Supplemental information for Mushinski *et al.*

Supplemental Methods

Southern blot analysis– Genomic DNA was isolated from PC-3 and PC-3M cells, digested overnight with EcoRI, BamHI or PstI and electrophoresed on a Tris acetate EDTA 1% agarose gel (15). A Southern blot of the gel was hybridized with a ^{32}P-labeled, nick-translated full-length MxA cDNA probe.

Phase contrast time-lapse microscopy– PC-3M-β-gal and PC-3M-MxA (clone #1) cells were seeded in complete medium in 25 cm^2 tissue culture flasks. After 24 h, the flasks were filled to the top with prewarmed RPMI 1640 plus 10%% fetal bovine serum. During the observation period, cells were maintained at 37°C using an ASI 400 Air Stream Incubator (Nevtek, Williamsville, VA). Cell motility was observed by phase-contrast microscopy using an Optronics cooled CCD camera mounted on a Leica DM IRB inverted microscope. Time-lapse video microscopy (250 minute recordings converted into 1 min of playing time) was converted into QuickTime movies using Adobe Premier 51 software.

Fluorescence time-lapse microscopy– PC-3M cells were transiently transfected with GFP-MxA using Lipofectamine Transfection Reagent, Invitrogen, Carlsbad, CA with and seeded in complete medium in 25 cm^2 tissue culture flasks. After 24 h incubation, the flasks were filled to the top with prewarmed RPMI 1640 plus 10% fetal bovine serum. During the observation period, cells were maintained at 37°C using an ASI 400 Air Stream Incubator (Nevtek, Williamsville, VA). The cells were observed using fluorescence microscopy on a Leica DM IRB inverted microscope. Time-lapse video microscopy (120 minute recordings converted into 1 min of playing time) was converted into QuickTime movies using Openlab software.

Supplemental Figure Legends

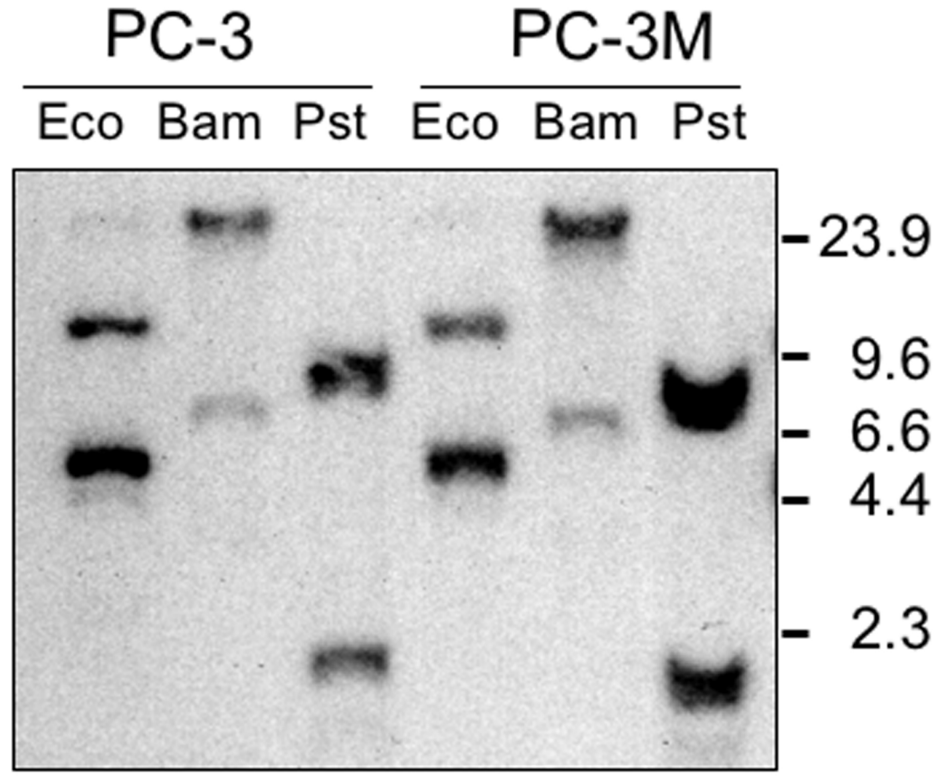
Supplemental Figure S1: Southern blot. Ten µg of genomic DNA from PC-3 and PC-3M were digested with EcoRI (Eco), BamHI (Bam), or PstI (Pst), electrophoresed on a 1% agarose gel, blotted onto a nylon membrane and probed with insert from MxA cDNA.

Supplemental Figure S2: Western blot of the level of MxA in PC-3M-MxA clone 1, PC-3M clone 2, and untransfected PC-3. Cell lysates were resolved on SDS-polyacrylamide gels, transferred to nitrocellulose filters and immunoblotted with anti-MxA antibody.

Supplemental Figure S3: **Time-lapse microscopy of motility of β-galactosidase- and MxA-expressing PC-3M cells observed by phase contrast microscopy.** *A*. PC-3M cells expressing exogenous β-galactosidase (control) and *B*. PC-3M cells expressing exogenous wild-type MxA (clone 1). MxA-expressing cells show a dramatic diminution in movement across the field. Compression: 250 min to one min video.

Supplemental Figure S4: **Time-lapse microscopy of GFP-MxA transfected PC-3M cells.** PC-3M cells were transiently transfected with GFP-MxA using Lipofectamine Transfection Reagent, Invitrogen, Carlsbad, CA). After incubation in complete medium for 24h in a 75 cm2 surface area flask the flask was filled with complete medium, sealed, and placed on a Leica inverted IM DRB (Leica) fluorescence microscope with a heated stage. A time-lapse recording of two cells representative of distinct cytoplasmic GFP-MxA distributions was prepared using OpenLab (Improvision/PerkinElmer, Waltham, MA) software and a compression of 120 min to one min.

Supplemental Figure S1



151 1

129 1

84 1

011 1

281 1

Bgal 2

Mxa 4

Mxa 4-2

PC-3

↑ Mxa