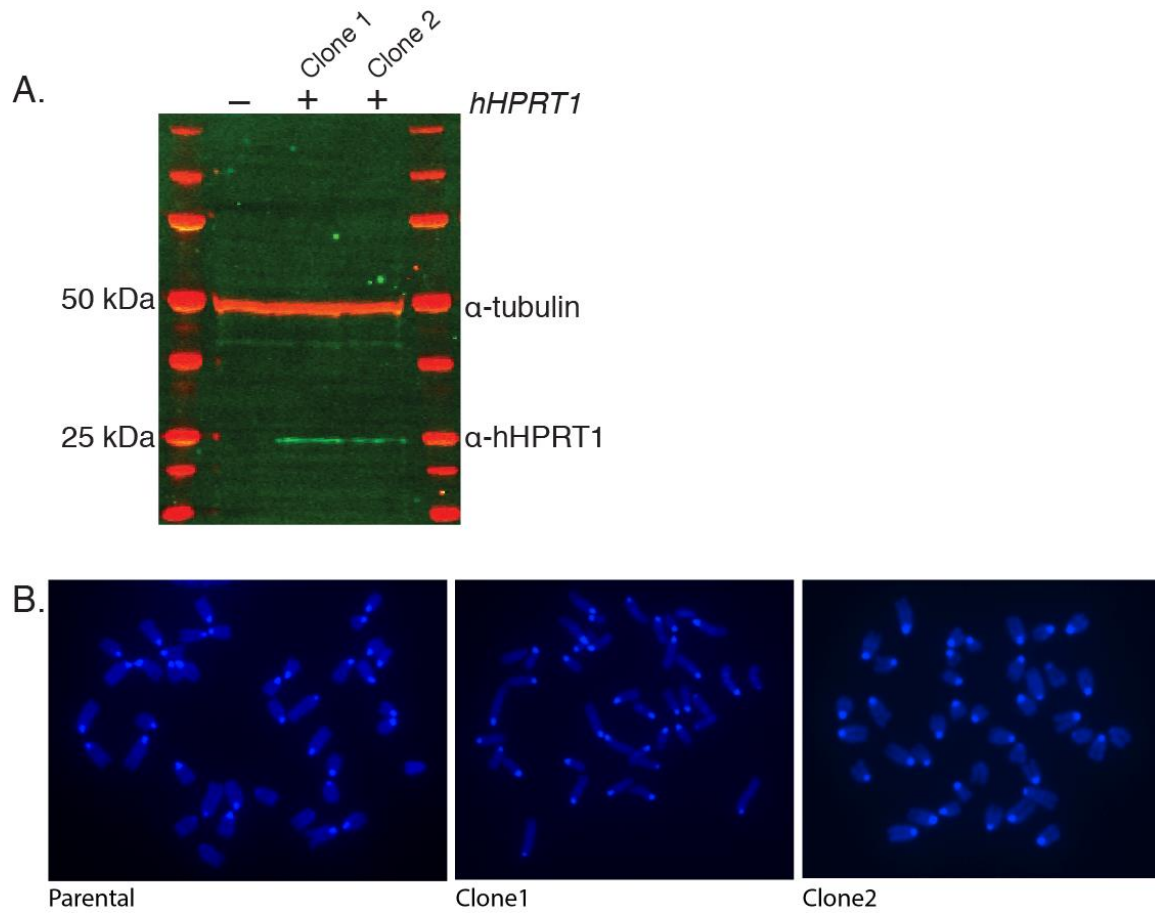


## Supplemental information



**Supplementary Fig. 1. *hHPRT1* clone characterization.** (a) Western blot analysis for *hHPRT1* protein expression in mouse ES cells using a human HPRT-specific monoclonal antibody. Parental mES (–) and *hHPRT1* clones 1 and 2 (+) were used. (b) Representative metaphase spreads from parental mESCs and *hHPRT1* clones 1 and 2.

**Supplemental Table 1: List of primers used to produce *HPRT1* amplicons**

For primer	Sequence	Rev primer	Sequence
HPR T.F1	gcggccgcTCCCATTACCATGGACAGTGTGCTT	HPRT .R1	gcggccgcGACCAGCATAATTCAGTACCCATGTT
HPR T.F2	gcggccgcGGTCAGTTACATGAAACAAGATGAAGTCA	HPRT .R2	gcggccgcTGTTTGTAATAGTTTCTAGGTACTATAATGGGA
HPR T.F3	gcggccgcGTCCATTAATTACTTCTTGATACTACTGACCT	HPRT .R3	gcggccgcGGGCTTGGAATCTTGACTAAAATTCTCC
HPR T.F4	gcggccgcCAGTAATATCCAGGGGTAATGGAGG	HPRT .R4	gcggccgcCAGTCACAAGTTAAGGTGAAAACA
HPR T.F5	gcggccgcGAAGGAAAGAGTACA AACTATTTGGTTCAG	HPRT .R5	gcggccgcGCACAATTCCTTCCTTGATGGT
HPR T.F6	gcggccgcATGATTCTGGGATACAAAATCCCTGG	HPRT .R6	gcggccgcTACAGTATCTTACACCCAAACCATCT
HPR T.F7	gcggccgcCCTAATTATTTCTAATACATGTCCTACCCC	HPRT .R7	gcggccgcGCTTAAATAATGCACCC TATCATTATTTAG
HPR T.F8	gcggccgcTCTTACATTGAGAAA AACTGTATTTTCAAT	HPRT .R8	gcggccgcCTTGTGTGTGTACTTTTCAATTAAGAAGTGA
HPR T.F9	gcggccgcCAGCTTCAGAATTCAACTTGCTTATAATTC	HPRT .R9	gcggccgcTACAGTGGTATAAGGTATGGGGACG
HPR T.F10	gcggccgcGTATGGCCTTAGACTATTCAGAACGTTA	HPRT .R10	gcggccgcacatgggggtgtaaaattgaaccataattgg
HPR T.F11	gcggccgcggggtcacagggaagactttg	HPRT .R11	gcggccgcACAGTACAGTCAGCAATGGA
HPR T.F12	gcggccgcGAAATGGTGTGCTGGAGCAAC	HPRT .R12	gcggccgcTTGGCAGAATTAAGTAAGTTGATGTTT
HPR T.F13	gcggccgcctcGAGTAATACCAGTTAAAAAATAGGCTAC	HPRT .R13	gcggccgcGAATGGGCAGAAATTGCTAGTTGG
HPR T.F14	gcggccgcCACAATTATCCAAGGAGATGGCG	HPRT .R14	gcggccgcATCTACCTTGATTAAGGCAGTGTGC
HPR T.F15	gcggccgcTATGCCTTTCCCACTAGATTTTAAGC	HPRT .R15	gcggccgcTATGCCAGATCCTCTAGGATTAATGC
HPR T.F16	gcggccgcAGCAAGGTTTGTATTTTCCTAGAACTTG	HPRT .R16	gcggccgcCAATCCCAAATCCAAACAGCCT
HPR T.F17	gcggccgcGATGACACTTCATGAGTTGACTATAATAATC	HPRT .R17	gcggccgcGCATATAGTATACATGCATAGCCAGTG
HPR T.F18	gcggccgcGTATTGAATGCTTGCA TTGTATGTCT	HPRT .R18	gcggccgcGCTTTGCTTATGTTTAA GATGTCATGC
HPR T.F19	gcggccgcATAAGATCAATTCTGAGTGGTAGAAATGc	HPRT .R19	gcggccgcCACCATAATGCAGACTAATTTTCCCTC
HPR T.F20	gcggccgcCCAAAAAACAACAAAGCCTCAGGA	HPRT .R20	gcggccgcTCAACCATTCTCTGTCC TTTAACTG
HPR T.F21	gcggccgcACCTGAGCATATGTCC TTTCATACTTA	HPRT .R21	gcggccgcCAAGGATATGTCCCTCAAAAGTCTAGC

HPR T.F22	gcggccgcCCATTGAATCTCCTGT AAGGGTTTTATTG	HPRT .R22	gcggccgcACTGCAGCCTATTGGTA GCCTA
HPR T.F23	gcggccgcTACTGGGACCTCATAC AAATGGGA	HPRT .R23	gcggccgcGGTCCAAGTGAATTGAA GAGGAAA
HPR T.F24	gcggccgcatCACTGTCTAACAGC CTCTCTTt	HPRT .R24	gcggccgcAACAGCATAGGTAAGG TGAGGAG
HPR T.F25	gcggccgcGATTCCAAAGCGGGT GAGGAAG	HPRT .R25	gcggccgcAACCCAAATGCTGCCTG TTGA
HPR T.F26	gcggccgcGCCCTTTCAGCTCTT TAAACATATA	HPRT .R26	gcggccgcCCAGTTTCACTAATGAC ACAAACATGC
HPR T.F27	gcggccgcAAGATACACTCCCCA AAAGTTACTGA	HPRT .R27	gcggccgcAGCCAGCAGAAAAATC TGAAGAG
HPR T.F28	gcggccgcAGTCCAGATGACTTGT ACATTAAACAC	HPRT .R28	gcggccgcGTGCCGAATTTGGTTAC TCCTTT
HPR T.F29	gcggccgcACCTGGCCTTTGGAAC TTGG	HPRT .R29	gcggccgcATCAGGGGGAAATGTT ATTTATCATGAA
HPR T.F30	gcggccgcaGTTTCTTCCAGGGTG GCTTCT	HPRT .R30	gcggccgcTGTCTTTGCCTGTGTTT TTAGGAA
HPR T.F31	gcggccgcAGCACCACAAAGTTA GAGGTCAA	HPRT .R31	gcggccgcAAGCATTATATCAGAAA CAGAAAAATAATCATC
HPR T.F32	gcggccgcCTCACTGAATGAGGC AGGTAGC	HPRT .R32	gcggccgcaagACTTATTTCTAGTAT TTTCTTCATGATCG
HPR T.F33	gcggccgcGGAGAGAGAAGGCAT AAACAATATTAATAA	HPRT .R33	gcggccgcaaaGCTGAGGAGAAAAA TAAAAAGAATAC
HPR T.F34	gcggccgcTTTGAGAAACAGCC CACCACC	HPRT .R34	gcggccgcaatGGTATGGGAGAATTG GGTTA
HPR T.F35	gcggccgctgCTGTGGATCGTTCA GCATGT	HPRT .R35	gcggccgccACGAAGCATAAATTTT TCCACAAA
HPR T.F36	gcggccgcGGTTATGTTCACTTCA TTTGGTTACAG	HPRT .R36	gcggccgcAAAGATAATAAAATAC CTCACATCATGAAtg
HPR T.F37	gcggccgcacGGTGTCTTAAATCTT TATGTGTTTG	HPRT .R37	gcggccgcCCATAATTAAATGTCTG TGTTCTGGTC
HPR T.F38	gcggccgcgcttcactctaaaatgatTGGAC CATg	HPRT .R38	gcggccgcCATGGTGAGCGAGGTG AGGC

**Supplemental Table 2: Yeast strains used in this study**

Yeast strain	Genotype	Description	Reference
BY4741	<i>MATa LYS2 met15Δ0 ura3Δ0 his3Δ1 leu2Δ0</i>	Wild-type lab strain for yeast assemblies	(BRACHMANN <i>et al.</i> 1998)
yLM1227	BY4741 + <i>HPRT1 step 1::URA3</i>	<i>HPRT1</i> assembly step 1, isolate 1	This study
yLM1228	BY4741 + <i>HPRT1 step 1::URA3</i>	<i>HPRT1</i> assembly step 1, isolate 2	This study
yLM1229	yLM1227 + <i>HPRT1 step 2::LEU2</i>	<i>HPRT1</i> assembly step 2, isolate 1	This study
yLM1231	yLM1228 + <i>HPRT1 step 2::LEU2</i>	<i>HPRT1</i> assembly step 2, isolate 2	This study
yLM1234	yLM1229 + <i>HPRT1 step3::URA3</i>	<i>HPRT1</i> assembly step 3, isolate 1	This study
yLM1235	yLM1231 + <i>HPRT1 step3::URA3</i>	<i>HPRT1</i> assembly step 3, isolate 2	This study

**Supplemental Table 3: Plasmids used in this study**

Plasmid	Description	Length (kb)	Reference
pLM453	eSwAP-In assembly vector	11	This study
pLM718, pLM719	<i>HPRT1</i> step 1, recovered from yLM1227 and yLM1228, respectively	46	This study
pLM747, pLM749	<i>HPRT1</i> step 2, recovered from yLM1229 and 1231, respectively	82	This study
pLM750, pLM751	<i>HPRT1</i> step 3, recovered from yLM1234 and yLM1235, respectively	112	This study
pLM707	ICE loxP-loxM cassette	4.5	(IACOVINO <i>et al.</i> 2011; IACOVINO <i>et al.</i> 2014)
pLM854	pLM453 + loxP-loxM cassette	13	This study
pLM848	pLM718 + loxP-loxM cassette	48	This study
pLM881	pLM747 + loxP-loxM cassette	84	This study
pLM886	pLM750 + loxP-loxM cassette	114	This study

**Supplemental Table 4: qRT-PCR primers**

Target	Forward primer	Rev primer
Mouse <i>Gapdh</i>	AGAACATCATCCCTGCATCC	CACATTGGGGGTAGGAACAC
Mouse <i>Nanog</i>	TTTCAGAAATCCCTTCCCTCG	TGATGAGGCGTTCCCAGAAT
Mouse <i>Esrrb</i>	CAGGCAAGGATGACAGACG	GAGACAGCACGAAGGACTGC
Mouse <i>Tcl1</i>	AAATTCCAGGTGATCTTGCG	TGTCCTTGGGGTACAGTTGC
Mouse <i>Gata6</i>	TTGCTCCGTAACAGCAGTG	GTGGTCGCTTGTGTAGAAGGA
Mouse <i>T</i>	GCTTCAAGGAGCTAACTAACGAG	CCAGCAAGAAAGAGTACATGGC
Mouse <i>Pax3</i>	GCAGCGCAGGAGCAGAACCA	GCACTCGGGCCTCGGTAAGC
Mouse <i>Hand1</i>	CCCCTCTTCCGTCCTCTTAC	CTGCGAGTGGTCACACTGAT
Human <i>HPRT1</i>	GACCAGTCAACAGGGGACAT	CCTGACCAAGGAAAGCAAAG

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