Supplemental information

Identification of a drug-response gene
in multiple myeloma through longitudinal
single-cell transcriptome sequencing

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Table S1. The number and percentage of total or MM cells at every time point, related to Figure 2

|               | P1     | P3     | P4     | P5     |
|---------------|--------|--------|--------|--------|
| Total (cells) | 66299  | 204582 | 224991 | 1203299|
| MM (cells)    | 1288   | 5182   | 2567   | 4818   |
| MM (%)        | 1.94   | 2.53   | 1.14   | 0.40   |
Table S2. The number of cell types in each cluster, related to Figure 2

| Cluster | 0  | 1  | 2  | 3  | 4  |
|---------|----|----|----|----|----|
| B cell  | 1785 | 751 | 324 | 78  | 35  |
| Pre-B cell | 0   | 1   | 0   | 0   | 1   |
| Pro-B cell | 1   | 0   | 0   | 0   | 0   |
| T cell  | 0   | 0   | 0   | 1   | 0   |
| NK cell | 2   | 0   | 0   | 0   | 0   |
| GMP\(^a\) | 1   | 0   | 0   | 0   | 0   |
| MEP\(^b\) | 2   | 0   | 0   | 0   | 0   |

\(^a\)GMP: granulocyte-monocyte progenitor; \(^b\)MEP: megakaryocyte-erythrocyte progenitor.
Table S3. Top20 DEGs in cluster 0 when comparing P1 and P5, related to Figure 3

| Genes     | P value     | Average log₂ fold change | P1 (%) | P5 (%) | Adjusted P value |
|-----------|-------------|--------------------------|--------|--------|------------------|
| CHASERR   | 7.91E-131   | -3.002259                | 0.0    | 99.4   | 1.20E-126        |
| GOLM2     | 2.29E-117   | -2.060659                | 0.0    | 93.2   | 3.48E-113        |
| JTB       | 9.26E-112   | -1.234871                | 98.7   | 100.0  | 1.41E-107        |
| BPNT2     | 5.40E-111   | -3.210023                | 0.0    | 90.0   | 8.19E-107        |
| ARHGEF2   | 9.21E-105   | -2.096255                | 65.4   | 99.0   | 1.40E-105        |
| FTL       | 6.62E-105   | -1.127746                | 99.7   | 100.0  | 1.01E-100        |
| MT-ND4    | 5.06E-103   | -0.724104                | 100.0  | 100.0  | 7.67E-99         |
| SRGN      | 5.74E-103   | -1.275119                | 98.7   | 100.0  | 8.71E-99         |
| IRAG2     | 7.23E-102   | -1.969907                | 0.0    | 85.4   | 1.10E-97         |
| JUNB      | 1.22E-101   | -2.482860                | 32.2   | 97.9   | 1.85E-97         |
| COMMD3    | 2.62E-97    | -1.810259                | 62.1   | 98.8   | 3.97E-93         |
| GNAS      | 7.43E-97    | -0.849370                | 100.0  | 100.0  | 1.13E-92         |
| JCHAIN    | 7.57E-93    | -1.349773                | 99.7   | 100.0  | 1.15E-88         |
| LILRB4    | 1.56E-90    | -2.002945                | 6.3    | 0.83   | 2.37E-86         |
| MT-CO1    | 2.91E-90    | -0.757605                | 100.0  | 100.0  | 4.42E-86         |
| GAPDH     | 1.08E-85    | -1.093327                | 84.4   | 98.8   | 1.64E-81         |
| RPS14     | 1.31E-82    | -0.971777                | 100.0  | 100.0  | 1.99E-78         |
| AZGP1     | 5.26E-81    | -1.588882                | 45.2   | 94.7   | 7.98E-77         |
| MTATP6P1  | 1.90E-80    | -0.701332                | 100.0  | 100.0  | 2.88E-76         |
| IER2      | 2.45E-80    | -1.701222                | 18.9   | 93.2   | 3.72E-76         |

*The Candidate genes reported as PI-resistant genes (Mosca et al., 2013; Fan et al., 2017) are highlighted in red.
Table S4. Top 20 DEGs in cluster 3, related to Figure 4

| Genes  | P value | Average log₂ fold change | Cluster 3 (%) | Others (%) | Adjusted P value |
|--------|---------|--------------------------|---------------|------------|-----------------|
| ELF3   | 1.58E-113 | 1.562214                | 39.2          | 1.2        | 2.39E-109       |
| PDE6G  | 4.44E-85  | 0.827875                | 34.2          | 1.3        | 6.74E-81        |
| BAMBI  | 5.72E-81  | 2.690407                | 94.9          | 18.6       | 8.67E-77        |
| EREG   | 5.79E-76  | 1.517543                | 32.9          | 1.5        | 8.79E-72        |
| PELI2  | 9.55E-61  | 1.707534                | 74.7          | 12.6       | 1.45E-56        |
| C2     | 6.12E-46  | 1.369921                | 65.8          | 12.0       | 9.28E-42        |
| WFDC2  | 7.10E-38  | 1.252782                | 81.0          | 25.1       | 1.08E-33        |
| SERPINE2 | 3.45E-26 | 1.315427                | 51.9          | 12.2       | 5.24E-22        |
| TMED10 | 1.04E-24  | 0.806174                | 100.0         | 97.4       | 1.58E-20        |
| KLF2   | 5.20E-24  | 0.90756                 | 100.0         | 99.4       | 7.90E-20        |
| PHLDA1 | 5.74E-22  | 1.791656                | 57.0          | 18.2       | 8.71E-18        |
| FCRL2  | 2.10E-21  | 0.862569                | 94.9          | 51.9       | 3.19E-17        |
| VCP    | 2.81E-21  | -0.91888                | 89.9          | 98.4       | 4.27E-17        |
| JCHAIN | 8.01E-21  | 0.600871                | 100.0         | 99.9       | 1.22E-16        |
| ARRD3C | 1.16E-20  | 0.721227                | 100.0         | 96.0       | 1.75E-16        |
| EPHX2  | 1.99E-20  | 1.259161                | 64.6          | 24.6       | 3.01E-16        |
| ARNT   | 2.12E-20  | 1.332001                | 78.5          | 35.4       | 3.22E-16        |
| KLF6   | 1.09E-19  | 1.062859                | 100.0         | 90.7       | 1.66E-15        |
| USP53  | 3.20E-19  | 0.922525                | 87.3          | 47.6       | 4.85E-15        |
| ZSCAN18| 4.40E-19  | 0.73587                 | 51.9          | 15.9       | 6.68E-15        |
Figure S1. Quality control using Seurat R package, related to Figure 2
Plots of quality control metrics which include the number of unique molecular identifiers (UMIs) (left panel), number of genes (middle panel), and percentage of transcripts mapping to the mitochondrial genome (right panel). Red horizontal lines in middle and right panels indicate the cut-off values.
Figure S2. Cell cycle scoring and regression, related to Figure 2

(A) UMAP plot depicting 2982 single bone marrow MM cells obtained at diagnosis and each relapse before regression by Seurat's cell cycle scoring. Each cluster is represented by a specific color and number. The dotted red circle indicates a cluster 3', which is composed of satellite cells with increased expression of G2/M/S phase markers. (B) Pie chart showing the proportions of predicted cell cycle phases (G1, G2/M, or S) across clusters by Seurat’s cell cycle scoring. In cluster 3', cell cycle scoring analysis shows particular bias toward a cell cycle-specific phase. (C) Bar charts showing the statistically significant enriched Reactome pathways in the list of DEGs in cluster 3'.
Figure S3. Cell type annotation, related to Figure 2
Overview of cell types identified. UMAP plot depicting MM cells obtained at diagnosis and each relapse. Each cluster is represented by a specific color and number (left panel). UMAP plot of MM cells colored by their seven cell types (right panel). GMP: granulocyte-monocyte progenitor; MEP: megakaryocyte-erythrocyte progenitor.
Figure S4. Cell composition dynamics at every time point, related to Figure 3
The colors represent the clusters identified. The x axis: fraction of clusters; y axis: sampling points.
Figure S5. Reactome pathways associated with DEGs in cluster 0 when comparing P1 and P5, related to Figure 3

Bar charts showing the significantly enriched Reactome pathways in the list of differentially expressed genes (DEGs) in cluster 0, when comparing P1 and P5.
Figure S6. Expression of *CD38* at every time point, related to Figure 3
Red dots indicate cells with expression level of *CD38* being higher than the cut-off value.
Figure S7. UMAP plots of Cells expressing PELI2, ELF3, and EREG, related to Figure 4
Uppermost column: UMAP plots from Figure 3A. Three lower columns: cells expressing of PELI2, ELF3, and EREG. Red dots indicate cells with expression levels being higher than the cut-off values.
Figure S8. Cell proliferation assay, related to Figure 5
The KMS-34 cells transfected with PEL12, ELF3, or EREG or mock-transfected cells were seeded (5 × 10^3 cells/well) in 96-well plates. Cell proliferation was measured at 24 h intervals up to 72 h. Data are expressed as mean values ± S.E.M of three independent experiments.
Figure S9. Expression of *PELI2* mRNA in MM patients in the database, related to Figure 6

Stacked area chart shows *PELI2* expression across MM patients (n = 2119) in MMRF-CoMMpass database which is generated using the UCSC Xena web-based tool.
Figure S10. Kaplan-Meier survival analysis of patients treated with dexamethasone monotherapy, related to Figure 6
Kaplan-Meier plot for patients with high and low *PELI2* expression. The MM patients treated with dexamethasone monotherapy (n = 76) are divided into two groups, according to the median expression level. The log-rank test is used for statistical significance.