

mRNA expression of CRBN and IKZF1 was analyzed in human myeloma cell lines (RPMI-8226, H929, MM.1S) and human CD34⁺ cells #1 and 2 by RT-PCR.



CD34⁺ cells were treated with either DMSO (0.01%), LEN (1 μ M) or POM (1 μ M) for 1d. Then wash them and culture only with culture medium, which were changed every 2 days. Cells lysates were analyzed for IKZF1 and CRBN expression by western blotting.



CD34⁺ cells were treated with DMSO (0.01%) or POM (1 μ M) for 4H, 8H or 24H and used for a colony formation assay to evaluate BFU-E colony formation. After 14 d, the colony numbers were counted under Leica microscope (25X).



CD34⁺ cells were transducted by lentivirus with control shRNA (shCNTL), CRBN-shRNA #1 (shCRBN #1) or CRBN-shRNA #3 (shCRBN #3). Transfected cells were sorted by GFP positive after 3 days of transduction. Cell lysates were analyzed by western blotting to compare the levels of CRBN. β -actin was used as control.



CD34⁺ cells were cultured with DMSO (0.01%), LEN (1µM) or POM (1µM) for 24h , and then cytoplasm and nuclear fractions were extracted separately . IKZF1, MEK $\frac{1}{2}$ and Histone H3 levels were compared by western blot.



Mice CD117⁺ cells were cultured with POM at the indicated concentrations for 24h. Cells lysates were analyzed for IKZF1 expression by western blotting.