

Regulation of hematogenous tumor metastasis by acid sphingomyelinase

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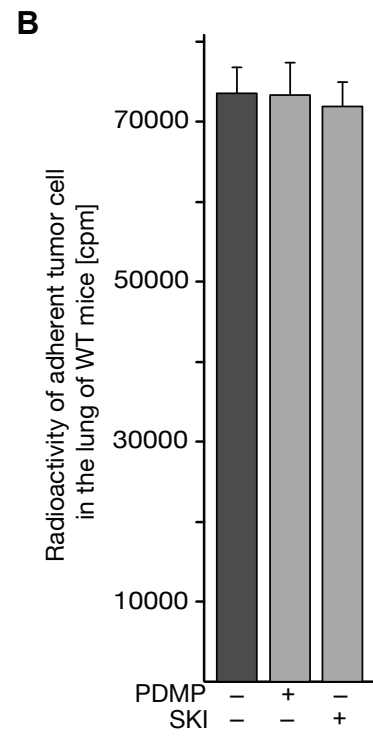
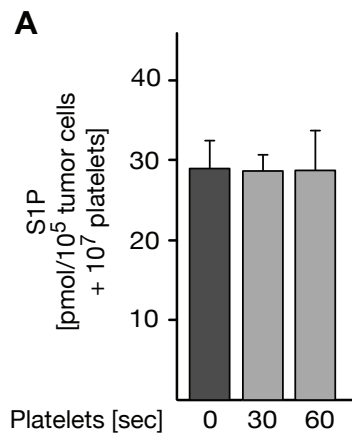
Supplementary Figure 1: Sphingosine 1-phosphate after co-incubation of B16F10 tumor cells with platelets

Co-incubation of B16F10 melanoma cells with wild type platelets does not result in formation of S1P as determined by mass spectrometry. Likewise, *in vivo* inhibition of glucosyltransferases or sphingosine kinases by application of PDMP or SKI does not alter B16F10 tumor metastasis. Shown is the mean \pm SD, from 4 independent samples, ANOVA.

Supplementary Figure 2: H⁺-ATPase clusters in ceramide-enriched membrane domains after co-incubation of tumor cells with platelets

H⁺-ATPase clusters in ceramide-enriched membrane domains on the surface of B16F10 cells after co-incubation of the tumor cells with platelets. Tumor cells were co-incubated with platelets for 60 seconds, fixed in 2% buffered PFA (pH 7.4) for 10 min, washed, blocked with H/S supplemented with 5% FCS and 0.01% Tween 20, washed, stained with anti-H⁺-ATPase antibodies (Santa Cruz Inc.) and anti-ceramide antibodies for 45 min, washed again and stained with FITC-labelled anti-goat and Cy3-labelled anti-mouse IgM antibodies. Finally, cells were washed, embedded in Mowiol and analysed by confocal microscopy. Shown are representative studies from 3 independent experiments.

Supplementary Figure 1



Supplementary Figure 2

H⁺-ATPase (FITC)

Ceramide (Cy3)

B16F10 +
WT-platelets

