Supporting Information

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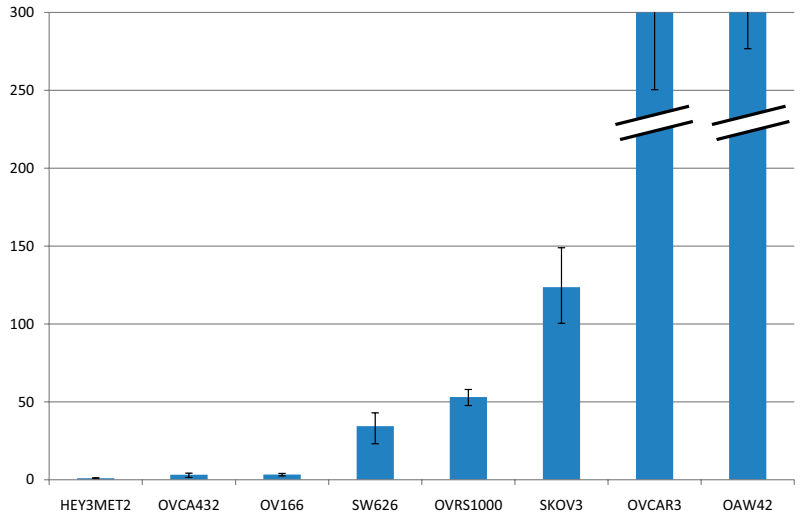

Fig. S1. Real-time RT-PCR analysis for relative quantification of the endogenous expression levels of RNASET2 in a panel of human ovarian cancer cell lines. The expression levels shown were normalized with those from the β-actin gene as an internal standard.

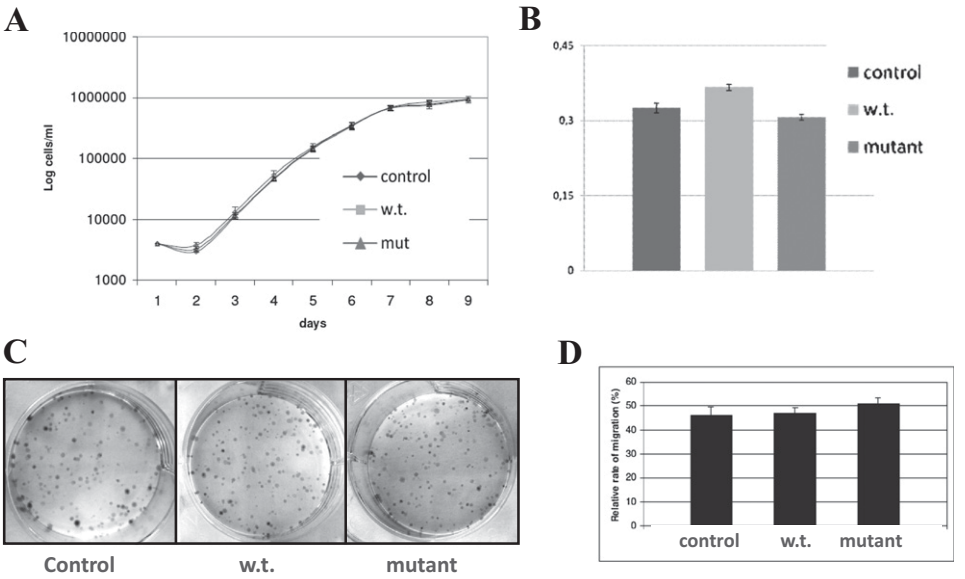

Fig. S2. RNASET2 overexpression in Hey3Met2 cells does not affect several in vitro cancer-related parameters. The panels show the results of proliferation (*A*), apoptotic rate (*B*), clonogenic (*C*), and migration (*D*) assays carried out on RNASET2-overexpressing clones and control, vector-only transfected clones. The experimental procedures for these assays are described in *Materials and Methods*.

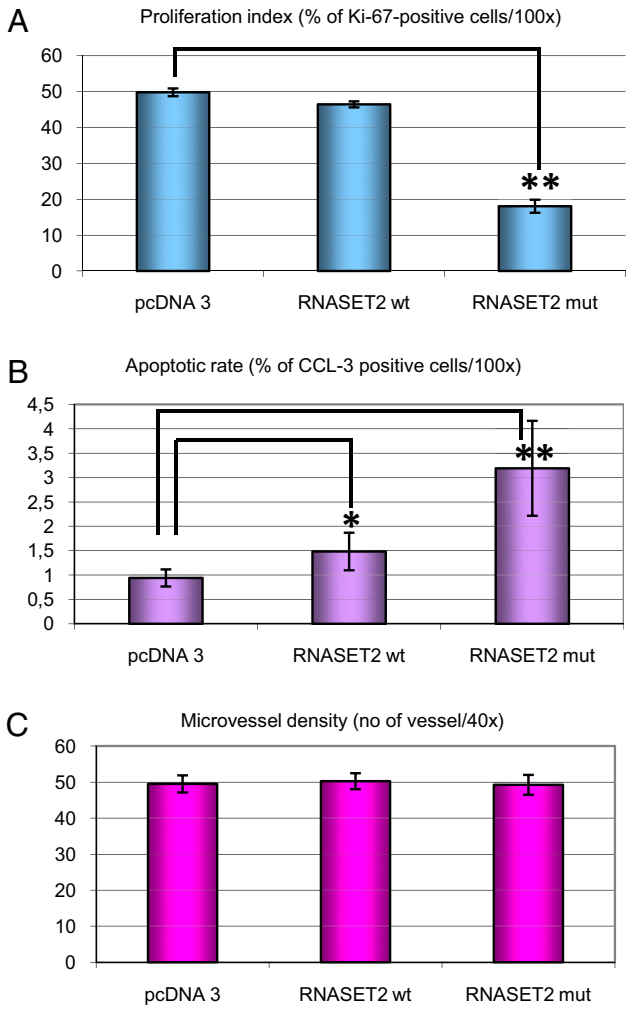

Fig. S3. Quantification of apoptotic, proliferating, and endothelial cells in RNASET2-expressing and control tumor samples following immunohistochemical (IHC) detection. Quantitative analysis of K_i-67 (*A*) and cleaved Caspase 3$^+$ (CCL-3$^+$) cells (*B*) and CD31$^+$ blood vessels (*C*) is shown. For K_i-67 and CCL-3 IHC, results are expressed as percentage of K_i-67$^+$ cells ± SE or percentage of CCL-3$^+$ apoptotic cells ± SE per total number of cells, respectively. A total of 10 100× fields were examined and counted from three tumors of each of the treatment groups. The values were initially subjected to one-way ANOVA and then compared using unpaired Student's *t* test. For CD-31 IHC, vessel density was estimated by counting CD31$^+$ vessel structures with a 40× magnification using a squaric grid. Counting in at least six fields was done separately. The values were initially subjected to one-way ANOVA and then compared using unpaired Student's *t* test. *$P < 0.05$; **$P < 0.01$.

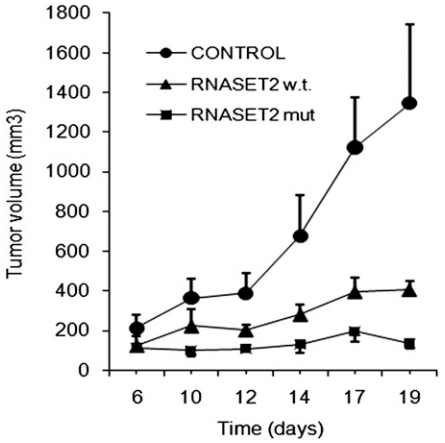

Fig. S4. Hey3Met2 clones stably transfected with control plasmid or RNASET2-expressing vectors were inoculated subcutaneously into Rag/γ-chain$^{-/-}$ double knock-out mice as described in *Materials and Methods*. Tumor growth was checked every 2 d until day 19. At least five mice were inoculated for each tested clone. Bars represent SD values.

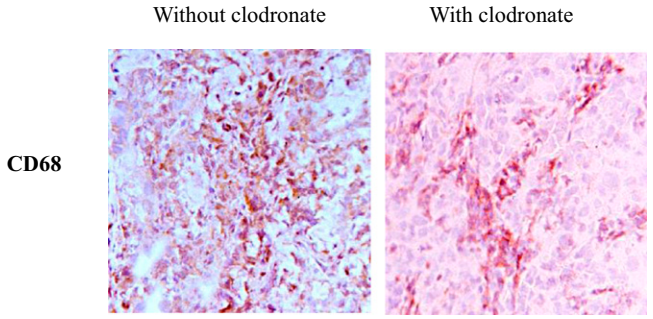

Fig. S5. Host macrophages are ineffectively depleted by clodronate following inoculation of mutant RNASET2-expressing Hey3Met2 cells. Rag2$^{-/-}\gamma_c^{-/-}$ mice were preatreated with clodronate liposomes, as described in *Material and Methods* before injecting Hey3Met2 cells expressing catalytically dead mutant RNASET2. The effectiveness of clodronate treatment was evaluated by IHC analysis of tumor sections with the macrophage lineage-specific anti-CD68 antibody. (Magnification: 40×.)

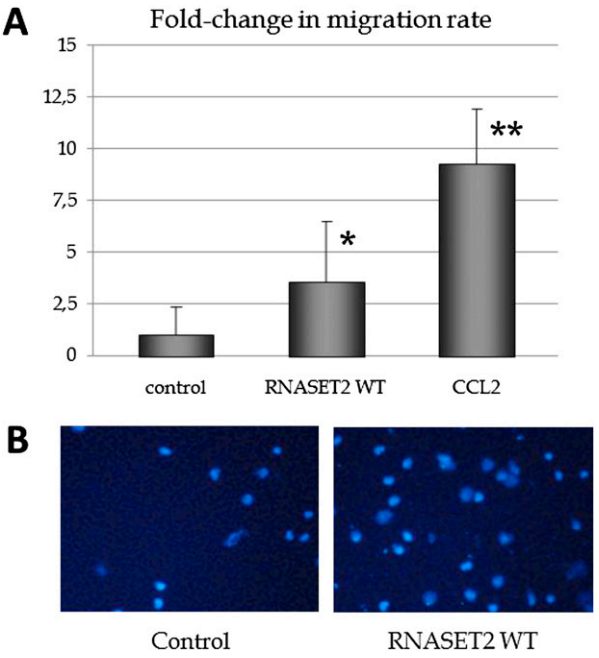

Fig. S6. Extracellular RNASET2 triggers monocyte chemotaxis in vitro. (*A*) Freshly prepared human monocytes were incubated in Boyden chambers with conditioned medium from control and wild-type RNASET2-expressing Hey3Met2 clones, as described in *Materials and Methods*. Recombinant CCL-2, a potent chemoattractor for human monocytes, was used as a positive control. The graph reports mean values for three independent Hey3Met2 clones. (*B*) A representative image of DAPI-stained nuclei from monocytes that have actively migrated through the filter.

Table S1. In vitro analysis of control and RNASET2 stably transfected Hey3Met2 clones

	pcDNA3 vector	pcDNA3 RNASET2 wt	pcDNA3 RNASET2 mut
Population doubling	1,160 ± 12 min	1,172 ± 15 min	1,148 ± 16 min
Clonogenic assay*	230 ± 25 clones	222 ± 19 clones	219 ± 20 clones
Growth in soft agar*	22 ± 5 clones	26 ± 8 clones	28 ± 9 clones
Cell motility[†]	100%	102%	105%
Matrigel adhesion test[‡]	0.32	0.36	0.29
Apoptosis[§]	2.5%	3.1%	2.8%

Four independent clones for each transected construct were analyzed according to the methods described in *Materials and Methods*. Data were obtained from at least three independent experiments.
*Values are expressed as average clone number/plate.
[†]Values are expressed as relative rate of migration with respect to the control clones.
[‡]Values expressed as average absorbance at 295 nm in three independent experiments.
[§]Values expressed as percentage of apoptotic cells detected with a TUNEL assay.