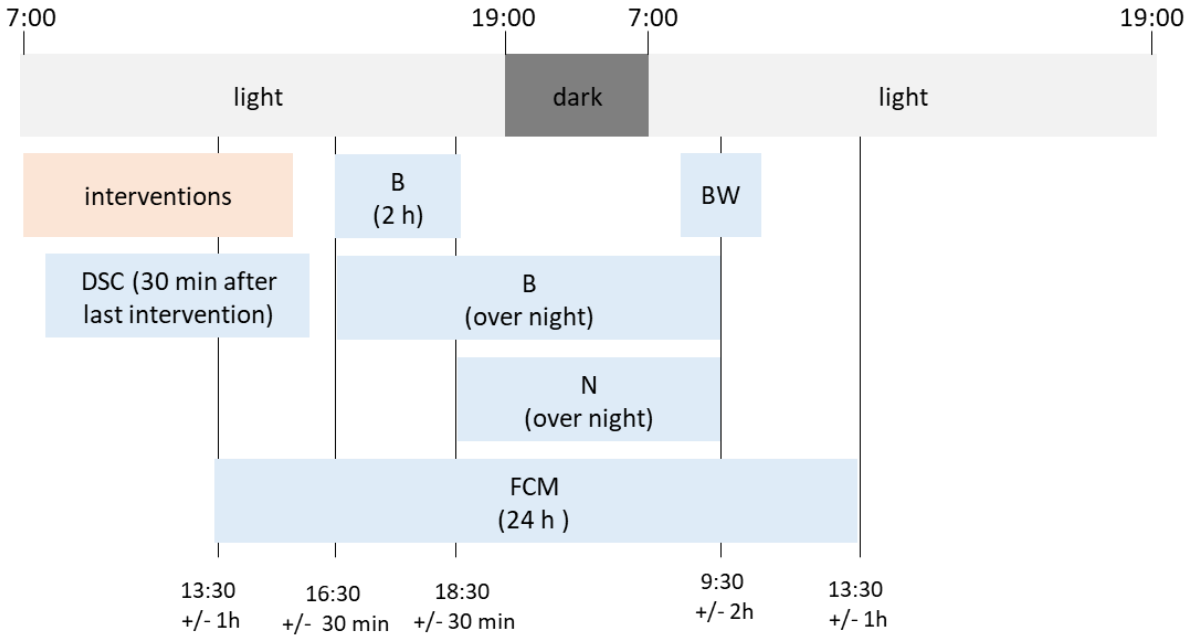
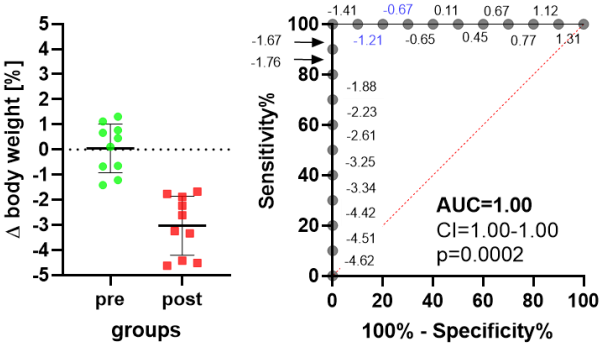


Figure S1. Timeframe of measuring animal distress. The distress score (DCS) was assessed 30 minutes after the last intervention (e.g. after the third cerulein injection, CCl4 injection, application of chemotherapy or after anesthesia plus surgical intervention). The burrowing assay (B) was started at 16:30 and evaluated after 2 hours and on the following morning. Nesting assay was started at 18:30 and also evaluated on the following morning. The mice were put in a new cage at 13:30 so that 24 hours later the fresh feces could be collected in order to assess fecal corticosterone metabolites (FCM). The body weight was measured on the following morning, because any reduction of the body weight in response to distress needs sufficient time.

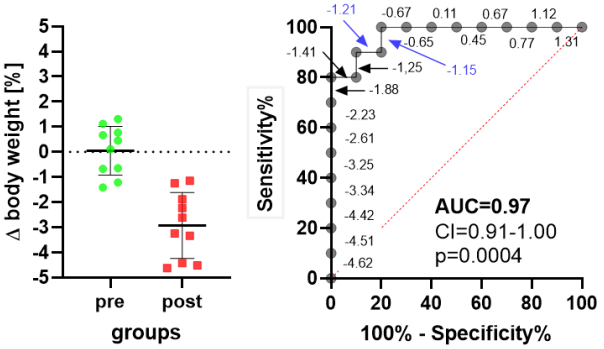
A

Pre (healthy)	Post (diseased)
1.31	-1.67
1.12	-1.76
0.77	-1.88
0.67	-2.23
0.45	-2.61
0.11	-3.25
-0.65	-3.34
-0.67	-4.42
-1.21	-4.51
-1.41	-4.62



B

Pre (healthy)	Post (diseased)
1.31	-1.15
1.12	-1.25
0.77	-1.88
0.67	-2.23
0.45	-2.61
0.11	-3.25
-0.65	-3.34
-0.67	-4.42
-1.21	-4.51
-1.41	-4.62



C

Pre (healthy)	Post (diseased)
1,31	1,31
1,12	1,12
0,77	0,77
0,67	0,67
0,45	0,45
0,11	0,11
-0,65	-0,65
-0,67	-0,67
-1,21	-1,21
-1,41	-1,41

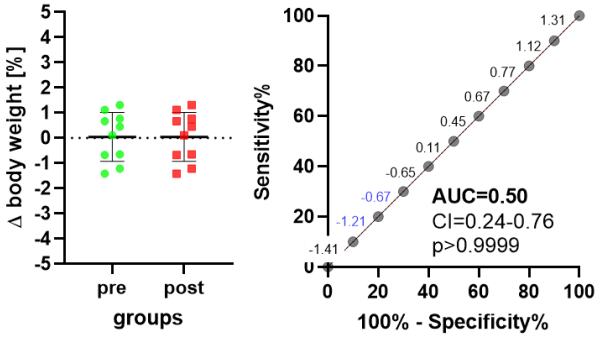


Figure S2. Example of ROC curve analysis. Fictional data before induction of a disease (pre = healthy) and after induction of a disease (post = diseased) are presented as data tables, dot blots and ROC curves in order to demonstrate how data are used to graph a ROC curve. In case all diseased animals loose more weight than healthy animals, measuring the body weight is the perfect method to differentiate between healthy and diseased animals (A). The sensitivity and specificity of various cut offs are presented in a ROC curve. For example, a cut-off between -1.21% and -0.67% body weight change allows to diagnose diseased animals with a sensitivity of 100% (because all diseased animals are diagnosed to be diseased) and a specificity of 80% (because only 8 out of 10 healthy animals are diagnosed to be healthy). Plotting sensitivity and 1-specificity for all other cut-offs results in a ROC curve with an area under the curve (AUC) of 1.00, a 95% confidence interval (CI) of 1.00-1.00 and a p-value of 0.0002. In case most diseased animals lose more weight than healthy animals, measuring the body weight is a good method to differentiate between healthy and diseased animals (B). For example, a cut-off between -1.21% and -1.15% body weight change allows to diagnose diseased animals with a sensitivity of 90% (because 9 out of 10 diseased animals are diagnosed to be diseased) and a specificity of 80% (because only 8 out of 10 healthy animals are diagnosed to be healthy). Plotting sensitivity and 1-specificity for all other cut-offs results in a ROC curve with an AUC of 0.97, a CI of 0.91-1.00 and a p-value of 0.0004. In case diseased animals have similar body weight change than healthy animals, this method cannot differentiate well between healthy and diseased animals (C). For example, a cut-off of between -1.21% and -0.67% body weight change allows to diagnose diseased animals with a sensitivity of 20% (because 2 out of 10 diseased animals are diagnosed to be diseased) and a specificity of 80% (because 8 out of 10 healthy animals are diagnosed to be healthy). A ROC curve based on all cut-offs has an AUC of 0.50, a CI of 0.24-0.76 and a p-value of > 0.9999.

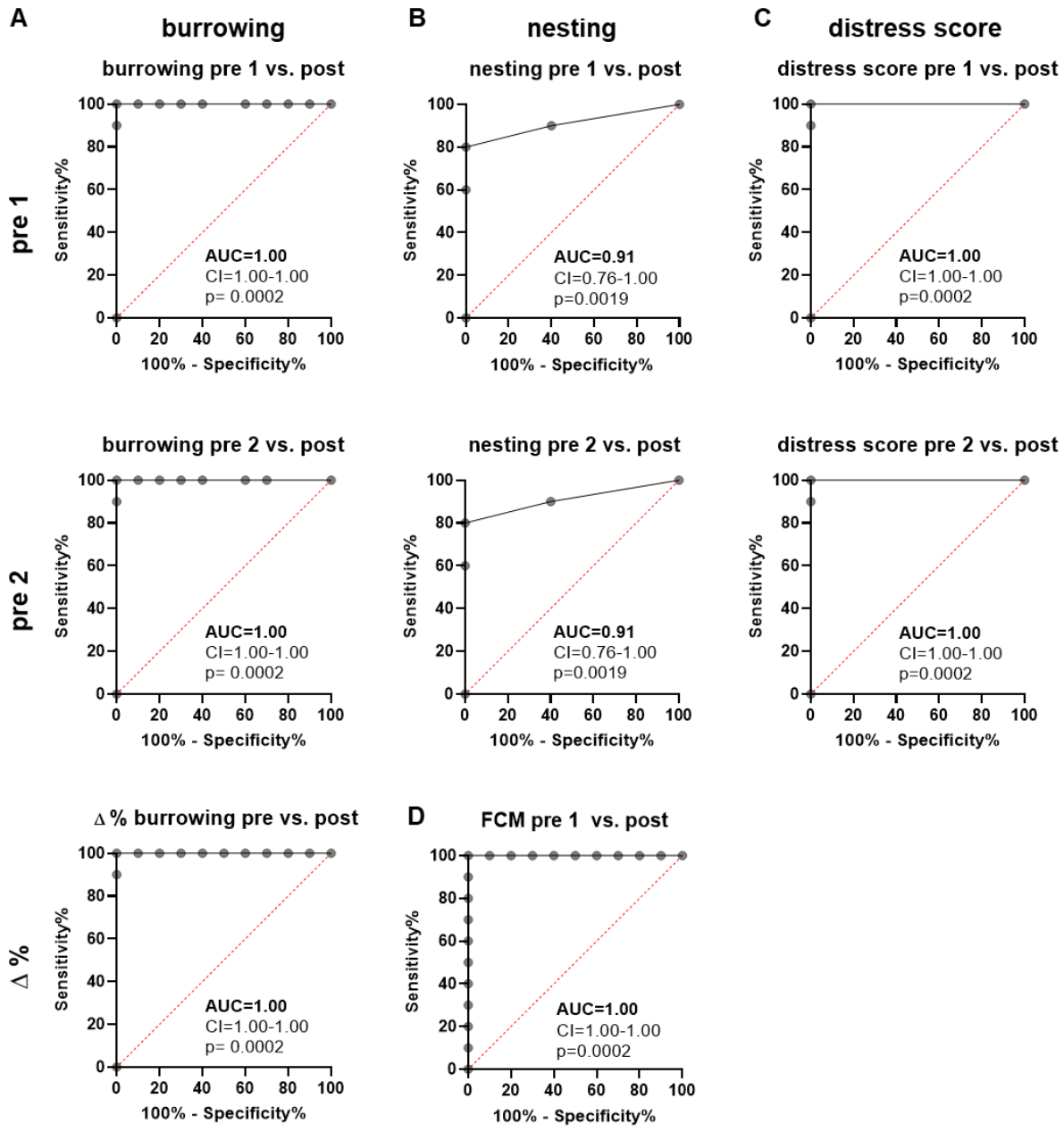


Figure S3. Presentation of classifier performance of various methods when differentiating between animals before and after implanting a telemetric transmitter. ROC curves describe changes in burrowing activity (A), nesting behavior (B) a distress score (C) or fecal corticosterone metabolites concentration (D). Burrowing activity, nesting behavior and distress score after transmitter implantation are compared to a timepoint, pre 1, or to another time point, pre 2, before implantation. In addition, the percentage in burrowing activity between the two days before transmitter implantation, is compared to the percentage in body weight change between the postoperative day and pre 1. The classifier performance of each method in differentiating between animals before and after implanting a transmitter was characterized by the area under the curve (AUC) the confidence interval (CI) and the p value indicating how significant the difference was to the reference line (red dotted line). n = 10 mice.

Table S1. Supplemental information to ROC curve analysis of figure 2. Analysis of various methods such as body weight (BW), burrowing activity (B), nesting behavior (N), a distress score (DSC) or fecal corticosterone metabolites concentration (FCM), when differentiating between animals before and after three surgical interventions. The table presents the number of animals (n) analyzed, the area under the curve (AUC), the confidence interval (CI), and the p value describing the significant difference to a reference line, which indicates no discriminative power and thus has an AUC of 0.5.

	BW	B	N	DSC	FCM
transm. impl. pre 1	n = 10 AUC = 0.8950 CI = 0.7462-1.000 p = 0.0028	n = 10 AUC = 1.000 CI = 1.000-1.000 p = 0.0002	n = 10 AUC = 0.9100 CI =0.7641-1.000 p = 0.0019	n = 10 AUC = 1.000 CI = 1.000-1.000 p = 0.0002	n = 10 AUC = 1.000 CI = 1.000-1.000 p = 0.0002
transm. impl. pre 2	n = 10 AUC = 8.8900 CI =0.7337-1.000 p = 0.0032	n = 10 AUC = 1.000 CI = 1.000-1.000 p = 0.0002	n = 10 AUC = 0.9100 CI = 0.7641-1.000 p = 0.0019	n = 10 AUC =1.000 CI = 1.000-1.000 p = 0.0002	not done
transm. impl. Δ %	n = 10 AUC = 1.000 CI = 1.000-1.000 p = 0.0002	n = 10 AUC = 1.000 CI = 1.000-1.000 p = 0.0002	not done	not done	not done
cell. inject. pre 1	n = 14 AUC = 0.5816 CI = 0.3650-0.7983 p = 0.4622	n = 14 AUC = 0.9184 CI = 0.8214-1.000 p = 0.0002	n = 14 AUC = 0.5179 CI = 0.2943-0.7414 p = 0.8722	n = 14 AUC = 0.500 CI = 0.2824-0.7176 p > 0.9999	n = 14 AUC = 0.8571 CI = 0.7092-1.000 p =0.0013
cell. inject. pre 2	n = 14 AUC = 0.5714 CI = 0.3559-0.7870 p =0.5201	n = 14 AUC = 0.8776 CI = 0.7405-1.000 p = 0.0007	n = 14 AUC = 0.6071 CI = 0.3928-0.8215 p = 0.3346	n = 14 AUC = 0.500 CI =0.2824-0.7176 p > 0.9999	not done
cell. inject. Δ %	n = 14 AUC = 0.5689 CI = 0.3510-0.7868 p = 0.5351	n = 14 AUC = 0.9184 CI = 0.8047-1.000 p = 0.0002	not done	not done	not done
BDL pre 1	n = 16 AUC = 0.5449 CI = 0.3417-0.7282 p = 0.6647	n = 16 AUC = 0.9434 CI = 0.8560-1.000 p < 0.0001	n = 14 AUC = 0.9898 CI = 0.645-1.000 p < 0.0001	n = 16 AUC = 0.9375 CI = 0.8392-1.000 p < 0.0001	not done
BDL pre 2	n = 16 AUC = 0.5957 CI = 0.3963-0.7951 p = 0.3558	n = 16 AUC = 0.8906 CI = 0.7755-1.000 p = 0.0002	n = 14 AUC = 0.9949 CI = 0.9784-1.000 p < 0.0001	n = 16 AUC = 0.9375 CI = 0.8392-1.000 p < 0.0001	not done
BDL Δ %	n = 16 AUC = 0.9141 CI = 0.8196-1.000 p < 0.0001	n = 16 AUC = 0.8867 CI = 0.7728-1.000 p = 0.0002	not done	not done	not done

Table S2. Supplemental information to ROC curve analysis of figure 3. Analysis of various methods such as percentage of body weight change (%BW), burrowing activity (B), nesting behavior (N), a distress score (DSC) or fecal corticosterone metabolites concentration (FCM), when differentiating between animals before and after two surgical interventions. The table presents the number of animals (n) analyzed, the area under the curve (AUC), the confidence interval (CI), and the p value describing the significant difference to a reference line, which indicates no discriminative power and thus has an AUC of 0.5.

	%BW	B	N	DSC	FCM
cell inject. (A)	n = 7 AUC = 0.6122 CI = 0.2928-0.9317 p = 0.4822	n = 7 AUC = 0.8571 CI = 0.6577-1.000 p = 0.0253	n = 7 AUC = 0.5612 CI =0,2448-0.8776 p = 0.7015	n = 7 AUC = 0.500 CI = 0.1870-0.8130 p > 0.9999	n = 7 AUC = 0.8367 CI = 0.6099-1.000 p = 0.0350
cell inject. (B)	n = 7 AUC = 0.5408 CI = 0.2050-0.8766 p = 0.7983	n = 7 AUC = 0.9592 CI = 0.8605-1.000 p = 0.0040	n = 7 AUC = 0.5918 CI = 0.2757-0.9079 p = 0.5653	n = 7 AUC = 0.5000 CI = 0.1870-0.8130 p > 0.9999	n = 7 AUC = 0.8367 CI = 0.5981-1.000 p = 0.0350
BDL (vehicle)	n = 9 AUC = 0.8642 CI = 0,6938-1.000 p = 0.0092	n = 9 AUC = 0.08557 CI = 0.7150-1.000 p = 0.0062	n = 7 AUC = 0.9898 CI = 0.9497-1.000 p = 0.0022	n = 9 AUC = 1.000 CI = 1.000-1.000 p = 0.0003	not done
BDL (MCC950)	n = 7 AUC = 0.9388 CI = 0.8067-1.000 p = 0.0060	n = 7 AUC = 1.000 CI = 1.000-1.000 p = 0.0017	n = 7 AUC = 0.9898 CI = 0.9497-1.000 p = 0.0022	n = 7 AUC = 0.8571 CI = 0.6381-1.000 p = 0.0253	not done

Table S3. Supplemental information to ROC curve analysis of figure 4. Analysis of various methods such as percentage of body weight change (%BW), burrowing activity (B), nesting behavior (N), a distress score (DSC) or fecal corticosterone metabolites concentration (FCM), when differentiating between animals before and after induction of a disease. The table presents the number of animals (n) analyzed, the area under the curve (AUC), the confidence interval (CI), and the p value describing the significant difference to a reference line, which indicates no discriminative power and thus has an AUC of 0.5.

	%BW	B	N	DSC	FCM
cholestasis (vehicle)	n = 9 AUC = 0.9218 CI = 0.8343-1.000 p = 0.0002	n = 9 AUC = 0.7716 CI = 0.5976-0.9456 p = 0.0159	n = 7 AUC = 0.8231 CI = 0.6586-0.9877 p = 0.0117	n = 9 AUC = 1.000 CI = 1.000-1.000 p < 0.0001	not done
cholestasis (MCC950)	n = 7 AUC = 0.9320 CI = 0.8327-1.000 p = 0.0008	n = 7 AUC = 0.9728 CI = 0.9129-1.000 p = 0.0002	n = 7 AUC = 0.8912 CI = 0.7240-1.000 p = 0.0023	n = 7 AUC = 1.000 CI = 1.000-1.000 p < 0.0001	not done
CCl4 intox. (vehicle)	increased	n = 3 AUC = 0.6111 CI = 0.2619-0.9603 p = 0.5791	n = 6 AUC = 0.8796 CI = 0.7437-1.000 p = 0.0063	n = 3 AUC = 0.5556 CI = 0.1838-0.9273 p = 0.7815	not done
CCl4 intox. (MCC950)	increased	n = 7 AUC = 0.8639 CI = 0.7200-1.000 p = 0.1762	n = 6 AUC = 0.9306 CI = 0.8216-1.000 p = 0.0019	n = 7 AUC = 0.5000 CI = 0.2487-0.7513 p > 0.9999	not done
cancer (vehicle)	n = 7 AUC = 0.7109 CI = 0.5109-0.9108 p = 0.1000	n = 7 AUC = 0.5272 CI = 0.2708-0.7836 p =0.8319	n = 7 AUC = 0.5306 CI = 0.2416-0.8197 p = 0.8113	n = 7 AUC = 0.5000 CI = 0.2487-0.7513 p > 0.9999	n = 7 AUC = 0.5034 CI = 0.2531-0.7537 p = 0.9788
cancer (CHC+Met)	n = 7 AUC = 0.7245 CI = 0.5299-0.9191 p = 0.0800	n = 7 AUC = 0.6190 CI = 0.4036-0.8345 p = 0.3532	n = 7 AUC = 0.6633 CI = 0.4417-0.8849 p = 0.2029	n = 7 AUC = 0.5714 CI = 0.3382-0.8047 p = 0.5775	n = 7 AUC = 0.5069 CI = 0.2786-0.7350 p = 0.9577
pancreatitis (vehicle)	n = 8 AUC = 0.9740 CI = 0.9272-1.000 p < 0.0001	n = 8 AUC = 0.8854 CI = 0.7678-1.000 p = 0.0013	n = 8 AUC = 0.7786 CI = 0.5826-0.9747 p = 0.0199	n = 8 AUC = 0.6042 CI = 0.3948-0.8135 p = 0.3841	n = 8 AUC = 0.7370 CI = 0.5676-0.9063 p = 0.0477
pancreatitis (miR-21 inhib.)	n = 8 AUC = 0.8568 CI = 0.7229-0.9906 p = 0.0029	n = 8 AUC = 0.8177 CI = 0.6503-0.9851 p = 0.0079	n = 8 AUC = 0.8880 CI = 0.7761-1.000 p = 0.0012	n = 8 AUC = 0.5208 CI = 0.2911-0.7506 p = 0.8618	n = 8 AUC = 0.6536 CI = 0.4491-0.8582 p = 0.1992

Table S4. Supplemental information to ROC curve analysis of figure 5. Analysis of various methods such as percentage of body weight change (%BW), burrowing activity (B), nesting behavior (N), a distress score (DSC) or fecal corticosterone metabolites concentration (FCM), when differentiating between animals before and at the early, middle or late stage of a disease. The table presents the number of animals (n) analyzed, the area under the curve (AUC), the confidence interval (CI), and the p value describing the significant difference to a reference line, which indicates no discriminative power and thus has an AUC of 0.5.

	BW	B	N	DSC	FCM
cholestasis early	n = 16 AUC = 0.8887 CI = 0.7626-1.000 p = 0.0002	n = 16 AUC = 0.7598 CI = 0.5913-0.9283 p = 0.0122	n = 14 AUC = 0.9617 CI = 0.8931-1.000 p < 0.0001	n = 16 AUC = 1.000 CI = 1.000-1.000 p < 0.0001	not done
cholestasis middle	n = 16 AUC = 0.9199 CI = 0.8215-1.000 p < 0.0001	n = 16 AUC = 0.7891 CI = 0.6147-0.9634 p =0.0053	n = 14 AUC = 0.8571 CI = 0.7048-1.000 p = 0.0013	n = 16 AUC = 1.000 CI = 1.000-1.000 p < 0.0001	not done
cholestasis late	n = 16 AUC = 0.9766 CI = 0.9322-1.000 p < 0.0001	n = 16 AUC = 0.9570 CI = 0.8915-1.000 p < 0.0001	n = 14 AUC = 0.8980 CI = 0.7774-1.000 p = 0.0003	n = 16 AUC = 1.000 CI = 1.000-1.000 p < 0.0001	not done
CCl4 intox. early	increased	n = 10 AUC = 0.5850 CI = 0.3277-0.8423 p = 0,5205	n = 12 AUC = 0.7257 CI = 0.5175-0.9339 p = 0.0606	n = 10 AUC = 0.5000 CI = 0.2407-0.7593 p > 0.9999	not done
CCl4 intox. middle	increased	n = 10 AUC = 0.6850 CI = 0.4422-0.9278 p = 0.1620	n = 12 AUC = 1.000 CI = 1.000-1.000 p < 0.0001	n = 10 AUC = 0.5500 CI = 0.2920-0.8080 p = 0.7055	not done
CCl4 intox. late	increased	n = 10 AUC = 0.6950 CI = 0.4565-0.9335 p = 0.1405	n = 12 AUC = 1.000 CI = 1.000-1.000 p < 0.0001	n = 10 AUC = 0.5000 CI = 0.2407-0.7593 p > 0.9999	not done
cancer early	n = 14 AUC = 0.5944 CI = 0.3768-0.8119 p = 0.3953	n = 14 AUC = 0.7883 CI = 0.6161-0.9604 p = 0.0094	n = 14 AUC = 0.6020 CI = 0.3885-0.8156 p = 0.3581	n = 14 AUC = 0.5357 CI = 0.3186-0.7528 p = 0.7477	n = 14 AUC = 0.5026 CI = 0.2761-0.7290 p = 0.9817
cancer middle	n = 14 AUC = 0.7398 CI = 0.5522-0.9274 p =0.0308	n = 14 AUC = 0.5051 CI = 0.2861-0.7241 p = 0.9634	n = 14 AUC = 0.5383 CI = 0.3203-0.7562 p = 0.7304	n = 14 AUC = 0.5357 CI = 0.3186-0.7528 p = 0.7477	n = 14 AUC = 0.5561 CI = 0.3308-0.7815 p = 0.6133
cancer late	n = 14 AUC = 0.7577 CI = 0.5700-0.9453 p = 0.0203	n = 14 AUC = 0.6582 CI = 0.4483-0.8680 p = 0.1543	n = 14 AUC = 0.5918 CI = 0.3788-0.8049 p =0.4082	n = 14 AUC = 0.5357 CI = 0.3186-0.7528 p = 0.7477	n = 14 AUC = 0.5255 CI = 0.3064-0.7446 p = 0.8183
pancreatitis early	n = 16 AUC = 0.8477 CI = 0.7099-0.9854 p = 0.0008	n = 16 AUC = 0.9102 CI = 0.8140-1.000 p = 0.0001	n = 16 AUC = 0.7109 CI = 0.5309-0.8909 p = 0.0418	n = 16 AUC = 0.5000 CI = 0.2969-0.7031 p > 0.9999	n = 16 AUC = 0.8359 CI = 0.6955-0.9764 p = 0.0012
pancreatitis middle	n = 16 AUC = 0.9395 CI = 0.8557-1.000 p < 0.0001	n = 16 AUC = 0.8008 CI = 0.6417-0.9598 p = 0.0037	n = 16 AUC = 0.9023 CI = 0.7901-1.000 p < 0.0001	n = 16 AUC = 0.5938 CI = 0.3942-0.7933 p = 0.3657	n = 16 AUC = 0.5547 CI = 0.3493-0.7600 p = 0.5977
pancreatitis late	n = 16 AUC = 0.9395 CI = 0.8589-1.000 p < 0.0001	n = 16 AUC = 0.8281 CI = 0.6775-0.9788 p = 0.0015	n = 16 AUC = 0.8789 CI = 0.7542-1.000 p = 0.0003	n = 16 AUC = 0.5938 CI = 0.3942-0.7933 p = 0.3657	n = 16 AUC = 0.7188 CI = 0.5296-0.9079 p = 0.0348