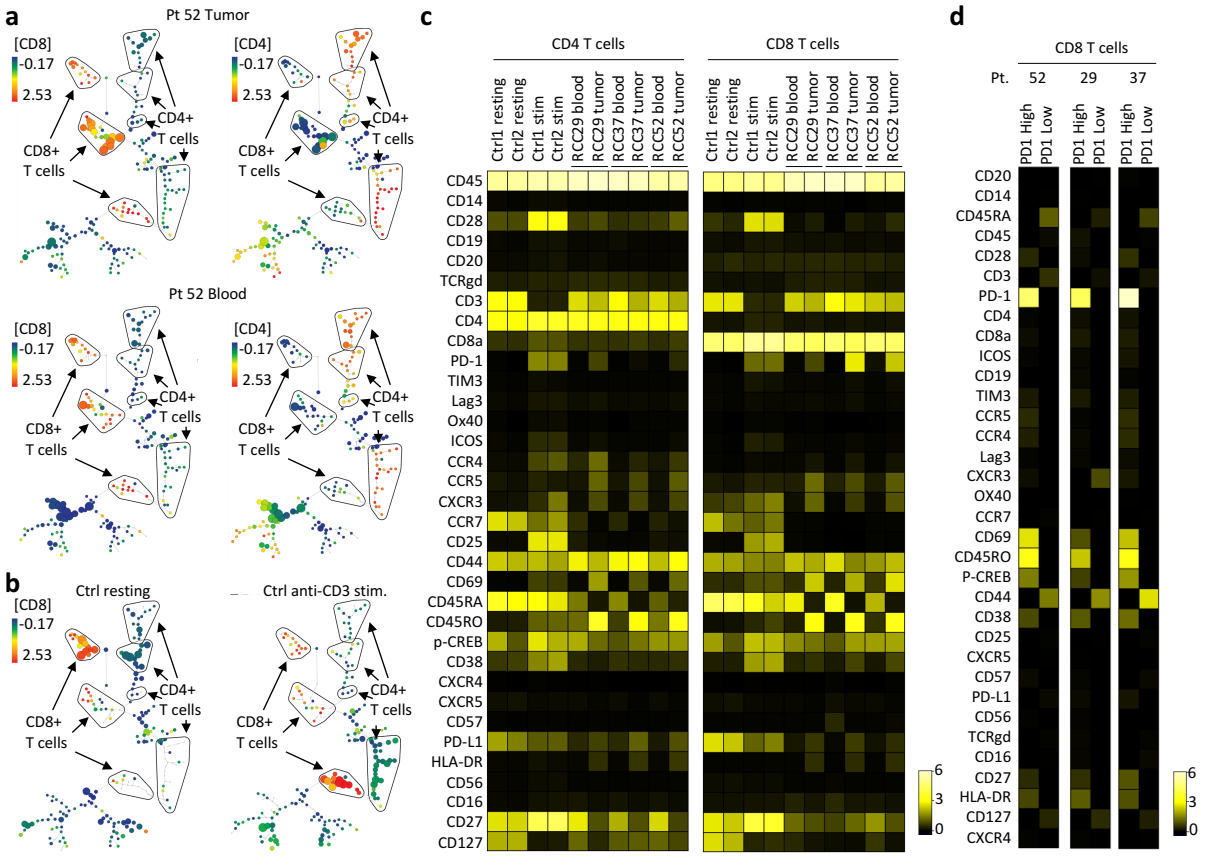
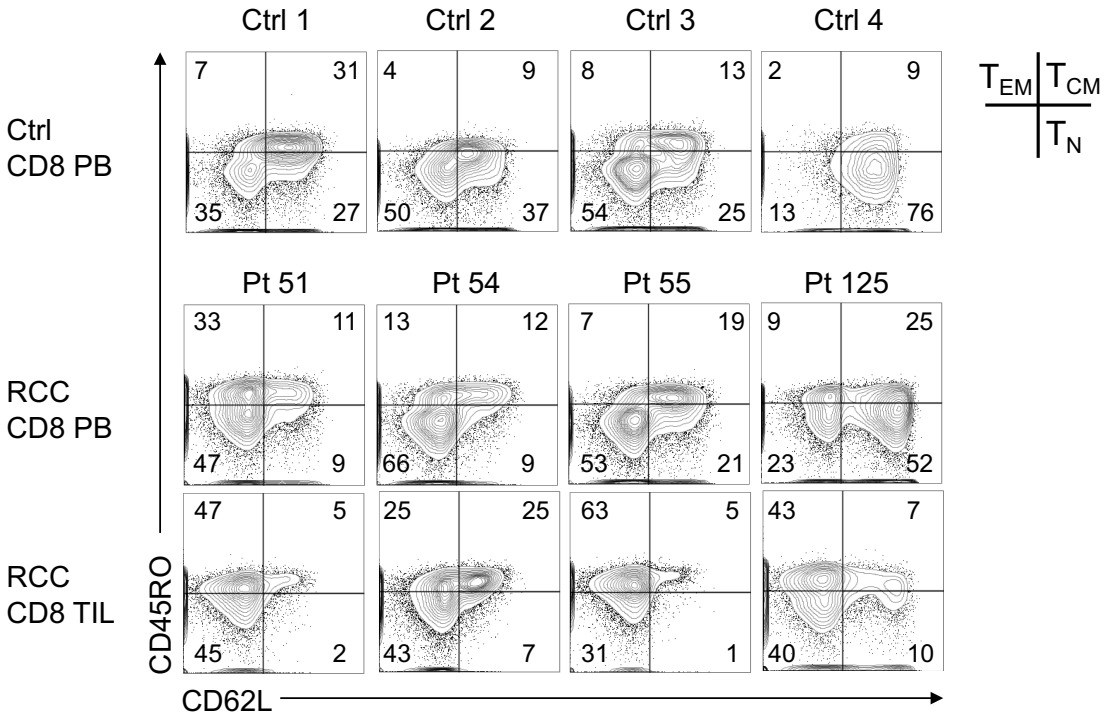
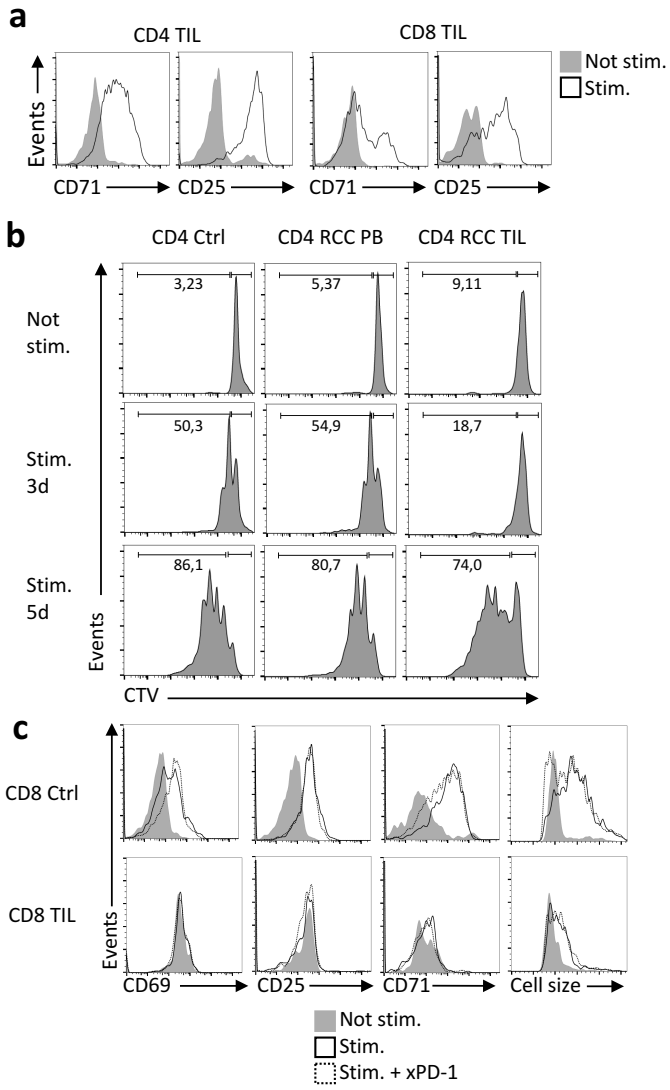
SUPPLEMENTAL FIGURES



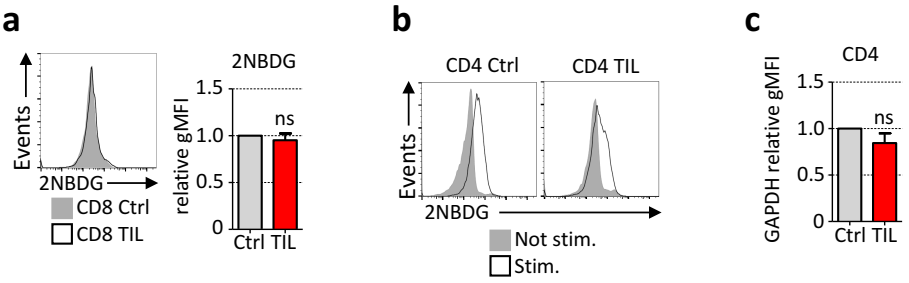
Supplementary Figure 1. Phenotype of RCC infiltrating T cells. (**a, b**) CD45+ Leukocytes from RCC patient peripheral blood or tumor and healthy donor resting or anti-CD3 stimulated peripheral blood mononuclear cells were analyzed by mass cytometry. Unsupervised clustering and SPADE diagram visualization was performed using Cytobank software. Markers used for clustering are presented as heatmap in (c). Data are representative of 3 RCC patient TIL and blood pairs and 2 healthy donor unstimulated and stimulated samples. (**c**) Heatmap depiction of markers measured in (a, b), gated on CD4 and CD8 T cells. (**d**) CD8 T cells from RCC patients were separated to PD-1^{hi} and PD-1^{low} and phenotype was assessed as in (a, b). Heatmap cell colors are normalized to row's minimum.



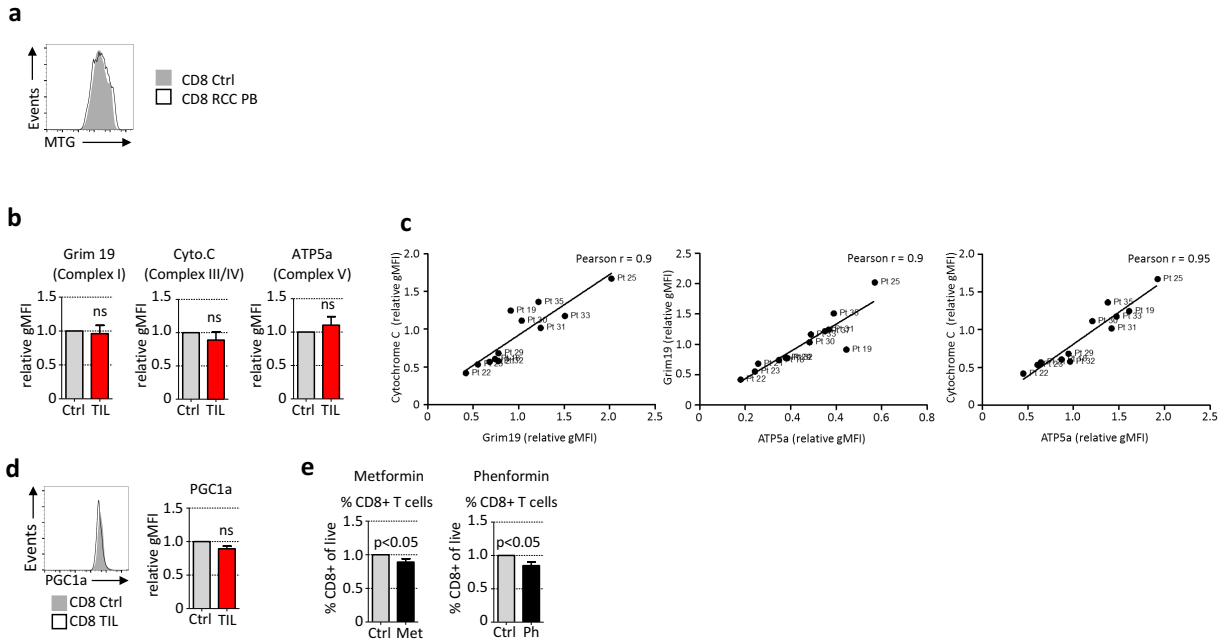
Supplementary Figure 2. CD8 T cell subset distribution in healthy and RCC samples. CD8 T cells from peripheral blood of healthy donors or RCC patients and CD8 RCC TIL from corresponding patients were analyzed for surface expression of CD45RO and CD62L to differentiate between T cell effector-memory (T_{EM}), central-memory (T_{CM}) and naïve T cells (T_N).



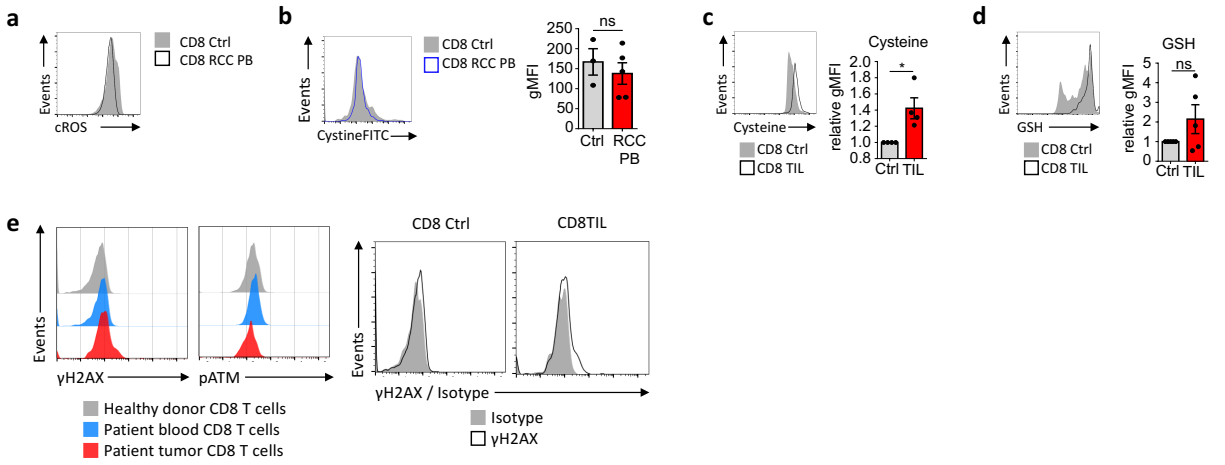
Supplementary Figure 3. Activation, proliferation and PD-1 blockade sensitivity of RCC TIL. (**a**) Tumor single cell suspensions were stimulated for 3 days and CD25 and CD71 expression was measured on CD4 and CD8 T cells. Data are representative for at least 3 independent experiments. (**b**) Cells were stimulated as in (a) for 3 or 5 days and proliferation of CD4 RCC TIL was assessed using Cell Trace Violet dilution. Data are representative of 4 RCC patients. (**c**) Cells were stimulated as in (a) with and without anti-PD-1 blocking antibodies and selected markers were measured on CD8 T cells. Data are representative of 4 independent experiments.



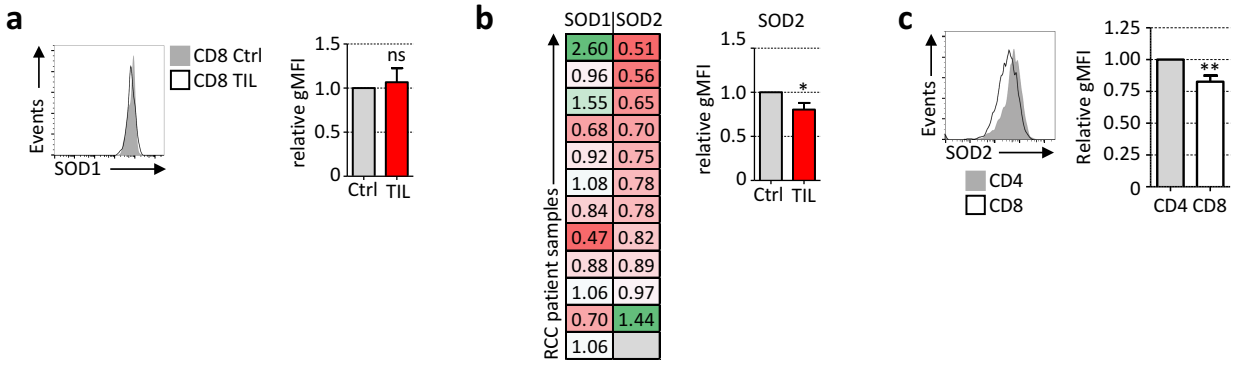
Supplementary Figure 4. CD4 and CD8 TIL glucose uptake and GAPDH expression. (**a**) Glucose uptake was measured using 2NBDG in freshly isolated CD8 TIL (n=5) and healthy controls (n=10). (**b**) ccRCC TIL were stimulated and glucose uptake measured on CD4 T cells using 2NBDG. Data are representative of 6 independent experiments. (**b**) GAPDH expression was measured on CD4 TIL from RCC patients (n=8) or healthy donor PBMC CD4 T cells (n=11).



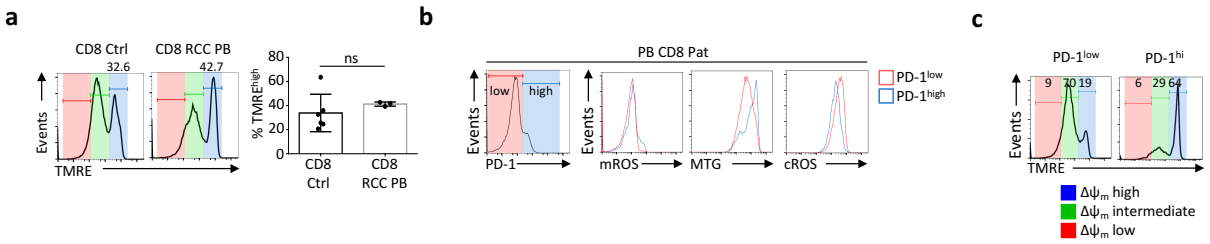
Supplementary Figure 5. Maintained mitochondrial integrity of RCC CD8 TIL. (**a**) Healthy control and RCC patient blood CD8 T cells were stained with Mitotracker green. Data are representative of 3 RCC patients. (**b**) Expression of selected mitochondrial membrane proteins was measured on 12 RCC patient samples. (**c**) Data from (b) were used to pair-correlate expression of selected markers. (**d**) Expression of PGC1a measured with flow cytometry on healthy controls or RCC patient samples. (**e**) Tumor suspensions from 4 RCC patients were anti-CD3 stimulated with selected drug concentrations for 3 days and expression of CD71 and percentage of CD8 T cells of live cells were assessed by flow cytometry. Error bars represent s.e.m.; * P < 0.05, ** P < 0.01 and *** P < 0.001.



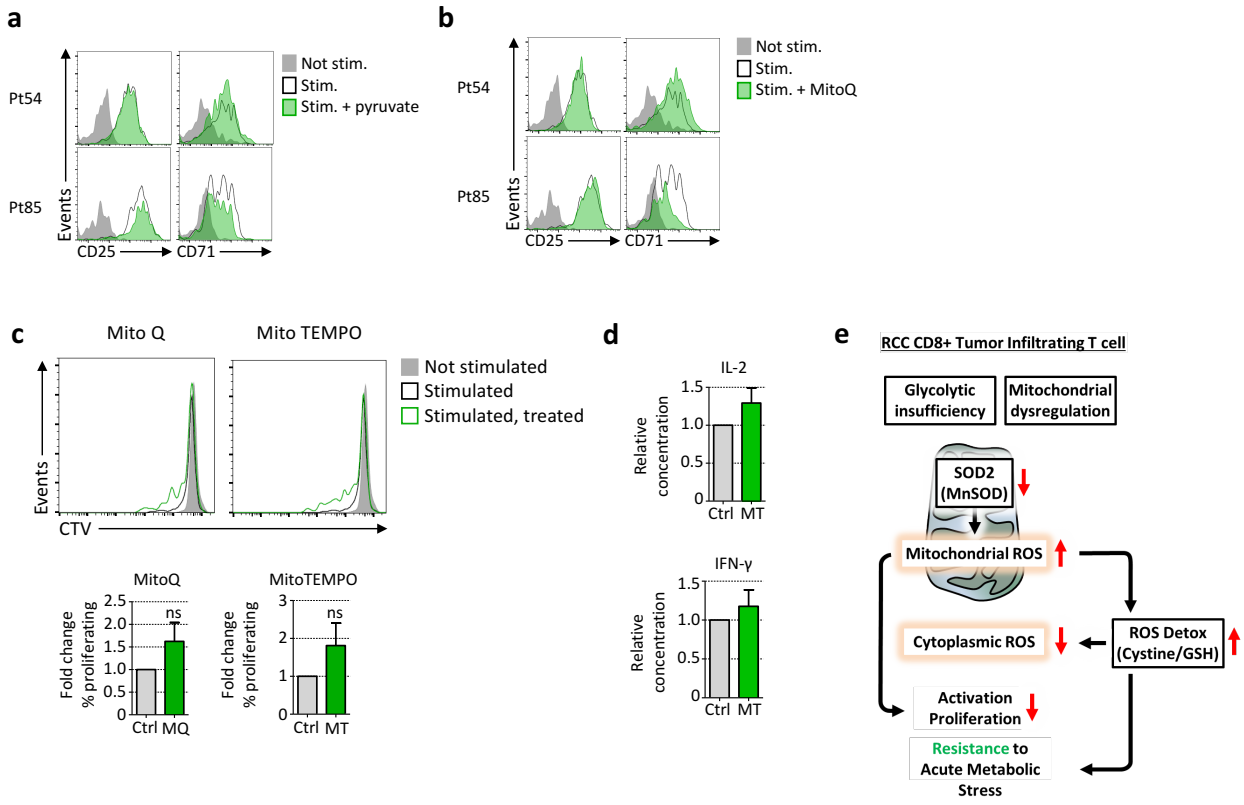
Supplementary Figure 6. Impact of ROS dysregulation in CD8 RCC TIL. (**a, b**) Accumulation of cytosolic ROS and uptake of cystine was assessed in CD8 T cells from healthy controls or from RCC patient blood. (**c**) Intracellular cysteine levels were measured with a cysteine sensitive dye on 4 RCC patient samples and 6 healthy donors. (**d**) Glutathione levels in 5 RCC patients were measured with Thioltracker. (**e**) DNA damage markers yH2AX and pATM measured with flow cytometry on healthy donor CD8 cells and CD8 cells from RCC patient blood or tumor. Data are representative of 7 independent experiments.



Supplementary Figure 7. SOD expression in RCC TIL. (a-c) SOD1 and SOD2 expression was assessed on CD4 and CD8 T cells from healthy controls (n=18) or RCC patient TIL (n=11-13).



Supplementary Figure 8. Mitochondrial phenotype of peripheral blood CD8 T cells from RCC patients. CD8 T cells from peripheral blood of RCC patients were analyzed for mitochondrial membrane potential, PD-1 expression, mitochondrial ROS (MitoSOX), mitochondrial membrane content/mitochondrial mass (MTG) and cytosolic ROS (DFCDA). Data are representative of 3-4 RCC patients.



Supplementary Figure 9. The role of SOD2 downregulation and mROS accumulation on CD8 TIL activation. (**a**) ccRCC patient samples (n=7) were stimulated with or without additional pyruvate and expression of activation markers was measured with flow cytometry. (**b**) Activation of ccRCC CD8 TIL was assessed as in (a) after treatment with MitoQ. (**c**) RCC samples were treated with indicated compounds and *in vitro* stimulated. Proliferation was measured using CTV after 5 days (n=5). (**d**) Effects of MitoTEMPO on RCC TIL activation was assessed by IL2 and IFNγ measurements using Luminex assay (n=3). (**e**) Model.

Supplemental Movies. **Mitochondrial morphology in CD8 T cells**. Movies of CD8 T cells from peripheral blood of healthy donors stained for CD8 (magenta) and Mitotracker (green). Mitotracker is shown alone on left and CD8 together with Mitotracker are shown on right. Movies show confocal imaging of z-stack series with movies 1-2 showing control CD8 T cells and movies 3-5 showing ccRCC CD8 TIL.

Supplemental Table 1. Patient charateristics.

ID	Disease	Subtype	Grade	Age	Sex	Race
3	Renal Cell Carcinoma	Clear Cell	4	NA	NA	NA
4	Renal Cell Carcinoma	Clear Cell	2	NA	NA	NA
5	Renal Cell Carcinoma	Clear Cell	3	NA	NA	NA
6	Renal Cell Carcinoma	NA	NA	NA	NA	NA
7	Renal Cell Carcinoma	NA	NA	NA	NA	NA
8	Renal Cell Carcinoma	Clear Cell	3	NA	NA	NA
9	Renal Cell Carcinoma	NA	NA	NA	NA	NA
11	Renal Cell Carcinoma	Clear Cell	2	NA	NA	NA
12	Renal Cell Carcinoma	Clear Cell	3	NA	NA	NA
13	Renal Cell Carcinoma	Clear Cell	3	NA	NA	NA
14	Renal Cell Carcinoma	NA	3	NA	NA	NA
17	Renal Cell Carcinoma	Clear Cell	2	NA	NA	NA
18	Renal Cell Carcinoma	Clear Cell	2	NA	NA	NA
19	Renal Cell Carcinoma	Clear Cell	2	NA	NA	NA
21	Renal Cell Carcinoma	NA	NA	NA	NA	NA
22	Renal Cell Carcinoma	NA	NA	NA	NA	NA
23	Renal Cell Carcinoma	NA	NA	NA	NA	NA
24	Renal Cell Carcinoma	NA	NA	NA	NA	NA
25	Renal Cell Carcinoma	NA	NA	NA	NA	NA
26	Renal Cell Carcinoma	Clear Cell	4	79	male	white
29	Renal Cell Carcinoma	Clear Cell	3	72	male	white
30	Renal Cell Carcinoma	Clear Cell Tubulopalillary Type	2	64	female	white
31	Renal Cell Carcinoma	Unclassified	3	35	female	black
33	Renal Cell Carcinoma	Clear Cell	2	77	female	white
34	Renal Cell Carcinoma	Clear Cell	2	62	female	white
36	Renal Cell Carcinoma	Clear Cell	3	62	male	white
37	Renal Cell Carcinoma	Clear Cell	2	54	male	white
38	Renal Cell Carcinoma	Clear Cell	3	57	male	white
40	Renal Cell Carcinoma	Clear Cell	3	51	female	white
41	Renal Cell Carcinoma	Clear Cell	3	65	male	white
42	Renal Cell Carcinoma	Clear Cell	4	62	female	white
43	Renal Cell Carcinoma	Clear Cell	2	82	female	white
46	Renal Cell Carcinoma	Clear Cell	2	78	female	white
49	Renal Cell Carcinoma	Clear Cell	3	73	male	white
51	Renal Cell Carcinoma	Clear Cell	3	79	male	white
52	Renal Cell Carcinoma	Clear Cell	3	45	male	white
53	Renal Cell Carcinoma	Clear Cell	2	51	female	white
54	Renal Cell Carcinoma	Clear Cell	3	69	male	white
55	Renal Cell Carcinoma	Clear Cell	2	55	male	white
56	Renal Cell Carcinoma	Clear Cell	2	40	male	white
58	Renal Cell Carcinoma	Clear Cell	3	58	male	black
60	Renal Cell Carcinoma	Clear Cell	2	60	female	white
61	Renal Cell Carcinoma	Clear Cell	2	45	male	white

62	Renal Cell Carcinoma	Clear Cell	3	43	white	male
63	Renal Cell Carcinoma	Clear Cell	2	50	male	white
76	Renal Cell Carcinoma	Clear Cell	3	65	female	white
84	Renal Cell Carcinoma	Clear Cell	2	77	male	white
85	Renal Cell Carcinoma	Clear Cell	3	81	male	black
89	Renal Cell Carcinoma	Clear Cell	2	56	male	white
91	Renal Cell Carcinoma	Clear Cell	2	49	female	white
93	Renal Cell Carcinoma	Clear Cell	2	44	male	white
94	Renal Cell Carcinoma	Clear Cell	NA	72	male	white
105	Renal Cell Carcinoma	Clear Cell	2	47	female	white
125	Renal Cell Carcinoma	Clear Cell	2	66	male	black