SUPPLEMENTARY TABLES

Supplementary table 1, ***Specificities of Pmel17-specific antibodies used in this study***

Antibody	Epitope	Specificity	References
Pmel-N	N-terminus (aa 24-40)	**Pulse-chase** (P1, P2, Mα-S-S-Mβ) **Immunoprecipitation** (P1, P2, Mα-S-S-Mβ) **Western blot** (reducing) (P1, P2 and Mα) **Immunofluorescence** (ER, Golgi)	(1,2)
Pep13h	C-terminus (aa 647-661)	**Pulse-chase** (P1, P2, Mα-S-S-Mβ) **Immunoprecipitation** (P1, P2, Mα-S-S-Mβ) **Western blot** (reducing) (P1, P2 and Mβ) **Immunofluorescence** (ER, Golgi, early endosomes)	(2,3)
HMB50	PKD-domain	**Pulse-chase** (P1, P2, Mα-S-S-Mβ) **Immunoprecipitation** (P1, P2, Mα-S-S-Mβ, and probably also the 7 kDa PKD-derived fibrillogenic fragment, although this has not been confirmed so far) **Immunofluorescence** (predominantly stage II melanosomes)	(2,4,5)
NKI-beteb	PKD-domain	**Pulse-chase** (P1, P2, Mα-S-S-Mβ) **Immunoprecipitation** (P1, P2, Mα-S-S-Mβ, and probably also the 7 kDa PKD-derived fibrillogenic fragment, although this has not been confirmed so far) **Immunofluorescence** (predominantly stage II melanosomes)	(2,5,6)
HMB45	RPT-domain	**Western blot** (reducing) (very weakly P2, Mα, RPT-derived fibrillogenic fragments) **Immunofluorescence** (predominantly stage II melanosomes)	(2,7-9)
I51	aa 206-220	**Western blot** (reducing) (7 kDa PKD-derived fibrillogenic fragment, P1, P2, Mα)	(10)

The table shows specificities and reactivities of the indicated Pmel17-specific antibodies as reported in the literature (see *References*) or in this study. Fragments (or organelles) recognized by the antibodies in the indicated applications are given in brackets.

SUPPLEMENTARY REFERENCES

1. Nichols, S. E., Harper, D. C., Berson, J. F., and Marks, M. S. (2003) *J Invest Dermatol* **121**(4), 821-830
2. Harper, D. C., Theos, A. C., Herman, K. E., Tenza, D., Raposo, G., and Marks, M. S. (2008) *J Biol Chem* **283**(4), 2307-2322
3. Berson, J. F., Harper, D. C., Tenza, D., Raposo, G., and Marks, M. S. (2001) *Mol Biol Cell* **12**(11), 3451-3464
4. Esclamado, R. M., Gown, A. M., and Vogel, A. M. (1986) *Am J Surg* **152**(4), 376-385
5. Hoashi, T., Tamaki, K., and Hearing, V. J. (2009) *Faseb J*
6. Vennegoor, C., Hageman, P., Van Nouhuijs, H., Ruiter, D. J., Calafat, J., Ringens, P. J., and Rumke, P. (1988) *Am J Pathol* **130**(1), 179-192
7. Gown, A. M., Vogel, A. M., Hoak, D., Gough, F., and McNutt, M. A. (1986) *Am J Pathol* **123**(2), 195-203
8. Chiamenti, A. M., Vella, F., Bonetti, F., Pea, M., Ferrari, S., Martignoni, G., Benedetti, A., and Suzuki, H. (1996) *Melanoma Res* **6**(4), 291-298
9. Hoashi, T., Muller, J., Vieira, W. D., Rouzaud, F., Kikuchi, K., Tamaki, K., and Hearing, V. J. (2006) *J Biol Chem* **281**(30), 21198-21208
10. Watt, B., van Niel, G., Fowler, D. M., Hurbain, I., Luk, K. C., Stayrook, S. E., Lemmon, M. A., Raposo, G., Shorter, J., Kelly, J. W., and Marks, M. S. (2009) *J Biol Chem*

SUPPLEMENTARY FIGURE LEGENDS

Supplementary figure S1, ***Expression levels of transfected Pmel17 in Mel220 cells are slightly higher than endogenous Pmel17-levels in melanoma cell line buf1280(A2).*** The amount of Pep13h-reactive Pmel17 in lysates (1 x 10^7 cells/ml) derived from either buf1280(A2)-cells (40 μl loaded), untransfected Mel220-cells (40 μl loaded) or Pmel17-transfected Mel220-cells (40, 20, 10 or 5 μl loaded) was compared by Western blotting. Endogenous Pmel17-levels in buf1280(A2) cells were found to be ~2-fold higher than levels of transfected Pmel17 in Mel220-cells.

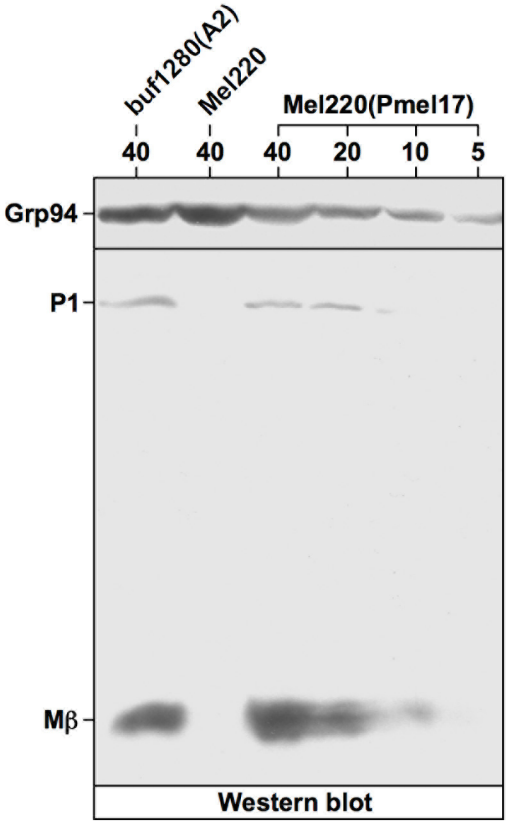
Supplementary figure S2, ***No evidence for excessive global misfolding of Δ190-208.*** **A) No unfolded protein response (UPR) induction in Mel220 cells expressing Δ190-208.** Status of UPR-induction in indicated Mel220 transfectants was analyzed by assessing XBP1-splicing. RT-PCR of XBP1 was performed using primers flanking the region that is spliced out when the UPR is induced (*left four lanes*). As this region contains a Pst I-site (*see schematic representation in bottom panel*) UPR-induction can be assessed by sensitivity of the amplified fragment to Pst I-cleavage (*right four lanes*). As a positive control the UPR was induced with tunicamycin. One unspecific cross-amplification product is labeled with an asterisk. **B) Δ190-208 has a half-life of more than 3h.** Mel220 transfectants stably expressing Δ190-208 were pulse-labeled for 30 min. with ^{35}S and subsequently chased for the indicated times. 2% Triton X-100 lysates were immunoprecipitated with Pmel17-specific antibody HMB50, eluted with 0.5% SDS under vigorous vortexing for 30 min. and analyzed by autoradiography (*top panel*). Quantitative phosphorimager analysis of the pulse-chase data with maximal levels set to 100% is shown (*bottom panel*).

Supplementary figure S3, ***IR-wt and H190P localize to a different subcellular compartment than wt-Pmel17.*** **A)** Schematic representation of the characteristic subcellular distribution pattern of wt-Pmel17 (*red*) in the cell. Pmel17 is mostly localized to a horseshoe-shaped broad band surrounding the perinuclear area. In contrast, LAMP1 (*green*) is mostly found in a condensed

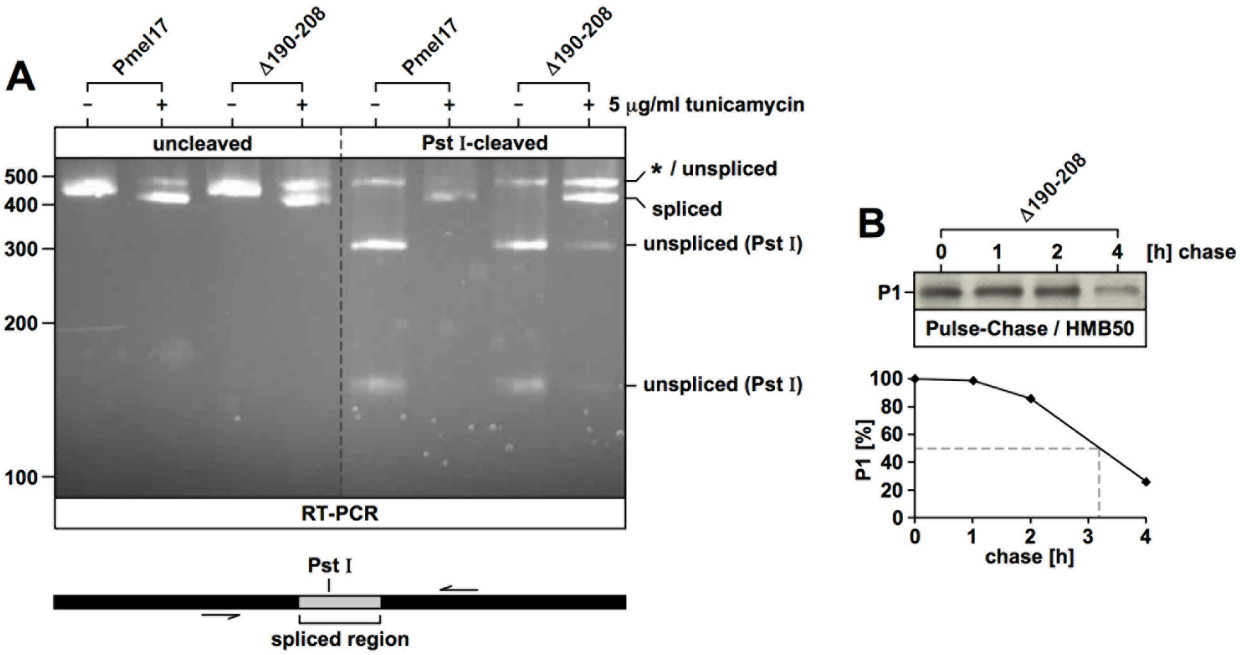
juxtanuclear pattern and only to a much lower extent in the Pmel17-positive horseshoe (*top panel*). Schematic representation of the fluorescence intensity distribution for Pmel17 (*red*) and LAMP1 (*green*) along the horizontal axis of a cell oriented with the nucleus to the right and the perinuclear area to the left (*bottom panel*). **B)** Quantification of the subcellular Pmel17- (*red*) and LAMP1- (*green*) distribution along the horizontal axis of similarly oriented Mel220 transfectants. Curves overlap in case that distribution is similar (*as for IR-wt and H190P*), but diverge from each other in case that distribution is different (*as for wt-Pmel17*). One example cell is shown for each cell line. The *average difference* between the Pmel17- and the LAMP1-profile was calculated as described in *Experimental procedures* and is depicted below the histograms.

Supplementary figure S4, *Wildtype Pmel17 expression does not rescue the subcellular distribution pattern of mutant IR-wt (in Mel220 bulk transfectants).* IR-wt was stably transfected into Mel220-cells already stably pre-expressing wt-Pmel17. Due to the vector combinations used, we were not able to select for transfected cells with an antibiotic, however, we typically observe transfection rates approaching 100% in this system (*data not shown*). Thus, we expect a very high percentage of cells to have received the construct. Strongly supporting this, when subsequently cloned, all 8 of 8 arbitrarily picked clones co-expressed wt-Pmel17 and IR-wt (Supplementary Fig. S5A). **A) After transfer of IR-wt into Mel220 cells stably pre-expressing wt-Pmel17, bulk transfectants show co-expression of both Pmel17 constructs.** cDNA was prepared from plain Mel220 cells or their derivatives expressing wt-Pmel17 alone or wt-Pmel17 and IR-wt together. Using this cDNA as a template, RT-PCR was performed with primers amplifying vector-derived wt-Pmel17 or IR-wt (*lanes 4-9 in upper panel*). Actin was used as a control (*lanes 1-3 in upper panel*). The positions of the Pmel17-specific primers relative to the gene are indicated in red in a schematic figure (*lower panel*). Wt-Pmel17- and IR-wt-derived fragments (both 2357 bp) can be distinguished from each other by sensitivity to the restriction endonuclease Pvu I (*lanes 7-9 in upper panel*) (only the IR-wt-derived fragment is cleaved by Pvu I and then gives rise to a 718 bp and a 1639 bp-fragment (*see lower panel*)). One unspecific cross-amplification product running very similar in size to the 1639 bp-fragment is labeled with an asterisk. **B) Introduction of IR-wt into Mel220 cells stably pre-expressing wt-Pmel17 does not impair processing of wt-Pmel17.** Membrane lysates were prepared as in Fig. 1B from the stable bulk transfectants shown in Supplementary Fig. S4A and analyzed by Western blot using Pmel17-specific antibodies. **C) Presence of wt-Pmel17 does not correct the subcellular distribution pattern of IR-wt.** Bulk Mel220 transfectants expressing IR-wt alone, wt-Pmel17 alone or co-expressing wt-Pmel17 and IR-wt were analyzed by immunofluorescence using antibodies against folded Pmel17 (HMB50) and LAMP1 (H4A3). The large pictures (*left panels*) show an overview over a group of cells. A selected subregion within these is highlighted by a white-framed rectangle and provided as a higher zoom image (*right panels*). Additionally, for better visualization a schematic picture of each of these subregions was generated (*second row in each of the right panels*) displaying the horseshoe pattern typical for wt-Pmel17 (areas that are $Pmel17^{high}/LAMP1^{low}$) in red and the typical juxtanuclear lysosomal pattern in green when it contained little or no Pmel17 (areas that are $Pmel17^{low}/LAMP1^{high}$) or in yellow, when it contained high levels of Pmel17 as in IR-wt-expressing cells (areas that are $Pmel17^{high}/LAMP1^{high}$). (These pictures are meant to give a rough idea of the distinct Pmel17-specific staining intensities observed in particular subcellular regions of the cells. Their color code does not reflect actual fluorescence intensity measurements). Note the presence of horseshoes in Mel220-cells expressing wt-Pmel17 independent of whether IR-wt is co-expressed (*third row*) or not (*second row*). Also, note the presence of juxtanuclear Pmel17-specific staining in Mel220-cells expressing IR-wt independent of whether wt-Pmel17 is co-expressed (*third row*) or not (*first row*).

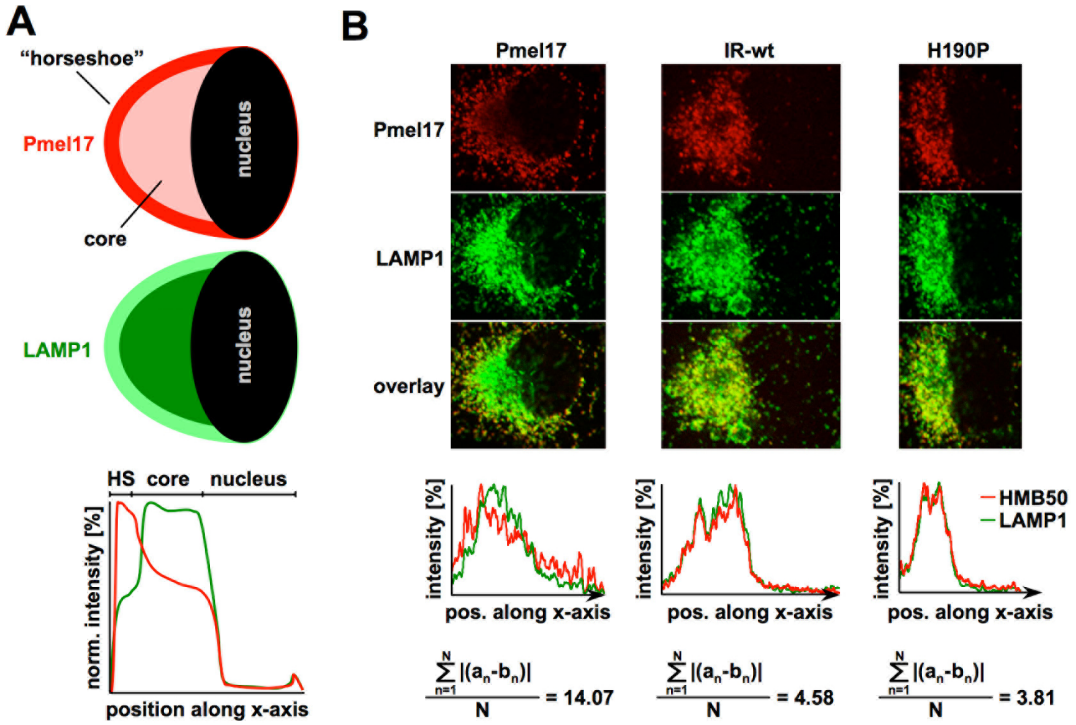
Supplementary figure S5, ***Wildtype Pmel17 expression does not rescue the subcellular distribution pattern of mutant IR-wt (in Mel220 cloned transfectants).*** **A)** Bulk Mel220 transfectants co-expressing wt-Pmel17 and IR-wt from Supplementary Fig. S4A were cloned by limiting dilution and eight clones were arbitrarily picked and analyzed for IR-wt-expression by RT-PCR as in Supplementary Fig. S4A. IR-wt-expression can be confirmed by appearance of a 1639 and a 718 bp-fragment after cleavage of the PCR-product with Pvu I (*lanes 1-8*). Uncleaved PCR product was run on the gel as a control (*lanes 9-16*). One unspecific cross-amplification product is labeled with an asterisk. All eight clones expressed IR-wt. **B)** Some Mel220 clones co-expressing wt-Pmel17 and IR-wt were arbitrarily picked and analyzed by immunofluorescence using Pmel17- or LAMP1-specific antibodies as in Supplementary Fig. S4C. Note that as in the bulk transfectants (*see Supplementary Fig. S4C*) many cells display Pmel17-specific labeling both in the "horseshoe"-area as well as in the juxtanuclear region.



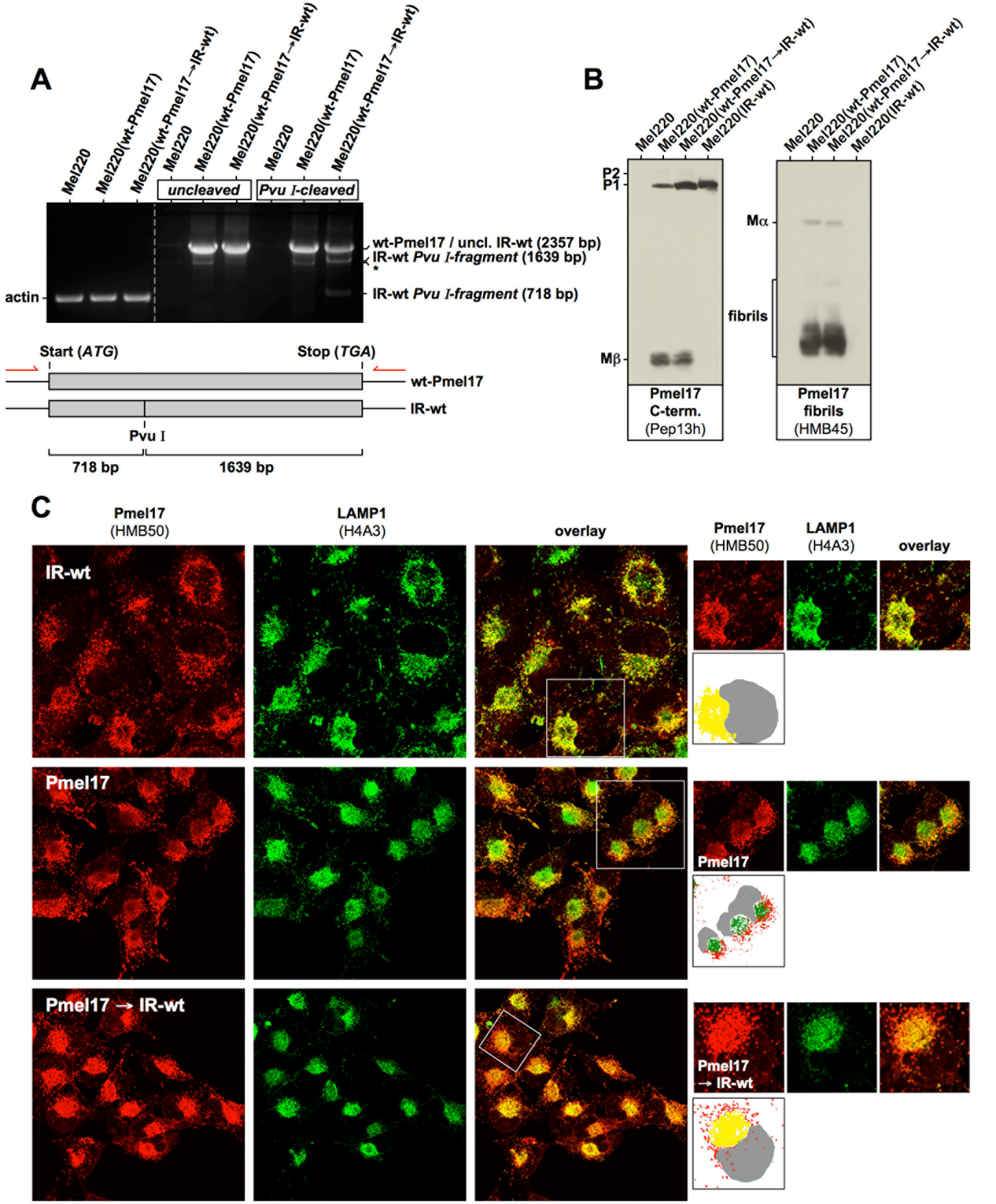
Supplementary Fig.S1



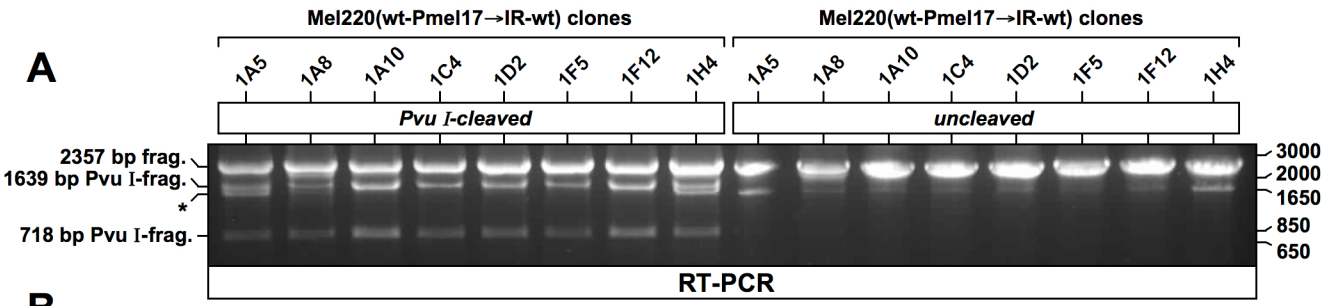
Supplementary Fig.S2

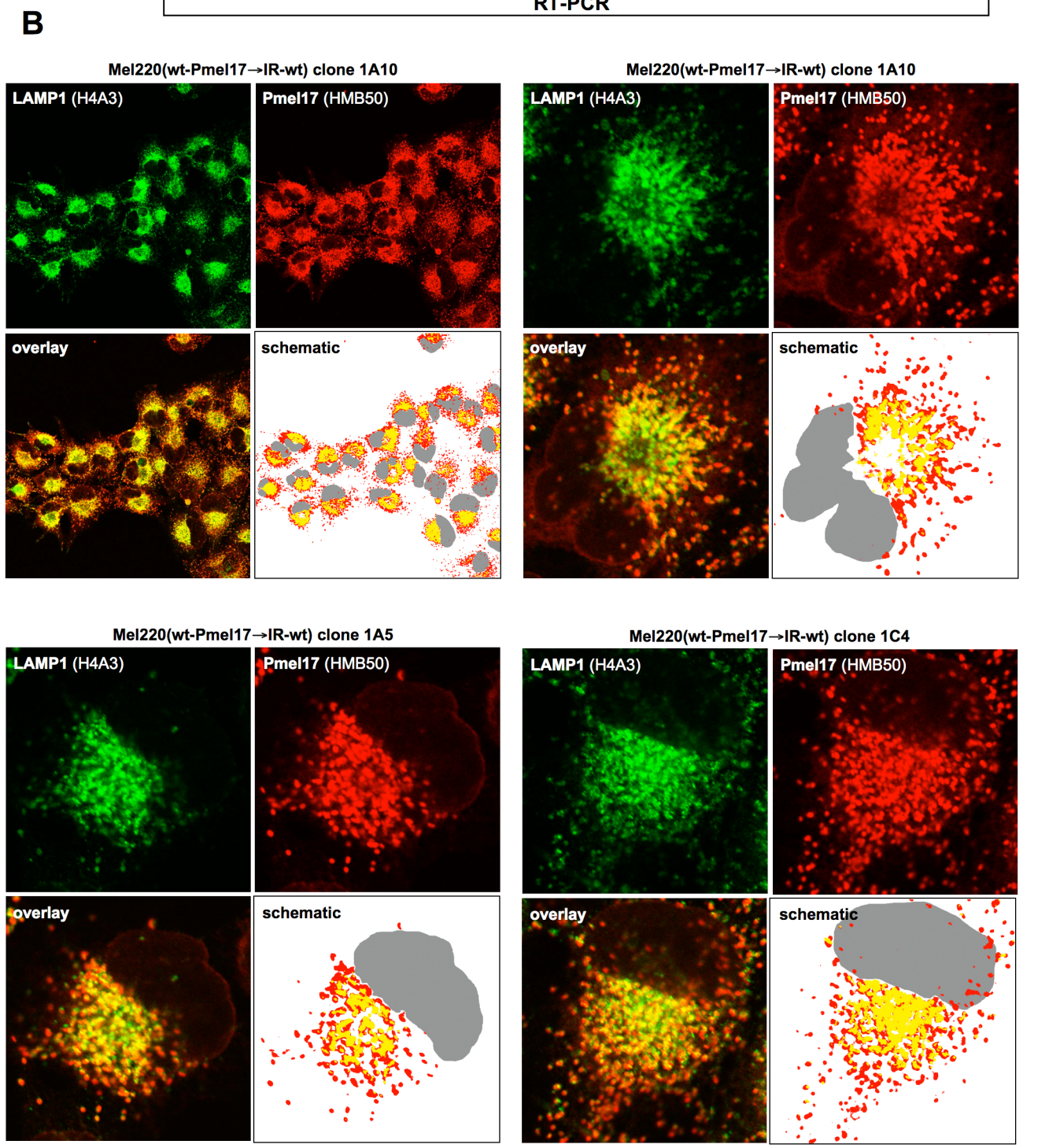


Supplementary Fig.S3



Supplementary Fig.S4





Supplementary Fig.S5