**Cell Reports Medicine, Volume *2***

## Supplemental information

# Increased p53 signaling impairs neural differentiation in HUWE1-promoted intellectual disabilities

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## SUPPLEMENTAL INFORMATION

# Increased p53 signaling impairs neural differentiation in HUWE1-promoted intellectual disabilities

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**SUPPLEMENTAL FIGURES**

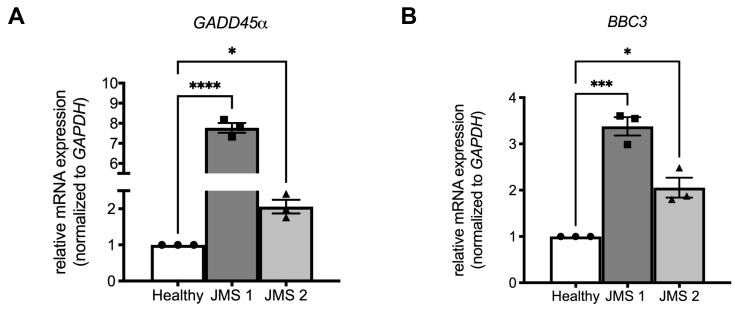


**Figure S1. Expression of p53 target genes in JMS patient cells, Related to Figure 2.** (**A and B**) mRNA levels of p53 target genes *GADD45α* (A) and *BBC3/PUMA* (B) in healthy control, JMS1 and JMS2 LCs, addressed by RT-qPCR. All error bars indicate mean ± SEM ($n \geq 3$, biological replicates). Statistic significance determined by one-way ANOVA with Bonferroni post-test; *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$, n.s $\geq 0.05$.

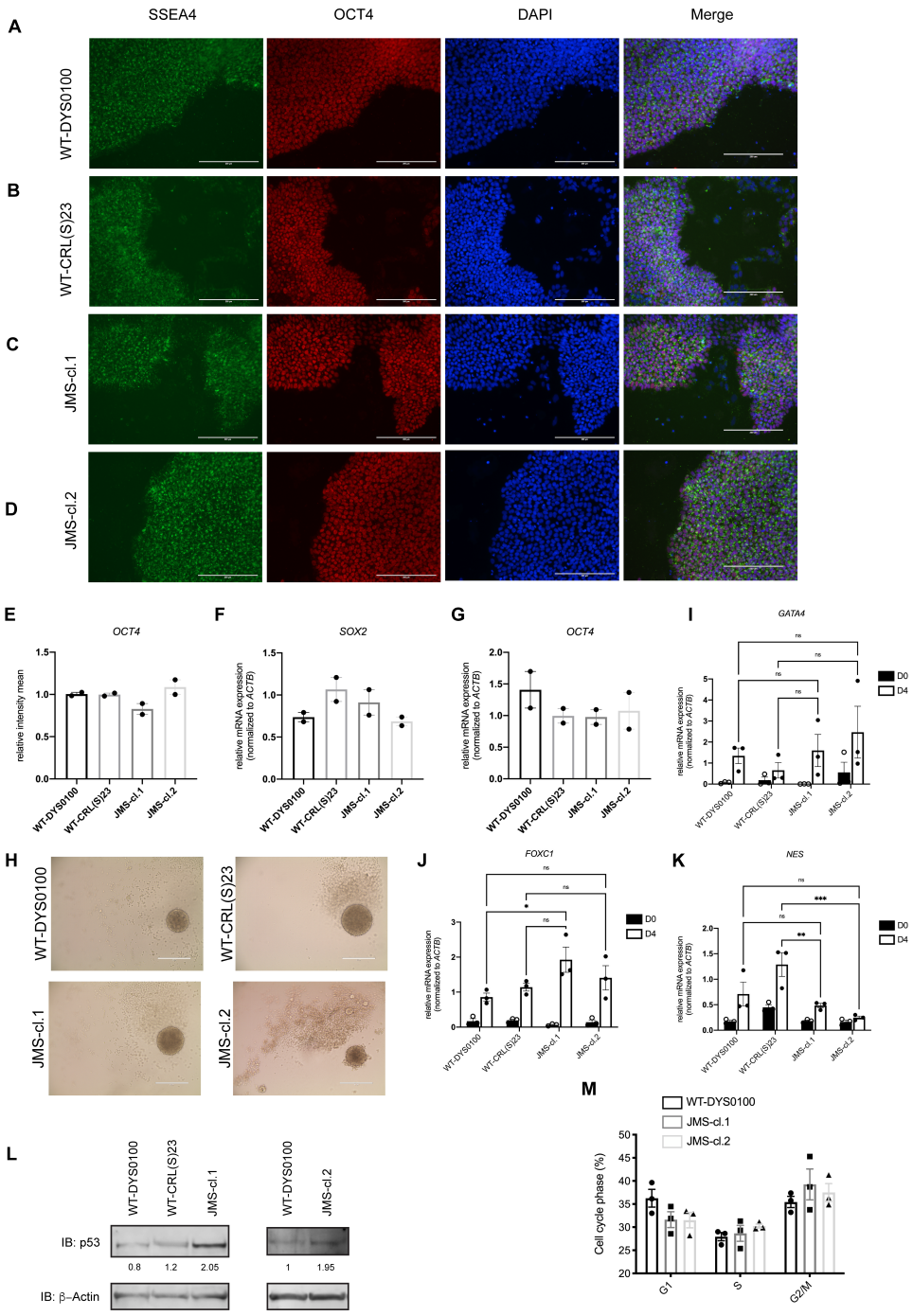


**Figure S2. Stemness markers and cell cycle progression are unaltered in JMS hiPSCs, Related to Figure 3.** (**A-D**) Expression of the pluripotency markers SSEA and OCT4 in (A) WT-DYS0100 (B) WT-CRL(S)23 (C) JMS clone 1 (JMS-cl.1) and (D) JMS-cl.2 hiPSCs by immunofluorescence. **(E)** Relative intensity mean of OCT4 signal in WT-DYS0100, WT-CRL(S)23, JMS-cl.1 and JMS-cl.2 hiPSCs (n=2, biological replicates). **(F-G)** RT-qPCR analysis of *SOX2* and *OCT4* expression (n=2, biological replicates). (**H**) Representative bright-field images of WT-DYS0100, WT-CRL(S)23, JMS-clone.1 and JMS-clone.2 EBs at day 3 after EB initiation (n=3, biological replicates). (**I-K**) RT-qPCR analysis of mRNA expression levels of *GATA4* (J), *FOXC1* (K) and *NES* (L) in WT and JMS EBs (collected at day (D) 0 and 4) (n = 3, biological replicates). (**L**) Immunoblot analysis of p53 levels in WT and JMS iPSCs. Protein levels relative to β-actin loading control are indicated. (**M**) Cell cycle distribution determined by flow cytometry of WT DYS0100, JMS-cl.1 and cl.2 iPSCs (n=3, biological replicates). Error bars indicate mean ± SEM; statistical significance was calculated using two-way ANOVA with Bonferroni post-test *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, n.s ≥ 0.05. Scale bar: 400µm.

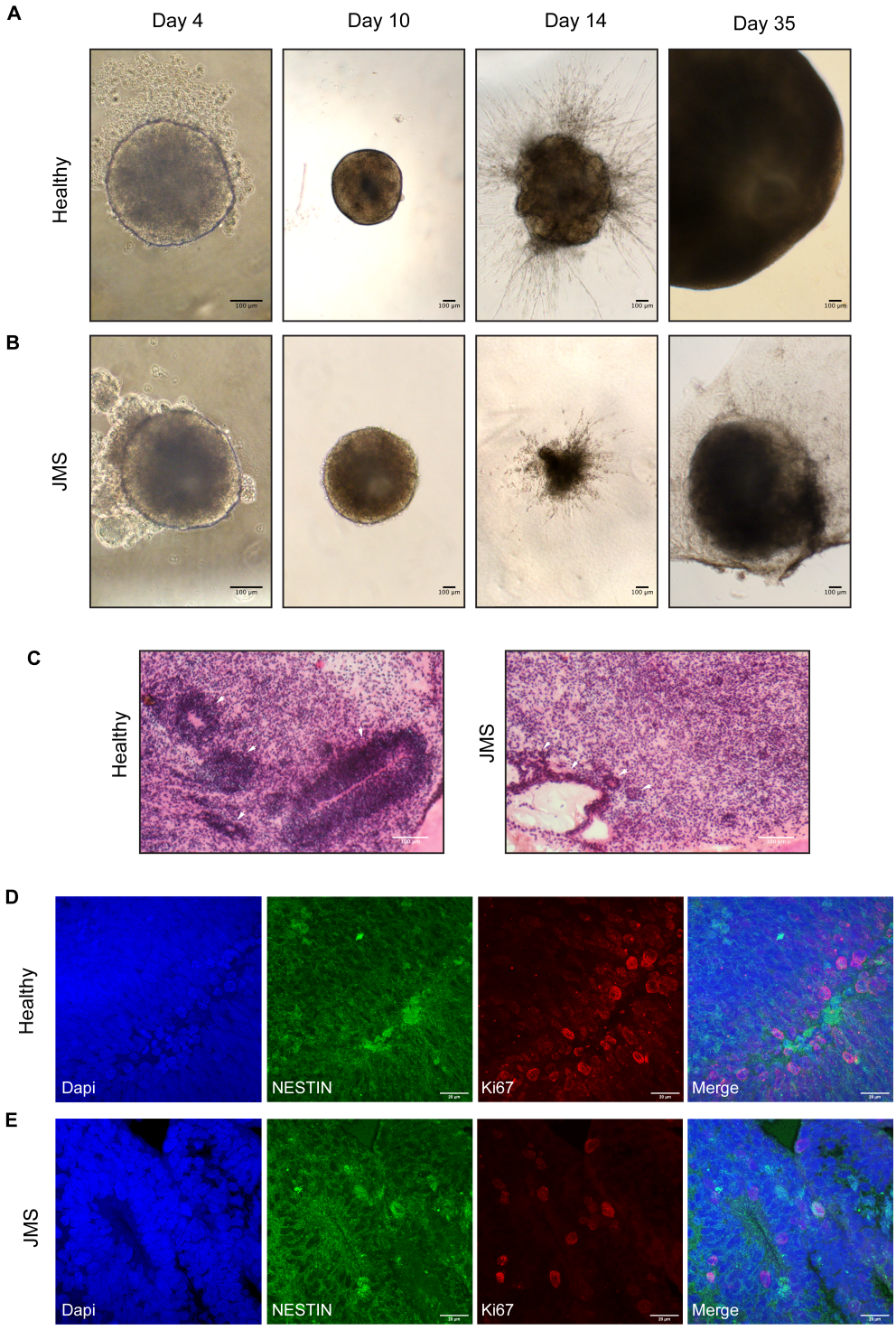


**Figure S3. Cerebral organoids derived from JMS hiPSCs are reduced in size and exhibit altered cellular organization, Related to Figure 3.** (**A and B**) Representative bright-field images of healthy control (A) and JMS (B) cerebral organoids at day 4, day 10, day 14 and day 35 of differentiation. Scale bar: 100µm. (**C**) Hematoxylin Eosin Saffron (HES) staining of 60 days old healthy and JMS cerebral organoid cryosections. Scale bar: 100 µm. (**D and E**) Representative immunofluorescence analysis of NESTIN and Ki67 in 60 days old healthy control (D) and JMS (E) cerebral organoids. Per batch five organoids were analyzed in healthy and two organoids in JMS condition (due to developmental failure in JMS). Scale bar: 20 µm.

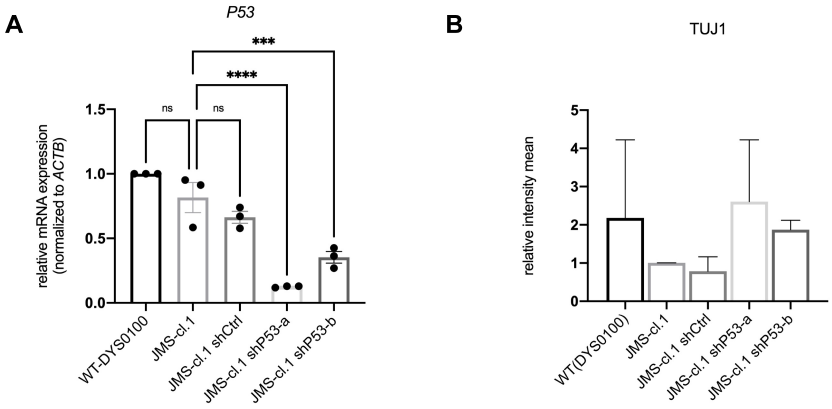


**Figure S4. Impact of p53 knock-down on neural differentiation of JMS hiPSCs, Related to Figure 4.** (**A**) Relative p53 mRNA levels in WT-DYS0100, JMS-cl.1. JMS-cl.1 expressing shRNA Control (shCtrl) or p53 targeting shRNA (shP53a and shP53b) (n=3, biological replicates). (**B)** Relative intensity of TUJ1 signal from experiments as the one in Figure 4E (n≥2, biological replicates). Error bars indicate mean ± SEM; statistical significance in (A) was calculated using one-way ANOVA with Dunett post-test; *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$, n.s $\geq 0.05$.

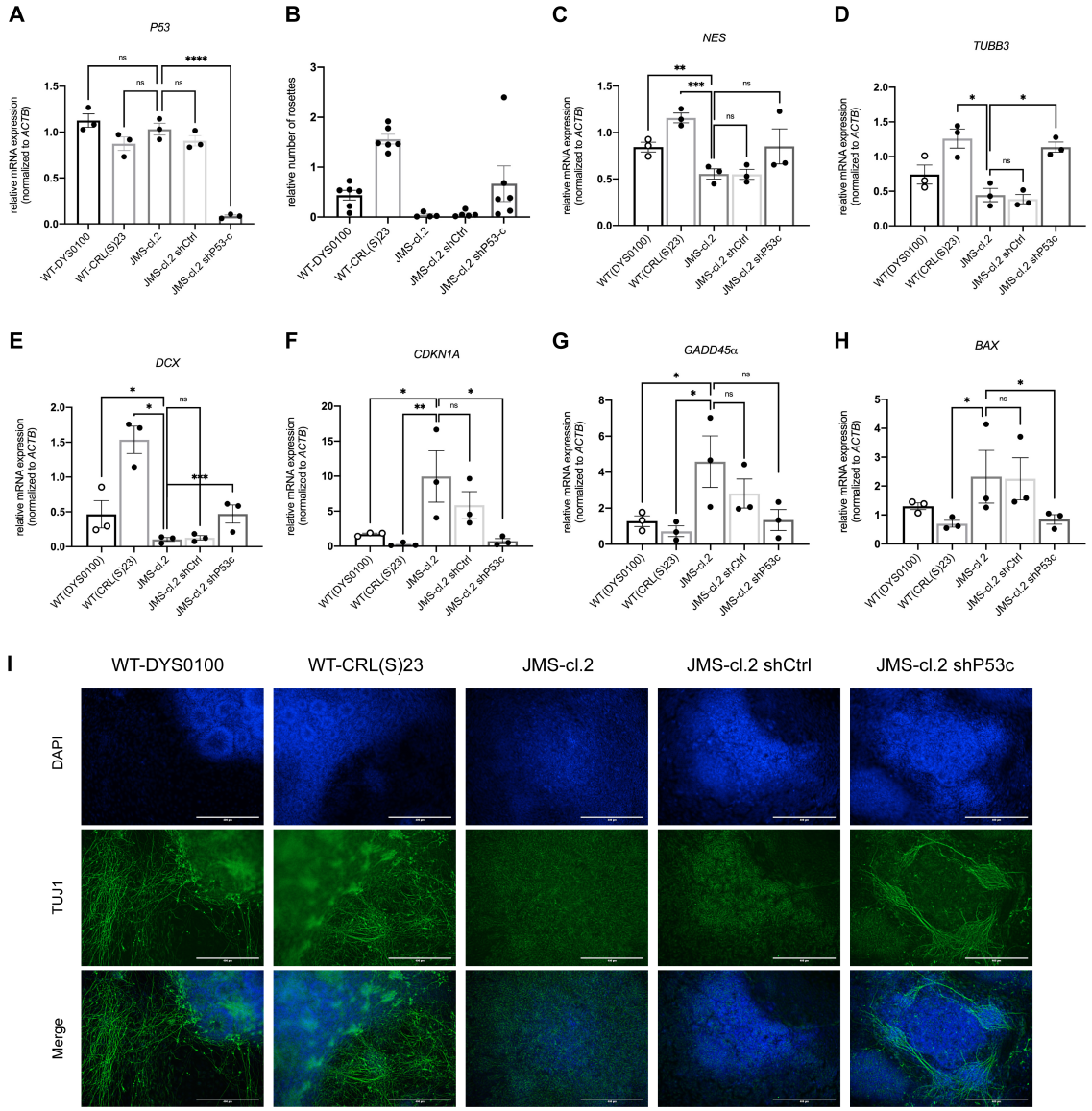


**Figure S5. p53 down-regulation rescues neurodevelopmental defects in XLID JMS, Related to Figure 4.** (**A**) Relative p53 mRNA levels in WT-DYS0100, WT-CRL(S)23, JMS-cl.2. JMS-cl.2 expressing shRNA Control (shCtrl) or p53 targeting shRNA (shP53c) (n=3, biological replicates). (**B**) Relative number of rosettes formed in WT-DYS0100 WT-CRL(S)23, JMS-cl.2 JMS-cl.2 shCtrl and JMS-cl.2 shP53c upon neural differentiation (n≥3, biological replicates). **(C-H)** RT-qPCR analysis of: *NES/NESTIN* (C), *TUBB3/TUJ1* (D), *DCX* (E) *CDKN1A/p21* (F), *GADD45α* (G) and *BAX* (H) upon neural differentiation of WT-DYS0100 WT-CRL(S)23, JMS-cl.2 JMS-cl.2 shCtrl and JMS-cl.2 shP53c (n=3, biological replicates). **(I)** Immunofluorescence analysis of TUJ1 in WT-DYS0100 WT-CRL(S)23, JMS-cl.2 JMS-cl.2 shCtrl and JMS-cl.2 shP53c at day 13 of neural differentiation. Error bars indicate mean ± SEM; statistical significance was calculated using one-way ANOVA with Dunett post-test (A); one-way ANOVA with Tukey post-test (C-H); *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$, n.s $\geq 0.05$.

**SUPPLEMENTAL TABLE**

**Table S1. Oligonucleotides used in the study, Related to Figure 1, 3, 4, S1, S2, S4 and S5.** (see STAR Method)

| **Sequence (indicated in 5'-3' direction)** | **Source** | ***Target*** |
|---|---|---|
| GGTACCCCTGCAACGCTTTGCTGC | Microsynth | *HECT_JMS Fw* |
| GCAGCAAAGCGTTGCAGGGGTACC | Microsynth | *HECT_JMS Rev* |
| GCAGGATCCTTCCATTGAGA | Microsynth | *GADD45α Fw* |
| AGCTCCTGCTCTTGGAGACC | Microsynth | *GADD45α Rev* |
| GTAAGGGCAGGAGTCCCAT | Microsynth | *BBC3/PUMA Fw* |
| GACGACCTCAACGCACAGTA | Microsynth | *BBC3/PUMA Rev* |
| TCTTTCCACCAGGCCCCCGGCTC | Microsynth | *OCT4 Fw* |
| TGCGGGCGGACATGGGGAGATCC | Microsynth | *OCT4 Rev* |
| GGCGCACCTCAAGATGTCC | Microsynth | *NES/NESTIN Fw* |
| CTTGGGGTCCTGAAAGCTG | Microsynth | *NES/NESTIN Rev* |
| GCAACTACGTGGGCGACT | Microsynth | *TUBB3/TUJ1 Fw* |
| TCGAGGCACGTACTTGTGAG | Microsynth | *TUBB3/TUJ1 Rev* |
| TCAGGGAGTGCGTTACATTTAC | Microsynth | *DCX Fw* |
| GTTGGGATTGACATTCTTGGTG | Microsynth | *DCX Rev* |
| CATGTTTTCTGACGGCAACTTC | Microsynth | *BAX Fw* |
| AGGGCCTTGAGCACCAGTTT | Microsynth | *BAX Rev* |
| GGCACTCAGAGGAGGCGCCAT | Microsynth | *CDKN1A/p21 Fw* |
| TAGCGCATCACAGTCGCGGC | Microsynth | *CDKN1A/p21 Rev* |
| GTGTCCCAGACGTTCTCAGTC | Sigma | *GATA4 Fw* |
| GGGAGACGCATAGCCTTGT | Sigma | *GATA4 Rev* |
| TGTTCGAGTCACAGAGGATCG | Sigma | *FOXC1 Fw* |
| ACAGTCGTAGACGAAAGCTCC | Sigma | *FOXC1 Rev* |
| GAGTCAACGGATTTGGTCGT | Microsynth | *GAPDH Fw* |
| TTGATTTTGGAGGGATCTCG | Microsynth | *GAPDH Rev* |
| GTTACAGGAAGTCCCTTGCCATCC | Microsynth | *ACTB/b-actin Fw* |
| CACCTCCCCTGTGTGGACTTGGG | Microsynth | *ACTB/b-actin Rev* |