**Supplementary Digital Content File**

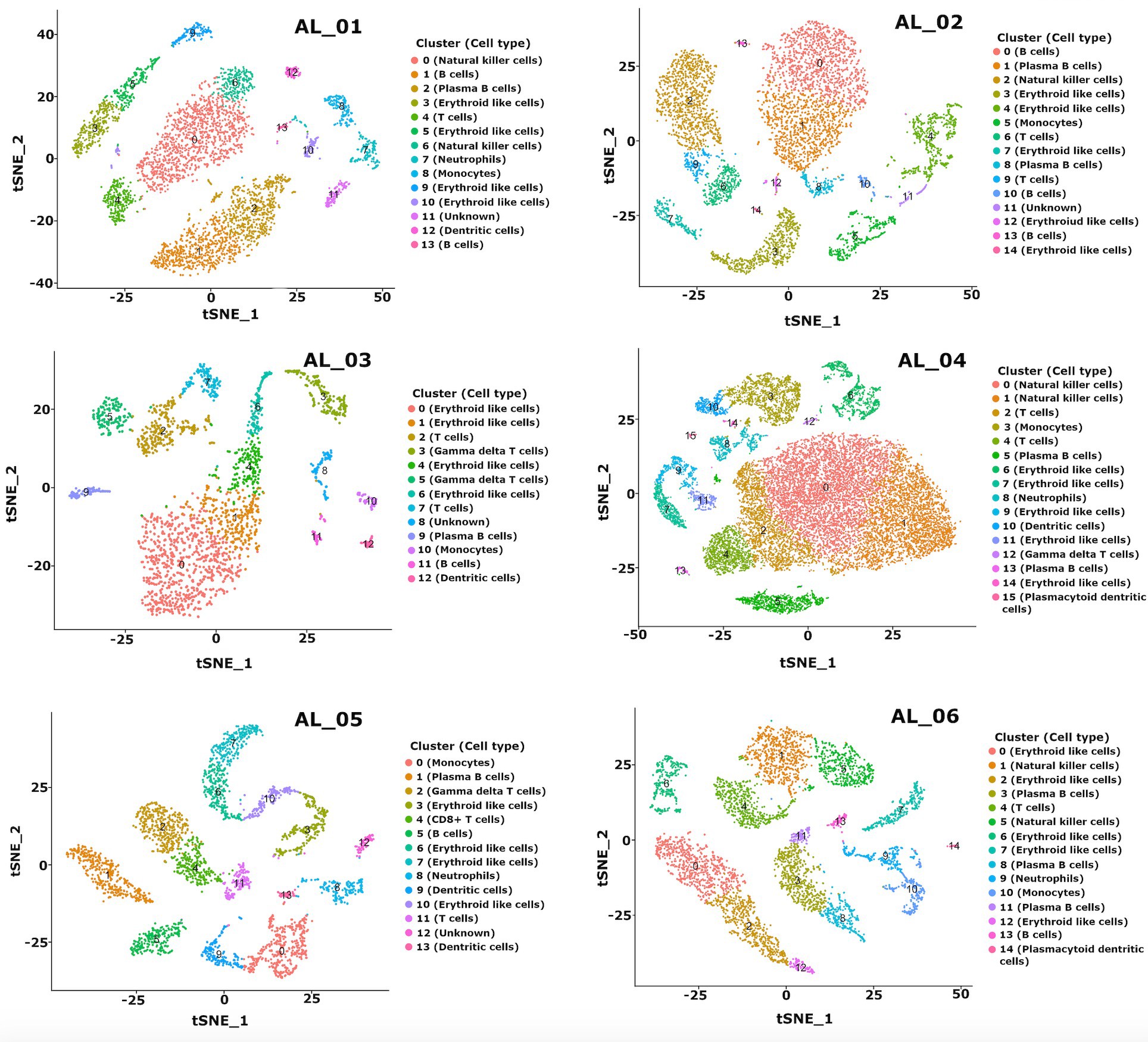
### **Manuscript Title:** The Peptide Drug Conjugate Melflufen Modulates the Unfolded Protein Response of Multiple Myeloma and Amyloidogenic Plasma Cells and Induces Cell Death

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Lehmann, Nina N. Nupponen, Sarah A. Holstein, and Caroline A. Heckman

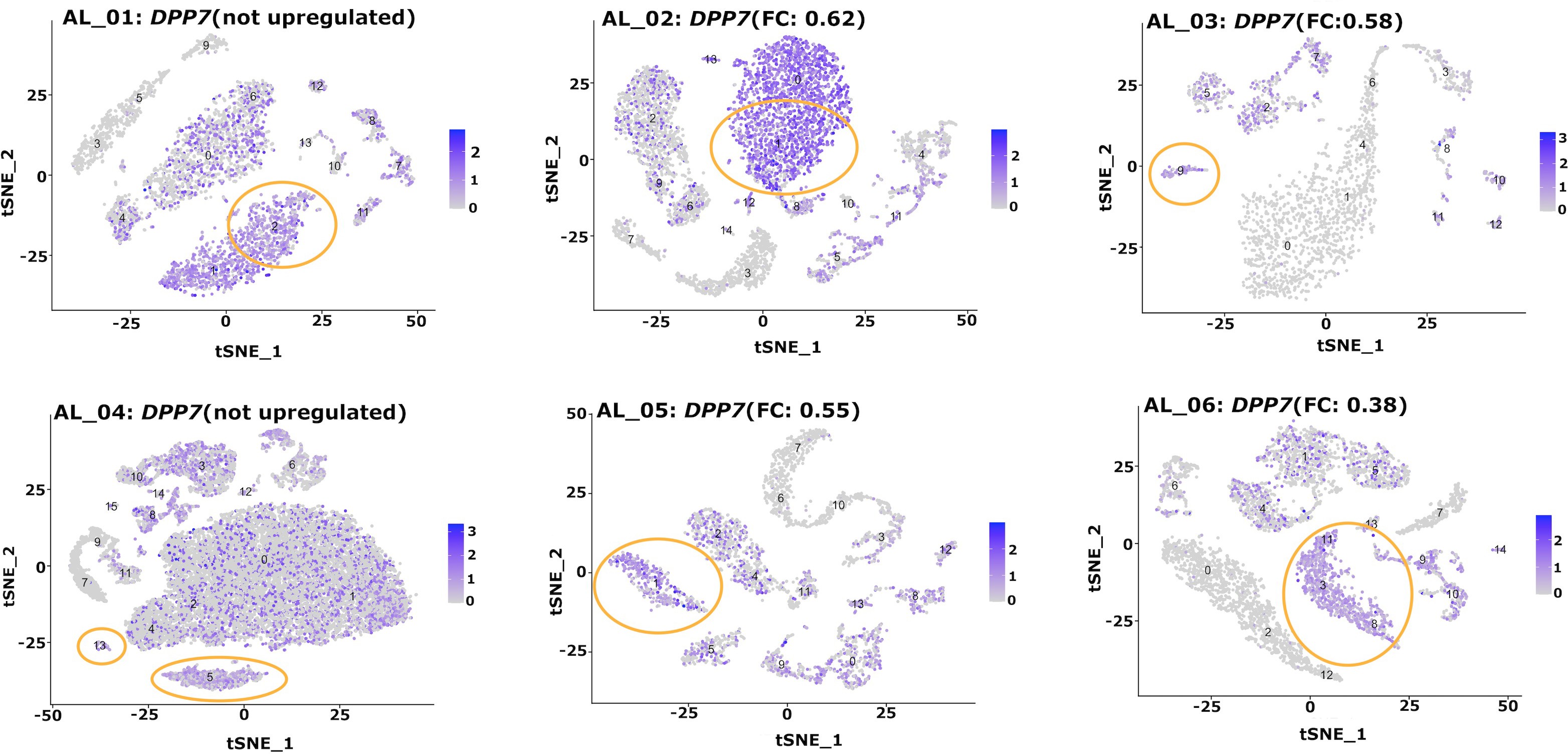
**Manuscript Citation:** *HemaSpher*e. 2022;6:e687. DOI: 10.1097/HS9.0000000000000687

**Supplementary Figure 1**



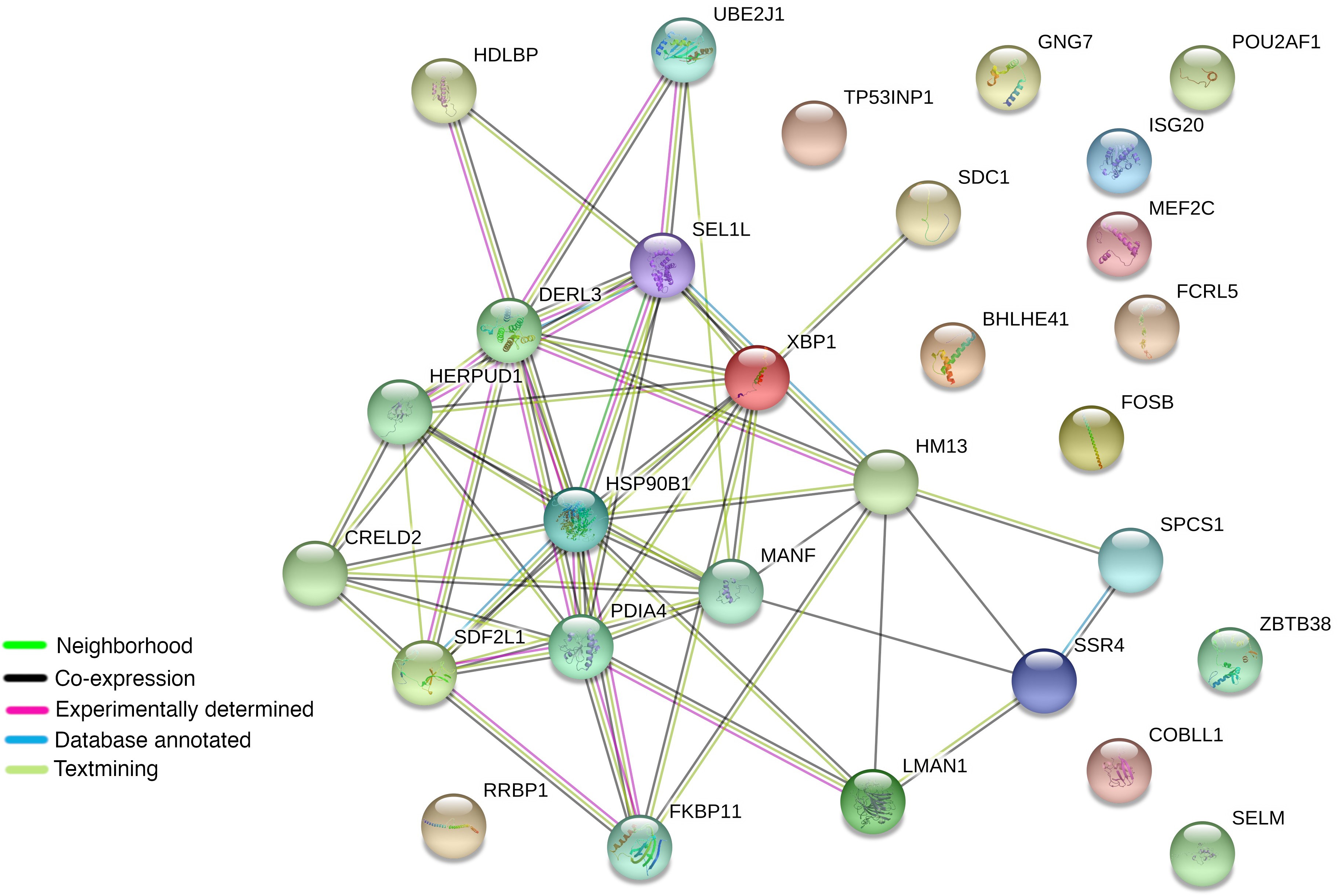
**Supplementary Figure 1**: Single cell RNA sequencing of BM-MNCs from amyloidosis patients revealing clusters of cells with identifiable plasma cell populations.

**Supplementary Figure 2**



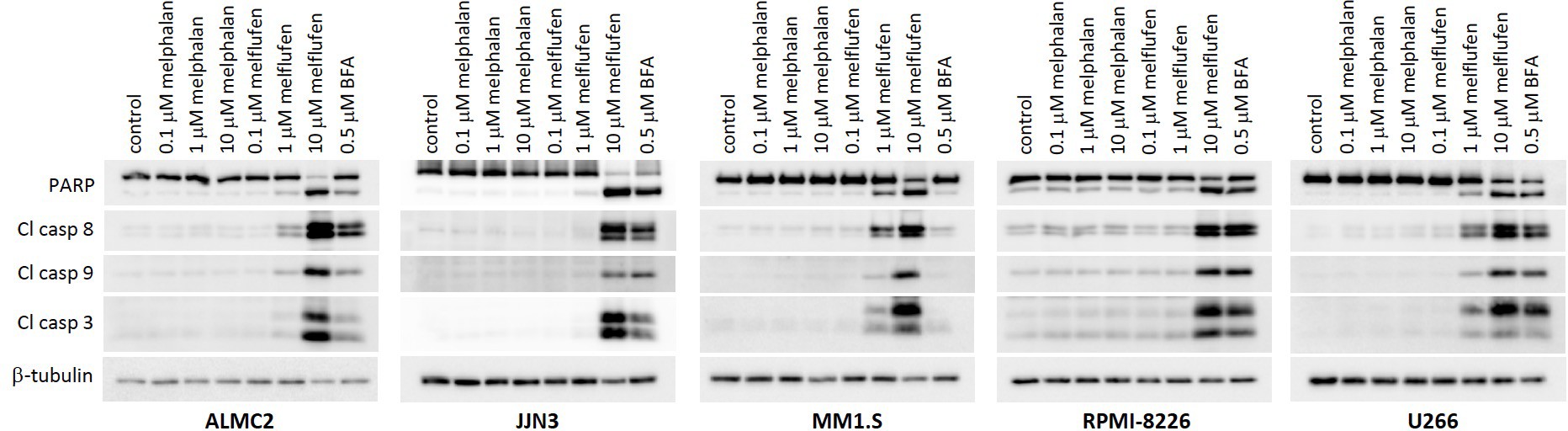
**Supplementary Figure 2**: Identification of over expression of *DPP7* in plasma cell clusters of 4 of the 6 samples of in plasma cell clusters. Sample AL\_01 and AL\_04 demonstrated no increased expression of *DPP7* in plasma cells compared to other cell clusters.

**Supplementary Figure 3**



**Supplementary Figure 3**: Protein-protein interaction (PPI) analysis from 30 genes found to be over expressed in plasma cell clusters by scRNAseq analysis revealed significant functional associations.

**Supplementary Figure 4**



**Supplementary Figure 4**: Melflufen mediates cellular events associated with apoptosis within 6 hours. The indicated cells were incubated in the presence of melphalan or melflufen (with Brefeldin A as a positive control) for 6 hours and cells were harvested and analyzed cleavage of PARP, or caspase 3, 8 and 9 as indicated, by immunoblot analysis, with β-tubulin as a loading control.

**Supplementary Table 1**

Characteristics of amyloidosis patients used for *ex vivo* analysis of melflufen and melphalan sensitivity.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample ID** | **Disease**  **status** | **Age** | **Gender** | **Light chain**  **type** | **FISH findings** |
| AL\_01 | Diagnosis | 61 | Female | lambda | t(11;14), Trisomy 11 and Del chr12 |
| AL\_02 | Diagnosis | 64 | Male | lambda | Del(13q) and Del(14q32) |
| AL\_03 | Diagnosis | 72 | Male | lambda | Del in the 14q32 region |
| AL\_04 | Diagnosis | 53 | Female | kappa | t(14;20) and Del(13q) |
| AL\_05 | Diagnosis | 81 | Male | lambda | Not checked |
| AL\_06 | Diagnosis | 63 | Female | kappa | Del(13q), Del(14q32), abnormal chr1 |

**Supplementary Table 2**

EC50 values (in uM) for melflufen and melphalan *ex vivo* sensitivity of plasma cells from amyloidosis patients.

|  |  |  |
| --- | --- | --- |
| **Sample ID** | **Melphalan**  **EC50** | **Melflufan**  **EC50** |
| AL\_01 | ~7842 | ~66364 |
| AL\_02 | NA | 19.12 |
| AL\_03 | 6886 | 22.55 |
| AL\_04 | ~11656 | 15.34 |
| AL\_05 | ~9897 | 0.1833 |
| AL\_06 | 9514 | 0.3601 |

**Supplementary Table 3**

EC50 values for melflufen and melphalan sensitivity of light chain producing human cell lines, JJN3, ALMC1 and ALMC2.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **ALMC-1** | **ALMC-2** | **JJN3** |
| Melphalan | ND | ND | 5.4 |
| Melflufen | 1.6 | 1.2 | 1.2 |

**Supplementary Table 4**

EC50 values for melflufen induction of apoptosis of plasma cells from cell lines. Melphalan treatment did not induce calculable EC50 values in any cell line tested.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **ALMC2** | **JJN3** | **MM.1S** | **RPMI 8226** | **U266** |
| EC50 (µM) | 0.77 | 1.84 | 2.14 | 1.54 | 2.5 |

**Supplementary Table 5**

PCR primers for CHOP quantification.

|  |  |
| --- | --- |
| **Gene** | **Primer sequence** |
| CHOP | F: TCTTCACCACTCTTGACCCTGCTT  R: GTTCTTTCTCCTTCATGCGCTGCT |
| β-actin | F: ACGTTGCTATCCAGGCTGTGCTAT  R: TAATGTCACGCACGATTTCCCGC |