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# **STEAP1 is overexpressed in cancers: a promising therapeutic target**

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## Abstract

The six-transmembrane epithelial antigen of prostate (STEAP) protein was identified in advanced prostate cancer and is up-regulated in multiple cancer cell lines, including prostate, bladder, colon, ovarian, and Ewing sarcoma. STEAP1 was described as a suitable antigen for T-cell-based or antibody-based immunotherapy.

We have investigated the expression of *STEAP1* in 40 human tumor types – brain, epithelial, lymphoid – and in their normal tissue counterparts using publicly available gene expression data, including the Oncomine Cancer Microarray database. *STEAP1* was found significantly overexpressed in 11 cancers. In addition, high *STEAP1* expression was associated with poor overall survival in colorectal cancer, diffuse large B cell lymphoma, acute myeloid leukemia and multiple myeloma.

Taken together, these data suggest that *STEAP1* is a potential therapeutic target for T-cell based immunotherapy or antibody therapy in a large panel of cancers.

## Introduction

The six-transmembrane epithelial antigen of prostate (STEAP) protein was identified in advanced prostate cancer[1]. *STEAP1* is highly expressed in human prostate cancer and is up-regulated in various cancers, including lung, bladder, colon, ovarian, and Ewing cancers [1,2,3]. Immunohistochemical analysis of clinical specimens demonstrates significant STEAP1 expression at the cell–cell junctions of the secretory epithelium of prostate and prostate cancer cells[1]. Little to no staining was detected at the plasma membranes of normal non-prostate human tissues, except for bladder tissue, which expressed low levels of STEAP1 at the cell membrane. Its cell-surface localization, together with its six-transmembrane topology, suggests STEAP1 may function as a channel/transporter protein in cell-cell junctions[1]. Given its increased expression in cancer tissues, STEAP1 could be a promising target for T-cell based or antibody immunotherapy. In prostate cancers, STEAP1-specific cytotoxic T lymphocytes (CTLs) were found to inhibit the growth of transplantable prostate tumor cells in murine models[2,4,5]. An immunization with recombinant DNA or modified vaccinia virus Ankara vector delivering STEAP1 antigen inhibited prostate cancer progression in a murine subcutaneous syngeneic tumor model[6]. Targeting STEAP1 appeared also useful for T-cell based immunotherapy in renal and bladder cancers[7]. Finally, STEAP1 was also described as an attractive target for antibody therapy in multiple solid tumors including prostate, renal and bladder cancers[3].

In this report, we have investigated the expression of *STEAP1* gene in 40 human tumor types – brain, epithelial, lymphoid – as well as in their normal tissue counterparts using publicly available gene expression data, including the Oncomine Cancer Microarray

database.

## Materials and Methods

### Databases and gene expression data

*STEAP1* gene expression in normal or malignant human tissues or cell lines was obtained from the Oncomine Cancer Microarray database (<http://www.oncomine.org>)[8], Amazonia database ([http:// amazonia.montp.inserm.fr/](http://amazonia.montp.inserm.fr/))[9], or ITTACA database (Integrated Tumor Transcriptome Array and Clinical data Analysis, <http://bioinfo-out.curie.fr/ittaca/>)[10]. Comparison of *STEAP1* expression between samples was performed only when gene expression data were from a same study, using the same methodology. Gene expression profiling (GEP) data are either two channel ratio data (from cDNA microarrays) or single channel intensity data (from Affymetrix microarrays). All data were log transformed, median centered per array, and the standard deviation was normalized to one per array[11].

MMCs were purified from 206 patients with newly-diagnosed MM after written informed consent was given at the University hospitals of Heidelberg (Germany) or Montpellier (France). The study was approved by the ethics boards of Heidelberg and Montpellier Universities. After Ficoll-density gradient centrifugation, plasma cells were purified using anti-CD138 MACS microbeads (Miltenyi Biotech, Bergisch Gladbach, Germany). Microarray experiments were performed in DNA microarray platform of the Institute of Research in Biotherapy at the Montpellier University Hospital (France) (<http://irb.montp.inserm.fr/en/index.php?page=Plateau&IdEquipe=6>). The .CEL files and MAS5 files have been deposited in the ArrayExpress public database (E-MTAB-372). We also used Affymetrix data of a cohort of 345 purified MMC from previously untreated MM patients from the University of Arkansas for Medical Sciences (UAMS, Little Rock,

AR). These data are publicly available via the online Gene Expression Omnibus (Gene Expression Profile of Multiple Myeloma, accession number GSE2658. <http://www.ncbi.nlm.nih.gov/geo/>). We also used Affymetrix data of a cohort of patients with colorectal carcinoma (N=177) (accession number GSE17538. <http://www.ncbi.nlm.nih.gov/geo/>) with diffuse large B cell lymphoma (N= 414) (accession number GSE10846. <http://www.ncbi.nlm.nih.gov/geo/>) and a cohort of patients with acute myeloid leukaemia (N=79) (accession number GSE12417. <http://www.ncbi.nlm.nih.gov/geo/>). Affymetrix gene expression profiles of 917 cancer cell lines was also investigated for *STEAP1* expression (accession number GSE36133). Patient cohorts were described in supplementary appendix 1.

### Statistical analysis

Statistical comparisons were done with student t-test. Gene expression data of MM patients were normalized with the MAS5 algorithm and analyzed with our bioinformatics platforms: RAGE (<http://rage.montp.inserm.fr/>)[12] and Amazonia (<http://amazonia.montp.inserm.fr/>)[9]. The prognostic value of *STEAP1* gene expression signal was determined using the MaxStat R function in R software (<http://www.r-project.org>). The statistical significance of differences in overall survival between groups of patients was calculated by the log-rank test. Multivariate analysis was performed using the Cox proportional hazards model. Survival curves were plotted using the Kaplan-Meier method. All these analyses have been done with R.2.10.1 (<http://www.r-project.org/>) and bioconductor version 2.5.

### Western blot analysis

Cells were lysed in 10 mM Tris-HCl (pH 7.05), 50 mM NaCl, 50 mM NaF, 30 mM sodium pyrophosphate (NaPPi), 1% triton X-100, 5  $\mu$ M ZnCl<sub>2</sub>, 100  $\mu$ M Na<sub>3</sub>VO<sub>4</sub>, 1 mM DTT, 20 mM  $\beta$ -glycerophosphate, 20 mM p-nitrophenolphosphate (PNPP), 20  $\mu$ g/ml aprotinin, 2.5  $\mu$ g/ml leupeptin, 0.5 mM PMSF, 0.5 mM benzamidine, 5  $\mu$ g/ml pepstatin, and 50 nM okadaic acid. Lysates were resolved on 12% sodium dodecyl sulfate-polyacrylamide by gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane (Schleicher and Schuell, Kassel, Germany). Membranes were blocked for 2 hours at room temperature in 140 mM NaCl, 3 mM KCl, 25 mM Tris-HCl (pH 7.4), 0.1% Tween 20 (TBS-T), 5% non-fat milk and human Ig (1 mg/ml), and then immunoblotted with a mouse anti-STEAP1 mAb (Sigma, St Louis, MO, clone 4F6-1F3). As a control for protein loading, we used a mouse monoclonal anti- $\beta$ -actin antibody (Sigma, St Louis, MO). The primary antibodies were visualized with goat anti-mouse (Bio-Rad, Hercules, CA) peroxidase-conjugated antibody by an enhanced chemiluminescence detection system. Blots were quantified by densitometry using acquisition into Adobe Photo Shop (Adobe Systems, San Jose, CA), and analyzing with the NIH Image J software (National Institutes of Health, Bethesda, MD, USA).

#### Immunofluorescence staining

A total of  $5 \times 10^4$  cells were deposited onto poly-L-lysine-coated slides, fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton. Non-specific staining was blocked by incubating the slides in 5% BSA-PBS, after which the slides were subjected to labeling with the anti-STEAP1 mAb for 1h in a moist chamber. Slides were then washed with PBS and incubated for 1h with an Alexafluor 488-conjugated rabbit anti-mouse antibody (Molecular probes, OR, USA) in a dark moist chamber. Slides were



washed and mounted with Vectashield containing 4'-6-Diamidino-2-phenylindole (DAPI) (Sigma Aldrich, St Louis, MO, USA), and examined under an epifluorescence microscope (Leica Microsystems, Wetzlar, Germany).

## Results and discussion

*STEAP1* expression was investigated in 40 tumor types including 36 solid tumors and 4 hematological malignancies and in the corresponding normal tissues (Table 1 and Figure 1). Overexpression of *STEAP1* was found in glioblastoma compared to normal brain ( $P = 4.5E-5$ )[13]; in esophageal cancer compared to normal esophagus in two independent studies ( $P = 4.5E-5$ ,  $7.4E-9$ )[14,15]; in pancreatic carcinoma compared to normal pancreas in three independent studies ( $P = 1.6E-13$ ,  $6.1E-5$ ,  $.007$ )[16,17,18]; in prostate carcinoma compared to normal prostate in three independent studies ( $P = 1.8E-6$ ,  $2.4E-4$ ,  $9.6E-4$ )[19,20,21]; in head and neck cancer compared to normal counterpart in three independent studies ( $P = 2.3E-18$ ,  $1.1E-5$ ,  $.001$ )[22,23,24]; in lung carcinoma compared to normal lung in six independent studies ( $P = 1.5E-4$ ,  $P = 2.3E-4$ ,  $P = 3.9E-4$ ,  $P = 5.1E-16$ ,  $P = 8.8E-16$ ,  $P = 4.5E-5$ ,  $P = 7.8E-5$  and  $P = .005$ )[25,26,27,28,29,30]. Overexpression of *STEAP1* was also found in primary effusion lymphoma compared to normal B cells ( $P = 3.1E-5$ )[31] and in various leukemia compared to normal bone marrow: T-cell acute lymphoblastic leukemia ( $P = 5.6E-9$ )[32], acute myeloid leukemia ( $P = 5.6E-9$ )[32]; B-cell acute lymphoblastic leukemia ( $P = 8.3E-12$ )[32]; and T-cell prolymphocytic leukemia ( $P = 5.1E-7$ )[33].

Given that *STEAP1* was shown to be expressed in prostate cancer metastases[3] and to be associated with invasive behaviour of Ewing tumors[34], an association between *STEAP1* expression and tumor progression and patients' survival was investigated. A high *STEAP1* expression was significantly associated with a reduced overall survival in patients with colorectal cancer ( $n=177$ ;  $P = .02$ ), with diffuse large B cell lymphoma ( $n=414$ ;  $P = .008$ ), with acute myeloid leukemia ( $n=79$ ;  $P = .0005$ ), or with multiple myeloma (MM) ( $n=206$   $P = .01$  and  $n=345$ ;  $P = .02$ ) (Figure 2). Patients were split into

two groups using Maxstat R function[35] according to *STEAP1* expression. In patients with colorectal cancer, a maximum difference in overall survival (OS) was obtained splitting patients in a high-risk group of 26% patients (*STEAP1* signal > 1155 (unlogged GEP data)) with a 50 months median OS and a low risk group of 74% patients (*STEAP1* signal  $\leq$  -1155) with 133 months median OS (Figure 2). In patients with DLBCL, a maximum difference in overall survival (OS) was obtained splitting patients in a high-risk group of 67.6% (*STEAP1* signal > 164 (unlogged GEP data)) with a 73 months median OS and a low risk group of 32.4% patients (*STEAP1* signal  $\leq$  164) with not reached median OS (Figure 2). In patients with cytogenetically normal AML, a maximum difference in overall survival (OS) was obtained splitting patients in a high-risk group of 10.1% (*STEAP1* signal > 6.07 (log transformed GEP data)) with a 4 months median OS and a low risk group of 89.9% patients (*STEAP1* signal  $\leq$  6.07) with 20 months median OS (Figure 2). In patients with MM, a maximum difference in overall survival (OS) was obtained splitting patients in a high-risk group of 31% with a 74 and 67 months median OS respectively in two independent cohorts of patients and a low risk group of 69% patients with not reached median OS (Figure 2). Furthermore, *STEAP1* is significantly overexpressed in malignant plasma cells compared to normal bone marrow plasma cells and in tumor cells of patients with AML compared to normal hematopoietic stem cells (Supplementary Figure 1). In MM, seven molecular groups of tumor cells have been identified in previously-untreated patients [36], and *STEAP1* was significantly overexpressed in the proliferation group and in the groups associated with Cyclin D1 or MMSET translocations (Figure 3), two of them (proliferation and MMSET) being associated with poor survival in MM[36]. Using Cox univariate analysis in colorectal cancer patient cohort, AJCC staging, colorectal cancer grading and *STEAP1* expression

had prognostic value. Comparing these prognostic factors two by two, *STEAP1* expression remained significant compared to AJCC staging, and colorectal cancer grading (Supplementary Table S1). When these parameters were tested together, *STEAP1* expression and AJCC staging kept prognostic value. In MM patient cohorts, UAMS-HRS, IFM-score and GPI (Growth proliferation index) had prognostic value as well as t(4;14), del17p,  $\beta$ 2m and ISS (Supplementary Table S2). Comparing these prognostic factors two by two, *STEAP1* expression remained significant compared to  $\beta$ 2m, ISS, IFM-score and GPI (Supplementary Table S2). When these parameters were tested together, only  $\beta$ 2m and t(4;14) kept prognostic value in the two cohorts. In cytogenetically normal AML patient cohort, *STEAP1*, *BAALC*, *ERG*, *MN1* and *EVI1* expression had prognostic value (Supplementary Table S3). Comparing these prognostic factors two by two, *STEAP1* expression remained significant compared to *BAALC*, *ERG*, and *EVI1* expression (Supplementary Table S3). When these parameters were tested together, only *STEAP1* and *BAALC* kept prognostic value. *STEAP1* gene expression was also confirmed at protein level in 5 human myeloma cell lines (HMCLs) and purified primary myeloma cells of 5 patients using western blot (Figure 4A&B). Normalized *STEAP1* protein expression was significantly correlated with *STEAP1* Affymetrix expression ( $r = .96$ ,  $P = .01$ ) (Figure 4A). *STEAP1* gene expression was also validated in HMCLs at protein level by immunofluorescence (Figure 4C). *STEAP1* was first identified in advanced prostate cancer. Comparing *STEAP1* expression in all of these cancer cell lines, we identified a significant overexpression of *STEAP1* in acute lymphoblastic T cell leukemia, diffuse large B cell lymphoma, Hodgkin lymphoma, multiple myeloma, oesophagus carcinoma and lung carcinoma cell lines compared to

prostate cancer cell lines (Supplementary Figure S2). No significant difference of *STEAP1* expression was noted between acute myeloid leukemia, acute lymphoblastic B cell leukemia, Burkitt lymphoma, glioma, pancreas carcinoma and pancreatic ductal carcinoma cell lines compared to prostate cancer cell lines (Supplementary Figure S2). Investigating genes correlated with *STEAP1* expression among various tumor samples, we identified 1313 genes, 1041 genes and 638 genes significantly correlated with *STEAP1* in colon cancer, DLBCL and MM respectively (Supplementary Tables S4, S5 and S6). Crossing the gene lists, 4 genes appeared correlated with *STEAP1* expression: dermatan sulfate epimerase (DSE), ubiquitin-conjugating enzyme E2T (UBE2T), atlastin GTPase 3 (ATL3) and CSL-type zinc finger-containing protein 2 (DPH3) (Supplementary Table S7). These genes are not linked with a specific biological pathway (Ingenuity and Reactome analyses).

Taken together, these data demonstrate that *STEAP1* could be an interesting target not only in solid tumors but also in haematological malignancies. Recent study described a function of *STEAP1* in mediating the transfer of small molecules between adjacent cells in culture, indicating its potential role in tumor cell intercellular communication [3]. Antibodies directed to *STEAP1* inhibited *STEAP1* induced intercellular communication of prostate cancer cells in a dose-dependent manner. Furthermore, anti *STEAP1* antibodies significantly inhibited tumor growth of patient derived prostate and bladder cancer cells in a mouse model[3]. *STEAP1* peptides have been recently demonstrated to induce antigen-specific CTLs that were able to recognize and destroy *STEAP1*-expressing tumor cells in vitro[2,5]. Active immunization against *STEAP1* using DNA prime/modified vaccinia virus Ankara boost strategy inhibits

prostate cancer progression in a murine model[6]. The immunogenicity of STEAP1 has led to the development of anti-STEAP1 antibody-drug conjugates to combine the specificity of anti-STEAP1 antibody to the cytotoxic potency of chemotherapeutic drugs[37].

Using a dye transfer assay, it was shown that STEAP1 expression in connexin deficient prostate cancer cells induces significant dye transport between cells, which is inhibited by specific STEAP1 siRNA. The predicted secondary structure of STEAP1 as a channel protein supports the possibility that STEAP1 acts as a transporter protein to directly transfer the dye between the cells[3]. More recently, Grunewald et al. demonstrated that STEAP1 is important for anchorage-independent colony-formation and invasiveness of Ewing tumor cells in vitro and for tumorigenicity and metastasis in vivo[34]. In Ewing tumors, STEAP1 expression correlates with increased cellular reactive oxygen species (ROS)-levels, which in turn induce the expression of redox-sensitive and pro-invasive genes. These results sustain previous data that STEAP1 overexpression promotes ROS-mediated hyperproliferation of thyroid epithelial cells[38]. Elevated ROS-levels activate pro-metastatic and pro-proliferative signaling in cancer cells[39,40]. STEAP1 overexpression could promote proliferation, invasiveness, tumorigenicity, and metastasis through ROS-levels upregulation in cancer. A recent study reported the interest of antibody-drug conjugates as promising therapeutic approach combining the STEAP1 antigen targeting specificity of monoclonal antibodies with the cytotoxic potency of chemotherapeutic drugs[37].

## **Conclusion**

The current analysis emphasizes that STEAP1 could be a good candidate for T-cell based or antibody-based immunotherapy in a large panel of solid and hematological cancers.

## **Competing interests**

The author(s) declare that they have no competing interests.

## **Authors' contributions**

JM designed the study, supported data analysis and wrote the paper.

AK was involved in the study design and supported data analysis.

DH participated in the design of the study.

BK was involved in the study design and wrote the paper.

All authors read and approved the final manuscript.

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## Figure legends

**Figure 1:** Increased *STEAP1* gene expression in cancer tissue compared to normal counterparts.

*STEAP1* gene expression in glioblastoma, esophageal adenocarcinoma, pancreatic carcinoma, prostate cancer, head and neck cancer, lung adenocarcinoma, primary effusion lymphoma, T-cell acute lymphoblastic leukemia, acute myeloid leukemia, B-cell acute lymphoblastic leukemia, T-cell prolymphocytic leukemia and the corresponding normal tissues Data sets in a given panel were from the same study. GEP data are log transformed and normalized as previously described[8].

**Figure 2:** Overall survival related to *STEAP1* gene expression in cancers

The prognostic value of *STEAP1* gene expression was determined using the MaxStat R function in R software. The overall survival of subgroups of patients was compared with the log-rank test and survival curves computed with the Kaplan-Meier method (R software).

**Figure 3:** *STEAP1* expression in the 7 molecular groups of malignant plasma cells documented in patients with previously-untreated multiple myeloma.

The expression of *STEAP1* gene was investigated in the 7 molecular groups of UAMS cohort of patients treated with Total therapy 2 protocol. PR: proliferation, LB: low bone disease, MS: MMSET, HY: hyperdiploid, CD1: Cyclin D1, D2: Cyclin D2, MF: MAF.

\* indicates a *P* value < .05 and \*\* indicates a *P* value < .01.

**Figure 4:** Validation of *STEAP1* expression. (A) Expression level of STEAP1 protein in HMCLs using Western blot and correlation with STEAP1 gene expression Affymetrix signal value. For each cell line, the ratios of STEAP1 and  $\beta$ -actin proteins were determined in order to compare STEAP1 protein expression between cell lines. (B) STEAP1 protein expression in purified primary myeloma cells using Western blot analysis. (C) STEAP1 protein expression in STEAP1 negative XG-12 and STEAP1 positive LP1 HMCLs using immunofluorescence analysis.

		Gene overexpression compared to normal tissue counterpart
Tissue	Datasets	<i>Steap1</i>
Myeloma	28	No
Leukemia	79	<u>Yes</u>
Lymphoma	48	<u>Yes</u>
Bladder	17	No
Brain	55	<u>Yes</u>
Breast	116	No
Cervical	13	No
Colorectal	69	No
Kidney	32	No
Esophageal	17	<u>Yes</u>
Gastric	17	No
Head & Neck	27	<u>Yes</u>
Liver	22	No
Lung	64	<u>Yes</u>
Melanoma	44	No
Ovarian	37	No
Pancreas	24	<u>Yes</u>
Prostate	52	<u>Yes</u>
Sarcoma	40	No

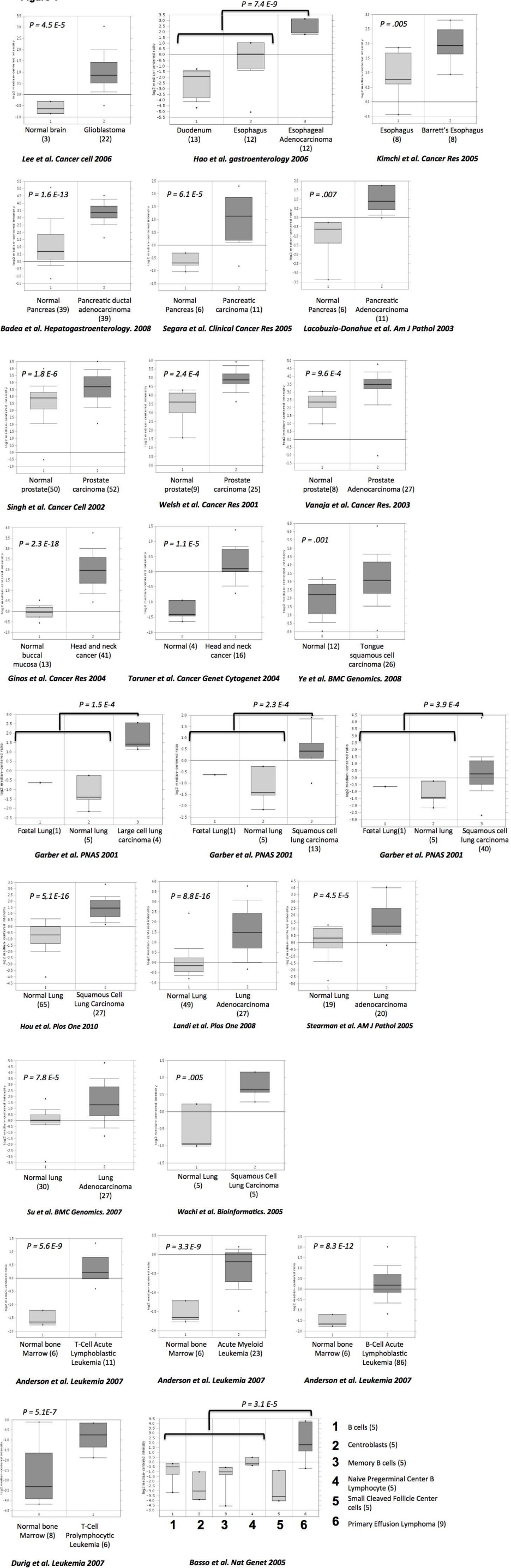
**Table 1:**

Expression of *STEAP1* gene in human tumors and in normal tissue

counterparts using publicly available gene expression data, including the

Oncomine Cancer Microarray database.

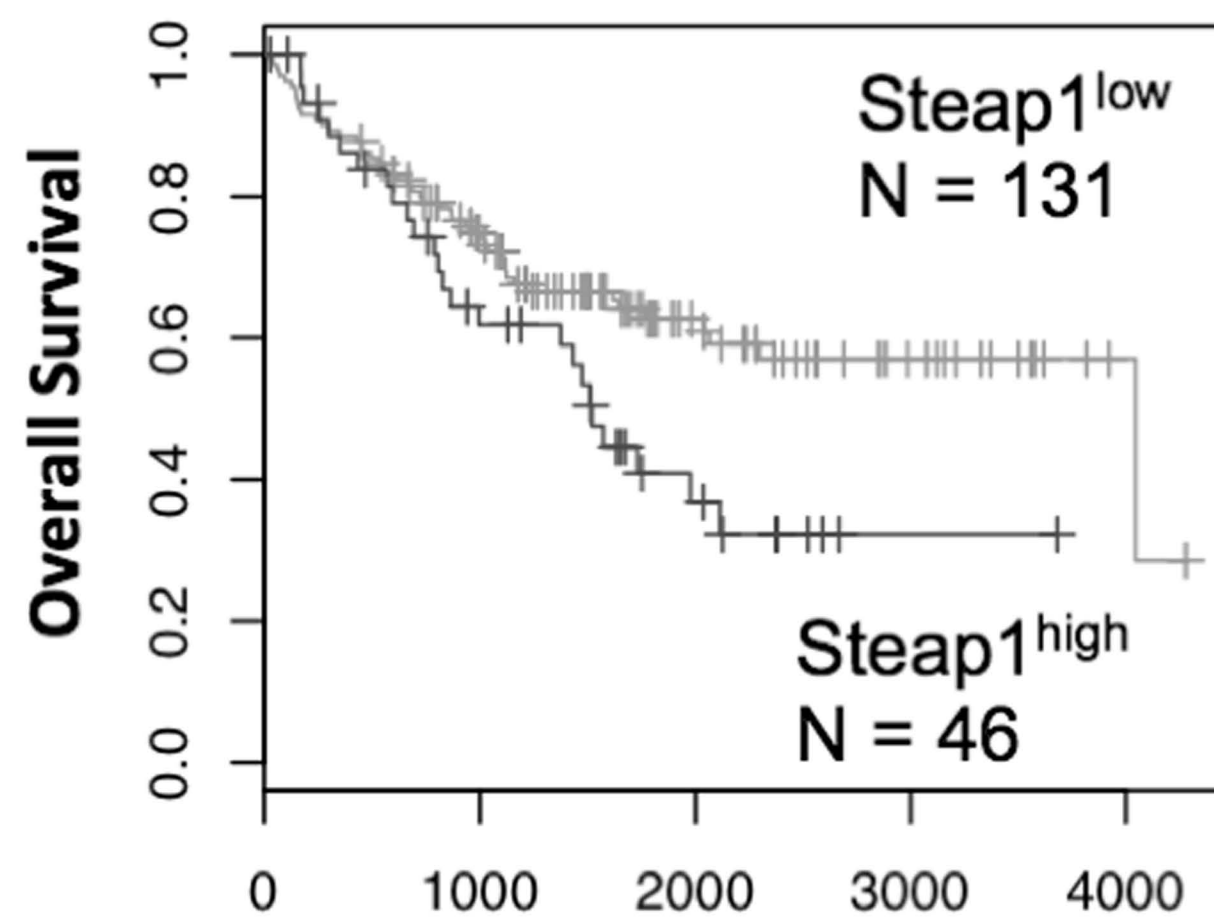
**Figure 1**





**Colorectal Cancer N=177**

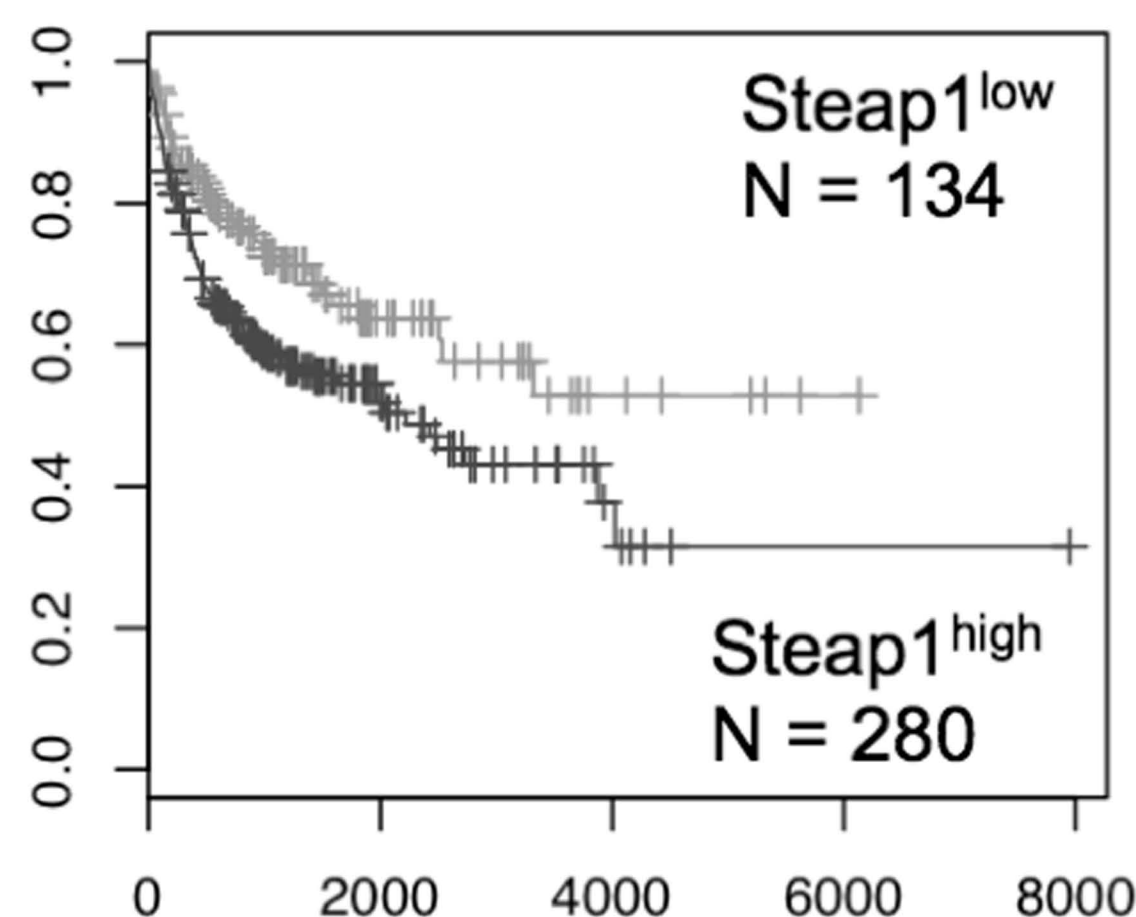
**$P = .02$**



Days from diagnosis

**Diffuse Large B cell Lymphoma N=414**

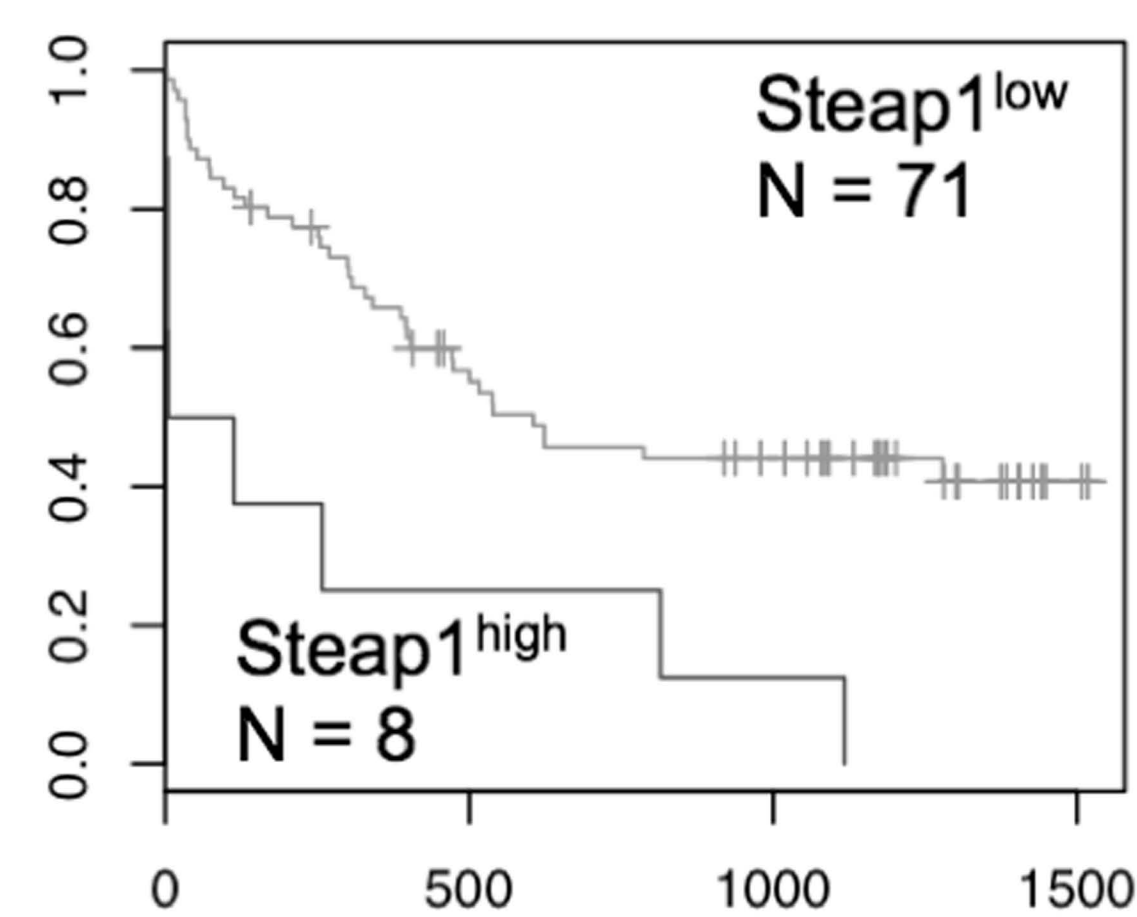
**$P = .008$**



Days from diagnosis

**Acute Myeloid Leukemia N=79**

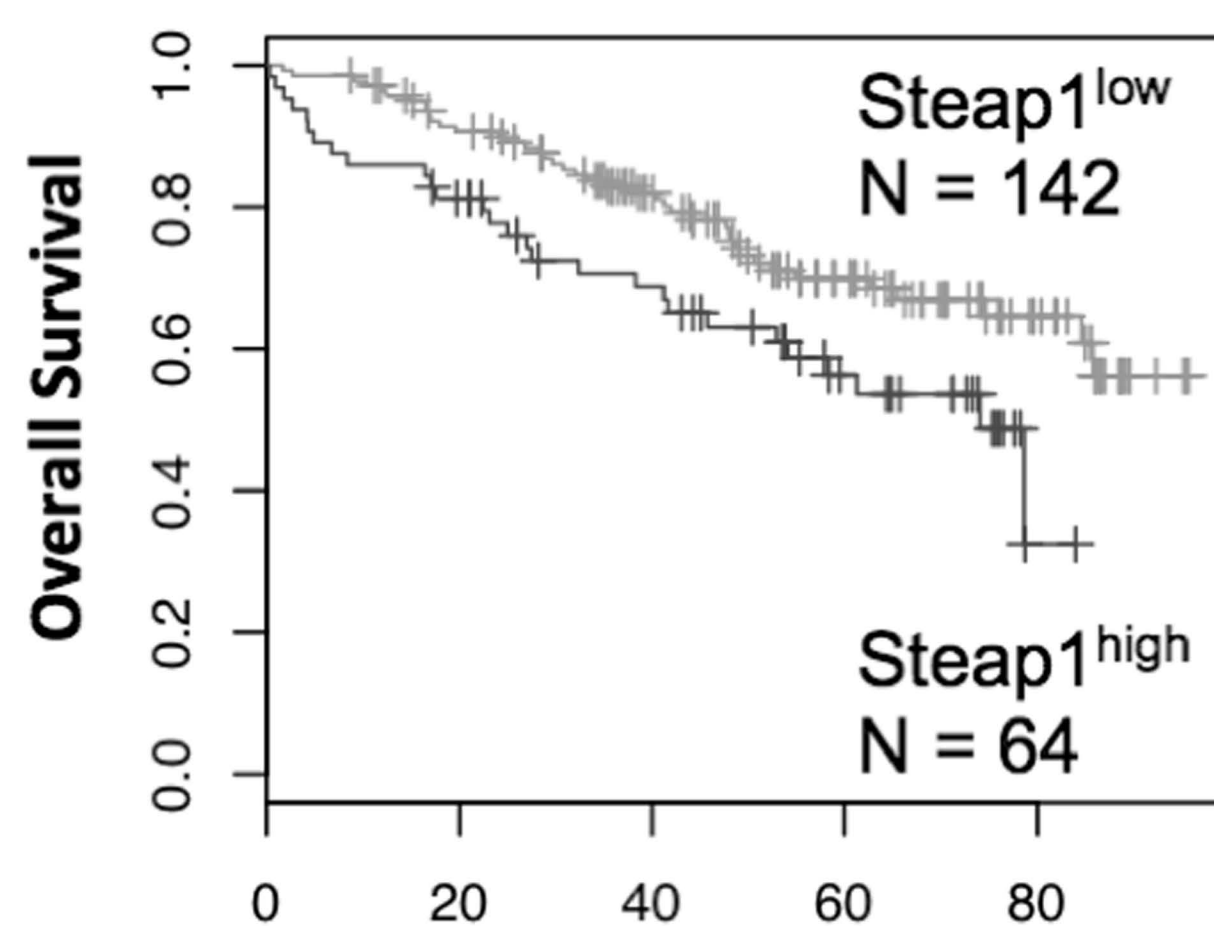
**$P = .0005$**



Days from diagnosis

**Multiple Myeloma N=206**

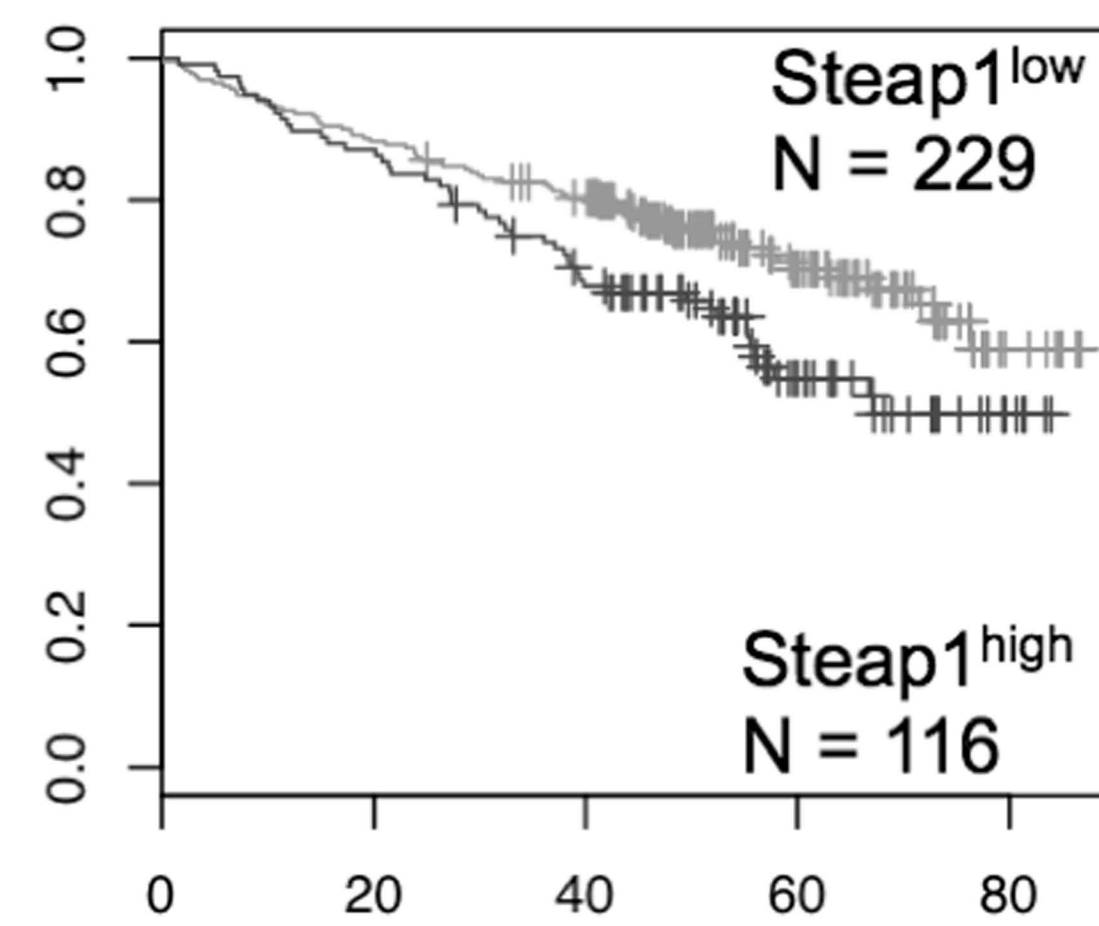
**$P = .01$**



Months from diagnosis

**Multiple Myeloma N=345**

**$P = .02$**



Months from diagnosis



**Figure 2**

**Figure 3**

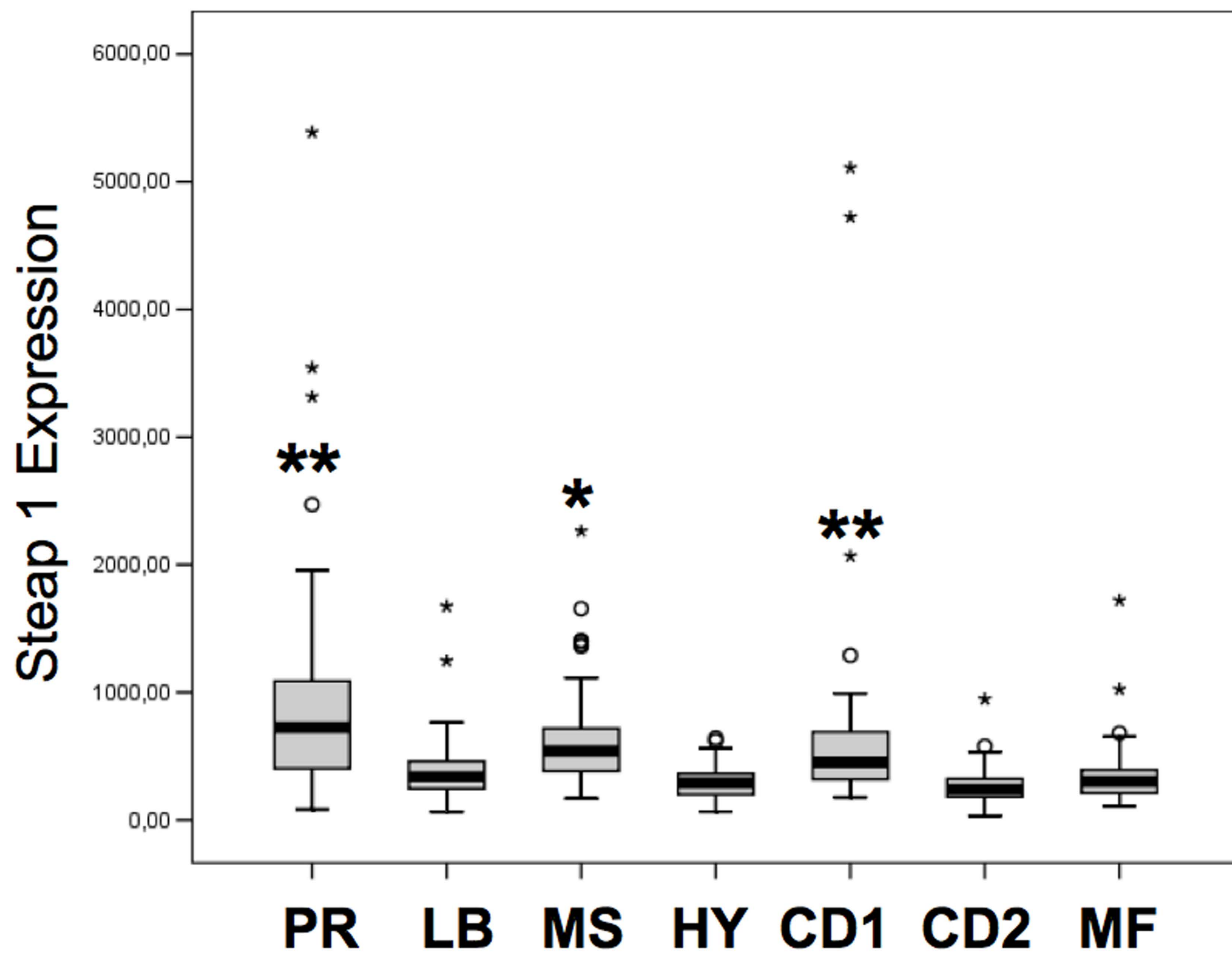


Figure 4A

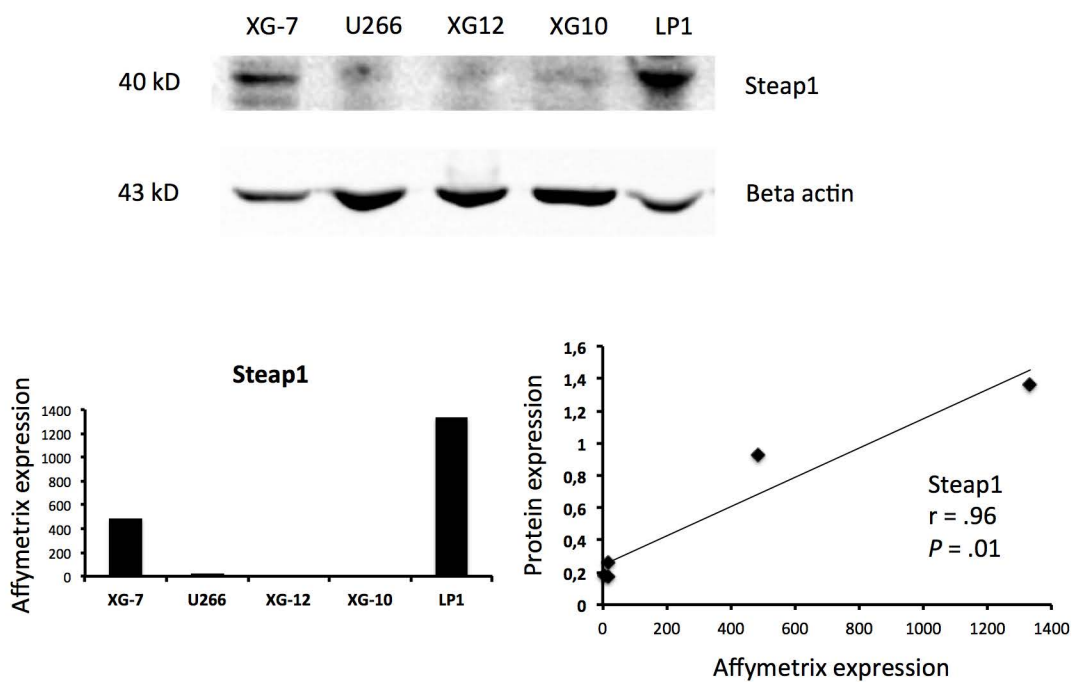
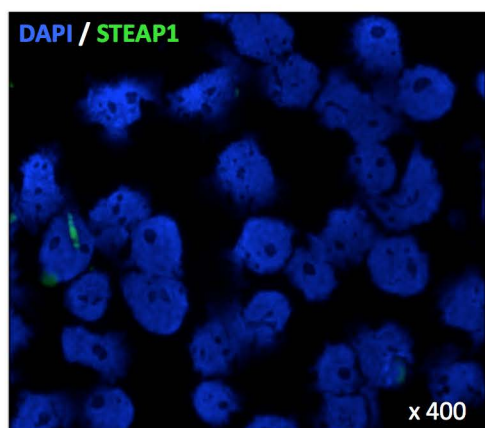
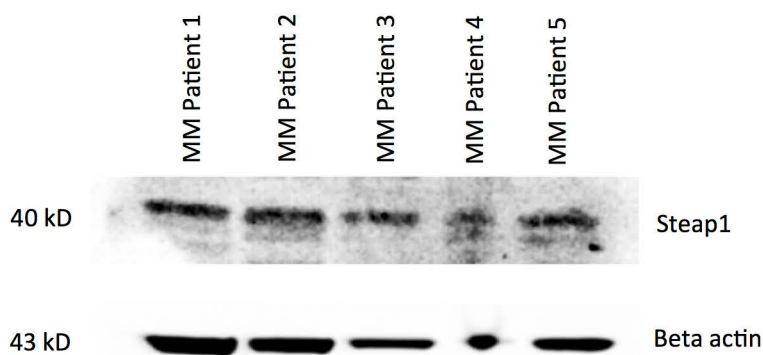
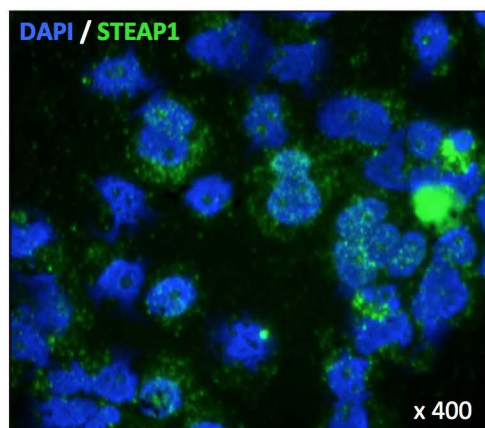


Figure 4B

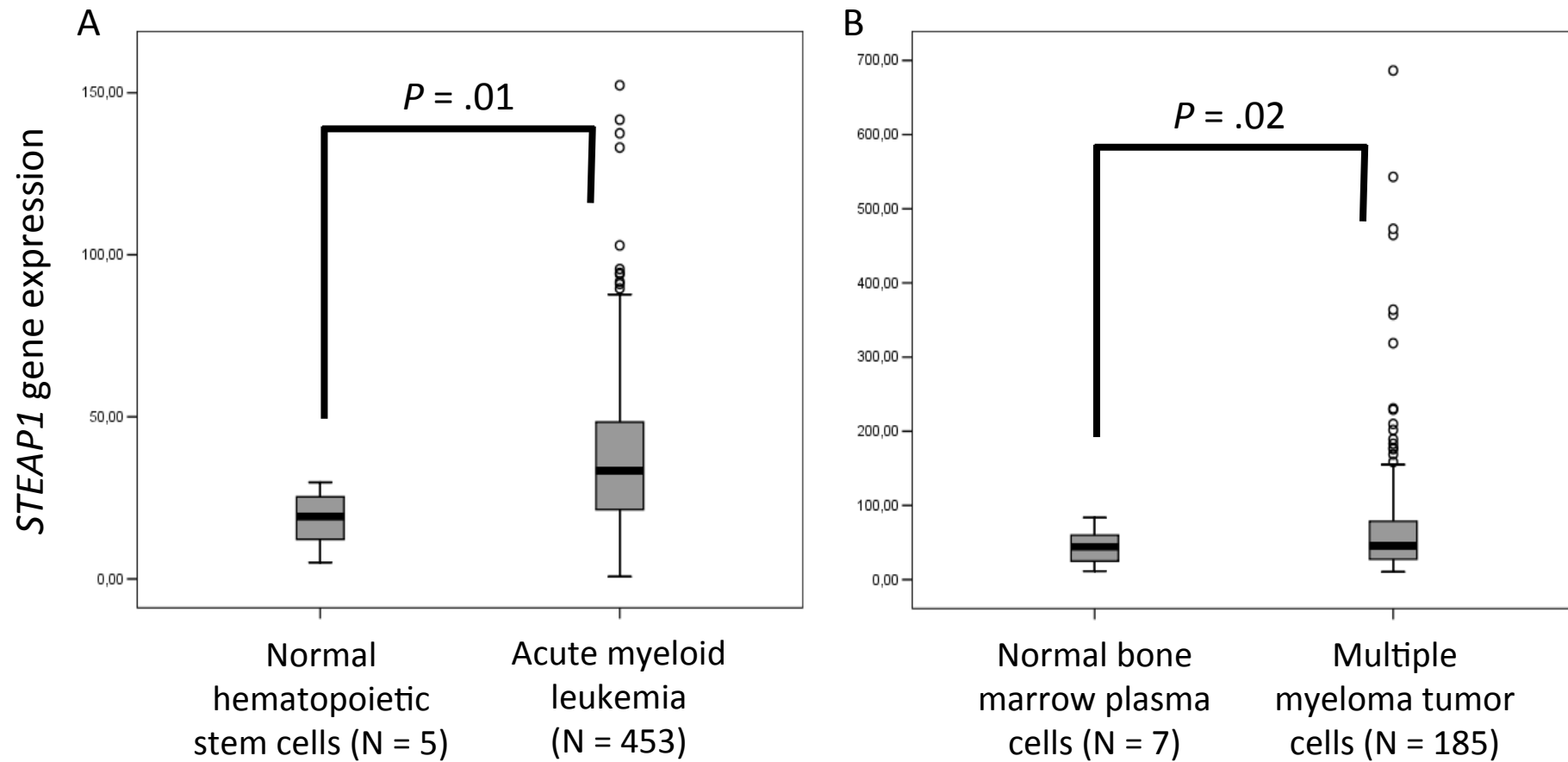


XG-12



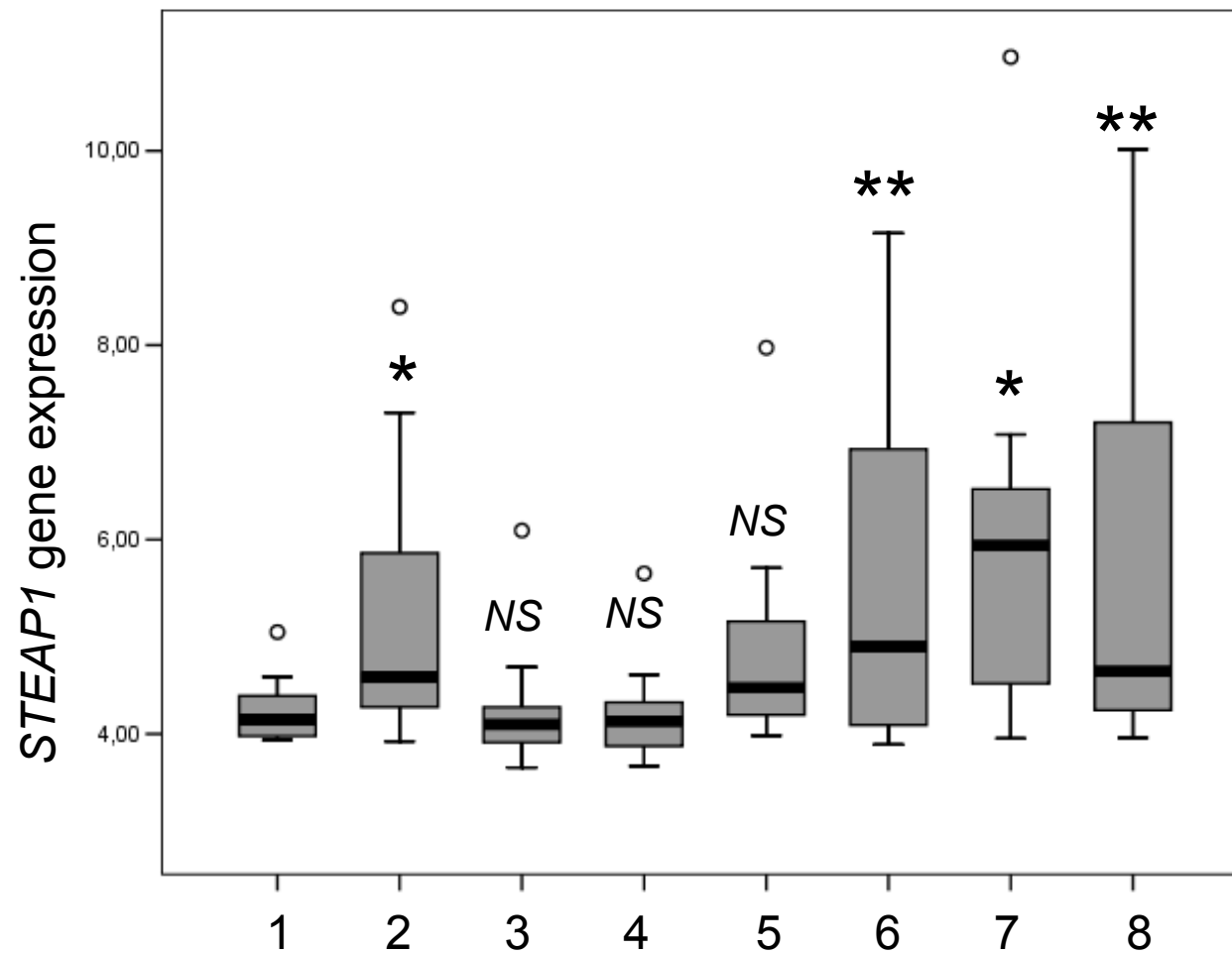
LP1

Figure 4C



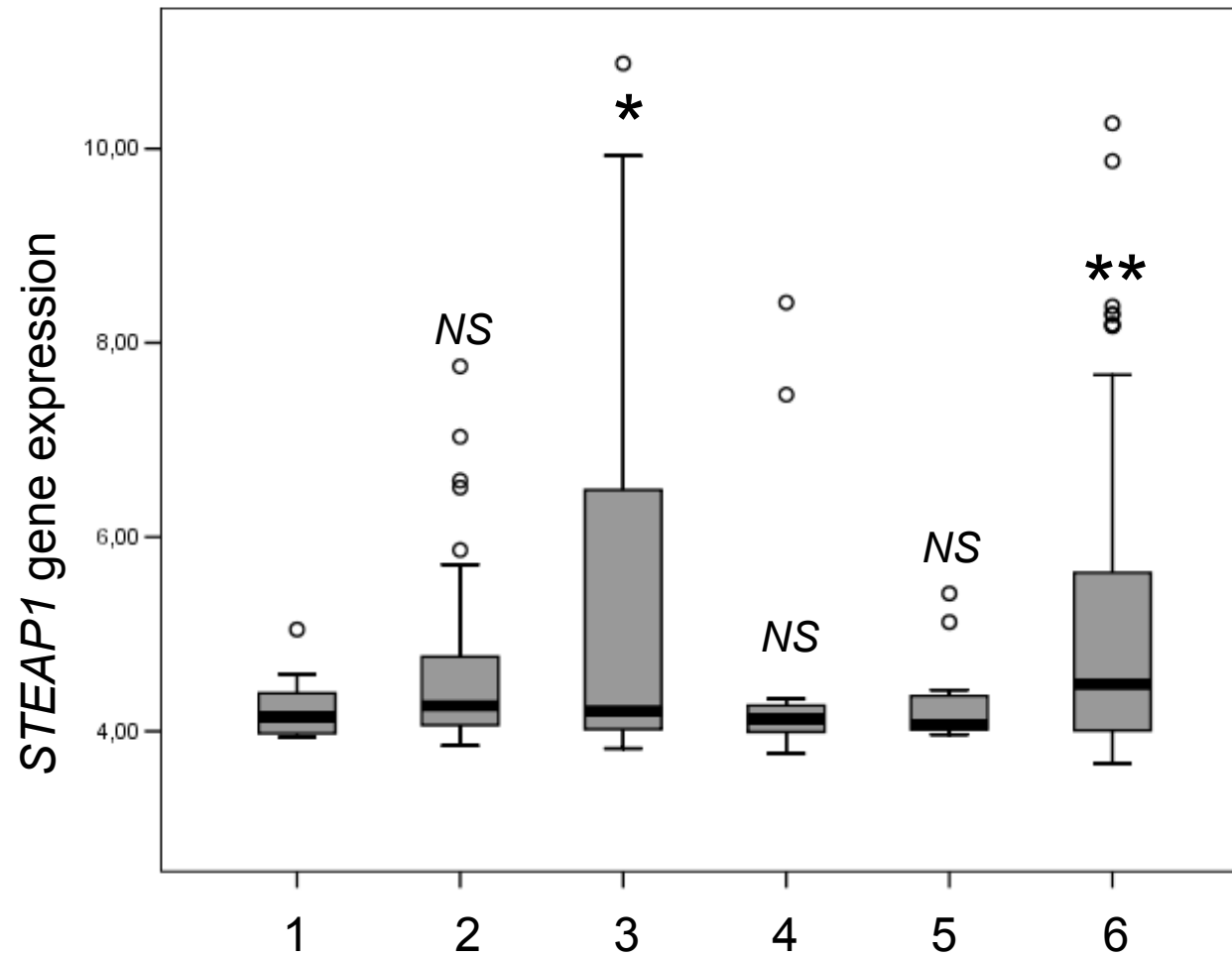
Supplementary Figure S1: STEAP1 expression in (A) AML tumor cells compared to normal hematopoietic stem cells and (B) in MM tumor cells compared to normal bone marrow plasma cells (Amazonia database ([http:// amazonia.montp.inserm.fr/](http://amazonia.montp.inserm.fr/))).

A



- 1 – Prostate carcinoma cell lines (N = 7)
- 2 – Acute lymphoblastic T cell leukemia cell lines (N = 13)
- 3 – Acute myeloid leukemia cell lines (N = 33)
- 4 – Acute lymphoblastic B cell leukemia cell lines (N = 12)
- 5 – Burkitt lymphoma cell lines (N = 10)
- 6 – Diffuse large B cell lymphoma cell lines (N = 16)
- 7 – Hodgkin lymphoma cell lines (N = 13)
- 8 – Multiple myeloma cell lines (N= 27)

B



- 1 – Prostate carcinoma cell lines (N = 7)
- 2 – Glioma cell lines (N = 27)
- 3 – Oesophagus carcinoma cell lines (N = 23)
- 4 – Pancreas carcinoma cell lines (N = 18)
- 5 – Pancreatic ductal carcinoma cell lines (N = 13)
- 6 – Lung carcinoma cell lines (N = 78)

Supplementary Figure S2: *STEAP1* gene expression in (A) acute lymphoblastic T cell leukemia, diffuse large B cell lymphoma, Hodgkin lymphoma, multiple myeloma, acute myeloid leukemia, acute lymphoblastic B cell leukemia, Burkitt lymphoma, (B) oesophagus carcinoma and lung carcinoma cell lines, glioma, pancreas carcinoma and pancreatic ductal carcinoma cell lines compared to prostate cancer cell lines.