**SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Figure S1: Comparison of different methods previously used to test the CD271-based cancer stem cell model in human melanoma.**

**Supplementary Figure S2: Clinico-pathological characteristics of patients who donated melanomas.**

**Supplementary Figure S3: Typical sorting strategy used to isolate viable melanoma cells from cell suspensions derived by dissociation from patient tumors.** After tumor dissociation (see Methods), cells were labelled with antibodies against human-HLA (for detecting human cells), CD45 (for detecting leucocytes), CD31 (for detecting endothelial cells), CD235a (glycophorin A; for detecting immature red blood cells) and 4',6-diamidino-2-phenylindole (DAPI; for detecting non-viable cells). To identify melanoma cells, a cell morphology gate was first applied, based on side-scatter area (SSC-A) and forward-scatter area (FSC-A) characteristics. Next, single cells were selected within the morphology gate by gating on side-scatter width (SSC-W) vs side-scatter height (SSC-H) and forward-scatter width (FSC-W) vs forward-scatter height (FSC-H), characteristics. Viable cells were then gated by virtue of DAPI exclusion, which is often only partial in melanoma cells compared to hematopoietic cells (gate on middle left dot plot includes events with low DAPI signal and excludes DAPIhi cells, which are non-viable, and DAPI- cells, which are mostly hematopoietic in origin (not shown)). In the lower plot, gated viable cells show heterogeneous expression for HLA and lineage (Lin; CD45/CD31/CD235a) markers. Melanoma cells were highly enriched (>95% (9)) in the Lin- gate. For sorting human melanoma cells from tumors grown in immunocompromised mice, the same general gating strategy was employed, except that lineage markers were mouse-specific.

**Supplementary Figure S4: Melanoma initiating cell frequency.** Individual tumorigenesis data (summarized in Figure 3b) from the subcutaneous transplantation into NSG mice of the indicated number of CD271-, CD271+ and Lin- cells dissociated side-by-side, according to published methods (Q, C and B), from seven patient melanomas (12-042, 12-272, 12-316, 12-1036, 12-1254,13-444, 13-1061). Tumorigenic cell frequencies were calculated using limit dilution analysis (15). Lines indicate average values.

**Supplementary Figure S5: Variable re-expression patterns in PDX tumors generated from CD271- and CD271+ cells.** Flow cytometrically purified CD271- and CD271+ cells from patient melanoma 12-1254, dissociated according to Q or C methods, were injected into NSG mice to form PDX tumors, which were then analyzed for CD271 expression. Top plots show CD271 expression in patient tumors at left and reanalyses of sorted cells at right. Bottom plots show CD271 expression in secondary PDX tumors (ST8, ST9, etc.) derived from injections of the indicated numbers of cells taken from the same pools of purified CD271+ (black plots) or CD271- (grey plots) cells.

**Supplementary Figure S6: Marker expression patterns in PDX tumors grown from CD271- and CD271+ cells.** Flow cytometrically purified CD271- and CD271+ cells from patient melanoma 12-1254 were injected into NSG mice to form secondary PDX tumors. These tumors were analyzed by immuno- histochemistry for expression of ALDH **(a)**, CD133 **(b)**, ABCB5 **(c)**, and JARID1B **(d)**. Percentages of positive cells are indicated in the top right corner. Positive staining indicated by arrows.

**Supplementary Figure S7: Genomic copy number differences among sibling PDX melanomas.** T-statistic analyses indicating genomic regions of copy number difference among pairs of secondary sibling PDX tumors (ST6/7, ST10/11, ST12/13; see Figure 5a). Double headed arrows indicate chr 8 in ST11 and ST10 tumors. Grey regions in heat maps indicate regions of difference. Tumors in each pair were grown in the same experiments from injections of cells that were obtained from the same patient melanomas and that had the same CD271 phenotype.

**Supplementary Figure S8: Genomic copy number differences among CD271- and CD271+ cells from PDX melanomas.** T-statistic analyses indicating genomic regions of copy number difference between CD271- and CD271+ cells in four PDX melanomas (404, 12-736, 12-1036, 12-1254). Double headed arrows indicate chr 12 of melanoma 12-1254. Grey regions in heat maps indicate regions of difference.