Importin-β facilitates nuclear import of human GW proteins and balances cytoplasmic gene silencing protein levels

Daniel Schraivogel, Susann G. Schindler, Johannes Danner, Elisabeth Kremmer, Janina Pfaff, Stefan Hannus, Reinhard Depping & Gunter Meister

Supplemental Figure legends

Figure S1: Ago2 localization in 26 different human cell lines from various tissue origins. (A) Confocal microscopy with anti-Ago2 and anti-Lsm4 antibody in 23 different cell lines. (B) Confocal microscopy with anti-Ago2 antibody in HPAEC, HPASMC and primary keratinozytes. (C) Ago2 signal quantification from nucleus and cytoplasm of cell lines shown in (A) and (B) were used to calculate nucleus-to-cytoplasm ratio in different cell lines. (C) Heterokaryon assays were performed (two biological replicates, n = 2) as described in Fig. 1C with a HEK 293T cell line stably transfected with inducible eGFP-Ago2. eGFP-Ago2 expression was induced 24 hours prior to addition of NIH 3T3. Data are represented as mean ± SEM. *, $p<0.05$. Related to Fig. 1.

Figure S2: Ago2 accumulates in the nucleus upon inhibition of Crm1-mediated export. HeLa cells were treated with Leptomycin B (lower panels) or left untreated (upper panels) and Ago2 was detected with monoclonal anti-Ago2 antibody. Shown are the same samples as in Fig. 1 with an expanded field of view. Related to Fig. 1.

Figure S3: Ago2 and TNRC6B accumulate in the nucleus upon knock down of Crm1. (A, B) Crm1 was knocked down and localization of endogenous Ago2 (A) or TNRC6B (B) was analyzed by confocal microscopy using monoclonal antibodies. (C) Crm1 protein levels in siCrm1 transfected cells (lane 1) or cells transfected with a control siRNA (lane 2) were detected by Western Blot with anti-Crm1 antibody. Error bars indicate standard error of the mean. *, $p<0.05$. Related to Fig. 1.

Figure S4: Nuclear TNRC6A foci co-localize with Ago2 but not with any nuclear protein tested. (A) to (E) HEK 293T cells with stable inducible myc-GFP-TNRC6A NES-mut were co-stained with different antibodies against RISC components, factors associated with protein degradation, nuclear compartments, transcription, double-strand break repair and others. Anti-TNRC6ABC clone 7A9 detects all three TNRC6 proteins. (F) Tabular summary of the data presented in (A) to (E). Related to Fig. 3.

Figure S5: Import of myc-GFP-TNRC6A NES-mut requires Impβ. Impβ was knocked down using two different siRNAs and localization of myc-GFP-TNRC6A NES-mut was determined by detection of GFP signal. Shown are the same cells as in Fig. 4 with an expanded field of view. Related to Fig. 4.

Figure S6: Importin-α binds to TNRC6A. *In vitro* transcribed and translated TNRC6A was incubated with recombinant GST (lane 1), GST-tagged Impα-family members (lanes 2 to 5) or Impβ-GST (lane 6). After GST-pulldown TNRC6A was detected by autoradiography, recombinant proteins by Coomassie staining. Lane 7 shows TNRC6A input. Related to Fig. 5.

Supplemental methods

Transfections and RNA interference

Plasmid transfections were done using Lipofectamine 2000 (Life Technologies) according to the manufacturer's forward transfection protocol: 2.5 µg DNA and 10 µl transfection reagent were used per 6-well well. For coimmunoprecipitations, HEK or HEK TRex FLP/IN myc-GFP-TNRC6A were transfected with plasmids using calcium phosphate method. For RNA interference experiments, siRNA transfections were done with Lipofectamine RNAiMAX (Life Technologies) according to the manufacturer's reverse transfection protocol: 40 nM final siRNA and 5 µl transfection reagent were used per 6-well well. Plasmid transfections were analyzed 48 h post-transfection. RNAi was analyzed 72 h post-transfection, except for knockdowns of 7SK snRNA, which were analyzed 24 h post-transfection.

All siRNAs were designed with Dharmacon siRNA Design Center (http://dharmacon.gelifesciences.com/design-center/) and Whitehead siRNA selection program (http://sirna.wi.mit.edu/). Importin siRNA library was synthesized as described before (1), all other siRNAs were ordered at Biomers. siPools were designed by and ordered at siTool Biotech (2). The siRNA sequences were: si1 Impβ (NM_002265) 5'-CUGAAUGAGCUAAGGGAAAUT, 5'-UUUCCCUUAGCUCAUUCAGUT; si2 Impβ 5'-GGAAAGAAGAGCCUAGUAAUT, 5'-UUACUAGGCUCUUCUUUCCUT; si1 Imp3 (NM_013433) 5'-GGGCAGAGAUGCAGCCUUAUT, 5'-UAAGGCUGCAUCUCUGCCCUT; si2 Imp3 5'-GGGAUGAAGUACUCGGAAAUT, 5'-UUUCCGAGUACUUCAUCCCUT; si Imp4 (NM_024658) 5'-UGCGAUACGCAUAGCUAUUUT, 5'-AAUACGUAUGCGUAUCGCAUT; si1 Imp5 (NM_002271) 5'-GAGAAAUGCACGAGGCAAUUT, 5'-AUUGCCUCGUGCAUUUCUCUT; si2 Imp5 5'-

GUGCAAAAUCCUUGGAAAAUT, 5’-UUUUCCAAGGAUUUUGCACUT; si1 Imp7 (NM_006391) 5’-GGAAAGAGGUACUGCAAAAUT, 5’-UUUUGCAGUACCUCUUUCCUT; si2 Imp7 5’-GGAAGAAGAUGAUGCUGAAUT, 5’-UUCAGCAUCAUCUUCUUCCUT; si1 Imp8 (NM_006390) 5’-ACAAUAGUGUGGAUGGAUAUT, 5’-UAUUCAUCCACACUAUUGUUT; si2 Imp8 5’-UGAGCUCAAUCUAAGAAAUUT, 5’-AUUUCUUAGAUUGAGCUCAUT; si1 Imp9 (NM_018085) 5’-CCUAAUGGGUUGAGAGAAUUT, 5’-AUUCUCUCAACCCAUUAGGUT; si2 Imp9 5’-CCACAGAUUUCCAGAAUGAUT, 5’-UCAUUCUGGAAAUCUGUGGUT; si1 Imp11 (NM_016338) 5’-GGAAGAUGGUUUAGAAUUAUT, 5’-UAAUUCUAAACCAUCUUCCUT; si2 Imp11 5’-GAUAAUGUGUGUAGAGAUAUT, 5’-UAUCUCUACACACAUUAUCUT; si1 Imp12 (NM_012470) 5’-ACGUGAAGAUUUAGACAAAUT, 5’-UUUGUCUAAAUCUUCACGUUT; si2 Imp12 5’-GCACAGAAAUUAUAGAAGAUT, 5’-UCUUCUAUAAUUUCUGUGCUT; si1 Imp13 (NM_014652) 5’-CCCUUCUGAUGAGGAAUAUUT, 5’-AUAUUCCUCAUCAGAAGGGUT; si2 Imp13 5’-GGGAAAGGUGGUACAGGAAUT, 5’-UUCCUGUACCACCUUUCCCUT; si1 Tnpo1 (NM_002270) 5’-GUAUAGAGAUGCAGCCUUAUT, 5’-UAAGGCUGCAUCUCUAUACUT; si2 Tnpo1 5’-GCCACAGGUAUUCAUUUAUUT, 5’-AUAAAUGAAUACCUGUGGCUT; si 7SK (NM_001445) 5‘-CCUCCAAACAAGCUCUCAAUT, 5‘-UUGAGAGCUUGUUUGGAGGUT; si Crm1 (NM_003400) 5‘-GGACAAGAGUCGACACAAUTT, 5‘-AUUGUGUCGACUCUUGUCCTT. Imp4 and Imp8 sequences were taken from (3).

Plasmids

Following plasmids have been previously published: pCS2-Ago2 (3), pCS2-GFP (4), pIRES-Flag/HA-Ago2 (5), pIRES-Flag/HA-Ago2 Y529E (6), pIRES-Flag/HA (5), pIRES-Flag/HA-FA (6), pIRES-Flag/HA-SV40NLS-Ago2 (3), pOG44 (Life Technologies), pmyc-GFP-TNRC6A (1709 aa TNRC6A,(7)), pmyc-GFP-TNRC6A NLS-mut (7), pmyc-GFP-TNRC6A NES-mut (7), pmyc-GFP-TNRC6A ΔGW (7), pmyc-GFP-TNRC6A ΔGW NES-mut (7), pIRES-Flag/HA-TNRC6B (TNRC6B 1723 aa variant, (8), pFRT/TO/FLAG/HA-DEST TNRC6C (9).

pIRES-Flag/HA-TNRC6A was cloned by amplifying TNRC6A open reading frame (ORF) from pmyc-GFP-TNRC6A and insertion into pIRES-Flag/HA-FA via FseI/AscI. pIRES-Flag/HA-TNRC6C was cloned by amplifying TNRC6C ORF from pFRT/TO/FLAG/HA-DEST TNRC6C and insertion into pIRES-Flag/HA via FseI/AscI. pIRES-Flag/HA-hnRNPA1 and pIRES-Flag/HA-hnRNPC were cloned by amplifying hnRNPA1 and hnRNPC from HeLa cDNA and cloning into pIRES-Flag/HA via NotI/BamHI. pcDNA5-FRT/TO-myc-GFP-TNRC6A NES-mut was cloned by amplifying myc-GFP-TNRC6A NES-mut ORF from pmyc-GFP-TNRC6A NES mut and insertion into pcDNA5-FRT/TO (Life Technologies) via BamHI/XhoI. pFastBac-HTa-HA-Ago2 was cloned by amplifying Ago2 from pIRES-Flag/HA-Ago2 and insertion via BamHI/NotI into pFastBac-HTa (Life Technologies) with forward primer introducing the HA-tag. This construct retains the N-terminal HA-tag after TEV cleavage. pET28a-TNRC6A and pET28a-TNRC6A NLS-mut were cloned by amplifying TNRC6A and TNRC6A NLS-mut ORFs from pmyc-GFP-TNRC6A and -TNRC6A NLS-mut. Inserts were ligated into pET28a via SacI/XhoI. pET28a-TNRC6B and pET28a-TNRC6C were cloned by amplifying TNRC6B and TNRC6C ORFs from pIRES-Flag/HA-TNRC6B and -TNRC6C. Inserts were ligated into pET28a via BamHI/NotI (TNRC6B) and SacI/XhoI (TNRC6C). pIRES-Flag/HA-Impβ was generated by amplifying Impβ ORF *de novo* from HeLa cDNA and insertion into pIRES-Flag/HA via

FseI/AscI. pET28a-Ran Q69L was cloned by inserting Ran Q69L ORF from pcDNA3.1-RanWT (3) into modified pET28a-FseI/AscI via FseI/AscI.

RNA preparation, cDNA synthesis and quantitative real-time PCR (qPCR)

RNA for qPCR was prepared from 1 x 10^6 cells with NucleoSpin RNA extraction Kit (Macherey-Nagel). 1 µg of isolated RNA was digested with DNaseI (Fermentas) and used for cDNA synthesis. cDNA was synthesized with First Strand cDNA synthesis Mix (Fermentas) using random hexamers and following the manufacturer's protocol. qPCR was done with Sso Fast Eva Green Mix (Bio-Rad), 0.4 µM forward and 0.4 µM reverse primer and cDNA from 50 ng RNA as template. The forward (fwd) and reverse (rev) sequences for qPCR were: Impβ, fwd 5'-CAGCAGAACAAGGACGGCCCC, rev 5'-TGCTGCTTTGCAGGGGTTCCA; Imp3, fwd 5'-CAAGGCCTTCTTATCCGACA, rev 5'-GAGTGGGTGGTCAAGGAGTC; Imp4, fwd 5'-TGTTGTCAGCCAGAATGAGG, rev 5'-GAGGACTTGGAGGAGTGGGT; Imp5, fwd 5'-TTCCAACTTGATCCTCAGGC, rev 5'-GATCAGGATACTTGCGGCAC; Imp7 fwd 5'-ATGTCGGAACAGCTGGATTTACCTG, rev 5'-CCCCTGGTGCTGTTTCTCGATCA; Imp8 fwd 5'-AGCCGAGAACGAGCTCAACCA, rev 5'-CGATCTGGCCAGTATTGTGTCACCA; Imp9 fwd 5'-GGCATCCGCACCCGCTCTAAG, rev 5'-CCTGGCGAGCGGCATTAGCC; Imp11 fwd 5'-TCCTGTTTCAGGATCTTCCG, rev 5'-CTTTCAGCTTTGGCTTTGCT; Imp12 fwd 5'-TTGGGGAGCTGCAGCGTTC, rev 5'-CATGAGAGTCTGTGGGGAGCTCA; Imp13 fwd 5'-TCGACAGCAGTGTGGAGGCCA, rev 5'-TTCTGCACTGCCTGCCGCAG; Tnpo1 fwd 5'-GGTTCTCTGGATGGTGGTGT, rev 5'-GTCTGGGATGGTGTGGGA. Ago1, fwd 5'-TTTCCTGGTTTCCCACTAGG, rev 5'-AACCACCACCTTCTCCAGTC; Ago2, fwd 5'-CGTGGTCATGAGGCACTTGCCA, rev 5'-GATGGAAGCCAAACCACACTTC; Ago3, fwd

5‘-ATGCAATATGAAACCAGCCA, rev 5‘-CTGCCAAGCAACTTGAGGTA; Ago4, fwd 5‘-GCTCGGAATAGTTGCACTGA, rev 5‘-ATCAGCCTTTGCACATTGAG. GAPDH primers were taken from (10). qPCRs were run on a CFX96 cycler (Bio-Rad) and data were analyzed using ΔΔCt method. RNA for Northern Blot was prepared using TRIzol reagent (Life Technologies) according to the manufacturer’s procedures.

Expression and purification of recombinant proteins

Expression of human Ago2 with Bacculo system was done essentially as described before (11) using Spodoptera frugiperda-21 cells in Sf-900 II SFM medium (Invitrogen) and pFastBac-HTa-HA-Ago2. 2.5 l culture was infected with viral stock and cultivated for 72 hours at 27.5 °C. Cells were harvested (300 g, 10 min, RT) and lyzed using 120 ml Buffer A [50 mM HEPES pH 7.5 / 1 M NaCl / 0.1 % NP-40 / 10 mM imidazole / 5 % glycerol / 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF) / 5 U per ml Benzonase] and additional sonification. Lysate was cleared by centrifugation (40000 g, 60 min, 4 °C) and filtered through a 45 µm filter. Cleared lysate was applied onto a Ni-loaded HiTrap IMAC FF 5 ml column (GE Healthcare). After binding, column was washed extensively with 10 column volumes of Buffer A and 5 column volumes 5 % Buffer B [50 mM HEPES pH 7.5 / 300 mM NaCl / 200 mM imidazole / 1 mM DTT]. Elution was done with 100 % Buffer B and eluate was collected from peak fractions, supplemented with tobacco etch virus (TEV) protease and incubated over night. Sample was loaded on a HiPrep 26/60 desalting column (GE Healthcare) using desalting buffer [50 mM HEPES pH 7.5 / 1 M NaCl / 10 mM Imidazole]. Peak fractions were collected and applied again onto Ni-loaded HiTrap IMAC FF 5 ml column and unbound flow-through was collected. Due to incomplete TEV cleavage, also bound peak fractions were collected again after

elution. Bound and unbound peak fractions were pooled, concentrated and resolved by size-exclusion chromatography with a HiLoad Superdex 200 26/60 column (GE Healthcare) in gelfiltration buffer [20 mM HEPES pH 7.5 / 200 mM KCl / 2 mM EDTA / 1 mM DTT / 5 % glycerol]. Fractions corresponding to His-HA-Ago2 and HA-Ago2 were collected, pooled and concentrated.

Recombinant human importins α1, α3, α4, α5, α7 and β were expressed as C-terminal GST-fusion proteins and purified as described before (12), Impβ was additionally expressed as C-terminal His-tagged protein. Briefly, transformed BL21/pRep4 were grown at 37 °C in LB medium. At mid-log phase of growth, protein overexpression was induced by 1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) for 4 h at 25 °C. Cells were collected and disintegrated in a French Press. Lysate was cleared by centrifugation (1 h, 22.000 rpm, SS 34 rotor) and incubated for 2 to 3 h at 4°C with glutathione sepharose (GE Healthcare). Sepharose was washed three times and protein was eluted by incubation with elution buffer [50 mM Tris–HCl pH 8.0 / 10 mM reduced glutathione]. Protein samples were dialyzed against dialysis buffer [50 mM HEPES-KOH pH 7.5 / 200 mM NaCl / 5% glycerol]. If required, protein was concentrated in centrifugal filter devices (Millipore). His-tagged Impβ and His-tagged Nucleoplasmin were separated from the protein extract by metal affinity chromatography using BD TALON metal affinity resin (Clontech) according to the manufacturer's instructions.

His-tagged Ran Q96L mutant was expressed in BL21. A 2 l culture was induced with IPTG for 9 h and lyzed in 100 ml lysis buffer [20 mM Tris pH 8.0 / 300 mM NaCl / 10 mM Imidazole / 1 mM AEBSF / 5 U/ml Benzonase (Novagen) / 5 µg/ml Lysozyme (Carl Roth)] by sonification. Lysates were cleared by centrifugation, supernatant was filtered and loaded onto a Ni^{2+} charged 5 ml HiTrap IMAC FF column. Column was washed with wash buffer [20 mM Tris pH 8.0 / 300

mM NaCl / 20 mM Imidazole]. Elution was done with 5 column volumes of elution buffer [20 mM Tris pH 8.0 / 300 mM NaCl / 250 mM Imidazole]. Protein containing fractions were pooled and dialyzed against dialysis buffer [150 mM KCl / 25 mM Tris-HCl pH 7.5 / 2 mM EDTA / 1 mM NaF / 0,5 % NP-40 / 0.5 mM DTT / 1 mM AEBSF / 10 % glycerol]. Dialysate was concentrated and cleared again by centrifugation.

Protein extract preparations and Western blotting

For Western Blotting, protein was extracted from cells with lysis buffer [25 mM Tris pH 7.5 / 150 mM KCl / 2 mM EDTA / 1 mM NaF / 0.5 % NP-40 / 0.5 mM DTT / 1 mM AEBSF] or RIPA buffer [50 mM Tris pH 7.4 / 1 % NP-40 / 0.5 % Na-deoxycholate / 0.1 % SDS / 150 mM NaCl / 2 mM EDTA / 0.5 mM DTT / 1 mM AEBSF]. Cleared lysates were supplemented with Laemmli buffer and heated to 95 °C or with LDS sample buffer [1x: 200 mM Tris pH 7.5 / 1 % lithium dodecyl sulfate (LDS) / 10 % glycerol / 0.5 mM EDTA / 0,006 % bromophenol blue / 12.5 mM DTT] and heated to 70 °C for 10 min. RIPA and LDS sample buffer were used for detection of TNRC6 proteins and 4-fold amount of sample was loaded for TNRC6 proteins. Western blotting was performed semi-dry at 13 V for 1.5 h or wet at 30 V for 16 h (TNRC6 proteins). Membranes were blocked with TBS + 0.1 % Tween-20 + 5 % w/v milk powder and antibodies were incubated for 2 h or o/n in TBS + 0.1 % Tween-20 + 2 % milk.

Nucleo-cytoplasmic fractionations

Nuclear and cytoplasmic extracts from HeLa cells were prepared using NE-PER Kit (Thermo Scientific) according to the manufacturer’s protocol with following modifications. Nuclear

extract was prepared by resuspension of nuclear pellet in RIPA buffer and centrifugation as described above. Whole cell extract was prepared as described above using RIPA buffer.

Co-immunoprecipitations

Co-immunoprecipitations (CoIPs) of myc-GFP-TNRC6A and Impβ were done from transiently transfected HEK 293T cells of HEK TRex FLP/IN myc-GFP-TNRC6A cells. Induction of myc-GFP-TNRC6A in HEK TRex FLP/IN myc-GFP-TNRC6A was started with plasmid transfection as described above. Two days post transfection, cytoplasmic extracts were isolated from two 80 % confluent 15 cm plates per CoIP: Cells were washed once with PBS and resuspended in 1.5 ml IP buffer [150 mM KCl / 25 mM Tris-HCl pH 7.5 / 2 mM EDTA / 1 mM NaF / 0.5 mM DTT / 1 mM AEBSF]. To lyze the cells, Digitonin (Calbiochem) was added to 200 µg/ml final concentration until 90 % of cell show Trypan blue positive staining. Cells were mixed by vortexing for 5 sec and incubated on ice for 10 min. Lysates were cleared by centrifugation at 17000 g for 10 min and supernatant was used as input for IP. 20 µl Dynabeads Protein A (Life Technologies) were coupled with 2 µg rabbit-anti-c-Myc antibody (Sigma Aldrich) in PBS + 0,02 % Tween-20 over night. Beads were washed twice with IP buffer, added to cytoplasmic extract and incubated rotating at 4 °C for 1 h. Beads were washed two to three times with IP wash buffer [300 mM NaCl / 50 mM Tris pH 7.5 / 1 mM NaF / 0.01 % NP-40 / 5 mM $MgCl_2$] and once with PBS. Elution was done by adding 25 µl 2x Laemmli buffer and incubation at 95 °C for 5 min. Recombinant His-Ran Q96L was mixed with 1.5 µl 100 mM GTP, preincubated for 15 min on ice and complete reaction was added to the CoIPs when indicated.

Northern Blot

Northern Blot was done from total RNA preparations essentially as described in (10). For detection of 7sk and U6 snRNA, RNA was run on a 6 % urea polyacrylamide gel and blotted semi-dry for 45 min at 20 V. RNA was subsequently cross linked using EDC and UV light. Hybridization was done with human U6 probe 5'-GAATTTGCGTGTCATCCTTGCGCAGGGGCCATGCTAA and 7sk probe 5'-ACTCGTATACCCTTGACCGAAGA. Signals were detected and quantified using Personal Molecular Imager System (Bio-Rad).

Supplemental references

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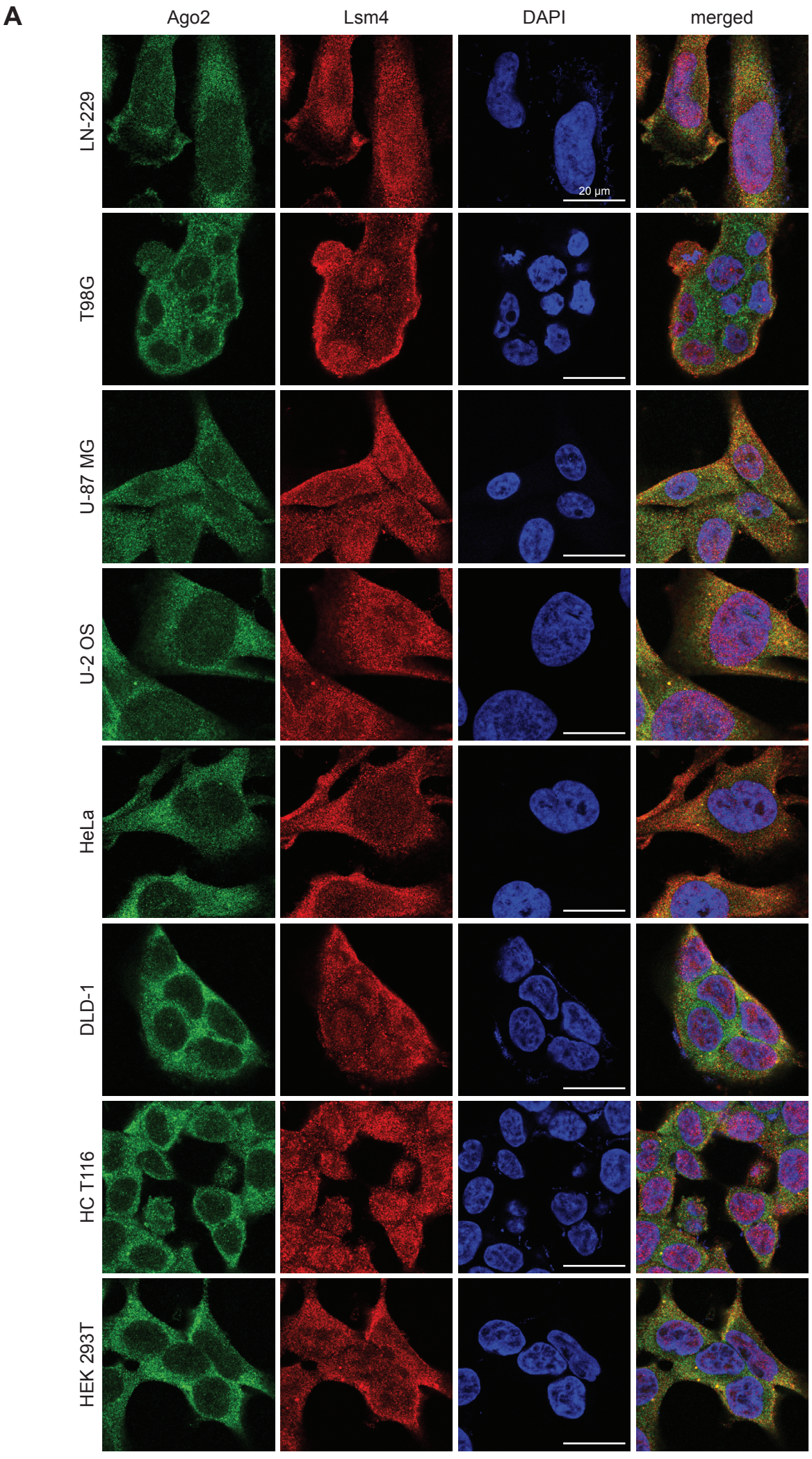
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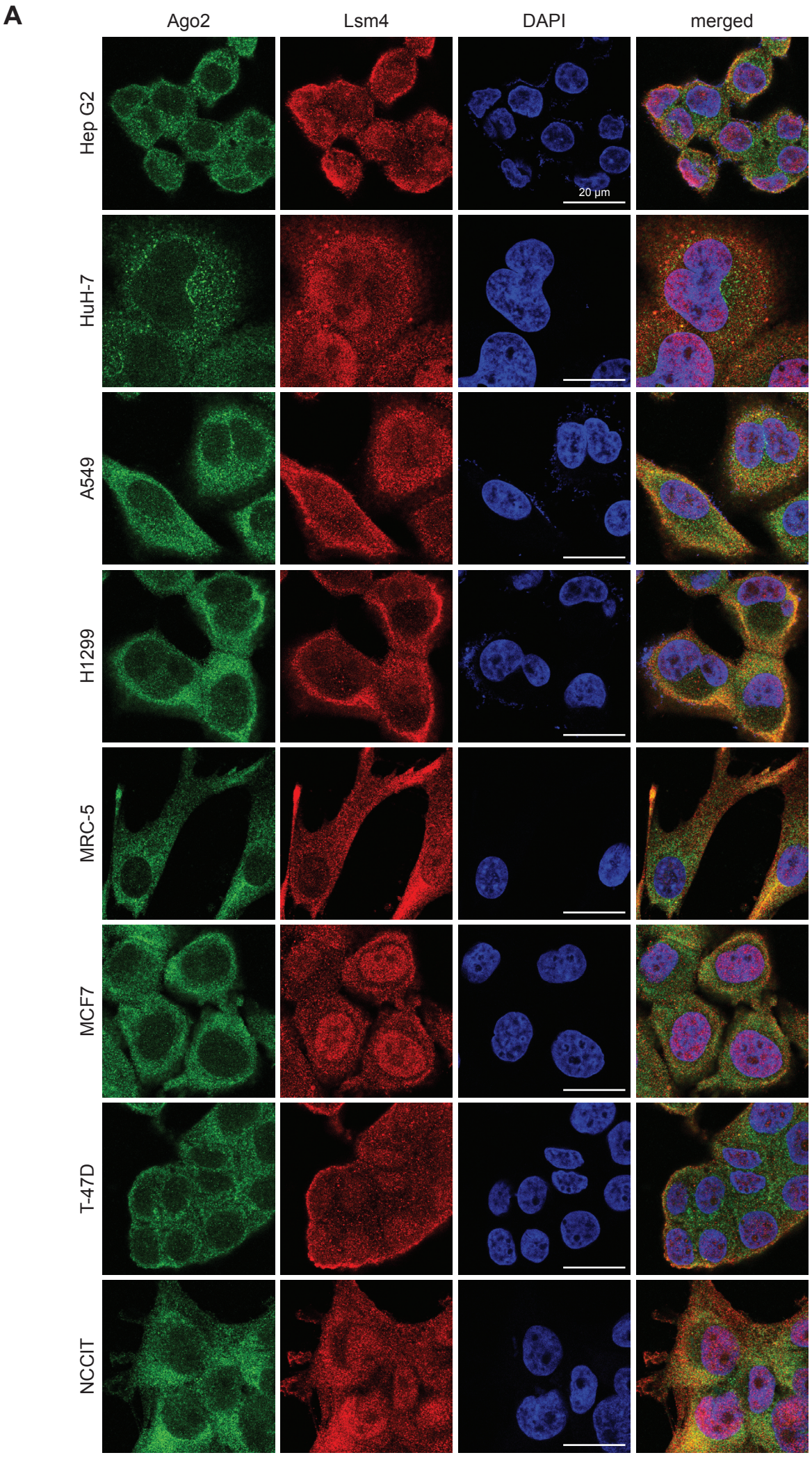
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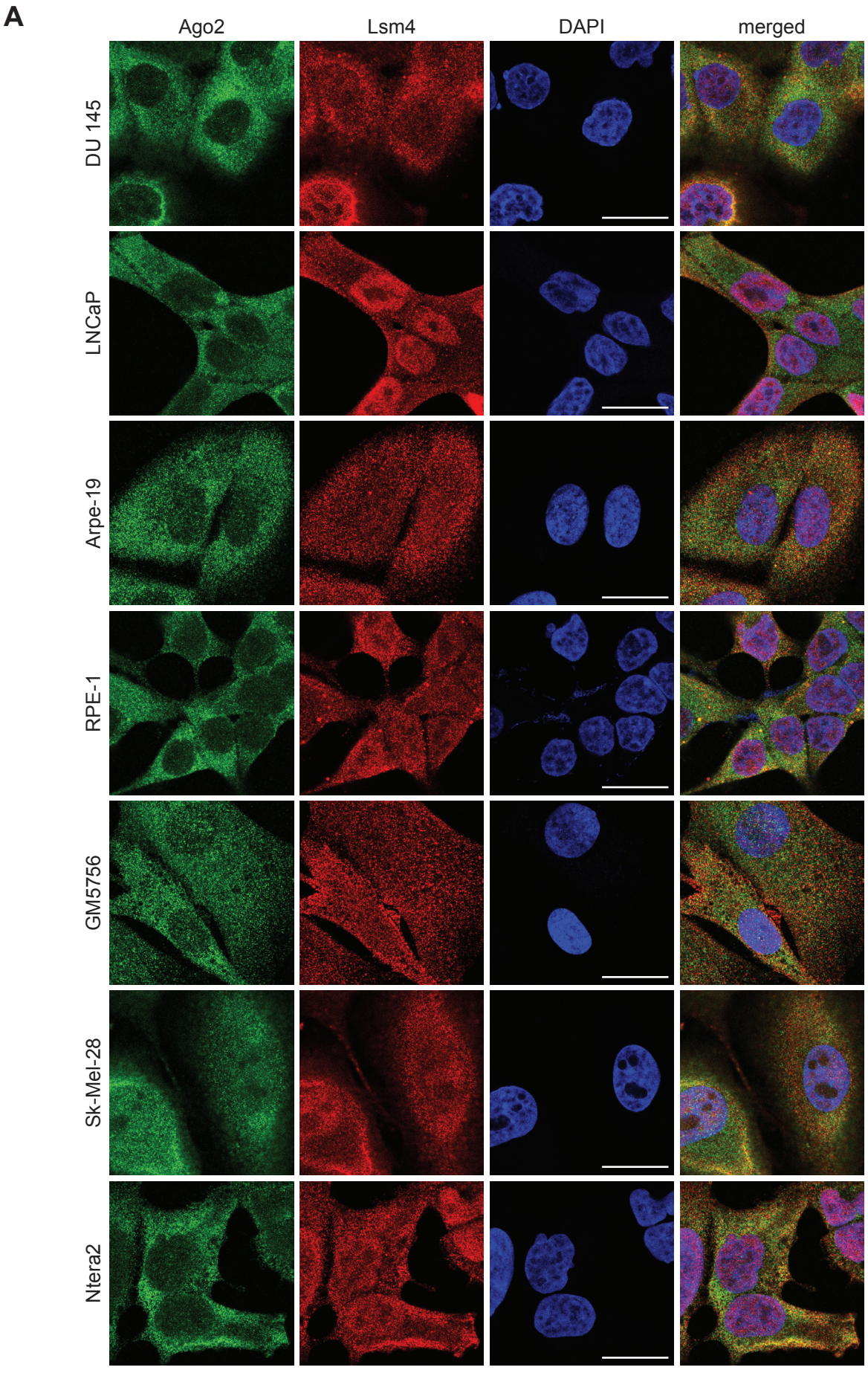
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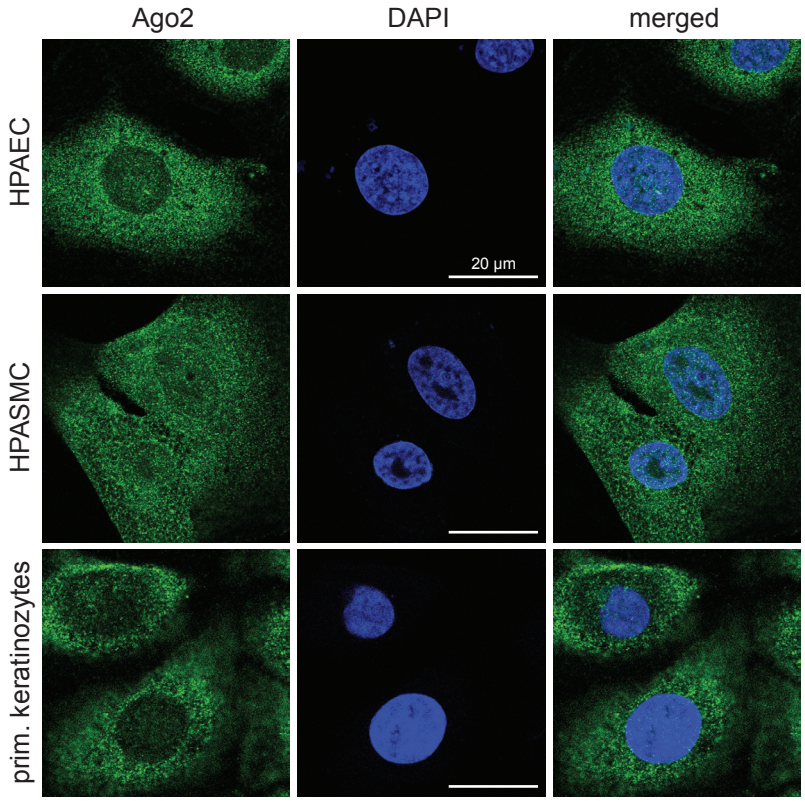
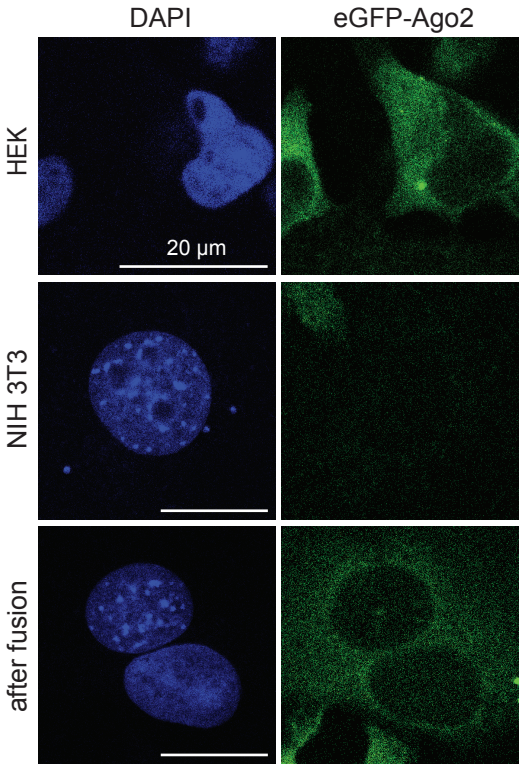


C

Tissue origin	Cell line	Ratio Nuc:Cyt	Disease
brain	LN-229	0.56 ± 0.05	glioblastoma
	T98G	0.52 ± 0.06	glioblastoma
	U-87 MG	0.74 ± 0.03	glioblastoma
bone	U-2 OS	0.58 ± 0.07	osteosarcoma
cervix	HeLa	0.45 ± 0.07	cervical cancer
colon	DLD-1	0.50 ± 0.08	colorectal carcinoma
	HCT116	0.46 ± 0.07	colorectal carcinoma
kidney	HEK 293T	0.38 ± 0.05	non-cancerous
liver	Hep G2	0.66 ± 0.09	hepatocellular carcinoma
	HuH-7	0.61 ± 0.04	hepatocellular carcinoma
lung	A549	0.54 ± 0.06	adenocarcinoma
	H1299	0.61 ± 0.15	non-small cell lung cancer
	MRC-5	0.42 ± 0.06	non-cancerous
mammary gland	MCF7	0.43 ± 0.04	ductal carcinoma
	T-47D	0.51 ± 0.03	ductal carcinoma
placenta	NCCIT	0.52 ± 0.09	pluripotent carcinoma
pulmonary artery	HPAEC	0.63 ± 0.06	non-cancerous
	HPASMC	0.85 ± 0.13	non-cancerous
prostate	DU 145	0.42 ± 0.04	adenocarcinoma
	LNCaP	0.54 ± 0.06	adenocarcinoma
retina	Arpe-19	0.52 ± 0.03	non-cancerous
	RPE-1	0.46 ± 0.08	non-cancerous
skin	GM5756	0.62 ± 0.07	non-cancerous
	Sk-Mel-28	0.81 ± 0.04	melanoma
	keratinozytes	0.41 ± 0.07	non-cancerous
testis	Ntera2	0.57 ± 0.06	carcinoma

D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HEK
NIH 3T3
after fusion
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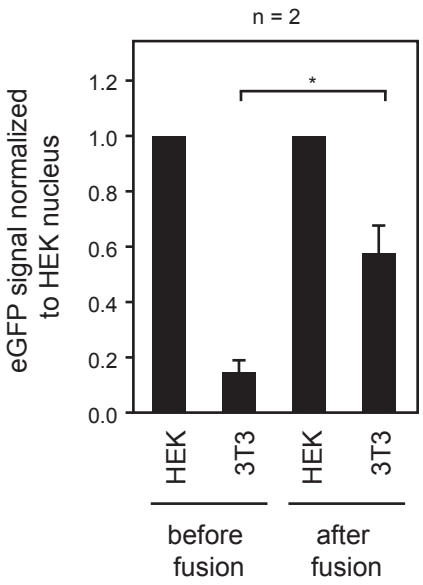
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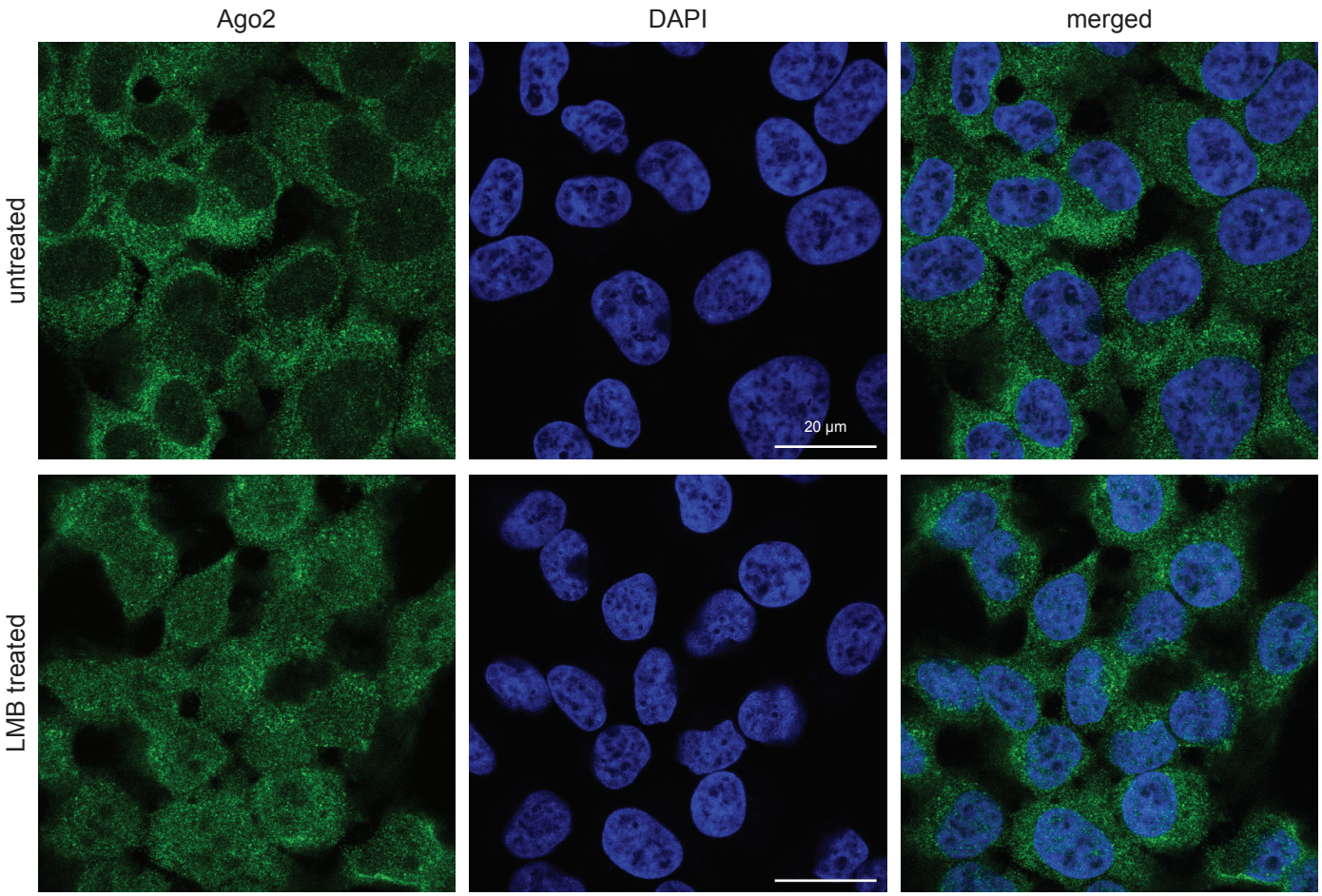
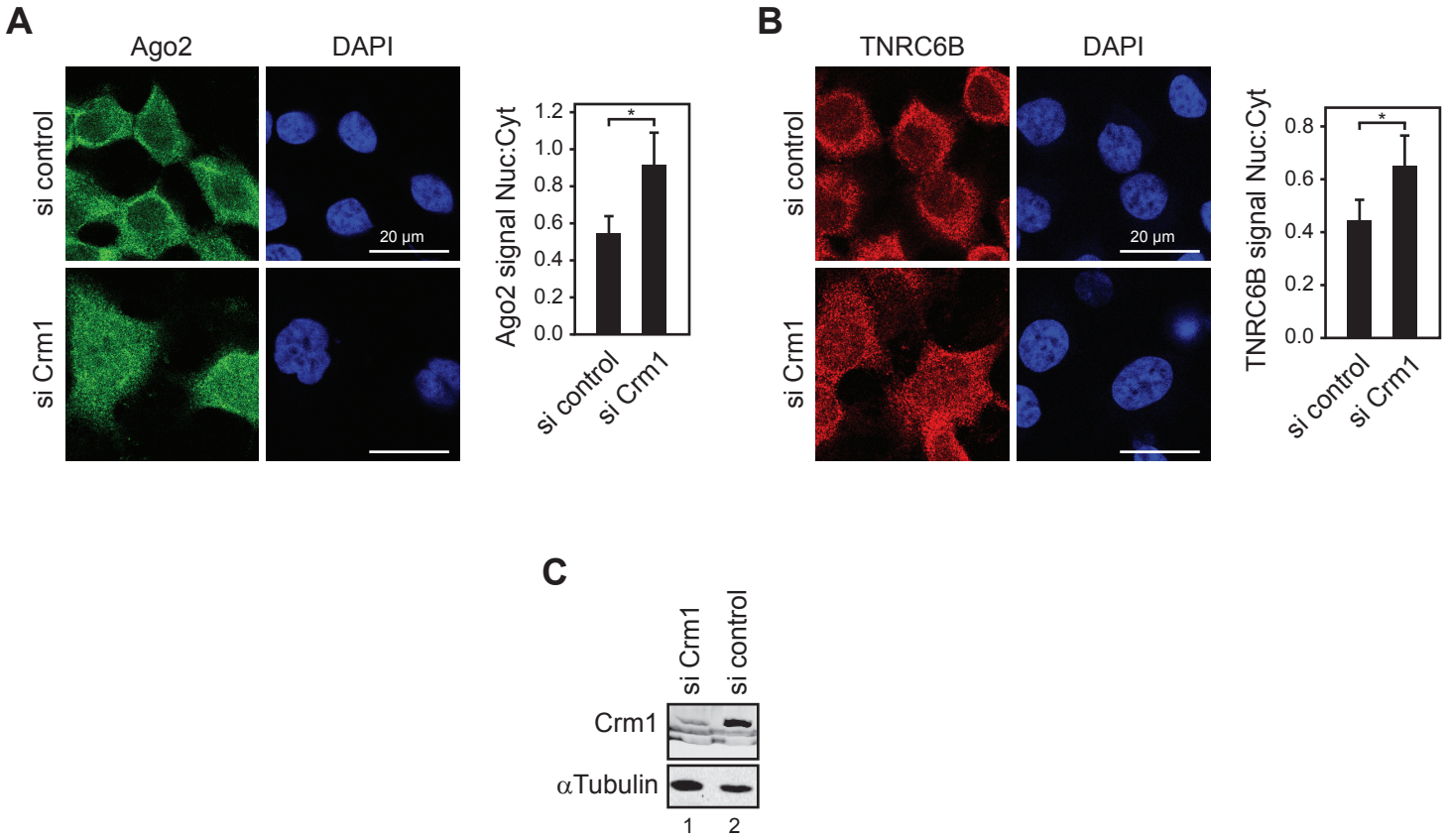
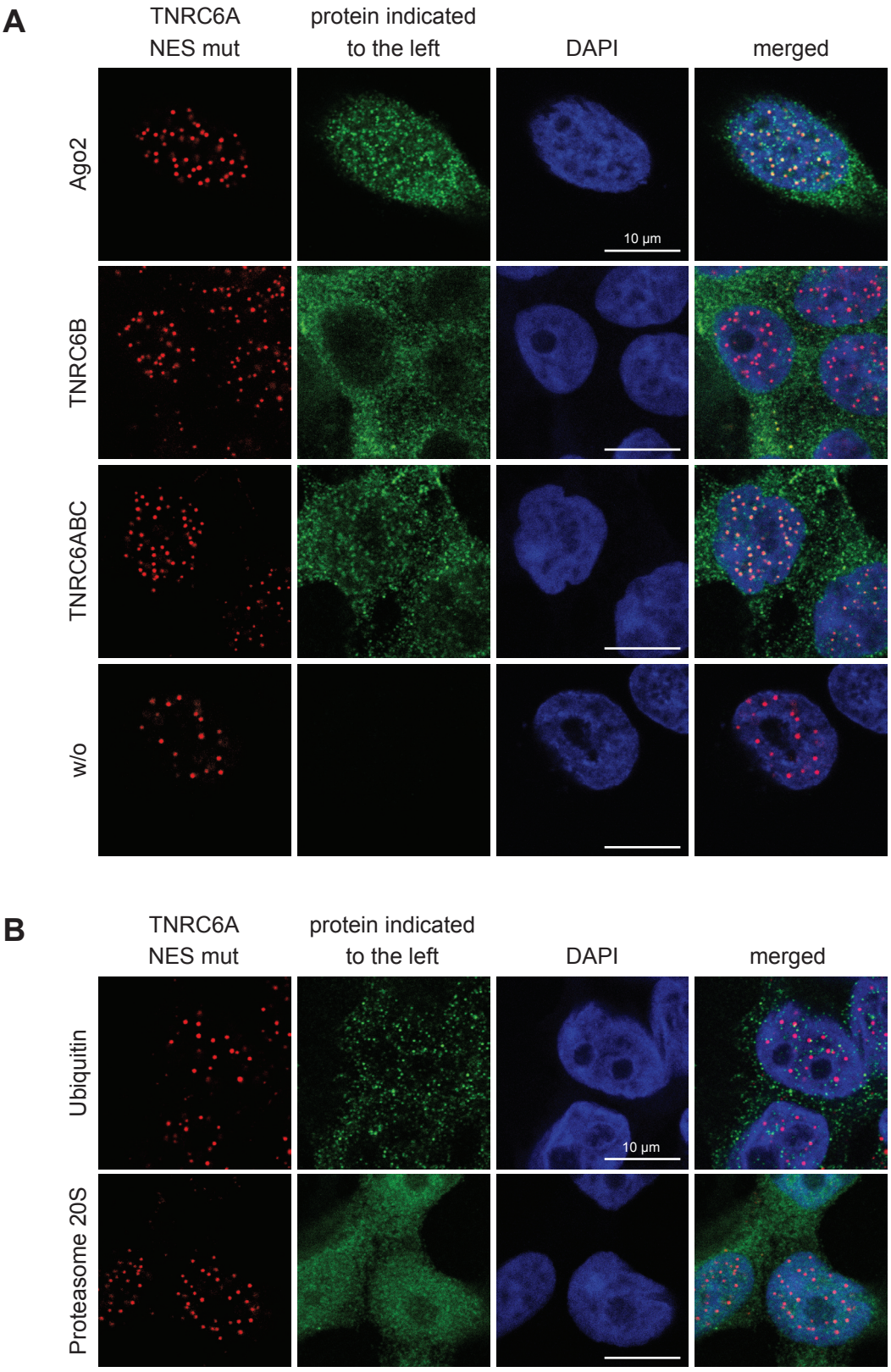
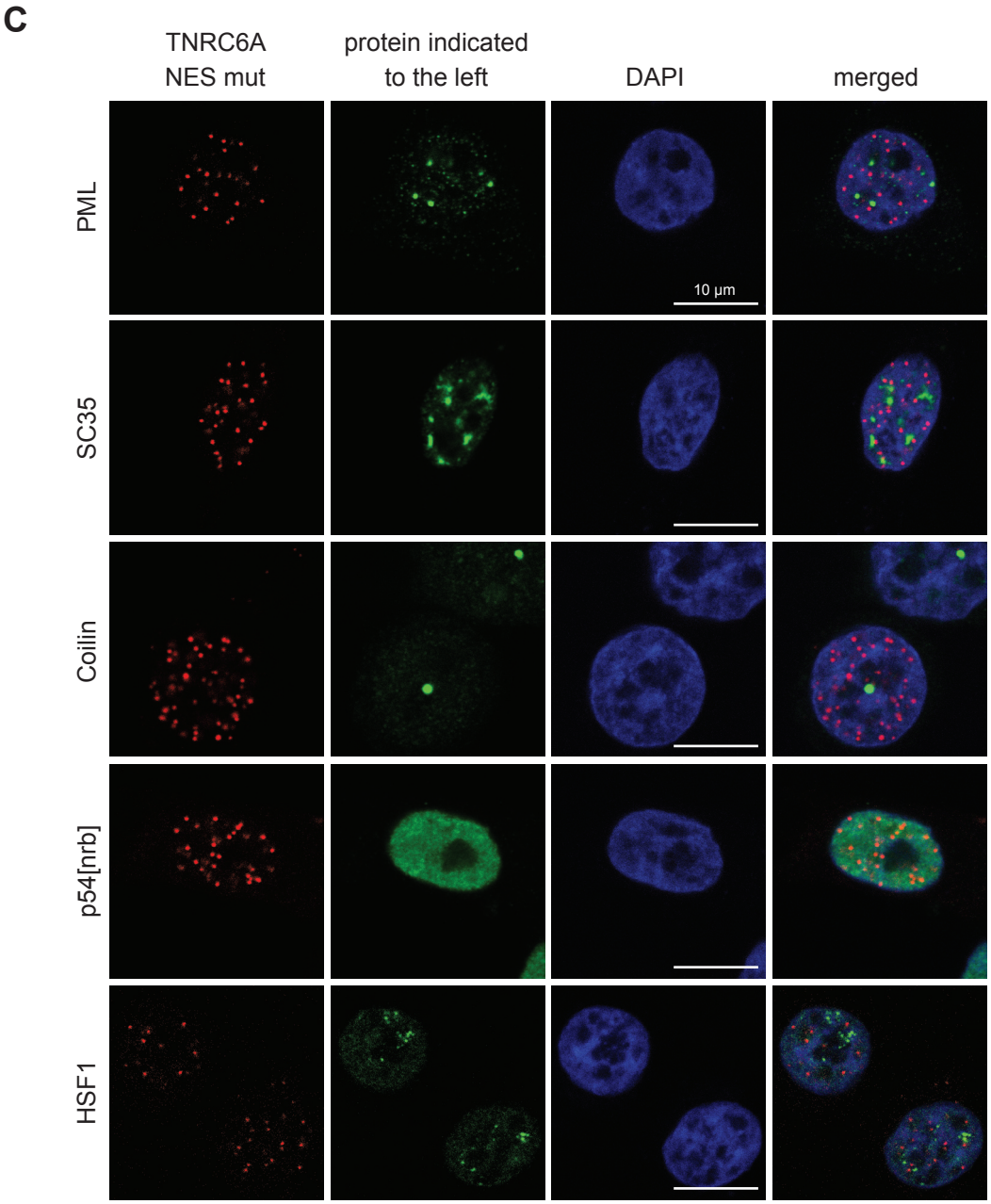
1.0
0.8
0.6
0.4
0.2
0.0
HEK
3T3
HEK
3T3
before fusion
after fusion

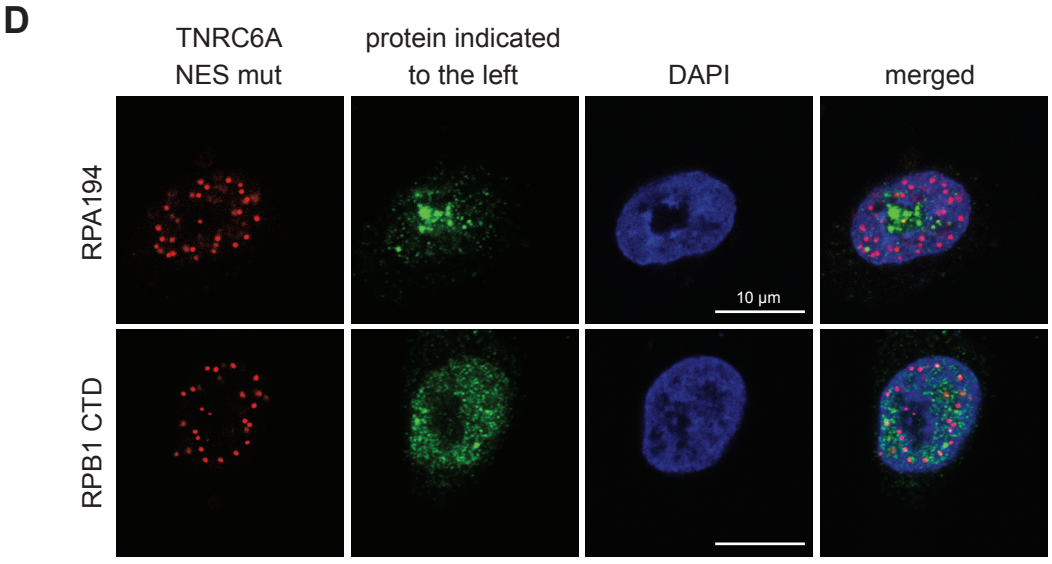

Ago2
DAPI
merged
untreated
LMB treated
20 µm

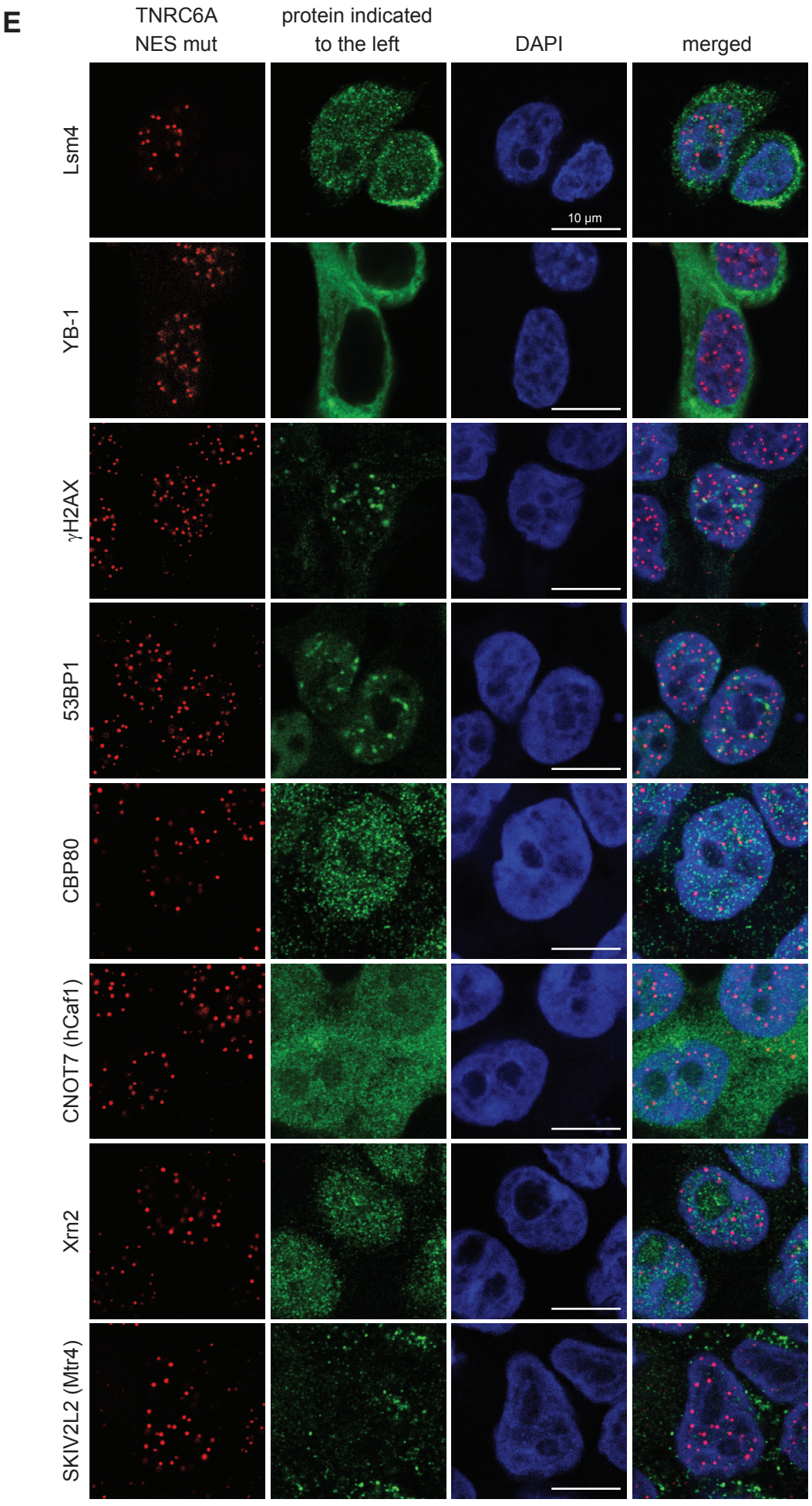

A
Ago2
DAPI
si control
si Crm1
20 µm
Ago2 signal Nuc:Cyt
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
si control
si Crm1
B
TNRC6B
DAPI
si control
si Crm1
20 µm
TNRC6B signal Nuc:Cyt
0.8
0.6
0.4
0.2
0.0
*
si control
si Crm1

C
si Crm1
si control
Crm1
αTubulin
1
2


A
TNRC6A NES mut
protein indicated to the left
DAPI
merged
Ago2
TNRC6B
TNRC6ABC
w/o
10 µm
B
TNRC6A NES mut
protein indicated to the left
DAPI
merged
Ubiquitin
Proteasome 20S
10 µm

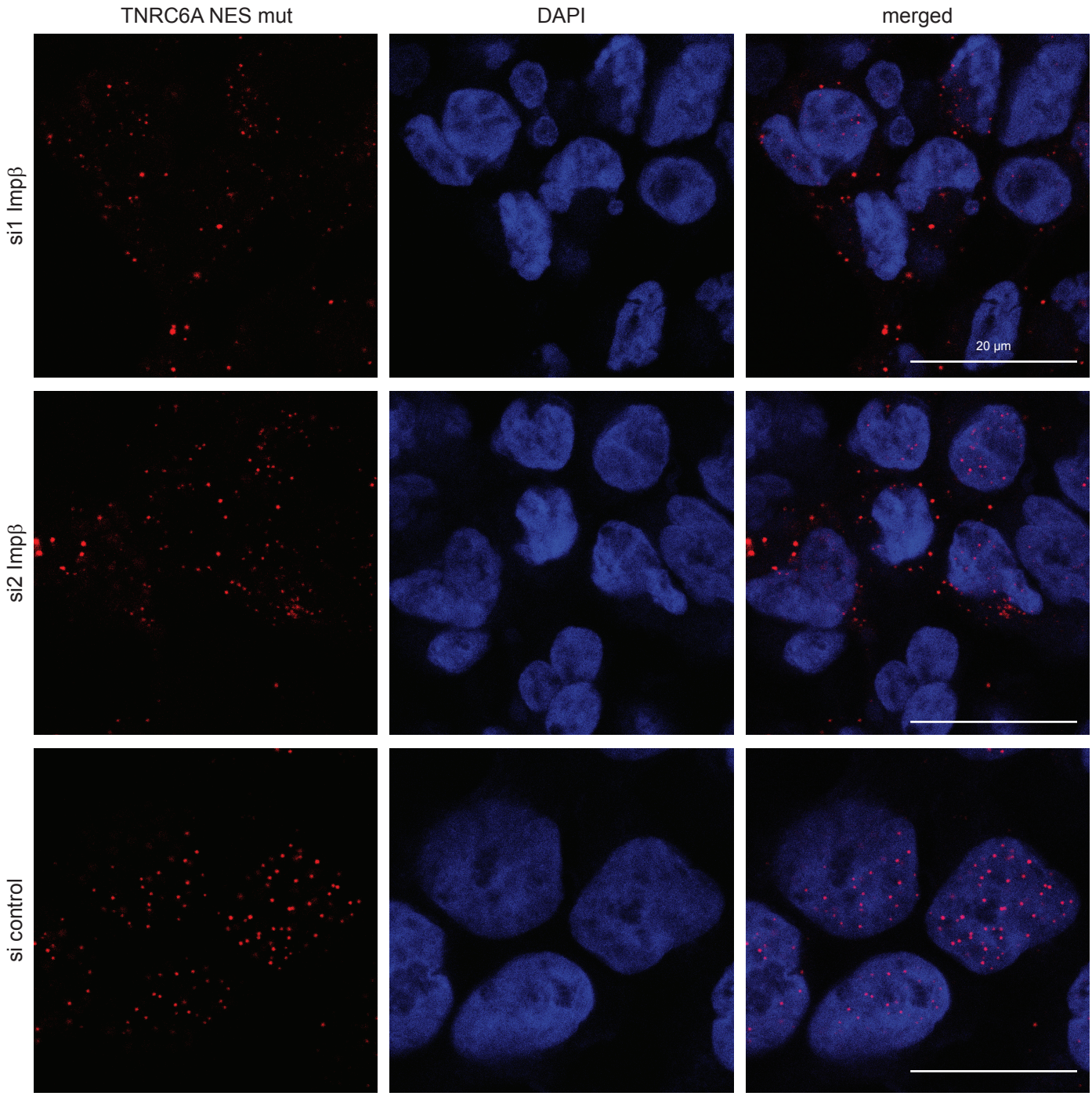

C
TNRC6A NES mut
protein indicated to the left
DAPI
merged
PML
SC35
Coilin
p54[nrb]
HSF1
10 µm

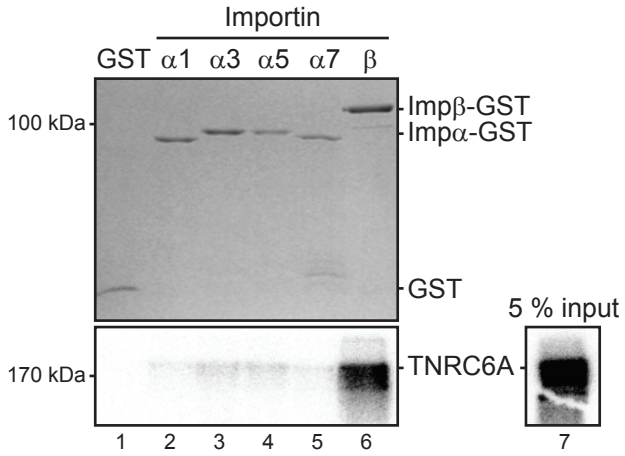

D
TNRC6A NES mut
protein indicated to the left
DAPI
merged
RPA194
RPB1 CTD
10 µm


E
TNRC6A NES mut
protein indicated to the left
DAPI
merged
Lsm4
YB-1
γH2AX
53BP1
CBP80
CNOT7 (hCaf1)
Xrn2
SKIV2L2 (Mtr4)
10 µm

F

	Marker	Antibody	Localization	Colocalization with TNRC6A NES mut
RISC	Ago2	Ago2 11A9	Cyt	yes
	TNRC6B	TNRC6B 6G3	Cyt	no
	TNRC6ABC	TNRC6ABC 7A9	Cyt	yes
Protein degradation	Ubiquitin	Ubiquitin	Cyt + Nuc	no
	Proteasome 20S	20S α123567	Cyt + Nuc	no
Nuclear compartments	PML body	PML	Nuc	no
	Nuclear speckles	SC35	Nuc	no
	Cajal bodies	Coilin	Nuc	no
	Paraspeckles	p54[nrb]	Nuc	no
	HSF1 granules	HSF1	Nuc	no
Transcription	RNA polymerase I	RPA194	Nuc	no
	RNA polymerase II	RPB1 CTD	Nuc	no
Others	Lsm complex	Lsm4	Cyt + Nuc	no
	YB-1	YB-1	Cyt	no
	DNA damage	γH2AX	Nuc	no
	DNA damage	53BP1	Nuc	no
	CBP80	CBP80	Cyt + Nuc	no
	Ccr4-Not complex	CNOT7 (hCaf1)	Cyt + Nuc	no
	5'-exonuclease	Xrn2	Nuc	no
	Exosome	SKIV2L2 (Mtr4)	Cyt + Nuc	no


TNRC6A NES mut
DAPI
merged
si1 Impβ
si2 Impβ
si control
20 µm


Importin
GST
α1
α3
α5
α7
β
100 kDa
Impβ-GST
Impα-GST
GST
5 % input
170 kDa
TNRC6A
1
2
3
4
5
6
7