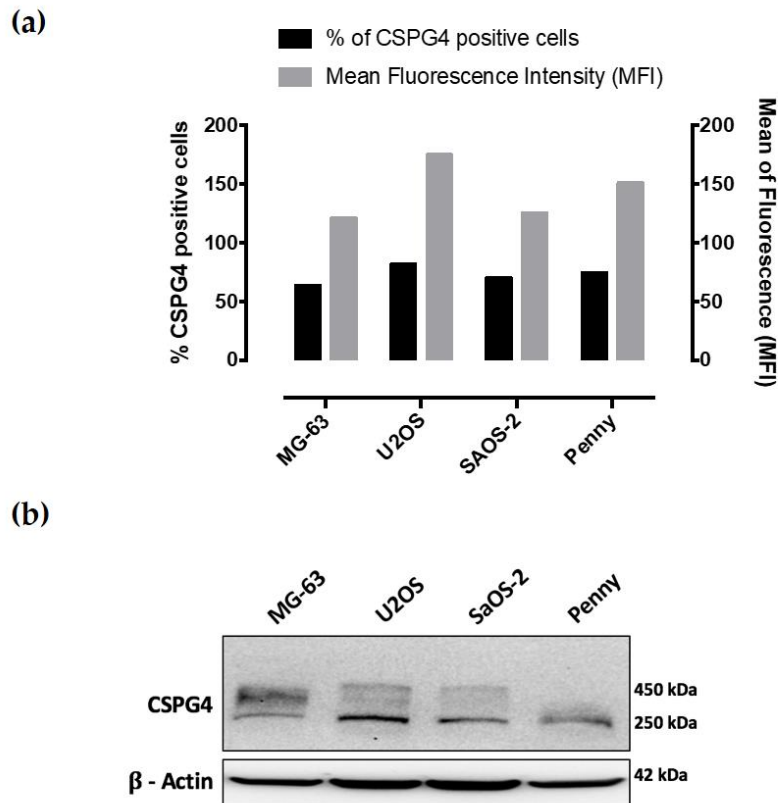


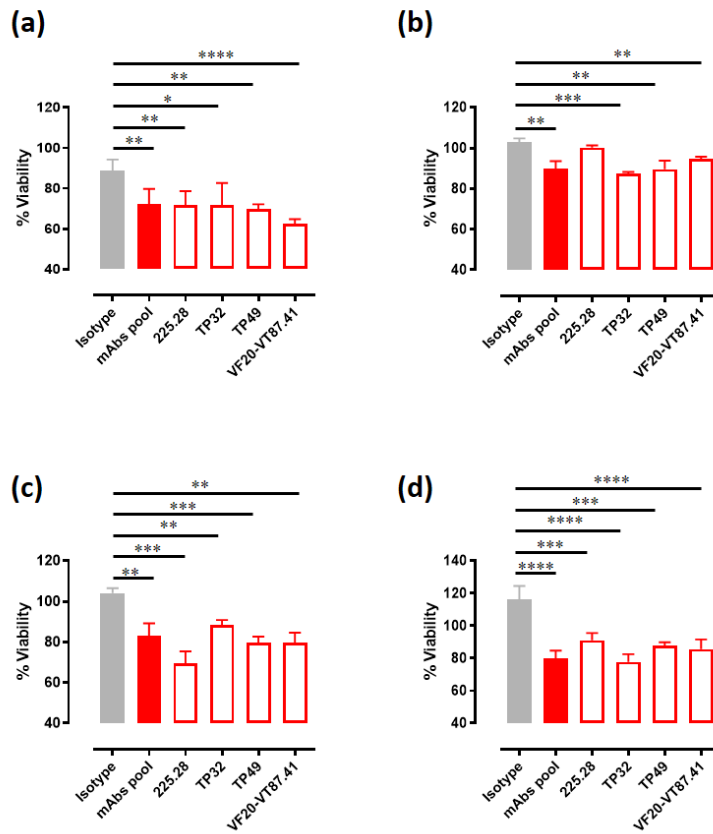
## Supplemental Figures

**Supplemental Figure S1.** CSPG4 mRNA expression in human and canine OSA cell lines. (a) CSPG4 expression levels in three human (MG-63, U2OS and SaOS-2) and one (Penny) canine OSA cell lines evaluated by flow cytometry analysis. Flow cytometry was performed with a FACS Verse and the results were analyzed with BDFacs Suite software. A representative staining of three independent experiments is reported. Both percentage of CSPG4 positive cells and the mean fluorescence intensity (MFI) are plotted on the graph. (b) Western Blot analysis of CSPG4 in human (MG-63, U2OS, SaOS-2) and canine (Penny) OSA cell lines.  $\beta$ -Actin expression was used as normalization control.



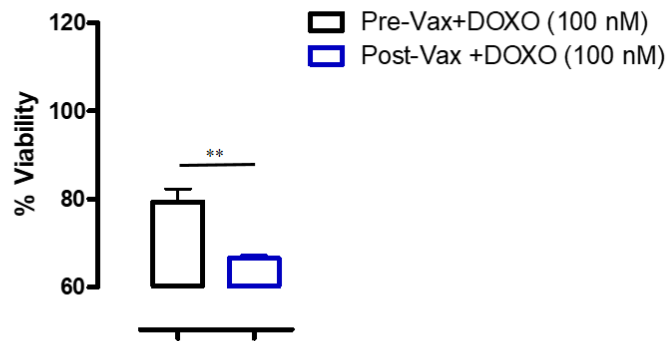
Supplementary Figure S1

**Supplemental Figure S2.** Effects of CSPG4 immune-targeting on OSA cell proliferation and osteosphere viability. The proliferation (a, b) and viability (c, d) of human MG-63 (a, c) and canine Penny (b, d) cells, incubated for 48 hrs with medium alone, control isotypes (25  $\mu\text{g}/\text{ml}$  final concentration), or anti-CSPG4 mAbs (225.28, TP32, TP49, VF20-VT87.41) was assessed by using the MTT assay. Anti-CSPG4 mAbs were mixed in a pool to a final concentration of 25  $\mu\text{g}/\text{ml}$  (mAbs pool) or used as single agents alone (25  $\mu\text{g}/\text{ml}$  final concentration). The results are expressed as the percentage (mean value  $\pm$  SD) of cell proliferation (a, b) and viability (c, d) in each condition respect to cells grown in the medium alone, considered as 100%. Student's t-test \*  $P < 0.01$ ; \*\*  $P < 0.006$ ; \*\*\*  $P < 0.002$ ; \*\*\*\*  $P < 0.0001$ .



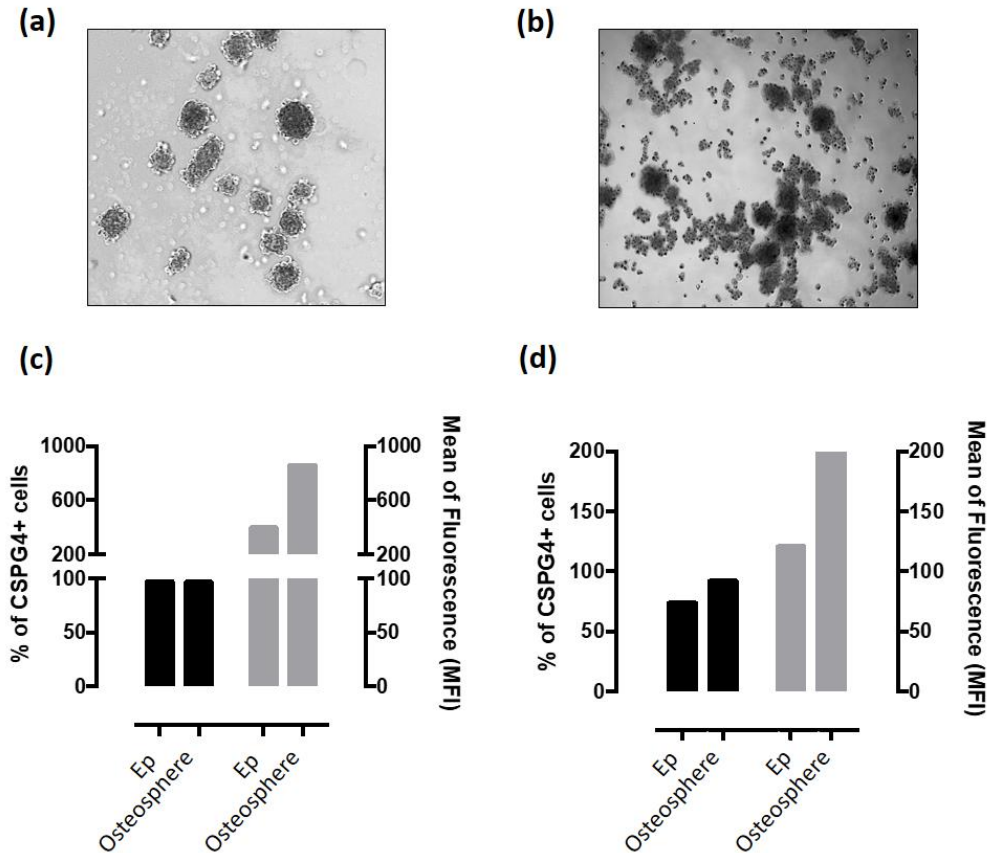
Supplementary Figure S2

**Supplemental Figure S3.** Effects of CSPG4 immune-targeting on human MG-63 OSA cell proliferation. Cell proliferation was assessed by using the MTT assay and the results are expressed as the percentage (mean value  $\pm$  SD) of cell viability in each condition respect to cells grown in the medium alone, considered as 100%. Cells were incubated with medium alone, Pre-Vax sera (black bars) or Post-Vax sera (blue bars) from 5 canine malignant melanoma (MM) patients after the fourth cycle of vaccination with the Hu-CSPG4 DNA vaccine in combination with 100 nM doxorubicin (DOXO). Student's t-test \*\* P = 0.0018.



Supplementary Figure S3

**Supplemental Figure S4.** CSPG4 generation of human and canine osteospheres. (a-b) Representative images of human MG-63 (a) and canine Penny (b)-derived osteospheres generated according the protocol described in Conti et al., 2013.<sup>24</sup> (c-d) Flow cytometry analysis of CSPG4 expression on epithelial and osteospheres-derived human MG-63 (c) and canine Penny (d) cells. Flow cytometry was performed with a FACS Verse and the results were analyzed with BDFacs Suite software. Results are representative of three independent experiments and are expressed as percentage (%) of positivity and mean fluorescence intensity (MFI).



Supplementary Figure S4