Supplementary material

Increasing the accuracy of proteomic typing by decellularisation of amyloid tissue biopsies

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Full datasets with protein and peptide identifications by Mascot (and LFQ intensity by MaxQuant) have been uploaded separately as supplementary files.
Supplementary Table 1. Proteomic MS analysis of untreated and decellularised cardiac and fat biopsies.

| Cardiac biopsy | Untreated | Decellularised |
|----------------|-----------|---------------|
| **Patient**    | **SCAFFOLD RESULTS** | **SCAFFOLD RESULTS** |
| **Amyloid Score** | **MASCOT** | **P (%)** | **EUP** | **EUS** | **TS** |
| **MASCOT** | **P (%)** | **EUP** | **EUS** | **TS** | **P (%)** | **EUP** | **EUS** | **TS** |
| k, 184        | >99       | 4      | 6      | 8      | k, 242  | >99   | 6     | 7     | 10      |
| TTR, 86       | >99       | 3      | 3      | 4      | TTR, 0  | ND    | 0     | 0     | 0       |
|               |           |        |        |        | TTR, 0  | ND    | 0     | 0     | 0       |
| *1            |           |        |        |        |         |       |       |       |         |
| λ, 180        | >99       | 3      | 5      | 7      | λ, 310  | >99   | 5     | 7     | 11      |
|               |           |        |        |        | λ, 87   | >99   | 5     | 6     | 7       |
|               |           |        |        |        | λ, 268  | >99   | 6     | 9     | 15      |
|               |           |        |        |        | k, 28   | ND    | 0     | 0     | 0       |
|               |           |        |        |        | k, 60   | ND    | 0     | 0     | 0       |
|               |           |        |        |        | k, 30   | ND    | 0     | 0     | 0       |
| *2            |           |        |        |        |         |       |       |       |         |
| k, 107        | ND        | 0      | 0      | 0      | k, 147  | >99   | 5     | 9     | 20      |
| TTR, 836      | >99       | 7      | 13     | 17     | TTR, 969| >99   | 9     | 14    | 27      |
| TTR, 700      | >99       | 9      | 11     | 18     | TTR, 976| >99   | 10    | 13    | 34      |
| TTR, 846      | >99       | 14     | 17     | 29     | TTR, 975| >99   | 10    | 13    | 30      |
|               |           |        |        |        |         |       |       |       |         |
| Fat aspirate  | Untreated | Decellularised |
| **Patient**    | **SCAFFOLD RESULTS** | **SCAFFOLD RESULTS** |
| **Amyloid Score** | **MASCOT** | **P (%)** | **EUP** | **EUS** | **TS** |
| **MASCOT** | **P (%)** | **EUP** | **EUS** | **TS** | **P (%)** | **EUP** | **EUS** | **TS** |
| λ, 809        | >99       | 10     | 14     | 28     | λ, 2682 | >99   | 7     | 12    | 79      |
| λ, 874        | >99       | 12     | 17     | 47     | λ, 1258 | >99   | 14    | 19    | 62      |
| λ, 781        | >99       | 10     | 15     | 36     | λ, 1424 | >99   | 8     | 12    | 52      |
| k, 560        | >99       | 8      | 11     | 15     | k, 427  | >99   | 6     | 9     | 11      |
| k, 782        | >99       | 9      | 13     | 27     | k, 603  | >99   | 5     | 9     | 20      |
| k, 586        | >99       | 8      | 12     | 25     | k, 587  | >99   | 5     | 9     | 17      |
| 4             |           |        |        |        |         |       |       |       |         |
| λ, 882        | >99       | 12     | 15     | 30     | λ, 1793 | >99   | 11    | 17    | 67      |
| λ, 921        | >99       | 15     | 21     | 47     | λ, 939  | >99   | 12    | 19    | 54      |
| λ, 669        | >99       | 9      | 14     | 43     | λ, 972  | >99   | 10    | 15    | 48      |
| TTR, 481      | >99       | 9      | 11     | 11     | TTR, 359| >99   | 8     | 9     | 9       |
| TTR, 401      | >99       | 7      | 9      | 12     | TTR, 402| >99   | 6     | 8     | 10      |
| TTR, 470      | >99       | 7      | 11     | 16     | TTR, 284| >99   | 6     | 8     | 8       |
| 5             |           |        |        |        |         |       |       |       |         |
| λ, 350        | >99       | 4      | 6      | 10     | λ, 513  | >99   | 9     | 12    | 26      |
| λ, 196        | >99       | 5      | 6      | 8      | λ, 399  | >99   | 5     | 7     | 17      |
| λ, 117        | >99       | 6      | 8      | 10     | λ, 224  | >99   | 5     | 5     | 12      |
| k, 60         | >99       | 2      | 2      | 3      | k, 0    | ND    | 0     | 0     | 0       |
| k, 58         | >99       | 2      | 2      | 2      | k, 0    | ND    | 0     | 0     | 0       |
| k, 0          | >99       | 0      | 0      | 0      | k, 0    | ND    | 0     | 0     | 0       |
| 6             |           |        |        |        |         |       |       |       |         |
| λ, 287        | >99       | 5      | 6      | 14     | λ, 997  | >99   | 5     | 9     | 29      |
| λ, 282        | >99       | 6      | 7      | 12     | λ, 413  | >99   | 6     | 8     | 21      |
| λ, 334        | >99       | 5      | 5      | 14     | λ, 376  | >99   | 11    | 12    | 24      |
| TTR, 132      | >99       | 2      | 2      | 3      | TTR, 132| ND    | 0     | 0     | 0       |
| TTR, 30       | ND        | 0      | 0      | 0      | TTR, 60 | >99   | 2     | 2     | 2       |
| TTR, 0        | ND        | 0      | 0      | 0      | TTR, 35 | >99   | 2     | 2     | 2       |
| 7             |           |        |        |        |         |       |       |       |         |
Three technical replicates shown for untreated and decellularised tissue biopsies except for untreated samples for patient 1 and 2 due to lack of material. Proteins identified in each sample with Mascot score probabilities and Scaffold software results: P: Protein identification probability; EUP: Exclusive Unique Peptide count (number of unique peptides only with this protein); EUS: Exclusive Unique Spectrum count (number of unique spectra only associated with this protein); TS: Total Spectrum count (number of total spectra associated with this protein including those shared with other proteins). ND: not detected.

**Supplementary Table 2.** MS identification of amyloid signature proteins in cardiac and fat tissue specimen.

| Patient | Untreated | Decellularised |
|---------|-----------|---------------|
|         | ApoA4 | ApoE | SAMP | CLU | VTNC | ApoA4 | ApoE | SAMP | CLU | VTNC |
| 1       | 218   | 62   | 147  |     |      | 51    | 88   | 76   |     |      |
| 2       | 123   | 64   |      |     |      | 62    | 103  | 35   |     |      |
| 3       | 273   | 110  | 302  | 186 |      | 108   | 335  | 241  | 375 |      |
| 4       | 1314  | 260  | 304  | 1324| 1388| 209   | 428  | 467  |     |      |
| 5       | 1612  | 578  | 1408 | 737 | 874 | 215   | 427  | 1622 |     |      |
| 6       | 321   | 169  | 92   | 59  | 315 | 113   | 242  | 200  |     |      |
| 7       | 551   | 234  | 372  | 282 | 891 | 301   | 335  | 477  |     |      |
| 8       | 716   | 495  | 389  | 1214| 297 | 265   | 711  |     |     |      |
| 9       | 447   | 133  | 331  | 1006| 342 | 1457  | 514  | 853  |     |      |
| 10      | 478   | 275  | 143  | 195 | 167 | 406   | 171  | 89   |     |      |

Identification of proteins associated with the presence of amyloid [1, 2] are shown with Mascot score probabilities (average of three technical replicates except for untreated samples from patients 1 and 2).
Supplementary Table 3. MaxQuant quantification of the area of the identified peptides (LFQ) in fat aspirate specimen.

| Patient | Protein | Untreated | Decellularised |
|---------|---------|-----------|---------------|
|         |         | LFQ intensity | LFQ intensity |
| 4       | λ       | 3492900000 | 4010100000   |
|         | λ       | 3713800000 | 3124900000   |
|         | λ       | 3254400000 | 2729600000   |
|         | κ       | 1205800000 | 224700000    |
|         | κ       | 1343000000 | 322380000    |
|         | κ       | 1663700000 | 299510000    |
| 5       | λ       | 3568200000 | 3073900000   |
|         | λ       | 2793200000 | 3531600000   |
|         | λ       | 2228500000 | 2650500000   |
|         | TTR     | 197700000  | 1832800000   |
|         | TTR     | 150340000  | 1482200000   |
|         | TTR     | 126730000  | 1395600000   |
| 6       | λ       | 3050100000 | 2682900000   |
|         | λ       | 3356600000 | 2725600000   |
|         | λ       | 2810800000 | 2505400000   |
|         | κ       | 43558000   | 0             |
|         | κ       | 45316000   | 0             |
|         | κ       | 54000000   | 0             |
| 7       | λ       | 647330000  | 1049500000    |
|         | λ       | 942160000  | 944390000     |
|         | λ       | 857270000  | 53109000      |
|         | TTR     | 0          | 0             |
|         | TTR     | 0          | 0             |
|         | TTR     | 0          | 0             |
| 8       | λ       | 237050000  | 69546000      |
|         | λ       | 273100000  | 54688000      |
|         | λ       | 178810000  | 48899000      |
|         | κ       | 56321000   | 0             |
|         | κ       | 86285000   | 0             |
|         | κ       | 83693000   | 0             |
| 9       | λ       | 292170000  | 2938000000    |
|         | λ       | 363270000  | 3820500000    |
|         | λ       | 439300000  | 4053300000    |
|         | κ       | 270020000  | 28283000      |
|         | κ       | 261410000  | 38332000      |
|         | κ       | 259610000  | 37839000      |
| 10      | λ       | 863980000  | 11603000      |
|         | λ       | 905900000  | 5365700       |
|         | λ       | 623670000  | 12154000      |
|         | κ       | 35173000   | 212450        |
|         | κ       | 36192000   | 209520        |
|         | κ       | 71379000   | 0             |
Label free quantification (LFQ) intensity values by MaxQuant software (see Methods) are given for each identified protein in three technical replicates per sample. Means of LFQ intensity were used to calculate ratios of soluble versus fibrillar protein before and after decellularisation (see Figure 2B).
Supplementary Table 4. Retrospective clinical and pathologic review of the cases presented in the manuscript.

| Patient | Gender | Age (y) | Clinical Diagnosis | Organ involvement | Genetic sequencing |
|---------|--------|---------|--------------------|-------------------|-------------------|
| 1       | M      | 73      | AL                 | Cardiac, soft tissue |                  |
| 2       | M      | 65      | AL                 | Peripheral and autonomic neuropathy |                  |
| 3       | M      | 67      | ATTR               | Cardiac, peripheral and autonomic neuropathy | TTR T60A |
| 4       | F      | 77      | AL                 | Cardiac            |                  |
| 5       | M      | 53      | AL                 | Cardiac, soft tissue |                  |
| 6       | M      | 60      | AL                 | Cardiac, renal, liver and autonomic neuropathy |                  |
| 7       | F      | 50      | AL                 | Cardiac, renal and liver |                  |
| 8       | F      | 54      | AL                 | Cardiac, renal and soft tissue |                  |
| 9       | M      | 83      | ATTR               | Cardiac            | Wild type TTR     |
| 10      | M      | 60      | ATTR               | Cardiac, peripheral and autonomic neuropathy | TTR A97S |

Supplementary references

[1] A. Dogan, Amyloidosis: Insights from Proteomics, Annu. Rev. Pathol. 12 (2016).
[2] P. Mollee, S. Boros, D. Loo, J.E. Ruelcke, V.A. Lakis, K.L. Cao, P. Renaut, M.M. Hill, Implementation and evaluation of amyloidosis subtyping by laser-capture microdissection and tandem mass spectrometry, Clin. Proteomics 13 (2016) 30.