PROTEOMICS

Supporting Information for Proteomics

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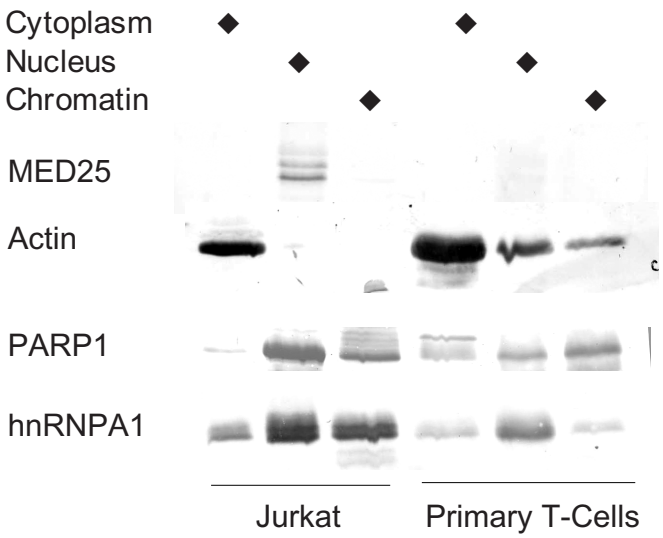
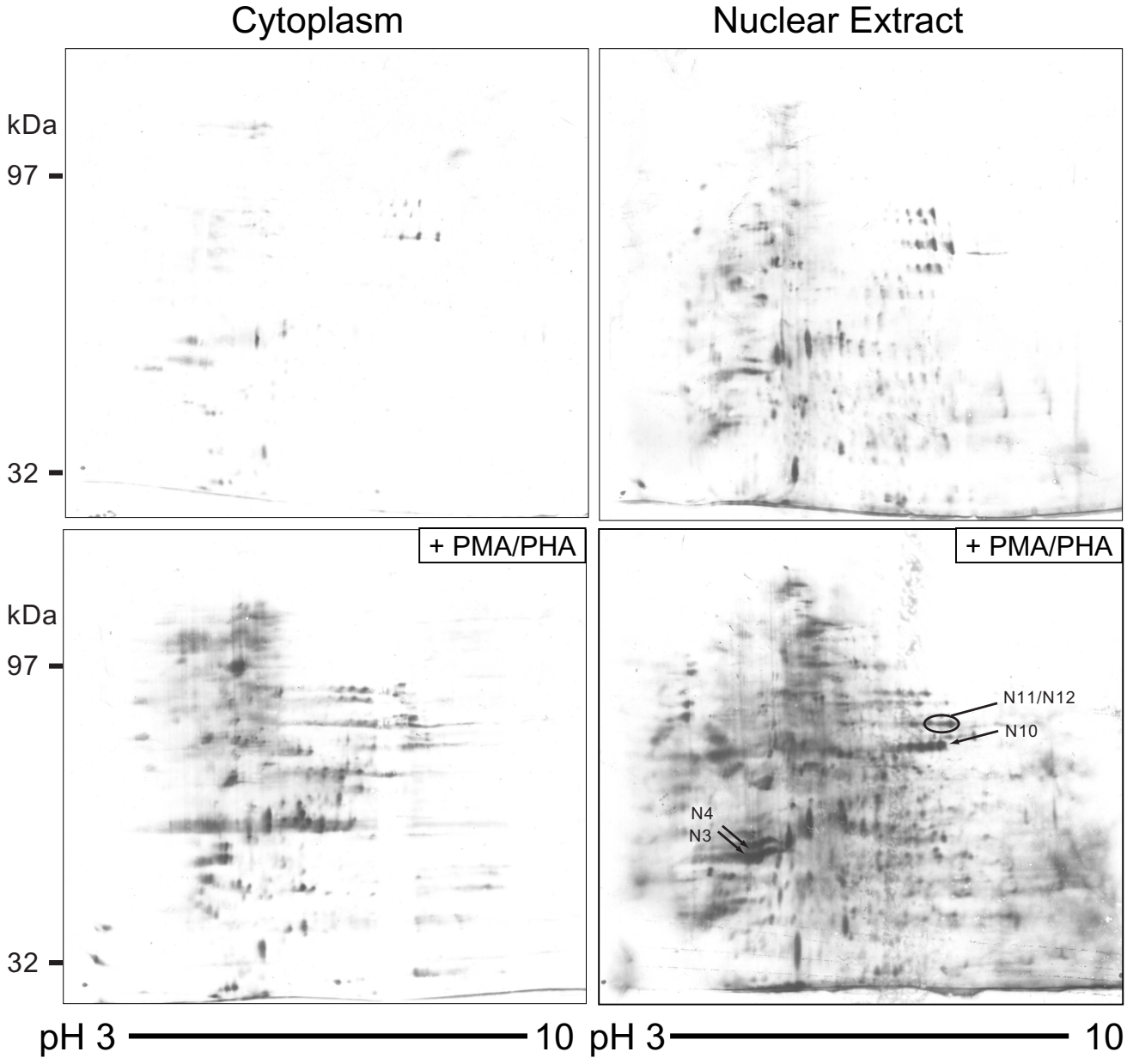
A functional proteomics approach for the detection of nuclear proteins based on derepressed importin alpha



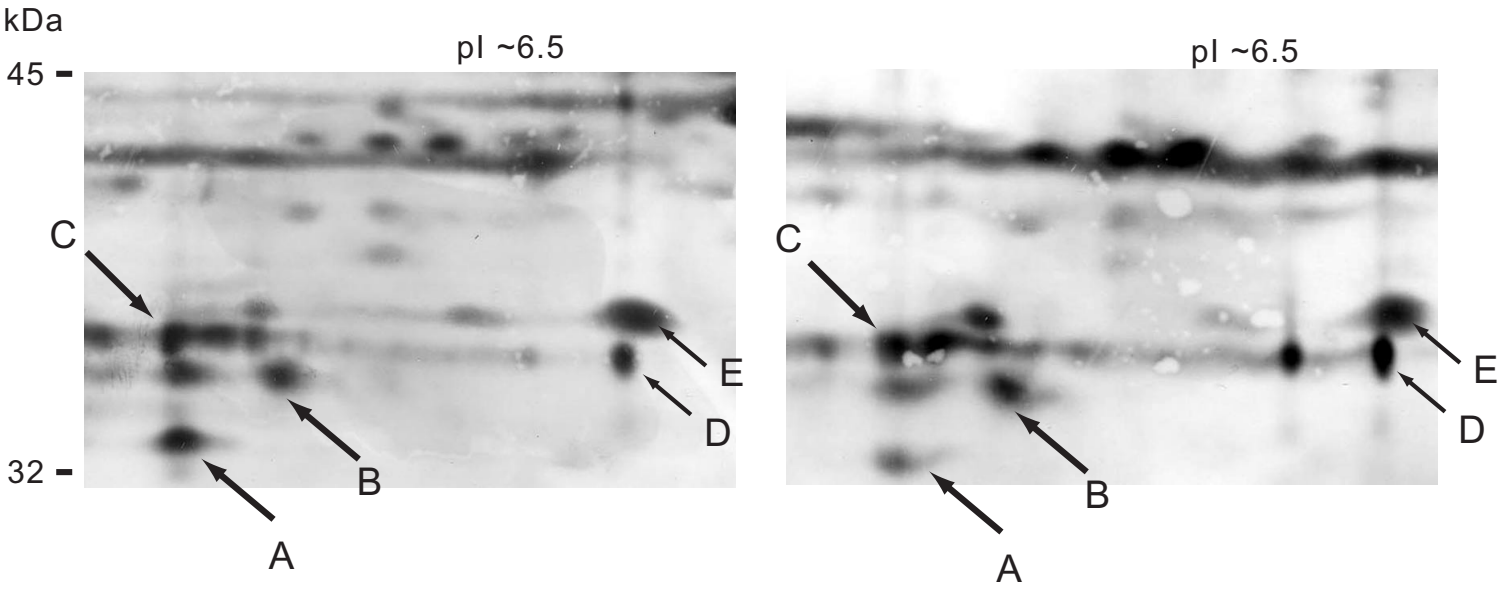
Separation of selected proteins during cell fractionation according to our protocols.: mammalian Mediator subunit MED25 (nuclear), actin (predominantly cytoplasmic), PARP1 (nuclear), hnRNPA1 (predominantly nuclear). The extraction protocols closely reproduce the reported localization (in parentheses) of the proteins tested.



DIRE analysis (2D far-Western blot) of T cell activation (PMA/PHA stimulation). The extracts and methods are comparable to Fig. 2 with extracts obtained from an independent biological sample. In contrast to Fig. 2 NLS-proteins in cytoplasmic fractions were also monitored. T cell activation leads to a significant increase of NLS-protein expression as seen in both cytoplasmic and nuclear fractions.

In contrast to the gels in Fig. 2 a linear gradient pH 3-10 and a lower percentage second dimension gel was used.

As an example of reproducibility some of the previously characterized proteins (N3/N4, N10, N11/N12, cp. Fig. 2) are identified by comparison with the patterns shown in Fig. 2, panels 4 and 6.



Comparison of 2-DE patterns generated from independent biological samples. DIRE 2-D far-Western blots were prepared from two independently prepared Jurkat nuclear extracts (as in Fig. 2). An enlarged section of the gels is shown demonstrating the reproducibility of DIRE on a number of proteins (examples A-E).