Supporting Information: Materials and Methods

Samples

DNA samples used were obtained from the National Cancer Institute; from The Johns Hopkins Brady Urological Institute Prostate Specimen Repository; and from the University of Michigan. DNAs were extracted by standard methods from fresh or frozen tissues. Formalin fixed and paraffin embedded (FFPE) tissue samples and tissue microarray slides for IHC were provided by The Johns Hopkins Brady Urological Institute Prostate Specimen Repository. All specimens were obtained following approval from the respective Institutional Review Boards.

Real-Time PCR

22Rv1, 293T and DH5α (*E. coli*) cell genomic DNAs (gDNAs) were extracted using the QIAamp DNA Mini kit (Qiagen). HeLa gDNA was purchased from New England Biolabs. AmpliTaq Gold polymerase and associated reagents (Life Technologies), and oligonucleotides from Integrated DNA Technologies, were used in the real-time PCR. The XMRV primer and probe sequences were designed to the VP62 XMRV (1) *pol* region: CGAGAGGCAGCCATGAAGG (nt 4552-4570)(XMRV_F); GATCTGTTTCGGTGTAATGGAAATG (nt 4627-4651)(XMRV_R) and 6-carboxyfluorescein ("FAM")-AGTTCTAGAAACCTCTACACTC-Black Hole Quencher (BHQ) 1 (nt 4572-4593)(XMRV_PR). CCR5 primer and probe sequences were: CCAGAAGAGCTGAGACATCCG (CCR5_F); GCCAAGCAGCTGAGAGGTTACT (CCR_R) and hexachlorofluorescein ("HEX")-TCCCCTACAAGAAACTCTCCCCGG-BHQ1 (CCR5_PR). The PCR volume was 50μL. A master mix of PCR reagents was

prepared such that final concentrations in the PCR reactions were 1xPCR buffer, 300nM dNTPs, 2.5mM $MgCl_2$, 100nM CCR5_F, 100nM CCR5_R, 100nM CCR5_PR, 500nM XMRV_F, 500nM XMRV_R, 100nM XMRV_PR, and 0.06 Units/µL polymerase. 5 or 10µL volumes of DNA were used as a template. To avoid cross-contamination, we handled 22Rv1 g DNA in one workspace and the negative controls and samples in another. For the prostate tumor DNA samples, between 25 and 1000ng of gDNA was used per reaction. 54 samples were tested at 25 - 35ng, 54 at 40 - 60ng, and 53 at 100ng or greater. PCR reactions were heated to 95°C for 10 minutes and then cycled 40 times through 95°C for 25 seconds, 55°C for 30 seconds and 60°C for 30 seconds. Real-time PCR, monitoring both FAM and HEX emission, was carried out using a DNA engine Opticon 2 instrument (MJ Research, now BioRad). Fluorescence profiles are presented on a log scale and are global minimum background subtracted as described by the Opticon Monitor 2 software. All DNA samples were tested at least twice.

Antisera

The antisera used in this study were rabbit polyclonal sera obtained following immunization with Moloney murine leukemia virus (Mo-MLV) $p30^{CA}$ (a cleavage product of the viral Gag polyprotein) or $gp70^{SU}$ (a cleavage product of the Env polyprotein); these proteins had been purified from Mo-MLV particles by reverse-phase high performance liquid chromatography. We refer to the sera as MLV30 and MLV70, respectively.

Immunoblotting

Antisera were tested for reactivity against viral proteins by immunoblotting, using virus produced by transient transfection of 293T cells with VP62 plasmid DNA. Results are shown in Fig. S1.

Immunohistochemistry

Cells were fixed in 10% neutral buffered formalin and processed into paraffin blocks. As an additional positive control, we similarly processed 22Rv1 cells (obtained from The American Type Culture Collection) into paraffin blocks. We also included two other prostate cancer cell lines (DU145 and PC3) that were found to be negative by PCR for XMRV. Slides containing sections of FFPE 293T cells, prostate cancer cell lines, tissue microarrays (TMAs), or prostate tissue were deparaffinized and steamed for 25 minutes in citrate-based unmasking solution for antigen retrieval (Vector Laboratories). Following peroxidase block, slides were incubated for 1 hour at room temperature with either MLV30 or MLV70 antisera. The PowerVision™ IHC detection system (ImmunoVision Technologies) was used as a secondary antibody. Staining was visualized using 3,3'-Diamino-benzidine (DAB) (Sigma) and slides were counterstained with hematoxylin.

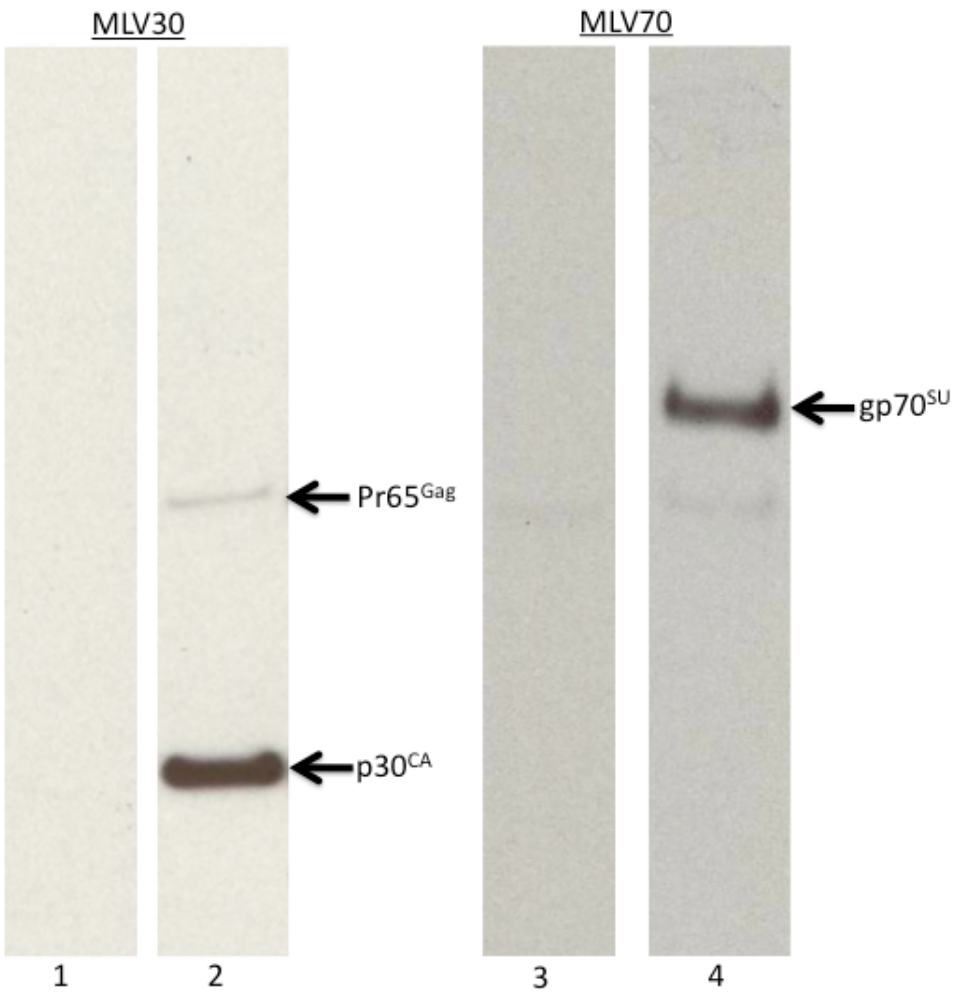

Figure S1. Western blot analysis of MLV30 and MLV70 antisera. Virus particles were pelleted from culture fluids of 293T cells transfected with pcDNA 3.1 (1 and 3) or VP62 (2 and 4). The XMRV $p30^{CA}$ is formed in the mature virus from the action of the viral protease on full length $Pr65^{Gag}$.