

SUPPLEMENTARY INFORMATION

Supplementary Table 1

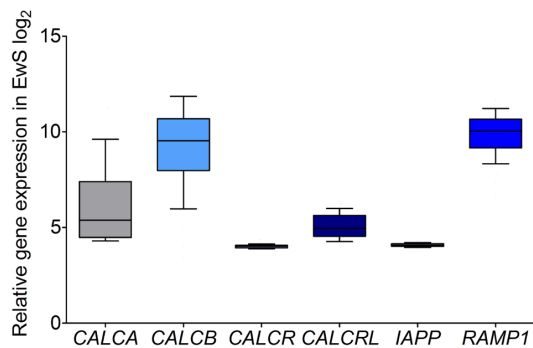
Microarray data accession codes

Supplementary Table 2

Mass spectrometric detection of CALCA and CALCB in EwS cell line supernatants

SUPPLEMENTARY FIGURES

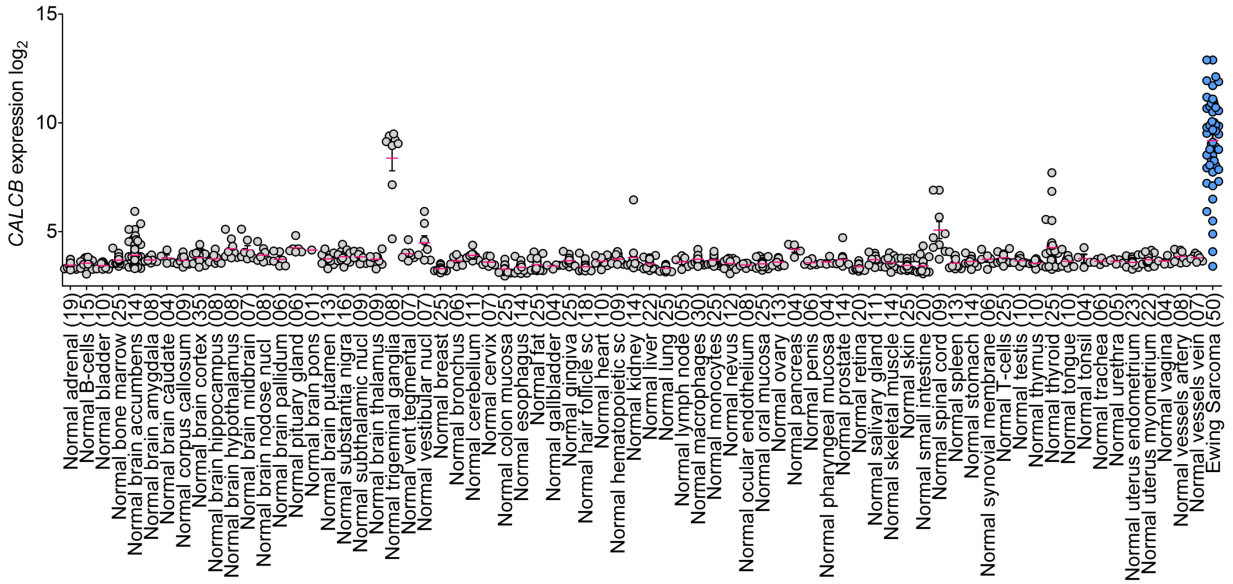
Supplementary Figure S1 Dallmayer *et al.*



Supplementary Figure S1: Comparison of *CALCA*, *CALCB*, *CALCR*, *CALCRL*, *IAPP*, and *RAMP1* expression levels in EwS

Analysis of expression levels of publicly available microarray data of 50 primary EwS tumors are shown in log₂-scale. Data are represented as box-plots. Horizontal bars indicate median expression levels, boxes the interquartile range. Whiskers indicate the 10th and 90th percentile, respectively.

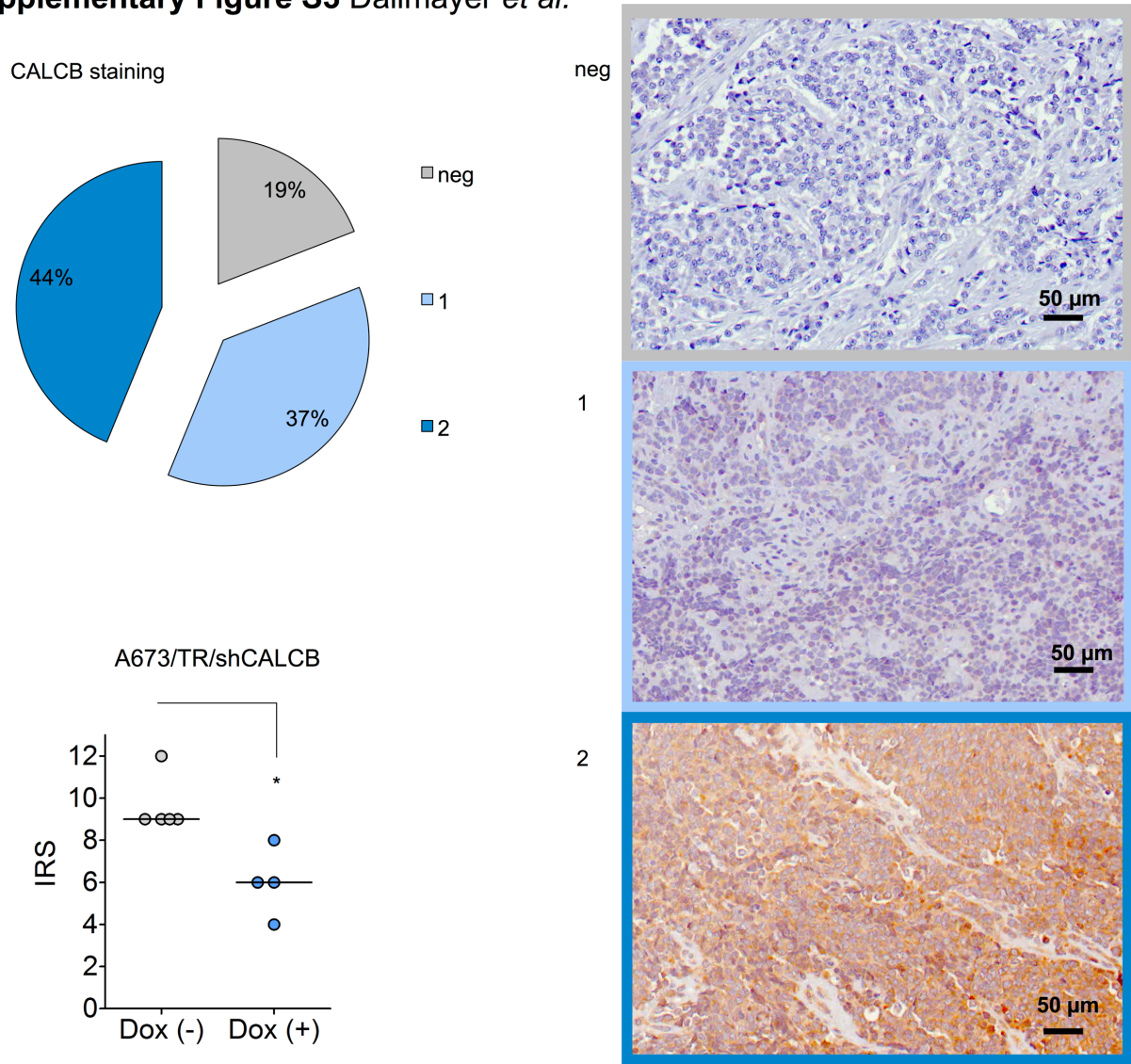
Supplementary Figure S2 Dallmayer *et al.*



Supplementary Figure S2: *CALCB* Expression in 71 normal tissue types and EwS

Publicly available microarray data are represented as scatter plot in log₂-scale with red bars representing means, and whiskers representing the SEM. Each dot represents one sample. Number of samples is given in parentheses. EwS is highlighted in blue color.

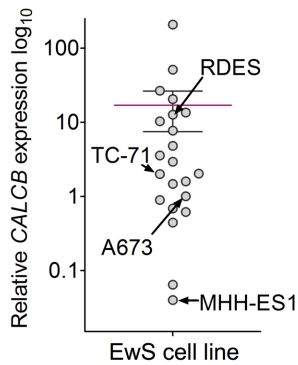
Supplementary Figure S3 Dallmayer *et al.*



Supplementary Figure S3: EwS TMA stained for CALCB

A EwS TMA comprising 89 primary EwS samples was stained with a specific antibody against human CALCB. The left circular chart shows proportion of staining intensities of the samples. Micrographs on the right show representative samples with CALCB staining intensity 0 (upper picture), 1 (middle picture), and 2 (lower picture). The left lower plot shows the IRS of CALCB staining of tissue from xenografted A673/TR/shCALCB EwS cells carrying a dox-inducible shRNA against *CALCB*. Each dot represents one sample ($n \geq 4$ per group), horizontal bars represent medians. Mann-Whitney-test. * $P \leq 0.05$

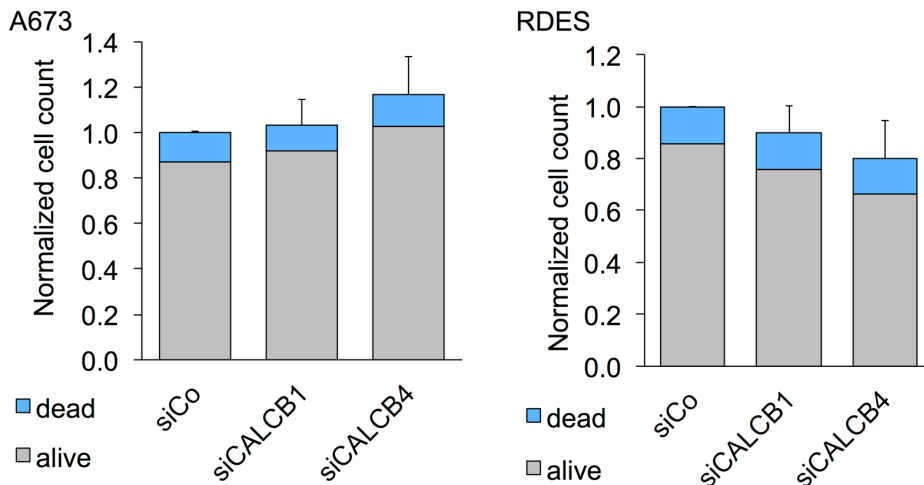
Supplementary Figure S4 Dallmayer *et al.*



Supplementary Figure S4: Analysis of relative *CALCB* expression in EwS cell lines

Relative *CALCB* expression levels of 21 EwS cell lines relative to the A673 EwS cell line were determined by qRT-PCR. Data are shown as scatter plot in \log_{10} scale. Each dot represents the mean *CALCB* expression from two independent replicates for each cell line. The horizontal bar represents the mean expression, whiskers indicate the SEM.

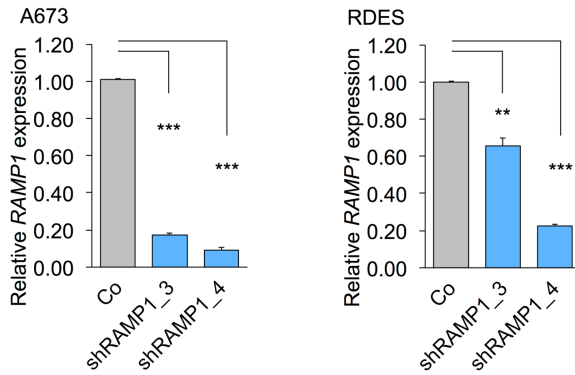
Supplementary Figure S5 Dallmayer *et al.*



Supplementary Figure S5: Analysis of short-term proliferation of A673 and RDES EwS cells after silencing of *CALCB*

Cells were transiently transfected with specific siRNAs against *CALCB* (siCALCB1 and siCALCB4) or non-targeting control siRNA (siCo). Cells were harvested after 48-72 h after transfection and the number of viable and dead cells was determined by standardized hemocytometry including cell culture supernatants. Data are represented as means and SEM ($n_{A673} = 2-4$; $n_{RDES} = 3$).

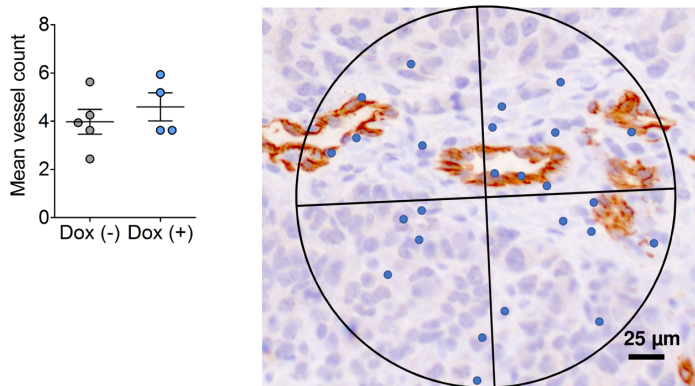
Supplementary Figure S6 Dallmayer *et al.*



Supplementary Figure S6: Validation of *RAMP1* knockdown

The graphs show knockdown of *RAMP1* as determined by qRT-PCR in A673 and RDES EwS cells carrying dox-inducible shRNAs against *RAMP1* obtained from *in vitro* assays. Data are means and SEM, $n \geq 3$; unpaired two-tailed Student's t-test. ** $P \leq 0.01$; *** $P \leq 0.001$

Supplementary Figure S7 Dallmayer *et al.*



Supplementary Figure S7: Microvessel density evaluation in CD31 stained xenografts

Left panel shows mean microvessel count evaluated in murine CD31 stained EwS Xenografts with/without silencing of *CALCB* in the tumors (dox +/-). Each dot represents mean microvessel density evaluated in 4 high power fields of one tumor. Horizontal bars indicate the mean, whiskers the SEM. Right panel: representative CD31 stained slide with Chalkley grid.