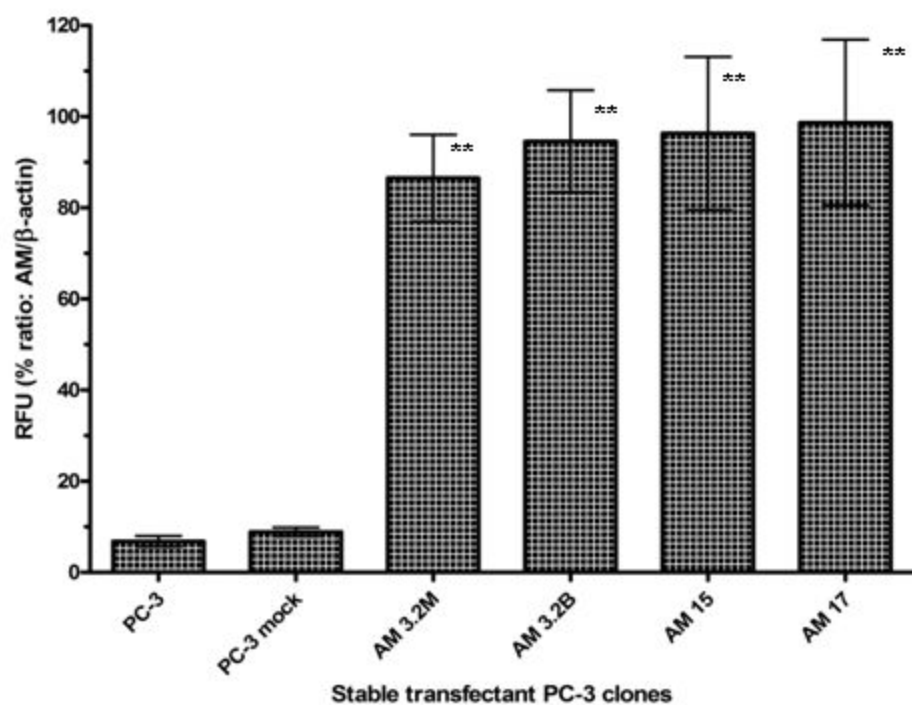
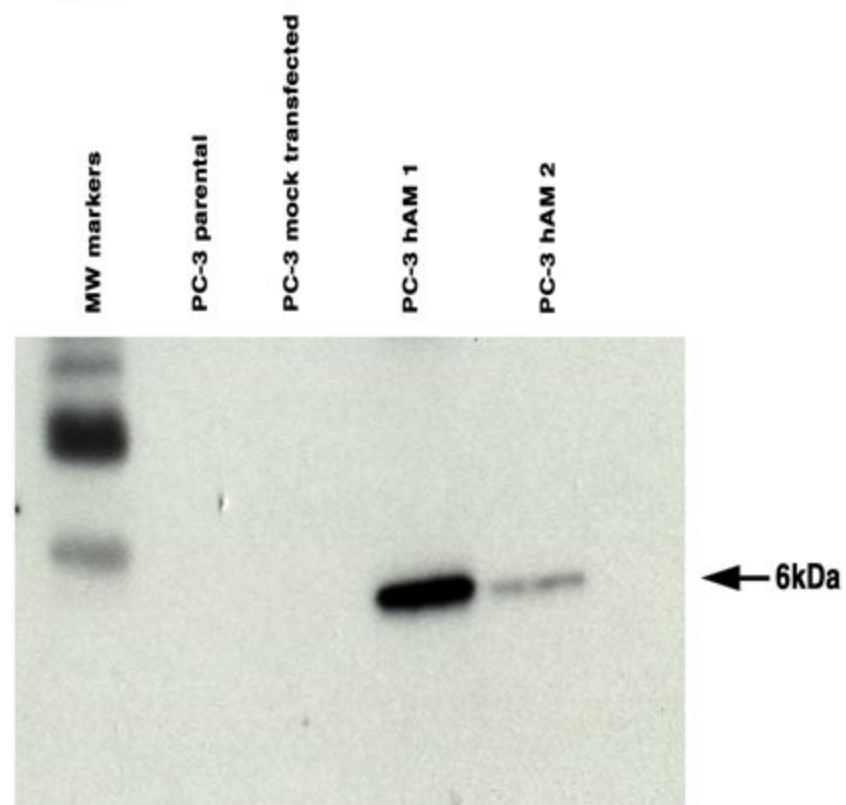
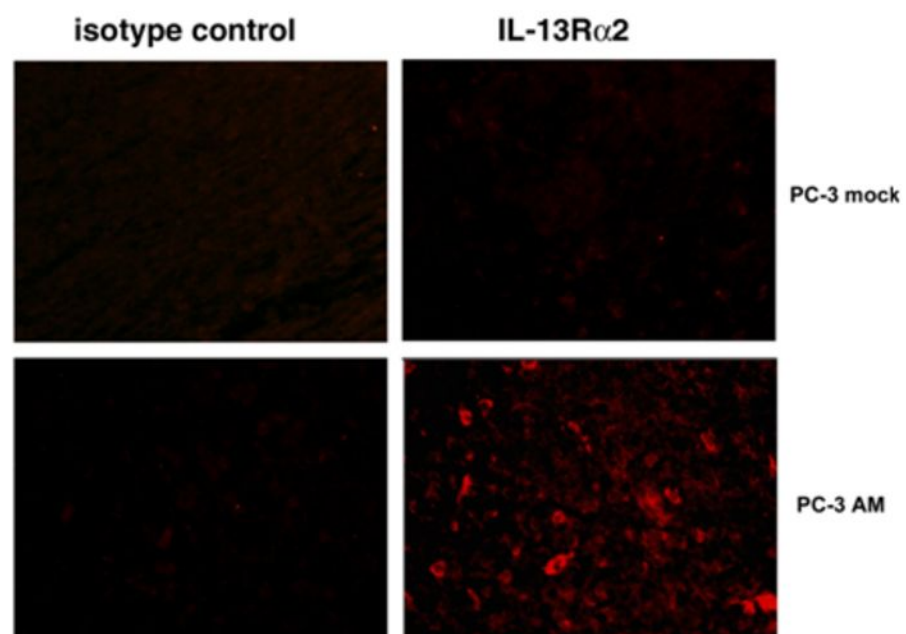
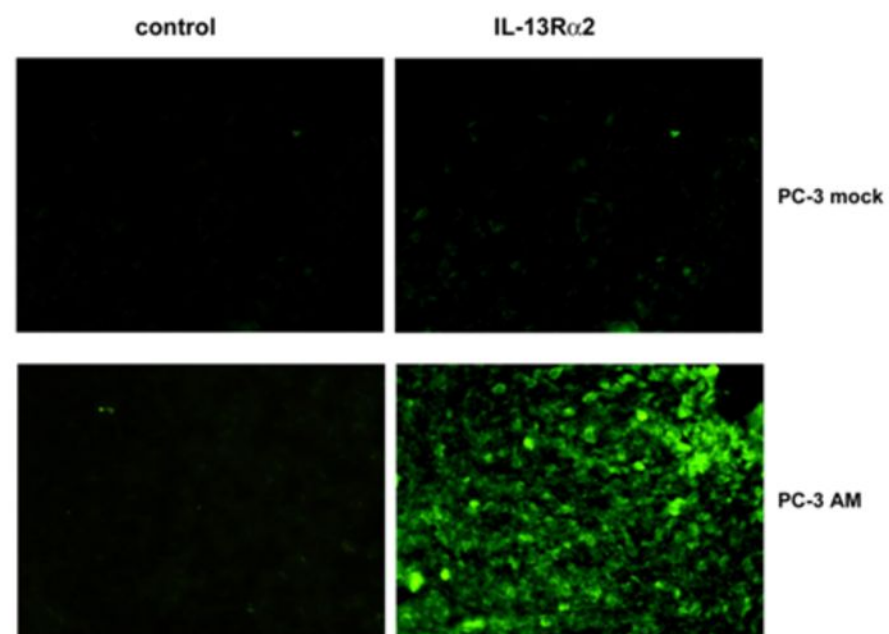


**A****mRNA****B****Protein****Supplementary Fig. 1**

**A****B****Supplementary Fig. 2**

## Supplementary Methods and Materials:

### Recombinant AM expression:

Briefly, full-length AM gene was cloned into a mammalian expression vector pcDNA3.1 with hygromycin as a selection antibiotic. After transfection of PC-3 with plasmid DNA and lipofectamine, the clones were selected (23). The transgene expression in transfected cells was evaluated by performing Western blot and Real-time PCR analyses. A clone strongly expressing AM was maintained in RPMI 1640 complete medium with 150µg/ml of hygromycin for the present set of experiments.  $0.5 \times 10^6$  cells were plated in a T-150 cm<sup>2</sup> flask and cultured with RPMI 1640 complete medium containing hygromycin. When the cells were 80-90% confluent, the medium was replaced with indicator free RPMI 1640 medium without serum and incubated further for an additional 24 hours to facilitate the secretion of intracellular AM into the medium. The cell-free supernatants from 10 T-150cm<sup>2</sup> flasks were pooled, centrifuged to remove any cell debris (2000 rpm, 10 minutes), purified by ion-exchanged chromatography column on FPLC. The purity of AM was examined by 16% SDS-PAGE that showed a single band of approximately 6 KD (data not shown) and also examined for its immunoreactivity using rabbit polyclonal antibody (Bachem Lab, San Carlos, CA, USA) by Western blot analysis. This rAM supernatant was used for treating the tumor cell lines (also referred as rAM treatment). A supernatant obtained from a mock-transfected cell line was used as a corresponding negative control (control medium). Both supernatants were aliquoted and stored frozen at -20°C.

### Supplementary Table 1

Molecular turn over of IL-13R $\alpha$ 2 mRNA transcripts and protein in adrenomedullin treated prostate tumor cell line<sup>a</sup>

Cell line	t <sub>1/2</sub> (hrs)			
	mRNA		Protein	
	control	AM	control	AM
PC-3	4.0 ± 1.5	11.0 ± 2.0**	6.0 ± 2.0	14.0 ± 2.0**

<sup>a</sup>mRNA and protein half-lives were determined by treating cells with act D or CHX, respectively and mRNA and protein levels were measured by RT-PCR or Western blot analyses.

\*\*p<0.01 compared to untreated or mock control

Figure Legends:

**Figure 1: Expression of AM in transfected PC-3 cells.** Real-time PCR analysis was performed for AM gene expression and relative expression of AM mRNA was calculated as RFU (relative fluorescence units) from three independent experiments performed in duplicate. The data are statistically significant (\*\*,  $p < 0.01$ ) (A). Supernatants obtained from parental, mock transfected and PC-3 cells transfected with AM gene were separated and immunoblotted with rabbit anti-AM antibody and chemiluminescence was developed by ECL reaction as described in materials and methods. Lane 1, molecular weight markers; lane 2, supernatant obtained from PC-3 parental cells; lane 3, supernatant obtained from PC-3 mock transfected cells; and 4 and 5, supernatant from a high and low expressing clones from stable AM transfected PC-3 cells (B).

**Fig. 2: Expression of IL-13R $\alpha$ 2 gene and protein *in vivo* in AM transfected tumors.**

IHC and ISH assays were performed on paraffin embedded tissue sections from PC-3 mock or PC-3 AM xenograft tumors. IHC with isotype control or IL-13R $\alpha$ 2 antibody stained sections were labeled with TRICT (red) for IL-13R $\alpha$ 2 (A); ISH assay for IL-13R $\alpha$ 2 RNA using anti-sense riboprobe were labeled with FITC (green) (B). Sense riboprobe was used a negative control (upper panel) (B). All sections were viewed at 200X magnification.