Supplementary Information

RanGTPase links nucleo-cytoplasmic transport to recruitment of cargoes into small extracellular vesicles

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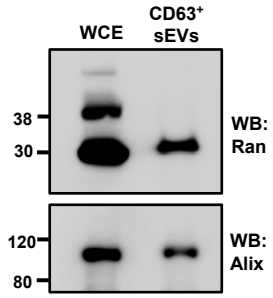

Fig. S1. Ran is present in CD63⁺ exosomes. HeLa sEVs isolated through ultracentrifugation (1,00,000 *g* pellet) were further subjected to immuno-affinity purification using Exosome-Human CD63 Isolation/Detection kit (Invitrogen) to obtain CD63-positive sEVs (CD63⁺ sEVs). The isolated vesicles were lysed and subjected to western blotting (WB) using indicated antibodies. Alix was used as sEV (exosome) marker. Molecular weight markers (in numbers) are as indicated.

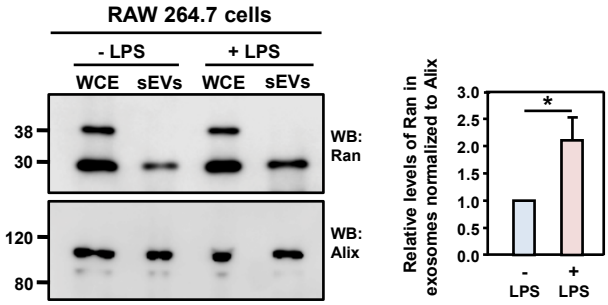

Fig. S2. Secretion of Ran through sEVs is dependent on extracellular signalling. RAW 264.7 cells were treated with 100 μM LPS for 36 h. Equal amount proteins from whole cell extract (WCE) and sEV fractions (sEVs) was loaded for western blotting. The relative levels of Ran sEVs derived from LPS untreated (-LPS) and treated (+LPS) cells was quantified after normalizing with the levels of Alix. Alix was used as sEV (exosome) marker (left panel). Graph represents quantitative data from three independent experiments with mean±SD (right panel). *P* value was calculated using Student's *t*-test. * indicates $P < 0.05$.

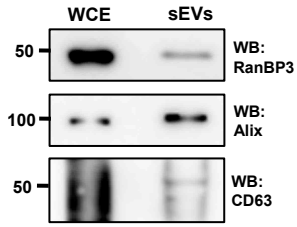

Fig. S3. RanBP3 is present in sEVs. Small extracellular vesicles (sEVs) isolated from HeLa cells were subjected to western blotting using indicated antibodies. WCE, whole cell extract. Alix and CD63 were used as sEVs (exosome) markers.

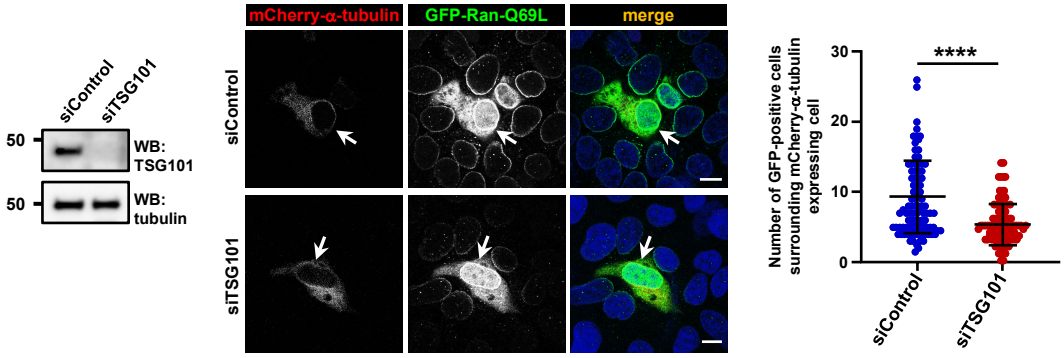

Fig. S4. Inter-cellular transfer of Ran depends on exosome biogenesis. HeLa cells were transfected with control (siControl) or TSG101-specific (siTSG101) siRNA (40 nM) for 48 h and re-transfected with each siRNA (40 nM) for 24 h. Then the cells were co-transfected with GFP-Ran-Q69L and mCherry-α-tubulin (transfection marker) for transient Ran transfer assay as described earlier (Khuperkar et al., 2015). The extent of TSG101 depletion was monitored by western blot analysis (left panel). The number of GFP-positive recipient cells (green) surrounding mCherry-α-tubulin expressing donor cells (red, indicated by arrow) were analyzed (middle panel). DNA was stained with Hoechst 33342 (blue). Scale bar, 10 μm. Relative extent of GFP-Ran-Q69L transferred to the recipient cells was calculated from three independent experiments (n=134 donor cells) and plotted (right panel). Mann-Whitney test was performed for statistical analysis. Data represented as mean±SD, **** P <0.0001.

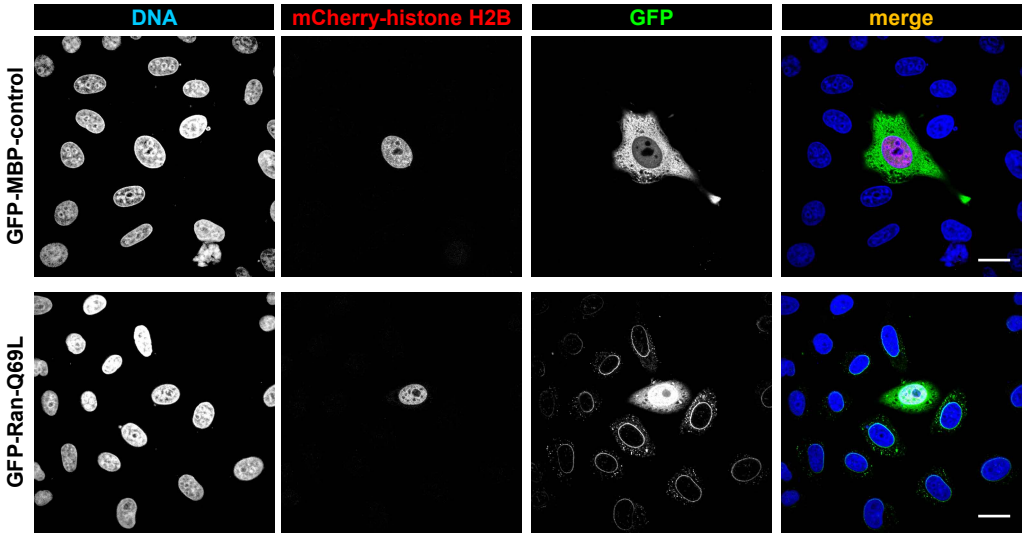

Fig. S5. Transient transfection assay for inter-cellular transfer of Ran using mCherry-histone H2B as a co-transfection marker. HeLa cells were co-transfected with GFP-MBP-control or GFP-Ran-Q69L and mCherry-H2B constructs for 10 h. Cells were fixed and immnuostained for GFP (G) with specific primary and fluorescently labelled secondary antibodies, whereas, mCherry (red) was detected by direct fluorescence. DNA was stained with Hoechst 33342 (blue). Scale bar, 20 µm.

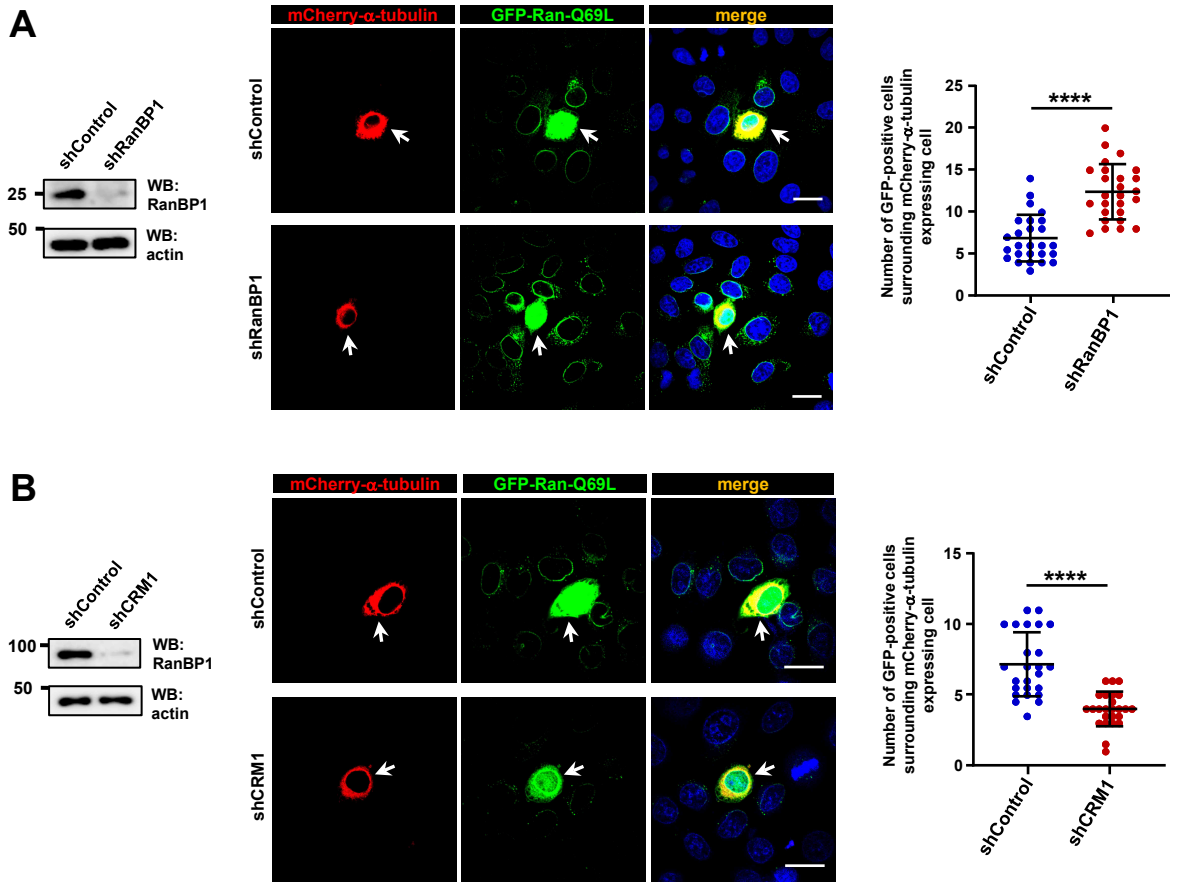

Fig. S6. Inter-cellular transfer of Ran depends on NCT. (A) HeLa cells harbouring inducible control (shControl) or RanBP1-specific shRNA (shRanBP1) were induced with doxycycline for 48 h. Then the cells were co-transfected with GFP-Ran-Q69L and mCherry-α-tubulin (transfection marker) for transient Ran transfer assay. The extent of RanBP1 depletion was monitored by western blot analysis (left panel). Actin was used as loading control. The number of GFP-positive recipient cells (green) surrounding mCherry-α-tubulin expressing donor cells (red, indicated by arrow) was analyzed (middle panel). DNA was stained with Hoechst 33342 (blue). Scale bar, 20 μm. Relative extent of GFP-Ran-Q69L transferred to the recipient cells was calculated from three independent experiments (*n*=25 donor cells) and plotted (right panel). Mann-Whitney test was performed for statistical analysis. Data represented as mean±SD, **** *P* <0.0001. (B) HeLa cells harbouring inducible control (shControl) or CRM1 shRNA (shCRM1) were induced with doxycycline for 72 h. The cells were then co-transfected with GFP-Ran-Q69L and mCherry-α-tubulin (transfection marker) for transient Ran transfer assay. The extent of CRM1 depletion was monitored by western blot analysis (left panel). Actin was used as loading control. The number of GFP-positive recipient cells (green) surrounding mCherry-α-tubulin expressing donor cells (red, indicated by arrow) were analyzed (middle panel). DNA was stained with Hoechst 33342 (blue). Scale bar, 20 μm. Relative extent of GFP-Ran-Q69L transferred to the recipient cells was calculated from three independent experiments (*n*=25 donor cells) and plotted (right panel). Mann-Whitney test was performed for statistical analysis. Data represented as mean±SD, **** *P* <0.0001.