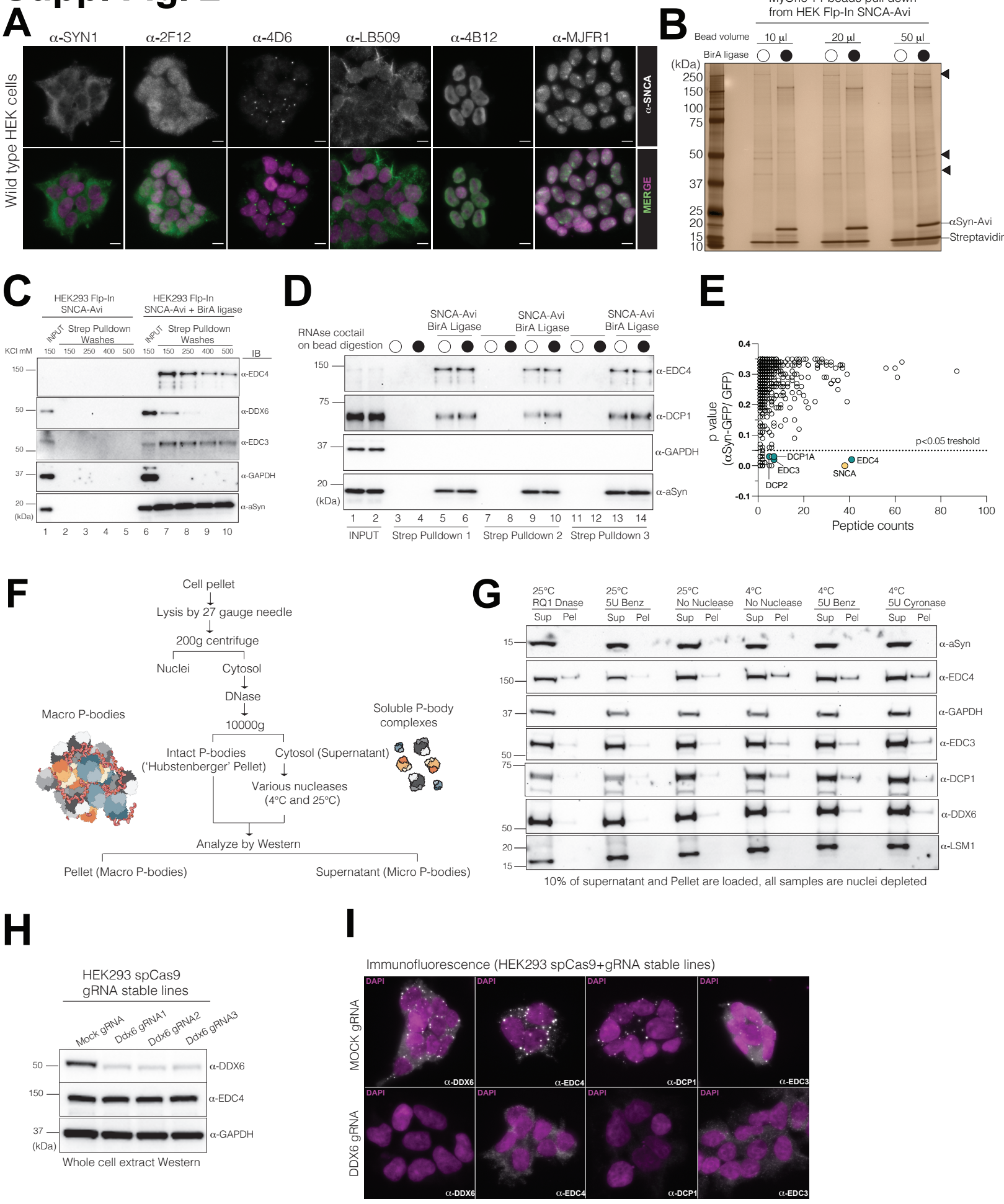
Supp. Fig. 2


A
α-SYN1
α-2F12
α-4D6
α-LB509
α-4B12
α-MJFR1
Wild type HEK cells
α-SNCA
MERGE
B
MyOne T1 beads pull down
from HEK Flp-In SNCA-Avi
Bead volume
10 µl
20 µl
50 µl
BirA ligase
(kDa)
250
150
100
75
50
37
25
20
15
10
αSyn-Avi
Streptavidin
C
HEK293 Flp-In
SNCA-Avi
HEK293 Flp-In
SNCA-Avi + BirA ligase
INPUT
Strep Pulldown
Washes
KCl mM
150 150 250 400 500 150 150 250 400 500
IB
α-EDC4
α-DDX6
α-EDC3
α-GAPDH
α-aSyn
(kDa)
1 2 3 4 5 6 7 8 9 10
D
RNAse coctail
on bead digestion
SNCA-Avi
BirA Ligase
α-EDC4
α-DCP1
α-GAPDH
α-aSyn
(kDa)
1 2 3 4 5 6 7 8 9 10 11 12 13 14
INPUT
Strep Pulldown 1
Strep Pulldown 2
Strep Pulldown 3
E
p value
(αSyn-GFP/ GFP)
p<0.05 treshold
DCP1A
EDC4
EDC3
SNCA
DCP2
Peptide counts
F
Cell pellet
Lysis by 27 gauge needle
200g centrifuge
Nuclei
Cytosol
DNase
10000g
Macro P-bodies
Intact P-bodies
('Hubstenberger' Pellet)
Cytosol (Supernatant)
Soluble P-body
complexes
Various nucleases
(4°C and 25°C)
Analyze by Western
Pellet (Macro P-bodies)
Supernatant (Micro P-bodies)
G
25°C
RQ1 Dnase
25°C
5U Benz
25°C
No Nuclease
4°C
No Nuclease
4°C
5U Benz
4°C
5U Cyronase
Sup Pel
α-aSyn
α-EDC4
α-GAPDH
α-EDC3
α-DCP1
α-DDX6
α-LSM1
10% of supernatant and Pellet are loaded, all samples are nuclei depleted
H
HEK293 spCas9
gRNA stable lines
Mock gRNA
Ddx6 gRNA1
Ddx6 gRNA2
Ddx6 gRNA3
α-DDX6
α-EDC4
α-GAPDH
(kDa)
Whole cell extract Western
I
Immunofluorescence (HEK293 spCas9+gRNA stable lines)
MOCK gRNA
DDX6 gRNA
DAPI
α-DDX6
α-EDC4
α-DCP1
α-EDC3