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Authentication of Key Biological and/or Chemical Resources

Cell Lines

Identification of Cell Lines:
Cancer cell line SNU-1041 obtained from KCLB Cat# 01041, RRID:CVCL_L085 from a human male and
Cancer cell line PaCaDD-161 obtained from DSMZ Cat# ACC-746, RRID:CVCL_M466, from a human female, will be used in the proposed studies.

To verify that this is not a false cell line, misidentified, or is known to be an authentic stock we will periodically check the Cellosaurus web pages as we understand that this resource houses the most up to date information on cell line misidentification and works closely with cell line repositories, example page http://web.expasy.org/cellosaurus/CVCL_L085

Authentication of Cell Lines is based on guidelines from the International Cell Line Authentication Committee (iclac.org):
Authentication testing will be performed on established cell lines regardless of the application, and the test method and results included in the Materials and Methods section. Testing will be done, at minimum, at the beginning and end of experimental work.
For SNU-1041 and PaCaDD-161 human cell lines, short tandem repeat (STR) profiling will be performed and compared to results from donor tissue, or to online databases of human cell line STR profiles (ANSI/ATCC ASN-0002-2011 Authentication of Human Cell Lines: Standardization of STR Profiling. ANSI eStandard Store.)
For non-human cell lines, best practice will vary with the species being tested. At minimum, species should be confirmed using an appropriate method such as karyotyping, isoenzyme analysis, or mitochondrial DNA typing (DNA barcoding).

The SNU-1041 cell line was described in detail by Kim K.-H., Chung P.-S., Park H.-M., Rhee C.-S., Park J.-G.; "Establishment and characterization of cell lines derived from squamous cell carcinoma of the head and neck."; Korean J. Head Neck Oncol. 12:181-187(1996).

[For Cell Lines without a detailed publication please include the growth medium used, including additives; any additional growth requirements, including special substrates and gas mixtures; and the passage number or population doubling level (PDL) used for experimental work.
Passage number is important when working with early passage or finite cultures, or cell lines where changes in phenotype have been documented with increasing passage. ICLAC recommends that laboratories freeze down stocks when they first receive a cell line and set a limit (e.g. 20 passages) to avoid overpassaging. More information can be found at http://iclac.org/resources/advice-scientists/]

Antibodies used in Immunoprecipitation

Antibody Identification: FoxP2 antibody Abcam Cat# ab16046, RRID:AB_2107107; we will monitor this record periodically http://antibodyregistry.org/AB_775817 to determine if other authors who used this antibody raised issues.

Validation Techniques were selected according to the antibody validation pillars (Uhlen et al, Nat.Meth.2016) [we are including known standard relevant validation techniques as examples below, *indicates best option but this may not possible in all cases, citing your previous publications showing the validation technique was successfully used by your group is also important]:

[*Genetic method]: Complete or significant reduction in antibody signal after gene disruption or knockdown in the 129S6/SvEvTac-Foxp2^{tm1Momo}/MomoRbrc Mus musculus Cat# RBRC04060 genetic mutant mouse from RBRC stock center at Riken, Japan.
[Independent antibodies]: Correlation between two antibodies to the same target with non-overlapping epitopes across several samples preferentially with differential expression of the target protein. We will immunopercipitate with the Abcam antibody and then use the second antibody in a IP-western. We have yet to identify the second antibody that will be used for this purpose, but we will make sure that the antibody is distinct from the Abcam antibody by verifying that the epitope that the second antibody recognizes is different and if this is not possible then we will ask the antibody vendor about the original manufacturer of both antibodies.
[Mass Spec and IP]: Target binding will be confirmed with mass spectrometry, but this does not normally give conclusive information about cross-reactivity, therefore we will confirm cross reactivity with Western Blot.

Antibodies used in Histology and Immunohistochemistry

Antibody Identification: FoxP2 antibody Abcam Cat# ab16046, RRID:AB_2107107; we will monitor this record periodically http://antibodyregistry.org/AB_775817 to determine if other authors who used this antibody raised issues, some of this type of data is available in the comments section of the antibodyregistry.

Validation Techniques [examples]:
[*Genetic method]: Complete or significant reduction in antibody signal after gene disruption or knockdown in the 129S6/SvEvTac-Foxp2^{tm1Momo}/MomoRbrc Mus musculus Cat# RBRC04060 genetic mutant mouse from RBRC stock center at Riken, Japan.
[*Endogenous expression of tagged proteins]: Target binding is confirmed by co-localization, often combined with the use of tag-specific antibody to identify recombinant protein versus endogenous protein target.
[Independent antibodies]: Correlation between two antibodies to the same target with non-overlapping epitopes across several samples preferentially with differential expression of the target protein. We have yet to identify the second antibody that will be used for this purpose, but we will make sure that the antibody is distinct from the Abcam antibody by verifying that the epitope that the second antibody recognizes is different and if this is not possible then we will ask the antibody vendor about the original manufacturer of both antibodies.
[Orthogonal methods]: Correlation of protein abundance using an antibody-independent method across several samples with differential expression of the target protein.

All histology and immunohistochemistry will be performed by the {University} Histology core, directed by {Dr. Smith}, using standard methodologies.

Data about validation problems including lack of specificity or cross reactivity will be included in any resulting manuscript, as part of the antibody validation methods, if no manuscript is written, then relevant data will be submitted to a public data repository such as FigShare or antibodyregistry.org, and the catalog number as well as the RRID will be included in all publications, even single figures so that other authors may know that an issue has been raised about this antibody.

Antibodies used in Western Blotting

Antibody Identification: FoxP2 antibody Abcam Cat# ab16046, RRID:AB_2107107; we will monitor this record periodically http://antibodyregistry.org/AB_775817 to determine if other authors who used this antibody raised issues, some of this type of data is available in the comments section of the antibodyregistry.

Validation Techniques [examples]:
[Genetic method]: Complete or significant reduction in antibody signal after gene disruption or knockdown in the 129S6/SvEvTac-Foxp2^{tm1Momo}/MomoRbrc Mus musculus Cat# RBRC04060 genetic mutant mouse from RBRC stock center at Riken, Japan.
[Independent antibodies]: Correlation between two antibodies to the same target with non-overlapping epitopes across several samples preferentially with differential expression of the target protein. We have yet to identify the second antibody that will be used for this purpose, but we will make sure that the antibody is distinct from the Abcam antibody by verifying that the epitope that the second antibody recognizes is different and if this is not possible then we will ask the antibody vendor about the original manufacturer of both antibodies.
[Endogenous expression of tagged proteins]: Target binding is confirmed by co-localization, often combined with the use of tag-specific antibody to identify recombinant protein versus endogenous protein target.
[Orthogonal methods]: Correlation of protein abundance using an antibody-independent method across several samples with differential expression of the target protein.

Data about validation problems including lack of specificity or cross reactivity will be included in any resulting manuscript, as part of the antibody validation methods, if no manuscript is written, then relevant data will be submitted to a public data repository such as FigShare or antibodyregistry.org, and the catalog number as well as the RRID will be included in all publications, even single figures so that other authors may know that an issue has been raised about this antibody.

Antibodies used in Flow Cytometry

Antibody Identification: FoxP2 antibody Abcam Cat# ab16046, RRID:AB_2107107; we will monitor this record periodically http://antibodyregistry.org/AB_775817 to determine if other authors who used this antibody raised issues, some of this type of data is available in the comments section of the antibodyregistry.

Validation Techniques:

[*Orthogonal methods]: Correlation of protein abundance using an antibody-independent method across several samples with differential expression of the target protein.
[Genetic method]: Complete or significant reduction in antibody signal after gene disruption or knockdown in the 129S6/SvEvTac-Foxp2^{tm1Momo}/MomoRbrc Mus musculus Cat# RBRC04060 genetic mutant mouse from RBRC stock center at Riken, Japan.
[Independent antibodies]: Correlation between two antibodies to the same target with non-overlapping epitopes across several samples preferentially with differential expression of the target protein. We have yet to identify the second antibody that will be used for this purpose, but we will make sure that the antibody is distinct from the Abcam antibody by verifying that the epitope that the second antibody recognizes is different and if this is not possible then we will ask the antibody vendor about the original manufacturer of both antibodies.
[Endogenous expression of tagged proteins]: Target binding is confirmed by co-localization, often combined with the use of tag-specific antibody to identify recombinant protein versus endogenous protein target.

Data about validation problems including lack of specificity or cross reactivity will be included in any resulting manuscript, as part of the antibody validation methods, if no manuscript is written, then relevant data will be submitted to a public data repository such as FigShare or antibodyregistry.org, and the catalog number as well as the RRID will be included in all publications, even single figures so that other authors may know that an issue has been raised about this antibody.

Antibodies used in Sandwich Assays

Antibody Identification: FoxP2 antibody Abcam Cat# ab16046, RRID:AB_2107107; we will monitor this record periodically http://antibodyregistry.org/AB_775817 to determine if other authors who used this antibody raised issues, some of this type of data is available in the comments section of the antibodyregistry.

Validation Techniques [examples]:
[Genetic method]: Complete or significant reduction in antibody signal after gene disruption or knockdown in the 129S6/SvEvTac-Foxp2^{tm1Momo}/MomoRbrc Mus musculus Cat# RBRC04060 genetic mutant mouse from RBRC stock center at Riken, Japan.
[Independent antibodies]: Correlation between two antibodies to the same target with non-overlapping epitopes across several samples preferentially with differential expression of the target protein. We have yet to identify the second antibody that will be used for this purpose, but we will make sure that the antibody is distinct from the Abcam antibody by verifying that the epitope that the second antibody recognizes is different and if this is not possible then we will ask the antibody vendor about the original manufacturer of both antibodies.
[Orthogonal methods]: Correlation of protein abundance using an antibody-independent method across several samples with differential expression of the target protein.

Data about validation problems including lack of specificity or cross reactivity will be included in any resulting manuscript, as part of the antibody validation methods, if no manuscript is written, then relevant data will be submitted to a public data repository such as FigShare or antibodyregistry.org, and the catalog number as well as the RRID will be included in all

publications, even single figures so that other authors may know that an issue has been raised about this antibody.

Antibodies used in Reverse phase protein arrays

Antibody Identification: FoxP2 antibody Abcam Cat# ab16046, RRID:AB_2107107; we will monitor this record periodically http://antibodyregistry.org/AB_775817 to determine if other authors who used this antibody raised issues, some of this type of data is available in the comments section of the antibodyregistry.

Validation Techniques [examples]:
[Genetic method]: Complete or significant reduction in antibody signal after gene disruption or knockdown in the 129S6/SvEvTac-Foxp2^{tm1Momo}/MomoRbrc Mus musculus Cat# RBRC04060 genetic mutant mouse from RBRC stock center at Riken, Japan.
[Independent antibodies]: Correlation between two antibodies to the same target with non-overlapping epitopes across several samples preferentially with differential expression of the target protein. We have yet to identify the second antibody that will be used for this purpose, but we will make sure that the antibody is distinct from the Abcam antibody by verifying that the epitope that the second antibody recognizes is different and if this is not possible then we will ask the antibody vendor about the original manufacturer of both antibodies.
[Orthogonal methods]: Correlation of protein abundance using an antibody-independent method across several samples with differential expression of the target protein.

Data about validation problems including lack of specificity or cross reactivity will be included in any resulting manuscript, as part of the antibody validation methods, if no manuscript is written, then relevant data will be submitted to a public data repository such as FigShare or antibodyregistry.org, and the catalog number as well as the RRID will be included in all publications, even single figures so that other authors may know that an issue has been raised about this antibody.

Key Chemical resources for all experiments

Identification of Key Chemical resources:
KAPA Library Amplification Kit (Kappa Biosystems Cat# KK2611)
SUPERase-In RNase Inhibitor (Life Technologies Cat# AM2694)
Illumina TruSeq small RNA cloning adapters and barcoded primers (Illumina Cat# RS-200-0012)

Validation of Key Chemical resources for all experiments

All new lots of specialty chemicals purchased from commercial vendors will be subjected to basic chemical validation methods, including Mass Spec, measuring melting point or boiling point, as appropriate, to determine if the chemical sold fulfills the purity stated in the material data sheet. Additionally, new batches of chemicals will be run along side of the existing batch for the same samples so that lot variability can be controlled for.

As our laboratory does not currently have all needed equipment, all Chemical validation experiments including Mass Spec will be performed by the chemical core facility at [University Name] by chemists.