
Supplementary information

**The NALCN channel regulates metastasis
and nonmalignant cell dissemination**

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Supplementary materials for

The NALCN channel regulates metastasis and non-malignant cell dissemination

Eric P. Rahrman¹, David Shorthouse², Amir Jassim¹, Linda P. Hu¹, Mariaestela Ortiz³, Betania Mahler-Araujo⁴, Peter Vogel⁵, Marta Paez-Ribes¹, Atefeh Fatemi¹, Gregory J Hannon¹, Radhika Iyer⁶, Jay A. Blundon⁷, Filipe C. Lourenço¹, Jonathan Kay⁸, Rosalynn M. Nazarian⁹, Benjamin A. Hall², Stanislav S. Zakharenko⁷, Douglas J Winton¹, Liqin Zhu¹⁰, Richard J. Gilbertson^{1,11*}

Correspondence should be addressed to RJG: Richard.Gilbertson@cruk.cam.ac.uk

**This PDF file includes:
Supplementary Figures 1, 2 and 3
Source Data for Supplementary Fig. 2**

Reagents, antibodies and software

A table of the source of all reagents, antibodies, kits, cell lines, chemical and software are included (Table SX)

Supplementary Figures, Tables:

Fig. S1. Single channel immunofluorescent images for all figures and extended data figures.

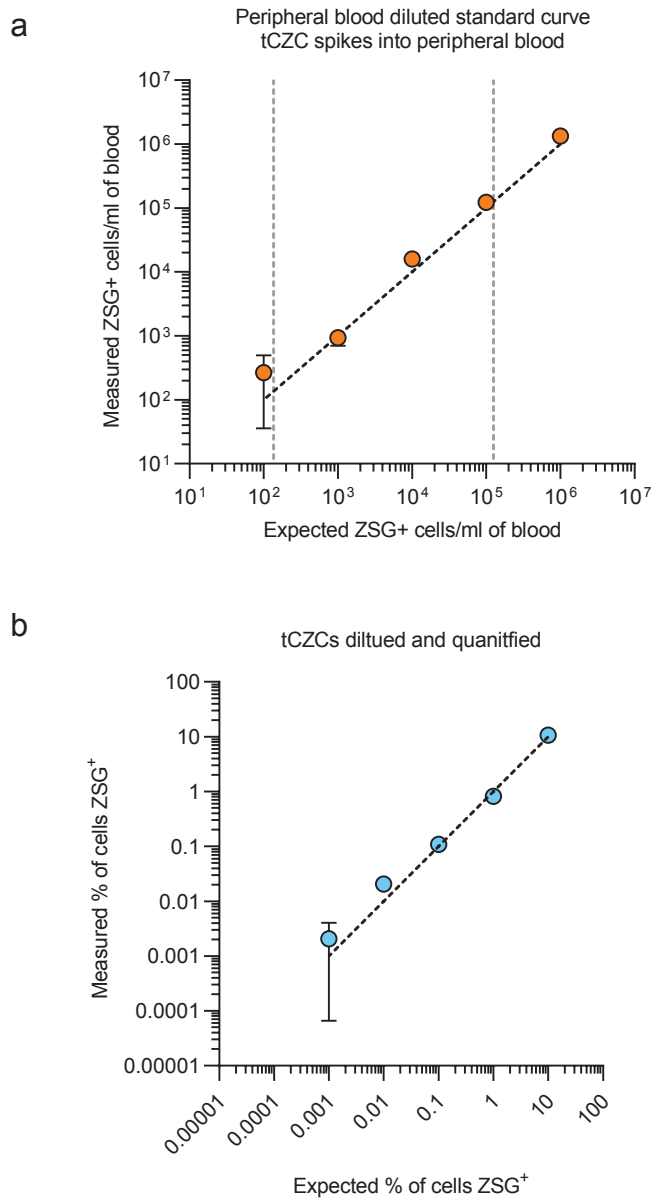
Fig. S2. CZC standard curves generated by two ‘spike-in’ control techniques.

Fig. S3. Supplementary Figure S3. Exemplar FACS gating strategies for isolating ZSG+ cells from peripheral blood samples.

Source data for Supplementary Figure S2.

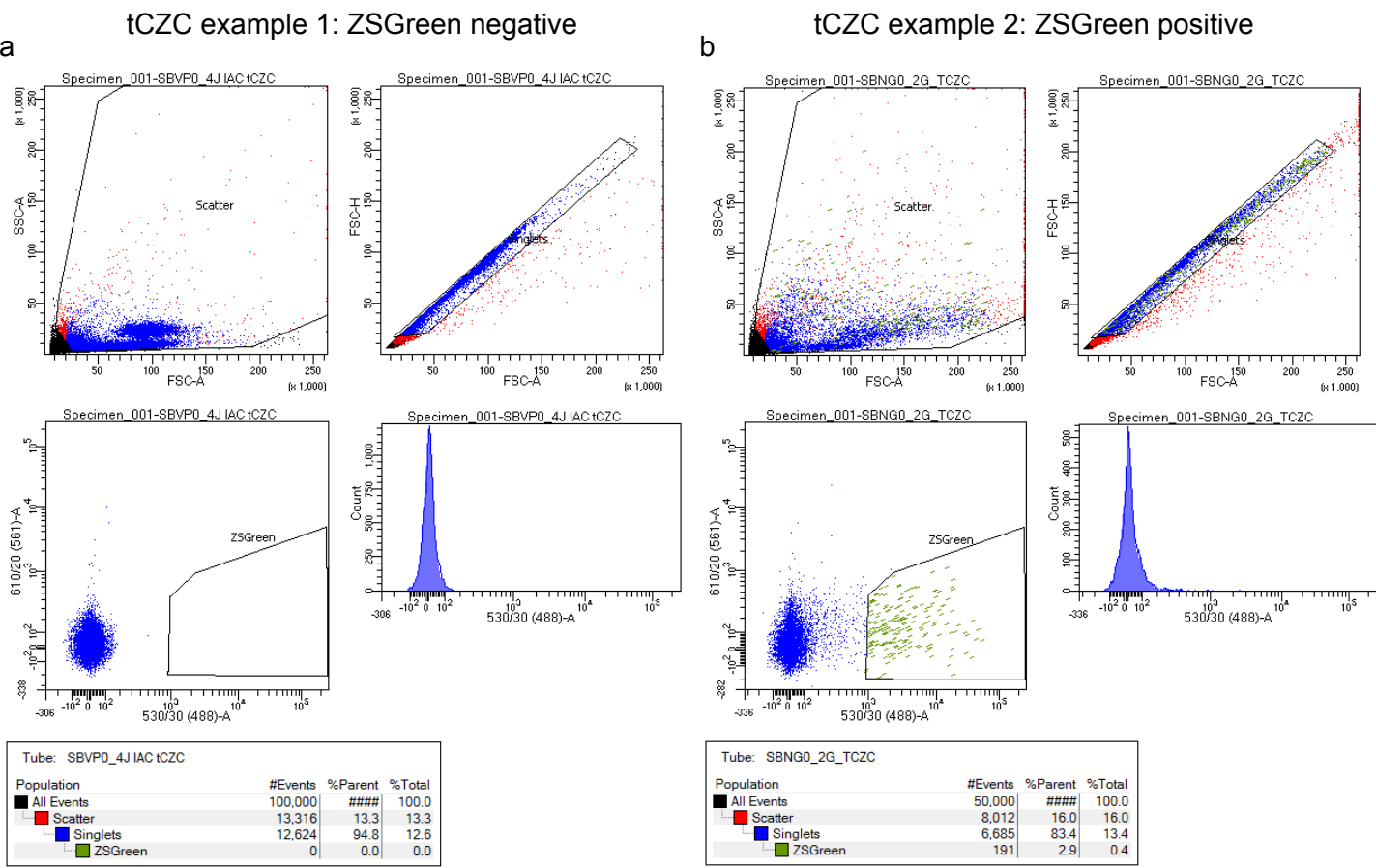


Supplementary Figure 2



Standard curves generated by two ‘spike-in’ control techniques. (a) Normal peripheral blood was harvested from adult *P1-KP* mice with gastric adenocarcinoma. Peripheral blood mononuclear cells (PBMCs) were isolated by ficoll gradient centrifugation. ZSG⁺ cells were enumerated manually and spiked into fresh peripheral blood to give the final number of actual ZSG⁺ cells/ml (x-axis). These samples were then subject to the same FACS protocol used in all blood isolation studies to provide the observed (y-axis) quantification. The vertical dotted lines represent the 25th and 75th percentile of observed CZCs/ml recorded in Supplementary Table 11. **(b)** Normal PBMCs used in (a) were also spiked at the indicated percentage of total PBMCs in buffered saline and quantified in the same manner as in (a). In both graphs, the hashed black line represents the ideal curve in which expected and observed results are the same.

Supplementary Figure 3



Supplementary Figure S3. Exemplar FACS gating strategies for isolating ZSG+ cells from peripheral blood samples. (a) Example of a tumour-bearing animal that did not have ZSG+ cells in the peripheral blood. (b) Example of a tumour-bearing animal with ZSG+ cells in the the peripheral blood.

Source data for Supplementary Fig. S2a and b.

Expected # of ZSG+ cells/ml blood	GAC (<i>Nalcn</i> ^{flx/+}) 1	GAC (<i>Nalcn</i> ^{flx/+}) 2	GAC (<i>Nalcn</i> ^{flx/+}) 3
1000000	1392000	1356000	1244000
100000	148800	107600	110400
10000	13960	16000	17640
1000	800	800	1200
100	400	0.001	400

Expected % of ZSG+ cells/ml blood	GAC (<i>Nalcn</i> ^{flx/+}) 1	GAC (<i>Nalcn</i> ^{flx/+}) 2	GAC (<i>Nalcn</i> ^{flx/+}) 3
10	10.4467	9.2553	12.0833
1	0.79	0.8013	0.8498
0.1	0.0992	0.0867	0.1404
0.01	0.0167	0.0213	0.0234
0.001	0.002	0.0041	0.0001