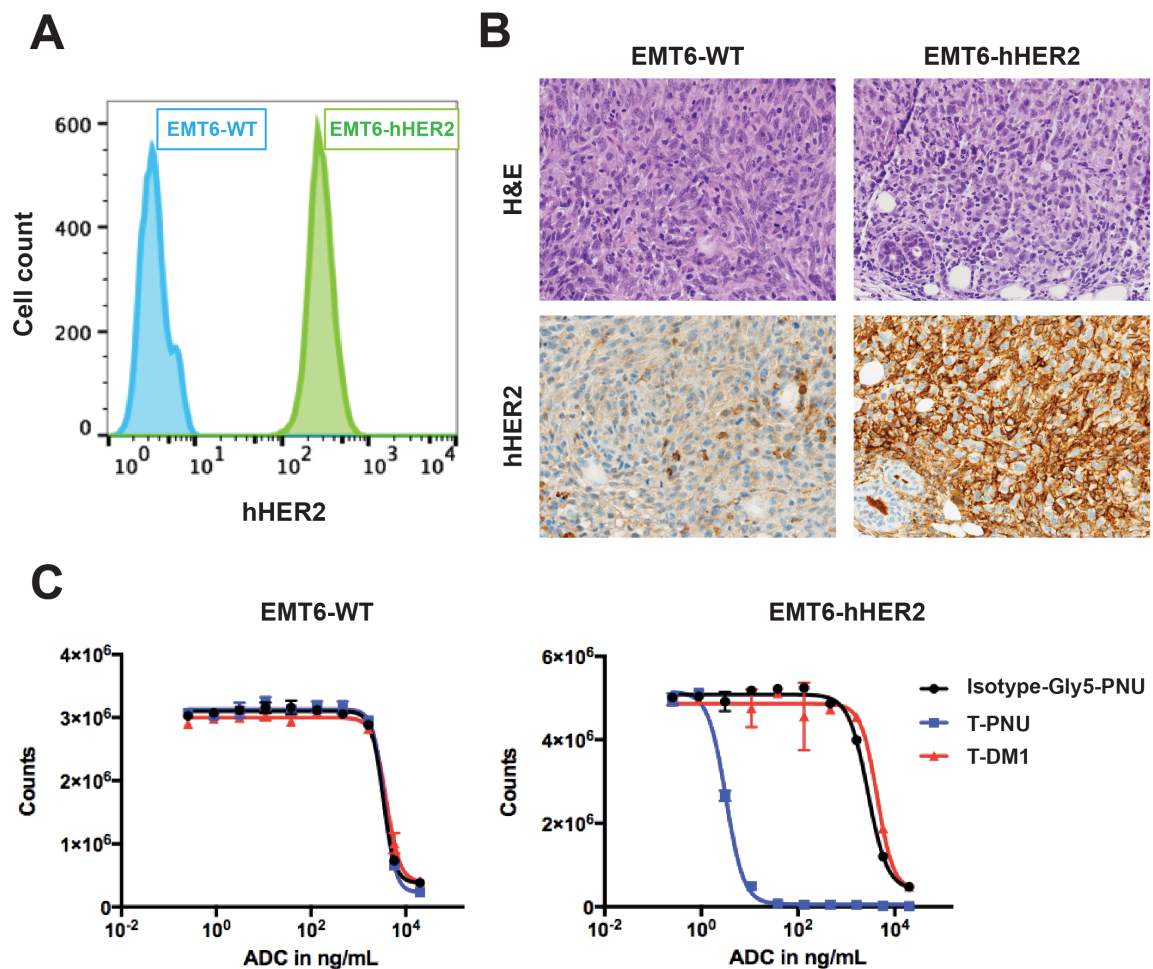


## Supplementary Material

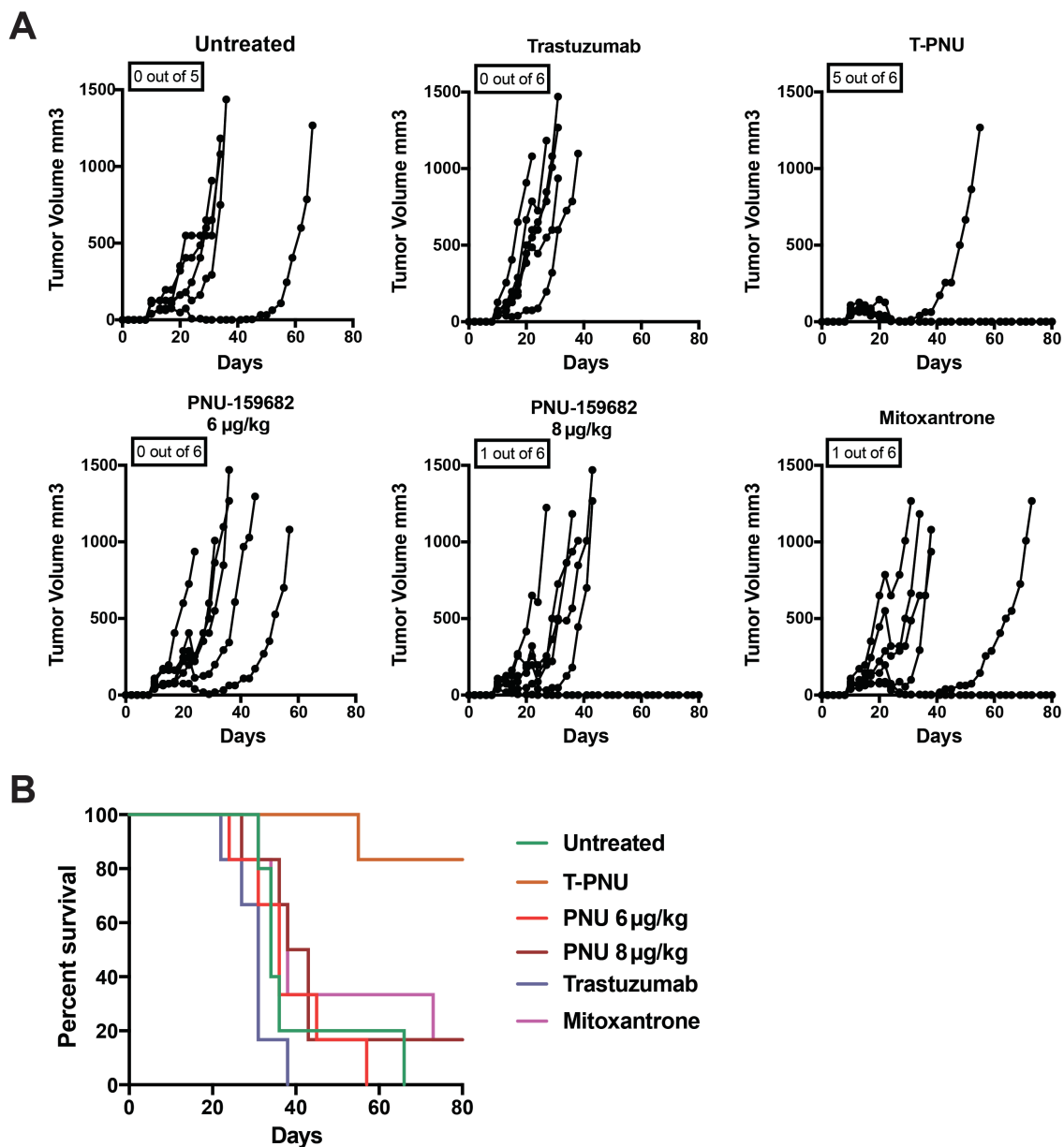
### Supplementary Figure 1.

Generation of a murine EMT6-hHER2 breast cancer cell line. **A**, Human-HER2 expression in EMT6-hHER2 or EMT6-WT tumor cell line determined by FACS. **B**, H&E (top panel) and  $\alpha$ -hHER2 (bottom panel) immunohistochemical staining of orthotopic EMT6-WT and EMT6-hHER2 tumors. Images were acquired at 400X magnification. **C**, Cell proliferation assay showing EMT6-WT and EMT6-hHER2 growth inhibition after T-PNU or T-DM1 treatment *in vitro*.



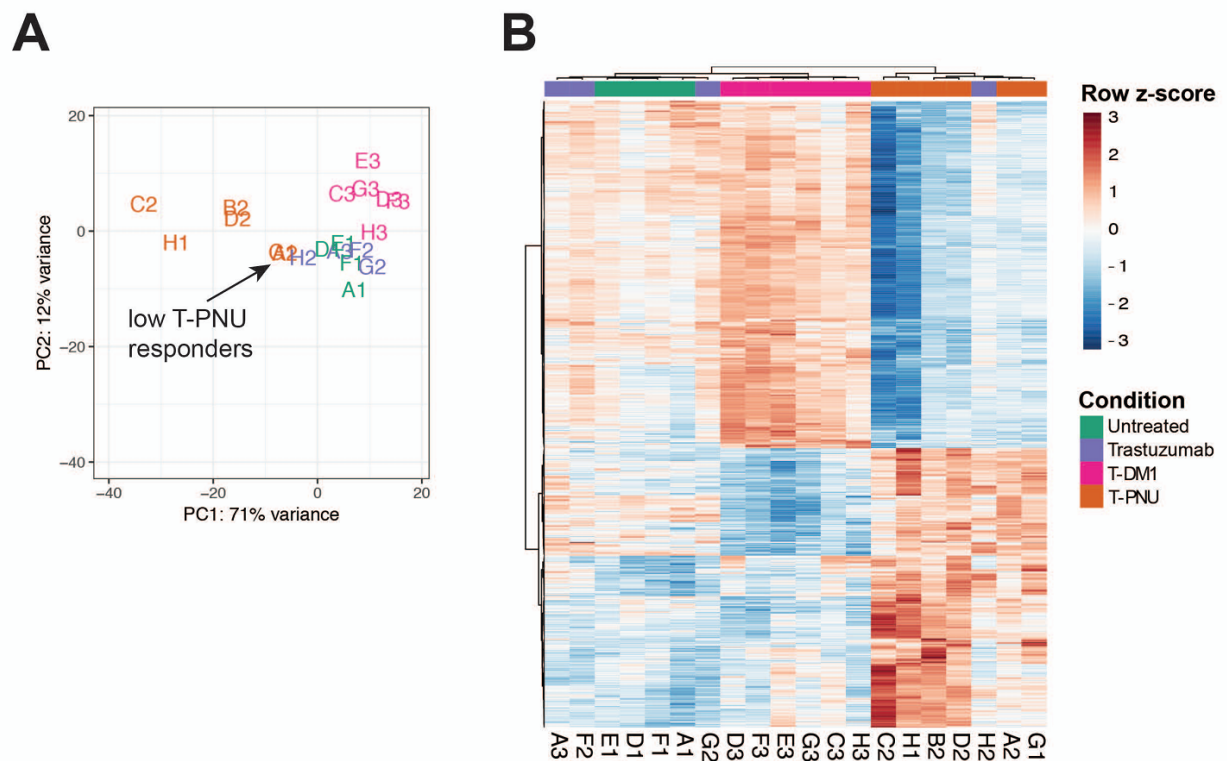
## Supplementary Figure 2.

Trastuzumab, free PNU or mitoxantrone treatment is ineffective in promoting EMT6-hHER2 tumor growth inhibition. **A**, Therapeutic response of EMT6-hHER2 tumors exposed to T-PNU (1 mg/kg, 2x), free PNU (6 and 8  $\mu$ g/kg, 2x), trastuzumab (20 mg/kg, 4x, once per week) and mitoxantrone (4 mg/kg 1x). One representative experiment is shown (n=number of cured mice out of treated animals as indicated). **B**, Survival curves of **A**.



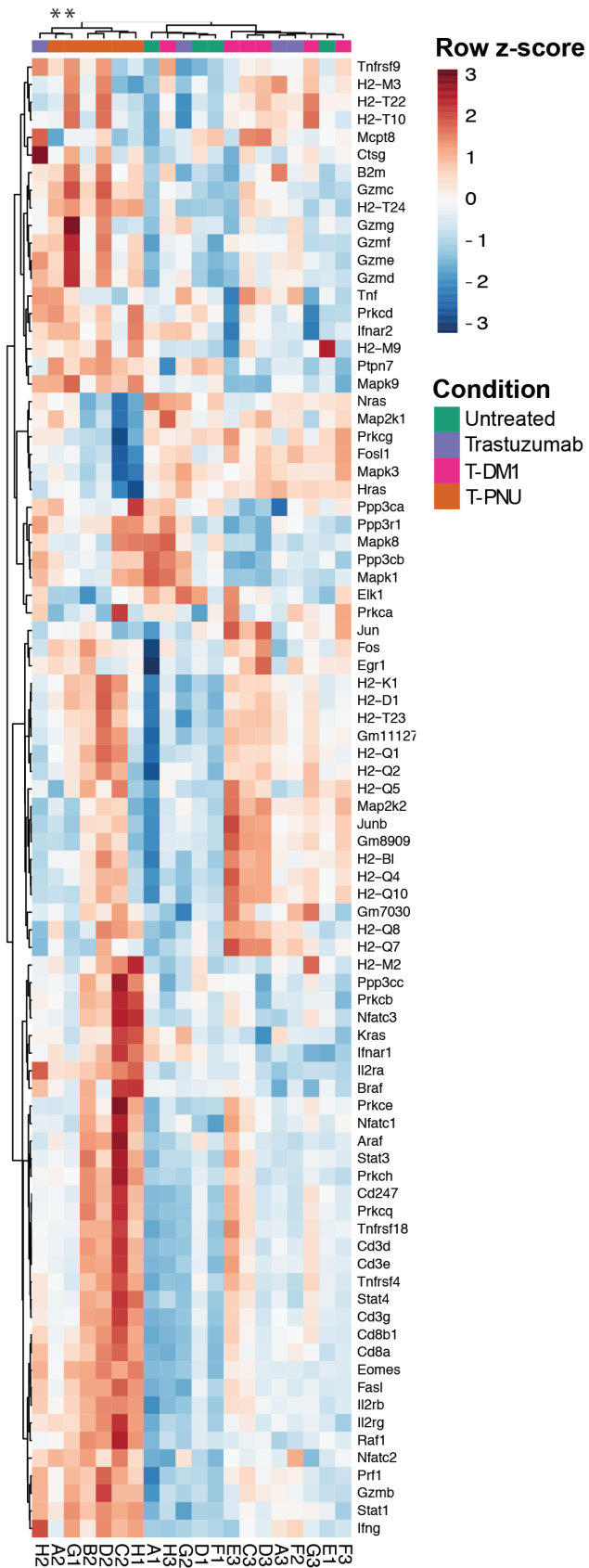
### Supplementary Figure 3.

T-PNU samples cluster apart from untreated, trastuzumab and T-DM1 treated samples based on gene expression. **A**, Sample similarity in a 2D projection by multi-dimensional scaling. Only the top 1,000 differentially expressed genes (DEGs) are taken into account. **B**, Heatmap of the gene expression vs sample matrix. Displayed are custom selected genes plus the top 1,000 DEGs (rows) of all samples (columns).



### Supplementary Figure 4.

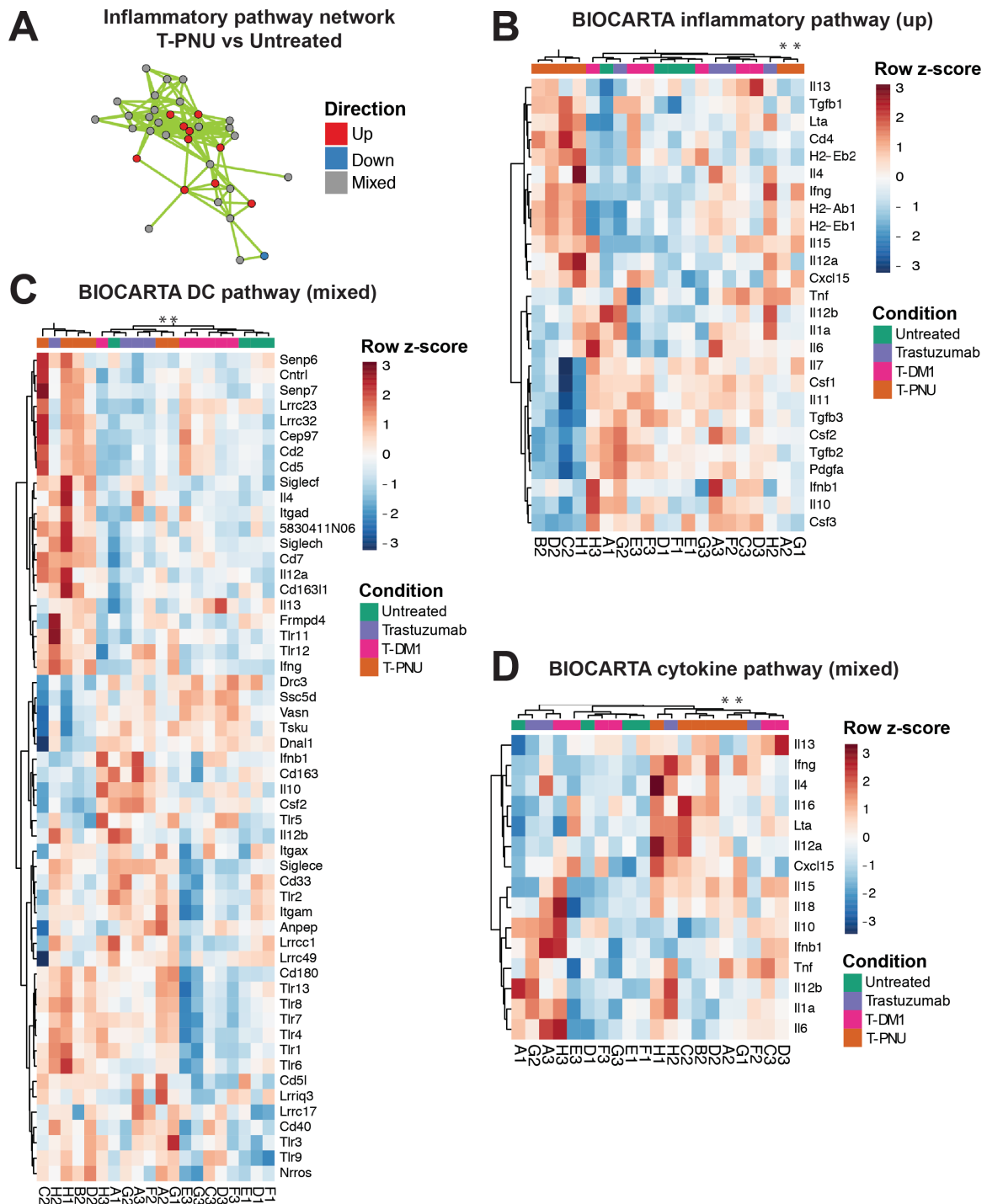
Heatmap PID CD8 TCR downstream pathway.





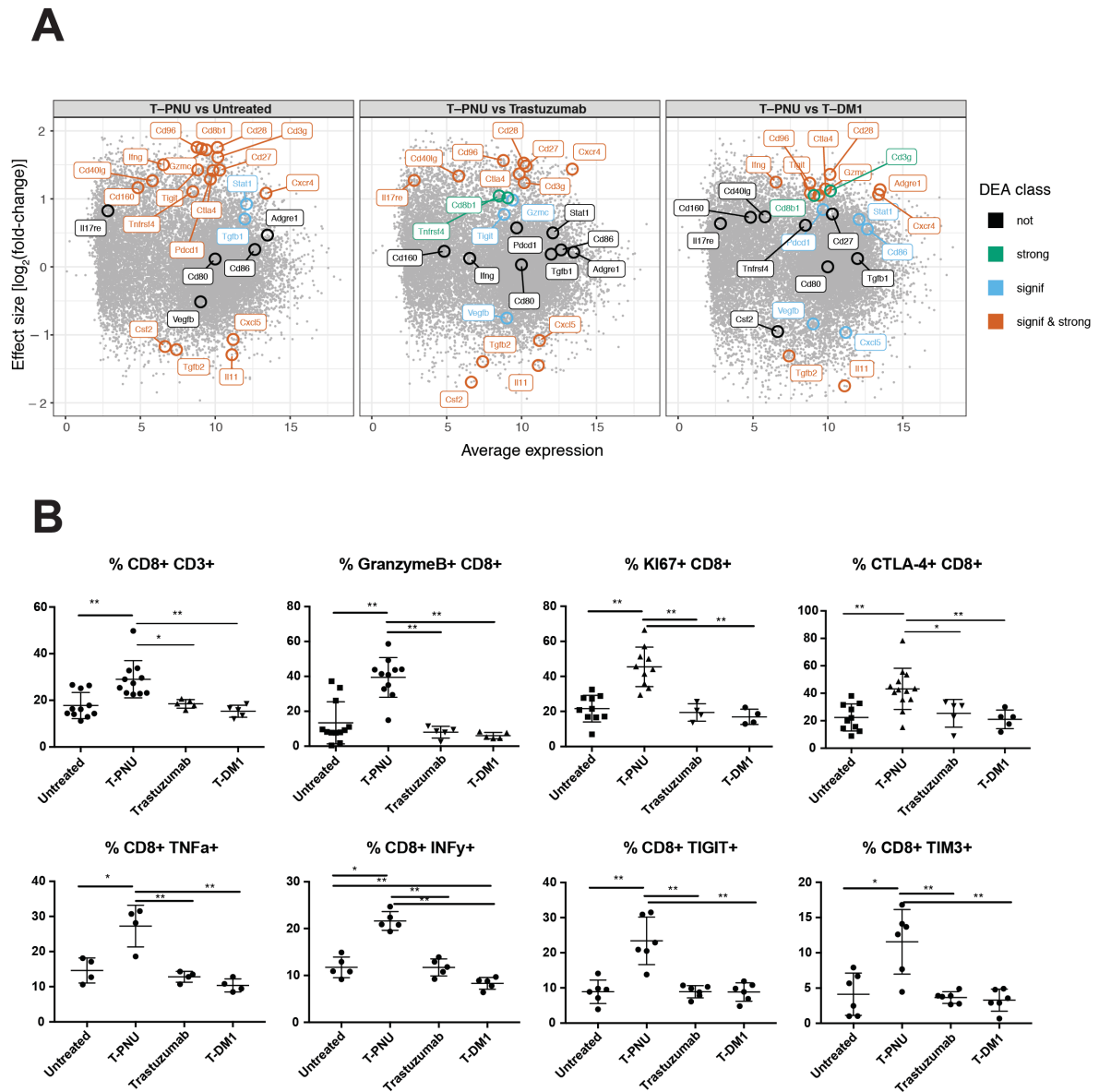
### Supplementary Figure 5.

Gene signatures of innate immune and cytokine pathways. **A**, Network cluster of gene sets with overlapping genes with shared function of inflammatory pathway. The network includes 36 gene sets and 7,551 genes (see Supplementary Table S2). **B-D**, Heatmaps of BIOCARTE inflammatory, dendritic cell (DC) and cytokine pathway, respectively. Asterisks denote low-responding T-PNU samples.



## Supplementary Figure 6.

Characterization of intratumoral T cells upon treatment. \* $p \leq 0.05$ , \*\* $p \leq 0.01$ . **A**, MvA plot depicting expression and effect size of selected key immune genes. For each comparison, the fold-change is set in relation to the average expression of each gene. The labels indicate genes of interest. **B**, Validation by FACS of selected CD8 T cell markers of functional activation and proliferation identified in **A** in between the comparisons.



**Supplementary Table 1.**  
Gene sets of network TCR pathway.

Name	NGenes	Direction	FDR.Mixed
BIOCARTA TCRA PATHWAY	36	Up	1.4E-05
REACTOME COSTIMULATION BY THE CD28 FAMILY	126	Mixed	2.5E-05
REACTOME CD28 CO STIMULATION	69	Mixed	3.5E-05
REACTOME CD28 DEPENDENT VAV1 PATHWAY	39	Mixed	1.2E-04
BIOCARTA CTLA4 PATHWAY	39	Mixed	5.2E-04
REACTOME CD28 DEPENDENT PI3K AKT SIGNALING	54	Mixed	5.3E-05
REACTOME REGULATION OF SIGNALING BY CBL	39	Mixed	2.3E-04
REACTOME TCR SIGNALING	105	Up	8.9E-06
REACTOME DOWNSTREAM TCR SIGNALING	69	Up	8.4E-06
REACTOME PHOSPHORYLATION OF CD3 AND TCR ZETA CHAINS	30	Mixed	7.0E-07
REACTOME PECAM1 INTERACTIONS	39	Mixed	4.4E-04
REACTOME TRANSLOCATION OF ZAP 70 TO IMMUNOLOGICAL SYNAPSE	27	Up	8.0E-07
REACTOME GENERATION OF SECOND MESSENGER MOLECULES	48	Up	1.5E-06
REACTOME PD1 SIGNALING	36	Up	8.0E-07
BIOCARTA CDC42RAC PATHWAY	39	Down	1.4E-05
PID EPHA2 FWD PATHWAY	60	Mixed	4.4E-06
BIOCARTA IL7 PATHWAY	45	Mixed	3.1E-04
PID ECADHERIN KERATINOCYTE PATHWAY	57	Mixed	1.6E-04
PID EPHRINB REV PATHWAY	75	Up	3.6E-05
REACTOME CTLA4 INHIBITORY SIGNALING	57	Mixed	1.4E-04
PID NFKAPPAB ATYPICAL PATHWAY	51	Mixed	4.3E-05
PID NETRIN PATHWAY	81	Mixed	7.0E-06
PID EPHA FWDPATHWAY	75	Up	6.3E-05
REACTOME REGULATION OF KIT SIGNALING	36	Mixed	1.5E-03
BIOCARTA EPHA4 PATHWAY	33	Mixed	2.1E-05
REACTOME DCC MEDIATED ATTRACTIVE SIGNALING	33	Mixed	4.8E-05
PID NEPHRIN NEPH1 PATHWAY	57	Mixed	7.7E-05
PID SYNDECAN 2 PATHWAY	114	Mixed	2.5E-05
BIOCARTA TCAPOPTOSIS PATHWAY	18	Up	9.0E-07
BIOCARTA SALMONELLA PATHWAY	27	Down	4.4E-05
BIOCARTA ACTINY PATHWAY	39	Down	4.1E-06
PID NECTIN PATHWAY	84	Mixed	6.4E-05
PID P38 ALPHA BETA PATHWAY	66	Mixed	3.4E-05
PID CDC42 PATHWAY	171	Mixed	1.0E-04
PID ARF6 PATHWAY	87	Mixed	1.1E-04
PID ECADHERIN NASCENT AJ PATHWAY	117	Mixed	6.5E-05
REACTOME NETRIN1 SIGNALING	57	Mixed	5.8E-05
BIOCARTA CBL PATHWAY	30	Mixed	1.1E-03
BIOCARTA RAC1 PATHWAY	54	Down	3.0E-06
BIOCARTA RHO PATHWAY	81	Mixed	6.6E-05
PID ECADHERIN STABILIZATION PATHWAY	105	Mixed	1.5E-04
PID NCADHERIN PATHWAY	102	Mixed	6.0E-05
PID RAC1 PATHWAY	129	Down	1.3E-05
REACTOME DSCAM INTERACTIONS	24	Down	6.1E-05

## Supplementary Table 2.

### Gene sets of network activated TLR pathway.

Name	NGenes	Direction	FDR.Mixed
BIOCARTA NFKB PATHWAY	57	Mixed	8.9E-05
BIOCARTA TOLL PATHWAY	84	Mixed	4.8E-05
REACTOME TAK1 ACTIVATES NFKB BY PHOSPHORYLATION AND ACTIVATION OF IKKS COMPLEX	39	Mixed	3.2E-05
REACTOME TRIF MEDIATED TLR3 SIGNALING	147	Up	3.9E-05
REACTOME NFKB AND MAP KINASES ACTIVATION MEDIATED BY TLR4 SIGNALING REPERTOIRE	135	Up	2.9E-05
REACTOME MYD88 MAL CASCADE INITIATED ON PLASMA MEMBRANE	156	Up	2.8E-05
REACTOME ACTIVATED TLR4 SIGNALLING	171	Mixed	3.8E-05
BIOCARTA IL1R PATHWAY	81	Mixed	3.5E-05
BIOCARTA NTHI PATHWAY	63	Mixed	3.0E-05
BIOCARTA STRESS PATHWAY	63	Down	1.5E-04
REACTOME TRAF6 MEDIATED INDUCTION OF NFKB AND MAP KINASES UPON TLR7 8 OR 9 ACTIVATION	156	Mixed	2.7E-05
KEGG TOLL LIKE RECEPTOR SIGNALING PATHWAY	177	Mixed	1.7E-05
BIOCARTA RELA PATHWAY	42	Mixed	7.3E-05
REACTOME RIP MEDIATED NFKB ACTIVATION VIA DAI	36	Mixed	1.0E-04
BIOCARTA CD40 PATHWAY	48	Mixed	8.7E-05
BIOCARTA TNFR2 PATHWAY	57	Mixed	7.3E-05
PID TOLL ENDOGENOUS PATHWAY	27	Down	6.3E-04
REACTOME MAP KINASE ACTIVATION IN TLR CASCADE	99	Up	2.4E-05
REACTOME TRAF6 MEDIATED NFKB ACTIVATION	45	Mixed	1.9E-04
BIOCARTA 41BB PATHWAY	39	Mixed	4.9E-05
REACTOME MAPK TARGETS NUCLEAR EVENTS MEDIATED BY MAP KINASES	66	Mixed	3.8E-05
REACTOME TRAF6 MEDIATED IRF7 ACTIVATION	33	Mixed	4.2E-04
REACTOME TRAF6 MEDIATED IRF7 ACTIVATION IN TLR7 8 OR 9 SIGNALING	21	Mixed	1.0E-04
REACTOME TRAF6 MEDIATED INDUCTION OF TAK1 COMPLEX	21	Up	5.6E-03
BIOCARTA RNA PATHWAY	36	Mixed	4.9E-05
BIOCARTA TALL1 PATHWAY	42	Mixed	1.9E-04
REACTOME NUCLEAR EVENTS KINASE AND TRANSCRIPTION FACTOR ACTIVATION	54	Mixed	2.8E-05
REACTOME ERK MAPK TARGETS	45	Mixed	1.0E-05
REACTOME IL1 SIGNALING	81	Mixed	7.4E-06
KEGG RIG I LIKE RECEPTOR SIGNALING PATHWAY	93	Mixed	1.4E-04
BIOCARTA DC PATHWAY	57	Mixed	6.1E-05
PID NFKAPPAB CANONICAL PATHWAY	45	Up	3.6E-04
REACTOME ACTIVATION OF IRF3 IRF7 MEDIATED BY TBK1 IKK EPSILON	21	Mixed	6.5E-04
BIOCARTA CYTOKINE PATHWAY	30	Mixed	2.7E-04
REACTOME NOD1 2 SIGNALING PATHWAY	48	Mixed	4.8E-05
REACTOME NUCLEOTIDE BINDING DOMAIN LEUCINE RICH REPEAT CONTAINING RECEPTOR NLR SIGNALING PATHWAYS	69	Mixed	3.1E-05
KEGG CYTOSOLIC DNA SENSING PATHWAY	81	Up	1.1E-04
BIOCARTA INFLAM PATHWAY	48	Up	1.3E-05
PID CD40 PATHWAY	81	Mixed	3.3E-05
REACTOME TRAFFICKING AND PROCESSING OF ENDOSOMAL TLR	39	Mixed	5.7E-05
REACTOME ERKS ARE INACTIVATED	27	Up	1.1E-06
REACTOME ACTIVATED TAK1 MEDIATES P38 MAPK ACTIVATION	24	Mixed	2.2E-04
PID IL1 PATHWAY	84	Up	2.3E-05
PID TNF PATHWAY	111	Mixed	1.1E-05
REACTOME JNK C JUN KINASES PHOSPHORYLATION AND ACTIVATION MEDIATED BY ACTIVATED HUMAN TAK1	24	Up	3.6E-04
KEGG NOD LIKE RECEPTOR SIGNALING PATHWAY	102	Up	1.3E-04
KEGG PATHOGENIC ESCHERICHIA COLI INFECTION	111	Mixed	5.6E-05
KEGG LEISHMANIA INFECTION	138	Mixed	2.1E-05
REACTOME REGULATION OF IFNA SIGNALING	27	Mixed	1.9E-05
REACTOME RIG I MDA5 MEDIATED INDUCTION OF IFN ALPHA BETA PATHWAYS	99	Mixed	2.2E-04
BIOCARTA TID PATHWAY	63	Up	8.7E-05
BIOCARTA GSK3 PATHWAY	75	Mixed	1.5E-05
REACTOME PLATELET SENSITIZATION BY LDL	42	Mixed	1.3E-04
REACTOME TRAF3 DEPENDENT IRF ACTIVATION PATHWAY	27	Mixed	2.0E-04
REACTOME NFKB ACTIVATION THROUGH FADD RIP1 PATHWAY MEDIATED BY CASPASE 8 AND10	21	Mixed	7.9E-04
BIOCARTA ATM PATHWAY	57	Mixed	1.6E-04
BIOCARTA RANKL PATHWAY	48	Mixed	5.8E-05
BIOCARTA EPONFKB PATHWAY	45	Up	2.2E-05
BIOCARTA ARENRF2 PATHWAY	18	Mixed	7.0E-04
BIOCARTA STEM PATHWAY	18	Up	2.5E-05
REACTOME CTNNB1 PHOSPHORYLATION CASCADE	36	Mixed	4.7E-05
REACTOME ADVANCED GLYCOSYLATION ENDPRODUCT RECEPTOR SIGNALING	15	Mixed	8.3E-04
REACTOME THE NLRP3 INFLAMMASOME	21	Mixed	5.0E-05
REACTOME INFLAMMASOMES	24	Mixed	3.5E-05
BIOCARTA ERYTH PATHWAY	27	Up	3.0E-04
PID TCR JNK PATHWAY	27	Mixed	6.1E-05
REACTOME IRAK1 RECRUITS IKK COMPLEX	18	Mixed	2.9E-03