

cAMP-mediated endothelial barrier stabilization is plakoglobin-independent

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Figure S1

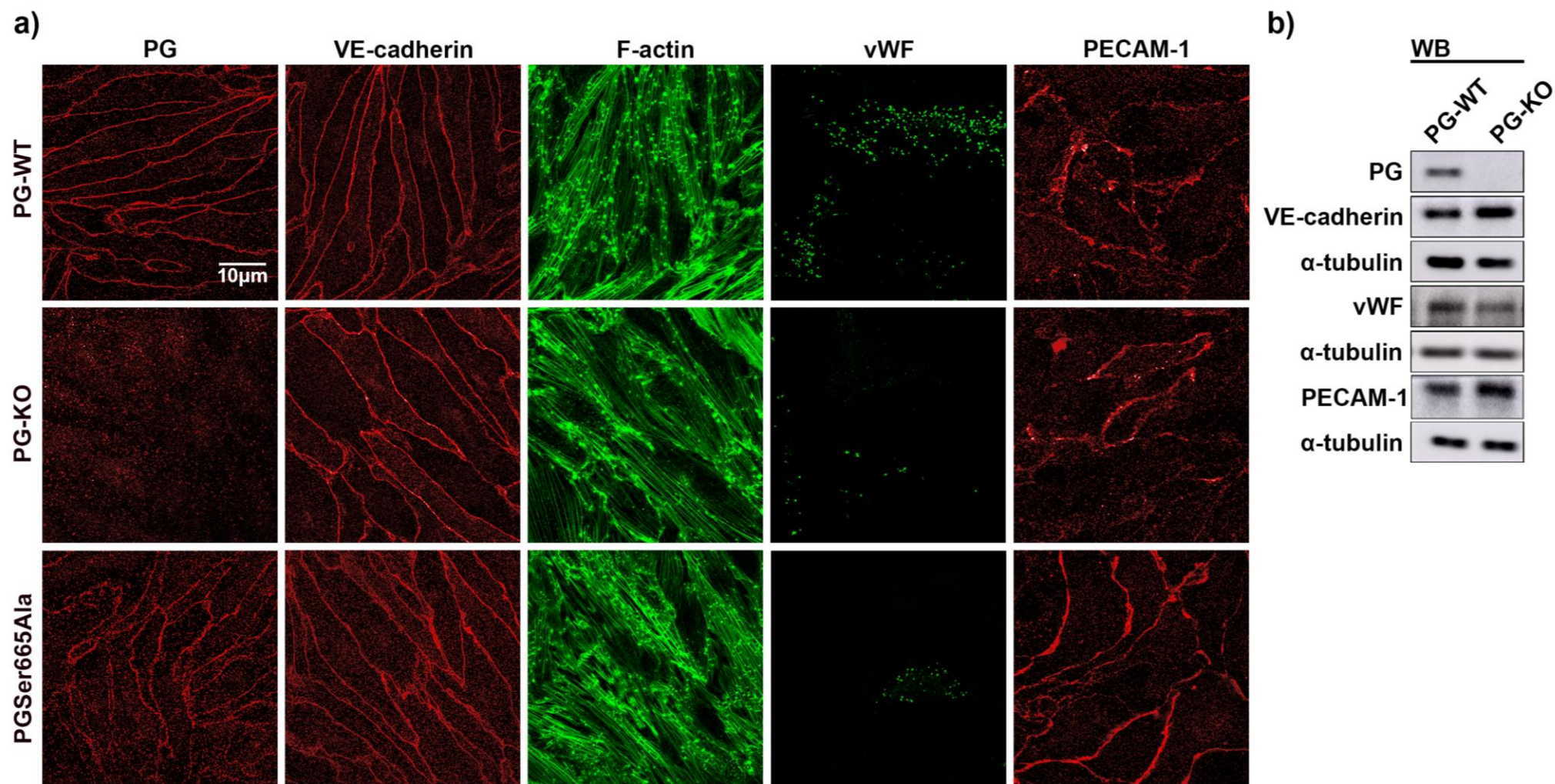


Figure S1

Cells derived from WT and PG-KO mice express typical endothelial markers

a) The presence of endothelial specific markers such as VE-cadherin, vWF and PECAM-1 was validated by immunostaining. Presence or lack of PG was also confirmed (the WB images for PG, VE-cadherin and respective loading control were also used in Figure 2). Actin cytoskeleton organization was visualized with Alexa Fluor®488 Phalloidin; N=3 b) Cellular abundance of the proteins presented in a) was proven by Western blot analyses. Equal loading was ensured by detection of α -tubulin; N≥5

Figure S2

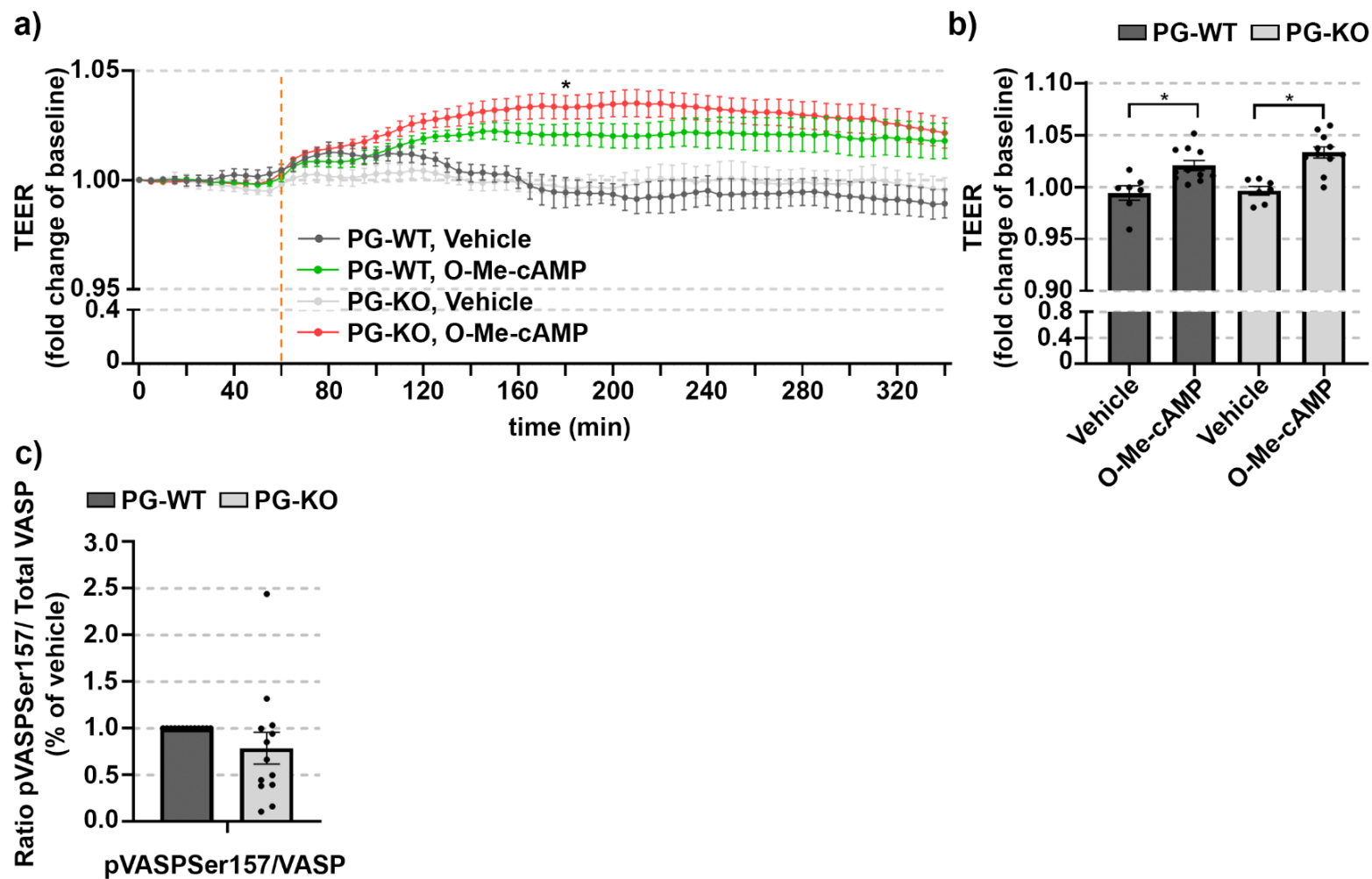
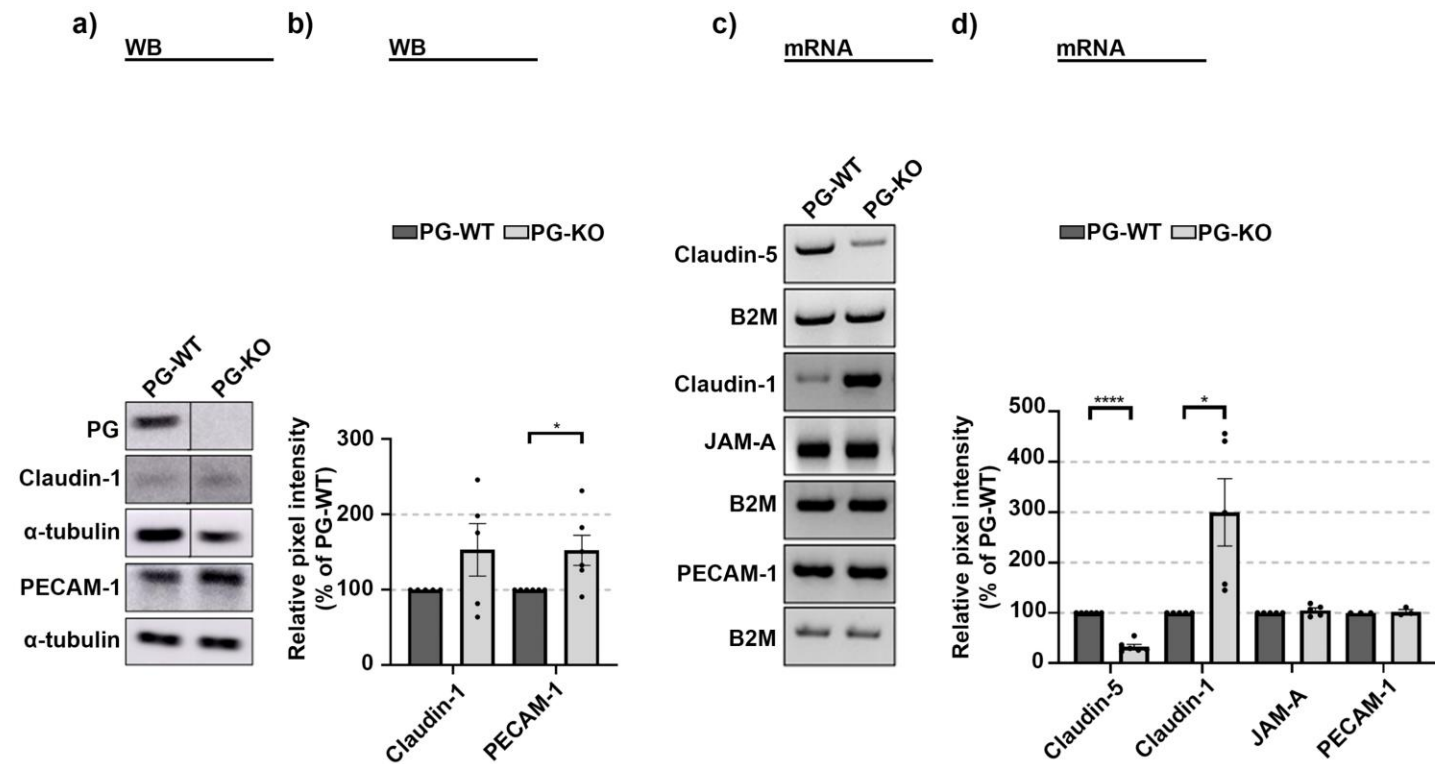


Figure S2

Barrier integrity behaviour in WT and PG-KO cells, exposed to O-Me-cAMP treatment

a) Time course of TEER measurements in WT and PG-depleted confluent monolayers treated with either Vehicle or O-Me-cAMP. The recorded data were normalized to the initial point of measure. The segmented orange line depicts the time of primary treatment; N=5 b) Bar diagram representing the significant differences in the average TEER measurements between Vehicle and O-Me-cAMP treated cells, in WT and PG-null cells, respectively. The analysis is done 2 h after initial application; N=5. c) Bar diagram illustrating the phosphorylation of VASP in WT and PG-depleted MyEnd cells under basal condition; N≥5. Data are presented as mean ± SEM; unpaired t-test; * p≤0,05

Figure S3



Supplementary Figure 3

Effect of PG deficiency on central junctional molecules

a) Representative Western blot analyses of claudin-1 and PECAM-1 in WT and PG-KO cells, α-tubulin was used as loading control. Non-contiguous bands for PG, claudin-1 and α-tubulin originating from the same membrane are distinct by a vertical black line. An equal exposure time was used for detection of the specific bands. Original blots are presented in Figure S4 a, s-t, provided in the Supplementary info file. The images, representing PECAM-1 protein expression in WT and PG-KO are the same used in Supplementary Figure 1; N≥5 b) Bar diagrams depicting the relative protein expression of each analyzed target of interest in PG-KO when compared to WT cells. c) PCR analysis, revealing the transcription levels of TJ-related claudin-1, -5, JAM-A and PECAM-1 in WT and PG-KO cells. Equal loading for the PCR analyses was validated by B2M expression. Original agarose gels are presented in Figure S5 h-k from the Supplementary info file; N≥3 c) d) Bar diagram presenting the mRNA expression in PG-KO, relative to WT cells. Data are presented as mean ± SEM; the following values were considered significant * p≤0,05; **** p≤0,0001.

Original western blot gel
images for basal condition

Figure S4 a)

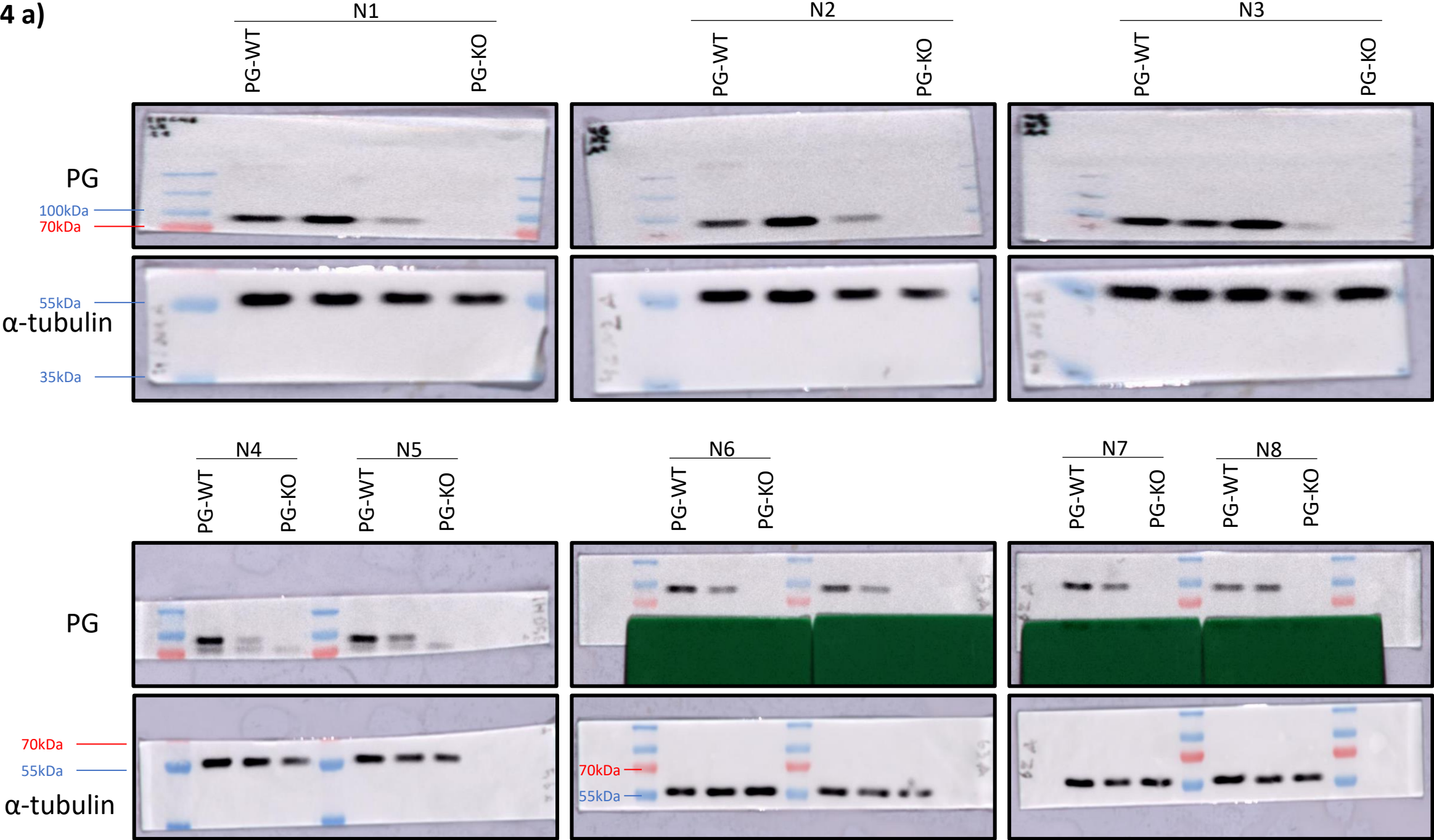


Figure S4 b)

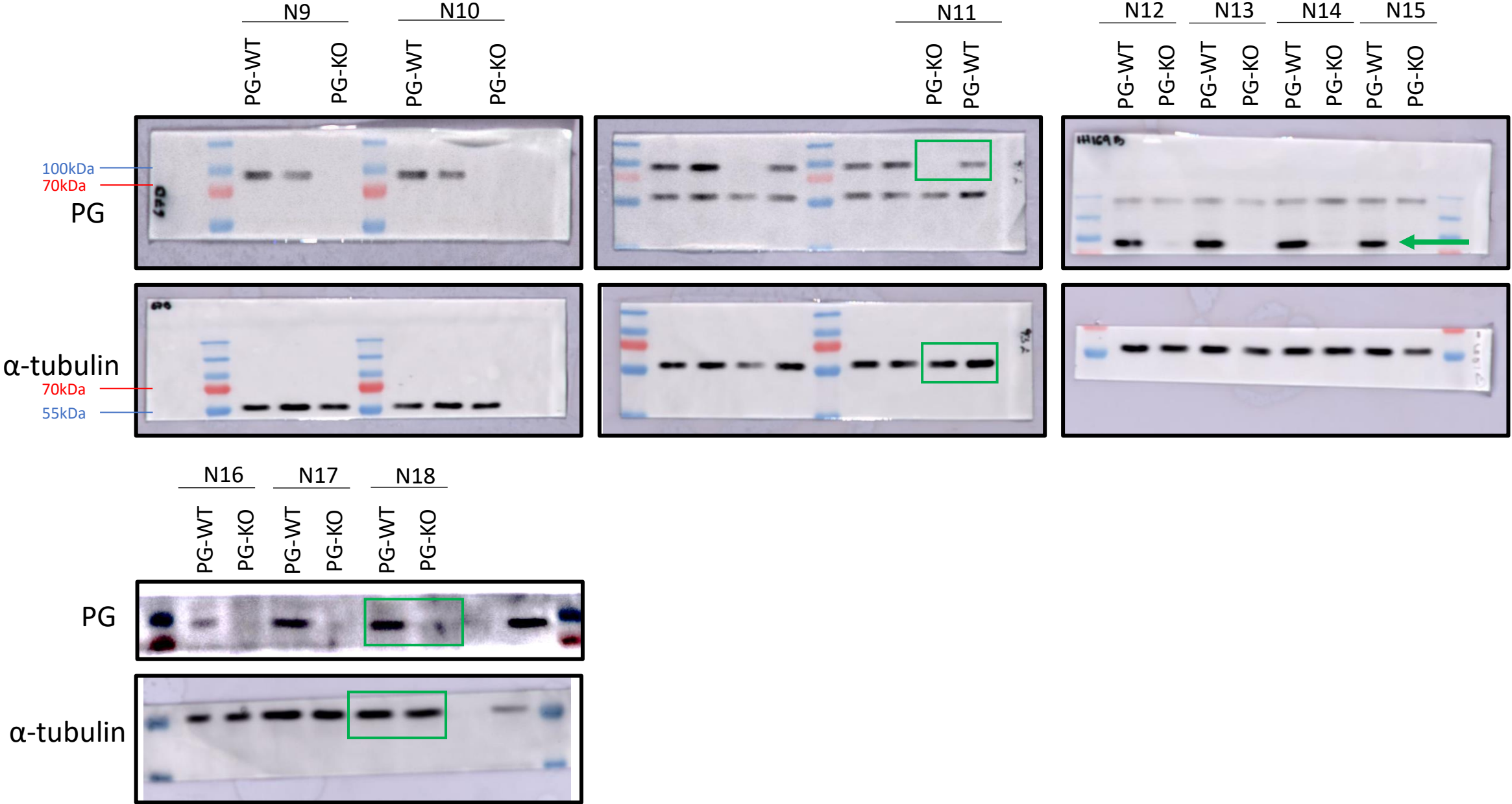


Figure S4 c)

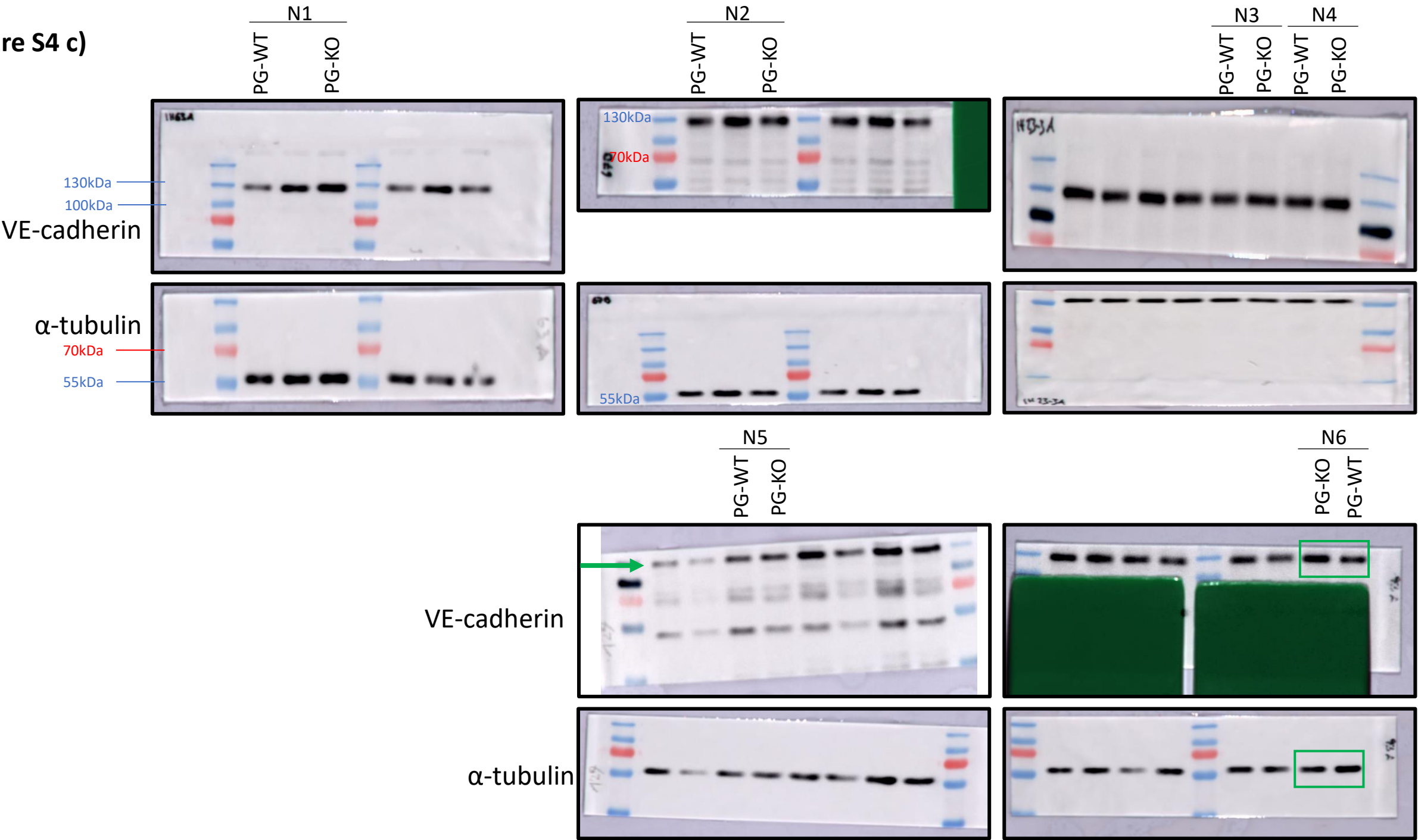


Figure S4 d)

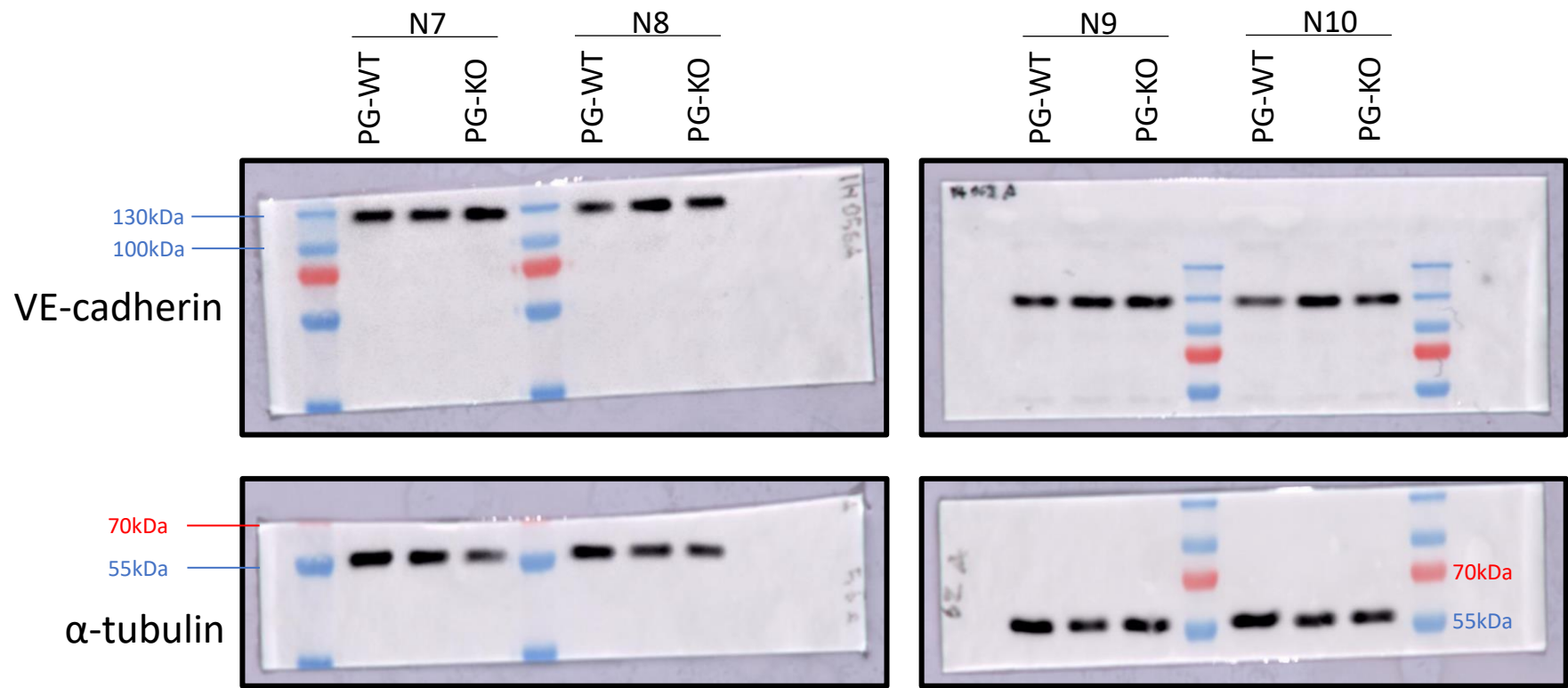


Figure S4, a-d) Original Western Blot gel images for basal condition. To prevent over exposure, the black or green cover was used. The green square determines the selected blot used in the figure 2 and S1.

Figure S4 e)

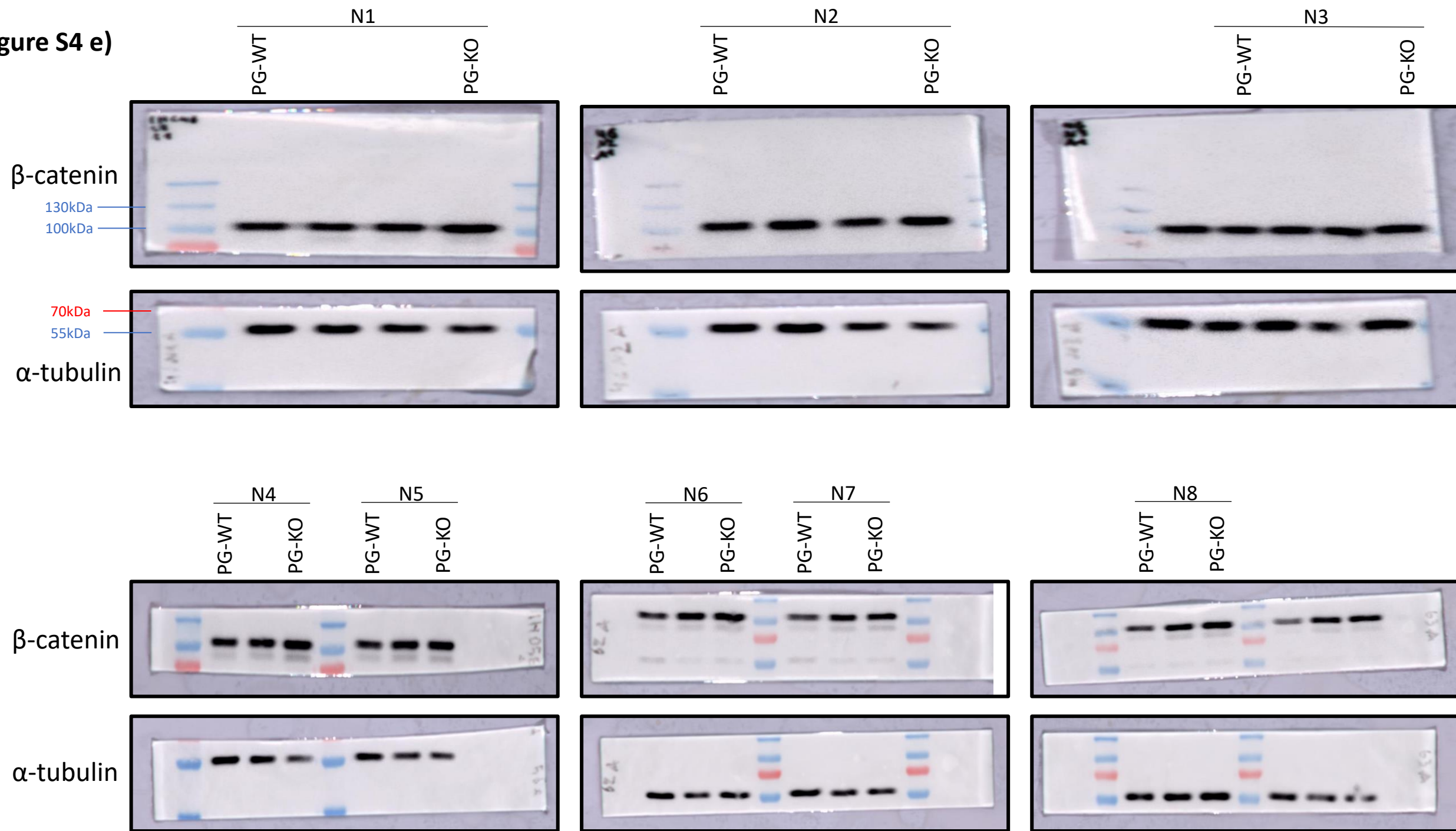


Figure S4 f)

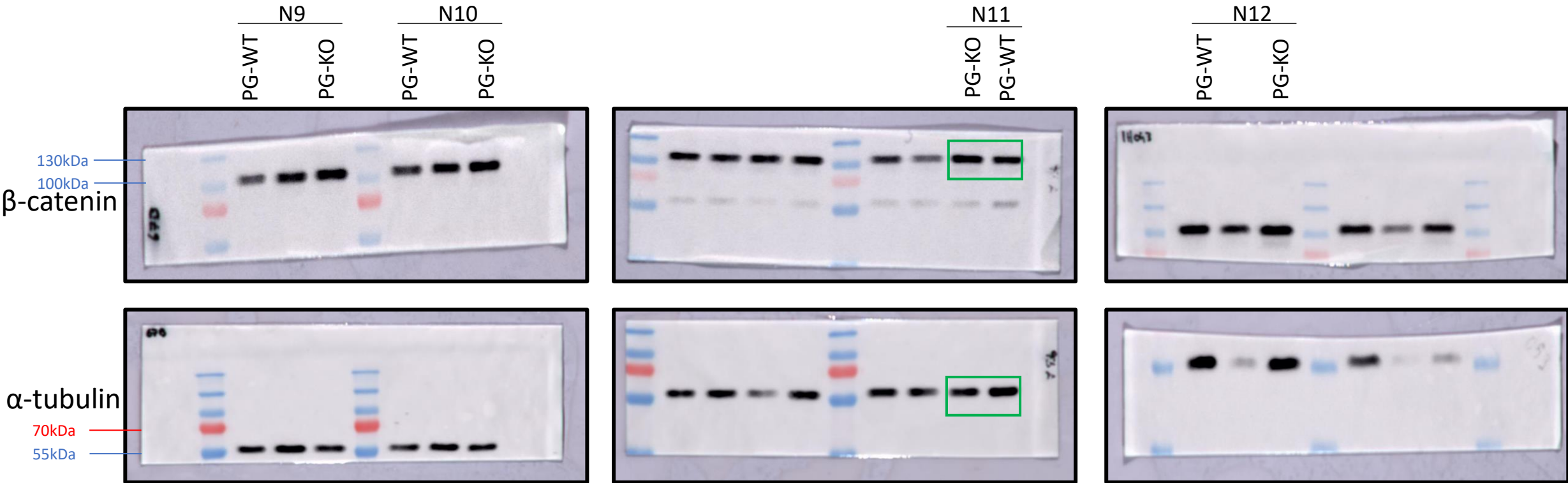


Figure S4 g)

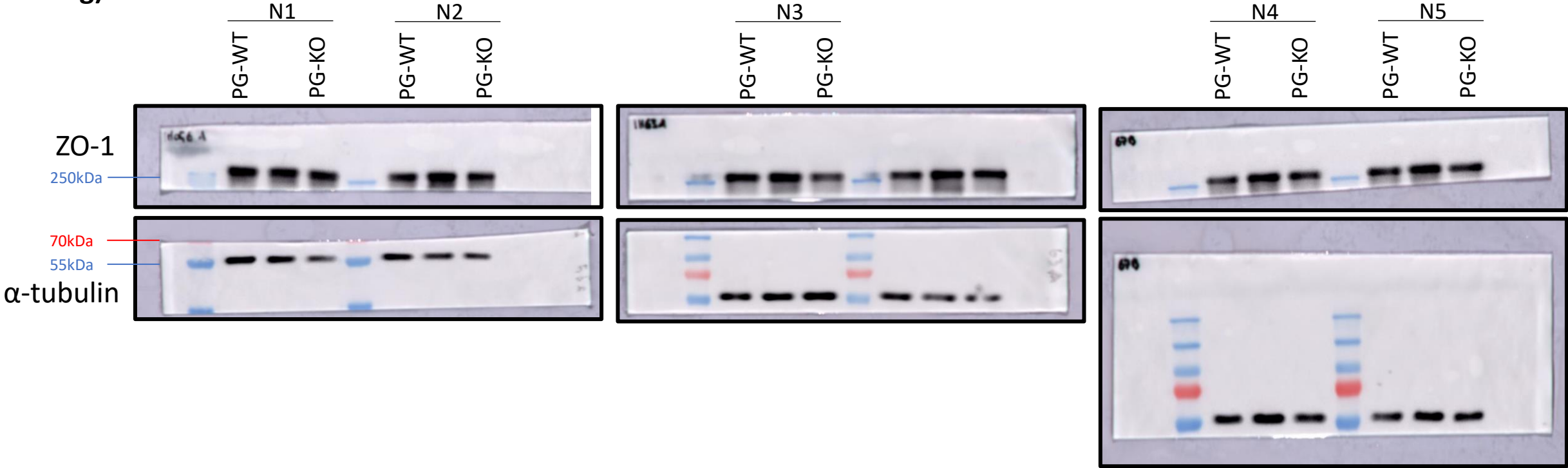


Figure S4 h)

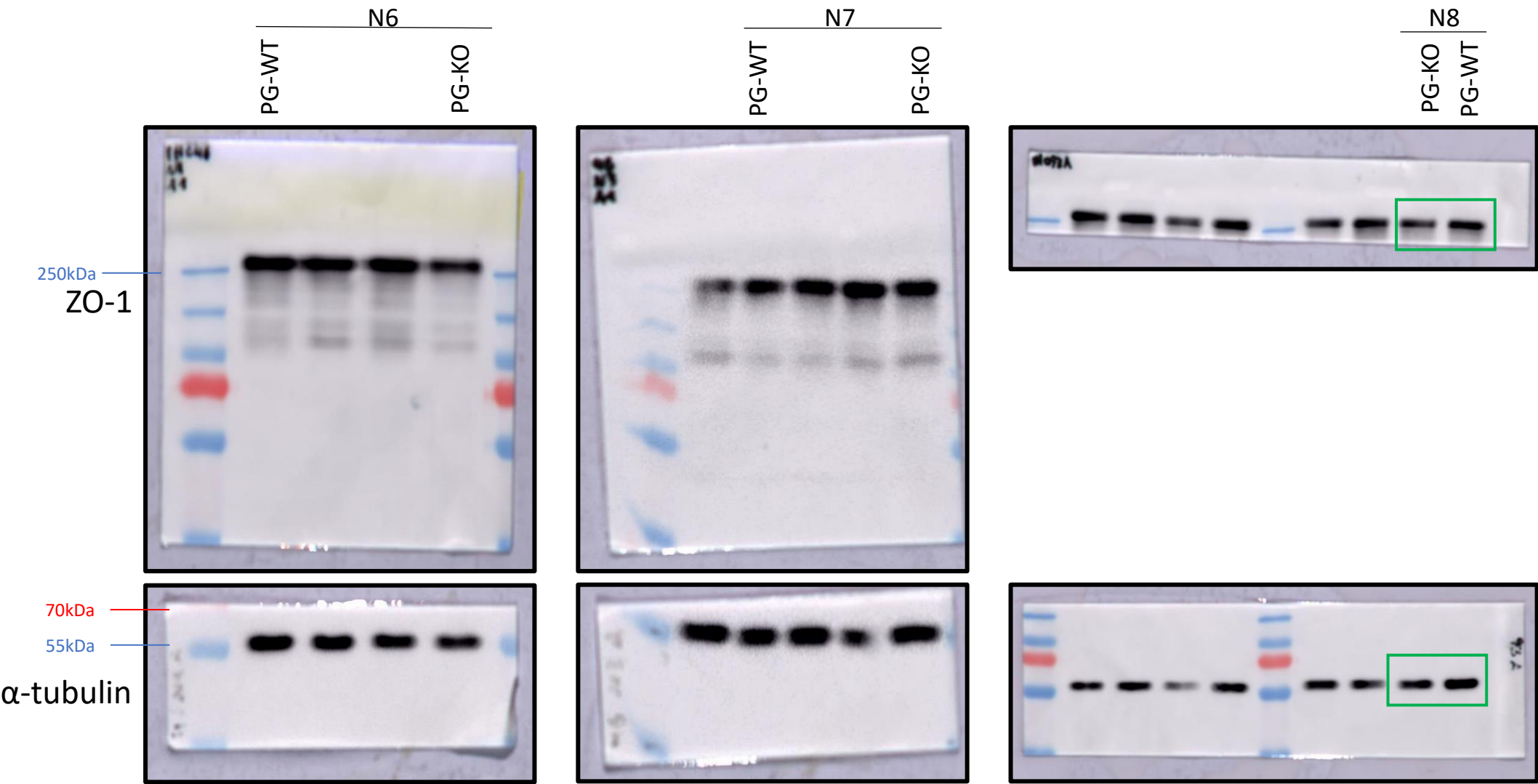


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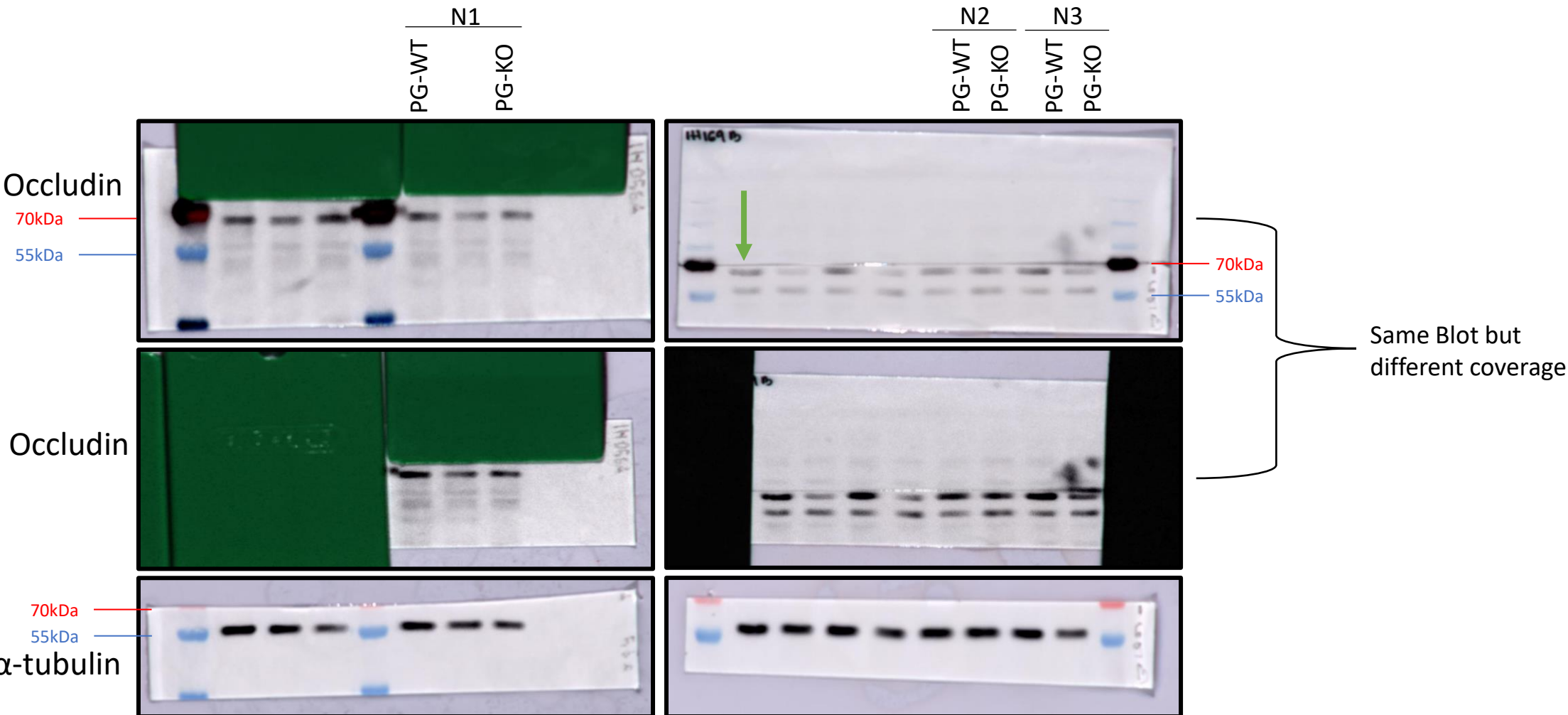


Figure S4 j)

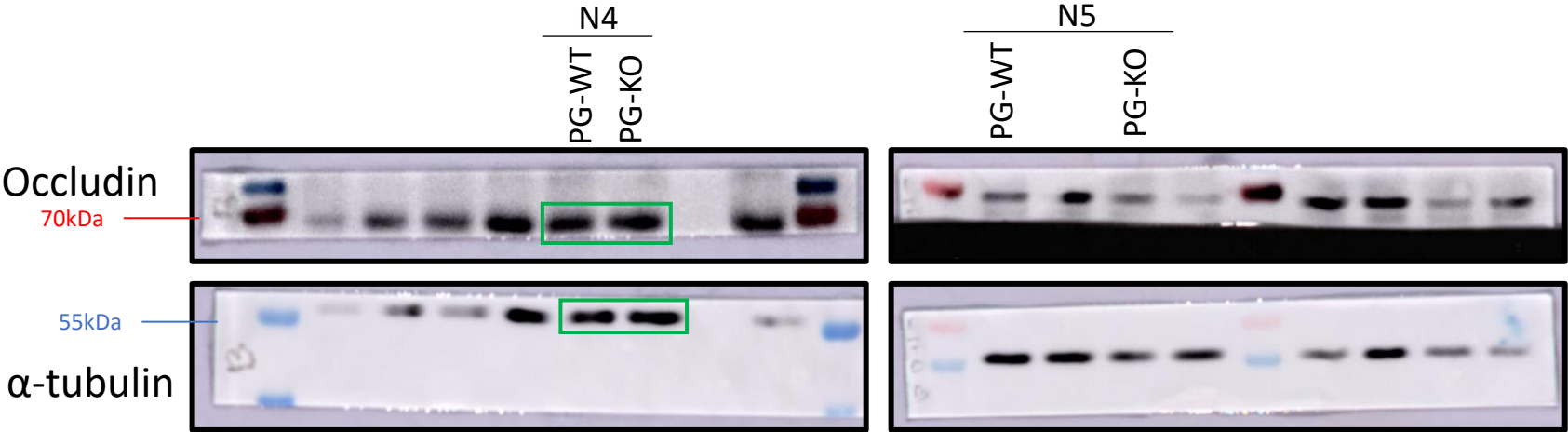


Figure S4 k)

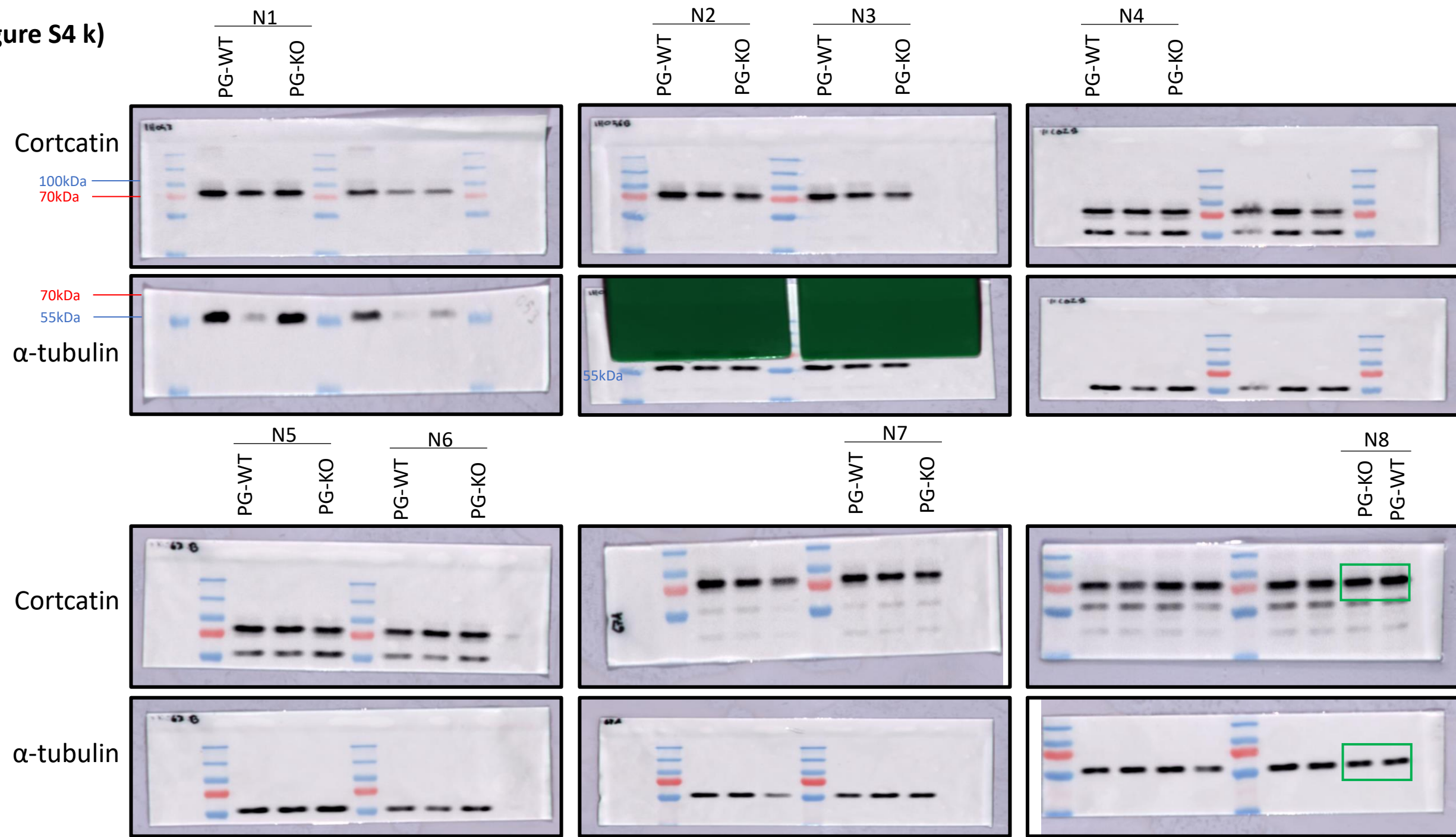


Figure S4 I)

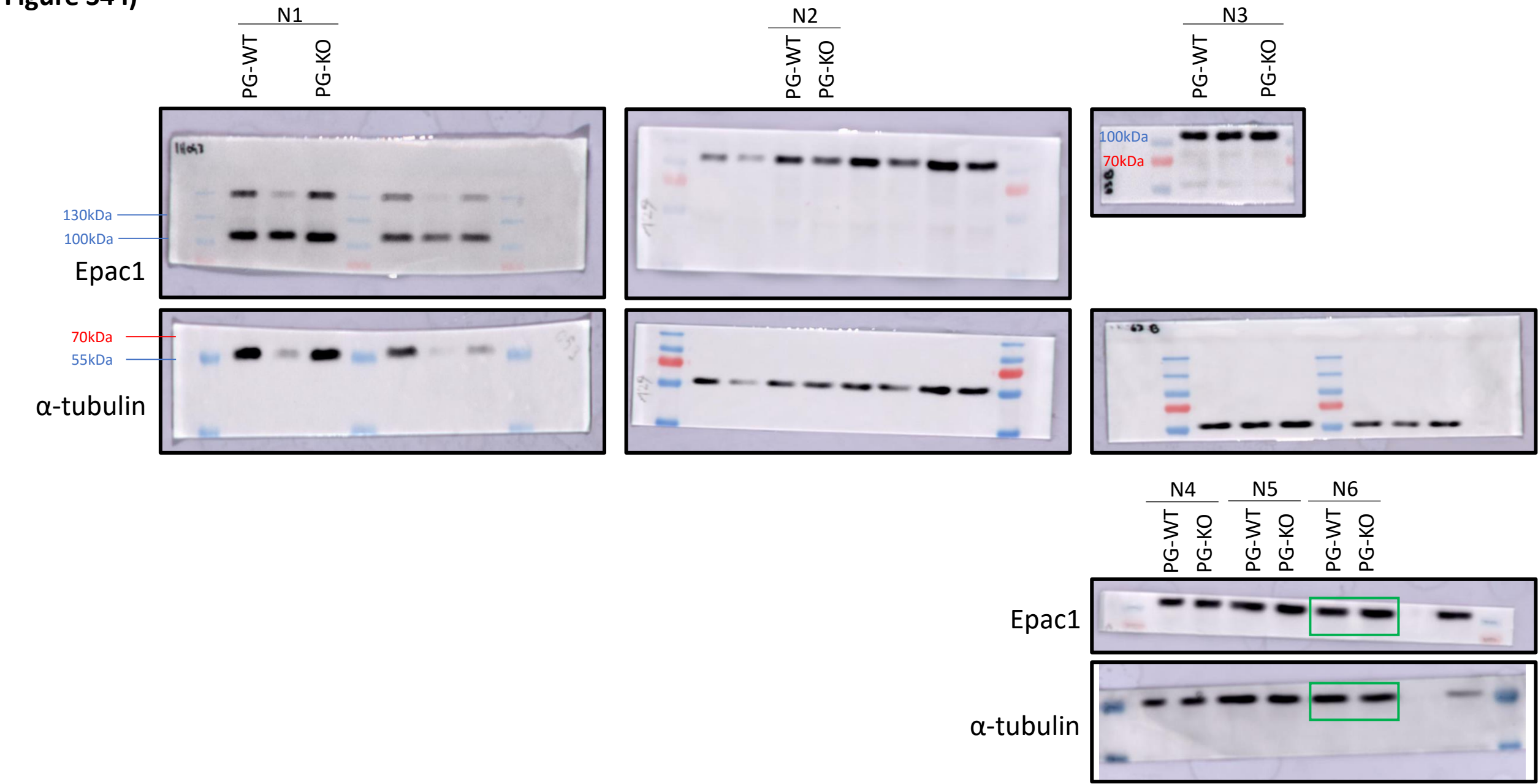


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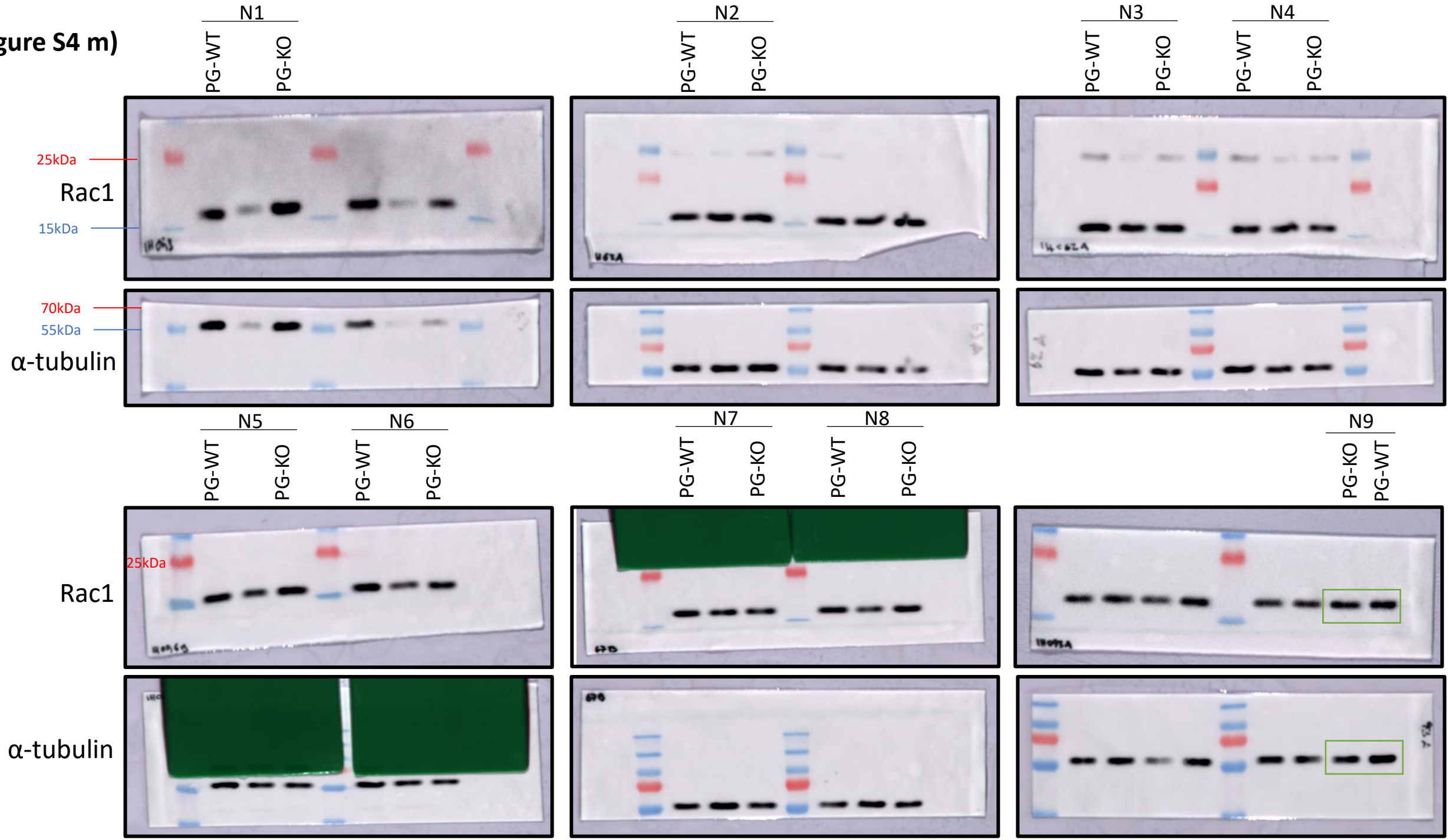


Figure S4 n)

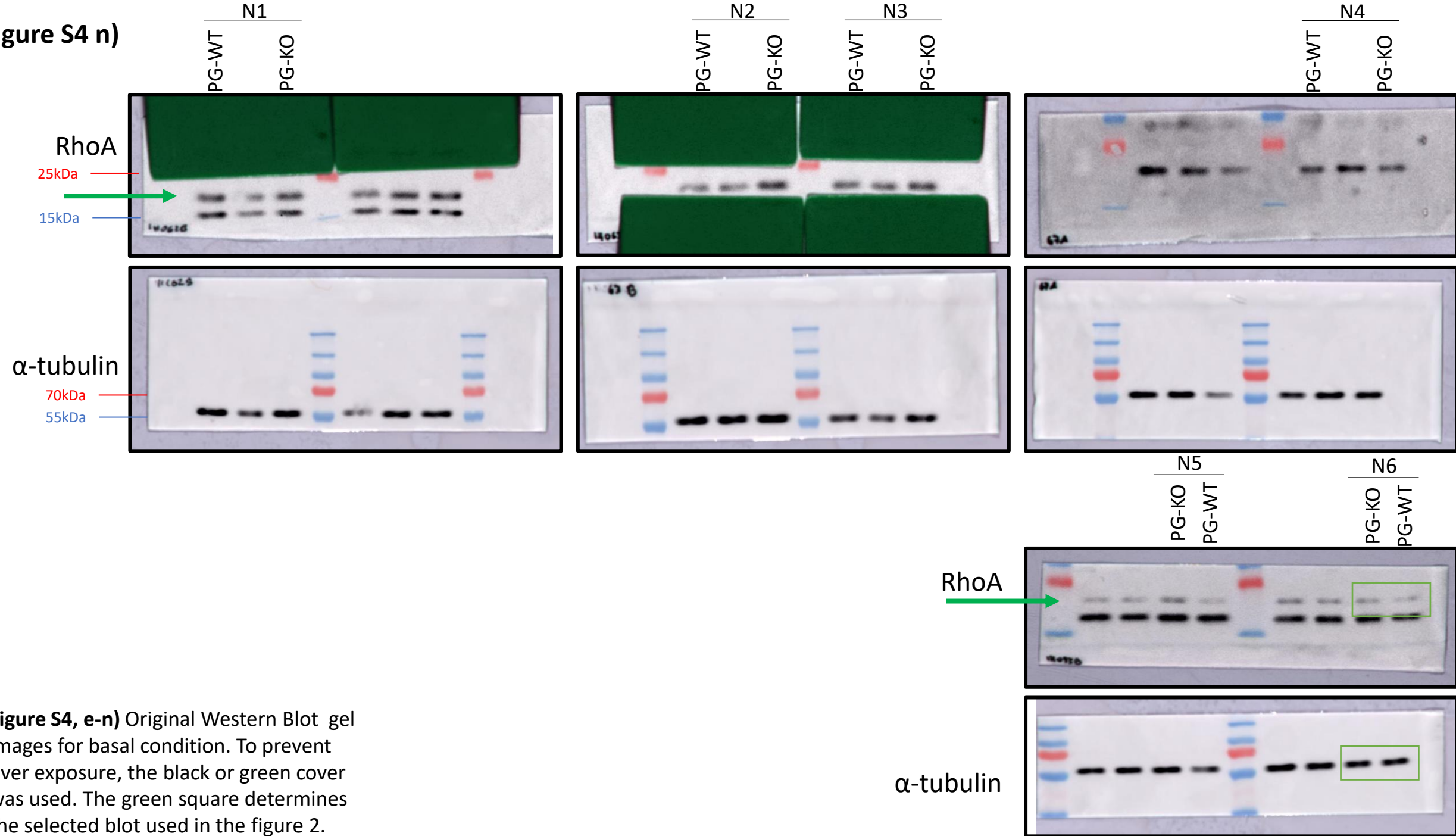


Figure S4, e-n) Original Western Blot gel images for basal condition. To prevent over exposure, the black or green cover was used. The green square determines the selected blot used in the figure 2.

Figure S4 o)

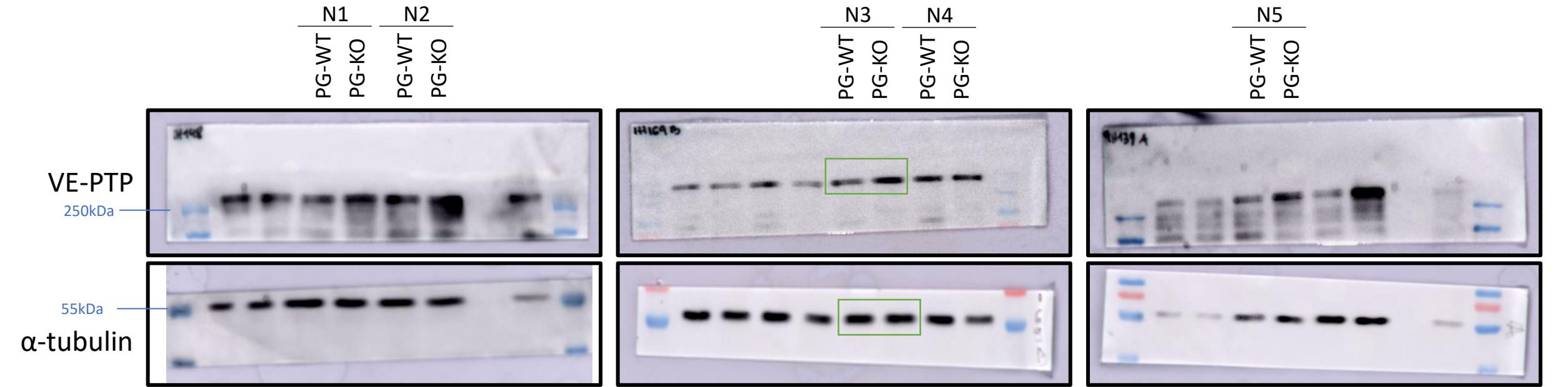


Figure S4 p)

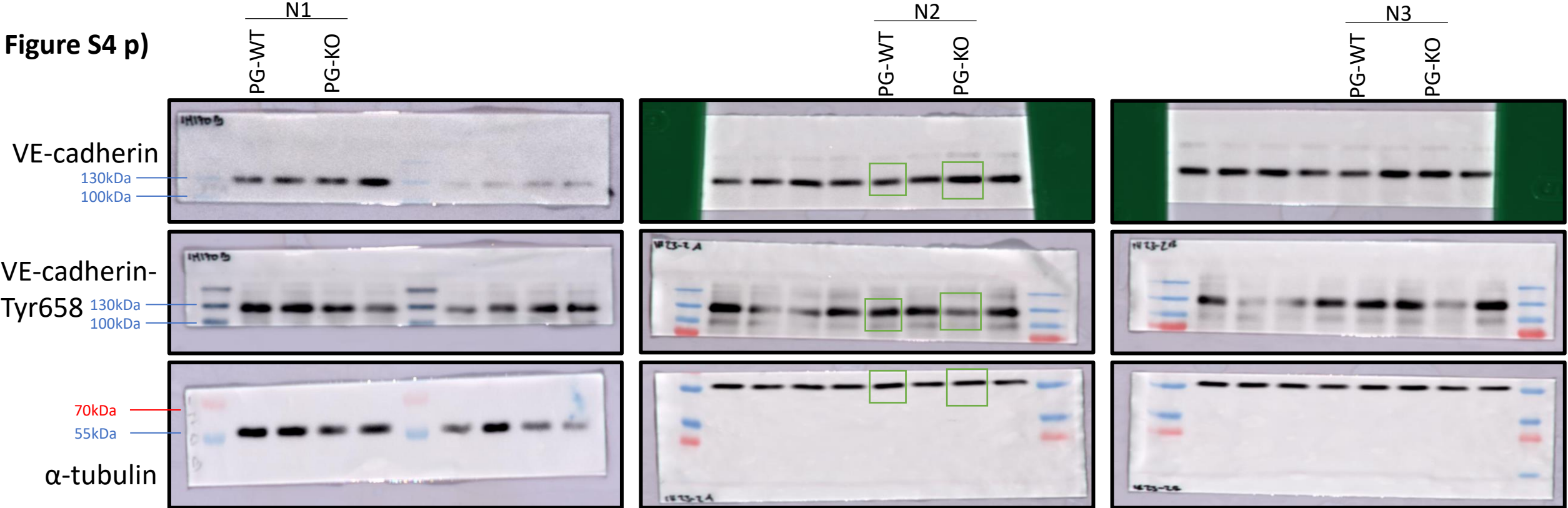


Figure S4 q)

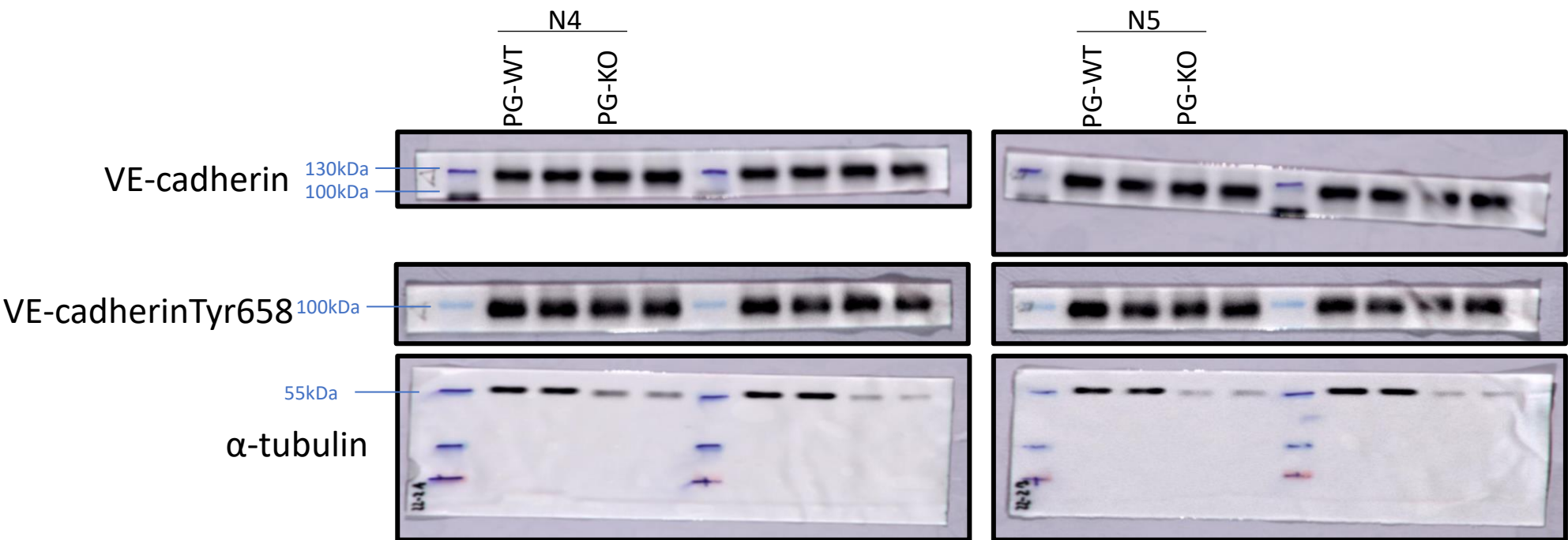


Figure S4, o-q) Original Western Blot gel images for basal condition. To prevent over exposure, the black or green cover was used. The green square determines the selected blot used in the figure 3.

Figure S4 r)

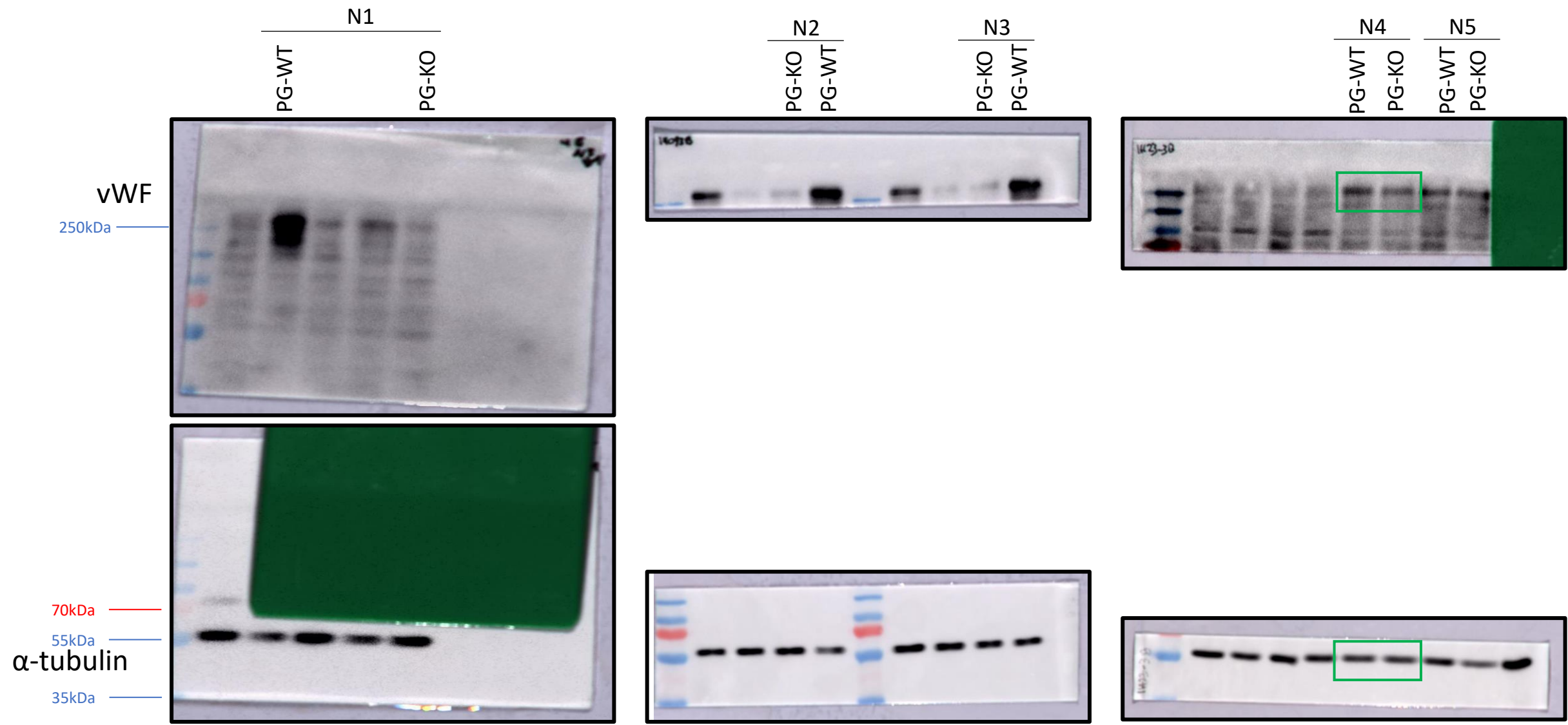


Figure S4 s)

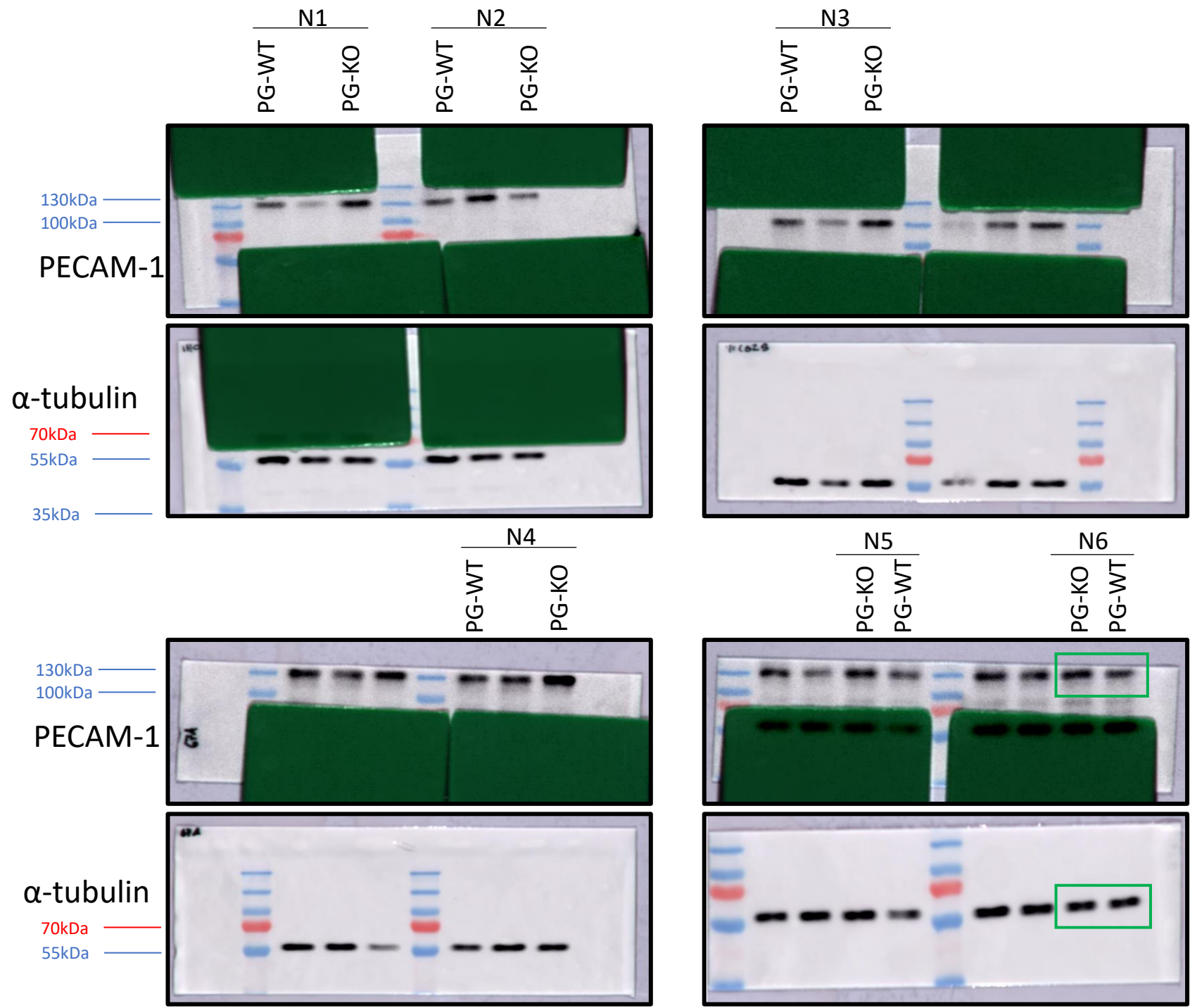


Figure S4, r-s) Original Western Blot gel images for basal condition. To prevent over exposure, the black or green cover was used. The green square determines the selected blot used in the figure S1.

Figure S4 t)

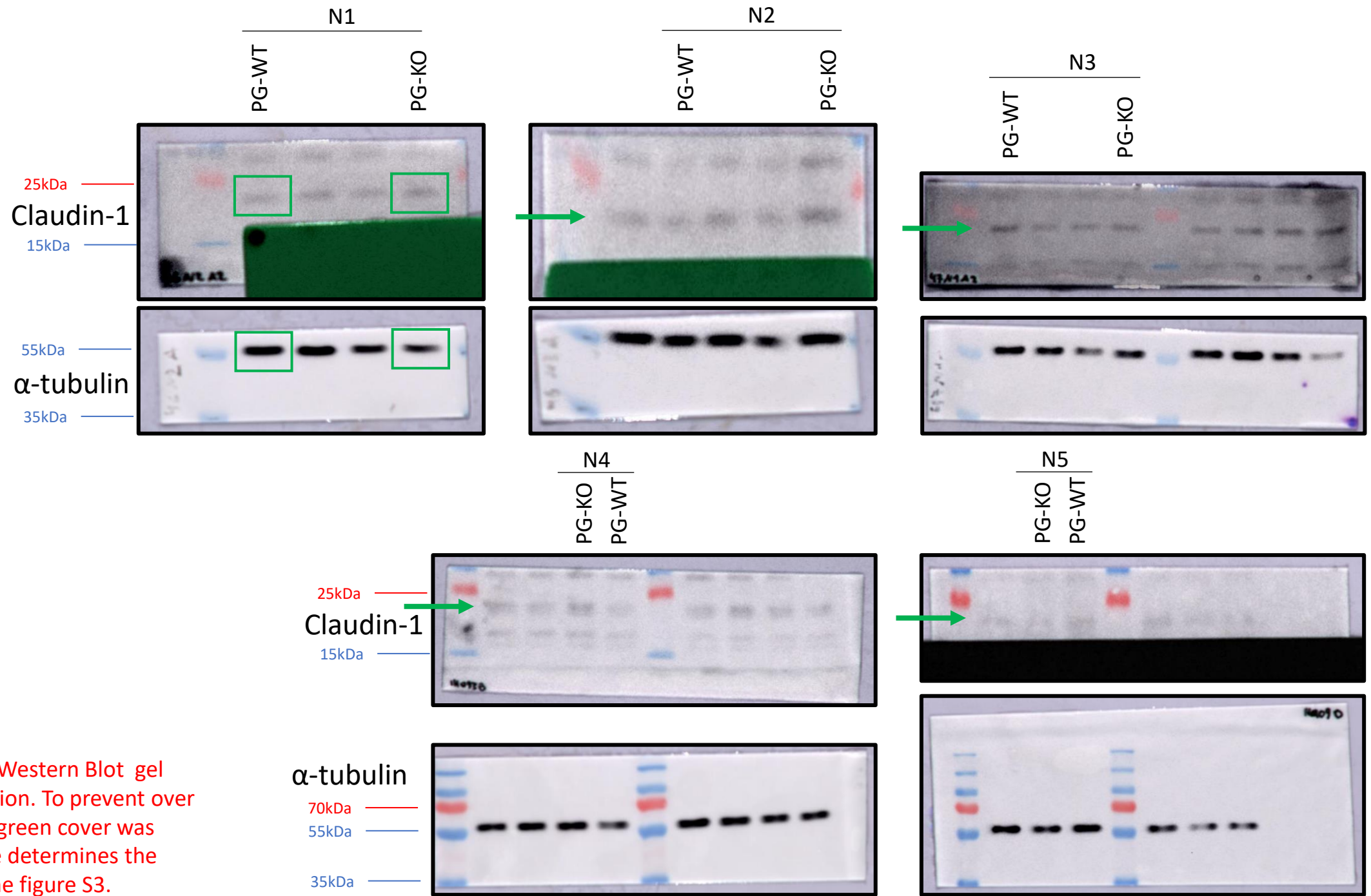
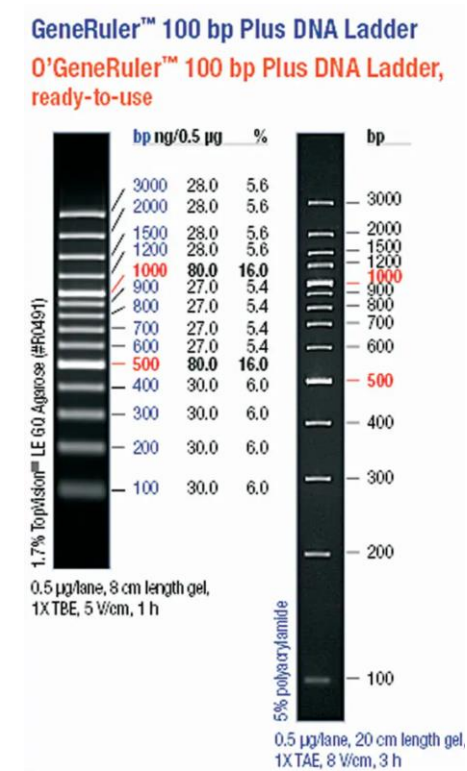


Figure S4, s-t) Original Western Blot gel images for basal condition. To prevent over exposure, the black or green cover was used. The green square determines the selected blot used in the figure S3.

Original PCR gel images

Figure S5 a)



PG

B2M

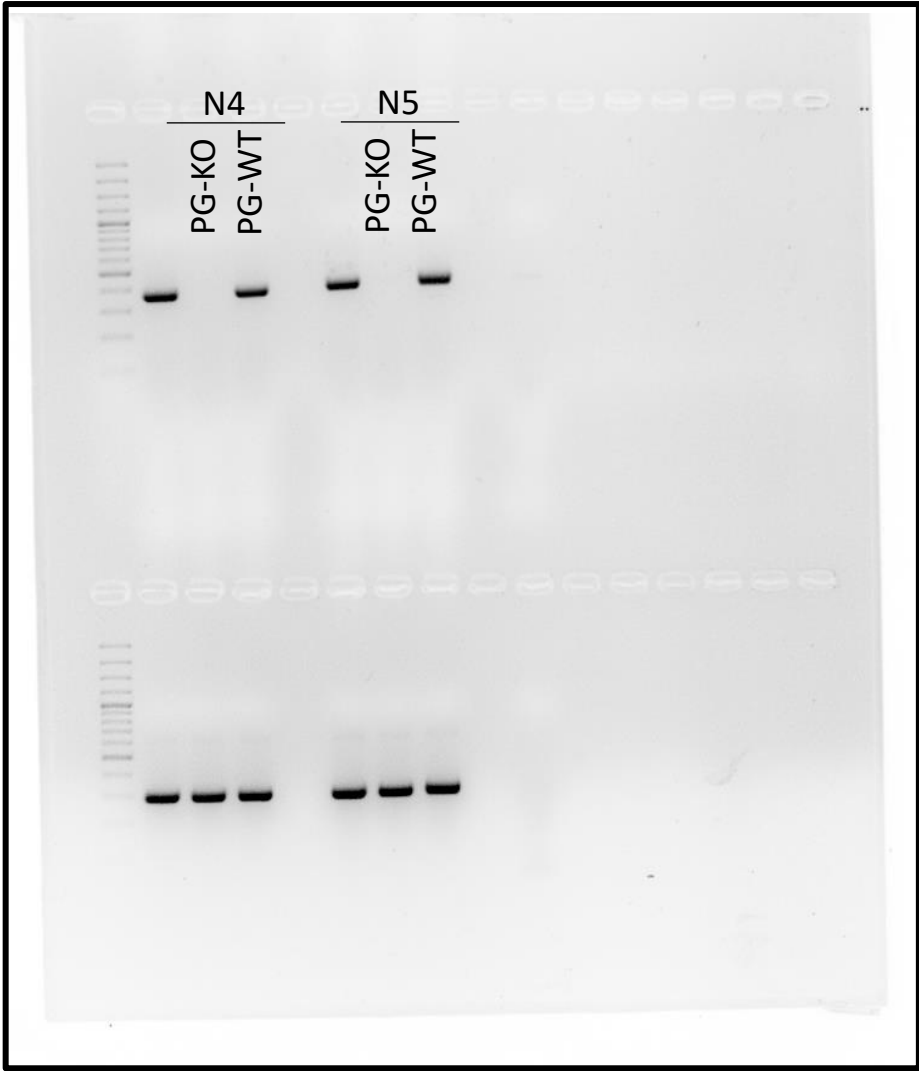
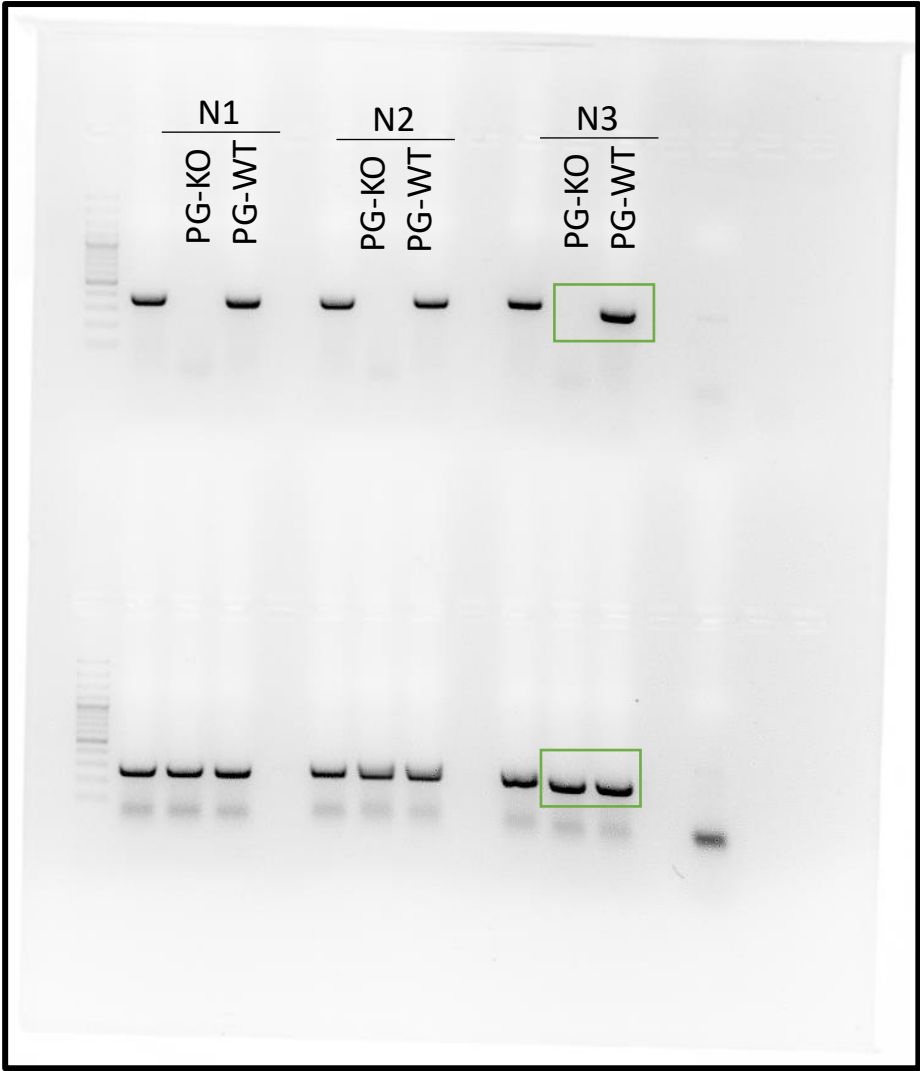


Figure S5 b)

VE-cadherin



B2M

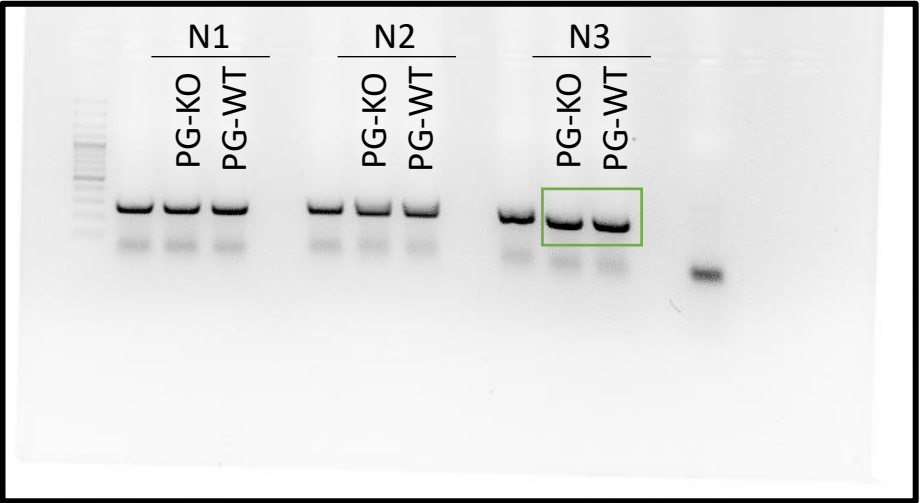


Figure S5 c)

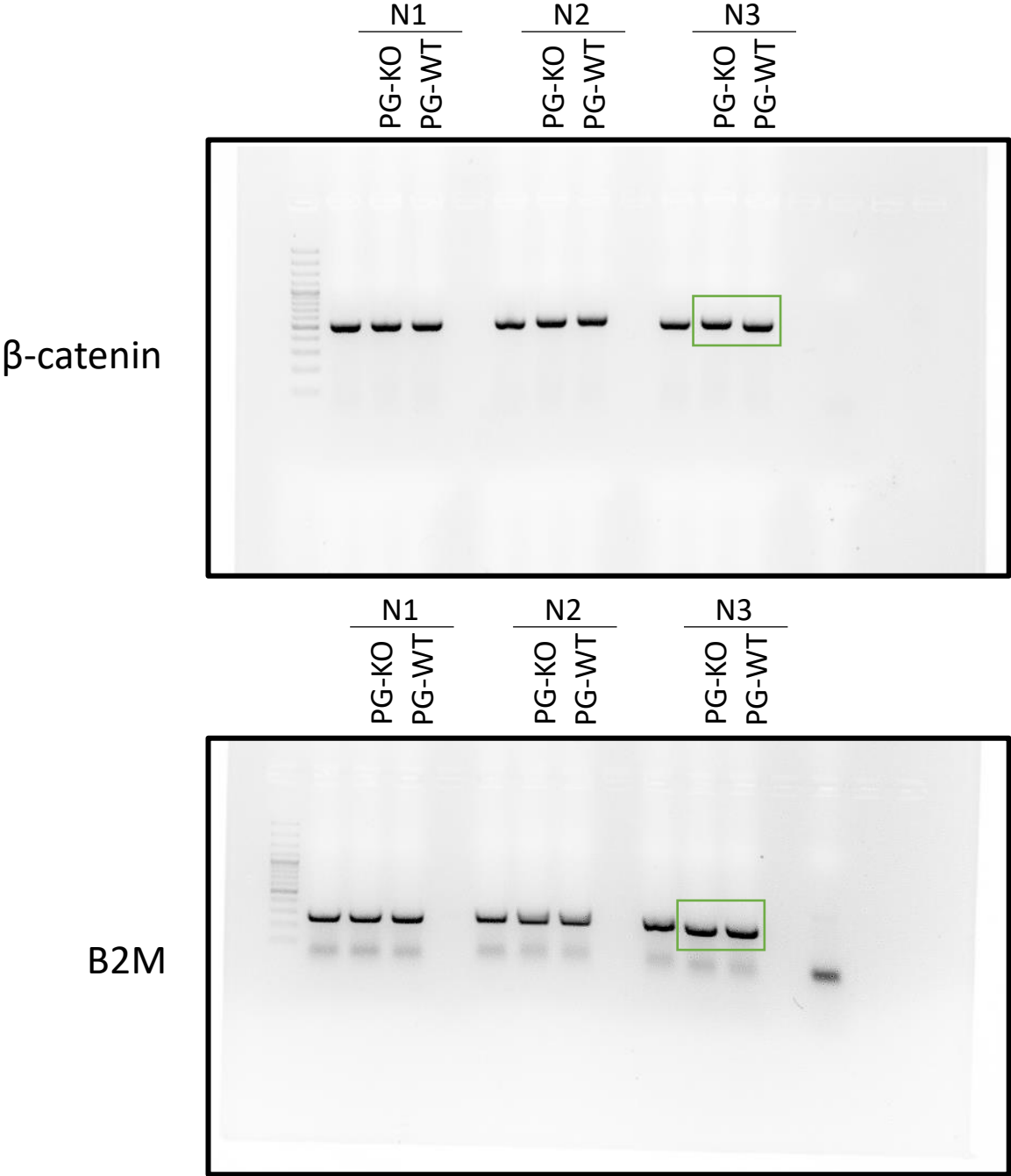


Figure S5 d)

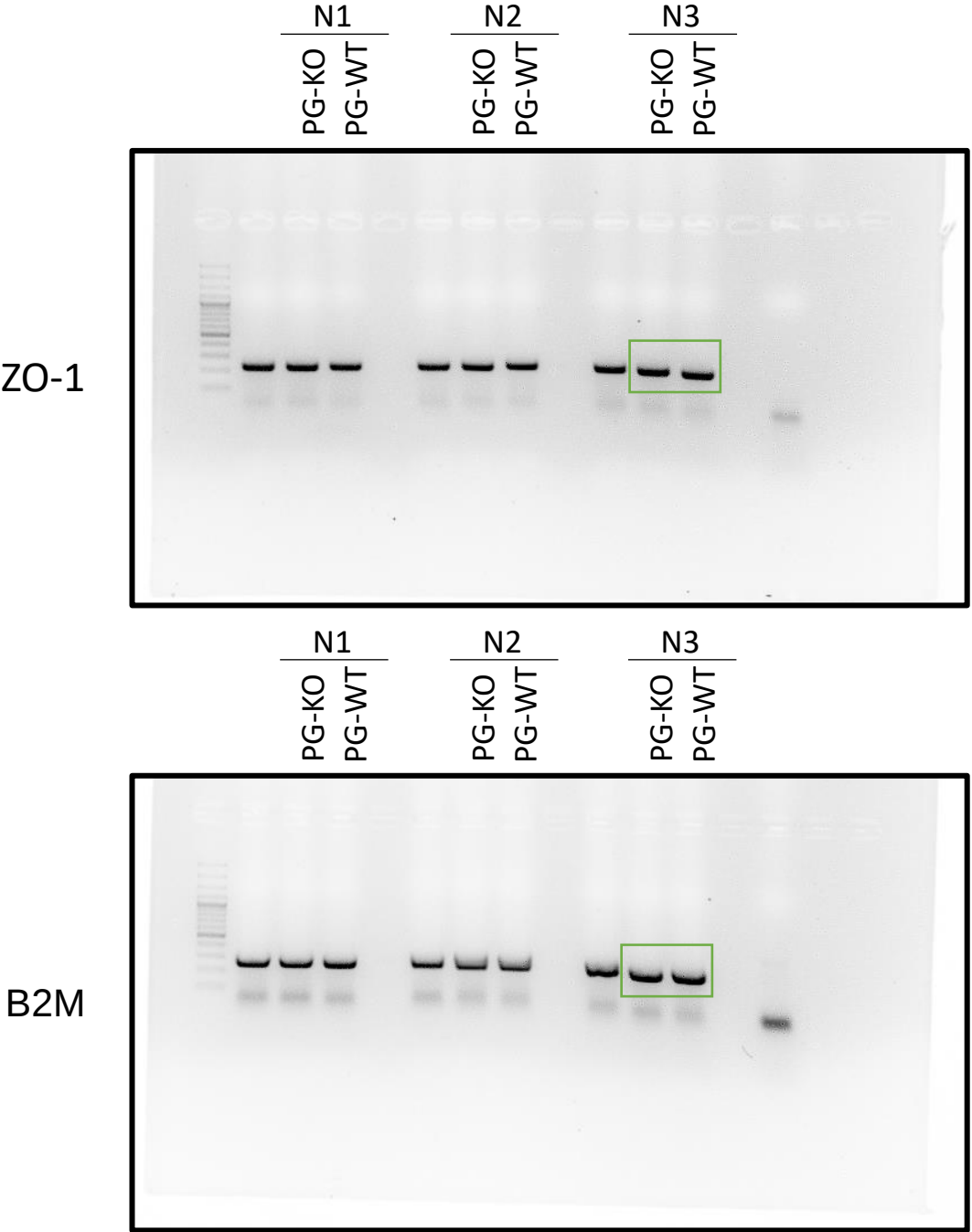
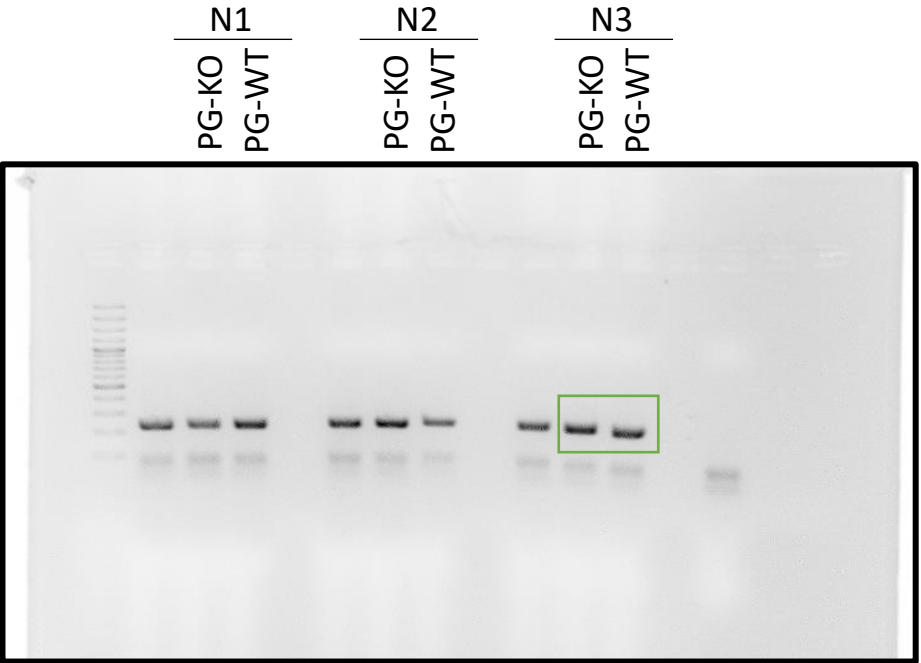


Figure S5 e)

Occludin



B2M

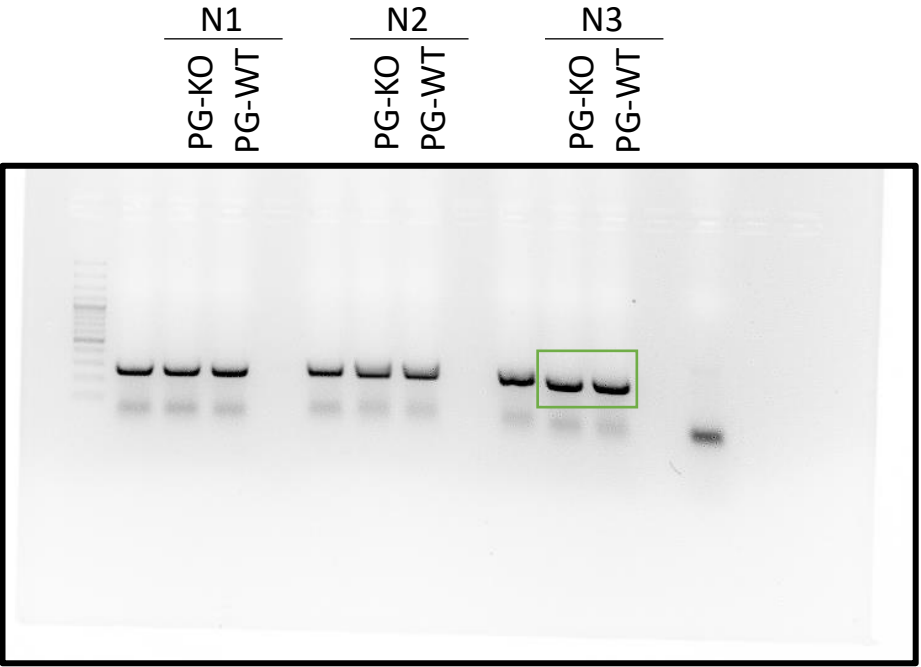


Figure S5 f)

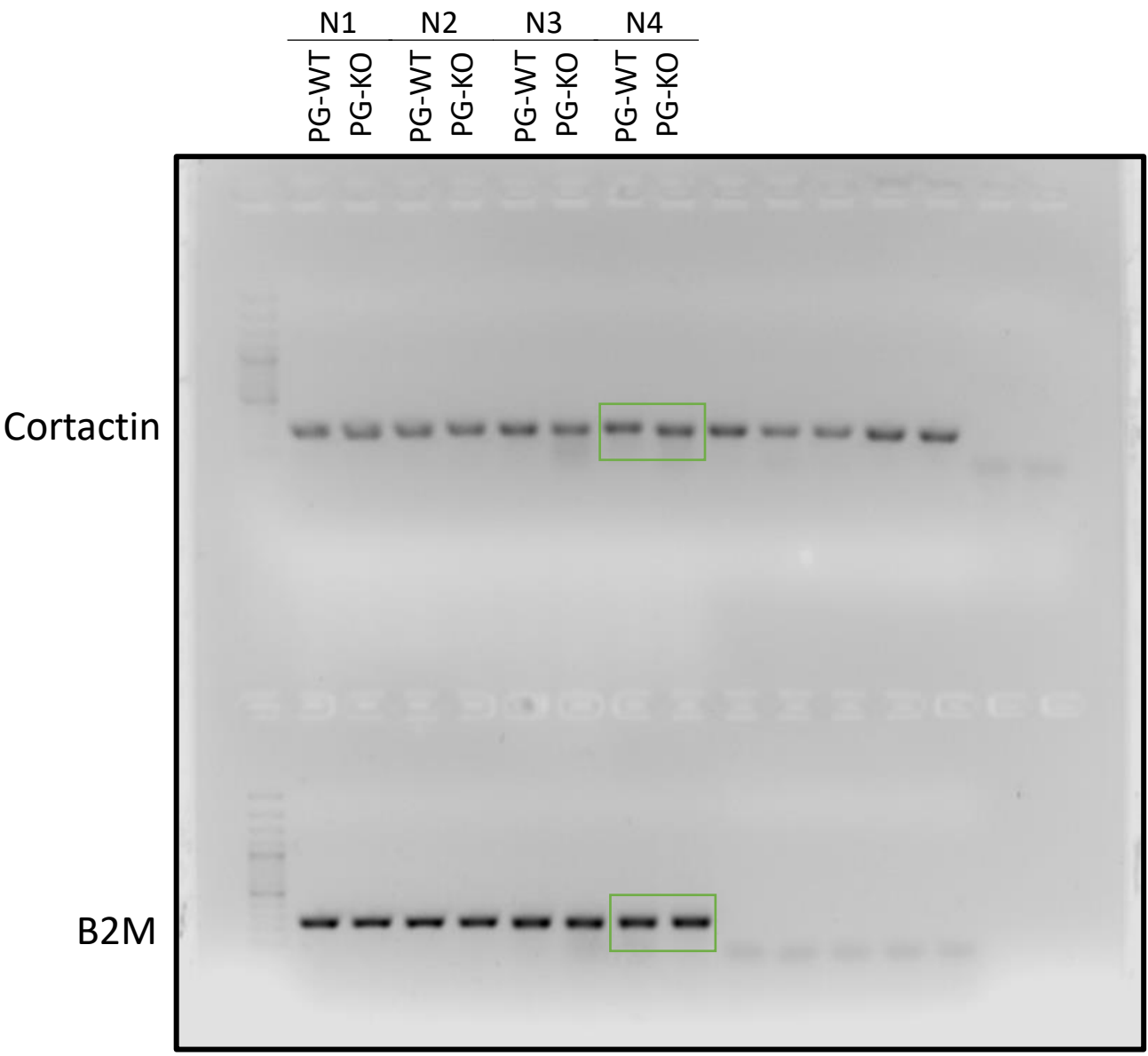


Figure S5, a-f) Original gel images for PCR. The green square determines the selected blot used in the figure 2.

Figure S5 g)

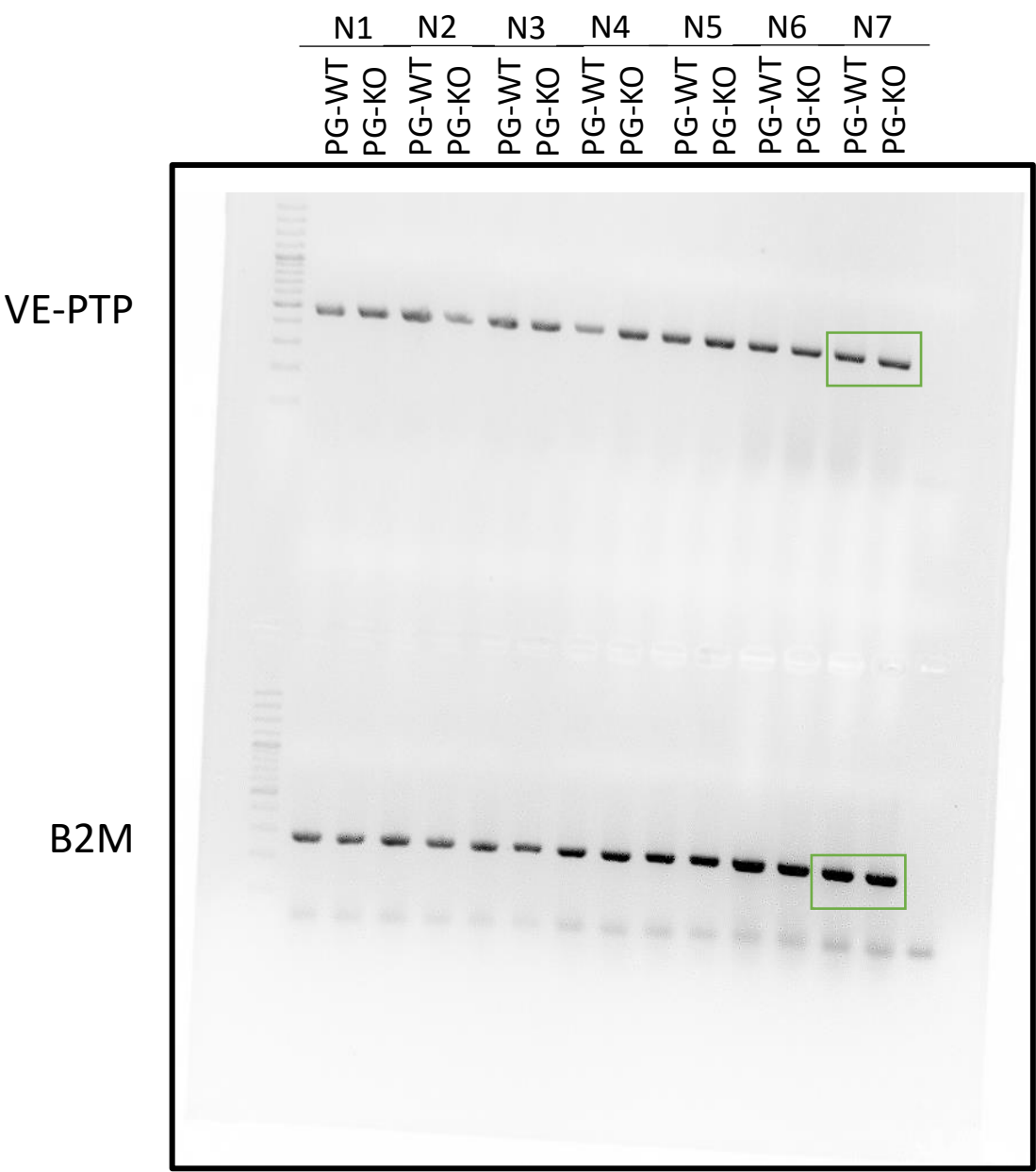


Figure S5, g) Original gel images for PCR. The green square determines the selected blot used in the figure 3.

Figure S5 h)

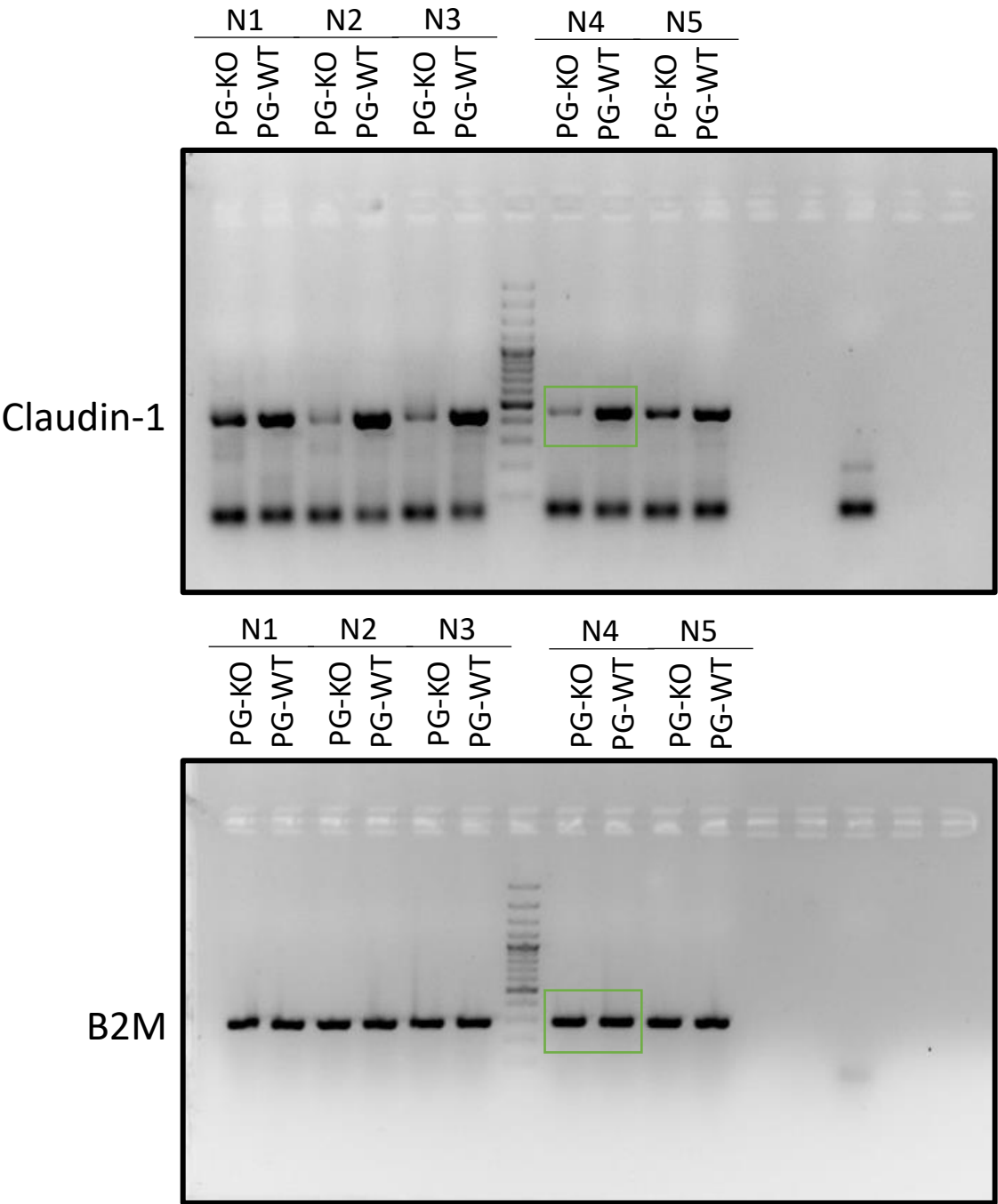
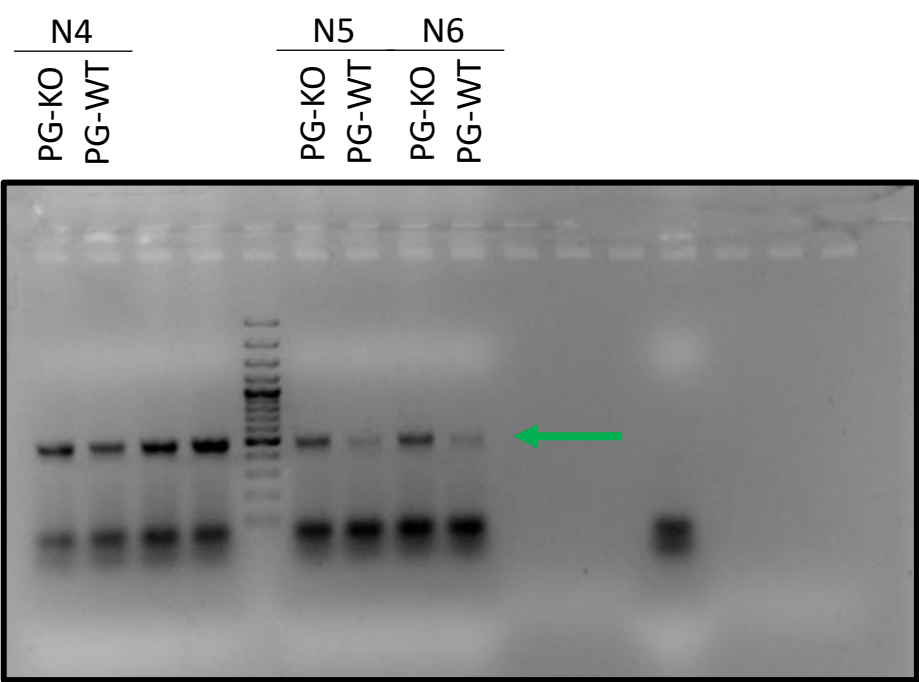
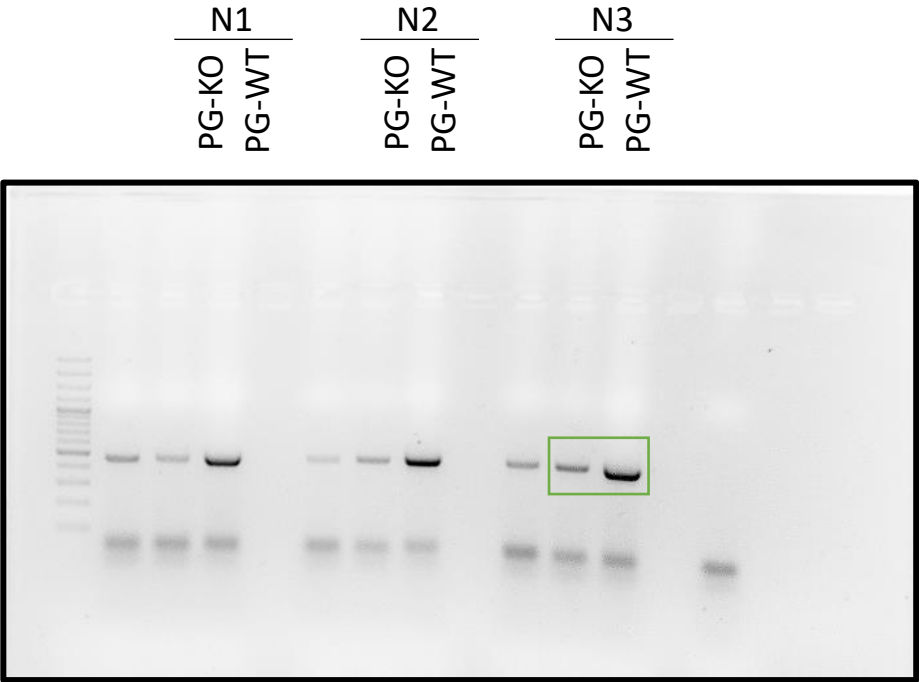


Figure S5 i)

Claudin-5



B2M

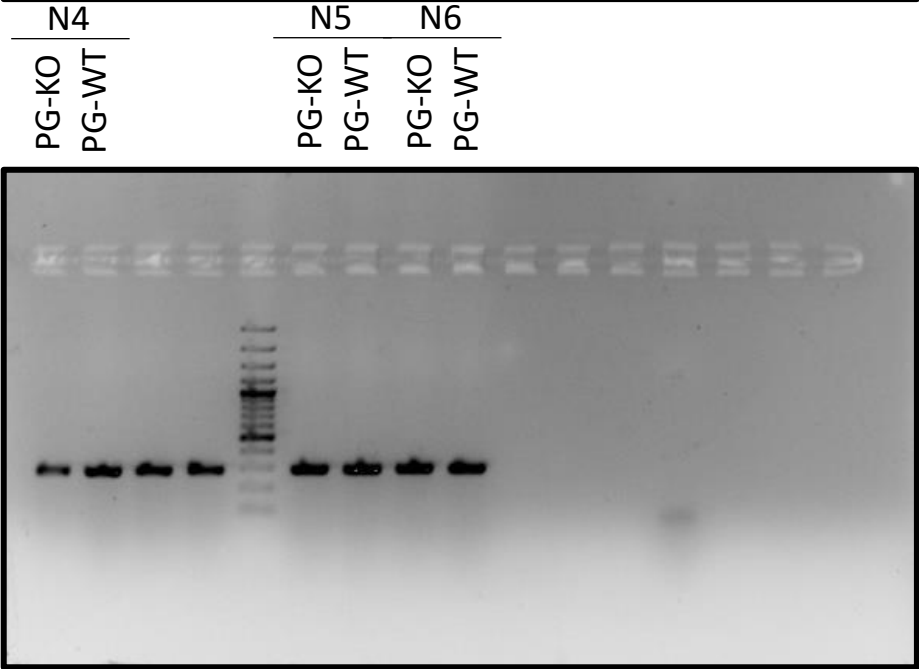
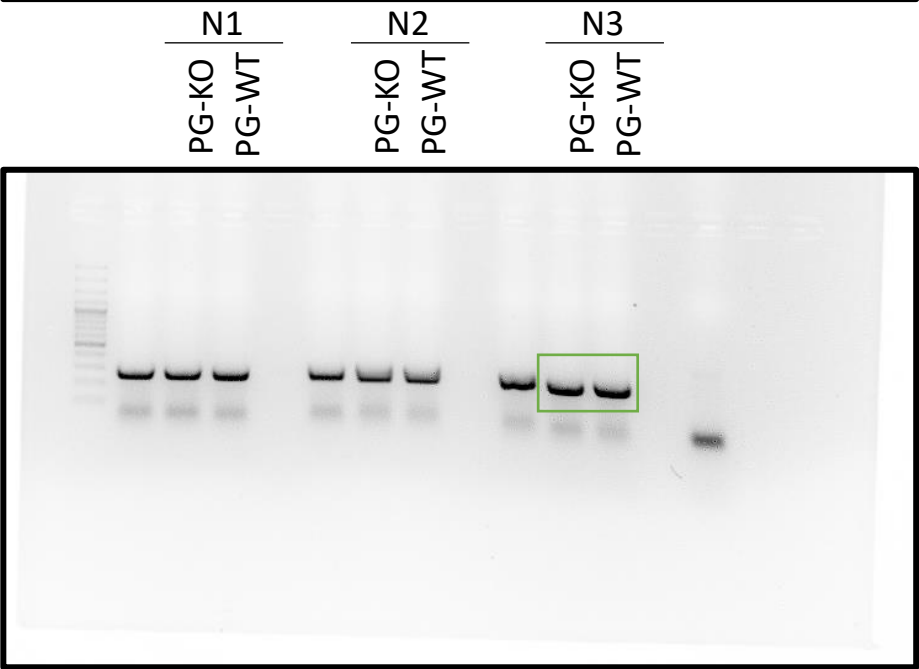


Figure S5 j)

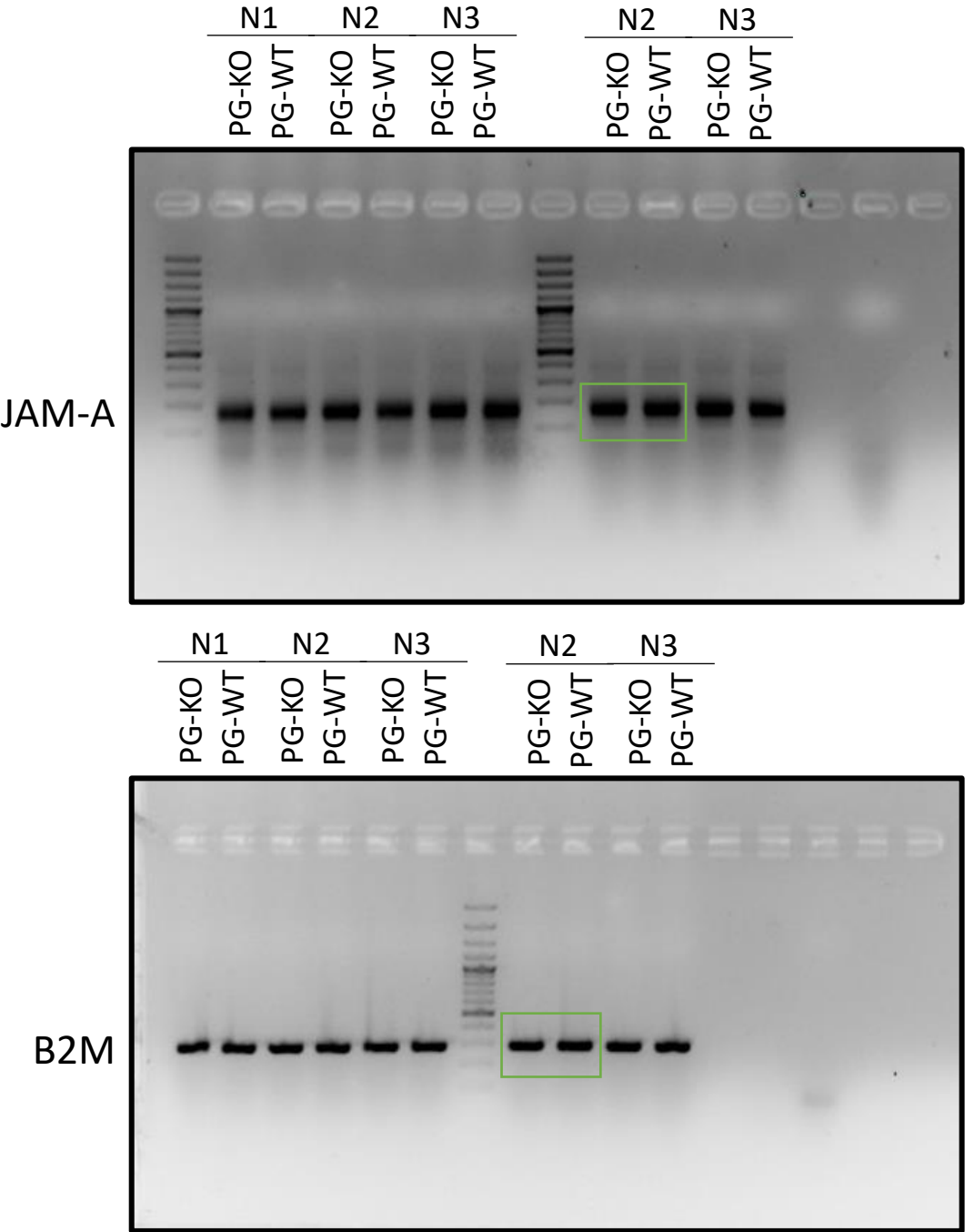


Figure S5 k)

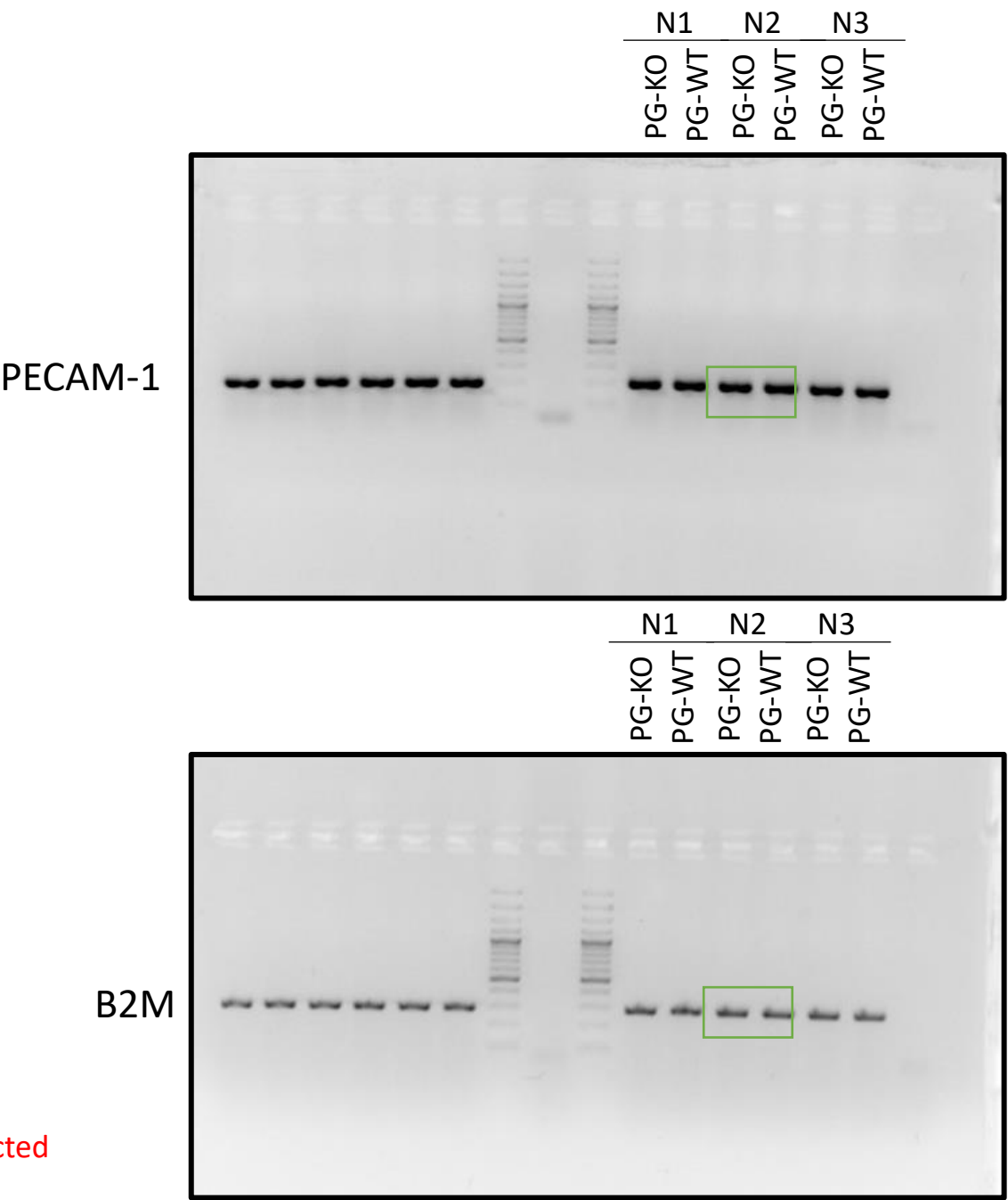


Figure S5, h-k) Original gel images for PCR. The green square determines the selected blot used in the figure S3.

Original western blot gel images
for vehicle or F/R treatment

Figure S6 a)

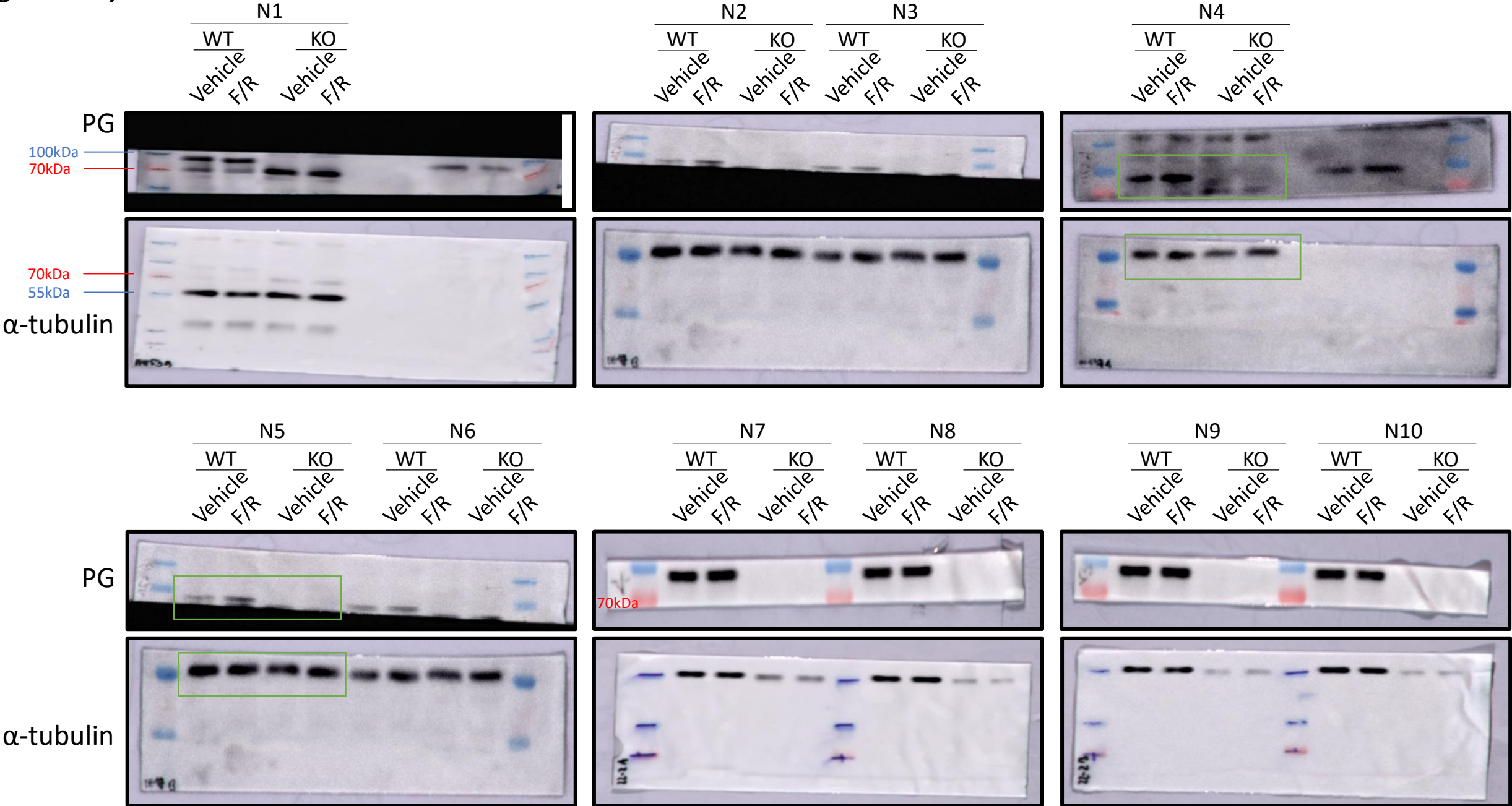


Figure S6 b)

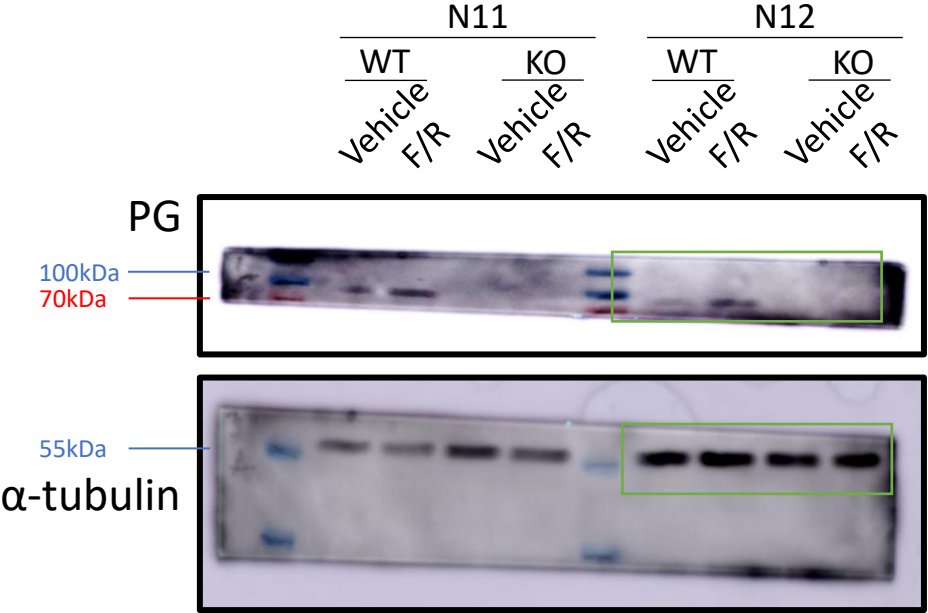


Figure S6 c)

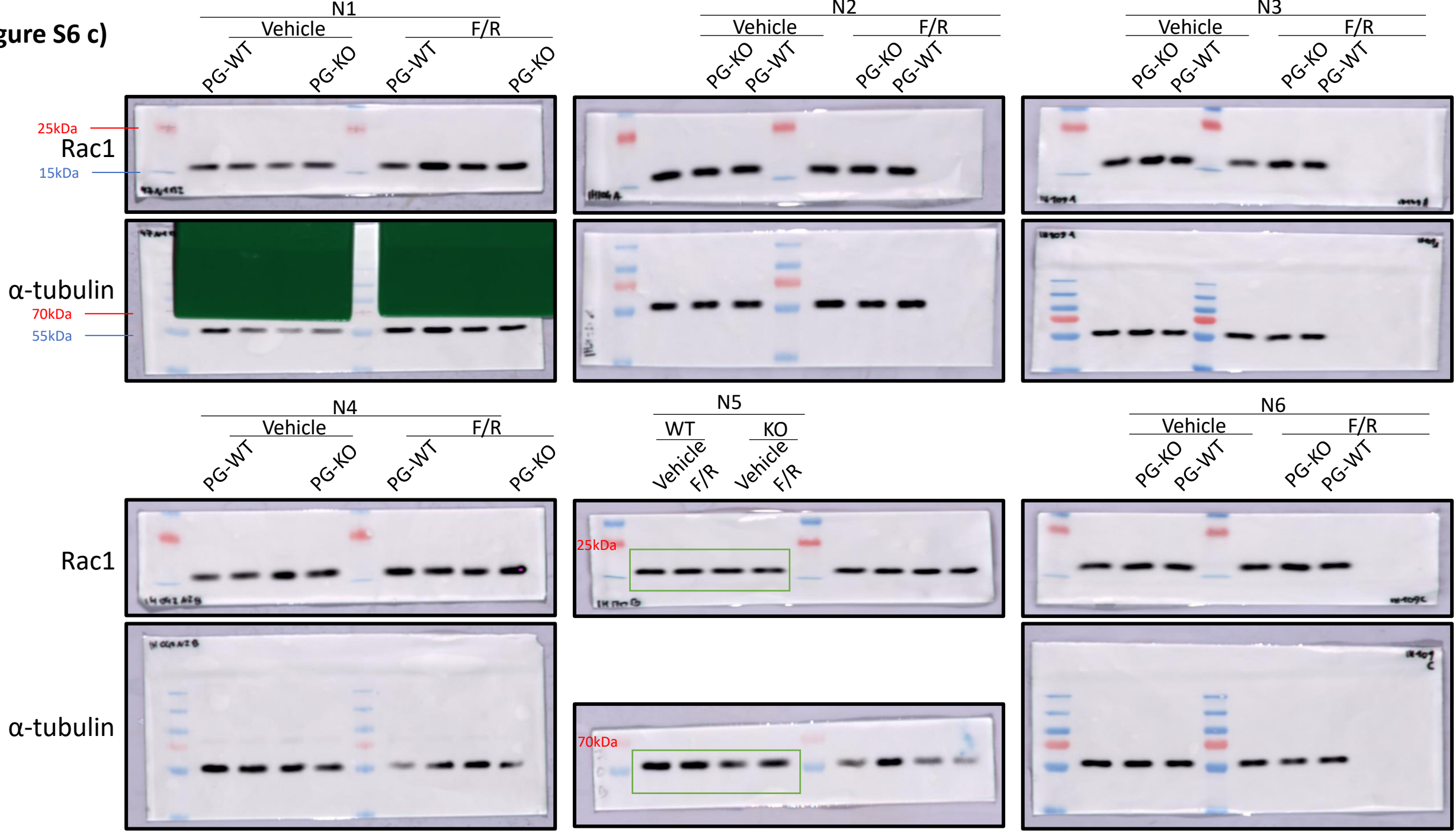


Figure S6 d)

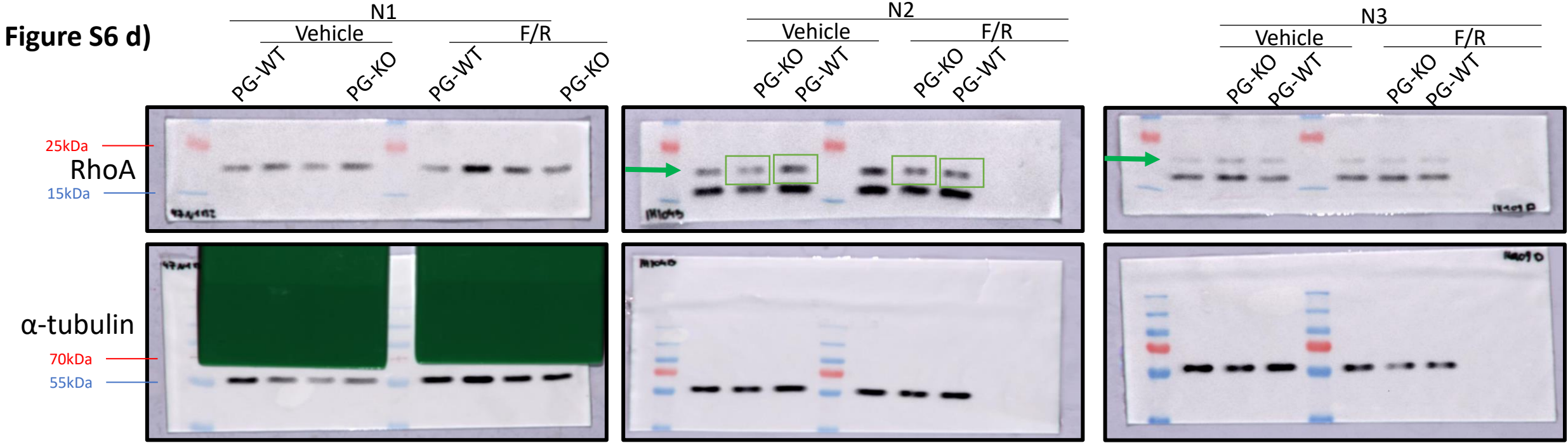


Figure S6 e)

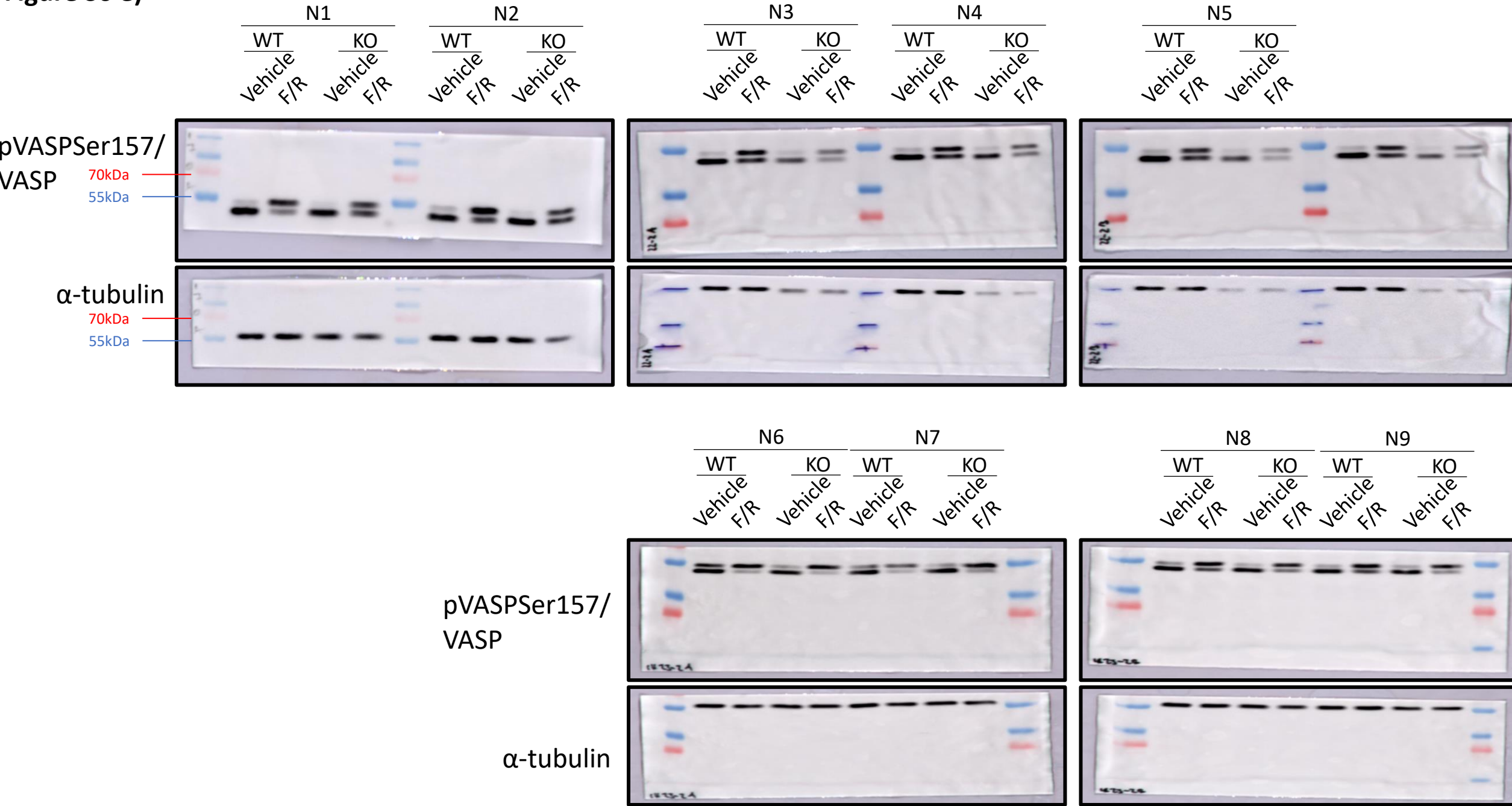


Figure S6 f)

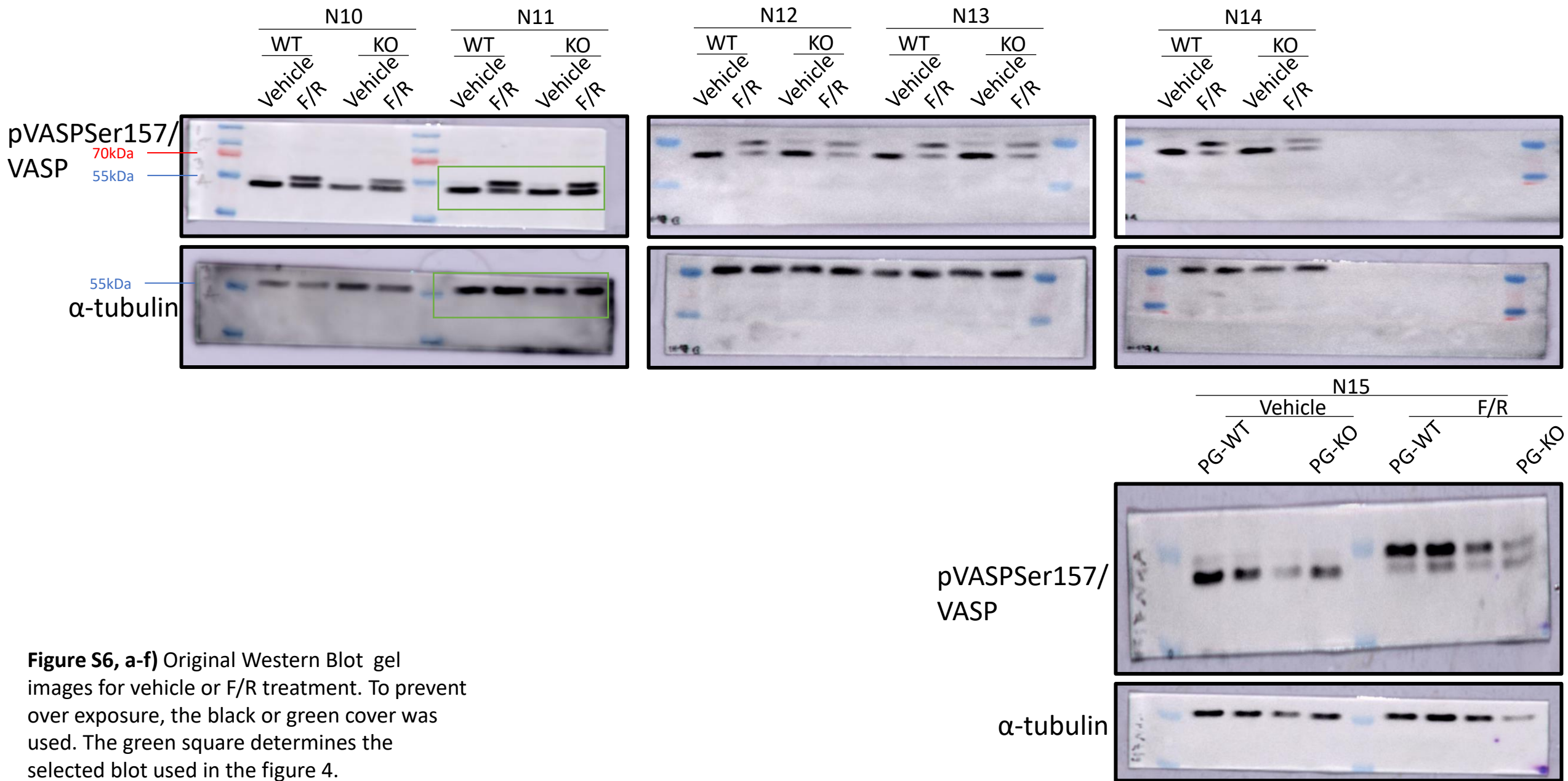


Figure S6, a-f) Original Western Blot gel images for vehicle or F/R treatment. To prevent over exposure, the black or green cover was used. The green square determines the selected blot used in the figure 4.

Figure S6 g)

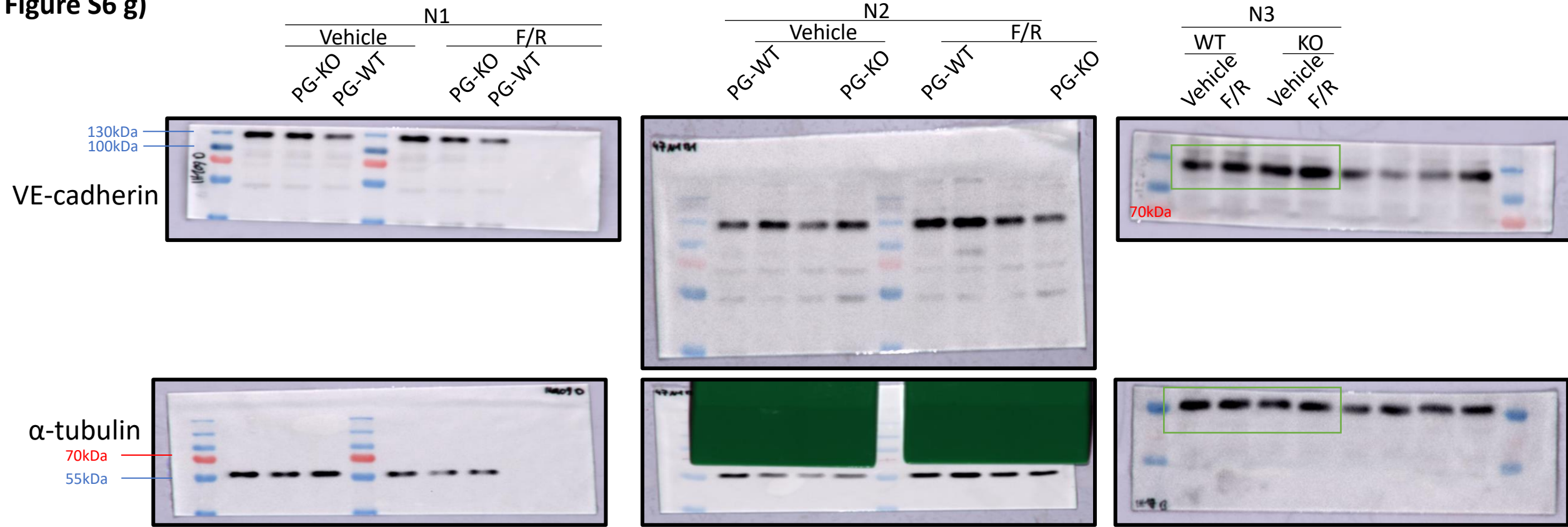


Figure S6 h)

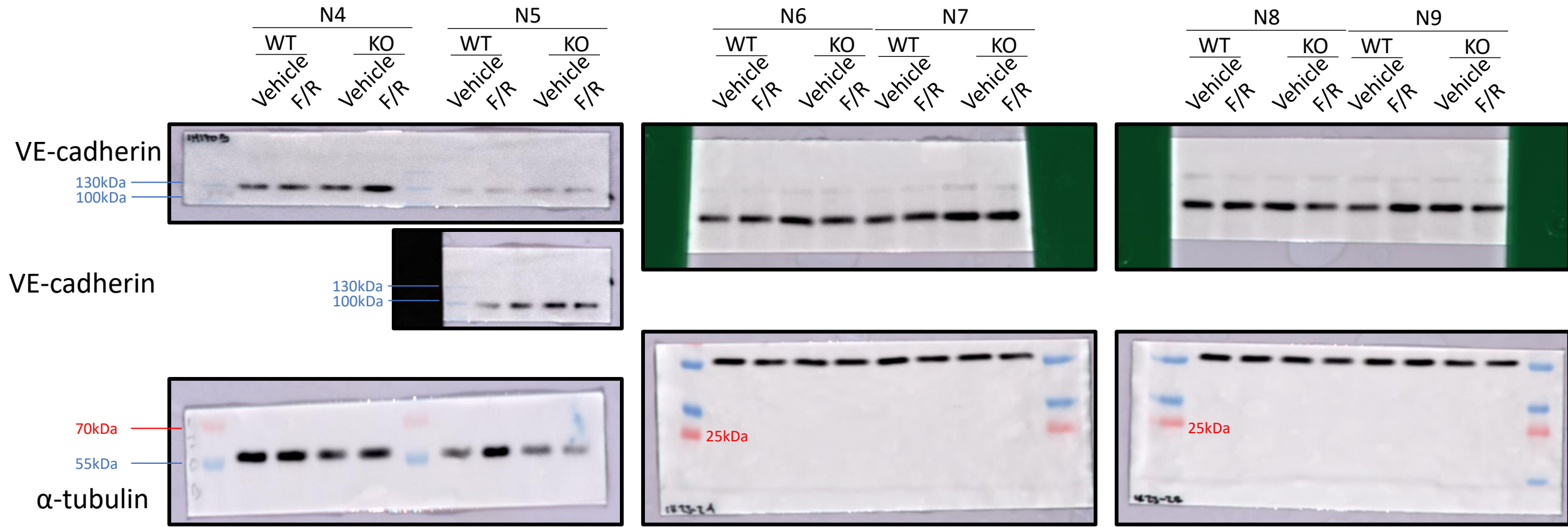


Figure S6 i)

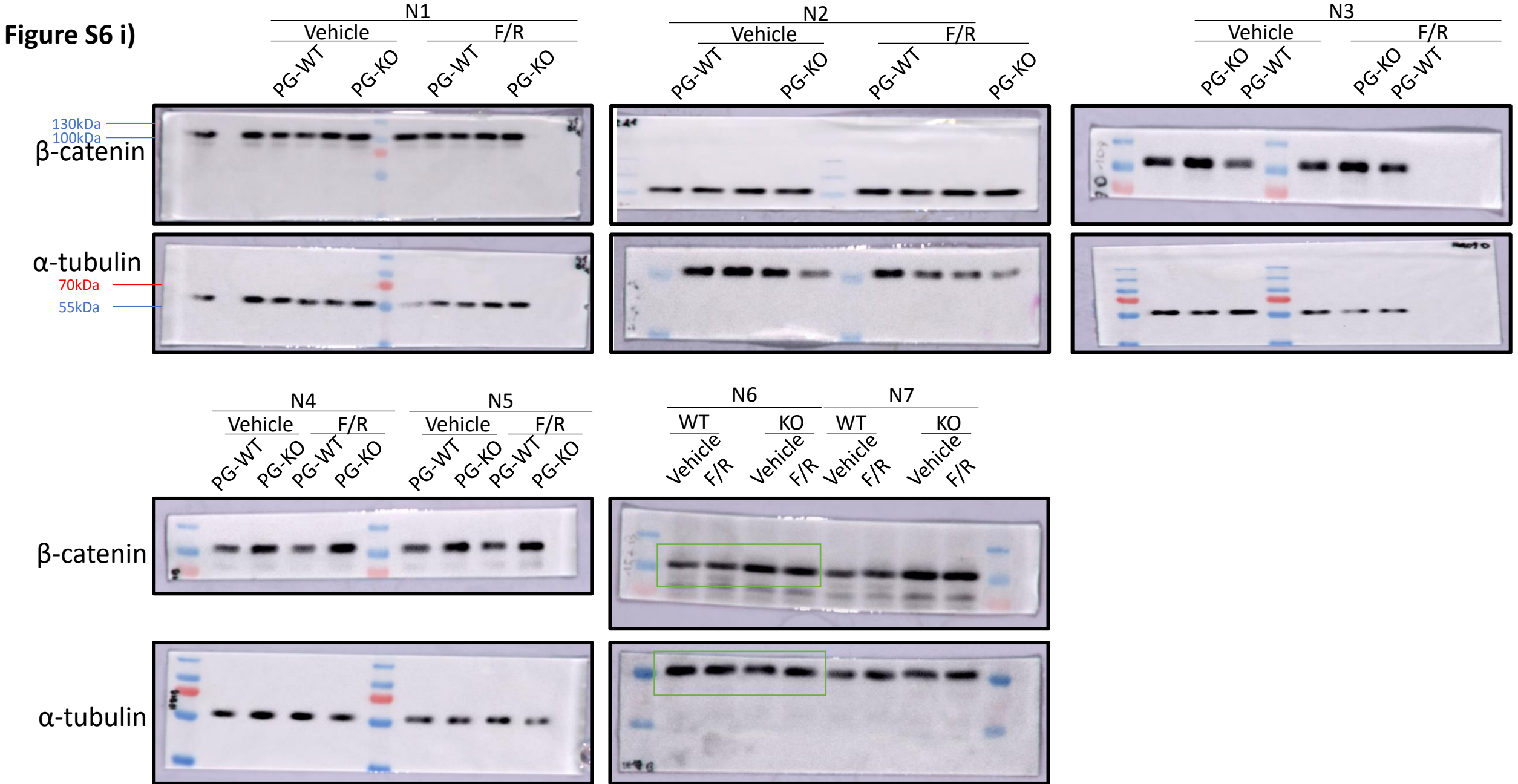


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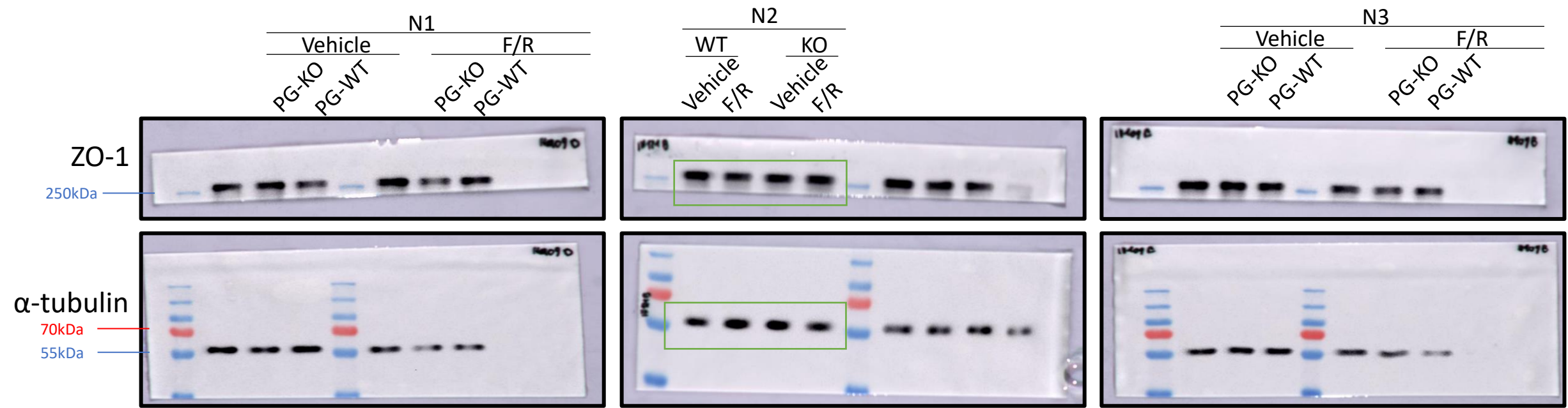


Figure S6 k)

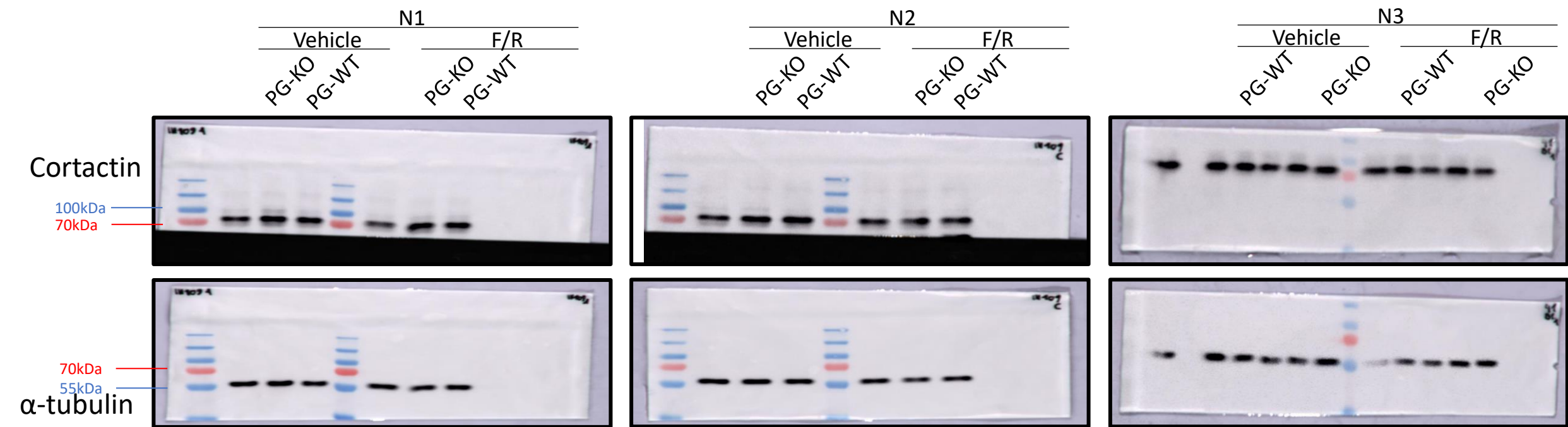


Figure S6 I)

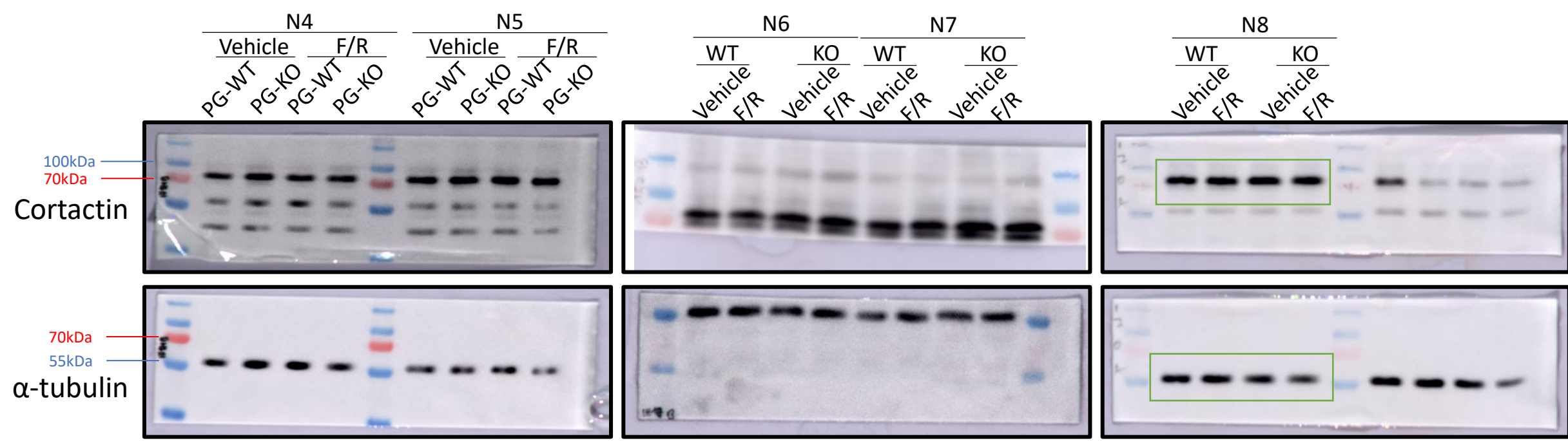


Figure S6, g-l) Original Western Blot gel images for vehicle or F/R treatment. To prevent over exposure, the black or green cover was used. The green square determines the selected blot used in the figure 6.

Figure S7 m)

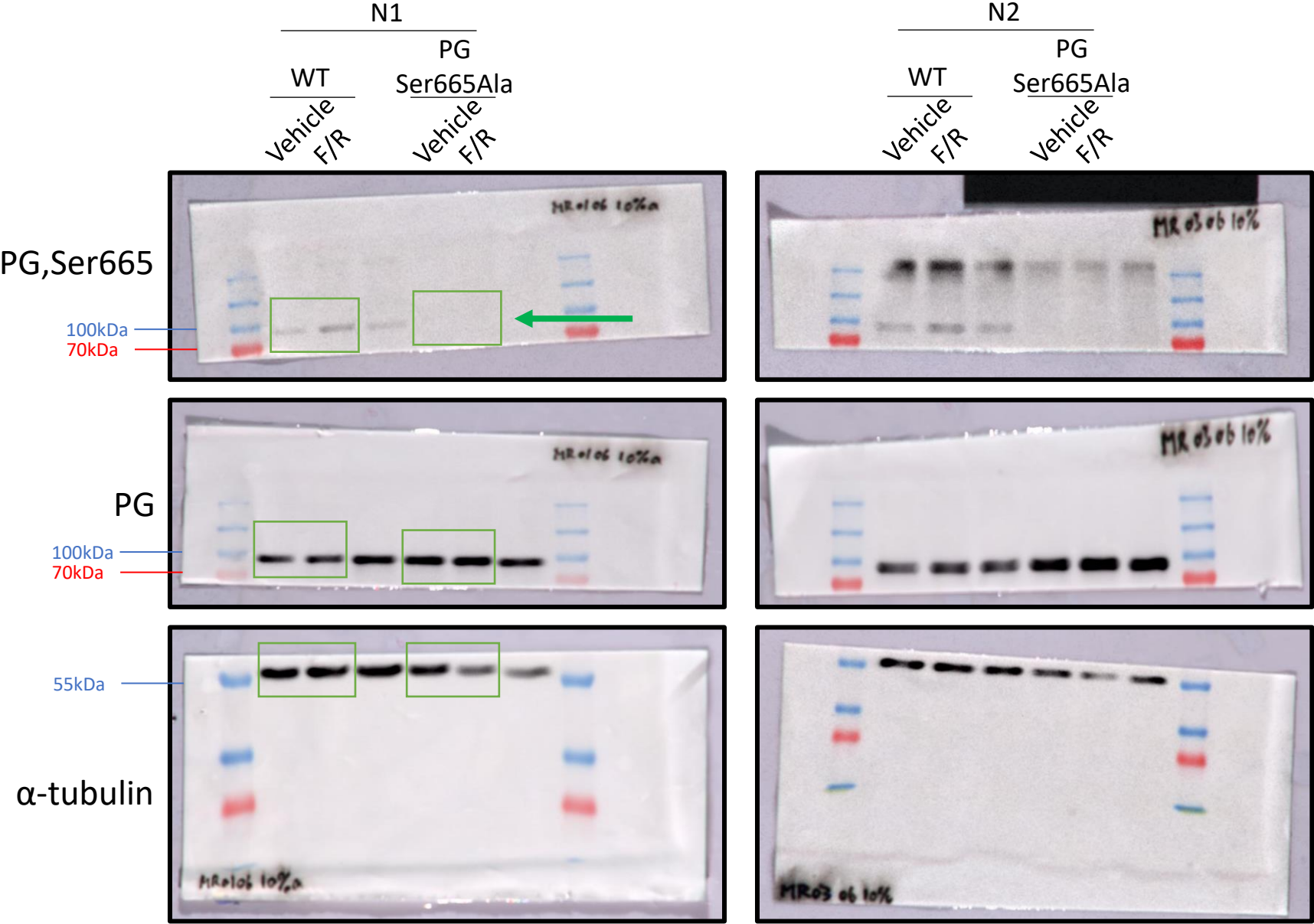


Figure S7 n)

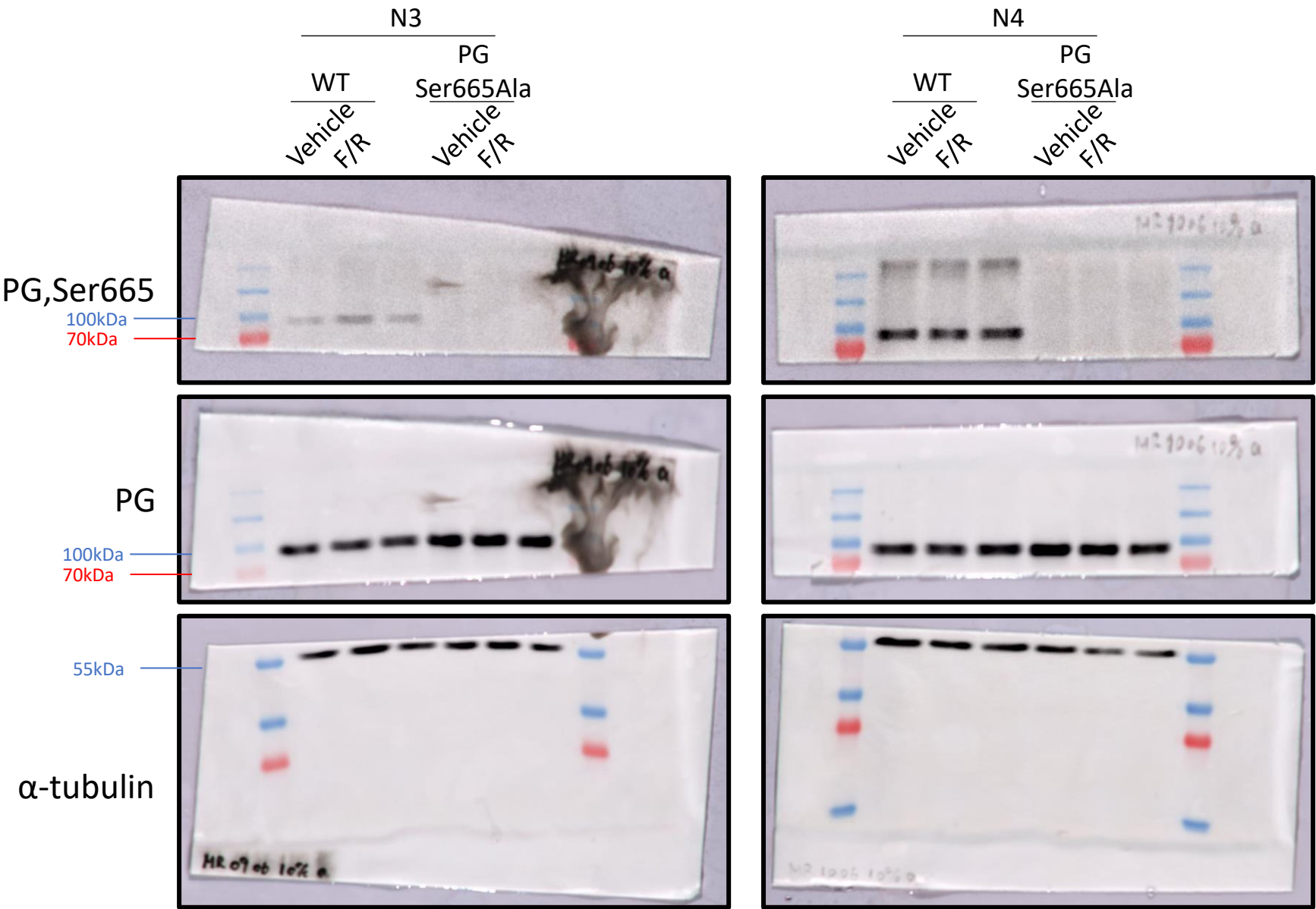


Figure S7, m-n) Original Western Blot gel images for vehicle or F/R treatment. To prevent over exposure, the black or green cover was used. The green square determines the selected blot used in the figure 7.

Original western blot gel
images for VE-cadherin IPs

Figure S8 a)

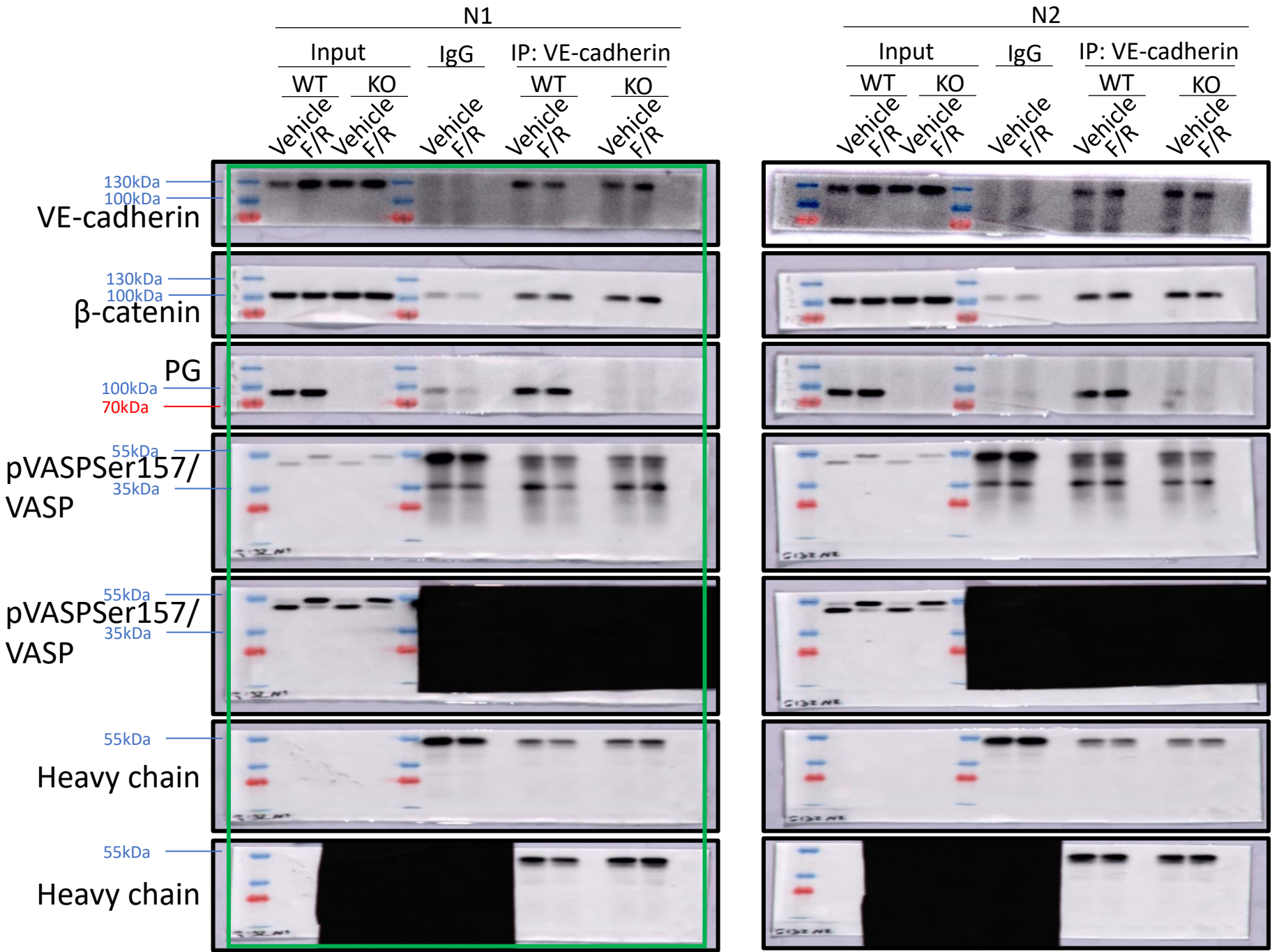


Figure S8 b)

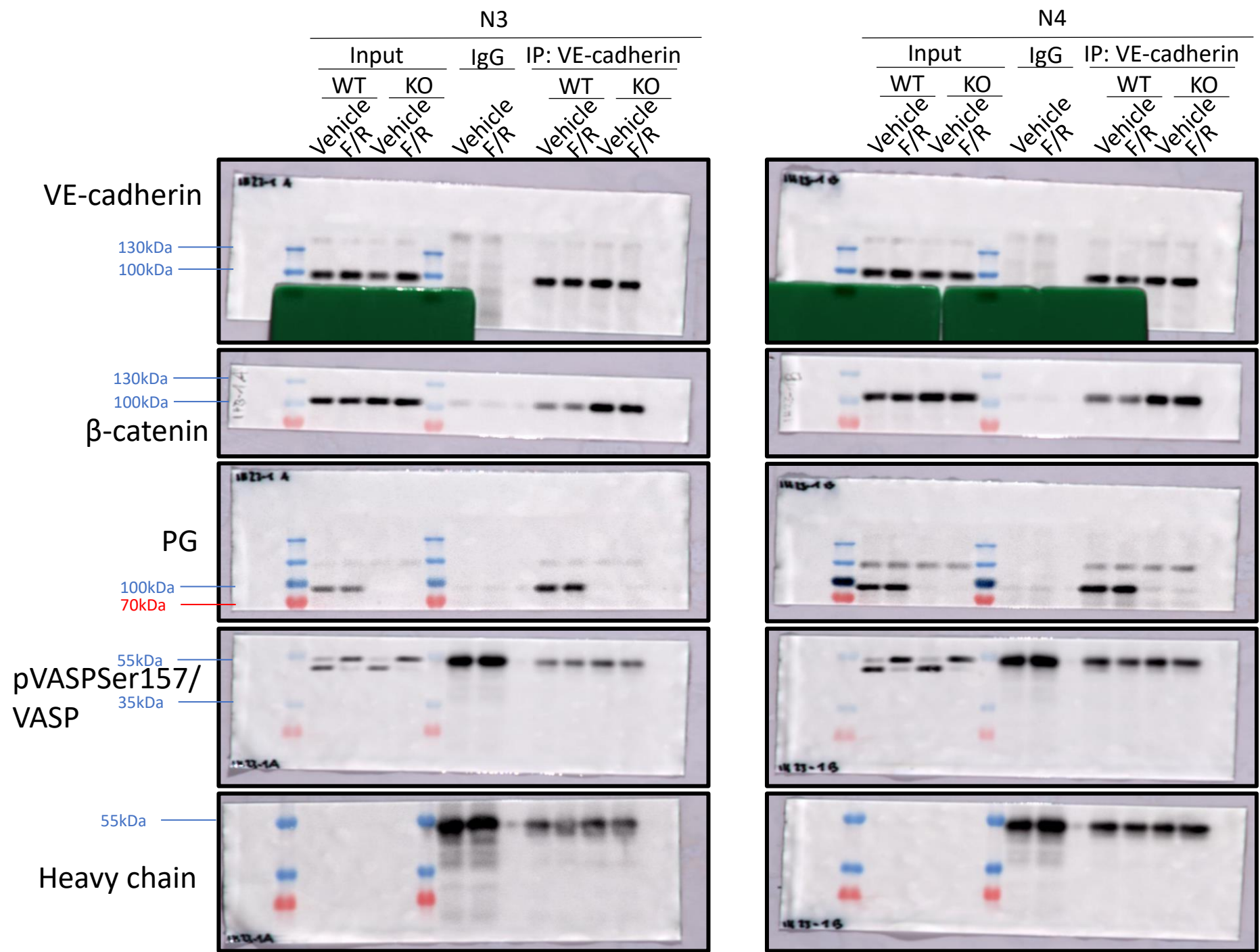


Figure S8 c)

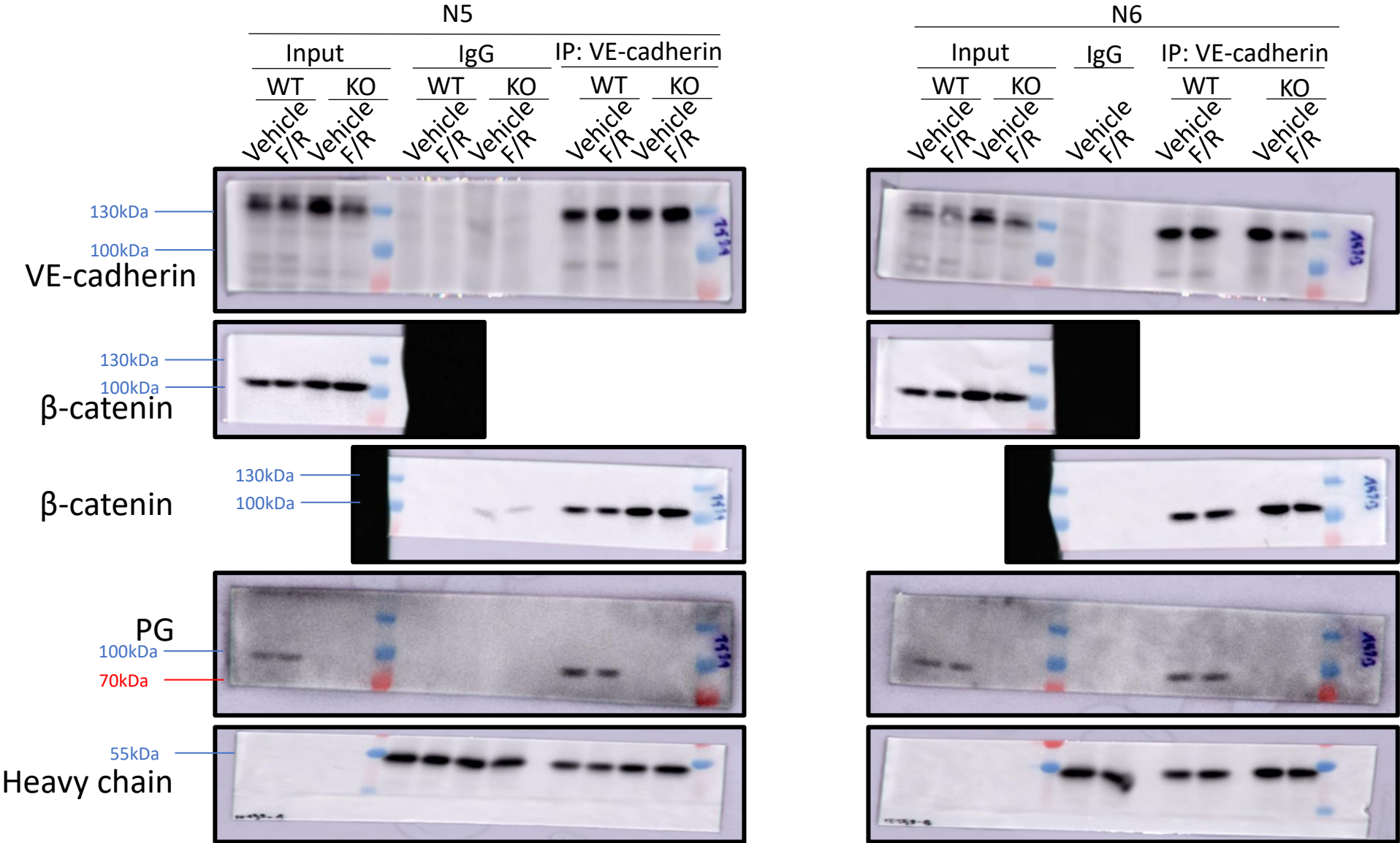


Figure S8, a-c) Original Western Blot gel images for VE-cadherin IPs. To prevent over exposure, the black or green cover was used. The green square determines the selected blot used in the figure 6.