

Supplementary Figures legend:

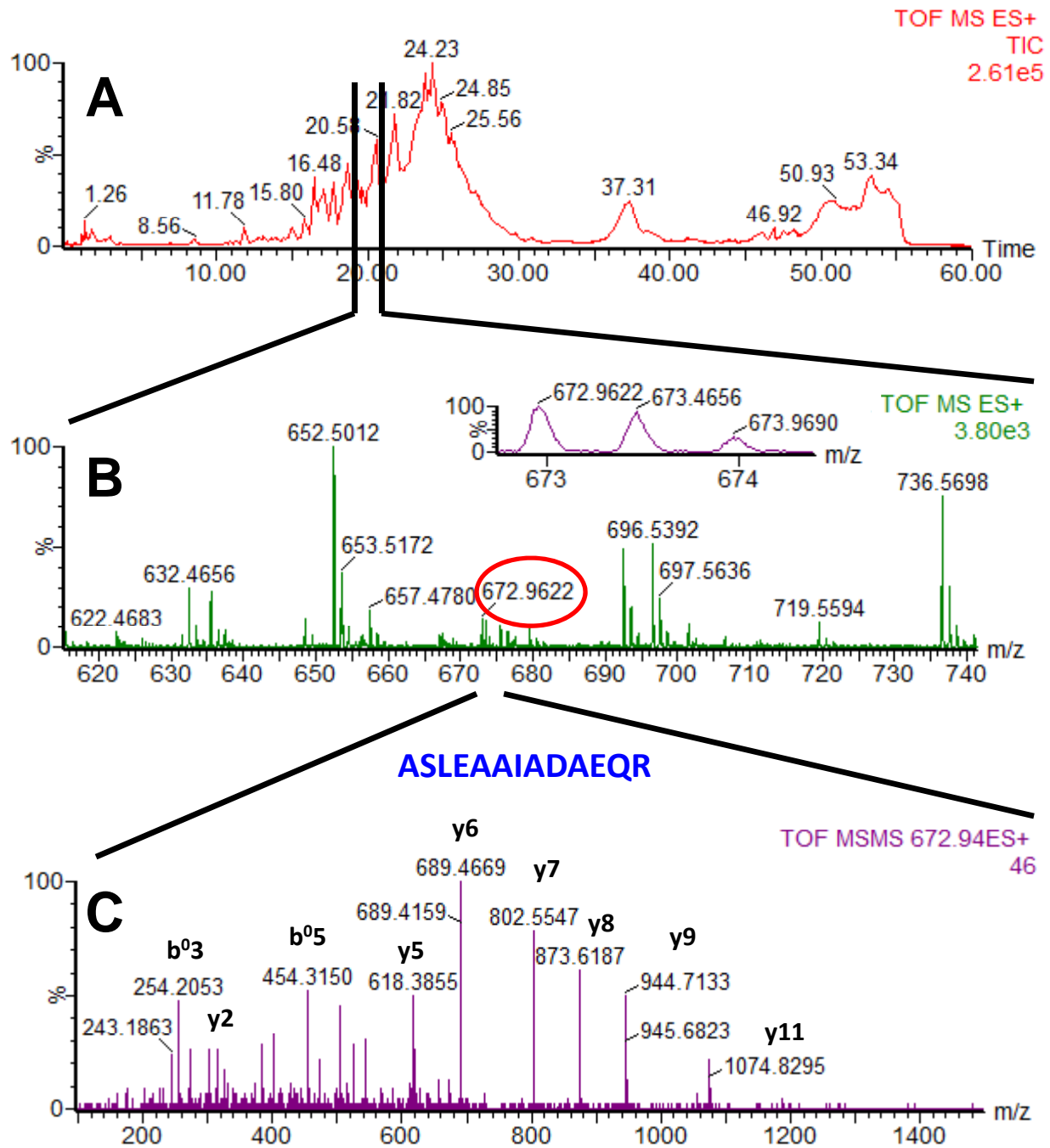
Supplemental Figure 1: LC-MS/MS analysis of an AP and MS control experiment in MCF7 cells. In the AP experiment, only agarose beads were used, without TDF-P1 cross-linked to them. The control AP eluate was separated on SDS-PAGE and the gel bands were excised and digested by trypsin. The resulting peptide mixture was separated on a C18 reverse phase column over an acetonitrile gradient. (A) TIC of the chromatogram. (B) MS survey mass spectrum, in which one double-charged peak (2+) at m/z of 672.96 (expanded in the inset) was fragmented by MS/MS and produced a MS/MS spectrum (C). The resulting peaks in the MS/MS spectrum correspond to a series of b and y ions that resulted from fragmentation of a peptide which was part of Cytokeratin 8. Data analysis of these peaks led to identification of the sequence shown in (C) and of Cytokeratin 8.

Supplemental Figure 2: LC-MS/MS analysis of an AP and MS control experiment in MCF7 cells. In the AP experiment, only agarose beads were used, without TDF-P1 crosslinked to them. The control AP eluate was separated on SDS-PAGE and the gel bands were excised and digested by trypsin. The resulting peptide mixture was separated on a C18 reverse phase column over an acetonitrile gradient. (A) TIC of the chromatogram. (B) MS survey mass spectrum, in which one double-charged (2+) peak at m/z of 521.35 (expanded in the inset) was fragmented by MS/MS and produced a MS/MS spectrum (C). The resulting peaks in the MS/MS spectrum correspond to a series of b and y ions that resulted from fragmentation of a peptide which was part of Cytokeratin 18. Data analysis of these peaks led to identification of the sequence shown in (C) and of Cytokeratin 18.

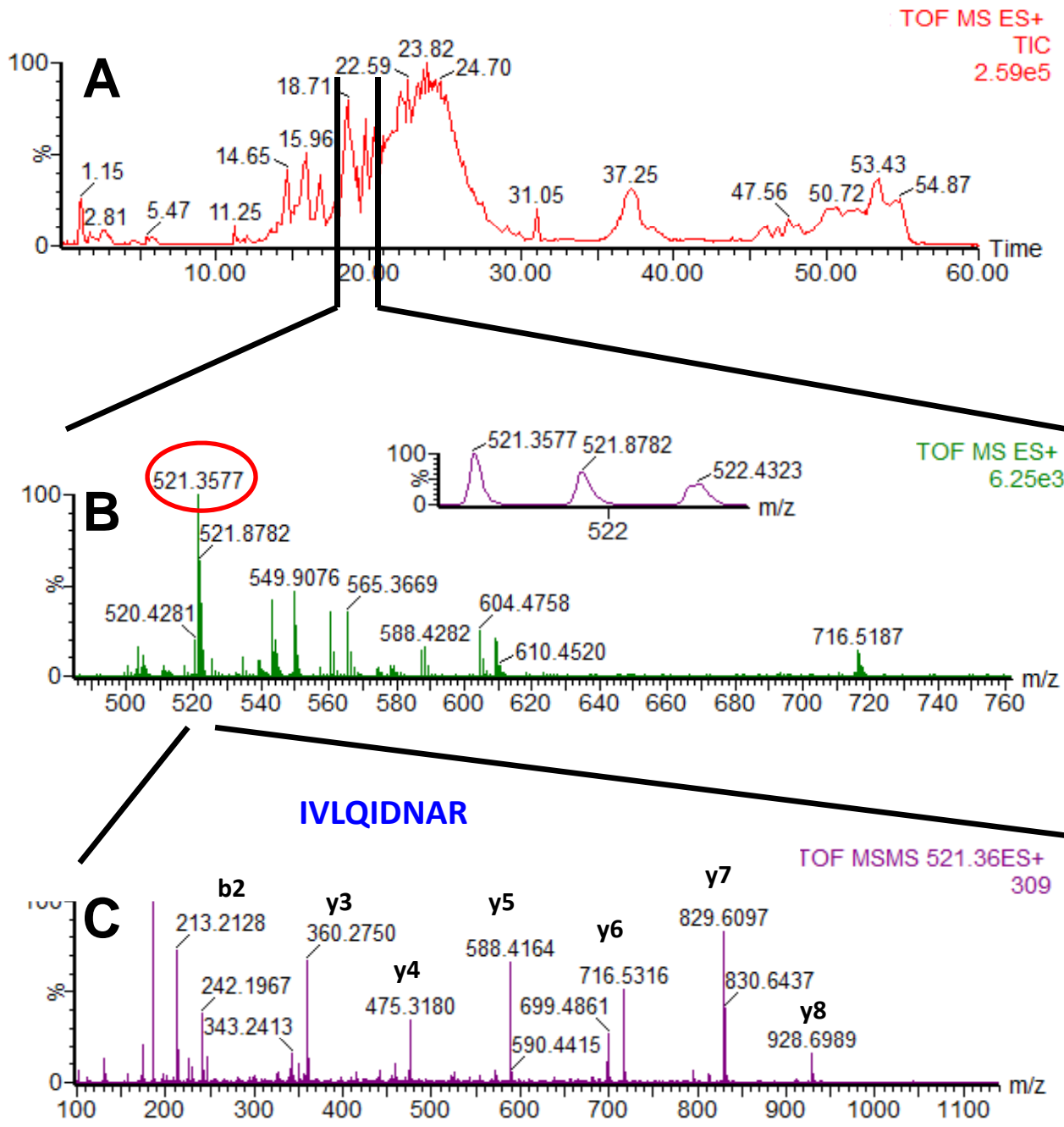
Supplemental Figure 3: Western blotting of the affinity purified TDF-R candidates. The TDF-R candidates were purified by AP using TDF-P1 peptide and the eluate investigated by WB using anti-GRP78 and anti-HSP70 antibodies. The cell lysate was prepared from MCF7 steroid-responsive cells (A), and from HeLa cancer cells (B). The molecular mass marker is shown (in kDa) on the left. Each WB contains input cell lysate (1), flow-through (2), eluate (3) and concentrated eluate (4) of the AP experiments.

Supplemental Figure 4: Investigation of the interaction of TDF-P1 with Myoglobin, BSA and GRP78/BiP proteins by ESI-MS. ESI-MS mass spectra of TDF-P1 (A), TDF-P1 in the presence of myoglobin (B), TDF-P1 in the presence of BSA (C) and TDF-P1 in the presence of recombinant GRP78/BiP protein (D). The peaks marked with a circle at m/z of 738.09 and 1106.59, represent triple- (3+) and double- (2+) charged TDF-P1, respectively. TDF-P1 at concentration 1 pM/ μ l was incubated for 2 hours at RT with 1 pM/ μ l of BSA - bovine serum albumin (Sigma, St. Louis, MO), 1 pM/ μ l Myoglobin (Waters Corporation, Milford, MA) and recombinant BiP protein. Proteins alone, TDF-P1 alone and mixture of proteins with TDF-P1 were adjusted to 0.1% formic acid and used for mass spectrometry analysis. Samples were injected with a flow rate of 5 μ l/min into Q-ToF Micro MS (Micromass/Waters, Milford, MA) to acquire positive ion mode ESI-MS spectra. GRP78/BiP protein was expressed in M15 *E. coli* bacteria containing QE-10 vector with cDNA from hamster (gift from Dr. Linda Hendershot). After 4 hours induction with 1mM IPTG, bacterial cells were lysed and recombinant BiP protein was purified by binding to Ni²⁺ agarose at pH 8.0 and eluting at pH 4.5. For MS experiments, GRP78/BiP protein was desalted using Amicon Ultra centrifugal filter devices with 10 kDa MWCO (Millipore Corp., Billerica, MA). No TDF-P1 peak was observed after 2 hours incubation of TDF-P1 with GRP78/BiP protein.

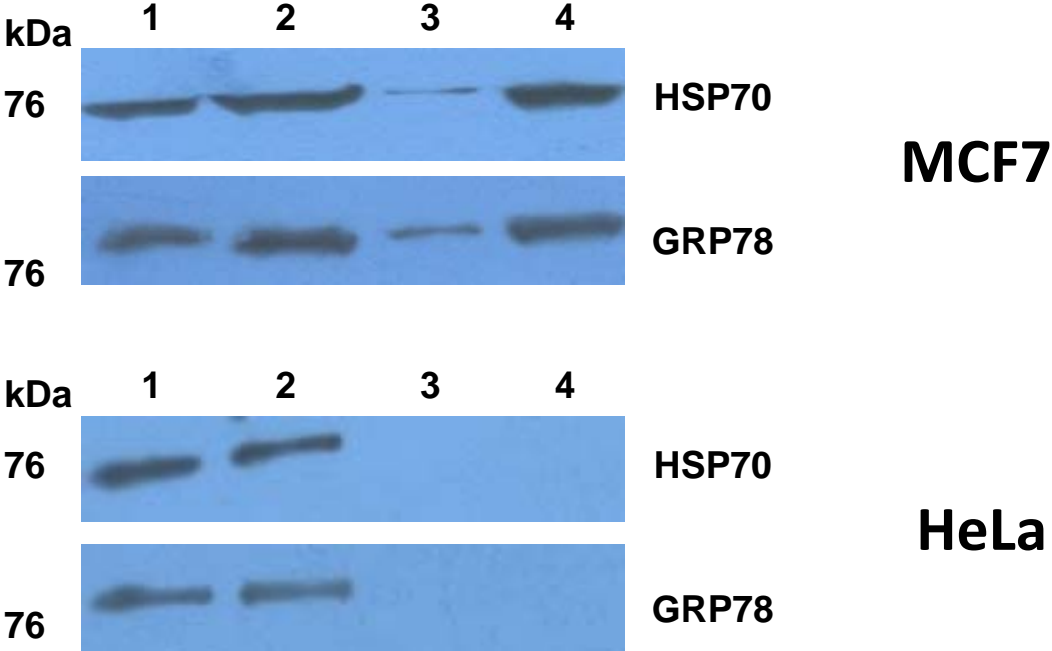
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4

