# Aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate

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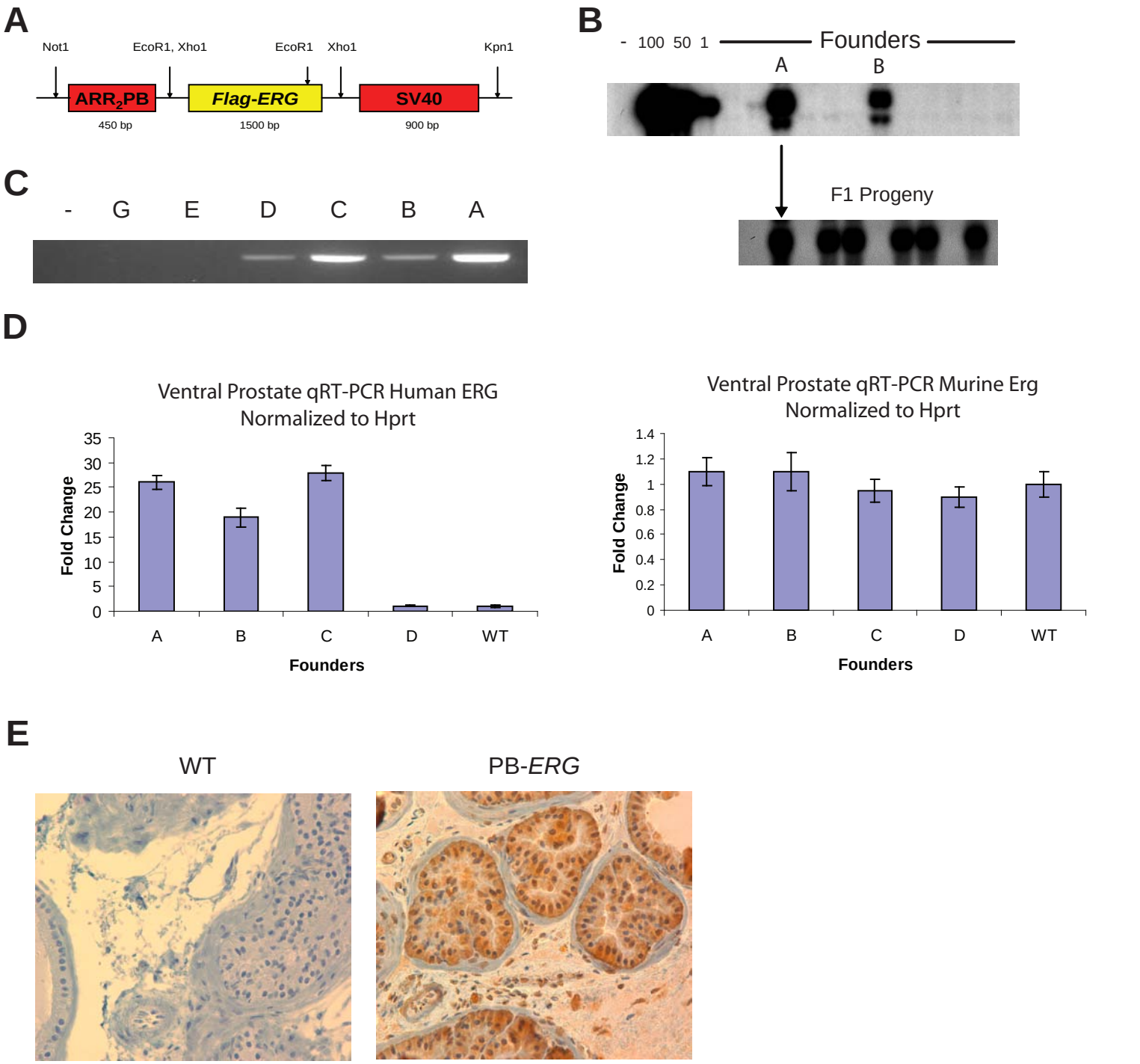
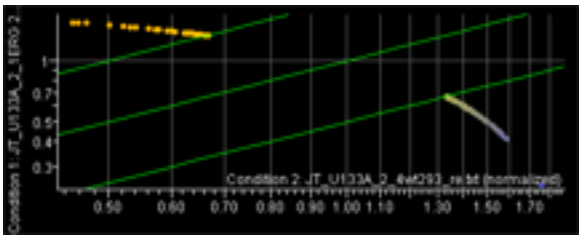



**Supplementary Figure 1. Generation of probasin-ERG mouse model**

Our *Flag-ERG* construct was subcloned into the probasin-SV40 construct utilizing a Xho1 multicloning site and linearized with Not1 and Kpn1 (A). Founders were identified using Southern blot analysis with EcoR1 restriction enzyme digest, demonstrating a positive band corresponding to the *ERG* transgene at 1500 bp, and F1 offspring were analyzed for equivalent germ line transmission of the transgene (B). mRNA expression analysis was performed in triplicate using quantitative RT-PCR for both the human and murine ERG transcripts (D). The bar graph demonstrates the fold change in mRNA level following normalization to Hprt and our lowest value, and the mean and standard deviations from 3 experiments are shown. Immunohistochemistry was performed on representative sections of paraffin embedded prostate glands with the Flag polyclonal antibody, demonstrating protein expression of the *ERG* transgene (E).

A



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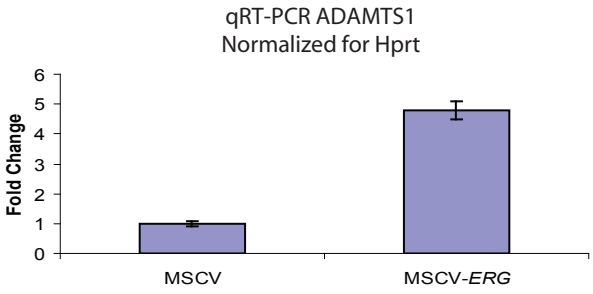


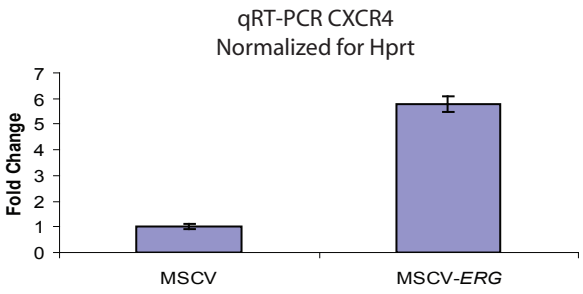


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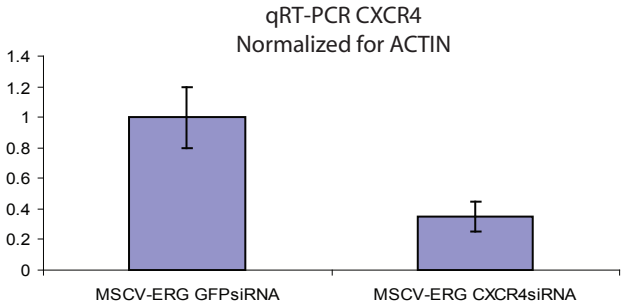
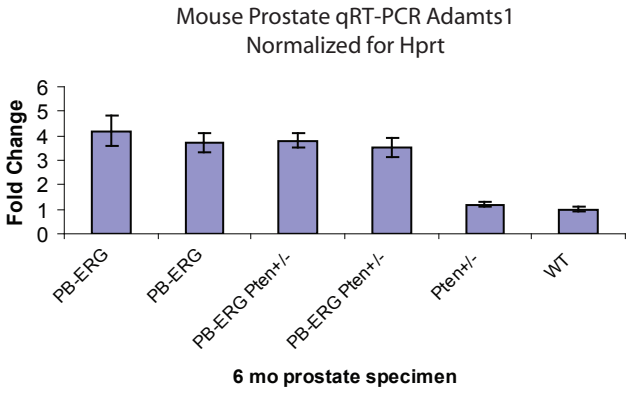



D




**Supplementary Figure 2. CXCR4 and ADAMTS1 regulation by ERG**

Microarray expression analysis of ERG over-expression in 293HEK cells compared to vector control demonstrated differential expression of 167 genes (A). Candidate genes, *CXCR4* and *ADAMTS1*, were further evaluated and found to be up-regulated in primary MEF over-expressing ERG compared to controls, validating our findings across species (B). The bar graphs demonstrate the fold change in mRNA level following normalization to Hprt and our lowest sample value, and the mean and standard deviations from 3 experiments are shown. Knock-down of CXCR4 with pooled siRNA against CXCR4 and control (C). Further evaluation revealed that the mRNA expression of murine *Adamts1* was up-regulated in the prostate specimens of *ERG* transgenic mice compared to controls (D). The bar graphs demonstrate the fold change in mRNA level following normalization to Hprt and our lowest sample value, and the mean and standard deviations from 3 experiments are shown.

Supplementary Table 1. Primer sequences used for cloning, genotyping, RNA, and DNA analyses.

| Primer Name | Primer Sequence |
|---|---|
| **Cloning** | |
| *ERG* forward | CTT GAT CGC ATT ATG GCC |
| *ERG* reverse | CCG CCA GGT CTT TAG TAG |
| **Genotyping** | |
| ARR2PB promoter forward | AGC AGG AAG CTA CTC TGC ACC |
| *ERG* 5' reverse | TTT GGC CAC ACT GCA TTC ATC |
| **qRT-PCR** | |
| human *ACTIN* forward | CAC GAG ACC ACC TTC AAC TC |
| human *ACTIN* reverse | CTT GAT CTT CAT GGT GCT GG |
| human *ERG* forward | TTA TCA GTT GTG AGT GAG GAC CA |
| human *ERG* reverse | AAG TCT GTC CAT AGT CGC TGG |
| human *CXCR4* forward | TAC ACC GAG GAA ATG GGC TCA |
| human *CXCR4* reverse | AGA TGA TGG AGT AGA TGG TGG G |
| human *ADAMTS1* forward | ACG AGG ACG AAG GGA CTG AG |
| human *ADAMTS1* forward | ATC GCT TCT TTC TTA TGC TTC CA |
| murine *Erg* forward | ACC TCA CCC CTC AGT CCA AA |
| murine *Erg* reverse | TGG TCG GTC CCA GGA TCT G |
| murine *Hprt* forward | CAC AGG ACT AGA ACA CCT GC |
| murine *Hprt* reverse | GCT GGT GAA AAG GAC CTC T |
| murine *Cxcr4* forward | GCT TCC GGG ATG AAA ACG TC |
| murine *Cxcr4* reverse | ACC AAT CCA TTG CCG ACT ATG |
| murine *Adamts1* forward | GAC CCG AGA GCC AGA ACA C |
| murine *Adamts1* reverse | CAC AAA TCG CTT CTT CCT TAT GC |
| **ChIP** | |
| *ADAMTS1* promoter forward | CTT TCC TCA TAT GTT CTC |
| *ADAMTS1* promoter reverse | AAC ATT GAC ATC CGC ACA CC |
| *CXCR4* promoter forward | GGC TGC GTC TGC TGA AAG C |
| *CXCR4* promoter reverse | GCT AGG AAC GCG TCT CTC |