Supplemental figure 1

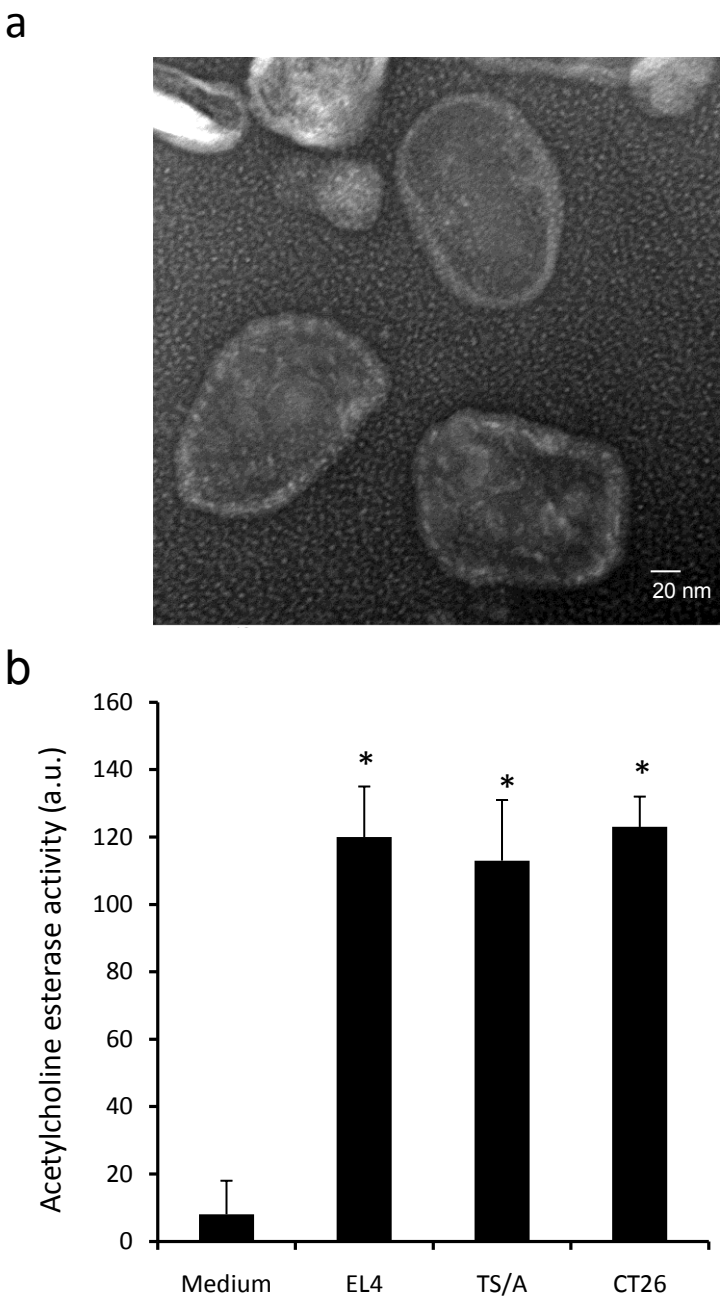

Figure S1
a : Picture from the ultracentrifuged pellet from culture supernatant of EL4.
b: Acetylcholine esterase activity in ultracentrifuged fraction (TDE).

Supplemental figure 2

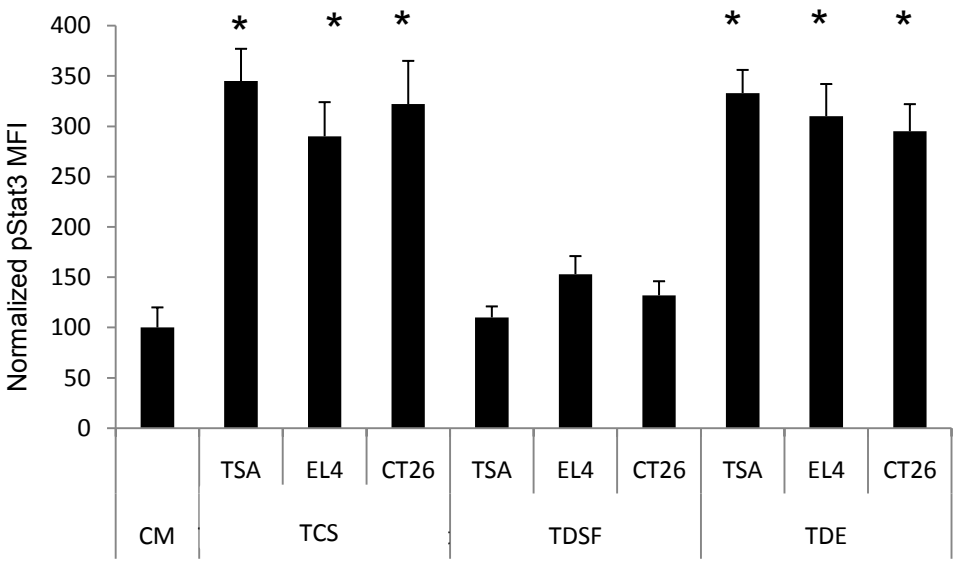

Figure S2 :Stat3 activation in purified MDSCs
Purified splenic MDSCs from naïve mice were treated with TCS or TDSF or TDE. pStat3 expression was determined by FACS analysis. Data are Mean ± s.d. fluorescence intensity (n=3)

Supplemental figure 3

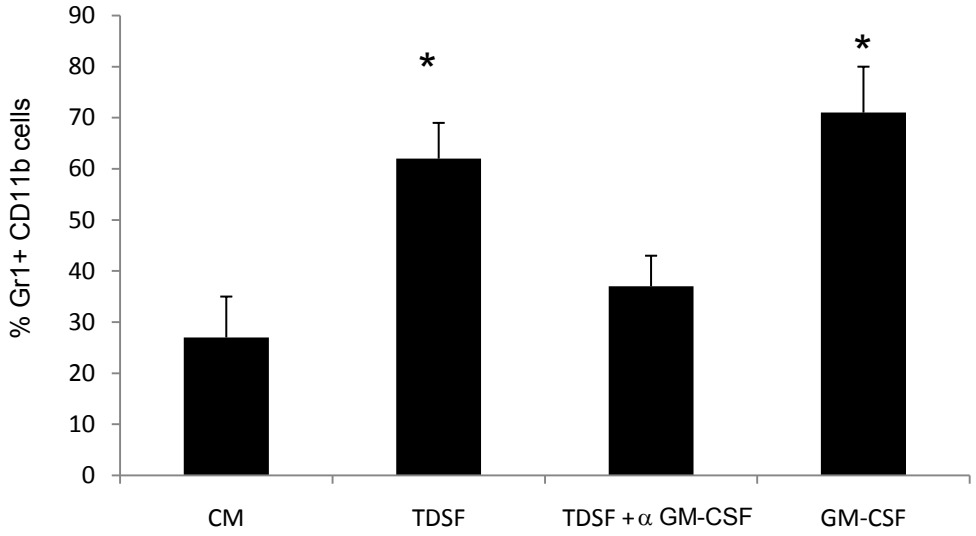

Figure S3. Expansion of MDSCs by GM-CSF

Bone marrow from naïve mice were cultured 3 days in complete medium alone or with TDSF or TDSF + blocking anti GM-CSF or rGM-CSF **.** The percentage of $Gr1^+$ $CD11b^+$ precursor cells ± s.d. was determined by flow cytometry.

Supplemental figure 4

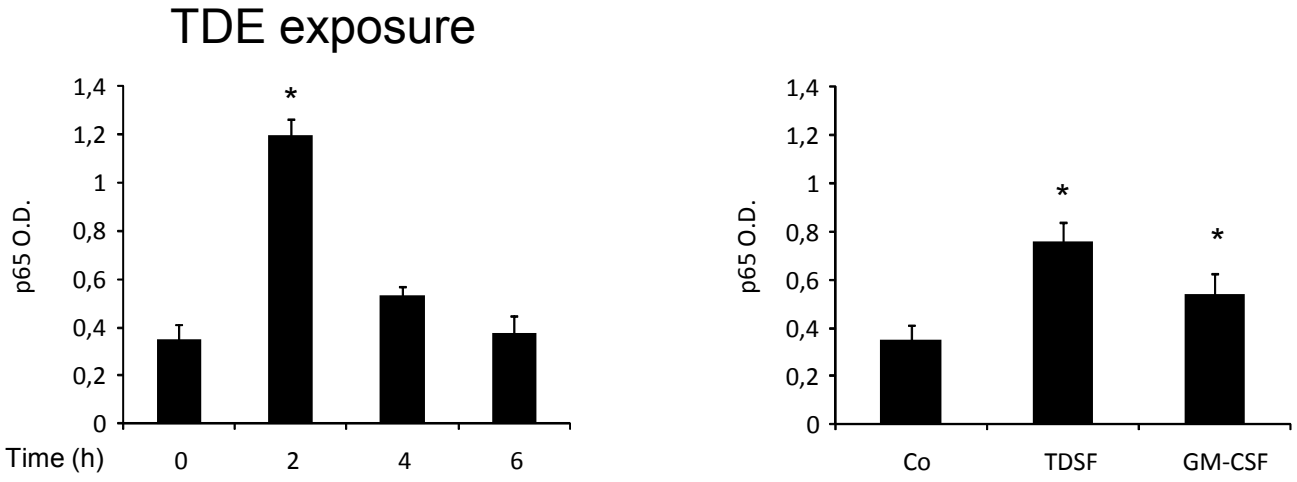

Figure S4. NFκB p65 transcriptional activition by TDE and TDSF

(Left panel) MDSC clone were stimulated with TDE for 2h, 4h or 6h . Then NFκB p65 transcriptional activity was determined with TransAm assay.

(Right panel) MDSC clone were stimulated with TDSF or GM-CSF for 2h. Then NFκB p65 transcriptional activity was determined with TransAm assay.

Supplemental figure 5

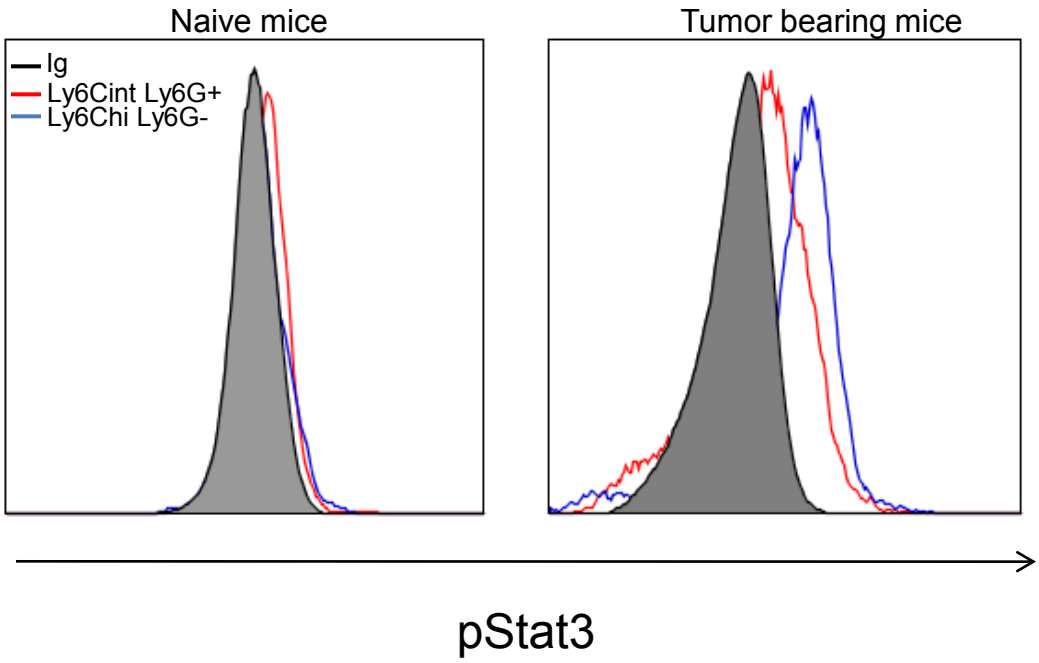

Figure S5 . pStat3 expression in MDSCs Subpopulations

MDSC from tumor-bearing mice were analysed for Ly6C Ly6G CD11b and pStat3 expression by cytometry.

Monocytic MDSC were characterized by $Ly6C^{high}Ly6G^{-}CD11b^{+}$; granulocytic MDSC by $Ly6C^{intermediate}$ $Ly6G^{+}$ $CD11b^{+}$.

Supplemental figure 6

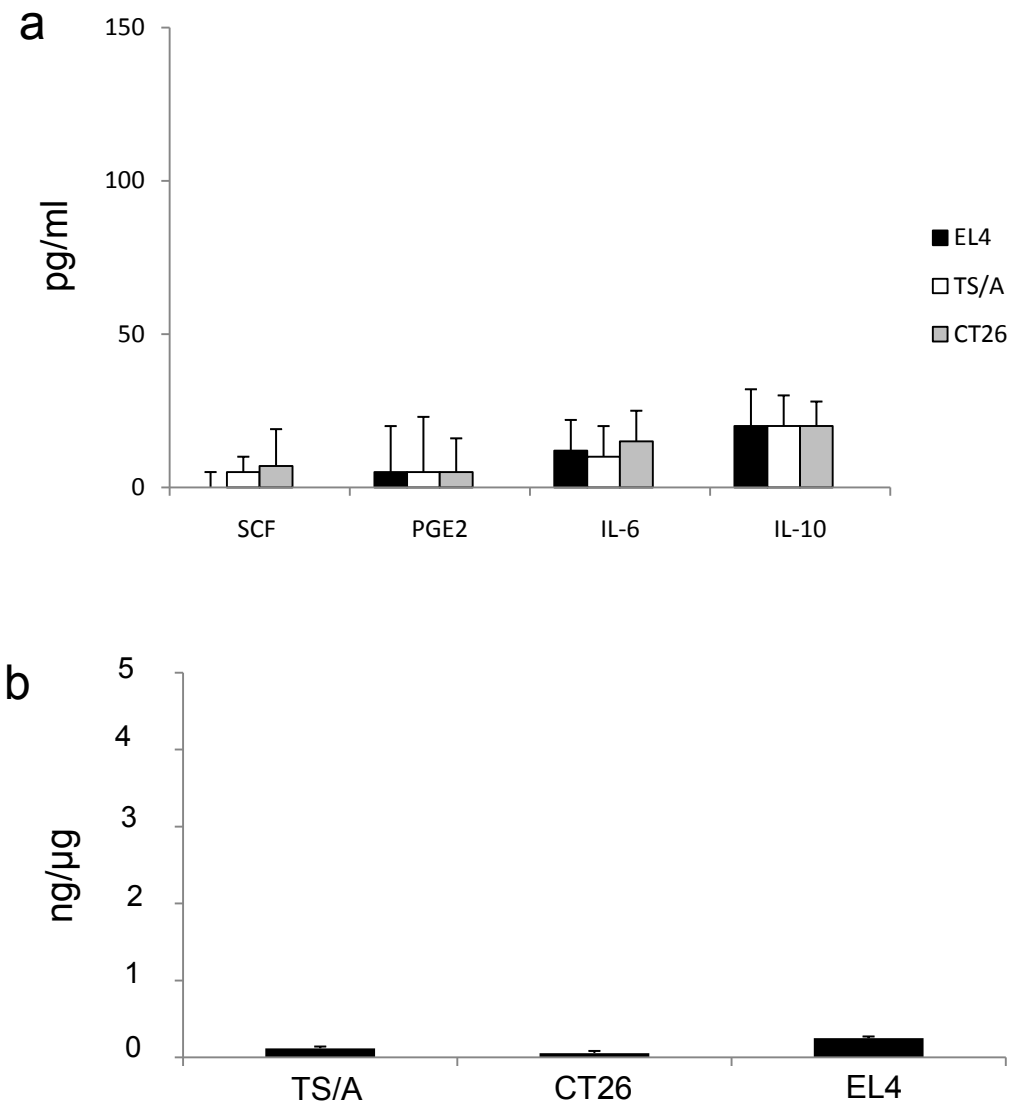

Figure S6. Cytokines production by tumor cells and TDE

A. Tumor cell supernatant SCF, PGE2, IL-10 and IL-6 were determined by ELISA.

B. PGE2 levels in TDE were determined by ELISA.

Supplementary figure 7

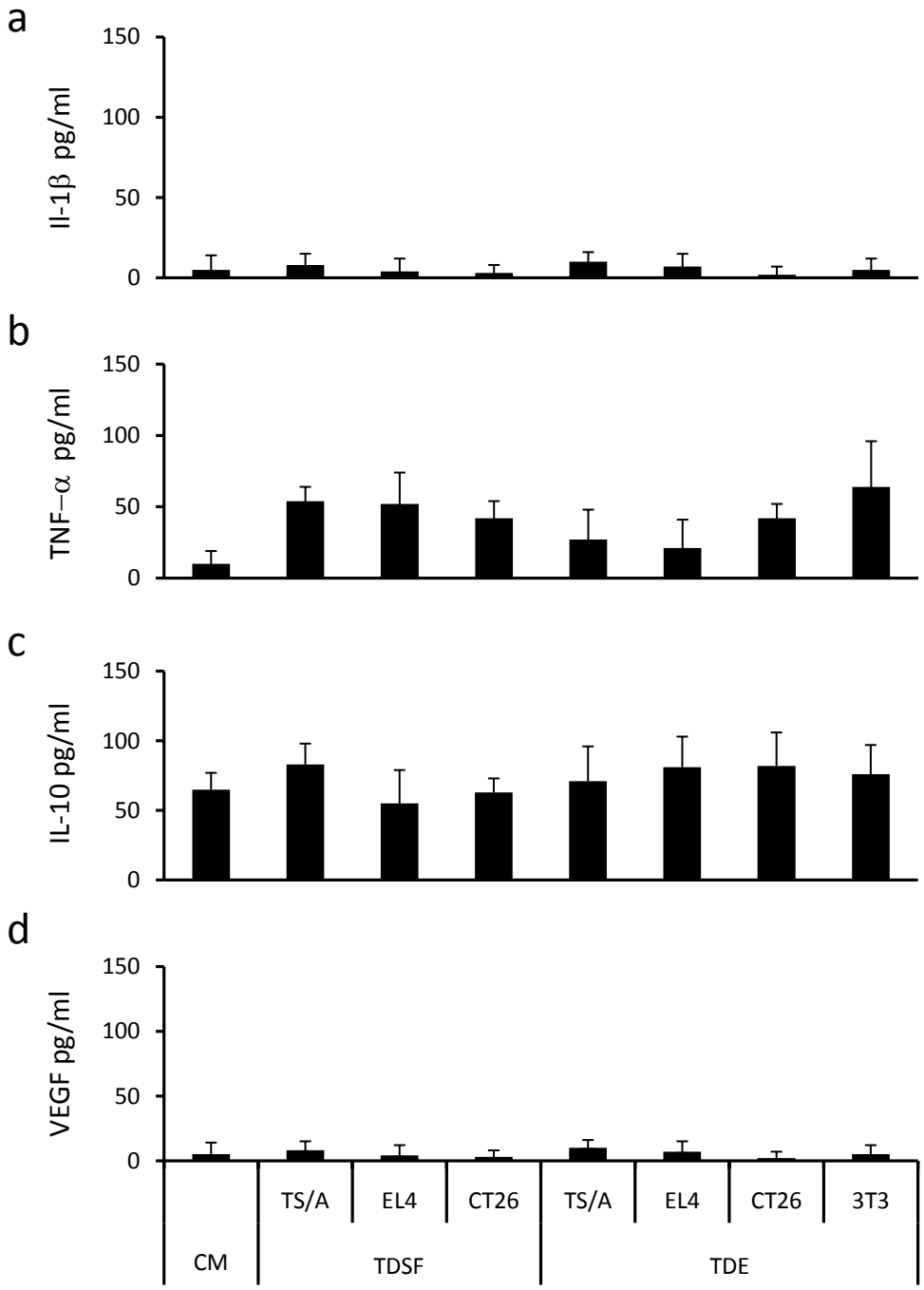

Figure S7. Cytokines production by MDSCs.
Purified MDSC from naïve mice were treated 24h with complete conditioned medium (CM) or soluble fraction (TDSF) or exosome (TDE) obtained from CT26, TS/A, EL4 and 3T3 cell cultures before and after ultracentrifugation (UC). IL-1β (a), TNF-α (b), IL-10 (c) and VEGF (d) concentrations were determined by ELISA.

Supplementary figure 8

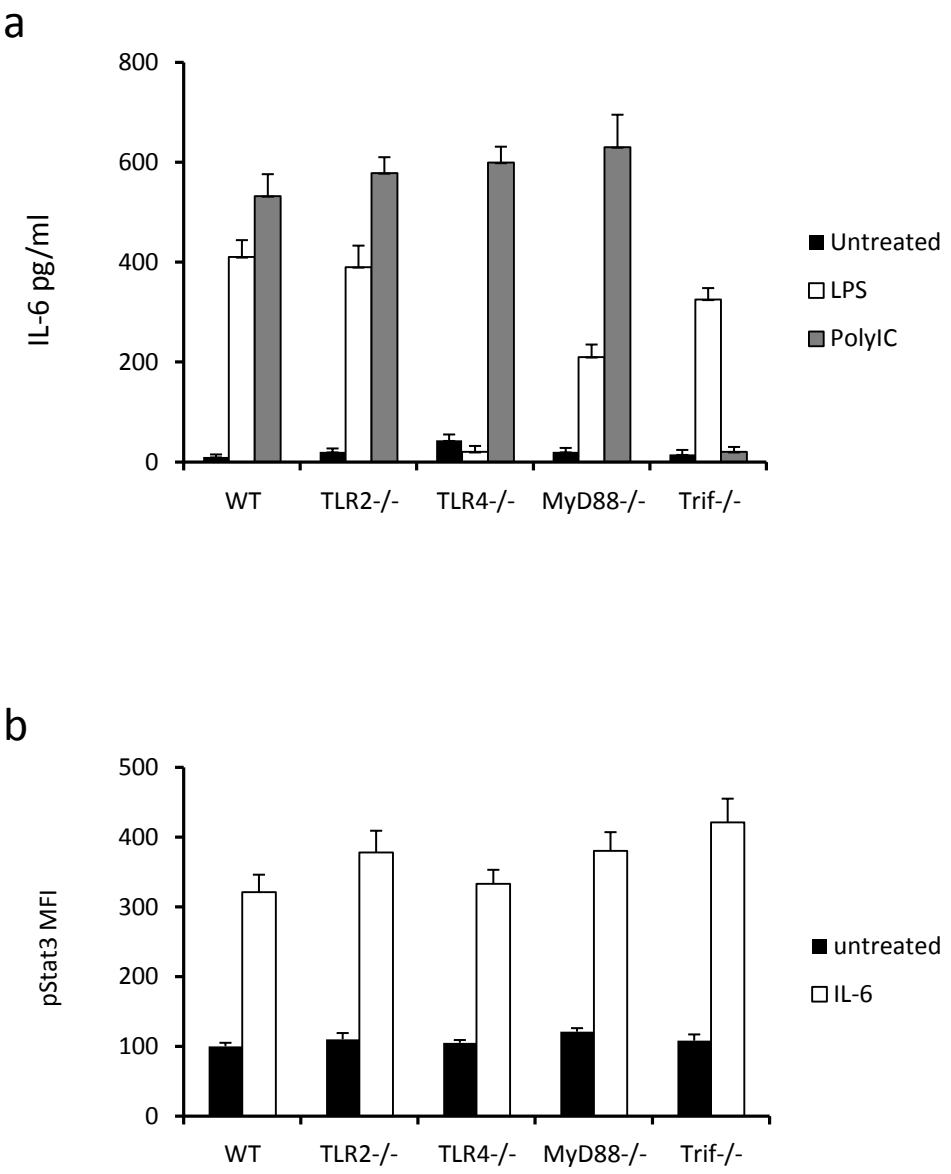

Figure S8. IL-6 production and pStat3 expression by MDSCs from wild type and TLR deficient mice

a. MDSCs from wild-type or TLR2-, TLR4-, MyD88- or TRIF-deficient tumor-free mice were cultured for 24h with PBS alone or supplemented with LPS (10 ng/mL) or Poly(I:C) (50µg/mL), then IL-6 concentrations were determined by ELISA in the supernatant.

b. MDSCs from wild-type or TLR2-, TLR4-, MyD88- or TRIF-deficient tumor-free mice were cultured for 24h with PBS alone or supplemented with rIL-6 (10ng/mL). pStat3 expression was determined by FACS analysis on MDSCs gated cells. MFI = mean fluorescence intensity ± s.d**.**

Supplemental figure 9

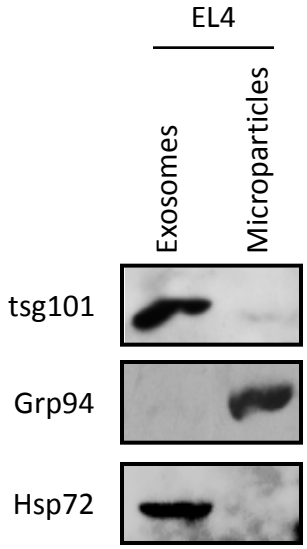

Figure S9. Expression of Hsp72, Tsg101 and Grp72 in exosome and other microparticules
EL4- exosome were separated from other microparticules by ultracentrifugation on 30% sucrose/D2O. Both fractions were blotted for tsg101 (exosomal protein), Grp94 (endoplasmic reticulum protein) and Hsp72.

Supplemental figure 10

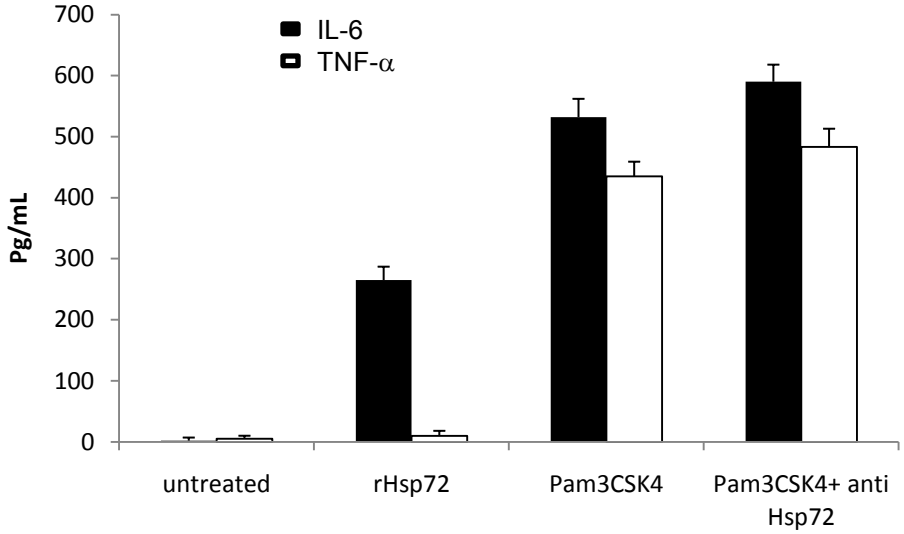

Figure S10. IL-6 and TNF-α production by MDSCs
Naïve MDSCs were stimulated 24h with rHsp72 or PAM3CSK4 or PAM3CSK4 plus a blocking anti Hsp72. IL-6 and TNF-α in the supernatant were determined by ELISA.

Supplemental figure 11

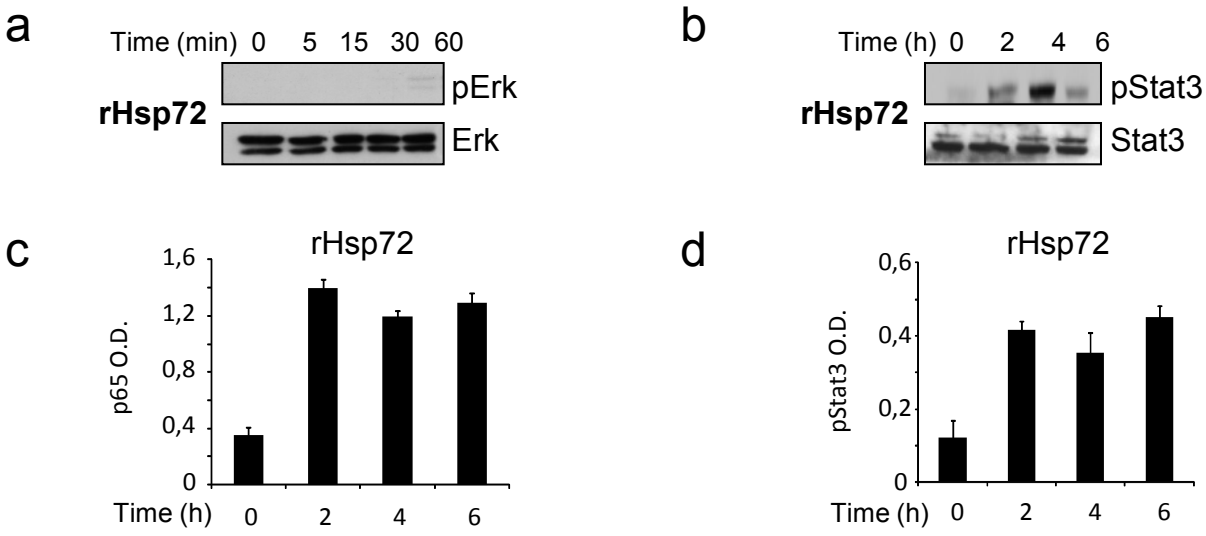

Figure S11. Erk ,Stat3 and NFκB activation by Hsp72 in MDSCs

MDSC clone were stimulated with rHsp72, pERK (a) and pStat3 (b) expression was determined western blotting.

MDSC clone were stimulated with rHsp72, then NFκB p65 (c) and Stat3 (d) transcriptional activity was determined with TransAm assay.

Supplemental figure 12

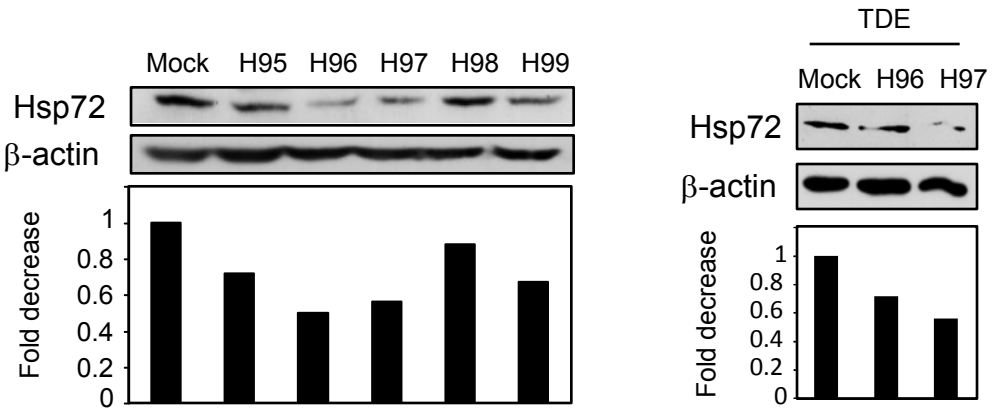

Figure S12. Hsp72 expression in CT26 clones transfected with ShRNA targeting Hsp72
Hsp72 expression was determined by western blotting in shRNA Mock CT26, and in 5 different shRNA Hsp72 CT26 clones. Hsp72 expression was also determined in TDE from shRNA Mock CT26 and shRNA Hsp72 CT26 clones H96 and H97.

Supplementary figure 13

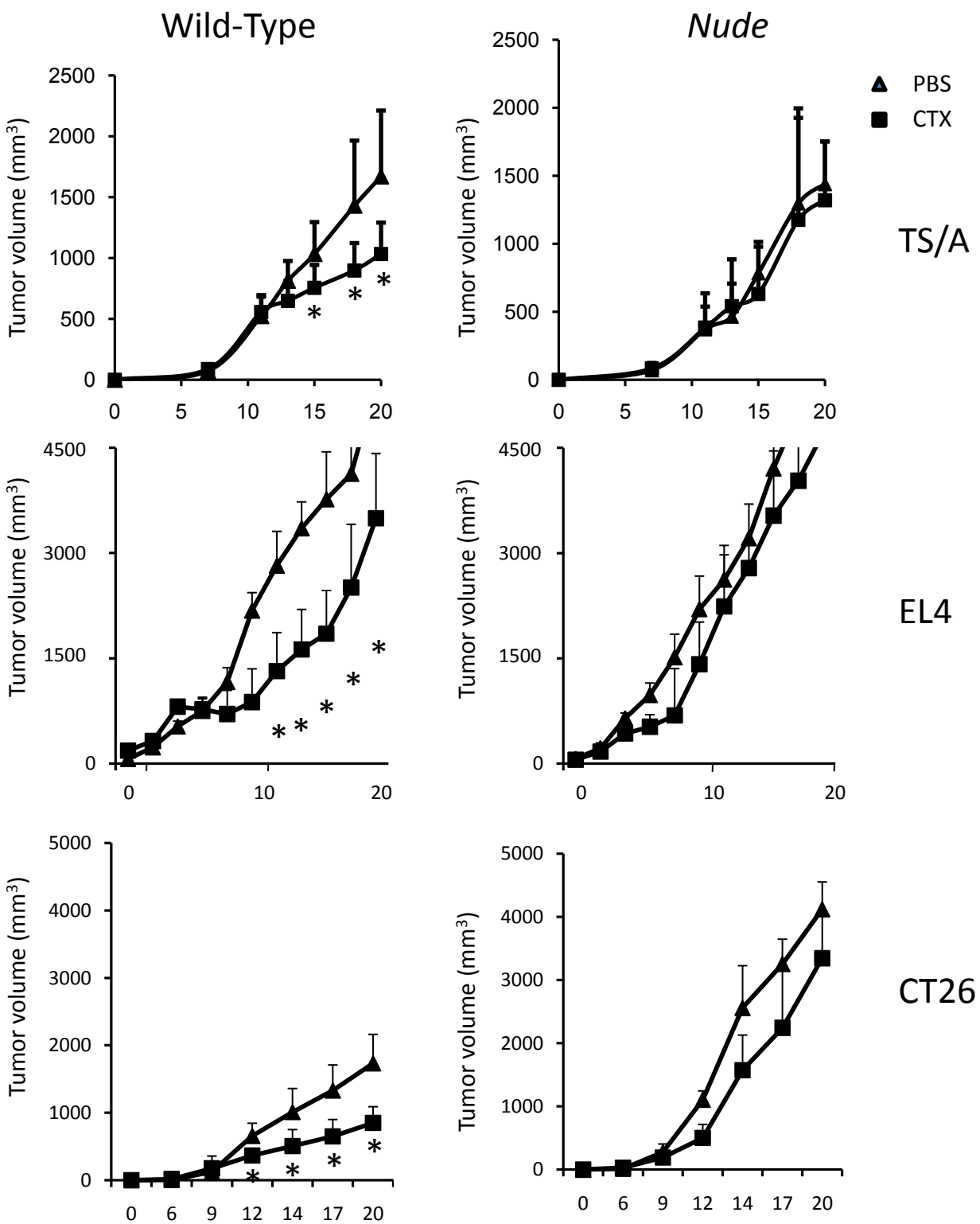

Figure S13. Tumor growth in wild type and *n ude* mice after cyclophosphamide treatment.
Wild-type and *nude* mice were subcutaneously injected with 1.10^6 TS/A, EL4 or CT26 tumor cells. 10 days later, mice were injected with PBS or cyclophosphamide (CTX) (100 mg/kg). Tumor volume was determined using a caliper.

Supplemental figure 14

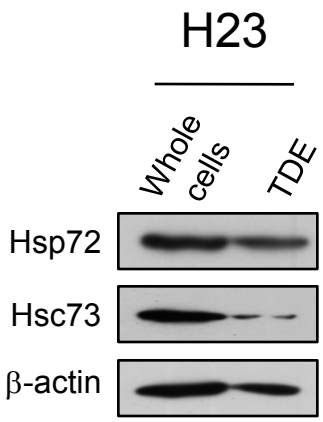

Figure S14.
Hsc73 and Hsp72 expression in H23 and H23 TDE was determined by western blotting.