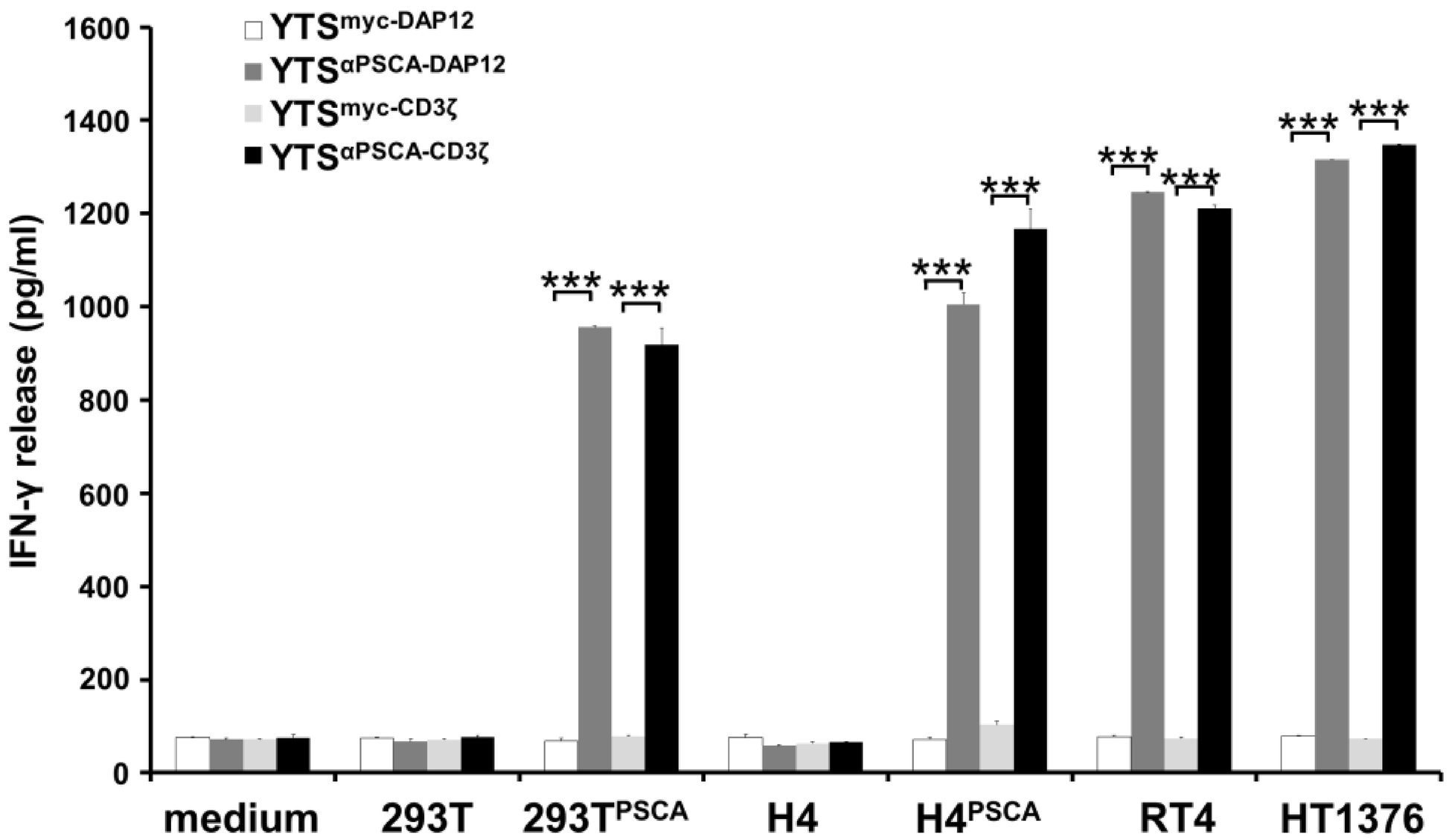
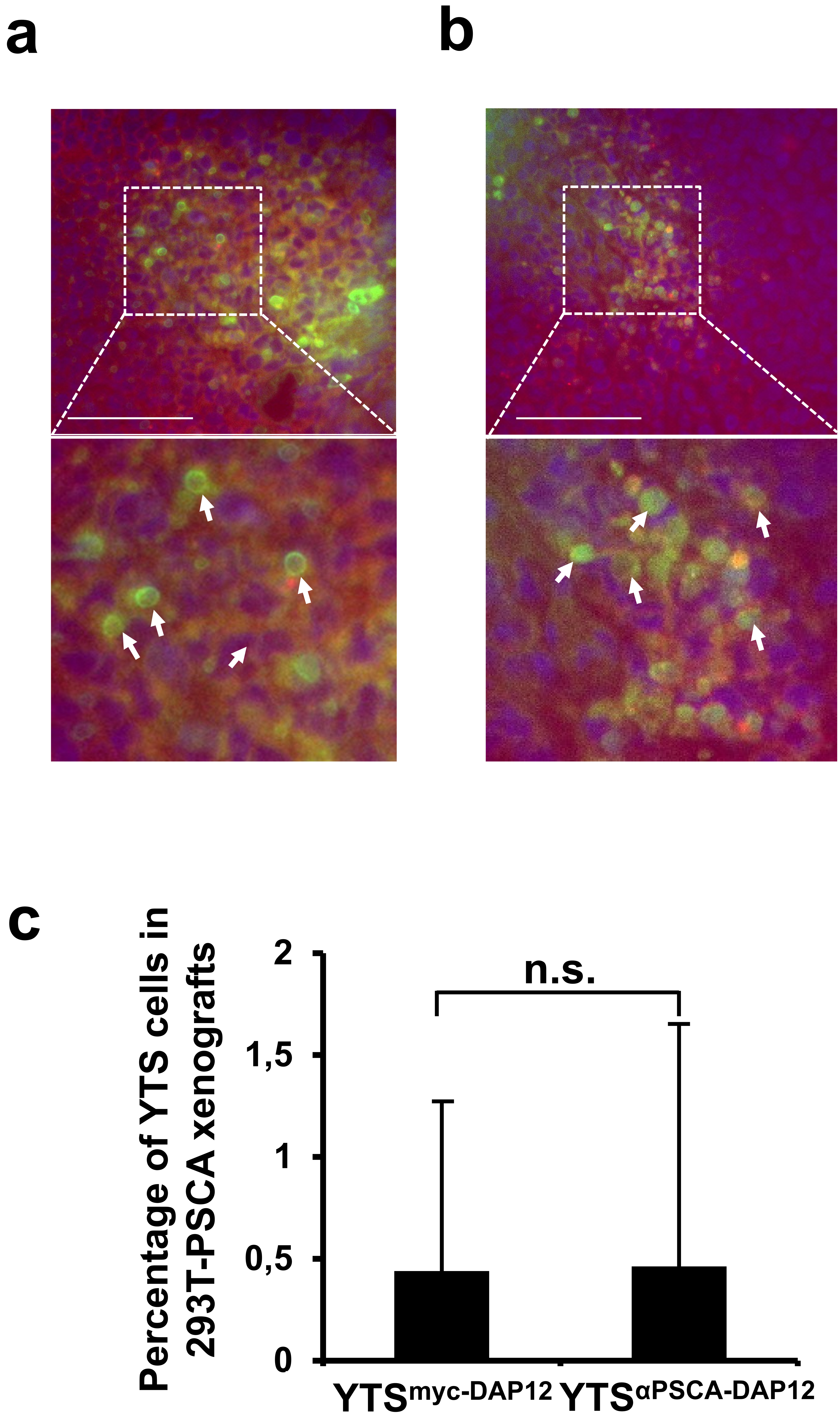
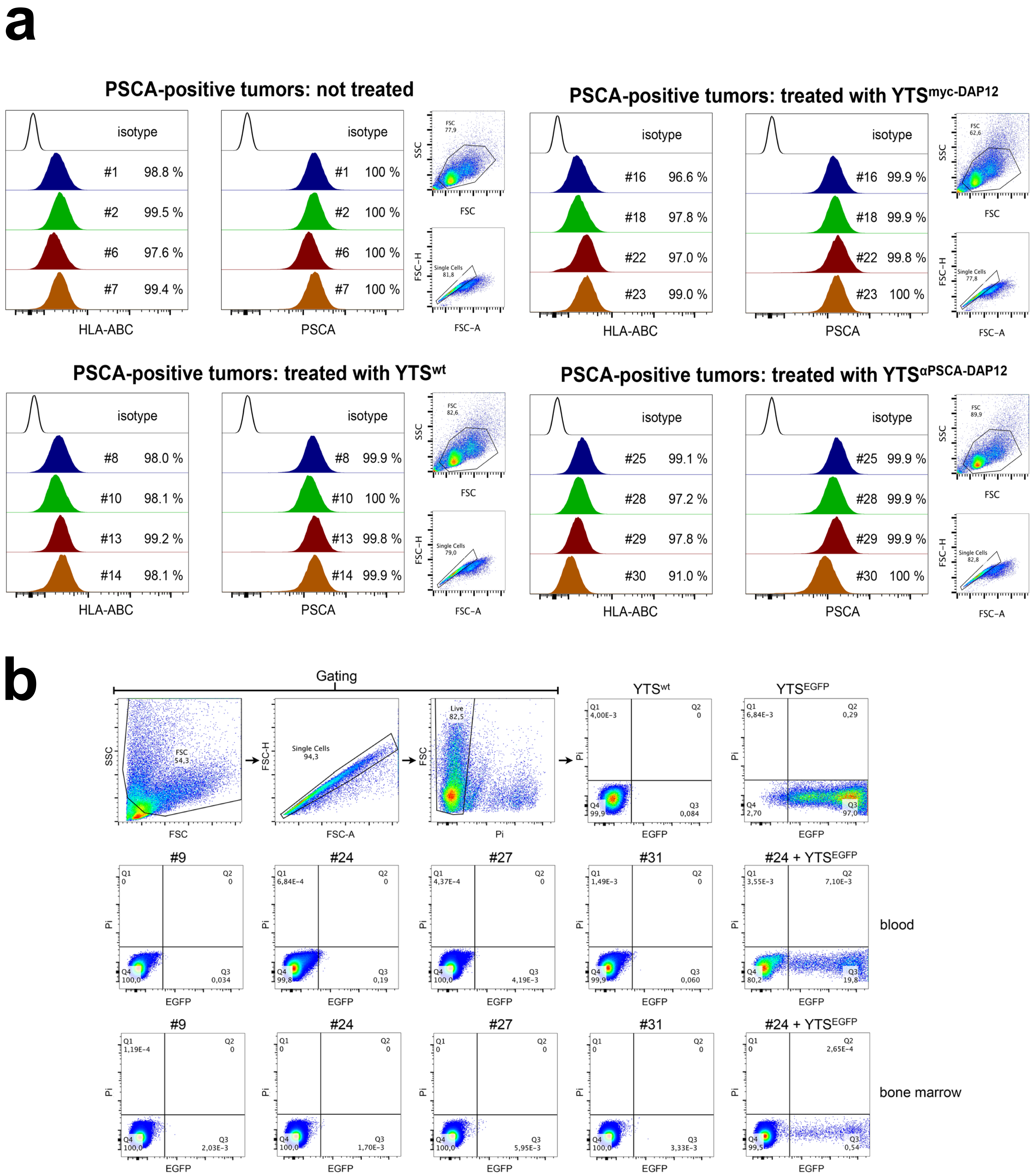
Supplementary data



Supplementary Fig. 1: YTS$^{\alpha PSCA\text{-}DAP12}$– and YTS$^{\alpha PSCA\text{-}CD3\zeta}$-engineered YTS release IFN-γ cells after contact with PSCA-positive cell lines: Transduced YTS$^{\alpha PSCA\text{-}DAP12}$, YTS$^{\alpha PSCA\text{-}CD3\zeta}$ and controls expressing only the DAP12 or CD3ζ signal adapter were incubated with PSCA-positive tumor cell lines PC3^{PSCA}, H4^{PSCA}, corresponding isogenic PSCA-negative controls and RT4 or HT1376 cells with endogenous expression of PSCA. After 18 h of incubation cell-free supernatant was harvested and the amount of released IFN-γ was measured by sandwich ELISA. Note the increase in IFN-γ release of YTS$^{\alpha PSCA\text{-}DAP12}$ (dark grey columns) and YTS$^{\alpha PSCA\text{-}CD3\zeta}$ cells (black columns) after co-cultivation with PSCA-positive target cells. Mean IFN-γ release of four single measurements and SEM is shown (***$p < 0.001$).

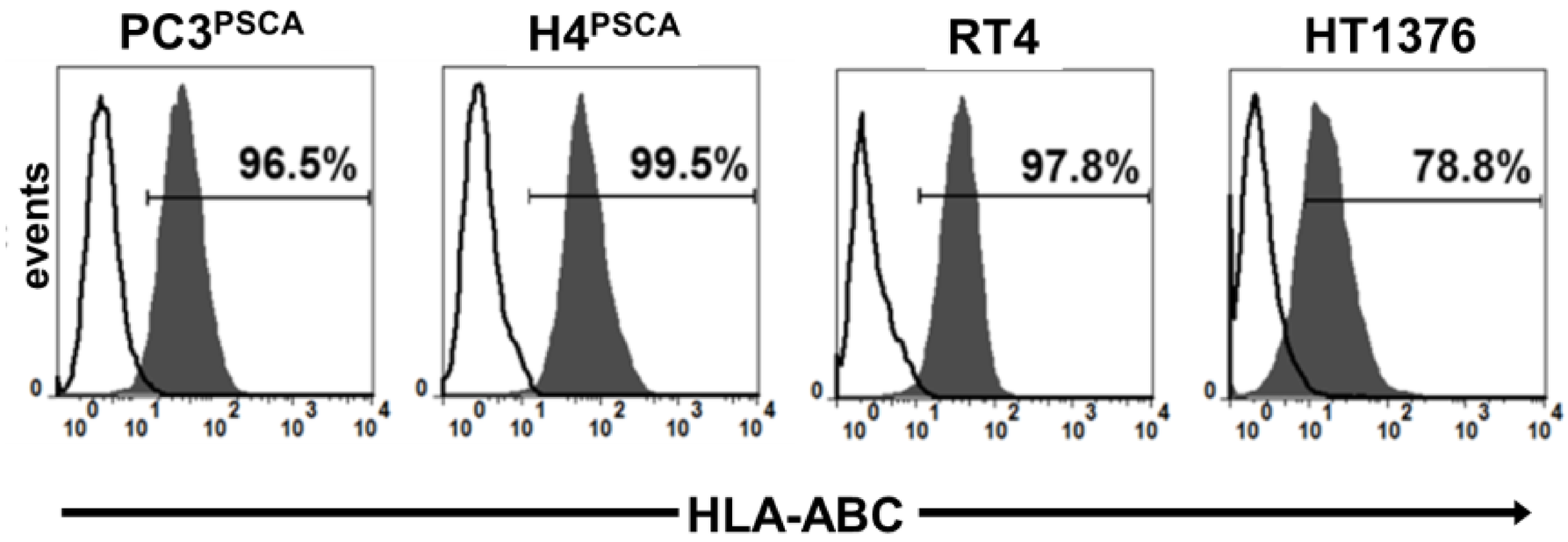


Supplementary figure S2: YTS-NK cells infiltrate PSCA-positive 293T xenografts: a: Image and magnified region of $293T^{PSCA/DsRed}$ tumor with infiltrated $YTS^{myc\text{-}DAP12}$ and **b:** image and magnified region showing tumor-infiltrating $YTS^{\alpha PSCA\text{-}DAP12}$ NK cells. EGFP-positive YTS cells are marked with arrows. **c:** quantification of YTS-NK cells in tumor slices reveled no differences in tumor infiltration rates between $YTS^{myc\text{-}DAP12}$ and $YTS^{\alpha PSCA\text{-}DAP12}$ NK cells. Magnification bars: 100 µm. n.s., not significant.



Supplementary figure S3: YTS-treated 293T^{PSCA} xenografts show no loss of PSCA antigen expression and YTS$^{αPSCA\text{-}DAP12}$ cells do not engraft in NMRI-Foxn1^{nu}/Foxn1^{nu} mice. a: Xenografted tumors were treated with YTS$^{αPSCA\text{-}DAP12}$ and different controls as indicated. When tumors sizes exceeded 18mm diameter four randomly selected tumors from each treatment group were excised and PSCA-expression was analyzed using flow cytometry. Depicted are measurements including isotype controls and the gating on viable cells and subsequent gating excluding cell doublets. Note, that all tumors from all treatment groups expressed HLA and showed no loss of PSCA antigen expression. **b:.** Blood and bone marrow from three mice showing complete and stable tumor regression (mice #24, #27, #31) after treatment with YTS$^{αPSCA\text{-}DAP12}$ NK cells and from one non-treated mouse (mouse #9) was prepared and analyzed for EGFP-positive NK cells. The first line depicts the gating strategy on viable cells, exclusion of cell doublets and measurement of EGFP-marked YTS cells. The EGFP-marked YTS cells were used to set up of a positive control of spiked peripheral blood and bone marrow cells, respectively. Note that the non-treated mouse (#9) as well the three YTS$^{αPSCA\text{-}DAP12}$ –treated survivors did not contain YTS cells in peripheral blood or bone marrow.

a



b

HLA genotypes

PC3	H4	RT4	HT1376
HLA-A*01	HLA-A*03:01	HLA-A*03:01	HLA-A*24:02
HLA-A*24	HLA-A*30:02	HLA-A*02:01	
HLA-B*13	HLA-B*08:01	HLA-B*44:02	HLA-B*15:01
HLA-B*55	HLA-B*18:01		
HLA-C*01	HLA-C*07:01	HLA-C*05:01	HLA-C*03:03
HLA-C*06	HLA-C*05:01		

Donor #1	Donor #2	Donor #3
HLA-A*11:AAAVB	HLA-A*02:ABPND	HLA-A*02:DFKP
HLA-A*25:AH	HLA-A*24:ABPNV	HLA-A*03:ECAM
HLA-B*51:AAAWC	HLA-B*18:ABUAP	HLA-B*13:02
HLA-B*18:TXYF	HLA-B*56:ABUBR	HLA-B*07:CZZS
HLA-C*12:ZAMS	HLA-C*01:ABRXZ	HLA-C*07:ENWG
HLA-C*15:ZAMV	HLA-C*05:ABRNK	HLA-C*06:02

Supplementary figure S4: HLA expression on tumor cells and genotyping of target and effector cells. a: Flow cytometry analysis showing expression of HLA-expression on target cells (filled histograms) using an HLA-ABC antibody detecting a monophorphic epitope present on HLA-A, -B, and –C. Isotype control stainings are included (open histograms). **b:** Results of HLA-genotyping of tumor cell lines and donors used for the study.