Supplementary Protocol 1.

Generation of hESC derived fibroblast-like cell (hdF) cultures:

Stewart et al. *Nature Methods*

1. hESC cultures that were composed of approximately 70% fibroblast-like cells (See Supplemental Figure 1 for culture morphologies) were treated for 20min with collagenase IV.
2. Collagenase IV was removed and wells were rinsed vigorously with KO-DMEM to collect the fibroblast-like cells.
   a. Fibroblast-like cells should peel off in a sheet leaving the hESC colonies still attached to the dish.
3. Supernatant containing the cells was centrifuged for 5min @ 250g, aspirated and the cell pellet resuspended in fresh MEFCM+8ng/ml and transferred to fresh matrigel coated wells.
4. The media was changed every other day and hdFs were passaged when confluent in the same manner as for hESCs.
5. If colonies are observed to arise in the hdF cultures, manually remove the colonies.
6. hdF cultures can be cultured for 8-10 passages before senesce occurs.
7. Freeze hdFs in same manner as for hESCs.

Illustrative overview of hdF culture generation