Supplementary Figure 1: Protein elution profiles for (a) MARS6 column; (b) MARS14 column; (c) PROT20 column. In (a) and (b) the blue trace represents the UV absorption at 214nm. In (c) the blue trace is UV absorption at 280nm. Representative traces are shown.

a. mAU 2410 UV absorption at 214nm 2000 1500 1000 500 12 933 15.518 Time (minutes) b. mAU 2000 UP absorption at 214nm 8.602 1500 1000 8 50 c. Time (minutes)



Time (minutes)

Supplementary Figure 2: Representative examples of 1D-SDS-PAGE analysis showing the reproducibility of immunodepletion of 6, 14 or 20 proteins. Peaks 1 and 2 are shown for each column type, on 12% silver stained SDS-PAGE gels with molecular weight (MW) markers. Peak 1 gels = 1 μ g/lane, peak 2 gels = 5 μ g/lane.



Supplementary Figure 3: Histograms showing distributions of the 2D-DIGE coefficients of variation for gel spot normalised volumes, against scaled frequency measurements (density) derived from 4 replicate gels of peak 1 immunodepleted material per column.



Supplementary Figure 4: Representative 2D gel images for depleted (peak 1; a-d) and bound (peak 2; e-h) material from each column and principal components analysis (PCA) plot for each of MARS6, MARS14, PROT20 columns respectively. On PCA plots, stars mark positions for MARS6 gels, squares for MARS14 and circles for Prot 20. 75µg protein was loaded per DIGE-labelling channel in each case.



Supplementary Figure 5: a. Peak 1 preparatory gel image with spots selected for cutting marked; **b.** Peak 2 preparatory gel image with spots selected for cutting marked. 150µg prtoein loaded. Both gels silver stained. Spot numbers refer to their designation in the Progenesis SameSpots software used for the DIGE analysis



