Figure S1. VL30 retrotransposition in isolated HC11 clones. Cells from either control HC11 or isolated single hygromycin B-resistant clones were flow cytometrically analyzed for EGFP-positivity. (A) Overlaid green circumscribed-solid blue and yellow histogram profiles represent fluorescence of control HC11 and HC11 cl.11 cells, respectively. M1 and M2 gates correspond to arbitrarily threshold settings for control auto-fluorescence. Percentage values shown inside the histogram panel of HC11 cl.11 cells, subtracted by 0.4% (false positive at M2), represents the net frequency of EGFP-positive cells as the mean ± SE of duplicate samples from three independent experiments. (B) Columns with SE indicated with bars ± SE, represent retrotransposition frequencies of isolated clones measured as in (A). Percentage of retrotransposition values shown, are the mean value of duplicate samples from three independent experiments. (C) 4 μl DNA lysates from hygromycin B-resistant clones were analyzed by PCR and their products separated in a 1.2% agarose gel containing ethidium bromide. Lanes C1 and C2 are control reactions either with pEGFP-N1 (absence of γ-globin intron) or pNVL-3*/EGFP-INT (contains the γ-globin intron, unable to be removed by splicing from the EGFP retrotransposition cassette), as positive substrates for the intron-less 342 bp and 1243 bp EGFP-PCR products of retrotransposition-positive clones. Lane N.T., non-transfected HC11 cells; Lanes cl.11, cl.12, cl.5 and cl.17, PCR analyses of respective clone DNA lysates; Lane M, 100 bp molecular mass-size markers; n.s., not significant.

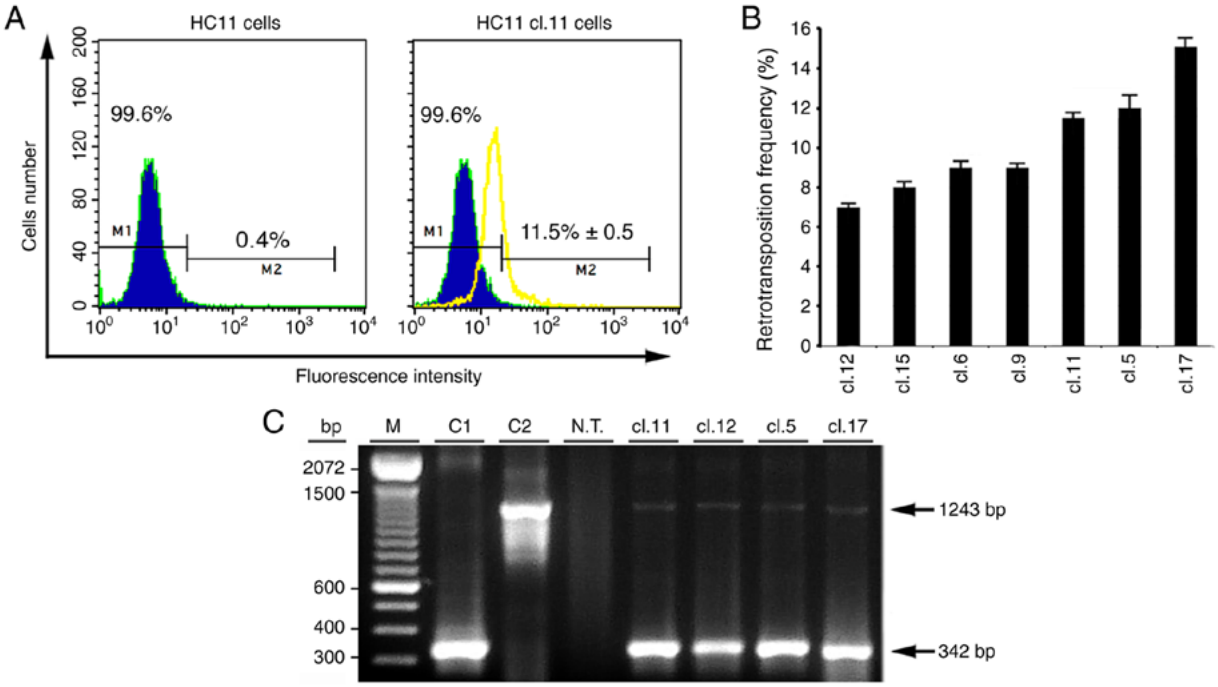

Figure S2. Flow cytometric analysis of retrotransposition-positive HC11 cl.11 cells. Control HC11 or HC11 cl.11 cells were analyzed by using flow cytometry for EGFP expression. Dot plots are presented for each sample. UL and UR contain retrotransposition-negative and retrotransposition-positive cells, respectively; 11.48% value in HC11 cl.11 data represents the net retrotransposition frequency, subtracted by a 0.44% of false-positive cells.

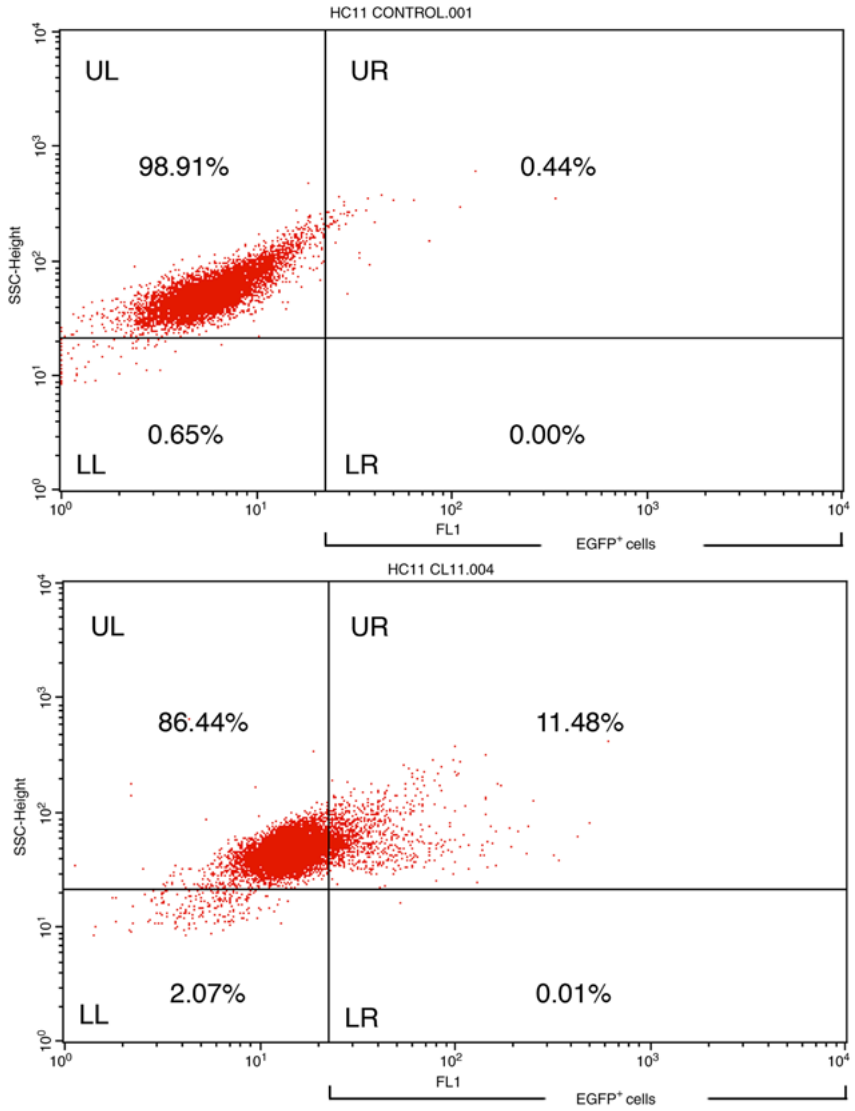

Figure S3. Induced epithelial-mesenchymal transition and multinucleation in VL30 retrotransposition-positive HC11 cells. (A) Normal HC11 and (B) retrotransposition-positive HC11 cl.11 cells in normal culture dishes; magnification, x20. HC11 cl.11 cells cultured on glass coverslips were fixed with 3.7% paraformaldehyde and images were captured under normal (C) or UV light (D); magnification, x40. White arrow (C) indicates a large multinucleated cell overlaid with EGFP retrotransposition-positive cells (D).

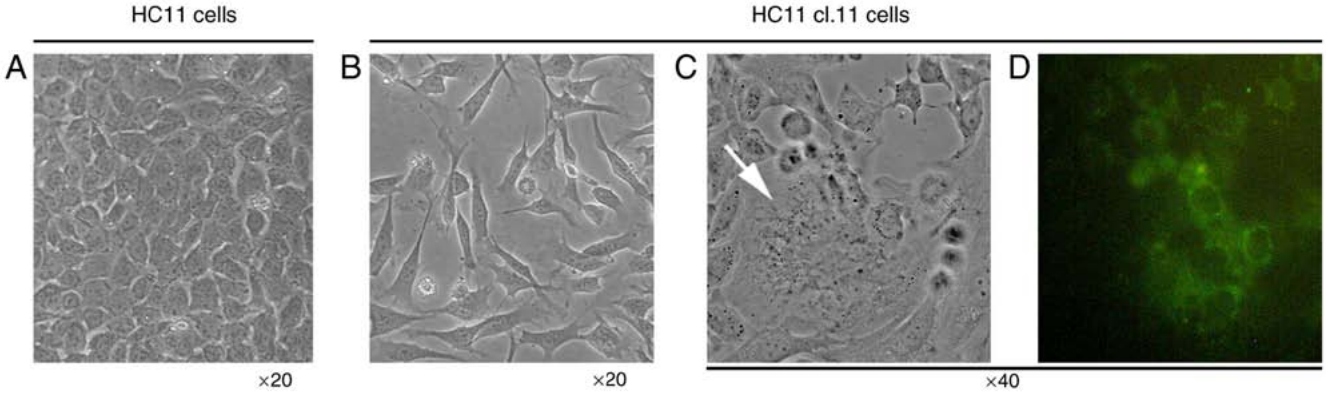

Figure S4. Tinzaparin inhibits serum starved- and VEGF-induced VL30 retrotransposition of mouse NIH3T3 fibroblast cells. Pcl.10 cells, harboring the recombinant plasmid NVL-3*/EGFP-INT, were treated as indicated. Results are presented as the mean ± SD of duplicate samples from three independent experiments. **P<0.01 and ***P<0.001 (one-way ANOVA followed by Tukey's test).