mSA1| Validation File

TARGET mouse SA1 (also known as STAG1)
CLONE NAME SUSI63B
DESCRIPTION Rat monoclonal
ANTIGEN USED mSA1-MBP recombinant protein (N terminal fragment 225 aa)
ISOTYPE IgG2a
SPECIES REACTIVITY mouse and human
LOCALIZATION nuclear
POSITIVE CONTROL MOLT4 cell line
STORAGE BUFFER Tissue culture supernatant: 0.02% sodium azide
Purified antibody: PBS plus 1%BSA and 0.02% sodium azide. MAb concentration: 1mg/ml
STORAGE Aliquot and store at 4ºC. Do not freeze

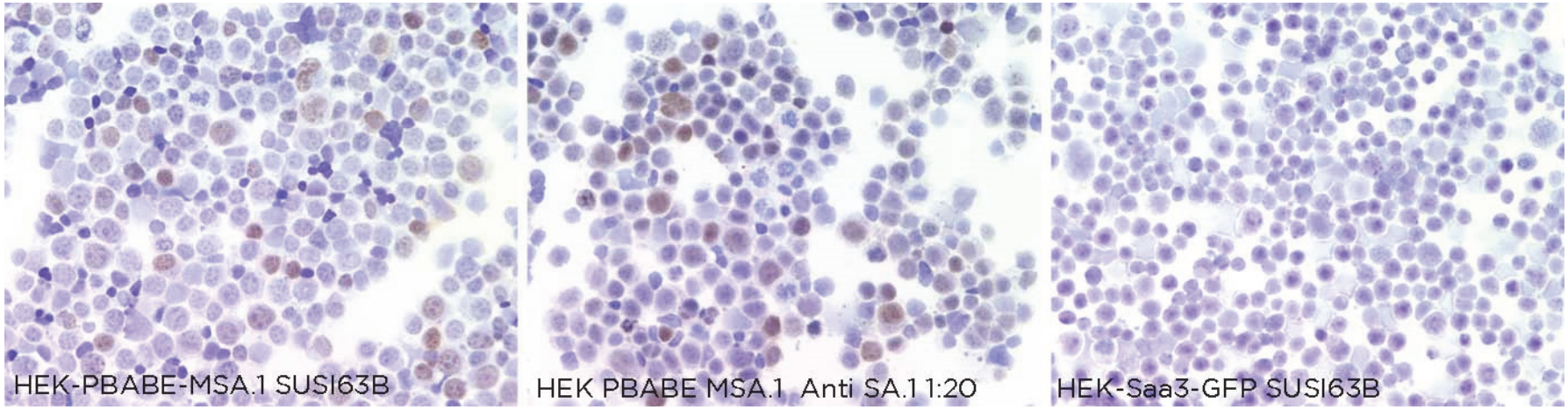
Recommended Inconclusive Not Recommended Not Tested

APPLICATIONS

| ICC | ***Immunocytochemistry***

SUSI63B is able to detect mouse SA1 protein in immunocytochemistry

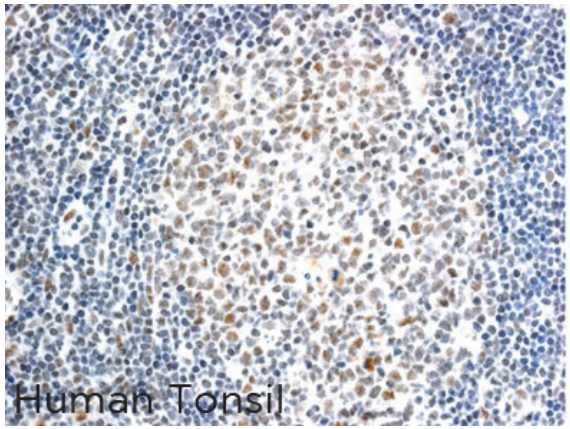
To confirm that SUSI63B mAb recognizes mouse SA1 protein, immunocytochemistry on frozen cytospin preparations of mouse SA1 expressed in HEK293T was performed. Anti-SA1 rabbit polyclonal antibody was used as a positive control. Cytospin preparation of human GFP-tagged Saa3 protein was used as a negative control.

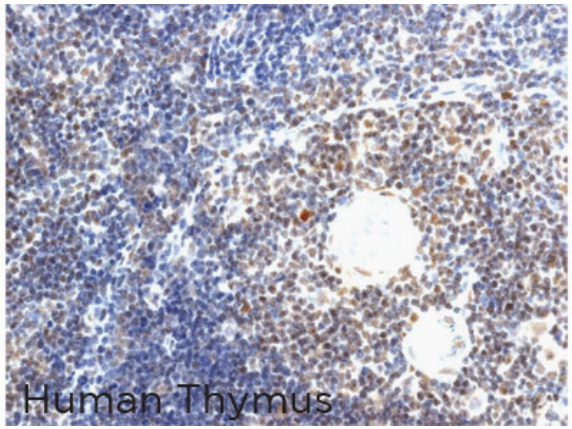


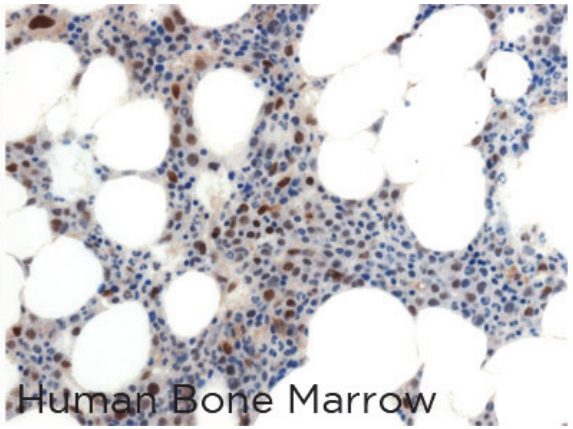
| IHC-P | ***Immunohistochemistry (paraffin)***

SUSI63B mAb can be used to detect SA1 protein in human paraffin tissues

TISSUE SAMPLE human tonsil, thymus and bone marrow
DILUTION neat supernatant
ANT. RETRIEVAL 20 minutes ER2 (Tris-EDTA)
DETECTION SYSTEM Novolink kit (BondMax Leica)





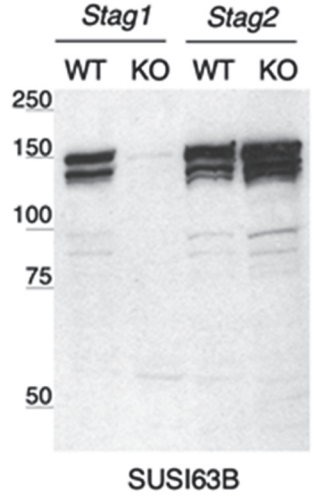


| WB | ***Western Blotting***

SUSI63B mAb is able to detect endogenous mouse and human SA1 protein by WB.
It does not recognize SA2

DILUTION No dilution (neat supernatant)

Predicted molecular weight: ***144 kDa***
Observed molecular weight: ***144 kDa***



LANES

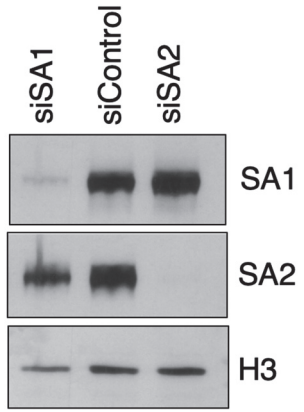
Lane 1 WMEF *Stag1* WT (10 µg) (+)
Lane 2 WMEF *Stag1* KO (10 µg) (-)
Lane 3 WMEF *Stag2* WT (10 µg) (+)
Lane 4 WMEF *Stag2* KO (10 µg) (+)

WMEF: Whole mouse embryo fibroblast extract

LANES

Lane 1 WEHC transfected with siRNA against SA1 (10 µg) (-)
Lane 2 WEHC treated with control siRNA (10 µg) (+)
Lane 3 WEHC treated with siRNA against SA2 (10 µg) (+)

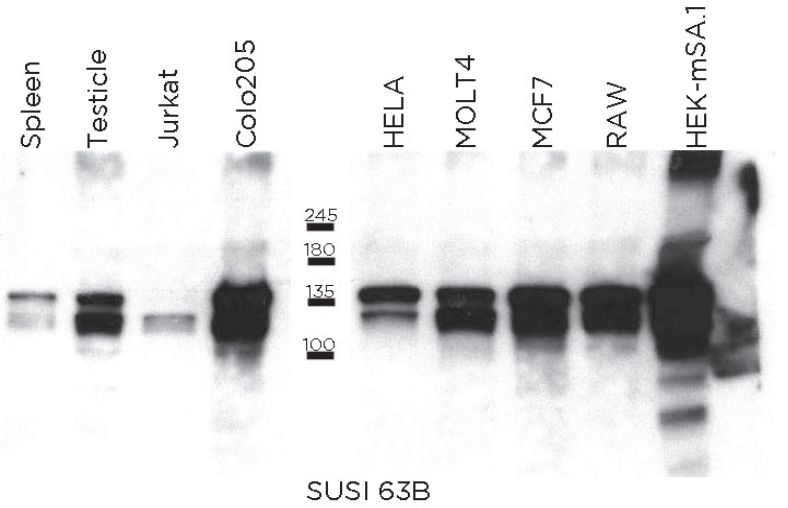
WEHC: Whole extract from HeLa cells



Membrane was probed with SUSI63B (undiluted) to detect SA1, a rabbit polyclonal antibody to detected SA2 and a rabbit polyclonal antibody against H3 (loading control)

LANES

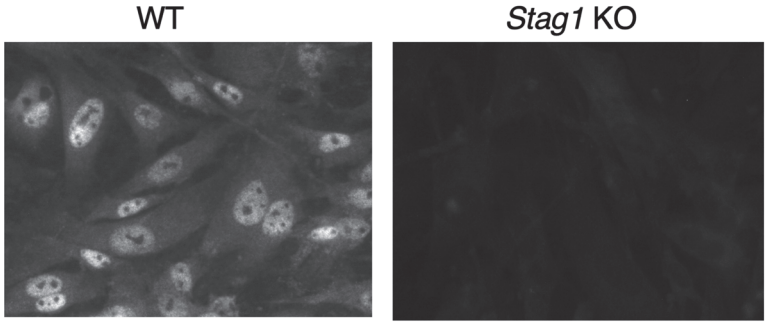
Lane 1 Human spleen	(100 µg) (+)
Lane 2 Human testicle	(100 µg) (+)
Lane 3 Jurkat cell line	(100 µg) (+)
Lane 4 Colo205 cell line	(100 µg) (+)
Lane 5 HeLa cell line	(100 µg) (+)
Lane 6 Molt-4 cell line	(100 µg) (+)
Lane 7 MCF-7 cell line	(100 µg) (+)
Lane 8 RAW cell line	(100 µg) (+)
Lane 9 HEK293-mSA1 transfected cells	(5 µg) (+)



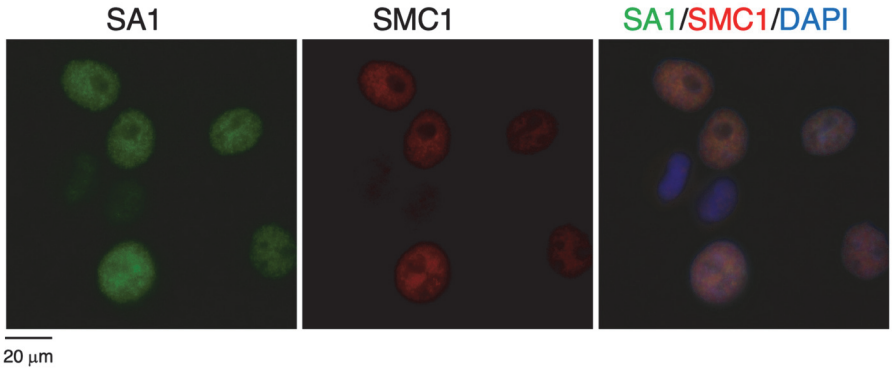
| IF | ***Immunofluorescence***

SUSI63B mAb can be used to detect SA1 protein by immunofluorescence in mouse and human cells.

SAMPLE Mouse embryo fibroblasts (MEFs) from wild type (WT) or *Stag1* KO embryos fixed in paraformaldehyde.
DILUTION No dilution (neat supernatant)



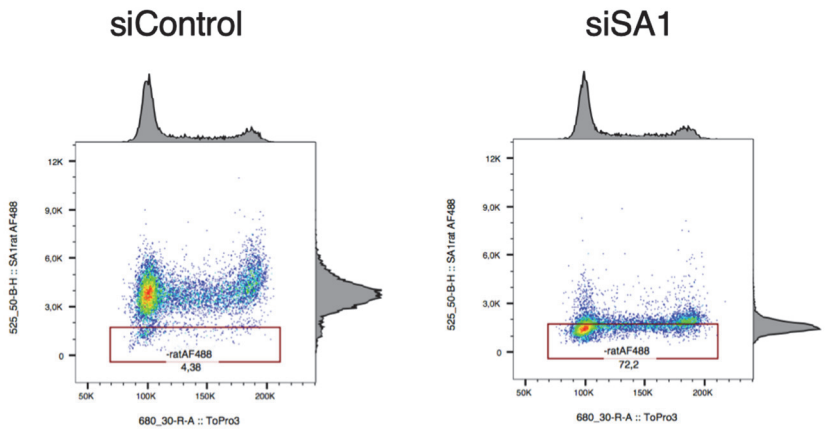
SAMPLE HeLa cells fixed with paraformaldehyde and permeabilized with 0.2% Triton X-100
DILUTION No dilution (neat supernatant)



| FC | ***Flow Cytometry***

SUSI63B mAb can be used to detect human SA1 protein by flow cytometry

SAMPLE HeLa cells mock transfected or transfected with siRNA against hSA1
DILUTION: No dilution (neat supernatant)



| IHC-F | ***Immunohistochemistry (frozen) Not tested***

| IP | ***Immunoprecipitation***

REFERENCES

De Koninck M, Lapi E, Badía-Careaga C, Cossío I, Giménez-Llorente D, Rodríguez-Corsino M, Andrada E, Hidalgo A, Manzanares M, Real FX, Losada A (2020). Essential Roles of Cohesin STAG2 in Mouse Embryonic Development and Adult Tissue Homeostasis.. Cell Rep 32, 108014.

Cuadrado A, Giménez-Llorente D, Kojic A, Rodríguez-Corsino M, Cuartero Y, Martin-Serrano G, Gómez-López G, Marti-Renom MA, Losada A (2019). Specific Contributions of Cohesin-SA1 and Cohesin-SA2 to TADs and Polycomb Domains in Embryonic Stem Cells.. Cell Rep 27, 3500-3510

Kojic A, Cuadrado A, De Koninck M, Giménez-Llorente D, Rodríguez-Corsino M, Gómez-López G, Le Dily F, Marti-Renom MA, Losada A (2018). Distinct roles of cohesin-SA1 and cohesin-SA2 in 3D chromosome organization. Nat Struct Mol Biol 25, 496-504.