Supplemental Figure legends

Figure S1, Related to Figure 1 **Embryonal carcinomas derived from iPS or ES p53-/- cells respond Omomyc^{ER} activation while differentiated tissues can tolerate MYC blockade**. **A**, Immunofluorescence for Nanog and DAPI on representative iPS colonies; **B**, FACS showed expression of the exogenous *OCT4, MYC, KLF4*, and *SOX2* linked to four different fluorescent markers (OCT4-GFP, KLF4-Cherry, MYC-Cerulean, SOX2-Citrine) in mouse embryonic fibroblast, the vectors are silenced in iPS cells. **C**, Quantitative RT-PCR measures expression of *KLF4, SOX2, MYC*, and *OCT4* in iPS and embryonic stem (ES) cells. This confirmed that the expression level of their endogenous counterparts is at or below the level seen in embryonic stem cells; **D,** Representative karyotype analysis of an iPS clone selected for Omomyc^{ER} transduction; **E**, Schematic diagram of Omomyc^{ER} lentiviral construct; FACS analysis of control and Omomyc^{ER} / citrine expressing mouse-iPS cells and human-iPS cells clone 5.10-omo10. **F**, Time to detection of reprogrammed (iPS) colonies from *p53*+/+ and *p53-/-* MEFS (mean ± SD); **G**, Kaplan-Meier analysis of latency to tumor development (~ 1cm^3) from xenografted iPS cells of indicated genotypes (60 days, $n_{(\text{each group})} = 6$, $p < 0.01$); **H**, Histology of embryonal carcinoma (Embryonal CA) derived from iPS p53-/- cells; **I,** Quantitative RT-PCR measuring relative expression of exogenous (human) and endogenous (murine) *Myc* expression in mouse embryo fibroblasts (MEF), MEFs expressing the transgenic (human) *MYC* (MEF + tMYC), and embryonal carcinomas (Embryonal CA). **J**, Quantification of apoptotic index TUNEL, proliferation fraction Ki67, yolk sac differentiation marker SALL4 in control and Omomyc^{ER} expressing embryonal carcinomas after TAM

treatment (mean ± SD); unpaired t-test was used to compare the samples. **K**, Quantitative RT-PCR measures p53 mRNA level in p53 wild type ES (ES), iPS cells derived from p53-/- MEFs (iPS p53-/-), and p53 wild type ES cells expressing a shRNA against p53 (ES shp53 and ES-OmomycER/shp53); **L**, Histology of embryonal carcinoma (Embryonal CA) derived from ES cells expressing shp53; **M**, Representative control and Omomyc^{ER} embryonal carcinoma derived from mouse ES-shp53 harvested after TAM treatment; **N**, Animals bearing embryonal carcinomas derived from mouse ES-shp53 cells expressing Omomyc^{ER} or control tumors after 2 weeks of tamoxifen treatment; **O**, Microscopic pathology of residual tissue from Omomyc^{ER} expressing embryonal carcinomas after TAM treatment. **P,** Representative microscopic pathology of Omomyc^{ER} expressing teratomas derived form mouse iPS cells harvested after TAM treatment; **Q and R,** Quantitative RT-PCR measuring relative expression of exogenous (human) (q) and endogenous (murine) (r) *Myc* expression in p53 wild type mouse embryo fibroblasts (MEF), p53 deficient MEFs (MEF/p53-/-), or expressing the transgenic MYC (tMYC), and teratomas. **S**, Representative control and Omomyc^{ER} iPS p53+/+ cell derived teratomas harvested after TAM treatment; **T**, Comparison of tumor weights of iPS p53+/+ cell derived teratomas following TAM treatment. **U**, Representative histology of benign teratomas derived from ES cells. **V**, Quantification of yolk sac differentiation marker (SALL4) in control and Omomyc^{ER} expressing teratomas after TAM treatment (mean ± SD); **W**, Animals bearing ES cell derived teratomas expressing Omomyc^{ER} or control vector (Control) after 3 weeks of tamoxifen treatment; **X,** Representative control and Omomyc^{ER} ES cell derived teratomas harvested after TAM treatment; **Y**, Comparison of tumor weights of ES cell derived teratomas following TAM treatment. **Z,**

Histology of benign ES-derived teratomas control and expressing Omomyc^{ER} including the indicated tissue types and stained as labeled. **A1,** Representative picture of iPS p53+/+ cells treated with vehicle or tamoxifen; **B1,** Apoptosis analysis of Omomyc^{ER} iPS p53+/+ cells treated with vehicle or tamoxifen measuring annexin positive cells by flow cytometry, **C1,** Representative Omomyc^{ER} iPS p53+/+ cell derived teratomas harvested after treatment with vehicle or tamoxifen. **D1** Comparison of tumor weights of iPS p53+/+ cell derived teratomas treated with vehicle or tamoxifen.

Figure S2, Related to Figure 2 **Pathological characterization of iPS-derived brain tumors. A**, H&E staining of coronal brain section showing a representative primitive neuroectodermal tumor (PNET); **B** Histology and indicated immunohistochemical stains of PNETs.

Figure S3, Related to Figure 4. **Additional analyses of metabolic changes in PNETs**. **A**, Immunohistochemical stain for glutaminase (GLS) in control and Omomyc^{ER} PNETs and human glioma as a positive control; **B**, PCR ratio of nuclear and mitochondrial DNA in human iPS cells (iPS), differentiated neurons (Neurons), and PNET tumors (PNET); **C and D**, Quantitative RT-PCR determination TFAM of and UCP2 expression in the indicated samples. **E,** Positive and negative immunohistochemical control staining for PKM2, LDHA and Phospho-Histon3 T11.

Supplementary Experimental Procedures

iPS cell characterization. For immunofluorescence analysis iPS cells were grown on glass cover slides and fixed with 4% paraformaldehyde for 10 min at room temperature. Fixed cells were incubated in PBS containing 20% goat serum for 30 min at 37°C in a humidified chamber and over-night at 4°C with anti-nanog (eBioscience 53-5761) diluted 1:1000 and anti SSEA1 (eBioscience 51-8813) antibodies in PBS containing 5% goat serum. After five washes in PBS, 0.05% Tween 20, nuclei were counterstained with DAPI (4',6'-diamino-2-phenylindole) 0.1mg/ml in SSC2X for 3'room temperature. Samples were then washed in PBS and mounted on glass slides in Vectashield (Vector Laboratories). Cell preparations were examined under a Leica. Fluorescence microscope equipped with a cooled camera (Coolsnap, Photometrics). iPS cells were characterized by flow cytometry analysis and karyotyping assay as previously described(*1*). For assessment of expression of pluripotency genes total RNA from iPS and ES cells was isolated with Trizol (Invitrogen). Reverse transcription was performed with Superscript III (Invitrogen) and qPCR was performed TaqMan probe by Applied Biosystems SOX2 (Mn03053810), OCT4 (Mn03053917), MYC (Mn00487804), KLF4 (Mn00516104). Reactions were carried out in duplicate in an ABI PRISM 7500 Sequence Detection System (Applied Biosystems). Expression was calculated by relative quantification using the ΔΔCt method.

Pathological tumor analyses. For teratoma formation assays, undifferentiated iPSCs 2×10^6 cells were injected subcutaneously into NOD-SCID *IL2Rg*-null mice (Jackson Laboratory). Four to six weeks later, the tumors were surgically dissected and fixed in 4% formaldehyde. All animal experiments were conducted in accordance with protocols approved by MSKCC Institutional Animal Care and Use Committee (IACUC) and following National Institutes of Health guidelines for animal welfare. All tumors were evaluated by an expert on germ cell tumor pathology at MSKCC (Victor Reuter), and the histological diagnosis was based on hematoxylin and eosin (H&E) staining and immunohistochemistry using antibodies against SALL4 (Abcam, cat# ab29112), OCT4 (Ventana, cat# 760-4392), CD30 (Dako, clone BH2 cat# mo751). In treatment studies tumors were collected one week after the last treatment, weighed, fixed and stained as above.

Generation of Chimeric mice with iPS Cells. Frozen iPS cells were thawed into 6-well plates containing irradiated mouse embryonic fibroblast (MEF) feeders. When cells reached confluency, they were trypsinized and expanded by passaging into larger culture vessels at a ratio of 1:4 - 1:6. When sufficient cells become available, they are frozen for future use and a small portion is submitted for karyotyping by the Institute's Cytogenetics Core Facility. In addition, some of the cells were analyzed for alkaline phosphatase (AP) expression. All cells were cultured in medium containing KnockOut™ DMEM with high glucose (GIBCO), supplemented with 15% Fetal Bovine Serum (Gemini), 0.1mM b-mercaptoethanol, 4mM L-glutamine, 1X Non-essential amino acids and 1000U/ml of LIF. During the week of injection, 2 million frozen iPS cells are thawed into a T12.5

flask containing feeders and cultured for two day to reach confluency. The day before injection, cells are passed 1:2 or 1:3 onto new T12.5 flasks, cultured overnight to provide exponentially growing cells for blastocysts injection to produce chimeras. Briefly, cells are trypsinized to give a single cell suspension just prior to injection. Blastocysts are obtained at day 3.5 of gestation from superovulated albino C57Bl/6J (B6(Cg)-*Tyrc-2J*/J, Jax # 000058) female mice mated to stud males of the same strain. This strain combination will allow us to identify the extent of contribution by the iPS cells to the chimeras based on coat color since the iPS cells are derived from a C57Bl/6J (black) mouse strain and the blastocysts obtained from an albino strain. For each blastocyst, an average of either 8 or 12 iPS cells are injected to produce the chimeric embryos. Chimeric blastocysts are then transferred into pseudo-pregnant females to permit development to term.

Differentiation of iPS cells

Neural rosettes

Control iPSCs and omomyc^{ER} iPSCs were differentiated towards a neural fate by a modified dual SMAD-inhibition protocol[31]. After 8 to 10 days of differentiation, iPSCs lines were subjected to WNT signaling inhibition (XAV939: 10 μM) to prevent non-CNS neural fates. 4 days after passaging neural cultures formed polarized columnar neuroepithelial structures known as neural rosettes. These cells were then dissociated, collected and a total of 200,000 cells were injected into the striatum of immunocompromised mouse host (see grafting section below).

Midbrain dopamine (DA) neurons

Midbrain dopaminergic neurons were derived as previously described(*2*). Briefly, iPS cell lines were exposed to activators of SHH (puromorphamine) and Wnt signaling (GSK-inhibitor: CHIR99021) to induce FOXA2+/LMX1A+ midbrain floor plate cells. These cells were further differentiated into to DA neurons in Neuralbasal/B27 medium supplemented with BDNF, ascorbic acid, GDNF, dibutyryl-cyclic AMP, TGFβ3. At day 23 of DA neuron differentiation, cells were infected with the omomyc^{ER} virus for 48h and then FACS-purified for citrine-positive cells. A total of 150,000 cells were injected into the striatum of immunocompromised mouse host (see grafting section below). Prior to transplantation, DA neuron identity and induction efficiency was validated by immunocytochemistry for co-expression of FOAX2/LMX1A/NURR1.

Grafting.

NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (The Jackson Laboratory) were housed and treated according to the MSKCC IACUC and NIH guidelines. At 6 to 8 weeks of age, mice were anesthetized with ketamine/xylazine (Fort Dodge) and unilateral stereotactic injections of hESC-derived cells were delivered to the striatum at the following coordinates: anteroposterior [AP], +0.5; mediolateral [ML], –1.8; dorsoventral [DV], –3.2. Transplantation of 150,000 (mDA) or 200,000 cells (rosettes), at a concentration of 100,000 cells/µl, was performed with a Hamilton syringe (26s gauge) at a rate of one µl per minute.

Immunohistochemistry.

Mice were given a lethal dose of pentobarbital (Nembutal, Abbot Laboratories) and transcardially perfused with PBS followed by 4% paraformaldehyde. Brains were removed and fixed overnight at 4°C and then transferred to 30% sucrose overnight. Brains were embedded in O.C.T. (Tissue-Tek) and stored at ‑80°C until serial cryosectioning at 30 microns in 5 bottles. For fluorescence immunohistochemistry, sections were blocked in 10% normal goat serum (NGS; Gibco) (1% BSA for FOXA2 staining) with 0.2% Triton X-100 for 30 minutes at room temperature. Primary antibodies were applied overnight in 2% NGS (.1% BSA for Sox2 and FOXA2 staining) at 4°C, followed by appropriate fluorochrome-conjugated secondary antibodies (Alexa Fluor conjugates; Molecular Probes) for 1 hour at room temperature. Slides were then washed and counterstained with Vectashield with DAPI (Vector Laboratories). Primary antibodies included mouse anti-nestin (1:500; Neuromics), goat anti-Sox2 (1:100; Santa Cruz), mouse anti-Ki67 (1:500; DAKO), rabbit anti-TH (1:500; Pel Freez Biologicals), goat anti-FOXA (1:100; Santa Cruz Biotechnology Inc.), mouse anti-hNCAM (ERIC-1; 1:100; Santa Cruz), anti-PKM2 (1:100 Cell Signaling), anti-LDHA (1:100 Cell Signaling), anti-GLS (1:100 Proteintech) anti-Histone H3 (phospho T11) (1:200 Abcam).

Quantitative expression analyses: Total RNA was extracted from iPS and mouse brain samples using the RecoverAll Total Nucleic Acid isolation (Ambion). cDNA synthesis and qRT-PCR and analysis by the ΔΔ Ct method as described (Oricchio et al 2011). Taqman Gene Expression Assays: GAPDH (Hs02758991 Applied Biosystems) MYC (Hs00153400 Applied Biosystems) Hexokinase II (Hs00606086 Applied Biosystems) LDHA (Hs01378790 Applied Biosystems) SLC1A5 (Hs01056542) and GLS

(Hs00248163). For PKM2 specific splicing form we used primers Forward 5'cagccaaaggggactatcct 3' and Reverse 5'caaataattgcaagtggtagatgg3' with Probe 75 (Universal probe library Roche cat 04688988001).

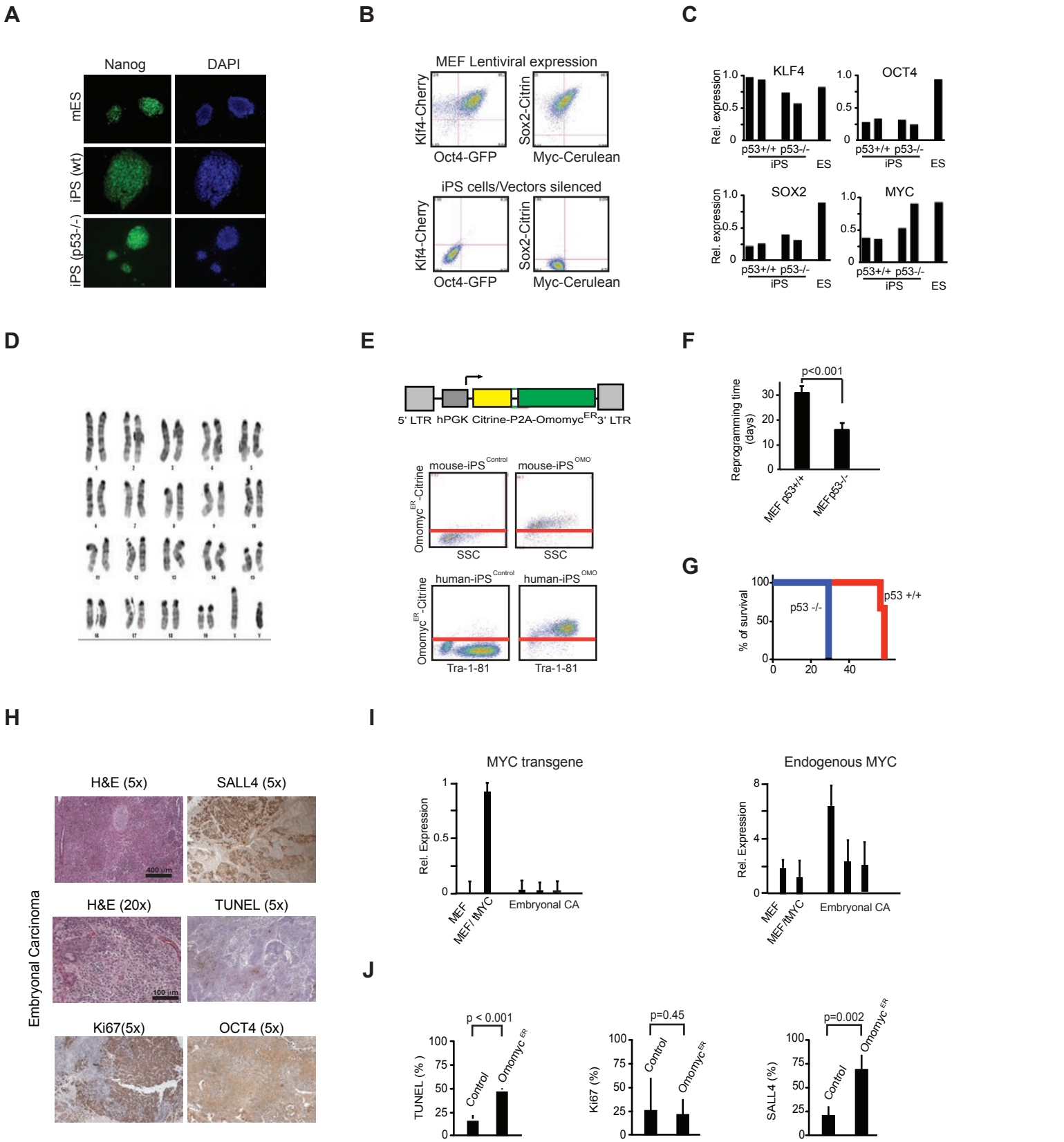
MRI imaging: All mouse brain MRI was carried out on a 200 MHz Bruker 4.7T Biospec scanner equipped with a 560 mT/m ID 12 cm gradient (Bruker Biospin MRI GmbH, Ettlingen, Germany; Resonance Research, Inc., Billerica, MA). RF excitation and acquisition was achieved by a custom-built quadrature birdcage resonator with ID of 36 mm (Stark Contrast MRI Coils Research Inc., Erlangen, Germany). The mice were immobilized with 1% isoflurane (Baxter Healthcare Corp., Deerfield, IL) gas in oxygen. Animal respiration was monitored with a small animal physiological monitoring system (SA Instruments, Inc., Stony Brook, New York). Scout images along three orthogonal orientations were first acquired for animal positioning. For mouse brain imaging, brain coronal T2-weighted images using fast spin-echo RARE sequence (Rapid Acquisition with Relaxation Enhancement) was acquired with TR 2.6s, TE 50 ms, RARE factor of 8, slice thickness of 0.7 mm, FOV 30 mm, in-plane resolution of 117 x 234 mm, and 28 averages.

References:

1. E. P. Papapetrou, M. J. Tomishima, S. M. Chambers, Y. Mica, E. Reed, J. Menon, V. Tabar, Q. Mo, L. Studer, M. Sadelain, Stoichiometric and temporal requirements of Oct4, Sox2, Klf4, and c-Myc expression for efficient human iPSC induction and differentiation. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 12759-12764 (2009).
2. S. Kriks, J. W. Shim, J. Piao, Y. M. Ganat, D. R. Wakeman, Z. Xie, L. Carrillo-Reid, G. Auyeung, C. Antonacci, A. Buch, L. Yang, M. F. Beal, D. J. Surmeier, J. H.

Kordower, V. Tabar, L. Studer, Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. *Nature* **480**, 547-551 (2011).

Figure S1
(related to Figure 1)



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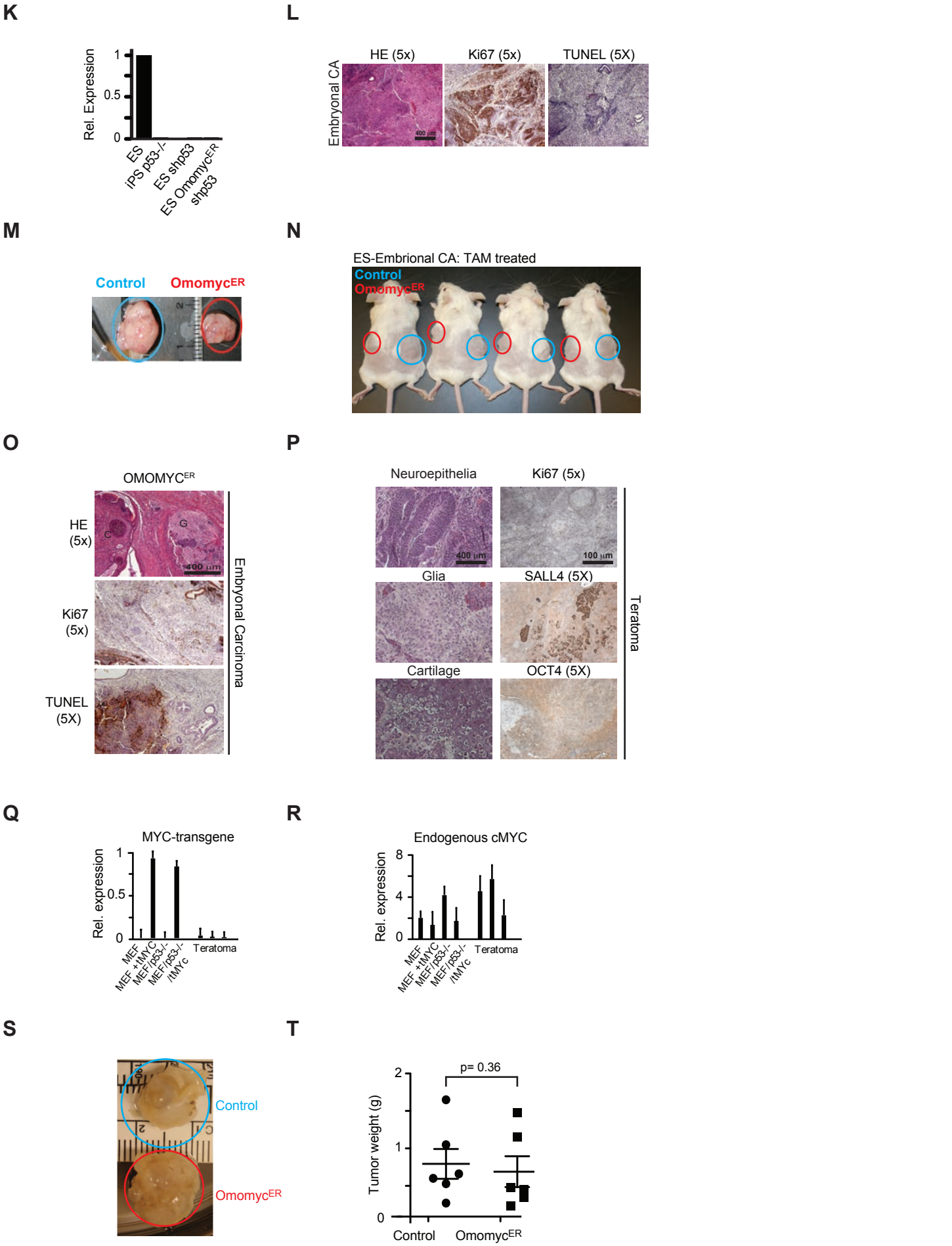

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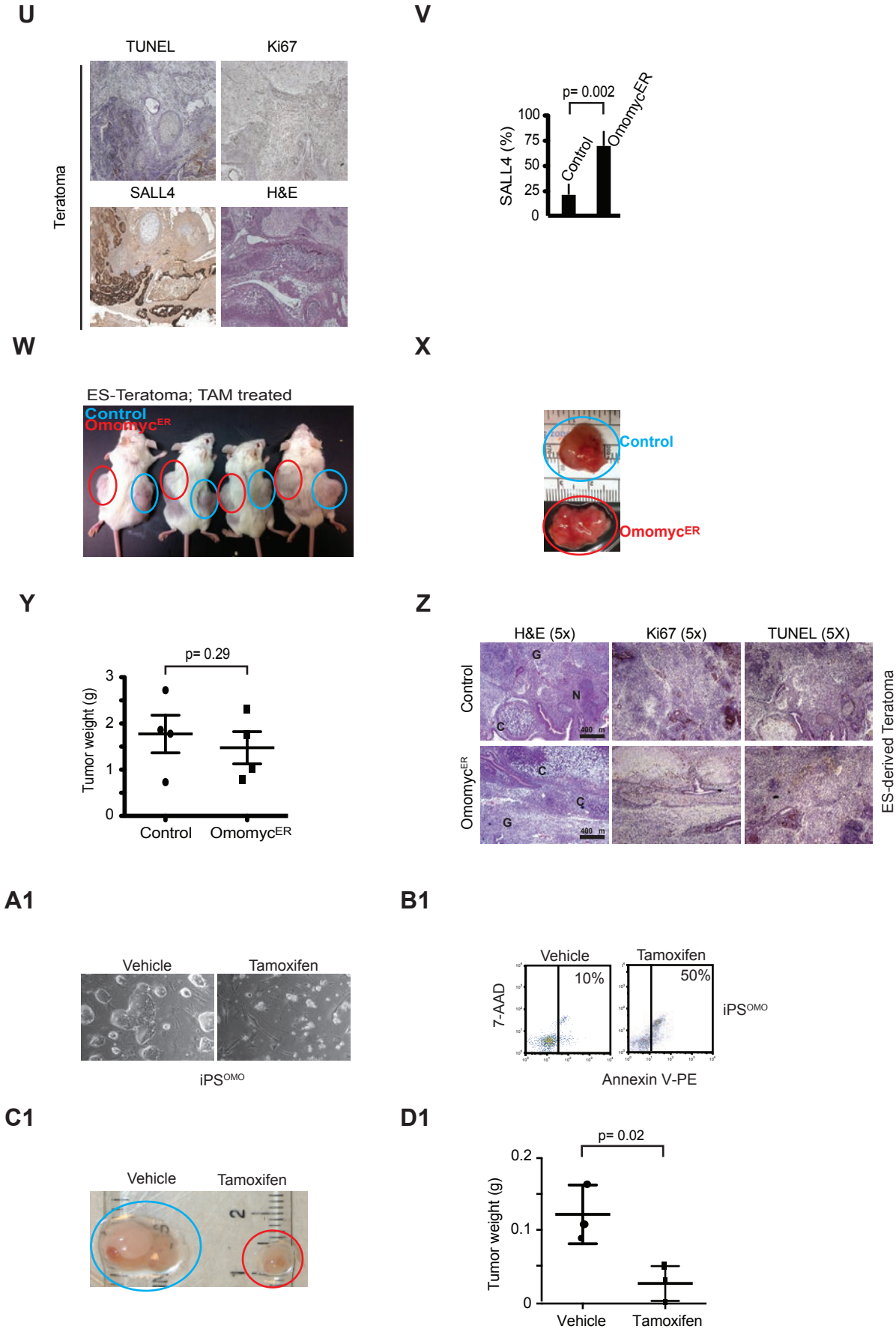

Figure S2
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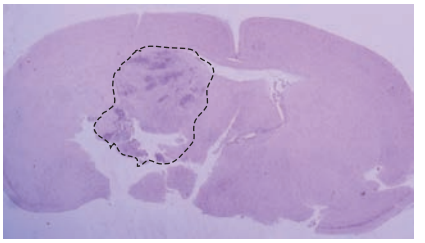
A

Primitive Neuroectodermal Tumors (PNET)



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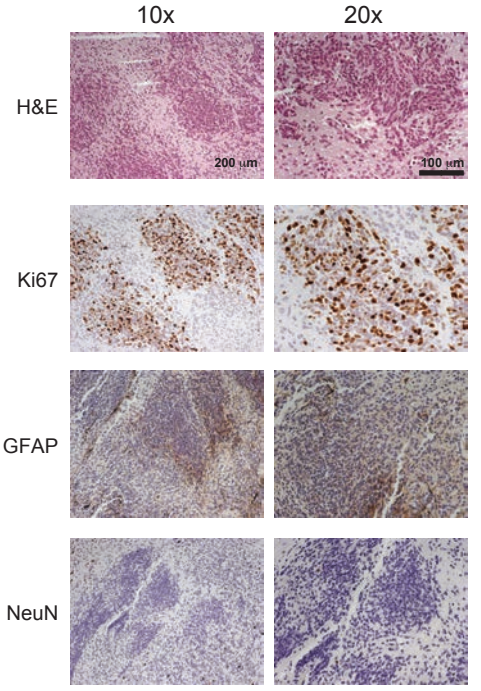

Figure S3
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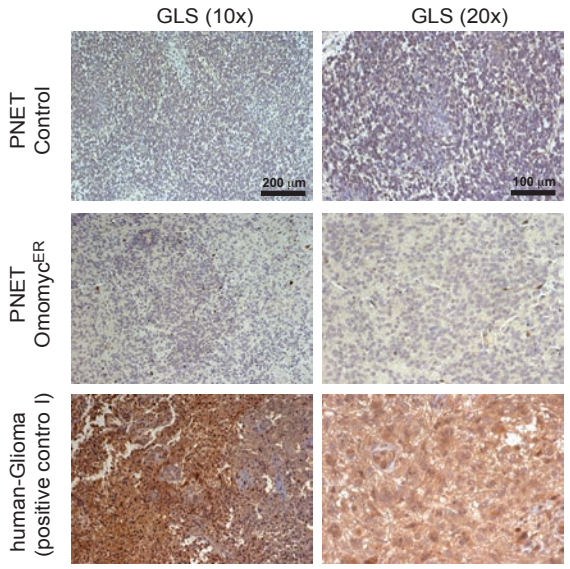

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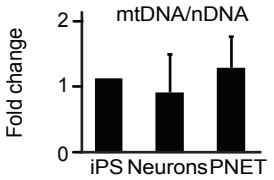

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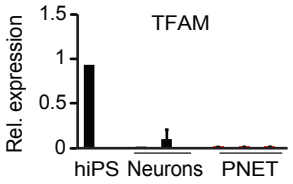

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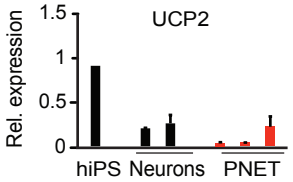

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