



**Supplemental figure7. Tumor-secreted NAMPT is responsible for the expression of SIRT1 on Naged, promoting pre-metastatic niche formation.**

(A) Schematic illustrating irradiation and hematopoietic stem cells (HSCs) transplantation. (B) Flow cytometry analysis (left panel) and quantification (right panel) of bone marrow (BM) -infiltrating CD45<sup>+</sup> cells from BM reconstruction mice. (C) Verification of the level of the SIRT1 protein in different bone marrow cells (BMCs) reconstruction *in vitro*. Mock, untreated HSCs. sh1-3, *SIRT1* knockdown in HSCs by shSIRT1 lentivirus targeting different regions. (D) Verification of the level of the SIRT1 protein in different BMCs reconstruction mice *in vivo*. (E) Flow cytometry analysis (left panel) and quantification (right panel) of lung-infiltrating neutrophils from 2-week tumor-bearing mice with BM reconstruction. (F) Analysis of the correlations between NAMPT expression in primary tumors with overall survival (OS) and relapse-free survival (RFS) in patients with breast cancer from the TCGA database. (G) Representative images of IHC scoring criteria. A specific description is provided in the *Methods*. (H) Representative images of NAMPT staining in tissues from patients with breast cancer and breast fibroadenoma. (I and L) Quantification of NAMPT staining in tissues (I) and serum NAMPT concentrations (L) from patients with breast adenoma and breast cancer (left panel) and patients with breast cancer during different age grades (right panel). (J) Representative images of NAMPT staining in breast cancer tissues. (K) Representative images of NAMPT expression in triple negative breast cancer (TNBC) and non-TNBC tissues. Scale bar, 50  $\mu$ m. (M) Analysis of the expression of the *SIRT1* mRNA in neutrophils from the PB of patients with breast fibroadenoma and the BM of naïve mice treated with rNAMPT *in vitro*. (N) Percentage of aged neutrophils in the PB of patients with breast adenoma and the BM of naïve mice treated with rNAMPT in the presence or absence of the SIRT1 inhibitor (EX527). (O) Expression of the *NAMPT* mRNA in tumor cells incubated with different culture media. (P) Levels of the NAMPT protein in cells (iNAMPT) and secreted NAMPT in the medium (eNAMPT) from the same plates were calculated.  $\sqrt$ , Complete Medium;  $\times$ , Serum-free Medium. (Q) Flow analysis of aged neutrophils from the PB of patients with breast adenoma and the BM of naïve mice treated with different conditioned tumor cell cultured supernatants (TCCS) in the presence or absence of the FK866 (NAMPT inhibitor) and EX527 (SIRT1 inhibitor). (R) Detection of cfDNA in different conditioned TCCS. Data are presented as the means  $\pm$  SD from one representative experiment. Similar results were obtained from three independent experiments, unless indicated otherwise. Statistical analysis was performed by two-tailed unpaired Student's t test (I, L, M, O and R), Mann-Whitney U-tests (I, L) and one-way ANOVA (B, C, D and E). ns, not significant, \* $p$ <0.05, \*\* $p$ <0.01, and \*\*\* $p$ <0.001.