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SUPPLEMENTARY MATERIAL AND METHODS

Plasmid description

The extension .dn3 indicates that the backbone plasmids are pcDNA3 (#1) from Invitrogen. The **pEGFP-C1** (#6) plasmid encodes the green fluorescent protein and is from Clontech (ref: 6084-1). The **pTK-Hyg** (#317) plasmid encodes for a protein that confers resistance to the hygromycin B (Roche; ref: 12653400) and is from Clontech (ref: 6153-1). **HA-hRasGAP[158-455].dn3** (#145) previously called HA-N2.dn3 (1), encodes the HA-tagged form of fragment N2. **GFP-HA-hRasGAP[158-455]** (#213), previously named GFP-HA-N2 (2) encodes an HA-tagged version of fragment N2 fused with a GFP-protein at the N-terminal. The **HA-hRasGAP[158-455].lti** (#769) used for lentiviral infection was described earlier (3).

Experimental metastasis assay

Experimental metastasis assays were performed as previously reported (4). Nude mice were used (Janvier; ref: Rj:NMRI-$^{Foxn1nu/Foxn1nu}$) and MDA-MB-231 firefly-luciferase expressing cell were injected. Peptide regimen was similar than with 4T1 model except that it last for 30 days. Alternatively, MDA-MB-231 were infected by lentiviruses encoding the RasGAP Fragment N2 (or its empty vector), then were injected in the tail vein and sacrificed after 46 days. Lungs were taken out. The blood that was remaining in the lungs was washed by PBS perfusion into the right cardiac ventricle. The left lobes were weighed, lyzed and 20 μg of lung lysate were analyzed for luciferase activity using the GloMax luminometer and according to the manufacturer's instructions (Promega; ref: E6521). The results were reported in relative light units (R.L.U.) and in number of cells as known number of cells were quantified *in vitro* for their bioluminescence.

TAT-RasGAP$_{317-326}$ synthesis

The TAT-RasGAP$_{317-326}$ peptide (GRKKRRQRRRGGWMWVTNLRTD) was described and synthesized as previously reported (5;6).

Radioiodination of the TAT-Y-RasGAP$_{317-326}$ peptide

Ten MBq Na[I^{125}]-Iodide (Perkin Elmer) were added to 4.8 µl of 1 mM TAT-Y-RasGAP$_{317-326}$ (GRKKRRQRRRG**Y**GWMWVTNLRTD) in a glass vial coated with 100 µg iodogen (Iodination reagent; Pierce; ref: 28600). The vial was placed on ice for 20 minutes. The mixture was diluted up to 1 ml with gelatin eluting buffer (0.25 % gelatin in PBS (w/v)) and then loaded on a PD Minitrap G-10 column (GE Healthcare; ref: 28-9180-10). The radiolabeled peptide was then eluted in 0.5 ml gelatin elution buffer.

TAT-RasGAP$_{317-326}$ biodistribution

Five Balb/c mice were injected with one hundred thousand 4T1 cells in the mammary fat pads. Fourteen days after cancer cell injection, the mice were injected with 1.6 mg/kg TAT-RasGAP$_{317-326}$ peptide (containing 5 % (v/v) of I^{125}-radiolabeled peptide). Six hours after, the mice were sacrificed and the 4T1 tumor and organ radioactivity were recorded in counts per minute (cpm). Since the radioactivity of the whole mouse was recorded, the percentage of the injected dose per organ could be calculated.

4T1 orthotopic model with TAT-RasGAP$_{317-326}$

We carried out orthotopic implantations as described in the material and methods. Mice treated with the RasGAP peptide were injected intraperitoneally with 1.6 mg/kg TAT-RasGAP$_{317-326}$ (or its vehicle: PBS) three times a week (every monday, wednesday and friday) and sacrificed after 25 days.

Boxplot description

Boxplots were performed as follows: the line in a box indicates the median; the box contains 50% of values (i.e. 25% of those above and 25% of those below the median); whisker's length corresponds to 1.5 time of the box's length (if shorter, the length of the whisker reaches the lowest or the highest value of the data set). Empty circles represent outlier values (i.e. outside the range covered by the box and the whiskers).

Reference List

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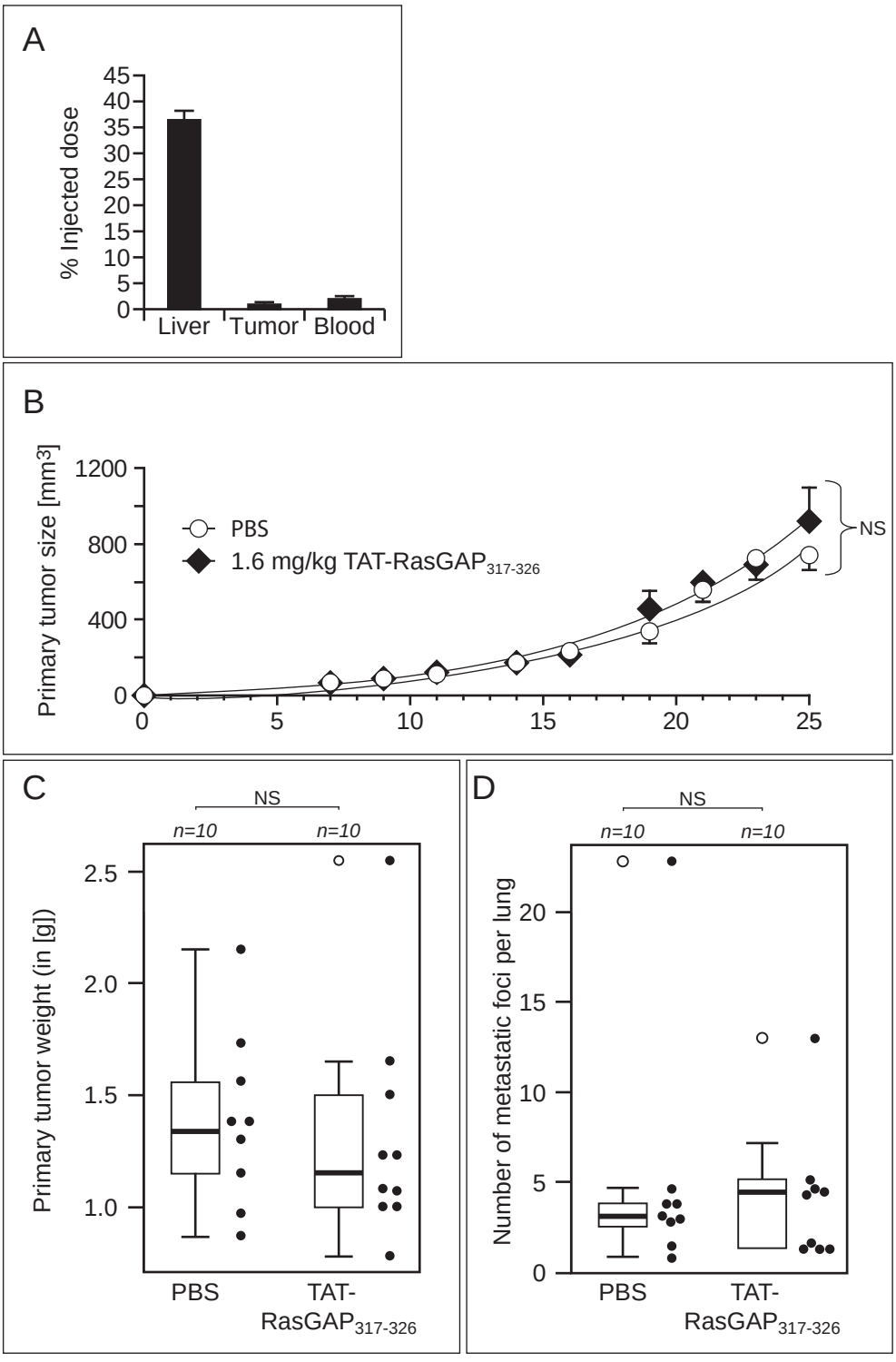
Supplementary Figure 1



Supplementary Figure 1. Effect of TAT-RasGAP$_{317-326}$ on 4T1 metastasis formation.
(**A**) The biodelivery of 1.6 mg/kg I^{125}-labelled TAT-RasGAP$_{317-326}$ in Balb/c bearing pre-established 4T1 tumors was reported in percentage of the injected dose (*n* = 5 mice).
(**B-D**) Balb/c mice were injected with 4T1 cells and then treated three times a week with 1.6 mg/kg TAT-RasGAP$_{317-326}$ for 25 days. The figure displays the tumor growth (**B**), the tumor weight after sacrifice (**C**) and the number of metastatic foci per lung (**D**). Ten mice per condition were analyzed. The results are shown as boxplots on the left while raw data points are displayed on the right.