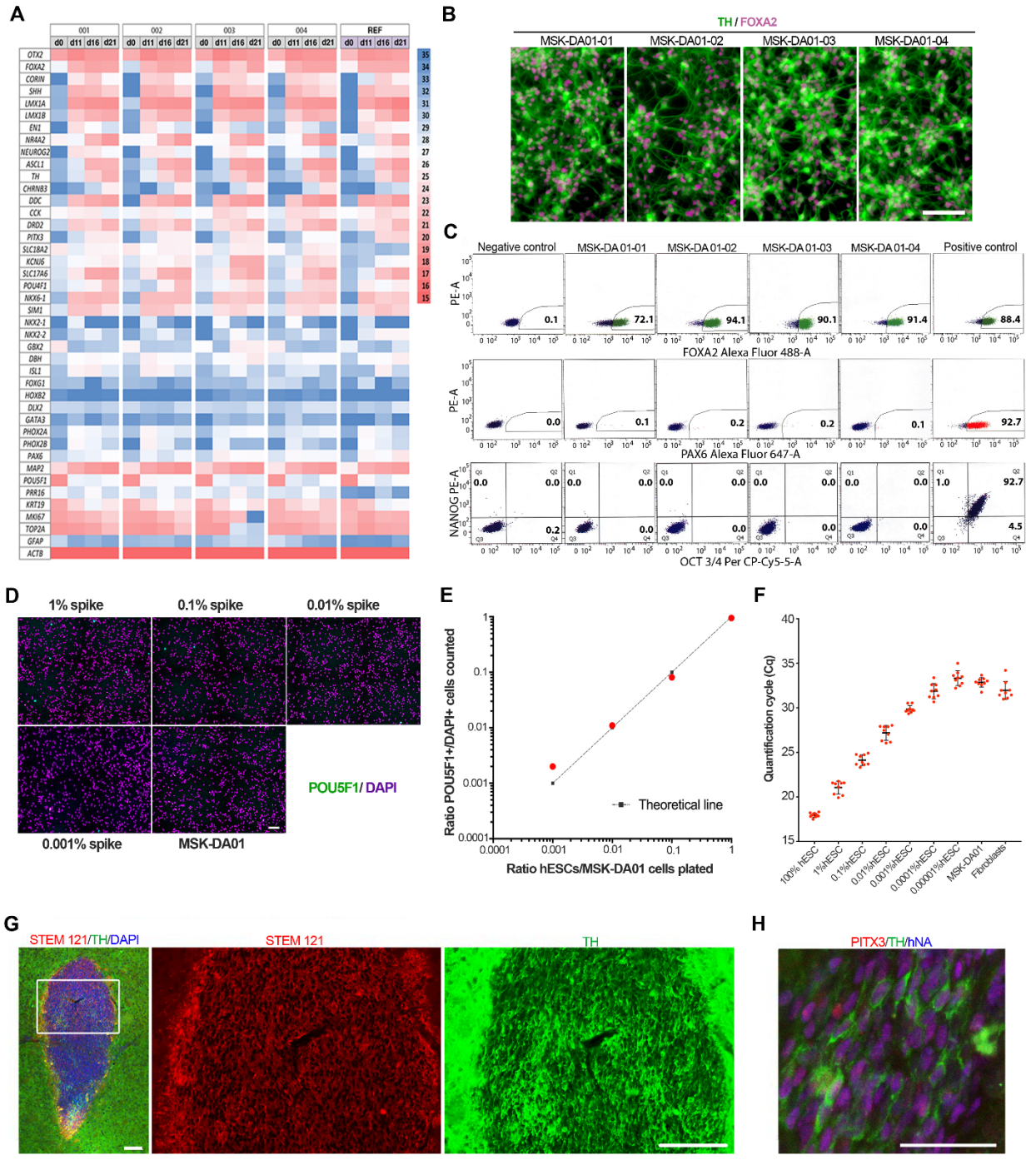

Figure S1. Characterization of MSK-DA01 (related to Figure 1). (A) qRT-PCR analysis of selected genes expressed on days 0, 11, 16, 21 during differentiation. The data are represented as quantification cycle values (Cq) and normalized with *ACTB*. REF=reference batch from previous successfully differentiated mDA cells. **(B)** Immunohistochemistry for FoxA2 and TH of re-plated thawed different lots of MSK-DA01 cells after additional 5 days of differentiation. **(C)** Flow cytometry analysis of FoxA2, Pax6 and Nanog expression in four lots of MSK-DA01. For FOXA2 flow, positive control is NB072715SZ, negative control is

hES-derived PAX6^{+} forebrain precursor bank; for PAX6 flow, positive control is forebrain precursor bank, negative control is PSCs; for OCT4/NANOG flow, positive control is undifferentiated ES cells, negative control is NB072715SZ. NB072715SZ is a previously validated batch of hES-derived differentiated dopamine neurons. **(D)** IHC images of immunostaining with anti-POU5F1 in MSK-DA01 spiked with different concentrations of hES. **(E)** The ratio of detected POU5F1^{+} cells versus total DAPI cells is obtained by high content imaging. Ratio of 1 would correspond to 100% POU5F1^{+} cells counted. **(F)** qRT-PCR analysis of *POU5F1* in MSK-DA01 spiked with different concentrations of hES. The data are represented as quantification cycle values (Cq) (n=9) and normalized with ACTB. **(G)** Representative IHC collage image of the graft which was identified by human specific marker STEM121 in host striatum three weeks after transplantation in a non-lesioned animal (short term in vivo studies). **(H)** Most human dopamine neurons (hNA^{+}TH^{+}) co-expressed PITX3 at three weeks post grafting. Scale bars=100 µm in B, D and G, and 50 µm in H.

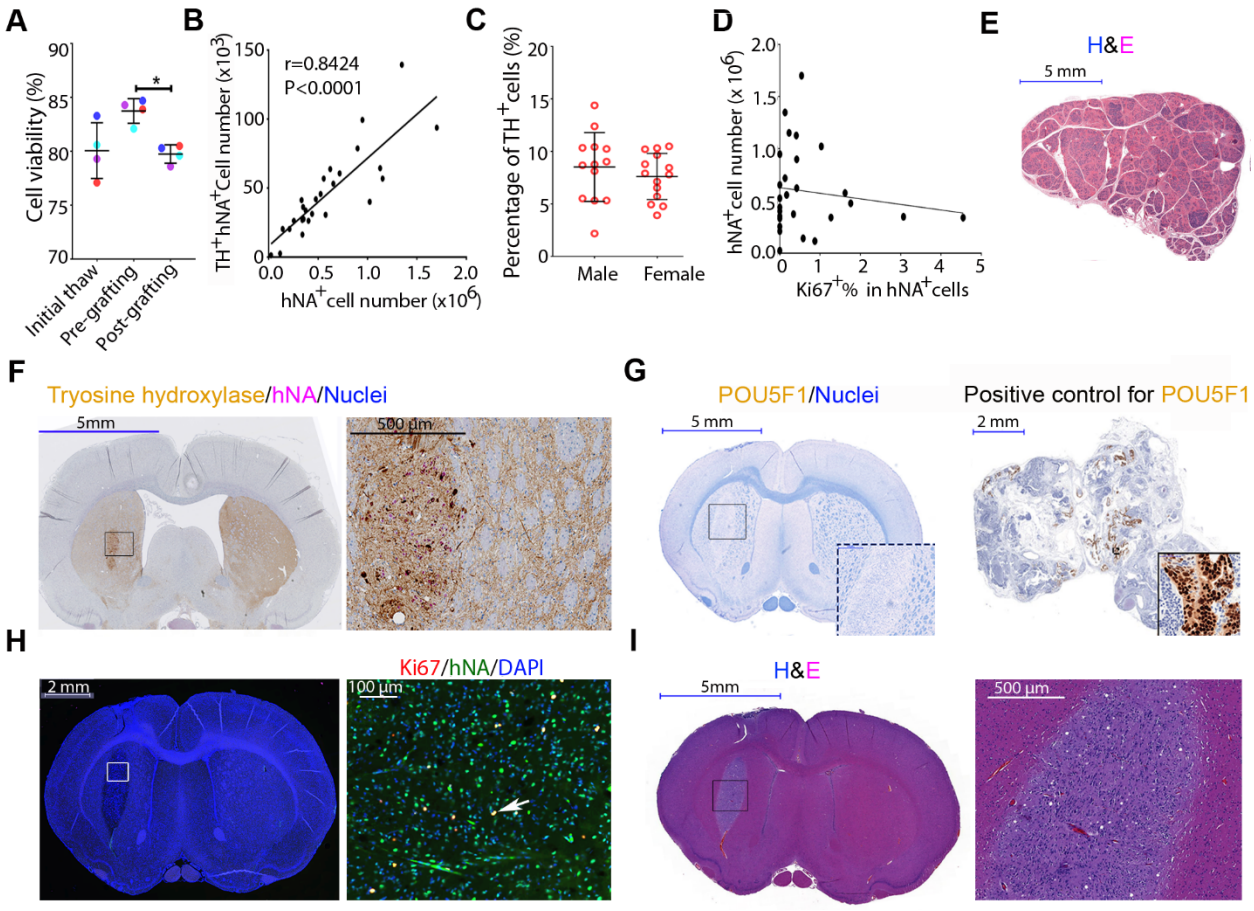

Figure S2. Characterization of MSK-DA01 grafts at the scheduled and early termination timepoints of the efficacy study (related to Figure 2). **(A)** The cell viability of the thawed MSK-DA01 in four consecutive days was monitored just after thawing (initial thaw), after suspension for transplantation (pre-grafting), and post-grafting. Cell viability at different stages on the same grafting day is plotted with the same color. Data are represented as Mean±SD. There is a statistically significant difference (*P<0.05) in cell viability of post-grafting with pre-grafting but not with initial thaw. **(B)** Graph representing the correlation between the total number of human cells and TH^+ cells in all rats (n=27). **(C)** TH^+ cells as a percentage of all human cells in male (n=13) or female (n=14) rat brains. **(D)** There is no clear correlation between the percentage of Ki67 and the number of hNA+ cells in all rats (r=0.06329, P=0.7538, n=27). **(E)** H&E staining of the flank mass in a rat shows features of mammary fibroadenoma. **(F)** MSK-DA01 graft in rats with early termination showed TH^+ cell bodies and processes. **(G)** $POU5F1^+$ cells were absent in MSK-DA01 graft of rats with early termination by immunohistochemistry (IHC). A positive control (a teratoma formed with human ES cells) for anti-POU5F1 is shown in the right panel. **(H)** Representative IHC images for the cell proliferation marker Ki67 and hNA in grafts of rats with early termination. The nuclei were counterstained with DAPI. The arrow points to co-labeled cells (indicating proliferating human cells). **(I)** A representative section of H&E stained graft in the striatum of rats with early termination. Note that no teratoma, abnormal structures or very large grafts were observed. In the images, areas outlined in white or black lines are shown with higher magnification images on the right panels. Scale bars are indicated on the individual images. Scale bar values are shown in the images.

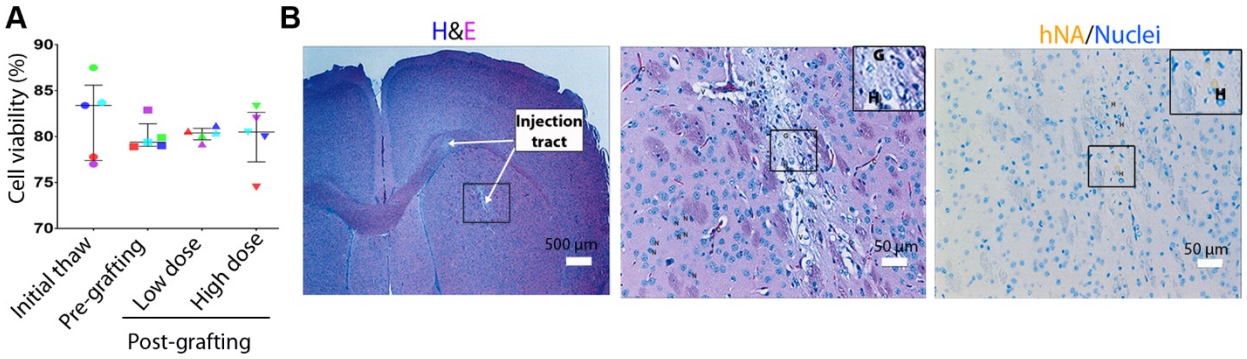

Figure S3. **No obvious adverse effects of MSK-DA01 in the biodistribution and toxicology study (related to Figure 3). (A)** In the biodistribution and toxicology studies, the cell viability of the thawed MSK-DA01 on five consecutive grafting days was monitored just after thawing (initial thaw), at time of suspension for transplantation (pre-grafting), and post-grafting. The cell viability at different stages on the same day is plotted with the same color. **(B)** Representative H&E stained section in vehicle group showing the injection tract. The two right panels are magnified images of the areas outlined in the left panel. A few gitter cells (G, middle panel) and hemosiderin-laden macrophages (H, right panel) are found within the region of the injection tract which is enlarged in the insets. Human nuclear antigen (hNA) IHC is expectedly negative. Scale bar value is 500 µm in left panel and 50 µm in right panels in B.

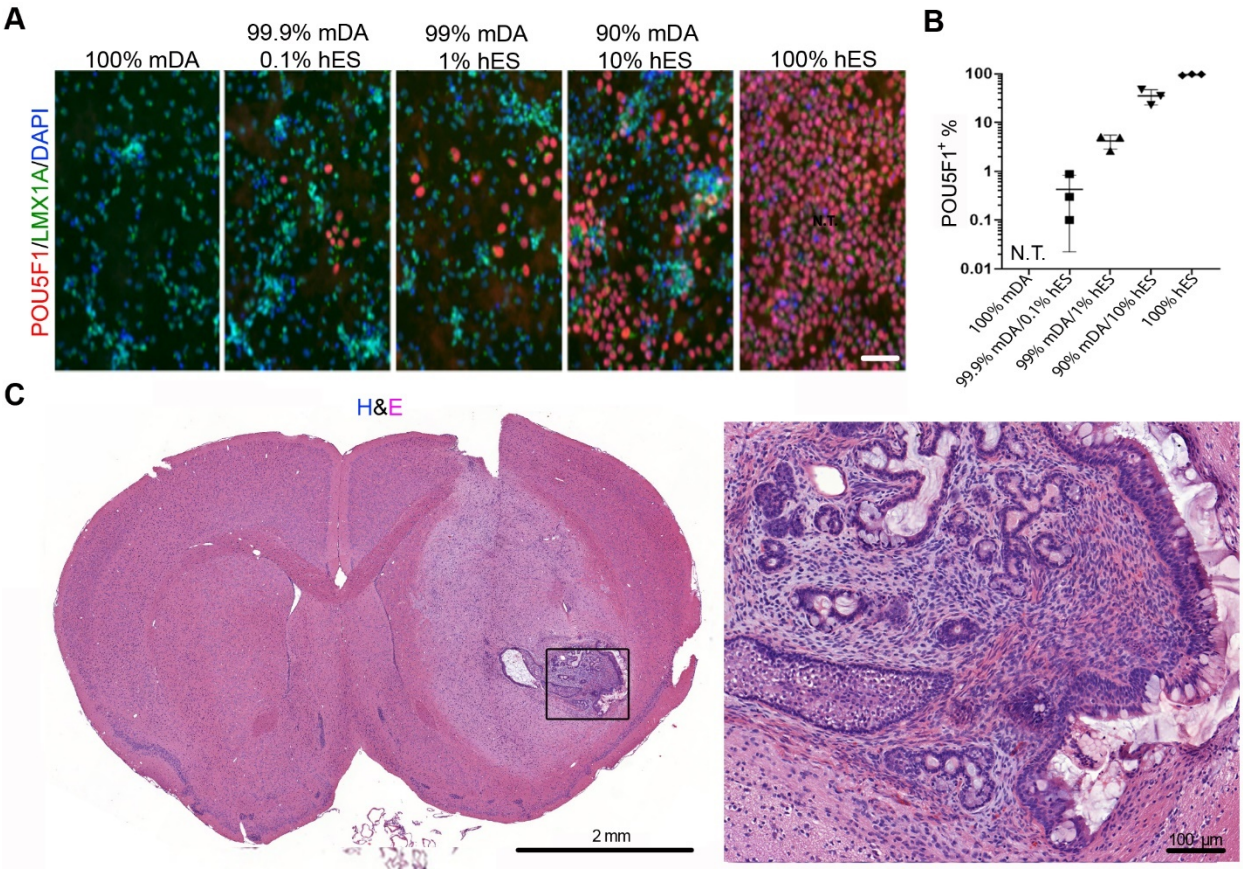

Figure S4. Midbrain dopamine neurons (mDA) cells were spiked with different concentration of hES cells for testing tumorigenicity (related to Figure 4). (A) Representative images of immunocytochemistry (IHC) staining with anti-POU5F1 and LMX1A on plated cells of 100% midbrain dopaminergic (mDA) cells, mDA spiked with 0.1% hES, mDA spiked with 1% hES, mDA spiked with 10% hES and 100% ES. The nuclei were counterstained with DAPI. Scale bar=50 µm. **(B)** The percentage of POU5F1^{+} was calculated from the cells during three consecutive grafting days. Note that POU5F1^{+} was absent in 100% mDA and increased with the spiked percentage of hES cells in different cell groups. N.T.=non-detected. Data are represented as mean±SD (n=3). **(C)** Representative image of H&E-stained teratoma formed by intra-striatal grafts of mDA with 1% hES cells. The area outlined with a rectangle is magnified on the right. Scale bar= 50µm in A and scale bar values are shown on the images in C.

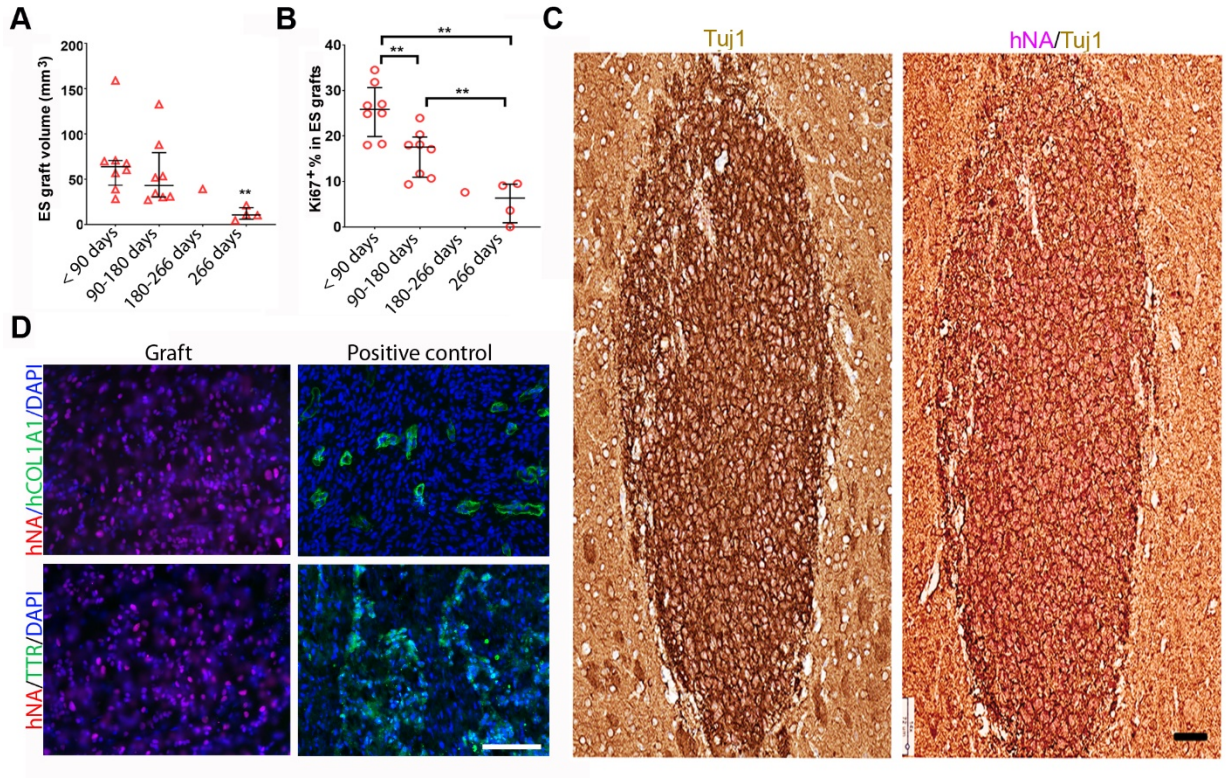

Figure S5. Evolution of grafts over time (related to Figure 5). (A) Volume of 100% hES cell grafts at <90 days (n=8), 90-180 days (n=8), 180-266 days (n=1), and 266 days (n=4). **(B)** The percentage of $Ki67^{+}$ cells in 100% ES grafts at different time ranges. **(C)** A representative image of sections of grafts at 30 days post transplantation. The left panel shows IHC for Tuj1. The right panel shows an adjacent section for hNA/Tuj1 double staining. **(D)** The grafts labeled by human nuclei antigen (hNA) did not contain human COL1A1(hCOL1A1) and transthyretin (TTR) expressing cells at 6 months post transplantation (left panel). Human glioblastoma tissue sections served as positive control (right panel). Data are represented as median with interquartile range. **P<0.01, ***P<0.001. Scale bars=70 µm in C and 100 µm in D.

Table S1. Quality control tests performed on WA09 cells (related to Figure 1).

Test Description	Specification	Test Result	Pass or Fail?
Post thaw % viability by Trypan blue dye exclusion	≥70% Viable	86% Viable	PASS
Karyotype by G-band analysis (20 metaphase spreads)	No clonal abnormalities or recurrent non-clonal abnormalities observed	Normal Karyotype. No clonal abnormalities were detected at the stated band resolution of 400-500	PASS
Sterility test	PASS	No bacterial or fungal growth	PASS
Endotoxin	≤10 EU/mL	<0.500EU/mL	PASS
Detection of Mycoplasma by PTC	Not detected (Negative)	Not detected (Negative)	PASS
Human ES cell marker expression (by flow cytometry)	SSEA1 TRA1-60 & TRA1-81 % Oct-4^+/SSEA-4^+ ≥90%	SSEA1: 0.62% TRA1-60: 67.14% TRA1-81:42.26% Oct-4^+/SSEA-4^+: 96.60%	PASS
Identity by STR	Results match donor	Results match human stem cell line of WA09	PASS
In vitro adventitious virus testing-inoculation of MRC5, Vero, and NIH-3T3 cell lines with cell lysates	Not detected	No viral contaminants detected	PASS
In vivo adventitious virus testing:suckling and adult mice, embryonated chicken eggs, and guinea pigs	Not detected	No adventitious viral contaminants detected	PASS
Retrovirus-Mus Dunni (PG4 S+L-)	Not detected	Not detected	PASS
Mouse antibody production (MAP)	Not detected	Not detected	PASS

Note: MRC5, Fibroblast cell Line; PTC, Points to Consider (regulatory guidance regarding mycoplasma testing); STR, Short Tandem Repeat

Table S2. Early death/termination and likely causes in efficacy study, biodistribution and toxicology studies (related to Figure 2 and 3).

Study	Group	Gender	Time of early death/termination after grafting	Cause of death and major pathological findings
Efficacy	Vehicle	Male	6 weeks	Dermatitis; Infection was observed in multiple organs.
Efficacy	Vehicle	Female	16 weeks	Dermatitis; Infection was observed in multiple organs.
Efficacy	Cell	Female	13 weeks	Pneumonia; Multifocal necrotizing and ulcerative dermatitis; Infection was observed in multiple organs. Brain:The graft was observed in the brain.
Efficacy	Cell	Male	16 weeks	Euthanasia was performed due to injury to the genital and stomach area inflicted by an attack by the cage mate. Brain: The graft was observed in the brain.
Toxicology, 30 days	Vehicle	Female	0 day	Surgical or anesthesia complications
Toxicology, 30 days	Vehicle	Female	3 days	More than 20% weight loss in 3 days after surgery, vascular injury or physical trauma as a complication of the stereotactic injection procedure
Biodistribution, 180 days	Vehicle	Male	0 day	Surgical or anesthesia complications
Toxicology, 180 days	Low dose	Female	53 days	Severe multi-organ lymphohistiocytic inflammation associated with the presence of bacterial emboli (coccoid bacteria) within small vessels and tissues throughout the body. Cause of death was bacteremia

Table S3. Histopathologic evaluation of selected visceral organs from male and female mice in different groups at the end time point of day 30 and 180 (related to Figure 3).

Time point	Day 30				Day 180			
Group	**Vehicle**	**High dose**	**Vehicle**	**High dose**	**Vehicle**	**High dose**	**Vehicle**	**High dose**
Gender	**Male**	**Male**	**Female**	**Female**	**Male**	**Male**	**Female**	**Female**
Animal number	**n=10**	**n=9**	**n=10**	**n=9**	**n=10**	**n=9**	**n=10**	**n=9**
Eyes (with optic nerve)	E (n=10)	E (n=9)	E (n=10)	E (n=9)	E (n=10)	E (n=9)	E (n=10)	E (n=9)
Eyes (with optic nerve), normal	N (n=10)	N (n=9)	N (n=10)	N (n=9)	N (n=10)	N (n=9)	N (n=10)	N (n=6), 0 (n=3)
Eyes, vacuolation with grey glass material, optic nerve (artifact of tissue fixation or processing)	0 (n=10)	0 (n=9)	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=9)	0 (n=10)	0 (n=6), P (n=3)
Kidney	E (n=10)	E (n=9)	E (n=10)	E (n=9)	E (n=10)	E (n=9)	E (n=10)	E (n=10)
Kidney, normal	N (n=7), 0 (n=3)	N (n=5), 0 (n=4)	N (n=8), 0 (n=2)	N (n=7), 0 (n=2)	N (n=5), 0 (n=5)	N (n=9)	N (n=9), 0 (n=1)	N (n=8), 0 (n=1)
Kidney, nephropathy	0 (n=8), 1F (n=2)	0 (n=5), 1F (n=3), 1M (n=1)	0 (n=8), 1F (n=2)	0 (n=6), 1F (n=2), 1M (n=1)	0 (n=6), 1F (n=3), 1M (n=1)	0 (n=9)	0 (n=9), 1F (n=1)	0 (n=8), 1F (n=1)
Kidney, cortical cyst	0 (n=9), 1F (n=1)	0 (n=9)	0 (n=10)	0 (n=9)	0 (n=9), 1F (n=1)	0 (n=9)	0 (n=10)	0 (n=9)
Adrenal gland	E (n=10)	E (n=9)	E (n=10)	E (n=9)	E (n=10)	E (n=9)	E (n=10)	E (n=9)
Adrenal gland, normal	N (n=5), 0 (n=5)	0 (n=7), N(n=2)	N (n=3), 0 (n=7)	0 (n=9)	N (n=2), 0 (n=8)	N(n=1), 0 (n=8),	0 (n=10)	0 (n=9)
Adrenal gland, cortical subcapsular hyperplasia	0 (n=5), 1F (n=4), 1M (n=1)	0 (n=1), 1F (n=3), 1M (n=4), 2M (n=1)	0 (n=3), 1F (n=2), 1M (n=3), 2M (n=2)	1F (n=1), 1M (n=4), 2M (n=4)	0 (n=2), 1F (n=3), 2F (n=1), 1M (n=4)	0 (n=1), 1F (n=2), 1M (n=6),	1M (n=3), 2M (n=7)	1M (n=3), 2M (n=6)
Liver	E (n=10)	E (n=9)	E (n=10)	E (n=9)	E (n=10)	E (n=9)	E (n=10)	E (n=9)
Liver, normal	N (n=10)	N (n=9)	N (n=10)	N (n=9)	N (n=9), 0 (n=1)	N (n=9)	N (n=10)	N (n=9)
Liver, necrosis, lobular, adjacent to gallbladder, with replacement by granulomatous inflammation and with intra histiocytic bile pile pigment	0 (n=10)	0 (n=9)	0 (n=10)	0 (n=9)	0 (n=9), 2L (n=1)	0 (n=9)	0 (n=10)	0 (n=9)
Stomach	E (n=10)	E (n=9)	E (n=10)	E (n=9)	E (n=10)	E (n=9)	E (n=10)	E (n=9)
Stomach, normal	N (n=9), 0 (n=1)	N (n=8), 0 (n=1)	N (n=10)	N (n=7), 0 (n=2)	N (n=10)	N (n=7), 0 (n=2)	N (n=9), 0 (n=1)	N (n=8), 0 (n=1)
Stomach, mineralization, serosa	0 (n=9), 1F (n=1)	0 (n=8), 2F (n=1)	0 (n=10)	0 (n=8), 1F (n=1)	0 (n=10)	0 (n=8), 1F (n=1)	0 (n=9), 1F (n=1)	0 (n=8), 1M (n=1)
Stomach, mineralization, tunica muscularis	0 (n=10)	0 (n=9)	0 (n=10)	0 (n=8), 1F (n=1)	0 (n=10)	0 (n=8), 2F (n=1)	0 (n=9), 1F (n=1)	0 (n=9)
Pancreas	E (n=10)	E (n=9)	E (n=10)	E (n=9)	E (n=10)	E (n=9)	E (n=10)	E (n=9)
Pancreas, normal	N (n=10)	N (n=9)	N (n=10)	N (n=9)	N (n=10)	N (n=8), 0 (n=1)	N (n=10)	N (n=9)
Pancreas, interstitial fibrosis	0 (n=10)	0 (n=9)	0 (n=10)	0 (n=9)	0 (n=10)	0 (n=8), 4F (n=1)	0 (n=10)	0 (n=9)
Skin	E (n=10)	E (n=9)	E (n=10)	E (n=9)	E (n=10)	E (n=9)	E (n=10)	E (n=9)

Skin, normal	N (n=10)	N (n=9)	N (n=10)	N (n=9)	N (n=10)	N (n=7), 0 (n=2)	N (n=10)	N (n=9)
Sternum (with marrow)	E (n=10)	E (n=9)	E (n=10)	E (n=9)	0 (n=10)	0 (n=9)	0 (n=9), P (n=1)	0 (n=9)
Sternum (with marrow), normal	N (n=10)	N (n=9)	N (n=10)	N (n=9)	0 (n=10)	0 (n=8), 3M (n=1)	0 (n=10)	0 (n=9)
Testes	E (n=10)	E (n=9)	NA (n=10)	NA (n=9)	E (n=10)	E (n=10)	NA (n=10)	NA (n=9)
Testes, normal	N (n=5), 0 (n=5)	N (n=7), 0 (n=2)	NA (n=10)	NA (n=9)	N (n=10)	N (n=8), 0 (n=1)	NA (n=10)	NA (n=9)
Testes, seminiferous epithelium, degeneration	0 (n=5), 1M (n=2), 1F (n=3)	0 (n=7), 1F (n=2)	NA (n=10)	NA (n=9)	0 (n=10)	0 (n=8), 2U (n=1)	NA (n=10)	NA (n=9)
Ovaries	NA (n=10)	NA (n=9)	E (n=10)	E (n=9)	NA (n=10)	NA (n=9)	E (n=10)	E (n=9)
Ovaries, normal	NA (n=10)	NA (n=9)	N (n=10)	N (n=9)	NA (n=10)	NA (n=9)	N (n=10)	N (n=8), 0 (n=1)
Ovary, cyst, epithelial	NA (n=10)	NA (n=9)	0 (n=10)	0 (n=9)	NA (n=10)	NA (n=9)	0 (n=10)	0 (n=8), P (n=1)
Uterus	NA (n=10)	NA (n=9)	E (n=10)	E (n=9)	NA (n=10)	NA (n=9)	E (n=10)	E (n=9)
Uterus, normal	NA (n=10)	NA (n=9)	N (n=10)	N (n=9)	NA (n=10)	NA (n=9)	N (n=10)	N (n=8), 0 (n=1)
Uterus, polyp, endometrial stromal, benign	NA (n=10)	NA (n=9)	0 (n=10)	0 (n=8)	NA (n=10)	NA (n=9)	0 (n=10)	0 (n=8), P (n=1)
Cervix	NA (n=10)	NA (n=9)	E (n=10)	E (n=9)	NA (n=10)	NA (n=9)	E (n=10)	E (n=9)
Cervix, normal	NA (n=10)	NA (n=9)	0 (n=10)	N (n=5), 0 (n=4)	NA (n=10)	NA (n=9)	N (n=6), 0 (n=4)	N (n=2), 0 (n=7)
Cervix, superficial epithelial inflammation, suppurative	NA (n=10)	NA (n=9)	0 (n=1), 1M (n=3), 2M (n=1), 3M (n=4), 4M (n=1)	0 (n=5), 1 M(n=2), 3M (n=2)	NA (n=10)	NA (n=9)	0 (n=6), 1F (n=2), 1M (n=2)	0 (n=2), 1 M (n=2), 2M (n=3), 3M (n=1)
Vagina	NA (n=10)	NA (n=9)	E (n=10)	E (n=8), NE (n=1)	NA (n=10)	NA (n=9)	E (n=10)	E (n=9)
Vagina, normal	NA (n=10)	NA (n=9)	N (n=3), 0 (n=7)	N (n=6), 0 (n=2), NE (n=1)	NA (n=10)	NA (n=9)	N (n=8), 0 (n=2)	N (n=3), 0 (n=6)
Vagina, superficial epithelial inflammation, suppurative	NA (n=10)	NA (n=9)	0 (n=3), 1M (n=1), 2M (n=3), 3M (n=2), 4M (n=1)	0 (n=6), 1M (n=1), 2M (n=1), NE (n=1)	NA (n=10)	NA (n=9)	0 (n=8), 1F (n=1), 1M (n=1)	0 (n=3), 1M (n=2), 2M (n=3), 3M (n=1)
Peri-thyroid/peri-parathyroid tissue, ciliated epithelial cyst	NA (n=10)	NA (n=9)	NA (n=10)	NA (n=10)	0 (n=10)	0 (n=6), P (n=3)	0 (n=10)	0 (n=9)

Note: There are incidental laboratory lesions presented in animals which are not related to grafts. Overall, the histological evaluation is comparable at the same age and gender between vehicle and MSK-DA01 cell group. E: examined; NE: not evaluated; NA: not available. Histologic findings are graded on a severity scale of 0 (absent), 1 (minimal/slight), 2 (mild), 3 (moderate), 4 (marked/severe), or noted as N (normal) or P (presence). Lesion distribution is denoted as M (multifocal), F (focal), L (lobular), D (diffuse), B (bilateral), and U (unilateral).

Table S4. Animal number in the studies (related to Figure 2, 3 and 4).

Study	Groups	Original animal number in group	Added animal number	Number of animals excluded from analysis due to early death	Number of animals excluded from analysis due to the absence of brain graft
Efficacy study	Cell	M (n=14); F (n=14)	F (n=1) was added*	M (n=1); F (n=1)	none
	Vehicle	M(n=8); F (n=8)	n=0	M (n=1); F (n=1)	N/A
Biodistribution study (30 days post grafting)	High dose cell	M (n=5); F (n=5)	N/A	none	M(n=0); F (n=0)
	Low dose cell	M (n=5); F (n=5)	N/A	none	M (n=0); F (n=1)
	Vehicle	M (n=5); F (n=5)	N/A	none	N/A
Biodistribution study (180 days post grafting)	High dose cell	M (n=5); F (n=5)	N/A	none	M (n=0); F (n=1)
	Low dose cell	M (n=5); F (n=5)	N/A	none	M(n=1); F (n=1)
	Vehicle	M (n=5); F (n=5)	N/A	M (n=1)[#]	N/A
Toxicology study (30 days post grafting)	High dose cell	M (n=10); F (n=10)	N/A	none	M (n=1); F (n=1)
	Low dose cell	M (n=10); F (n=10)	N/A	none	M(n=0); F(n=0)
	Vehicle	M (n=10); F (n=10)	N/A	F (n=2)[#]	N/A
Toxicology study (180 days post grafting)	High dose cell	M (n=10); F (n=10)	N/A	none	M (n=1); F (n=1)
	Low dose cell	M (n=10); F (n=10)	N/A	F (n=1)[#]	M (n=0); F (n=0)
	Vehicle	M (n=10); F (n=10)	N/A	none	N/A
Tumorigenicity study	100% MSK-DA01	M (n=22); F (n=22)	N/A	none	M (n=4); F (n=2)
	MSK-DA01 with 0.01% hESC	M (n=22); F (n=22)	N/A	none	M (n=1); F (n=1)
	MSK-DA01 with 0.1% hESC	M (n=22); F (n=22)	N/A	none	M (n=5); F (n=2)
	100% hESC	M (n=12); F (n=12)	N/A	none	M (n=1); F (n=1)
	Vehicle	M (n=12); F (n=12)	N/A	none	N/A

Note: M, male; F, female; * due to premature withdrawal of the injection needle in one female during the procedure. [#]replaced by equal number of mice from predetermined replacement group.