Title: Monoclonal antibody K312-based depletion of pluripotent cells from differentiated stem cell progeny prevents teratoma formation

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Running Title: Use of an antibody to isolate teratogenic cells

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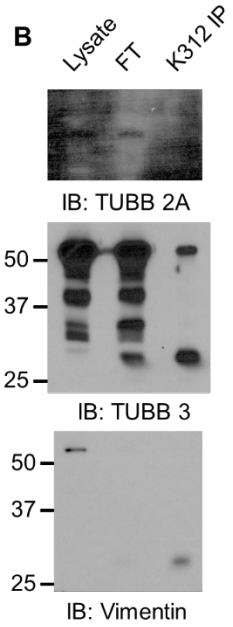
A

Identified Proteins (peptide sequence coverage, %)	Peptide sequence coverage (%)	Protein percentage of total spectra (%)
Tubulin beta-2A chain (TUBB 2A)	70	0.46
Tubulin beta chain	69	0.14
Actin, cytoplasmic 1	68	0.30
Tubulin beta-4B chain	66	0.015
Eukaryotic initiation factor 4A-I	62	0.18
Tubulin alpha-1B chain	61	0.31
Tubulin beta-3 chain (TUBB 3)	51	0.044
Elongation factor 1-alpha 1	49	0.22
cDNA FLJ11352 fis, clone HEMBA1000020, highly similar to Tubulin beta-2C chain	48	0.029
Poly(rC)-binding protein 1	44	0.080
Vimentin	33	0.087
Spliceosome RNA helicase DDX39B	32	0.058
Tubulin beta-6 chain	31	0.029
ATP synthase subunit beta, mitochondrial	29	0.073
Isoform 2 of Poly(rC)-binding protein 2	28	0.022
Alpha-enolase	23	0.044
Keratin, type I cytoskeletal 18	21	0.029
D-3-phosphoglycerate dehydrogenase	20	0.058

B



Supplemental Figure 1. Mass spectrometry analysis for identification of the K312 target protein. H9 hESC lysates were immunoprecipitated with the K312 antibody and a protein band visualized in the SDS-PAGE gel was analyzed by mass spectrometry. (A) A list of proteins identified from the analyzed peptide sequences. Candidate proteins are listed in order of highest peptide coverage. From the list, TUBB (Tubulin beta class I) 2A, TUBB 3, and vimentin were selected as candidate targets based on the molecular weight (~60 kDa). (B) H9 hESC lysates were immunoprecipitated with the K312 antibody, followed by immunoblotting analysis using an HRP-labeled secondary antibody targeting each candidate. None of the candidate proteins precipitated with K312 (FT: flow-through, K312 IP: immunoprecipitated with K312). The detected protein bands, about ~50 and ~25 kDa in size, are expected to be heavy and light chains of K312, respectively.

A

Identified Proteins (peptide sequence coverage, %)	Peptide sequence coverage (%)	Protein percentage of total spectra (%)
Tubulin beta-2B chain	62	0.37
Tubulin beta-2A chain	62	0.019
Tubulin beta-4B chain	59	0.029
Tubulin beta chain	56	0.029
Tubulin beta-4B chain	54	0.029
Tubulin alpha-1B chain	50	0.29
Tubulin beta-3 chain	35	0.038
Tubulin beta-6 chain	31	0.038
Vimentin	26	0.15
Elongation factor 1-alpha 1	23	0.077
ATP synthase subunit beta, mitochondrial	19	0.077

B

Identified Proteins (peptide sequence coverage, %)	Peptide sequence coverage (%)	Protein percentage of total spectra (%)
Tubulin beta-2B chain	52	0.37
Tubulin beta chain	52	0.019
Eukaryotic initiation factor 4A-I	47	0.029
Elongation factor 1-alpha 1	45	0.029
Alpha-enolase	45	0.029
Tubulin beta-3 chain	42	0.29
Tubulin alpha-1B	39	0.038
Actin, cytoplasmic 1	38	0.038
Tubulin beta-6 chain	34	0.15
ATP-dependent RNA helicase DDX39A	33	0.077
26S proteasome regulatory subunit 6B	32	0.077

Supplemental Figure 2. Additional mass spectrometry analysis for identification of the K312 target protein. (A and B) A list of proteins identified by mass spectrometry. Two additional mass spectrometry analyses were performed using protein bands immunoprecipitated with K312.

Supplemental Methods

Mass spectrometry analysis

To identify the target of K312, H9 hESCs (10^8 cells) were lysed using RIPA buffer and immunoprecipitated with K312-bound protein G agarose beads (Merck Millipore). K312-bound proteins were fractionated by SDS-PAGE and stained with Coomassie brilliant blue G-250 (BIO-RAD). The protein bands about ~60 kDa in size were carefully cut and fully destained with 30% methyl alcohol. Thereafter, the gel fragments were dehydrated in pure acetonitrile for 10 minutes and desiccated in a vacuum centrifuge. All fragments were further digested with trypsin (Promega) in 50 mM ammonium bicarbonate for 16 hours at 37 ˚C. Trypsinized proteins were concentrated using C18ZipTips (Millipore) and eluted with 50% (v/v) acetonitrile water. Peptide fragment mass spectrometry was carried out using a Q-TOF MS (Micromass), equipped with a nano-ESI source. The solution of protein fragments was sprayed at a 2 kV potential, leading to the production of molecular ions. To acquire peptide ions, the collision energy was raised from 10 eV to 30 eV for the collision-induced dissociation process. Argon was introduced as a collision gas at 10 psi. The Masslynx program (Micromass) was used for data trimming, and the MS-Tag searching program was used for identifying proteins corresponding to the fragment sequences.