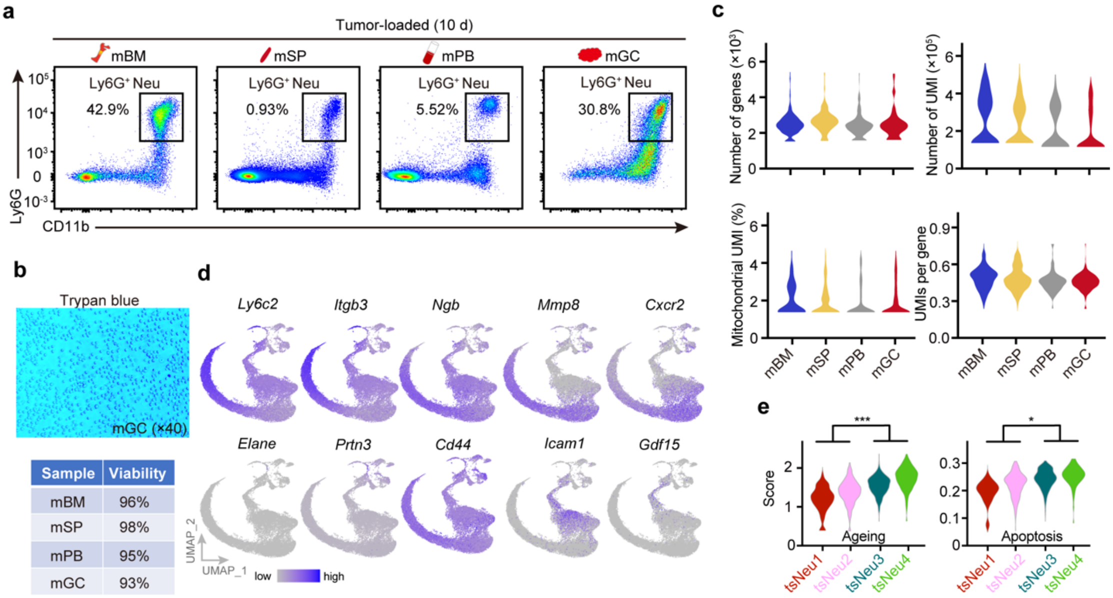
SUPPLEMENTAL INFORMATION

Single-Cell Analysis of Tumor-Specific Neutrophils During Gastric Cancer Progression Inspired a Hippo Targeted Antitumor Therapy

Pingping Nie, Weihong Zhang, Yan Meng, Fenghua Guo, Hui Zhang, Zhenzhu Tong, Meng Wang, Fan Chen, Liwei An, Yi Han, Wenjia Wang, Linxin Wei, Zhaocai Zhou, Shi Jiao

SUPPLEMENTAL FIGURES



Sample	Viability
mBM	96%
mSP	98%
mPB	95%
mGC	93%

Figure S1 Neutrophil Displays Transcriptomic Heterogeneity. (**a**) Ly6G-positive Neu in BM, SP, PB and GC sorted from three tumor-bearing mice according to the gating strategy. (**b**) The viability of Neutrophil from panel A was measured and then loaded on a 10×Chromium Single Cell Controller. (**c**) Violin plots of the number of genes, number of UMIs, mitochondria UMI percentage, and UMI per gene of all QC-passed cells in the indicated samples. (**d**) Transcriptional pattern of the indicated gene projected on the UMAP plot. (**e**) Violin plots of aging score and apoptosis score for the indicated cluster.

Related to **Figure 1**

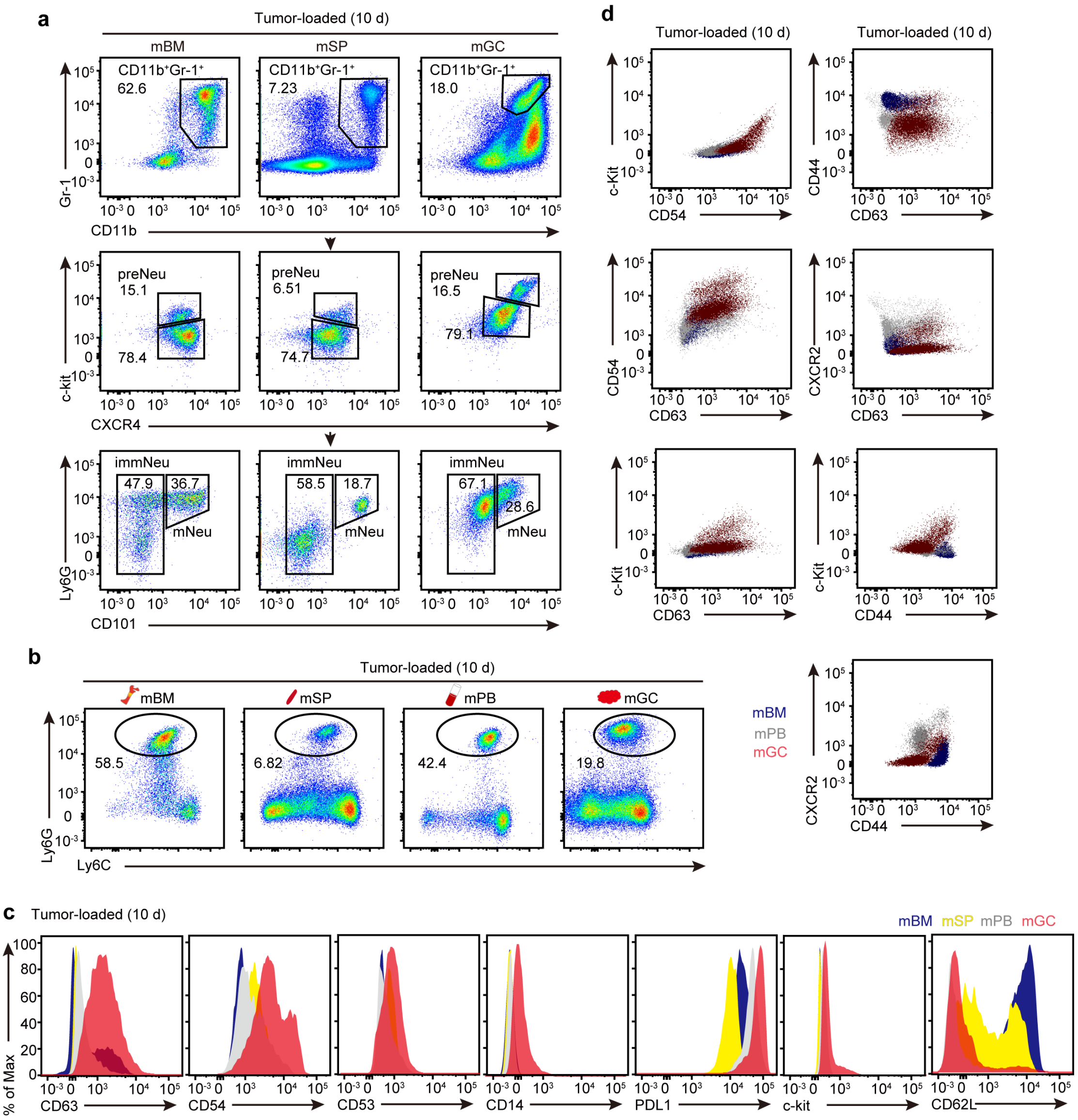


Figure S2 Neutrophil Displays Morphological and Functional Heterogeneity. (**a**) Representative FACS plots of preNeu, immNeu and mNeu from tumor-bearing mice according to the indicated gating strategy. (**b**) Representative FACS plots of total Neu (Ly6G$^+$Ly6C^{lo}) in BM, SP, PB and GC from tumor-bearing mice. (**c**) Histograms of the indicated surface marker in total Neu from the indicated samples. (**d**) Differential expression represented by plotting the indicated marker. Related to **Figure 2**

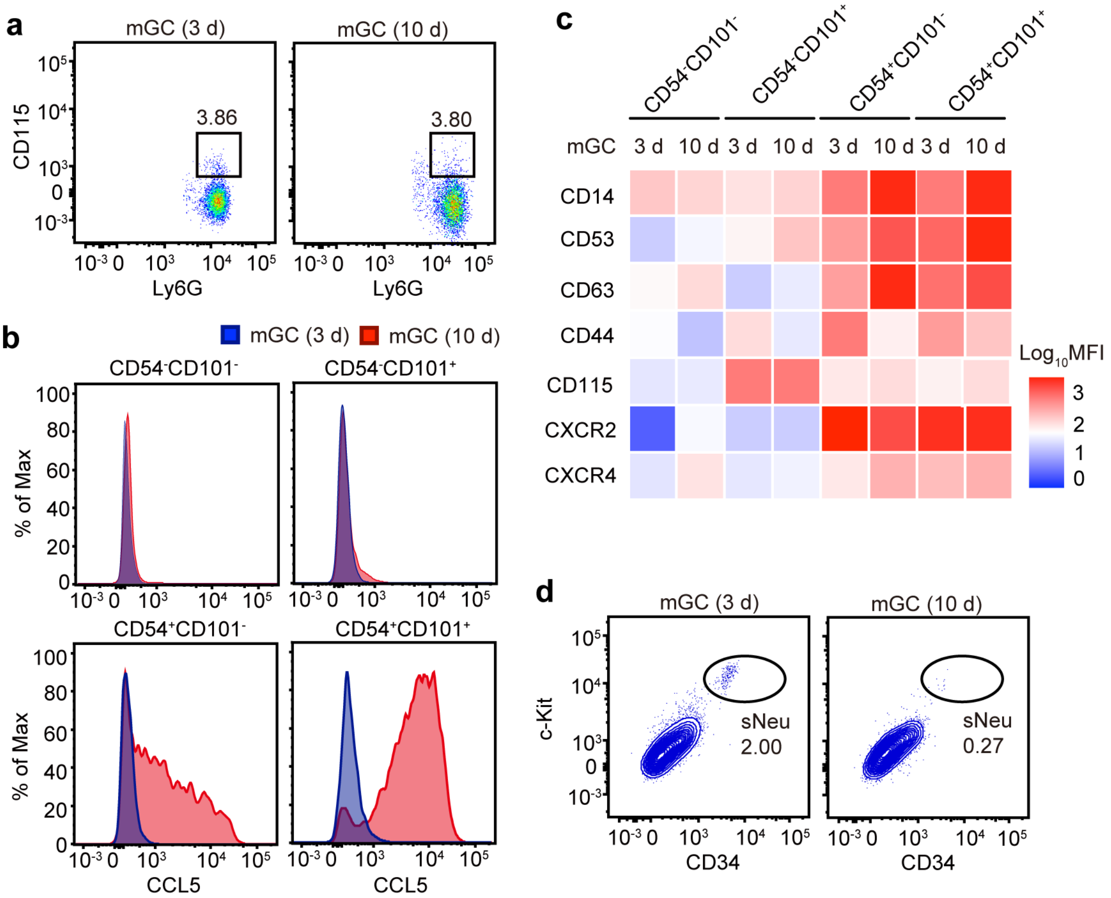


Figure S3 Trancing Neu Subpopulations in Tumor. (**a**) Expression of CD115 in Ly6G$^+$ cells. (**b**) Histograms of CCL5 in the various tsNeu subpopulations from mice bearing tumors for 3 days (blue) or 10 days (red). (**c**) Heatmap showing the expression of the indicated markers in the tsNeus from murine tumor tissues (3d or 10 d). (**d**) Representative FACS plots of sNeu from the indicated samples.

Related to **Figure 3**

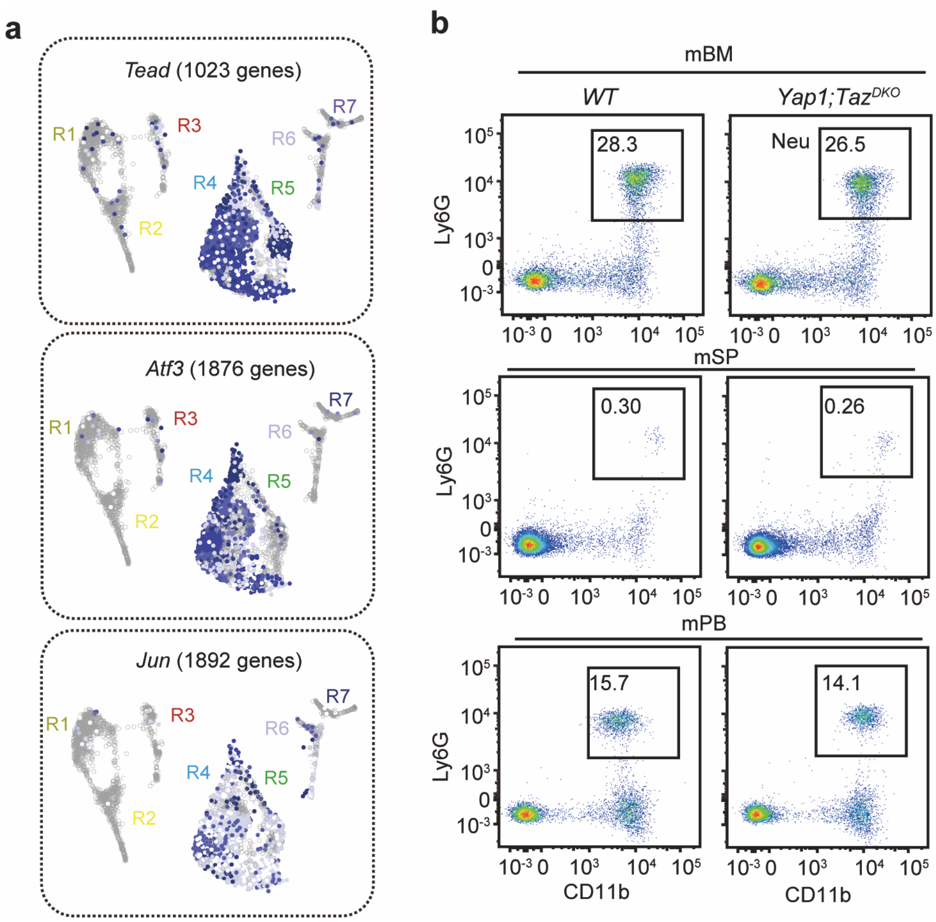


Figure S4 YAP/TAZ Deficiency Has no Influence on the Neutrophil Development in Bone Marrow, Spleen and Peripheral Blood. (**a**) Activities of the three identified tsNeu-associated regulons. Activities of *Teads*, *Arf3* and *Jun* projected on the UMAP plots. (**b**) CD11b$^+$Ly6G$^+$ Neu in bone marrow (mBM), spleen (mSP) and peripheral blood (mPB) from the indicated tumor-bearing mice.

Related to **Figure 4**

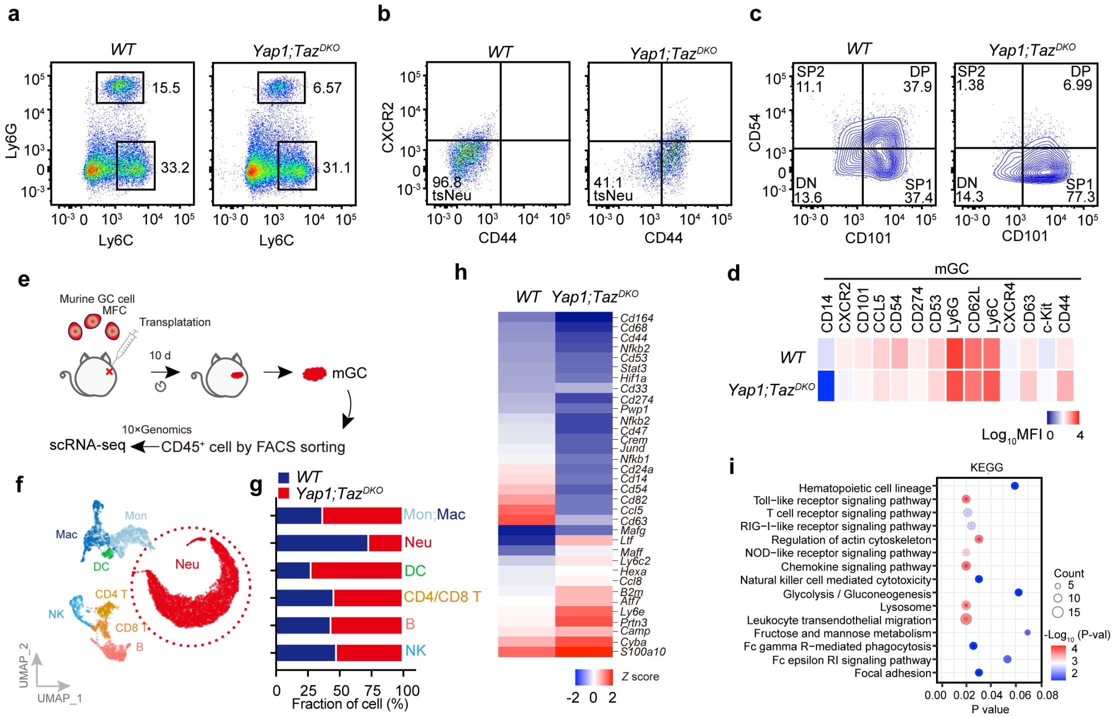


Figure S5 YAP/TAZ Deficiency Impairs the Development of tsNeu and Its Upstream Neutrophil Populations in Tumor. (**a**) CD11b^{+}Ly6G^{+} Neus in the tumor tissues from the *Yap1*$^{flox/flox}$*Taz*$^{flox/flox}$*LyzM*$^{cre/cre}$ mice and control mice. (**b**) preNeus in the tumor from the tumor-bearing *Yap1*$^{flox/flox}$*Taz*$^{flox/flox}$*LyzM*$^{cre/cre}$ mice and control mice. (**c**) GMPs (Lin^{-}c-Kit^{hi}CD11b^{-}CD34^{+}CD16/32^{+}), preNeu (Lin^{-}CD115^{-}Siglec^{-}c-Kit^{+}CXCR4^{+}), immNeu (Ly6G^{lo}CD101^{-}) and mNeu (Ly6G^{lo}CD101^{+}) were gated from tumor-bearing *Yap1*$^{flox/flox}$*Taz*$^{flox/flox}$*LyzM*$^{cre/cre}$ mice and control mice. (**d**) UMAP plots of CD45^{+} immune cells colored by the indicated mice (**e**) UMAP plot showing Neu subsets in the tumor tissues. (**f**) Dot plot showing the frequency of Neu populations from the indicated mice.

Related to **Figure 5**

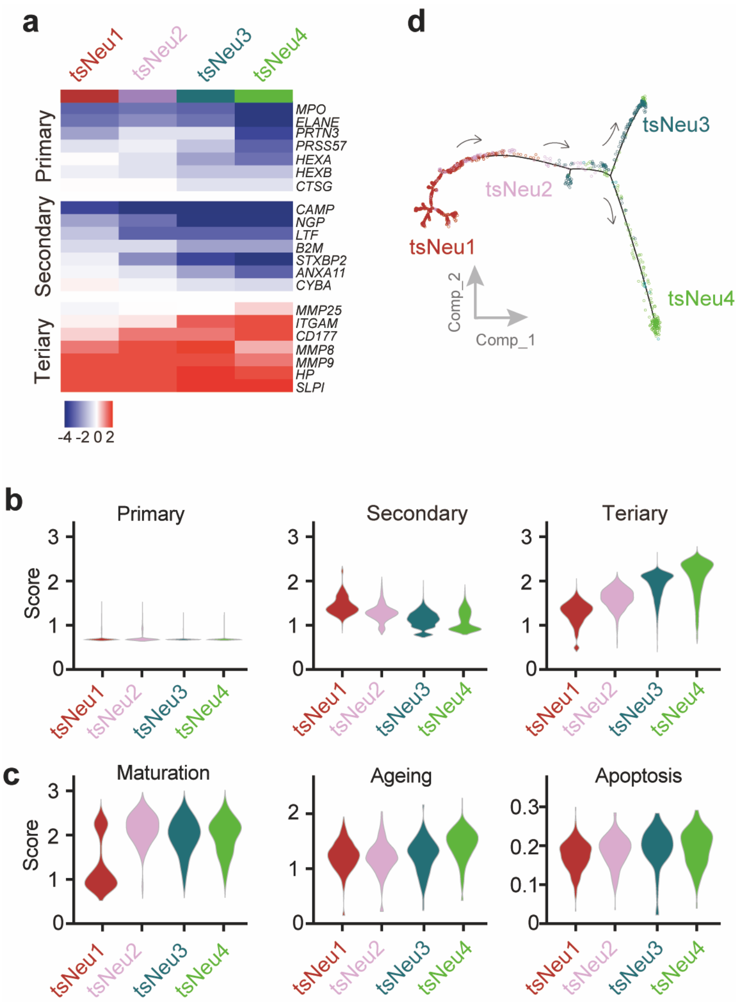


Figure S6 sc-Seq of Human GC Samples Characterizes Four tsNeu Subsets. (**a**) Heatmap showing the expression of selected functionally relevant gene. (**b,c**) Violin plots of primary (azurophil) score, secondary (specific) score, teriary (gelatinase score and secretory) score, mature score, aging score and apoptosis score for the Neu clusters. (**d**) Trajectory prediction for the tsNeu subsets.

Related to **Figure 6**

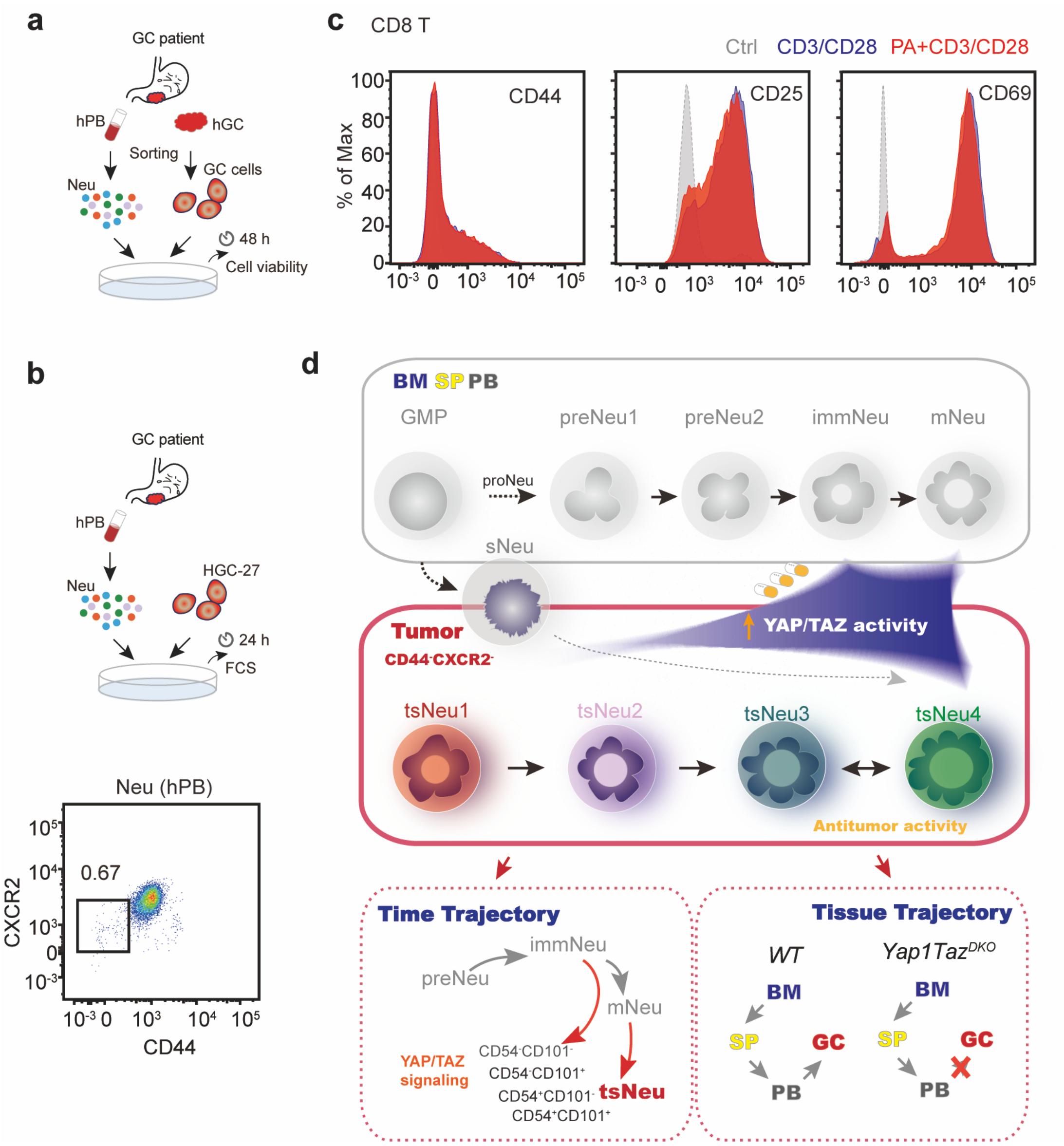


Figure S7 Hippo Agonist Reprograms Neutrophils for Anti-tumor Immunotherapy. (**a**) Experimental workflow of *in vitro* human neutrophil tumor-killing assay after incubation with PA. (**b**) $CD44^-CXCR2^-$ Neu in PA-treated peripheral blood from GC patient. (**c**) CD3/CD28-induced T activation in the splenic $CD8^+$ T cells after treatment with PA for 3 days. (**d**) A Schematic Illustration for the Neu Therapy. Our study defines $CXCR2^{neg}CD44^{neg}$ neutrophils as tsNeus, which can be further classified/separated into four subsets according to their expression of CD54 and CD101. Molecular dissection of tsNeus identified the Hippo pathway transcriptional coactivators YAP/TAZ as essential factors for the generation and function of teNeus. By pharmacological targeting the Hippo pathway, we show a proof of concept being able to reprogram neutrophils for anti-tumor immunotherapy.

Related to **Figure 7**