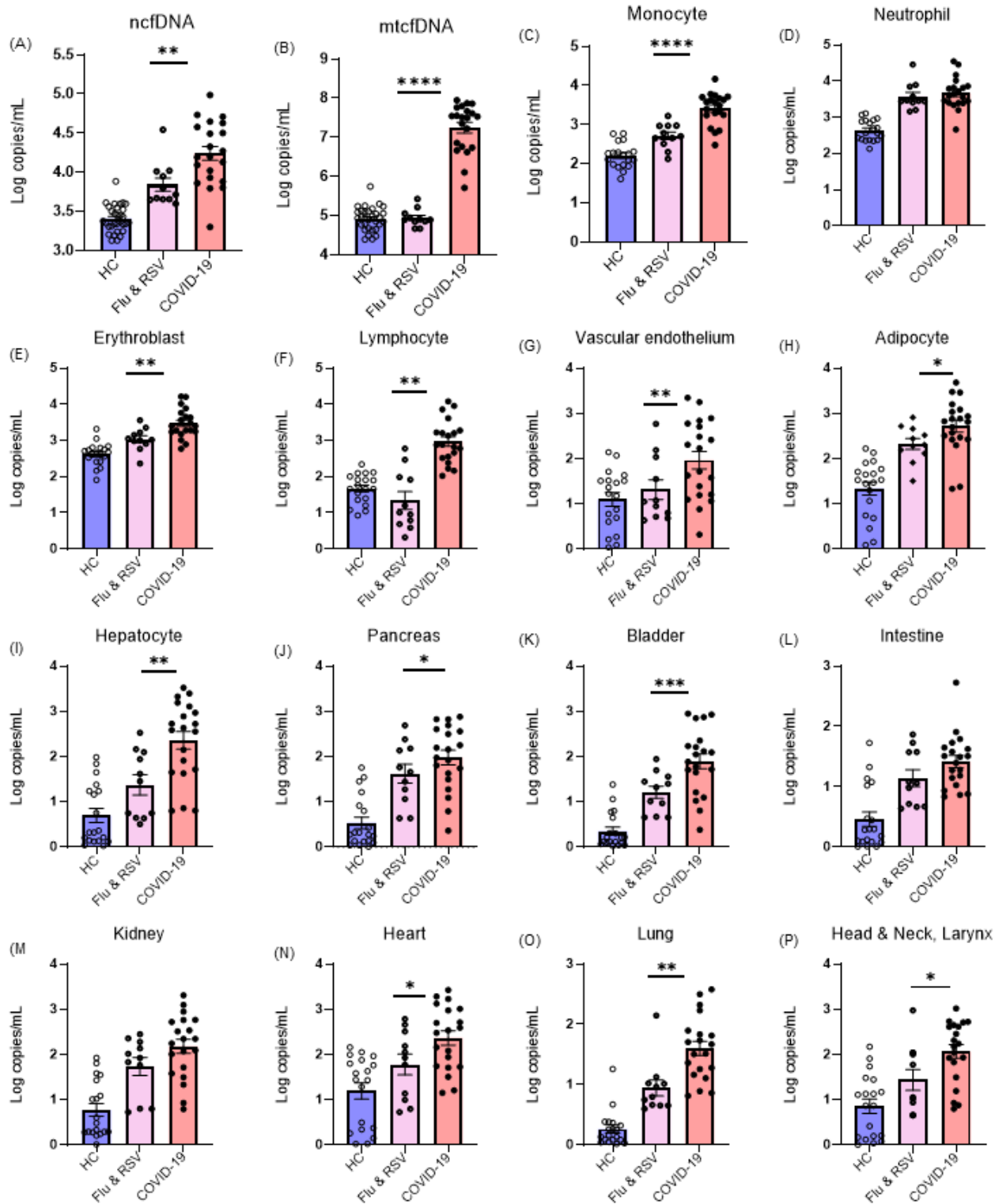


# Supplementary Material

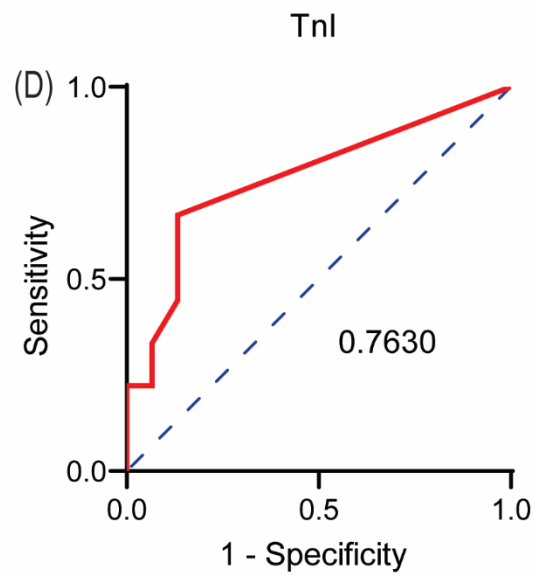
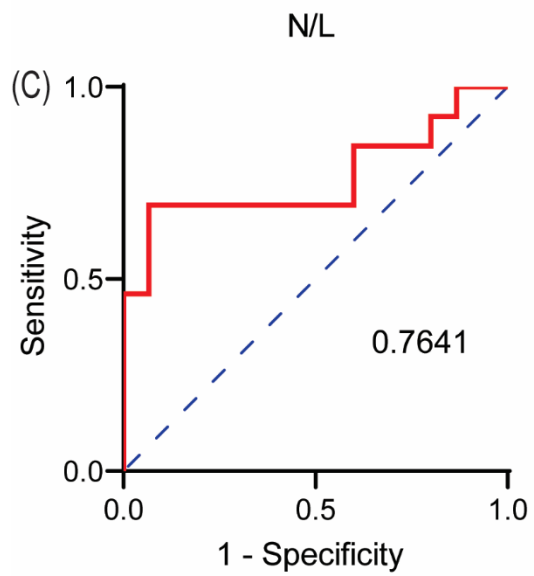
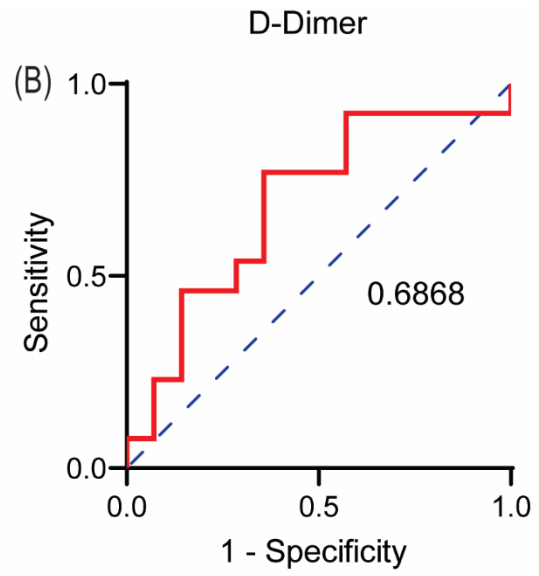
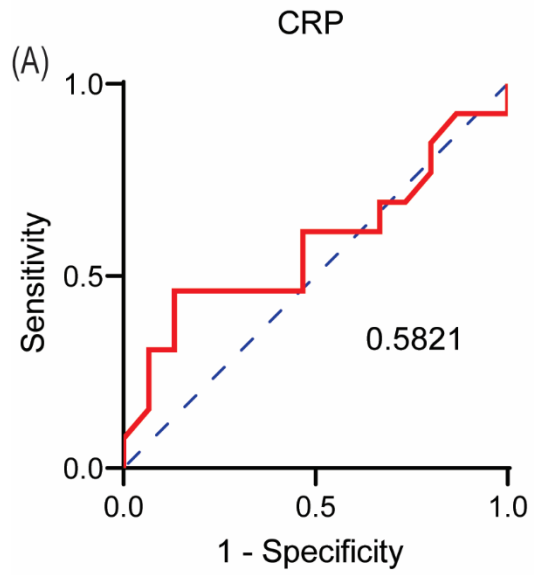
## **Cell-free DNA maps COVID-19 tissue injury and risk of death, and propagates tissue injury**

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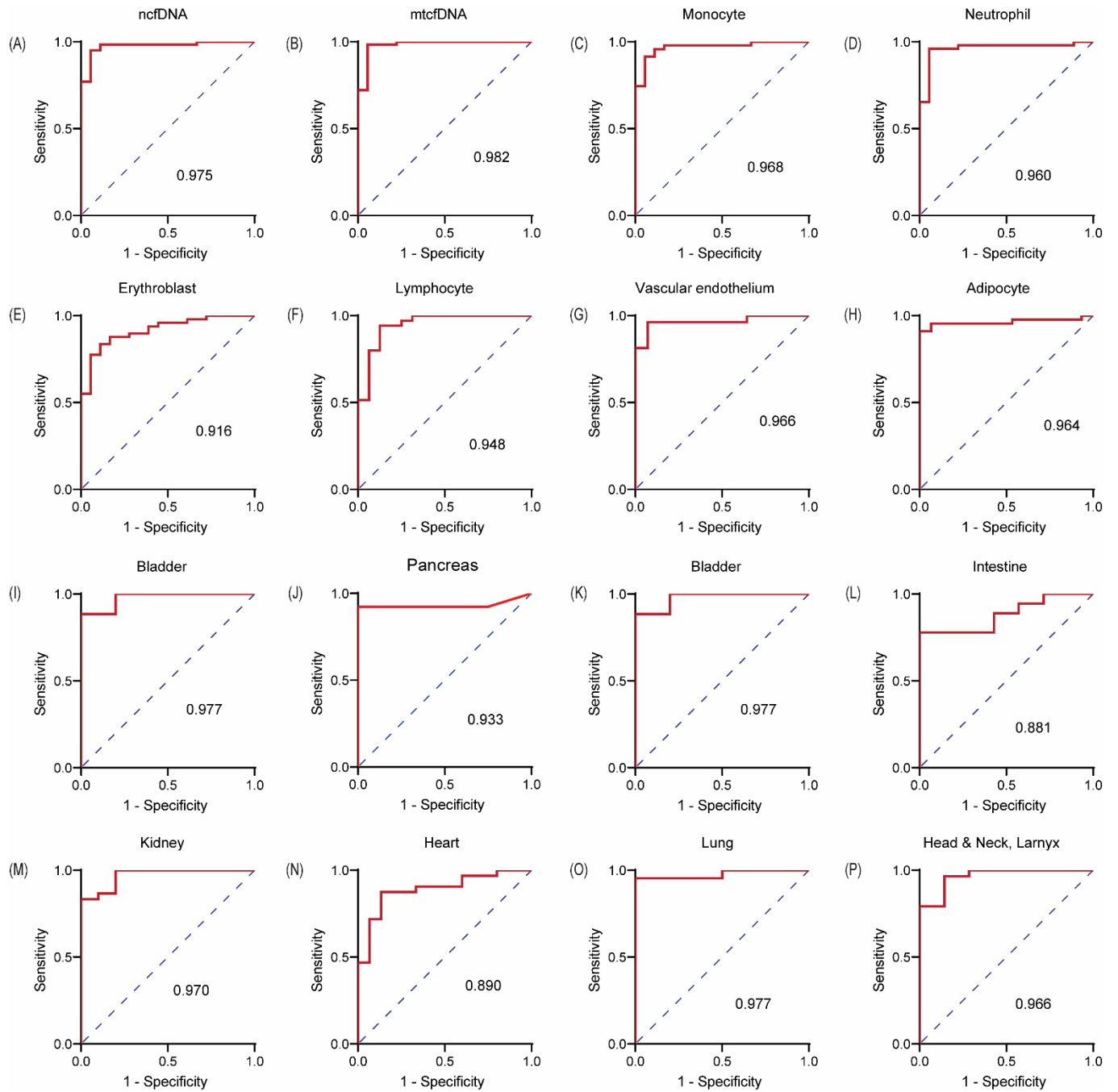


**Supplementary Figure 1.** cfDNA profiles in COVID-19 patients compared with WHO-scale-matched Flu & RSV patients.

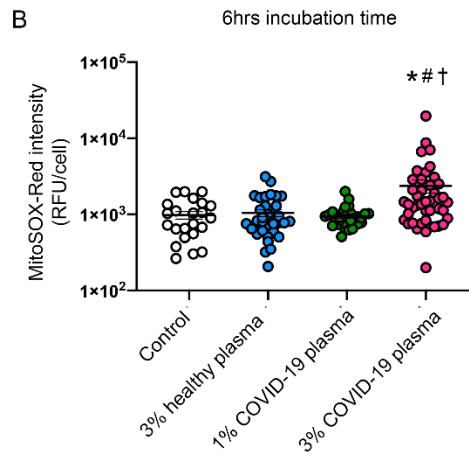
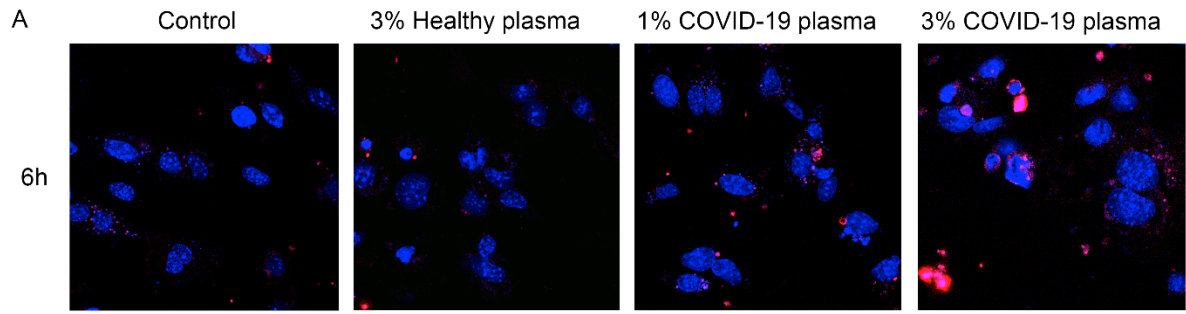
Total plasma cell-free nuclear (A) and mitochondrial cfDNA (B) from Healthy controls, blue (n=31), hospitalized non-ICU Influenza and Respiratory Syncytial Virus (Flu & RSV) infected patients, orange (n=12), and hospitalized non-ICU COVID-19 patients, black (n= 20). (C-P) quantification of cell/tissue-specific cfDNA; (C) Monocytes, (D) Neutrophils, (E) Erythroblasts, (F) Lymphocytes, (G) Vascular endothelial cells, (H) adipocytes, (I) Hepatocytes, (J) Pancreas, (K) Bladder, (L) intestine, (M) Kidney, (N) Heart, (O) Lung and (P) Head and neck larynx-derived cfDNA from HCs, brown (n=19), Flu & RSV patients, yellow (n=12), and COVID-19 patients, red (n=20). Statistical significances were determined by unpaired 2-sided student's t-test. Bar graphs expressed as mean  $\pm$  standard error of the mean (SEM). P-values less than 0.05 were considered statistically significant; \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001.



**Supplementary Figure 2.** ROC analysis showing performance traditional inflammatory markers to identify hospitalised patients who subsequently require ICU versus who did not: A) CRP, (B) D-Dimer, (C) Neutrophil to Lymphocyte ration (N/L) and (D) Troponin.



**Supplementary Figure 3.** ROC analysis testing the performance of cfDNA to distinguished hospitalized patients from patients with mild COVID-19 not requiring hospitalization: (A)ncfDNA, (B) mtcfDNA , (C) Monocyte-, (D) Neutrophil-, (E) Erythroblast-, (F) Lymphocytes, (G) Vascular endothelium- (H) adipocyte-, (I) Hepatocyte-, (J) Pancreas, (K) Bladder-, (L) intestine-, (M) Kidney-, (N) Heart-, (O) Lung- and (P) Head and neck larynx-derived cfDNA.





**Supplementary Figure 4.** Mitochondrial reactive oxygen species (mtROS) in mouse primary proximal tubular cells (mPPTCs) treated with COVID-19 patient B plasma of day 1.

(A) Representative images of mitochondrial superoxide, as detected by MitoSOX-Red staining and visualized under confocal microscopy. mPPTCs were untreated (control) or treated with 3% healthy volunteer plasma, 1% or 3% COVID-19 patient plasma after 6 hours of incubation. Red: MitoSOX-Red representing mitochondrial superoxide, blue: Hoechst3342 representing nuclei. Original magnification: x400. (B) MitoSOX-Red intensity in mPPTCs (n=23-44 cells from 3-5 fields/group) of each group after 6 hours of incubation. The bar in plots are expressed as mean  $\pm$  standard error of the mean (SEM). Statistical significance was determined using Tukey's multiple comparison after two-way ANOVA. P-values less than 0.05 were considered statistically significant; \*p<0.05: vs control, #p<0.05: vs 3% healthy plasma, †p<0.05: vs 1% COVID-19 plasma.

Supplementary Table 1. Demographic and clinical data's of Flu and RSV patients.

	Influenza (n=16)	RSV (n=9)	RSV and Influenza combined (n=21)
Sex			
Male (%)	9 (43.8)	2 (22.2)	11 (52.4)
Female (%)	7 (56.3)	7 (77.8)	10 (47.6)
Median Age (years)(IQR)	55 (25.75-62)	53 (31-61.5)	56 (31-62)
Race			
White	14 (87.5)	7 (77.8)	18 (85.7)
Black	2 (12.5)	2 (22.2)	3 (14.3)
Mean BMI (SE)	26.9 (1.4)	27.5 (0.8)	27.0 (0.95)
Obesity (%)	1 (7.7)	0 (0)	1 (5.9)
Hypertension (%)	6 (42.9)	3 (37.5)	8 (44.4)
Diabetes (%)	7 (50)	4 (50)	9 (50)
Coronary Artery Disease	2 (16.7)	1 (12.5)	3 (18.8)
Heart Failure	1 (9.1)	1 (12.5)	2 (13.3)

Supplementary Table 2. Multivariable Analysis of Nuclear cell-free DNA and Outcome (deceased vs recovered)

Variable	Odds ratio	95% Confidence Interval	p-value
ncfDNA	13.3	2.2-82.1	0.0053
Sex (Male vs Female)	1.1	0.99-1.1	0.0731
Age	1.2	0.2-6.4	0.8086
Obesity (Yes vs No)	1.6	0.3-8.4	0.6068
Cancer (Yes vs No)	0.8	0.1-12.2	0.8692

ncfDNA: nuclear cell-free DNA

Supplementary Table 3. Adjusted t-test for multiple comparison using Hommel procedure.

Figure Panel	Outcome (Hospitalized Recovered vs. Deceased)		Trajectories					
	t-test, Unadjusted	t-test, Adjusted	t-test, Unadjusted			t-test, Adjusted		
			HC vs. Mild	Mild vs. Moderate	Moderate vs. Severe	HC vs. Mild	Mild vs. Moderate	Moderate vs. Severe
ncfDNA	1.08E-05	0	0.492024215	3.92E-08	3.08E-09	0.492	0	0
mtcfDNA	0.529143322	0.5291	2.17E-06	2.91E-12	0.589810172	0	0	0.5898
Monocytes	0.034047696	0.034	0.001117393	1.46E-08	0.000242643	0.0011	0	5.00E-04
Neutrophils	0.001859509	0.0019	0.002194376	3.89E-06	1.08E-10	0.0022	0	0
Erythroblast	0.018271506	0.0183	0.001281169	0.000216636	9.89E-06	0.0013	4.00E-04	0
Lymphocyte	0.790886224	0.7909	0.501921301	4.55E-06	0.830440885	0.8304	0	0.8304
Lung	0.008952128	0.009	0.016324583	1.76E-08	2.49E-07	0.0163	0	0
Heart	0.026058338	0.0261	0.052275097	0.023461693	0.005773749	0.0523	0.0469	0.0173
Hepatocytes	0.046924027	0.0469	0.025306252	4.04E-05	0.012074128	0.0253	1.00E-04	0.0241
Kidney	0.121531541	0.1215	0.470366462	1.50E-06	0.006715796	0.4704	0	0.0134
Adipocytes	0.008586377	0.0086	0.081170915	1.11E-05	0.000295646	0.0812	0	6.00E-04
Pancreas	0.014490903	0.0145	0.06668904	0.031891273	0.000765462	0.0633	0.0319	0.0023
Vascular endothelium	0.001881479	0.0019	0.051222829	5.73E-07	0.001610231	0.0512	0	0.0032
Bladder	0.015742999	0.0157	0.987042441	9.96E-09	0.002071943	0.987	0	0.0041
GIT	0.056808039	0.0568	0.054544692	0.001345637	6.97E-09	0.0545	0.0027	0
Head & Neck, larynx	0.201998734	0.202	0.763553593	9.15E-06	0.005058013	0.7636	0	0.0101