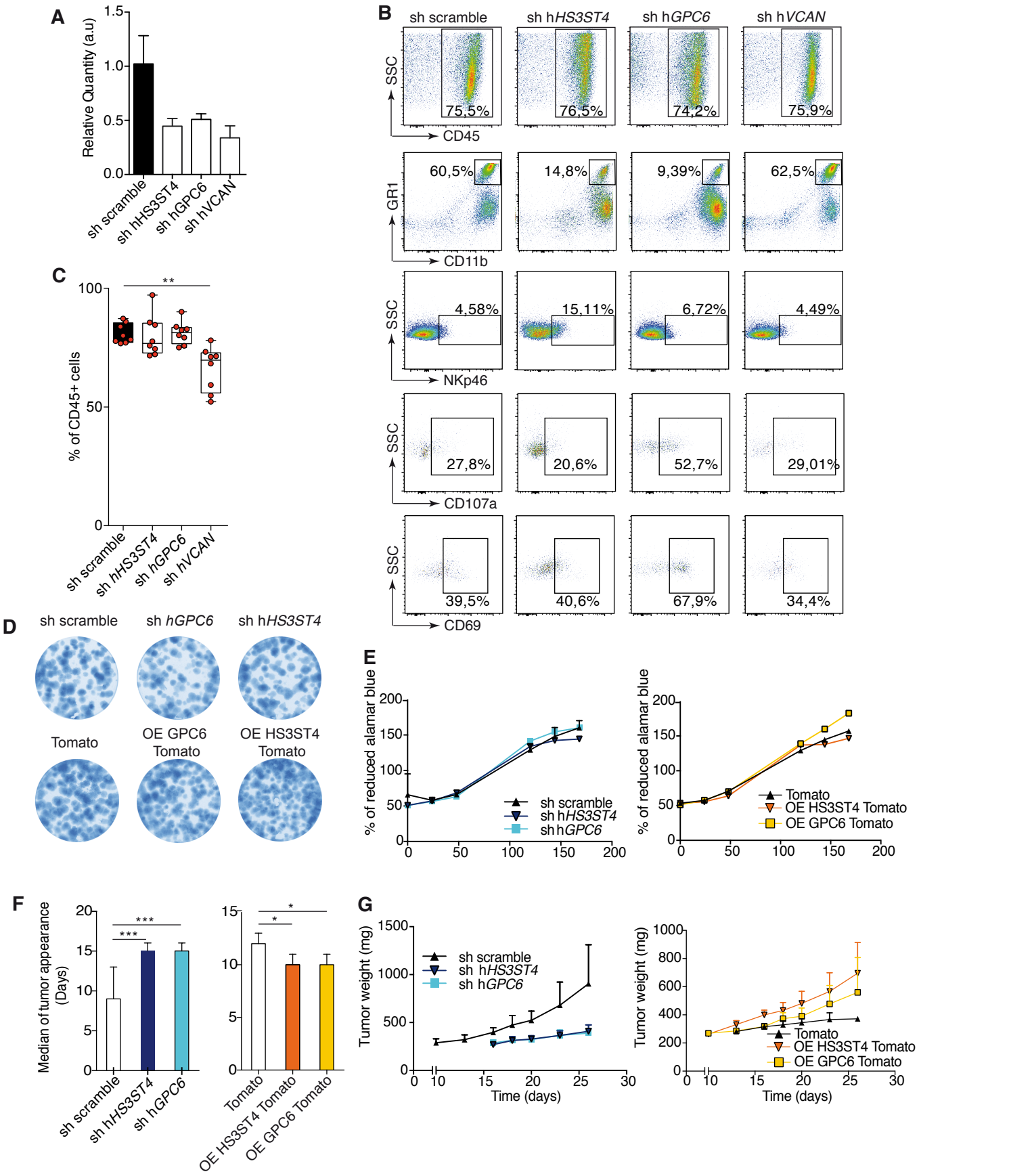
Appendix Table of Content

Appendix S1: HS3ST4, GPC6, and VCAN are effectors of the immunosuppressive and oncogenic properties of TRF2. Related to Fig 5

Appendix S2: TRF2 enhances glycocalyx stiffness, which activates MDSCs. Related to Fig 6

Appendix S3: TRF2 upregulation in human malignancies is associated with a poor prognosis. Related to Fig 7

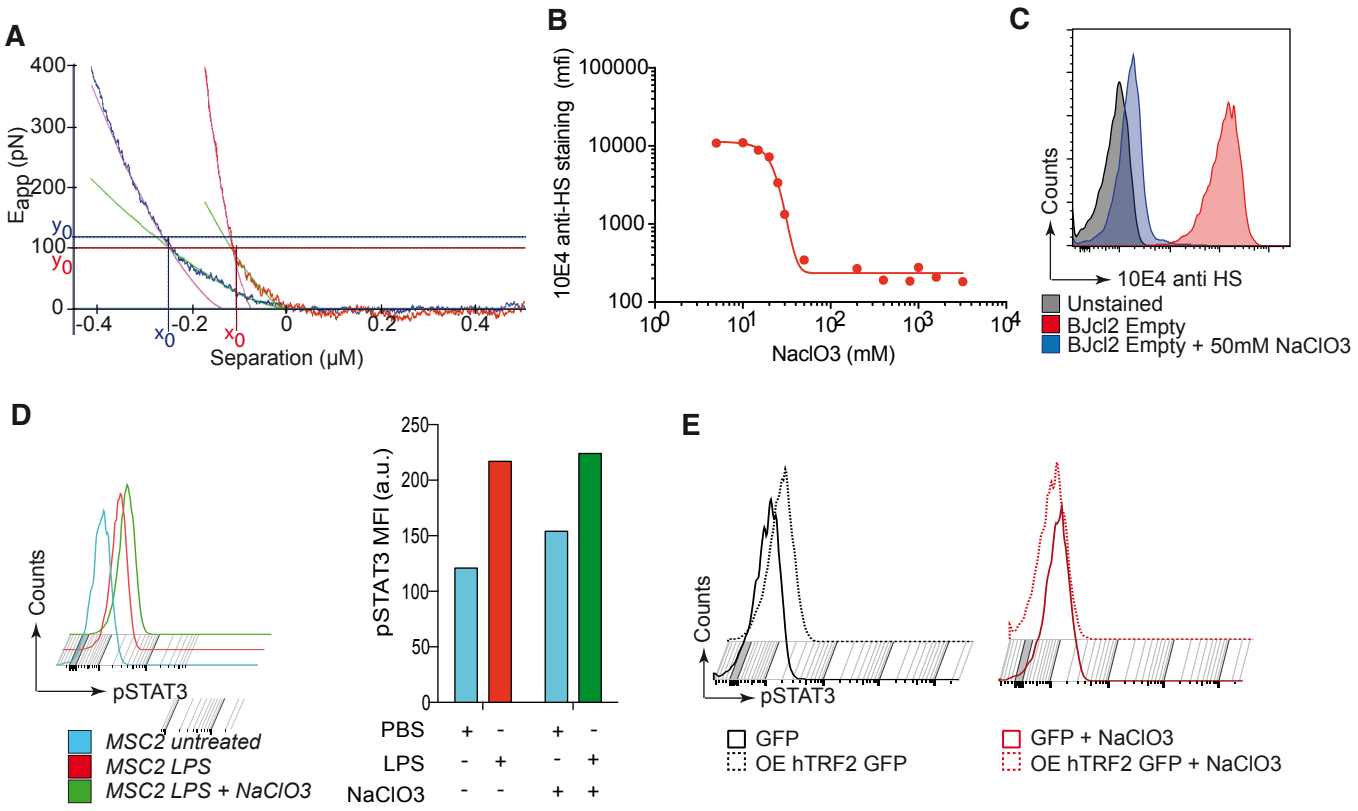
Appendix S4: TRF2 upregulation in human malignancies is associated with high expression of glycocalyx genes and MDSC infiltration. Related to Fig 8



Appendix S1: HS3ST4, GPC6, and VCAN are effectors of the immunosuppressive and oncogenic properties of TRF2. Related to Fig 5

A	qPCR analysis of the expression of TRF2 target genes following TRF2 knockdown.
B	Density plot of total immune cell infiltration (CD45), MDSCs, NK cell infiltration, and NK cell degranulation (CD107a) and activation (CD69), in relation to the box plots presented in Fig 5B.
C	Box plots presenting total immune cell infiltration (CD45+ cells) following TRF2 target gene knockdown.
D	Colony assay following TRF2 target gene knockdown or overexpression.
E	Growth curves obtained by AlamarBlue assay with BJcl2 cells overexpressing or knock down for HS3ST4 or GPC6.
F-G	Tumor growth experiment BJcl2 cells overexpressing or knock down for HS3ST4 or GPC6. Histograms presenting the median of tumor appearance (F) or the tumor weight over time after implantation (G).

Data information: data are presented as Box plot Min to Max showing all points with median or mean +/- SEM. (n = 8 mice per group; *p < 0.05, **p < 0.01, and ***p < 0.001; Mann–Whitney test).

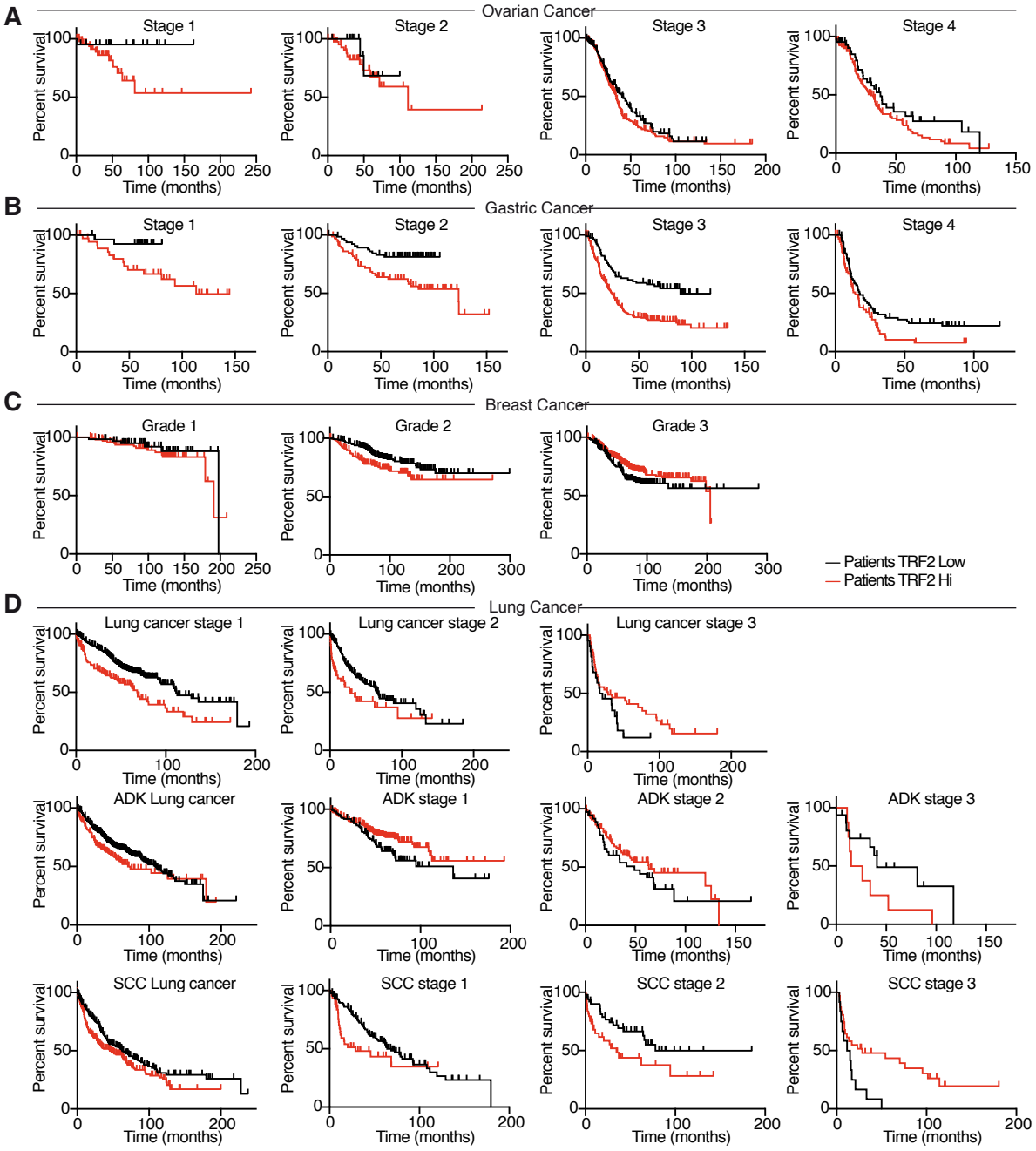


Appendix S2: TRF2 enhances glycocalyx stiffness, which activates MDSCs. Related to Fig 6.

A Atomic force microscopic determination of glycocalyx and cell stiffnesses and glycocalyx length.

B-C Dose-response analysis of Heparan sulfate (HS) synthesis inhibition by FACS using the 10E4 antibody. Increasing doses of NaClO3 were applied on BJcl2 cells and the expression level of HS at cell surface were determined by FACS.

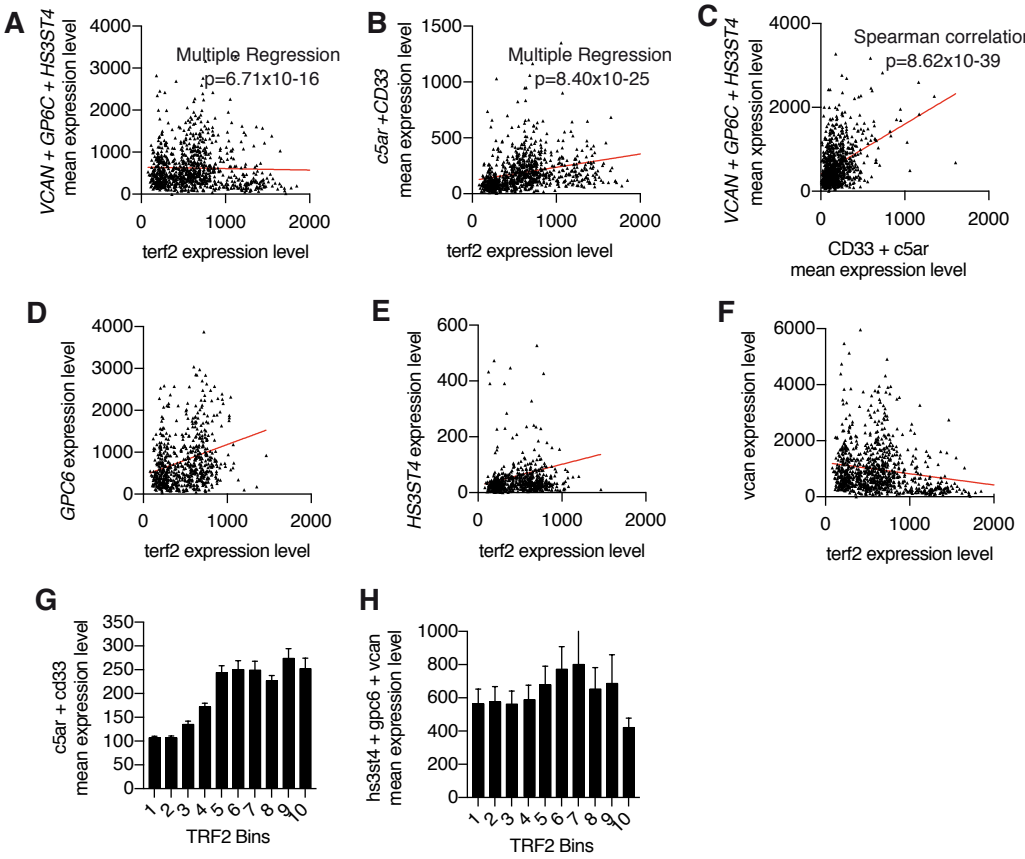
D-E Impact of HS synthesis inhibition by NaClO3 on STAT3 phosphorylation. Histograms (D, left panels) presenting unstained cells or cells incubated with the isotype control antibody in the FACS analysis; histograms of the mean MFIs for MSC2 cells untreated, LPS-treated (10 μM), or LPS- and sodium chlorate (NaClO3)-treated (D, right panel) or after co-culture with TRF2 overexpressing or compromised BJcl2 (E).



Appendix S3: TRF2 upregulation in human malignancies is associated with a poor prognosis

A–D Down-staging analysis of Kaplan-Meier curves for overall survival of ovarian (A), gastric (B), breast (C), and lung cancer (D) patients.

Data information: In (A-B), the cancer genome atlas (TCGA) data were analyzed via the CbioPortal website (http://bit.ly/2tfFAtK). http://bit.ly/2tfFAtK). In (C-F), the optimal cut-off is determined on KMplot. The p value (log Rank test), the Hazard Ratio and number of patients are indicated in Table 6.



I

CD33 C5AR analysis	Chi square	df	p value	Significant
all	66.67	3	<0,0001	****
CD33+ C5AR+ LOLO vs HIHI	47.44	1	<0,0001	****
CD33+ C5AR+ LOLO vs LoHI	1.627	1	0.2021	ns
CD33+ C5AR+ LOLO vs HILO	20.83	1	<0,0001	****
CD33+ C5AR+ HILO vs HIHI	0.4308	1	0.5116	ns
CD33+ C5AR+ LOHI vs HIHI	27.35	1	<0,0001	****
CD33+ C5AR+ LOHI vs HILO	10.25	1	0.0014	**

J

TRF2 + Target genes + MDSC signature	Chi square	df	p value	Significant
all	86.4	5	<0,0001	****
Target gene Hi mdsc hi vs TRF2 hi Target gene Hi MDSC hi	0.1172	1	0.7321	ns
Target gene Hi mdsc hi vs Target gene Lo MDSC Lo	29.07	1	<0,0001	****
Target gene Hi mdsc hi vs TRF2 Lo Target gene Lo MDSC Lo	32.01	1	<0,0001	****
TRF2 Hi Target gene Hi mdsc hi vs TRF2 Lo Target gene Lo MDSC Lo	30.13	1	<0,0001	****
TRF2 Hi Target gene Hi mdsc hi vs Target gene Lo MDSC Lo	27.19	1	<0,0001	****
Target gene Lo mdsc Lo vs TRF2 Lo Target gene Lo MDSC Lo	0.2456	1	0.6202	ns

Appendix S4: TRF2 upregulation in human malignancies is associated with high expression of glycocalyx genes and MDSC infiltration

A-f Analysis of the correlation between TRF2 expression value and the expression value of TRF2 target gene (HS3ST4 or GPC6 or VCAN) or the mean expression level of HS3ST4, GPC6 and VCAN or the mean expression level of CD33 and C5AR (MDSC signature) or the correlation between the two means.

G-H Representation of the expression value of the mean expression level of CD33 and C5AR (MDSC signature) or the mean expression level of HS3ST4, GPC6 and VCAN depending on TRF2 bins.

I-J Chi2 analysis using GraphPad (Prisme).

Data informations: In (G-H), data comes from KM-plotter, http://kmplot.com/analysis/index.php?p=background.
In (K-P), multiple regression was determined to compare TRF2 expression level and the means or Pearsonn correlation to compare the two means (impossibility to use multiple regression to compare 2 means). p values and test type are indicated.