

Supplementary Figure 1: Principle and quantification of the V-well adhesion assay of fluorescently labeled myeloma cells adapted by Weetall et al. 2001. A: Sample: Subsequent rounds of centrifugation and removal of cell pellet yielded the size of adhesive subpopulations. Fluorescently stained INA-6 cells were added to an hMSC monolayer. Non-adherent INA-6 cells (V2) were pelleted in the well-tip. Pellets were quantified by fluorescence brightness and isolated by pipetting. Immobile INA-6 cells (V3) were manually detached by forceful pipetting. Reference: Omitting adhesive hMSC-layer yielded ~100% non-adherent cells (V1) after the first centrifugation step; Background: hMSC monolayer was used as background signal. B: Calculation of the population size relative to total cells starting with pellet intensity. The shown example is the pellet gained by centrifuging mobile subpopulation (V2) after 1 h of co-culture. (see Fig. 2 for context): Intensity values from pellet images were summarized. After subtracting the unlabeled hMSC signal and normalization by a full-size pellet (reference), the resulting values represented the fraction of the adhesive subpopulation. C: One of three biological replicates summarized in Fig. 2. Line range shows the standard deviation of four technical replicates. Non.Adh. Rem.: Fluorescence signal after removal of V2. D: Example images of myeloma cell lines (INA-6, U266, MM.1S) pelleted in the tip of V-wells. The leftmost image shows the recorded area in a complete V-well. Scale bar = 200 µm. E: Results from (D) comparing adhesion strength of three myeloma cell lines to hMSC. Error bars represent technical deviation. MM=Multiple Myeloma.