**FBXL11A-p044**

**Growth method**:

Medium:

Virus amplification: Sf900 III (Gibco) + 2% FBS.

Expression: Insect-Xpress (Lonza).

2 x 1L of Sf9 insect cells in 3L glass non-baffled flasks were infected with 3mL of virus P2 per flask.Cell density at infection time: 2e6 /mL. Protein was expressed for 72h at 27°C with 100rpm shaking.

**Extraction buffers**:

Lysis buffer: 50 mM HEPES-KOH, 0.3 M KCl, 5% glycerol, 10mM imidazole pH 7.4, protease inhibitors cocktail set III (Calbiochem) and 50U of benzonase (EMD Milipore).

**Extraction procedure**:

Fresh cell pellets were resuspended in a total volume of 50 mL of lysis buffer. Cells were broken by dounce homogenisation, approx 30 strokes. Cell debris were removed by centrifugation for 60 minutes at 55 000 xg.

**Purification buffers**:

Ni-affinity

lysis/wash10: 50mM HEPES, 0.3 KCl 5% glycerol, 10mM imidazole, pH 7.5 wash: 50mM HEPES, 0.3M KCl, 5% glycerol, 40mM imidazole, pH 7.5 elution: 50mM HEPES, 0.3M KCl. 5% glycerol, 250mM imidazole,pH 7.5.

IEX

low salt: 25mM HEPES, 50mM NaCl, 5% glycerol, pH 7.5

high salt: 25mM HEPES, 1M NaCl, 5% glycerol pH 7.5

dilution buffer: 25mM HEPES pH 7.5

**Purification procedure**:

Column 1: Proteins were batch bound to 2mL of Ni-sepharose 6 FF resin (GE) for one hour at 4°C. Resin was spun at 500 x g and supernatant removed. Resin was redissolved in 20 column volumes of binding buffer and transferred to gravity column. Column was washed with 10 volumes of wash buffer and proteins were eluted with elution buffer containing protease inhibitors. Fractions containing proteins were analysed on SDS-page and pooled together.

Column 2: Ion exchange, Q FF 1ml HiTrap column (GE) attached to Akta-Xpress system. Pooled fractions were diluted 10x with dilution buffer and loaded on HiTrap column at flow of 2mL/min. Column was washed with 20 volumes of low salt buffer and protein were eluted with 20CV 50%B gradient.

Protein elutes between

350mM -500mM NaCl concentration.

Purification procedure has to be performed during one day due to fast degradation and loss of activity.

**Protein stock concentration**:

The protein was concentrated using an Amicon Ultracel centrifugal concentrator (50 kDa MWCO) to 1.7 mg/ml by A280 and extinction coefficient. Aliquots were snap frozen and kept in -80°C.

**Mass spec**:

The molecular weight was confirmed (62 589) and protein shows specific demethylase activity by alpha screen assay.