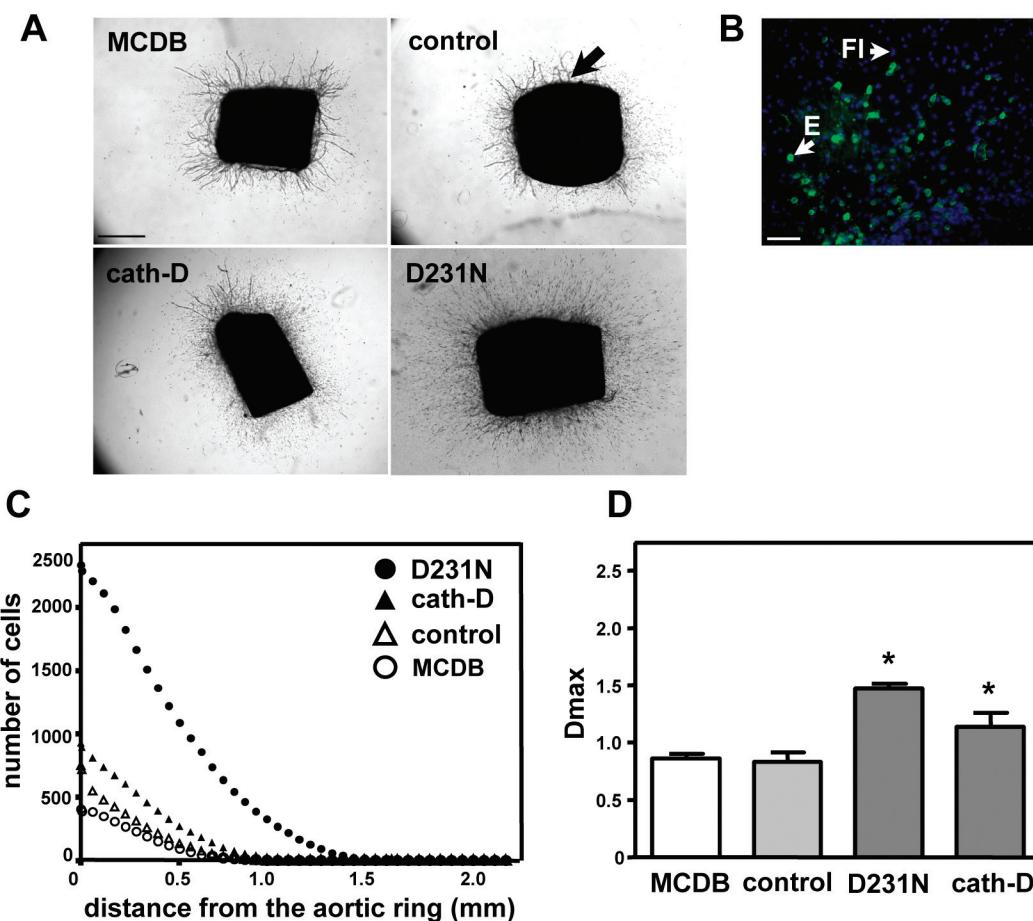


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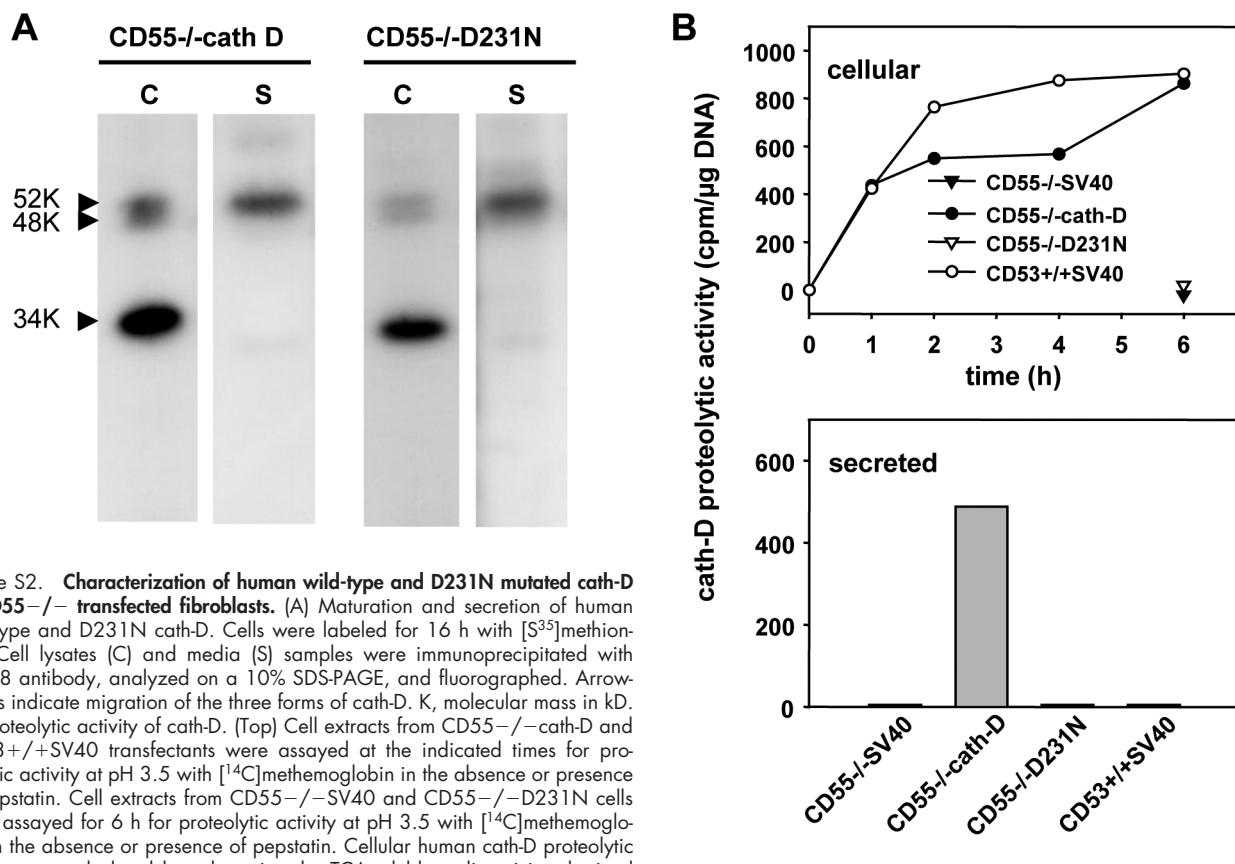
## Supplemental materials and methods

### Aortic ring assays

Rat aorta rings prepared as described previously (Blacher et al., 2001) were maintained for 9 d at 37°C in MCDB131 (Life Technologies) and conditioned media from mock-transfected, cath-D, and D231N transfected 3Y1-Ad12 cancer cells were prepared as described above (1:1). The cultures were examined by phase contrast microscopy with a microscope (Olympus) at the appropriate magnification. Image analysis was performed on a Sun SPARC30 WorkStation, using the Visilog5.0 Noesis software. Images were digitized in 760 × 540 pixels with 256 gray levels. Gray level image transformation and binary image processing have been carrying out using traditional tools and mathematical morphology (Blacher et al., 2001). To identify endothelial cells, gels containing aorta were fixed in 70% EtOH. Dried gels were incubated with 1.5% milk in PBS, with biotinylated isolectin IB4 that have a strong affinity for endothelial cells (5 µg / ml in 1 mM CaCl<sub>2</sub>, pH 7.2; Molecular Probes) for 2 h, with streptavidin-FITC (dilution 1:40 in PBS; Amersham Biosciences) for 30 min and were counter stained with bisbenzimide (10 µg/ml in PBS).



**Figure S1. Paracrine action of pro-cath-D on fibroblasts in an aortic ring.** (A) Photomicrographs of aortic rings cultured for 9 d in the absence (MCDB) or presence of media conditioned by 3Y1-Ad12 cancer cell lines secreting no human cath-D (control), 30 nM human wild-type (cath-D) or 10 nM D231N cath-D (D231N). Points correspond to isolated cells spreading around aorta rings. Arrow indicates a micro-vessel. Bar, 1 mm. (B) Representative illustration of cells spreading around the aortic ring in presence of D231N CM. Endothelial cells stained in green were identified by their capacity to bind to isolectin IB4 as described in Materials and methods. Nuclei were stained in blue with bisbenzimide. Arrows indicate endothelial cells (E) and fibroblast-like cells (Fl). Bar, 100 µm. (C) Quantification of isolated cells escaping aorta rings. Cell number was quantified by computer-assisted image analysis and plotted as a function of the distance to the ring. (D) Quantification of maximal distance of isolated cell migration (Dmax) from ring. Dmax was quantified by computer-assisted image analysis. \*P < 0.01 versus control (ANOVA analysis; P values < 0.05 were considered significant). Each experiment was performed with three rings in each condition and in triplicates. Similar results were obtained in another experiment performed with different conditioned media.



**Figure S2. Characterization of human wild-type and D231N mutated cath-D in CD55<sup>-/-</sup> transfected fibroblasts.** (A) Maturation and secretion of human wild-type and D231N cath-D. Cells were labeled for 16 h with [<sup>35</sup>S]methionine. Cell lysates (C) and media (S) samples were immunoprecipitated with M1G8 antibody, analyzed on a 10% SDS-PAGE, and fluorographed. Arrowheads indicate migration of the three forms of cath-D. K, molecular mass in kD. (B) Proteolytic activity of cath-D. (Top) Cell extracts from CD55<sup>-/-</sup>cath-D and CD53<sup>+/+</sup>SV40 transfectants were assayed at the indicated times for proteolytic activity at pH 3.5 with [<sup>14</sup>C]methemoglobin in the absence or presence of pepstatin. Cell extracts from CD55<sup>-/-</sup>-SV40 and CD55<sup>-/-</sup>-D231N cells were assayed for 6 h for proteolytic activity at pH 3.5 with [<sup>14</sup>C]methemoglobin in the absence or presence of pepstatin. Cellular human cath-D proteolytic activity was calculated by subtracting the TCA-soluble radioactivity obtained with pepstatin and was expressed as cpm per microgram of DNA. (Bottom) Conditioned media from transfected fibroblasts containing equivalent amount of proteins secreted by 24 h were assayed for 16 h for proteolytic activity. Secreted cath-D proteolytic activity was obtained by subtracting the TCA soluble radioactivity obtained with pepstatin.

#### Proteolytic activity of cath-D

Conditioned media (1 ml) were collected after a 24-h incubation of transfected cells in 10% FCS medium in 6-well plates and cell extracts were prepared as described previously (Glondu et al., 2001). Total proteins from conditioned media (100 μl) or from cell extracts (75 μg) were incubated with 30,000 cpm [<sup>14</sup>C]methemoglobin (NEN Life Science Products), 2 μg of unlabeled methemoglobin (Sigma-Aldrich), in a citrate reaction buffer, pH 3.5, and in the absence or presence of 2 mM pepstatin (Sigma-Aldrich; Cappy et al., 1987). The proteolytic activity was calculated from the TCA-soluble radioactivity per microgram of DNA extracted from the corresponding cells.

#### References

- Blacher, S., L. Devy, M.F. Burridge, G. Roland, G. Tucker, A. Noël, and J.M. Foidart. 2001. Improved quantification of angiogenesis in the rat aortic ring assay. *Angiogenesis*. 4:133–142.