Expanded View Figures

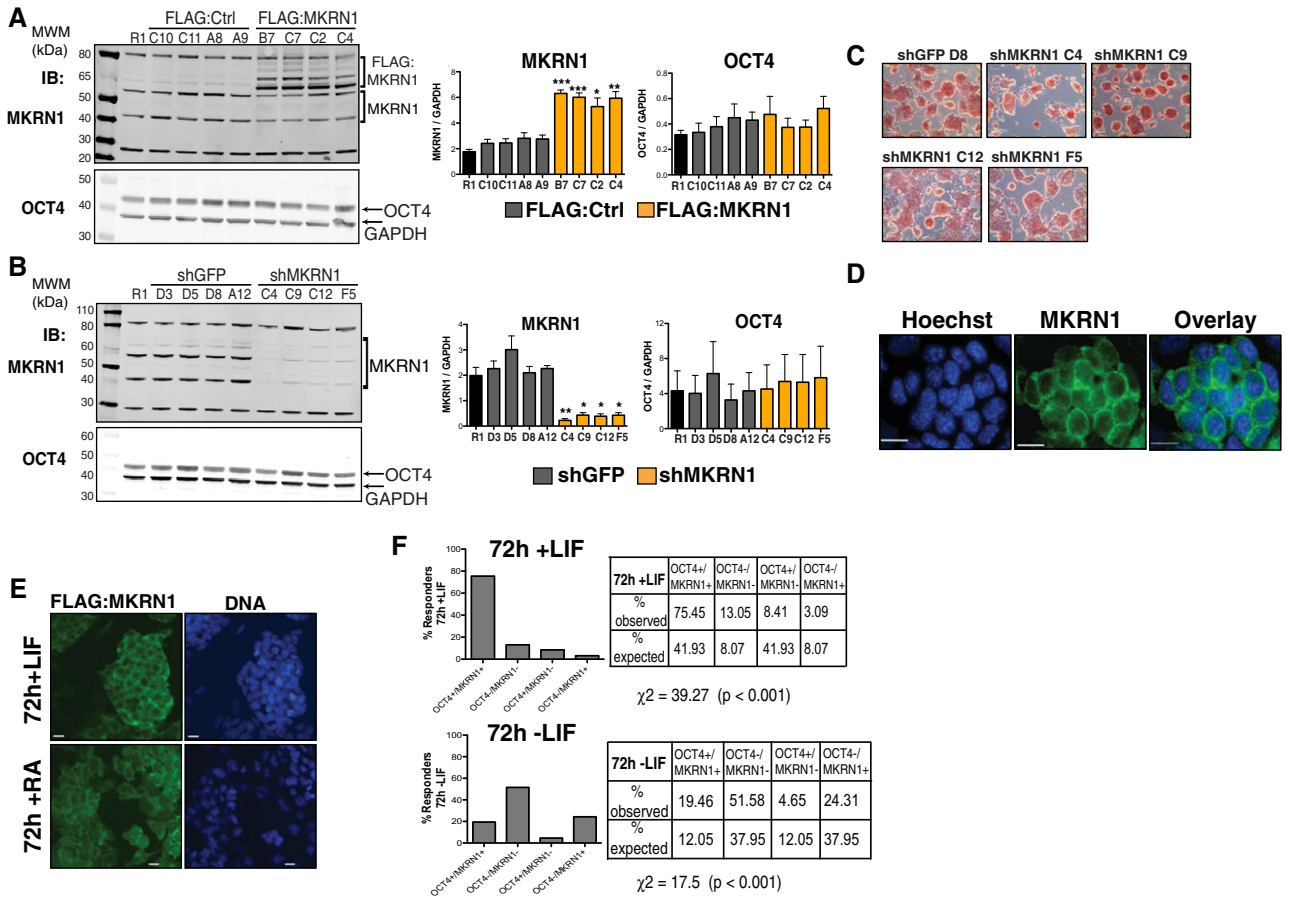

Figure EV1. Validation of MKRN1 overexpression and knockdown transgenic ESC clones.

A, B Twenty micrograms of total protein from whole-cell lysates derived from undifferentiated MKRN1 overexpression (FLAG:MKRN1) (A) and MKRN1 knockdown (shMKRN1) clones (B) and their respective controls (including R1 parental ESCs) was resolved with SDS–PAGE. Blots were probed with commercial antibodies directed against MKRN1 (top) to test the specificity of the antibody, and OCT4 (bottom) to verify that lysates were obtained from undifferentiated ESC populations. The MKRN1 antibody (ab72054) detects two MKRN1-specific bands at ~53 and ~42 kDa, and two non-specific bands of size 80 and 25 kDa. Both the 53-kDa and 42-kDa bands were depleted in stable MKRN1 knockdown ESC lysates, but not the non-specific 80-kDa and 25-kDa bands (B). The 53-kDa band corresponds to the full-length endogenous MKRN1 protein and the second ~42-kDa band may represent a degraded by-product of the full-length 53-kDa MKRN1, or potentially the putative MKRN1 splice variant, *Mkrn1-004*, which encodes a putative protein of 47.3 kDa. The MKRN1 antibody also detects an additional ~63-kDa band exclusively in FLAG:MKRN1 ESCs that corresponds to recombinant FLAG:MKRN1 (A). Total MKRN1 and OCT4 protein expression was quantified and reported relative to GAPDH. OCT4 protein abundance was comparable between MKRN1 transgenic and control clones (A, B). Total MKRN1 expression was highest in FLAG:MKRN1 clones B7 and C7 (***$P < 0.001$), followed by C4 (**$P < 0.01$) and C2 (*$P < 0.05$), whereas MKRN1 was depleted to the greatest extent in shMKRN1 clones C4 (**$P < 0.01$) followed by C12, F5, and C9 (*$P < 0.01$). Data are means ± SEM from three biological replicate experiments (one-way ANOVA).

C Alkaline phosphatase-positive ESC colonies formed from stable shGFP control and stable MKRN1 knockdown ESC clones cultured in LIF + serum in feeder-free conditions. Scale bars: 60 μm.

D R1 ESCs cultured in LIF + serum immunostained with anti-MKRN1 antibodies and counterstained with Hoechst. Scale bars: 10 μm.

E FLAG:MKRN1 ESCs cultured for 72 h in self-renewal (top) or RA-induced differentiation conditions (bottom) were immunostained with anti-FLAG antibodies and counterstained with Hoechst. Scale bars: 10 μm.

F Distribution of MKRN1$^+$ and MKRN1$^-$ cells in the OCT4$^+$ and OCT4$^-$ fraction of ESC cultures as determined by high-content imaging analysis. Association between OCT4 and MKRN1 expression in individual cells was significant in both self-renewal (72 h +LIF) and differentiation (72 h −LIF) conditions ($P < 0.001$; chi-square test).

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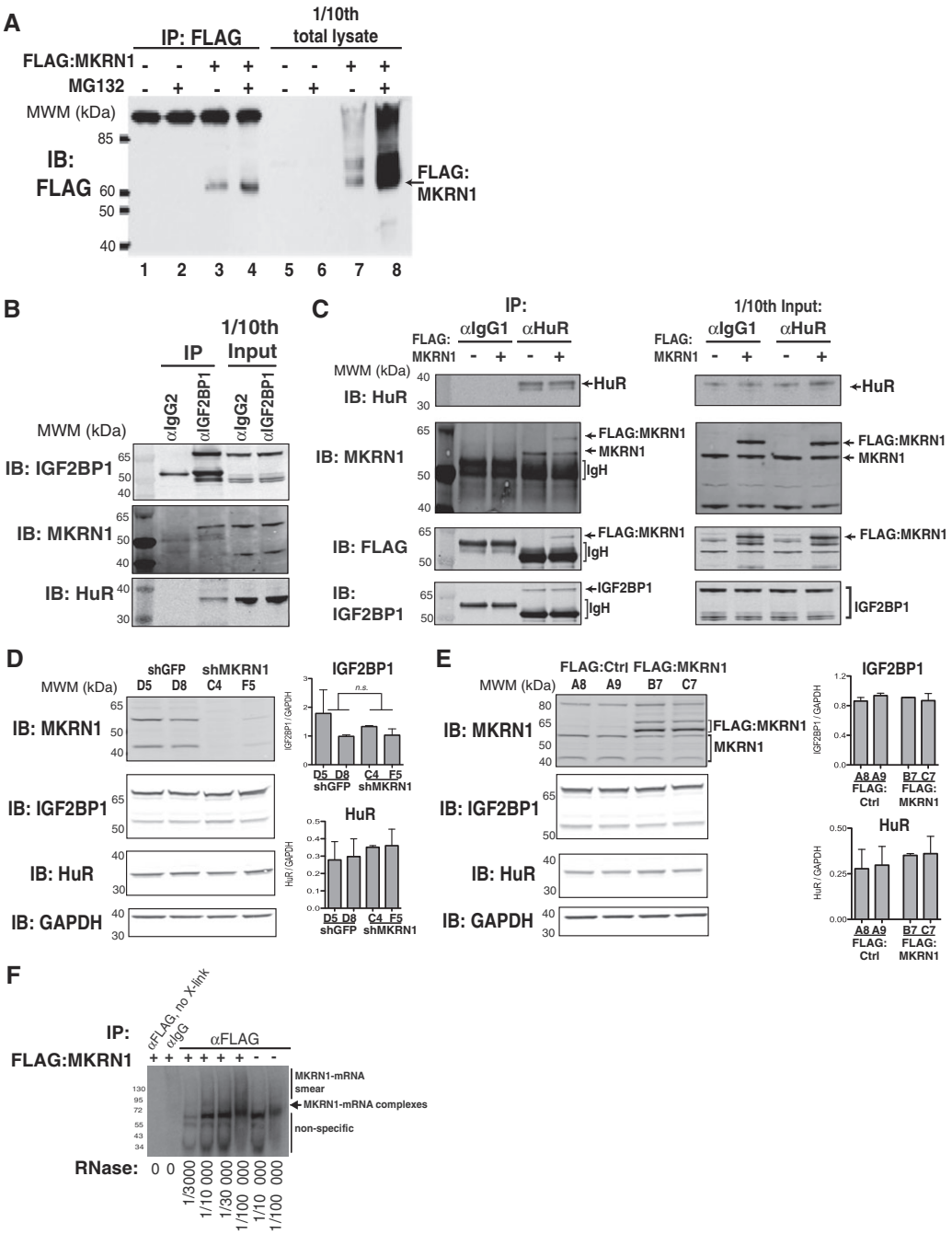

Figure EV2. Validation of the FLAG:MKRN1 AP-MS data set.

A Anti-FLAG immunoblot of representative anti-FLAG IP immunoprecipitates (lanes 1–4) and total protein lysates (lanes 5–8) derived from MG132-treated or untreated ESCs expressing recombinant FLAG:MKRN1 protein or an empty FLAG vector. Anti-FLAG immunoprecipitates purified in this experiment were subjected to LC-MS/MS analysis.

B, C Reciprocal anti-IGF2BP1 (B) and anti-HuR (C) IPs from undifferentiated ESC lysates verify the presence of MKRN1 in the respective mRNP complexes.

D, E Steady-state abundance of MKRN1-associated proteins, IGF2BP1 and HuR, in MKRN1 knockdown (D) and MKRN1 overexpression (E) ESC populations. Quantified protein abundance is reported relative to GAPDH. Neither IGF2BP1 nor HuR protein abundance was affected by the manipulation of MKRN1 expression. Data are means ± SEM from two biological replicate experiments.

F Autoradiogram of ^{32}P-labeled RNA obtained by CLIP with either anti-FLAG antibodies or IgG isotype control antibodies from FLAG:MKRN1 or FLAG:Ctrl ESC lysates, as indicated. Prior to lysis, ESCs were either UV cross-linked, or left uncross-linked (no X-link). Data represent findings from two biological replicate experiments.

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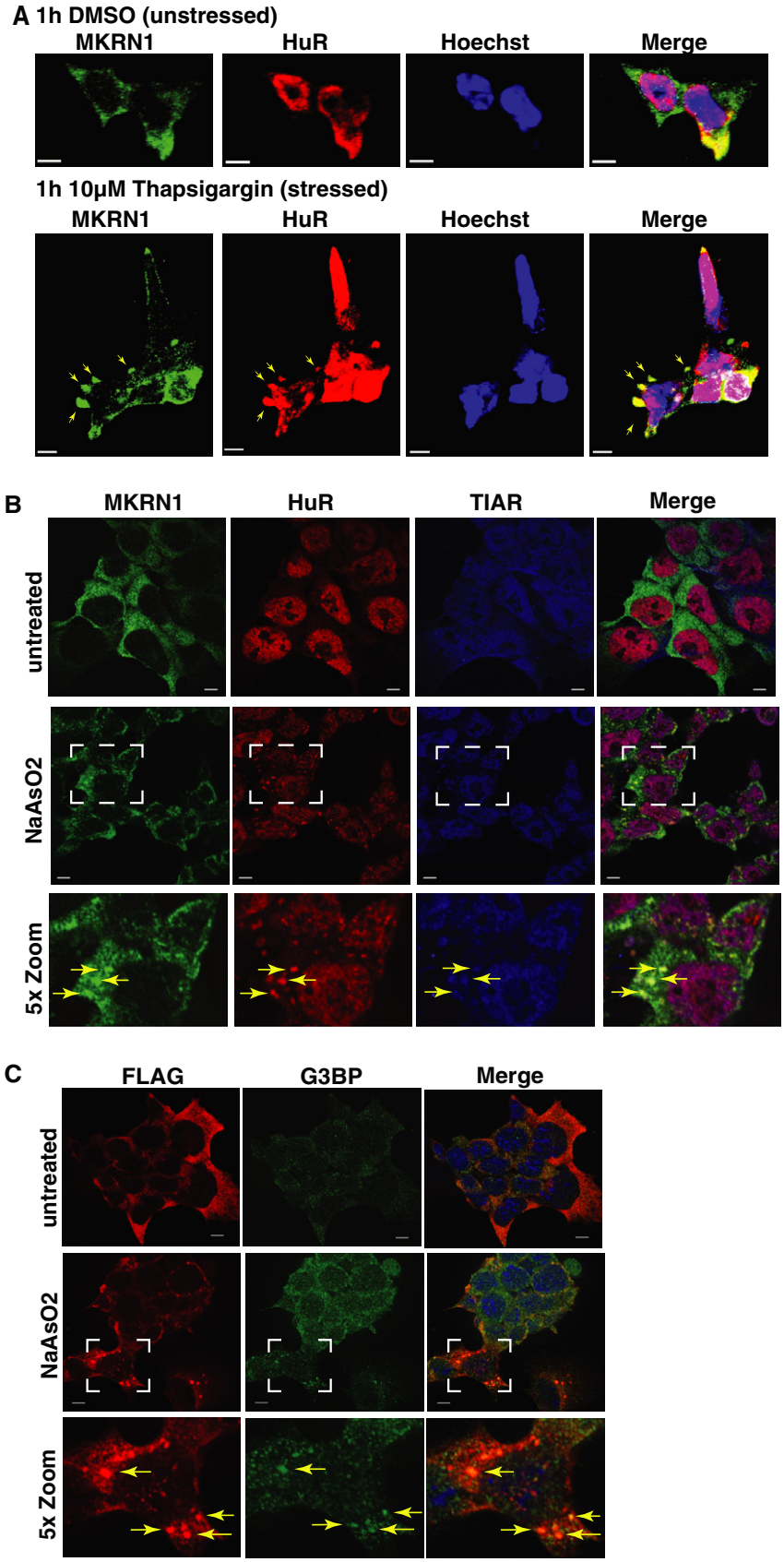

Figure EV3. MKRN1 is mobilized to SGs in response to environmental stress.

A Endogenous MKRN1 is targeted to SGs in ESCs following 1-h treatment with 10 μM thapsigargin. Yellow arrows indicate MKRN1/HuR-positive SGs. Scale bars: 10 μm.

B Overexpression of MKRN1 does not spontaneously induce SG assembly in unstressed ESCs and does not impair SG formation in $NaAsO_2$-stressed ESCs. Yellow arrows indicate MKRN1/HuR/TIAR-positive SGs. Scale bars: 10 μm. Enlargements of boxed regions are indicated as 5× zoom.

C Ectopic FLAG:MKRN1 is localized to SGs upon $NaAsO_2$-induced environmental stress. Yellow arrows indicate G3BP-positive SGs in $NaAsO_2$-treated FLAG:MKRN1 ESC populations. Scale bars: 10 μm. Enlargements of boxed regions are indicated as 5× zoom.

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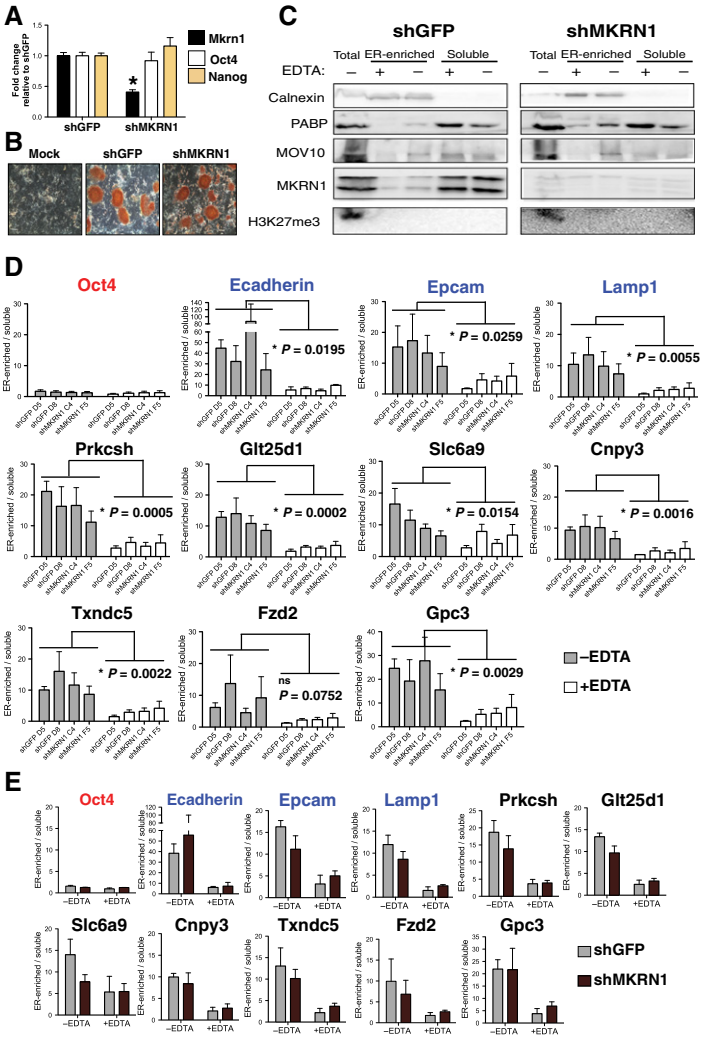

Figure EV4. Subcellular fractionation analysis of MKRN1-associated mRNAs predicted to localize to the ER.

A qPCR analysis verifies that *Mkrn1* expression was repressed in shMKRN1-transfected ESC populations relative to shGFP-transfected ESC populations prior to performing microarray analysis. Data are means ± SEM from four biological replicate experiments (*$P < 0.0001$, two-tailed *t*-test).

B Alkaline phosphatase-stained, shRNA-transfected ESC populations following four days of puromycin selection. Only positively transfected cells remained following puromycin selection as indicated by the absence of cells in the mock-transfected population. Transient knockdown of *Mkrn1* did not induce spontaneous differentiation of ESCs cultured in LIF + serum. Scale bars: 100 μm.

C Immunoblot analysis of the ER-enriched and the soluble (cytoplasmic) fractions derived from stable MKRN1 knockdown and shGFP control ESC populations. Calnexin, an ER luminal protein marker, was identified exclusively in the ER-enriched fraction of both shGFP and MKRN1 knockdown lysates irrespective of EDTA treatment, whereas trimethylated H3K27 (a nuclear marker) was not detected in ER-enriched or soluble fractions. EDTA treatment evoked a marked release of mRNPs from membrane-bound ribosomes as evident by the decrease of PABP and the RNA helicase, MOV10, in the ER-enriched fraction. MKRN1 is present in ER-enriched fractions, yet comparatively it is more abundant in the cytosolic fraction of undifferentiated ESC lysates. Similar to PABP and MOV10, MKRN1 is sensitive to EDTA treatment, suggesting that MKRN1 localization to ER membranes is dependent on its association with mSMERPs. These data confirm that our subcellular fractionation technique accurately partitioned each subcellular compartment and, furthermore, could distinguish between ribosome-dependent and ribosome-independent mRNP translocation events.

D Subcellular ER-to-cytosol distribution of seven representative MKRN1 target mSMERPs in shMKRN1 and shGFP ESC lysates. mRNA from the ER-enriched and soluble fraction was quantified with qPCR. The seven representative MKRN1 target mSMERPs are labeled in black. *E-cadherin*, *Epcam*, and *Lamp1* are non-MKRN1 target mRNAs encoding signal-peptide-containing proteins (positive controls; blue labels). *Oct4* mRNA is a non-MKRN1 target mRNA encoding a soluble nuclear protein (negative control; red label). Data are means ± SEM from three biological replicate experiments. Asterisks indicate the transcripts whose association in the ER-enriched fraction was sensitive to EDTA treatment (two-way ANOVA).

E qPCR data comparing the relative distribution of the indicated mRNAs (labels are consistent with D) in MKRN1 knockdown and shGFP control ESC lysates. Data are means of two MKRN1 knockdown clones (C4 and F5) and two shGFP control clones (D5 and D8) in each group ± SEM from three biological replicate experiments. Differences in relative distributions between MKRN1 knockdown and shGFP control clones are not significant.

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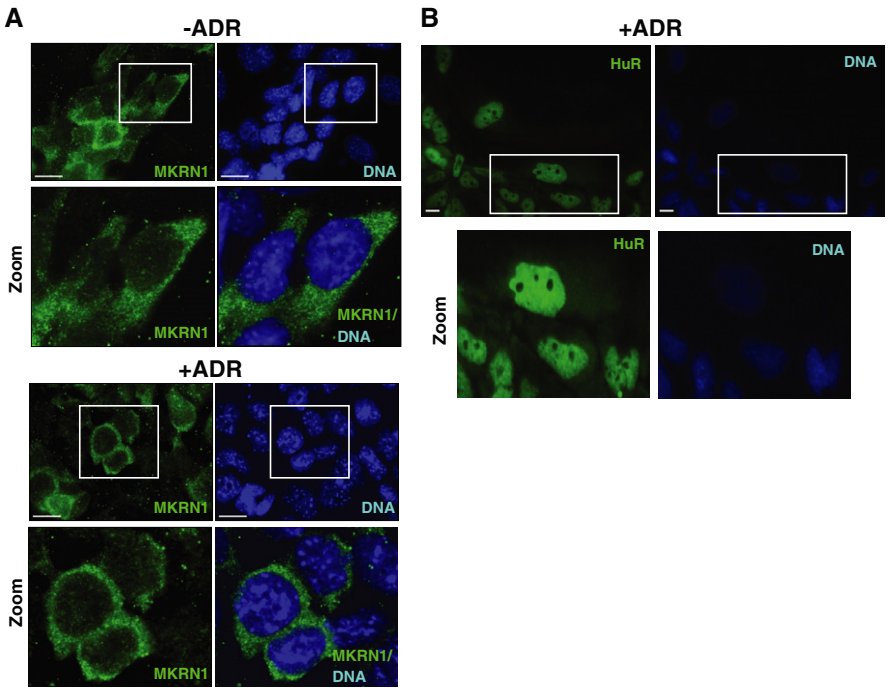

Figure EV5. SGs do not form in response to ADR-induced genotoxic stress.

A, B Wild-type R1 ESCs treated for 4 h with 0.5 μM ADR (+ADR) or left untreated (−ADR). Cells were immunostained with either anti-MKRN1 (A) or anti-HuR (B) antibodies and counterstained with Hoechst. MKRN1 does not mobilize to stress granules upon ADR-induced genotoxic stress in ESCs (A), nor does the known SG-resident protein, HuR (B). Scale bars: 10 μm. Enlargements of boxed regions are indicated as zoom.

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