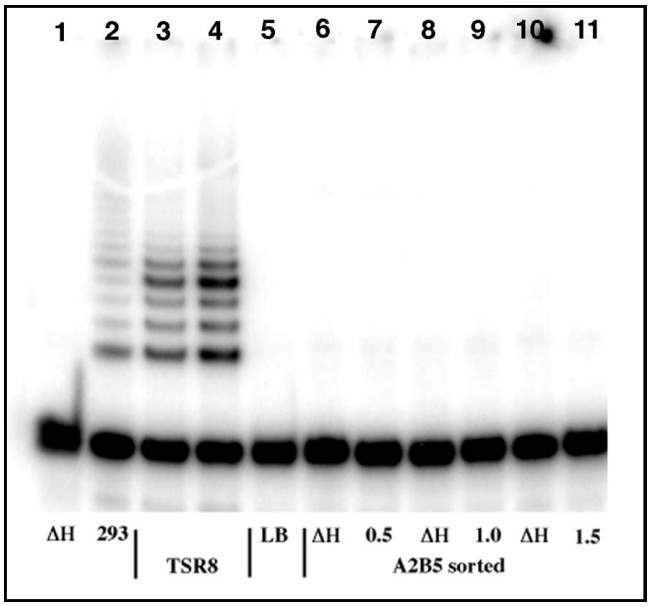
1 2 3 4 5 6 7 8 9 10 11
ΔH
293
TSR8
LB
ΔH
0.5
ΔH
1.0
ΔH
1.5
A2B5 sorted

Supplemental Fig. 1

WMPCs lack telomerase activity

Second passage spheres maintained in culture for three months were assessed for their telomerase activity using the TRAP assay. Lane 2 shows telomerase activity by 293 cells used as a positive control. Lanes 7, 9 and 11 show the absence of telomerase activity by matched WMPCs. Lanes 1, 6, 8 and 10 are heat inactivated controls of each sample. Lanes 3 and 4 are positive primer template controls, at concentrations of 0.1 M and 0.3 M.

Methods: Telomerase activity was determined using the TRAP assay[23,24]. Cells were solubilized in 100 (l of CHAPS lysis buffer, and 500 ng of protein assayed. For controls, cell lysates were preincubated at 85(C for 10 min to inactivate telomerase. The samples were added to a mixture of TRAP buffer, dNTP, Taq polymerase (Promega), $^{32}$P-end-labeled TS primer (5'–AATCCGTCGAGCAGAGTT-3′), reverse primer (Intergen, Gaithersburg, MD), and T4 gene-32 protein (Boehringer Mannheim, Indianapolis). To test assay specificity, 0.5 (g/50 (l DNase-free RNase A (Promega), heat-inactivated at 65$^{o}$C for 20 min, was added to the reaction mixture. After a 30 min incubation at 30(C for telomerase-mediated extension of TS primer, the samples were amplified by PCR, and the products run on a 12.5% non-denaturing PAGE gel, then autoradiographed.