Protocol for Pull-down experiment using the biotin labelling with APEX2 system

1. Add doxycycline (5 µg/ml) to the culture media 24 to 48h prior to cell lysis.
2. Add biotin-phenol (500 µM) (biotin tyramide, Iris Biotech) to the culture media 1h before lysis. Make sure the biotin-phenol has dissolved correctly. Incubate for 1h at 37°C.
3. Add hydrogen peroxide (2 mM) to the media. Incubate for 2 minutes at RT while rocking the dishes.
4. Wash the cells 3 times using ice cold PBS with added inhibitors.
5. Add ice cold RIPA lysis buffer with added inhibitors in the dishes (1 ml for 100mm dish). Keep on ice during lysis.
6. Scrape the cells off the dish and put into microcentrifuge tube.
7. Incubate the cells for 10-30 minutes on ice.
8. Sonicate the cells 3x10s (25% intensity) with 10s intervals.
9. Centrifuge the lysate at 20,000 x g for 10 minutes at 4°C.
10. Carefully remove the supernatant and put in a fresh microcentrifuge tube without disturbing the pellet. Store on ice or at -20°C.
11. Add 25 µl/100mm dish of streptavidin Sepharose bead (80% slurry)(Streptavidin-sepharose high performance, GE Healthcare), incubate 2h at 4°C with agitation. Centrifuge at 2,000 x g for 5 min at 4°C (use slow deceleration). Carefully remove the supernatant.
12. Wash the beads 3 times with ice cold RIPA lysis buffer with added inhibitors.
13. Transfer the beads in lobind tube, wash the beads 5 times with 20mM NH_4HCO_3 and proceed with on-beads digestion protocol.

PBS 1X with added inhibitors

-PBS 1X pH 7.4

-10 mM Sodium Azide (Freshly made and added)

-10 mM Sodium Ascorbate (Freshly made and added)

-5 mM Trolox (Freshly made and added)

RIPA with added inhibitors

-50 mM Tris pH 7.5

-150 mM NaCl

-0.1% SDS

-5 mg/ml Sodium deoxycholate

-1% Triton X-100

-1 mM PMSF (Freshly added)

-10 mM Sodium Azide (Freshly made and added)

-10 mM Sodium Ascorbate (Freshly made and added)

-5 mM Trolox (Freshly made and added)