FIGURE S1. **Immunochemical detection of endogenous Rab32 and Rab38.** A total extract from MNT-1 cells was divided into aliquots and immunoprecipitated (IP) using irrelevant rabbit IgG (lane 2) or rabbit affinity-purified antibodies against Rab32 (lane 3) or Rab38 (lane 4). Bound proteins, along with a total MNT-1 cellular extract (lane 1), were analyzed by immunoblotting (IB) using rabbit or rat affinity-purified antibodies against Rab38 (top two panels) or Rab32 (bottom two panels). Both rabbit and rat Rab38 and Rab32 antibodies are sufficiently sensitive to detect the corresponding polypeptide in total extracts and recognize only a single band (note that Rab32 mobility is slightly slower than Rab38 due to larger size) (lane 1). Immunoprecipitated Rab32 (lane 3) is detected only in immunoblots for Rab32 and not in Rab38 blots. Similarly, immunoprecipitated Rab38 (lane 4) is detected only in immunoblots for Rab38 and not for Rab32. * indicate bands from the rabbit antibody used in the IP step that is detected by the anti-rabbit secondary antibody in the IB analysis.

FIGURE S2. **siRNA treatment in MNT-1 cells efficiently and specifically silenced the corresponding targets.** The following targets were knocked down in MNT-1 cells by siRNA nucleofection: the γ subunit of AP-1 (lane 2), the δ subunit of AP-3 (lane 3), the HPS6 subunit of BLOC-2 (lane 4), Rab32 (lane 6), Rab38 (lane 7), and both Rab32 and Rab38 (lane 8). Control 1 (lane 1) represents mock nucleofected cells and Control 2 cells nucleofected with an irrelevant siRNA (lane 5). Total extracts were normalized by total protein content and analyzed by immunoblotting (IB) using the indicated antibodies.

FIGURE S3. **Rab32 partially colocalizes to proteins required in the trafficking from early endosomes to melanosomes.** MNT-1 cells were fixed/permeabilized and costained with antibodies against Rab32 (*A, D, G, J*) and the δ subunit of AP-3 (*B*), the γ subunit of AP-1 (*E*), the HPS6 subunit of BLOC-2 (*H*), or the clathrin heavy chain (*K*). Cells were imaged by confocal fluorescence microscopy and Rab32 was mainly found on small structures distributed throughout the cells. Numerous AP-3 (*B*), AP-1 (*E*), BLOC-2 (*H*), and Clathrin (*K*) labeled structures show colocalization with Rab32 in the merged images (*C, F, I,* and *L*), although to a lesser extent than with Rab38. Boxed areas are shown in the magnified insets where arrowheads indicate sites of colocalization. Scale bars indicate 10 μm.

FIGURE S4. **Validation of antibodies against Rab32 and Rab38 for immunofluorescence microscopy.** MNT1 cells were subjected to Rab32 or Rab38 siRNA treatment, fixed/permeabilized, and stained with affinity-purified antibodies against Rab32 or Rab38 generated in both rat and rabbit. Cells were imaged by confocal fluorescence microscopy and the Slidebook software was used to generate automated masks covering numerous individual cells and total fluorescence intensity was quantified and represented relative to control stainings.

FIGURE S5. **Partial rescue of tyrosinase, Tyrp1, and Tyrp2 steady state levels by leupeptin treatment on Rab32- and Rab38-deficient cells.** Control MNT-1 cells and cells deficient for Rab32, Rab38, or both Rab32 and Rab38 were incubated with leupeptin for 6 hours, total cell extracts were prepared, and the total abundance of tyrosinase (black), Tyrp1 (grey), and Tyrp2 (white) analyzed by immunoblotting. Results correspond to three independent experiments normalized to number of cells, represented relative to control cells, and compared using the *t* test, * $p<0.05$, ** $p<0.01$. Notice the higher abundance of tyrosinase, Tyrp1, and Tyrp2 (rescue) compared to knockdown cells not treated with leupeptin (Figure 8).

FIGURE S6. **MNT-1 cells express similar amounts of Rab32 and Rab38.** MNT-1 total extracts (77 μg) were analyzed by immnublotting using the corresponding rabbit anti-Rab32 or anti-Rab38 antibodies together with His-Rab32 or His-Rab38 samples containing the indicated mass of protein. The intensity of the bands was determined by chemifluorescent detection and the amount of Rab32 and Rab38 in MNT-1 total extract was calculated by comparison with the His-Rab32 and His-Rab38 calibration curves, respectively.

Figure S1

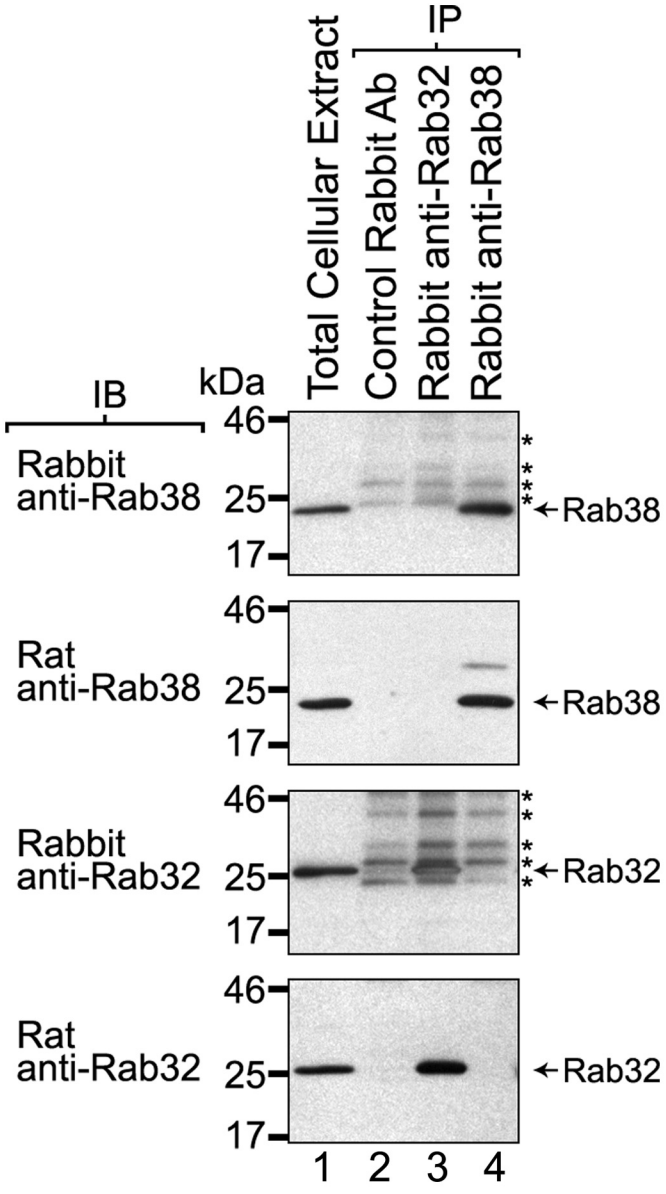

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Figure S2

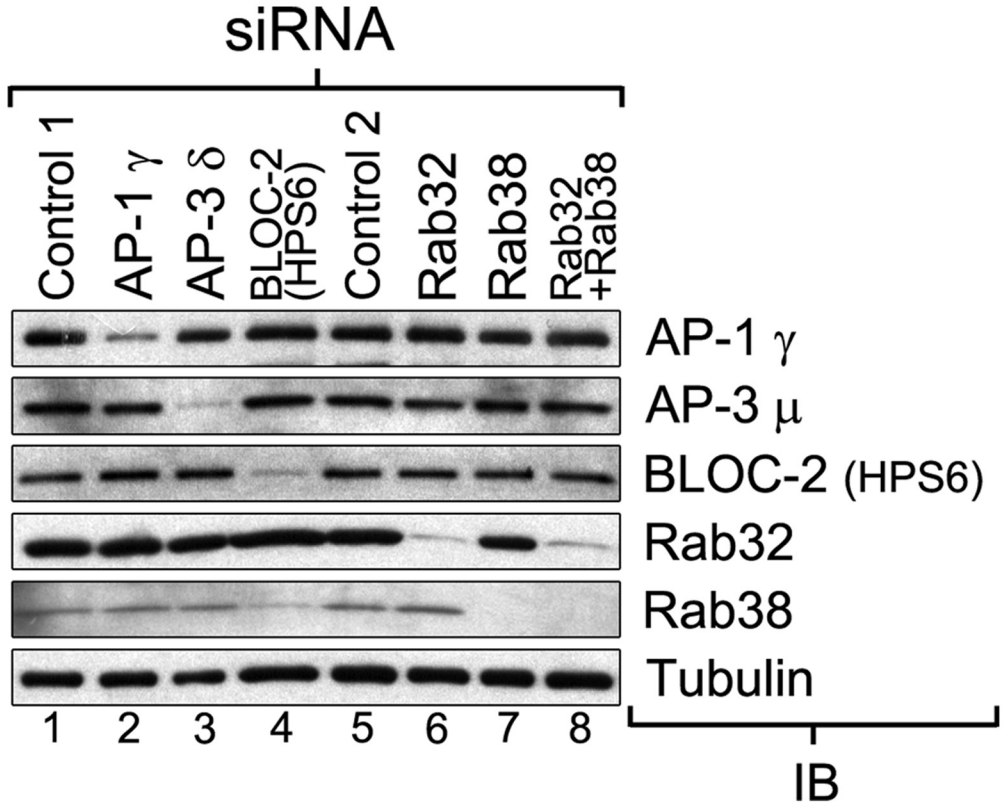

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Figure S3

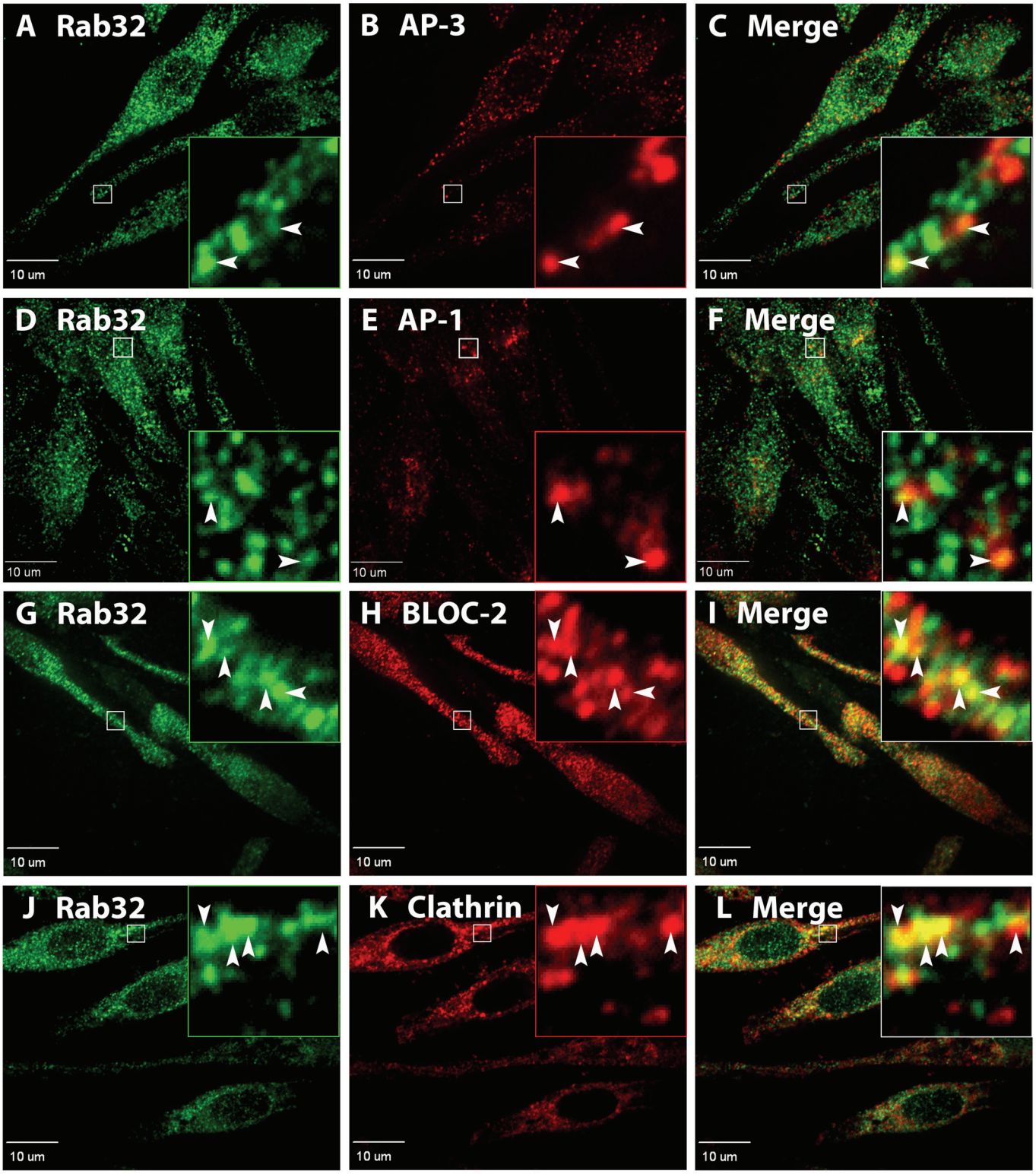

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Figure S4

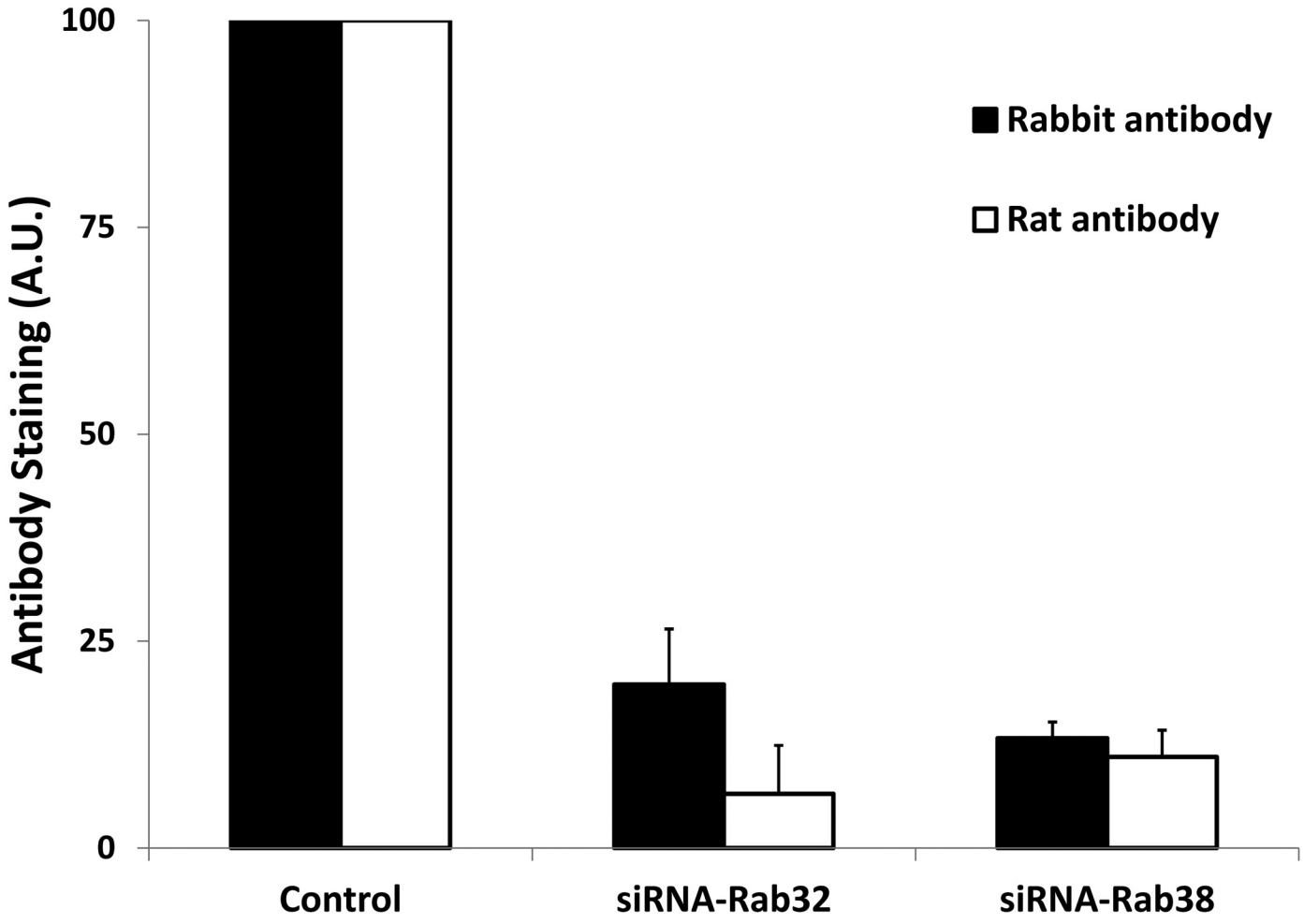

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Figure S5

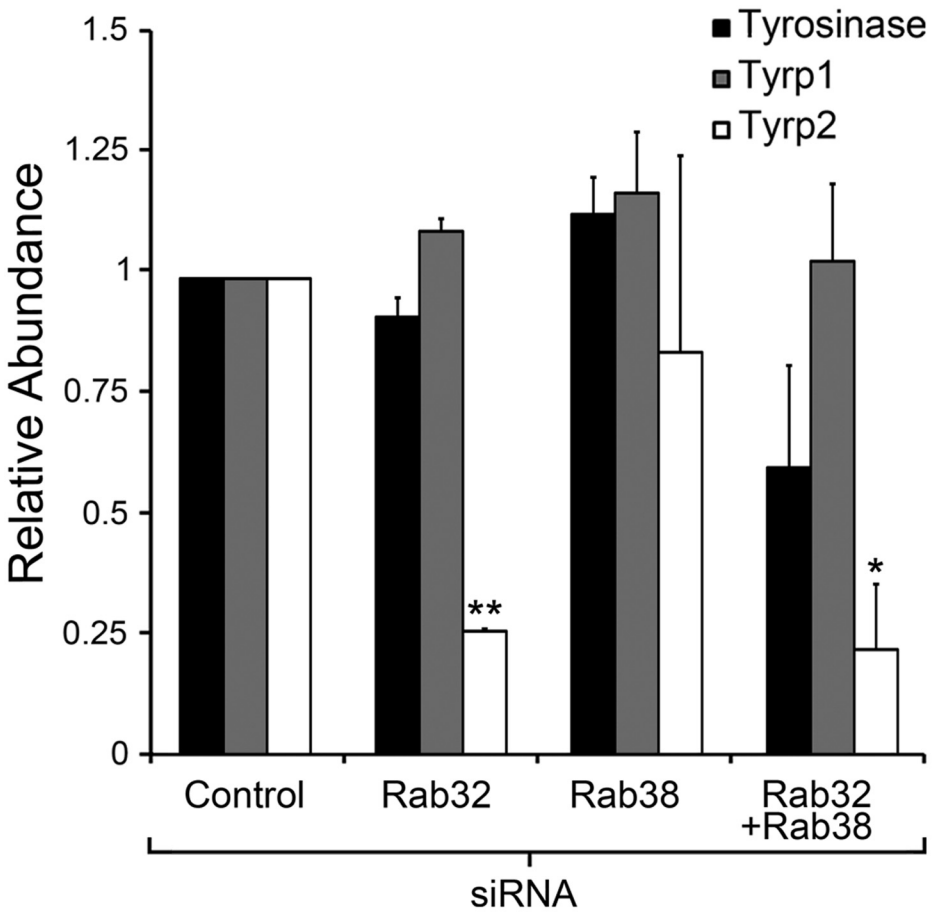

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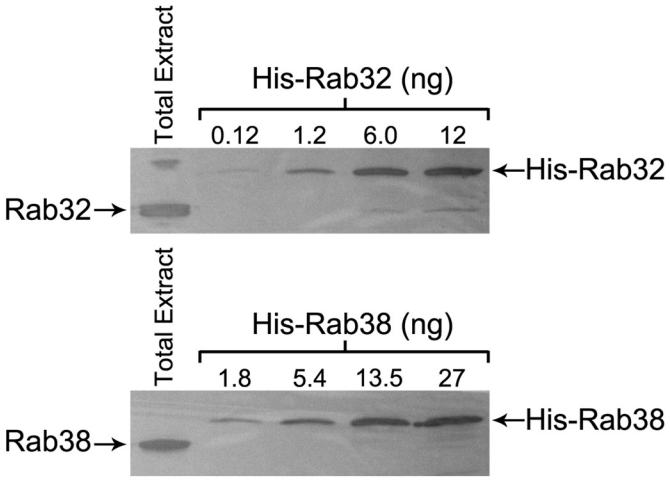
Figure S6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His-Rab32 (ng)
0.12
1.2
6.0
12
Rab32
His-Rab32
Total Extract
His-Rab38 (ng)
1.8
5.4
13.5
27
Rab38
His-Rab38