

Two *in vivo* Well-differentiated G1/G2 PanNET Preclinical Mouse Models

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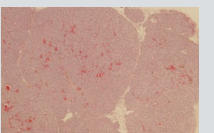
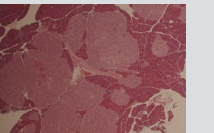
Abstract (Click)

To improve clinical outcomes in pancreatic neuroendocrine tumors (PanNETs), an effective preclinical mouse model that closely resembles human disease is necessary to be developed. Here we generated two genetically engineered mouse models (GEMM) using Cre-Lox system, with insulin-specific biallelic inactivation of *Men1* and *Pten*. These mice developed well-differentiated G1/G2 PanNETs by three months of age. The first model with rat insulin 2 promoter (RIP) driving Cre expression developed pituitary neuroendocrine tumors (PitNETs) as well in the same mouse by two to three months of age. The second model with mouse insulin 1 promoter driving Cre expression developed only PanNETs, which should provide a more specific and practical model for *in vivo* preclinical therapeutic study for human PanNETs. The histology of the PanNETs in these two models was similar to patients with MEN1 related tumors. Treatments with the mTOR inhibitor - rapamycin prolonged survival and delayed development of both types of NETs. Collectively, these are the first mouse models that underscored the cooperative roles of *Men1* and *Pten* in cancer, in particular in NETs. These are the first mouse models that developed human-like well-differentiated PanNETs within three months. These models provide a platform to study novel therapeutic opportunities for NETs through targeting PI3K/AKT/mTOR and MENIN pathways.

Conclusion

- we developed two effective preclinical mouse models that closely resemble human *Men1* disease with biallelic inactivation of *Men1* and *Pten* in β cells.
- MPR and MPM mouse models developed well-differentiated G1/G2 PanNETs
- Treatments of mTOR inhibitor-rapamycin prolonged the survival and delayed development of both PanNETs and PitNETs.
- Our genetics data indicated that with deficiency of *Pten* or *Men1*, dosage of the other tumor suppressor is critical.
- Menin pathway plays a key role in development of PanNETs while PI3K/AKT/mTOR pathway plays a key role in development of PitNETs.
- Both mouse models provide a platform to study novel therapeutic opportunities for NETs through targeting PI3K/AKT/mTOR and Menin pathways.

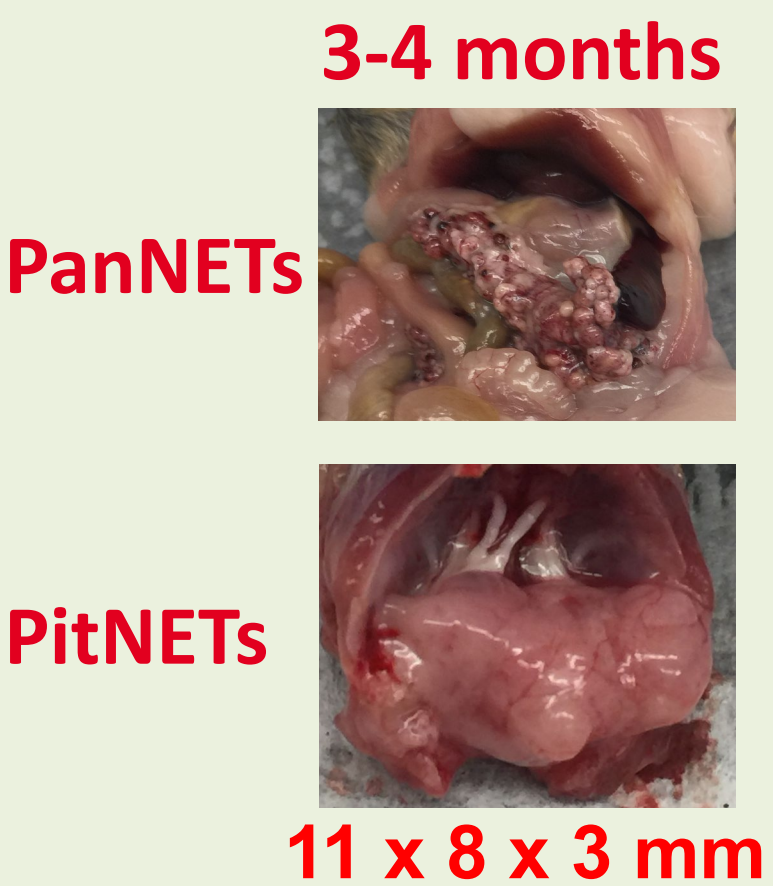
Hypothesis

Genes	PanNETs	Floxed mouse available	Histology of Pancreas in the deletion mice
<i>MEN1</i> (Multiple Endocrine Neoplasia 1)	44%	Yes, <i>Men1^{fl/fl}</i>	 9-12 months
<i>DAXX</i> , <i>ATRX</i>	43%	NA	NA
Genes in mTOR pathway (<i>PTEN</i> , <i>TSC2</i> , <i>PIK3CA</i> , etc)	15%	Yes, <i>Pten^{fl/fl}</i>	 8-10 months

Jiao Y., et al Science, 2011

Pten cooperates with Menin to suppress NE tumorigenesis

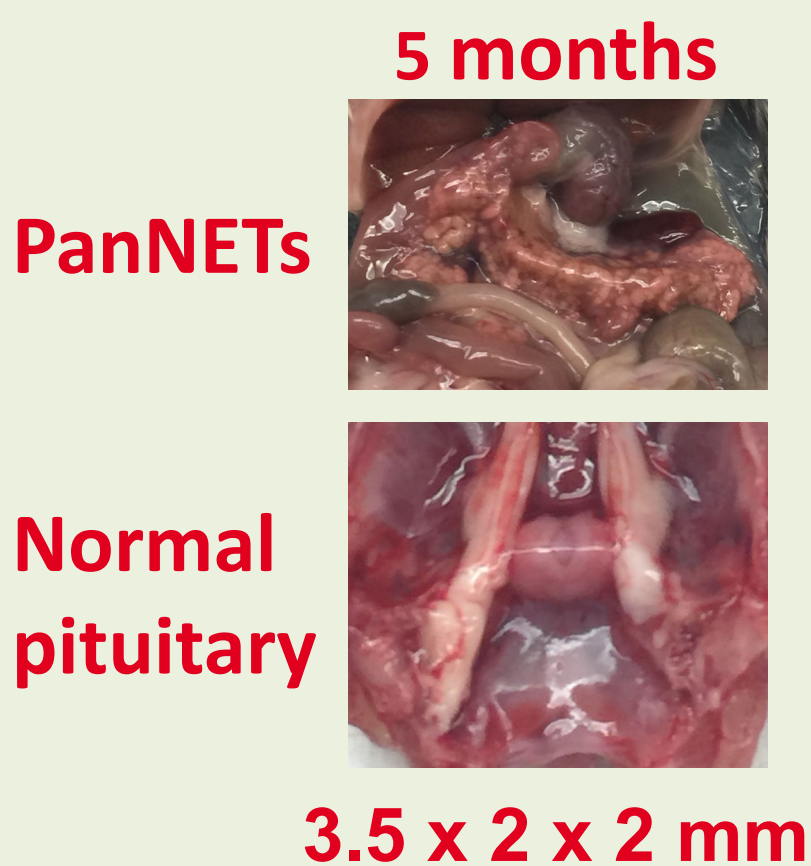
MPR mouse model:
Men1^{flox/flox} Pten^{flox/flox}
Rip-Cre



Results (Click)

- MPR mouse model with conditional deletion of *Men1* and *Pten* in β cells with rat insulin 2 promoter driving Cre expression (RIP-Cre) developed both PanNETs and PitNETs in one mouse (left figure)
- MPM mouse model with conditional deletion of *Men1* and *Pten* in β cells with mouse insulin 1 promoter driving Cre expression (MIP-Cre) developed only PanNETs and PitNETs in one mouse (right figure)

MPM mouse model:
Men1^{flox/flox} Pten^{flox/flox}
Mip-Cre



Click Headings to View More Information

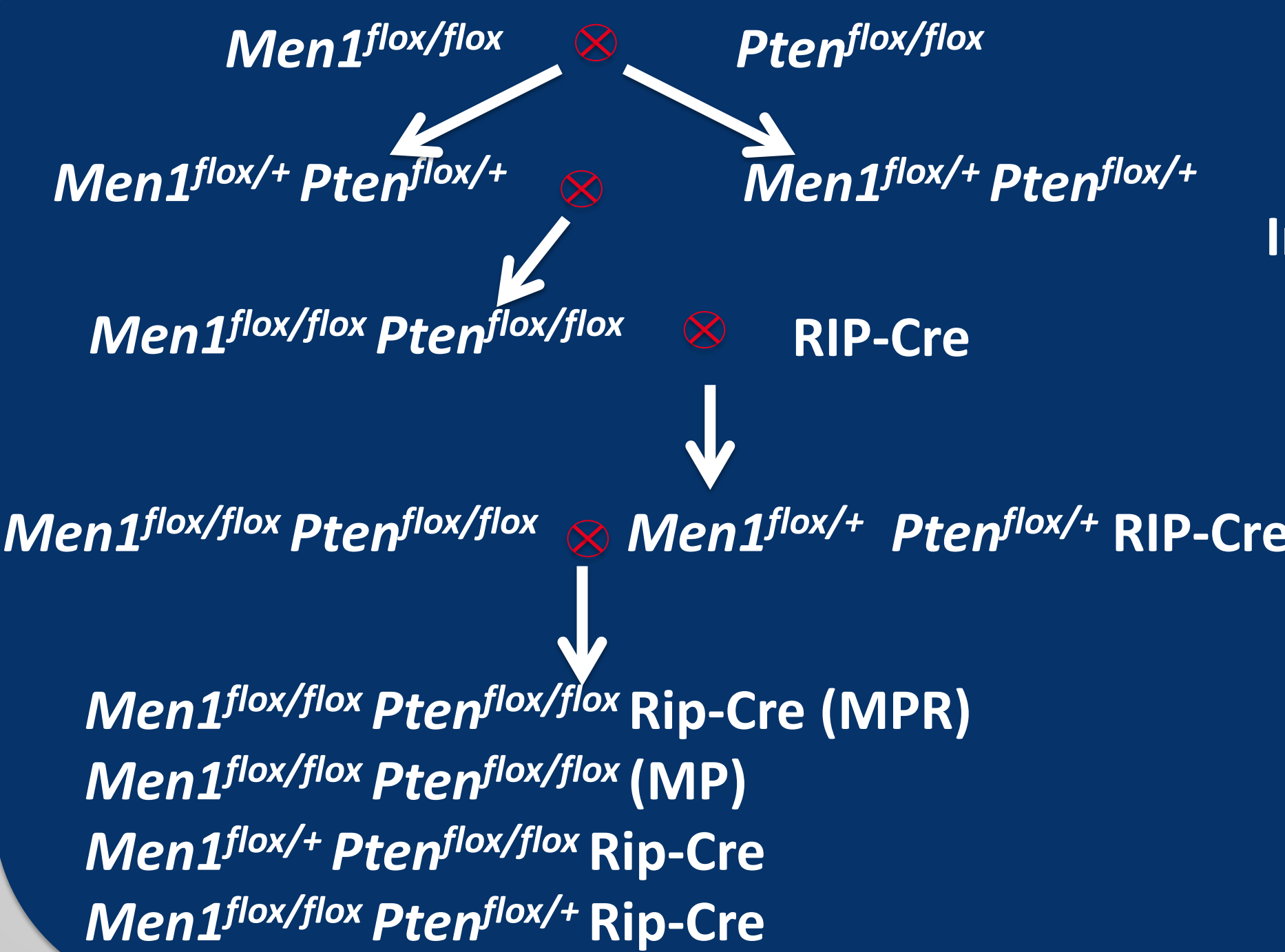
Materials and Methods

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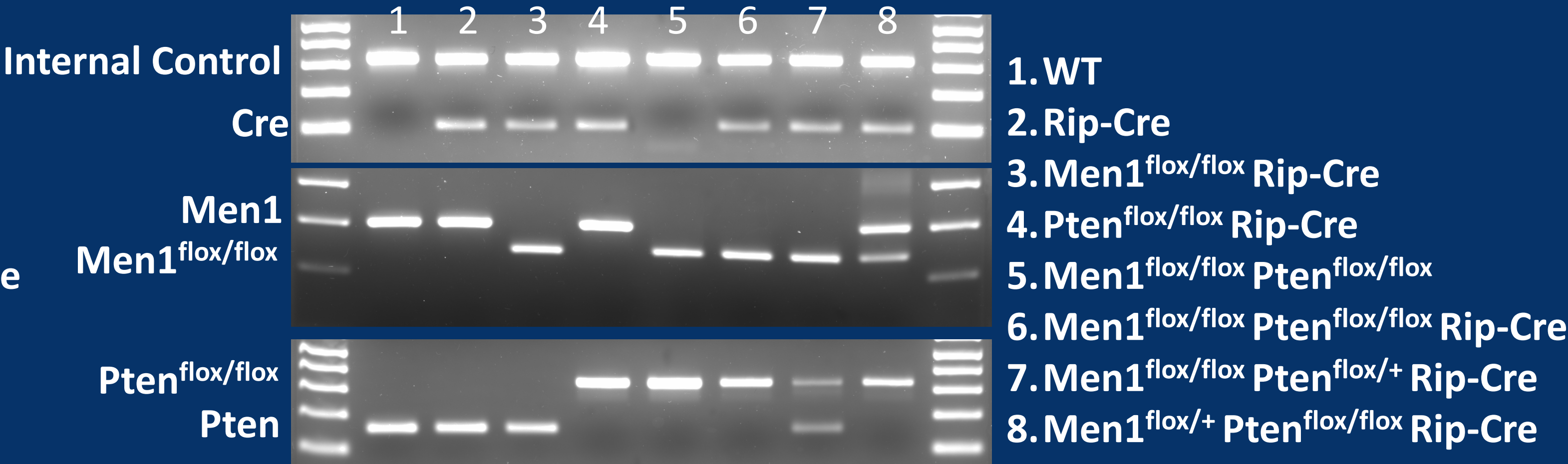
Material & Methods (Click)

- Mouse genetics on synthetic tumor suppressor deletions in β cells
- Characteristics of islets at hyperplastic and neoplastic stages
- Histopathology and Immunohistochemistry on pancreas and pituitary in mice
- Computational analysis of islets numbers and areas, and pancreas areas
- Survival analysis
- Loss of heterozygosity analysis of WT *Men1* and *Pten* alleles
- Western blotting and quantitative mRNA analysis
- PCR on genomic DNA
- Rapamycin treatment on mice
- Statistical analysis

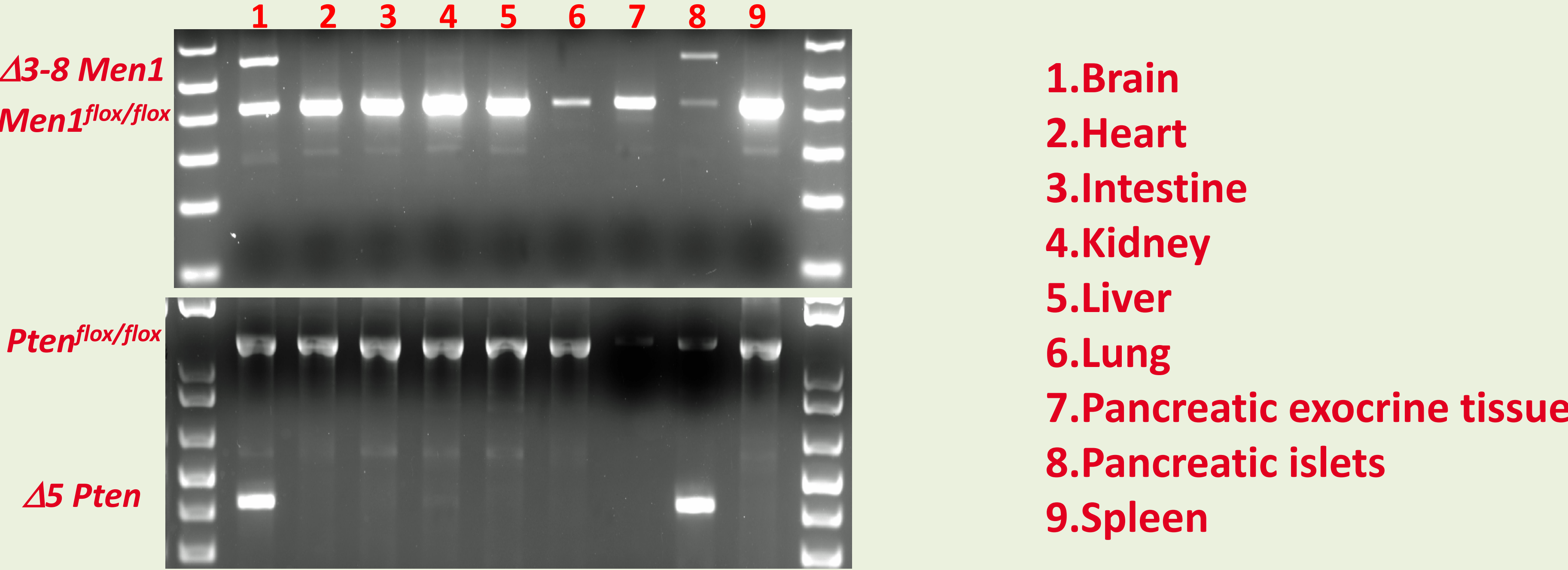
Breeding Strategy



Genotyping Results



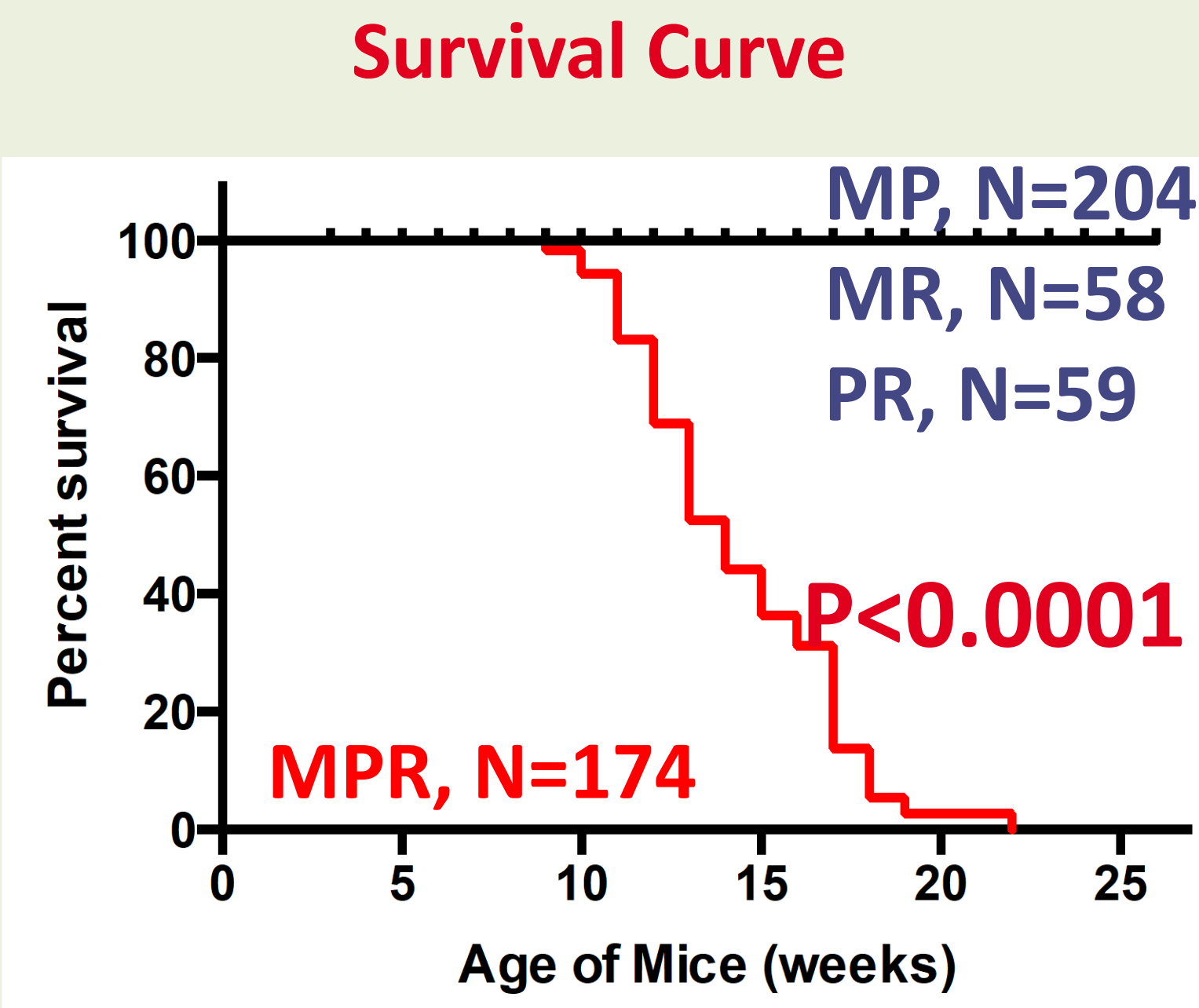
Tissue - specific deletion of *Men1* and *Pten* in *Men1*^{flox/flox} *Pten*^{flox/flox} *Rip-Cre* mice



Results

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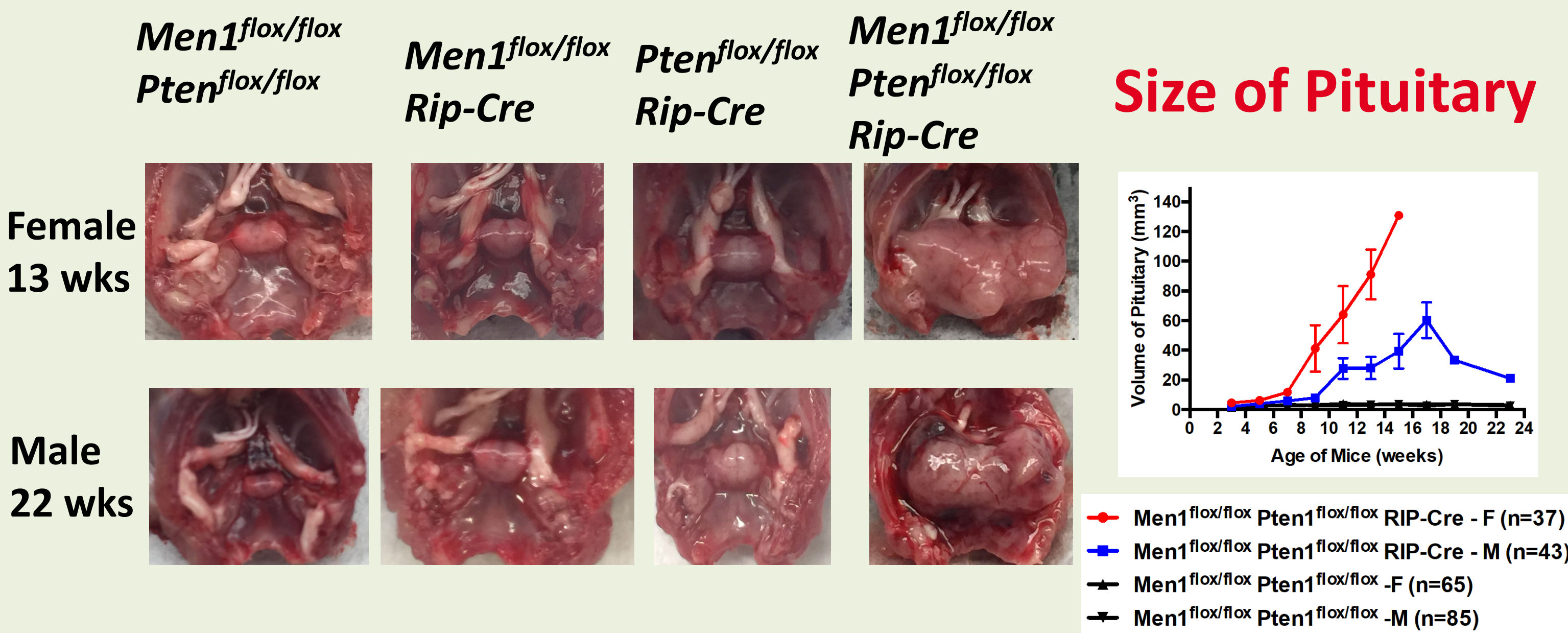
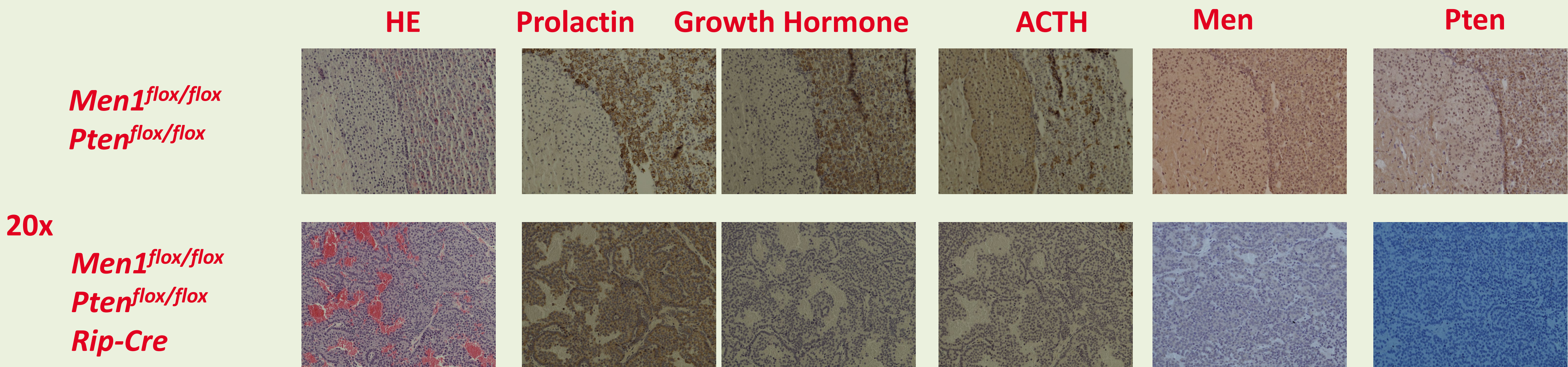
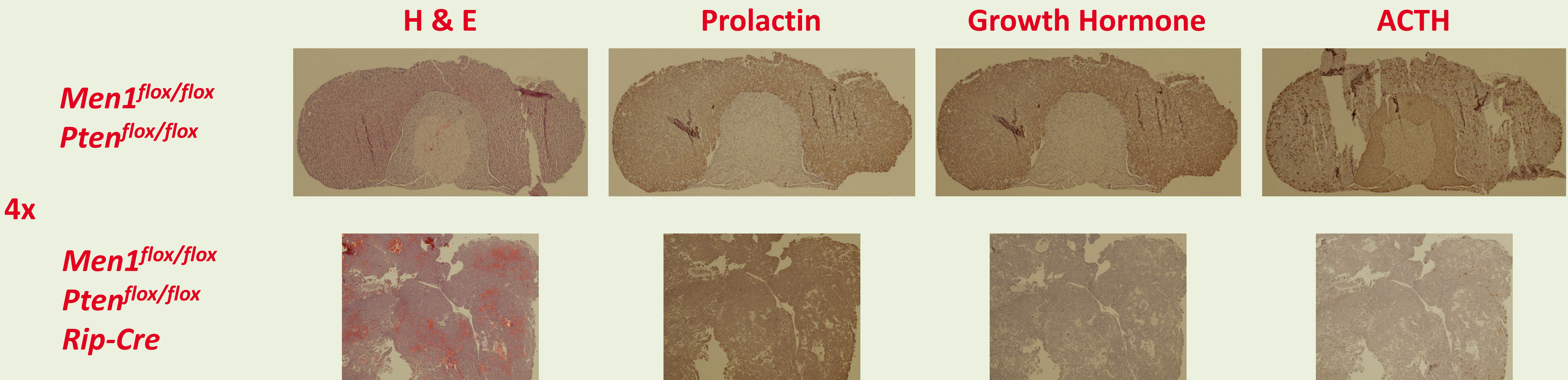
Figure 1. PTEN loss decreases survival and accelerates pituitary adenoma in deficiency of Menin in MPR mouse model



Symptoms at Death

Total # of sick mice	Found dead	Loss of balance, vision, hind leg paralysis	Sickness
70	5 (3F/2 M)	49 (31F/18M) (70%)	16 (6F/10M)

PitNETs developed in MPR mouse model are prolactinoma

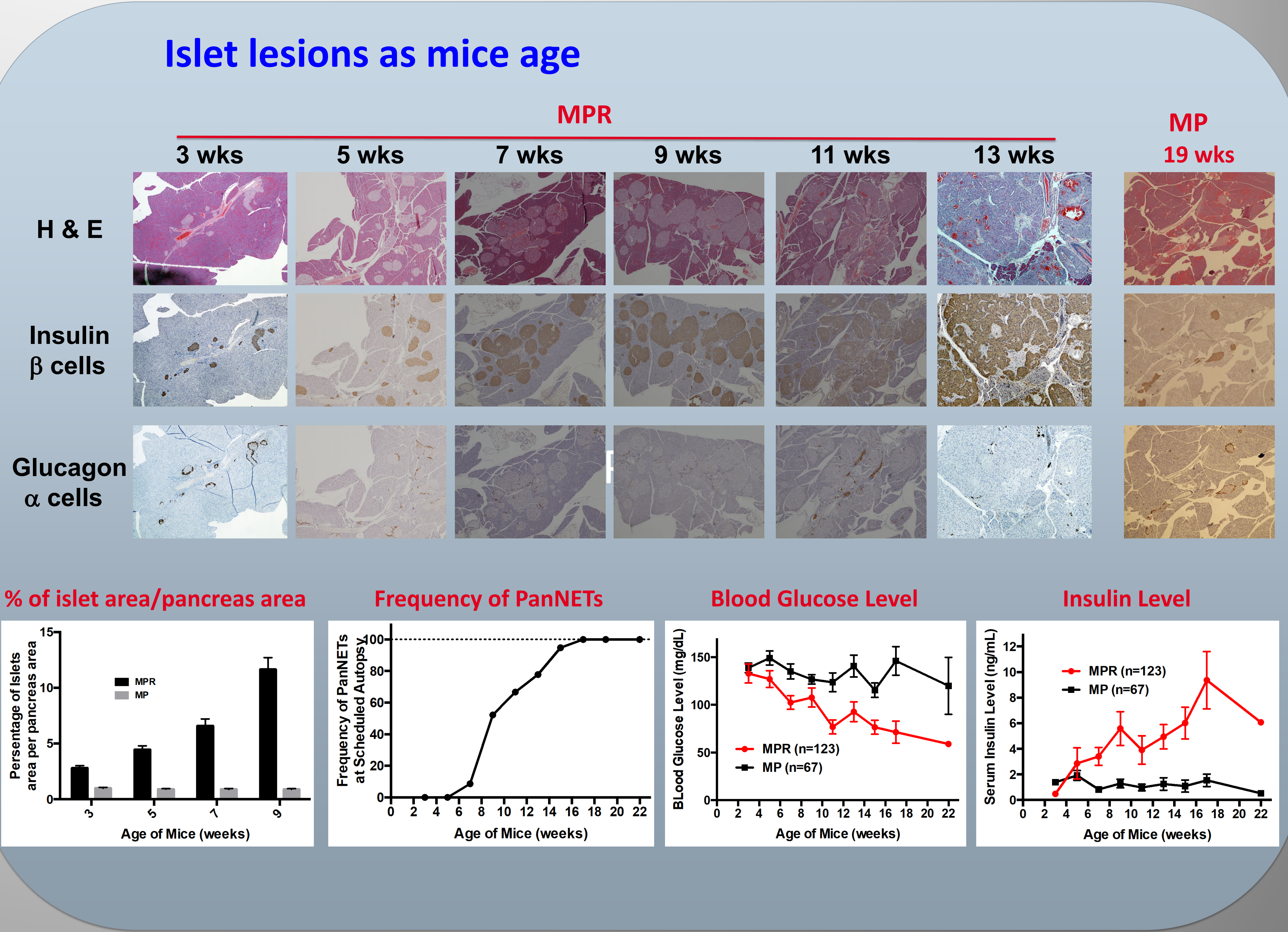
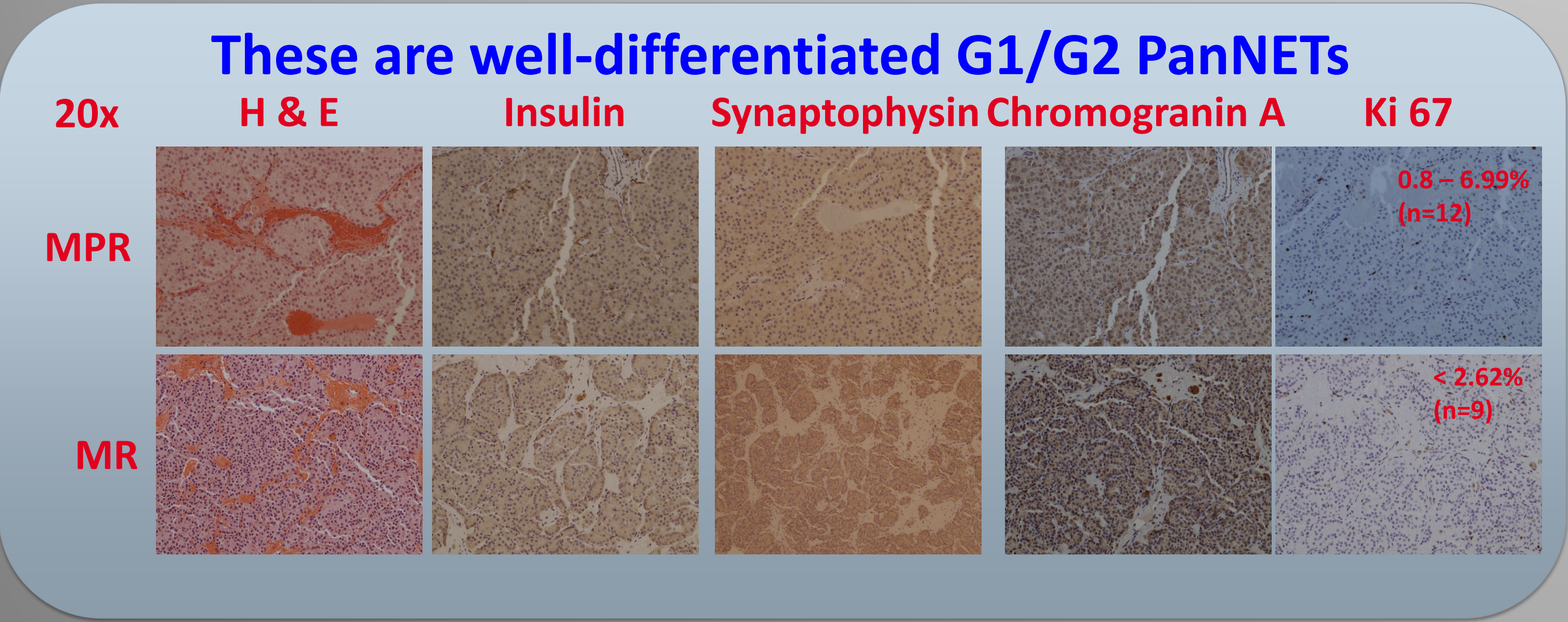
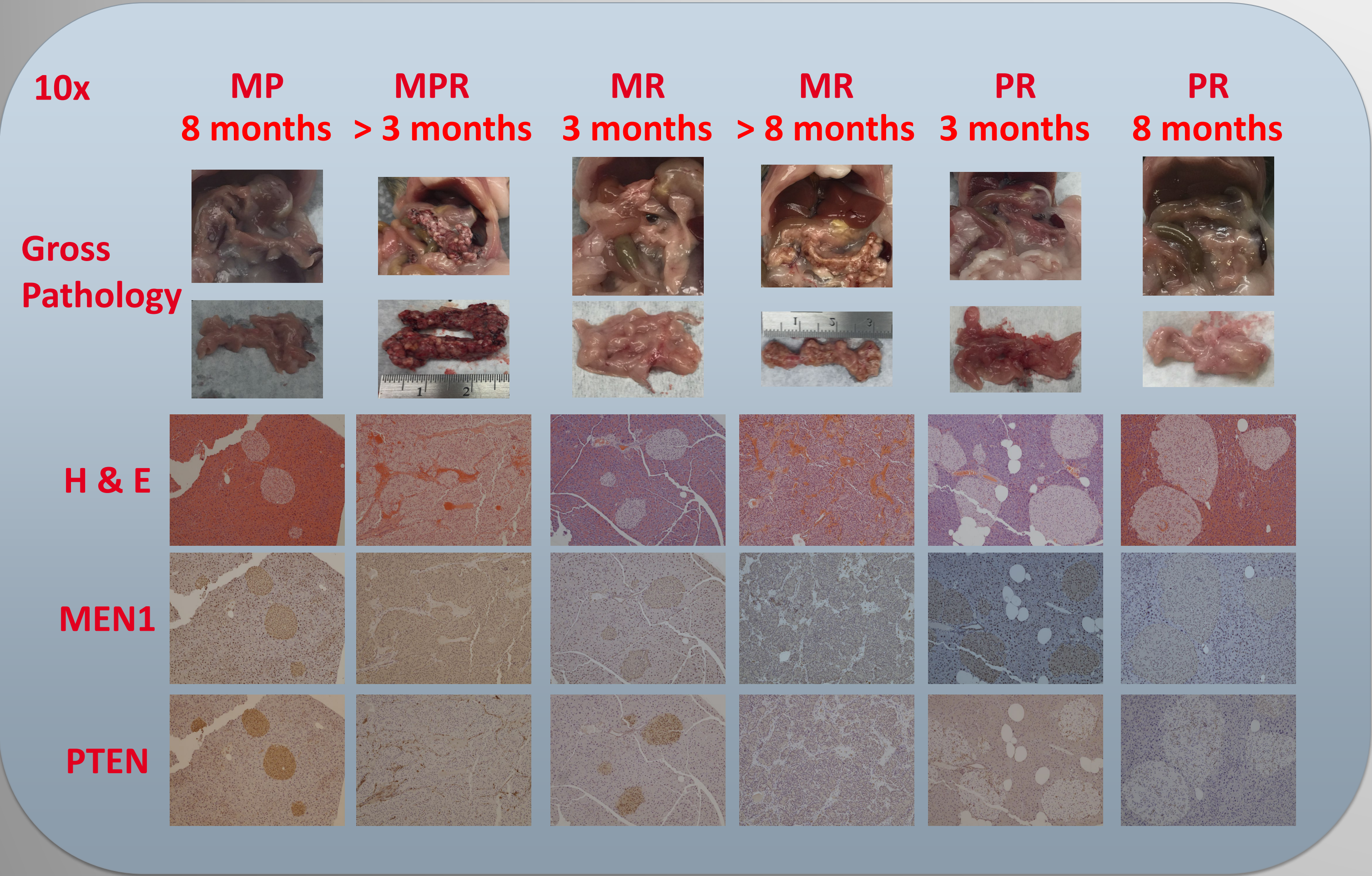


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Figure 2. PTEN loss accelerates PanNETs in deficiency of Menin in MPR mouse model.

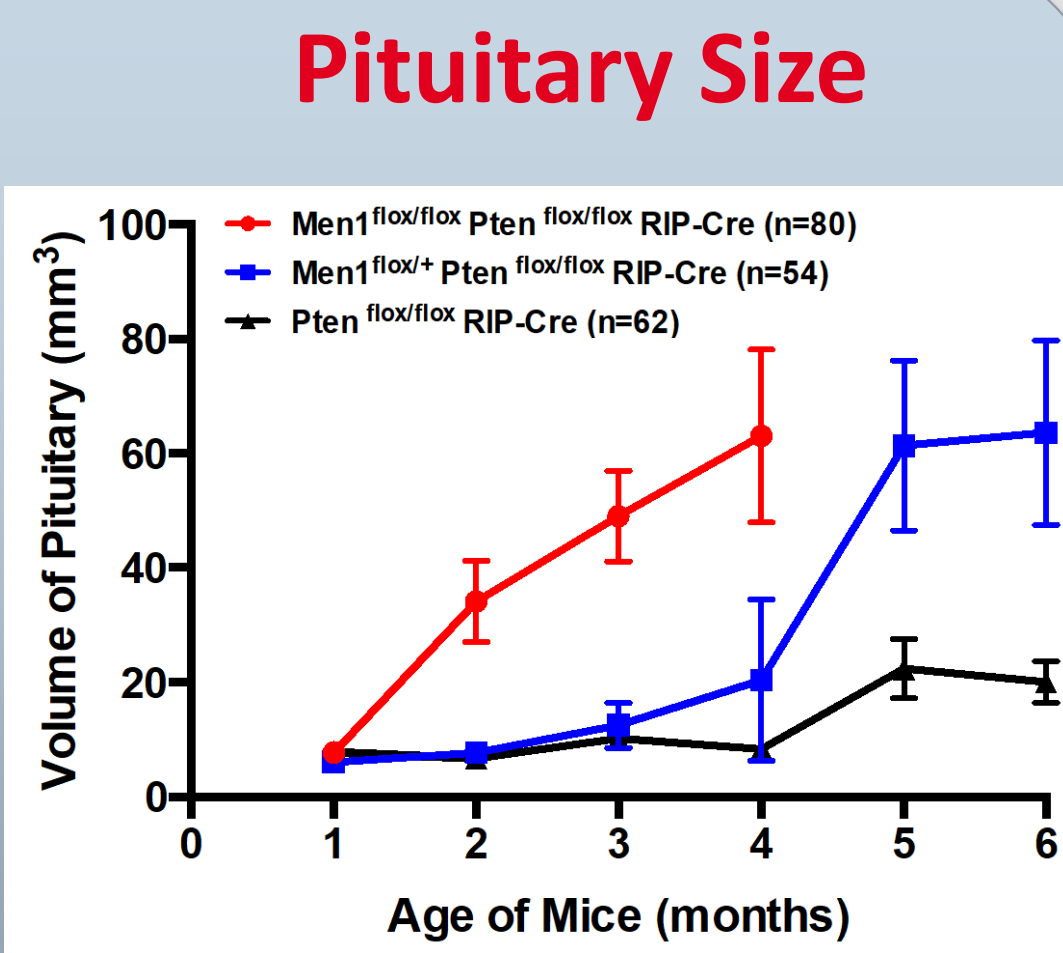
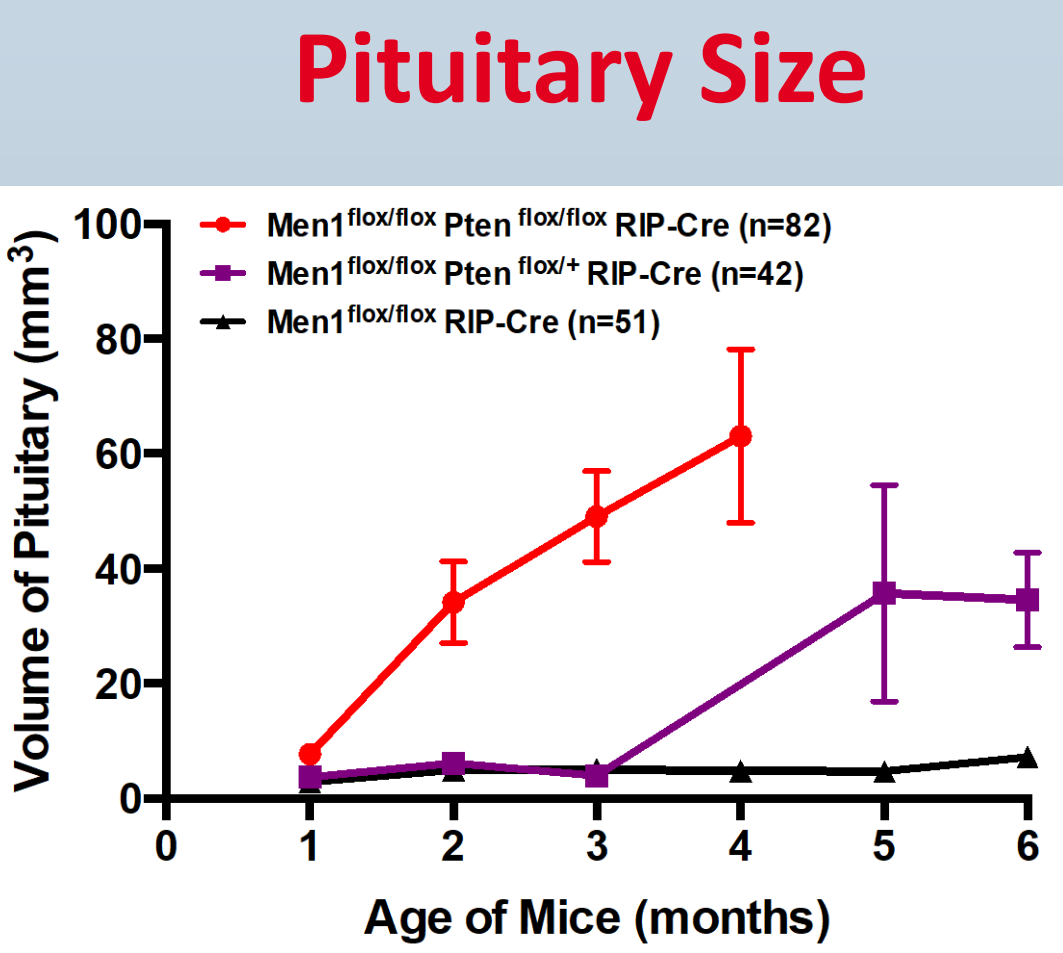
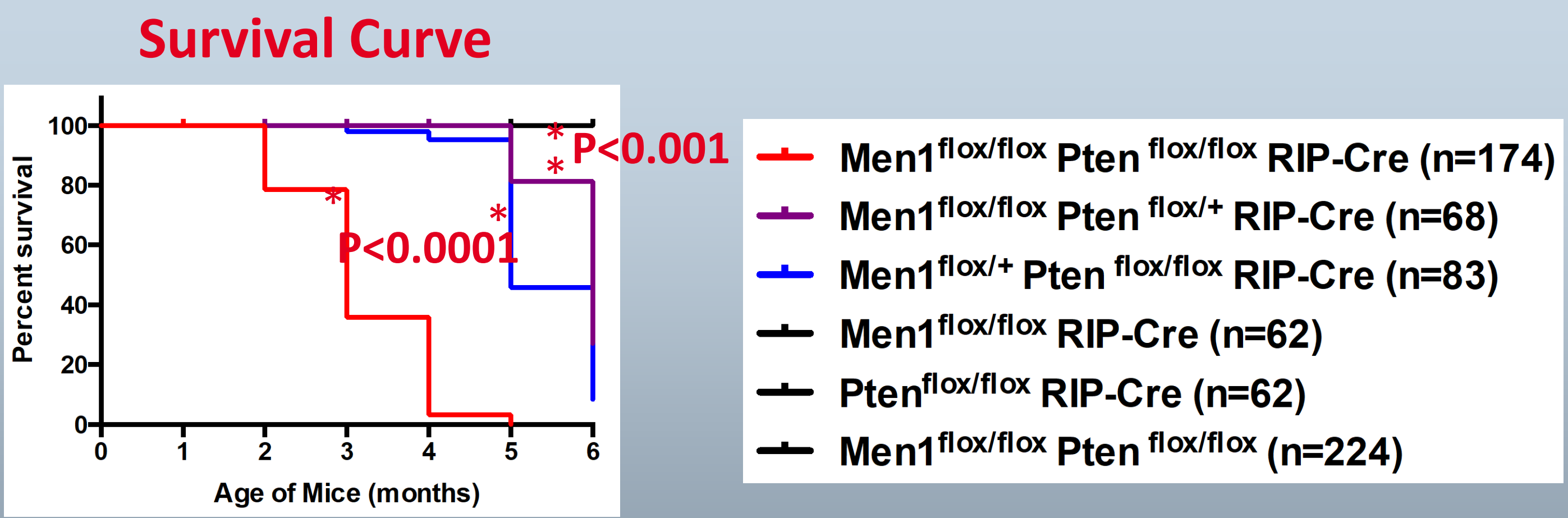


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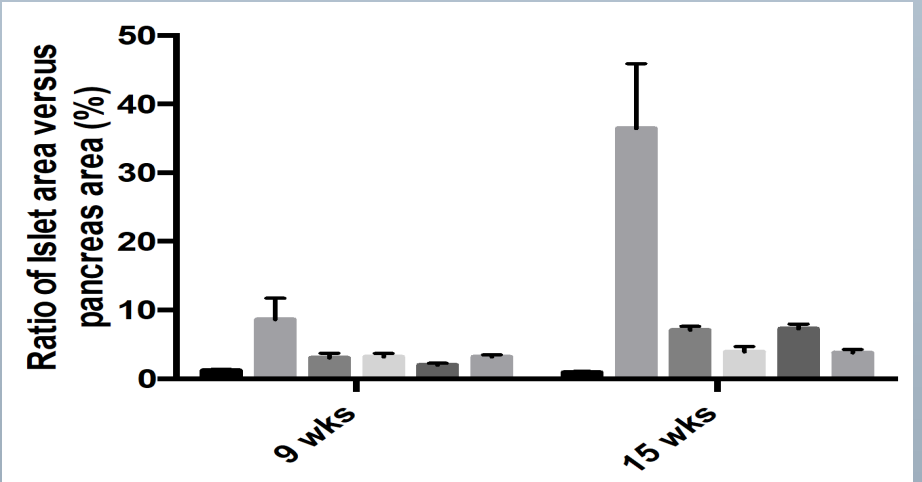
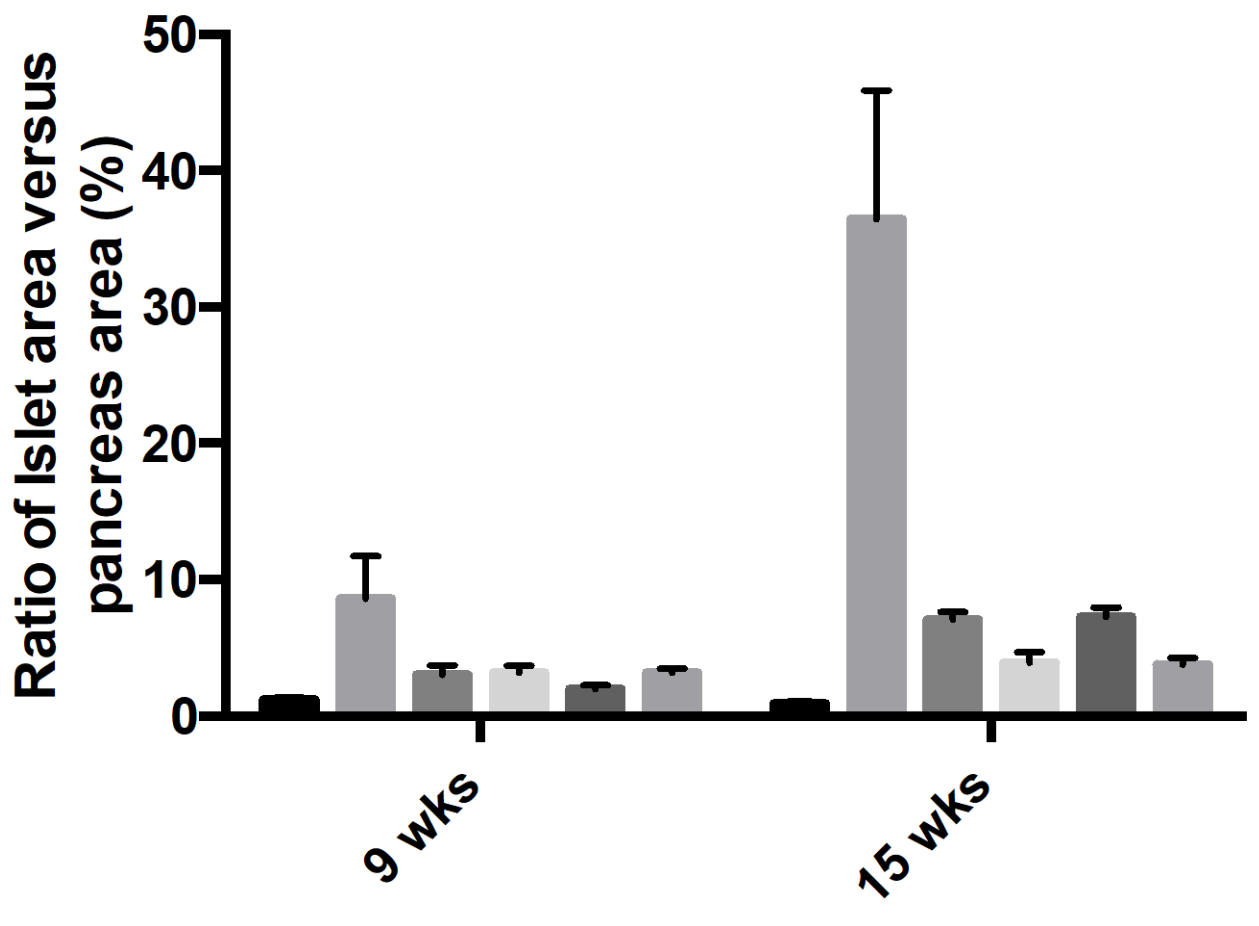
Figure 3. Gene dosage-dependent NE tumor suppression in Men1 Pten mice



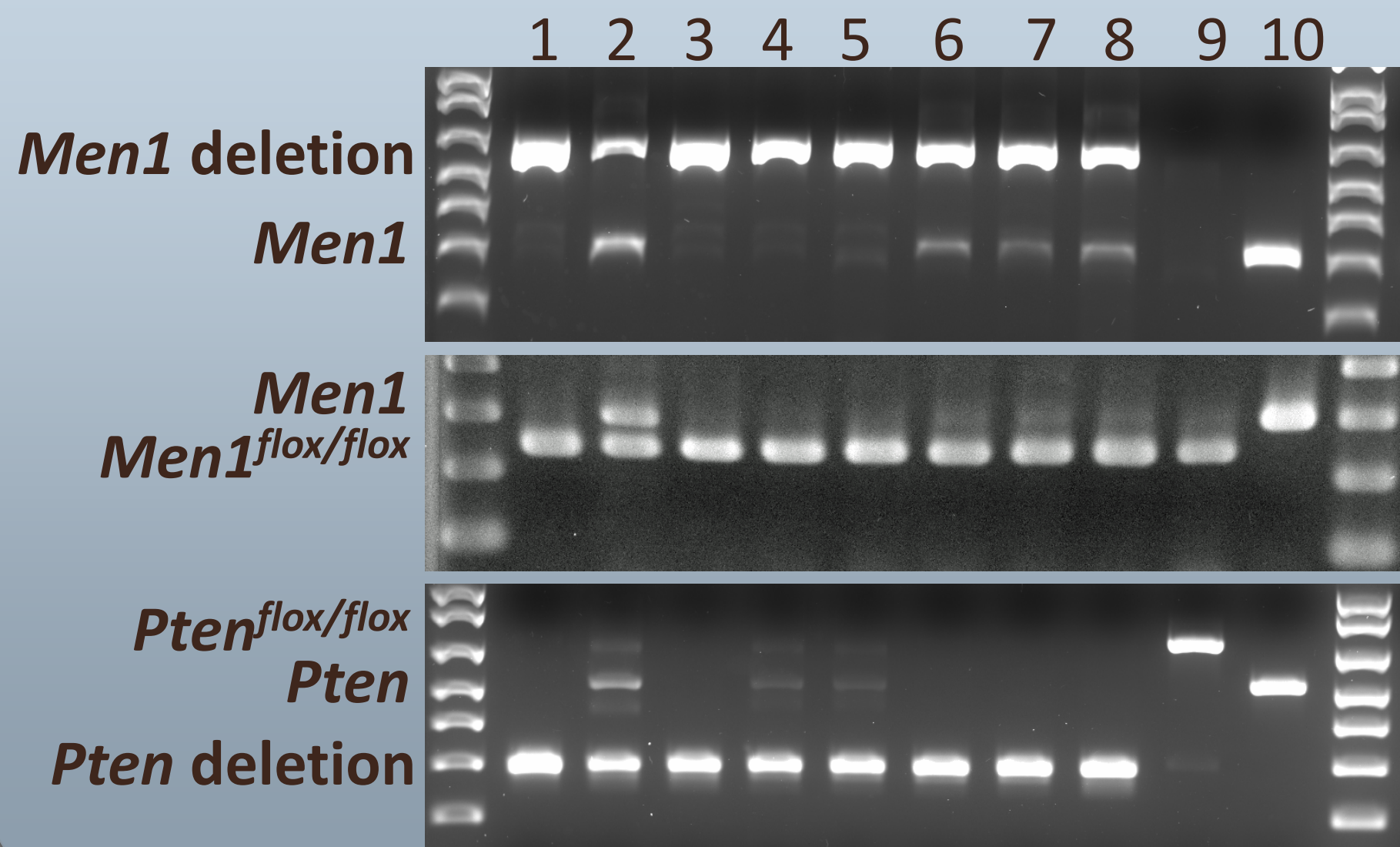
Life Span due to development of PitNETs:
MPR < Men1^{+/-flox} Pten^{flox/flox} RIP-Cre < Men1^{flox/flox} Pten^{+/-flox} RIP-Cre < Men1^{flox/flox} RIP-Cre or Pten^{flox/flox} RIP-Cre

Biallelic deletion of Pten promoted islets lesions greatly than heterozygous deletion of Pten in deficiency of Men1;
Similarly, biallelic deletion of Men1 promoted islets lesions greatly than heterozygous deletion of Men1 in deficiency of Pten.

% of islet area versus pancreas area



Loss of heterozygosity (LOH) of WT Men1 or Pten resulted in earlier onset of PitNETs in Men1^{+/-flox} Pten^{flox/flox} RIP-Cre or Men1^{flox/flox} Pten^{+/-flox} RIP-Cre, respectively



- 1.MPR
- 2.Men1^{+/-flox} Pten^{+/-flox} RIP-Cre
- 3.Men1^{+/-flox} Pten^{flox/flox} RIP-Cre
- 4.Men1^{+/-flox} Pten^{flox/flox} RIP-Cre
- 5.Men1^{+/-flox} Pten^{flox/flox} RIP-Cre
- 6.Men1^{flox/flox} Pten^{+/-flox} RIP-Cre
- 7.Men1^{flox/flox} Pten^{+/-flox} RIP-Cre
- 8.Men1^{flox/flox} Pten^{+/-flox} RIP-Cre
- 9.Men1^{flox/flox} Pten^{flox/flox}
- 10.RIP-Cre

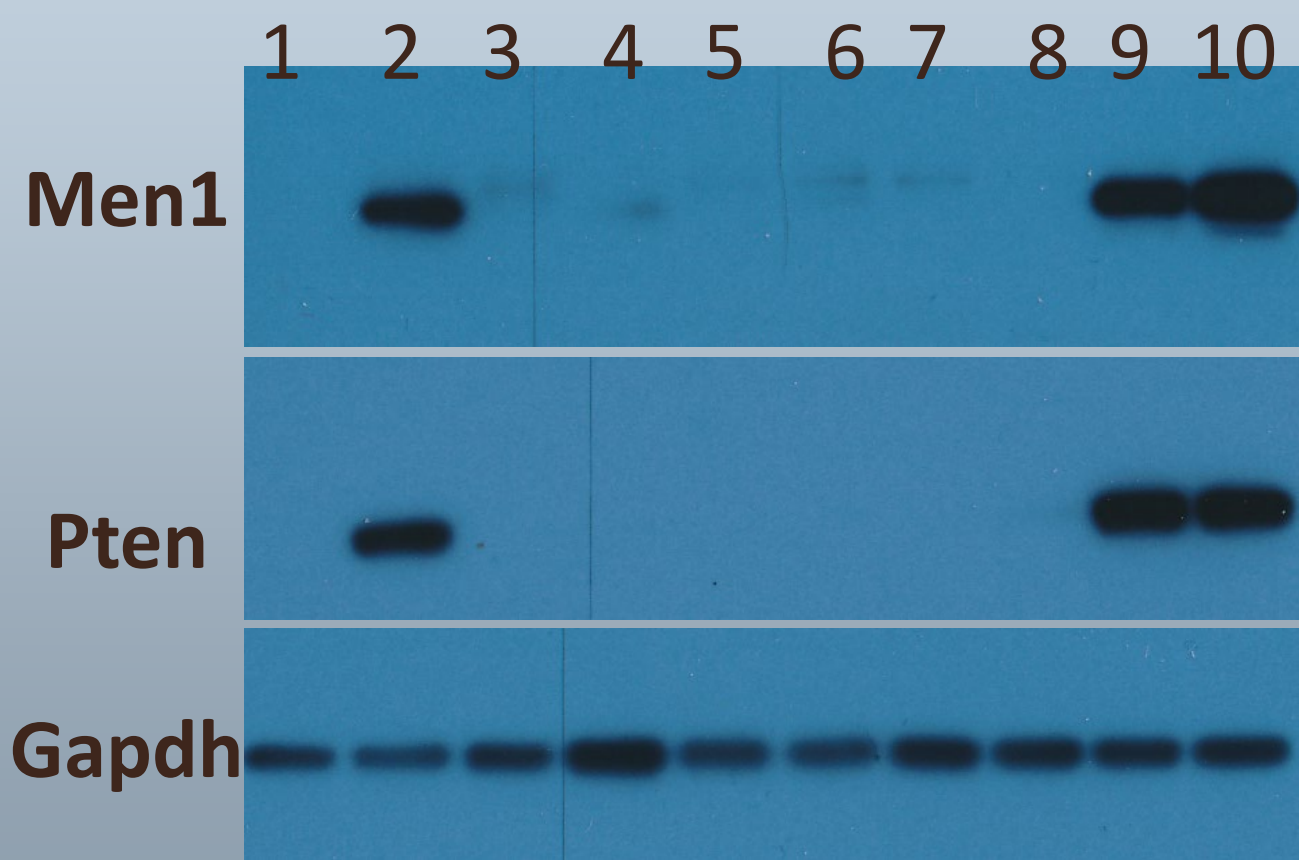


Figure 4. Rapamycin treatment results in delayed growth of PanNETs and PitNETs in MPM mouse model

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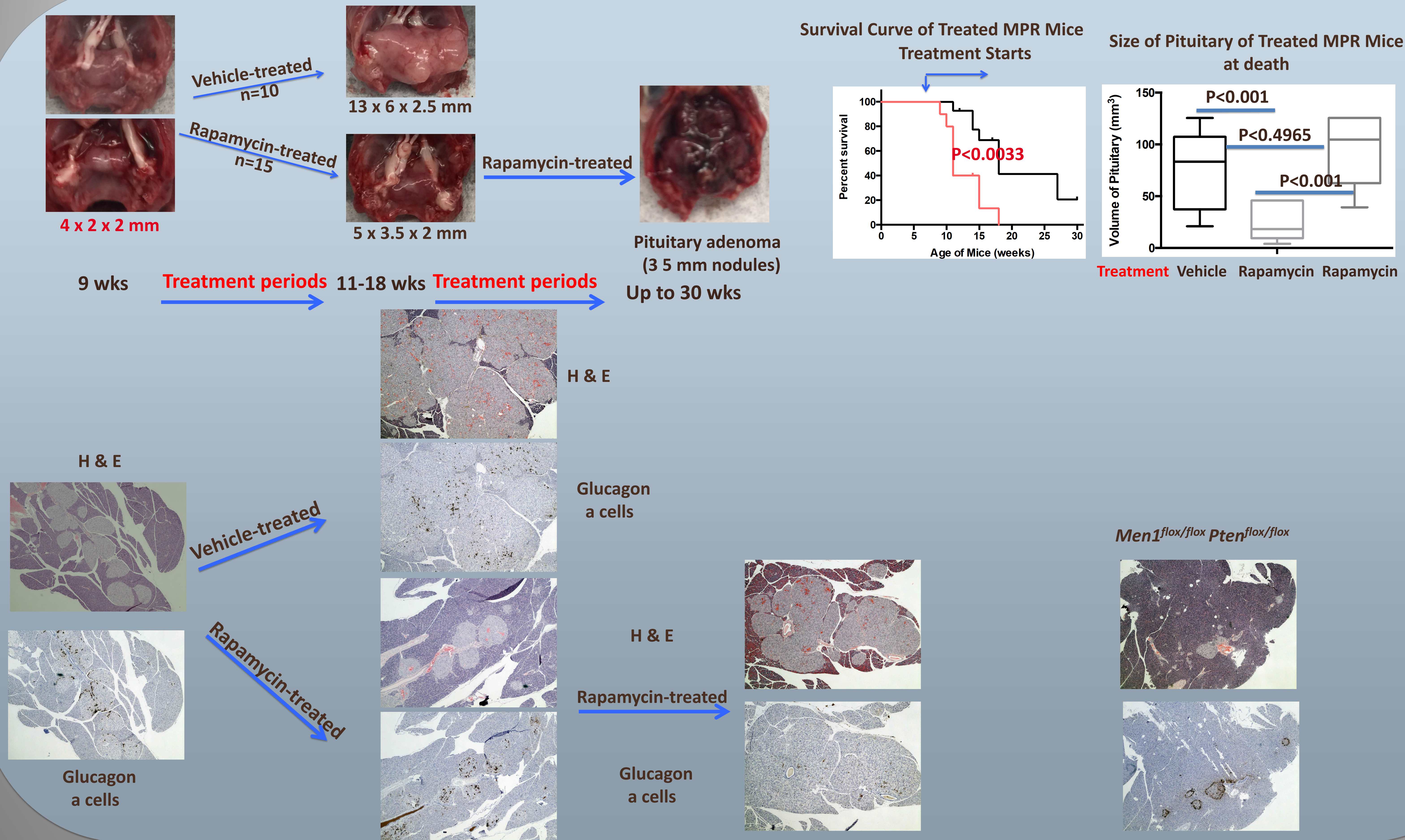
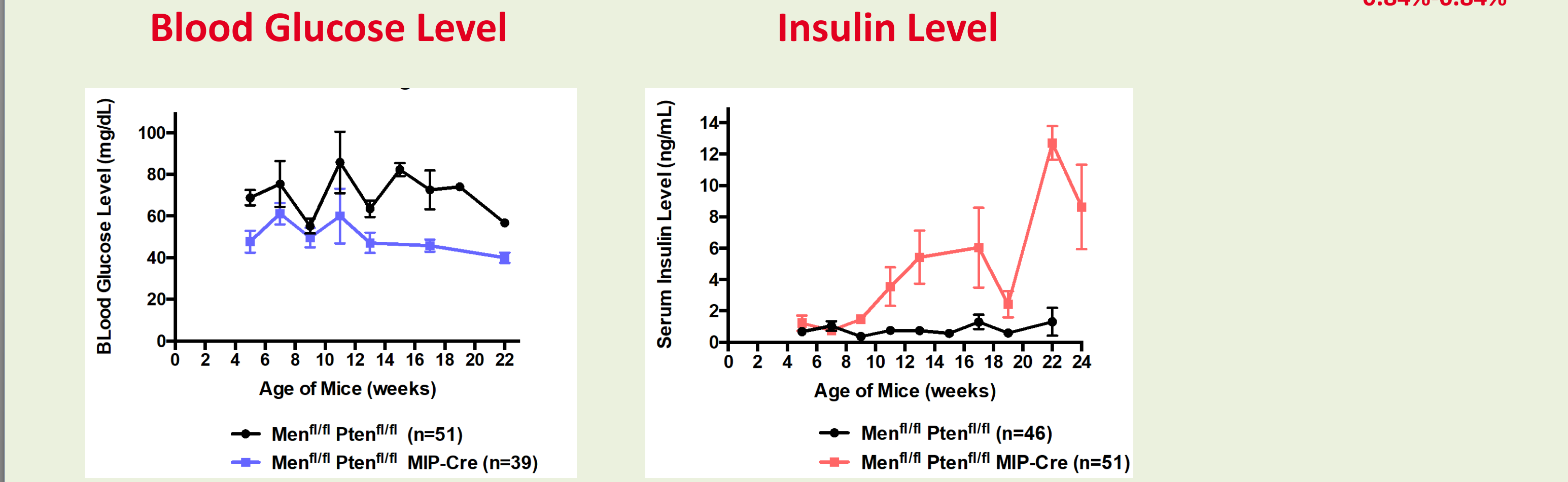
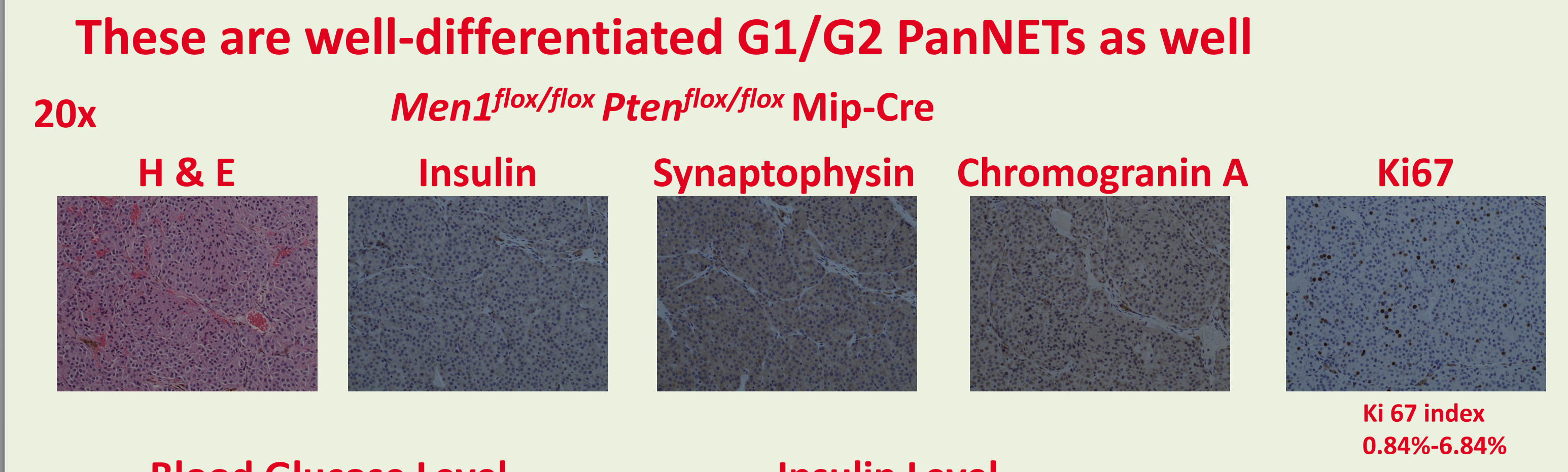
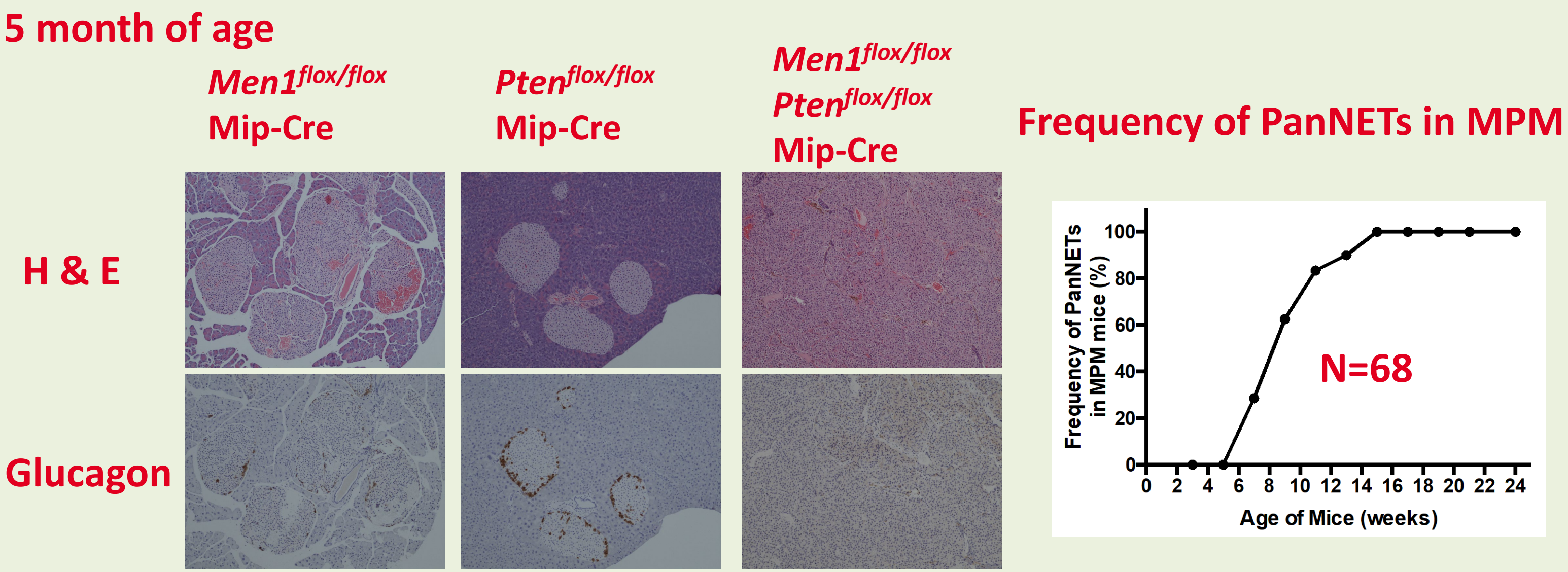
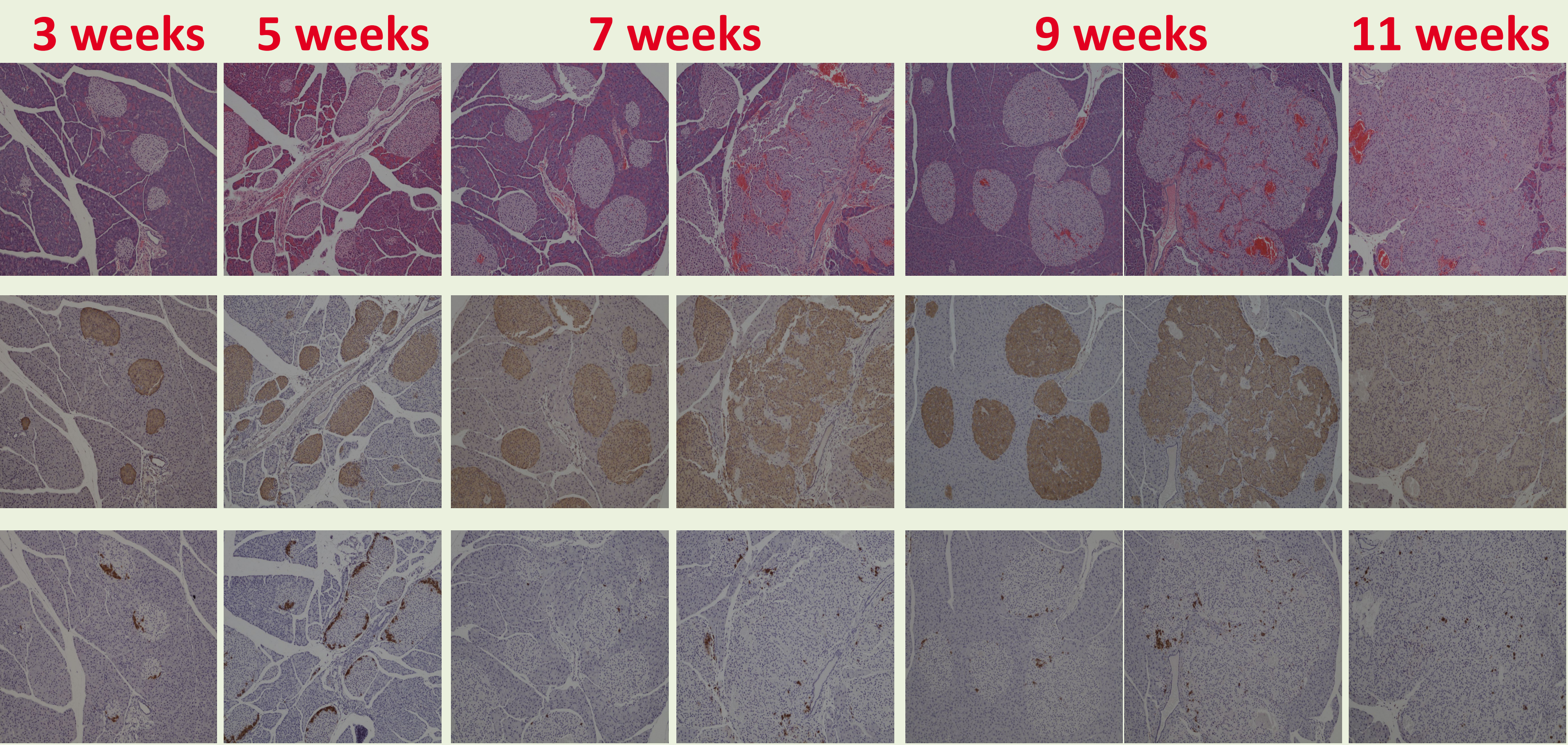


Figure 5. PTEN loss accelerates PanNETs in deficiency of Menin in MPM mouse model.



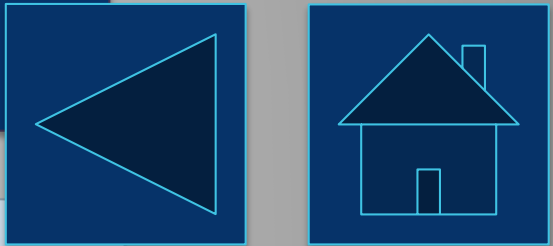
Islet lesions as mice age



RIP-Tag & Men1 Mouse Modles vs.
MPR/MPM Mouse Models

	RIP-Tag	Men1	MPR/MPM
Tumors	WD- PanNET G3 or PD- PanNETs	WD-PanNETs G1	WD-PanNETs G1/G2
Deletion of Tumor Suppressors	Rb, P53 and others	Men1 and others	Men1 and Pten
Co-mutations of tumor suppressors in human PanNETs	None	Yes, common	Yes, but rare
Onset of tumor	10-14 weeks of age	23 weeks of age	7-9 weeks of age
Mimic human disease	No	Yes	Yes
Preclinical trial feasibility	Yes	No	Yes

Models for function of Men1 and Pten

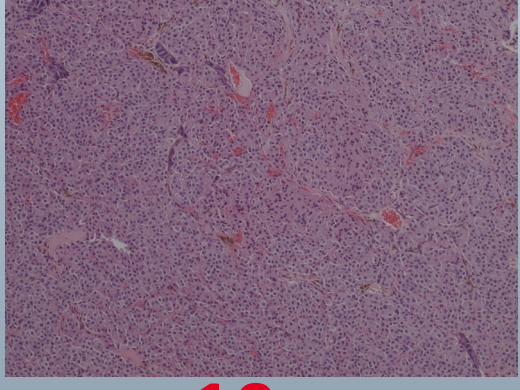
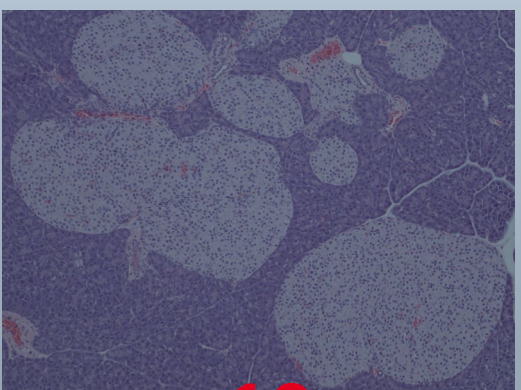
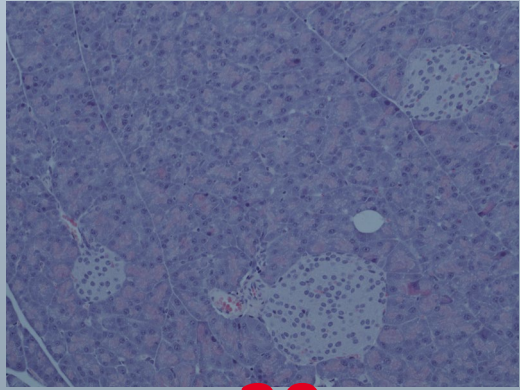


Model for PanNET development

Normal islets

Hyperplastic islets

Neoplastic islets



20x

10x

10x

Normal stage

adenomatosis stage

Tumor stage

