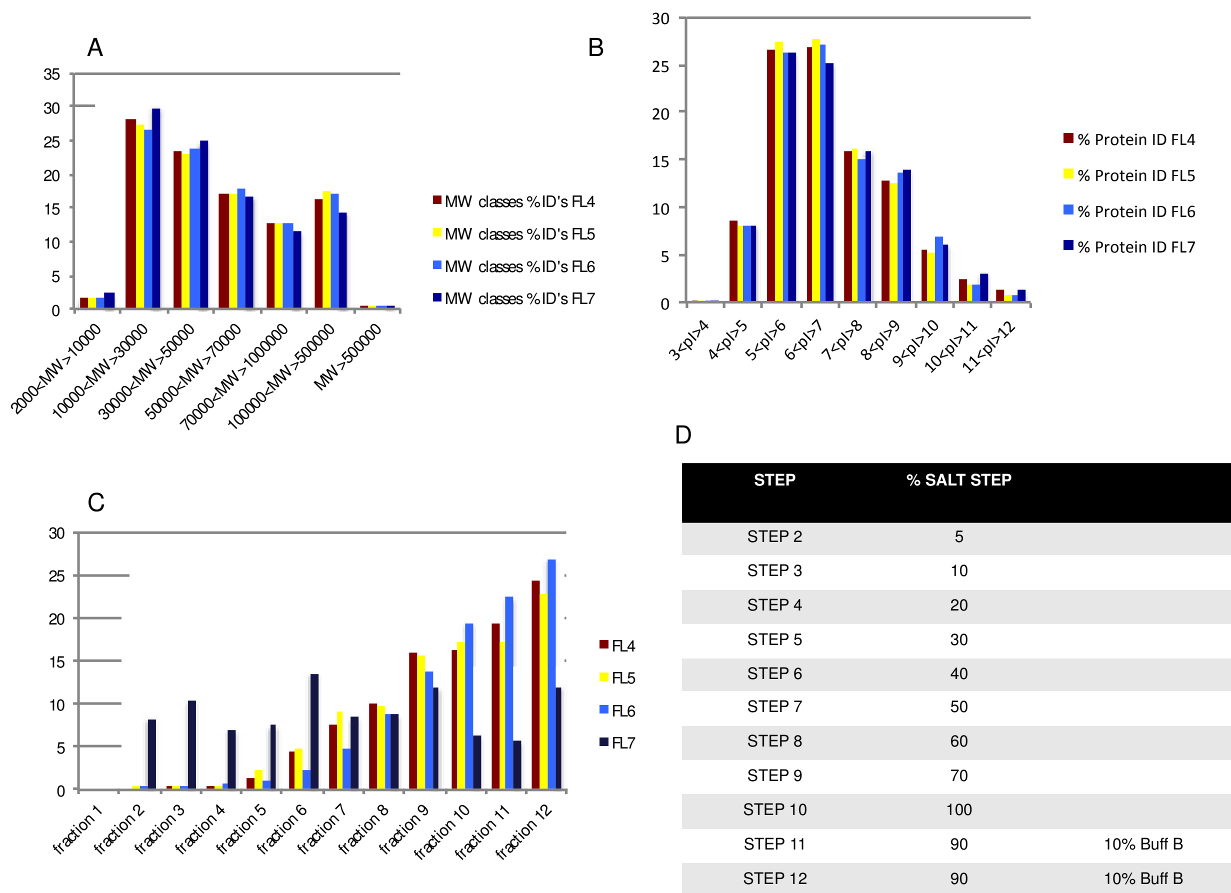
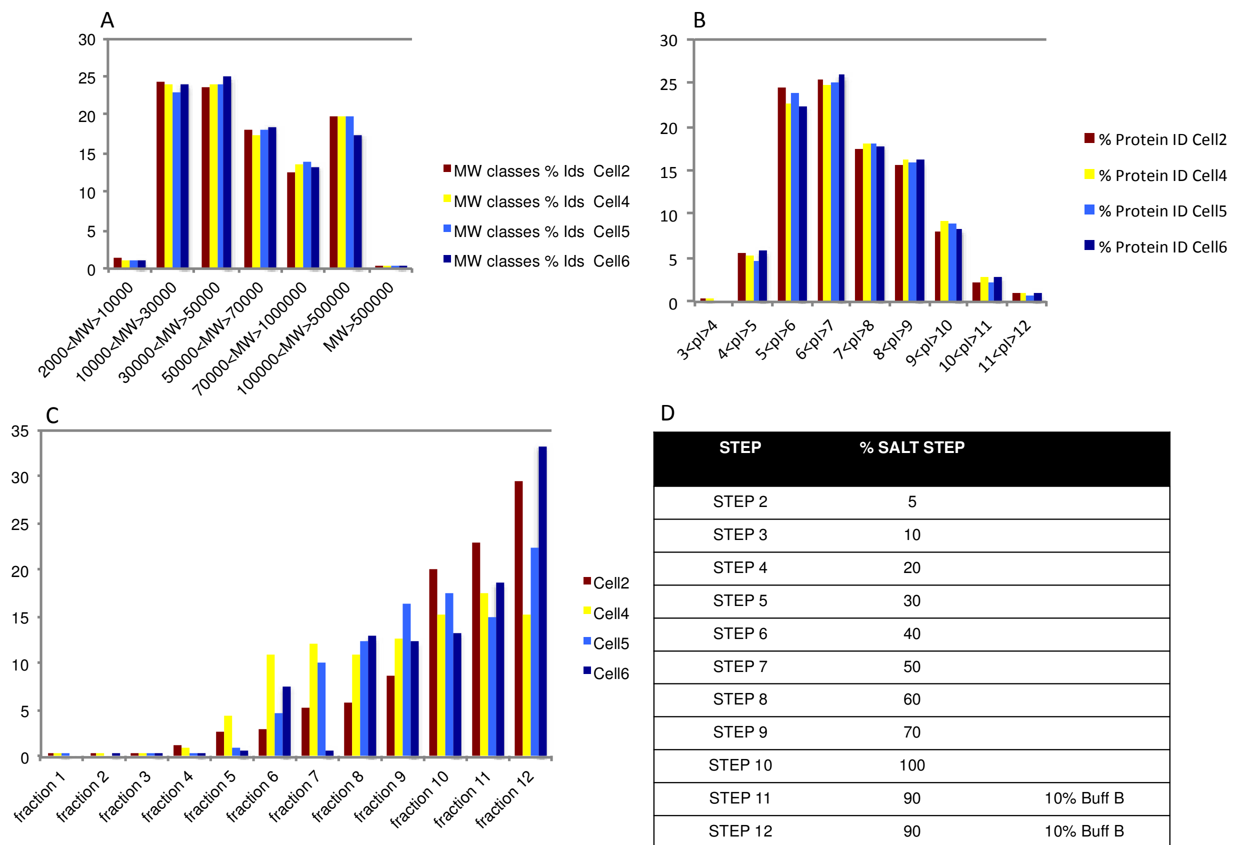
Supporting Information



STEP	% SALT STEP	
STEP 2	5	
STEP 3	10	
STEP 4	20	
STEP 5	30	
STEP 6	40	
STEP 7	50	
STEP 8	60	
STEP 9	70	
STEP 10	100	
STEP 11	90	10% Buff B
STEP 12	90	10% Buff B

Figure 1s. Interstitial fluids samples. Performance evaluation in terms of, respectively, MW IDs % (A), pI IDs, % (B), Peptides *per* Fraction (C), chromatographic salt steps in MudPIT (D). FL4 to FL7 are the markers of the samples for the replicate analyses. After the TmT labeling step, samples were combined (called FL1), divided in 6 aliquotes (called FL2-7) and subsequently stored at -80℃. For our study we have analyzed 4 aliquotes, the remaining two (Fl2 and FL3) were kept as a backup in case of necessity.



STEP	% SALT STEP	
STEP 2	5	
STEP 3	10	
STEP 4	20	
STEP 5	30	
STEP 6	40	
STEP 7	50	
STEP 8	60	
STEP 9	70	
STEP 10	100	
STEP 11	90	10% Buff B
STEP 12	90	10% Buff B

Figure 2s. Primary cancer cells samples. Performance evaluation in terms of, respectively, Mw IDs % (A), pI IDs, % (B), % of Peptides *per* MudPIT Fraction (C), chromatographic salt steps in MudPIT (D). Cell2-6 are the markers of the samples for the replicate analysis. After the TmT labeling step, samples combined (called Cell1), divided in 6 aliquotes (called Cell 2-7) and subsequently stored at -80°C. For our study we have analyzed 4 aliquotes, the remaining two (Cell3 and Cell7) were kept as a backup in case of necessity.

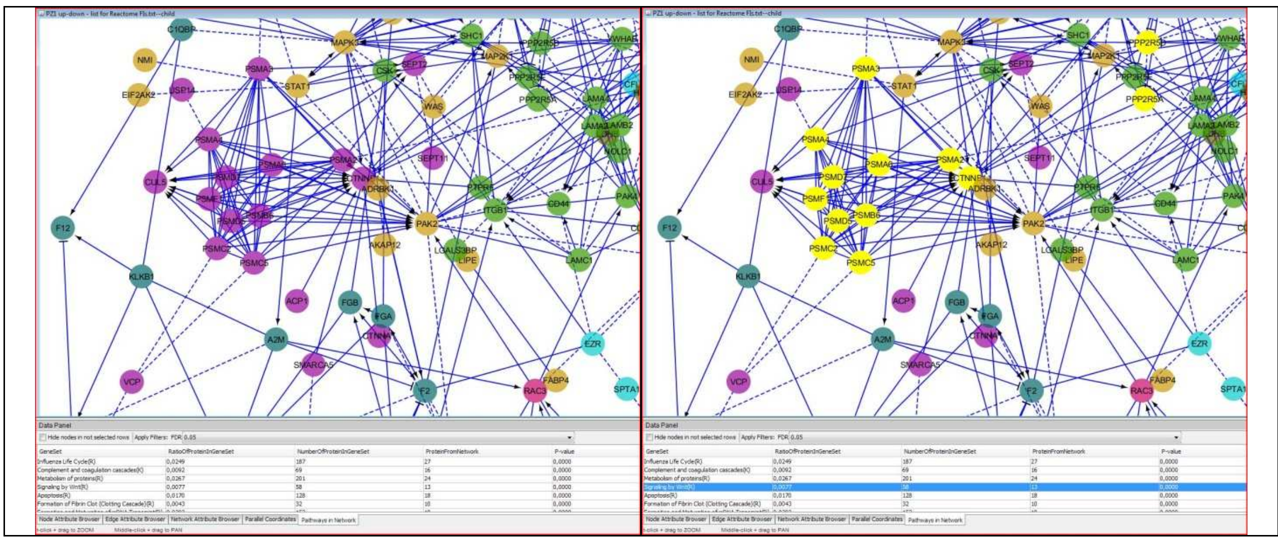


Figure 3s. Data panel extraction for Pt1, representing information deriving from both plugins, Reactome FIs and CentiScaPe. The data were obtained on the basis of topological properties, clustering the different modules on the network, applying the pathway enrichment tool to the modules, and selecting the specific pathway on the data panel, highlighting the protein included in the module.

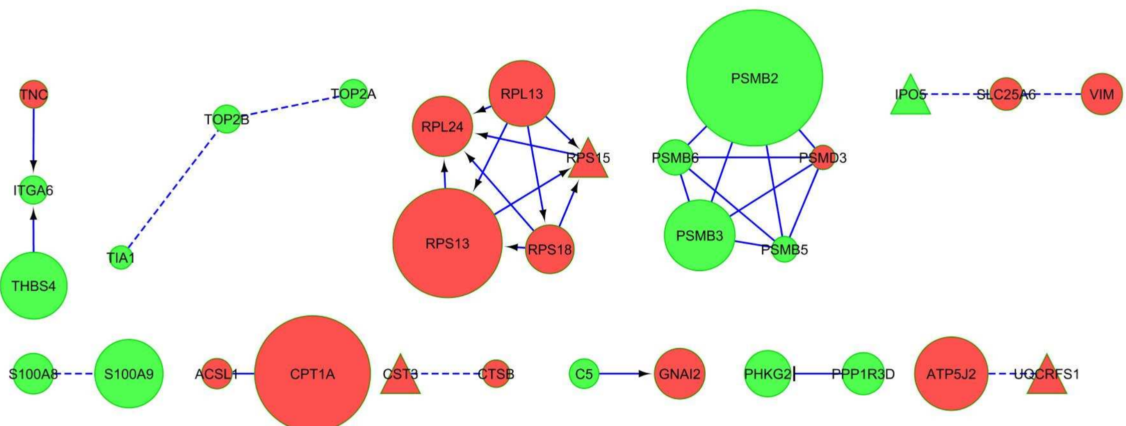


Figure. 4s. Cytoscape diagram of the functional interaction network representing proteins modulation in Pt1 primary cells. Colors and markers code is the same used in Figure 3.

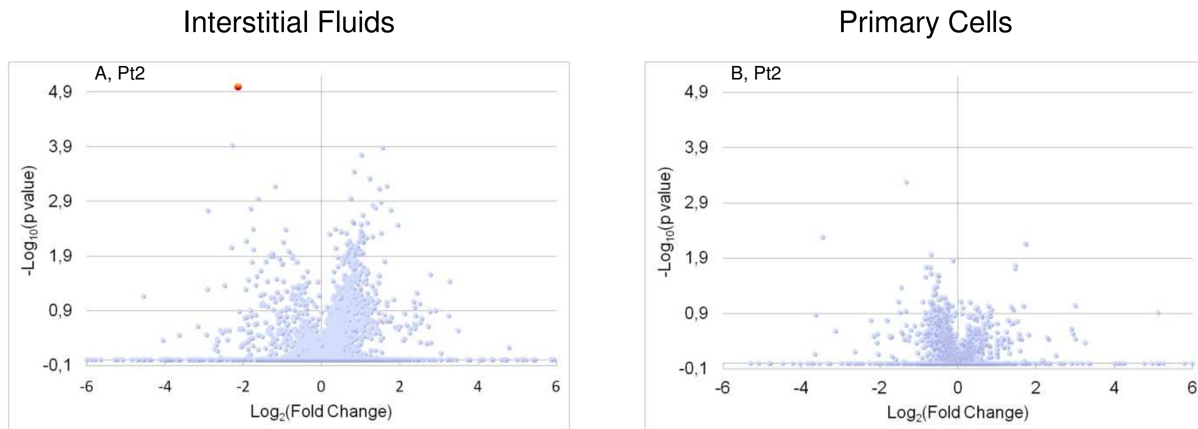


Figure 5s. Volcano plot for Pt2, respectively interstitial fluids (A) and primary cancer cells (B), represented as $-log_{10}$ (p-value) on y-axes, and log_2 (fold change) on the x-axes. Colors code is the same used in Figure 2.

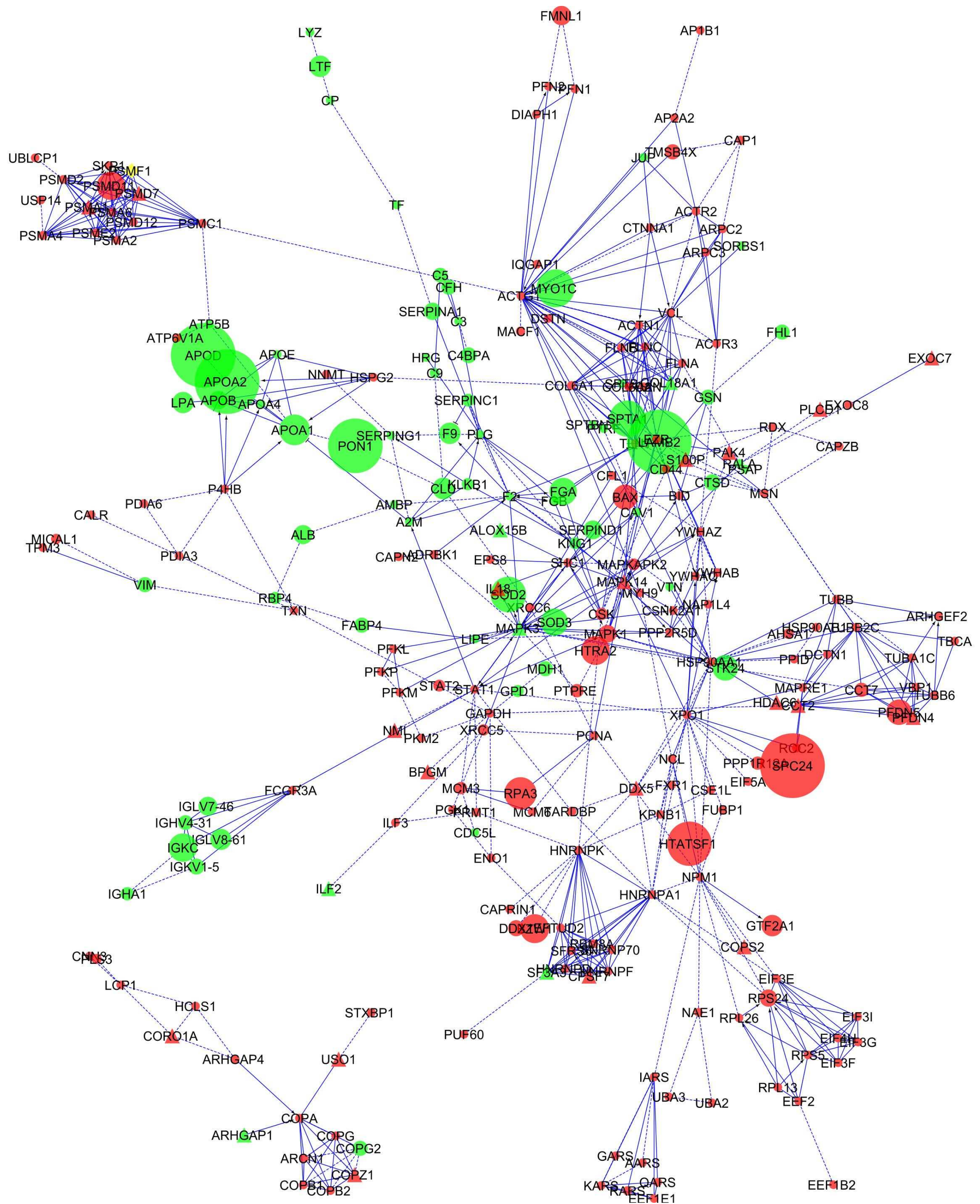


Figure 6s. Cytoscape diagram of the functional interaction network representing proteins modulation in Pt3 interstitial fluids. Colors and markers code is the same used in Figure 3.

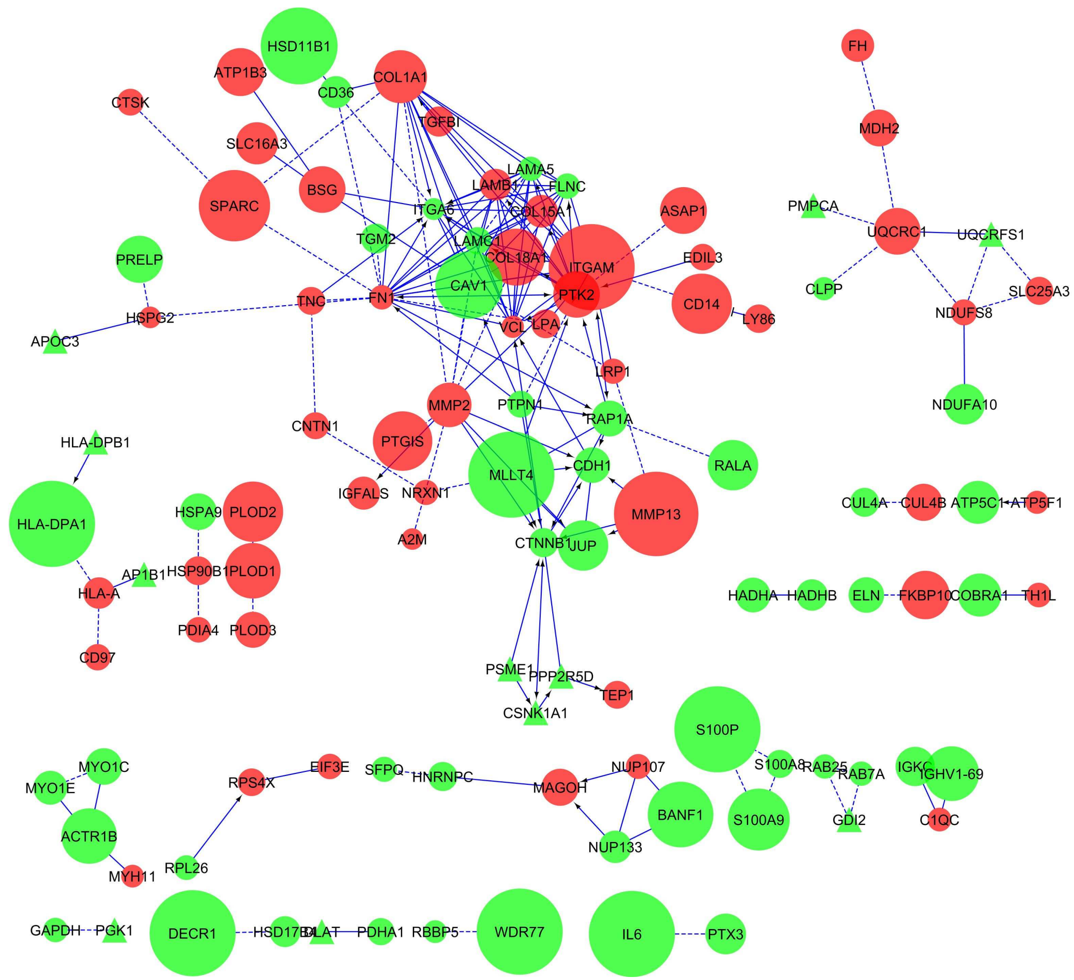


Figure 7s. Cytoscape diagram of the functional interaction network representing proteins modulation in Pt3 primary cells. Colors and markers code is the same used in Figure 3.

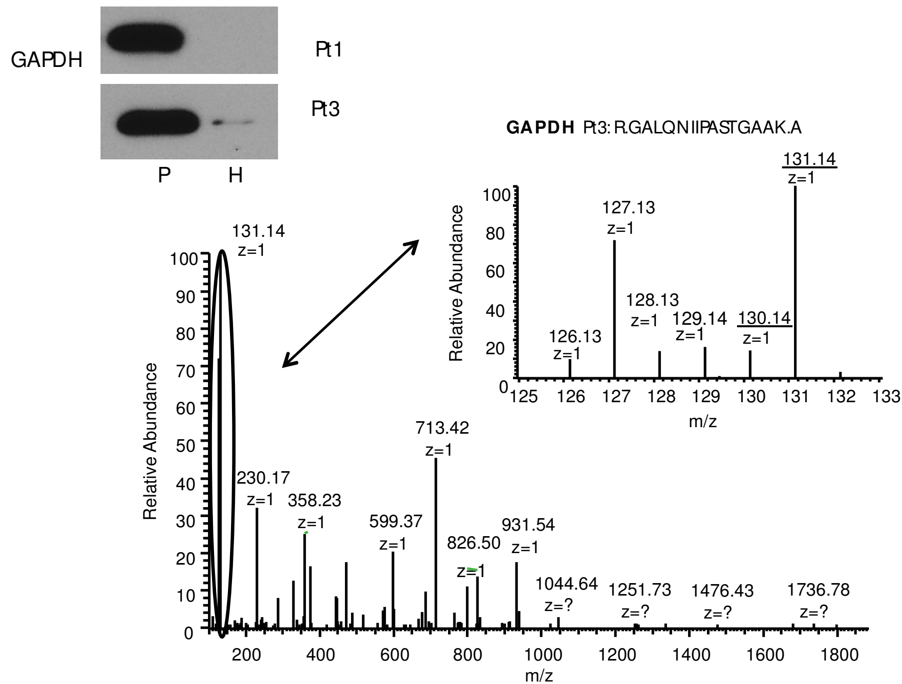


Figure 8s. Validation of the differential expression of GAPDH protein in Pt3, pathological (P) vs healthy (H) samples, by western blotting. Below the western blot picture, the MS/MS spectrum for the GAPDH tryptic peptide with sequence GALQNIIPASTGAAK and a zoomed in view on the corresponding TmT reporter ions is shown. The underlined TmT reporter ions correspond to the specific sample in which the modulation is statistically validated (Pt3). In Pt1, GAPDH western blots showed the same pattern, although the protein was not found significantly differentially expressed by the mass spectrometry data analysis

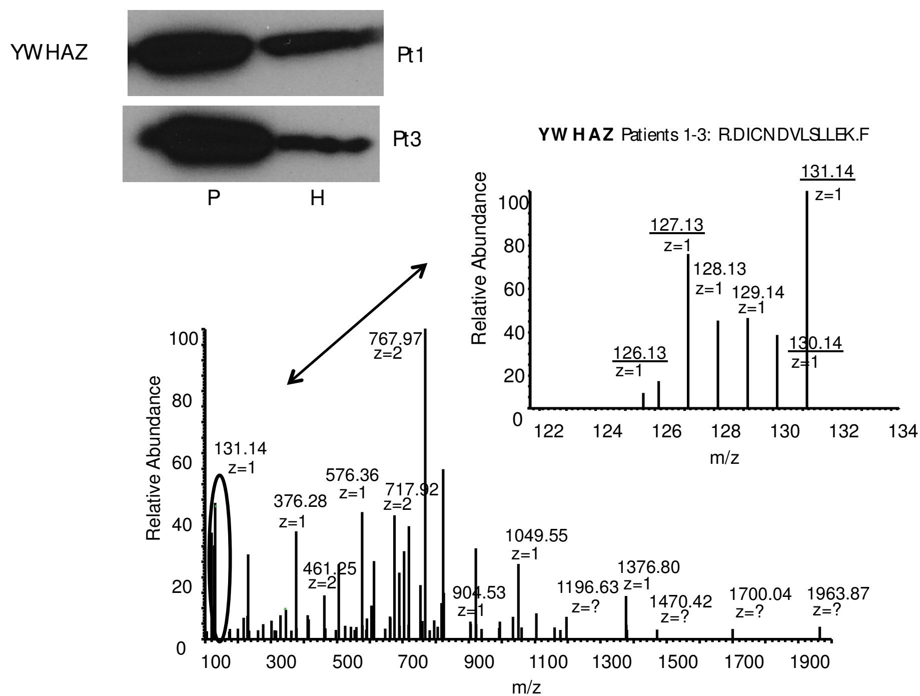


Figure 9s. Validation of the differential expression of YWHAZ protein in two patients, pathological (P) vs healthy (H) samples, by western blotting. Below the western blot picture, the MS/MS spectrum for the YWHAZ tryptic peptide with sequence DICNDVLSLLEK and a zoomed in view on the corresponding TmT reporter ions is shown. The underlined TmT reporter ions denote that the modulation is statistically validated in both patients.

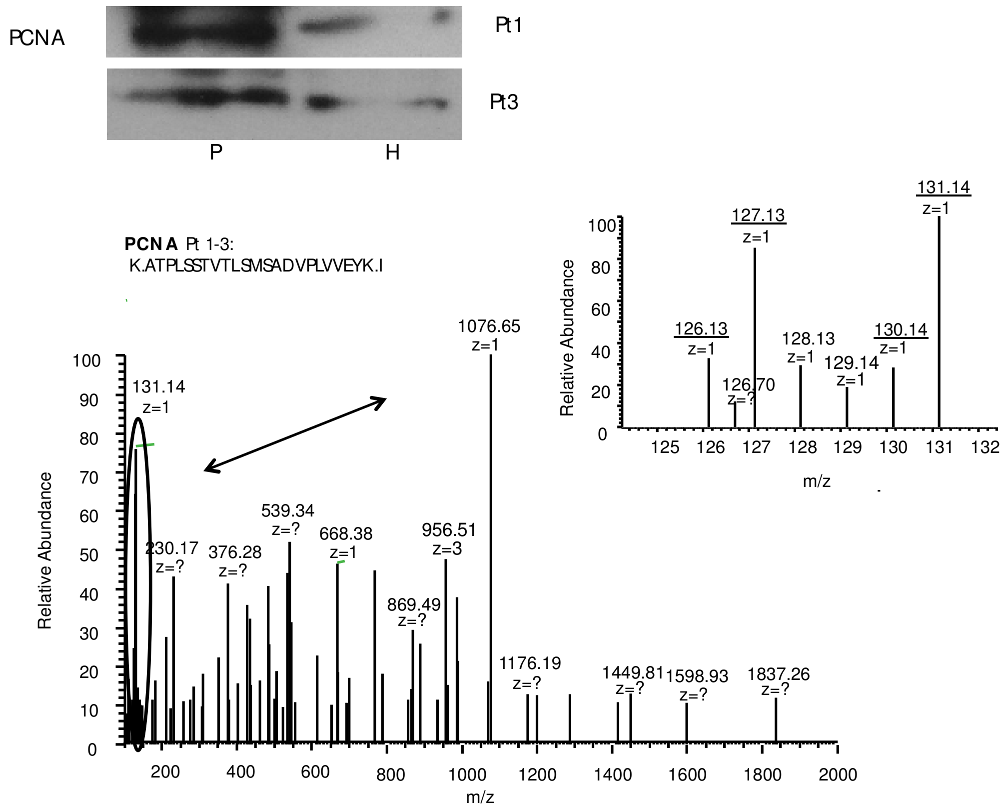


Figure 10s. Validation of the differential expression of PCNA protein in two patients, pathological (P) vs healthy (H) sample, by western blotting. Below the western blot picture, the MS/MS spectrum for the PCNA tryptic peptide with sequence ATPLSSTVTLSMSADVPLVVEYK and a zoomed in view on the corresponding TmT reporter ions is shown. The underlined TmT reporter ions denote that the protein is statistically validated in both patients.

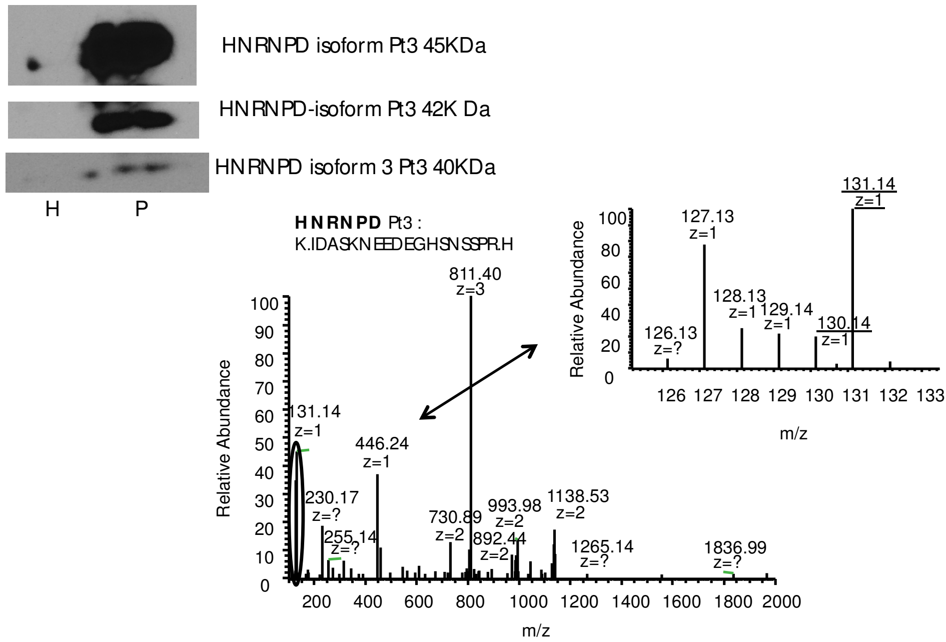


Figure 11s. Validation of the differential expression of HNRNPD protein in Pt3, pathological (P) vs healthy (H) samples, by western blotting. Below the western blot picture, the MS/MS spectrum for the HNRNPD tryptic peptide with sequence IDASKNEEDEGHSSPR and a zoomed in view on the corresponding TmT reporter ions is shown. The underlined TmT reporter ions correspond to the specific sample in which the modulation is statistically validated (Pt3).

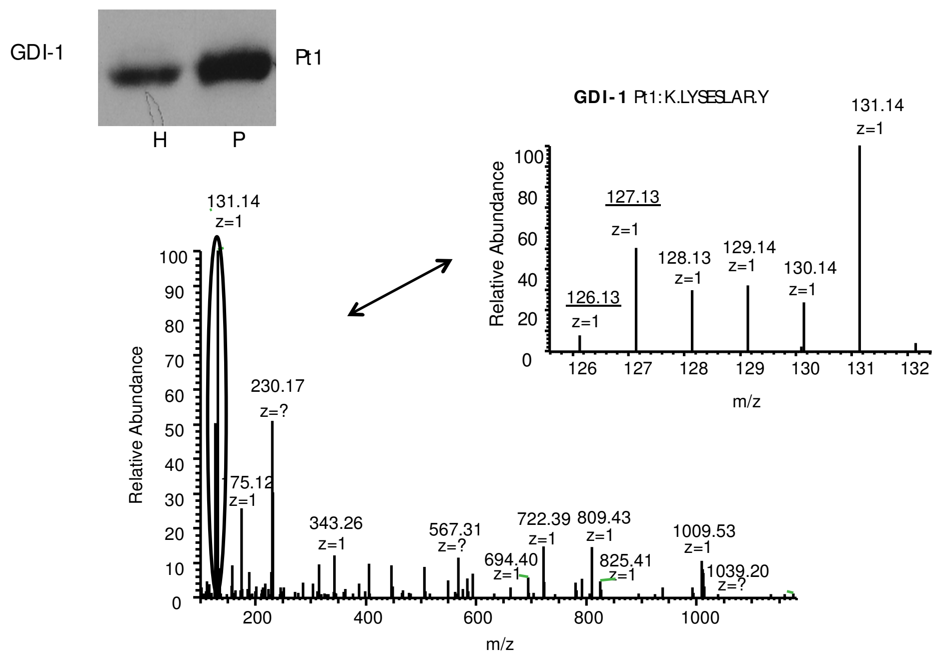


Figure 12s. Validation of the differential expression of GDI-1 protein in Pt1, pathological (P) vs healthy (H) samples, by western blotting. Below the western blot picture, the MS/MS spectrum for the HNRNPD tryptic peptide with sequence LYSESLAR and a zoomed in view on the corresponding TmT reporter ions is shown. The underlined TmT reporter ions correspond to the specific sample in which the modulation is statistically validated (Pt1).