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Oltean *et al.* 10.1073/pnas.0603090103.

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- [Supporting Table 1](#)
- [Supporting Figure 7](#)
- [Supporting Figure 8](#)

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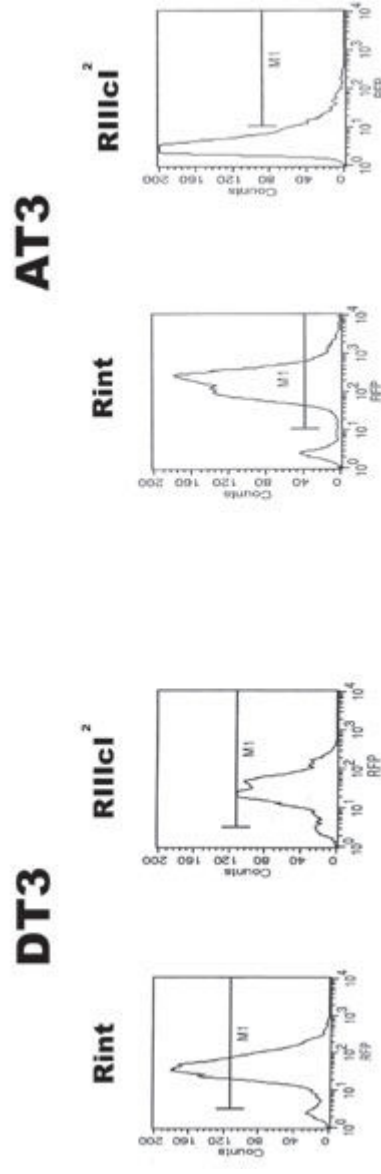


Fig. 6. Fluorescence cytometry analysis of pRint and pRIIIc1² stably transfected DT3 cells (left two images) and AT3 cells (right two images). M1 represents a marker indicating fluorescence positive readings (i.e., above the background fluorescence for untransfected cells). The expression of red fluorescent protein (RFP) is relatively homogeneous, although it is clear that a small fraction of the Rint cells may have lost the reporter.

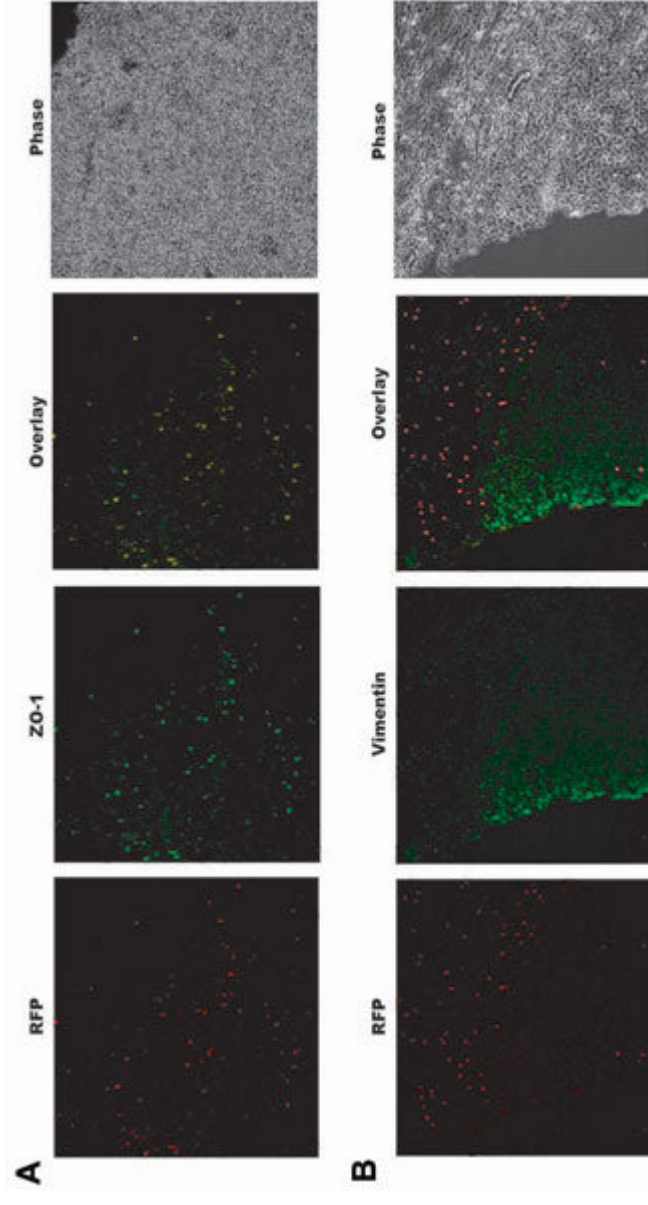


Fig. 7. Mesenchymal-epithelial transitions (MET) in tumors. (A) Zonula occludens protein-1 (ZO-1) counterstaining is consistent with MET. Example of a section from R111cI² tumors shows that the red fluorescent cells also stained positive for ZO-1. Images were acquired at $\times 200$ magnification. (B) Vimentin counterstaining. Example of a section from R111cI² tumors shows that the red fluorescent cells have no or have low vimentin expression. The RFP+ cells are within a larger tumor region that has low vimentin staining. Images were acquired at $\times 200$ magnification.

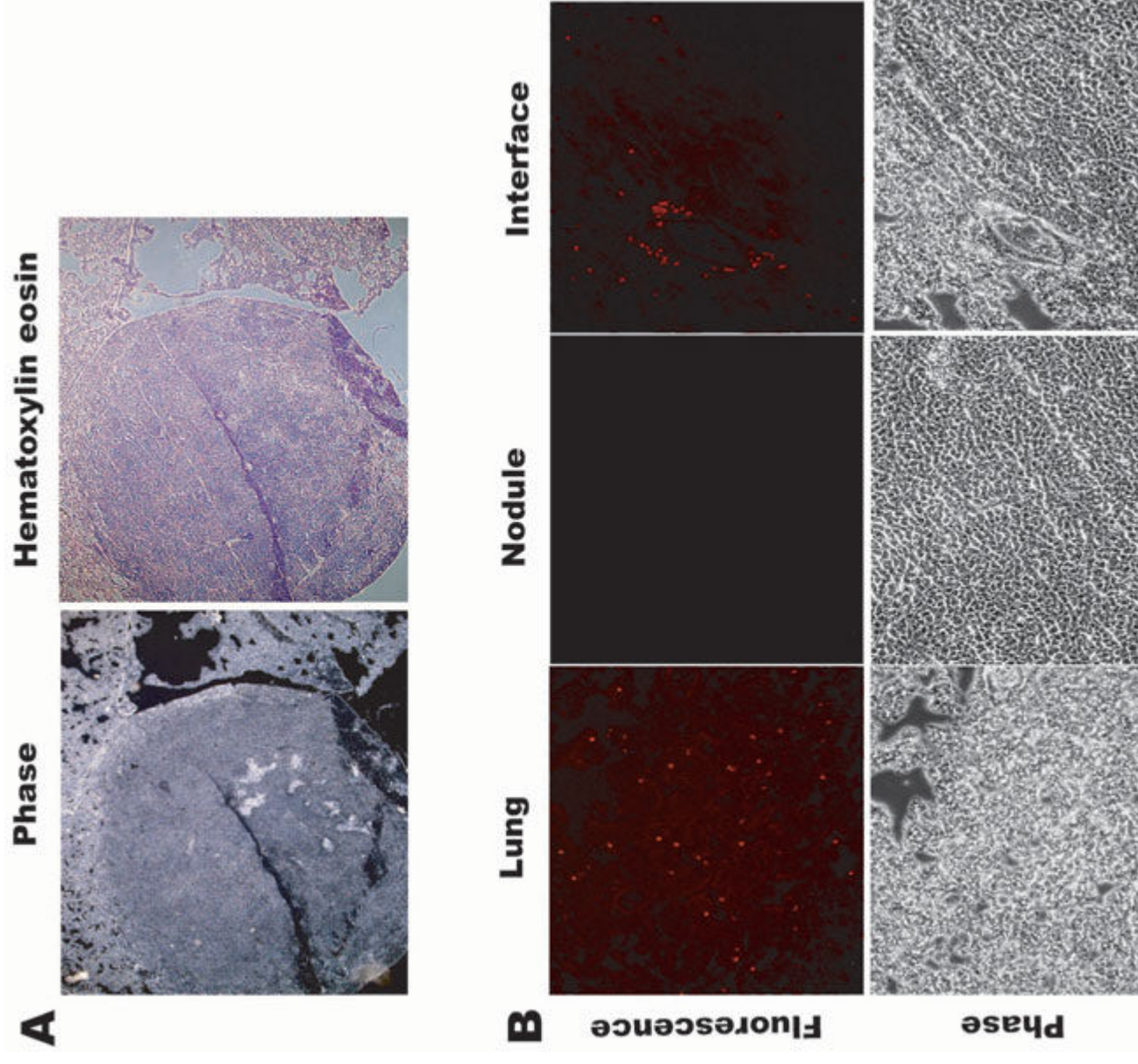


Fig. 8. A metastatic nodule does not express RFP, whereas in the lung tissue around it there are RFP-expressing metastatic cells. (A) Phase-contrast and hematoxylin/eosin staining of a large metastatic nodule in the lung of an animal bearing an AT3-RIIIc1² tumor ($\times 40$ magnification). (B) (Upper) Fluorescence pictures of lung tissue in the vicinity of the nodule, middle of the nodule, and at the interface of the nodule with the lung tissue. (Lower) Phase-contrast images of the same fields. Images were acquired at $\times 400$ magnification.

Table 1. RFP+ clusters in tumors and in metastases in lungs: Frequency and characterization

Tumors (animals)	Frequency of tumor sections with at least one RFP+ cluster*	Frequency of RFP+ clusters that express E-cadherin [†]	Frequency of RFP+ clusters that are present in stromal regions [‡]	RFP+ cells in lungs	Frequency of RFP+ metastasis that express E-cadherin [§]
AT3-RIIIc1 ² tumors (males)					
01	1/6	0/1	ND	N	NA
02	4/8	4/4	4/4	Y	1/2
03	3/7	0/2	0/2	Y	2/2
04	4/7	2/2	2/2	Y	2/2
05	2/5	1/1	ND	Y	0/2
06	1/5	1/1	ND	ND	NA
07	1/3	1/1	ND	Y	2/2

08	3/6	2/3	2/2	Y	1/1
09	0/8	NA ⁵	ND	Y	2/2
Male totals	19/55	11/15	8/10	NA	10/13
AT3-RIIIc1 ² tumors (females)					
01	1/8	1/1	ND	Y	ND
02	0/8	NA	ND	Y	2/2
03	3/3	ND	ND	Y	1/1
04	5/8	1/1	4/4	Y	2/2
05	2/6	1/1	2/2	Y	0/2
Female totals	11/33	3/3	6/6	NA	5/7
Total AT3-RIIIc1 ² tumors	30/88	14/18	14/16	NA	15/20
AT3-RΔ,Δ tumors (males)					
01	0/4	NA	NA	NA	NA
02	0/4	NA	NA	NA	NA
03	0/4	NA	NA	NA	NA
04	0/4	NA	NA	NA	NA

Total RA,Δ tumors	0/16	NA	NA	NA
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*Frequency = No. of sections with at least one RFP+ island/no. of sections examined. Note that 12 of 14 tumors had at least one RFP+ cluster.

†Analysis of tumor sections with one RFP+ island that were costained with E-cadherin (see Fig. 3).

‡Stromal regions were identified by using Masson’s Trichrome stain (see Fig. 4).

§Analysis of lung sections with one RFP+ island that were costained with E-cadherin (see Fig. 5).

¶NA, not applicable; ND, not determined.

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