Cell Reports, Volume *32*

Supplemental Information

Extensive SUMO Modification of Repressive Chromatin Factors Distinguishes Pluripotent from Somatic Cells

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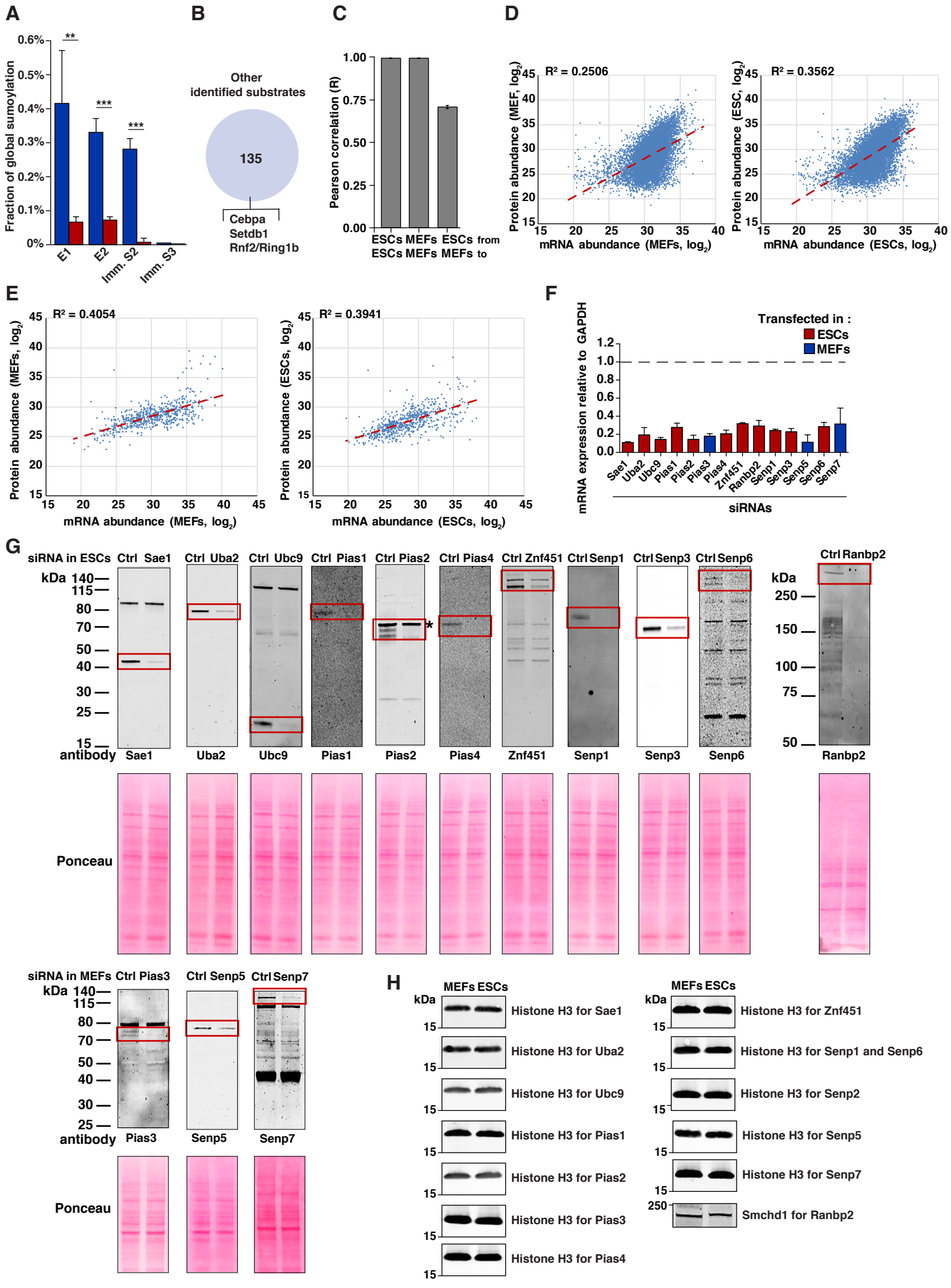
A
Fraction of global sumoylation
0.6%
0.5%
0.4%
0.3%
0.2%
0.1%
0%
**
***
***
E1
E2
Imm. S2
Imm. S3
B
Other identified substrates
135
Cebpa
Setdb1
Rnf2/Ring1b
C
Pearson correlation (R)
1.00
0.75
0.50
0.25
0.00
ESCs MEFs
ESCs MEFs
ESCs from MEFs to
D
R² = 0.2506
Protein abundance (MEF, log2)
mRNA abundance (MEFs, log2)
R² = 0.3562
Protein abundance (ESC, log2)
mRNA abundance (ESCs, log2)
E
R² = 0.4054
Protein abundance (MEFs, log2)
mRNA abundance (MEFs, log2)
R² = 0.3941
Protein abundance (ESCs, log2)
mRNA abundance (ESCs, log2)
F
mRNA expression relative to GAPDH
Transfected in :
ESCs
MEFs
Sae1
Uba2
Ubc9
Pias1
Pias2
Pias3
Pias4
Znf451
Ranbp2
Senp1
Senp3
Senp5
Senp6
Senp7
siRNAs
G
siRNA in ESCs
Ctrl Sae1
Ctrl Uba2
Ctrl Ubc9
Ctrl Pias1
Ctrl Pias2
Ctrl Pias4
Ctrl Znf451
Ctrl Senp1
Ctrl Senp3
Ctrl Senp6
Ctrl Ranbp2
kDa
140
115
80
70
50
40
30
25
15
antibody
Sae1
Uba2
Ubc9
Pias1
Pias2
Pias4
Znf451
Senp1
Senp3
Senp6
kDa
250
150
100
75
50
Ranbp2
Ponceau
siRNA in MEFs
Ctrl Pias3
Ctrl Senp5
Ctrl Senp7
kDa
140
115
80
70
50
40
30
25
antibody
Pias3
Senp5
Senp7
Ponceau
H
MEFs ESCs
kDa
15
Histone H3 for Sae1
Histone H3 for Uba2
Histone H3 for Ubc9
Histone H3 for Pias1
Histone H3 for Pias2
Histone H3 for Pias3
Histone H3 for Pias4
MEFs ESCs
kDa
15
250
Histone H3 for Znf451
Histone H3 for Senp1 and Senp6
Histone H3 for Senp2
Histone H3 for Senp5
Histone H3 for Senp7
Smchd1 for Ranbp2

Figure S1 SUMOylome analysis between MEFs and ESCs. Related to Figures 1-3.
(A) The histogram is a detail of the Figure 2A. Quantification of the SUMO2/3 equilibrium in MEFs and ESCs, visualizing the fraction of total SUMO existing as conjugated to certain target proteins, or as immature free SUMO. S2 = SUMO2, S3 = SUMO3, Imm. = Immature. Error bars indicate mean + SD, n=4 cell culture replicates.
(B) Number and examples of relevant SUMO substrates that were quantified in less than 4 replicates in MEFs and/or ESCs.
(C) Visualization of average Pearson correlation between MEF and ESC total proteomes. Error bars represent SD, n=4 cell culture replicates.
(D) Correlation between mRNA abundance (x-axis) (Cossec et al., 2018) and the abundance of the corresponding proteins (y-axis) in MEFs (left) and ESCs (right) for all proteins identified in the total proteome analysis. The dotted line corresponds to the regression line.
(E) Correlation between mRNA abundance (x-axis) (Cossec et al., 2018) and the abundance of the corresponding proteins (y-axis) in MEFs (left) and ESCs (right) for all SUMO targets. The dotted line corresponds to the regression line.
(F) Knockdown efficiency for SUMO enzyme transcripts as detected by RT-qPCR upon transfection of the indicated siRNAs in MEFs or ESCs. The mRNA levels were normalized against GAPDH and expressed relative to the control siRNA (dotted line). n=3
(G) Knockdown efficiency for SUMO enzymes as detected by western blotting upon transfection of the indicated siRNAs in MEFs or ESCs. Ponceau staining was used as a loading control. The asterisk indicates a non-specific band. Ctrl = Control.
(H) Immunoblots for histone H3 used as loading controls corresponding to each individual blot as shown in Figure 3E. SMCHD1 was used, instead of H3, for Ranbp2 due to Ranbp2 high molecular weight. Signals were obtained from the same blots as used to detect SUMO enzymes.

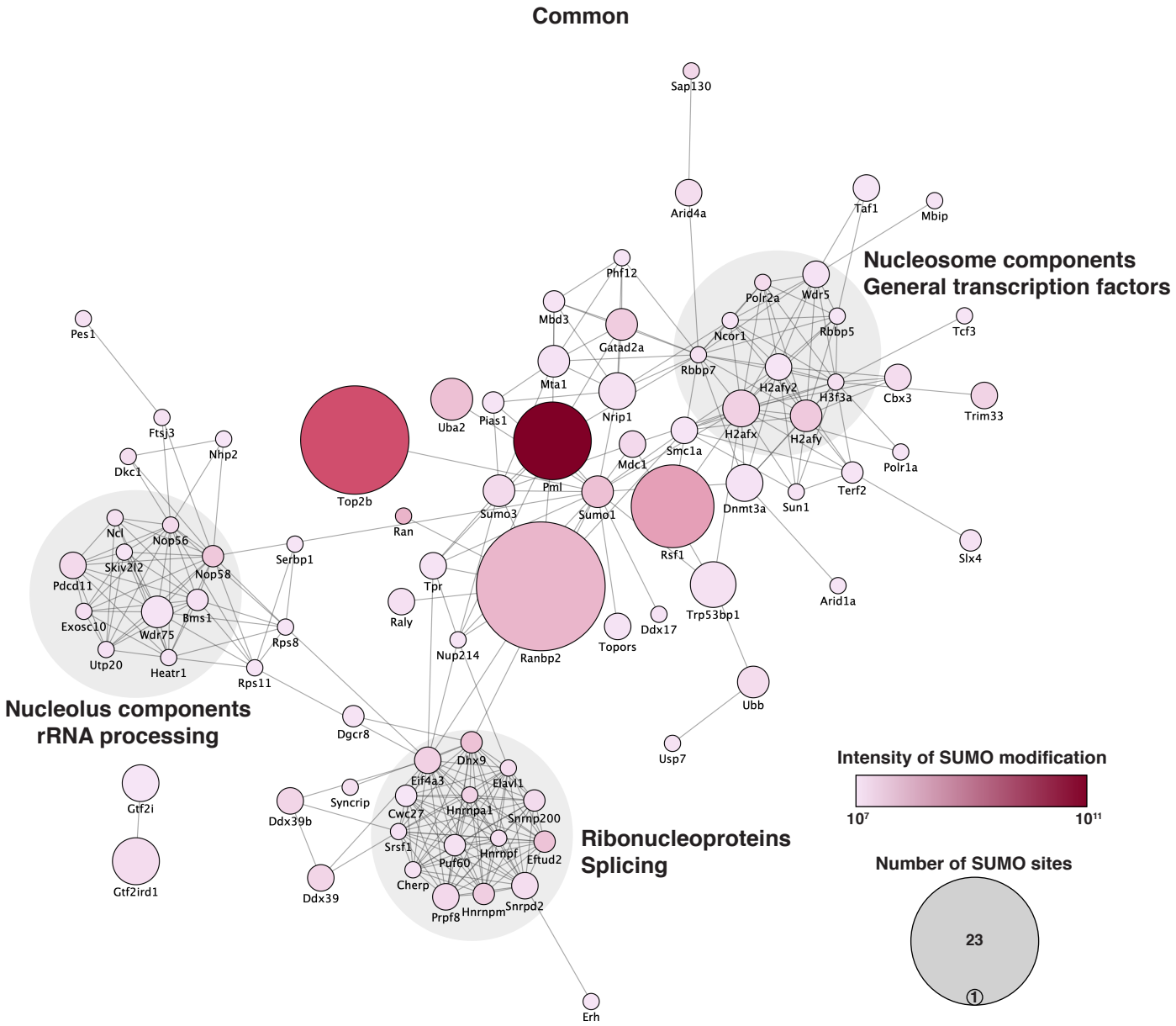

Figure S2 Network of the common SUMO targets in MEFs and ESCs. Related to Figure 4. STRING-network analysis of proteins equally SUMOylated in MEFs and ESCs. The size of the individual proteins corresponds to the number of SUMOylation sites identified in the proteins and the color to the intensity of SUMOylation of the protein.

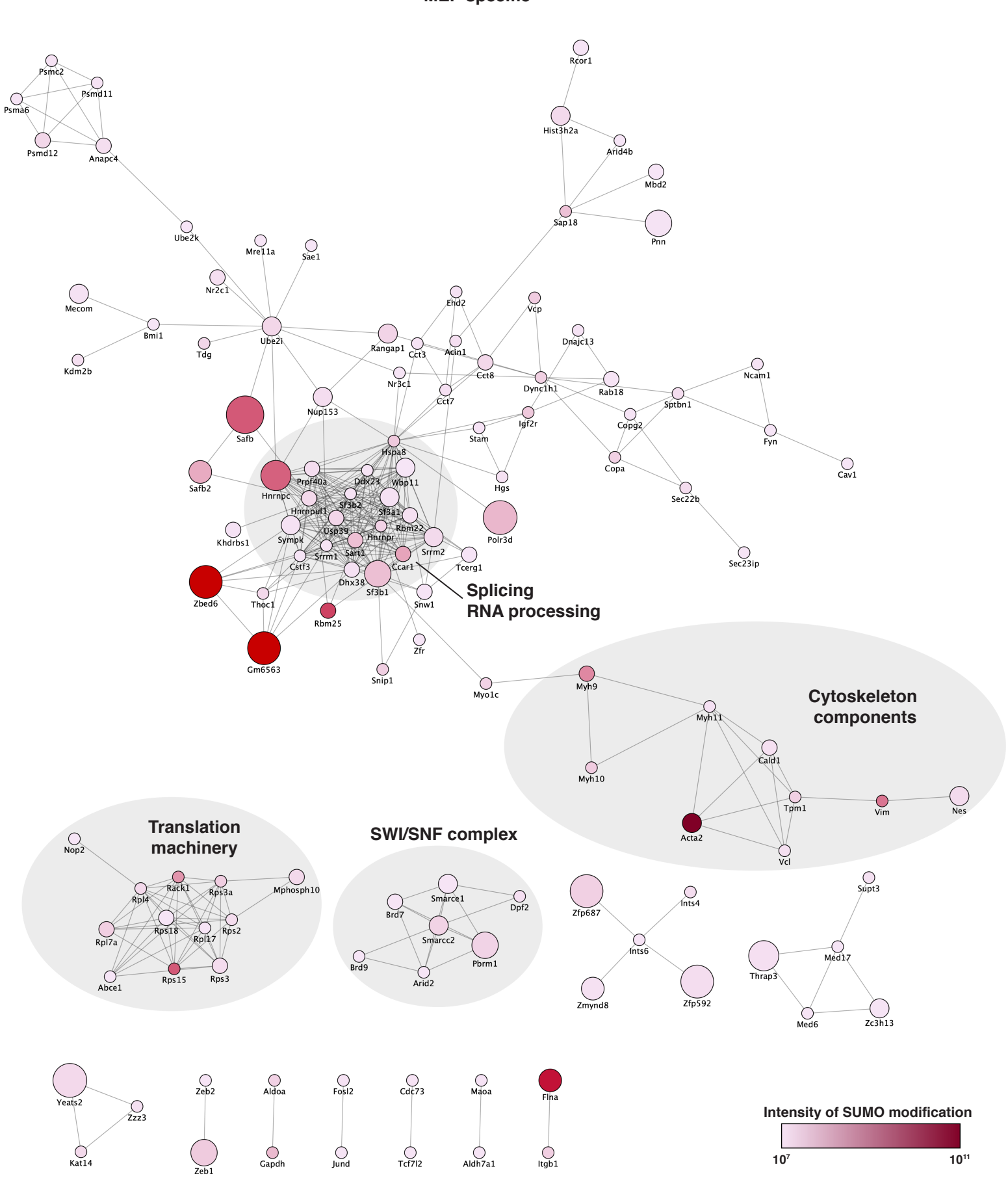

Figure S3 Network of the specific SUMO targets in MEFs. Related to Figure 4.
STRING-network analysis of proteins preferentially SUMOylated in MEFs. The size of the individual proteins corresponds to the number of SUMOylation sites identified in the proteins and the color to the intensity of SUMOylation of the protein.

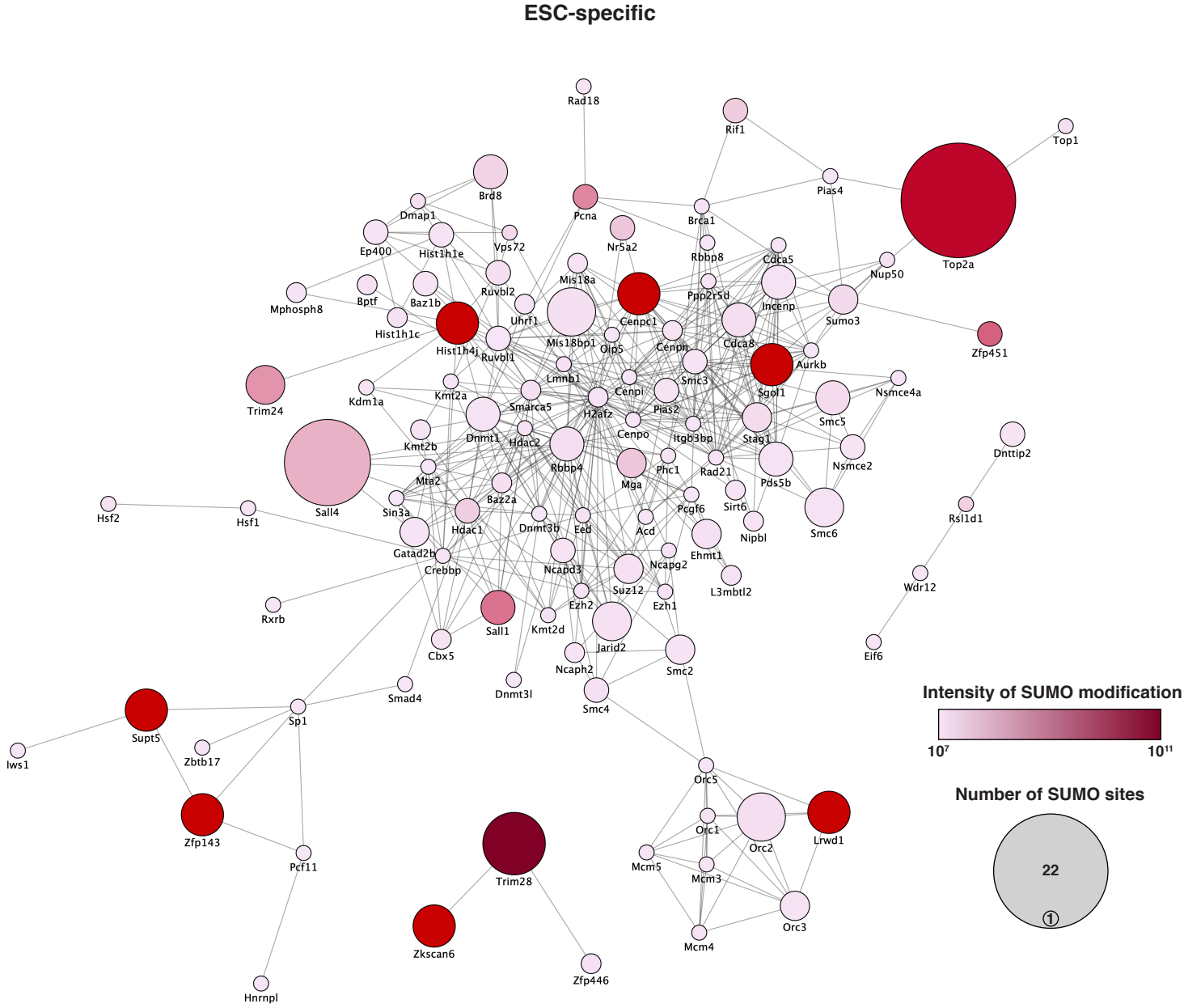

Figure S4 Network of the specific SUMO targets in ESCs. Related to Figure 4.
STRING-network analysis of proteins preferentially SUMOylated in ESCs. The size of the individual proteins corresponds to the number of SUMOylation sites identified in the proteins and the color to the intensity of SUMOylation of the protein.

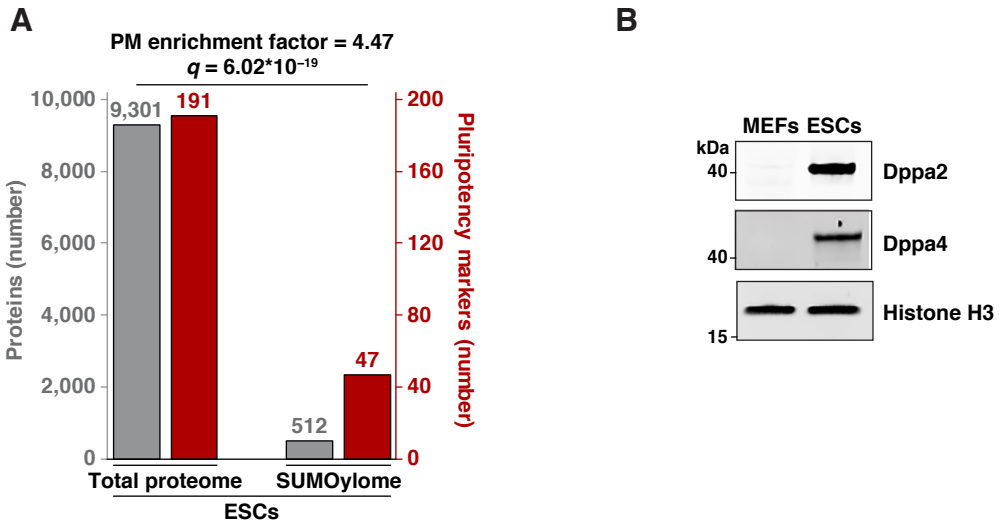

Figure S5 SUMOylation targets Dppa2 and Dppa4 among other pluripotency factors in ESCs. Related to Figure 5.

(A) Number of pluripotency factors within the total set of identified proteins in ESCs and among the identified SUMO targets. The relative enrichment factor was determined via Fisher's exact testing. (B) Immunoblots for Dppa2 and Dppa4 in MEFs and ESCs. Whole cell lysates from the same number of cells were loaded for the two cell-types. Histone H3 was used as a loading control given the comparable histone/chromatin content per cell, regardless of cell type. Signals were obtained from the same blot. Representative example, n=2.

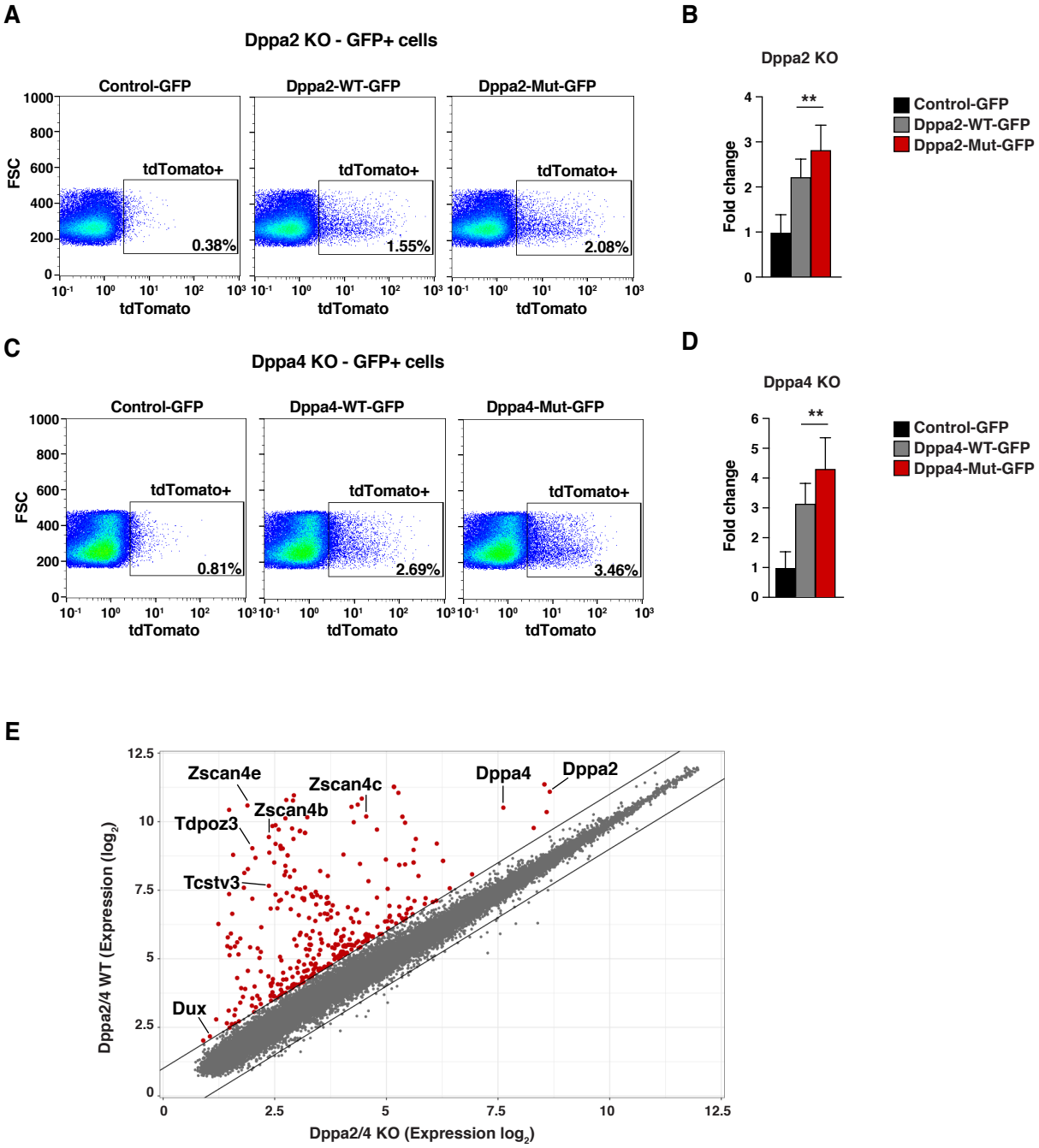

Figure S6 Individual SUMO-deficient versions of Dppa2 and Dppa4 increases the conversion towards the 2C-like state. Related to Figure 6.

(A) Flow cytometry profiles of GFP-positive cells 2 days after transfection of GFP (left), Dppa2-WT-GFP (middle) or Dppa2-Mut-GFP (right) in Dppa2 KO ESCs. The population and percentage of tdTomato-positive cells are shown in the square. Representative example, n=6.

(B) Percentage of tdTomato positive cells in Dppa2 KO ESCs complemented with GFP, Dppa2-WT-GFP or Dppa2-Mut-GFP. n=6, error bars indicate mean + SD.

(C) Flow cytometry profiles of GFP-positive cells 2 days after transfection of GFP (left), Dppa4-WT-GFP (middle) or Dppa4-Mut-GFP (right) in Dppa4 KO ESCs. The population and percentage of tdTomato-positive cells are shown in the square. Representative example, n=6.

(D) Percentage of tdTomato positive cells in Dppa4 KO ESCs complemented with GFP, Dppa4-WT-GFP or Dppa4-Mut-GFP. n=6, error bars indicate mean + SD.

(E) Scatter plot comparing gene expression of double KO ESCs complemented with GFP (Control) or Dppa2-WT-GFP + Dppa4-WT-GFP. Cells were sorted for GFP expression in both conditions. The genes dependent on Dppa2 and Dppa4 expression are colored in red (Fold Change>2). n=3.