Supplementary Figure S1: A3B expression does not activate the DNA damage response in SMF-A3B cells and tumors. (A-B) Immunofluorescence staining for γH2AX on control and APOBEC tumors growing in wildtype mice from Figure 2B. Representative images are shown in (A) and quantification of $\gamma H2AX^+$ foci (number of foci/number of cells per field of view) is shown in (B). 5 tumors per cohort were analyzed and 8 fields of view were averaged per tumor. DAPI is in blue and γH2AX is in green. Error bars denote mean ± SEM and statistical significance was determined by unpaired Student's t-test. **(C)** Western blot analysis of HA-epitope tagged A3B, γH2AX, and cleaved PARP in SMF-A3B cells treated with or without dox for 2 weeks. α-Tubulin and histone H2A are shown as loading controls. ns > 0.05

Supplementary Figure S2: Flow cytometry gating strategy. (A) Gating strategy for PD-1 and PD-L1 expression on immune cells and tumor cells. **(B)** Gating strategy for macrophages, natural killer cells, granzyme B^+ immune cells, interferon-γ^+ immune cells, $CD8^+$ T cells, $CD4^+$ T cells, type-2 T helper cells, T regulatory cells, and $CD103^+$ dendritic cells.

Supplementary Figure S3: Representative FACS plots showing immune cell infiltration in APOBEC tumors. (A) Staining for $CD8^+$ T cells ($CD8^+CD3^+$) and CD3 fluorescence minus one (FMO) control without αCD3 antibody. (B) Staining for $CD4^+$ T cells ($CD4^+CD3^+$) and CD3 FMO. (C) Staining for CD103 expression on dendritic cells ($CD103^+CD11c^+$) and CD103 FMO. (D) Staining for macrophages ($F4/80^+CD11c^{low}$). (E) Staining for T regulatory cells ($CD4^+FOXP3^+$) and FOXP3 FMO. (F) Staining for Type-2 T helper cells ($CD4^+GATA3^+$) and GATA3 FMO.

Supplementary Figure S4: Tumor growth inhibition and increased immune infiltration in APOBEC tumors is reproducible. **(A)** Tumor flow cytometry from mice in Figure 2B showing that APOBEC tumors have a trend towards increased IFNγ on $CD8^+$ T cells and NK cells, as well as GZMB on NK cells. **(B)** Bilateral tumor-draining inguinal lymph nodes (TDLN) were harvested from mice in Figure 2B and aggregated from 4 mice per cohort for flow cytometry. APOBEC TDLNs show increased $CD8^+$ T cells and $CD103^+$ DCs compared to control TDLNs. Error bars denote mean ± SEM and statistical significance was determined by unpaired Student's t-test. **(C)** Tumor volume (mm^3) over time for unilateral control tumors (n=5), and APOBEC tumors (n=5) generated from SMF-A3B cells in wildtype mice in an independent experiment, demonstrating that the growth defect of APOBEC tumors is reproducible in an independent experiment. Error bars denote mean ± SEM. **(D)** Control tumors (n=5) and APOBEC tumors (n=5) from (C) were harvested and immune profiled by flow cytometry. Quantification shows the APOBEC tumors had increased leukocytes, $CD103^+$ DCs, and tumor cell PD-L1 expression (MFI, mean fluorescence intensity), while T regulatory cells (Tregs) and type-2 T helper (Th2) cells were reduced in APOBEC tumors. These results demonstrate that immune infiltration in APOBEC tumors is reproducible in an independent experiment. Error bars denote mean ± SEM and statistical significance was determined by unpaired Student's t-test. **(E)** Additional images of immunohistochemistry (IHC) staining for the T cell marker CD3 in the tumor core of control and APOBEC tumors as in Figure 2H. The top row is control tumors (n=3) and bottom row is APOBEC tumors (n=3). Scale bar indicates 100 μm. * $p < 0.05$, ** $p < 0.01$

Supplementary Figure S5: APOBEC tumor immunogenicity is generalizable to other mammary tumor models. **(A)** qRT-PCR analysis for APOBEC3B (A3B) expression in NDL^{UCD}-A3B cells cultured with or without dox for 2 days. Left: A3B expression relative to 0 μg/mL dox

condition. Right: A3B expression relative to BT474 cells. Results show 3 technical replicates and error bars depict mean ± SD. Significance was determined using unpaired Student's t-test. **(B)** qRT-PCR analysis for A3B expression in EMT6-A3B cells cultured with increasing concentrations of dox for 2 days. 1+/- indicates cells cultured with 1 µg/mL dox for 2 days followed by removal of dox for 3 days prior to analysis to demonstrate reversibility. Left: A3B expression relative to 0 µg/mL dox condition. Right: A3B expression relative to BT474 cells. Results show 3 biological replicates and error bars depict mean ± SEM. Significance was determined using a one-way ANOVA and Tukey's multiple comparisons test. **(C)** NDL^{UCD}-A3B cells were cultured as in (A) and cell lysates harvested for *in vitro* deaminase activity assay. **(D)** EMT6-A3B cells were cultured as in (B) and cell lysates harvested for *in vitro* deaminase activity assay. Deaminase activity is comparable to that of human cell line, BT474. **(E)** Tumor volume curves for control (-dox; n=16) and APOBEC (+dox; n=16) tumors derived from EMT-A3B cells orthotopically implanted in the mammary gland of syngeneic BALB/c mice. Error bars denote mean ± SEM and statistical significance was determined by two-way repeated-measures ANOVA. **(F)** The fraction of control and APOBEC EMT6 tumors that grew or spontaneously regressed following tumor cell injection. Fisher's exact test, p=0.0659. **(G)** Flow cytometry quantification of leukocytes in control (n=6) and APOBEC (n=6) EMT6 tumors from (E). Error bars denote mean ± SEM and statistical significance was determined by unpaired Student's t-test. **(H)** Tumor volume curves for control (-dox; n=16) and APOBEC (+dox; n=16) tumors derived from NDL^{UCD}-A3B cells orthotopically implanted in the mammary gland of syngeneic FVB mice. Error bars denote mean ± SEM and statistical significance was determined by two-way repeated-measures ANOVA. **(I)** CellTiter-Glo assay showing growth curves of NDL^{UCD}-A3B cells cultured with or without

dox. Error bars depict mean ± SD and statistical significance was determined by two-way ANOVA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

Supplementary Figure S6: The APOBEC tumor growth defect is dependent on the immune system and requires the catalytic activity of A3B. (A) Tumor volume (mm^3) over time for control (n=16) and APOBEC tumors (+dox in drinking water; n=16) growing in NSG mice. Error bars denote mean ± SEM. Statistical significance was determined by two-way repeated-measures ANOVA and Tukey's multiple comparisons test with the same control cohort as in Supplementary Fig. S6K. **(B)** qRT-PCR of A3B gene expression in SMF-$A3B^{inactive}$ cells treated with 1 μg/mL dox for 5 days. Error bars denote mean ± SD for 3 technical replicates and statistical significance was determined by unpaired Student's t-test. **(C)** Immunofluorescence staining for HA epitope-tagged A3B in control tumors (-dox) and tumors expressing $A3B^{inactive}$ (+dox) from (E). **(D)** *In vitro* deaminase activity assay in SMF-$A3B^{inactive}$ cells treated with dox. SMF-A3B cells are shown as a control. **(E)** Tumor volume (mm^3) over time for control tumors (-dox; n=14) and tumors expressing $A3B^{inactive}$ (+dox; n=14) in wildtype mice. Error bars denote mean ± SEM. Statistical significance was determined by two-way repeated-measures ANOVA. **(F)** Quantification of IHC staining for CD45 (left) or CD3 (right) in control tumors (n=5) and tumors expressing $A3B^{inactive}$ (n=5) from (E). Four fields of view were quantified for each tumor. Error bars denote mean ± SD. Statistical significance was determined by unpaired Student's t-test. **(G)** Growth curves for control and *in vitro* APOBEC mutagenized cells. Data are shown as mean ± SD of 4 replicates. **(H)** Schematic showing experimental design for tumor growth experiment. SMF-A3B cells were cultured with or without dox for 2 weeks, then dox was removed for 2 weeks. These *in vitro* APOBEC mutagenized cells or control cells were orthotopically implanted in the mammary gland of mice in the absence

of dox. **(I)** Tumor volume (mm^3) over time for control (n=14) and *in vitro* APOBEC mutagenized tumors (n=14) in wildtype mice. Error bars denote mean ± SEM. Statistical significance was determined by two-way repeated-measures ANOVA. **(J)** qRT-PCR analysis for Granzyme A (*Gzma*), Perforin-1 (*Prf-1*), and T-bet (*Tbx21*), in control (n=6) and *in vitro* APOBEC mutagenized tumors (n=6) from (I). All genes showed a trend toward increased expression in the *in vitro* APOBEC mutagenized cohort that did not reach statistical significance. **(K)** Tumor volume (mm^3) over time for control (n=16) and *in vitro* APOBEC mutagenized tumors (n=16) in NSG mice. Error bars denote mean ± SEM. Statistical significance was determined by two-way repeated-measures ANOVA and Tukey's multiple comparisons test. Note that control mice are the same as in (A). ns $p > 0.05$, ** $p < 0.01$, *** $p < 0.001$

Supplementary Figure S7: Gene expression analysis of control and APOBEC tumors. (A) All statistically significant main GO biological processes upregulated in APOBEC tumors from wildtype mice (FDR adjusted $p < 0.05$ by Fisher's test). Fold enrichment is shown for each. **(B)** All statistically significant GSEA Hallmark pathways positively enriched in APOBEC tumors from wildtype mice (FDR adjusted $p < 0.25$). Normalized enrichment score (NES) is shown for each. **(C)** All statistically significant GSEA Hallmark pathways positively enriched in APOBEC tumors from NSG mice (FDR adjusted $p < 0.25$). Normalized enrichment score (NES) is shown for each.

Supplementary Figure S8: CD4+/CD8+ T cell depletion but not CD8+ T cell depletion alone rescues the growth defect of APOBEC tumors. (A) Flow cytometry showing depletion of $CD8^+$ T cells in peripheral blood following intraperitoneal injection of an *in vivo* CD8 depleting antibody

(300 μg/dose) or isotype-control antibody twice weekly. Peripheral blood was assayed on day 9 post tumor inoculation. **(B)** Quantification of $CD8^+$ T cells in peripheral blood of isotype-control antibody treated mice (n=7) and αCD8 antibody treated mice (n=7) as in (A). Error bars denote mean ± SEM. **(C)** qRT-PCR for CD8 expression in tumors from the indicated cohorts: control tumors + αIgG (n=7); APOBEC tumors + αIgG (n=7); APOBEC tumors + αCD8 (n=7). Error bars denote mean ± SEM. **(D)** Tumor volume (mm^3) over time for control + αIgG (n=14), APOBEC + αIgG (n=14), and APOBEC + αCD8 (n=14) tumors in wildtype mice. Error bars denote mean ± SEM. **(E)** Flow cytometry showing depletion of $CD8^+$ and $CD4^+$ T cells in peripheral blood following intraperitoneal injection of CD8 and CD4 depleting antibodies (200 μg αCD8 and 200 μg αCD4/dose) or isotype-control antibody twice weekly. Peripheral blood was assayed on day 25 post tumor inoculation. **(F)** Quantification of $CD4^+$ and $CD8^+$ T cells in peripheral blood of isotype-control antibody treated mice (n=7) and αCD8/αCD4 antibody treated mice (n=7) as in (E). Error bars denote mean ± SEM. **(G)** Flow cytometry quantification of $CD8^+$ and $CD4^+$ T cells in APOBEC tumors treated with isotype-control antibody or αCD8/αCD4 depleting antibodies. Error bars denote mean ± SEM.

Supplementary Figure S9: APOBEC activity renders HER2-driven mammary tumors responsive to anti-CTLA-4 therapy, but not anti-PD-1 monotherapy. (A) Response of control and APOBEC tumors to combination αPD-1/αCTLA-4 therapy. Number of tumors with no response or CR/PR are depicted. Statistical significance was determined by Fisher's exact test. **(B)** Kaplan-Meier curves showing tumor-free survival (100 mm^3 endpoint) of mice treated with isotype control or combination αPD-1/αCTLA-4 therapy. Statistical significance was determined by log-rank test. Mantel-Haenszel hazard ratio and 95% confidence interval are indicated. **(C)**

Response of control and APOBEC tumors to αPD-1 monotherapy. Number of tumors with no response or CR/PR are depicted. Statistical significance was determined by Fisher's exact test. **(D)** Kaplan-Meier curves showing tumor-free survival (100 mm^3 endpoint) of mice treated with isotype control or αPD-1 monotherapy. Statistical significance was determined by log-rank test. Mantel-Haenszel hazard ratio and 95% confidence interval are indicated. **(E)** Response of control and APOBEC tumors to αCTLA-4 monotherapy. Number of tumors with no response or CR/PR are depicted. Statistical significance was determined by Fisher's exact test. **(F)** Response of control and APOBEC tumors to combination αCTLA-4/αHER2 therapy. Number of tumors with no response or CR/PR are depicted. Statistical significance was determined by Fisher's exact test. **(G)** Kaplan-Meier curves showing tumor-free survival (100 mm^3 endpoint) of mice treated with isotype control, αCTLA-4 monotherapy, or combination αCTLA-4/αHER2 therapy. Statistical significance was determined by log-rank test. Mantel-Haenszel hazard ratio and 95% confidence interval are indicated.CR, complete response; PR, partial response. HR; hazard ratio. ns $p > 0.05$, * $p < 0.05$, *** $p < 0.001$

Supplementary Figure S10: Immune-mediated suppression of tumor growth for an additional APOBEC clone. (A-B) *In vitro* growth curves of single-cell clones derived from control SMF-A3B cells (control) or SMF-A3B cells treated with dox for 2 weeks (APOBEC). The growth curves of the polyclonal parental cells (parental control or parental APOBEC) are shown as a control. Error bars denote mean ± SD of 4 replicates.

Supplementary Figure S11: APOBEC-low TCGA tumors and immune signature clusters. (A) Heatmap showing the relative expression of immune cell gene signatures from TCGA RNA-

seq data in APOBEC-low tumors, grouped by breast cancer subtype. Columns are individual patient tumors and rows are different immune cell gene signatures. Legend shows colors corresponding to relative expression levels (red, row max; blue, row min). Hierarchical clustering segregated tumors into 2 main clusters in the HER2-enriched subtype and 2 clusters in the basal-like subtype. **(B)** APOBEC enrichment score plotted for Basal-like and HER2-enriched tumors from each cluster. Boxplots show 25th percentile, median, and 75th percentile, while whiskers show minimum to maximum values excluding outliers. Statistical significance was determined by one-way ANOVA and Sidak's multiple comparisons test. **(C)** Schematic of a model showing APOBEC mutagenesis increases immune activation, infiltration, and immunotherapy response in mouse and human breast tumors. But ongoing APOBEC mutagenesis can also generate subclonal diversification, which leads to increased subclonal mutations and decreased immune activation. ns $p > 0.05$

Supplementary Table S1: Spreadsheet of gene lists for immune cell gene signatures for analyses in Figure 6 and Supplementary Figure S9. The genes that were absent from the TCGA-BRCA RNA-seq dataset are colored in red.

Supplementary Table S2: Spreadsheet containing TCGA-BRCA patient ID and data used for analyses in Figure 6 and Supplementary Figure S9. Column descriptions:

Sample_ID – TCGA sample identifier

Cluster_Number – APOBEC-high or -low immune cluster number (e.g. "APOBEC-high HER2-1" refers to APOBEC-high HER2 subtype Immune Cluster 1)

Age_Median – patient age

ER.Status – clinical ER status

PR.Status – clinical PR status

Her2.Status – clinical HER2 status

PAM50 – PAM50 subtype

Pathologic_stage – clinical pathologic stage

Histological_type – clinical histological type

n_C_mut – number of C>T/G (or G>A) mutations

n_C_con – number of C (or G) within the 41-nucleotide region centered on the C>T/G (or G>C/A) mutations

n_TCW_mut – number of C>T/G (or G>C/A) mutations in TCW (or WGA) motifs

n_TCW_con – number of TCW (or WGA) motifs within the 41-nucleotide region centered on the mutated motifs, TCW to TTW/TGW (or WGA to WAA/WCA).

APOBEC – APOBEC enrichment score

Number_of_Subclonal_Mutations – number of subclonal mutations from Raynaud et al. 2018

The remaining columns are principal component analysis (PCA)-collapsed log2 normalized gene expression of immune cell gene signatures from RNA-seq data.

Supplementary Table S3: Spreadsheet of correlations between APOBEC enrichment score and immune cell signatures used for analyses in Figure 6. Column descriptions:

PAM50 – PAM50 subtype

rho – Spearman's rho value from correlation analysis

pvalue – p-value from correlation analysis

adjusted_pvalue – adjusted p-value from correlation analysis