SUPPLEMENTARY DATA FOR:

Sprouty genes regulate proliferation and survival of human embryonic stem cells

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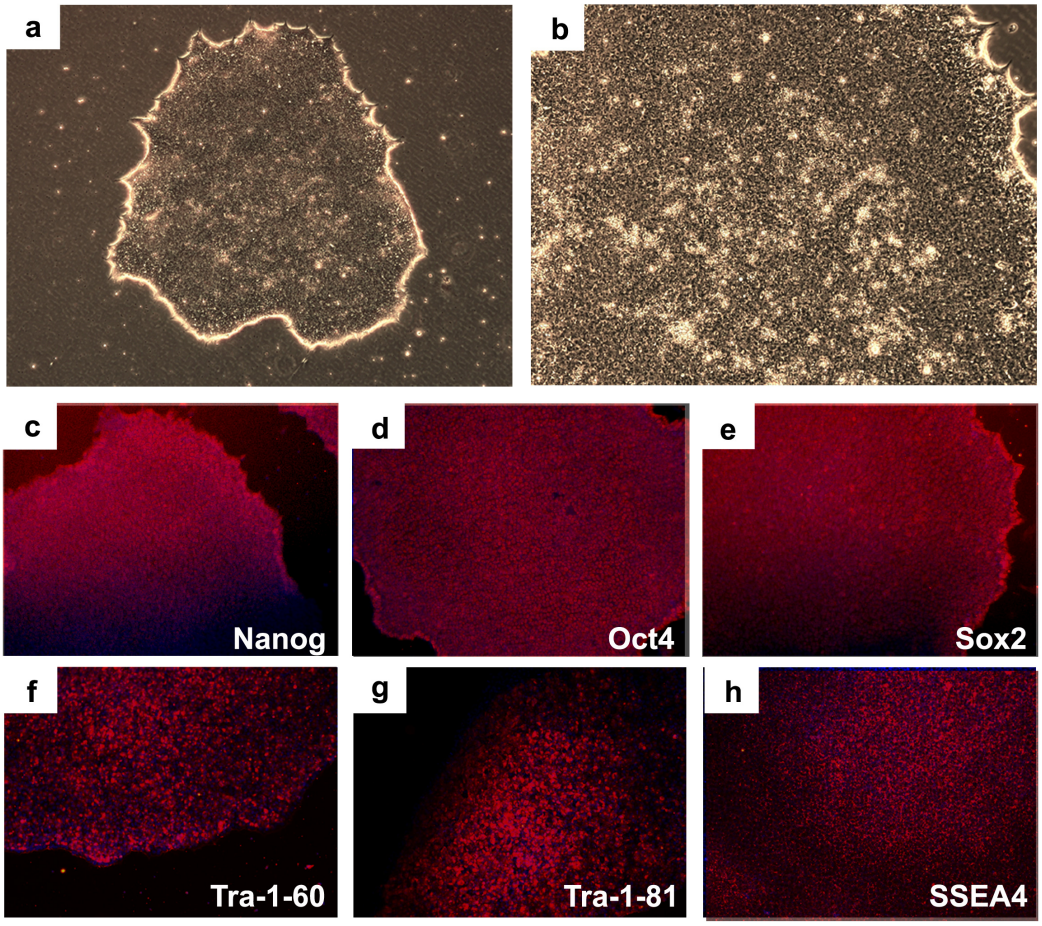
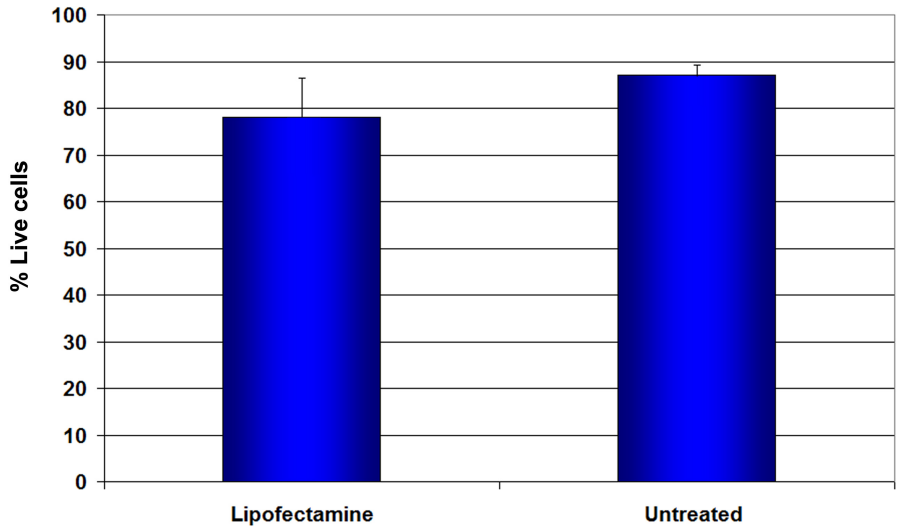
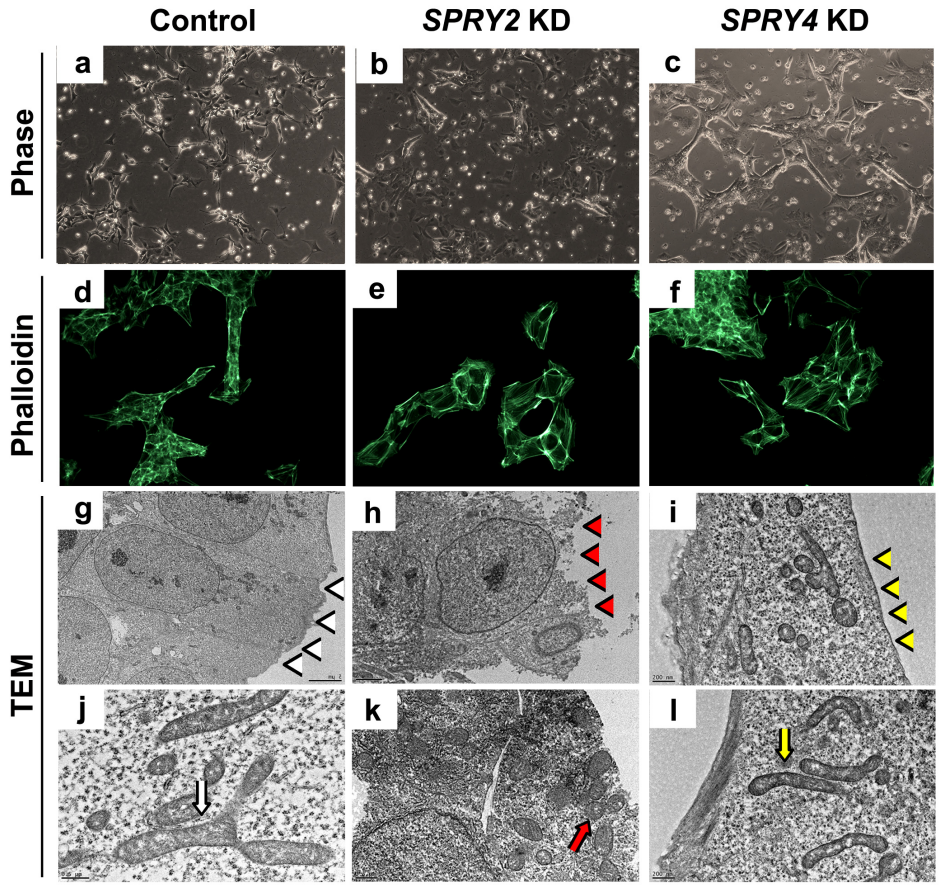


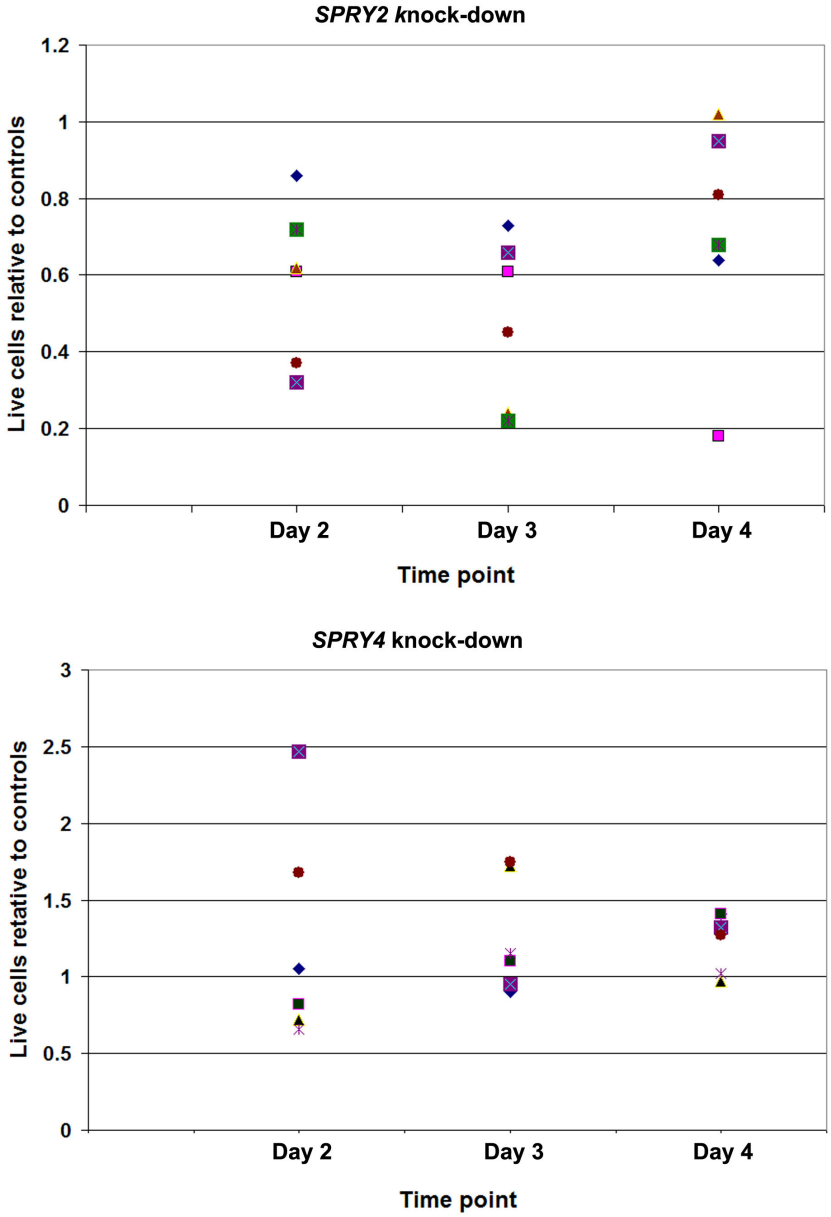
Supplementary Figure 1: Colony characteristics of the UCSF4 hESC. The UCSF4 hESC line was grown feeder free and exhibited typical colony morphology of hESCs (a, b). These cells also expressed common markers for hESCs, as stained by immunocytochemistry and observed by fluorescent microscope: Nanog (c), Oct4 (d), Sox2 (e), Tra-1-60 (f), Tra-1-81 (g), SSEA4 (h). Magnification: a, c-h, 100X; b, 200X.



Supplementary Figure 2: Percentage of live cells with and without lipofectamine. To assess the level of lipofectamine toxicity, the percentage of live cells was measured by flow cytometry 24h after adding lipofectamine to cells (without siRNA), and compared with untreated controls.



Supplementary Figure 3: Morphological analysis of cells after knock-down (KD). (a-c) Phase contrast of control, *SPRY2* KD, and *SPRY4* KD cells. (d-f) Actin cytoskeleton staining using phalloidin. (g-i) Transmission electron microscopy (TEM) showed that mitochondria in controls are large and elongated (g, j, black arrow); mitochondria in all *SPRY4* KD (l) cells were comparable to controls (yellow arrow), whereas in *SPRY2* KD cells, while most surviving cells were comparable to controls, in some cells (k) mitochondria were small and round (yellow arrow). Some *SPRY2* KD cells also showed membrane damage (h, red arrowheads), whereas in all *SPRY4* KD cells (yellow arrowheads) and controls (white arrowheads), cell membranes were intact.



Supplementary Figure 4: Percentage of live cells after knock-down. Knock-down experiments were performed and the percentage of live cells was measured at three time points afterwards for both SPRY2 (top) and SPRY4 (bottom). After SPRY2 knock-down, the percentage of live cells was lower at Day 2 and increased progressively afterwards, while in SPRY4 the percentage of live cells was higher at all three time points.