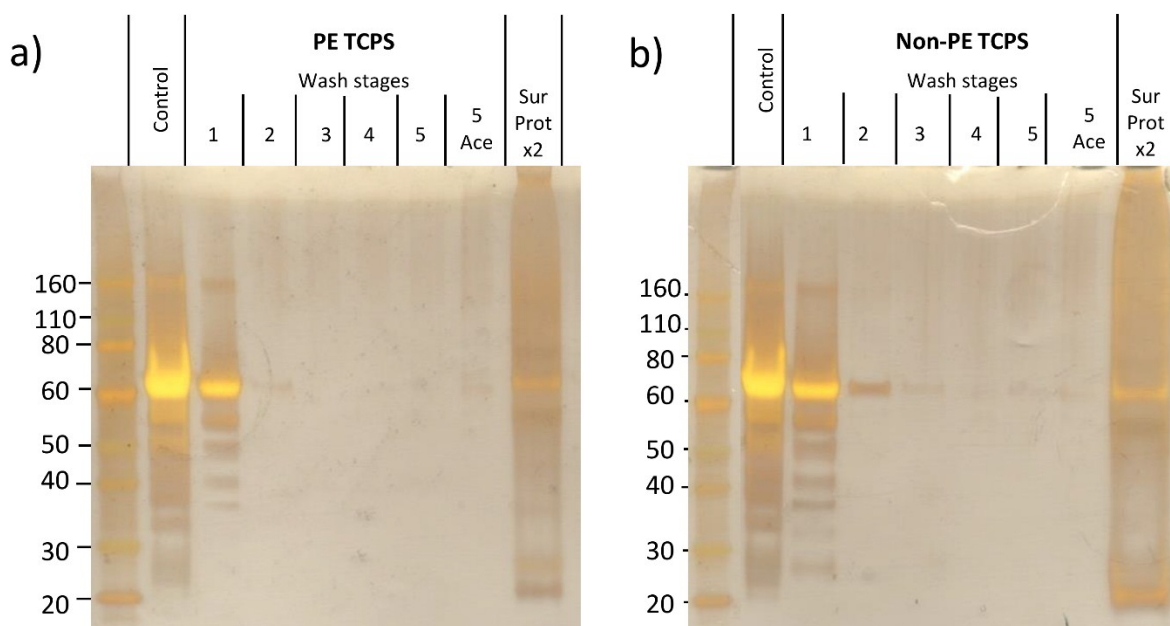
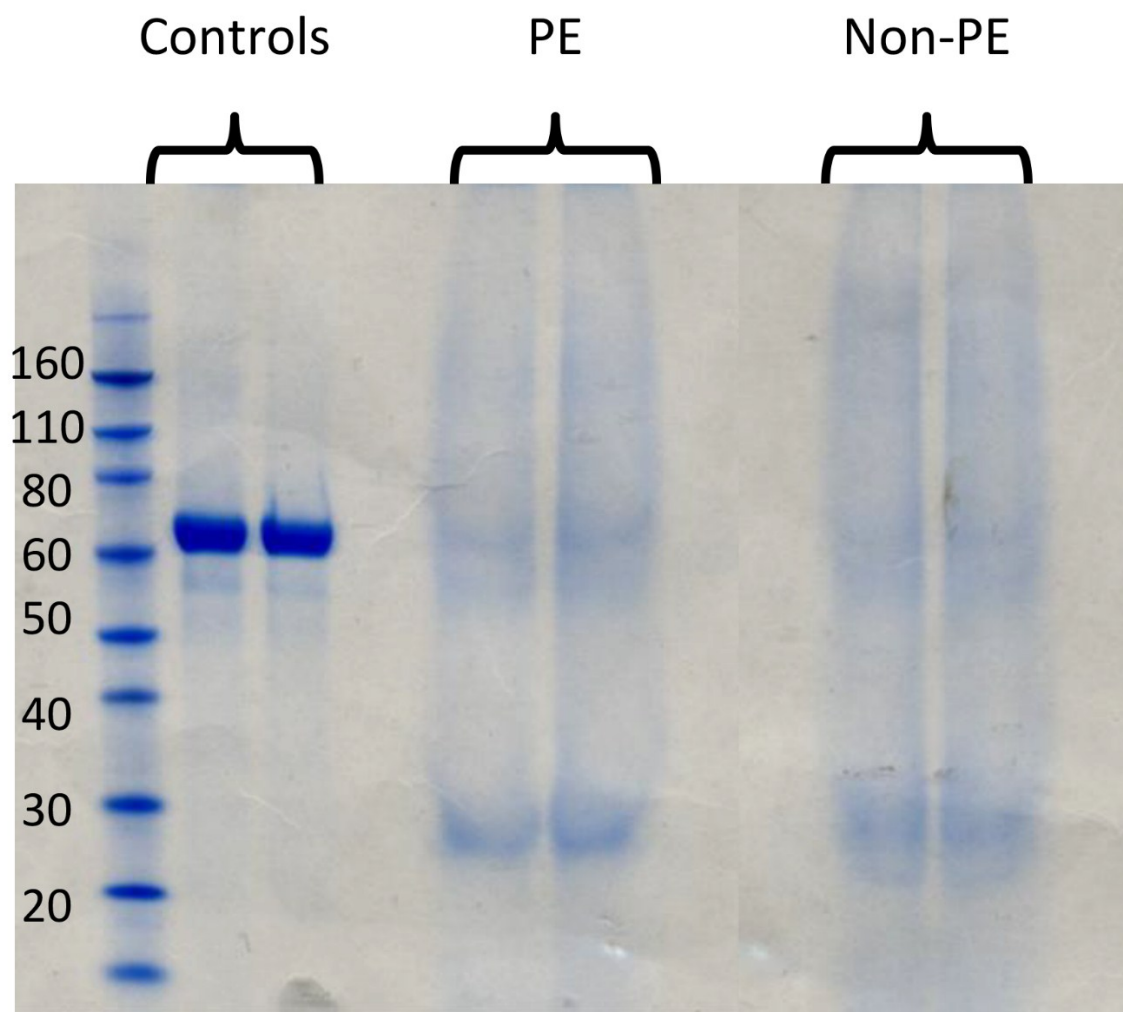


## 1. Proteomics



**Figure 1** In order to analyse adsorbed proteins from the surface of TCPS plates it was necessary to remove all soluble proteins that may have been left behind from the MEF medium and it was found that a wash procedure consisting of five PBS rinses was sufficient to remove the majority of soluble proteins from the surface. The optimal extraction solvent was determined to be 1M NaCl, 8M urea, 1% TX-100 and 50% isopropanol, which was found to be sufficient to remove surface bound proteins from PE (A) and non-PE (B) treated TCPS plates.



**Figure 2** An intensity of the proteins from 12 wells was found to be needed for proteomics analysis, which could be observed using standard Coomassie staining.

| Biomolecule                              | Molecular weight | Concentration when printing at 1 fmol | Source                      |
|--|------------------|---------------------------------------|-----------------------------|
| Platelet factor 4                        | 29 kDa           | 8.055 µg/mL                           | Abcam (ab80477)             |
| Tetranectin                              | 25 kDa           | 6.94 µg/mL                            | R&D Systems (5170-CL-050)   |
| Serum amyloid P-component                | 786 kDa          | 218 µg/mL                             | Anaspec (62922)             |
| Beta-lactoglobulin                       | 19,883 Da        | 5.522 µg/mL                           | Mybiosource.com (MBS717034) |
| Alpha-2-antiplasmin                      | 70,000 Da        | 19.44 µg/mL                           | Abcam (ab77936)             |
| Fibronectin                              | 440 kDa          | 125 µg/mL                             | Sigma Aldrich (F2006)       |
| Desmoplakin                              | 331,774 Da       | 92.16 µg/mL                           | Abcam (ab71689)             |
| Heat shock protein 1-like                | 70.46 kDa        | 19.57 µg/mL                           | Abnova (H00003305-T01)      |
| Glyceraldehyde-3-phosphate dehydrogenase | 36 kDa           | 10 µg/mL                              | Mybiosource.com (MBS203254) |
| Reticulon 4 interacting protein 1        | 43.6 kDa         | 12.11 µg/mL                           | Abnova (H00084816-T02)      |
| Agrin                                    | 110 kDa          | 30.56 µg/mL                           | R&D Systems (6624-AG-050)   |
| Ubiquitin                                | 8565 Da          | 2.38 µg/mL                            | Abcam (ab51097)             |
| Heat shock protein 90                    | 86.8 kDa         | 24.11 µg/mL                           | Mybiosource.com (MBS203032) |

**Table 1 Identity of proteins and concentrations used when spotting at 1 fmol.**

Table 1 displays the biomolecules used in this experiment. Proteins used differ from those identified by the proteomic work. Human proteins were used where possible with the exception of beta-lactoglobulin (bovine). Human desmoplakin I + II peptide was also used instead of desmoplakin. Reticulon 4 interacting protein 1 was used in place of reticulon 4 protein. Mini-agrin is a murine protein and therefore human agrin was used as an alternative. Heat shock protein 90 was also used in place of heat shock protein 90 beta member 1. Proteomics analysis also identified ubiquitin subunit 1, but full length ubiquitin was used instead.

## 2. Primary screen Print

|                  |               |                  |                 |                 |                 |              |               |               |               |                  |              |
|------------------|---------------|------------------|-----------------|-----------------|-----------------|--------------|---------------|---------------|---------------|------------------|--------------|
| BL/A<br>2A       | DPK/<br>A2A   | FN/A<br>2A       | GAPDH<br>/A2A   | GAPDH<br>/A2A   | HSP90/<br>A2A   | MA/A<br>2A   | PF4/A<br>2A   | RTU/A<br>2A   | SAP/A<br>2A   | TN/A<br>2A       | UQ/A<br>2A   |
| BL               | DPK/<br>BL    | FN/B<br>L        | GAPDH<br>/BL    | GAPDH<br>/BL    | HSP90/<br>BL    | MA/B<br>L    | PF4/B<br>L    | RTU/B<br>L    | SAP/B<br>L    | TN/B<br>L        | UQ/B<br>L    |
| BL/D<br>PK       | DPK           | FN/D<br>PK       | GAPDH<br>/DPK   | GAPDH<br>/DPK   | HSP90/<br>DPK   | MA/D<br>PK   | PF4/D<br>PK   | RTU/D<br>PK   | SAP/D<br>PK   | TN/D<br>PK       | UQ/D<br>PK   |
| BL/F<br>N        | DPK/<br>FN    | FN               | GAPDH<br>/FN    | GAPDH<br>/FN    | HSP90/<br>FN    | MA/F<br>N    | PF4/F<br>N    | RTU/F<br>N    | SAP/F<br>N    | TN/F<br>N        | UQ/F<br>N    |
| BL/G<br>APD<br>H | BL/G<br>APDH  | FN/G<br>APD<br>H | GAPDH           | HSP/G<br>APDH   | HSP90/<br>GAPDH | MA/G<br>APDH | PF4/G<br>APDH | RTU/G<br>APDH | SAP/G<br>APDH | TN/G<br>APD<br>H | UQ/G<br>APDH |
| BL/H<br>SP       | DPK/<br>HSP   | FN/H<br>SP       | GAPDH<br>/HSP   | HSP             | HSP90/<br>HSP   | MA/H<br>SP   | PF4/H<br>SP   | RTU/H<br>SP   | SAP/H<br>SP   | TN/H<br>SP       | UQ/H<br>SP   |
| BL/H<br>SP90     | DPK/<br>HSP90 | FN/H<br>SP90     | GAPDH<br>/HSP90 | GAPDH<br>/HSP90 | HSP90           | MA/H<br>SP90 | PF4/H<br>SP90 | RTU/H<br>SP90 | SAP/H<br>SP90 | TN/H<br>SP90     | UQ/H<br>SP90 |
| BL/M<br>A        | DPK/<br>MA    | FN/M<br>A        | GAPDH<br>/MA    | GAPDH<br>/MA    | HSP90/<br>MA    | MA           | PF4/M<br>A    | RTU/<br>MA    | SAP/<br>MA    | TN/M<br>A        | UQ/M<br>A    |
| BL/P<br>F4       | DPK/<br>PF4   | FN/P<br>F4       | GAPDH<br>/PF4   | GAPDH<br>/PF4   | HSP90/<br>PF4   | MA/P<br>F4   | PF4           | RTU/P<br>F4   | SAP/P<br>F4   | TN/P<br>F4       | UQ/P<br>F4   |
| BL/R<br>TU       | DPK/<br>RTU   | FN/R<br>TU       | GAPDH<br>/RTU   | GAPDH<br>/RTU   | HSP90/<br>RTU   | MA/R<br>TU   | PF4/R<br>TU   | RTU           | SAP/R<br>TU   | TN/R<br>TU       | UQ/R<br>TU   |
| BL/S<br>AP       | DPK/<br>SAP   | FN/S<br>AP       | GAPDH<br>/SAP   | GAPDH<br>/SAP   | HSP90/<br>SAP   | MA/S<br>AP   | PF4/S<br>AP   | RTU/S<br>AP   | SAP           | TN/S<br>AP       | UQ/S<br>AP   |
| BL/T<br>N        | DPK/<br>TN    | FN/T<br>N        | GAPDH<br>/TN    | GAPDH<br>/TN    | HSP90/<br>TN    | MA/T<br>N    | PF4/T<br>N    | RTU/T<br>N    | SAP/T<br>N    | TN               | UQ/T<br>N    |
| BL/U<br>Q        | DPK/<br>UQ    | FN/U<br>Q        | GAPDH<br>/UQ    | GAPDH<br>/UQ    | HSP90/<br>UQ    | MA/U<br>Q    | PF4/U<br>Q    | RTU/U<br>Q    | SAP/U<br>Q    | TN/U<br>Q        | UQ           |

**Table 2** Layout of array plan, co-adsorbed mixtures were prepared by spotting 3 drops of one protein followed by spotting 7 drops of another protein. Each cell is 1 x 7 polymer spots. Two further arrays were printed on the same slide as proteins were spotted at 3 different concentrations. BL = Beta-lactoglobulin, A2A = alpha-2-antiplasmin, TN = tetranectin, PF4 = platelet factor 4, GAPDH = glyceraldehyde-3-phosphate dehydrogenase, MA = agrin, UQ = ubiquitin, HSP90 = heat shock protein 90, DPK = desmoplakin, HSP = heat shock protein-1-like, SAP = serum amyloid P, Fn = fibronectin and RTU = reticulon 4 interacting protein 1

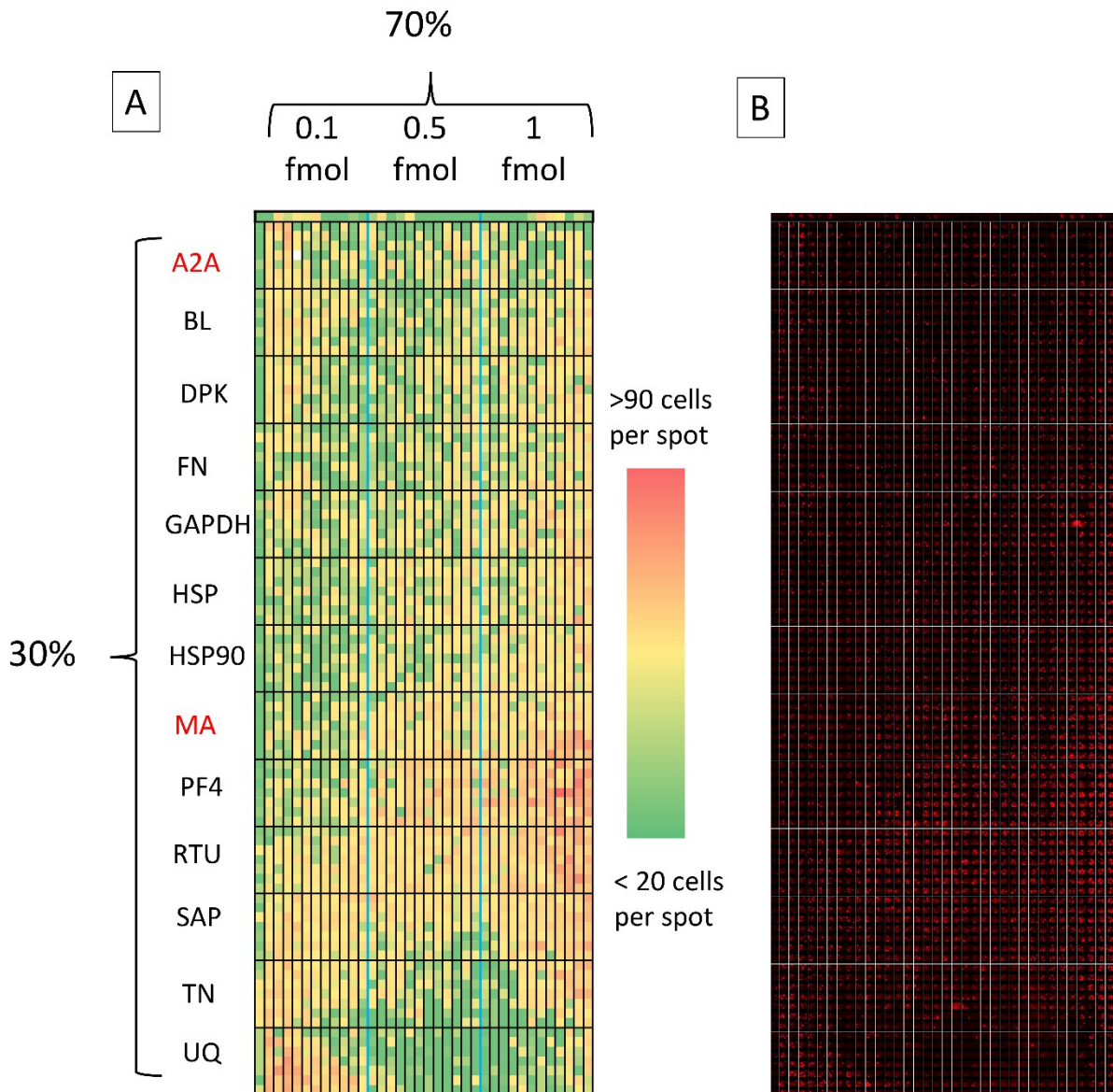
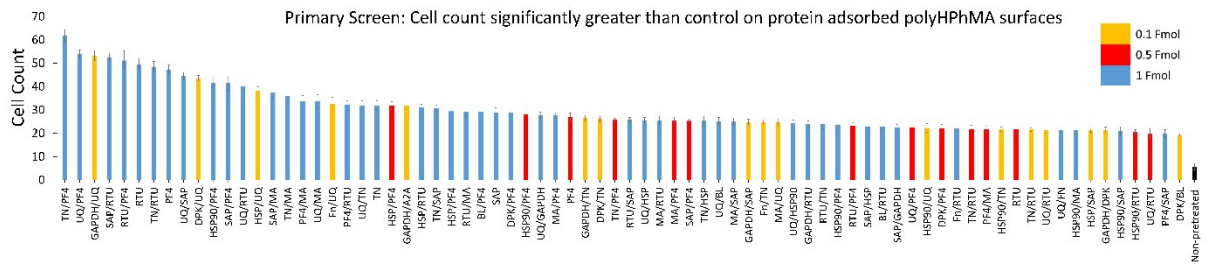


Figure 3 **A)** Collated images of the preliminary screen array. From left to right: heat map representative of cell attachment is shown (red signifies > 90 cells per spot, green signifies < 20 cells per spot). Each spot acquired separately and stitched together. ‘Blocks’ are separated by light blue lines, the ‘block’ on the left signifies cell attachment on surfaces spotted with 0.1 fmol protein, the centre ‘block’ shows cell attachment on surfaces spotted with 0.5 fmol, and the ‘block’ on the right shows cell adherence on surfaces spotted with 1 fmol. Columns (signified by bold black lines) represent different proteins spotted at 7 drops (from left to right these are BL, DPK, FN, GAPDH, HSP, HSP90, MA, PF4, RTU, SAP, TN and UQ). A2A columns did not print at all due to a blocked PDC nozzle head. Rows are separated by horizontal black lines and the order is shown above. MA did not spot due to a blocked PDC nozzle. The top row depicts cell adherence on non-spotted N-(4-Hydroxyphenyl) methacrylamide polymer spots. **B)** Detection of Oct-4 expressing cells on the array using Cy3 labelled antibody



**Figure 4** HUES7 cell count to protein adsorbed surfaces, only samples with cell adhesion significantly greater than control ( $p < 0.0004$ ) are shown. Error bars are standard error of mean. HUES7 cell adherence to samples from preliminary screening array,  $n=7$  from one microarray, raw data in Figure 2B. Bars are colour coded to represent concentration of spotted proteins. Yellow: 0.1 fmol, red: 0.5 fmol, and cyan: 1 fmol. The non-pretreated surface is shown in black.

### 3. Secondary screen Print

The ratios were used to assess in parallel the effect of cell adherence to biomolecules spotted as a major, minor or equivalent component on the surface.

|        | 0.1 fmol  | 0.5 fmol  | 1 fmol    | 2 fmol    | 4 fmol    |
|--------|-----------|-----------|-----------|-----------|-----------|
| Ratio% | Bare      | Bare      | Bare      | Bare      | Bare      |
| 100    | BL        | BL        | BL        | BL        | BL        |
| 100    | Fn        | Fn        | Fn        | Fn        | Fn        |
| 100    | GAPDH     | GAPDH     | GAPDH     | GAPDH     | GAPDH     |
| 100    | HSP       | HSP       | HSP       | HSP       | HSP       |
| 100    | HSP90     | HSP90     | HSP90     | HSP90     | HSP90     |
| 100    | MA        | MA        | MA        | MA        | MA        |
| 100    | PF4       | PF5       | PF6       | PF7       | PF8       |
| 100    | RTU       | RTU       | RTU       | RTU       | RTU       |
| 100    | SAP       | SAP       | SAP       | SAP       | SAP       |
| 100    | TN        | TN        | TN        | TN        | TN        |
| 100    | UQ        | UQ        | UQ        | UQ        | UQ        |
| 30/70  | GAPDH/SAP | GAPDH/SAP | GAPDH/SAP | GAPDH/SAP | GAPDH/SAP |
| 50/50  | GAPDH/SAP | GAPDH/SAP | GAPDH/SAP | GAPDH/SAP | GAPDH/SAP |
| 70/30  | GAPDH/SAP | GAPDH/SAP | GAPDH/SAP | GAPDH/SAP | GAPDH/SAP |
| 30/70  | HSP/HSP90 | HSP/HSP90 | HSP/HSP90 | HSP/HSP90 | HSP/HSP90 |
| 50/50  | HSP/HSP90 | HSP/HSP90 | HSP/HSP90 | HSP/HSP90 | HSP/HSP90 |
| 70/30  | HSP/HSP90 | HSP/HSP90 | HSP/HSP90 | HSP/HSP90 | HSP/HSP90 |
| 30/70  | HSP/FN    | HSP/FN    | HSP/FN    | HSP/FN    | HSP/FN    |
| 50/50  | HSP/FN    | HSP/FN    | HSP/FN    | HSP/FN    | HSP/FN    |
| 70/30  | HSP/FN    | HSP/FN    | HSP/FN    | HSP/FN    | HSP/FN    |
| 30/70  | PF4/GAPDH | PF4/GAPDH | PF4/GAPDH | PF4/GAPDH | PF4/GAPDH |
| 50/50  | PF4/GAPDH | PF4/GAPDH | PF4/GAPDH | PF4/GAPDH | PF4/GAPDH |
| 70/30  | PF4/GAPDH | PF4/GAPDH | PF4/GAPDH | PF4/GAPDH | PF4/GAPDH |
| 30/70  | PF4/HSP   | PF4/HSP   | PF4/HSP   | PF4/HSP   | PF4/HSP   |
| 50/50  | PF4/HSP   | PF4/HSP   | PF4/HSP   | PF4/HSP   | PF4/HSP   |
| 70/30  | PF4/HSP   | PF4/HSP   | PF4/HSP   | PF4/HSP   | PF4/HSP   |

**Table 3** Secondary array plan. Cells with 'Bare' represent 7 x 1 spots. All other cells represent 7 x 4 spots (making for 28 replicates per co-adsorbed sample). BL = Beta-lactoglobulin, TN = tetranectin, PF4 = platelet factor 4, GAPDH = glyceraldehyde-3-phosphate dehydrogenase, MA = agrin, UQ = ubiquitin, HSP90 = heat shock protein 90, HSP = heat shock protein-1-like, SAP = serum amyloid P, Fn = fibronectin and RTU = reticulon 4 interacting protein 1