Supplemental Data

Figure S1

14.7K does not block CCP formation of activated TNF-R1 complexes, but prevents receptor internalization.

NIH 3T3 PM-14.7K and NIH 3T3 14.7K cells were labeled with biotin-TNF for 2 hr at 4ºC followed by incubation with streptavidin-microbeads and biotin-gold (5nm) particles. Electron micrographs were taken either from cells maintained at 4º C (**a**, **b**) or from cells incubated for additional 60 min at 37ºC (**c**-**f**).

Figure S2

Internalization of transferrin receptor is not inhibited by 14.7K.

PM-14.7K cells (**a**) and 14.7K NIH 3T3 cells (**b**) were incubated with Alexa-488 labeled-transferrin for 15 minutes at 4ºC, and temperature was then increased to 37ºC to allow internalization of transferrin receptor. Reactions were stopped by formaldehyde fixation and the intracellular distribution of transferring receptors was visualized by confocal microscopy at indicated time points.

Figure S3

14.7K inhibits TNF-R1 internalization in HeLa cells.

Cells were labeled with biotin-TNF/strepavidin-FITC complexes at 4°C for 240 minutes (**a-c**), and TNF-R1 endocytosis was monitored after a temperature shift to 37°C for additional 15 to 60 minutes by confocal laser microscopy (**d-l**). Labeled TNF-R1 complexes were detected after at 4°C on the cell surface of the cells (**a-c**). After a temperature shift for the indicated time points only in HeLa parental (d, g, j) and HeLa vector cells (**e**, **h**, **k**) TNF-R1 endocytosis was detected whereas in HeLa

14.7-expressing cells (**f**, **i**, **l**) the labeled receptor complexes remained on the cell surface indicating that TNF-R1 internalization was inhibited.

Figure S4

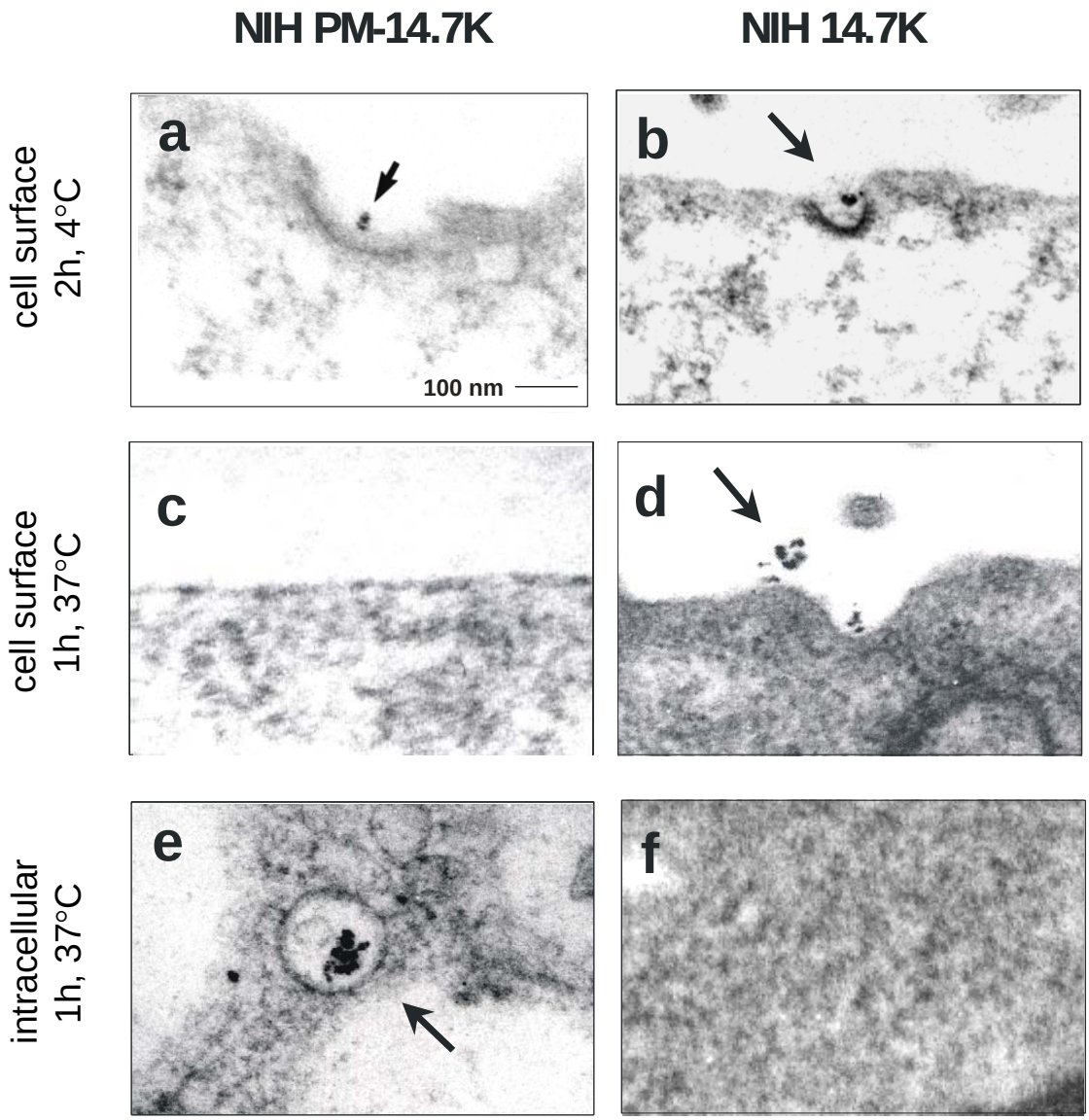
Posttranlational modifications in TNF-R1 associated TRADD in murine NIH 3T3 and C127 cells

Magnetic fractions harboring labeled TNF-TNF-R1 complexes were purified from PM-14.7K and 14.7 cells and immunoblotted with anti-TRADD antibodies. In magnetic fractions isolated from NIH PM-14.7K cells, 34 kD TRADD and two higher molecular weight proteins are detected, probably representing ubiquitinylated TRADD.

Figure S5

14.7K protein prevents DISC formation in TNF-R1 magnetic fractions purified from HeLa cells.

TNF-R1 magnetic fractions were purified from HeLa parental cells and HeLa cells stably transduced with the empty vector or 14.7K gene and immunobloted with the antibodies indicated. In HeLa parental and HeLa vector cells recruitment of TRADD, FADD, activated caspase-8, RIP-1 and TRAF-2 was detected after 30 minutes of TNF stimulation. In HeLa 14.7K cells TRADD, FADD, and caspase-8 were not detectable after 30 minutes of TNF treatment, but recruitment of RIP-1 and TRAF-2 was clearly visible. Rab5 and dynamin 2 were only recruited to TNF-R1 magnetic fractions in TNF-treated HeLa and HeLa vector cells and were absent in preparations from HeLa 14.7K cells.



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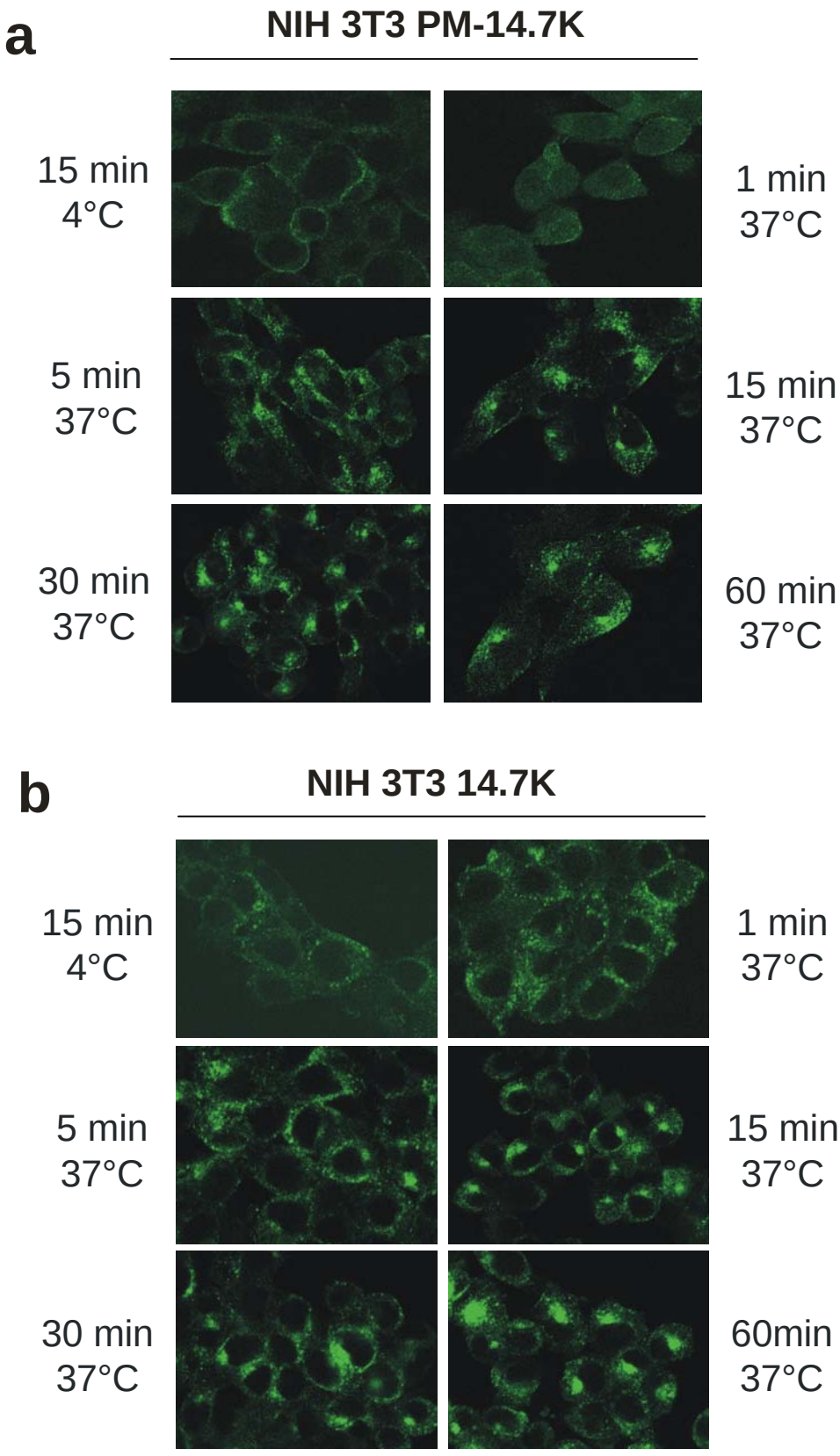
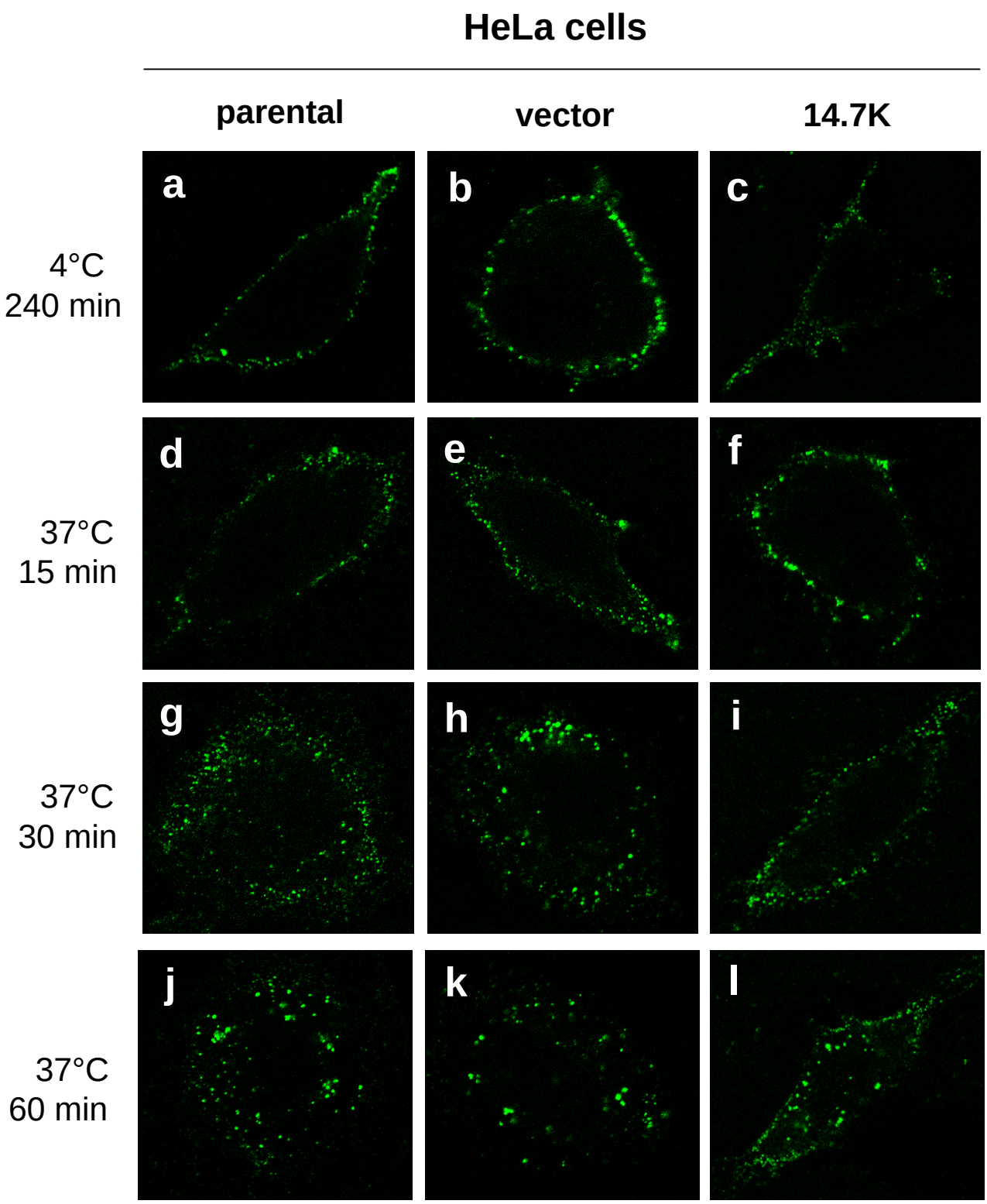
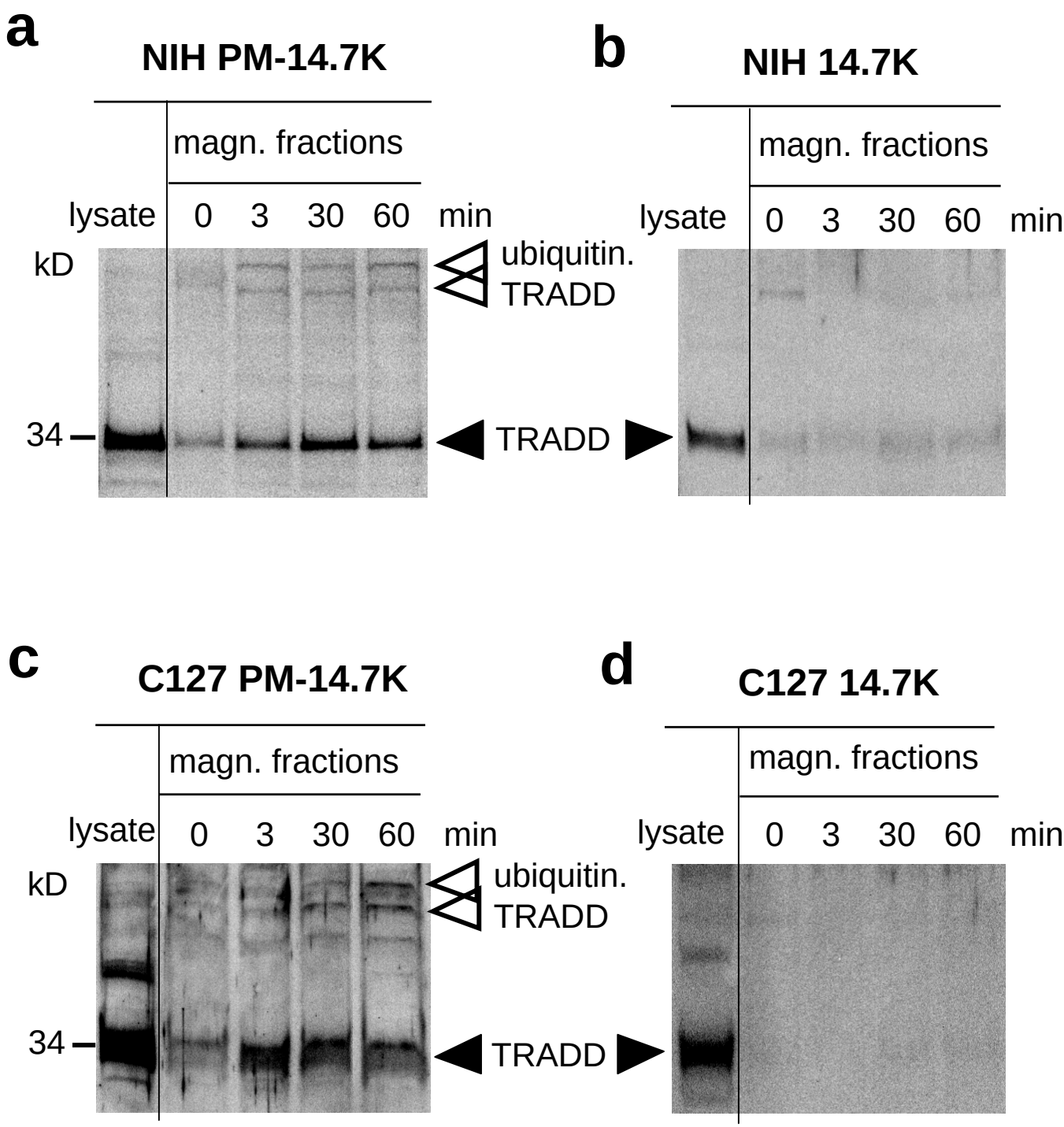
Supplemental Figure S1



Schneider-Brachert et al. 2006
Supplemental Figure S2



Schneider-Brachert et al. 2006
Supplemental Figure S3



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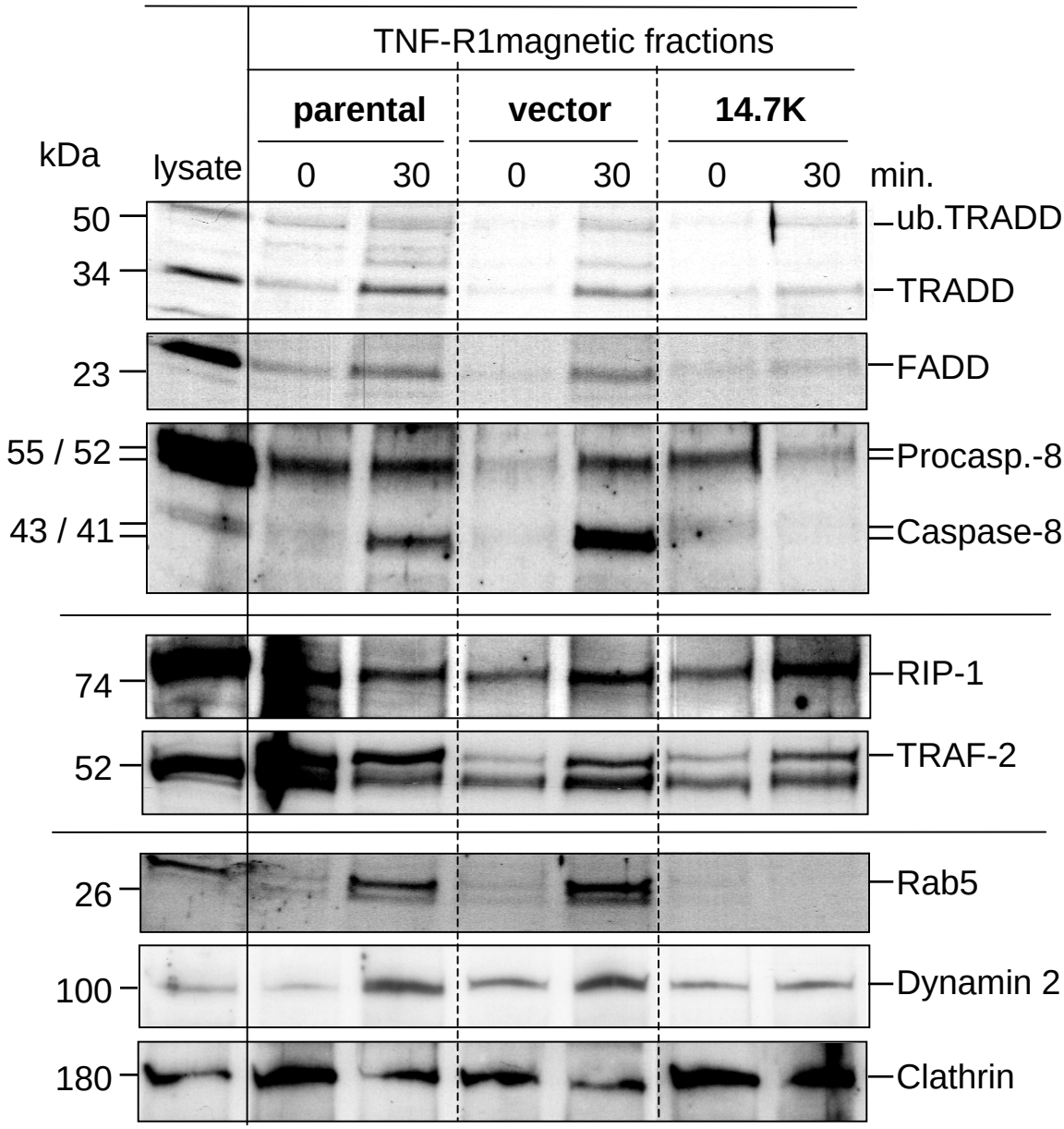
Supplemental Figure S4

HeLa cells



Schneider-Brachert et al. 2006
Supplemental Figure S5