in metastatic PCa tumors compared to localized primary PCa tumors. This study provides novel insights into the role of the *TMPRSS2:ERG* fusion in PCa metastasis.

Key words: Prostate cancer, *TMPRSS2:ERG* fusion, Migration, Invasion, *MMP9*, *PLXNA2*.

INTRODUCTION

Prostate cancer (PCa) is one of the most prevalent malignancies and a leading cause of cancer-related deaths affecting men in Western countries (1). Recent PCa cytogenetic and genomic studies have highlighted several critical genomic alterations involved in disease onset and/or progression (2). Of these, genomic rearrangements leading to the aberrant expression of the E-twenty-six (*ETS*) family of transcription factors in PCa is considered as a hallmark (3, 4). The most frequent genomic rearrangement in PCa is the fusion of the *ETS*-related gene (*ERG*) with 5' regulatory elements of the androgen receptor (*AR*)-responsive transmembrane protease serine 2 (*TMPRSS2*), which has been identified in 40% to 70% of PCa patient samples (3). The *TMPRSS2:ERG* gene fusion leads to aberrant expression of full length and/or N-terminal-truncated *ERG* transcription factors in PCa (referred to as *TMPRSS2:ERG* protein in this study) (5).

The association between *TMPRSS2:ERG* fusion and PCa clinical outcome has not yet been clearly established. Some studies have shown that this gene fusion is not significantly associated with PCa disease progression or prognosis (6-8); however, other studies have demonstrated that it is associated with favorable (9, 10) or poor clinical outcomes (11-16).

Functional studies on the role of *TMPRSS2:ERG* in PCa have been performed using PCa cell lines as well as mouse models. Although some reports have demonstrated that ectopic *TMPRSS2:ERG* expression in the prostate is sufficient to induce prostatic intraepithelial neoplasia (PIN) in mice, a pre-malignancy form of PCa (17, 18), others have highlighted how *TMPRSS2:ERG* expression cooperates with other genomic alterations, such as *PTEN* haploinsufficiency, constitutive PI3K/AKT activation or *AR* signaling, to promote PCa cell migration, invasion and disease progression (19-21). Using next-generation sequencing technologies, recent studies aiming to identify *TMPRSS2:ERG* direct target genes in established PCa cell models have provided further evidence that *ERG* can modulate the

output of AR target genes (22-24). These studies provide functional insights into the role of *TMPRSS2:ERG* in PCa initiation and/or progression.

Nevertheless, PCa is considered as a multifocal disease, because primary PCa tumors are mainly composed of multiple, genetically distinct cancer cells (2, 25-27). Regarding *TMPRSS2:ERG* status and the corresponding *TMPRSS2:ERG* protein expression profile, a significant prevalence of inter- and/or intra-focal heterogeneity has been also observed in several PCa cohorts (28-31). This raises the possibility that the differentially expressed *TMPRSS2:ERG* protein can control variable transcriptional programs in genetically distinct PCa cells. It is therefore necessary to perform studies on different PCa cell models.

In this study, we examined the role of *TMPRSS2:ERG* using an established PCa cell model, PC3c, which was previously isolated from AR and *TMPRSS2:ERG* double-negative PC3 cells (32). *In vivo* studies have revealed that PC3c cells can induce mixed lesions in bone (32), a commonly observed complication in PCa patients with advanced disease (33). Here, we demonstrate that ectopic expression of *TMPRSS2:ERG* fusion increases PC3c cell migration and invasion in a dose-dependent manner. High-throughput transcriptomic studies revealed that a set of genes related to cell motility and invasiveness is deregulated in *TMPRSS2:ERG*-expressing cells in a dose-dependent manner. We further demonstrate that *TMPRSS2:ERG* directly and positively regulates MMP9 (Metalloproteinase 9) and PLXNA2 (Plexin A2) expression in PC3c cells, and that PLXNA2 upregulation contributes to the *TMPRSS2:ERG*-mediated PC3c cell migration and invasion.

RESULTS

Ectopic TMPRSS2:ERG expression leads to phenotype changes in PC3c cells.

Previous studies have identified diverse *TMPRSS2:ERG* fusion transcripts in human PCa samples, and the most frequent variant is composed of exon 1 of the *TMPRSS2* gene (NM_005656) fused to exon 4 of the *ERG* gene (NM_004449) (5, 34). In addition, the presence of a 72bp exon in fusion gene transcripts may contribute to a more aggressive phenotype (35). We therefore used the *TMPRSS2:ERG* fusion isoform that includes exon 1 of *TMPRSS2* fused to exon 4 of *ERG* with the presence of the 72bp exon (Figure 1a).

Three *TMPRSS2:ERG*-expressing clones were included in this study, designated as H clone (high), M clone (moderate) and L clone (low), according to their *ERG* mRNA expression levels (Figure 1b). *ERG* mRNA levels in H cells were 2-fold and 10-fold higher than in M cells and in L cells respectively, whereas no endogenous *ERG* mRNA was found in pcDNA control cells or in parental PC3c cells. In particular, *ERG* expression in H cells was only about 50% of that in VCaP cells (endogenous *TMPRSS2:ERG*-expressing PCa cells), and comparable to endogenous full-length *ERG* expression in HUVEC cells, suggesting that *TMPRSS2:ERG* expression levels in our cell models were not excessive. As expected, high, moderate and low expression levels of *TMPRSS2:ERG* protein were only found respectively in H, M and L cells, and not in control cells or in parental PC3c cells (Figure 1c). In addition, compared to VCaP cells, *TMPRSS2:ERG* expression in H cells was lower (Supplementary Figure S1).

A comparison of H, M and L cell morphology to that of control cells showed that, where control cells were round and clustered, H cells were more elongated and scattered (Figure 2a). This morphological change was also observed, to a lesser extent, in M cells, but nearly absent in L cells (Figure 2a). Next, we examined whether the ectopic *TMPRSS2:ERG* expression could have an impact on PC3c cell proliferation. Surprisingly, no significant cell proliferation

changes were observed in H, M and L cells compared to control cells (Figure 2b), suggesting that *TMPRSS2:ERG* fusion contributes to PCa initiation and/or progression in a different way. Accordingly, ectopic *TMPRSS2:ERG* expression significantly increased PC3c cell migration in a membrane-based assay system (Figure 2c), as well as its invasiveness in a Matrigel-based system (Figure 2d). Interestingly, among H, M and L cells, the enhancements of cell motility and invasiveness seemed to depend on *TMPRSS2:ERG* expression levels: compared to control cells, H cells showed the most significant induction of cell migration (3.5 fold) and invasion (7.5 fold); whereas M cell migration (1.7 fold) and invasion (3.4 fold) were higher than those of L cells (migration: 1.2 fold; invasion: 1.2 fold) (Figure 2c and 2d). These results provide *in vitro* evidence that *TMPRSS2:ERG* promotes, in a dose-dependent manner, PC3c cell migration and invasion, two crucial attributes of cancer cell metastasis.

Genome-wide expression analysis reveals potential *TMPRSS2:ERG* target genes and their associated cell motility and invasiveness functions.

To decipher the molecular mechanisms that underlie *TMPRSS2:ERG*-mediated PC3c cell migration and invasion enhancements, we investigated global gene expression changes in H, M and L cells using Agilent 44k Whole Human Genome Expression arrays. Compared to control cells, 1673 genes showed significant differential expression (adjusted P-value <0.01) in H cells (809 upregulated genes and 864 downregulated genes) (Figure 3a), and 446 genes were differentially expressed in M cells (267 upregulated genes and 179 downregulated genes), whereas only 191 genes with differential expression were identified in L cells (101 upregulated genes and 90 downregulated genes). Therefore, the quantity of deregulated genes identified in H, M and L cells also appeared to rely on *TMPRSS2:ERG* expression levels in PC3c cells.

A comparison of H, M and L cell transcriptomic profiles revealed that 126 genes were commonly deregulated in H, M and L cells (hereafter referred to as “common deregulated

genes”), including 64 common upregulated genes and 62 common downregulated genes (Figure 3b, Supplementary Table S2 and S3). In particular, about 50% of these 126 common deregulated genes showed high, moderate and low expression levels in *TMPRSS2:ERG*-expressing H, M, L cells, respectively (Figure 3b and Supplementary Table S2 and S3). The “dose-dependent” expression profile of these genes suggested that they could be direct target genes of *TMPRSS2:ERG*. Among these genes, we noted the striking upregulation of *PLAT* (*Tissue-type plasminogen activator*), a well-known *TMPRSS2:ERG* target gene in PCa (4, 36, 37). Moreover, the over-expression of some of these genes has been shown to be associated with PCa progression and poor clinical outcomes, such as *CD44* (38), *MMP9* (39) and *FSCN1* (*Fascin homolog 1*) (40). To understand the overall functional significance of these common 126 deregulated genes, we used the DAVID gene ontology analysis (41). Among the top Gene Ontology (GO) terms, we noted that “*cell motion*”, “*cell migration*” and “*metalloendopeptidase activity*”, were upregulated, whereas “*transcription*” and “*regulation of apoptosis*” were downregulated (Figure 3c). Taken together, these transcriptomic studies were in agreement with the observed enhancements of PC3c cell migration and invasion, and further highlighted the *TMPRSS2:ERG* role in the PCa cell metastasis process.

Turning to the identification of *TMPRSS2:ERG* direct target genes in PCa, we focused on two candidate target genes in the following studies. The first one was *MMP9*, also known as *Gelatinase B*, which has been shown to be associated with PCa progression (39), and also regulated by other ETS transcription factors in diverse types of cancer cells including PCa (42, 43). However, it was not clear whether *TMPRSS2:ERG* could directly regulate *MMP9* expression in PCa. The second potential target gene was *PLXNA2*, a type I trans-membrane glycoprotein that functions as a receptor for Semaphorins. Together with other Plexins, it was initially considered to mediate axon guidance during development (44). However, recent studies have revealed Semaphorin-Plexin signaling outside of the nervous system, providing

additional information about this signaling in cancer (45). For instance, *PLXNB1* (*Plexin B1*) gene mutations in human PCa samples have been shown to be associated with PCa cell motility and invasion enhancements (46). These findings, together with the phenotype observed in H, M and L cells, prompted us to further investigate whether *TMPRSS2:ERG* could directly control *MMP9* and *PLXNA2* expression to increase PCa cell migration and invasion.

MMP9 and PLXNA2 expression levels were associated with TMPRSS2:ERG expression in human PCa samples

To investigate whether *MMP9* and *PLXNA2* are genuine target genes of *TMPRSS2:ERG* in PCa, we first examined their expression in human PCa samples. We used RNA from a PCa cohort composed of 52 human prostatectomy specimens from the Tumor Tissue Bank, C2RC, Lille University Hospital (CHRU Lille) (Supplementary Table S4). Among these PCa samples, we found, using *TMPRSS2:ERG*-specific primers (4), 32 samples that showed detectable *TMPRSS2:ERG* fusion transcripts (61.5%). In this PCa cohort, *MMP9* and *PLXNA2* expression levels were significantly higher in *TMPRSS2:ERG*-positive PCa samples than in *TMPRSS2:ERG*-negative samples ($P=0.0055$ and $P<0.0001$ respectively) (Figures 4a and 4b).

To further examine the associations between *TMPRSS2:ERG* and its potential targets, *MMP9* and *PLXNA2*, we performed a two-tailed non-parametric Spearman correlation analysis. Given the heterogeneity of *TMPRSS2:ERG* transcripts detected in PCa samples (5, 34), it is difficult to design universal specific primers to accurately quantify total *TMPRSS2:ERG* expression. We therefore used *ERG*-specific primers that could detect not only *ERG* transcripts, but also *TMPRSS2:ERG* transcripts. Importantly, high *ERG* expression was detected only in *TMPRSS2:ERG*-positive PCa samples, whereas *ERG* expression in fusion negative PCa samples was very low (Supplementary Figure S2), suggesting that, in this PCa

cohort, high-level *ERG* transcripts may be a direct consequence of the presence of *TMPRSS2:ERG* fusion. Spearman correlation analysis showed that *MMP9* expression was significantly correlated with *ERG* expression ($R=0.4287$, $P=0.0015$) (Figure 4c), as was *PLXNA2* expression ($R=0.6178$, $P<0.001$) (Figure 4d). Thus, *MMP9* and *PLXNA2* expression levels were upregulated in *TMPRSS2:ERG*-positive PCa specimens, and their expression levels were correlated with *ERG* expression in PCa samples.

TMPRSS2:ERG directly and positively regulates MMP9 expression in PCa cells

To determine whether *MMP9* gene expression was directly controlled by *TMPRSS2:ERG* in PCa cells, we first performed a chromatin immunoprecipitation assay (ChIP) in H cells and control cells. By analyzing the promoter region of the *MMP9* gene, we found a putative ETS-binding site (EBS) within 1 kb from the *MMP9* transcription start site (TSS) (Figure 5a, top panel). ChIP assay showed significant binding of *TMPRSS2:ERG* on this EBS in H cells (Figure 5a, bottom panel), whereas no *TMPRSS2:ERG* binding was found on this EBS in control cells, or in the promoter region of *GAPDH* gene which does not contain any functional EBS, suggesting direct and specific *TMPRSS2:ERG* binding to the *MMP9* promoter region in PC3c cells.

Next, dose-dependent upregulation of *MMP9* gene expression was confirmed by RT-qPCR in H, M and L cells (Figure 5b). In addition, we found that ectopic expression of *TMPRSS2:ERG* in DU145 cells, another AR and fusion double-negative PCa cell model, also resulted in increased *MMP9* expression (Figure 5c and Supplementary Figure S3). Because it has been shown that secreted *MMP9* has an important role in extracellular matrix remodeling during cancer progression (47), we further investigated secreted *MMP9* levels in *TMPRSS2:ERG*-expressing cells using a gelatin zymography assay. As expected, compared to control cells, more secreted *MMP9* was found in *TMPRSS2:ERG*-expressing H and M cell conditioned mediums (Figure 5d). In particular, the cell gelatin degradation assay highlighted

the association between increased MMP9 secretion and the enhancement of gelatin matrix degradation in *TMPRSS2:ERG*-expressing H cells (Figure 5e and Supplementary Figure S4). Moreover, the knockdown of *TMPRSS2:ERG* in H cells using pooled *ERG* siRNA (Supplementary Figure S5) led to over 40% reduction of *MMP9* expression (Figure 5f), as well as decreased MMP9 secretion (Figure 5g). Similar results were also observed in M cells (Supplementary Figure S6) as well as in *TMPRSS2:ERG*-expressing DU145 cells (Supplementary Figure S7). Taken together, these results indicate that *TMPRSS2:ERG* is capable to directly and positively regulate MMP9 in PCa cells.

PLXNA2 is directly and positively regulated by TMPRSS2:ERG in PCa cells

Plexin A2 (PLXNA2) is another candidate *TMPRSS2:ERG* target gene identified by our transcriptomic studies (Figure 3b), and its expression was found to be upregulated in *TMPRSS2:ERG*-positive PCa samples and correlated with *ERG* expression in PCa specimens. A putative EBS, located within 1 kb from the *PLXNA2* gene TSS, was found in the *PLXNA2* promoter region (Figure 6a, top panel). The ChIP assay showed significant *TMPRSS2:ERG* binding on this EBS in H cells, but not in control cells (Figure 6a, bottom panel). Moreover, this binding was absent in the irrelevant GAPDH promoter region, indicating *TMPRSS2:ERG* directly and specifically binds to the *PLXNA2* promoter in PC3c cells.

The dose-dependent increase of *PLXNA2* expression was then confirmed in *TMPRSS2:ERG*-expressing H, M and L cells compared to control cells (Figure 6b). In addition, *PLXNA2* upregulation was also observed in *TMPRSS2:ERG*-expressing DU145 cells (Figure 6c and Supplementary Figure S3). An immunofluorescence assay also showed *PLXNA2* protein induction in *TMPRSS2:ERG*-expressing H cells (Figure 6e, left panel). Importantly, *TMPRSS2:ERG* knockdown in H cells resulted in about 50% reduction of *PLXNA2* mRNA levels (Figure 6d), as well as *PLXNA2* protein immunostaining (Figure 6e, right panel). Similar results were observed in M cells (Supplementary Figure S6) and *TMPRSS2:ERG*-

expressing DU145 cells (Supplementary Figure S7). These results therefore highlight that *TMPRSS2:ERG* can directly and positively regulate *PLXNA2* expression in PCa cells.

PLXNA2, but not MMP9, contributes to TMPRSS2:ERG-induced PC3c cell migration and invasion in vitro.

To further explore whether *MMP9* and *PLXNA2* were direct mediators of *TMPRSS2:ERG*-induced pro-motility and pro-invasion phenotypes observed in PCa cells, we performed migration and invasion assays using H cells in which *ERG*, *MMP9* and *PLXNA2* were knocked down, respectively. The pooled *MMP9* siRNA efficiently knocked down *MMP9* expression (Supplementary Figure S8a) as well as *MMP9* secretion in H cells (Supplementary Figure S8b). Efficient *PLXNA2* knockdown effects were as well observed using pooled *PLXNA2* siRNA in H cells (Figure 7a and 7b).

In H cells, *ERG* knockdown dramatically decreased cell migration by over 50% (Figure 7c), and cell invasion by 70% (Figure 7d), indicating that the inductions of cell migration and invasion are specific to ectopic *TMPRSS2:ERG* expression. Surprisingly, *MMP9* knockdown had no significant effect on cell migration or invasion in H cells (Supplementary Figure S8c and S8d), whereas *PLXNA2* knockdown in H cells resulted in a 30% decrease in cell migration (Figure 7c) and a 20% decrease in cell invasion (Figure 7d). Hence, these findings demonstrate that *PLXNA2* upregulation, but not *MMP9* upregulation, may contribute, at least in part, to *TMPRSS2:ERG*-mediated induction of PC3c cell migration and invasion *in vitro*.

Furthermore, increased cell motility and invasiveness are considered as attributes commonly acquired by PCa cells during metastatic progression (48). Whether *PLXNA2* expression is associated with metastatic PCa remains unclear. We therefore analyzed *PLXNA2* expression in microarray datasets from three independent PCa cohorts (49-51). As shown in Figures 7e, 7f and 7g, compared to primary PCa samples, *PLXNA2* expression levels were significantly higher in the metastatic PCa, substantiating its potential role in PCa metastasis.

DISCUSSION

Although the presence of chromosomal abnormalities have commonly been observed in hematologic malignancies and sarcomas (52), it was only in 2005, using conceptually new bioinformatics approaches, that the first gene fusions were identified in a large proportion of PCa (4). While the gene fusions initially found in PCa were *ETS* transcription factors (*ERG* and *ETV1*) fused to the 5' part of the prostate-specific gene *TMPRSS2*, it is now clear that they may involve other *ETS* family members (i.e., *Fli-1*, *ELK4*), novel 5' partners and a class of non-ETS-based fusions (3). However, the *TMPRSS2:ERG* fusion remains the most prevalent chromosomal rearrangement in PCa (3). To date, this fusion has been identified not only in androgen-responsive PCa, but also in androgen-refractory PCa (16).

Here, using three AR-negative PC3c cell clones with different *TMPRSS2:ERG* expression levels, we demonstrated that ectopic expression of *TMPRSS2:ERG* can induce, in a dose-dependent manner, PC3c cell motility and invasiveness. These findings are in agreement with the role of the *TMPRSS2:ERG* fusion as revealed using various PCa cell models (4, 18, 21, 35, 53). Importantly, inhibition of endogenous *TMPRSS2:ERG* expression in AR-positive VCaP cells significantly reduced cell invasiveness (17, 18). Given the diverse genetic backgrounds of PCa cell models used in studies, the common function of *TMPRSS2:ERG* fusion is to improve PCa cell motility and invasiveness. Furthermore, since *TMPRSS2:ERG*-induced cell migration and invasion have been shown in AR-positive and -negative PCa cells, this function does not seem to be associated with the AR status of PCa cells. Increased cell motility and invasiveness are the essential hallmarks of cancer, as well as prerequisites for local tumor progression and metastasis (48). Interestingly, recent clinical studies also revealed the presence of *TMPRSS2:ERG* fusion in circulating tumor cells isolated from PCa patients with metastatic disease and in castration-resistant PCa patients (54, 55). In addition, *TMPRSS2:ERG*-positive foci in multifocal PCa have a greater predilection for lymph node

metastasis (56, 57). These clinical findings highlight the potential role of *TMPRSS2:ERG* in PCa metastasis.

Increased cell motility and invasiveness are also associated with cells adopting a mesenchymal phenotype (58). The epithelial-to-mesenchymal transition (EMT) is considered to be one of key steps for cells to acquire metastatic adaptation (59). Three previous studies have also demonstrated that EMT is one of the consequences of *TMPRSS2:ERG* expression in PCa cell models (37, 53, 60). Accordingly, PC3c cells with high *TMPRSS2:ERG* expression (H cells) demonstrated a more elongated and scattered mesenchymal morphology. However, the absence of differential expression of EMT markers, such as *E-Cadherin*, *Vimentin*, *Twist1* and *Snail* in our cell models (Supplementary Figure S9), raised two questions that need to be addressed in future studies: 1) Among our cell models (H, M and L cells), the highest *TMPRSS2:ERG* expression was only 50% of that found in VCaP cells; is there a threshold of *ERG* expression necessary to induce EMT in PCa cells? 2) Is *TMPRSS2:ERG*-induced EMT independent of AR signaling?

MMP9 expression has been shown to be correlated with increased invasive and metastatic phenotypes in various tumor types including prostate cancer (39, 61, 62). Here, we showed that MMP9 was positively and directly regulated by *TMPRSS2:ERG* in PC3c cells and its expression was correlated with *ERG* expression in PCa samples. In fact, several transcription factors, such as NF- κ B, SP-1, AP-1 and ETS have been shown to control MMP9 expression in physiological and pathological conditions (63-65). Although it has been reported that ETS-1 promotes the invasiveness of paclitaxel-resistant and hormone-refractory PCa cells by increasing MMP9 expression (66), a recent study reported that SPDEF, another ETS transcription factor, suppresses prostate cancer cell invasion through the repression of MMP9 expression (42). Together with our results, these findings highlight the importance of the functional specificity of ETS transcription factors in controlling MMP9 expression in PCa.

Furthermore, although increased secretion of MMP9 and corresponding gelatin matrix degradation were observed in our cell model, *MMP9* knockdown did not impair TMPRSS2:ERG-induced cell migration or invasion in the membrane-based transwell assays. Similar results have also been observed in *TMPRSS2:ERG* or *ERG*-expressing RWPE-1 and BPH-1 cells treated with the pan-MMP inhibitor or a MMP2/9 specific inhibitor (4, 18), suggesting that, under membrane-based transwell assay systems, increased MMP9 expression is not essential for TMPRSS2:ERG-induced cell migration and invasion. Of particular interest, MMP9 has been shown to trigger the release of the matrix-bound VEGF, thus promoting tumor angiogenesis (67), and remodeling the pro-metastatic microenvironment of the metastatic niche (47, 68). Therefore, future studies are required to determine the role of MMP9 in *TMPRSS2:ERG*-positive PCa cells using *in vivo* models.

Plexin receptors and their Semaphorin ligands were initially identified as evolutionarily conserved axon-guidance cues in the nervous system (69). However, increasing evidence highlights their role outside the nervous system, and particularly in cancer metastasis and progression (45). In particular, there is supporting evidence that somatic aberrations of Plexins and Semaphorins are involved in pancreatic carcinogenesis (70). Although their functions are poorly understood in PCa, Plexin receptors and its Semaphorin ligands have been shown to be expressed in diverse PCa cell lines and tissues (71). The overexpression of *PLXNB1* with somatic missense mutations is functionally associated with increased PCa cell motility and invasiveness, as well as disease metastatic progression (46, 72). We showed in the present study that that TMPRSS2:ERG positively and directly regulates PLXNA2 expression in PC3c cells. Interestingly, during mouse (73, 74) and chicken development (75, 76), the *Erg* expression profile overlaps with that of *Plxna2* in migrating neural crest cells and aorta, suggesting the possible direct regulation of *Plxna2* by the *Erg* transcription factor in development process. Moreover, other transcription factors may also control PLXNA2

expression. For example, GATA6 has been shown to directly regulate PLXNA2 expression during human cardiac outflow tract development (77). Furthermore, the pleiotropic signals mediated by Semaphorin-Plexin have been shown to positively or negatively control multiple functions in tumor cells, including their migration and invasion abilities (45). We showed that the upregulation of PLXNA2 contributes, at least in part, to *TMPRSS2:ERG*-induced PC3c cell migration and invasion. In agreement with these findings *in vitro*, analysis of microarray datasets provided evidence that *PLXNA2* expression is up-regulated in metastatic PCa, supporting the potential involvement of PLXNA2 in PCa metastatic spread.

In summary, using an AR-negative PCa model, we confirmed the important role of *TMPRSS2:ERG* in PCa cell migration and invasion, and identified two “cell motility”- and “invasiveness”-related direct target genes, *MMP9* and *PLXNA2*, providing novel insights into the role of *TMPRSS2:ERG* in PCa metastasis.

MATERIALS AND METHODS

Cell culture and Chemical reagents

PC3c cells (32) and DU145 cells (ATCC) were cultured in DMEM supplemented with 10% fetal bovine serum and sub-cultured every 3-4 days. Unless otherwise stated, all chemical reagents were from Sigma-Aldrich.

Generation of plasmids and retrovirus constructs

The *TMPRSS2:ERG* cDNA was isolated from a fusion-positive PCa patient sample, and sub-cloned into a pcDNA3.1(+) (Invitrogen) or retrovirus pLPCX vector (Clontech). The primers used for cloning are listed in Supplementary Table S1. Amplified plasmids were purified on NucleoBond Xtra Midi endotoxin-free columns (Macherey-Nagel) and sequenced prior to use.

Generation of *TMPRSS2:ERG*-expressing cells

Low passage PC3c cells were transfected with a *TMPRSS2:ERG* expression vector or empty pcDNA vector using FuGENE[®] 6 Reagent (Roche) according to the manufacturer's instruction, then selected in routine culture medium containing 400 mg/mL G418 antibiotic (Life Technologies) for 3 weeks. The pLPCX- or pLPCX/*TMPRSS2:ERG*-containing retroviruses were transduced into DU145 cells to generate *TMPRSS2:ERG*-expressing cells according to the manufacturer's instructions (Clontech).

Short interfering RNAs (siRNA) transfection

Pre-designed pooled siRNA were obtained from Dharmaco-Termo Scientific. Cells were transfected with the siRNA (50 nM) using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions. Gene knockdown effects were evaluated after 72 h of transfection. The siRNA used in this study were siERG (ON-TARGETplus SMARTpool L-003886-00), siMMP9 (ON-TARGETplus SMARTpool L-005970-00), siPLXNA2 (ON-TARGETplus SMARTpool L-021532-01) and the control siRNA (ON-TARGETplus Non-targeting Pool D-001810-10).

Protein extraction and immunoblotting

Proteins were extracted and immunoblotted (Western Blotting) as described previously (78). Antibodies used in immunoblotting were: rabbit monoclonal anti-ERG (Epitomics 2085, dilution 1:1 000), mouse monoclonal anti β -ACTIN (Sigma Clone AC-15, dilution 1:10 000), rabbit polyclonal anti HPRT (Santa Cruz sc-20975, dilution 1:1 000), Rabbit IgG-HRP (GE Healthcare NA934V, dilution 1:10 000) and Mouse IgG-HRP (Santa Cruz, 1:10 000).

Cell proliferation, migration and invasion assay

Cell proliferation assays were performed in quadruplicate using CellTiter-Glo® kit (Promega) in 96-well plate (2 500 cells/well) according to the manufacturer's recommendations, and luminescence was measured using a Centro LB 960 microplate luminometer (Berthold) at indicated times.

Cell migration assays and cell invasion assays were performed with 100 000 cells in triplicate using a FluoroBlok™ 8 μ m pores 24-well insert system (BD Biosciences) and 24-well BioCoat™ Tumor Invasion System (BD Biosciences) according to the manufacturer's instructions. After 16 h of incubation, cells were labeled with 10 μ g/mL DilC₁₂(3) fluorescent dye (BD Biosciences), and the migrated or invaded cells were measured using a FLUOstar OPTIMA lecturer (BMG Labtech).

Gelatin degradation assay and gelatin zymography analysis

Gelatin degradation assay was performed as described previously (79). To perform the gelatin zymography assay, cells were rinsed and incubated with medium without FBS for 24 h. Then the conditioned mediums (CM) were collected, and total protein concentrations were measured and used to adjust loading quantity of CM. Equal amount of CMs were mixed with non-denaturing Laemmli blue loading dye, then loaded on a 7% SDS-PAGE gel containing 0.1% (w/v) gelatin. After migration, the gel was incubated with zymogram renaturing buffer and zymogram developing buffer. Finally, the gel was stained with 0.5% (w/v) Coomassie

Blue R-250 and destained with a solution containing 40% (v/v) ethanol and 10% (v/v) acetic acid.

Cell total RNA preparation and RT-qPCR

Cell total RNA was purified using the NucleoSpin[®] RNA II kit (Macherey Nagel) according to the manufacturer's instructions. Then, 1 µg total RNA was used to generate cDNA strand using the High Capacity RNA-to-cDNA kit (Applied Biosystems). Quantitative polymerase chain reactions (qPCRs) were performed using the Power SYBR Green PCR Master kit (Applied Biosystems) on a Stratagene Mx3005P qPCR System according to manufacturer's instructions. Individual gene relative expression level was calculated using the $2^{-\Delta\Delta CT}$ method and normalized to that of the housekeeping gene *18S*. Optimal primer specificity and efficiency were validated according to the Mx3005P qPCR System user's guide. The primers used in this study can be found in Supplementary Table S1.

Immunofluorescence

Immunofluorescence (IF) assays were performed as described (78). The antibodies used in this study were monoclonal rabbit anti-ERG (Epitomics 2805, dilution 1:200), monoclonal rat anti-PLXNA2 (R&D Systems, MAB5486, 10 µg/mL), Alexa Fluo[®]-488 conjugated anti-rabbit IgG (Invitrogen, dilution 1:500) and Alexa Fluo[®]-555 anti-rat IgG (Invitrogen, dilution 1:500). Images were acquired using an LSM 710 confocal microscopy system and ZEN 2010 software (Carl Zeiss).

Microarray analysis

Microarray analyses were conducted following the Two-Color Microarray-Based Expression Analysis Protocol (Agilent Technologies). For each sample, 1 µg of total RNA was divided into two equal aliquots to enable technical replication known as "dye-swap hybridization". The reverse transcription and the labeling procedure were performed using the protocol recommended by Agilent Technologies (Low RNA Input Fluorescent Linear Amplification

Kit). Hybridizations were performed on Agilent Whole Human Genome 44K microarrays for 17 hours at 65°C using the Agilent Gene Expression Hybridization kit. Arrays were washed and scanned using Innoscan 700 (Innopsys) and the raw data were processed and normalized using the Limma package (Linear Models for Microarray Data) running under the R environment. Gene Ontology analysis was performed using the DAVID program as described (41).

Chromatin immunoprecipitation (ChIP)

ChIP assay was performed as described (80). Antibodies used were polyclonal rabbit anti ERG antibody (Santa Cruz sc-353) or control rabbit IgG antibody (Santa Cruz). The genomic DNA was purified using NucleoSpin® Clean-up columns (Macherey-Nagel) according to protocol recommended by the manufacturer. Immunoprecipitated genomic DNA was analyzed by PCR and details of the primers used can be found in Supplementary Table S1.

Human PCa samples

Human PCa samples (n=52) were obtained from the local tumor tissue bank, C2RC (Lille, France) after approval by the internal review board (CSTMT-042, 27/07/2009). Sampled and subsequently frozen tumor tissues originated from radical prostatectomies or trans-urethral prostatic resections performed at Lille University Hospitals (CHRU de Lille). All patients were informed and consent was obtained by the referring physician. To extract RNA, tissue samples were first homogenized in TRIzol® (Invitrogen), and total RNA was prepared using NucleoSpin® RNA II columns (Macherey-Nagel). The RNA quality and quantity were assessed by the Agilent 2100 Bioanalyser system. Pathological findings of these PCa samples can be found in Supplementary Table S4.

Image treatment and Statistical analysis

All images treatments were carried out using Image J or Photoshop CS software. Statistical analysis was performed using GraphPad Prism software. Statistical methods used in this study

are indicated in the corresponding figure legends. Unless otherwise indicated, all values in the figures are expressed as means \pm SEM.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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FIGURE LEGENDS

Figure 1 Stable ectopic expression of the *TMPRSS2:ERG* gene fusion in PC3c cells. (a) Schematic illustration of the *TMPRSS2:ERG* gene fusion expression plasmid construction. (b) Relative *ERG* expression was evaluated using qPCR in three *TMPRSS2:ERG*-expressing PC3c clone cells (H: PC3c cells with high *ERG* expression, M: PC3c cells with medium *ERG* expression and L: PC3c cells with low *ERG* expression), in control pcDNA clone cells, in HUVEC cells, as well as in VCaP cells. The expression levels of *ERG* in VCaP cells were normalized to 1. Results were obtained from three independent triplicate experiments. (c) Detection of *TMPRSS2:ERG* protein products (N-terminal-truncated *ERG* protein < 55 kDa) in H, M, L cells, control cells and parental PC3c cells. Total cell lysates were analyzed using Western blotting with *ERG* antibody, and human HPRT immunoblotting was used as a loading control.

Figure 2 Phenotype characterization of PC3c cells with ectopic *TMPRSS2:ERG* expression. (a) Phase contrast micrographs of H, M, L cells and control cells. Scale bar, 50 μm . (b) Cell proliferation assays were performed using the CellTiter-Glo kit (Promega) in quadruplicate for 5 days. Three independent experiments were performed and results are presented as relative luminescence unit (RLU) fold change compared to the RLU measured on Day 0. (c) Cell migration assays were performed using the FluoroBlok™ 8 μm pore 24-well insert system (BD Biosciences) in triplicate according to the manufacturer's recommendations. Results are presented as relative fluorescence unit (RFU) fold change compared to control cells after 16 h. *** $P < 0.001$, * $P < 0.05$, one-way analysis of variance (ANOVA) with Dunnett's multiple comparison test. (d) Cell invasion assays were performed in triplicate using the 8 μm pore 24-well BioCoat™ Tumor Invasion system (BD Biosciences) according to the manufacturer's instructions. Results are presented as RFU fold change

compared to control cells measured after 16 hours. *** $P < 0.001$, one-way analysis of variance (ANOVA) with Dunnett's multiple comparison test.

Figure 3 Transcriptomic studies of PC3c H, M and L cells. (a) Red Venn diagram shows 809 genes, 267 genes and 101 genes are upregulated in *TMPRSS2:ERG*-expressing H, M and L cells, respectively (left panel). Comparison of upregulated genes in three clones shows an overlap of 64 genes commonly upregulated in *TMPRSS2:ERG*-expressing PC3c cells compared to control cells. Green Venn diagram illustrates 864 genes, 179 genes and 90 genes downregulated in H, M and L cells, respectively (right panel). The 62 genes located in the overlap are common downregulated genes in *TMPRSS2:ERG*-expressing PC3c cells compared to control cells. (b) Common genes associated with *TMPRSS2:ERG* ectopic expression in H, M, and L cells. Left panel: 64 upregulated genes; right panel: 62 downregulated genes. The gene expression level is presented in color and average linkage hierarchical clustering was used to ordinate profiles. (c) Top-ranked Gene Ontology (GO) terms analyzed using the DAVID algorithm. GO term significances are presented as histograms. Red: GO terms of the 64 common upregulated genes in H, M and L cells; Green: GO terms of the 62 common downregulated genes in H, M and L cells.

Figure 4 Upregulation of *MMP9* and *PLXNA2* in *TMPRSS2:ERG*-positive human PCa samples. (a) *MMP9* mRNA expression in *TMPRSS2:ERG*-positive ($n=32$) and -negative ($n=20$) PCa samples was evaluated using RT-qPCR in triplicate. Two independent experiments were performed. $P=0.0055$, two-tailed Mann-Whitney test. (b) *PLXNA2* mRNA expression in *TMPRSS2:ERG*-positive ($n=32$) or -negative ($n=20$) PCa samples using RT-qPCR. Two independent triplicate experiments were performed. $P < 0.0001$, two-tailed Mann-Whitney test. (c) Scatter-plot showing relative *MMP9* mRNA expression and its corresponding *ERG* mRNA expression in primary PCa samples. $n=52$, $R=0.4287$, $P=0.0015$, two-tailed non-parametric Spearman correlation test with $\alpha=0.05$. (d) Scatter-plot

showing the relative *PLXNA2* mRNA expression and its corresponding *ERG* mRNA expression in primary PCa samples. $n=52$, $R=0.6178$, $P<0.0001$, two-tailed non-parametric Spearman correlation test with $\alpha=0.05$.

Figure 5 *TMPRSS2:ERG* directly and positively regulates *MMP9* expression in PCa cells. (a) A putative ETS binding site (EBS) (black oval) was identified in the *MMP9* gene promoter region (top panel), and ChIP assays were performed to examine *ERG* binding in H cells and in control cells. The irrelevant *GAPDH* gene promoter (without EBS) was used for negative controls (bottom panel). The positions of primers used in ChIP assay are represented by black arrows in the top panel. (b) *MMP9* expression was analyzed in the PC3c clone cells using RT-qPCR. (c) *MMP9* gene expression was analyzed using RT-qPCR in DU145 cells with ectopic *TMPRSS2:ERG* expression. (d) Gelatin zymography was performed to analyze secreted *MMP9* levels in PC3c clone cell conditioned mediums. Loading quantity was adjusted to total conditioned medium concentration. Three independent experiments were performed and a representative experiment is shown. (e) Gelatin degradation assays were performed on coverslips pre-coated with FITC-gelatin for 6 h. Ten images were taken of each coverslip and degradation surfaces were analyzed by Image J, then divided by cell number after counting DAPI- and F-Actin-positive cells to calculate relative degradation surface per cell. Results are presented as fold change of the degradation surface per cell relative to control pcDNA cells set to equal 1. Mean and SEM were calculated from two independent experiments with two coverslips each. $***P<0.001$, two-tailed Mann-Whitney test. (f) *MMP9* gene expression was analyzed in H cells treated with control or *ERG* siRNA. The expression level in cells treated with control siRNA was normalized to 1. (g) Gelatin zymography showing a reduction of secreted *MMP9* levels in the conditioned medium of H cells treated with *ERG* siRNA. Two independent experiments were performed and a representative experiment is shown.

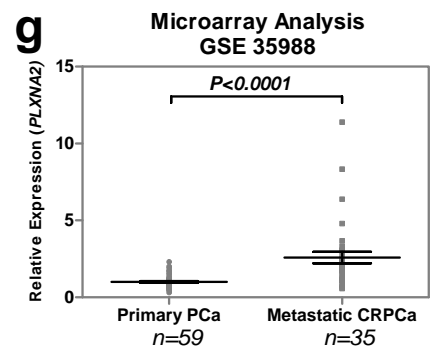
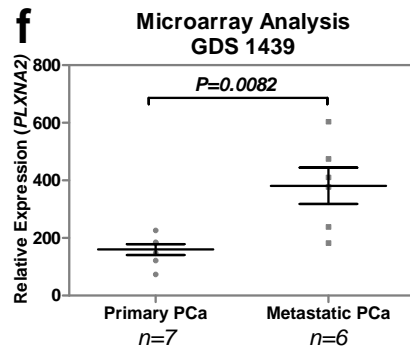
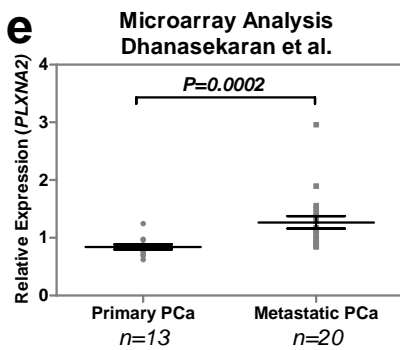
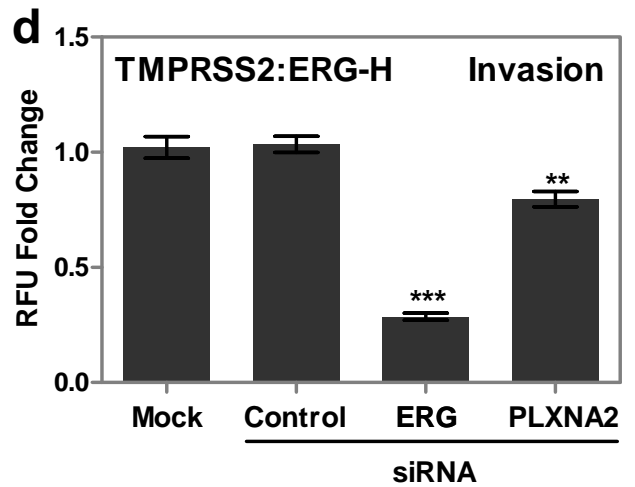
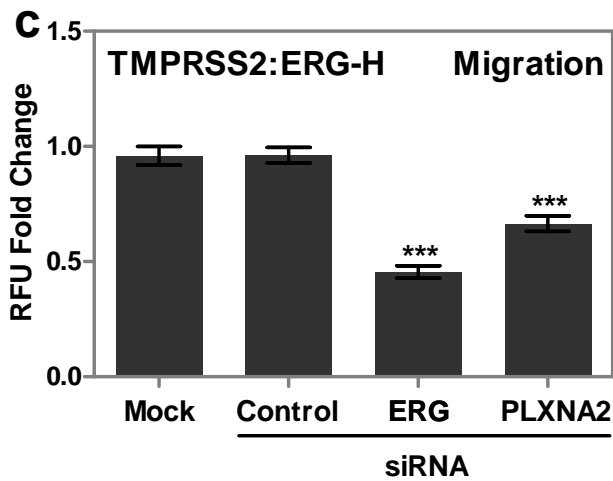
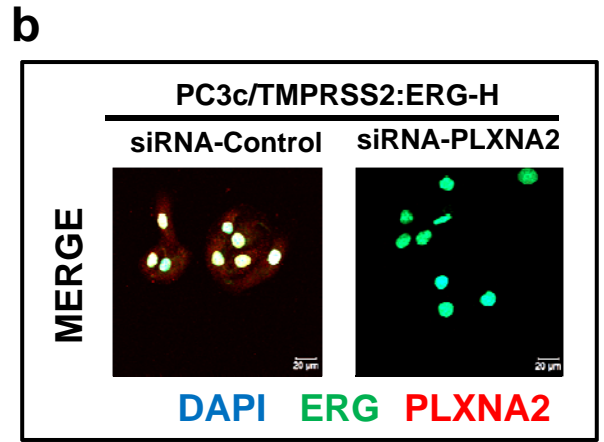
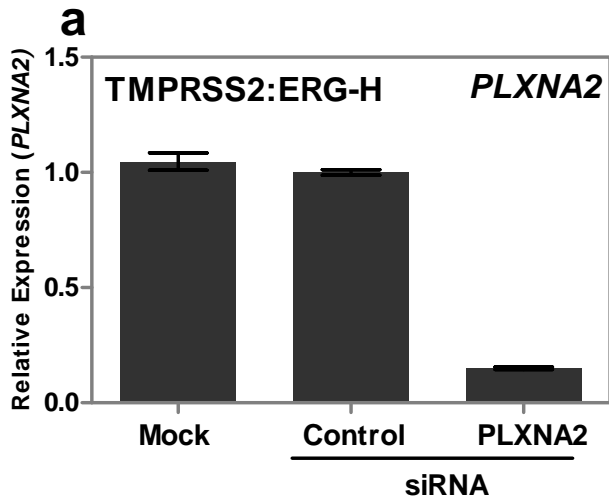
Figure 6 *PLXNA2* is directly and positively regulated by *TMPRSS2:ERG* in PCa cells.

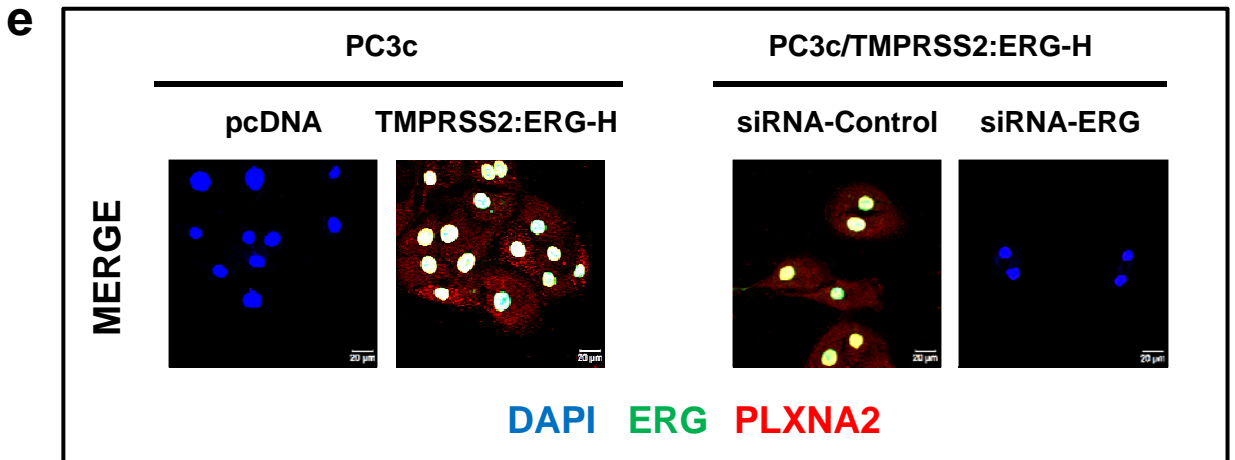
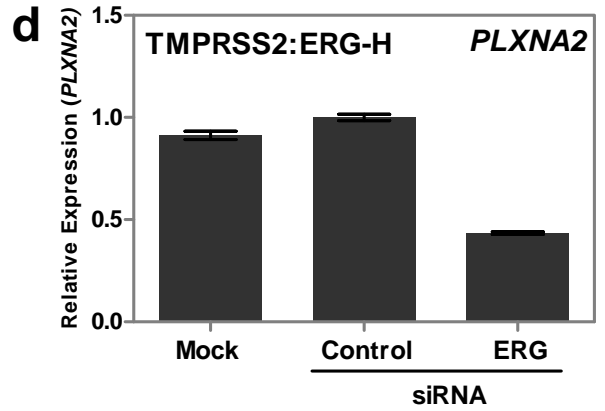
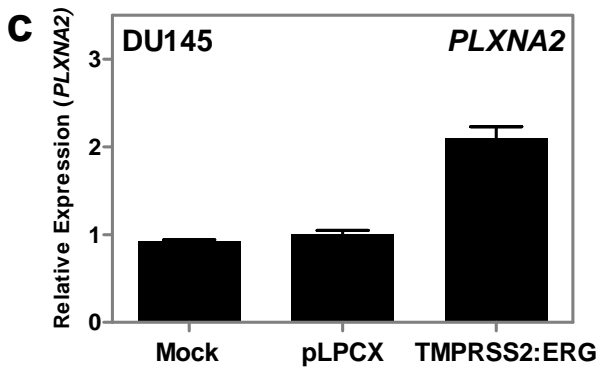
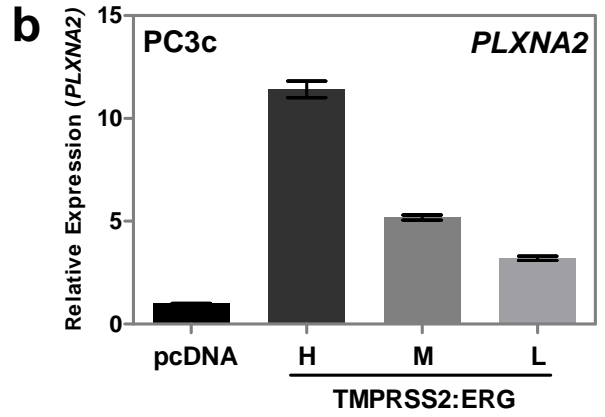
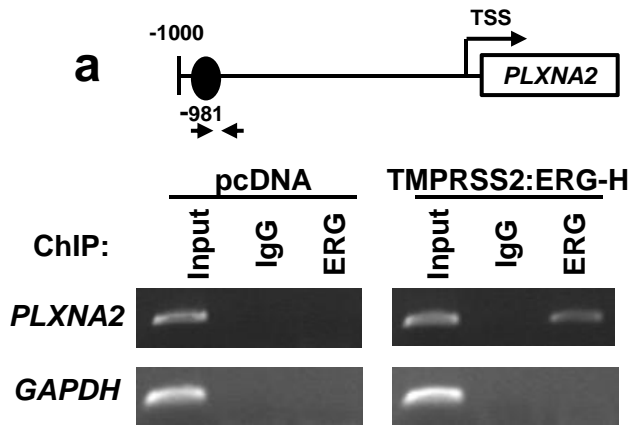
(a) A putative EBS was identified in the *PLXNA2* gene promoter (black oval) (top panel). ChIP assay was performed to examine ERG binding on this EBS in *TMPRSS2:ERG*-expressing H cells or in *TMPRSS2:ERG*-negative control cells. The irrelevant *GAPDH* gene promoter (without EBS) was used for negative controls (bottom panel). The positions of primers used in ChIP assay are represented by black arrows in the top panel. (b) Real-time PCR detection of *PLXNA2* expression in PC3c clone cells. (c) *PLXNA2* expression was analyzed in DU145 with ectopic *TMPRSS2:ERG* expression. (d) Analysis of *PLXNA2* expression in H cells treated with either pooled control or pooled *ERG* siRNA. The expression level in H cells treated with control siRNA was normalized to 1. (e) Immunofluorescent analysis (IF) of ERG and *PLXNA2* expression in H cells or control cells (left panel), and in H cells treated with either pooled control siRNA or pooled *ERG* siRNA (right panel). Scale bar, 20 μ m.

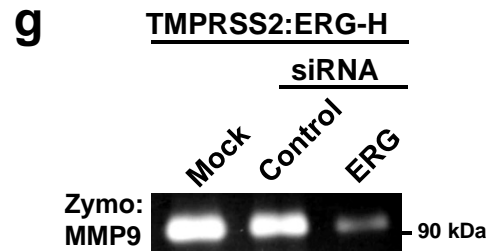
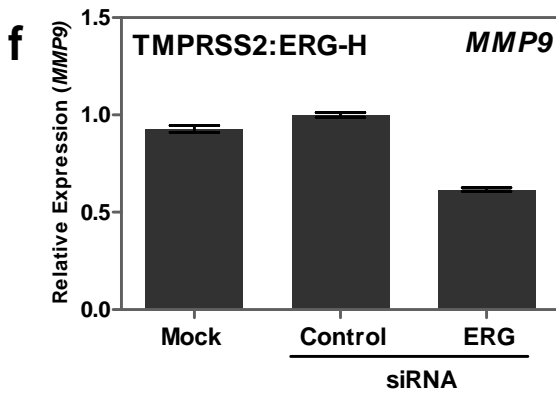
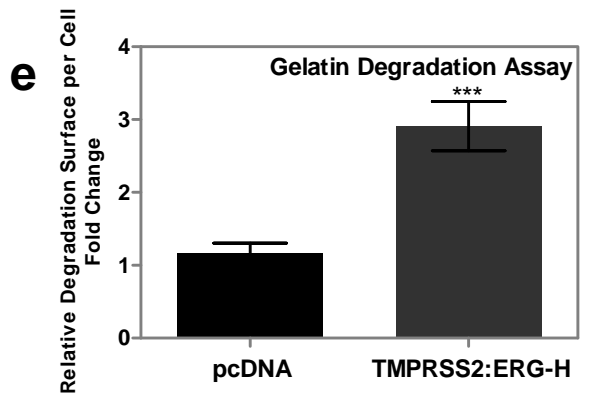
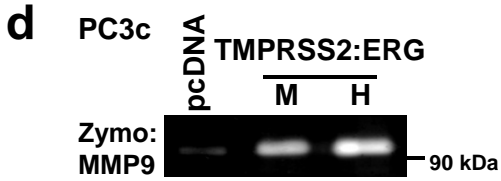
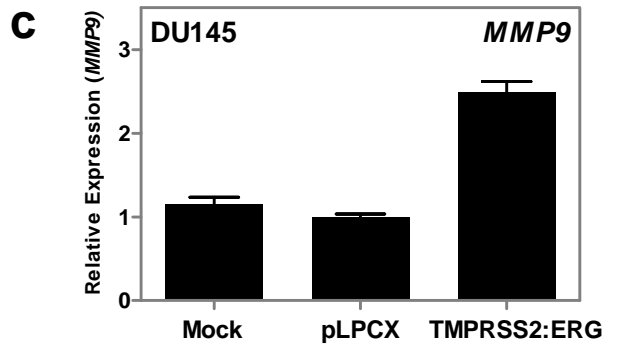
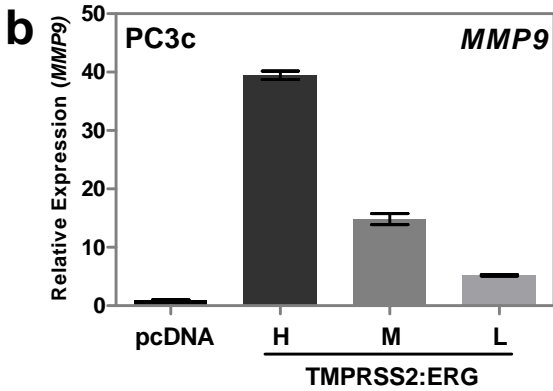
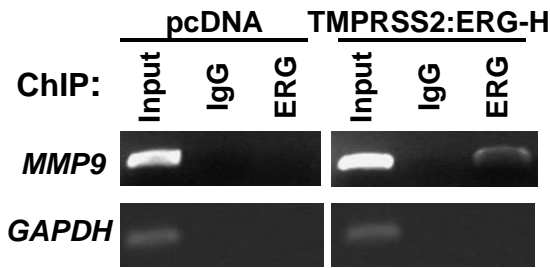
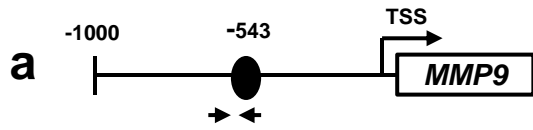
Figure 7 *PLXNA2* contributes to *TMPRSS2:ERG*-induced PC3c migration and invasion.

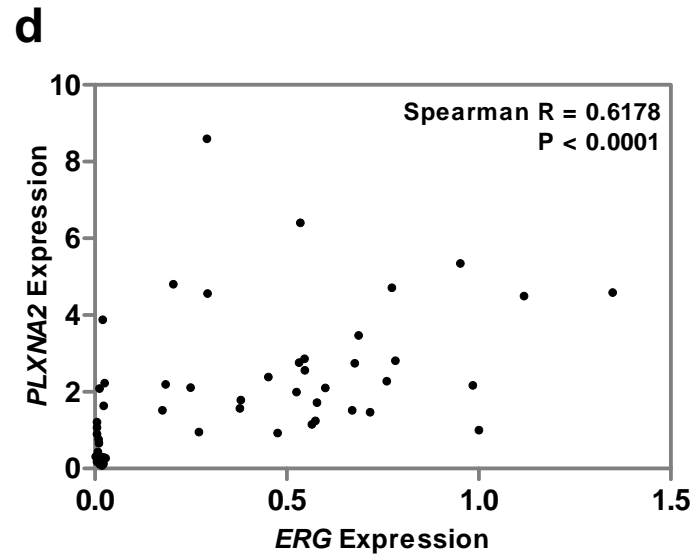
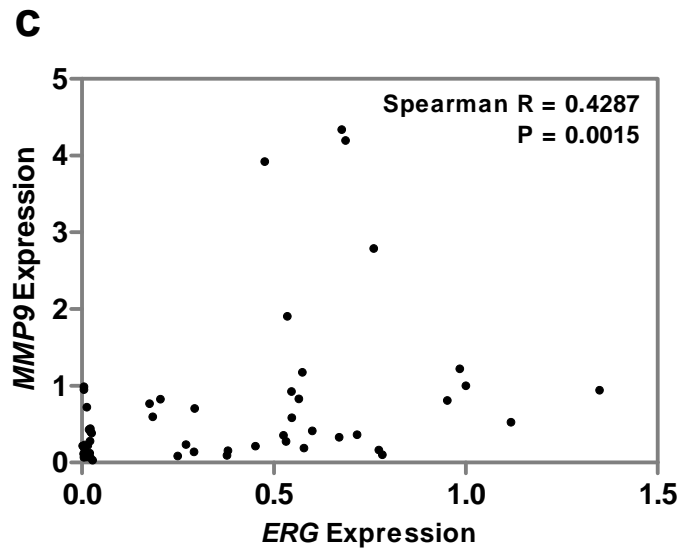
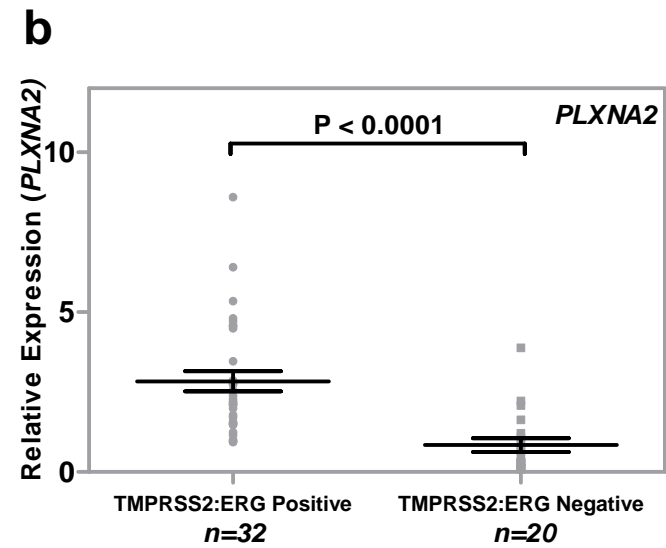
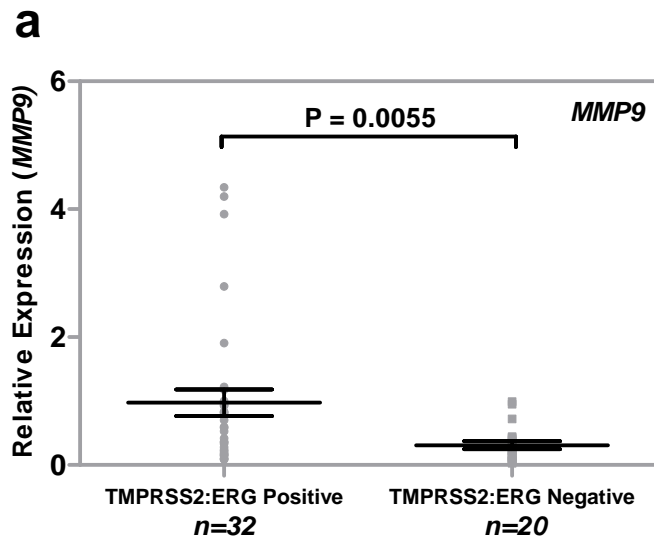
(a) H cells were treated with pooled control or *PLXNA2*-specific siRNA, and *PLXNA2* gene expression was analyzed by RT-qPCR. The expression level in H cells treated with control siRNA was normalized to 1. (b) IF analysis showing ERG and *PLXNA2* expression in H cells transfected with control or *PLXNA2* siRNA. Scale bar, 20 μ m. (c) Cell migration assays were performed using the FluoroBlok™ 8 μ m pore 24-well insert system (BD Biosciences) in triplicate. *TMPRSS2:ERG*-expressing H cells were treated with control, *ERG* or *PLXNA2* siRNA for 48 h, then migration was analyzed by measuring fluorescence 16 h later. Results are presented as RFU fold change compared to cells treated with control siRNA. *** $P < 0.001$, One-way analysis of variance (ANOVA) with Dunnett's multiple comparison test. (d) Cell invasion assays were performed in triplicate on H cells, pre-treated with control, *ERG* or *PLXNA2* siRNA for 48 h, using the 8 μ m pore 24 well BioCoat™

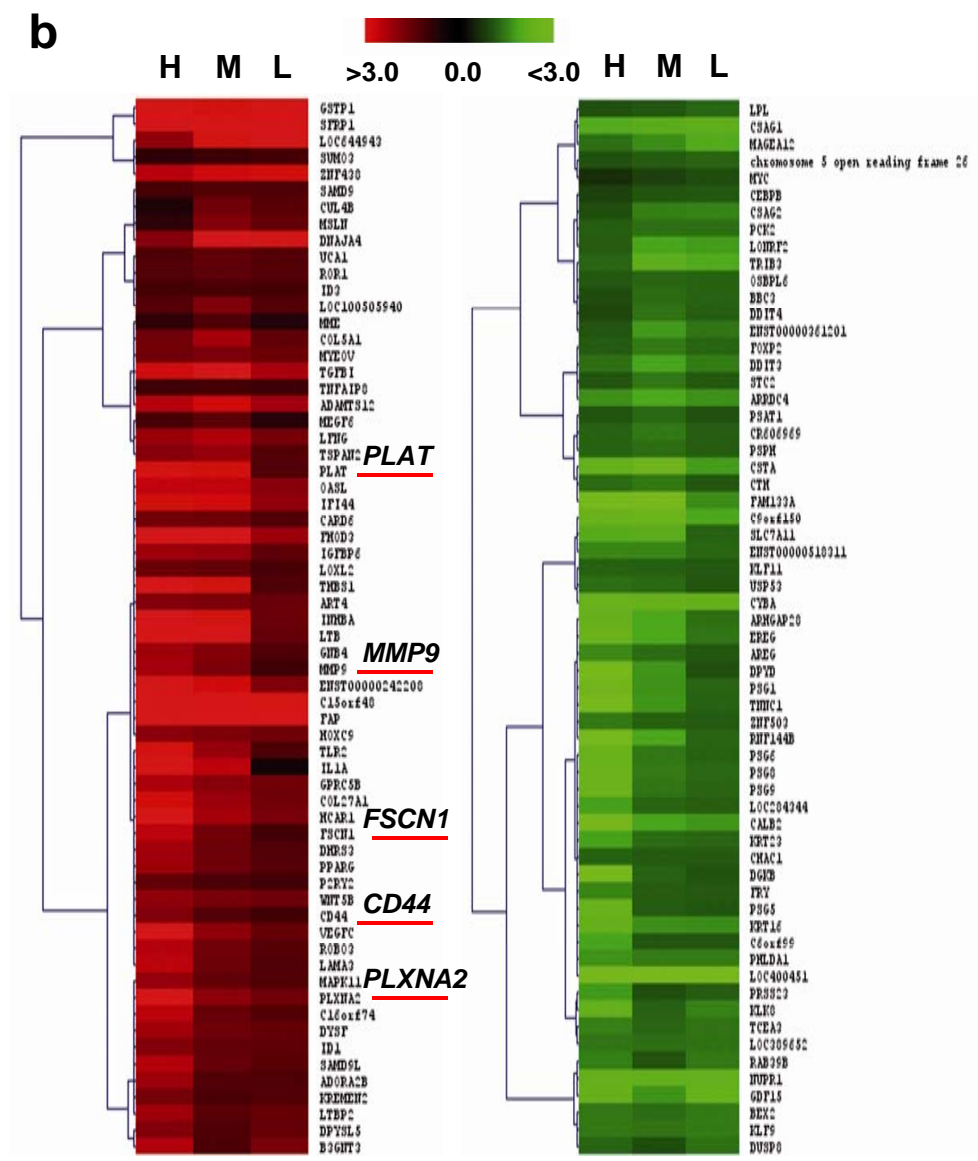
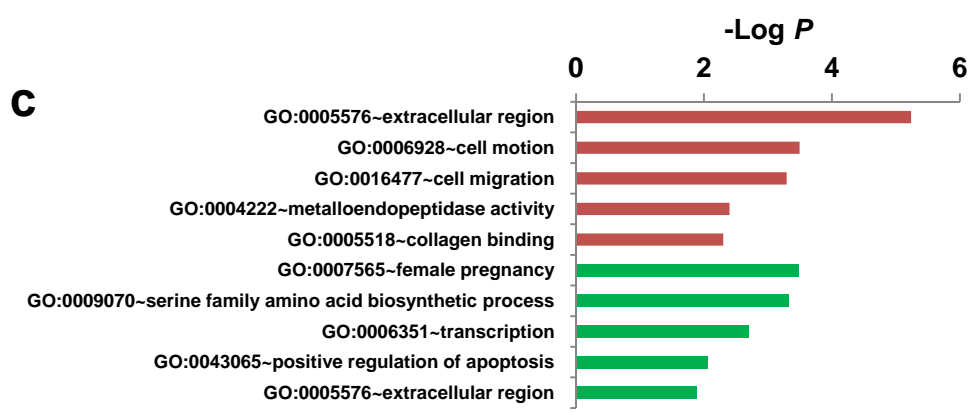
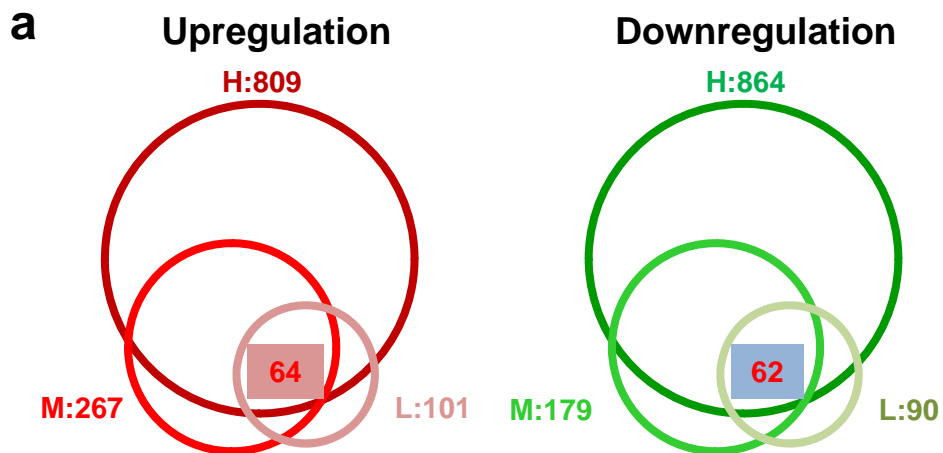
Tumor Invasion System (BD Bioscience). Results are also presented as RFU fold change compared to cells treated with control siRNA measured at 16 h later. *** $P < 0.001$, ** $P < 0.01$, one-way analysis of variance (ANOVA) with Dunnett's multiple comparison test. **(e)** Comparison of *PLXNA2* expression profile in primary PCa (n=13) and in metastatic PCa (n=20) using the dataset from Dhanasekaran *et al.* $P = 0.0002$, two-tailed Mann-Whitney test. **(f)** Comparison of *PLXNA2* expression profile in primary PCa (n=7) and in metastatic PCa (n=6) using the GDS1439 dataset. $P = 0.0082$, two-tailed Mann-Whitney test. **(g)** Comparison of *PLXNA2* expression profile in primary PCa (n=59) and metastatic PCa (n=35) using the GSE35988 dataset. $P < 0.0001$, two-tailed Mann-Whitney test.

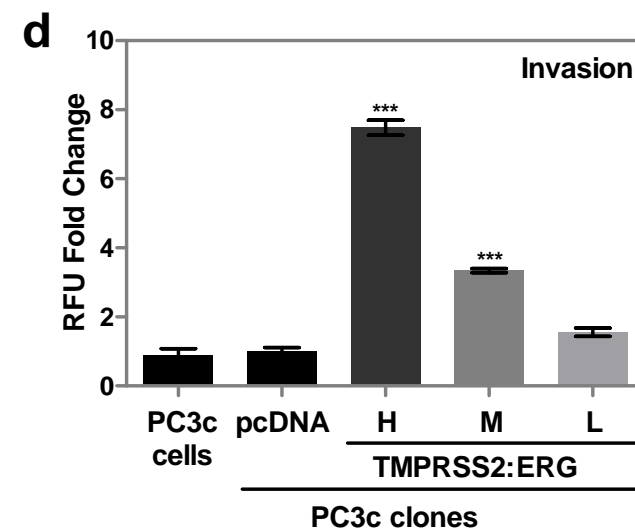
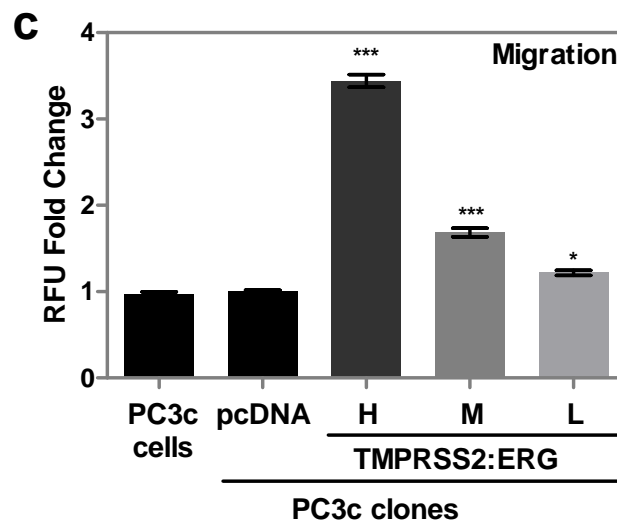
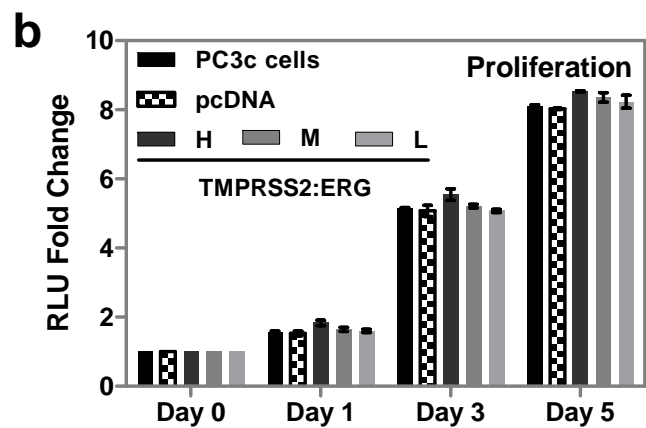
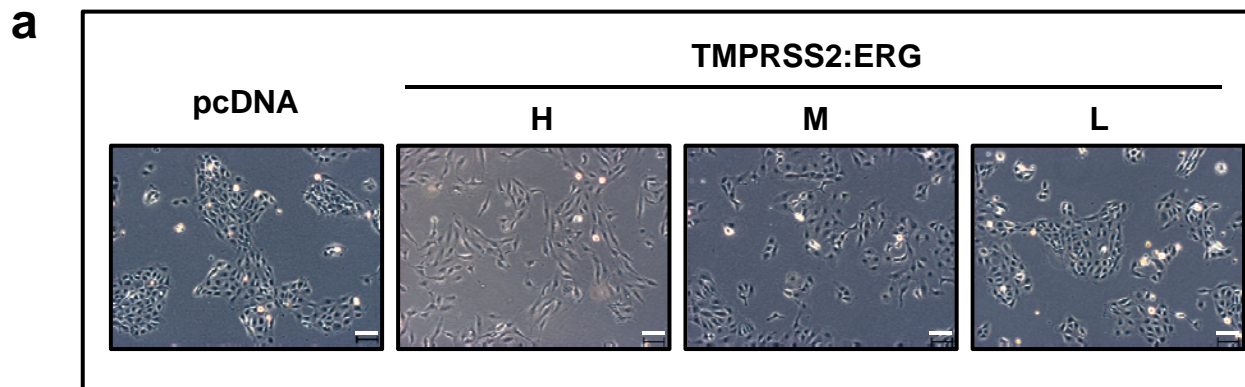


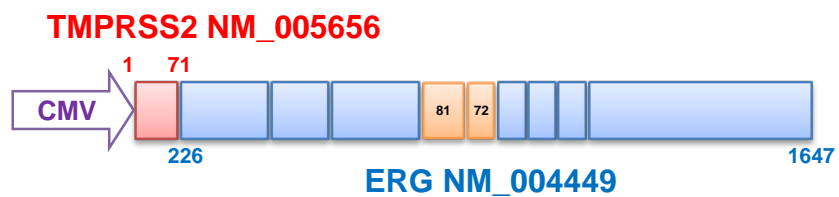
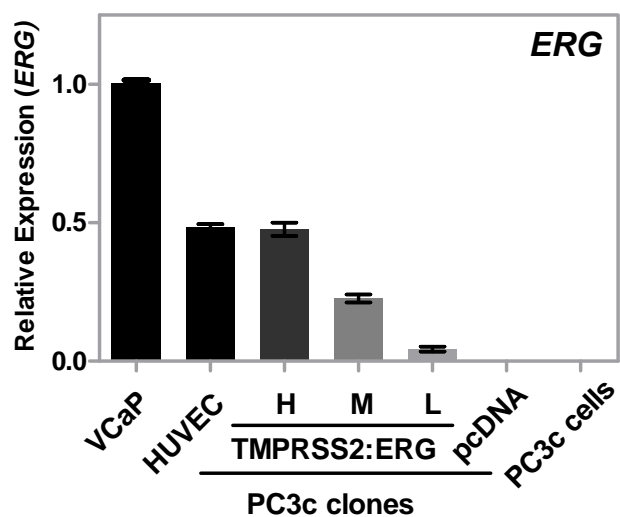
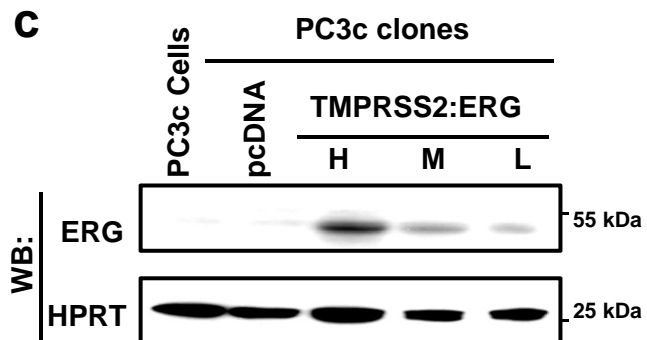










a**b****c**

Supplementary Table S4: Histo-pathological Characterization of PCa Samples (n=52)

PCa Samples	TNM classification			Gleason Score	Pre-surgery PSA (ng/mL)	TMPRSS2:ERG fusion
	T	N	M			
1	T2	N0	M0	4+3	7.7	-
2	T2	N0	M0	3+3	4.51	+
3	T2	N0	M0	3+3	2.7	+
4	T2	N0	M0	3+4	4.4	+
5	T2	N0	M0	3+4	7.5	+
6	T2	N0	M0	3+4	7.21	+
7	T2	N0	M0	4+3	6.54	+
8	T2	N0	M0	3+4	9.5	+
9	T2	N0	M0	3+4	5.4	+
10	T2	N0	M0	3+3	5.38	+
11	T2	N0	M0	3+4	9.65	-
12	T2	N0	M0	3+4	7.48	+
13	T2	N0	M0	3+3	7.35	+
14	T2	N0	M0	3+3	6.26	+
15	T2	N0	M0	3+3	2.13	+
16	T2	N0	M0	3+3	5.5	-
17	T2	N0	M0	3+3	5.5	+
18	T2	N0	M0	3+3	6	+
19	T2	N0	M0	3+3	2.3	-
20	T2	N1	M0	3+3	4.79	-
21	T2	N0	M0	4+5	45.1	+
22	T3	N0	M0	4+3	6.54	+
23	T3	N0	M0	4+3	5.5	+
24	T3	N0	M0	4+5	13.1	+
25	T3	N0	M0	5+3	5.9	+
26	T3	N0	M0	3+4	224	-
27	T3	N0	M0	3+4	10	+
28	T3	N0	M0	4+3	6.9	+
29	T3	N0	M0	4+3	5	-
30	T3	N0	M0	3+4	27	-
31	T3	N0	M0	4+3	7.86	+
32	T3	N0	M0	3+4	5.9	+
33	T3	N0	M0	3+4	7.5	-
34	T3	N0	M0	3+4	5.4	+
35	T3	N0	M0	3+4	4.8	+
36	T3	N1	M1	4+5	120	-
37	T3	N1	MX	5+4	125	+
38	T3	N1	M1	5+5	563	-
39	T3	N1	M1	4+4	432	+
40	T3	N0	M0	3+3	4.72	+
41	T3	N0	M0	4+4	8.2	+
42	T3	N1	M0	4+5	169	+
43	T3	N0	M0	4+5	101	-
44	T3	N0	M0	4+5	60	-
45	T3	N1	M0	4+5	80.1	-
46	T3	N0	M0	4+5	59.2	-
47	T3	N0	M0	4+5	66.1	+
48	T3	N0	M0	4+5	101	-
49	T3	N0	M0	4+5	34	-
50	T4	N0	M0	4+5	429	-
51	T4	N1	M1	4+5	50	-
52	T4	N0	M0	5+5	79.1	-

Supplementary Table S3: 62 common downregulated genes in TMPRSS2:ERG-expressing PC3c cells

Gene Name	Probe	Gene Symbol	Annotation	H		M		L	
				Log ₂ FC	P value	Log ₂ FC	P value	Log ₂ FC	P value
BF210146	A_32_P158376	LOC389652	BF210146	-1,41791868	7,54E-05	-1,35322104	1,24E-03	-1,38671454	8,74E-03
CR606969	A_32_P150040	CR606969	CR606969	-1,2314649	2,66E-04	-1,44763614	4,24E-04	-1,17659944	6,79E-03
NM_032523	A_32_P220762	OSBPL6	Oxysterol Binding protein-like 6	-1,12058145	2,04E-03	-1,28085046	2,33E-03	-1,23966881	9,11E-03
ENST0000036	A_24_P101651	ENST0000036	ENST00000361201	-1,10286513	2,13E-03	-1,75148013	1,48E-04	-1,41629755	2,88E-03
NM_002781	A_32_P219970	PSG6	pregnancy specific beta-1-glycoprotein 5	-2,47159202	8,91E-05	-1,14207606	1,63E-03	-1,09511648	7,84E-03
ENST0000051	A_32_P49284	ENST0000051	ENST00000518311	-1,58372159	1,52E-04	-1,58382846	2,74E-04	-1,27794328	4,25E-03
NM_000101	A_23_P163506	CYBA	cytochrome b-245, alpha polypeptide	-2,44913037	7,14E-06	-2,4178692	1,31E-05	-2,36437005	3,98E-04
NM_000110	A_23_P135548	DPYD	dihydropyrimidine dehydrogenase	-3,03162486	3,29E-06	-1,69897541	3,16E-04	-1,05290523	9,43E-03
NM_000237	A_23_P146233	LPL	lipoprotein lipase	-1,06797162	2,24E-04	-1,12289186	1,81E-03	-1,28554578	3,96E-03
NM_0010100	A_24_P579356	ARHGAP28	Rho GTPase activating protein 28	-2,43072177	1,33E-05	-1,89550863	1,77E-04	-1,36599581	4,48E-03
NM_0010424	A_24_P270728	NUPR1	nuclear protein, transcriptional regulator, 1	-2,29528964	1,07E-04	-2,25895311	4,80E-05	-2,29344887	2,35E-03
NM_0010808	A_24_P79529	CSAG2	CSAG family, member 2; CSAG family, member 3; similar to CSAG family, member 2	-1,01216228	3,60E-03	-1,5151696	4,19E-04	-1,54606854	4,05E-03
NM_0011950	A_23_P257497	C6orf99	Homo sapiens chromosome 6 open reading frame 99	-1,85372067	3,74E-05	-1,11099989	2,04E-03	-1,09733037	8,08E-03
NM_001206	A_23_P415401	KLF9	Kruppel-like factor 9	-1,4574446	2,88E-05	-1,3958477	5,18E-04	-1,48405439	2,58E-03
NM_001432	A_23_P41344	EREG	epiregulin	-2,55901413	1,70E-05	-1,90571418	1,10E-04	-1,41329737	4,51E-03
NM_001657	A_23_P259071	AREG	amphiregulin; amphiregulin B	-1,62107875	2,37E-05	-1,33552944	1,18E-03	-1,12549374	8,48E-03
NM_001740	A_23_P21092	CALB2	calbindin 2	-2,92119518	4,78E-06	-1,85880197	1,16E-04	-1,72202989	1,66E-03
NM_001902	A_23_P126103	CTH	cystathionase (cystathionine gamma-lyase)	-1,32233196	3,58E-05	-1,4561616	4,15E-04	-1,11619076	7,13E-03
NM_002467	A_23_P215956	MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	-0,63748745	1,47E-02	-0,88129184	7,71E-03	-1,02104782	1,18E-02
NM_002782	A_23_P108170	PSG6	pregnancy specific beta-1-glycoprotein 6	-2,49631972	5,45E-06	-1,44671338	4,25E-04	-1,22506964	6,07E-03
NM_002784	A_23_P39309	PSG9	pregnancy specific beta-1-glycoprotein 9	-2,57434014	7,45E-06	-1,41470487	1,05E-03	-1,25086787	5,20E-03
NM_003196	A_24_P327886	TCEA3	transcription elongation factor A (SII), 3	-1,54030146	1,13E-04	-1,26681864	9,45E-04	-1,38941773	4,47E-03
NM_003280	A_23_P166823	TNNC1	troponin C type 1 (slow)	-3,17514396	1,17E-06	-1,72710138	3,69E-04	-1,22870263	5,73E-03
NM_003597	A_23_P44505	KLF11	Kruppel-like factor 11	-1,22256751	7,22E-04	-1,20407056	1,22E-03	-1,13637723	7,18E-03
NM_003714	A_23_P110686	STC2	stanniocalcin 2	-1,10457472	2,16E-04	-1,43759507	4,65E-04	-1,14723848	7,13E-03
NM_004080	A_23_P61919	DGKB	diacylglycerol kinase, beta 90kDa	-4,03766126	1,87E-06	-1,23710948	1,02E-03	-1,07648412	9,22E-03
NM_004083	A_23_P21134	DDIT3	DNA-damage-inducible transcript 3	-1,3660376	7,93E-04	-1,85167646	2,16E-04	-1,48448283	2,99E-03
NM_004420	A_23_P390528	DUSP8	dual specificity phosphatase 8	-1,255972708	3,35E-03	-1,01322655	3,26E-03	-1,38583012	4,30E-03
NM_004563	A_23_P140207	PCK2	phosphoenolpyruvate carboxykinase 2 (mitochondrial)	-1,18098063	6,73E-04	-1,37479311	5,52E-04	-1,35664248	3,35E-03
NM_004577	A_23_P251984	PSPH	phosphoserine phosphatase-like; phosphoserine phosphatase	-1,2359542	2,30E-04	-1,35077206	6,05E-04	-1,1716885	5,79E-03
NM_004864	A_23_P16523	GDF15	growth differentiation factor 15	-2,42366784	8,20E-05	-1,69858499	4,38E-04	-2,39238588	4,29E-04
NM_005194	A_23_P411296	CEBPB	CCAAT/enhancer binding protein (C/EBP), beta	-0,87343114	2,89E-03	-1,10199141	2,75E-03	-1,12832902	7,56E-03
NM_005213	A_23_P170233	CSTA	cystatin A (stefin A)	-2,28500829	3,30E-05	-2,66372862	8,38E-06	-1,78687604	1,39E-03
NM_005367	A_23_P252928	MAGEA12	melanoma antigen family A, 12	-1,35694933	1,31E-03	-1,64465938	3,13E-04	-2,00564759	5,07E-04
NM_005557	A_23_P38537	KRT16	keratin 16; keratin type 16-like	-2,35504973	1,18E-05	-1,65904918	3,56E-04	-1,63495607	2,17E-03
NM_006905	A_23_P50697	PSG1	pregnancy specific beta-1-glycoprotein 1	-2,84056045	3,20E-06	-1,69333899	1,85E-04	-1,2473813	4,48E-03
NM_007173	A_24_P937405	PRSS23	protease, serine, 23	-1,76082169	1,96E-05	-1,03313193	2,87E-03	-1,17759475	5,71E-03
NM_007350	A_23_P76450	PHLDA1	pleckstrin homology-like domain, family A, member 1	-1,69903751	3,40E-05	-1,4769497	3,89E-04	-1,4811118	2,35E-03
NM_014331	A_32_P165477	SLC7A11	solute carrier family 7, (cationic amino acid transporter, y+ system) member 11	-2,08047468	6,45E-06	-2,14489415	3,86E-05	-1,23562508	4,67E-03
NM_014417	A_23_P382775	BBC3	BCL2 binding component 3	-1,00732159	4,17E-04	-1,33654378	6,77E-04	-1,23073552	4,85E-03
NM_015515	A_23_P78248	KRT23	keratin 23 (histone deacetylase inducible)	-1,83350968	1,85E-05	-1,32441993	6,83E-04	-1,26026537	4,35E-03
NM_019050	A_24_P137522	USP53	ubiquitin specific peptidase 53	-1,35699793	1,87E-04	-1,29991961	8,52E-04	-1,09717863	9,11E-03
NM_019058	A_23_P104318	DDIT4	DNA-damage-inducible transcript 4	-0,92606048	3,03E-04	-1,3922176	5,19E-04	-1,19533261	5,31E-03
NM_021158	A_24_P305541	TRIB3	tribbles homolog 3 (Drosophila)	-1,26830874	3,14E-03	-2,22619136	1,48E-04	-1,99878018	1,79E-03

Supplementary Table S3: 62 common downregulated genes in TMPRSS2:ERG-expressing PC3c cells

Gene Name	Probe	Gene Symbol	Annotation	H		M		L	
				Log ₂ FC	P value	Log ₂ FC	P value	Log ₂ FC	P value
NM_023037	A_23_P105862	FRY	<i>furry homolog (Drosophila)</i>	-1,58604354	1,74E-04	-1,15620552	1,97E-03	-1,14045508	7,02E-03
NM_024111	A_23_P14863	CHAC1	<i>ChaC, cation transport regulator homolog 1 (E. coli)</i>	-1,23800755	9,10E-05	-1,14978915	1,88E-03	-1,14312725	6,96E-03
NM_032621	A_23_P22735	BEX2	<i>brain expressed X-linked 2</i>	-1,43746385	1,97E-05	-1,33027821	6,84E-04	-1,46674838	2,53E-03
NM_032772	A_23_P202484	ZNF503	<i>zinc finger protein 503</i>	-1,46634134	3,01E-04	-1,2218935	1,21E-03	-1,14919162	6,32E-03
NM_058179	A_23_P259692	PSAT1	<i>chromosome 8 open reading frame 62; phosphoserine aminotransferase 1</i>	-1,09216315	3,70E-04	-1,2851445	1,33E-03	-1,06783684	9,49E-03
NM_144505	A_23_P369343	KLK8	<i>kallikrein-related peptidase 8</i>	-2,3099324	7,71E-04	-1,23426937	1,08E-03	-1,54530491	2,88E-03
NM_148898	A_24_P38316	FOXP2	<i>forkhead box P2</i>	-1,16257205	3,45E-04	-1,46460559	4,79E-04	-1,27051843	5,70E-03
NM_153478	A_24_P11061	CSAG1	<i>chondrosarcoma associated gene 1</i>	-2,00142153	1,89E-04	-2,09838797	3,03E-04	-2,23168982	2,25E-04
NM_171998	A_24_P408603	RAB39B	<i>RAB39B, member RAS oncogene family</i>	-1,52450725	2,86E-04	-1,09278857	2,07E-03	-1,45376668	2,98E-03
NM_173698	A_23_P345808	FAM133A	<i>family with sequence similarity 133, member A</i>	-4,08705351	1,87E-06	-5,63321118	8,88E-06	-1,64580833	1,77E-03
NM_182707	A_24_P392110	PSG8	<i>pregnancy specific beta-1-glycoprotein 7; pregnancy specific beta-1-glycoprotein 8; pregnancy specific b</i>	-2,52811923	4,24E-06	-1,47525321	4,15E-04	-1,2884775	3,96E-03
NM_182757	A_24_P406060	RNF144B	<i>ring finger protein 144B</i>	-3,98861218	1,23E-06	-1,88007321	1,21E-04	-1,25415931	7,31E-03
NM_183376	A_23_P339818	ARRDC4	<i>arrestin domain containing 4</i>	-1,6682404	1,67E-03	-1,93675889	7,42E-05	-1,68108966	2,36E-03
NM_198461	A_32_P190303	LONRF2	<i>LON peptidase N-terminal domain and ring finger 2</i>	-1,16022619	1,47E-03	-1,86532483	1,88E-04	-1,77632568	1,17E-03
NM_203403	A_23_P83007	C9orf150	<i>chromosome 9 open reading frame 150</i>	-2,51188422	3,88E-04	-2,60612569	9,67E-06	-1,9019306	1,29E-03
NM_207446	A_23_P100001	LOC400451	<i>family with sequence similarity 174, member B</i>	-4,1202078	7,05E-06	-3,04001545	5,89E-06	-3,07287002	5,25E-05
NR_015370	A_23_P58538	chromosome	<i>chromosome 5 open reading frame 26</i>	-1,05905921	1,95E-04	-1,17500604	1,40E-03	-1,24873723	4,90E-03
NR_033888	A_24_P882959	LOC284344	<i>Homo sapiens uncharacterized LOC284344 (LOC284344), non-coding RNA</i>	-1,81421343	7,95E-05	-1,26512158	1,25E-03	-1,18898504	9,21E-03

Supplementary Table S2: 64 common upregulated genes in TMPRSS2:ERG-expressing PC3c cells

Gene Name	Probe	Gene Symbol	Annotation	H		M		L	
				Log ₂ FC	P value	Log ₂ FC	P value	Log ₂ FC	P value
ENST0000024	A_24_P535256	ENST0000024	ENST00000242208	3,4495517	9,72E-06	2,75864448	1,05E-05	1,82524492	0,00217015
NM_005012	A_32_P4018	ROR1	receptor tyrosine kinase-like orphan receptor 1	1,30283884	0,0010761	1,4226011	0,00084521	1,32838946	0,0034485
NM_032554	A_23_P394246	HCAR1	hydroxycarboxylic acid receptor 1	3,13840381	1,40E-05	2,27924136	7,35E-05	1,74639699	0,00119794
NM_032888	A_23_P158096	COL27A1	collagen, type XXVII, alpha 1	2,81024944	3,29E-06	2,12891847	5,87E-05	1,63171763	0,00252617
NM_000093	A_23_P158593	COL5A1	collagen, type V, alpha 1	1,64029408	0,00014023	2,26156607	2,37E-05	1,44663007	0,00334751
NM_000358	A_23_P156327	TGFBI	transforming growth factor, beta-induced, 68kDa	2,80880325	3,68E-05	3,81845776	3,82E-06	2,3140582	0,00021414
NM_000428	A_23_P218144	LTBP2	latent transforming growth factor beta binding protein 2	2,193703	0,00032987	1,36893566	0,0006058	1,50946873	0,00447424
NM_000575	A_23_P72096	IL1A	interleukin 1, alpha	4,86449141	1,87E-06	2,52702853	1,05E-05	0,49369335	0,2022043
NM_000610	A_23_P24870	CD44	CD44 molecule (Indian blood group)	1,82579518	4,54E-05	1,29534947	0,00079437	1,06257404	0,00973226
NM_000676	A_23_P55477	ADORA2B	hypothetical LOC100131909; adenosine A2b receptor	2,15785283	7,05E-06	1,27540873	0,00117234	1,21517596	0,00497849
NM_000852	A_23_P202658	GSTP1	glutathione S-transferase pi 1	3,20785566	3,34E-05	2,89560413	1,15E-05	5,56786645	6,37E-06
NM_000930	A_23_P82868	PLAT	plasminogen activator, tissue	2,92014795	2,23E-06	2,86176488	5,44E-06	1,26695125	0,00445263
NM_010401	A_23_P8452	LFNG	LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase	2,14012398	1,47E-05	2,36719695	1,70E-05	1,70620911	0,00132041
NM_010805	A_32_P52414	LOC644943	Ras association (RalGDS/AF-6) domain family (N-terminal) member 10	2,01940409	0,00123561	2,90719901	1,06E-05	3,4273124	5,12E-05
NM_001409	A_24_P926960	MEGF6	multiple EGF-like-domains 6	1,1308263	0,00118809	1,38102082	0,00074941	0,74476166	0,04080211
NM_002165	A_23_P252306	ID1	inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	1,85010354	1,62E-05	1,4674131	0,00047408	1,40446158	0,00295129
NM_002167	A_23_P137381	ID3	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	1,13698227	0,00014229	1,17194042	0,00168606	1,13781983	0,00659747
NM_002178	A_23_P139912	IGFBP6	insulin-like growth factor binding protein 6	2,17737016	4,40E-06	2,08349049	5,67E-05	1,42906818	0,00312489
NM_002192	A_23_P122924	INHBA	inhibin, beta A	3,34695573	4,45E-06	2,91142459	5,44E-06	1,55485994	0,0020654
NM_002318	A_23_P111995	LOXL2	lysyl oxidase-like 2	1,57115991	3,56E-05	1,48596967	0,00039221	1,16303028	0,00597385
NM_002341	A_23_P93348	LTB	lymphotoxin beta (TNF superfamily, member 3)	4,38910213	2,83E-06	3,43222314	5,44E-06	1,50379139	0,00306967
NM_002751	A_23_P502274	MAPK11	mitogen-activated protein kinase 11	2,06921509	1,64E-05	1,46188256	0,00041398	1,26296329	0,00593456
NM_003012	A_23_P10127	SFRP1	secreted frizzled-related protein 1	3,38233813	7,65E-06	3,29062341	5,44E-06	5,14603198	6,37E-06
NM_003088	A_23_P168531	FSCN1	fascin homolog 1, actin-bundling protein (Strongylocentrotus purpuratus)	2,50677701	3,46E-05	1,62291075	0,00035597	1,09130434	0,00876387
NM_003246	A_23_P206212	THBS1	thrombospondin 1	3,37607996	1,95E-05	2,91622092	5,44E-06	1,22455685	0,0063206
NM_003264	A_23_P92499	TLR2	toll-like receptor 2	2,904175	3,24E-05	2,08635311	0,00013044	1,21870916	0,00489826
NM_003494	A_23_P39925	DYSF	dysferlin, limb girdle muscular dystrophy 2B (autosomal recessive)	2,1937892	1,40E-05	1,63096776	0,00025711	1,49991476	0,00236278
NM_003588	A_23_P422178	CUL4B	cullin 4B	0,64342805	0,00745917	1,62356929	0,00028589	1,3598221	0,00312222
NM_003733	A_23_P139786	OASL	2'-5'-oligoadenylate synthetase-like	2,57422695	8,44E-05	2,54860934	9,02E-06	1,91915364	0,00095544
NM_004460	A_23_P56746	FAP	fibroblast activation protein, alpha	5,39496843	9,05E-07	4,77699852	2,16E-06	4,02339409	1,85E-05
NM_004753	A_23_P33759	DHRS3	dehydrogenase/reductase (SDR family) member 3	2,20445645	3,34E-05	1,60821553	0,00027451	1,27020485	0,00428201
NM_004994	A_23_P40174	MMP9	matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	2,24730803	2,83E-06	1,77389168	0,00015004	1,04873192	0,00940379
NM_005429	A_23_P167096	VEGFC	vascular endothelial growth factor C	3,1671143	2,71E-06	2,00963852	0,00024478	1,52382508	0,00285125
NM_005725	A_23_P201193	TSPAN2	tetraspanin 2	1,90595797	5,32E-05	2,10056978	5,06E-05	1,31110511	0,00370411
NM_005823	A_23_P77529	MSLN	mesothelin	0,80726104	0,00547454	1,8557777	0,00013593	1,48933316	0,00283113
NM_006417	A_23_P23074	IFI44	interferon-induced protein 44	2,87859612	1,06E-05	2,79779509	5,89E-06	2,03208076	0,00044277
NM_006897	A_23_P25150	HOXC9	homeobox C9	1,94806845	0,00023853	1,80660472	0,0002962	1,63448271	0,0036561
NM_006936	A_23_P102937	SUMO3	SMT3 suppressor of mif two 3 homolog 2 (S. cerevisiae) pseudogene; SMT3 suppressor of mif two	0,8361159	0,00410646	1,01719451	0,00367611	1,117685	0,00712696
NM_007289	A_24_P260101	MME	membrane metallo-endopeptidase	0,8168381	0,00693129	1,24306003	0,00105343	0,7019343	0,05419134
NM_014256	A_23_P78980	B3GNT3	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 3	2,39803785	7,14E-06	1,21272689	0,00125079	1,77586456	0,00121201
NM_014350	A_32_P219520	TNFAIP8	tumor necrosis factor, alpha-induced protein 8	1,06953295	0,00114313	1,09599389	0,00331229	1,05042516	0,00941858
NM_016235	A_23_P324327	GPRC5B	G protein-coupled receptor, family C, group 5, member B	2,32040176	3,83E-05	1,92159657	7,94E-05	1,6169327	0,00543052
NM_017654	A_23_P355244	SAMD9	sterile alpha motif domain containing 9	1,15433736	0,00031326	1,27265962	0,00087128	1,2459451	0,00629024
NM_018602	A_23_P206140	DNAJA4	DnaJ (Hsp40) homolog, subfamily A, member 4	1,90273976	0,00250654	4,03422988	3,13E-06	2,96510379	0,00015874

Supplementary Table S2: 64 common upregulated genes in TMPRSS2:ERG-expressing PC3c cells

Gene Name	Probe	Gene Symbol	Annotation	H		M		L	
				Log ₂ FC	P value	Log ₂ FC	P value	Log ₂ FC	P value
NM_020134	A_23_P210224	DPYSL5	dihydropyrimidinase-like 5	1,84146402	1,64E-05	1,22727201	0,00111669	1,47884867	0,00234838
NM_021071	A_23_P116902	ART4	ADP-ribosyltransferase 4 (Dombrock blood group)	1,849363	0,00787111	1,77604652	0,00031777	1,53876511	0,00306597
NM_021629	A_32_P171313	GNB4	guanine nucleotide binding protein (G protein), beta polypeptide 4	2,18971734	1,85E-05	1,87336873	0,00010921	1,2461904	0,00447424
NM_022370	A_23_P356581	ROBO3	roundabout, axon guidance receptor, homolog 3 (Drosophila)	2,4008704	4,54E-05	1,64022292	0,00047275	1,33342014	0,00343007
NM_025135	A_32_P34444	FHOD3	formin homology 2 domain containing 3	4,10351737	1,07E-05	3,86856762	5,44E-06	2,15478103	0,00166461
NM_025179	A_23_P46618	PLXNA2	plexin A2	3,19528483	5,96E-06	1,96905162	6,77E-05	1,61377018	0,00287726
NM_030775	A_23_P53588	WNT5B	wingless-type MMTV integration site family, member 5B	2,03949498	0,00055932	1,74557366	0,00038816	1,61532611	0,0016596
NM_030955	A_32_P103220	ADAMTS12	ADAM metalloproteinase with thrombospondin type 1 motif, 12	2,44605665	2,05E-05	2,74427443	5,93E-06	2,21406233	0,00022686
NM_032413	A_23_P26024	C15orf48	chromosome 15 open reading frame 48	5,8889039	9,05E-07	4,59532803	2,16E-06	2,95210513	5,25E-05
NM_032587	A_23_P41854	CARD6	caspase recruitment domain family, member 6	1,63727421	7,72E-05	1,59568454	0,00038816	1,26003906	0,0063206
NM_138711	A_23_P252062	PPARG	peroxisome proliferator-activated receptor gamma	2,178288	1,14E-05	1,60375259	0,00038238	1,32264733	0,00376812
NM_138768	A_23_P360240	MYEOV	myeloma overexpressed (in a subset of t(11;14) positive multiple myelomas)	1,6338799	0,00027	1,78456231	0,00013592	1,5658368	0,00267157
NM_152703	A_23_P145874	SAMD9L	sterile alpha motif domain containing 9-like	2,40778834	8,87E-06	1,5074545	0,00037234	1,34942225	0,00699606
NM_172229	A_23_P77612	KREMEN2	kringle containing transmembrane protein 2	1,68625263	1,81E-05	1,21097049	0,00114599	1,27440774	0,00543052
NM_176072	A_23_P24903	P2RY2	purinergic receptor P2Y, G-protein coupled, 2	1,47009792	4,60E-05	1,1897628	0,00125637	1,06418491	0,00917063
NM_182755	A_23_P161156	ZNF438	zinc finger protein 438	2,47978681	5,85E-05	2,72025567	0,00013848	2,83450713	6,26E-05
NM_198129	A_24_P687302	LAMA3	laminin, alpha 3	2,51712884	2,07E-05	1,58203896	0,00099137	1,22698646	0,01184986
NM_206967	A_24_P289178	C16orf74	chromosome 16 open reading frame 74	2,50235533	2,32E-06	1,51491943	0,000344	1,27585318	0,00478496
NR_015379	A_32_P200238	UCA1	urothelial cancer associated 1	1,2258268	8,12E-05	1,45323115	0,00041949	1,28564612	0,00412749
XR_110477	A_32_P158019	LOC10050594	PREDICTED: Homo sapiens uncharacterized LOC100505940	1,3192231	0,00046213	1,83220714	0,0002962	1,29626969	0,00643079

Supplementary Table S1: Oligonucleotide Primers

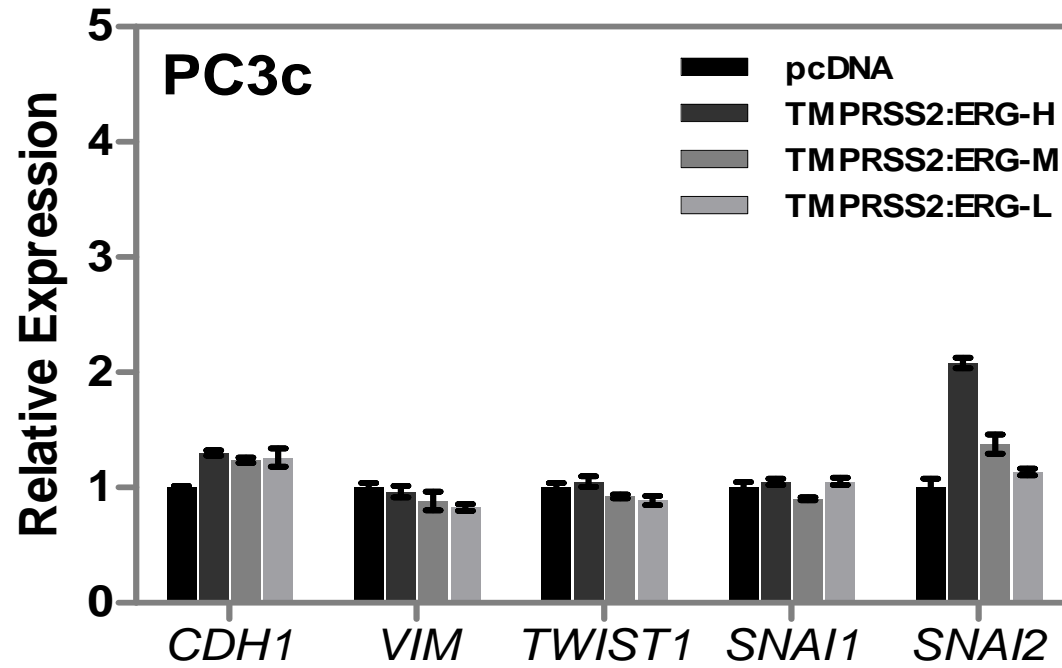
Primer	Direction	Sequence	Reference
<i>TMPRSS2 :ERG</i> (Cloning)	Forward	5'- CGCGAGCTAAGCAGGAGGC -3'	This study
	Reverse	5'- CCTCCGCCAGGTCTTTAGTA -3'	
<i>TMPRSS2:ERG</i> (Classical PCR)	Forward	5'- CGCGAGCTAAGCAGGAGGC -3'	(1)
	Reverse	5'- GTAGGCACACTCAAACAACGACTGG- 3'	
<i>ERG</i> (qPCR)	Forward	5'- AACGAGCGCAGAGTTATCGT -3'	This study (Effeciency: 99.8%)
	Reverse	5'- GTGAGCCTCTGGAAGTCGTC -3'	
<i>MMP9</i> (qPCR)	Forward	5'- AAGTGGCACCACCACAACATC -3'	(2) (Effeciency: 98.3%)
	Reverse	5'- CAAAGGCGTCGTCAATCACC -3'	
<i>PLXNA2</i> (qPCR)	Forward	5'-GGAGCCACTCTTCATGCTATACTGT-3'	(3) (Effeciency: 99.1%)
	Reverse	5'- GTTGACGCAGTTCAGGATCA-3'	
<i>18s</i> (qPCR)	Forward	5'- CAGCTTCCGGGAAACCAAAGTC-3'	(4)
	Reverse	5'- AATTAAGCCGCAGGCTCCACTC-3'	
<i>pMMP9</i> (ChIP)	Forward	5'- TCGGGACTIONACAACCTACAGTG-3'	(5)
	Reverse	5'- TCTTTGACTCAGCTTCTCTCC-3'	
<i>pPLXNA2</i> (ChIP)	Forward	5'- CCGCTTCCGGAAAGCCGTGT-3'	This study
	Reverse	5'- GTCCCGACTTGCATGGGGGC-3'	
<i>pGAPDH</i> (ChIP)	Forward	5'- GCGTGCCAGTTGAACCA-3'	(6)
	Reverse	5'- CGCCCGTAAAACCGTAGT-3'	
<i>CHD1</i> (qPCR)	Forward	5'- CCCACCACGTACAAGGGTC-3'	* (Effeciency: 99.3%)
	Reverse	5'- CTGGGGTATTGGGGGCAT-3'	
<i>VIM</i> (qPCR)	Forward	5'- GAGAACTTTGCCGTTGAAGC-3'	* (Effeciency: 97.6%)
	Reverse	5'- GCTTCCTGTAGGTGGCAATC-3'	
<i>TWIST1</i> (qPCR)	Forward	5'-GCAGGACGTGTCCAGCTC-3'	* (Effeciency: 99.1%)
	Reverse	5'- CTGGCTCTTCTCGCTGTT-3'	

Primer	Direction	Sequence	Reference
<i>SNAI1/SNAI1</i> (qPCR)	Forward	5'- AATCGGAAGCCTAACTACAGCG-3'	* (Effeciency: 96.3%)
	Reverse	5'- GTCCCAGATGAGCATTGGCAA-3'	
<i>SNAI2/SLUG</i> (qPCR)	Forward	5'- ATACCACAACCAGAGATCCTCA-3'	* (Effeciency: 99.8%)
	Reverse	5'- GACTCACTCGCCCCAAAGATG-3'	

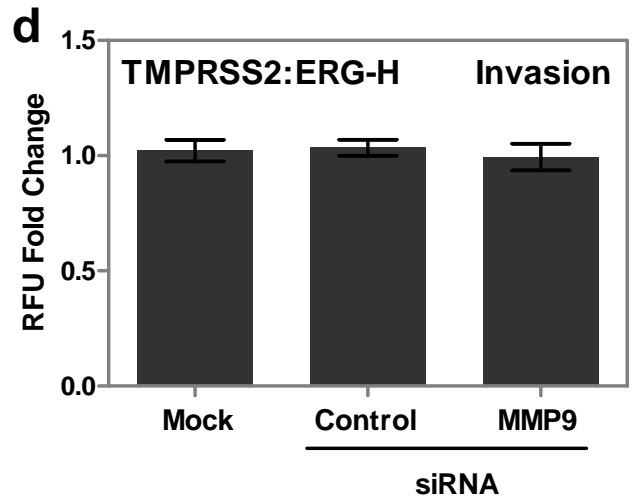
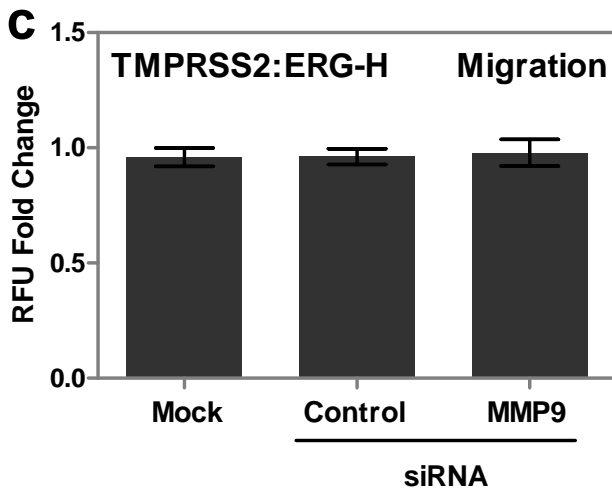
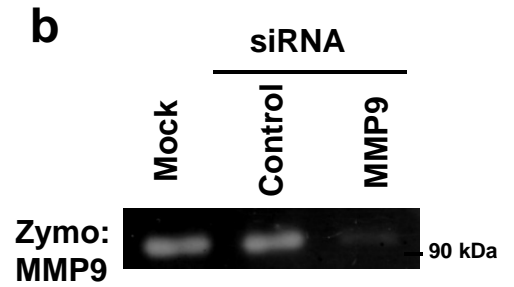
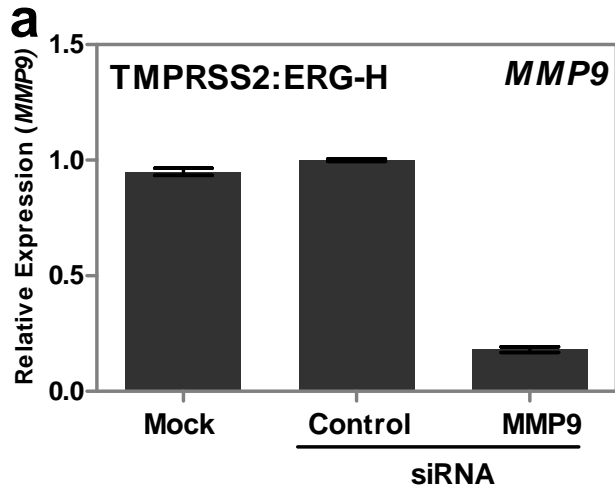
*** These primers have been cited by multiple publications.**

1. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science*. 2005 Oct 28;310(5748):644-8.
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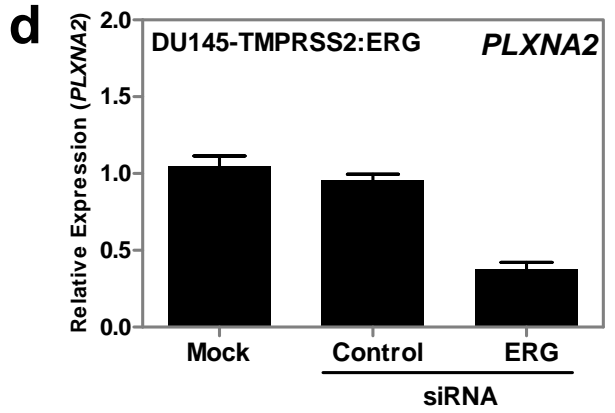
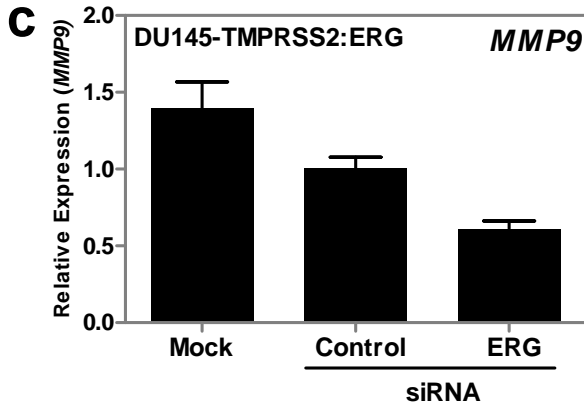
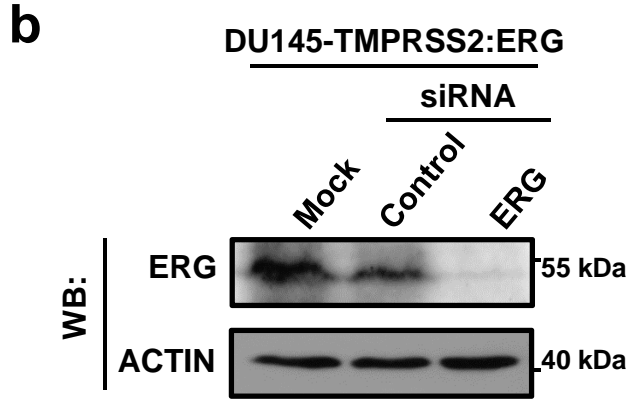
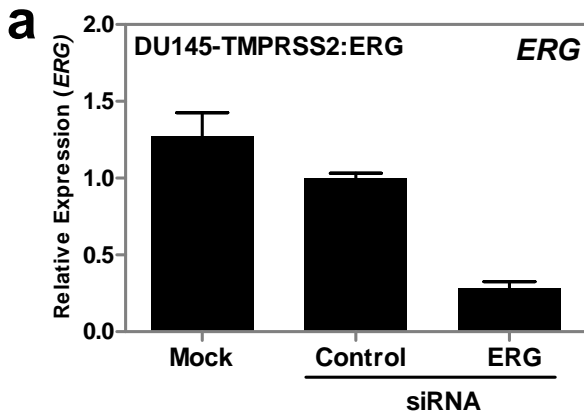
Supplementary Figure S9 Tian et al.



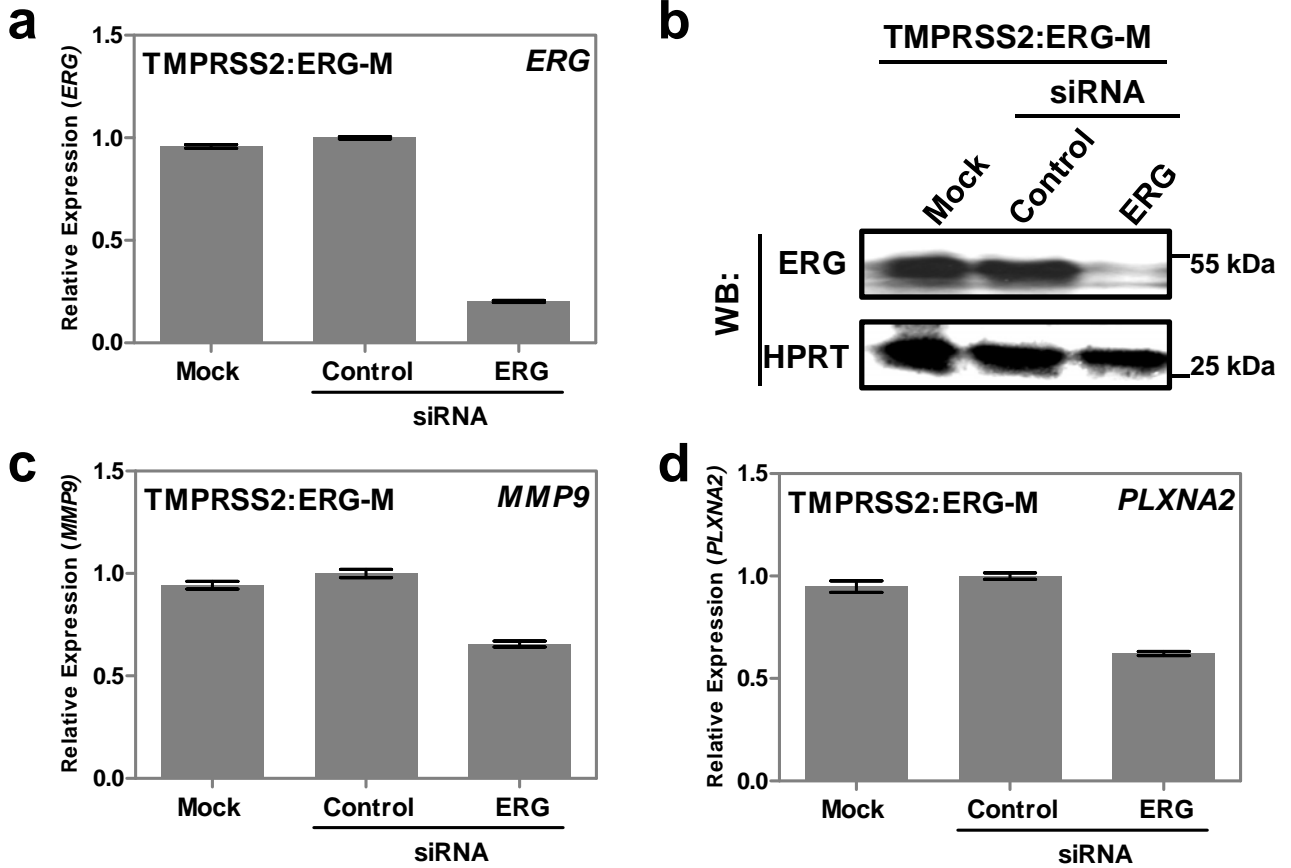
Supplementary Figure S8 Tian et al.



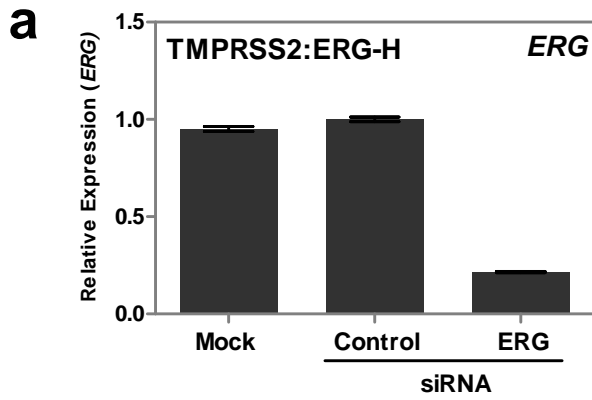
Supplementary Figure S7 Tian et al.



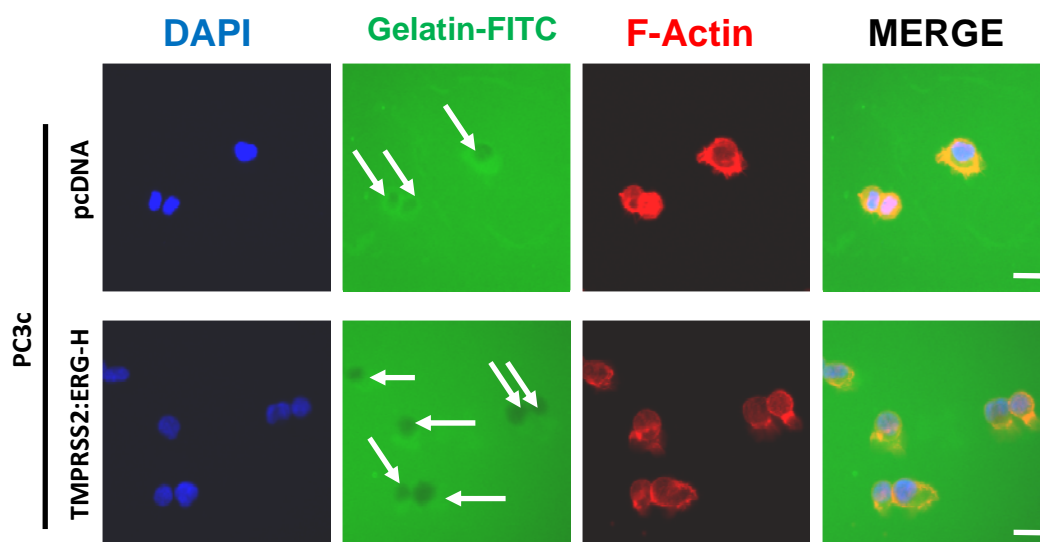
Supplementary Figure S6 Tian et al.



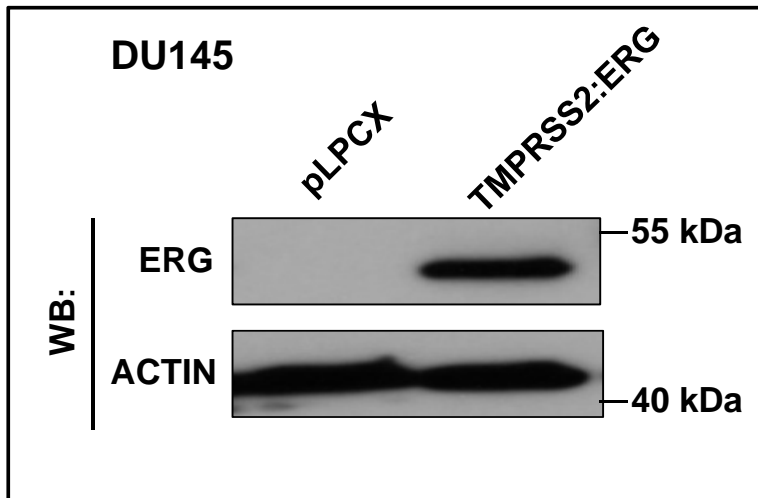
Supplementary Figure S5 Tian et al.



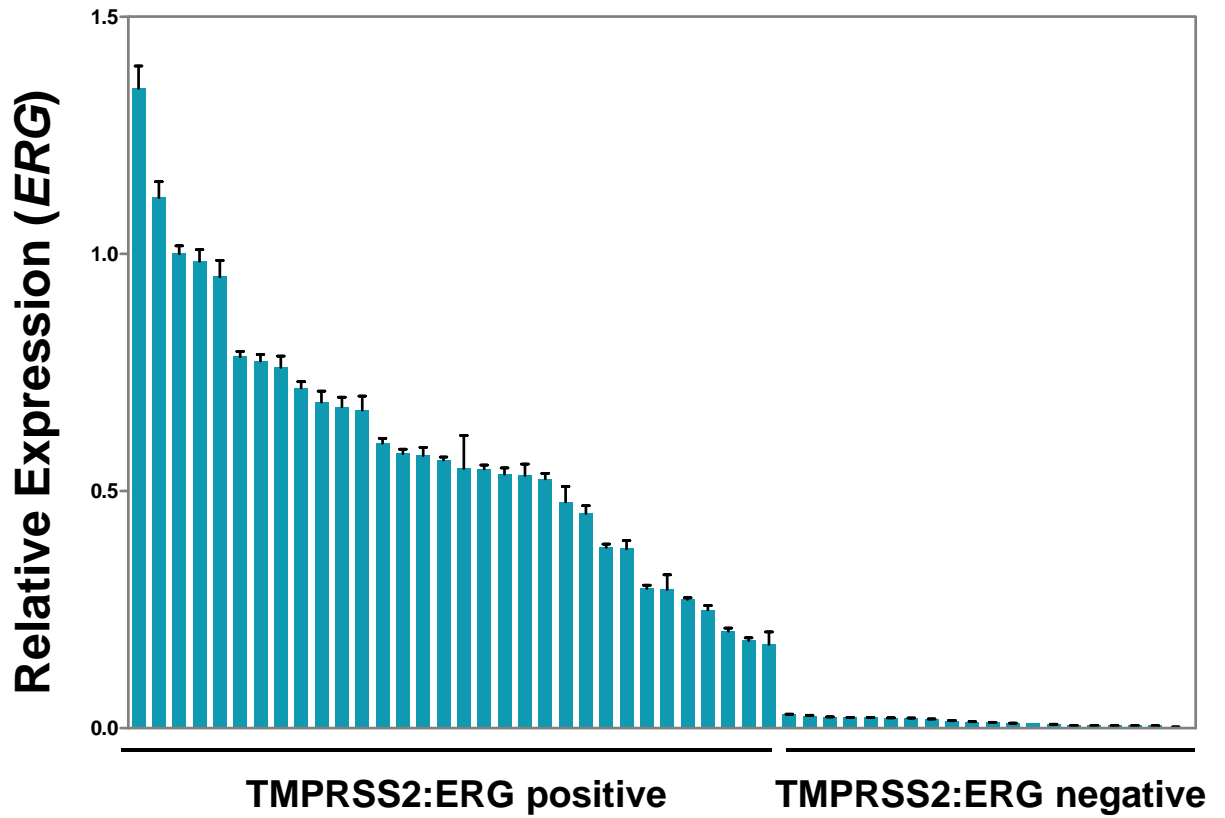
Supplementary Figure S4 Tian et al.



Supplementary Figure S3 Tian et al.



Supplementary Figure S2 Tian et al.



Supplementary Figure S1 Tian et al.

