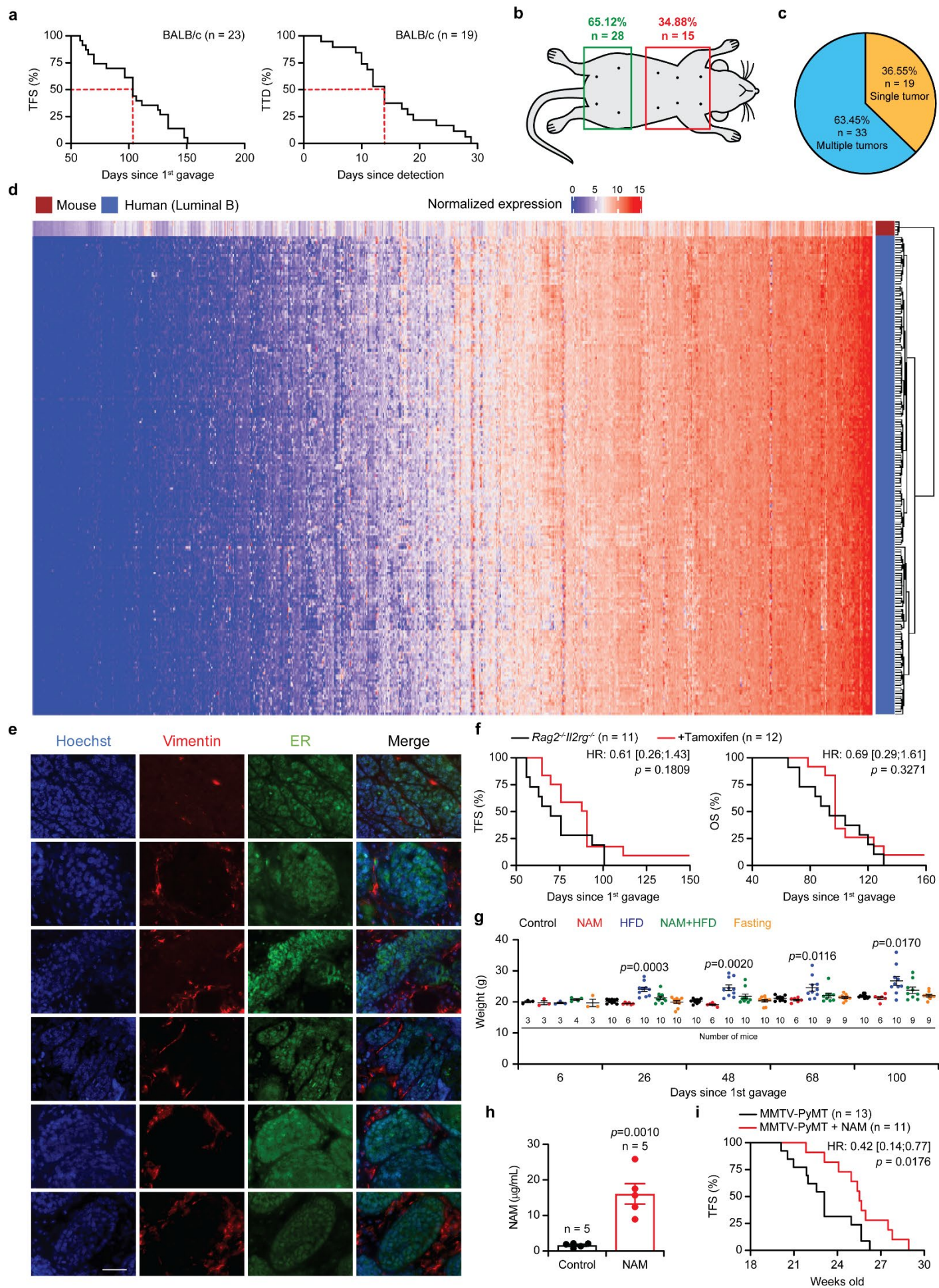


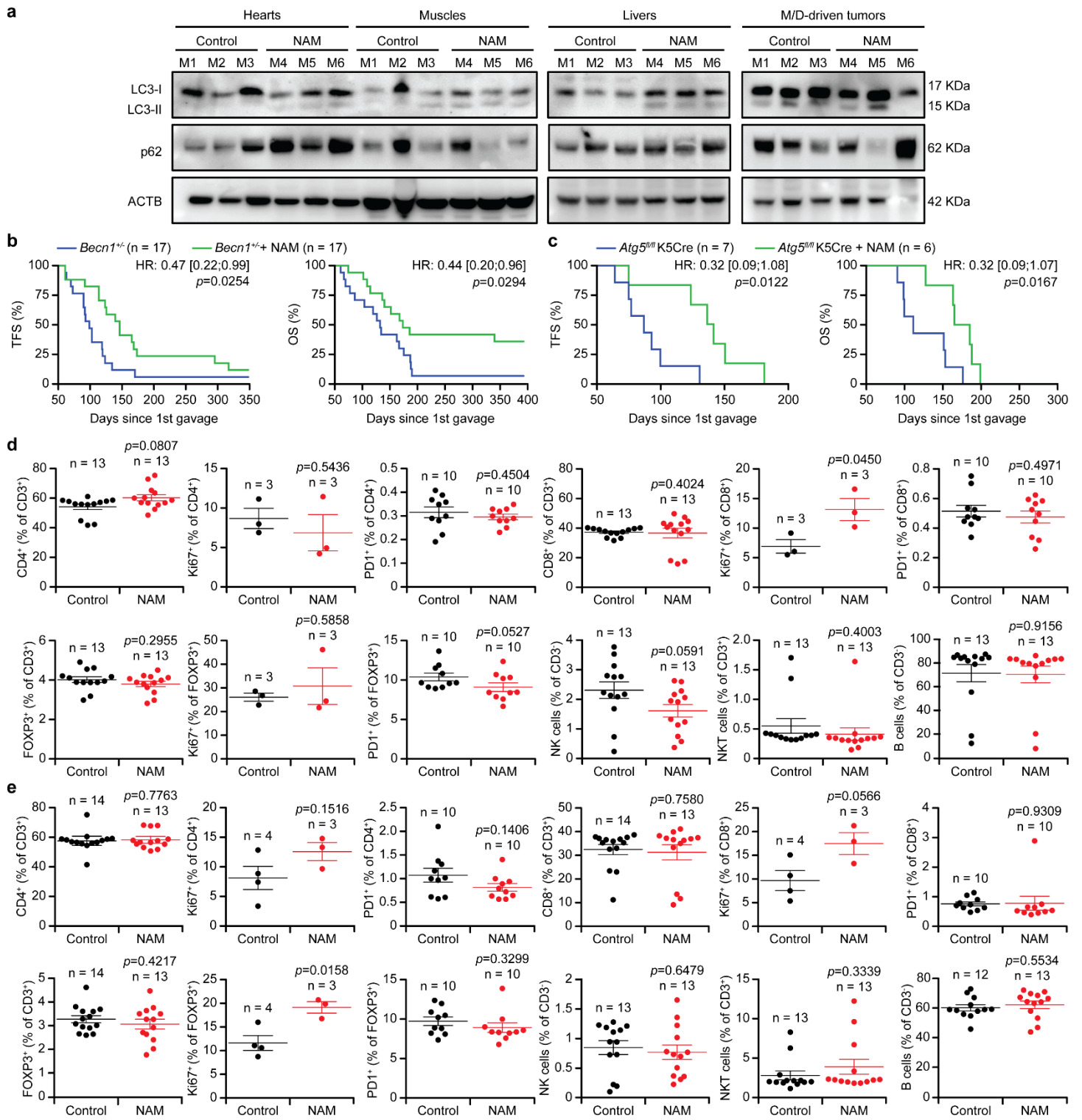
Immunoprophylactic and immunotherapeutic control of hormone-receptor positive breast cancer

Buqué et al.

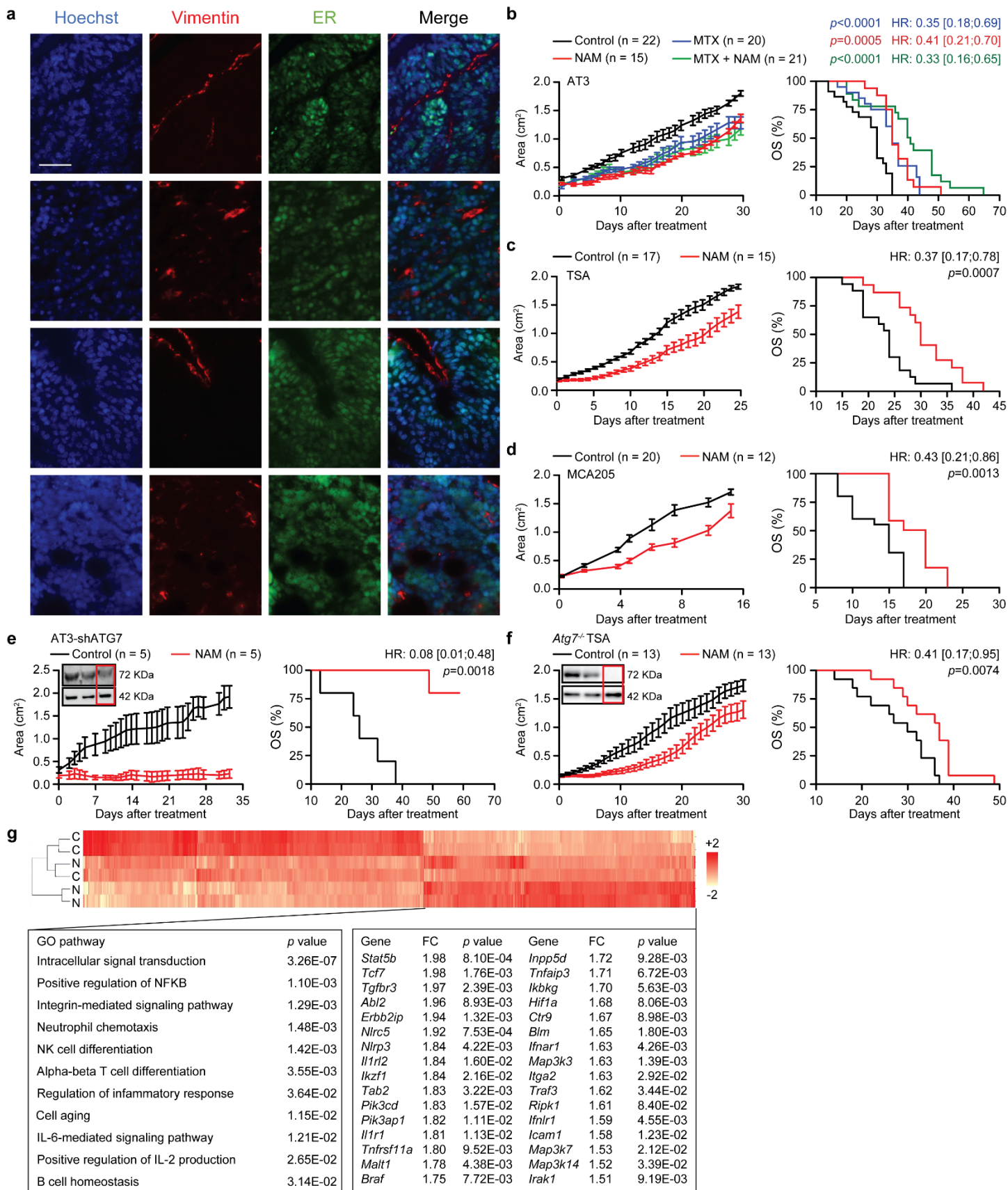
Supplementary Figures



Supplementary Figure 1. M/D-driven tumors as a model of HR⁺ BC sensitive to nutritional interventions. **a.** Tumor-free survival (TFS) and time-to-death (TTD) of WT BALB/c mice subjected to M/D-driven oncogenesis. Number of mice is reported. **b.** Anatomical localization of M/D-driven tumors evolving in WT C57BL/6 mice. **c.** Percentage of multifocal vs focal disease upon M/D-driven carcinogenesis in WT C57BL/6 mice. **d.** Hierarchical clustering of orthologous human and mouse genes from M/D-driven tumors evolving in WT C57BL/6 mice (n = 6) and luminal B breast cancer (BC) patients included in the TCGA database (n = 187). **e.** Molecular phenotype of M/D-driven tumors evolving in WT C57BL/6 mice, as assessed by immunofluorescence for estrogen receptor 1 (ESR1, best known as ER) and vimentin expression. Hoechst 33258 was employed for nuclear counterstaining. Scale bar = 40 μ m. **f.** TFS and overall survival (OS) of *Rag2^{-/-}Il2rg^{-/-}* mice subjected to M/D-driven oncogenesis in control conditions or along with tamoxifen administration with the drinking water. Number of mice, hazard ratio (HR) and *p* values (two-sided log-rank) are reported. **g.** Body weight of C57BL/6 mice subjected to M/D-driven carcinogenesis in control conditions or in the context of the indicated nutritional interventions. Results are means $\pm$ SEM and individual data points. Number of mice and *p* values are reported (unpaired, two-sided Student's *t*, as compared to age-matched mice maintained in control conditions). HFD, high-fat diet. **h.** Circulating levels of NAM in C57BL/6 mice maintained in control housing conditions or receiving NAM supplementation with the drinking water for 14 days. Results are means $\pm$ SEM and individual data points. Number of mice and *p* value are reported (unpaired, two-sided Student's *t*). **i.** TFS of MMTV-PyMT mice maintained in control conditions or subjected to NAM supplementation with the drinking water. Number of mice, HR and *p* values (two-sided log-rank) are reported.

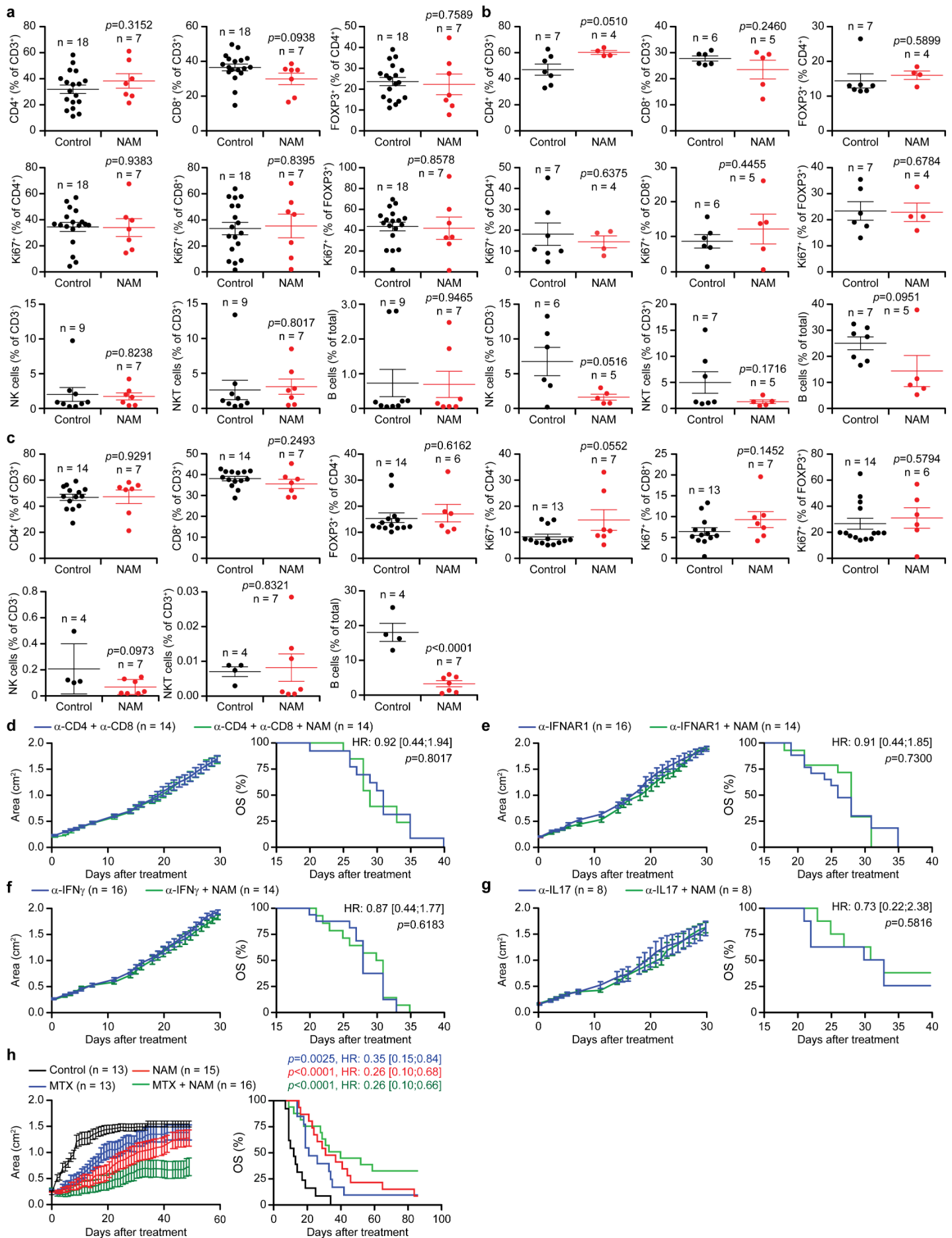


Supplementary Figure 2. Mechanisms of NAM-mediated oncoprevention. **a.** LC3 lipidation and p62 levels in different organs from C57BL/6 mice subjected to M/D-driven carcinogenesis in control conditions or in the context of 0.5% w/w NAM supplementation with the drinking water. ACTB levels were assessed as loading control. Uncropped immunoblots are provided as a Source Data file. **b, c.** Tumor-free survival (TFS) and overall survival (OS) of *Becn1*^{+/+} mice (**b**) and *Atg5*^{fl/fl} K5Cre mice (**c**) subjected to M/D-driven oncogenesis in control conditions or along with 0.5% w/w NAM supplementation with the drinking water. Number of mice, hazard ratio (HR) and *p* values (two-sided log-rank) are reported. **d, e.** Relative abundance and proliferative potential (based on Ki67 positivity) of CD8⁺ T cells, CD4⁺ T cells, CD4⁺CD25⁺FOXP3⁺ T cells, CD3⁺CD56⁺ NK cells, CD3⁺CD56⁺ NKT cells, and CD19⁺B220⁺ B cells in the spleen (**d**) and lymph nodes (**e**) of C57BL/6 mice maintained in control conditions or receiving 0.5% w/w NAM supplementation with the drinking water for 14 days. Results are means ± SEM and individual data points. Number of mice and *p* values (unpaired, two-sided Student's *t*) are reported.



Supplementary Figure 3. Mechanistic insights into the therapeutic activity of NAM. **a.** Molecular phenotype of M/D-driven tumors established in WT C57BL/6 mice and progressing despite NAM supplementation with the drinking water, as assessed at ethical endpoint by immunofluorescence for estrogen receptor 1 (ESR1, best known as ER) and vimentin expression. Scale bar = 40 μ m. **b-d.** Tumor growth and overall survival (OS) in C57BL/6 or BALB/c mice bearing established AT3 (**b**), TSA (**c**) or MCA205 (**d**) tumors that were left untreated or received NAM supplementation with the drinking water, intraperitoneal MTX treatment or both (as indicated). Tumor

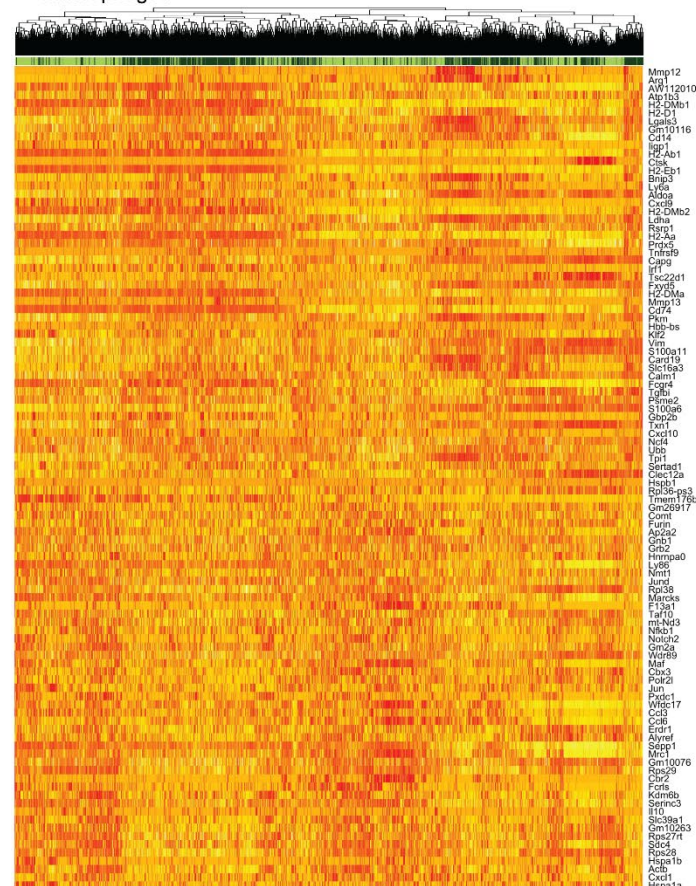
growth results are means $\pm$ SEM. Number of mice, hazard ratio (HR) and *p* values (two-sided log-rank for OS) are reported. **e, f.** Tumor growth and OS in C57BL/6 or BALB/c mice bearing established ATG7-depleted AT3 tumors (**e**) or *Atg7*^{-/-} TSA tumors (**f**) that were maintained in control conditions or subjected to NAM supplementation with the drinking water. Tumor growth results are means $\pm$ SEM. Number of mice, HR and *p* values (two-sided log-rank for OS) are reported. Insets depict ATG7 levels (top blots, 72 KDa) in ATG7-competent (wild type or control-transfected, left and central lanes) vs ATG7-deficient (right lanes) cells. Actin levels were monitored as loading control (bottom blots, 42 KDa). Uncropped immunoblots are provided as a Source Data file. **g.** Unsupervised hierarchical clustering of genes differentially expressed in untreated (*n* = 3) vs NAM-treated (*n* = 3) AT3 tumors. Top upregulated genes, fold change (FC) and adjusted *p* values are reported. Gene Ontology (GO) analysis and adjusted, two-sided *p* values for enrichment are indicated.



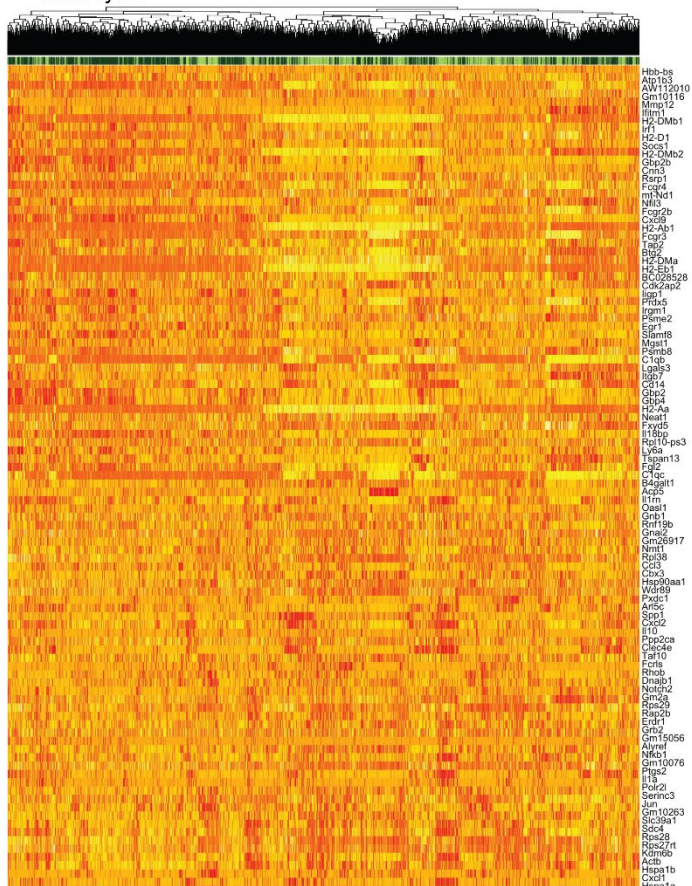
Supplementary Figure 4. Immunostimulatory effects of NAM. **a-c.** Relative abundance and proliferative potential (based on Ki67 positivity) of CD8⁺ T cells, CD4⁺ T cells, CD4⁺CD25⁺FOXP3⁺ T cells, CD3⁺CD56⁺ NK cells, CD3⁺CD56⁺ NKT cells, and CD19⁺B220⁺ B cells in the tumor (**a**), spleen (**b**) and lymph nodes (**c**) of C57BL/6 mice with established M/D-driven tumors that were maintained in control conditions or subjected to NAM supplementation with the drinking water until lesions reached 70-100 mm² surface area. Results are means $\pm$ SEM and individual data points. Number of mice and *p* values (unpaired, two-sided Student's *t*) are reported. **d-g.** Tumor growth and overall survival (OS) in C57BL/6 mice bearing established AT3 tumors that received NAM supplementation with the drinking water alone or along with co-depletion of CD4⁺ and CD8⁺ T cells (**d**) or the neutralization of IFNAR1 (**e**), IFNG (**f**) or IL17 (**g**). Tumor growth results are means $\pm$ SEM. Number of mice, HR and *p* values (two-sided log-rank) are reported. **h.** Tumor growth and OS in C57BL/6 mice bearing established M/D-driven tumors that were left untreated or received NAM supplementation with the drinking water, intraperitoneal mitoxantrone (MTX) treatment or both. Number of mice, Tumor growth results are means $\pm$ SEM. Number of mice, HR and *p* values (two-sided log-rank) are reported.



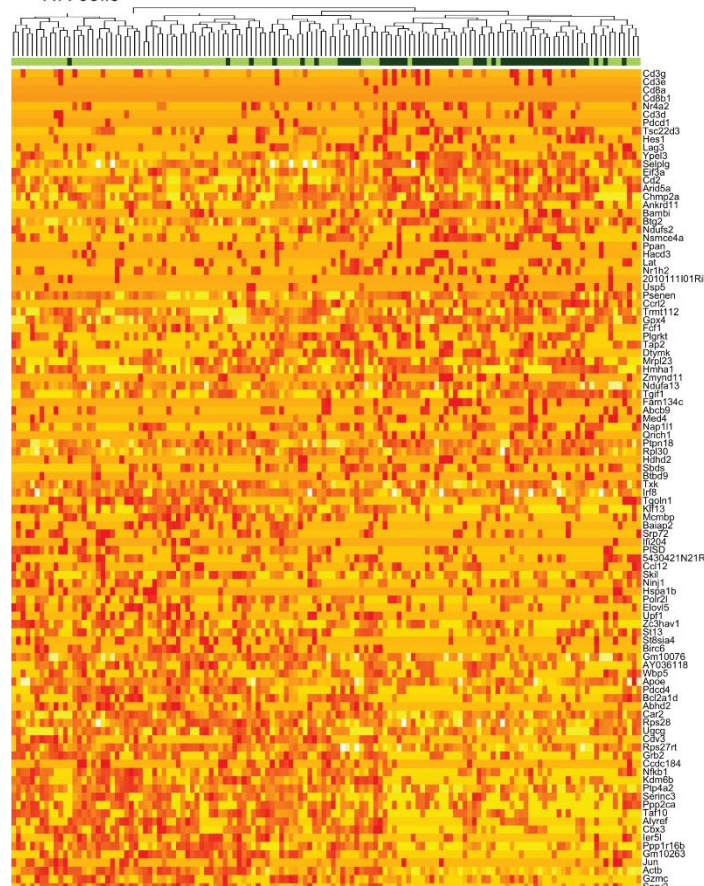
a Macrophages



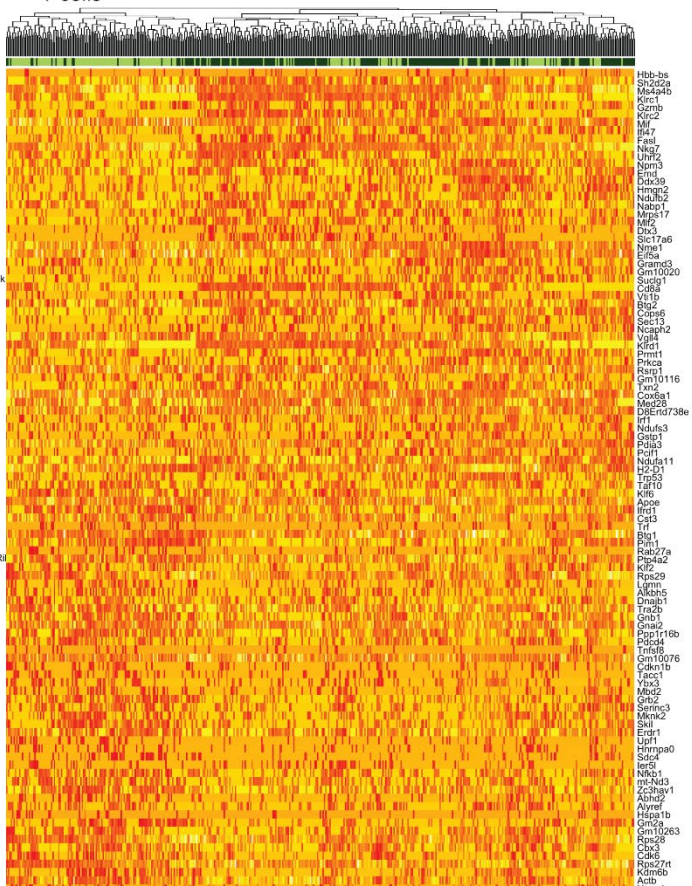
b Monocytes



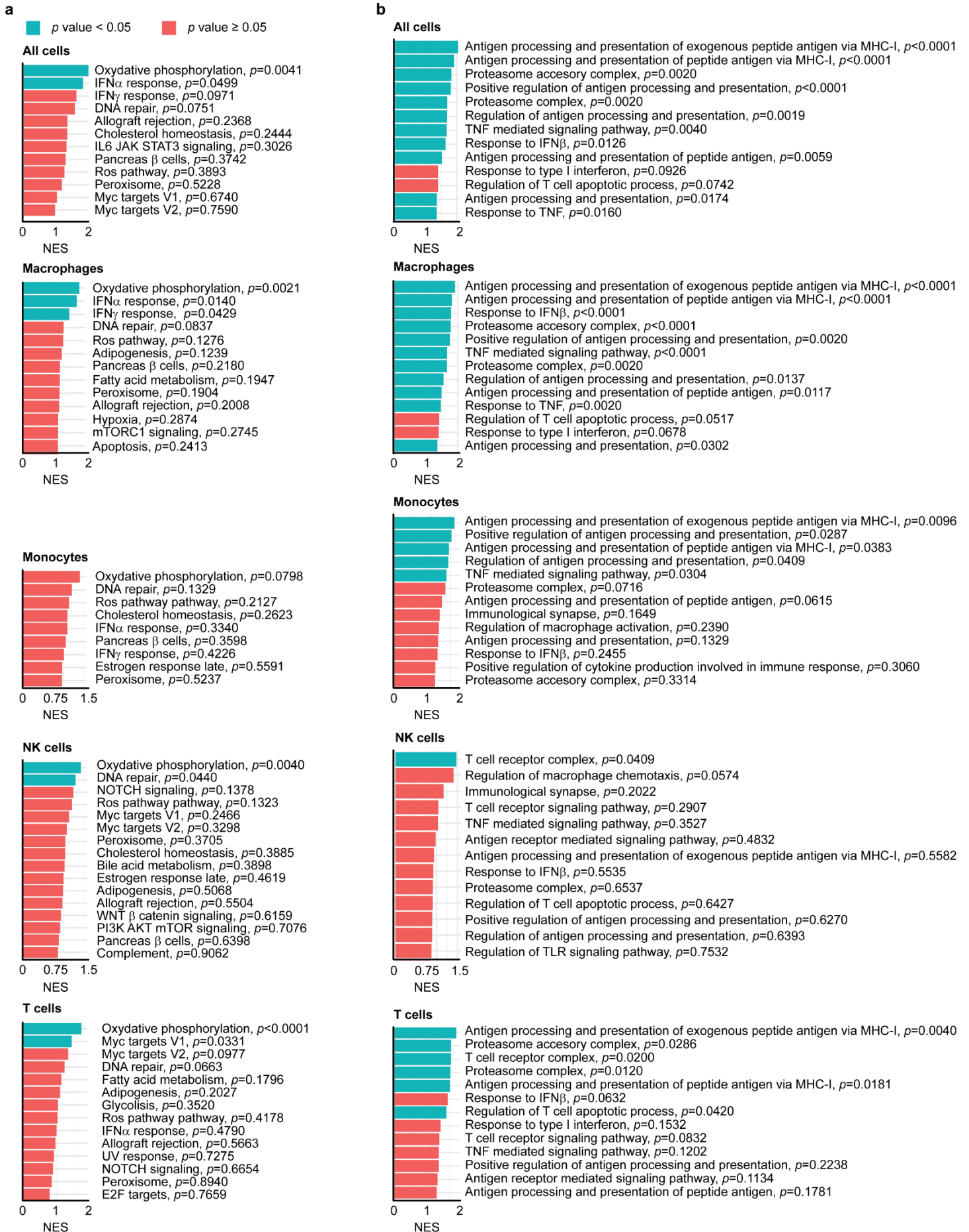
c NK cells



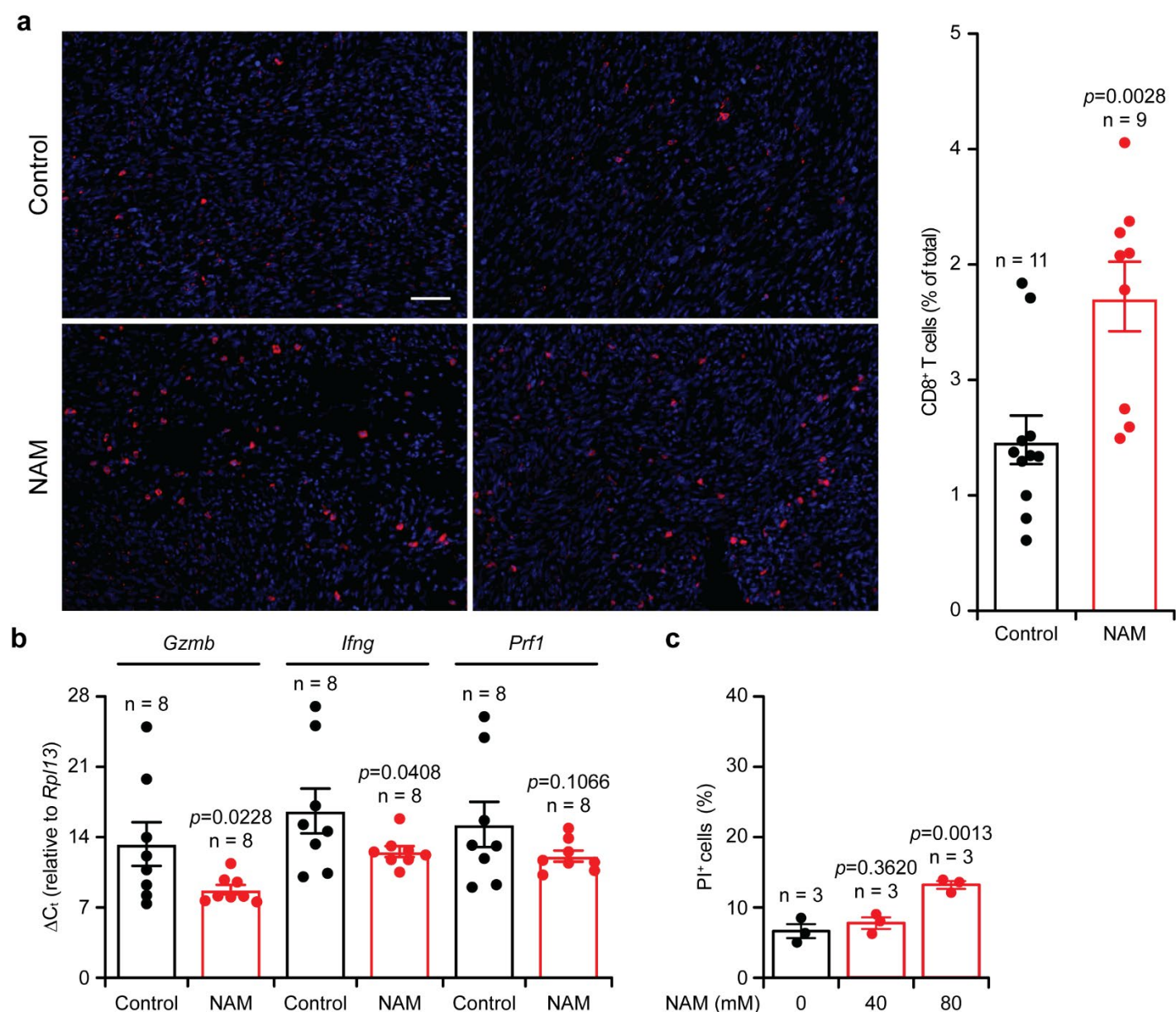
d T cells



Supplementary Figure 5. Transcriptional alterations induced by NAM in tumor-infiltrating macrophages, monocytes, T cells and NK cells. a-d. Unsupervised hierarchical clusters of the top 100 differentially expressed genes in tumor-infiltrating macrophages (a), monocytes (b), T cells (c) and NK cells (d) from TSA tumors established in BALB/c mice that (at tumor detection) were either left untreated (control) or subjected to NAM supplementation with the drinking water for 10 consecutive days. Gene names, fold change and unadjusted, one-sided p values (based on the MAST model) are available in **Supplementary Data 1**.

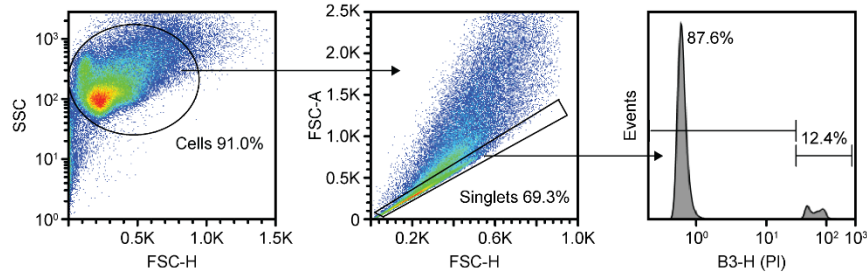


Supplementary Figure 6. NAM-treated tumors exhibit improved antigen presentation and superior cytotoxic functions. a, b. Gene Ontology (GO, **a**) and Hallmark (**b**) gene set enrichment analysis of differentially expressed genes in the indicated immune cell populations from TSA tumors established in BALB/c mice that were maintained in control conditions or subjected to NAM supplementation with the drinking water for 10 consecutive days after tumor detection. Only GO terms linked to immunity plus all Hallmarks terms were tested, and only those enriched in NAM-treated tumors are reported. Statistical significance was determined by fast gene set enrichment analysis (FGSEA). Unadjusted, two-sided p values for enrichment are reported. NES, normalized enrichment score.

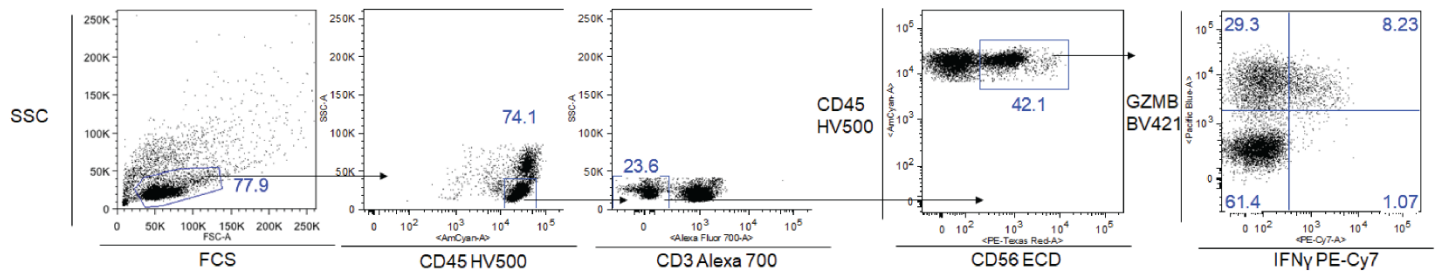


Supplementary Figure 7. Tumor infiltration by immune effectors driven by NAM. **a.** CD8⁺ T cell infiltration in TSA tumors established in BALB/c mice that were maintained in control conditions or subjected to NAM supplementation with the drinking water for 7 consecutive days upon tumor detection, as assessed by immunofluorescence microscopy with a CD8-specific antibody (red) and automated image analysis. Representative images (scale bar = 100 μ m) and quantitative data (means $\pm$ SEM and individual data points) are reported. Number of images (collected from 3 independent samples per condition) and p values (unpaired, two-sided Student's t) are reported. **b.** Transcriptional levels of *GZMB*, *IFNG* and *PRF1* in M/D-driven tumors established in C57BL/6 mice that were maintained in control conditions or subjected to NAM supplementation in the drinking water for 7 consecutive days upon tumor detection. Please note that results are reported as ΔC_t values as compared to *Rpl13* C_t values for each tumor, because $\Delta\Delta C_t$ values cannot be calculated in the absence of uniform control conditions. In this setting, lower ΔC_t correspond to higher transcript levels. Results are mean $\pm$ SEM plus individual data points. Number of biologically independent samples collected over three independent experiments and p values are reported (one way-ANOVA plus Fisher LSD, as compared to the same transcript in M/D-driven tumors from untreated mice). **c.** Percentage of dead (PI⁺) TSA cells upon culture in control conditions or in the presence of NAM at the indicated concentrations for 48 hours. Results are mean $\pm$ SEM plus individual data points. Number of biologically independent samples and p values (one way-ANOVA plus Fisher LSD, as compared to untreated cells) are reported.

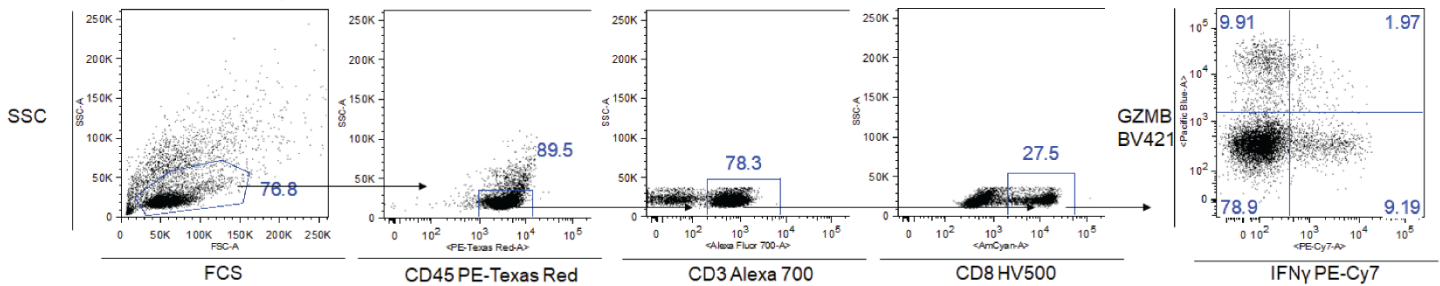
In vitro - Cell death (Suppl. Fig. 7c)



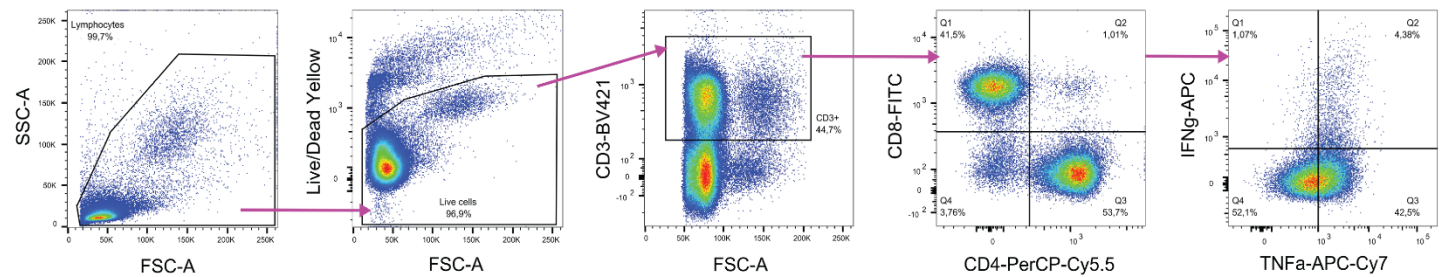
In vitro - NK cell activation (Fig. 8d)



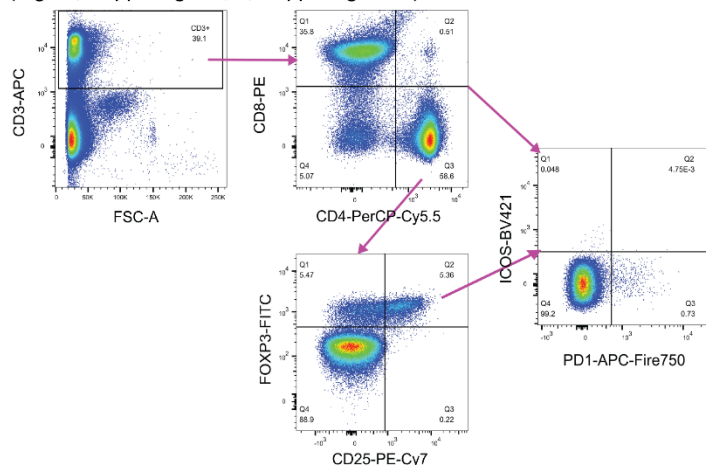
In vitro - T cell activation (Fig. 8c)



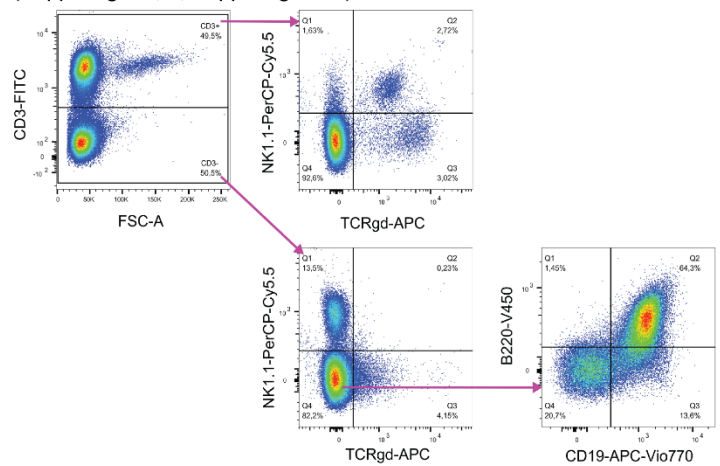
In vivo - Infiltrate phenotyping - Procedure 1 (Fig. 6f)



In vivo - Infiltrate phenotyping - Procedure 2
(Fig. 1i; Suppl. Fig. 2d, e; Suppl. Fig. 4a-c)



In vivo - Infiltrate phenotyping - Procedure 3
(Suppl. Fig. 2d, e; Suppl. Fig. 4a-c)



Supplementary Figure 8. Gating strategies for flow cytometry employed in this study.