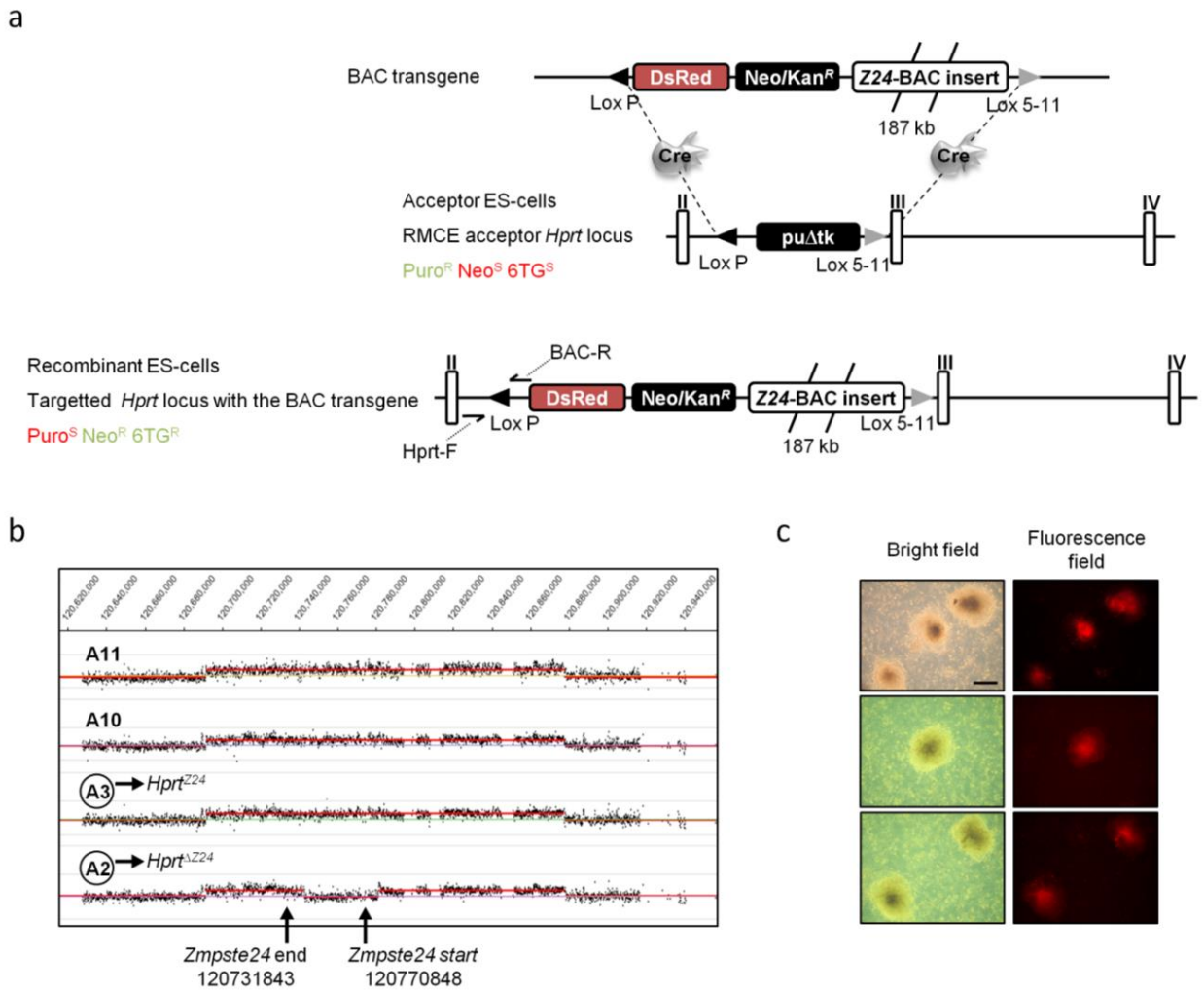
Supplementary Information; de la Rosa *et al.*

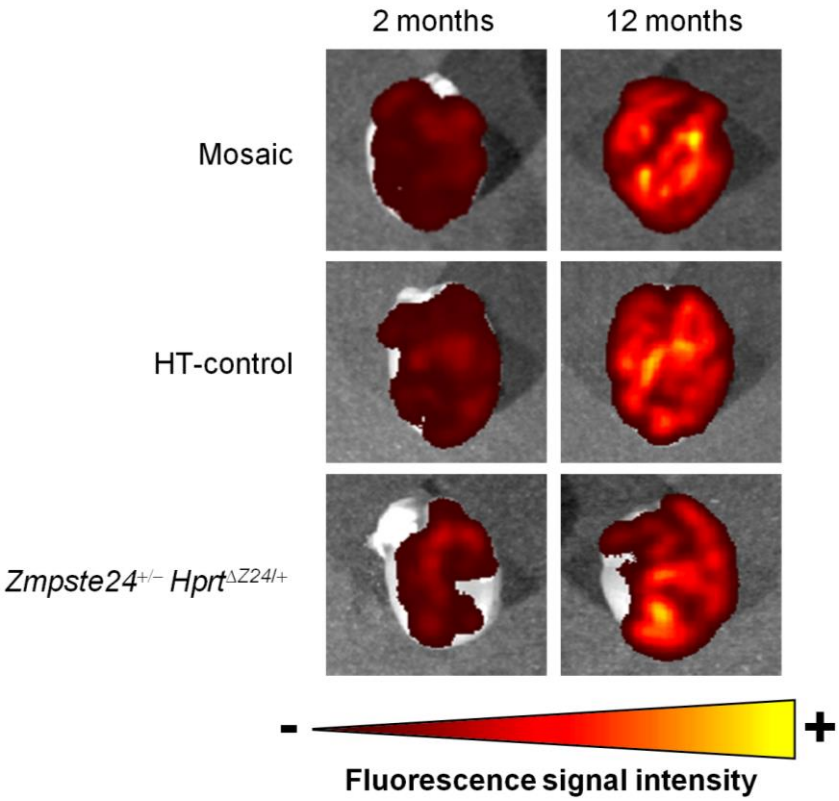
Supplementary Figures

Supplementary Figure S1



Supplementary Figure S1. Generation of the *Hprt*$^{Z24/+}$ and *Hprt*$^{\Delta Z24}$ alleles by RMCE.
(**a**) Schematic representation of the BAC transgene, the RMCE acceptor *Hprt* locus and the targeted *Hprt* locus with the inserted BAC transgene. Drug sensitivities (superscript S) and resistances (superscript R) of ES cell clones to puromycin (Puro), G418 (Neo), and 6-TG are indicated. puΔTK: cassette conferring resistance to puromycin and sensitivity to gancyclovir. Neo/Kan^R: cassette conferring dual resistance to G418 and kanamycin. DsRed: cassette driving expression of the fluorescent DsRed protein under the control of the CAGGS promoter. Hprt-F and BAC-R indicate the positions of the oligonucleotides used for PCR-based genotyping. (**b**) CGH-array based analysis of BAC integrity in ES cell clones. A region of chromosome 4 corresponding to the sequence of the *Z24*-BAC insert plus 60 kbp of 3´ and 5´ adjacent sequences were analyzed. The baseline corresponds to 2N. The signal from the whole *Z24*-BAC insert corresponds to 3N in clones A11, A10 and A3 (2N for the two copies of chromosome 4 plus 1N corresponding to the BAC insert in chromosome X), whereas a 40 kb region including most of the *Zmpste24* gene is deleted in the *Z24*-BAC insert from clone A2. A3 and A2 clones were selected for the generation of genetically modified *Hprt*$^{Z24/+}$ and *Hprt*$^{\Delta Z24}$mice, respectively. (**c**) Red fluorescence in recombinant ES cells with the BAC transgene in culture. Scale bar represents 500 µm.

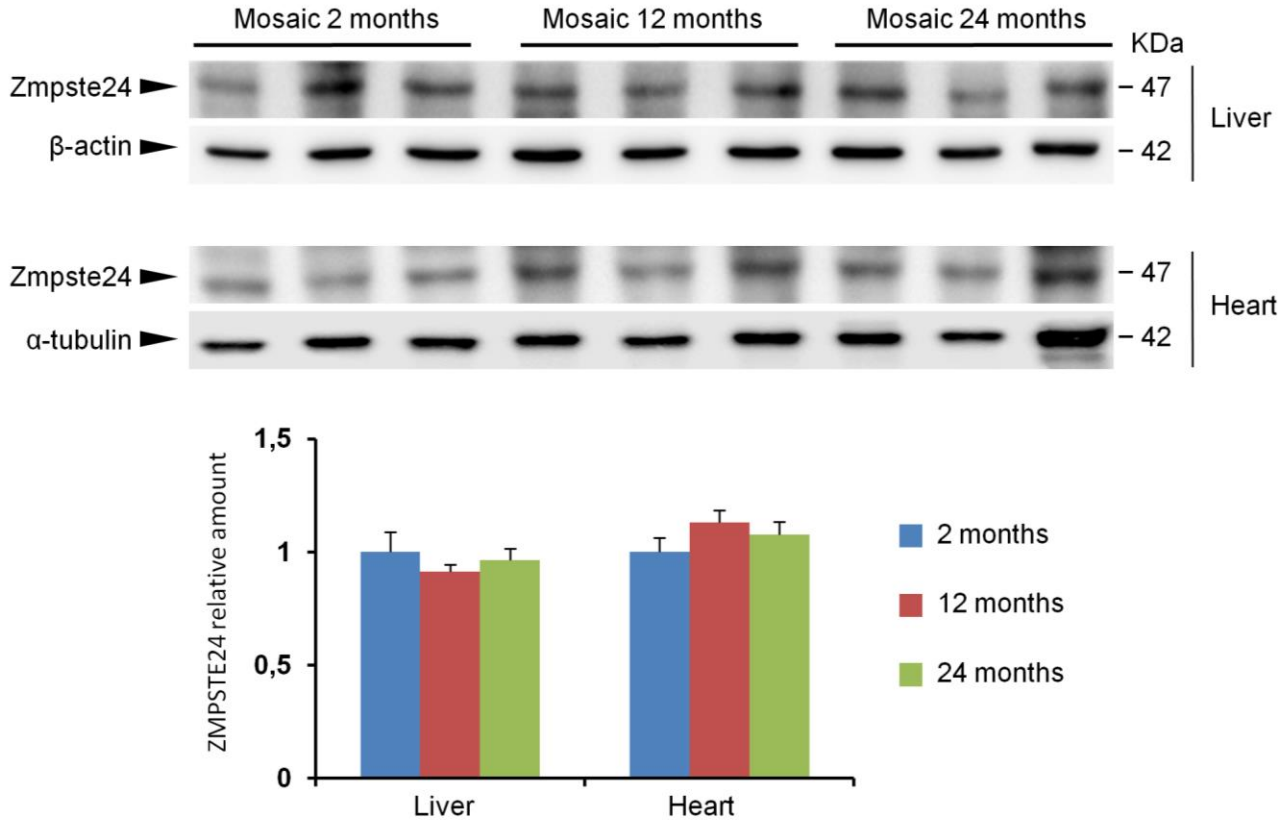
Supplementary Figure S2



Supplementary Figure S2. IVIS imaging of hearts from mosaic, HT-control and *Zmpste24*$^{+/-}$ *Hprt*$^{\Delta Z24/+}$ mice.

Similar changes in red fluorescence with age are detected in hearts from mosaic, HT-control and *Zmpste24*$^{+/-}$*Hprt*$^{\Delta Z24/+}$ mice, indicating that this increase is unrelated to the absence of Zmpste24.

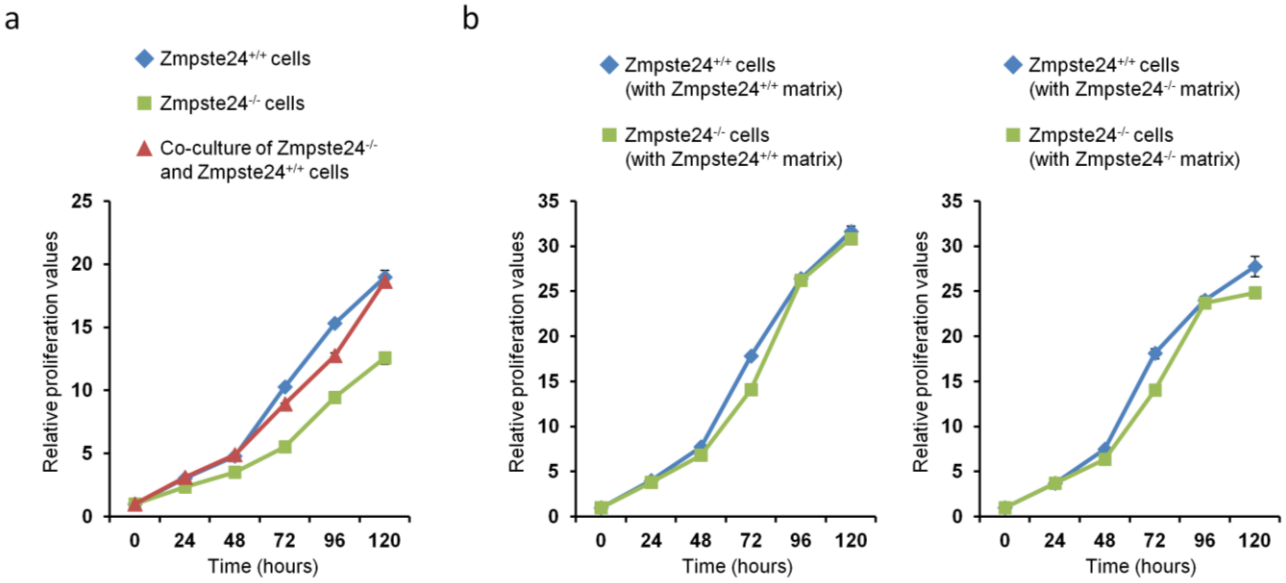
Supplementary Figure S3



Supplementary Figure S3. Analysis of Zmpste24 protein level in tissues from mosaic mice at different ages.

Western blot detection of Zmpste24 was performed in liver and heart from 2-, 12- and 24-month-old mosaic mouse. Densitometry analysis of the immunoblots is also shown. Error bars represent s.e.m. n=3.

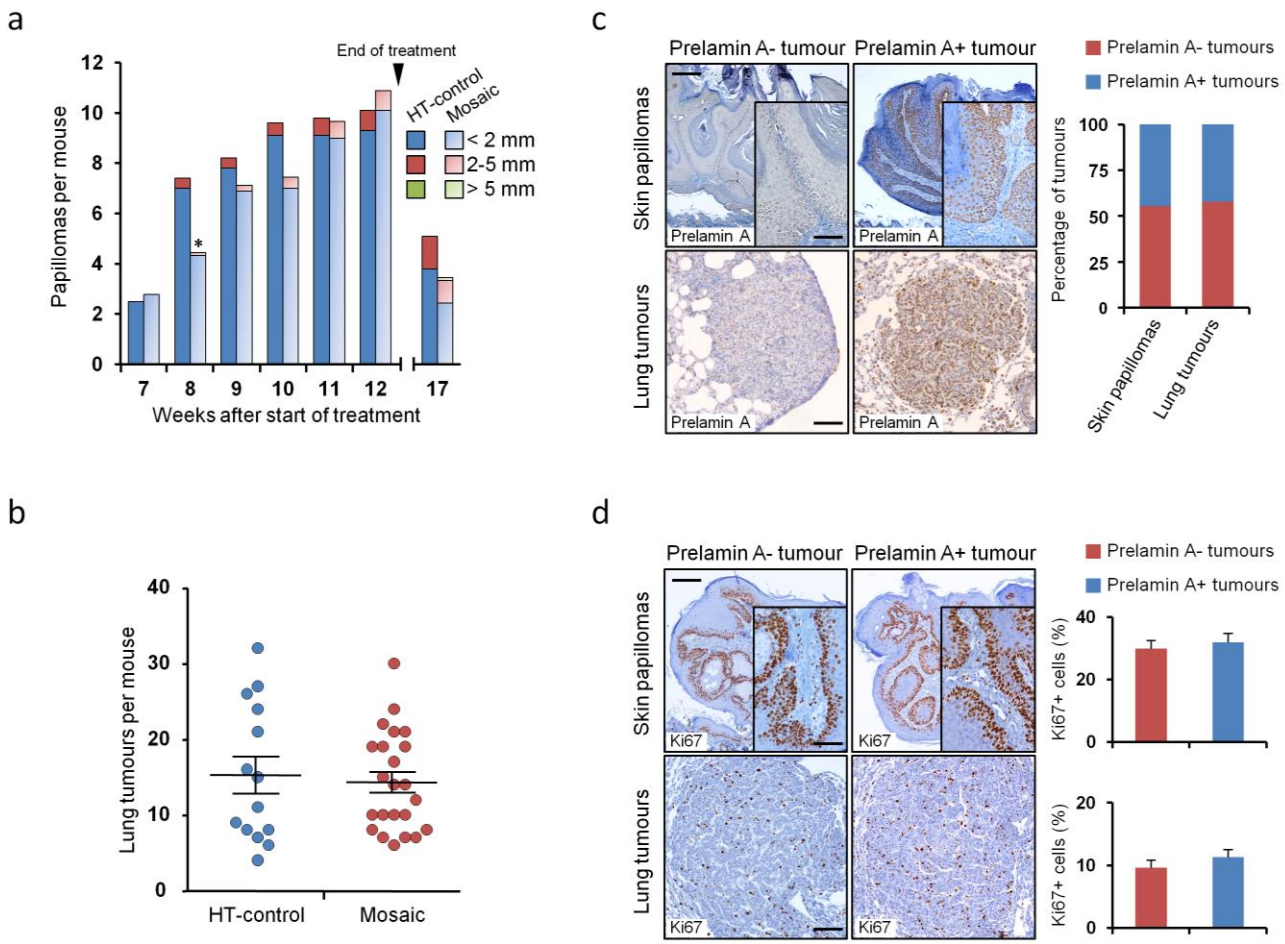
Supplementary Figure S4



Supplementary Figure S4. Evaluation of the proliferative potential of Zmpste24$^{-/-}$ cells cultured in the presence of Zmpste24$^{+/+}$ cells or ECM.

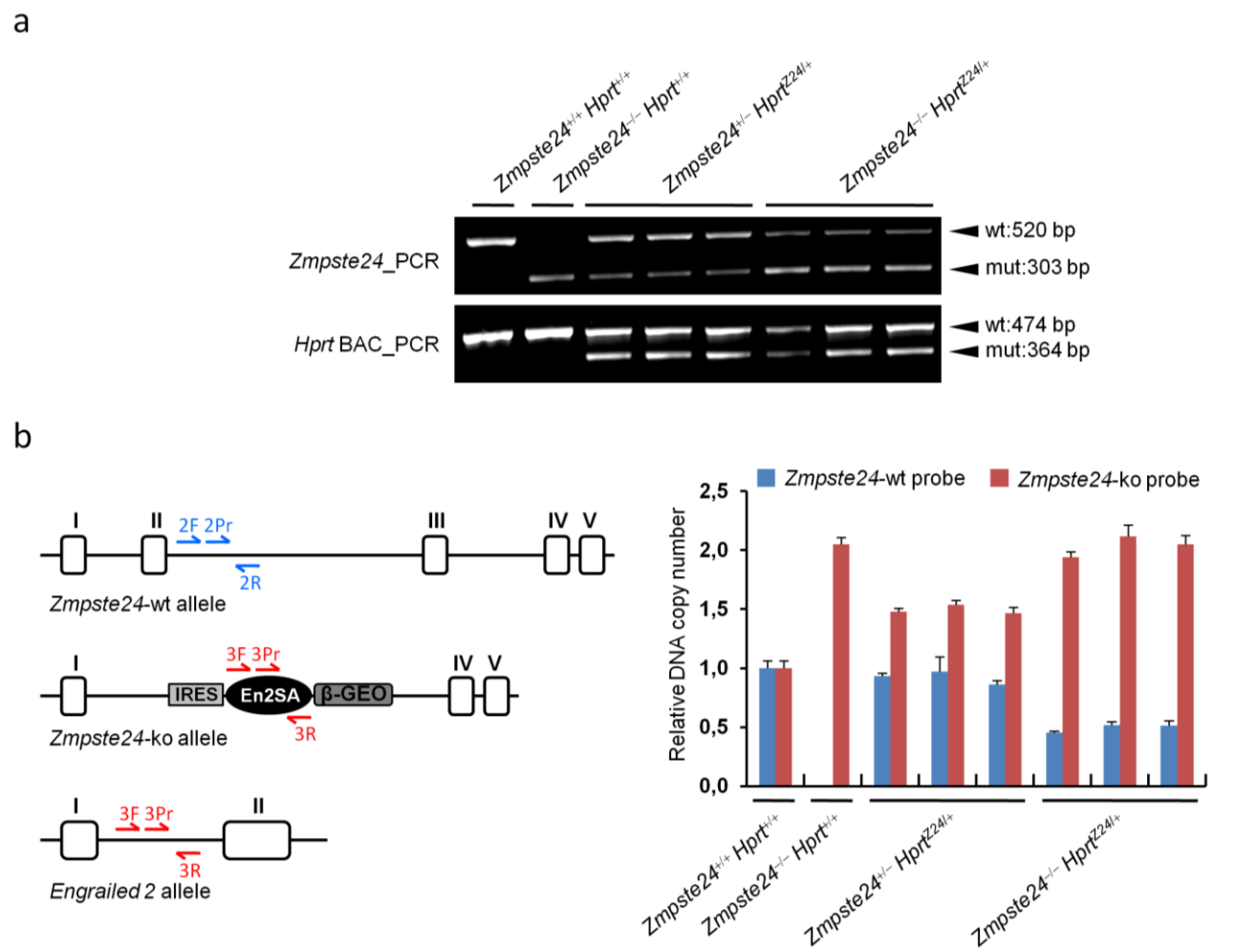
(**a**) MTT cell proliferation assay of Zmpste24$^{-/-}$ and Zmpste24$^{+/+}$ AFs cultured in separate or together. (**b**) MTT proliferation assay of Zmpste24$^{-/-}$ and Zmpste24$^{+/+}$ AFs in the presence of ECM deposited by Zmpste24$^{+/+}$ (left panel) or Zmpste24$^{-/-}$ (right panel) cells. A representative experiment done with the pair of cells showing the largest difference between their proliferative ability in the absence of ECM is shown. Data represent average values from 8 wells for each condition. Values are means ± s.e.m. normalized by the 0 h time point.

Supplementary Figure S5



Supplementary Figure S5. Analysis of the effects of prelamin A on tumour susceptibility.
(**a**) Average number of DMBA/TPA-induced papillomas in mosaic (n=10) and HT-control (n=9) mice. The mean numbers of papillomas per mouse of the indicated size ranges are plotted over time after DMBA treatment. (**b**) Number of lung tumours per mouse at time of sacrifice in HT-control (n=14) and mosaic (n=23) mice. In (b), each circle represents a single mouse, horizontal lines represent the average number of tumours per mouse in each group and error bars correspond to s.e.m. (**c**) Representative photomicrographs of positive (right column) and negative (left column) prelamin A staining in skin and lung tumours of mosaic mice. The percentage of prelamin A positive and negative tumours for both tumour induction protocols is plotted. Scale bars represent 250 µm (large panels) and 50 µm (insets). (**d**) Ki67 staining of *Zmpste24*-proficient (prelamin A-) and -deficient (prelamin A+) tumours from mosaic mice. n=16 (8 of each type) for skin papillomas and n=28 (14 of each type) for lung tumors. For the papillomas, the number of positive cells per 150 µm-basal layer are plotted. Scale bars represent 250 µm (large panels) and 50 µm (insets). Error bars represent s.e.m. (*), $p < 0.05$; two tailed Student's t test.

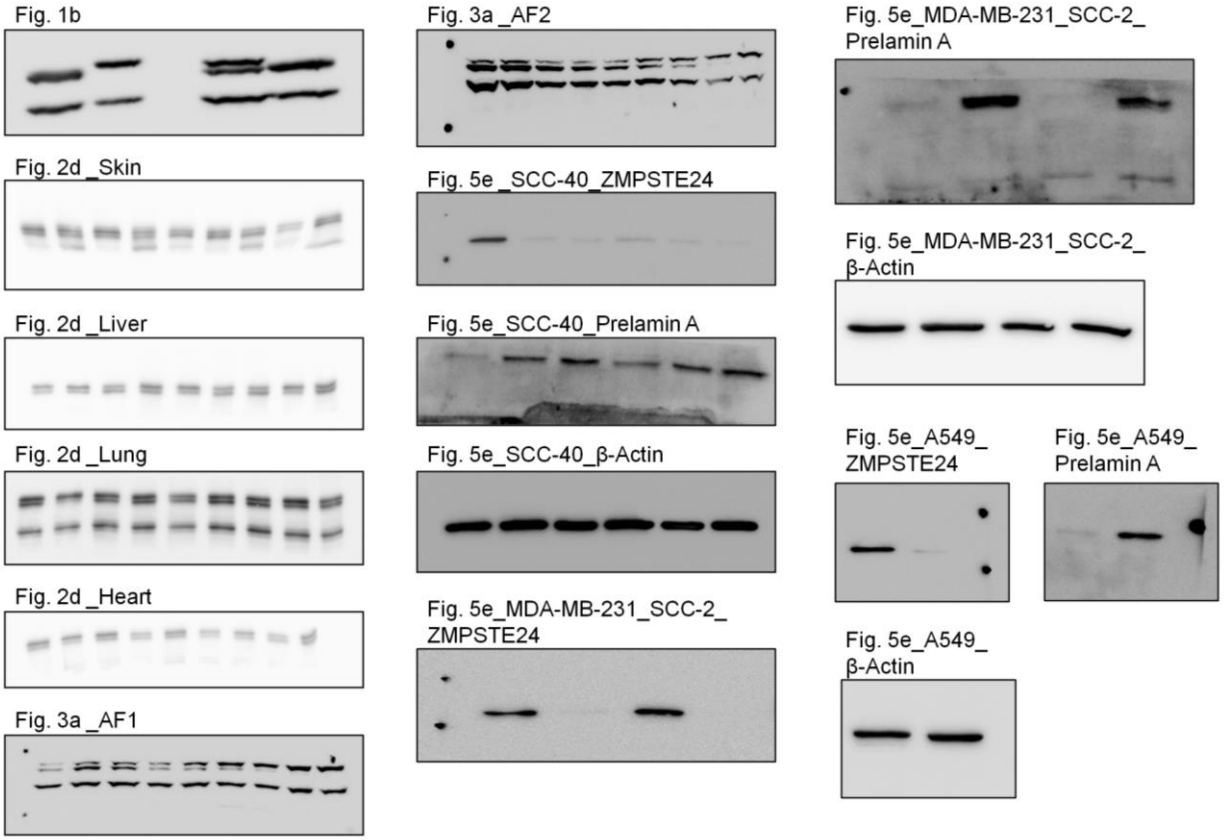
Supplementary Figure S6



Supplementary Figure S6. Genotyping of *Zmpste24*$^{+/-}$*Hprt*$^{Z24/+}$ (HT-control) and *Zmpste24*$^{-/-}$ *Hprt*$^{Z24/+}$ (mosaic) mice by PCR and qPCR.

(**a**) Genotyping PCR for the *Zmpste24* (upper panel) and the *Hprt* loci (lower panel). Same size bands are obtained from *Zmpste24*$^{+/-}$*Hprt*$^{Z24/+}$ and *Zmpste24*$^{-/-}$*Hprt*$^{Z24/+}$ genomic DNA. However, *Zmpste24*$^{+/-}$*Hprt*$^{Z24/+}$ DNA samples produce a more intense band for the *Zmpste24* wild-type allele, whereas *Zmpste24*$^{-/-}$*Hprt*$^{Z24/+}$ DNA samples produce a more intense band for the *Zmpste24*-deleted allele. (**b**) Genotyping qPCR for the *Zmpste24* locus. A pair of primers and a TaqMan probe were designed that are specific for the *Zmpste24* wild-type allele, thus determining the presence of 2 copies in *Zmpste24*$^{+/-}$*Hprt*$^{Z24/+}$ mice and 1 copy in *Zmpste24*$^{-/-}$ *Hprt*$^{Z24/+}$ mice. In addition, a different set of primers and TaqMan probe was specifically designed for intronic region of the splice acceptor sequence used in the generation of the *Zmpste24*-deleted allele [4]. This splice acceptor site comprises part of the Engrailed 2 mouse gene sequence. Therefore, this qPCR allows to distinguish between the 3 copies present in the *Zmpste24*$^{+/-}$ *Hprt*$^{Z24/+}$ mice and the 4 copies of the *Zmpste24*$^{-/-}$*Hprt*$^{Z24/+}$ mice. 2F, 2Pr, 2R, 3F, 3Pr and 3R indicate the positions of the oligonucleotides and Taqman probes used (for sequences see Supplementary Table S4). n=3. Error bars represent s.d.

Supplementary Figure S7



Supplementary Figure S7. Original blots corresponding to main figures 1, 2, 3 and 5.
Each blot is labelled with its own panel identification tag. For figure 1b, lanes 1, 2 and 4 were used. Membranes corresponding to blots in figure 2d were cut before any antibody incubation to avoid tissue immunoglobulin interference. For figure 5e, SCC40-ZMPSTE24, -Prelamin A and -β-Actin, lanes 1 and 2 were used. In Fig. 5e, MDA-MB-231 panels were built with lanes 1 and 2 of the corresponding blots, whereas SCC-2 panels were done using lanes 3 and 4.

Supplementary Tables

Supplementary Table S1. Top five gene sets most significantly enriched upon *ZMPSTE24* silencing in SCC-40 SCC-2 MDA-MB-231 and A549 cancer cells

SCC-40 Gene Set	p	SCC-2 Gene Set	p	MDA-MB-231 Gene Set	p	A549 Gene Set	p
KEGG Glycosaminoglycan Biosynthesis: Chondroitin Sulfate	0.000	KEGG Glycosaminoglycan Biosynthesis: Chondroitin Sulfate	0.000	KEGG Glycosphingolipid Biosysnthesis Ganglio Series	0.000	REACTOME Signalling to Ras	0.000
KEGG ECM-Receptor Interaction	0.000	KEGG CytrateCycle.TCA Cycle	0.000	REACTOME Muscle Contraction	0.000	REACTOME Signalling to ERKs	0.000
KEGG Integrin-Cell Surface Interactions	0.000	REACTOME Cell-ECM Interactions	0.000	BIOCARTA MEF2D Pathway	0.008	KEGG Glycosaminoglycan Biosynthesis: HeparanSulfate	0.002
KEGG Glycosaminoglycan Biosynthesis: Heparan Sulphate	0.002	KEGG ECM-Receptor Interaction	0.000	KEGG Glycosaminoglycan Biosynthesis: HeparanSulfate	0.008	KEGG Glycosaminoglycan Biosynthesis: Chondroitin Sulfate	0.004
KEGG ABC Transporters	0.002	REACTOME Zinc Transportation	0.004	KEGG Glycosaminoglycan Biosynthesis: Chondroitin Sulfate	0.012	KEGG Glycosphingolipid Biosysnthesis Ganglio Series	0.008

Dark Grey: chondroitin sulfate and heparan sulfate synthesis pathways. Light Grey: ECM-cell surface interaction pathways. p-values were calculated as in Subramanian *et al.* [51].

Supplementary Table S2. Expression changes in genes from the KEGG ECM Receptor Interaction Pathway significantly altered upon ZMPSTE24 silencing in SCC-40 and SCC-2 cells and their corresponding values in ZMPSTE24-silenced MDA-MB-231 and A549 cells

KEGG ECM RECEPTOR INTERACTION								
	SCC-40		SCC-2		MDA-MB-231		A549	
Gene Symbol	FC	p	FC	p	FC	p	FC	p
CD47	1,267	0,001	1,497	0,000	1,313	0,000	1,312	0,000
COL1A1	1,114	0,044	2,064	0,000	-1,027	0,600	1,090	0,101
COL3A1	1,142	0,023	1,725	0,000	1,070	0,230	-1,053	0,359
COL5A1	1,222	0,004	1,148	0,037	1,049	0,449	1,976	0,000
COL5A2	1,755	0,000	2,242	0,000	1,071	0,266	1,183	0,010
DAG1	1,633	0,000	1,692	0,000	1,318	0,000	1,175	0,011
ITGA2	1,713	0,000	-1,387	0,000	-1,385	0,000	1,143	0,025
ITGA5	1,368	0,002	1,398	0,001	1,254	0,017	1,430	0,000
LAMB2	1,196	0,003	1,194	0,003	1,079	0,176	1,021	0,700
LAMC1	1,223	0,002	1,276	0,000	-1,007	0,908	1,079	0,200
THBS1	1,221	0,001	1,334	0,000	1,361	0,000	-1,196	0,002
THBS2	1,413	0,000	1,159	0,028	-1,044	0,504	-1,061	0,358

Red figures: significant upregulation. Blue figures: significant downregulation. Black figures: no significant changes. p-values were calculated as in Subramanian *et al.*[51].

Supplementary Table S3. Expression changes in genes from the KEGG Chondroitin Sulfate and KEGG Heparan Sulfate biosynthesis pathways significantly altered upon ZMPSTE24 silencing in SCC-40 SCC-2 MDA-MB-231 and A549 cell lines

KEGG GLYCOSAMINOGLYCAN BIOSYNTHESIS. CHONDROITIN SULFATE								
	SCC-40		SCC-2		MDA-MB-231		A549	
Gene Symbol	FC	p	FC	p	FC	p	FC	p
CHPF	1,670	0,000	1,818	0,000	1,380	0,000	1,169	0,010
CHST12	1,342	0,000	1,242	0,005	1,189	0,021	1,311	0,001
CHST14	1,520	0,000	1,449	0,000	1,197	0,004	1,271	0,000
CHST15	1,228	0,003	1,586	0,000	1,709	0,000	1,900	0,000
CHSY1	-1,423	0,000	-1,431	0,000	-1,385	0,000	-1,219	0,002
CSGALNACT2	1,609	0,000	1,289	0,000	1,428	0,000	1,244	0,002
XYLT2	1,220	0,002	1,417	0,000	1,306	0,000	1,308	0,000
KEGG GLYCOSAMINOGLYCAN BIOSYNTHESIS. HEPARAN SULFATE								
	SCC-40		SCC-2		MDA-MB-231		A549	
Gene Symbol	FC	p	FC	p	FC	p	FC	p
HS2ST1	1,311	0,000	1,295	0,000	1,540	0,000	1,253	0,001
HS3ST1	3,286	0,000	1,150	0,028	1,225	0,002	1,131	0,050
NDST1	1,242	0,002	1,225	0,003	1,165	0,021	1,265	0,001
XYLT2	1,220	0,002	1,417	0,000	1,306	0,000	1,308	0,000

Red figures: significant upregulation. Blue figures: significant downregulation. p-values were calculated as in Subramanian *et al.* [51].

Supplementary Table S4. Primers used in this study

Primer	Product size	Sequence
Genotyping PCR for the *Hprt* locus		
Hprt-F	wt:474 bp; mut: 364 bp	5´-GACAAGTAAAAATCACTGGTCAAGG-3´
Hprt-R		5´-CTGGACTGTAATCATAATCCTTGTCTCTAC-3´
BAC-R		5´-GTTAGCAATTTAACTGTGATAAACTACCG-3´
Genotyping PCR for the *Zmpste24* locus		
F1-01	wt: 520 bp; mut: 303 bp	5´-GCTGGCCTTGTTGCTGGAAT-3´
F1/F2-02		5´-CTTCCGGAGCGGATCTCAAA-3´
F1-03		5´-GCTTCCTCCCTGAGCCAACC-3´
Genotyping qPCR for the *Zmpste24* locus		
2F	-	5´-AGGTTGGCTCAGGGAGGAA-3´
2Pr		5´-FAM-CATCAATGGTGTATGCGC-TAMRA -3´
2R		5´-ATCAGGTCCATCTGCAATGCT-3´
3F	-	5´-TGACCGTGGGTACACTTAAAACA-3´
3Pr		5´-FAM-ATCTGCACTGTCCCGGATGTCCTCTG-TAMRA-3´
3R		5´-GGCAAACCCAAAAGGGTCTT-3´
β-actin-F	-	5´-TTCAACACCCCAGCCATGTA -3´
β-actin-Pr		5´-FAM-TAGCCATCCAGGCTGTGCTGTCCC -TAMRA -3´
β-actin-R		5´-TGTGGTACGACCAGAGGCATAC-3´
Pyrosequencing analysis of the *Zmpste24* promoter		
1F	212 bp	5´-GGGGTTTATTGTTTTTTTTGGTAATT-3’
1R		5’-ACTCTTCAAAAACCTACTTATTTCTACTA-3’ (biotinylated)
S1	-	5´-AATTGGATTTTTGTATATAGAATT-3’ (sequencing primer)

Supplementary Methods

Bac selection.

BAC clone RP23-227I2, with a 186,658 bp insert corresponding to coordinates chr4:120691503-120878160 of the mouse genome (NCB1m37 version) was selected using Ensembl. This BAC contains the whole *Zmpste24* locus (chr4:120731842-120770846), besides 107,296 bp and 40,339 bp of 5' and 3' genomic flanking regions, respectively. The 3' flanking sequence contains the whole *Col9a2* locus, whereas the 5' flanking region contains the complete *Tmco2* locus and the last 7 exons of the *Rlf* locus. This BAC is based on the pBAC3.6 vector, in which a loxP and a lox511 site flank the *sacBII* gene and the multiple cloning site used to introduce the genomic DNA insert.

Cell culture.

ES cell culture, electroporation, and the generation of chimeric and germ line transgenic mice were described previously [52]. *Hprt*$^{tm(rmce1)Brd}$ pluripotent ES cells were used as recipients of the BAC insert, following the RMCE protocol previously described [20]. The double selection applied with G418 (resistance provided by the Neo cassette) and 6-thioguanine (resistance obtained by interruption of the *Hprt* locus), together with the efficiency of the Cre/Lox mediated RMCE process, produces a high yield of correct recombinant events. Accordingly, virtually all resistant ES cell clones contain the insert properly introduced into the *Hprt* locus (Supplementary Fig. S1a). The establishment and culture of mouse fibroblasts was done as previously described [11]. The two established human HNSCC cell lines used in this study (SCC-40 and SCC-2) were kindly provided by Dr. R. Grénman (Turku University, Finland). Cells other than ES were grown in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% fetal bovine serum (Gibco), 100 units/ml penicillin, 200 µg/ml streptomycin. In the case of SCC-40 and SCC-2 cells, this medium was also supplemented with 2 mM L-glutamine, 20 mM Hepes pH 7.3 and 100 µM non-essential amino acids.

SDS-PAGE and Western blot.

Cultured cells and tissues were homogenized in SDS lysis buffer containing 100 mM Tris-HCl pH 7.4, 2% SDS, 50 mM EDTA pH 8 and "Complete" protease inhibitor cocktail (Roche Applied Science). Protein concentration was evaluated with the bicinchoninic acid assay (Pierce BCA Protein Assay Kit). Equal amounts of proteins were loaded onto 8% SDS-polyacrylamide gels. After electrophoresis, gels were electrotransferred onto nitrocellulose membranes or Immobilon-FL polyvinylidene fluoride membranes (Millipore), blocked with 5% nonfat dry milk in TBS-T buffer (20 mM Tris pH 7.4, 150 mM NaCl, and 0.05% Tween 20) for 1 hour at room temperature

and incubated overnight at 4°C with various primary antibodies: monoclonal anti–lamin A/C (Manlac-1, provided by Dr. G. Morris, 1:500), goat polyclonal anti-prelamin A (sc-6214, Santa Cruz, 1:500), monoclonal anti-ZMPSTE24 (Daiichi Chemical), anti–α-tubulin (T6074, Sigma, 1:10,000) or anti–β-actin (AC-40, Sigma, 1:10,000). Finally, we incubated the blots with 1:10,000 of goat anti-mouse, rabbit anti-goat or donkey anti-rabbit horseradish peroxidase-conjugated secondary antibodies (Jackson Immuno Research) in 1.5% nonfat dry milk in TBS-T, washed them and developed the immunoreactive bands with Immobilon Western chemiluminescent HRP substrate (Millipore). For analysis of MUPs, 1 µl of urine was loaded in each lane of a 10% SDS-polyacrilamide gel. After electrophoresis, the proteins in the gel were stained with Coomasie blue. Original images of immunoblots are shown in Supplementary Figure S7.

Immunohistochemistry.
Formalin-fixed paraffin-embedded tissue sections were cut at 5 µM for immunohistochemical detection of DsRed, prelamin A and Ki67 on a Discover automated immunostainer (Ventana Medical Systems). Deparaffinization and heat-induced antigen retrieval were performed directly on the stainer. Antigen retrieval procedures were as follows: DsRed: no retrieval required; prelamin A: retrieval performed with CC1 solution (Ventana) for 90 min at 95 ^{o}C; Ki67: retrieval performed with CC2 solution (Ventana) for 30 min at 95 ^{o}C. Primary antibody incubation was perfomed for 1 h at 37 ^{o}C, using the following antibodies: rabbit polyclonal anti-DsRed (632496, Clontech, 1:250), goat polyclonal anti-prelamin A (sc-6214, Santa Cruz, 1:250) and rabbit polyclonal anti-Ki67 (ab833, Abcam, 1:100), respectively. For prelamin A signal amplification, sections were incubated with a rabbit anti-goat IgG (NB7373, Novus Biologicals, 1:500) for 32 min at 37 ^{o}C. Finally, in all cases, HRP-conjugated antibody (OmniMap anti-Rb HRP, Ventana) was applied for 16 min at 37 ^{o}C. Staining was visualized by using Chromo Map DAB kit (Ventana). Cells were counterstained with hematoxylin and visualized by light microscopy.

Tissue preparation and microdissection.
Tissue blocks were embedded in Tissue Tek OCT compound medium and stored at -80 ^{o}C. Frozen tissue sections (5 µm) were mounted on glass PEN membrane slides (Leica), air dried for 15 min and fixed in 75% ethanol for 3 min. After fixation, the slides were dehydrated in 70% ethanol for 1 min, 95% ethanol for 1 min and 100% ethanol for 1 min. Once air dried for at least 30 min, sections were visualized under fluorescence microscopy and DsRed fluorescent and non-fluorescent cells were separately microdissected using a Laser Microdissection System (Leica LMD6000). Approximately 5 x 10^6 µm^2 of tissue were obtained from each sample for subsequent gene expression analysis by qRT-PCR.

RNA preparation.
Total RNA was extracted using the TRIzol reagent (Invitrogen) according to the manufacturer, with the only modification that an additional chlorophorm extraction was performed (2 extractions in total). The upper phase from this extraction was further processed through alcohol precipitation. RNA pellets were then washed in cold 75% ethanol, resuspended in nuclease-free water (Ambion), and the samples were quantified and evaluated for purity (260 nm/280 nm ratio) using a NanoDrop ND-1000 spectrophotometer.

Quantitative RT-PCR analysis.
cDNA was synthesized with Thermoscript RT-PCR (Invitrogen), using either 1-4 µg of total RNA from whole organs or 0.2-0.3 µg of total RNA from microdissected tissue sections. Quantitative RT-PCR (qRT-PCR) was carried out in triplicate for each sample using 20 ng of cDNA per reaction, TaqMan Universal PCR Master Mix (Applied Biosystems), and 1 µL of the specific TaqMan custom gene expression assay for the gene of interest (Applied Biosystems). As an internal control for the amount of template cDNA used, gene expression was normalized to the mouse PGK1 endogenous control. Relevant primers are listed in Supplementary Table S4.

Proliferation assay.
To quantify cell proliferation, a Cell Titer 96 Non Radioactive cell proliferation kit was used following the manufacturer's instructions (Promega Corp.). Briefly, Zmpste24$^{+/+}$ and Zmpste24$^{-/-}$ AFs were seeded into 96-well plates in the presence or absence of ECM deposited by either wt or Zmpste24-deficient AFs (see Beacham et al., 2007, for a description of dish coating with cell-

derived ECM[53]), at a density of 2,000 cells per well (100 µl). For co-culture experiments, 1,000 cells of each type were seeded into the same non-precoated wells. Cells were incubated at 37 ^{o}C, 5% CO_2 for 5 days. Proliferation was monitored by measuring the conversion of a tetrazolium salt into formazan in metabolically active cells. At the designated time periods (0 h, 24 h, 48 h, 72 h, 96 h and 120 h), 15 µl of Dye solution was added into each well (n=8) and cells were incubated at 37 ^{o}C for 2 h. Then, 100 µl of solubilization/stop mixture was added into each well. After 1 h of incubation at 37 ^{o}C the absorbance was measured at 570 nm with a Power Wave XS Microplate reader (Biotek).

Statistical analysis.

We used the Fisher's exact test for comparing tumour incidence between groups. The remaining analyses were perfomed using a two-tailed Student's t test, except for p-values in Supplementary Tables S1-S3, which were calculated as in Subramanian *et al.* [51]. We used Microsoft Excel or GraphPad Prism software for calculations. All experimental data are reported as mean and the error bars represent the standard error of the mean (s.e.m.), except for the results of the qPCRs used for genotyping, where the error bars represent standard deviation (s.d.).

Supplementary References

52. Ramírez-Solís, R., Davis, A.C. & Bradley, A. Gene targeting in embryonic stem cells. *Methods Enzymol*. **225**, 855-878 (1993).
53. Beacham, D.A., Amatangelo, M.D. & Cukierman, E. Preparaton of extracellular matrices produced by cultured and primary fibroblasts. *Curr. Prot. Cell. Biol.* **33**, 10.9.1-10.9.21 (2007).