Immunohistochemistry, blood analysis and flow cytometry. (IHC) was carried out by the pathology core at City of Hope using the Ventana Discovery Ultra autostainer (Ventana Medical Systems, Roche Diagnostics, Indianapolis, USA) and the ChromoMap DAB detection system according to manufacturer's recommendations. Briefly, the tissue samples were collected and fixed in 4% paraformaldehyde for 3 days and then stored in 70% EtOH. The sampled were blocked in paraffin, sectioned at a thickness of 5 μm and put on positively charged glass slides. For hematoxylin & eosin (H&E) stains, the slides were deparaffinized, rehydrated and stained with Modified Mayer's Hematoxylin and Eosin Y Stain (America MasterTech Scientific) on a H&E Auto Stainer (Prisma Plus Auto Stainer, SAKURA) according to standard laboratory procedures. For the specific immunohistochemistry stains, the slides were loaded on the machine, deparaffinization, rehydration, endogenous peroxidase activity inhibition and antigen retrieval were first performed. Then, each primary antibody was incubated following by DISCOVERY anti-Rabbit HQ and DISCOVERY anti-HQ-HRP incubation. The stains were visualized with DISCOVERY ChromoMap DAB Kit, counterstained with hematoxylin (Ventana) and cover slipped. For humanized anti-human M5A antibody stains, Goat anti-human IgG antibody (H+L), biotinylated (Vector Laboratories) was used and followed by VECTOASTAIN ABC kits (HRP) and DAB. H&E or IHC stained slides were digitalized and documented by iScan HT (Roche) scanner.

Hematologic analysis was done using the VETSCAN HM5 Hematology Analyzer. Blood (50-100 μl) was collected and stored in EDTA tubes until analyzed, 1-2 hours post collection. For kidney and liver toxicity, about 400 μl blood was collected in lithium heparin tubes and spun down to separate RBCs and plasma. Plasma was collected and stored at -80C until analysis on the VETSCAN VS2 Chemistry Analyzer, using the Preventive Care Profile Plus rotors (Zoetis).

Tissues and blood were collected at time points indicated above, and leukocyte populations were analyzed by flow cytometry using gating strategy as published before. Blood samples were analyzed after red cells lysis using Red Blood Cell Lysis Buffer Hybri-Max (Sigma). Tumor draining lymph nodes and spleens were pushed through a 40μm cell strainer (Corning) and red cells were lysed. Tumors were dissociated by enzymatic digestion using gentleMacs Octo Dissociator and dissociation kit following the manufacture's protocol (Miltenyi Biotec). Cell suspensions were stained with different combinations of fluorochrome-coupled antibodies to CD3, CD4, CD8, CD19, CD11b, Ly6C, Ly6G, CD11c, F4/80, PD-1, CTLA-4, Tim-3 (BioLegend). For IFNγ production cells from all tissues were re-stimulated using Cell Activation Cocktail containing Brefeldin A(BioLegend) in 10% FBS IMDM media for 4 hours in 37ºC. Next, cells were stained for surface markers and viability marker (Zombie UV, BioLegend) and fixed and permeabilized using Foxp3 Transcription Factor Fixation/Permeabilization kit (ThermoFisher) following the manufacture's protocol. Finally, cells were stained for intracellular IFNγ (BioLegend) and analyzed by flow cytometry. For FoxP3 expression cells were stained for surface markers, fixed and permeabilized as described above and stained with anti-FoxP3 antibody (ThermoFisher).

Supplemental Table 1. Statistical analyses of tumor treatments[1]

Fig 1A	3.7kBq	7.4kBq	11.1kBq
Untr.	n.s.	***	***
3.7kBq		***	***
7.4kBq			***

Fig 1B	3.7kBq	7.4kBq	11.1kBq
Untr.	*	**	**
3.7kBq		**	**
7.4kBq			**

Fig 2A	TAT	ICK	ICK+TAT	TAT+ICK
Untr.	***	***	***	***
TAT		n.s.	n.s.	**
ICK+TAT		n.s.		***
TAT+ICK		*		

Fig 2B	TAT	ICK	ICK+TAT	TAT+ICK
Untr.	**	*	**	**
TAT		n.s.	n.s.	**
ICK+TAT		n.s.		**
TAT+ICK		*		

Fig 3A	TAT	ICK	TAT+ICK5d	TAT+ICK10d
Untr.	**	**	***	***
TAT		n.s.	**	**
ICK			***	**
TAT+ICK5d				n.s.

Fig 3B	TAT	ICK	TAT+ICK5d	TAT+ICK10d
Untr.	*	*	**	**
TAT		n.s.	**	**
ICK			**	**
TAT+ICK5d				n.s.

Fig 4A	3.7kBq	7.4kBq	11.1kBq
Untr.	n.s.	**	***

3.7kBq		***	***
7.4kBq			**

Fig 4B	3.7kBq	7.4kBq	11.1kBq
Untr.	n.s.	**	**
3.7kBq		**	**
7.4kBq			**

Fig 4C	14.8kBq	2x7.4kBq
Untr.	***	***
14.8kBq		n.s.

Fig 4D	14.8kBq	2x7.4kBq
Untr.	**	**
14.8kBq		n.s.

Fig 4E	ICK	TAT	TAT+ICK
Untr.	***	***	***
3.7kBq		n.s.	***
7.4kBq			**

Fig 4F	ICK	TAT	TAT+ICK
Untr.	**	***	***
3.7kBq		n.s.	***
7.4kBq			*

[1] Statistical analysis between all treatment groups using two-way ANOVA test. Statistical analysis results of survival curves using Log-rank (Mantel-Cox) test. n.s. – not significant; *** $p<0.001$; ** $p<0.01$; * $p<0.05$.

Supplemental Table 2: Kidney and liver toxicity associated with TAT and ICK therapies in a E0771/CEA breast cancer model[1].

	Kidney		Liver	
	BUN (mg/dL)	**CRE** (mg/dL)	**ALT** (U/L)	**AST** (U/L)
Murine Normal	22±5	0.5±0.17	40±21	141±67
Saline	23±4	0.30±0.13	36±6	266±85
3.7kBq ^{225}Ac-M5A	22±3	0.45±0.13	39±6	352±138
7.4kBq ^{225}Ac-M5A	24.1±4	0.30±0.08	41±2	354±88
11.1kBq ^{225}Ac-M5A	25±5	0.50±0.15	41±3	369±73
ICK	26±2	0.3±0.17	34±5	168±24
ICK+7.4kBq TAT	21±1	0.2±0.07	37±3	292±62
7.4kBq TAT+ICK	25±3	0.2±0.14	46±10	303±94

[1] Liver Toxicity: alanine aminotransferase (ALT)/ aspartate aminotransferase (AST); Kidney Toxicity: Blood Urea Nitrogen (BUN)/ creatinine (CRE). Plasma analyzed at end point of each mouse. Average of N=4.

Supplemental Table 3: Median Survival of E0771/CEA Experiments[1].

	Saline	**3.7kBq TAT**	**7.4kBq TAT***	**11.1kBq TAT**	**ICK**	**ICK+ 7.4kBq TAT**	**7.4kBq TAT+ ICK(18d)**	**7.4kBq TAT+ ICK(13d)**
Median Survival	21.8	24.0	28.9	36.0	31.2	30.2	43.7	45.4

[1] Average of all groups.

Supplemental Table 4: Median survival of TAT and ICK therapies in a Colon Cancer MC38/CEA Model[1].

	Saline	3.7kBq TAT	7.4kBq TAT	11.1kBq TAT	ICK	7.4kBq TAT+ ICK	7.4kBq TAT x 2	14.8kBq TAT
Median Survival	29.1	32.2	42.3	50.2	40.6	57.3	51.5	65.3

[1]Average of all groups.

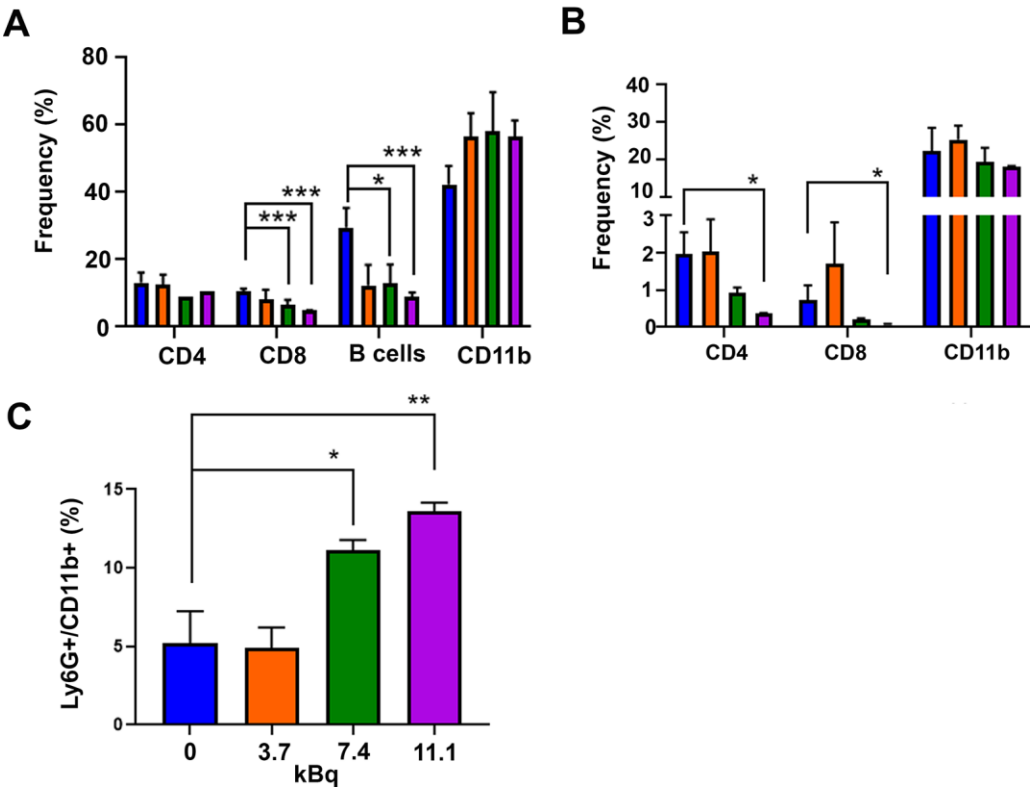
Supplemental Table 5: Kidney and Liver Toxicity associated with TAT and ICK therapies in a Colon Cancer MC38/CEA Model[1].

	BUN (mg/dL)	CRE (mg/dL)	ALT (U/L)	AST (U/L)
Murine Normal	22±5	0.5±0.17	40±21	141±67
Saline	30±2	0.22±0.05	24±6	198±81
7.4kBq TAT+ICK	26±3	0.22±0.04	96±20	264±67
7.4kBq ^{225}Ac-M5A x2	27±3	0.25±0.06	35±11	185±9
14.8kBq ^{225}Ac-M5A	24±2	0.3±0.14	36±13	154±32

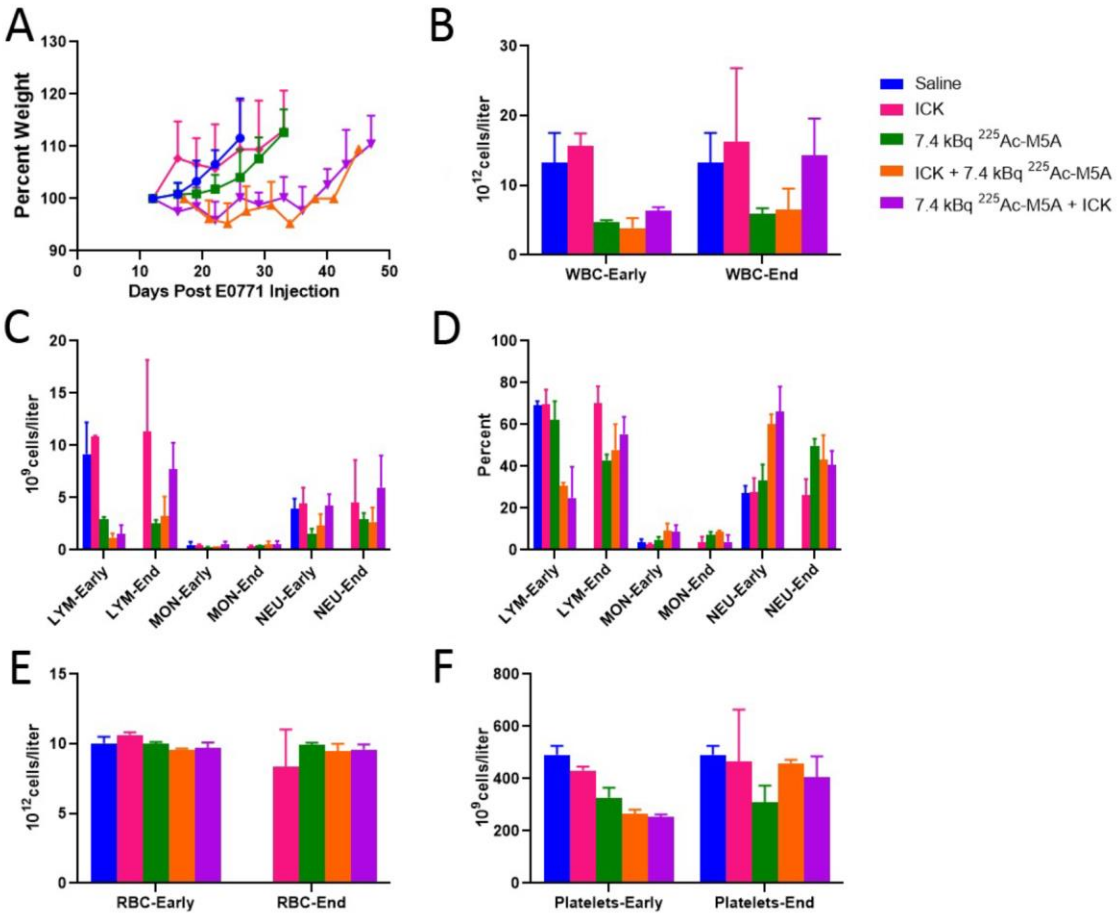
[1] Liver Toxicity: alanine aminotransferase (ALT)/ aspartate aminotransferase (AST); Kidney Toxicity: Blood Urea Nitrogen (BUN)/ creatinine (CRE). Plasma analyzed at end point of each mouse. N=4-5

SUPPLEMENTAL FIGURES

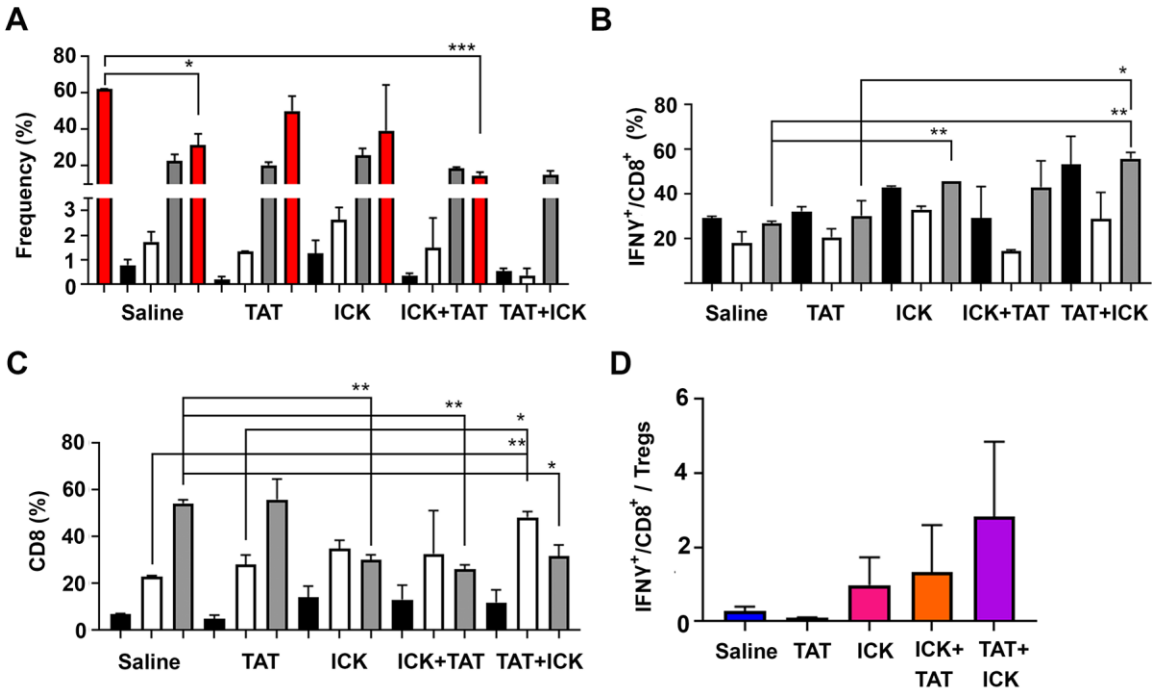
Supplemental Figure 1. Immunophenotyping of ^{225}Ac-DOTA-M5A TAT in an orthotopic breast cancer model. Analysis of CD4, CD8, B and CD11b cells in the blood (**A**) and tumor (**B**). Tumor infiltrating neutrophils to CD11b cell ratio (**C**). P values vs untreated controls, *** p<0.001; ** p<0.01; * p<0.05.



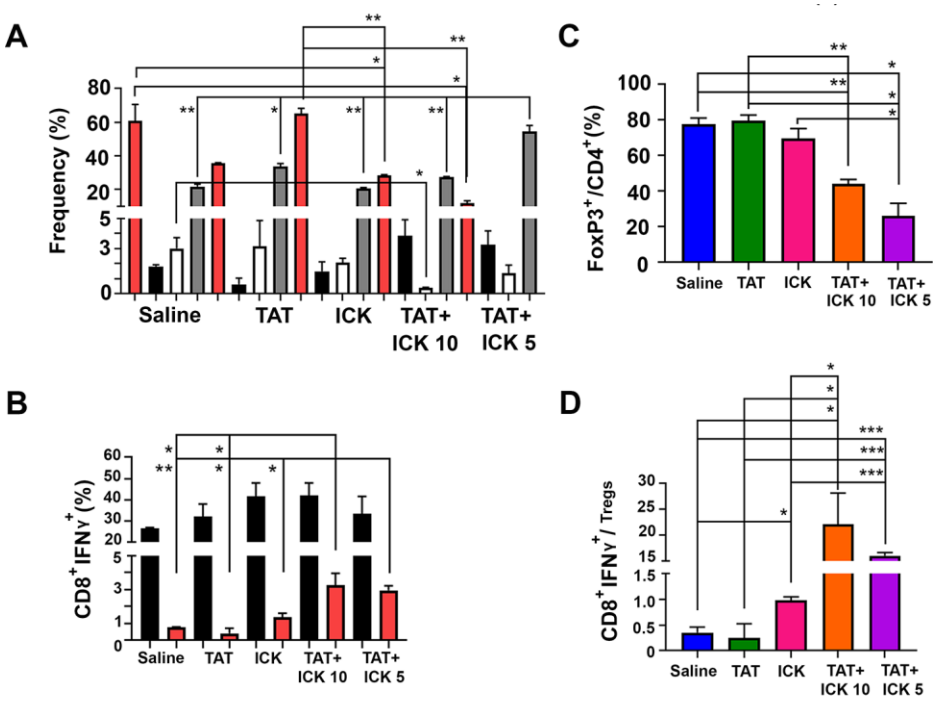
Supplemental Figure 2. Whole body toxicity and hematologic analysis of combination therapy, varying order of therapy, in E0771/CEA mice. (**A**) Whole body weights, a measure of toxicity. (**B-F**) Early time point was collected 21-22 days post E0771/CEA injection, N=2. End time point was collected as each mouse reached the 1500mm^3 end point, N=4. (**B**) White Blood Cell counts. (**C**) Leukocytes counts. (**D**) Leukocyte percents. (**E**) Red Blood Cell counts. (**F**) Platelets counts.



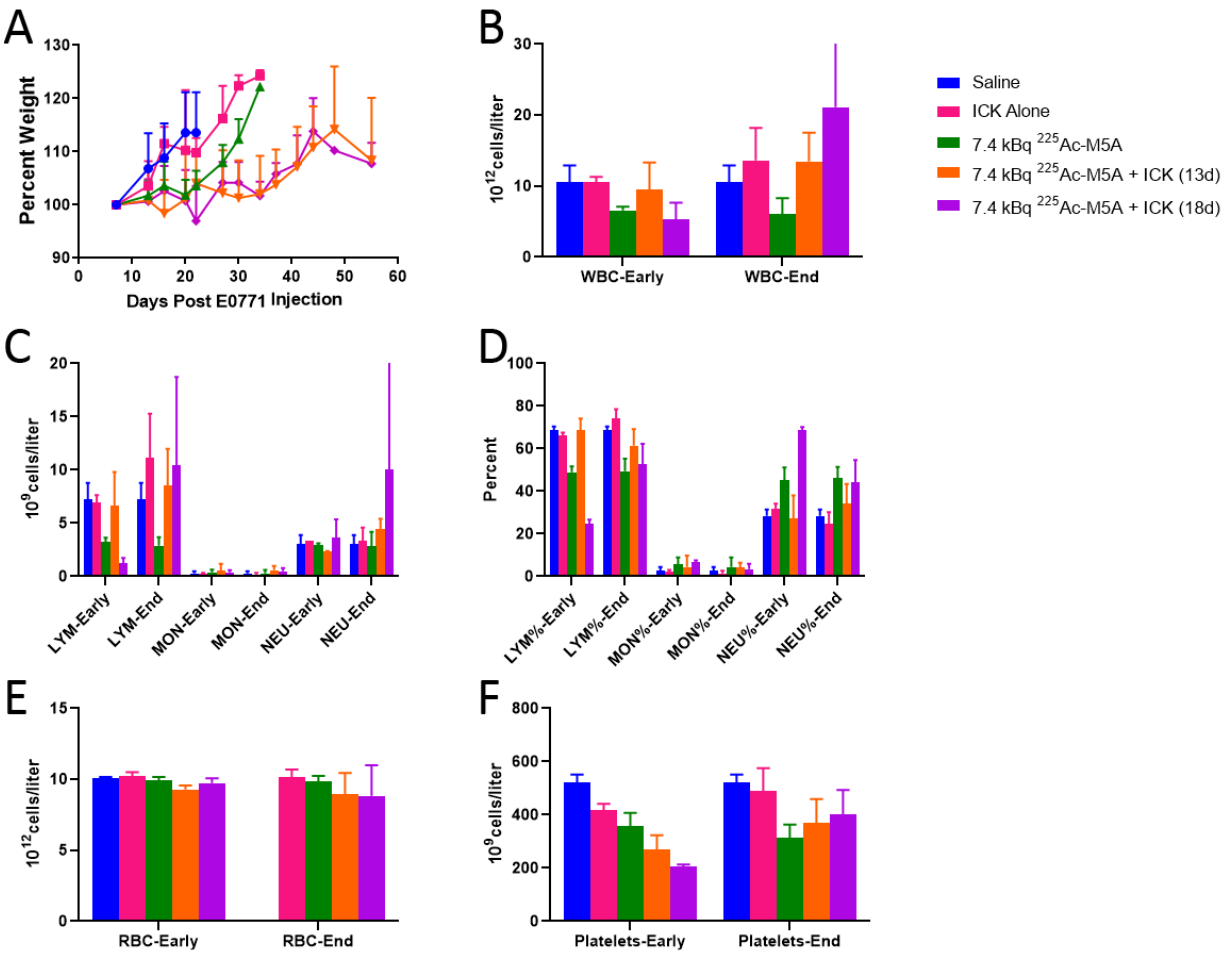
Supplemental Figure 3. Immunophenotyping of ICK first vs TAT first in sequential therapy in a breast cancer model. Analysis of CD4 (white) and CD11b (grey) cells in tumors (**A).** Percent of IFNγ^{+} /CD8^{+} T-cells in spleen (black), TDLNs (white) and tumor (grey) (**B**). Percent of IFNγ^{+}/PD-1^{-} (black), IFNγ^{+}/PD-1^{+} (white) and IFNγ^{-}/PD-1^{+} (grey) populations of tumor infiltrating CD8^{+} T-cells (**C**). Ratio of IFNγ^{+}/CD8^{+} T-cells to Foxp3^{+}/CD4^{+} regulatory T-cells in tumors (**D**). P values vs untreated controls, *** p<0.001; ** p<0.01; * p<0.05.



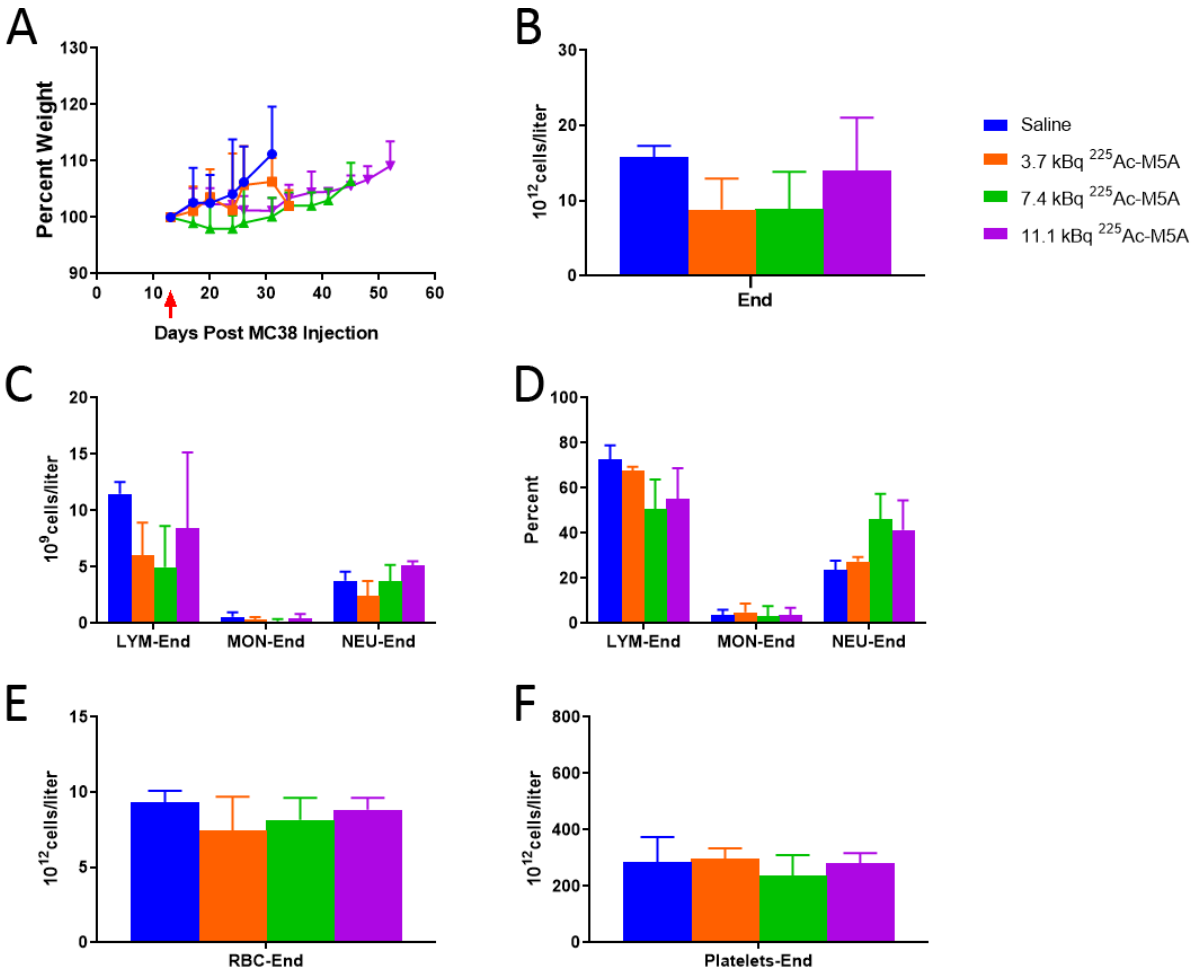
Supplemental Figure 4. Immunophenotyping of TAT followed by ICK, 5 or 10 days later in a breast cancer model. Analysis of cell viability (red), and frequency of CD8 (black), CD4 (white) and CD11b (grey) cells in tumors (**A**). Percent of FoxP3$^+$ /CD4$^+$ T-cells for different treatment groups (**B**). Percent of IFNγ$^+$ CD8$^+$ as % of CD8 cells (black) or % of viable cells (red) (**C**). Ratio of IFNγ$^+$/CD8$^+$ T-cells to Foxp3$^+$/CD4$^+$ regulatory T-cells in tumors (**D**). P values vs untreated controls, *** p<0.001; ** p<0.01; * p<0.05.



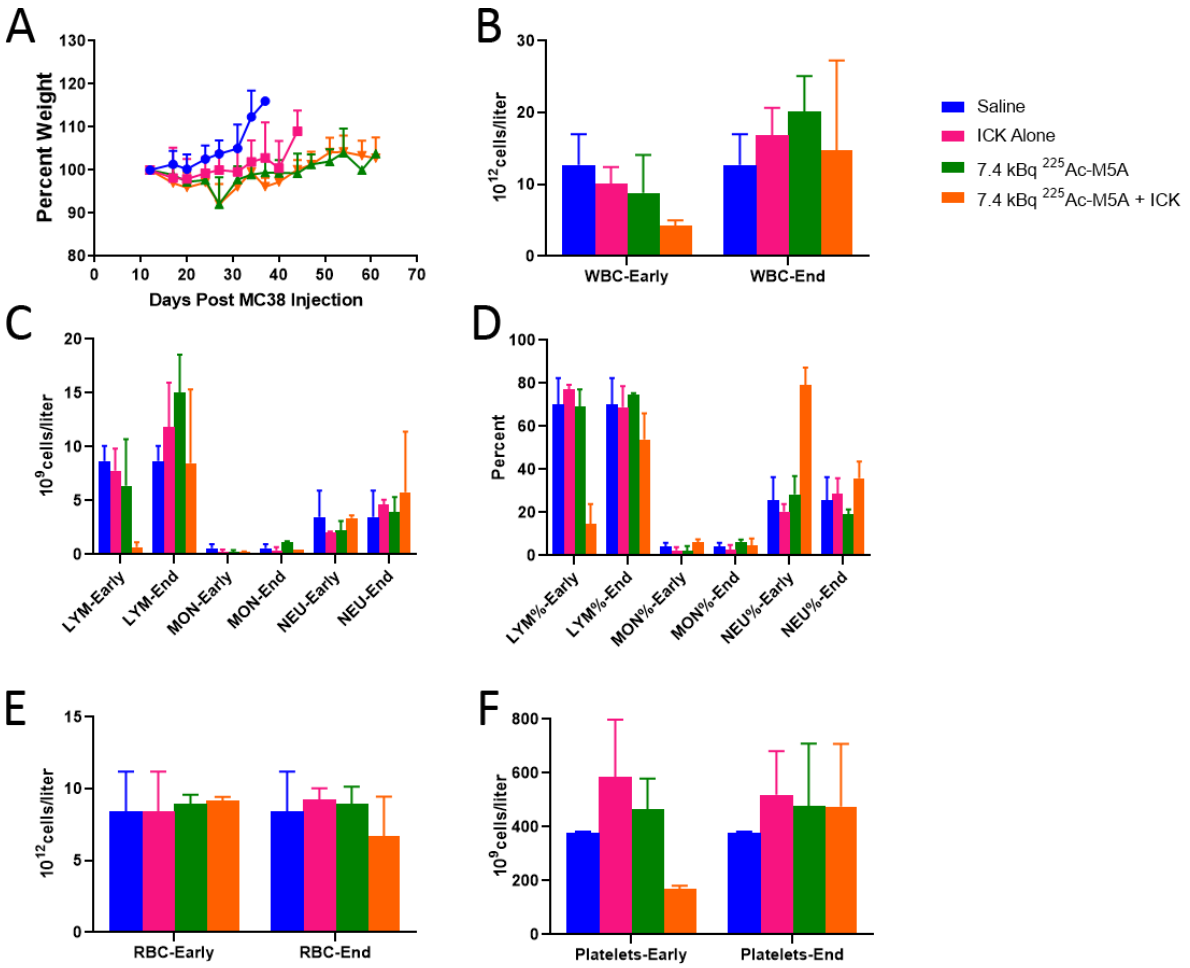
Supplemental Figure 5. Whole body toxicity and hematologic analysis of combination therapy, with ICK 5 or 10 days post TAT, in E0771/CEA mice. (**A**) Whole body weights, a measure of toxicity. (**B-F**) Early time point was collected 22 days post E0771/CEA injection, N=2. End time point was collected as each mouse reached the 1500mm^3 end point, N=4. (**B**) White Blood Cell counts. (**C**) Leukocytes counts. (**D**) Leukocyte percents. (**E**) Red Blood Cell counts. (**F**) Platelets counts.



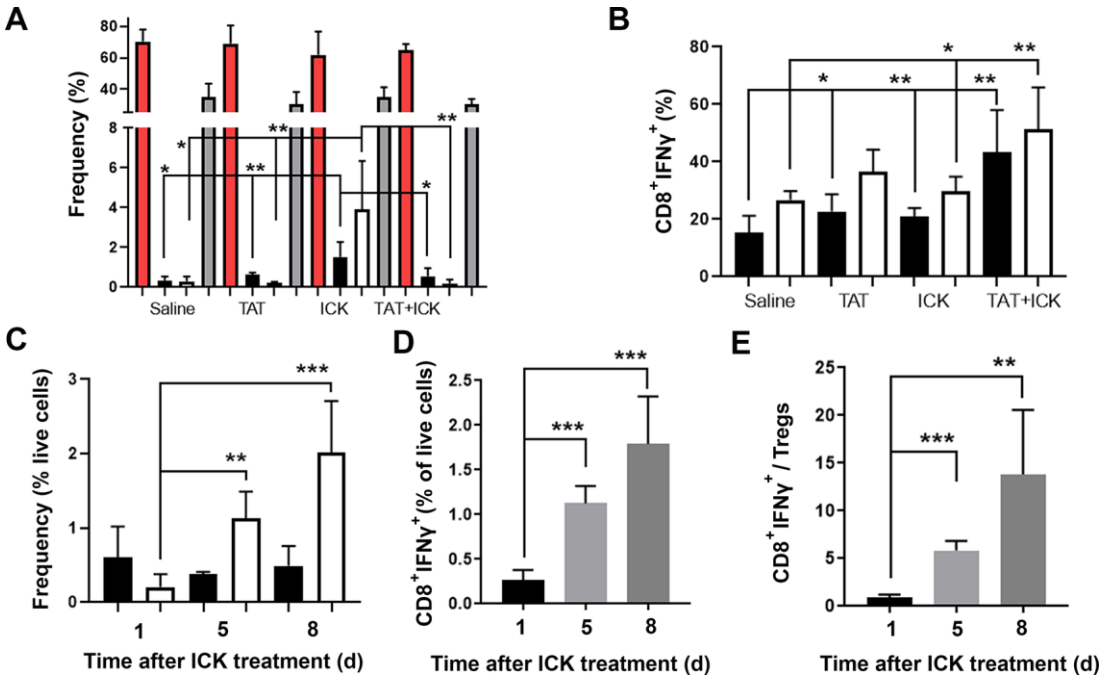
Supplemental Figure 6. Whole body toxicity and hematologic analysis of dose escalation study in MC38/CEA engrafted mice. (**A**) Whole body weights, a measure of toxicity. (**B-F**) Early time point was collected 22 days post E0771/CEA injection, N=2. End time point was collected as each mouse reached the 1500mm^3 end point, N=4. (**B**) White Blood Cell counts. (**C**) Leukocytes counts. (**D**) Leukocyte percents. (**E**) Red Blood Cell counts. (**F**) Platelets counts.



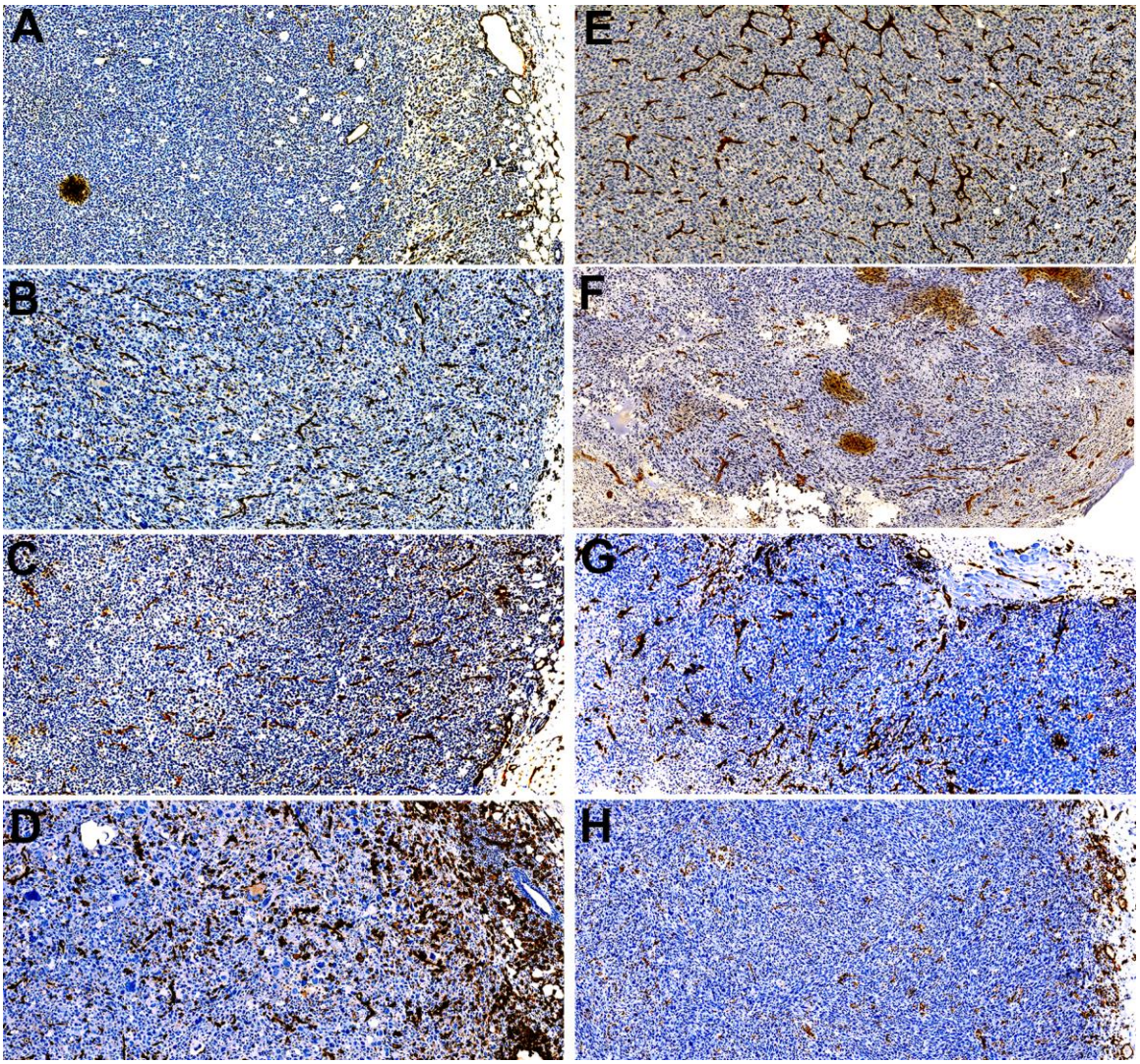
Supplemental Figure 7. Whole body toxicity and hematologic analysis of combination therapy in MC38/CEA engrafted mice. (**A**) Whole body weights, a measure of toxicity. (**B-F**) Early time point was collected 22 days post E0771/CEA injection, N=2-3. End time point was collected as each mouse reached the 1500mm^3 end point, N=2-3. (**B**) White Blood Cell counts. (**C**) Leukocytes counts. (**D**) Leukocyte percents. (**E**) Red Blood Cell counts. (**F**) Platelets counts.



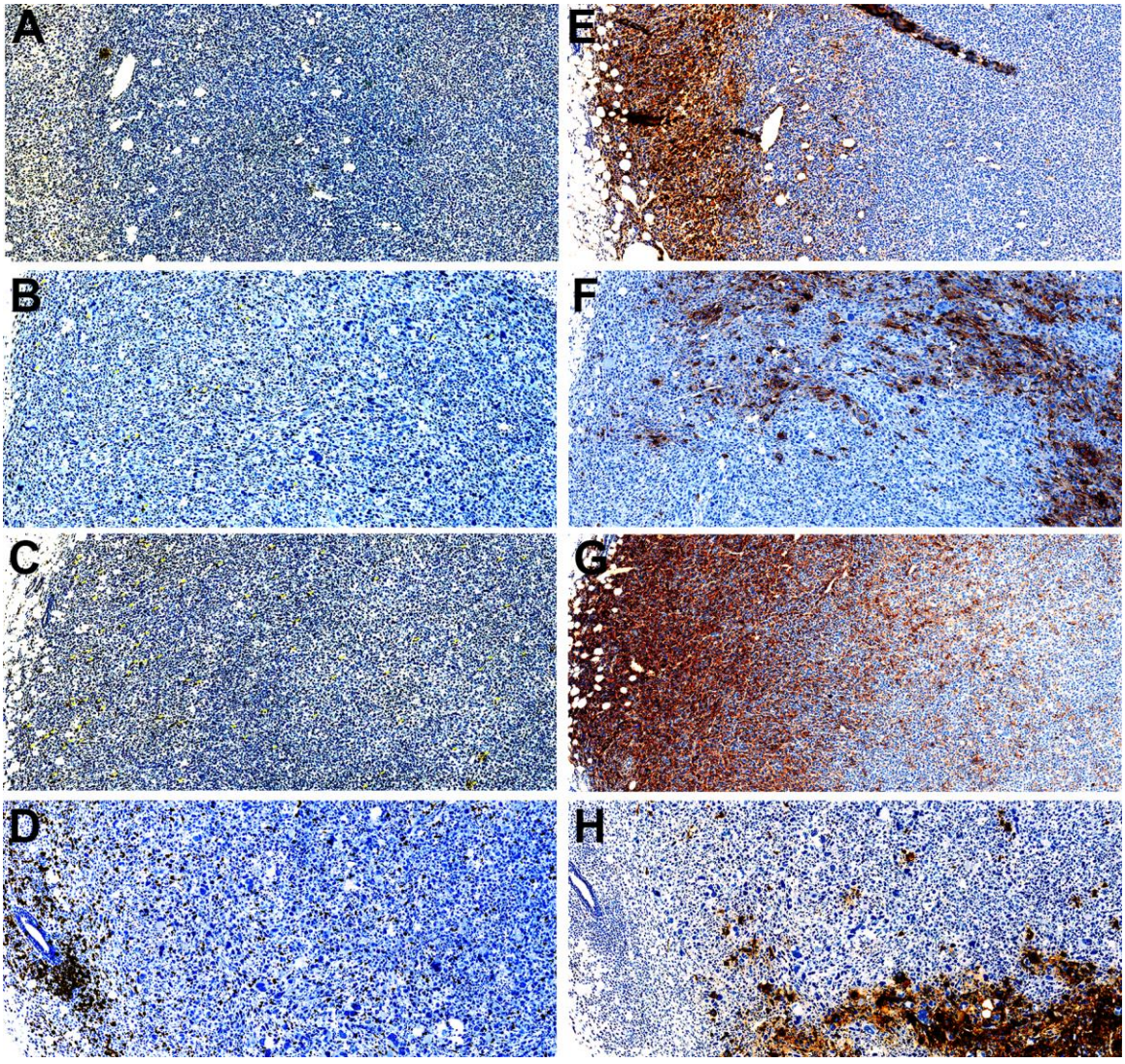
Supplemental Figure 8. Phenotyping of TAT first followed by ICK therapy in a colon cancer model. Analysis of Live cells (red), CD4 (black), CD8 (white) and CD11b (grey) cells in tumors analyzed by flow cytometry at day 27 (**A**). IFNγ$^+$ CD4$^+$ (black) and CD8$^+$ (white) cells in tumors analyzed by flow cytometry at day 27 (**B**). CD4 (black) and CD8 (white) cells in tumors analyzed at days 1, 5 and 8 after last dose of ICK in TAT+ICK group (**C**). Tumor infiltrating IFNγ$^+$ CD8$^+$ cells at days 1, 5 and 8 after last dose of ICK in TAT+ICK group (**D**). Ratio of IFNγ$^+$ CD8$^+$ T-cells to regulatory T-cells in tumors at days 1, 5 and 8 after last dose of ICK in TAT+ICK group (**E**). P values vs untreated controls, *** p<0.001; ** p<0.01; * p<0.05



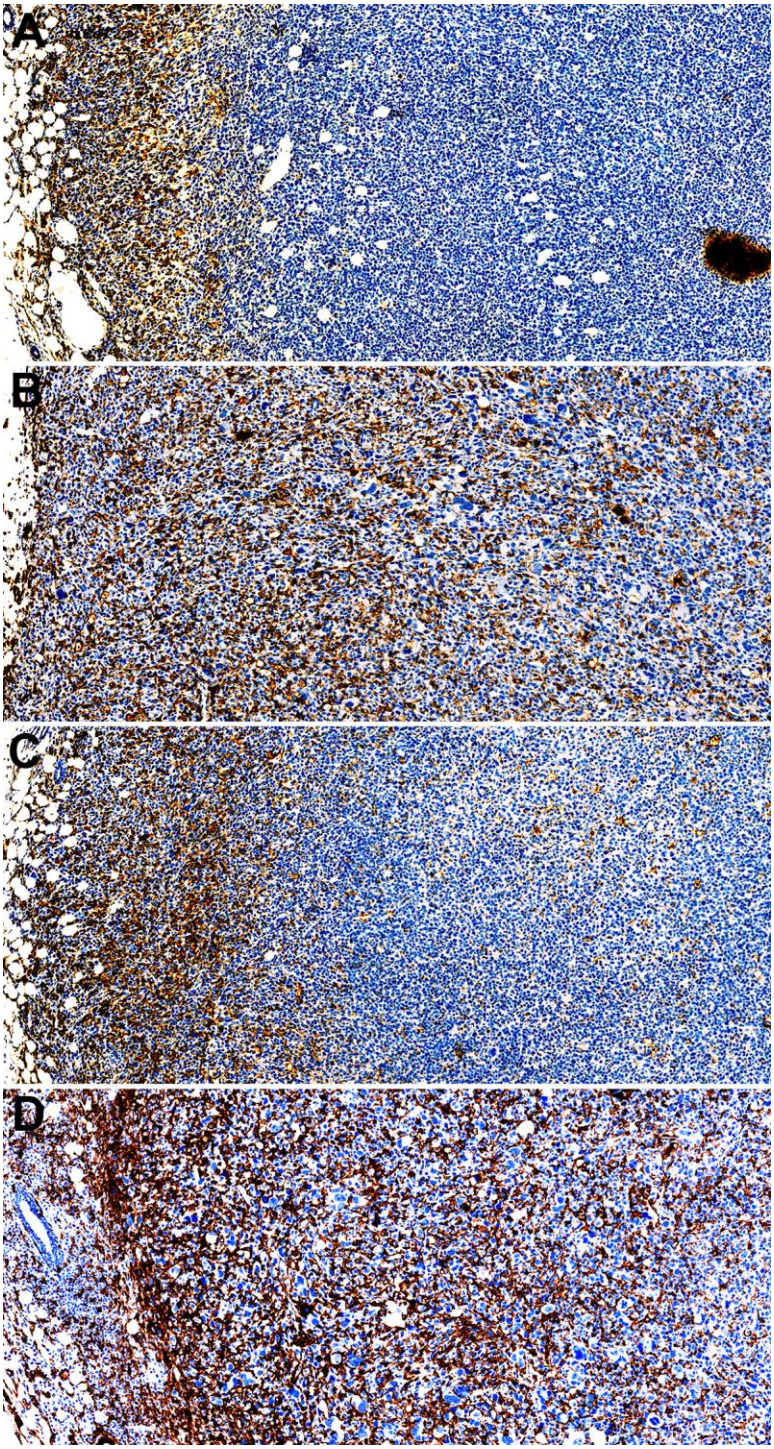
Supplemental Figure 9. CD31 staining of E0771/CEA breast and MC38/CEA colon cancer tumors. A-D, CD31 staining for E0771/CEA breast cancer tumors in untreated controls (**A**), TAT only (**B**), ICK only (**C**), and combination therapy (**D**). **E- F**, CD31 staining for MC38/CEA colon tumors in untreated controls (**E**), TAT only (**F**), ICK only (**G**), and combination therapy (**H**). 10x magnification.



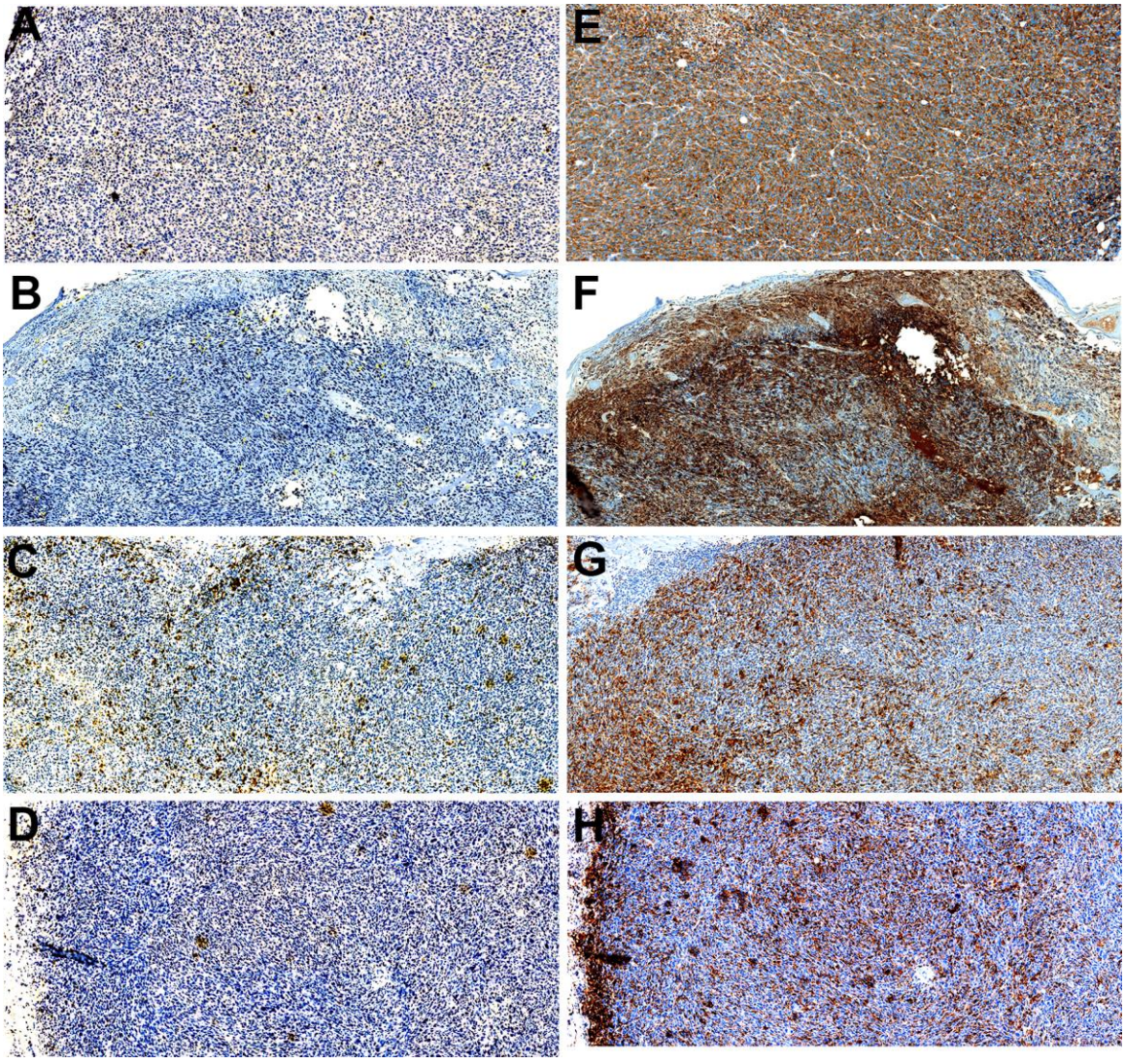
Supplemental Figure 10. CD8 and CEA staining of E0771/CEA breast cancer tumors. A-D, CD8 staining in untreated controls (**A**), TAT only (**B**), ICK only (**C**), and combination therapy (**D**). **E- F**, CEA staining in untreated controls (**E**), TAT only (**F**), ICK only (**G**), and combination therapy (**H**). 10x magnification.



Supplemental Figure 11. F4-80 staining of E0771/CEA breast cancer tumors. F4-80 staining in untreated controls (**A**), TAT only (**B**), ICK only (**C**), and combination therapy (**D**). 10x magnification.



Supplemental Figure 12. CD8 and CEA staining of MC38/CEA colon cancer tumors. A-D, CD8 staining in untreated controls (**A**), TAT only (**B**), ICK only (**C**), and combination therapy (**D**). **E- F**, CEA staining in untreated controls (**E**), TAT only (**F**), ICK only (**G**), and combination therapy (**H**). 10x magnification.



Supplemental Figure 13. F4-80 staining of MC38/CEA colon cancer tumors. F4-80 staining in untreated controls (**A**), TAT only (**B**), ICK only (**C**), and combination therapy (**D**). 10x magnification.